Development and Function of Dendritic Cell subsets

Ken Murphy

AAI Advanced Course 2022

https://sites.wustl.edu/murphylab/

Outline:

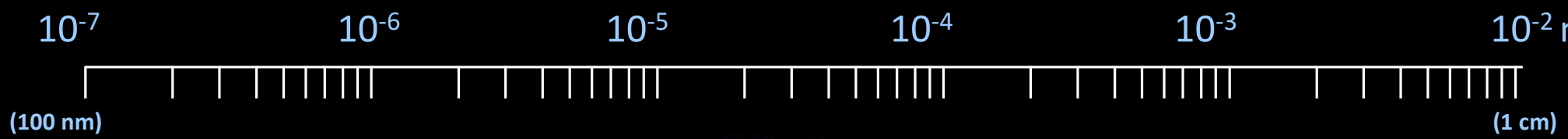
Background and History

Development of cDC subsets –transcriptional basis

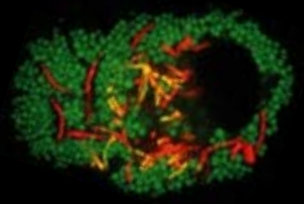
Function of different subsets – an emerging area

- cDC1, CD8 responses and IL-12/Th1
- Cross-presentation and Help
- cDC2 (heterogeneous) Th17, Th2, ??

Defense against pathogens requires diverse effector functions



Viruses

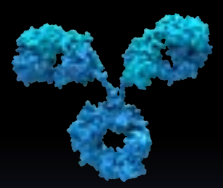


Intracellular Bacteria

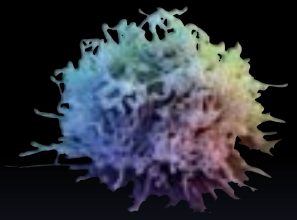
Extracellular Bacteria

Fungi

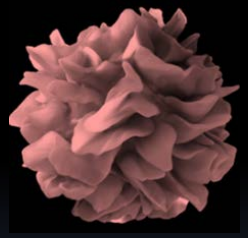
Parasites



Antibody
B cells

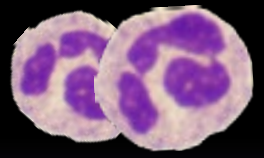


NK cells
CD8 T cells
CD4 T cells

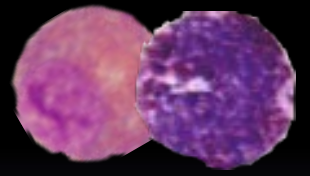


Dendritic cells

Macrophages



Neutrophils



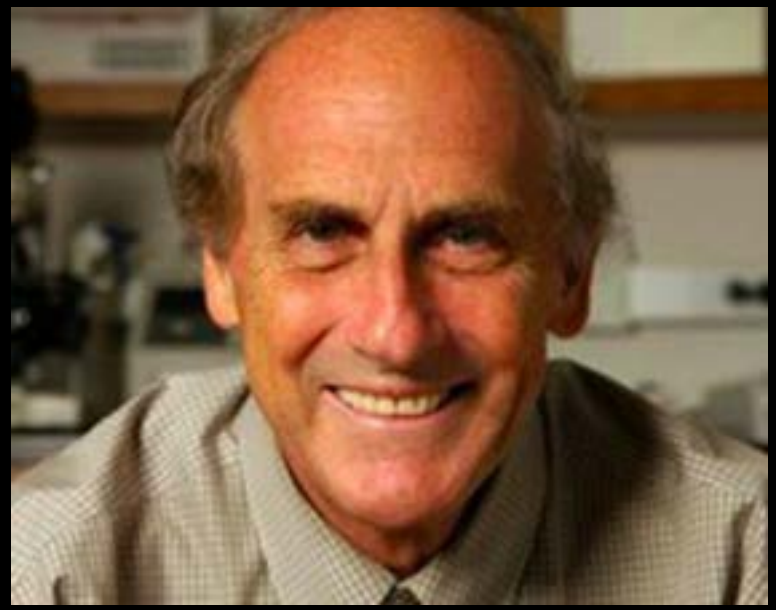
Mucosal immunity
Eosinophils/Basophils

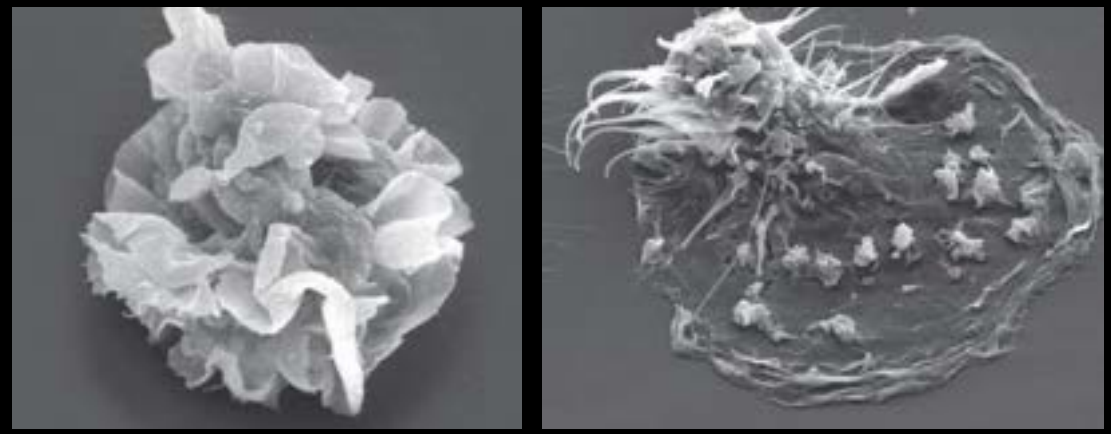
Innate Immunity

Adaptive Immunity

Dendritic cells are powerful APCs

Ralph M. Steinman MD
2011 Nobel Prize





IDENTIFICATION OF A NOVEL CELL TYPE IN PERIPHERAL LYMPHOID ORGANS OF MICE

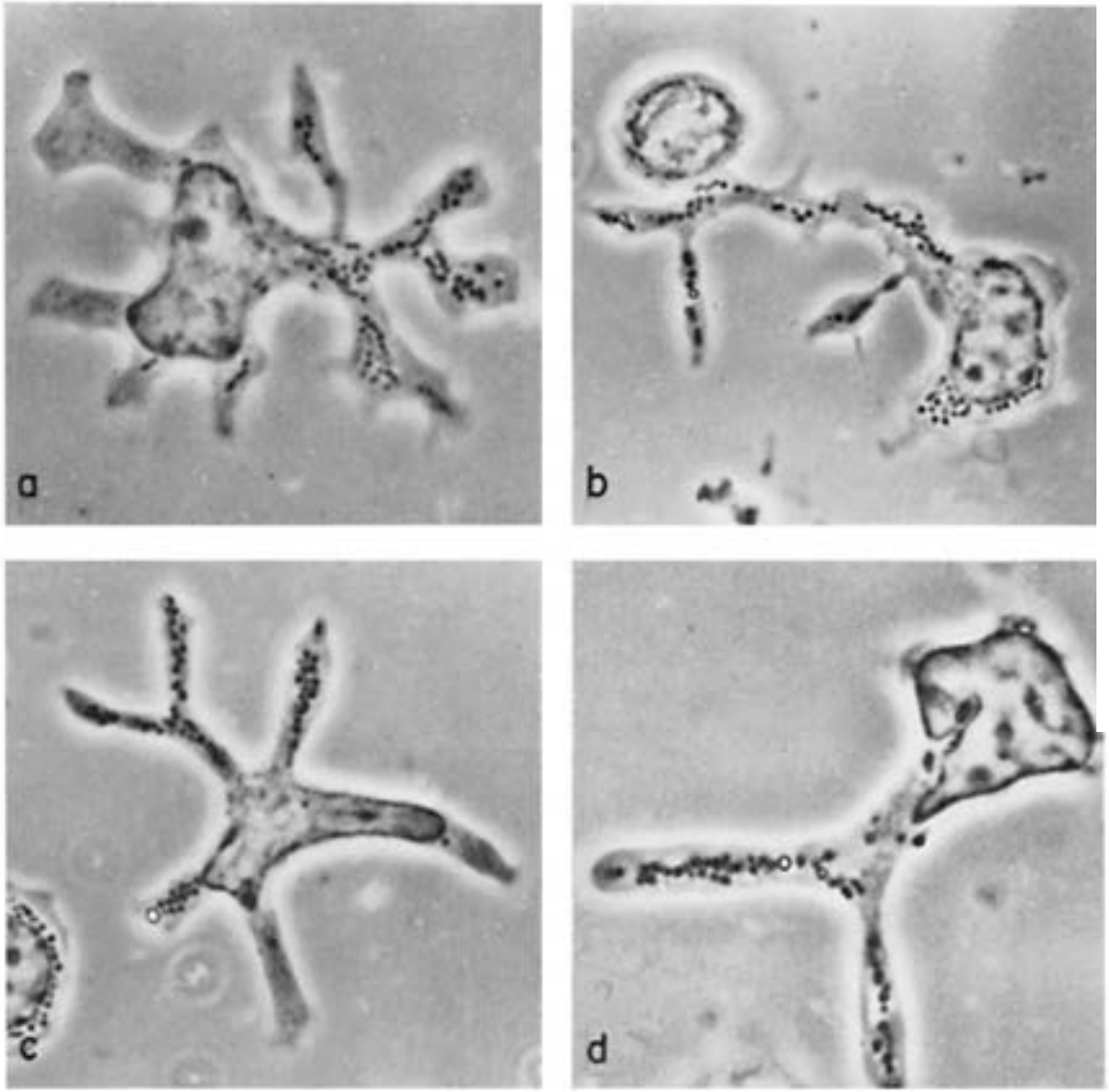
I. Morphology, Quantitation, Tissue Distribution*

By RALPH M. STEINMAN‡ and ZANVIL A. COHN

(*From The Rockefeller University, New York 10021*)

(Received for publication 19 January 1973)

During the course of observations on the cells of mouse spleen that adhere to glass and plastic surfaces, it was clear that this population was quite heterogeneous. In addition to mononuclear phagocytes, granulocytes, and lymphocytes, we noticed a large stellate cell with distinct properties from the former cell types. In this paper, we describe the morphology, quantitation, and tissue distribution of this novel cell as identified in vitro. In following papers, we will further characterize it with respect to its functional properties in vitro, as well as its localization and properties *in situ*.



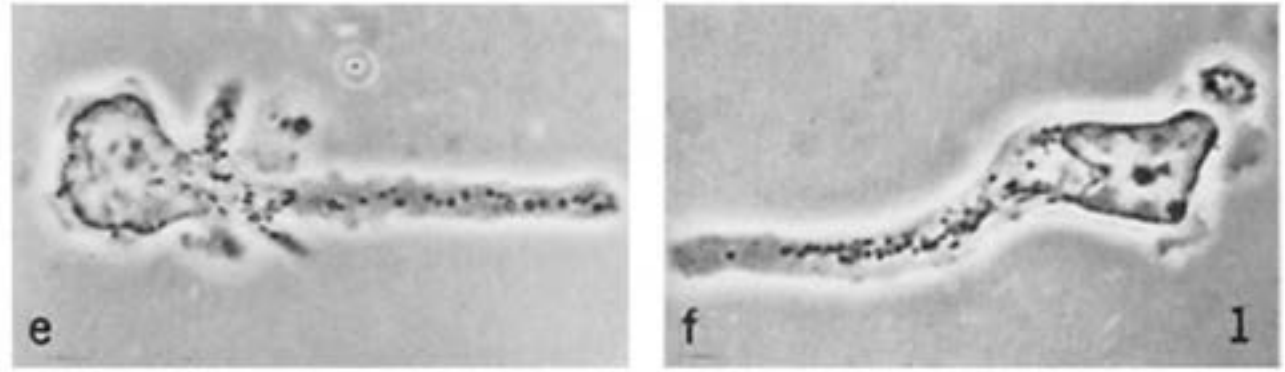


FIG. 1. Phase-contrast micrographs of dendritic cells isolated from peripheral lymphoid organs and fixed in glutaraldehyde. Figs. 1 *a–d* are from spleen, (*e*) from cervical lymph node, and (*f*) from Peyer's patch. The nucleus is large, irregular in shape, and has a refractile quality. The cytoplasm is arranged in processes of varying sizes and shapes, many of which contain spherical phase-dense mitochondria. Occasional refractile lipid granules are also present. A medium size lymphocyte in Fig. 1 *b* can be used as a size comparison. (*a*) × 4,500; (*b*) × 3,500; (*c*) × 3,200; (*d*) × 4,600; (*e*) × 3,200; (*f*) × 3,200.

Discovery of dendritic cells by Steinman and first few papers

First report, rapid turnover and BM origin

1. Steinman, R. M., D. S. Lustig, and Z. A. Cohn. 1974. Identification of a novel cell type in peripheral lymphoid organs of mice. 3. Functional properties in vivo. *J Exp.Med* 139:1431-1445.

Distinct from other cells

2. Steinman, R. M. and Z. A. Cohn. 1974. Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro. *J Exp.Med* 139:380-397.

Present in mouse spleen

3. Steinman, R. M., J. C. Adams, and Z. A. Cohn. 1975. Identification of a novel cell type in peripheral lymphoid organs of mice. IV. Identification and distribution in mouse spleen. *J Exp.Med* 141:804-820.

Potent in primary MLR

4. Steinman, R. M. and M. D. Witmer. 1978. Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. *Proc.Natl Acad.Sci.U S A* 75:5132-5136.

High MHC-II expression

5. Steinman, R. M., G. Kaplan, M. D. Witmer, and Z. A. Cohn. 1979. Identification of a novel cell type in peripheral lymphoid organs of mice. V. Purification of spleen dendritic cells, new surface markers, and maintenance in vitro. *J Exp.Med* 149:1-16.

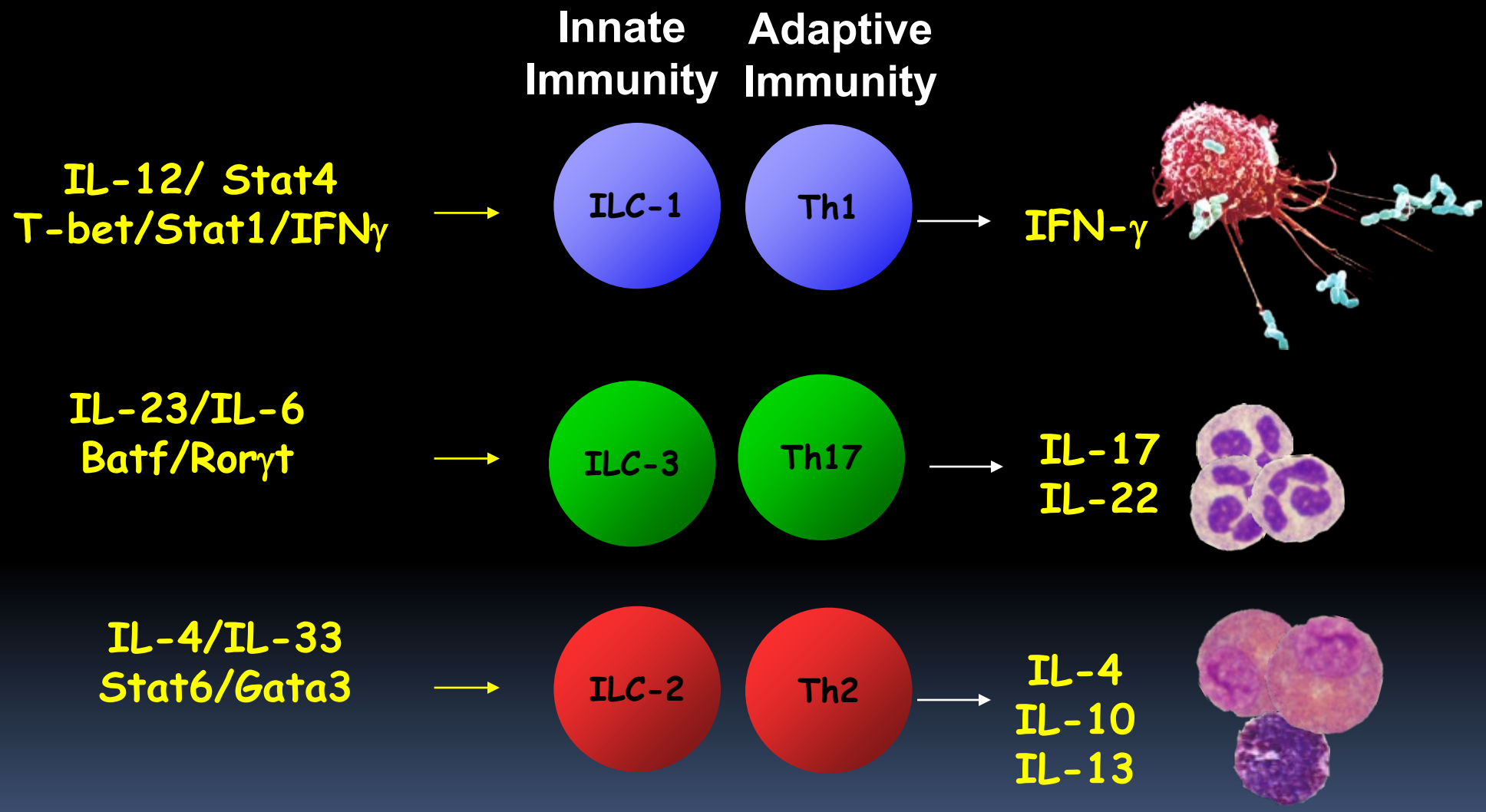
Syngeneic MLR

6. Nussenzweig, M. C. and R. M. Steinman. 1980. Contribution of dendritic cells to stimulation of the murine syngeneic mixed leukocyte reaction. *J Exp.Med* 151:1196-1212.

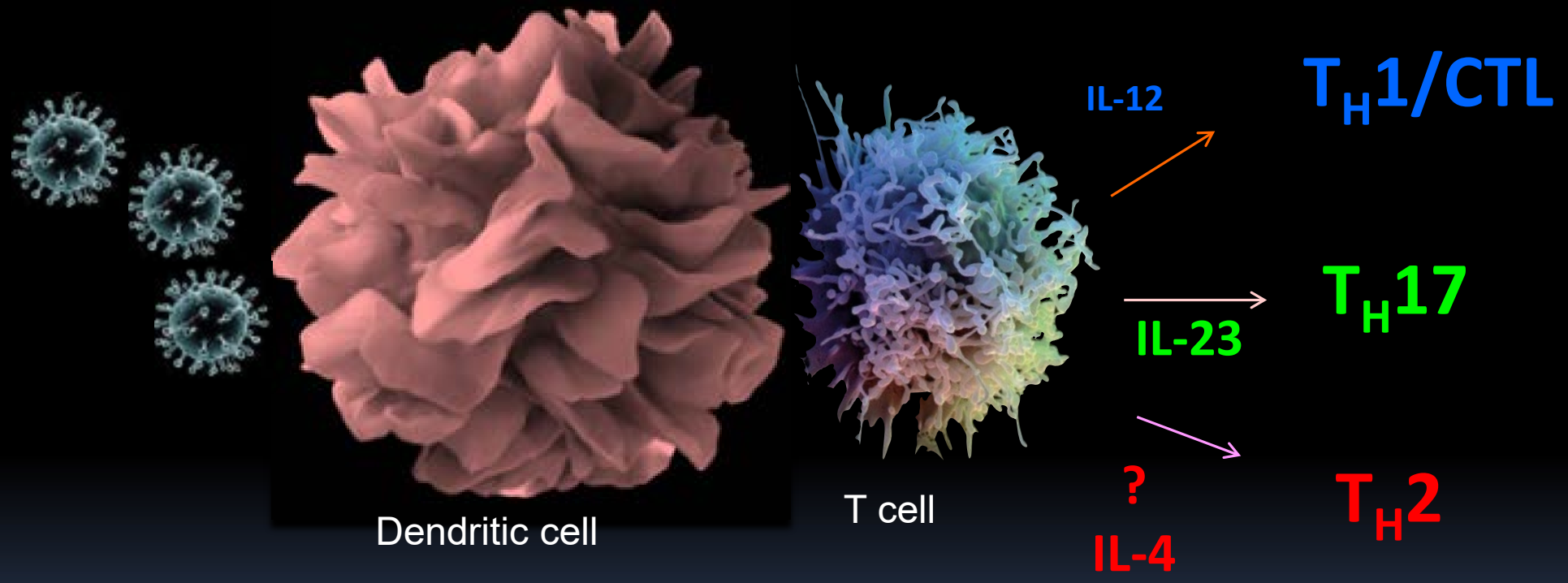
APCs for real antigens

7. Nussenzweig, M. C., R. M. Steinman, B. Gutchinov, and Z. A. Cohn. 1980. Dendritic cells are accessory cells for the development of anti-trinitrophenyl cytotoxic T lymphocytes. *J.Exp.Med.* 152:1070-1084.

Pathogen-induced T cell/ILC modules rely on *instructive* cues



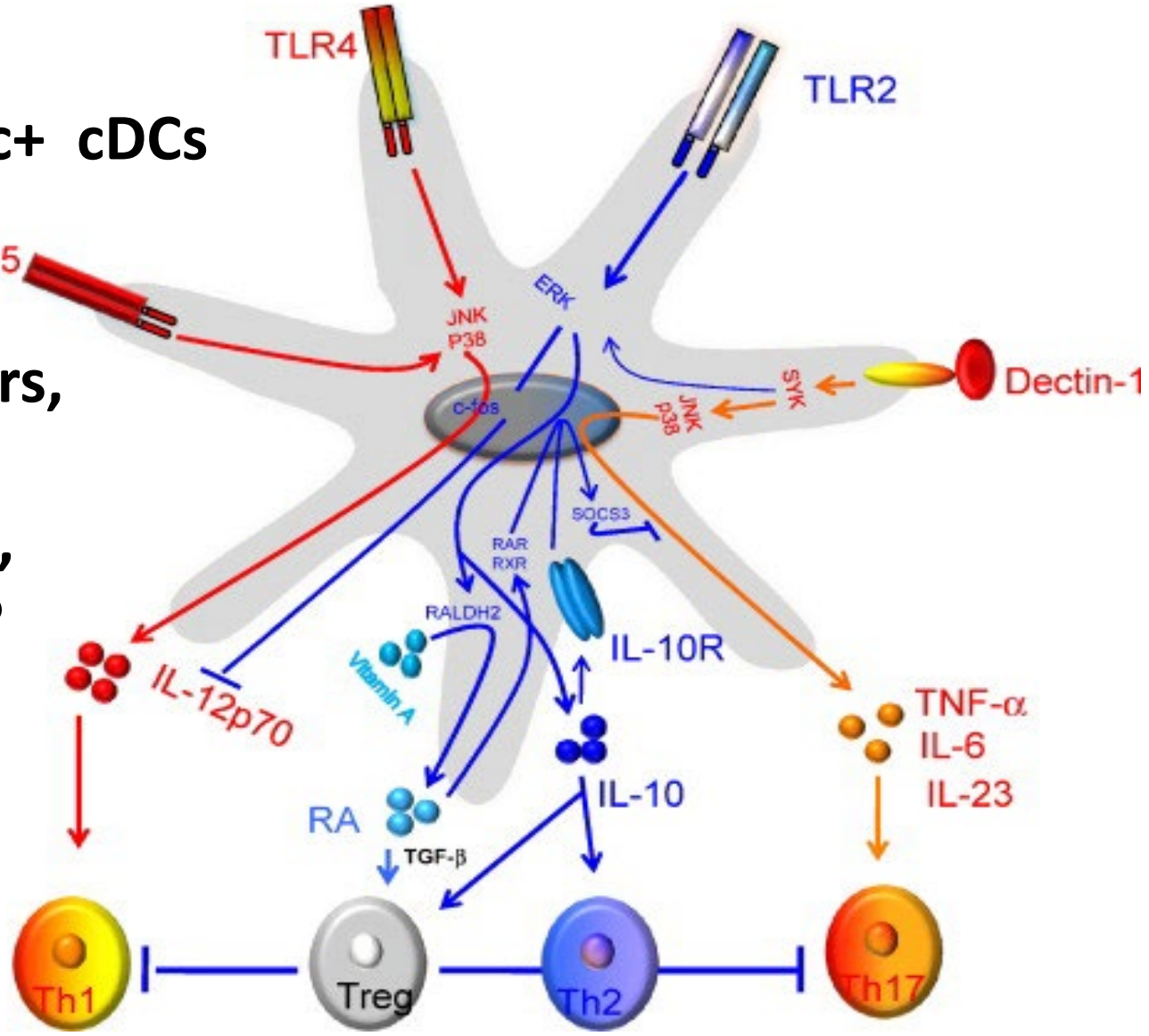
How do DCs choose the appropriate instructive signal?



Can one DC make all the decisions?

CD11c+ cDCs

Express all sensors, all cytokines, in all locations, and calculate?

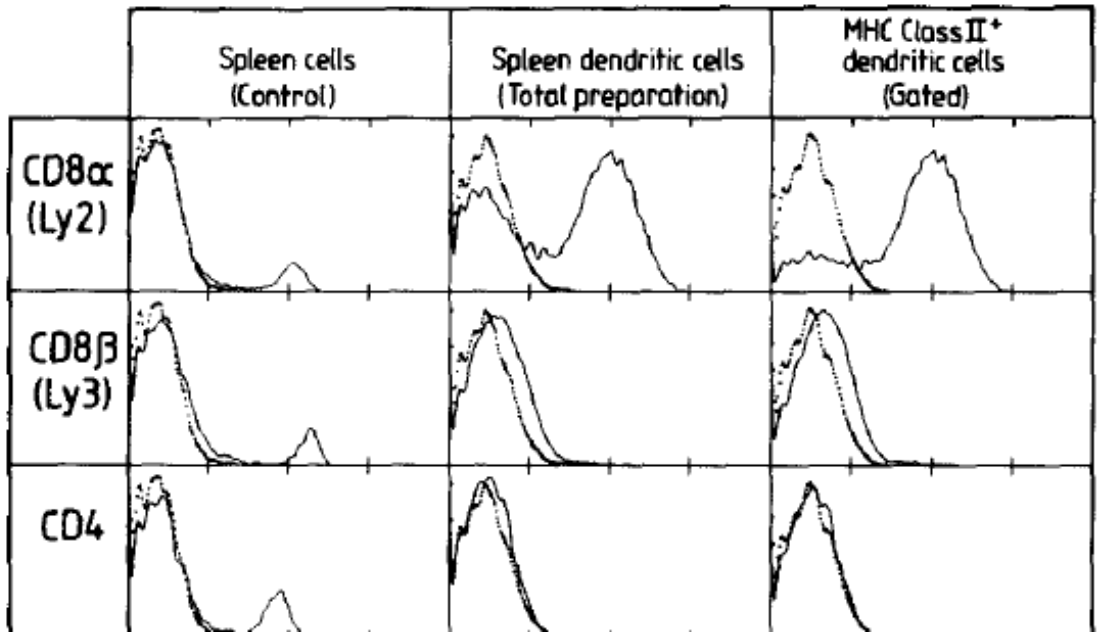


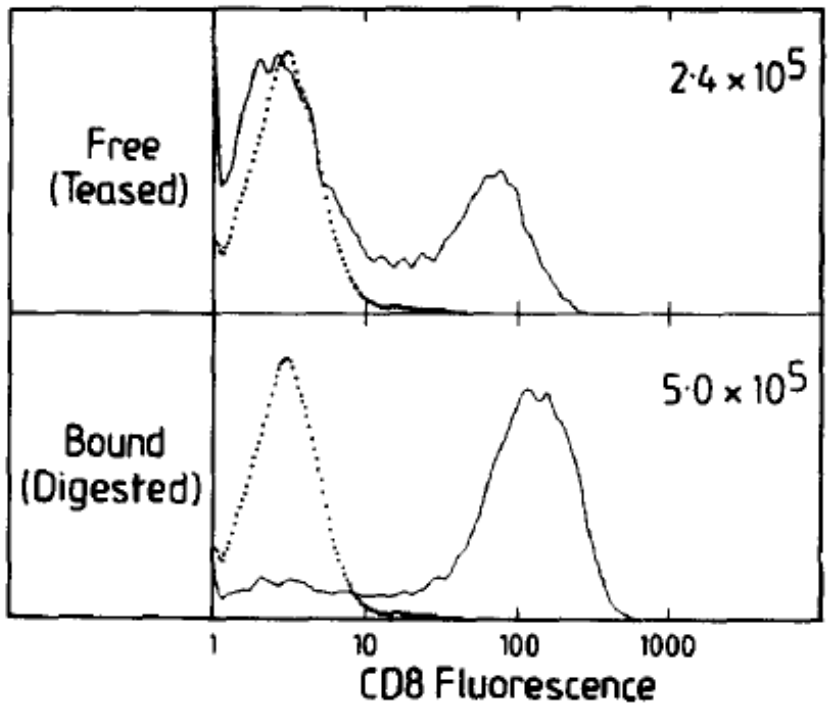
Seminars in Immunology Volume 21, Issue 4 2009 185 - 193

1992 JEM

The Surface Phenotype of Dendritic Cells Purified from Mouse Thymus and Spleen: Investigation of the CD8 Expression by a Subpopulation of Dendritic Cells

By David Vremec, Michelle Zorbas, Roland Scollay, Dolores J. Saunders, Carlos F. Ardavin, Li Wu, and Ken Shortman





Types of DCs

Plasmacytoid DCs

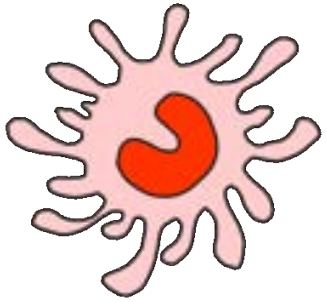
E2-2 dependent
Irf8^{hi} Irf4^{lo}

B220^{+} SiglecH^{+} Bst2^{+} (CD317)

anti-viral
IFNα/β

pDC specific deletion
BDCA2-DTR

cDC1



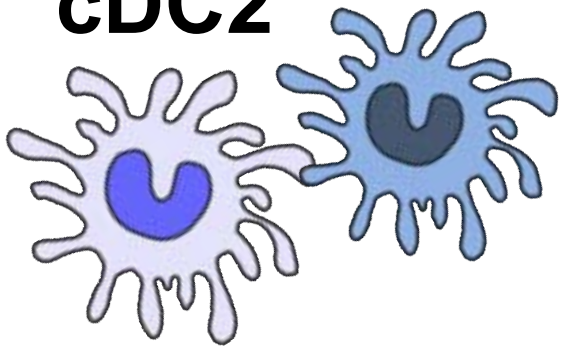
IRF8^{high}

Xcr1, *Clec9a*, *Tlr3*

Intracellular pathogens, tumor
IL-12 production, Th1 induction
Cross-presentation

cDC1 specific deletion
(Xcr1-Cre, Batf3$^{-/-}$ mice, Irf8 32$^{-/-}$)

cDC2



IRF4$^{low/int}$

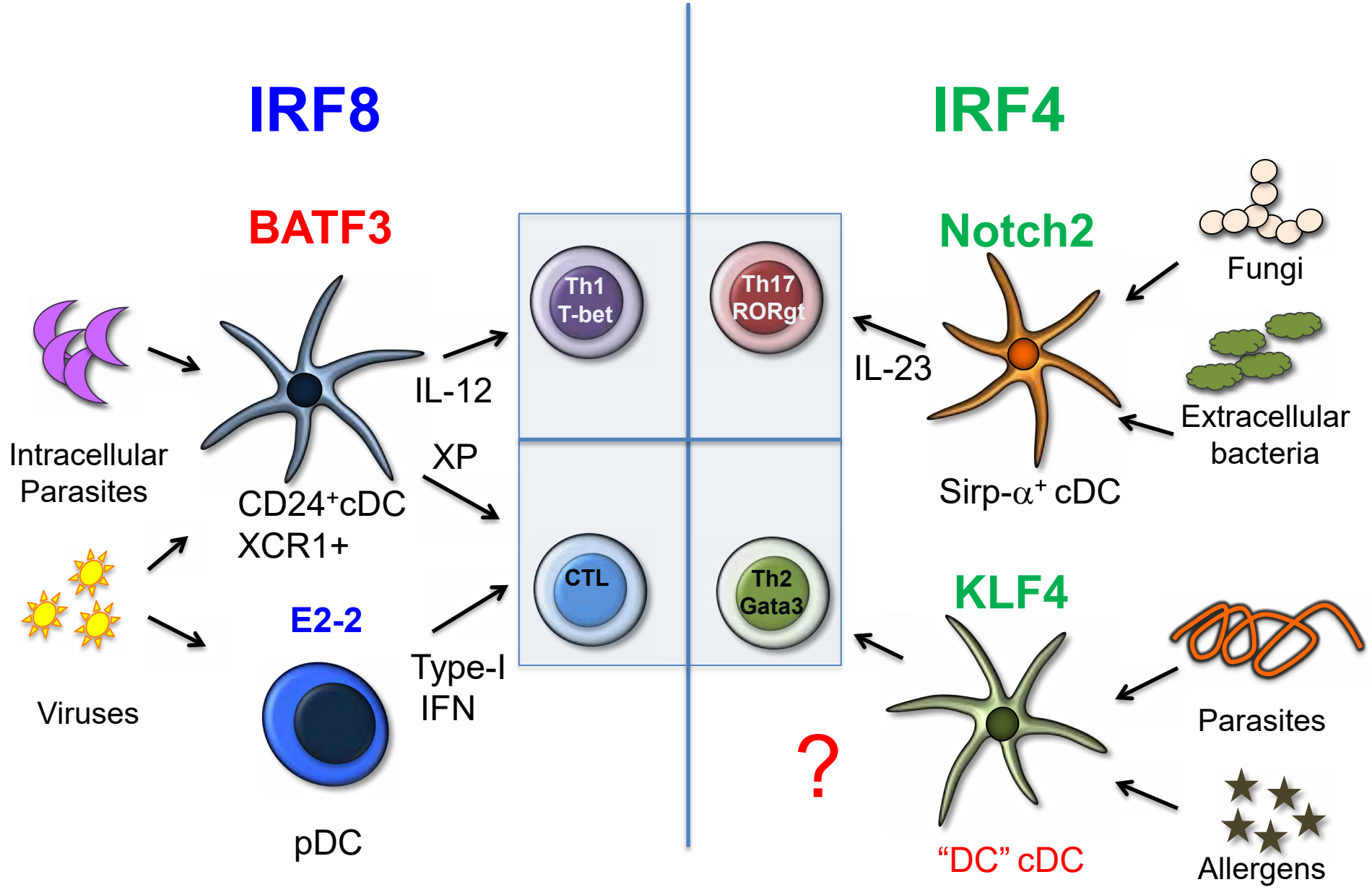
CD4, Sirp-α (CD172a), ESAM

Fungi, extracellular bacteria,
parasites ??
IL-23 production
Th2, Th17 induction

So far only non-specific deletion
(CD11c-Cre or,germline *Irf4*, Mgl2-DTR)

Hildner and Murphy_Science (2008), Satpathy and Murphy_Nat Immunol (2013)
Tussiwand and Murphy_Immunity (2015), Grajales-Reyes and Murphy_Nat Immunol (2015), Murphy and Murphy,_Ann Rev Immunol (2016)

A developing framework for DC diversity



Short list of references for development of cDCs

Identification of the MDP/CDP (not the only one)
Onai, N., A. Obata-Onai, M. A. Schmid, T. Ohteki, D. Jarrossay, and M. G. Manz. 2007. Identification of clonogenic common Flt3(+) M-CSFR+ plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow. *Nat.* 8:1207-1216.

Discovery of the requirement of BATF3 in cDC1 development
Hildner, K., B. T. Edelson, W. E. Purtha, M. Diamond, H. Matsushita, M. Kohyama, B. Calderon, B. U. Schraml, E. R. Unanue, M. S. Diamond, R. D. Schreiber, T. L. Murphy, and K. M. Murphy. 2008. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science* 322:1097-1100.

Identification of pregenitors for cDC1 and cDC2
- Grajales-Reyes, G. E., A. Iwata, J. Albring, X. Wu, R. Tussiwand, W. KC, N. M. Kretzer, C. G. Briseno, V. Durai, P. Bagadia, M. Haldar, J. Schonheit, F. Rosenbauer, T. L. Murphy, and K. M. Murphy. 2015. Batf3 maintains autoactivation of Irf8 for commitment of a CD8alpha(+) conventional DC clonogenic progenitor. *Nat Immunol* 16:708-717.

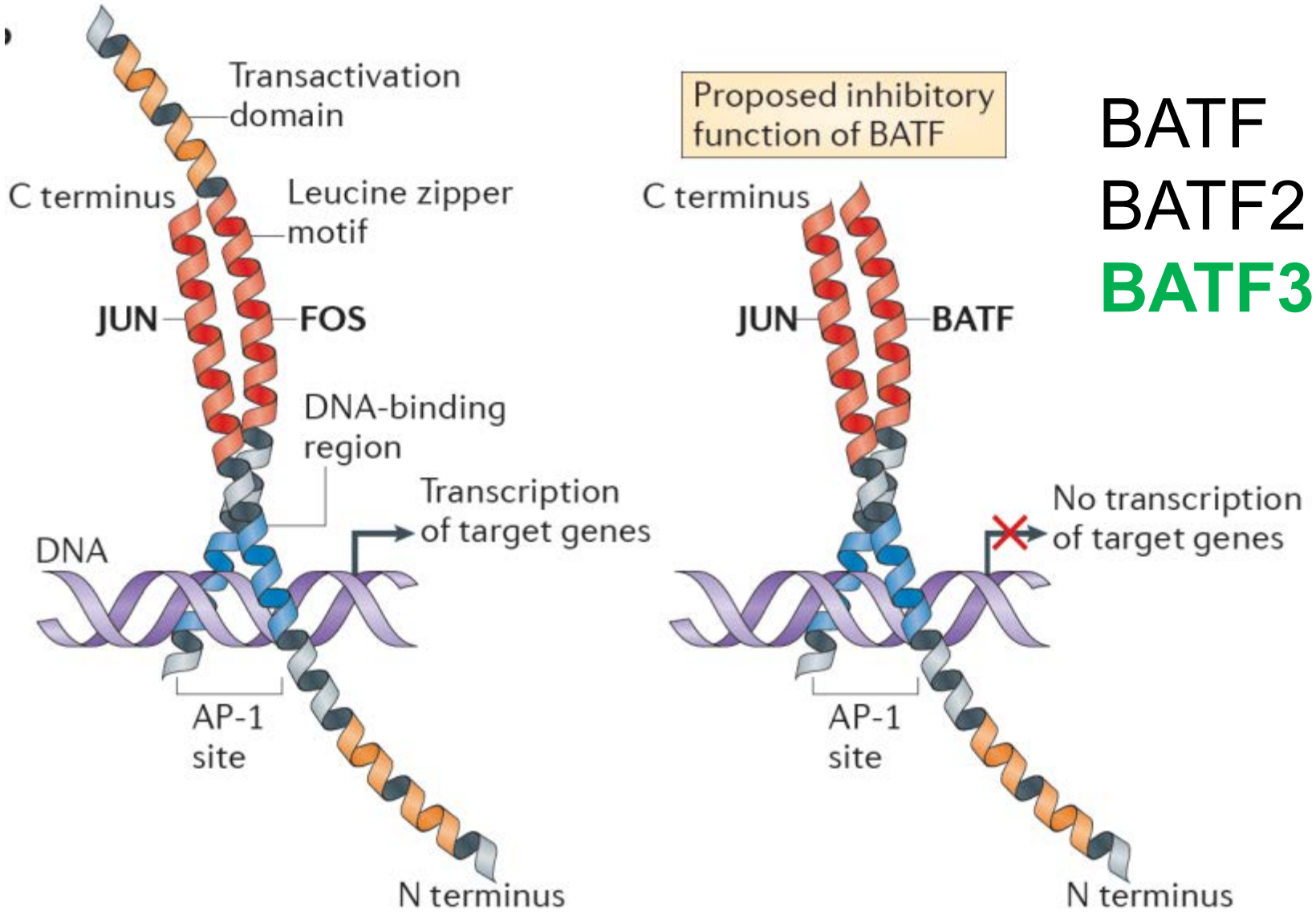
- Schlitzer, A., N. McGovern, and F. Ginhoux. 2015. Dendritic cells and monocyte-derived cells: Two complementary and integrated functional systems. *Semin Cell Dev.Biol*.

