공학박사 학위논문

Clinical Application of Multi-protein Biomarker Assay in Classifying Risk Subgroups of Pancreatic Ductal Adenocarcinomas using Quantitative Proteomics

정량 단백체학 기술을 이용한 췌관선암 조직에서의 위험군 예측용 다중 바이오마커 어세이 개발

2021년 8월

서울대학교 대학원
협동과정 바이오엔지니어링 전공

손 민 수

Ph.D. Dissertation of Engineering

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by

Minsoo Son

August 2021

Interdisciplinary Program in Bioengineering
The Graduate School
Seoul National University

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Abstract

Clinical Application of Multi-protein Biomarker Assay in Classifying Risk Subgroups of Pancreatic Ductal Adenocarcinomas using Quantitative Proteomics

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Purpose:

Pancreatic ductal adenocarcinoma (PDAC) subtypes have been identified using various methodologies. However, it is a challenge to develop classification system applicable to routine clinical evaluation. We aimed to identify risk subgroups based on molecular features and develop a classification model that was more suited for clinical applications.

Experimental Design:

We collected whole dissected specimens from 225 patients who underwent surgery at Seoul National University Hospital, between October 2009 and February 2018, in Korea. Target proteins with potential relevance to tumor progression or prognosis were quantified with robust quality controls. We used hierarchical clustering analysis to identify risk subgroups. A random forest classification model was developed to predict the identified risk subgroups, and the model was validated using transcriptomic datasets from external cohorts (N = 700), with survival analysis.

Results:

We identified 24 protein features that could classify the four risk subgroups associated with patient outcomes: Stable; Exocrine-like; Activated; and Extracellular matrix (ECM)-Remodeling. The “Stable” risk subgroup was characterized by proteins that were associated with differentiation and tumor suppressors. “Exocrine-like” tumors highly expressed pancreatic enzymes. Two high-risk subgroups, “Activated” and “ECM-Remodeling,” were enriched in such terms as cell cycle, angiogenesis, immuno-competence, tumor invasion-metastasis, and metabolic reprogramming. The classification model that included these features made prognoses with relative accuracy and precision in multiple cohorts.

Conclusions:

We proposed PDAC risk subgroups and developed a classification model that may potentially be useful for routine clinical implementations, at the individual level. This clinical system may improve the accuracy of risk prediction and treatment guidelines.

Keyword : Pancreatic Ductal Adenocarcinomas; Risk Subgroups; Prognostic Biomarkers; Classification model; Method Validation; MRM-MS

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List of Abbreviation

ADEX, aberrantly differentiated endocrine-exocrine
AU, approximately unbiased
AUC, area under the curve
CI, confidence interval
CPTAC, Clinical Proteomic Tumor Analysis Consortium
CV, coefficient of variance
DEPs, differentially expressed proteins
DFS, disease-free survival
ECM, extracellular matrix
FFPE, formalin-fixed paraffin-embedded
IHC, immunohistochemistry
IPMN, intraductal papillary mucinous neoplasia
LLOQ, lower limit of quantitation
MRM-MS, multiple reaction monitoring-mass spectrometry
OS, overall survival
PCA, principal component analysis
PDAC, pancreatic ductal adenocarcinoma
PEI, pancreatic exocrine insufficiency
PERT, pancreatic enzyme replacement therapy
QC, quality control
RF, random forest
RTKs, receptor tyrosine kinases
SDC, sodium deoxycholate
SIS, stable isotope-labeled standard
TCGA, The Cancer Genome Atlas
WGCNA, weighted gene correlation network analysis

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General Introduction

Advances in medical technology and research have improved treatment outcomes for many cancers; however, pancreatic ductal adenocarcinoma (PDAC) prognosis remains dismal, with an overall survival rate of approximately 10%(1). The low survival rate associated with PDAC can primarily be attributed to the difficulty performing early diagnosis, which results in only 20% of patients being eligible for resection at the time of diagnosis, even when including borderline cases and locally advanced post-chemotherapy pancreatic cancer cases(2-4). Additionally, PDAC is prone to recurrence and metastasis, even after surgery, due to its tumorigenic biology. Although radiologic images and pathological data are currently the primary methods used to determine treatment strategies and identify tumor characteristics, the development of superior methods for characterizing tumors and facilitating the early diagnosis of cancer are urgently necessary to improve the PDAC survival outcome(5,6).

Protein biomarkers are important in the pathogenesis of various diseases. However, they are rarely used in clinical practice due to their low diagnostic power (7,8). Based on the wide variety of individual circumstances (physiological, environmental, and clinical factors) and the complexity of disease-inducing processes, single-protein markers are unable to diagnose specific diseases accurately (9,10). These issues can be remedied through the use of several protein markers, improving their diagnostic power and accuracy by increasing sensitivity and specificity (11-13).

Multiple reaction monitoring-mass spectrometry (MRM-MS), a high-throughput '-omics' technologies can simultaneously measure the abundance of protein targets on the order of thousands and assess their feasibility as biomarkers (14,15). Although hundreds of MRM-MS assays have been published, the protocols for characterizing their performance have not been standardized. Further, their reproducibility and transferability

across various platforms and sites have not been demonstrated. To address these problems, the Clinical Proteomic Tumor Analysis Consortium of the National Cancer Institute (NCI-CPTAC) has evaluated the intra- and interlaboratory analytical performance of the MRM-MS assay (16-18) and developed guidelines for analytical validation using a fit-for-purpose approach (19). CPTAC assay characterization guidelines were established to evaluate clinical assays by observing quantitative changes across various targets, and the CPTAC assay portal was designed as a public repository of well-characterized, MS-based, targeted proteomics assays to help users of different instruments between laboratories developing new assays (20).

Clinical applications of risk prediction for pancreatic cancers have remained stagnant due to some obstacles. The hundreds of genes used to define the subtypes can cause overfitting that hindered prediction and transferability of subtypes. In addition, the methodologies, such as micro-array, RNA-Seq, isobaric-tagging, and high-pH fractionations, are clinically unsuitable due to their high levels of complexity, expensive costs, and long run-times. A clinical assay must be developed, similar to the MammaPrint(21) and PAM50(22) assays that have been developed for the classification of breast cancer risks based on dozens of genes, and utilize a simple and affordable quantitation platform. Furthermore, predicting the risk for an individual patient has been difficult because most classification methodologies are based on clustering analyses.

In the present study, we characterized method validation of protein quantitation using MRM-MS for clinical application, based on the CPTAC Assay Characterization Guidance Document. Then, using the methodology, we identified risk subgroups and developed a classification model, based on 24 carefully selected proteins, to address the current limitations.

Chapter 1

Method Validation by CPTAC Guidelines for Multi-protein Marker Assays Using Multiple Reaction Monitoring-Mass Spectrometry

1.1 Introduction

For nearly a decade, hundreds of millions of dollars have been spent annually on the discovery of putative protein biomarkers for human diseases (23). Changes in protein expression in biospecimens are reflective of specific disease states (24). Thus, *in vitro* diagnostic tests that use protein biomarkers are useful for the early detection of human diseases, and the prediction of patient prognosis (25,26).

Traditionally, the most frequently used technique for quantifying proteins has been the enzyme-linked immunosorbent assay (ELISA). Its high sensitivity, specificity, and compatibility with standard clinical laboratory equipment render ELISA suitable for clinical practice (27,28). However, this method is expensive and laborious due to the time that is needed to develop antibodies. Moreover, multiplex ELISA encounters difficulties quantitating proteins in parallel due to the crossreactivity that results from multiple antibodies interacting with one another (29,30).

In contrast, high-throughput '-omics' technologies can simultaneously measure the abundance of protein targets on the order of thousands and assess their feasibility as biomarkers (14,15). One such method is multiple reaction monitoring-mass spectrometry (MRM-MS). It is a highly selective and sensitive approach for quantitating targeted proteins in samples—a potential alternative tool for screening diseases. MRM-MS is a targeted proteomics technology that can simultaneously measure more than 300 protein targets in a cost-effective manner (31). In addition, MRM-MS does not require antibodies and can circumvent the issue of crossreactivity altogether, unlike multiplex ELISA (31,32).

Here, we report the MRM-MS assays that were characterized, based on the 5 criteria in the CPTAC Assay Characterization Guidance Document, established to evaluate clinical assays by observing quantitative changes across various targets. The assays cover 29 disease-related proteins, consisting of US Food and Drug Administration (FDA)-approved and

Laboratory Development Test (LDT) markers that are used in quantitative assays. The performance of the assays was verified by comparison with techniques in the CPTAC ASSAY PORTAL (https://assays.cancer.gov). This study demonstrates that our MRM-MS assay is a reproducible and transferrable analytical platform for targeted proteomics analysis that has potential for preclinical applications.

1.2 Materials and Methods

1.2.1 Reagents and Materials

Dithiothreitol was purchased from Merck (Darmstadt, Germany). Iodoacetamide was purchased from Sigma-Aldrich (St. Louis, MO, USA), Rapigest™ SF Surfactant was obtained from Waters (Manchester, UK). Sequencing-grade modified trypsin was obtained from Promega (Madison, WI, USA). High-performance liquid chromatography (HPLC)-grade water, formic acid, and acetonitrile were purchased from Thermo Fisher Scientific (Bremen, Germany). The digested samples were desalted using OASIS HLB 1 cc (30 mg) extraction cartridges (Waters Corp., Milford, MA). Semi-absolute stable isotope-labeled standard (SIS) peptides (isotope labeled at the C-terminus of ^{13}C and ^{15}N) were synthesized by JPT (Berlin, Germany) (85% to 95% purity, according to the manufacturer).

1.2.2 Human serum samples

A set of 6 individual normal serum samples and pooled normal serum were purchased from an FDA-approved facility (Innovative Research, MI, USA). All samples tested negative for human immunodeficiency virus (HIV)-1 ribonucleic acid (RNA), anti-HIV 1/2, hepatitis C virus (HCV), HCV RNA, hepatitis B virus surface antigen (HbsAg), and syphilis using FDA-approved methods by the provider.

1.2.3 Sample preparation for MRM-MS analysis

All samples were depleted of high-abundance proteins on a Multiple Affinity Removal System Human-7 affinity column that was interfaced with an HPLC system (Shimadzu, Kyoto, Japan). Depleted serum samples were concentrated, and 100 µg of depleted serum was denatured with 0.1% Rapigest. After the trypsin digestion and desalting steps, the eluted samples were lyophilized on a vacuum centrifuge. All serum digests were resolubilized in 0.1% formic acid/water prior to analysis via MRM-MS (more details are given in Supporting Information).

1.2.4 Quantitative MRM-MS analysis

We used a 5-µL sample loop (100-µm inner diameter capillary tubing) in an autosampler and performed full-loop injection to reduce void volume. For the MRM-MS analysis, a 150-mm homemade analytical column was used—an IntegraFrit capillary (ID, 75 µm; OD, 360 µm) was packed with ReproSil-Pur C18-AQ (5 µm, 200 Å, Dr.Maisch GmbH, Ammerbuch, Germany). We injected 5 µL (1 µg desalted samples) of prepared sample with Sol A (97% distilled water, 3% acetonitrile, and 0.1% formic acid) and Sol B (97% acetonitrile, 3% distilled water, and 0.1% formic acid) directly into the analytical column; the flow rate was set to 300 nL/min, and exponential gradient elution was performed with a 55-min gradient, consisting of 0% to 7% Sol B for 3 min, 7% to 37% sol B for 35 min, 37% to 87% sol B for 1 min, 87% sol B for 5 min, and 0% sol B for 9 min, to equilibrate the column for the following run.

MRM-MS analyses were performed on a hybrid triple quadrupole/ion trap mass spectrometer (QTRAP 4000, AB SCIEX, Foster City, CA, USA) that was coupled to a tempo multidimensional liquid chromatography system (Tempo MDLC, Applied Biosystems, CA, USA). The optimal parameters for the mass spectrometer that was interfaced with a nanospray source were set as follows: ion spray, 2400 V; source temperature, 180°C; high collision

gas, approximately 4×10^{-5} torr; and curtain gas, 25 μL/min. MS parameters for declustering potential were determined using Skyline, and collision energy parameters were generated by optimization in ramping collision energy mode (step size 1, step count 5).

1.2.5 CPTAC assay guidance

The CPTAC assay guidelines consist of 5 experimental items: response curve, repeatability, selectivity, stability, and reproducibility. Under these guidelines, only the first 2 experimental items are required to be uploaded to the CPTAC portal, and the first 4 experimental items should be completed for an assay to be recognized with a higher level of qualification in the CPTAC program (20).

The purpose and criteria of the 5 itemized experiments were as follows. The “response curve” evaluates the linearity of the target peptides and assesses several analytical properties, including limit of detection (LOD), limit of quantitation (LOQ), and lower limit of quantitation (LLOQ). “Repeatability” examines intra- and interday variability using 3 quality control (QC) samples (low, medium, and high). “Selectivity” evaluates whether endogenous components interfere with the detection of the target peptides using 3 QC samples (blank, 1/2 medium, and medium) in 6 different biological matrices of the same matrix type. “Stability” assesses the stability of target peptides under 4 storage conditions (0 h, 6 h, 24 h after at 4°C, and 28 d after at -80°C) and up to 2 freeze-thaw cycles. Finally, “Reproducibility” measures the reproducibility of the overall process of quantifying an endogenous analyte.

1.2.6 Data processing

The raw MRM-MS data were processed to calculate the peak areas of transitions using Skyline, ver. 3.6 (MacCoss Lab, University of Washington, USA) (33). Peak integration was performed manually. Figures and tables were created from the peak area ratios of stable isotope-labeled standard (SIS) peptides to endogenous peptides using Excel, ver. 2013 (Microsoft Corp., WA, USA) and R, ver. 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria). Interference screening of peptides was performed using the automated detection of inaccurate and imprecise transitions (AuDIT) algorithm (34).

1.2.7 Data comparison with public repository data

The assays in our study were compared with those that have been uploaded to the CPTAC ASSAY PORTAL (https://assays.cancer.gov) for 2 experimental items: Response curves and Repeatability. A total of 1582 assays for 830 unique proteins and 1503 unique peptides have been uploaded since the portal was launched. We downloaded and used data on the assays for 193 unique proteins and 350 unique peptides, which were validated using plasma or serum specimens and 4 assay platforms and laboratories: the Broad institute (Broad), Fred Hutchison Cancer Research Center (FHCRC), Johns Hopkins University (JHU), and University of Victoria Genome British Columbia Proteomics Centre (UVGPC).

1.3 Results

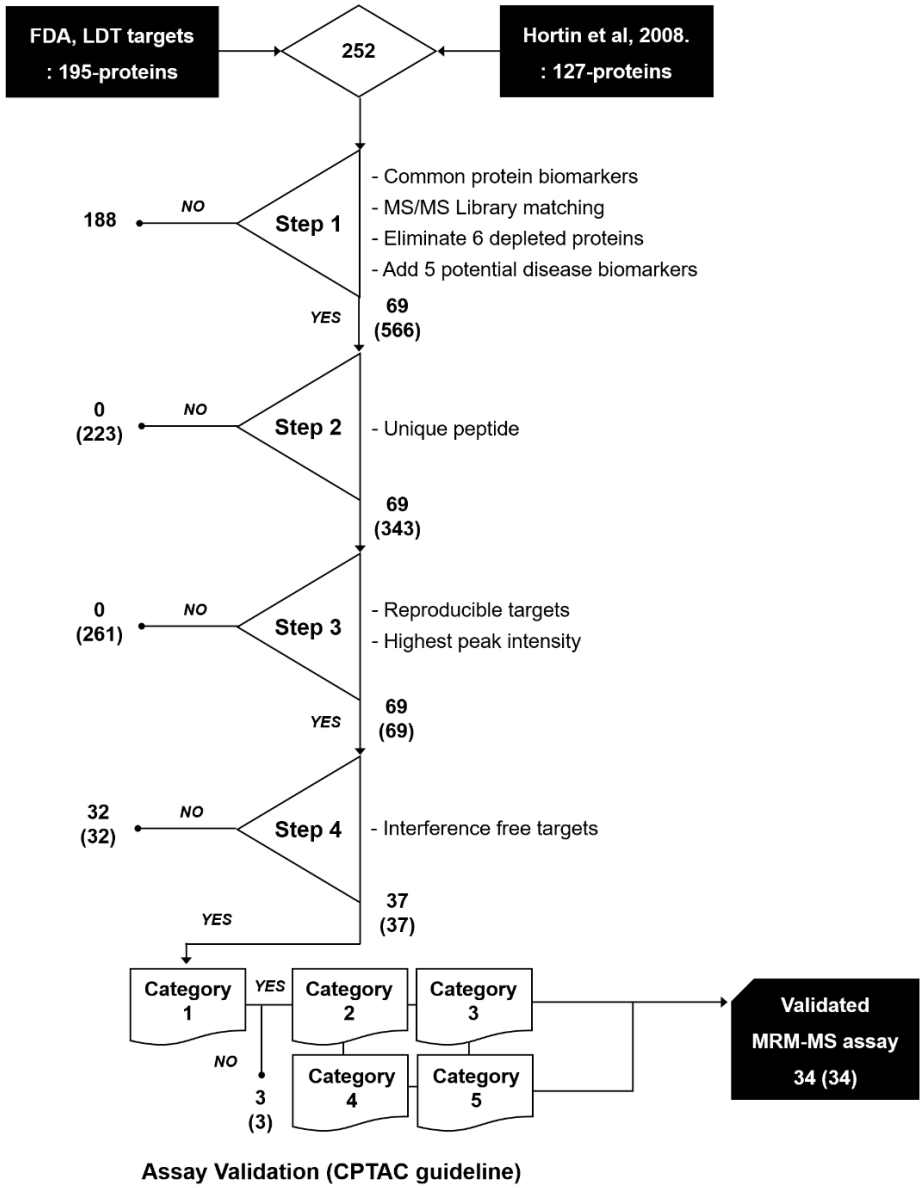
1.3.1 Target peptide selection

We selected the 195 protein biomarkers that are FDA-approved or Laboratory Development Test (LDT)-approved as the initial targets (7). Five additional proteins that were not on the lists were included with the initial targets to characterize novel targets in the diagnosis of the disease. We decided not to synthesize unlabeled synthetic peptide (internal standard), which could have increased the intensity of the endogenous peptide (or light peptide). Therefore, of the 200 initial targets, 75 high-abundance proteins that had high-intensity light peptides were selected to minimize the influence of light peptides on the variability in peak area ratio (PAR) (35). Six proteins that were influenced by depletion were removed from the 75 proteins (Table 1-1) (36). Peptides that were derived from the 69 initial targets were filtered by empirical evidence of MS detectability, based on cross-referencing of MS/MS spectra in the National Institute of Standards and Technology MS/MS libraries (37). Up to 10 proteolytic peptides were selected per protein, and a total of 566 peptides were selected to represent the 69 proteins in subsequent analyses. Unique peptides were chosen by aligning the target peptide sequences to the corresponding regions of source proteins with the BLAST tool, National Center for Biotechnology Information (38). As a result, 69 proteins and 343 unique peptides were selected for further analysis.

The next step involved high-throughput quantification to establish sensitive and accurate assays for peptide measurements. We were unable to afford SIS peptides for all 343 surrogate peptides. Thus, to select the best peptides for quantitative analysis, a pooled normal serum sample without an internal standard was analyzed in triplicate, which led to the selection of 1 peptide (69 peptides) per protein with high reproducibility [coefficient of variation (CV) < 20%] and peak intensity. Ultimately, 69 SIS peptides were synthesized prior to the MRM-MS assay development. Of the 69 peptides

that were surveyed, 37 peptides, corresponding to 37 proteins, were verified to be interference-free in the pooled normal serum sample. (Table 1-2). The other 32 peptides did not meet the criteria of the AuDIT algorithm (34). The overall scheme is presented in Figure 1-1, and more details are supplied in Supporting Information.

Figure 1-1. Workflow for selecting targets.



Workflow for building and validating the MRM-MS assay with the appropriate targets following CPTAC guidelines, showing the number of proteins (peptides). Of the 252 proteins from Anderson's group (FDA or LDT targets) and Hortin's group (high concentrations in the blood), the 64 proteins representing the 548 tryptic peptides were common to both references. The 69 unique peptides were selected depending on reproducible detection and highest peak intensity. With the 37 targets detected by interference free-transition, the analytical method was validated following CPTAC guidelines, comprising 5 experimental categories. The 34 proteins were finally validated by MRM-MS assay.

Table 1-1. List of target proteins for MRM-MS assay.

N.	Gene Symbol	Uniprot ID	Uniprot Accession	Protein name	FDA or LDT targets (195)	Hortin et al, 2008 (127)	Initial targets (75)
1	A1BG	A1BG	P04217	Alpha-1B-glycoprotein	N	Y	N
2	A2M	A2MG	P01023	Alpha-2-macroglobulin	Y	Y	Y
3	ACE	ACE	P12821	Angiotensin-converting enzyme	Y	N	N
4	ACPP	PPAP	P15309	Prostatic acid phosphatase	Y	N	N
5	ADA	ADA	P00813	Adenosine deaminase	Y	N	N
6	ADAMTS13	ATS13	Q76LX8	A disintegrin and metalloproteinase with thrombospondin motifs 13	Y	N	N
7	ADIPOQ	ADIPO	Q15848	Adiponectin	Y	Y	Y
8	AFM	AFAM	P43652	Afamin	N	Y	N
9	AFP	FETA	P02771	Alpha-fetoprotein	Y	N	N
10	AGT	ANGT	P01019	Angiotensinogen	Y	Y	Y
11	AHSG	FETUA	P02765	Alpha-2-HS-glycoprotein	Y	Y	Y
12	ALB	ALBU	P02768	Serum albumin	Y	Y	Y
13	ALDOA	ALDOA	P04075	Fructose-bisphosphate aldolase A	Y	N	N
14	ALPL	PPBT	P05186	Alkaline phosphatase, tissue-nonspecific isozyme	Y	N	N
15	AMBP	AMBP	P02760	Protein AMBP [Cleaved into: Alpha-1-microglobulin	N	Y	Y[a]
16	AMH	MIS	P03971	Muellerian-inhibiting factor	Y	N	N
17	AMY2A	AMYP	P04746	Pancreatic alpha-amylase	Y	N	N
18	APCS	SAMP	P02743	Serum amyloid P-component	N	Y	N
19	APOA1	APOA1	P02647	Apolipoprotein A-I	N	Y	Y
20	APOA2	APOA2	P02652	Apolipoprotein A-II	N	Y	Y[a]
21	APOA4	APOA4	P06727	Apolipoprotein A-IV	N	Y	N
22	APOB	APOB	P04114	Apolipoprotein B-100	Y	Y	Y
23	APOC1	APOC1	P02654	Apolipoprotein C-I	N	Y	N
24	APOC2	APOC2	P02655	Apolipoprotein C-II	N	Y	N
25	APOC3	APOC3	P02656	Apolipoprotein C-III	N	Y	N
26	APOC4	APOC4	P55056	Apolipoprotein C-IV	N	Y	N
27	APOD	APOD	P05090	Apolipoprotein D	N	Y	N
28	APOE	APOE	P02649	Apolipoprotein E	N	Y	Y[a]
29	APOF	APOF	Q13790	Apolipoprotein F	N	Y	N
30	APOH	APOH	P02749	Beta-2-glycoprotein 1	N	Y	Y[a]
31	APOL1	APOL1	O14791	Apolipoprotein L1	N	Y	N
32	APOM	APOM	O95445	Apolipoprotein M	N	Y	N
33	APP	A4	P05067	Amyloid beta A4 protein	Y	N	N
34	AZGP1	ZA2G	P25311	Zinc-alpha-2-glycoprotein	N	Y	N
35	B2M	B2MG	P61769	Beta-2-microglobulin	Y	Y	Y
36	BCHE	CHLE	P06276	Cholinesterase	Y	Y	Y
37	BGLAP	OSTCN	P02818	Osteocalcin	Y	N	N
38	BTD	BTD	P43251	Biotinidase	Y	N	N

39	C1QA	C1QA	P02745	Complement C1q subcomponent subunit A	Y	Y	Y
40	C1QB	C1QB	P02746	Complement C1q subcomponent subunit B	Y	Y	Y
41	C1QC	C1QC	P02747	Complement C1q subcomponent subunit C	Y	Y	Y
42	C1R	C1R	P00736	Complement C1r subcomponent	Y	Y	Y
43	C1S	C1S	P09871	Complement C1s subcomponent	Y	Y	Y
44	C2	CO2	P06681	Complement C2	Y	Y	Y
45	C3	CO3	P01024	Complement C3	Y	Y	Y
46	C4A	CO4A	P0C0L4	Complement C4-A	Y	Y	Y
47	C4B	CO4B	P0C0L5	Complement C4-B	N	Y	N
48	C4BPA	C4BPA	P04003	C4b-binding protein alpha chain	N	Y	N
49	C4BPB	C4BPB	P20851	C4b-binding protein beta chain	N	Y	N
50	C5	CO5	P01031	Complement C5	Y	Y	Y
51	C6	CO6	P13671	Complement component C6	Y	Y	Y
52	C7	CO7	P10643	Complement component C	Y	Y	Y
53	C8A	CO8A	P07357	Complement component C8 alpha chain	Y	Y	Y
54	C8B	CO8B	P07358	Complement component C8 beta chain	Y	Y	Y
55	C8G	CO8G	P07360	Complement component C8 gamma chain	Y	Y	Y
56	C9	CO9	P02748	Complement component C9	Y	Y	Y
57	CALCA	CALC	P01258	Calcitonin	Y	N	N
58	CCK	CCKN	P06307	Cholecystokinin	Y	N	N
59	CEACAM5	CEAM5	P06731	Carcinoembryonic antigen-related cell adhesion molecule 5	Y	N	N
60	CFB	CFAB	P00751	Complement factor B	Y	Y	Y
61	CFD	CFAD	P00746	Complement factor D	N	Y	N
62	CFH	CFAH	P08603	Complement factor H	Y	Y	Y
63	CFHR1	FHR1	Q03591	Complement factor H-related protein 1	N	Y	N
64	CFI	CFAI	P05156	Factor I, heavy chain	N	Y	N
65	CFP	PROP	P27918	Properdin	N	Y	N
66	CGA	GLHA	P01215	Glycoprotein hormones alpha chain	Y	N	N
67	CGB3	CGB3	P0DN86	Choriogonadotropin subunit beta 3	Y	N	N
68	CHGA	CMGA	P10645	Chromogranin-A	Y	N	N
69	CKB	KCRB	P12277	Creatine kinase B-type	Y	N	N
70	CKM	KCRM	P06732	Creatine kinase M-type	Y	N	N
71	CLEC3B	TETN	P05452	Tetranectin	N	Y	N
72	CLU	CLUS	P10909	Clusterin	N	Y	N
73	CP	CERU	P00450	Ceruloplasmin	Y	Y	Y
74	CPB2	CBPB2	Q96IY4	Carboxypeptidase B2	N	Y	N
75	CR1L	CR1L	Q2VPA4	Complement component receptor 1-like protein	Y	N	N
76	CRP	CRP	P02741	C-reactive protein	Y	Y	Y
77	CSF2	CSF2	P04141	Granulocyte-macrophage colony-stimulating factor	Y	N	N
78	CSF3	CSF3	P09919	Granulocyte colony-stimulating factor	Y	N	N
79	CSH1	Q6PF11	Q6PF11	Chorionic somatomammotropin hormone 1	Y	N	N
80	CST3	CYTC	P01034	Cystatin-C	Y	Y	Y
81	CXCL8	IL8	P10145	Interleukin-8	Y	N	N

82	DDC	DDC	P20711	Aromatic-L-amino-acid decarboxylase	Y	N	N
83	EGF	EGF	P01133	Pro-epidermal growth factor	Y	N	N
84	EGFR	EGFR	P00533	Epidermal growth factor receptor	Y	N	N
85	ELA3A	Q96QL8	Q96QL8	Elastase 3A, pancreatic	Y	N	N
86	ENGASE	ENASE	Q8NFI3	Cytosolic endo-beta-N-acetylglucosaminidase	Y	N	N
87	ENO2	ENOG	P09104	Gamma-enolase	Y	N	N
88	EPO	EPO	P01588	Erythropoietin	Y	N	N
89	ERBB2	ERBB2	P04626	Receptor tyrosine-protein kinase erbB-2	Y	N	N
90	F10	FA10	P00742	Coagulation factor X	Y	Y	Y
91	F11	FA11	P03951	Coagulation factor XI	Y	Y	Y
92	F12	FA12	P00748	Coagulation factor XII	Y	Y	Y
93	F13A1	F13A	P00488	Coagulation factor XIII A chain	Y	Y	Y
94	F13B	F13B	P05160	Coagulation factor XIII B chain	Y	Y	Y
95	F2	THRB	P00734	Prothrombin	Y	Y	Y
96	F5	FA5	P12259	Coagulation factor V	Y	Y	Y
97	F7	FA7	P08709	Coagulation factor VII	Y	N	N
98	F8	FA8	P00451	Coagulation factor VIII	Y	N	N
99	F9	FA9	P00740	Coagulation factor IX	Y	Y	Y
100	FBLN1	FBLN1	P23142	Fibulin-1	N	Y	N
101	FCN2	FCN2	Q15485	Ficolin-2	N	Y	N
102	FCN3	FCN3	O75636	Ficolin-3	N	Y	N
103	FGA	FIBA	P02671	Fibrinogen alpha chain	Y	Y	Y
104	FGB	FIBB	P02675	Fibrinogen beta chain	Y	Y	Y
105	FGF2	FGF2	Q60487	Fibroblast growth factor 2	Y	N	N
106	FGG	FIBG	P02679	Fibrinogen gamma chain	Y	Y	Y
107	FN1	FINC	P02751	Fibronectin	Y	Y	Y
108	FSHB	FSHB	P01225	Follitropin subunit beta	Y	N	N
109	FTH1	FRIH	P02794	Ferritin heavy chain	Y	N	N
110	FTL	FRIL	P02792	Ferritin light chain	Y	N	N
111	GC	VTDB	P02774	Vitamin D-binding protein	Y	Y	Y
112	GGT1	GGT1	P19440	Gamma-glutamyltranspeptidase 1	Y	N	N
113	GH1	SOMA	P01241	Somatotropin	Y	N	N
114	GHR	GHR	P10912	Growth hormone receptor	Y	N	N
115	GHRH	SLIB	P01286	Somatoliberin	Y	N	N
116	GIP	GIP	P09681	Gastric inhibitory polypeptide	Y	N	N
117	GLA	AGAL	P06280	Alpha-galactosidase A	Y	N	N
118	GOT1	AATC	P17174	Aspartate aminotransferase, cytoplasmic	Y	N	N
119	GPI	G6PI	P06744	Glucose-6-phosphate isomerase	Y	N	N
120	GPLD1	PHLD	P80108	Phosphatidylinositol-glycan-specific phospholipase D	N	Y	N
121	GPT	ALAT1	P24298	Alanine aminotransferase 1	Y	N	N
122	GPX1	GPX1	P07203	Glutathione peroxidase 1	Y	N	N
123	GPX3	GPX3	P22352	Glutathione peroxidase 3	N	Y	N
124	GSN	GELS	P06396	Gelsolin	N	Y	Y[a]

125	GUSB	BGLR	P08236	Beta-glucuronidase	Y	N	N
126	HABP2	HABP2	Q14520	Hyaluronan-binding protein 2	N	Y	N
127	HBA1	HBA	P69905	Hemoglobin subunit alpha	Y	Y	Y
128	HBB	HBB	P68871	Hemoglobin subunit beta	Y	Y	Y
129	HEXA	HEXA	P06865	Beta-hexosaminidase subunit alpha	Y	N	N
130	HEXB	HEXB	P07686	Beta-hexosaminidase subunit beta	Y	N	N
131	HP	HPT	P00738	Haptoglobin	Y	Y	Y
132	HPR	HPTR	P00739	Haptoglobin-related protein	N	Y	N
133	HPX	HEMO	P02790	Hemopexin	Y	Y	Y
134	HRG	HRG	P04196	Histidine-rich glycoprotein	N	Y	N
135	ICAM1	ICAM1	P05362	Intercellular adhesion molecule 1	Y	N	N
136	IFNG	IFNG	P01579	Interferon gamma	Y	N	N
137	IGF1	IGF1	P05019	Insulin-like growth factor I	Y	N	N
138	IGF2	IGF2	P01344	Insulin-like growth factor II	Y	N	N
139	IGFBP1	IBP1	P08833	Insulin-like growth factor-binding protein 1	Y	N	N
140	IGFBP2	IBP2	P18065	Insulin-like growth factor-binding protein 2	Y	N	N
141	IGFBP3	IBP3	P17936	Insulin-like growth factor-binding protein 3	Y	Y	Y
142	IGHG2	IGHG2	P01859	Immunoglobulin heavy constant gamma 2	N	Y	N
143	IGHM	IGHM	P01871	Immunoglobulin heavy constant mu	N	Y	N
144	IGLC2	IGLC2	P0DOY2	Immunoglobulin lambda constant 2	N	Y	N
145	IL10	IL10	P22301	Interleukin-10	Y	N	N
146	IL11	IL11	P20809	Interleukin-11	Y	N	N
147	IL12A	IL12A	P29459	Interleukin-12 subunit alpha	Y	N	N
148	IL12B	IL12B	P29460	Interleukin-12 subunit beta	Y	N	N
149	IL13	IL13	P35225	Interleukin-13	Y	N	N
150	IL15	IL15	P40933	Interleukin-15	Y	N	N
151	IL16	Q9UME6	Q9UME6	Pro-interleukin-16 [Cleaved into: Interleukin-16	Y	N	N
152	IL18	IL18	Q14116	Interleukin-18	Y	N	N
153	IL1A	IL1A	P01583	Interleukin-1 alpha	Y	N	N
154	IL1B	IL1B	P01584	Interleukin-1 beta	Y	N	N
155	IL1R2	IL1R2	P27930	Interleukin-1 receptor type 2	Y	N	N
156	IL1RN	IL1RA	P18510	Interleukin-1 receptor antagonist protein	Y	N	N
157	IL2	IL2	P60568	Interleukin-2	Y	N	N
158	IL2RA	IL2RA	P01589	Interleukin-2 receptor subunit alpha	Y	N	N
159	IL3	IL3	P08700	Interleukin-3	Y	N	N
160	IL4	IL4	P05112	Interleukin-4	Y	N	N
161	IL5	IL5	P05113	Interleukin-5	Y	N	N
162	IL6	IL6	P05231	Interleukin-6	Y	N	N
163	IL7	IL7	P13232	Interleukin-7	Y	N	N
164	IL9	IL9	P15248	Interleukin-9	Y	N	N
165	INHA	INHA	P05111	Inhibin alpha chain	Y	N	N
166	INHBA	INHBA	P08476	Inhibin beta A chain	Y	N	N
167	INHBB	INHBB	P09529	Inhibin beta B chain	Y	N	N

168	INS	INS	P01308	Insulin	Y	N	N
169	ITIH1	ITIH1	P19827	Inter-alpha-trypsin inhibitor heavy chain H1	N	Y	N
170	ITIH2	ITIH2	P19823	Inter-alpha-trypsin inhibitor heavy chain H2	N	Y	N
171	ITIH3	ITIH3	Q06033	Inter-alpha-trypsin inhibitor heavy chain H3	N	Y	N
172	ITIH4	ITIH4	Q14624	Inter-alpha-trypsin inhibitor heavy chain H4	N	Y	N
173	JCHAIN	IGJ	P01591	Immunoglobulin J chain	N	Y	N
174	KLK3	KLK3	P07288	Prostate-specific antigen	Y	N	N
175	KLKB1	KLKB1	P03952	Plasma kallikrein	N	Y	N
176	KNG1	KNG1	P01042	Kininogen-1	Y	Y	Y
177	KRT19	K1C19	P08727	Keratin, type I cytoskeletal 19	Y	N	N
178	LBP	LBP	P18428	Lipopolysaccharide-binding protein	N	Y	N
179	LDHA	LDHA	P00338	L-lactate dehydrogenase A chain	Y	N	N
180	LDHB	LDHB	P07195	L-lactate dehydrogenase B chain	Y	N	N
181	LEP	LEP	P41159	Leptin	Y	N	N
182	LHB	LSHB	P01229	Lutropin subunit beta	Y	N	N
183	LPA	APOA	P08519	Apolipoprotein A	Y	Y	Y
184	LRG1	A2GL	P02750	Leucine-rich alpha-2-glycoprotein	N	Y	N
185	LTA	TNFB	P01374	Lymphotoxin-alpha	Y	N	N
186	LTF	TRFL	P02788	Lactotransferrin	Y	N	N
187	LYZ	LYSC	P61626	Lysozyme C	Y	Y	Y
188	MB	MYG	P02144	Myoglobin	Y	N	N
189	MBL2	MBL2	P11226	Mannose-binding protein C	Y	Y	Y
190	MPO	PERM	P05164	Myeloperoxidase	Y	N	N
191	MSLN	MSLN	Q13421	Mesothelin	Y	N	N
192	MUC1	MUC1	P15941	Mucin-1	Y	N	N
193	MUC16	MUC16	Q8WXI7	Mucin-16	Y	N	N
194	NAGLU	ANAG	P54802	Alpha-N-acetylglucosaminidase	Y	N	N
195	NPPB	ANFB	P16860	Natriuretic peptides B	Y	N	N
196	ORM1	A1AG1	P02763	Alpha-1-acid glycoprotein 1	Y	Y	Y
197	ORM2	A1AG2	P19652	Alpha-1-acid glycoprotein 2	N	Y	N
198	OXT	NEU1	P01178	Oxytocin-neurophysin 1	Y	N	N
199	PGC	PEPC	P20142	Gastricsin	Y	N	N
200	PLA2G1B	PA21B	P04054	Phospholipase A2	Y	N	N
201	PLAT	TPA	P00750	Tissue-type plasminogen activator	Y	N	N
202	PLAU	UROK	P00749	Urokinase-type plasminogen activator	Y	N	N
203	PLG	PLMN	P00747	Plasminogen	Y	Y	Y
204	PNLIP	LIPP	P16233	Pancreatic triacylglycerol lipase	Y	N	N
205	PON1	PON1	P27169	Serum paraoxonase/arylesterase 1	N	Y	N
206	PPBP	CXCL7	P02775	Platelet basic protein	Y	N	N
207	PRL	PRL	P01236	Prolactin	Y	N	N
208	PROC	PROC	P04070	Vitamin K-dependent protein C	Y	Y	Y
209	PROS1	PROS	P07225	Vitamin K-dependent protein S	Y	Y	Y
210	PROZ	PROZ	P22891	Vitamin K-dependent protein Z	N	Y	N

211	PRSS1	TRY1	P07477	Trypsin-1	Y	N	N
212	PTH	PTHY	P01270	Parathyroid hormone	Y	N	N
213	RBP4	RET4	P02753	Retinol-binding protein 4	Y	Y	Y
214	REN	RENI	P00797	Renin	Y	N	N
215	RNASE3	ECP	P12724	Eosinophil cationic protein	Y	N	N
216	S100A8	S10A8	P05109	Protein S100-A8	Y	Y	Y
217	S100A9	S10A9	P06702	Protein S100-A9	Y	Y	Y
218	S100B	S100B	P04271	Protein S100-B	Y	N	N
219	SAA1	SAA1	P0DJI8	Serum amyloid A-1 protein	N	Y	N
220	SAA4	SAA4	P35542	Serum amyloid A-4 protein	N	Y	N
221	SERPINA1	A1AT	P01009	Alpha-1-antitrypsin	Y	Y	Y
222	SERPINA3	AACT	P01011	Alpha-1-antichymotrypsin	N	Y	N
223	SERPINA4	KAIN	P29622	Kallistatin	N	Y	N
224	SERPINA5	IPSP	P05154	Plasma serine protease inhibitor	N	Y	N
225	SERPINA6	CBG	P08185	Corticosteroid-binding globulin	Y	Y	Y
226	SERPINA7	THBG	P05543	Thyroxine-binding globulin	Y	Y	Y
227	SERPINB3	SPB3	P29508	Serpin B3	Y	N	N
228	SERPINC1	ANT3	P01008	Antithrombin-III	Y	Y	Y
229	SERPIND1	HEP2	P05546	Heparin cofactor 2	Y	Y	Y
230	SERPINE1	PAI1	P05121	Plasminogen activator inhibitor 1	Y	N	N
231	SERPINF1	PEDF	P36955	Pigment epithelium-derived factor	N	Y	N
232	SERPINF2	A2AP	P08697	Alpha-2-antiplasmin	Y	Y	Y
233	SERPING1	IC1	P05155	Plasma protease C1 inhibitor	Y	Y	Y
234	SHBG	SHBG	P04278	Sex hormone-binding globulin	Y	Y	Y
235	SORD	DHSO	Q00796	Sorbitol dehydrogenase	Y	N	N
236	TF	TRFE	P02787	Serotransferrin	Y	Y	Y
237	TFRC	TFR1	P02786	Transferrin receptor protein 1	Y	Y	Y
238	TG	THYG	P01266	Thyroglobulin	Y	N	N
239	TGFB1	TGFB1	P01137	Transforming growth factor beta-1	Y	N	N
240	TNF	TNFA	P01375	Tumor necrosis factor	Y	N	N
241	TNFRSF1A	TNR1A	P19438	Tumor necrosis factor receptor superfamily member 1A	Y	N	N
242	TNFRSF1B	TNR1B	P20333	Tumor necrosis factor receptor superfamily member 1B	Y	N	N
243	TNFRSF8	TNR8	P28908	Tumor necrosis factor receptor superfamily member 8	Y	N	N
244	TNNI3	TNNI3	P19429	Troponin I, cardiac muscle	Y	N	N
245	TNNT2	TNNT2	P45379	Troponin T, cardiac muscle	Y	N	N
246	TRH	TRH	P20396	Pro-thyrotropin-releasing hormone	Y	N	N
247	TSHB	TSHB	P01222	Thyrotropin subunit beta	Y	N	N
248	TTR	TTHY	P02766	Transthyretin	Y	Y	Y
249	VEGFA	VEGFA	P15692	Vascular endothelial growth factor A	Y	N	N
250	VTN	VTNC	P04004	Vitronectin	N	Y	N
251	VWF	VWF	P04275	Von Willebrand Factor	Y	Y	Y
252	WFDC2	WFDC2	Q14508	WAP four-disulfide core domain protein 2	Y	N	N

Table 1-2. Lists of interference-free product ions examined by AuDIT analysis.

Information of target protein and peptide							AuDIT analysis	
N.	Gene symbol	Uniprot ID	Uniprot Accession	Protein Name	Peptide Sequence	Quantification ion[a]	*P* value	CV
1	AMBP	AMBP	P02760	Protein AMBP	ETLLQDFR	2.y6.1	0.2475	0.12
						2.y4.1	0.7607	0.04
						2.y3.1	0.8590	0.09
2	APOA2	APOA2	P02652	Apolipoprotein A2	SPELQAEAK	2.y6.1	0.1597	0.01
						2.y4.1	0.2184	0.02
						2.y8.2	0.9417	0.01
						2.b2.1	0.8037	0.03
3	A2M	A2MG	P01023	Alpha-2-macroglobulin	FEVQVTVPK	2.b2.1	0.5558	0.04
						2.y2.1	0.8219	0.03
						2.y4.1	0.9663	0.05
						2.y5.1	0.8625	0.12
						2.y6.1	0.8452	0.09
						2.y7.1	0.7624	0.09
4	APOB	APOB	P04114	Apolipoprotein B-100	ALVDTLK	2.b2.1	0.8989	0.03
						2.b3.1	0.9889	0.08
						2.y3.1	0.8733	0.13
						2.y4.1	0.7054	0.06
						2.y5.1	0.9873	0.09
						2.y6.1	0.9941	0.13

5	APOE	APOE	P02649	Apolipoprotein E	LAVYQAGAR	2.y7.1	0.1785	0.04
						2.y6.1	0.4967	0.04
						2.y5.1	0.7550	0.03
						2.y4.1	0.0406	0.04
6	APOH	APOH	P02749	Apolipoprotein H	ATVVYQGER	2.y7.1	0.9676	0.04
						2.y6.1	0.3132	0.02
						2.y5.1	0.0122	0.02
						2.b3.1	0.0886	0.01
7	B2M	B2MG	P61769	Beta-2-microglobulin	VNHVTLSQPK	3.b3.1	0.8816	0.08
						3.b4.1	0.8615	0.13
						3.b5.1	0.8937	0.14
						3.y5.1	0.9900	0.08
						3.y6.1	0.9864	0.15
						3.y7.1	0.1545	0.19
8	BCHE	CHLE	P06276	Cholinesterase	IFFPGVSEFGK	2.b2.1	0.9261	0.09
						2.y5.1	1.0000	0.16
						2.y7.1	0.8531	0.08
						2.y8.1	0.7717	0.09
						2.y8.2	0.8399	0.14
						2.y9.1	0.9464	0.11
9	C1QA	C1QA	P02745	Complement C1q subcomponent subunit A	SLGFCDTTNK	2.b2.1	0.9845	0.08
						2.b3.1	0.6955	0.16
						2.y4.1	0.1842	0.11
						2.y5.1	0.6495	0.14

						2.y6.1	1.0000	0.10
						2.y8.1	0.9465	0.06
10	C2	CO2	P06681	Complement C2	LNINLK	2.b2.1	0.9967	0.19
						2.y1.1	0.8968	0.13
						2.y2.1	0.0187	0.15
						2.y3.1	0.3665	0.19
						2.y4.1	1.0000	0.15
						2.y5.1	1.0000	0.17
11	C3	CO3	P01024	Complement C3	DFDFVPPVVR	2.b2.1	0.5193	0.04
						2.b3.1	0.8248	0.05
						2.y5.1	0.1130	0.02
						2.y6.1	0.2954	0.04
						2.y7.1	0.2659	0.06
						2.y8.1	0.0298	0.08
12	C5	CO5	P01031	Complement C5	LQGTLPVEAR	2.b2.1	0.8622	0.06
						2.y2.1	0.9559	0.06
						2.y5.1	0.9605	0.02
						2.y6.1	0.9980	0.13
						2.y7.1	0.2553	0.06
						2.y8.1	0.9967	0.10
13	C6	CO6	P13671	Complement component C6	ALQEYAAK	2.b2.1	0.9866	0.15
						2.b3.2	0.6244	0.04
						2.y2.1	0.2904	0.08
						2.y4.1	0.8143	0.05

						2.y5.1	0.7735	0.15
						2.y6.1	0.6488	0.11
14	C7	CO7	P10643	Complement component C	LSGNVLSYTFQVK	2.b2.1	0.9129	0.08
						2.b4.1	1.0000	0.15
						2.b5.1	0.8935	0.19
						2.y7.1	0.9449	0.05
						2.y8.1	0.9997	0.14
						2.y6.1	0.9962	0.04
15	C8A	CO8A	P07357	Complement component C8 alpha chain	LYYGDDEK	2.b2.1	0.7556	0.09
						2.b4.2	0.9607	0.08
						2.y5.1	0.9992	0.07
						2.y6.1	0.9989	0.07
						2.y7.1	0.9533	0.15
16	C8B	CO8B	P07358	Complement component C8 beta chain	YEFILK	2.b2.1	0.4644	0.11
						2.y1.1	0.7428	0.14
						2.y2.1	0.8635	0.16
						2.y4.1	0.6131	0.13
						2.y4.2	0.0053	0.09
						2.y5.1	0.1309	0.18
17	C9	CO9	P02748	Complement component C9	VVEESELAR	2.b2.1	0.9089	0.07
						2.y2.1	0.8351	0.18
						2.y3.1	0.7394	0.17
						2.y5.1	0.6830	0.07
						2.y6.1	0.6030	0.08

						2.y7.1	0.9246	0.10
18	CFB	CFAB	P00751	Complement factor B	EELLPAQDIK	2.b2.1	0.8074	0.02
						2.b3.1	0.1088	0.05
						2.y2.1	0.0061	0.06
						2.y5.1	0.0251	0.09
						2.y6.1	0.2405	0.04
						2.y7.1	0.7291	0.03
19	CFH	CFAH	P08603	Complement factor H	VGEVLK	2.b2.1	0.2287	0.00
						2.b3.1	0.2661	0.07
						2.y1.1	0.0044	0.04
						2.y3.1	0.0011	0.07
						2.y4.1	0.1411	0.04
						2.y5.1	0.0908	0.03
20	CP	CERU	P00450	Ceruloplasmin	GAYPLSIEPIGVR	2.y10.1	0.0939	0.06
						2.y5.1	0.0006	0.17
						2.y6.1	0.0002	0.09
						2.y7.1	0.5150	0.13
						2.y8.1	0.7508	0.11
						2.y9.1	0.4158	0.12
21	CRP	CRP	P02741	C-reactive protein	ESDTSYVSLK	2.b2.1	1.0000	0.18
						2.y1.1	0.8434	0.12
						2.y3.1	0.9720	0.18
						2.y4.1	0.8565	0.12
						2.y5.1	0.1432	0.18

						2.y6.1	0.8422	0.18
22	CST3	CYTC	P01034	Cystatin-C	ALDFAVGEYNK	2.b2.1	0.0414	0.06
						2.b3.1	0.1042	0.18
						2.y5.1	0.0469	0.10
						2.y6.1	0.5433	0.17
						2.y7.1	0.4308	0.11
						2.y9.1	0.2989	0.08
23	F2	THRB	P00734	Prothrombin	ELLESYIDGR	2.b2.1	0.4727	0.08
						2.y4.1	0.3316	0.12
						2.y5.1	0.9522	0.16
						2.y6.1	0.9694	0.06
						2.y7.1	0.7167	0.09
						2.y8.1	1.0000	0.18
24	F9	FA9	P00740	Coagulation factor IX	SALVLQYLR	2.b2.1	0.9643	0.19
						2.b3.1	0.7667	0.01
						2.y4.1	0.3665	0.05
						2.y5.1	0.9711	0.07
						2.y6.1	0.9635	0.13
25	F10	FA10	P00742	Coagulation factor X	TGIVSGFGR	2.b2.1	0.0425	0.16
						2.b3.1	0.5520	0.12
						2.y4.1	0.8154	0.13
						2.y5.1	0.8076	0.07
						2.y6.1	0.5761	0.11
						2.y7.1	0.0366	0.10

26	F11	FA11	P03951	Coagulation factor XI	VVSGFSLK	2.b2.1	0.5930	0.13
						2.y3.1	0.2553	0.20
						2.y5.1	0.2932	0.16
						2.y6.1	0.8633	0.10
						2.y7.1	0.0002	0.11
27	GSN	GELS	P06396	Gelsolin	AGALNSNDAFVLK	2.y9.1	0.5243	0.09
						2.y8.1	0.6882	0.16
						2.b3.1	0.0761	0.10
28	IGFBP3	IBP3	P17936	Insulin-like growth factor-binding protein 3	FLNVLSPR	2.b2.1	0.4577	0.12
						2.b4.1	0.5577	0.01
						2.y3.1	0.0083	0.09
						2.y4.1	0.7284	0.08
						2.y5.1	1.0000	0.07
						2.y6.1	0.7592	0.07
29	LYZ	LYSC	P61626	Lysozyme C	WESGYNTR	2.b2.1	0.9843	0.11
						2.y5.1	0.3790	0.06
						2.y6.1	0.8789	0.09
30	PLG	PLMN	P00747	Plasminogen	LFLEPTR	2.b2.1	0.5997	0.05
						2.b3.1	0.3179	0.06
						2.y3.1	0.2521	0.11
						2.y4.1	0.4726	0.04
						2.y5.1	0.9868	0.05
						2.y6.1	0.8828	0.02
31	PROC	PROC	P04070	Vitamin K-dependent protein C	TFVLNFIK	2.b2.1	0.9564	0.19

						2.y3.1	0.6758	0.08
						2.y4.1	0.8704	0.19
						2.y5.1	1.0000	0.19
						2.y6.1	1.0000	0.04
32	PROS1	PROS	P07225	Vitamin K-dependent protein S	YLVCLR	2.b2.1	0.7924	0.12
						2.y3.1	0.4293	0.07
						2.y4.1	0.2902	0.04
						2.y5.1	0.8178	0.18
33	SERPINC1	ANT3	P01008	Antithrombin-III	FDTISEK	2.b2.1	0.2474	0.04
						2.b3.1	0.0332	0.11
						2.y3.1	0.0866	0.04
						2.y4.1	0.1437	0.09
						2.y5.1	0.4035	0.04
						2.y6.1	0.5806	0.05
34	SERPINF2	A2AP	P08697	Alpha-2-antiplasmin	LGNQEPGGQTALK	2.b3.1	0.9694	0.12
						2.b4.1	0.9198	0.09
						2.y1.1	0.9875	0.09
						2.y7.1	0.9974	0.16
						2.y8.1	0.6086	0.03
						2.y9.1	0.5700	0.02
35	SERPING1	IC1	P05155	Plasma protease C1 inhibitor	LLDSLPSDTR	2.b2.1	0.9683	0.17
						2.b3.1	0.8575	0.16
						2.y2.1	0.7104	0.12
						2.y5.1	0.4496	0.03

						2.y7.1	0.8073	0.11
						2.y8.1	0.9136	0.07
36	SHBG	SHBG	P04278	Sex hormone-binding globulin	IALGGLLFPASNLR	2.b2.1	0.9995	0.06
						2.b5.1	0.2687	0.06
						2.y1.1	0.0040	0.15
						2.y6.1	0.2755	0.01
						2.y7.1	0.8184	0.04
						2.y8.1	0.8715	0.11
37	TTR	TTHY	P02766	Transthyretin	VLDAVR	2.b2.1	0.2619	0.08
						2.y1.1	0.0182	0.18
						2.y3.1	0.1358	0.13
						2.y4.1	0.0852	0.04
						2.y5.1	0.0141	0.05

AuDIT, automated detection of inaccurate and imprecise transitions; CV, coeffcient of variance.

[a] The column title indicates the information of quantification ion, followed by the precursor ion charge, production ion type, and product ion charge.

1.3.2 Reverse response curves of targets in normal serum

Reverse response curves were generated to determine the limit of detection (LOD), limit of quantitation (LOQ), lower limit of quantitation (LLOQ), upper limit of quantitation (ULOQ), and linear range of the target peptides. These curves were constructed with a series of diluted SIS peptide mixtures (8–13 concentration points, 3 orders of magnitude) with pooled normal serum as matrices and analyzed at each concentration point in triplicate over 3 days. The peak area ratio (y) of SIS peptide to endogenous peptide was fit by linear regression using the “1/y” weighting option. The LOD and LOQ values were determined, based on the averaged peak area ratio of blank (matrix without SIS peptides) measurements plus 3 and 10 times the standard deviation, respectively. The LLOQ and ULOQ values were determined as the lowest and highest concentrations that satisfied the linearity ($R^2 > 0.99$) and precision (CV < 20%) estimates, respectively.

Of the 37 peptides, 36 peptides, except LYZ (WESGYNTR), met the aforementioned criteria (linearity and precision). A2M (FEVQVTPK) and C7 (LSGNVLSYTFQVK) fulfilled the criteria but were excluded because they were unsuitable for preparing medium-QC and high-QC samples due to their high LLOQ values (about 1928.9 and 412.95 ng/mL, respectively). The characteristics of the reverse response curves for 112 transitions that represented 34 peptides were determined according to CPTAC guidelines (Table 1-3 and 1-4); information on these transitions is listed in Table 1-5. The reverse response curves of the 34 peptides, which had endogenous concentrations that spanned over 4 orders of magnitude (39.3 ng/mL–118.2 μg/mL) in the pooled normal serum, were generated. The median LOD, LOQ, LLOQ, and ULOQ values were 0.12 fmol/μL (8.01 ng/mL), 0.40 fmol/μL (21.4 ng/mL), 0.37 fmol/μL (16.0 ng/mL), and 249.37 fmol/μL (13.2 μg/mL), respectively.

Table 1-3. Results of response curve analysis.

N.	Information of Target Protein and Peptide						Results of response curve														Concentration of QC samples					
							Curve fit (Normal-scale)			Curve fit (Log-scale)				fmol/μL of peptide				ng/mL of protein[b]								
	Gene symbol	Uniprot ID	Uniprot Accession	Protein Name	Peptide Sequence	Quanification ion[a]	Slope	Intercept	R square	Slope	Intercept	R square	Points no.	LOD	LOQ	LLOQ	ULOQ	LOD	LOQ	LLOQ	ULOQ	Low QC (fmol/μL)	Medium QC (fmol/μL)	High QC (fmol/μL)	Low QC (ng/mL)	Medium QC (ng/mL)	High QC (ng/mL)
1	AMBP	AMBP	P02760	Protein AMBP	ETLLQDFR	2.y6.1	0.0598	0.0133	0.9993	3.2475	-3.9238	0.9993	10	0.07	0.41	0.24	250.04	2.78	16.01	9.52	9751.30	0.49	48.84	225.04	19.05	1904.55	8776.17
						2.y4.1	0.0464	-0.0007	1.0000	1.0049	-1.3517	0.9992	12	0.05	0.09	0.12	250.04	2.01	3.49	4.76	9751.30						
						2.y3.1	0.0271	-0.0039	0.9999	1.0175	-1.6000	0.9998	10	0.21	0.31	0.24	250.04	8.06	12.09	9.52	9751.30						
2	APOA2	APOA2	P02652	Apolipoprotein A2	SPELQAEAK	2.y6.1	0.0018	0.0005	0.9997	0.9909	-2.7331	0.9998	10	0.33	1.36	1.55	792.00	3.69	15.18	17.29	8850.60	3.09	154.69	714.19	34.57	1728.63	7981.10
						2.y4.1	0.0018	-0.0002	1.0000	0.9878	-2.7117	0.9992	11	0.45	0.90	0.77	792.00	5.05	10.07	8.64	8850.60						
						2.y8.2	0.0021	-0.0006	0.9999	3.3030	-8.8593	0.9983	11	0.71	1.40	0.77	792.00	7.94	15.69	4.32	8850.60						
						2.b2.1	0.0021	0.0023	1.0000	3.0817	-8.3817	0.9981	11	0.11	0.94	0.77	792.00	1.18	10.55	8.64	8850.60						
3	APOB	APOB	P04114	Apolipoprotein B-100	ALVDTLK[c]	2.y5.1	0.0045	-0.0005	0.9995	0.9976	-2.3546	0.9997	11	0.29	0.56	0.35	360.00	151.20	290.12	181.27	185617.80	5.63	181.41	329.06	2900.28	93533.97	169666.27
						2.y4.1	0.0087	0.0070	0.9997	0.9276	-1.9223	0.9956	8	1.71	5.29	2.81	360.00	881.28	2725.55	362.53	185617.80						
						2.b2.1	0.0155	0.0282	0.9996	0.9229	-1.6465	0.9984	7	1.66	2.63	5.63	360.00	855.94	1357.71	2900.28	185617.80						
4	APOE	APOE	P02649	Apolipoprotein E	LAVYQAGAR	2.y7.1	0.0735	-0.0114	0.9995	0.9405	-1.0707	0.9974	12	0.02	0.19	0.09	192.88	0.76	6.88	3.40	6973.24	0.38	18.84	173.59	13.62	680.98	6275.92
						2.y6.1	0.0867	0.0024	0.9996	0.9714	-1.0463	0.9990	12	0.03	0.10	0.09	192.88	1.15	3.70	3.40	6973.24						
						2.y5.1	0.0771	-0.0029	0.9999	0.9601	-1.0510	0.9994	11	0.12	0.22	0.19	192.88	4.30	8.08	6.81	6973.24						
						2.y4.1	0.0720	0.0177	0.9999	0.9412	-1.0545	0.9976	11	0.06	0.52	0.09	192.88	2.04	18.71	3.40	6973.24						
5	APOH	APOH	P02749	Apolipoprotein H	ATVVYQGER	2.y7.1	0.0030	0.0003	0.9999	0.9961	-2.5165	0.9983	11	0.81	2.50	1.16	594.00	31.11	95.76	22.22	22749.01	2.32	116.02	535.64	88.86	4443.17	20514.10
						2.y6.1	0.0029	-0.0007	1.0000	0.9792	-2.5041	0.9990	10	0.41	0.66	1.16	594.00	15.85	25.09	44.43	22749.01						
						2.y5.1	0.0036	0.0005	1.0000	0.9722	-2.3967	0.9993	12	0.19	0.71	0.29	594.00	7.38	27.08	11.11	22749.01						
						2.b3.1	0.0034	-0.0011	0.9997	3.2412	-8.0805	0.9966	11	1.00	1.51	1.16	594.00	38.17	58.01	22.22	22749.01						
6	B2M	B2MG	P61769	Beta-2-microglobulin	VNHVTLSQPK	3.y6.1	0.1794	0.0229	0.9965	0.9674	-0.7105	0.9982	10	0.11	0.50	0.49	248.69	1.52	6.86	6.66	3410.80	0.97	48.57	224.26	13.32	666.17	3075.71
						3.y5.1	0.1892	0.1121	0.9983	0.9888	-0.6966	0.9983	10	0.14	1.23	0.49	248.69	1.96	16.84	6.66	3410.80						
						3.b3.1	0.1852	0.0738	0.9996	0.9806	-0.6671	0.9975	10	0.13	0.85	0.49	248.69	1.85	11.61	6.66	3410.80						
7	BCHE	CHLE	P06276	Cholinesterase	IFFPGVSEFGK	2.y8.1	0.3204	-0.0468	0.9989	1.0956	-0.6503	0.9967	9	0.31	0.45	0.48	123.83	20.94	30.96	8.27	8472.50	0.97	62.16	111.89	16.55	1063.20	7655.03
						2.y7.1	0.8994	0.1439	0.9988	1.0870	-0.1748	0.9984	10	0.06	0.26	0.24	123.83	4.07	17.48	16.55	8472.50						
						2.b2.1	0.7867	-0.0020	0.9992	1.1184	-0.2888	0.9980	11	0.03	0.06	0.12	123.83	1.94	3.80	8.27	8472.50						
8	C1QA	C1QA	P02745	Complement C1q subcomponent subunit A	SLGFCDTTNK	2.y8.1	0.0110	0.0021	0.9989	0.9566	-1.8908	0.9984	10	0.03	0.35	0.42	216.70	0.65	8.99	11.01	5637.86	1.69	84.65	195.79	44.05	2202.29	5093.89
						2.y6.1	0.0224	0.0048	0.9999	1.0039	-1.6425	0.9987	9	0.36	1.30	0.85	216.70	9.30	33.80	22.02	5637.86						
						2.b2.1	0.0427	0.0200	0.9969	0.9676	-1.3108	0.9985	10	0.02	0.82	0.42	216.70	0.48	21.42	11.01	5637.86						
9	C2	CO2	P06681	Complement C2	LNINLK[c]	2.y5.1	0.0786	0.0594	0.9991	0.9777	-1.0567	0.9980	9	0.07	1.36	0.94	241.50	5.56	113.46	78.55	20108.94	2.83	121.22	218.20	235.65	10093.74	18168.74
						2.y4.1	0.0730	-0.0141	0.9992	0.9868	-1.1164	0.9988	10	0.37	0.62	0.47	241.50	30.61	51.65	39.28	20108.94						
						2.b2.1	0.2887	0.0188	0.9998	0.9708	-0.5137	0.9992	10	0.10	0.38	0.47	241.50	8.33	31.65	39.28	20108.94						
10	C3	CO3	P01024	Complement C3	DFDFVPPVVR	2.y8.1	0.0059	-0.0094	0.9997	1.1312	-2.5277	0.9975	8	1.63	1.69	3.09	396.00	305.35	315.81	72.37	74110.61	3.09	77.34	357.79	72.37	1809.34	66960.09
						2.y6.1	0.0044	-0.0062	0.9997	1.1437	-2.6680	0.9981	9	1.48	1.57	1.55	396.00	276.75	293.94	144.75	74110.61						
						2.y5.1	0.0035	-0.0043	0.9984	1.1265	-2.7350	0.9977	9	1.26	1.29	1.55	396.00	236.20	240.61	72.37	74110.61						
						2.b2.1	0.0105	-0.0165	0.9960	1.1365	-2.2802	0.9983	8	1.63	1.70	3.09	396.00	304.42	318.83	72.37	74110.61						
						2.b3.1	0.0117	-0.0166	0.9991	3.7670	-7.4128	0.9981	9	1.47	1.53	1.55	396.00	275.12	286.32	36.19	74110.61						
11	C5	CO5	P01031	Complement C5	LQGTLPVEAR	2.y8.1	0.0889	0.0062	0.9997	0.9981	-1.0631	0.9987	11	0.07	0.16	0.34	344.54	12.68	30.90	63.36	64878.17	1.35	67.29	310.69	253.43	12671.52	58504.39
						2.y6.1	0.0597	-0.0004	0.9995	0.9868	-1.2025	0.9996	10	0.10	0.17	0.67	344.54	19.62	31.78	126.72	64878.17						
						2.y5.1	0.0609	0.0006	0.9995	0.9793	-1.1953	0.9978	13	0.08	0.17	0.08	344.54	15.27	32.63	15.84	64878.17						
						2.b2.1	0.0898	0.0004	0.9997	0.9822	-1.0256	0.9998	11	0.05	0.08	0.34	344.54	8.91	15.85	63.36	64878.17						
12	C6	CO6	P13671	Complement component C6	ALQEYAAK[c]	2.y6.1	0.0124	0.0007	0.9990	0.9751	-1.8511	0.9979	9	0.11	0.31	0.83	211.31	11.72	32.49	86.49	22141.83	4.95	82.54	190.92	518.95	8649.15	20005.49
						2.b2.1	0.2499	0.0041	0.9995	0.9503	-0.5275	0.9981	7	1.18	2.57	1.65	105.65	123.30	268.89	86.49	11070.91						
						2.b3.2	0.1459	0.0283	0.9994	0.9750	-0.7982	0.9988	10	0.24	0.81	0.41	211.31	24.70	85.11	21.62	22141.83						
13	C8A	CO8A	P07357	Complement component C8 alpha chain	LYYGDDEK[c]	2.y6.1	0.0155	0.0066	0.9991	0.9650	-1.7480	0.9995	9	0.12	1.14	1.02	261.67	11.23	106.42	95.59	24471.02	6.13	102.22	237.34	573.54	9558.99	22195.98
						2.b2.1	0.0535	-0.0186	0.9971	0.9802	-1.2712	0.9979	9	0.56	0.86	1.02	261.67	52.15	80.17	95.59	24471.02						
						2.b4.2	0.0663	-0.0106	0.9997	0.9807	-1.1636	0.9986	10	0.22	0.23	2.04	261.67	20.89	21.46	191.18	24471.02						

14	C8B	CO8B	P07358	Complement component C8 beta chain	YEFILK[c]	2.y2.1	0.1567	0.0050	0.99821.0234	-0.8493	0.9993	10	0.120.32	0.38197.08	7.76	21.35	25.81	13213.53	2.31	98.73	178.06	154.85	6619.67	11938.63
						2.y4.2	0.1091	0.0058	0.99910.9924	-0.9532	0.9978	12	0.050.23	0.10197.08	3.23	15.70	6.45	13213.53						
						2.b2.1	0.1771	-0.0475	0.99991.0171	-0.7812	0.9994	9	0.390.58	0.77197.08	26.29	38.97	25.81	13213.53						
15	C9	CO9	P02748	Complement component C9	VVEESELAR	2.y7.1	0.0471	-0.0061	0.99730.9711	-1.3240	0.9994	12	0.170.24	0.17355.69	10.64	15.28	10.97	22469.72	0.35	17.37	320.27	21.94	1097.15	20232.62
						2.y6.1	0.0623	-0.0042	0.99760.9891	-1.2017	0.9992	12	0.130.22	0.17355.69	7.97	13.78	10.97	22469.72						
						2.b2.1	0.0865	0.0106	0.99993.0797	-3.2246	0.9970	13	0.060.30	0.09355.69	3.57	18.82	5.49	22469.72						
16	CFB	CFAB	P00751	Complement factor B	EELLPAQDIK	2.y6.1	0.0021	0.0002	1.00000.9891	-2.6531	0.9998	12	0.050.24	0.24486.00	4.34	20.80	20.30	41569.04	0.95	47.46	437.83	81.19	4059.48	37448.67
						2.b2.1	0.0421	0.0056	0.99950.9698	-1.3217	0.9994	11	0.020.20	0.47486.00	1.65	17.51	40.59	41569.04						
						2.b3.1	0.0287	0.0048	0.99980.9326	-1.4227	0.9941	11	0.170.54	0.47486.00	14.63	46.22	40.59	41569.04						
17	CFH	CFAH	P08603	Complement factor H	VGEVLK	2.y5.1	0.0129	0.0074	0.99960.9223	-1.7438	0.9988	10	0.321.06	0.90461.02	44.40	147.15	125.25	64125.95	1.80	90.04	415.73	250.49	12524.60	57826.07
						2.y4.1	0.0085	0.0017	0.99880.9874	-2.0552	0.9989	11	0.080.58	0.45461.02	11.05	80.09	62.62	64125.95						
						2.b3.1	0.0452	0.0009	0.99950.9853	-1.3204	0.9988	11	0.260.57	0.45461.02	35.63	78.78	62.62	64125.95						
18	CP	CERU	P00450	Ceruloplasmin	GAYPLSIEPIGVR	2.y10.1	0.0047	-0.0081	0.99971.1001	-2.5705	0.9988	11	0.401.19	0.46468.00	48.53	418.45	55.85	57191.94	1.83	91.41	422.02	223.41	11170.30	51573.28
						2.y8.1	0.0025	-0.0031	0.99933.7360	-9.5714	0.9981	10	0.641.93	0.91468.00	78.55	124.57	55.85	57191.94						
						2.y6.1	0.0052	0.0010	0.99951.0832	-2.4677	0.9984	11	0.040.32	0.46468.00	4.49	38.74	55.85	57191.94						
						2.y5.1	0.0034	-0.0008	0.99951.0845	-2.6573	0.9989	11	0.410.64	0.46468.00	49.59	78.64	27.93	57191.94						
19	CRP	CRP	P02741	C-reactive protein	ESDTSYVSLK	2.y6.1	0.3163	0.0242	0.99621.0002	-0.5015	0.9992	9	0.040.22	0.64163.77	1.10	5.51	16.02	4100.68	1.28	63.97	147.97	32.04	1601.83	3705.03
						2.y5.1	0.3351	0.0042	0.99680.9995	-0.4919	0.9981	11	0.090.16	0.16163.77	2.30	4.04	4.00	4100.68						
						2.y3.1	0.1834	-0.0056	0.99913.2499	-2.3487	0.9981	9	0.220.52	0.64163.77	5.48	12.94	16.02	4100.68						
20	CST3	CYTC	P01034	Cystatin-C	ALDFAVGEYNK[c]	2.y6.1	0.4733	-0.8167	0.99443.4198	-1.3531	0.9991	7	0.551.18	1.82116.39	8.69	18.59	28.73	1838.89	1.36	45.47	105.16	21.55	718.32	1661.46
						2.b2.1	0.5163	0.0917	0.99781.0258	-0.2985	0.9985	9	0.190.67	0.45116.39	3.01	10.62	7.18	1838.89						
						2.b3.1	0.5936	0.0816	0.99973.3307	-0.7477	0.9972	11	0.080.43	0.23116.39	1.28	6.84	3.59	1838.89						
21	F2	THRB	P00734	Prothrombin	ELLESYIDGR	2.y6.1	0.0523	-0.0318	1.00003.6708	-4.9217	0.9991	10	0.310.88	0.44226.48	21.61	61.54	15.49	15861.98	0.88	44.23	204.23	30.98	1549.02	14303.67
						2.y5.1	0.0647	-0.0268	0.99971.1121	-1.4001	0.9987	10	0.420.79	0.44226.48	29.10	55.22	15.49	15861.98						
						2.b2.1	0.0869	0.0092	0.99931.0895	-1.2206	0.9992	11	0.050.19	0.22226.48	3.63	13.22	15.49	15861.98						
22	F9	FA9	P00740	Coagulation factor IX	SALVLQYLR	2.y5.1	0.2793	-0.0194	0.99971.0552	-0.6350	0.9988	11	0.090.12	0.10 98.28	4.67	6.46	4.97	5088.57	0.38	19.19	88.62	19.88	993.86	4588.66
						2.b2.1	0.8954	-0.0166	0.99641.0432	-0.1162	0.9985	10	0.110.24	0.19 98.28	5.82	12.23	9.94	5088.57						
						2.b3.1	0.7865	-0.0425	0.99903.5396	-0.6471	0.9983	10	0.070.08	0.19 98.28	3.47	4.16	9.94	5088.57						
23	F10	FA10	P00742	Coagulation factor X	TGIVSGFGR	2.y6.1	0.3063	0.0060	0.99930.9759	-0.4759	0.9993	11	0.070.22	0.10 98.30	4.07	11.90	5.25	5380.13	0.38	19.20	88.64	21.02	1050.81	4851.57
						2.y5.1	0.2552	0.0115	0.99980.9945	-0.5888	0.9991	11	0.060.18	0.10 98.30	3.51	10.07	5.25	5380.13						
						2.y4.1	0.1588	0.0090	0.99850.9267	-0.7222	0.9958	10	0.050.21	0.19 98.30	2.94	11.61	10.51	5380.13						
						2.b3.1	0.2738	0.0157	0.99820.9624	-0.5196	0.9988	11	0.060.24	0.10 98.30	3.51	12.91	5.25	5380.13						
24	F11	FA11	P03951	Coagulation factor XI	VVSGFSLK	2.y6.1	0.2866	-0.0230	0.99911.0094	-0.5553	0.9984	10	0.150.26	0.24120.55	10.78	18.40	16.51	8451.48	0.47	23.54	108.70	33.01	1650.68	7621.19
						2.b2.1	1.0483	0.0890	0.99900.9624	0.0717	0.9984	12	0.050.29	0.06120.55	3.20	20.11	4.13	8451.48						
25	GSN	GELS	P06396	Gelsolin	AGALNSNDAFVLK	2.y9.1	0.0318	0.0007	0.99961.0091	-1.5195	0.9987	12	0.070.19	0.18358.64	5.62	16.01	15.01	30734.40	0.70	35.02	323.09	60.03	3001.41	27687.98
						2.y8.1	0.0309	-0.0058	0.99991.0210	-1.5600	0.9992	11	0.290.39	0.35358.64	24.55	33.52	30.01	30734.40						
						2.b3.1	0.0383	0.0101	0.99991.0125	-1.4455	0.9989	11	0.110.59	0.35358.64	9.06	50.21	30.01	30734.40						
26	IGFBP3	IBP3	P17936	Insulin-like growth factor-binding protein 3	FLNVLSPR	2.y6.1	0.2810	-0.0089	0.99981.0202	-0.5787	0.9994	11	0.060.09	0.14145.58	1.79	2.99	4.50	4611.22	0.28	14.22	131.15	9.01	450.31	4154.15
						2.y4.1	0.3110	0.0029	0.99931.0182	-0.5323	0.9995	11	0.020.07	0.14145.58	0.71	2.15	4.50	4611.22						
						2.y3.1	0.3681	0.0172	0.99791.0386	-0.4991	0.9992	10	0.020.13	0.28145.58	0.67	3.99	9.01	4611.22						
27	PLG	PLMN	P00747	Plasminogen	LFLEPTR	2.y6.1	0.0243	0.0013	0.99920.9760	-1.5903	0.9956	12	0.010.08	0.14286.43	0.72	7.62	12.67	25941.35	0.56	27.97	258.04	50.67	2533.33	23370.01
						2.y5.1	0.0167	0.0006	1.00000.9805	-1.7546	0.9996	12	0.110.24	0.14286.43	9.93	22.13	12.67	25941.35						
						2.y4.1	0.0190	0.0022	0.99980.9941	-1.7081	0.9989	11	0.090.35	0.28286.43	8.10	31.46	25.33	25941.35						
						2.y3.1	0.0255	0.0002	0.99990.9715	-1.5519	0.9992	11	0.150.32	0.28286.43	13.40	28.93	25.33	25941.35						
						2.b2.1	0.0267	0.0004	0.99993.2559	-5.1392	0.9998	12	0.090.24	0.14286.43	8.39	21.89	12.67	25941.35						
28	PROC	PROC	P04070	Vitamin K-dependent protein C	TFVLNFIK	2.y6.1	0.1648	-0.0048	0.99981.0839	-0.8990	0.9945	10	0.090.18	0.16 79.59	4.43	9.31	8.09	4144.18	0.62	15.54	71.91	32.38	809.41	3744.33
						2.y5.1	0.3194	-0.0488	0.99911.0845	-0.6250	0.9987	9	0.180.23	0.31 79.59	9.60	11.78	8.09	4144.18						
						2.b2.1	0.6298	-0.0327	0.99771.0912	-0.2701	0.9991	10	0.070.08	0.16 79.59	3.57	3.98	8.09	4144.18						
29	PROS1	PROS	P07225	Vitamin K-dependent protein S	YLVCLR	2.y5.1	0.1371	-0.0125	0.99780.9703	-0.4086	0.9987	9	0.180.35	0.31 79.59	13.41	26.26	23.35	5978.82	0.62	31.09	71.91	46.71	2335.48	5401.96
						2.y4.1	0.1052	0.0001	0.99901.0074	-0.5683	0.9994	11	0.080.20	0.08 79.59	5.74	14.76	5.84	5978.82						
						2.y3.1	0.1262	0.0364	0.99810.9491	-0.3766	0.9965	9	0.281.01	0.31 79.59	20.85	75.83	23.35	5978.82						
						2.b2.1	0.2507	0.0183	0.99970.9899	-0.1638	0.9990	9	0.030.19	0.31 79.59	2.10	14.40	23.35	5978.82						
30	SERPINC1	ANT3	P01008	Antithrombin-III	FDTISEK	2.y6.1	0.0156	-0.0012	0.99860.9975	-1.8087	0.9971	9	0.210.42	0.94241.82	10.97	21.87	49.69	12720.41	2.83	94.46	218.49	149.07	4968.91	11493.09
						2.y5.1	0.0135	-0.0011	0.99860.9839	-1.8640	0.9982	9	0.230.43	0.94241.82	12.09	22.41	49.69	12720.41						
						2.b2.1	0.0337	0.0496	0.99970.9263	-1.3175	0.9986	9	0.664.61	0.94241.82	34.76	242.38	49.69	12720.41						
31	SERPINF2	A2AP	P08697	Alpha-2-antiplasmin	LGNQEPGGQTALK	2.y8.1	0.0261	0.0023	0.99980.9839	-1.5641	0.9993	13	0.050.19	0.07273.62	2.55	10.61	3.65	14930.45	2.14	106.88	247.22	116.64	5832.21	13489.90
						2.y7.1	0.0617	0.0134	0.99950.9956	-1.1858	0.9993	9	0.241.07	1.07273.62	12.85	58.50	58.32	14930.45						
						2.b4.1	0.0731	0.0045	0.99890.9322	-1.0298	0.9966	11	0.090.26	0.27273.62	4.93	14.35	14.58	14930.45						
32	SERPING1	IC1	P05155	Plasma protease C1 inhibitor	LLDSLPSDTR[c]	2.y7.1	0.0120	0.0169	0.99970.9755	-1.8634	0.9991	8	0.402.34	2.81360.00	21.81	128.82	155.12	19855.44	5.63	181.41	326.53	310.24	10005.28	18009.50
						2.y5.1	0.0116	-0.0015	0.99971.0181	-1.9727	0.9993	11	0.280.40	0.35360.00	15.21	21.87	19.39	19855.44						
						2.b2.1	0.0158	0.0168	0.99633.3644	-6.0090	0.9989	11	0.252.54	0.35360.00	13.59	140.33	19.39	19855.44						
33	SHBG	SHBG	P04278	Sex hormone-binding globulin	IALGGLLFPASNLF	2.y8.1	0.2906	-0.1897	0.99931.2067	-0.7816	0.9982	11	0.130.50	0.27278.79	5.74	21.96	11.92	12205.08	1.09	54.45	251.40	47.68	2383.80	11006.03
						2.y7.1	0.2903	-0.1329	0.99951.2015	-0.7692	0.9981	11	0.130.78	0.27278.79	5.60	34.13	11.92	12205.08						

							2.b5.1	0.7138	-0.3585	0.9983	1.2042	-0.3955	0.9979	10	0.20	1.62	0.54	278.79	8.71	70.79	23.84	12205.08						
34	TTR	TTHY	P02766	Transthyretin		VLDAVR	2.y5.1	0.2971	-0.0515	0.9996	0.9970	-0.5500	0.9988	10	0.25	0.36	0.39	201.75	3.98	5.66	6.26	3205.19	1.58	78.81	182.28	25.04	1252.03	2895.94
							2.y4.1	0.2141	0.0011	0.9998	0.9687	-0.6450	0.9991	13	0.05	0.14	0.05	201.75	0.78	2.25	0.78	3205.19						
							2.y3.1	0.1765	0.0955	0.9997	0.9644	-0.6913	0.9988	9	0.03	1.08	0.79	201.75	0.42	17.08	12.52	3205.19						

LOD, limit of detection; LOQ, limit of quantitation; LLOQ, lower limit of quantitation; ULOQ, upper limit of quantitation; QC, quality control.

