서울대학교 시스템면역의학연구소

제4회 국제 학술 대회

The 4th International Symposium of Wide River Institute of Immunology

Innate immune cells in the pathogenesis

▶일시: 2017년 10월 13일 (금) 13:30~18:00

▶장소: 서울대학교 시스템면역의학연구소 볼룸

서울대학교 시스템면역의학연구소

인 사 말

안녕하십니까?

서울대학교 시스템면역의학연구소의 개소 3주년을 기념하며 개최하는 제4회 국제 학술대회에 참석하여 주신 여러분께 깊은 감사의 말씀을 전합니다. 또한 강의를 수락하여 주신 연자분들께도 특별한 감사의 말씀을 드립니다.

서울대학교 시스템면역의학연구소는 종양 및 다양한 면역 질환의 기전 및 치료법에 대한 연구를 수행해 왔습니다. 또한 의생명과학 분야 여러 핵심 기술들을 지원하는 Lab on a Cloud를 구축하여 지난 해 동안 80여 팀과 공동협력 연구를 진행하였습니다. 이처럼 활발한 협력 연구를 통해 건강한 미래사회를 구현하기 위해 최선의 노력을 다하겠습니다. 앞으로도 아낌없는 격려와 관심 부탁드립니다.

이번 국제학술대회에서는 선천면역세포 연구를 선도하는 세계 각국의 연구자를 초청하여 감염, 자가면역 등 다양한 질환에서의 선천면역세포 질병연관성관련된 최신 연구를 소개하는 자리를 마련하였습니다. 본 학회를 통하여 참석자분들의 학문증진 및 상호 교류를 유도하여 연구의 결실이 보다 풍성하게 무르익을 수 있기를 기원합니다.

제4회 국제학술대회가 성공적으로 마무리 될 수 있도록 여러분의 적극적인 성원을 부탁드리며 다시 한 번 감사의 말씀을 드립니다.

서울대학교 시스템면역의학연구소장 **성 승 용**

제4회 서울대학교 시스템면역의학연구소 국제 학술 대회

The 4th International Symposium of Wide River Institute of Immunology

Frontiers in Immune Regulation

Scientific Program

12:30~13:30 Registration

13:30~13:40 Opening remark
Seung-Yong Seong (Seoul National Univiersity, Korea)

13:40~14:20 Mast cells are crucial for induction of group 2 innate lymphoid cells and clearance of helminth infections
Hiroshi Ohno (RIKEN, Japan)

14:20~15:00 NFAT5 is Essential to Rheumatoid Inflammation
Wan-Uk Kim (The Catholic University of Korea, Korea)

15:00~15:40 Critical Role of Commensal Microbiota in Shaping Antiviral Immunity
Heung Kyu Lee (KAIST, Korea)

15:40~16:00 Break

16:00~16:40 A Role of STAT3 in Barrier Integrity and Microbiota Composition of the Skin
Masato Kubo (RIKEN, Japan)

16:40~17:20 Estrogen-related Receptor α and Innate Immune Regulation
Eun-Kyeong Jo (Chungnam National University, Korea

17:20~18:00 Probing contributions of macrophages to organismal homeostasis
Steffen Jung (Weizmann Institute of Science, Israel)

18:00 Closing

Mast cells are crucial for induction of group 2 innate lymphoid cells and clearance of helminth infections

Hiroshi Ohno

RIKEN, Japan

Mast cells are important for eradication of intestinal nematodes; however, the precise mechanisms of action have remained elusive, especially in the early phase of infection. We found that Spi-B-deficient mice (Spi-B-KO) had an increased number of mast cells and rapidly expelled the *Heligmosomoides polygyrus* (Hp) nematode. This was accompanied by the induction of IL-13-producing group 2 innate lymphoid cells (ILC2) and goblet cell hyperplasia. Immediately after Hp infection, mast cells were rapidly activated to produce IL-33 in response to ATP, which was released from apoptotic intestinal epithelial cells. *In vivo* inhibition of the P2X7 ATP receptor on mast cells rendered the Spi-B-KO mice more susceptible to Hp, concomitant with elimination of mast-cell activation and IL-13-producing ILC2 induction. These results uncover a previously unknown role for mast cells in innate immunity in that activation of mast cells by ATP orchestrates the development of protective type 2 immune responses by producing IL-33 crucial for ILC2 activation.

CURRICULUM VITAE

Hiroshi Ohno, MD/PhD

Group Director

Laboratory for Intestinal Ecosystem

RIKEN Center for Integrative Medical Sciences (IMS)

1-7-22 Suehiro, Tsurumi, Yokohama 230-0045, Japan

E-mail: hiroshi.ohno@riken.jp

Education and Appointment

1983	M.D., Chiba University School of Medicine
1991	Ph.D. (Dr. of Medical Science), Chiba University

Professional Training and Employment

1983	passed the Examination of National Board for Medicine
1983-1987	Clinical Fellow in Anesthesiology, Chiba University School of Medicine, Chiba, Japan.
1987-1991	Graduate student in Immunology, Chiba University School of Medicine, Chiba, Japan.
1991-1994	Research Fellow, Division of Molecular Genetics, Center for Biomedical Science, Chiba University School of Medicine, Chiba, Japan.
1994-1997	Visiting Fellow, CBMB, NICHD, NIH, USA.
1997-1999	Associate Professor, Division of Molecular Genetics, Center for Biomedical Science, Chiba University School of Medicine, Chiba, Japan.
1999-2004	Professor, Division of Molecular Membrane Biology, Cancer Research Institute, Kanazawa University, Kanazawa, Japan
2003-2013	Team Leader, Laboratory for Epithelial Immunobioloby, Research Center for Allergy and Immunology, RIKEN, Yokohama, Japan
2005-present	Visiting Professor, Yokohama City University, Yokohama, Japan
2007-present	Visiting Professor, Chiba University, Chiba, Japan
2013-present	Group Director, Laboratory for Intestinal Ecocystem, RIKEN Center for Integrative Medical Sciences, Yokoyama, Japan

Awards

1996	NIH Fellows Award for Research Excellence
1998	Praemium Academiae Inohanae Chibae
2015	Grand Prize, Momofuku Ando Award
2016	Bälz Award

Laboratory for Intestinal Ecosystem

The 4th International Symposium of Wide Rive Institute of Immunology : Innate immune cells in the pathogenesis

October 13, 2017

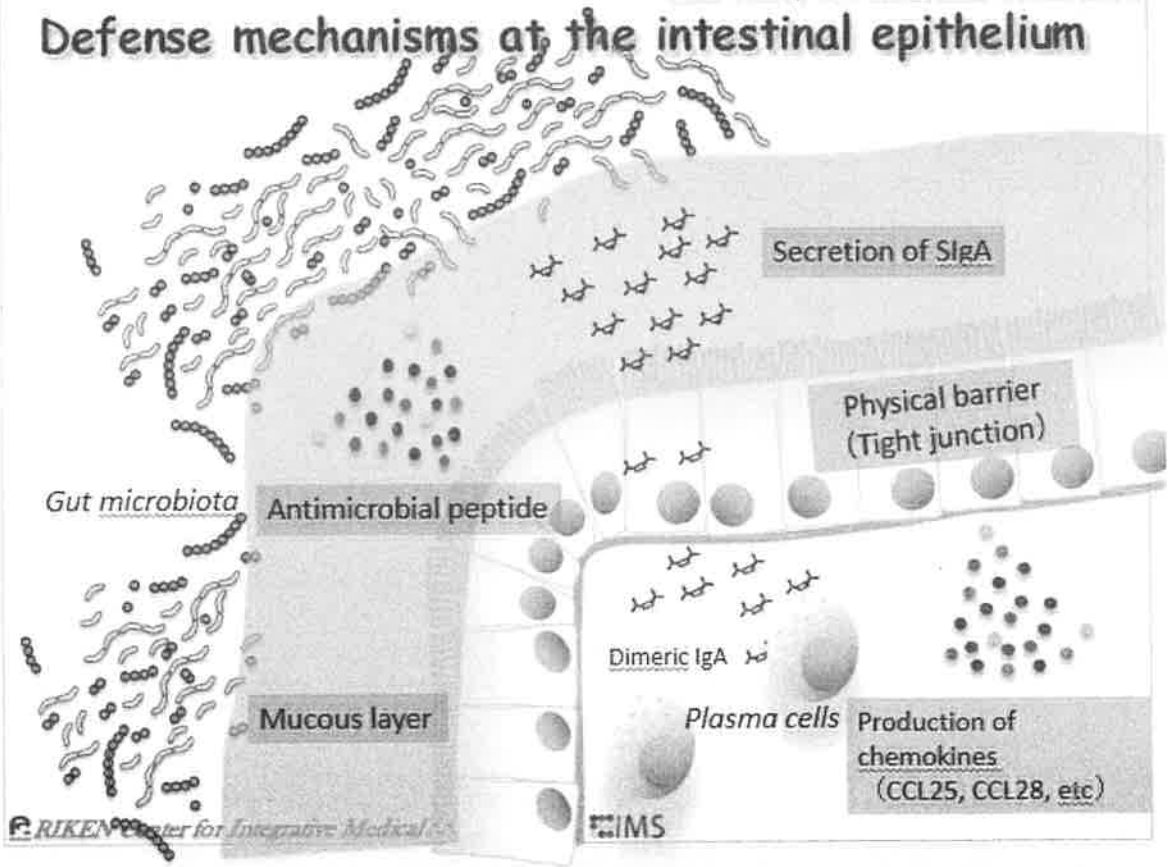
Mast cell-group 2 innate lymphoid cell interaction is important for clearance of helminth infection

Chikako Shimokawa
Hiroshi Ohno
Laboratory for Intestinal Ecosystem
RIKEN Center for Integrative Medical Sciences (IMS)

RIKEN Center for Integrative Medical Sciences (IMS) IMS

Laboratory for Intestinal Ecosystem

Defense mechanisms at the intestinal epithelium

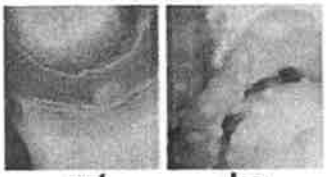


IgA is important for containing gut microbiota

Fagarasan et al., Science 298: 1424-1427, 2002

AID (activation-induced cytidine deaminase) KO mice
→ no class switch → no IgA
→ no somatic hypermutation → no high affinity Ab

B-cell Hyperplasia of GALT in AID-KO mice

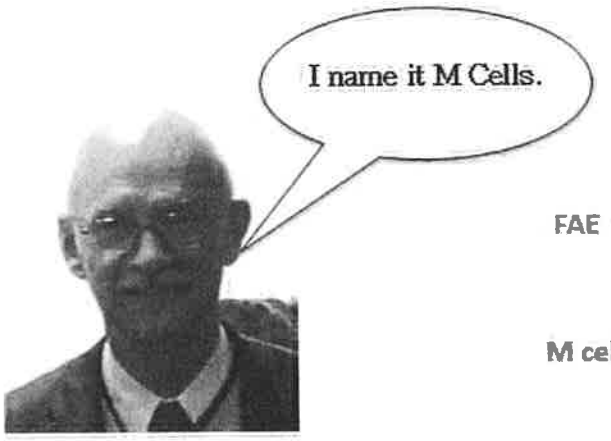


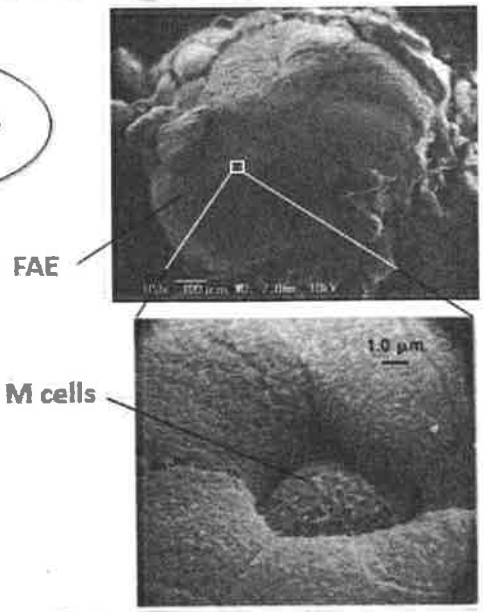
wt ko

Drastic increase of gut bacteria in AID-KO mice

Reversal of lymphatic hyperplasia in AID-KO mice by antibiotics

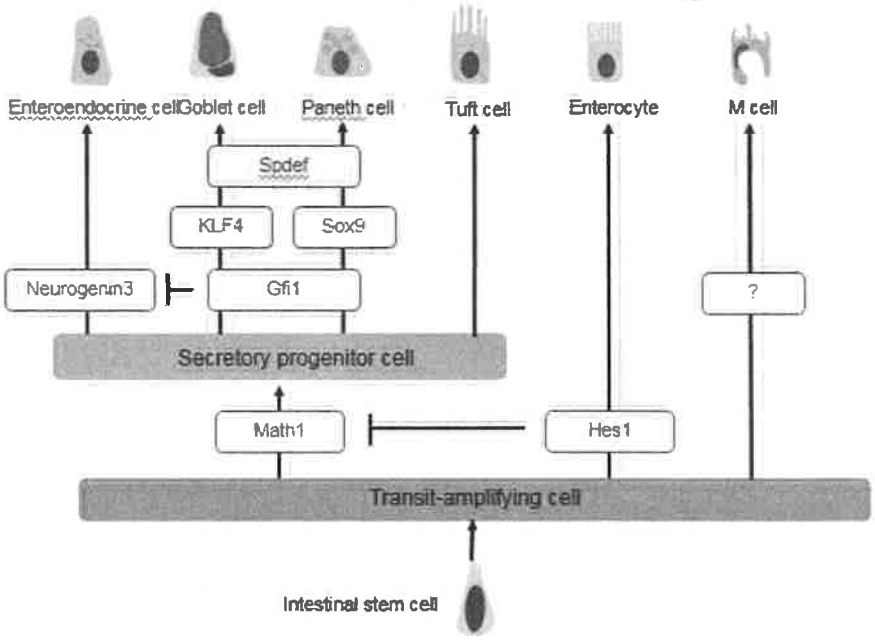
M cells in Follicle-associated epithelium (FAE) is responsible for mucosal antigen-uptake



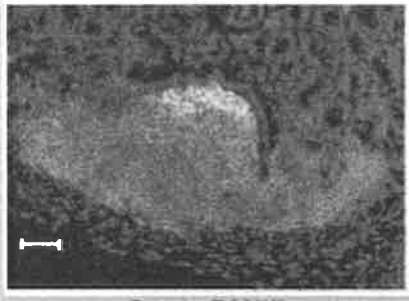


Bockman, D.E. and Cooper, M.D., *Am. J Anat.* 1973
Owen, R.L. and Jones A.L., *Gastroenterology* 1974

Cell-intrinsic regulation for the differentiation of intestinal epithelial lineages

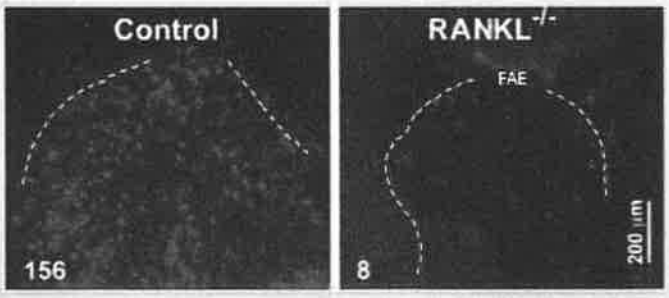


RANKL promotes M-cell differentiation

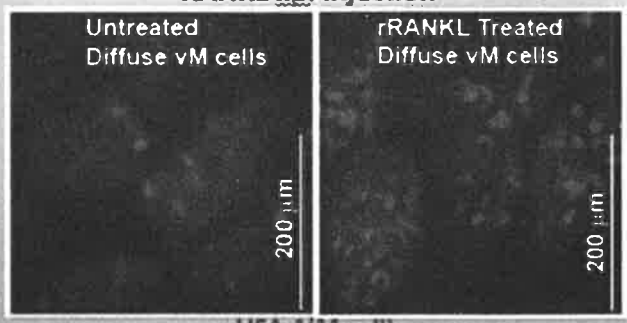


Green: RANKL

Taylor *et al.*, J. Immunol., 2007



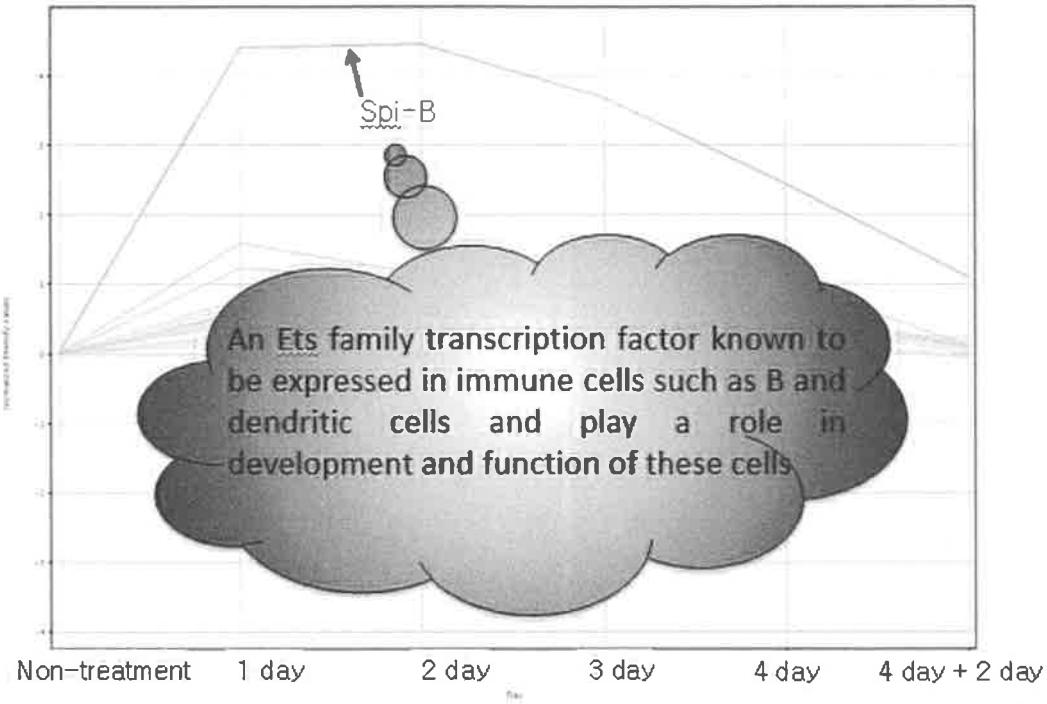
RANKL i.p. injection



UEA-1(M cell)

Knoop *et al.* J. Immunol., 2009

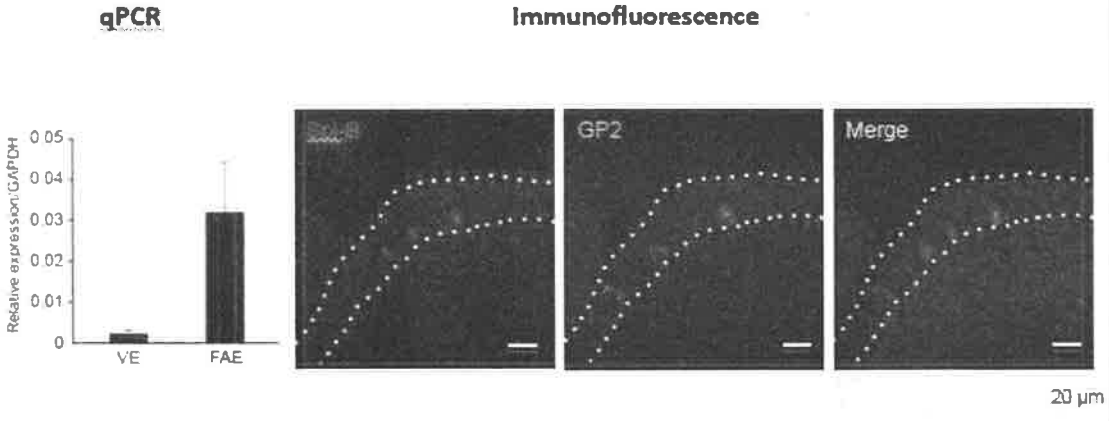
Spi-B is highly induced in villi after RANKL treatment



Kanaya *et al.*, **Nat. Immunol**, 2012

Laboratory for Intestinal Ecosystem

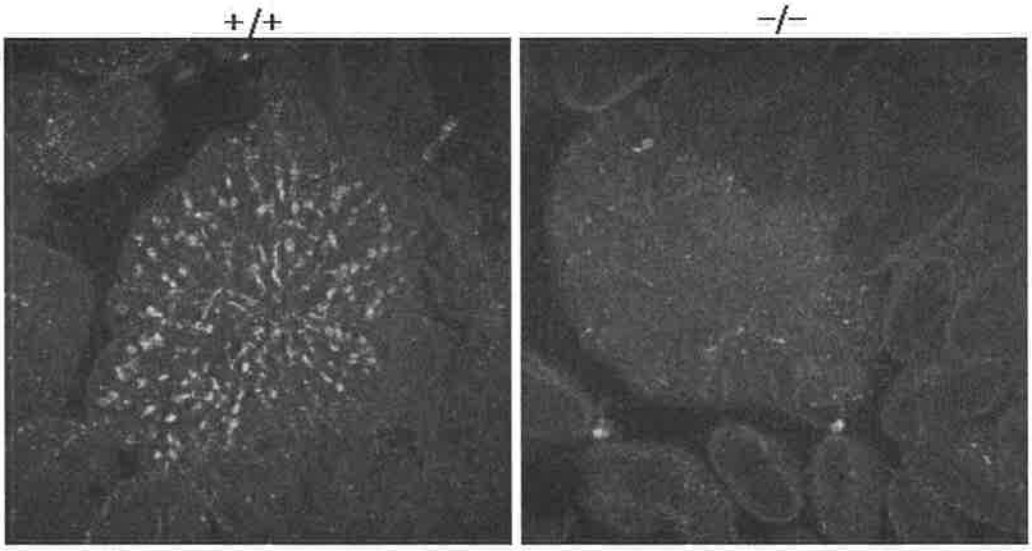
Spi-B is specifically expressed in FAE M cells



Kanaya *et al.*, **Nat. Immunol**, 2012

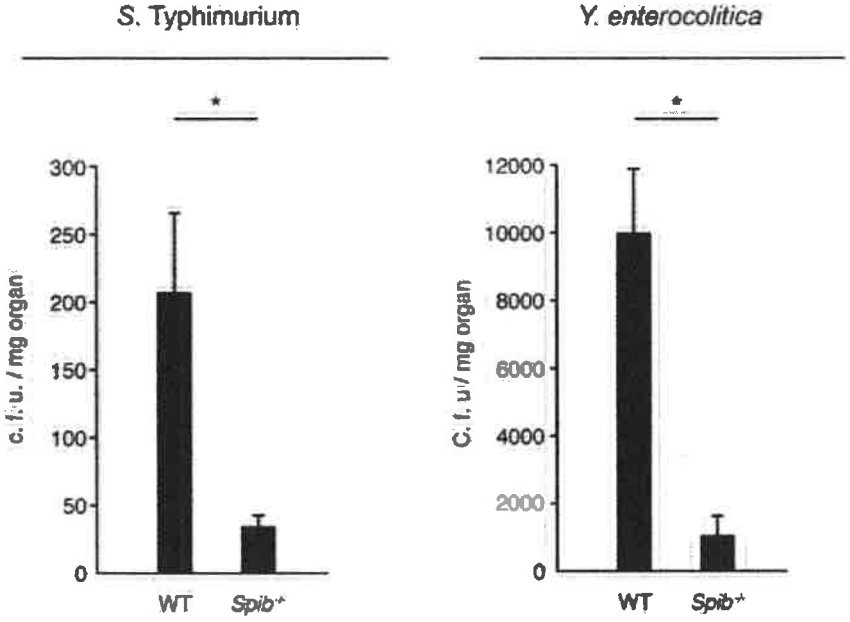
RIKEN Center for Integrative Medical Sciences (IMS) IMS

Whole mount staining reveals no GP2 expression in Spi-B KO mice



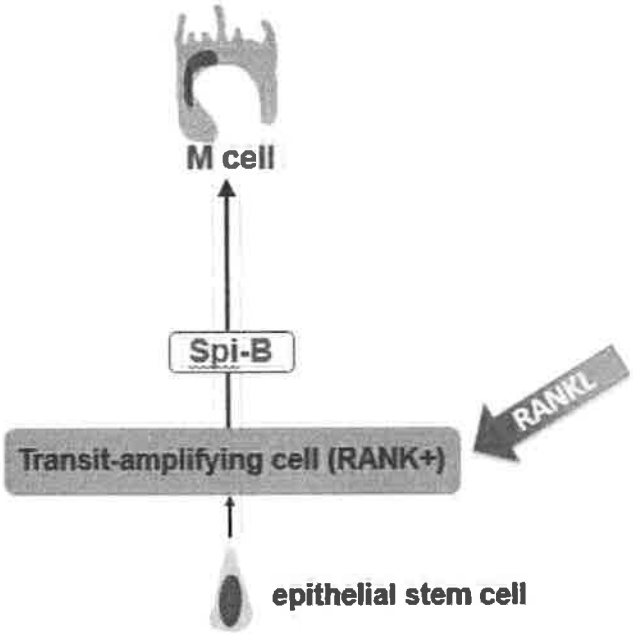
Kanaya *et al.*, **Nat. Immunol**, 2012

Bacterial translocation to PP is impaired in Spi-B KO mice

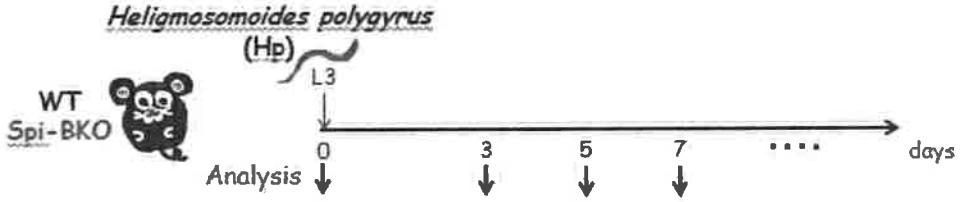


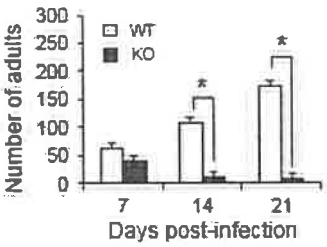
Kanaya et al., *Nat. Immunol*, 2012

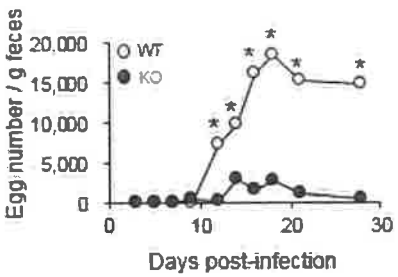
RANK-RANKL dependent Spi-B expression induces M-cell differentiation

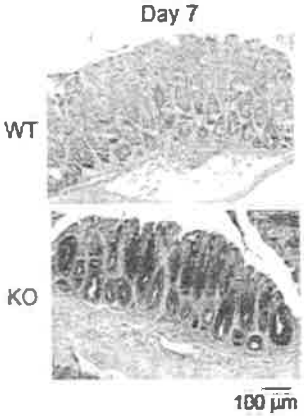


Spi-B-KO mice efficiently eradicate Hp



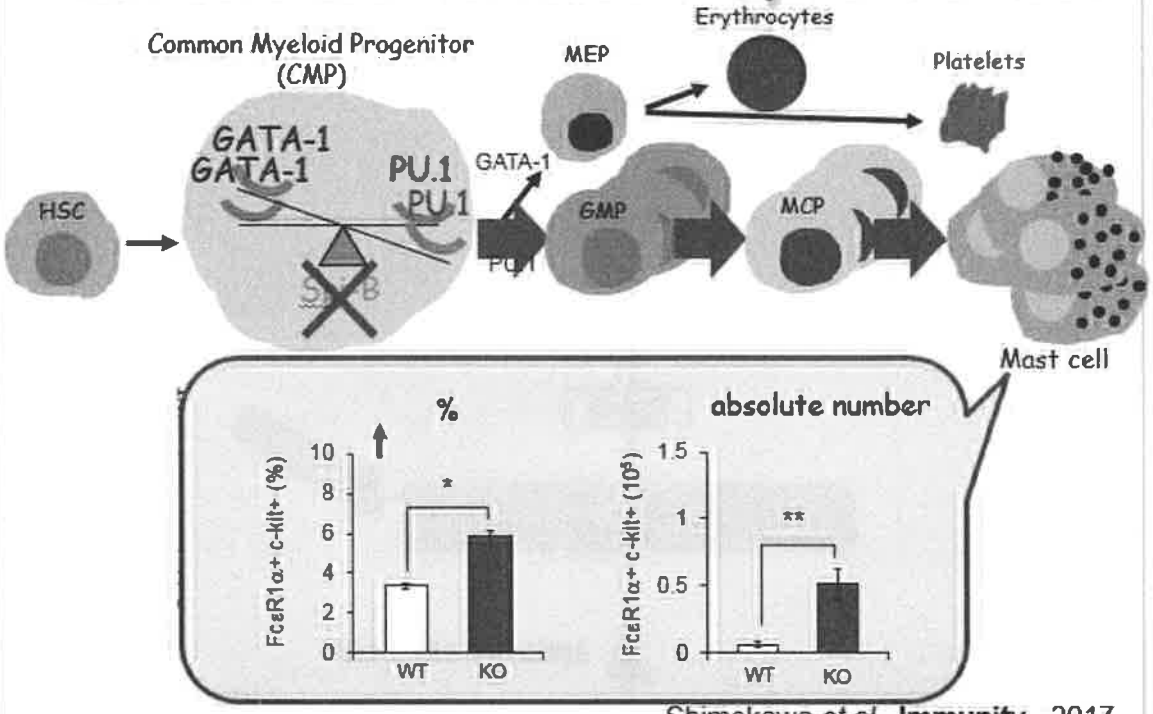






Shimokawa *et al.*, **Immunity**, 2017

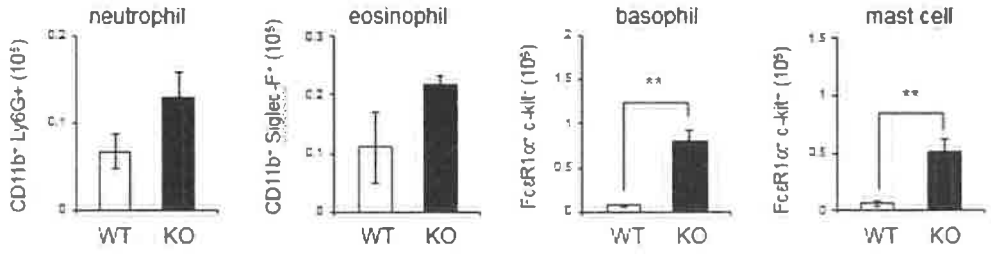
Mast cells are increased in Spi-B-KO mice



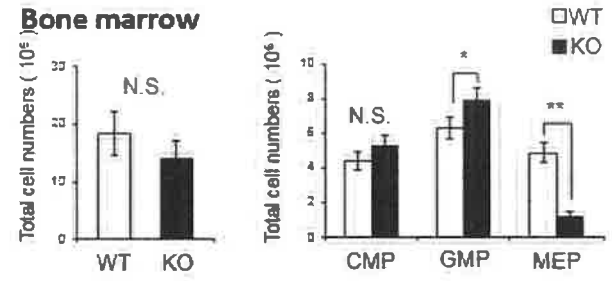
Shimokawa *et al.*, **Immunity** , 2017

Granulocytosis and altered hematopoiesis in Spi-B-KO mice

Mesenteric lymph node

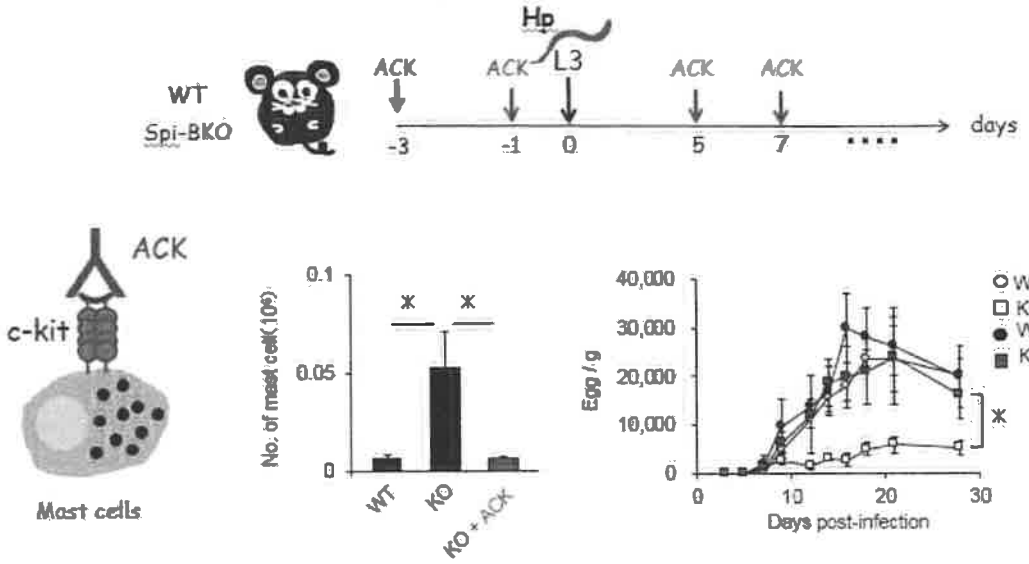


Bone marrow



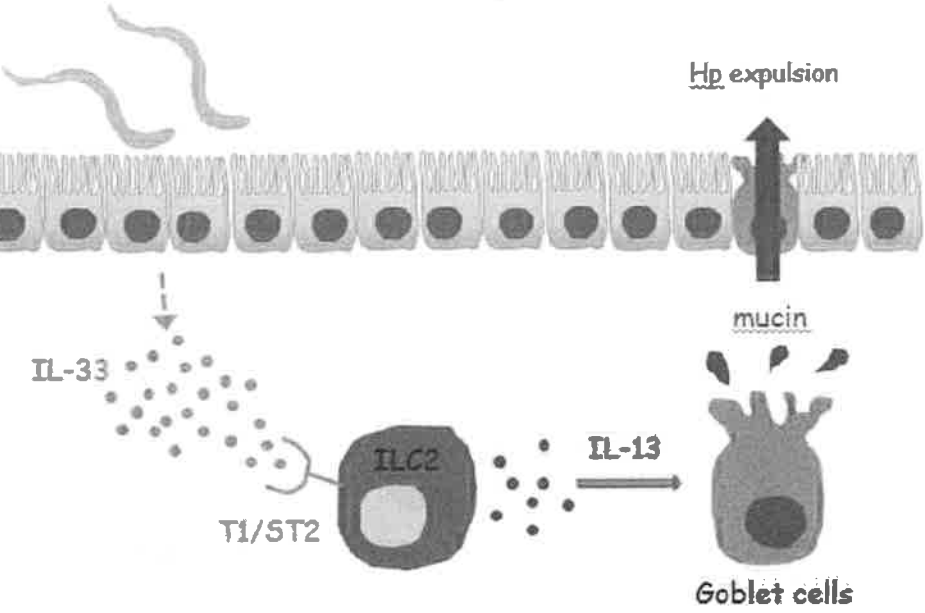
Shimokawa *et al.*, **Immunity** , 2017

Spi-B-KO mice depleted of mast cells were susceptible to Hp infection

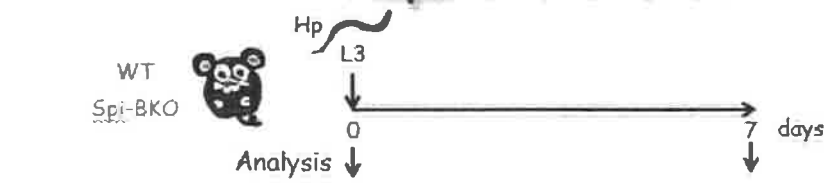


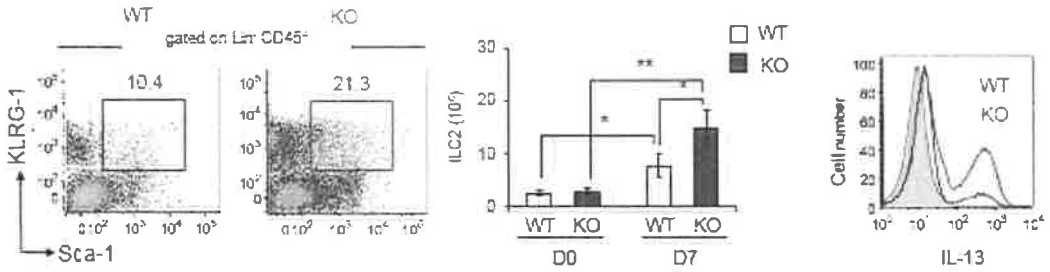
Shimokawa *et al.*, **Immunity** , 2017

ILC2 is important for expulsion of helminths



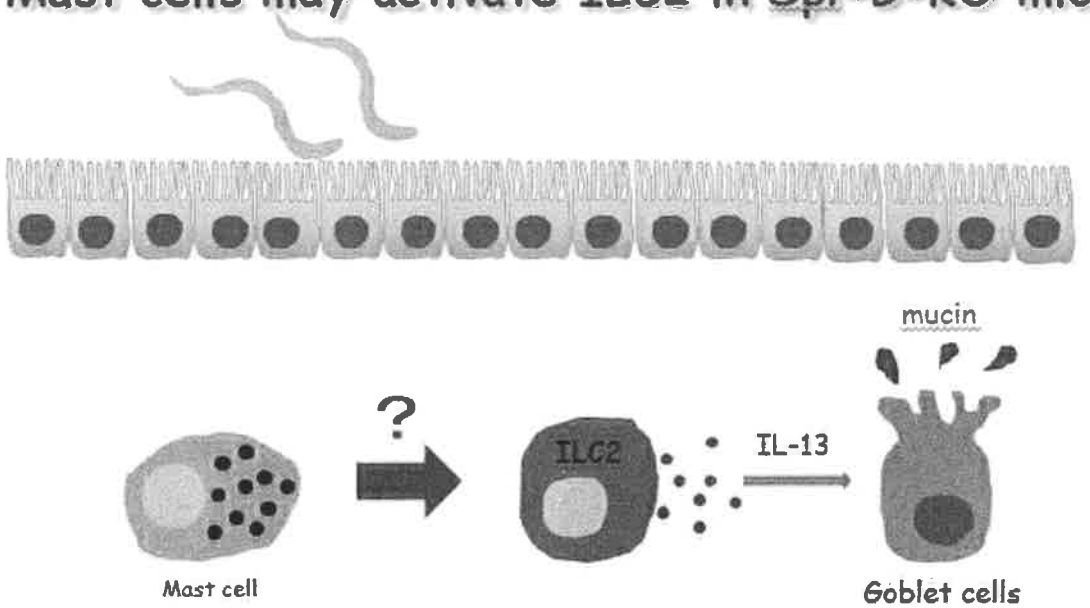
ILC2 is likely responsible for Hp eradication in Spi-B-KO mice