Basic distinction between cDC1 and cDC2 transcriptional programs
Kim, S., P. Bagadia, D. A. Anderson, III, T. T. Liu, X. Huang, D. J. Theisen, K. W. O'Connor, R. A. Ohara, A. Iwata, T. L. Murphy, and K. M. Murphy. 2020. High Amount of Transcription Factor IRF8 Engages AP1-IRF Composite Elements in Enhancers to Direct Type 1 Conventional Dendritic Cell Identity. *Immunity* 53:1-16.

Enhancers of IRF8 gene required for cDC1 development – explains BATF3 requirement.
Durai, V., P. Bagadia, J. M. Granja, A. T. Satpathy, D. H. Kulkarni, J. T. Davidson, R. Wu, S. J. Patel, A. Iwata, T. T. Liu, X. Huang, C. G. Briseno, G. E. Grajales-Reyes, M. Wohner, H. Tagoh, B. L. Kee, R. D. Newberry, M. Busslinger, H. Y. Chang, T. L. Murphy, and K. M. Murphy. 2019. Cryptic activation of an Irf8 enhancer governs cDC1 fate specification. *Nat Immunol* 20:1161-1173.

Beginning of the circuitry for CDP divergence
Bagadia, P., X. Huang, T. T. Liu, V. Durai, G. E. Grajales-Reyes, M. Nitschke, Z. Modrusan, J. M. Granja, A. T. Satpathy, C. G. Briseno, M. Gargaro, A. Iwata, S. Kim, H. Y. Chang, A. S. Shaw, T. L. Murphy, and K. M. Murphy. 2019. An Nfil3-Zeb2-Id2 pathway imposes Irf8 enhancer switching during cDC1 development. *Nat Immunol* 20:1174-1185.

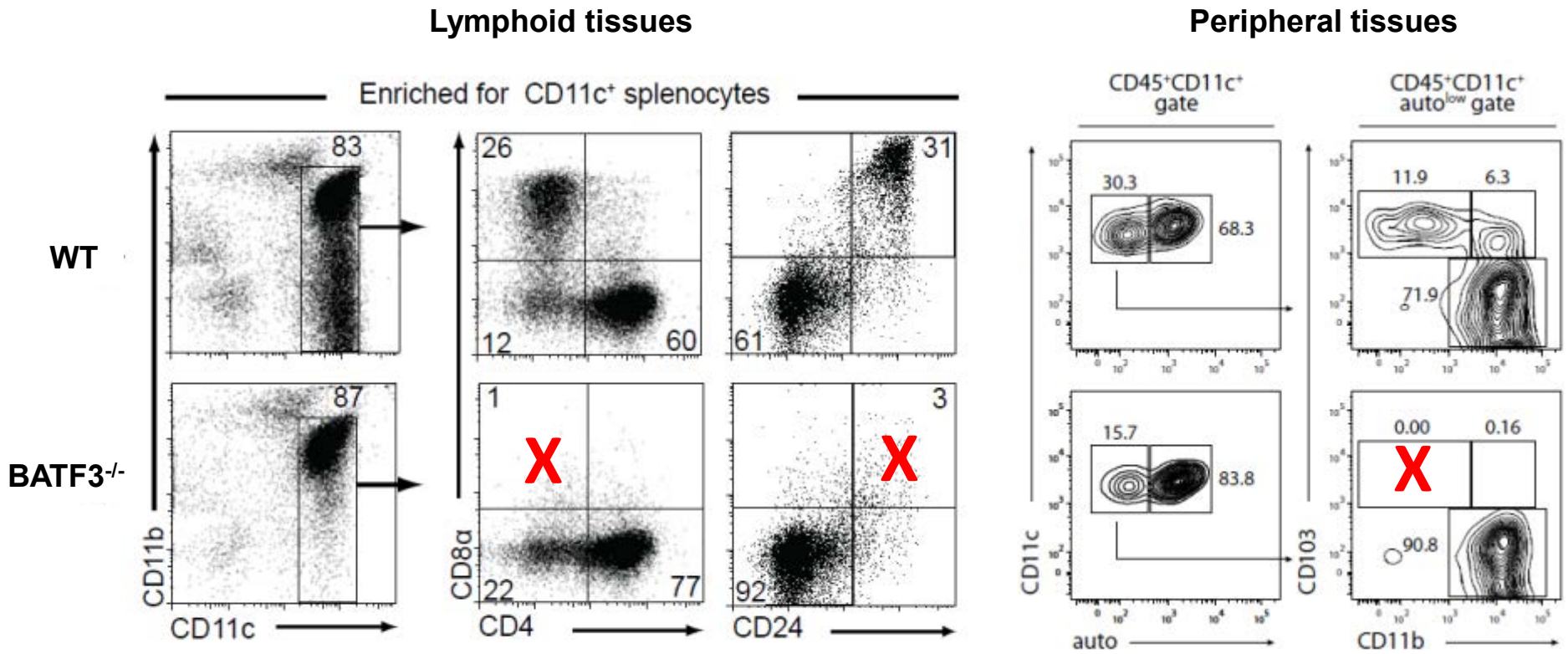
BATF3 is an AP-1 factor expressed uniquely in DCs



BATF
BATF2
BATF3

Murphy et al., Nature Reviews Immunology, 2013

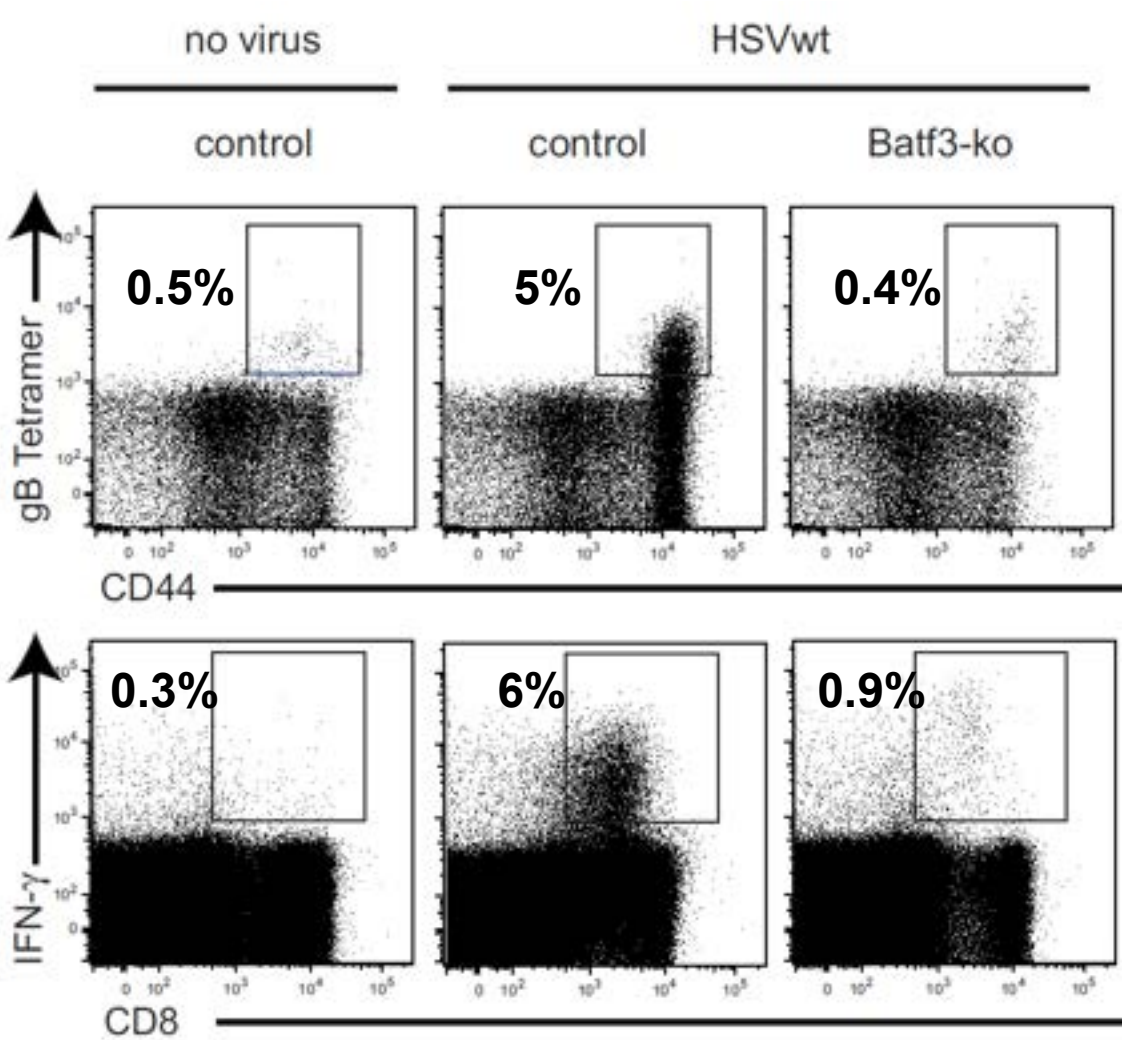
Batf3 controls development of CD8α⁺ and CD103⁺ DCs

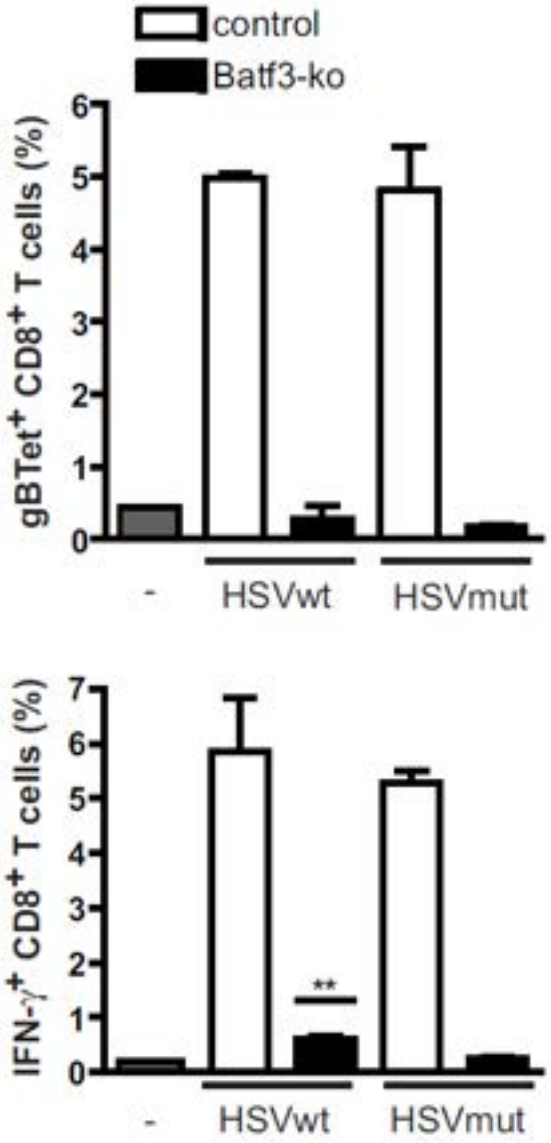


Hildner K at al., 2008,

Edelson et al., 2010

Batf3$^{-/-}$ mice fail to prime CD8 T cells to HSV



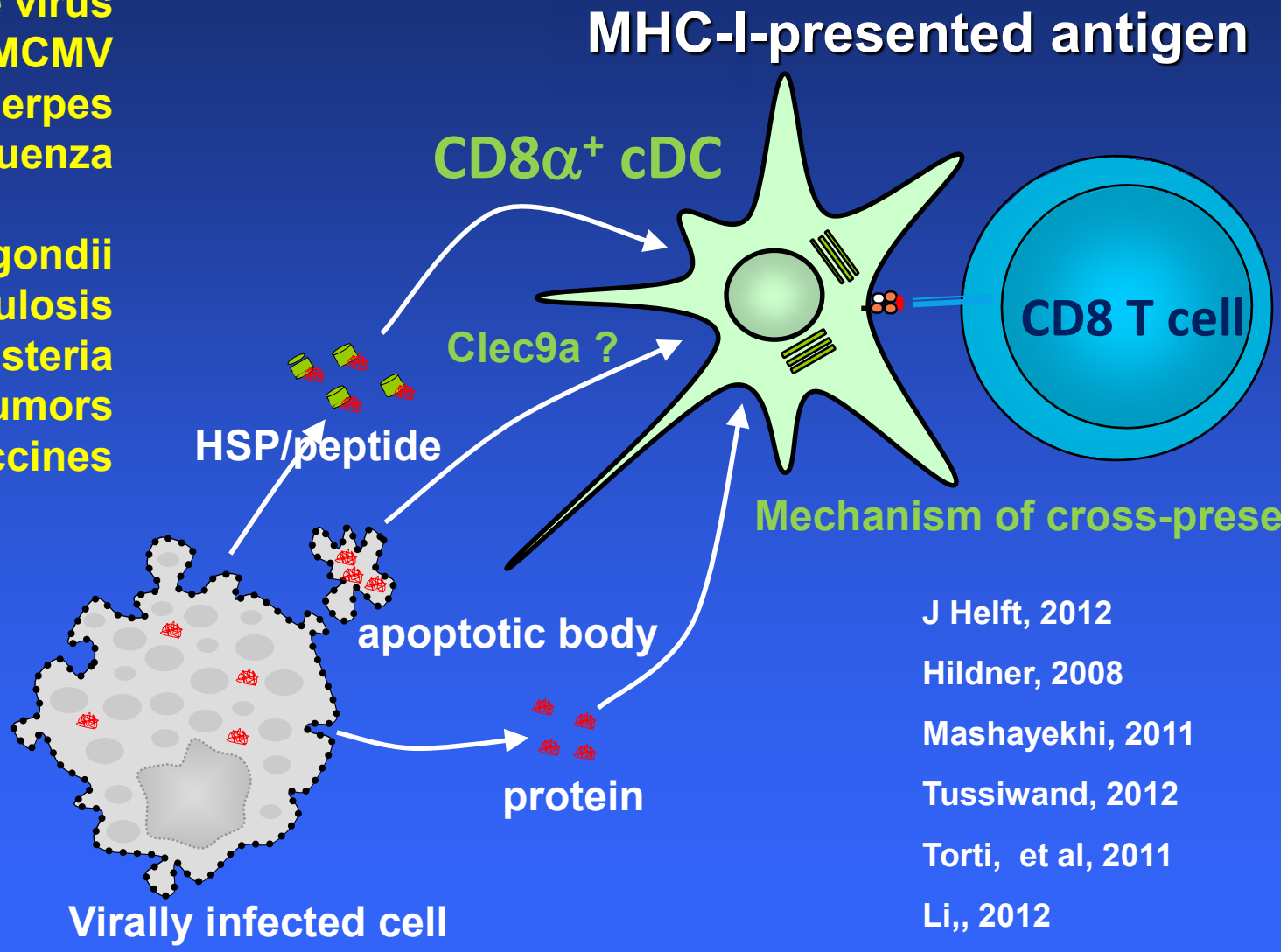


Nopora et al., 2012

Cross presentation - Mechanisms?

West Nile virus
MCMV
Herpes
Influenza

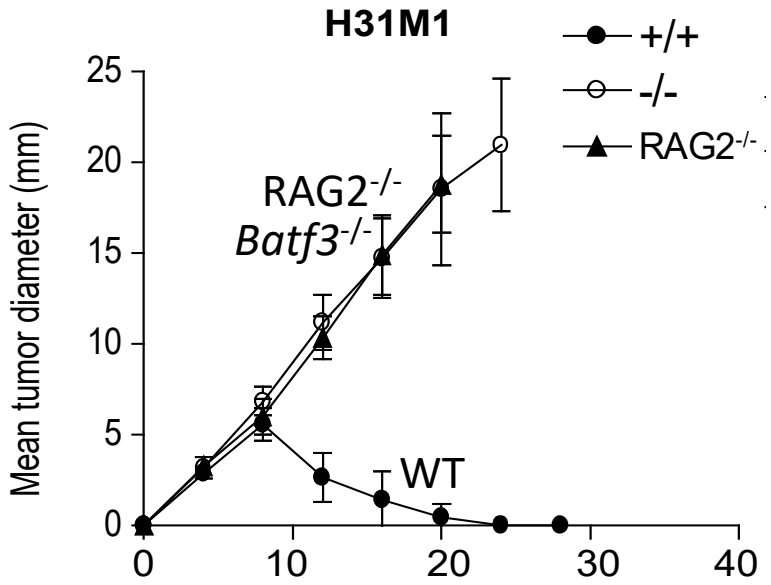
T. gondii
M. tuberculosis
Listeria
Tumors
DNA vaccines



Mechanism of cross-presentation?

J Helft, 2012
Hildner, 2008
Mashayekhi, 2011
Tussiwand, 2012
Torti, et al, 2011
Li,, 2012
Edelson, 2011

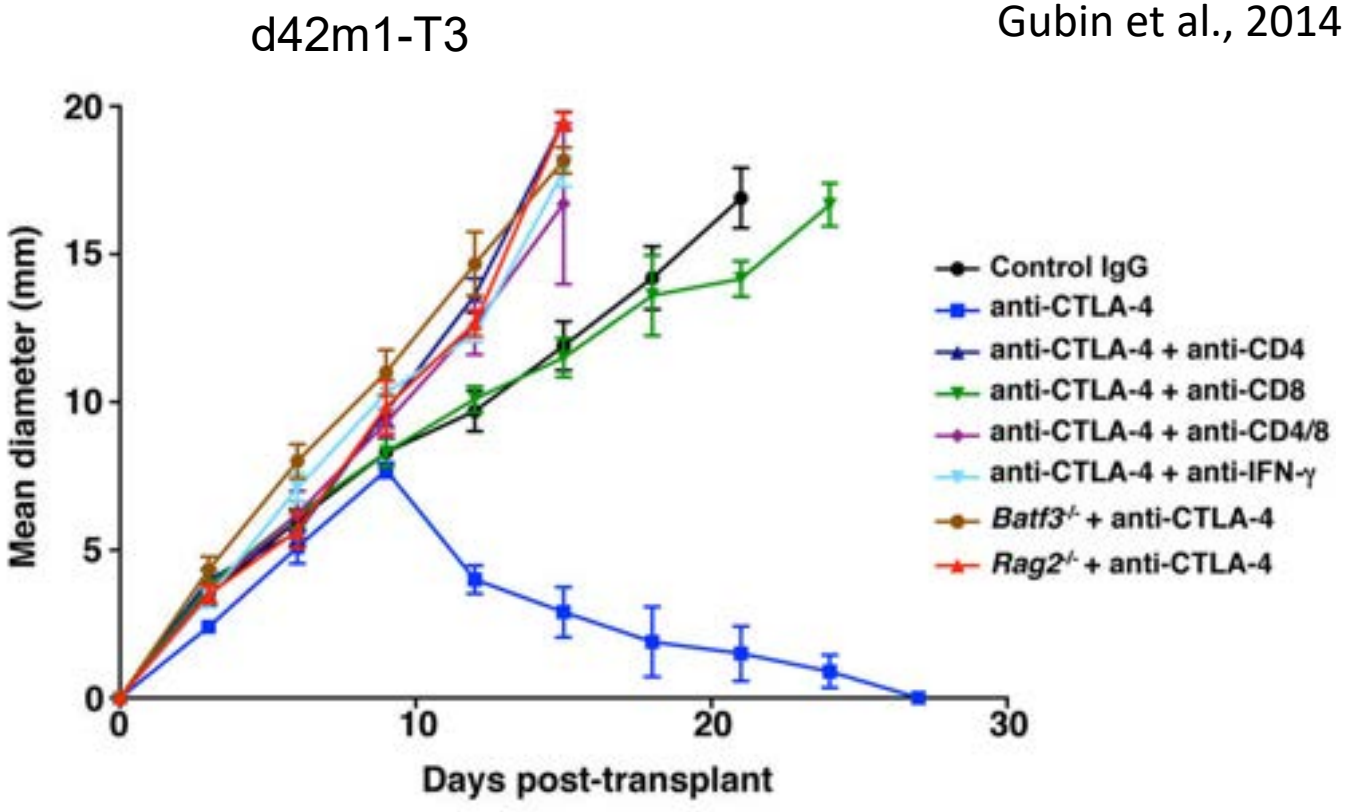
CD8α⁺ DCs (cDC1) are required for anti-tumor immunity



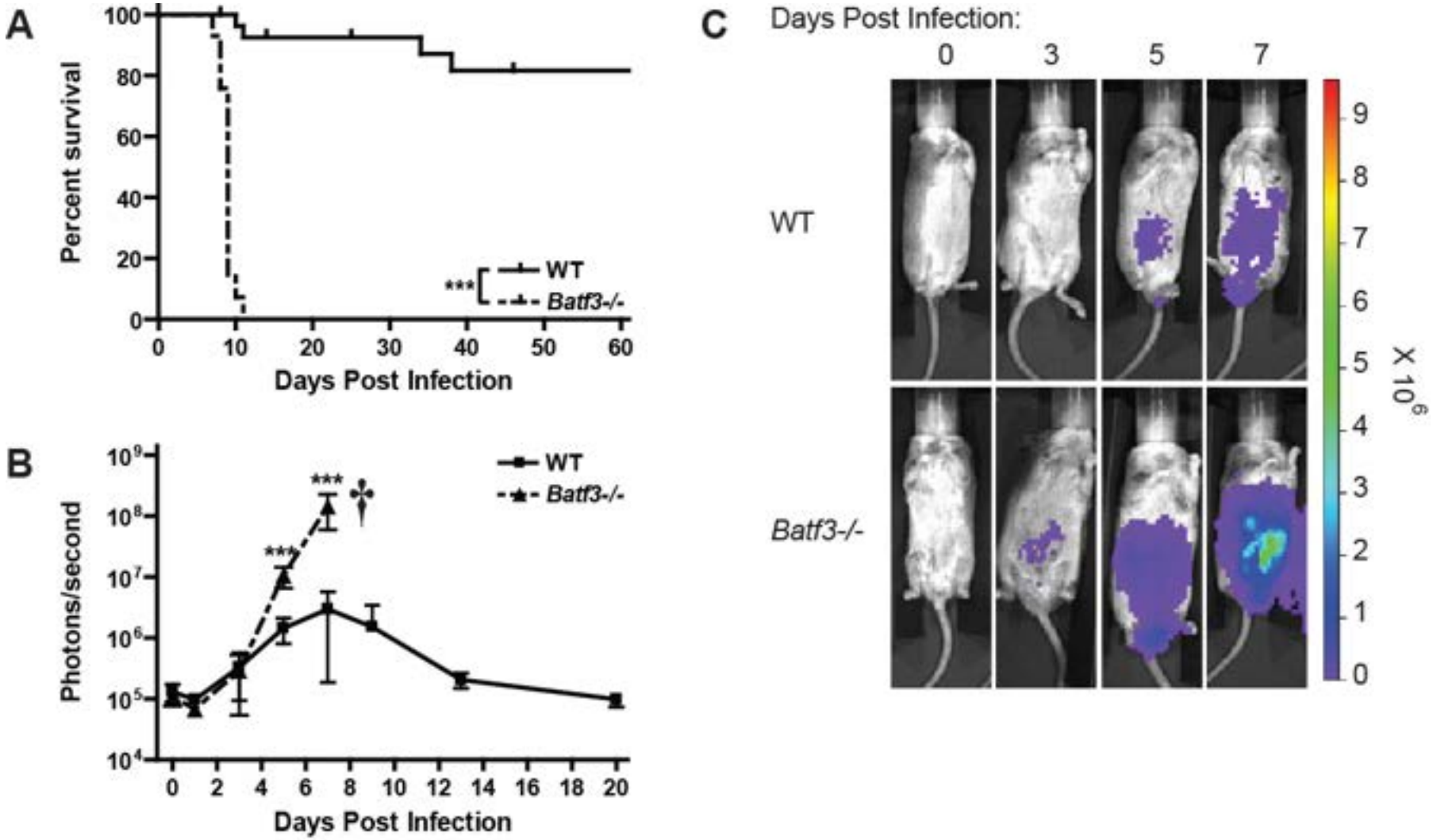
Hildner et al.,, 2008

... and for checkpoint blockade

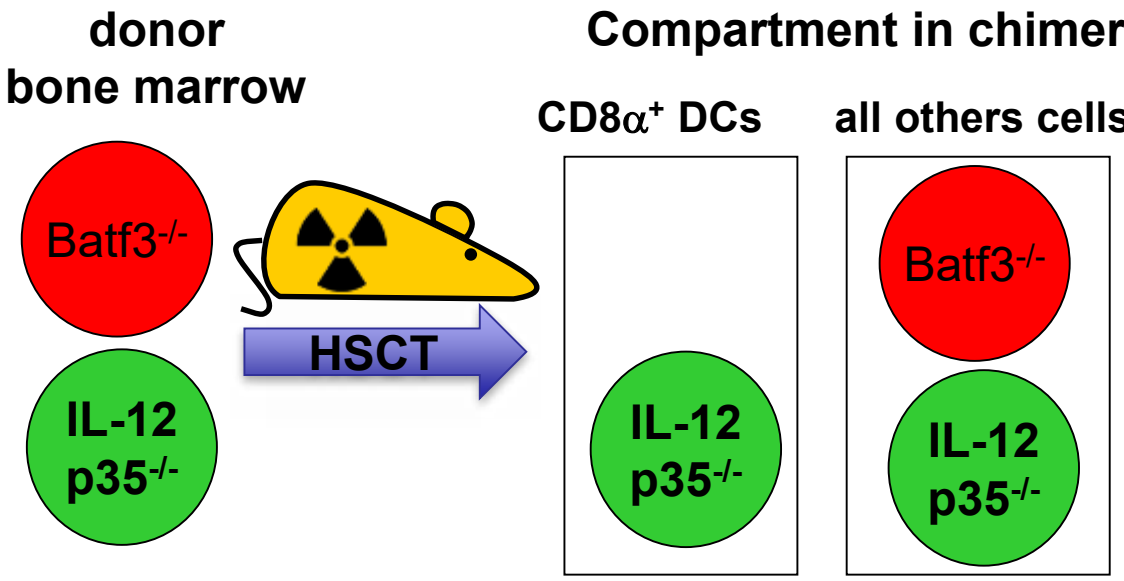
Gubin et al., 2014

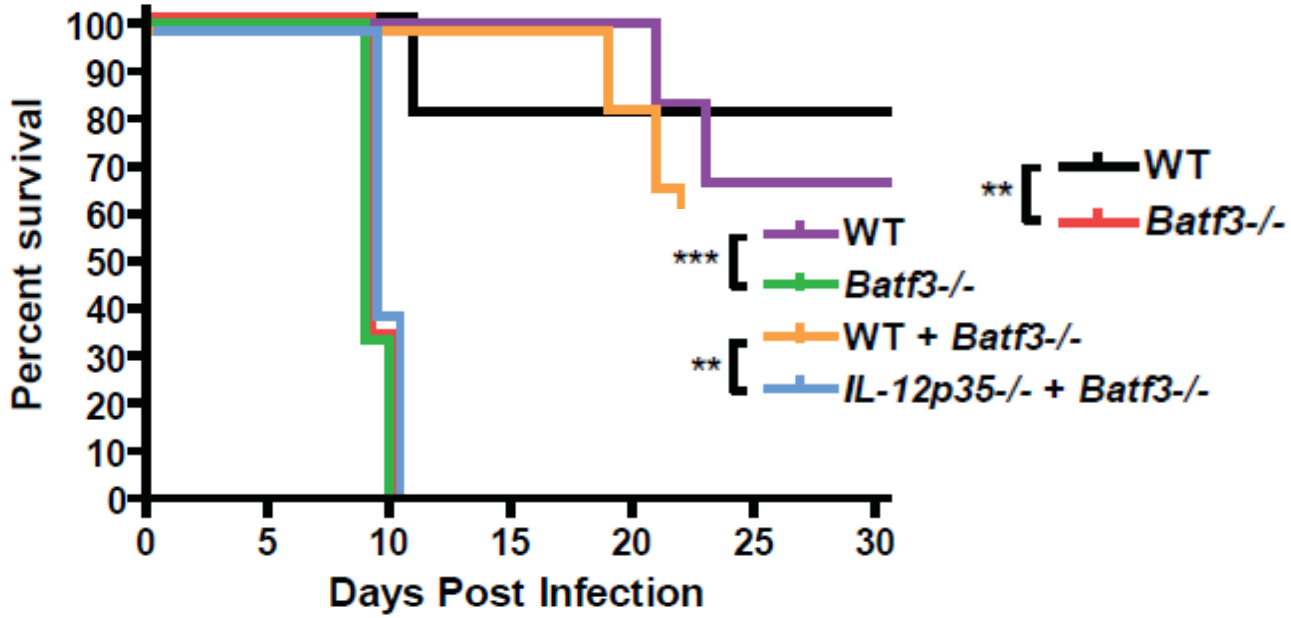


Batf3$^{-/-}$ mice die rapidly after *T. gondii* infection and cannot control parasite replication

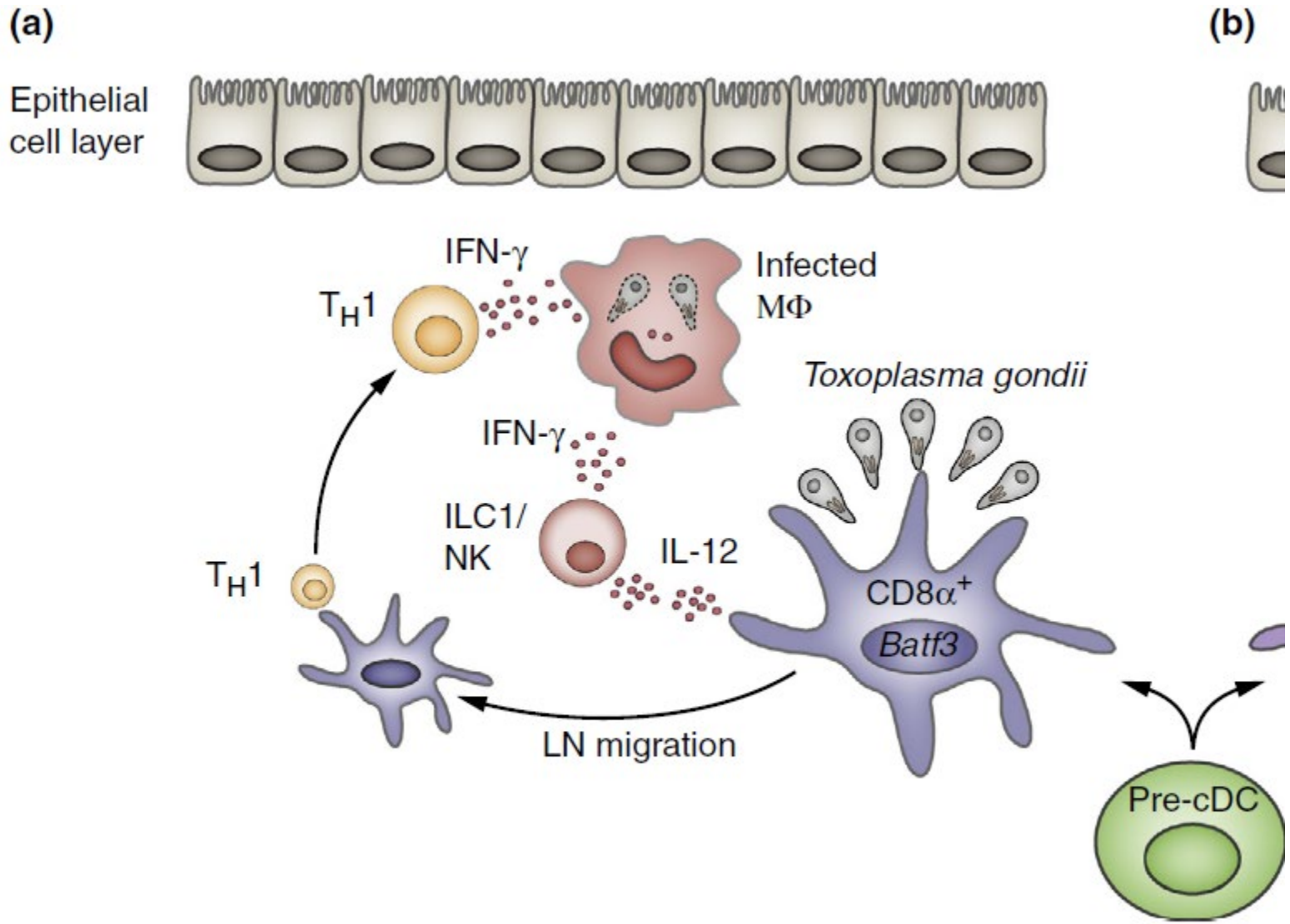


IL-12 from CD8α⁺ DCs is required in *T. gondii*

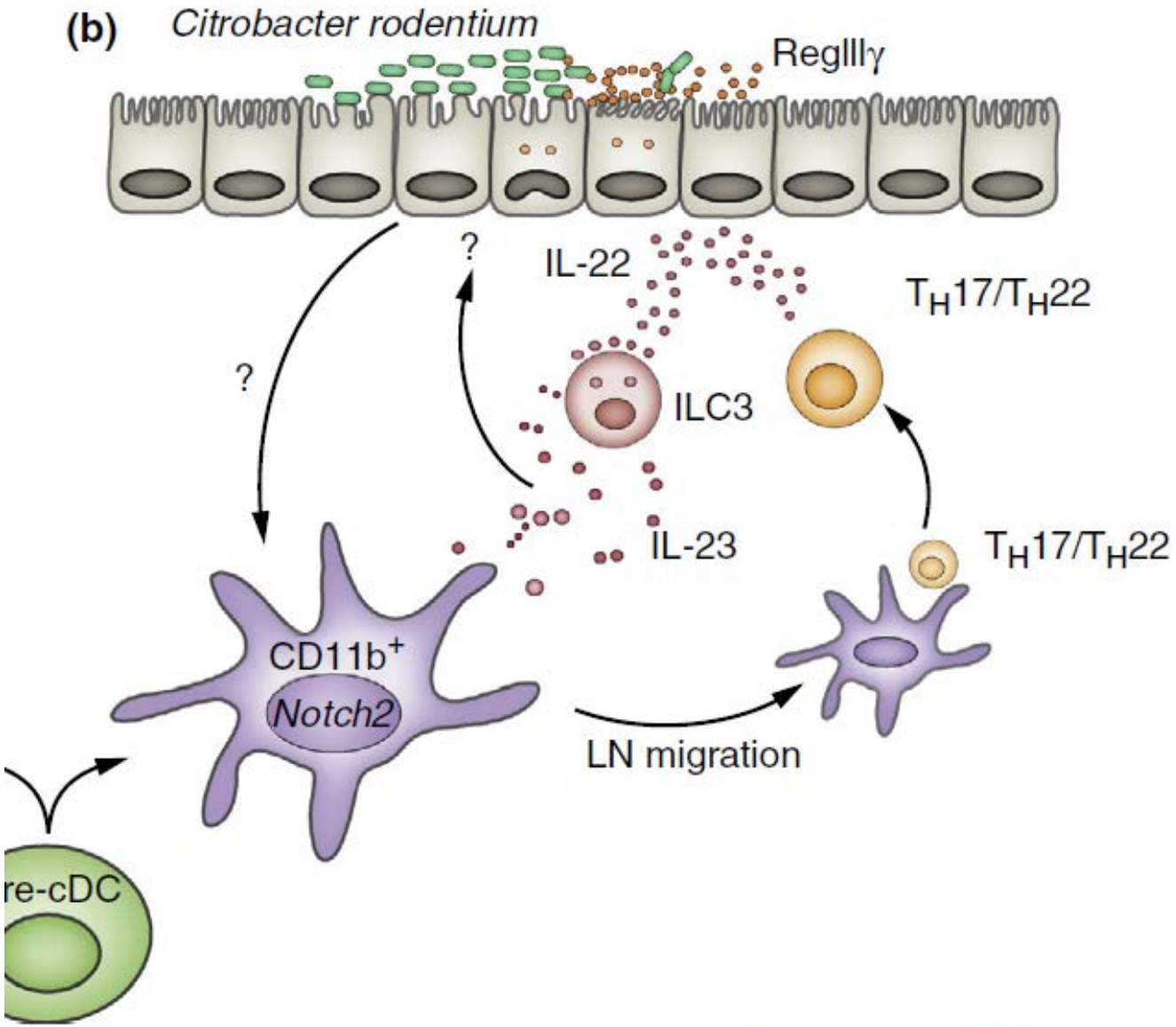




cDC1 are useful in defense against *Toxoplasma gondii*



cDC2 are useful in defense against *Citrobacter rodentium*



Summary – cDC1 Part 1

What we know.
BATF3 is required for cDC1 development.
cDC1 are required to prime CD8 T cell responses to viruses and tumors
cDC1 provide defense against *T. gondii* by sensing (TLR11/12) and producing IL-12 to activate NK cells.

What we don't know.
-It is still unclear why cDC1 and cDC2 seem to have different capacity for IL-12 and IL-23 production.
-We don't know for sure that the defense is ONLY due to TLR expression.
- no work on subset-specific TLR in cDC1/cDC2 activity

What distinguishes cDC1 and cDC2 gene programs?