[a] The column title indicates the information of quantification ion, followed by the precursor ion charge, production ion type, and product ion charge.

[b] Calculated as "peptide concentration (fmol/μL) × molecular weight (g/mol) / 1000".

[c] The concentration of low-QC is detemined as three times of LLOQ, not two times.

Table 1-4. Spike concentrations of heavy peptides for response curves.

N.	Information of target protein and peptide					Theoretical concentration of curve point samples (fmol/μL)[a]													Endogenous level (fmol/μL)[b]
	Gene symbol	Uniprot ID	Uniprot Accession	Protein Name	Peptide Sequence	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Point 8	Point 9	Point 10	Point 11	Point 12	Point 13	
1	AMBP	AMBP	P02760	Protein AMBP	ETLLQDFR	1389.11	694.55	347.28	173.64	86.82	43.41	21.70	10.85	5.43	2.71	1.36	0.68	0.34	17.36
2	APOA2	APOA2	P02652	Apolipoprotein A2	SPELQAEAK	4400.00	2200.00	1100.00	550.00	275.00	137.50	68.75	34.38	17.19	8.59	4.30	2.15	1.07	438.76
3	APOB	APOB	P04114	Apolipoprotein B-100	ALVDTLK	360.00	180.00	90.00	45.00	22.50	11.25	5.63	2.81	1.41	0.70	0.35	0.18	0.09	229.27
4	APOE	APOE	P02649	Apolipoprotein E	LAVYQAGAR	1071.53	535.77	267.88	133.94	66.97	33.49	16.74	8.37	4.19	2.09	1.05	0.52	0.26	13.39
5	APOH	APOH	P02749	Apolipoprotein H	ATVVYQGER	3300.00	1650.00	825.00	412.50	206.25	103.13	51.56	25.78	12.89	6.45	3.22	1.61	0.81	324.56
6	B2M	B2MG	P61769	Beta-2-microglobulin	VNHVTLSQPK	248.69	124.35	62.17	31.09	15.54	7.77	3.89	1.94	0.97	0.49	0.24	0.12	0.06	5.83
7	BCHE	CHLE	P06276	Cholinesterase	IFFPGVSEFGK	123.83	61.92	30.96	15.48	7.74	3.87	1.93	0.97	0.48	0.24	0.12	0.06	0.03	3.92
8	C1QA	C1QA	P02745	Complement C1q subcomponent subunit A	SLGFCDTTNK	216.70	108.35	54.17	27.09	13.54	6.77	3.39	1.69	0.85	0.42	0.21	0.11	0.05	22.63
9	C2	CO2	P06681	Complement C2	LNINLK	241.50	120.75	60.37	30.19	15.09	7.55	3.77	1.89	0.94	0.47	0.24	0.12	0.06	13.53
10	C3	CO3	P01024	Complement C3	DFDFVPPVVR	396.00	198.00	99.00	49.50	24.75	12.38	6.19	3.09	1.55	0.77	0.39	0.19	0.10	267.88
11	C5	CO5	P01031	Complement C5	LQGTLPVEAR	344.54	172.27	86.13	43.07	21.53	10.77	5.38	2.69	1.35	0.67	0.34	0.17	0.08	11.62
12	C6	CO6	P13671	Complement component C6	ALQEYAAK	211.31	105.65	52.83	26.41	13.21	6.60	3.30	1.65	0.83	0.41	0.21	0.10	0.05	3.30
13	C8A	CO8A	P07357	Complement component C8 alpha chain	LYYGDDEK	202.79	101.39	50.70	25.35	12.67	6.34	3.17	1.58	0.79	0.40	0.20	0.10	0.05	15.36
14	C8B	CO8B	P07358	Complement component C8 beta chain	YEFILK	197.08	98.54	49.27	24.63	12.32	6.16	3.08	1.54	0.77	0.38	0.19	0.10	0.05	9.13
15	C9	CO9	P02748	Complement component C9	VVEESELAR	355.69	177.84	88.92	44.46	22.23	11.12	5.56	2.78	1.39	0.69	0.35	0.17	0.09	11.14
16	CFB	CFAB	P00751	Complement factor B	EELLPAQDIK	486.00	243.00	121.50	60.75	30.38	15.19	7.59	3.80	1.90	0.95	0.47	0.24	0.12	33.53
17	CFH	CFAH	P08603	Complement factor H	VGEVLK	461.02	230.51	115.25	57.63	28.81	14.41	7.20	3.60	1.80	0.90	0.45	0.23	0.11	77.75
18	CP	CERU	P00450	Ceruloplasmin	GAYPLSIEPIGVR	468.00	234.00	117.00	58.50	29.25	14.63	7.31	3.66	1.83	0.91	0.46	0.23	0.11	217.08

19	CRP	CRP	P02741	C-reactive protein	ESDTSYVSLK	163.77	81.89	40.94	20.47	10.24	5.12	2.56	1.28	0.64	0.32	0.16	0.08	0.04	2.56
20	CST3	CYTC	P01034	Cystatin-C	ALDFAVGEYNK	116.39	58.20	29.10	14.55	7.27	3.64	1.82	0.91	0.45	0.23	0.11	0.06	0.03	2.49
21	F2	THRB	P00734	Prothrombin	ELLESYIDGR	226.48	113.24	56.62	28.31	14.16	7.08	3.54	1.77	0.88	0.44	0.22	0.11	0.06	21.92
22	F9	FA9	P00740	Coagulation factor IX	SALVLQYLR	98.28	49.14	24.57	12.28	6.14	3.07	1.54	0.77	0.38	0.19	0.10	0.05	0.02	4.00
23	F10	FA10	P00742	Coagulation factor X	TGIVSGFGR	98.30	49.15	24.57	12.29	6.14	3.07	1.54	0.77	0.38	0.19	0.10	0.05	0.02	3.07
24	F11	FA11	P03951	Coagulation factor XI	VVSGFSLK	120.55	60.27	30.14	15.07	7.53	3.77	1.88	0.94	0.47	0.24	0.12	0.06	0.03	3.55
25	GSN	GELS	P06396	Gelsolin	AGALNSNDAFVLK	1992.42	996.21	498.11	249.05	124.53	62.26	31.13	15.57	7.78	3.89	1.95	0.97	0.49	49.81
26	IGFBP3	IBP3	P17936	Insulin-like growth factor-binding protein 3	FLNVLSPR	145.58	72.79	36.40	18.20	9.10	4.55	2.27	1.14	0.57	0.28	0.14	0.07	0.04	3.69
27	PLG	PLMN	P00747	Plasminogen	LFLEPTR	286.43	143.21	71.61	35.80	17.90	8.95	4.48	2.24	1.12	0.56	0.28	0.14	0.07	61.59
28	PROC	PROC	P04070	Vitamin K-dependent protein C	TFVLNFIK	79.59	39.79	19.90	9.95	4.97	2.49	1.24	0.62	0.31	0.16	0.08	0.04	0.02	1.24
29	PROS1	PROS	P07225	Vitamin K-dependent protein S	YLVCLR	217.63	108.82	54.41	27.20	13.60	6.80	3.40	1.70	0.85	0.43	0.21	0.11	0.05	3.40
30	SERPINC1	ANT3	P01008	Antithrombin-III	FDTISEK	241.82	120.91	60.46	30.23	15.11	7.56	3.78	1.89	0.94	0.47	0.24	0.12	0.06	26.44
31	SERPINF2	A2AP	P08697	Alpha-2-antiplasmin	LGNQEPGGQTALK	273.62	136.81	68.41	34.20	17.10	8.55	4.28	2.14	1.07	0.53	0.27	0.13	0.07	38.89
32	SERPING1	IC1	P05155	Plasma protease C1 inhibitor	LLDSLPSDTR	360.00	180.00	90.00	45.00	22.50	11.25	5.63	2.81	1.41	0.70	0.35	0.18	0.09	86.62
33	SHBG	SHBG	P04278	Sex hormone-binding globulin	IALGGLLFPASNLR	278.79	139.39	69.70	34.85	17.42	8.71	4.36	2.18	1.09	0.54	0.27	0.14	0.07	4.36
34	TTR	TTHY	P02766	Transthyretin	VLDAVR	201.75	100.87	50.44	25.22	12.61	6.30	3.15	1.58	0.79	0.39	0.20	0.10	0.05	4.63

ITR, Ion transition ratio; %mean df, percentage of mean difference.

[a] The concentration of samples for injection is marked and was calculated considering the endogenous level and purity of the SIS peptide.

[b] The endogenous level was calculated using the most intense ion of target peptide.

Table 1-5. Target transitions and their parameters for MRM-MS assays with related diseases.

Gene symbol	Uniprot ID	Uniprot Accession	Protein Name	Peptide Sequence	Light peptide		Heavy peptide		Ion charge state		Product ion type	RT	DP	CE	Related disease information	
					Precursor ion (m/z)	Product ion (m/z)	Precursor ion (m/z)	Product ion (m/z)	Precursor ion	Product ion					Diseases	References
AMBP[a]	AMBP	P02760	Protein AMBP	ETLLQDFR	511.3	791.4	516.3	801.4	++	+	y6	31.268.4	22.9		Not specifically but highly related disease	64, 65
					511.3	565.3	516.3	575.3	++	+	y4	31.268.4	24.9			
					511.3	437.2	516.3	447.2	++	+	y3	31.268.4	24.9			
APOA2[a]	APOA2	P02652	Apolipoprotein A2	SPELQAEAK	486.8	659.4	490.8	667.4	++	+	y6	19.866.6	23.5		Hypercholesterolemia; metabolic syndrome; autoimmune pancreatitis	66, 67
					486.8	418.2	490.8	426.2	++	+	y4	19.866.6	25.5			
					486.8	443.2	490.8	447.2	++	++	y8	19.866.6	27.5			
					486.8	185.1	490.8	185.1	++	+	b2	19.866.6	27.5			
APOB	APOB	P04114	Apolipoprotein B-100	ALVDTLK	380.2	575.3	384.2	583.4	++	+	y5	23.758.8	19.4		Cardiovascular disease (CVD), type II diabetes	49, 51
					380.2	476.3	384.2	484.3	++	+	y4	23.758.8	17.4			
					380.2	185.1	384.2	185.1	++	+	b2	23.758.8	21.4			
APOE[a]	APOE	P02649	Apolipoprotein E	LAVYQAGAR	474.8	764.4	479.8	774.4	++	+	y7	21.465.7	22.8		Cardiovascular disease (CVD); Alzheimer disease (AD)	68, 69
					474.8	665.3	479.8	675.3	++	+	y6	21.465.7	22.8			
					474.8	502.3	479.8	512.3	++	+	y5	21.465.7	20.8			
					474.8	374.2	479.8	384.2	++	+	y4	21.465.7	20.8			
APOH[a]	APOH	P02749	Apolipoprotein H	ATVVYQGER	511.8	850.4	516.8	860.5	++	+	y7	20.268.4	22.9		Antiphospholipid syndrome; autoimmune diseases	70, 71
					511.8	751.4	516.8	761.4	++	+	y6	20.268.4	22.9			
					511.8	652.3	516.8	662.3	++	+	y5	20.268.4	22.9			
					511.8	272.2	516.8	272.2	++	+	b3	20.268.4	22.9			
B2M	B2MG	P61769	Beta-2-microglobulin	VNHVTLSQPK	374.9	673.4	377.6	681.4	+++	+	y6	19.858.4	20.7		Multiple myeloma; leukemia; lymphoma; kidney damage	53, 57, 60
					374.9	572.3	377.6	580.4	+++	+	y5	19.858.4	20.7			
					374.9	351.2	377.6	351.2	+++	+	b3	19.858.4	20.7			
BCHE	CHLE	P06276	Cholinesterase	IFFPGVSEFGK	614.3	820.4	618.3	828.4	++	+	y8	35.175.9	24.8		Pseudocholinesterase deficiency	59
					614.3	723.4	618.3	731.4	++	+	y7	35.175.9	26.8			
					614.3	261.2	618.3	261.2	++	+	b2	35.175.9	34.8			
C1QA	C1QA	P02745	Complement C1q subcomponent subunit A	SLGFCDTTNK	571.8	942.4	575.8	950.4	++	+	y8	23.072.8	24.3		Systemic lupus erythematosus (SLE); non-HAE C1 inhibitor deficiency	32, 33, 34
					571.8	738.3	575.8	746.3	++	+	y6	23.072.8	24.3			
					571.8	201.1	575.8	201.1	++	+	b2	23.072.8	28.3			
C2	CO2	P06681	Complement C2	LNINLK	357.7	601.4	361.7	609.4	++	+	y5	24.557.2	18.1		Systemic Lupus Erythematosus (SLE)	32, 33, 34
					357.7	487.3	361.7	495.3	++	+	y4	24.557.2	18.1			
					357.7	228.1	361.7	228.1	++	+	b2	24.557.2	26.1			
C3	CO3	P01024	Complement C3	DFDFVPPVVR	595.8	928.5	600.8	938.5	++	+	y8	34.674.6	23.7		Systemic lupus erythematosus (SLE); nephritis; partial lipodystrophy	32, 33, 34, 35
					595.8	666.4	600.8	676.4	++	+	y6	34.674.6	27.7			
					595.8	567.4	600.8	577.4	++	+	y5	34.674.6	27.7			
					595.8	263.1	600.8	263.1	++	+	b2	34.674.6	21.7			
					595.8	378.1	600.8	378.1	++	+	b3	34.674.6	21.7			
C5	CO5	P01031	Complement C5	LQGTLPVEAR	542.3	842.5	547.3	852.5	++	+	y8	23.170.7	26.6		Neisserial (Meningococcal/Gonococcal) susceptible	37
					542.3	684.4	547.3	694.4	++	+	y6	23.170.7	26.6			
					542.3	571.3	547.3	581.3	++	+	y5	23.170.7	26.6			
					542.3	242.2	547.3	242.2	++	+	b2	23.170.7	26.6			
C6	CO6	P13671	Complement component C6	ALQEYAAK	447.2	709.4	451.2	717.4	++	+	y6	20.163.7	19.2		Neisserial (Meningococcal/Gonococcal) susceptible	37
					447.2	185.1	451.2	185.1	++	+	b2	20.163.7	29.2			
					447.2	157.1	451.2	157.1	++	++	b3	20.163.7	29.2			
C8A	CO8A	P07357	Complement component C8 alpha chain	LYYGDDEK	501.7	726.3	505.7	734.3	++	+	y6	21.067.7	22.3		Neisserial (Meningococcal/Gonococcal) susceptible	37
					501.7	277.2	505.7	277.2	++	+	b2	21.067.7	24.3			
					501.7	249.1	505.7	249.1	++	++	b4	21.067.7	24.3			
C8B	CO8B	P07358	Complement component C8 beta chain	YEFILK	406.7	260.2	410.7	268.2	++	+	y2	29.660.8	18.9		Neisserial (Meningococcal/Gonococcal) susceptible	37
					406.7	260.7	410.7	264.7	++	++	y4	29.660.8	26.9			
					406.7	293.1	410.7	293.1	++	+	b2	29.660.8	26.9			
C9	CO9	P02748	Complement component C9	VVEESELAR	516.3	833.4	521.3	843.4	++	+	y7	21.068.8	23.2		Neisserial (Meningococcal/Gonococcal) susceptible	37
					516.3	704.4	521.3	714.4	++	+	y6	21.068.8	23.2			

					516.3	199.1	521.3	199.1	++	+	b2	21.068.8	25.2		
CFB	CFAB	P00751	Complement factor B	EELLPAQDIK	578.3	671.4	582.3	679.4	++	+	y6	26.173.3	24.7	Secondary C3 deficiency; partial lipodystrophy	36
					578.3	259.1	582.3	259.1	++	+	b2	26.173.3	18.7		
					578.3	372.2	582.3	372.2	++	+	b3	26.173.3	18.7		
CFH	CFAH	P08603	Complement factor H	VGEVLK	322.7	545.3	326.7	553.3	++	+	y5	20.154.6	16.1	Nephritis; atypical hemolytic-uremic syndromes (HUS)	38
					322.7	488.3	326.7	496.3	++	+	y4	20.154.6	16.1		
					322.7	286.1	326.7	286.1	++	+	b3	20.154.6	18.1		
CP	CERU	P00450	Ceruloplasmin	GAYPLSIEPIGVR	686.4	1080.6	691.4	1090.6	++	+	y10	31.981.2	28.9	Wilson disease	56
					686.4	870.5	691.4	880.5	++	+	y8	31.981.2	34.9		
					686.4	670.4	691.4	680.4	++	+	y6	31.981.2	38.9		
					686.4	541.3	691.4	551.4	++	+	y5	31.981.2	38.9		
CRP	CRP	P02741	C-reactive protein	ESDTSYVSLK	564.8	696.4	568.8	704.4	++	+	y6	23.172.3	23.9	Cardiovascular disease (CVD); arthritis; autoimmune disease	46, 47, 48
					564.8	609.4	568.8	617.4	++	+	y5	23.172.3	23.9		
					564.8	347.2	568.8	355.2	++	+	y3	23.172.3	25.9		
CST3	CYTC	P01034	Cystatin-C	ALDFAVGEYNK	613.8	709.4	617.8	717.4	++	+	y6	28.775.9	26.7	Acute kidney syndrome; Chronic renal failure	54, 55
					613.8	185.1	617.8	185.1	++	+	b2	28.775.9	24.7		
					613.8	300.2	617.8	300.2	++	+	b3	28.775.9	24.7		
F2	THRB	P00734	Prothrombin	ELLESYIDGR	597.8	710.3	602.8	720.4	++	+	y6	30.274.7	27.8	Hypopro-thrombinemia	45
					597.8	623.3	602.8	633.3	++	+	y5	30.274.7	27.8		
					597.8	243.1	602.8	243.1	++	+	b2	30.274.7	25.8		
F9	FA9	P00740	Coagulation factor IX	SALVLQYLR	531.8	692.4	536.8	702.4	++	+	y6	32.869.9	24.0	Hemophilia B	46
					531.8	159.1	536.8	159.1	++	+	y5	32.869.9	22.0		
					531.8	272.2	536.8	272.2	++	+	b2	32.869.9	22.0		
					447.2	622.3	452.3	632.3	++	+	b3	24.863.7	19.2		
F10	FA10	P00742	Coagulation factor X	TGIVSGFGR	447.2	523.3	452.3	533.3	++	+	y5	24.863.7	21.2	Stuart-Prower factor deficiency	42
					447.2	436.2	452.3	446.2	++	+	y4	24.863.7	21.2		
					447.2	272.2	452.3	272.2	++	+	b3	24.863.7	21.2		
F11	FA11	P03951	Coagulation factor XI	VVSGFSLK	418.7	638.4	422.8	646.4	++	+	y6	25.561.6	21.6	Hemophilia C; plasma thromboplastin antecedent deficiency	46, 40
					418.7	199.1	422.8	199.1	++	+	b2	25.561.6	19.6		
GSN[a]	GELS	P06396	Gelsolin	AGALNSNDAFVLK	660.4	1007.5	664.4	1015.5	++	+	y9	28.679.3	27.4	Multiple organ dysfunction syndrome; antagonistic sepsis	72, 73
					660.4	893.5	664.4	901.5	++	+	y8	28.679.3	27.4		
					660.4	200.1	664.4	200.1	++	+	b3	28.679.3	31.4		
IGFBP3	IBP3	P17936	Insulin-like growth factor-binding protein 3	FLNVLSPR	473.3	685.4	478.3	695.4	++	+	y6	29.765.6	20.7	Growth hormone deficiency	61
					473.3	472.3	478.3	482.3	++	+	y4	29.765.6	22.7		
					473.3	359.2	478.3	369.2	++	+	y3	29.765.6	22.7		
PLG	PLMN	P00747	Plasminogen	LFLEPTR	438.3	762.4	443.3	772.4	++	+	y6	26.763.1	22.7	Congenital plasminogen deficiency; thrombosis	44
					438.3	615.3	443.3	625.4	++	+	y5	26.763.1	22.7		
					438.3	502.3	443.3	512.3	++	+	y4	26.763.1	20.7		
					438.3	373.2	443.3	383.2	++	+	y3	26.763.1	20.7		
					438.3	261.2	443.3	261.2	++	+	b2	26.763.1	20.7		
PROC	PROC	P04070	Vitamin K-dependent protein C	TFVLNFIK	491.3	733.5	495.3	741.5	++	+	y6	35.766.9	19.7	Deep vein thrombosis (DVT)	43
					491.3	634.4	495.3	642.4	++	+	y5	35.766.9	19.7		
					491.3	249.1	495.3	249.1	++	+	b2	35.766.9	23.7		
PROS1	PROS	P07225	Vitamin K-dependent protein S	YLVCLR	412.2	660.4	417.2	670.4	++	+	y5	26.561.2	23.2	Deep vein thrombosis (DVT)	43
					412.2	547.3	417.2	557.3	++	+	y4	26.561.2	23.2		
					412.2	448.2	417.2	458.2	++	+	y3	26.561.2	19.2		
					412.2	277.2	417.2	277.2	++	+	b2	26.561.2	21.2		
SERPINC1	ANT3	P01008	Antithrombin-III	FDTISEK	420.2	692.3	424.2	700.4	++	+	y6	20.861.8	21.7	Deep vein thrombosis (DVT)	43
					420.2	577.3	424.2	585.3	++	+	y5	20.861.8	21.7		
					420.2	263.1	424.2	263.1	++	+	b2	20.861.8	19.7		
SERPINF2	A2AP	P08697	Alpha-2-antiplasmin	LGNQEPGGQTALK	656.8	771.4	660.9	779.5	++	+	y8	20.379.0	31.2	Liver cirrhosis (LC); disorder of fibrinolysis	41
					656.8	674.4	660.9	682.4	++	+	y7	20.379.0	33.2		
					656.8	413.2	660.9	413.2	++	+	b4	20.379.0	37.2		
SERPING1	IC1	P05155	Plasma protease C1 inhibitor	LLDSLPSDTR	558.8	775.4	563.8	785.4	++	+	y7	24.771.9	25.6	Hereditary angioedema (HAE)	39
					558.8	575.3	563.8	585.3	++	+	y5	24.771.9	27.6		
					558.8	227.2	563.8	227.2	++	+	b2	24.771.9	23.6		
SHBG	SHBG	P04278	Sex hormone-binding globulin	IALGGLLFPASNLR	721.4	917.5	726.4	927.5	++	+	y8	39.383.7	34.9	Testosterone deficiency; type II diabetes	50, 52
					721.4	804.4	726.4	814.4	++	+	y7	39.383.7	34.9		
					721.4	412.3	726.4	412.3	++	+	b5	39.383.7	32.9		
TTR	TTHY	P02766	Transthyretin	VLDAVR	336.7	573.3	341.7	583.3	++	+	y5	20.355.7	14.9	Senile systemic amyloidosis (SSA); Alzheimer disease	58, 62
					336.7	460.3	341.7	470.3	++	+	y4	20.355.7	14.9		
					336.7	345.2	341.7	355.2	++	+	y3	20.355.7	14.9		

1.3.3 Intra-assay and inter-assay repeatability

Three QC samples were prepared at low, medium, and high concentrations to evaluate the repeatability of the intraday and interday quantification assays. Low- and medium-QC samples were prepared by spiking SIS peptides at 2 or 3 times and 50-100 times the LLOQ, respectively. The high-QC samples were prepared to be less than 90% of the ULOQ and over 100 times of the LLOQ (see Table 1-3). Three types of QC samples were analyzed in triplicate each day (≥ 16 h apart) over 5 days.

The intra-assay repeatability (CV_{intra}) was calculated as follows: first, 3 replicates of the low QC sample were analyzed each day for 5 days. The CV values of each replicate for a given day were calculated. Subsequently, mean CV values for each day were averaged to calculate the intra-assay repeatability. The first replicates from each day were averaged to calculate the inter-assay repeatability (CV_{inter}). This process was repeated for all remaining QC samples. CV_{intra} and CV_{inter} values were used to calculate the total CV (CV_{total}) by determining the magnitude: ($\sqrt{(CV_{\mathrm{intra}})^2 + (CV_{\mathrm{inter}})^2}$).

The median CV_{intra}, CV_{inter}, and CV_{total} values were 11.6%, 13.4%, and 18.2% in low-QC; 7.7%, 9.4%, and 12.2% in medium-QC; and 7.2%, 7.9%, and 10.6% in high-QC samples, respectively (Figure 1-2A). Of the 112 transitions, 80 (71.4%), 103 (92.0%), and 111 (99.1%) had a CV < 20% in the low-, medium-, and high-QC samples, respectively, corresponding to 34 proteins (100%) (Table 1-6). For the low-, medium-, and high-QC samples in 45 measurements (3 QC samples × 3 replicates × 5 days), the mean %difference from the mean ion ratio ($\frac{2nd\ most\ intense\ ion}{most\ intense\ ion}$), which indicates the specificity of targets, was 13.1%, 4.34%, and 3.51%, respectively. The 34 proteins (100%), corresponding to 104 transitions (92.9%), were within 30% of the total mean ion ratio.

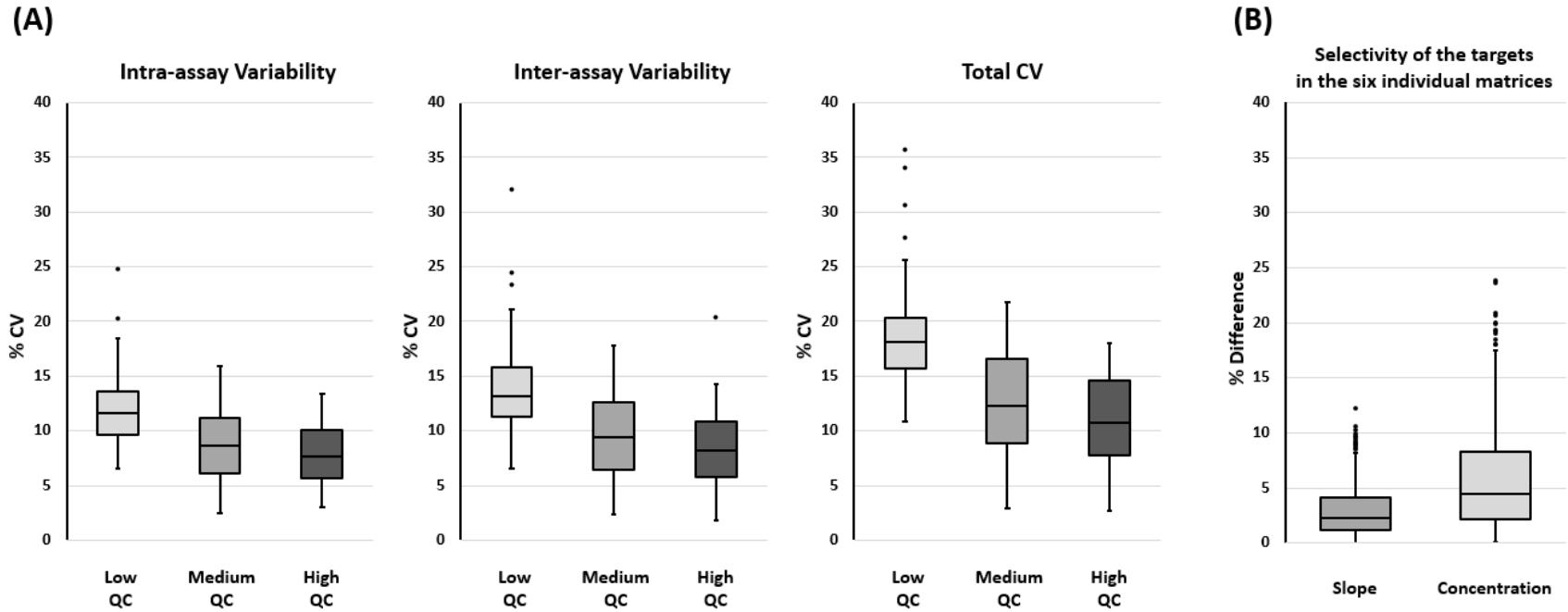
1.3.4 Selectivity of target peptides in six matrices

The 6 individual (men and women of 3 races) normal serum samples were used as matrices. Three QC samples (zero-QC, half medium-QC, and medium-QC) were prepared on the same day for each matrix: “zero-QC,” which did not contain SIS peptides; “half-medium-QC,” which was spiked with half of the concentration of the medium-QC; and “medium-QC,” which was spiked with the same concentration as the medium-QC. These QC samples were analyzed in duplicate, which were then averaged for plotting on a linear scale.

First, the 3 QC samples of each matrix were used to generate a linear fit. The resulting slopes of the 6 matrices were then averaged. Then, the percent difference between the mean slope and individual slope was calculated for all 6 matrices. A total of 110 of 112 transitions (98.2%), representing all 34 proteins, were within 10% of the difference between the mean slope of all matrices and the slopes of the 6 individual matrices (Figure 1-2B and Table 1-7).

Then, the predicted values of the “half-medium-QC,” which was one-half of the concentration value that was obtained by converting the peak area ratio (SIS/endogenous peptide) from the “medium-QC” sample, were compared with the actual values. Only 53 of 112 transitions (47.3%), representing 27 of 34 proteins (79.4%), passed the criterion of a difference of less than 10% (Figure 1-2B and Table 1-7)

Figure 1-2. Variability of assays and the difference in the response of analytes in six individual matrices



The intra- and interday assays analyzing 3 QC samples (low, middle, and high QC) were performed over 5 days in triplicate per day. The differences in slope among the 6 matrices are represented as %mean difference, and their distribution is shown in a box-whisker plot. For observing overall Repeatability (A) and Selectivity (B) of the target peptides, the box-whisker plots show the distribution of the CV results in experiment 2 and the %difference results in experiment 3. The bottom and top of the box are the first and third quartiles, and the band inside of the box is the median (second quartile). The outliers are indicated as lower than the first quartile minus 1.5 interquartile range (IQR) or higher than the third quartile plus 1.5 IQR. The criterion is that the variabilities must be lower than 20% CV.

Table 1-6. Intra- and inter-assay analytical repeatability of target peptides.

N.	Information of target protein and peptide						Average Intra-assay CV (%)			Average Inter-assay CV (%)			Total-CV (%)			ITR[b] (% mean df)
	Gene symbol	Uniprot ID	Uniprot Accession	Protein Name	Peptide Sequence	Quantification ion[a]	Low	Medium	High	Low	Medium	High	Low	Medium	High	
1	AMBP	AMBP	P02760	Protein AMBP	ETLLQDFR	2.y6.1	17.4	15.5	12.0	20.8	12.7	12.8	27.1	20.0	17.5	13.4 ± 13.5
						2.y4.1	10.6	13.0	8.4	14.7	13.2	8.1	18.1	18.6	11.7	5.6 ± 5.9
						2.y3.1	25.5	11.2	11.8	24.2	11.9	11.3	35.2	16.4	16.4	9.1 ± 12.2
2	APOA2	APOA2	P02652	Apolipoprotein A2	SPELQAEAK	2.y6.1	15.0	2.1	3.2	12.6	2.4	4.5	19.6	3.1	5.5	4.5 ± 5.6
						2.y4.1	9.4	3.6	2.3	16.6	4.5	2.5	19.1	5.8	3.4	3.7 ± 3.6
						2.y8.2	11.2	3.0	2.8	11.1	2.9	2.8	15.7	4.1	3.9	3.3 ± 3.7
						2.b2.1	13.1	1.7	2.9	12.5	2.4	3.3	18.1	2.9	4.4	5.6 ± 5.7
3	APOB	APOB	P04114	Apolipoprotein B-100	ALVDTLK	2.y5.1	14.2	2.5	4.0	10.5	2.7	5.4	17.6	3.7	6.7	27.3 ± 13.4
						2.y4.1	10.4	6.8	9.0	19.4	7.7	9.2	22.0	10.3	12.9	6.6 ± 4.9
						2.b2.1	7.2	5.8	4.0	12.1	5.6	4.8	14.1	8.1	6.2	9.6 ± 5.4
4	APOE	APOE	P02649	Apolipoprotein E	LAVYQAGAR	2.y7.1	8.5	3.5	2.6	21.6	4.1	3.3	23.2	5.3	4.2	4 ± 5.1
						2.y6.1	11.5	4.5	5.1	16.2	5.7	4.7	19.9	7.3	6.9	7.7 ± 10
						2.y5.1	12.1	5.1	3.3	14.7	5.8	6.3	19.1	7.7	7.1	11.6 ± 11.7
						2.y4.1	18.0	4.2	5.2	19.2	5.9	7.5	26.3	7.2	9.1	12.6 ± 17.7
5	APOH	APOH	P02749	Apolipoprotein H	ATVVYQGER	2.y7.1	14.1	3.5	4.2	17.9	6.2	4.5	22.8	7.2	6.1	9.9 ± 10
						2.y6.1	12.6	2.9	2.6	13.0	3.3	2.8	18.2	4.4	3.8	4.4 ± 5.2
						2.y5.1	9.9	2.4	2.1	8.5	2.5	1.8	13.0	3.4	2.7	2.6 ± 2.9
						2.b3.1	10.2	4.4	2.3	16.8	3.9	5.1	19.7	5.9	5.6	11.6 ± 9.2
6	B2M	B2MG	P61769	Beta-2-microglobulin	VNHVTLSQPK	3.y6.1	13.7	12.6	6.1	18.1	14.4	7.1	22.7	19.1	9.3	8.2 ± 7.6
						3.y5.1	17.3	9.2	10.7	14.6	9.4	12.1	22.6	13.2	16.2	8.2 ± 9.6
						3.b3.1	8.7	8.7	8.1	17.9	8.3	7.6	19.9	12.0	11.1	4.8 ± 7.6
7	BCHE	CHLE	P06276	Cholinesterase	IFFPGVSEFGK	2.y8.1	8.6	13.7	10.2	6.6	13.8	10.9	10.8	19.5	14.9	9.7 ± 10.8
						2.y7.1	7.8	14.7	11.7	8.3	18.4	14.8	11.4	23.6	18.9	16.5 ± 17.1
						2.b2.1	8.1	14.5	10.1	7.8	14.1	10.3	11.2	20.2	14.5	6 ± 5.7
8	C1QA	C1QA	P02745	Complement C1q subcomponent subunit A	SLGFCDTTNK	2.y8.1	13.7	5.2	5.3	14.4	7.2	5.4	19.9	8.9	7.6	6.1 ± 8.1
						2.y6.1	10.0	6.8	4.5	13.5	7.9	6.3	16.8	10.4	7.7	5.6 ± 5.6
						2.b2.1	8.1	5.3	5.7	8.5	5.6	7.0	11.8	7.8	9.0	2.6 ± 2.6
9	C2	CO2	P06681	Complement C2	LNINLK	2.y5.1	14.6	12.4	11.0	16.6	14.2	14.4	22.1	18.9	18.2	8.2 ± 6.3
						2.y4.1	13.0	12.3	10.6	17.6	14.3	11.1	21.9	18.9	15.3	10.1 ± 9.5
						2.b2.1	12.9	10.5	12.4	13.6	12.6	14.3	18.8	16.4	18.9	4.6 ± 4.4
10	C3	CO3	P01024	Complement C3	DFDFVPPVVR	2.y8.1	7.3	8.7	6.4	9.1	8.3	6.9	11.6	12.0	9.4	5.2 ± 4.1
						2.y6.1	11.0	6.6	4.9	12.3	8.3	5.7	16.5	10.6	7.5	3 ± 2.4
						2.y5.1	9.1	8.6	5.0	12.7	11.0	5.6	15.6	14.0	7.5	2.1 ± 1.5
						2.b2.1	9.6	8.6	6.2	8.1	12.1	6.8	12.6	14.9	9.2	4.1 ± 3.5
						2.b3.1	6.5	10.1	5.5	9.0	12.5	8.2	11.1	16.1	9.9	4 ± 3.9
11	C5	CO5	P01031	Complement C5	LQGTLPVEAR	2.y8.1	10.3	6.4	6.9	12.5	5.7	9.1	16.2	8.6	11.4	2.5 ± 1.8
						2.y6.1	7.8	6.6	7.1	9.0	6.2	7.5	11.9	9.0	10.4	4.2 ± 3.6
						2.y5.1	8.4	3.5	5.9	9.2	4.6	4.6	12.5	5.8	7.5	3.1 ± 2.9
						2.b2.1	9.6	7.2	5.3	7.6	7.3	5.1	12.2	10.3	7.4	3.1 ± 2.6
12	C6	CO6	P13671	Complement component C6	ALQEYAAK	2.y6.1	18.3	7.4	4.8	24.5	9.7	6.4	30.6	12.3	8.0	11.1 ± 15.3

						2.b2.1	9.2	7.3	10.2	12.9	9.7	14.9	15.9	12.2	18.0	13.4 ± 9.1
						2.b3.2	13.2	6.1	5.3	12.0	6.9	4.4	17.8	9.2	6.9	6.3 ± 3.8
13	C8A	CO8A	P07357	Complement component C8 alpha chain	LYYGDDEK	2.y6.1	13.1	7.5	4.0	17.9	6.9	3.8	22.2	10.2	5.5	5.8 ± 7.1
						2.b2.1	10.0	6.9	10.3	15.4	9.2	10.1	18.3	11.5	14.4	9.9 ± 14
						2.b4.2	11.1	5.3	3.0	9.0	5.2	3.5	14.3	7.5	4.7	4.4 ± 5.4
14	C8B	CO8B	P07358	Complement component C8 beta chain	YEFILK	2.y2.1	13.0	10.7	11.2	13.1	10.8	12.4	18.5	15.2	16.7	5.6 ± 5.8
						2.y4.2	13.1	11.3	11.1	12.0	10.5	14.4	17.7	15.4	18.2	5.6 ± 6.8
						2.b2.1	12.3	11.5	9.9	13.0	14.4	10.2	17.9	18.4	14.2	5.5 ± 6.2
15	C9	CO9	P02748	Complement component C9	VVEESELAR	2.y7.1	11.6	5.0	5.5	11.4	5.6	5.7	16.3	7.5	7.9	5.7 ± 7.3
						2.y6.1	18.4	6.9	5.0	17.0	5.6	5.1	25.0	8.9	7.2	8.1 ± 9.6
						2.b2.1	10.5	4.6	4.6	15.0	4.6	5.2	18.3	6.5	6.9	3.8 ± 4.2
16	CFB	CFAB	P00751	Complement factor B	EELLPAQDIK	2.y6.1	20.2	6.6	10.1	10.7	9.6	9.6	22.9	11.6	13.9	7.9 ± 10.2
						2.b2.1	11.4	11.9	8.6	18.2	16.9	10.6	21.4	20.7	13.7	11.7 ± 14.3
						2.b3.1	8.5	12.6	10.1	11.0	13.6	13.6	13.9	18.5	16.9	11.5 ± 15.5
17	CFH	CFAH	P08603	Complement factor H	VGEVLK	2.y5.1	7.0	7.1	7.5	13.6	13.2	12.3	15.3	15.0	14.4	3.6 ± 2.7
						2.y4.1	16.2	6.1	6.0	14.2	7.3	5.7	21.6	9.5	8.3	20.6 ± 15.3
						2.b3.1	10.6	4.5	5.2	13.5	8.1	8.3	17.2	9.3	9.8	7.8 ± 5.8
18	CP	CERU	P00450	Ceruloplasmin	GAYPLSIEPIGVR	2.y10.1	9.7	4.9	6.1	11.8	8.4	6.3	15.3	9.7	8.8	6.6 ± 5
						2.y8.1	10.2	9.3	7.8	21.6	11.1	9.7	23.9	14.5	12.5	9.8 ± 9.1
						2.y6.1	12.1	9.7	9.3	15.0	12.5	8.7	19.3	15.8	12.7	5.2 ± 5.3
						2.y5.1	12.4	13.4	8.1	12.8	13.6	7.3	17.8	19.1	10.9	7.2 ± 7.9
19	CRP	CRP	P02741	C-reactive protein	ESDTSYVSLK	2.y6.1	11.2	7.7	12.9	14.9	11.6	13.1	18.6	14.0	18.4	5.4 ± 7
						2.y5.1	10.1	8.7	10.6	14.6	12.0	11.0	17.7	14.8	15.3	5.7 ± 6.2
						2.y3.1	14.4	11.4	11.6	12.6	11.7	10.7	19.1	16.4	15.8	10 ± 12.4
20	CST3	CYTC	P01034	Cystatin-C	ALDFAVGEYNK	2.y6.1	9.9	13.2	10.0	11.3	13.3	15.7	15.0	18.7	18.6	10.8 ± 13.2
						2.b2.1	15.1	11.6	13.0	15.8	16.7	12.6	21.9	20.3	18.2	7.5 ± 10.3
						2.b3.1	16.9	15.9	11.5	15.8	16.4	11.0	23.1	22.9	15.9	16.3 ± 19.3
21	F2	THRB	P00734	Prothrombin	ELLESYIDGR	2.y6.1	11.5	13.3	8.8	11.2	14.5	6.3	16.0	19.7	10.8	5.9 ± 4.8
						2.y5.1	11.7	12.9	8.6	14.5	11.1	8.6	18.6	17.0	12.2	9.6 ± 12
						2.b2.1	12.9	10.6	10.6	13.6	11.6	12.3	18.8	15.7	16.3	6.6 ± 5.9
22	F9	FA9	P00740	Coagulation factor IX	SALVLQYLR	2.y5.1	16.1	13.2	9.5	17.2	12.0	10.5	23.5	17.8	14.1	7.1 ± 7.8
						2.b2.1	15.8	14.7	11.4	32.1	14.6	20.3	35.7	20.8	23.3	11.8 ± 11.3
						2.b3.1	14.3	9.5	13.3	13.3	12.7	9.8	19.5	15.9	16.5	7.5 ± 7.8
23	F10	FA10	P00742	Coagulation factor X	TGIVSGFGR	2.y6.1	10.7	5.9	3.3	11.0	7.3	6.0	15.4	9.4	6.8	3.3 ± 3.9
						2.y5.1	12.9	5.1	6.5	16.4	5.9	5.7	20.9	7.8	8.7	5.4 ± 6.9
						2.y4.1	10.0	12.0	7.0	12.4	11.8	8.8	15.9	16.8	11.2	5.4 ± 6
						2.b3.1	11.4	7.4	8.5	12.2	5.6	11.9	16.7	9.3	14.6	7.2 ± 5.9
24	F11	FA11	P03951	Coagulation factor XI	VVSGFSLK	2.y6.1	11.6	8.7	6.5	13.0	11.1	5.7	17.4	14.1	8.7	5.7 ± 5.7
						2.b2.1	13.8	12.7	7.5	16.4	13.0	7.2	21.4	18.1	10.4	2.3 ± 2.3
25	GSN	GELS	P06396	Gelsolin	AGALNSNDAFVLK	2.y9.1	10.8	14.8	6.9	11.3	12.6	8.7	15.7	19.5	11.1	4.7 ± 4.9
						2.y8.1	12.4	10.2	6.4	14.8	10.2	8.3	19.3	14.4	10.5	6 ± 5.6
						2.b3.1	9.3	10.4	7.7	10.5	13.1	7.8	14.0	16.7	11.0	4.5 ± 5.7
26	IGFBP3	IBP3	P17936	Insulin-like growth factor-binding protein 3	FLNVLSPR	2.y6.1	15.7	10.2	9.9	21.2	11.1	10.9	26.4	15.1	14.7	5.9 ± 7.7
						2.y4.1	13.8	13.3	10.1	14.9	14.9	11.5	20.3	20.0	15.3	5 ± 6.1
						2.y3.1	11.3	11.9	11.5	11.4	13.9	11.9	16.0	18.3	16.5	8.6 ± 10.2
27	PLG	PLMN	P00747	Plasminogen	LFLEPTR	2.y6.1	16.2	6.6	7.4	15.6	7.8	6.4	22.5	10.3	9.8	9.7 ± 10.9
						2.y5.1	12.2	4.1	5.9	12.3	3.4	5.0	17.3	5.4	7.8	4.2 ± 3.9
						2.y4.1	9.2	6.2	6.7	15.9	6.4	6.9	18.4	8.9	9.7	6 ± 7.3
						2.y3.1	14.3	6.0	7.6	13.3	6.6	8.3	19.5	8.9	11.3	6 ± 6.8
						2.b2.1	13.0	5.6	6.4	14.3	7.1	6.2	19.4	9.1	8.9	4.6 ± 4.2
28	PROC	PROC	P04070	Vitamin K-dependent protein C	TFVLNFIK	2.y6.1	12.2	6.8	6.4	19.6	7.2	7.6	23.1	9.9	9.9	6.1 ± 6.5
						2.y5.1	10.3	9.2	9.5	17.1	12.2	8.4	20.0	15.3	12.7	8.8 ± 11.3
						2.b2.1	12.6	5.9	4.0	13.1	9.3	8.8	18.2	11.0	9.6	4.1 ± 4.7

29	PROS1	PROS	P07225	Vitamin K-dependent protein S	YLVCLR	2.y5.1	12.1	13.5	9.8	21.6	16.0	14.3	24.8	21.0	17.4	10.8 ± 10
						2.y4.1	11.5	10.8	8.3	12.8	12.5	6.9	17.2	16.5	10.8	5.4 ± 5.8
						2.y3.1	14.4	7.6	6.3	12.3	8.1	8.3	18.9	11.1	10.4	9.7 ± 9.5
						2.b2.1	9.6	8.8	10.9	11.5	8.8	10.8	15.0	12.4	15.4	7.5 ± 6.7
30	SERPINC1	ANT3	P01008	Antithrombin-III	FDTISEK	2.y6.1	13.0	2.7	4.2	11.9	4.8	6.5	17.6	5.5	7.7	4 ± 4.9
						2.y5.1	7.8	3.5	3.1	15.4	3.7	4.4	17.3	5.1	5.3	5.6 ± 6.3
						2.b2.1	7.9	2.8	3.9	11.1	3.0	4.3	13.6	4.1	5.8	2.4 ± 2.3
31	SERPINF2	A2AP	P08697	Alpha-2-antiplasmin	LGNQEPGGQTALK	2.y8.1	11.2	4.5	3.3	12.8	5.3	4.9	17.0	6.9	5.9	4.4 ± 5.3
						2.y7.1	24.8	8.0	7.6	23.3	11.1	10.2	34.0	13.7	12.8	16.7 ± 20.8
						2.b4.1	9.0	9.5	8.1	15.8	9.7	9.4	18.2	13.6	12.4	17.1 ± 24.5
32	SERPING1	IC1	P05155	Plasma protease C1 inhibitor	LLDSLPSDTR	2.y7.1	17.4	10.9	9.6	19.9	12.6	11.0	26.4	16.6	14.6	25.3 ± 20.1
						2.y5.1	9.4	6.4	7.2	9.3	8.2	6.2	13.3	10.4	9.5	5.1 ± 6.5
						2.b2.1	12.5	9.0	11.1	14.5	11.2	11.3	19.1	14.4	15.9	11.3 ± 14.3
33	SHBG	SHBG	P04278	Sex hormone-binding globulin	IALGGLLFPASNLR	2.y8.1	8.6	6.4	7.3	8.9	7.1	7.5	12.4	9.6	10.5	3.3 ± 2.8
						2.y7.1	6.7	6.1	6.9	9.5	6.4	7.6	11.6	8.8	10.3	2.6 ± 2.3
						2.b5.1	8.8	8.9	9.9	8.8	9.5	10.0	12.5	13.0	14.0	3.5 ± 2.8
34	TTR	TTHY	P02766	Transthyretin	VLDAVR	2.y5.1	12.2	10.0	9.6	14.5	13.3	11.0	18.9	16.6	14.6	13.9 ± 13.9
						2.y4.1	12.5	5.2	4.3	10.6	6.4	4.3	16.4	8.3	6.1	3.5 ± 3.4
						2.y3.1	18.5	10.6	9.5	20.5	13.1	8.9	27.6	16.8	13.0	15.7 ± 18.3

ITR, Ion transition ratio; %mean df, percentage of mean difference.

[a] The column title indicates the information of quantification ion, followed by the precursor ion charge, production ion type, and product ion charge.

[b] Mean ± standard deviation.

Underline indicates that the value did not meet the criteria.

Table 1-7. Response of the targets in 6 different matrices.

N.	Information of target protein and peptide						Evaluation Items		6 individual samples						ITR[b] (%mean df)
	Gene symbol	Uniprot ID	Uniprot Accession	Protein Name	Peptide Sequence	Quantification ion[a]			A	B	C	D	E	F	
1	AMBP	AMBP	P02760	Protein AMBP	ETLLQDFR	2.y6.1	Difference (%)	Slope	1.1	3.8	2.6	5.7	1.9	2.2	3.6 ± 2.8
								Concentration	0.9	1.2	3.0	5.1	8.5	6.2	
						2.y4.1	Difference (%)	Slope	1.4	2.1	1.1	0.8	1.2	1.3	1.5 ± 1.2
								Concentration	1.9	3.2	0.3	3.8	3.9	2.4	
						2.y3.1	Difference (%)	Slope	0.1	0.9	2.3	2.2	0.6	1.7	2.5 ± 1.8
								Concentration	2.2	6.7	2.6	1.3	9.6	3.7	
2	APOA2	APOA2	P02652	Apolipoprotein A2	SPELQAEAK	2.y6.1	Difference (%)	Slope	0.7	0.2	0.8	0.1	1.0	0.8	2.4 ± 2.3
								Concentration	8.0	1.5	6.2	3.4	2.8	6.3	
						2.y4.1	Difference (%)	Slope	0.6	1.1	2.1	0.7	3.3	0.2	3.0 ± 2.4
								Concentration	0.1	2.6	9.0	1.9	2.2	6.7	
						2.y8.2	Difference (%)	Slope	0.6	0.9	3.6	1.0	0.1	4.2	2.2 ± 1.8
								Concentration	4.1	0.8	5.3	14.3	0.3	0.6	
						2.b2.1	Difference (%)	Slope	0.2	3.0	0.9	0.6	1.0	2.7	2.3 ± 1.0
								Concentration	0.5	6.5	4.9	18.5	9.8	3.8	
3	APOB	APOB	P04114	Apolipoprotein B-100	ALVDTLK	2.y5.1	Difference (%)	Slope	3.8	5.4	3.2	0.2	4.0	5.8	3.3 ± 3.2
								Concentration	6.1	2.9	4.3	9.0	14.2	3.5	
						2.y4.1	Difference (%)	Slope	0.4	3.8	2.1	4.9	1.2	0.5	6.4 ± 4.1
								Concentration	4.1	0.1	20.7	3.2	4.5	6.1	
						2.b2.1	Difference (%)	Slope	1.8	5.7	1.1	6.1	6.7	6.5	2.2 ± 1.6
								Concentration	3.3	1.8	0.6	9.5	1.7	9.5	
4	APOE	APOE	P02649	Apolipoprotein E	LAVYQAGAR	2.y7.1	Difference (%)	Slope	6.8	2.7	2.2	0.7	2.0	3.2	3.1 ± 2.4
								Concentration	8.8	6.0	25.6	3.6	13.0	7.1	
						2.y6.1	Difference (%)	Slope	4.0	0.4	0.2	2.0	0.5	6.7	2 ± 1.8
								Concentration	1.3	9.0	4.5	8.6	1.2	0.5	
						2.y5.1	Difference (%)	Slope	3.2	9.1	4.4	0.1	0.7	2.4	4 ± 2.9
								Concentration	3.5	7.2	16.8	3.0	6.8	16.2	
						2.y4.1	Difference (%)	Slope	1.6	3.8	5.3	2.7	0.8	0.3	3.6 ± 2.9
								Concentration	1.1	1.1	5.9	0.1	5.4	9.5	
5	APOH	APOH	P02749	Apolipoprotein H	ATVVYQGER	2.y7.1	Difference (%)	Slope	1.5	6.3	6.3	3.9	2.1	4.6	4.6 ± 3.3
								Concentration	3.9	14.4	2.4	7.9	9.1	3.6	
						2.y6.1	Difference (%)	Slope	0.4	1.0	5.1	4.7	1.0	0.8	2.5 ± 1.8
								Concentration	10.5	11.4	2.1	1.6	2.6	1.5	
						2.y5.1	Difference (%)	Slope	1.9	0.2	0.5	5.0	0.7	2.1	1.5 ± 1.1
								Concentration	5.3	0.8	3.8	8.5	1.8	1.5	
						2.b3.1	Difference (%)	Slope	0.1	5.7	3.8	2.1	0.8	1.1	4.2 ± 2.4
								Concentration	10.5	3.7	3.7	20.0	1.5	10.1	
6	B2M	B2MG	P61769	Beta-2-microglobulin	VNHVTLSQPK	3.y6.1	Difference (%)	Slope	5.0	0.1	3.3	1.9	1.5	2.0	4.5 ± 3.7
								Concentration	5.7	3.7	1.3	17.5	12.6	9.7	
						3.y5.1	Difference (%)	Slope	7.8	8.7	1.0	1.9	2.8	3.0	3.9 ± 3.6
								Concentration	9.3	5.6	4.8	9.8	1.3	12.1	
						3.b3.1	Difference (%)	Slope	7.1	0.9	5.0	0.8	7.4	5.4	2.7 ± 2.4

7	BCHE	CHLE	P06276	Cholinesterase	IFFPGVSEFGK	2.y8.1	Difference (%)	Concentration	2.7	11.5	5.2	0.3	9.2	4.5	2.4 ± 1.6
								Slope	6.9	6.4	0.2	3.3	5.1	2.5	
						2.y7.1	Difference (%)	Concentration	2.6	0.4	1.6	6.8	15.6	2.3	2.7 ± 2.1
								Slope	8.8	4.9	0.7	4.0	4.5	3.7	
						2.b2.1	Difference (%)	Concentration	4.3	1.0	5.1	5.7	0.8	2.8	1.2 ± 0.9
								Slope	7.1	7.3	0.3	2.9	5.7	2.9	
8	C1QA	C1QA	P02745	Complement C1q subcomponent subunit A	SLGFCDTTNK	2.y8.1	Difference (%)	Concentration	9.6	6.8	0.1	8.9	12.2	4.4	6.0 ± 3.4
								Slope	0.6	3.9	2.7	2.3	2.3	0.6	
						2.y6.1	Difference (%)	Concentration	1.7	0.3	8.3	6.5	8.9	4.1	5.8 ± 3.9
								Slope	3.0	0.9	3.0	4.9	2.1	1.8	
						2.b2.1	Difference (%)	Concentration	3.5	2.6	6.0	4.4	10.8	3.3	2.0 ± 1.4
								Slope	0.6	3.1	2.2	0.9	5.8	4.6	
9	C2	CO2	P06681	Complement C2	LNINLK	2.y5.1	Difference (%)	Concentration	0.1	3.0	7.6	5.6	9.0	1.1	4.1 ± 3.2
								Slope	3.1	5.9	1.6	0.8	4.6	5.0	
						2.y4.1	Difference (%)	Concentration	13.9	15.2	7.8	1.2	8.7	3.4	3.6 ± 2.4
								Slope	6.2	1.1	1.9	3.1	0.9	0.9	
						2.b2.1	Difference (%)	Concentration	6.4	2.7	1.2	2.0	1.7	6.9	2.5 ± 1.5
								Slope	9.7	3.8	1.5	2.3	5.5	1.3	
10	C3	CO3	P01024	Complement C3	DFDFVPPVVR	2.y8.1	Difference (%)	Concentration	20.9	3.0	3.7	9.7	10.2	3.5	2.3 ± 2.1
								Slope	2.7	2.0	2.1	0.9	5.7	6.2	
						2.y6.1	Difference (%)	Concentration	9.4	8.4	10.9	12.1	19.4	6.7	1.2 ± 1.4
								Slope	2.4	2.9	0.7	0.7	5.9	5.1	
						2.y5.1	Difference (%)	Concentration	9.8	9.1	8.1	8.1	8.6	9.8	0.8 ± 0.6
								Slope	1.3	0.6	0.4	0.1	4.4	3.3	
						2.b2.1	Difference (%)	Concentration	7.4	9.9	6.4	6.0	5.5	9.2	0.9 ± 0.7
								Slope	1.4	2.7	0.3	0.3	4.2	6.1	
						2.b3.1	Difference (%)	Concentration	9.2	10.5	12.9	1.5	4.8	6.5	1.0 ± 0.7
								Slope	2.1	1.8	1.0	1.1	2.7	4.5	
11	C5	CO5	P01031	Complement C5	LQGTLPVEAR	2.y8.1	Difference (%)	Concentration	10.5	8.4	9.9	1.5	10.8	8.4	3.1 ± 2.4
								Slope	4.3	0.2	2.9	3.7	3.2	2.1	
						2.y6.1	Difference (%)	Concentration	8.9	1.9	7.8	11.7	6.5	15.8	5.7 ± 3.9
								Slope	1.9	0.4	1.2	1.5	3.0	5.1	
						2.y5.1	Difference (%)	Concentration	5.6	0.7	15.1	16.7	1.7	4.5	3.9 ± 3.5
								Slope	4.7	2.7	0.8	2.1	1.7	2.8	
						2.b2.1	Difference (%)	Concentration	15.2	4.3	2.3	8.5	12.5	2.6	3.9 ± 4.0
								Slope	12.2	6.1	2.1	3.5	10.6	2.1	
12	C6	CO6	P13671	Complement component C6	ALQEYAAK	2.y6.1	Difference (%)	Concentration	8.3	0.7	7.2	6.2	1.1	9.7	5.6 ± 7.2
								Slope	6.9	0.9	1.0	1.4	2.7	2.8	
						2.b2.1	Difference (%)	Concentration	9.8	12.1	18.0	5.3	4.4	0.1	3.0 ± 3.1
								Slope	2.7	2.7	2.7	1.5	2.4	1.8	
						2.b3.2	Difference (%)	Concentration	9.5	3.5	2.5	13.0	5.0	6.7	1.6 ± 1.5
								Slope	5.1	0.3	3.5	0.2	4.8	3.8	
13	C8A	CO8A	P07357	Complement component C8 alpha chain	LYYGDDEK	2.y6.1	Difference (%)	Concentration	4.2	13.1	1.2	2.7	12.6	14.2	3.0 ± 2.2
								Slope	5.0	0.9	1.7	1.7	1.9	1.2	
						2.b2.1	Difference (%)	Concentration	3.5	4.7	0.6	0.4	7.8	17.5	3.0 ± 2.5
								Slope	5.0	1.9	1.0	3.0	4.8	5.7	
						2.b4.2	Difference (%)	Concentration	5.9	5.6	4.3	4.9	7.5	9.4	1.6 ± 1.2
								Slope	0.3	6.5	1.6	1.7	2.3	0.6	
14	C8B	CO8B	P07358	Complement component C8 beta chain	YEFILK	2.y2.1	Difference (%)	Concentration	0.4	4.9	1.9	11.1	7.3	3.7	1.3 ± 0.9
								Slope	6.5	0.8	2.7	1.4	1.9	3.6	
						2.y4.2	Difference (%)	Concentration	6.9	1.6	0.9	9.9	3.3	7.4	1.6 ± 1.3
								Slope	5.9	2.4	6.0	0.3	3.3	1.0	
								Concentration	6.3	5.4	0.2	3.9	11.9	7.2	

						2.b2.1	Difference (%)	Slope	7.1	0.4	4.0	1.9	1.5	3.0	1.3 ± 1.1
								Concentration	2.2	3.2	2.7	1.3	4.7	2.3	
15	C9	CO9	P02748	Complement component C9	VVEESELAR	2.y7.1	Difference (%)	Slope	3.9	1.7	1.5	6.8	3.1	2.7	3.3 ± 2.3
								Concentration	14.1	6.5	1.5	4.8	2.3	5.9	
						2.y6.1	Difference (%)	Slope	0.2	2.3	0.3	0.5	0.8	1.5	4.2 ± 2.9
								Concentration	7.6	3.5	11.8	6.0	4.8	2.6	
						2.b2.1	Difference (%)	Slope	2.6	1.4	7.1	2.6	1.2	0.8	1.6 ± 1.0
								Concentration	4.4	2.5	7.9	3.1	4.5	8.2	
16	CFB	CFAB	P00751	Complement factor B	EELLPAQDIK	2.y6.1	Difference (%)	Slope	4.4	1.3	1.2	4.2	0.6	0.6	1.7 ± 1.3
								Concentration	0.3	5.8	4.6	5.0	0.9	2.4	
						2.b2.1	Difference (%)	Slope	3.0	2.4	2.1	1.4	0.8	2.9	2.7 ± 1.8
								Concentration	2.8	10.3	2.3	3.7	5.4	1.9	
						2.b3.1	Difference (%)	Slope	5.0	1.5	1.6	4.3	3.2	0.8	2.2 ± 1.7
								Concentration	2.0	2.4	4.4	1.1	8.5	7.9	
17	CFH	CFAH	P08603	Complement factor H	VGEVLK	2.y5.1	Difference (%)	Slope	6.3	0.9	7.1	0.7	1.8	0.6	1.6 ± 1.3
								Concentration	2.6	1.4	12.0	9.5	13.8	11.7	
						2.y4.1	Difference (%)	Slope	2.4	2.1	6.0	6.8	1.4	0.3	4.0 ± 2.8
								Concentration	2.9	8.5	15.2	0.3	3.8	2.1	
						2.b3.1	Difference (%)	Slope	1.5	1.8	1.8	0.3	7.4	2.7	3.3 ± 2.5
								Concentration	19.3	7.0	0.2	7.0	10.5	3.6	
18	CP	CERU	P00450	Ceruloplasmin	GAYPLSIEPIGVR	2.y10.1	Difference (%)	Slope	4.4	1.0	2.7	0.9	8.2	4.6	1.3 ± 1.2
								Concentration	5.7	2.4	1.5	1.6	1.7	0.1	
						2.y8.1	Difference (%)	Slope	2.8	1.9	2.6	0.2	6.1	4.3	1.9 ± 1.7
								Concentration	5.7	3.9	3.0	3.4	2.6	10.1	
						2.y6.1	Difference (%)	Slope	3.3	2.6	4.3	1.5	9.0	5.7	1.9 ± 1.7
								Concentration	2.0	3.3	0.6	0.9	2.4	5.7	
						2.y5.1	Difference (%)	Slope	3.4	0.6	2.5	2.2	8.1	5.5	1.3 ± 0.9
								Concentration	2.3	8.4	3.8	0.6	10.0	4.9	
19	CRP	CRP	P02741	C-reactive protein	ESDTSYVSLK	2.y6.1	Difference (%)	Slope	3.2	1.5	7.4	2.4	1.6	1.6	2.5 ± 1.7
								Concentration	0.5	4.1	6.6	0.7	11.4	8.3	
						2.y5.1	Difference (%)	Slope	0.5	2.1	6.7	2.2	5.2	1.9	2.4 ± 1.9
								Concentration	1.6	6.2	19.0	6.5	48.2	1.5	
						2.y3.1	Difference (%)	Slope	4.8	0.2	4.3	0.4	3.1	4.1	5.7 ± 4.0
								Concentration	0.6	8.3	11.8	13.9	2.1	1.0	
20	CST3	CYTC	P01034	Cystatin-C	ALDFAVGEYNK	2.y6.1	Difference (%)	Slope	4.6	4.9	1.2	1.8	2.4	2.2	1.5 ± 1.2
								Concentration	7.8	10.8	7.4	4.4	2.1	9.6	
						2.b2.1	Difference (%)	Slope	4.4	1.9	3.8	2.8	2.0	0.6	1.2 ± 0.9
								Concentration	6.8	7.4	17.0	19.9	0.4	1.3	
						2.b3.1	Difference (%)	Slope	1.3	1.8	2.8	1.5	1.6	3.3	1.9 ± 1.6
								Concentration	6.9	6.2	10.5	9.7	6.4	17.2	
21	F2	THRB	P00734	Prothrombin	ELLESYIDGR	2.y6.1	Difference (%)	Slope	0.2	3.7	0.2	0.2	5.8	2.3	1.2 ± 1.1
								Concentration	6.6	1.9	3.6	1.9	0.6	7.8	
						2.y5.1	Difference (%)	Slope	0.4	0.8	0.6	2.0	5.6	1.9	2.2 ± 1.6
								Concentration	4.7	2.6	4.1	11.1	10.5	0.7	
						2.b2.1	Difference (%)	Slope	0.6	2.0	0.5	2.6	5.4	0.6	1.4 ± 0.9
								Concentration	5.3	4.3	0.7	3.3	2.9	8.2	
22	F9	FA9	P00740	Coagulation factor IX	SALVLQYLR	2.y5.1	Difference (%)	Slope	4.6	0.3	0.0	2.0	1.2	4.1	1.2 ± 1.2
								Concentration	0.7	3.8	3.2	1.6	8.7	0.7	
						2.b2.1	Difference (%)	Slope	3.0	2.0	1.2	2.9	0.9	0.2	2.0 ± 1.3
								Concentration	2.3	3.0	3.7	6.6	1.5	10.1	
						2.b3.1	Difference (%)	Slope	5.4	0.8	0.7	1.3	1.1	3.7	1.0 ± 0.7
								Concentration	8.7	6.3	1.4	8.1	3.6	0.4	
23	F10	FA10	P00742	Coagulation factor X	TGIVSGFGR	2.y6.1	Difference (%)	Slope	1.8	0.1	3.2	1.0	2.4	0.0	1.1 ± 0.9

								Concentration	5.7	2.1	0.7	0.8	1.4	13.1	
						2.y5.1	Difference (%)	Slope	1.7	2.4	4.1	1.1	2.1	3.2	1.5 ± 1.1
								Concentration	2.9	2.1	1.4	7.4	1.3	7.1	
						2.y4.1	Difference (%)	Slope	2.0	0.3	3.4	0.4	3.6	3.0	2.6 ± 2.4
								Concentration	2.8	1.7	3.9	3.1	2.1	9.5	
						2.b3.1	Difference (%)	Slope	0.7	2.1	2.3	3.1	1.8	5.4	2.6 ± 2.0
								Concentration	2.3	0.6	3.7	3.6	7.7	3.9	
24	F11	FA11	P03951	Coagulation factor XI	VVSGFSLK	2.y6.1	Difference (%)	Slope	0.3	1.4	1.3	0.4	0.5	2.4	2.3 ± 1.7
								Concentration	3.0	8.2	8.1	5.6	7.3	7.0	
						2.b2.1	Difference (%)	Slope	0.5	1.7	2.1	2.6	0.5	1.1	0.8 ± 0.5
								Concentration	1.4	3.7	1.0	0.5	5.9	2.3	
25	GSN	GELS	P06396	Gelsolin	AGALNSNDAFVLK	2.y9.1	Difference (%)	Slope	5.8	1.5	3.0	3.4	5.8	0.9	1.9 ± 1.6
								Concentration	0.5	1.6	0.7	2.5	6.4	10.9	
						2.y8.1	Difference (%)	Slope	3.0	2.3	2.0	4.7	3.9	0.5	2.1 ± 2.0
								Concentration	0.4	3.2	0.6	8.2	8.7	0.2	
						2.b3.1	Difference (%)	Slope	2.9	3.5	0.7	3.6	1.5	0.7	1.1 ± 1.1
								Concentration	0.1	1.7	2.3	0.0	11.2	9.5	
26	IGFBP3	IBP3	P17936	Insulin-like growth factor-binding protein 3	FLNVLSPR	2.y6.1	Difference (%)	Slope	9.6	2.4	4.9	2.1	1.2	3.8	1.8 ± 1.1
								Concentration	1.7	5.3	0.7	2.5	0.7	2.3	
						2.y4.1	Difference (%)	Slope	10.3	0.8	3.0	4.4	0.4	4.1	1.5 ± 1.1
								Concentration	1.0	0.6	2.1	4.0	5.8	9.1	
						2.y3.1	Difference (%)	Slope	9.1	3.0	4.2	2.0	0.3	5.5	3.0 ± 2.0
								Concentration	2.1	1.8	4.8	3.1	3.1	0.2	
27	PLG	PLMN	P00747	Plasminogen	LFLEPTR	2.y6.1	Difference (%)	Slope	0.9	0.6	1.7	0.0	2.2	2.0	2.0 ± 1.3
								Concentration	1.6	3.0	1.7	4.2	9.9	7.0	
						2.y5.1	Difference (%)	Slope	2.6	1.8	2.3	0.1	2.8	1.2	0.6 ± 0.5
								Concentration	1.0	1.0	2.3	0.8	2.4	5.3	
						2.y4.1	Difference (%)	Slope	1.6	0.9	3.3	0.0	2.0	1.2	1.2 ± 1.0
								Concentration	1.7	2.8	6.2	3.9	0.3	6.3	
						2.y3.1	Difference (%)	Slope	2.5	1.7	1.8	0.8	2.1	1.8	1.2 ± 0.9
								Concentration	1.7	6.2	2.4	4.0	7.3	3.7	
						2.b2.1	Difference (%)	Slope	1.6	0.1	1.9	0.1	0.3	0.4	0.6 ± 0.5
								Concentration	2.7	5.1	0.3	0.2	3.0	1.4	
28	PROC	PROC	P04070	Vitamin K-dependent protein C	TFVLNFIK	2.y6.1	Difference (%)	Slope	2.7	7.3	2.3	3.8	1.9	4.1	1.6 ± 1.4
								Concentration	0.7	2.5	4.6	1.7	5.6	2.1	
						2.y5.1	Difference (%)	Slope	2.5	1.7	1.8	0.8	2.1	1.8	2.3 ± 1.8
								Concentration	1.7	6.2	2.4	4.0	7.3	3.7	
						2.b2.1	Difference (%)	Slope	1.6	0.1	1.9	0.1	0.3	0.4	0.8 ± 0.7
								Concentration	2.7	5.1	0.3	0.2	3.0	1.4	
29	PROS1	PROS	P07225	Vitamin K-dependent protein S	YLVCLR	2.y5.1	Difference (%)	Slope	6.0	2.2	3.3	2.2	1.9	4.6	2.3 ± 1.7
								Concentration	3.2	3.7	8.8	1.2	10.3	18.0	
						2.y4.1	Difference (%)	Slope	8.1	0.7	3.9	0.6	1.3	5.6	1.3 ± 1.0
								Concentration	2.7	2.8	1.1	2.0	2.5	4.5	
						2.y3.1	Difference (%)	Slope	8.5	2.1	2.3	3.0	1.8	2.9	1.9 ± 1.7
								Concentration	2.2	0.6	2.1	14.3	1.6	5.9	
						2.b2.1	Difference (%)	Slope	4.3	1.8	3.3	0.1	0.9	2.0	1.8 ± 1.1
								Concentration	1.6	1.6	9.3	1.6	3.6	7.4	
30	SERPINC1	ANT3	P01008	Antithrombin-III	FDTISEK	2.y6.1	Difference (%)	Slope	3.0	3.5	0.8	6.5	3.5	2.8	2.4 ± 2.1
								Concentration	1.0	1.8	1.8	9.0	0.2	7.2	
						2.y5.1	Difference (%)	Slope	1.4	0.9	1.8	2.7	0.1	3.1	2.1 ± 1.6
								Concentration	2.1	7.5	3.5	6.2	9.7	5.1	
						2.b2.1	Difference (%)	Slope	2.8	0.9	0.1	6.5	2.0	2.7	1.3 ± 1.2
								Concentration	1.0	3.3	1.9	10.5	11.3	4.9	

31	SERPINF2	A2AP	P08697	Alpha-2-antiplasmin	LGNQEPGGQTALK	2.y8.1	Difference (%)	Slope	3.1	0.9	2.3	0.8	0.5	0.2	1.8 ± 1.3
								Concentration	4.9	14.8	4.9	0.2	3.3	10.3	
						2.y7.1	Difference (%)	Slope	7.6	0.2	8.0	5.3	0.6	6.1	7.2 ± 5.1
								Concentration	7.5	3.0	11.2	11.5	23.9	9.8	
						2.b4.1	Difference (%)	Slope	1.6	2.6	3.3	2.7	7.4	0.4	5.8 ± 3.8
								Concentration	12.1	2.4	9.0	8.3	0.8	0.2	
32	SERPING1	IC1	P05155	Plasma protease C1 inhibitor	LLDSLPSDTR	2.y7.1	Difference (%)	Slope	4.7	5.8	3.4	1.7	4.5	1.0	4.0 ± 3.4
								Concentration	10.1	2.6	3.6	8.7	5.5	6.5	
						2.y5.1	Difference (%)	Slope	5.9	1.3	4.1	1.9	1.0	4.0	1.9 ± 1.4
								Concentration	0.7	3.3	1.0	2.1	2.6	0.9	
						2.b2.1	Difference (%)	Slope	2.4	2.9	0.2	0.3	1.4	1.0	4.0 ± 2.9
								Concentration	1.7	5.1	1.3	1.4	3.0	4.4	
33	SHBG	SHBG	P04278	Sex hormone-binding globulin	IALGGLLFPASNLR	2.y8.1	Difference (%)	Slope	3.2	0.3	5.7	1.7	5.4	9.9	0.9 ± 0.6
								Concentration	9.9	11.3	10.2	3.9	11.3	2.1	
						2.y7.1	Difference (%)	Slope	4.1	0.5	5.8	2.2	6.0	9.4	0.7 ± 0.3
								Concentration	13.5	13.7	14.2	14.6	11.4	12.7	
						2.b5.1	Difference (%)	Slope	4.7	0.5	6.7	1.2	6.4	9.2	0.8 ± 0.5
								Concentration	5.5	10.9	3.2	11.3	7.3	4.2	
34	TTR	TTHY	P02766	Transthyretin	VLDAVR	2.y5.1	Difference (%)	Slope	0.5	0.1	5.4	1.9	1.3	4.3	2.8 ± 2.0
								Concentration	4.8	9.2	15.1	23.6	11.2	9.7	
						2.y4.1	Difference (%)	Slope	0.9	2.0	6.9	4.3	0.6	2.1	0.7 ± 0.4
								Concentration	3.0	14.0	5.2	2.1	2.8	5.7	
						2.y3.1	Difference (%)	Slope	6.2	6.6	6.5	3.1	4.3	5.0	8.0 ± 5.4
								Concentration	7.9	1.0	6.0	3.2	1.0	9.5	

ITR, Ion transition ratio; %mean df, percentage of mean difference.

[a] The column title indicates the information of quantification ion, followed by the precursor ion charge, production ion type, and product ion charge.

[b] Mean ± standard deviation.

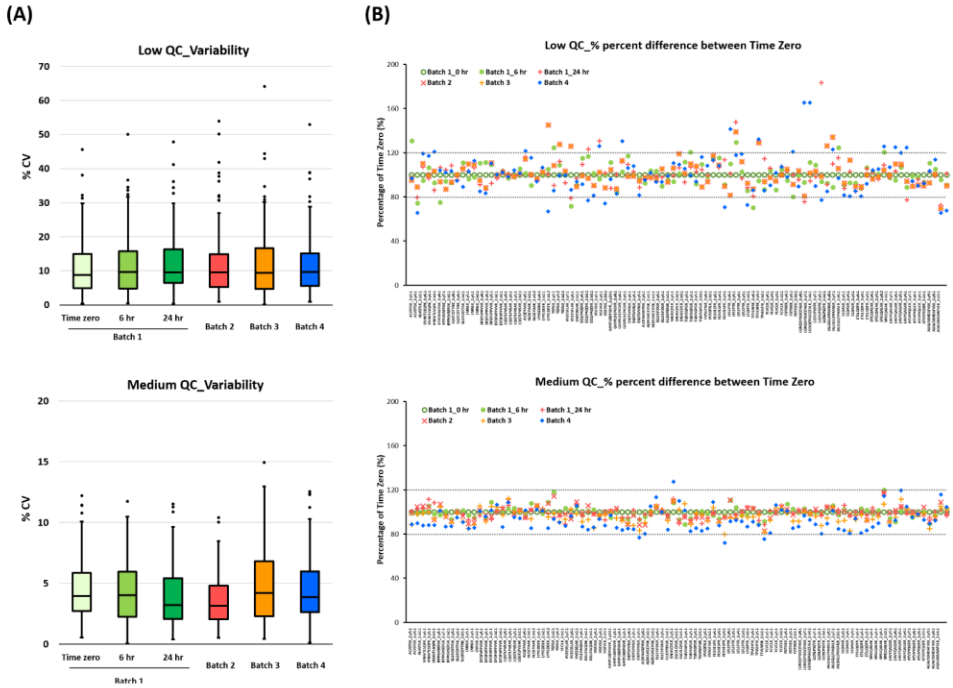
Underline indicates that the value did not meet the criteria.

1.3.5 Stability of target peptides under conditions for storage or freeze-thawing

Pooled normal serum was used as a matrix to prepare 12 aliquots of low- and medium-QC samples. Three of the 12 aliquots were analyzed immediately (first batch, 0 h) in the autosampler (always at 4°C), after 6 h (first batch, 6 h), or after 24 h (first batch, 24 h). The remaining 9 aliquots were frozen in a deep-freezer at -80°C. Six of the 9 aliquots were thawed slowly on ice, 3 of which were promptly analyzed (second batch, 1 freeze-thaw cycle). The remaining 3 thawed aliquots were refrozen and stored in a deep-freezer prior to rethawing and analysis (third batch, 2 freeze-thaw cycles). The last 3 aliquots were stored frozen for 4 weeks and then slowly thawed on ice and analyzed (fourth batch). All analyses were repeated in duplicate.

The variability in measurements of low- and medium-QC was similar under the 6 conditions (Figure 1-3A). The median CVs at 0 h, 6 h, and 24 h at 4°C, 1 and 2 freeze-thaw cycles, and 4 weeks at -70°C were 8.8%, 9.7%, 9.6%, 9.5%, 9.4%, and 9.6% in low-QC and 4.0%, 4.0%, 3.2%, 3.2%, 4.2%, and 3.9% in medium-QC samples. When the CV values of the 6 conditions were compared with the total CV from the Repeatability evaluations, 68 transitions (60.7%) in low-QC and 102 transitions (91.1%) in medium-QC had CV values that were lower (Table 1-8). Then, the peak area ratios of time zero (first batch, 0 h) were compared with those of the other 5 conditions. The 78 transitions (69.6%) in low-QC and 108 transitions (96.4%) in medium-QC were within 80% to 120% of the percentage of the time zero sample. In total, 59 of 112 transitions (52.7%), representing 27 of 34 proteins (79.4%), were considered to be stable targets (Figure 1-3B).

Figure 1-3. Variability of analytes in six different conditions related to stability.