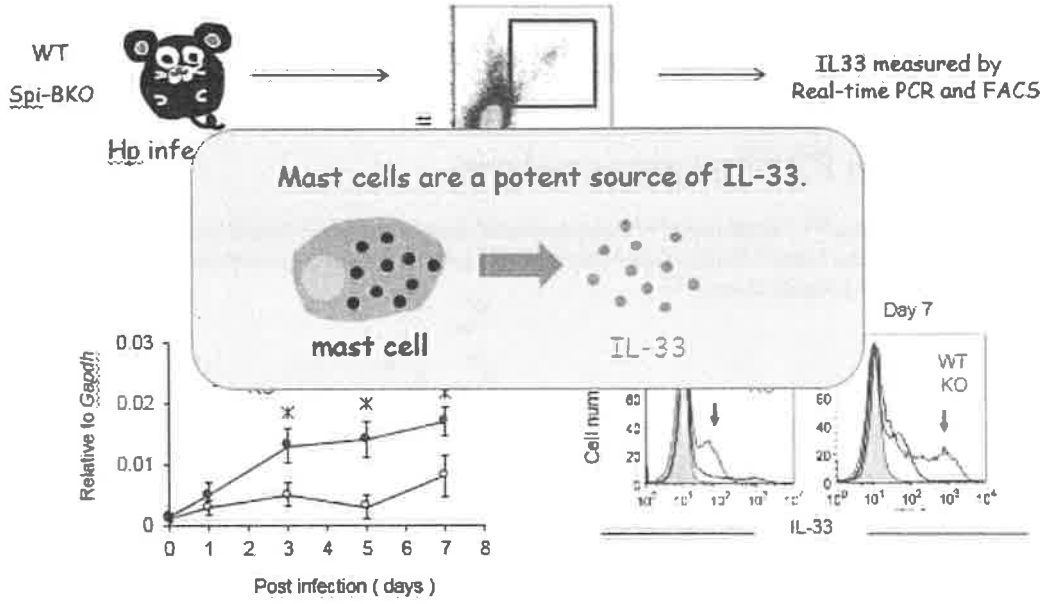


Shimokawa *et al.*, **Immunity** , 2017

Mast cells may activate ILC2 in Spi-B-KO mice

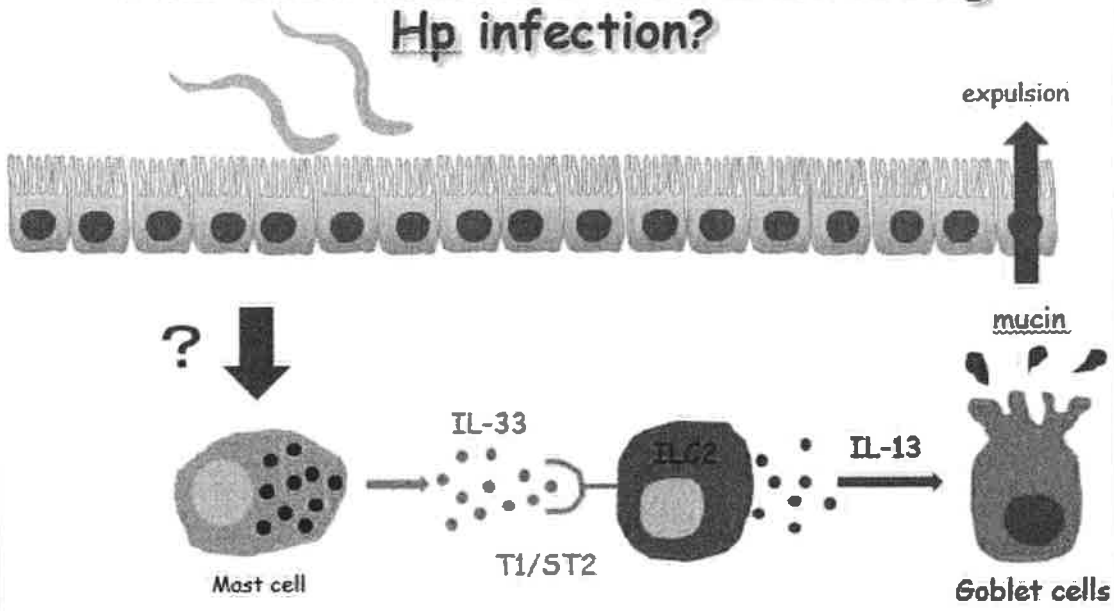


Mast cells produce IL-33 upon Hp infection



Shimokawa *et al.*, **Immunity** , 2017

How mast cells are activated during Hp infection?



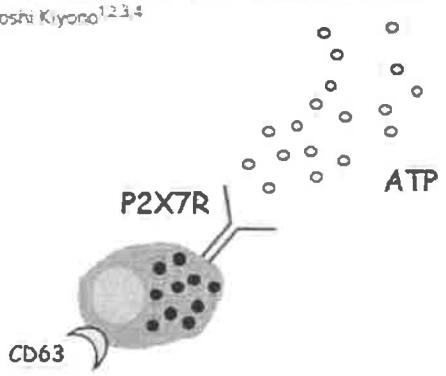
Mast cells are activated by ATP

ARTICLE

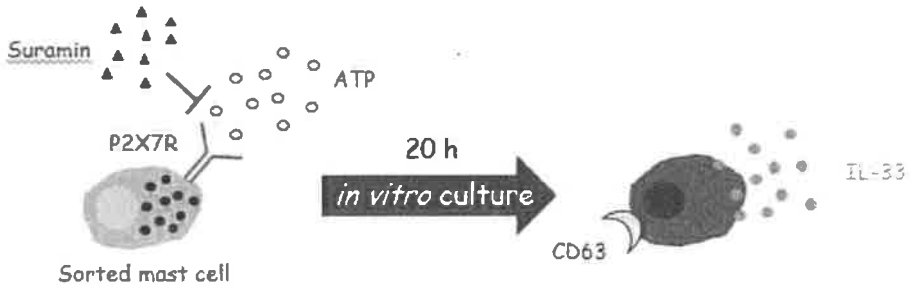
Received 12 Apr 2012 | Accepted 27 Jul 2012 | Published 4 Sep 2012

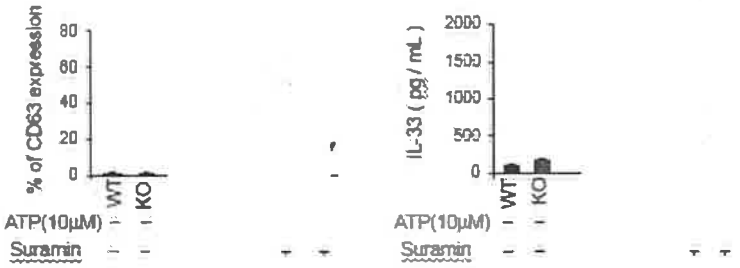
Extracellular ATP mediates mast cell-dependent intestinal inflammation through P2X7 purinoceptors

Yosuke Kurashima[1,2,3], Takeaki Amiya[1,3,4], Tomonori Nochi[7], Kumiko Fujisawa[1,3], Takeshi Haraguchi[5], Hideo Iba[5], Hiroko Tsutsui[6], Shintaro Sato[1,3], Sachiko Nakajima[7], Hideki Iijima[7], Masato Kubo[8,9], Jun Kunisawa[1,4] & Hiroshi Kiyono[1,2,3,4]



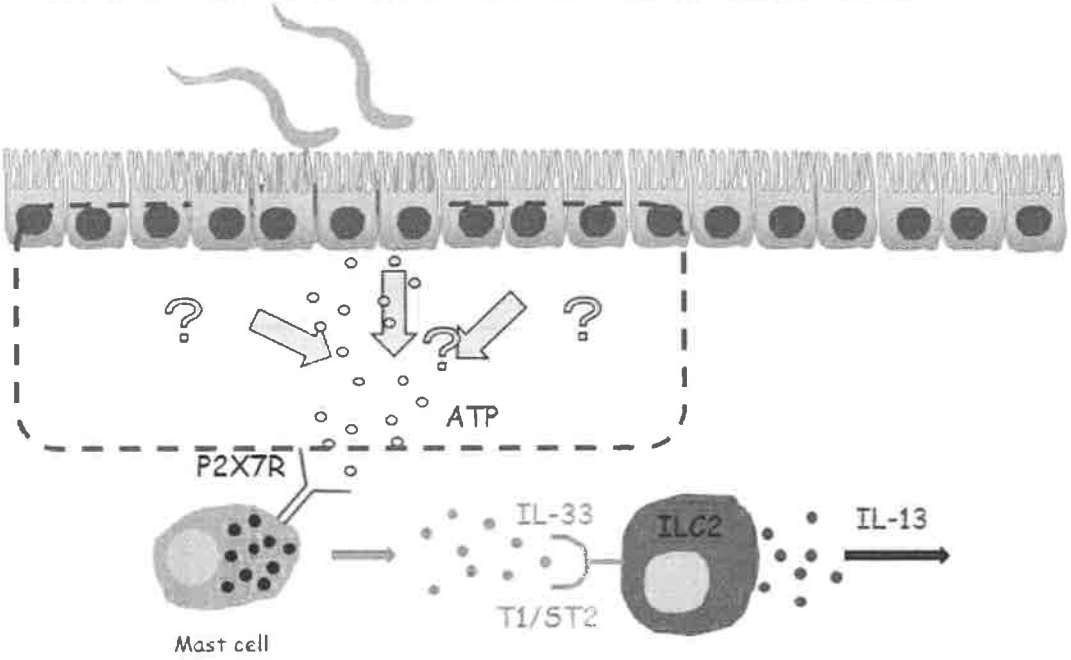
ATP activates mast cells in a P2XR-dependent manner *in vitro*



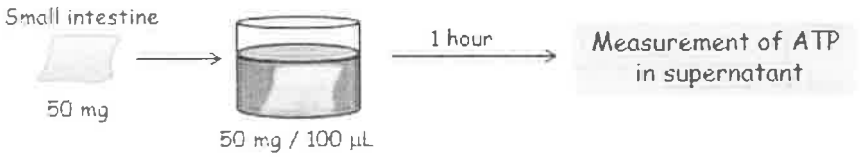


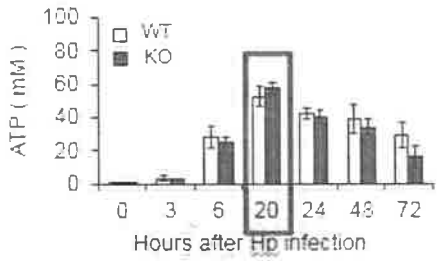
Shimokawa *et al.*, **Immunity** , 2017

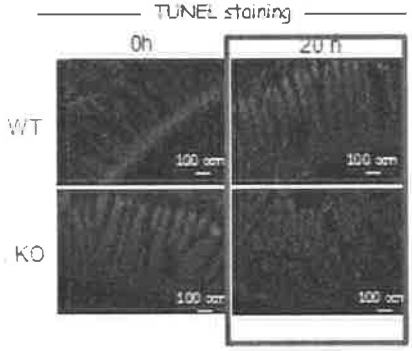
What is the source of extracellular ATP?



ATP is released from intestinal epithelium in association with cell death



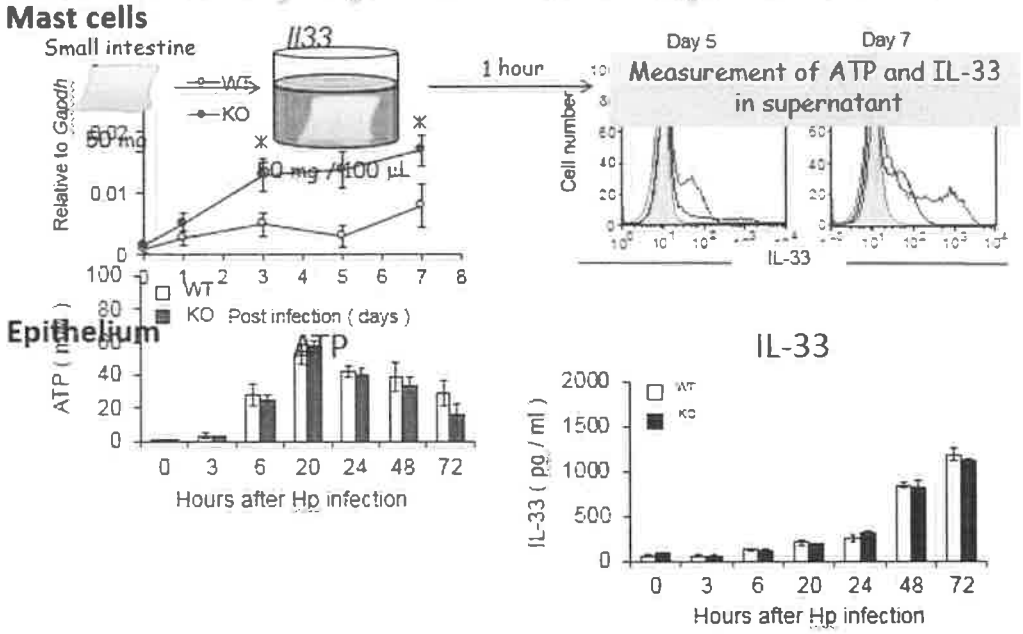




Shimokawa *et al.*, **Immunity** , 2017

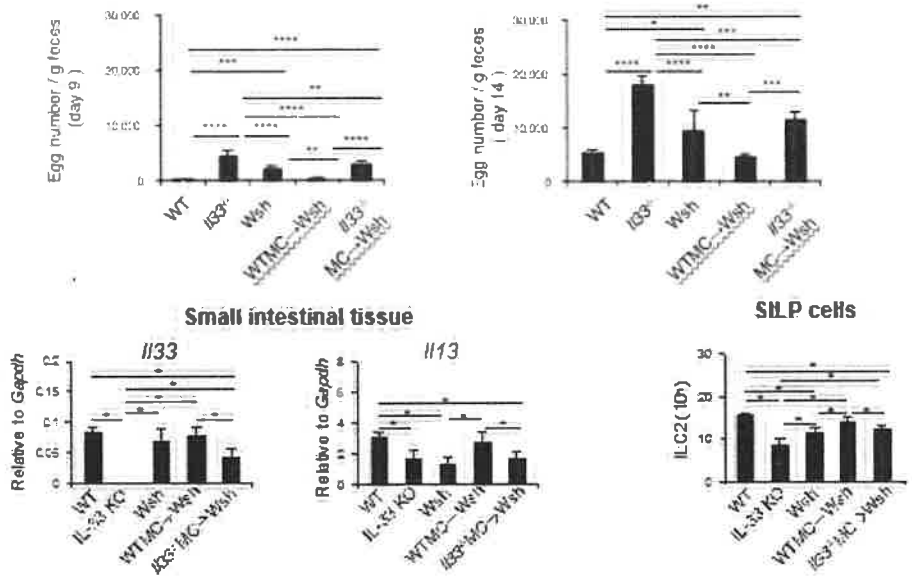
Differential IL-33 secretion by mast cells but not by epithelium in Spi-B-KO mice

Mast cells



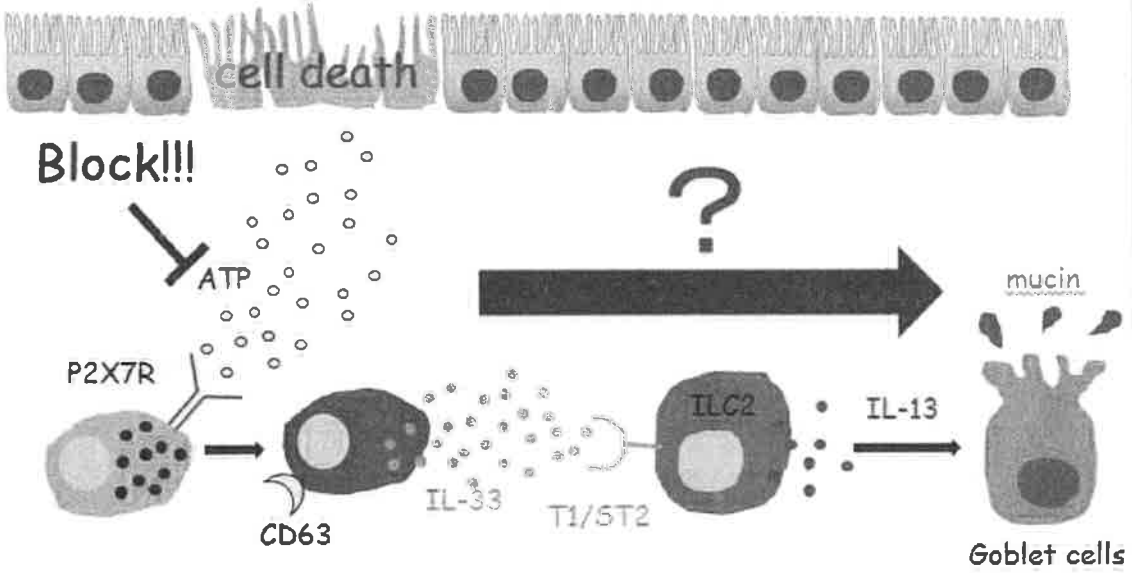
Shimokawa *et al.*, **Immunity** , 2017

Mast cell-derived IL-33 contribute to protect mice from Hp infection

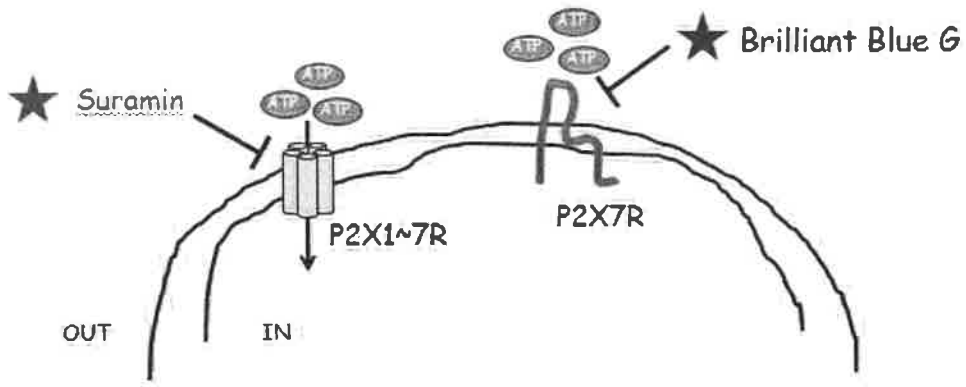


Shimokawa *et al.*, **Immunity** , 2017

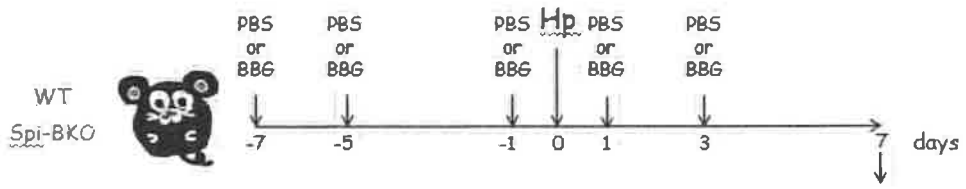
Is mast cell-activation by ATP essential for Hp eradication?

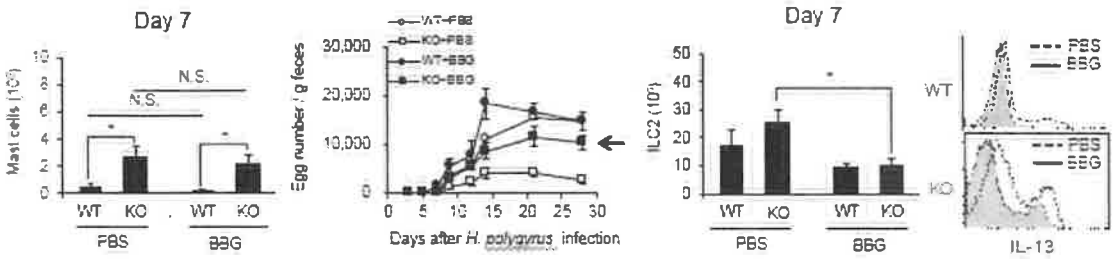


Brilliant blue G (BBG) specifically blocks P2X7R and can be used *in vivo*



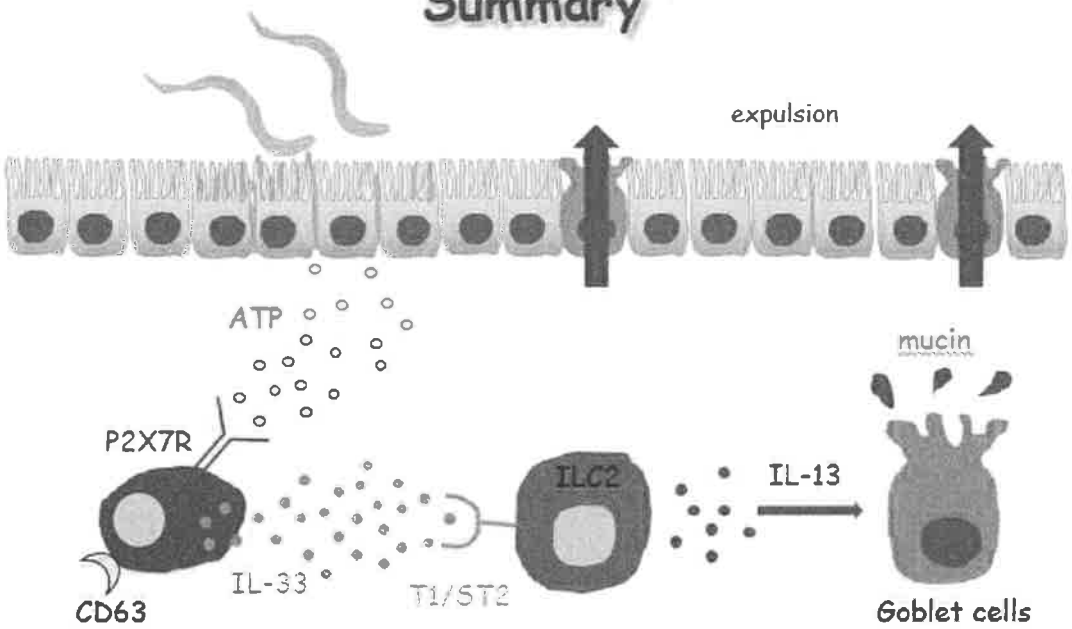
BBG-treated Spi-BKO mice were less resistant to Hp infection





Shimokawa *et al.*, **Immunity** , 2017

Summary



Shimokawa *et al.*, **Immunity** , 2017

Acknowledgment

RIKEN (IMS)

Laboratory for Intestinal Ecosystem

Chikako Shimokawa

Takashi Kanaya

Masami Hachisuka

Naoko Tachibana

Sayuri Sakakibara

The Jikei University School of Medicine

Kenji Ishiwata

Gunma University

Hajime Hisaeda

The University of Tokyo

Yosuke Kurashima

Hiroshi Kiyono

Hyogo College of Medicine

Koubun Yasuda

Tomohiro Yoshimoto

Wakayama Medical University

Tsuneyasu Kaisho

NFAT5 is Essential to Rheumatoid Inflammation

Wan-Uk Kim

The Catholic University of Korea, Korea

Apoptotic death of activated macrophages is important for controlling chronic inflammation and its defect in these cells has been implicated in the pathogenesis of rheumatoid arthritis (RA). However, the molecular signatures defining apoptotic resistance of RA macrophages have not been fully understood. Here, global transcriptome profiling of RA macrophages revealed that nuclear factor of activated T-cells 5 (NFAT5), an osmoprotective transcription factor, is one of the critical regulators for a wide range of pathologic processes of synovial macrophages, including cell cycle, apoptosis, and proliferation. Analysis of transcriptomes in NFAT5-deficient macrophages demonstrated the molecular networks defining cell survival and proliferation. Proinflammatory M1 polarizing stimuli and hypoxic conditions were responsible for enhanced NFAT5 expression in RA macrophages. An *in vitro* functional study demonstrated that NFAT5-deficient macrophages were more susceptible to apoptotic death. Specifically, chemokine ligand 2 (CCL2) was secreted in an NFAT5-dependent fashion and it bestowed RA macrophages apoptotic resistance. In mice, NFAT5-deficient macrophages were more susceptible to apoptosis and were less efficient in promoting joint destruction than NFAT5-sufficient macrophages when injected intra-articularly. Moreover, when recombinant CCL2 was administered into one of the affected joints of NFAT5 (+/-) mice, joint destruction as well as macrophage infiltration was significantly increased, demonstrating the essential role of NFAT5-CCL2 axis in arthritis progression in vivo. Conclusively, NFAT5 regulates macrophage survival by inducing CCL2 secretion. Our results provide the first evidence that NFAT5 expression in macrophages enhances chronic arthritis by conferring apoptotic resistance to activated macrophages.

CURRICULUM VITAE

Wan-Uk Kim, MD/PhD

Professor, Director

Center for Integrative Rheumatid Transcriptomics and Dyanmics (CiRad)

Department of Internal Medicine, Seoul St. Mary's Hospital,

College of Medicine, The Catholic University of Korea

Seoul 137-701

E-mail: wan725@catholic.ac.kr

Education and Appointment

1985-91	M.D. School of Medicine, Catholic University of Korea, Feb 1991
1995-02	PhD, School of Medicine, Catholic University of Korea, Aug 2002
2003-07	Assistant Professor of Internal Medicine, Catholic University of Korea, Seoul
2007-08	Visiting Scholar of Internal Medicine, Yale University, New Haven, CT
2009-10	Director, Institute of Immuno-biology, Catholic University of Korea, Seoul
2012-	Professor of Internal Medicine, Catholic University of Korea, Seoul
2016-	Director, National Creative Research Center

Professional Service:

2012-	Member of Board of Directors, Korean College of Rheumatology (KCR)
2014-	Member of Board of Directors, Korean Association of Immunologists (KAI)
2014-2016	President, Korean Society of Synovitis Research (KSSR)
2012-2013	Advisory Editor, Arthritis and Rheumatism
2013-	Associate Editor, Arthritis and Rheumatology (A&R)
2016-	Editorial Board, Experimental and Molecular Medicine (EMM)
2017-	Chairman of Scientific Committee of the Korean Association of Immunologists (KAI)

Specialty and Research Field of Interest

Systems Approach to Rheumatoid Arthritis

Synoviocyte biology

Selected publications since 2012

1) Choi S, You S, Kim D, Cho SY3, Kwon HM, Kim HS, Hwang D, Park YJ, Cho CS, Kim WU. NFAT5 Promotes Macrophage Survival in Rheumatoid Arthritis. *J. Clin. Invest.* 2017:127:954-69.

2) Hwang D, Kim WU. Modelling cytokine signaling networks for rheumatoid arthritis. *Nature Rev. Rheumatol.* 2017;13:5-6.

3) Yoo SA, Kong JS, Yoon HJ, Cho CS, Kim WU*, Bucala R*. MIF Allele-dependent Regulation of the MIF Co-receptor CD44 and Role in Rheumatoid Arthritis. *Proc. Natl. Acad. Sci. USA.* 2016; 113: E7917-E7926. *Co-corresponding authors.

4) Hwang SH, Jung SH, Lee S, Choi S, Yoo SA, Park JH, Hwang D, Shim SC, Sabbagh L, Kim KJ, Park SH, Cho CS, Kim BS, Leng L, Montgomery RR, Bucala R, Chung YJ, Kim WU. Leukocyte-specific protein 1 regulates T-cell migration in rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA.* 2015; 112: E6535-43.

5) You S, Yoo SA, Choi S, Kim JY, Park SJ, Ji JD, Kim TH, Kim KJ, Cho CS, Hwang DH, Kim WU. Identification of key regulators for migration and invasion of rheumatoid synoviocytes through a systems approach. *Proc. Natl. Acad. Sci. USA.* 2014;111:550-5.

6) Yoo SA, You SY, Yoon HJ, Kim DH, Kim HS, Lee K, Ahn JH, Hwang D, Lee AS, Kim KJ, Park YJ, Cho CS, Kim WU. A novel pathogenic role of the ER chaperone GRP78/BiP in rheumatoid arthritis. *J. Exp. Med.* 2012;209:871-86.

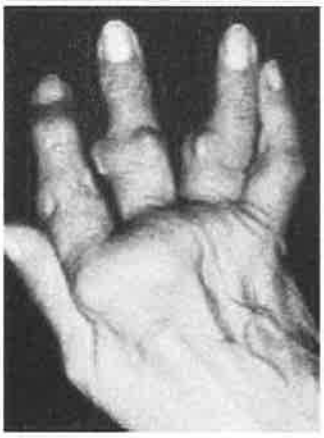
NFAT5 is Essential to Rheumatoid Inflammation

Department of Internal Medicine
The Catholic University of Korea
Seoul St. Mary Hospital

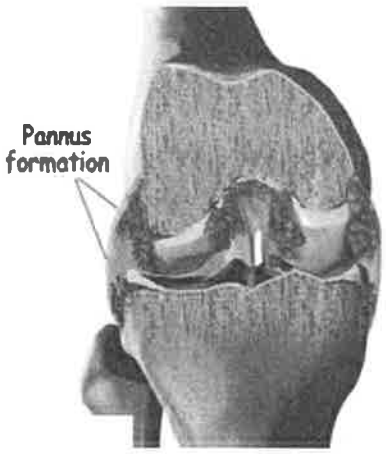
Wan-Uk Kim, Professor

CiRaD

Pathology of Rheumatoid Arthritis (RA)

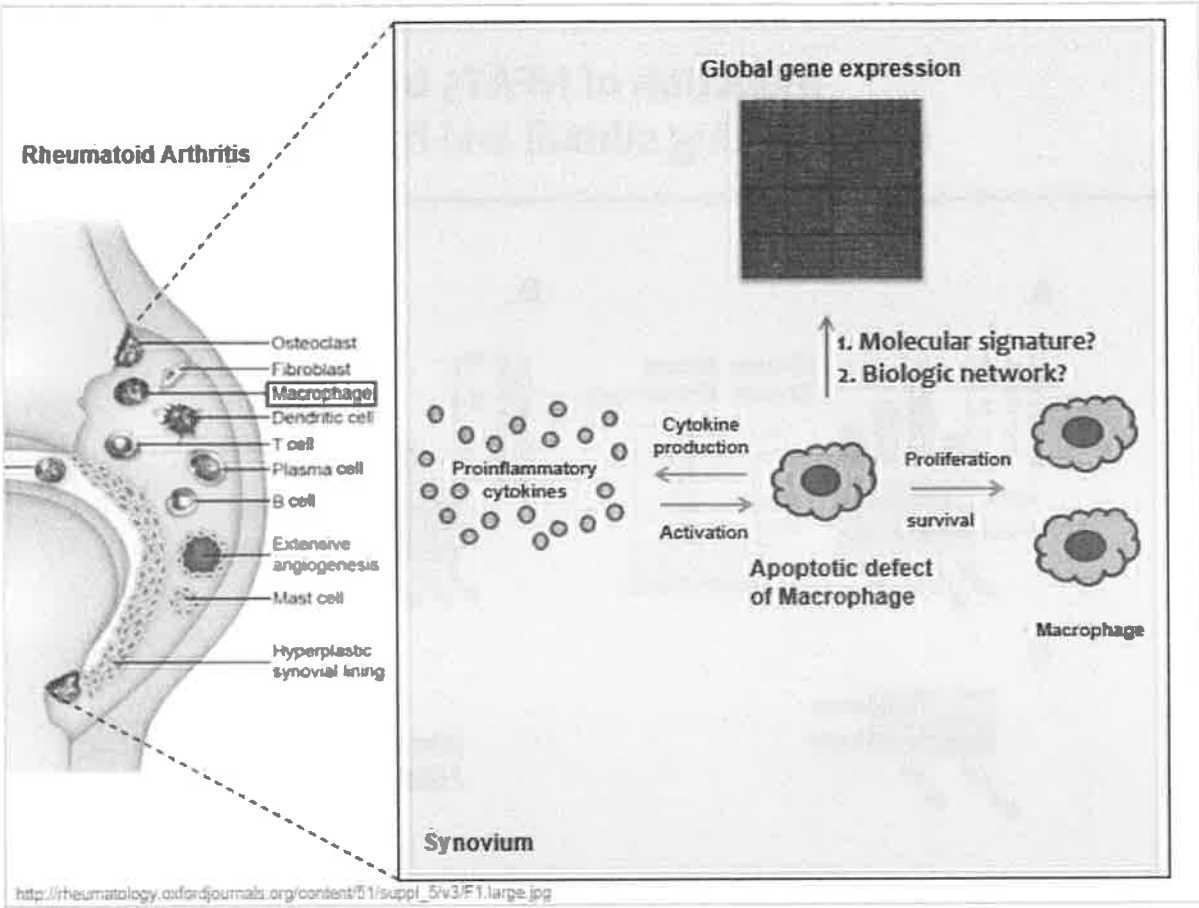


RA hand

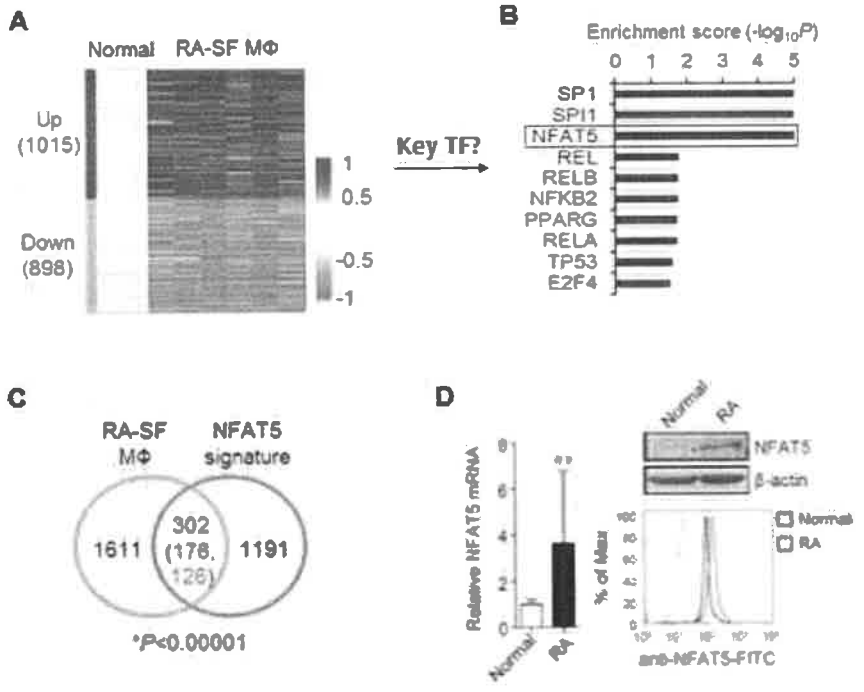


rheumatoid joint

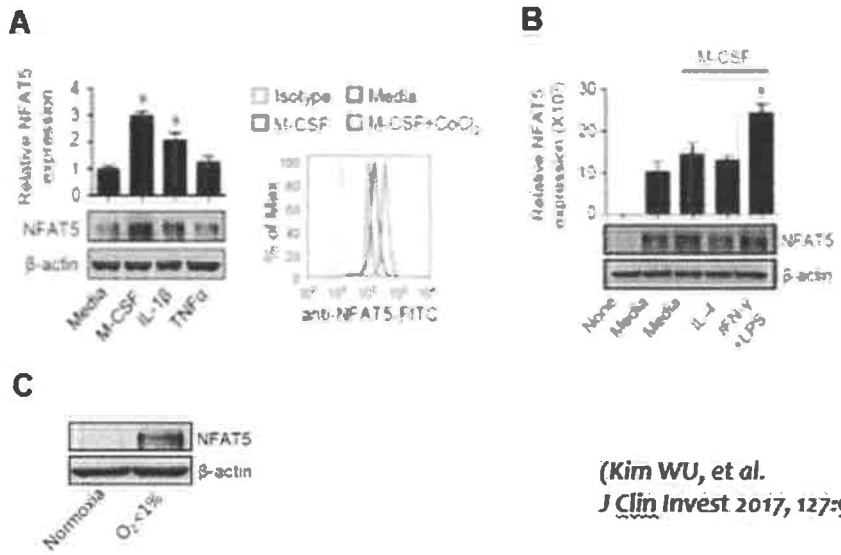
"synovial hyperplasia"



