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Chitin-based Polysaccharides Enhance the Immunomodulating Potential of Human Mesenchymal Stem Cell-derived Extracellular Vesicles

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Eun Seo Kim

Department of International Agricultural Technology

Graduate School of International Agricultural Technology

Seoul National University

Chitin-based Polysaccharides Enhance the Immunomodulating Potential of Human Mesenchymal Stem Cell-derived Extracellular Vesicles

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By
Eun Seo Kim

Supervised by
Prof. Joonghoon Park

Major of Applied Animal Science
Department of International Agricultural Technology
Graduate School of International Agricultural Technology
Seoul National University

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Approved as a qualified thesis
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by the committee members

Chairman	**Su Cheong Yeom, Ph.D.**
Member	**Joonghoon Park, Ph.D.**
Member	**Tae Min Kim, Ph.D.**

Abstract

Mesenchymal stem cell (MSC) transplantation is a promising therapy for regenerative medicine. However, MSCs grown under two-dimensional (2D) culture conditions differ significantly from those in the body, especially with different cell shape, downregulated stemness genes and secretion of paracrine factors.

Here, I evaluated the effect of 3D culture using chitin-based polysaccharides on the characteristics of human Wharton's jelly-derived MSCs (hMSCs). Chitin-based polysaccharides significantly increased cell proliferation after retrieval. Transcriptome analyses suggested that genes involved in cell stemness, migration ability, and extracellular vesicle (EV) production were enhanced by 3D culture.

Subsequent biochemical analyses showed that the expression levels of stemness genes including OCT4, NANOG, and SSEA4 were upregulated and migration capacity was elevated in 3D-cultured hMSCs. In addition, EV production was significantly elevated in 3D cells, which contained a distinct protein profile from 2D cells.

Gene and drug connectivity analyses revealed that the 2D and 3D EVs had similar functions as immunomodulators; however, 3D EVs had completely distinct therapeutic profiles for various diseases based on activation of disease-associated pathways. Therefore, EVs from chitin-based polysaccharides primed hMSCs offer a new treatment for immune and metabolic diseases.

Keywords: Chitin-based polysaccharides, MSC, 3D culture, EV, Immunomodulator

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List of Abbreviations

2D – Two dimensional

3D – Three dimensional

ADSCs – Adipose Derived Mesenchymal Stem Cells

AQP1 – Aquaporin 1

ATC – Anatomical Therapeutic Chemical

ATP – Adenosine Triphosphate

AUC – Area Under Curve

BICC1 – BicC Family RNA Binding Protein 1

BSA – Bovine Serum Albumin

C9 – Complement Component 9

CAMs – Cell Adhesion Molecules

CCL2 – C-C motif Chemokine Ligand 2

CCL5 – C-C Motif Chemokine Ligand 5

CD – Cytochalasin D

CDA – Cytidine Deaminase

DAPI – 4′,6-diamidino-2-phenylindole

DEGs – Differentially Expressed Genes

DEPs – Differentially Expressed Proteins

DPBS – Dulbecco's Phosphate Buffered Saline

ECM – Extracellular Matrix

EDTA – Ethylenediaminetetraacetic Acid

ELISA – Enzyme Linked Immunosorbent Assay

ENG – Endoglin

EVs – Extracellular vesicles

FABP4 – Fatty Acid Binding protein 4

FACS – Fluorescence Activated Cell Sorting

FBS – Fetal Bovine Serum

FDR – False Discovery Rate

GAPDH – Glyceraldehyde 3-Phosphate Dehydrogenase

GDNF – Glial Cell-Derived Neurotrophic Factor

GPRC5B – G Protein-Coupled Receptor Class C Group 5 Member B

GSEA – Gene Set Enrichment Analysis

HAS2 – Hyaluronan Synthase 2

HCl – Hydrogen Chloride

HGF – Hepatocyte growth factor

HLA-DR – Human Leukocyte Antigen DR

HRP – Horseradish Peroxidase

ICAM1 – Intercellular Adhesion Molecule 1

IL17 – Interleukin 17

JAG1 – Jagged 1

KEGG – Kyoto Encyclopedia of Gene and Genome

KIAA1324L - KIAA1324-like

KIT – V-Kit Hardy-Zuckerman 4 Feline Sarcoma Viral Oncogene Homolog

NOTCH3 – Notch Receptor 3

NTA – Nanoparticle Tracking Analysis

MAPK – Mitogen-Activated Protein Kinase

MDCK – Madin Darby Canine Kidney

MEMα – Minimum Essential Medium α

MFGE8 – Milk Fat Globule-EGF Factor 8 Protein

MME – Membrane Metalloendopeptidase

MSC – Mesenchymal stem cell

MSigDb – Molecular Signature Database

MTT – 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide

NANOG – Nanog Homeobox

OCT4 – POU Class 5 Homeobox 1

PDGF – Platelet Derived Growth Factor

PI3K – Phosphoinositide 3-Kinase

PPP4R4 – Protein Phosphatase 4 Regulatory Subunit 4

PVDF – Polyvinylidene Fluoride

RIN – RNA Integrity Number

RMA – Robust Multi-array Average

SD – Standard Deviation

SDS – Sodium Dodecyl Sulphate

SSEA4 – Stage-specific Embryonic Antigen-4

TEK – TEK Tyrosine Kinase

TEM – Transmission Electron Microscopy

TNFRSF10D – Tumor Necrosis Factor Receptor Superfamily Member 10d

TNFSF4 – Tumor Necrosis Factor Superfamily Member 4

TSG101 – Tumor Susceptibility 101

VCAM1 – Vascular Cell Adhesion Molecule 1

WJ – Wharton's Jelly

ZNF836 – Zinc Finger Protein 836

Introduction

1. Mesenchymal stem cell (MSC) therapy

MSCs are characterized by spontaneous adherence to plastic. They exhibit typical surface markers including CD44, CD73, CD90, and CD105, and absence of CD14, CD34, CD45, and major histocompatibility complex (MHC) II cell surface receptor (HLA-DR). MSCs also have the potential to differentiate into a variety of cell types like osteocytes, adipocytes, and chondrocytes. MSCs derived from different tissue sources have varying cellular compositions, self-renewal capabilities, and lineage-specific differentiation capacity. Therefore, MSCs are a potential tool for cell therapy and tissue regeneration [1, 2].

MSCs derived from Wharton's jelly of the umbilical cord (WJ-MSCs) seems to have great clinical utility because of their limited heterogeneity and unique properties, such as ease of their isolation and culture, immunomodulatory properties, capacity to self-regenerate, differentiate into several cell lineages, and lack of ethical considerations associated with their usage [3, 4].

The use of MSCs in cell therapy is being intensely investigated within the last years. MSCs are used in plastic surgeries, cardiovascular disorders, endocrine and neurological diseases, cell transplantation, repair of injured musculoskeletal tissues, treatment of degenerative changes in joints, and to restore bones and cartilage [5]. New clinical uses are being tested because of the unique characteristics of these cells, such as their fast proliferation, high

differentiation ability, and capacity to migrate into the damaged site [4].

However, while there is a lot of knowledge about the in vitro characteristics of MSCs, much less knowledge about their in vivo behaviors. They can act indirectly by generating and secreting a wide variety of paracrine factors that enhance the regenerative potential of injured tissue [6]. Many researches confirmed that those paracrine factors actually ameliorate the symptoms of a wide variety of diseases [7].

Therefore, MSC transplantation is a promising therapy for the repair and regeneration of organs. These therapeutic effects appear to be mediated by paracrine factors in part.

2. Therapeutic potential of extracellular vesicles (EVs)

EVs are membrane vesicles secreted from a wide variety of mammalian cell types [8]. They are about ~200 nm in diameter and contain components such as microRNA and mRNA [9, 10]. Hence, they play an important role in intercellular communication as well as disease development and treatment [11, 12].

The use of EVs, a paracrine factor that may be used as a replacement for MSCs, is a new method of stem cell therapy. The paracrine effect of EVs as a therapeutic vector acts without the direct participation of cells. They are released from stem cells and deliver various components to the target region, including mRNA, DNA, and proteins [13, 14]. Recently, EVs secreted from

MSCs were reported to show the same treatment efficacy as MSCs [15]. Notably, EVs may provide relatively safe treatment in terms of adaptive and innate immune responses and tumorigenicity [16]. Furthermore, EVs have demonstrated promising regenerative benefits in many disease models, including myocardial infarction, renal injury, and neurological injury [17]. For example, in a rat myocardial infarction model, infarct size was reduced and the long-term cardiac function was restored after injecting of MSC-EVs [18]. Therefore, they hold promise as a future regenerative medicine.

To produce large amounts of EVs for use as medicine, it is essential to scale up the culture volume of MSCs. Several methods are used for large-scale cultivation of MSCs, and some companies seek to cultivate them at the liter scale [19, 20]. Nevertheless, no gold standard of large-scale MSC culture has yet been established. In addition, the components contained in EVs can differ due to culture conditions such as cell integrity, aging, and especially the growth environment [21]. In this respect, the development of a well-organized and reliable system of cell culture is a prerequisite for the production high-quality and large scale EVs.

3. Three-dimensional (3D) culture for the EV production

When MSCs are cultured using a conventional tissue culture plate or micro-carrier, the cells adhere to the plastic surface in a spindle shape, which differs significantly from the cell shape in the body. Furthermore, large differences in cytoskeletal structure exist between cells grown on plastic surfaces and those in the body, and these differences have important effects on cell characteristics.

In MSCs cultured under 2D culture conditions, the cell diameter increases with each passage, resulting in decreases in the growth rate, expression of stemness genes, and secretion of paracrine factors [22-24].

3D culture is one of the priming approaches that aim to increase MSC therapeutic potential. In comparison to monolayer culture, 3D cultured MSC showed significant differences in cellular phenotype and biological response. MSC function is dependent on the microenvironment, and experiments about cell aggregation reveal that cell-cell interaction is essential, as well as cell polarity [25]. In other words, 3D culture allows for more cell-cell contact and cell-ECM interaction so it can imitate the in vivo environment for cell development and signaling activity, therefore, increase therapeutic properties of MSC [26]. MSC cultured in spheroids have been shown better cell survival following infusion and have enhanced angiogenic, anti-inflammatory, and regenerative effects following infusion [27].

Several 3D culture methods are being developed to produce MSC spheroids, including the hanging drop technique, utilization of low-attachment surface materials, and forced aggregation [28]. For MSC spheroids, previous studies have shown that the secretion of certain paracrine factors and stemness of MSCs increase significantly when MSCs are cultured in 3D compared to 2D cultivation [29, 30]. However, these culture methods only support spheroid generation, not MSC expansion, which makes them poorly suited for mass production of EVs at low cost.

MSCs require scaffolds for attachment and growth [31], so scaffolds must be

in a culture medium to prevent apoptosis (anoikis). For the continuous production of EVs from MSCs with high yield, a robust culture method that not only maintains the quality of MSCs, but also enables easy proliferation of MSCs under 3D culture conditions is essential.

Taken together, it is necessary to find an appropriate 3D culture method that supports MSC expansion without losing the characters of MSC for the mass production of EVs.

4. Chitin-based polysaccharides as a 3D culture material

Chitin is a biopolymer derived from animals, primarily invertebrates. It is the major component of crustacean such as shrimp, crab, and lobster, and it also be found in the exoskeletons of mollusks and insects [32].

Chitin has unique biochemical properties that can influence many biological functions, including immune response and antibacterial activity. These features have made chitin a desirable biomaterial in a variety of applications, including scaffolds for 3D cell growth [33]. Studies on 3D culture using chitin are being conducted. After 7 days of hMSCs culture on the chitin scaffolds, the number of cells was increased and these cells were able to differentiate into the chondrogenic, adipogenic, and osteogenic lineages, respectively [34].

Therefore, in this study, human MSCs are 3D cultured using chitin-based polysaccharides named Cellhesion VP as a scaffold. Cellhesion VP is a water-insoluble fiber developed by Nissan Chemical Corporation, which has been

proved to be a 3D culture material suitable for large-scale cultivation of adherent cells such as Madin-Darby Canine Kidney (MDCK) cells. A unique feature of Cellhesion VP is that this scaffold material allows cells to adhere and proliferate on the scaffold in a round shape similar to *in vivo* conditions or other 3D culture materials, rather than the spindle shape usually observed in 2D cultivation.

Although the MSCs are well studied as therapeutic agents, additional investigations of MSCs-derived EVs are still needed. It is difficult to increase the yield of EVs and analyze in-depth. Also, in terms of 3D culture materials, chitosan which is a chitin derivative has been studied a lot, but chitin is not.

In this study, I assumed that the yield of EVs can be enhanced by 3D culture due to the differences of the cellular microenvironment of 2D and 3D. Furthermore, as the therapeutic properties of human MSCs are improved in 3D culture, the therapeutic potential of EVs can be improved as well by the molecular changes of EVs. Therefore, I evaluated the effects of 3D culture using chitin-based polysaccharides on the characteristics of hMSCs and EVs.

Materials and Methods

hMSC culture

For 2D culture, hMSCs from Wharton's jelly at passage 7 were cultured in MEMα medium (Gibco, Grand Island, NY, USA) supplemented with 10% (v/v) FBS and 1% (v/v) antibiotic-antimycotic solution (2D medium) under 5% CO_2 and 37 °C conditions, and the medium was changed every 3 days. When the cells reached 80% confluence, cells were passaged using 0.25% (w/v) trypsin-EDTA (Gibco). For 3D culture of hMSCs, 3D medium was prepared by supplementing MEMα medium described above with 0.05% (v/v) chitin-based polysaccharide; Cellhesion VP (Nissan Chemical Corporation, Tokyo, Japan) according to the manufacturer's instruction. Cells at passage 9 were seeded at a density of 1×10^6 cells/flask in 10 mL of 3D medium in a 125 mL non-adherent flask (Corning, Corning, NY, USA) and incubated on a rocker (LABOGENE, Seoul, Korea) at 9 rpm with or without mechanical dissociation daily up to 15 days under 5% CO2 and 37 °C conditions. The medium was changed every 3 days.

Nine days after 3D culture, primed hMSCs were retrieved by transferring the cell-Cellhesion VP aggregates to a tissue culture plate. The aggregates were spin-down at 1,500 rpm for 3 min and the supernatant was discarded. The aggregates were resuspended by pipetting in 5 mL of 2D medium, transferred to the 100 mm tissue culture plate (Corning), and subjected to cell retrieval from the aggregates. The retrieved cells were maintained in 2D culture condition until used.

For the cell culture in U-bottom plate, hMSCs were seeded into 96-well Clear

Round Bottom Ultra-Low Attachment Microplate (Corning) at 1.5×10^4 cells in 300 μL of MEMα medium (Gibco) supplemented with 10% (v/v) FBS and 1% (v/v) antibiotic-antimycotic solution. Adipose-Derived Mesenchymal Stem Cells (ADSCs; CellSource Co., Ltd, Tokyo, Japan) were seeded into U-bottom plate (Corning) at 5×10^2 cells in 100 μL of MEMα medium (Gibco) supplemented with 17% (v/v) FBS and 1% (v/v) antibiotic-antimycotic solution.

ATP assay

Cell number was calculated by measuring the intracellular ATP content using CellTiter-Glo® 3D cell viability assay kit according to the manufacturer's instruction (Promega, Madison, WI, USA).

MTT assay

The viability of cells was examined by using the CyQUANT™ MTT Cell Viability Assay (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After EV or drug treatment for 6 hrs, cells were washed with DPBS and incubated with a mixture of 10 μL of the MTT and 100 μL of phenol red-free culture medium. After 4 hrs of incubation at 37 °C, 100 μL of 0.01M HCl was added. The absorbance of each sample was measured at 570 nm after 4 hrs of HCl addition.

Flow cytometry analysis

To determine the expression of the cell surface antigens, hMSCs were incubated with mouse monoclonal anti-Oct-3/4 antibody (1:250; Santa Cruz Biotechnology, Dallas, TX, USA), rabbit monoclonal anti-Nanog antibody (1:250; Cell Signaling, Danvers, MA, USA), mouse monoclonal anti-SSEA4 antibody (1:250; Santa Cruz Biotechnology), followed by FITC-conjugated secondary anti-mouse IgG (1:500; Invitrogen) or anti-rabbit IgG (1:500; Invitrogen). hMSCs were harvested and resuspended in PBS containing 1% (w/v) BSA (Sigma-Aldrich, St. Louise, MI, USA). To measure the expression of OCT4 and NANOG, cells were permeabilized by BD Cytofix/Cytoperm™ solution (BD Biosciences, San Jose, CA, USA) according to the manufacturer's protocol. After staining, the cells were subjected to FACS Aria III (BD Biosciences), and the flow cytometry data were analyzed using FlowJo software (Tree Star, San Carlos, CA, USA).

Cell migration assay

hMSCs for migration assay were prepared from 2D cultured and the retrieved 3D cells. Cells were suspended in 2D medium at 150,000 cells/mL and 70 μL of the cell suspension was pipetted into each silicon culture insert (Ibidi, Munich, Germany) in a 24-well cell culture plate (Corning). After overnight culture, the silicon inserts were removed and cells were washed with DPBS. Each well of the plate was treated with 50 ng/mL of PDGF-BB (Thermo Fisher Scientific, Waltham, MA, USA) in 500 uL of MEMα medium supplemented with 0.1% (v/v) FBS and 1% (v/v) antibiotic-antimycotic solution. Cytochalasin D at 0.2 μM (Thermo Fisher Scientific) was used as a negative control. The area filled by cell migration was observed with an inverted

microscope (Leica, Wetziar, Germany) until 24 hours after the inserts were removed. Images were taken at 50 × magnification and the cell-free area was determined by using the AxioVision SE64 (Carl Zeiss, Oberkochen, Germany).

qRT-PCR

Total RNA from 2D and 3D cultured hMSCs was prepared using a RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. During RNA preparation, DNase (Qiagen) was applied to prevent the genomic DNA contamination. cDNA was prepared using Super Script III Reverse Transcription Reagents (Invitrogen) and qRT-PCR was performed with Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) on a StepOnePlus Real-Time PCR System (Applied Biosystems). qRT-PCR was carried out with primer sets as followed: *OCT4* (forward) 5'-TTC AGC CAA ACG ACC ATC TG-3', (reverse) 5'-CAC GAG GGT TTC TGC TTT GC-3' [35], *NANOG* (forward) 5'-GCA GAT GCA AGA ACT CTC CAA C-3', (reverse) 5'-CTG CGT CAC ACC ATT GCT ATT C-3' [36], *CCL2* (forward) 5'-CAG CCA GAT GCA ATC AAT GCC-3', (reverse) 5'-TGG AAT CCT GAA CCC ACT TCT-3', *ZNF836* (forward) 5'-GGG GAA GCC ATA TCA ATG TGG-3', (reverse) 5'-GCA TGA GCC TTG TTT GAA GGT T-3', and *GAPDH* (forward) 5'-AGG GCT GCT TTT AAC TCT GGT-3', (reverse) 5'-CCC CAC TTG ATT TTG GAG GGA-3' [37]. Relative expression levels of each mRNA were analyzed by the $\Delta\Delta C_T$ method, normalized to *GAPDH* mRNA and shown as fold change in expression over control.

Preparation of EVs

To prepare 2D EVs, 1×10^6 hMSCs at passage 9 were seeded on 150 mm tissue culture plate (Corning) in 20 mL of MEMα medium supplemented with 10% (v/v) FBS and 1% (v/v) antibiotic-antimycotic solution. Upon reaching 80% confluency 3 days after culture, the media was replaced with 20 mL of MEMα medium supplemented with 10% (v/v) EV-depleted FBS and 1% (v/v) antibiotic-antimycotic solution. After 3 days of additional culture, media was harvested for 2D EV preparation. To prepare 3D EVs, 1×10^6 hMSCs at passage 9 were seeded in 125 mL non-adherent flask (Corning) and incubated on a rocker (LABOGENE) at 25 rpm with mechanical dissociation daily up to 3 days, and then the media were replaced with 20 mL of fresh MEMα medium supplemented with 10% (v/v) EV-depleted FBS and the cells were subsequently cultured for additional 3 days. Media were harvest for 3D EV preparation. Harvested media were spin downed at 300 g for 10 min, and the supernatant was harvested after additional centrifuge at 2,000 g for 10 min. The supernatant was filtered through 0.2 μm syringe filter (Sartorius, Goettingen, Germany), and subjected to EV isolation using the Exo2D reagent (ExosomePlus, Gyeonggi-do, Korea). The isolated EVs were resuspended in 100 μL DPBS (Thermo Fisher Scientific). The size distribution and particle number of EVs was measured using nanoparticle tracking analysis (NTA; Malvern, Worcestershire, UK). The isolated EVs were kept -80 °C until use. All relevant data of my experiments have been submitted to the EV-TRACK knowledgebase [38] (EV-TRACK ID: EV200183).

Transmission Electron Microscopy (TEM)

Isolated EVs at 5 μL were absorbed on a 200 mesh carbon-coated grid (Electron Microscopy Sciences, Hatfield, PA, USA) and negatively stained with 2% (v/v) uranyl acetate. EVs were analyzed under the Energy Filtering Transmission Electron Microscopes ZEISS (Carl Zeiss), operated at 300 kV. Images were taken with an Orius SC200W CCD camera (GATAN, Pleasanton, CA, USA).

Western blotting

EVs and cell lysates were prepared using RIPA buffer (Thermo Fisher Scientific). After transfer, PVDF membranes were incubated in TBST supplemented with 5% (w/v) BSA (Sigma-Aldrich) and one of each anti-TSG101 antibody (1:200, clone C-2; Santa Cruz Biotechnology), anti-CD81 antibody (1:500, clone EPR4244; Abcam, Cambridge, MA, USA), anti-CD9 antibody (1:1000, clone EPR2949; Abcam) or anti-Calnexin antibody (1:1000; Abcam) at 4 °C overnight. After washing three times with TBST, the PVDF membranes were incubated with HRP-conjugated goat anti-mouse IgG secondary antibody (1:5000) or anti-rabbit IgG secondary antibody (1:5000; Thermo Fisher Scientific) for 1 hr at room temperature and washed three times with TBST for 15 min. Blots were detected using an enhanced chemiluminescence kit (BioRad, Inc., Berkeley, CA, USA).

Enzyme-linked immunosorbent assay (ELISA)

EVs were quantified by using PS Capture Exosome ELISA Kit (Wako, Osaka, Japan) using cell culture media according to the manufacturer's instructions.

Anti-CD63 antibody (1:100; provided in the kit) and anti-CD81 antibody (1:50, clone M38; Novus Biologicals, Centennial, CO, USA) were used. Absorbance was measured at 450 nm with reference wavelength of 620 nm using Cytation 5 (BioTek, Milton, VT, USA).

Drugs

Brompheniramine maleate (MedChemExpress, Monmouth Junction, NJ, USA) and fenbendazole (Sigma-Aldrich) were purchased and used.

EV uptake

hMSCs were seeded on chamber slides (Thermo Fisher Scientific) at 1×10^3 cells/slide. 2D and 3D EVs were labeled with PKH26 Red Fluorescent Cell Linker Kit (Sigma-Aldrich). After 6 hrs treatment of various concentrations of the labeled EVs ranging from 0 to 57 μg/mL, cells were stained with Alexa Fluor® 488 Phalloidin (Thermo Fisher Scientific) for 1 hr, and counterstained with DAPI (Maravai Life Sciences, San Diego, CA, USA) for another 1 hr. EV uptake was measured by counting the red fluorescence observed in individual cells (> 40 cells per treatment) using Cytation 5 (BioTek).

Microarray

For transcriptome analysis, RNA was prepared from 2D and 3D cultured hMSCs, DPBS-treated, 2D EV- or 3D EV-treated cells as well as Fenbendazole or Brompheniramine-treated cells. Treatment was performed for 6 hrs. All of

the treatment was independently carried out in triplicate. Total RNA extracted from the cells was evaluated quantitatively and qualitatively with BioAnalyzer (Agilent, Santa Clara, CA, USA). To identify differentially expressed genes (DEGs) induced by 3D culture and EV treatment, triplicated RNA samples with a RNA integrity number (RIN) ≥ 7.6 and A_{260}/A_{280} values ≥ 2.0 were pooled into one sample per group. To compare the similarity of transcriptome responses to EV and drug treatment, triplicated RNA samples with a RIN ≥ 9.7 and A260/A280 values ≥ 2.0 were used individually. Qualified RNA samples were then applied to the GeneChip WT cDNA synthesis and amplification kit (Applied Biosystems) for cDNA synthesis. The cDNA was then fragmented and labeled with biotin using GeneChip WT terminal labeling kit (Applied Biosystems). Approximately 5.5 μg of labeled cDNA was hybridized to the Affymetrix GeneChip Human Gene 2.0 ST Array (Affymetrix, Santa Clara, CA, USA) at 45 °C for 16 h. After hybridization, the arrays were scanned on a GCS3000 Scanner (Affymetrix) and data analyses were carried out with the GeneChip Command Console Software (Affymetrix). Expression data were normalized using the robust multi-array average (RMA) approach.

Proteome analysis

EVs were precipitated using cold acetone and dissolved with 5% SDS solubilization buffer (5% SDS, 50 mM TEAB, pH 7.55). EV proteins were digested using S-TrapTM mini spin columns (Protifi, Farmingdale, NY, USA) according to the manufacture's instruction. Digested peptides were labeled with TMTproTM 16plex label reagent (Thermo Fisher Scientific) and separated using the reversed-phase fractionation liquid chromatography

system (Agilent Technologies). Fractionated peptide samples resuspended in 0.1% (v/v) aqueous formic acid solution were subjected to Q Exactive™ hybrid quadrupole-orbitrap mass spectrometer (Thermo Fisher Scientific) coupled with an Ultimate™ 3000 RSLCnano system (Thermo Fisher Scientific). The peptides were loaded onto a trap column (100 μm × 2 cm) packed with Acclaim PepMap100 C18 resin, separated by the analytical column (EASY-Spray column, 75 μm × 50 cm, Thermo Fisher Scientific), and sprayed into nano-ESI source. A top 10 data-dependent method was used to operate the Q Exactive™ mass analyzer. Full MS scans were acquired over the m/z range between 350 and 1,500 with mass resolution of 70,000 at m/z 200 and 3×10^6 of AGC target value. In the higher-energy collisional dissociation (HCD) collision cell with a normalized collision energy of 34, the ten most intense peaks with charge state ≥ 2 were fragmented and tandem mass spectra were acquired with a mass resolution of 35,000 at m/z 200 in the Orbitrap mass analyzer. All LC-MS/MS raw data were analyzed using Proteome Discoverer 2.4 software (Thermo Fisher Scientific) for protein identification and reporter ion-based quantitation. SEQUEST-HT was used for human protein search (https://www.uniprot.org/) with at least two unique peptides and high protein confidence at the false discovery rate (FDR) of 1%.

Bioinformatic analysis

Protein-coding differentially expressed genes (DEGs) in 3D-cultured hMSCs were identified based on a fold change cutoff of 1.5 in comparison with 2D-cultured hMSCs. DEGs were then subjected to the gene set enrichment analysis (GSEA) [39] to validate the effect on the biological process in hMSCs

by 3D culture with MSigDb (Molecular Signature Database) using an FDR q-value cutoff of 0.05. EV-induced DEGs were identified between untreated, 2D EV-treated, and 3D EV-treated cells at fold change cutoff of 1.5. DEGs were applied to the Kyoto Encyclopedia of Gene and Genome (KEGG) analysis and significantly altered signaling pathways by EV treatment were identified at p-value cutoff of 0.05. Expression profiles of 2D and 3D EVs, Fenbendazole, and Brompheniramine were subjected to hierarchical clustering based on Spearman rank correlation analysis with average linkage method using MORPHEUS (https://software.broadinstitute.org/Morpheus).

Differentially expressed proteins (DEPs) in 3D EVs were identified based on a fold change cutoff of 2 and p-value cutoff of 0.05. Top 100 proteins often identified in EVs were referred to ExoCarta (http://www.exocarta.org) and Vesiclepedia (http://microvesicles.org). Functional enrichment and membership search of the 3D EV-induced DEPs were conducted using Metascape (https://metascape.org) [40] with Reactome gene sets. Significant pathways were enriched with minimum overlap of 3, p-value cutoff of 0.01, and minimum enrichment of 1.5.

Among the EV-induced DEGs, the top 30 up-regulated genes and top 30 down-regulated genes were used as an EV signature for drug and disease connectivity analysis (https://clue.io) [41]. Drugs identified as similar to the EV signature were annotated with anatomical therapeutic chemical (ATC) codes (https://www.whocc.no/atc_ddd_index). Anti-infectives were classified with antiinfectives for systemic use, antiparasitic products, insecticides and repellents at ATC level 1, metabolic drugs with alimentary tract and metabolism, cardiovascular system at ATC level 1 and lipid modifying agents, drugs used in diabetes, antiobesity preparations excluding diet products at ATC

level 2, immune modulators with respiratory system, musculo-skeletal system, antineoplastic and immunomodulating agents at ATC level 1, anti-inflammatory and antirheumatic products, immunosuppressants, antihistamines for systemic use, drug for obstructive airway diseases at ATC level 1, and cardiovascular drugs with cardiovascular system at ATC level 1, agents acting on the renin-angiotensin system, calcium channel blockers, antihypertensives, beta blocking agents at ATC level 2.

Statistical analysis

With the exception of the bioinformatic analysis, statistical analysis was conducted using GraphPad Prism 8 (GraphPad Software, Inc., La Jolla, USA). Parametric data were analyzed with two-way Student t-tests or ANOVA followed by post-hoc Dunnett's multiple comparison test after evaluation of the homogeneity of variance of the data ($p < 0.05$). Data with $p < 0.05$ were considered to indicate statistical significance. All measurements are reported as means ± standard deviation (SD).

Results

1. Characterization of 3D-cultured hMSCs using Cellhesion VP

To verify the basic characteristics of hMSCs, I analyzed the expression of MSC markers including CD90, CD29, CD73, CD44, and CD105. All of these were highly expressed in hMSCs, at levels over 95.8%. By contrast, there was negligible expression (1.6%) of the hematopoietic marker CD34 (Fig. 1A). Therefore, hMSCs were used to evaluate the priming effect of 3D culture using Cellhesion VP.

In 3D culture, hMSCs began to form spheroid-like aggregates with Cellhesion VP. On day 3, aggregates became visible and these gradually increased in size with time. The aggregates typically had a diameter greater than 1 mm. DAPI staining demonstrated that the cell number increased proportionally with aggregate size (Fig. 1B). To compare cell growth between 2D- and 3D-cultured hMSCs, 2×10^4 cells were seeded on adherent tissue culture plates or non-adherent tissue culture flasks, and the cell number was measured every 3 days for 15 days. On day 6, the mean cell numbers were 8.6×10^4 in 2D culture and 8.8×10^4 in 3D culture with periodic dissociation. The cell number in 3D culture without dissociation was 5.7×10^4, which was significantly reduced compared to 2D culture ($p < 0.001$), and it was constant until the end of the culture period. On day 9, the cell number in the 3D culture with dissociation was 12.3×10^4, which was significantly lower than that of 16.3×10^4 in 2D culture ($p < 0.001$). This difference remained until the end of the culture period, but cell growth remained exponential. On day 15, the mean cell number was 55.6×10^4 in 2D culture, 44.9×10^4 in 3D culture with dissociation, and 16.8

$\times 10^4$ in 3D culture without dissociation (Fig. 1C). On day 9, the spheroid-like aggregates were transferred to adherent tissue culture plates, where the cells began outgrowth on day 2 and reached confluence on day 6 (Fig. 1D). The retrieved cells were harvested through trypsinization and their growth was compared to that of 2D cells; the 3D cells grew significantly faster (Fig. 1E). Thus, 3D culture of hMSCs using Cellhesion VP appeared to require manipulation such as dissociation to control the excessive size of the aggregates. Compared to 2D culture, 3D culture led to substantially fewer cells, but their proliferation rate after retrieval was greater than that of conventional 2D cells. These results indicate that 3D culture using Cellhesion VP changes the cytological properties of hMSCs.

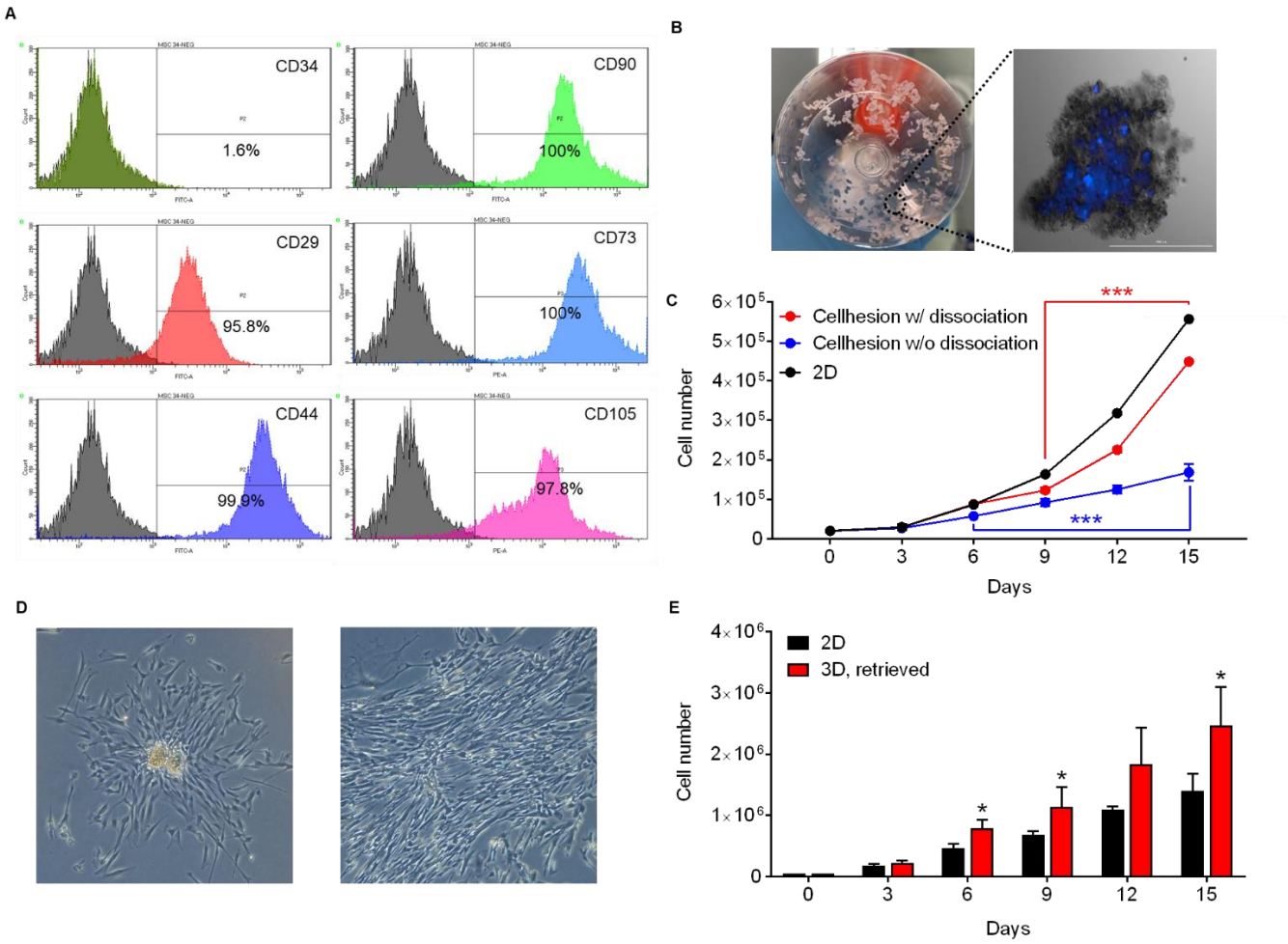

A
CD34
1.6%
CD90
100%
CD29
95.8%
CD73
100%
CD44
99.9%
CD105
97.8%
B
C
Cellhesion w/ dissociation
Cellhesion w/o dissociation
2D
Cell number
Days
***
***
D
E
2D
3D, retrieved
Cell number
Days
*
*
*

Figure 1. Characterization of 3D-cultured hMSCs

A. FACS analysis using MSC markers including CD34 (negative marker), CD90, CD29, CD73, CD44, and CD105 (positive markers). Percentage of MSC marker-positive cell population was denoted. **B**. Morphology of chitin-based polysaccharide (Cellhesion VP) and hMSC aggregates. Blue signal indicates the DAPI-stained cell nuclei. Scale bar is 1000 μm. **C**. Growth comparison between 2D- vs. 3D-cultured hMSCs. Plots at each time point indicate mean ± SD (n = 3). Black dots with connecting line indicate the growth of conventional 2D-cultured cells. Red dots with connecting line indicate the growth of 3D-cultured cells with periodic dissociation. Blue dots with connecting line indicate the growth of 3D-cultured cells without dissociation. ***$p < 0.001$ vs. 2D. **D**. Growth performance and cell morphology of 3D cells on 2 days and 6 days after retrieval. Magnification is × 50. **E**. Growth comparison between 2D- vs. retrieved cells of 3D-cultured hMSCs. Bars indicate mean ± SD (n = 3). Black bars indicate the growth of conventional 2D-cultured cells. Red bars indicate the growth of the retrieved cells of 3D-cultured hMSCs. *$p < 0.05$ vs. 2D.

2. Effects on global gene expression in cells under 3D culture conditions

To analyze the putative priming effects of 3D culture of hMSCs using Cellhesion VP, global gene expression analyses were conducted with the retrieved cells. Gene expression levels were compared, and 694 differentially expressed genes (DEGs) were identified in 3D cells compared to 2D cells at a fold change cutoff of 1.5. Among these genes, 292 were upregulated in 3D cells, including vascular cell adhesion molecule 1 (*VCAM1*), tumor necrosis factor receptor superfamily member 10d (*TNFRSF10D*), membrane metalloendopeptidase (*MME*), cytidine deaminase (*CDA*), and BicC family RNA binding protein 1 (*BICC1*); 402 genes were downregulated, including TEK tyrosine kinase (*TEK*), fatty acid binding protein 4 (*FABP4*), KIAA1324-like (*KIAA1324L*), protein phosphatase 4, regulatory subunit 4 (*PPP4R4*), and tumor necrosis factor (ligand) superfamily member 4 (*TNFSF4*) (Fig. 2A, Table 1). Biological processes affected by 3D-induced DEGs were identified through gene set enrichment analyses. Among the affected processes, those related to cell migration, such as cell motility (q = 4.91E-04), cell projection organization (q = 6.54E-03), cell secretion and its regulation (q = 5.80E-03), and import into the cell (q = 2.33E-02), were significantly enriched in upregulated genes. By contrast, cell differentiation-related processes such as regulation of cell differentiation (q = 1.97E-05) and mesenchymal cell differentiation (q = 2.18E-05) were significantly downregulated (Fig. 2B, Table 2). These findings indicated that cell stemness, cell motility, and EV are the major biological processes primed by Cellhesion VP. Then I performed a literature search to identify individual genes involved in these biological processes. Evidence in the literature for genes related to cell stemness was

identified using the search terms ‘stemness,’ ‘pluripotency,’ ‘dedifferentiation,’ and ‘stem cell,’ which showed that 15 out of 56 genes enriched in cell differentiation-related biological processes had strong literature support. Likewise, cell motility was searched using ‘cell motility,’ ‘migration,’ and ‘wound healing,’ and EV using ‘extracellular vesicle’ and ‘exosome,’ which showed that 33 of 51 genes and 25 of 58 genes, respectively, had evidence in the literature of association with the corresponding processes (Table 3). The genes related to cell stemness included endoglin (*ENG*), hepatocyte growth factor (*HGF*), glial cell-derived neurotrophic factor (*GDNF*), jagged 1 (*JAG1*), and *NOTCH3*, which were all downregulated in 3D cells. Meanwhile, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (*KIT*), chemokine (C-C motif) ligand 2 (*CCL2*), chemokine (C-C motif) ligand 5 (*CCL5*), and hyaluronan synthase 2 (*HAS2*), related to cell motility, were all upregulated in 3D cells. Genes associated with EVs such as intercellular adhesion molecule 1 (*ICAM1*), aquaporin 1 (*AQP1*), and *VCAM1* were upregulated, whereas milk fat globule-EGF factor 8 protein (*MFGE8*), complement component 9 (*C9*), and G protein-coupled receptor class C group 5 member B (*GPRC5B*) were downregulated in 3D cells (Fig. 2C).

Thus, I observed that 3D culture of hMSCs using Cellhesion VP induced a wide range of changes in gene expression. In particular, genes involved in cell stemness, migration ability, and EV production appeared to be dysregulated in 3D culture.

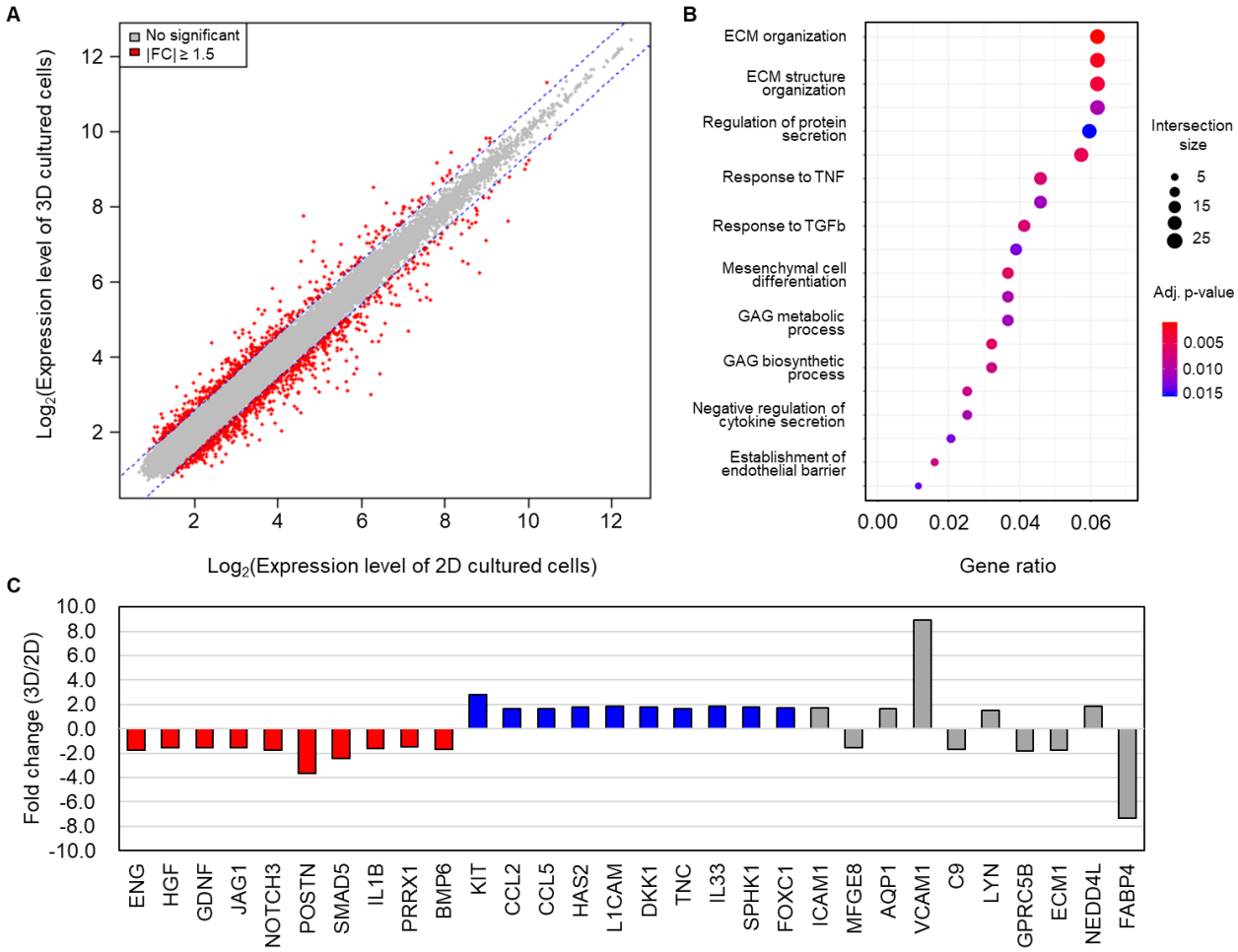

A
No significant
|FC| ≥ 1.5
Log2(Expression level of 3D cultured cells)
Log2(Expression level of 2D cultured cells)
B
ECM organization
ECM structure organization
Regulation of protein secretion
Response to TNF
Response to TGFb
Mesenchymal cell differentiation
GAG metabolic process
GAG biosynthetic process
Negative regulation of cytokine secretion
Establishment of endothelial barrier
Gene ratio
Intersection size
Adj. p-value
C
Fold change (3D/2D)
ENG
HGF
GDNF
JAG1
NOTCH3
POSTN
SMAD5
IL1B
PRRX1
BMP6
KIT
CCL2
CCL5
HAS2
L1CAM
DKK1
TNC
IL33
SPHK1
FOXC1
ICAM1
MFGE8
AQP1
VCAM1
C9
LYN
GPRC5B
ECM1
NEDD4L
FABP4

Figure 2. Transcriptomic comparison between 2D- and 3D-cultured hMSCs

A. Scatter plots comparing gene expression levels between 2D- and 3D-cultured hMSCs. Red spots depict differentially expressed genes in 3D over 2D cells with a 1.5-fold change. **B**. Gene Ontology enrichment of differentially expressed genes in 3D cultured cells (adjusted $p < 0.05$). Gene ratio is intersection size over query size. **C**. Relative expression levels of cell differentiation-related genes (red bars), cell motility-related genes (blue bars), and EV-related genes (gray bars).

Table 1. Differentially expressed genes (DEGs) between 3D- and 2D- cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
16667702	VCAM1	7412	NM_001078	NM_001078	vascular cell adhesion molecule 1	4.602	7.757	8.907
17075448	TNFRSF10D	8793	NM_003840	NM_003840	crosis factor receptor superfamily, member 10d, decoy with truncated death	6.276	8.512	4.710
16947173	MME	4311	NM_000902	NM_000902	membrane metallo-endopeptidase	4.863	7.068	4.614
16660360	CDA	978	NM_001785	NM_001785	cytidine deaminase	4.817	6.823	4.017
16705089	BICC1	80114	NM_001080512	NM_001080512	BicC family RNA binding protein 1	4.546	6.535	3.970
16714399	LINC01468	101928687	NR_120641	NR_120641	long intergenic non-protein coding RNA 1468	2.402	4.357	3.876
16691314	TSPAN2	10100	NM_001308315	NM_001308315	tetraspanin 2	3.885	5.812	3.804
17043727	SCIN	85477	NM_001112706	NM_001112706	scinderin	3.539	5.254	3.284
16844604	KRTAP4-12	83755	NM_031854	NM_031854	keratin associated protein 4-12	2.967	4.643	3.196
16917400	KIF16B	55614	NM_001199865	NM_001199865	kinesin family member 16B	3.146	4.723	2.984
16972835	STOX2	56977	XM_011532131	XM_011532131	storkhead box 2	3.671	5.223	2.933
16990572	SH3RF2	153769	NM_152550	NM_152550	SH3 domain containing ring finger 2	3.637	5.125	2.804
17019805	TNFRSF21	27242	NM_014452	NM_014452	tumor necrosis factor receptor superfamily, member 21	5.520	7.004	2.797
16824352	XYLT1	64131	NM_022166	NM_022166	xylosyltransferase I	6.604	8.086	2.793
16943944	CD200	4345	NM_001004196	NM_001004196	CD200 molecule	3.425	4.903	2.785
16705329	MYPN	84665	NM_001256267	NM_001256267	myopalladin	5.064	6.542	2.785
16966855	KIT	3815	NM_000222	NM_000222	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	3.152	4.628	2.783
16819213	MT1L	4500	NR_001447	NR_001447	metallothionein 1L (gene/pseudogene)	3.072	4.504	2.700
16688799	ADGRL4	64123	NM_022159	NM_022159	adhesion G protein-coupled receptor L4	4.047	5.470	2.681
16950440	OXTR	5021	NM_000916	NM_000916	oxytocin receptor	7.807	9.180	2.589
16937137	LMCD1	29995	NM_001278233	NM_001278233	LIM and cysteine-rich domains 1	6.115	7.465	2.549
16928428	ADRBK2	157	NM_005160	NM_005160	adrenergic, beta, receptor kinase 2	1.991	3.316	2.505
17015084	SERPINB1	1992	NM_030666	NM_030666	serpin peptidase inhibitor, clade B (ovalbumin), member 1	4.529	5.846	2.491
16908197	IGFBP5	3488	NM_000599	NM_000599	insulin like growth factor binding protein 5	8.141	9.452	2.480
16972229	ANXA10	11199	NM_007193	NM_007193	annexin A10	5.126	6.408	2.431
16961501	TNIK	23043	NM_001161560	NM_001161560	TRAF2 and NCK interacting kinase	5.946	7.210	2.402
16690704	SLC16A4	9122	NM_001201546	NM_001201546	solute carrier family 16, member 4	4.014	5.244	2.346
16783602	SSTR1	6751	NM_001049	NM_001049	somatostatin receptor 1	4.771	5.980	2.313
16976599	SULT1B1	27284	NM_014465	NM_014465	sulfotransferase family 1B member 1	2.456	3.648	2.285
17012632	ENPP1	5167	NM_006208	NM_006208	ectonucleotide pyrophosphatase/phosphodiesterase 1	4.046	5.234	2.279
16911804	SLC24A3	57419	NM_020689	NM_020689	solute carrier family 24 (sodium/potassium/calcium exchanger), member 3	3.338	4.516	2.263
16911493	SPTLC3	55304	NM_018327	NM_018327	serine palmitoyltransferase, long chain base subunit 3	2.751	3.918	2.245
16841634	CDRT1	374286	NM_001282540	NM_001282540	CMT1A duplicated region transcript 1	2.659	3.819	2.236
16949914	LINC00969	440993	AK128346	AK128346	long intergenic non-protein coding RNA 969	2.807	3.944	2.200
16875083	ZNF600	162966	NM_198457	NM_198457	zinc finger protein 600	2.780	3.901	2.176
17106997	STK26	51765	NM_001042452	NM_001042452	serine/threonine protein kinase 26	4.915	6.009	2.134
16819252	MT1F	4494	NM_001301272	NM_001301272	metallothionein 1F	2.041	3.094	2.075
16987766	C5orf30	90355	NM_033211	NM_033211	chromosome 5 open reading frame 30	6.788	7.836	2.068
16798422	SNORD115-39	100033813	NR_003354	NR_003354	small nucleolar RNA, C/D box 115-39	3.115	4.162	2.066
16742384	LRRC32	2615	NM_001128922	NM_001128922	leucine rich repeat containing 32	5.097	6.120	2.032
16924620	ADAMTS5	11096	NM_007038	NM_007038	ADAM metallopeptidase with thrombospondin type 1 motif 5	5.484	6.503	2.027
16725459	LOC105369324	105369324	XR_913156	XR_913156	uncharacterized LOC105369324	1.566	2.576	2.015
16798270	SNORD115-5	100033442	NR_003297	NR_003297	small nucleolar RNA, C/D box 115-5	2.845	3.851	2.009
16798286	SNORD115-9	100033446	NR_003301	NR_003301	small nucleolar RNA, C/D box 115-9	2.845	3.851	2.009
16798303	SNORD115-11	100033448	NR_003303	NR_003303	small nucleolar RNA, C/D box 115-11	2.845	3.851	2.009

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
16798305	SNORD115-9	100033446	NR_003301	NR_003301	small nucleolar RNA, C/D box 115-9	2.845	3.851	2.009
16798402	SNORD115-11	100033448	NR_003303	NR_003303	small nucleolar RNA, C/D box 115-11	2.845	3.851	2.009
16798416	SNORD115-11	100033448	NR_003303	NR_003303	small nucleolar RNA, C/D box 115-11	2.845	3.851	2.009
16798430	SNORD115-11	100033448	NR_003303	NR_003303	small nucleolar RNA, C/D box 115-11	2.845	3.851	2.009
16798258	SNORD115-1	338433	NR_001291	NR_001291	small nucleolar RNA, C/D box 115-1	3.451	4.439	1.983
16798333	SNORD115-16	100033454	NR_003308	NR_003308	small nucleolar RNA, C/D box 115-16	3.451	4.439	1.983
16716478	ANKRD1	27063	NM_014391	NM_014391	ankyrin repeat domain 1 (cardiac muscle)	7.003	7.991	1.983
16798228	SNORD116-26	100033438	NR_003340	NR_003340	small nucleolar RNA, C/D box 116-26	2.184	3.165	1.974
16860168	ZNF431	170959	NM_133473	NM_133473	zinc finger protein 431	3.360	4.331	1.960
16868000	CD209	30835	NM_001144893	NM_001144893	CD209 molecule	2.964	3.934	1.960
16852858	SERPINB7	8710	NM_001040147	NM_001040147	serpin peptidase inhibitor, clade B (ovalbumin), member 7	6.500	7.468	1.956
16952782	TMEM158	25907	NM_015444	NM_015444	transmembrane protein 158 (gene/pseudogene)	6.360	7.325	1.953
16818501	SLC6A10PB	653562	XR_951352	XR_951352	solute carrier family 6 (neurotransmitter transporter), member 8 pseudogene	5.281	6.247	1.953
16693375	SPRR2F	6705	NM_001014450	NM_001014450	small proline-rich protein 2F	2.411	3.371	1.945
17094893	ALDH1A1	216	NM_000689	NM_000689	aldehyde dehydrogenase 1 family, member A1	7.220	8.174	1.938
16762413	LMNTD1	160492	NM_001145727	NM_001145727	lamin tail domain containing 1	2.123	3.074	1.932
17051626	MEST	4232	NM_001253900	NM_001253900	mesoderm specific transcript	5.452	6.400	1.929
16745693	ESAM	90952	NM_138961	NM_138961	endothelial cell adhesion molecule	4.176	5.100	1.898
16886717	GALNT5	11227	NM_014568	NM_014568	polypeptide N-acetylgalactosaminyltransferase 5	6.121	7.039	1.889
16821377	CDH13	1012	NM_001220488	NM_001220488	cadherin 13	7.006	7.913	1.875
17104947	SLC16A2	6567	NM_006517	NM_006517	solute carrier family 16, member 2 (thyroid hormone transporter)	7.711	8.611	1.866
16852573	NEDD4L	23327	NM_001144964	NM_001144964	ursor cell expressed, developmentally down-regulated 4-like, E3 ubiquitin pr	6.535	7.433	1.864
16961449	SLC7A14	57709	NM_020949	NM_020949	solute carrier family 7, member 14	4.097	4.995	1.863
16857886	ANGPTL4	51129	NM_001039667	NM_001039667	angiopoietin like 4	4.892	5.788	1.861
16947955	LOC102724479	102724479	XR_924707	XR_924707	uncharacterized LOC102724479	2.194	3.090	1.860
17015540	TFAP2A	7020	NM_001032280	NM_001032280	transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)	4.248	5.141	1.856
16775758	LOC105370309	105370309	XR_915178	XR_915178	uncharacterized LOC105370309	1.544	2.430	1.848
16997399	SNORA47	677828	NR_003014	NR_003014	small nucleolar RNA, H/ACA box 47	3.754	4.639	1.847
16939479	ENTPD3	956	NM_001248	NM_001248	ectonucleoside triphosphate diphosphohydrolase 3	3.163	4.044	1.841
17115271	L1CAM	3897	NM_000425	NM_000425	L1 cell adhesion molecule	6.508	7.384	1.836
17051553	CPA4	51200	NM_001163446	NM_001163446	carboxypeptidase A4	10.433	11.301	1.825
17083433	IL33	90865	NM_001199640	NM_001199640	interleukin 33	3.636	4.504	1.825
16768341	ATP2B1	490	NM_001001323	NM_001001323	ATPase, Ca++ transporting, plasma membrane 1	6.976	7.837	1.816
16855182	SNORD58A	26791	NR_002571	NR_002571	small nucleolar RNA, C/D box 58A	4.353	5.211	1.813
16705011	DKK1	22943	NM_012242	NM_012242	dickkopf WNT signaling pathway inhibitor 1	8.101	8.957	1.810
16838059	SPHK1	8877	NM_001142601	NM_001142601	sphingosine kinase 1	5.228	6.082	1.808
16707196	IFIT1	3434	NM_001270927	NM_001270927	interferon-induced protein with tetratricopeptide repeats 1	6.517	7.371	1.807
16864181	RCN3	57333	NM_020650	NM_020650	reticulocalbin 3, EF-hand calcium binding domain	6.402	7.256	1.807
17100201	CLIC3	9022	NM_004669	NM_004669	chloride intracellular channel 3	4.364	5.205	1.790
17080648	HAS2	3037	NM_005328	NM_005328	hyaluronan synthase 2	8.980	9.818	1.788
16778274	TRPC4	7223	NM_001135955	NM_001135955	transient receptor potential cation channel, subfamily C, member 4	6.190	7.026	1.784
16838017	GALR2	8811	NM_003857	NM_003857	galanin receptor 2	2.963	3.796	1.781
17045078	BMPER	168667	NM_133468	NM_133468	BMP binding endothelial regulator	6.338	7.162	1.771
17044193	LOC101927841	101927841	XR_252181	XR_252181	uncharacterized LOC101927841	2.264	3.087	1.769
16817630	QPRT	23475	NM_014298	NM_014298	quinolinate phosphoribosyltransferase	7.153	7.974	1.766