Batch 1 was left on the autosampler for 0 h, 6 h, and 24 h (at 4°C) before analysis. Each batch was prepared under various conditions (eg, adjusting freeze-thaw cycles and storage duration) following guidelines. To validate whether target peptides are stable, (A) the CV values of targets at 2 concentrations should be lower than 20%, and the variability of every condition should not exceed that of 0 h. (B) Also, the peak area ratios should be constant across batches. Percentages of time 0 between each condition for all transitions are presented, and the range from 80% to 120% is marked by dashed lines. The outliers were determined as in Experiment 2.

Table 1-8. Stability of the targets under different conditions of storage.

Information of target protein and peptide						Low QC												Medium QC											
						Comparing with the variability of repeatability (%CV)						Comparing with Time Zero (%)						Comparing with the variability of repeatability (%CV)						Comparing with Time Zero (%)					
Gene symbol	Uniprot ID	Uniprot Accession	Protein Name	Peptide Sequence	Quantification ion	Batch 1			Batch 2	Batch 3	Batch 4	CV of repeatability	Batch 1		Batch 2	Batch 3	Batch 4	Batch 1			Batch 2	Batch 3	Batch 4	CV of repeatability	Batch 1		Batch 2	Batch 3	Batch 4
						0 h	6 h	24 h					6 h	24 h				0 h	6 h	24 h					6 h	24 h			
AMBP	AMBP	P02760	Protein AMBP	ETLLQDFR	2.y6.1	28.3	28.4	8.4	15.9	19.0	27.6	27.1	90.9	98.0	100.9	90.1	67.7	9.1	6.3	1.1	1.0	5.7	3.7	20.0	101.2	96.5	99.2	103.5	104.6
					2.y4.1	14.0	10.4	10.3	10.9	6.7	5.6	18.1	96.6	118.9	105.9	113.3	104.8	2.7	2.7	4.0	1.5	1.9	4.0	18.6	104.6	101.6	103.0	98.3	97.3
					2.y3.1	9.6	17.1	34.5	9.6	3.5	26.7	35.2	83.1	110.9	110.2	112.9	97.2	3.4	6.0	3.1	2.6	4.2	1.8	16.4	101.0	101.5	99.2	98.8	92.8
APOA2	APOA2	P02652	Apolipoprotein A2	SPELQAEAK	2.y6.1	7.6	0.8	16.9	6.3	4.6	16.1	19.6	97.5	93.9	112.3	84.4	89.3	2.0	0.2	5.4	6.4	4.2	4.4	3.1	99.2	100.4	105.4	111.4	117.7
					2.y4.1	3.1	5.4	9.5	18.9	0.7	10.0	19.1	104.3	101.4	94.5	106.2	91.5	3.1	2.8	8.9	4.4	5.9	8.1	5.8	95.2	101.6	100.4	108.8	113.3
					2.y8.2	4.8	3.2	3.1	6.9	6.4	9.8	15.7	102.9	101.3	100.4	105.6	103.5	2.7	0.2	6.5	5.2	1.8	2.9	4.1	95.4	97.4	102.3	111.5	112.8
					2.b2.1	8.9	8.9	3.5	17.1	13.8	12.9	18.1	99.9	97.1	99.5	101.4	98.6	2.6	5.4	1.5	3.5	6.9	4.6	2.9	96.8	98.5	98.0	112.4	105.3
APOB	APOB	P04114	Apolipoprotein B-100	ALVDTLK	2.y5.1	10.5	14.5	8.6	4.1	10.3	13.0	17.6	130.6	120.9	97.5	95.2	96.7	3.6	2.8	4.1	2.4	2.3	5.9	3.7	98.9	100.6	99.2	88.5	88.7
					2.y4.1	16.8	13.1	9.6	30.7	12.8	18.0	22.0	74.4	66.9	79.5	89.0	65.6	5.1	2.1	4.5	3.2	3.2	7.2	10.3	98.6	102.8	104.6	98.1	89.8
					2.b2.1	1.8	8.5	1.0	5.2	7.4	6.1	14.1	94.7	100.4	107.1	110.1	119.2	1.4	5.1	2.3	2.5	6.4	0.9	8.1	101.2	103.2	105.3	98.4	87.9
APOE	APOE	P02649	Apolipoprotein E	LAVYQAGAR	2.y7.1	17.6	2.7	22.3	30.7	16.7	6.3	19.9	118.6	115.7	91.5	103.1	95.8	9.3	8.2	3.8	3.5	7.0	12.6	7.3	93.8	94.1	96.7	99.6	119.6
					2.y6.1	18.2	4.3	15.9	1.1	22.8	20.5	23.2	103.5	101.5	108.3	99.9	99.5	5.0	5.3	5.5	1.5	4.4	5.7	5.3	95.4	98.3	96.1	107.1	118.3
					2.y5.1	29.8	32.5	27.0	22.6	23.2	10.3	19.1	119.6	106.6	82.1	121.4	112.0	6.3	2.3	2.0	8.2	3.4	3.6	7.7	92.2	96.7	94.0	100.3	99.7
					2.y4.1	27.5	14.6	36.2	23.6	8.2	4.8	26.3	83.7	92.1	70.5	69.2	72.8	3.4	4.6	7.9	5.1	13.0	3.7	7.2	96.1	102.0	98.5	109.3	119.2
APOH	APOH	P02749	Apolipoprotein H	ATVVYQGER	2.y7.1	32.2	14.6	16.5	23.0	3.5	8.6	22.8	114.6	95.9	136.2	107.6	118.1	2.2	4.8	2.1	2.8	3.9	12.4	7.2	97.0	96.8	100.3	93.0	99.9
					2.y6.1	4.9	11.0	6.5	12.5	9.2	12.8	18.2	98.1	100.8	106.3	98.6	99.5	4.2	1.6	3.2	2.3	1.7	4.1	4.4	97.1	98.0	106.0	99.9	104.0
					2.y5.1	4.9	7.7	11.1	10.5	1.6	1.1	13.0	106.9	99.5	96.1	97.2	86.9	4.1	0.7	5.9	1.9	5.1	3.2	3.4	103.5	103.1	99.8	100.9	97.8
					2.b3.1	12.9	3.0	4.6	32.1	30.0	8.6	19.7	100.1	92.5	81.2	82.7	100.2	3.8	5.5	8.0	6.0	3.7	2.5	5.9	97.0	97.3	98.4	101.0	104.3
B2M	B2MG	P61769	Beta-2-microglobulin	VNHVTLSQPK	3.y6.1	3.8	6.1	22.9	36.4	16.6	16.9	22.7	106.6	109.3	96.7	99.5	117.2	11.4	4.4	10.9	13.6	16.5	6.3	19.1	105.5	111.4	104.7	100.0	88.2
					3.y5.1	25.4	7.7	11.3	10.1	7.8	6.3	22.6	92.8	74.1	86.3	103.2	120.7	3.1	8.1	1.6	5.5	4.3	15.8	13.2	98.1	108.3	93.0	93.5	88.1
					3.b3.1	12.2	19.6	18.2	11.1	9.4	8.0	19.9	74.9	77.3	106.7	93.6	104.2	7.6	2.2	1.4	3.0	7.7	3.3	12.0	97.4	101.6	107.0	104.2	101.0
BCHE	CHLE	P06276	Cholinesterase	IFFPGVSEFGK	2.y8.1	6.7	11.0	5.4	12.0	12.3	11.5	10.8	94.2	93.3	104.4	86.8	101.9	3.3	3.1	0.8	1.7	4.5	0.1	19.5	100.7	96.8	95.6	96.5	86.9
					2.y7.1	4.6	15.6	4.4	12.3	18.8	12.0	11.4	92.9	100.7	108.4	93.3	103.4	9.4	3.2	9.0	8.2	1.2	4.0	23.6	93.4	90.3	91.3	90.3	86.9
					2.b2.1	16.2	9.5	7.2	3.6	8.8	8.0	11.2	100.5	98.7	95.5	100.0	94.6	2.1	5.7	3.0	2.8	3.1	3.3	20.2	101.2	99.4	100.0	98.4	94.8
C1QA	C1QA	P02745	Complement C1q subcomponent subunit A	SLGFCDTTNK	2.y8.1	15.0	9.6	14.4	16.9	16.7	15.8	19.9	111.1	107.1	106.5	105.5	89.0	1.6	8.1	3.4	3.6	5.7	2.4	8.9	101.4	97.8	96.0	91.4	90.8
					2.y6.1	13.0	4.5	8.5	9.4	13.1	5.2	16.8	96.3	109.6	103.3	109.7	103.1	7.4	3.0	7.2	6.8	6.1	3.0	10.4	97.0	92.7	89.3	85.1	85.2
					2.b2.1	5.2	3.2	3.4	6.3	4.9	9.7	11.8	98.1	102.9	106.2	109.1	112.6	7.4	2.3	2.1	4.8	1.2	5.7	7.8	94.9	94.1	90.4	88.3	85.7
C2	CO2	P06681	Complement C2	LNINLK	2.y5.1	17.8	4.5	14.0	12.8	4.6	13.3	22.1	110.4	114.6	90.9	94.6	85.1	6.8	4.3	6.9	7.8	1.1	15.0	18.9	99.6	96.4	101.6	105.4	100.8
					2.y4.1	18.5	23.6	13.1	9.5	9.7	37.0	21.9	111.3	111.5	86.7	88.4	83.4	4.3	5.6	5.8	4.4	7.1	3.9	18.9	95.0	92.6	97.0	91.2	96.0
					2.b2.1	6.6	15.7	11.1	13.3	17.4	8.8	18.8	109.1	87.2	92.3	110.9	110.1	5.0	9.6	5.0	5.2	7.0	3.6	16.4	109.0	93.5	95.7	105.0	101.4
C3	CO3	P01024	Complement C3	DFDFVPPVVR	2.y8.1	2.1	4.5	5.3	4.9	3.3	3.8	11.6	100.2	101.5	96.9	99.6	99.9	2.9	3.3	0.4	2.4	0.7	1.6	12.0	101.1	100.9	100.4	104.4	106.6
					2.y6.1	2.8	2.0	2.3	2.5	3.0	1.6	16.5	103.4	102.9	103.7	102.0	102.0	2.1	2.3	1.6	1.8	1.5	2.0	10.6	101.4	103.9	103.0	97.2	86.2
					2.y5.1	1.9	2.2	0.7	2.4	0.8	2.5	15.6	97.0	102.2	100.7	99.1	98.4	2.1	1.4	1.3	2.0	0.4	2.6	14.0	103.1	106.1	110.0	112.1	108.6
					2.b2.1	0.7	0.8	0.9	0.9	3.3	2.9	12.6	97.8	104.3	99.1	98.6	104.1	2.2	3.0	3.2	1.7	3.8	3.1	14.9	103.3	100.3	98.6	100.1	95.3
					2.b3.1	2.0	5.8	2.6	1.1	3.9	5.0	11.1	105.4	101.9	101.2	101.0	101.4	5.5	4.1	0.9	1.4	1.4	1.8	16.1	101.9	101.2	101.5	100.1	94.3
C5	CO5	P01031	Complement C5	LQGTLPVEAR	2.y8.1	2.1	6.1	5.4	2.7	6.3	2.4	16.2	101.3	104.2	116.2	114.1	121.4	2.8	5.1	2.1	4.2	2.2	1.3	8.6	96.8	94.8	95.2	99.9	101.4
					2.y6.1	5.7	3.6	7.3	2.2	7.4	6.1	11.9	90.3	101.0	99.4	100.3	115.5	5.9	4.1	4.3	2.5	6.0	2.6	9.0	108.0	101.8	101.6	93.6	85.4
					2.y5.1	9.5	3.9	8.7	5.4	7.9	2.4	12.5	95.6	94.0	95.5	96.3	93.8	4.3	2.6	5.2	5.2	5.3	2.5	5.8	104.7	105.1	105.6	105.6	102.2
					2.b2.1	1.1	2.4	5.4	4.7	5.9	3.0	12.2	100.5	105.8	102.2	102.5	106.5	0.5	2.9	1.9	2.0	2.6	9.9	10.3	98.8	103.1	101.1	102.0	101.0
C6	CO6	P13671	Complement component C6	ALQEYAAK	2.y6.1	10.2	36.6	10.2	16.7	27.0	18.1	30.6	105.0	118.3	100.4	145.0	66.9	5.3	10.2	11.3	1.2	10.6	10.3	12.3	109.0	98.5	107.8	95.3	85.2
					2.b2.1	10.8	33.7	21.6	20.8	24.3	27.1	15.9	124.6	116.0	90.4	108.2	85.5	5.9	16.7	3.5	10.0	8.8	7.0	12.2	118.0	95.2	114.3	99.0	93.5
					2.b3.2	6.1	23.1	3.9	10.8	13.0	21.6	17.8	108.7	126.0	112.2	127.6	99.3	13.4	8.3	5.6	3.4	3.4	9.3	9.2	99.5	96.7	97.1	91.1	90.4
C8A	CO8A	P07357	Complement component C8 alpha chain	LYYGDDEK	2.y6.1	38.1	16.3	5.0	11.7	16.0	17.9	22.2	100.6	93.8	92.8	103.2	99.7	7.8	9.1	2.1	3.9	8.4	7.2	10.2	103.5	89.0	98.1	93.5	87.3
					2.b2.1	9.5	50.0	41.2	23.0	0.9	28.8	18.3	71.5	73.7	79.0	125.9	86.5	1.0	3.8	5.9	11.0	8.7	3.4	11.5	95.7	98.3	93.7	102.8	96.3

					2.b4.2	4.6	12.1	4.9	3.9	7.4	2.2	14.3	90.8	95.4	96.6	89.1	93.9	4.9	1.1	5.5	1.7	4.2	5.6	7.5	104.7	106.6	109.1	98.2	105.4
C8B	CO8B	P07358	Complement component C8 beta chain	YEFILK	2.y2.1	14.7	6.9	9.3	15.3	34.8	38.7	18.5	115.1	110.8	109.3	99.1	91.5	2.7	5.7	2.4	3.1	2.4	6.6	15.2	97.1	96.3	99.0	91.3	86.6
					2.y4.2	16.3	19.6	18.5	53.9	2.1	23.7	17.7	116.8	112.0	123.0	96.6	76.7	5.8	0.9	3.0	2.6	2.2	1.4	15.4	101.0	100.4	105.5	93.1	84.0
					2.b2.1	13.6	8.4	23.7	27.0	26.8	52.9	17.9	101.3	93.3	89.8	90.2	81.3	3.8	6.4	4.5	3.3	0.6	12.3	18.4	102.1	98.3	98.2	85.6	85.9
C9	CO9	P02748	Complement component C9	VVEESELAR	2.y7.1	31.3	23.0	27.6	13.6	29.5	21.4	16.3	96.3	112.9	130.7	102.3	126.2	6.5	10.5	2.7	2.2	6.8	4.1	7.5	96.5	97.9	95.2	97.3	93.7
					2.y6.1	14.5	31.7	24.8	11.6	44.4	20.8	25.0	99.9	94.1	101.4	88.1	74.2	2.7	0.1	4.7	3.0	6.9	7.9	8.9	99.7	92.6	100.0	97.0	87.7
					2.b2.1	6.7	5.3	17.0	3.4	10.7	12.6	18.3	111.2	90.6	87.8	104.5	95.8	4.0	1.3	2.8	2.9	4.3	3.2	6.5	94.8	101.3	100.4	99.4	97.0
CFB	CFAB	P00751	Complement factor B	EELLPAQDIK	2.y6.1	10.5	0.9	6.8	5.3	15.5	5.6	22.9	83.7	86.4	100.2	87.2	82.9	4.0	3.7	1.5	3.2	1.9	4.5	11.6	101.9	102.3	103.2	94.4	85.5
					2.b2.1	15.7	11.6	8.3	12.4	22.5	9.9	21.4	102.8	115.2	111.5	112.9	130.5	3.8	8.7	2.4	1.1	3.7	4.8	20.7	94.2	94.8	93.3	92.1	83.7
					2.b3.1	6.9	8.5	7.3	4.9	11.9	13.6	13.9	117.2	98.0	109.5	98.4	106.1	2.9	4.5	2.9	2.7	5.2	4.7	18.5	93.4	95.1	88.9	89.3	85.0
CFH	CFAH	P08603	Complement factor H	VGEVLK	2.y5.1	3.4	4.6	6.6	3.4	7.6	3.9	15.3	100.4	92.1	102.5	98.9	108.0	3.9	4.6	7.9	8.4	10.0	2.6	15.0	98.0	101.6	96.9	87.6	84.2
					2.y4.1	4.1	10.9	9.6	9.1	19.3	25.4	21.6	96.2	95.6	88.6	94.3	81.5	4.1	5.2	8.8	3.6	4.0	7.3	9.5	93.3	93.4	88.2	83.2	76.9
					2.b3.1	10.3	9.7	6.4	1.0	3.0	3.9	17.2	92.4	95.6	96.6	97.4	95.2	3.0	4.0	2.3	2.7	8.8	3.9	9.3	95.6	97.6	88.2	90.2	80.3
CP	CERU	P00450	Ceruloplasmin	GAYPLSIEPIGVR	2.y10.1	5.1	6.6	0.4	10.6	1.6	6.7	15.3	103.7	109.1	96.6	97.8	101.8	2.0	3.9	2.2	3.9	4.4	1.0	9.7	97.6	97.9	98.5	100.5	105.1
					2.y8.1	6.8	16.4	12.0	14.8	1.0	9.2	23.9	103.3	94.5	98.6	95.7	93.5	4.3	1.4	2.2	1.6	4.8	1.7	14.5	104.9	103.6	105.0	105.5	113.2
					2.y6.1	4.7	15.0	13.6	5.5	1.8	5.7	19.3	89.7	96.3	97.6	105.2	93.5	4.5	0.6	1.3	0.8	1.9	7.0	15.8	101.5	102.3	99.9	100.5	99.9
					2.y5.1	3.1	11.2	15.1	8.6	9.4	1.4	17.8	95.5	91.4	98.9	93.2	98.8	5.6	0.9	3.2	0.8	4.1	2.4	19.1	95.9	97.6	95.3	92.2	83.9
CRP	CRP	P02741	C-reactive protein	ESDTSYVSLK	2.y6.1	5.7	6.0	4.6	8.7	3.8	9.5	18.6	93.7	107.1	99.1	106.1	110.6	7.5	6.4	0.5	6.7	9.4	6.7	14.0	108.2	111.7	109.8	109.9	127.3
					2.y5.1	8.1	12.7	9.5	10.3	21.9	7.6	17.7	100.9	103.0	106.2	118.9	109.2	6.2	10.2	6.4	3.4	6.8	8.0	14.8	93.3	89.3	91.9	102.3	110.0
					2.y3.1	4.6	11.5	11.6	11.2	11.9	9.9	19.1	111.7	108.5	93.3	101.3	105.4	0.7	3.9	12.1	10.4	10.3	5.7	16.4	88.8	95.7	95.4	107.5	101.4
CST3	CYTC	P01034	Cystatin-C	ALDFAVGEYNK	2.y6.1	11.1	17.2	17.9	31.8	10.8	30.3	15.0	120.6	114.1	107.2	109.7	99.5	10.1	1.1	5.1	3.5	3.7	6.2	18.7	94.2	92.3	91.6	89.0	82.7
					2.b2.1	22.9	19.3	7.6	9.3	16.0	19.7	21.9	91.7	101.0	103.0	107.6	93.9	1.3	13.4	8.6	3.4	12.9	5.5	20.3	98.4	93.9	96.7	99.0	85.4
					2.b3.1	13.9	8.6	11.1	1.3	7.5	12.6	23.1	109.5	92.4	104.5	88.9	116.0	12.2	9.3	3.7	1.3	8.6	5.9	22.9	100.7	89.6	95.3	97.5	82.9
F2	THRB	P00734	Prothrombin	ELLESYIDGR	2.y6.1	7.5	1.6	7.9	6.0	5.6	4.4	16.0	98.4	104.2	99.3	93.7	108.4	5.2	2.5	1.4	4.0	0.7	3.6	19.7	98.5	94.4	93.2	92.0	84.8
					2.y5.1	24.2	9.5	20.6	6.4	19.4	3.2	18.6	114.1	110.2	112.9	117.1	113.5	5.6	5.5	1.0	3.4	3.7	2.3	17.0	96.9	98.8	98.8	103.9	109.1
					2.b2.1	5.1	6.3	14.4	10.3	15.9	11.2	18.8	115.5	109.6	106.5	108.8	108.2	7.6	4.0	4.5	1.5	3.4	5.0	15.7	94.0	90.6	91.6	90.3	87.8
F9	FA9	P00740	Coagulation factor IX	SALVLQYLR	2.y5.1	20.4	9.9	25.8	4.7	12.0	11.9	23.5	91.2	87.3	113.7	89.5	70.4	3.6	3.8	5.7	3.7	1.5	11.2	17.8	96.6	94.6	95.4	79.8	71.9
					2.b2.1	9.2	25.7	11.4	18.7	30.8	11.2	35.7	110.0	66.4	100.9	81.2	141.4	17.6	1.7	16.4	1.2	6.7	3.1	20.8	110.8	95.7	110.7	94.5	91.3
					2.b3.1	7.7	21.6	23.9	6.1	7.0	17.9	19.5	129.5	102.0	147.9	138.7	117.9	2.3	11.7	3.0	3.1	3.5	6.4	15.9	104.2	97.7	95.4	94.3	92.7
F10	FA10	P00742	Coagulation factor X	TGIVSGFGR	2.y6.1	6.7	17.0	12.4	15.9	18.2	13.0	15.4	102.8	113.2	96.7	111.9	118.9	3.1	4.7	3.3	4.5	9.0	6.8	9.4	96.9	99.1	100.6	96.5	91.6
					2.y5.1	9.4	10.1	10.3	50.2	13.8	31.8	20.9	85.7	85.9	88.3	92.5	72.8	3.6	2.4	3.9	3.2	2.4	5.5	7.8	97.7	104.3	103.8	95.2	86.8
					2.y4.1	27.5	19.7	17.5	37.7	64.1	13.4	15.9	70.4	86.4	80.6	87.3	86.1	3.6	8.6	2.7	2.5	4.1	7.6	16.8	103.2	103.7	103.1	95.5	91.3
					2.b3.1	15.0	19.5	8.0	38.8	8.1	3.4	16.7	94.5	131.7	129.4	129.0	132.1	3.8	7.2	3.4	5.2	4.5	9.9	9.3	89.2	92.2	99.5	97.3	87.9
F11	FA11	P03951	Coagulation factor XI	VVSGFSLK	2.y6.1	4.8	15.9	15.8	9.4	10.4	15.2	17.4	86.6	87.5	114.6	93.8	85.8	8.3	7.2	2.1	2.9	4.3	3.0	14.1	91.5	88.6	82.8	81.6	75.3
					2.b2.1	17.0	7.1	9.5	9.6	6.5	7.4	21.4	104.1	88.9	106.0	97.3	90.6	5.3	9.0	1.0	0.7	8.9	6.1	18.1	99.3	100.8	94.3	92.2	80.9
GSN	GELS	P06396	Gelsolin	AGALNSNDAFVLK	2.y9.1	7.6	5.9	11.0	2.7	1.7	7.5	15.7	96.0	106.8	111.1	109.9	92.3	2.5	2.2	0.9	4.2	1.8	6.2	19.5	100.0	101.9	105.0	90.7	87.0
					2.y8.1	19.0	0.5	2.9	6.4	3.0	4.1	19.3	95.9	92.0	104.2	110.7	96.6	2.0	6.2	5.8	0.9	2.0	3.8	14.4	104.4	104.9	105.2	101.6	96.0
					2.b3.1	9.2	8.0	7.2	2.8	2.3	8.3	14.0	95.7	99.3	102.4	109.8	98.8	2.4	0.5	2.4	1.5	2.7	4.9	16.7	94.7	98.2	97.1	97.6	94.1
IGFBP3	IBP3	P17936	IGF binding protein-3	FLNVLSPR	2.y6.1	4.0	0.7	6.8	8.4	10.1	8.5	26.4	86.5	98.7	93.6	94.3	101.6	3.5	1.6	3.3	2.8	7.4	1.0	15.1	100.8	101.9	99.0	104.0	106.3
					2.y4.1	7.6	6.2	6.8	8.2	11.4	9.0	20.3	104.7	107.5	107.3	99.7	107.4	2.1	5.4	2.7	4.3	3.4	3.8	20.0	102.5	105.6	104.5	103.3	101.7
					2.y3.1	3.4	13.5	7.3	12.8	6.9	12.3	16.0	94.9	110.2	103.3	104.9	97.9	1.2	2.0	3.9	2.6	4.9	2.5	18.3	107.1	101.5	102.3	99.2	100.6
PLG	PLMN	P00747	Plasminogen	LFLEPTR	2.y6.1	3.8	3.3	20.3	11.0	22.7	6.8	22.5	80.1	91.7	99.7	91.4	120.7	4.2	1.1	7.0	3.0	2.2	3.9	10.3	102.9	98.8	97.4	91.6	85.4
					2.y5.1	8.8	3.7	12.0	5.1	2.9	10.6	17.3	98.9	101.5	95.3	103.5	89.5	2.2	1.2	2.2	0.8	3.3	1.5	5.4	99.8	102.2	100.6	91.8	86.9
					2.y4.1	7.4	9.5	15.2	5.2	9.4	8.1	18.4	93.1	100.1	75.6	80.7	165.4	3.3	4.8	1.0	1.3	0.9	1.8	8.9	103.0	101.0	101.4	99.8	96.8
					2.y3.1	19.3	21.0	18.1	7.5	9.5	17.5	19.5	111.4	103.5	94.3	101.5	165.3	2.7	2.4	1.4	1.0	2.0	3.6	8.9	98.1	98.8	98.4	98.9	92.9
					2.b2.1	11.3	13.6	4.6	6.1	10.5	6.4	19.4	108.8	112.4	98.4	103.4	89.6	2.9	2.2	1.9	1.2	2.0	2.6	9.1	99.4	100.5	102.4	96.3	88.9
PROC	PROC	P04070	Vitamin K-dependent protein C	TFVLNFIK	2.y6.1	27.4	15.0	32.7	20.7	0.1	38.9	23.1	93.9	96.3	183.3	98.3	77.0	5.6	3.7	2.4	2.9	0.5	3.9	9.9	98.9	96.6	98.7	87.7	80.3
					2.y5.1	45.6	34.5	27.5	5.9	30.1	16.4	20.0	92.3	92.6	91.3	126.2	84.9	5.7	7.8	2.4	6.4	4.9	3.6	15.3	104.3	104.9	104.3	94.4	89.3
					2.b2.1	11.5	10.8	16.3	19.3	25.1	12.7	18.2	88.8	112.2	109.8	134.1	123.0	0.9	3.1	3.0	4.8	3.5	4.1	11.0	105.7	103.8	103.5	103.5	101.1
PROS1	PROS	P07225	Vitamin K-dependent protein S	YLVCLR	2.y5.1	12.9	26.5	20.7	10.9	13.8	7.6	24.8	124.7	120.1	115.4	105.7	96.9	9.9	4.4	2.4	4.9	5.3	4.8	21.0	97.4	102.0	98.8	91.3	84.7
					2.y4.1	0.5	1.2	8.3	7.8	10.0	6.3	17.2	90.9	103.1	87.7	92.4	81.7	3.8	4.2	1.8	1.2	4.0	9.6	16.5	102.5	106.1	100.7	92.4	83.3
					2.y3.1	8.8	10.7	14.1	7.6	8.7	5.2	18.9	92.8	112.5	103.1	113.1	80.6	5.3	4.1	2.3	2.9	6.1	1.2	11.1	97.2	96.0	97.9	82.9	80.5
					2.b2.1	6.7	13.9	13.7	9.8	5.4	2.9	15.0	92.5	102.2	85.0	89.4	85.2	5.2	7.5	4.7	8.5	8.9	3.3	12.4	98.3	99.8	95.5	93.2	94.0
SERPINC1	ANT3	P01008	Antithrombin-III	FDTISEK	2.y6.1	5.7	8.1	3.3	7.5	4.6	14.7	17.6	87.8	90.0	87.4	89.3	80.4	3.2	0.1	2.5	2.5	7.8	5.8	5.5	103.0	98.8	100.6	97.1	81.0
					2.y5.1	0.9	2.8	7.7	11.8	4.2	6.1	17.3	106.7	117.0	105.3	100.0	91.8	4.2	9.8	1.3	0.5	1.6	5.8	5.1	101.9	97.1	92.8	92.4	83.2
					2.b2.1	6.5	3.1	8.3	3.3	6.1	5.6	13.6	103.2	101.2	101.9	96.0	104.1	3.5	3.9	6.6	5.1	4.7	3.0	4.1	98.3	103.5	99.8	93.3	86.2
SERPINF2	A2AP	P08697	Alpha-2-antiplasmin	LGNQEPGGQTALK	2.y8.1	6.1	5.7	2.5	4.5	5.1	4.9	17.0	100.7	94.5	95.9	98.2	105.2	1.8	0.2	4.4	5.0	4.9	3.7	6.9	101.3	95.9	98.0	95.7	89.7
					2.y7.1	15.4	28.4	29.8	16.8	43.0	13.4	34.0	120.4	88.1	108.8	104.2	125.6	7.4	6.0	11.5	7.8	14.9	2.2	13.7	119.7	117.3	116.9	107.1	114.4
					2.b4.1	13.9	16.5	14.6	41.9	22.2	5.3	18.2	97.2	99.9	97.5	100.0	107.0	11.4	7.8	4.8	4.7	8.2	5.0	13.6	94.3	91.7	99.1	89.9	87.8
SERPING1	IC1	P05155	Plasma protease C1 inhibitor	LLDSLPSDTR	2.y7.1	23.0	34.6	26.6	16.0	17.5	17.3	26.4	95.1	111.4	103.3	109.7	124.9	1.9	4.6	2.1	3.1	9.2	5.8	16.6	91.1	98.7	101.7	95.0	92.6
					2.y5.1	7.3	4.8	6.6	11.0	13.2	0.9	13.3	106.7	104.5	108.7	109.4	119.7	3.7	3.6	4.2	1.6	1.9	2.8	10.4	101.3	104.7	105.0	111.3	119.5
					2.b2.1	5.9	9.9	3.0	8.2	1.7	13.1	19.1	88.7	94.2	77.3	94.1	124.7	6.3	3.0	1.3	6.0	5.8	2.8	14.4	100.7	98.0	100.0	103.5	104.8
SHBG	SHBG	P04278	Sex hormone-binding globulin	IALGGLLFPASNLR	2.y8.1	1.1	3.5	4.9	2.8	5.1	11.5	12.4	98.7	93.9	93.7	89.7	94.3	5.4	1.3	0.4	3.4	1.0	2.0	9.6	95.2	98.3	100.1	96.9	95.3

						2.y7.1	0.3	0.5	6.4	3.0	3.7	3.2	11.6	97.8	97.0	95.9	90.6	90.1	3.9	4.5	3.8	2.2	4.9	0.8	8.8	97.4	98.8	97.9	98.1	98.0
						2.b5.1	3.8	3.9	4.2	2.9	3.2	3.1	12.5	93.5	94.4	93.3	90.1	92.0	7.1	5.1	4.7	5.1	0.9	3.4	13.0	100.5	96.9	104.2	99.0	93.0
TTR	TTHY	P02766	Transthyretin	VLDAVR		2.y5.1	12.4	13.0	12.5	1.8	16.7	15.0	18.9	110.8	105.6	102.8	92.7	103.4	8.3	1.1	9.6	6.4	11.2	1.1	16.6	89.5	94.8	91.4	84.9	89.4
						2.y4.1	4.0	6.8	6.4	5.3	4.1	2.0	16.4	105.3	102.5	99.2	100.4	113.6	6.5	2.1	4.1	4.6	2.4	2.4						
						2.y3.1	25.6	13.9	47.8	11.9	31.7	8.9	27.6	95.6	61.7	72.5	69.5	65.5	10.8	9.7	8.7	5.0	23.6	2.5						

QC, quality control; %CV, percentage of coefficient of variation.

[a] The column title indicates the information of quantification ion, followed by the precursor ion charge, production ion type, and product ion charge.

Underline indicates that the value did not meet the criteria.

1.3.6 Evaluating reproducibility of the assay in the overall workflow

The variability of the entire MRM-MS assay workflow was evaluated by preparing and analyzing aliquots of the same sample on different days. Five aliquots (medium-QCs) were freshly prepared each day (16 h apart) and analyzed in triplicate over 5 days.

With the exception of IALGGLLFPASNLR (derived from SHBG), the CV_{intra}, CV_{inter}, and CV_{total} values of all target peptides were less than 20% (Figure 1-4A). The median CV_{intra}, CV_{inter}, and CV_{total} were 6.1%, 7.3%, and 9.8%, respectively. The CV_{intra} and CV_{inter} of all transitions were less than 20% (Figure 1-4B & Table 1-9). Although 3 of 112 transitions (2.7%) had a CV_{total} above 20% [y6+ ion of SPELQAEAK (APOA2), b5+ ion of IALGGLLFPASNLR (SHBG), y5+ ion of VLDAVR (TTR)], they were lower than 25%.

1.3.7 Comparison of assay performance with representative laboratories

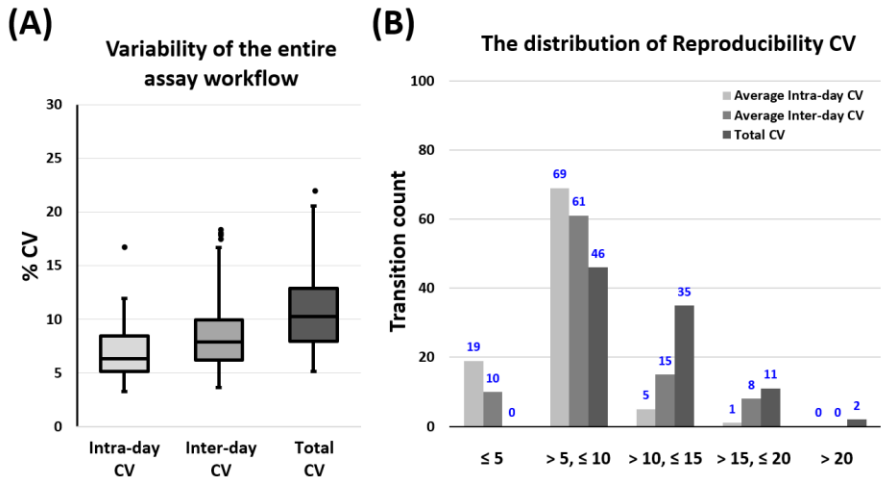
We compared the performance of our assay with that of 4 representative laboratories (Johns Hopkins University, JHU; Broad Institute, Broad; Fred Hutchinson Cancer Research Center, FHCRC; UVic-Genome BC Proteomics Centre, UVGPC), uploaded to the CPTAC ASSAY PORTAL (Figure 1-5). Three of the 4 laboratories analyzed the same type of peptides as we did; the JHU laboratory analyzed glycosylated peptides. None of 34 target proteins was in common with those of FHCRC. We compared the results of 2 items (response curve and repeatability), provided by CPTAC ASSAY PORTAL for 24 proteins, which overlapped between our study and 2 laboratories (Broad and UVGPC). The differences in the specifications of the interlaboratory assay platforms are described in Table 1-10.

The results of the comparison of the 23 proteins that overlap between UVGPC and our study are shown in Figure 1-6 and Table 1-11. We

selected the top 3 transitions of each peptide for the data that were provided by the PORTAL. The median LOD in the response curve was 0.60 fmol and 0.68 fmol in our study and the UVGPC report, respectively (Figure 1-6A). The LOQ in our study (median LOQ, 1.75 fmol) was higher than that of the UVGPC study (median LOQ, 0.69 fmol). In the UVGPC study, the median CV values for the repeatability of the 3 QC samples was slightly lower than in our study (Figure 1-6B). The median CV_{total} values in low-, medium-, and high-QC samples were 18.2%, 10.9%, and 10.4% for our study and 14.1%, 9.7%, and 8.0% for the UVGPC study, respectively. Eleven of the 23 proteins were quantified by peptides that were shared in our study and the UVGPC study. The median LOD and LOQ of 11 shared peptides were 0.60 fmol and 1.42 fmol for our study and 0.65 fmol and 0.65 fmol for the UVGPC study, respectively. The median CV_{total} values in low-, medium-, and high-QC samples were 19.3%, 12.6%, and 10.9% for our study and 15.3%, 10.7%, and 8.8% for the UVGPC study.

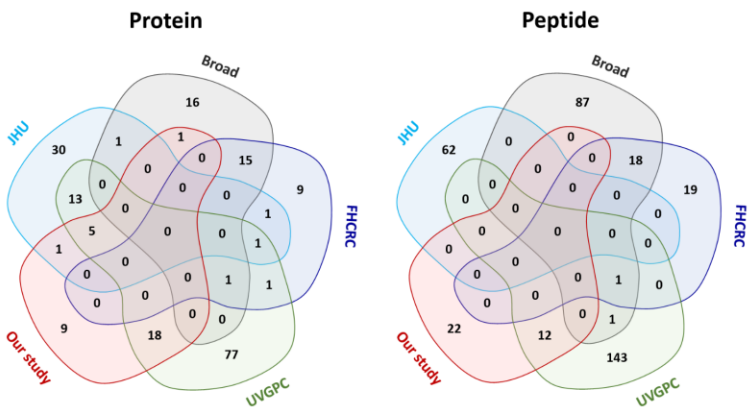
In the Broad study and our study, CRP protein was analyzed with the GYSIFSYATK and ESDTSYVSLK peptides, respectively. The LOD and LOQ range of the Broad study was 1.20–2.30 fmol in 3 transitions (Table 1-11). The LOD and LOQ range in our study was 0.22–1.10 fmol and 0.81–2.58 fmol, respectively (Table 1-3). The median CV_{total} values for 3 transitions of the low-, medium-, and high-QC samples were 18.6%, 14.8%, and 15.8% and 8.70%, 5.40%, and 3.40%, respectively, for our study and the Broad study (Table 1-6 and 1-11).

Figure 1-4. Reproducible detection of endogenous analytes.



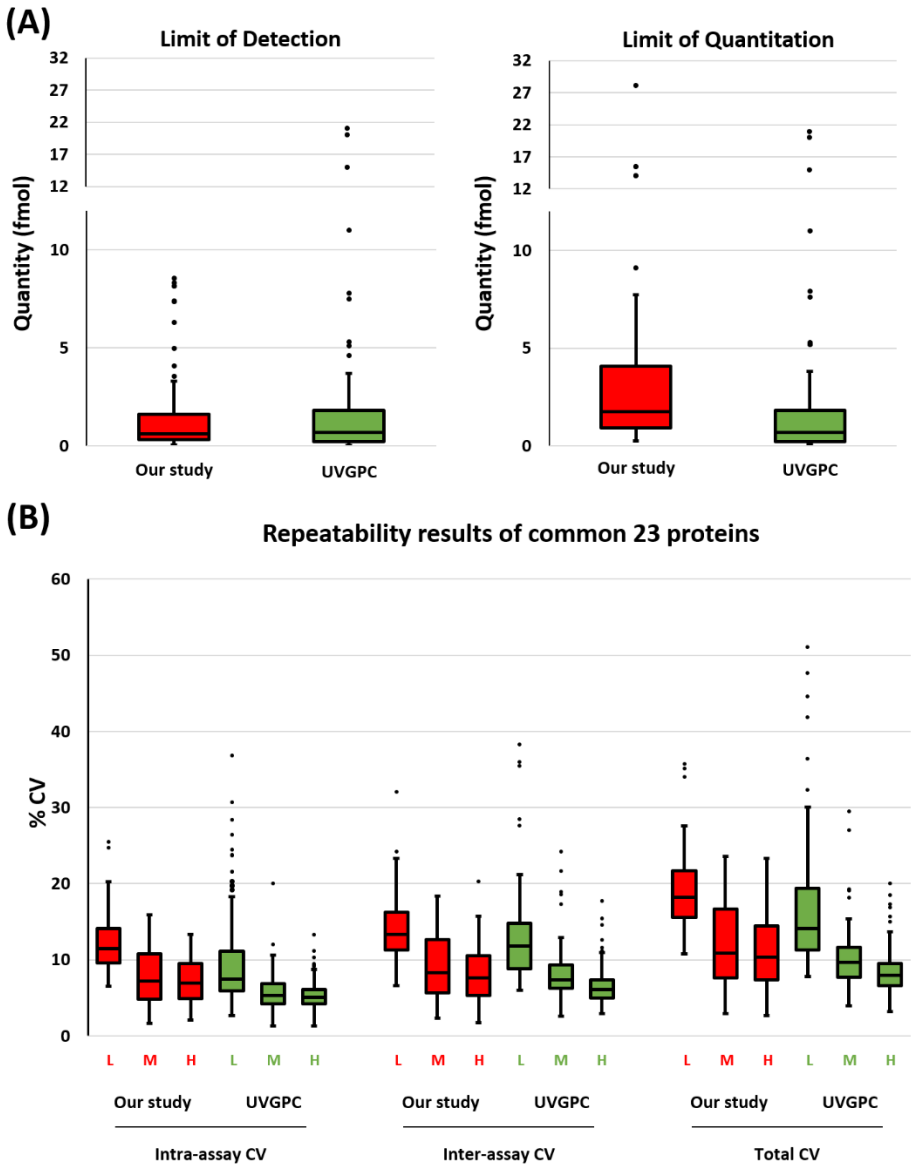
The 5 aliquots of normal pooled serum were digested and desalted on each of 5 days. The prepared samples were mass-analyzed on that day. (A) The distribution of the CV results is shown in the box-whisker plot. Comparing the variability in the validation of repeatability (Category 2), the entire assay workflow, including the sample preparation, influenced the variability to a lesser extent. (B) Averaged intra-, inter-, and total CV values were obtained from 5 analyses on each of 5 days for 34 targets. Only the total CVs of SHBG (IALGGLLFPASNLR) and TTR (VLDAVR) were above 20% (24.7% and 20.6%, respectively). The values above the bars indicate the number of target peptides within the range of CVs, on the x-axis.

Figure 1-5. The protein assays uploaded to the CPTAC Assay portal and validated in this study.



A total of 1582 assays for 830 unique proteins and 1503 unique peptides have been uploaded since the CPTAC Assay portal was launched. The assays for 193 unique proteins and 350 unique peptides were validated using plasma or serum specimens on 4 different assay platforms and laboratories: Broad Institute (Broad), Fred Hutchison Cancer Research Center (FHCRC), Johns Hopkins University (JHU), and University of Victoria Genome British Columbia Proteomics Centre (UVGPC). JHU validated the assays for 62 peptides derived from 51 proteins by N-linked glycosylation enrichment and parallel-reaction monitoring mass spectrometry (PRM-MS), another targeted MS technique. Broad and FHCRC quantified 34 (107) and 28 (38) proteins (peptides). To increase the sensitivity for the protein assays, they depleted high-abundance proteins using a MARS-14 column or by SISCAPA technique. UVGPC developed the assays for 164 peptides derived from 118 proteins using ultrahigh-pressure liquid chromatography (uHPLC, flow rate: 0.4 mL/min), unlike the nano-flow LC system of other groups.

Figure 1-6. Comparing the performance of the assays for 23 proteins in UVGPC and our study.



To confirm that the validated assays of this study were proper to be submitted to the portal, their performance was compared with that of the assays for common targets in UVGPC. The top 3 transitions per peptide was selected to compare the validation results. (A) With regard to Response curve, the distribution of LOD values was similar in both groups—the median LOD was 0.60 fmol in our study and 0.68 fmol in UVGPC. The LOQ in our study (median LOQ, 1.75 fmol) was higher than in UVGPC (median LOQ, 0.69 fmol). (B) The median total CVs in three-QC samples (low, medium, and high-QC) were 19.3%, 12.6%, and 10.9% in our study and 15.3%, 10.7%, and 8.8% in UVGPC. The percentage of transitions, that had a total CV lower than 20% at the concentrations of the low-, medium-, and high-QC was 71.3%, 90.0%, and 98.8% (57, 72, and 79 of 80 transitions) in our study and 78.6%, 98.3%, and 100% (92, 115, and 117 of 117 transitions) in UVGPC.

Table 1-9. Reproducible detection of target analytes in overall workflow.

Information of target protein and peptide							Average intra-day CV (%)	Average inter-day CV (%)	Total CV (%)
N.	Gene symbol	Uniprot ID	Uniprot Accession	Protein Name	Peptide Sequence	Quantification ion[a]			
1	AMBP	AMBP	P02760	Protein AMBP	ETLLQDFR	2.y6.1	4.6	4.9	6.7
						2.y4.1	3.4	3.5	4.8
						2.y3.1	3.9	4.6	6.0
2	APOA2	APOA2	P02652	Apolipoprotein A2	SPELQAEAK	2.y6.1	8.7	19.3	21.2
						2.y4.1	6.7	17.6	18.9
						2.y8.2	6.8	18.5	19.7
						2.b2.1	5.7	16.7	17.6
3	APOB	APOB	P04114	Apolipoprotein B-100	ALVDTLK	2.y5.1	5.0	5.1	7.1
						2.y4.1	5.7	6.8	8.9
						2.b2.1	5.4	6.9	8.7
4	APOE	APOE	P02649	Apolipoprotein E	LAVYQAGAR	2.y7.1	4.8	6.4	8.0
						2.y6.1	7.0	7.7	10.4
						2.y5.1	5.1	5.8	7.7
						2.y4.1	5.9	7.1	9.2
5	APOH	APOH	P02749	Apolipoprotein H	ATVVYQGER	2.y7.1	4.6	6.6	8.0
						2.y6.1	4.0	4.3	5.8
						2.y5.1	3.8	4.3	5.7
						2.b3.1	5.4	5.5	7.7
6	B2M	B2MG	P61769	Beta-2-microglobulin	VNHVTLSQPK	3.y6.1	11.2	11.4	16.0
						3.y5.1	9.6	8.9	13.1
						3.b3.1	12.0	11.2	16.4
7	BCHE	CHLE	P06276	Cholinesterase	IFFPGVSEFGK	2.y8.1	8.0	7.9	11.3
						2.y7.1	8.4	8.8	12.1
						2.b2.1	8.6	9.0	12.5
8	C1QA	C1QA	P02745	Complement C1q subunit A	SLGFCDTTNK	2.y8.1	7.2	7.8	10.6
						2.y6.1	5.3	6.1	8.0
						2.b2.1	4.9	7.0	8.5
9	C2	CO2	P06681	Complement C2	LNINLK	2.y5.1	9.4	8.7	12.8
						2.y4.1	8.8	9.4	12.9
						2.b2.1	7.9	8.1	11.3
10	C3	CO3	P01024	Complement C3	DFDFVPPVVR	2.y8.1	9.0	15.6	18.1
						2.y6.1	9.2	15.5	18.0
						2.y5.1	9.2	15.8	18.2
						2.b2.1	9.0	16.2	18.5
						2.b3.1	9.1	15.9	18.3
11	C5	CO5	P01031	Complement C5	LQGTLPVEAR	2.y8.1	6.5	7.1	9.6
						2.y6.1	5.8	5.5	7.9
						2.y5.1	6.8	8.1	10.6
						2.b2.1	6.2	6.2	8.8
12	C6	CO6	P13671	Complement component C6	ALQEYAAK	2.y6.1	8.7	9.9	13.2
						2.b2.1	6.3	11.0	12.7
						2.b3.2	6.2	11.8	13.3
13	C8A	CO8A	P07357	Complement C8 alpha chain	LYYGDDEK	2.y6.1	4.7	6.2	7.8
						2.b2.1	8.7	9.9	13.2
						2.b4.2	6.4	7.6	9.9
14	C8B	CO8B	P07358	Complement C8 beta chain	YEFILK	2.y2.1	5.4	4.6	7.1
						2.y4.2	5.2	5.9	7.9
						2.b2.1	6.9	6.7	9.6
15	C9	CO9	P02748	Complement component C9	VVEESELAR	2.y7.1	6.4	7.5	9.9
						2.y6.1	7.0	7.1	10.0
						2.b2.1	7.6	8.2	11.2
16	CFB	CFAB	P00751	Complement factor B	EELLPAQDIK	2.y6.1	4.7	6.2	7.8
						2.b2.1	6.1	7.0	9.3
						2.b3.1	4.8	6.5	8.1
17	CFH	CFAH	P08603	Complement factor H	VGEVLK	2.y5.1	5.1	8.1	9.6
						2.y4.1	5.1	6.4	8.1
						2.b3.1	6.3	6.5	9.0
18	CP	CERU	P00450	Ceruloplasmin	GAYPLSIEPIGVR	2.y10.1	4.0	4.0	5.7
						2.y8.1	3.7	3.6	5.2
						2.y6.1	3.3	3.9	5.1
						2.y5.1	3.7	4.3	5.7
19	CRP	CRP	P02741	C-reactive protein	ESDTSYVSLK	2.y6.1	5.1	6.0	7.8
						2.y5.1	7.1	8.0	10.7
						2.y3.1	9.3	9.6	13.4
20	CST3	CYTC	P01034	Cystatin-C	ALDFAVGEYNK	2.y6.1	4.2	8.6	9.6
						2.b2.1	10.1	9.0	13.5
						2.b3.1	9.2	9.1	12.9
21	F2	THRB	P00734	Prothrombin	ELLESYIDGR	2.y6.1	4.6	10.5	11.5
						2.y5.1	5.8	10.6	12.1
						2.b2.1	5.4	11.3	12.5

22	F9	FA9	P00740	Coagulation factor IX	SALVLQYLR	2.y5.1	4.3	5.1	6.7
						2.b2.1	7.7	7.1	10.5
						2.b3.1	6.9	8.1	10.7
23	F10	FA10	P00742	Coagulation factor X	TGIVSGFGR	2.y6.1	4.6	5.1	6.9
						2.y5.1	5.0	5.9	7.8
						2.y4.1	7.6	7.2	10.5
						2.b3.1	5.8	6.2	8.5
24	F11	FA11	P03951	Coagulation factor XI	VVSGFSLK	2.y6.1	3.5	4.9	6.0
						2.b2.1	5.6	4.8	7.3
25	GSN	GELS	P06396	Gelsolin	AGALNSNDAFVLK	2.y9.1	4.8	5.4	7.3
						2.y8.1	3.6	3.2	4.8
						2.b3.1	3.3	5.1	6.1
26	IGFBP3	IBP3	P17936	IGF-binding protein 3	FLNVLSPR	2.y6.1	4.8	5.2	7.1
						2.y4.1	4.4	4.6	6.3
						2.y3.1	5.4	6.2	8.3
27	PLG	PLMN	P00747	Plasminogen	LFLEPTR	2.y6.1	5.8	7.4	9.4
						2.y5.1	3.7	5.3	6.5
						2.y4.1	4.7	6.4	7.9
						2.y3.1	3.4	4.8	5.9
						2.b2.1	3.2	4.6	5.6
28	PROC	PROC	P04070	Vitamin K-dependent protein C	TFVLNFIK	2.y6.1	7.7	9.2	12.0
						2.y5.1	9.7	10.4	14.2
						2.b2.1	6.9	8.0	10.6
29	PROS1	PROS	P07225	Vitamin K-dependent protein S	YLVCLR	2.y5.1	7.2	8.9	11.5
						2.y4.1	5.9	6.0	8.4
						2.y3.1	5.4	5.1	7.5
						2.b2.1	7.3	6.9	10.0
30	SERPINC1	ANT3	P01008	Antithrombin-III	FDTISEK	2.y6.1	6.7	10.0	12.0
						2.y5.1	5.5	8.0	9.7
						2.b2.1	5.9	10.6	12.1
31	SERPINF2	A2AP	P08697	Alpha-2-antiplasmin	LGNQEPGGQTALK	2.y8.1	5.7	5.5	7.9
						2.y7.1	8.5	9.0	12.4
						2.b4.1	6.6	6.3	9.1
32	SERPING1	IC1	P05155	Plasma protease C1 inhibitor	LLDSLPSDTR	2.y7.1	8.5	12.4	15.0
						2.y5.1	6.2	12.1	13.6
						2.b2.1	7.7	10.7	13.2
33	SHBG	SHBG	P04278	Sex hormone-binding globulin	IALGGLLFPASNLR	2.y8.1	11.8	11.7	16.6
						2.y7.1	10.0	11.1	14.9
						2.b5.1	16.8	18.0	24.7
34	TTR	TTHY	P02766	Transthyretin	VLDAVR	2.y5.1	9.6	18.2	20.6
						2.y4.1	7.9	14.2	16.2
						2.y3.1	10.2	15.3	18.4

QC, quality control

[a] The column title indicates the information of quantification ion, followed by the precursor ion charge, production ion type, and product ion charge.

Underline indicates that the value did not meet the criteria.

Table 1-10. Specifications for the Experiments 1 and 2 of the assays in 3 laboratories.

Information	Broad Institute (Broad)	UVic-Genome BC Proteomics Centre (UVGPC)	Seuol National University (our study)
Liquid chromatography	Easy NanoLC1000 (Thermo)	1290 LC (Agilent)	Eksigent 2D MDLC (ABsciex)
Mass spectrometer (vender)	Quantiva TSQ (Thermo)	6490 Triple Quad (Agilent)	4000 QTRAP (ABsciex)
Column specifications	Reprosil C18, 3 µm, 120A (0.075 x 120 mm)	Zorbax Eclipse Plus C18, 1.8 µm (2.1 x 150 mm)	Reprosil C18, 5 µm, 200A (0.075 x 150 mm)
flow rate of LC	300 nL/min	400 µL/min	300 nL/min
Matrix type	Depleted digested plasma	plasma	Depleted digested serum
Injection volume	1 µL	10 µL	5 µL
Response curve type	Forward Curve	Reverse Curve	Reverse Curve
Concentration range of curves	0.001 fmol/µL - 100 fmol/µL (Light peptide)	12.5 amol/µL - 1 pmol/µL (Heavy peptide)	Different depending upon targets (Heavy peptide)
Number of response curve points	9	11	13
Spiked concentration of synthetic peptide as internal standard	10 fmol/µL (Heavy peptide)	20 fmol/µL (Light peptide)	No spike
Concentration of matrix	0.5 µg/µL	1.0 µg/µL	0.2 µg/µL
Low QC	0.9 fmol/µL	LLOQ x 3	LLOQ x 2 or 3
Medium QC	15 fmol/µL	LLOQ x 50	LLOQ x 50–100, 50% of linear range
High QC	80 fmol/µL	LLOQ x 500	> LLOQ x 100, 90% of linear range

Table 1-11. Performance of assays in the portal for common target proteins with this study.

Information of target protein and peptide							Experiment 1 (Response curve)								Experiment 2 (Repeatability)					
							Curve fit			fmol of peptide[b]		Average Intra-assay CV (%)			Average Inter-assay CV (%)			Total-CV (%)		
No.	Gene symbol	Uniprot ID	Uniprot Accession	Protein Name	Peptide Sequence	Quantification[a]	Slope	Intercept	R square	LOD	LOQ	Low	Medium	High	Low	Medium	High	Low	Medium	High
1	AMBP	AMBP	P02760	Protein AMBP	ETLLQDFR	2.y3.1	2.28	0.20	1.00	0.51	0.52	7.1	5.1	5.9	11.1	7.0	5.9	13.2	8.7	8.3
						2.y4.1	2.43	0.28	1.00	0.22	0.23	4.0	5.2	5.1	8.6	6.3	6.1	9.5	8.2	8.0
						2.y5.1	2.19	0.19	1.00	0.55	0.55	7.5	4.6	3.7	6.0	5.4	3.8	9.6	7.1	5.3
					HHGPTITAK	3.b2.1	1.96	0.08	1.00	1.30	1.30	7.5	7.3	6.9	12.0	10.4	11.2	14.2	12.7	13.2
						3.b3.1	1.73	0.11	1.00	2.80	2.80	8.2	5.3	5.8	11.8	10.3	14.6	14.4	11.6	15.7
						3.y3.1	1.93	0.02	1.00	1.60	1.60	6.4	4.8	6.1	8.7	6.1	7.6	10.8	7.8	9.7
2	APOA2	APOA2	P02652	Apolipoprotein A2	EQLTPLIK	2.y2.1	1.25	0.06	1.00	2.60	2.60	5.3	3.3	5.0	8.6	3.9	5.9	10.1	5.1	7.7
						2.y4.1	1.31	0.07	1.00	0.40	0.40	6.7	5.1	6.1	9.7	7.2	4.7	11.8	8.8	7.7
						2.y5.1	1.35	0.07	1.00	1.30	1.30	5.4	4.7	5.0	8.8	8.3	5.4	10.3	9.5	7.4
					SPELQAEAK	2.y2.1	1.11	0.06	1.00	3.00	3.00	7.4	4.3	5.8	10.8	4.1	8.4	13.1	5.9	10.2
						2.y7.1	1.17	0.04	1.00	1.80	1.80	7.4	7.8	5.0	12.6	9.3	6.2	14.6	12.1	8.0
						2.y8.2	1.10	0.07	1.00	0.91	0.91	5.8	5.0	5.7	8.8	7.3	7.0	10.5	8.8	9.0
3	APOB	APOB	P04114	Apolipoprotein B-100	ATLYALSHAVNNYHK	4.y12.2	5.70	2.38	1.00	0.07	0.08	6.0	6.2	3.6	7.8	8.8	5.1	9.8	10.8	6.2
						4.y11.2	4.67	1.03	1.00	0.10	0.11	5.8	6.0	5.6	8.1	5.9	4.8	10.0	8.4	7.4
						4.y12.3	4.20	0.92	1.00	0.07	0.08	5.5	4.0	4.5	6.9	6.0	6.5	8.8	7.2	7.9
					FPEVDVLTK	2.y8.1	3.22	0.14	1.00	0.91	0.91	10.2	4.4	3.8	12.6	5.3	5.0	16.2	6.9	6.3
						2.y7.1	3.31	0.13	1.00	0.34	0.35	4.5	3.6	3.4	9.5	6.5	3.7	10.5	7.4	5.0
						2.y2.1	3.38	0.16	1.00	0.70	0.70	13.9	3.8	3.6	13.9	6.2	3.3	19.7	7.3	4.9
					TGISPLALIK	2.y7.1	3.62	0.42	1.00	0.08	0.09	6.2	5.1	4.0	9.4	5.8	6.5	11.3	7.7	7.6
						2.y6.1	3.51	0.37	1.00	0.19	0.20	5.8	7.7	5.2	11.8	6.9	5.6	13.1	10.3	7.6
						2.b3.1	3.33	0.33	1.00	0.45	0.46	8.7	2.4	4.8	8.5	3.2	5.1	12.2	4.0	7.0
4	APOE	APOE	P02649	Apolipoprotein E	LGPLVEQGR	2.y4.1	2.55	0.08	1.00	0.34	0.35	6.1	3.6	2.8	9.9	6.4	4.6	11.6	7.3	5.4
						2.y5.1	2.49	0.05	1.00	0.34	0.34	7.1	3.9	6.1	11.5	5.5	5.7	13.5	6.7	8.3
						2.y7.2	2.51	0.06	1.00	0.13	0.14	8.9	3.9	4.2	10.7	7.8	4.9	13.9	8.7	6.5
5	APOH	APOH	P02749	Apolipoprotein H	ATVVYQGER	2.y5.1	1.48	0.07	1.00	1.20	1.20	7.7	7.3	8.2	8.6	8.5	6.7	11.7	11.2	10.6
						2.y6.1	1.53	0.06	1.00	0.65	0.65	10.6	8.8	4.8	12.9	11.5	6.4	16.7	14.5	8.0
						2.y7.1	1.52	0.07	1.00	1.30	1.30	12.6	7.4	4.6	15.4	7.3	6.5	19.9	10.4	8.0
6	BCHE	CHLE	P06276	Cholinesterase	YLTLNTESTR	2.y8.1	20.90	0.35	1.00	0.12	0.13	14.1	6.4	5.3	13.9	8.2	7.0	19.8	10.4	8.8
						2.y6.1	20.10	0.46	1.00	0.12	0.14	10.8	6.9	4.8	13.8	11.1	7.9	17.5	13.1	9.2
						2.b2.1	21.00	0.49	1.00	0.11	0.12	12.1	6.2	4.9	17.1	7.7	9.4	20.9	9.9	10.6
7	C2	CO2	P06681	Complement C2	HAFILQDTK	3.y4.1	8.07	0.47	1.00	0.18	0.19	11.7	6.9	4.4	6.6	7.8	7.7	13.4	10.4	8.9
						3.b3.1	7.01	0.34	1.00	1.20	1.20	10.7	8.9	6.8	14.6	7.7	7.4	18.1	11.8	10.0
						3.b4.1	7.63	0.47	1.00	0.12	0.12	10.2	7.4	5.8	8.7	8.7	4.7	13.4	11.4	7.5
8	C3	CO3	P01024	Complement C3	SGIPIVTSPYQIHFTK	3.y13.2	1.23	0.40	1.00	0.31	0.31	6.0	4.5	3.6	13.2	7.4	5.8	14.5	8.7	6.8
						3.y13.3	1.35	0.42	1.00	0.44	0.44	5.9	3.4	4.3	13.5	7.1	5.5	14.7	7.9	7.0
						3.b3.1	1.19	0.22	1.00	2.20	2.20	5.5	3.0	2.7	14.3	7.1	6.0	15.3	7.7	6.6
					TGLQEVEVK	2.y6.1	1.19	0.03	1.00	0.94	0.95	6.3	4.2	5.0	11.7	9.5	6.1	13.3	10.4	7.9
						2.y2.1	1.13	0.03	1.00	1.50	1.50	4.9	4.5	4.5	8.8	6.5	4.2	10.1	7.9	6.2
						2.y7.2	1.28	0.05	1.00	1.60	1.60	6.5	4.8	5.0	13.0	6.4	6.6	14.5	8.0	8.3
					VHQYFNVELIQPGAVK	3.y7.1	1.22	0.21	1.00	1.30	1.30	9.4	6.9	3.6	17.0	8.2	5.3	19.4	10.7	6.4

						3.y6.1	1.25	0.12	1.00	2.50	2.50	6.2	4.8	5.4	17.0	9.8	5.7	18.1	10.9	7.9
						3.b13.3	1.24	0.19	1.00	1.90	1.90	11.9	5.9	6.3	17.4	7.2	7.1	21.1	9.3	9.5
9	C5	CO5	P01031	Complement C5	FQNSAILTIQPK	2.y10.1	3.96	0.32	1.00	1.90	1.90	30.7	6.4	4.2	28.5	8.7	7.0	41.9	10.8	8.2
						2.y2.1	4.04	0.24	1.00	1.90	1.90	36.8	7.3	5.1	35.5	9.3	6.0	51.1	11.8	7.9
						2.b2.1	4.00	0.37	1.00	1.20	1.20	19.2	6.4	4.5	19.2	8.1	4.6	27.2	10.3	6.4
					VFQFLEK	2.y6.1	3.79	0.37	1.00	0.10	0.14	15.1	5.9	6.4	17.4	7.1	7.6	23.0	9.2	9.9
						2.y5.1	3.71	0.36	1.00	0.07	0.11	14.1	5.6	3.6	15.9	5.3	4.6	21.3	7.7	5.8
						2.b2.1	3.62	0.35	1.00	0.13	0.17	18.3	6.7	7.2	19.8	7.4	6.5	27.0	10.0	9.7
10	C9	CO9	P02748	Complement component C9	VVEESELAR	2.y7.1	3.79	0.23	1.00	0.22	0.23	10.8	7.3	8.7	11.6	9.6	9.3	15.8	12.1	12.7
						2.y5.1	3.68	0.31	1.00	2.40	2.40	28.4	6.0	5.4	38.3	9.0	7.5	47.7	10.8	9.2
						2.y3.1	3.43	0.13	1.00	5.10	5.20	21.6	9.8	13.3	14.7	8.8	11.0	26.1	13.2	17.3
					LSPIYNLVPVK	2.y9.2	3.70	0.67	1.00	0.06	0.07	2.7	4.2	3.5	8.8	4.9	3.3	9.2	6.5	4.8
						2.y3.1	3.52	0.42	1.00	0.32	0.33	6.3	5.4	2.9	11.2	6.4	3.2	12.9	8.4	4.3
						2.b2.1	2.93	0.23	1.00	0.83	0.85	5.2	5.9	6.1	7.0	5.8	5.7	8.7	8.3	8.3
					TSNFNAAISLK	2.y7.1	2.80	0.16	1.00	1.80	1.90	8.7	6.6	5.3	9.4	9.9	5.5	12.8	11.9	7.6
						2.y3.1	3.21	0.14	1.00	1.90	1.90	5.7	8.3	5.8	15.7	9.1	6.2	16.7	12.3	8.5
						2.y9.2	3.26	0.17	1.00	3.70	3.80	11.2	6.9	7.5	15.9	8.6	9.7	19.4	11.0	12.3
11	CFB	CFAB	P00751	Complement factor B	EELLPAQDIK	2.y6.1	2.06	0.11	1.00	0.22	0.22	10.8	6.0	5.3	13.4	9.2	7.7	17.2	11.0	9.3
						2.b2.1	1.98	0.11	1.00	0.96	0.97	15.4	7.6	5.8	17.3	7.5	6.5	23.2	10.7	8.7
						2.b3.1	2.04	0.10	1.00	1.40	1.40	20.3	9.5	5.0	21.2	11.1	7.2	29.4	14.6	8.8
12	CFH	CFAH	P08603	Complement factor H	SSQESYAHGTK	3.y3.1	1.39	0.16	1.00	21.00	21.00	10.1	7.3	5.6	13.5	8.0	7.7	16.9	10.8	9.5
						3.y9.2	1.20	0.10	1.00	7.80	7.90	3.9	3.2	2.2	8.9	6.0	3.2	9.7	6.8	3.9
						3.y7.2	1.50	0.19	1.00	15.00	15.00	23.7	12.0	9.3	27.6	24.2	17.7	36.4	27.0	20.0
13	CP	CERU	P00450	Ceruloplasmin	GAYPLSIEPIGVR	2.y10.1	1.25	0.14	1.00	2.30	2.30	9.2	2.1	4.6	10.9	4.3	3.3	14.3	4.8	5.7
						2.y11.2	1.39	0.14	1.00	2.00	2.00	9.5	5.2	5.0	12.0	7.3	6.4	15.3	9.0	8.1
						2.y10.2	1.71	0.29	1.00	0.26	0.26	7.3	3.6	4.7	11.2	6.2	4.6	13.4	7.2	6.6
					IYHSHIDAPK	3.y2.1	1.37	0.04	1.00	3.70	3.70	4.5	4.0	4.0	10.4	6.6	5.9	11.3	7.7	7.1
						3.y9.2	1.36	0.10	1.00	2.10	2.10	5.8	3.7	3.8	14.1	9.9	4.6	15.2	10.6	6.0
						3.y8.2	1.41	0.08	1.00	1.10	1.10	5.3	3.6	3.4	8.0	6.4	3.8	9.6	7.3	5.1
					NNEGTYYSPNYNPQSR	3.y4.1	1.29	0.10	1.00	11.00	11.00	19.8	5.7	5.4	19.8	9.4	5.4	28.0	11.0	7.6
						3.y9.2	1.16	0.06	1.00	20.00	20.00	19.7	7.6	6.2	18.5	8.8	8.0	27.0	11.6	10.1
						3.y8.2	1.12	0.07	1.00	4.60	4.60	9.6	3.5	4.7	12.9	2.6	7.1	16.1	4.4	8.5
14	CRP	CRP	P02741	C-reactive protein	GYSIFSYATK[a]	2.y8.1	1.06	0.02	1.00	1.20	1.20	5.1	1.4	1.3	7.1	5.6	2.9	8.7	5.8	3.2
						2.y6.1	1.05	0.02	1.00	1.20	1.20	3.2	1.9	1.5	7.1	4.9	3.1	7.8	5.3	3.4
						2.y5.1	0.99	0.02	1.00	2.30	2.30	4.7	1.3	1.8	8.6	5.2	3.1	9.8	5.4	3.6
15	CST3	CYTC	P01034	Cystatin-C	ALDFAVGEYNK	2.y9.1	18.00	1.56	1.00	0.13	0.17	4.7	5.3	4.0	7.2	11.5	8.1	8.6	12.7	9.0
						2.y7.1	19.00	1.39	1.00	0.11	0.15	14.5	6.5	5.5	13.9	9.9	5.6	20.1	11.8	7.8
						2.y6.1	18.00	1.35	1.00	0.10	0.15	10.7	7.1	5.5	13.1	11.2	8.4	16.9	13.3	10.0
16	F9	FA9	P00740	Coagulation factor IX	SALVLQYLR	2.y5.1	24.70	2.72	1.00	0.05	0.08	6.1	4.5	10.3	13.1	6.7	15.4	14.5	8.1	18.5
						2.y4.1	25.90	2.56	1.00	0.11	0.13	6.2	7.6	9.5	14.8	11.4	11.6	16.0	13.7	15.0
						2.y7.2	25.70	3.79	1.00	0.09	0.12	9.9	5.9	11.2	14.4	9.4	12.6	17.5	11.1	16.9
17	F10	FA10	P00742	Coagulation factor X	MLEVPYVDR	2.y5.1	9.02	0.62	1.00	0.10	0.12	5.5	3.4	4.9	12.6	7.3	6.9	13.7	8.1	8.5
						2.b3.1	8.97	0.56	1.00	0.20	0.22	10.3	4.4	6.2	16.2	6.4	8.9	19.2	7.8	10.8
						2.b4.1	10.71	0.46	1.00	0.20	0.20	8.4	6.4	2.9	10.2	9.8	6.9	13.2	11.7	7.5
18	GSN	GELS	P06396	Gelsolin	AGALNSNDAFVLK	2.b3.1	2.32	0.39	1.00	0.68	0.69	6.0	4.3	3.6	7.5	4.3	4.0	9.6	6.1	5.4
						2.y8.1	2.53	0.30	1.00	1.10	1.10	5.0	6.5	5.4	11.7	6.9	5.4	12.7	9.5	7.6
						2.y9.1	2.46	0.39	1.00	0.98	0.99	4.7	4.5	3.9	9.7	5.4	4.8	10.8	7.0	6.2
					TGAQELLR	2.b3.1	2.92	0.11	1.00	0.64	0.65	13.6	7.7	5.9	17.1	8.6	6.5	21.8	11.5	8.8
						2.y4.1	3.05	0.11	1.00	0.28	0.29	14.9	9.0	6.8	15.1	10.2	9.8	21.2	13.6	11.9
						2.y5.1	3.03	0.09	1.00	0.24	0.24	8.2	10.6	6.1	11.3	11.2	6.0	14.0	15.4	8.6
19	PLG	PLMN	P00747	Plasminogen	LFLEPTR	2.y5.1	6.45	0.29	1.00	0.04	0.05	10.2	5.1	7.7	12.2	8.2	5.9	15.9	9.7	9.7
						2.y3.1	6.22	0.22	1.00	0.09	0.10	8.5	7.6	6.7	8.8	9.0	6.7	12.2	11.8	9.5
						2.b2.1	6.50	0.30	1.00	0.04	0.05	6.1	5.4	6.0	6.3	7.5	6.3	8.8	9.2	8.7

20	PROS1	PROS	P07225	Vitamin K-dependent protein S	SFQTGLFTAAR	2.y5.1	3.92	0.31	1.00	0.95	0.98	5.9	5.9	6.1	8.8	7.4	7.8	10.6	9.5	9.9
						2.y4.1	3.77	0.31	1.00	1.70	1.80	6.3	6.4	9.1	12.5	8.6	10.3	14.0	10.7	13.7
						2.y9.2	3.82	0.25	1.00	0.65	0.68	11.1	5.3	6.3	17.3	8.4	9.0	20.6	9.9	11.0
21	SERPINC1	ANT3	P01008	Antithrombin-III	DDLYVSDAFHK	3.y9.2	1.73	0.17	1.00	0.72	0.72	4.5	4.0	3.0	6.6	5.5	3.7	8.0	6.8	4.8
						3.y8.2	1.68	0.19	1.00	0.60	0.61	4.0	2.6	3.4	8.1	5.7	3.6	9.0	6.3	5.0
						3.b2.1	1.56	0.13	1.00	0.91	0.92	4.3	1.8	2.9	6.9	4.9	3.1	8.1	5.2	4.2
					FATTFYQHLADSK	3.y2.1	1.79	0.37	1.00	0.63	0.65	5.1	4.2	4.4	10.0	9.3	5.0	11.2	10.2	6.7
						3.y11.2	1.74	0.45	1.00	0.32	0.35	7.4	7.5	5.8	8.3	10.1	6.5	11.1	12.6	8.7
						3.b2.1	1.81	0.42	1.00	0.44	0.46	5.9	6.3	3.6	7.4	7.4	4.0	9.5	9.7	5.4
					TSDQIHFFFAK	3.y6.1	1.77	0.57	1.00	0.85	0.86	6.4	4.1	5.5	12.6	5.4	5.1	14.1	6.8	7.5
						3.y10.2	1.77	0.78	1.00	0.19	0.20	4.8	4.7	5.4	11.6	7.3	5.6	12.6	8.7	7.8
						3.y9.2	1.56	0.38	1.00	0.52	0.53	6.7	4.8	4.3	10.7	5.9	4.6	12.6	7.6	6.3
22	SERPINF2	A2AP	P08697	Alpha-2-antiplasmin	LGNQEPGGQTALK	2.y8.1	2.15	0.13	1.00	0.58	0.59	12.9	6.7	7.0	17.8	7.4	7.0	22.0	10.0	9.9
						2.b4.1	2.01	0.09	1.00	7.50	7.60	26.4	20.0	9.3	36.0	21.7	8.5	44.6	29.5	12.6
						2.b5.1	1.93	0.06	1.00	2.80	2.80	24.5	7.6	4.6	21.1	12.9	5.8	32.3	15.0	7.4
23	SERPING1	IC1	P05155	Plasma protease C1 inhibitor	FQPTLLTLPR	2.y8.1	1.67	0.42	1.00	0.28	0.29	6.1	4.2	5.7	11.0	5.1	5.3	12.6	6.6	7.8
						2.y4.1	1.38	0.17	1.00	2.00	2.00	7.3	4.4	5.5	12.4	6.8	6.2	14.4	8.1	8.3
						2.y8.2	1.67	0.39	1.00	0.30	0.31	7.2	4.5	4.8	11.5	6.7	5.0	13.6	8.1	6.9
					GVTSVSQIFHSPDLAIR	3.y15.2	1.35	0.40	1.00	0.57	0.59	21.6	5.1	4.7	17.2	11.5	5.3	27.6	12.6	7.1
						3.y12.2	1.36	0.82	1.00	0.03	0.05	17.3	6.6	2.9	17.0	9.9	4.9	24.3	11.9	5.7
						3.y15.3	1.24	0.21	1.00	3.70	3.80	23.8	6.4	4.8	18.4	10.5	6.2	30.1	12.3	7.8
24	TTR	TTHY	P02766	Transthyretin	GSPAINVAVHVFR	3.y3.1	1.68	0.21	1.00	5.30	5.30	8.6	4.1	8.6	12.2	18.9	7.7	14.9	19.3	11.5
						3.y11.2	2.12	0.67	1.00	0.28	0.30	7.8	5.5	7.5	10.5	17.3	8.2	13.1	18.2	11.1
						3.y9.2	1.94	0.34	1.00	1.80	1.80	9.1	4.3	7.6	8.2	18.6	6.8	12.2	19.1	10.2

Transition marked with "P" were content with criteria of the items, "NP" were not content with, and "Y" were qualified in all the experiments.
Exp1 to Exp5 indicate the 5 experimental categories in the guidelines.
[a] The column title indicates the information of quantification ion, followed by the precursor ion charge, production ion type, and product ion charge.

1.4 Discussion

Three tiers of targeted MS assays were defined at a workshop that was sponsored by the National Institutes of Health (NIH) under the auspices of the National Cancer Institute-Clinical Proteomic Tumor Analysis Consortium (NCI-CPTAC) and National Heart, Lung, and Blood Institute (NHLBI) as “fit-for-purpose” with regard to validation strategies (19). Tier 1 and 2 assays have 2 properties that differentiate them from Tier 3 assays: (i) the ability to repeatedly measure sets of analytes of interest within and across samples and experiments and (ii) the ability to incorporate internal standards for each analyte for confident detection and precise quantification.

Tier 1 assays are designed to provide actionable information to medical practitioners. To ensure the credibility of these assays, the targets must be rigorously analyzed and validated in thousands of clinical samples in accordance with guidelines that are provided by organizations, such as the US FDA (39) and the CLSI (40). Hundreds to thousands of clinical samples must be analyzed reliably for a small number of targets that have been proven to be clinically useful.

Conversely, the purpose of Tier 2 assays is to narrow the range of potential targets that have clinical value by observing quantitative changes across various targets that have been identified in previous studies of tens or hundreds of clinical samples. To accomplish this, Tier 2 assays require high selectivity and repeatability, moderate sensitivity and reproducibility, and multiple analytical verification steps to establish confident and precise assays. The 5 experiments that are described in our study provide such an analytical validation of Tier 2 assays for preclinical applications (20).

The 34 protein markers used to develop MRM-MS assays are relevant to a variety of diseases (Table 1-5). Eleven of them (37.9%) were the components of the complement cascade, a part of the immune system. In Systemic Lupus Erythematosus (SLE), an autoimmune disease, serum

levels of C1q, C2 or C3 can be lower than normal (41-44). The deficiency of components of the Membrane Attack Complex (MAC): C5, C6, C8 and C9 are potentially associated with continuous bacterial infections (45). CFB, CFH, or SERPING1, which were complement regulatory proteins, were related to diseases such as Partial lipodystrophy, Nephritis, Atypical Hemolytic-uremic Syndromes (HUS), or Hereditary Angioedema (HAE) (46-48). Nine proteins (F2, F9, F10, F11, PLG, PROC, PROS1, SERPINC1, and SERPINF2; 31.0%) were components of the coagulation cascade and each of them were deficient in different diseases like Hemophilia, or disorders related to Thrombosis or Fibrinolysis (49-55). Another 9 proteins (31.0%) were mainly associated with Cardio-Vascular Diseases (CVD; APOB, CRP) (55-58), Type-II diabetes (APOB, SHBG) (59-61), Renal diseases (B2M, CST3) (62-64), or another several diseases (65-71). AMBP (72,73), APOA2 (74,75), APOE (76,77), APOH (78,79), and GSN (80,81) have also been studied as biomarkers of diseases, such as autoimmune disease, CVD, and organ dysfunction syndrome, despite their exclusion from the FDA lists. Quantifying the serum levels of the aforementioned markers in patients may serve a complementary role in aiding diagnosis. The disease relevance of the serum markers supports our finding that MRM-MS assays are a reproducible and transferrable analytical platform and also has pre-clinical potential.

Among 112 assays that represented 34 proteins, only 26 assays, representing 17 proteins, passed all 5 experiments (Table 1-12). Although 76 assays (67.9%) that quantified all 34 proteins were qualified by the first 2 experiments (Response curves and Repeatability), 50 of 76 assays (65.8%) and 17 proteins failed to pass all of the experiments, primarily due to Selectivity and Stability. However, the FLNVLSPR peptide (derived from IGFBP3), which had the lowest low QC concentration (LLOQ*2) of the 34 targets, with a value of 0.28 fmol/μL (9.01 ng/mL), was reliably quantified using the y3+ ion, as evidenced by its ability to pass all of the criteria. These results confirm that the protein assay is quantitatively reproducible within a sub-fmol/μL (or a single ng/mL) by MRM-MS.