NFAT5 is a Key TF in RA macrophages

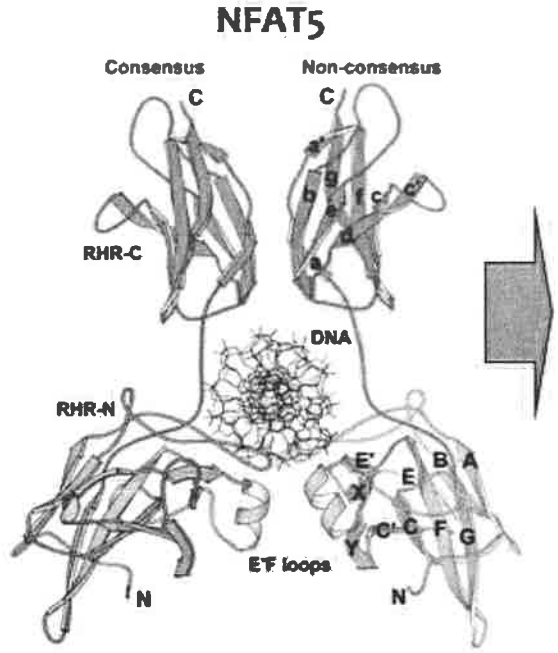


Induction of NFAT5 by M1 polarizing stimuli and hypoxia



(Kim WU, et al. J Clin Invest 2017, 127:954-969)

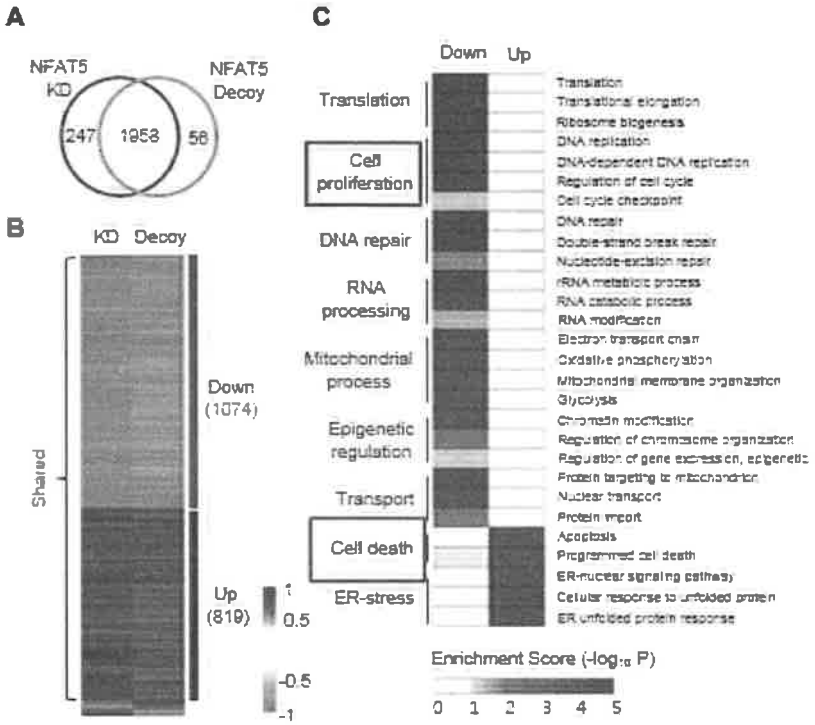
Biological role of NFAT5



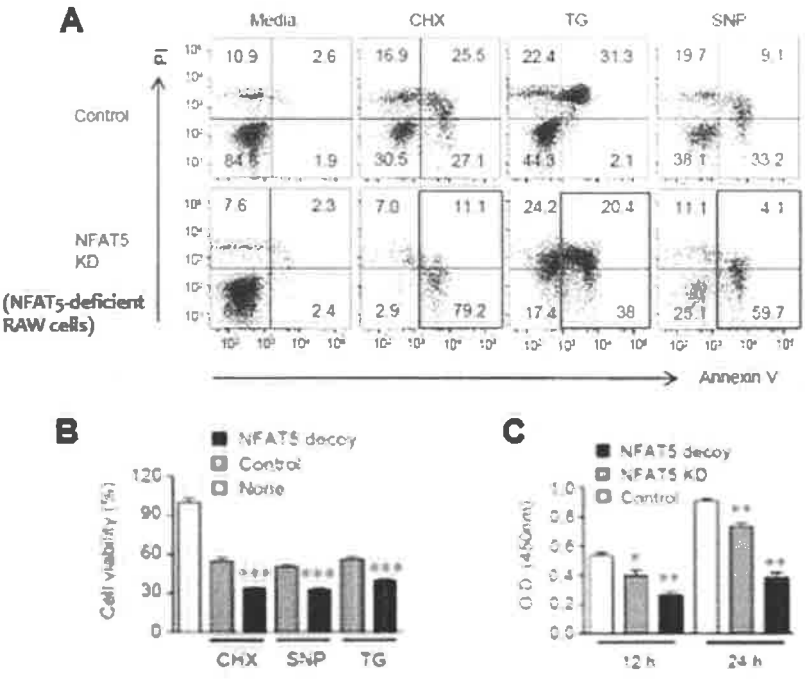
1. Osmo-protective function
2. T cell proliferation
3. Cancer invasion & meta
4. Rheumatoid inflammation ?
   Macrophage survival?

Nat Struct Biol 9: 90 (2002)

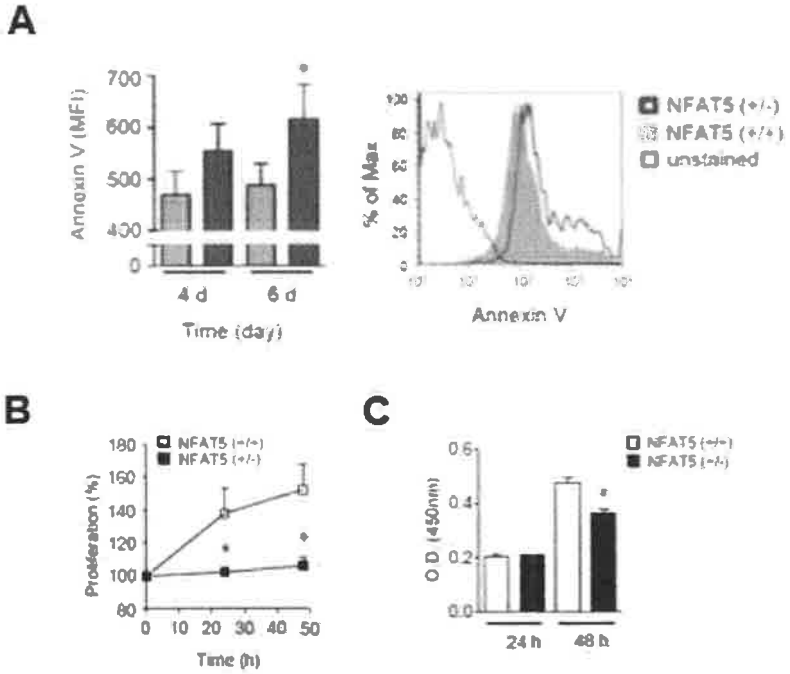
Gene expression profile in NFAT5-deficient RAW 264.7 cells



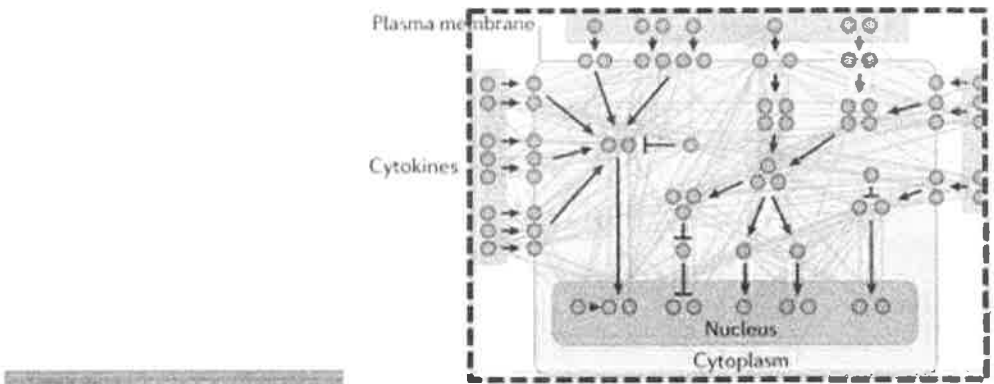
NFAT5 regulates macrophage survival and proliferation (1)



NFAT5 regulates macrophage survival and proliferation (2)

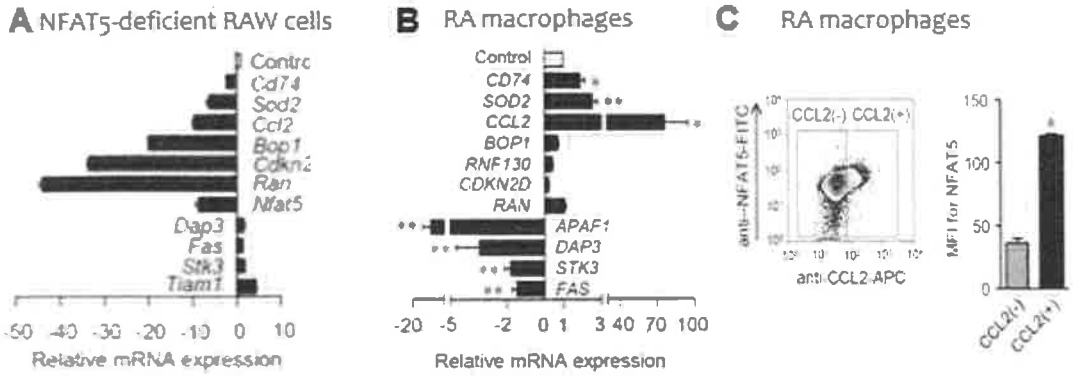


Modelling of molecular networks in RA synovia



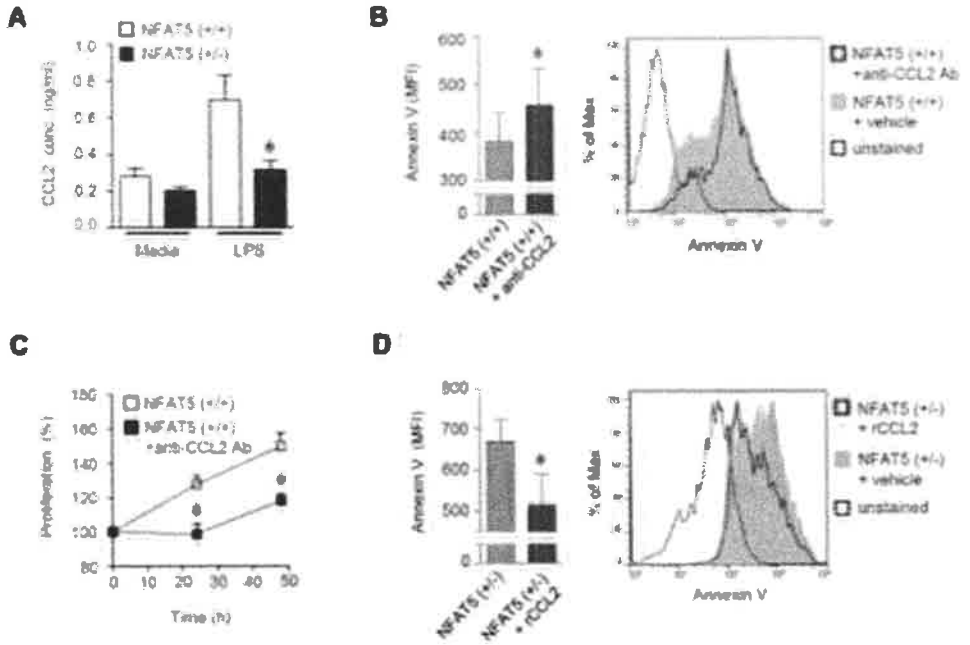
(Kim wu, et al. Nature Rev Rheum, 2016)

NFAT5 expression in CCL2(+) versus CCL2(-) subpopulations of RA-SF macrophages

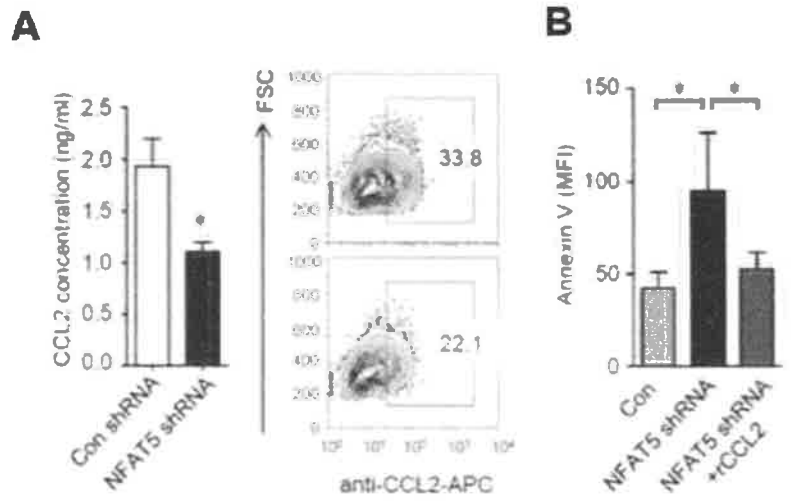


(Kim WU, et al.
J Clin Invest 2017, 127:954-969)

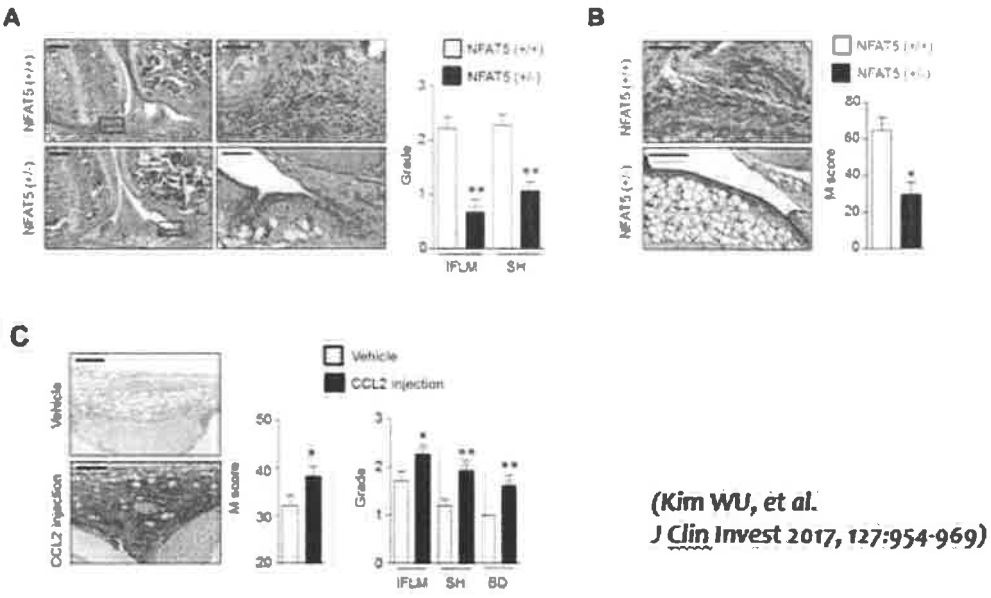
NFAT5 promotes survival of primary mouse macrophages by inducing CCL2 (MCP-1) secretion



CCL2 restores survival of NFAT5-deficient RA macrophages

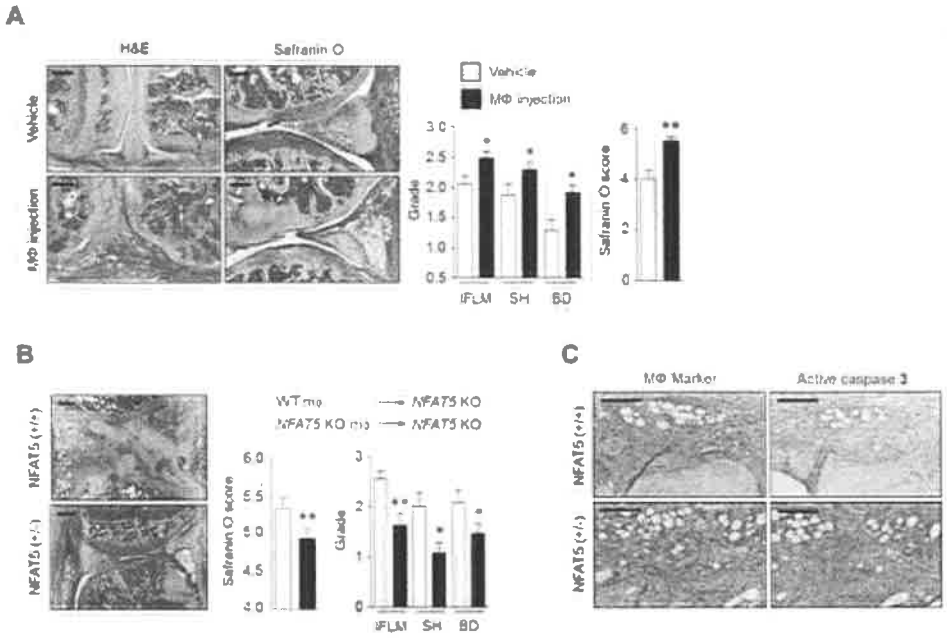


Essential role of NFAT5-CCL2 axis in macrophage infiltration and arthritis progression *in vivo*.

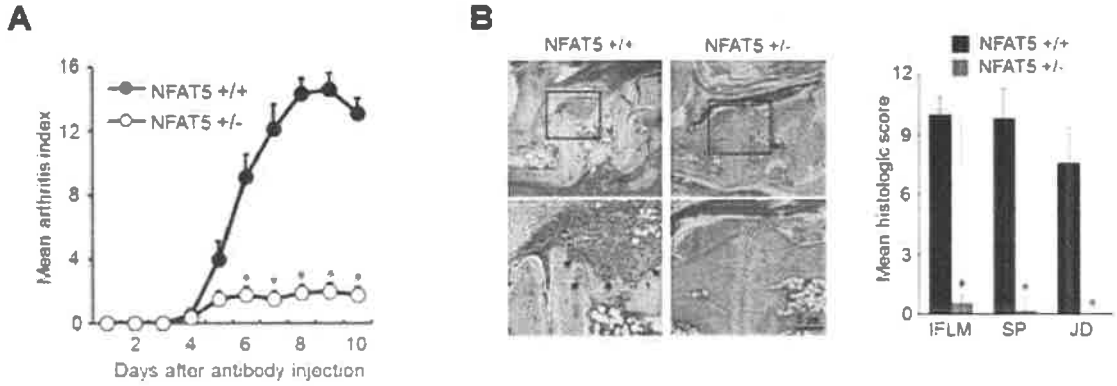


(Kim WU, et al. J Clin Invest 2017, 127:954-969)

Direct *in vivo* effect of NFAT5 in macrophages on the progression of arthritis.



Marked Reduction of anti-CII antibody-induced arthritis in NFAT5 haplo-insufficient mice



(Kim WU, et al.
Arthritis Rheum 2011, 63:1843-52)

Development of a new NFAT5 inhibitor for RA treatment : small molecules

- HTS
- Chemical modification
- Early ADME/T
- Chemical Library
- Synthesis

Korea Research Institute of Chemical Technology

- NFAT5 specificity
- Drug Mechanism
- NFAT5 target genes
- RA animal model
- RA clinical trials

Catholic University

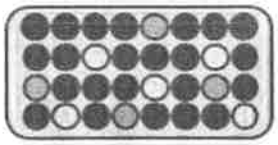
First in class!!

The discovery of NFAT5 inhibitor using HTS

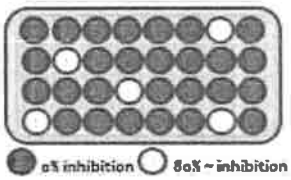
> 150,000 small molecules

Griess reaction (NO)

HTS

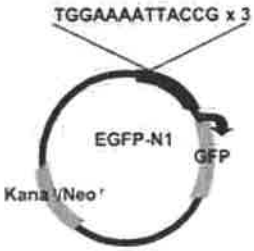


1st Selection

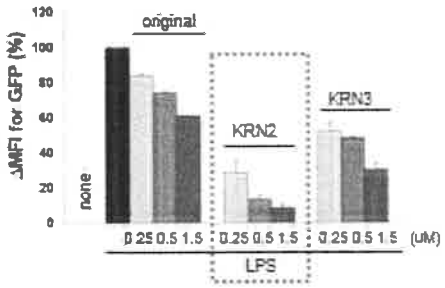


0% inhibition 80% ~ inhibition

> 200_{ea} Primary hits



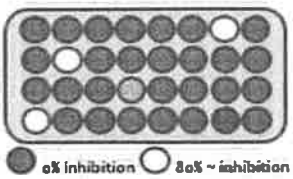
90% ~ inhibitory activity



KRN2 (IC50= 100 nM)

Minimal toxicity

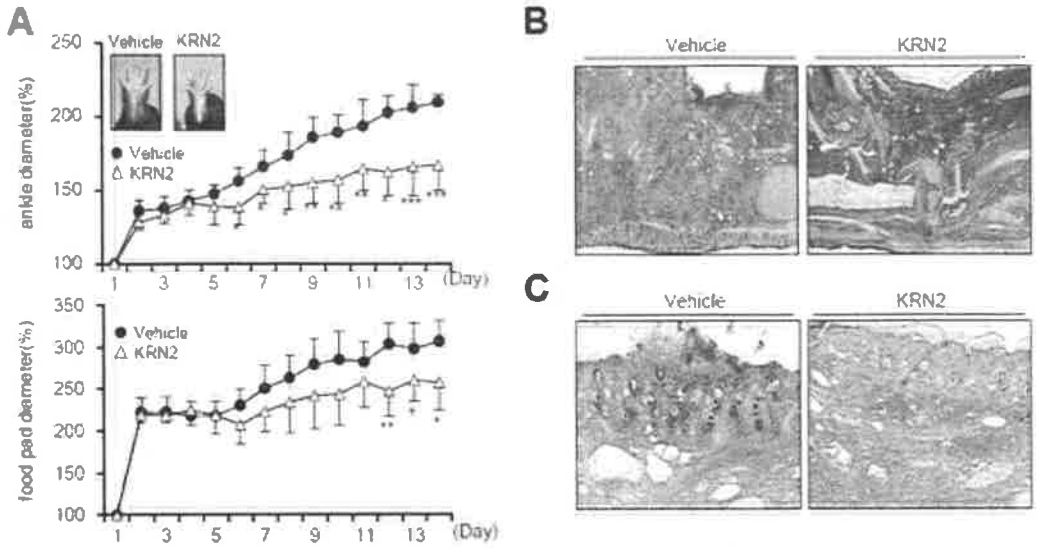
2nd Selection



0% inhibition 80% ~ inhibition

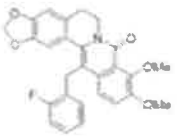
5_{ea} Compounds

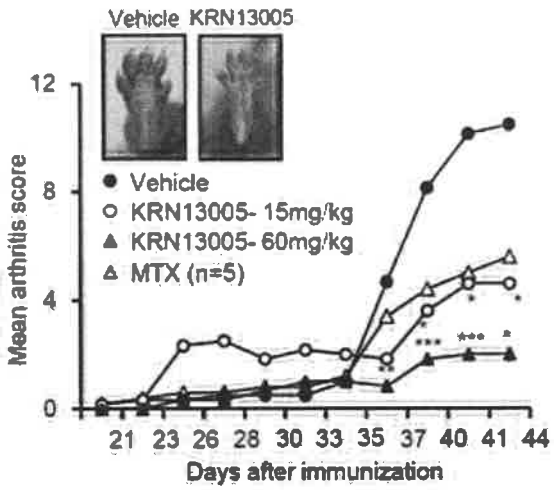
KRN2 suppresses CFA-induced arthritis and macrophage infiltration in arthritic joints

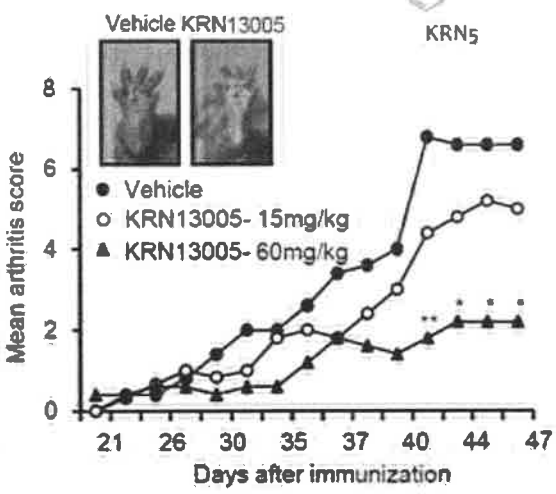


(Kim WU, et al. EBioMedicine 2017;18:261-73)

Modified Scaffold : KRN5







(Kim WU, et al. EBioMedicine 2017;18:261-73)

Summary

- Global transcriptome profiling of RA macrophages revealed that NFAT5, an osmo-protective transcription factor, is one of the critical regulators for a wide range of synovial macrophages, including cell cycle, apoptosis, and proliferation.

- Functional studies demonstrated that NFAT5 prevents apoptotic death of both human and murine macrophages, promoting macrophage-induced arthritis in mice.

- Specifically, CCL2, a chemokine, is critically involved in macrophage survival as a representative downstream target of NFAT5 *in vitro* and *in vivo*.

- We discovered novel NFAT5 suppressors, KRN2 and KRN5, to selectively inhibit NFAT5 expression.

Acknowledgement

Catholic University of Korea
National Creative Research Center

Susanna Choi, PhD Candidate
Eun-Jin Han, MS
Hyung-Ju Yoon, PhD
Seung-Ah Yoo, PhD
Naeun Lee, PhD
Jin-Sun Kong, PhD candidate

DGIST

Sungyoung You, PhD
Daehee Hwang, PhD

KRICT 한국화학연구원 Korea Research Institute of Chemical Technology

Heeyeong Cho, PhD

Critical Role of Commensal Microbiota in Shaping Antiviral Immunity

Heung Kyu Lee

KAIST, Korea

Commensal microbiota are well known to play an important role in antiviral immunity by providing immune inductive signals; however, the consequence of dysbiosis on antiviral immunity remains unclear. We demonstrate that dysbiosis caused by oral antibiotic treatment directly impairs antiviral immunity following viral infection of the vaginal mucosa. Antibiotic-treated mice succumbed to mucosal herpes simplex virus type 2 infection more rapidly than water-fed mice, and also showed delayed viral clearance at the site of infection. However, innate immune responses including type I interferon and proinflammatory cytokine production at infection sites, as well as induction of virus-specific CD4 and CD8 T cell responses in draining lymph nodes, were not impaired in antibiotic-treated mice. By screening the factors controlling antiviral immunity, we found that interleukin-33, an alarmin released in response to tissue damage, was secreted from vaginal epithelium after the depletion of commensal microbiota. This cytokine suppresses local antiviral immunity by blocking the migration of effector T cells to the vaginal tissue, thereby inhibiting the production of interferon-γ, a critical cytokine for antiviral defense, at local infection sites. These findings provide insight into the mechanisms of homeostasis maintained by commensal bacteria, and reveal a deleterious consequence of dysbiosis in antiviral immune defense.

CURRICULUM VITAE

Heung Kyu Lee, Ph.D

BioMedical Research Center, Room 5106

291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Korea

tel: +82-42-350-4241,

E-mail: heungkyu.lee@kaist.ac.kr

Education

Sep/2004-May/2009 **Doctor of Philosophy** in Immunobiology

Yale University, New Haven, CT, USA

Thesis title: Dendritic cells, Autophagy and Antiviral immunity

Sep/1996-Aug/1998 **Master of Science** in Biotechnology

Yonsei University, Seoul, Korea

Mar/1992-Aug/1995 **Bachelor of Science in Engineering** in Food Science and Technology

Dongguk University, Seoul, Korea

Positions and Employment

Sep/2016-Present **Associate Professor** of Graduate School of Medical Science and Engineering, KAIST, Daejeon, Korea

Nov/2009-Aug/2016 **Assistant Professor** of Graduate School of Medical Science and Engineering, KAIST, Daejeon, Korea

Mar/2009-Jan/2010 **Post-doctoral Associate** of Immunobiology, Yale University School of Medicine, New Haven, CT

Sep/2004-Feb/2009 **Graduate Student** of Immunobiology,

Yale University, New Haven, CT (PI: Akiko Iwasaki, Ph.D.)

Sep/2003-Aug/2004 **Graduate Research Assistant** of Immunology,

University of Texas M.D. Anderson Cancer Center, Houston, TX (PI: Yong-Jun Liu, M.D., Ph.D.)

Jun/2001-Oct/2001 **Researcher** of Immunology and Transplantation,

Asan Institute for Life Sciences and Asan Medical Center, Seoul, Korea (PI: Duck-Jong Han, M.D., Ph.D.)

Oct/1998-Dec/2000 **Military service** of Republic of Korea Army

Yong In, Korea

Sep/1996-Aug/1998 **Graduate Research Assistant** of Biotechnology,

Yonsei University, Seoul, Korea (PI: Sang-Kyou Lee, Ph.D.)

Publications

1. Oh DS, Oh JE, Jung HE, **Lee HK**. Transient Depletion of CD169+ Cells Contributes to Impaired Early Protection and Effector CD8+ T Cell Recruitment against Mucosal Respiratory Syncytial Virus Infection. Frontiers in Immunology. 2017 Jul 13;8:819

2. Oh DS, Kim H, Oh JE, Jung HE, Lee YS, Park JH, **Lee HK**. Intratumoral depletion of regulatory T cells using CD25-targeted photodynamic therapy in a mouse melanoma model induces antitumora

3. Oh JE, Oh DS, J HE, **Lee HK**. A mechanism for the induction of type 2 immune responses by a protease allergen in the genital tract. Proc. Natl. Acad. Sci. U. S. A. 2017 Jan 30. Ahead of print doi:10.1073/pnas.1612997114

4. Ryu JK, Kim SJ, Rah SH, Kang JI, Jung HE, Lee D, **Lee HK**, Lee JO, Park BS, Yoon TY, Kim HM. Reconstruction of LPS Transfer Cascade Reveals Structural Determinants within LBP, CD14, and TLR4-MD2 for Efficient LPS Recognition and Transfer. Immunity. 2017 Jan 17. 46(1):38-50

5. Hong GH, Kwon HS, Moon KA, Park SY, Park S, Lee KY, Ha EH, Kim TB, Moon HB, **Lee HK**, Cho YS. Clusterin Modulates Allergic Airway Inflammation by Attenuating CCL20-Mediated Dendritic Cell Recruitment. Journal of Immunology 2016 Mar 1. 1;196(5):2021-30

6. Oh JE, Kim B, Chang DH, Kwon M, Lee SY, Kang D, Kim JY, Hwang I, Yu JW, Nakae N, **Lee HK**. Dysbiosis-induced IL-33 contributes to impaired antiviral immunity in the genital mucosa. Proc. Natl. Acad. Sci. U. S. A. 2016 Feb 9;113(6):E762-71

Introduced as highlight journal in "**In This Issue**" section of PNAS 2016 Feb 9;113(6)

7. Oh JE, Lee MS, Kim YJ, **Lee HK**. OASL1 deficiency promotes antiviral protection against genital herpes simplex virus type 2 infection by enhancing type I interferon production. Scientific Reports *2016 Jan. 11.*

8. Park S, Won JH, Hwang I, Hong S, **Lee HK**, Yu JW. Defective mitochondrial fission augments NLRP3 inflammasome activation. Scientific Reports 2015 Oct 22;5:15489. doi: 10.1038/srep15489

9. Chang DH, Rhee MS, Ahn S, Bang BH, Oh JE, **Lee HK**, Kim BC. Faecalibaculum rodentium gen. nov., sp. nov., isolated from the faeces of a laboratory mouse. Antonie Van Leeuwenhoek. 2015 Sep 8[Epub ahead of print]

10. Shim YR, **Lee HK**. Caspase-1 independent viral clearance and adaptive immunity against mucosal respiratory syncytial virus infection. Immune Network. 2015 Apr 15(2):73-82 Invited Review Article

11. Oh JE, **Lee HK**. Pattern recognition receptors and autophagy. Frontiers in Immunology. 2014 Jun 25;5:300 Invited Review Article

12. Kim TH, **Lee HK**. Differential roles of lung dendritic cell subsets against respiratory virus infection. Immune Network. 2014 Jun 14(3):128-137 Invited Review Article

13. Nish SA, Schenten D, Wunderlich FT, Pope SD, Gao Y, Hoshi N, Yu S, Yan X, **Lee HK**, Pasman L, Brodsky I, Yordy B, Zhao H, Bruning J, Medzhitov R. T cell-intrinsic role of IL-6 signaling in primary and memory responses. Elife. 2014 May 19;3:e01949

14. Kim TH, **Lee HK**. Innate immune recognition of respiratory syncytial virus infection. BMB Reports. 2014 Apr;47(4):184-191

15. Schenten D, Nish SA, Yu S, Yan X, **Lee HK**, Brodsky I, Pasman L, Yordy B, Wuderlich FT, Bruning JC, Zhao H, Medzhitov R. Signaling through the adaptor molecule MyD88 in CD4+ T cells is required to overcome suppression by regulatory T cells. Immunity. 2014 Jan 16;40(1):78-90

16. Oh JE and **Lee HK**. 2013. Autophagy as an innate immune modulator. Immune Network. 2013 Feb;13(1):1-9 Invited Review Article

17. Shin JH, Min SH, Kim SJ, Kim YI, Park J, **Lee HK***, Yoo OJ*. 2013. TAK1 regulates autophagic cell death by suppressing the phosphorylation of p70 S6 kinase 1. Scientific Reports. 2013 Mar 27;3:1561 (* Co-corresponding author)

18. **Lee HK**, Bae YS. 2013. 12th International Dendritic Cell Symposium, October 7-11, 2012; Daegu, Korea: New Paradigm of Dendritic Cell Science and application. Oncoimmunology. 2013 Feb 1;2(2) Invited Review Article

19. Oh JE and **Lee HK**. 2012. Modulation of pathogen recognition by autophagy. Frontiers in Immunology. **3**:44. doi: 10.3389/fimmu.2012.00044 Invited Review Article

20. Oh JE, **Lee HK**. 2012. Autophagy in innate recognition of pathogens and adaptive immunity. Yonsei Medical Journal. 53(2):241-247 Invited Review Article

21. Lee DH, Kim SH, Kang W, Choi YS, Lee SH, Lee SR, You S, **Lee HK**, Chang KT, Shin EC. 2011. Adjuvant effect of bacterial outer membrane vesicles with penta-acylated lipopolysaccharide on antigen-specific T cell priming. Vaccine. 26;29 (46):8293-301

22. Choi MS, Min SH, Jung H, Lee JD, **Lee HK***, Yoo OJ*. 2011. The essential role of FKBP38 in regulating phosphatase of regenerating liver 3 (PRL-3) protein stability. Biochemical and Biophysical Research Communications. 406, 305-309 (Co-corresponding)

23. Lee S, Nishio H, Nishio S, Choi BS, **LEE HK**, Cantley LG. 2011. Macrophage heterogeneity regulates renal injury and repair. Journal of the American Society of Nephrology. 22 (2):317-326

24. Shin MJ, Shim JH, Lee JY, Chae WJ, **Lee HK**, Morio T, Park JH, Chang EJ, Lee SK. 2010. Qualitative and quantitative differences in the intensity of Fas-mediated intracellular signals determine life and death in T cells. International Journal of Hematology. 92(2):262-70

25. **Lee HK**, Seinberg BE, Alberts P, Lee YH, Chervonsky A, Mizushima N, Grinstein S, Iwasaki A. 2010. In vivo requirements for atg5 in antigen presentation by dendritic cells. Immunity 32 (2):227-239

26. Tal MC, Sasai M, **Lee HK**, Yordy B, Shadel GS, Iwasaki A. 2009. Absence of autophagy results in reactive oxygen species-dependent amplification of RLR signaling. Proc Natl Acad Sci U S A 106, 2770-2775

27. **Lee HK**, Zamora M, Linehan MM, Iijima N, Gonzales D, Haberman A, Iwasaki A. 2009. Differential role of migratory and resident DCs in T cell priming after mucosal vs. skin HSV-1 infection. Journal of Experimental Medicine, 206, 359-370

28. Takeshi I, **Lee HK**, Ogura Y, Flavell RM, Iwasaki A. 2009. The requirement of inflammasome in the generation of adaptive immunity against influenza virus infection. Journal of Experimental Medicine, 206, 79-87

29. **Lee HK**, Iwasaki A. 2008. Autophagy and antiviral immunity. Current Opinion in Immunology, 20(1):23-29.