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
16966152	LOC401127	401127	NR_026854	NR_026854	WD repeat domain 5 pseudogene	3.420	4.240	1.766
16970762	PCDH10	57575	NM_020815	NM_020815	protocadherin 10	5.845	6.664	1.763
16667094	EPHX4	253152	NM_173567	NM_173567	epoxide hydrolase 4	2.320	3.130	1.754
16979845	KRT18P54	100418792	OTTHUMT00000364203	OTTHUMT00000364203	keratin 18 pseudogene 54	7.429	8.239	1.753
16831442	TRPV2	51393	NM_016113	NM_016113	transient receptor potential cation channel, subfamily V, member 2	4.746	5.553	1.749
16815735	ABAT	18	NM_000663	NM_000663	4-aminobutyrate aminotransferase	4.327	5.133	1.749
16875026	ZNF616	90317	NM_178523	NM_178523	zinc finger protein 616	2.533	3.335	1.743
17015143	TUBB2A	7280	NM_001069	NM_001069	tubulin, beta 2A class IIa	4.949	5.750	1.742
17025844	THBS2	7058	NM_003247	NM_003247	thrombospondin 2	8.040	8.841	1.742
17010522	IRAK1BP1	134728	NM_001010844	NM_001010844	interleukin 1 receptor associated kinase 1 binding protein 1	3.004	3.804	1.741
16916270	PCMTD2	55251	ENST00000299468	ENST00000299468	rotein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing	2.534	3.335	1.741
16822919	RPS2	6187	ENST00000343262	ENST00000343262	ribosomal protein S2	3.216	4.011	1.735
16832336	TBC1D3P5	440419	NR_033892	NR_033892	TBC1 domain family, member 3 pseudogene 5	3.056	3.849	1.732
16781354	OR11H12	440153	NM_001013354	NM_001013354	olfactory receptor, family 11, subfamily H, member 12	1.289	2.081	1.731
16798345	SNORD115-22	100033799	NR_003314	NR_003314	small nucleolar RNA, C/D box 115-22	2.888	3.678	1.729
16844600	KRTAP4-11	653240	NM_033059	NM_033059	keratin associated protein 4-11	2.127	2.916	1.729
16977396	SCD5	79966	NM_001037582	NM_001037582	stearoyl-CoA desaturase 5	6.272	7.059	1.726
17005018	LOC101928331	101928331	XR_251968	XR_251968	uncharacterized LOC101928331	1.652	2.439	1.726
16668375	GSTM1	2944	NM_000561	NM_000561	glutathione S-transferase mu 1	1.954	2.741	1.725
17043843	TSPAN13	27075	NM_014399	NM_014399	tetraspanin 13	7.383	8.168	1.723
16858137	ICAM1	3383	NM_000201	NM_000201	intercellular adhesion molecule 1	8.108	8.890	1.720
16735332	NLRP10	338322	NM_176821	NM_176821	NLR family, pyrin domain containing 10	3.806	4.586	1.717
16874970	ZNF649	65251	NM_023074	NM_023074	zinc finger protein 649	3.727	4.507	1.716
16866065	ZNF773	374928	NM_001304334	NM_001304334	zinc finger protein 773	4.755	5.534	1.716
16935607	NFAM1	150372	NM_145912	NM_145912	NFAT activating protein with ITAM motif 1	3.077	3.852	1.711
16881189	LINC01143	104355141	NR_126384	NR_126384	long intergenic non-protein coding RNA 1143	3.468	4.240	1.708
16674589	KIAA1614	57710	NM_020950	NM_020950	KIAA1614	2.131	2.902	1.706
17004208	FOXC1	2296	NM_001453	NM_001453	forkhead box C1	4.316	5.086	1.705
16870925	ZNF724P	440519	NR_045525	NR_045525	zinc finger protein 724, pseudogene	1.988	2.752	1.698
16751900	HOXC13	3229	NM_017410	NM_017410	homeobox C13	2.831	3.594	1.696
17093031	MOB3B	79817	NM_024761	NM_024761	MOB kinase activator 3B	3.335	4.096	1.696
16700911	ERO1B	56605	NM_019891	NM_019891	endoplasmic reticulum oxidoreductase beta	2.602	3.363	1.695
16689384	GBP4	115361	NM_052941	NM_052941	guanylate binding protein 4	4.798	5.552	1.686
16986583	JMY	133746	NM_152405	NM_152405	junction mediating and regulatory protein, p53 cofactor	4.270	5.023	1.685
16773552	ATP5EP2	432369	NR_002162	NR_002162	nthase, H+ transporting, mitochondrial F1 complex, epsilon subunit pseudo	2.433	3.179	1.678
16665621	CACHD1	57685	NM_001293274	NM_001293274	cache domain containing 1	5.042	5.785	1.674
17025191	EZR	7430	NM_001111077	NM_001111077	ezrin	8.321	9.060	1.670
16678247	ZNF678	339500	NM_178549	NM_178549	zinc finger protein 678	2.164	2.904	1.670
16706522	SFTPA1	653509	NM_001093770	NM_001093770	surfactant protein A1	1.847	2.585	1.669
16833204	CCL2	6347	NM_002982	NM_002982	chemokine (C-C motif) ligand 2	7.394	8.131	1.667
16979985	MGARP	84709	NM_032623	NM_032623	mitochondria localized glutamic acid rich protein	7.966	8.701	1.665
16788606	SNORD113-6	767566	NR_003234	NR_003234	small nucleolar RNA, C/D box 113-6	1.787	2.520	1.663
16869060	ZNF878	729747	NM_001080404	NM_001080404	zinc finger protein 878	3.887	4.620	1.661
16664868	LOC105378727	105378727	XR_947351	XR_947351	uncharacterized LOC105378727	3.335	4.067	1.661
16815355	IL32	9235	NM_001012631	NM_001012631	interleukin 32	3.409	4.138	1.658

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
17019892	LOC101927048	101927048	NR_110843	NR_110843	uncharacterized LOC101927048	2.343	3.072	1.657
16859199	OR10H3	26532	NM_013938	NM_013938	olfactory receptor, family 10, subfamily H, member 3	1.473	2.195	1.649
16661687	EPB41	2035	NM_001166005	NM_001166005	erythrocyte membrane protein band 4.1	5.205	5.924	1.646
16999421	MARCH3	115123	NM_178450	NM_178450	membrane associated ring finger 3	5.501	6.217	1.642
16833373	RASL10B	91608	NM_033315	NM_033315	RAS-like, family 10, member B	4.529	5.244	1.641
16846672	LRRC59	55379	ENST00000503118	ENST00000503118	leucine rich repeat containing 59	3.963	4.673	1.636
16876147	ZNF132	7691	NM_003433	NM_003433	zinc finger protein 132	3.630	4.340	1.636
16843511	CCL5	6352	NM_001278736	NM_001278736	chemokine (C-C motif) ligand 5	4.054	4.763	1.635
17016486	HIST1H2BL	8340	NM_003519	NM_003519	histone cluster 1, H2bl	3.097	3.804	1.633
17044862	AQP1	358	NM_001185060	NM_001185060	aquaporin 1 (Colton blood group)	3.921	4.626	1.631
17097661	TNC	3371	NM_002160	NM_002160	tenascin C	6.665	7.370	1.629
17052685	TRBV3-1	28619	BC070387	BC070387	T cell receptor beta variable 3-1	3.881	4.585	1.629
16835386	SNX11	29916	NM_013323	NM_013323	sorting nexin 11	5.527	6.227	1.624
16792420	MDGA2	161357	NM_001113498	NM_001113498	MAM domain containing glycosylphosphatidylinositol anchor 2	3.388	4.088	1.624
17113860	MRRFP1	286423	OTTHUMT00000058139	OTTHUMT00000058139	mitochondrial ribosome recycling factor pseudogene 1	2.926	3.625	1.623
16663051	ZFP69B	65243	NM_023070	NM_023070	ZFP69 zinc finger protein B	2.773	3.471	1.622
16894422	SNORA80B	100302743	NR_028374	NR_028374	small nucleolar RNA, H/ACA box 80B	3.685	4.381	1.620
17095307	C9orf64	84267	NM_032307	NM_032307	chromosome 9 open reading frame 64	3.554	4.249	1.620
16881863	LINC01291	102724515	NR_125792	NR_125792	long intergenic non-protein coding RNA 1291	2.182	2.878	1.619
16952414	CSRNP1	64651	NM_033027	NM_033027	cysteine-serine-rich nuclear protein 1	4.346	5.041	1.618
16998682	EFNA5	1946	NM_001962	NM_001962	ephrin-A5	4.270	4.965	1.618
17052538	MGAM2	93432	NM_001293626	NM_001293626	maltase-glucoamylase 2 (putative)	2.917	3.609	1.616
17058121	ZNF680	340252	NM_001130022	NM_001130022	zinc finger protein 680	1.486	2.175	1.612
16976074	CLOCK	9575	NM_001267843	NM_001267843	clock circadian regulator	7.589	8.277	1.612
17058002	LOC650226	650226	NR_029420	NR_029420	ankyrin repeat domain 26 pseudogene	1.836	2.522	1.609
16943983	GTPBP8	29083	NM_014170	NM_014170	GTP-binding protein 8 (putative)	2.960	3.646	1.608
16717272	LOXL4	84171	NM_032211	NM_032211	lysyl oxidase-like 4	4.829	5.514	1.607
16897226	LOC105374582	105374582	XR_925608	XR_925608	uncharacterized LOC105374582	2.487	3.171	1.606
16829085	SLC7A5	8140	NM_003486	NM_003486	lute carrier family 7 (amino acid transporter light chain, L system), member	8.832	9.514	1.605
16923738	LINC00334	114042	AF426268	AF426268	long intergenic non-protein coding RNA 334	2.845	3.525	1.601
17021092	FAM46A	55603	NM_017633	NM_017633	family with sequence similarity 46, member A	5.244	5.923	1.601
16797427	IGHD2-8	28504	OTTHUMT00000325967	OTTHUMT00000325967	immunoglobulin heavy diversity 2-8	1.678	2.356	1.600
16897493	LOC105377627	105377627	XR_940082	XR_940082	uncharacterized LOC105377627	3.242	3.919	1.599
16971737	GUCY1B3	2983	NM_000857	NM_000857	guanylate cyclase 1, soluble, beta 3	4.498	5.175	1.598
16944559	ARGFX	503582	NM_001012659	NM_001012659	arginine-fifty homeobox	1.625	2.301	1.598
16962277	EHHADH	1962	NM_001166415	NM_001166415	enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase	3.215	3.890	1.596
16968447	GPAT3	84803	NM_001256421	NM_001256421	glycerol-3-phosphate acyltransferase 3	3.481	4.155	1.596
16832133	LOC100996813	100996813	XM_011524139	XM_011524139	multidrug and toxin extrusion protein 2-like	3.064	3.736	1.594
16987992	SNORA13	654322	NR_002922	NR_002922	small nucleolar RNA, H/ACA box 13	3.204	3.876	1.594
16700989	ZP4	57829	NM_021186	NM_021186	zona pellucida glycoprotein 4	1.862	2.532	1.591
17087508	KRT8P11	347265	OTTHUMT00000053395	OTTHUMT00000053395	keratin 8 pseudogene 11	3.578	4.247	1.590
16673934	LOC105371617	105371617	XR_918083	XR_918083	uncharacterized LOC105371617	3.109	3.778	1.589
16831219	LINC00670	284034	NR_034144	NR_034144	long intergenic non-protein coding RNA 670	5.572	6.240	1.589
16810341	CA12	771	NM_001218	NM_001218	carbonic anhydrase XII	5.961	6.628	1.588
16939934	ZNF35	7584	NM_003420	NM_003420	zinc finger protein 35	4.963	5.630	1.588

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
16870746	ZNF506	440515	NM_001099269	NM_001099269	zinc finger protein 506	3.072	3.737	1.586
16726416	KCNK4	50801	uc001nzk.1	uc001nzk.1	potassium channel, two pore domain subfamily K, member 4	4.919	5.584	1.586
17076481	RNF5P1	286140	NR_003129	NR_003129	ring finger protein 5, E3 ubiquitin protein ligase pseudogene 1	3.013	3.676	1.583
17002194	ADAM19	8728	NM_033274	NM_033274	ADAM metallopeptidase domain 19	9.063	9.724	1.581
16750595	ANP32D	23519	NM_012404	NM_012404	acidic nuclear phosphoprotein 32 family member D	1.419	2.078	1.579
16885736	LOC150776	150776	NR_026922	NR_026922	elin phosphodiesterase 4, neutral membrane (neutral sphingomyelinase-3) p	4.577	5.235	1.578
17104098	PAGE2	203569	NM_207339	NM_207339	P antigen family, member 2 (prostate associated)	2.198	2.855	1.577
16968237	LINC01094	100505702	NR_038303	NR_038303	long intergenic non-protein coding RNA 1094	3.206	3.863	1.577
17060820	LOC102724094	102724094	NR_120519	NR_120519	uncharacterized LOC102724094	3.104	3.760	1.577
16979825	PABPC4L	132430	NM_001114734	NM_001114734	poly(A) binding protein, cytoplasmic 4-like	1.891	2.547	1.575
16927787	IGLV3-27	28791	OTTHUMT00000321641	OTTHUMT00000321641	immunoglobulin lambda variable 3-27	2.953	3.608	1.575
16998510	LOC100652833	100652833	XM_011543783	XM_011543783	putative POM121-like protein 1-like	1.703	2.358	1.574
16834151	KRTAP9-8	83901	NM_031963	NM_031963	keratin associated protein 9-8	3.627	4.281	1.574
16721684	TUB	7275	NM_003320	NM_003320	tubby bipartite transcription factor	3.935	4.589	1.573
16927821	IGLV2-11	28816	OTTHUMT00000321841	OTTHUMT00000321841	immunoglobulin lambda variable 2-11	5.158	5.810	1.572
16843417	SLFN12	55106	NM_001289009	NM_001289009	schlafen family member 12	4.970	5.621	1.571
16824258	MYH11	4629	NM_001040113	NM_001040113	myosin, heavy chain 11, smooth muscle	3.031	3.682	1.571
16968213	ANXA3	306	NM_005139	NM_005139	annexin A3	4.929	5.580	1.571
17063832	TRBV6-4	28603	OTTHUMT00000351239	OTTHUMT00000351239	T cell receptor beta variable 6-4	1.453	2.104	1.570
16887702	ITGA6	3655	NM_000210	NM_000210	integrin alpha 6	7.205	7.855	1.569
16871846	ZNF607	84775	NM_001172677	NM_001172677	zinc finger protein 607	1.940	2.588	1.567
16941052	CYB561D2	11068	NM_001291284	NM_001291284	cytochrome b561 family, member D2	4.975	5.623	1.567
16995281	NADK2	133686	NM_001085411	NM_001085411	NAD kinase 2, mitochondrial	6.981	7.627	1.565
16874985	ZNF615	284370	NM_001199324	NM_001199324	zinc finger protein 615	2.636	3.282	1.565
16757589	LOC105370006	105370006	XR_914552	XR_914552	uncharacterized LOC105370006	3.725	4.371	1.565
16927874	GNAZ	2781	NM_002073	NM_002073	guanine nucleotide binding protein (G protein), alpha z polypeptide	5.989	6.634	1.564
16961610	GHSR	2693	NM_004122	NM_004122	growth hormone secretagogue receptor	1.782	2.425	1.562
16699529	LOC105372937	105372937	XR_920814	XR_920814	uncharacterized LOC105372937	2.159	2.802	1.562
16797498	IGHV1-24	28467	OTTHUMT00000325192	OTTHUMT00000325192	immunoglobulin heavy variable 1-24	1.419	2.060	1.559
16719882	FUOM	282969	NM_001098483	NM_001098483	fucose mutarotase	3.813	4.454	1.559
16834603	TMEM106A	113277	NM_001291586	NM_001291586	transmembrane protein 106A	6.858	7.499	1.559
16876024	FKBP1AP1	2282	NR_024162	NR_024162	FK506 binding protein 1A pseudogene 1	3.615	4.253	1.557
16972919	SNX25	83891	NM_031953	NM_031953	sorting nexin 25	5.681	6.318	1.556
16736672	LOC100126784	100126784	NR_015384	NR_015384	uncharacterized LOC100126784	3.407	4.044	1.555
17063838	TRBV30	28557	OTTHUMT00000352519	OTTHUMT00000352519	T cell receptor beta variable 30 (gene/pseudogene)	1.080	1.717	1.555
16691965	PDE4DIP	9659	BC152553	BC152553	phosphodiesterase 4D interacting protein	3.166	3.803	1.554
17108953	VCX2	51480	NM_016378	NM_016378	variable charge, X-linked 2	4.361	4.996	1.553
16698234	FMOD	2331	NM_002023	NM_002023	fibromodulin	3.378	4.013	1.552
16776160	ITGBL1	9358	NM_001271754	NM_001271754	integrin beta like 1	4.097	4.731	1.552
17016490	HIST1H2AJ	8331	NM_021066	NM_021066	histone cluster 1, H2aj	1.879	2.513	1.552
17082250	FAM83H	286077	NM_198488	NM_198488	family with sequence similarity 83, member H	4.012	4.646	1.551
16997199	ENC1	8507	NM_001256574	NM_001256574	ectodermal-neural cortex 1 (with BTB domain)	6.531	7.163	1.550
16962579	RTP2	344892	NM_001004312	NM_001004312	receptor (chemosensory) transporter protein 2	1.296	1.926	1.549
17067748	LOC105379362	105379362	XR_949653	XR_949653	uncharacterized LOC105379362	3.874	4.504	1.548
16831890	LGALS9C	654346	NM_001040078	NM_001040078	lectin, galactoside-binding, soluble, 9C	3.525	4.155	1.548

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
17049167	LOC105375425	105375425	XR_927807	XR_927807	uncharacterized LOC105375425	2.438	3.067	1.547
16893704	FAM110C	642273	NM_001077710	NM_001077710	family with sequence similarity 110, member C	1.317	1.946	1.547
16734818	OR51A4	401666	NM_001005329	NM_001005329	olfactory receptor, family 51, subfamily A, member 4	1.513	2.142	1.546
16669983	LOC101060254	101060254	XM_011508668	XM_011508668	myomegalin-like	3.756	4.385	1.546
16901388	LOC100506473	100506473	XR_109933	XR_109933	uncharacterized LOC100506473	2.473	3.100	1.545
16777794	UBL3	5412	NM_007106	NM_007106	ubiquitin-like 3	6.879	7.506	1.544
16984562	PARP8	79668	NM_001178055	NM_001178055	poly(ADP-ribose) polymerase family member 8	2.662	3.288	1.543
16696824	ABL2	27	NM_001136000	NM_001136000	ABL proto-oncogene 2, non-receptor tyrosine kinase	6.584	7.210	1.543
16769386	TTC41P	253724	NR_027249	NR_027249	tetratricopeptide repeat domain 41, pseudogene	2.122	2.746	1.542
16925312	LOC105372788	105372788	XR_920472	XR_920472	uncharacterized LOC105372788	2.134	2.755	1.538
16862904	ZNF223	7766	NM_013361	NM_013361	zinc finger protein 223	2.344	2.965	1.537
17010196	KRT19P1	441160	OTTHUMT00000041169	OTTHUMT00000041169	keratin 19 pseudogene 1	1.424	2.045	1.537
16910312	LOC105373970	105373970	XR_924061	XR_924061	uncharacterized LOC105373970	2.586	3.206	1.537
16932706	SLC7A4	6545	NM_004173	NM_004173	solute carrier family 7, member 4	2.583	3.203	1.537
17007738	SCUBE3	222663	NM_001303136	NM_001303136	signal peptide, CUB domain, EGF-like 3	7.377	7.996	1.536
16689354	GBP2	2634	NM_004120	NM_004120	guanylate binding protein 2, interferon-inducible	5.914	6.532	1.535
16667530	PALMD	54873	NM_017734	NM_017734	palmdelphin	2.551	3.167	1.533
16966721	SNORA26	677810	NR_003016	NR_003016	small nucleolar RNA, H/ACA box 26	2.167	2.782	1.532
16864401	SIGLEC16	400709	NR_002825	NR_002825	sialic acid binding Ig-like lectin 16 (gene/pseudogene)	1.684	2.299	1.532
16697004	RNASEL	6041	NM_021133	NM_021133	ribonuclease L (2,5-oligoisoadenylate synthetase-dependent)	2.171	2.785	1.530
16805804	FAM138E	100124412	NR_026819	NR_026819	family with sequence similarity 138, member E	4.136	4.749	1.530
16849400	SOCS3	9021	NM_003955	NM_003955	suppressor of cytokine signaling 3	5.250	5.862	1.529
17107462	SPANXN4	441525	NM_001009613	NM_001009613	SPANX family, member N4	1.791	2.403	1.528
16703251	KIAA1217	56243	NM_001098500	NM_001098500	KIAA1217	2.452	3.064	1.528
16927863	IGLJ5	28829	OTTHUMT00000321640	OTTHUMT00000321640	immunoglobulin lambda joining 5 (non-functional)	3.098	3.709	1.527
16775229	OR7E156P	283491	NR_002171	NR_002171	olfactory receptor, family 7, subfamily E, member 156 pseudogene	2.744	3.354	1.527
17013650	ULBP2	80328	NM_025217	NM_025217	UL16 binding protein 2	3.580	4.191	1.527
16919508	TOMM34	10953	NM_006809	NM_006809	translocase of outer mitochondrial membrane 34	6.947	7.557	1.526
16746203	ADAMTS8	11095	NM_007037	NM_007037	ADAM metallopeptidase with thrombospondin type 1 motif 8	3.488	4.097	1.526
16921724	NCAM2	4685	NM_004540	NM_004540	neural cell adhesion molecule 2	4.835	5.444	1.526
16797524	IGHV3-35	28432	OTTHUMT00000325174	OTTHUMT00000325174	immunoglobulin heavy variable 3-35 (non-functional)	2.386	2.995	1.526
16942100	LOC105377104	105377104	XR_937496	XR_937496	uncharacterized LOC105377104	3.072	3.681	1.526
16660713	LYPLA2	11313	XM_005245728	XM_005245728	lysophospholipase II	4.206	4.815	1.525
16696013	CREG1	8804	NM_003851	NM_003851	cellular repressor of E1A-stimulated genes 1	2.964	3.573	1.525
16793671	HIF1A-AS1	100750246	NR_047116	NR_047116	HIF1A antisense RNA 1	3.593	4.201	1.524
16843296	ASIC2	40	ENST00000583395	ENST00000583395	acid sensing ion channel 2	2.452	3.059	1.523
17085082	ALDH1B1	219	NM_000692	NM_000692	aldehyde dehydrogenase 1 family, member B1	7.448	8.052	1.520
17069063	LYN	4067	NM_001111097	NM_001111097	LYN proto-oncogene, Src family tyrosine kinase	5.920	6.523	1.519
17005871	HIST1H2BN	8341	ENST00000449538	ENST00000449538	histone cluster 1, H2bn	3.005	3.608	1.519
16900108	IGKV3-7	28915	OTTHUMT00000323360	OTTHUMT00000323360	immunoglobulin kappa variable 3-7 (non-functional)	4.086	4.689	1.519
17003193	MXD3	83463	NM_001142935	NM_001142935	MAX dimerization protein 3	3.072	3.674	1.517
16998309	ELL2	22936	NM_012081	NM_012081	elongation factor, RNA polymerase II, 2	8.671	9.272	1.517
16929648	LOC105377199	105377199	XR_938223	XR_938223	uncharacterized LOC105377199	2.746	3.347	1.516
16884523	SLC20A1	6574	NM_005415	NM_005415	solute carrier family 20 (phosphate transporter), member 1	8.135	8.735	1.515
17117397	LOC400743	400743	AK127830	AK127830	uncharacterized LOC400743	3.038	3.638	1.515

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
16662942	LOC105378668	105378668	XR_947223	XR_947223	uncharacterized LOC105378668	3.385	3.985	1.515
16837172	CACNG4	27092	NM_014405	NM_014405	calcium channel, voltage-dependent, gamma subunit 4	2.918	3.517	1.515
17046528	INTS4P1	285905	XR_928285	XR_928285	integrator complex subunit 4 pseudogene 1	1.270	1.869	1.515
17068344	LOC105379386	105379386	XR_949698	XR_949698	uncharacterized LOC105379386	1.652	2.251	1.515
16804188	LOC103171574	103171574	NR_120379	NR_120379	uncharacterized LOC103171574	1.303	1.901	1.514
16721184	OR51E1	143503	NM_152430	NM_152430	olfactory receptor, family 51, subfamily E, member 1	1.534	2.131	1.513
17046586	SNORA22	677807	NR_002961	NR_002961	small nucleolar RNA, H/ACA box 22	5.855	6.452	1.513
17044199	SNORD93	692210	NR_003075	NR_003075	small nucleolar RNA, C/D box 93	1.473	2.069	1.512
16977868	ABCG2	9429	NM_001257386	NM_001257386	ATP binding cassette subfamily G member 2 (Junior blood group)	3.039	3.634	1.511
16971705	LOC105377503	105377503	XR_939374	XR_939374	uncharacterized LOC105377503	1.716	2.310	1.509
16696094	ANKRD36BP1	84832	NR_026844	NR_026844	ankyrin repeat domain 36B pseudogene 1	3.921	4.514	1.509
17106688	GRIA3	2892	NM_000828	NM_000828	glutamate receptor, ionotropic, AMPA 3	2.536	3.130	1.509
17042728	LOC101927000	101927000	NR_132384	NR_132384	uncharacterized LOC101927000	2.513	3.103	1.505
16811085	ITGA11	22801	NM_001004439	NM_001004439	integrin alpha 11	3.925	4.513	1.504
17045169	LOC105375233	105375233	XR_927174	XR_927174	uncharacterized LOC105375233	3.119	3.707	1.503
16773946	RFC3	5983	NM_002915	NM_002915	replication factor C subunit 3	4.980	5.568	1.503
16713309	FZD8	8325	NM_031866	NM_031866	frizzled class receptor 8	4.948	5.535	1.503
16734877	HBE1	3046	NM_005330	NM_005330	hemoglobin, epsilon 1	3.308	3.896	1.503
16901793	LOC400997	400997	NR_122074	NR_122074	uncharacterized LOC400997	1.223	1.810	1.502
17082947	ZNF252P	286101	NR_023392	NR_023392	zinc finger protein 252, pseudogene	3.477	4.064	1.501
16967197	LOC105377249	105377249	XR_938808	XR_938808	uncharacterized LOC105377249	1.270	1.856	1.501
16805471	LOC105371007	105371007	XR_916738	XR_916738	uncharacterized LOC105371007	1.426	2.011	1.500
16843156	OMG	4974	ENST00000580156	ENST00000580156	oligodendrocyte myelin glycoprotein	2.095	1.510	-1.500
17016366	HIST1H2AB	8335	NM_003513	NM_003513	histone cluster 1, H2ab	3.347	2.761	-1.501
16958432	SLC12A8	84561	NM_001195483	NM_001195483	solute carrier family 12, member 8	4.602	4.016	-1.501
17088991	MIR181A2HG	100379345	NR_038975	NR_038975	MIR181A2 host gene	3.206	2.619	-1.502
16718263	SORCS1	114815	NM_001013031	NM_001013031	sortilin-related VPS10 domain containing receptor 1	4.091	3.504	-1.502
16782082	TRAJ21	28734	OTTHUMT00000410977	OTTHUMT00000410977	T cell receptor alpha joining 21	1.840	1.253	-1.502
16888031	HNRNPA3	220988	NM_194247	NM_194247	heterogeneous nuclear ribonucleoprotein A3	2.280	1.693	-1.502
17062402	IQUB	154865	NM_001282855	NM_001282855	IQ motif and ubiquitin domain containing	2.452	1.865	-1.502
16730976	ZC3H12C	85463	NM_033390	NM_033390	zinc finger CCCH-type containing 12C	4.978	4.390	-1.503
16817537	LAT	27040	NM_001014987	NM_001014987	linker for activation of T-cells	4.510	3.922	-1.503
16844312	TOP2A	7153	NM_001067	NM_001067	topoisomerase (DNA) II alpha	6.674	6.086	-1.504
16962916	GP5	2814	NM_004488	NM_004488	glycoprotein V (platelet)	2.602	2.013	-1.504
16890915	RQCD1	9125	NM_001271634	NM_001271634	RCD1 required for cell differentiation1 homolog (S. pombe)	5.525	4.936	-1.504
17086728	LOC105376143	105376143	XR_930110	XR_930110	uncharacterized LOC105376143	2.372	1.782	-1.505
16979875	PCDH18	54510	NM_001300828	NM_001300828	protocadherin 18	7.660	7.069	-1.505
16713729	SYT15	83849	NM_031912	NM_031912	synaptotagmin XV	4.776	4.185	-1.506
16842147	CCDC144B	284047	NR_036647	NR_036647	coiled-coil domain containing 144B (pseudogene)	3.836	3.245	-1.506
16904573	LOC105373730	105373730	XR_923551	XR_923551	uncharacterized LOC105373730	3.488	2.897	-1.506
16852069	KIAA1328	57536	XR_935236	XR_935236	KIAA1328	2.545	1.954	-1.506
17059355	SEMA3D	223117	NM_152754	NM_152754	omain, immunoglobulin domain (Ig), short basic domain, secreted, (semapho	6.223	5.632	-1.507
16800707	SEMA6D	80031	NM_001198999	NM_001198999	domain, transmembrane domain (TM), and cytoplasmic domain, (semaphori	7.251	6.659	-1.507
17080444	LOC105375721	105375721	XR_928577	XR_928577	uncharacterized LOC105375721	4.803	4.211	-1.507
16786492	YLPM1	56252	NM_019589	NM_019589	YLP motif containing 1	5.514	4.921	-1.508

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
16673636	PRRX1	5396	NM_006902	NM_006902	paired related homeobox 1	6.871	6.278	-1.508
16863593	C5AR2	27202	NM_001271749	NM_001271749	complement component 5a receptor 2	3.525	2.932	-1.509
16983254	ANKRD33B	651746	NM_001164440	NM_001164440	ankyrin repeat domain 33B	5.499	4.905	-1.509
16833292	LIG3	3980	ENST00000378526	ENST00000378526	ligase III, DNA, ATP-dependent	5.200	4.606	-1.510
16891494	LOC105373888	105373888	XR_923924	XR_923924	uncharacterized LOC105373888	3.002	2.407	-1.511
16788701	SNORD114-30	767611	NR_003223	NR_003223	small nucleolar RNA, C/D box 114-30	3.921	3.325	-1.511
16834566	NBR2	10230	NR_003108	NR_003108	neighbor of BRCA1 gene 2 (non-protein coding)	4.026	3.431	-1.511
16787236	SPATA7	55812	NM_001040428	NM_001040428	spermatogenesis associated 7	4.040	3.444	-1.512
17080440	LOC105375721	105375721	XR_928579	XR_928579	uncharacterized LOC105375721	2.048	1.451	-1.513
16665501	LOC105378770	105378770	XR_947454	XR_947454	uncharacterized LOC105378770	2.335	1.736	-1.514
17092115	GLIS3	169792	XM_011517768	XM_011517768	GLIS family zinc finger 3	3.477	2.879	-1.514
16788622	SNORD114-4	767580	NR_003196	NR_003196	small nucleolar RNA, C/D box 114-4	3.933	3.335	-1.514
16668109	SCARNA2	677766	NR_003023	NR_003023	small Cajal body-specific RNA 2	4.842	4.242	-1.515
17117545	LOC100131541	100131541	AY358248	AY358248	uncharacterized LOC100131541	3.380	2.781	-1.515
17080486	TNFRSF11B	4982	NM_002546	NM_002546	tumor necrosis factor receptor superfamily, member 11b	7.498	6.898	-1.516
17013507	SAMD5	389432	NM_001030060	NM_001030060	sterile alpha motif domain containing 5	6.663	6.062	-1.517
16921461	LOC101930100	101930100	XR_424059	XR_424059	uncharacterized LOC101930100	6.136	5.535	-1.517
16782028	TRAJ48	28707	OTTHUMT00000410950	OTTHUMT00000410950	T cell receptor alpha joining 48	2.735	2.131	-1.519
16725041	FAM111B	374393	NM_001142703	NM_001142703	family with sequence similarity 111, member B	3.206	2.602	-1.520
16781536	PNP	4860	NM_000270	NM_000270	purine nucleoside phosphorylase	7.061	6.457	-1.520
16881802	DOK1	1796	NM_001197260	NM_001197260	docking protein 1	3.538	2.933	-1.520
17010517	MEI4	101928601	NM_001282136	NM_001282136	meiotic double-stranded break formation protein 4	1.623	1.017	-1.522
16806538	GOLGA8R	101059918	NM_001282484	NM_001282484	golgin A8 family, member R	4.178	3.573	-1.522
16874935	HAS1	3036	NM_001297436	NM_001297436	hyaluronan synthase 1	3.880	3.274	-1.522
16850541	SMCHD1	23347	NM_015295	NM_015295	structural maintenance of chromosomes flexible hinge domain containing 1	7.229	6.622	-1.524
16947925	CLDN11	5010	NM_001185056	NM_001185056	claudin 11	4.504	3.896	-1.524
16941687	SNORD19	692089	NR_003047	NR_003047	small nucleolar RNA, C/D box 19	2.217	1.610	-1.524
17010354	CD109	135228	NM_001159587	NM_001159587	CD109 molecule	6.734	6.125	-1.524
16929615	APOL6	80830	NM_030641	NM_030641	apolipoprotein L, 6	5.632	5.024	-1.524
17001901	FAT2	2196	NM_001447	NM_001447	FAT atypical cadherin 2	2.962	2.353	-1.525
17023514	SAMD3	154075	XM_011535487	XM_011535487	sterile alpha motif domain containing 3	3.206	2.596	-1.527
16865782	RFPL4AL1	729974	NM_001277397	NM_001277397	ret finger protein-like 4A-like 1	2.369	1.755	-1.530
16733516	ADAMTS15	170689	NM_139055	NM_139055	ADAM metallopeptidase with thrombospondin type 1 motif 15	5.296	4.682	-1.530
16673036	C1orf226	400793	NM_001085375	NM_001085375	chromosome 1 open reading frame 226	3.402	2.787	-1.531
17011496	CEP57L1	285753	NM_001083535	NM_001083535	centrosomal protein 57kDa-like 1	3.949	3.335	-1.531
16696533	SNORD75	692195	NR_003941	NR_003941	small nucleolar RNA, C/D box 75	5.055	4.439	-1.532
17066065	SLC7A2	6542	NM_001008539	NM_001008539	olute carrier family 7 (cationic amino acid transporter, y+ system), member	6.163	5.547	-1.532
17024775	SYNE1	23345	NM_033071	NM_033071	spectrin repeat containing, nuclear envelope 1	5.274	4.658	-1.533
16995461	GDNF	2668	NM_000514	NM_000514	glial cell derived neurotrophic factor	4.026	3.408	-1.534
17059165	HGF	3082	NM_000601	NM_000601	hepatocyte growth factor (hepapoietin A; scatter factor)	9.282	8.664	-1.535
16821790	ZNF469	84627	NM_001127464	NM_001127464	zinc finger protein 469	5.365	4.747	-1.535
16917183	JAG1	182	NM_000214	NM_000214	jagged 1	8.254	7.636	-1.535
17052532	MGAM2	93432	NM_001293626	NM_001293626	maltase-glucoamylase 2 (putative)	2.721	2.101	-1.536
16811246	LOC101929151	101929151	XR_243177	XR_243177	uncharacterized LOC101929151	4.602	3.983	-1.537
16749747	KIAA1551	55196	NM_018169	NM_018169	KIAA1551	4.425	3.804	-1.538

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
17017641	TNXB	7148	NM_019105	NM_019105	tenascin XB	4.200	3.577	-1.540
17104049	SNORA11	677799	NR_002953	NR_002953	small nucleolar RNA, H/ACA box 11	5.127	4.504	-1.540
16994618	MYO10	4651	NM_012334	NM_012334	myosin X	8.052	7.428	-1.540
16974358	LOC105374484	105374484	XR_925392	XR_925392	uncharacterized LOC105374484	2.072	1.447	-1.541
16760953	MFAP5	8076	NM_001297709	NM_001297709	microfibrillar associated protein 5	5.574	4.950	-1.542
17117424	LOC100287497	100287497	XR_159115	XR_159115	uncharacterized LOC100287497	3.828	3.203	-1.542
16813058	MFGE8	4240	ENST00000560553	ENST00000560553	milk fat globule-EGF factor 8 protein	6.352	5.727	-1.542
17095703	NFIL3	4783	NM_001289999	NM_001289999	nuclear factor, interleukin 3 regulated	4.599	3.971	-1.546
16976815	PF4	5196	NM_002619	NM_002619	platelet factor 4	5.312	4.683	-1.546
16767324	SNORA70G	100379132	NR_033335	NR_033335	small nucleolar RNA, H/ACA box 70G	3.462	2.833	-1.547
16953997	C3orf84	646498	NM_001080528	NM_001080528	chromosome 3 open reading frame 84	2.028	1.398	-1.547
16779049	LOC105370201	105370201	XR_914986	XR_914986	uncharacterized LOC105370201	2.181	1.550	-1.548
16681845	PRAMEF11	440560	NM_001146344	NM_001146344	PRAME family member 11	2.770	2.138	-1.549
16957636	ZBTB20	26137	NM_001164342	NM_001164342	zinc finger and BTB domain containing 20	4.792	4.160	-1.550
16797599	IGHV3-72	28410	OTTHUMT00000324210	OTTHUMT00000324210	immunoglobulin heavy variable 3-72	2.111	1.478	-1.551
16842183	ZNF286B	729288	NM_001145045	NM_001145045	zinc finger protein 286B	4.991	4.358	-1.551
17016499	HIST1H1B	3009	NM_005322	NM_005322	histone cluster 1, H1b	3.565	2.932	-1.551
16877562	GDF7	151449	NM_182828	NM_182828	growth differentiation factor 7	3.701	3.067	-1.552
16773759	FRY	10129	NM_023037	NM_023037	FRY microtubule binding protein	7.080	6.445	-1.553
16929920	H1F0	3005	NM_005318	NM_005318	H1 histone family, member 0	6.394	5.759	-1.553
16959148	ASTE1	28990	NM_001288950	NM_001288950	asteroid homolog 1 (Drosophila)	3.694	3.059	-1.553
16952349	SCN11A	11280	NM_001287223	NM_001287223	sodium channel, voltage gated, type XI alpha subunit	3.094	2.459	-1.553
16732891	NRGN	4900	NM_001126181	NM_001126181	neurogranin (protein kinase C substrate, RC3)	5.577	4.941	-1.554
16919044	RBL1	5933	NM_002895	NM_002895	retinoblastoma-like 1	5.035	4.399	-1.555
16843374	RAD51D	5892	ENST00000345365	ENST00000345365	RAD51 paralog D	4.672	4.033	-1.557
17004721	GCNT2	2651	NM_001491	NM_001491	glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (I blood group)	4.529	3.889	-1.558
17117724	PSMD3	5709	ENST00000485835	ENST00000485835	proteasome 26S subunit, non-ATPase 3	3.975	3.335	-1.559
16943433	PDCL3P4	285359	NR_002941	NR_002941	phosducin-like 3 pseudogene 4	5.973	5.331	-1.560
16882744	IGKV1D-27	28898	OTTHUMT00000323279	OTTHUMT00000323279	immunoglobulin kappa variable 1D-27 (pseudogene)	5.505	4.864	-1.560
16893143	TWIST2	117581	NM_057179	NM_057179	twist family bHLH transcription factor 2	4.706	4.062	-1.563
16798351	SNRPN	6638	AF400499	AF400499	small nuclear ribonucleoprotein polypeptide N	5.236	4.591	-1.565
16748126	LOC642846	642846	NR_024374	NR_024374	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11-like	3.033	2.386	-1.566
17024079	MAP3K5	4217	NM_005923	NM_005923	mitogen-activated protein kinase kinase kinase 5	6.066	5.419	-1.567
16706562	LOC642361	642361	NR_029407	NR_029407	uncharacterized LOC642361	4.986	4.338	-1.567
16744125	KDELC2	143888	NM_153705	NM_153705	KDEL (Lys-Asp-Glu-Leu) containing 2	7.557	6.909	-1.567
16967779	CXCL6	6372	NM_002993	NM_002993	chemokine (C-X-C motif) ligand 6	3.549	2.899	-1.569
16995029	LOC105374715	105374715	XR_925901	XR_925901	uncharacterized LOC105374715	3.980	3.330	-1.569
17047268	GTF2IRD2B	389524	NM_001003795	NM_001003795	GTF2I repeat domain containing 2B	3.771	3.120	-1.571
16997275	GCNT4	51301	NM_016591	NM_016591	glucosaminyl (N-acetyl) transferase 4, core 2	4.343	3.692	-1.571
16701007	LINC01139	339535	NR_015407	NR_015407	long intergenic non-protein coding RNA 1139	2.751	2.099	-1.572
16661192	HMGN2	3151	NM_005517	NM_005517	high mobility group nucleosomal binding domain 2	2.676	2.023	-1.572
16671082	LCE1A	353131	NM_178348	NM_178348	late cornified envelope 1A	2.291	1.637	-1.573
16659395	PRAMEF18	391003	NM_001099850	NM_001099850	PRAME family member 18	4.226	3.572	-1.573
16760760	CD163L1	283316	NM_001297650	NM_001297650	CD163 molecule-like 1	3.840	3.185	-1.574
17046750	LINC01372	101929736	NR_108104	NR_108104	long intergenic non-protein coding RNA 1372	2.002	1.347	-1.575

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
16705507	SRGN	5552	NM_002727	NM_002727	serglycin	10.484	9.827	-1.576
16939668	NKTR	4820	NM_005385	NM_005385	natural killer cell triggering receptor	5.500	4.843	-1.578
17105393	TMEM35	59353	NM_021637	NM_021637	transmembrane protein 35	7.532	6.871	-1.581
17085685	TJP2	9414	NM_001170414	NM_001170414	tight junction protein 2	7.736	7.075	-1.582
17086270	SPATA31D4	389761	NM_001145197	NM_001145197	SPATA31 subfamily D, member 4	3.120	2.459	-1.582
16942511	KBTBD8	84541	NM_032505	NM_032505	kelch repeat and BTB (POZ) domain containing 8	5.033	4.370	-1.584
16862385	CYP2B6	1555	NM_000767	NM_000767	cytochrome P450, family 2, subfamily B, polypeptide 6	2.943	2.277	-1.586
16838101	SNORD1B	677849	NR_004396	NR_004396	small nucleolar RNA, C/D box 1B	2.636	1.971	-1.586
16853671	TMEM200C	645369	NM_001080209	NM_001080209	transmembrane protein 200C	3.917	3.251	-1.586
17110372	LOC401585	401585	NR_125365	NR_125365	uncharacterized LOC401585	3.897	3.229	-1.588
16768270	KITLG	4254	NM_000899	NM_000899	KIT ligand	7.938	7.270	-1.589
17103303	PORCN	64840	NM_001282167	NM_001282167	porcupine homolog (Drosophila)	7.208	6.539	-1.590
17108490	FAM223B	286967	AY168775	AY168775	family with sequence similarity 223, member B (non-protein coding)	1.696	1.026	-1.592
16967843	EREG	2069	NM_001432	NM_001432	epiregulin	3.420	2.749	-1.592
17062955	LINC-PINT	378805	NR_015431	NR_015431	long intergenic non-protein coding RNA, p53 induced transcript	3.728	3.057	-1.593
16777384	SACS	26278	NM_001278055	NM_001278055	sacsin molecular chaperone	8.228	7.554	-1.595
16927767	LL22NC03-63E9.3	648691	NR_027426	NR_027426	uncharacterized LOC648691	2.527	1.853	-1.595
16869639	PTGER1	5731	NM_000955	NM_000955	prostaglandin E receptor 1	5.662	4.988	-1.595
17098896	LOC105376287	105376287	XR_930378	XR_930378	uncharacterized LOC105376287	1.959	1.285	-1.596
16901986	IL1B	3553	NM_000576	NM_000576	interleukin 1 beta	4.548	3.872	-1.597
17098477	ANGPTL2	23452	NM_012098	NM_012098	angiopoietin like 2	6.634	5.958	-1.598
17046559	INTS4P2	644619	NR_027392	NR_027392	integrator complex subunit 4 pseudogene 2	5.654	4.977	-1.598
16667492	LOC729987	729987	NR_046088	NR_046088	uncharacterized LOC729987	2.938	2.261	-1.599
16902809	FAR2P2	100216479	NR_046258	NR_046258	fatty acyl-CoA reductase 2 pseudogene 2	3.593	2.915	-1.599
17057066	COA1	55744	NM_018224	NM_018224	cytochrome c oxidase assembly factor 1 homolog	5.112	4.434	-1.600
16859166	CYP4F12	66002	NM_023944	NM_023944	cytochrome P450, family 4, subfamily F, polypeptide 12	2.926	2.246	-1.602
16817677	PRRT2	112476	NM_001256442	NM_001256442	proline-rich transmembrane protein 2	2.743	2.062	-1.603
16842850	DHRS13	147015	NM_144683	NM_144683	dehydrogenase/reductase (SDR family) member 13	3.886	3.206	-1.603
16921464	LOC101930100	101930100	XR_424059	XR_424059	uncharacterized LOC101930100	3.814	3.133	-1.604
16773478	RPL21	6144	ENST00000466550	ENST00000466550	ribosomal protein L21	3.544	2.861	-1.606
16682333	MFAP2	4237	NM_001135247	NM_001135247	microfibrillar associated protein 2	4.929	4.245	-1.606
17075628	EBF2	64641	NM_022659	NM_022659	early B-cell factor 2	3.154	2.468	-1.608
17118152	QIQN5815	100129033	AY358807	AY358807	uncharacterized LOC100129033	3.282	2.593	-1.612
16685165	CLSPN	63967	NM_001190481	NM_001190481	claspin	4.046	3.356	-1.614
17117186	RBMY2EP	159125	NR_001574	NR_001574	RNA binding motif protein, Y-linked, family 2, member E pseudogene	2.891	2.200	-1.615
16977537	HPSE	10855	NM_001098540	NM_001098540	heparanase	2.751	2.059	-1.616
16806844	KATNBL1	79768	NM_024713	NM_024713	katanin p80 subunit B-like 1	3.732	3.039	-1.617
17051943	TMEM140	55281	NM_018295	NM_018295	transmembrane protein 140	4.295	3.602	-1.617
16738704	OR5A2	219981	NM_001001954	NM_001001954	olfactory receptor, family 5, subfamily A, member 2	3.425	2.731	-1.618
17118030	SEPT11	55752	ENST00000512778	ENST00000512778	septin 11	4.406	3.709	-1.621
17005865	HIST1H2BM	8342	NM_003521	NM_003521	histone cluster 1, H2bm	6.579	5.881	-1.622
16706906	ADIRF	10974	NM_006829	NM_006829	adipogenesis regulatory factor	4.380	3.682	-1.622
16898359	LOC101927402	101927402	XM_005264677	XM_005264677	uncharacterized proline-rich protein-like	3.444	2.744	-1.624
16866002	ZNF543	125919	NM_213598	NM_213598	zinc finger protein 543	4.394	3.694	-1.625
16682219	LOC100132147	100132147	BC036435	BC036435	uncharacterized LOC100132147	1.820	1.117	-1.628

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
16990534	ARHGAP26-IT1	100874372	NR_046816	NR_046816	ARHGAP26 intronic transcript 1	2.068	1.364	-1.629
17101193	CD99	4267	XM_005274530	XM_005274530	CD99 molecule	4.097	3.393	-1.629
17059119	SEMA3C	10512	NM_006379	NM_006379	omain, immunoglobulin domain (Ig), short basic domain, secreted, (semapho	9.107	8.401	-1.632
16962661	CLDN1	9076	NM_021101	NM_021101	claudin 1	6.059	5.349	-1.636
16821015	LOC105371342	105371342	XR_917460	XR_917460	uncharacterized LOC105371342	2.177	1.466	-1.637
16816704	OTOA	146183	NM_001161683	NM_001161683	otoancorin	2.781	2.070	-1.637
16927850	IGLJ2	28832	OTTHUMT00000321848	OTTHUMT00000321848	immunoglobulin lambda joining 2	5.081	4.368	-1.639
16900724	LOC100506123	100506123	uc002sxv.4	uc002sxv.4	uncharacterized LOC100506123	3.921	3.206	-1.641
16675398	CFH	3075	NM_000186	NM_000186	complement factor H	6.059	5.344	-1.641
16848123	ABCA8	10351	NM_001288985	NM_001288985	ATP binding cassette subfamily A member 8	4.843	4.126	-1.644
16987377	GPR150	285601	NM_199243	NM_199243	G protein-coupled receptor 150	3.257	2.539	-1.645
16788618	SNORD114-2	767578	NR_003194	NR_003194	small nucleolar RNA, C/D box 114-2	4.426	3.707	-1.646
16797467	IGHD	3495	BC021276	BC021276	immunoglobulin heavy constant delta	1.915	1.194	-1.648
16691766	ANKRD20A12P	100874392	AK090412	AK090412	ankyrin repeat domain 20 family, member A12, pseudogene	2.570	1.849	-1.649
16706382	KCNMA1-AS1	101929328	NR_120655	NR_120655	KCNMA1 antisense RNA 1	3.238	2.515	-1.651
17004657	BMP6	654	NM_001718	NM_001718	bone morphogenetic protein 6	5.641	4.917	-1.652
17096379	LOC105376171	105376171	XR_930164	XR_930164	uncharacterized LOC105376171	2.721	1.997	-1.652
16774405	DNAJC15	29103	NM_013238	NM_013238	DnaJ (Hsp40) homolog, subfamily C, member 15	7.910	7.184	-1.653
16863682	SNORD23	692091	NR_003048	NR_003048	small nucleolar RNA, C/D box 23	5.212	4.487	-1.654
16864575	CLEC11A	6320	NM_002975	NM_002975	C-type lectin domain family 11, member A	7.073	6.346	-1.655
16688992	LPAR3	23566	NM_012152	NM_012152	lysophosphatidic acid receptor 3	5.344	4.616	-1.656
16869427	CACNA1A	773	NM_000068	NM_000068	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	4.445	3.716	-1.658
16751655	IGFBP6	3489	NM_002178	NM_002178	insulin like growth factor binding protein 6	7.448	6.718	-1.658
16768528	UBE2N	7334	NM_003348	NM_003348	ubiquitin conjugating enzyme E2N	3.425	2.695	-1.659
16797494	ZCWPW2	152098	AB087877	AB087877	zinc finger, CW type with PWWP domain 2	3.867	3.136	-1.660
16774271	NAA16	79612	NM_001110798	NM_001110798	N(alpha)-acetyltransferase 16, NatA auxiliary subunit	5.838	5.106	-1.662
16962536	MASP1	5648	NM_001031849	NM_001031849	binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactiv	6.315	5.581	-1.663
16995629	C9	735	NM_001737	NM_001737	complement component 9	3.335	2.600	-1.665
16863715	SNAR-C4	100170219	NR_024218	NR_024218	small ILF3/NF90-associated RNA C4	6.383	5.646	-1.667
16863719	SNAR-C3	100170226	NR_024221	NR_024221	small ILF3/NF90-associated RNA C3	6.383	5.646	-1.667
16792954	NID2	22795	NM_007361	NM_007361	nidogen 2 (osteonidogen)	9.538	8.801	-1.667
17118076	LOC100128340	100128340	XR_430664	XR_430664	uncharacterized LOC100128340	4.064	3.326	-1.668
16874214	NTF4	4909	NM_006179	NM_006179	neurotrophin 4	4.422	3.682	-1.670
17060167	TMEM130	222865	NM_001134450	NM_001134450	transmembrane protein 130	5.821	5.077	-1.675
16996956	NAIP	4671	XM_011546977	XM_011546977	NLR family, apoptosis inhibitory protein	4.348	3.601	-1.678
17112149	XIST	7503	NR_001564	NR_001564	X inactive specific transcript (non-protein coding)	7.258	6.510	-1.679
16725485	PGA3	643834	NM_001079807	NM_001079807	pepsinogen 3, group I (pepsinogen A)	2.287	1.539	-1.680
16875508	LENG8-AS1	104355426	NR_126418	NR_126418	LENG8 antisense RNA 1	4.543	3.795	-1.680
16794313	LOC105370555	105370555	XR_915753	XR_915753	uncharacterized LOC105370555	2.088	1.336	-1.685
16879385	PKDCC	91461	NM_138370	NM_138370	protein kinase domain containing, cytoplasmic	4.874	4.122	-1.685
16798130	SNRPN	6638	AF400485	AF400485	small nuclear ribonucleoprotein polypeptide N	4.723	3.967	-1.688
17043588	GLCCI1	113263	NM_138426	NM_138426	glucocorticoid induced 1	4.090	3.335	-1.688
16949882	SDHAP2	727956	NR_003265	NR_003265	succinate dehydrogenase complex subunit A, flavoprotein pseudogene 2	4.542	3.786	-1.689
16876242	KIR2DL2	3803	NM_014219	NM_014219	ller cell immunoglobulin-like receptor, two domains, long cytoplasmic tail,	2.941	2.184	-1.689
17102352	GK	2710	NM_000167	NM_000167	glycerol kinase	2.527	1.769	-1.691