Also, our validated assays were well-characterized and transferrable targe ted proteomics assays, based on the comparison with those that have been uploaded to the CPTAC assay portal (Figure 1-6). The analytical p latforms from UVGPC and our study performed similarly with regard to sensitivity and precision. But, our platform included MARS depletion and nano-flow LC, and we could claim following strengths in comparison wit h UVGPC. Twenty-four assays were validated in all 5 experiments in th e CPTAC assay guidelines, in contrast to the UVGPC assays, which we re validated only in the first 2 experiments (Response curve and Repea tability). Fourteen of 23 proteins passed all 5 experiments, and the rem aining 9 proteins passed at least 4 experiments. Our platform may be more transferrable to many laboratories that use nano-flow LC for MS a nalysis. The analytical performance was influenced significantly by the fl ow rate of the LC system; thus, having a similar flow rate and operatin g procedure is important in transferring established assays. Also, our pl atform could quantify low-abundance and moderate–high abundance pro teins simultaneously due to MARS depletion.

Our study has limitations. The quantification of proteins by MRM-MS assay is typically performed using 2 or more peptide assays, when possible, to improve the accuracy of the quantitation (82). However, only 1 peptide per protein was selected for analysis due to financial constraints. If we had used 2 or more peptides to validate them, the assays that represented more proteins could have been qualified in all of the experiments in the guidelines.

Another limitation is that homemade analytical columns were used for the method validation, which could have affected the reproducibility of the assays. A single column was used for all experiments in this study to minimize this concern. Although we used self-pack columns for our experiments, the conditions of the established assays must be optimized between lab settings with moderate effort if pre-packed columns with identical specifications are purchased from the same supplier that we used.

Also, serum proteins were quantified using nano-flow LC [0.1–1 μL/min], which typically improves analytical sensitivity at the expense of throughput and robustness. If we had used a capillary-flow [1–100 μL/min] or standard-flow [> 100 μL/min] LC system, both of which have higher throughput and are more robust, the results would have been more reproducible, and more targets would have met the performance specifications in the CPTAC guideline (83).

We have developed a quantitative MRM-MS assay that can quantify 29 FDA-approved and LDT target proteins and 5 potential disease biomarkers. Among them, 26 assays (14 proteins) were validated with regard to all 5 items and thus transferable to the research community using the MRM-MS assay platform. Throughout the experiments, we confirmed the LOD, LOQ, LLOQ, ULOQ, and linear range of target peptides using our MRM-MS assay. The precision was good (CV < 20%) for the 3 QCs (low, medium, and high), and our MRM-MS assay was repeatable in the overall linear range. The protein assays were also stable and minimally affected by temperature and freeze-thaw cycles. In addition, we verified the reproducibility of the overall process of the MRM-MS assay. The results of this study indicate that our MRM-MS assay has the advantages of being highly validated, transferrable, and able to quantify high- to low-abundance proteins and has potential for use as a preclinical verification method

Table 1-12. Protein assays qualified by 5 categories.

N.	Information of target protein and peptide						Experiment					Pass
	Gene symbol	Uniprot ID	Uniprot Accession	Protein Name	Peptide Sequence	Quantification ion[a]	Exp1	Exp2	Exp3	Exp4	Exp5	
1	AMBP	AMBP	P02760	Protein AMBP	ETLLQDFR	2.y6.1	P	NP	P	P	P	
						2.y4.1	P	P	P	P	P	Y
						2.y3.1	P	NP	P	P	P	
2	APOA2	APOA2	P02652	Apolipoprotein A2	SPELQAEAK	2.y6.1	P	P	P	NP	NP	
						2.y4.1	P	P	P	P	P	Y
						2.y8.2	P	P	NP	P	P	
						2.b2.1	P	P	NP	P	P	
3	APOB	APOB	P04114	Apolipoprotein B-100	ALVDTLK	2.y5.1	P	NP	NP	NP	P	
						2.y4.1	P	NP	NP	NP	P	
						2.b2.1	P	P	P	P	P	Y
4	APOE	APOE	P02649	Apolipoprotein E	LAVYQAGAR	2.y7.1	P	NP	NP	NP	P	
						2.y6.1	P	P	P	P	P	Y
						2.y5.1	P	P	NP	NP	P	
						2.y4.1	P	NP	P	NP	P	
5	APOH	APOH	P02749	Apolipoprotein H	ATVVYQGER	2.y7.1	P	NP	NP	NP	P	
						2.y6.1	P	P	NP	P	P	
						2.y5.1	P	P	P	P	P	Y
						2.b3.1	P	P	NP	NP	P	
6	B2M	B2MG	P61769	Beta-2-microglobulin	VNHVTLSQPK	3.y6.1	P	NP	NP	NP	P	
						3.y5.1	P	NP	NP	NP	P	
						3.b3.1	P	P	NP	NP	P	
7	BCHE	CHLE	P06276	Cholinesterase	IFFPGVSEFGK	2.y8.1	P	P	NP	NP	P	
						2.y7.1	P	NP	P	NP	P	
						2.b2.1	P	NP	NP	NP	P	
8	C1QA	C1QA	P02745	Complement C1q subcomponent subunit A	SLGFCDTTNK	2.y8.1	P	P	P	P	P	Y
						2.y6.1	P	P	NP	P	P	
						2.b2.1	P	P	P	P	P	Y
9	C2	CO2	P06681	Complement C2	LNINLK	2.y5.1	P	NP	NP	P	P	
						2.y4.1	P	NP	P	NP	P	
						2.b2.1	P	P	NP	P	P	
10	C3	CO3	P01024	Complement C3	DFDFVPPVVR	2.y8.1	P	P	NP	P	P	
						2.y6.1	P	P	P	P	P	Y
						2.y5.1	P	P	P	P	P	Y
						2.b2.1	P	P	NP	P	P	
						2.b3.1	P	P	NP	P	P	
11	C5	CO5	P01031	Complement C5	LQGTLPVEAR	2.y8.1	P	P	NP	NP	P	
						2.y6.1	P	P	NP	P	P	

						2.y5.1	P	P	NP	P	P	
						2.b2.1	P	P	NP	P	P	
12	C6	CO6	P13671	Complement component C6	ALQEYAAK	2.y6.1	P	NP	NP	NP	P	
						2.b2.1	P	P	NP	NP	P	
						2.b3.2	P	P	NP	NP	P	
13	C8A	CO8A	P07357	Complement component C8 alpha chain	LYYGDDEK	2.y6.1	P	NP	NP	NP	P	
						2.b2.1	P	P	P	NP	P	
						2.b4.2	P	P	NP	P	P	
14	C8B	CO8B	P07358	Complement component C8 beta chain	YEFILK	2.y2.1	P	P	P	NP	P	
						2.y4.2	P	P	NP	NP	P	
						2.b2.1	P	P	P	NP	P	
15	C9	CO9	P02748	Complement component C9	VVEESELAR	2.y7.1	P	P	NP	NP	P	
						2.y6.1	P	NP	NP	NP	P	
						2.b2.1	P	P	P	P	P	Y
16	CFB	CFAB	P00751	Complement factor B	EELLPAQDIK	2.y6.1	P	NP	P	P	P	
						2.b2.1	P	NP	NP	NP	P	
						2.b3.1	P	P	P	P	P	Y
17	CFH	CFAH	P08603	Complement factor H	VGEVLK	2.y5.1	P	P	NP	P	P	
						2.y4.1	P	NP	NP	NP	P	
						2.b3.1	P	P	NP	P	P	
18	CP	CERU	P00450	Ceruloplasmin	GAYPLSIEPIGVR	2.y10.1	P	P	P	P	P	Y
						2.y8.1	P	NP	NP	P	P	
						2.y6.1	P	P	P	P	P	Y
						2.y5.1	P	P	P	P	P	Y
19	CRP	CRP	P02741	C-reactive protein	ESDTSYVSLK	2.y6.1	P	P	NP	NP	P	
						2.y5.1	P	P	NP	NP	P	
						2.y3.1	P	P	NP	P	P	
20	CST3	CYTC	P01034	Cystatin-C	ALDFAVGEYNK	2.y6.1	P	P	NP	NP	P	
						2.b2.1	P	NP	NP	NP	P	
						2.b3.1	P	NP	NP	P	P	
21	F2	THRB	P00734	Prothrombin	ELLESYIDGR	2.y6.1	P	P	P	P	P	Y
						2.y5.1	P	P	NP	NP	P	
						2.b2.1	P	P	P	P	P	Y
22	F9	FA9	P00740	Coagulation factor IX	SALVLQYLR	2.y5.1	P	NP	P	NP	P	
						2.b2.1	P	NP	NP	NP	P	
						2.b3.1	P	P	P	NP	P	
23	F10	FA10	P00742	Coagulation factor X	TGIVSGFGR	2.y6.1	P	P	NP	NP	P	
						2.y5.1	P	NP	P	NP	P	
						2.y4.1	P	P	P	NP	P	
						2.b3.1	P	P	P	NP	P	
24	F11	FA11	P03951	Coagulation factor XI	VVSGFSLK	2.y6.1	P	P	P	NP	P	
						2.b2.1	P	NP	P	P	P	
						2.b2.1	P	NP	P	P	P	
25	GSN	GELS	P06396	Gelsolin	AGALNSNDAFVLK	2.y9.1	P	P	NP	P	P	
						2.y8.1	P	P	P	P	P	Y

						2.b3.1	P	P	P	P	P	Y
26	IGFBP3	IBP3	P17936	Insulin-like growth factor-binding protein 3	FLNVLSPR	2.y6.1	P	NP	P	P	P	
						2.y4.1	P	NP	NP	P	P	
						2.y3.1	P	P	P	P	P	Y
27	PLG	PLMN	P00747	Plasminogen	LFLEPTR	2.y6.1	P	NP	P	NP	P	
						2.y5.1	P	P	P	P	P	Y
						2.y4.1	P	P	P	NP	P	
						2.y3.1	P	P	P	NP	P	
						2.b2.1	P	P	P	P	P	Y
28	PROC	PROC	P04070	Vitamin K-dependent protein C	TFVLNFIK	2.y6.1	P	NP	P	NP	P	
						2.y5.1	P	NP	P	NP	P	
						2.b2.1	P	P	P	NP	P	
29	PROS1	PROS	P07225	Vitamin K-dependent protein S	YLVCLR	2.y5.1	P	NP	NP	NP	P	
						2.y4.1	P	P	P	P	P	Y
						2.y3.1	P	P	NP	P	P	
						2.b2.1	P	P	P	P	P	Y
30	SERPINC1	ANT3	P01008	Antithrombin-III	FDTISEK	2.y6.1	P	P	P	P	P	Y
						2.y5.1	P	P	P	P	P	Y
						2.b2.1	P	P	NP	P	P	
31	SERPINF2	A2AP	P08697	Alpha-2-antiplasmin	LGNQEPGGQTALK	2.y8.1	P	P	NP	P	P	
						2.y7.1	P	NP	NP	NP	P	
						2.b4.1	P	NP	NP	NP	P	
32	SERPING1	IC1	P05155	Plasma protease C1 inhibitor	LLDSLPSDTR	2.y7.1	P	NP	NP	NP	P	
						2.y5.1	P	P	P	P	P	Y
						2.b2.1	P	P	P	NP	P	
33	SHBG	SHBG	P04278	Sex hormone-binding globulin	IALGGLLFPASNLR	2.y8.1	P	P	NP	P	P	
						2.y7.1	P	P	NP	P	P	
						2.b5.1	P	P	NP	P	NP	
34	TTR	TTHY	P02766	Transthyretin	VLDAVR	2.y5.1	P	P	NP	P	NP	
						2.y4.1	P	P	NP	P	P	
						2.y3.1	P	NP	P	NP	P	

Transition marked with "P" were content with criteria of the items, "NP" were not content with, and "Y" were qualified in all the experiments.
Exp1 to Exp5 indicate the 5 experimental categories in the guidelines.
a The column title indicates the information of quantification ion, followed by the precursor ion charge, production ion type, and product ion charge.

Chapter 2

A Clinically Applicable 24-Protein Model for Classifying Risk Subgroups in Pancreatic Ductal Adenocarcinomas using Multiple Reaction Monitoring-Mass Spectrometry

1.1 Introduction

Several studies have recently reported that classifying PDAC characteristics can stratify patient prognosis and improve therapeutic responses. Transcriptomic subtypes are the most frequently investigated with regard to classifying PDACs. Notably, Collisson et al. (2011) defined a classification system, comprising 3 subtypes: classical, quasi-mesenchymal, and exocrine-like(84). In another study, Moffitt et al. (2015) proposed an alternative stratification method that categorizes PDACs into tumor cell (classical and basal-like) and stromal subtypes (normal and activated)(85). In 2016, Bailey et al. and the International Cancer Genome Consortium (ICGC) identified 4 subtypes: pancreatic progenitor, squamous, ADEX, and immunogenic(6). The Cancer Genome Atlas (TCGA) consortium (2017) reproduced these subtypes and compiled several subtypes (exocrine-like or ADEX and immunogenic) with low cellularity(86). Puleo et al. (2018) proposed another system, composed of pure classical, immune classical, desmoplastic, stroma activated, and pure basal-like subtypes, using a larger number of formalin-fixed paraffin-embedded (FFPE) specimens from multiple cohorts(87).

Although not as extensively as transcriptomic studies, PDAC subtypes have also been examined by proteomics and metabolomics(88). Daemen et al. (2015) discovered 3 metabolic subtypes (slow proliferating, glycolytic, and lipogenic) using 38 PDAC cell lines(89). Recently, Law et al. (2019) identified 4 proteomic microenvironment subtypes (metabolic, progenitor-like, proliferative, and inflammatory), based on their therapeutic response(90). Although many stratification systems have been proposed, the consensus leans toward 3 distinct subtypes—basal-like, classical, and exocrine-like—based on epithelial tumor content. The basal-like and classical subtypes have also been identified in other solid tumors, such as breast cancer and colorectal cancer(91).

Over the past decades, various hallmarks of cancer have been verified across multiple studies, including the avoidance of immune reactions, the activation of invasion and metastasis, and the factors associated with these mechanisms, and may represent credible biomarker candidates(92). In addition, various prognostic protein biomarkers have been discovered in tissues by immunohistochemistry (IHC) that have been correlated with the outcomes of resected PDAC and now play instrumental roles in the management of PDAC(93). Moreover, some of these proteins have been investigated as novel therapeutic targets during ongoing clinical trials(94). Molecular subtypes developed on the basis of these biomarkers and proteomics would be useful for predicting and improving the survival outcomes of PDAC patients.

In the present study, we identified risk subgroups and developed a classification model, based on 24 carefully selected proteins, to address the current limitations. The classification model is based on multiple-reaction monitoring-mass spectrometry (MRM-MS), a highly selective and reproducible technique for the simultaneous quantification of hundreds of proteins(31), to assess the levels of protein risk factors in PDAC tissue specimens. The model was validated using transcriptomic datasets from external cohorts, which implies the transferable potential to other methodologies. These proteomics-based risk subgroups showed association with transcriptomic subtypes, but displayed significantly differential survival outcome both in classical and basal-like tumors. Moreover, in the high purity dataset from an external cohort, the three risk subgroups, “Stable”, “Activated”, and “ECM-Remodeling”, show upregulation of different signaling pathways that are targeted in ongoing clinical trials of anticancer therapies. The implementation of such a model could be used to predict the prognosis of patients and facilitate the identification of patients who are likely to benefit from various therapeutic regimens.

1.2 Materials and Methods

1.2.1 Patient Cohort and Data Collection

A total of 557 patients, who were diagnosed with pancreatic cancer and underwent surgery at Seoul National University Hospital (SNUH) between October 2009 and February 2018, were assessed for eligibility, and their data were reviewed. The inclusion criteria were as follows: (i) patients with pancreatic ductal adenocarcinoma, based on their final pathology reports; (ii) patients whose tissue was available via a biobank; and (iii) patients who provided written informed consent for the study. Among them, 300 patients, who had other malignancies; were histologically determined to have non-PDAC, such as intraductal papillary mucinous neoplasm (IPMN) and pancreatic neuroendocrine tumor (PNET); had no postoperatively retrieved tissue; or declined to enroll in the study, were excluded. Ultimately, 257 patients were included. All patients had sufficient clinical, radiological, and pathological information. This study was conducted in accordance with the guidance principles of the Declaration of Helsinki. This study protocols were approved by the internal review board of the SNUH, in Seoul, Korea (H-1705-031-852), and written informed consent was obtained from all participants who contributed biospecimens.

Clinicopathologic data were prospectively collected, using a web-based database, which included clinicopathological variables, such as gender, age at diagnosis, tumor site, preoperative assessment of clinical disease stage, type of operative procedure, tumor stage according to the American Joint Committee on Cancer, tumor, node, metastasis (TNM) classification, histologic grade, presence of lymphovascular invasion, perineural invasion, and relevant outcome parameters.

1.2.2 Tissue Protein Extraction and Sample Preparation

A total of 257 fresh-frozen, whole-tissue samples collected from the cohort that satisfied the inclusion criteria were selected for protein extraction. The samples were prepared in block-randomized batches, with respect to gender, age, and prognosis (good vs. poor; median recurrence time, 12 months). The tissue samples were weighed before lysis. An average wet weight of 23.2 mg (95% CI, 15.4 to 30.9 mg) of each tumor sample was lysed in 300 μL 5% sodium deoxycholate (SDC) buffer, except for those tissue specimens that weighed less than 2 mg, which were lysed in 200 μL 5% SDC buffer. Samples were dissolved using Bioruptor Pico (Diagenode Inc. Denville, NJ, USA), utilizing a bath-based rotor. The total extracted protein concentrations were determined using the Pierce™ BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA), and tissue lysates with concentrations below 1.67 μg/μL were excluded from trypsin digestion, due to projected deficiencies in peptide yields. The extracted proteins from 244 tissue samples were digested into peptides and purified using OASIS HLB (1 ml, 30 mg, Waters, Milford, MA, USA) cartridges. A volume of 5 μL crude stable isotope-labeled standard (SIS) peptide mixture (JPT Peptide Technology, Acton, MA, USA) was spiked into each 45 μL peptide sample, for MRM-MS analysis (See details in the Supplementary Information).

1.2.3 Protein Quantitation using MRM-MS

Each individual sample was analyzed in triplicate, and each replicate was distributed into block-randomized batches, based on gender, age, and prognosis (good vs. poor; median recurrence time, 12 months). Protein levels were calculated as the average peak area ratio of each triplicate, multiplied by the SIS standard quantity in each sample. Targets with interference signals were excluded, according to the AuDIT algorithm(34). The analytical quality of each protein was assessed based on its response curve and repeatability, according to the Clinical Proteomic Tumor Analysis

Consortium (CPTAC) assay guidance document(95) and the bioanalytical method validation guidance for industry from U.S. Food and Drug Administration (https://www.fda.gov/media/70858/download). Variations between analytical batches were examined using the triplicate analyses. Additionally, 19 samples were excluded from the study due to poor MS spectra data. See the Supplementary Information for details regarding MRM-MS data processing, quantitation, and quality verification.

1.2.4 Proteomic Data Processing and Analysis

The levels of 115 qualified proteins were determined in 225 tumor samples, using MRM-MS. Univariate and multivariate survival analyses were performed in the survival package (Version 3.1-8). Differences of protein levels by standard prognostic factors were determined using ANOVA and Student's t-tests, in IBM SPSS Statistics (Version 23). The χ^2 test was used to verify associations between risk subgroups and standard prognostic factors or molecular subtypes identified by other studies. The proteomic risk subgroups were defined using hierarchical clustering analysis of principal components in the FactoMineR package (Version 2.2 in R) and factorextra package (Version 1.0.7 in R). Functional annotations of proteins enriched in each risk subgroup were identified using DAVID Bioinformatics Resources (Version 6.8). Principal component analysis (PCA) corresponding factor analysis was performed to compare the original transcriptomic subtypes, using protein levels in IBM SPSS Statistics (Version 23). The risk subgroups based on the tumor samples were obtained using a random forest (RF) model, developed by 10-round, 5-fold, cross-validation in the randomForest package (Version 4.6-14) in R. The MRM-MS raw files for all 225 tumors were deposited to PeptideAtlas (http://www.peptideatlas.org), along with the quantitation target lists (Dataset identifier: PASS01611; Password: SJ4748du). Details regarding the proteomic data processing and analysis protocols are available in the Supplementary Information, with external validation using published datasets.

1.3 Results

1.3.1 Characteristics of the Patient Population

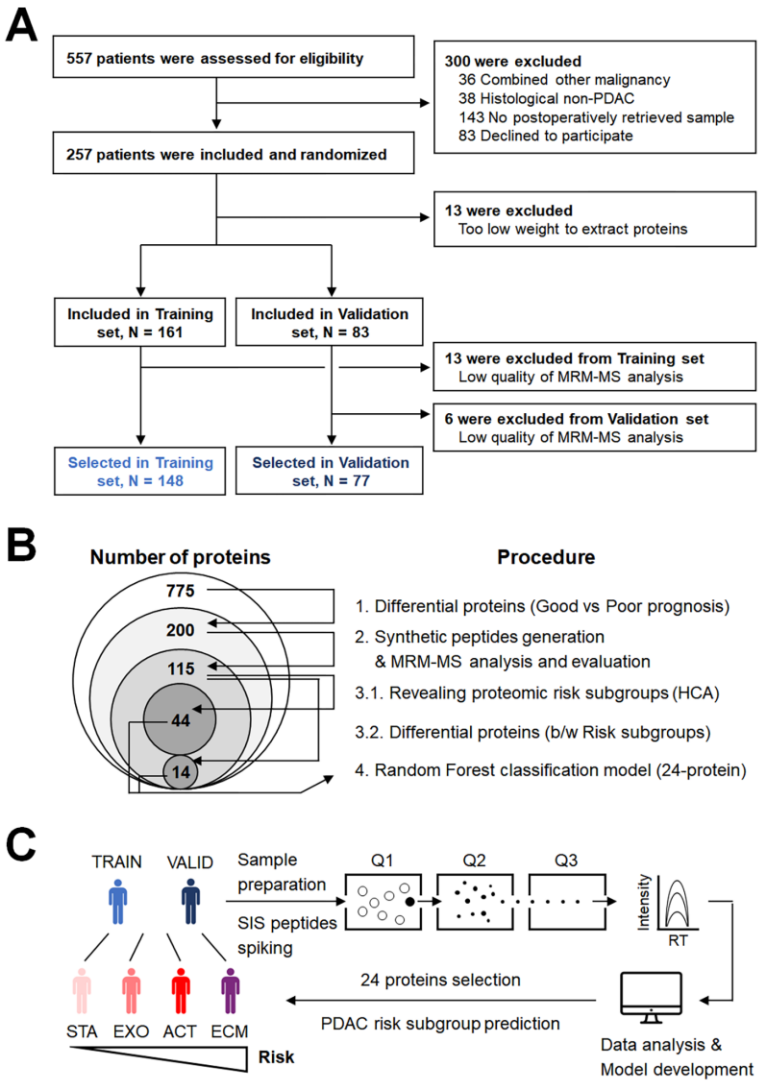
Study sets were selected using the following steps (Figure 2-1A). (i) First, the total population of 557 prospectively collected patients was assessed for eligibility, and 257 patients were included in the study. Tumor purity was not considered, to facilitate the identification of purity-independent risk subgroups among PDAC patients, based only on the protein assay platform. (ii) Prior to analysis, 13 tumor samples were removed due to insufficient protein amounts. (iii) The 244 tumor samples were distributed into training and validation sets, each with similar characteristics, and the tissue protein levels were independently estimated. (iv) The data analysis was limited to 148 samples in the training set and 77 samples in the validation set, after the exclusion of 13 and 6 outliers, respectively, following the MRM-MS analysis quality control. The median follow-up time was 27.0 months (95% CI, 21.8-32.1) in the training set and 35.0 months (95% CI, 24.5-45.5) in the validation set, and 77 (52.0%) and 43 (55.8%) death events were observed during the overall follow-up period, respectively. The demographic and clinical characteristics of the 225 patients included in both the training and validation sets are summarized in Table 2-1, and the detailed clinicopathological characteristics of the cohort matched the general population of resectable PDAC patients (Table 2-2).

1.3.2 Overall Workflow of Target Protein Selection

Highly quantitative MRM-MS assays for protein biomarkers were developed, as shown in Figure 2-1B (See details in Figure 2-2). To identify those proteins whose expression patterns correlated with prognosis, 775 detectable proteins were selected from the following sources: (i) a compiled list of markers known to be associated with tumor development or progression, retrieved from the Cancer Genome Interpreter(96) and the

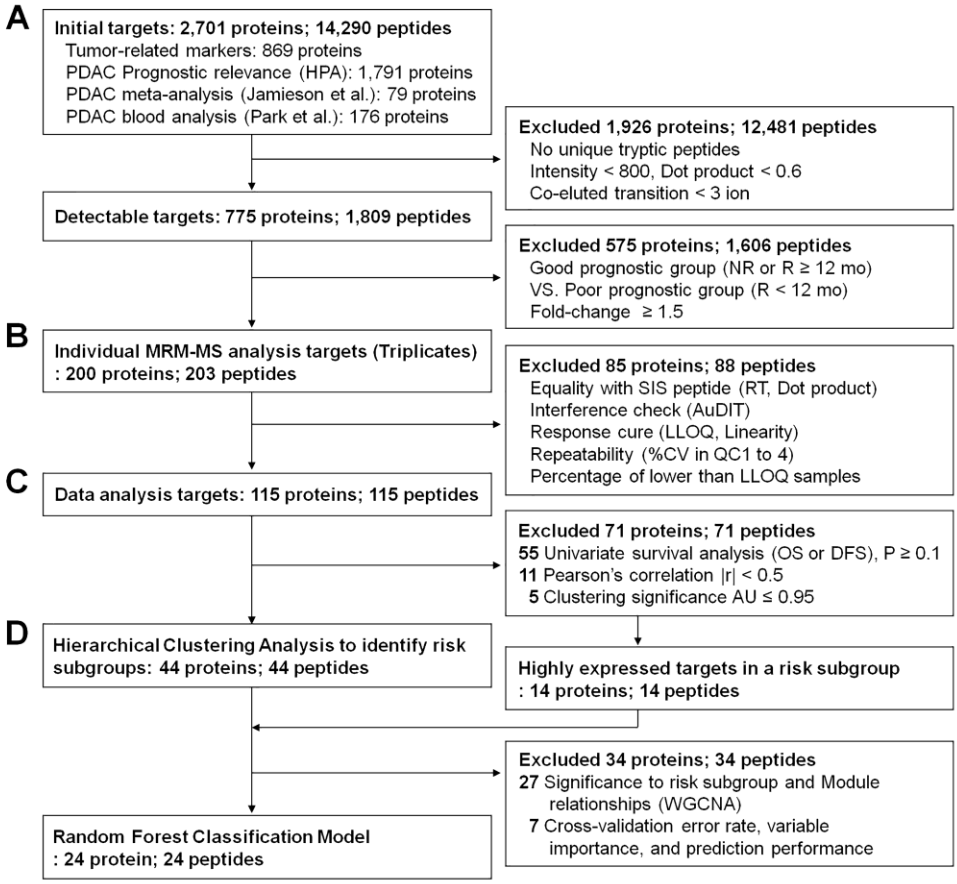
Early Detection Research Network (https://edrn.nci.nih.gov/biomarkers); (ii) targets related to tumor prognosis, from the Human Protein Atlas(97); (iii) prognostic biomarkers associated with PDAC(93); and (iv) a target list of marker candidates, derived from our previous study(5) (Table 2-3). Among these sources, 200 differentially expressed proteins (DEPs) between the good and poor prognostic pooled sample groups (median recurrence time, 12 months) were selected (see details in Table 2-4). A total of 115 of the 200 proteins met the criteria for quantification, based on their response curves and repeatability tests, according to the authorized guidelines, and were verified in individual samples (Table 2-5). The results of the response curve and repeatability analyses are detailed in Figures 2-3 and 2-4. Of the 115 verified proteins, 44 proteins were included in the hierarchical clustering analysis for the identification of proteomic risk subgroups. In addition, 14 DEPs between risk subgroups were selected for the development of the classification model. Subsequently, 24 of 58 (the 44 verified proteins and the 14 additional DEPs) proteins were included in the final Random Forest (RF) model, for the prediction of risk subgroups (Figure 2-1C).

Figure 2-1. Cohort and target protein selection.



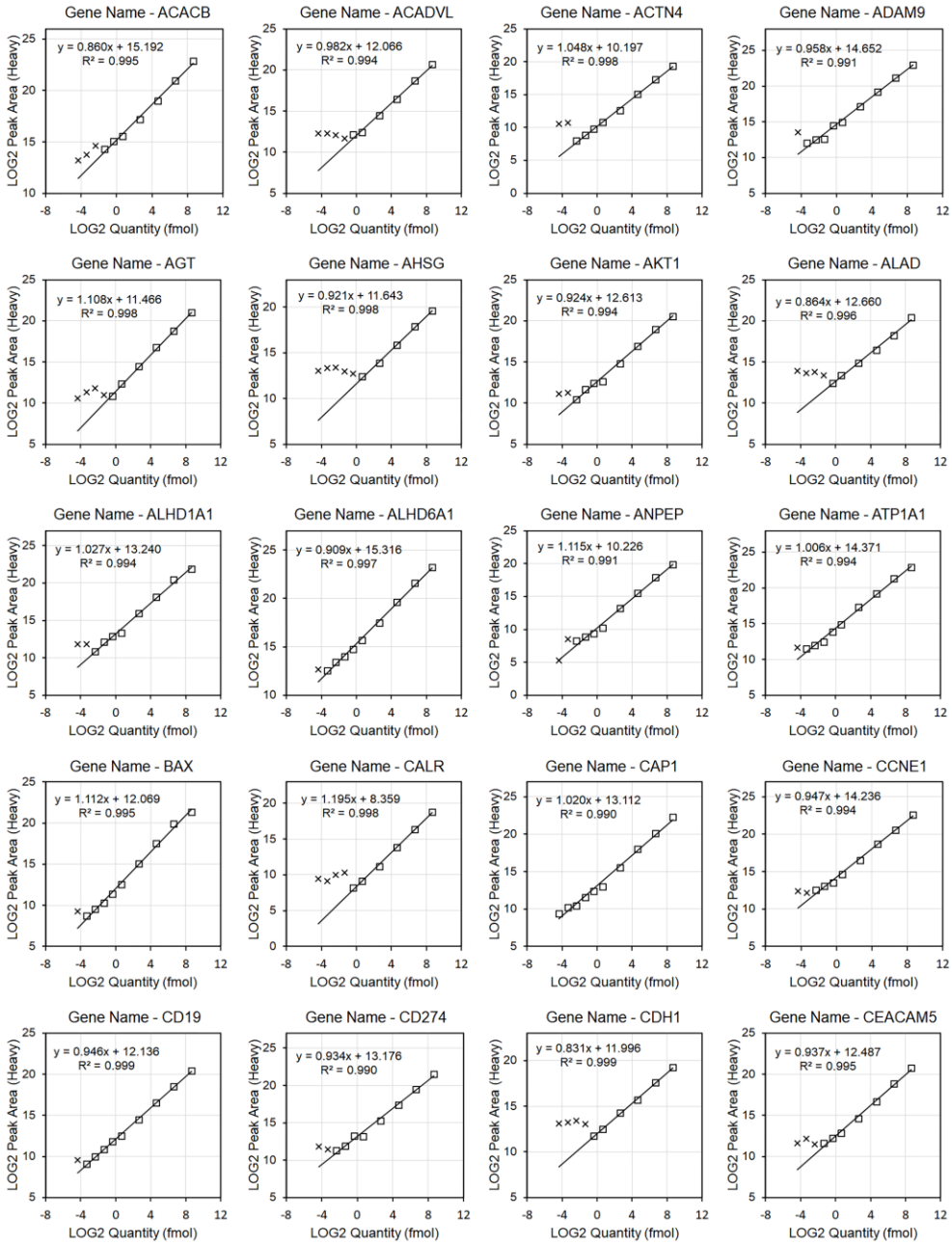
(A) Sample inclusion diagram. The 148-sample training set and the 77-sample validation set were finally selected from among the 557 patients in the cohort. (B) Target selection workflow for prognostic and quantitative analysis. A total of 115 differentially expressed proteins between good and poor prognostic groups were verified by quantitative quality control. (C) Schematic workflow for developing risk subgroup classification model. PDAC, pancreatic ductal adenocarcinoma; NR, no recurrence; R, recurrence; mo, months; SIS, stable isotope-labeled standard; RT, retention time; AuDIT, automated detection of inaccurate and imprecise transitions; LLOQ, lower limit of quantitation; CV, coefficient of variance; QC, quality control.

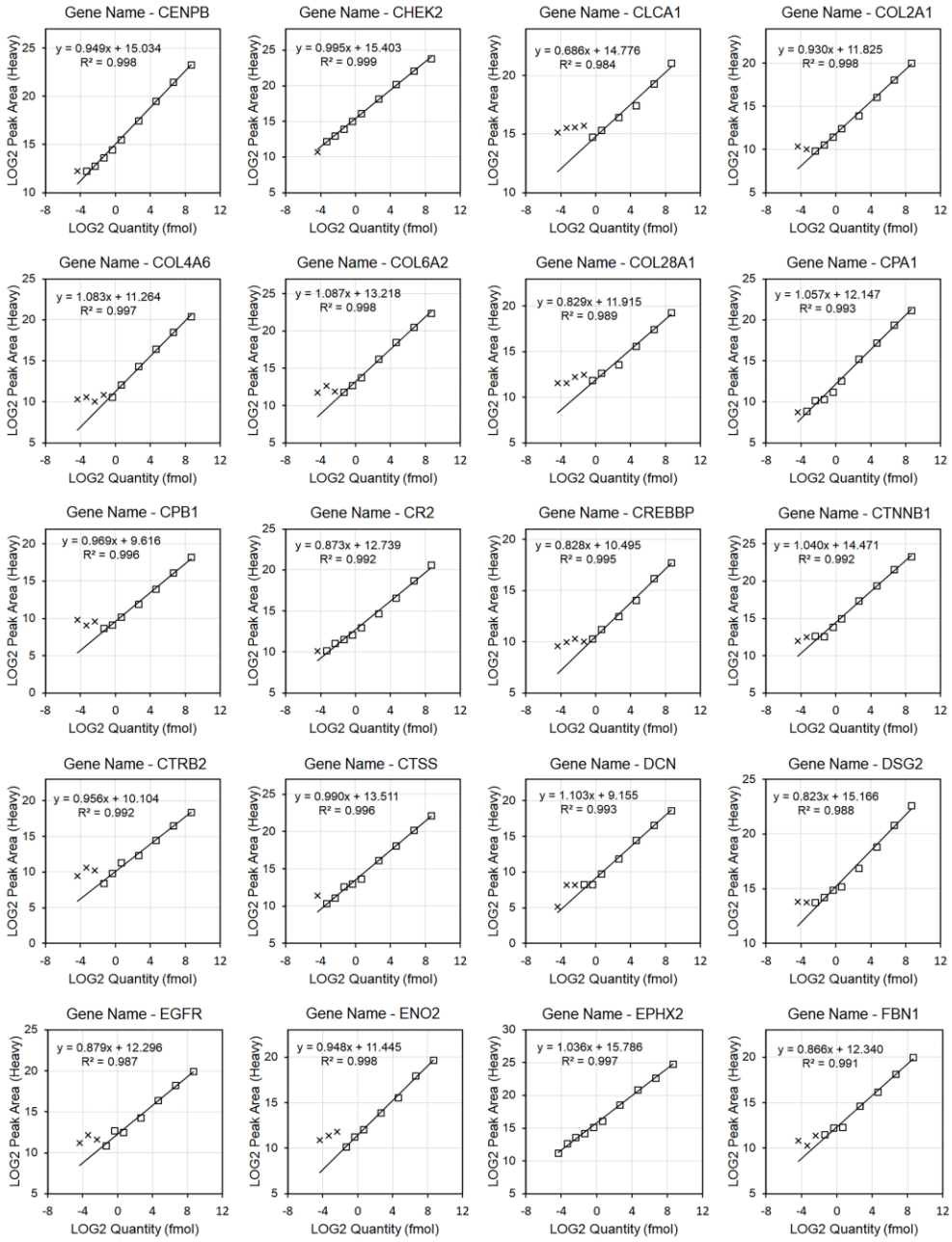
Figure 2-2. Target protein selection workflow.



A. A total of 2,701 proteins were compiled from the four resources, and 775 proteins that were detectable by the MRM-MS analysis were selected. To reduce the number of analytical targets, pooled tissue lysates in groups with similar prognosis (median 12-month disease-free survival) were analyzed. A total of 200 differentially expressed proteins were selected to synthesize stable isotope-labeled standard (SIS) peptides for quantitation in individual samples. B. Through the quantitation quality check, based on the SIS peptide interference analysis, Response curve, Repeatability, and the number of samples above the lower limit of quantitation (LLOQ), 115 proteins were selected for data analysis. C. To define proteomic risk subgroups with protein clusters, 55 proteins with ward test p-values above 0.1 during the univariate survival analysis were filtered out, and 44 of 60 proteins were included in the final clustering analysis. D. To improve the performance of the random forest classification model, 14 proteins enriched in a risk subgroup were added, despite not being included in the hierarchical clustering analysis. Finally, 24 proteins were included in the classification model, through feature selection using the WGCNA and randomForest packages.

Figure 2-3. Quality Control of 115 proteins for response curve generation.

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R² = 0.999
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y = 0.686x + 14.776
R² = 0.984
Gene Name - COL2A1
y = 0.930x + 11.825
R² = 0.998
Gene Name - COL4A6
y = 1.083x + 11.264
R² = 0.997
Gene Name - COL6A2
y = 1.087x + 13.218
R² = 0.998
Gene Name - COL28A1
y = 0.829x + 11.915
R² = 0.989
Gene Name - CPA1
y = 1.057x + 12.147
R² = 0.993
Gene Name - CPB1
y = 0.969x + 9.616
R² = 0.996
Gene Name - CR2
y = 0.873x + 12.739
R² = 0.992
Gene Name - CREBBP
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R² = 0.995
Gene Name - CTNNB1
y = 1.040x + 14.471
R² = 0.992
Gene Name - CTRB2
y = 0.956x + 10.104
R² = 0.992
Gene Name - CTSS
y = 0.990x + 13.511
R² = 0.996
Gene Name - DCN
y = 1.103x + 9.155
R² = 0.993
Gene Name - DSG2
y = 0.823x + 15.166
R² = 0.988
Gene Name - EGFR
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R² = 0.987
Gene Name - ENO2
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R² = 0.998
Gene Name - EPHX2
y = 1.036x + 15.786
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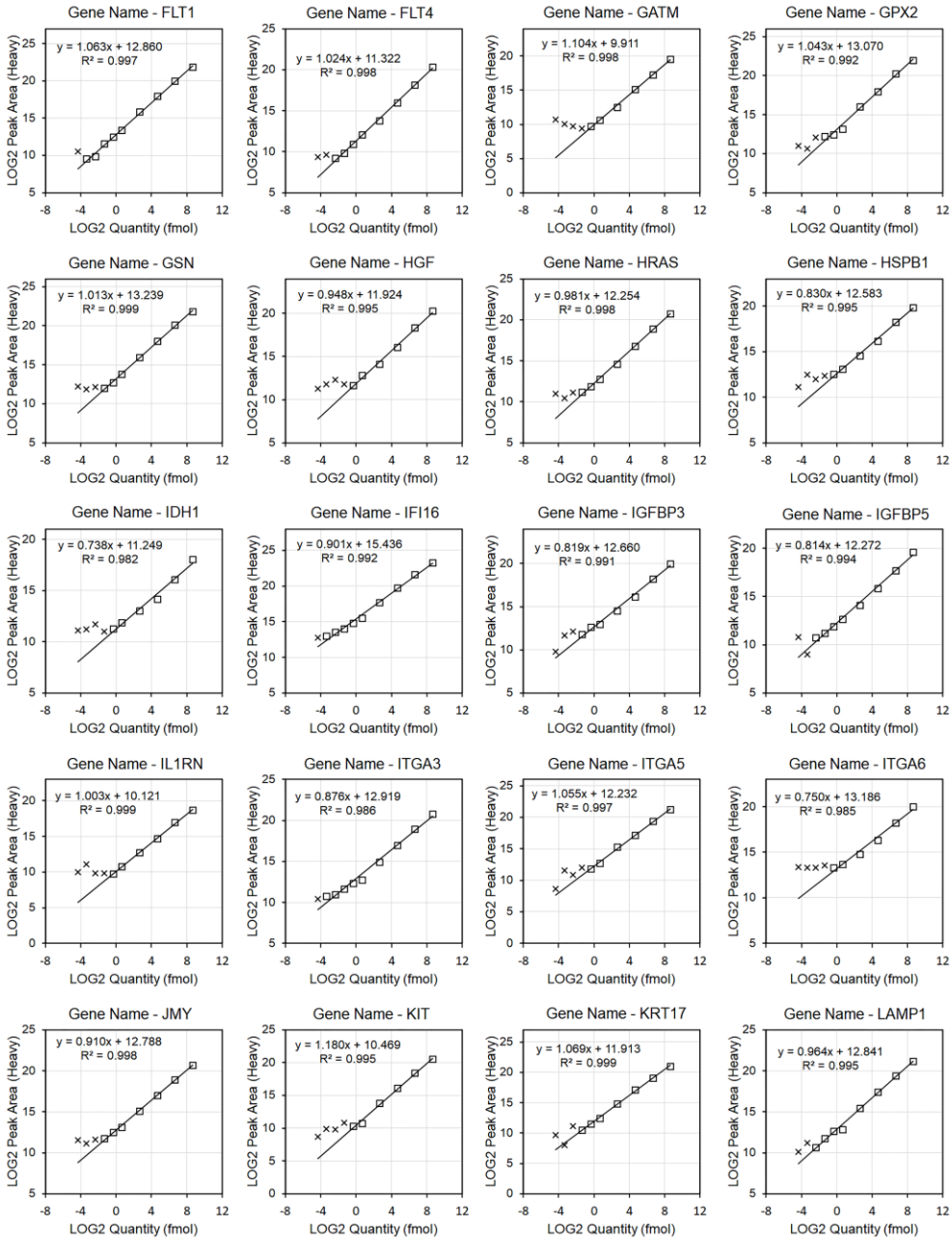
LOG2 Quantity (fmol)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y = 1.043x + 13.070
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Gene Name - GSN
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Gene Name - IDH1
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Gene Name - IFI16
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Gene Name - IL1RN
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R² = 0.999
Gene Name - ITGA3
y = 0.876x + 12.919
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Gene Name - ITGA5
y = 1.055x + 12.232
R² = 0.997
Gene Name - ITGA6
y = 0.750x + 13.186
R² = 0.985
Gene Name - JMY
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R² = 0.998
Gene Name - KIT
y = 1.180x + 10.469
R² = 0.995
Gene Name - KRT17
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Gene Name - LAMP1
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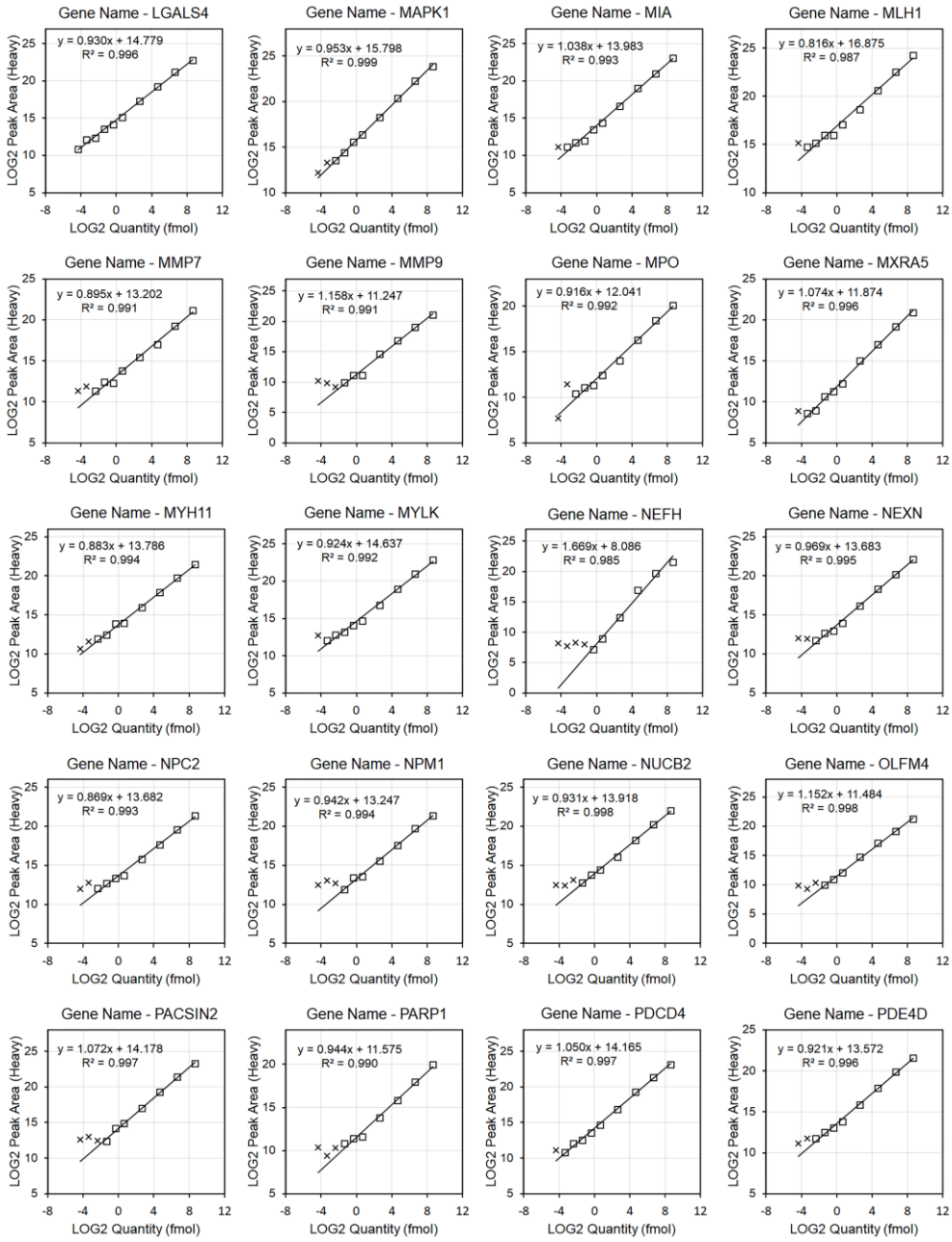
LOG2 Quantity (fmol)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y = 0.816x + 16.875
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Gene Name - MMP7
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Gene Name - MMP9
y = 1.158x + 11.247
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Gene Name - MPO
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R² = 0.992
Gene Name - MXRA5
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Gene Name - MYH11
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Gene Name - NEXN
y = 0.969x + 13.683
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Gene Name - PACSIN2
y = 1.072x + 14.178
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Gene Name - PARP1
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Gene Name - PDCD4
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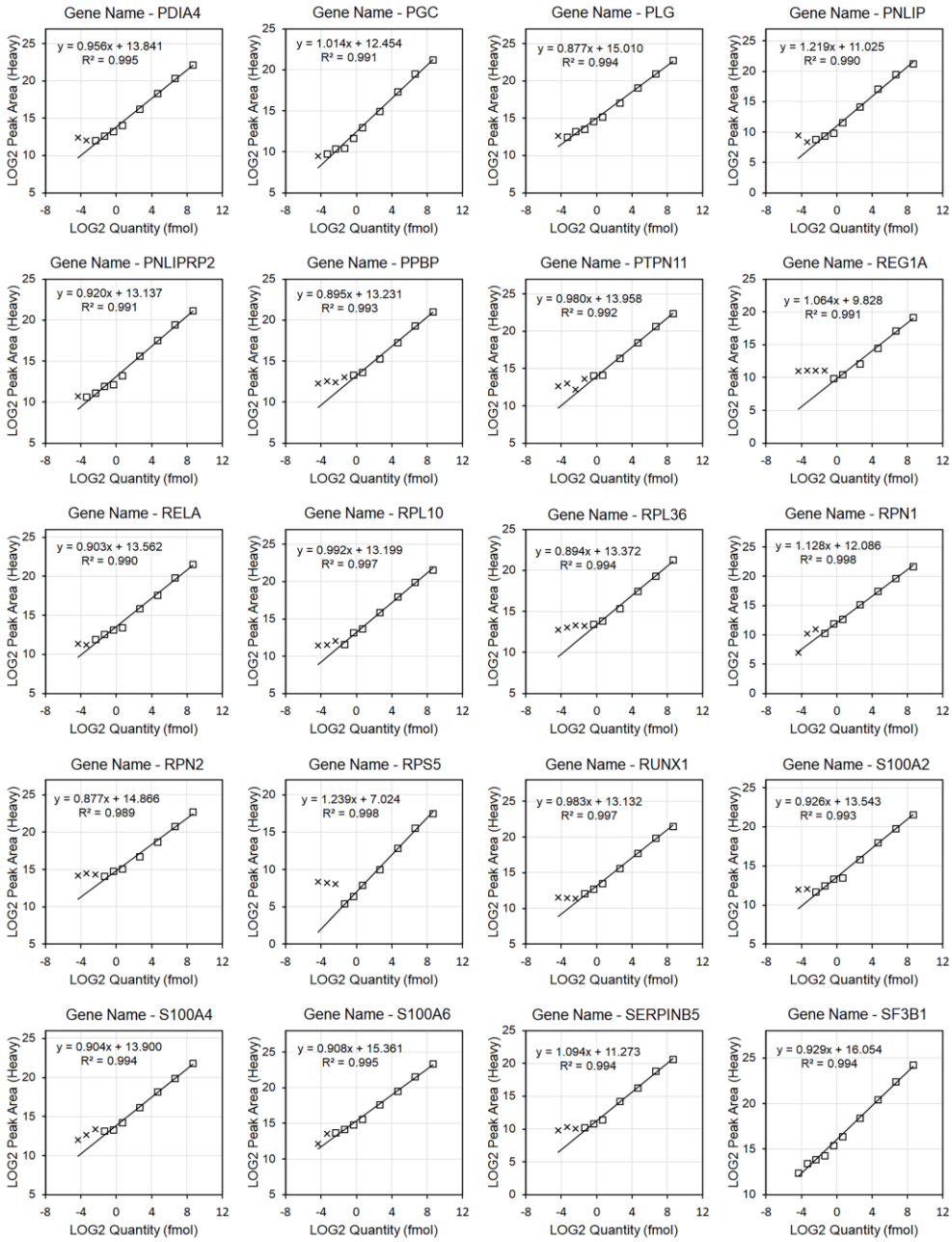
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R² = 0.990
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Gene Name - PTPN11
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R² = 0.992
Gene Name - REG1A
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y = 0.903x + 13.562
R² = 0.990
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R² = 0.997
Gene Name - RPL36
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R² = 0.994
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R² = 0.998
Gene Name - RPN2
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Gene Name - RUNX1
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Gene Name - S100A2
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Gene Name - S100A4
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Gene Name - S100A6
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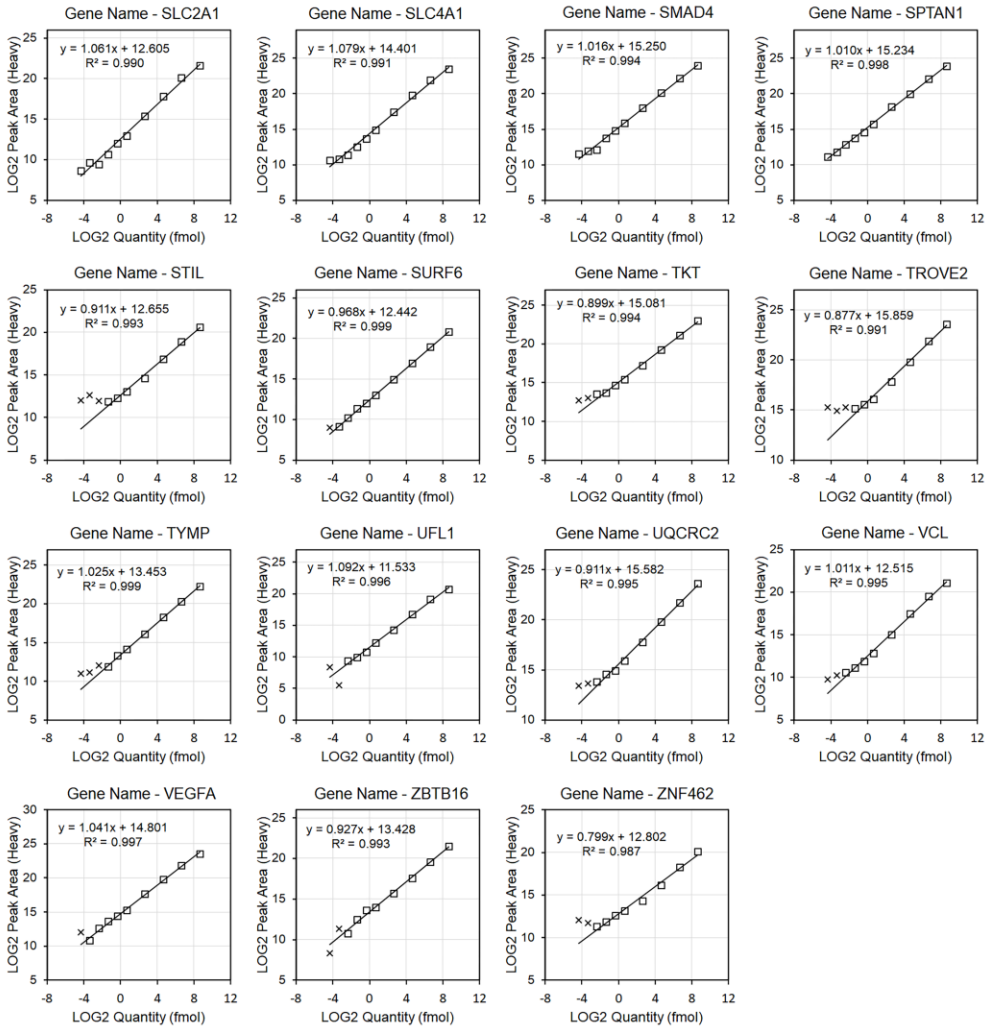
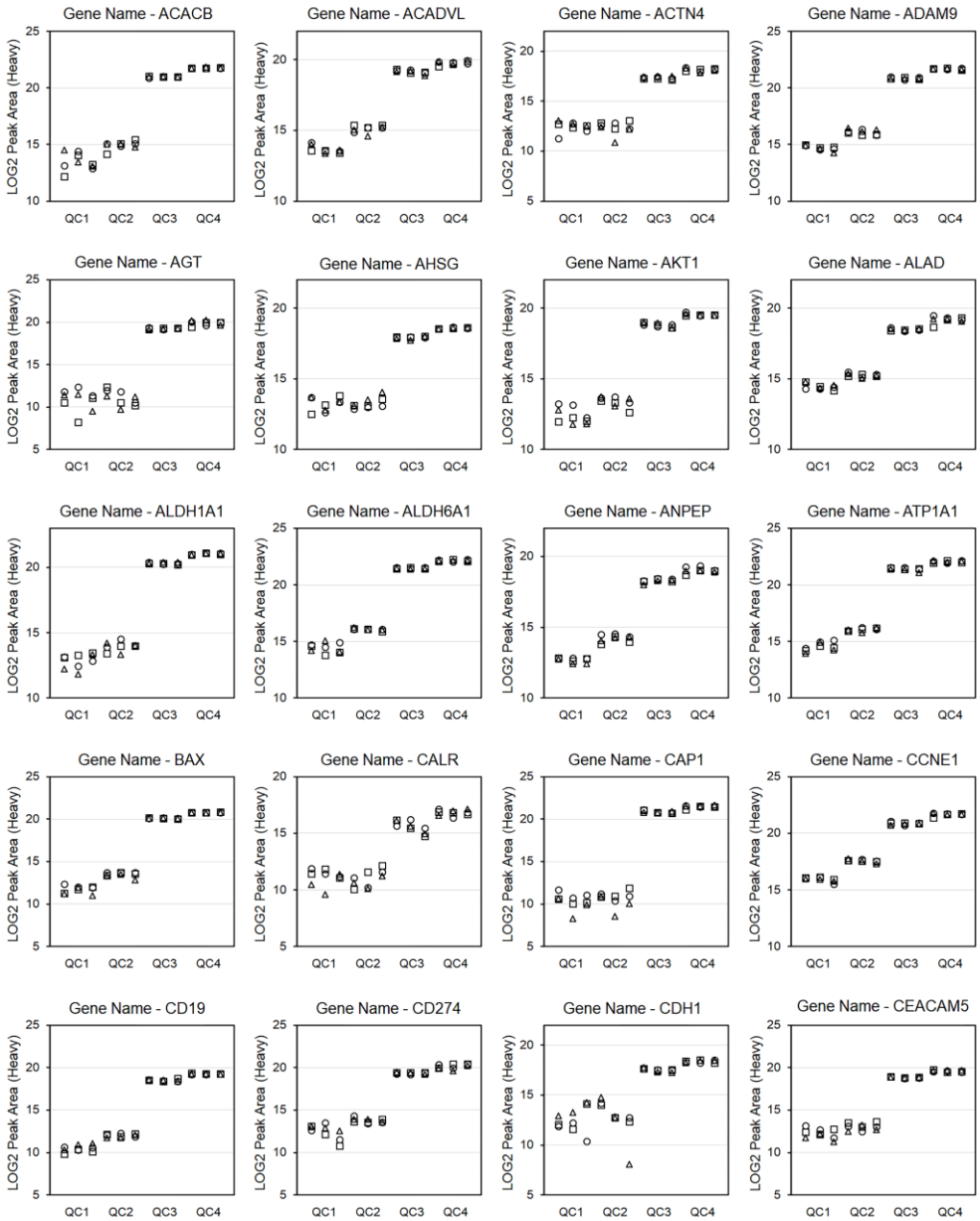
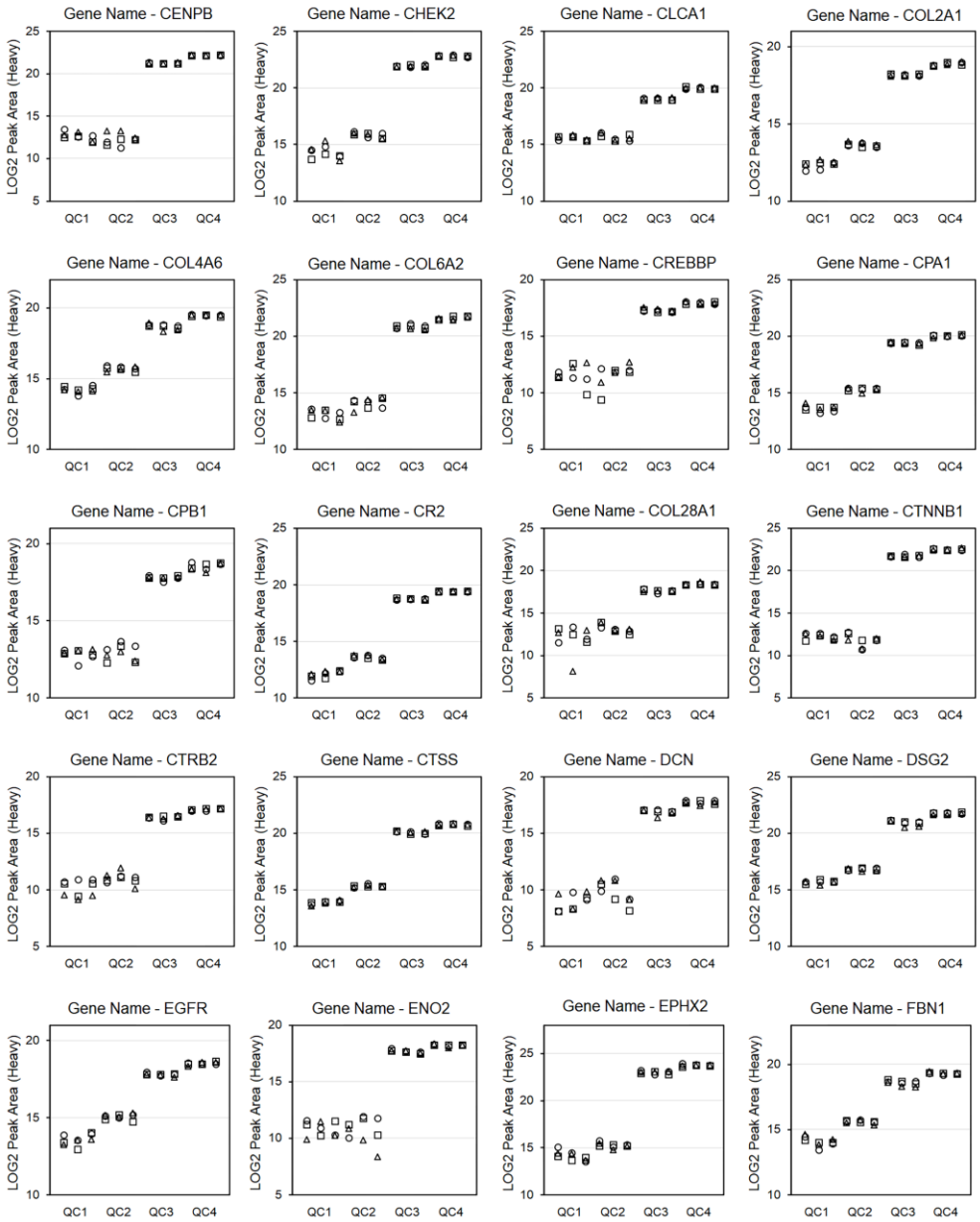
LOG2 Quantity (fmol)



The response curves for each protein generated with continuous quantity of standard (femto-mole). Log2-transformed values were used to obtain a better view in the lower quantity of curves. Squared dots represent the points used to fit linear regression curves, and “x” marks show the points that do not meet the acceptance criteria for linearity (R squared > 0.98) or precision in the triplicate analysis (Coefficient of variance < 20%).

Figure 2-4. Quality Control of 115 proteins for repeatability analysis.

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Gene Name - CPB1
Gene Name - CR2
Gene Name - COL28A1
Gene Name - CTNNB1
Gene Name - CTRB2
Gene Name - CTSS
Gene Name - DCN
Gene Name - DSG2
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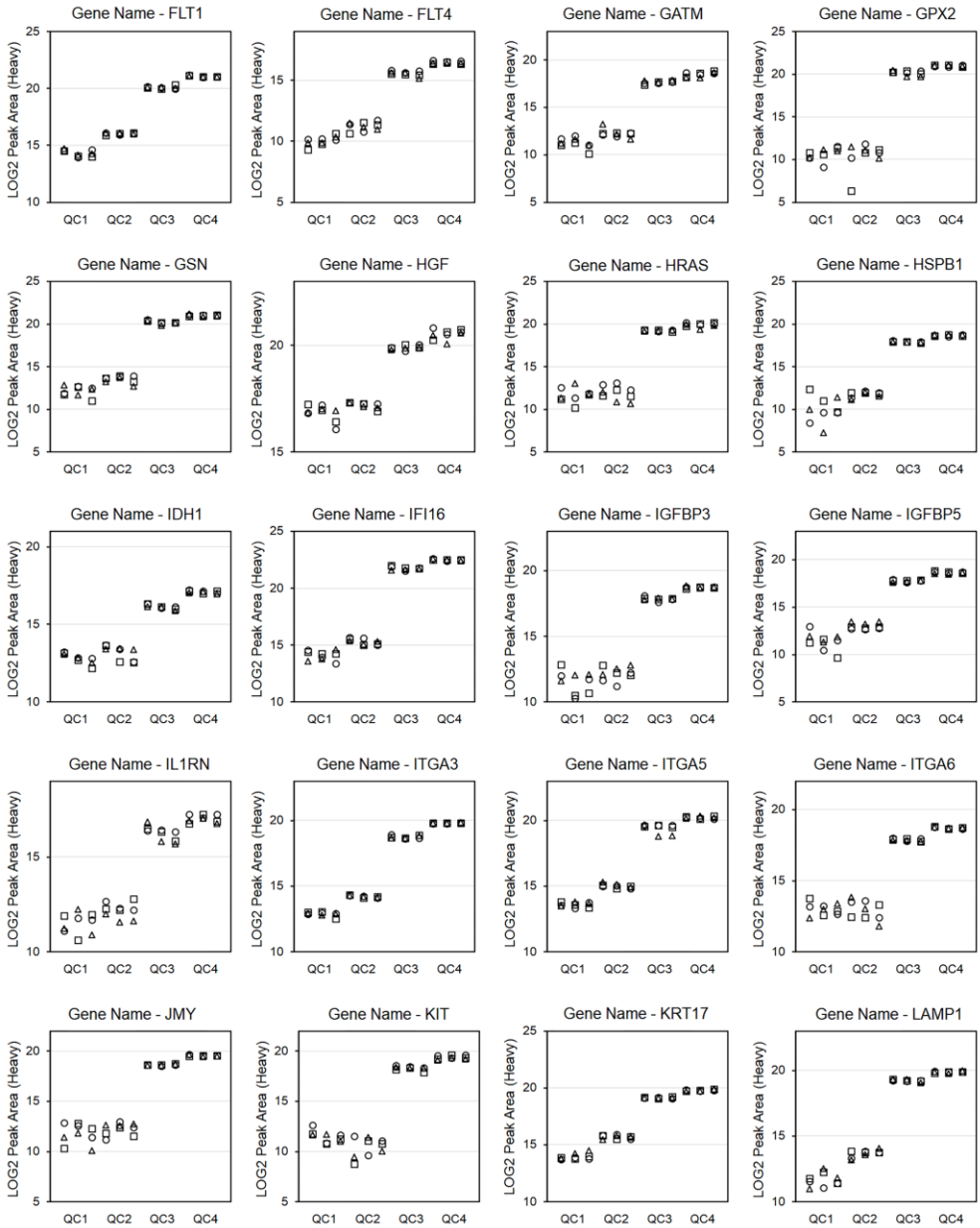
LOG2 Peak Area (Heavy)
QC1
QC2
QC3
QC4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Gene Name - IFI16
Gene Name - IGFBP3
Gene Name - IGFBP5
Gene Name - IL1RN
Gene Name - ITGA3
Gene Name - ITGA5
Gene Name - ITGA6
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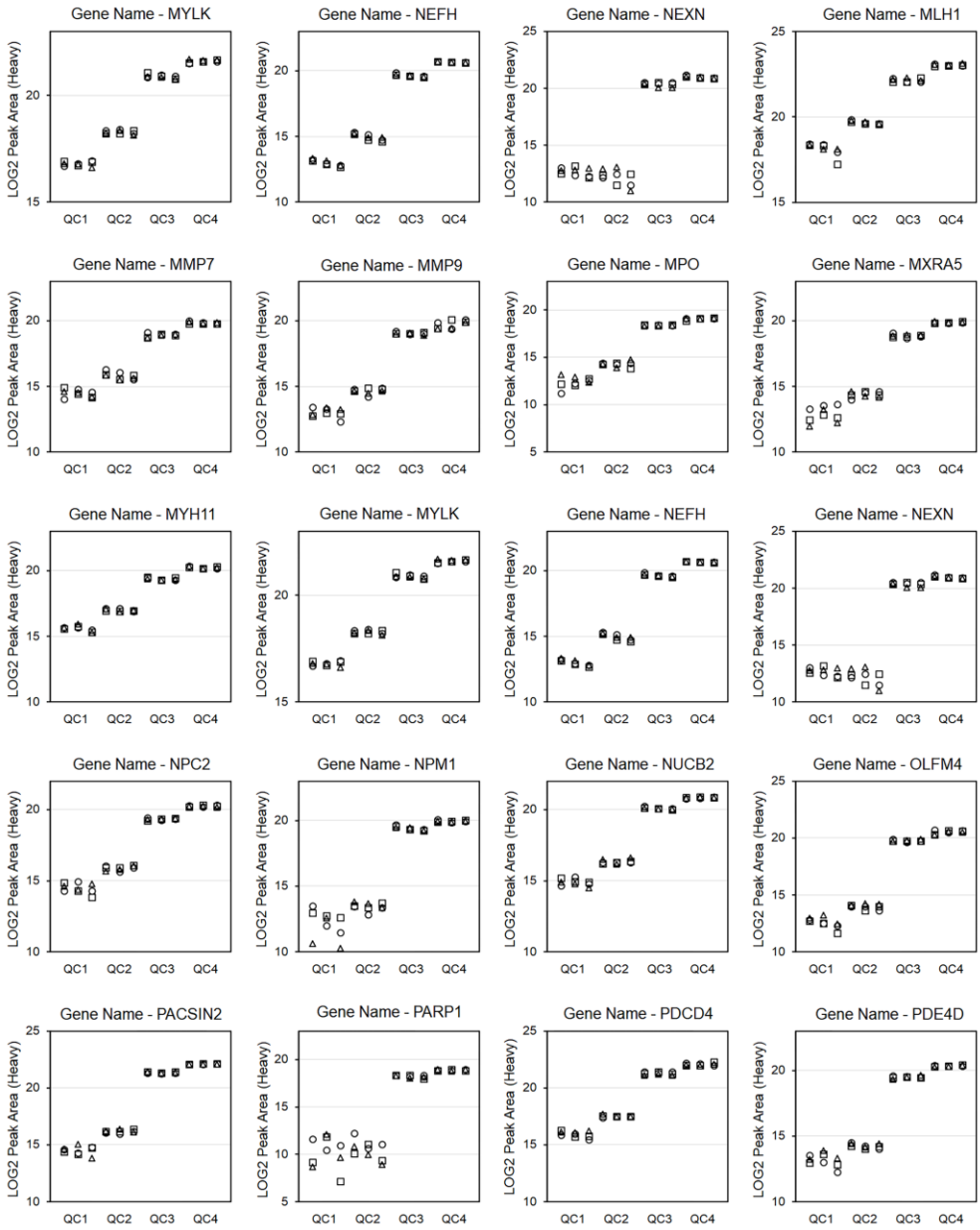
LOG2 Peak Area (Heavy)
QC1
QC2
QC3
QC4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Gene Name - NEFH
Gene Name - NEXN
Gene Name - NPC2
Gene Name - NPM1
Gene Name - NUCB2
Gene Name - OLFM4
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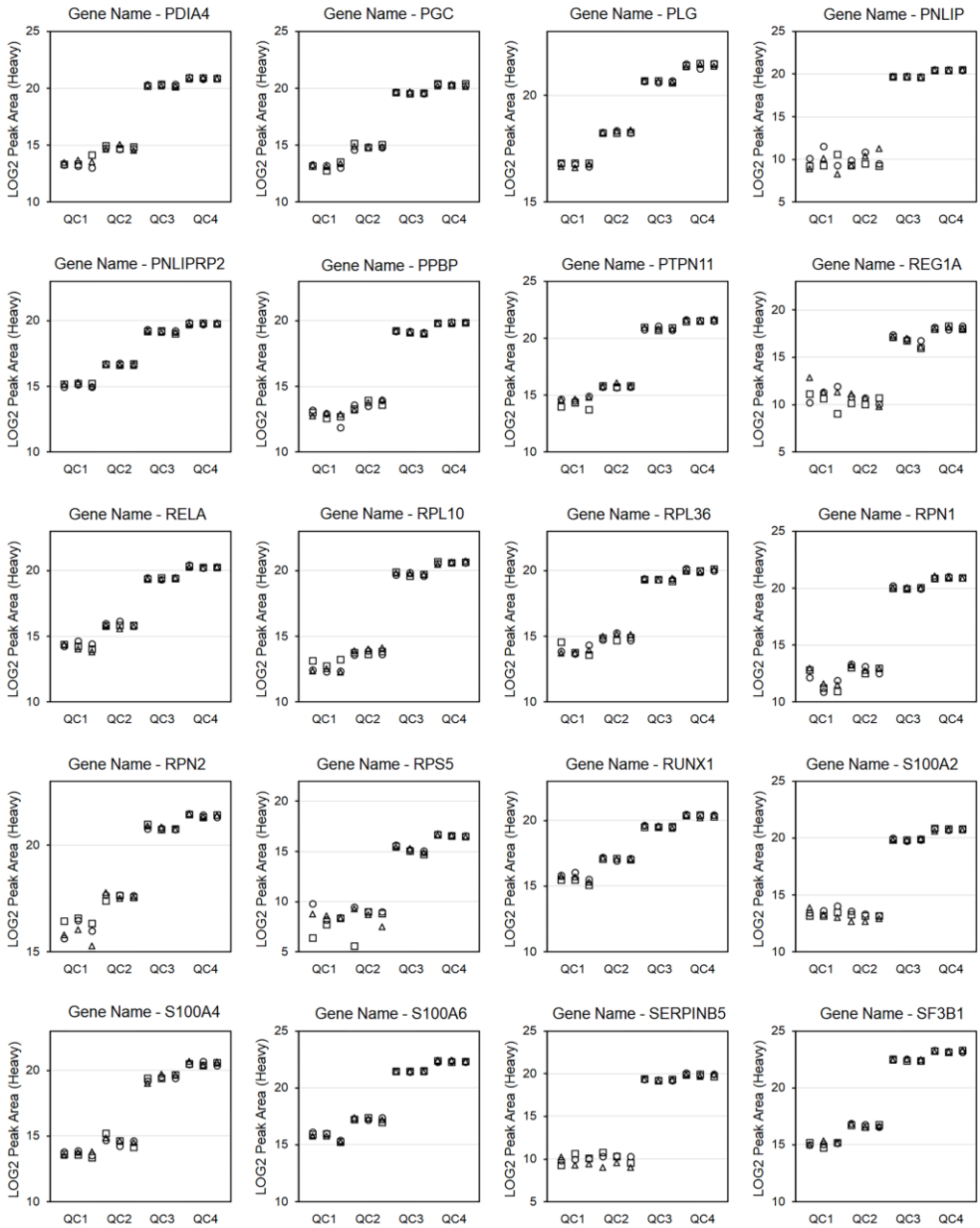
LOG2 Peak Area (Heavy)
QC1
QC2
QC3
QC4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Gene Name - RPL10
Gene Name - RPL36
Gene Name - RPN1
Gene Name - RPN2
Gene Name - RPS5
Gene Name - RUNX1
Gene Name - S100A2
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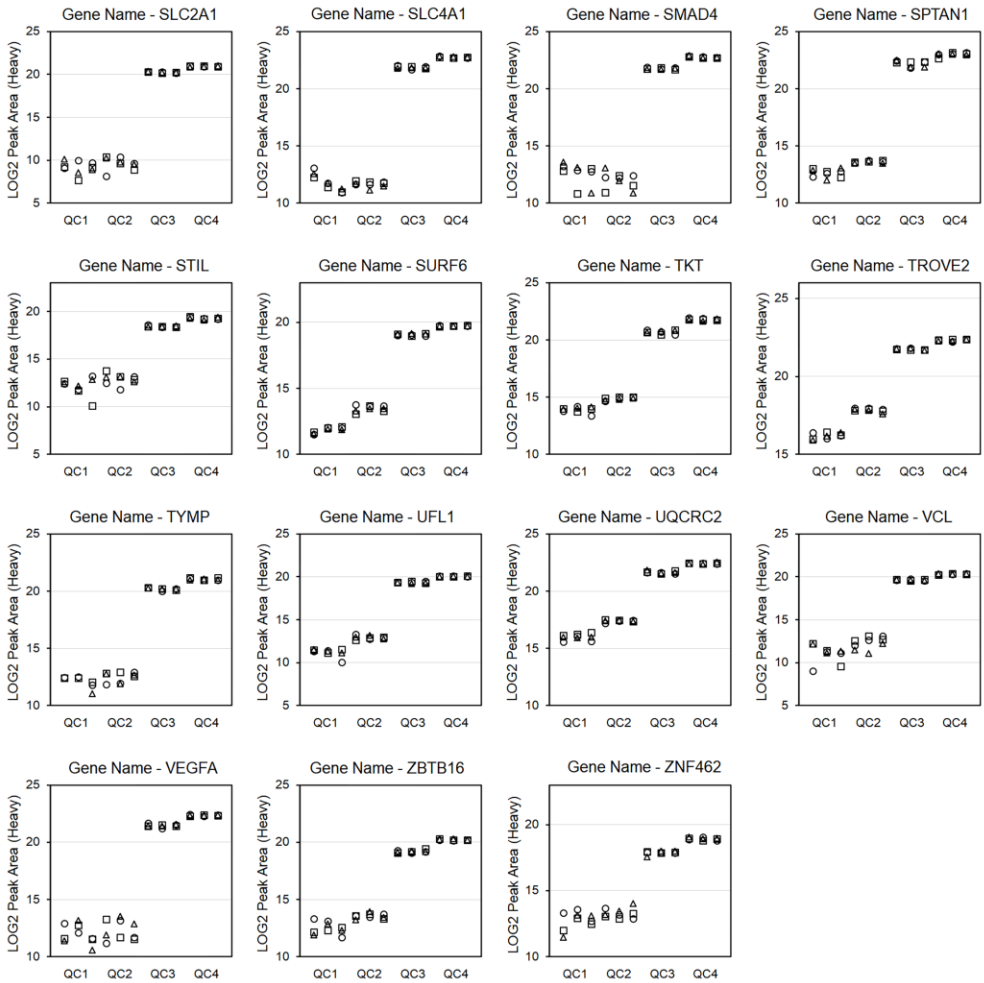
LOG2 Peak Area (Heavy)
QC1
QC2
QC3
QC4



Distribution of the peak areas of the signals for intra- and inter-day assays performed for each protein. The scatter plots represent the triplicate analysis (circles, squares, and triangles) on each of 3 days (from left to right, in each QC). Quality check for repeatability was performed using concentrations of QC1 (1.5 x LLOQ), QC2 (3.0 x LLOQ), QC3 (midway between LLOQ and the high end of the range), and QC4 (high end of the range).

Table 2-1. Summary of clinicopathological information in training and validation sets.

Descriptions	Training set (N = 148)	Validation set (N = 77)	P value
	Mean (range)		
Age, years	64.2 (40-83)	64.3 (41-87)	0.954
	Number of patients, n (%percent)		
Gender			0.596
Female	68 (45.9%)	39 (50.6%)	
Male	80 (54.1%)	38 (49.4%)	
Tumor location			0.089#
Head	92 (62.4%)	39 (50.6%)	
Body + Tail	42 (28.2%)	33 (42.9%)	
Diffused	14 (9.4%)	5 (6.5%)	
Resection margin			0.937
R0	129 (87.2%)	67 (87.0%)	
R1	16 (10.8%)	8 (10.4%)	
R2	3 (2.0%)	2 (2.6%)	
Differentiation			0.936
Well differentiated	12 (8.1%)	5 (6.5%)	
Moderately differentiated	108 (73.0%)	58 (75.3%)	
Poorly differentiated	23 (15.5%)	12 (15.6%)	
Undifferentiated	1 (0.7%)	1 (1.3%)	
Unknown	4 (2.7%)	1 (1.3%)	
Lymphatic invasion			0.502
No	74 (50.0%)	41 (53.2%)	
Yes	73 (49.3%)	32 (41.6%)	
Unknown	1 (0.7%)	4 (5.2%)	
Venous invasion			0.172
No	76 (51.4%)	45 (58.4%)	
Yes	71 (48.0%)	27 (35.1%)	
Unknown	1 (0.7%)	5 (6.4%)	
Perineural invasion			0.782
No	23 (15.5%)	10 (13.0%)	
Yes	125 (84.5%)	66 (85.7%)	
Unknown	0 (0.0%)	1 (1.3%)	
TNM T			0.269
T1	27 (18.2%)	10 (13.0%)	
T2	82 (55.4%)	38 (49.4%)	
T3	36 (24.3%)	28 (36.4%)	
T4	3 (2.0%)	1 (1.3%)	
TNM N			0.004**
N0	69 (46.6%)	36 (46.8%)	
N1	46 (31.1%)	36 (46.8%)	
N2	33 (22.3%)	5 (6.5%)	
Adjuvant chemotherapy			0.896
No	15 (10.1%)	9 (11.7%)	
Yes	133 (89.9%)	68 (88.3%)	
Adjuvant radiotherapy			0.591
No	82 (55.4%)	39 (50.6%)	
Yes	66 (44.6%)	38 (49.4%)	
Tumor size			0.742
≤3 cm	76 (51.4%)	37 (48.1%)	
>3 cm	72 (48.6%)	40 (51.9%)	
Death			0.686
No	71 (48.0%)	34 (44.2%)	
Yes	77 (52.0%)	43 (55.8%)	
Recurrence			0.582
No	55 (37.2%)	25 (32.5%)	
Yes	93 (62.8%)	52 (67.5%)	
Recurrence type			0.145
Local	20 (21.5%)	5 (9.6%)	
Systemic	73 (78.5%)	47 (90.4%)	

Table 2-2. Demographic and clinical overview of the study cohort.