30. Miller BC, Zhao Z, Stephenson LM, Cadwell K, Pua HH, **Lee HK**, Mizushima NN, Iwasaki A, He YW, Swat W, Virgin HW. 2007. The autophagy gene Atg5 plays an essential role in B lymphocyte development. Autophagy, 24;4(3)

31. Joshi NS, Cui W, Chandele A, **Lee HK**, Urso D, Hagman J, Gapin L, Kaech SM. 2007. Inflammation directs a gradient of T-bet expression that specifies memory precursor and short-lived effector CD8 T cells. Immunity 27:281-295

32. **Lee HK**, Lund JM, Ramanathan B, Mizushima N, Iwasaki A. 2007. Autophagy-dependent viral recognition by plasmacytoid dendritic cells. Science 315:1398-401

Introduced in Perspective: Reis e Sousa C. 2007. Eating in to avoid infection. Science 315:1376-7

33. **Lee HK**, Iwasaki A. 2007. Innate control of adaptive immunity: dendritic cells and beyond. Seminars in Immunology 19:48-55

34. Watanabe N, Wang YH, **Lee HK**, Ito T, Wang YH, Cao W, Liu YJ. 2005. Hassall's corpuscles instruct dendritic cells to induce CD4+CD25+ regulatory T cells in human thymus. Nature 436:1181-5

35. Chae WJ, **Lee HK**, Han JH, Kim SW, Bothwell AL, Morio T, Lee SK. Qualitatively Differential Regulation of T cell Activation and Apoptosis by T cell receptor ζ chain ITAMs and their Tyrosine residues, International Immunology. 2004, 16(9):1225-1236

36. Han DJ, Jeong YL, Wee YM, Lee AY, **Lee HK**, Ha JC, Lee SK, Kim SC. 2003. Tautomycetin as a novel immunosuppressant in transplantation. Transplantation Proceedings 35:547

37. Shim JH*, **Lee HK***, Chang EJ, Chae WJ, Han JH, Han DJ, Morio T, Yang JJ, Bothwell A, Lee SK. 2002. Immunosuppressive effects of tautomycetin in vivo and in vitro via T cell-specific apoptosis induction. Proc Natl Acad Sci U S A 99:10617-22 (equally contributed)

38. **Lee HK**, Cho KM, Chun HS, Son HJ, Lee SK. 1998. Immunosuppressive effects of tautomycetin on T cells, Korean J. Immunology 20, 2: 85-90.

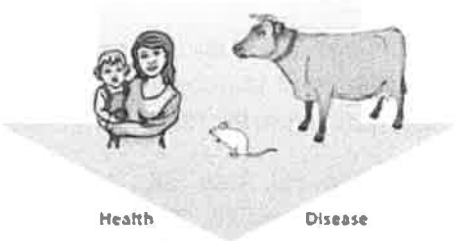
Critical roles of commensal microbiota in shaping antiviral immunity

Heung Kyu Lee, Ph.D.

Graduate School of Medical Science and Engineering
Korea Advanced Institute of Science and Technology

What are the commensal microbiota?

- Microbial cells > 10x human cells
- Generally not harmful, in fact essential for maintaining health
- Roles of commensal microbiota
  - ✓ Produce some vitamins
  - ✓ Break down our food to extract nutrients
  - ✓ Teach our immune systems
- Changes in the composition correlate with numerous disease states



Health	Disease
Angiogenesis	Allergy
Digestion	Asthma
Enteric nerve function	Cancer
Epithelial homeostasis	Diabetes
Fat metabolism	IBD
Immune cell homeostasis	Obesity
Resistance to infection	

Hill, David A and Artis, David. Annual review of immunology (2010) Vol. 28

KAIST

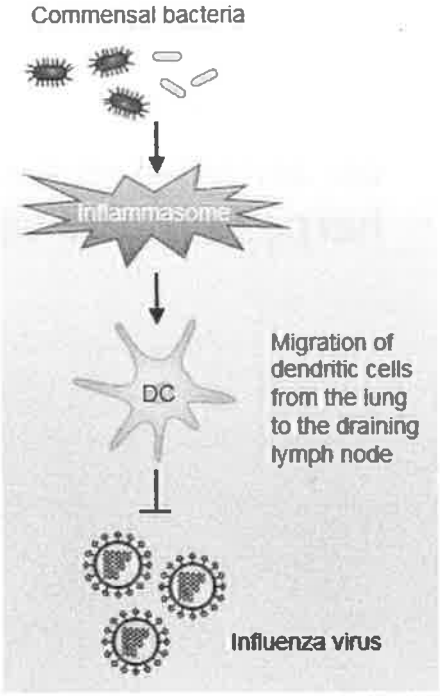
Commensal bacteria supply signals for inflammasome activation

Microbiota regulates immune defense against respiratory tract influenza A virus infection

Takeshi Ichinohe[a,b,1], Iris K. Pang[a,1], Yosuke Kumamoto[a], David R. Peaper[c], John H. Ho[a], Thomas S. Murray[a,d], and Akiko Iwasaki[a,2]

Ichinohe et al. PNAS (2011) Vol. 108

- Immune responses to respiratory influenza virus infection are diminished by antibiotic treatment.
- Neomycin-sensitive commensal bacteria are required.
- Local or distal TLR stimulation restores immune response to influenza virus infection in antibiotic-treated mice.
- Commensal bacteria supply signal 1 for IL-1β and IL-18 secretion.
- Antibiotic treatment impairs DC homeostasis and migration by reducing priming signals for inflammasome-dependent cytokines.



Modified from Wang J & Sokolovska T. PLoS Pathog (2012) Vol. 8

KAIST

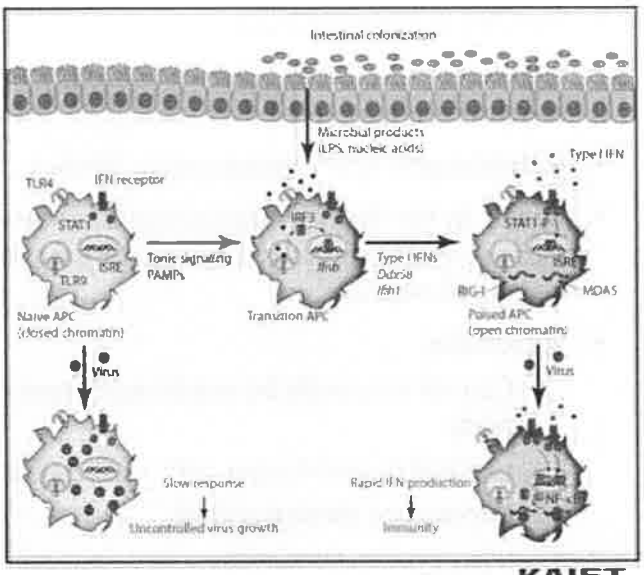
Commensal-derived signals provide tonic immune stimulation

Commensal Bacteria Calibrate the Activation Threshold of Innate Antiviral Immunity

Michael C. Abt,[1] Lisa C. Osborne,[1] Lauren A. Monticelli,[1] Travis A. Doering,[1] Theresa Alenghat,[1] Gregory F. Sonnenberg,[1] Michael A. Paley,[1] Marcelo Antonus,[1] Katie L. Williams,[1] Jan Erikson,[1] E. John Wherry,[1,2] and David Artis[1,2]

Abt et al. Immunity (2012) Vol. 37

- Commensal bacteria augment immunity against systemic or mucosal viral infection.
- Commensal-depleted mice exhibit impaired innate and adaptive antiviral immunity.
- Expression of antiviral genes is reduced in macrophages from antibiotic-treated mice.
- Commensal-derived signals tune the activation threshold of the innate immune system.



Jeremy P. McAleer, Jay K. Kolls. Immunity (2012) Vol. 37

KAIST

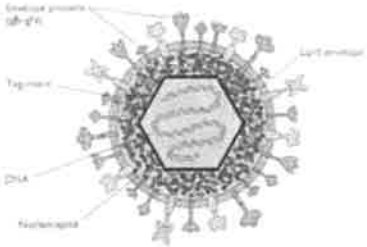
Purpose

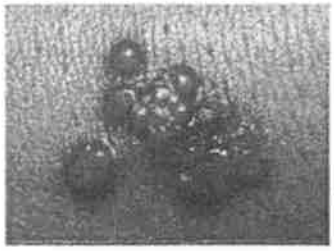
To examine the role of commensal microbiota on antiviral immunity against genital mucosal herpes simplex virus type 2 (HSV-2) infection

KAIST

Genital herpes is a common, life-long viral infection

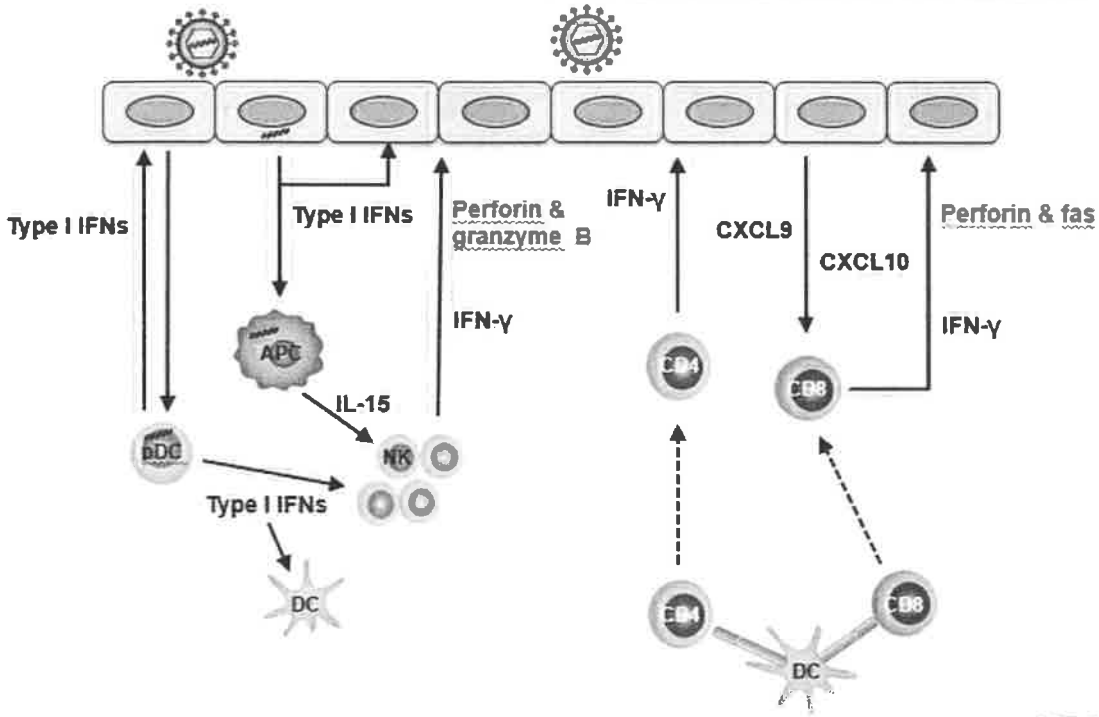
- 15.5 % of persons aged 14-49 years
- Caused by HSV-2 > HSV-1
- Vesicles or painful ulcers
- Significant risk factor for other sexually transmitted infections such as HIV-1
- Neurotrophic and neuroinvasive viruses
- Persist in the body by becoming latent and hiding from the immune system in the cell bodies of neurons
- Treatment
  - Cannot currently be eradicated from the body
  - Limited to interfering with viral replication
  - No vaccine treatment yet





KAIST

Mechanisms of innate and adaptive immunity against HSV-2 infection



Modified from Kumamoto & Iwasaki. Curr Opin Immunol. 2012;24:411-416 and Chan et al. J Reprod Immunol. 2011;88:210-218

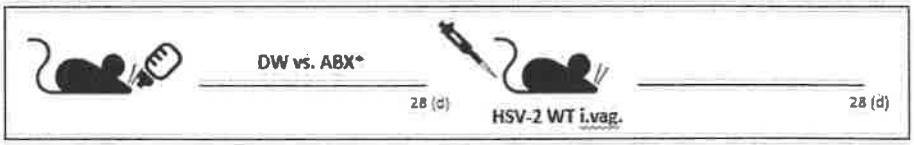
KAIST

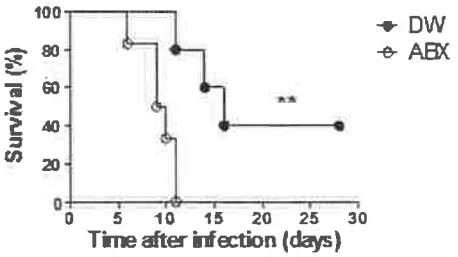
Question I

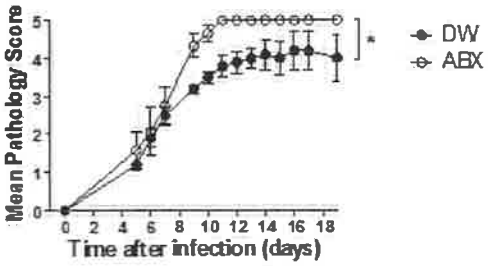
Does commensal microbiota influence on antiviral protection against genital mucosal HSV-2 infection?

KAIST

ABX-treated mice are more susceptible to mucosal HSV-2 infection



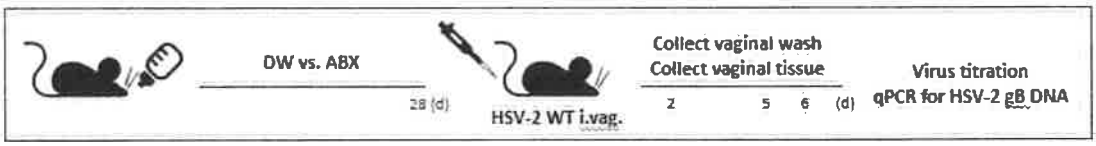


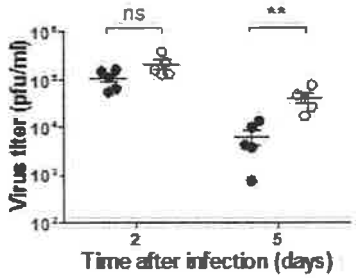


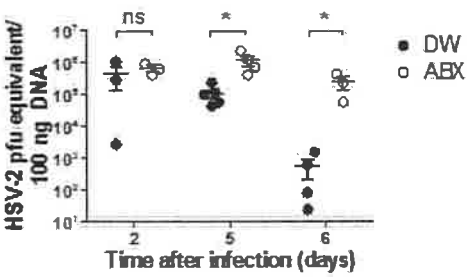
* ABX: ampicillin (G+/G-), vancomycin (G+), neomycin sulfate (G->G+), gentamicin (G->G+), metronidazole (anaerobe/protozoa)

KAIST

ABX-treated mice show delayed viral clearance at the site of infection







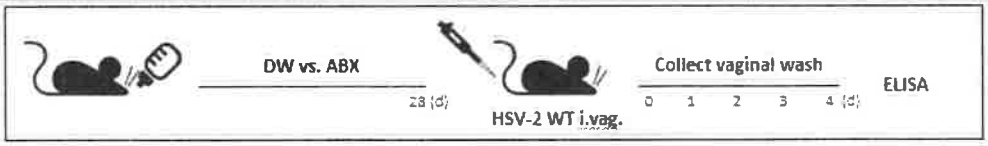
KAIST

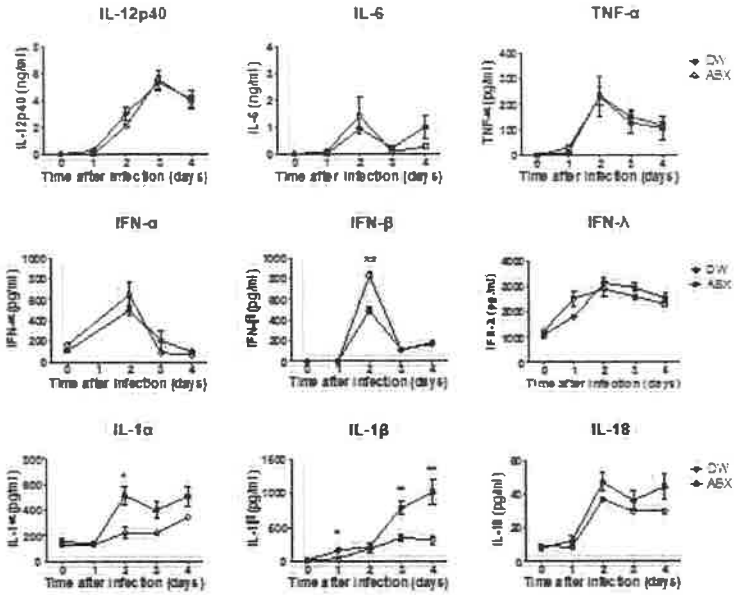
Question II

How can commensal microbiota support immune protection against genital mucosal HSV-2 infection?

KAIST

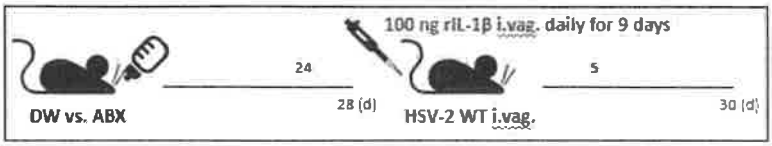
Production of innate cytokines except IL-1 family cytokines in ABX-treated mice is comparable to control mice after HSV-2 infection

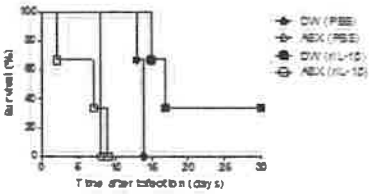


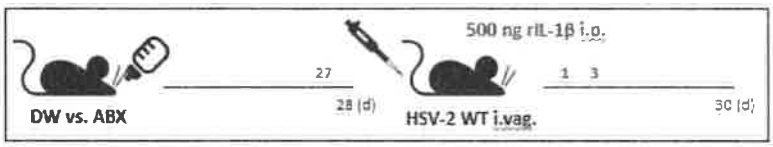


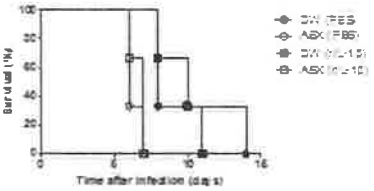
KAIST

IL-1β treatment does not restore immune protection against mucosal HSV-2 infection in ABX-treated mice



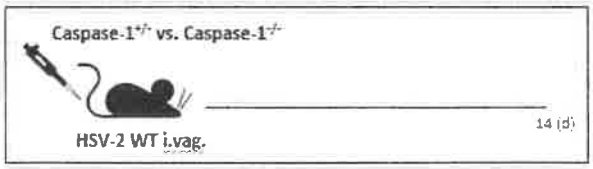


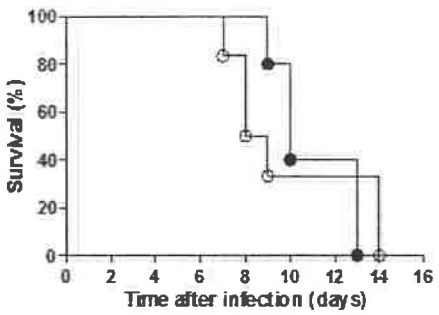


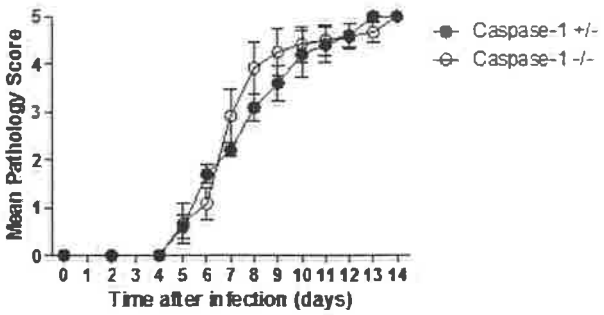


KAIST

Caspase-1 deficiency does not affect the susceptibility to mucosal HSV-2 infection

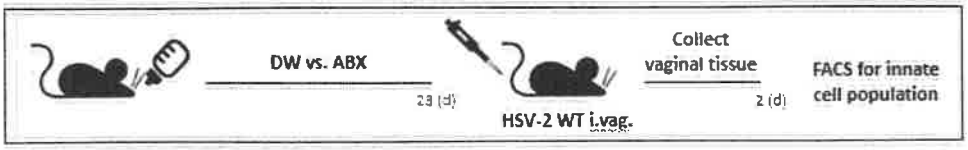


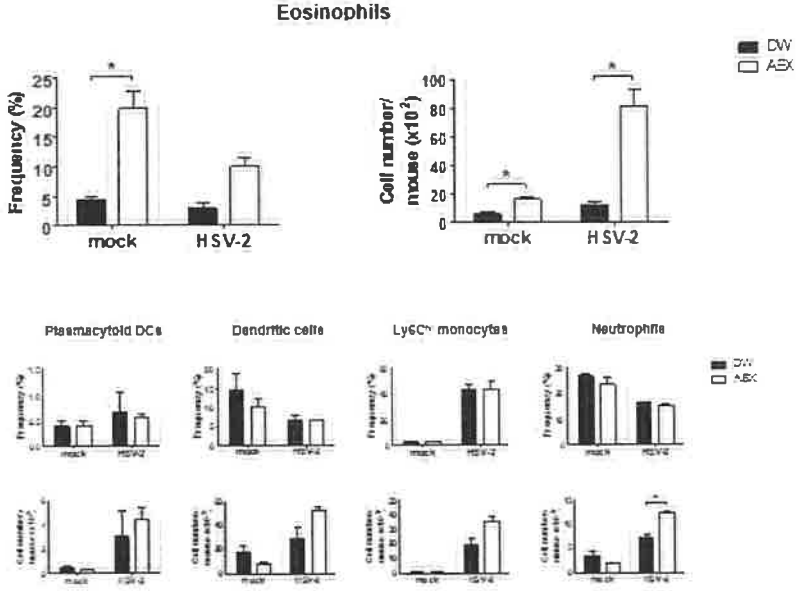




KAIST

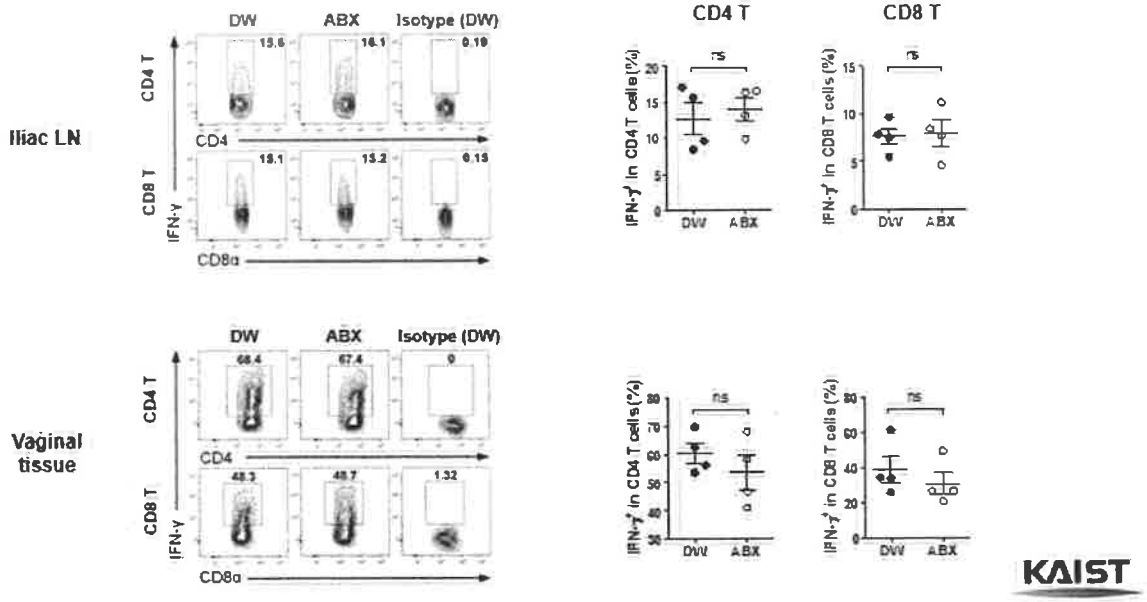
Eosinophils are markedly increased in vaginal tissue in ABX-treated mice





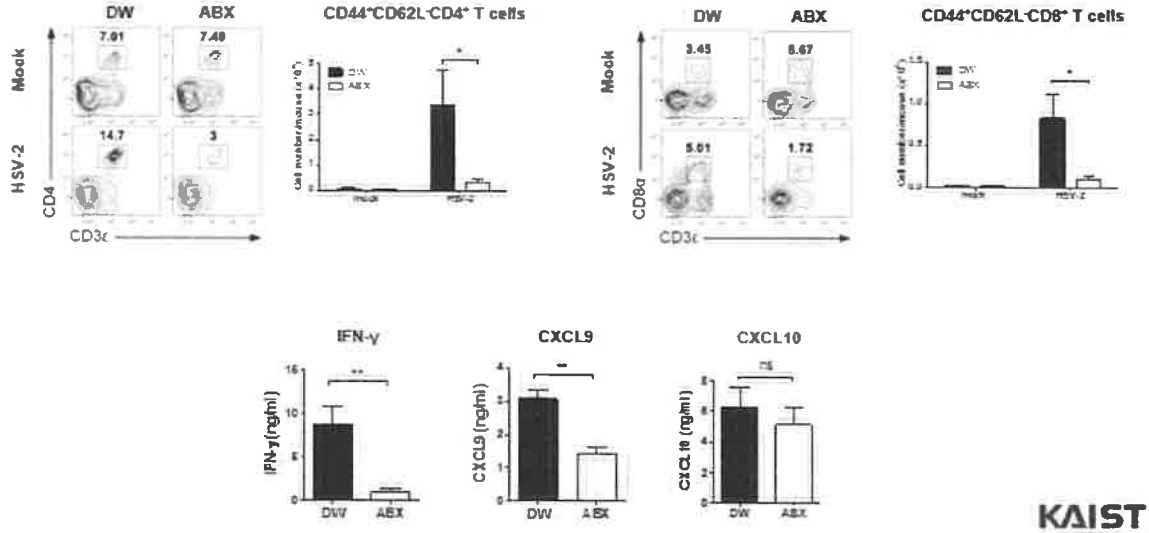
KAIST

IFN-γ-producing capacity of T cells in draining LN and vaginal tissue is not impaired in ABX-treated mice



KAIST

IFN-γ production at the local infection site is impaired in ABX-treated mice after mucosal HSV-2 infection due to defective T cell migration



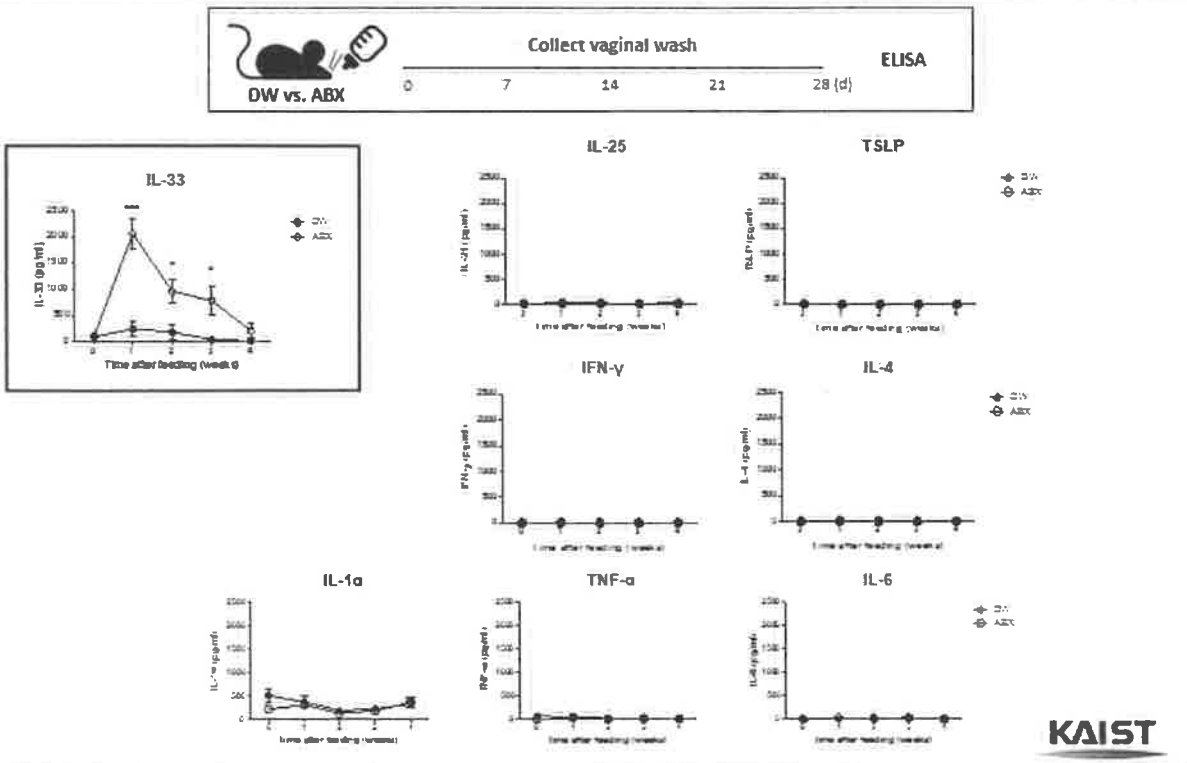
KAIST

Question III

Which factors do modulate the defects in local immune defense against genital mucosal HSV-2 infection after antibiotic treatment?

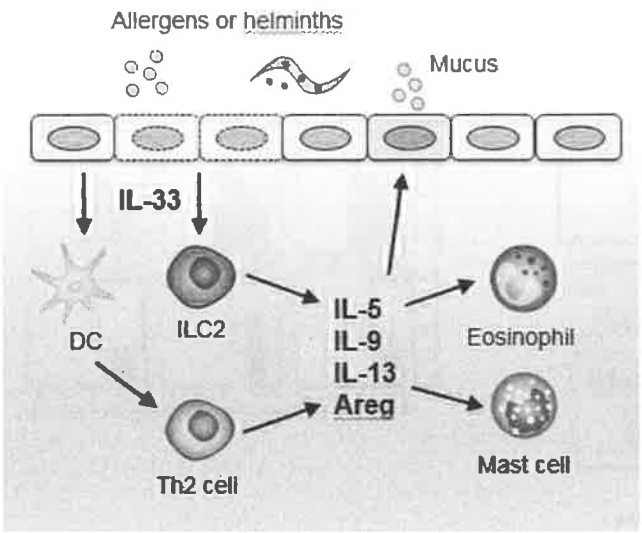
KAIST

IL-33 secretion is induced in vaginal mucosa during antibiotic treatment



KAIST

The role of IL-33 in Th2 immune response

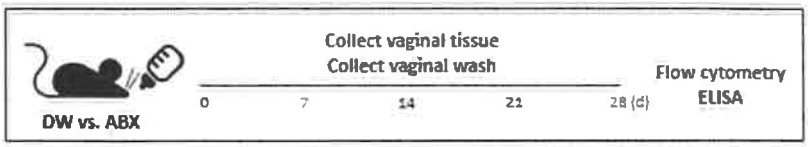


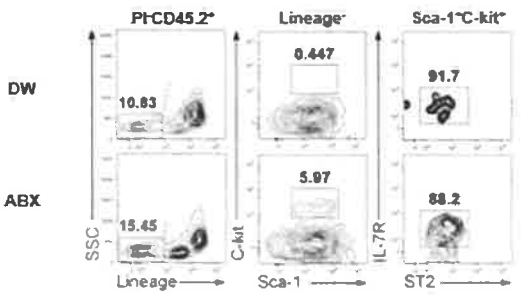
Modified from Licona-Limón et al. Nat Immunol. 2013;14:536-542

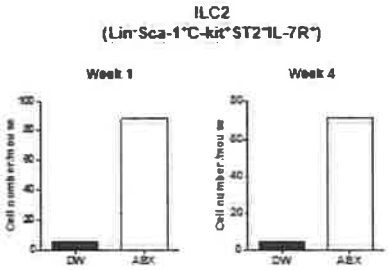
- IL-33 (or IL-25, TSLP) activates type 2 innate lymphoid cells (ILC2), which directly secrete type 2 cytokines.
- IL-33 (or IL-25, TSLP) activates DCs, which induce Th2 response.
- Type 2 cytokines feed back on the epithelium to induce mucus secretion (IL-13) and tissue repair (amphiregulin).
- IL-9 and IL-5 induced by ILC2 cells lead to the recruitment and activation of mast cells and eosinophils.