Immunity

CellPress

Article

High Amount of Transcription Factor IRF8 Engages AP1-IRF Composite Elements in Enhancers to Direct Type 1 Conventional Dendritic Cell Identity

Sunkyung Kim,[1] Prachi Bagadia,[1,3] David A. Anderson III,[1] Tian-Tian Liu,[1] Xiao Huang,[1] Derek J. Theisen,[1] Kevin W. O'Connor,[1] Ray A. Ohara,[1] Arifumi Iwata,[1,4] Theresa L. Murphy,[1] and Kenneth M. Murphy[1,2,5,*]

[1]Department of Pathology and Immunology, Washington University in St. Louis, School of Medicine, St. Louis, MO 63110, USA

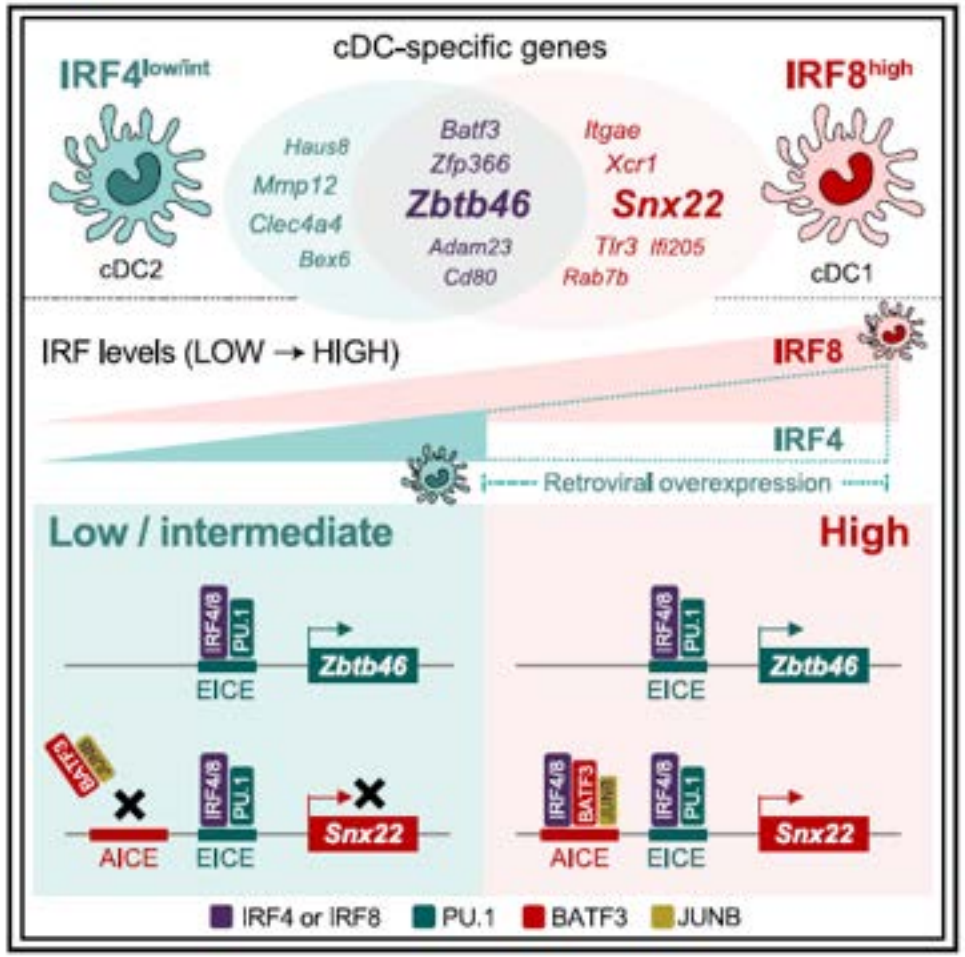
[2]Howard Hughes Medical Institute, Washington University in St. Louis, School of Medicine, St. Louis, MO 63110, USA

[3]Present address: Department of Oncology, Amgen Inc., 1120 Veterans Boulevard, South San Francisco, CA 94080, USA

[4]Present address: Department of Allergy and Clinical Immunology, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan

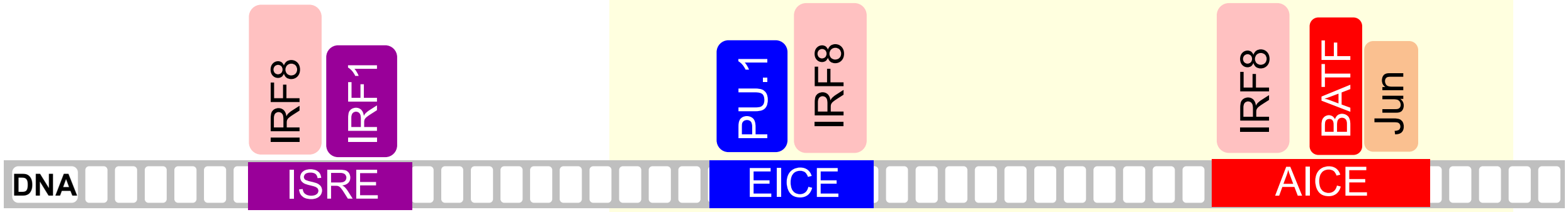
[5]Lead Contact

Graphical Abstract



IRF4 and IRF8 can bind DNA in three ways.

IRF4/8-specific interactions



IFN-**S**timulated **R**esponse **E**lement

---**AA**nn**GAAA**---

Levy and Darnell
Gene and Dev. 1988

Ets-**I**RF **C**omposite **E**lement

---**GGAA**nn**GAAA**---

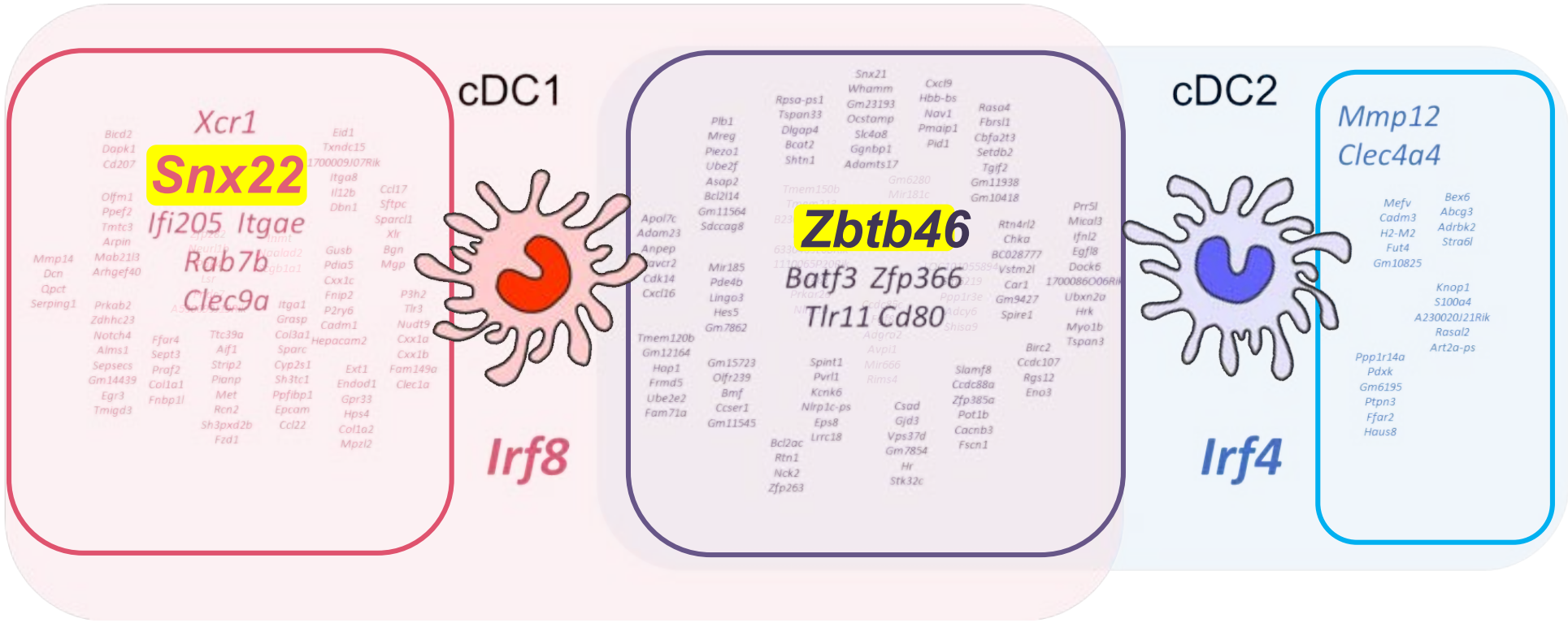
Eisenbeis and Storb
Gene and Dev. 1995

Ap1-**I**RF **C**omposite **E**lement

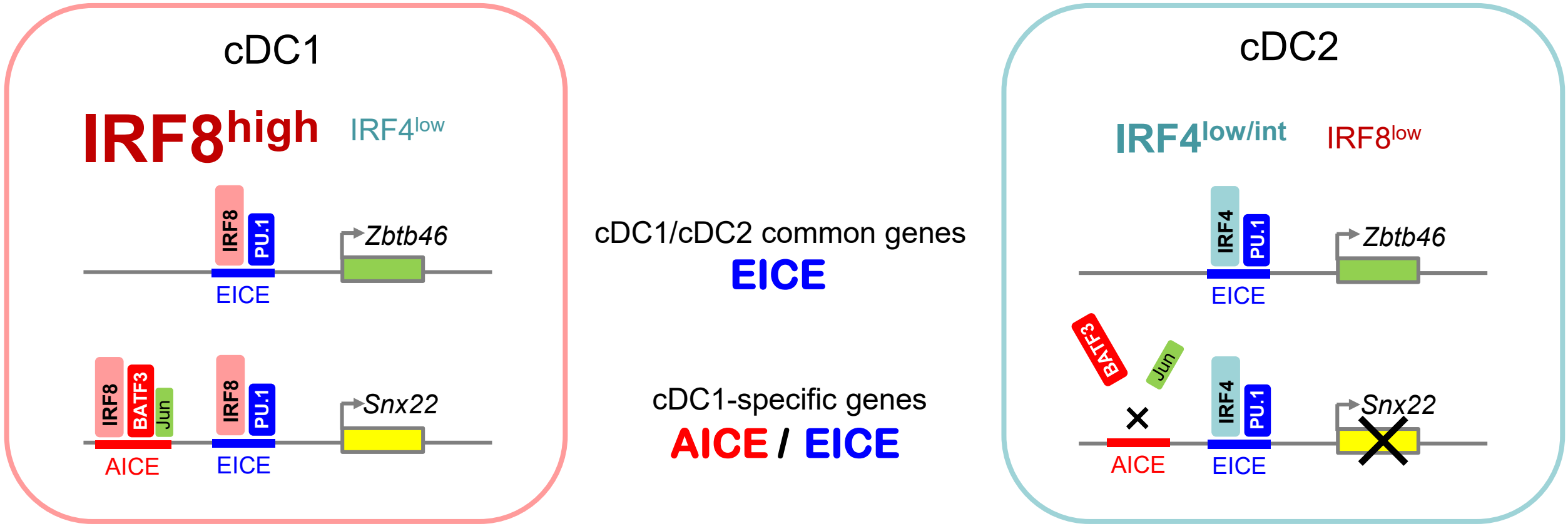
AICE1 ---**TTTC**nnnn**TGA**n**TCA**---
AICE2 ---**GAAATGA**n**TCA**---

Tussiwand Nature 2012
Ciofani Cell 2012
Glasmacher Science 2012

cDC1 engage an AICE-dependent gene program.



High IRF8 activated the cDC1-specific AICE gene program.

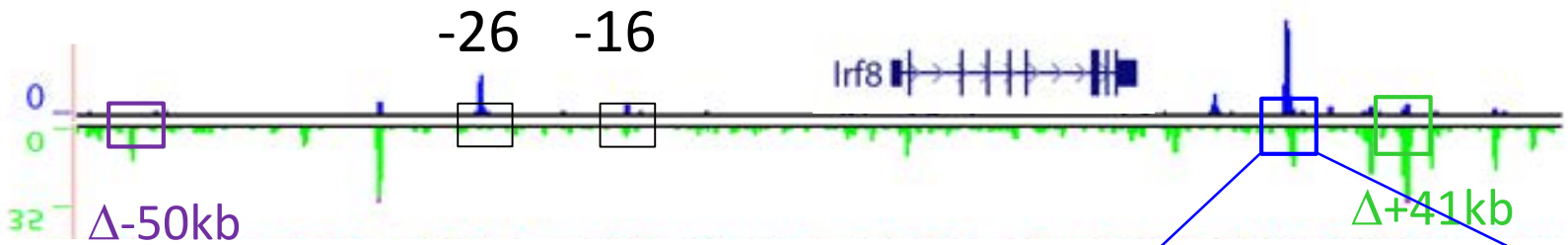


Summary

What we know.
cDC1 express HIGH levels of IRF8, cDC2 express low IRF8/IRF4
cDC1-specific genes are controlled by AICEs and EICEs
BATF3 has virtually no known functions in cDC2.
cDC1-specific genes require HIGH level of IRF8 to occupy AICEs

What we don't know.
How is cDC2-specific gene expression imparted?
Are there IRF4-specific targets? Why is Batf3 expressed in cDC2?
Are there subsets of cDC2, probably, but how?
How is Batf3 required for cDC1 development? this we will now address.

The *Irf8* +32 kb enhancer contains AICEs binding Batf3 and Irf8

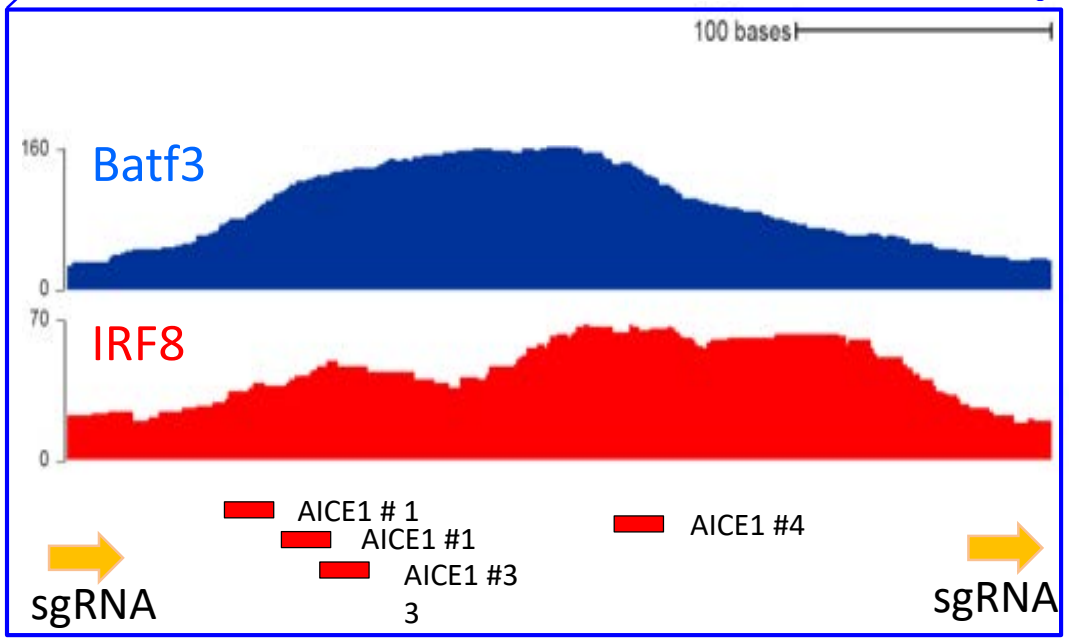


Ap1-**I**RF **C**omposite **E**lement

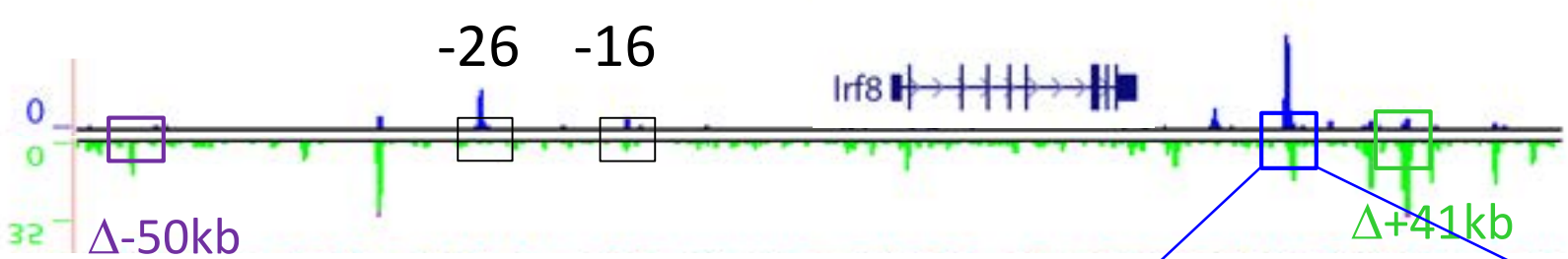
AICE1 ---**TTTC**nnnn**TGA**n**TCA**---
AICE2 ---**GAAATGA**n**TCA**---

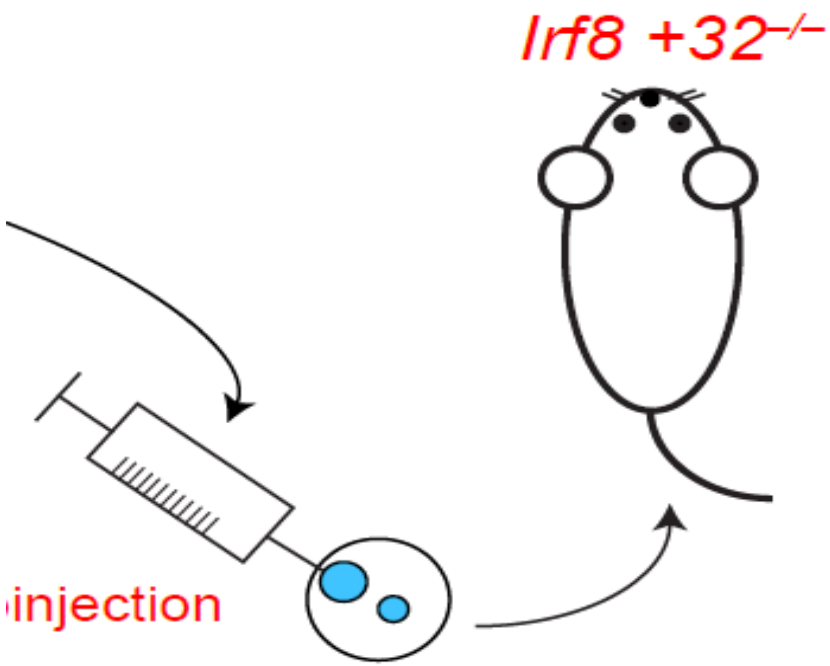
Tussiwand Nature 2012
Ciofani Cell 2012
Glasmacher Science 2012

+32kb enhancer

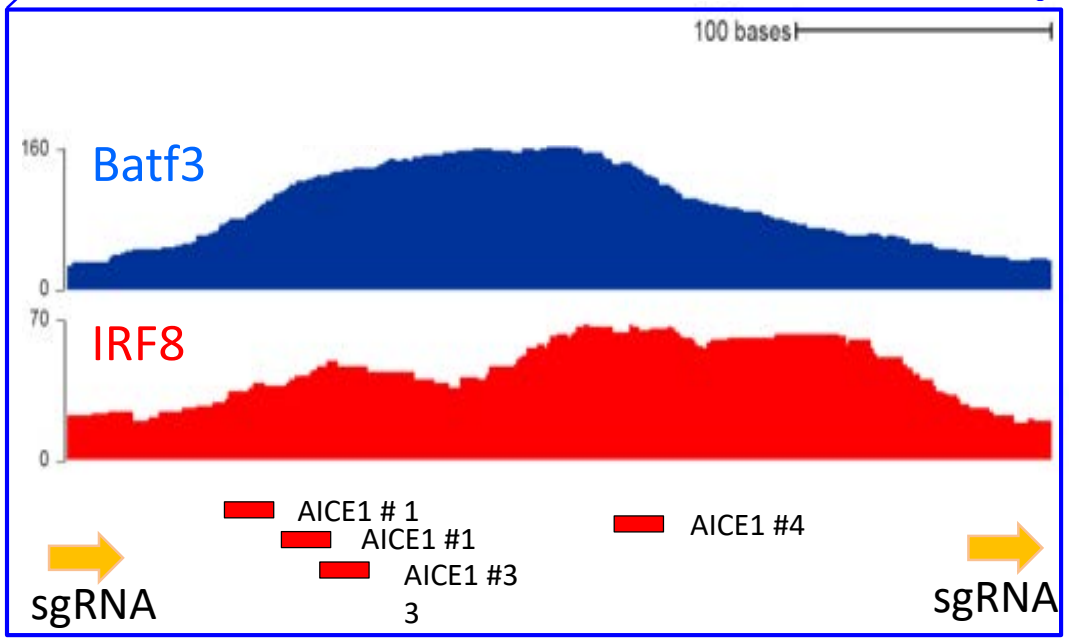


The *Irf8* +32 kb enhancer contains AICEs binding Batf3 and Irf8



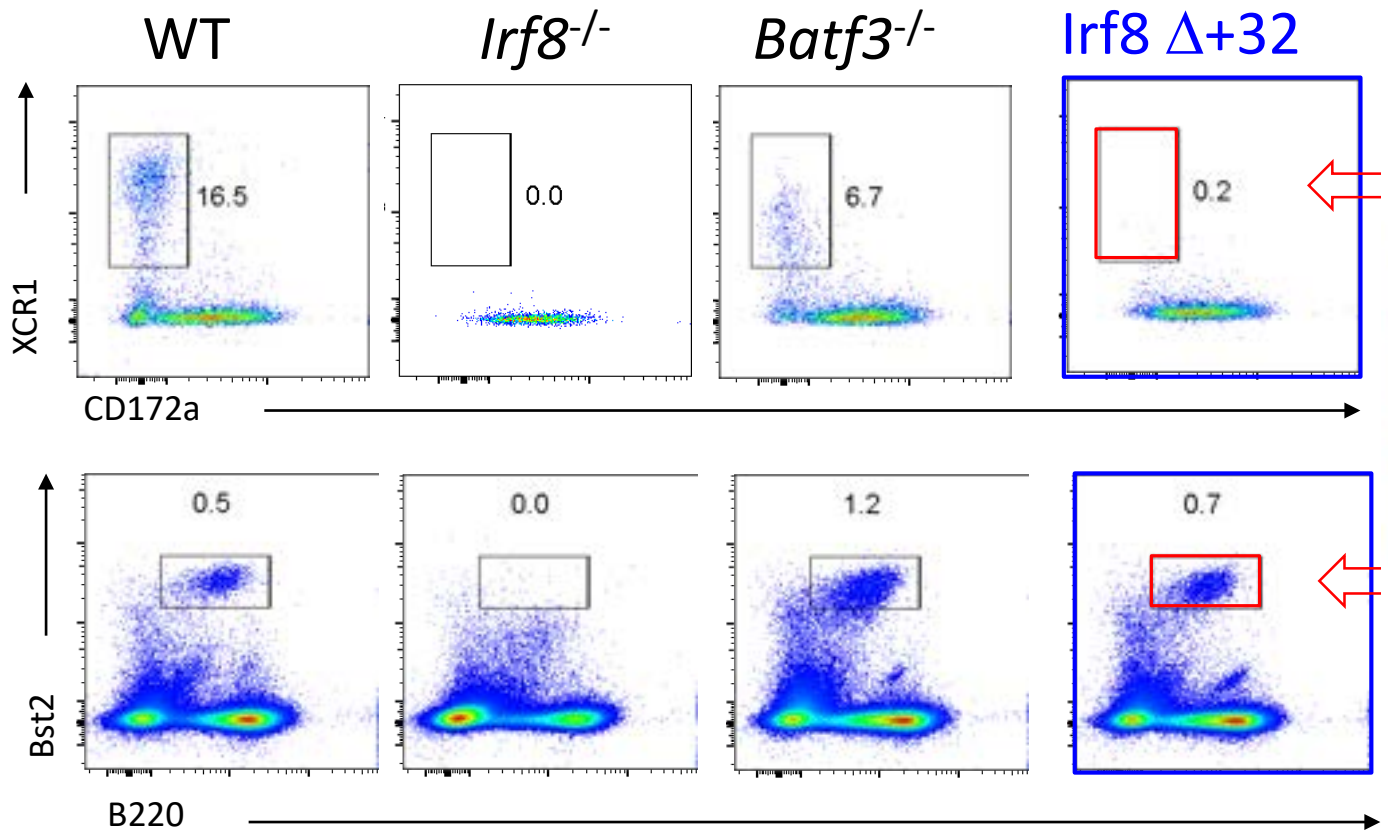


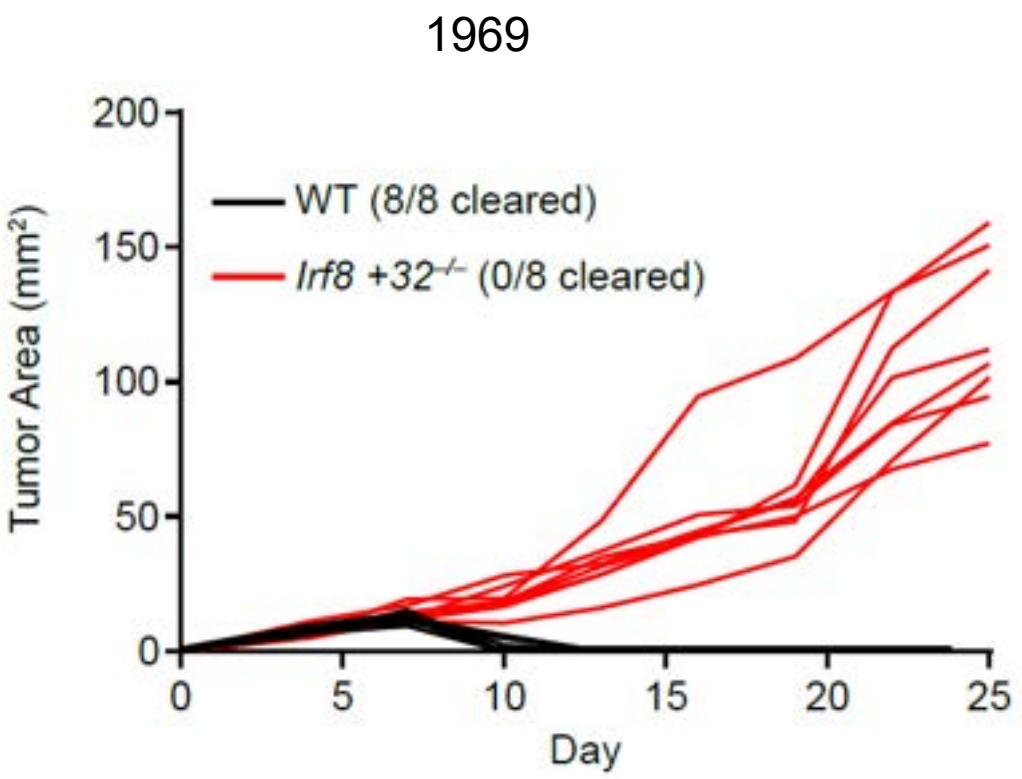
+32kb enhancer



+32 kb *Irf8* enhancer is absolutely required for cDC1 development

AUTOACTIVATION- IRF8 drives IRF8 with help from BATF3





Durai, V., P. Bagadia, accepted at *Nat. Immunol.* 2019

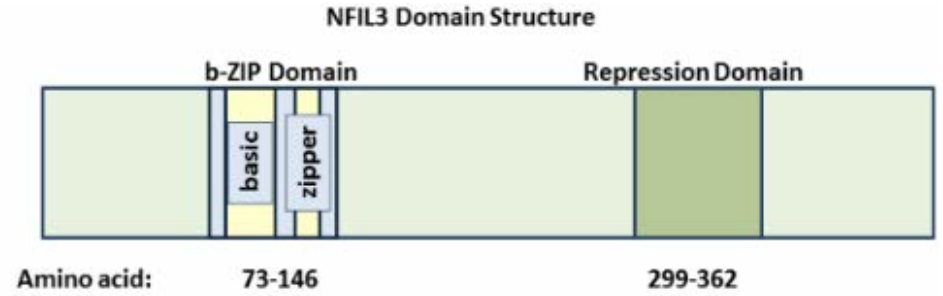
ARTICLES
https://doi.org/10.1038/s41590-019-0449-3
nature immunology

An *Nfil3–Zeb2–Id2* pathway imposes *Irf8* enhancer switching during cDC1 development

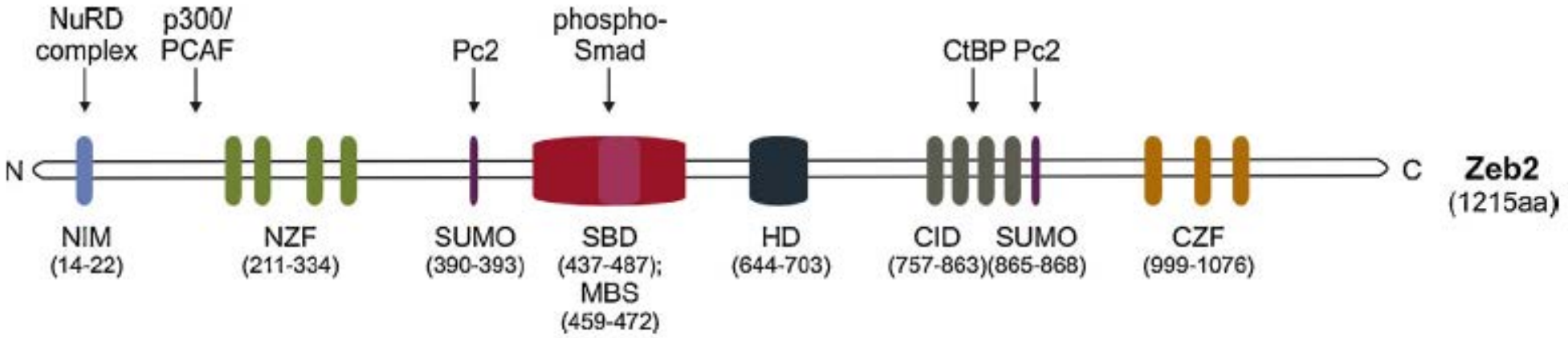
2019

Prachi Bagadia[1,13], Xiao Huang[1,13], Tian-Tian Liu[1,2,13],

NFIL3



Zeb2



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An *Nfil3–Zeb2–Id2* pathway imposes *Irf8* enhancer switching during cDC1 development

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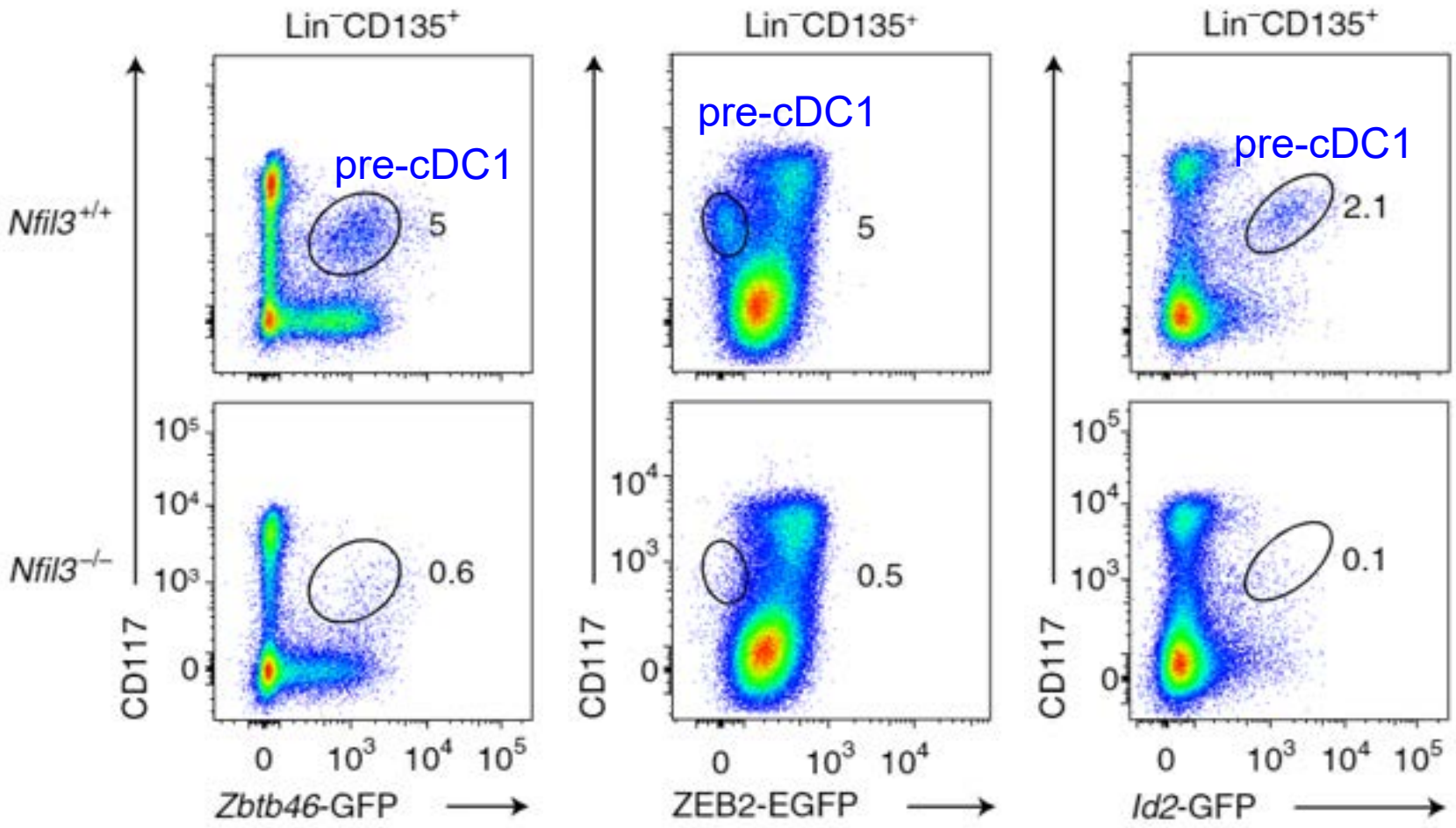
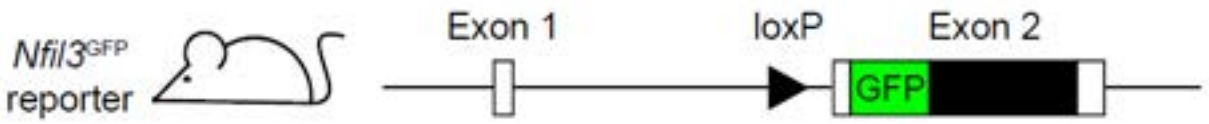
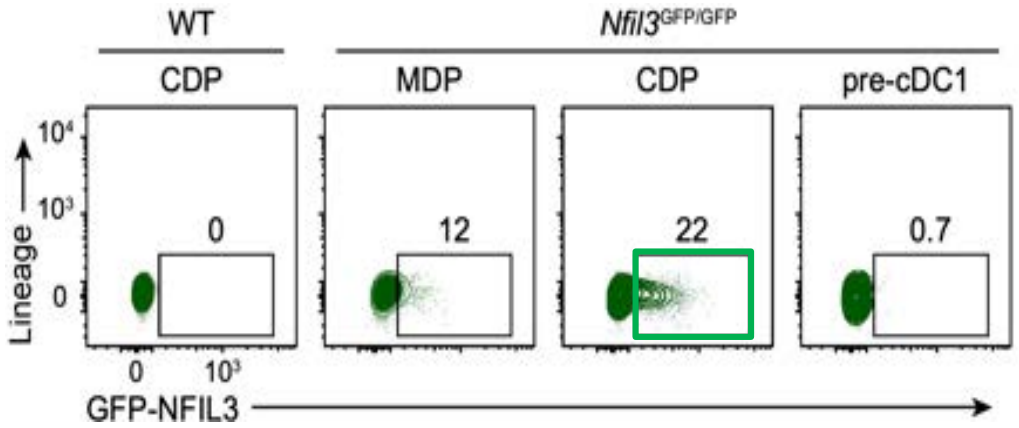
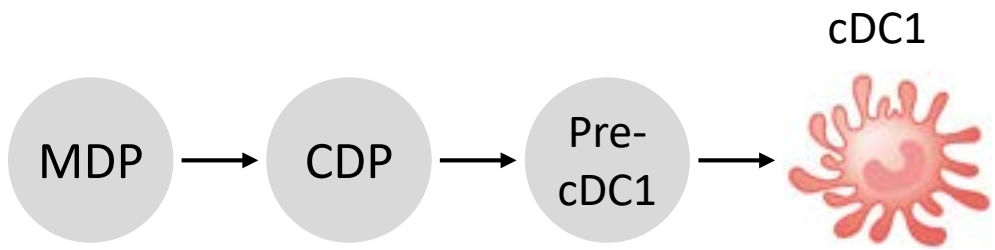


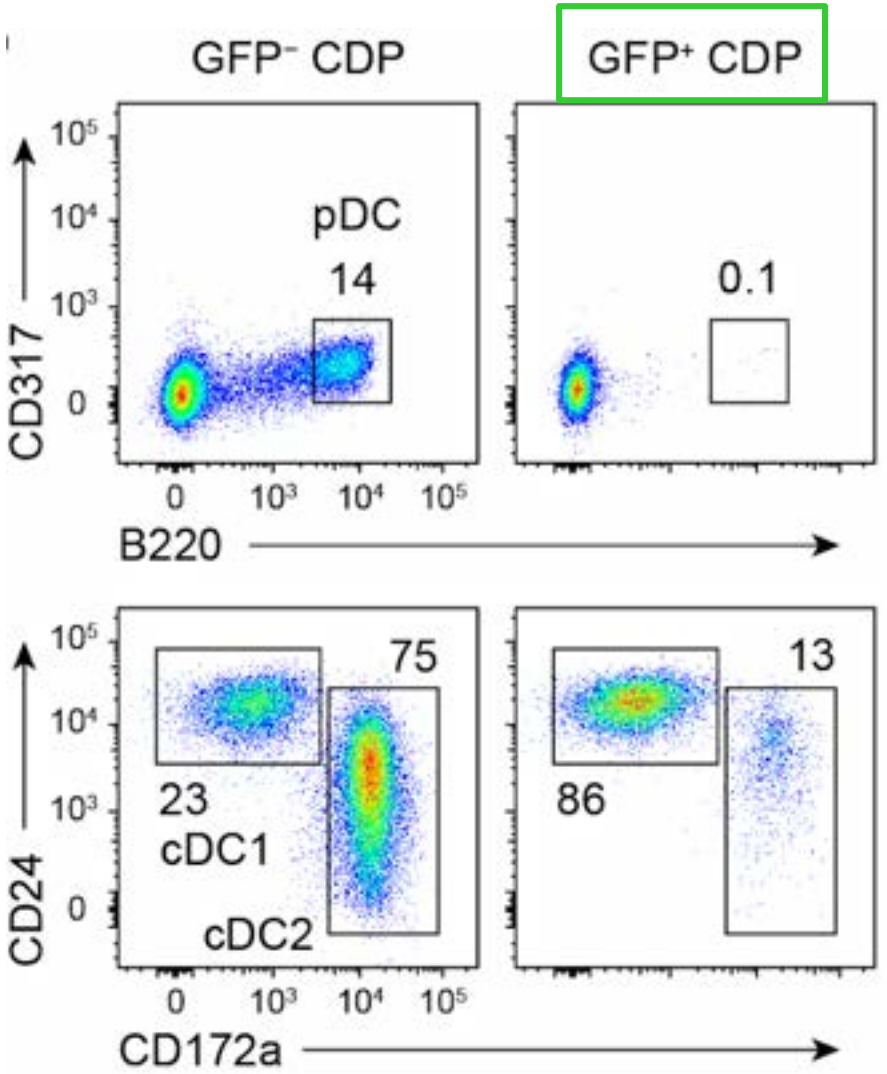
A transient pulse of NFIL3 induces cDC1 specification