Patient ID	Tumor Sample ID	Study Set	Sample Preparation Batch Set	MS Analysis Replicate 1 Batch Set	MS Analysis Replicate 2 Batch Set	MS Analysis Replicate 3 Batch Set	Total Protein Concentration (μg/μL)	Gender	Age	Operation Date	Operation Name	Resection Margin	Diagnosis	TNM T	TNM N	TNM M	Differentiation
11-14-061	SNU-PnBL-PDAC-T001	Training	G	A	D	F	4.2223	Female	66	26-Sep-14	Distal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-15-016	SNU-PnBL-PDAC-T002	Training	G	C	E	G	1.4850	Male	64	17-Apr-15	Distal pancreatectomy	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-17-051	SNU-PnBL-PDAC-T003	Training	B	C	F	F	1.3511	Female	70	28-Jul-17	Whipple	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-17-053	SNU-PnBL-PDAC-T004	Training	C	A	C	G	1.2119	Male	58	4-Jul-17	PPPD	R0	Pancreas cancer	T1	N0	M0	Poorly Differentiated
11-10-013	SNU-PnBL-PDAC-T005	Training	F	B	C	B	1.1019	Male	63	12-Mar-10	PPPD	R0	Pancreas cancer	T3	N2	M0	Moderately Differentiated
11-14-046	SNU-PnBL-PDAC-T006	Training	B	B	A	C	1.0957	Female	59	22-Jul-14	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-17-101	SNU-PnBL-PDAC-T007	Training	E	G	A	A	1.3882	Female	71	15-Dec-17	PPPD	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-18-022	SNU-PnBL-PDAC-T008	Training	G	F	E	C	1.6354	Female	72	20-Feb-18	Whipple	R1	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-17-097	SNU-PnBL-PDAC-T009	Training	E	A	F	C	1.2584	Female	76	28-Nov-17	Whipple	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-14-049	SNU-PnBL-PDAC-T010	Training	A	B	E	E	0.9086	Female	75	5-Aug-14	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-14-052	SNU-PnBL-PDAC-T011	Training	A	E	F	F	2.0079	Male	76	22-Aug-14	PPPD	R0	Pancreas cancer	T2	N0	M0	Well Differenetiated
11-17-008	SNU-PnBL-PDAC-T012	Training	G	G	G	E	1.2780	Female	58	3-Feb-17	Distal pancreatectomy	R0	Pancreas cancer	T1	N1	M0	Undifferentiated
11-17-041	SNU-PnBL-PDAC-T013	Training	F	E	E	B	1.4589	Female	58	29-Jun-17	Whipple	R1	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-15-028	SNU-PnBL-PDAC-T014	Training	E	D	A	G	2.3400	Female	57	6-May-15	Whipple	R0	Pancreas cancer	T2	N2	M0	Poorly Differentiated
11-15-046	SNU-PnBL-PDAC-T015	Training	B	D	F	E	3.1629	Female	59	14-Aug-15	PPPD	R0	Pancreas cancer	T1	N1	M0	Moderately Differentiated
11-13-004	SNU-PnBL-PDAC-T016	Training	D	B	E	E	3.7561	Male	59	22-Jan-13	PPPD	R0	Pancreas cancer	T1	N0	M0	Poorly Differentiated
11-17-084	SNU-PnBL-PDAC-T017	Training	E	B	C	B	2.2292	Female	70	31-Oct-17	PPPD	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-13-092	SNU-PnBL-PDAC-T018	Training	E	A	D	G	1.2483	Female	60	13-Dec-13	Distal pancreatectomy	R0	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-14-082	SNU-PnBL-PDAC-T019	Training	D	A	B	C	0.7930	Male	54	17-Dec-14	Whipple	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-16-063	SNU-PnBL-PDAC-T020	Training	E	D	B	B	1.1197	Male	53	18-Nov-16	Distal pancreatectomy	R0	Pancreas cancer	T1	N1	M0	Poorly Differentiated
11-17-075	SNU-PnBL-PDAC-T021	Training	E	E	F	C	1.3204	Male	60	10-Oct-17	Distal pancreatectomy	R2	Pancreas cancer	T2	N1	M1	Poorly Differentiated
11-15-012	SNU-PnBL-PDAC-T022	Training	D	D	C	G	0.8139	Female	69	25-Mar-15	Subtotal pancreatectomy	R0	Pancreas cancer	T1	N1	M0	Moderately Differentiated
11-16-066	SNU-PnBL-PDAC-T023	Training	B	C	G	G	1.3578	Female	66	29-Nov-16	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-16-021	SNU-PnBL-PDAC-T024	Training	D	A	G	C	1.7706	Male	74	20-Apr-16	Distal pancreatectomy	R0	Pancreas cancer	T2	N2	M0	Moderately Differentiated
11-13-002	SNU-PnBL-PDAC-T025	Training	C	C	D	G	1.4898	Male	54	11-Jan-13	Total pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-18-017	SNU-PnBL-PDAC-T026	Training	A	D	F	E	1.8705	Female	64	6-Feb-18	Distal pancreatectomy	R2	Pancreas cancer	T3	N1	M1	Moderately Differentiated
11-18-030	SNU-PnBL-PDAC-T027	Training	A	A	E	D	2.0735	Male	78	6-Mar-18	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-15-033	SNU-PnBL-PDAC-T028	Training	A	D	C	E	1.5353	Male	56	30-Jun-15	Whipple	R0	Pancreas cancer	T4	N0	M0	Moderately Differentiated
11-15-004	SNU-PnBL-PDAC-T029	Training	B	E	C	F	2.9692	Male	80	3-Feb-15	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-16-049	SNU-PnBL-PDAC-T030	Training	E	C	B	D	1.1531	Female	65	6-Sep-16	Total pancreatectomy	R0	Pancreas cancer	T3	N1	M0	Poorly Differentiated
11-18-012	SNU-PnBL-PDAC-T031	Training	B	C	G	D	1.3888	Male	56	25-Jan-18	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-17-072	SNU-PnBL-PDAC-T032	Training	D	D	E	D	1.9681	Female	74	29-Sep-17	Distal pancreatectomy	R0	Pancreas cancer	T1	N0	M0	Well Differenetiated
11-18-032	SNU-PnBL-PDAC-T033	Training	G	G	E	F	1.7608	Female	54	9-Mar-18	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-18-036	SNU-PnBL-PDAC-T034	Training	C	C	A	D	2.2114	Female	75	13-Mar-18	PPPD	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-16-009	SNU-PnBL-PDAC-T035	Training	D	E	B	F	1.3749	Female	66	16-Feb-16	Distal pancreatectomy	R0	Pancreas cancer	T1	N0	M0	Well Differenetiated
11-12-066	SNU-PnBL-PDAC-T036	Training	A	B	C	A	4.0321	Female	59	21-Dec-12	Total pancreatectomy	R0	Pancreas cancer	T3	N2	M0	Moderately Differentiated
11-14-003	SNU-PnBL-PDAC-T037	Training	D	B	C	B	4.9938	Male	75	10-Jan-14	PPPD	R0	Pancreas cancer	T2	N0	M0	Poorly Differentiated
11-18-020	SNU-PnBL-PDAC-T038	Training	E	E	G	D	1.2788	Female	51	13-Feb-18	Total pancreatectomy	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-13-016	SNU-PnBL-PDAC-T039	Training	B	F	F	E	1.2310	Male	60	12-Mar-13	Whipple	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-12-062	SNU-PnBL-PDAC-T040	Training	B	E	B	D	3.5148	Male	50	27-Nov-12	PPPD	R0	Pancreas cancer	T3	N1	M0	Well Differenetiated
11-14-060	SNU-PnBL-PDAC-T041	Training	B	A	G	A	2.8302	Male	59	26-Sep-14	PPPD	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-17-050	SNU-PnBL-PDAC-T042	Training	E	E	C	D	1.4033	Male	62	25-Jul-17	Whipple	R1	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-16-001	SNU-PnBL-PDAC-T043	Training	G	E	F	D	0.8148	Male	76	5-Jan-16	Distal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-17-028	SNU-PnBL-PDAC-T044	Training	G	B	D	A	1.9365	Female	64	19-May-17	Whipple	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-15-025	SNU-PnBL-PDAC-T045	Training	D	D	E	A	1.1538	Female	78	19-May-15	Subtotal pancreatectomy	R1	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-17-077	SNU-PnBL-PDAC-T046	Training	F	E	F	C	1.9425	Male	59	13-Oct-17	Whipple	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-16-071	SNU-PnBL-PDAC-T047	Training	D	B	C	D	1.4470	Female	58	6-Dec-16	Whipple	R0	Pancreas cancer	T4	N2	M0	Moderately Differentiated
11-12-030	SNU-PnBL-PDAC-T048	Training	B	F	D	C	1.0096	Male	49	8-Jun-12	Subtotal pancreatectomy	R0	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-17-099	SNU-PnBL-PDAC-T049	Training	C	F	D	B	1.4185	Male	74	5-Dec-17	Distal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-13-032	SNU-PnBL-PDAC-T050	Training	C	D	A	E	1.2513	Male	69	24-May-13	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-15-059	SNU-PnBL-PDAC-T051	Training	A	C	F	D	2.4529	Female	76	13-Oct-15	PPPD	R0	Pancreas cancer	T2	N2	M0	Moderately Differentiated
11-17-039	SNU-PnBL-PDAC-T052	Training	C	F	G	F	1.4099	Male	64	27-Jun-17	Whipple	R1	Pancreas cancer	T3	N2	M0	Moderately Differentiated
11-17-055	SNU-PnBL-PDAC-T053	Training	F	F	A	G	1.5135	Female	77	2-Aug-17	PPPD	R0	Pancreas cancer	T2	N2	M0	Moderately Differentiated
11-15-058	SNU-PnBL-PDAC-T054	Training	D	F	C	C	0.7169	Male	64	13-Oct-15	Distal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-15-022	SNU-PnBL-PDAC-T055	Training	E	D	F	A	1.2624	Female	71	28-Apr-15	PPPD	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-17-058	SNU-PnBL-PDAC-T056	Training	D	F	A	F	2.0293	Male	75	9-Aug-17	Distal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Poorly Differentiated

11-13-001	SNU-PnBL-PDAC-T057	Training	A	G	D	B	1.7563	Male	56	4-Jan-13	Subtotal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-13-096	SNU-PnBL-PDAC-T058	Training	C	A	B	C	1.6677	Male	63	27-Dec-13	PPPD	R0	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-18-014	SNU-PnBL-PDAC-T059	Training	F	C	B	B	1.4115	Female	65	2-Feb-18	PPPD	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-16-065	SNU-PnBL-PDAC-T060	Training	A	B	G	A	1.3350	Female	69	25-Nov-16	Distal pancreatectomy	R0	Pancreas cancer	T2	N2	M0	Moderately Differentiated
11-15-026	SNU-PnBL-PDAC-T061	Training	G	G	D	G	0.8630	Female	68	6-May-15	PPPD	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-15-029	SNU-PnBL-PDAC-T062	Training	B	C	A	E	1.4706	Female	55	29-May-15	Distal pancreatectomy	R0	Pancreas cancer	T3	N0	M0	Poorly Differentiated
11-15-030	SNU-PnBL-PDAC-T063	Training	A	G	B	B	1.3046	Male	40	2-Jun-15	PPPD	R0	Pancreas cancer	T2	N2	M0	Poorly Differentiated
11-17-034	SNU-PnBL-PDAC-T064	Training	G	C	F	D	1.8215	Male	79	13-Jun-17	Whipple	R0	Pancreas cancer	T2	N0	M0	Poorly Differentiated
11-17-037	SNU-PnBL-PDAC-T065	Training	F	D	D	F	1.7882	Male	70	20-Jun-17	Whipple	R0	Pancreas cancer	T3	N2	M0	Moderately Differentiated
11-13-089	SNU-PnBL-PDAC-T066	Training	B	E	F	G	1.9559	Male	55	11-Dec-13	PPPD	R0	Pancreas cancer	T2	N0	M0	Poorly Differentiated
11-14-057	SNU-PnBL-PDAC-T067	Training	B	G	B	A	0.9965	Male	71	16-Sep-14	Whipple	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-17-047	SNU-PnBL-PDAC-T068	Training	G	G	D	F	2.2078	Female	56	12-Jul-17	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-15-003	SNU-PnBL-PDAC-T069	Training	F	D	C	A	1.8312	Male	61	23-Jan-15	Distal pancreatectomy	R1	Pancreas cancer	T3	N1	M0	Poorly Differentiated
11-17-070	SNU-PnBL-PDAC-T070	Training	C	G	E	C	1.3818	Male	70	27-Sep-17	Whipple	R0	Pancreas cancer	T3	N2	M0	Moderately Differentiated
11-15-011	SNU-PnBL-PDAC-T071	Training	G	E	C	C	0.9041	Male	67	24-Mar-15	Whipple	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-13-054	SNU-PnBL-PDAC-T072	Training	C	F	F	E	2.0830	Male	48	7-Aug-13	Central pancreatectomy	R0	Pancreas cancer	T1	N0	M0	Well Differenetiated
11-13-059	SNU-PnBL-PDAC-T073	Training	E	G	A	G	3.5382	Female	52	13-Aug-13	PPPD	R1	Pancreas cancer	T2	N2	M0	Moderately Differentiated
11-14-065	SNU-PnBL-PDAC-T074	Training	B	G	A	A	3.2743	Male	65	30-Sep-14	Whipple	R0	Pancreas cancer	T2	N2	M0	Moderately Differentiated
11-15-074	SNU-PnBL-PDAC-T075	Training	C	F	B	C	1.5510	Male	55	29-Dec-15	Total pancreatectomy	R0	Pancreas cancer	T3	N0	M0	Well Differenetiated
11-17-052	SNU-PnBL-PDAC-T076	Training	B	C	C	C	1.6141	Male	59	28-Jul-17	Distal pancreatectomy	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-14-079	SNU-PnBL-PDAC-T077	Training	A	B	G	B	1.3108	Male	72	26-Nov-14	PPPD	R1	Pancreas cancer	T2	N1	M0	Not Applicable
11-13-010	SNU-PnBL-PDAC-T078	Training	F	F	C	E	1.6758	Female	42	15-Feb-13	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-14-050	SNU-PnBL-PDAC-T079	Training	G	E	F	E	2.7618	Male	77	13-Aug-14	PPPD	R0	Pancreas cancer	T2	N0	M0	Well Differenetiated
11-18-029	SNU-PnBL-PDAC-T080	Training	D	A	E	D	1.4551	Female	53	2-Mar-18	PPPD	R0	Pancreas cancer	T3	N1	M0	Not Applicable
11-18-013	SNU-PnBL-PDAC-T081	Training	A	C	G	A	0.9875	Male	61	26-Jan-18	Distal pancreatectomy	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-14-075	SNU-PnBL-PDAC-T082	Training	F	A	C	D	2.3058	Male	59	27-Oct-14	PPPD	R0	Pancreas cancer	T2	N2	M0	Moderately Differentiated
11-13-047	SNU-PnBL-PDAC-T083	Training	A	A	E	G	2.1448	Male	71	23-Jul-13	Whipple	R0	Pancreas cancer	T2	N2	M0	Poorly Differentiated
11-13-084	SNU-PnBL-PDAC-T084	Training	A	A	E	B	3.7110	Male	51	19-Nov-13	PPPD	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-17-062	SNU-PnBL-PDAC-T085	Training	D	E	E	B	1.6805	Female	83	30-Aug-17	Whipple	R0	Pancreas cancer	T2	N2	M0	Not Applicable
11-16-068	SNU-PnBL-PDAC-T086	Training	E	B	B	B	1.3138	Female	71	2-Dec-16	PPPD	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-12-022	SNU-PnBL-PDAC-T087	Training	A	A	G	A	1.8643	Female	70	20-Apr-12	Distal pancreatectomy	R0	Pancreas cancer	T3	N1	M0	Poorly Differentiated
11-17-089	SNU-PnBL-PDAC-T088	Training	A	F	B	B	1.9311	Male	56	15-Nov-17	Distal pancreatectomy	R0	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-13-023	SNU-PnBL-PDAC-T089	Training	C	D	G	G	4.0926	Male	70	16-Apr-13	Distal pancreatectomy	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-15-005	SNU-PnBL-PDAC-T090	Training	B	E	G	B	2.7348	Male	69	6-Feb-15	PPPD	R0	Pancreas cancer	T2	N2	M0	Poorly Differentiated
11-14-064	SNU-PnBL-PDAC-T091	Training	C	E	F	G	0.6831	Female	75	30-Sep-14	Whipple	R1	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-17-094	SNU-PnBL-PDAC-T092	Training	A	A	C	B	1.3323	Male	61	21-Nov-17	Whipple	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-12-016	SNU-PnBL-PDAC-T093	Training	B	B	A	C	1.0847	Female	76	13-Mar-12	Whipple	R0	Pancreas cancer	T3	N1	M0	Well Differenetiated
11-15-023	SNU-PnBL-PDAC-T094	Training	G	E	G	F	1.4016	Female	67	8-May-15	Subtotal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Well Differenetiated
11-17-105	SNU-PnBL-PDAC-T095	Training	A	B	F	A	1.3942	Male	76	27-Dec-17	Distal pancreatectomy	R0	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-17-076	SNU-PnBL-PDAC-T096	Training	F	A	B	G	1.8750	Male	46	10-Oct-17	PPPD	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-18-025	SNU-PnBL-PDAC-T097	Training	F	E	A	D	2.0469	Female	64	28-Feb-18	Subtotal pancreatectomy	R0	Pancreas cancer	T2	N2	M0	Moderately Differentiated
11-17-013	SNU-PnBL-PDAC-T098	Training	F	D	B	C	1.4353	Female	70	28-Feb-17	Distal pancreatectomy	R0	Pancreas cancer	T1	N0	M0	Well Differenetiated
11-15-018	SNU-PnBL-PDAC-T099	Training	C	D	G	A	1.9366	Female	72	17-Feb-15	Distal pancreatectomy	R1	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-17-065	SNU-PnBL-PDAC-T100	Training	G	B	B	B	2.0078	Male	67	8-Sep-17	Distal pancreatectomy	R1	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-17-107	SNU-PnBL-PDAC-T101	Training	B	C	F	G	1.6976	Male	56	29-Dec-17	PPPD	R1	Pancreas cancer	T2	N2	M0	Not Applicable
11-15-007	SNU-PnBL-PDAC-T102	Training	F	B	E	G	4.9938	Female	74	2-Mar-15	PPPD	R1	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-18-004	SNU-PnBL-PDAC-T103	Training	A	C	B	A	1.3917	Male	72	10-Jan-18	Whipple	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-17-060	SNU-PnBL-PDAC-T104	Training	F	C	A	E	1.4512	Female	72	18-Aug-17	PPPD	R0	Pancreas cancer	T2	N2	M0	Moderately Differentiated
11-17-038	SNU-PnBL-PDAC-T105	Training	D	F	A	G	1.4155	Female	47	23-Jun-17	PPPD	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-17-035	SNU-PnBL-PDAC-T106	Training	G	D	E	D	1.4090	Female	69	16-Jun-17	Distal pancreatectomy	R0	Pancreas cancer	T3	N2	M0	Moderately Differentiated
11-16-034	SNU-PnBL-PDAC-T107	Training	C	F	E	B	1.2162	Male	62	5-Jul-16	PPPD	R0	Pancreas cancer	T2	N2	M0	Moderately Differentiated
11-17-048	SNU-PnBL-PDAC-T108	Training	G	A	D	A	1.5726	Male	54	18-Jul-17	PPPD	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-15-020	SNU-PnBL-PDAC-T109	Training	G	A	D	C	3.4922	Male	71	27-Feb-15	Whipple	R0	Pancreas cancer	T2	N2	M0	Moderately Differentiated
11-14-066	SNU-PnBL-PDAC-T110	Training	A	E	D	E	3.8085	Male	62	1-Oct-14	PPPD	R0	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-14-045	SNU-PnBL-PDAC-T111	Training	D	C	A	F	1.0177	Male	61	18-Jul-14	Distal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-14-037	SNU-PnBL-PDAC-T112	Training	D	B	G	A	1.0031	Male	64	11-Jun-14	PPPD	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-17-103	SNU-PnBL-PDAC-T113	Training	E	G	C	C	1.2989	Female	73	20-Dec-17	PPPD	R1	Pancreas cancer	T1	N0	M0	Poorly Differentiated
11-18-023	SNU-PnBL-PDAC-T114	Training	D	A	B	G	1.3699	Female	69	23-Feb-18	Remnant Distal pancreatectomy	R0	pancreas cancer	T2	N1	M0	Moderately Differentiated
11-13-018	SNU-PnBL-PDAC-T115	Training	D	C	D	E	1.0661	Female	65	15-Mar-13	PPPD	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-16-070	SNU-PnBL-PDAC-T116	Training	E	A	C	D	1.2986	Female	80	2-Dec-16	Distal pancreatectomy	R0	Pancreas cancer	T4	N2	M0	Moderately Differentiated
11-15-015	SNU-PnBL-PDAC-T117	Training	D	D	D	E	1.1633	Male	64	10-Apr-15	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-18-019	SNU-PnBL-PDAC-T118	Training	C	B	A	F	1.2667	Female	54	13-Feb-18	Total pancreatectomy	R0	Pancreas cancer	T2	N2	M0	Poorly Differentiated

11-17-032	SNU-PnBL-PDAC-T119	Training	F	D	A	B	1.4951	Male	54	30-May-17	Distal pancreatectomy	R0	Pancreas cancer	T2	N2	M0	Well Differenetiated
11-14-058	SNU-PnBL-PDAC-T120	Training	A	G	C	F	0.9135	Male	59	16-Sep-14	Total pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-18-024	SNU-PnBL-PDAC-T121	Training	C	G	G	E	1.6046	Male	73	23-Feb-18	PPPD	R0	Pancreas cancer	T2	N2	M0	Poorly Differentiated
11-13-058	SNU-PnBL-PDAC-T122	Training	F	D	D	F	3.0662	Female	60	13-Aug-13	PPPD	R0	Pancreas cancer	T2	N2	M0	Moderately Differentiated
11-14-081	SNU-PnBL-PDAC-T123	Training	B	C	B	A	1.0467	Male	65	16-Dec-14	PPPD	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-14-067	SNU-PnBL-PDAC-T124	Training	F	A	B	F	2.1629	Female	70	7-Oct-14	Whipple	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-15-032	SNU-PnBL-PDAC-T125	Training	G	G	F	C	2.6378	Female	64	30-Jun-15	PPPD	R0	Pancreas cancer	T3	N2	M0	Poorly Differentiated
11-13-062	SNU-PnBL-PDAC-T126	Training	F	A	D	F	1.1508	Male	73	21-Aug-13	Distal pancreatectomy	R0	Pancreas cancer	T3	N2	M0	Moderately Differentiated
11-13-094	SNU-PnBL-PDAC-T127	Training	A	F	F	F	1.3766	Male	72	24-Dec-13	Whipple	R0	Pancreas cancer with IPMN	T1	N0	M0	Moderately Differentiated
11-17-042	SNU-PnBL-PDAC-T128	Training	E	F	A	C	1.4333	Male	74	30-Jun-17	Distal pancreatectomy	R1	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-13-025	SNU-PnBL-PDAC-T129	Training	G	G	A	D	0.8227	Female	50	7-May-13	Whipple	R0	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-14-071	SNU-PnBL-PDAC-T130	Training	C	G	E	C	2.5237	Male	68	17-Oct-14	PPPD	R0	Pancreas cancer	T2	N1	M0	Poorly Differentiated
11-14-026	SNU-PnBL-PDAC-T131	Training	D	A	G	F	3.9883	Male	69	22-Apr-14	Distal pancreatectomy	R0	Pancreas cancer	T3	N1	M0	Poorly Differentiated
11-11-034	SNU-PnBL-PDAC-T132	Training	E	B	A	F	1.1099	Female	65	27-Jun-11	Whipple	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-17-057	SNU-PnBL-PDAC-T133	Training	E	F	D	E	1.4089	Male	70	8-Aug-17	Distal pancreatectomy	R2	Pancreas cancer	T3	N0	M1	Moderately Differentiated
11-17-021	SNU-PnBL-PDAC-T134	Training	E	F	D	A	2.1038	Male	70	4-Apr-17	PPPD	R1	Pancreas cancer	T2	N2	M0	Moderately Differentiated
11-17-091	SNU-PnBL-PDAC-T135	Training	E	G	D	B	2.1475	Female	60	14-Nov-17	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-17-096	SNU-PnBL-PDAC-T136	Training	G	C	F	A	1.3908	Male	53	24-Nov-17	Distal pancreatectomy	R0	Pancreas cancer	T3	N2	M0	Moderately Differentiated
11-17-081	SNU-PnBL-PDAC-T137	Training	F	G	E	B	1.2610	Male	65	26-Oct-17	Total pancreatectomy	R0	Pancreas cancer	T1	N1	M0	Moderately Differentiated
11-14-051	SNU-PnBL-PDAC-T138	Training	C	B	E	A	2.3973	Female	57	19-Aug-14	Distal pancreatectomy	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-18-009	SNU-PnBL-PDAC-T139	Training	C	D	C	D	1.4432	Female	76	19-Jan-18	PPPD	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-15-006	SNU-PnBL-PDAC-T140	Training	E	G	B	B	1.9353	Male	54	17-Feb-15	Distal pancreatectomy	R1	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-17-071	SNU-PnBL-PDAC-T141	Training	C	F	A	E	1.4118	Male	55	28-Sep-17	PPPD	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-15-038	SNU-PnBL-PDAC-T142	Training	B	E	G	G	4.0442	Male	67	28-Jul-15	Distal pancreatectomy	R0	Pancreas cancer	T2	N1	M0	Poorly Differentiated
11-18-034	SNU-PnBL-PDAC-T143	Training	D	B	F	C	1.3627	Female	41	13-Mar-18	Whipple	R0	Pancreas cancer	T2	N0	M0	Well Differenetiated
11-13-042	SNU-PnBL-PDAC-T144	Training	C	F	C	D	1.8625	Female	60	9-Jul-13	Distal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-14-069	SNU-PnBL-PDAC-T145	Training	G	G	D	E	2.8215	Female	63	14-Oct-14	PPPD	R0	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-15-068	SNU-PnBL-PDAC-T146	Training	B	D	G	A	1.5628	Female	71	27-Nov-15	PPPD	R0	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-17-102	SNU-PnBL-PDAC-T147	Training	B	E	C	G	1.3363	Female	61	15-Dec-17	Distal pancreatectomy	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-15-070	SNU-PnBL-PDAC-T148	Training	F	G	B	F	1.1065	Male	53	4-Dec-15	PPPD	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-17-025	SNU-PnBL-PDAC-V001	Validation	J	J	H	K	1.3687	Male	55	25-Apr-17	PPPD	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-17-080	SNU-PnBL-PDAC-V002	Validation	I	K	J	K	1.2828	Male	75	20-Oct-17	Distal pancreatectomy	R1	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-12-010	SNU-PnBL-PDAC-V003	Validation	H	J	I	J	1.4375	Male	67	21-Feb-12	PPPD	R1	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-12-043	SNU-PnBL-PDAC-V004	Validation	J	H	K	I	1.1727	Male	73	22-Aug-12	Whipple	R0	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-13-039	SNU-PnBL-PDAC-V005	Validation	J	H	H	J	2.0302	Female	51	2-Jul-13	Whipple	R1	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-18-007	SNU-PnBL-PDAC-V006	Validation	I	H	K	K	1.6115	Male	61	16-Jan-18	Distal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-17-093	SNU-PnBL-PDAC-V007	Validation	J	J	I	J	1.1774	Male	67	21-Nov-17	Distal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-13-037	SNU-PnBL-PDAC-V008	Validation	I	I	I	J	1.7788	Female	76	18-Jun-13	PPPD	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-13-033	SNU-PnBL-PDAC-V009	Validation	H	J	J	H	2.0174	Female	78	28-May-13	PPPD	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-14-077	SNU-PnBL-PDAC-V010	Validation	J	J	I	H	2.3445	Female	77	14-Nov-14	Distal pancreatectomy	R0	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-14-056	SNU-PnBL-PDAC-V011	Validation	K	I	K	K	2.0429	Female	87	12-Sep-14	Distal pancreatectomy	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-17-074	SNU-PnBL-PDAC-V012	Validation	I	I	K	J	1.4016	Female	51	20-Sep-17	PPPD	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-16-025	SNU-PnBL-PDAC-V013	Validation	J	I	I	I	0.6846	Female	58	13-May-16	PPPD	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-17-085	SNU-PnBL-PDAC-V014	Validation	J	J	K	I	1.3957	Male	80	31-Oct-17	Distal pancreatectomy	R0	Pancreas cancer	T3	N0	M0	Poorly Differentiated
11-13-056	SNU-PnBL-PDAC-V015	Validation	K	K	J	K	1.5917	Female	47	9-Aug-13	Distal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-17-036	SNU-PnBL-PDAC-V016	Validation	H	H	H	H	1.3609	Male	67	20-Jun-17	Distal pancreatectomy	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-15-027	SNU-PnBL-PDAC-V017	Validation	H	I	I	H	3.9590	Male	55	22-Apr-15	Whipple	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-13-052	SNU-PnBL-PDAC-V018	Validation	K	J	K	I	2.8191	Male	42	6-Aug-13	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-16-072	SNU-PnBL-PDAC-V019	Validation	H	H	I	K	1.9860	Male	50	13-Dec-16	PPPD	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-16-020	SNU-PnBL-PDAC-V020	Validation	K	K	J	H	1.2021	Female	73	20-Apr-16	Distal pancreatectomy	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-14-059	SNU-PnBL-PDAC-V021	Validation	K	H	J	I	3.2623	Male	54	23-Sep-14	PPPD	R0	Pancreas cancer	T2	N2	M0	Poorly Differentiated
11-11-074	SNU-PnBL-PDAC-V022	Validation	H	I	H	K	1.2022	Male	75	20-Dec-11	Distal pancreatectomy	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-11-040	SNU-PnBL-PDAC-V023	Validation	I	K	K	H	0.8414	Male	67	22-Jul-11	Distal pancreatectomy	R0	Pancreas cancer	T2	N1	M0	Poorly Differentiated
11-17-043	SNU-PnBL-PDAC-V024	Validation	I	I	H	J	1.3597	Male	63	30-Jun-17	Distal pancreatectomy	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-13-074	SNU-PnBL-PDAC-V025	Validation	H	K	I	H	1.9177	Female	64	22-Oct-13	Whipple	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-13-009	SNU-PnBL-PDAC-V026	Validation	I	I	J	I	1.3905	Male	72	13-Feb-13	Whipple	R0	Pancreas cancer	T2	N2	M0	Moderately Differentiated
11-12-001	SNU-PnBL-PDAC-V027	Validation	K	K	K	K	1.0798	Male	65	4-Jan-12	Distal pancreatectomy	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-17-082	SNU-PnBL-PDAC-V028	Validation	K	I	I	H	1.7039	Female	68	27-Oct-17	Distal pancreatectomy	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-13-007	SNU-PnBL-PDAC-V029	Validation	J	I	K	I	1.3999	Male	54	1-Feb-13	Distal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Well Differenetiated
11-16-064	SNU-PnBL-PDAC-V030	Validation	H	H	K	I	1.2804	Female	61	23-Nov-16	Distal pancreatectomy	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-15-071	SNU-PnBL-PDAC-V031	Validation	H	K	I	K	1.3863	Male	52	8-Dec-15	Distal pancreatectomy	R0	Pancreas cancer	T3	N1	M0	Poorly Differentiated
11-18-035	SNU-PnBL-PDAC-V032	Validation	H	I	H	H	1.4512	Female	80	13-Mar-18	PPPD	R0	Pancreas cancer	T2	N2	M0	Poorly Differentiated
11-14-080	SNU-PnBL-PDAC-V033	Validation	I	K	K	J	1.7819	Male	58	9-Dec-14	PPPD	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated

11-17-063	SNU-PnBL-PDAC-V034	Validation	H	H	I	H	1.4471	Female	62	1-Sep-17	PPPD	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-13-040	SNU-PnBL-PDAC-V035	Validation	J	H	H	K	2.8249	Female	64	2-Jul-13	Total pancreatectomy	R1	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-17-045	SNU-PnBL-PDAC-V036	Validation	I	K	J	I	1.2962	Male	52	27-Jun-17	Distal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Undifferentiated
11-14-054	SNU-PnBL-PDAC-V037	Validation	I	I	J	J	3.1145	Male	58	29-Aug-14	PPPD	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-12-049	SNU-PnBL-PDAC-V038	Validation	J	J	I	J	0.7387	Female	75	18-Sep-12	Distal pancreatectomy	R1	Pancreas cancer	T4	N2	M0	Poorly Differentiated
11-15-024	SNU-PnBL-PDAC-V039	Validation	I	J	I	I	2.1681	Female	70	19-May-15	PPPD	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-12-004	SNU-PnBL-PDAC-V040	Validation	H	H	H	J	1.1460	Male	52	10-Jan-12	Whipple	R1	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-14-084	SNU-PnBL-PDAC-V041	Validation	I	H	H	I	3.5382	Female	62	24-Dec-14	Whipple	R0	Mucinous noncystic carcinoma	T3	N0	M0	Not Applicable
11-13-087	SNU-PnBL-PDAC-V042	Validation	J	H	J	H	2.2848	Male	65	6-Dec-13	Distal pancreatectomy	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-11-066	SNU-PnBL-PDAC-V043	Validation	J	J	H	H	1.8077	Female	43	15-Nov-11	Whipple	R0	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-17-104	SNU-PnBL-PDAC-V044	Validation	I	I	H	I	1.4771	Female	78	27-Dec-17	Subtotal pancreatectomy	R2	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-14-085	SNU-PnBL-PDAC-V045	Validation	K	K	J	I	0.7675	Female	79	26-Dec-14	Distal pancreatectomy	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-12-007	SNU-PnBL-PDAC-V046	Validation	I	J	J	J	1.0577	Male	66	13-Jan-12	Distal pancreatectomy	R0	Pancreas cancer	T3	N2	M0	Moderately Differentiated
11-10-017	SNU-PnBL-PDAC-V047	Validation	I	I	J	K	1.2630	Female	75	31-Mar-10	Distal pancreatectomy	R0	Pancreas cancer	T3	N0	M0	Moderately Differentiated
11-09-029	SNU-PnBL-PDAC-V048	Validation	H	K	J	H	1.4299	Male	64	16-Oct-09	PPPD	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-13-076	SNU-PnBL-PDAC-V049	Validation	J	H	K	J	2.6956	Female	63	25-Oct-13	Total pancreatectomy	R1	Pancreas cancer	T1	N0	M0	Well Differenetiated
11-12-061	SNU-PnBL-PDAC-V050	Validation	I	H	H	I	3.1902	Male	61	13-Nov-12	Distal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Well Differenetiated
11-14-083	SNU-PnBL-PDAC-V051	Validation	K	J	K	H	1.8118	Female	64	23-Dec-14	Distal pancreatectomy	R0	Pancreas cancer	T3	N0	M0	Poorly Differentiated
11-13-078	SNU-PnBL-PDAC-V052	Validation	K	I	K	K	2.5679	Male	45	29-Oct-13	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-12-028	SNU-PnBL-PDAC-V053	Validation	K	H	J	J	1.1825	Male	72	23-May-12	Whipple	R0	Pancreas cancer	T3	N1	M0	Poorly Differentiated
11-15-014	SNU-PnBL-PDAC-V054	Validation	K	H	H	H	2.9280	Female	70	10-Apr-15	Distal pancreatectomy	R0	Pancreas cancer	T1	N0	M0	Moderately Differentiated
11-13-068	SNU-PnBL-PDAC-V055	Validation	H	K	J	I	2.1290	Male	62	25-Sep-13	Whipple	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-13-028	SNU-PnBL-PDAC-V056	Validation	J	K	K	H	1.9682	Female	62	15-May-13	Whipple	R0	Pancreas cancer	T1	N0	M0	Well Differenetiated
11-17-059	SNU-PnBL-PDAC-V057	Validation	K	H	J	K	1.4551	Male	75	16-Aug-17	Distal pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-13-048	SNU-PnBL-PDAC-V058	Validation	J	K	K	J	2.6201	Male	70	25-Jul-13	Distal pancreatectomy	R0	Pancreas cancer	T3	N1	M0	Poorly Differentiated
11-13-075	SNU-PnBL-PDAC-V059	Validation	H	K	I	I	1.3660	Female	44	22-Oct-13	Distal pancreatectomy	R0	Pancreas cancer	T3	N1	M0	Well Differenetiated
11-17-061	SNU-PnBL-PDAC-V060	Validation	I	I	H	J	1.5066	Male	74	22-Aug-17	Total pancreatectomy	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-17-078	SNU-PnBL-PDAC-V061	Validation	K	J	H	J	1.5837	Female	51	17-Oct-17	Subtotal pancreatectomy	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-17-049	SNU-PnBL-PDAC-V062	Validation	I	J	I	I	1.5066	Female	51	18-Jul-17	Whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-15-019	SNU-PnBL-PDAC-V063	Validation	K	J	K	K	2.3358	Female	61	25-Feb-15	PPPD	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-17-046	SNU-PnBL-PDAC-V064	Validation	J	I	J	J	1.9431	Female	70	11-Jul-17	Distal pancreatectomy	R0	Pancreas cancer	T1	N1	M0	Moderately Differentiated
11-15-051	SNU-PnBL-PDAC-V065	Validation	I	H	K	K	2.5522	Male	68	9-Sep-15	whipple	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-13-003	SNU-PnBL-PDAC-V066	Validation	H	H	I	I	2.5267	Male	41	15-Jan-13	PPPD	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-17-067	SNU-PnBL-PDAC-V067	Validation	H	H	H	H	2.1801	Female	60	15-Sep-17	Distal pancreatectomy	R2	Pancreas cancer	T3	N1	M1	Poorly Differentiated
11-14-055	SNU-PnBL-PDAC-V068	Validation	K	K	H	J	1.7479	Male	79	12-Sep-14	Whipple	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-11-051	SNU-PnBL-PDAC-V069	Validation	J	J	H	H	0.7256	Female	64	18-Aug-11	PPPD	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-14-074	SNU-PnBL-PDAC-V070	Validation	K	I	I	K	1.5930	Female	69	24-Oct-14	PPPD	R0	Pancreas cancer	T2	N0	M0	Moderately Differentiated
11-17-004	SNU-PnBL-PDAC-V071	Validation	K	K	I	J	1.2804	Male	69	31-Jan-17	Whipple	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-15-002	SNU-PnBL-PDAC-V072	Validation	J	J	H	I	4.8247	Female	78	21-Jan-15	Distal pancreatectomy	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-11-033	SNU-PnBL-PDAC-V073	Validation	K	K	J	H	1.0217	Female	74	15-Jun-11	Whipple	R0	Pancreas cancer	T3	N0	M0	Poorly Differentiated
11-16-016	SNU-PnBL-PDAC-V074	Validation	H	K	J	K	1.6084	Male	55	3-May-16	Whipple	R0	Pancreas cancer	T3	N1	M0	Moderately Differentiated
11-14-072	SNU-PnBL-PDAC-V075	Validation	H	H	J	K	3.5825	Female	60	21-Oct-14	PPPD	R0	Pancreas cancer	T2	N0	M0	Poorly Differentiated
11-17-017	SNU-PnBL-PDAC-V076	Validation	J	J	J	K	1.3295	Female	73	24-Mar-17	Whipple	R0	Pancreas cancer	T2	N1	M0	Moderately Differentiated
11-17-022	SNU-PnBL-PDAC-V077	Validation	H	J	K	H	2.1108	Female	75	7-Apr-17	Distal pancreatectomy	R1	Pancreas cancer	T3	N1	M0	Moderately Differentiated

Table 2-3. The 775 detectable protein target candidates and target selection for MRM-MS analysis.

Gene Name	Uniprot Entry	Uniprot Entry name	Protein Name	Tumor marker (201)	Human Protein Atlas (479)	Jamieson et al. (61)	Park et al. (148)	Fold Change > 1.5 (212)	Selected Targets (200)	Final targets (115)
A1CF	Q9NQ94	A1CF_HUMAN	APOBEC1 complementation factor	Yes	No	No	No	Yes	Yes	No
AASS	Q9UDR5	AASS_HUMAN	Alpha-aminoadipic semialdehyde synthase, mitochondrial	No	Yes	No	No	No	No	No
ABCB9	Q9NP78	ABCB9_HUMAN	ATP-binding cassette sub-family B member 9	No	Yes	No	No	No	No	No
ABHD12	Q8N2K0	ABD12_HUMAN	Lysophosphatidylserine lipase ABHD12	No	Yes	No	No	No	No	No
ABHD14A	Q9BUJ0	ABHEA_HUMAN	Protein ABHD14A	No	Yes	No	No	No	No	No
ABL1	P00519	ABL1_HUMAN	Tyrosine-protein kinase ABL1	Yes	No	No	No	No	No	No
ABL2	P42684	ABL2_HUMAN	Tyrosine-protein kinase ABL2	No	Yes	No	No	No	No	No
ABR	Q12979	ABR_HUMAN	Active breakpoint cluster region-related protein	No	Yes	No	No	No	No	No
ACACB	O00763	ACACB_HUMAN	Acetyl-CoA carboxylase 2	No	Yes	No	No	Yes	Yes	Yes
ACADM	P11310	ACADM_HUMAN	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial	No	Yes	No	No	No	No	No
ACADS	P16219	ACADS_HUMAN	Short-chain specific acyl-CoA dehydrogenase, mitochondrial	No	Yes	No	No	No	No	No
ACADVL	P49748	ACADV_HUMAN	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	No	Yes	No	No	Yes	Yes	Yes
ACAT1	P24752	THIL_HUMAN	Acetyl-CoA acetyltransferase, mitochondrial	No	Yes	No	No	No	No	No
ACE	P12821	ACE_HUMAN	Angiotensin-converting enzyme	Yes	No	No	No	No	No	No
ACO1	P21399	ACOC_HUMAN	Cytoplasmic aconitate hydratase	No	Yes	No	No	Yes	Yes	No
ACSL5	Q9ULC5	ACSL5_HUMAN	Long-chain-fatty-acid--CoA ligase 5	No	Yes	No	No	No	No	No
ACSS1	Q9NUB1	ACS2L_HUMAN	Acetyl-coenzyme A synthetase 2-like, mitochondrial	No	Yes	No	No	No	No	No
ACTN1	P12814	ACTN1_HUMAN	Alpha-actinin-1	No	No	No	Yes	No	No	No
ACTN4	O43707	ACTN4_HUMAN	Alpha-actinin-4	No	Yes	Yes	Yes	Yes	Yes	Yes
ACY1	Q03154	ACY1_HUMAN	Aminoacylase-1	No	Yes	No	No	No	No	No
ADAM9	Q13443	ADAM9_HUMAN	Disintegrin and metalloproteinase domain-containing protein 9	No	Yes	Yes	No	Yes	Yes	Yes
ADAM12	O43184	ADA12_HUMAN	Disintegrin and metalloproteinase domain-containing protein 12	Yes	No	No	Yes	No	No	No
ADAM19	Q9H013	ADA19_HUMAN	Disintegrin and metalloproteinase domain-containing protein 19	No	Yes	No	No	No	No	No
ADAMTS12	P58397	ATS12_HUMAN	A disintegrin and metalloproteinase with thrombospondin motifs 12	No	No	No	Yes	No	No	No
ADGRA2	Q96PE1	AGRA2_HUMAN	Adhesion G protein-coupled receptor A2	No	No	No	Yes	No	No	No
ADH1B	P00325	ADH1B_HUMAN	All-trans-retinol dehydrogenase	No	No	No	Yes	No	No	No
ADIPOQ	Q15848	ADIPO_HUMAN	Adiponectin	Yes	No	No	Yes	No	No	No
AEBP1	Q8IUX7	AEBP1_HUMAN	Adipocyte enhancer-binding protein 1	No	No	No	Yes	No	No	No
AFDN	P55196	AFAD_HUMAN	Afadin	No	Yes	No	No	No	No	No
AFP	P02771	FETA_HUMAN	Alpha-fetoprotein	Yes	No	No	No	No	No	No
AGA	P20933	ASPG_HUMAN	N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase	No	Yes	No	No	No	No	No
AGT	P01019	ANGT_HUMAN	Angiotensinogen	No	No	No	Yes	Yes	Yes	Yes
AGXT	P21549	SPYA_HUMAN	Serine--pyruvate aminotransferase	No	Yes	No	No	No	No	No
AHNAK2	Q8IVF2	AHNK2_HUMAN	Protein AHNAK2	No	Yes	No	Yes	No	No	No
AHSG	P02765	FETUA_HUMAN	Alpha-2-HS-glycoprotein	No	Yes	No	No	Yes	Yes	Yes
AKAP9	Q99996	AKAP9_HUMAN	A-kinase anchor protein 9	No	Yes	No	No	Yes	Yes	No
AKAP12	Q02952	AKA12_HUMAN	A-kinase anchor protein 12	Yes	No	No	Yes	No	No	No
AKT1	P31749	AKT1_HUMAN	RAC-alpha serine/threonine-protein kinase	Yes	No	Yes	No	Yes	Yes	Yes
ALAD	P13716	HEM2_HUMAN	Delta-aminolevulinic acid dehydratase	No	Yes	No	No	Yes	Yes	Yes
ALDH1A1	P00352	AL1A1_HUMAN	Retinal dehydrogenase 1	No	Yes	No	No	Yes	Yes	Yes
ALDH4A1	P30038	AL4A1_HUMAN	Delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial	No	Yes	No	No	No	No	No
ALDH6A1	Q02252	MMSA_HUMAN	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial	No	No	No	Yes	Yes	Yes	Yes
ALDOA	P04075	ALDOA_HUMAN	Fructose-bisphosphate aldolase A	No	Yes	No	No	No	No	No
ALG2	Q9H553	ALG2_HUMAN	Alpha-1,3/1,6-mannosyltransferase ALG2	No	Yes	No	No	No	No	No
ALPL	P05186	PPBT_HUMAN	Alkaline phosphatase, tissue-nonspecific isozyme	Yes	No	No	No	No	No	No
AMBP	P02760	AMBP_HUMAN	Protein AMBP [Cleaved into: Alpha-1-microglobulin	Yes	No	No	No	No	No	No
AMPH	P49418	AMPH_HUMAN	Amphiphysin	No	Yes	No	No	No	No	No

ANG	P03950	ANGI_HUMAN	Angiogenin	No	Yes	No	No	No	No	No
ANK2	Q01484	ANK2_HUMAN	Ankyrin-2	No	Yes	No	No	No	No	No
ANLN	Q9NQW6	ANLN_HUMAN	Anillin	No	Yes	No	No	No	No	No
ANO1	Q5XXA6	ANO1_HUMAN	Anoctamin-1	Yes	Yes	No	No	No	No	No
ANPEP	P15144	AMPN_HUMAN	Aminopeptidase N	No	No	Yes	Yes	Yes	Yes	Yes
ANXA1	P04083	ANXA1_HUMAN	Annexin A1	Yes	No	No	Yes	No	No	No
AOX1	Q06278	AOXA_HUMAN	Aldehyde oxidase	No	Yes	No	No	No	No	No
AP2B1	P63010	AP2B1_HUMAN	AP-2 complex subunit beta	No	Yes	No	No	No	No	No
AP3S2	P59780	AP3S2_HUMAN	AP-3 complex subunit sigma-2	No	Yes	No	No	No	No	No
APC	P25054	APC_HUMAN	Adenomatous polyposis coli protein	Yes	No	No	No	Yes	Yes	No
APLP1	P51693	APLP1_HUMAN	Amyloid-like protein 1	No	Yes	No	No	No	No	No
APOA1	P02647	APOA1_HUMAN	Apolipoprotein A-I	Yes	No	No	No	Yes	No	No
APOB	P04114	APOB_HUMAN	Apolipoprotein B-100	Yes	No	No	No	No	No	No
APOC1	P02654	APOC1_HUMAN	Apolipoprotein C-I	No	No	No	Yes	Yes	No	No
APOC3	P02656	APOC3_HUMAN	Apolipoprotein C-III	No	Yes	No	No	No	No	No
APOH	P02749	APOH_HUMAN	Beta-2-glycoprotein 1	No	Yes	No	No	No	No	No
APOL1	O14791	APOL1_HUMAN	Apolipoprotein L1	No	Yes	No	No	No	No	No
APP	P05067	A4_HUMAN	Amyloid-beta precursor protein	No	Yes	No	No	No	No	No
APPL1	Q9UKG1	DP13A_HUMAN	DCC-interacting protein 13-alpha	No	Yes	No	No	No	No	No
ARFGAP3	Q9NP61	ARFG3_HUMAN	ADP-ribosylation factor GTPase-activating protein 3	No	Yes	No	No	No	No	No
ARHGAP24	Q8N264	RHG24_HUMAN	Rho GTPase-activating protein 24	No	Yes	No	No	No	No	No
ARID1A	O14497	ARI1A_HUMAN	AT-rich interactive domain-containing protein 1A	Yes	No	No	No	No	No	No
ARL6IP4	Q66PJ3	AR6P4_HUMAN	ADP-ribosylation factor-like protein 6-interacting protein 4	No	Yes	No	No	Yes	Yes	No
ARMCX3	Q9UH62	ARMX3_HUMAN	Armadillo repeat-containing X-linked protein 3	No	Yes	No	No	No	No	No
ARPC1A	Q92747	ARC1A_HUMAN	Actin-related protein 2/3 complex subunit 1A	No	No	No	Yes	No	No	No
ARPC1B	O15143	ARC1B_HUMAN	Actin-related protein 2/3 complex subunit 1B	No	Yes	No	No	No	No	No
ARPIN	Q7Z6K5	ARPIN_HUMAN	Arpin	No	Yes	No	No	No	No	No
ARSB	P15848	ARSB_HUMAN	Arylsulfatase B	No	Yes	No	No	No	No	No
AS3MT	Q9HBK9	AS3MT_HUMAN	Arsenite methyltransferase	No	No	No	Yes	No	No	No
ASPN	Q9BXN1	ASPN_HUMAN	Asporin	No	No	No	Yes	No	No	No
ASPSCR1	Q9BZE9	ASPC1_HUMAN	Tether containing UBX domain for GLUT4	No	Yes	No	No	No	No	No
ASS1	P00966	ASSY_HUMAN	Argininosuccinate synthase	No	Yes	No	No	No	No	No
ATG4A	Q8WYN0	ATG4A_HUMAN	Cysteine protease ATG4A	No	Yes	No	No	No	No	No
ATM	Q13315	ATM_HUMAN	Serine-protein kinase ATM	Yes	No	No	No	No	No	No
ATP1A1	P05023	AT1A1_HUMAN	Sodium/potassium-transporting ATPase subunit alpha-1	No	Yes	No	No	Yes	Yes	Yes
ATP2A3	Q93084	AT2A3_HUMAN	Sarcoplasmic/endoplasmic reticulum calcium ATPase 3	No	Yes	No	No	No	No	No
ATP2B4	P23634	AT2B4_HUMAN	Plasma membrane calcium-transporting ATPase 4	No	Yes	No	No	No	No	No
ATP5F1A	P25705	ATPA_HUMAN	ATP synthase subunit alpha, mitochondrial	No	No	No	Yes	No	No	No
ATP8A1	Q9Y2Q0	AT8A1_HUMAN	Phospholipid-transporting ATPase IA	No	Yes	No	No	No	No	No
ATR	Q13535	ATR_HUMAN	Serine/threonine-protein kinase ATR	Yes	Yes	No	No	No	No	No
AXL	P30530	UFO_HUMAN	Tyrosine-protein kinase receptor UFO	No	Yes	No	No	No	No	No
B2M	P61769	B2MG_HUMAN	Beta-2-microglobulin	Yes	No	No	No	No	No	No
BAX	Q07812	BAX_HUMAN	Apoptosis regulator BAX	No	No	Yes	No	Yes	Yes	Yes
BBOF1	Q8ND07	BBOF1_HUMAN	Basal body-orientation factor 1	No	Yes	No	No	No	No	No
BCHE	P06276	CHLE_HUMAN	Cholinesterase	Yes	No	No	No	No	No	No
BCL2	P10415	BCL2_HUMAN	Apoptosis regulator Bcl-2	Yes	No	Yes	No	Yes	Yes	No
BCL3	P20749	BCL3_HUMAN	B-cell lymphoma 3 protein	No	Yes	No	No	No	No	No
BCR	P11274	BCR_HUMAN	Breakpoint cluster region protein	No	Yes	No	No	No	No	No
BICD1	Q96G01	BICD1_HUMAN	Protein bicaudal D homolog 1	No	No	No	Yes	No	No	No
BIRC3	Q13489	BIRC3_HUMAN	Baculoviral IAP repeat-containing protein 3	No	Yes	No	No	No	No	No
BLVRA	P53004	BIEA_HUMAN	Biliverdin reductase A	No	Yes	No	No	No	No	No
BRAF	P15056	BRAF_HUMAN	Serine/threonine-protein kinase B-raf	Yes	No	No	No	No	No	No
BRCA1	P38398	BRCA1_HUMAN	Breast cancer type 1 susceptibility protein	Yes	No	No	No	No	No	No

BRCA2	P51587	BRCA2_HUMAN	Breast cancer type 2 susceptibility protein	Yes	No	No	No	No	No	No
BTD	P43251	BTD_HUMAN	Biotinidase	No	Yes	No	No	Yes	Yes	No
BTK	Q06187	BTK_HUMAN	Tyrosine-protein kinase BTK	Yes	No	No	No	No	No	No
C1QB	P02746	C1QB_HUMAN	Complement C1q subcomponent subunit B	No	Yes	No	No	No	No	No
C1QC	P02747	C1QC_HUMAN	Complement C1q subcomponent subunit C	No	Yes	No	No	No	No	No
C1R	P00736	C1R_HUMAN	Complement C1r subcomponent	No	No	No	Yes	Yes	No	No
C1S	P09871	C1S_HUMAN	Complement C1s subcomponent	No	No	No	Yes	No	No	No
C3	P01024	CO3_HUMAN	Complement C3	Yes	No	No	No	No	No	No
C4A	P0C0L4	CO4A_HUMAN	Complement C4-A	No	No	No	Yes	No	No	No
C4BPA	P04003	C4BPA_HUMAN	C4b-binding protein alpha chain	No	No	No	Yes	Yes	No	No
C5	P01031	CO5_HUMAN	Complement C5	No	No	No	Yes	Yes	No	No
C6	P13671	CO6_HUMAN	Complement component C6	No	No	No	Yes	Yes	No	No
C7	P10643	CO7_HUMAN	Complement component C7	Yes	No	No	Yes	No	No	No
C8B	P07358	CO8B_HUMAN	Complement component C8 beta chain	No	Yes	No	No	No	No	No
C8G	P07360	CO8G_HUMAN	Complement component C8 gamma chain	No	Yes	No	No	No	No	No
CACHD1	Q5VU97	CAHD1_HUMAN	VWFA and cache domain-containing protein 1	No	No	No	Yes	No	No	No
CACNA2D1	P54289	CA2D1_HUMAN	Voltage-dependent calcium channel subunit alpha-2/delta-1	No	Yes	No	No	No	No	No
CALD1	Q05682	CALD1_HUMAN	Caldesmon	No	No	No	Yes	No	No	No
CALR	P27797	CALR_HUMAN	Calreticulin	Yes	No	No	No	Yes	Yes	Yes
CAMK1D	Q8IU85	KCC1D_HUMAN	Calcium/calmodulin-dependent protein kinase type 1D	No	Yes	No	No	No	No	No
CANX	P27824	CALX_HUMAN	Calnexin	No	Yes	No	No	No	No	No
CAP1	Q01518	CAP1_HUMAN	Adenylyl cyclase-associated protein 1	No	No	No	Yes	Yes	Yes	Yes
CAPN1	P07384	CAN1_HUMAN	Calpain-1 catalytic subunit	No	Yes	No	Yes	No	No	No
CAT	P04040	CATA_HUMAN	Catalase	No	Yes	No	No	No	No	No
CAV3	P56539	CAV3_HUMAN	Caveolin-3	No	No	Yes	No	No	No	No
CBL	P22681	CBL_HUMAN	E3 ubiquitin-protein ligase CBL	Yes	No	No	No	No	No	No
CCDC69	A6NI79	CCD69_HUMAN	Coiled-coil domain-containing protein 69	Yes	No	No	No	No	No	No
CCDC149	Q6ZUS6	CC149_HUMAN	Coiled-coil domain-containing protein 149	No	Yes	No	No	No	No	No
CCDC154	A6NI56	CC154_HUMAN	Coiled-coil domain-containing protein 154	No	Yes	No	No	Yes	Yes	No
CCNB1	P14635	CCNB1_HUMAN	G2/mitotic-specific cyclin-B1	No	Yes	No	No	Yes	Yes	No
CCND2	P30279	CCND2_HUMAN	G1/S-specific cyclin-D2	Yes	Yes	No	No	No	No	No
CCNE1	P24864	CCNE1_HUMAN	G1/S-specific cyclin-E1	Yes	No	Yes	No	Yes	Yes	Yes
CD2AP	Q9Y5K6	CD2AP_HUMAN	CD2-associated protein	Yes	Yes	No	No	No	No	No
CD5L	O43866	CD5L_HUMAN	CD5 antigen-like	No	Yes	No	No	No	No	No
CD14	P08571	CD14_HUMAN	Monocyte differentiation antigen CD14	Yes	No	No	No	No	No	No
CD19	P15391	CD19_HUMAN	B-lymphocyte antigen CD19	Yes	No	No	No	Yes	Yes	Yes
CD40	P25942	TNR5_HUMAN	Tumor necrosis factor receptor superfamily member 5	No	Yes	No	No	No	No	No
CD55	P08174	DAF_HUMAN	Complement decay-accelerating factor	No	Yes	No	No	No	No	No
CD68	P34810	CD68_HUMAN	Macrosialin	No	Yes	No	No	No	No	No
CD74	P04233	HG2A_HUMAN	HLA class II histocompatibility antigen gamma chain	No	No	Yes	No	No	No	No
CD274	Q9NZQ7	PD1L1_HUMAN	Programmed cell death 1 ligand 1	Yes	No	Yes	No	Yes	Yes	Yes
CDC20	Q12834	CDC20_HUMAN	Cell division cycle protein 20 homolog	No	Yes	No	No	No	No	No
CDH1	P12830	CADH1_HUMAN	Cadherin-1	Yes	No	Yes	No	Yes	Yes	Yes
CDH3	P22223	CADH3_HUMAN	Cadherin-3	Yes	Yes	No	No	No	No	No
CDH11	P55287	CAD11_HUMAN	Cadherin-11	No	No	No	Yes	No	No	No
CDH17	Q12864	CAD17_HUMAN	Cadherin-17	No	No	Yes	No	Yes	Yes	No
CDH23	Q9H251	CAD23_HUMAN	Cadherin-23	No	Yes	No	No	No	No	No
CDKN1B	P46527	CDN1B_HUMAN	Cyclin-dependent kinase inhibitor 1B	Yes	No	Yes	No	Yes	Yes	No
CEACAM5	P06731	CEAM5_HUMAN	Carcinoembryonic antigen-related cell adhesion molecule 5	Yes	No	No	Yes	Yes	Yes	Yes
CENPB	P07199	CENPB_HUMAN	Major centromere autoantigen B	No	Yes	No	No	Yes	Yes	Yes
CENPF	P49454	CENPF_HUMAN	Centromere protein F	No	Yes	No	No	Yes	Yes	No
CEP55	Q53EZ4	CEP55_HUMAN	Centrosomal protein of 55 kDa	No	Yes	No	No	No	No	No
CFB	P00751	CFAB_HUMAN	Complement factor B	Yes	No	No	No	No	No	No

CFH	P08603	CFAH_HUMAN	Complement factor H	No	No	No	Yes	Yes	No	No
CFI	P05156	CFAI_HUMAN	Complement factor I	No	No	No	Yes	No	No	No
CFL1	P23528	COF1_HUMAN	Cofilin-1	No	Yes	No	No	No	No	No
CFLAR	O15519	CFLAR_HUMAN	CASP8 and FADD-like apoptosis regulator	No	No	No	Yes	No	No	No
CGNL1	Q0VF96	CGNL1_HUMAN	Cingulin-like protein 1	No	Yes	No	No	Yes	Yes	No
CHAD	O15335	CHAD_HUMAN	Chondroadherin	No	Yes	No	No	No	No	No
CHD7	Q9P2D1	CHD7_HUMAN	Chromodomain-helicase-DNA-binding protein 7	No	No	No	Yes	No	No	No
CHDH	Q8NE62	CHDH_HUMAN	Choline dehydrogenase, mitochondrial	No	No	No	Yes	No	No	No
CHEK2	O96017	CHK2_HUMAN	Serine/threonine-protein kinase Chk2	Yes	No	No	No	Yes	Yes	Yes
CHGA	P10645	CMGA_HUMAN	Chromogranin-A	Yes	Yes	No	No	Yes	Yes	No
CHI3L1	P36222	CH3L1_HUMAN	Chitinase-3-like protein 1	Yes	No	No	Yes	Yes	Yes	No
CHN2	P52757	CHIO_HUMAN	Beta-chimaerin	Yes	No	No	No	No	No	No
CIRBP	Q14011	CIRBP_HUMAN	Cold-inducible RNA-binding protein	No	Yes	No	No	No	No	No
CKB	P12277	KCRB_HUMAN	Creatine kinase B-type	No	Yes	No	No	No	No	No
CKM	P06732	KCRM_HUMAN	Creatine kinase M-type	Yes	No	No	No	No	No	No
CLCA1	A8K7I4	CLCA1_HUMAN	Calcium-activated chloride channel regulator 1	No	Yes	No	No	Yes	Yes	Yes
CLDN18	P56856	CLD18_HUMAN	Claudin-18	No	No	Yes	No	No	No	No
CLIC1	O00299	CLIC1_HUMAN	Chloride intracellular channel protein 1	No	No	No	Yes	No	No	No
CLU	P10909	CLUS_HUMAN	Clusterin	No	No	No	Yes	Yes	No	No
COBLL1	Q53SF7	COBL1_HUMAN	Cordon-bleu protein-like 1	No	Yes	No	No	No	No	No
COL1A1	P02452	CO1A1_HUMAN	Collagen alpha-1(I) chain	No	No	No	Yes	Yes	Yes	No
COL2A1	P02458	CO2A1_HUMAN	Collagen alpha-1(II) chain	Yes	No	No	No	Yes	Yes	Yes
COL3A1	P02461	CO3A1_HUMAN	Collagen alpha-1(III) chain	No	No	No	Yes	No	No	No
COL4A1	P02462	CO4A1_HUMAN	Collagen alpha-1(IV) chain	No	No	No	Yes	Yes	Yes	No
COL4A2	P08572	CO4A2_HUMAN	Collagen alpha-2(IV) chain	Yes	No	No	Yes	Yes	Yes	No
COL4A3	Q01955	CO4A3_HUMAN	Collagen alpha-3(IV) chain	No	Yes	No	No	No	No	No
COL4A6	Q14031	CO4A6_HUMAN	Collagen alpha-6(IV) chain	Yes	No	No	No	Yes	Yes	Yes
COL5A1	P20908	CO5A1_HUMAN	Collagen alpha-1(V) chain	No	No	No	Yes	No	No	No
COL5A2	P05997	CO5A2_HUMAN	Collagen alpha-2(V) chain	No	No	No	Yes	No	No	No
COL6A2	P12110	CO6A2_HUMAN	Collagen alpha-2(VI) chain	No	No	No	Yes	Yes	Yes	Yes
COL6A3	P12111	CO6A3_HUMAN	Collagen alpha-3(VI) chain	No	No	No	Yes	No	No	No
COL11A1	P12107	COBA1_HUMAN	Collagen alpha-1(XI) chain	No	No	No	Yes	No	No	No
COL12A1	Q99715	COCA1_HUMAN	Collagen alpha-1(XII) chain	No	No	No	Yes	No	No	No
COL17A1	Q9UMD9	COHA1_HUMAN	Collagen alpha-1(XVII) chain	No	Yes	No	No	No	No	No
COL18A1	P39060	COIA1_HUMAN	Collagen alpha-1(XVIII) chain	Yes	No	No	Yes	No	No	No
COL28A1	Q2UY09	COSA1_HUMAN	Collagen alpha-1(XXVIII) chain	Yes	No	No	No	Yes	Yes	Yes
COMP	P49747	COMP_HUMAN	Cartilage oligomeric matrix protein	No	Yes	No	No	No	No	No
COMT	P21964	COMT_HUMAN	Catechol O-methyltransferase	No	Yes	No	No	No	No	No
CP	P00450	CERU_HUMAN	Ceruloplasmin	Yes	No	No	No	No	No	No
CPA1	P15085	CBPA1_HUMAN	Carboxypeptidase A1	No	No	No	Yes	Yes	Yes	Yes
CPB1	P15086	CBPB1_HUMAN	Carboxypeptidase B	Yes	No	No	Yes	Yes	Yes	Yes
CPD	O75976	CBPD_HUMAN	Carboxypeptidase D	No	No	No	Yes	No	No	No
CPM	P14384	CBPM_HUMAN	Carboxypeptidase M	Yes	No	No	No	No	No	No
CPS1	P31327	CPSM_HUMAN	Carbamoyl-phosphate synthase [ammonia], mitochondrial	No	Yes	No	No	No	No	No
CPT2	P23786	CPT2_HUMAN	Carnitine O-palmitoyltransferase 2, mitochondrial	No	Yes	No	No	No	No	No
CR2	P20023	CR2_HUMAN	Complement receptor type 2	No	Yes	No	No	Yes	Yes	Yes
CREBBP	Q92793	CBP_HUMAN	CREB-binding protein	No	Yes	No	No	Yes	Yes	Yes
CRP	P02741	CRP_HUMAN	C-reactive protein	Yes	No	No	No	No	No	No
CRYAB	P02511	CRYAB_HUMAN	Alpha-crystallin B chain	Yes	No	No	No	No	No	No
CST3	P01034	CYTC_HUMAN	Cystatin-C	No	Yes	No	No	No	No	No
CSTB	P04080	CYTB_HUMAN	Cystatin-B	No	Yes	No	No	No	No	No
CTNNB1	P35222	CTNB1_HUMAN	Catenin beta-1	Yes	No	No	No	Yes	Yes	Yes
CTRB2	Q6GPI1	CTRB2_HUMAN	Chymotrypsinogen B2	No	Yes	No	No	Yes	Yes	Yes

CTSD	P07339	CATD_HUMAN	Cathepsin D	No	No	No	Yes	No	No	No
CTSF	Q9UBX1	CATF_HUMAN	Cathepsin F	No	Yes	No	No	No	No	No
CTSH	P09668	CATH_HUMAN	Pro-cathepsin H	No	No	No	Yes	No	No	No
CTSS	P25774	CATS_HUMAN	Cathepsin S	No	No	No	Yes	Yes	Yes	Yes
CXCR4	P61073	CXCR4_HUMAN	C-X-C chemokine receptor type 4	No	No	Yes	No	No	No	No
CYP1A2	P05177	CP1A2_HUMAN	Cytochrome P450 1A2	Yes	No	No	No	Yes	Yes	No
CYP3A5	P20815	CP3A5_HUMAN	Cytochrome P450 3A5	No	Yes	No	No	No	No	No
DAD1	P61803	DAD1_HUMAN	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit DAD1	No	Yes	No	No	No	No	No
DCN	P07585	PGS2_HUMAN	Decorin	No	No	No	Yes	Yes	Yes	Yes
DCXR	Q7Z4W1	DCXR_HUMAN	L-xylulose reductase	No	Yes	No	No	Yes	Yes	No
DEK	P35659	DEK_HUMAN	Protein DEK	No	Yes	No	No	No	No	No
DES	P17661	DESM_HUMAN	Desmin	No	Yes	No	No	No	No	No
DHTKD1	Q96HY7	DHTK1_HUMAN	Probable 2-oxoglutarate dehydrogenase E1 component DHKTD1, mitochondrial	No	Yes	No	No	No	No	No
DIAPH3	Q9NSV4	DIAP3_HUMAN	Protein diaphanous homolog 3	No	Yes	No	No	No	No	No
DLG5	Q8TDM6	DLG5_HUMAN	Disks large homolog 5	No	No	No	Yes	No	No	No
DNAJB4	Q9UDY4	DNJB4_HUMAN	DnaJ homolog subfamily B member 4	No	Yes	No	No	No	No	No
DNAJC15	Q9Y5T4	DJC15_HUMAN	DnaJ homolog subfamily C member 15	Yes	No	No	No	No	No	No
DPT	Q07507	DERM_HUMAN	Dermatopontin	No	Yes	No	No	No	No	No
DPYD	Q12882	DPYD_HUMAN	Dihydropyrimidine dehydrogenase	Yes	Yes	No	No	No	No	No
DSC2	Q02487	DSC2_HUMAN	Desmocollin-2	Yes	No	No	No	No	No	No
DSG1	Q02413	DSG1_HUMAN	Desmoglein-1	No	Yes	No	No	Yes	Yes	No
DSG2	Q14126	DSG2_HUMAN	Desmoglein-2	Yes	Yes	No	No	Yes	Yes	Yes
DSG3	P32926	DSG3_HUMAN	Desmoglein-3	No	Yes	No	No	No	No	No
DSP	P15924	DESP_HUMAN	Desmoplakin	No	Yes	No	No	Yes	Yes	No
DTNA	Q9Y4J8	DTNA_HUMAN	Dystrobrevin alpha	No	Yes	No	No	No	No	No
ECI2	O75521	ECI2_HUMAN	Enoyl-CoA delta isomerase 2, mitochondrial	No	Yes	No	No	Yes	Yes	No
ECM1	Q16610	ECM1_HUMAN	Extracellular matrix protein 1	Yes	Yes	No	Yes	No	No	No
ECT2	Q9H8V3	ECT2_HUMAN	Protein ECT2	No	Yes	No	No	No	No	No
EEF1B2	P24534	EF1B_HUMAN	Elongation factor 1-beta	No	Yes	No	No	No	No	No
EEF1D	P29692	EF1D_HUMAN	Elongation factor 1-delta	No	Yes	No	No	No	No	No
EEF2	P13639	EF2_HUMAN	Elongation factor 2	No	No	No	Yes	No	No	No
EGFR	P00533	EGFR_HUMAN	Epidermal growth factor receptor	Yes	No	Yes	No	Yes	Yes	Yes
EIF2AK3	Q9NZJ5	E2AK3_HUMAN	Eukaryotic translation initiation factor 2-alpha kinase 3	Yes	No	No	No	Yes	Yes	No
EIF3G	O75821	EIF3G_HUMAN	Eukaryotic translation initiation factor 3 subunit G	No	Yes	No	No	No	No	No
ELN	P15502	ELN_HUMAN	Elastin	No	Yes	No	No	No	No	No
ELP1	O95163	ELP1_HUMAN	Elongator complex protein 1	No	Yes	No	No	No	No	No
ENG	P17813	EGLN_HUMAN	Endoglin	Yes	No	No	No	No	No	No
ENO2	P09104	ENOG_HUMAN	Gamma-enolase	Yes	No	No	No	Yes	Yes	Yes
EPB41L4B	Q9H329	E41LB_HUMAN	Band 4.1-like protein 4B	No	Yes	No	No	No	No	No
EPB41L5	Q9HCM4	E41L5_HUMAN	Band 4.1-like protein 5	No	Yes	No	No	Yes	Yes	No
EPCAM	P16422	EPCAM_HUMAN	Epithelial cell adhesion molecule	Yes	No	No	No	No	No	No
EPHX2	P34913	HYES_HUMAN	Bifunctional epoxide hydrolase 2	No	Yes	No	No	Yes	Yes	Yes
ERBB2	P04626	ERBB2_HUMAN	Receptor tyrosine-protein kinase erbB-2	Yes	Yes	Yes	No	No	No	No
ERBB3	P21860	ERBB3_HUMAN	Receptor tyrosine-protein kinase erbB-3	Yes	No	No	No	No	No	No
ERCC1	P07992	ERCC1_HUMAN	DNA excision repair protein ERCC-1	Yes	No	Yes	No	Yes	Yes	No
ERCC2	P18074	ERCC2_HUMAN	General transcription and DNA repair factor IIH helicase subunit XPD	Yes	Yes	No	No	No	No	No
ERO1A	Q96HE7	ERO1A_HUMAN	ERO1-like protein alpha	No	Yes	No	Yes	No	No	No
ETV6	P41212	ETV6_HUMAN	Transcription factor ETV6	No	Yes	No	No	No	No	No
EXOSC10	Q01780	EXOSX_HUMAN	Exosome component 10	No	Yes	No	No	No	No	No
EXT1	Q16394	EXT1_HUMAN	Exostosin-1	No	Yes	No	No	Yes	Yes	No
EZH2	Q15910	EZH2_HUMAN	Histone-lysine N-methyltransferase EZH2	Yes	No	No	No	No	No	No
EZR	P15311	EZRI_HUMAN	Ezrin	No	Yes	Yes	No	No	No	No
F2	P00734	THRB_HUMAN	Prothrombin	No	Yes	No	No	No	No	No

F5	P12259	FA5_HUMAN	Coagulation factor V	No	Yes	No	No	No	No	No
F9	P00740	FA9_HUMAN	Coagulation factor IX	No	Yes	No	No	No	No	No
F10	P00742	FA10_HUMAN	Coagulation factor X	No	Yes	No	No	Yes	No	No
F12	P00748	FA12_HUMAN	Coagulation factor XII	No	Yes	No	No	No	No	No
F13A1	P00488	F13A_HUMAN	Coagulation factor XIII A chain	No	Yes	No	No	Yes	No	No
F13B	P05160	F13B_HUMAN	Coagulation factor XIII B chain	No	Yes	No	No	No	No	No
FAM13A	O94988	FA13A_HUMAN	Protein FAM13A	No	Yes	No	No	Yes	Yes	No
FAM174B	Q3ZCQ3	F174B_HUMAN	Membrane protein FAM174B	No	Yes	No	No	No	No	No
FAP	Q12884	SEPR_HUMAN	Prolyl endopeptidase FAP	No	No	No	Yes	No	No	No
FAS	P25445	TNR6_HUMAN	Tumor necrosis factor receptor superfamily member 6	Yes	No	No	Yes	Yes	Yes	No
FAT1	Q14517	FAT1_HUMAN	Protocadherin Fat 1	Yes	No	No	No	No	No	No
FBL	P22087	FBRL_HUMAN	rRNA 2'-O-methyltransferase fibrillarin	No	Yes	No	No	No	No	No
FBN1	P35555	FBN1_HUMAN	Fibrillin-1	No	Yes	No	No	Yes	Yes	Yes
FBN2	P35556	FBN2_HUMAN	Fibrillin-2	No	Yes	No	No	No	No	No
FBXO16	Q8IX29	FBX16_HUMAN	F-box only protein 16	No	Yes	No	No	No	No	No
FCGBP	Q9Y6R7	FCGBP_HUMAN	IgGFc-binding protein	No	No	No	Yes	No	No	No
FCGR3A	P08637	FCG3A_HUMAN	Low affinity immunoglobulin gamma Fc region receptor III-A	No	Yes	No	No	No	No	No
FECH	P22830	HEMH_HUMAN	Ferrochelatase, mitochondrial	No	Yes	No	No	No	No	No
FGF1	P05230	FGF1_HUMAN	Fibroblast growth factor 1	No	No	Yes	No	Yes	Yes	No
FGFR3	P22607	FGFR3_HUMAN	Fibroblast growth factor receptor 3	Yes	No	No	No	No	No	No
FGFR4	P22455	FGFR4_HUMAN	Fibroblast growth factor receptor 4	Yes	No	No	No	Yes	Yes	No
FGL1	Q08830	FGL1_HUMAN	Fibrinogen-like protein 1	No	Yes	No	No	No	No	No
FIP1L1	Q6UN15	FIP1_HUMAN	Pre-mRNA 3'-end-processing factor FIP1	No	Yes	No	No	No	No	No
FLNA	P21333	FLNA_HUMAN	Filamin-A	Yes	No	No	Yes	No	No	No
FLT1	P17948	VGFR1_HUMAN	Vascular endothelial growth factor receptor 1	Yes	No	Yes	No	Yes	Yes	Yes
FLT4	P35916	VGFR3_HUMAN	Vascular endothelial growth factor receptor 3	Yes	No	No	No	Yes	Yes	Yes
FMR1	Q06787	FMR1_HUMAN	Synaptic functional regulator FMR1	No	Yes	No	No	No	No	No
FN1	P02751	FINC_HUMAN	Fibronectin	No	Yes	No	No	No	No	No
FNDC3A	Q9Y2H6	FND3A_HUMAN	Fibronectin type-III domain-containing protein 3A	Yes	No	No	No	No	No	No
FTCD	O95954	FTCD_HUMAN	Formimidoyltransferase-cyclodeaminase	No	Yes	No	No	No	No	No
FTH1	P02794	FRIH_HUMAN	Ferritin heavy chain	No	Yes	No	No	No	No	No
FTL	P02792	FRIL_HUMAN	Ferritin light chain	No	Yes	No	No	No	No	No
G6PD	P11413	G6PD_HUMAN	Glucose-6-phosphate 1-dehydrogenase	Yes	No	No	No	No	No	No
GALNT2	Q10471	GALT2_HUMAN	Polypeptide N-acetylgalactosaminyltransferase 2	No	Yes	No	No	No	No	No
GATA4	P43694	GATA4_HUMAN	Transcription factor GATA-4	Yes	No	No	No	No	No	No
GATM	P50440	GATM_HUMAN	Glycine amidinotransferase, mitochondrial	No	Yes	No	No	Yes	Yes	Yes
GBP1	P32455	GBP1_HUMAN	Guanylate-binding protein 1	No	Yes	No	No	No	No	No
GC	P02774	VTDB_HUMAN	Vitamin D-binding protein	No	Yes	No	No	No	No	No
GGA2	Q9UJY4	GGA2_HUMAN	ADP-ribosylation factor-binding protein GGA2	No	Yes	No	No	No	No	No
GIPC2	Q8TF65	GIPC2_HUMAN	PDZ domain-containing protein GIPC2	No	Yes	No	No	No	No	No
GLA	P06280	AGAL_HUMAN	Alpha-galactosidase A	No	Yes	No	No	No	No	No
GLB1	P16278	BGAL_HUMAN	Beta-galactosidase	No	Yes	No	No	No	No	No
GMPS	P49915	GUAA_HUMAN	GMP synthase	No	Yes	No	No	No	No	No
GNAZ	P19086	GNAZ_HUMAN	Guanine nucleotide-binding protein G(z) subunit alpha	No	Yes	No	No	No	No	No
GPD1L	Q8N335	GPD1L_HUMAN	Glycerol-3-phosphate dehydrogenase 1-like protein	No	Yes	No	No	No	No	No
GPI	P06744	G6PI_HUMAN	Glucose-6-phosphate isomerase	Yes	No	No	No	No	No	No
GPR137B	O60478	G137B_HUMAN	Integral membrane protein GPR137B	No	Yes	No	No	No	No	No
GPT	P24298	ALAT1_HUMAN	Alanine aminotransferase 1	No	Yes	No	No	No	No	No
GPX2	P18283	GPX2_HUMAN	Glutathione peroxidase 2	No	Yes	No	No	Yes	Yes	Yes
GPX3	P22352	GPX3_HUMAN	Glutathione peroxidase 3	No	Yes	No	No	No	No	No
GRB7	Q14451	GRB7_HUMAN	Growth factor receptor-bound protein 7	No	Yes	No	No	No	No	No
GRHPR	Q9UBQ7	GRHPR_HUMAN	Glyoxylate reductase/hydroxypyruvate reductase	No	Yes	No	No	No	No	No
GRN	P28799	GRN_HUMAN	Progranulin	Yes	No	No	No	No	No	No