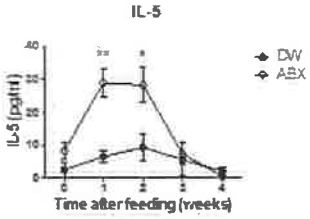
KAIST

Antibiotic treatment induces ILC2 recruitment and IL-5 production in the vagina





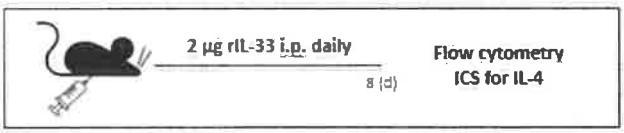


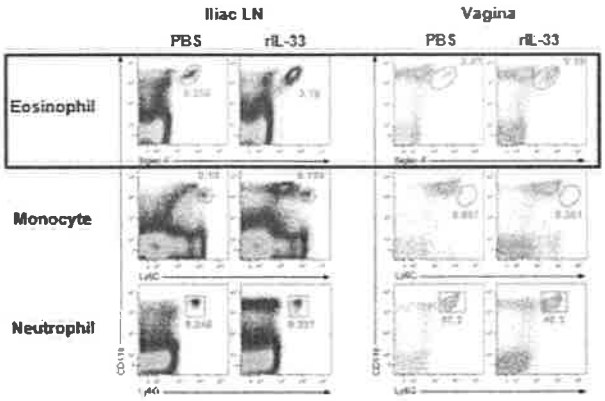


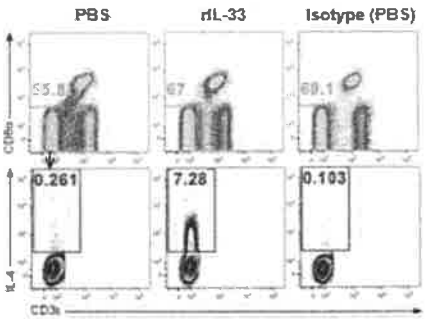
lineage: CD3ε/B220/CD11b/CD11c/NK1.1/FcεRI

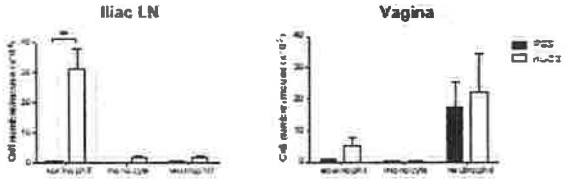
KAIST

IL-33 treatment induces eosinophil recruitment and IL-4 production



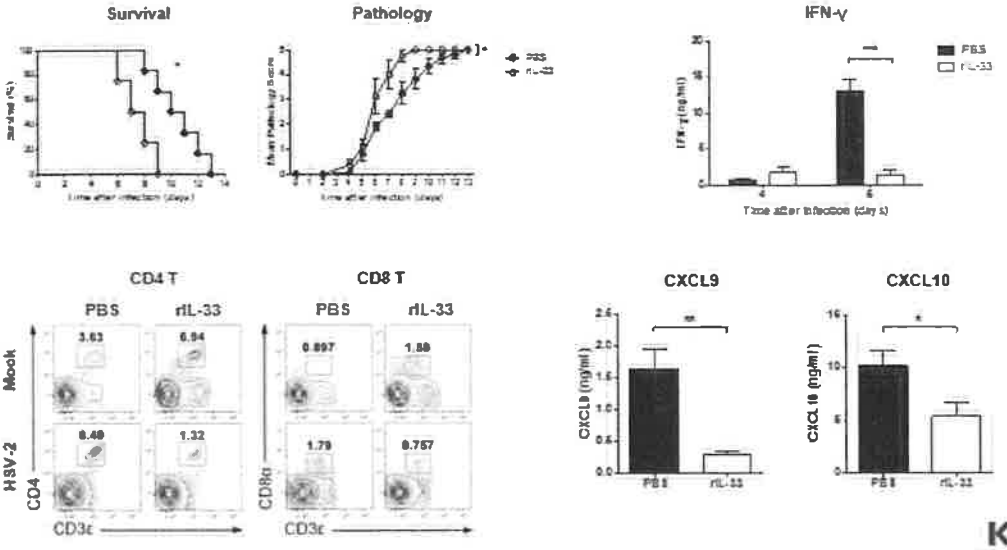






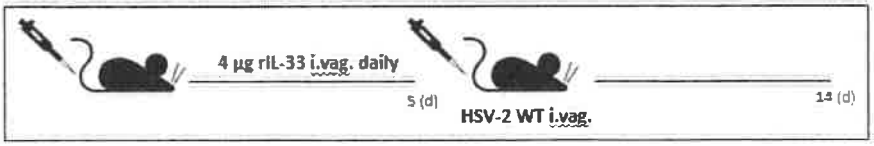
KAIST

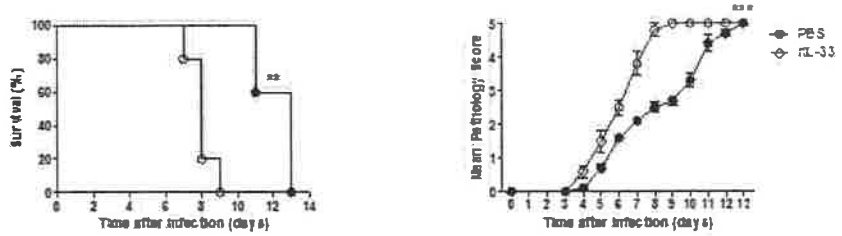
Systemic rIL-33 treatment impairs antiviral immunity to mucosal HSV-2 infection



KAIST

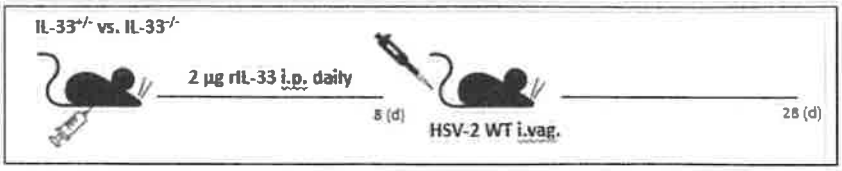
Local rIL-33 treatment induces mice susceptible to mucosal HSV-2 infection

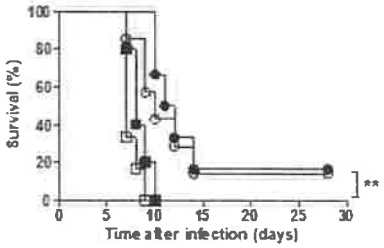


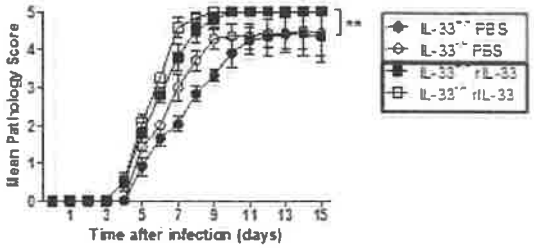


KAIST

Exogenous, but not endogenous, IL-33 modulates antiviral immunity to mucosal HSV-2 infection

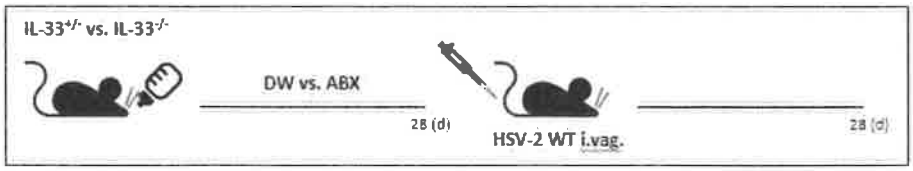


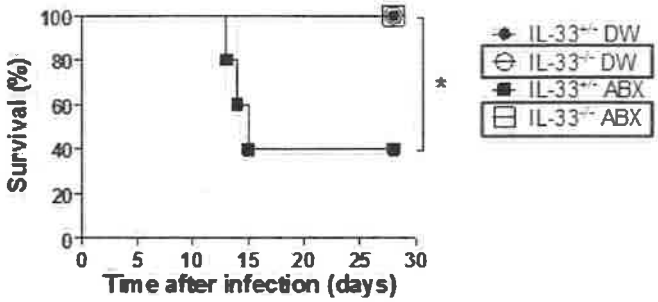




KAIST

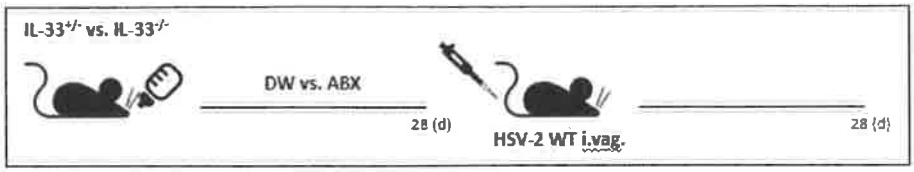
IL-33-deficient mice treated with antibiotics show comparable survival rates with water treated mice

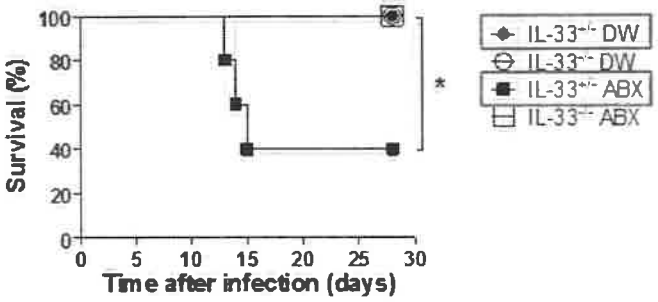




KAIST

IL-33-deficient mice treated with antibiotics show comparable survival rates with water treated mice





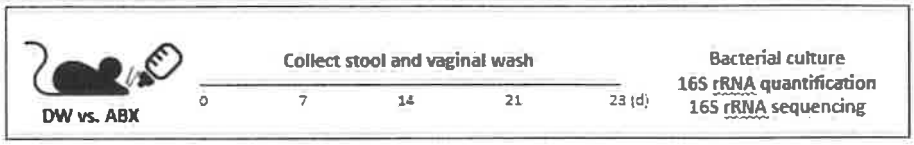
KAIST

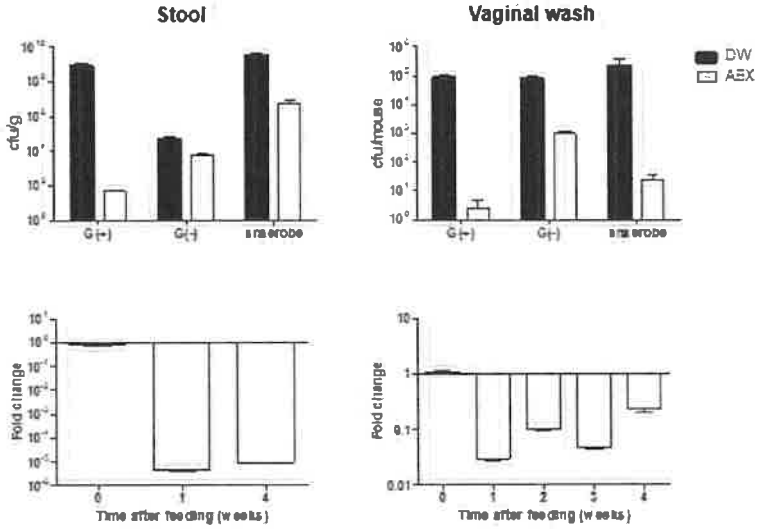
Question IV

What does trigger IL-33 secretion in the vagina after antibiotic treatment?

KAIST

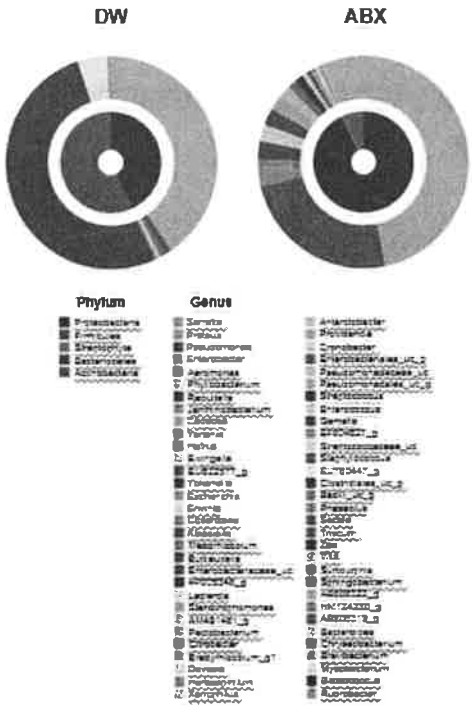
Decreased bacterial colonization after antibiotic treatment

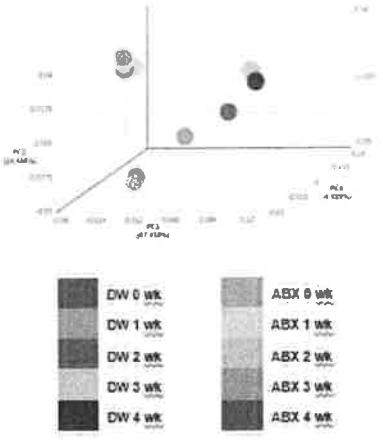




KAIST

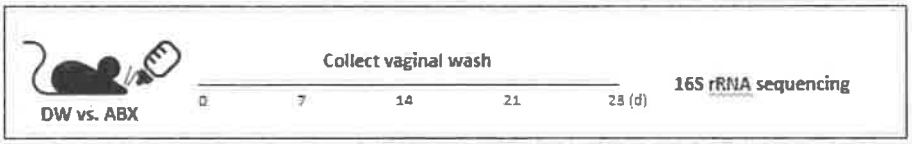
Increased bacterial diversity after antibiotic treatment

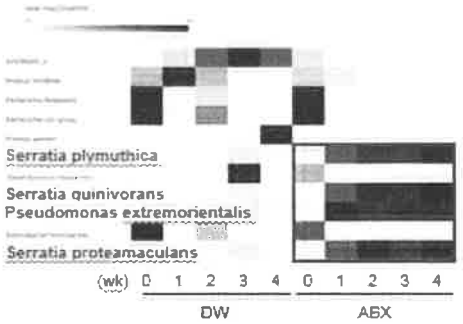


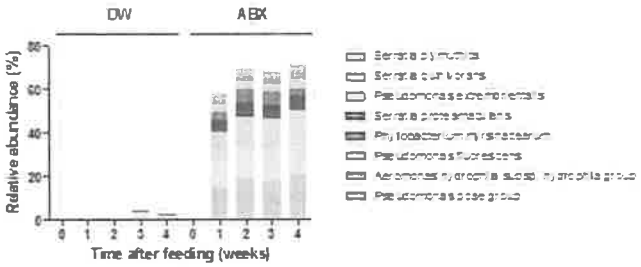


KAIST

Relative abundance of pathogenic bacteria is increased in ABX-treated mice







KAIST

Some strains of *Serratia* and *Pseudomonas* produce proteases, as a major factor of pathogenesis of these bacteria

Characterization of 73 kDa Thiol Protease from *Serratia marcescens* and Its Effect on Plasma Proteins[1]

Akhteruzzaman Molla,* Tetsuro Yamamoto,** and Hiroshi Maeda*,†

*Department of Microbiology and **Department of Allergy, Institute for Medical Immunology, Kumamoto University Medical School, Kumamoto, Kumamoto 860

Mechanisms involved in the evasion of the host defence by *Pseudomonas aeruginosa*

Arsalan Kharazmi

Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark

Isolation and Properties of *Serratia proteamaculans* 94 Cysteine Protease

N. V. Mozhina[a], O. A. Burmistrova[a], D. V. Pupov[a], G. N. Rudenskaya[a,b], Ya. E. Dunaevsky[a], I. V. Demiduk[b], and S. V. Kostrov[b]

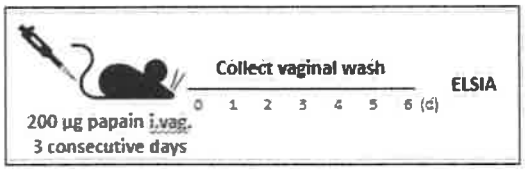
[a] Faculty of Chemistry, Moscow State University, Vorob'evy gory, Moscow, 119992 Russia

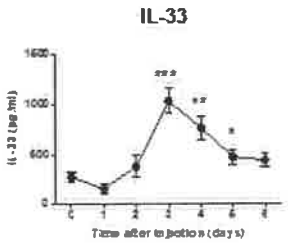
[b] Institute of Molecular Genetics, Russian Academy of Sciences, pl. Akademika Kurchatova 46, Moscow, 123182 Russia

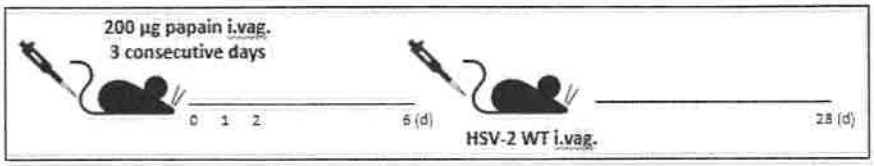
Received July 3, 2007; in final form, October 8, 2007

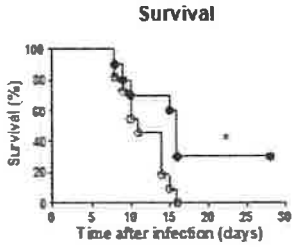
KAIST

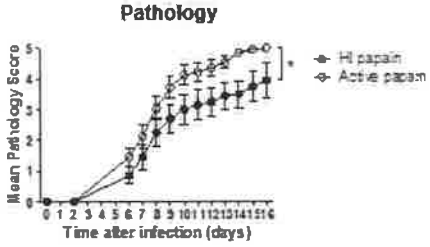
Proteases induce IL-33 secretion and contributes to impaired antiviral immunity to mucosal HSV-2 infection











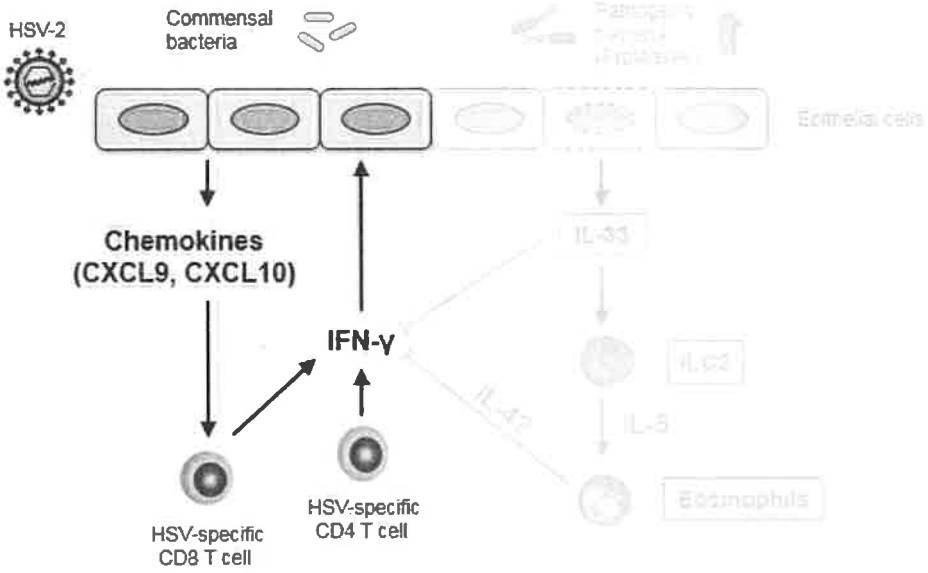
KAIST

Summary

- Antibiotic-treated mice rapidly succumb to mucosal HSV-2 infection.
- IFN-γ production is severely impaired at local infection site in antibiotic-treated mice due to defective migration of effector T cells.
- IL-33 is secreted from the vagina after depletion of commensal bacteria.
- IL-33 contributes to impaired antiviral immunity against mucosal HSV-2 infection.
- Antibiotic treatment results in an imbalance in the microbial composition of the vagina.
- Proteases, such as those induced by dysbiosis, induce IL-33 secretion and impair antiviral immunity to mucosal HSV-2 infection.

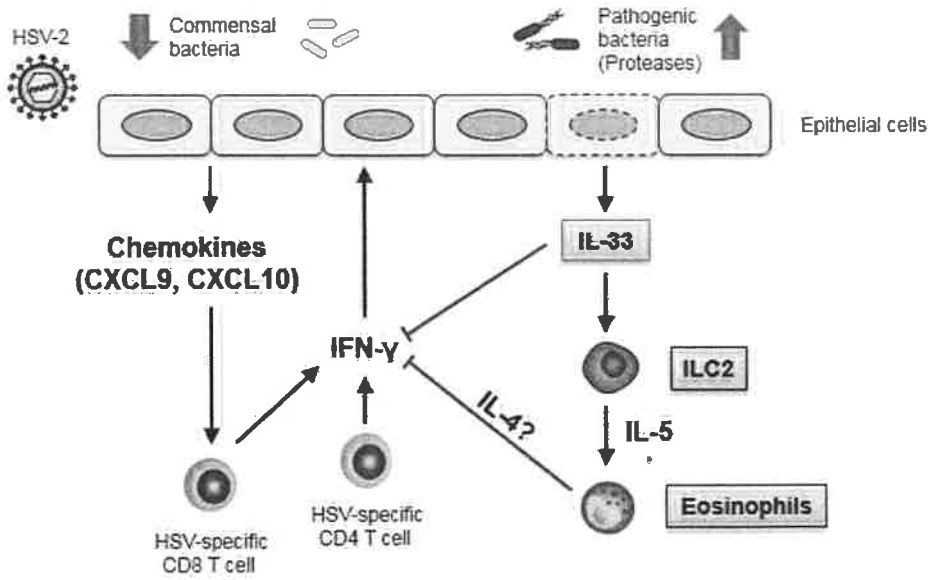
KAIST

Summary



KAIST

Summary



KAIST

Acknowledgements

Lab of Host Defenses

Ji Eun Oh, M.D., Ph.D.

한국생명공학연구원
Korea Research Institute of Bioscience and Biotechnology

Byoung-Chan Kim, Ph.D.
Dong-Ho Chang

Je-Wook Yu, Ph.D.
Inhwa Hwang

KRISS 한국표준과학연구원

Dukjin Kang, Ph.D.
Meehyang Kwon
Sun Young Lee

東京大学 THE UNIVERSITY OF TOKYO

Susumu Nakae, Ph.D.

KBSI 한국기초과학지원연구원 KOREA BASIC SCIENCE INSTITUTE

Jin Young Kim, Ph.D.

NRF 한국연구재단

KAIST

A Role of STAT3 in Barrier Integrity and Microbiota Composition of the Skin

Masato Kubo

RIKEN, Japan

Atopic dermatitis (AD) is the most common inflammatory skin disease. Stat3 mutation is a major cause of hyper IgE syndrome (HIES), which consistently represent AD like eczematous dermatitis. However, how Stat3 deficiency contributes to the dermatitis symptom remains unclear. We found that *Stat3* deficiency in the skin caused spontaneous development of eczematous dermatitis dependent on T cells and IL-4 receptor. Based on multi-dimensional transcriptome analysis in pre- and post-flares skin, dermatitis phenotype was controlled by sequential two steps of Stat3 deficiency and environmental pathogenic stimuli. The Stat3 deficiency determined the barrier integrity that increased threshold of inflammation, but this step was not sufficient to form pathogenicity. Transcriptome data indicated that emergence of dermatitis phenotype need to trigger robust activation of NF kB pathway and T_H2 cells. Continuous colonization of Staphylococcus aureus was an environmental stimulus to increase the activation threshold of T_H2 inflammation in the skin. Therefore, STAT3 was a homeostasis switch in the skin controlling barrier integrity and microbiota composition. The STAT3 mouse model provides coherent biomarkers to explain how synergistic regulation with the genetic factor and environmental stimuli were necessary for onset of dermatitis in T_H2 mediated AD patients.

CURRICULUM VITAE

Masato Kubo, Ph.D.

Professor, Department of Molecular Pathology, Research Institute for Biomedical Sciences, Tokyo University of Science

Laboratory head, Laboratory for cytokine regulation, Center for Integrated Medical Science, RIKEN Yokohama Institute

E-mail: masato.kubo@riken.jp

Education and Appointment

1984/3/31	Master, Department of Animal Breeding, Tokyo University of Agriculture, Tokyo
1991/3/31	Ph. D, Department of Immunology, Faculty of Medicine, University of Tokyo, Tokyo
1987-89	Research Fellow of Institute of Immunology, University of Toronto, Canada.
1991-93	Researcher at The Department of Molecular Immunology, Syntex Discovery Research, Palo Alto, U.S.A
1993-95	Researcher at Nihon Syntex Discovery Research, Ibaragi, Japan
1995-00	Research Associate & Assistant Professor at Division of Immunobiology, Research Institute for Biological Sciences, Science University of Tokyo, Chiba, Japan
2000-04	Associate Professor at Division of Immunobiology, Research Institute for Biological Sciences, Science University of Tokyo, Chiba, Japan
2003-11	Laboratory head, Laboratory for Signal Network, RIKEN Research, Center for Allergy and Immunology (RCAI), RIKEN Yokohama Institute
2008-11	Adjunct Professor, Graduate School of Biomedical Science, Tokyo Medical and Dental University
2009-present	Professor, Department of Molecular Pathology, Research Institute for Biomedical Sciences, Tokyo University of Science
2013-present	Laboratory head, Laboratory for cytokine regulation, Center for Integrated Medical Science, RIKEN Yokohama Institute

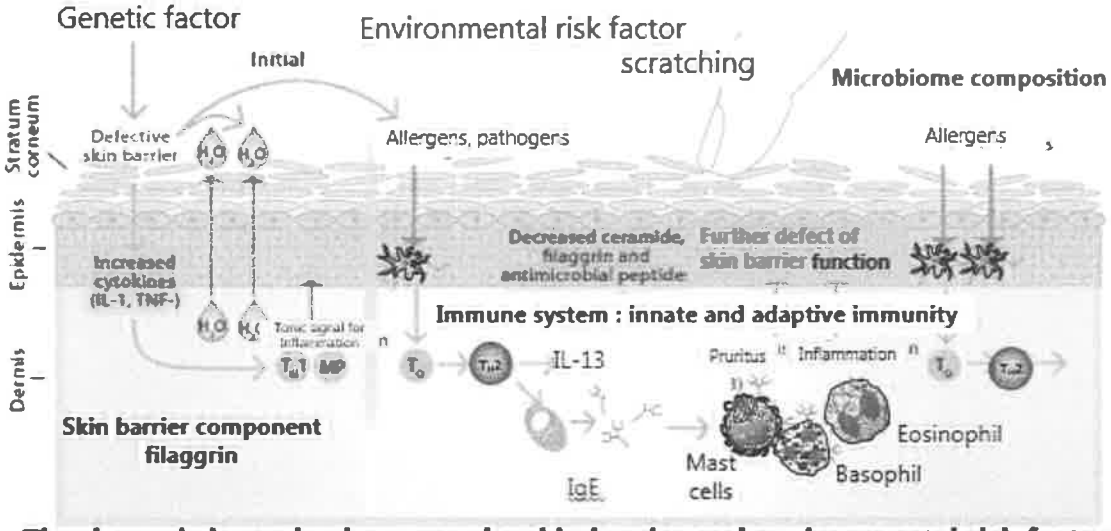
2017-present	Project Reader, RIKEN program for Drag Discovery and Medical Technology

Atopic dermatitis (AD)

Skin is a complex system to maintain the homeostasis between the inner and outer environments in the body

AD is a popular chronic skin disease. This disease has been thought to be a severe skin inflammation as a consequence of allergic immune reaction. The disease usually associate with scratching.

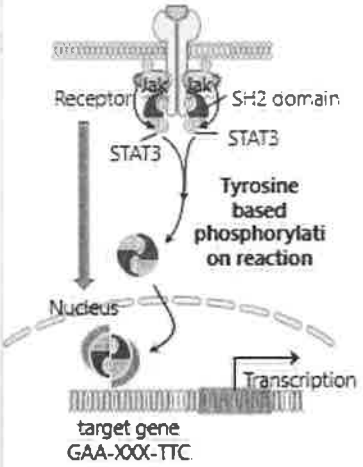
Loss-of-function variants of the epidermal barrier protein filaggrin become a major risk factor for AD. Nat Genet 2006



The dynamic interplay between the skin barrier and environmental risk factors may critical process to compose of AD symptom.

What is a role of STAT3 in skin homeostasis?

IL-6, IL-23, IL-10 .....



STAT3 is a transcriptional activator

Stat3$^{flox/flox}$ K5-cre

- Keratinocyte-specific ablation of Stat3 impaired skin remodeling, but does not affect skin morphogenesis. ***EMBO J.*** 18, 4657–4668, 1999
- Enhanced Apoptosis by Disruption of the STAT3-IkB-ζ Signaling Pathway in Epithelial Cells Induces Sjogren's Syndrome-like Autoimmune Disease, ***Immunity*** 38, 1–11, 2013

  Stat3$^{flox/flox}$ K5-cre mice also develop Sjogren's syndrome-like symptom, including periocular dermatitis.

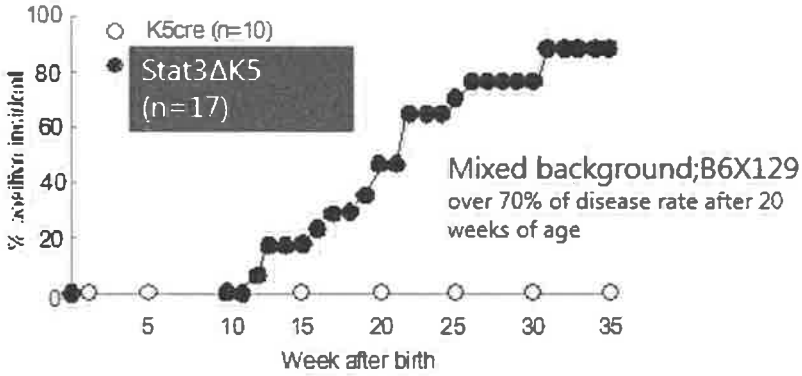
Hyper-IgE Syndrome (HIES or Job's syndrome)

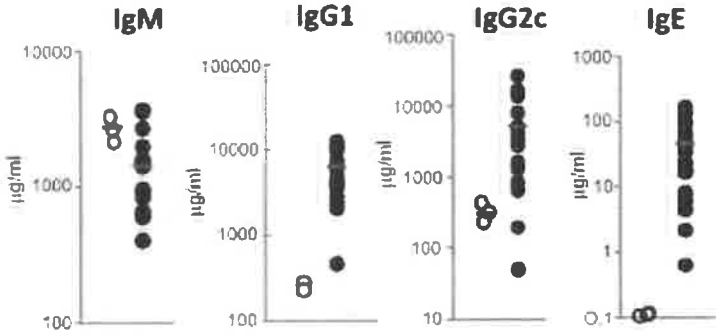
a rare primary immunodeficiency disease characterized by eczema, recurrent staphylococcal skin abscesses, recurrent lung infections, eosinophilia and high serum titer of IgE.

Autosomal dominant (AD) a heterozygous mutation in the *STAT3* gene

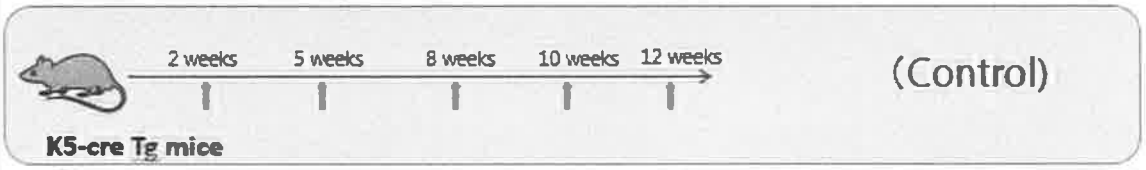
Autosomal recessive (AR) mutations and deletions in the *DOCK8* gene

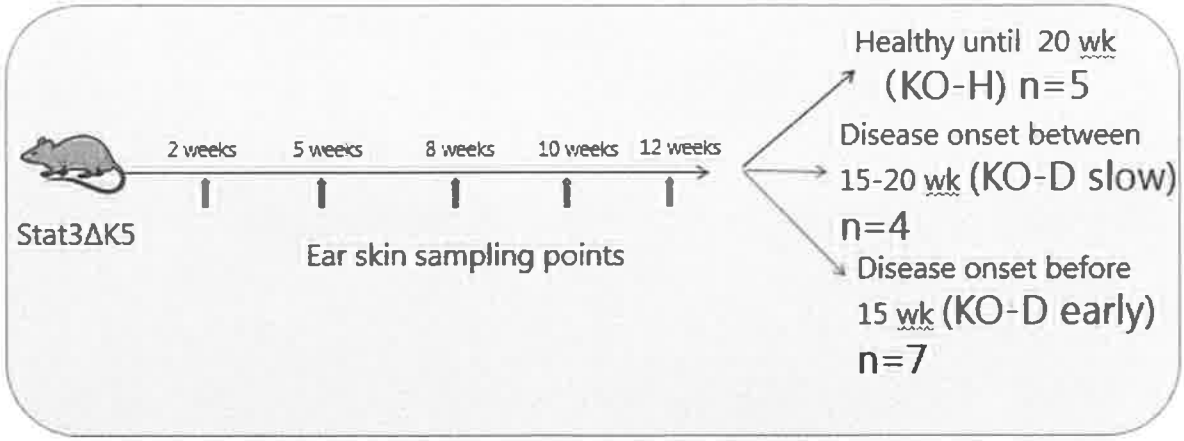
Stat3 deficiency in keratinocyte caused dermatitis incident followed by high titer of IgE production and scratching





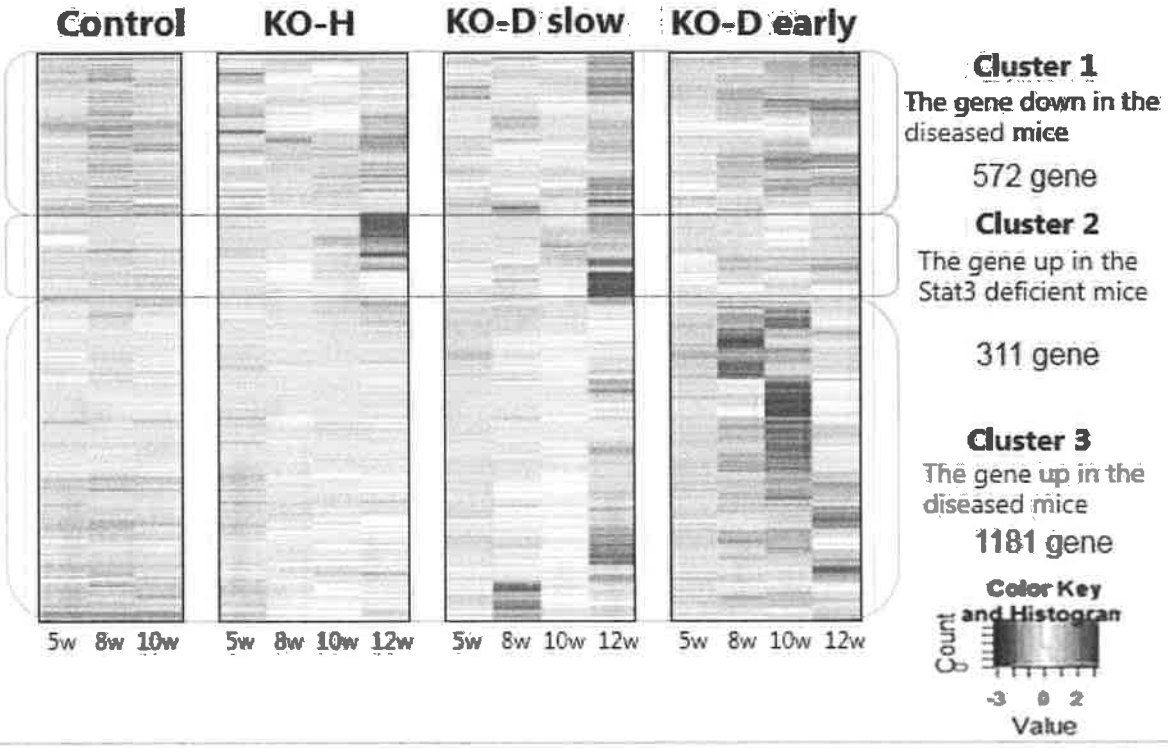
How dose the Stat3 defect link to the dermatitis phenotype in keratinocyte specific *Stat3* deficient mice?





Time-course RNA-sequencing analysis

Kinetic change of the heat map profile in the transcriptome results



Estrogen-related Receptor α and Innate Immune Regulation

Eun-Kyeong Jo

Chungnam National University, Korea

Nuclear receptors (NRs) are critically involved in various physiological responses through the regulation of numerous target genes. Orphan NRs are a subset of NR superfamily which ligands and functions have not been fully characterized. Emerging evidence has accumulated that several orphan NRs play critical roles in regulation of innate immunity to prevent harmful inflammatory responses in the host. The orphan NR estrogen-related receptor α (ERRα; NR3B1) is the first identified orphan NR that plays an important role in regulation of energy metabolism and mitochondrial biogenesis. We found that ERRα was a novel regulator of the toll-like receptor-induced inflammatory response, with the unique capacity to modulate Tnfaip3 transcriptional induction and p65 acetylation through metabolic reprogramming via enhancement of mitochondrial function. In addition, I will discuss our recent findings showing that ERRα, operating in a feed-forward loop with sirtuin 1, in activation of autophagy and anti-mycobacterial responses, via both transcriptional and post-translational control of autophagy genes. Unveiling the new functions of ERRα could accelerate develop and improve novel strategies against human inflammatory and infectious diseases.

CURRICULUM VITAE

Eun-Kyeong Jo, Ph.D

Professor; Director, Infection Control Convergence Research Center

and Department of Microbiology, Chungnam National University School of Medicine,

266 Munhwa-ro, Jung-gu, Daejeon 35015, Korea

E-mail: hayoungj@cnu.ac.kr

Field of Expertise

- Autophagy and innate immune responses in mycobacterial infection
- Identification of new regulators and the molecular mechanisms in innate immune signaling
- Development of therapeutic modalities to control infection and inflammation

Education

1991. 2.	M.D. from College of Medicine, Chungnam National University (CNU), Korea
1996. 2.	Ph.D. in Department of Microbiology, College of Medicine, CNU, Korea

Professional Experience

1997 - 2003	Full-time instructor (1997-1999) and Assistant Professor (1999-2003), Dept. of Microbiology, College of Medicine, CNU
2002	Visiting Scientist, Tokyo Medical and Dental University
2003 - 2004	Research Associate, Imperial College London, U. K.
2004 - 2008	Associate Professor, Dept. of Microbiology, College of Medicine, CNU
2008 - present	Professor, Dept. of Microbiology, College of Medicine, CNU
2007 - present	Director, Medical Research Center (ISNRC), CNU

Honors

2006	Research Award for Young Medical Scientist of Societies for Korean Basic Medical Sciences
2008	Eui-Dang Research Award, Korean Association of Medical Doctors

2008 Award of Excellent Papers in Science and Technology, Korean Association of Scientists

2010 Award of KUN-IL, Korean Association of Woman Medical Doctors

2012 Pfeizer's Research Award for Basic Medicine

2015 Wunsch Medical Award

Selected Publications

1. Kim SY, Yang CS, Lee HM, Kim JK, Kim YS, Kim YR, Kim JS, Kim TS, Yuk JM, Dufour CR, Lee SH, Kim JM, Choi HS, Giguère V, Jo EK*. Estrogen-related receptor-alpha is a key coordinator of transcriptional and post-translational activation of autophagy to promote innate host defense. *Autophagy* 2017, Accepted.

2. Kim JK, Lee HM, Park KS, Shin DM, Kim TS, Kim YS, Suh HW, Kim SY, Kim IS, Kim JM, Son JW, Sohn KM, Jung SS, Chung C, Han SB, Yang CS, Jo EK*. MIR144* inhibits antimicrobial responses against Mycobacterium tuberculosis in human monocytes and macrophages by targeting the autophagy protein DRAM2. *Autophagy* 2017 Feb;13(2):423-441

3. Yuk JM, Kim TS, Kim SY, Lee HM, Han J, Dufour CR, Kim JK, Jin HS, Yang CS, Park KS, Lee CH, Kim JM, Kweon GR, Choi HS, Vanacker J-M, Moore DD, Giguère V, Jo EK*. Orphan nuclear receptor ERRα controls macrophage metabolic signaling and A20 expression to negatively regulate TLR-induced inflammation. *Immunity* 2015 Jul;43(1):80-91.

4. Yang CS, Kim JJ, Kim TS, Lee PY, Kim SY, Lee HM, Shin DM, Nguyen LT, Lee MS, Jin HS, Kim KK, Lee CH, Kim MH, Park SG, Kim JM, Choi HS, Jo EK*. Small heterodimer partner interacts with NLRP3 and negatively regulates activation of the NLRP3 inflammasome. *Nat Commun.* 2015 Feb 6;6:6115.

5. Kim JK, Yuk JM, Kim SY, Kim TS, Jin HS, Yang CS, Jo EK*. MicroRNA-125a inhibits autophagy activation and antimicrobial responses during mycobacterial infection. *J Immunol.* 2015 Jun 1;194(11):5355-65.

6. Yang CS, Kim JJ, Lee HM, Jin HS, Lee SH, Park JH, Kim SJ, Kim JM, Han YM, Lee MS, Kweon GR, Shong M, Jo EK*. The AMPK-PPARGC1A pathway is required for antimicrobial host defense through activation of autophagy. *Autophagy* 2014 May;10(5):785-802.

7. Lee HM, Kim JJ, Kim HJ, Shong M, Ku BJ, Jo EK*. Upregulated NLRP3 inflammasome activation in patients with type 2 diabetes. *Diabetes* 2013; 62(1):194-204.