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
16850663	DLGAP1-AS2	84777	NR_119377	NR_119377	DLGAP1 antisense RNA 2	3.206	2.448	-1.691
16816599	LOC105371120	105371120	XR_950899	XR_950899	uncharacterized LOC105371120	2.318	1.559	-1.692
16824637	LOC105371120	105371120	XR_950899	XR_950899	uncharacterized LOC105371120	2.318	1.559	-1.692
16927581	RIMBP3B	440804	NM_001128635	NM_001128635	RIMS binding protein 3B	3.915	3.155	-1.693
17016400	HIST1H3F	8968	NM_021018	NM_021018	histone cluster 1, H3f	4.274	3.514	-1.694
16881242	OR7E91P	79315	NR_002185	NR_002185	olfactory receptor, family 7, subfamily E, member 91 pseudogene	2.783	2.023	-1.694
16805218	LINC01578	100507217	NR_037600	NR_037600	long intergenic non-protein coding RNA 1578	5.151	4.390	-1.694
16782108	TRAJ7	28748	OTTHUMT00000410991	OTTHUMT00000410991	T cell receptor alpha joining 7	2.268	1.506	-1.696
17022691	FYN	2534	NM_002037	NM_002037	FYN proto-oncogene, Src family tyrosine kinase	4.196	3.433	-1.696
17112560	NAP1L3	4675	NM_004538	NM_004538	nucleosome assembly protein 1-like 3	5.117	4.354	-1.697
17062127	WNT2	7472	NM_003391	NM_003391	wingless-type MMTV integration site family member 2	4.637	3.874	-1.697
17052552	MGAM2	93432	NM_001293626	NM_001293626	maltase-glucoamylase 2 (putative)	2.309	1.544	-1.699
17049904	LRRC17	10234	NM_001031692	NM_001031692	leucine rich repeat containing 17	8.731	7.965	-1.700
16969648	LOC105377359	105377359	XR_939060	XR_939060	uncharacterized LOC105377359	3.335	2.569	-1.700
16818466	IGHV2OR16-5	100129631	OTTHUMT00000328352	OTTHUMT00000328352	immunoglobulin heavy variable 2/OR16-5 (non-functional)	4.257	3.491	-1.700
16706985	LOC102723794	102723794	XR_946168	XR_946168	uncharacterized LOC102723794	4.536	3.769	-1.701
16960054	PCOLCE2	26577	NM_013363	NM_013363	procollagen C-endopeptidase enhancer 2	5.383	4.612	-1.706
16788667	SNORD114-16	767594	NR_003209	NR_003209	small nucleolar RNA, C/D box 114-16	4.963	4.188	-1.711
16802497	PAQR5	54852	NM_001104554	NM_001104554	progestin and adipoQ receptor family member V	6.170	5.395	-1.711
16729035	SPCS2	9789	NM_014752	NM_014752	signal peptidase complex subunit 2	3.540	2.765	-1.711
16878947	LTBP1	4052	NM_000627	NM_000627	latent transforming growth factor beta binding protein 1	10.005	9.229	-1.712
17047881	DMTF1	9988	NM_001142326	NM_001142326	cyclin D binding myb-like transcription factor 1	6.887	6.111	-1.712
17051827	AKR1B10	57016	NM_020299	NM_020299	aldo-keto reductase family 1, member B10 (aldose reductase)	5.924	5.147	-1.714
16683470	SRSF10	10772	ENST00000341154	ENST00000341154	serine/arginine-rich splicing factor 10	2.763	1.986	-1.714
16759652	ZNF140	7699	NM_001300776	NM_001300776	zinc finger protein 140	3.229	2.452	-1.714
17023799	SLC2A12	154091	NM_145176	NM_145176	solute carrier family 2 (facilitated glucose transporter), member 12	6.396	5.618	-1.715
16837348	MAP2K6	5608	NM_002758	NM_002758	mitogen-activated protein kinase kinase 6	3.231	2.452	-1.717
17094389	CNTNAP3B	728577	OTTHUMT00000143893	OTTHUMT00000143893	contactin associated protein-like 3B	4.463	3.683	-1.717
16887589	CYBRD1	79901	NM_001127383	NM_001127383	cytochrome b reductase 1	9.926	9.144	-1.719
16889653	FAM117B	150864	NM_173511	NM_173511	family with sequence similarity 117, member B	8.426	7.644	-1.719
17050797	CPED1	79974	NM_001105533	NM_001105533	cadherin-like and PC-esterase domain containing 1	7.135	6.353	-1.720
16917849	THBD	7056	NM_000361	NM_000361	thrombomodulin	5.057	4.273	-1.722
16840442	ALOX12-AS1	100506713	ENST00000570562	ENST00000570562	ALOX12 antisense RNA 1	3.519	2.734	-1.724
16939768	KRBOX1	100506243	NM_001205272	NM_001205272	KRAB box domain containing 1	3.436	2.647	-1.728
16819926	CES4A	283848	NM_001190201	NM_001190201	carboxylesterase 4A	3.682	2.890	-1.731
16952118	TRANK1	9881	NM_014831	NM_014831	tetratricopeptide repeat and ankyrin repeat containing 1	4.178	3.386	-1.731
16860203	ZNF429	353088	NM_001001415	NM_001001415	zinc finger protein 429	2.602	1.810	-1.732
17013773	AKAP12	9590	NM_005100	NM_005100	A kinase (PRKA) anchor protein 12	6.953	6.156	-1.737
17105269	FAM133A	286499	NM_001171109	NM_001171109	family with sequence similarity 133, member A	3.437	2.640	-1.738
16670574	ECM1	1893	NM_001202858	NM_001202858	extracellular matrix protein 1	5.425	4.626	-1.740
17050251	SLC26A4	5172	NM_000441	NM_000441	solute carrier family 26 (anion exchanger), member 4	3.692	2.891	-1.742
16828964	FENDRR	400550	NR_033925	NR_033925	FOXF1 adjacent non-coding developmental regulatory RNA	6.707	5.904	-1.745
16863705	SNAR-C2	100170218	NR_024217	NR_024217	small ILF3/NF90-associated RNA C2	5.717	4.913	-1.747
16863711	SNAR-C2	100170218	NR_024217	NR_024217	small ILF3/NF90-associated RNA C2	5.717	4.913	-1.747
16863721	SNAR-C2	100170218	NR_024217	NR_024217	small ILF3/NF90-associated RNA C2	5.717	4.913	-1.747

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
16798479	GABRA5	2558	NM_000810	NM_000810	gamma-aminobutyric acid (GABA) A receptor, alpha 5	4.177	3.372	-1.747
16896816	THUMPD2	80745	NM_025264	NM_025264	THUMP domain containing 2	3.877	3.072	-1.747
17066112	LOC105379299	105379299	XR_949534	XR_949534	uncharacterized LOC105379299	2.558	1.753	-1.747
16814619	TPSAB1	7177	NM_003294	NM_003294	tryptase alpha/beta 1	4.334	3.528	-1.748
16768723	USP44	84101	NM_001042403	NM_001042403	ubiquitin specific peptidase 44	4.718	3.912	-1.749
16659936	FAM231A	729574	NM_001282321	NM_001282321	family with sequence similarity 231, member A	2.500	1.693	-1.750
16993390	LOC105377763	105377763	XR_941317	XR_941317	uncharacterized LOC105377763	5.120	4.313	-1.750
16962523	RPL39L	116832	NM_052969	NM_052969	ribosomal protein L39-like	4.453	3.646	-1.750
16777996	N4BP2L2-IT2	116828	NR_026928	NR_026928	N4BPL2 intronic transcript 2	3.346	2.538	-1.751
16741725	PDE2A	5138	NM_001143839	NM_001143839	phosphodiesterase 2A, cGMP-stimulated	4.630	3.819	-1.755
16933437	MN1	4330	NM_002430	NM_002430	meningioma (disrupted in balanced translocation) 1	5.711	4.899	-1.755
17072110	COLEC10	10584	NM_006438	NM_006438	collectin sub-family member 10 (C-type lectin)	6.679	5.863	-1.761
16808871	CEP152	22995	XR_931770	XR_931770	centrosomal protein 152kDa	2.702	1.883	-1.764
17098594	ENG	2022	NM_000118	NM_000118	endoglin	9.056	8.236	-1.765
16745503	BLID	414899	NM_001001786	NM_001001786	BH3-like motif containing, cell death inducer	4.728	3.907	-1.766
16756202	EID3	493861	NM_001008394	NM_001008394	EP300 interacting inhibitor of differentiation 3	5.443	4.622	-1.768
16860183	ZNF493	284443	NM_001076678	NM_001076678	zinc finger protein 493	2.394	1.571	-1.769
16979330	LINC01061	401149	NR_037596	NR_037596	long intergenic non-protein coding RNA 1061	5.120	4.296	-1.771
16880168	RPL23AP32	56969	NR_002229	NR_002229	ribosomal protein L23a pseudogene 32	3.072	2.246	-1.772
16869769	NOTCH3	4854	NM_000435	NM_000435	notch 3	6.438	5.612	-1.773
17099076	PTGES	9536	NM_004878	NM_004878	prostaglandin E synthase	5.895	5.066	-1.777
16768183	MKRN9P	400058	NR_033410	NR_033410	makorin ring finger protein 9, pseudogene	3.335	2.503	-1.780
16806870	SLC12A6	9990	NM_001042494	NM_001042494	solute carrier family 12 (potassium/chloride transporter), member 6	6.670	5.837	-1.781
16711258	LOC101927880	101927880	XR_930569	XR_930569	uncharacterized LOC101927880	2.984	2.151	-1.781
17077191	LOC100507516	100507516	XR_928879	XR_928879	uncharacterized LOC100507516	4.362	3.527	-1.783
16782811	LTB4R2	56413	ENST00000528054	ENST00000528054	leukotriene B4 receptor 2	4.002	3.167	-1.784
16847343	WFDC21P	645638	NR_030732	NR_030732	WAP four-disulfide core domain 21, pseudogene	3.593	2.756	-1.786
17055937	OSBPL3	26031	NM_015550	NM_015550	oxysterol binding protein-like 3	7.839	7.001	-1.788
17102951	CHST7	56548	NM_019886	NM_019886	carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 7	5.620	4.780	-1.789
16788669	SNORD114-17	767595	NR_003210	NR_003210	small nucleolar RNA, C/D box 114-17	5.965	5.124	-1.791
17095887	ASPN	54829	NM_001193335	NM_001193335	asporin	2.599	1.753	-1.798
16813974	TM2D3	80213	NM_001307960	NM_001307960	TM2 domain containing 3	4.632	3.784	-1.799
16788673	SNORD114-19	767597	NR_003212	NR_003212	small nucleolar RNA, C/D box 114-19	2.755	1.907	-1.799
16773165	TNFRSF19	55504	NM_001204458	NM_001204458	tumor necrosis factor receptor superfamily, member 19	4.334	3.486	-1.799
16852564	LOC100505549	100505549	NM_001242804	NM_001242804	uncharacterized LOC100505549	2.726	1.876	-1.802
16975890	OCIAD2	132299	NM_001014446	NM_001014446	OCIA domain containing 2	5.267	4.416	-1.804
16706180	PLAU	5328	NM_001145031	NM_001145031	plasminogen activator, urokinase	7.345	6.491	-1.806
16677898	LOC100287497	100287497	XR_159115	XR_159115	uncharacterized LOC100287497	2.213	1.359	-1.807
16963220	SDHAP1	255812	NR_003264	NR_003264	succinate dehydrogenase complex subunit A, flavoprotein pseudogene 1	5.095	4.240	-1.808
17056973	LOC101928716	101928716	XR_242166	XR_242166	uncharacterized LOC101928716	2.751	1.897	-1.808
16768265	LOC105369884	105369884	XR_914322	XR_914322	uncharacterized LOC105369884	5.082	4.227	-1.809
17087413	GALNT12	79695	NM_024642	NM_024642	polypeptide N-acetylgalactosaminyltransferase 12	6.436	5.579	-1.811
16698816	PLXNA2	5362	NM_025179	NM_025179	plexin A2	6.349	5.487	-1.817
17066083	PDGFRL	5157	NM_006207	NM_006207	platelet-derived growth factor receptor-like	8.770	7.907	-1.819
16725664	RPLP0P2	113157	NR_002775	NR_002775	ribosomal protein, large, P0 pseudogene 2	4.728	3.864	-1.820

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
16802232	SMAD6	4091	NM_005585	NM_005585	SMAD family member 6	4.528	3.663	-1.821
16976827	CXCL5	6374	NM_002994	NM_002994	chemokine (C-X-C motif) ligand 5	5.536	4.669	-1.824
16824572	GPRC5B	51704	NM_001304771	NM_001304771	G protein-coupled receptor, class C, group 5, member B	7.113	6.245	-1.826
16816897	NPIPB4	440345	OTTHUMT00000402426	OTTHUMT00000402426	nuclear pore complex interacting protein family, member B4	7.234	6.357	-1.838
16996135	LOC102467080	102467080	NR_104658	NR_104658	uncharacterized LOC102467080	4.892	4.009	-1.844
16986246	LOC105379040	105379040	XR_948485	XR_948485	uncharacterized LOC105379040	4.297	3.409	-1.850
16927955	LOC102724679	102724679	XR_430421	XR_430421	uncharacterized LOC102724679	4.124	3.236	-1.850
16867766	CD70	970	NM_001252	NM_001252	CD70 molecule	5.133	4.233	-1.866
16738393	OR5M1	390168	NM_001004740	NM_001004740	olfactory receptor, family 5, subfamily M, member 1	3.225	2.313	-1.881
16824865	NPIPB5	100132247	OTTHUMT00000403182	OTTHUMT00000403182	nuclear pore complex interacting protein family, member B5	7.272	6.359	-1.884
16738630	LPXN	9404	NM_001143995	NM_001143995	leupaxin	8.541	7.626	-1.885
16949264	LOC344887	344887	NR_033752	NR_033752	NmrA-like family domain containing 1 pseudogene	4.437	3.518	-1.890
17118372	GVQW1	101362076	AK098413	AK098413	GVQW motif containing 1	3.072	2.149	-1.895
17118264	DMTF1	9988	XM_011516731	XM_011516731	cyclin D binding myb-like transcription factor 1	5.982	5.059	-1.896
16960355	TM4SF1	4071	NM_014220	NM_014220	transmembrane 4 L six family member 1	9.917	8.993	-1.897
16877795	EFR3B	22979	NM_014971	NM_014971	EFR3 homolog B	5.245	4.305	-1.919
16701037	GREM2	64388	NM_022469	NM_022469	gremlin 2, DAN family BMP antagonist	4.343	3.403	-1.919
17087610	MURC	347273	NM_001018116	NM_001018116	muscle-related coiled-coil protein	4.542	3.595	-1.928
16672489	LINC01133	100505633	NR_038849	NR_038849	long intergenic non-protein coding RNA 1133	6.677	5.727	-1.932
17118094	LOC100506217	100506217	XM_011514191	XM_011514191	putative POM121-like protein 1-like	3.593	2.636	-1.941
17113195	COL4A6	1288	NM_001287758	NM_001287758	collagen, type IV, alpha 6	5.414	4.455	-1.945
16786060	LOC145474	145474	NR_027046	NR_027046	uncharacterized LOC145474	2.957	1.986	-1.960
16826140	ANKRD26P1	124149	NR_026556	NR_026556	ankyrin repeat domain 26 pseudogene 1	4.676	3.700	-1.967
16747158	NTF3	4908	NM_001102654	NM_001102654	neurotrophin 3	4.375	3.387	-1.983
17115913	CD99P1	401577	ENST00000414513	ENST00000414513	CD99 molecule pseudogene 1	4.040	3.050	-1.986
17062491	GPR37	2861	NM_005302	NM_005302	G protein-coupled receptor 37 (endothelin receptor type B-like)	5.139	4.148	-1.987
16726880	NEAT1	283131	NR_131012	NR_131012	nuclear paraspeckle assembly transcript 1 (non-protein coding)	8.774	7.783	-1.987
16996969	GUSBP3	653188	AK124130	AK124130	glucuronidase, beta pseudogene 3	3.053	2.057	-1.995
16911108	SMOX	54498	NM_001270691	NM_001270691	spermine oxidase	5.928	4.919	-2.013
17019820	PTCHD4	442213	NM_001013732	NM_001013732	patched domain containing 4	4.046	3.033	-2.018
17047918	CROT	54677	NM_001143935	NM_001143935	carnitine O-octanoyltransferase	6.470	5.456	-2.020
16916901	RASSF2	9770	NM_014737	NM_014737	Ras association (RalGDS/AF-6) domain family member 2	7.495	6.476	-2.026
16970435	SPRY1	10252	NM_001258038	NM_001258038	sprouty RTK signaling antagonist 1	5.495	4.465	-2.043
16777441	ANKRD20A19P	400110	NR_073430	NR_073430	ankyrin repeat domain 20 family, member A19, pseudogene	4.353	3.308	-2.064
17058142	ZNF117	51351	NM_015852	NM_015852	zinc finger protein 117	3.211	2.164	-2.066
16788649	SNORD114-7	767583	NR_003199	NR_003199	small nucleolar RNA, C/D box 114-7	4.155	3.099	-2.078
17075168	CSGALNACT1	55790	NM_001130518	NM_001130518	chondroitin sulfate N-acetylgalactosaminyltransferase 1	4.303	3.240	-2.088
16739479	LRRN4CL	221091	NM_203422	NM_203422	LRRN4 C-terminal like	5.616	4.540	-2.108
16749782	FGD4	121512	NM_001304480	NM_001304480	FYVE, RhoGEF and PH domain containing 4	6.136	5.060	-2.108
16698923	IRF6	3664	NM_001206696	NM_001206696	interferon regulatory factor 6	4.827	3.745	-2.117
16813206	ANPEP	290	NM_001150	NM_001150	alanyl (membrane) aminopeptidase	5.542	4.439	-2.148
16986913	VCAN	1462	NM_001126336	NM_001126336	versican	8.384	7.278	-2.153
17052348	TMEM178B	100507421	NM_001195278	NM_001195278	transmembrane protein 178B	5.582	4.475	-2.155
16902933	LYPD1	116372	NM_001077427	NM_001077427	LY6/PLAUR domain containing 1	8.656	7.546	-2.157
16929442	TIMP3	7078	NM_000362	NM_000362	TIMP metallopeptidase inhibitor 3	8.573	7.460	-2.163

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
16906749	HECW2	57520	NM_001304840	NM_001304840	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 2	6.011	4.898	-2.164
16955939	LRIG1	26018	NM_015541	NM_015541	leucine-rich repeats and immunoglobulin-like domains 1	7.575	6.450	-2.181
16688332	DIRAS3	9077	NM_004675	NM_004675	DIRAS family, GTP-binding RAS-like 3	5.176	4.050	-2.183
16805161	SLCO3A1	28232	NM_001145044	NM_001145044	solute carrier organic anion transporter family, member 3A1	4.766	3.637	-2.187
17071640	CTHRC1	115908	NM_001256099	NM_001256099	collagen triple helix repeat containing 1	5.880	4.751	-2.187
16754134	LGR5	8549	NM_001277226	NM_001277226	leucine-rich repeat containing G protein-coupled receptor 5	4.255	3.118	-2.200
16983611	LINC01021	643401	NR_038848	NR_038848	long intergenic non-protein coding RNA 1021	4.539	3.398	-2.205
17112918	BEX1	55859	NM_018476	NM_018476	brain expressed X-linked 1	3.200	2.054	-2.213
16979917	SLC7A11	23657	NM_014331	NM_014331	carrier family 7 (anionic amino acid transporter light chain, xc- system), mer	8.745	7.598	-2.214
16874508	SNAR-B2	100170217	NR_024230	NR_024230	small ILF3/NF90-associated RNA B2	6.807	5.655	-2.222
16874510	SNAR-B2	100170217	NR_024230	NR_024230	small ILF3/NF90-associated RNA B2	6.807	5.655	-2.222
16788570	SNORD113-1	767561	NR_003229	NR_003229	small nucleolar RNA, C/D box 113-1	5.417	4.240	-2.261
16806817	LOC101929988	101929988	XR_253363	XR_253363	uncharacterized LOC101929988	4.343	3.161	-2.269
16738628	OR5B21	219968	NM_001005218	NM_001005218	olfactory receptor, family 5, subfamily B, member 21	3.787	2.587	-2.296
17077974	CPA6	57094	NM_020361	NM_020361	carboxypeptidase A6	4.170	2.970	-2.298
16780893	FAM155A-IT1	100874375	NR_046848	NR_046848	FAM155A intronic transcript 1	4.886	3.682	-2.303
17117684	NPIPB11	728888	XM_011546005	XM_011546005	nuclear pore complex interacting protein family, member B11	7.149	5.937	-2.316
17025523	PDE10A	10846	NM_001130690	NM_001130690	phosphodiesterase 10A	6.054	4.841	-2.318
16887062	SCN2A	6326	NM_001040142	NM_001040142	sodium channel, voltage gated, type II alpha subunit	5.628	4.393	-2.353
16852025	FHOD3	80206	NM_001281739	NM_001281739	formin homology 2 domain containing 3	5.416	4.170	-2.371
16668785	WNT2B	7482	NM_001291880	NM_001291880	wingless-type MMTV integration site family, member 2B	5.954	4.707	-2.373
16904514	SCN3A	6328	NM_001081676	NM_001081676	sodium channel, voltage gated, type III alpha subunit	8.141	6.880	-2.398
16989544	SMAD5	4090	ENST00000513418	ENST00000513418	SMAD family member 5	3.255	1.986	-2.411
16970536	HSPA4L	22824	NM_014278	NM_014278	heat shock 70kDa protein 4-like	5.088	3.805	-2.433
16785938	TTC9	23508	NM_015351	NM_015351	tetratricopeptide repeat domain 9	4.220	2.922	-2.459
16705701	H2AFY2	55506	NM_018649	NM_018649	H2A histone family, member Y2	5.221	3.916	-2.472
16920156	PTGIS	5740	NM_000961	NM_000961	prostaglandin I2 (prostacyclin) synthase	6.730	5.409	-2.497
17061099	RASA4B	100271927	ENST00000488284	ENST00000488284	RAS p21 protein activator 4B	3.826	2.490	-2.523
16675197	PLA2G4A	5321	NM_001311193	NM_001311193	phospholipase A2, group IVA (cytosolic, calcium-dependent)	7.181	5.838	-2.536
16909319	DNER	92737	NM_139072	NM_139072	delta/notch like EGF repeat containing	4.144	2.765	-2.600
16965252	BST1	683	NM_004334	NM_004334	bone marrow stromal cell antigen 1	6.086	4.688	-2.636
16995035	LOC105374715	105374715	XR_925901	XR_925901	uncharacterized LOC105374715	4.505	3.051	-2.740
16740725	CD248	57124	NM_020404	NM_020404	CD248 molecule, endosialin	6.846	5.386	-2.751
16883107	ANKRD36	375248	NM_001164315	NM_001164315	ankyrin repeat domain 36	4.498	3.028	-2.769
16723058	FIBIN	387758	NM_203371	NM_203371	fin bud initiation factor homolog (zebrafish)	6.905	5.359	-2.920
17108816	MXRA5	25878	NM_015419	NM_015419	matrix-remodelling associated 5	5.769	4.205	-2.958
16854466	DSC3	1825	NM_001941	NM_001941	desmocollin 3	7.336	5.768	-2.965
16767851	E2F7	144455	NM_203394	NM_203394	E2F transcription factor 7	7.555	5.932	-3.080
16763032	PKP2	5318	NM_001005242	NM_001005242	plakophilin 2	6.557	4.891	-3.173
16755712	ANO4	121601	NM_001286615	NM_001286615	anoctamin 4	4.892	3.158	-3.327
16874512	SNAR-D	100170227	NR_024243	NR_024243	small ILF3/NF90-associated RNA D	5.639	3.891	-3.359
16806097	NDN	4692	NM_002487	NM_002487	necdin, MAGE family member	4.448	2.604	-3.591
17087374	FOXE1	2304	NM_004473	NM_004473	forkhead box E1	6.265	4.395	-3.656
16672214	PEAR1	375033	NM_001080471	NM_001080471	platelet endothelial aggregation receptor 1	5.205	3.335	-3.657
16852179	SLC14A1	6563	NM_001128588	NM_001128588	solute carrier family 14 (urea transporter), member 1 (Kidd blood group)	9.505	7.629	-3.672

(*cont'd*) Table 1. DEGs between 3D and 2D cultured hMSCs

ProbeID	Gene_Symbol	Gene_ID	Gene Accession	mRNA Accession	Gene Description	Expression level		Fold change
						log2(2Dcell)	log2(3Dcell)	3Dcell/2Dcell
16944410	PLA1A	51365	NM_001206960	NM_001206960	phospholipase A1 member A	8.428	6.550	-3.675
16778241	POSTN	10631	NM_001135934	NM_001135934	periostin, osteoblast specific factor	6.474	4.592	-3.685
16873645	SNAR-E	100170220	NR_024258	NR_024258	small ILF3/NF90-associated RNA E	7.409	5.523	-3.697
17085760	MAMDC2	256691	NM_153267	NM_153267	MAM domain containing 2	8.992	7.095	-3.724
16728287	ANO1	55107	NM_018043	NM_018043	anoctamin 1, calcium activated chloride channel	7.551	5.588	-3.900
17072135	NOV	4856	NM_002514	NM_002514	nephroblastoma overexpressed	6.401	4.417	-3.956
16831550	FAM106CP	100129396	NR_026810	NR_026810	family with sequence similarity 106, member C, pseudogene	4.083	2.037	-4.129
16774303	RGCC	28984	NM_014059	NM_014059	regulator of cell cycle	4.564	2.507	-4.161
16978779	DKK2	27123	NM_014421	NM_014421	dickkopf WNT signaling pathway inhibitor 2	8.557	6.488	-4.195
17111725	EDA2R	60401	NM_001199687	NM_001199687	ectodysplasin A2 receptor	7.635	5.520	-4.333
17069173	LINC01602	100505477	NR_130934	NR_130934	long intergenic non-protein coding RNA 1602	5.870	3.673	-4.584
17013851	MYCT1	80177	NM_025107	NM_025107	myc target 1	5.417	3.179	-4.717
16855358	CCDC68	80323	NM_001143829	NM_001143829	coiled-coil domain containing 68	5.815	3.557	-4.785
16733319	ST3GAL4	6484	XM_011542962	XM_011542962	ST3 beta-galactoside alpha-2,3-sialyltransferase 4	5.641	3.352	-4.887
16696425	TNFSF4	7292	NM_001297562	NM_001297562	tumor necrosis factor (ligand) superfamily, member 4	7.116	4.688	-5.379
16787831	PPP4R4	57718	NM_020958	NM_020958	protein phosphatase 4, regulatory subunit 4	5.655	3.106	-5.854
17059388	KIAA1324L	222223	NM_001142749	NM_001142749	KIAA1324-like	8.832	6.226	-6.088
17078592	FABP4	2167	NM_001442	NM_001442	fatty acid binding protein 4, adipocyte	6.431	3.560	-7.316
17084130	TEK	7010	NM_000459	NM_000459	TEK tyrosine kinase, endothelial	6.225	2.983	-9.462

Table 2. Biological process enrichment of DEGs in 3D cultured hMSCs

Up-regulated					
Gene Set Name	**No. of Genes in Gene Set**	**Description**	**No. of Genes in Overlap (k)**	**p-value**	**FDR q-value**
LOCOMOTION	1943	Self-propelled movement of a cell or organism from one location to another	36	3.90E-08	7.16E-05
POSITIVE REGULATION OF MULTICELLULAR ORGANISMAL PROCESS	1795	Any process that activates or increases the frequency, rate or extent of an organismal process, any of the proces	33	1.82E-07	2.68E-04
CELL MOTILITY	1719	Any process involved in the controlled self-propelled movement of a cell that results in translocation of the cell	31	6.69E-07	4.91E-04
EXTRACELLULAR STRUCTURE ORGANIZATION	421	A process that is carried out at the cellular level which results in the assembly, arrangement of constituent parts,	14	1.22E-06	7.07E-04
LEUKOCYTE MIGRATION	488	The movement of a leukocyte within or between different tissues and organs of the body	15	1.35E-06	7.07E-04
MEMBRANE TO MEMBRANE DOCKING	5	The initial attachment of a membrane to a target membrane, mediated by proteins protruding from the two mem	3	2.98E-06	1.22E-03
NEUROGENESIS	1599	Generation of cells within the nervous system	28	4.22E-06	1.52E-03
REGULATION OF PEPTIDE SECRETION	477	Any process that modulates the frequency, rate, or extent of peptide secretion	14	5.16E-06	1.52E-03
TRANSMEMBRANE TRANSPORT	1587	The process in which a solute is transported across a lipid bilayer, from one side of a membrane to the other	27	1.06E-05	2.78E-03
PEPTIDE SECRETION	597	The controlled release of a peptide from a cell or a tissue	15	1.51E-05	3.58E-03
REGULATION OF SECRETION	788	Any process that modulates the frequency, rate or extent of the controlled release of a substance from a cell or	17	2.87E-05	5.70E-03
ENDOCYTOSIS	642	A vesicle-mediated transport process in which cells take up external materials or membrane constituents by the	15	3.48E-05	6.54E-03
CELL PROJECTION ORGANIZATION	1512	A process that is carried out at the cellular level which results in the assembly, arrangement of constituent parts,	25	3.65E-05	6.54E-03
NEURON DIFFERENTIATION	1348	The process in which a relatively unspecialized cell acquires specialized features of a neuron	23	4.71E-05	7.75E-03
REGULATION OF CELLULAR COMPONENT MOVEMENT	1077	Any process that modulates the frequency, rate or extent of the movement of a cellular component	20	4.75E-05	7.75E-03
CELL ADHESION MEDIATED BY INTEGRIN	68	The attachment of a cell, either to another cell or to an underlying substrate such as the extracellular matrix, via	5	9.77E-05	1.20E-02
POSITIVE REGULATION OF LOCOMOTION	580	Any process that activates or increases the frequency, rate or extent of locomotion of a cell or organism	13	1.74E-04	1.69E-02
ACTIN FILAMENT BASED PROCESS	744	Any cellular process that depends upon or alters the actin cytoskeleton, that part of the cytoskeleton comprising	15	1.77E-04	1.69E-02
NEGATIVE REGULATION OF SECRETION	230	Any process that stops, prevents, or reduces the frequency, rate or extent of the controlled release of a substanc	8	1.79E-04	1.69E-02
REGULATION OF CELL PROJECTION ORGANIZATION	667	Any process that modulates the frequency, rate or extent of a process involved in the formation, arrangement of	14	1.93E-04	1.80E-02
IMPORT INTO CELL	782	The directed movement of some substance from outside of a cell into the cytoplasmic compartment. This may o	15	3.01E-04	2.33E-02

(*cont'd*) Table 2. Biological process enrichment of DEGs in 3D cultured hMSCs

Down-regulated					
Gene Set Name	**No. of Genes in Gene Set**	**Description**	**No. of Genes in Overlap (k)**	**p-value**	**FDR q-value**
REGULATION OF CELL DIFFERENTIATION	1863	Any process that modulates the frequency, rate or extent of cell differentiation, the process in which relatively u	44	8.20E-09	1.97E-05
MESENCHYMAL CELL DIFFERENTIATION	217	The process in which a relatively unspecialized cell acquires specialized features of a mesenchymal cell. A mese	14	1.48E-08	2.18E-05
OSSIFICATION	385	The formation of bone or of a bony substance, or the conversion of fibrous tissue or of cartilage into bone or a	17	1.15E-07	9.41E-05
LOCOMOTION	1943	Self-propelled movement of a cell or organism from one location to another	40	1.41E-06	5.47E-04
REGULATION OF OSSIFICATION	194	Any process that modulates the frequency, rate or extent of ossification, the formation of bone or of a bony sub	11	1.88E-06	6.82E-04
TAXIS	636	The directed movement of a motile cell or organism in response to an external stimulus	20	1.95E-06	6.82E-04
CELL MOTILITY	1719	Any process involved in the controlled self-propelled movement of a cell that results in translocation of the cell	36	3.38E-06	9.93E-04
POSITIVE REGULATION OF MULTICELLULAR ORGANISMAL PROCESS	1795	Any process that activates or increases the frequency, rate or extent of an organismal process, any of the proces	37	3.51E-06	9.93E-04
EPITHELIAL TO MESENCHYMAL TRANSITION	139	A transition where an epithelial cell loses apical/basolateral polarity, severs intercellular adhesive junctions, degr	9	5.56E-06	1.36E-03
POSITIVE REGULATION OF CELL DIFFERENTIATION	985	Any process that activates or increases the frequency, rate or extent of cell differentiation	24	1.46E-05	2.61E-03
REGULATION OF OSTEOBLAST DIFFERENTIATION	122	Any process that modulates the frequency, rate or extent of osteoblast differentiation	8	1.69E-05	2.95E-03
NEURAL CREST CELL DIFFERENTIATION	89	The process in which a relatively unspecialized cell acquires specialized features of a neural crest cell	7	1.76E-05	3.01E-03
POSITIVE REGULATION OF EPITHELIAL TO MESENCHYMAL TRANSITION	50	Any process that increases the rate, frequency, or extent of epithelial to mesenchymal transition. Epithelial to m	5	9.14E-05	9.78E-03
EPITHELIAL CELL DIFFERENTIATION	767	The process in which a relatively unspecialized cell acquires specialized features of an epithelial cell, any of the	19	9.18E-05	9.78E-03
REGULATION OF NEURON DIFFERENTIATION	650	Any process that modulates the frequency, rate or extent of neuron differentiation	17	1.13E-04	1.13E-02
POSITIVE REGULATION OF OSSIFICATION	86	Any process that activates or increases the frequency, rate or extent of ossification, the formation of bone or of	6	1.38E-04	1.35E-02
EXTRACELLULAR STRUCTURE ORGANIZATION	421	A process that is carried out at the cellular level which results in the assembly, arrangement of constituent parts,	13	1.46E-04	1.39E-02
APOPTOTIC PROCESS	1980	A programmed cell death process which begins when a cell receives an internal (e.g. DNA damage) or external	35	1.48E-04	1.39E-02
REGULATION OF CELLULAR COMPONENT MOVEMENT	1077	Any process that modulates the frequency, rate or extent of the movement of a cellular component	23	1.58E-04	1.45E-02
OSTEOBLAST DIFFERENTIATION	214	The process whereby a relatively unspecialized cell acquires the specialized features of an osteoblast, a mesoder	9	1.64E-04	1.49E-02
NEURAL CREST CELL MIGRATION	57	The characteristic movement of cells from the dorsal ridge of the neural tube to a variety of locations in a verteb	5	1.72E-04	1.50E-02
CARDIAC EPITHELIAL TO MESENCHYMAL TRANSITION	32	A transition where a cardiac epithelial cell loses apical/basolateral polarity, severs intercellular adhesive junction	4	1.98E-04	1.61E-02

Table 3. Literature mining of DEGs in 3D cultured hMSCs

	No. of literature related with stemness					No. of literature related with cell mobility				No. of literature related with extracellular vesicle		
PubMatrix	stemness	pluripotency	dedifferentiation	stem cell	PubMatrix	Cell motility	Migration	Wound healing	PubMatrix	extracellular vesicle	exosome
WNT2	2	2	1	64	ITGA6	36	47	7	UBL3	2	2
CCN3	2	0	0	28	IGFBP5	25	39	8	SLC26A4	2	2
KITLG	0	2	1	98	CD200	17	27	10	PLAU	2	2
TNFSF4	0	0	0	11	TRPV2	18	21	6	ALDH1A1	1	3
RGCC	1	1	0	4	ABL2	21	20	4	ATP2B1	2	1
PLXNA2	0	0	0	9	SLC7A5	15	18	4	EZR	2	1
GCNT2	0	1	0	4	ANXA3	10	13	4	SCIN	1	1
PNP	0	0	0	41	ANKRD1	6	10	7	LOXL4	1	1
PKP2	0	2	0	18	MYH11	16	13	2	IRF6	1	1
OMG	0	0	0	3	EZR	15	14	1	CFH	1	1
PF4	0	0	1	89	GHSR	16	13	1	ADIRF	1	1
GDF7	0	0	0	16	ESAM	12	11	1	MME	2	0
NTF3	0	0	0	13	ITGA11	6	8	2	CDH13	0	0
NTF4	0	0	0	1	TNIK	5	8	1	STK26	0	0
SEMA3C	0	1	0	14	TNFRSF21	5	5	1	MYH11	0	0
CTHRC1	0	0	4	13	GALR2	3	3	1	QPRT	0	0
PKDCC	0	0	0	1	TSPAN2	4	2	1	MEST	0	0
SEMA6D	0	0	0	3	TNFRSF10D	2	2	1	ANXA3	1	0
SEMA3D	0	0	0	4	EFNA5	1	3	1	TUBB2A	0	0
CD109	1	0	0	24	CDH13	7	18	0	CREG1	0	0
PSMD3	0	0	0	1	ITGBL1	5	4	0	SERPINB1	0	0
RASSF2	0	0	1	2	BMPER	6	10	0	RPS2	0	1
MAP3K5	0	0	0	21	IGLV3-27	0	0	0	CLIC3	0	0
PRAMEF11	0	0	0	0	IGLV2-11	0	0	0	LYPLA2	0	0
H2AFY2	0	1	0	3	STK26	1	1	0	GP5	0	0
GPRC5B	0	0	1	3	C5orf30	0	2	2	BST1	0	0
HIST1H3F	0	0	0	0	FAM110C	1	1	0	MXRA5	0	0
LPAR3	0	0	0	3	SH3RF2	0	1	0	IGHD	0	0
HECW2	0	1	0	4	FAM83H	4	8	0	NAA16	0	0
ADIRF	0	0	0	0	JMY	15	10	0	TNXB	0	0
LRRC17	0	0	0	3	MYPN	1	0	0	SEMA3C	0	0
SPRY1	3	2	0	42	NCAM2	0	3	0	CYBRD1	0	0
KBTBD8	0	1	0	3	NEDD4L	5	10	0	ANPEP	0	0
SLC7A11	2	0	0	32	LYPLA2	1	1	0	CD70	0	0
CLDN1	3	0	7	22	ENC1	3	3	0	NID2	0	0
DSC3	0	0	0	5	SCIN	3	6	0	UBE2N	0	1
E2F7	1	2	0	6	EPB41	0	3	2	FAT2	0	0
PDE2A	0	1	0	4					CD248	0	0
IRF6	2	0	0	14					H2AFY2	0	0
TJP2	0	0	0	8					SCN11A	0	0
LCE1A	0	0	0	0					PGA3	0	0
CEP152	0	1	0	2					PNP	2	0
									PSMD3	0	1
									ANGPTL2	1	0

3. Priming effect of 3D culture of hMSCs on stemness, migration ability, and EV production

To verify the putative priming effects on hMSCs of Cellhesion VP, I first evaluated the expression levels of stemness markers such as POU Class 5 Homeobox 1 (*POU5F1*; *OCT4*), Nanog Homeobox (*NANOG*), and stage-specific embryonic antigen-4 (SSEA4). Compared to 2D cells (day 0), 3D cells showed levels of *OCT4* mRNA that were 1.5 times higher on day 6 and 3.8 times higher on day 9 ($p < 0.01$). Likewise, *NANOG* mRNA showed a 4.9-fold increase on day 6 and 7.1-fold increase on day 9 relative to 2D cells ($p < 0.05$) (Fig. 3A). Fluorescence-activated cell sorting (FACS) analyses of stemness marker expression was consistent with the results from mRNA. The OCT4-positive proportion of the cell population was 83.9% in 2D cells and 95.2% in the retrieved 3D cells. NANOG-positive cells made up 28.3% of 2D cells and 73.2% of 3D cells. In addition, SSEA4-positive cells accounted for 5.09% of 2D cells and 9.36% of 3D cells (Fig. 3B). These results demonstrate that 3D culture using Cellhesion VP allowed hMSCs to gain enhanced stemness, and this change was persistent even after retrieval under 2D culture conditions

Next, I evaluated the migration ability of the retrieved cells compared to 2D cells under serum starvation conditions. The retrieved cells from 3D culture migrated faster than 2D cells beginning 6 h after platelet-derived growth factor (PDGF) treatment, and filled the wound completely at 30 h. By contrast, 2D cells showed moderate migration and reached complete healing at 48 h. Cytochalasin D (CD) at 0.2 μM prevented cell migration until the end of the assay (Fig. 4A). The area recovered was 81% for 2D cells and 0.08% for CD-treated retrieved cells throughout the assay period, indicating significantly slowed migration ($p < 0.01$, Fig. 4B). Representative images of cell migration

taken at 0, 12, 24, and 36 h of culture of each cell type are displayed in Figure 4C. As shown in the figure, 3D culture using Cellhesion VP appeared to cause enhanced cell motility of hMSCs, and this improvement was persistent even after retrieval under conventional 2D culture conditions.

To explore the enhanced capacity of the 3D cells for EV-related functions, I evaluated the primary characteristics of EVs secreted from them, such as the size distribution, quantity, and protein content of EVs compared to those derived from 2D cells.

Before that, I first checked the protein of media and EV-free FBS to be free from the problem of contamination during exosome preparations. Proteins of EVs derived from media that grew 2D cells, only the media and EV-free FBS without cells in the same conditions were analyzed. Western blot analysis showed that canonical EV marker proteins including Tumor susceptibility 101 (TSG101), CD81, and CD9 were only expressed in 2D EVs (Fig. 5A).

The average size of EVs from the 3D cells was 174.9 ± 14.6 nm and the size of those from 2D-grown cells was 157.7 ± 9.3 nm, and no significant differences were found between these values (Fig. 6A). The small peak observed at about 30 nm was confirmed to be a polysaccharide derived from EV isolation reagent. The NTA assay demonstrated that $3.73 \pm 0.25 \times 10^9$ EVs were recovered from 3D cells. In comparison, $2.37 \pm 1.00 \times 10^9$ EVs were obtained from the same number and culture period of 2D cells. Therefore, 3D cells released 1.6 times more amount of EVs than 2D cells with a significant difference ($p < 0.05$, Fig. 6B). To validate the isolated EVs and their purity, TEM, Western blot analysis as well as ELISA were applied. TEM data revealed that all 2D and 3D cultured hMSCs released a homogenous mixture of cup-shaped, rounded EVs with diameters around 100 nm (Fig. 6C). Western blot

analysis showed that canonical EV marker proteins including TSG101, CD81, and CD9 were expressed in 2D and 3D cultured hMSCs as well as EVs from them; however, Calnexin, an integral protein of the endoplasmic marker protein, was only expressed in 2D or 3D cultured hMSCs, not in EVs (Fig. 6D, Fig. 7). ELISA confirmed that 2D and 3D EVs had substantial quantities of CD63 and CD81, and that 3D EVs had 3.4 and 4.6 times greater quantities of CD63 and CD81, respectively ($p < 0.001$, Fig. 6E).

Then I analyzed the protein contents of the EVs, and identified 2,158 proteins both in 2D and 3D EVs. Among them, 567 proteins were significantly up-regulated and 141 proteins were down-regulated in 3D EVs (fold change $\geq |2|$, $p < 0.05$, Table 4, Fig. 6F). When I compared the EV proteins with the top 100 marker proteins from ExoCarta and Vesiclepedia, I found that of the 100 markers provided in the two databases, more than 74 proteins were observed in the EVs from 2D and 3D cultured hMSCs, those included CD9, CD63, CD81, Syntenin-1, and Flotillin-1 (Fig. 6G). The 567 proteins up-regulated in 3D EVs compared with 2D EVs were subjected to functional enrichment and membership search with Reactome gene sets. Pathway enrichment revealed that the up-regulated proteins in 3D EVs were enriched in vesicle-mediated transport ($p = 1.41 \times 10^{-29}$), metabolism of carbohydrate ($p = 9.08 \times 10^{-19}$), cellular response to stress ($p = 1.09 \times 10^{-15}$), hemostasis ($p = 7.80 \times 10^{-13}$), response to mycobacterium tuberculosis to phagocytosis ($p = 1.01 \times 10^{-8}$), disease of metabolism ($p = 3.40 \times 10^{-8}$), and Golgi associated vesicle biogenesis ($p = 1.66 \times 10^{-7}$) (Fig. 6H). These results demonstrate that Cellhesion VP in the 3D culture of hMSC affects the biogenesis and secretion of EVs as well as their protein composition.

Cell proliferation, production of EV, and stemness gene expression were compared using WJ-hMSCs and ADSCs to find out what difference the 3D culture of cells using Cellhesion VP shows from other 3D culture method. For this purpose, I performed cell culture using Cellhesion VP and U-bottom plate (Fig. 8). When 3D culture of WJ-hMSCs using U-bottom plate, it was confirmed that the size of the WJ-hMSC sphere decreased as the culture period extended. In addition, EV production using Cellhesion VP increased by about 17% compared to U-bottom ($p < 0.05$), and the mRNA levels of *OCT4*, *NANOG*, and *SOX2* decreased by 6.3 times, 17 times and 6.4 times, respectively. The inhibitory effect of cell proliferation using U-bottom plate was also confirmed in ADSC culture, and no significant difference was observed between the two 3D culture methods in the mRNA levels of *OCT4* and *SOX2*. Therefore, the 3D culture of hMSCs using Cellhesion VP appears to be advantageous in terms of cell proliferation and EV production, and it was confirmed that the expression levels of stemness genes would be different depending on the type of cell used.

According to global gene expression profiling and functional enrichment analyses, I predicted that enhanced stemness, cell migration ability, and EV production in hMSCs are priming effects of Cellhesion VP-mediated 3D culture. Subsequent biochemical and cellular assays revealed that the 3D cultured hMSCs had significantly increased levels of OCT4, NANOG, and SSEA4 expression, as well as enhanced wound healing ability and EV biogenesis with significantly different protein composition compared to 2D cells. Therefore, Cellhesion VP appears to improve the potential of hMSCs for therapeutic applications.

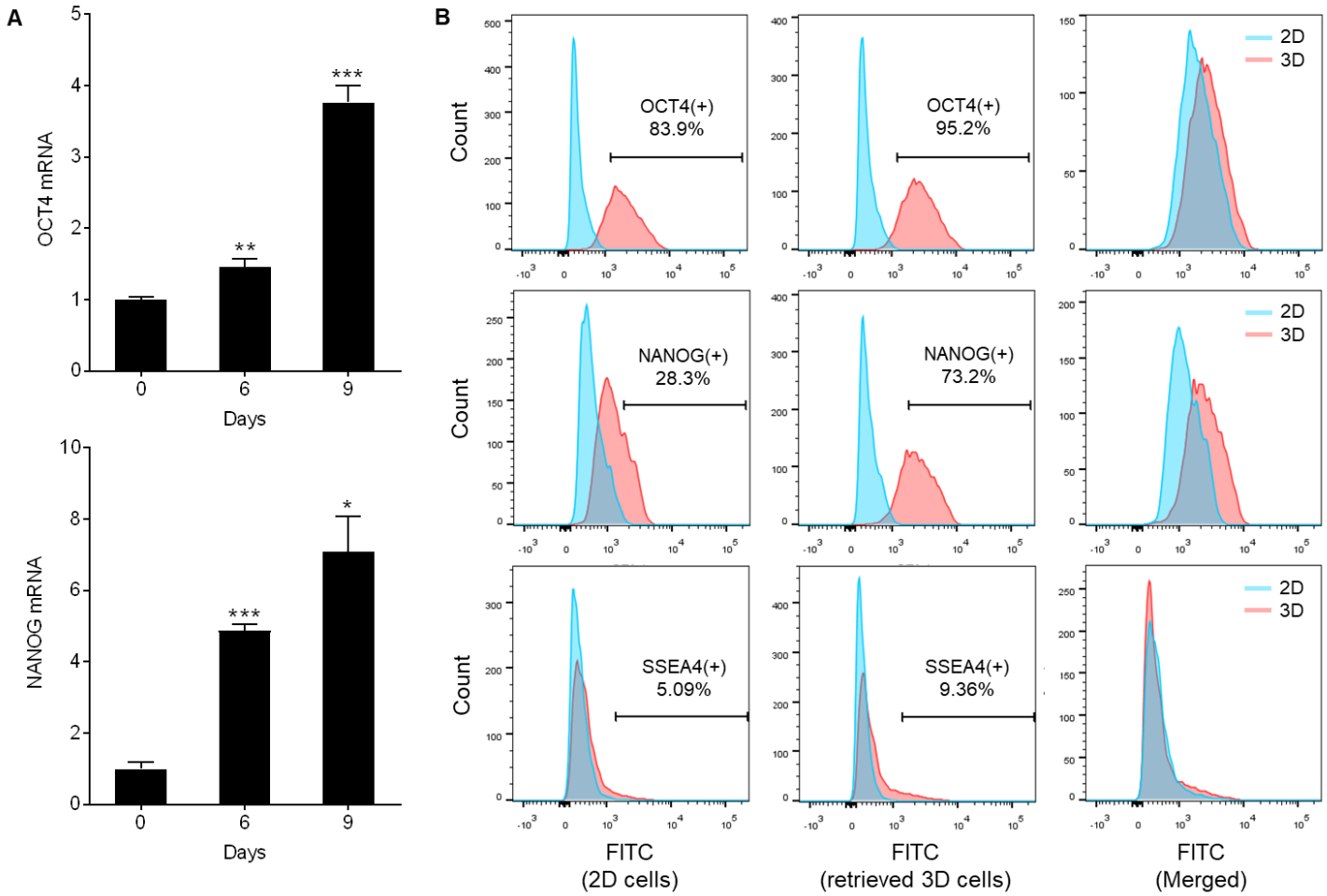
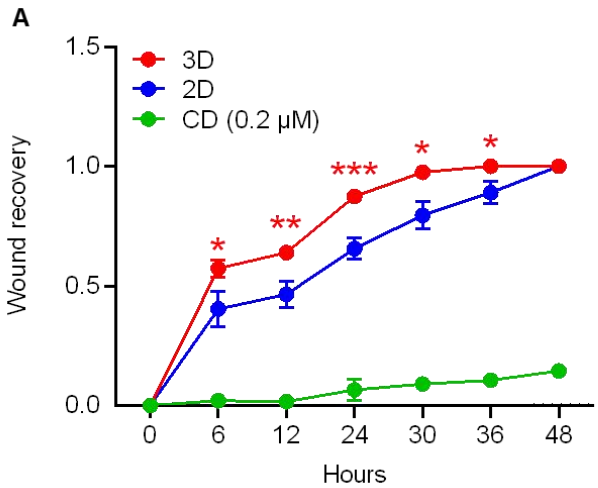
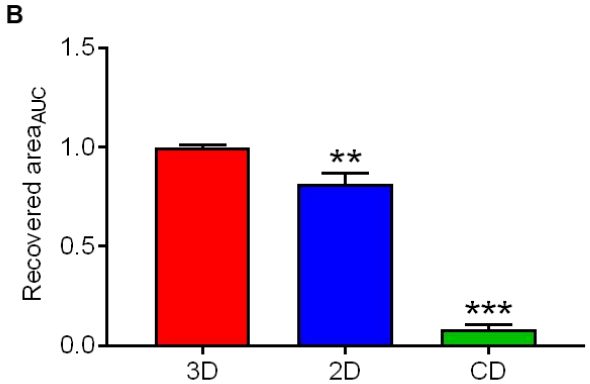

A
OCT4 mRNA
Days
NANOG mRNA
Days
B
Count
OCT4(+)
83.9%
OCT4(+)
95.2%
NANOG(+)
28.3%
NANOG(+)
73.2%
SSEA4(+)
5.09%
SSEA4(+)
9.36%
2D
3D
FITC
(2D cells)
FITC
(retrieved 3D cells)
FITC
(Merged)

Figure 3. Expression levels of stemness-related genes

A. Relative mRNA levels of OCT4 and NANOG during 3D culture. Expression levels were normalized as Day 0 = 1. Bars indicate mean ± SD (n = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. Day 0. **B**. Flow cytometry analysis of stemness marker-positive cell population between 2D and retrieved 3D cells. Left column indicates stemness markers-positive 2D cell population, middle column of the retrieved 3D cells, and right column of merged 2D and retrieved 3D cell population. Percentage of the marker positive cells is depicted under the marker symbol.


A
3D
2D
CD (0.2 μM)
Wound recovery
1.5
1.0
0.5
0.0
0
6
12
24
30
36
48
Hours
*
**
***
*
*


B
Recovered area$_{AUC}$
1.5
1.0
0.5
0.0
**
***
3D
2D
CD

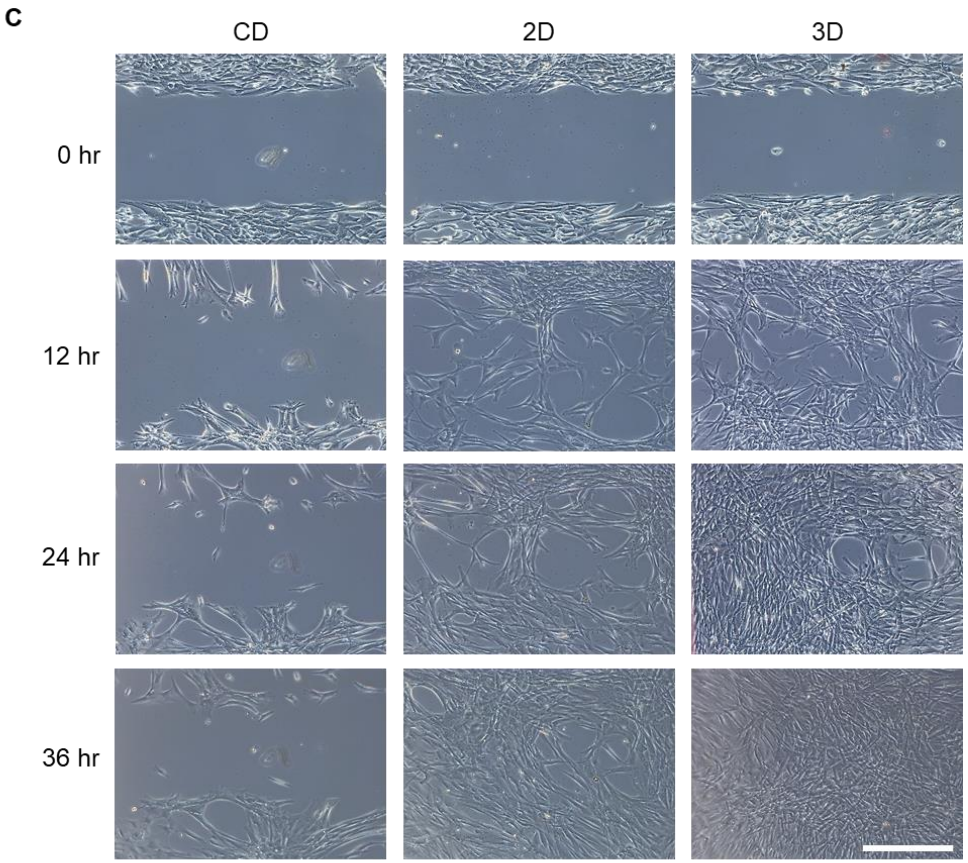

C
CD
2D
3D
0 hr
12 hr
24 hr
36 hr

Figure 4. Cell migration ability between 2D- and 3D-cultured hMSCs

A. Wound recovery of 2D and retrieved 3D cells. Red plots with connecting line indicate retrieved 3D cells. Blue indicates 2D cells. Green indicates cytochalasin (CD) treated 2D cells. Plots indicates mean ± SD (n = 3). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs. 2D. **B**. Area recovered by each type of cell is depicted as area under curve (AUC). Bars indicate mean ± SD (n = 3). **$p < 0.01$ and ***$p < 0.001$ vs. 3D. **C**. Representative images of cell migration up to 36 hrs of culture. Scale bar is 400 μm.