GSN	P06396	GELS_HUMAN	Gelsolin	No	Yes	No	No	Yes	Yes	Yes
GSTO1	P78417	GSTO1_HUMAN	Glutathione S-transferase omega-1	No	No	No	Yes	No	No	No
GSTP1	P09211	GSTP1_HUMAN	Glutathione S-transferase P	No	No	No	Yes	No	No	No
H1-0	P07305	H10_HUMAN	Histone H1.0	No	Yes	No	No	No	No	No
H2AFY	O75367	H2AY_HUMAN	Core histone macro-H2A.1	No	Yes	No	No	No	No	No
HARS	P12081	SYHC_HUMAN	Histidine--tRNA ligase, cytoplasmic	No	Yes	No	No	No	No	No
HDGF	P51858	HDGF_HUMAN	Hepatoma-derived growth factor	No	No	Yes	No	No	No	No
HDLBP	Q00341	VIGLN_HUMAN	Vigilin	No	Yes	No	No	No	No	No
HEXA	P06865	HEXA_HUMAN	Beta-hexosaminidase subunit alpha	No	Yes	No	No	Yes	Yes	No
HGF	P14210	HGF_HUMAN	Hepatocyte growth factor	Yes	No	No	No	Yes	Yes	Yes
HIF1A	Q16665	HIF1A_HUMAN	Hypoxia-inducible factor 1-alpha	Yes	No	Yes	Yes	Yes	Yes	No
HLA-B	P01889	HLAB_HUMAN	HLA class I histocompatibility antigen, B alpha chain	No	Yes	No	No	No	No	No
HMGA1	P17096	HMGA1_HUMAN	High mobility group protein HMG-I/HMG-Y	No	Yes	Yes	No	No	No	No
HNRNPK	P61978	HNRPK_HUMAN	Heterogeneous nuclear ribonucleoprotein K	No	No	No	Yes	No	No	No
HOOK1	Q9UJC3	HOOK1_HUMAN	Protein Hook homolog 1	No	Yes	No	No	No	No	No
HOXB3	P14651	HXB3_HUMAN	Homeobox protein Hox-B3	No	Yes	No	No	No	No	No
HOXB7	P09629	HXB7_HUMAN	Homeobox protein Hox-B7	No	No	No	Yes	No	No	No
HP	P00738	HPT_HUMAN	Haptoglobin	No	Yes	No	No	No	No	No
HPX	P02790	HEMO_HUMAN	Hemopexin	No	Yes	No	No	No	No	No
HRAS	P01112	RASH_HUMAN	GTPase Hras	Yes	No	Yes	No	Yes	Yes	Yes
HRG	P04196	HRG_HUMAN	Histidine-rich glycoprotein	No	No	No	Yes	No	No	No
HSP90AA1	P07900	HS90A_HUMAN	Heat shock protein HSP 90-alpha	Yes	No	No	No	Yes	Yes	No
HSP90B1	P14625	ENPL_HUMAN	Endoplasmin	Yes	No	No	No	No	No	No
HSPA5	P11021	BIP_HUMAN	Endoplasmic reticulum chaperone BiP	No	Yes	No	No	No	No	No
HSPB1	P04792	HSPB1_HUMAN	Heat shock protein beta-1	Yes	No	No	Yes	Yes	Yes	Yes
HSPG2	P98160	PGBM_HUMAN	Basement membrane-specific heparan sulfate proteoglycan core protein	Yes	No	No	Yes	No	No	No
HYI	Q5T013	HYI_HUMAN	Putative hydroxypyruvate isomerase	No	Yes	No	No	No	No	No
ICA1	Q05084	ICA69_HUMAN	Islet cell autoantigen 1	No	Yes	No	No	No	No	No
ICAM1	P05362	ICAM1_HUMAN	Intercellular adhesion molecule 1	Yes	No	No	Yes	No	No	No
IDH1	O75874	IDHC_HUMAN	Isocitrate dehydrogenase [NADP], cytoplasmic	Yes	No	No	No	Yes	Yes	Yes
IDH2	P48735	IDHP_HUMAN	Isocitrate dehydrogenase [NADP], mitochondrial	Yes	No	No	Yes	No	No	No
IDUA	P35475	IDUA_HUMAN	Alpha-L-iduronidase	No	Yes	No	No	No	No	No
IFI16	Q16666	IF16_HUMAN	Gamma-interferon-inducible protein 16	No	Yes	No	No	Yes	Yes	Yes
IFRD1	O00458	IFRD1_HUMAN	Interferon-related developmental regulator 1	No	No	No	Yes	No	No	No
IGF1R	P08069	IGF1R_HUMAN	Insulin-like growth factor 1 receptor	Yes	No	No	No	No	No	No
IGF2	P01344	IGF2_HUMAN	Insulin-like growth factor II	Yes	No	No	No	No	No	No
IGFBP1	P08833	IBP1_HUMAN	Insulin-like growth factor-binding protein 1	Yes	No	No	No	No	No	No
IGFBP2	P18065	IBP2_HUMAN	Insulin-like growth factor-binding protein 2	Yes	No	No	No	No	No	No
IGFBP3	P17936	IBP3_HUMAN	Insulin-like growth factor-binding protein 3	Yes	No	No	Yes	Yes	Yes	Yes
IGFBP5	P24593	IBP5_HUMAN	Insulin-like growth factor-binding protein 5	No	Yes	No	No	Yes	Yes	Yes
IL1B	P01584	IL1B_HUMAN	Interleukin-1 beta	Yes	No	No	No	No	No	No
IL1RN	P18510	IL1RA_HUMAN	Interleukin-1 receptor antagonist protein	No	Yes	Yes	No	Yes	Yes	Yes
IL4R	P24394	IL4RA_HUMAN	Interleukin-4 receptor subunit alpha	No	Yes	No	No	Yes	Yes	No
IL5	P05113	IL5_HUMAN	Interleukin-5	Yes	No	No	No	No	No	No
IL6	P05231	IL6_HUMAN	Interleukin-6	Yes	No	No	No	No	No	No
ILF2	Q12905	ILF2_HUMAN	Interleukin enhancer-binding factor 2	No	Yes	No	No	No	No	No
INHBA	P08476	INHBA_HUMAN	Inhibin beta A chain	No	Yes	No	No	No	No	No
INSR	P06213	INSR_HUMAN	Insulin receptor	No	Yes	No	No	Yes	Yes	No
INTS10	Q9NVR2	INT10_HUMAN	Integrator complex subunit 10	No	Yes	No	No	No	No	No
IQGAP1	P46940	IQGA1_HUMAN	Ras GTPase-activating-like protein IQGAP1	No	Yes	No	Yes	No	No	No
IQGAP2	Q13576	IQGA2_HUMAN	Ras GTPase-activating-like protein IQGAP2	No	Yes	No	No	No	No	No
ITGA2B	P08514	ITA2B_HUMAN	Integrin alpha-lib	Yes	No	No	No	Yes	Yes	No
ITGA3	P26006	ITA3_HUMAN	Integrin alpha-3	No	Yes	No	No	Yes	Yes	Yes

ITGA5	P08648	ITA5_HUMAN	Integrin alpha-5	Yes	No	Yes	No	Yes	Yes	Yes
ITGA6	P23229	ITA6_HUMAN	Integrin alpha-6	No	No	Yes	No	Yes	Yes	Yes
ITIH2	P19823	ITIH2_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H2	No	No	No	Yes	No	No	No
ITIH4	Q14624	ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4	Yes	No	No	Yes	No	No	No
ITPRID2	P28290	ITPI2_HUMAN	Protein ITPRID2	No	Yes	No	No	No	No	No
IVD	P26440	IVD_HUMAN	Isovaleryl-CoA dehydrogenase, mitochondrial	No	Yes	No	No	No	No	No
JADE1	Q6IE81	JADE1_HUMAN	Protein Jade-1	No	Yes	No	No	No	No	No
JAK2	O60674	JAK2_HUMAN	Tyrosine-protein kinase JAK2	Yes	No	No	No	No	No	No
JCHAIN	P01591	IGJ_HUMAN	Immunoglobulin J chain	No	No	No	Yes	Yes	No	No
JMY	Q8N9B5	JMY_HUMAN	Junction-mediating and -regulatory protein	No	Yes	No	No	Yes	Yes	Yes
JUP	P14923	PLAK_HUMAN	Junction plakoglobin	No	Yes	No	No	No	No	No
KCNK3	O14649	KCNK3_HUMAN	Potassium channel subfamily K member 3	No	Yes	No	No	No	No	No
KDR	P35968	VGFR2_HUMAN	Vascular endothelial growth factor receptor 2	Yes	No	No	No	No	No	No
KIF1A	Q12756	KIF1A_HUMAN	Kinesin-like protein KIF1A	Yes	No	No	No	No	No	No
KIF2C	Q99661	KIF2C_HUMAN	Kinesin-like protein KIF2C	No	Yes	No	No	Yes	Yes	No
KIRREL1	Q96J84	KIRR1_HUMAN	Kin of IRRE-like protein 1	No	Yes	No	No	No	No	No
KIT	P10721	KIT_HUMAN	Mast/stem cell growth factor receptor Kit	Yes	No	No	No	Yes	Yes	Yes
KLB	Q86Z14	KLOTB_HUMAN	Beta-klotho	Yes	No	No	No	Yes	Yes	No
KLHDC2	Q9Y2U9	KLDC2_HUMAN	Kelch domain-containing protein 2	No	Yes	No	No	No	No	No
KLKB1	P03952	KLKB1_HUMAN	Plasma kallikrein	No	No	No	Yes	No	No	No
KNG1	P01042	KNG1_HUMAN	Kininogen-1	No	Yes	No	No	No	No	No
KPNA2	P52292	IMA1_HUMAN	Importin subunit alpha-1	No	Yes	No	No	Yes	Yes	No
KRAS	P01116	RASK_HUMAN	GTPase Kras	Yes	Yes	No	Yes	Yes	Yes	No
KRT1	P04264	K2C1_HUMAN	Keratin, type II cytoskeletal 1	Yes	No	No	No	No	No	No
KRT5	P13647	K2C5_HUMAN	Keratin, type II cytoskeletal 5	No	Yes	No	No	No	No	No
KRT7	P08729	K2C7_HUMAN	Keratin, type II cytoskeletal 7	Yes	Yes	No	Yes	No	No	No
KRT9	P35527	K1C9_HUMAN	Keratin, type I cytoskeletal 9	Yes	No	No	No	No	No	No
KRT14	P02533	K1C14_HUMAN	Keratin, type I cytoskeletal 14	No	Yes	No	No	No	No	No
KRT17	Q04695	K1C17_HUMAN	Keratin, type I cytoskeletal 17	No	Yes	No	No	Yes	Yes	Yes
KRT18	P05783	K1C18_HUMAN	Keratin, type I cytoskeletal 18	No	Yes	No	Yes	No	No	No
KRT19	P08727	K1C19_HUMAN	Keratin, type I cytoskeletal 19	Yes	Yes	No	Yes	No	No	No
KRT20	P35900	K1C20_HUMAN	Keratin, type I cytoskeletal 20	No	No	Yes	Yes	Yes	Yes	No
LAMA2	P24043	LAMA2_HUMAN	Laminin subunit alpha-2	No	No	No	Yes	No	No	No
LAMA4	Q16363	LAMA4_HUMAN	Laminin subunit alpha-4	No	No	No	Yes	No	No	No
LAMB3	Q13751	LAMB3_HUMAN	Laminin subunit beta-3	No	Yes	No	No	No	No	No
LAMC2	Q13753	LAMC2_HUMAN	Laminin subunit gamma-2	No	Yes	Yes	Yes	No	No	No
LAMP1	P11279	LAMP1_HUMAN	Lysosome-associated membrane glycoprotein 1	No	Yes	No	No	Yes	Yes	Yes
LDB3	O75112	LDB3_HUMAN	LIM domain-binding protein 3	Yes	No	No	No	Yes	Yes	No
LDHA	P00338	LDHA_HUMAN	L-lactate dehydrogenase A chain	No	Yes	No	No	No	No	No
LDHB	P07195	LDHB_HUMAN	L-lactate dehydrogenase B chain	No	No	No	Yes	No	No	No
LDLR	P01130	LDLR_HUMAN	Low-density lipoprotein receptor	No	Yes	No	No	No	No	No
LEMD1	Q68G75	LEMD1_HUMAN	LEM domain-containing protein 1	No	Yes	No	No	No	No	No
LEP	P41159	LEP_HUMAN	Leptin	Yes	No	No	No	Yes	Yes	No
LEPR	P48357	LEPR_HUMAN	Leptin receptor	Yes	No	No	No	No	No	No
LGALS3	P17931	LEG3_HUMAN	Galectin-3	No	No	Yes	No	No	No	No
LGALS4	P56470	LEG4_HUMAN	Galectin-4	No	Yes	No	No	Yes	Yes	Yes
LIFR	P42702	LIFR_HUMAN	Leukemia inhibitory factor receptor	No	No	No	Yes	No	No	No
LMNA	P02545	LMNA_HUMAN	Prelamin-A/C	Yes	No	No	No	No	No	No
LRG1	P02750	A2GL_HUMAN	Leucine-rich alpha-2-glycoprotein	Yes	No	No	Yes	No	No	No
LRIG1	Q96JA1	LRIG1_HUMAN	Leucine-rich repeats and immunoglobulin-like domains protein 1	No	Yes	No	No	No	No	No
LTBP2	Q14767	LTBP2_HUMAN	Latent-transforming growth factor beta-binding protein 2	Yes	No	No	Yes	No	No	No
LTF	P02788	TRFL_HUMAN	Lactotransferrin	No	Yes	No	No	No	No	No
LYZ	P61626	LYSC_HUMAN	Lysozyme C	No	Yes	No	No	No	No	No

MACROD2	A1Z1Q3	MACD2_HUMAN	ADP-ribose glycohydrolase MACROD2	Yes	No	No	No	Yes	Yes	No
MAG	P20916	MAG_HUMAN	Myelin-associated glycoprotein	Yes	No	No	No	Yes	Yes	No
MALT1	Q9UDY8	MALT1_HUMAN	Mucosa-associated lymphoid tissue lymphoma translocation protein 1	Yes	No	No	No	Yes	Yes	No
MAML2	Q8IZL2	MAML2_HUMAN	Mastermind-like protein 2	No	Yes	No	No	No	No	No
MAN1A2	O60476	MA1A2_HUMAN	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IB	No	Yes	No	No	No	No	No
MAP2K1	Q02750	MP2K1_HUMAN	Dual specificity mitogen-activated protein kinase kinase 1	Yes	No	Yes	No	No	No	No
MAP2K2	P36507	MP2K2_HUMAN	Dual specificity mitogen-activated protein kinase kinase 2	Yes	No	No	No	No	No	No
MAP4K4	O95819	M4K4_HUMAN	Mitogen-activated protein kinase kinase kinase kinase 4	No	No	Yes	Yes	Yes	Yes	No
MAPK1	P28482	MK01_HUMAN	Mitogen-activated protein kinase 1	No	No	Yes	No	Yes	Yes	Yes
MAPK8IP1	Q9UQF2	JIP1_HUMAN	C-Jun-amino-terminal kinase-interacting protein 1	No	Yes	No	No	No	No	No
MAST2	Q6P0Q8	MAST2_HUMAN	Microtubule-associated serine/threonine-protein kinase 2	No	Yes	No	No	No	No	No
MBL2	P11226	MBL2_HUMAN	Mannose-binding protein C	No	No	No	Yes	No	No	No
MBOAT2	Q6ZWT7	MBOA2_HUMAN	Lysophospholipid acyltransferase 2	No	Yes	No	No	No	No	No
MCCC1	Q96RQ3	MCCA_HUMAN	Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial	No	Yes	No	No	No	No	No
MCM6	Q14566	MCM6_HUMAN	DNA replication licensing factor MCM6	No	Yes	No	No	No	No	No
MDK	P21741	MK_HUMAN	Midkine	No	No	Yes	No	No	No	No
MECP2	P51608	MECP2_HUMAN	Methyl-CpG-binding protein 2	No	Yes	No	No	Yes	Yes	No
MELK	Q14680	MELK_HUMAN	Maternal embryonic leucine zipper kinase	No	Yes	No	No	No	No	No
MET	P08581	MET_HUMAN	Hepatocyte growth factor receptor	Yes	Yes	No	No	No	No	No
MGAT4A	Q9UM21	MGT4A_HUMAN	Alpha-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase A	No	Yes	No	No	Yes	Yes	No
MGLL	Q99685	MGLL_HUMAN	Monoglyceride lipase	No	Yes	No	No	No	No	No
MIA	Q16674	MIA_HUMAN	Melanoma-derived growth regulatory protein	No	Yes	No	No	Yes	Yes	Yes
MICAL2	O94851	MICA2_HUMAN	[F-actin]-monooxygenase MICAL2	No	Yes	No	No	No	No	No
MKI67	P46013	KI67_HUMAN	Proliferation marker protein Ki-67	Yes	Yes	Yes	No	Yes	Yes	No
MLH1	P40692	MLH1_HUMAN	DNA mismatch repair protein Mlh1	No	Yes	No	No	Yes	Yes	Yes
MLLT1	Q03111	ENL_HUMAN	Protein ENL	No	Yes	No	No	No	No	No
MLLT3	P42568	AF9_HUMAN	Protein AF-9	No	Yes	No	No	No	No	No
MLPH	Q9BV36	MELPH_HUMAN	Melanophilin	No	No	No	Yes	No	No	No
MLYCD	O95822	DCMC_HUMAN	Malonyl-CoA decarboxylase, mitochondrial	No	Yes	No	No	No	No	No
MMP1	P03956	MMP1_HUMAN	Interstitial collagenase	Yes	No	No	No	No	No	No
MMP2	P08253	MMP2_HUMAN	72 kDa type IV collagenase	Yes	No	No	Yes	No	No	No
MMP7	P09237	MMP7_HUMAN	Matrilysin	Yes	No	Yes	No	Yes	Yes	Yes
MMP9	P14780	MMP9_HUMAN	Matrix metalloproteinase-9	Yes	No	No	Yes	Yes	Yes	Yes
MMP11	P24347	MMP11_HUMAN	Stromelysin-3	No	Yes	No	No	Yes	Yes	No
MMP14	P50281	MMP14_HUMAN	Matrix metalloproteinase-14	No	No	No	Yes	No	No	No
MPI	P34949	MPI_HUMAN	Mannose-6-phosphate isomerase	No	Yes	No	No	No	No	No
MPO	P05164	PERM_HUMAN	Myeloperoxidase	Yes	No	No	No	Yes	Yes	Yes
MPP1	Q00013	EM55_HUMAN	55 kDa erythrocyte membrane protein	No	Yes	No	No	No	No	No
MPP6	Q9NZW5	MPP6_HUMAN	MAGUK p55 subfamily member 6	No	Yes	No	No	No	No	No
MPP7	Q5T2T1	MPP7_HUMAN	MAGUK p55 subfamily member 7	No	Yes	No	No	No	No	No
MRTFA	Q969V6	MRTFA_HUMAN	Myocardin-related transcription factor A	No	Yes	No	No	No	No	No
MSANTD3	Q96H12	MSD3_HUMAN	Myb/SANT-like DNA-binding domain-containing protein 3	No	Yes	No	No	Yes	Yes	No
MSH2	P43246	MSH2_HUMAN	DNA mismatch repair protein Msh2	No	Yes	No	No	No	No	No
MSH6	P52701	MSH6_HUMAN	DNA mismatch repair protein Msh6	No	Yes	No	No	No	No	No
MSLN	Q13421	MSLN_HUMAN	Mesothelin	Yes	Yes	No	Yes	No	No	No
MSN	P26038	MOES_HUMAN	Moesin	No	No	No	Yes	No	No	No
MTDH	Q86UE4	LYRIC_HUMAN	Protein LYRIC	No	Yes	No	No	No	No	No
MTHFR	P42898	MTHR_HUMAN	Methylenetetrahydrofolate reductase	No	Yes	No	No	No	No	No
MUC1	P15941	MUC1_HUMAN	Mucin-1	Yes	Yes	No	No	No	No	No
MUC16	Q8WXI7	MUC16_HUMAN	Mucin-16	Yes	No	No	Yes	No	No	No
MXRA5	Q9NR99	MXRA5_HUMAN	Matrix-remodeling-associated protein 5	Yes	No	No	Yes	Yes	Yes	Yes
MYBPC3	Q14896	MYPC3_HUMAN	Myosin-binding protein C, cardiac-type	No	Yes	No	No	Yes	Yes	No
MYH6	P13533	MYH6_HUMAN	Myosin-6	No	Yes	No	No	Yes	Yes	No

MYH9	P35579	MYH9_HUMAN	Myosin-9	No	No	No	Yes	No	No	No
MYH11	P35749	MYH11_HUMAN	Myosin-11	No	Yes	No	No	Yes	Yes	Yes
MYL3	P08590	MYL3_HUMAN	Myosin light chain 3	No	Yes	No	No	No	No	No
MYLK	Q15746	MYLK_HUMAN	Myosin light chain kinase, smooth muscle	No	Yes	No	No	Yes	Yes	Yes
MYO1E	Q12965	MYO1E_HUMAN	Unconventional myosin-Ie	No	Yes	No	No	No	No	No
MYOF	Q9NZM1	MYOF_HUMAN	Myoferlin	No	Yes	No	Yes	Yes	Yes	No
MYRIP	Q8NFW9	MYRIP_HUMAN	Rab effector MyRIP	No	Yes	No	No	No	No	No
NAGLU	P54802	ANAG_HUMAN	Alpha-N-acetylglucosaminidase	No	Yes	No	No	No	No	No
NAT1	P18440	ARY1_HUMAN	Arylamine N-acetyltransferase 1	No	Yes	No	No	No	No	No
NCF4	Q15080	NCF4_HUMAN	Neutrophil cytosol factor 4	No	Yes	No	No	No	No	No
NDC80	O14777	NDC80_HUMAN	Kinetochore protein NDC80 homolog	No	Yes	No	No	No	No	No
NEFH	P12036	NFH_HUMAN	Neurofilament heavy polypeptide	No	Yes	No	No	Yes	Yes	Yes
NEFL	P07196	NFL_HUMAN	Neurofilament light polypeptide	No	Yes	No	No	No	No	No
NEXN	Q0ZGT2	NEXN_HUMAN	Nexilin	No	Yes	No	No	Yes	Yes	Yes
NF1	P21359	NF1_HUMAN	Neurofibromin	Yes	No	No	No	No	No	No
NGF	P01138	NGF_HUMAN	Beta-nerve growth factor	No	Yes	No	No	No	No	No
NIBAN1	Q9BZQ8	NIBA1_HUMAN	Protein Niban 1	No	Yes	No	No	No	No	No
NMU	P48645	NMU_HUMAN	Neuromedin-U	Yes	No	No	No	No	No	No
NOP53	Q9NZM5	NOP53_HUMAN	Ribosome biogenesis protein NOP53	No	Yes	No	No	No	No	No
NOP56	O00567	NOP56_HUMAN	Nucleolar protein 56	No	Yes	No	No	No	No	No
NPC1	O15118	NPC1_HUMAN	NPC intracellular cholesterol transporter 1	No	Yes	No	No	No	No	No
NPC2	P61916	NPC2_HUMAN	NPC intracellular cholesterol transporter 2	Yes	No	No	No	Yes	Yes	Yes
NPM1	P06748	NPM_HUMAN	Nucleophosmin	Yes	No	No	No	Yes	Yes	Yes
NR1D2	Q14995	NR1D2_HUMAN	Nuclear receptor subfamily 1 group D member 2	No	Yes	No	No	No	No	No
NRAS	P01111	RASN_HUMAN	GTPase Nras	Yes	Yes	No	No	Yes	Yes	No
NRP1	O14786	NRP1_HUMAN	Neuropilin-1	No	No	No	Yes	No	No	No
NRP2	O60462	NRP2_HUMAN	Neuropilin-2	No	Yes	No	No	No	No	No
NT5DC1	Q5TFE4	NT5D1_HUMAN	5'-nucleotidase domain-containing protein 1	No	Yes	No	No	No	No	No
NUCB2	P80303	NUCB2_HUMAN	Nucleobindin-2	No	Yes	No	No	Yes	Yes	Yes
NUF2	Q9BZD4	NUF2_HUMAN	Kinetochore protein Nuf2	No	Yes	No	No	No	No	No
NUP210	Q8TEM1	PO210_HUMAN	Nuclear pore membrane glycoprotein 210	No	Yes	No	No	No	No	No
NUP214	P35658	NU214_HUMAN	Nuclear pore complex protein Nup214	No	Yes	No	No	No	No	No
OLFM4	Q6UX06	OLFM4_HUMAN	Olfactomedin-4	No	Yes	No	No	Yes	Yes	Yes
ORC6	Q9Y5N6	ORC6_HUMAN	Origin recognition complex subunit 6	No	Yes	No	No	No	No	No
ORM1	P02763	A1AG1_HUMAN	Alpha-1-acid glycoprotein 1	Yes	No	No	No	No	No	No
OSBPL10	Q9BXB5	OSB10_HUMAN	Oxysterol-binding protein-related protein 10	Yes	Yes	No	No	No	No	No
OXCT1	P55809	SCOT1_HUMAN	Succinyl-CoA:3-ketoacid coenzyme A transferase 1, mitochondrial	No	Yes	No	No	No	No	No
P4HB	P07237	PDIA1_HUMAN	Protein disulfide-isomerase	No	No	No	Yes	No	No	No
PABPC4	Q13310	PABP4_HUMAN	Polyadenylate-binding protein 4	No	No	No	Yes	No	No	No
PACSIN2	Q9UNF0	PACN2_HUMAN	Protein kinase C and casein kinase substrate in neurons protein 2	No	Yes	No	No	Yes	Yes	Yes
PARD3	Q8TEW0	PARD3_HUMAN	Partitioning defective 3 homolog	No	Yes	No	No	No	No	No
PARD6A	Q9NPB6	PAR6A_HUMAN	Partitioning defective 6 homolog alpha	No	Yes	No	No	Yes	Yes	No
PARP1	P09874	PARP1_HUMAN	Poly [ADP-ribose] polymerase 1	No	Yes	No	No	Yes	Yes	Yes
PARP4	Q9UKK3	PARP4_HUMAN	Protein mono-ADP-ribosyltransferase PARP4	No	Yes	No	No	No	No	No
PAWR	Q96IZ0	PAWR_HUMAN	PRKC apoptosis WT1 regulator protein	No	No	No	Yes	No	No	No
PCBP2	Q15366	PCBP2_HUMAN	Poly(rC)-binding protein 2	No	Yes	No	No	No	No	No
PCDH1	Q08174	PCDH1_HUMAN	Protocadherin-1	No	Yes	No	No	No	No	No
PDCD4	Q53EL6	PDCD4_HUMAN	Programmed cell death protein 4	Yes	No	No	Yes	Yes	Yes	Yes
PDE4D	Q08499	PDE4D_HUMAN	cAMP-specific 3',5'-cyclic phosphodiesterase 4D	No	Yes	No	No	Yes	Yes	Yes
PDE4DIP	Q5VU43	MYOME_HUMAN	Myomegalin	No	No	No	Yes	No	No	No
PDGFB	P01127	PDGFB_HUMAN	Platelet-derived growth factor subunit B	Yes	No	No	No	No	No	No
PDGFRA	P16234	PGFRA_HUMAN	Platelet-derived growth factor receptor alpha	Yes	No	No	No	No	No	No
PDGFRB	P09619	PGFRB_HUMAN	Platelet-derived growth factor receptor beta	No	Yes	No	No	No	No	No

PDIA4	P13667	PDIA4_HUMAN	Protein disulfide-isomerase A4	No	Yes	No	No	Yes	Yes	Yes
PDX1	P52945	PDX1_HUMAN	Pancreas/duodenum homeobox protein 1	No	No	Yes	No	Yes	Yes	No
PER1	O15534	PER1_HUMAN	Period circadian protein homolog 1	Yes	No	No	No	Yes	Yes	No
PFKP	Q01813	PFKAP_HUMAN	ATP-dependent 6-phosphofructokinase, platelet type	No	Yes	No	No	No	No	No
PFN1	P07737	PROF1_HUMAN	Profilin-1	No	Yes	No	No	No	No	No
PGC	P20142	PEPC_HUMAN	Gastricsin	Yes	No	No	No	Yes	Yes	Yes
PGD	P52209	6PGD_HUMAN	6-phosphogluconate dehydrogenase, decarboxylating	No	Yes	No	No	No	No	No
PGM3	O95394	AGM1_HUMAN	Phosphoacetylglucosamine mutase	No	Yes	No	No	No	No	No
PHGDH	O43175	SERA_HUMAN	D-3-phosphoglycerate dehydrogenase	No	Yes	No	No	No	No	No
PHYH	O14832	PAHX_HUMAN	Phytanoyl-CoA dioxygenase, peroxisomal	No	Yes	No	No	No	No	No
PICALM	Q13492	PICAL_HUMAN	Phosphatidylinositol-binding clathrin assembly protein	No	Yes	No	No	No	No	No
PIEZO1	Q92508	PIEZ1_HUMAN	Piezo-type mechanosensitive ion channel component 1	No	Yes	No	No	No	No	No
PIGR	P01833	PIGR_HUMAN	Polymeric immunoglobulin receptor	No	Yes	No	No	No	No	No
PIK3CB	P42338	PK3CB_HUMAN	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit beta isoform	Yes	Yes	No	No	No	No	No
PKHD1	P08F94	PKHD1_HUMAN	Fibrocystin	No	Yes	No	No	No	No	No
PKM	P14618	KPYM_HUMAN	Pyruvate kinase PKM	No	Yes	No	Yes	No	No	No
PKP2	Q99959	PKP2_HUMAN	Plakophilin-2	No	Yes	No	No	No	No	No
PLA2R1	Q13018	PLA2R_HUMAN	Secretory phospholipase A2 receptor	No	Yes	No	No	No	No	No
PLAAT5	Q96KN8	PLAT5_HUMAN	Phospholipase A and acyltransferase 5	No	Yes	No	No	No	No	No
PLAT	P00750	TPA_HUMAN	Tissue-type plasminogen activator	No	Yes	No	No	No	No	No
PLEC	Q15149	PLEC_HUMAN	Plectin	Yes	No	No	Yes	No	No	No
PLEK2	Q9NYT0	PLEK2_HUMAN	Pleckstrin-2	No	Yes	No	No	No	No	No
PLEKHG2	Q9H7P9	PKHG2_HUMAN	Pleckstrin homology domain-containing family G member 2	No	Yes	No	No	No	No	No
PLEKHM1	Q9Y4G2	PKHM1_HUMAN	Pleckstrin homology domain-containing family M member 1	No	Yes	No	No	No	No	No
PLG	P00747	PLMN_HUMAN	Plasminogen	No	Yes	No	No	Yes	Yes	Yes
PLS1	Q14651	PLSI_HUMAN	Plastin-1	No	Yes	No	No	No	No	No
PLS3	P13797	PLST_HUMAN	Plastin-3	No	Yes	No	Yes	No	No	No
PLTP	P55058	PLTP_HUMAN	Phospholipid transfer protein	Yes	No	No	No	No	No	No
PMM2	O15305	PMM2_HUMAN	Phosphomannomutase 2	No	Yes	No	No	No	No	No
PNLIP	P16233	LIPP_HUMAN	Pancreatic triacylglycerol lipase	No	Yes	No	No	Yes	Yes	Yes
PNLIPRP1	P54315	LIPR1_HUMAN	Inactive pancreatic lipase-related protein 1	No	No	No	Yes	No	No	No
PNLIPRP2	P54317	LIPR2_HUMAN	Pancreatic lipase-related protein 2	No	Yes	No	No	Yes	Yes	Yes
POLR2H	P52434	RPAB3_HUMAN	DNA-directed RNA polymerases I, II, and III subunit RPABC3	No	Yes	No	No	No	No	No
PON1	P27169	PON1_HUMAN	Serum paraoxonase/arylesterase 1	No	Yes	No	No	No	No	No
PPBP	P02775	CXCL7_HUMAN	Platelet basic protein	Yes	No	No	No	Yes	Yes	Yes
PPIB	P23284	PPIB_HUMAN	Peptidyl-prolyl cis-trans isomerase B	No	Yes	No	No	No	No	No
PPOX	P50336	PPOX_HUMAN	Protoporphyrinogen oxidase	No	Yes	No	No	Yes	Yes	No
PPP2R1B	P30154	2AAB_HUMAN	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform	No	Yes	No	No	No	No	No
PPT1	P50897	PPT1_HUMAN	Palmitoyl-protein thioesterase 1	No	Yes	No	No	No	No	No
PRC1	O43663	PRC1_HUMAN	Protein regulator of cytokinesis 1	No	Yes	No	No	No	No	No
PRDX1	Q06830	PRDX1_HUMAN	Peroxiredoxin-1	No	Yes	No	No	No	No	No
PRDX2	P32119	PRDX2_HUMAN	Peroxiredoxin-2	No	No	No	Yes	No	No	No
PRL	P01236	PRL_HUMAN	Prolactin	Yes	No	No	No	No	No	No
PRODH	O43272	PROD_HUMAN	Proline dehydrogenase 1, mitochondrial	No	Yes	No	No	Yes	Yes	No
PROS1	P07225	PROS_HUMAN	Vitamin K-dependent protein S	Yes	No	No	Yes	No	No	No
PSMB8	P28062	PSB8_HUMAN	Proteasome subunit beta type-8	No	Yes	No	No	No	No	No
PSMD6	Q15008	PSMD6_HUMAN	26S proteasome non-ATPase regulatory subunit 6	No	Yes	No	No	No	No	No
PTBP1	P26599	PTBP1_HUMAN	Polypyrimidine tract-binding protein 1	No	Yes	No	No	No	No	No
PTGS2	P35354	PGH2_HUMAN	Prostaglandin G/H synthase 2	No	Yes	No	No	No	No	No
PTK7	Q13308	PTK7_HUMAN	Inactive tyrosine-protein kinase 7	Yes	No	No	Yes	No	No	No
PTMA	P06454	PTMA_HUMAN	Prothymosin alpha	No	Yes	No	No	Yes	Yes	No
PTPN11	Q06124	PTN11_HUMAN	Tyrosine-protein phosphatase non-receptor type 11	Yes	No	No	No	Yes	Yes	Yes
PTPRE	P23469	PTPRE_HUMAN	Receptor-type tyrosine-protein phosphatase epsilon	Yes	No	No	No	No	No	No

PTPRJ	Q12913	PTPRJ_HUMAN	Receptor-type tyrosine-protein phosphatase eta	No	No	No	Yes	No	No	No
PTPRN2	Q92932	PTPR2_HUMAN	Receptor-type tyrosine-protein phosphatase N2	No	Yes	No	No	No	No	No
QSOX2	Q6ZRP7	QSOX2_HUMAN	Sulfhydryl oxidase 2	No	Yes	No	No	No	No	No
RACK1	P63244	RACK1_HUMAN	Receptor of activated protein C kinase 1	No	Yes	No	No	No	No	No
RAD50	Q92878	RAD50_HUMAN	DNA repair protein RAD50	Yes	No	No	No	No	No	No
RAF1	P04049	RAF1_HUMAN	RAF proto-oncogene serine/threonine-protein kinase	Yes	No	No	No	No	No	No
RANBP17	Q9H2T7	RBP17_HUMAN	Ran-binding protein 17	Yes	No	No	No	Yes	Yes	No
RAP1GAP	P47736	RPGP1_HUMAN	Rap1 GTPase-activating protein 1	No	Yes	No	No	Yes	Yes	No
RB1	P06400	RB_HUMAN	Retinoblastoma-associated protein	Yes	No	No	No	Yes	Yes	No
RBM15	Q96T37	RBM15_HUMAN	RNA-binding protein 15	No	Yes	No	No	No	No	No
RBP4	P02753	RET4_HUMAN	Retinol-binding protein 4	No	Yes	No	No	No	No	No
RECQL5	O94762	RECQ5_HUMAN	ATP-dependent DNA helicase Q5	No	Yes	No	No	No	No	No
REG1A	P05451	REG1A_HUMAN	Lithostathine-1-alpha	No	Yes	No	No	Yes	Yes	Yes
RELA	Q04206	TF65_HUMAN	Transcription factor p65	No	No	Yes	No	Yes	Yes	Yes
REN	P00797	RENI_HUMAN	Renin	No	Yes	No	No	No	No	No
RET	P07949	RET_HUMAN	Proto-oncogene tyrosine-protein kinase receptor Ret	Yes	No	No	No	No	No	No
RFC1	P35251	RFC1_HUMAN	Replication factor C subunit 1	No	Yes	No	No	No	No	No
RFC4	P35249	RFC4_HUMAN	Replication factor C subunit 4	No	Yes	No	No	No	No	No
RGN	Q15493	RGN_HUMAN	Regucalcin	No	Yes	No	No	No	No	No
RNF144A	P50876	R144A_HUMAN	E3 ubiquitin-protein ligase RNF144A	No	Yes	No	No	No	No	No
TROVE2	P10155	RO60_HUMAN	60 kDa SS-A/Ro ribonucleoprotein	No	Yes	No	No	Yes	Yes	Yes
RPL5	P46777	RL5_HUMAN	60S ribosomal protein L5	No	Yes	No	No	Yes	Yes	No
RPL6	Q02878	RL6_HUMAN	60S ribosomal protein L6	No	Yes	No	No	No	No	No
RPL7A	P62424	RL7A_HUMAN	60S ribosomal protein L7a	No	Yes	No	No	No	No	No
RPL10	P27635	RL10_HUMAN	60S ribosomal protein L10	No	Yes	No	No	Yes	Yes	Yes
RPL10A	P62906	RL10A_HUMAN	60S ribosomal protein L10a	No	Yes	No	No	No	No	No
RPL12	P30050	RL12_HUMAN	60S ribosomal protein L12	No	Yes	No	No	No	No	No
RPL36	Q9Y3U8	RL36_HUMAN	60S ribosomal protein L36	No	Yes	No	No	Yes	Yes	Yes
RPLP0	P05388	RLA0_HUMAN	60S acidic ribosomal protein P0	No	Yes	No	No	No	No	No
RPN1	P04843	RPN1_HUMAN	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	No	Yes	No	No	Yes	Yes	Yes
RPN2	P04844	RPN2_HUMAN	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2	No	Yes	No	No	Yes	Yes	Yes
RPS2	P15880	RS2_HUMAN	40S ribosomal protein S2	No	No	No	Yes	No	No	No
RPS4X	P62701	RS4X_HUMAN	40S ribosomal protein S4, X isoform	No	Yes	No	No	No	No	No
RPS5	P46782	RS5_HUMAN	40S ribosomal protein S5	No	Yes	No	No	Yes	Yes	Yes
RPS7	P62081	RS7_HUMAN	40S ribosomal protein S7	No	Yes	No	No	No	No	No
RPS13	P62277	RS13_HUMAN	40S ribosomal protein S13	No	Yes	No	No	No	No	No
RPS15A	P62244	RS15A_HUMAN	40S ribosomal protein S15a	No	Yes	No	No	No	No	No
RPS18	P62269	RS18_HUMAN	40S ribosomal protein S18	No	Yes	No	No	No	No	No
RPS23	P62266	RS23_HUMAN	40S ribosomal protein S23	No	Yes	No	No	No	No	No
RPSA	P08865	RSSA_HUMAN	40S ribosomal protein SA	Yes	No	No	No	No	No	No
RRBP1	Q9P2E9	RRBP1_HUMAN	Ribosome-binding protein 1	No	No	No	Yes	No	No	No
RRM1	P23921	RIR1_HUMAN	Ribonucleoside-diphosphate reductase large subunit	No	No	Yes	No	No	No	No
RRM2	P31350	RIR2_HUMAN	Ribonucleoside-diphosphate reductase subunit M2	No	Yes	No	No	No	No	No
RUNX1	Q01196	RUNX1_HUMAN	Runt-related transcription factor 1	No	Yes	No	No	Yes	Yes	Yes
S100A2	P29034	S10A2_HUMAN	Protein S100-A2	No	Yes	Yes	No	Yes	Yes	Yes
S100A4	P26447	S10A4_HUMAN	Protein S100-A4	Yes	No	Yes	No	Yes	Yes	Yes
S100A6	P06703	S10A6_HUMAN	Protein S100-A6	Yes	No	Yes	No	Yes	Yes	Yes
S100A9	P06702	S10A9_HUMAN	Protein S100-A9	No	Yes	No	No	No	No	No
SCG5	P05408	7B2_HUMAN	Neuroendocrine protein 7B2	No	Yes	No	No	No	No	No
SCGN	O76038	SEGN_HUMAN	Secretagogin	No	Yes	No	No	Yes	Yes	No
SDF4	Q9BRK5	CAB45_HUMAN	45 kDa calcium-binding protein	No	No	No	Yes	No	No	No
SDK1	Q7Z5N4	SDK1_HUMAN	Protein sidekick-1	No	Yes	No	No	No	No	No
SEC63	Q9UGP8	SEC63_HUMAN	Translocation protein SEC63 homolog	No	Yes	No	No	No	No	No

SEL1L	Q9UBV2	SE1L1_HUMAN	Protein sel-1 homolog 1	No	No	No	Yes	Yes	Yes	No
SELENOP	P49908	SEPP1_HUMAN	Selenoprotein P	No	Yes	No	No	No	No	No
SEMA6D	Q8NFY4	SEM6D_HUMAN	Semaphorin-6D	No	Yes	No	No	No	No	No
SEPTIN9	Q9UHD8	SEPT9_HUMAN	Septin-9 (MLL septin-like fusion protein MSF-A)	No	Yes	No	No	No	No	No
SERPINA1	P01009	A1AT_HUMAN	Alpha-1-antitrypsin	No	Yes	No	No	No	No	No
SERPINA4	P29622	KAIN_HUMAN	Kallistatin	No	Yes	No	No	No	No	No
SERPINA5	P05154	IPSP_HUMAN	Plasma serine protease inhibitor	No	Yes	No	No	No	No	No
SERPINA6	P08185	CBG_HUMAN	Corticosteroid-binding globulin	Yes	No	No	No	Yes	Yes	No
SERPINB5	P36952	SPB5_HUMAN	Serpin B5	No	Yes	Yes	No	Yes	Yes	Yes
SERPINC1	P01008	ANT3_HUMAN	Antithrombin-III	No	No	No	Yes	No	No	No
SERPIND1	P05546	HEP2_HUMAN	Heparin cofactor 2	No	Yes	No	No	No	No	No
SERPINE1	P05121	PAI1_HUMAN	Plasminogen activator inhibitor 1	Yes	No	No	No	No	No	No
SERPINE2	P07093	GDN_HUMAN	Glia-derived nexin	No	No	No	Yes	No	No	No
SERPINF1	P36955	PEDF_HUMAN	Pigment epithelium-derived factor	No	No	Yes	No	No	No	No
SERPINF2	P08697	A2AP_HUMAN	Alpha-2-antiplasmin	No	Yes	No	No	No	No	No
SF3B1	O75533	SF3B1_HUMAN	Splicing factor 3B subunit 1	Yes	No	No	No	Yes	Yes	Yes
SFN	P31947	1433S_HUMAN	14-3-3 protein sigma	Yes	Yes	Yes	No	No	No	No
SFRP1	Q8N474	SFRP1_HUMAN	Secreted frizzled-related protein 1	No	Yes	No	No	No	No	No
SFTPB	P07988	PSPB_HUMAN	Pulmonary surfactant-associated protein B	No	No	No	Yes	No	No	No
SGPP2	Q8IWX5	SGPP2_HUMAN	Sphingosine-1-phosphate phosphatase 2	No	Yes	No	No	No	No	No
SGSH	P51688	SPHM_HUMAN	N-sulphoglucosamine sulphohydrolase	No	Yes	No	No	No	No	No
SH3BP4	Q9P0V3	SH3B4_HUMAN	SH3 domain-binding protein 4	No	Yes	No	No	No	No	No
SHISA5	Q8N114	SHSA5_HUMAN	Protein shisa-5	No	Yes	No	No	No	No	No
SHMT2	P34897	GLYM_HUMAN	Serine hydroxymethyltransferase, mitochondrial	No	No	No	Yes	No	No	No
SIK2	Q9H0K1	SIK2_HUMAN	Serine/threonine-protein kinase SIK2	No	Yes	No	No	No	No	No
SKP2	Q13309	SKP2_HUMAN	S-phase kinase-associated protein 2	No	No	Yes	No	No	No	No
SLC1A4	P43007	SATT_HUMAN	Neutral amino acid transporter A	No	Yes	No	No	No	No	No
SLC2A1	P11166	GTR1_HUMAN	Solute carrier family 2, facilitated glucose transporter member 1	No	Yes	Yes	No	Yes	Yes	Yes
SLC3A1	Q07837	SLC31_HUMAN	Neutral and basic amino acid transport protein rBAT	No	Yes	No	No	No	No	No
SLC4A1	P02730	B3AT_HUMAN	Band 3 anion transport protein	No	Yes	No	No	Yes	Yes	Yes
SLC25A20	O43772	MCAT_HUMAN	Mitochondrial carnitine/acylcarnitine carrier protein	No	Yes	No	No	Yes	Yes	No
SLC25A4	P12235	ADT1_HUMAN	ADP/ATP translocase 1	No	Yes	No	Yes	No	No	No
SLC28A3	Q9HAS3	S28A3_HUMAN	Solute carrier family 28 member 3	No	No	Yes	No	No	No	No
SLC29A1	Q99808	S29A1_HUMAN	Equilibrative nucleoside transporter 1	Yes	No	Yes	No	No	No	No
SLC30A2	Q9BRI3	ZNT2_HUMAN	Zinc transporter 2	No	Yes	No	No	No	No	No
SLC38A1	Q9H2H9	S38A1_HUMAN	Sodium-coupled neutral amino acid transporter 1	No	Yes	No	No	No	No	No
SLC39A14	Q15043	S39AE_HUMAN	Zinc transporter ZIP14	No	Yes	No	No	Yes	Yes	No
SLC43A1	O75387	LAT3_HUMAN	Large neutral amino acids transporter small subunit 3	No	Yes	No	No	No	No	No
SLK	Q9H2G2	SLK_HUMAN	STE20-like serine/threonine-protein kinase	Yes	Yes	No	No	No	No	No
SMAD4	Q13485	SMAD4_HUMAN	Mothers against decapentaplegic homolog 4	No	No	Yes	No	Yes	Yes	Yes
SMPD1	P17405	ASM_HUMAN	Sphingomyelin phosphodiesterase	No	Yes	No	No	No	No	No
SMPD4	Q9NXE4	NSMA3_HUMAN	Sphingomyelin phosphodiesterase 4	No	Yes	No	No	No	No	No
SND1	Q7KZF4	SND1_HUMAN	Staphylococcal nuclease domain-containing protein 1	No	Yes	No	No	No	No	No
SNRNP70	P08621	RU17_HUMAN	U1 small nuclear ribonucleoprotein 70 kDa	No	Yes	No	No	No	No	No
SNRPB	P14678	RSMB_HUMAN	Small nuclear ribonucleoprotein-associated proteins B and B'	No	Yes	No	No	No	No	No
SNTB1	Q13884	SNTB1_HUMAN	Beta-1-syntrophin	Yes	No	No	No	Yes	Yes	No
SNX22	Q96L94	SNX22_HUMAN	Sorting nexin-22	No	Yes	No	No	No	No	No
SOD3	P08294	SODE_HUMAN	Extracellular superoxide dismutase	Yes	No	No	No	No	No	No
SP1	P08047	SP1_HUMAN	Transcription factor Sp1	No	Yes	Yes	No	No	No	No
SPAG4	Q9NPE6	SPAG4_HUMAN	Sperm-associated antigen 4 protein	No	Yes	No	No	No	No	No
SPATA13	Q96N96	SPT13_HUMAN	Spermatogenesis-associated protein 13	No	Yes	No	No	No	No	No
SPTAN1	Q13813	SPTN1_HUMAN	Spectrin alpha chain, non-erythrocytic 1	No	Yes	No	No	Yes	Yes	Yes
SRGAP2	O75044	SRGP2_HUMAN	SLIT-ROBO Rho GTPase-activating protein 2	No	Yes	No	No	No	No	No

SRPRA	P08240	SRPRA_HUMAN	Signal recognition particle receptor subunit alpha	No	Yes	No	No	No	No	No
SRPRB	Q9Y5M8	SRPRB_HUMAN	Signal recognition particle receptor subunit beta	No	Yes	No	No	No	No	No
SSB	P05455	LA_HUMAN	Lupus La protein	No	Yes	No	No	No	No	No
STAT1	P42224	STAT1_HUMAN	Signal transducer and activator of transcription 1-alpha/beta	Yes	Yes	No	No	Yes	Yes	No
STAT2	P52630	STAT2_HUMAN	Signal transducer and activator of transcription 2	Yes	No	No	No	No	No	No
STAT3	P40763	STAT3_HUMAN	Signal transducer and activator of transcription 3	No	No	No	Yes	No	No	No
STIL	Q15468	STIL_HUMAN	SCL-interrupting locus protein	No	Yes	No	No	Yes	Yes	Yes
STOX2	Q9P2F5	STOX2_HUMAN	Storkhead-box protein 2	Yes	No	No	No	Yes	Yes	No
STS	P08842	STS_HUMAN	Steryl-sulfatase	No	Yes	No	No	No	No	No
SULF2	Q8IWU5	SULF2_HUMAN	Extracellular sulfatase Sulf-2	No	Yes	No	No	No	No	No
SUMF1	Q8NBK3	SUMF1_HUMAN	Formylglycine-generating enzyme	No	Yes	No	No	No	No	No
SURF6	O75683	SURF6_HUMAN	Surfeit locus protein 6	No	Yes	No	No	Yes	Yes	Yes
SYTL2	Q9HCH5	SYTL2_HUMAN	Synaptotagmin-like protein 2	No	Yes	No	No	No	No	No
SZT2	Q5T011	SZT2_HUMAN	KICSTOR complex protein SZT2	No	Yes	No	No	No	No	No
TACSTD2	P09758	TACD2_HUMAN	Tumor-associated calcium signal transducer 2	No	No	Yes	No	No	No	No
TAL1	P17542	TAL1_HUMAN	T-cell acute lymphocytic leukemia protein 1	No	No	No	Yes	No	No	No
TAP1	Q03518	TAP1_HUMAN	Antigen peptide transporter 1	No	Yes	No	No	No	No	No
TBL1XR1	Q9BZK7	TBL1R_HUMAN	F-box-like/WD repeat-containing protein TBL1XR1	No	Yes	No	No	No	No	No
TBX2	Q13207	TBX2_HUMAN	T-box transcription factor TBX2	No	No	No	Yes	No	No	No
TC2N	Q8N9U0	TAC2N_HUMAN	Tandem C2 domains nuclear protein	No	Yes	No	No	No	No	No
TCAP	O15273	TELT_HUMAN	Telethonin	No	Yes	No	No	Yes	Yes	No
TCEA3	O75764	TCEA3_HUMAN	Transcription elongation factor A protein 3	No	Yes	No	No	No	No	No
TDRD3	Q9H7E2	TDRD3_HUMAN	Tudor domain-containing protein 3	No	Yes	No	No	No	No	No
TEP1	Q99973	TEP1_HUMAN	Telomerase protein component 1	No	Yes	No	No	Yes	Yes	No
TERT	O14746	TERT_HUMAN	Telomerase reverse transcriptase	Yes	No	No	No	No	No	No
TF	P02787	TRFE_HUMAN	Serotransferrin	Yes	No	No	No	Yes	No	No
TFE3	P19532	TFE3_HUMAN	Transcription factor E3	Yes	No	No	No	No	No	No
TFF3	Q07654	TFF3_HUMAN	Trefoil factor 3	No	Yes	No	No	No	No	No
TFRC	P02786	TFR1_HUMAN	Transferrin receptor protein 1	No	Yes	No	No	No	No	No
TGFB1	P01137	TGFB1_HUMAN	Transforming growth factor beta-1 proprotein	No	No	Yes	No	No	No	No
TGM2	P21980	TGM2_HUMAN	Protein-glutamine gamma-glutamyltransferase 2	No	Yes	No	Yes	No	No	No
THBD	P07204	TRBM_HUMAN	Thrombomodulin	Yes	No	No	No	Yes	Yes	No
THBS1	P07996	TSP1_HUMAN	Thrombospondin-1	Yes	No	No	Yes	No	No	No
TKT	P29401	TKT_HUMAN	Transketolase	No	Yes	No	No	Yes	Yes	Yes
TMED2	Q15363	TMED2_HUMAN	Transmembrane emp24 domain-containing protein 2	No	Yes	No	No	No	No	No
TMEM131L	A2VDJ0	T131L_HUMAN	Transmembrane protein 131-like	No	Yes	No	No	No	No	No
TNC	P24821	TENA_HUMAN	Tenascin	No	No	No	Yes	No	No	No
TNFRSF11B	O00300	TR11B_HUMAN	Tumor necrosis factor receptor superfamily member 11B	Yes	No	No	No	No	No	No
TNFRSF21	O75509	TNR21_HUMAN	Tumor necrosis factor receptor superfamily member 21	Yes	No	No	No	No	No	No
TNNC1	P63316	TNNC1_HUMAN	Troponin C, slow skeletal and cardiac muscles	Yes	No	No	No	Yes	Yes	No
TNS4	Q8IZW8	TENS4_HUMAN	Tensin-4	No	Yes	No	No	No	No	No
TOP1	P11387	TOP1_HUMAN	DNA topoisomerase 1	No	No	No	Yes	No	No	No
TOP2A	P11388	TOP2A_HUMAN	DNA topoisomerase 2-alpha	Yes	Yes	No	Yes	No	No	No
TP53	P04637	P53_HUMAN	Cellular tumor antigen p53	Yes	No	Yes	No	Yes	Yes	No
TP63	Q9H3D4	P63_HUMAN	Tumor protein 63	Yes	No	No	No	No	No	No
TPD52L1	Q16890	TPD53_HUMAN	Tumor protein D53	Yes	No	No	No	Yes	Yes	No
TPM1	P09493	TPM1_HUMAN	Tropomyosin alpha-1 chain	No	No	No	Yes	No	No	No
TPMT	P51580	TPMT_HUMAN	Thiopurine S-methyltransferase	Yes	No	No	No	No	No	No
TPP1	O14773	TPP1_HUMAN	Tripeptidyl-peptidase 1	No	Yes	No	No	No	No	No
TPX2	Q9ULW0	TPX2_HUMAN	Targeting protein for Xklp2	No	Yes	No	Yes	No	No	No
TRAP1	Q12931	TRAP1_HUMAN	Heat shock protein 75 kDa, mitochondrial	No	Yes	No	No	No	No	No
TRHDE	Q9UKU6	TRHDE_HUMAN	Thyrotropin-releasing hormone-degrading ectoenzyme	No	Yes	No	No	No	No	No
TRIM29	Q14134	TRI29_HUMAN	Tripartite motif-containing protein 29	No	Yes	No	Yes	No	No	No

TRPS1	Q9UHF7	TRPS1_HUMAN	Zinc finger transcription factor Trps1	No	Yes	No	Yes	No	No	No
TTN	Q8WZ42	TITIN_HUMAN	Titin	No	No	No	Yes	No	No	No
TTR	P02766	TTHY_HUMAN	Transthyretin	Yes	No	No	No	No	No	No
TXNL1	O43396	TXNL1_HUMAN	Thioredoxin-like protein 1	No	Yes	No	No	No	No	No
TYMP	P19971	TYPH_HUMAN	Thymidine phosphorylase	No	No	Yes	No	Yes	Yes	Yes
TYMS	P04818	TYSY_HUMAN	Thymidylate synthase	No	No	Yes	No	Yes	Yes	No
UBE2C	O00762	UBE2C_HUMAN	Ubiquitin-conjugating enzyme E2 C	No	Yes	No	No	No	No	No
UBE2T	Q9NPD8	UBE2T_HUMAN	Ubiquitin-conjugating enzyme E2 T	No	Yes	No	No	No	No	No
UBE4B	O95155	UBE4B_HUMAN	Ubiquitin conjugation factor E4 B	No	Yes	No	No	No	No	No
UCHL1	P09936	UCHL1_HUMAN	Ubiquitin carboxyl-terminal hydrolase isozyme L1	Yes	No	Yes	No	No	No	No
UCHL5	Q9Y5K5	UCHL5_HUMAN	Ubiquitin carboxyl-terminal hydrolase isozyme L5	No	Yes	No	No	Yes	Yes	No
UFL1	O94874	UFL1_HUMAN	E3 UFM1-protein ligase 1	Yes	No	No	No	Yes	Yes	Yes
UGT1A1	P22309	UD11_HUMAN	UDP-glucuronosyltransferase 1-1	Yes	No	No	No	No	No	No
UMPS	P11172	UMPS_HUMAN	Uridine 5'-monophosphate synthase	No	No	Yes	No	No	No	No
UQCRC2	P22695	QCR2_HUMAN	Cytochrome b-c1 complex subunit 2, mitochondrial	No	Yes	No	No	Yes	Yes	Yes
UROD	P06132	DCUP_HUMAN	Uroporphyrinogen decarboxylase	No	Yes	No	No	No	No	No
USP2	O75604	UBP2_HUMAN	Ubiquitin carboxyl-terminal hydrolase 2	No	Yes	No	No	No	No	No
UTRN	P46939	UTRO_HUMAN	Utrophin	No	Yes	No	No	No	No	No
VASP	P50552	VASP_HUMAN	Vasodilator-stimulated phosphoprotein	No	Yes	No	No	No	No	No
VCL	P18206	VINC_HUMAN	Vinculin	No	Yes	No	Yes	Yes	Yes	Yes
VCP	P55072	TERA_HUMAN	Transitional endoplasmic reticulum ATPase	No	No	Yes	No	No	No	No
VEGFA	P15692	VEGFA_HUMAN	Vascular endothelial growth factor A	Yes	No	Yes	No	Yes	Yes	Yes
VEPH1	Q14D04	MELT_HUMAN	Ventricular zone-expressed PH domain-containing protein homolog 1	No	Yes	No	No	No	No	No
VIM	P08670	VIME_HUMAN	Vimentin	No	No	No	Yes	No	No	No
VLDLR	P98155	VLDLR_HUMAN	Very low-density lipoprotein receptor	No	Yes	No	No	No	No	No
VPS51	Q9UID3	VPS51_HUMAN	Vacuolar protein sorting-associated protein 51 homolog	No	Yes	No	No	Yes	Yes	No
VWF	P04275	VWF_HUMAN	von Willebrand factor	Yes	No	No	No	No	No	No
WDR60	Q8WVS4	WDR60_HUMAN	WD repeat-containing protein 60	Yes	No	No	No	Yes	Yes	No
XBP1	P17861	XBP1_HUMAN	X-box-binding protein 1	Yes	No	No	No	No	No	No
YIF1B	Q5BJH7	YIF1B_HUMAN	Protein YIF1B	No	Yes	No	No	No	No	No
YWHAE	P62258	1433E_HUMAN	14-3-3 protein epsilon	Yes	No	No	No	No	No	No
YWHAZ	P63104	1433Z_HUMAN	14-3-3 protein zeta/delta	No	Yes	No	No	No	No	No
ZAP70	P43403	ZAP70_HUMAN	Tyrosine-protein kinase ZAP-70	No	Yes	No	No	No	No	No
ZBED3	Q96IU2	ZBED3_HUMAN	Zinc finger BED domain-containing protein 3	No	Yes	No	No	No	No	No
ZBTB16	Q05516	ZBT16_HUMAN	Zinc finger and BTB domain-containing protein 16	Yes	No	No	No	Yes	Yes	Yes
ZEB1	P37275	ZEB1_HUMAN	Zinc finger E-box-binding homeobox 1	No	No	No	Yes	No	No	No
ZNF22	P17026	ZNF22_HUMAN	Zinc finger protein 22	No	Yes	No	No	No	No	No
ZNF185	O15231	ZN185_HUMAN	Zinc finger protein 185	No	Yes	No	Yes	No	No	No
ZNF462	Q96JM2	ZN462_HUMAN	Zinc finger protein 462	No	Yes	No	No	Yes	Yes	Yes

Table 2-4. The 200 differentially expressed proteins and target selection information.

Gene Name	Uniprot Entry	Uniprot Entry name	Protein Name	Peptide Name	Retention Time , Mean±SD (Min)	Good Prognostic Group , Mean±SD (Peak Area)	Poor Prognostic Group , Mean±SD (Peak Area)	Fold Change (Poor/Good)
A1CF	Q9NQ94	A1CF_HUMAN	APOBEC1 complementation factor	LFVGGIPK	25.83±0.11	2657±1348	1725±1343	0.649
ACACB	O00763	ACACB_HUMAN	Acetyl-CoA carboxylase 2	IPAPVEK	15.64±0.06	41803±18460	63133±62099	1.510
ACADVL	P49748	ACADV_HUMAN	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	AGLGSGLSLSGLVHPELSR	27.26±0.28	2773±775	1730±852	0.624
ACO1	P21399	ACOC_HUMAN	Cytoplasmic aconitate hydratase	GPFLLGIK	25.06±0.11	31444±20281	20283±4130	0.645
ACTN4	O43707	ACTN4_HUMAN	Alpha-actinin-4	NVNVQNFHISWK	23.73±0.18	1181±1353	2652±2038	2.246
ADAM9	Q13443	ADAM9_HUMAN	Disintegrin and metalloproteinase domain-containing protein 9	EVPIYANR	16.51±1.05	2223±1999	3631±3442	1.633
AGT	P01019	ANGT_HUMAN	Angiotensinogen	ALQDQLVLVAAK	23.63±0.09	4099±1815	6649±1888	1.622
AHSG	P02765	FETUA_HUMAN	Alpha-2-HS-glycoprotein	EHAVEGDCDFQLLK	22.02±0.31	5549±1360	3552±832	0.640
AKAP9	Q99996	AKAP9_HUMAN	A-kinase anchor protein 9	QQNQALEK	25.46±0.24	1167±924	593±263	0.508
AKT1	P31749	AKT1_HUMAN	RAC-alpha serine/threonine-protein kinase	NDGTFIGYK	18.84±0.56	7215±3807	11244±2280	1.558
ALAD	P13716	HEM2_HUMAN	Delta-aminolevulinic acid dehydratase	CVLIFGVPSR	26.31±0.19	4793±1209	2904±808	0.606
ALDH1A1	P00352	AL1A1_HUMAN	Retinal dehydrogenase 1	ELGEYGFHEYTEVK	20.27±0.12	20898±9674	12996±1682	0.622
ALDH6A1	Q02252	MMSA_HUMAN	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial	AISFVGSNK	18.21±0.13	32592±21001	21597±9828	0.663
ANPEP	P15144	AMPN_HUMAN	Aminopeptidase N	YLSYTLNPDLIR	26.20±0.10	1654±1417	1060±1142	0.641
APC	P25054	APC_HUMAN	Adenomatous polyposis coli protein	VTSHTELTSNQQSANK	25.20±0.12	1485±386	894±356	0.602
ARL6IP4	Q66PJ3	AR6P4_HUMAN	ADP-ribosylation factor-like protein 6-interacting protein 4	GDGEVLEEIVTK	23.53±0.12	8733±3493	5596±1911	0.641
ATP1A1	P05023	AT1A1_HUMAN	Sodium/potassium-transporting ATPase subunit alpha-1	IVEIPFNSTNK	21.65±0.11	12755±6023	7613±1545	0.597
BAX	Q07812	BAX_HUMAN	Apoptosis regulator BAX	TGALLLQGFIQDR	29.09±0.09	2384±1510	4099±2222	1.720
BCL2	P10415	BCL2_HUMAN	Apoptosis regulator Bcl-2	FATVVEELFR	9.55±0.24	569±418	956±531	1.681
BTD	P43251	BTD_HUMAN	Biotinidase	VDLITFDTPFAGR	23.78±0.13	9223±4233	5277±1687	0.572
CALR	P27797	CALR_HUMAN	Calreticulin	GQTLVVQFTVK	24.09±0.10	3866±3556	2294±836	0.593
CAP1	Q01518	CAP1_HUMAN	Adenylyl cyclase-associated protein 1	LSDLLAPISEQIK	26.82±0.14	8535±3562	5154±859	0.604
CCDC154	A6NI56	CC154_HUMAN	Coiled-coil domain-containing protein 154	ALQGQHEVGPGGR	24.13±0.13	2268±1444	1141±466	0.503
CCNB1	P14635	CCNB1_HUMAN	G2/mitotic-specific cyclin-B1	DIYAYLR	18.27±0.15	94256±35283	61266±6793	0.650
CCNE1	P24864	CCNE1_HUMAN	G1/S-specific cyclin-E1	DSLDLLDK	21.78±0.32	5850±3702	3457±748	0.591
CD19	P15391	CD19_HUMAN	B-lymphocyte antigen CD19	SSEGPSSPSGK	8.12±0.07	4364±2196	2784±1274	0.638
CD274	Q9NZQ7	PD1L1_HUMAN	Programmed cell death 1 ligand 1	LFNVTSTLR	22.82±0.42	2197±1410	4208±5805	1.915
CDH1	P12830	CADH1_HUMAN	Cadherin-1	TIFFCER	22.67±0.65	55847±19350	87932±12293	1.575
CDH17	Q12864	CAD17_HUMAN	Cadherin-17	DPEGLDISYSLR	29.04±0.07	1459±637	911±284	0.624
CDKN1B	P46527	CDN1B_HUMAN	Cyclin-dependent kinase inhibitor 1B	YEWQEVEK	35.06±0.12	186±135	282±208	1.518
CEACAM5	P06731	CEAM5_HUMAN	Carcinoembryonic antigen-related cell adhesion molecule 5	SDLVNEEATGQFR	18.86±0.11	11468±3034	21599±12625	1.883
CEACAM5	P06731	CEAM5_HUMAN	Carcinoembryonic antigen-related cell adhesion molecule 5	QIIGYVIGTQQATPGPAYSGR	22.32±0.12	7786±3650	13970±7031	1.794
CENPB	P07199	CENPB_HUMAN	Major centromere autoantigen B	AAGLPVK	16.12±0.08	63174±31666	31460±8782	0.498
CENPF	P49454	CENPF_HUMAN	Centromere protein F	HQASSSVFSWQQEK	17.40±0.35	15482±7758	9451±6309	0.610
CGNL1	Q0VF96	CGNL1_HUMAN	Cingulin-like protein 1	LTIEVAELQR	18.51±0.20	3481±1710	5410±1731	1.554
CHEK2	O96017	CHK2_HUMAN	Serine/threonine-protein kinase Chk2	LLVVDPK	18.52±0.48	32232±22699	19606±4200	0.608
CHGA	P10645	CMGA_HUMAN	Chromogranin-A	GYPEEK	18.73±0.21	14686±11716	27036±14284	1.841
CHI3L1	P36222	CH3L1_HUMAN	Chitinase-3-like protein 1	GTTGHHSPLFR	32.12±0.20	1327±1267	784±1004	0.591
CLCA1	A8K7I4	CLCA1_HUMAN	Calcium-activated chloride channel regulator 1	SIQLESK	16.00±0.08	50421±45424	29506±6411	0.585
COL1A1	P02452	CO1A1_HUMAN	Collagen alpha-1(I) chain	DGEAGAQGPPGPAGPAGER	9.69±0.11	2802±833	1780±1129	0.635
COL2A1	P02458	CO2A1_HUMAN	Collagen alpha-1(II) chain	GPEGAQGPR	8.33±0.19	4859±4581	2296±2022	0.473
COL4A1	P02462	CO4A1_HUMAN	Collagen alpha-1(IV) chain	GSPGFPGEPGYPGLIGR	23.81±0.17	2221±1038	1362±588	0.613
COL4A2	P08572	CO4A2_HUMAN	Collagen alpha-2(IV) chain	GQTGFPGLTGPPGSQGELGR	9.61±0.06	3638±1755	2159±1090	0.593
COL4A6	Q14031	CO4A6_HUMAN	Collagen alpha-6(IV) chain	GLPGFPGLPGK	25.42±0.08	9620±1009	6078±2463	0.632
COL6A2	P12110	CO6A2_HUMAN	Collagen alpha-2(VI) chain	VFAVVITDGR	22.84±0.13	6292±2323	3472±1938	0.552
COL28A1	Q2UY09	COSA1_HUMAN	Collagen alpha-1(XXVIII) chain	IGIINYSHK	17.60±0.29	2645±1790	4596±2521	1.738
CPA1	P15085	CBPA1_HUMAN	Carboxypeptidase A1	TEPVPDQDELDQLSK	21.22±0.07	20208±24965	11526±7557	0.570
CPB1	P15086	CBPB1_HUMAN	Carboxypeptidase B	ATCEETFLAIK	24.13±0.13	15252±8442	9896±6700	0.649

CR2	P20023	CR2_HUMAN	Complement receptor type 2	VAACEATGR	8.87±0.09	17117±10575	11215±6069	0.655
CREBBP	Q92793	CBP_HUMAN	CREB-binding protein	DAAYYSYQNR	16.61±0.27	1048±614	1941±1742	1.852
CTNNB1	P35222	CTNB1_HUMAN	Catenin beta-1	LHYGLPVVVK	22.11±0.16	8804±3144	14457±5931	1.642
CTRB2	Q6GPI1	CTRB2_HUMAN	Chymotrypsinogen B2	TCSTTTPAVYAR	16.10±0.94	79866±68133	47534±38802	0.595
CTSS	P25774	CATS_HUMAN	Cathepsin S	YTELPYGR	18.14±0.33	1894±965	3322±1090	1.754
CYP1A2	P05177	CP1A2_HUMAN	Cytochrome P450 1A2	DTTLNGFYIPK	19.35±0.12	17333±3222	11304±2043	0.652
DCN	P07585	PGS2_HUMAN	Decorin	DLPPDTTLLDLQNNK	24.84±0.14	3486±929	2113±715	0.606
DCXR	Q7Z4W1	DCXR_HUMAN	L-xylulose reductase	AVTNHSVYCSTK	25.80±0.11	25810±9155	16716±4832	0.648
DSG1	Q02413	DSG1_HUMAN	Desmoglein-1	EQYGQYALAVR	23.12±0.38	631±175	1392±1070	2.207
DSG2	Q14126	DSG2_HUMAN	Desmoglein-2	AWITAPVALR	25.47±0.16	4510±1467	7085±2147	1.571
DSP	P15924	DESP_HUMAN	Desmoplakin	QHLEIELK	9.58±0.07	31577±5188	59547±58738	1.886
ECI2	O75521	ECI2_HUMAN	Enoyl-CoA delta isomerase 2, mitochondrial	WLSDECTNAVVNFLSR	21.95±0.18	2523±677	1485±570	0.588
EGFR	P00533	EGFR_HUMAN	Epidermal growth factor receptor	CNLLEGEPR	18.46±0.31	4436±1181	2943±1390	0.663
EIF2AK3	Q9NZJ5	E2AK3_HUMAN	Eukaryotic translation initiation factor 2-alpha kinase 3	GQLYLQSSVR	25.71±0.12	16616±3159	10751±1986	0.647
ENO2	P09104	ENOG_HUMAN	Gamma-enolase	IEEELGDEAR	16.08±1.02	7809±5059	14605±6321	1.870
EPB41L5	Q9HCM4	E41L5_HUMAN	Band 4.1-like protein 5	LPGLGEPEVEYETLK	27.64±0.17	6303±5820	4068±1733	0.645
EPHX2	P34913	HYES_HUMAN	Bifunctional epoxide hydrolase 2	FLLDTLK	24.80±0.08	15718±13955	10174±3395	0.647
ERCC1	P07992	ERCC1_HUMAN	DNA excision repair protein ERCC-1	LEQDFVSR	23.43±0.18	6655±3135	4062±2137	0.610
EXT1	Q16394	EXT1_HUMAN	Exostosin-1	NSLIWNK	9.49±0.21	23276±12687	15414±7166	0.662
FAM13A	O94988	FA13A_HUMAN	Protein FAM13A	DFEDNFFR	18.23±0.20	3303±2554	5663±1165	1.714
FAS	P25445	TNR6_HUMAN	Tumor necrosis factor receptor superfamily member 6	NDNVQDTAEQK	18.57±0.12	15169±5801	9496±2074	0.626
FBN1	P35555	FBN1_HUMAN	Fibrillin-1	GFIPNIR	23.06±0.19	115383±25538	174036±62341	1.508
FGF1	P05230	FGF1_HUMAN	Fibroblast growth factor 1	NWFVGLK	9.64±0.13	1449±523	900±842	0.621
FGFR4	P22455	FGFR4_HUMAN	Fibroblast growth factor receptor 4	YNYLLDVLER	26.73±0.22	2901±1371	4572±2028	1.576
FLT1	P17948	VGFR1_HUMAN	Vascular endothelial growth factor receptor 1	GFIISNATYK	21.22±0.52	1959±1205	4315±3614	2.203
FLT4	P35916	VGFR3_HUMAN	Vascular endothelial growth factor receptor 3	SGVDLADSNQK	18.29±0.15	3954±1050	5989±2613	1.515
GATM	P50440	GATM_HUMAN	Glycine amidinotransferase, mitochondrial	AENACVPPFTIEVK	24.17±0.13	27295±19879	15337±11271	0.562
GPX2	P18283	GPX2_HUMAN	Glutathione peroxidase 2	LIIWSPVR	26.50±0.20	3377±3522	1682±682	0.498
GSN	P06396	GELS_HUMAN	Gelsolin	EPGLQIWR	24.04±0.22	6117±858	3601±1056	0.589
HEXA	P06865	HEXA_HUMAN	Beta-hexosaminidase subunit alpha	DFYIVEPLAFEGTPEQK	20.05±0.18	5182±1846	3357±1234	0.648
HGF	P14210	HGF_HUMAN	Hepatocyte growth factor	DYEAWLGIHDVHGR	23.16±0.32	410575±508759	271521±262813	0.661
HIF1A	Q16665	HIF1A_HUMAN	Hypoxia-inducible factor 1-alpha	GQVTTGQYR	17.17±0.94	2690±1164	4092±1205	1.521
HRAS	P01112	RASH_HUMAN	GTPase Hras	SYGIPYIETSAK	22.93±0.34	30362±19461	50242±24149	1.655
HSP90AA1	P07900	HS90A_HUMAN	Heat shock protein HSP 90-alpha	CLELFTELAEDK	23.21±0.28	1824±1404	1176±655	0.645
HSPB1	P04792	HSPB1_HUMAN	Heat shock protein beta-1	QLSSGVSEIR	17.43±0.10	29865±7268	46012±9081	1.541
IDH1	O75874	IDHC_HUMAN	Isocitrate dehydrogenase [NADP], cytoplasmic	GQETSTNPIASIFAWTR	34.25±0.57	2026±1315	1324±1158	0.653
IFI16	Q16666	IF16_HUMAN	Gamma-interferon-inducible protein 16	SLLSNDLK	19.86±0.28	52856±26470	34438±8810	0.652
IGFBP3	P17936	IBP3_HUMAN	Insulin-like growth factor-binding protein 3	GLCVNASAVSR	17.18±0.09	7391±1455	11827±2287	1.600
IGFBP5	P24593	IBP5_HUMAN	Insulin-like growth factor-binding protein 5	GICWCVDK	21.21±0.12	5241±1898	8298±5636	1.583
IL1RN	P18510	IL1RA_HUMAN	Interleukin-1 receptor antagonist protein	LQLEAVNITDLSENR	25.35±0.47	2271±1202	1489±622	0.656
IL4R	P24394	IL4RA_HUMAN	Interleukin-4 receptor subunit alpha	EWWDQIPNPAR	23.52±0.19	2156±1781	6192±10842	2.871
INSR	P06213	INSR_HUMAN	Insulin receptor	IELQACNQDTPEER	24.66±0.56	901±754	1995±1469	2.214
ITGA2B	P08514	ITA2B_HUMAN	Integrin alpha-lib	ASVQLLVQDSLNPAVK	21.31±0.27	5337±2641	3545±750	0.664
ITGA3	P26006	ITA3_HUMAN	Integrin alpha-3	SQPSETER	7.93±0.24	1650±867	2735±1300	1.657
ITGA5	P08648	ITA5_HUMAN	Integrin alpha-5	SLQWFGATVR	26.21±0.23	10837±3213	16769±5474	1.547
ITGA6	P23229	ITA6_HUMAN	Integrin alpha-6	CYVLSQNLR	19.52±0.71	3492±2009	7553±3385	2.163
JMY	Q8N9B5	JMY_HUMAN	Junction-mediating and -regulatory protein	SSAWAEGGSPR	16.28±0.17	9718±8985	17344±5512	1.785
KIF2C	Q99661	KIF2C_HUMAN	Kinesin-like protein KIF2C	LFDLLNK	21.85±0.62	4428±2679	11456±2710	2.587
KIT	P10721	KIT_HUMAN	Mast/stem cell growth factor receptor Kit	HGLSNSIYVFVR	23.62±0.24	4675±2078	7112±4503	1.521
KLB	Q86Z14	KLOTB_HUMAN	Beta-klotho	LAVIPWGVR	26.21±0.07	5575±2075	9793±1382	1.756
KPNA2	P52292	IMA1_HUMAN	Importin subunit alpha-1	ASLSLIEK	11.68±0.82	25030±8020	16349±8056	0.653
KRAS	P01116	RASK_HUMAN	GTPase Kras	SFEDIHHYR	20.77±0.11	6399±2101	9913±2177	1.549
KRT17	Q04695	K1C17_HUMAN	Keratin, type I cytoskeletal 17	ASLEGNLAETENR	18.40±0.14	2895±1615	4688±2087	1.619