8. Kim JJ, Lee HM, Shin DM, Kim W, Yuk JM, Jin HS, Lee SH, Cha GH, Kim JM, Lee ZW, Shin SJ, Yoo H, Park YK, Park JB, Chung J, Yoshimori T, Jo EK*. Host cell autophagy activated by antibiotics is required for their effective antimycobacterial drug action. *Cell Host Microbe* 2012 May 17;11(5):457-68.

9. Kim KH, An DR, Song J, Yoon JY, Kim HS, Yoon HJ, Im HN, Kim J, Kim DJ, Lee SJ, Kim KH, Lee HM, Kim HJ, Jo EK, Lee JY, Suh SW. *Mycobacterium tuberculosis* Eis protein initiates suppression of host immune responses by acetylation of DUSP16/MKP-7. *Proc Natl Acad Sci USA.* 2012 May 15;109(20):7729-34

10. Yuk JM, Shin DM, Lee HM, Kim JJ, Kim SW, Jin HS, Yang CS, Park KA, Chanda D, Kim DK, Huang SM, Lee SK, Lee CH, Kim JM, Song CH, Lee SY, Hur GM, Moore DD, Choi HS, Jo EK*. The orphan nuclear receptor SHP acts as a negative regulator in inflammatory signaling triggered by Toll-like receptors. *Nat Immunol.* 2011 Jul 3;12(8):742-51

11. Fabri M, Stenger S, Shin DM, Yuk JM, Liu PT, Realegeno S, Lee HM, Krutzik SR, Schenk M, Sieling PA, Teles R, Montoya D, Iyer SS, Bruns H, Lewinsohn DM, Hollis BW, Hewison M, Adams JS, Steinmeyer A, Zügel U, Cheng G, Jo EK, Bloom BR, Modlin RL. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. *Sci Transl Med.* 2011 Oct 12;3(104):104ra102.

12. Lee HM, Shin DM, Yuk JM, Shi G, Choi DK, Lee SH, Huang SM, Kim JM, Kim CD, Lee JH, Jo EK*. Autophagy Negatively Regulates Keratinocyte Inflammatory Responses via Scaffolding Protein p62/SQSTM1. *J Immunol.* 2011 Jan 15;186(2):1248-58.

13. Fabri M, Realegeno SE, Jo EK, Modlin RL. Role of autophagy in the host response to microbial infection and potential for therapy. *Curr Opin Immunol.* 2011 Feb;23(1):65-70.

14. Shin DM, Jeon BY, Lee HM, Jin HS, Yuk JM, Song CH, Lee SH, Lee ZW, Cho SN, Kim JM, Friedman RL, Jo EK*. *Mycobacterium tuberculosis* Eis Regulates Autophagy, Inflammation, and Cell Death through Redox-dependent Signaling. *PLoS Pathog.* 2010 Dec 16;6(12):e1001230.

15. Yuk JM, Shin DM, Song KS, Lim K, Kim KH, Lee SH, Kim JM, Lee JS, Paik TH, Kim JS, Jo EK*. Bacillus calmette-guerin cell wall cytoskeleton enhances colon cancer radiosensitivity through autophagy. *Autophagy.* 2010; 6: 46-60.

16. Kim D, You B, Jo EK, Han SK, Simon MI, Lee SJ. NADPH oxidase 2-derived reactive oxygen species in spinal cord microglia contribute to peripheral nerve injury-induced neuropathic pain. *Proc Natl Acad Sci U S A.* 2010; 107: 14851-6.

17. Lee HM, Yuk JM, Shin DM, Yang CS, Kim KK, Choi DK, Liang ZL, Kim JM, Jeon BH, Kim CD, Lee JH, Jo EK*. Apurinic/Apyrimidinic Endonuclease1 Is a Key Modulator of Keratinocyte Inflammatory Responses. *J Immunol* 2009 Nov 15;183(10):6839-48

18. Yuk JM, Shin DM, Lee HM, Yang CS, Jin HS, Kim KK, Lee ZW, Lee SH, Kim JM, Jo EK*. Vitamin D3 Induces Autophagy in Human Monocytes/Macrophages via Cathelicidin. *Cell Host Microbe* 2009; 6: 231-43.

19. Yuk JM, Yang CS, Shin DM, Kim KK, Lee SK, Song YJ, Lee HM, Cho CH, Jeon BH, Jo EK*. A dual regulatory role of apurinic/apyrimidinic endonuclease 1/redox factor-1 in HMGB1-induced inflammatory responses. *Antioxid Redox Signal* 2009;11(3):575-88..

20. Jo EK*. Mycobacterial interaction with innate receptors: TLRs, C-type lectins, and NLRs. *Curr Opin Infect Dis* 2008 Jun;21(3):279-86

21. Yang CS, Lee DS, Song CH, An SJ, Li S, Kim JM, Kim CS, Yoo DG, Jeon BH, Yang HY, Lee TH, Lee ZW, El-Benna J, Yu DY, Jo EK*. Roles of peroxiredoxin II in the regulation of proinflammatory responses to LPS and protection against endotoxin-induced lethal shock. *J Exp Med* 2007; 204: 583-94.

충남대학교

Estrogen-related receptor-α and innate immune regulation

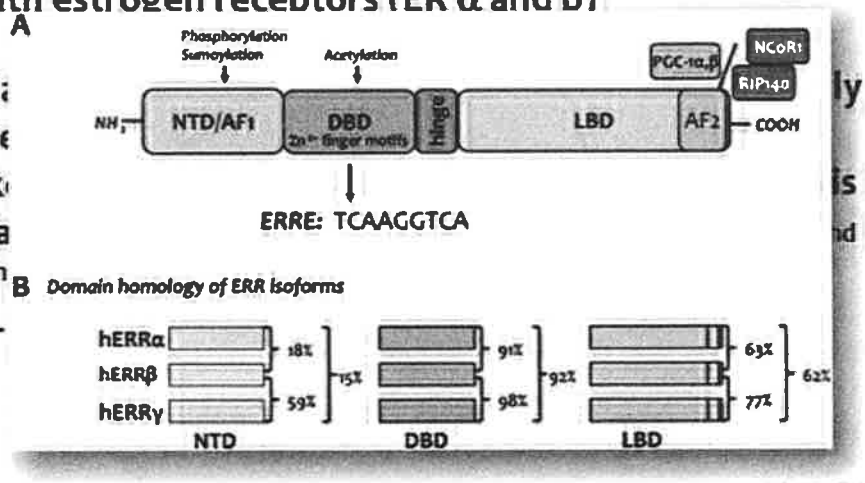
Eun-Kyeong Jo, M.D., Ph.D.

Infection Signaling Network Research Center
Chungnam National University School of Medicine,
Korea

Estrogen-related Receptor α (NR3B1)

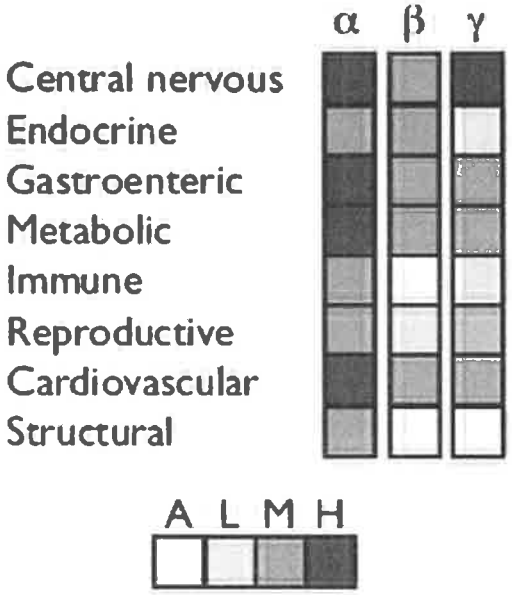
✓ The first orphan nuclear receptor to be identified (Giguere et al., 1988)
✓ Share characteristics with, but are distinct from, with estrogen receptors (ER α and β)

✓ Displ… identifie…
✓ Unlik… amenda… deprivation …



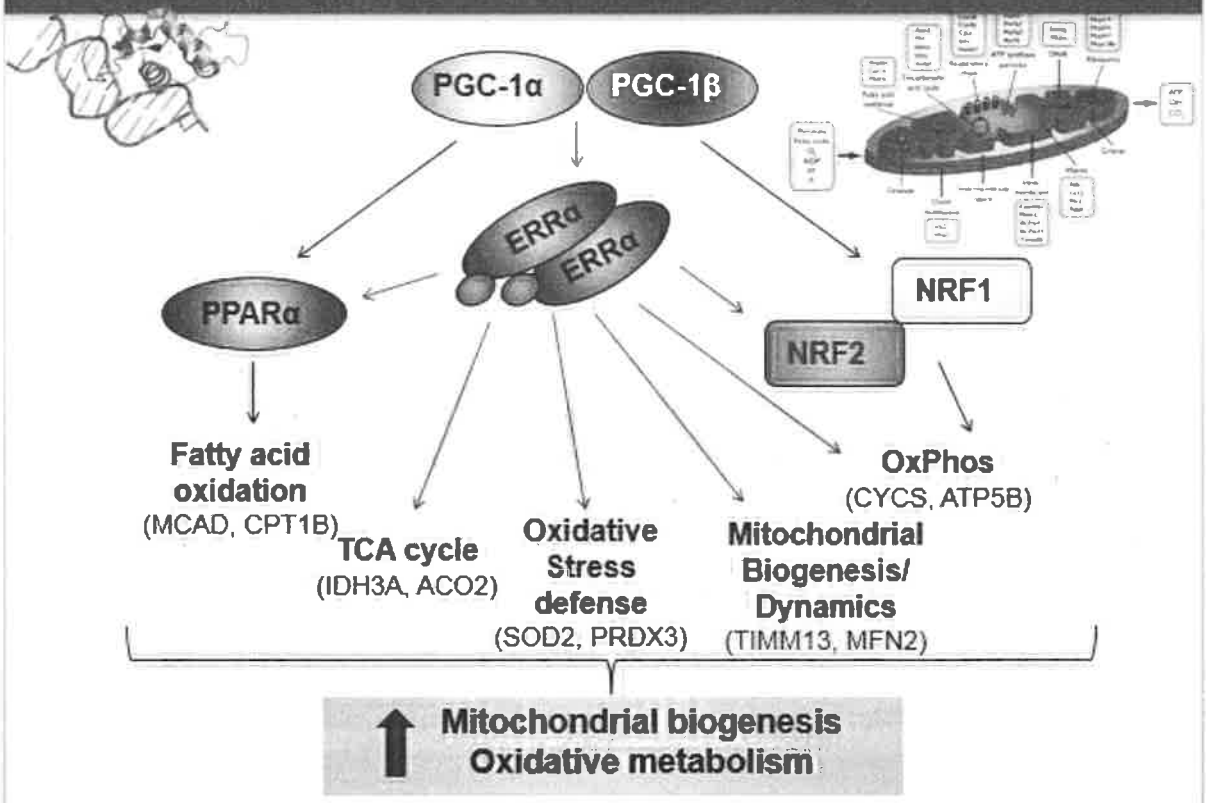
Huss et al., BBA, 2015

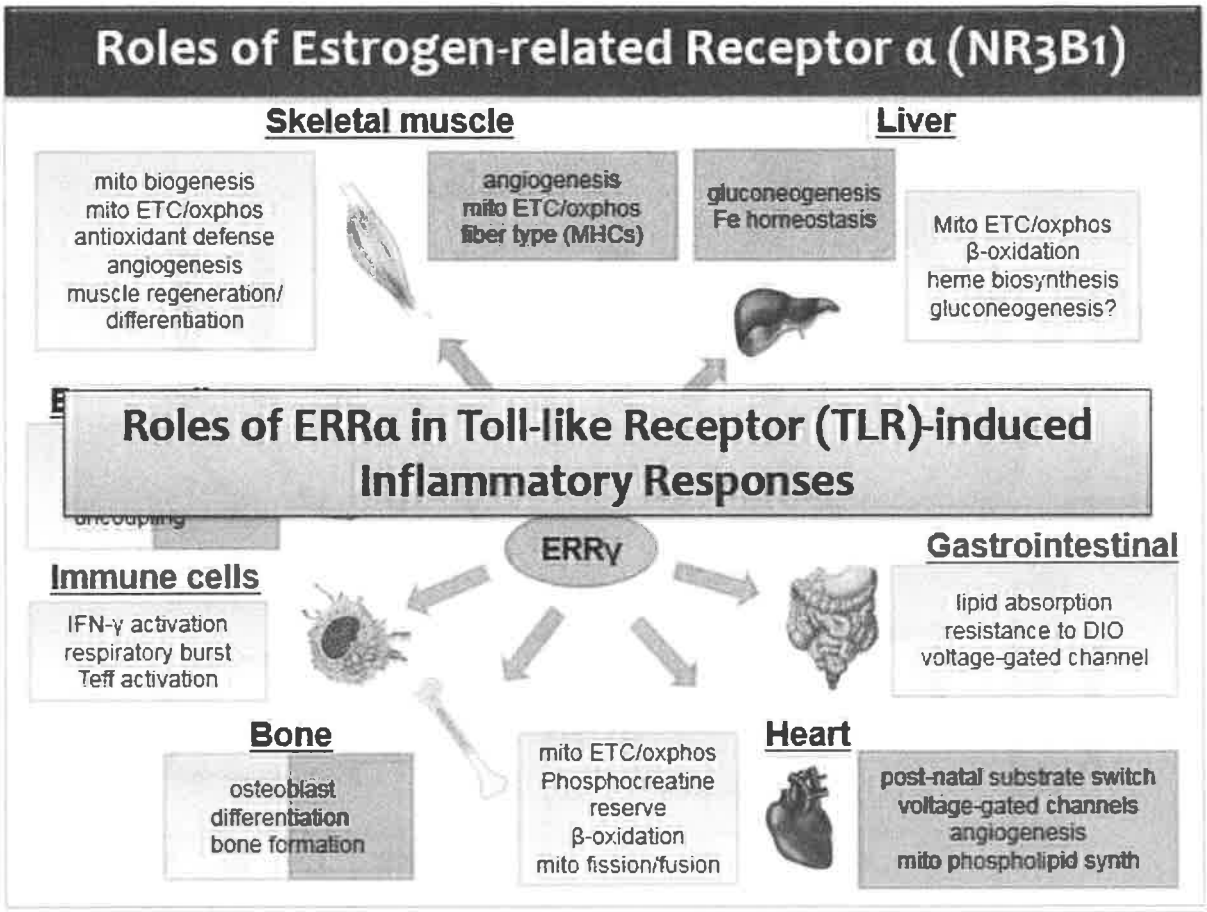
Distribution of ERR mRNA expression according to a functional grouping of tissues

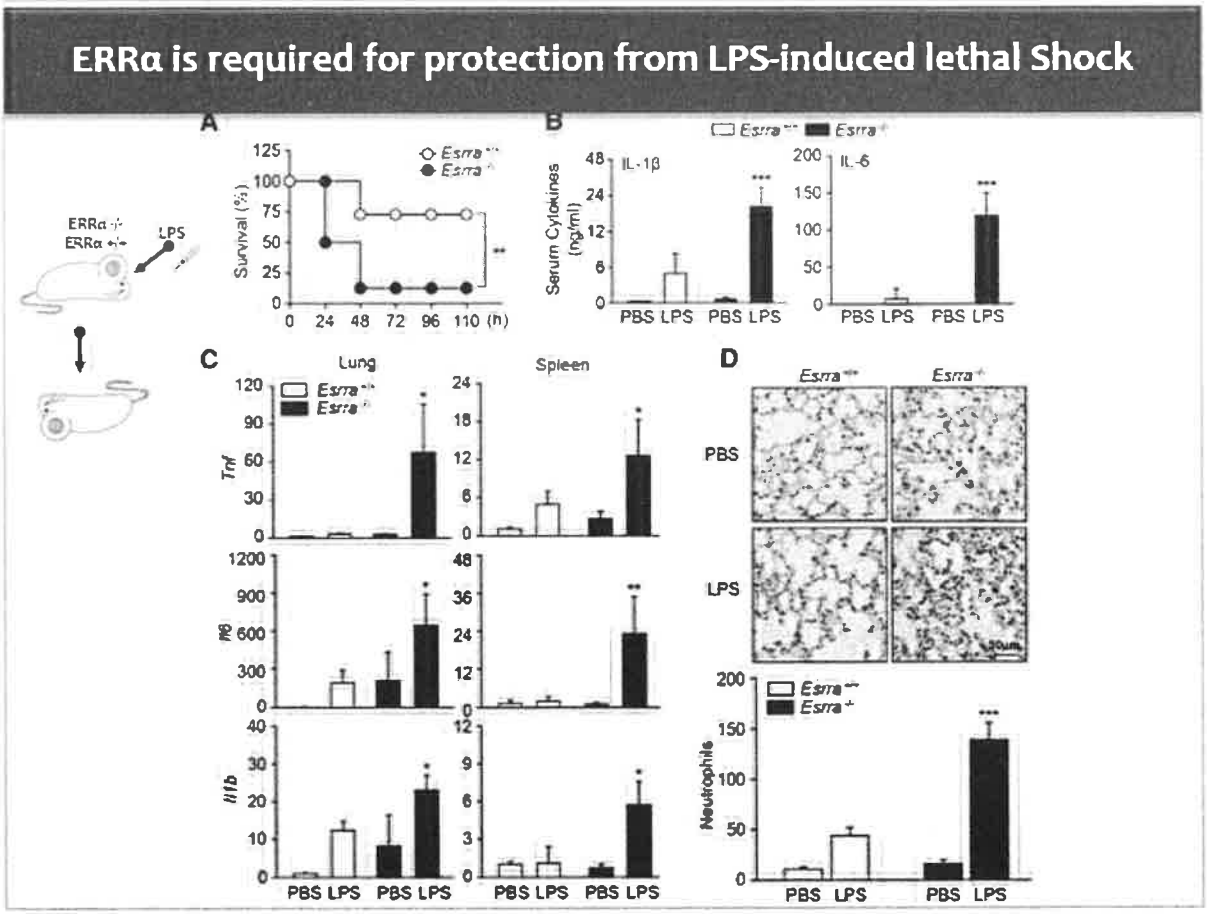


- Expressed in tissues in all major physiological systems (CNS, endocrine, metabolic, gastrointestinal, immune, reproductive, cardiovascular, respiratory and structural)

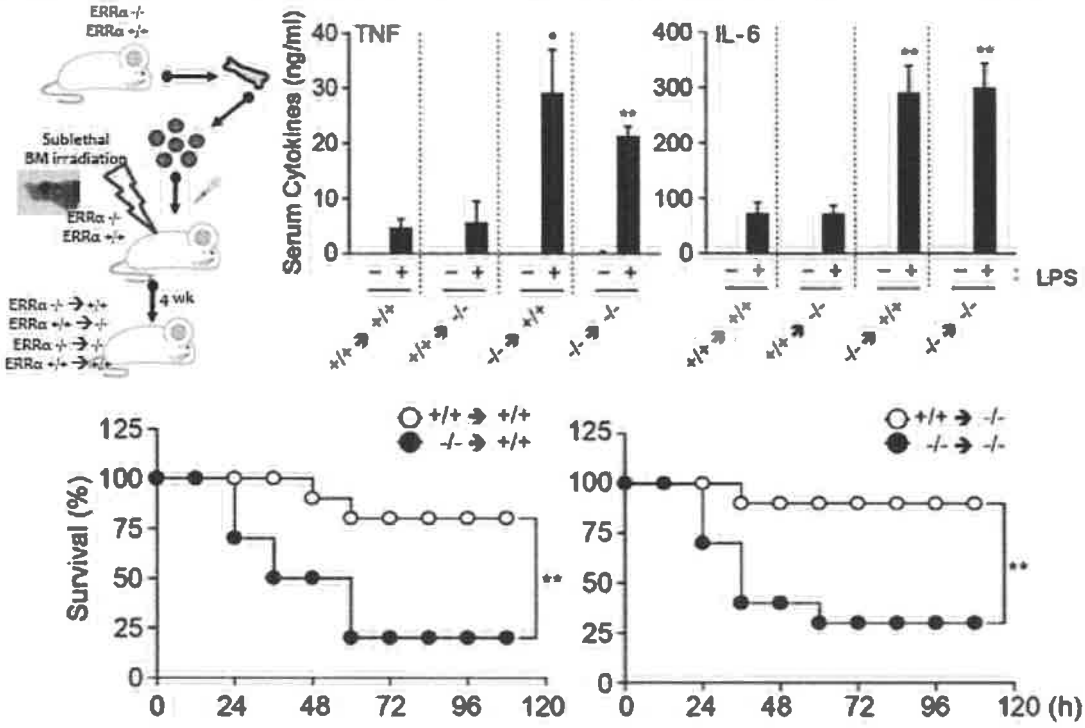
How ERRα is regulated?



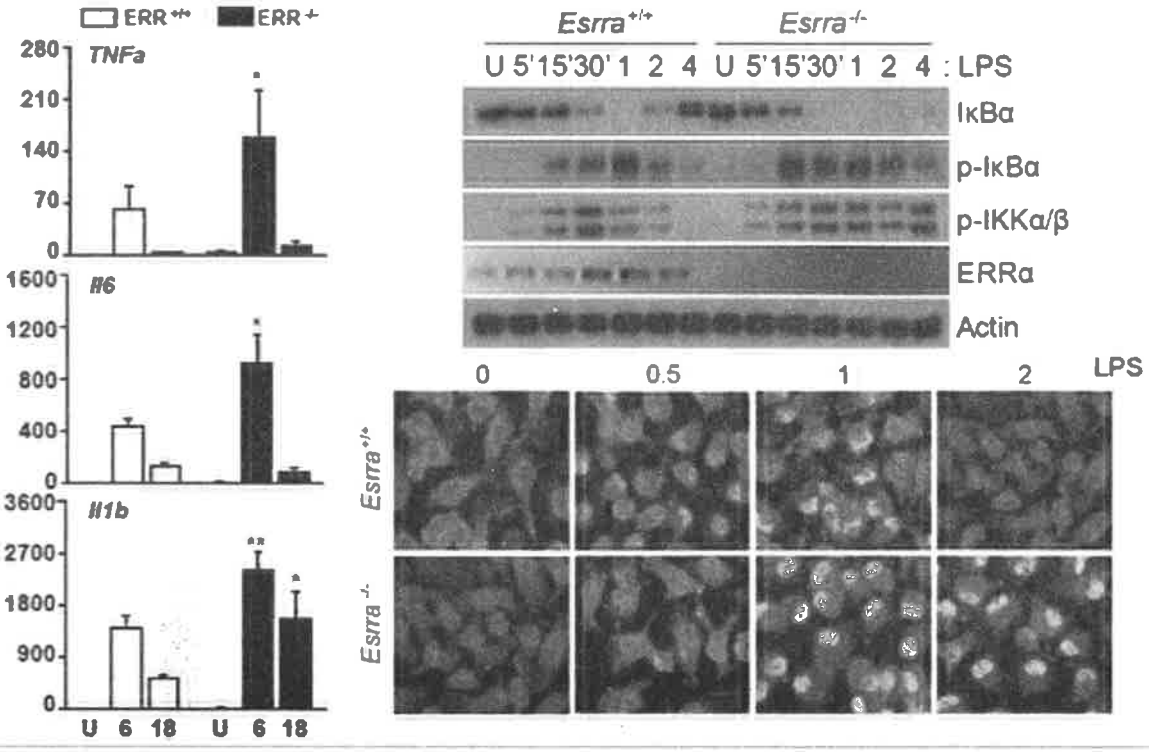

Roles of Estrogen-related Receptor α (NR3B1)
Skeletal muscle
mito biogenesis
mito ETC/oxphos
antioxidant defense
angiogenesis
muscle regeneration/
differentiation
angiogenesis
mito ETC/oxphos
fiber type (MHCs)
Liver
gluconeogenesis
Fe homeostasis
Mito ETC/oxphos
β-oxidation
heme biosynthesis
gluconeogenesis?
Roles of ERRα in Toll-like Receptor (TLR)-induced Inflammatory Responses
ERRγ
Immune cells
IFN-γ activation
respiratory burst
Teff activation
Gastrointestinal
lipid absorption
resistance to DIO
voltage-gated channel
Bone
osteoblast
differentiation
bone formation
mito ETC/oxphos
Phosphocreatine
reserve
β-oxidation
mito fission/fusion
Heart
post-natal substrate switch
voltage-gated channels
angiogenesis
mito phospholipid synth


ERRα is required for protection from LPS-induced lethal Shock
A
B
C
D
LPS
Survival (%)
Serum Cytokines (ng/ml)
IL-1β
IL-6
Lung
Spleen
Tnf
Il6
Il1b
Neutrophils
PBS
LPS
PBS LPS PBS LPS

Bone marrow-derived ERRα contributes to LPS-induced septic shock and inflammation

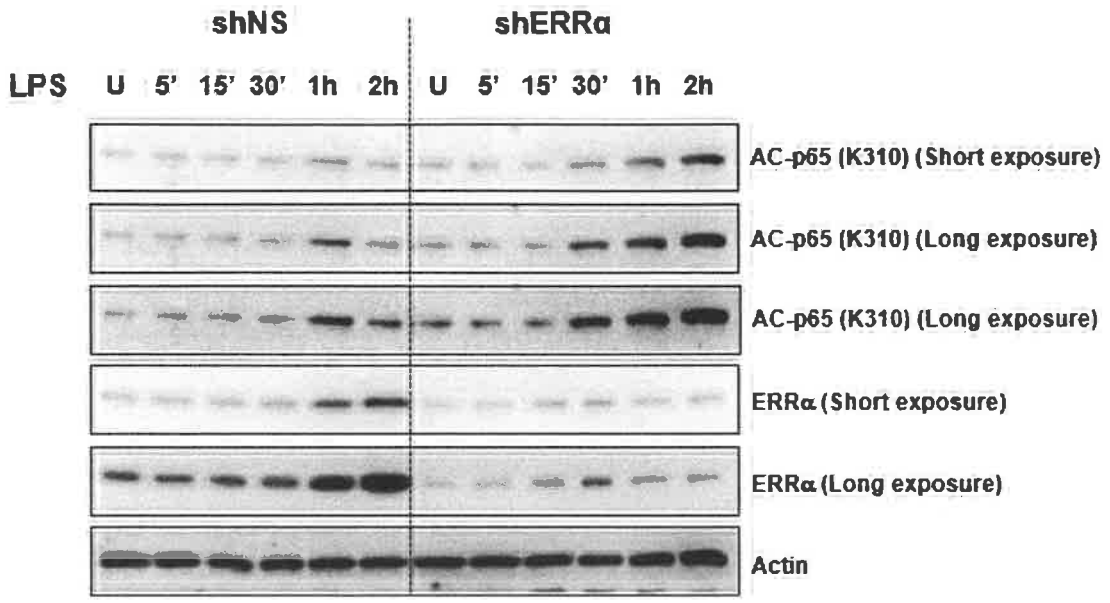


ERRα attenuates TLR4-induced inflammatory responses by regulation of NF-κB signaling in macrophages

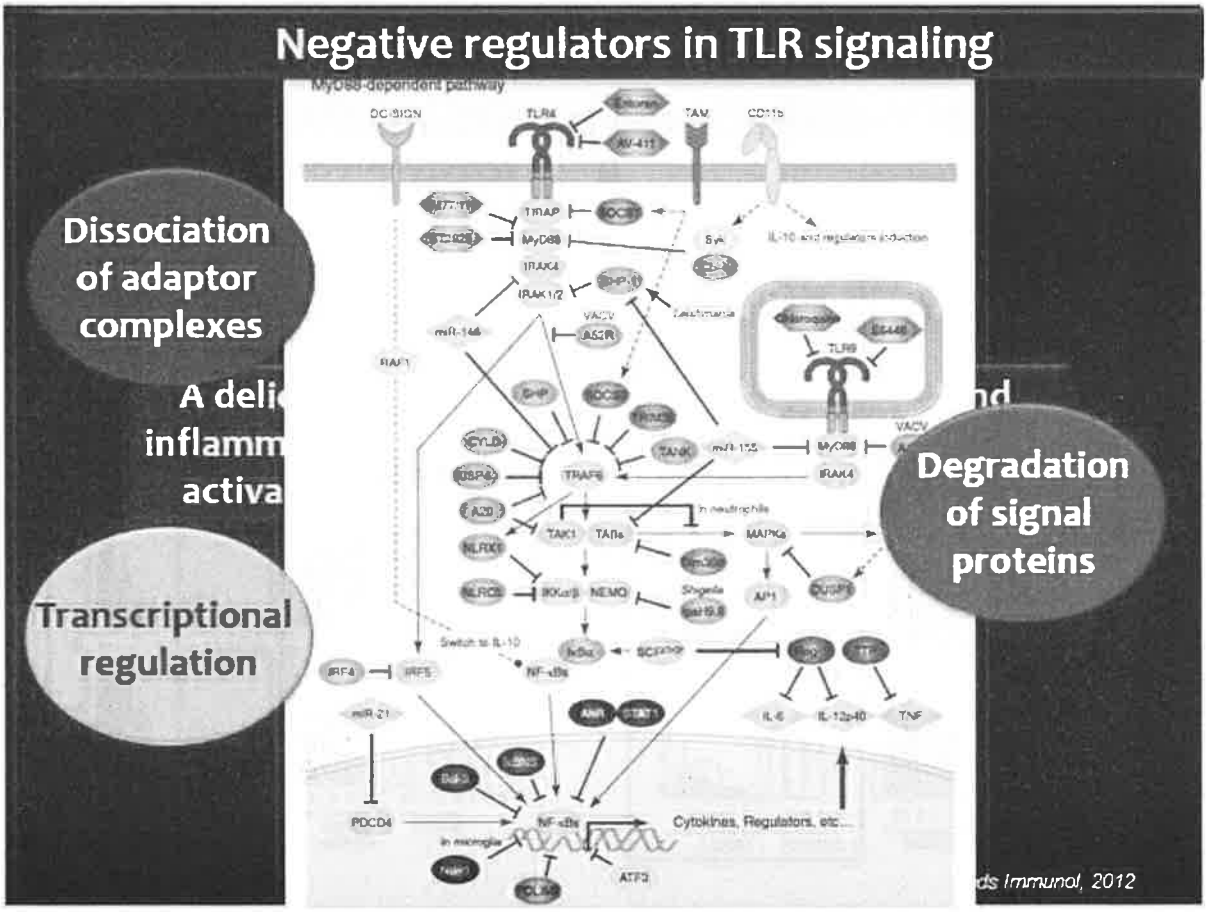


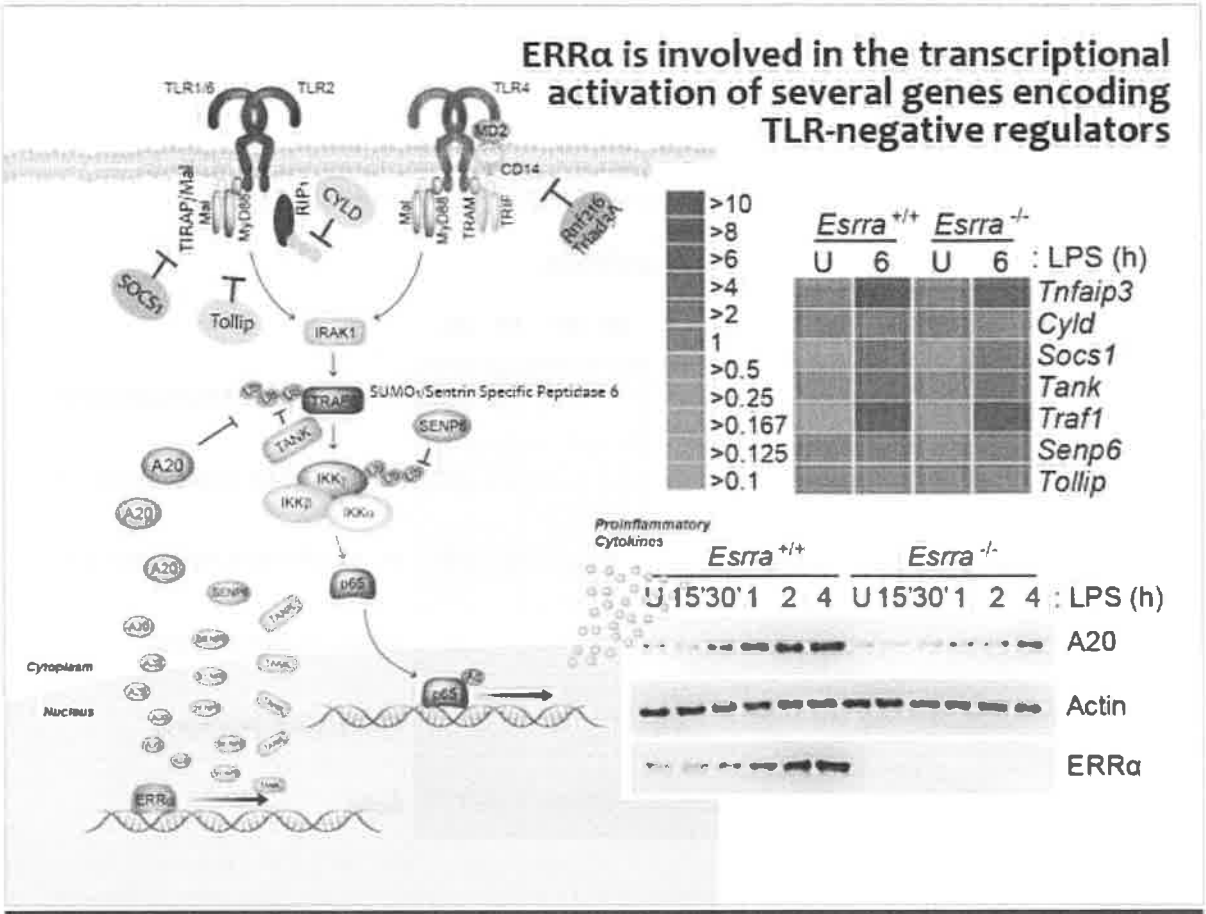
LPS stimulation results in increased acetylation of NF-κB p65 in *Esrra*$^{-/-}$ macrophages

Posttranslational modification of NF-κB is crucial for enhancement of DNA-binding activity of NF-κB p65 and gene activation of pro-inflammatory mediators (Huang et al., 2010; Perkins, 2006).