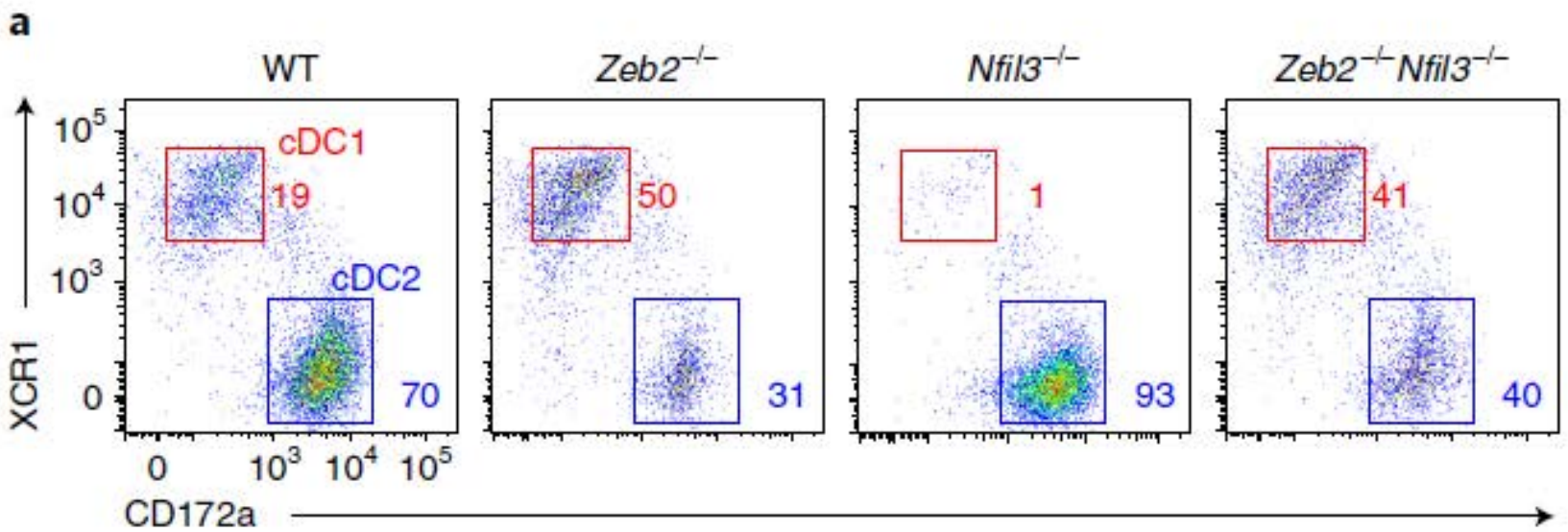


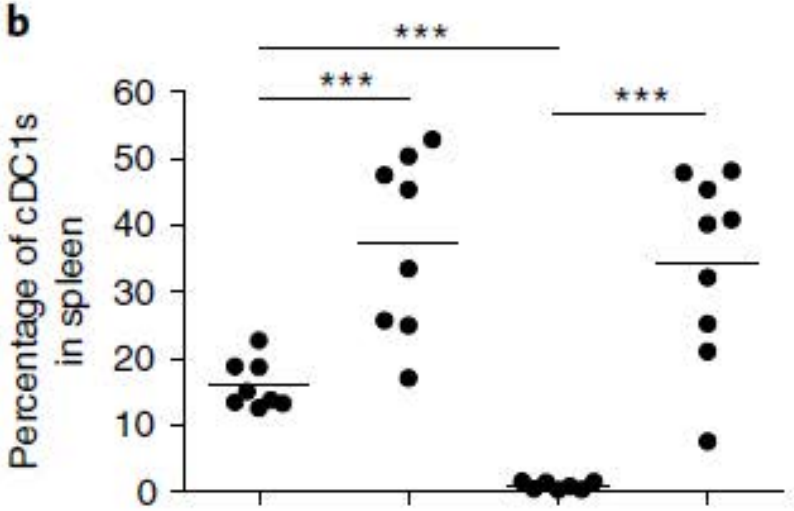


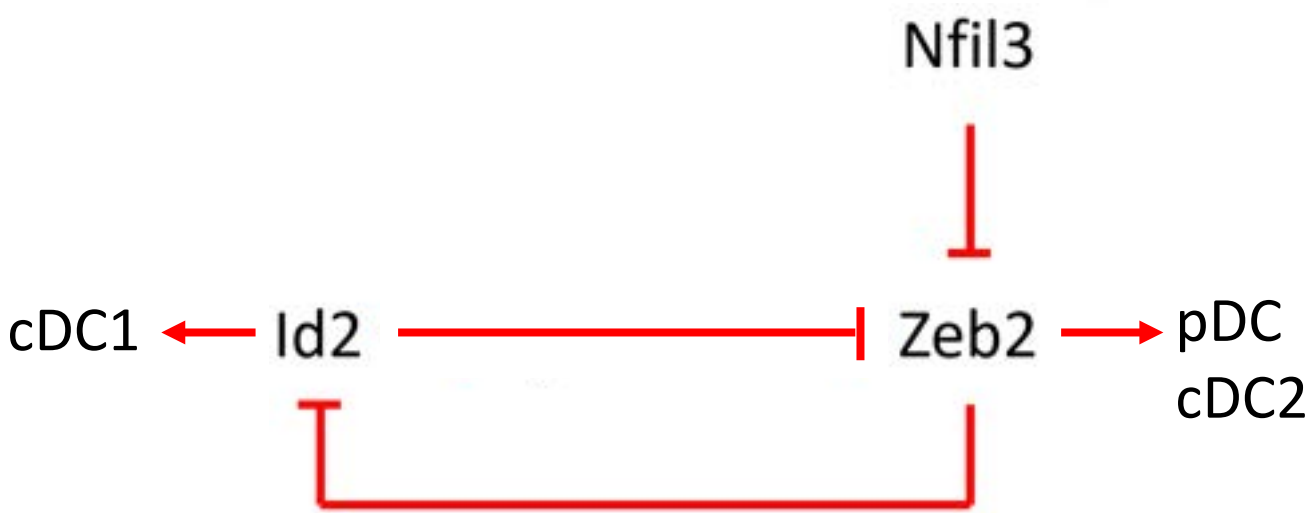
Sorted cells cultured in Flt3L



Nfil3 acts upstream of Zeb2 in cDC1 development

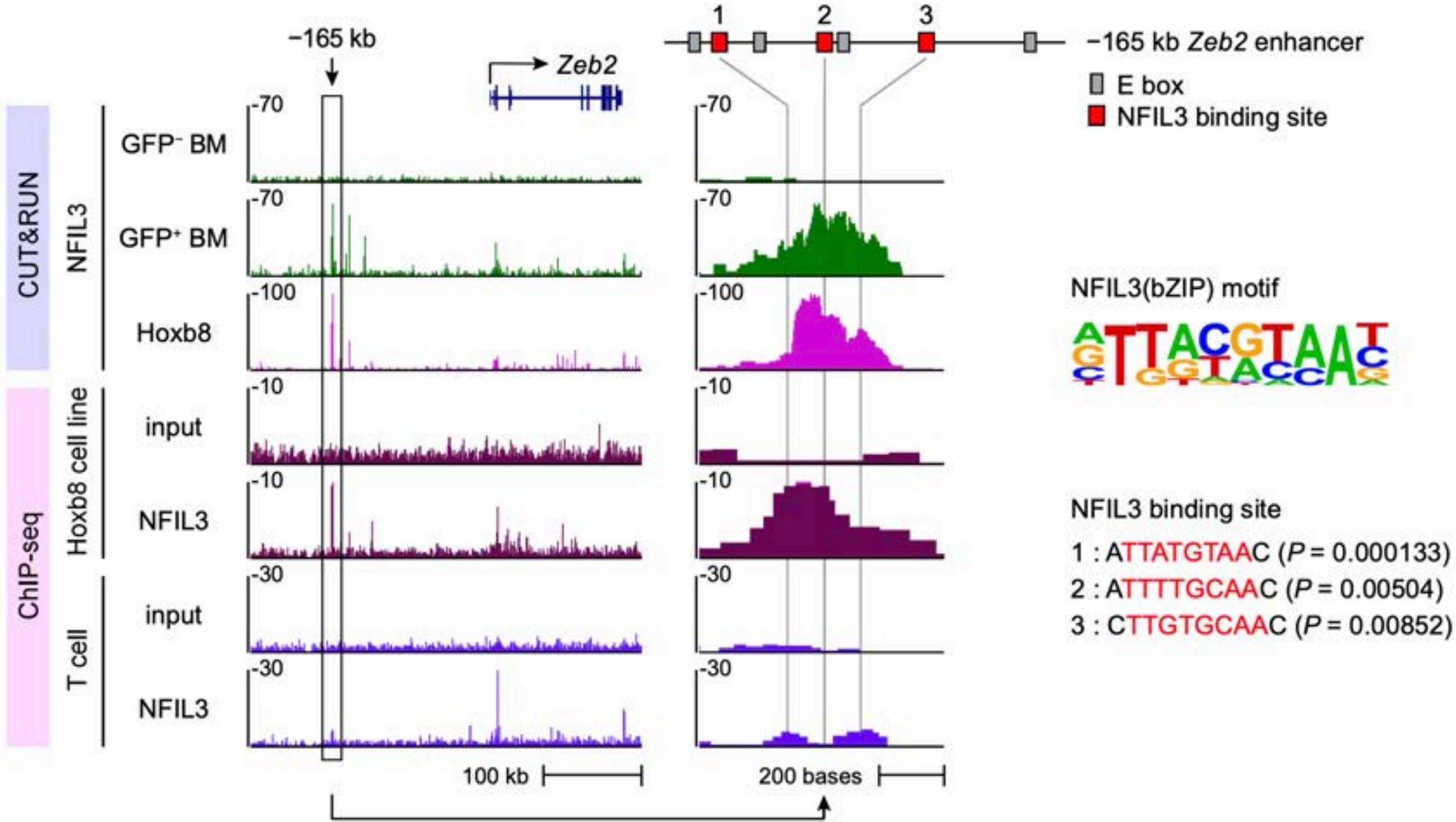






Bagadia, Huang, Liu *et al*. *NI*. 2019

NFIL3 binds to −165 kb *Zeb2* enhancer



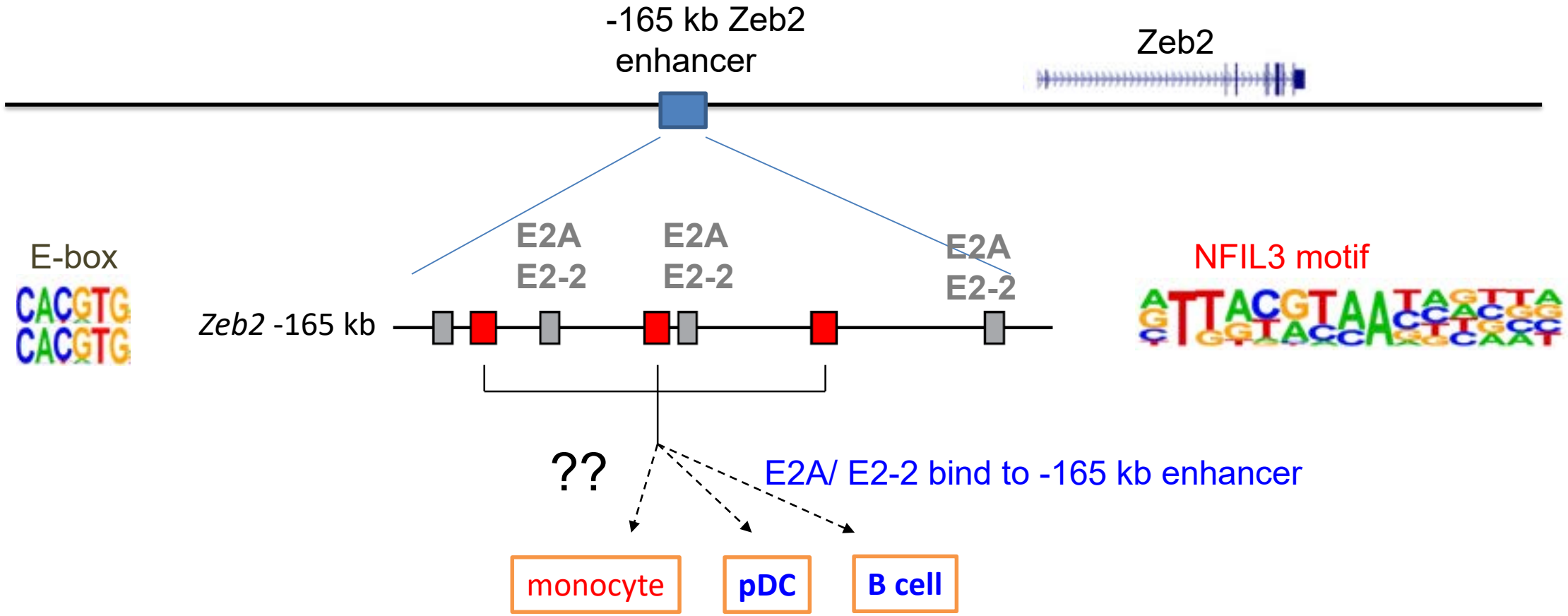
The -165 kb Zeb2 enhancer is required for B cells, pDCs and monocytes

Immunity July 2021

Article

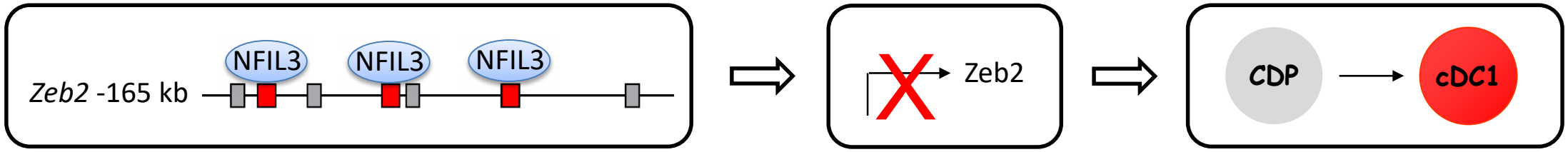
Differential usage of transcriptional repressor *Zeb2* enhancers distinguishes adult and embryonic hematopoiesis

Xiao Huang,[1] Stephen T. Ferris,[1] Sunkyung Kim,

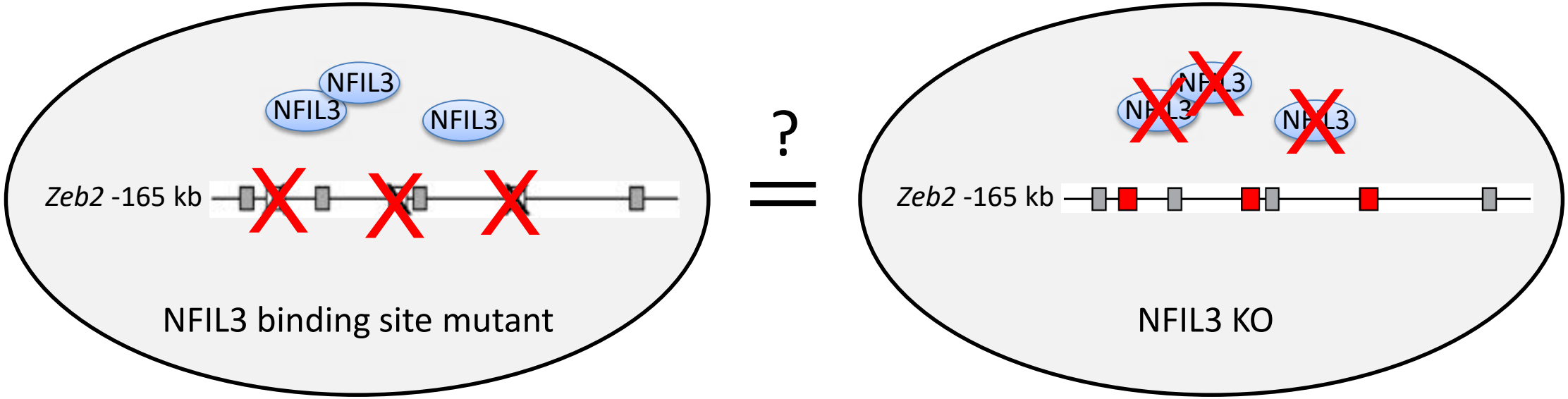


Hypothesis

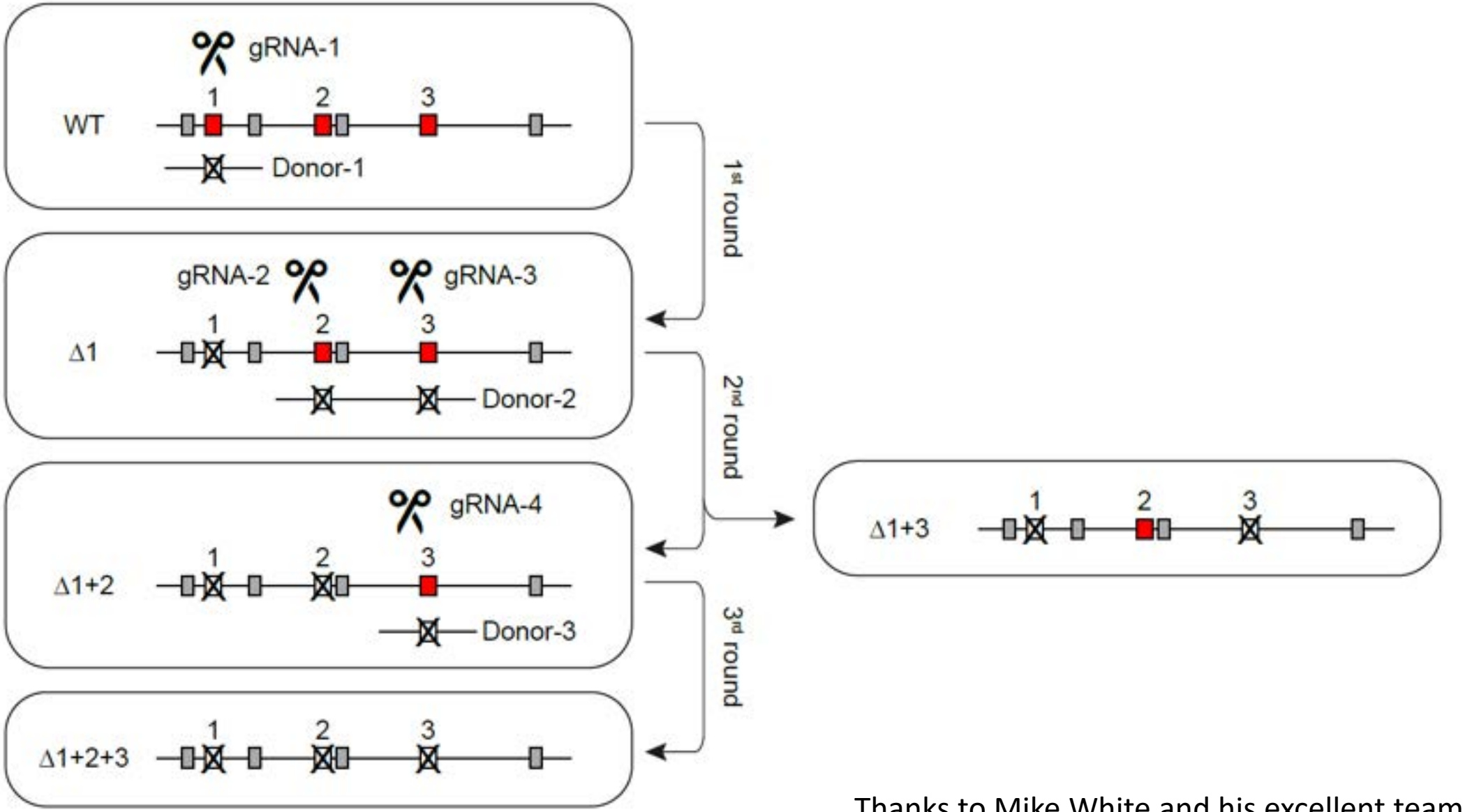
NFIL3 directly represses the *Zeb2* -165kb enhancer to drive cDC1 specification



Test: Does mutation of the NFIL3 binding sites eliminate cDC1 development?

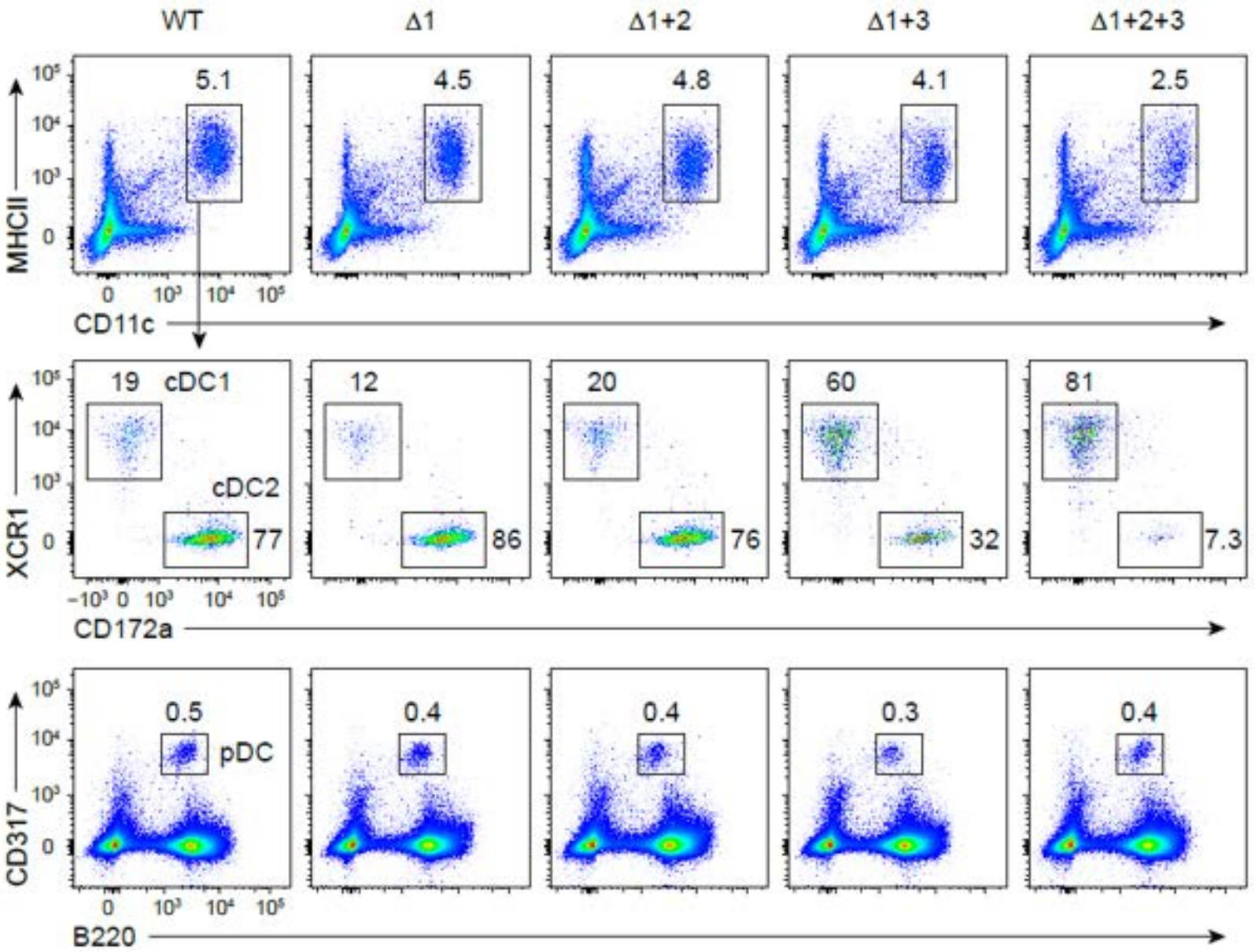


Sequential mutation of sites 1, 2 and 3 in combinations

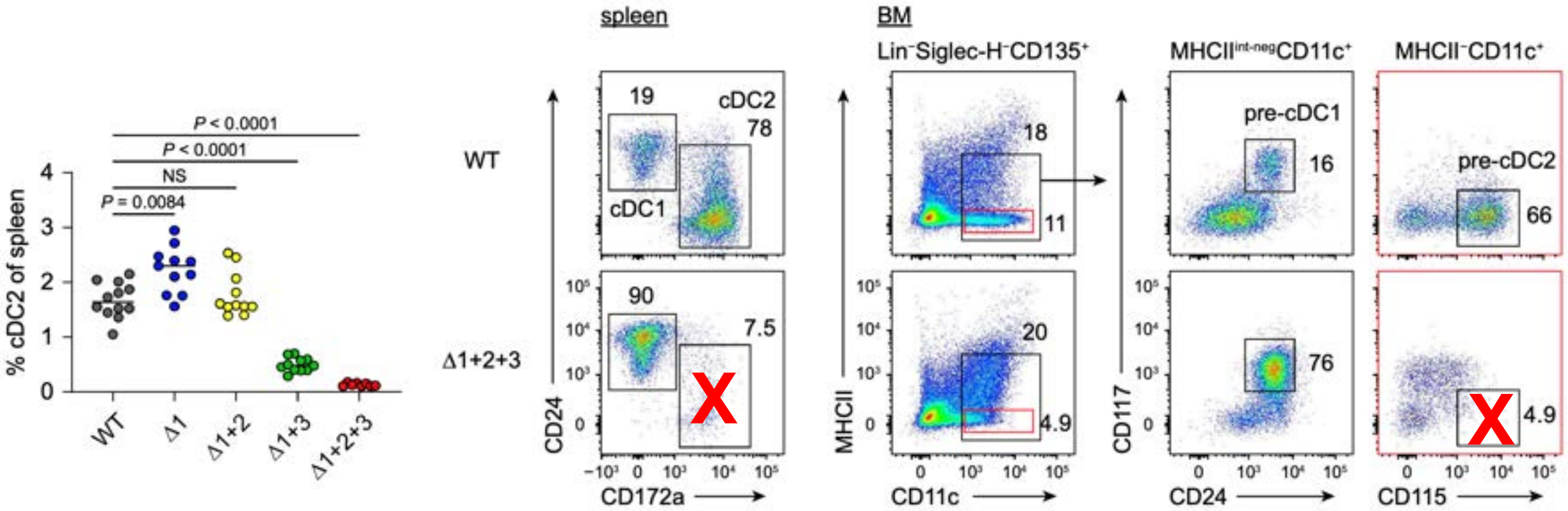


Thanks to Mike White and his excellent team

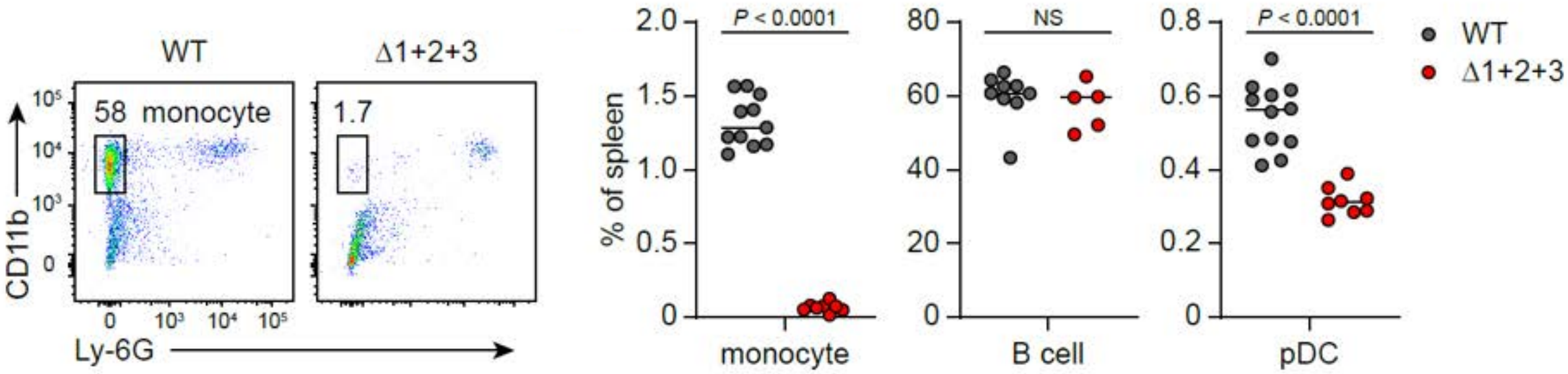
Sequential mutation of sites 1, 2 and 3 in combinations



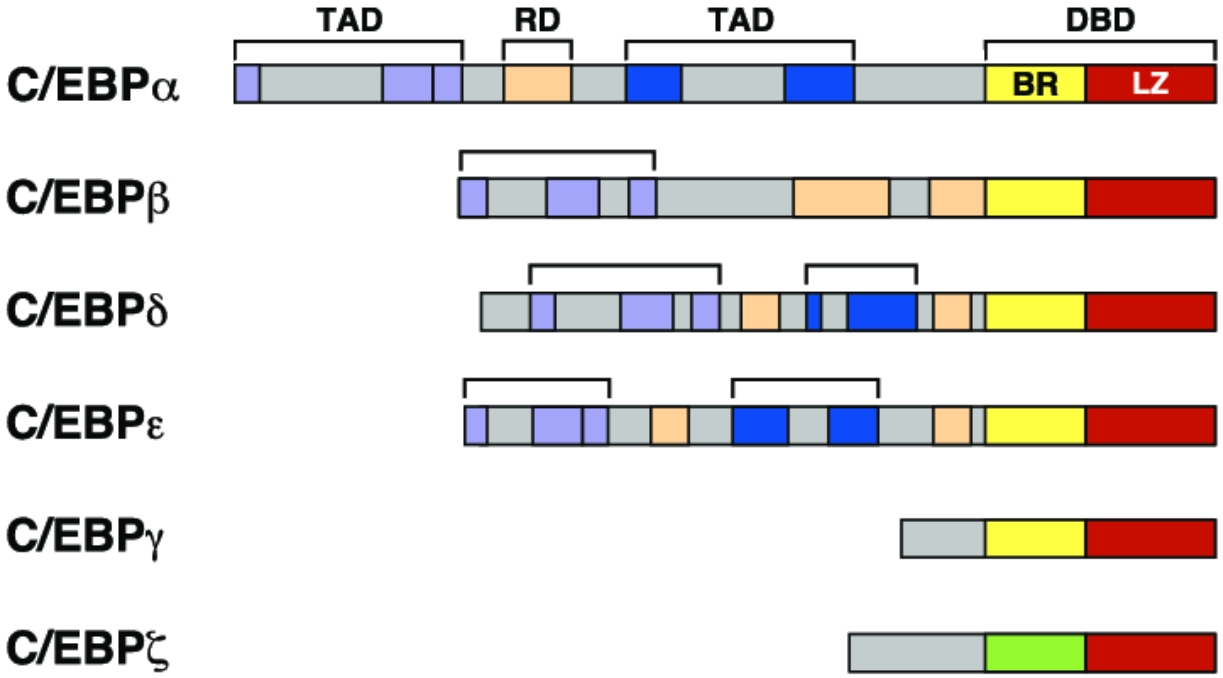
We got exactly the opposite of our prediction – No cDC2 development!

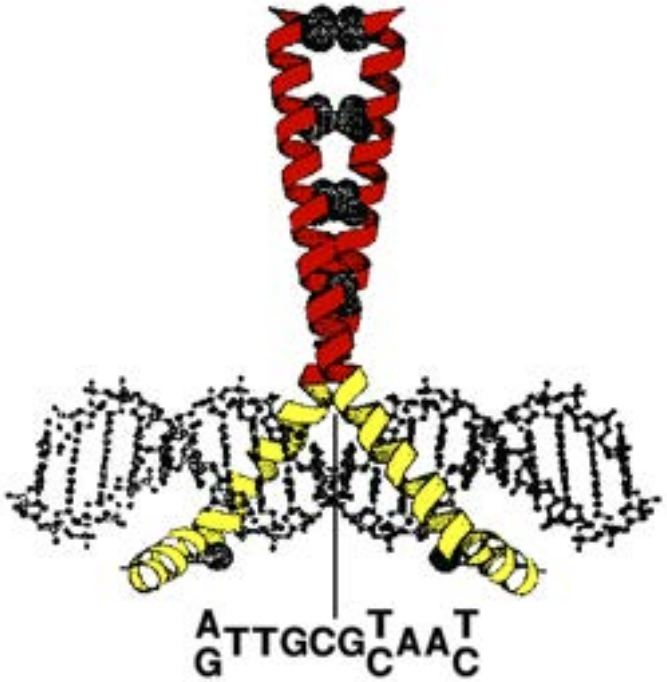


Δ1+2+3 mice lack monocytes but have pDC and B cells



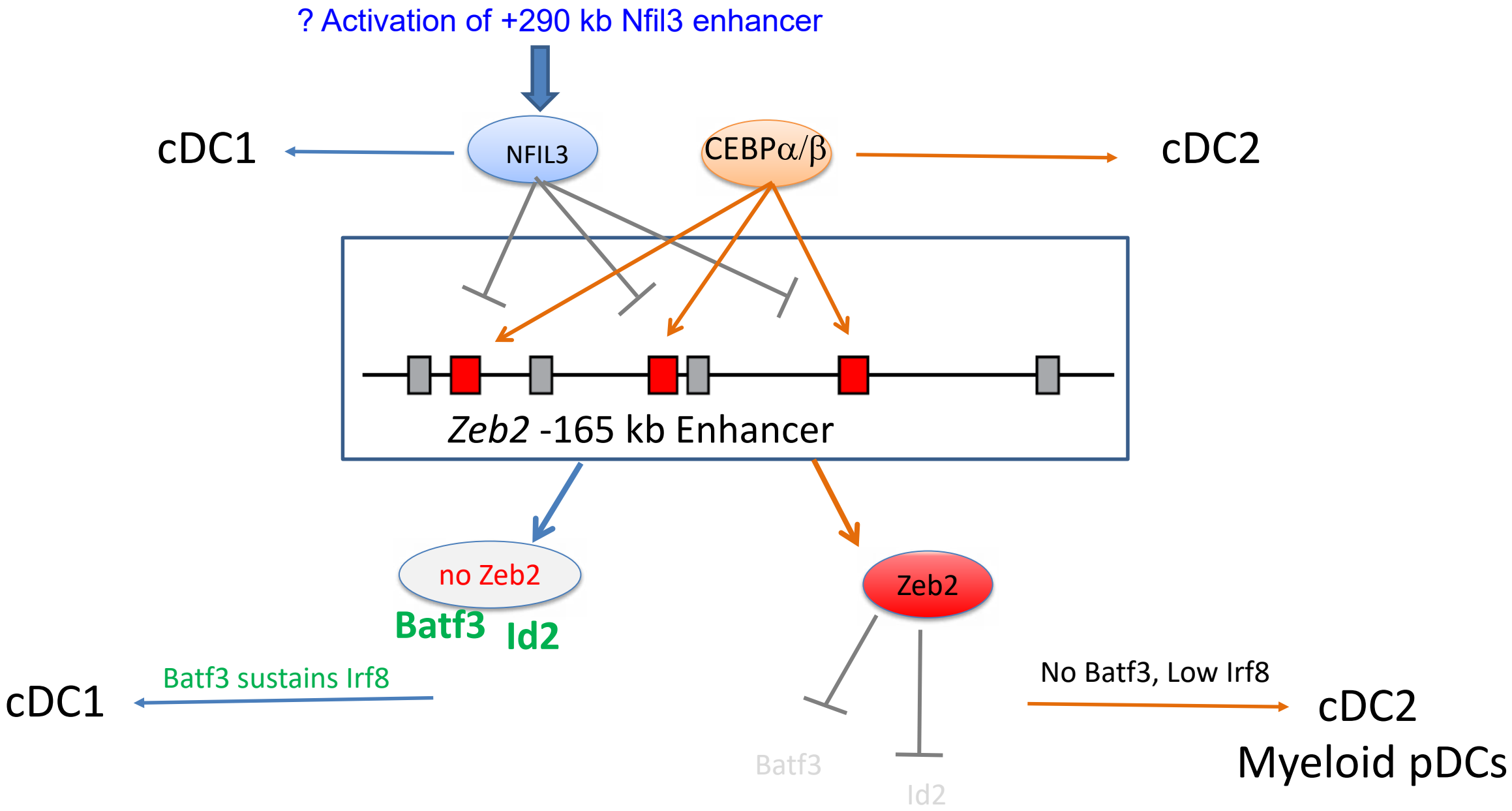
C/EBP family of transcription factors





Peter Johnson J. Cell Science 2005

C/EBP and NFIL3 compete for support or repression of Zeb2



Summary

What we know.
cDC1 and cDC2 split from CDPs based on NFIL3 expression.
NFIL3 transient induction drives cDC1 specification.
NFIL3 inhibits Zeb2 expression at C/EBP sites in the -165kb enhancer.
cDC1 specification leads to induction of Id2 and BATF3
BATF3 maintains HIGH IFR8 expression at the +32kb enhancer

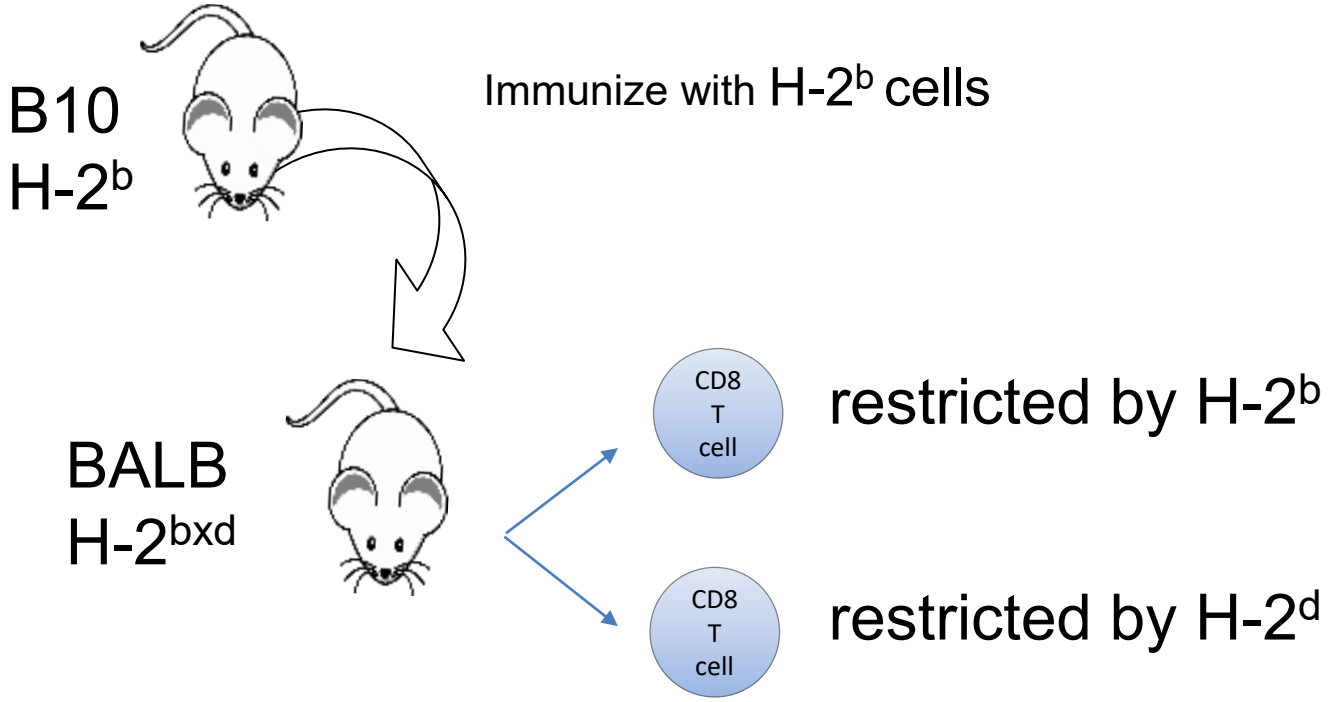
What we don’t know.
How is NFIL3 induced? Timing, Niche, Cytokines? a therapy?
How does Id2 act in cDC1 development? Block E protein? Kill Zeb2?
Does Zeb2 directly repress Batf3 and Id2 expression? Where?