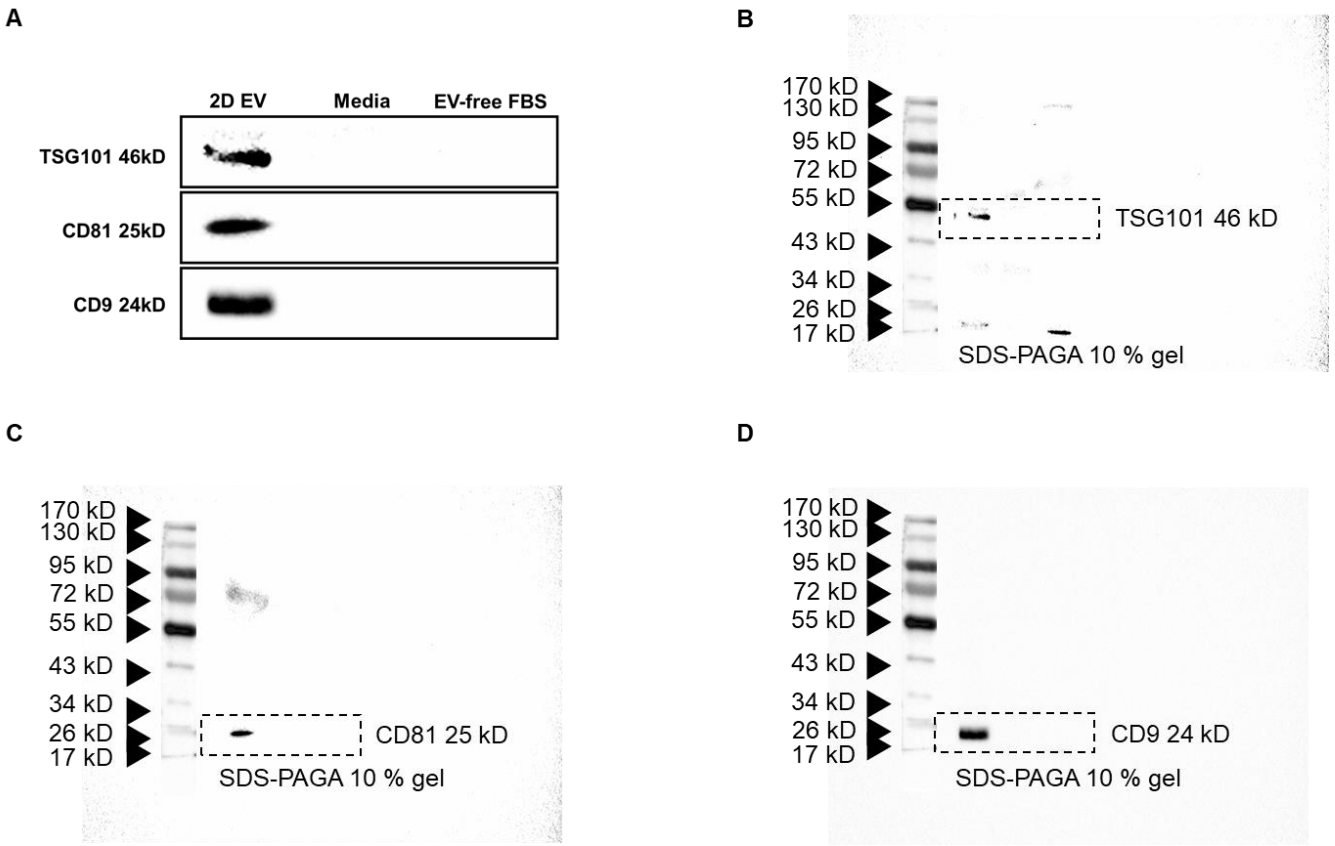


Figure 5. Demonstration of depleted EV contaminants

A. Western blot of EV marker proteins. **B.** TSG101 at 46 kD. **C.** CD81 at 25 kD. **D.** CD9 at 24 kD

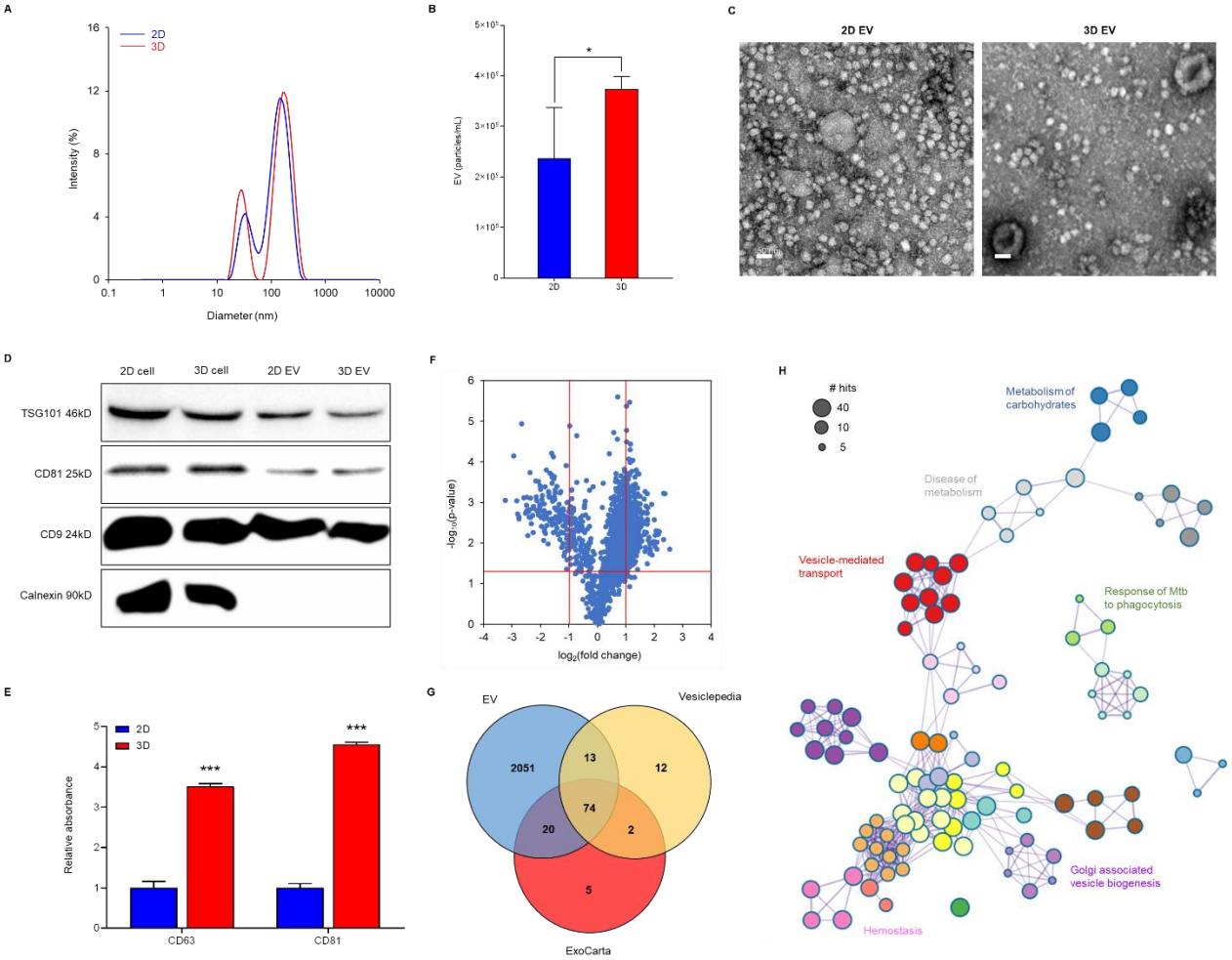

A
2D
3D
Intensity (%)
Diameter (nm)
B
EV (particles/mL)
2D
3D
C
2D EV
3D EV
D
2D cell
3D cell
2D EV
3D EV
TSG101 46kD
CD81 25kD
CD9 24kD
Calnexin 90kD
E
2D
3D
Relative absorbance
CD63
CD81
F
log2(fold change)
G
EV
Vesiclepedia
ExoCarta
2051
13
12
74
20
2
5
H
hits
40
10
5
Metabolism of carbohydrates
Disease of metabolism
Vesicle-mediated transport
Response of Mtb to phagocytosis
Golgi associated vesicle biogenesis
Hemostasis

Figure 6. Characterization of 2D and 3D EVs

A. The size distribution of EVs. The blue line indicates the size distribution of 2D cell-derived EVs and red indicates that of 3D cell-derived EVs (n = 4). **B**. The particle concentration of EVs. Blue bar indicates the particle concentration of 2D cell-derived EVs and red indicates that of 3D cell-derived EVs. Bars indicate mean ± SD (n = 3). *$p < 0.05$ vs. 2D. **C**. TEM images of 2D and 3D EVs. Scale bar = 50 nm. **D**. Western blot of EV marker proteins. **E**. Quantification of EV marker proteins by ELISA. Blue bars indicate the relative quantities of EV markers in 2D EVs, and red indicate those of 3D EVs. Bars indicate mean ± SD (n = 6). ***$p < 0.001$ vs. 2D. **F**. Identification of differentially expressed proteins in 3D EVs. Horizontal axis indicates $\log_2$-transformed relative abundance of 3D EV proteins, and vertical axis indicates $-\log_{10}$-transformed p-value. Horizontal red line indicates p-value cutoff = 0.05 and vertical red line indicates fold change cutoff = 2 (n = 3). **G**. Identification of canonical EV markers. Blue circle indicates EV proteins, yellow indicates the top 100 marker proteins from Vesiclepedia, and red indicates those of ExoCarta. **H**. Pathway enrichment of up-regulated proteins in 3D EVs. Each colored node represents each cluster of pathways. The node size is proportional to the number of proteins belonging to the pathway and the edge thickness (similarity) to the numbers of proteins common in the pathways.

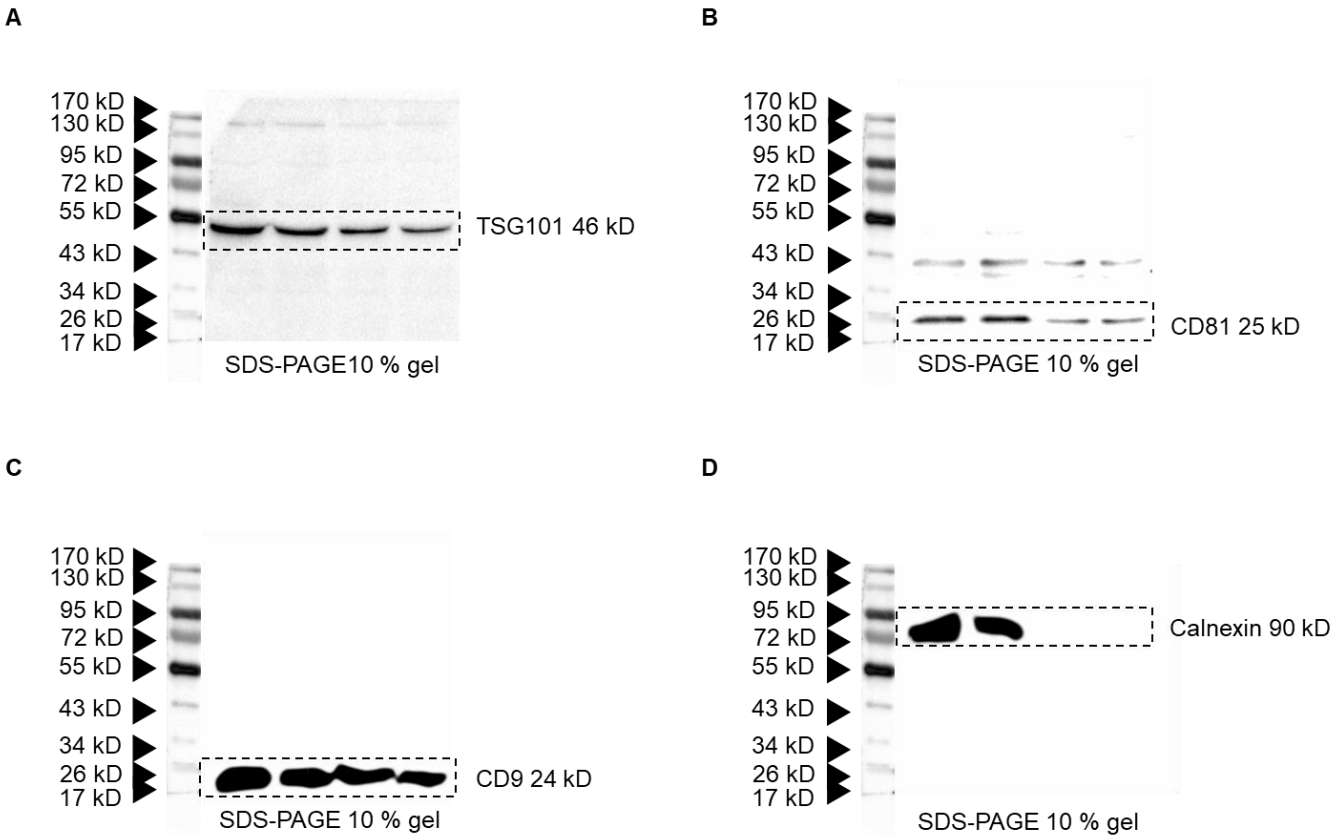


Figure 7. Uncropped images of Western blots

A. TSG101 at 46 kD. **B.** CD81 at 25 kD. **C**. CD9 at 24 kD. **D.** Calnexin at 90kD.

Table 4. Differentially expressed proteins in 3D EVs

Up regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	P16402	H1-3	Generic binc	0	29.429	24	6	12	2	221	22.3	11.02	28.73	2.55	0.01434441	31.1	168.9
High	O75874	IDH1	Generic enz	0	109.318	40	16	41	16	414	46.6	7.01	110.5	2.38	0.00061201	32.3	167.7
High	P10412	H1-4	Generic binc	0	35.339	29	8	15	3	219	21.9	11.03	34.52	2.32	0.00817823	33.9	166.1
High	O60218	AKR1B10	Generic enz	0	17.974	13	4	5	4	316	36	7.84	14.16	2.32	0.00060139	32.6	167.4
High	Q5QNW6	H2BC18	Generic binc	0	66.245	53	8	39	2	126	13.9	10.32	119.58	2.17	0.005227	36.4	163.6
High	P84243	H3-3A	Generic binc	0	36.395	50	6	12	2	136	15.3	11.27	40.58	2.14	0.00517074	36.9	163.1
High	P16104	H2AX	Generic binc	0	21.766	38	6	13	2	143	15.1	10.74	34.35	2.14	0.00612274	37.2	162.8
High	P06865	HEXA	Generic enz	0.004	3.917	3	2	2	2	529	60.7	5.16	4.59	2.1	0.00944845	35.8	164.2
High	P02647	APOA1	Receptor lig	0	25.629	11	3	37	2	267	30.8	5.76	123.59	2.09	0.01421595	44.8	155.2
High	P14550	AKR1A1	Generic enz	0	33.003	21	6	11	5	325	36.6	6.79	30.98	2.07	0.00493007	40.5	159.5
High	Q16778	H2BC21	Generic binc	0	64.575	53	8	35	2	126	13.9	10.32	107.77	2.03	0.00644315	39.2	160.8
High	P62805	H4C1	Generic binc	0	59.908	53	8	30	8	103	11.4	11.36	93.8	2.02	0.00919886	39.6	160.4
High	Q71UI9	H2AZ2	Generic binc	0	16.43	31	4	11	2	128	13.5	10.58	30.19	2.02	0.01210075	42.6	157.4
High	P48723	HSPA13	Generic binc	0	66.047	20	10	18	10	471	51.9	5.76	60.97	2	0.002679	43.5	156.5
High	Q92688	ANP32B	Generic binc	0	19.175	16	4	7	3	251	28.8	4.06	17.26	1.95	0.00921346	40.2	159.8
High	P98088	MUC5AC	Generic binc	0	9.153	1	3	3	3	5654	585.2	7.02	4.71	1.93	0.019642	42.5	157.5
High	P15121	AKR1B1	Generic enz	0	33.386	29	8	10	7	316	35.8	6.98	29.34	1.91	0.00112233	44.5	155.5
High	O94919	ENDOD1	Generic binc	0	8.831	5	2	4	2	500	55	5.71	5.61	1.86	0.01534953	41.9	158.1
High	Q15818	NPTX1	Generic binc	0	128.134	51	19	44	19	432	47.1	6.55	142.11	1.85	0.01838949	43.5	156.5
High	P16401	H1-5	Generic binc	0	26.477	25	6	13	3	226	22.6	10.92	26.79	1.85	0.01045777	43.9	156.1
High	Q9BZZ5	API5	Generic binc	0	7.129	4	2	3	2	524	59	7.34	4.74	1.85	0.01376714	45.7	154.3
High	P51572	BCAP31	Generic binc	0.001	5.375	7	2	2	2	246	28	8.44	5.28	1.82	0.00717838	42.6	157.4
High	P16870	CPE	Generic prot	0	24.611	9	3	5	3	476	53.1	5.14	16.13	1.79	0.00833945	44.8	155.2
High	P49755	TMED10	Generic binc	0	12.497	12	2	2	2	219	25	7.44	8.08	1.77	0.0013669	45.1	154.9
High	P49888	SULT1E1	Generic enz	0.002	4.28	6	2	2	2	294	35.1	6.62	5.43	1.76	0.00260668	47.5	152.5
High	P37837	TALDO1	Generic enz	0	56.284	40	15	22	15	337	37.5	6.81	51.29	1.75	0.00279523	47.2	152.8
High	Q15493	RGN	Generic binc	0	46.239	18	5	23	5	299	33.2	6.25	70.67	1.75	0.00289866	45.3	154.7
High	P05387	RPLP2	Generic binc	0	44.945	43	3	7	3	115	11.7	4.54	28.31	1.75	0.03256615	47.8	152.2
High	O75339	CILP	Generic binc	0	34.005	8	10	19	10	1184	132.5	8.41	42.56	1.75	0.00342369	45.8	154.2
High	O75367	MACROH2	Generic binc	0	15.982	12	4	6	4	372	39.6	9.79	13.56	1.75	0.00061667	45.7	154.3
High	P48506	GCLC	Generic enz	0	6.965	4	3	3	3	637	72.7	6.09	5.04	1.75	0.00184335	47.9	152.1
High	P13798	APEH	Generic prot	0	23.533	10	6	10	6	732	81.2	5.48	22.84	1.73	0.00178523	46.1	153.9
High	Q9BRF8	CPPED1	Generic pho	0	9.982	11	3	3	3	314	35.5	6.2	5.73	1.72	0.0054518	44.3	155.7
High	Q9BVK6	TMED9	Generic binc	0	18.06	23	4	4	4	235	27.3	8.02	13.4	1.71	0.00121881	47.4	152.6
High	Q5GJ75	TNFAIP8L3	Protein	0	10.514	10	2	2	2	292	32.6	8.59	4.57	1.7	0.00183906	44.4	155.6
High	Q9Y5C1	ANGPTL3	Receptor lig	0	42.546	15	8	21	8	460	53.6	6.7	59.03	1.69	0.0129188	51.1	148.9
High	P09455	RBP1	Generic binc	0	26.804	47	6	9	6	135	15.8	5.11	17.68	1.67	0.00053468	49.6	150.4
High	Q12888	TP53BP1	Generic binc	0	6.088	1	2	2	2	1972	213.4	4.7	2.26	1.67	0.01630805	40.7	159.3
High	P15559	NQO1	Generic enz	0	21.124	23	8	9	8	274	30.8	8.88	18.96	1.66	0.00540563	48.1	151.9
High	Q9Y3B3	TMED7	Protein	0	8.71	10	2	2	2	224	25.2	6.89	5.99	1.66	0.00327955	48.4	151.6
High	P11413	G6PD	Generic enz	0	78.834	46	20	31	20	515	59.2	6.84	73.85	1.65	0.00082571	48.3	151.7
High	Q5T2E6	ARMH3	Protein	0.006	3.329	2	2	3	2	689	78.7	6.6	4.94	1.65	0.00623144	49.1	150.9
High	Q96NU7	AMDHD1	Generic enz	0	40.281	23	7	10	7	426	46.7	6.61	29.58	1.62	0.00294635	49	151

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Up regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	Q92820	GGH	Generic prot	0	25.352	18	5	15	5	318	35.9	7.11	28.61	1.62	0.00173571	49	151
High	Q92563	SPOCK2	Generic binc	0	47.744	29	10	12	10	424	46.7	4.83	35.55	1.56	0.00889542	50.6	149.4
High	Q9HC38	GLOD4	Protein	0	10.496	10	3	3	3	313	34.8	5.6	9.3	1.55	0.00187966	54	146
High	Q6ZN66	GBP6	Generic enz	0.001	4.979	4	2	5	2	633	72.4	6.37	2.02	1.54	0.0089951	51.8	148.2
High	P21283	ATP6V1C1	Generic cha	0.005	3.433	5	2	2	2	382	43.9	7.46	0	1.54	0.00250255	51.7	148.3
High	P35625	TIMP3	Generic binc	0	27.871	39	8	10	8	211	24.1	8.72	26.83	1.53	0.00390841	51.4	148.6
High	Q13247	SRSF6	Generic binc	0.002	4.165	6	2	2	2	344	39.6	11.43	4.58	1.52	0.00692557	48.7	151.3
High	P08621	SNRNP70	Generic binc	0.006	3.098	4	2	2	2	437	51.5	9.94	0	1.52	0.00394011	51.1	148.9
High	Q13228	SELENBP1	Generic binc	0	32.999	14	6	10	6	472	52.4	6.37	27.44	1.51	0.00071349	51.9	148.1
High	P16278	GLB1	Generic enz	0	9.889	5	3	4	3	677	76	6.57	5.22	1.51	0.01258686	51.9	148.1
High	P60903	S100A10	Generic binc	0.001	5.37	18	2	3	2	97	11.2	7.37	5.38	1.5	0.00108222	51.6	148.4
High	Q93088	BHMT	Generic enz	0	81.162	29	8	20	8	406	45	7.03	80.92	1.49	0.00309416	52.6	147.4
High	Q7Z7M9	GALNT5	Generic enz	0	10.456	4	3	3	3	940	106.2	9.47	7.5	1.49	0.00343177	54	146
High	P52895	AKR1C2	Generic enz	0	10.097	5	3	7	3	323	36.7	7.49	18.53	1.49	0.00146192	52.3	147.7
High	P53041	PPP5C	Protein phos	0.001	5.527	4	2	2	2	499	56.8	6.28	2.64	1.49	0.00344795	52.9	147.1
High	Q9Y383	LUC7L2	Generic binc	0	20.092	14	5	7	5	392	46.5	10.01	12.75	1.48	0.01039801	52.8	147.2
High	P14324	FDPS	Generic enz	0	11.624	8	4	5	4	419	48.2	6.15	12.45	1.48	0.00399501	57	143
High	Q9P2E9	RRBP1	Generic rec	0	220.632	51	52	82	50	1410	152.4	8.6	209.68	1.47	0.00166152	53.1	146.9
High	Q14195	DPYSL3	Generic binc	0	74.33	29	12	19	10	684	73.9	6.35	65.9	1.47	0.00637963	51.6	148.4
High	P08567	PLEK	Generic binc	0	47.888	13	4	7	4	350	40.1	8.28	34.64	1.47	0.00440887	54.1	145.9
High	Q3LXA3	TKFC	Generic kina	0	38.618	15	7	8	7	575	58.9	7.49	29.42	1.47	0.00354104	53.4	146.6
High	P0DMV8	HSPA1A	Generic binc	0	261.877	57	29	104	23	641	70	5.66	323.59	1.46	0.00170871	55.4	144.6
High	P19338	NCL	Generic binc	0	38.236	16	13	17	13	710	76.6	4.7	32.69	1.46	0.00873018	53.2	146.8
High	Q14108	SCARB2	Generic rec	0	7.22	6	3	3	3	478	54.3	5.14	7.07	1.46	0.0002	53.4	146.6
High	P06732	CKM	Generic kina	0	41.468	31	10	13	9	381	43.1	7.25	35.2	1.45	0.00304286	56.9	143.1
High	P17096	HMGA1	Transcriptio	0	6.595	31	3	3	3	107	11.7	10.32	6.97	1.45	0.00259324	53.6	146.4
High	O95232	LUC7L3	Generic binc	0.005	3.548	3	2	2	2	432	51.4	9.79	2.06	1.45	0.00594967	53.6	146.4
High	P15169	CPN1	Metalloprot	0	18.346	9	3	5	3	458	52.3	7.34	17.03	1.44	0.00514743	57.1	142.9
High	O75915	ARL6IP5	Generic binc	0	10.427	16	2	3	2	188	21.6	9.77	7.41	1.44	0.00338224	51.7	148.3
High	O00425	IGF2BP3	Generic binc	0.002	4.438	5	2	2	2	579	63.7	8.87	2.69	1.44	0.0040968	52.8	147.2
High	O43390	HNRNPR	Generic binc	0	32.872	15	8	11	4	633	70.9	8.13	30.41	1.43	0.01009002	54.2	145.8
High	O95379	TNFAIP8	Generic binc	0	13.194	17	2	2	2	198	23	7.93	8.43	1.43	0.01005095	59.6	140.4
High	P29762	CRABP1	Generic binc	0.002	4.58	14	2	2	2	137	15.6	5.38	2.08	1.43	0.00107373	55.8	144.2
High	P13929	ENO3	Generic enz	0	114.855	38	13	60	8	434	47	7.71	208.09	1.42	0.00810689	55.8	144.2
High	Q96GK7	FAHD2A	Generic enz	0	24.393	22	5	5	5	314	34.6	8.24	15.57	1.42	0.00070841	56.6	143.4
High	Q9Y617	PSAT1	Generic enz	0	22.287	13	5	12	5	370	40.4	7.66	24.45	1.42	0.00019246	55.4	144.6
High	Q08752	PPID	Generic enz	0	15.176	11	4	5	4	370	40.7	7.21	10.45	1.42	0.00238205	52.8	147.2
High	P08397	HMBS	Generic enz	0	13.949	17	5	6	5	361	39.3	7.18	15.09	1.42	0.00543756	55.8	144.2
High	Q7Z4W1	DCXR	Generic enz	0	12.982	9	2	5	2	244	25.9	8.1	11.57	1.42	0.00043796	54.2	145.8
High	Q96KP4	CNDP2	Generic prot	0	54.957	20	8	16	8	475	52.8	5.97	46.92	1.41	0.00061812	55.5	144.5
High	Q02790	FKBP4	Generic binc	0	16.289	10	5	8	5	459	51.8	5.43	21.1	1.41	0.00764311	53.6	146.4
High	Q07666	KHDRBS1	Generic binc	0	11.558	7	4	5	4	443	48.2	8.66	7.14	1.41	0.01604241	54.7	145.3
High	P52849	NDST2	Generic enz	0.001	5.678	3	2	2	2	883	100.8	8.63	2.33	1.41	0.04494452	64.1	135.9

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Up regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	P47712	PLA2G4A	Generic pho	0	48.337	19	10	13	10	749	85.2	5.38	40.24	1.39	0.00143865	55.3	144.7
High	Q14914	PTGR1	Generic enz	0	11.665	7	2	3	2	329	35.8	8.29	7.34	1.39	0.00104408	56.4	143.6
High	Q9Y5X3	SNX5	Generic bind	0	46.491	34	12	15	12	404	46.8	6.76	33.62	1.38	0.00369655	59	141
High	P14678	SNRPB	Generic bind	0	8.794	10	2	2	2	240	24.6	11.19	5.82	1.38	0.01363824	52.6	147.4
High	Q9BSL1	UBAC1	Generic bind	0	6.909	5	2	3	2	405	45.3	4.92	5.99	1.38	0.01160074	61.7	138.3
High	Q96FN4	CPNE2	Protein	0	6.896	4	2	2	2	548	61.2	6.07	5.91	1.38	0.00226469	55.6	144.4
High	Q9NPR2	SEMA4B	Generic rece	0.004	3.875	2	2	3	2	832	92.1	6.95	7.1	1.38	0.01716663	57.2	142.8
High	Q12906	ILF3	Transcriptio	0	29.69	13	9	9	9	894	95.3	8.76	20.28	1.37	0.00629348	58	142
High	P30566	ADSL	Generic enz	0	22.353	20	9	9	9	484	54.9	7.11	15.44	1.37	0.0001573	55.9	144.1
High	Q6P996	PDXDC1	Generic bind	0	18.869	6	4	6	4	788	86.7	5.38	10.35	1.37	0.00312484	55.9	144.1
High	P11279	LAMP1	Protein	0	14.511	10	4	6	4	417	44.9	8.75	14	1.37	0.00713333	55.9	144.1
High	P07910	HNRNPC	Generic bind	0	13.17	14	4	4	4	306	33.7	5.08	8.03	1.37	0.01377405	55.8	144.2
High	Q9UK55	SERPINA10	Generic bind	0	9.873	4	2	5	2	444	50.7	8.27	13.84	1.37	0.00677941	55.7	144.3
High	P09211	GSTP1	Generic enz	0	81.987	55	9	26	9	210	23.3	5.64	79.12	1.36	0.02074162	62.2	137.8
High	Q9HB71	CACYBP	Generic enz	0	12.74	18	4	5	3	228	26.2	8.25	13.44	1.36	0.00902915	62.9	137.1
High	Q15363	TMED2	Generic bind	0	12.364	18	3	4	3	201	22.7	5.17	7.19	1.36	0.00126718	56.1	143.9
High	Q96IU4	ABHD14B	Generic enz	0	10.923	15	3	5	3	210	22.3	6.4	14.63	1.36	0.01515258	58.1	141.9
High	P50454	SERPINH1	Generic bind	0	120.719	56	21	41	21	418	46.4	8.69	129.04	1.35	0.00883656	56.3	143.7
High	P01023	A2M	Receptor lig	0	59.464	5	8	213	6	1474	163.2	6.46	552.95	1.35	0.00741115	56.2	143.8
High	O60749	SNX2	Transporter	0	40.688	23	10	14	9	519	58.4	5.12	33.17	1.35	0.00409761	60.3	139.7
High	Q15233	NONO	Transcriptio	0	10.325	8	4	4	3	471	54.2	8.95	10.09	1.35	0.00931645	53.4	146.6
High	Q07955	SRSF1	Generic enz	0	10.101	15	4	4	4	248	27.7	10.36	8.06	1.35	0.00496237	54.6	145.4
High	Q9UIK4	DAPK2	Protein kina	0	31.174	20	6	9	6	370	42.9	6.92	25.2	1.34	0.00035329	56.7	143.3
High	Q9UBQ7	GRHPR	Generic enz	0	23.993	16	6	9	6	328	35.6	7.39	22.62	1.34	0.00245971	57.1	142.9
High	Q14258	TRIM25	Generic enz	0	7.219	5	3	3	3	630	70.9	8.09	4.56	1.34	0.00659689	59.5	140.5
High	P17677	GAP43	Generic bind	0	6.365	9	2	2	2	238	24.8	4.72	4.73	1.34	0.01075833	56.6	143.4
High	P06733	ENO1	Generic enz	0	225.008	73	26	103	22	434	47.1	7.39	307.03	1.33	0.00973778	59.6	140.4
High	Q9Y4G6	TLN2	Generic bind	0	159.16	8	20	73	2	2542	271.4	5.57	214.5	1.33	0.00070851	56.9	143.1
High	P23471	PTPRZ1	Generic pho	0.001	5.436	1	2	3	2	2315	254.4	4.88	7.97	1.33	0.02830089	56.9	143.1
High	O60701	UGDH	Generic enz	0	186.665	57	20	44	20	494	55	7.12	160.13	1.32	0.00092473	57.2	142.8
High	P07954	FH	Generic enz	0	37.003	17	7	13	7	510	54.6	8.76	34.24	1.32	0.00183292	57.8	142.2
High	P09467	FBP1	Generic pho	0	19.674	12	4	13	4	338	36.8	6.99	25.28	1.32	0.00075574	59.1	140.9
High	P62316	SNRPD2	Generic bind	0.001	5.827	16	2	2	2	118	13.5	9.91	3.06	1.32	0.00821781	57.1	142.9
High	P52943	CRIP2	Generic bind	0.001	5.64	7	2	4	2	208	22.5	8.72	3.08	1.32	0.00023562	56.7	143.3
High	O60763	USO1	Transporter	0	127.117	27	20	33	20	962	107.8	4.91	107.1	1.31	0.00145421	59.9	140.1
High	P37802	TAGLN2	Protein	0	120.622	65	13	44	13	199	22.4	8.25	139.87	1.31	0.03193574	60.7	139.3
High	P31939	ATIC	Generic enz	0	111.559	39	17	33	17	592	64.6	6.71	101.17	1.31	0.0021161	59.2	140.8
High	P47755	CAPZA2	Protein	0	58.303	48	9	16	7	286	32.9	5.85	55.6	1.31	0.00195587	57.6	142.4
High	O43681	GET3	Generic cha	0	28.278	19	5	8	5	348	38.8	4.91	13.5	1.31	0.0049065	60.4	139.6
High	P36543	ATP6V1E1	Generic cha	0	22.703	37	7	7	7	226	26.1	8	18.54	1.31	0.00677401	60	140
High	P53004	BLVRA	Generic enz	0	20.694	15	4	5	4	296	33.4	6.44	15.36	1.31	0.00063784	59	141
High	Q96DG6	CMBL	Generic enz	0	11.063	12	3	6	3	245	28	7.18	12.55	1.31	0.00178268	58.7	141.3
High	O60610	DIAPH1	Generic bind	0	73.906	13	14	19	14	1272	141.3	5.41	52.9	1.3	0.00306852	60.9	139.1

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Up regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	Q709C8	VPS13C	Generic binc	0	67.511	5	17	20	17	3753	422.1	6.83	46.63	1.3	0.00161346	59	141
High	Q96G03	PGM2	Generic enz	0	22.002	12	7	8	7	612	68.2	6.73	19.79	1.3	0.00205532	57.8	142.2
High	O75410	TACC1	Generic binc	0	9.514	3	3	3	3	805	87.7	4.88	4.82	1.3	0.02140503	58.4	141.6
High	Q92841	DDX17	Generic enz	0	26.785	13	7	8	3	650	72.3	8.59	18.51	1.29	0.01547918	57.5	142.5
High	P62304	SNRPE	Generic binc	0	10.434	25	2	3	2	92	10.8	9.44	9.07	1.29	0.00770142	58	142
High	O15400	STX7	Transporter	0	8.963	9	2	2	2	261	29.8	5.55	6.94	1.29	0.00736779	58	142
High	Q9NVG8	TBC1D13	Regulators (	0	7.941	9	3	3	3	400	46.5	5.24	4.29	1.29	0.02016294	60.8	139.2
High	Q9H0P0	NT5C3A	Generic pho	0	7.488	7	2	3	2	297	33.9	5.86	8.81	1.29	0.01300361	59	141
High	P52209	PGD	Generic enz	0	137.062	41	16	43	15	483	53.1	7.23	145.45	1.28	0.00039513	59.5	140.5
High	Q6P3W7	SCYL2	Protein kina	0	11.552	4	3	3	3	929	103.6	8.22	8.42	1.28	0.00784789	59	141
High	Q9P2D3	HEATR5B	Generic binc	0	5.961	1	2	2	2	2071	224.2	7.17	3.39	1.28	0.01197923	66.2	133.8
High	O95352	ATG7	Generic enz	0.002	4.592	3	2	2	2	703	77.9	6.24	4.65	1.28	0.02194772	64.2	135.8
High	Q8TBF2	PRXL2B	Protein	0.005	3.523	12	2	3	2	198	21.2	6.67	4.69	1.28	0.01455203	58.3	141.7
High	Q01581	HMGCS1	Generic enz	0	71.12	31	11	19	11	520	57.3	5.41	53.04	1.27	0.00728089	58.5	141.5
High	Q13177	PAK2	Protein kina	0	48.331	27	10	12	10	524	58	5.96	41.77	1.27	0.00360099	61.2	138.8
High	Q9NTK5	OLA1	Generic binc	0	47.933	34	11	13	11	396	44.7	7.81	42.01	1.27	0.00224322	57.7	142.3
High	P00492	HPRT1	Generic enz	0	26.297	28	5	7	5	218	24.6	6.68	20.11	1.27	0.01285817	60.3	139.7
High	Q15185	PTGES3	Generic enz	0	20.63	38	5	13	5	160	18.7	4.54	37.05	1.27	0.00668696	58.5	141.5
High	Q01130	SRSF2	Generic pro	0	13.698	15	4	4	4	221	25.5	11.85	10.75	1.27	0.01971088	58.7	141.3
High	O75396	SEC22B	Generic binc	0	12.335	18	3	5	3	215	24.6	6.92	10.13	1.27	0.00593963	58.6	141.4
High	Q13867	BLMH	Generic enz	0	11.051	10	4	5	4	455	52.5	6.27	11.48	1.27	0.00116428	58.7	141.3
High	Q9H2J4	PDCL3	Generic binc	0.001	4.868	8	2	2	2	239	27.6	4.84	4.82	1.27	0.00644056	58.8	141.2
High	P61326	MAGOH	Generic binc	0.001	4.795	17	2	2	2	146	17.2	6.11	4.81	1.27	0.00050512	58.7	141.3
High	O00429	DNM1L	Generic enz	0	174.919	53	30	50	30	736	81.8	6.81	154.63	1.26	0.00239031	60.1	139.9
High	O75891	ALDH1L1	Generic enz	0	117.881	26	21	41	21	902	98.8	5.94	108.59	1.26	0.00944412	57.5	142.5
High	Q86VS8	HOOK3	Generic binc	0	29.341	11	6	6	6	718	83.1	5.17	17.03	1.26	0.00582641	59.2	140.8
High	O75351	VPS4B	Generic binc	0	26.879	14	5	8	5	444	49.3	7.23	19.45	1.26	0.00228442	61.8	138.2
High	Q9BXS5	AP1M1	Transporter	0	14.195	9	4	5	4	423	48.6	7.3	12.62	1.26	0.01033994	58.8	141.2
High	P38919	EIF4A3	Generic enz	0	13.587	13	4	5	2	411	46.8	6.73	11.39	1.26	0.02213155	59	141
High	Q8N335	GPD1L	Generic enz	0	13.373	11	4	5	2	351	38.4	7.02	13.04	1.26	0.00153033	61.1	138.9
High	O43175	PHGDH	Generic enz	0	106.789	25	12	27	12	533	56.6	6.71	90.05	1.25	0.01462803	59.4	140.6
High	P31150	GDI1	Regulators (	0	89.719	43	14	22	6	447	50.6	5.14	69.37	1.25	0.00037633	59.2	140.8
High	Q00839	HNRNPU	Generic binc	0	29.709	12	8	12	8	825	90.5	6	23.57	1.25	0.00992987	57.8	142.2
High	O76094	SRP72	Protein	0	27.153	14	8	9	8	671	74.6	9.26	18.25	1.25	0.00485852	60.1	139.9
High	O95394	PGM3	Generic enz	0	25.24	13	7	9	7	542	59.8	6.25	24.58	1.25	9.1837E-05	59.1	140.9
High	P10768	ESD	Generic enz	0	16.683	13	3	15	3	282	31.4	7.02	35.12	1.25	0.00234134	59.1	140.9
High	Q13641	TPBG	Generic binc	0	12.756	9	4	5	4	420	46	6.83	10.16	1.25	0.00244942	59.7	140.3
High	P06703	S100A6	Generic binc	0.001	5.796	24	3	3	3	90	10.2	5.48	6.52	1.25	0.01088343	65.8	134.2
High	P38606	ATP6V1A	Generic cha	0	78.178	29	14	24	14	617	68.3	5.52	64.47	1.24	0.00580922	59.5	140.5
High	P23381	WARS1	Generic enz	0	62.659	38	14	19	14	471	53.1	6.23	49.84	1.24	0.00412041	57.8	142.2
High	Q12882	DPYD	Generic enz	0	45.116	12	10	13	10	1025	111.3	7.05	31.74	1.24	0.00468655	59.4	140.6
High	P11172	UMPS	Generic enz	0	8.425	4	3	3	3	480	52.2	7.24	7.8	1.24	0.00159312	59.3	140.7
High	Q92890	UFD1	Generic pro	0.001	4.89	7	2	2	2	343	38.7	6.34	5.78	1.24	0.00836221	65.1	134.9

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Up regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	P48444	ARCN1	Generic binc	0	67.031	27	13	23	13	511	57.2	6.21	50.88	1.23	0.00510343	60.4	139.6
High	P69891	HBG1	Generic binc	0	12.779	17	3	43	2	147	16.1	7.2	120.01	1.23	0.0144519	65.9	134.1
High	P06276	BCHE	Generic enz	0	11.982	5	3	5	3	602	68.4	7.42	12.31	1.23	0.03500198	59.8	140.2
High	Q8NES3	LFNG	Generic enz	0	11.263	6	2	3	2	379	41.7	9.17	7.6	1.23	0.00120777	59.2	140.8
High	Q9Y3Z3	SAMHD1	Generic enz	0	8.824	4	2	2	2	626	72.2	7.14	4.17	1.23	0.01286586	59.9	140.1
High	Q96GD0	PDXP	Generic pho	0	8.36	8	2	2	2	296	31.7	6.55	6.75	1.23	0.00118372	57.2	142.8
High	Q16769	QPCT	Generic enz	0	6.723	7	2	4	2	361	40.9	6.61	6.72	1.23	0.00187566	61.3	138.7
High	P47895	ALDH1A3	Generic enz	0.001	5.039	4	2	2	2	512	56.1	7.25	4.67	1.23	0.00415617	59.8	140.2
High	P69905	HBA1	Generic binc	0	52.074	35	5	157	5	142	15.2	8.68	340.67	1.22	0.01358702	64.5	135.5
High	P13010	XRCC5	Generic binc	0	27.707	8	4	5	4	732	82.7	5.81	17.71	1.22	0.01874963	57.3	142.7
High	O95810	CAVIN2	Generic binc	0	17.49	12	4	4	4	425	47.1	5.21	12.62	1.22	0.00771429	64.3	135.7
High	P34913	EPHX2	Generic enz	0	16.576	2	2	6	2	555	62.6	6.28	15.29	1.22	0.01485799	60.1	139.9
High	Q16629	SRSF7	Generic binc	0	7.232	10	2	5	2	238	27.4	11.82	12.05	1.22	0.0010292	60	140
High	Q9C0C2	TNKS1BP1	Generic binc	0.001	5.15	2	2	2	2	1729	181.7	4.86	0	1.22	0.02107805	62.9	137.1
High	P78344	EIF4G2	Generic binc	0.001	4.971	3	3	3	3	907	102.3	7.14	0	1.22	0.02547096	66.3	133.7
High	Q15042	RAB3GAP1	Regulators (	0.002	4.379	2	2	2	2	981	110.5	5.55	2.95	1.22	0.00718648	62.6	137.4
High	P23526	AHCY	Generic enz	0	138.403	43	16	61	15	432	47.7	6.34	188.04	1.21	0.00811177	60.2	139.8
High	P05388	RPLP0	Generic binc	0	65.367	40	10	19	10	317	34.3	5.97	60.19	1.21	0.01597469	61.9	138.1
High	P32754	HPD	Generic enz	0	28.562	23	7	8	7	393	44.9	7.01	18.53	1.21	0.00628702	61.9	138.1
High	Q9UHB9	SRP68	Protein	0	26.198	12	7	7	7	627	70.7	8.56	17.27	1.21	0.00280658	58.1	141.9
High	P30043	BLVRB	Generic enz	0	22.828	15	3	7	3	206	22.1	7.65	27.07	1.21	0.00651557	59	141
High	Q16772	GSTA3	Generic enz	0	22.265	18	3	6	2	222	25.3	9.2	22.18	1.21	0.00346807	64.2	135.8
High	Q12905	ILF2	Transcriptio	0	21.123	20	5	5	5	390	43	5.26	15.02	1.21	0.00737763	57.9	142.1
High	P21695	GPD1	Generic enz	0	19.629	13	5	7	3	349	37.5	6.18	16.89	1.21	0.01339875	61.2	138.8
High	P52790	HK3	Generic kina	0	18.241	7	5	5	3	923	99	5.4	12.55	1.21	0.00202263	60.8	139.2
High	Q9Y224	RTRAF	Generic binc	0	17.372	16	3	6	3	244	28.1	6.65	17.46	1.21	0.01463171	66.8	133.2
High	Q00796	SORD	Generic enz	0	12.652	12	3	5	3	357	38.3	7.97	10.87	1.21	0.00017129	61	139
High	Q8TC07	TBC1D15	Regulators (	0	9.813	5	3	3	3	691	79.4	5.67	7.86	1.21	0.00135996	60.4	139.6
High	Q9NUM4	TMEM106E	Protein	0	9.393	9	2	2	2	274	31.1	6.99	6.11	1.21	0.00452136	62.9	137.1
High	Q15257	PTPA	Generic binc	0	7.046	5	2	2	2	358	40.6	5.94	5.49	1.21	0.00659513	58.9	141.1
High	P62308	SNRPG	Generic binc	0	6.501	25	2	3	2	76	8.5	8.88	5.53	1.21	0.02428301	59.3	140.7
High	Q9Y490	TLN1	Generic binc	0	1412.687	66	140	535	122	2541	269.6	6.07	1868.43	1.2	0.01226178	61.6	138.4
High	Q86VP6	CAND1	Generic binc	0	300.378	47	50	87	50	1230	136.3	5.78	253.9	1.2	0.00412708	60.5	139.5
High	P55786	NPEPPS	Generic prot	0	110.132	30	24	37	24	919	103.2	5.72	101.63	1.2	0.0035689	60.6	139.4
High	P54652	HSPA2	Generic binc	0	90.688	28	17	43	3	639	70	5.74	125.01	1.2	0.01235127	60.6	139.4
High	P54577	YARS1	Generic enz	0	37.178	23	13	18	13	528	59.1	7.05	33.29	1.2	0.00839396	66.8	133.2
High	P20339	RAB5A	RAS - super	0	34.715	29	5	13	2	215	23.6	8.15	36.71	1.2	0.0070669	65	135
High	P33908	MAN1A1	Generic enz	0	28.827	10	7	13	6	653	72.9	6.47	32.3	1.2	0.00631073	60.6	139.4
High	P12956	XRCC6	Generic binc	0	21.483	12	6	8	6	609	69.8	6.64	15.58	1.2	0.0134983	56.5	143.5
High	P52597	HNRNPF	Generic binc	0	19.054	12	4	5	3	415	45.6	5.58	13.97	1.2	0.01004656	60.5	139.5
High	Q6VY07	PACS1	Generic binc	0	11.913	4	4	5	4	963	104.8	7.74	2.27	1.2	0.0018062	60.6	139.4
High	P61011	SRP54	Protein	0	10.086	8	3	3	3	504	55.7	8.75	5.68	1.2	0.01589407	60.6	139.4
High	P49756	RBM25	Generic binc	0	8.912	4	2	2	2	843	100.1	6.32	6.16	1.2	0.00294292	60	140

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Up regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	Q14376	GALE	Generic enz	0	7.576	10	3	4	3	348	38.3	6.73	7.17	1.2	0.00205794	63.4	136.6
High	Q08945	SSRP1	Generic binc	0	6.588	3	2	2	2	709	81	6.87	4.51	1.2	0.0028253	61.3	138.7
High	A8TX70	COL6A5	Generic binc	0.009	2.623	1	2	2	2	2615	289.7	6.95	4.2	1.2	0.00919381	60.5	139.5
High	Q15181	PPA1	Generic pho	0	36.421	34	6	10	6	289	32.6	5.86	26.15	1.19	0.00355352	64.4	135.6
High	Q04760	GLO1	Generic enz	0	22.64	18	4	8	4	184	20.8	5.31	23	1.19	0.00246386	64.6	135.4
High	Q12904	AIMP1	Receptor lig	0	19.642	14	3	5	3	312	34.3	8.43	15.89	1.19	0.00652726	60.9	139.1
High	Q14117	DPYS	Generic enz	0	16.342	8	4	6	3	519	56.6	7.27	17.25	1.19	0.00150698	61.3	138.7
High	Q9P0V9	SEPTIN10	Generic binc	0	15.042	12	4	6	3	454	52.6	6.8	7.6	1.19	0.00596272	61.6	138.4
High	Q9BYF1	ACE2	Generic prot	0	12.12	6	2	2	2	805	92.4	5.54	7.53	1.19	0.00050901	60.5	139.5
High	Q16186	ADRM1	Generic binc	0	10.147	13	5	5	5	407	42.1	5.07	7.18	1.19	0.00100727	61.1	138.9
High	P62314	SNRPD1	Generic binc	0	8.445	28	2	2	2	119	13.3	11.56	7.32	1.19	0.00663313	60	140
High	Q9BS26	ERP44	Generic binc	0	34.344	24	9	14	8	406	46.9	5.26	36.62	1.18	0.00249043	61.5	138.5
High	P02549	SPTA1	Generic binc	0	30.38	3	7	10	7	2419	279.8	5.05	21.16	1.18	0.00747422	61.1	138.9
High	Q92598	HSPH1	Generic binc	0	26.591	10	7	8	5	858	96.8	5.39	19.84	1.18	0.00194375	62.6	137.4
High	Q6P1N9	TATDN1	Generic enz	0	20.873	20	6	8	6	297	33.6	6.96	17.83	1.18	0.00257338	60.2	139.8
High	P20645	M6PR	Generic rece	0	14.929	19	3	4	3	277	31	5.83	10.49	1.18	0.00142206	62.2	137.8
High	A5YKK6	CNOT1	Generic binc	0	13.112	2	3	3	3	2376	266.8	7.11	7.63	1.18	0.00105627	61.6	138.4
High	P30039	PBLD	Generic enz	0	12.732	11	3	7	3	288	31.8	6.52	20.17	1.18	0.00182085	62.6	137.4
High	Q5QJ74	TBCEL	Generic binc	0	11.689	13	4	4	4	424	48.2	5.38	5.99	1.18	0.00052624	60.2	139.8
High	Q9NXJ5	PGPEP1	Generic prot	0	8.39	11	2	2	2	209	23.1	5.9	7.52	1.18	0.00584429	62.6	137.4
High	O75822	EIF3J	Protein	0.005	3.436	7	2	2	2	258	29	4.83	2.35	1.18	0.01317481	65.4	134.6
High	Q9NZN3	EHD3	Generic binc	0	196.135	56	25	64	8	535	60.8	6.57	204.39	1.17	0.0010588	61.6	138.4
High	Q15172	PPP2R5A	Generic binc	0	43.747	21	9	12	8	486	56.2	6.71	32.89	1.17	0.00259073	65.3	134.7
High	P42357	HAL	Generic enz	0	34.348	15	9	15	9	657	72.7	6.95	38.93	1.17	0.01927742	61.5	138.5
High	O60271	SPAG9	Generic binc	0	16.667	5	5	5	5	1321	146.1	5.15	9.78	1.17	0.00869812	62.5	137.5
High	P51857	AKR1D1	Generic enz	0	13.554	12	3	4	3	326	37.4	7.5	13.61	1.17	0.00053154	60.7	139.3
High	Q8N1G4	LRRC47	Generic binc	0	9.365	7	3	3	3	583	63.4	8.28	7.93	1.17	0.01046323	66.3	133.7
High	Q07889	SOS1	Regulators (	0	8.429	2	2	2	2	1333	152.4	6.84	5.39	1.17	0.01909848	61.4	138.6
High	P26639	TARS1	Generic enz	0	57.322	25	16	19	16	723	83.4	6.67	50.16	1.16	0.01090103	62	138
High	Q9UQ16	DNM3	Protein	0	47.744	17	13	13	8	869	97.7	8.35	35.39	1.16	3.6063E-05	61.9	138.1
High	O43150	ASAP2	Regulators (	0	39.488	9	8	9	7	1006	111.6	6.68	23.74	1.16	0.00932268	64.7	135.3
High	Q9UBF2	COPG2	Generic binc	0	27.805	7	6	8	3	871	97.6	5.81	20.01	1.16	0.00188267	61.3	138.7
High	Q08211	DHX9	Generic enz	0	23.786	7	6	6	6	1270	140.9	6.84	17.13	1.16	0.00806611	61.7	138.3
High	P53999	SUB1	Generic binc	0	15.268	27	3	5	3	127	14.4	9.6	12.8	1.16	0.01487289	57.4	142.6
High	O75976	CPD	Generic prot	0	13.429	4	4	4	4	1380	152.8	6.05	5.65	1.16	0.00105671	63.3	136.7
High	Q13043	STK4	Protein kina	0	11.736	7	3	3	3	487	55.6	5.07	8.63	1.16	0.00197384	60.4	139.6
High	P11245	NAT2	Generic enz	0.002	4.481	9	2	2	2	290	33.5	5.86	5.27	1.16	0.00073515	61.9	138.1
High	Q9NQC3	RTN4	Receptor lig	0	60.777	34	9	21	9	373	40.3	4.78	67.79	1.15	0.00689103	62.1	137.9
High	P09104	ENO2	Generic enz	0	59.255	19	6	29	2	434	47.2	5.03	112.64	1.15	0.02669776	62	138
High	P29692	EEF1D	Generic binc	0	58.745	37	8	18	7	281	31.1	5.01	56.11	1.15	0.01810957	64.5	135.5
High	Q04446	GBE1	Generic enz	0	33.272	14	10	11	10	702	80.4	6.32	20.42	1.15	0.00864822	61.7	138.3
High	P10644	PRKAR1A	Generic binc	0	31.893	19	6	8	6	381	43	5.35	21.27	1.15	0.00010519	62.1	137.9
High	O00194	RAB27B	RAS - super	0	30.851	29	6	8	4	218	24.6	5.52	21.86	1.15	0.00900575	62	138