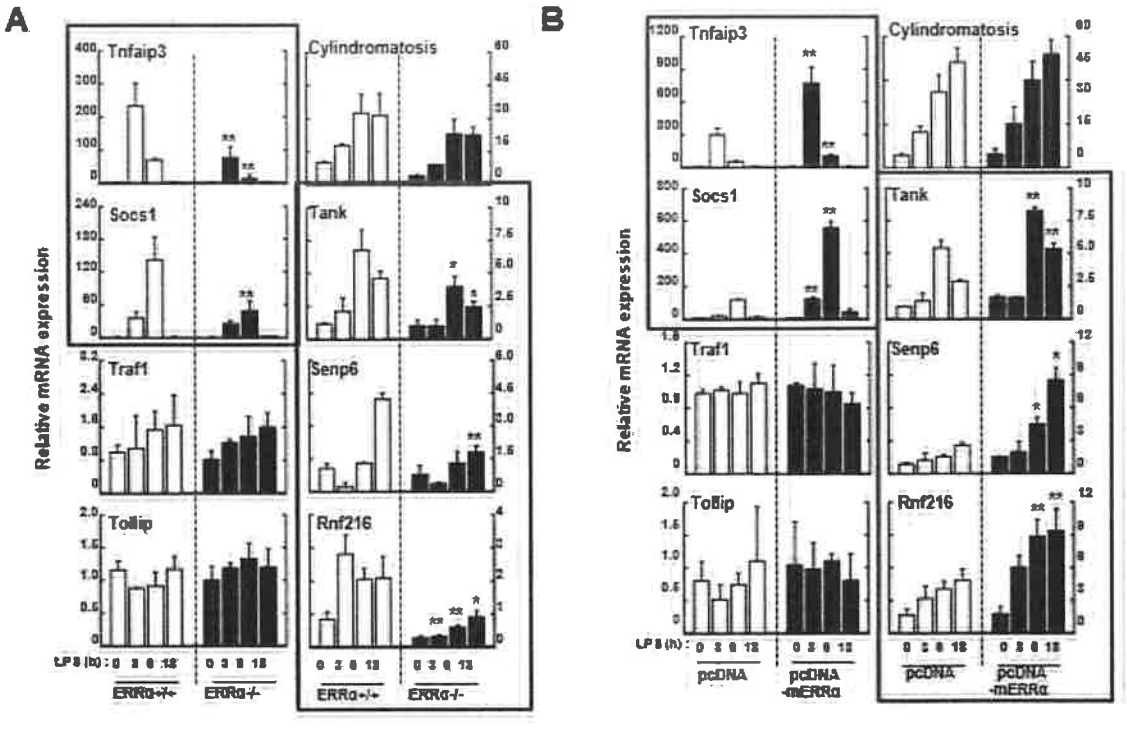


Negative regulators in TLR signaling

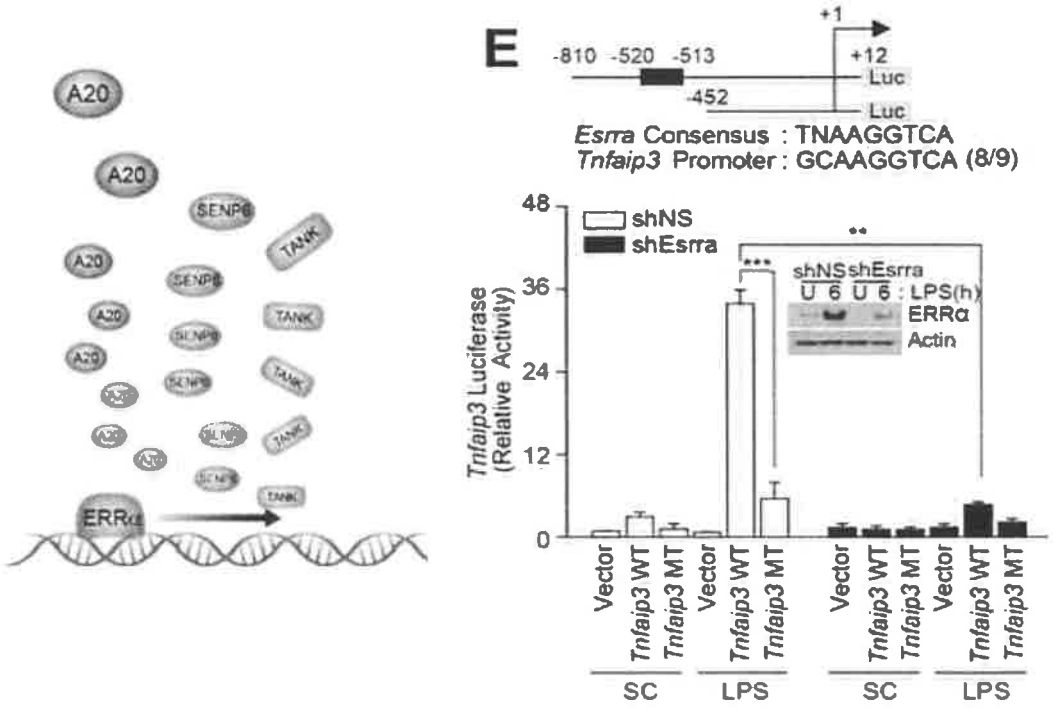




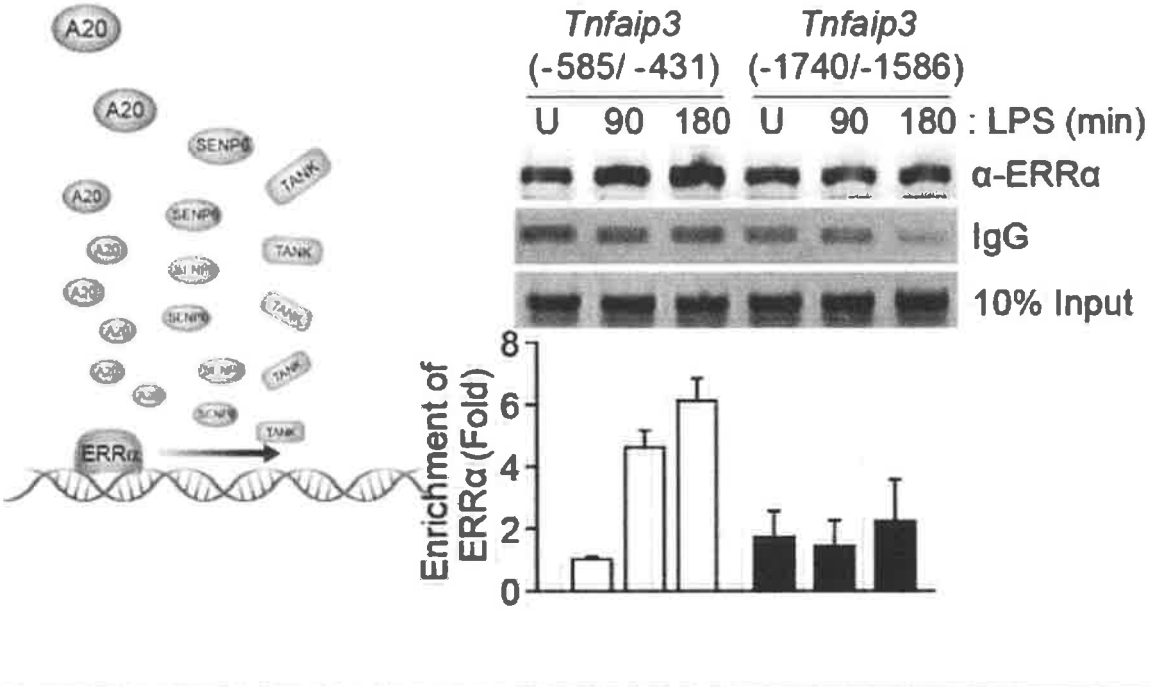
ERRα is involved in the transcriptional activation of genes encoding TLR-negative regulators including A20 (*Tnfaip3*)



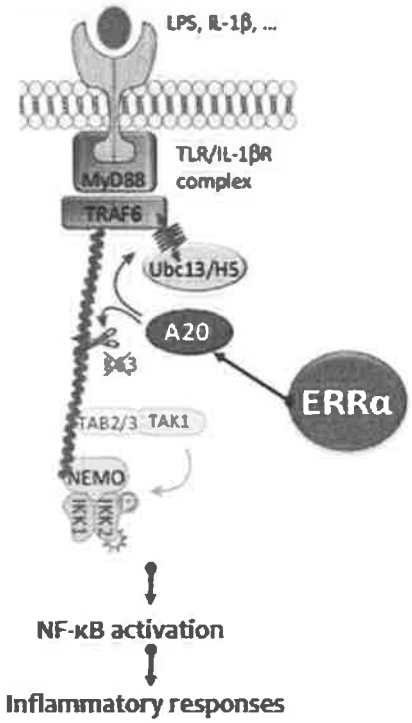
ERRα enhances A20 promoter activity and recruits to the A20 promoter region (-520/-513) in response to LPS

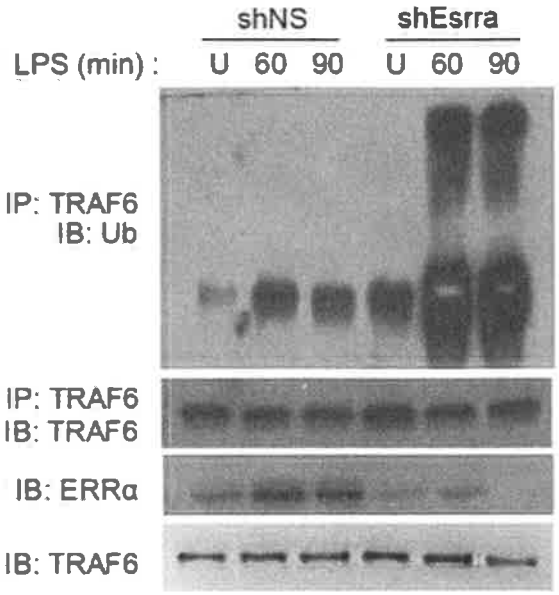


ERRα recruits to the A20 promoter region (-520/-513) in response to LPS

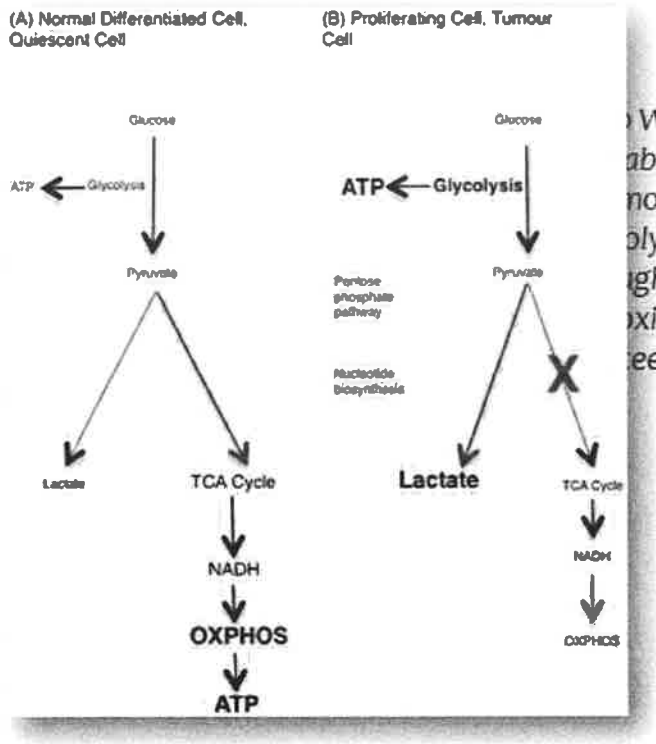


ERRα inhibits TRAF6 ubiquitination in response to LPS



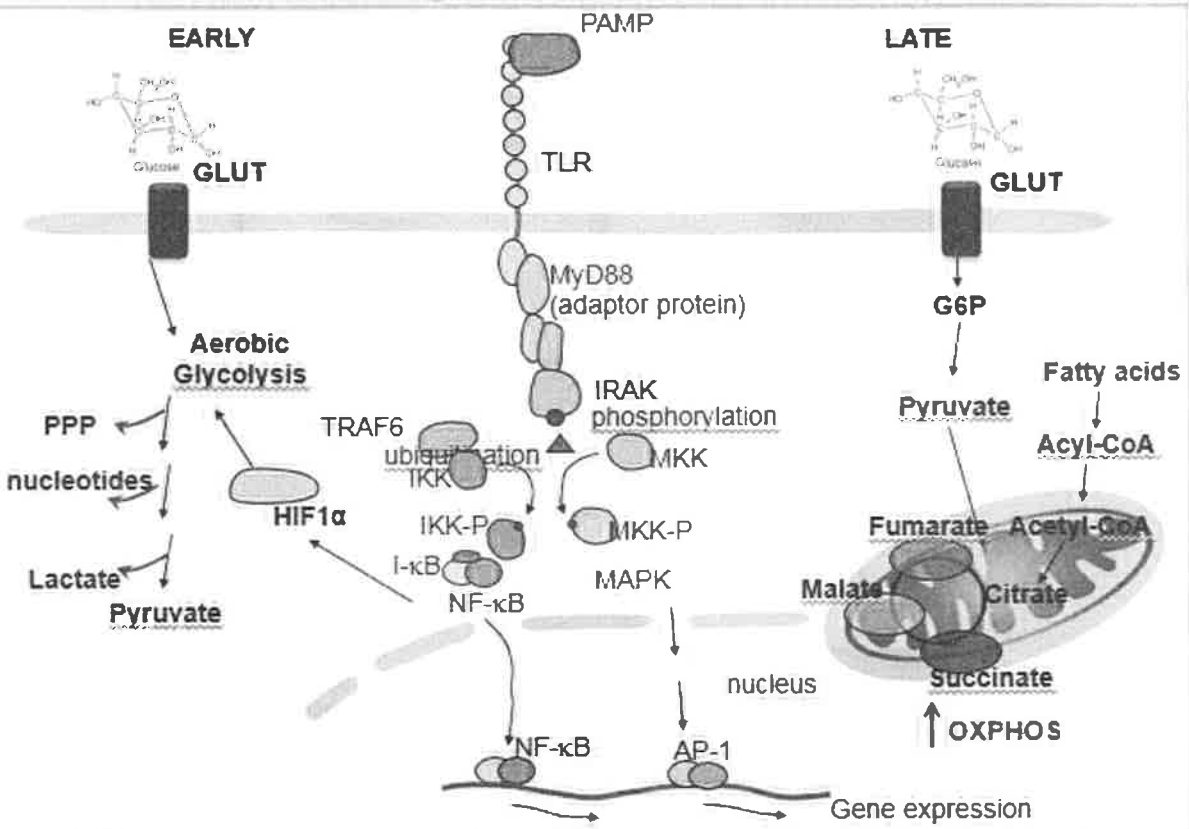


Warburg effect



Warburg described a abolic profile of tumors in noxic conditions, in which olysis predominates even gh there is oxygen available xidative metabolism to eed

Metabolic changes in innate immune responses



Metabolic reprogramming in innate immune responses

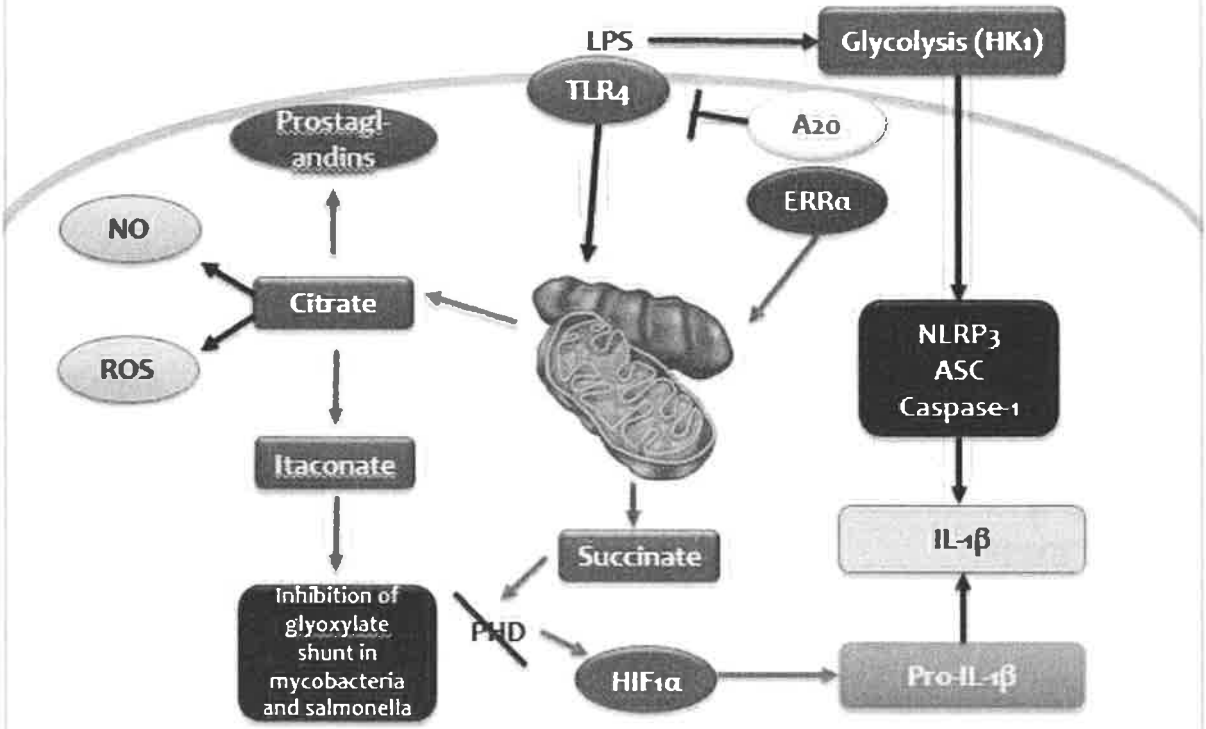
- In response to pathogenic or dangerous stimuli, immune cells undergo metabolic reprogramming that shapes the innate immune responses to invading pathogens or tissue damage (Kelly and O'Neill, Cell Res 2015; 25:771-784; Annu Rev Immunol. 2014, 32:609-634; Cell Metab. 2013, 17:895-900)

- During inflammation, the early phase responses require glycolysis, whereas fatty acid oxidation, via NAD^+-dependent processes, plays a more dominant role in the later phases (Nat Immunol. 2014, 15:323-332; J Biol Chem. 2012, 287:25758-25769; Liu et al., J Biol Chem. 2011, 286:9856-9864)

- In M1 macrophages, the metabolic shift increases glycolytic flux, and production of key M1 products such as acetyl CoA, succinate, and nitric oxide. M2 polarization activates glutamine catabolism and UDP-GlcNAc-associated modules; M1 macrophages have TCA cycle fragmentation, through a metabolic break at Idh (Tannahill et al., Nature., 2013; Jha et al., Immunity, 2015)

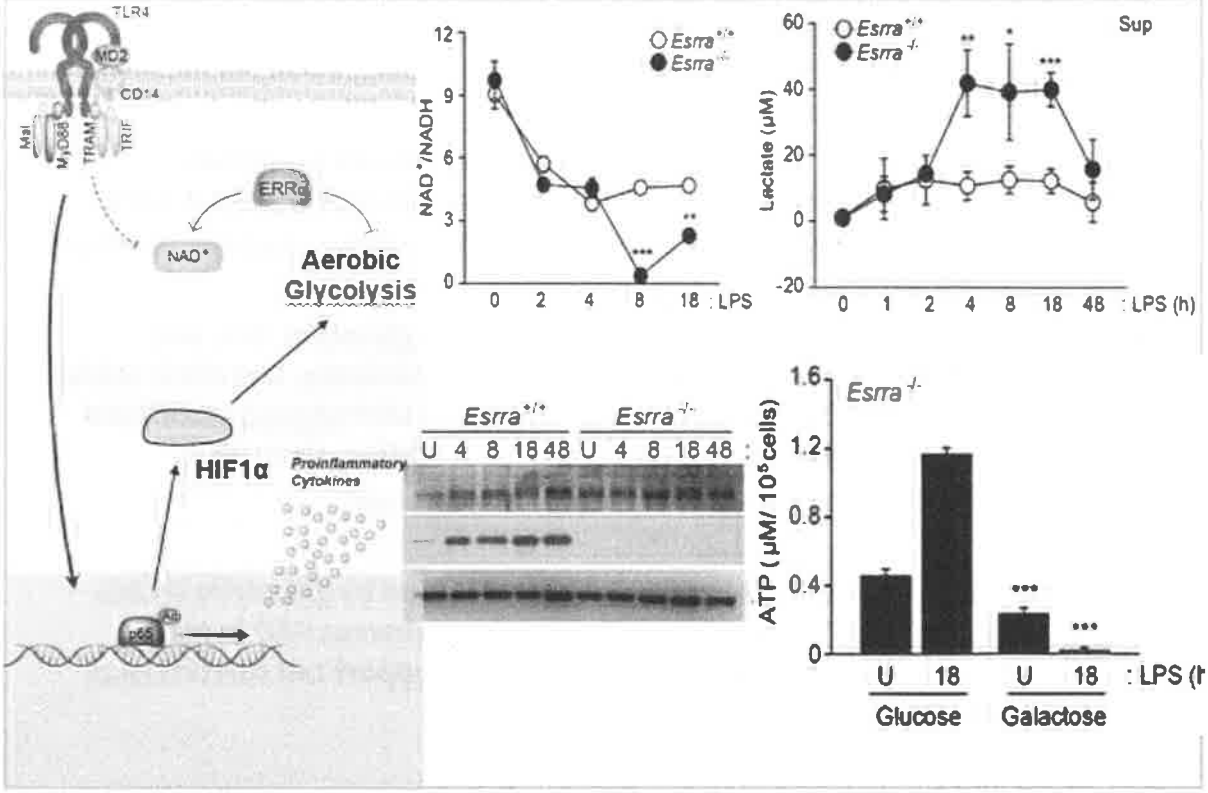
- In early phase, rapid, short-term bursts of activation by glycolytic switch are required at sites of infection or inflammation, whereas FAO in M2 macrophages may be better able to energetically support cell survival (O'Neill and Pearce., J Exp Med, 2016).

Immunometabolism governs innate immune function

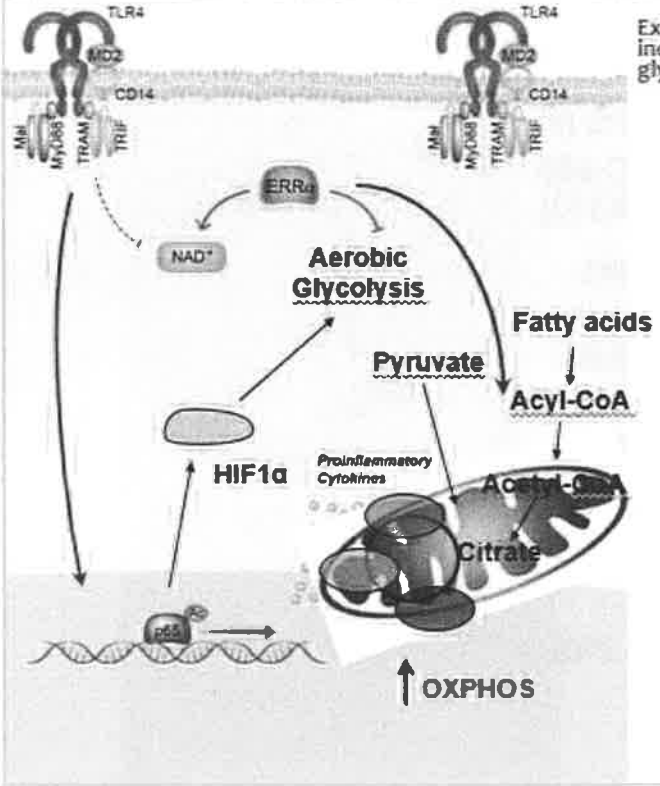


Modified from O'Neill and Pearce, J Exp Med 2016

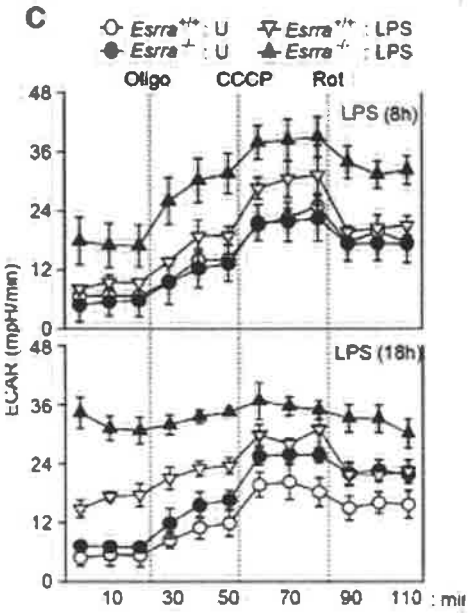
ERRα Regulates TLR4-Mediated NAD levels and Glycolytic Metabolism



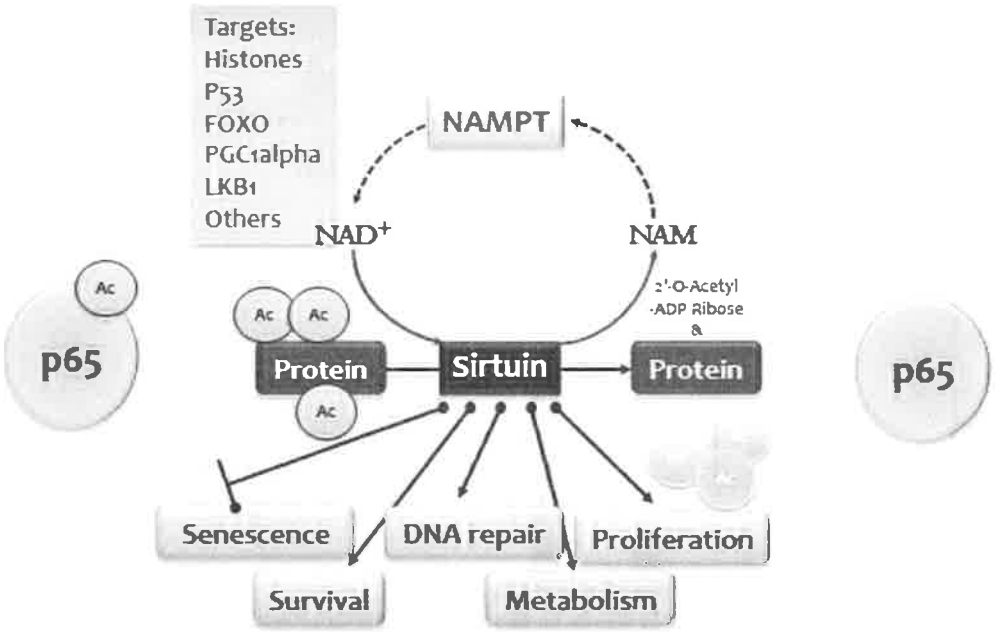
ERRα regulates TLR4-mediated glycolytic metabolism and enhances mitochondrial respiration



Extracellular acidification rate (ECAR), an indirect indicator of lactate production and enhanced glycolytic metabolism

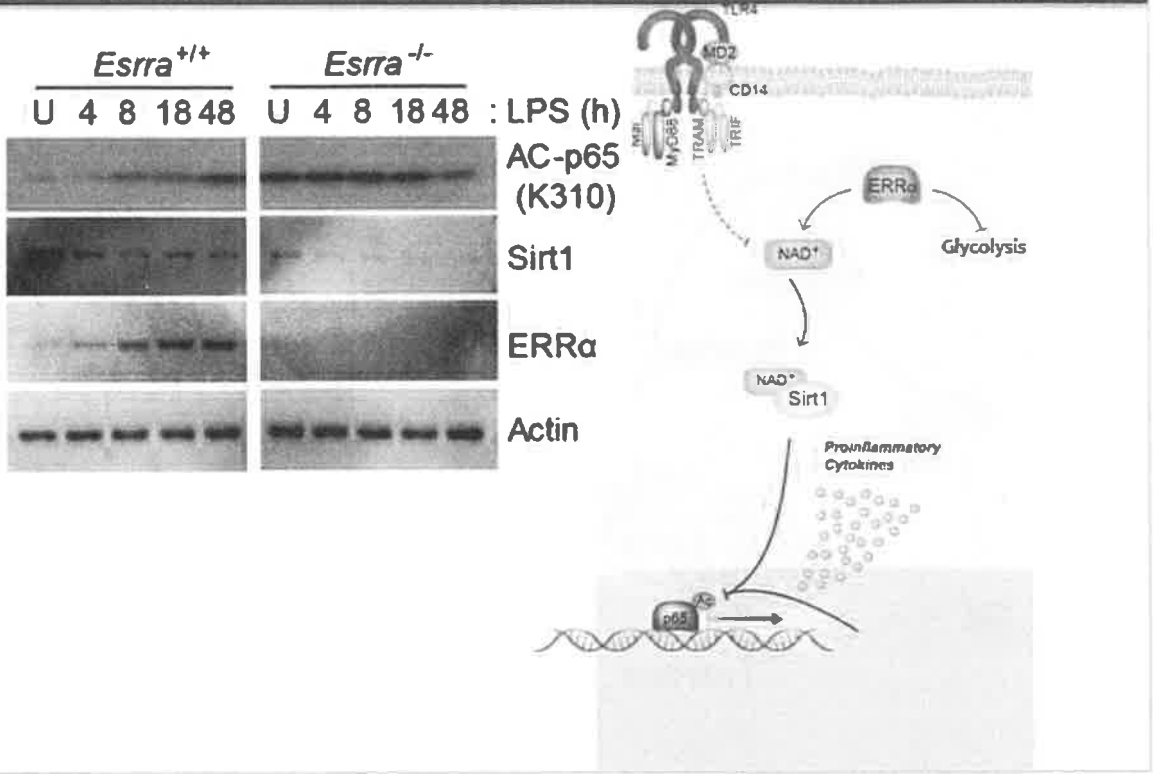


Sirtuin 1 (SIRT1), an NAD+-dependent deacetylase

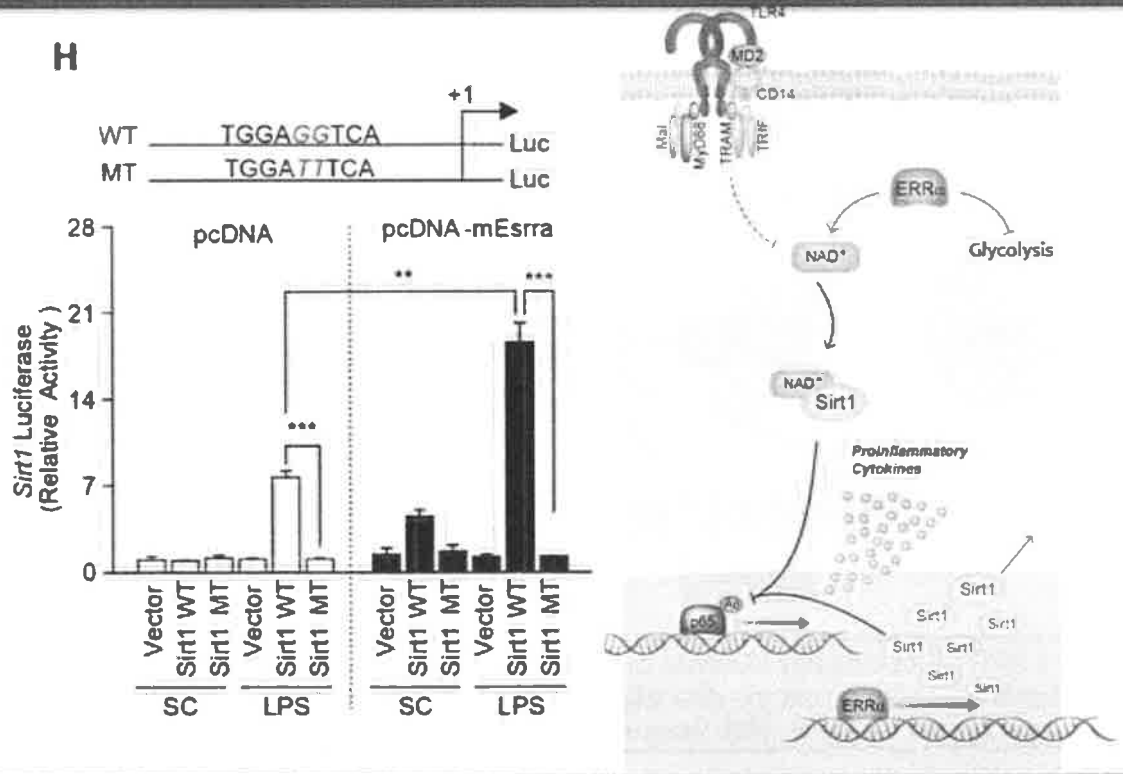


Sirtuin 1 (SIRT1) is critically involved in the regulation of NF-κB-mediated inflammatory responses via the deacetylation of p65/RelA on lysine 310 (Chen et al., 2005; Yang et al., 2012; Yang et al., 2006; Yeung et al., 2004).

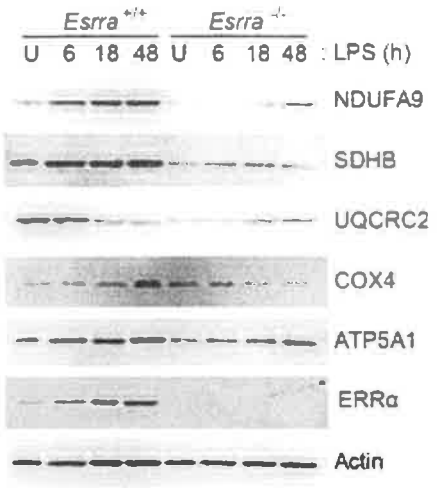
ERRα Regulates TLR4-Mediated NF-kB p65 Acetylation and Sirtuin 1 Expression in Macrophages

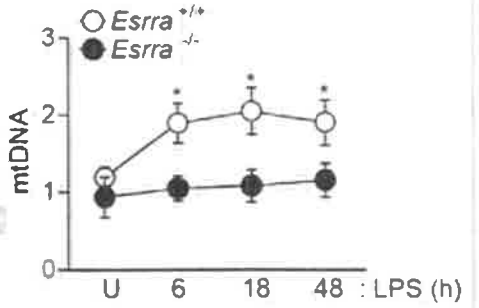


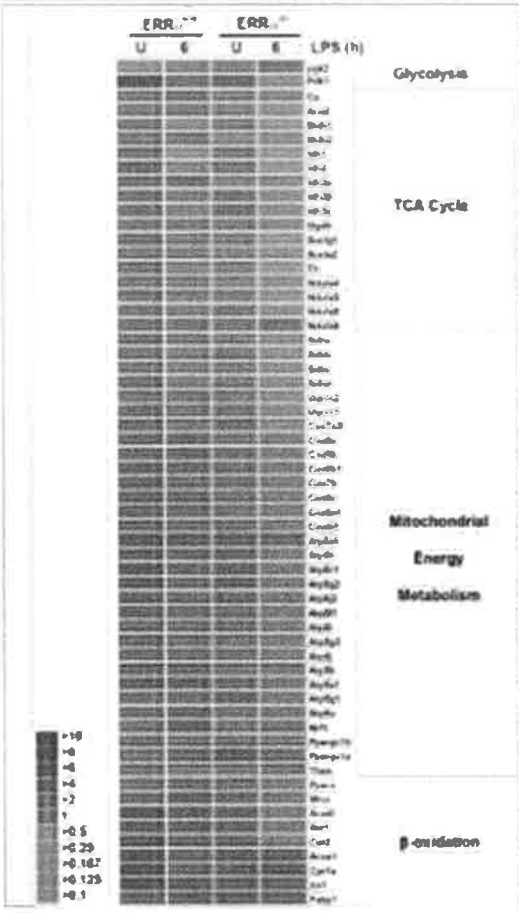
ERRα Regulates TLR4-mediated Sirtuin 1 Gene Expression in Macrophages in Response to LPS Stimulation



ERRα Deficiency Impairs Mitochondrial Respiration and Biogenesis during TLR4 Signaling

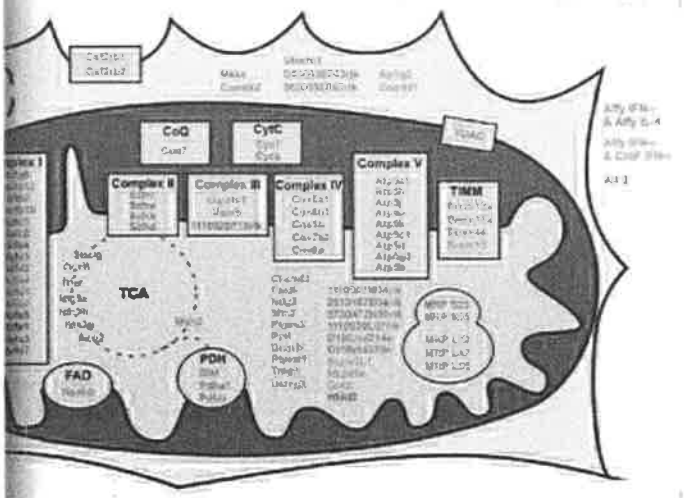


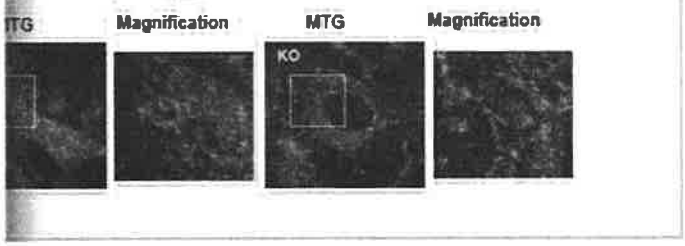




ochondrial Respiration and Biogenesis TLR4 Signaling

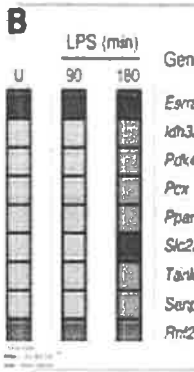
Sonoda et al., Genes Dev 2007; 21: 1909



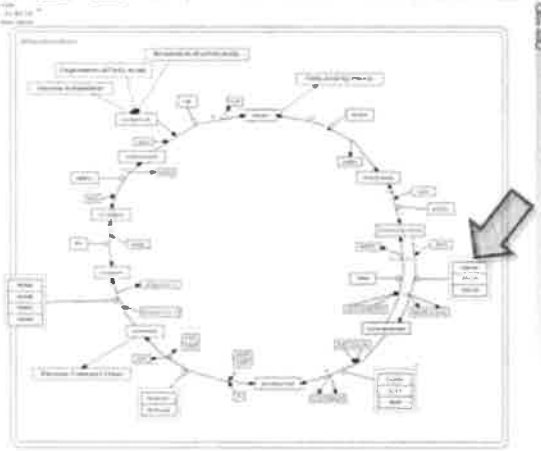


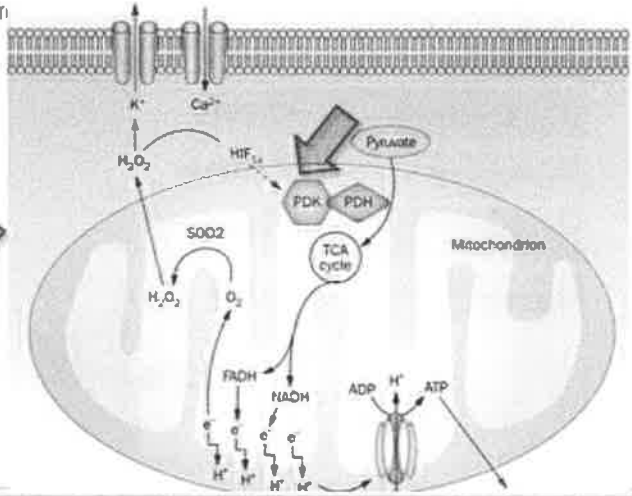
Standard ChIP of promoter and enhancer elements confirms direct regulation of metabolic genes by ERRα in macrophages

B



Gene	Distance to TSS	ERRE	Function	Expression in BMDM
Esrra	-528/-551	TCAAGGTCA/TGAAGGTCA	Transcription	↑ with LPS
Idh3a	+750/+825	TCAAGGTCA/CCAAGGTCA	TCA cycle	↓ in ERRα KO
Pdk4	-360	ACAAGGACA	Inhibits conversion of pyruvate to acetyl-CoA	↓ with LPS in WT but ↑ with LPS in ERRα KO
Pcx	+3547	TCAAGGTCA	Conversion of pyruvate to oxaloacetate	Greater ↑ with LPS in WT vs ERRα KO
Ppargc1b	-16318	TCAAGGTCA	Transcription	Greater ↑ with LPS in WT vs ERRα KO
Slc2a1	+7372	TGAAGGTCA	Glucose transporter	Greater LPS induction in ERRα KO vs WT
Tank	+8429	TCAAGGTCA	Negative regulator of TLR signaling	Greater ↑ with LPS in WT vs ERRα KO
Serpb6	-34524	GGAAGGTCA		
Rnf216	-14111/-14099	ACAAGGTCA/ACAAGGTI		

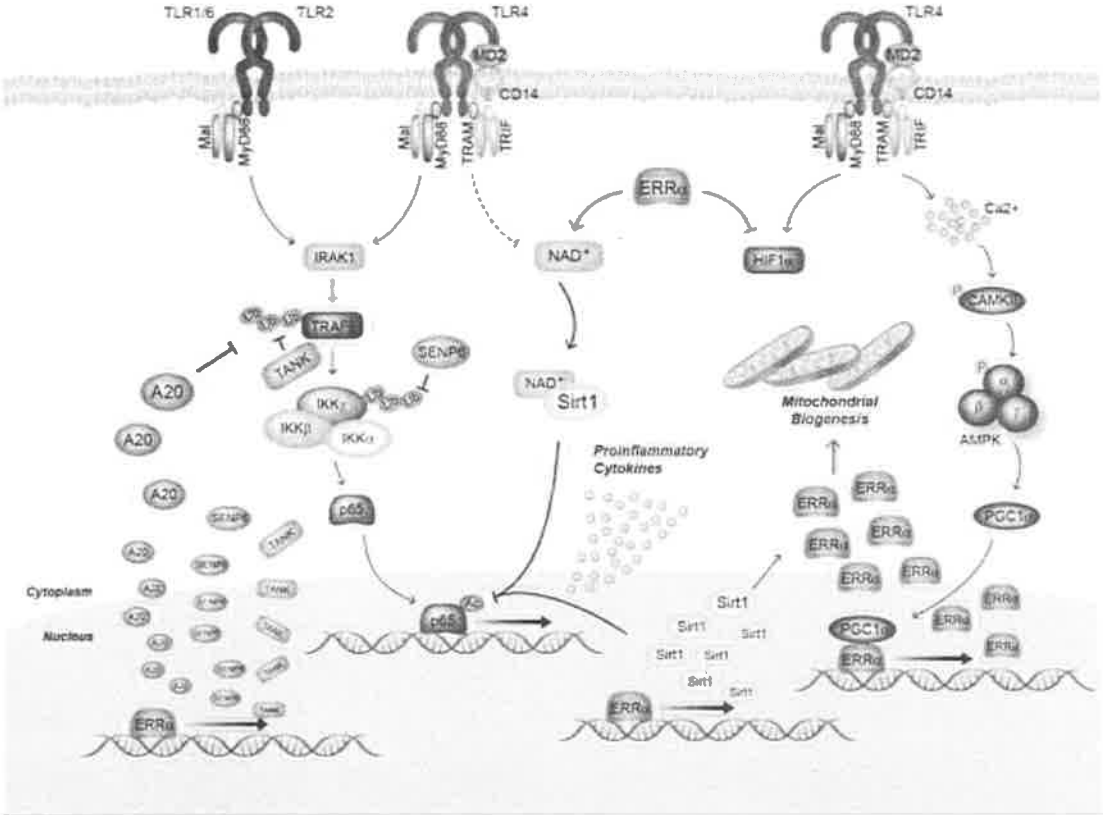




Summary

- ERRα-deficient (ERRα$^{-/-}$) mice showed increased susceptibility to endotoxin-induced septic shock, leading to more severe pro-inflammatory responses than control mice.
- ERRα regulated macrophage inflammatory responses by directly binding the promoter region of Tnfaip3, a deubiquitinating enzyme in TLR signaling
- In addition, ERRα$^{-/-}$ macrophages showed an increased glycolysis, but impaired mitochondrial respiratory function and biogenesis
- Further, ERRα was required for the regulation of NF-κB signaling by controlling p65 acetylation via maintenance of NAD^+ levels and sirtuin 1 activation.
- These findings unravel a previously unappreciated role for ERRα as a negative regulator of TLR-induced inflammatory responses through inducing Tnfaip3 transcription and controlling the metabolic reprogramming.