Cross-presentation loads exogenous antigens onto MHC class I

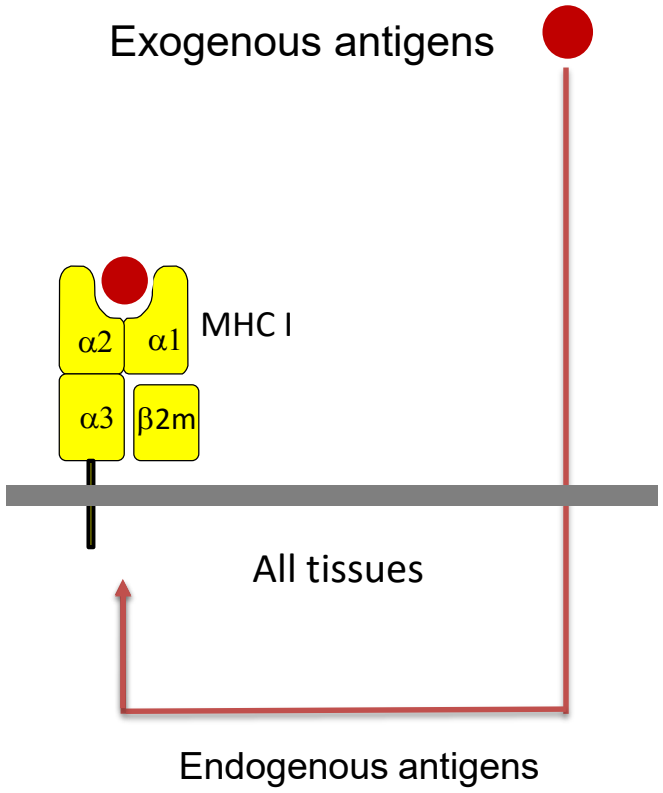
1978

CROSS-PRIMING FOR A SECONDARY CYTOTOXIC RESPONSE TO MINOR H ANTIGENS WITH *H-2* CONGENIC CELLS WHICH DO NOT CROSS-REACT IN THE CYTOTOXIC ASSAY*

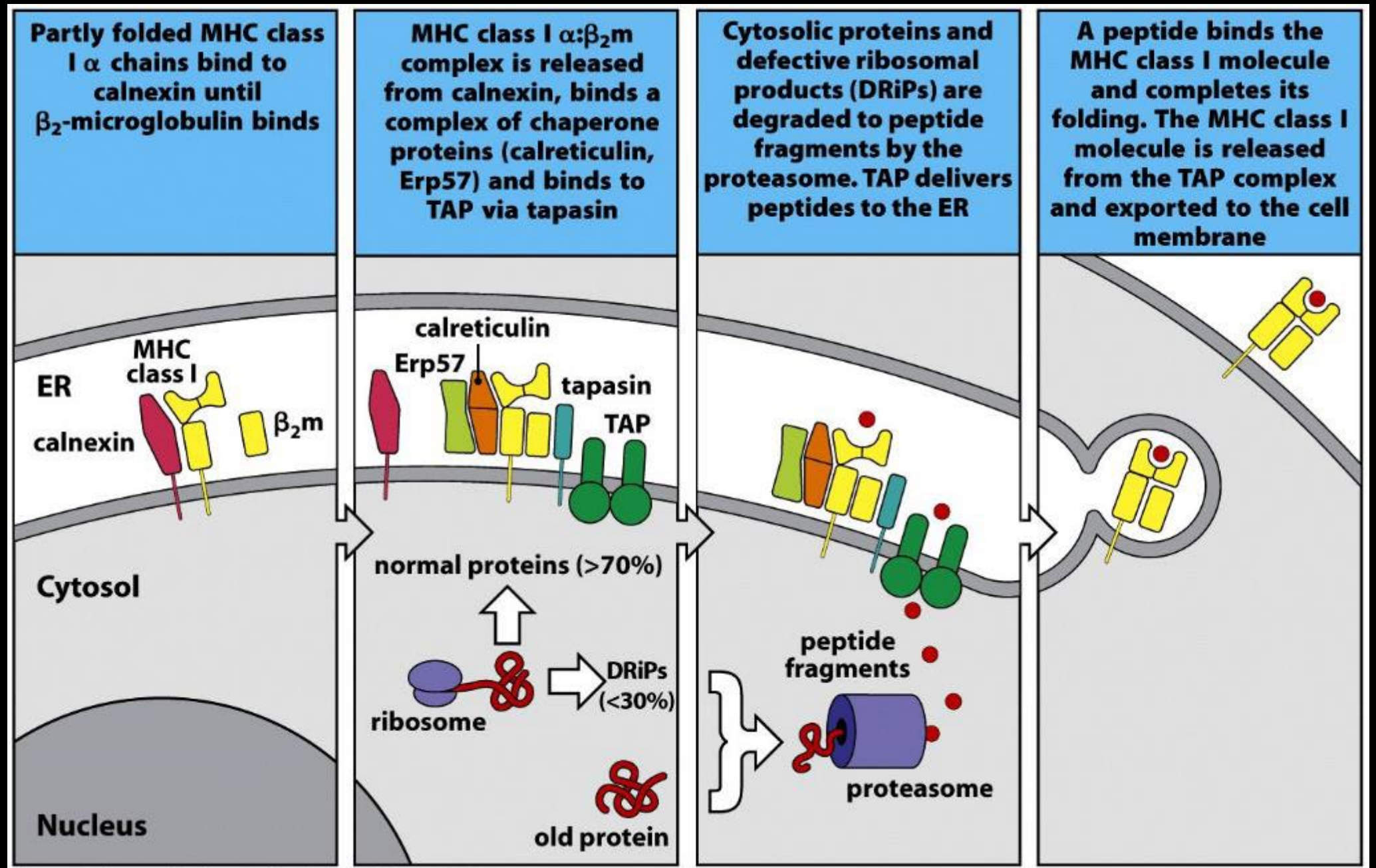
By MICHAEL JOHN BEVAN



Cross-presentation



Dogma says that only cytosolic proteins are loaded onto class I MHC molecules



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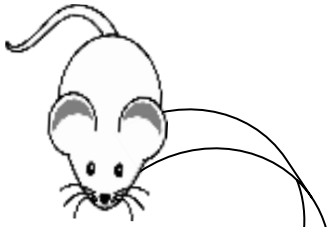
CROSS-PRIMING FOR A SECONDARY CYTOTOXIC RESPONSE TO MINOR H ANTIGENS WITH *H-2* CONGENIC CELLS WHICH DO NOT CROSS-REACT IN THE CYTOTOXIC ASSAY*

By MICHAEL JOHN BEVAN

B10
H-2^{b}

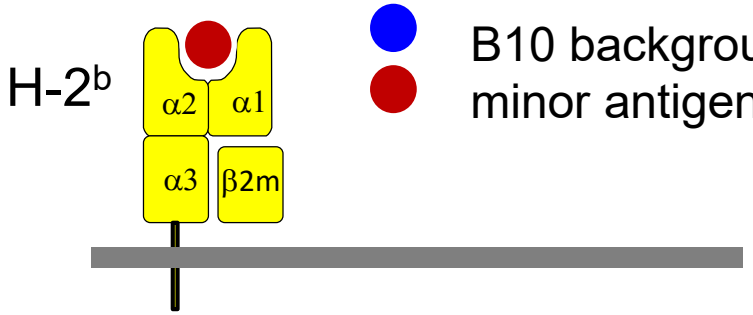


Immunize with H-2^{b} cells

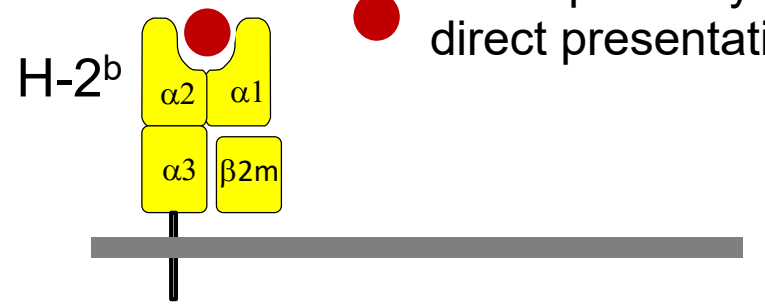
BALB
H-2^{bxd}

CD8 T cell — restricted by H-2^{b}

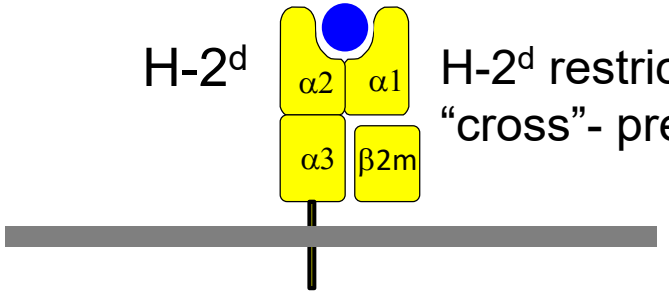
CD8 T cell — restricted by H-2^{d}



B10 background minor antigens

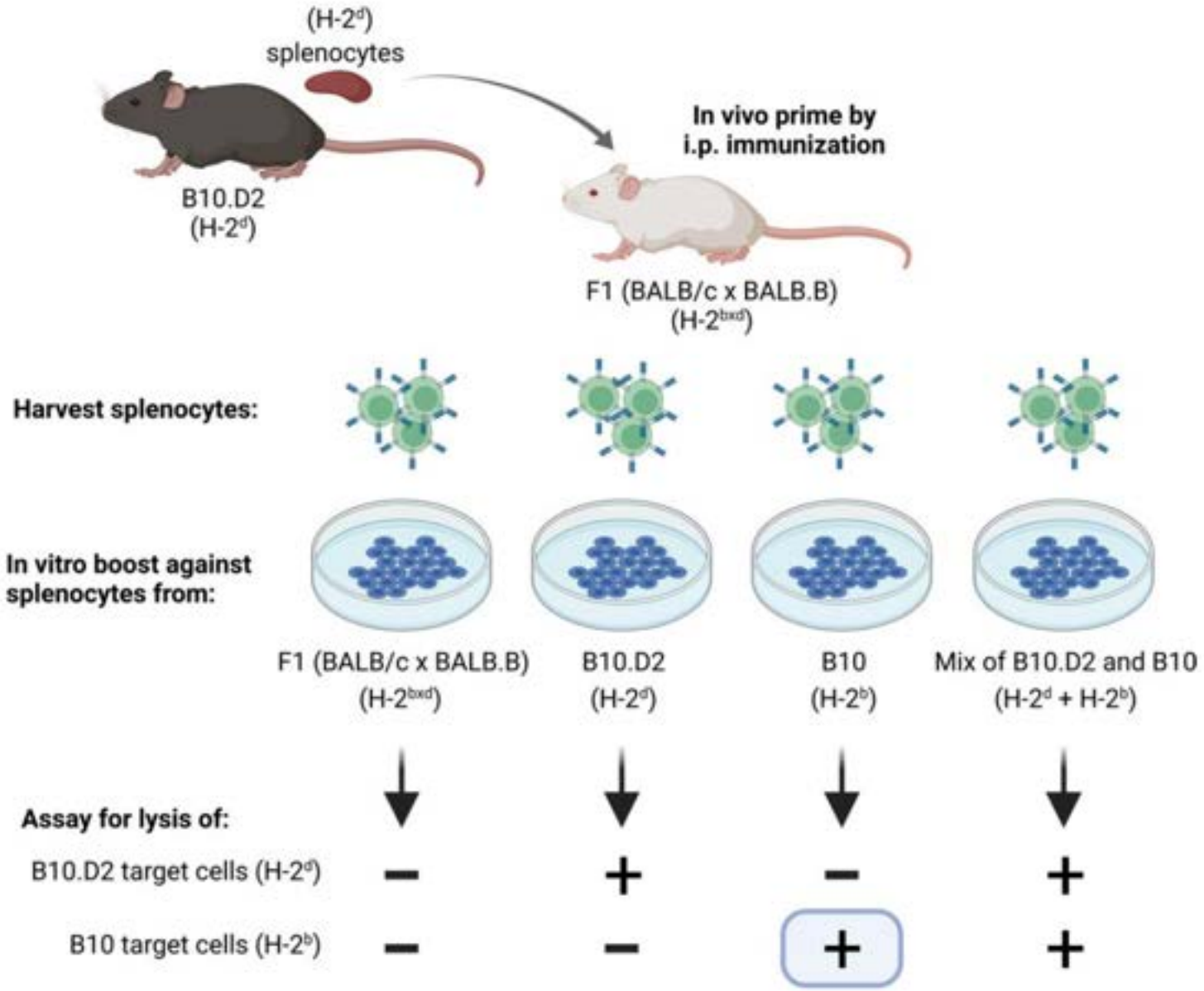


Could prime by direct presentation



H-2^{d} restricted Ag "cross"- presentation

Original Bevan discovery of Cross-priming.



Endogenous antigens only are loaded onto MHC class I

1987

DIFFERENCES IN ANTIGEN PRESENTATION TO MHC CLASS I- AND CLASS II-RESTRICTED INFLUENZA VIRUS-SPECIFIC CYTOLYTIC T LYMPHOCYTE CLONES

By LYNDA A. MORRISON, ARON E. LUKACHER, VIVIAN L. BRACIALE, DAVID P. FAN,* AND THOMAS J. BRACIALE

TABLE VI

Effect of Chloroquine on Target Cell Sensitization by Infectious Virus

Clone	Percent specific ^{51}Cr-release from A20-1.11 targets*		
	Uninfected	A/JAP infected	A/JAP infected + chloroquine‡
14-1	4§	64	66
14-7	2	66	62
G1	4	68	14
U-12	2	67	7

* As in Table I. Spontaneous ^{51}Cr-release from all target groups was <10%. E/T ratio is 5:1.

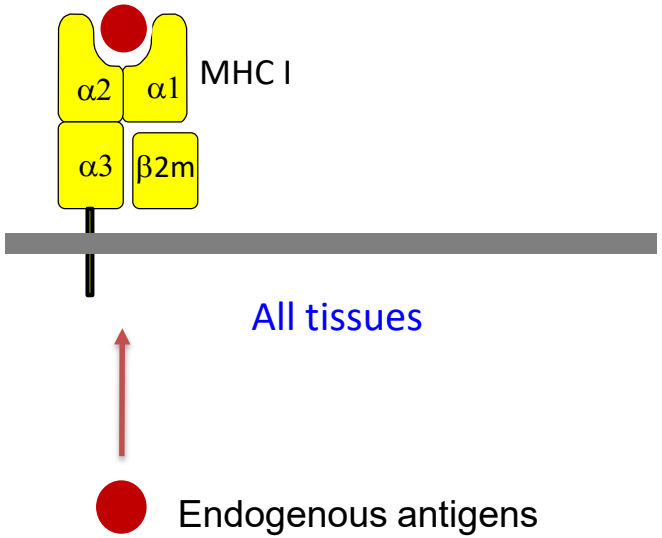
‡ Target cells were exposed to infectious A/JAP/57 virus (10–50 infectious units per cell) in the absence or presence of 5×10^{-5} M chloroquine. Chloroquine was then maintained at a lower concentration (5×10^{-6} M) throughout the course of the assay as described (see Materials and Methods).

§ As in Table I.

Interpreting a negative result

Concluded class I MHC processing does not involve exogenous antigens

But was NOT examining dendritic cells

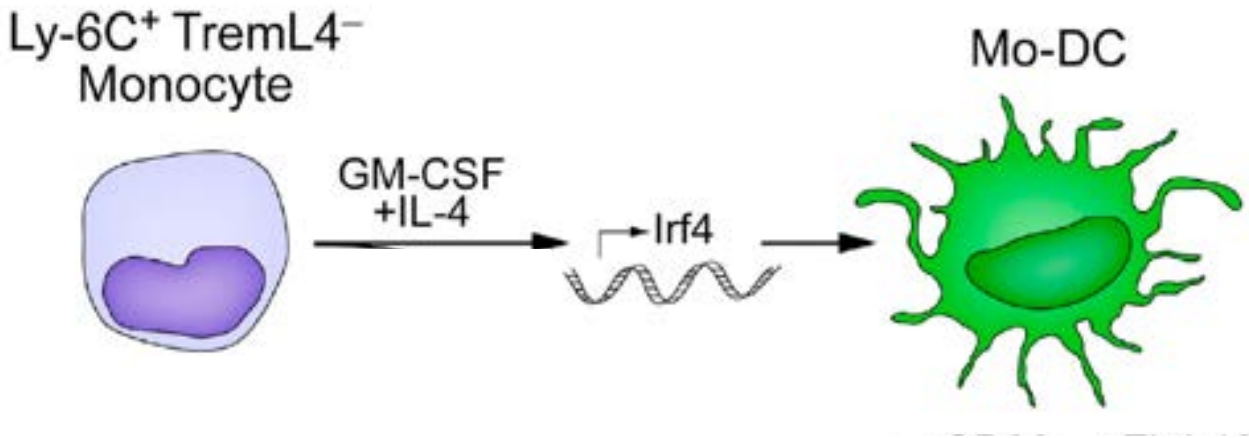


Cross-presentation mechanisms derived from analysis of MoDCs

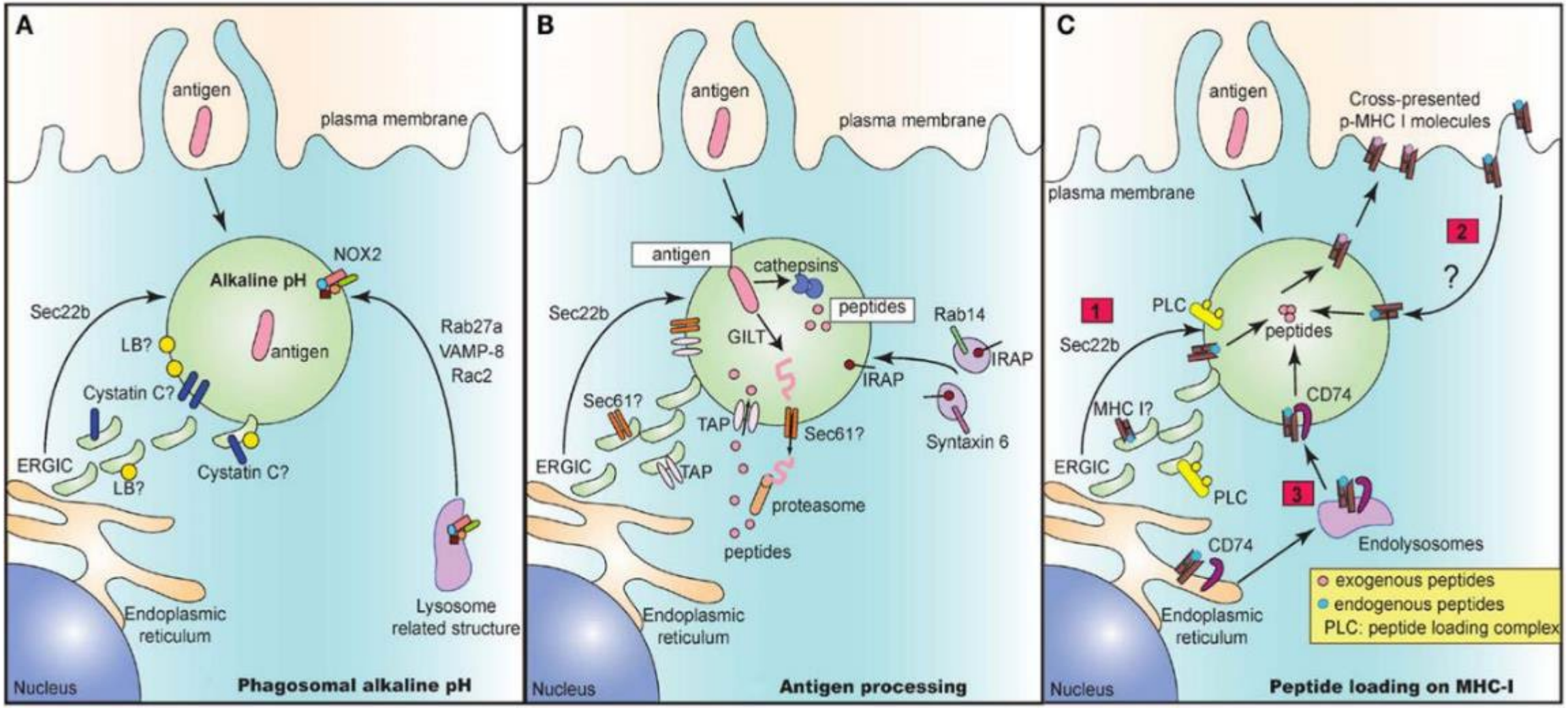
1994

Dendritic Cells Use Macropinocytosis and the Mannose Receptor to Concentrate Macromolecules in the Major Histocompatibility Complex Class II Compartment: Downregulation by Cytokines and Bacterial Products

By Federica Sallusto,*‡ Marina Cella,* Carlo Danieli,* and Antonio Lanzavecchia*



The model according to “MoDCs”



Nair-Gupta and Blander, Front. Immunol. 2013

Cross-presentation mechanisms derived from analysis of MoDCs

Genes identified as controlling cross-presentation in MoDCs

Nox2 (gp91),
Rac2, Rab27a,
IRAP, Rab3b/c,
Mannose receptor,
Rab34,
TFEB,

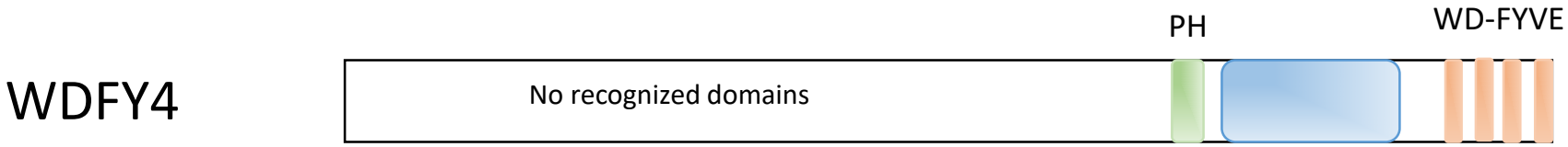
Sec22b

Cross-presentation mechanisms derived from analysis of MoDCs?

Genes never confirmed *in vivo* for controlling cross-presentation:

Nox2 (gp91),
Rac2, Rab27a,
IRAP, Rab3b/c,
Mannose receptor,
Rab34,
TFEB

Wdfy4 is a gene of unknown function

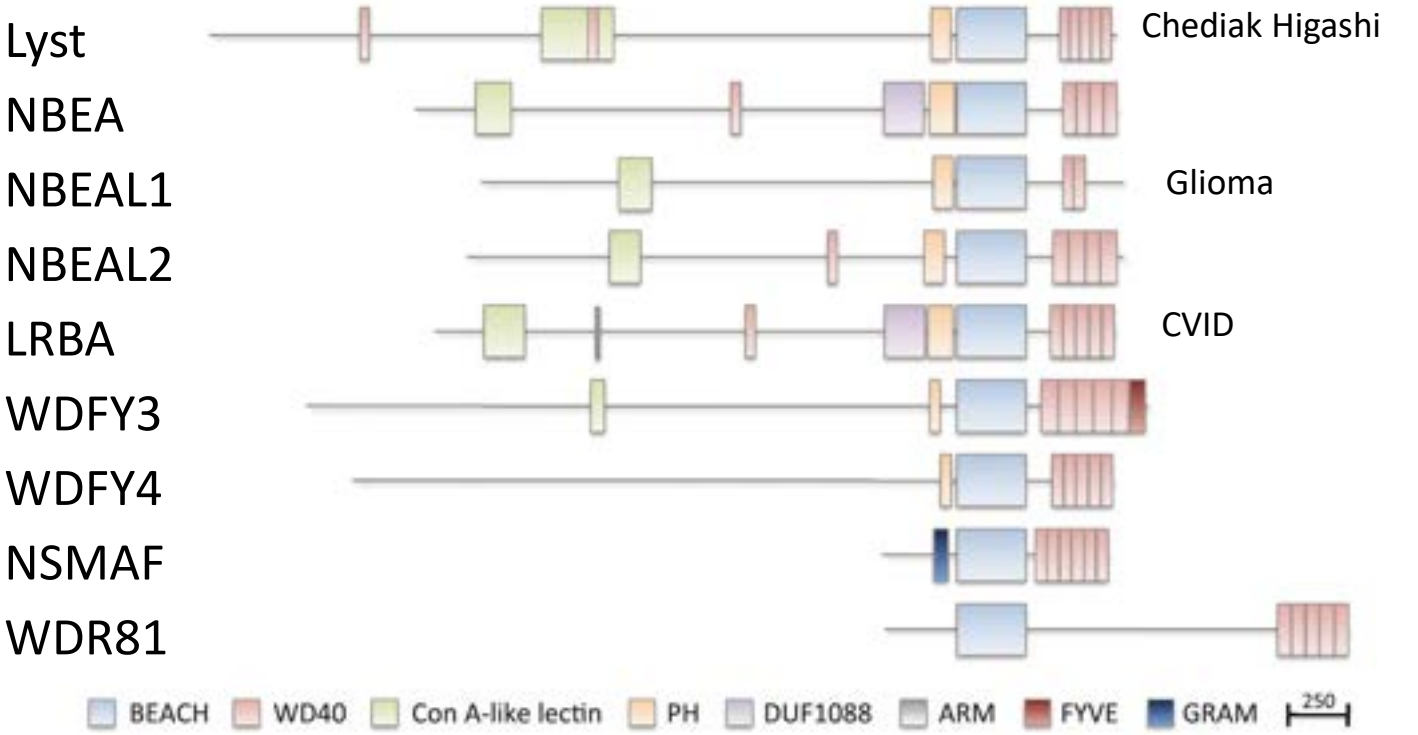


56 exons encoding a protein of 3014 amino acids

Beige and Chediak-Higashi

BEACH domain proteins

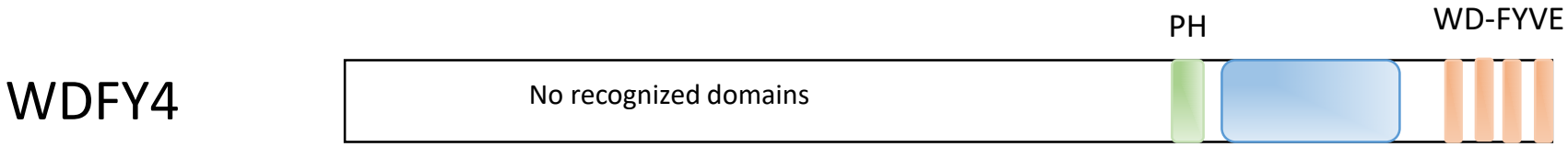
Disease Association



Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy

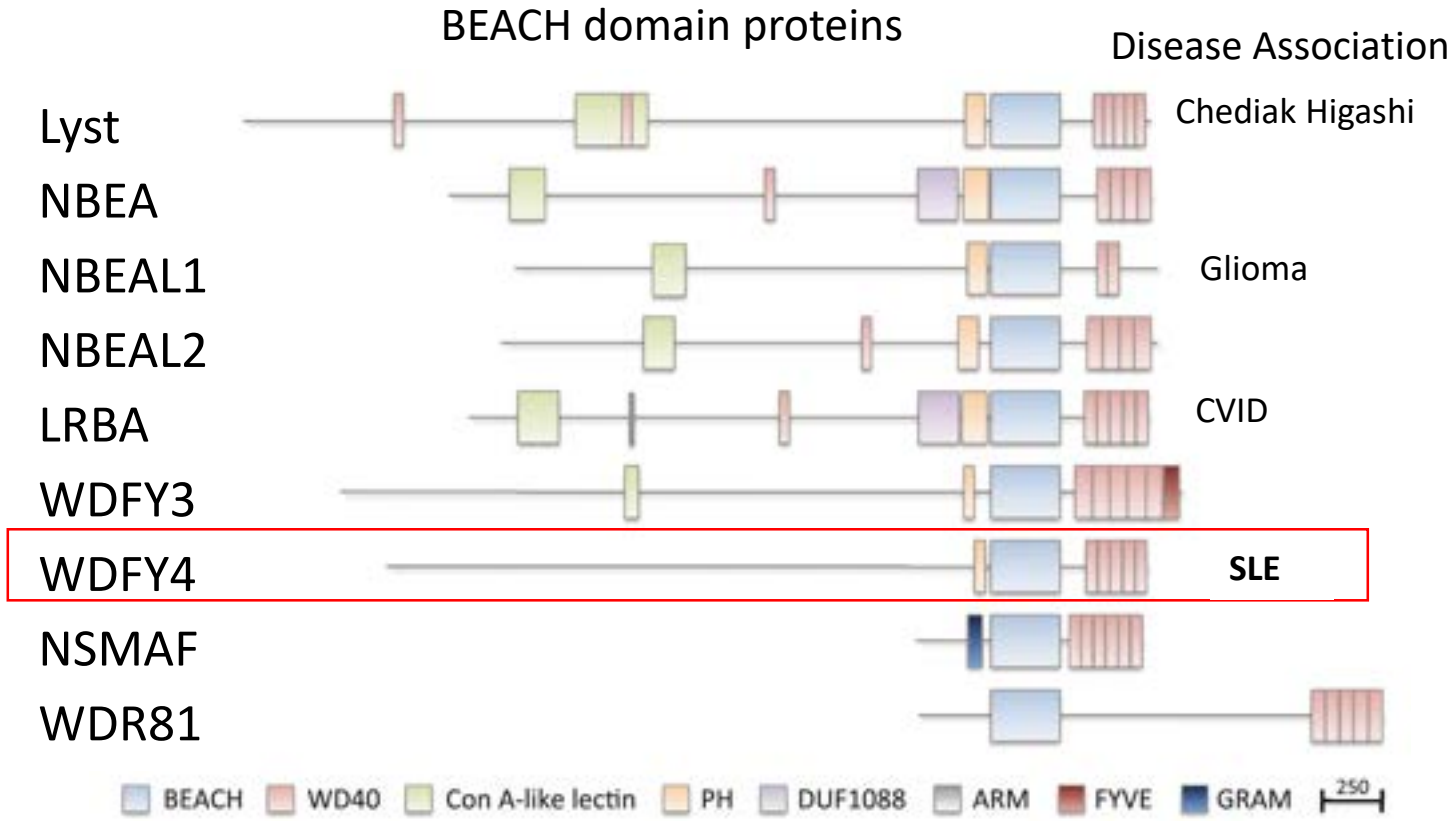
Bernice Lo,[1,2*] Kejian Zhang,[3*] Wei Lu,[1,2] Lixin Zheng,[1,2] Qian Zhang,[2,4] Chrysi Kanellopoulou,[1,2] Yu Zhang,[2,4] Zhiduo Liu,[5] Jill M. Fritz,[1,2] Rebecca Marsh,[6] Ammar Husami,[5] Diane Kissell,[5] Shannon Nortman,[5] Vijaya Chaturvedi,[6] Hilary Haines,[7] Lisa R. Young,[8] Jun Mo,[9] Alexandra H. Filipovich,[6] Jack J. Bleesing,[6] Peter Mustillo,[10] Michael Stephens,[11] Cesar M. Rueda,[12] Claire A. Chougnet,[12] Kasper Hoebe,[12] Joshua McElwee,[13] Jason D. Hughes,[13] Elif Karakoc-Aydiner,[14] Helen F. Matthews,[1,2] Susan Price,[1,2] Helen C. Su,[2,4] V. Koneti Rao,[1,2] Michael J. Lenardo,[1,2†] Michael B. Jordan[6,12†‡]

Wdfy4 is a gene of unknown function

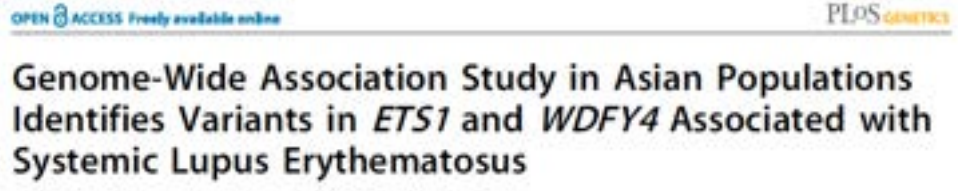


56 exons encoding a protein of 3014 amino acids

Beige and Chediak-Higashi



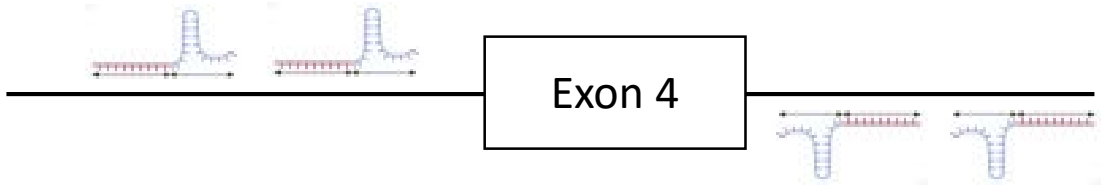
6 GWAS studies link WDFY4 to systemic lupus erythematosus (SLE) risk in Asian populations.


OPEN ACCESS Freely available online PLoS GENETICS

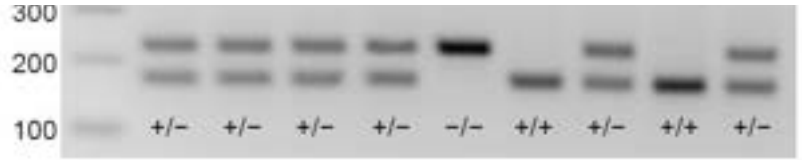
Genome-Wide Association Study in Asian Populations Identifies Variants in *ETS1* and *WDFY4* Associated with Systemic Lupus Erythematosus

Wdfy4$^{-/-}$ mice develop DC1 and are resistant to *Toxoplasma gondii*

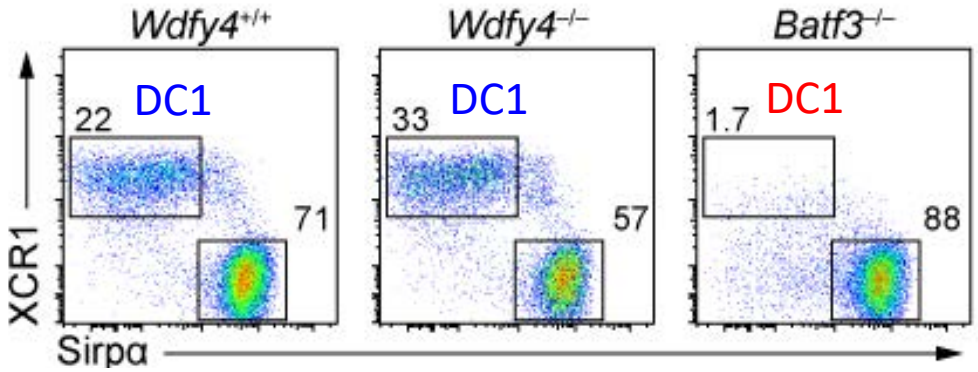
CRISPR deletion of exon 4 alters reading frame between exons and terminates after aa 146.



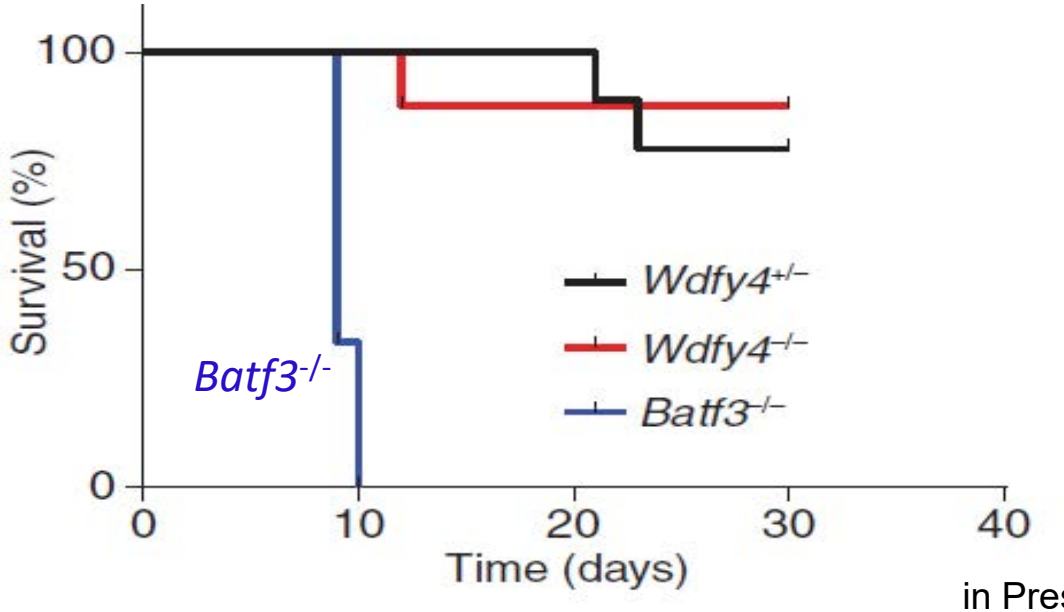
Wdfy4$^{-/-}$ mice are viable



Normal DC1 development in *Wdfy4*$^{-/-}$ mice



Wdfy4$^{-/-}$ mice are resistant to *Toxoplasma gondii*



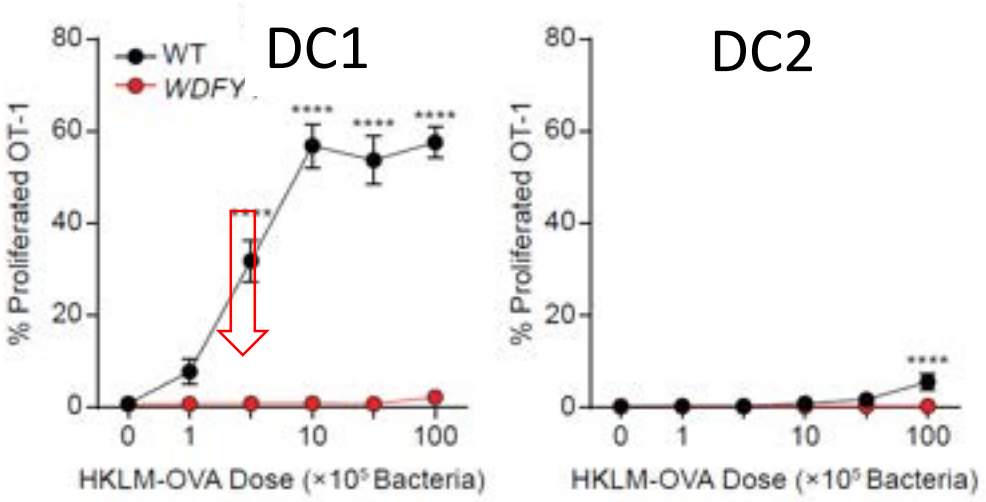
CD8α$^+$ Dendritic Cells Are the Critical Source of Interleukin-12 that Controls Acute Infection by *Toxoplasma gondii* Tachyzoites

Mona Mashayekhi,[1] Michelle M. Sandau,[1,7] Ildiko R. Dunay,[2,8] Eva M. Frickel,[4,9] Asis Khan,[2] Romina S
Alan Sher,[5] Hidde L. Ploegh,[4] Theresa L. Murphy,[1] L. David Sibley,[2] and Kenneth M. Murphy[1,3,*]