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Up regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	P49419	ALDH7A1	Generic enz	0	30.156	15	6	13	6	539	58.5	7.99	30.58	1.15	0.00395043	64.2	135.8
High	P07738	BPGM	Generic enz	0	29.615	24	7	20	7	259	30	6.54	41.56	1.15	0.00284708	63.6	136.4
High	O75533	SF3B1	Generic bind	0	17.594	5	4	4	4	1304	145.7	7.09	9.33	1.15	0.01628688	60.6	139.4
High	Q14012	CAMK1	Protein kina	0	15.392	16	5	6	5	370	41.3	5.29	10.03	1.15	0.00904839	68.3	131.7
High	P22352	GPX3	Generic enz	0	13.658	8	2	5	2	226	25.5	8.13	16.25	1.15	5.102E-05	62.2	137.8
High	Q9H4A6	GOLPH3	Protein	0	9.836	9	2	2	2	298	33.8	6.44	7.62	1.15	0.00575509	62.4	137.6
High	P51674	GPM6A	Generic cha	0	8.712	10	3	3	3	278	31.2	5.27	7.21	1.15	0.00816981	62.1	137.9
High	Q00653	NFKB2	Transcriptio	0.001	5.035	4	2	2	2	900	96.7	6.25	2.6	1.15	0.00777371	56.5	143.5
High	P14625	HSP90B1	Generic bind	0	226.826	43	34	85	32	803	92.4	4.84	262.28	1.14	0.00730688	67.4	132.6
High	Q10567	AP1B1	Transporter	0	81.304	23	22	29	10	949	104.6	5.06	71.77	1.14	0.00465413	67.1	132.9
High	P78527	PRKDC	Protein kina	0	78.112	7	24	25	24	4128	468.8	7.12	52.02	1.14	0.01271681	62.1	137.9
High	P11766	ADH5	Generic enz	0	42.845	27	9	18	9	374	39.7	7.49	50.92	1.14	0.00355667	62.2	137.8
High	P08243	ASNS	Generic enz	0	24.756	18	7	8	7	561	64.3	6.86	12.56	1.14	0.00462001	62.5	137.5
High	P10619	CTSA	Generic prot	0	19.727	9	4	5	4	480	54.4	6.61	14.7	1.14	0.02662013	64.2	135.8
High	Q9UKG1	APPL1	Generic bind	0	16.145	6	4	4	4	709	79.6	5.41	11.73	1.14	0.01418602	65.3	134.7
High	Q8WUX9	CHMP7	Transporter	0	13.256	8	2	2	2	453	50.9	5.35	7.34	1.14	0.00597013	63	137
High	Q8TAT6	NPLOC4	Generic bind	0	13.169	7	4	5	4	608	68.1	6.38	11.56	1.14	0.03666893	62.4	137.6
High	P15291	B4GALT1	Generic enz	0	10.031	7	2	2	2	398	43.9	8.65	7.09	1.14	0.00020949	62	138
High	P05455	SSB	Generic bind	0	8.555	6	2	2	2	408	46.8	7.12	6.83	1.14	0.01327026	62.5	137.5
High	P01266	TG	Generic bind	0	7.044	1	3	3	3	2768	304.6	5.58	7.09	1.14	0.00794247	67.7	132.3
High	Q16775	HAGH	Generic enz	0	6.527	6	2	3	2	308	33.8	8.12	6.8	1.14	0.01519008	66.4	133.6
High	Q13148	TARDBP	Transcriptio	0	6.424	5	2	2	2	414	44.7	6.19	5.87	1.14	0.00995155	62.4	137.6
High	O00170	AIP	Generic bind	0	6.308	5	2	2	2	330	37.6	6.29	5.28	1.14	0.01234878	66.6	133.4
High	P30153	PPP2R1A	Generic bind	0	135.396	45	23	43	23	589	65.3	5.11	124.94	1.13	0.00723584	64.7	135.3
High	Q86UX7	FERMT3	Generic bind	0	93.313	27	18	43	18	667	75.9	6.98	114.11	1.13	0.00446532	63.9	136.1
High	P48147	PREP	Generic prot	0	70.658	33	19	21	19	710	80.6	5.86	48.85	1.13	0.00610075	62.6	137.4
High	Q15029	EFTUD2	Generic enz	0	49.654	15	9	9	9	972	109.4	5	29.85	1.13	0.00574314	62.7	137.3
High	P11940	PABPC1	Generic bind	0	42.26	23	13	16	9	636	70.6	9.5	32.22	1.13	0.00461732	62.7	137.3
High	Q9NR99	MXRA5	Generic bind	0	30.871	4	10	10	10	2828	312	8.32	19.24	1.13	0.00776586	59.4	140.6
High	Q9BQ16	SPOCK3	Generic bind	0	30.633	17	7	15	5	433	49	5.06	36.54	1.13	0.02332586	62.8	137.2
High	Q9UNH7	SNX6	Transporter	0	29.466	20	8	10	8	406	46.6	6.16	26.12	1.13	0.00226995	62.6	137.4
High	Q96A65	EXOC4	Generic bind	0	28.869	10	8	8	8	974	110.4	6.49	19.47	1.13	0.00113438	63	137
High	P20618	PSMB1	Generic prot	0	28.319	27	4	8	4	241	26.5	8.13	20.06	1.13	0.0283054	61.6	138.4
High	Q13564	NAE1	Generic bind	0	24.984	13	4	4	4	534	60.2	5.4	16.38	1.13	0.01000766	63.1	136.9
High	A0AVT1	UBA6	Generic enz	0	18.506	5	5	5	5	1052	117.9	6.14	12.85	1.13	0.00561218	62.9	137.1
High	P14866	HNRNPL	Generic bind	0	16.635	12	6	7	6	589	64.1	8.22	12.93	1.13	0.00887913	61.8	138.2
High	Q13155	AIMP2	Generic bind	0	14.583	9	3	5	3	320	35.3	8.22	13.97	1.13	0.00757569	59.4	140.6
High	P55212	CASP6	Generic prot	0	7.823	6	2	3	2	293	33.3	6.93	6.96	1.13	0.00622772	61.1	138.9
High	Q9HAV4	XPO5	Transporter	0.001	5.611	2	2	2	2	1204	136.2	5.8	2.9	1.13	3.441E-06	62.7	137.3
High	Q9Y6V0	PCLO	Generic bind	0.006	3.302	0	2	4	2	5065	552.9	6.51	8.47	1.13	0.03666068	65.7	134.3
High	P00352	ALDH1A1	Generic enz	0	53.931	19	10	21	10	501	54.8	6.73	54.58	1.12	0.00293402	63.1	136.9
High	Q99733	NAP1L4	Generic bind	0	41.079	23	8	12	7	375	42.8	4.69	40.18	1.12	0.00587365	67.6	132.4
High	O43747	AP1G1	Transporter	0	38.293	13	10	11	10	822	91.3	6.8	35.56	1.12	0.00088853	62.4	137.6

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Up regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	A4D1P6	WDR91	Protein	0	21.789	8	5	5	5	747	83.3	6.58	16.24	1.12	0.01691167	67.9	132.1
High	Q96T51	RUFY1	Transporter	0	20.374	10	7	8	7	708	79.8	5.74	16.1	1.12	0.00342815	63.4	136.6
High	Q6P2Q9	PRPF8	Generic binc	0	18.321	3	7	7	7	2335	273.4	8.84	12.65	1.12	0.00600872	62.2	137.8
High	Q99627	COPS8	Generic enz	0	16.839	19	3	4	3	209	23.2	5.38	12.91	1.12	0.00243751	64.2	135.8
High	P04424	ASL	Generic enz	0	12.764	9	4	6	4	464	51.6	6.48	10.76	1.12	0.00553621	62.9	137.1
High	Q9NRY5	FAM114A2	Generic binc	0	11.619	7	3	3	3	505	55.4	4.88	6.78	1.12	0.0024338	64.7	135.3
High	Q9UHX3	ADGRE2	GPCR	0	9.123	3	2	3	2	823	90.4	6.87	4.92	1.12	0.03965932	62.9	137.1
High	Q9BQS7	HEPH	Generic binc	0	7.866	2	2	2	2	1158	130.4	5.99	5.27	1.12	0.00380917	62.1	137.9
High	Q9NR46	SH3GLB2	Generic binc	0	7.298	9	3	3	3	395	43.9	5.99	5.18	1.12	0.00886947	65.3	134.7
High	Q8TCE6	DENND10	Protein	0.001	5.147	6	2	2	2	357	40.5	6.61	3.98	1.12	0.01446178	68.8	131.2
High	P07900	HSP90AA1	Generic binc	0	389.349	60	44	177	27	732	84.6	5.02	553.66	1.11	0.00056478	65	135
High	Q13885	TUBB2A	Generic binc	0	203.532	60	20	102	3	445	49.9	4.89	244.72	1.11	0.02318604	70.1	129.9
High	P11586	MTHFD1	Generic enz	0	96.644	25	21	31	21	935	101.5	7.3	88.84	1.11	0.00160137	62.9	137.1
High	Q7L1Q6	BZW1	Generic binc	0	72.801	47	17	23	16	419	48	5.92	62.12	1.11	0.00148093	64.8	135.2
High	O00571	DDX3X	Generic enz	0	70.001	29	15	22	14	662	73.2	7.18	48.5	1.11	0.00811584	62.5	137.5
High	P49189	ALDH9A1	Generic enz	0	64.849	24	10	23	10	494	53.8	5.87	67.73	1.11	0.00426887	63.4	136.6
High	Q07960	ARHGAP1	Regulators (	0	50.3	31	13	18	13	439	50.4	6.29	44.38	1.11	0.00516795	65.9	134.1
High	O95747	OXSR1	Protein kina	0	44.221	19	8	9	8	527	58	6.43	33.67	1.11	0.00967908	67.1	132.9
High	Q12907	LMAN2	Generic binc	0	22.605	18	5	7	5	356	40.2	6.95	17.96	1.11	0.00274484	66.4	133.6
High	Q6UX71	PLXDC2	Generic rece	0	19.797	12	5	7	5	529	59.5	6.46	20.61	1.11	0.00778574	68	132
High	Q07065	CKAP4	Generic binc	0	19.355	13	5	5	5	602	66	5.92	10.21	1.11	0.00533325	63.2	136.8
High	P36959	GMPR	Generic enz	0	14.462	16	4	4	3	345	37.4	7.06	11.75	1.11	0.00893081	63.3	136.7
High	Q9BXD5	NPL	Generic enz	0	14.406	13	3	3	3	320	35.1	5.57	12.2	1.11	0.00436911	63.4	136.6
High	Q6UWE0	LRSAM1	Generic enz	0	13.882	5	3	3	3	723	83.5	5.94	6.78	1.11	0.0083807	63.4	136.6
High	Q9NT62	ATG3	Generic binc	0	11.891	17	3	4	3	314	35.8	4.74	11.62	1.11	0.01028012	68.6	131.4
High	Q9H9C1	VIPAS39	Generic binc	0.006	2.868	3	2	2	2	493	57	7.4	0	1.11	0.00951791	63.5	136.5
High	Q16555	DPYSL2	Generic binc	0	118.477	48	19	34	18	536	58.1	6.15	105.97	1.1	0.00644021	62.6	137.4
High	Q16181	SEPTIN7	Generic binc	0	93.823	52	20	35	19	437	50.6	8.63	95.41	1.1	0.00536932	62.2	137.8
High	Q92499	DDX1	Generic enz	0	86.191	33	18	20	18	740	82.4	7.23	58.14	1.1	0.0039789	64	136
High	Q6ZNJ1	NBEAL2	Generic binc	0	85.526	8	19	22	18	2754	302.3	6.38	72.42	1.1	0.01161325	63.8	136.2
High	P29401	TKT	Generic enz	0	46.75	21	11	16	11	623	67.8	7.66	42.82	1.1	0.00114523	64.6	135.4
High	P09960	LTA4H	Generic enz	0	43.18	17	9	13	9	611	69.2	6.18	37.59	1.1	0.00257264	63.6	136.4
High	P00450	CP	Generic enz	0	43.098	11	11	19	11	1065	122.1	5.72	54.06	1.1	0.00336777	67.3	132.7
High	Q13126	MTAP	Generic enz	0	40.693	28	6	14	6	283	31.2	7.18	37.96	1.1	0.00042445	63.9	136.1
High	P55263	ADK	Generic kina	0	34.768	28	10	17	10	362	40.5	6.7	44.24	1.1	0.00042019	63.9	136.1
High	Q6IBS0	TWF2	Generic binc	0	19.733	14	3	4	3	349	39.5	6.84	11.21	1.1	8.4226E-05	63.8	136.2
High	P48163	ME1	Generic enz	0	19.684	10	5	5	5	572	64.1	6.13	12.84	1.1	0.0033731	62.6	137.4
High	P00387	CYB5R3	Generic enz	0	16.934	13	3	4	3	301	34.2	7.59	11.82	1.1	0.0008513	63.7	136.3
High	Q9BWD1	ACAT2	Generic enz	0	15.087	8	4	9	4	426	44.6	8.46	19.74	1.1	0.00759475	66.5	133.5
High	Q99961	SH3GL1	Generic binc	0	15.065	11	5	7	5	368	41.5	5.43	14.95	1.1	0.0050505	67.6	132.4
High	P37235	HPCAL1	Generic binc	0	12.668	23	4	5	4	193	22.3	5.35	8.8	1.1	0.01506615	63.7	136.3
High	P23258	TUBG1	Generic binc	0	8.397	6	2	2	2	451	51.1	6.14	4.07	1.1	0.01433615	63.7	136.3
High	Q13363	CTBP1	Generic binc	0	6.644	7	2	2	2	440	47.5	6.77	5	1.1	0.02117605	63.6	136.4

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Up regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	Q14457	BECN1	Generic binc	0.001	4.745	4	2	2	2	450	51.9	4.89	2.34	1.1	0.00988755	64.9	135.1
High	P00558	PGK1	Generic kina	0	194.551	68	24	64	24	417	44.6	8.1	220.5	1.09	0.00042661	64.1	135.9
High	P31146	CORO1A	Generic binc	0	109.627	35	12	31	12	461	51	6.68	107.28	1.09	0.00553723	64	136
High	O14980	XPO1	Transporter	0	108.528	26	23	32	23	1071	123.3	6.06	94.64	1.09	0.00094805	63.9	136.1
High	P16112	ACAN	Generic binc	0	81.663	6	16	34	16	2415	250	4.18	99.32	1.09	0.02992438	65.6	134.4
High	P11277	SPTB	Generic binc	0	53.073	8	18	21	13	2137	246.3	5.27	38.83	1.09	0.00058371	63.7	136.3
High	P16152	CBR1	Generic enz	0	53.06	32	8	20	8	277	30.4	8.32	61.33	1.09	0.00096928	65.2	134.8
High	Q8WVM8	SCFD1	Generic binc	0	40.993	17	9	10	9	642	72.3	6.27	24.63	1.09	0.00549154	64	136
High	Q92905	COPS5	Generic enz	0	32.087	25	7	11	7	334	37.6	6.54	27.55	1.09	0.00691499	63.2	136.8
High	P27361	MAPK3	Protein kina	0	25.8	16	5	10	2	379	43.1	6.74	27.69	1.09	0.00531794	64.2	135.8
High	O15260	SURF4	Generic binc	0	18.727	12	2	3	2	269	30.4	7.78	12.16	1.09	0.01404175	59.1	140.9
High	Q09028	RBBP4	Protein	0	16.084	13	4	4	2	425	47.6	4.89	11.75	1.09	0.00797976	65.4	134.6
High	O00743	PPP6C	Protein phos	0	15.805	14	4	6	4	305	35.1	5.69	14.77	1.09	0.00187699	65	135
High	P62318	SNRPD3	Generic binc	0	15.766	25	2	5	2	126	13.9	10.32	15.38	1.09	0.01809771	63.8	136.2
High	P23470	PTPRG	Generic rece	0	14.603	3	4	4	4	1445	161.9	6.42	11.99	1.09	0.02698586	70.1	129.9
High	Q5W0V3	FAM160B1	Protein	0	6.201	4	2	2	2	765	86.5	5.29	2.97	1.09	0.00026573	63.9	136.1
High	Q9H8Y8	GORASP2	Generic binc	0.002	4.618	4	2	2	2	452	47.1	4.82	4.53	1.09	0.01124439	69.3	130.7
High	Q9H270	VPS11	Generic binc	0.004	3.594	3	2	2	2	941	107.8	7.05	0	1.09	0.00215923	65.7	134.3
High	P18669	PGAM1	Generic pho	0	106.574	69	14	45	14	254	28.8	7.18	130.02	1.08	0.00833908	67.4	132.6
High	P13591	NCAM1	Generic binc	0	70.509	16	10	21	10	848	93.3	4.87	64.39	1.08	0.03175706	67.1	132.9
High	O95834	EML2	Generic binc	0	60.288	18	10	36	10	649	70.6	6.32	83.69	1.08	0.00691311	64.2	135.8
High	P21281	ATP6V1B2	Generic cha	0	59.469	32	12	16	12	511	56.5	5.81	55.22	1.08	0.00317419	64.1	135.9
High	P56192	MARS1	Generic enz	0	50.01	18	10	12	10	900	101.1	6.16	38.17	1.08	0.00523742	62.8	137.2
High	Q14651	PLS1	Generic binc	0	39.877	16	8	11	6	629	70.2	5.41	28.29	1.08	0.00089011	65	135
High	P30613	PKLR	Generic kina	0	30.261	10	7	17	6	574	61.8	7.74	33.01	1.08	0.00296768	64.3	135.7
High	Q96EK6	GNPNAT1	Generic enz	0	23.715	35	5	6	5	184	20.7	7.99	17.84	1.08	0.00346209	66.8	133.2
High	O43815	STRN	Generic binc	0	21.034	9	6	7	5	780	86.1	5.27	15.78	1.08	0.00286483	64.2	135.8
High	P00749	PLAU	Generic prot	0	16.438	15	5	5	5	431	48.5	8.41	10.74	1.08	0.00623329	66	134
High	Q08257	CRYZ	Generic enz	0	13.254	16	5	6	5	329	35.2	8.44	8.36	1.08	0.00019218	64.2	135.8
High	Q16853	AOC3	Generic enz	0	8.709	3	3	6	3	763	84.6	6.52	17.7	1.08	0.01618238	63.8	136.2
High	Q1KMD3	HNRNPUL2	Generic binc	0	7.716	4	3	3	3	747	85.1	4.91	2.6	1.08	0.01122538	59.4	140.6
High	Q9NY97	B3GNT2	Generic enz	0	7.501	8	3	5	3	397	46	8.54	10.16	1.08	0.00181246	65.8	134.2
High	O14920	IKBKB	Protein kina	0	6.294	3	2	2	2	756	86.5	5.78	4.9	1.08	0.01314035	64.2	135.8
High	P00374	DHFR	Generic enz	0.006	3.124	8	2	3	2	187	21.4	7.42	6.61	1.08	0.00161796	63.7	136.3
High	P53621	COPA	Generic binc	0	191.539	38	35	53	35	1224	138.3	7.66	167.89	1.07	0.01386426	64.5	135.5
High	P41252	IARS1	Generic enz	0	161.173	32	31	49	31	1262	144.4	6.15	144.4	1.07	0.00693983	63.7	136.3
High	P06748	NPM1	Generic binc	0	93.316	36	10	20	10	294	32.6	4.78	67.11	1.07	0.03359267	64.4	135.6
High	Q13561	DCTN2	Generic binc	0	67.991	41	14	21	14	401	44.2	5.21	69.14	1.07	0.0032591	66.2	133.8
High	Q9NQW7	XPNPEP1	Metalloprot	0	67.036	24	12	15	12	623	69.9	5.67	55.33	1.07	0.00784873	64.5	135.5
High	Q15366	PCBP2	Generic binc	0	43.762	22	7	13	2	365	38.6	6.79	34.7	1.07	0.00607946	66.4	133.6
High	Q15057	ACAP2	Regulators (	0	35.712	15	9	10	9	778	88	6.8	21.33	1.07	0.00165214	64.4	135.6
High	P22694	PRKACB	Protein kina	0	35.213	22	7	10	2	351	40.6	8.78	30.25	1.07	0.00387607	65.7	134.3
High	Q86X55	CARM1	Generic enz	0	33.005	14	8	10	8	608	65.8	6.73	27.35	1.07	0.00503757	63.7	136.3

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Up regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	Q13492	PICALM	Generic binc	0	32.234	14	8	11	8	652	70.7	7.9	26.63	1.07	0.00990876	63.6	136.4
High	Q9H9T3	ELP3	Generic binc	0	19.804	10	5	7	5	547	62.2	8.88	17.13	1.07	0.01566912	65.4	134.6
High	P48507	GCLM	Generic enz	0	19.789	17	4	8	4	274	30.7	6.02	22.71	1.07	0.00221148	63.7	136.3
High	Q9Y6D6	ARFGEF1	Regulators (	0	19.593	4	6	6	3	1849	208.6	5.85	9.45	1.07	0.00568211	64.4	135.6
High	Q9H3P7	ACBD3	Generic binc	0	15.293	9	3	3	3	528	60.6	5.06	12.22	1.07	0.02177875	60.2	139.8
High	Q9NPH3	IL1RAP	Generic rece	0	12.049	4	3	5	3	570	65.4	8.12	13.16	1.07	0.04080062	68.5	131.5
High	Q7Z5N4	SDK1	Generic binc	0	11.578	2	3	3	3	2213	242	6.39	8.7	1.07	0.00694148	65.1	134.9
High	Q9NTU7	CBLN4	Protein	0	11.079	16	3	3	3	201	21.8	8.73	9.08	1.07	0.01905164	67	133
High	Q9Y4E1	WASHC2C	Generic pho	0	9.512	6	4	4	4	1318	144.6	4.79	5.11	1.07	0.00650996	62.8	137.2
High	Q9UPN7	PPP6R1	Generic binc	0	8.694	4	2	2	2	881	96.7	4.55	3.74	1.07	0.00207619	63.6	136.4
High	P06454	PTMA	Protein	0	6.184	13	2	2	2	111	12.2	3.78	2.68	1.07	0.02126258	67.6	132.4
High	P05106	ITGB3	Generic rece	0	173.76	36	23	70	23	788	87	5.24	194.35	1.06	0.02212644	64.9	135.1
High	P62333	PSMC6	Generic binc	0	127.762	59	19	43	19	389	44.1	7.49	127.45	1.06	0.00743486	65	135
High	Q7Z6Z7	HUWE1	Generic enz	0	109.367	7	27	34	27	4374	481.6	5.22	83.13	1.06	0.0024429	67.1	132.9
High	P52907	CAPZA1	Protein	0	79.648	50	11	24	9	286	32.9	5.69	88.99	1.06	0.00200146	64.7	135.3
High	Q16401	PSMD5	Generic binc	0	63.19	26	10	18	10	504	56.2	5.48	61.34	1.06	0.00231101	64.9	135.1
High	Q9NVA2	SEPTIN11	Generic binc	0	46.106	30	11	24	5	429	49.4	6.81	47.89	1.06	0.00216718	64.8	135.2
High	Q7Z460	CLASP1	Generic binc	0	45.451	9	10	11	10	1494	164.5	8.47	26.04	1.06	0.00652638	68	132
High	P21266	GSTM3	Generic enz	0	22.755	29	6	8	5	225	26.5	5.54	15.79	1.06	0.00125876	66.8	133.2
High	Q9UJY5	GGA1	Generic binc	0	22.664	10	4	5	4	639	70.3	5.29	15.25	1.06	0.04391227	67.1	132.9
High	P08514	ITGA2B	Generic rece	0	22.644	3	3	9	3	1039	113.3	5.38	23.02	1.06	0.01883311	64.9	135.1
High	P19784	CSNK2A2	Protein kina	0	15.895	15	4	4	4	350	41.2	8.56	8.92	1.06	0.00537693	64.7	135.3
High	Q15370	ELOB	Generic enz	0	15.705	42	4	6	4	118	13.1	4.88	13.61	1.06	0.0031895	65.1	134.9
High	Q9NZI8	IGF2BP1	Generic binc	0	14.36	6	3	3	3	577	63.4	9.2	9.45	1.06	0.00341461	62.9	137.1
High	P49589	CARS1	Generic enz	0	14.113	6	4	4	4	748	85.4	6.76	9.89	1.06	0.0099729	64.1	135.9
High	P06727	APOA4	Receptor lig	0	13.783	6	3	7	3	396	45.4	5.38	20.46	1.06	0.03777007	64.9	135.1
High	Q10469	MGAT2	Generic enz	0	13.378	9	4	4	4	447	51.5	8.76	12.27	1.06	0.01059682	71.2	128.8
High	O00533	CHL1	Generic binc	0	12.916	4	4	4	4	1208	135	5.76	10.05	1.06	0.02572769	65.9	134.1
High	Q00765	REEP5	Transporter	0	12.484	20	5	6	5	189	21.5	8.1	13.28	1.06	0.01533321	64.2	135.8
High	Q96C24	SYTL4	Generic binc	0	11.476	5	3	4	3	671	76	8.98	7.93	1.06	0.00448304	66.9	133.1
High	Q9NPB8	GPCPD1	Generic enz	0	10.253	4	3	4	3	672	76	5.53	8.51	1.06	0.01201093	64.7	135.3
High	Q8TAG9	EXOC6	Protein	0	9.054	3	2	2	2	804	93.7	6.2	6.89	1.06	0.00807977	64.9	135.1
High	Q6UWP8	SBSN	Protein	0	7.171	12	2	2	2	590	60.5	7.01	0	1.06	0.00507998	62.7	137.3
High	Q0VDG4	SCRN3	Generic prot	0	6.783	5	2	2	2	424	48.5	5.55	3.06	1.06	0.00313057	64.7	135.3
High	Q8IUI8	CRLF3	Protein	0.001	5.5	6	3	3	3	442	49.7	5.14	4.53	1.06	0.00490871	66.2	133.8
High	Q99490	AGAP2	Regulators (	0.001	5.167	3	3	3	2	1192	124.6	9.89	0	1.06	0.02077955	69.9	130.1
High	P17980	PSMC3	Generic binc	0	153.66	60	22	47	22	439	49.2	5.24	126.28	1.05	0.00427291	65	135
High	P55060	CSE1L	Transporter	0	135.601	35	30	43	30	971	110.3	5.77	113.35	1.05	0.00082054	66.2	133.8
High	O75955	FLOT1	Generic binc	0	128.798	55	19	43	19	427	47.3	7.49	128.95	1.05	0.01432944	65.2	134.8
High	P09525	ANXA4	Generic binc	0	122.579	71	21	36	3	319	35.9	6.13	113.9	1.05	0.00799309	65.1	134.9
High	P43686	PSMC4	Generic binc	0	119.761	61	20	37	19	418	47.3	5.21	102.14	1.05	0.01172278	65.1	134.9
High	Q9UKU9	ANGPTL2	Receptor lig	0	74.744	36	18	24	16	493	57.1	7.53	66.72	1.05	0.01188035	65.4	134.6
High	Q13642	FHL1	Generic binc	0	37.392	30	7	18	7	280	31.9	8.32	32.71	1.05	0.00616148	65.1	134.9

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Up regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	Q9NQ79	CRTAC1	Generic binc	0	25.265	9	4	6	4	661	71.4	5.12	22.41	1.05	0.00425618	66.3	133.7
High	P50502	ST13	Generic binc	0	24.396	18	6	11	6	369	41.3	5.27	20.46	1.05	0.00075709	65.1	134.9
High	O75643	SNRNP200	Generic enz	0	22.659	5	7	7	7	2136	244.4	6.06	14.64	1.05	0.01725553	65.2	134.8
High	P00491	PNP	Generic enz	0	21.307	17	4	5	4	289	32.1	6.95	15.23	1.05	0.00322694	66.1	133.9
High	Q96PD5	PGLYRP2	Generic rece	0	18.233	5	2	6	2	576	62.2	7.55	24.07	1.05	0.01480609	68	132
High	Q32P51	HNRNPA1L	Generic binc	0	17.514	13	4	5	3	320	34.2	9	11.75	1.05	0.01711166	65.1	134.9
High	Q13201	MMRN1	Receptor lig	0	15.962	2	3	7	3	1228	138	7.93	16.37	1.05	0.01095322	63.1	136.9
High	P08575	PTPRC	Generic rece	0	15.127	4	5	5	5	1304	147.2	6.15	10.65	1.05	0.02321393	65.2	134.8
High	P13473	LAMP2	Generic binc	0	13.454	14	6	7	6	410	44.9	5.69	16.15	1.05	0.00060414	66.2	133.8
High	P05107	ITGB2	Generic rece	0	10.717	5	3	3	2	769	84.7	6.95	6.5	1.05	0.00246054	64.8	135.2
High	P17405	SMPD1	Generic enz	0	9.856	4	2	3	2	629	69.7	7.28	6.88	1.05	0.00742248	65.4	134.6
High	Q9BQG0	MYBBP1A	Generic binc	0	7.011	3	3	3	3	1328	148.8	9.28	3.13	1.05	0.0148792	63.9	136.1
High	Q99719	SEPTIN5	Generic binc	0.002	4.327	5	2	8	2	369	42.8	6.67	14.78	1.05	0.03368806	62.7	137.3
High	Q9HA65	TBC1D17	Regulators (	0.004	3.609	3	2	2	2	648	72.7	5.19	3.61	1.05	0.00795699	62.8	137.2
High	P60709	ACTB	Generic binc	0	327.914	63	23	377	11	375	41.7	5.48	1169.84	1.04	0.00108494	65.5	134.5
High	P23921	RRM1	Generic enz	0	92.836	30	19	26	19	792	90	7.15	67.37	1.04	0.01253637	65.1	134.9
High	P28482	MAPK1	Protein kina	0	68.806	40	13	25	10	360	41.4	6.98	67.22	1.04	0.00850493	65.5	134.5
High	Q15365	PCBP1	Generic binc	0	61.529	37	10	17	5	356	37.5	7.09	46.78	1.04	0.00486821	68.8	131.2
High	P04040	CAT	Generic enz	0	43.54	16	10	13	10	527	59.7	7.39	39.65	1.04	0.0054617	65.4	134.6
High	P55036	PSMD4	Generic binc	0	32.423	21	5	8	4	377	40.7	4.79	25.65	1.04	0.00615094	68.1	131.9
High	Q03135	CAV1	Generic binc	0	32.412	44	7	12	7	178	20.5	6.02	32.15	1.04	0.00216613	65.4	134.6
High	Q9UBW8	COPS7A	Generic enz	0	25.597	21	5	5	5	275	30.3	8.22	17.78	1.04	0.00244852	65.5	134.5
High	Q9UMS4	PRPF19	Generic binc	0	22.497	12	5	6	5	504	55.1	6.61	18.5	1.04	0.01757169	63.7	136.3
High	Q8IXL6	FAM20C	Protein kina	0	20.286	8	4	6	4	584	66.2	7.74	14.81	1.04	0.00507186	65.9	134.1
High	P17050	NAGA	Generic enz	0	17.706	9	3	5	3	411	46.5	5.19	15.95	1.04	0.02991048	61.5	138.5
High	O76003	GLRX3	Generic enz	0	16.801	18	5	6	5	335	37.4	5.39	17.78	1.04	0.00049372	65.5	134.5
High	O75935	DCTN3	Protein	0	13.681	15	2	5	2	186	21.1	5.47	16.27	1.04	0.00085078	65.7	134.3
High	A8K7I4	CLCA1	Generic binc	0	10.96	4	3	3	3	914	100.2	6.37	8.76	1.04	0.02822676	63.1	136.9
High	Q9NRV9	HEBP1	Generic binc	0	10.544	17	2	4	2	189	21.1	5.8	12.47	1.04	0.00489031	65	135
High	O14828	SCAMP3	Transporter	0	10.06	8	2	2	2	347	38.3	7.64	6.45	1.04	0.01671589	65.1	134.9
High	Q9BWP8	COLEC11	Generic binc	0	9.634	12	3	9	3	271	28.6	5.41	18.3	1.04	0.02676455	65.5	134.5
High	Q5T5C0	STXBP5	Generic binc	0	9.026	2	2	2	2	1151	127.5	7.28	4.71	1.04	0.00034438	65.8	134.2
High	Q7Z3E5	ARMC9	Generic binc	0.001	5.414	3	2	2	2	817	91.7	6.2	0	1.04	0.01914131	64.3	135.7
High	P14618	PKM	Generic kina	0	425.565	77	42	187	7	531	58	7.71	596.93	1.03	0.00364355	65.7	134.3
High	P47756	CAPZB	Generic binc	0	77.981	47	14	29	14	272	30.6	6	76.23	1.03	0.00342719	69	131
High	P06858	LPL	Generic binc	0	51.689	17	7	19	7	475	53.1	8.15	58.31	1.03	0.00125689	65.3	134.7
High	Q15084	PDIA6	Generic enz	0	50.957	38	11	13	11	440	48.1	5.08	41.23	1.03	0.03852785	72.3	127.7
High	Q13619	CUL4A	Generic enz	0	48.611	17	11	14	4	759	87.6	8.13	43.32	1.03	0.00110612	66	134
High	Q00266	MAT1A	Generic enz	0	48.384	22	6	12	4	395	43.6	6.29	41.76	1.03	0.00586368	63.7	136.3
High	P05543	SERPINA7	Transporter	0	39.908	12	5	19	5	415	46.3	6.3	57.6	1.03	0.03208437	69.1	130.9
High	P36507	MAP2K2	Protein kina	0	38.857	20	8	10	4	400	44.4	6.55	27.81	1.03	0.00157633	68.2	131.8
High	Q13838	DDX39B	Generic enz	0	31.043	22	6	9	3	428	49	5.67	25.07	1.03	0.00091459	67.3	132.7
High	Q03001	DST	Generic binc	0	30.956	1	7	7	5	7570	860.1	5.25	17.41	1.03	0.00800044	66.7	133.3

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Up regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	Q5T447	HECTD3	Generic enz	0	30.926	10	6	9	6	861	97.1	5.64	27.7	1.03	0.00730103	65.6	134.4
High	Q9H3U1	UNC45A	Generic bind	0	29.59	9	8	11	8	944	103	6.07	20.71	1.03	0.00562256	65.7	134.3
High	P51570	GALK1	Generic kina	0	29.032	15	6	18	6	392	42.2	6.46	52.61	1.03	0.00389059	66.2	133.8
High	P16885	PLCG2	Generic pho	0	28.871	10	11	11	11	1265	147.8	6.64	20.2	1.03	0.00607408	67	133
High	O15067	PFAS	Generic enz	0	27.656	4	5	12	5	1338	144.6	5.76	26.17	1.03	0.02497642	65.6	134.4
High	Q9Y4E8	USP15	Generic pro	0	27.556	9	8	9	7	981	112.3	5.22	17.78	1.03	0.01547754	65.5	134.5
High	O60502	OGA	Generic enz	0	25.576	8	5	5	5	916	102.8	4.91	17.76	1.03	0.01116908	65.6	134.4
High	Q8NI99	ANGPTL6	Generic bind	0	25.121	10	4	8	3	470	51.7	8.53	29.3	1.03	0.00873995	63	137
High	O94915	FRYL	Generic bind	0	18.967	3	7	8	7	3013	339.4	5.58	13.81	1.03	0.00432697	66.5	133.5
High	Q6DD88	ATL3	Generic bind	0	18.311	12	5	6	5	541	60.5	5.66	13.06	1.03	0.00267271	67.3	132.7
High	O75608	LYPLA1	Generic enz	0	13.22	15	3	3	3	230	24.7	6.77	9.05	1.03	0.00041682	64.6	135.4
High	Q92572	AP3S1	Transporter	0	12.228	24	3	3	3	193	21.7	5.39	7.92	1.03	0.01451306	65.9	134.1
High	O43143	DHX15	Generic enz	0	11.733	6	3	3	3	795	90.9	7.46	6.79	1.03	0.0126828	65.8	134.2
High	O15305	PMM2	Generic enz	0	10.683	15	3	4	3	246	28.1	6.77	3.24	1.03	4.2229E-06	65.7	134.3
High	Q9UDY2	TJP2	Generic bind	0	9.7	4	4	4	4	1190	133.9	7.4	4.82	1.03	0.00931448	62.6	137.4
High	Q9Y5B9	SUPT16H	Metalloprot	0	7.454	3	3	3	3	1047	119.8	5.66	2.56	1.03	0.03692621	65.7	134.3
High	Q8WYJ6	SEPTIN1	Generic bind	0	7.383	7	2	2	2	367	41.9	5.8	3.56	1.03	0.00492209	63.4	136.6
High	P32929	CTH	Generic enz	0	6.246	6	2	3	2	405	44.5	6.7	1.99	1.03	1.6876E-05	65.8	134.2
High	O75436	VPS26A	Generic bind	0.001	5.175	7	2	2	2	327	38.1	6.57	3.08	1.03	0.00222101	65.5	134.5
High	Q02952	AKAP12	Generic bind	0.005	3.479	1	2	2	2	1782	191.4	4.41	0	1.03	0.0026325	65.9	134.1
High	P21399	ACO1	Generic bind	0	123.836	29	21	38	21	889	98.3	6.68	113.42	1.02	0.00828704	66.1	133.9
High	P55209	NAP1L1	Generic bind	0	113.602	46	12	30	3	391	45.3	4.46	106.63	1.02	0.00649256	70.6	129.4
High	P35606	COPB2	Generic bind	0	109.731	40	25	34	25	906	102.4	5.27	83.54	1.02	0.01863868	64.8	135.2
High	P49591	SARS1	Generic enz	0	95.425	47	21	29	21	514	58.7	6.43	73.55	1.02	0.00448948	66.2	133.8
High	Q5VYK3	ECPAS	Generic bind	0	77.872	11	16	19	16	1845	204.2	7.12	54.49	1.02	0.00148012	68.5	131.5
High	O00232	PSMD12	Generic bind	0	73.964	32	13	21	13	456	52.9	7.65	63.75	1.02	0.01235242	65.9	134.1
High	P54136	RARS1	Generic enz	0	63.854	25	14	19	14	660	75.3	6.68	49.25	1.02	0.00500888	65.4	134.6
High	P13861	PRKAR2A	Generic bind	0	56.398	31	11	19	9	404	45.5	5.07	50.23	1.02	0.00413086	66.2	133.8
High	P50570	DNM2	Protein	0	49.253	20	14	17	9	870	98	7.44	39.99	1.02	3.2291E-05	66	134
High	Q15833	STXBP2	Generic bind	0	46.867	22	11	15	10	593	66.4	6.55	37.35	1.02	0.00065624	66.1	133.9
High	P21964	COMT	Generic enz	0	33.237	21	6	7	6	271	30	5.47	21.29	1.02	0.00587555	65.9	134.1
High	P62495	ETF1	Generic bind	0	30.815	26	9	11	9	437	49	5.71	24.88	1.02	0.0019647	66.1	133.9
High	P68400	CSNK2A1	Protein kina	0	26.667	21	6	10	6	391	45.1	7.74	22.96	1.02	0.00657148	65.5	134.5
High	Q9UN36	NDRG2	Protein	0	23.593	19	5	8	5	371	40.8	5.21	20.44	1.02	0.00824578	68.2	131.8
High	Q14738	PPP2R5D	Generic bind	0	21.698	11	6	7	6	602	69.9	8.13	14.36	1.02	0.00178517	65.2	134.8
High	P22626	HNRNPA2E	Generic bind	0	17.82	7	3	4	2	353	37.4	8.95	13.18	1.02	0.01643813	68.2	131.8
High	P10809	HSPD1	Generic bind	0	14.356	8	2	3	2	573	61	5.87	7.31	1.02	0.02463747	68.5	131.5
High	Q9NR19	ACSS2	Generic enz	0	13.389	4	3	5	3	701	78.5	6.46	13.94	1.02	0.00580801	66.2	133.8
High	O95466	FMNL1	Generic bind	0	11.638	3	2	2	2	1100	121.8	5.72	8.13	1.02	0.00031297	65.3	134.7
High	Q6UWP2	DHRS11	Generic enz	0	11.521	12	3	4	3	260	28.3	6.64	12.44	1.02	0.00166074	66.7	133.3
High	O60234	GMFG	Generic bind	0	10.78	24	3	3	2	142	16.8	5.26	9.49	1.02	0.00530594	68.4	131.6
High	P09488	GSTM1	Generic enz	0	10.202	19	4	5	3	218	25.7	6.7	12.69	1.02	0.00638153	67	133

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Up regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	Q8NEU8	APPL2	Generic binc	0	9.69	4	2	3	2	664	74.4	4.94	8.97	1.02	0.03032162	65.1	134.9
High	O60826	CCDC22	Protein	0	8.471	5	3	3	3	627	70.7	6.74	6.52	1.02	0.04649458	65.5	134.5
High	O00423	EML1	Generic pho	0	7.29	4	3	3	3	815	89.8	7.06	2.94	1.02	0.00401029	65.4	134.6
High	Q8N584	TTC39C	Generic binc	0	7.032	4	2	2	2	583	65.8	6.99	2.75	1.02	0.00354476	65.3	134.7
High	Q9Y6Y8	SEC23IP	Generic binc	0	6.543	4	4	4	4	1000	111	5.54	4.81	1.02	0.00964662	67.3	132.7
High	Q9H7D7	WDR26	Protein	0	6.267	4	2	2	2	661	72.1	6.16	4.32	1.02	0.01490724	66.7	133.3
High	Q9Y2X3	NOP58	Generic enz	0.001	4.981	6	2	2	2	529	59.5	8.92	2.16	1.02	0.00036565	66.1	133.9
High	Q12929	EPS8	Generic binc	0.002	4.196	3	2	2	2	822	91.8	7.5	0	1.02	0.00172906	68.5	131.5
High	P11217	PYGM	Generic enz	0	107.703	32	25	36	14	842	97	7.03	90.28	1.01	0.00796403	66.3	133.7
High	Q96F07	CYFIP2	Generic binc	0	80.445	16	18	22	2	1278	148.3	7.31	67.32	1.01	0.00256922	67.8	132.2
High	Q9Y5P6	GMPPB	Generic enz	0	29.145	22	6	9	6	360	39.8	6.61	25.06	1.01	0.00049885	67.3	132.7
High	Q92835	INPP5D	Generic pho	0	24.228	6	5	5	5	1189	133.2	7.59	17.08	1.01	0.00145525	66.5	133.5
High	P00439	PAH	Generic enz	0	22.482	12	5	10	5	452	51.8	6.6	23.79	1.01	0.03833393	66.3	133.7
High	P35813	PPM1A	Protein phos	0	22.44	17	5	7	5	382	42.4	5.36	20.09	1.01	0.01101752	71.8	128.2
High	P50851	LRBA	Generic binc	0	20.802	3	8	9	7	2863	318.9	5.6	12.55	1.01	0.00541527	66.2	133.8
High	Q9Y6E0	STK24	Protein kina	0	19.873	14	5	5	3	443	49.3	5.69	11.98	1.01	0.01755904	68.6	131.4
High	P08237	PFKM	Generic kina	0	18.598	7	4	4	2	780	85.1	7.99	13.39	1.01	0.00862517	67.8	132.2
High	Q05209	PTPN12	Protein phos	0	17.163	9	7	7	7	780	88.1	5.62	11.83	1.01	0.0072121	63.4	136.6
High	P31323	PRKAR2B	Generic binc	0	15.359	11	4	4	2	418	46.3	4.92	13.28	1.01	0.01477346	64	136
High	Q96KP1	EXOC2	Generic binc	0	15.234	5	4	4	4	924	104	6.9	13.56	1.01	0.00096353	66.5	133.5
High	Q14515	SPARCL1	Generic binc	0	14.121	7	4	6	4	664	75.2	4.81	16.91	1.01	0.00096371	67	133
High	Q9Y3P9	RABGAP1	Regulators (	0	12.074	4	4	4	4	1069	121.7	5.25	7.19	1.01	0.00338709	66.3	133.7
High	P49641	MAN2A2	Generic enz	0	10.17	3	3	5	3	1139	128.8	8.05	8.56	1.01	0.01881431	65.3	134.7
High	Q9UNW1	MINPP1	Generic pho	0	9.613	7	3	3	3	487	55	7.81	8.76	1.01	0.00031804	66.8	133.2
High	Q05086	UBE3A	Generic enz	0	7.894	4	2	2	2	875	100.6	5.22	5.6	1.01	0.02037676	67.2	132.8
High	Q13275	SEMA3F	Generic rece	0	6.082	3	2	2	2	785	88.3	8.27	5.35	1.01	0.00886513	69	131
High	P49792	RANBP2	Generic cha	0.005	3.678	1	2	2	2	3224	358	6.2	2.21	1.01	0.00466718	66.1	133.9
High	P16050	ALOX15	Generic enz	0.006	2.927	3	2	2	2	662	74.8	6.58	4.1	1.01	0.0255654	63.7	136.3
High	P53618	COPB1	Generic binc	0	170.814	39	32	49	32	953	107.1	6.05	137.95	1	0.0133018	65.7	134.3
High	O76074	PDE5A	Protein	0	128.442	29	23	36	23	875	99.9	6.09	106.76	1	0.00238582	66.7	133.3
High	P05771	PRKCB	Protein kina	0	66.761	24	14	20	14	673	77	7.02	52.5	1	0.01036065	66.3	133.7
High	Q8TD16	BICD2	Generic binc	0	48.161	15	11	12	11	824	93.5	5.44	38.93	1	0.02121966	65.2	134.8
High	P52294	KPNA1	Transporter	0	43.283	22	8	10	4	538	60.2	5.01	25.32	1	0.00104779	68.5	131.5
High	Q9Y613	FHOD1	Generic binc	0	40.583	7	7	11	7	1164	126.5	6.39	28.38	1	0.00069825	68	132
High	P51692	STAT5B	Transcriptio	0	35.251	15	11	14	11	787	89.8	6.05	25.64	1	0.00143573	66.7	133.3
High	Q13098	GPS1	Generic enz	0	28.271	17	8	9	8	491	55.5	6.74	22.66	1	0.0087217	66.7	133.3
High	Q9UI12	ATP6V1H	Generic cha	0	25.519	15	6	6	6	483	55.8	6.48	16.42	1	0.00205795	65.9	134.1
High	P62906	RPL10A	Generic binc	0	19.828	31	7	8	7	217	24.8	9.94	15.61	1	0.00798565	67.7	132.3
High	Q13576	IQGAP2	Generic binc	0	19.267	4	6	7	4	1575	180.5	5.64	18.53	1	0.00143034	67.3	132.7
High	P19801	AOC1	Generic enz	0	15.579	4	4	9	4	751	85.3	7.09	20.87	1	0.03381372	65.8	134.2
High	Q9UPY8	MAPRE3	Generic binc	0	15.164	20	5	6	3	281	32	5.54	13.03	1	0.018314	66.8	133.2
High	Q9P2T1	GMPR2	Generic enz	0	14.679	18	6	6	5	348	37.9	7.23	10.44	1	0.00142664	66.9	133.1

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Up regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	Q9NRF8	CTPS2	Generic enz	0	14.391	8	4	4	2	586	65.6	6.9	12.33	1	0.00504172	66.8	133.2
High	P46976	GYG1	Generic bind	0	13.972	16	6	7	6	350	39.4	5.53	13.65	1	0.00510852	66.7	133.3
High	Q9NV70	EXOC1	Protein	0	13.333	7	6	6	6	894	101.9	6.61	9.71	1	0.00023995	66.8	133.2
High	Q9BTE6	AARSD1	Generic bind	0	12.587	10	4	5	4	412	45.5	6.42	10.42	1	0.00429315	67.2	132.8
High	P42892	ECE1	Generic prot	0	12.249	5	3	3	3	770	87.1	5.88	7.6	1	0.00023976	66.7	133.3
High	Q8IYI6	EXOC8	Generic bind	0	7.892	3	2	2	2	725	81.7	5.49	6	1	0.01139321	61.7	138.3
High	P0C862	C1QTNF9	Protein	0	7.751	8	2	3	2	333	34.7	8.46	8.31	1	0.00233252	63.1	136.9
High	Q93009	USP7	Generic prot	0.002	4.665	2	2	2	2	1102	128.2	5.55	2.59	1	0.0092145	64.9	135.1
High	Q92945	KHSRP	Generic bind	0.002	4.485	3	2	2	2	711	73.1	7.3	2.49	1	0.00981024	66.9	133.1
High	Q9P258	RCC2	Protein	0.004	3.633	3	2	2	2	522	56	8.78	2.2	1	0.0035239	66.5	133.5

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Down regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	Q7Z5L7	PODN	Generic binc	0	66.865	28	15	19	15	613	68.9	6.99	48.45	-1	0.00170236	135.9	64.1
High	Q15389	ANGPT1	Receptor lig	0	42.431	20	10	16	10	498	57.5	6.76	39.51	-1	0.00642425	134.3	65.7
High	P48509	CD151	Generic rece	0	25.63	24	7	15	7	253	28.3	7.47	35.69	-1.01	0.01306028	132.5	67.5
High	Q9UKP4	ADAMTS7	Metalloprot	0	8.085	2	4	4	4	1686	184	6.2	4.82	-1.01	0.01491904	133.8	66.2
High	Q86Y38	XYLT1	Generic enz	0	71.715	21	15	21	15	959	107.5	9.22	61.74	-1.03	0.00097353	134.4	65.6
High	Q15043	SLC39A14	Transporter	0	26.154	9	3	5	3	492	54.2	5.33	18.88	-1.03	0.00463482	135.7	64.3
High	Q7Z304	MAMDC2	Generic binc	0	152.951	41	22	56	22	686	77.5	5.16	163.06	-1.04	0.01004071	134.6	65.4
High	P35613	BSG	Generic binc	0	86.024	35	11	25	11	385	42.2	5.66	65.49	-1.05	0.00229103	132.1	67.9
High	P29317	EPHA2	Receptor wi	0	50.596	22	15	19	14	976	108.2	6.23	36.67	-1.06	0.00636855	135.2	64.8
High	P04216	THY1	Generic binc	0	42.775	25	4	14	4	161	17.9	8.73	54.7	-1.06	0.00101736	135.2	64.8
High	P23560	BDNF	Receptor lig	0	20.843	24	4	6	4	247	27.8	8.79	17.6	-1.06	0.00320004	134.3	65.7
High	P20809	IL11	Receptor lig	0	12.608	12	2	4	2	199	21.4	10.62	11.02	-1.06	0.02577602	135.1	64.9
High	P17936	IGFBP3	Generic binc	0	48.076	42	10	17	10	291	31.7	8.69	47.06	-1.07	0.01328081	140	60
High	Q96L58	B3GALT6	Generic enz	0.002	4.598	5	2	2	2	329	37.1	9.66	2.1	-1.08	0.00466581	134.2	65.8
High	Q13438	OS9	Generic binc	0	67.355	21	12	19	12	667	75.5	4.87	56.13	-1.09	0.00777262	133.8	66.2
High	P07951	TPM2	Generic binc	0	40.966	31	11	19	4	284	33	4.67	36.36	-1.09	0.04356751	135.8	64.2
High	P80723	BASP1	Generic binc	0	101.223	83	13	38	13	227	22.7	4.63	100.98	-1.1	0.00012261	136.4	63.6
High	P04156	PRNP	Generic binc	0	16.802	17	4	7	4	253	27.6	9	13.49	-1.12	0.00239596	136.1	63.9
High	Q4LDE5	SVEP1	Generic binc	0	711.277	40	99	256	99	3571	389.9	5.5	764.63	-1.14	0.00361076	137.7	62.3
High	Q14112	NID2	Generic binc	0	554.992	56	60	246	2	1274	140.8	5.33	794.03	-1.14	0.01686436	136.4	63.6
High	O00391	QSOX1	Generic enz	0	214.588	53	35	67	35	747	82.5	8.92	205.29	-1.14	0.00276953	138.4	61.6
High	P35555	FBN1	Receptor lig	0	1276.55	58	129	731	123	2871	312	4.93	1968.69	-1.15	0.00373036	138	62
High	P09486	SPARC	Generic binc	0	137.808	61	15	48	15	303	34.6	4.84	159.22	-1.17	0.02069805	140.1	59.9
High	Q99985	SEMA3C	Generic rece	0	75.86	28	19	25	18	751	85.2	8.69	66.73	-1.18	0.00208794	138.8	61.2
High	Q9HCB6	SPON1	Generic binc	0	92.081	27	16	36	16	807	90.9	6.11	97.76	-1.19	0.00153957	139.8	60.2
High	Q9Y4F1	FARP1	Regulators (	0	92.684	28	23	27	23	1045	118.6	8.15	70	-1.2	0.00142798	139.5	60.5
High	Q9BQT9	CLSTN3	Generic binc	0	17.652	7	5	5	5	956	106	5.44	12.13	-1.2	0.01091763	139.2	60.8
High	Q9UBI6	GNG12	G betta/gam	0	15.995	44	3	5	3	72	8	8.97	14.64	-1.2	0.00566858	139.4	60.6
High	Q9H8M9	EVA1A	Protein	0.001	5.494	13	2	2	2	152	17.5	6.93	4.42	-1.2	0.00376941	139.9	60.1
High	Q12841	FSTL1	Generic binc	0	112.59	70	20	49	20	308	35	5.52	121.08	-1.21	0.01018777	139.9	60.1
High	Q96AY3	FKBP10	Generic binc	0	33.95	18	8	9	8	582	64.2	5.62	24.78	-1.25	0.0128381	140.2	59.8
High	P24593	IGFBP5	Generic binc	0	28.434	39	7	12	7	272	30.6	8.21	32.07	-1.25	0.00561412	144.1	55.9
High	Q14766	LTBP1	Generic binc	0	418.833	44	61	190	11	1721	186.7	5.96	492.5	-1.28	0.00219502	141.6	58.4
High	Q06481	APLP2	Generic binc	0	157.813	36	24	56	22	763	86.9	4.79	135.35	-1.28	0.00054729	140.7	59.3
High	O60565	GREM1	Generic binc	0	126.779	60	12	68	12	184	20.7	9.39	187.8	-1.29	0.00170652	141.4	58.6
High	Q9BTY2	FUCA2	Generic enz	0	20.024	11	5	7	5	467	54	6.25	12.78	-1.29	0.00543001	141.8	58.2
High	Q8WUJ3	CEMIP	Protein	0	114.34	25	28	41	28	1361	152.9	7.85	94.98	-1.31	0.00169189	142.7	57.3
High	O95633	FSTL3	Generic binc	0	11.242	18	4	6	4	263	27.6	6.77	6.5	-1.31	0.00818696	145.7	54.3
High	Q92819	HAS2	Generic enz	0	8.678	5	2	2	2	552	63.5	8.6	5.04	-1.31	0.00289188	141.6	58.4
High	Q9UHI8	ADAMTS1	Metalloprot	0	320.653	55	35	79	35	967	105.3	6.83	269.65	-1.32	0.00270558	143.6	56.4
High	Q32P28	P3H1	Generic enz	0	117.035	39	20	36	19	736	83.3	5.14	100.42	-1.32	0.00058789	142.8	57.2
High	O95965	ITGBL1	Generic rece	0	16.901	16	5	5	5	494	53.9	5.64	9.23	-1.34	0.00218675	143.9	56.1
High	Q99988	GDF15	Receptor lig	0	59.064	48	10	18	10	308	34.1	9.66	52.19	-1.35	0.00172472	143.7	56.3