KRT20	P35900	K1C20_HUMAN	Keratin, type I cytoskeletal 20	DAQLQNAR	16.51±1.02	2223±1999	3631±3442	1.633
LAMP1	P11279	LAMP1_HUMAN	Lysosome-associated membrane glycoprotein 1	ALQATVGNSYK	16.34±0.96	14251±6396	22645±6637	1.589
LDB3	O75112	LDB3_HUMAN	LIM domain-binding protein 3	SWHPEEFTCAYCK	9.88±0.10	6212±1030	9946±5256	1.601
LEP	P41159	LEP_HUMAN	Leptin	DLLHVLAFSK	23.35±0.34	2280±1267	1487±735	0.652
LGALS4	P56470	LEG4_HUMAN	Galectin-4	VVFNTLQGGK	18.98±0.25	43170±38398	23072±8932	0.534
MACROD2	A1Z1Q3	MACD2_HUMAN	ADP-ribose glycohydrolase MACROD2	YVIHTVGPIAR	9.63±0.10	2154±1199	1009±489	0.469
MAG	P20916	MAG_HUMAN	Myelin-associated glycoprotein	SLELPFQGAHR	21.79±0.18	3877±1897	2438±813	0.629
MALT1	Q9UDY8	MALT1_HUMAN	Mucosa-associated lymphoid tissue lymphoma translocation protein 1	NAVDEFLLLLDK	23.66±0.15	31743±22025	21126±8579	0.666
MAP4K4	O95819	M4K4_HUMAN	Mitogen-activated protein kinase kinase kinase kinase 4	EDWIAYISR	18.11±0.12	10900±2518	7266±1443	0.667
MAPK1	P28482	MK01_HUMAN	Mitogen-activated protein kinase 1	LFPNADSK	16.69±0.29	13635±7562	40063±66746	2.938
MECP2	P51608	MECP2_HUMAN	Methyl-CpG-binding protein 2	GGSLESDGCPK	24.13±0.24	21198±6274	13282±2983	0.627
MGAT4A	Q9UM21	MGT4A_HUMAN	Alpha-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase A	SSELNTIVQQFK	21.09±0.41	4219±1988	1792±1220	0.425
MIA	Q16674	MIA_HUMAN	Melanoma-derived growth regulatory protein	LGYFPSSIVR	24.92±0.34	1873±753	3894±3212	2.079
MKI67	P46013	KI67_HUMAN	Proliferation marker protein Ki-67	EEAQALEDLTGFK	23.80±0.14	26238±11123	17446±3197	0.665
MLH1	P40692	MLH1_HUMAN	DNA mismatch repair protein Mlh1	LVESTSLR	16.20±0.37	1020±554	2085±557	2.043
MMP7	P09237	MMP7_HUMAN	Matrilysin	EIPLHFR	19.96±0.12	2637±1125	3981±1278	1.510
MMP9	P14780	MMP9_HUMAN	Matrix metalloproteinase-9	QLSLPETGELDSATLK	23.73±0.15	893±174	514±120	0.576
MMP11	P24347	MMP11_HUMAN	Stromelysin-3	FPVHAALVWGPEK	23.78±0.14	5066±1416	3330±560	0.657
MPO	P05164	PERM_HUMAN	Myeloperoxidase	FPTDQLTPDQER	18.99±0.21	5993±2950	11687±5800	1.950
MPO	P05164	PERM_HUMAN	Myeloperoxidase	VVLEGGIDPILR	24.20±0.29	15663±5449	27756±14941	1.772
MSANTD3	Q96H12	MSD3_HUMAN	Myb/SANT-like DNA-binding domain-containing protein 3	TWQALAHEYNSQPSVSLR	23.68±0.29	24348±16650	16174±8337	0.664
MXRA5	Q9NR99	MXRA5_HUMAN	Matrix-remodeling-associated protein 5	VLVQSPSTQPAEK	15.87±0.14	6783±3104	4427±2451	0.653
MYBPC3	Q14896	MYPC3_HUMAN	Myosin-binding protein C, cardiac-type	DQAVFK	16.55±0.55	3005±2644	5514±3579	1.835
MYH6	P13533	MYH6_HUMAN	Myosin-6	LELQSALEEAEASLEHEEGK	22.81±0.39	6596±2806	4246±767	0.644
MYH11	P35749	MYH11_HUMAN	Myosin-11	QLVSNLEK	16.93±0.27	14677±18968	5348±4904	0.364
MYLK	Q15746	MYLK_HUMAN	Myosin light chain kinase, smooth muscle	DLEVVEGSAAR	18.34±0.26	5487±972	10246±5432	1.867
MYLK	Q15746	MYLK_HUMAN	Myosin light chain kinase, smooth muscle	QISEGVEYIHK	9.68±0.03	14522±5941	9491±2001	0.654
MYOF	Q9NZM1	MYOF_HUMAN	Myoferlin	EDIVPQLK	9.64±0.08	15254±8047	23019±8400	1.509
NEFH	P12036	NFH_HUMAN	Neurofilament heavy polypeptide	IGFGPIPFSLPEGLPK	33.21±0.29	22653±15709	14379±7105	0.635
NEXN	Q0ZGT2	NEXN_HUMAN	Nexilin	LTFEELER	23.09±0.32	7431±1819	11806±5124	1.589
NPC2	P61916	NPC2_HUMAN	NPC intracellular cholesterol transporter 2	SGINCPIQK	15.81±0.23	8970±4412	15604±9572	1.740
NPM1	P06748	NPM_HUMAN	Nucleophosmin	VDNDENEHQLSLR	16.76±0.75	9843±8463	14814±3542	1.505
NRAS	P01111	RASN_HUMAN	GTPase Nras	SFADINLYR	22.58±0.62	1698±649	1103±389	0.650
NUCB2	P80303	NUCB2_HUMAN	Nucleobindin-2	ADIEEIK	16.57±0.37	29674±15507	16721±6335	0.563
OLFM4	Q6UX06	OLFM4_HUMAN	Olfactomedin-4	GLYWVAPLNTDGR	26.47±0.27	16534±8637	25813±13446	1.561
PACSIN2	Q9UNF0	PACN2_HUMAN	Protein kinase C and casein kinase substrate in neurons protein 2	HLDLSNVAGYK	19.30±0.15	7972±4029	5023±960	0.630
PARD6A	Q9NPB6	PAR6A_HUMAN	Partitioning defective 6 homolog alpha	VAPQGLER	17.01±0.15	16752±9163	9546±6029	0.570
PARP1	P09874	PARP1_HUMAN	Poly [ADP-ribose] polymerase 1	GGAAVDPDSGLEHSAHVLEK	18.23±0.31	3010±1113	1425±981	0.473
PDCD4	Q53EL6	PDCD4_HUMAN	Programmed cell death protein 4	DLPELALDTPR	24.59±0.12	7402±4780	4603±2026	0.622
PDE4D	Q08499	PDE4D_HUMAN	cAMP-specific 3',5'-cyclic phosphodiesterase 4D	TEQEDVLAK	16.09±0.06	45285±17871	27295±18815	0.603
PDIA4	P13667	PDIA4_HUMAN	Protein disulfide-isomerase A4	VDATAETDLAK	15.58±0.56	21221±23655	38640±20511	1.821
PDX1	P52945	PDX1_HUMAN	Pancreas/duodenum homeobox protein 1	VQLPFPWMK	9.61±0.15	2006±352	1234±899	0.615
PER1	O15534	PER1_HUMAN	Period circadian protein homolog 1	AVLSLHTQK	24.15±0.36	2970±1086	4715±1588	1.588
PGC	P20142	PEPC_HUMAN	Gastricsin	GLLGEFLR	29.08±0.31	170431±236012	11094±17362	0.065
PLG	P00747	PLMN_HUMAN	Plasminogen	HSIFTPETNPR	17.84±0.26	21994±5192	33278±12040	1.513
PNLIP	P16233	LIPP_HUMAN	Pancreatic triacylglycerol lipase	FIWYNNVINPTLPR	29.28±0.25	21495±28293	8646±12134	0.402
PNLIPRP2	P54317	LIPR2_HUMAN	Pancreatic lipase-related protein 2	IALYGSNENSK	17.32±0.19	6004±6893	1882±1463	0.313
PPBP	P02775	CXCL7_HUMAN	Platelet basic protein	ICLDPDAPR	18.09±0.10	6581±1759	4220±1694	0.641
PPOX	P50336	PPOX_HUMAN	Protoporphyrinogen oxidase	TLLLVSELGLDSEVLPVR	21.05±0.29	7655±1597	4473±1085	0.584
PRODH	O43272	PROD_HUMAN	Proline dehydrogenase 1, mitochondrial	QLLWLELLR	26.62±0.11	812±440	2020±977	2.489
PTMA	P06454	PTMA_HUMAN	Prothymosin alpha	EVVEEAENGR	20.67±0.46	2489±2051	4334±2945	1.742
PTPN11	Q06124	PTN11_HUMAN	Tyrosine-protein phosphatase non-receptor type 11	SNPGDFTLSVR	21.55±0.23	13496±3042	20832±3687	1.544
RANBP17	Q9H2T7	RBP17_HUMAN	Ran-binding protein 17	GIAFALNTK	9.86±0.05	18113±11779	9524±5706	0.526

RAP1GAP	P47736	RPGP1_HUMAN	Rap1 GTPase-activating protein 1	LPYTEGDAQQLQR	16.59±0.28	2965±2757	4774±1724	1.610
RB1	P06400	RB_HUMAN	Retinoblastoma-associated protein	FPSSPLR	9.59±0.15	2616±728	1490±1059	0.569
REG1A	P05451	REG1A_HUMAN	Lithostathine-1-alpha	ESGTDDFNVWIGLHDPK	26.32±0.31	3709±3088	2341±1628	0.631
RELA	Q04206	TF65_HUMAN	Transcription factor p65	DLEQAISQR	18.69±0.17	2757±1720	4164±3205	1.510
TROVE2	P10155	RO60_HUMAN	60 kDa SS-A/Ro ribonucleoprotein	YLEAVEK	16.46±0.05	6879±2642	12798±10466	1.860
RPL5	P46777	RL5_HUMAN	60S ribosomal protein L5	EFNAEVHR	9.73±0.10	10689±9694	6925±815	0.648
RPL10	P27635	RL10_HUMAN	60S ribosomal protein L10	EHVIEALR	16.68±0.20	25369±7658	16906±3253	0.666
RPL36	Q9Y3U8	RL36_HUMAN	60S ribosomal protein L36	EVCGFAPYER	19.28±0.11	10326±5901	6586±939	0.638
RPN1	P04843	RPN1_HUMAN	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	SEDLLDYGPFR	27.51±0.31	10181±5091	6699±1841	0.658
RPN2	P04844	RPN2_HUMAN	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2	FELDTSER	18.58±0.11	10340±5217	6550±1608	0.633
RPS5	P46782	RS5_HUMAN	40S ribosomal protein S5	TIAECLADELINAAK	32.52±0.15	1277±796	681±498	0.533
RUNX1	Q01196	RUNX1_HUMAN	Runt-related transcription factor 1	LSELEQLR	19.42±0.14	97175±35173	227234±149704	2.338
S100A2	P29034	S10A2_HUMAN	Protein S100-A2	ELPSFVGEK	20.97±0.13	20088±7731	30384±6991	1.513
S100A4	P26447	S10A4_HUMAN	Protein S100-A4	TDEAAFQK	15.42±0.09	45832±15945	71420±17057	1.558
S100A6	P06703	S10A6_HUMAN	Protein S100-A6	ELTIGSK	16.33±0.18	61296±11322	96876±22782	1.580
SCGN	O76038	SEGN_HUMAN	Secretagogin	LDLNDLAR	21.82±0.48	2674±1400	5483±2039	2.050
SEL1L	Q9UBV2	SE1L1_HUMAN	Protein sel-1 homolog 1	AASQGYTVAR	24.53±0.09	5582±3104	3618±1352	0.648
SERPINA6	P08185	CBG_HUMAN	Corticosteroid-binding globulin	GTWTQPFDLASTR	9.64±0.08	2343±953	1524±842	0.650
SERPINB5	P36952	SPB5_HUMAN	Serpin B5	ILVVNAAYFVGK	26.78±0.25	6510±1360	9911±3221	1.522
SF3B1	O75533	SF3B1_HUMAN	Splicing factor 3B subunit 1	LTPILK	18.69±0.15	14680±9731	24188±8135	1.648
SLC2A1	P11166	GTR1_HUMAN	Solute carrier family 2, facilitated glucose transporter member 1	VTILELFR	31.80±0.08	4159±1407	2732±765	0.657
SLC4A1	P02730	B3AT_HUMAN	Band 3 anion transport protein	VLLPLIFR	32.63±0.07	16084±4589	9852±4175	0.613
SLC25A20	O43772	MCAT_HUMAN	Mitochondrial carnitine/acylcarnitine carrier protein	LYQEFGIR	9.83±0.08	4402±2021	2909±684	0.661
SLC39A14	Q15043	S39AE_HUMAN	Zinc transporter ZIP14	YGEGDSLTLQQLK	23.47±0.15	19467±5634	8196±2556	0.421
SMAD4	Q13485	SMAD4_HUMAN	Mothers against decapentaplegic homolog 4	IYPSAYIK	19.44±0.06	86766±26513	135391±31822	1.560
SNTB1	Q13884	SNTB1_HUMAN	Beta-1-syntrophin	QELGGLGISIK	25.06±0.15	23031±4389	14850±5666	0.645
SPTAN1	Q13813	SPTN1_HUMAN	Spectrin alpha chain, non-erythrocytic 1	FQFFQR	23.98±0.18	18381±7688	11241±2516	0.612
STAT1	P42224	STAT1_HUMAN	Signal transducer and activator of transcription 1-alpha/beta	NFPFWLWIESILELIK	25.86±0.10	23273±8377	15441±5681	0.663
STIL	Q15468	STIL_HUMAN	SCL-interrupting locus protein	HSPEVEAGEPSLR	16.04±0.62	1988±730	4022±1387	2.023
STOX2	Q9P2F5	STOX2_HUMAN	Storkhead-box protein 2	LVQHHGAEPSSLDK	16.60±0.19	3299±2812	1900±1749	0.576
SURF6	O75683	SURF6_HUMAN	Surfeit locus protein 6	TQGSETAGPPK	8.45±0.08	6828±2203	4265±1867	0.625
TCAP	O15273	TELT_HUMAN	Telethonin	HETYHQQGQCQVLVQR	24.01±0.29	2079±2175	1345±658	0.647
TEP1	Q99973	TEP1_HUMAN	Telomerase protein component 1	TCDADASGTFR	27.11±0.71	5056±4951	3128±3117	0.619
THBD	P07204	TRBM_HUMAN	Thrombomodulin	HIGTDCDSGK	21.62±0.51	6832±2102	4385±500	0.642
TKT	P29401	TKT_HUMAN	Transketolase	AVELAANTK	15.68±0.39	49033±16798	32349±10900	0.660
TNNC1	P63316	TNNC1_HUMAN	Troponin C, slow skeletal and cardiac muscles	NADGYIDLDELK	25.85±0.09	49868±14013	33176±9455	0.665
TP53	P04637	P53_HUMAN	Cellular tumor antigen p53	LGFLHSGTAK	9.88±0.04	56201±12940	37391±5223	0.665
TPD52L1	Q16890	TPD53_HUMAN	Tumor protein D53	THETLSHAGQK	18.02±0.29	5184±5748	13864±5295	2.675
TYMP	P19971	TYPH_HUMAN	Thymidine phosphorylase	VAAALDDGSALGR	17.44±0.14	11983±2233	18530±11599	1.546
TYMS	P04818	TYSY_HUMAN	Thymidylate synthase	GVLEELLWFIK	27.48±0.16	265±98	803±752	3.030
UCHL5	Q9Y5K5	UCHL5_HUMAN	Ubiquitin carboxyl-terminal hydrolase isozyme L5	TLAEHQQLIPLVEK	27.35±0.08	2954±1153	1910±886	0.647
UFL1	O94874	UFL1_HUMAN	E3 UFM1-protein ligase 1	TQSTWVDSFFR	29.56±0.08	5242±1411	7997±2413	1.526
UQCRC2	P22695	QCR2_HUMAN	Cytochrome b-c1 complex subunit 2, mitochondrial	LTSSLTTK	15.65±1.02	223549±209963	113846±21254	0.509
VCL	P18206	VINC_HUMAN	Vinculin	AQQVSQGLDVLTAK	21.49±0.12	10150±1352	16237±4114	1.600
VEGFA	P15692	VEGFA_HUMAN	Vascular endothelial growth factor A	FMDVYQR	19.03±0.56	7215±3807	11244±2280	1.558
VPS51	Q9UID3	VPS51_HUMAN	Vacuolar protein sorting-associated protein 51 homolog	CVELGAYGQAVR	19.83±0.10	7171±2164	4677±1039	0.652
WDR60	Q8WVS4	WDR60_HUMAN	WD repeat-containing protein 60	IGELSLK	9.61±0.07	68110±55521	42397±26737	0.622
ZBTB16	Q05516	ZBT16_HUMAN	Zinc finger and BTB domain-containing protein 16	LGELAVGMK	19.68±0.14	14752±7043	24209±10138	1.641
ZNF462	Q96JM2	ZN462_HUMAN	Zinc finger protein 462	HTEELDSHLR	15.55±0.05	21028±9533	13247±5702	0.630

Table 2-5. Individual sample analysis in the training set and the validation set.

Gene Name	Response Curve , LLOQ (fmol)	Reference Range , 1st Quantile (fmol)	Reference Range , 2nd Quantile (fmol)	Reference Range , 3rd Quantile (fmol)	Triplicate Variance , 1st Quantile (CV%)	Triplicate Variance , 2nd Quantile (CV%)	Triplicate Variance , 3rd Quantile (CV%)	Percentage of Samples , Above LLOQ (%)	Percentage of Samples , no missing value (%)	Skewness Training Set	Skewness Validation Set
ACACB	0.3906	0.6951	1.0444	1.5693	9.8	19.5	28.8	91.1	100.0	-0.513	-0.177
ACADVL	0.7813	1.7993	2.4842	3.4211	10.0	16.7	24.5	95.6	100.0	0.241	-1.375
ACTN4	0.1953	4.9498	7.7940	13.6380	16.3	26.0	36.9	100.0	100.0	-0.329	-0.249
ADAM9	0.0977	0.2049	0.3148	0.5198	10.4	17.7	29.4	96.9	100.0	-0.359	-0.211
AGT	0.7813	1.4597	2.0499	2.9122	11.8	19.2	25.9	93.3	100.0	-0.430	-0.363
AHSG	1.5625	5.5482	7.6917	9.8452	12.8	19.5	28.3	100.0	100.0	-0.195	0.254
AKT1	0.1953	2.2679	2.9538	4.4139	16.0	24.6	35.4	100.0	100.0	-0.029	-0.648
ALAD	0.7813	3.2571	6.1026	8.9976	8.6	13.6	22.0	98.7	100.0	-0.391	-0.241
ALDH1A1	0.1953	3.6164	6.2592	9.4540	8.5	14.8	23.7	100.0	100.0	-0.445	-0.498
ALDH6A1	0.0977	0.8602	1.4568	2.1437	8.3	13.7	19.6	100.0	100.0	0.323	-0.224
ANPEP	0.1953	0.5960	0.9497	1.5801	14.0	23.0	35.0	96.9	100.0	0.471	-0.427
ATP1A1	0.0977	0.6770	1.0047	1.6030	14.6	23.7	35.3	100.0	100.0	-0.034	-0.233
BAX	0.0977	0.7374	0.9885	1.3850	10.5	17.8	26.9	100.0	100.0	-0.247	-0.565
CALR	0.7813	6.4307	12.4074	26.9824	18.2	29.7	40.9	100.0	100.0	-0.105	0.027
CAP1	0.0488	1.4641	2.9174	5.1160	11.6	19.5	28.7	100.0	100.0	-0.474	-0.484
CCNE1	0.1953	0.3073	0.4217	0.6059	12.6	19.9	27.0	95.6	100.0	-0.009	0.329
CD19	0.0977	0.1289	0.2094	0.3434	12.8	22.4	33.1	88.0	100.0	0.902	0.965
CD274	0.1953	0.4243	0.8225	1.3236	16.1	25.3	42.3	91.1	100.0	-0.500	-0.678
CDH1	0.7813	2.2582	3.2034	4.7503	12.0	21.5	31.6	99.6	100.0	0.185	-0.430
CEACAM5	0.3906	0.6145	2.6794	8.4019	10.5	17.2	25.0	83.6	99.6	-0.010	-0.019
CENPB	0.0977	0.1474	0.1963	0.2742	15.9	23.4	33.5	93.8	100.0	-0.060	-0.162
CHEK2	0.0977	0.2673	0.3303	0.4222	9.9	15.7	24.7	99.6	100.0	-0.164	0.510
CLCA1	0.7813	5.1657	6.8213	9.9869	8.3	13.5	19.9	100.0	100.0	-0.721	-0.017
COL2A1	0.1953	1.0252	1.4730	2.1563	15.0	23.3	32.9	100.0	100.0	1.291	0.710
COL4A6	0.7813	1.1609	1.5222	2.2127	10.7	17.2	27.7	88.9	100.0	-0.251	0.102
COL6A2	0.3906	1.2554	2.0940	3.6635	15.4	25.7	39.0	97.8	100.0	0.564	0.230
COL28A1	0.7813	2.5429	3.5476	5.3065	11.8	18.9	26.8	99.6	100.0	-0.307	0.095
CPA1	0.0977	0.3001	0.8001	4.7130	4.5	6.8	14.4	94.7	98.5	0.715	0.510
CPB1	0.3906	4.5441	6.4309	15.4905	9.5	15.2	25.5	100.0	100.0	1.297	1.082
CR2	0.0977	0.5363	0.7191	0.9848	16.1	26.1	44.3	100.0	100.0	-0.525	0.336
CREBBP	0.7813	5.6414	8.8387	17.3452	12.9	22.2	33.0	100.0	100.0	0.171	-0.380
CTNNB1	0.1953	0.3179	0.5576	1.0761	10.8	16.5	23.3	95.1	100.0	0.179	-0.249
CTRB2	0.3906	6.1292	12.2402	77.0468	10.1	19.4	37.8	99.6	100.0	0.699	0.599
CTSS	0.0977	0.3817	0.5537	0.8922	13.8	22.3	32.2	99.1	100.0	-0.078	-0.537
DCN	0.3906	8.1950	13.3145	21.2885	15.3	24.3	33.7	100.0	100.0	-0.031	-0.024
DSG2	0.1953	0.3326	0.6222	1.1530	6.3	9.9	17.0	92.4	100.0	-0.204	-0.259
EGFR	0.3906	15.1509	22.0130	31.1466	13.5	22.0	34.6	100.0	100.0	-0.359	0.105
ENO2	0.3906	2.1069	3.3340	5.3589	6.5	10.2	19.8	100.0	100.0	0.129	-0.443
EPHX2	0.0488	0.1326	0.1952	0.2927	16.1	30.8	44.9	99.6	100.0	0.926	0.786
FBN1	0.3906	75.8340	130.6415	284.2478	7.2	15.2	27.4	100.0	100.0	-0.256	-0.397
FLT1	0.0977	0.1774	0.2536	0.3814	18.3	29.6	45.1	96.4	100.0	0.038	-0.237
FLT4	0.1953	0.2141	0.4196	0.8491	13.1	23.7	33.2	79.1	99.5	-1.041	-0.573
GATM	0.7813	3.8991	8.1599	28.3969	11.7	24.5	38.6	98.2	100.0	0.779	0.365
GPX2	0.1953	0.3851	0.8183	1.6314	13.8	22.5	31.8	91.6	100.0	-0.053	-0.286
GSN	0.3906	2.4706	3.8559	5.5758	10.9	17.1	27.5	100.0	100.0	-0.440	-0.303

HGF	0.7813	3.1369	4.2756	5.7087	13.5	20.6	29.1	100.0	100.0	1.409	1.114
HRAS	0.3906	1.1017	1.6365	2.2933	11.5	17.6	27.6	99.1	100.0	0.092	-0.534
HSPB1	0.7813	17.4589	26.6933	43.4496	7.8	12.4	18.0	100.0	100.0	0.320	-0.436
IDH1	0.7813	4.0147	6.2299	8.5392	15.3	27.7	38.6	100.0	100.0	-0.714	0.070
IFI16	0.0977	0.5268	0.8141	1.2486	11.2	16.3	23.8	100.0	100.0	0.050	-0.534
IGFBP3	0.3906	1.8275	2.4911	3.4647	18.7	27.7	39.4	100.0	100.0	-0.821	-0.259
IGFBP5	0.1953	1.4684	2.4477	3.4458	12.2	18.9	28.8	100.0	100.0	-0.139	-0.245
IL1RN	0.7813	4.1646	6.2659	9.4454	10.8	17.8	29.9	99.1	100.0	-0.628	0.174
ITGA3	0.0977	0.4483	0.7116	1.1167	11.4	19.0	30.1	100.0	100.0	-0.524	0.036
ITGA5	0.7813	1.0628	1.5876	2.3221	10.7	18.0	29.0	87.6	100.0	0.046	-0.700
ITGA6	0.7813	2.4220	3.5940	4.9280	16.6	25.5	39.7	100.0	100.0	0.720	-0.002
JMY	0.3906	2.1098	3.5634	5.8488	12.7	19.5	28.3	100.0	100.0	0.160	0.096
KIT	0.7813	0.8790	1.3175	1.9812	17.1	26.1	43.6	79.6	100.0	1.093	0.723
KRT17	0.3906	0.5644	1.3222	3.6758	11.8	19.6	31.0	86.7	99.6	1.014	0.409
LAMP1	0.1953	4.8591	7.6344	10.5657	11.0	17.5	27.7	100.0	100.0	0.067	-0.643
LGALS4	0.0488	0.5952	1.3585	3.8176	11.1	17.4	27.7	99.6	100.0	-0.235	-0.133
MAPK1	0.1953	0.9370	1.5052	2.3895	8.1	13.1	21.6	100.0	100.0	-0.004	-0.319
MIA	0.0977	0.1353	0.2705	0.7009	18.5	29.4	42.0	86.2	100.0	0.070	-0.014
MLH1	0.0977	0.3941	0.7448	1.2973	8.9	16.0	25.4	97.8	100.0	-0.420	-0.262
MMP7	0.1953	1.5802	2.3302	3.7923	14.1	22.3	33.3	100.0	100.0	0.288	0.371
MMP9	0.3906	0.4034	0.7406	1.2942	12.2	18.4	27.9	76.9	94.7	0.202	0.013
MPO	0.1953	2.6538	4.9425	14.0439	12.0	18.6	28.5	100.0	100.0	0.715	-0.266
MXRA5	0.0977	0.4122	0.5796	0.8124	14.3	25.4	40.4	100.0	99.6	-0.334	-0.129
MYH11	0.1953	1.0149	1.6344	2.7118	15.0	24.1	32.6	99.6	100.0	0.959	-0.040
MYLK	0.0977	0.9389	1.2916	1.8766	14.3	24.7	35.7	100.0	100.0	1.421	0.137
NEFH	0.1953	0.3098	0.5692	0.8568	8.3	15.2	24.0	92.4	100.0	-0.340	0.217
NEXN	0.1953	1.9677	2.6070	3.8944	9.8	17.2	24.0	100.0	100.0	0.157	-0.652
NPC2	0.1953	6.1003	9.3074	13.4392	7.7	13.7	19.6	100.0	100.0	0.065	-0.674
NPM1	0.3906	5.3338	7.8229	12.3840	10.8	16.5	26.2	100.0	100.0	0.747	-0.427
NUCB2	0.3906	1.5264	2.2847	3.1937	9.1	14.6	20.8	100.0	100.0	0.914	-0.143
OLFM4	0.3906	0.7704	2.4312	7.7542	12.9	21.1	33.4	87.6	100.0	0.552	0.156
PACSIN2	0.3906	0.6508	0.9092	1.2099	21.4	30.8	42.3	95.6	100.0	0.314	-0.485
PARP1	0.3906	1.7925	2.4092	3.2809	20.6	29.1	41.8	100.0	100.0	-0.331	-0.216
PDCD4	0.0977	0.4220	0.6030	1.0333	10.9	17.7	27.0	100.0	100.0	0.700	-0.383
PDE4D	0.1953	1.3288	1.7252	2.2052	13.9	24.7	43.7	100.0	100.0	-0.992	-0.993
PDIA4	0.1953	3.5128	5.0177	7.6475	7.9	12.3	17.4	100.0	100.0	0.298	-0.441
PGC	0.0977	0.2103	0.4554	2.0906	10.9	19.2	30.8	97.8	100.0	1.433	0.948
PLG	0.0977	3.2857	4.7240	6.6106	10.8	18.3	31.8	100.0	100.0	0.828	-0.096
PNLIP	0.1953	0.4616	1.1945	3.8387	10.9	22.0	37.1	96.9	100.0	0.923	0.573
PNLIPRP2	0.0977	0.1635	0.2592	0.5346	12.0	21.2	31.5	94.7	98.7	1.215	0.593
PPBP	0.7813	2.0844	2.9409	4.8304	12.2	19.7	30.1	99.1	100.0	0.594	0.425
PTPN11	0.7813	0.9339	1.3053	1.8039	10.6	17.7	26.1	86.2	100.0	-0.113	-0.748
REG1A	0.7813	0.8963	2.0363	9.0869	11.1	16.8	25.2	78.7	99.6	0.709	0.300
RELA	0.1953	1.7117	2.0739	2.5443	12.2	17.0	23.4	100.0	100.0	-0.095	-0.073
RPL10	0.3906	0.9796	1.3893	2.1989	15.2	23.1	30.9	96.9	100.0	-0.860	-0.287
RPL36	0.7813	2.4099	3.5902	5.1556	11.0	18.4	27.6	97.8	100.0	-0.391	-0.223
RPN1	0.3906	1.1319	1.9441	3.0100	11.8	20.3	27.5	96.0	100.0	-0.610	-0.520
RPN2	0.3906	0.8401	1.2023	1.8159	13.9	25.6	40.7	97.3	100.0	-0.097	-0.189
RPS5	0.3906	14.8578	24.2220	39.3538	12.3	19.4	28.2	100.0	100.0	-0.547	-0.262
RUNX1	0.3906	1.5223	1.7624	1.9866	14.5	24.5	36.4	100.0	100.0	-0.640	0.331
S100A2	0.1953	0.4912	0.8859	1.7854	5.1	9.1	18.8	100.0	100.0	1.160	0.244
S100A4	0.3906	17.0969	27.5602	46.0929	4.8	8.4	21.6	100.0	100.0	0.695	-0.119
S100A6	0.1953	159.6607	252.7924	417.2014	3.6	9.6	20.6	100.0	100.0	-0.224	-0.812

SERPINB5	0.3906	0.9163	1.8133	3.3976	12.3	18.9	28.0	93.3	100.0	-0.098	-0.117
SF3B1	0.0488	0.4617	0.6983	1.0927	9.8	14.2	21.6	100.0	100.0	-0.377	-0.590
SLC2A1	0.0488	0.3345	0.6858	1.5749	12.7	23.7	37.8	97.3	100.0	-0.079	-0.528
SLC4A1	0.0488	0.6191	1.1558	2.0158	6.2	12.7	30.6	99.1	100.0	0.297	0.047
SMAD4	0.0488	0.2710	0.3757	0.5662	11.0	17.0	25.6	100.0	100.0	0.002	-0.143
SPTAN1	0.0488	1.7329	2.4316	3.5893	6.9	12.8	18.6	100.0	100.0	0.006	-0.871
STIL	0.3906	1.9559	2.6342	4.1541	12.7	21.7	28.9	100.0	100.0	-0.062	-0.438
SURF6	0.0977	0.1552	0.2646	0.3644	8.8	14.2	20.1	87.6	100.0	-0.883	-0.275
TKT	0.1953	4.4790	7.4575	11.3030	5.0	7.9	12.1	99.6	100.0	-0.772	-1.007
TROVE2	0.3906	0.5881	0.9815	1.5620	16.7	30.6	45.3	90.7	100.0	-0.206	-0.447
TYMP	0.3906	2.3734	3.7130	5.6881	8.5	14.5	22.5	99.1	100.0	-0.486	-0.469
UFL1	0.1953	0.4507	0.7503	1.2595	10.3	18.0	27.6	95.1	100.0	0.114	-0.462
UQCRC2	0.1953	1.4013	2.0534	3.1302	9.1	14.3	21.3	100.0	100.0	-0.306	-0.232
VCL	0.1953	3.8083	5.3108	7.9039	10.0	14.9	24.9	100.0	100.0	-0.067	-0.620
VEGFA	0.0977	0.2752	0.4170	0.5926	11.5	19.5	27.0	98.7	100.0	-0.054	-0.395
ZBTB16	0.1953	1.1668	1.4468	1.7330	16.7	29.8	44.2	100.0	100.0	-0.034	0.599
ZNF462	0.1953	8.0621	12.8977	25.5465	15.3	23.1	35.1	100.0	100.0	1.295	0.770

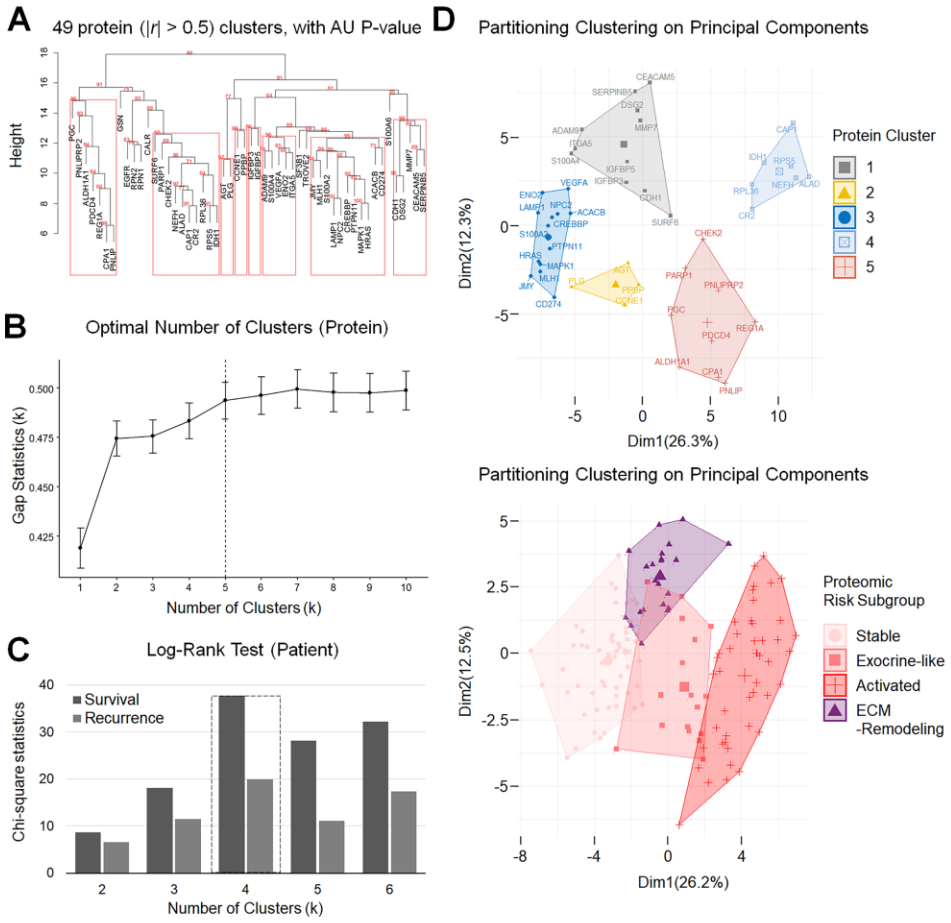
1.3.3 Revealing Proteomic Risk Subgroups, using Prognostic Biomarkers

To establish PDAC subtypes, co-expressed proteins associated with prognosis were chosen to classify PDAC tumors according to risk. Hazard ratios (for both recurrence and death) were calculated for the 115 proteins that were derived from the training set, comprised of 148 patients (Table 2-6). Of the 60 proteins identified by the univariate Cox regression with P-values less than 0.1, for either recurrence or death, 49 proteins had correlation coefficients (Pearson |r|) greater than 0.5, with at least one another protein subjected to hierarchical clustering. When the 44 proteins which composed significant clusters [Approximately Unbiased (AU) > 0.95] (Figure 2-5A) were clustered, 5 protein clusters and 4 subgroups of patients were identified, characterized by high correlations with prognosis and tumorigenesis (Figure 2-6A). The optimal cluster numbers were verified by determining the gap statistics for proteins (Figure 2-5B) and the significance of the log-rank test for patients (Figure 2-5C). The clusters demonstrated clear separation for both dimensions of principal components (Figure 2-5D).

The protein clusters and risk subgroups were characterized by activated biological functions (Figure 2-7A) and prognostic relevance. The risk subgroup designations were chosen according to their differentially enriched signatures (Figure 2-7B). The right-most subgroup (N = 67) exhibited elevated expression levels in protein cluster-4 and cluster-5, whose members showed lower hazard ratios, associated with early-stage tumors and the low overall prevalence of death or recurrence. Hence, this group was named “Stable” because stable disease corresponds with a low prevalence of death or recurrence. The left-most subgroup (N = 17), which was named “Exocrine-like”, was associated with signaling pathways that are activated in aggressive tumors (Cluster-3) and with high levels of pancreatic enzymes (Cluster-5). The second subgroup (N = 44) was enriched in proteins associated with tumorigenic programs (Cluster-2 and Cluster-3) and the interaction and organization of the extracellular matrix (ECM, Cluster-1). The third subgroup (N = 20) exhibited the high

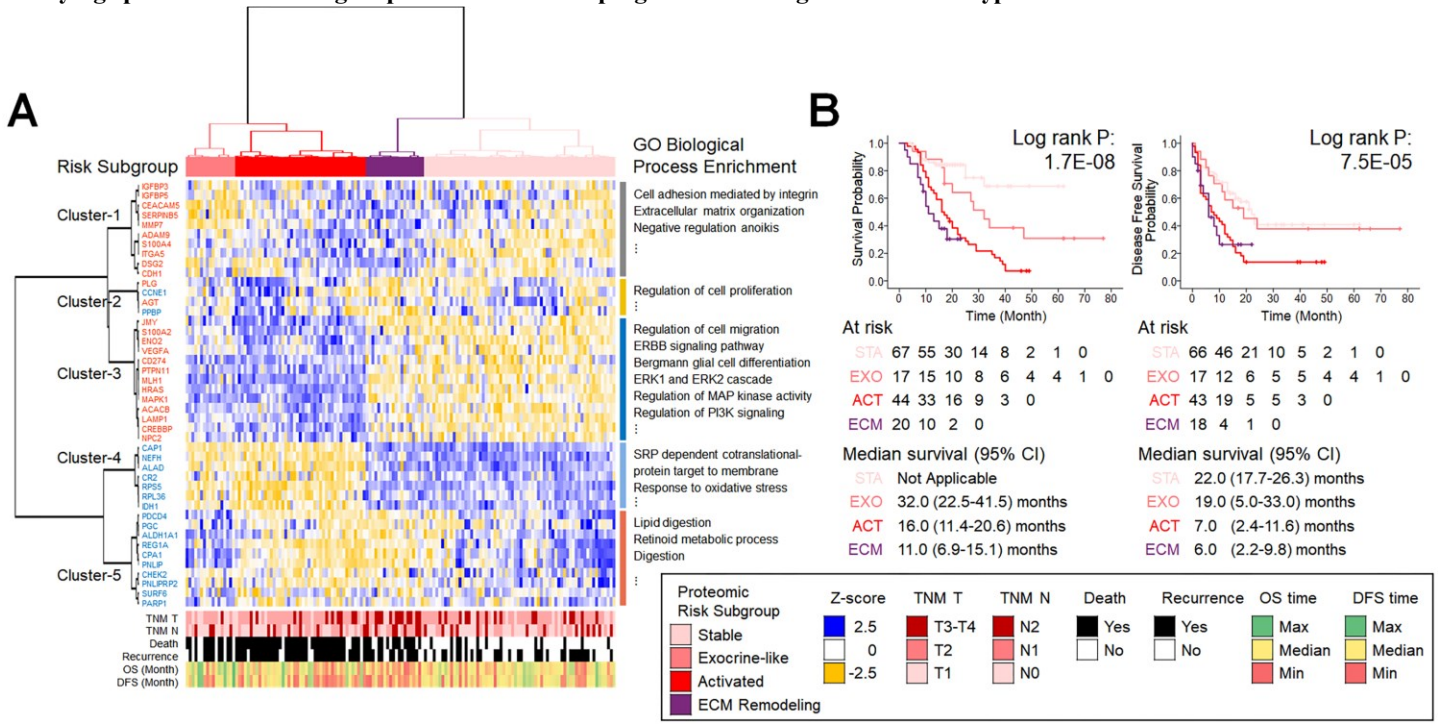
expression of Cluster-1 proteins and the downregulation of proteins in Cluster-2 and Cluster-3. This subgroup was primarily associated with advanced-stage tumors and poor outcomes, despite its association with Cluster-4. Because the third subgroup was significantly enriched in the Cluster-1 proteins compared with the second subgroup (Figure 2-7C), the second and third subgroups were named “Activated” and “ECM-Remodeling”, respectively. Kaplan-Meier estimates of cumulative survival showed that the risk subgroups were associated with patient outcomes [Log-rank test, P = 1.7E-08 for overall survival (OS), P = 7.5E-05 for disease-free survival (DFS), Figure 2-6B]. Patients in the “ECM-Remodeling” group had shorter median OS (11 months [95% CI, 6.9-15.1]) than the patients in the other subgroups. No patient in the “ECM-Remodeling” subgroup survived beyond 23 months. All risk subgroups were associated with T and N stages, and the T3-4 and N2 stages comprised 60% and 40% of the “ECM-Remodeling” subgroup, respectively (Table 2-7). In the multivariate survival analysis that included the most relevant standard prognostic factors, the identified risk subgroups remained significantly associated with survival, along with resection margin, T stage, and lymphatic invasion (Table 2-8).

Figure 2-5. Evaluating hierarchical clustering analysis for proteins and proteomic risk subgroups.



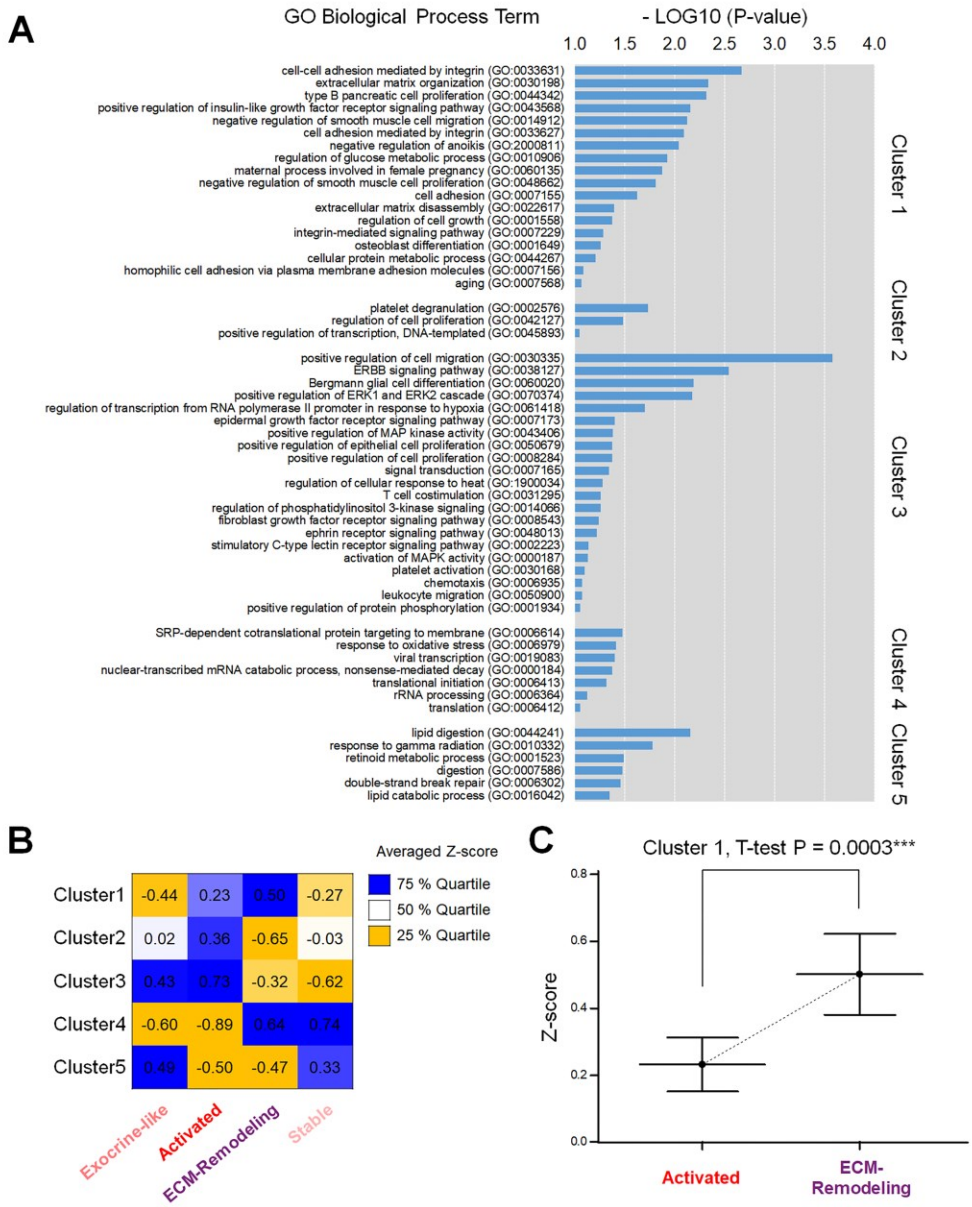
A. Dendrogram, generated after hierarchical clustering, using multiscale bootstrap resampling (n = 1,000). The 49 of 60 proteins that were correlated with at least one another protein (Pearson's |r| > 0.5) were included in the clustering analysis. Red values on the edges of the clustering are AU (Approximately Unbiased) P-values. The 44 proteins in the clusters that were significantly supported by the data (Red boxes, P < 0.05) were selected for the final clustering analysis. B. The optimal number of protein clusters for hierarchical clustering, using Gap statistics. Five protein clusters appear to be optimal. C. Log-rank test for recurrence and survival based on the number of clusters for PDAC patients. Four clusters of samples (risk subgroups) were the most significantly related to prognosis. D. Partitioning clusters, based on principal components, to validate interactions among the proteins in each of the five protein clusters or between individuals in each of the four risk subgroups.

Figure 2-6. Identifying proteomic risk subgroups associated with prognosis and original tumor subtypes.



(A) Hierarchical clustering analysis (HCA), using 44 proteins in the training set, identified 4 risk subgroups (X-axis of the heatmap) of tumors associated with 5 protein clusters (Y-axis of the heatmap). Protein names are colored according to significant associations with increased risk (Red, HR > 1) or reduced risk (Blue, HR < 1). Enriched representative biological processes, according to Gene Ontology analysis, in the five protein clusters (right side of the heatmap) and the prognostic information for individual patients (below the heatmap) are also presented. (B) Kaplan-Meier survival curves for death and recurrence in each risk subgroup. “ECM-remodeling” had the worst outcomes, followed by “Activated”, “Exocrine-like”, and “Stable” based on the numbers at risk, the median survival rate, and the log-rank test. “Not Applicable” indicates that the number of patients who experienced the event did not reach 50% in the overall follow-up.

Figure 2-7. Gene enrichment analysis and the defining signatures of risk subgroups.



A. Gene ontology functional analysis for biological process, according to the proteins in each cluster. Fisher's exact test P-values (EASE score < 0.1) are shown. B. Averaged Z-scores of the twenty expression blocks formed by proteins and individual sample clusters. The highly expressed blocks were considered to be signatures and were used to name the risk subgroups, according to the enriched biological processes. C. Mean difference (with 95% CI bar) between cluster 1 protein expression levels between the "Activated" and "ECM-remodeling" subgroups.

Table 2-6. Association of 115 protein levels with recurrence, death, and prognostic factors.

Gene Name	DFS, Hazard Ratio	DFS, 95% CI	DFS, P-value	OS, Hazard Ratio	OS, 95% CI	OS, P-value	Resection Margin, Expression	Resection Margin, P-value	TNM T, Expression	TNM T, P-value	TNM N, Expression	TNM N, P-value	Differentiation, Expression	Differentiation, P-value	Lymphatic Invasion, Expression	Lymphatic Invasion, P-value	Venous Invasion, Expression	Venous Invasion, P-value	Perineural Invasion, Expression	Perineural Invasion, P-value	Reccurence Type, Expression	Recurrence Type, P-value
ACACB	3.6	1.3 to 9.7	0.011	5.8	1.9 to 18	0.0023		0.095		0.83		0.49		0.57		0.21		0.65		0.31	Elevated	0.0099
ACADVL	0.90	0.3 to 2.7	0.86	1.0	0.32 to 3.3	0.96		0.21		0.56		0.71	Elevated	0.02		0.20		0.47		0.95		0.15
ACTN4	1.7	0.74 to 3.9	0.21	1.7	0.62 to 4.4	0.31		0.66	Elevated	0.00014	Not Linear	0.043	Elevated	0.02		0.19	Elevated	0.049		0.051		0.12
ADAM9	3.3	1.5 to 7.4	0.0034	6.1	2.4 to 15	0.00010		0.63	Elevated	0.050		0.29		0.07		0.10		0.15		0.098	Elevated	0.0093
AGT	1.6	0.52 to 4.9	0.42	2.7	0.75 to 9.7	0.095		0.51		0.23		0.43	Not Linear	0.00		0.99		0.22	Elevated	0.0028		0.86
AHSG	0.78	0.24 to 2.6	0.69	0.82	0.22 to 3	0.76		0.88		0.17		0.12		0.08		0.22		0.79	Elevated	0.031		0.72
AKT1	1.6	0.47 to 5.7	0.43	2.0	0.5 to 7.8	0.33		0.35		0.16		0.37		0.52		0.55		0.63		0.20		0.060
ALAD	0.37	0.17 to 0.79	0.011	0.30	0.13 to 0.72	0.0065		0.57	Elevated	0.0053		0.30		0.05		0.38		0.097		0.47	Not Linear	0.024
ALDH1A1	0.46	0.22 to 0.94	0.033	0.51	0.24 to 1.1	0.082		0.31		0.43		0.49	Down	0.03		0.65		0.37		0.61		0.31
ALDH6A1	1.2	0.57 to 2.6	0.62	1.3	0.59 to 3	0.49		0.31		0.56		0.68		0.82		0.90		0.90		0.55		0.14
ANPEP	0.49	0.25 to 0.99	0.046	0.48	0.22 to 1	0.057		0.35	Elevated	0.034		0.30	Elevated	0.00		0.33		0.30		0.58		0.22
ATP1A1	1.2	0.45 to 3.3	0.70	0.46	0.15 to 1.4	0.16		0.85	Elevated	0.0024		0.23		0.26		0.062		0.23		0.25		0.47
BAX	2.1	0.63 to 7.1	0.23	1.8	0.46 to 7.2	0.39		0.53		0.25		0.20		0.27		0.85		0.71		0.15		0.060
CALR	0.63	0.35 to 1.1	0.12	0.46	0.23 to 0.88	0.020		0.84	Elevated	6.71E-05	Elevated	0.046		0.26		0.44		0.22		0.25		0.19
CAP1	0.55	0.3 to 1	0.066	0.39	0.19 to 0.78	0.0079		0.59	Elevated	0.00049	Elevated	0.035	Elevated	0.00		0.072		0.055	Elevated	0.031		0.19
CCNE1	0.41	0.14 to 1.2	0.097	0.45	0.15 to 1.3	0.13		0.19		0.15		0.20		0.54		0.21		0.11		0.33		0.76
CD19	0.44	0.25 to 0.78	0.0049	0.37	0.19 to 0.74	0.0051		0.37		0.63		0.77		0.19		0.52		0.47		0.27		0.28
CD274	2.2	1.2 to 4.1	0.0086	2.7	1.4 to 5.3	0.0046	Down	0.0038		0.98		0.87		0.82		0.71		0.65		0.66	Elevated	0.00009
CDH1	2.2	0.85 to 5.9	0.098	2.3	0.82 to 6.3	0.11		0.68		0.099	Elevated	0.020		0.55		0.13		0.55		0.43	Elevated	0.021
CEACAM5	1.3	1 to 1.7	0.047	1.3	0.98 to 1.8	0.067		0.17	Elevated	0.027		0.077		0.13		0.76		0.70	Elevated	0.0035	Not Linear	0.042
CENPB	2.0	0.6 to 7	0.26	0.93	0.25 to 3.4	0.92		0.22		0.49		0.21		0.98		0.84		0.59		0.14		0.068
CHEK2	0.67	0.18 to 2.5	0.55	0.26	0.059 to 1.2	0.076		0.87	Down	0.0070		0.11		0.59		0.28		0.17		0.29		0.89
CLCA1	0.94	0.32 to 2.8	0.91	0.96	0.29 to 3.2	0.95		0.97		0.074		0.56		0.09		0.20		0.33	Elevated	0.013		0.64
COL2A1	0.38	0.17 to 0.83	0.015	0.30	0.13 to 0.7	0.0052		0.66	Elevated	0.00022		0.79		0.81		0.88		0.11		0.41		0.37
COL4A6	1.3	0.38 to 4.6	0.67	0.70	0.18 to 2.7	0.60		0.91	Not Linear	0.00017		0.18	Elevated	0.01	Elevated	0.015		0.20		0.18		0.22
COL6A2	0.55	0.29 to 1	0.061	0.48	0.25 to 0.91	0.026		0.73	Elevated	3.71E-05		0.54		0.30		0.79		0.098		0.38		0.95
COL28A1	0.75	0.29 to 1.9	0.54	1.2	0.42 to 3.4	0.74		0.75		0.19		0.96		0.29		0.24		0.55	Elevated	0.011		0.60
CPA1	0.86	0.69 to 1.1	0.16	0.73	0.57 to 0.93	0.012		0.32	Down	0.0032		0.29		0.55		0.21	Down	0.023	Down	0.015	Not Linear	0.030
CPB1	0.75	0.49 to 1.1	0.18	0.69	0.42 to 1.1	0.14		0.46	Down	0.013		0.60		0.71		0.40	Down	0.020	Down	0.0018	Not Linear	0.029
CR2	0.40	0.15 to 1.1	0.069	0.22	0.077 to 0.61	0.0036		0.24	Elevated	0.0040		0.055		0.81		0.63		0.43		0.93		0.52
CREBBP	4.7	43872	0.00028	4.9	1.9 to 13	0.00084		0.20	Not Linear	0.0077		0.32		0.16		0.17		0.42	Elevated	0.029	Elevated	6.55E-05
CTNNB1	1.6	0.72 to 3.5	0.26	1.5	0.63 to 3.6	0.36		0.40		0.31		0.26		0.50		0.30		0.86		0.19		0.11
CTRB2	0.96	0.74 to 1.2	0.76	0.95	0.71 to 1.3	0.70		0.13	Down	0.0070		0.20		0.55		0.71	Down	0.044	Down	0.0053	Not Linear	0.030
CTSS	1.0	0.43 to 2.5	0.94	1.7	0.66 to 4.5	0.26		0.35		0.39		0.13	Elevated	0.04		0.41		0.75	Elevated	0.032		0.57
DCN	0.62	0.32 to 1.2	0.16	0.65	0.33 to 1.3	0.21		0.91	Elevated	0.034		0.68		0.09		0.72		0.079		0.092		0.62
DSG2	2.2	1.1 to 4.4	0.018	1.9	0.88 to 4.1	0.10		0.44	Elevated	0.0018	Not Linear	0.021		0.10	Elevated	0.027		0.19	Elevated	0.027		0.067
EGFR	0.39	0.16 to 0.96	0.040	0.27	0.1 to 0.71	0.0080		0.98	Elevated	0.018		0.11		0.15		0.27		0.77		0.82		0.099
ENO2	3.6	1.6 to 8.4	0.0026	7.0	2.7 to 18	0.000067		0.12		0.57		0.15	Elevated	0.03		0.68		0.32	Elevated	0.045	Elevated	4.82E-05
EPHX2	1.0	0.45 to 2.3	0.98	0.79	0.33 to 1.9	0.59		0.34		0.44		0.86		0.09		0.46		0.31		0.094		0.78
FBN1	1.1	0.63 to 1.8	0.83	1.3	0.72 to 2.2	0.43		0.96		0.23		0.57	Not Linear	0.00		0.70		0.53		0.24		0.95
FLT1	1.6	0.65 to 3.7	0.26	2.0	0.77 to 5.1	0.11		0.36		0.74		0.50		0.15		0.88		0.79		0.058		0.12
FLT4	1.4	0.88 to 2.10	0.17	1.1	0.69 to 1.91	0.60		0.42		0.11		0.80		0.89		0.29		0.35		0.815		0.16
GATM	0.90	0.66 to 1.2	0.52	0.91	0.65 to 1.3	0.57	Down	0.071		0.11		0.64		0.35		0.85		0.20		0.13	Not Linear	0.0037
GPX2	1.1	0.66 to 1.9	0.70	1.2	0.7 to 2.2	0.47		0.20		0.052		0.29		0.34		0.91		0.77		0.052		0.77
GSN	0.63	0.26 to 1.5	0.31	0.26	0.096 to 0.73	0.011		0.71	Elevated	0.00017		0.18	Elevated	0.05		0.86		0.54	Elevated	0.032		0.80
HGF	0.54	0.19 to 1.6	0.26	0.40	0.12 to 1.4	0.15		0.20		0.65		0.48		0.62		0.90		0.097		0.53	Down	0.039
HRAS	1.6	0.61 to 4	0.35	2.9	43838	0.044		0.29		0.36		0.96		0.91		0.37		0.84		0.41	Elevated	0.023
HSPB1	1.9	0.84 to 4.1	0.12	1.7	0.75 to 4	0.20		0.11		0.062		0.17	Down	0.02		0.14		0.33		0.43		0.24
IDH1	0.29	0.11 to 0.73	0.0093	0.27	0.097 to 0.78	0.015		0.62	Elevated	0.0014	Elevated	0.049		0.38	Elevated	0.027		0.43		0.053	Not Linear	0.034
IFI16	2.1	0.8 to 5.7	0.13	0.91	0.31 to 2.6	0.86		0.50		0.083		0.11	Elevated	0.03		0.54		0.50		0.17	Not Linear	0.025
IGFBP3	2.8	0.86 to 9.1	0.088	4.8	1.3 to 18	0.020		0.85	Elevated	0.00090		0.32	Not Linear	0.03		0.32		0.22	Elevated	0.033		0.22
IGFBP5	3.1	1.4 to 6.8	0.0063	2.8	1.1 to 7	0.024		0.68	Elevated	0.041		0.26		0.29		0.70		0.22	Elevated	0.018	Not Linear	0.035
IL1RN	1.1	0.46 to 2.5	0.87	0.76	0.3 to 1.9	0.55		1.00	Not Linear	0.00093		0.58		0.32		0.26		0.70		0.78		0.062
ITGA3	1.3	0.62 to 2.8	0.47	1.3	0.54 to 2.9	0.60		0.67		0.99		0.61		0.79		0.24		0.97		0.67	Elevated	0.0043
ITGA5	4.4	1.7 to 12	0.0025	4.3	1.4 to 13	0.0098		0.74	Not Linear	0.00073	Not Linear	0.026	Elevated	0.00		0.070		0.081	Elevated	0.0017	Elevated	4.80E-05

ITGA6	2.0	0.73 to 5.4	0.18	1.4	0.48 to 4.3	0.51		0.49	Not Linear	0.00022	Elevated	0.032	Elevated	0.04		0.092		0.14		0.45		0.11
JMY	2.5	1.3 to 4.6	0.0047	3.5	1.8 to 7	0.00030		0.84		0.46		0.77		0.41		0.77		0.55		0.40	Elevated	0.00065
KIT	1.2	0.58 to 2.4	0.64	1.4	0.64 to 3.3	0.38		0.65		0.21		0.24		0.65		0.39		0.76		0.12		0.67
KRT17	1.6	1.1 to 2.2	0.0078	1.3	0.87 to 1.9	0.21		0.29	Elevated	9.28E-07		0.15	Elevated	0.01		0.16	Elevated	0.010	Elevated	0.034		0.054
LAMP1	2.9	1 to 8.3	0.041	4.6	1.5 to 14	0.0083	Down	0.046		0.96		0.30		0.91		0.80		0.83	Elevated	0.017	Not Linear	3.12E-05
LGALS4	0.98	0.66 to 1.5	0.94	1.0	0.64 to 1.6	0.99		0.81	Elevated	0.035		0.52	Not Linear	0.03		0.31		0.80	Elevated	0.026		0.82
MAPK1	3.1	1.1 to 8.9	0.032	3.7	1.1 to 12	0.035		0.38		0.43		0.63		0.20		0.39		0.45		0.12	Elevated	0.0092
MIA	1.3	0.83 to 2	0.26	1.5	0.93 to 2.5	0.091		0.57		0.59		0.81		0.85		0.98		0.55		0.12	Elevated	0.0047
MLH1	1.9	1.1 to 3.3	0.031	2.9	1.5 to 5.6	0.0012		0.28		0.79		0.75		0.99		0.97		0.17		0.73	Elevated	0.020
MMP7	2.1	0.96 to 4.5	0.065	2.1	0.86 to 5	0.11		0.54	Elevated	7.74E-05		0.25	Elevated	0.03		0.096		0.41	Elevated	0.00096	Elevated	0.014
MMP9	0.93	0.55 to 1.6	0.78	1.1	0.62 to 1.9	0.76		0.91	Not Linear	0.031	Elevated	0.021		0.10	Elevated	0.022		0.19	Elevated	0.0031		0.42
MPO	1.0	0.63 to 1.7	0.88	1.1	0.61 to 1.9	0.79		0.86		0.058		0.065	Elevated	0.04		0.58		0.35	Elevated	0.014		0.092
MXRA5	1.4	0.59 to 3.5	0.42	1.3	0.49 to 3.3	0.61		0.26		0.086		0.16		0.81		0.52		0.88		0.071		0.072
MYH11	0.75	0.38 to 1.5	0.41	0.88	0.42 to 1.9	0.73		0.57		0.16		0.84		0.10		0.34		0.32	Elevated	0.013		0.63
MYLK	1.0	0.49 to 2.1	0.97	1.3	0.57 to 3	0.54		0.96		0.44		0.97		0.30		0.46		0.17	Elevated	0.019		0.19
NEFH	0.39	0.18 to 0.82	0.014	0.21	0.09 to 0.5	0.00043		0.30	Elevated	0.0011		0.43		0.17		0.35		0.35		0.40	Not Linear	0.039
NEXN	1.4	0.38 to 4.8	0.64	1.7	0.4 to 7	0.49		0.52		0.22		0.41		0.26		0.96		0.52		0.17		0.056
NPC2	2.1	0.85 to 5.2	0.11	3.6	1.4 to 9.1	0.0073		0.47		0.73		0.20		0.13		0.44		0.72		0.18	Elevated	0.027
NPM1	1.3	0.44 to 3.7	0.67	0.88	0.26 to 2.9	0.84		0.77	Not Linear	0.0045	Not Linear	0.025	Elevated	0.04		0.16		0.094		0.13		0.36
NUCB2	0.91	0.36 to 2.3	0.83	0.84	0.3 to 2.4	0.75		0.58		0.48		0.24		0.65		0.51		0.70		0.83		0.18
OLFM4	0.97	0.7 to 1.3	0.87	1.0	0.72 to 1.5	0.90		0.86	Elevated	0.00081	Elevated	0.014	Elevated	0.04	Elevated	0.042		0.42	Elevated	0.012		0.35
PACSIN2	0.82	0.26 to 2.6	0.74	0.78	0.21 to 2.9	0.71		0.89	Elevated	0.038		0.085		0.32		0.24		0.078		0.21		0.75
PARP1	0.74	0.23 to 2.4	0.62	0.24	0.07 to 0.83	0.024		0.77	Not Linear	0.013		0.23		0.54		0.81		0.89		0.41		0.10
PDCD4	0.43	0.18 to 1	0.054	0.35	0.13 to 0.9	0.029		0.22		0.34		0.79		0.57		0.83		0.75		0.93		0.38
PDE4D	1.2	0.4 to 3.8	0.73	0.97	0.29 to 3.2	0.95		0.20		0.12	Not Linear	0.041		0.50		0.42		0.098		0.085		0.51
PDIA4	1.7	0.71 to 4.1	0.23	2.0	0.82 to 5	0.13		0.39		0.95		0.70		0.24		0.31		0.81		0.52	Elevated	0.0048
PGC	0.79	0.59 to 1.1	0.11	0.69	0.49 to 0.97	0.033		0.56		0.75		0.65	Down	0.0016		0.49		0.10		0.49		0.15
PLG	3.0	1.3 to 6.8	0.010	2.5	0.99 to 6.2	0.052		0.81		0.43		0.41		0.10		0.37		0.35		0.27		0.21
PNLIP	0.73	0.54 to 0.99	0.043	0.64	0.46 to 0.91	0.013		0.15	Down	0.0090		0.46		0.05		0.45	Down	0.024	Down	0.00061	Not Linear	0.0020
PNLIPRP2	0.80	0.51 to 1.3	0.34	0.57	0.33 to 0.99	0.046		0.77	Elevated	0.049		0.13		0.61		0.41		0.15		0.35		0.29
PPBP	0.54	0.26 to 1.1	0.083	0.78	0.35 to 1.7	0.54		0.50		0.17	Down	0.039		0.95		0.43	Down	0.0098		0.63		0.058
PTPN11	6.3	1.7 to 23	0.0056	6.6	1.5 to 29	0.012		0.15		0.26		0.33		0.30		0.43		0.93		0.30	Elevated	0.00053
REG1A	0.74	0.53 to 1	0.065	0.60	0.41 to 0.88	0.0093		0.46		0.15		0.16		0.092		0.95		0.085		0.078	Not Linear	0.0075
RELA	2.0	0.28 to 14	0.49	1.7	0.19 to 16	0.63		0.56		0.37		0.074		0.21		0.55		0.87	Elevated	0.010	Elevated	0.012
RPL10	1.4	0.57 to 3.7	0.44	0.83	0.32 to 2.2	0.71		0.46	Not Linear	0.044		0.22		0.46		0.46		0.82		0.17		0.087
RPL36	1.0	0.43 to 2.5	0.92	0.41	0.16 to 1.1	0.064		0.47	Not Linear	0.029		0.14		0.32		0.20		0.37		0.51		0.35
RPN1	0.67	0.29 to 1.5	0.34	0.30	0.12 to 0.75	0.010		0.72	Not Linear	0.033		0.081	Elevated	0.021		0.055		0.16		0.51		0.55
RPN2	0.49	0.16 to 1.5	0.21	0.28	0.083 to 0.97	0.044		0.97	Elevated	0.00077	Elevated	0.041	Elevated	0.028		0.092		0.11	Elevated	0.035		0.66
RPS5	0.58	0.27 to 1.2	0.15	0.34	0.15 to 0.75	0.0072		0.80	Elevated	0.0076		0.056		0.054		0.13		0.32		0.41		0.073
RUNX1	0.42	0.043 to 4.1	0.46	3.4	0.24 to 49	0.37		0.92	Not Linear	0.0011		0.91		0.87		0.65		0.36		0.15		0.21
S100A2	2.9	1.6 to 5.1	0.00026	2.9	1.6 to 5.3	0.00048		0.98	Not Linear	0.044		0.23	Not Linear	0.028		0.65		0.40		1.00	Not Linear	0.00023
S100A4	2.1	1.1 to 3.9	0.016	2.4	1.3 to 4.6	0.0075		0.36		0.58		0.30	Elevated	0.0013		0.38		0.34	Elevated	0.033	Not Linear	0.0016
S100A6	2.0	0.95 to 4.2	0.067	2.3	0.98 to 5.3	0.054		0.38		0.54		0.29		0.42		0.69		0.31	Elevated	0.0091		0.14
SERPINB5	1.7	1 to 2.6	0.033	1.6	1 to 2.7	0.050		0.17	Elevated	0.0069	Elevated	0.029		0.097		0.085		0.16	Elevated	0.020		0.18
SF3B1	2.3	0.86 to 6.1	0.095	1.1	0.36 to 3.3	0.89		0.19	Not Linear	0.047		0.23		0.46		0.13		0.32		0.10	Elevated	0.012
SLC2A1	1.5	0.86 to 2.5	0.16	1.3	0.72 to 2.3	0.41		0.88	Elevated	9.59E-05	Not Linear	0.0083	Elevated	0.00051	Elevated	0.035	Elevated	0.0099		0.12		0.85
SLC4A1	0.68	0.4 to 1.2	0.16	0.68	0.36 to 1.3	0.21		0.89		0.97		0.55		0.36		0.43		0.52		0.31		0.48
SMAD4	2.3	0.67 to 7.8	0.19	1.9	0.5 to 7.6	0.33		0.29		0.57		0.94		0.40		0.70		0.98		0.72	Elevated	0.012
SPTAN1	0.43	0.14 to 1.3	0.14	0.61	0.18 to 2.1	0.44		1.00		0.13		0.28		0.15		0.95		0.27		0.24		0.48
STIL	2.0	0.76 to 5.5	0.16	2.5	0.86 to 7.3	0.091		0.57		0.22		0.34		0.96		0.44		0.15		0.45	Elevated	0.0090
SURF6	0.64	0.31 to 1.3	0.23	0.47	0.23 to 0.99	0.046		0.60		0.95		0.12		0.98	Elevated	0.022		0.82		0.78		0.82
TKT	1.0	0.41 to 2.5	1.00	1.0	0.39 to 2.7	0.96		0.60		0.93		0.40		0.092		0.11		0.60		0.46		0.25
TROVE2	2.4	1 to 5.8	0.045	2.4	0.88 to 6.7	0.086	Down	0.033		0.23		0.45		0.55		0.71		0.98		0.18	Elevated	1.00E-04
TYMP	1.8	0.8 to 4	0.16	1.3	0.52 to 3.5	0.54		0.95		0.065		0.063	Elevated	2.75E-05		0.088		0.12	Elevated	0.046		0.20
UFL1	1.5	0.71 to 3.3	0.27	1.4	0.58 to 3.2	0.48		0.76		0.11		0.59		0.22		0.18		0.37		0.27		0.46
UQCRC2	0.82	0.33 to 2	0.66	0.66	0.25 to 1.8	0.40		0.33		0.69		0.73		0.68		0.51		0.57		0.47		0.30
VCL	1.8	0.63 to 5.2	0.27	2.0	0.59 to 7	0.26		0.67	Elevated	0.029		0.32	Elevated	0.00091		0.22	Elevated	0.041	Elevated	0.028		0.31
VEGFA	4.1	1.6 to 11	0.0038	4.6	1.6 to 13	0.0043		0.64	Elevated	0.031		0.22		0.45		0.65		0.44	Elevated	0.032	Elevated	0.0017
ZBTB16	4.2	0.78 to 22	0.094	4.6	0.67 to 31	0.12		0.69	Elevated	0.0016		0.40		0.31		0.91		0.75	Elevated	0.047		0.13
ZNF462	0.91	0.6 to 1.4	0.67	0.85	0.53 to 1.3	0.48		0.77	Elevated	6.49E-06		0.61		0.83		0.86		0.13		0.24		0.23

Table 2-7. Clinicopathological characteristics of the four classified subgroups in the training set.

Description	Stable (N = 67)	Exocrine-like (N = 17)	Activated (N = 44)	ECM-Remodeling (N = 20)	P-value
Age, years [Mean (95% CI)]	64.3(62.2-66.5)	60.0(56.6-63.4)	65.3(62.7-67.9)	64.7(59.8-69.6)	0.295
Gender					0.035*
Female	35 52.2%	3 17.6%	18 40.9%	12 60.0%	
Male	32 47.8%	14 82.4%	26 59.1%	8 40.0%	
TNM T					0.003**
T1	12 17.9%	7 41.2%	6 13.6%	2 10.0%	
T2	39 58.2%	9 52.9%	28 63.6%	6 30.0%	
T3	15 22.4%	1 5.9%	10 22.7%	10 50.0%	
T4	1 1.5%	0 0.0%	0 0.0%	2 10.0%	
TNM N					0.083#
N0	35 52.2%	10 58.8%	21 47.7%	3 15.0%	
N1	18 26.9%	3 17.6%	15 34.1%	9 45.0%	
N2	14 20.9%	4 23.5%	8 18.2%	8 40.0%	
Differentiation					0.847
Well differentiated	7 10.8%	1 5.9%	3 7.0%	1 5.3%	
Moderately differentiated	50 76.9%	13 76.5%	32 74.4%	13 68.4%	
Poorly differentiated	7 10.8%	3 17.6%	8 18.6%	5 26.3%	
Un-differentiated	1 1.5%	0 0.0%	0 0.0%	0 0.0%	
Lymphatic invasion					0.620
No	35 52.2%	9 52.9%	23 52.3%	7 36.8%	
Yes	32 47.8%	8 47.1%	21 47.7%	12 63.2%	
Venous invasion					0.159
No	38 56.7%	11 64.7%	21 47.7%	6 31.6%	
Yes	29 43.3%	6 35.3%	23 52.3%	13 68.4%	
Perineural invasion					0.539
No	12 17.9%	4 23.5%	5 11.4%	2 10.0%	
Yes	55 82.1%	13 76.5%	39 88.6%	18 90.0%	
N events (%percent)					
Death	13 19.4%	11 64.7%	40 90.9%	13 65.0%	
Recurrence	32 47.8%	10 58.8%	38 86.4%	13 65.0%	
Median survival time (95% CI)					
OS (Month)	NA(NA)	32(22.5-41.5)	16(11.4-20.6)	11(6.8-15.1)	
DFS (Month)	22(17.7-26.3)	19(5.0-33.0)	7(2.4-11.6)	6(2.1-9.8)	

Significance level is represented as "#" for P-value < 0.10; "*" for P-value < 0.05; and "**" for P-value < 0.01; Numerical variable (Age) was analyzed using Levene's Test for equality of variance, and Categorical variables were analyzed using $\chi 2$ Test.

Table 2-8. Univariate and multivariate survival analysis for risk subgroup classification in the training set.

Covariate		Univariate Analysis (Overall Survival)					Multivariate Analysis (Overall Survival)				
		β	Standard Error	P-value	Exp(β)	95% CI of Exp(β)	β	Standard Error	P-value	Exp(β)	95% CI of Exp(β)
Age		0.008	0.014	0.5352	1.01	0.9821 to 1.0355					
Gender	Female					Reference					
	Male	0.181	0.233	0.4373	1.20	0.7609 to 1.8873					
Resection margin	R0					Reference					Reference
	R1	0.686	0.345	0.0471	1.99	1.0124 to 3.8923	0.926	0.379	0.0146	2.52	1.2054 to 5.2882
	R2	0.560	1.012	0.5798	1.75	0.2434 to 12.5970	1.817	1.059	0.0861	6.16	0.7806 to 48.5466
TNM T	T1					Reference					Reference
	T2	0.887	0.374	0.0176	2.43	1.1714 to 5.0358	1.020	0.417	0.0144	2.77	1.1832 to 6.0753
	T3	1.068	0.393	0.0066	2.91	1.3520 to 6.2572	1.140	0.446	0.0105	3.13	1.2901 to 7.6186
	T4	3.051	0.689	<0.0001	21.14	5.5136 to 81.0522	3.300	0.796	<0.0001	27.12	3.2045 to 81.3347
TNM N	N0					Reference					
	N1	0.205	0.277	0.4593	1.23	0.7154 to 2.1054					
	N2	0.826	0.284	0.0037	2.28	1.3114 to 3.9757					
Differentiation	Well differentiated					Reference					
	Moderately diffenrentiated	0.831	0.518	0.1085	2.30	0.8364 to 6.3016					
	Pooly differentiated	1.002	0.574	0.0809	2.72	0.8891 to 8.3471					
Lymphatic invasion	No					Reference					Reference
	Yes	0.545	0.233	0.0194	1.72	1.0946 to 2.7147	0.415	0.248	0.0947	1.51	0.9331 to 2.4572
Venous invasion	No					Reference					
	Yes	0.748	0.236	0.0015	2.11	1.3333 to 3.3482					
Perineural invasion	No					Reference					
	Yes	0.574	0.355	0.1063	1.77	0.8878 to 3.5480					
Risk subgroup	Stable					Reference					Reference
	Exocrine-like	0.789	0.414	0.0564	2.20	0.9827 to 4.9346	1.291	0.446	0.0038	3.64	1.5232 to 8.6747
	Activated	1.562	0.320	<0.0001	4.77	2.5564 to 8.8943	1.645	0.332	<0.0001	5.18	2.7113 to 9.9068
	ECM-Remodeling	1.912	0.400	<0.0001	6.76	3.0989 to 14.7626	1.531	0.470	0.0011	4.62	1.8500 to 11.5576

1.3.4 Classification Model Development and Prognostic Relevance of Risk Subgroups

To assess risk subgroups of patients based on protein levels, in an objective manner, a random forest classification model was established using the training set (148 patients), by performing 10 iterations of 5-fold cross-validation. Fourteen DEPs (Bonferroni post hoc test, $P < 0.05$) identified in at least one subgroup were added to a list of features in addition to 44 proteins that were used in a clustering analysis to improve model performance (Figure 2-8). In summary, 33 of 58 proteins were selected, according to associations with risk subgroups and the eigenproteins of each module in weighted gene correlation network analysis (WGCNA) (Figure 2-9A and B, and Table 2-9). Considering the variable importance and error rates of cross-validation, a final 24-protein model was developed (Figure 2-9C-F). The fully detailed methods are described in the Supplementary Information.

We confirmed the model using an internal validation set (77 patients) and an external validation set, from Puleo et al.(87), which represents the largest (N = 309) PDAC cohort of FFPE tissue samples, to date. The four risk subgroups were represented in all three sets (training, validation, and Puleo et al.), along with the differential expression of the 24-protein model (Figure 2-10A). The risk subgroups in the validation set were significantly correlated with differentiation (χ^2 test, $P = 0.017$) and were associated with both T stage and N stage; however, the stage-based associations (χ^2 test, $P = 0.056$ and 0.059, respectively) did not reach significance (Table 2-10). In the Puleo et al. set, the risk subgroups were associated with differentiation (χ^2 test, $P = 0.022$) but were not associated with T stage or N stage (χ^2 test, $P = 0.805$ and 0.170, respectively) due to the high proportion of late stages (81.6% in T3 stage and 74.8% in N1 stage). Moreover, the risk subgroups showed strong prognostic relevance to survival (Log-rank test, P = 3.0E-09 for training set, 4.6E-04 for validation set, and 3.2E-12 for Puleo et al.) and recurrence (Log-rank test, P = 9.0E-06 for training set,

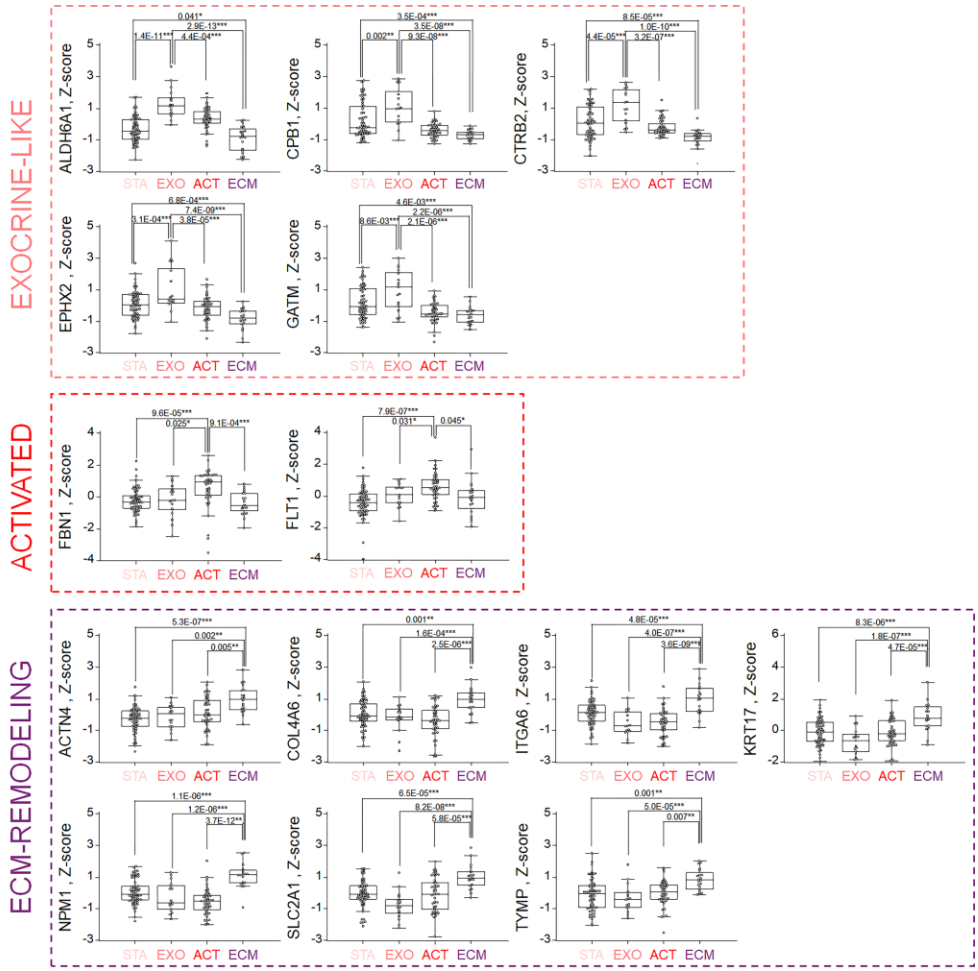
4.5E-05 for validation set, and 3.5E-10 for Puleo et al.) (Figure 2-10B and C).