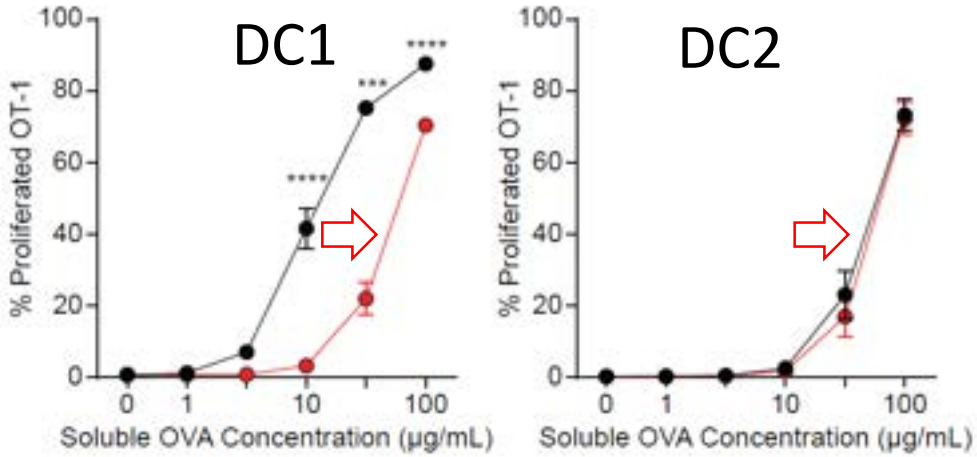
in Press

Wdfy4$^{-/-}$ mice have a selective failure in DC1 cross-presentation

Absent cross-presentation to cell-associated antigen by *Wdfy4*$^{-/-}$ DC1

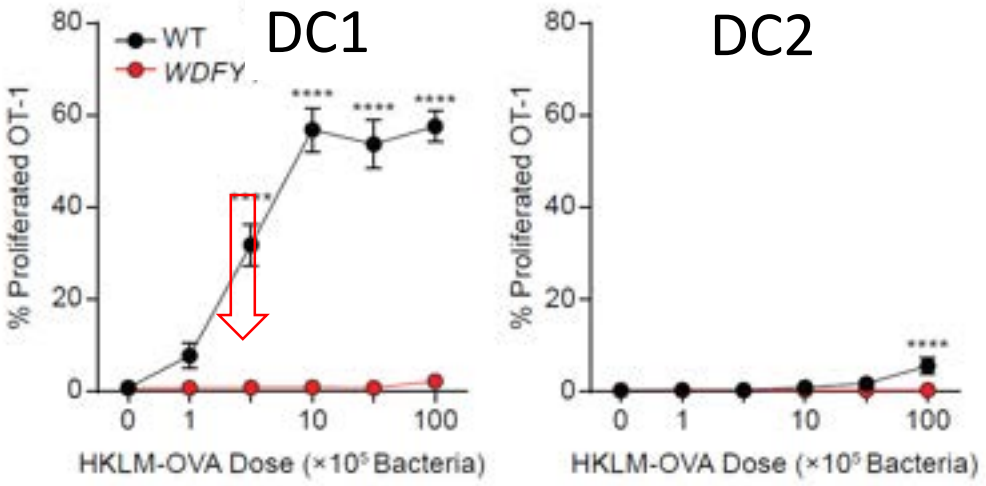


Impaired cross-presentation to soluble antigen by *Wdfy4*$^{-/-}$ DC1

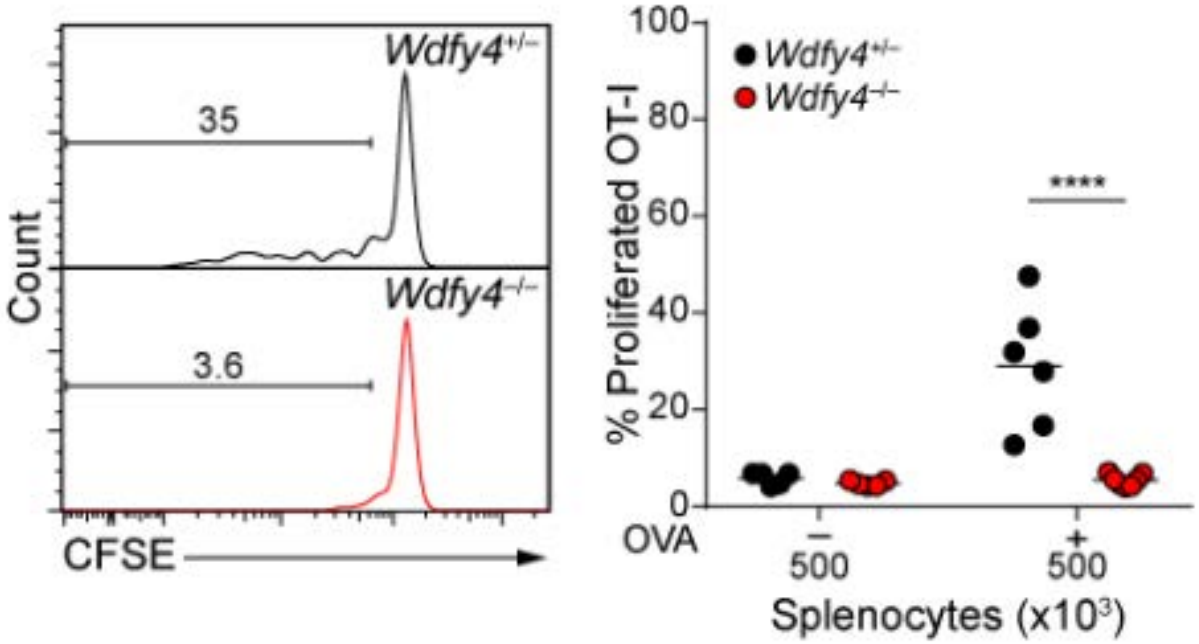


Wdfy4$^{-/-}$ mice have a selective failure in DC1 cross-presentation

Absent cross-presentation to cell-associated antigen by *Wdfy4*$^{-/-}$ DC1

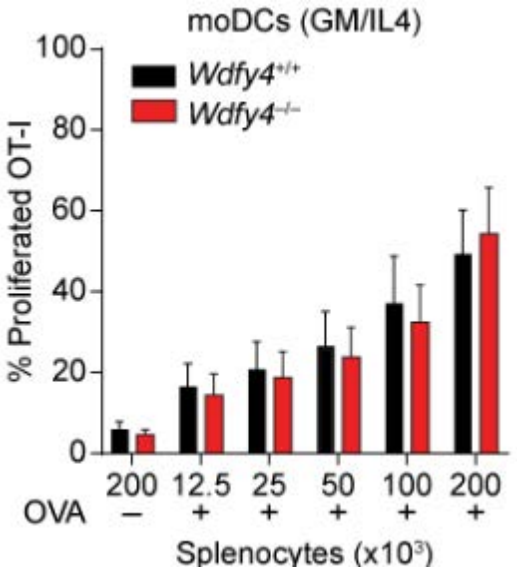


in vivo Cross-Presentation

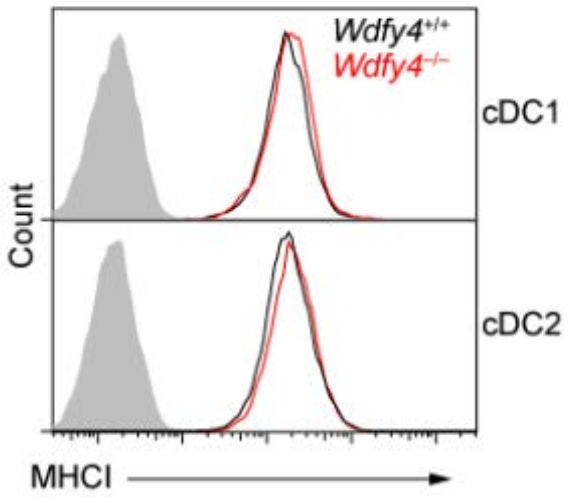


Wdfy4$^{-/-}$ mice have normal moDCs and direct presentation on MHCI

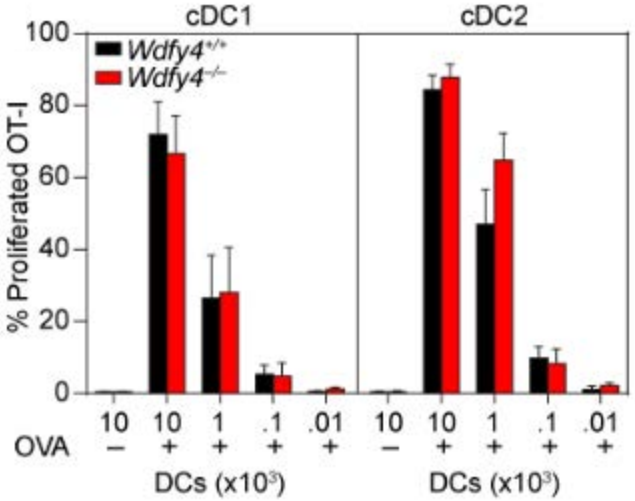
MoDCs (GM-CSF/IL-4)



MHCI Expression

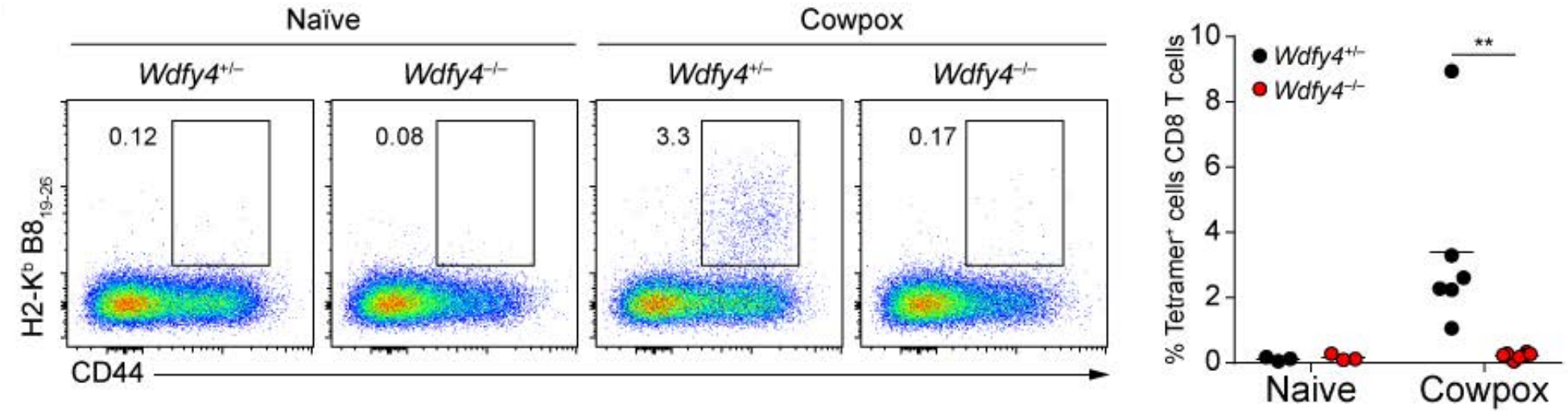


Direct Presentation



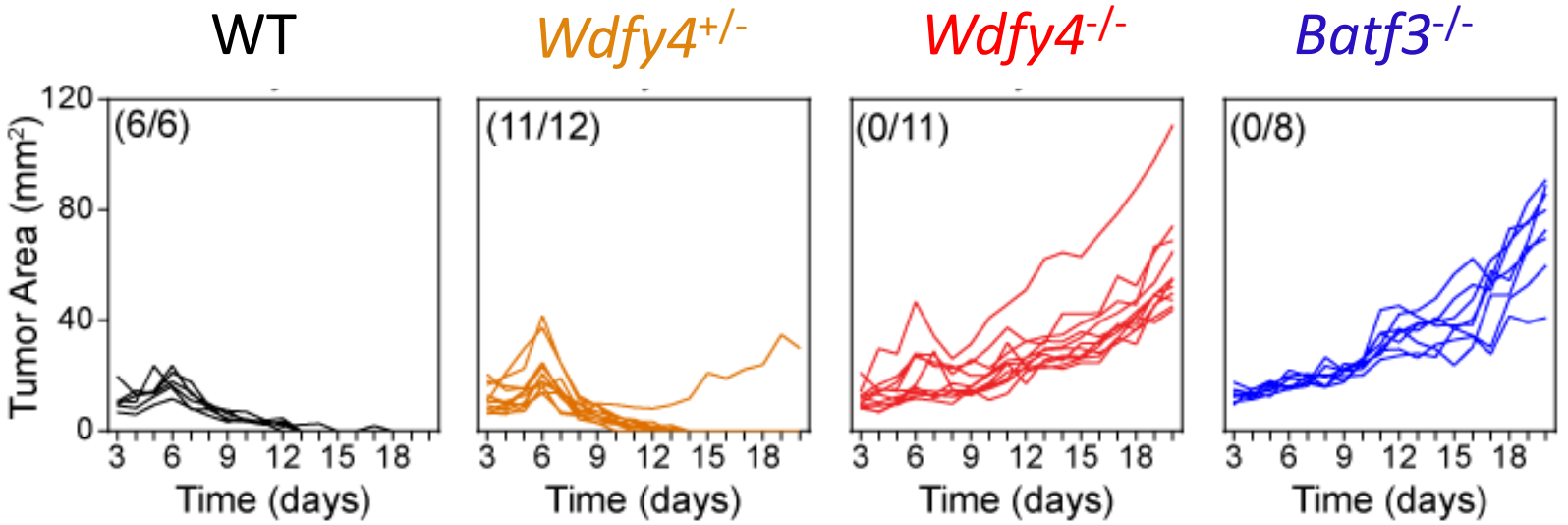
Wdfy4$^{-/-}$ mice cannot mount a response to cowpox virus

Lung



Wdfy4$^{-/-}$ mice cannot reject immunogenic tumors

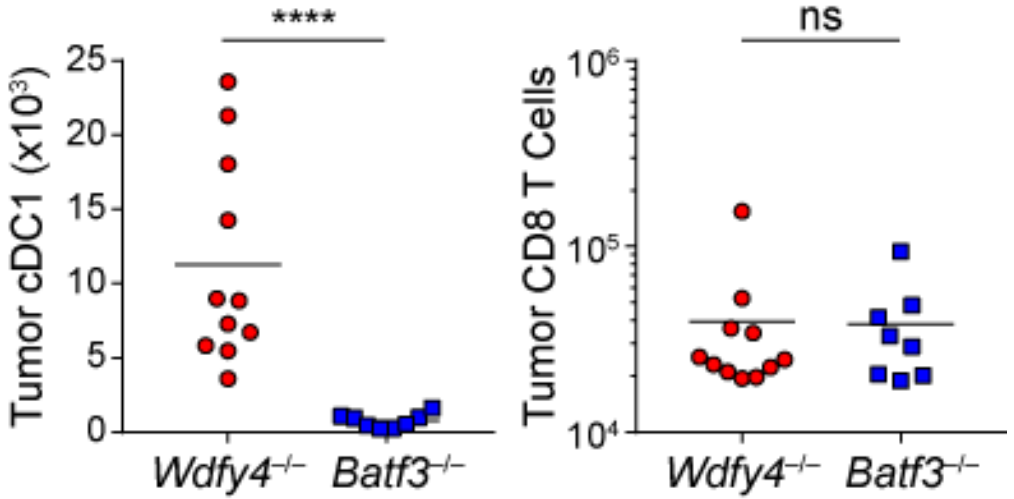
Response to regressor fibrosarcoma



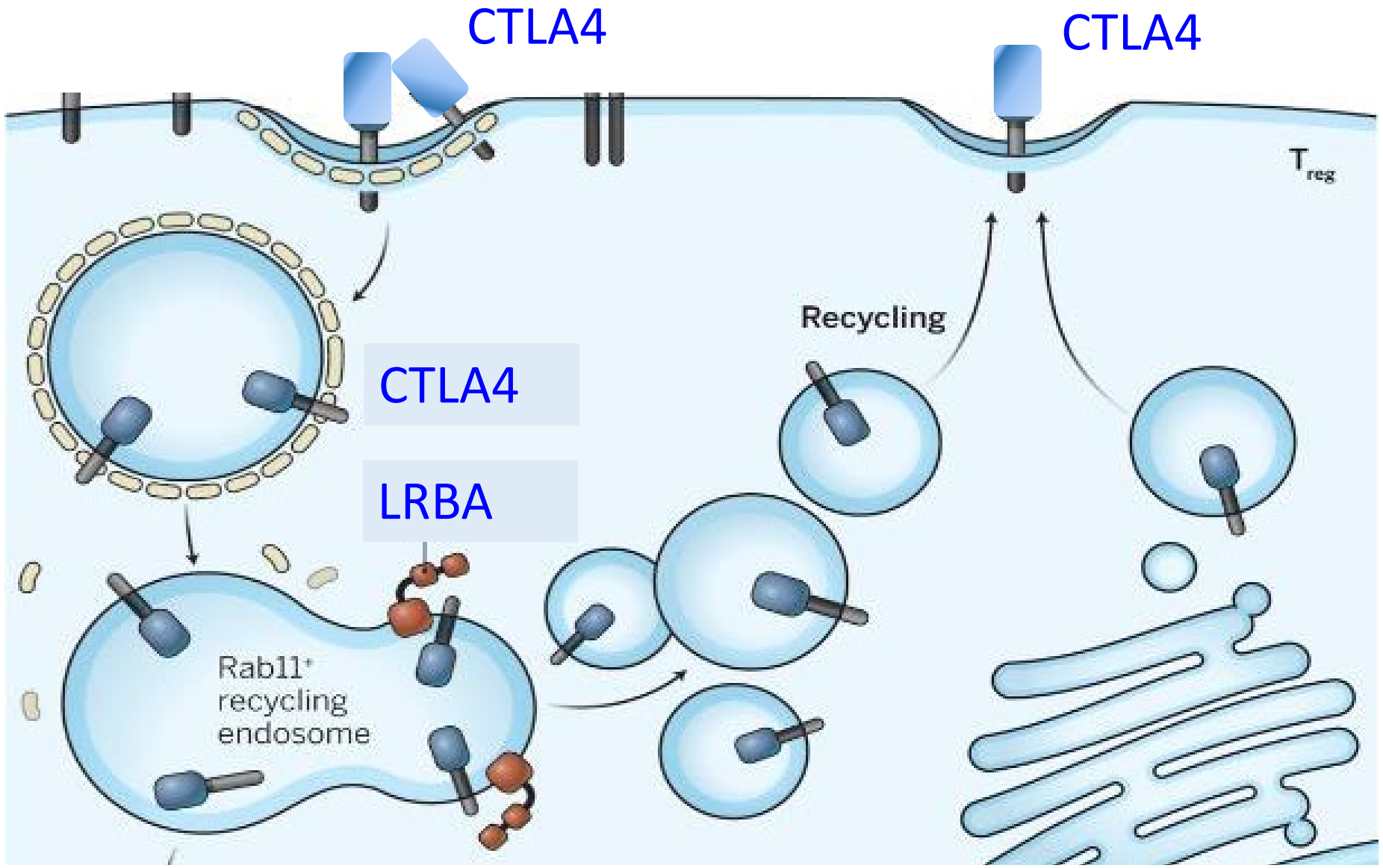
Regressor fibrosarcoma

DC1 enter in tumor

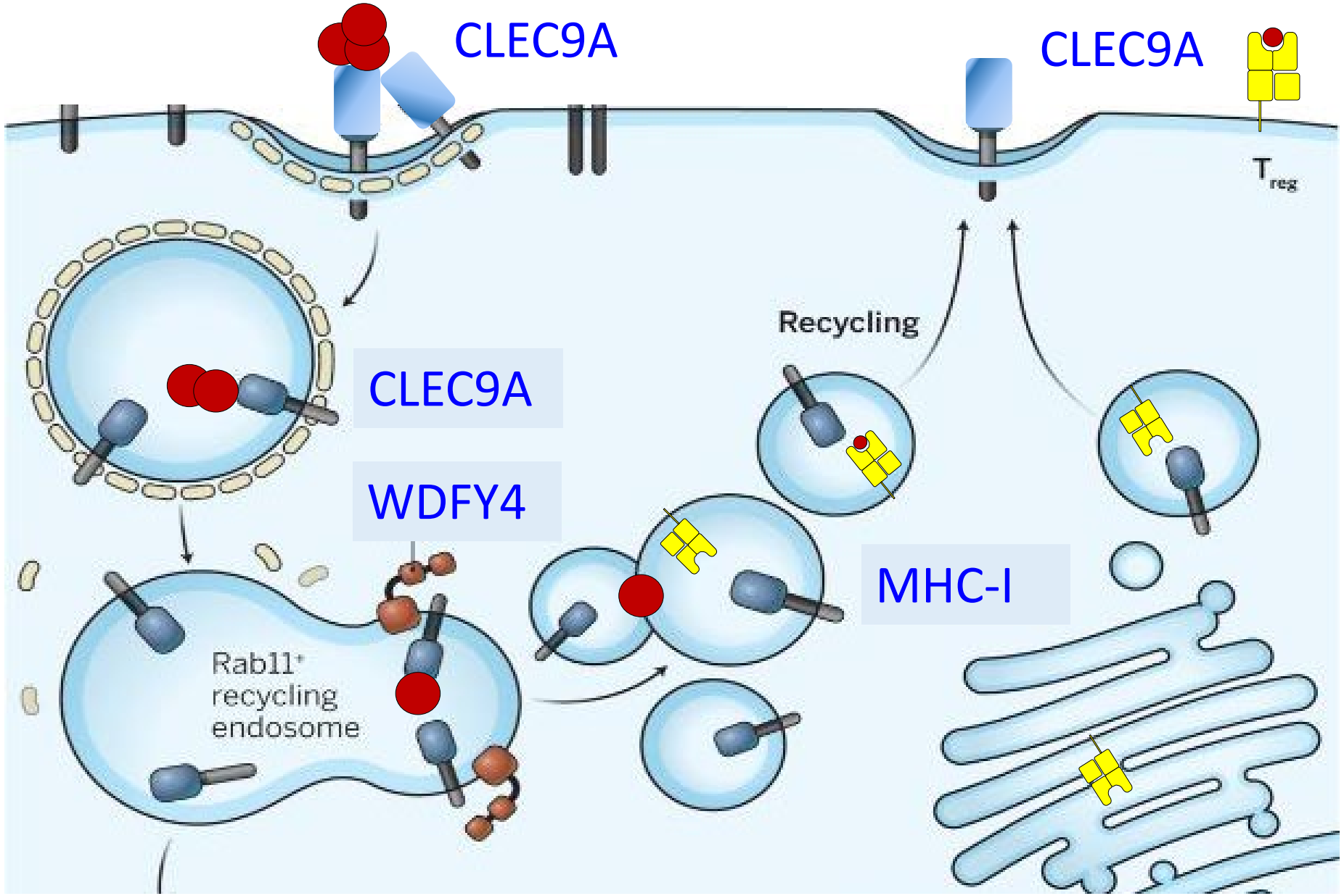
No CD8 T cell entry in tumor



How does Wdfy4 control cross-presentation by DC1?



How does Wdfy4 control cross-presentation by DC1?



Summary

What we know.

WDFY4 is required for cross-presentation of cell-associated antigens.
WDFY4 knockout mice fail to mount anti-viral or anti-tumor CD8 T cells responses.
Sec22b and Rab43 also contribute some to cross-presentation.
Real cDC1 in vivo cross-present by different pathways than MoDCs.
CLEC9a is not required for tumor rejection. Redundant receptor?

What we don’t know.

No clue how WDFY4 works in the cell.
Are there redundant receptors to capture cell-associated antigens?
No clue on the mechanism for Sec22b or Rab43.
Can cDC2 be induced to XP?
Does direct vs. indirect priming induce qualitative changes in T cells?

REVIEW

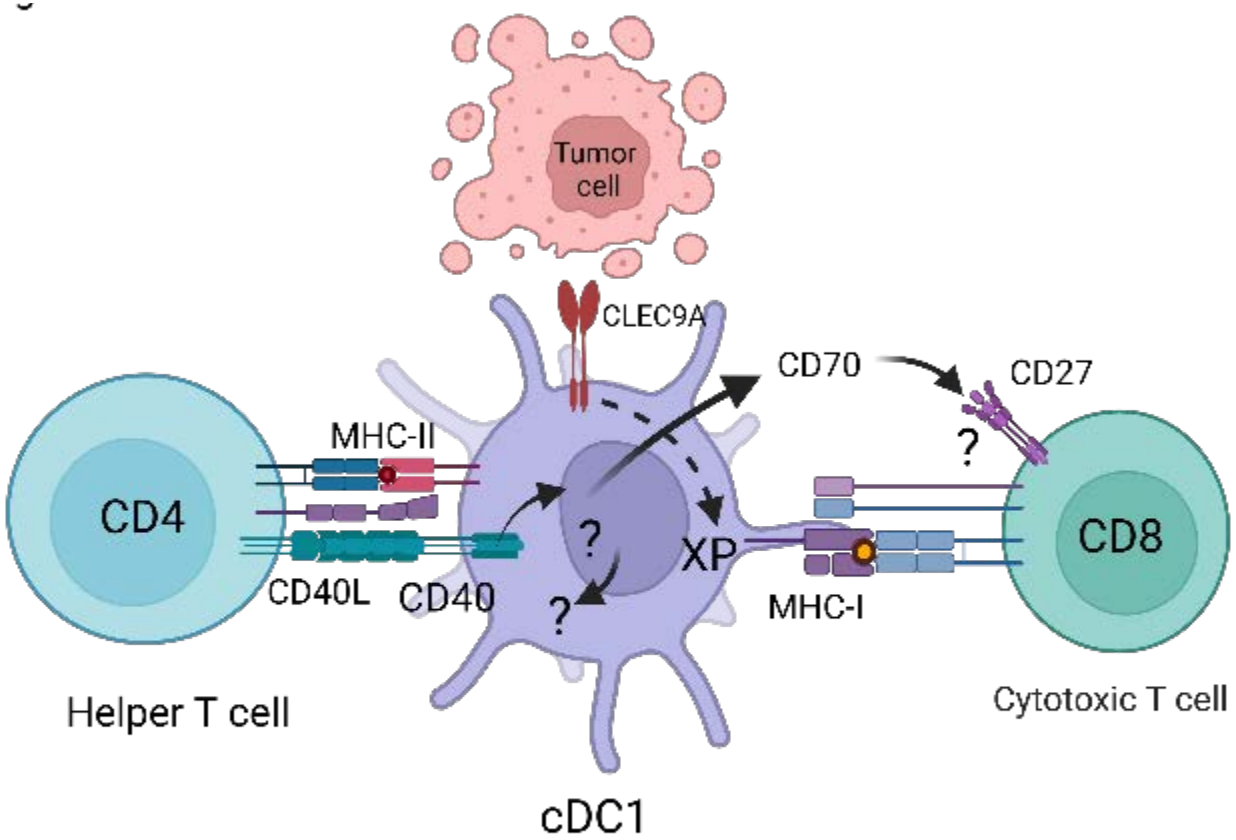
Innate Immunity Focus

DCs at the center of help: Origins and evolution of the three-cell-type hypothesis

Renee Wu[1] and Kenneth M. Murphy[1]

JEM 2022, vol 219

https://pubmed.ncbi.nlm.nih.gov/35543702/



cDC1 (vs cDC2)

MHC-II processing

MHC-I processing

CD40 mediated cDC1 licensing

FUNCTIONAL SUBCLASSES OF T LYMPHOCYTES BEARING DIFFERENT Ly ANTIGENS

II. Cooperation Between Subclasses of Ly$^+$ Cells in the Generation of Killer Activity*

By H. CANTOR and E. A. BOYSE (1975)

HELPER ACTIVITY IS REQUIRED FOR THE IN VIVO GENERATION OF CYTOTOXIC T LYMPHOCYTES*

By JO-ANN KEENE and JAMES FORMAN

From the Department of Microbiology and the Immunology Graduate Program, University of Texas Health Science Center, Dallas, Texas, 75235

Eur. J. Immunol. 1987. *17:* 1579–1583

N. Avrion Mitchison and Christine O'Malley

Imperial Cancer Research Fund, London

Three-cell-type clusters of T cells with antigen-presenting cells best explain the epitope linkage and noncognate requirements of the *in vivo* cytolytic response

1996

Ligation of CD40 on Dendritic Cells Triggers Production of High Levels of Interleukin-12 and Enhances T Cell Stimulatory Capacity: T-T Help via APC Activation

By Marina Cella,* Doris Scheidegger,* Kathrin Palmer-Lehmann,‡ Peter Lane,* Antonio Lanzavecchia,* and Gottfried Alber‡

*From the *Basel Institute for Immunology, CH-4005 Basel, Switzerland; and ‡Hoffmann-La Roche AG, CH-4002 Basel, Switzerland*

1998

T-cell help for cytotoxic T lymphocytes is mediated by CD40–CD40L interactions

Stephen P. Schoenberger*†‡, Rene E. M. Toes*†, Ellen I. H. van der Voort*, Rienk Offringa* & Cornelis J. M. Melief*

Help for cytotoxic-T-cell responses is mediated by CD40 signalling

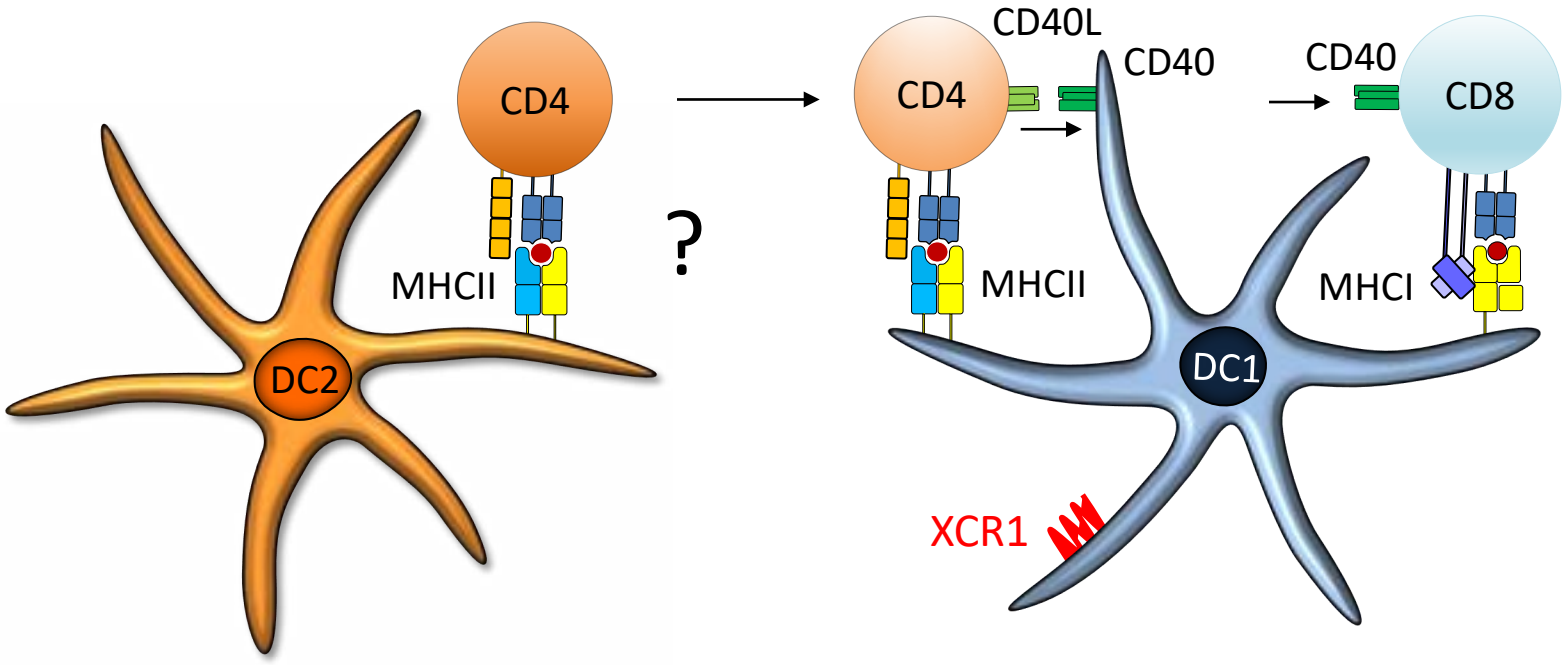
Sally R. M. Bennett*†, Francis R. Carbone‡, Freda Karamalis*†, Richard A. Flavell§, Jacques F. A. P. Miller* & William R. Heath*

How do CD4 T cells help CD8 responses?

CD4 T cells help CD8 T cells
Cantor and Boyse 1975
Buller and Morse 1987
Bennett and Heath 1998

Activation of CD40 on APC?
Bennett and Heath 1998
Schoenberger and Melief 1998

Activation of CD40 on CD8 T cells?
Bourgeois and Tanchot 2002



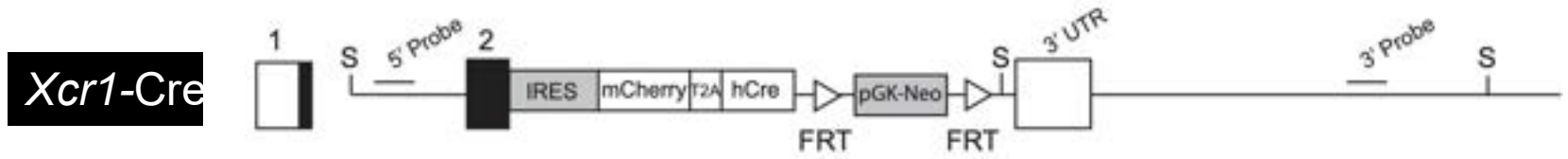
Do DC1 uniquely activate CD8 T cells *in vivo*?

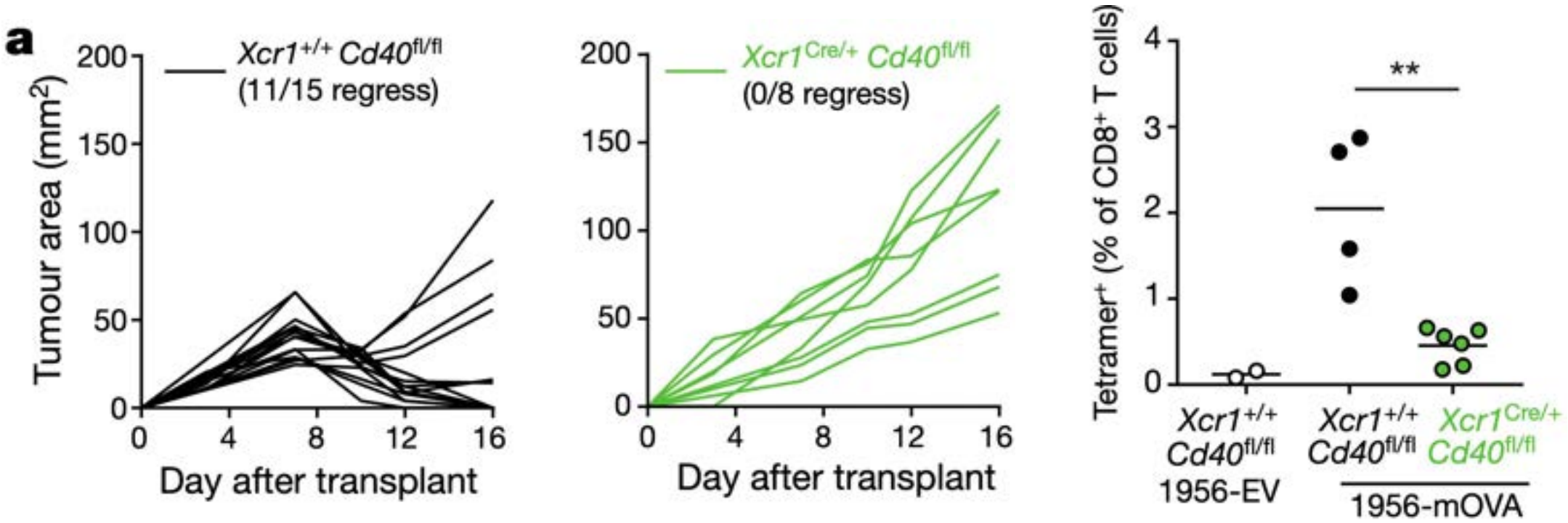
What is the function of MHC class II on DC1 *in vivo*?

What is mechanism of CD4 help through DC1?
TRAIL, IL-15, CD40, CD80/86. LAG3, IFNAR, CD70.
Janssen and Schoenberger 2005 vs. Sacks and Bevan 2008

Targeted XCR1-Cre allele

CD40 specifically in cDC1 is required for anti-tumor immunit



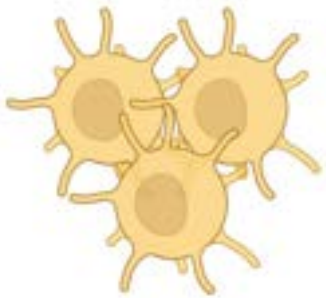


How does CD40 in cDC1 mediate help?