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Down regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	P34741	SDC2	Generic rece	0	17.622	16	4	5	4	201	22.1	4.86	11.56	-1.35	0.00111473	143.7	56.3
High	O15230	LAMA5	Receptor lig	0	958.295	50	125	279	123	3695	399.5	7.02	926.88	-1.36	0.00162589	143.5	56.5
High	Q99542	MMP19	Metalloprot	0	53.945	26	9	14	9	508	57.3	7.59	44.74	-1.36	0.00306041	143.1	56.9
High	Q9UKZ9	PCOLCE2	Generic bind	0	53.269	26	9	19	9	415	45.7	8.47	45.94	-1.37	0.00624882	144.3	55.7
High	P21810	BGN	Generic bind	0	210.494	54	18	124	17	368	41.6	7.52	478.37	-1.4	0.00139416	148.1	51.9
High	P39060	COL18A1	Receptor lig	0	237.004	27	26	60	26	1519	153.9	5.68	237.42	-1.42	0.00256058	145.8	54.2
High	Q8N6Y2	LRRC17	Generic bind	0	101.602	47	20	40	20	441	51.8	8.24	114.53	-1.42	0.00430026	146.2	53.8
High	P08253	MMP2	Metalloprot	0	198.98	53	28	67	28	660	73.8	5.47	207.64	-1.43	0.00040466	146.2	53.8
High	Q14767	LTBP2	Generic bind	0	303.47	32	49	114	49	1821	194.9	5.19	331.42	-1.44	0.00232334	146.8	53.2
High	Q86UD1	OAF	Protein	0	21.171	19	5	10	5	273	30.7	6.84	24.62	-1.44	0.00154325	146.1	53.9
High	O60687	SRPX2	Generic bind	0	22.228	15	6	8	6	465	52.9	7.25	22.28	-1.46	0.00336624	147	53
High	Q9H1J7	WNT5B	Receptor lig	0	101.449	50	15	34	11	359	40.3	8.4	99.21	-1.47	0.00225702	146.9	53.1
High	P20908	COL5A1	Generic bind	0	221.099	21	30	80	27	1838	183.4	5.06	243.16	-1.48	0.00057976	147.2	52.8
High	Q16394	EXT1	Generic enz	0	186.82	57	32	65	31	746	86.2	9.04	199.68	-1.48	0.00049151	146	54
High	Q8IX30	SCUBE3	Generic bind	0	122.476	33	22	30	21	993	109.2	7.56	78.94	-1.49	0.0053262	148.3	51.7
High	P28300	LOX	Generic enz	0	76.148	38	10	23	10	417	46.9	8.09	81.2	-1.5	0.00084653	149.5	50.5
High	P51693	APLP1	Generic bind	0	14.68	8	5	6	5	650	72.1	5.8	10.9	-1.5	0.00136654	147.2	52.8
High	P05121	SERPINE1	Receptor lig	0	325.793	71	24	458	24	402	45	7.2	1526.78	-1.51	0.00023072	148.1	51.9
High	Q9ULF5	SLC39A10	Transporter	0	22.783	10	8	11	8	831	94.1	6.76	15.91	-1.52	0.00073211	148.3	51.7
High	O75508	CLDN11	Generic bind	0.001	4.823	8	2	2	2	207	22	7.9	4.06	-1.53	0.00026524	148.6	51.4
High	Q15198	PDGFRL	GPCR	0	33.295	21	5	7	5	375	41.8	8.5	22.37	-1.54	7.5564E-05	148.5	51.5
High	P02751	FN1	Generic bind	0	2362.749	69	140	4374	7	2477	272.2	5.5	14840.48	-1.55	0.00554224	150.2	49.8
High	O43854	EDIL3	Receptor lig	0	186.633	56	25	62	25	480	53.7	7.28	193.25	-1.55	0.00254819	149	51
High	P12034	FGF5	Receptor lig	0	8.32	15	3	3	3	268	29.5	10.54	6.68	-1.57	0.00085291	149.5	50.5
High	P29966	MARCKS	Generic bind	0	75.87	46	11	26	11	332	31.5	4.45	72.6	-1.58	0.00093069	151.3	48.7
High	P08572	COL4A2	Generic bind	0	354.849	33	35	126	35	1712	167.4	8.66	429.84	-1.59	0.00023457	149.6	50.4
High	P21741	MDK	Receptor lig	0	21.381	43	5	8	5	143	15.6	9.79	21.45	-1.59	0.00358572	150.4	49.6
High	Q14393	GAS6	Receptor lig	0	10.332	6	4	4	4	721	79.6	6.21	6.08	-1.59	0.00302834	151.2	48.8
High	P24821	TNC	Receptor lig	0	865.013	59	90	337	2	2201	240.7	4.89	1118.02	-1.6	0.00033718	150.3	49.7
High	Q13219	PAPPA	Metalloprot	0	399.154	46	56	135	56	1627	180.9	6.18	430.73	-1.61	0.00021002	150.4	49.6
High	Q8N8U9	BMPER	Generic bind	0	68.655	27	15	21	15	685	75.9	7.77	55.18	-1.61	0.00076226	150.7	49.3
High	O75718	CRTAP	Generic bind	0	66.911	38	14	22	14	401	46.5	5.73	59.2	-1.61	6.0898E-05	150.3	49.7
High	P98160	HSPG2	Generic bind	0	1772.2	62	178	849	178	4391	468.5	6.51	2854.32	-1.62	0.00255427	151.6	48.4
High	P13726	F3	Generic rece	0	14.682	15	3	4	3	295	33	7.03	11.17	-1.65	0.00284283	151.8	48.2
High	P03956	MMP1	Metalloprot	0	163.147	57	24	64	24	469	54	6.96	194.11	-1.67	0.00287922	153	47
High	Q8NFJ5	GPRC5A	GPCR	0	15.416	9	2	5	2	357	40.2	8.15	15.92	-1.67	0.00019007	151.4	48.6
High	Q92626	PXDN	Generic enz	0	830.535	68	79	380	79	1479	165.2	7.17	1266.66	-1.68	0.00178285	152.5	47.5
High	Q93063	EXT2	Generic enz	0	306.684	53	33	105	33	718	82.2	6.55	327	-1.68	0.00074089	152.3	47.7
High	P05997	COL5A2	Generic bind	0	272.058	40	41	120	39	1499	144.8	6.46	320.14	-1.71	0.00061701	153.2	46.8
High	P78539	SRPX	Generic bind	0	76.073	39	16	25	16	464	51.5	8.66	65.2	-1.71	0.00071154	153.9	46.1
High	Q9GZM7	TINAGL1	Generic prot	0	77.148	46	15	24	15	467	52.4	6.99	65.07	-1.72	0.00054013	153.3	46.7
High	Q08397	LOXL1	Generic enz	0	123.926	44	19	41	19	574	63.1	7.52	129.92	-1.74	0.01044862	157.7	42.3
High	Q9HB63	NTN4	Generic bind	0	66.905	26	12	22	12	628	70	8.05	75.63	-1.76	0.00188598	155.8	44.2

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Down regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	P35579	MYH9	Generic binc	0	1149.428	64	140	465	114	1960	226.4	5.6	1373.63	-1.78	0.00244014	155	45
High	Q08431	MFGE8	Receptor lig	0	207.891	83	26	95	26	387	43.1	8.15	294.52	-1.78	0.00367459	154.8	45.2
High	Q9NS15	LTBP3	Receptor lig	0	230.283	39	36	75	35	1303	139.3	6.07	212.4	-1.8	0.00214131	155.8	44.2
High	Q08629	SPOCK1	Generic binc	0	174.639	63	22	81	20	439	49.1	6.1	240.44	-1.8	0.00015773	155.6	44.4
High	P21246	PTN	Receptor lig	0	32.82	36	5	9	5	168	18.9	9.6	25.61	-1.83	0.00574561	156.5	43.5
High	Q16270	IGFBP7	Generic binc	0	119.206	52	15	45	15	282	29.1	7.9	133.17	-1.84	0.00245028	155.9	44.1
High	O95084	PRSS23	Generic prot	0	84.135	49	15	30	15	383	43	9.42	79.74	-1.84	0.00041109	156.4	43.6
High	Q9NVM1	EVA1B	Generic binc	0	12.029	32	2	2	2	165	18.4	4.73	7.82	-1.86	0.00465711	156.3	43.7
High	P56730	PRSS12	Generic prot	0	7.742	5	3	3	3	875	97	8.03	5.05	-1.86	0.00524988	156.8	43.2
High	Q9HDB5	NRXN3	Generic rece	0	16.86	9	4	4	4	637	69.3	7.21	13.58	-1.87	0.00256243	154.9	45.1
High	P17948	FLT1	Receptor wi	0	128.776	20	27	57	2	1338	150.7	8.38	147.18	-1.88	0.00108022	155.8	44.2
High	Q07092	COL16A1	Generic binc	0	12.164	3	3	6	3	1604	157.7	7.84	10.58	-1.88	0.00158006	157.2	42.8
High	Q08380	LGALS3BP	Generic rece	0	268.722	52	24	105	24	585	65.3	5.27	363.25	-1.89	0.00864577	157.6	42.4
High	Q5FYB0	ARSJ	Generic enz	0.001	5.696	4	2	2	2	599	67.2	9.01	2.74	-1.89	0.00189905	157.5	42.5
High	O00469	PLOD2	Generic enz	0	150.612	41	26	45	26	758	87	6.71	130.73	-1.94	0.0006803	160.3	39.7
High	P35052	GPC1	Generic rece	0	262.595	55	24	103	24	558	61.6	7.3	330.66	-1.95	0.00085063	158.7	41.3
High	P58397	ADAMTS12	Metalloprot	0	176.607	24	32	61	32	1594	177.6	7.87	162.37	-1.96	0.00386392	158.1	41.9
High	P26022	PTX3	Generic binc	0	266.98	58	19	365	19	381	41.9	5.01	880.83	-1.98	0.00237637	160.3	39.7
High	P10646	TFPI	Generic binc	0	46.862	27	7	25	7	304	35	8.25	62.73	-2	0.00047844	160.7	39.3
High	Q99715	COL12A1	Generic binc	0	1393.763	61	148	665	148	3063	332.9	5.53	2054.74	-2.01	0.00118497	160.1	39.9
High	P35556	FBN2	Receptor lig	0	1094.126	52	112	455	106	2912	314.6	4.86	1315.22	-2.01	0.00156709	160.2	39.8
High	Q02809	PLOD1	Generic enz	0	311.363	63	37	117	37	727	83.5	6.95	372.71	-2.02	0.00038616	159.6	40.4
High	O94985	CLSTN1	Generic binc	0	180.323	39	31	61	31	971	108.6	4.93	173.53	-2.03	0.0013079	160.7	39.3
High	P10124	SRGN	Generic binc	0	83.246	32	6	44	6	158	17.6	4.96	127.5	-2.03	0.00110617	159.1	40.9
High	O94813	SLIT2	Receptor lig	0	352.971	40	50	104	50	1529	169.8	7.06	321.89	-2.07	0.00203646	160.4	39.6
High	P08476	INHBA	Receptor lig	0	155.39	56	27	68	27	426	47.4	8.03	160.4	-2.07	0.00030098	161.9	38.1
High	P29279	CCN2	Receptor lig	0	161.752	63	22	73	22	349	38.1	8	205.94	-2.13	0.00157762	162.9	37.1
High	P16035	TIMP2	Generic binc	0	86.863	44	12	27	12	220	24.4	7.49	74.65	-2.14	0.00088788	162	38
High	P05067	APP	Generic rece	0	255.161	41	29	142	27	770	86.9	4.82	385.71	-2.15	0.00073818	162.1	37.9
High	O76061	STC2	Generic binc	0	82.342	48	8	22	8	302	33.2	7.3	73.03	-2.16	0.00029338	163.5	36.5
High	Q14112	NID2	Generic binc	0	560.317	53	60	248	2	1375	151.2	5.29	820.77	-2.24	0.0012551	165.2	34.8
High	P02461	COL3A1	Generic binc	0	487.327	52	62	275	61	1466	138.5	6.61	773.79	-2.24	0.00126754	165	35
High	P08123	COL1A2	Generic binc	0	1017.893	75	79	970	79	1366	129.2	8.95	3277.49	-2.26	0.00140344	166.2	33.8
High	Q6UXH8	CCBE1	Generic binc	0	63.77	34	12	23	12	406	44.1	7.58	59.36	-2.26	0.00548845	164.8	35.2
High	O00468	AGRN	Generic binc	0	834.928	53	78	340	78	2045	214.7	6.4	1083.3	-2.28	0.00207231	166.1	33.9
High	Q8N6G6	ADAMTSL1	Generic prot	0	109.802	20	27	37	27	1762	193.3	7.8	97.11	-2.33	0.0033201	166.8	33.2
High	Q9Y4K0	LOXL2	Generic binc	0	344.228	55	35	160	35	774	86.7	6.38	501.48	-2.36	0.00079101	167.1	32.9
High	Q8N474	SFRP1	Generic binc	0	72.86	49	10	22	10	314	35.4	8.85	83.9	-2.36	0.00232976	166.2	33.8
High	P31431	SDC4	Generic rece	0	39.445	25	5	25	5	198	21.6	4.5	72.06	-2.37	0.00018274	167.5	32.5
High	P27658	COL8A1	Generic binc	0	102.498	27	17	44	17	744	73.3	9.61	115.86	-2.38	0.0014917	167.2	32.8
High	P07585	DCN	Receptor lig	0	198.624	52	21	161	20	359	39.7	8.54	466.66	-2.4	0.00228616	169.1	30.9
High	P35442	THBS2	Receptor lig	0	563.753	55	50	301	46	1172	129.9	4.83	960.31	-2.42	0.0016798	168.6	31.4

(*cont'd*) Table 4. Differentially expressed proteins in 3D EVs

Down regulated in 3D EVs																	
FDR confidence	**UniProt Accession**	**Gene symbol**	**Object type**	**q-value**	**Sum PEP Score**	**Coverage [%]**	**# Peptides**	**# PSMs**	**# Unique Peptides**	**# AAs**	**MW [kDa]**	**Calc. pI**	**Sequest HT**	**Abundance Ratio = log_2(3D EV/2D EV)**	**Abundance Ratio P-Value**	**Abundances (Grouped): 2D EV**	**Abundances (Grouped): 3D EV**
High	Q9UI42	CPA4	Generic prot	0	67.232	37	9	18	9	421	47.3	6.7	52.97	-2.44	0.00664309	171.3	28.7
High	P49767	VEGFC	Receptor lig	0	138.121	54	20	59	20	419	46.9	7.52	166.7	-2.47	0.00074003	169.4	30.6
High	O00622	CCN1	Receptor lig	0	168.063	70	21	61	21	381	42	8.21	188.51	-2.48	0.0015789	169.5	30.5
High	Q15113	PCOLCE	Generic bind	0	249.103	80	26	107	26	449	47.9	7.43	331.4	-2.49	0.00117337	171.2	28.8
High	P02452	COL1A1	Generic bind	0	1193.44	84	94	1177	92	1464	138.9	5.8	3581.99	-2.52	0.00181785	171.1	28.9
High	Q7Z7G0	ABI3BP	Generic bind	0	212.369	30	24	75	24	1075	118.6	9.44	244.3	-2.58	0.00075897	172.2	27.8
High	P43026	GDF5	Receptor lig	0	28.673	18	8	9	8	501	55.4	9.79	24.21	-2.66	1.1457E-05	172.7	27.3
High	Q76M96	CCDC80	Generic bind	0	421.247	50	45	223	45	950	108.1	9.72	572.56	-2.75	0.00231269	174.4	25.6
High	Q8IWU6	SULF1	Generic enz	0	150.453	30	24	72	24	871	101	9.09	179.25	-2.76	0.00255661	173.8	26.2
High	O00339	MATN2	Generic bind	0	351.348	49	40	121	40	937	104.7	6.39	399.21	-2.81	0.00192076	175.2	24.8
High	O94907	DKK1	Receptor lig	0	90.765	52	11	58	11	266	28.7	8.4	153.87	-2.84	0.00090224	175.5	24.5
High	P19883	FST	Generic bind	0	171.079	67	22	82	22	344	38	5.67	230.77	-2.95	7.0675E-05	177.2	22.8
High	P61812	TGFB2	Receptor lig	0	92.502	46	15	38	15	414	47.7	8.53	107.1	-3.23	0.00090137	179.8	20.2

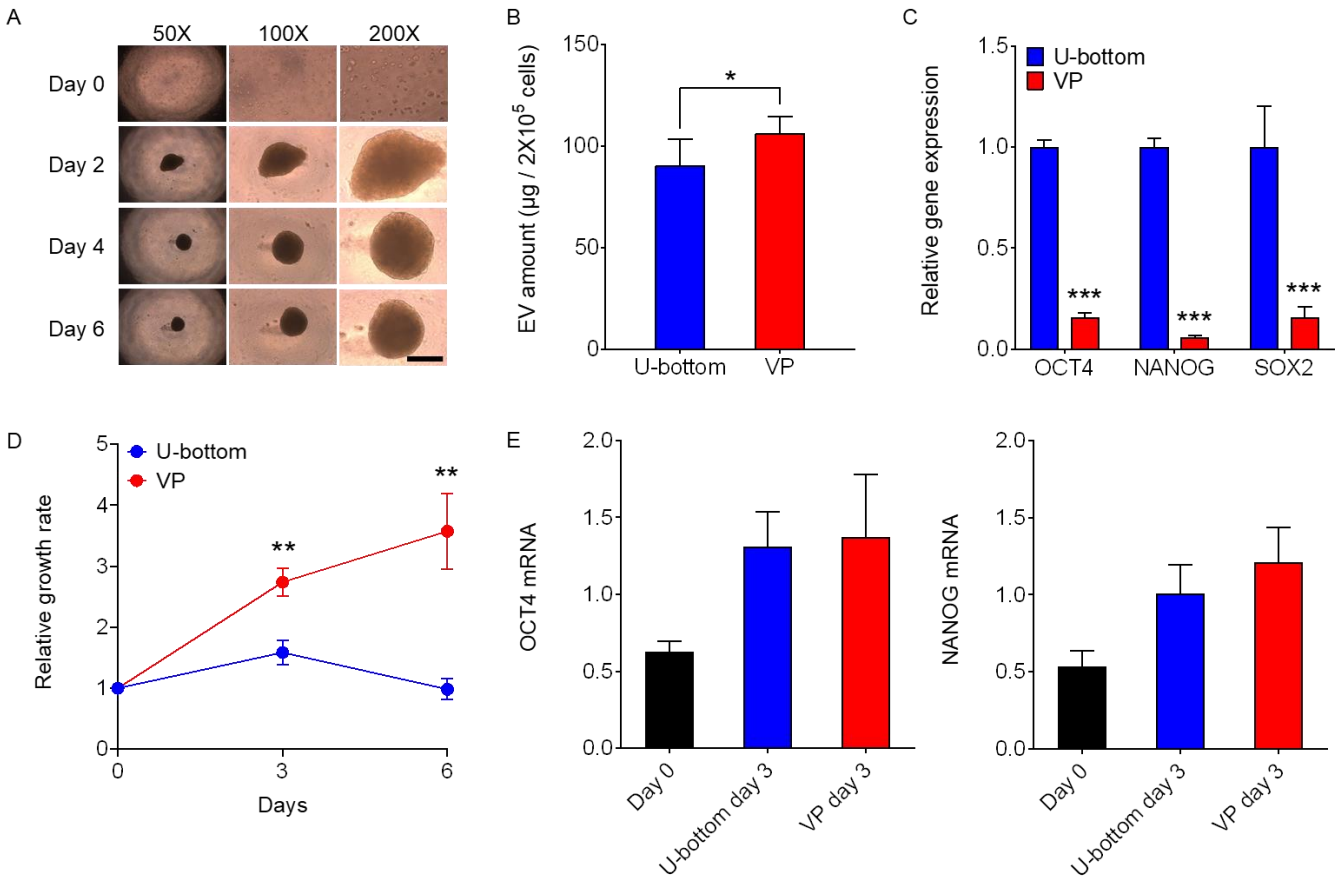

A
50X
100X
200X
Day 0
Day 2
Day 4
Day 6
B
EV amount (µg / 2X10^5 cells)
150
100
50
0
*
U-bottom
VP
C
Relative gene expression
1.5
1.0
0.5
0.0
U-bottom
VP
***
***
***
OCT4
NANOG
SOX2
D
Relative growth rate
5
4
3
2
1
0
U-bottom
VP
**
**
0
3
6
Days
E
OCT4 mRNA
2.0
1.5
1.0
0.5
0.0
Day 0
U-bottom day 3
VP day 3
NANOG mRNA
2.0
1.5
1.0
0.5
0.0
Day 0
U-bottom day 3
VP day 3

Figure 8. Comparison between Cellhesion VP and U-bottom plate-mediated 3D culture of hMSCs

A. Morphology of WJ-hMSC sphere in U-bottom plate. Scale bar is 400 μm. **B.** EV amount from WJ-hMSCs cultured with different 3D culture methods, VP (Chitin-based Polysaccharides) and U-bottom plate. *$p < 0.05$ vs. U-bottom ($n = 5$). **C.** Relative mRNA levels of stemness genes from WJ-hMSCs cultured with different 3D. culture methods. ***$p < 0.001$ vs. U-bottom ($n = 3$). **D.** ADSC growth rate with different 3D culture methods. **$p < 0.01$ vs. U-bottom ($n = 3$). **E.** Relative mRNA levels of stemness genes from ADSCs cultured with different 3D culture methods ($n = 3$). Bars indicate mean ± SD.

4. Therapeutic potential of EVs derived from cells under different culture conditions

Next, I investigated the altered therapeutic potential of EVs derived from 3D hMSCs compared to those from 2D cells.

First, performed the MTT assay to determine the concentration of EVs for cell treatment. As a result, it was observed that 2D EVs was tolerable up to 19 μg/mL and 3D EV up to 171 μg/mL as a result of treatment with hMSCs for 6 hours (Fig. 9). In addition, we investigated cellular uptake by EV concentration (Fig. 10A). Cellular uptake of EVs increased significantly in proportion to the treatment concentration ($p < 0.001$), and no significant difference was observed between 2D and 3D EVs (Fig. 10B). Accordingly, the EV concentration for cell treatment was determined to be 19 μg/mL.

Global transcription profiling analyses showed that the treatment of EVs derived from 2D cells increased expression of 118 genes and decreased expression of 164 genes at a fold-change cutoff of 1.5 relative to naïve hMSCs. Likewise, EVs from 3D cells increased expression of 173 genes and decreased that of 282 genes. A total of 197 DEGs were observed between 2D and 3D EVs, among which 94 were upregulated and 103 were downregulated in 3D EVs compared to 2D EVs (Table 5).

Subsequent pathway enrichment analyses of DEGs based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) showed that both types of EVs showed changes in the signaling pathways related to immune responses and human diseases, in particular infectious diseases, and these changes were significantly greater in 3D EVs (Fig. 11). For example, signaling pathways involved in immune responses, such as the Toll-like receptor signaling pathway, NOD-like receptor signaling pathway, Th1 and Th2 cell differentiation, Th17

cell differentiation, and IL-17 signaling pathway, were significantly dysregulated in 3D EVs ($p < 0.001$). In addition, signaling pathways related to immune diseases such as systemic lupus erythematosus and rheumatoid arthritis were significantly dysregulated ($p < 0.001$). Pathways related to various infectious diseases, in particular viral infections, were enriched with 3D EV treatment, including those for human T-cell leukemia virus 1, human immunodeficiency virus 1, measles, influenza A, hepatitis B and C, herpes simplex virus 1, human cytomegalovirus, and Epstein-Barr virus ($p < 0.001$). Interestingly, 3D EV treatment dysregulated significantly several signaling pathways related to the endocrine system and cardiovascular disease. These pathways include relaxin signaling and renin secretion ($p < 0.01$), and fluid shear stress and atherosclerosis ($p < 0.001$), respectively.

To obtain therapeutic insights into the signaling pathways altered by EVs, I applied the DEGs with EVs to the Connectivity map and identified the distinct connections of drug profiles between 2D and 3D EVs (Fig. 10C, Table 6). For example, fenbendazole was the secondly similar anti-infective to 3D EVs (C-score = 97.45), but showed very low similarity to 2D EVs (C-score = 4.71). Conversely, ofloxacin (C-score = 96.58) and mecillinam (C-score = 94.0) were observed to be most similar to 2D EVs, but were not observed among drugs similar to 3D EVs. Likewise, in metabolic and cardiovascular agent analyses, nateglinide, an anti-diabetic drug, was the most similar drug to 3D EVs (C-score = 95.73), but its similarity to 2D EVs was low (C-score = 53.71), while the hypertensive drug urapidil was highly similar to 3D EVs (C-score = 90.28) but was not among the drugs similar to 2D EVs. In immunomodulation analyses, the antihistamine drug brompheniramine showed high similarity to both 2D (C-score = 91.58) and 3D EVs (C-score = 88.22).

To evaluate the pharmacological similarity between 3D EVs and connected drugs, I selected candidate drugs based on the mRNA markers of 3D EVs. Among DEGs induced by 3D EVs, C-C motif chemokine ligand 2 (*CCL2*) was used as an up-regulated mRNA marker by 3D EVs, and zinc finger protein 836 (*ZNF836*) as a down-regulated mRNA marker. Among the selected candidate drugs, fenbendazole and brompheniramine, which showed similar patterns of expression of *CCL2* and *ZNF836* to 3D EVs, were identified (Fig. 10D). Each drug was subjected to MTT assay to determine the appropriate cell treatment concentration, and as a result, it was confirmed that fenbendazole was tolerable when treated up to 8 μM and brompheniramine up to 0.05 mM for 6 hours (Fig. 12). The pharmacological similarity between the selected drugs and EVs was investigated through the comparison of transcriptome responses after cell treatment. Hierarchical clustering revealed that 2D EVs, 3D EVs, and fenbendazole-treated groups excluding brompheniramine formed clusters for each treatment group. In addition, it was observed that fenbendazole formed the same cluster as 3D EVs, and 2D EVs belonged to the same cluster as vehicle control (Fig. 10E). Therefore, through the similarity analysis of the global transcriptome response, it was confirmed that 3D EVs induces a mostly similar pharmacological response to fenbendazole.

Taken together, these results show that different types of EVs derived from hMSCs function similarly in immunomodulation, and in particular, that 3D EVs can be expected to have greater therapeutic potential against rheumatoid arthritis. In addition, 2D and 3D EVs were expected to have completely distinct therapeutic profiles in various infectious and metabolic diseases, indicating that the therapeutic potential of EVs was dramatically altered by 3D culture of hMSCs.

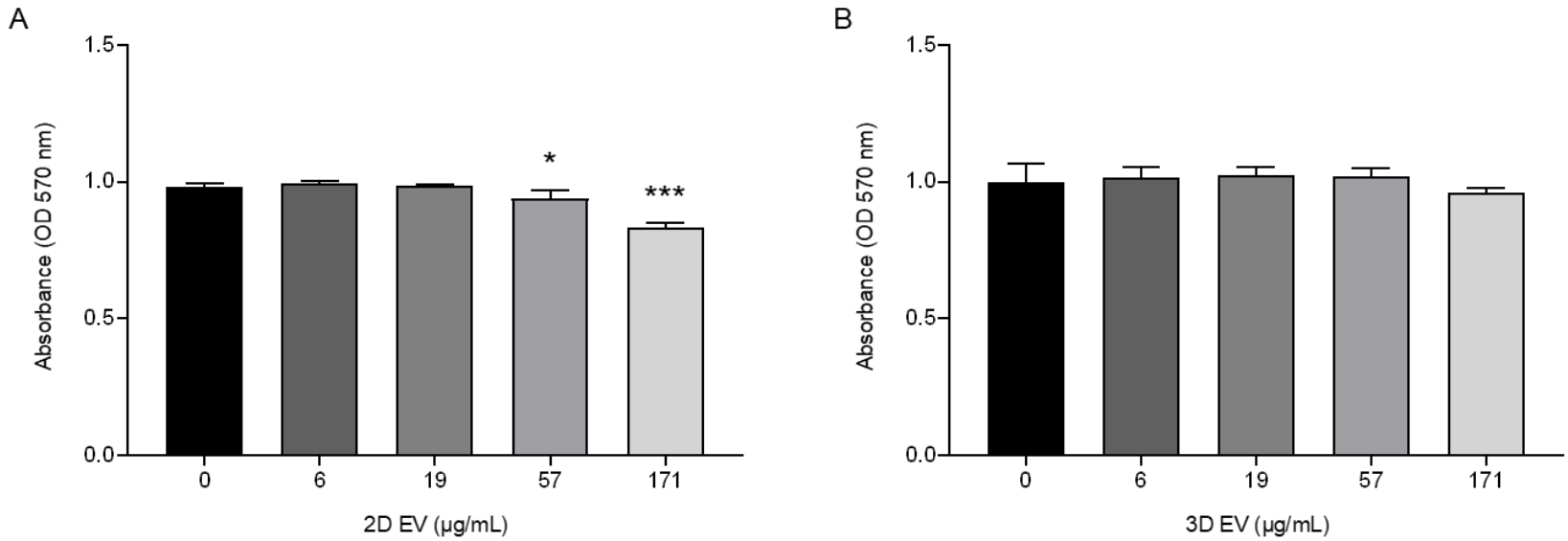


Figure 9. Range finding of EVs

A. Range finding of 2D EVs. *$p < 0.05$, ***$p < 0.001$ vs. 0 µg/mL (n = 4). **B.** Range finding of 3D EVs (n = 4). Bars indicate mean ± SD.

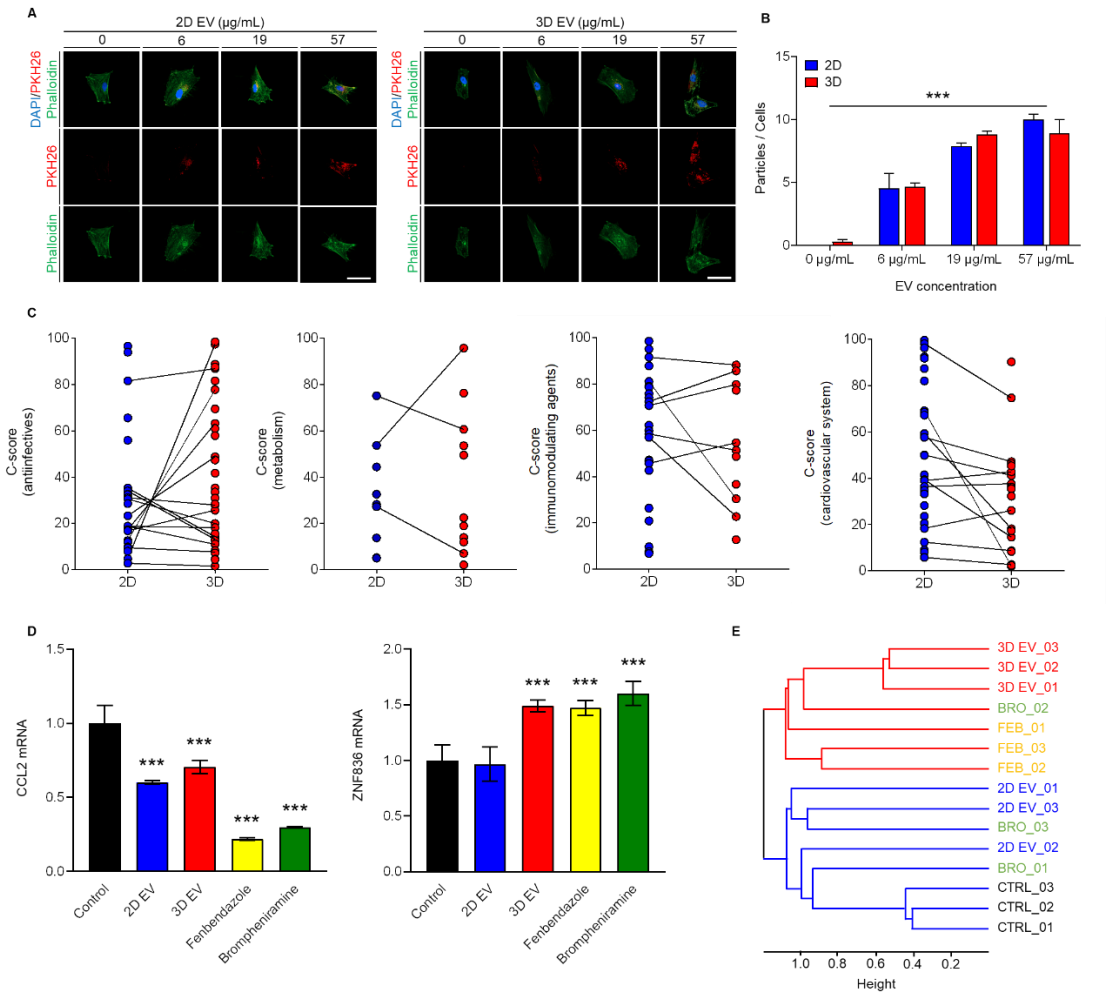

A
2D EV (µg/mL)
0
6
19
57
3D EV (µg/mL)
DAPI/PKH26
Phalloidin
PKH26
B
2D
3D
***
Particles / Cells
0 µg/mL
6 µg/mL
19 µg/mL
57 µg/mL
EV concentration
C
C-score (antiinfectives)
C-score (metabolism)
C-score (immunomodulating agents)
C-score (cardiovascular system)
2D
3D
D
CCL2 mRNA
ZNF836 mRNA
Control
2D EV
3D EV
Fenbendazole
Brompheniramine
E
3D EV_03
3D EV_02
3D EV_01
BRO_02
FEB_01
FEB_03
FEB_02
2D EV_01
2D EV_03
BRO_03
2D EV_02
BRO_01
CTRL_03
CTRL_02
CTRL_01
Height

Figure 10. Functional enrichment of DEGs by EVs and their connection to approved drugs

A. Concentration dependent EV uptake in hMSCs. EVs were labeled with PKH26 (red) and cells were counterstained with Phalloidin-488 (green) and DAPI (blue). Bar scale is 50 µm. **B**. Number of EVs uptaken in cells. Blue bars indicate the number of 2D EV particles in cells and red of 3D EV particles in cells. Bars represent mean ± SD (n = 71 averaged cells). ***$p < 0.001$ vs. 0 µg/mL EVs. **C**. Scatter plots of connectivity scores between EVs and approved drugs. Drugs are classified into anti-infectives, anti-metabolic disorders, immunosuppressants, and anti-cardiovascular disorders from left to right. Blue circles indicate drugs connected to 2D EV, and red indicate drugs connected to 3D EV. The same drugs in each group were connected by solid lines. **D**. *CCL2* and *ZNF836* mRNA levels by selected drugs. Bars represent mean ± SD (n = 4). ***$p < 0.001$ vs. vehicle control. **E**. Hierarchical clustering analysis of gene expression pattern in EV or drug-treated cells. BRO indicates Brompheniramine and FEB indicates Fenbendazole. Each treatment was independently triplicated.

Table 5. EV-induced DEGs in hMSCs

Fold change				Fold change				Fold change			
Up-regulated		Down-regulated		Up-regulated		Down-regulated		Up-regulated		Down-regulated	
Gene_Symbol	**3Dexo/2Dexo**	**Gene_Symbol**	**3Dexo/2Dexo**	**Gene_Symbol**	**2Dexo/CTRL**	**Gene_Symbol**	**2Dexo/CTRL**	**Gene_Symbol**	**3Dexo/CTRL**	**Gene_Symbol**	**3Dexo/CTRL**
VCX2	3.202	RNY4P13	-2.444	IGLJ5	2.696	CRYM-AS1	-3.333	TRAV12-1	2.460	CRYM-AS1	-4.076
IGHG3	3.056	HIST1H3I	-2.341	BTN3A3	2.419	CYP2B7P	-2.885	OR2A1	2.454	ANGPTL1	-3.280
TRBV6-4	2.254	GEM	-2.286	FAM209A	2.037	ANGPTL1	-2.729	NLGN4Y-AS1	2.363	SCN11A	-3.162
SPANXC	2.121	ZNF138	-2.269	ERVK13-1	1.940	KCNE1	-2.569	ZNF480	2.362	EGR1	-2.988
IGKV1-8	2.066	IGKV3D-15	-2.229	LOC105371371	1.925	SNRPN	-2.549	IGHG3	2.333	KCNE1	-2.946
NPIPB8	1.991	HIST1H3G	-2.191	PDE4DIP	1.912	TNFRSF17	-2.383	SCX	2.309	SUGT1P3	-2.723
ZNF836	1.977	HIST1H2BF	-2.184	KRT8P11	1.904	IFNA1	-2.341	IGLJ5	2.288	IFNA1	-2.719
GOLGA6L7P	1.969	YAE1D1	-2.139	ZNF480	1.887	SUGT1P3	-2.231	CCL2	2.159	GEM	-2.715
VTRNA1-3	1.949	NIF3L1	-2.035	LCE3E	1.882	MLANA	-2.186	OR2A14	2.105	CPEB3	-2.682
DNLZ	1.943	POU5F1	-1.940	POU5F1	1.881	VCX2	-2.174	OR7D2	2.087	LSMEM1	-2.585
MAP1LC3B2	1.943	KIR3DL2	-1.926	SIGLEC11	1.854	VIP	-2.124	ZNF836	2.048	CPOX	-2.445
BIRC7	1.856	ZNF260	-1.890	POTEH	1.853	MB21D1	-2.109	IGKV1-8	2.030	CFLAR	-2.442
KRT16	1.849	SDHAP2	-1.886	TRIM16L	1.832	CPEB3	-2.107	TUBA3C	1.992	OR5B2	-2.396
TUBA3FP	1.842	KGFLP2	-1.878	SPATA31D5P	1.827	FOS	-2.104	WASH4P	1.980	TNFRSF17	-2.383
EDN1	1.841	ZNF107	-1.877	IGLV2-18	1.821	LURAP1L-AS1	-2.104	DKK1	1.956	YAE1D1	-2.363
TPTE	1.841	SCN11A	-1.862	WASH4P	1.813	OR5AU1	-2.101	GH2	1.945	KBTBD8	-2.322
DMTF1	1.833	NUFIP1	-1.852	HUNK	1.805	VTRNA1-3	-2.080	HLA-DRA	1.942	BTK	-2.317
HOXD4	1.829	BMS1P4	-1.850	KRT83	1.805	BCAR4	-2.070	GOLGA6L7P	1.932	DLGAP1-AS3	-2.316
TRBV7-3	1.828	FAM157B	-1.849	HIST1H2BL	1.797	SCARNA9L	-2.042	TRBV7-3	1.915	NEAT1	-2.253
TRAV12-1	1.817	FAT2	-1.835	OR2T12	1.796	CDH17	-2.030	DNLZ	1.882	LHFPL3-AS1	-2.237
NBPF20	1.782	IGKV1-12	-1.760	GH2	1.789	LHFPL3-AS1	-2.010	SPAG1	1.877	GDF9	-2.230
CYP4F3	1.779	LRIG3	-1.756	TRAJ32	1.783	ACRC	-2.007	LOC440300	1.867	PM20D2	-2.211
LOC440300	1.774	SLC9B1P3	-1.747	RNY4P13	1.776	ABL2	-1.995	AOC3	1.850	ATAD3B	-2.205
BAZ2B	1.773	CAPNS2	-1.745	STAG3L1	1.766	KMT2E-AS1	-1.962	CDKN2B	1.847	DLGAP1-AS2	-2.192
NLGN4Y-AS1	1.757	LOC100507334	-1.730	FAM230A	1.759	FAM24B	-1.949	FAM209A	1.844	HIST1H3G	-2.191
TMIE	1.723	CHST6	-1.725	IGKV3D-15	1.740	EGR1	-1.945	TPTE	1.841	DNAH12	-2.172
RPL13AP6	1.717	ZNF841	-1.708	SLC6A10PB	1.736	FYB	-1.935	HOXD4	1.827	VIP	-2.154
NUTM2F	1.714	ZNF791	-1.700	TCEA2	1.733	OR51A2	-1.923	RNF126	1.808	ABCC2	-2.145
OR6P1	1.712	ATAD5	-1.699	ZNRD1-AS1	1.726	RNU4ATAC	-1.917	TCF19	1.802	CDH17	-2.129
TMEM236	1.707	TXNIP	-1.698	SSX8	1.725	DNAH12	-1.916	HERC2P4	1.802	SCARNA9L	-2.119
ULK4P3	1.702	HIST2H2AB	-1.696	TRBV5-6	1.723	MEG3	-1.915	MT1L	1.801	AHI1	-2.117
SNRPN	1.691	MKRN9P	-1.694	SLC25A52	1.711	BTK	-1.884	IL7R	1.800	MB21D1	-2.109
KCNJ2	1.687	NFIL3	-1.684	EP300-AS1	1.711	IFNA2	-1.874	LOC105371371	1.799	LURAP1L-AS1	-2.104
IGHV3-20	1.680	FAM86C2P	-1.681	MKRN9P	1.694	LOC646652	-1.864	HS6ST2	1.790	ATAD5	-2.093
GH1	1.678	CFLAR	-1.681	CREG1	1.690	ABCC2	-1.855	CYP2B6	1.769	TET1	-2.083
OR6C70	1.671	MAP3K12	-1.678	FAM72A	1.686	HIST1H2BM	-1.847	KRT83	1.764	HIST1H2BM	-2.078
HERC2P4	1.671	PLEKHM1P	-1.667	GLYATL1P2	1.684	HELB	-1.843	TRDJ3	1.757	AK7	-2.077
RNU6-63P	1.670	KRT7	-1.659	TCAP	1.683	GLCCI1	-1.835	MAP1LC3B2	1.752	RNU5E-1	-2.073
ZNF416	1.668	WNT3	-1.659	KRT72	1.680	SNAR-G1	-1.815	TRAJ7	1.749	GLCCI1	-2.067
TUBA3C	1.662	RPL39L	-1.641	PLEKHA6	1.672	RPL21P44	-1.815	CASC16	1.747	RELN	-2.049
FAM150A	1.662	CATSPER2P1	-1.629	TRAJ27	1.669	ZNF681	-1.814	SSX8	1.746	ABL2	-2.034
PDCL3	1.661	CCHCR1	-1.625	OR2T34	1.667	RASA4B	-1.807	SLC6A10PB	1.745	BCAR4	-2.031
TGIF2LY	1.650	FAM230A	-1.620	FAM157B	1.667	MAP2K4	-1.786	CLC	1.736	MLANA	-2.009
PNRC2	1.641	SNAR-F	-1.619	TCF19	1.665	ZNRF2P1	-1.783	HIST1H2BC	1.732	OR5AU1	-2.005

(*cont'd*) Table 5. EV-induced DEGs in hMSCs

Fold change				Fold change				Fold change			
Up-regulated		Down-regulated		Up-regulated		Down-regulated		Up-regulated		Down-regulated	
Gene_Symbol	**3Dexo/2Dexo**	**Gene_Symbol**	**3Dexo/2Dexo**	**Gene_Symbol**	**2Dexo/CTRL**	**Gene_Symbol**	**2Dexo/CTRL**	**Gene_Symbol**	**3Dexo/CTRL**	**Gene_Symbol**	**3Dexo/CTRL**
HLA-DRA	1.641	MYPN	-1.615	MC3R	1.659	CPOX	-1.782	CGB1	1.731	LHFPL3-AS2	-1.988
ZNF594	1.637	BAGE2	-1.605	ERICH1-AS1	1.646	RELN	-1.780	CORO1A	1.724	CD200	-1.982
KRTAP6-3	1.636	ZNF32-AS3	-1.602	TRDJ2	1.644	YTHDC1	-1.778	TMIE	1.723	KMT2E-AS1	-1.980
LOC100130428	1.635	NAIP	-1.601	GGT8P	1.640	PRAMEF2	-1.773	CGB2	1.713	USP38	-1.971
PMS2P1	1.631	MRPL54	-1.601	SCX	1.632	OR6B2	-1.773	TRAJ58	1.699	MKLN1-AS	-1.965
FRG2DP	1.629	SIGLEC11	-1.598	OR7E91P	1.631	CYP4F3	-1.767	KCNIP2	1.695	CUTC	-1.951
SIT1	1.628	RNASEL	-1.592	CXorf40B	1.631	ATAD3B	-1.764	TREX2	1.690	FAM24B	-1.949
HIST1H3A	1.624	HUNK	-1.590	APOLD1	1.631	C3orf35	-1.763	SFTPC	1.689	CYP2B7P	-1.949
OR2A1	1.606	ZNF426	-1.588	RPS7P5	1.629	SPINK8	-1.760	TRBV7-6	1.681	HIST1H3I	-1.949
IGKV1D-13	1.605	ZNF320	-1.587	SLC9B1P3	1.629	C9	-1.753	TPSAB1	1.675	IFRD1	-1.949
RFPL3S	1.601	ZNF587	-1.585	RNMTL1	1.627	IFNE	-1.748	EP300-AS1	1.673	LOC100507334	-1.946
SEMA6D	1.595	CEACAM7	-1.585	SOD2	1.619	MAP3K15	-1.743	RNU6-63P	1.670	LOC729080	-1.930
LOC105376731	1.594	WDR89	-1.585	B3GNTL1	1.619	CKMT2-AS1	-1.740	FAM150A	1.664	OR51A2	-1.923
CGB2	1.586	RNF144B	-1.584	KRTAP2-1	1.617	FRG2DP	-1.734	H3F3C	1.662	FAT2	-1.921
RNVU1-15	1.585	OAS3	-1.583	ADPGK-AS1	1.610	OR5B2	-1.724	KRTAP6-3	1.661	UCN2	-1.893
NXNL1	1.584	FCGR3A	-1.580	SPRR2A	1.607	C3P1	-1.717	MC3R	1.659	ZNRF2P1	-1.883
PTP4A3	1.582	SIRPG	-1.569	SLC1A7	1.607	SUZ12P1	-1.715	NF1P2	1.651	WDR89	-1.880
FAM153B	1.573	SLC25A52	-1.568	LRRC4B	1.605	NPFFR1	-1.714	LOC102723796	1.650	IFNA2	-1.874
LILRA4	1.570	DSTYK	-1.561	RNVU1-19	1.603	FIZ1	-1.714	OR1S1	1.649	SUZ12P1	-1.869
FAM72B	1.556	GK5	-1.561	ZNF587B	1.603	CSH1	-1.713	TMOD4	1.645	SNRPN	-1.867
TRBV7-6	1.548	ZNF888	-1.561	ZNF32-AS3	1.602	OR9I1	-1.709	OR2T34	1.645	TESK2	-1.864
LOC102723796	1.544	OR2T12	-1.559	SS18L2	1.602	SHTN1	-1.704	KCTD21-AS1	1.642	ANKUB1	-1.862
CYP3A7	1.543	GPCPD1	-1.556	OR2A14	1.597	KHDC1L	-1.703	TUBA3FP	1.640	NIF3L1	-1.858
RFPL2	1.542	NAP1L3	-1.555	IGKV1D-17	1.594	LHFPL3-AS2	-1.700	CYTL1	1.640	NUFIP1	-1.852
KCNIP2	1.539	LOC100652833	-1.552	ZNF252P	1.593	SCN11A	-1.698	ADAMTSL4-AS1	1.639	RIC1	-1.848
ANGPTL4	1.536	ZKSCAN7	-1.548	GANC	1.592	SCARNA10	-1.698	OR6C70	1.639	NFIL3	-1.848
PMS2P9	1.532	DUSP16	-1.548	ATP1A1-AS1	1.587	EFTUD1P1	-1.694	FLJ45248	1.634	HELB	-1.847
TRIM51EP	1.529	ZNF449	-1.548	C21orf62-AS1	1.582	PM20D2	-1.693	TRGV8	1.633	DNAH3	-1.843
SFTPC	1.529	ZNF280C	-1.547	LLPH-AS1	1.582	LOC407835	-1.693	ZNF587B	1.631	SLC28A1	-1.833
ANXA8L1	1.525	KLHL11	-1.546	ZNF878	1.581	FAM26F	-1.687	IGHM	1.626	SCARNA10	-1.825
TSSK3	1.525	TCEB3CL2	-1.546	MSTO1	1.579	SDHAP1	-1.683	HMHA1	1.626	TXNIP	-1.825
LOC100286922	1.525	HP	-1.537	CAPNS2	1.575	LSMEM1	-1.682	OR2A2	1.625	CALML4	-1.821
C9	1.522	CCDC163P	-1.537	YIPF1	1.571	SCARNA5	-1.677	EDN1	1.623	FEM1C	-1.813
CCL2	1.521	EGR1	-1.537	ZNF436	1.569	OR7E37P	-1.675	RNU1-13P	1.622	ASPRV1	-1.810
ATRIP	1.520	LSMEM1	-1.536	HIST1H3H	1.568	C1orf220	-1.675	ZNF225	1.621	ADRB2	-1.807
DKK1	1.520	ARG1	-1.535	TMEM115	1.560	FAM95A	-1.673	EIF5A	1.620	SFTPA2	-1.803
TRAJ7	1.518	ANKUB1	-1.534	MIS12	1.556	ERVW-1	-1.670	B3GNTL1	1.619	OR6B2	-1.801
APOA1	1.514	NEAT1	-1.533	DHH	1.554	ZNF416	-1.668	MAPK8IP1	1.618	LRIG3	-1.800
OR7D2	1.510	MKLN1-AS	-1.532	CLC	1.554	TPT1	-1.668	BTN3A3	1.614	C3orf35	-1.798
APOBEC3B-AS1	1.510	TRPC4	-1.532	CHCHD10	1.547	LOC102725179	-1.663	HAND1	1.613	FYB	-1.789
SLC18A3	1.510	RNF219	-1.532	PLEKHM1P	1.544	ITGAX	-1.660	SLC1A7	1.607	SNRPN	-1.779
RILP	1.510	APOBEC3D	-1.530	KGFLP2	1.541	MSANTD3	-1.657	ZNF878	1.605	UBE2Q2L	-1.779
FIZ1	1.508	ZNF239	-1.529	PDCD6IPP2	1.540	NUTM2B-AS1	-1.655	AMPH	1.603	FOS	-1.778
SORCS2	1.508	ZNF266	-1.529	CBWD6	1.537	MCTS2P	-1.655	AMIGO2	1.601	RASA4B	-1.771

(*cont'd*) Table 5. EV-induced DEGs in hMSCs

Fold change			
Up-regulated		Down-regulated	
Gene_Symbol	3Dexo/2Dexo	Gene_Symbol	3Dexo/2Dexo
SIX2	1.507	NAPEPLD	-1.528
LOC653653	1.506	TTI2	-1.528
NNAT	1.505	KIF20B	-1.525
CT47B1	1.505	PCDHB10	-1.525
TRBV20-1	1.501	ZNF267	-1.521
TCF23	1.501	PRICKLE4	-1.520
		IGHV4-61	-1.514
		SLC35G6	-1.510
		SLC24A3	-1.509
		SLC1A6	-1.509
		TRGV3	-1.509
		ZNF287	-1.508
		TDH	-1.505
		HSD17B1	-1.501
		LURAP1L	-1.501

Fold change			
Up-regulated		Down-regulated	
Gene_Symbol	2Dexo/CTRL	Gene_Symbol	2Dexo/CTRL
HP	1.537	KBTBD8	-1.653
CGB	1.537	KRT16	-1.653
PJA1	1.535	SLC28A1	-1.649
TIMM9	1.535	THEMIS	-1.649
ZNF181	1.534	TRBV6-4	-1.643
ANKRD20A11P	1.533	GDF9	-1.639
KRT7	1.532	DNAH3	-1.638
WNT3	1.531	CRYM	-1.638
TRBV5-5	1.528	ZNF705E	-1.636
OR2A1	1.528	PMS2P9	-1.634
DPY19L1P1	1.527	OR5B21	-1.630
OR8B8	1.526	AHI1	-1.630
TNFAIP6	1.523	CDRT1	-1.629
IL7R	1.522	TET1	-1.617
CAPN10	1.521	GKN2	-1.610
CYTL1	1.520	DLGAP1-AS2	-1.599
PTTG3P	1.520	OR5B17	-1.598
OSTCP1	1.519	MFSD11	-1.592
NHS-AS1	1.518	ZNF34	-1.589
GAS6-AS2	1.517	TRAJ5	-1.588
CTSO	1.516	DLGAP1-AS3	-1.588
CLYBL	1.514	MUC22	-1.587
GCAT	1.510	AK7	-1.582
ZNF479	1.507	GSTT2	-1.582
LOC389906	1.506	LOC100131131	-1.581
SF3B5	1.506	THOC6	-1.580
TDH	1.505	CAHM	-1.580
HSBP1L1	1.503	ERVE-1	-1.579
INPP5J	1.502	OR5K3	-1.578
ESPNP	1.501	NABP1	-1.578
		DCDC1	-1.577
		RNVU1-15	-1.575
		PDXDC2P	-1.575
		NPIPB8	-1.575
		MT4	-1.573
		ADAM32	-1.570
		TMEM200C	-1.566
		TBC1D19	-1.565
		SCART1	-1.564
		WHAMMP2	-1.563
		ARHGAP32	-1.560
		ZNF184	-1.560
		SMAD5	-1.554
		HESX1	-1.548

Fold change			
Up-regulated		Down-regulated	
Gene_Symbol	3Dexo/CTRL	Gene_Symbol	3Dexo/CTRL
CACNG8	1.597	EGOT	-1.770
ZNF252P	1.593	ZNF841	-1.770
RFPL2	1.593	SLC9B1	-1.768
PPIAL4A	1.591	TPT1	-1.768
RPL32	1.589	SLC25A3	-1.753
FAM153B	1.577	ZCWPW2	-1.753
MT1E	1.576	ZNF440	-1.747
NXF5	1.573	NPIPB4	-1.747
SEMA6D	1.572	INTS2	-1.743
LMAN1L	1.571	MAP3K15	-1.743
YIPF1	1.571	CKMT2-AS1	-1.740
PLEKHA6	1.571	MURC	-1.735
ZNF733P	1.568	ACRC	-1.731
INPP5J	1.567	IGKV1-39	-1.728
PMS2P1	1.562	THOC6	-1.722
LOC100286922	1.559	CRYM	-1.720
BCAS2	1.558	CUTC	-1.720
RNU6-56P	1.556	MYPN	-1.716
CAPN10	1.556	MSTO2P	-1.715
MYH4	1.556	HAS2	-1.714
GNAS-AS1	1.555	RNU4ATAC	-1.714
HIST1H3A	1.554	BACH1	-1.713
NUAK2	1.554	CSH1	-1.713
PITX3	1.552	LOC100652833	-1.706
SIPA1L2	1.550	HERC2P3	-1.704
FAM27E3	1.549	SHTN1	-1.704
UCHL3	1.548	SCARNA22	-1.701
GALNT8	1.546	ZNF266	-1.699
OR6P1	1.545	ASB5	-1.697
PMCH	1.543	ZNF426	-1.696
LRRTM2	1.541	PDXDC2P	-1.695
PDCD6IPP2	1.540	RNU6-59P	-1.691
SNX4	1.539	IRF6	-1.689
ANXA8L1	1.539	SMG1P3	-1.688
OR7E5P	1.538	VCX3B	-1.688
PJA1	1.537	PRAMEF2	-1.687
TRBV5-6	1.537	FAM26F	-1.687
HES3	1.536	NPFFR1	-1.685
CTRB1	1.535	CENPF	-1.684
NECAB3	1.535	ANKRD45	-1.682
LLPH-AS1	1.532	LURAP1L	-1.679
CT47B1	1.532	MAP3K12	-1.678
ZNF709	1.532	HLA-DQA1	-1.677
CTSE	1.531	SCARNA5	-1.677