Similar to the clustering analysis results, the “ECM-Remodeling” subgroup had the worst outcome, with median OS values of 10.0, 8.0, and 11.9 months and survival rates of 25.0%, 20.0%, and 30.0% in the training, validation, and Puleo et al. sets, respectively. The “Stable” subgroup had a better prognosis than the other subgroups, with median OS values of NA (did not reach survival probability of 50%), 55.0, and 33.6 months and survival rates of 77.8%, 69.7%, and 41.3% in the training, validation, and Puleo et al. sets, respectively. The “Stable” risk subgroup in Puleo et al. experienced earlier and higher mortality than the training and validation sets, due to its higher proportion of late-stage cases and longer overall follow-up time (over 140 months).

The inclusion of our risk subgroups into a multivariate Cox regression, in addition to the subtypes identified by Puleo et al., improved the predictive power (OS, Wald test, P = 4.2E-09 to 5.3E-11), which implied that the two classification systems were independent predictors of survival and were highly associated with one another. In addition, the prognostic relevance of the predicted risk subgroups was verified in the external validation sets of Bailey et al.(6), Moffitt et al.(85), and TCGA(86) (Figure 2-11).

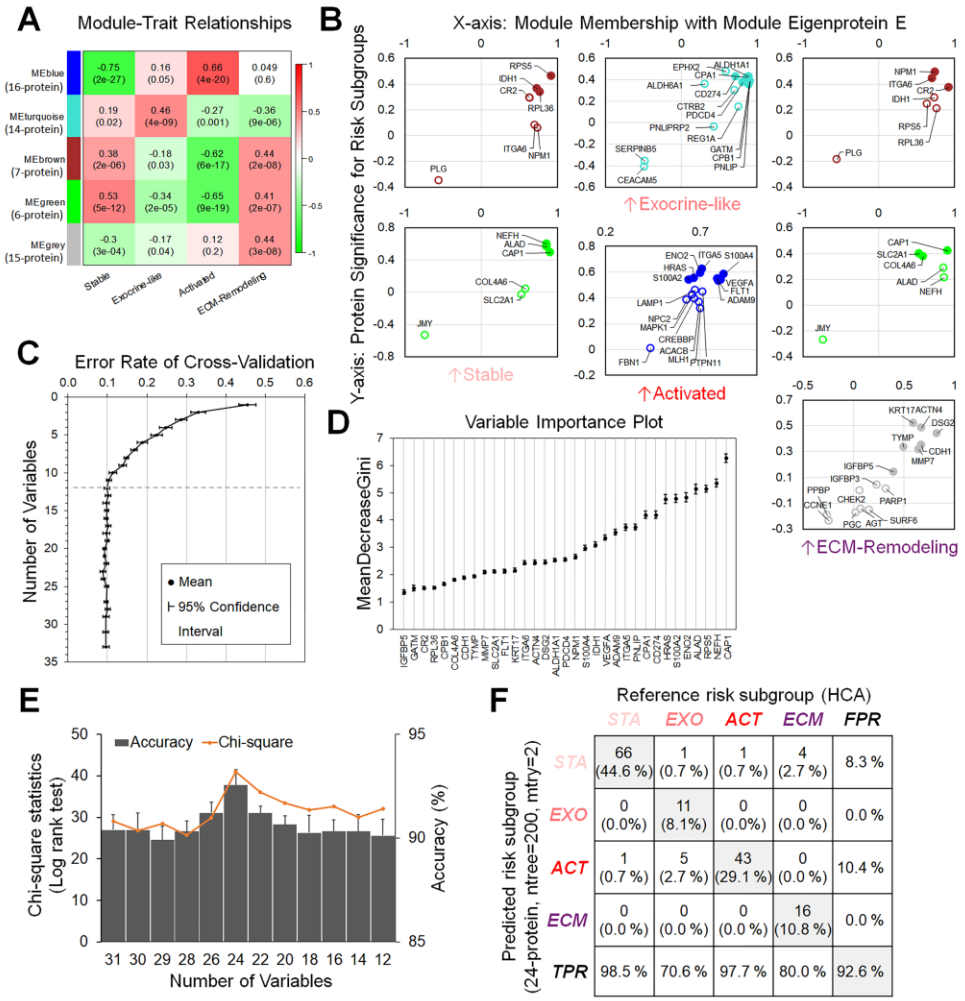
Further, we characterized the risk subgroups using 8 cases of FFPE tissues, matched with whole tumor tissues. The 4 representative markers for poor prognosis [NSE (ENO2), HRAS, GLUT1 (encoded by SLC2A1), and ACTN4] showed differential expression between risk subgroups by IHC (Figure 2-12A and B). Protein expression by MRM-MS correlated significantly between whole fresh frozen and FFPE tissues with regard to the 115 quantified proteins (Pearson r = 0.337, P = 1.4E-27) and the 24 proteins in the RF model (Pearson r = 0.493, P = 3.7E-13). The expression pattern was replicated in FFPE tissues, which was apparent when comparing the heatmaps of the training set, validation set, and Puleo et al. dataset in Figure 2-10A (Figure 2-12C and D). In addition, the RF model classified risk subgroups using the 24-marker expression of FFPE tissues with discriminatory predictive ability between risk subgroups (Figure 2-12E).

Figure 2-8. Selection of additional proteins that are highly expressed in each risk subgroup, to improve the performance of the classification model.



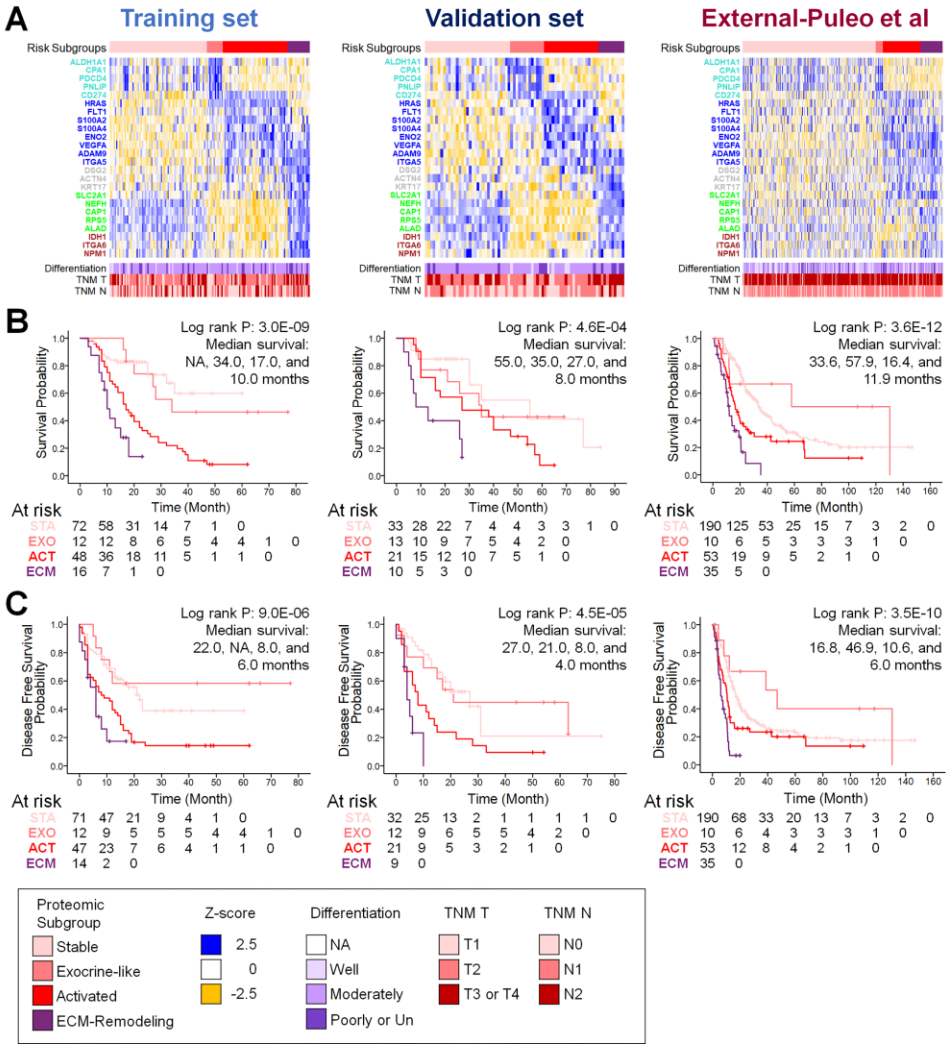
Fourteen proteins, which were not included in hierarchical clustering analysis due to P-values higher than 0.1 during the univariate survival analysis or Pearson's |r| lower than 0.5, were selected to improve the performance of the random forest classification model. Differences in protein expression between subgroups were measured by Bonferroni's post hoc test, and the fourteen proteins with significantly high expression ($P < 0.05$) in any risk subgroup were selected. Significance levels are represented as * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$.

Figure 2-9. Development of the Random Forest classification model.



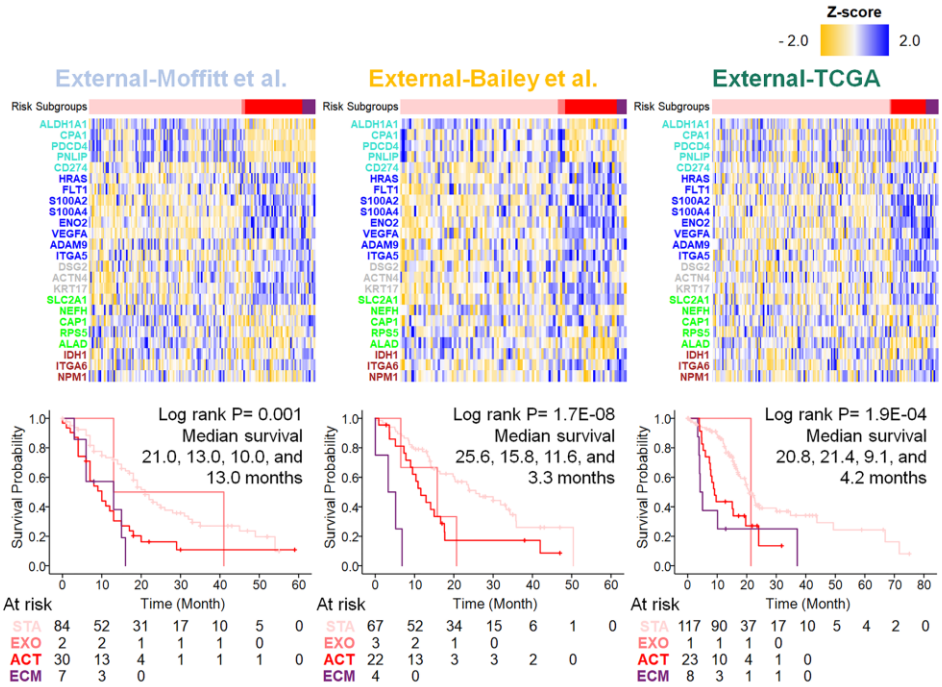
A. Student's asymptotic P-value for the correlation between protein modules and risk subgroups (Trait), for feature selection. Five protein modules were identified, using 58 proteins, and each module was significantly associated with binarized risk subgroups. B. Important genes in the modules were selected according to the significance of each protein associated with traits, and with membership with the eigenprotein of the module. Then, 33 (56.9%) proteins were selected to develop the random forest model. C. A 5-fold, cross-validated prediction performance, with sequentially reduced numbers of variables, ranked by variable importance. D. Similar error rates were derived using between 12 and 31 variables. E. The 24-protein model, which showed most high accuracy and the best chi-square value in the log-rank test, was finally selected. F. Confusion matrix of the risk subgroup classification, based on the 24-protein optimized model parameters (ntree = 200, mtry = 2).

Figure 2-10. Prediction of proteomic risk subgroups.



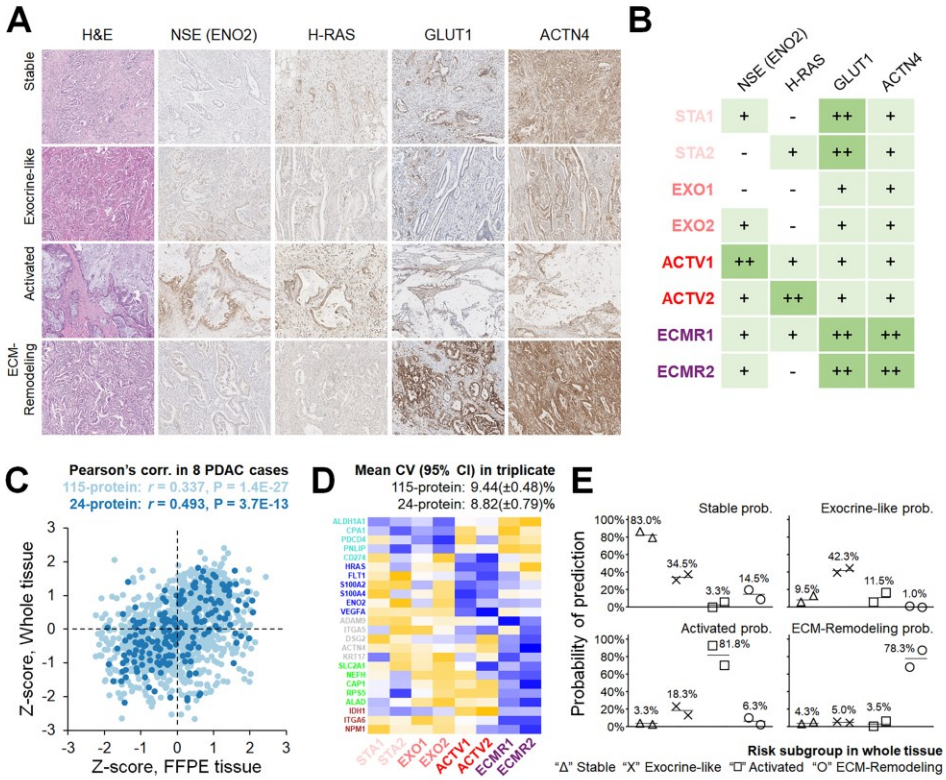
(A) Heatmap, showing the 24 proteins identified for subgroup classification, in the training set (N = 148), the internal validation set (N = 77), and the external Puleo et al. set (N = 309). Prognostic factors (T stage, N stage, and differentiation) are plotted below the heatmap. Samples are ordered by their OS time and death events, in each subgroup (low- to high-risk, from the left). The colors of the proteins are equal to the module colors in WGCNA. Kaplan-Meier curves of the predicted risk subgroups in all three cohorts are shown for OS (B) and DFS (C). "Not Applicable" indicates that the number of patients who experienced the event did not reach 50% in the overall follow-up.

Figure 2-11. External validation of the Random Forest classification model.



Additional model evaluation, using the three external PDAC subtype studies. Heatmap represented the expression of the 24 proteins for subgroup classification, in the Moffitt et al. (N = 145), Bailey et al. (N = 96), and TCGA (N = 150). Kaplan-Meier curves of the predicted risk subgroups are shown for OS. The predicted risk subgroups presented similar survival outcomes using external datasets as with our study cohort, despite the low proportion of the “Exocrine-like” subgroup.

Figure 2-12. Risk subgroups in formalin-fixed paraffin-embedded tissues.



A. Histology and immunohistochemistry (IHC) images of risk subgroup tumors. Representative images were demonstrated for NSE, HRAS, GLUT1 and ACTN4 immunohistochemistry. B. Summary of the immunohistochemical stain results for the 8 PDAC cases. C. Correlation between protein expression of whole resected tissues and protein expression of matched FFPE tissue samples in 8 PDAC cases. Pearson correlation was observed using all 115 quantified proteins and the 24 proteins in the RF model. D. Heatmap of expression of 24 proteins in FFPE tissue from 8 PDAC cases. Protein-wise z-score normalization was performed. E. Risk subgroup prediction using FFPE samples in 8 PDAC cases. Probabilities of prediction are represented for risk subgroups. Mean probability in each risk subgroup tumors is represented by a horizontal line.

Table 2-9. Protein features in the modules from WGCNA.

Proteins in MEblue	Proteins in MEturquoise	Proteins in MEbrown	Proteins in MEgreen	Proteins in MEgrey
ACACB	ALDH1A1	CR2	ALAD	ACTN4
ADAM9	ALDH6A1	IDH1	CAP1	AGT
CREBBP	CD274	ITGA6	COL4A6	CCNE1
ENO2	CEACAM5	NPM1	JMY	CDH1
FBN1	CPA1	PLG	NEFH	CHEK2
FLT1	CPB1	RPL36	SLC2A1	DSG2
HRAS	CTRB2	RPS5		IGFBP3
ITGA5	EPHX2			IGFBP5
LAMP1	GATM			KRT17
MAPK1	PDCD4			MMP7
MLH1	PNLIP			PARP1
NPC2	PNLIPRP2			PGC
PTPN11	REG1A			PPBP
S100A2	SERPINB5			SURF6
S100A4				TYMP
VEGFA				

Table 2-10. Clinicopathological characteristics of the four classified subgroups in the validation set.

Description	Stable (N = 33)		Exocrine-like (N = 13)		Activated (N = 21)		ECM-Remodeling (N = 10)		P-value
Age, years [Mean (95% CI)]	64.2	(60.8-67.6)	68.3	(61.6-75.0)	59.0	(54.0-63.9)	70.2	(65.2-75.2)	0.369
Gender									0.648
Female	14	42.4%	7	53.8%	12	57.1%	6	60.0%	
Male	19	57.6%	6	46.2%	9	42.9%	4	40.0%	
TNM T									0.056 #
T1	4	12.1%	3	23.1%	3	14.3%	0	0.0%	
T2	16	48.5%	8	61.5%	12	57.1%	2	20.0%	
T3	13	39.4%	2	15.4%	6	28.6%	7	70.0%	
T4	0	0.0%	0	0.0%	0	0.0%	1	10.0%	
TNM N									0.059 #
N0	16	50.0%	7	53.8%	9	42.9%	4	40.0%	
N1	16	50.0%	5	38.5%	12	57.1%	3	30.0%	
N2	0	0.0%	1	7.7%	0	0.0%	3	30.0%	
Differentiation									0.017 *
Well differentiated	0	0.0%	2	15.4%	3	14.3%	0	0.0%	
Moderately differentiated	29	90.6%	9	69.2%	15	71.4%	5	50.0%	
Poorly or Un-differentiated	4	12.5%	2	15.4%	2	9.5%	5	50.0%	
Lymphatic invasion									0.637
No	18	58.1%	9	69.2%	9	47.4%	5	50.0%	
Yes	13	41.9%	4	30.8%	10	52.6%	5	50.0%	
Venous invasion									0.451
No	20	66.7%	9	69.2%	12	63.2%	4	40.0%	
Yes	10	33.3%	4	30.8%	7	36.8%	6	60.0%	
Perineural invasion									0.186
No	4	12.1%	4	30.8%	1	5.0%	1	10.0%	
Yes	29	87.9%	9	69.2%	19	95.0%	9	90.0%	
N events (%percent)									
Death	10	30.3%	7	53.8%	18	85.7%	8	80.0%	
Recurrence	17	51.5%	8	61.5%	19	90.5%	8	80.0%	
Median survival time (95% CI)									
OS (Month)	55.0	(23.0-87.0)	35.0	(20.2-49.8)	27.0	(0.0-56.9)	8.0	(0.0-17.3)	
DFS (Month)	27.0	(15.1-38.9)	21.0	(11.0-31.0)	8.0	(5.0-11.0)	4.0	(2.1-5.9)	

Significance level is represented as "#" for P-value < 0.10; and "*" for P-value < 0.05; Numerical variable (Age) was analyzed using Levene's Test for equality of variance, and Categorical variables were analyzed using χ^2 Test.

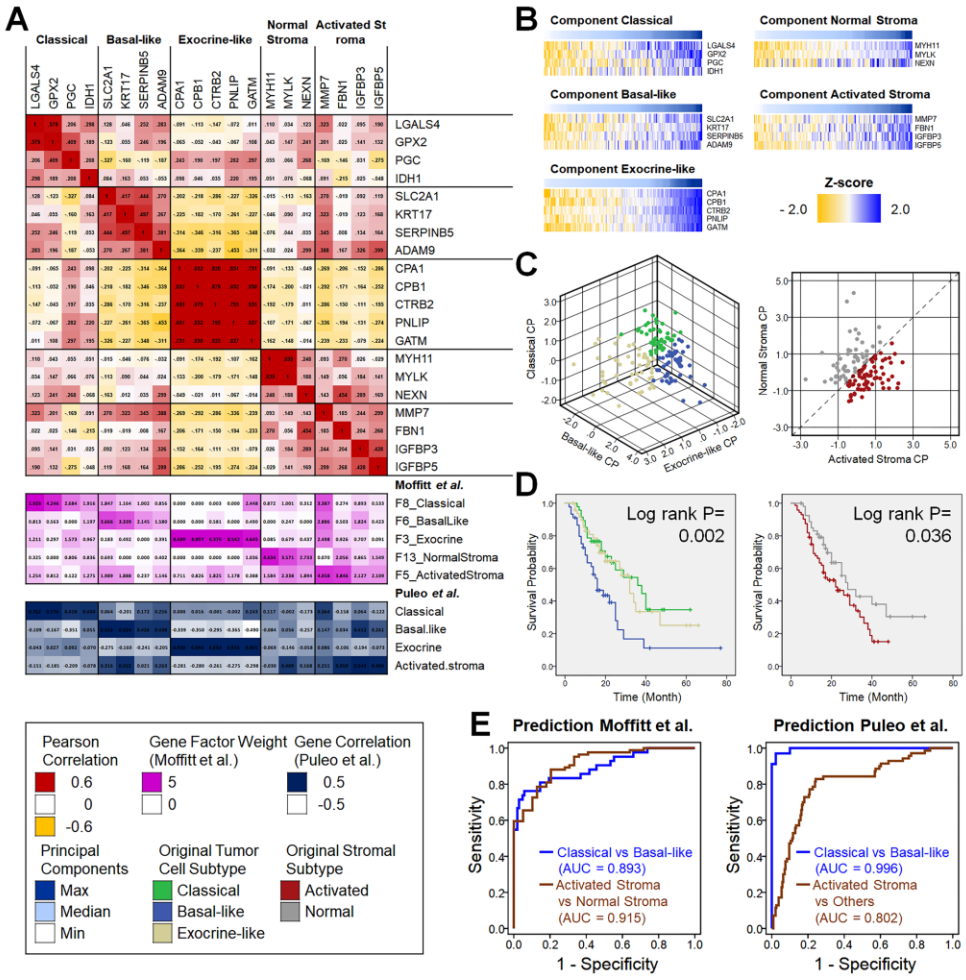
1.3.5 Association of Risk Subgroups with Original Transcriptomic Subtypes

To identify associations between the risk subgroups defined in this study and the original transcriptomic subtypes (classical, exocrine-like, basal-like, normal stroma, and activated stroma), 20 out 115 proteins were selected to represent each transcriptomic subtype, according to the subtype scores of the relevant genes, which were retrieved from the studies by Moffitt et al. and Puleo et al. (Figure 2-13A). The original subtypes were classified using subtype components, which were determined based on factor analysis, using principal components (Figure 2-13B and C). Both tumor cell subtypes (OS, Log-rank test, P = 0.002) and stromal subtypes (OS, Log-rank test, P = 0.036) were significantly associated with survival (Figure 2-13D). The associations between subtype components and the original subtypes (classical versus basal-like and activated stroma versus normal stroma) were verified by logistic regression, using the data from Moffitt et al. and Puleo et al. [area under the curve (AUC) > 0.8 for all comparisons] (Figure 2-13E).

After the inclusion of the original subtype classifications in our dataset, the following observations were made (Figure 2-14A). The exocrine-like subtype was associated with our defined “Stable” and “Exocrine-like” subgroups, whereas the basal-like subtype was associated with our “Activated” and “ECM-Remodeling” subgroups, in both the training and validation sets (χ^2 test, P < 1.0E-4 for all comparisons). In contrast, the classical subtype showed no association with any risk subgroups (χ^2 test, P = 0.6352 for training set and 0.4235 for validation set). Stromal subtypes (both activated and normal) showed weaker relevance for defining risk subgroups than tumor cell subtypes (χ^2 test, P = 0.004 for training set and 0.102 for validation set). These identified associations between predicted risk subgroups and the originally defined subtypes were repeated in all four external validation sets and high-purity tumors were found to be significantly enriched in the “Activated” and “ECM-Remodeling” subgroups

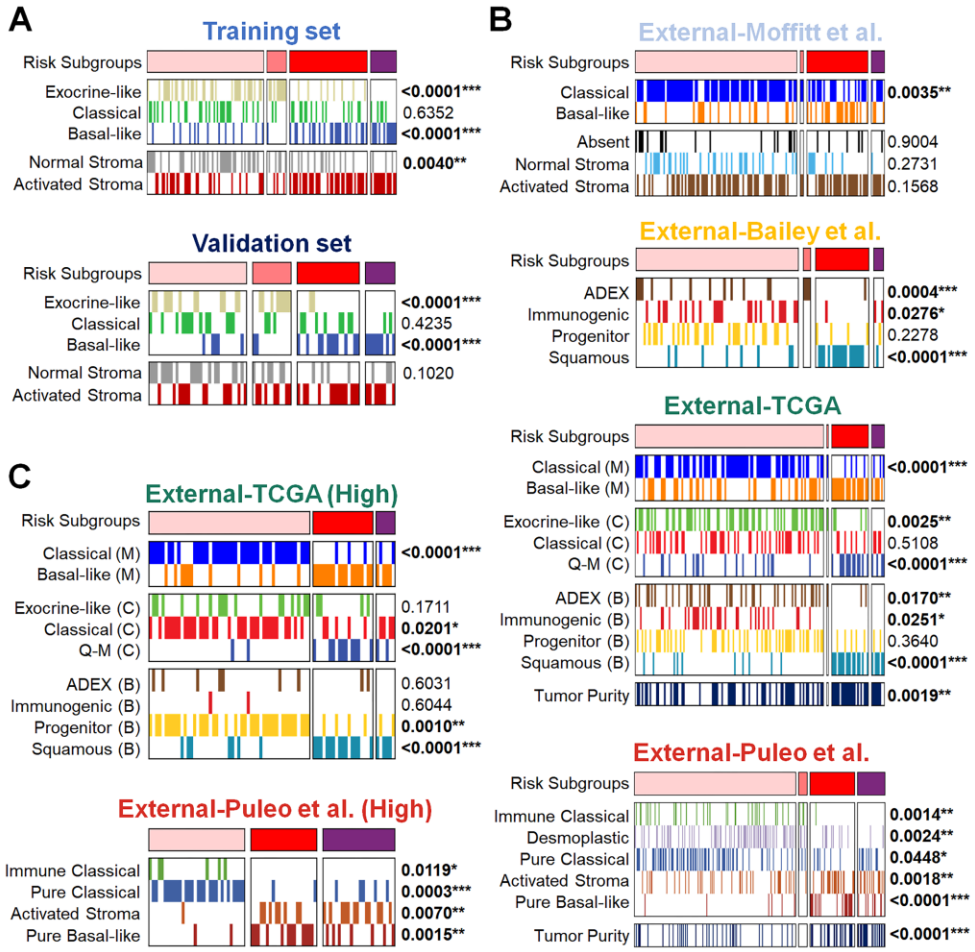
(Figure 2-14B). Moreover, among the high-purity datasets from TCGA and Puleo et al., we observed that classical subtypes were associated with “Stable” subgroups, whereas the association of exocrine-like subtypes with risk subgroups was insignificant (Figure 2-14C). By cross-referencing the original subtypes, the risk subgroups could be defined as follows (Figure 2-14 and Figure 2-15A). First, the two high-risk subgroups (“Activated” and “ECM-Remodeling”) had high tumor contents and were associated with basal-like subtypes and activated stroma subtypes. Second, the “Stable” subgroups overlapped with classical subtypes and tumor microenvironment subtypes, such as the immunogenic, immune classical, and desmoplastic subtypes defined in other studies. Third, our “Exocrine-like” subgroups appeared to have low tumor contents, with high levels of pancreatic enzymes, such as pancreatic lipase (PNLIP) and carboxypeptidase A1 (CPA1), which were absent from high-purity tumor sets. Thus, poor prognosis-associated basal-like subtypes were mainly categorized in the “Activated” and “ECM-Remodeling” subgroups, whereas subtypes that contain contents from the microenvironment were categorized in the “Stable” subgroup, along with a significant proportion of classical subtypes.

Figure 2-13. Principal component analysis for the classification of original transcriptomic PDAC subtypes based on protein expression.



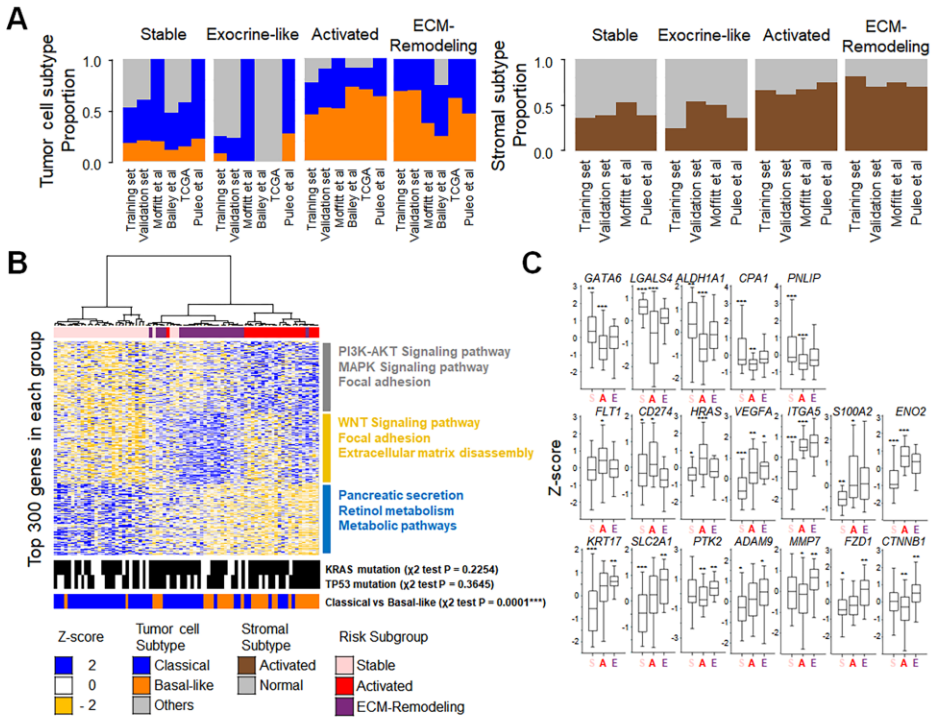
A. Pearson's correlation analysis between the 20 proteins selected for the classification of transcriptomic subtypes, according to the subtype scores reported by Moffitt et al. and Puleo et al.. B. The five subtype components (CPs) in a heatmap, obtained using the 20 proteins in the training set. Samples are ordered by their weight in each of the five components (first raw, from low [white] to high [blue]). C. 3D scatter plot of the CPs used to classify the three tumor cell subtypes, and a simple scatter plot showing the CPs for the classification of the two stromal subtypes. D. Kaplan-Meier curves for the original tumor cell or stromal cell subtypes in the training set. "Basal-like" tumor cell subtypes and "Activated stroma" subtypes are associated with higher death rates than other subtypes, which is identical to the results of previous studies. E. The prediction of subtypes in the data sets reported by Moffitt et al. and Puleo et al., based on the logistic regression model, using the principal components of subtypes.

Figure 2-14. Comparison between risk subgroups and original subtypes.



A. Heatmap showing the risk subgroups and their associations with the original tumor cell and stromal cell subtypes, classified according to subtype components (CPs). B. Predicted risk subgroups in the four external validation sets and their associations with the original subtypes classified in each study. In the external-TCGA set, the sample groups were classified into three subtypes, [Moffitt et al.(M), Collisson et al.(C), and Bailey et al.(B)], which were used for comparison. Tumor purity classifications (Low and High) were available in the external-TCGA and the external-Puleo et al. subsets. C. Predicted risks subgroups in the high-purity samples of the external-TCGA and the external-Puleo et al. data sets and their associations with the original subtypes that were classified in each study. Desmoplastic did not remain in the high-purity set from Puleo et al. Significance levels of the chi-square test is represented as * for P < 0.05, ** for P < 0.01, and *** for P < 0.001.

Figure 2-15. Molecular features of the proteomic risk subgroups.



(A) Associations between risk subgroups and the original tumor cell subtypes (classical, basal-like, and others) and the stromal subtypes (activated stroma and others). (B) Hierarchical clustering of the three risk subgroups ("Stable", "Activated", and "ECM-Remodeling") in the high-purity tumor sets from Puleo et al. The top 300 genes in each subgroup were used to perform clustering and for gene enrichment analysis (right side of the heatmap). KRAS mutation, TP53 mutation, and high-purity classifications based on Puleo et al. are shown below the heatmap. (C) Prognostic markers or therapeutic targets representative of the risk subgroups. Asterisks denote significant differences compared with the other subgroups, according to Student's t-test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

1.3.6 Molecular Characterization of Risk Subgroups

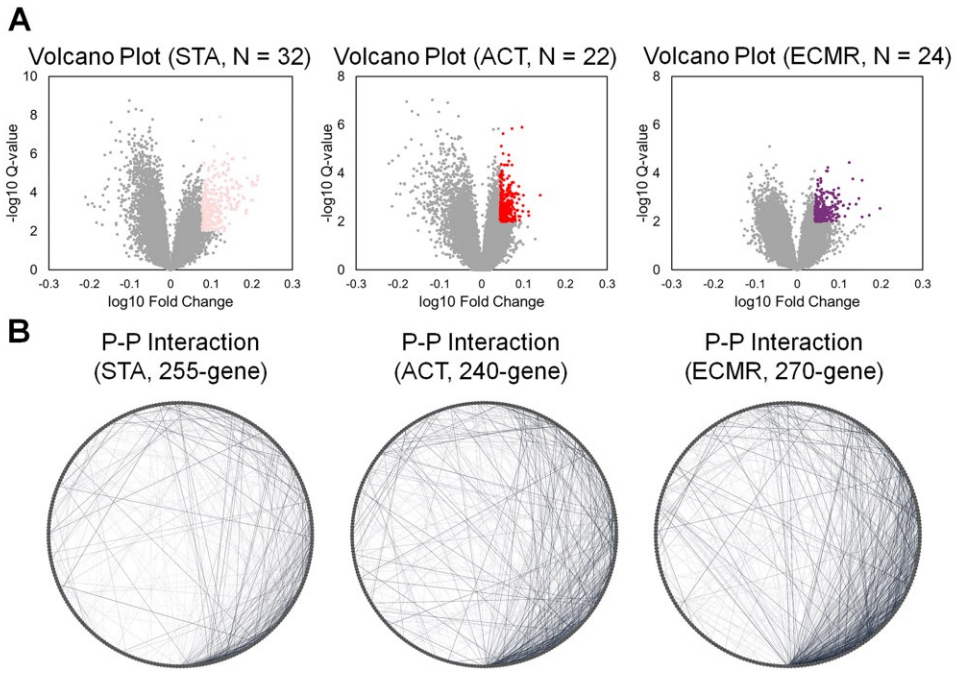
To define the molecular mechanisms of tumor progression, the top 300 genes, according to expression, in each risk subgroup (Q-value < 0.01 and ordered by fold change) were compiled in the high-purity data set from Puleo et al. (Figure 2-15B and Figure 2-16). The hierarchical clustering analysis, using the expression of 900 genes (300 unique genes from each risk subgroup), was able to classify the three risk subgroups with high significance (χ^2 test, P = 4.1E-25). The driver gene mutations in KRAS and TP53 occurred evenly in all three risk subgroups (χ^2 test, P = 0.2254 and 0.3645, respectively). In the gene enrichment analysis, performed using the DAVID functional annotation tool(98), different biological terms were enriched in each risk subgroup. The two high-risk subgroups ("Activated" and "ECM-Remodeling"), which overlapped primarily with basal-like subtypes, were associated with very distinct pathways. The phosphoinositide-3 kinase (PI3K)-protein kinase B (AKT) and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathways were highly enriched in the "Activated" subgroup, whereas the WNT/β-Catenin and NOTCH signaling pathways were enriched in the "ECM-Remodeling" subgroup. In addition, transcriptional network analysis was performed, using the genes in these pathways differentially expressed between the risk subgroups (Q-value < 0.05), and the upregulation of these processes were verified in the "Activated" and "ECM-Remodeling" subgroups (Figure 2-17).

In contrast, gene ontology terms related to numerous metabolic pathways, such as retinol metabolism, xenobiotic metabolic process, and steroid hormone-mediated signaling, consistent with the pancreatic progenitors identified by Bailey et al., and pancreatic secretion or digestion were enriched in the "Stable" subgroups (Figure 2-18A). Among the four categories of proteins, classical marker proteins, retinol metabolism proteins, exocrine-like marker protein, and pancreatic secretion proteins, the pancreatic secretion proteins were the only category that did not

correlate with the other categories of proteins (Figure 2-18B). Meanwhile, classical subtype tumors with higher levels of pancreatic enzymes were associated with a better prognosis than the other groups (Log-rank test, P = 0.073, HR [95%CI]: 0.42 [0.14-1.25]) (Figure 2-18C).

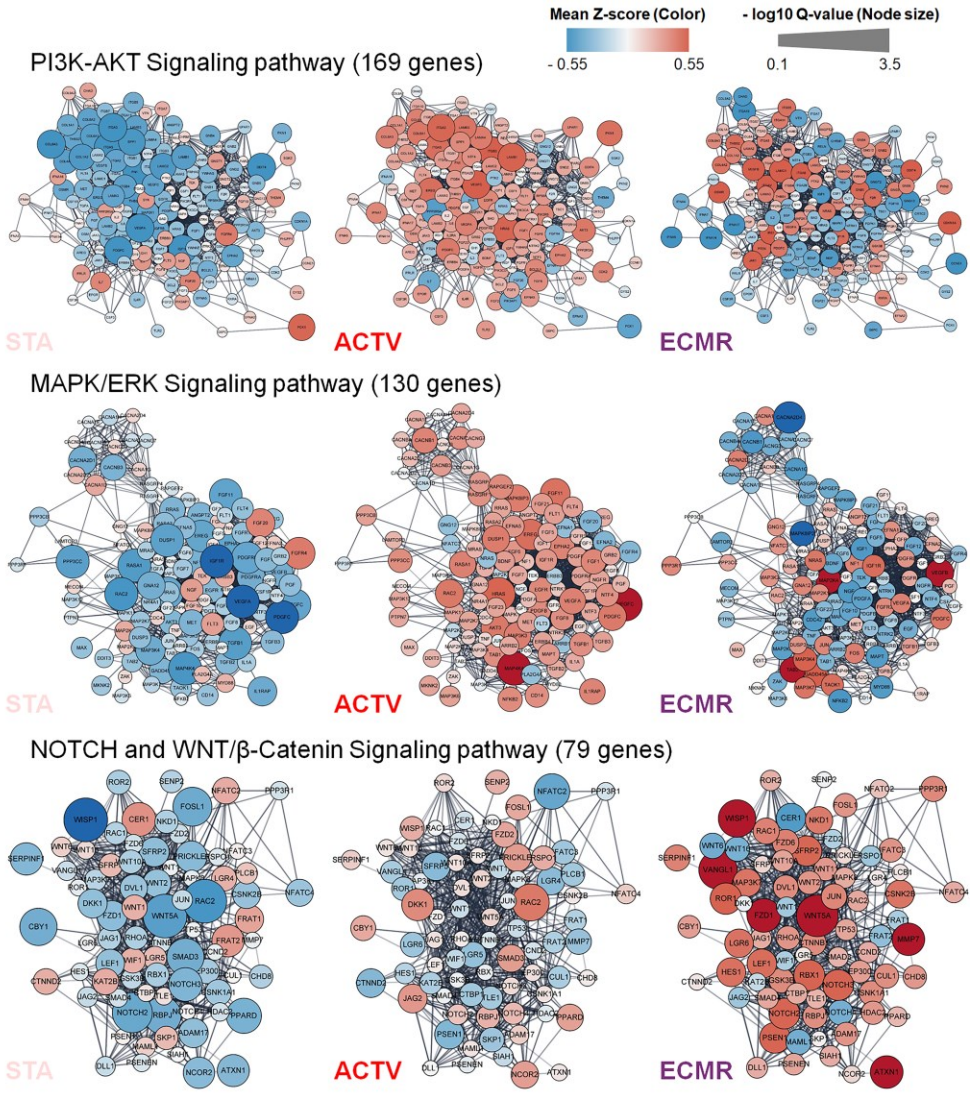
Furthermore, the risk subgroups were associated with PDAC prognostic markers and therapeutic targets (Figure 2-15C and Table 2-11). We observed a significant association between the classical subtype biomarkers [GATA-binding protein 6 (GATA6) and galectin 4 (LGALS4)], aldehyde dehydrogenase 1 A1 (ALDH1A1), pancreatic enzymes (PNLIP and CPA1), and the “Stable” risk subgroup. The proteins involved in the PI3K-AKT and MAPK/ERK signaling pathway [Fms-related receptor tyrosine kinase 1 (FLT1), CD274, HRAS, vascular endothelial growth factor A (VEGFA), and integrin alpha 5 (ITGA5)] were expressed at higher levels in the “Activated” risk subgroup, along with markers of poor prognosis [S100 calcium-binding protein A2 (S100A2) and enolase 2 (ENO2)]. The “ECM-Remodeling” risk subgroup displayed the high enrichment of proteins related to the cytoskeleton [keratin 17 (KRT17)], glucose transport [solute carrier 2A1 (SLC2A1)], ECM disassembly [protein tyrosine kinase 2 (PTK2), disintegrin and metalloproteinase domain-containing protein 9 (ADAM9), and matrix metalloproteinase 7 (MMP7)], and WNT/β-Catenin signaling [frizzled 1 (FZD1) and catenin beta 1 (CTNNB1)].

Figure 2-16. Re-defining risk subgroups and transcriptional networks in the high-purity tumor sets.



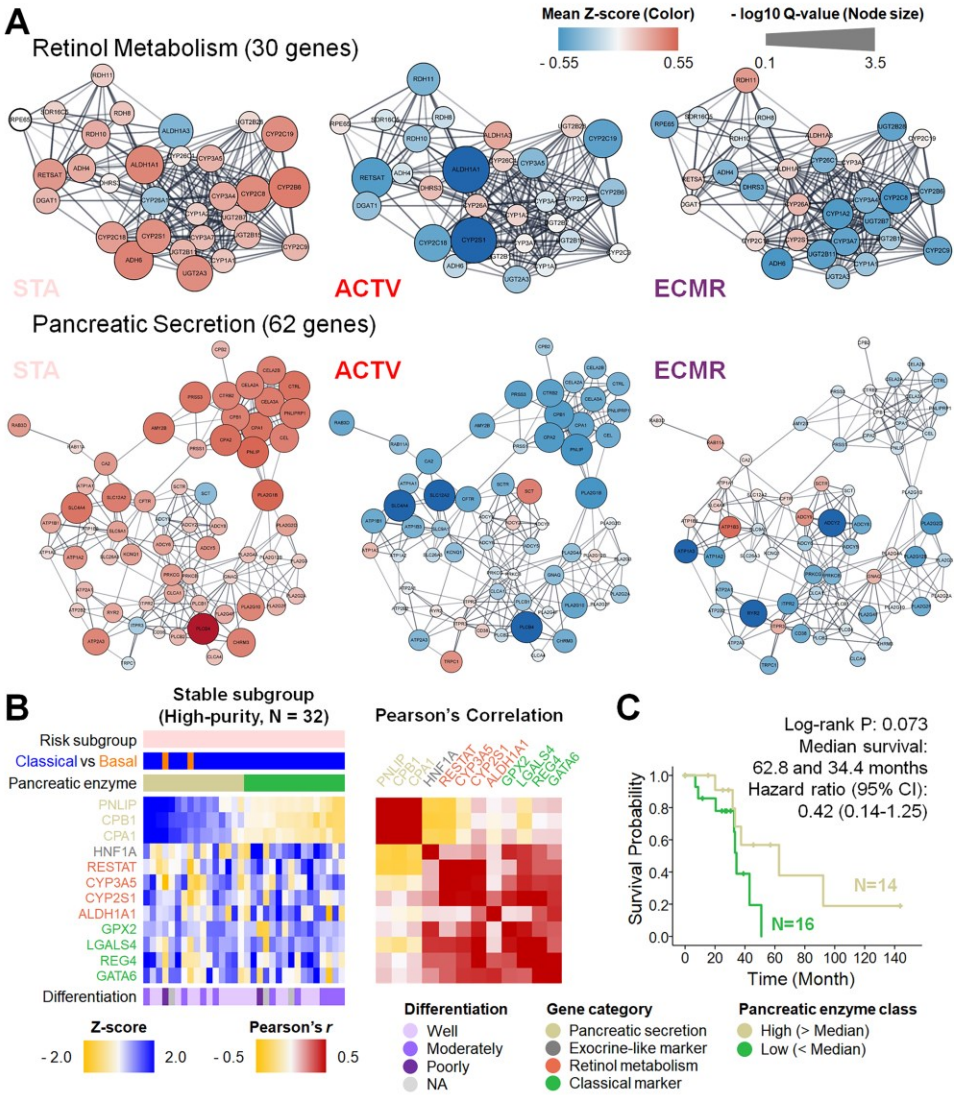
A. Volcano plots for the transcriptomic expression in the three risk subgroups. Colored dots represent the top 300 genes. B. Protein-Protein (P-P) interaction networks involving the top overexpressed proteins in the three risk subgroups. A line between nodes signifies that the corresponding proteins encoded by genes could interact physically (STRING database score > 0.3).

Figure 2-17. Sub-network of differentially expressed genes between the two high-risk subgroups.



The listed gene sets from the KEGG pathway analysis associated with the PI3K-AKT, MAPK/ERK, NOTCH and WNT/β-Catenin signaling pathways. The differentially expressed genes among the three risk subgroups (Q-value < 0.05 for at least one risk subgroup) were included in the gene network analysis, using Cytoscape (Ver 3.8.0). Node colors denote the mean Z-score of gene expression in the risk subgroups, and node sizes denote the significance of the difference between a given risk subgroup and other risk subgroups (-log10 Q-value). A line between nodes signifies that the corresponding proteins encoded by the genes could interact physically (STRING database score > 0.5).

Figure 2-18. Molecular characterization of the stable-risk subgroups.



A. Sub-networks of differentially expressed genes in the stable-risk subgroups. B. The expression pattern (ordered according to the average expression levels of three pancreatic secretion genes) and correlations among the genes from the four categories (pancreatic secretion, markers of exocrine-like subtype, retinol metabolism, and markers of classical subtype). C. Prognostic relevance of pancreatic enzyme concentrations in the classical tumors associated with the stable-risk subgroups. Pancreatic enzyme classes were determined by the median level of three-gene expression averages.

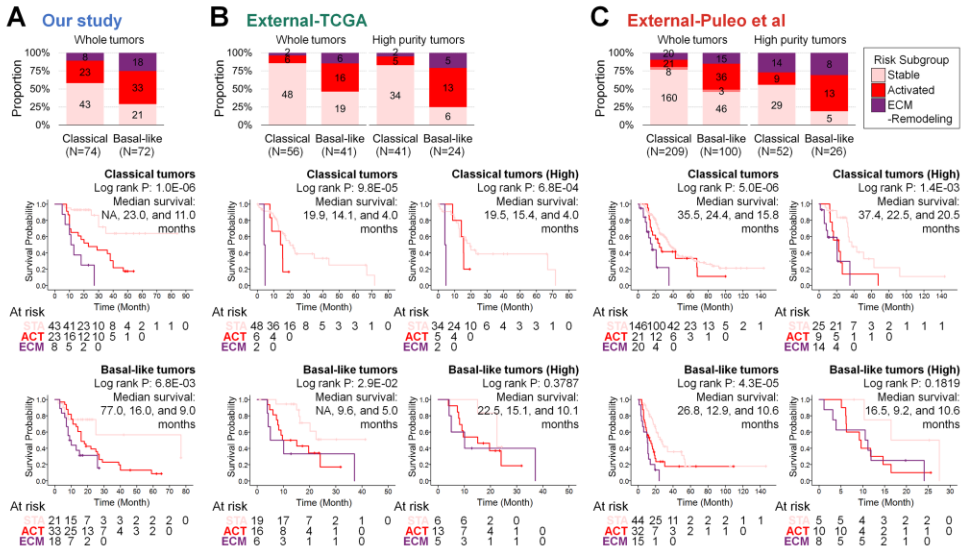
Table 2-11. Completed or ongoing clinical trials for pancreatic cancer associated with the highly expressed proteins in the risk subgroups.

Drug name	Company	Target protein(s)	ClinicalTrials.gov Identifier	Recuitment status	Sponsor	Combination partner(s)	Study phase
Bevacizumab	Genetech	VEGFA	NCT00614653	Completed	M.D. Anderson Cancer Center	Erlotinib and Capecitabine	
			NCT00047710	Completed	M.D. Anderson Cancer Center	Capecitabine with Radiotherapy	
			NCT00460174	Completed	Northwestern University	Gemcitabine with Radiotherapy	I
			NCT00365144	Completed	University of California, San Francisco	Erlotinib	I
			NCT00417976	Completed	Tony Bekaii-Saab	Gemcitabine and Infusional 5-Fluorouracil	I
			NCT00112528	Completed	Alliance for Clinical Trials in Oncology	Gemcitabine and Oxaliplatin	I
			NCT00602602	Active, and not recruiting	Abramson Cancer Center of the University of Pennsylvania	Gemcitabine, Fluorouracil, and Oxaliplatin with Radiotherapy	I
			NCT00126633	Completed	University of California, San Francisco	Gemcitabine and Cisplatin	I
			NCT00366457	Completed	Massachusetts General Hospital	Gemcitabine and Erlotinib	I
			NCT00222469	Completed	University of Oklahoma	Gemcitabine and Oxaliplatin	I
			NCT00066677	Completed	Fox Chase Cancer Center	Docetaxel	I
			NCT00114179	Completed	National Cancer Institute (NCI)	Gemcitabine and Capecitabine with Radiotherapy	I
			NCT00260364	Completed	Royal Marsden NHS Foundation Trust	Gemcitabine, Capecitabine, and Erlotinib	/II
			NCT00028834	Completed	National Cancer Institute (NCI)	Gemcitabine	I
			NCT00925769	Completed	Hoffmann-La Roche	Capecitabine and Erlotinib	
			NCT01214720	Completed	Hoffmann-La Roche	None	II
			NCT00100815	Completed	Roswell Park Cancer Institute	Gemcitabine and Capecitabine	I
			NCT00091026	Completed	National Cancer Institute (NCI)	Gemcitabine, Erlotinib, Cetuximab	I
			NCT00088894	Completed	National Cancer Institute (NCI)	Gemcitabine	II
			NCT00305877	Completed	National Cancer Institute (NCI)	Gemcitabine, Capecitabine and Cetuximab with Radiotherapy	I
			NCT03351296	Recruiting	Gustave Roussy, Cancer Campus, Grand Paris	LV5FU2, Streptozocin, Capecitabine, and Temzolomide	I
			NCT00336648	Completed	M.D. Anderson Cancer Center	Gemcitabine with Radiotherapy	I
			NCT02620800	Completed	Celgene	5-FU, Nab-paclitaxel, calcium leucovorin, oxaliplatin	
			NCT03127124	Recruiting	NantPharma, LLC	NANT-008, Fluorouracil, Leucovorin, and Eloxatin	/II
			NCT00735306	Completed	Duke University	Erlotinib	
Axitinib	Pfizer	RTKs (VGFR1-3, c-KIT, PDGFR)	NCT00471146	Completed	Pfizer	Gemcitabine	II
			NCT00219557	Completed	Pfizer	Gemcitabine	I
Sunitinib	Pfizer	RTKs (PDGFRs, VGFRs)	NCT00967603	Completed	IRCCS San Raffaele	None	I
			NCT00673504	Completed	Central European Society for Anticancer Drug Research	Gemcitabine	I
			NCT00397787	Completed	National Cancer Institute (NCI)	None	I
			NCT00462553	Completed	National Cancer Institute (NCI)	Gemcitabine	
Sorafenib	Bayer/Onyx	RTKs (VGFRs, PDGFRs, Rad kinases (c-Raf, BRAF))	NCT00837876	Completed	Vanderbilt-Ingram Cancer Center	Erlotinib	I
			NCT00789763	Completed	Grupo Espanol Multidisciplinario del Cancer Digestivo	Gemcitabine with Radiotherapy	
			NCT00696696	Completed	NYU Langone Health	Gemcitabine and Erlotinib	I
			NCT00375310	Completed	Indiana University School of Medicine	Gemcitabine with Radiotherapy	
			NCT00095966	Completed	National Cancer Institute (NCI)	Gemcitabine	I
			NCT00981162	Completed	Roswell Park Cancer Institute	Everolimus (mTOR inhibitor)	
			NCT00634751	Completed	University of Wisconsin, Madison	Oxaliplatin or Capecitabine	/II
Tipifarnib	Kura Oncology	Ras	NCT00005832	Completed	Southwest Oncology Group	None	I
			NCT00005648	Completed	Johnson & Johnson Pharmaceutical Research & Development, L.L.C.	Gemcitabine	II
			NCT00026104	Completed	National Cancer Institute (NCI)	Gemcitabine and Paclitaxel with Radiation therapy	I
			NCT00005843	Completed	National Cancer Institute (NCI)	None	I
Atezolizumab	Roche	PD-L1	NCT04161755	Recruiting	Memorial Sloan Kettering Cancer Center	RO7198457 and mFOLFIRINOX	
			NCT03915678	Not yet recruiting	Institut Bergonié	G100+Radiotherapy	I
			NCT03829501	Recruiting	Kymab Limited	KY1044	I
Avelumab	Pfizer	PD-L1	NCT03637491	Recruiting	Pfizer	Binimetinib (MEK inhibitor) and Talazoparib	I
Durvalumab	AstraZeneca	PD-L1	NCT03572400	Recruiting	Do-Youn Oh	Gemcitabine	I
			NCT02734160	Completed	Eli Lilly	Galunisertib (TGFβ antagonist)	
			NCT04156087	Not yet recruiting	Baki Topal	Gemcitabine and Tremelimumab(CTLA-4 inhibitor)	I
			NCT02311361	Active, and not recruiting	National Cancer Institute (NCI)	Tremelimumab (CTLA-4 inhibitor)	/II
			NCT02639026	Recruiting	Abramson Cancer Center of the University of Pennsylvania	Tremelimumab (CTLA-4 inhibitor)	
			NCT02403271	Completed	Pharmacyclics LLC	Ibrutinib (BTK inhibitor)	/II
			NCT02777710	Active, and not recruiting	Centre Leon Berard	Pexidartinib (CSF1R inhibitor)	
			NCT01693562	Active, and not recruiting	MedImmune LLC	None	/II
PRI-724	PRISM	CBP/β-Catenin	NCT01764477	Completed	PRISM	Gemcitabine	
Vantictumab	OncoMed	FZD receptors (FZD-1, -2, -5, and -7)	NCT02050178	Completed	OncoMed	Nab-Paclitaxel and Gemcitabine	
Defactinib	Verastem	FAK/BTK	NCT02546531	Active, and not recruiting	Washington University School of Medicine	Pembrolizumab and Gemcitabine	
			NCT02758587	Recruiting	NHS Greater Glasgow and Clyde	Pembrolizumab	/II
GSK2256098	GlaxoSmithKline	FAK/BTK	NCT02428270	Active, and not recruiting	University Health Network, Toronto	Trametinib	I
PF-00562271	Verastem	FAK	NCT00666926	Completed	Verastem, Inc.	None	

1.3.7 Prognostic Prediction Features of Risk Subgroups

High-risk subgroups were identified in both basal-like and classical tumors (Figure 2-19). To demonstrate the strength of risk subgroups for predicting survival outcomes, survival analysis was performed for each original subtype (classical and basal-like), subdivided into risk subgroups, for our study cohort and two external data sets (TCGA and Puleo et al.), including the classification of high-purity tumors (See details in Supplementary Information). Interestingly, the “Activated” and “ECM-Remodeling” risk subgroups consistently displayed significantly poor prognosis in classical (Log-rank test, P = 1.0E-06 for our study, 9.8E-05 for TCGA, and 5.0E-06 for Puleo et al.) and basal-like tumor subtypes (Log-rank test, P = 6.8E-03 for our study, 2.9E-02 for TCGA, and 4.3E-05 for Puleo et al.). Those prognostic associations were modestly replicated among high-purity classical tumors (Log-rank test, P = 6.8E-04 for TCGA and 1.4E-03 for Puleo et al.), but not among high-purity basal-like tumors (Log-rank test, P = 0.3787 for TCGA and 0.1819 for Puleo et al.).

Figure 2-19. Prognostic comparisons between original tumor cell subtypes and proteomic risk subgroups.



(A) The proportions of risk subgroups among classical and basal-like tumors and their Kaplan-Meier curves, in our study cohort. Exocrine-like subtypes were excluded from this analysis. (B) The proportions of the risk subgroups among classical and basal-like tumors and their Kaplan-Meier curves, in the TCGA cohort. Both whole-tumor and high-purity tumor classifications were considered. Other subtypes, such as exocrine-like/ADEX and immunogenic, were excluded from this analysis. (C) The proportions of the risk subgroups among classical and basal-like tumors and their Kaplan-Meier curves, in the Puleo et al. cohort. Both whole-tumor and high-purity tumor classifications were considered. All tumors were classified as either classical or basal-like tumors (N = 309), using the two centroid classifiers for classical and basal-like subtypes and Spearman's rank correlation method. "Not Applicable" indicates that the number of patients who experienced the event did not reach 50% in the overall follow-up.

1.4 Discussion

To date, numerous approaches have been attempted to improve the outcomes of pancreatic cancer treatment. Neoadjuvant chemotherapy has been attempted in resectable pancreatic cancer, and various radiation therapies have been introduced(99-101). In addition, paradigm shifts in treatment options have occurred, such as the performance of conversion surgery in locally advanced pancreatic cancer and metastatic pancreatic cancer, which were previously deemed impossible(4). In these circumstances, determining the subtype and tumor biology of PDAC can facilitate the design of personalized therapies. Collisson et al.(84) initially proposed three PDAC subtypes, and several investigations have reported that the stratification of patients based on subtypes can have clinical value when choosing a treatment strategy. The COMPASS trial, performed in Canada, used a tumor classification scheme that separated classical from basal-like subtypes and found that basal-like tumors were less likely to respond to first-line therapy(102). Recently, a study by Puleo et al.(87) proposed novel subtypes in a large prospective cohort, based on previous studies, to improve the clinical applicability of tumor categorization.

However, the application of tumor classifications during routine clinical implementation has been hampered by inapplicable classification platform. In addition, conventional tumor cell subtypes have generally been classified into either classical or basal-like tumors; however, the discovery of risk subgroups demonstrates the complexity of PDAC, which displays a level of heterogeneity that cannot be fully captured by the current classification methods. This notion has been supported by previous studies, which have indicated that PDAC progress from IPMN, through several different pathways(103,104). Furthermore, subpopulations of malignant ductal cells that were analyzed using single-cell RNA-seq revealed that components of multiple tumor-related pathways were differentially expressed during PDAC progression(105,106).

Our study proposed a random forest model, using 24 protein expression patterns that could be used to classify four PDAC risk subgroups, “Stable”, “Exocrine-like”, “Activated”, and “ECM-Remodeling”. This approach differs from previous approaches due to its increased emphasis on previous prognostic studies and the quality controls that were performed during model development. To develop an accurate and consistent risk subgroup and an MRM-MS-based model, we utilized biomarkers that have been thoroughly reviewed by numerous studies. We conducted a rigorous proteomic analysis, using MRM-MS, and validated the quality of the quantitation and the precision of the protein assays while satisfying standard guidelines, which is crucial for providing a reliable diagnostic evaluation in clinical settings. As advances in proteomics have rendered sample preparation protocols simpler and more economical, an MS-based proteomic assay might serve as an alternative platform and complement existing transcriptome-based assays by producing results faster—with several hours of measuring time and a total turnaround of several days(107).

Moreover, although our classification shows similarities with the originally defined subtypes, our subgroups possess distinct features that differ from the original classification groups. For instance, the “Stable” risk subgroup approximately encompassed classical tumor subtypes and lower-purity tumors that included contents from the tumor microenvironment, such as immune, stroma, and pancreatic enzymes. Puleo et al. suggested that the exocrine-like/ADEX subtype is more likely to be derived from normal acinar cells, which predominantly constitute the pancreas and produce digestive enzymes(87). Pancreatic enzymes (PNLIP and CPA1) that were enriched in the “Stable” and “Exocrine-like” subgroups were associated with good prognosis in our study and the high-purity tumors reported by Puleo et al. (Table 2-6 and Figure 2-18C). During pancreatic diseases, pancreatic acinar cells are prone to apoptosis, induced by endoplasmic reticulum stress(108), and pancreatic enzyme replacement therapy (PERT) could improve the prognosis of patients with pancreatic exocrine insufficiency

(PEI) due to chronic pancreatitis(109). Thus, the identification of low-purity tumors that are highly enriched in PNLIP and CPA1 in the “Stable” and “Exocrine-like” risk subgroups may be able to predict risk among PDAC patients. In addition, better response to first-line chemotherapy (FOLFIRINOX or Gemcitabine) in patients with classical subtype has been observed in other earlier studies(102). Hence, improved survival outcome could be observed in first-line chemotherapy treated “Stable” risk subgroups which mainly overlapped with classical subtypes.

The two high-risk subgroups, “Activated” and “ECM-Remodeling”, were distinguished by differences in signal transduction, associated with tumor progression, such as the PI3K-AKT, MAPK/ERK, and WNT/β-Catenin signaling pathways (Figure 2-17). In addition, the mechanisms for ECM disassembly appeared to be more activated in the “ECM-Remodeling” subgroup. Understanding tumor characteristics may improve the prediction of drug response in completed or ongoing PDAC treatment trials (Table 2-11). Clinical trials examining FDA-approved, targeted therapeutic agents, such as bevacizumab(110) and axitinib(111), which are in phase III, and sunitinib(112) and sorafenib(113), which are in phase II, have been conducted for advanced-stage PDAC, but none of these treatments have demonstrated significant improvements in OS. A phase III clinical trial for tipifarnib, which targets “Ras” proteins, was discontinued in 2005, for similar reasons(114). Drugs targeting programmed death-ligand 1 (PD-L1) are currently in clinical trials. These drugs all target receptor tyrosine kinases (RTKs) that function in the PI3K-AKT and MAPK/ERK signaling pathways and may improve the survival outcomes of the “Activated” subgroup by hindering RTKs during tumor progression. Drugs that target WNT/β-Catenin signaling, such as PRI-724(115) and vantictumab(116), could improve the survival outcomes of the “ECM-Remodeling” subgroup. Moreover, detactinib, GSK2256098, and PF-00562271, which target focal adhesion kinase (FAK), may hinder interactions between “ECM-Remodeling” tumors and the ECM.

Many prognostic markers for PDAC have been studied, especially by IHC,

and have been instrumental in the clinic(93,117). In this study, we have proposed 24 protein markers that are associated with the survival of PDAC patients. Twenty of 24 proteins were reported by prior studies as prognostic markers, and their survival patterns were closely replicated in our data (Table 2-12). Moreover, the 20 proteins are hallmarks of cancer that influence its prognosis, such as angiogenesis, differentiation, cell cycle progression, immune competence, metabolic reprogramming, tumor suppression, tumor invasion, and metastasis(92). In addition, the expression of well-known prognostic markers, such as cyclin E1 (CCNE1), E-cadherin (CDH1), carcinoembryonic antigen (CEACAM5), MutL-homolog 1 (MLH1), matrilysin (MMP7), poly [ADP-ribose] polymerase 1 (PARP1), and thymidine phosphorylase (TYMP), defined our risk subgroups. Further study of the correlation of the risk subgroups with conventional markers that are associated with tumor suppressors, apoptosis, and DNA mismatch repair would provide insights into the mechanism of standard chemotherapeutic agents, such as 5-fluorouracil, cisplatin, and gemcitabine.

Our study primarily used surgically resected, whole, fresh-frozen tissues, without considering tumor purity, in a single-assay platform, rendering our method applicable to (FNA) samples (118). Also, the small amount of samples (1 μg of protein) that was required for our method further supports its applicability to FNA samples, identifying and adding cell type-specific markers that improve the panel's performance(119). In addition, the results of the panel on FFPE tissue samples were comparable with those of whole, fresh-frozen tissues(120) (Figure 2-12), and the dataset of Puleo et al. (Figure 2-10A) validates our method beyond whole, fresh-frozen tissue.

Using previously reported datasets, the 24 proteins that were discovered in our study correlated highly with the corresponding mRNA in cancer cell lines, human tissues, and PDAC PDX tissues (Table 2-13). Considering the highly heterogeneous nature of PDAC tumors, which could hinder the identification of the driving factors of cancers, comparing proteomic- and transcriptomic-based methods is important(121), and high correlations between protein and transcript levels support the reliability of our markers

and risk subgroups. However, the concern over FNA samples having low cellularity and the high intratumoral heterogeneity of PDAC remains, necessitating testing of our method in a large cohort of FNA samples.

Various treatments, such as neoadjuvant therapy(3), FOLFIRINOX(122), and stereotactic ablative radiotherapy(123), have been administered to improve the prognosis but have had varying responses between patients. If a prospective treatment-responding risk subgroup is determined, especially at the time of diagnosis, and if a predictive model for the projected treatment response for each risk subgroup is developed, these achievements could form the basis of patient-specific treatment and drastically improve the survival outcomes of pancreatic cancer patients.

In conclusion, we proposed novel PDAC risk subgroups and a classification model that has the potential for clinical applications at an individual level. Our risk subgroup identification indicates the importance of incorporating the findings of previous studies and original prognostic biomarkers, by sub-classifying groups associated with higher death risk among classical or basal-like subtypes. This risk classification model may improve the accuracy with which clinicians identify patients who are likely to benefit from strenuous first-line therapy or targeted therapy.

Table 2-12. Prognostic markers in the Random Forest classification model.

Gene Name	Tumorigenic function	Prognostic marker study	DOI	Cancer type	Quantitation method	Reference group (Case)	HR for OS	95% CI	P-value	Log rank test, P-value	Sample size
ACTN4	Tissue invasion and metastasis	Kikuchi et al. 2008	10.1158/1078-0432.CCR-08-0075	Pancreatic cancer	IHC	Negative (Positive)	2.27	1.57 to 3.27	< 0.001***	< 0.001***	173
ACTN4	Tissue invasion and metastasis	Watanabe et al. 2015	10.1038/bjc.2014.623	Pancreatic cancer	IHC	Negative (Positive)	1.53	0.92 to 2.53	0.1004	0.116	91
ADAM9	Tissue invasion and metastasis	Grützmann et al. 2004	10.1038/sj.bjc.6601645	Pancreatic cancer	IHC	Negative (Positive)	3.94	1.72 to 9.05	< 0.001***	< 0.001***	51
ALDH1A1	Differentiation	Kahlert et al. 2011[a]	10.1186/1471-2407-11-275	Pancreatic cancer	IHC	Low (High)	0.40	0.22 to 0.71	0.002**	0.012*	97
CAP1	Tissue invasion and metastasis	Yamazaki et al. 2009	10.1038/labinvest.2009.5	Pancreatic cancer	IHC	Low (High)	Not available; Poor prognosis in with high	Not available	Not available	0.021*	73
CAP1	Tissue invasion and metastasis	Bergqvist et al. 2020	10.1186/s13058-020-01307-5	Breast cancer	IHC	High (Moderate; Low)	0.73; 0.54	0.55 to 0.99; 0.38 to 0.78	0.04*; 0.001**	0.002**	718
CD274	Immunocompetence	Nomi et al. 2007[a]	10.1158/1078-0432.CCR-06-2746	Pancreatic cancer	IHC	Negative (Positive)	2.30	Not available	0.022*	0.016*	51
DSG2	Tissue invasion and metastasis	Ormanns et al. 2015	10.1038/bjc.2015.362	Pancreatic cancer	IHC	Low (High)	1.24	0.82 to 1.88	0.31	0.029*	165
FLT1	Angiogenesis	Chung et al. 2006	10.1002/cncr.21783	Pancreatic cancer	TMA	Bottom (Top)	4.43	1.53 to 12.86	0.0062**	0.0038**	76
IDH1	Metabolic reprogramming	Liu et al. 2018[b]	10.1186/s13058-018-0953-7	Breast cancer	IHC	High (Low)	1.64	1.14 to 2.38	0.008**	0.008**	249
IDH1	Metabolic reprogramming	Laba et al. 2018	10.1186/s12885-018-4747-1	Renal cell carcinoma	TMA	Low (High)	0.50	0.25 to 0.99	0.046*	0.004**	285
ITGA5	Tissue invasion and metastasis	Kuninty et al. 2019	10.1126/sciadv.aax2770	Pancreatic cancer	IHC	Low (High)	1.87	1.27 to 2.76	0.002**	0.008**	137
ITGA6	Tissue invasion and metastasis	Sawai et al. 2006[c]	10.1186/1471-2121-7-8	Pancreatic cancer	IHC	Without strong (With strong)	Not available; Poor prognosis in with strong	Not available	Not available	< 0.001***	42
KRT17	Tissue invasion and metastasis	Roa-Peña et al. 2019	10.1038/s41598-019-47519-4	Pancreatic cancer	IHC	Low (High)	2.96	Not available	0.008**	Not available	74
NPM1	Cell cycle progression	Qin et al. 2020	10.1038/s41467-020-15364-z	Breast cancer	IHC	Low (High)	Not available; Poor prognosis in high	Not available	Not available	0.002**	133
NPM1	Cell cycle progression	Liu et al. 2012	10.1186/1423-0127-19-53	Colon cancer	IHC	Negative (Positive)	Not available; Poor prognosis in positive	Not available	Not available	0.017*	31
PDCD4	Tumor suppressor	Nagao et al. 2012	10.1038/modpathol.2011.142	Pancreatic cancer	IHC	Negative (Positive)	0.44	0.28 to 0.69	< 0.001***	Not available	65
S100A2	Cell cycle progression	Biankin et al. 2009[b]	10.1053/j.gastro.2009.04.009	Pancreatic cancer	IHC	Negative (Positive)	1.87	1.25 to 2.81	0.002**	< 0.001***	296
S100A4	Cell cycle progression	Oida et al. 2006	PubMed ID: *16865243*	Pancreatic cancer	IHC	Negative (Positive)	1.81	1.01 to 3.27	0.048*	0.014*	72
SLC2A1	Metabolic reprogramming	Sharen et al. 2017	10.18632/oncotarget.15035	Pancreatic cancer	IHC	Negative (Positive)	1.79	1.19 to 2.70	0.005**	Not available	538
VEGFA	Angiogenesis	Ikeda et al. 1999	10.1038/sj.bjc.6690248	Pancreatic cancer	IHC	Positive (Negative)	0.17	0.03 to 0.96	0.044*	Not available	40
ALAD	Differentiation	Ge et al. 2017	10.1111/cas.13180	Breast cancer	mRNA expression	Low (High)	Not available; Poor prognosis in low	Not available	Not available	< 0.001***	188
ENO2	Metabolic reprogramming	Daemen et al. 2015	10.1073/pnas.1501605112	Pancreatic cancer	mRNA expression	Lipogenic (Glycolytic)	Not available; Poor prognosis in glycolytic	Not available	Not available	Not available	38
NEFH	Tumor suppressor	van Vlodrop et al. 2016	10.1158/1078-0432.CCR-16-1236	Renal cell carcinoma	Gene methylation	Unmethylated (Methylated) in population-based series	2.80	1.7 to 4.6	< 0.001***	Not available	185
NEFH	Tumor suppressor	Huang et al. 2018	10.1002/ijc.31580	Ovarian cancer	Gene methylation	L-methyl (H-methyl)	2.37	1.17 to 4.83	0.017*	0.014*	84

IHC, immunohistochemistry; TMA, tissue microarray; HR, hazard ratio; 95% CI, 95% confidence interval; * P-value < 0.05; ** P-value < 0.01; *** P-value < 0.001; [a] Relative risk was presented instead of HR; [b] Disease specific survival was used to survival analysis instead of overall survival; [c] Wilcoxon test was used to calculate significance of difference between groups in Kaplan-Meier curves

Table 2-13. Correlation of 24 markers between mass spectrometry-based protein expression and RNA-seq-based mRNA expression.

Gene Name	375 cell-lines from CCLE[a], Pearson's correlation (*r*)	29 human tissues from HPA[b], Pearson's correlation (*r*)	29 human tissues from HPA[b], Pearson's correlation (P-value)	10 PDAC PDXs from Le Large et al.[c] and Dijk et al.[d], Pearson's correlation (*r*)	10 PDAC PDXs from Le Large et al.[c] and Dijk et al.[d], Pearson's correlation (P-value)
ACTN4	0.750	0.335	0.075	0.828	0.0058
ADAM9	0.840	0.436	0.018	0.395	0.29
ALAD	0.640	0.719	1.1E-05	0.675	0.046
ALDH1A1	0.840	0.874	5.7E-10	0.913	6.0E-04
CAP1	0.692	0.718	1.1E-05	0.919	5.0E-04
CD274	0.789	0.521	0.068	Not Available	Not Available
CPA1	Not Available	0.758	0.011	Not Available	Not Available
DSG2	0.788	0.741	1.5E-05	0.481	0.19
ENO2	0.697	0.841	3.9E-08	Not Available	Not Available
FLT1	0.566	0.784	1.1E-05	Not Available	Not Available
HRAS	0.308	0.067	0.74	Not Available	Not Available
IDH1	0.692	0.813	8.4E-08	-0.332	0.38
ITGA5	0.781	0.571	0.0012	0.642	0.062
ITGA6	0.789	0.723	9.3E-06	0.865	0.0026
KRT17	0.577	0.678	0.031	0.783	0.013
NEFH	0.200	0.523	0.18	Not Available	Not Available
NPM1	0.448	0.342	0.070	0.913	6.0E-04
PDCD4	0.713	0.720	1.1E-05	0.828	0.0059
PNLIP	Not Available	0.878	0.0018	Not Available	Not Available
RPS5	0.276	-0.090	0.64	0.422	0.26
S100A2	0.724	0.841	6.1E-04	0.492	0.18
S100A4	0.762	0.584	8.9E-04	0.948	1.0E-04
SLC2A1	0.760	0.465	0.013	0.727	0.027
VEGFA	0.511	0.594	0.054	Not Available	Not Available

CCLE, Cancer Cell Line Encyclopedia; HPA, Human Protein Atlas; PDAC, Pancreatic Ductal Adenocarcinomas; PDXs, Patient Derived Xenografts; [a] Nusinow DP, Szpyt J, Ghandi M, Rose CM, McDonald ER 3rd, Kalocsay M, et al. Quantitative Proteomics of the Cancer Cell Line Encyclopedia. *Cell* 2020;180(2):387-402. **doi: 10.1016/j.cell.2019.12.023**; [b] Wang D, Eraslan B, Wieland T, Hallström B, Hopf T, Zolg DP, et al. A deep proteome and transcriptome abundance atlas of 29 healthy human tissues. *Mol Syst Biol* 2019 Feb 18;15(2):e8503. **doi: 10.15252/msb.20188503**; [c] Le Large TY, Mantini G, Meijer LL, Pham TV, Funel N, van Grieken NC, et al. Microdissected pancreatic cancer proteomes reveal tumor heterogeneity and therapeutic targets. *JCI insight* 2020;5(15) doi 10.1172/jci.insight.138290; [d] Dijk F, Veenstra VL, Soer EC, Dings MPG, Zhao L, Halfwerk JB, et al. Unsupervised class discovery in pancreatic ductal adenocarcinoma reveals cell-intrinsic mesenchymal features and high concordance between existing classification systems. *Scientific reports* 2020;10(1):337 **doi 10.1038/s41598-019-56826-9**.

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Abstract in Korean

국문 초록

연구 목적: 췌장선도암(PDAC)의 아형은 현재까지 다양한 방법론을 사용하여 확인되고 있다. 그러나 일상적으로 임상에 적용 가능한 분류 체계를 확립하는 일이 아직까지 어려운 실정이다. 본 연구에서는 분자적 특성을 기반으로 췌장암 위험군을 식별하고 임상적 응용이 더 적합한 분류 모델을 개발하는 것을 목표로 하였다.

연구 방법: 2009년 10월부터 2018년 2월까지 서울대학교 병원에서 수술을 받은 225명의 환자를 대상으로 췌장암 절제 표본을 수집하였다. 종양의 진행 및 예후와 연관된 단백질들을 엄격한 정량분석 검정을 통하여 정량 하였다. 계층적 군집분석을 통하여 췌장암 위험군을 식별하였다. 확인된 위험군을 예측하기 위하여 랜덤 포레스트(Random Forest) 알고리즘을 사용한 모델을 개발하였으며, 생존분석과 더불어 전사체 외부 코호트(N = 700)에서 모델을 검증하였다.

연구 결과: 본 연구에서는 24 개 단백질 정량변수를 사용하여 네 가지 위험군인 Stable; Exocrine-like; Activated; Extracellular matrix(ECM)-Remodeling 을 분류할 수 있는 것을 확인하였다. “Stable” 위험군은 종양 분화와 종양억제인자와 연관된 단백질들로 특징지어졌다. “Exocrine-like” 위험군은 췌장 효소를 높게 발현하는 것을 확인하였다. 두 가지 고위험군인 “Activated”와 “ECM-Remodeling” 위험군은 세포주기, 혈관신생, 면역회피, 종양 침윤-전이 및 대사 변화 등 다양한 기능들이 발현되는 것을 확인하였다. 이러한 기능을 포괄하는 위험군 분류 모델은 다중 코호트에서 상대적으로 높은 정확도와 정밀도를 보이는 것을 확인하였다.

결론: 본 연구에서 췌장선도암 위험군을 제안하고, 개별환자 수준에서 임상적인 사용에 잠재적으로 유용할만한 분류 모델을 개발할 수 있었다. 이 임상 체계는 췌장선도암 환자의 예후 예측 및 치료 지침의 효과를 향상시킬 수 있을 것으로 기대한다.