ERRα negatively regulates Toll-like receptor (TLR)-induced inflammation by promoting Tnfaip3 transcription and fine-tuning of metabolic reprogramming in macrophages



Probing contributions of macrophages to organismal homeostasis

Steffen Jung

Weizmann Institute of Science, Israel

Macrophages are myeloid immune cells that are strategically positioned throughout the organism. As professional phagocytes, they ingest and degrade debris and foreign material, including pathogens, and orchestrate inflammatory processes. Macrophages can be generated from two sources: an early transient hematogenic wave commencing in the yolk sac and a pathway involving hematopoietic stem cells, that persists throughout adult life. Most tissue macrophage compartments are established prenatally, and develop independent from each other in their respective host tissue under the influence of the local microenvironment (Amit et al., 2016; Lavin et al., 2014; Varol et al., 2015). Recent studies revealed critical contributions of tissue macrophages to organ development and homeostasis. Organismal homeostasis is critical for health, establishing the dynamic equilibrium that preserves life by resisting outside forces. Specific contributions of macrophages to homeostasis maintenance remain for most tissues however incompletely defined.

Here I will report on our recent efforts to employ conditional macrophage mutagenesis (Yona et al., 2013) to investigate contributions of these cells to health and disease. Here we used constitutive and inducible mutagenesis to delete the nuclear transcription regulator methyl-CpG binding protein 2 (Mecp2) in defined tissue macrophages. Animals lacking the Rett syndrome-associated gene in macrophages did not show signs of a neurodevelopmental disorder, but displayed spontaneous obesity, which we could link to impaired brown adipose tissue (BAT) function. Specifically, mutagenesis of a BAT-resident Cx_3cr1^+ macrophage subpopulation compromised homeostatic, though not acute cold-induced thermogenesis. Mechanistically, BAT malfunction of pre-obese mice harboring mutant macrophages was associated with decreased sympathetic innervation and local norepinephrine titers, resulting in reduced adipocyte expression of two key thermogenic factors Ucp1 and Dio2. Using a 'ribotag approach' to retrieve translatomes, we show that *Mecp2*-mutant BAT macrophages over-expressed PlexinA4, a receptor known to respond to axon guidance cues and serve as ligand repelling Sema6A-expressing sympathetic axons. Collectively, we provide evidence for a unique role of macrophages in maintaining the sympathetic innervation of brown adipose fat tissue that is critical for balanced homeostatic energy expenditure in the adult (Wolf et al., 2017).

CURRICULUM VITAE

Steffen Jung, Ph.D

PhD, Professor

Head, Department of Immunology, Weizmann Institute of Science, Rehovot 76100, Israel

E-mail: s.jung@weizmann.ac.il

Personal Statement

I was born in Homburg/ Saar, Germany. After undergraduate studies at the University of Bonn, I moved to the Institute of Genetics in Cologne. In the Department of Immunology headed by Prof.

Klaus Rajewsky, I performed my PhD under the guidance of Prof. Andreas Radbruch. Specifically,

I used the then newly developed gene targeting approach to define cis-acting control elements driving non-coding 'sterile' transcripts in immunoglobulin class switch recombination. In 1993, I moved for post-doctoral training to Israel and joined the laboratory of Prof. Yinon Ben-Neriah at

the Lautenberg Center (Hebrew University, Jerusalem) studying transcription factors and kinases

in T cell signaling. In 1997, I went to New York for a post-doc in the laboratory of Prof. Dan Littman at the Skirball Institute for Molecular Pathogenesis, NYU Medical Center. My studies there

focused on the then newly discovered chemokine receptor CX3CR1 and its membrane-tethered ligand CX3CL1/ fractalkine. I generated CX3CR1gfp mice that became as reporter strain instrumental to define murine monocyte subsets and study brain microglia. Furthermore, I developed in collaboration with Prof. Richard Lang at the Skirball Institute a novel diphtheria toxin

receptor-based cell ablation strategy and a mouse model that allowed the study of dendritic cells

(DC) in their in vivo context by their conditional ablation (CD11c-DTR mice). In 2002, I returned to Israel and joined the faculty of the Department of Immunology at the Weizmann Institute, where

I received tenure in 2009 and full professorship in 2015. Current work of the Jung lab aims at

elucidating in vivo aspects of mononuclear phagocytes, including the definition of developmental pathways and differential functions of monocytes, DC and macrophages. Specifically, the team applies intra-vital imaging, conditional cell and gene ablation and precursor graft-mediated reconstitution, combined with advanced genomic analysis to investigate the biology of these cells in physiological context in health and disease. Recent work of the Jung laboratory focuses on the study of monocyte-derived intestinal macrophages, embryonic-derived brain microglia and lymph node DC, as well as the role of macrophages in metabolic disorders.

Academic Appointments

2002-09	Senior Scientist, Weizmann Institute of Science, Dpt. of Immunology
2009-15	Associate Professor, Weizmann Institute of Science, Dpt. of Immunology
2015-present	Full Professor, Weizmann Institute of Science Korea,
2017-present	Head, Department of Immunology

Awards and Honors

1993-	Post-doctoral Fellowship of European Molecular Biology Organization
1995-	Post-doctoral Fellowship of MINERVA Society
1997-	Associate of Howard Hughes Medical Institute
1999-	Special Fellow Award of Leukemia & Lymphoma Society
2002-	The Yigal Alon Scholarship ("Milgat Alon")
2002-	Scholar of the Benoziyo Center for Molecular Medicine.
2002-	Incumbent of the Pauline Recanati Career Development Chair

Contribution to Science

(1) During my PhD at the University of Cologne, Germany, I showed I was the first to provide direct evidence for the need of so-called 'sterile' transcripts to allow for the recombination of switch regions located upstream of C_H genes. Specifically, I used a Flp/FRT-based strategy to delete the promoter element driving transcription through the murine S 1 switch region and showed that the resulting mice had a deficiency in IgG1 production (Männ et al., 1993).

During my post-doctoral studies at the Skirball Institute for Molecular Pathogenesis, NYU medical Center, New York, US, I generated two novel mouse models that became critical tools for subsequent studies by myself and many other researchers.

(2) To study the physiological role of the CX_3CR1 chemokine receptor I generated CX_3CR1^{gfp} mice carrying a targeted insertion of a gene encoding green fluorescent protein in the CX_3CR1 locus (Jung et al., 2000). These mice were instrumental for our identification of murine Ly6C+

and Ly6- monocyte subsets (Geissmann et al., 2003), a seminal report that triggered subsequent efforts by many colleagues to investigate these intriguing blood cells and their contributions to inflammation and pathologies in the mouse. Moreover, through collaborative work we established the value of CX_3CR1^{gfp} mice for the back then emerging intra-vital imaging community, by demonstrating dynamics of intestinal macrophages (*Niess et al., 2005*) and brain microglia (*Davalos et al., 2005*).

(3) To probe for the role of dendritic cells in the initiation of in vivo T cell responses I employed, together with the group of Richard Lang, a novel conditional cell ablation strategy, that is based on rendering murine cells sensitive to diphtheria toxin (DT) by cell type-restricted expression of a primate DT receptor (DTR). These animals allowed me to corroborate the unrivaled potential of DC for the priming of naive T cells in intact animals, extending the seminal *in vitro* studies by Steinman and colleagues (*Jung et al., 2002*). CD11c-DTR mice and the DTR approach have become standard tools in modern immunological research.

Major contributions, since the establishment of my independent laboratory at the Weizmann Institute include

(4) Using a combination of cell ablation and adoptive monocyte transfers, we established that splenic classical DC derive from non-monocytic origin (*Varol et al., 2007*). Moreover, in the same study and a follow up (*Varol et al., 2009*), we showed that $Ly6C^+$ monocytes are precursors of intestinal macrophages residing in the lamina propria. Combined with the concomitant identification of precursor cells, such as MDPs (*Fogg et al., 2006*), our studies critically contributed to the realization that our current understanding of mononuclear phagocyte development.

(5) Taking advantage of the prominent expression of CX_3CR1 in monocytes and specific macrophage populations, we generated animals that harbor transgenes encoding conditional and inducible Cre recombinases under the CX_3CR1 promoter (*Yona et al., 2013*). CX_3CR1^{cre} and CX_3CR1^{creER} mice allow us and others to study functions of specific tissue macrophages, including intestinal, heart, adipose tissue and brain (*Goldmann et al., 2016; Molawi et al., 2014; Zigmond et al., 2014; Wolf et al., 2017*) Moreover, the animals enabled us to show, that most tissue macrophage compartments are established before birth and in the healthy adult organism largely maintained independent from monocyte input (*Yona et al., 2013*). Together with the work of others and our own recent transcriptome and epigenome profiling efforts (*Lavin et al., 2014*), this study contributed to a paradigm shift and a focus on differential functions of monocyte and embryo-derived tissue macrophages in health and pathology *(Amit et al., 2016; Ginhoux and Jung, 2014*).

Steffen Jung is an author on **159** peer-reviewed publications, consisting of **5 first-author, 29 senior-author** and **90 co-author papers**, and **32 reviews, book chapters and invited editorials**, consisting of **7 first-author, 20 senior-author** and **5 co-author publications**. His citation scores are: **H-index: 66** and **total citations (excluding self-citations): 14,768**.

References

Amit, I., Winter, D.R., and Jung, S. (2016). The role of the local environment and epigenetics in

shaping macrophage identity and their effect on tissue homeostasis. Nat Immunol *17*, 18–25.

Davalos, D., Grutzendler, J., Yang, G., Kim, J.V., Zuo, Y., Jung, S., Littman, D.R., Dustin, M.L., and Gan, W.-B. (2005). ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci *8*, 752–758.

Fogg, D.K., Sibon, C., Miled, C., Jung, S., Aucouturier, P., Littman, D.R., Cumano, A., and Geissmann, F. (2006). A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. Science *311*, 83–87.

Geissmann, F., Jung, S., and Littman, D.R. (2003). Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity *19*, 71–82.

Ginhoux, F., and Jung, S. (2014). Monocytes and macrophages: developmental pathways and tissue homeostasis. Nature Publishing Group *14*, 392–404.

Goldmann, T., Wieghofer, P., Jordao, M.J.C., Prutek, F., Hagemeyer, N., Frenzel, K., Amann, L., Staszewski, O., Kierdorf, K., Krueger, M., et al. (2016). Origin, fate and dynamics of macrophages at central nervous system interfaces. Nat Immunol *17*, 797–805.

Jung, S., Aliberti, J., Graemmel, P., Sunshine, M.J., Kreutzberg, G.W., Sher, A., and Littman, D.R. (2000). Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. Molecular and Cellular Biology *20*, 4106–4114.

Jung, S., Unutmaz, D., Wong, P., Sano, G.-I., De los Santos, K., Sparwasser, T., Wu, S., Vuthoori, S., Ko, K., Zavala, F., et al. (2002). In vivo depletion of CD11c+ dendritic cells abrogates priming of CD8+ T cells by exogenous cell-associated antigens. Immunity *17*, 211–220.

Lavin, Y., Winter, D., Blecher-Gonen, R., David, E., Keren-Shaul, H., Merad, M., Jung, S., and Amit, I. (2014). Tissue-Resident Macrophage Enhancer Landscapes Are Shaped by the Local Microenvironment. Cell *159*, 1312–1326.

Männ, L., Kochupurakkal, N., Martin, C., Verjans, E., Klingberg, A., Sody, S., Kraus, A., Dalimot, J., Bergmuller, E., Voortman, S., et al. (1993). Shutdown of class switch recombination by deletion of a switch region control element. Science *259*, 984–987.

Molawi, K., Wolf, Y., Kandalla, P.K., Favret, J., Hagemeyer, N., Frenzel, K., Pinto, A.R., Klapproth, K., Henri, S., Malissen, B., et al. (2014). Progressive replacement of embryo-derived cardiac macrophages with age. J. Exp. Med. *10*, 1538.

Niess, J.H., Brand, S., Gu, X., Landsman, L., Jung, S., McCormick, B.A., Vyas, J.M., Boes, M., Ploegh, H.L., Fox, J.G., et al. (2005). CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. Science *307*, 254–258.

Varol, C., Landsman, L., Fogg, D.K., Greenshtein, L., Gildor, B., Margalit, R., Kalchenko, V., Geissmann, F., and Jung, S. (2007). Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. J. Exp. Med. *204*, 171–180.

Varol, C., Vallon-Eberhard, A., Elinav, E., Aychek, T., Shapira, Y., Luche, H., Fehling, H.J., Hardt, W.-D., Shakhar, G., and Jung, S. (2009). Intestinal lamina propria dendritic cell subsets have different origin and functions. Immunity *31*, 502–512.

Wolf, Y., Boura-Halfon, S., Cortese, N., Haimon, Z., Shalom, H.S., Kuperman, Y., Kalchenko,

V., Brandis, A., David, E., Segal-Hayoun, Y., et al. (2017). Brown-adipose-tissue macrophages control tissue innervation and homeostatic energy expenditure. Nat Immunol *137*, 495.

Yona, S., Kim, K.-W., Wolf, Y., Mildner, A., Varol, D., Breker, M., Strauss-Ayali, D., Viukov, S., Guilliams, M., Misharin, A., et al. (2013). Fate Mapping Reveals Origins and Dynamics of Monocytes and Tissue Macrophages under Homeostasis. Immunity *38*, 79-91.

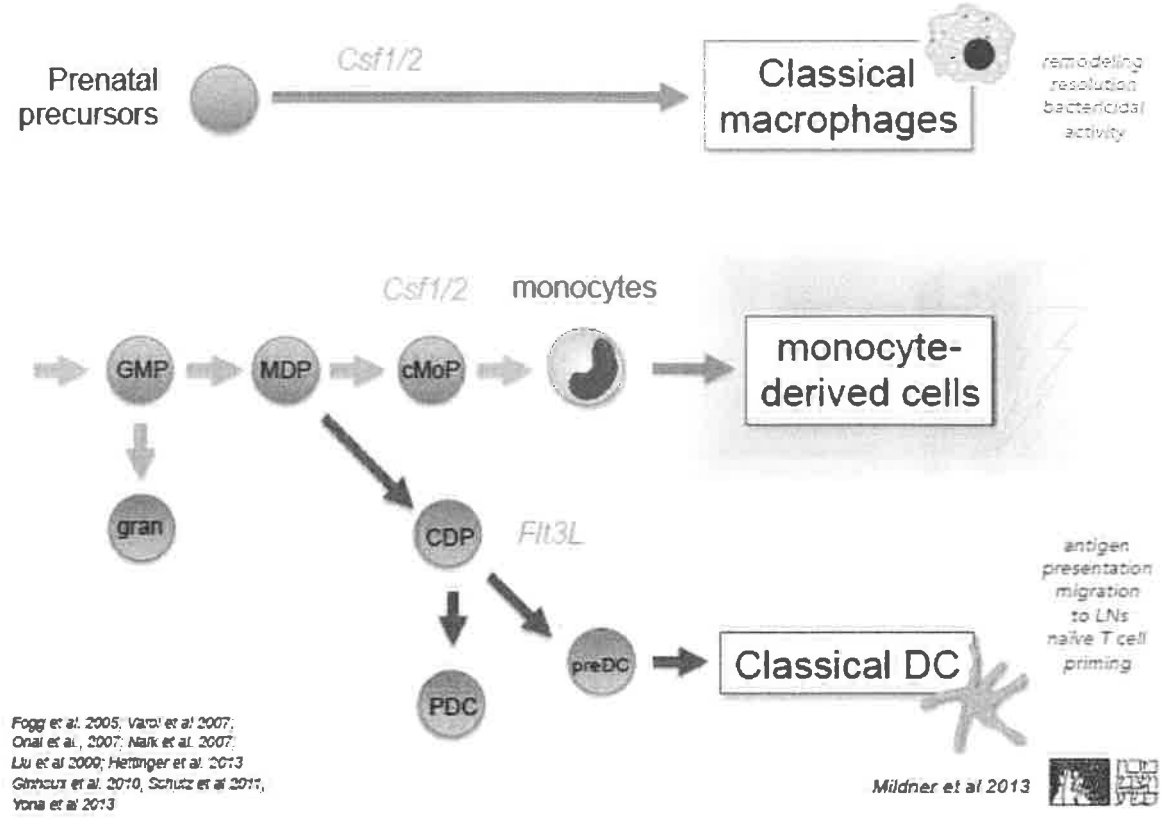
Zigmond, E., Bernshtein, B., Friedlander, G., Walker, C.R., Yona, S., Kim, K.-W., Brenner, O., Krauthgamer, R., Varol, C., Müller, W., et al. (2014). Macrophage-restricted interleukin-10 receptor deficiency, but not IL-10 deficiency, causes severe spontaneous colitis. Immunity *40*, 720-733.

Macrophages and organismal homeostasis

Wide River Institute of Immunology, October 2017

Steffen Jung,
Department of Immunology
The Weizmann Institute of Science

The Mononuclear Phagocyte System 2017



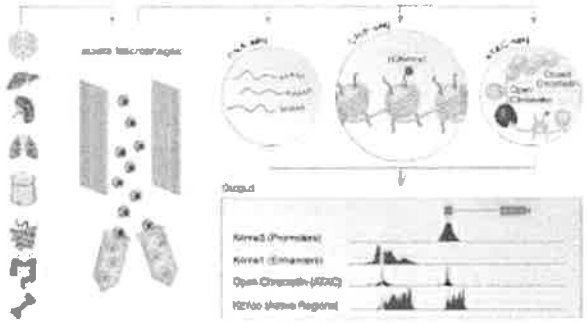
Tissue Macrophages - Development and Specialization

Tissue macrophage compartments

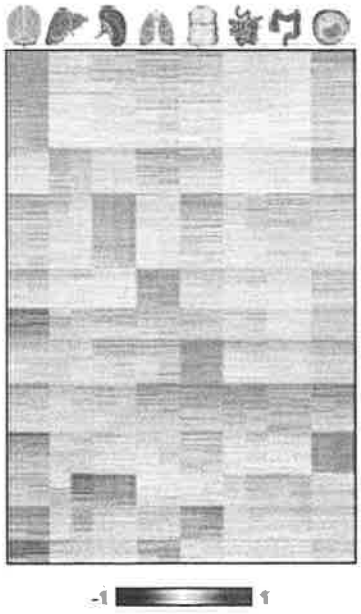
- are established prenatally
- develop locally, independent from each other

Ginhoux 2010, Hoeffel et al. 2012, Schulz et al. 2012, Yona et al. 2013, Perdiguero et al. 2014, Hoeffel et al 2015

Tissue Macrophage heterogeneity - *transcriptomes*



collaboration of Amit, Merad and Jung laboratories



3348 genes differentially expressed among < 2 populations

see also Gautier et al. 2012, Immgen consortium

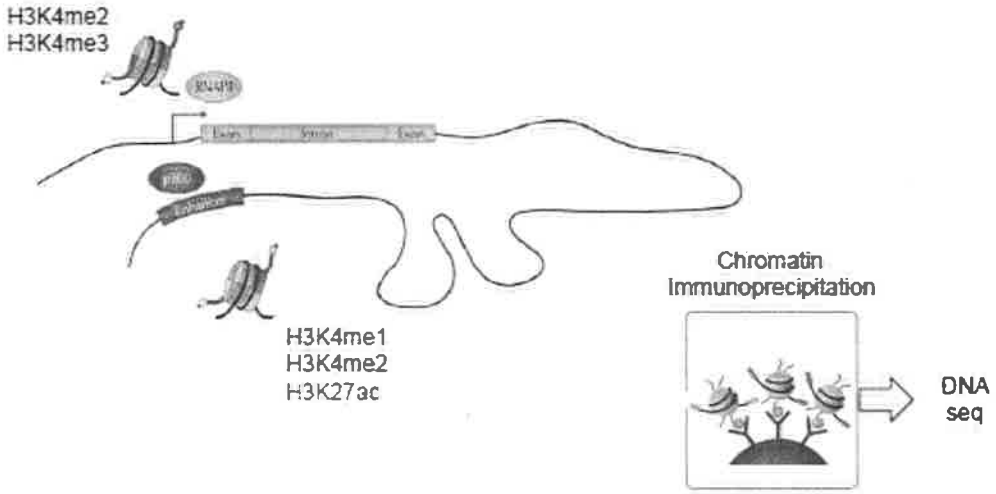
Lavin et al. 2014

Tissue Macrophage heterogeneity - *enhancer landscapes*

ChipSeq

- **~ 20.000 genes**
- **~ 400,000 genomic sites** with enhancer-like features

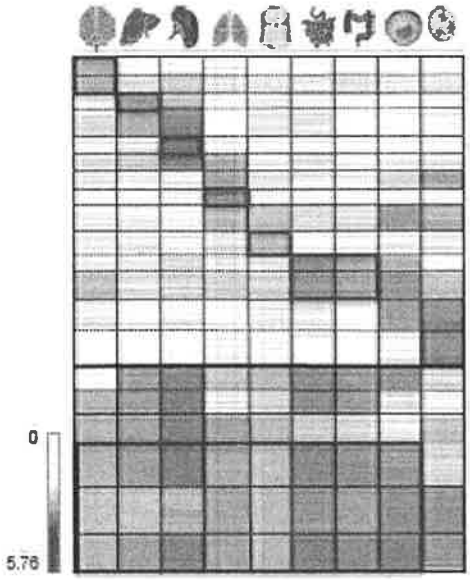
- read-out : **histon modifications**



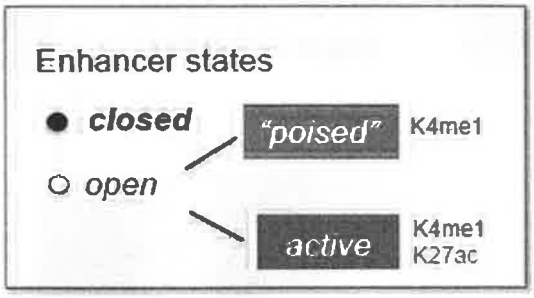
Zhou, Goren, and Bernstein 2011

Tissue Macrophage heterogeneity - *enhancer landscapes*

ChipSeq

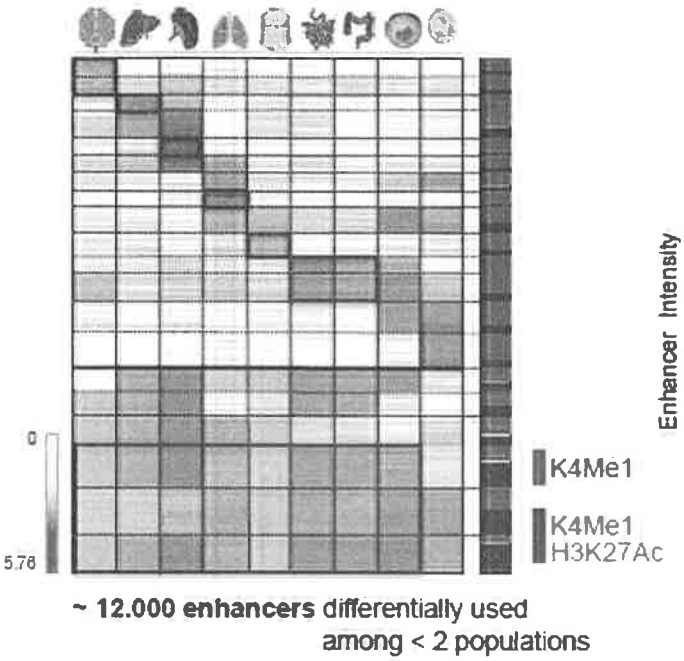


~ 12.000 enhancers differentially used among < 2 populations

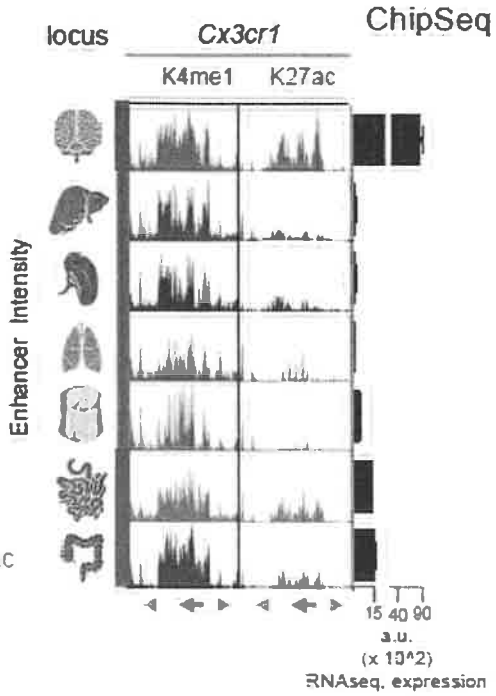


Lavin et al. 2014

Tissue Macrophage heterogeneity = *enhancer landscapes*



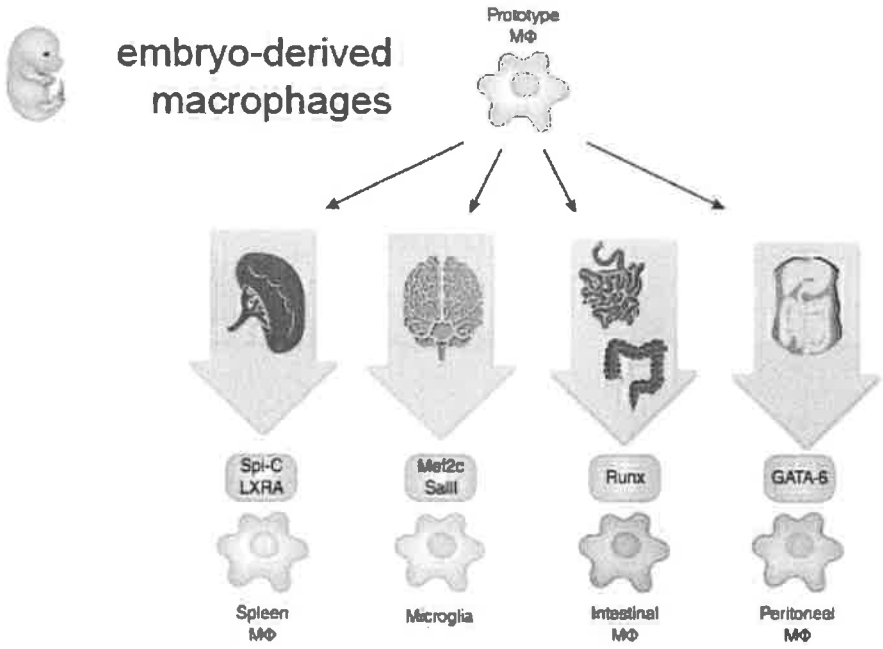
~ 12.000 enhancers differentially used among < 2 populations



macrophage heterogeneity includes differential potential to respond

Lavin et al. 2014

Tissue Macrophage heterogeneity



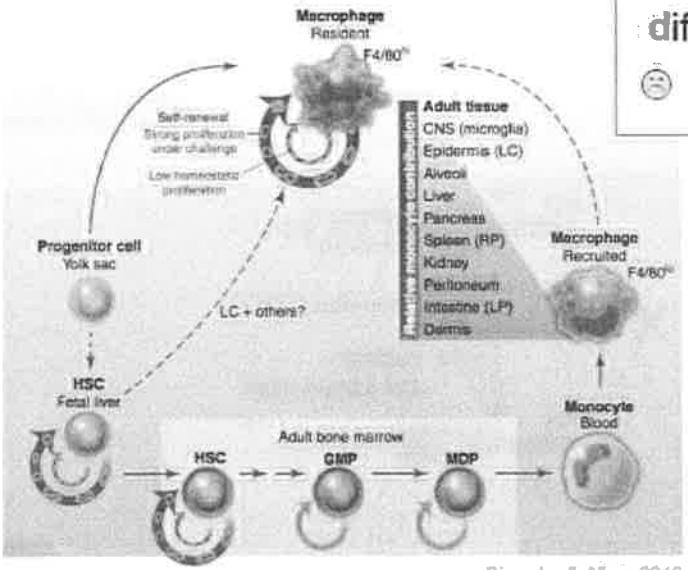
Lavin et al. 2014
Amit et al. 2015

Tissue Macrophage heterogeneity

embryo-derived macrophages ⟺ monocyte-derived macrophages

differential functions ?

☹ *aging vs. rejuvenation* ☺



Sieweke & Allen. 2013

Macrophage functions

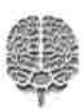
- immune sentinels
- contributions to organ development and homeostasis

surfactant clearance

Suzuki et al. 2008

heme & iron recycling

Kohyama et al. 2009

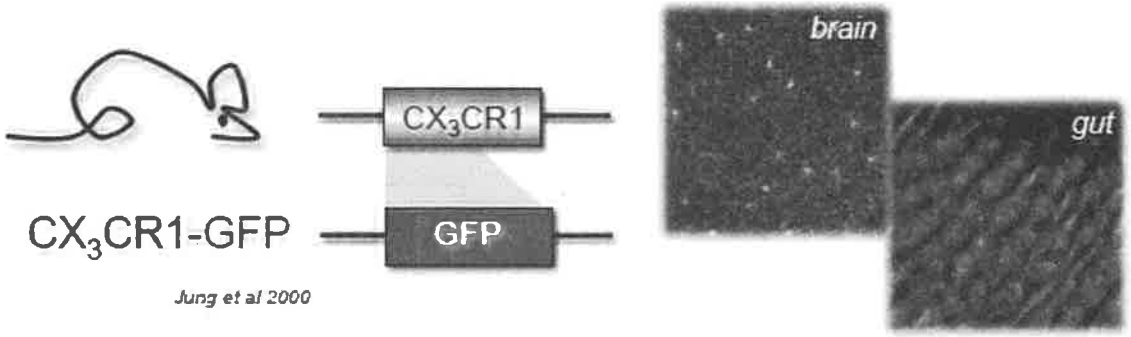


synaptic pruning

Tremblay et al 2010, Schafer et al., 2012

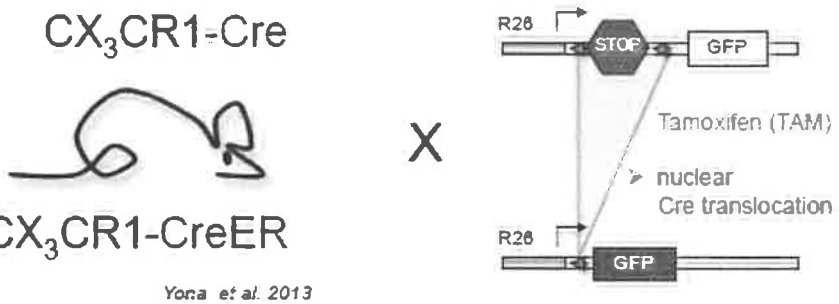
Varol, Mildner and Jung, Ann Rev Immunol 2015

Probing tissue macrophage functions



CX_3CR1-GFP

Jung et al 2000



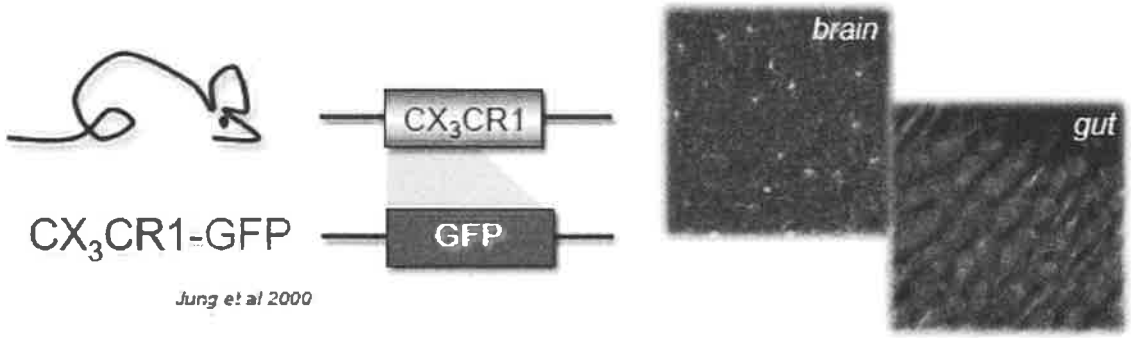
CX_3CR1-Cre

X

CX_3CR1-CreER

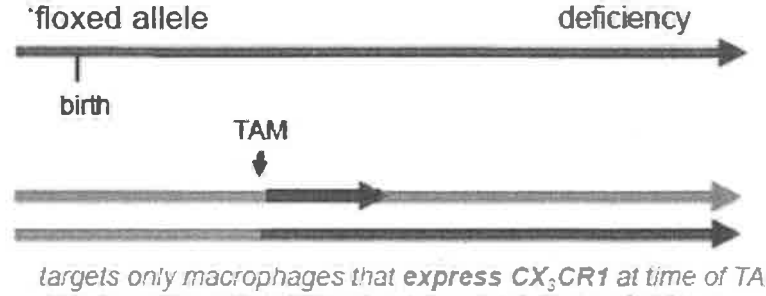
Yona et al. 2013

Probing tissue macrophage functions



CX_3CR1-GFP

Jung et al 2000



CX_3CR1-Cre

CX_3CR1-CreER

Yona et al. 2013

*targets only macrophages that **express CX_3CR1** at time of TAM **and are long-lived** (excluding monocytes and derivatives)*

Yona et al. 2013
Goldmann, Wolf et al. 2014

Macrophage-specific contributions to monogenic disorders