(*cont'd*) Table 5. EV-induced DEGs in hMSCs

Fold change				Fold change				Fold change			
Up-regulated		Down-regulated		Up-regulated		Down-regulated		Up-regulated		Down-regulated	
Gene_Symbol	3Dexo/2Dexo	Gene_Symbol	3Dexo/2Dexo	Gene_Symbol	2Dexo/CTRL	Gene_Symbol	2Dexo/CTRL	Gene_Symbol	3Dexo/CTRL	Gene_Symbol	3Dexo/CTRL
						TPTE2P3	-1.548	CYP2A6	1.530	NEK10	-1.677
						TXNDC8	-1.544	TRAV13-1	1.528	LZTS1-AS1	-1.677
						CPO	-1.538	GAL3ST2	1.528	HIST2H2AB	-1.676
						PDCL3	-1.538	GAS6-AS2	1.527	ANKRD30B	-1.676
						ASPRV1	-1.535	KCNK7	1.525	IGLJ2	-1.675
						CCL7	-1.534	WFDC21P	1.525	RNU11	-1.674
						NEK10	-1.531	RPL21P28	1.524	SCARNA21	-1.673
						PDE4C	-1.531	GNG5P2	1.523	ZBTB24	-1.672
						CEP295NL	-1.529	ZNF415	1.523	NPIPB5	-1.670
						LOC389705	-1.528	SEPT11	1.522	ANKRD12	-1.669
						HIST1H2BE	-1.527	FBXW10	1.522	KIF20A	-1.666
						SNRPN	-1.526	PTTG3P	1.520	FAM86FP	-1.664
						SNHG12	-1.526	ZNF436	1.519	XIST	-1.658
						TSSK3	-1.525	RNU6-57P	1.518	FAM86C2P	-1.656
						ZNF300P1	-1.525	KRTAP19-5	1.516	NSD1	-1.655
						SLC2A2	-1.522	TACR2	1.516	ZNF267	-1.654
						TRNAU1AP	-1.522	HEXIM2	1.515	SLC26A4	-1.652
						ZNF250	-1.521	EGFEM1P	1.515	THSD1	-1.647
						ATRIP	-1.520	FAM90A27P	1.515	ZNF791	-1.646
						LECT2	-1.514	CCL4L1	1.514	MCEE	-1.644
						TAS2R31	-1.513	GGT8P	1.514	ANKRD20A5P	-1.644
						GOLGA8A	-1.513	ETV7	1.512	NPIPB11	-1.644
						TRANK1	-1.512	MAP1LC3B2	1.512	BCL6	-1.639
						LZTS1-AS1	-1.511	GBP6	1.510	ZNF681	-1.638
						UCN2	-1.507	GCAT	1.510	NPIPB3	-1.635
						ROCK1P1	-1.506	TXN2	1.508	TRGV3	-1.630
						HNF4A-AS1	-1.506	SORCS2	1.508	TMEM156	-1.629
						SPANXC	-1.505	MRAS	1.508	TRBV10-2	-1.628
						GH1	-1.504	FAM223B	1.507	OR5B21	-1.625
						ULBP1	-1.504	PRR15	1.507	TXNDC8	-1.624
						RSPH10B2	-1.502	SF3B5	1.506	TRNAU1AP	-1.622
						FAR2P1	-1.501	RBPJL	1.505	SLC9C2	-1.620
								APOLD1	1.505	XAGE3	-1.616
								FNDC9	1.505	KLHL21	-1.612
								HSBP1L1	1.503	MRS2P2	-1.612
								MYL10	1.502	DUSP16	-1.610
								ESPNP	1.501	PPP1R15A	-1.610
								POMZP3	1.501	SDHAP1	-1.606
								PRB2	1.501	NPIPB5	-1.604
								TCF23	1.501	C17orf105	-1.601
								SLC26A4-AS1	1.500	MCF2	-1.601
										GK5	-1.599
										LOC100130964	-1.599
										OR5B17	-1.598

(*cont'd*) Table 5. EV-induced DEGs in hMSCs

Fold change				Fold change				Fold change			
Up-regulated		Down-regulated		Up-regulated		Down-regulated		Up-regulated		Down-regulated	
Gene_Symbol	3Dexo/2Dexo	Gene_Symbol	3Dexo/2Dexo	Gene_Symbol	2Dexo/CTRL	Gene_Symbol	2Dexo/CTRL	Gene_Symbol	3Dexo/CTRL	Gene_Symbol	3Dexo/CTRL
										RPL21P44	-1.598
										RCAN1	-1.596
										NFKBIA	-1.595
										LOC102725179	-1.594
										JMJD1C	-1.593
										RNASEL	-1.592
										NABP1	-1.592
										ZKSCAN1	-1.592
										MLLT10	-1.590
										MAP2K4	-1.590
										SPRY2	-1.589
										KIAA1683	-1.588
										GSKIP	-1.588
										ZNF320	-1.587
										ZNF215	-1.585
										DSTYK	-1.582
										LOC100131131	-1.581
										SUCLA2-AS1	-1.580
										TSC22D2	-1.579
										ZNF280C	-1.574
										CCL22	-1.574
										TCP11L2	-1.574
										BRD2	-1.573
										ZSWIM6	-1.573
										NPIPB4	-1.572
										ADAM32	-1.570
										LAMC2	-1.567
										TMEM200C	-1.566
										KHK	-1.566
										MGAM2	-1.566
										MEG3	-1.564
										SNHG24	-1.563
										HERC2P2	-1.558
										THEMIS	-1.556
										ZNF287	-1.555
										LECT2	-1.554
										ZNF385A	-1.550
										TBC1D8	-1.550
										OR5H15	-1.550
										GKN2	-1.549
										KIF20B	-1.549
										HESX1	-1.548
										TPTE2P3	-1.548
										ZKSCAN7	-1.548

(*cont'd*) Table 5. EV-induced DEGs in hMSCs

Fold change				Fold change				Fold change			
Up-regulated		Down-regulated		Up-regulated		Down-regulated		Up-regulated		Down-regulated	
Gene_Symbol	3Dexo/2Dexo	Gene_Symbol	3Dexo/2Dexo	Gene_Symbol	2Dexo/CTRL	Gene_Symbol	2Dexo/CTRL	Gene_Symbol	3Dexo/CTRL	Gene_Symbol	3Dexo/CTRL
										SNAR-G1	-1.548
										HMBOX1	-1.547
										OAS3	-1.547
										PLEKHA8P1	-1.547
										KLHL11	-1.546
										NAPEPLD	-1.545
										SYT16	-1.544
										BCOR	-1.543
										PDE4C	-1.542
										SLFN12	-1.542
										BMS1P4	-1.541
										PSG10P	-1.540
										MTF1	-1.540
										SLC3A2	-1.540
										NLRC4	-1.540
										MSANTD3	-1.537
										USP1	-1.532
										ANKRD36B	-1.531
										BRI3	-1.531
										LOC646652	-1.531
										TLR1	-1.531
										APOBEC3D	-1.530
										PLB1	-1.530
										GPCPD1	-1.530
										AKNAD1	-1.530
										ERVW-1	-1.530
										ZNF239	-1.529
										GEMIN4	-1.527
										SUOX	-1.526
										TBX3	-1.526
										ZNF699	-1.526
										LOC100289673	-1.526
										ERVE-1	-1.526
										RNU5B-1	-1.525
										SCART1	-1.525
										WHAMMP2	-1.525
										NPIP	-1.524
										TNKS	-1.523
										LOC105374086	-1.522
										CCL7	-1.519
										IFNE	-1.519
										CEACAM16	-1.517
										ZNF138	-1.517
										DLGAP1-AS1	-1.515

(*cont'd*) Table 5. EV-induced DEGs in hMSCs

Fold change				Fold change				Fold change			
Up-regulated		Down-regulated		Up-regulated		Down-regulated		Up-regulated		Down-regulated	
Gene_Symbol	3Dexo/2Dexo	Gene_Symbol	3Dexo/2Dexo	Gene_Symbol	2Dexo/CTRL	Gene_Symbol	2Dexo/CTRL	Gene_Symbol	3Dexo/CTRL	Gene_Symbol	3Dexo/CTRL
										AHR	-1.515
										TRPC4	-1.514
										CCNF	-1.514
										TAS2R31	-1.513
										GOLGA8A	-1.513
										JUN	-1.512
										TRANK1	-1.512
										NYX	-1.511
										PWAR5	-1.511
										ADAMTS1	-1.510
										TRIM69	-1.509
										DUSP5	-1.508
										RAP2A	-1.508
										HIST1H2BF	-1.504
										ITGAX	-1.503
										OR9I1	-1.502
										HSD17B1	-1.501
										KLHL9	-1.501

Category	Sub category	KEGG term	3D vs. 2D	2D vs. CTRL	3D vs. CTRL
Environmental information processing	Signal transduction	MAPK signaling pathway	3.9E-01	4.8E-01	4.7E-05
		Wnt signaling pathway	2.9E-02	1.0E+00	4.9E-01
		Jak-STAT signaling pathway	1.0E+00	2.0E-06	7.4E-04
		NF-kappa B signaling pathway	1.0E+00	1.0E+00	9.6E-03
		TNF signaling pathway	1.5E-02	2.2E-01	1.0E-05
		cAMP signaling pathway	1.0E+00	8.3E-02	4.5E-05
		PI3K-Akt signaling pathway	1.0E+00	1.5E-04	4.4E-03
Cellular processes	Cell growth and death	Apoptosis	2.0E-01	2.6E-01	3.0E-03
		Necroptosis	2.4E-01	3.0E-01	3.3E-02
Organismal systems	Immune system	Toll-like receptor signaling pathway		1.8E-03	0.0E+00
		NOD-like receptor signaling pathway	2.4E-04	3.3E-01	0.0E+00
		RIG-I-like receptor signaling pathway		1.1E-02	3.7E-03
		Cytosolic DNA-sensing pathway		9.2E-03	1.9E-04
		C-type lectin receptor signaling pathway			1.2E-03
		Natural killer cell mediated cytotoxicity	2.0E-01	3.5E-02	4.3E-01
		Th1 and Th2 cell differentiation	1.0E+00	1.0E+00	5.9E-05
		Th17 cell differentiation	1.0E+00	1.0E+00	1.2E-04
		IL-17 signaling pathway	1.0E+00	1.9E-01	7.8E-04
		B cell receptor signaling pathway	1.0E+00	1.7E-01	5.6E-03
		Intestinal immune network for IgA production	1.0E+00	1.0E+00	2.0E-02
		Chemokine signaling pathway	1.0E+00	1.0E+00	9.4E-03
	Endocrine system	GnRH signaling pathway	1.0E+00	1.9E-01	7.9E-03
		Oxytocin signaling pathway	1.0E+00	1.0E+00	4.5E-03
		Relaxin signaling pathway	1.0E+00	2.5E-01	2.6E-03
		Parathyroid hormone synthesis, secretion and action	1.0E+00	2.4E-02	7.6E-02
		Renin secretion	1.1E-01		3.6E-02
Human diseases	Immune disease	Systemic lupus erythematosus	0.0E+00	2.5E-04	0.0E+00
		Rheumatoid arthritis	1.4E-01	1.0E+00	7.2E-04
		Autoimmune thyroid disease	1.0E+00	1.1E-01	1.7E-03
		Inflammatory bowel disease (IBD)	1.0E+00		3.3E-02
	Cardiovascular disease	Fluid shear stress and atherosclerosis	2.1E-01	3.9E-02	3.7E-05
		Viral myocarditis	1.0E+00	1.0E+00	2.8E-02
	Endocrine and metabolic disease	AGE-RAGE signaling pathway in diabetic complications	1.2E-02	1.0E+00	9.6E-03
	Infectious disease: bacterial	Epithelial cell signaling in Helicobacter pylori infection		1.0E+00	3.7E-02
		Salmonella infection	1.0E+00	1.0E+00	6.4E-03
		Yersinia infection	1.0E+00	3.1E-02	2.0E-04
		Pertussis		1.0E+00	4.6E-03
	Infectious disease: viral	Human T-cell leukemia virus 1 infection	3.1E-01	8.6E-02	5.2E-05
		Human immunodeficiency virus 1 infection	1.0E+00	1.2E-02	4.3E-05
		Measles	1.0E+00	3.9E-02	3.6E-04
		Influenza A	2.8E-03	3.1E-01	0.0E+00
		Hepatitis B		6.1E-03	7.6E-04
		Hepatitis C	2.8E-02	2.9E-01	6.1E-04
		Herpes simplex virus 1 infection	0.0E+00	0.0E+00	0.0E+00
		Human cytomegalovirus infection	1.0E+00	9.0E-02	4.6E-04
		Kaposi sarcoma-associated herpesvirus infection		8.6E-03	1.9E-05
		Epstein-Barr virus infection	2.8E-01	1.1E-02	0.0E+00
		Human papillomavirus infection	1.0E+00	3.8E-02	1.4E-02
		Toxoplasmosis	1.7E-01		1.3E-02
	Infectious disease: parasitic	Leishmaniasis	1.2E-01	1.0E+00	3.4E-04
		Chagas disease (American trypanosomiasis)	1.6E-01	2.1E-01	9.8E-05

None
$p > 0.05$
$p \leq 0.05$
$p \leq 0.01$
$p \leq 0.001$

Figure 11. Heat map of KEGG pathway enrichment of EV-induced DEGs

Subsequent pathway enrichment analyses of DEGs based on the Kyoto Encyclopedia of Genes and Genomes (KEGG). There are four large categories including environmental information processing, cellular processes, organismal systems, human diseases and several sub categories included in each large category. Gene expression changes between control, 2D EV treated cell, 3D EV treated cell are compared in each KEGG term. Different degree of color denotes enrichment map test p-value.

Table 6. Connectivity Map analysis of EV-induced DEGs

Antiinfectives connected to 2D EVs											
id	name	description	target	belongs_to	score	ATC					
						code	L1	L2	L3	L4	
BRD-A24228527	ofloxacin	antibiotic that inhibits DNA gyrase, topoisomeras	TOP2A		96.58	J01MA01	ANTIINFECTIVES	ANTIBACTERIAL	QUINOLONE ANT	Fluoroquinolones [DG:DG01549]	
BRD-K41051431	mecillinam	cell wall synthesis inhibitor			94	J01CA11	ANTIINFECTIVES	ANTIBACTERIAL	BETA-LACTAM A	Penicillins with extended spectrum [DG:DG01780	
BRD-K51485625	ritonavir	HIV protease inhibitor, cytochrome P450 inhibito	CYP3A4, CYP	HIV protease inhi	81.6	J05AE03	ANTIINFECTIVES	ANTIVIRALS FOR	DIRECT ACTING	Protease inhibitors [DG:DG01647]	
BRD-A48570745	ivermectin	GABA receptor agonist, acetylcholine receptor a	CHRNA7, GA		65.62	P02CF01	ANTIPARASITIC	ANTHELMINTICS	ANTINEMATODA	Avermectines	Avermectines
BRD-K71059170	cefixime	cell wall synthesis inhibitor			55.93	J01DD08	ANTIINFECTIVES	ANTIBACTERIAL	OTHER BETA-LA	Third-generation cephalosporins [DG:DG01776]	
BRD-K15502390	nevirapine	non-nucleoside reverse transcriptase inhibitor, rev	CYP2B6, CYP	Reverse transcript	35.3	J05AG01	ANTIINFECTIVES	ANTIVIRALS FOR	DIRECT ACTING	Non-nucleoside reverse transcriptase inhibitors [D	
BRD-K20338176	cefaclor	cell wall synthesis inhibitor			34.03	J01DC04	ANTIINFECTIVES	ANTIBACTERIAL	OTHER BETA-LA	Second-generation cephalosporins [DG:DG01775	
BRD-A74980173	gatifloxacin	topoisomerase inhibitor, DNA gyrase inhibitor		Bacterial DNA gyr	32.56	J01MA16	ANTIINFECTIVES	ANTIBACTERIAL	QUINOLONE ANT	Fluoroquinolones [DG:DG01549]	
BRD-K96471533	nitazoxanide	pyruvate ferredoxin oxidoreductase inhibitor, pyruvate synthase inhibitor			32.52	P01AX11	ANTIPARASITIC	ANTIPROTOZOA	AGENTS AGAINS	Other agents against amoebiasis and other protozo	
BRD-M47937986	cefatrizine	cell wall synthesis inhibitor			32.45	J01DB07	ANTIINFECTIVES	ANTIBACTERIAL	OTHER BETA-LA	First-generation cephalosporins [DG:DG01774]	
BRD-A84493640	atovaquone	cytochrome b-c1 complex inhibitor, dihydroorota	DHODH		31.15	P01AX06	ANTIPARASITIC	ANTIPROTOZOA	AGENTS AGAINS	Other agents against amoebiasis and other protozo	
BRD-K29113274	ketoconazole	14-alpha demethylase inhibitor, androgen recepto	AR, CYP19A1	Sterol demethylase	30.55	J02AB02	ANTIINFECTIVES	ANTIMYCOTICS	ANTIMYCOTICS	Imidazole derivatives	
BRD-K66876909	linezolid	50S ribosomal subunit inhibitor, protein synthesis	MAOA		28.58	J01XX08	ANTIINFECTIVES	ANTIBACTERIAL	OTHER ANTIBAC	Other antibacterials	
BRD-A47829399	artesunate	DNA synthesis inhibitor			23.33	P01BE03	ANTIPARASITIC	ANTIPROTOZOA	ANTIMALARIALS	Artemisinin and derivatives, plain	
BRD-K28346421	rifapentine	DNA directed RNA polymerase inhibitor, DNA d	CYP2C8, CYP		18.84	J04AB05	ANTIINFECTIVES	ANTIMYCOBACT	DRUGS FOR TRE	Antibiotics	Antibiotics
BRD-K40645748	mefloquine	acidifying agent non gastric, adenosine receptor a	ADORA2A, H		18.72	P01BC02	ANTIPARASITIC	ANTIPROTOZOA	ANTIMALARIALS	Methanolquinolines	
BRD-K86003836	flubendazole	acetylcholinesterase inhibitor, microtubule inhibit	TUBBTUBB	Tubulin inhibitor	17.31	P02CA05	ANTIPARASITIC	ANTHELMINTICS	ANTINEMATODA	Benzimidazole derivatives	
BRD-K54472332	elvitegravir	HIV integrase inhibitor, HIV inhibitor			16.8	J05AX11	ANTIINFECTIVES	ANTIVIRALS FOR	DIRECT ACTING	Other antivirals	Other antivirals
BRD-A55913614	primaquine	antimalarial agent, DNA inhibitor	KRT7, NQO2		12.6	P01BA03	ANTIPARASITIC	ANTIPROTOZOA	ANTIMALARIALS	Aminoquinolines	Aminoquinolines
BRD-K46435977	valaciclovir	DNA directed DNA polymerase inhibitor, DNA polymerase inhib		DNA polymerase i	11.99	J05AB11	ANTIINFECTIVES	ANTIVIRALS FOR	DIRECT ACTING	Nucleosides and nucleotides excl. reverse transcri	
BRD-K45542189	diethylcarbamazin	lipoxygenase inhibitor	ALOX5, PTG		9.86	P02CB02	ANTIPARASITIC	ANTHELMINTICS	ANTINEMATODA	Piperazine and derivatives	
BRD-A21858158	praziquantel	membrane permeability enhancer, schistosomicide			9.52	P02BA01	ANTIPARASITIC	ANTHELMINTICS	ANTITREMATOD	Quinoline derivatives and related substances	
BRD-K56614220	clofazimine	GK0582 inhibitor			8.03	J04BA01	ANTIINFECTIVES	ANTIMYCOBACT	DRUGS FOR TRE	Drugs for treatment of lepra	
BRD-K51318897	fenbendazole	cytochrome P450 inhibitor, tubulin inhibitor	CYP2C19, CY	Tubulin inhibitor	4.71	P02CA06	ANTIPARASITIC	ANTHELMINTICS	ANTINEMATODA	Benzimidazole derivatives	
BRD-K09963420	saquinavir	HIV protease inhibitor, peptidase inhibitor	CYP3A4, CYP		2.78	J05AE01	ANTIINFECTIVES	ANTIVIRALS FOR	DIRECT ACTING	Protease inhibitors [DG:DG01647]	

(*cont'd*) Table 6. Connectivity Map analysis of EV-induced DEGs

Antiinfectives connected to 3D EVs

id	name	description	target	belongs_to	score	ATC code	ATC L1	ATC L2	ATC L3	ATC L4
BRD-K77987382	mebendazole	acetylcholinesterase inhibitor, microtubule inhibit	TUBA1A, TU	Tubulin inhibitor	98.45	P02CA01	ANTIPARASITIC	ANTHELMINTICS	ANTINEMATODA	Benzimidazole derivatives
BRD-K51318897	fenbendazole	cytochrome P450 inhibitor, tubulin inhibitor	CYP2C19, CY	Tubulin inhibitor	97.45	P02CA06	ANTIPARASITIC	ANTHELMINTICS	ANTINEMATODA	Benzimidazole derivatives
BRD-A67981824	cefotaxime	cell wall synthesis inhibitor		Bacterial cell wall	88.81	J01DD01	ANTIINFECTIVES	ANTIBACTERIAL	OTHER BETA-LA	Third-generation cephalosporins [DG:DG01776]
BRD-K79131256	albendazole	tubulin inhibitor, acetylcholinesterase inhibitor, m	CYP1A2, CYP	Tubulin inhibitor	87.61	P02CA03	ANTIPARASITIC	ANTHELMINTICS	ANTINEMATODA	Benzimidazole derivatives
BRD-A03323675	ritonavir	HIV protease inhibitor, cytochrome P450 inhibito	CYP3A4, CYP		86.89	J05AE03	ANTIINFECTIVES	ANTIVIRALS FOR	DIRECT ACTING	Protease inhibitors [DG:DG01647]
BRD-K99063460	didanosine	nucleoside reverse transcriptase inhibitor, reverse	PNPPNPPNP	Nucleoside reverse	81.64	J05AF02	ANTIINFECTIVES	ANTIVIRALS FOR	DIRECT ACTING	Nucleoside and nucleotide reverse transcriptase in
BRD-A55913614	primaquine	antimalarial agent, DNA inhibitor	KRT7, NQO2		77.79	P01BA03	ANTIPARASITIC	ANTIPROTOZOA	ANTIMALARIALS	Aminoquinolines Aminoquinolines
BRD-K55034111	pefloxacin	topoisomerase inhibitor	TOP2A		69.49	J01MA03	ANTIINFECTIVES	ANTIBACTERIAL	QUINOLONE ANT	Fluoroquinolones [DG:DG01549]
BRD-K86003836	flubendazole	acetylcholinesterase inhibitor, microtubule inhibit	TUBBTUBB	Tubulin inhibitor	63.21	P02CA05	ANTIPARASITIC	ANTHELMINTICS	ANTINEMATODA	Benzimidazole derivatives
BRD-K66412701	pazufloxacin	topoisomerase inhibitor			61.08	J01MA18	ANTIINFECTIVES	ANTIBACTERIAL	QUINOLONE ANT	Fluoroquinolones [DG:DG01549]
BRD-A41450521	tosufloxacin	topoisomerase inhibitor			57.93	J01MA22	ANTIINFECTIVES	ANTIBACTERIAL	QUINOLONE ANT	Fluoroquinolones [DG:DG01549]
BRD-A47829399	artesunate	DNA synthesis inhibitor			48.82	P01BE03	ANTIPARASITIC	ANTIPROTOZOA	ANTIMALARIALS	Artemisinin and derivatives, plain
BRD-A04352665	maraviroc	CC chemokine receptor antagonist, CC chemokin	CCR5, CYP3A		47.42	J05AX09	ANTIINFECTIVES	ANTIVIRALS FOR	DIRECT ACTING	Other antivirals Other antivirals
BRD-K86873305	piperacillin	cell wall synthesis inhibitor			41.74	J01CA12	ANTIINFECTIVES	ANTIBACTERIAL	BETA-LACTAM A	Penicillins with extended spectrum [DG:DG01780
BRD-A75479906	rimantadine	acetylcholine channel inhibitor, unidentified pharmacological activity			35.37	J05AC02	ANTIINFECTIVES	ANTIVIRALS FOR	DIRECT ACTING	Cyclic amines Cyclic amines
BRD-K06557128	voriconazole	cell wall synthesis inhibitor, cytochrome P450 inh	CYP2C19, CY		33.56	J02AC03	ANTIINFECTIVES	ANTIMYCOTICS	ANTIMYCOTICS	Triazole derivatives
BRD-A74500471	ethambutol	bacterial arabinosyl transferase inhibitor		Bacterial cell wall	31.01	J04AK02	ANTIINFECTIVES	ANTIMYCOBACT	DRUGS FOR TRE	Other drugs for treatment of tuberculosis
BRD-K08206212	entecavir	DNA directed DNA polymerase inhibitor, DNA polymerase inhib		Reverse transcript	28.41	J05AF10	ANTIINFECTIVES	ANTIVIRALS FOR	DIRECT ACTING	Nucleoside and nucleotide reverse transcriptase in
BRD-A84493640	atovaquone	cytochrome b-c1 complex inhibitor, dihydroorota	DHODH		27.97	P01AX06	ANTIPARASITIC	ANTIPROTOZOA	AGENTS AGAINS	Other agents against amoebiasis and other protozo
BRD-K54472332	elvitegravir	HIV integrase inhibitor, HIV inhibitor			25.7	J05AX11	ANTIINFECTIVES	ANTIVIRALS FOR	DIRECT ACTING	Other antivirals Other antivirals
BRD-K29113274	ketoconazole	14-alpha demethylase inhibitor, androgen recepto	AR, CYP19A1	Sterol demethylase	19.97	J02AB02	ANTIINFECTIVES	ANTIMYCOTICS	ANTIMYCOTICS	Imidazole derivatives
BRD-K40645748	mefloquine	acidifying agent non gastric, adenosine receptor a	ADORA2A, H		18.25	P01BC02	ANTIPARASITIC	ANTIPROTOZOA	ANTIMALARIALS	Methanolquinolines
BRD-K66019333	oxantel	fumarate reductase inhibitor			15.56	P02CC02	ANTIPARASITIC	ANTHELMINTICS	ANTINEMATODA	Tetrahydropyrimidine derivatives
BRD-K15502390	nevirapine	non-nucleoside reverse transcriptase inhibitor, rev	CYP2B6, CYP	Reverse transcript	14.3	J05AG01	ANTIINFECTIVES	ANTIVIRALS FOR	DIRECT ACTING	Non-nucleoside reverse transcriptase inhibitors [D
BRD-K20338176	cefaclor	cell wall synthesis inhibitor			13.1	J01DC04	ANTIINFECTIVES	ANTIBACTERIAL	OTHER BETA-LA	Second-generation cephalosporins [DG:DG01775
BRD-A87387433	cefpodoxime	cell wall synthesis inhibitor			12.63	J01DD13	ANTIINFECTIVES	ANTIBACTERIAL	OTHER BETA-LA	Third-generation cephalosporins [DG:DG01776]
BRD-K37848908	ceforanide	cell wall synthesis inhibitor, penicillin binding protein inhibitor			12.47	J01DC11	ANTIINFECTIVES	ANTIBACTERIAL	OTHER BETA-LA	Second-generation cephalosporins [DG:DG01775
BRD-K01612348	meropenem	cell wall synthesis inhibitor			11.18	J01DH02	ANTIINFECTIVES	ANTIBACTERIAL	OTHER BETA-LA	Carbapenems [DG:DG01458]
BRD-K28346421	rifapentine	DNA directed RNA polymerase inhibitor, DNA d	CYP2C8, CYP		10.97	J04AB05	ANTIINFECTIVES	ANTIMYCOBACT	DRUGS FOR TRE	Antibiotics Antibiotics
BRD-U94846492	quinine	cytochrome P450 inhibitor, hemozoin biocrystalli	KCNN4, ABC		10.95	P01BC01	ANTIPARASITIC	ANTIPROTOZOA	ANTIMALARIALS	Methanolquinolines
BRD-K30563334	rifabutin	DNA directed DNA polymerase inhibitor, DNA d	CYP3A4, HSP		8.3	J04AB04	ANTIINFECTIVES	ANTIMYCOBACT	DRUGS FOR TRE	Antibiotics Antibiotics
BRD-A21858158	praziquantel	membrane permeability enhancer, schistosomicide			7.7	P02BA01	ANTIPARASITIC	ANTHELMINTICS	ANTITREMATOD	Quinoline derivatives and related substances
BRD-A70083328	secnidazole	acetylcholinesterase inhibitor, microtubule inhibitor			7.27	P01AB07	ANTIPARASITIC	ANTIPROTOZOA	AGENTS AGAINS	Nitroimidazole derivatives
BRD-K93880783	stavudine	DNA directed DNA polymerase inhibitor, nucleoside reverse tran		Nucleoside reverse	4.59	J05AF04	ANTIINFECTIVES	ANTIVIRALS FOR	DIRECT ACTING	Nucleoside and nucleotide reverse transcriptase in
BRD-A34817987	itraconazole	cell wall synthesis inhibitor, cytochrome P450 inh	CYP3A5, CYP		4.44	J02AC02	ANTIINFECTIVES	ANTIMYCOTICS	ANTIMYCOTICS	Triazole derivatives
BRD-K09963420	saquinavir	HIV protease inhibitor, peptidase inhibitor	CYP3A4, CYP		1.59	J05AE01	ANTIINFECTIVES	ANTIVIRALS FOR	DIRECT ACTING	Protease inhibitors [DG:DG01647]

(*cont'd*) Table 6. Connectivity Map analysis of EV-induced DEGs

Metabolic agents connected to 2D EVs

id	name	description	target	belongs_to	score	ATC code	ATC L1	ATC L2	ATC L3	ATC L4
BRD-A82307304	atorvastatin	HMGCR inhibitor, dipeptidyl peptidase inhibitor,	HMGCR, DPP		75.19	C10AA05	CARDIOVASCUL	LIPID MODIFYIN	LIPID MODIFYIN	HMG CoA reductase inhibitors [DG:DG01660]
BRD-K84658672	nateglinide	insulin secretagogue, ATP-sensitive potassium ch	ABCC8, KCN		53.71	A10BX03	ALIMENTARY TR	DRUGS USED IN	BLOOD GLUCOS	Other blood glucose lowering drugs, excl. insulins
BRD-K81169441	cerivastatin	HMGCR inhibitor	HMGCR, CYP	HMGCR inhibitor	44.4	C10AA06	CARDIOVASCUL	LIPID MODIFYIN	LIPID MODIFYIN	HMG CoA reductase inhibitors [DG:DG01660]
BRD-K42693031	glimepiride	insulin secretagogue, ATP channel blocker, ATP-	ABCC8, KCN		32.64	A10BB12	ALIMENTARY TR	DRUGS USED IN	BLOOD GLUCOS	Sulfonylureas Sulfonylureas
BRD-K46018455	bezafibrate	PPAR receptor agonist, 11-beta hydroxysteroid d	PPARA, PPAR	PPAR receptor ag	28.3	C10AB02	CARDIOVASCUL	LIPID MODIFYIN	LIPID MODIFYIN	Fibrates Fibrates Fibrates
BRD-K09416995	lovastatin	HMGCR inhibitor	HMGCR, CYP	HMGCR inhibitor	27.28	C10AA02	CARDIOVASCUL	LIPID MODIFYIN	LIPID MODIFYIN	HMG CoA reductase inhibitors [DG:DG01660]
BRD-K36927236	glibenclamide	sulfonylurea, ATP channel blocker, ATP-sensitiv	KCNJ11, ABC		13.78	A10BB01	ALIMENTARY TR	DRUGS USED IN	BLOOD GLUCOS	Sulfonylureas Sulfonylureas
BRD-K72029282	probucol	atherogenesis inhibitor, cholesterol inhibitor, redu	ABCA1, ABC		5.23	C10AX02	CARDIOVASCUL	LIPID MODIFYIN	LIPID MODIFYIN	Other lipid modifying agents
BRD-K45252063	clofibrate	PPAR receptor agonist	PPARA, LPL		5.08	C10AB01	CARDIOVASCUL	LIPID MODIFYIN	LIPID MODIFYIN	Fibrates Fibrates Fibrates

Metabolic agents connected to 3D EVs

id	name	description	target	belongs_to	score	ATC code	ATC L1	ATC L2	ATC L3	ATC L4
BRD-K84658672	nateglinide	insulin secretagogue, ATP-sensitive potassium ch	ABCC8, KCN		95.73	A10BX03	ALIMENTARY TR	DRUGS USED IN	BLOOD GLUCOS	Other blood glucose lowering drugs, excl. insulins
BRD-K44779798	miglitol	glucosidase inhibitor, sodium/glucose cotransport	GAA, MGAM		76.3	A10BF02	ALIMENTARY TR	DRUGS USED IN	BLOOD GLUCOS	Alpha glucosidase inhibitors [DG:DG01803]
BRD-A82307304	atorvastatin	HMGCR inhibitor, dipeptidyl peptidase inhibitor,	HMGCR, DPP		60.66	C10AA05	CARDIOVASCUL	LIPID MODIFYIN	LIPID MODIFYIN	HMG CoA reductase inhibitors [DG:DG01660]
BRD-K44276885	acarbose	glucosidase inhibitor	GAA, MGAM		53.57	A10BF01	ALIMENTARY TR	DRUGS USED IN	BLOOD GLUCOS	Alpha glucosidase inhibitors [DG:DG01803]
BRD-A48430263	pioglitazone	PPAR receptor agonist, insulin sensitizer, adipon	PPARG, INS,	PPAR receptor ag	49.55	A10BG03	ALIMENTARY TR	DRUGS USED IN	BLOOD GLUCOS	Thiazolidinediones [DG:DG01683]
BRD-K82941592	rosuvastatin	HMGCR inhibitor	HMGCR	HMGCR inhibitor	22.46	C10AA07	CARDIOVASCUL	LIPID MODIFYIN	LIPID MODIFYIN	HMG CoA reductase inhibitors [DG:DG01660]
BRD-A81772229	simvastatin	HMGCR inhibitor	HMGCR, CYP	HMGCR inhibitor	19.01	C10AA01	CARDIOVASCUL	LIPID MODIFYIN	LIPID MODIFYIN	HMG CoA reductase inhibitors [DG:DG01660]
BRD-K63343048	orlistat	cholesterol inhibitor, diacylglycerol lipase inhibito	PNLIP, DAGL		13.96	A08AB01	ALIMENTARY TR	ANTIOBESITY PR	ANTIOBESITY PR	Peripherally acting antiobesity products
BRD-A49358627	ciprofibrate	PPAR receptor agonist, lipase clearing factor inhi	PPARA, LPL		11.97	C10AB08	CARDIOVASCUL	LIPID MODIFYIN	LIPID MODIFYIN	Fibrates Fibrates Fibrates
BRD-K09416995	lovastatin	HMGCR inhibitor	HMGCR, CYP	HMGCR inhibitor	7.1	C10AA02	CARDIOVASCUL	LIPID MODIFYIN	LIPID MODIFYIN	HMG CoA reductase inhibitors [DG:DG01660]
BRD-K22134346	simvastatin	HMGCR inhibitor	HMGCR, CYP		2.92	C10AA01	CARDIOVASCUL	LIPID MODIFYIN	LIPID MODIFYIN	HMG CoA reductase inhibitors [DG:DG01660]
BRD-K60511616	pravastatin	HMGCR inhibitor	HMGCR, SLC		2.11	C10AA03	CARDIOVASCUL	LIPID MODIFYIN	LIPID MODIFYIN	HMG CoA reductase inhibitors [DG:DG01660]

(*cont'd*) Table 6. Connectivity Map analysis of EV-induced DEGs

Immunomodulators connected to 2D EVs

id	name	description	target	belongs_to	score	ATC code	ATC L1	ATC L2	ATC L3	ATC L4
BRD-K99260425	etodolac	cyclooxygenase inhibitor, TRPV agonist	PTGS2, PTGS		98.53	M01AB08	MUSCULO-SKEL	ANTIINFLAMMA	ANTIINFLAMMA	Acetic acid derivatives and related substances
BRD-K65261396	tacrolimus	calcineurin inhibitor, FK506-binding protein inhib	FKBP1A, CYP		95.13	L04AD02	ANTINEOPLASTI	IMMUNOSUPPRE	IMMUNOSUPPRE	Calcineurin inhibitors
BRD-A68723818	brompheniramine	histamine receptor antagonist	HRH1, CHRM		91.58	R06AB01	RESPIRATORY S	ANTIHISTAMINE	ANTIHISTAMINE	Substituted alkylamines
BRD-A07780951	orciprenaline	adrenergic receptor agonist	ADRB2	Beta-adrenergic re	87.84	R03AB03	RESPIRATORY S	DRUGS FOR OBS	ADRENERGICS, I	Non-selective beta-adrenoreceptor agonists
BRD-K32821942	azathioprine	dehydrogenase inhibitor, immunosuppressant, pur	HPRT1, IMPD	IMPDH inhibitor	81.12	L04AX01	ANTINEOPLASTI	IMMUNOSUPPRE	IMMUNOSUPPRE	Other immunosuppressants
BRD-A13946108	sulindac	ABC transporter expression enhancer, cyclooxyg	AKR1B1, PTG		79.04	M01AB02	MUSCULO-SKEL	ANTIINFLAMMA	ANTIINFLAMMA	Acetic acid derivatives and related substances
BRD-A82238138	budesonide	glucocorticoid receptor agonist, glucocordicoid r	NR3C1, CYP3	Glucocorticoid rec	78.65	R03BA02	RESPIRATORY S	DRUGS FOR OBS	OTHER DRUGS F	Glucocorticoids Glucocorticoids
BRD-K43764301	dexketoprofen	cyclooxygenase inhibitor	CXCR1, PTGS		75.89	M01AE17	MUSCULO-SKEL	ANTIINFLAMMA	ANTIINFLAMMA	Propionic acid derivatives
BRD-A49225603	alimemazine	histamine receptor ligand	HRH1HRH1		74.15	R06AD01	RESPIRATORY S	ANTIHISTAMINE	ANTIHISTAMINE	Phenothiazine derivatives
BRD-A87479750	tenidap	cyclooxygenase inhibitor, lipoxygenase inhibitor,	ALOX5, KCN		72.44	M01AX23	MUSCULO-SKEL	ANTIINFLAMMA	ANTIINFLAMMA	Other antiinflammatory and antirheumatic agents,
BRD-A57382968	piroxicam	cyclooxygenase inhibitor	PTGS2, PTGS		70.71	M01AC01	MUSCULO-SKEL	ANTIINFLAMMA	ANTIINFLAMMA	Oxicams Oxicams Oxicams
BRD-A60571864	budesonide	glucocorticoid receptor agonist, glucocordicoid r	NR3C1, CYP3		68.68	R03BA02	RESPIRATORY S	DRUGS FOR OBS	OTHER DRUGS F	Glucocorticoids Glucocorticoids
BRD-A34299591	budesonide	glucocorticoid receptor agonist, glucocordicoid r	NR3C1, CYP3		67.47	R03BA02	RESPIRATORY S	DRUGS FOR OBS	OTHER DRUGS F	Glucocorticoids Glucocorticoids
BRD-K25394294	oxaprozin	cyclooxygenase inhibitor	PTGS1, PTGS		62.2	M01AE12	MUSCULO-SKEL	ANTIINFLAMMA	ANTIINFLAMMA	Propionic acid derivatives
BRD-A93255169	thalidomide	angiogenesis inhibitor, cereblon inhibitor, tumor n	TNF, CRBN,		59.93	L04AX02	ANTINEOPLASTI	IMMUNOSUPPRE	IMMUNOSUPPRE	Other immunosuppressants
BRD-K12513978	fenbufen	cyclooxygenase inhibitor	PTGS1, PTGS	Cyclooxygenase in	58.45	M01AE05	MUSCULO-SKEL	ANTIINFLAMMA	ANTIINFLAMMA	Propionic acid derivatives
BRD-A22684332	procaterol	adrenergic receptor agonist	ADRB2	Beta-adrenergic re	56.99	R03AC16	RESPIRATORY S	DRUGS FOR OBS	ADRENERGICS, I	Selective beta-2-adrenoreceptor agonists
BRD-K89595132	chlorphenamine	histamine receptor antagonist	HRH1, SLC6A		47.18	R06AB04	RESPIRATORY S	ANTIHISTAMINE	ANTIHISTAMINE	Substituted alkylamines
BRD-K96862998	pirfenidone	FGFR antagonist, p38 MAPK inhibitor, TGF bet	TNF, FURIN,		46.85	L04AX05	ANTINEOPLASTI	IMMUNOSUPPRE	IMMUNOSUPPRE	Other immunosuppressants
BRD-K84937637	sirolimus	mTOR inhibitor, CCR expression inhibitor, cell c	MTOR, FKBP		45.71	L04AA10	ANTINEOPLASTI	IMMUNOSUPPRE	IMMUNOSUPPRE	Selective immunosuppressants
BRD-A31312900	montelukast	leukotriene receptor antagonist	CYSLTR1, AL		42.7	R03DC03	RESPIRATORY S	DRUGS FOR OBS	OTHER SYSTEMI	Leukotriene receptor antagonists
BRD-A84174393	meloxicam	cyclooxygenase inhibitor, prostanoid receptor inh	PTGS2, PTGS		26.39	M01AC06	MUSCULO-SKEL	ANTIINFLAMMA	ANTIINFLAMMA	Oxicams Oxicams Oxicams
BRD-K69608737	tacrolimus	calcineurin inhibitor, FK506-binding protein inhib	FKBP1A, CYP		22.41	L04AD02	ANTINEOPLASTI	IMMUNOSUPPRE	IMMUNOSUPPRE	Calcineurin inhibitors
BRD-A29426959	carbinoxamine	histamine receptor antagonist, L-type calcium cha	HRH1HRH1		20.87	R06AA08	RESPIRATORY S	ANTIHISTAMINE	ANTIHISTAMINE	Aminoalkyl ethers Aminoalkyl ethers
BRD-A79768653	sirolimus	mTOR inhibitor, CCR expression inhibitor, cell c	MTOR, FKBP		13.37	L04AA10	ANTINEOPLASTI	IMMUNOSUPPRE	IMMUNOSUPPRE	Selective immunosuppressants
BRD-K12994359	valdecoxib	cyclooxygenase inhibitor	PTGS2, CA12		9.71	M01AH03	MUSCULO-SKEL	ANTIINFLAMMA	ANTIINFLAMMA	Coxibs Coxibs Coxibs
BRD-K69600043	thiethylperazine	dopamine receptor antagonist	DRD1, DRD2,	Dopamine recepto	7.58	R06AD03	RESPIRATORY S	ANTIHISTAMINE	ANTIHISTAMINE	Phenothiazine derivatives
BRD-A01320529	salmeterol	adrenergic receptor agonist	ADRB2	Beta-adrenergic re	6.8	R03AC12	RESPIRATORY S	DRUGS FOR OBS	ADRENERGICS, I	Selective beta-2-adrenoreceptor agonists

Immunomodulators connected to 3D EVs

id	name	description	target	belongs_to	score	ATC code	ATC L1	ATC L2	ATC L3	ATC L4
BRD-A68723818	brompheniramine	histamine receptor antagonist	HRH1, CHRM		88.22	R06AB01	RESPIRATORY S	ANTIHISTAMINE	ANTIHISTAMINE	Substituted alkylamines
BRD-A87479750	tenidap	cyclooxygenase inhibitor, lipoxygenase inhibitor,	ALOX5, KCN		85.74	M01AX23	MUSCULO-SKEL	ANTIINFLAMMA	ANTIINFLAMMA	Other antiinflammatory and antirheumatic agents,
BRD-A57382968	piroxicam	cyclooxygenase inhibitor	PTGS2, PTGS		79.82	M01AC01	MUSCULO-SKEL	ANTIINFLAMMA	ANTIINFLAMMA	Oxicams Oxicams Oxicams
BRD-K11742128	triprolidine	histamine receptor antagonist	HRH1HRH1		77.34	R06AX07	RESPIRATORY S	ANTIHISTAMINE	ANTIHISTAMINE	Other antihistamines for systemic use
BRD-K84937637	sirolimus	mTOR inhibitor, CCR expression inhibitor, cell c	MTOR, FKBP		54.68	L04AA10	ANTINEOPLASTI	IMMUNOSUPPRE	IMMUNOSUPPRE	Selective immunosuppressants
BRD-K12513978	fenbufen	cyclooxygenase inhibitor	PTGS1, PTGS	Cyclooxygenase in	51.38	M01AE05	MUSCULO-SKEL	ANTIINFLAMMA	ANTIINFLAMMA	Propionic acid derivatives
BRD-A06352418	terfenadine	histamine receptor antagonist	HRH1, KCNH		48.74	R06AX12	RESPIRATORY S	ANTIHISTAMINE	ANTIHISTAMINE	Other antihistamines for systemic use
BRD-A50157456	terbutaline	adrenergic receptor agonist	ADRB2	Beta-adrenergic re	36.71	R03AC03	RESPIRATORY S	DRUGS FOR OBS	ADRENERGICS, I	Selective beta-2-adrenoreceptor agonists
BRD-K32821942	azathioprine	dehydrogenase inhibitor, immunosuppressant, pur	HPRT1, IMPD	IMPDH inhibitor	30.44	L04AX01	ANTINEOPLASTI	IMMUNOSUPPRE	IMMUNOSUPPRE	Other immunosuppressants
BRD-A22684332	procaterol	adrenergic receptor agonist	ADRB2	Beta-adrenergic re	22.74	R03AC16	RESPIRATORY S	DRUGS FOR OBS	ADRENERGICS, I	Selective beta-2-adrenoreceptor agonists
BRD-A50311610	meclozine	constitutive androstane receptor (CAR) agonist	NR1I3, HRH1		12.78	R06AE05	RESPIRATORY S	ANTIHISTAMINE	ANTIHISTAMINE	Piperazine derivatives

(*cont'd*) Table 6. Connectivity Map analysis of EV-induced DEGs

Cardiovascular agents connected to 2D EVs											
id	name	description	target	belongs_to	score	ATC					
						code	L1	L2	L3	L4	
BRD-K60038276	irbesartan	angiotensin receptor antagonist, liver bile acid tra	AGTR1, JUN,	Angiotensin recept	99.58	C09CA04	CARDIOVASCUL	AGENTS ACTING	ANGIOTENSIN II	Angiotensin II receptor blockers, plain [DG:DG01	
BRD-A96107863	nisoldipine	calcium channel blocker, L-type calcium channel	CACNA1C, C	Calcium channel b	98.12	C08CA07	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Dihydropyridine derivatives [DG:DG01928]	
BRD-K52930707	rescinnamine	angiotensin converting enzyme inhibitor	ACEACE		96.29	C02AA01	CARDIOVASCUL	ANTIHYPERTEN	ANTIADRENERG	Rauwolfia alkaloids [DG:DG01737]	
BRD-K08806317	timolol	adrenergic receptor antagonist	ADRB2, ADR		96.27	C07AA06	CARDIOVASCUL	BETA BLOCKING	BETA BLOCKING	Beta blocking agents, non-selective	
BRD-K95921201	reserpine	vesicular monoamine transporter inhibitor	SLC18A2, SL		92.51	C02AA02	CARDIOVASCUL	ANTIHYPERTEN	ANTIADRENERG	Rauwolfia alkaloids [DG:DG01737]	
BRD-K96354014	nifedipine	calcium channel blocker, L-type calcium channel	CACNA1C, C		91.83	C08CA05	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Dihydropyridine derivatives [DG:DG01928]	
BRD-A48631911	mibefradil	T-type calcium channel blocker, angiogenesis inh	CACNA1G, C		87.4	C08CX01	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Other selective calcium channel blockers with mai	
BRD-A52282606	lacidipine	calcium channel blocker, L-type calcium channel	CACNA1C	Calcium channel b	82.03	C08CA09	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Dihydropyridine derivatives [DG:DG01928]	
BRD-K73109821	diazoxide	ATP channel activator, potassium channel activat	ATP1A1, CA1		69.14	C02DA01	CARDIOVASCUL	ANTIHYPERTEN	ARTERIOLAR SM	Thiazide derivatives	
BRD-K32830106	guanfacine	adrenergic receptor agonist, HCN (hyperpolariza	ADRA2A, AD		68.48	C02AC02	CARDIOVASCUL	ANTIHYPERTEN	ANTIADRENERG	Imidazoline receptor agonists	
BRD-A90799790	isradipine	calcium channel blocker, L-type calcium channel	CACNA1C, C		67.24	C08CA03	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Dihydropyridine derivatives [DG:DG01928]	
BRD-A64297288	amlodipine	breast cancer resistance protein inhibitor, calcium	CACNA1C, C	Calcium channel b	59.26	C08CA01	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Dihydropyridine derivatives [DG:DG01928]	
BRD-A19633847	perhexiline	carnitine palmitoyltransferase binder, carnitine pa	CPT1A, CPT2		57.72	C08EX02	CARDIOVASCUL	CALCIUM CHAN	NON-SELECTIVE	Other non-selective calcium channel blockers	
BRD-K81029756	diltiazem	L-type calcium channel blocker, calcium channel	CACNA1C, C		50.05	C08DB01	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Benzothiazepine derivatives	
BRD-A63445921	nicardipine	calcium channel blocker, L-type calcium channel	CACNA1C, A		49.9	C08CA04	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Dihydropyridine derivatives [DG:DG01928]	
BRD-K09549677	mibefradil	T-type calcium channel blocker, angiogenesis inh	CACNA1G, C	T-type calcium ch	47.38	C08CX01	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Other selective calcium channel blockers with mai	
BRD-K74763371	bosentan	endothelin receptor antagonist	EDNRB, EDN		41.42	C02KX01	CARDIOVASCUL	ANTIHYPERTEN	OTHER ANTIHYP	Antihypertensive for pulmonary arterial hypertens	
BRD-K01638814	rilmenidine	adrenergic receptor agonist, imidazoline receptor	NISCH	Imidazoline ligand	39.38	C02AC06	CARDIOVASCUL	ANTIHYPERTEN	ANTIADRENERG	Imidazoline receptor agonists	
BRD-A20239487	atenolol	adrenergic receptor antagonist	ADRB1, ADR		38.96	C07AB03	CARDIOVASCUL	BETA BLOCKING	BETA BLOCKING	Beta blocking agents, selective	
BRD-A65739223	ramipril	angiotensin converting enzyme inhibitor	ACEACE		36.39	C09AA05	CARDIOVASCUL	AGENTS ACTING	ACE INHIBITORS	ACE inhibitors, plain [DG:DG01501]	
BRD-A02759312	betaxolol	adrenergic receptor antagonist	ADRB1, ADR		34.75	C07AB05	CARDIOVASCUL	BETA BLOCKING	BETA BLOCKING	Beta blocking agents, selective	
BRD-A97701745	pindolol	adrenergic receptor antagonist	ADRB1, ADR		33.24	C07AA03	CARDIOVASCUL	BETA BLOCKING	BETA BLOCKING	Beta blocking agents, non-selective	
BRD-A07875874	cilnidipine	calcium channel blocker, L-type calcium channel	CACNA1B, C		28.21	C08CA14	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Dihydropyridine derivatives [DG:DG01928]	
BRD-A30977374	nifedipine	calcium channel blocker, L-type calcium channel	CACNA1C, C		24.47	C08CA05	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Dihydropyridine derivatives [DG:DG01928]	
BRD-K49671696	ketanserin	serotonin receptor antagonist, collagen stimulant	HTR2A, HTR		23.6	C02KD01	CARDIOVASCUL	ANTIHYPERTEN	OTHER ANTIHYP	Serotonin antagonists	
BRD-A10977446	carvedilol	adrenergic receptor antagonist, reducing agent, ry	ADRB1, ADR		20.56	C07AG02	CARDIOVASCUL	BETA BLOCKING	BETA BLOCKING	Alpha and beta blocking agents	
BRD-A12560204	nitrendipine	calcium channel blocker, L-type calcium channel	CACNA1C, C	Calcium channel b	18.36	C08CA08	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Dihydropyridine derivatives [DG:DG01928]	
BRD-A09533288	verapamil	calcium channel blocker, L-type calcium channel	CACNA1C, C		12.39	C08DA01	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Phenylalkylamine derivatives	
BRD-A77722753	hydralazine	smooth muscle relaxant	AOC3, P4HA1		9.12	C02DB02	CARDIOVASCUL	ANTIHYPERTEN	ARTERIOLAR SM	Hydrazinophthalazine derivatives [DG:DG02046]	
BRD-K77771411	moxonidine	imidazoline receptor agonist, adrenergic receptor	NISCH, ADR		8.14	C02AC05	CARDIOVASCUL	ANTIHYPERTEN	ANTIADRENERG	Imidazoline receptor agonists	
BRD-A89175223	bisoprolol	adrenergic receptor antagonist	ADRB1, ADR		5.82	C07AB07	CARDIOVASCUL	BETA BLOCKING	BETA BLOCKING	Beta blocking agents, selective	
BRD-K89348303	ramipril	angiotensin converting enzyme inhibitor	ACEACE		1.75	C09AA05	CARDIOVASCUL	AGENTS ACTING	ACE INHIBITORS	ACE inhibitors, plain [DG:DG01501]	

(*cont'd*) Table 6. Connectivity Map analysis of EV-induced DEGs

Cardiovascular agents connected to 3D EVs											
id	name	description	target	belongs_to	score	ATC					
						code	L1	L2	L3	L4
BRD-K73196317	urapidil	adrenergic receptor antagonist, serotonin recepto	HTR1A		90.28	C02CA06	CARDIOVASCUL	ANTIHYPERTEN	ANTIADRENERG	Alpha-adrenoreceptor antagonists
BRD-A96107863	nisoldipine	calcium channel blocker, L-type calcium channel	CACNA1C, C	Calcium channel b	74.68	C08CA07	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Dihydropyridine derivatives [DG:DG01928]
BRD-A19633847	perhexiline	carnitine palmitoyltransferase binder, carnitine pa	CPT1A, CPT2		47.17	C08EX02	CARDIOVASCUL	CALCIUM CHAN	NON-SELECTIVE	Other non-selective calcium channel blockers
BRD-K62996583	lidoflazine	calcium channel antagonist	SCN1A, SCN3		46.4	C08EX01	CARDIOVASCUL	CALCIUM CHAN	NON-SELECTIVE	Other non-selective calcium channel blockers
BRD-A58207013	pinacidil	ATP channel activator, potassium channel agonis	ABCC9, KCN		45.36	C02DG01	CARDIOVASCUL	ANTIHYPERTEN	ARTERIOLAR SM	Guanidine derivatives
BRD-A20239487	atenolol	adrenergic receptor antagonist	ADRB1, ADR		42.81	C07AB03	CARDIOVASCUL	BETA BLOCKING	BETA BLOCKING	Beta blocking agents, selective
BRD-K81029756	diltiazem	L-type calcium channel blocker, calcium channel	CACNA1C, C		41.23	C08DB01	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Benzothiazepine derivatives
BRD-A65739223	ramipril	angiotensin converting enzyme inhibitor	ACEACE		37.65	C09AA05	CARDIOVASCUL	AGENTS ACTING	ACE INHIBITORS	ACE inhibitors, plain [DG:DG01501]
BRD-A24891640	nimodipine	calcium channel blocker, L-type calcium channel	CACNA1C, N		36.24	C08CA06	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Dihydropyridine derivatives [DG:DG01928]
BRD-A51382177	fosinopril	angiotensin converting enzyme inhibitor, breast c	ACE, ABCG2		35.49	C09AA09	CARDIOVASCUL	AGENTS ACTING	ACE INHIBITORS	ACE inhibitors, plain [DG:DG01501]
BRD-A91008255	bepridil	calcium channel blocker, L-type calcium channel	ATP1A1, CAC		32.26	C08EA02	CARDIOVASCUL	CALCIUM CHAN	NON-SELECTIVE	Phenylalkylamine derivatives
BRD-A12560204	nitrendipine	calcium channel blocker, L-type calcium channel	CACNA1C, C	Calcium channel b	26.11	C08CA08	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Dihydropyridine derivatives [DG:DG01928]
BRD-A64297288	amlodipine	breast cancer resistance protein inhibitor, calcium	CACNA1C, C	Calcium channel b	18.16	C08CA01	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Dihydropyridine derivatives [DG:DG01928]
BRD-A07440155	labetalol	adrenergic receptor antagonist	ADRB1, ADR		17.32	C07AG01	CARDIOVASCUL	BETA BLOCKING	BETA BLOCKING	Alpha and beta blocking agents
BRD-M62162320	rilmenidine	adrenergic receptor agonist, imidazoline receptor	NISCH		14.57	C02AC06	CARDIOVASCUL	ANTIHYPERTEN	ANTIADRENERG	Imidazoline receptor agonists
BRD-A09533288	verapamil	calcium channel blocker, L-type calcium channel	CACNA1C, C		8.7	C08DA01	CARDIOVASCUL	CALCIUM CHAN	SELECTIVE CAL	Phenylalkylamine derivatives
BRD-A33168282	sotalol	adrenergic receptor antagonist, polarization inhib	ADRB1, ADR		8.39	C07AA07	CARDIOVASCUL	BETA BLOCKING	BETA BLOCKING	Beta blocking agents, non-selective
BRD-A89175223	bisoprolol	adrenergic receptor antagonist	ADRB1, ADR		2.69	C07AB07	CARDIOVASCUL	BETA BLOCKING	BETA BLOCKING	Beta blocking agents, selective
BRD-K73999723	telmisartan	angiotensin receptor antagonist, PPAR receptor a	AGTR1, PPAR		2.34	C09CA07	CARDIOVASCUL	AGENTS ACTING	ANGIOTENSIN II	Angiotensin II receptor blockers, plain [DG:DG01
BRD-K73109821	diazoxide	ATP channel activator, potassium channel activat	ATP1A1, CA1		1.98	C02DA01	CARDIOVASCUL	ANTIHYPERTEN	ARTERIOLAR SM	Thiazide derivatives

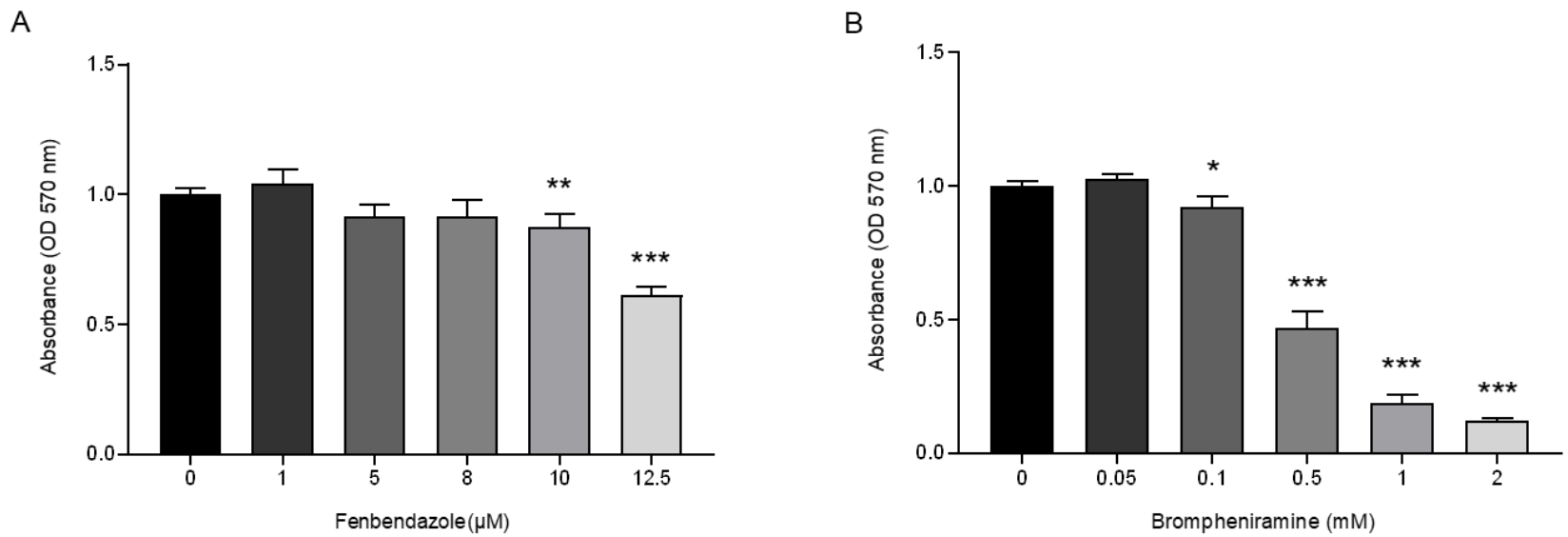


Figure 12. Range finding of drugs

A. Range finding of Fenbendazole. **p < 0.01, ***p < 0.001 vs. 0 μM (n = 4). **B.** Range finding of Brompheniramine (n = 4). Bars indicate mean ± SD.