Ferris, Durai, and Wu et al. *Nature* 20

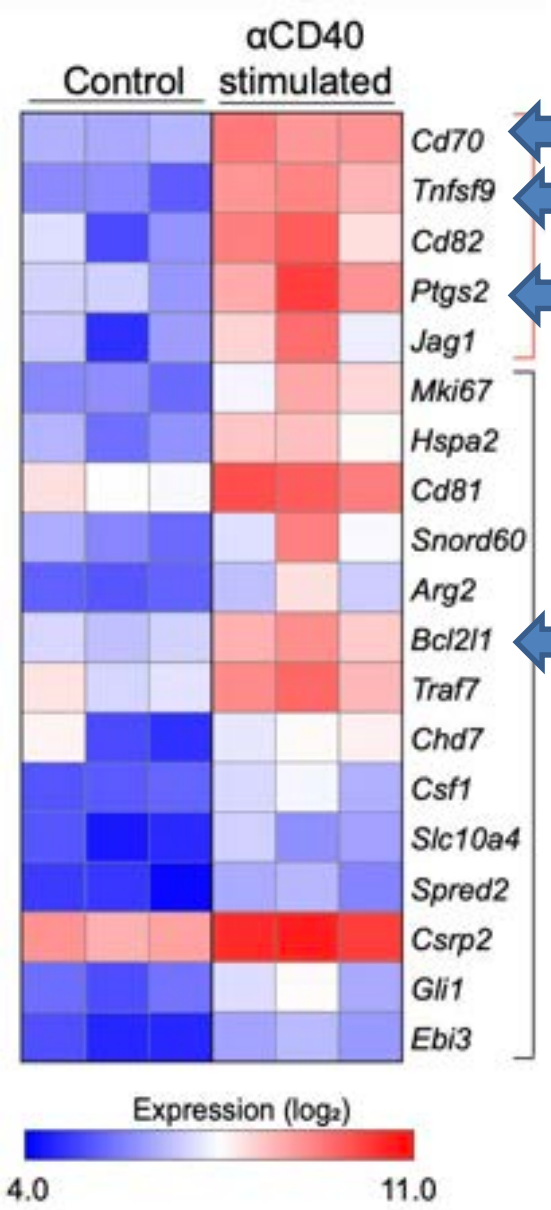
What are the transcriptional targets of CD40 signaling in cDC

Isolate CD40+ cDC1s



24 h

Stimulate ex vivo with agonistic anti-CD40

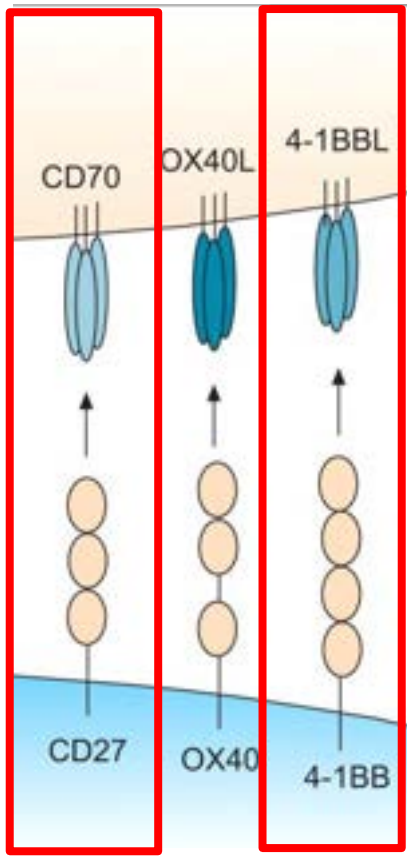


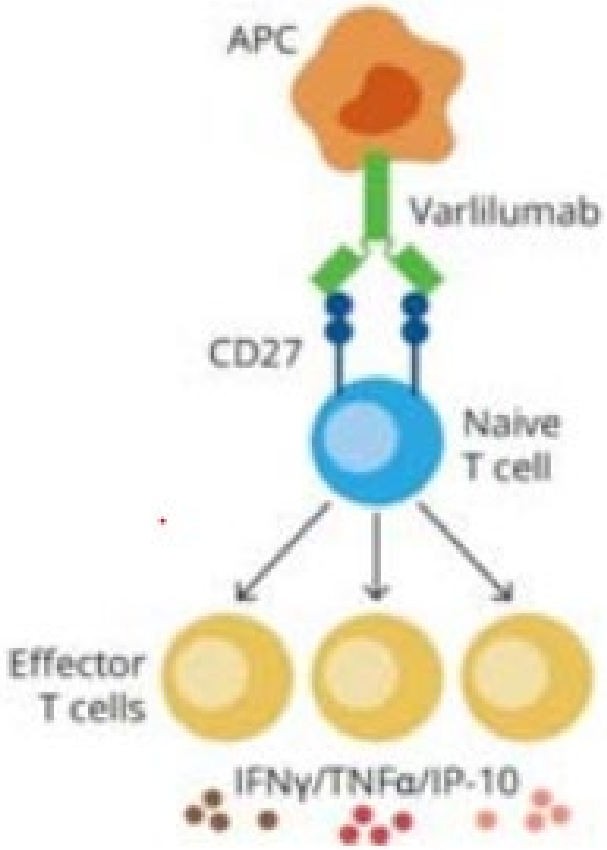
CD70 Partial

4-1BBL Partial

Cox2 Partial

Bcl-XL Partial





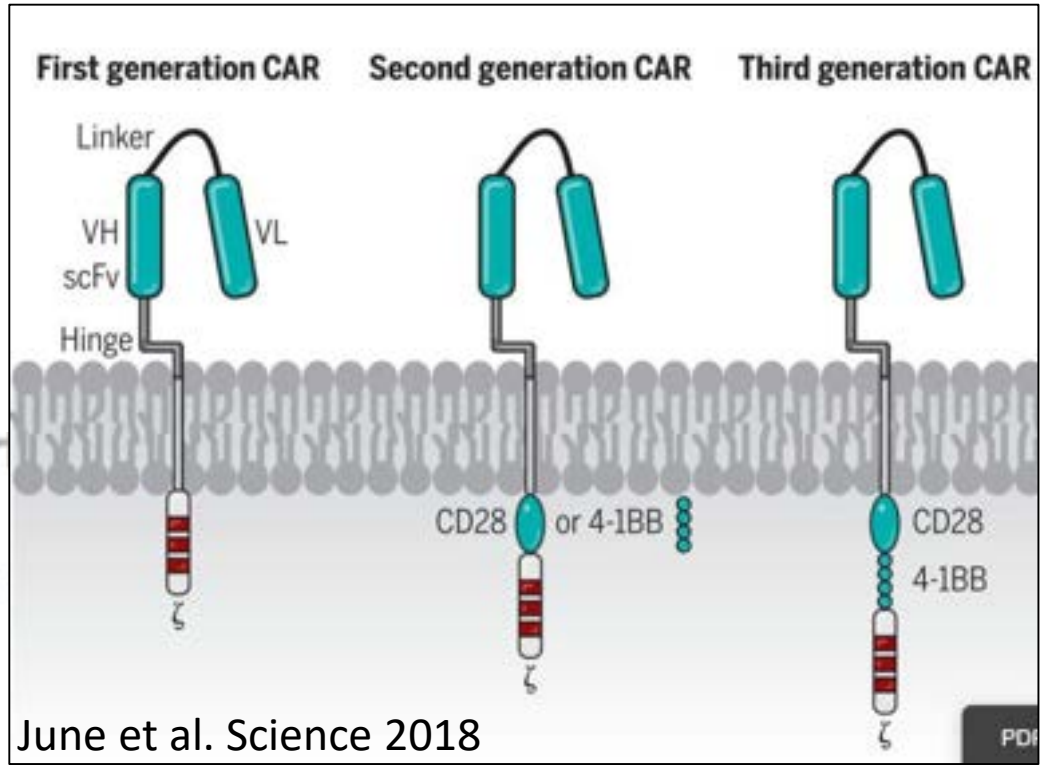
Cancer Therapy: Preclinical
Clinical Cancer Research

PD-1 Blockade and CD27 Stimulation Activate Distinct Transcriptional Programs That Synergize for CD8$^+$ T-Cell–Driven Antitumor Immunity

Sarah L. Buchan[1], Mohannad Fallatah[1], Stephen M. Thirdborough[2], Vadim Y. Taraban[1],

Microenvironment and Immunology
Cancer Research

CD27 Agonism Plus PD-1 Blockade Recapitulates CD4$^+$ T-cell Help in Therapeutic Anticancer Vaccination



Cancer Cell

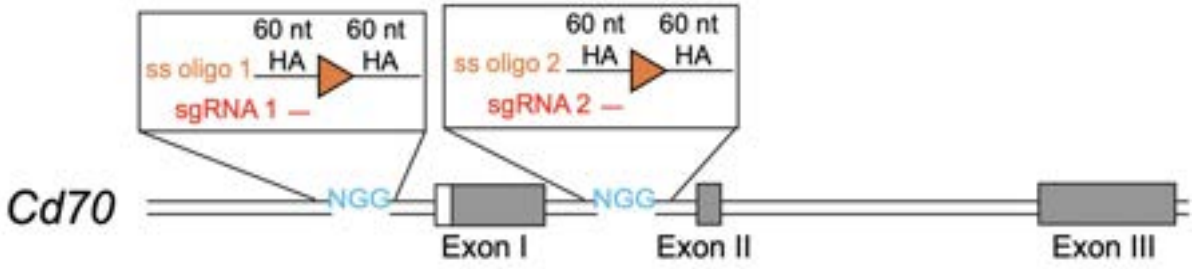
Antibody Tumor Targeting Is Enhanced by CD27 Agonists through Myeloid Recruitment

June et al. Science 2018

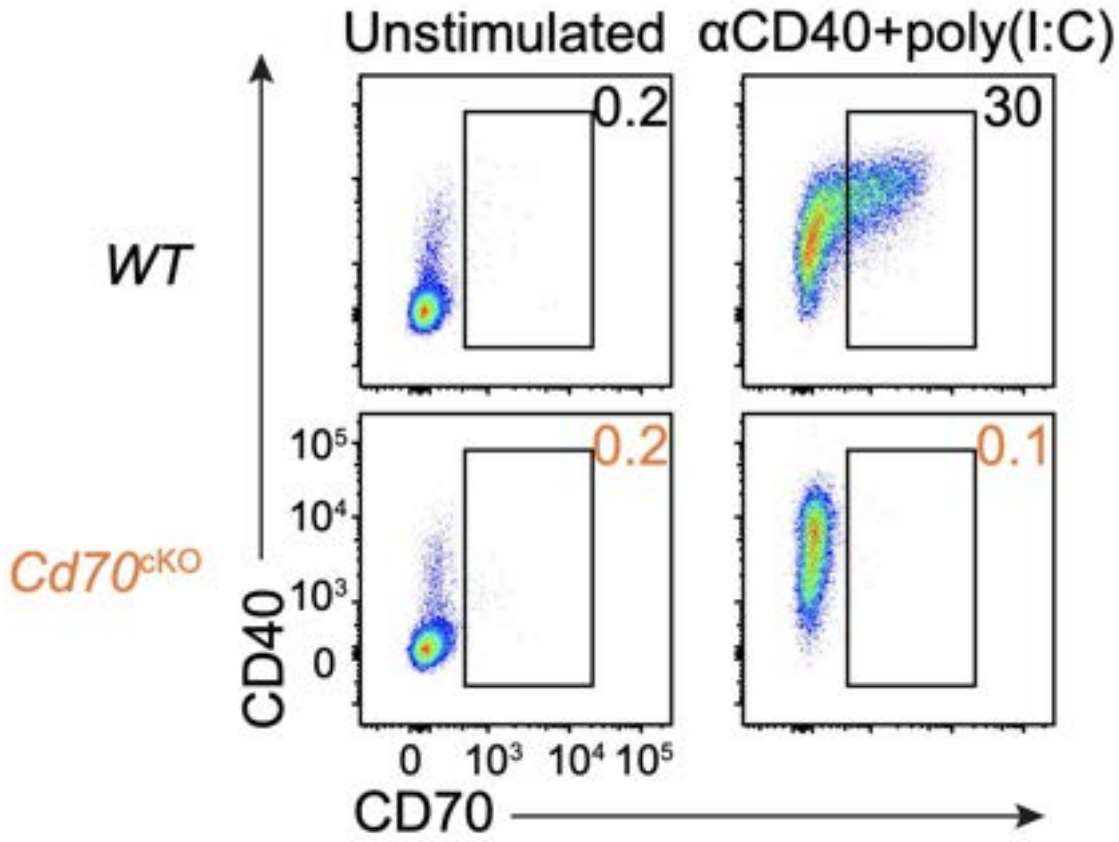
Does CD70 mediate CD40 help to cDC1 during tumor challenge?

- CD70 induced on several cell types: activated DCs, macrophages, B cells

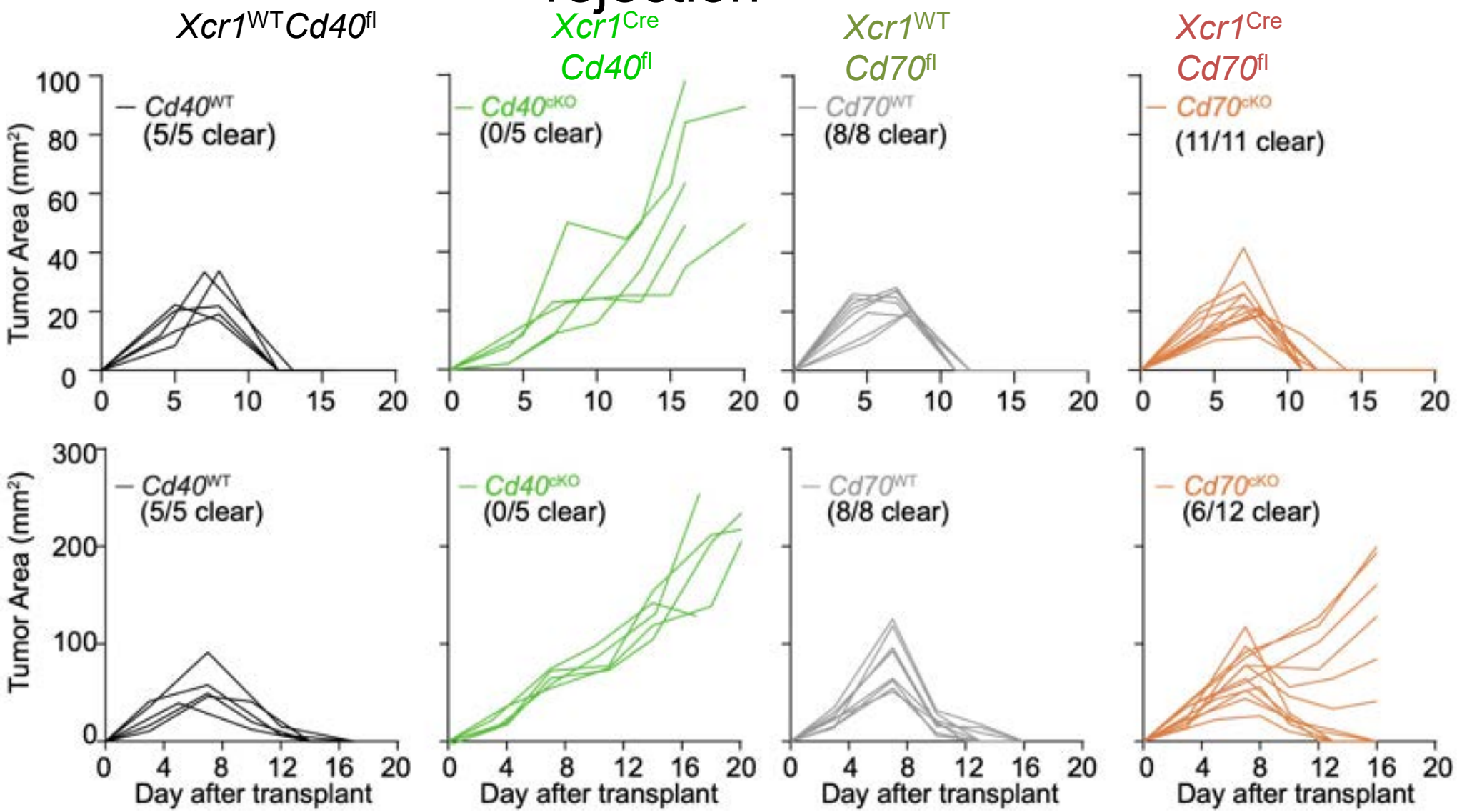
- Generated CD70 conditional KO



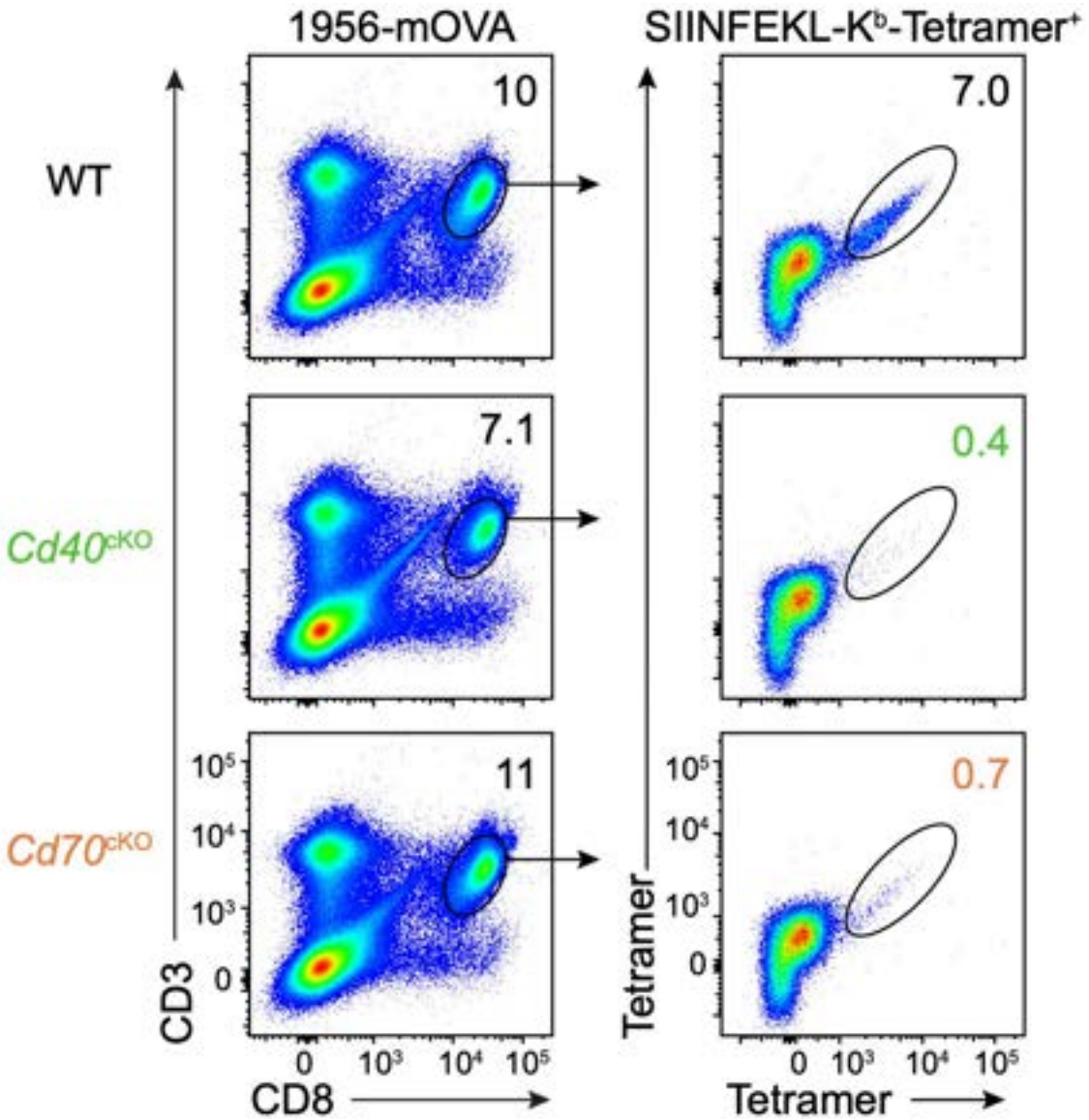
- Crossed *Cd70*$^{fl/fl}$ to *Xcr1*^{Cre}→ *Cd70*^{cKO}

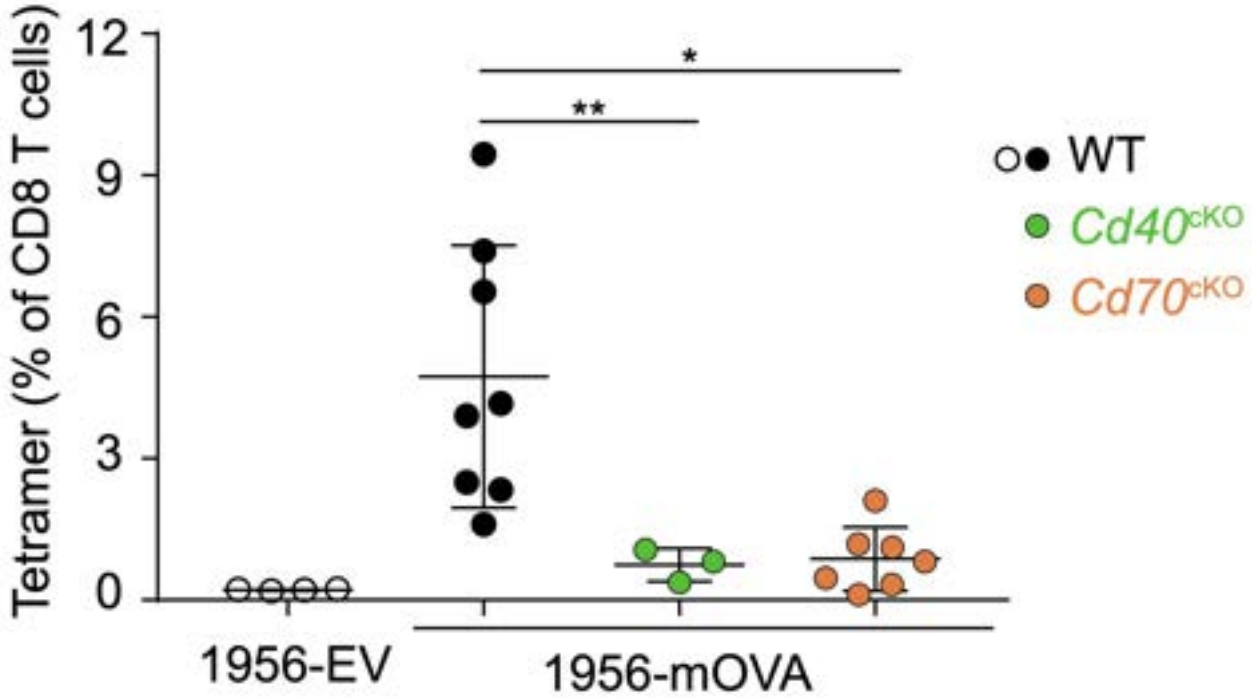


Cd70 in cDC1 partially contributes to CD40-dependent tumor rejection

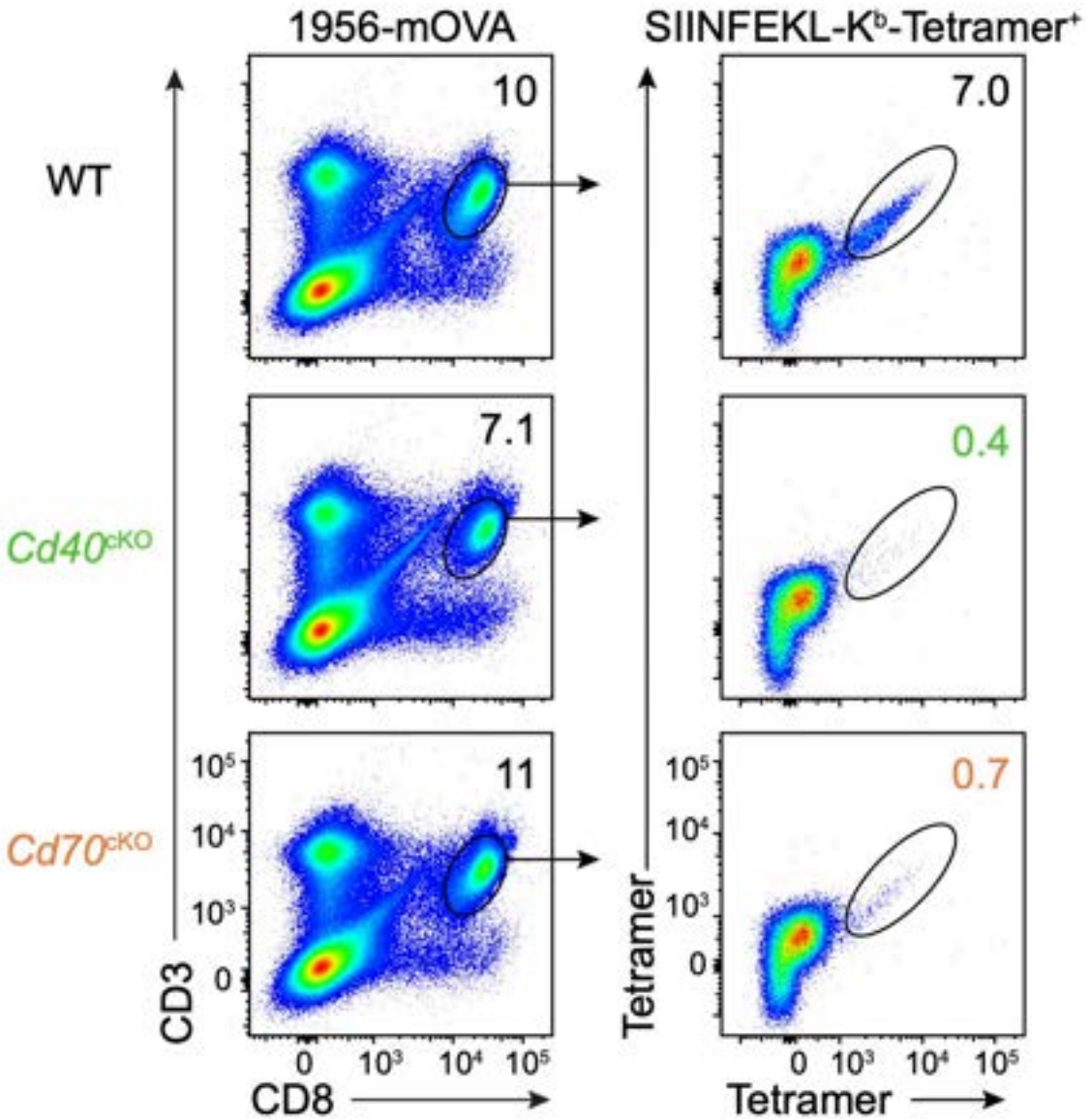


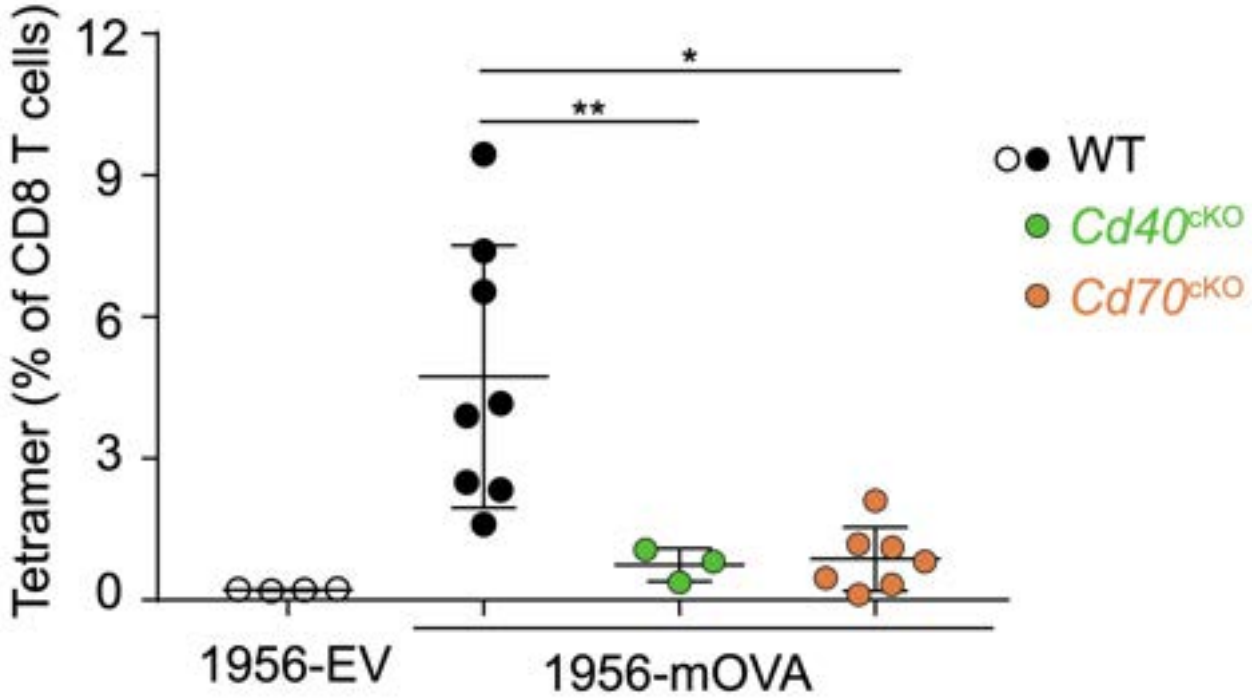
Loss of CD70 on cDC1 reduces anti-tumor CD8 T cell expans



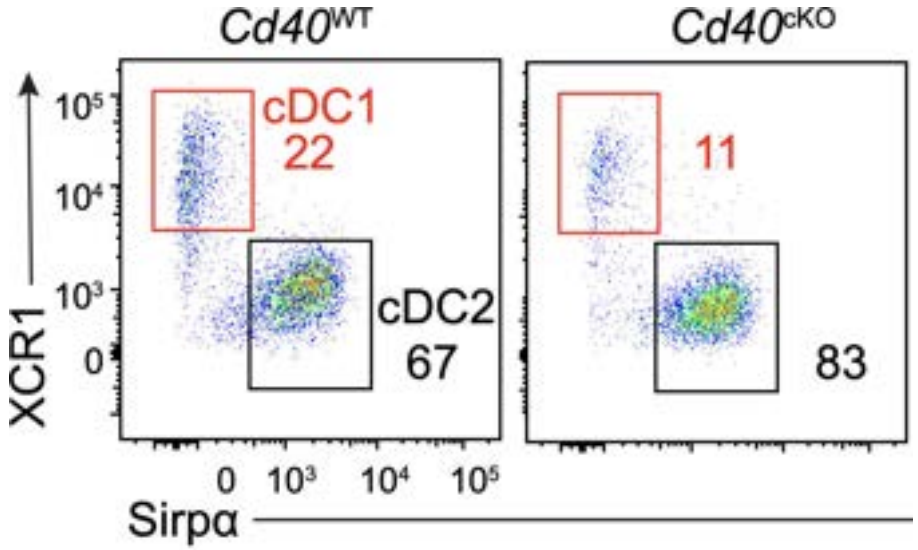


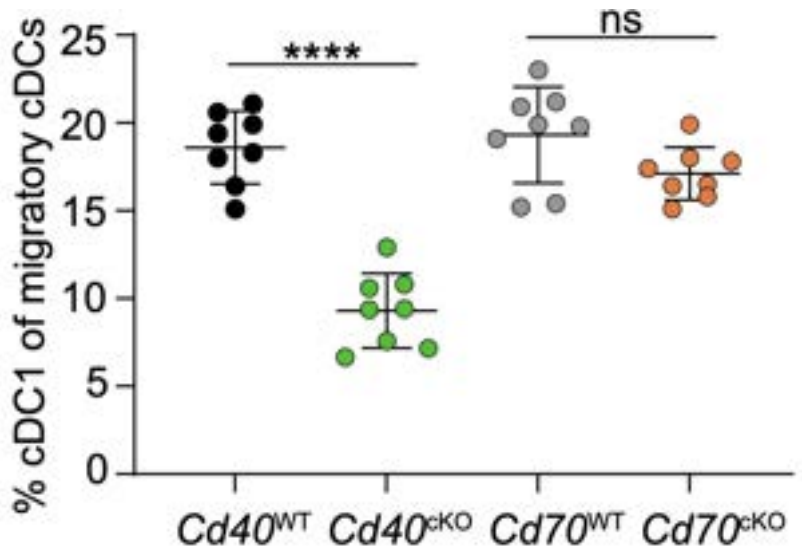
Loss of CD70 on cDC1 reduces anti-tumor CD8 T cell expans

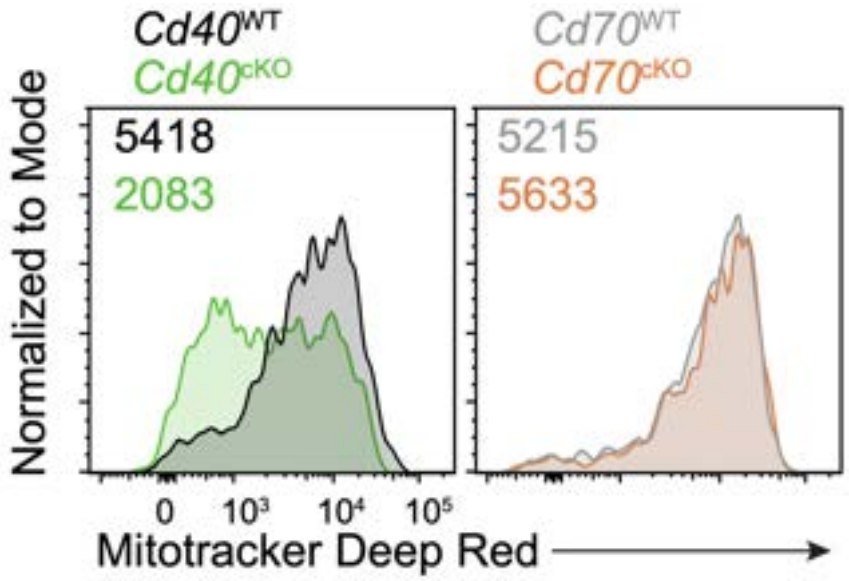


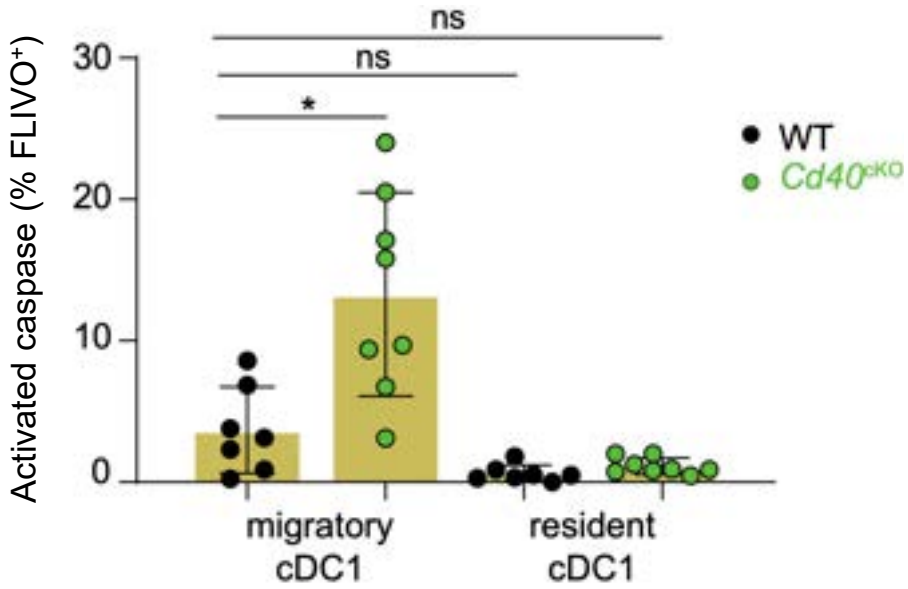


Loss of CD40 signaling reduces migratory cDC1 during tumor res

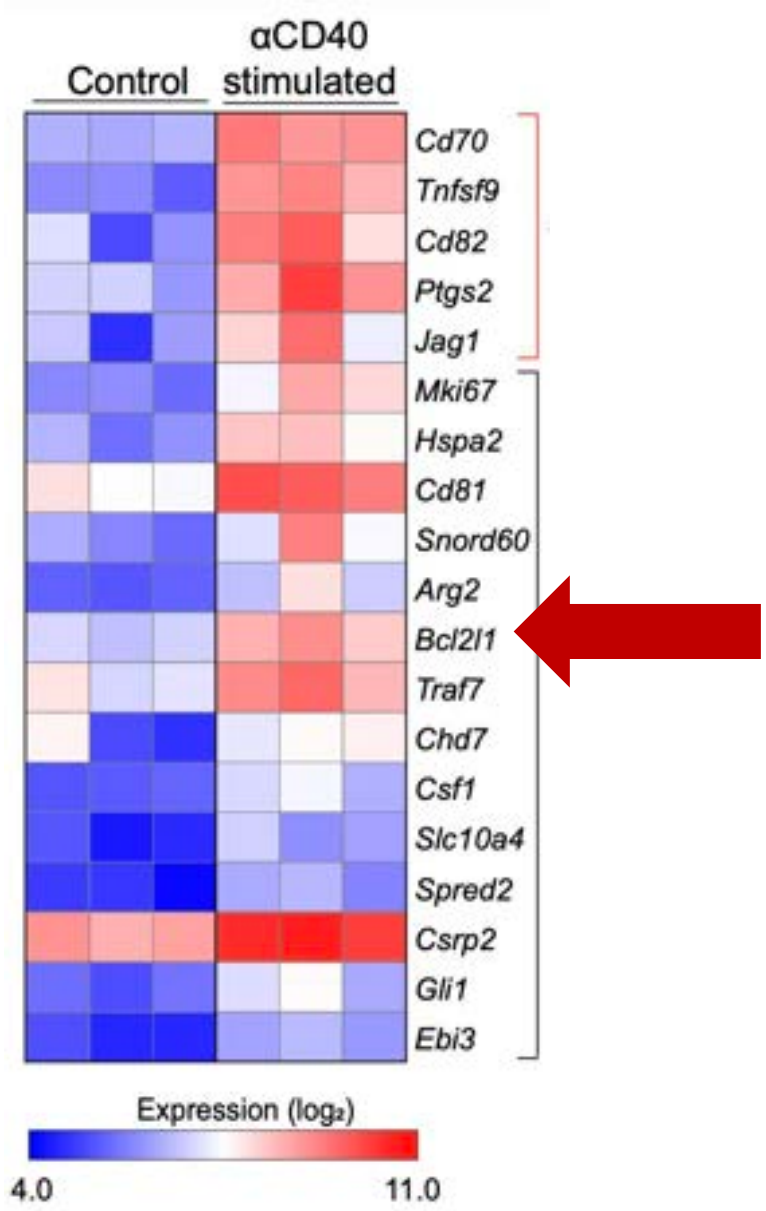


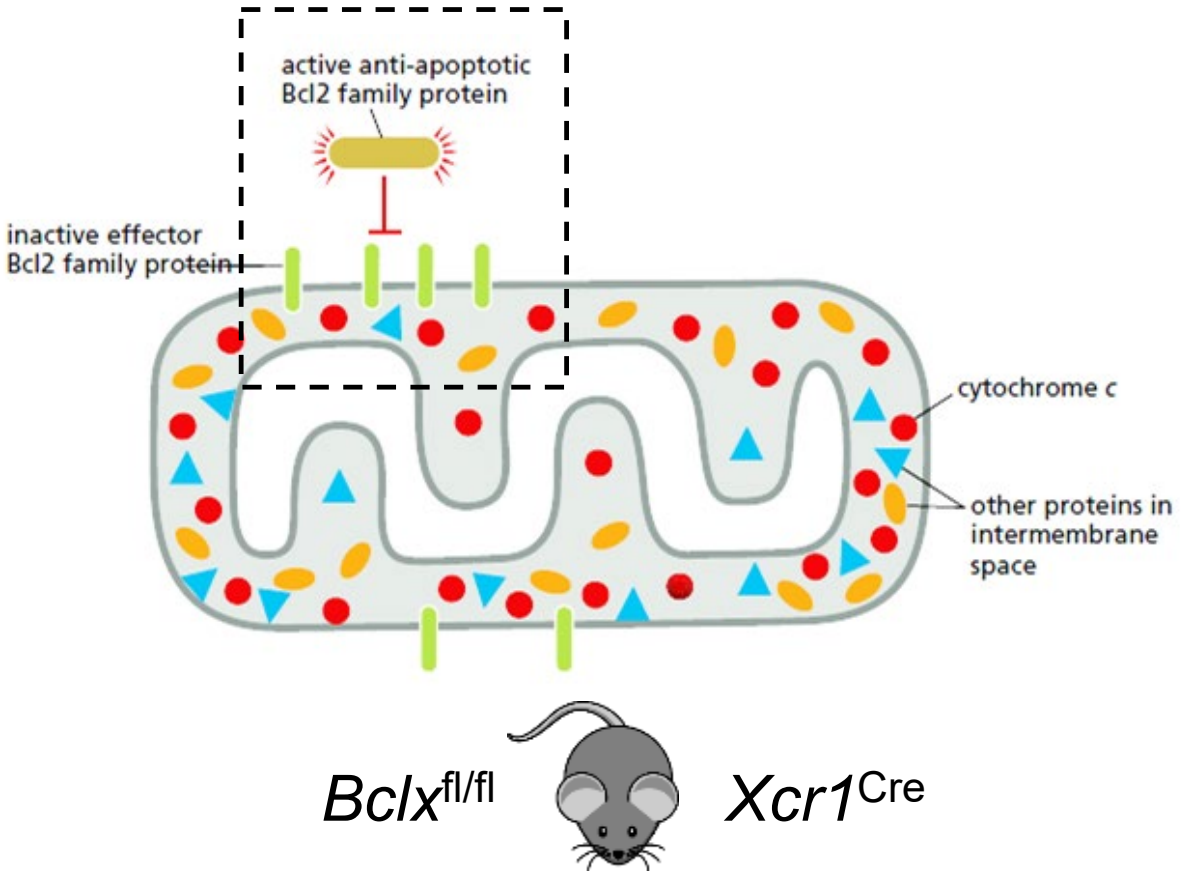






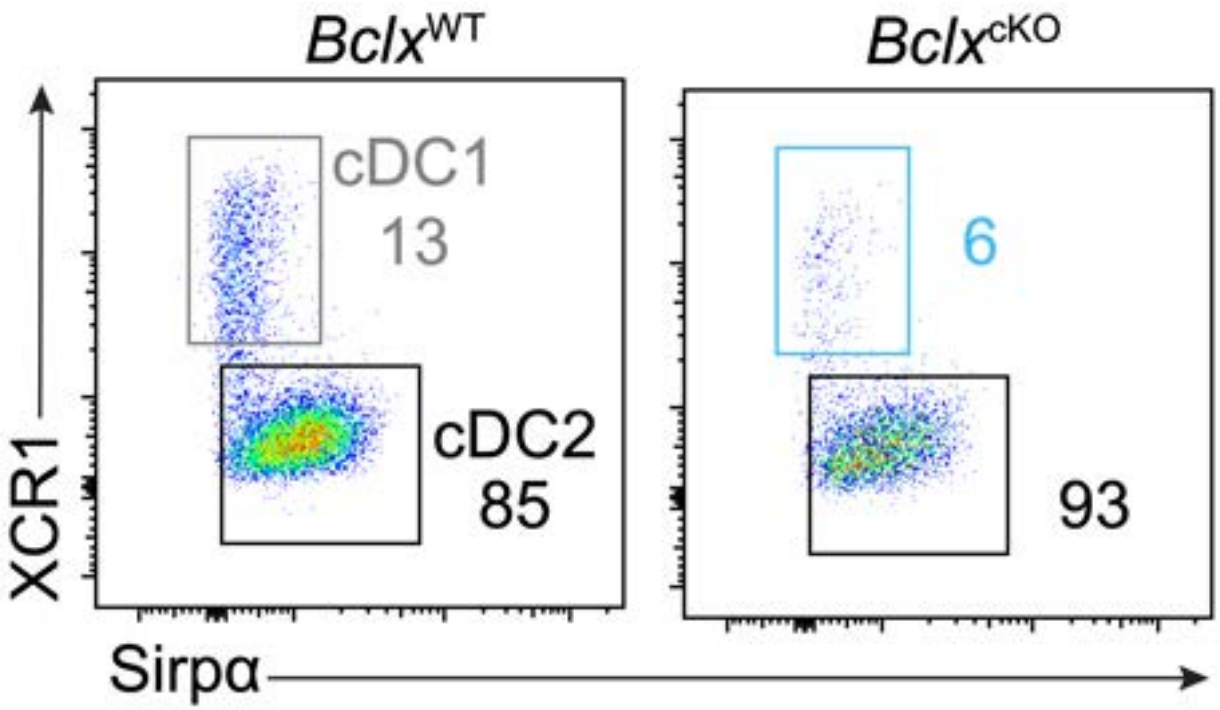
CD40 signaling induces Bcl-xL, an anti-apoptotic Bcl2 family mem

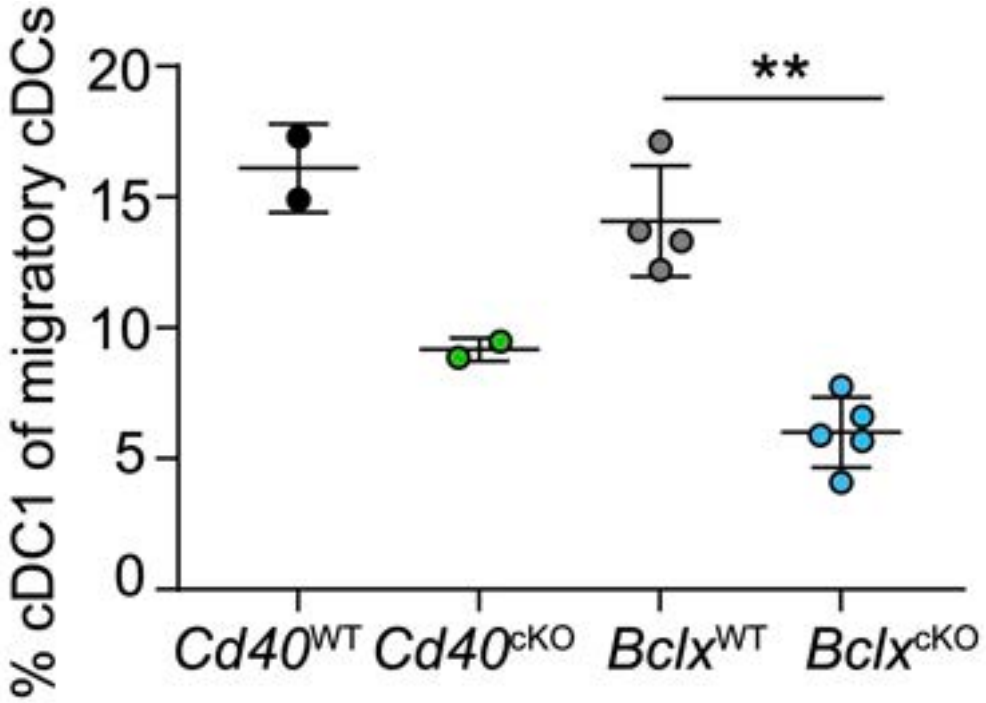




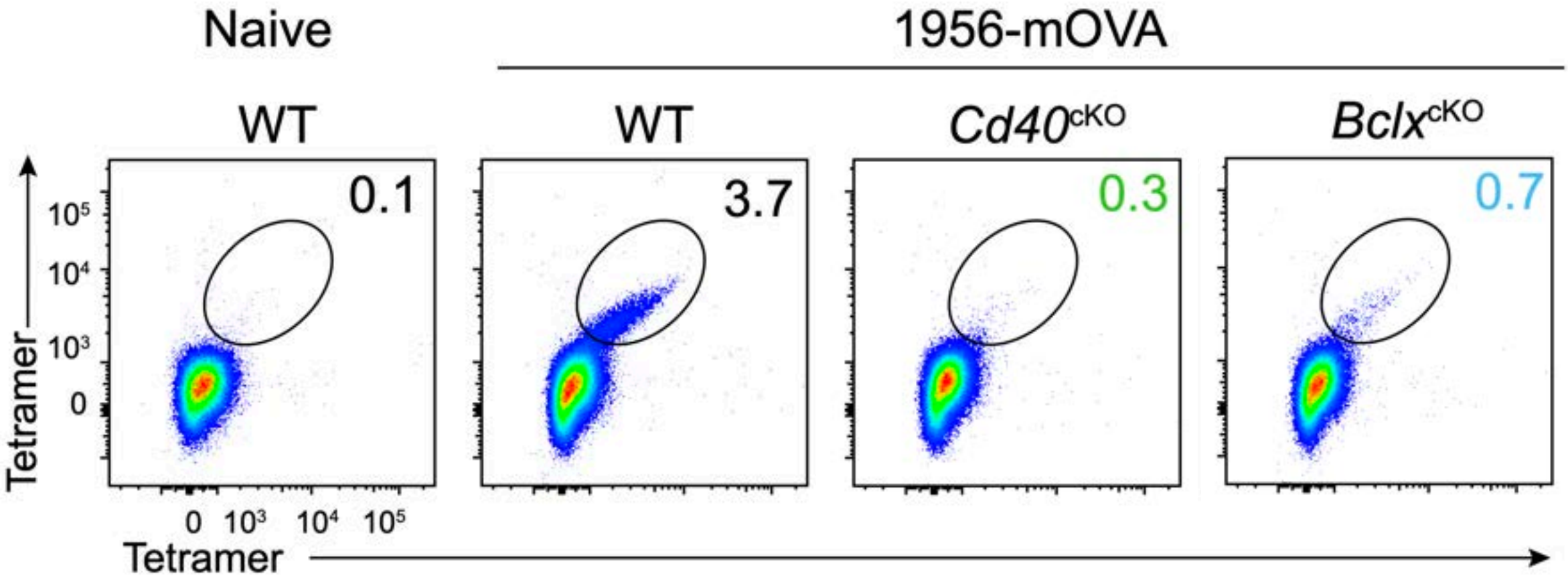
Is Bcl-xL required in cDC1 for anti-tumor immunity?