Table 7. Enriched KEGG pathways with DEGs by 3D EVs over 2D EVs

MapID	MapName	No. of SigGenes	Gene IDs	No. of GenesInKEGG	PValue	Bonferroni	FDR
01100	Metabolic pathways	37	10846, 10855, 11227, 133686, 1555, 18, 1962, 216,	1432	9.19E-14	2.21E-11	2.21E-11
05168	Herpes simplex virus 1 infection	22	125919, 162966, 170959, 284370, 340252, 353088,	492	1.82E-12	4.37E-10	2.19E-10
04060	Cytokine-cytokine receptor interaction	17	151449, 27242, 3553, 4982, 5196, 55504, 60401, 63	294	2.93E-11	7.02E-09	2.34E-09
04010	MAPK signaling pathway	14	1946, 2069, 27092, 3082, 3553, 3815, 4217, 4254, 4	295	2.41E-08	5.78E-06	1.03E-06
04151	PI3K-Akt signaling pathway	15	1288, 1946, 2069, 22801, 23566, 3082, 3371, 3655,	354	2.54E-08	6.09E-06	1.03E-06
04668	TNF signaling pathway	10	182, 3383, 3553, 4217, 5608, 6347, 6352, 6374, 741	110	2.59E-08	6.21E-06	1.03E-06
04014	Ras signaling pathway	12	100271927, 1946, 27, 27040, 3082, 3815, 4254, 490	232	1.46E-07	3.51E-05	5.01E-06
04514	Cell adhesion molecules (CAMs)	10	1462, 3383, 3655, 3897, 4267, 4685, 5010, 7412, 90	146	2.52E-07	6.06E-05	7.57E-06
04350	TGF-beta signaling pathway	8	151449, 2331, 4052, 4090, 4091, 5933, 64388, 654	94	1.72E-06	4.12E-04	4.58E-05
05165	Human papillomavirus infection	12	1288, 182, 22801, 3371, 3655, 4854, 5933, 7058, 71	330	4.03E-06	9.67E-04	9.67E-05
04360	Axon guidance	9	10512, 151449, 1946, 223117, 2534, 3897, 5362, 72	181	1.23E-05	2.96E-03	2.53E-04
05144	Malaria	6	3082, 3383, 3553, 6347, 7058, 7412	49	1.33E-05	3.18E-03	2.53E-04
04512	ECM-receptor interaction	7	1288, 22801, 2814, 3371, 3655, 7058, 7148	86	1.37E-05	3.28E-03	2.53E-04
05322	Systemic lupus erythematosus	8	55506, 735, 8331, 8335, 8340, 8341, 8342, 8968	133	1.49E-05	3.58E-03	2.56E-04
05323	Rheumatoid arthritis	7	3383, 3553, 6347, 6352, 6372, 6374, 7010	91	1.85E-05	4.44E-03	2.94E-04
05418	Fluid shear stress and atherosclerosis	8	2944, 3383, 3553, 4217, 5608, 6347, 7056, 7412	139	1.96E-05	4.70E-03	2.94E-04
04640	Hematopoietic cell lineage	7	2814, 290, 3553, 3655, 3815, 4254, 4311	97	2.61E-05	6.25E-03	3.63E-04
05205	Proteoglycans in cancer	9	10855, 117581, 3082, 5328, 7078, 7430, 7472, 7482	203	2.72E-05	6.54E-03	3.63E-04
04064	NF-kappa B signaling pathway	7	27040, 3383, 3553, 4067, 5328, 60401, 7412	100	3.07E-05	7.36E-03	3.87E-04
04730	Long-term depression	6	2781, 2892, 2983, 4067, 5321, 773	60	3.29E-05	7.90E-03	3.95E-04
00760	Nicotinate and nicotinamide metabolism	5	133686, 23475, 4860, 5167, 683	30	3.88E-05	9.30E-03	4.43E-04
04670	Leukocyte transendothelial migration	7	3383, 4267, 5010, 7412, 7430, 9076, 90952	112	5.60E-05	1.34E-02	6.11E-04
05164	Influenza A	8	3383, 3553, 6041, 6347, 6352, 7177, 9021, 90865	167	6.00E-05	1.44E-02	6.19E-04
05200	Pathways in cancer	13	1288, 182, 23566, 2944, 3082, 3655, 3815, 4254, 48	530	6.19E-05	1.48E-02	6.19E-04
05020	Prion diseases	5	2534, 3553, 4685, 6352, 735	35	6.73E-05	1.61E-02	6.46E-04
04621	NOD-like receptor signaling pathway	8	115361, 2634, 3553, 4671, 51393, 6041, 6347, 6352	178	8.81E-05	2.12E-02	8.14E-04
04510	Focal adhesion	8	1288, 22801, 2534, 3082, 3371, 3655, 7058, 7148	199	1.72E-04	4.12E-02	1.52E-03
04015	Rap1 signaling pathway	8	1946, 23566, 27040, 3082, 3815, 4254, 5608, 7010	210	2.36E-04	5.66E-02	2.02E-03
04666	Fc gamma R-mediated phagocytosis	6	27040, 4067, 4651, 5321, 85477, 8877	94	2.44E-04	5.87E-02	2.02E-03
04061	Viral protein interaction with cytokine and cytokine recepto	6	5196, 6347, 6352, 6372, 6374, 8793	100	3.21E-04	7.71E-02	2.49E-03
04933	AGE-RAGE signaling pathway in diabetic complications	6	1288, 3383, 3553, 6347, 7056, 7412	100	3.21E-04	7.71E-02	2.49E-03
04310	Wnt signaling pathway	7	22943, 27123, 64840, 7472, 7482, 8325, 8549	160	3.61E-04	8.67E-02	2.71E-03
04530	Tight junction	7	23327, 4217, 4629, 5010, 7430, 9076, 9414	170	4.93E-04	1.18E-01	3.58E-03
05034	Alcoholism	7	55506, 8331, 8335, 8340, 8341, 8342, 8968	180	6.58E-04	1.58E-01	4.65E-03
04664	Fc epsilon RI signaling pathway	5	2534, 27040, 4067, 5321, 5608	68	7.28E-04	1.75E-01	4.99E-03
04080	Neuroactive ligand-receptor interaction	9	23566, 2558, 2693, 2892, 5021, 56413, 5731, 6751,	338	7.91E-04	1.90E-01	5.27E-03
04062	Chemokine signaling pathway	7	157, 4067, 5196, 6347, 6352, 6372, 6374	190	8.64E-04	2.07E-01	5.61E-03
00230	Purine metabolism	6	10846, 2983, 4860, 5138, 5167, 956	130	1.01E-03	2.42E-01	6.36E-03
00410	beta-Alanine metabolism	4	18, 1962, 219, 54498	33	1.08E-03	2.58E-01	6.49E-03
03320	PPAR signaling pathway	5	1962, 2167, 2710, 51129, 79966	76	1.08E-03	2.60E-01	6.49E-03
04610	Complement and coagulation cascades	5	3075, 5328, 5648, 7056, 735	79	1.24E-03	2.98E-01	7.26E-03
04742	Taste transduction	5	2558, 40, 6326, 6328, 773	83	1.48E-03	3.54E-01	8.44E-03
05224	Breast cancer	6	182, 3815, 4854, 7472, 7482, 8325	147	1.70E-03	4.09E-01	9.46E-03
04072	Phospholipase D signaling pathway	6	23566, 2534, 3815, 4254, 5321, 8877	148	1.75E-03	4.21E-01	9.46E-03
05206	MicroRNAs in cancer	8	3371, 406911, 407014, 4854, 5328, 7078, 7148, 743	299	1.77E-03	4.26E-01	9.46E-03

(*cont'd*) Table 7. Enriched KEGG pathways with DEGs by 3D EVs over 2D EVs

MapID	MapName	No. of SigGenes	Gene IDs	No. of GenesInKEGG	PValue	Bonferroni	FDR
05032	Morphine addiction	5	10846, 157, 2558, 5138, 773	91	2.04E-03	4.89E-01	1.06E-02
04217	Necroptosis	6	3553, 5321, 55506, 8331, 8335, 90865	162	2.56E-03	6.15E-01	1.31E-02
04750	Inflammatory mediator regulation of TRP channels	5	3553, 40, 51393, 5321, 5608	100	2.83E-03	6.80E-01	1.42E-02
04916	Melanogenesis	5	3815, 4254, 7472, 7482, 8325	101	2.93E-03	7.04E-01	1.44E-02
05130	Pathogenic Escherichia coli infection	4	2534, 7280, 7430, 9076	55	4.25E-03	1.00E+00	2.04E-02
04740	Olfactory transduction	9	143503, 157, 219968, 219981, 26532, 390168, 4016	448	4.37E-03	1.00E+00	2.06E-02
00240	Pyrimidine metabolism	4	4860, 5167, 956, 978	57	4.68E-03	1.00E+00	2.16E-02
04020	Calcium signaling pathway	6	490, 5021, 56413, 5731, 773, 8877	193	5.28E-03	1.00E+00	2.39E-02
00561	Glycerolipid metabolism	4	219, 2710, 57016, 84803	61	5.60E-03	1.00E+00	2.49E-02
04611	Platelet activation	5	2534, 2814, 2983, 4067, 5321	124	5.93E-03	1.00E+00	2.59E-02
00590	Arachidonic acid metabolism	4	1555, 5321, 5740, 9536	63	6.10E-03	1.00E+00	2.61E-02
05203	Viral carcinogenesis	6	4067, 5933, 8340, 8341, 8342, 85477	201	6.22E-03	1.00E+00	2.62E-02
04380	Osteoclast differentiation	5	2534, 3553, 4982, 5608, 9021	128	6.60E-03	1.00E+00	2.72E-02
00532	Glycosaminoglycan biosynthesis - chondroitin sulfate / derm	3	55790, 56548, 64131	20	6.69E-03	1.00E+00	2.72E-02
04650	Natural killer cell mediated cytotoxicity	5	2534, 27040, 3383, 3803, 80328	131	7.13E-03	1.00E+00	2.85E-02
04024	cAMP signaling pathway	6	10846, 2693, 2892, 490, 5021, 6751	214	8.00E-03	1.00E+00	3.15E-02
05133	Pertussis	4	3553, 6372, 6374, 653509	76	1.00E-02	1.00E+00	3.87E-02
05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	4	22801, 27092, 3655, 5318	77	1.03E-02	1.00E+00	3.94E-02
04390	Hippo signaling pathway	5	151449, 654, 7472, 7482, 8325	154	1.22E-02	1.00E+00	4.58E-02
05160	Hepatitis C	5	3434, 5010, 6041, 9021, 9076	155	1.25E-02	1.00E+00	4.60E-02

Discussion

The 3D culture of cells induces cell–cell and cell–extracellular matrix (ECM) interactions that differ from those of 2D-cultured cells, affecting the tension and polarity of cells [42]. These changes alter the expression and composition of cell–cell interacting proteins and influence the activity of signaling pathways related to lineage specification and other cellular functions. In the present study, these changes were accompanied by enhancement of signaling pathways including those of cell adhesion molecules (CAMs), ECM-receptor interactions, the MAPK signaling pathway, and the PI3K-Akt signaling pathway under 3D culture conditions (Table 7). Local gradients of oxygen and cell growth factors may be main causes of changes in cell properties related to 3D culture [43]. In this study, I observed spheroid-like aggregates of hMSCs in 3D cultures grown using Cellhesion VP, and these increased in size to more than 100 μm during the prolonged culture period. A difference in oxygen concentration between the surface and the inside of the spheroid is inevitable in such a tight cellular structure, and the inside of these spheroids is expected to be hypoxic [44]. Insufficient delivery of oxygen and culture medium could induce cell necrosis within the spheroid, which may have caused the significant reduction of hMSC numbers in 3D culture compared to 2D culture. By contrast, adequate hypoxia has a beneficial impact on the properties of MSCs. For example, hMSCs grown under hypoxic conditions have higher expression levels of OCT4, c-MYC, and NANOG than their normoxic counterparts [45]. The improved proliferation rate and upregulated OCT4 expression of retrieved hMSCs from 3D culture observed in this study are consistent with previous

findings, indicating that appropriate hypoxia contributes to the enhanced growth and stemness of MSCs [46, 47].

Cellhesion VP is a water-insoluble material composed of chitin-based polysaccharide fibers. Chitin is a natural polysaccharide containing β (1–4)-linked D-glucosamine residues with a variable number of N-acetyl-glucosamine groups [48], and is a biodegradable, biocompatible, and bioadhesive material that has been approved by the US Food and Drug Administration (FDA) for human use [49]. Various cell types have been evaluated on chitin-based polymers. For example, chitosan, a deacetylated derivative of chitin, promotes spheroid formation in various cell types [44], enhances the pluripotency of adipose-derived stem cells [44], and branching morphogenesis of progenitor salivary tissue [50]. In accordance with previous results, I observed similar effects in Cellhesion VP. hMSCs formed spheroid-like aggregates in Cellhesion VP-mediated 3D culture, along with substantial increases in OCT4, NANOG, and SSEA4 expression, as well as migration ability. Mobility is a feature of MSCs that is essential to their therapeutic potency [51, 52]. Moreover, these characteristic improvements persisted even after retrieval of 3D cells under conventional 2D culture conditions. The molecular mechanisms of these changes remain unclear, but they may be associated with increased cell–cell and cell–ECM interactions due to Cellhesion VP. Cells in spheroid form are in close contact with each other, and show a high degree of confluency due to their rounded shape. This feature of 3D cells is similar to those in vivo, and allows them to exhibit distinct gene regulation compared to 2D cells [44]. Chitin derivatives is known to exhibit higher levels of gene expression for wound healing and ECM formation through spheroid formation [49]; my results are in line with those findings. In

addition, chitin-based polymers mediate biological functions through their polycationic properties, enabling interactions with various ECM molecules such as collagen, dextran sulfate, heparin, and hyaluronic acid and the establishment of cell–ECM network structures [50]. The molecular weight of a chitin-based polymer is correlated with its biological activity, and high-molecular-weight chitin, such as that in Cellhesion VP, may have better biological functions [50]. Therefore, the improved properties of hMSCs due to 3D culture in this study are expected to be derived from chitin, the main component of Cellhesion VP, as the high-molecular-weight chitin used in Cellhesion VP would improve the 3D culture performance of hMSCs.

Multipotent MSCs have been isolated and studied extensively for the past 30 years. Given their regenerative, tissue engineering, and immune-modulatory properties, MSCs continue to be cultivated for clinical applications. The most commonly used MSCs originate from human bone marrow [53, 54] and adipose tissue [55]. Recently, the umbilical cord [56] and placenta [57] have been recognized as prominent sources. As of 2019, 10 MSC-based products have received regulatory approval worldwide, including Alofisel® (www.takeda.com) and Cupistem® (www.anterogen.com) for Crohn's fistulas, Ortho-ACITM (www.orthocell.com.au) and Spherox (www.medthority.com) for cartilage damage, Ossgrow® (www.avnindia.co.in) to treat bone defects in avascular necrosis, Stempeucel® (www.stempeutics.com) for critical limb ischemia, Prochymal® (www.osiris.com) and Temcell®HS (www.jcrpharm.co.jp) for management of acute graft-versus-host disease, Neuronata-R® (www.corestem.com) for amyotrophic lateral sclerosis, and Hearticellgram®-AMI (www.pharmicell.com) for myocardial infarction. MSCs are advantageous over other stem cells for a variety of reasons,

including immune privilege, ease of expansion, engrafting ability, and ethical acceptance [58]. During the early phase of development, cell therapies using MSCs were studied for regenerative purposes by replacing damaged cells with newly introduced cells. However, due to the complex mechanism of tissue repair and the limited number of MSCs introduced, no clinical evidence of tissue regeneration or cell replacement with the injected cells was obtained. Instead, alternative mechanisms have been suggested for the therapeutic potential of MSCs, including the production of paracrine factors such as proteins and hormones, and their loading into EVs for delivery of bioactive ingredients [59].

The therapeutic efficacy of MSC-derived EVs has been demonstrated in animal models of liver diseases such as fibrosis, cirrhosis, and acute liver injury [60, 61]; kidney diseases such as ischemic reperfusion injury, inflammation, and acute tubular injury [62, 63], cardiovascular diseases such as acute myocardial infarction, pulmonary hypertension, and septic cardiomyopathy [64], neurological processes such as stroke and cognition [65, 66]; and immunological disorders such as chronic graft-versus-host disease and type 1 diabetes [67, 68]. Based on these findings, several clinical trials are underway to assess the therapeutic efficacy of MSC-derived EVs in humans for the treatment of cerebrovascular disorders at phase 1/2, macular holes at phase 1, type 1 diabetes at phase 3, and non-small cell lung cancer at phase 2 (http://clinicaltrials.gov). In this study, the clinical prospects of 2D and 3D EVs from hMSCs were assessed for anti-infective, anti-metabolic disorders, and anti-cardiovascular disorders, as well as immunosuppressant function, similar to the functions being tested in current clinical trials. Interestingly, 2D and 3D EVs were predicted to have similar clinical indications but distinct therapeutic

profiles. In particular, the dysregulation or enhancement by 3D EVs of specific signaling pathways associated with immune modulation, infection, and cardiovascular complications provides a molecular basis for the differentiated therapeutic profiles of 3D EVs.

Conclusions

This study demonstrated that hMSCs primed using chitin-based polysaccharides had increased cell proliferation rates and upregulated transcriptional pathways related to stemness, cell mobility, and the production of EVs. Subsequent cellular and biochemical experiments confirmed a substantial increase in the expression levels of OCT4, NANOG, and SSEA4, an improvement in wound healing capacity, and an increase in EV production along with distinct protein composition relative to 2D EVs. EVs from 3D-cultured hMSCs appeared to activate signaling pathways in the target cells related to immune, infection, and metabolic diseases, supporting their application as a therapeutic agent for these conditions. Efficacy studies for predicted clinical applications were performed in this study, and the pharmacological mechanisms and predicted applications based on connectivity map analyses supported the high therapeutic potential of 3D EVs for certain conditions. Therefore, I propose that EVs from hMSCs primed using chitin-based polysaccharides can be applied as novel therapeutic agents for treatment of immune and metabolic diseases.

Abstract in Korean

중간엽 줄기세포 이식은 재생의학에서 유망한 치료법입니다. 그러나 2차원 배양 조건에서 자란 중간엽 줄기세포는 체내에서의 중간엽 줄기세포와 세포 모양이 크게 다르고, 줄기세포능 관련 유전자 및 측분비인자의 분비가 하향 조절됩니다.

본 연구에서는 키틴 기반 다당류 섬유를 사용한 3차원 배양이 인간 와튼제대교질 유래 중간엽 줄기세포의 특성에 미치는 효과를 평가했습니다. 키틴 기반 다당류에서 3차원 배양한 후 다시 2차원으로 옮긴 세포는 증식이 크게 증가했습니다. 전사체 분석 결과는 줄기세포능, 이동 능력 및 세포 외 소포체 생산에 관련된 유전자의 발현이 3차원 배양에 의해 향상되었음을 나타냈습니다.

후속 생화학 분석은 3차원 배양된 인간 중간엽 줄기세포에서 OCT4, NANOG 및 SSEA4를 포함한 줄기세포능 관련 유전자의 발현과 이동 능력, 그리고 세포 외 소포체 생산 모두 크게 증가했음을 보여주었습니다. 또한, 3차원 배양된 세포에서 분비된 세포 외 소포체는 2차원 배양된 세포의 것과는 차별되는 단백질 발현 정보를 담고 있었습니다.

유전자 및 약물 연결성 분석을 통해 2차원 및 3차원 배양 세포의 세포 외 소포체가 면역 조절제와 유사한 기능을 가지고

있음을 확인했습니다. 그러나 3차원 배양 세포의 세포 외 소포체를 질병 관련 신호전달 경로의 활성화를 기반으로 분석했을 때 여러 감염성 및 대사성 질병에 대해 2차원 배양 세포의 세포 외 소포체와는 완전히 다른 치료 프로파일을 가진 것으로 나타났습니다.

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References

[1] J. Galipeau, M. Krampera, J. Barrett, F. Dazzi, R.J. Deans, J. DeBruijn, M. Dominici, W.E. Fibbe, A.P. Gee, J.M. Gimble, P. Hematti, M.B. Koh, K. LeBlanc, I. Martin, I.K. McNiece, M. Mendicino, S. Oh, L. Ortiz, D.G. Phinney, V. Planat, Y. Shi, D.F. Stroncek, S. Viswanathan, D.J. Weiss, L. Sensebe, International Society for Cellular Therapy perspective on immune functional assays for mesenchymal stromal cells as potency release criterion for advanced phase clinical trials, Cytotherapy 18(2) (2016) 151-9.
[2] N.C. Noronha, A. Mizukami, C. Caliári-Oliveira, J.G. Cominal, J.L.M. Rocha, D.T. Covas, K. Swiech, K.C.R. Malmegrim, Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies, Stem Cell Res Ther 10(1) (2019) 131.
[3] A. Bongso, C.Y. Fong, The therapeutic potential, challenges and future clinical directions of stem cells from the Wharton's jelly of the human umbilical cord, Stem Cell Rev Rep 9(2) (2013) 226-40.
[4] A. Musiał-Wysocka, M. Kot, M. Majka, The Pros and Cons of Mesenchymal Stem Cell-Based Therapies, Cell Transplant 28(7) (2019) 801-812.
[5] M.B. Murphy, K. Moncivais, A.I. Caplan, Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine, Exp Mol Med 45(11) (2013) e54.
[6] I.R. Murray, C.C. West, W.R. Hardy, A.W. James, T.S. Park, A. Nguyen, T. Tawonsawatruk, L. Lazzari, C. Soo, B. Péault, Natural history of mesenchymal stem cells, from vessel walls to culture vessels, Cell Mol Life Sci 71(8) (2014) 1353-74.
[7] C. Akyurekli, Y. Le, R.B. Richardson, D. Fergusson, J. Tay, D.S. Allan, A systematic review of preclinical studies on the therapeutic potential of mesenchymal stromal cell-derived microvesicles, Stem Cell Rev Rep 11(1) (2015) 150-60.
[8] T. Katsuda, N. Kosaka, F. Takeshita, T. Ochiya, The therapeutic potential of mesenchymal stem cell-derived extracellular vesicles, Proteomics 13(10-11) (2013) 1637-53.
[9] C. Thery, K.W. Witwer, E. Aikawa, M.J. Alcaraz, J.D. Anderson, R. Andriantsitohaina, A. Antoniou, T. Arab, F. Archer, G.K. Atkin-Smith, D.C. Ayre, J.M. Bach, D. Bachurski, H. Baharvand, L. Balaj, S. Baldacchino, N.N. Bauer, A.A. Baxter, M. Bebawy, C. Beckham, A. Bedina Zavec, A. Benmoussa, A.C. Berardi, P. Bergese, E. Bielska, C. Blenkiron, S. Bobis-Wozowicz, E. Boilard, W. Boireau, A. Bongiovanni, F.E. Borras, S. Bosch, C.M. Boulanger, X. Breakefield, A.M. Breglio, M.A. Brennan, D.R. Brigstock, A. Brisson, M.L. Broekman, J.F. Bromberg, P. Bryl-Gorecka, S. Buch, A.H. Buck, D. Burger, S. Busatto, D. Buschmann, B. Bussolati, E.I. Buzas, J.B. Byrd, G. Camussi, D.R. Carter,

S. Caruso, L.W. Chamley, Y.T. Chang, C. Chen, S. Chen, L. Cheng, A.R. Chin, A. Clayton, S.P. Clerici, A. Cocks, E. Cocucci, R.J. Coffey, A. Cordeiro-da-Silva, Y. Couch, F.A. Coumans, B. Coyle, R. Crescitelli, M.F. Criado, C. D'Souza-Schorey, S. Das, A. Datta Chaudhuri, P. de Candia, E.F. De Santana, O. De Wever, H.A. Del Portillo, T. Demaret, S. Deville, A. Devitt, B. Dhondt, D. Di Vizio, L.C. Dieterich, V. Dolo, A.P. Dominguez Rubio, M. Dominici, M.R. Dourado, T.A. Driedonks, F.V. Duarte, H.M. Duncan, R.M. Eichenberger, K. Ekstrom, S. El Andaloussi, C. Elie-Caille, U. Erdbrugger, J.M. Falcon-Perez, F. Fatima, J.E. Fish, M. Flores-Bellver, A. Forsonits, A. Frelet-Barrand, F. Fricke, G. Fuhrmann, S. Gabrielsson, A. Gamez-Valero, C. Gardiner, K. Gartner, R. Gaudin, Y.S. Gho, B. Giebel, C. Gilbert, M. Gimona, I. Giusti, D.C. Goberdhan, A. Gorgens, S.M. Gorski, D.W. Greening, J.C. Gross, A. Gualerzi, G.N. Gupta, D. Gustafson, A. Handberg, R.A. Haraszti, P. Harrison, H. Hegyesi, A. Hendrix, A.F. Hill, F.H. Hochberg, K.F. Hoffmann, B. Holder, H. Holthofer, B. Hosseinkhani, G. Hu, Y. Huang, V. Huber, S. Hunt, A.G. Ibrahim, T. Ikezu, J.M. Inal, M. Isin, A. Ivanova, H.K. Jackson, S. Jacobsen, S.M. Jay, M. Jayachandran, G. Jenster, L. Jiang, S.M. Johnson, J.C. Jones, A. Jong, T. Jovanovic-Talisman, S. Jung, R. Kalluri, S.I. Kano, S. Kaur, Y. Kawamura, E.T. Keller, D. Khamari, E. Khomyakova, A. Khvorova, P. Kierulf, K.P. Kim, T. Kislinger, M. Klingeborn, D.J. Klinke, 2nd, M. Kornek, M.M. Kosanovic, A.F. Kovacs, E.M. Kramer-Albers, S. Krasemann, M. Krause, I.V. Kurochkin, G.D. Kusuma, S. Kuypers, S. Laitinen, S.M. Langevin, L.R. Languino, J. Lannigan, C. Lasser, L.C. Laurent, G. Lavieu, E. Lazaro-Ibanez, S. Le Lay, M.S. Lee, Y.X.F. Lee, D.S. Lemos, M. Lenassi, A. Leszczynska, I.T. Li, K. Liao, S.F. Libregts, E. Ligeti, R. Lim, S.K. Lim, A. Line, K. Linnemannstons, A. Llorente, C.A. Lombard, M.J. Lorenowicz, A.M. Lorincz, J. Lotvall, J. Lovett, M.C. Lowry, X. Loyer, Q. Lu, B. Lukomska, T.R. Lunavat, S.L. Maas, H. Malhi, A. Marcilla, J. Mariani, J. Mariscal, E.S. Martens-Uzunova, L. Martin-Jaular, M.C. Martinez, V.R. Martins, M. Mathieu, S. Mathivanan, M. Maugeri, L.K. McGinnis, M.J. McVey, D.G. Meckes, Jr., K.L. Meehan, I. Mertens, V.R. Minciacchi, A. Moller, M. Moller Jorgensen, A. Morales-Kastresana, J. Morhayim, F. Mullier, M. Muraca, L. Musante, V. Mussack, D.C. Muth, K.H. Myburgh, T. Najrana, M. Nawaz, I. Nazarenko, P. Nejsum, C. Neri, T. Neri, R. Nieuwland, L. Nimrichter, J.P. Nolan, E.N. Nolte-'t Hoen, N. Noren Hooten, L. O'Driscoll, T. O'Grady, A. O'Loghlen, T. Ochiya, M. Olivier, A. Ortiz, L.A. Ortiz, X. Osteikoetxea, O. Ostergaard, M. Ostrowski, J. Park, D.M. Pegtel, H. Peinado, F. Perut, M.W. Pfaffl, D.G. Phinney, B.C. Pieters, R.C. Pink, D.S. Pisetsky, E. Pogge von Strandmann, I. Polakovicova, I.K. Poon, B.H. Powell, I. Prada, L. Pulliam, P. Quesenberry, A. Radeghieri, R.L. Raffai, S. Raimondo, J. Rak, M.I. Ramirez, G. Raposo, M.S. Rayyan, N. Regev-Rudzki, F.L. Ricklefs, P.D. Robbins, D.D. Roberts, S.C.

Rodrigues, E. Rohde, S. Rome, K.M. Rouschop, A. Rughetti, A.E. Russell, P. Saa, S. Sahoo, E. Salas-Huenuleo, C. Sanchez, J.A. Saugstad, M.J. Saul, R.M. Schiffelers, R. Schneider, T.H. Schoyen, A. Scott, E. Shahaj, S. Sharma, O. Shatnyeva, F. Shekari, G.V. Shelke, A.K. Shetty, K. Shiba, P.R. Siljander, A.M. Silva, A. Skowronek, O.L. Snyder, 2nd, R.P. Soares, B.W. Sodar, C. Soekmadji, J. Sotillo, P.D. Stahl, W. Stoorvogel, S.L. Stott, E.F. Strasser, S. Swift, H. Tahara, M. Tewari, K. Timms, S. Tiwari, R. Tixeira, M. Tkach, W.S. Toh, R. Tomasini, A.C. Torrecilhas, J.P. Tosar, V. Toxavidis, L. Urbanelli, P. Vader, B.W. van Balkom, S.G. van der Grein, J. Van Deun, M.J. van Herwijnen, K. Van Keuren-Jensen, G. van Niel, M.E. van Royen, A.J. van Wijnen, M.H. Vasconcelos, I.J. Vechetti, Jr., T.D. Veit, L.J. Vella, E. Velot, F.J. Verweij, B. Vestad, J.L. Vinas, T. Visnovitz, K.V. Vukman, J. Wahlgren, D.C. Watson, M.H. Wauben, A. Weaver, J.P. Webber, V. Weber, A.M. Wehman, D.J. Weiss, J.A. Welsh, S. Wendt, A.M. Wheelock, Z. Wiener, L. Witte, J. Wolfram, A. Xagorari, P. Xander, J. Xu, X. Yan, M. Yanez-Mo, H. Yin, Y. Yuana, V. Zappulli, J. Zarubova, V. Zekas, J.Y. Zhang, Z. Zhao, L. Zheng, A.R. Zheutlin, A.M. Zickler, P. Zimmermann, A.M. Zivkovic, D. Zocco, E.K. Zuba-Surma, Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines, J Extracell Vesicles 7(1) (2018) 1535750.

[10] H. Valadi, K. Ekstrom, A. Bossios, M. Sjostrand, J.J. Lee, J.O. Lotvall, Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, Nat Cell Biol 9(6) (2007) 654-9.

[11] A. Hoshino, B. Costa-Silva, T.L. Shen, G. Rodrigues, A. Hashimoto, M. Tesic Mark, H. Molina, S. Kohsaka, A. Di Giannatale, S. Ceder, S. Singh, C. Williams, N. Soplop, K. Uryu, L. Pharmer, T. King, L. Bojmar, A.E. Davies, Y. Ararso, T. Zhang, H. Zhang, J. Hernandez, J.M. Weiss, V.D. Dumont-Cole, K. Kramer, L.H. Wexler, A. Narendran, G.K. Schwartz, J.H. Healey, P. Sandstrom, K.J. Labori, E.H. Kure, P.M. Grandgenett, M.A. Hollingsworth, M. de Sousa, S. Kaur, M. Jain, K. Mallya, S.K. Batra, W.R. Jarnagin, M.S. Brady, O. Fodstad, V. Muller, K. Pantel, A.J. Minn, M.J. Bissell, B.A. Garcia, Y. Kang, V.K. Rajasekhar, C.M. Ghajar, I. Matei, H. Peinado, J. Bromberg, D. Lyden, Tumour exosome integrins determine organotropic metastasis, Nature 527(7578) (2015) 329-35.

[12] B. Costa-Silva, N.M. Aiello, A.J. Ocean, S. Singh, H. Zhang, B.K. Thakur, A. Becker, A. Hoshino, M.T. Mark, H. Molina, J. Xiang, T. Zhang, T.M. Theilen, G. Garcia-Santos, C. Williams, Y. Ararso, Y. Huang, G. Rodrigues, T.L. Shen, K.J. Labori, I.M. Lothe, E.H. Kure, J. Hernandez, A. Doussot, S.H. Ebbesen, P.M. Grandgenett, M.A. Hollingsworth, M. Jain, K. Mallya, S.K. Batra, W.R. Jarnagin, R.E. Schwartz, I. Matei, H. Peinado, B.Z.

Stanger, J. Bromberg, D. Lyden, Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver, Nat Cell Biol 17(6) (2015) 816-26.
[13] A. La Greca, C. Solari, V. Furmento, A. Lombardi, M.C. Biani, C. Aban, L. Moro, M. García, A.S. Guberman, G.E. Sevlever, S.G. Miriuka, C. Luzzani, Extracellular vesicles from pluripotent stem cell-derived mesenchymal stem cells acquire a stromal modulatory proteomic pattern during differentiation, Exp Mol Med 50(9) (2018) 1-12.
[14] K. Sabin, N. Kikyo, Microvesicles as mediators of tissue regeneration, Transl Res 163(4) (2014) 286-95.
[15] R.C. Lai, F. Arslan, M.M. Lee, N.S. Sze, A. Choo, T.S. Chen, M. Salto-Tellez, L. Timmers, C.N. Lee, R.M. El Oakley, G. Pasterkamp, D.P. de Kleijn, S.K. Lim, Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury, Stem Cell Res 4(3) (2010) 214-22.
[16] S. Rani, A.E. Ryan, M.D. Griffin, T. Ritter, Mesenchymal Stem Cell-derived Extracellular Vesicles: Toward Cell-free Therapeutic Applications, Mol Ther 23(5) (2015) 812-823.
[17] K. Popowski, H. Lutz, S. Hu, A. George, P.U. Dinh, K. Cheng, Exosome therapeutics for lung regenerative medicine, J Extracell Vesicles 9(1) (2020) 1785161.
[18] Z. Zhang, J. Yang, W. Yan, Y. Li, Z. Shen, T. Asahara, Pretreatment of Cardiac Stem Cells With Exosomes Derived From Mesenchymal Stem Cells Enhances Myocardial Repair, J Am Heart Assoc 5(1) (2016) e002856.
[19] K.M. Panchalingam, S. Jung, L. Rosenberg, L.A. Behie, Bioprocessing strategies for the large-scale production of human mesenchymal stem cells: a review, Stem Cell Res Ther 6 (2015) 225.
[20] I.K. Yan, N. Shukla, D.A. Borrelli, T. Patel, Use of a Hollow Fiber Bioreactor to Collect Extracellular Vesicles from Cells in Culture, Methods Mol Biol 1740 (2018) 35-41.
[21] J. Phan, P. Kumar, D. Hao, K. Gao, D. Farmer, A. Wang, Engineering mesenchymal stem cells to improve their exosome efficacy and yield for cell-free therapy, J Extracell Vesicles 7(1) (2018) 1522236.
[22] Y. Zhou, H. Chen, H. Li, Y. Wu, 3D culture increases pluripotent gene expression in mesenchymal stem cells through relaxation of cytoskeleton tension, J Cell Mol Med 21(6) (2017) 1073-1084.
[23] S. Sethe, A. Scutt, A. Stolzing, Aging of mesenchymal stem cells, Ageing Res Rev 5(1) (2006) 91-116.
[24] K. Ksiazek, A comprehensive review on mesenchymal stem cell growth and

senescence, Rejuvenation Res 12(2) (2009) 105-16.
[25] G.D. Kusuma, J. Carthew, R. Lim, J.E. Frith, Effect of the Microenvironment on Mesenchymal Stem Cell Paracrine Signaling: Opportunities to Engineer the Therapeutic Effect, Stem Cells Dev 26(9) (2017) 617-631.
[26] J.E. Frith, B. Thomson, P.G. Genever, Dynamic three-dimensional culture methods enhance mesenchymal stem cell properties and increase therapeutic potential, Tissue Eng Part C Methods 16(4) (2010) 735-49.
[27] Z. Cesarz, K. Tamama, Spheroid Culture of Mesenchymal Stem Cells, Stem Cells Int 2016 (2016) 9176357.
[28] Y. Petrenko, E. Sykova, S. Kubinova, The therapeutic potential of three-dimensional multipotent mesenchymal stromal cell spheroids, Stem Cell Res Ther 8(1) (2017) 94.
[29] T.J. Bartosh, J.H. Ylostalo, N. Bazhanov, J. Kuhlman, D.J. Prockop, Dynamic compaction of human mesenchymal stem/precursor cells into spheres self-activates caspase-dependent IL1 signaling to enhance secretion of modulators of inflammation and immunity (PGE2, TSG6, and STC1), Stem Cells 31(11) (2013) 2443-56.
[30] L. Guo, Y. Zhou, S. Wang, Y. Wu, Epigenetic changes of mesenchymal stem cells in three-dimensional (3D) spheroids, J Cell Mol Med 18(10) (2014) 2009-19.
[31] J. Feng, S. Yang, L. Xu, H. Tian, L. Sun, X. Tang, Role of caspase-3 inhibitor in induced anoikis of mesenchymal stem cells in vitro, J Huazhong Univ Sci Technolog Med Sci 27(2) (2007) 183-5.
[32] S. Islam, M.A.R. Bhuiyan, M.N. Islam, Chitin and Chitosan: Structure, Properties and Applications in Biomedical Engineering, Journal of Polymers and the Environment 25(3) (2017) 854-866.
[33] S. Afewerki, A. Sheikhi, S. Kannan, S. Ahadian, A. Khademhosseini, Gelatin-polysaccharide composite scaffolds for 3D cell culture and tissue engineering: Towards natural therapeutics, Bioeng Transl Med 4(1) (2019) 96-115.
[34] V.V. Mutsenko, V.V. Bazhenov, O. Rogulska, D.N. Tarusin, K. Schütz, S. Brüggemeier, E. Gossla, A.R. Akkineni, H. Meißner, A. Lode, S. Meschke, A. Ehrlich, S. Petović, R. Martinović, M. Djurović, A.L. Stelling, S. Nikulin, S. Rodin, A. Tonevitsky, M. Gelinsky, A.Y. Petrenko, B. Glasmacher, H. Ehrlich, 3D chitinous scaffolds derived from cultivated marine demosponge Aplysina aerophoba for tissue engineering approaches based on human mesenchymal stromal cells, Int J Biol Macromol 104(Pt B) (2017) 1966-1974.
[35] H. Lu, D. Samanta, L. Xiang, H. Zhang, H. Hu, I. Chen, J.W. Bullen, G.L. Semenza,

Chemotherapy triggers HIF-1-dependent glutathione synthesis and copper chelation that induces the breast cancer stem cell phenotype, Proc Natl Acad Sci U S A 112(33) (2015) E4600-9.
[36] S.B. Shivakumar, D. Bharti, S.J. Jang, S.C. Hwang, J.K. Park, J.K. Shin, J.H. Byun, B.W. Park, G.J. Rho, Cryopreservation of Human Wharton's Jelly-derived Mesenchymal Stem Cells Following Controlled Rate Freezing Protocol Using Different Cryoprotectants; A Comparative Study, Int J Stem Cells 8(2) (2015) 155-69.
[37] J.F. Liu, Y.T. Tsao, C.H. Hou, Amphiregulin enhances intercellular adhesion molecule-1 expression and promotes tumor metastasis in human osteosarcoma, Oncotarget 6(38) (2015) 40880-95.
[38] E.-T. Consortium, J. Van Deun, P. Mestdagh, P. Agostinis, O. Akay, S. Anand, J. Anckaert, Z.A. Martinez, T. Baetens, E. Beghein, L. Bertier, G. Berx, J. Boere, S. Boukouris, M. Bremer, D. Buschmann, J.B. Byrd, C. Casert, L. Cheng, A. Cmoch, D. Daveloose, E. De Smedt, S. Demirsoy, V. Depoorter, B. Dhondt, T.A. Driedonks, A. Dudek, A. Elsharawy, I. Floris, A.D. Foers, K. Gartner, A.D. Garg, E. Geeurickx, J. Gettemans, F. Ghazavi, B. Giebel, T.G. Kormelink, G. Hancock, H. Helsmoortel, A.F. Hill, V. Hyenne, H. Kalra, D. Kim, J. Kowal, S. Kraemer, P. Leidinger, C. Leonelli, Y. Liang, L. Lippens, S. Liu, A. Lo Cicero, S. Martin, S. Mathivanan, P. Mathiyalagan, T. Matusek, G. Milani, M. Monguio-Tortajada, L.M. Mus, D.C. Muth, A. Nemeth, E.N. Nolte-'t Hoen, L. O'Driscoll, R. Palmulli, M.W. Pfaffl, B. Primdal-Bengtson, E. Romano, Q. Rousseau, S. Sahoo, N. Sampaio, M. Samuel, B. Scicluna, B. Soen, A. Steels, J.V. Swinnen, M. Takatalo, S. Thaminy, C. Thery, J. Tulkens, I. Van Audenhove, S. van der Grein, A. Van Goethem, M.J. van Herwijnen, G. Van Niel, N. Van Roy, A.R. Van Vliet, N. Vandamme, S. Vanhauwaert, G. Vergauwen, F. Verweij, A. Wallaert, M. Wauben, K.W. Witwer, M.I. Zonneveld, O. De Wever, J. Vandesompele, A. Hendrix, EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research, Nat Methods 14(3) (2017) 228-232.
[39] A. Subramanian, P. Tamayo, V.K. Mootha, S. Mukherjee, B.L. Ebert, M.A. Gillette, A. Paulovich, S.L. Pomeroy, T.R. Golub, E.S. Lander, J.P. Mesirov, Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles, Proc Natl Acad Sci U S A 102(43) (2005) 15545-50.
[40] Y. Zhou, B. Zhou, L. Pache, M. Chang, A.H. Khodabakhshi, O. Tanaseichuk, C. Benner, S.K. Chanda, Metascape provides a biologist-oriented resource for the analysis of systems-level datasets, Nat Commun 10(1) (2019) 1523.
[41] A. Subramanian, R. Narayan, S.M. Corsello, D.D. Peck, T.E. Natoli, X. Lu, J. Gould,

J.F. Davis, A.A. Tubelli, J.K. Asiedu, D.L. Lahr, J.E. Hirschman, Z. Liu, M. Donahue, B. Julian, M. Khan, D. Wadden, I.C. Smith, D. Lam, A. Liberzon, C. Toder, M. Bagul, M. Orzechowski, O.M. Enache, F. Piccioni, S.A. Johnson, N.J. Lyons, A.H. Berger, A.F. Shamji, A.N. Brooks, A. Vrcic, C. Flynn, J. Rosains, D.Y. Takeda, R. Hu, D. Davison, J. Lamb, K. Ardlie, L. Hogstrom, P. Greenside, N.S. Gray, P.A. Clemons, S. Silver, X. Wu, W.N. Zhao, W. Read-Button, X. Wu, S.J. Haggarty, L.V. Ronco, J.S. Boehm, S.L. Schreiber, J.G. Doench, J.A. Bittker, D.E. Root, B. Wong, T.R. Golub, A Next Generation Connectivity Map: L1000 Platform and the First 1,000,000 Profiles, Cell 171(6) (2017) 1437-1452 e17.

[42] B. Follin, M. Juhl, S. Cohen, A.E. Pedersen, J. Kastrup, A. Ekblond, Increased Paracrine Immunomodulatory Potential of Mesenchymal Stromal Cells in Three-Dimensional Culture, Tissue Eng Part B Rev 22(4) (2016) 322-9.

[43] S. Sart, A.C. Tsai, Y. Li, T. Ma, Three-dimensional aggregates of mesenchymal stem cells: cellular mechanisms, biological properties, and applications, Tissue Eng Part B Rev 20(5) (2014) 365-80.

[44] N.C. Cheng, S. Wang, T.H. Young, The influence of spheroid formation of human adipose-derived stem cells on chitosan films on stemness and differentiation capabilities, Biomaterials 33(6) (2012) 1748-58.

[45] S.Y. Kwon, S.Y. Chun, Y.S. Ha, D.H. Kim, J. Kim, P.H. Song, H.T. Kim, E.S. Yoo, B.S. Kim, T.G. Kwon, Hypoxia Enhances Cell Properties of Human Mesenchymal Stem Cells, Tissue Eng Regen Med 14(5) (2017) 595-604.

[46] Y. Guo, L. Einhorn, M. Kelley, K. Hirota, J. Yodoi, R. Reinbold, H. Scholer, H. Ramsey, R. Hromas, Redox regulation of the embryonic stem cell transcription factor oct-4 by thioredoxin, Stem Cells 22(3) (2004) 259-64.

[47] A. Mishra, R.K. Ganesan, A. Dhali, I.J. Reddy, Interaction of apoptosis and pluripotency related transcripts for developmental potential of ovine embryos produced in vitro at different oxygen concentrations, Anim Biotechnol (2020) 1-9.

[48] S.J. Lin, S.H. Jee, W.C. Hsaio, S.J. Lee, T.H. Young, Formation of melanocyte spheroids on the chitosan-coated surface, Biomaterials 26(12) (2005) 1413-22.

[49] H.J. Shao, Y.T. Lee, C.S. Chen, J.H. Wang, T.H. Young, Modulation of gene expression and collagen production of anterior cruciate ligament cells through cell shape changes on polycaprolactone/chitosan blends, Biomaterials 31(17) (2010) 4695-705.

[50] T.L. Yang, T.H. Young, The specificity of chitosan in promoting branching morphogenesis of progenitor salivary tissue, Biochem Biophys Res Commun 381(4)

(2009) 466-70.
[51] Y. Mabuchi, S. Morikawa, S. Harada, K. Niibe, S. Suzuki, F. Renault-Mihara, D.D. Houlihan, C. Akazawa, H. Okano, Y. Matsuzaki, LNGFR(+)THY-1(+)VCAM-1(hi+) cells reveal functionally distinct subpopulations in mesenchymal stem cells, Stem Cell Reports 1(2) (2013) 152-65.
[52] H. Katagiri, Y. Kushida, M. Nojima, Y. Kuroda, S. Wakao, K. Ishida, F. Endo, K. Kume, T. Takahara, H. Nitta, H. Tsuda, M. Dezawa, S.S. Nishizuka, A Distinct Subpopulation of Bone Marrow Mesenchymal Stem Cells, Muse Cells, Directly Commit to the Replacement of Liver Components, Am J Transplant 16(2) (2016) 468-83.
[53] S.E. Haynesworth, J. Goshima, V.M. Goldberg, A.I. Caplan, Characterization of cells with osteogenic potential from human marrow, Bone 13(1) (1992) 81-8.
[54] M.F. Pittenger, A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, D.R. Marshak, Multilineage potential of adult human mesenchymal stem cells, Science 284(5411) (1999) 143-7.
[55] P.A. Zuk, M. Zhu, H. Mizuno, J. Huang, J.W. Futrell, A.J. Katz, P. Benhaim, H.P. Lorenz, M.H. Hedrick, Multilineage cells from human adipose tissue: implications for cell-based therapies, Tissue Eng 7(2) (2001) 211-28.
[56] Y.A. Romanov, V.A. Svintsitskaya, V.N. Smirnov, Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord, Stem Cells 21(1) (2003) 105-10.
[57] P.S. In 't Anker, S.A. Scherjon, C. Kleijburg-van der Keur, G.M. de Groot-Swings, F.H. Claas, W.E. Fibbe, H.H. Kanhai, Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta, Stem Cells 22(7) (2004) 1338-45.
[58] M.F. Pittenger, D.E. Discher, B.M. Peault, D.G. Phinney, J.M. Hare, A.I. Caplan, Mesenchymal stem cell perspective: cell biology to clinical progress, NPJ Regen Med 4 (2019) 22.
[59] K. Yin, S. Wang, R.C. Zhao, Exosomes from mesenchymal stem/stromal cells: a new therapeutic paradigm, Biomark Res 7 (2019) 8.
[60] T. Li, Y. Yan, B. Wang, H. Qian, X. Zhang, L. Shen, M. Wang, Y. Zhou, W. Zhu, W. Li, W. Xu, Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis, Stem Cells Dev 22(6) (2013) 845-54.
[61] G. Lou, Z. Chen, M. Zheng, Y. Liu, Mesenchymal stem cell-derived exosomes as a new therapeutic strategy for liver diseases, Exp Mol Med 49(6) (2017) e346.
[62] X. Zou, D. Gu, X. Xing, Z. Cheng, D. Gong, G. Zhang, Y. Zhu, Human mesenchymal stromal cell-derived extracellular vesicles alleviate renal ischemic

reperfusion injury and enhance angiogenesis in rats, Am J Transl Res 8(10) (2016) 4289-4299.
[63] A. Eirin, X.Y. Zhu, A.S. Puranik, H. Tang, K.A. McGurren, A.J. van Wijnen, A. Lerman, L.O. Lerman, Mesenchymal stem cell-derived extracellular vesicles attenuate kidney inflammation, Kidney Int 92(1) (2017) 114-124.
[64] E. Suzuki, D. Fujita, M. Takahashi, S. Oba, H. Nishimatsu, Therapeutic Effects of Mesenchymal Stem Cell-Derived Exosomes in Cardiovascular Disease, Adv Exp Med Biol 998 (2017) 179-185.
[65] H. Xin, M. Katakowski, F. Wang, J.Y. Qian, X.S. Liu, M.M. Ali, B. Buller, Z.G. Zhang, M. Chopp, MicroRNA cluster miR-17-92 Cluster in Exosomes Enhance Neuroplasticity and Functional Recovery After Stroke in Rats, Stroke 48(3) (2017) 747-753.
[66] G.H. Cui, J. Wu, F.F. Mou, W.H. Xie, F.B. Wang, Q.L. Wang, J. Fang, Y.W. Xu, Y.R. Dong, J.R. Liu, H.D. Guo, Exosomes derived from hypoxia-preconditioned mesenchymal stromal cells ameliorate cognitive decline by rescuing synaptic dysfunction and regulating inflammatory responses in APP/PS1 mice, FASEB J 32(2) (2018) 654-668.
[67] P. Lai, X. Chen, L. Guo, Y. Wang, X. Liu, Y. Liu, T. Zhou, T. Huang, S. Geng, C. Luo, X. Huang, S. Wu, W. Ling, X. Du, C. He, J. Weng, A potent immunomodulatory role of exosomes derived from mesenchymal stromal cells in preventing cGVHD, J Hematol Oncol 11(1) (2018) 135.
[68] T. Shigemoto-Kuroda, J.Y. Oh, D.K. Kim, H.J. Jeong, S.Y. Park, H.J. Lee, J.W. Park, T.W. Kim, S.Y. An, D.J. Prockop, R.H. Lee, MSC-derived Extracellular Vesicles Attenuate Immune Responses in Two Autoimmune Murine Models: Type 1 Diabetes and Uveoretinitis, Stem Cell Reports 8(5) (2017) 1214-1225.