Loss of Bcl-xL reduces migratory cDC1 in tumor-draining LN



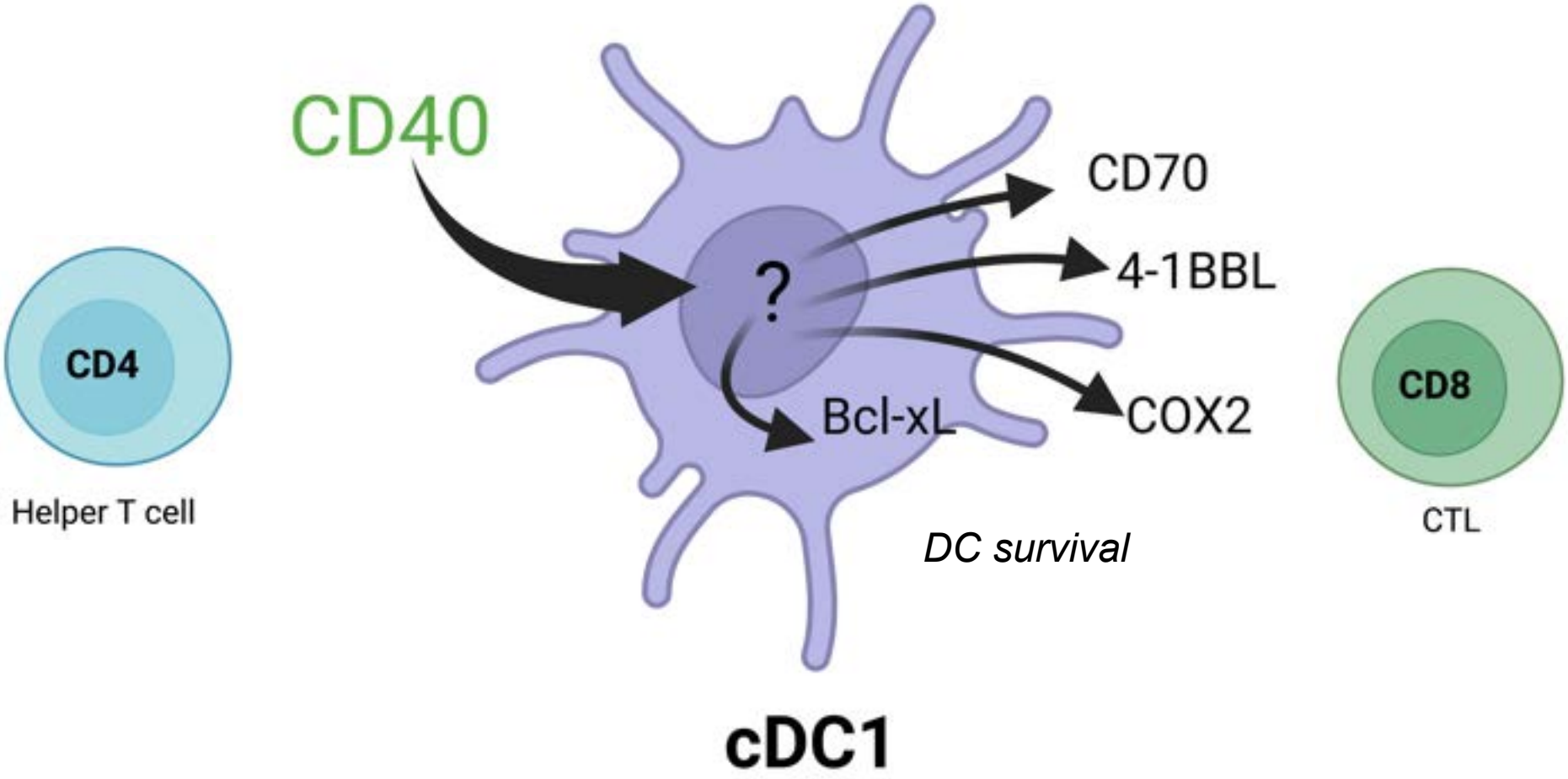


Loss of Bcl-xL in cDC1 reduces anti-tumor CD8 T cell expa



Conclusion:

CD40 signaling acts as a control hub for licensing cDC1



Summary

What we know.
CD40 signaling in cDC1 has a HUGE impact on CD8 T cells responses.
CD40 is a transcription HUB that controls several target genes.
All identified CD40 targets have smaller impacts on CD8 responses than CD40.
Bcl-xL induction in cDC1 contributes to increased ‘vitality’ of cDC1.

What we don’t know.
Is there another way to activate CD40 in cDC1 besides the CD4 T cell? (NKT?)
Could multiple CD8 T cell clones combine to ‘help’ the cDC1.
What kind of CD4 T cell licenses the cDC1? T_{FH}? T_H1? Etc.
What APC normally activates the CD4? cDC1 can, but do they always?

What about cDC2 functions?

IL-23

ILC3

IL-22

epithelium

Lu *et al*, *Am J Clin Nut* 2001

The different functions of cDC2 are still being worked out.

Plasmacytoid DCs

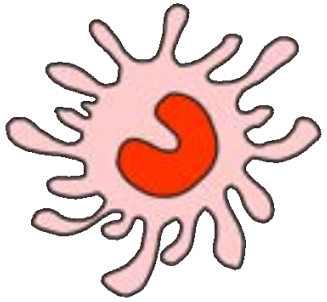
E2-2 dependent
Irf8^{hi} Irf4^{lo}

B220^{+} SiglecH^{+} Bst2^{+} (CD317)

anti-viral
IFNα/β

pDC specific deletion
BDCA2-DTR

cDC1



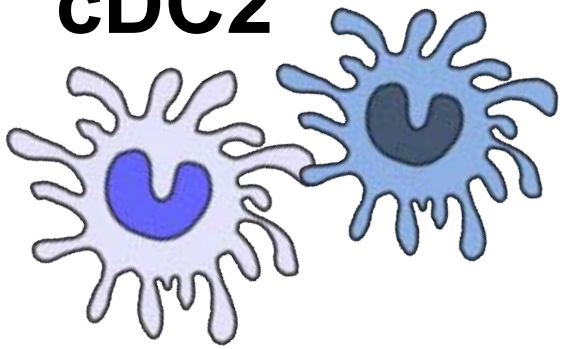
IRF8^{high}

Xcr1, *Clec9a*, *Tlr3*

Intracellular pathogens, tumor
IL-12 production, Th1 induction
Cross-presentation

cDC1 specific deletion
(Xcr1-Cre, Batf3$^{-/-}$ mice, Irf8 32$^{-/-}$)

cDC2



IRF4$^{low/int}$

CD4, Sirp-α (CD172a), ESAM

Fungi, extracellular bacteria,
parasites ??
IL-23 production
Th2, Th17 induction

So far only non-specific deletion
(CD11c-Cre or,germline *Irf4*, Mgl2-DTR)

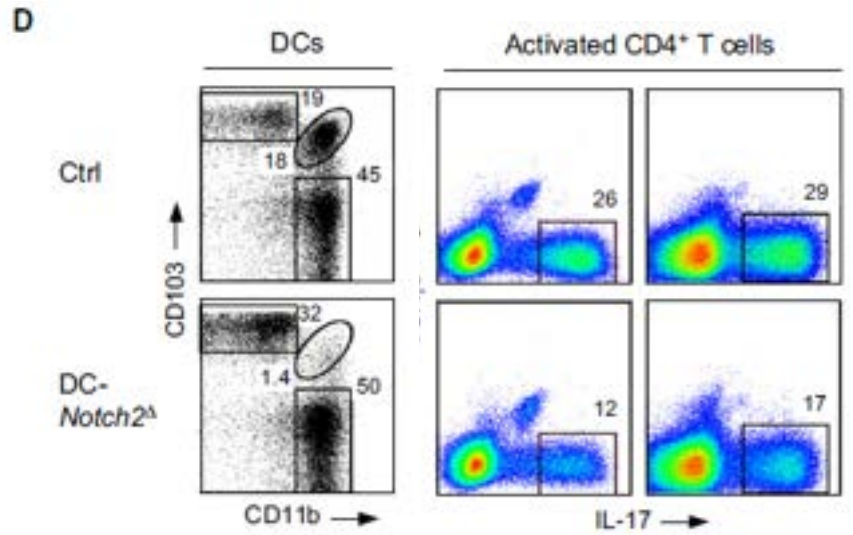
Hildner and Murphy_Science (2008), Satpathy and Murphy_Nat Immunol (2013)
Tussiwand and Murphy_Immunity (2015), Grajales-Reyes and Murphy_Nat Immunol (2015), Murphy and Murphy,_Ann Rev Immunol (2016)

IRF4/Notch2-dependent DCs influence IL-17/IL-22 responses

No Infection model (SFB⁺ colony?)

Notch2 Receptor Signaling Contr
Functional Differentiation of Dend
in the Spleen and Intestine

Kanako L. Lewis,[1] Michele L. Caton,[1] Milena Bogunovic,[2] Melanie Greter,[2]
Apostolos Klinakis,[4] Israel F. Charo,[5] Steffen Jung,[6] Jennifer L. Gommerma
and Boris Reizis[1,*]



No Infection model

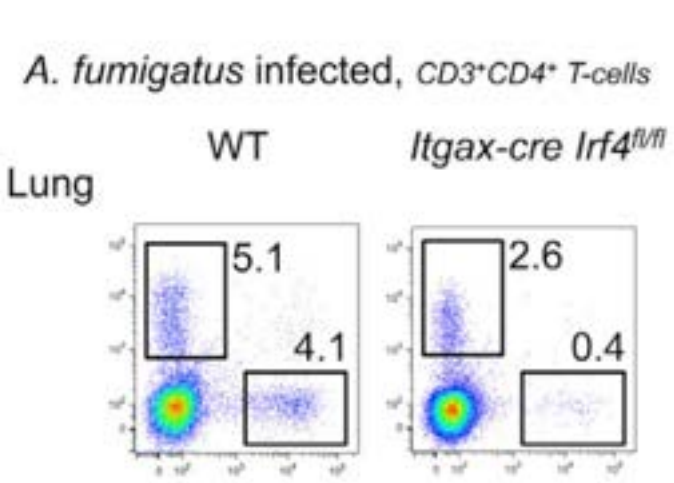
IRF4 Transcription-Factor-Dependent CD103⁺CD11b⁺ Dendritic Cells Drive Mucosal T Helper 17 Cell Differentiation

Emma K. Persson,[1] Heli Uronen-Hansson,[1] Monika Semmrich,[1] Aymeric Rivollier,[1] Karin Hägi
Sigurdur Gudjonsson,[3] Ulf Håkansson,[3] Boris Reizis,[4] Knut Kotarsky,[1] and William W. Agace[1]

Aspergillus fumigatus

IRF4 Transcription Factor-Dependent CD11b⁺ Dendritic Cells in Human and Mou Control Mucosal IL-17 Cytokine Response

Andreas Schlitzer,[1,9] Naomi McGovern,[2,9] Pearline Teo,[1] Teresa Zelante,[1] Koji Atarashi,[3] Dono
Peter See,[1] Amanda Shin,[1] Pavandip Singh Wasan,[1] Guillaume Hoeffel,[1] Benoit Malleret,[1] Ale
Samantha Chew,[1] Laura Jardine,[2] Harriet A. Purvis,[2] Catharien M.U. Hilkens,[2] John Tam,[5,6] M
E. Richard Stanley,[7] Anne B. Krug,[4] Laurent Renia,[1] Baalasubramanian Sivasankar,[8] Lai Guar
Paola Ricciardi-Castagnoli,[1] Kenya Honda,[3] Muzlifah Haniffa,[2] and Florent Ginhoux[1,*]



cDC2 support TH17 type responses

IRF4 Transcription Factor-Dependent CD11b$^+$ Dendritic Cells in Human and Mouse Control Mucosal IL-17 Cytokine Responses

Andreas Schlitzer,[1,9] Naomi McGovern,[2,9] Pearline Teo,[1] Teresa Zelante,[1] Koji Atarashi,[3] Donovan Low,[1] Adrian W.S. Ho,[1] Peter See,[1] Amanda Shin,[1] Pavandip Singh Wasan,[1] Guillaume Hoeffel,[1] Benoit Malleret,[1] Alexander Heiseke,[4] Samantha Chew,[1] Laura Jardine,[2] Harriet A. Purvis,[2] Catharien M.U. Hilkens,[2] John Tam,[5,6] Michael Poidinger,[1] E. Richard Stanley,[7] Anne B. Krug,[4] Laurent Renia,[1] Baalasubramanian Sivasankar,[8] Lai Guan Ng,[1] Matthew Collin,[2] Paola Ricciardi-Castagnoli,[1] Kenya Honda,[3] Muzlifah Haniffa,[2] and Florent Ginhoux[1,*]

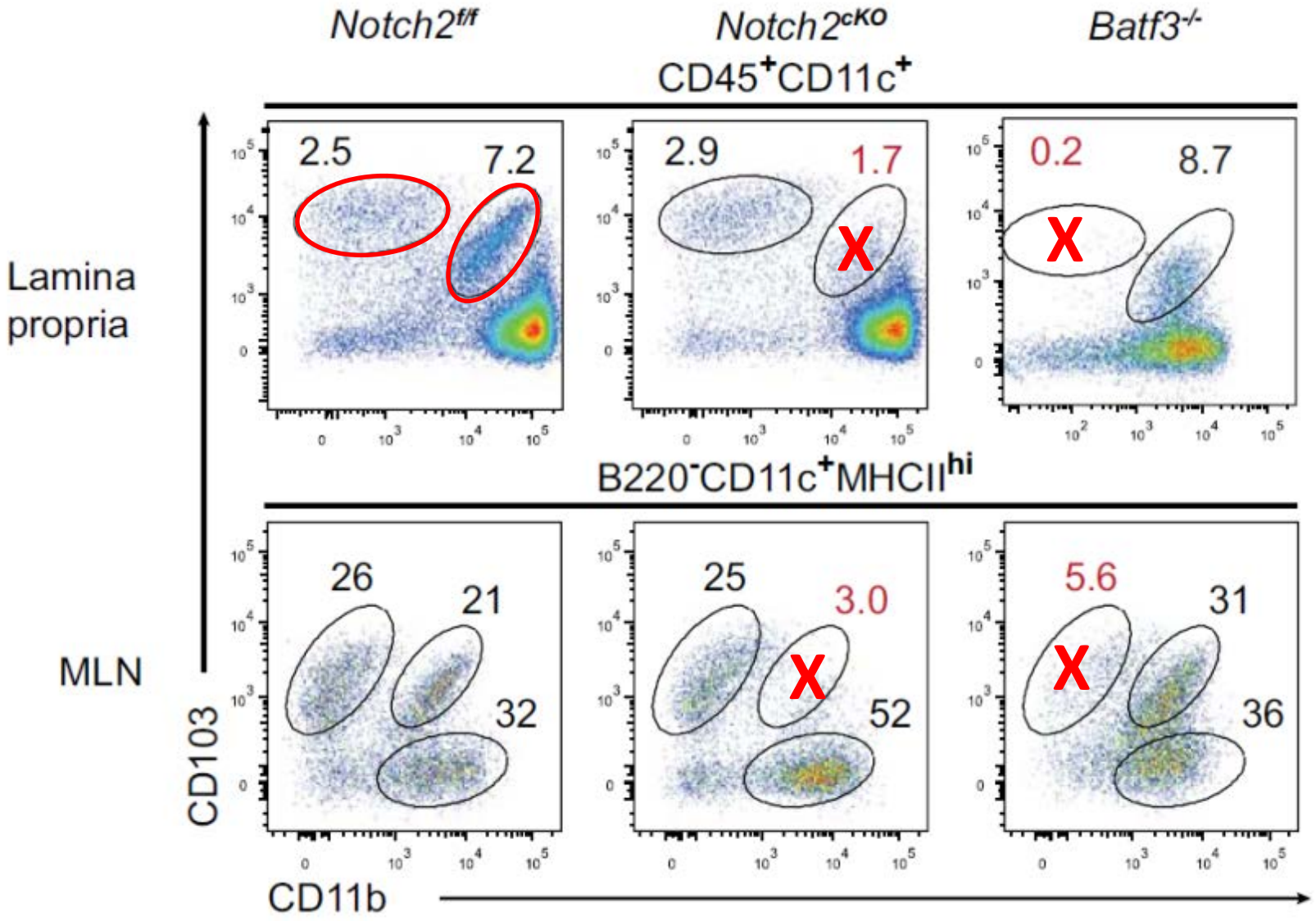
2013

Notch2-dependent classical dendritic cells orchestrate intestinal immunity to attaching-and-effacing bacterial pathogens

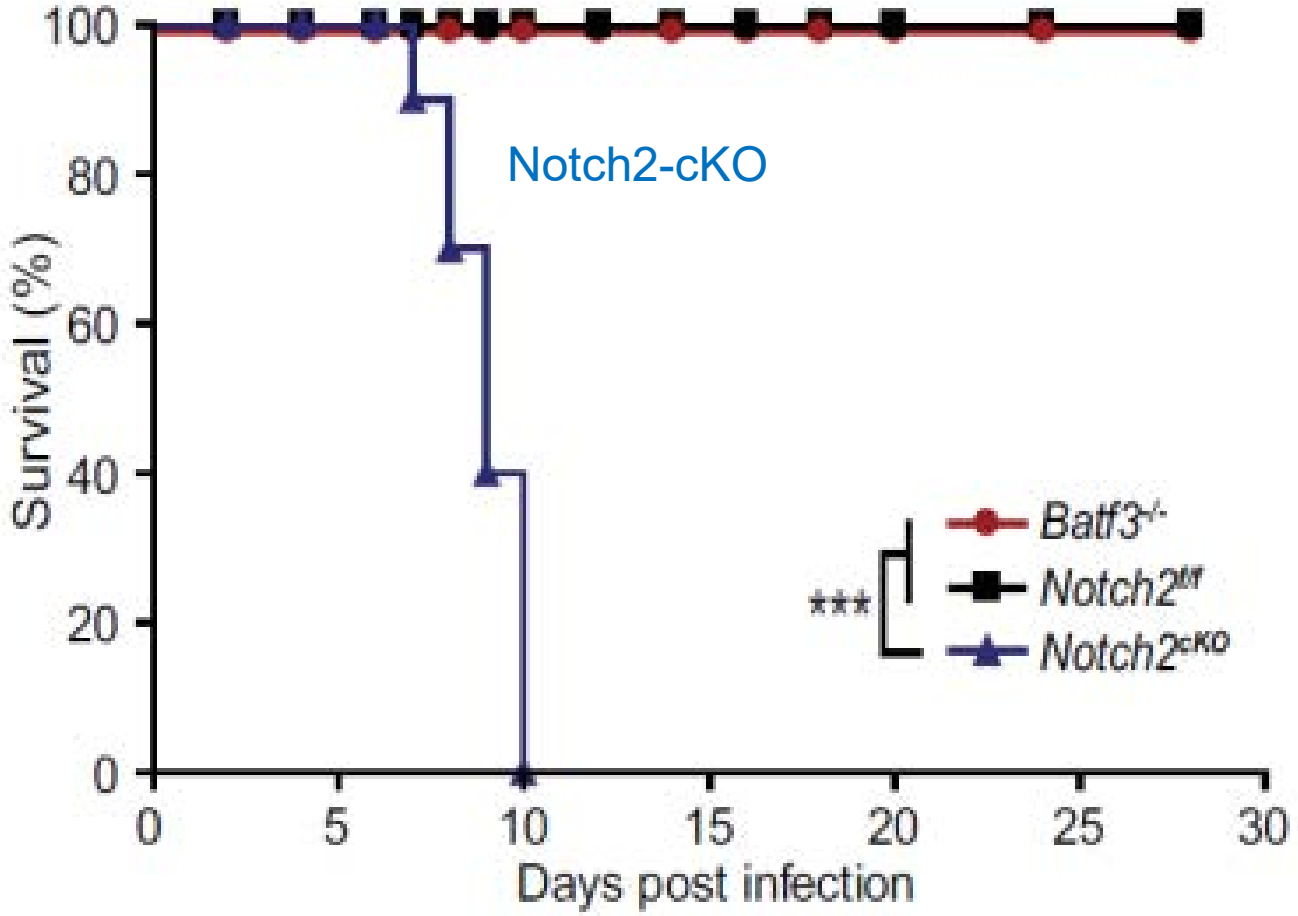
Ansuman T Satpathy[1], Carlos G Briseño[1], Jacob S Lee[1], Dennis Ng[2], Nicholas A Manieri[1], Wumesh KC[1], Xiaodi Wu[1], Stephanie R Thomas[1], Wan-Ling Lee[1], Mustafa Turkoz[3], Keely G McDonald[4], Matthew M Meredith[5], Christina Song[1], Cynthia J Guidos[2,6], Rodney D Newberry[4], Wenjun Ouyang[7], Theresa L Murphy[1], Thaddeus S Stappenbeck[1], Jennifer L Gommerman[2], Michel C Nussenzweig[5,8], Marco Colonna[1], Raphael Kopan[3] & Kenneth M Murphy[1,9]

2013

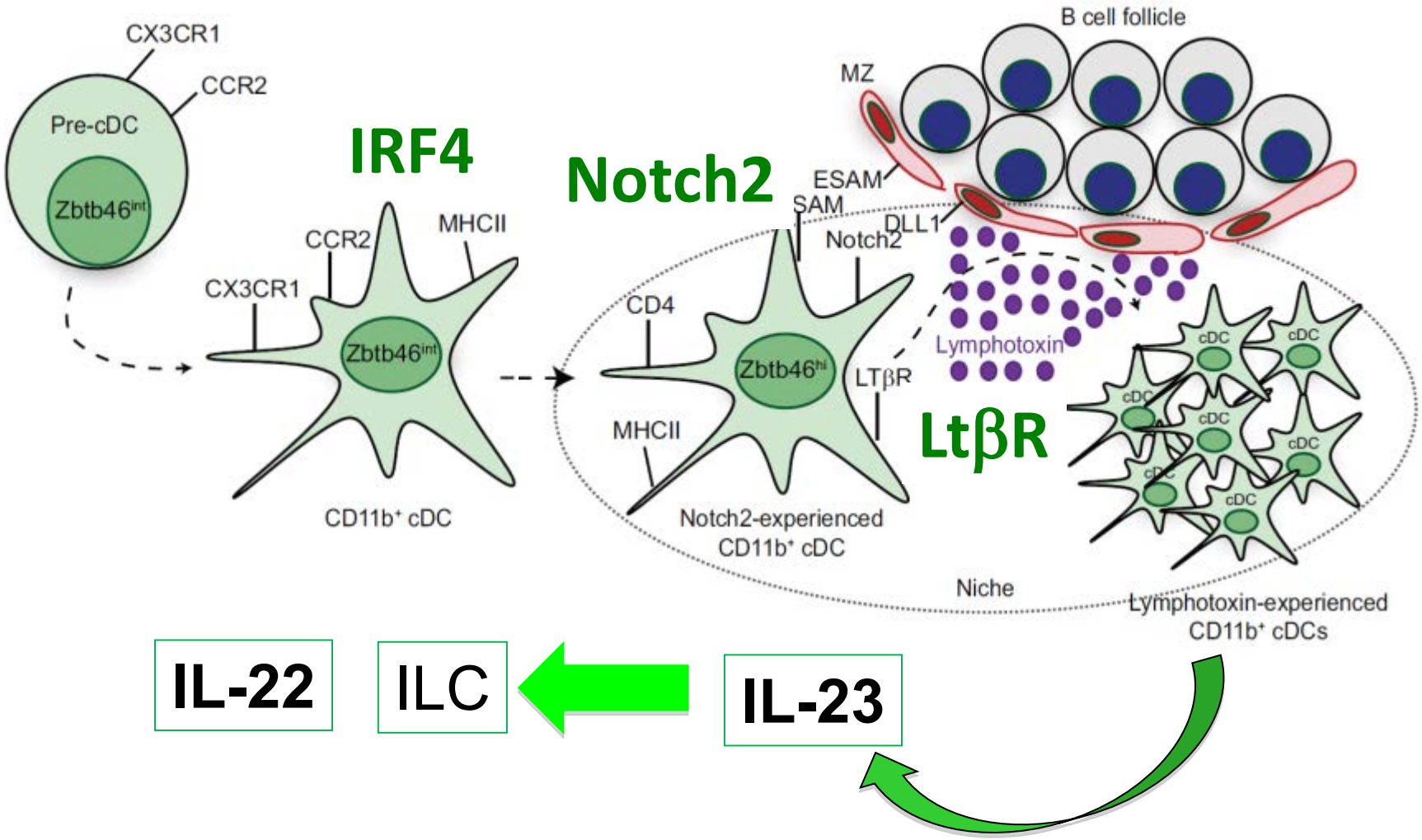
Intestinal CD11b^{+} CD103^{+} cDCs are Notch2-dependent



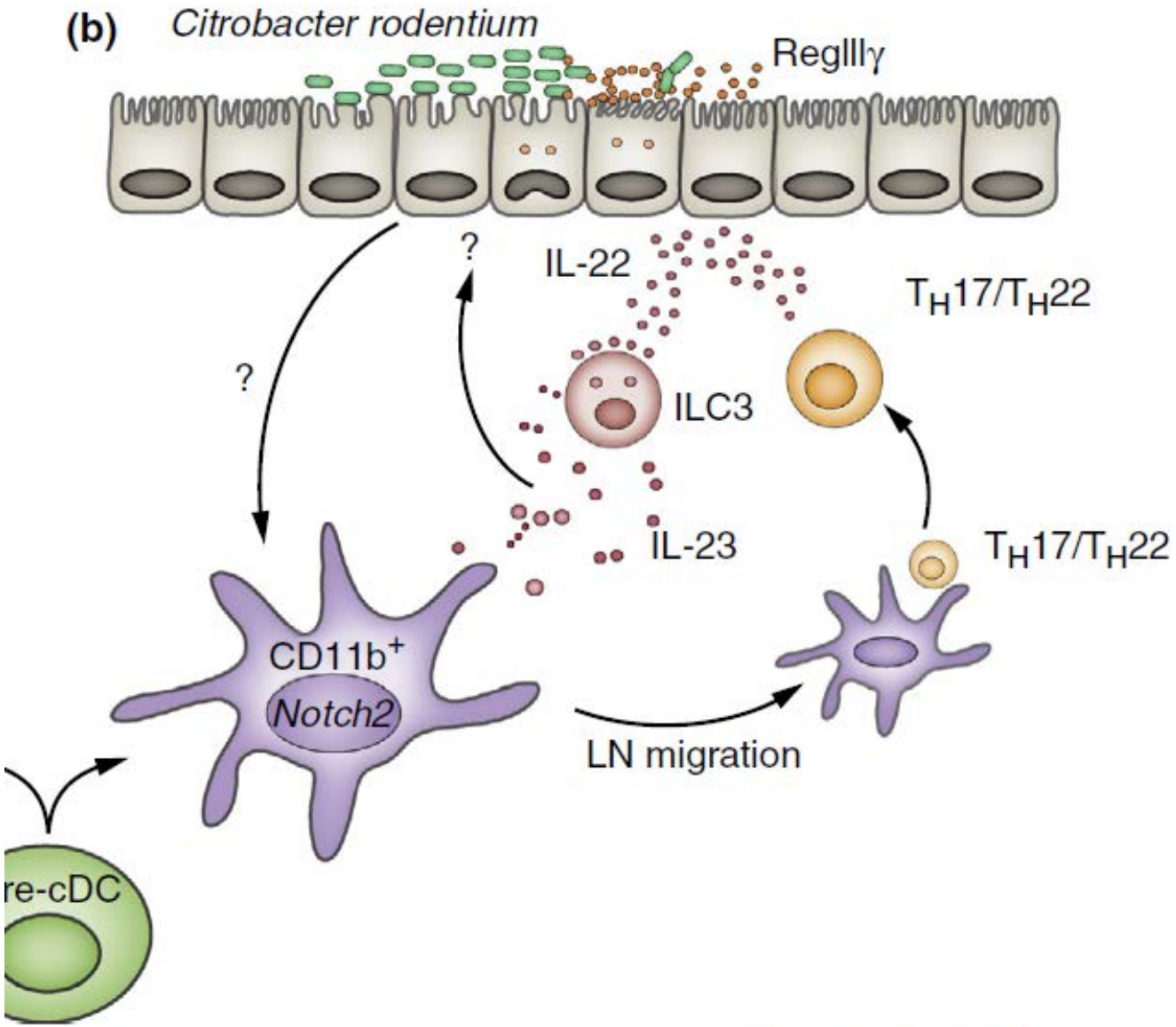
Notch2-dependent CD11b^{+} DCs are required in C. *rodentium* for IL-23



Development and maturation of CD11b^{+} cDCs



cDC2 are useful in defense against *Citrobacter rodentium*

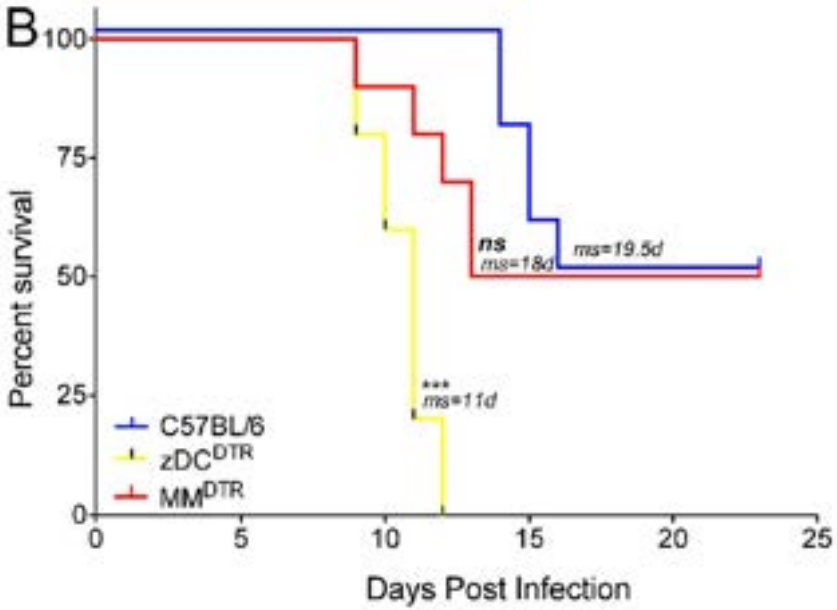


DCs act earlier than Mono/Macs in *C. rodentium*

C. rodentium **zDC-DTR system**
MM-DTR system

Intestinal monocytes and macrophages are required for T cell polarization in response to *Citrobacter rodentium*

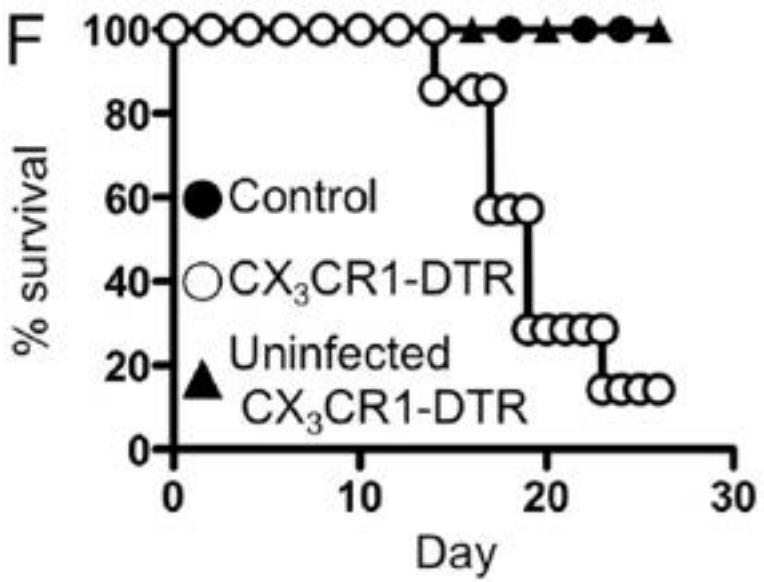
Heidi A. Schreiber,[1] Jakob Loschko,[1] Roos A. Karssemeijer,[2] Amelia Escolano,[1] Matthew M. Meredith,[1] Daniel Mucida,[2] Pierre Guermonprez,[1,4] and Michel C. Nussenzweig[1,3]



C. rodentium **CX3CR1-DTRsystem**

CX_3CR1^+ mononuclear phagocytes support colitis-associated innate lymphoid cell production of IL-22

Randy S. Longman,[1,3] Gretchen E. Diehl,[1] Daniel A. Victorio,[1,3] Jun R. Huh,[1] Carolina Galan,[1] Emily R. Miraldi,[1,5,6] Arun Swaminath,[4] Richard Bonneau,[5,6] Ellen J. Scherl,[3,4] and Dan R. Littman[1,2]

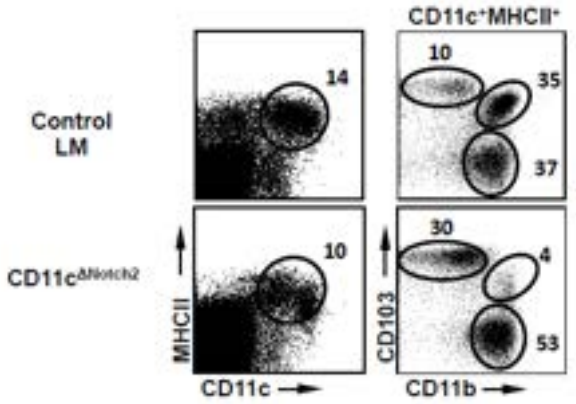


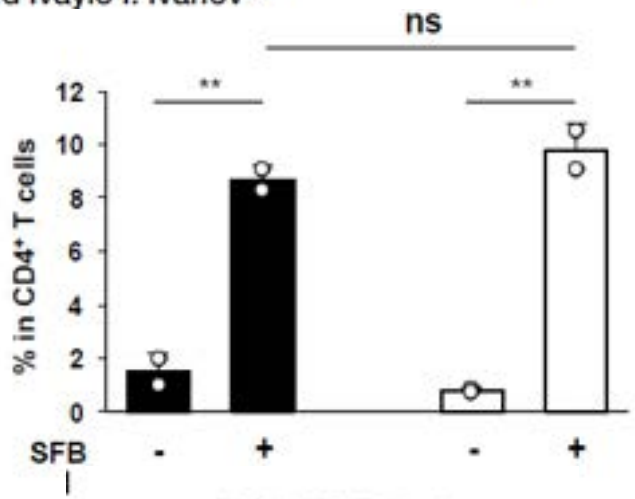
T_H17 to SFB may use MACs, not DCs.

Intestinal Monocyte-Derived Macrophages Control Commensal-Specific Th17 Responses

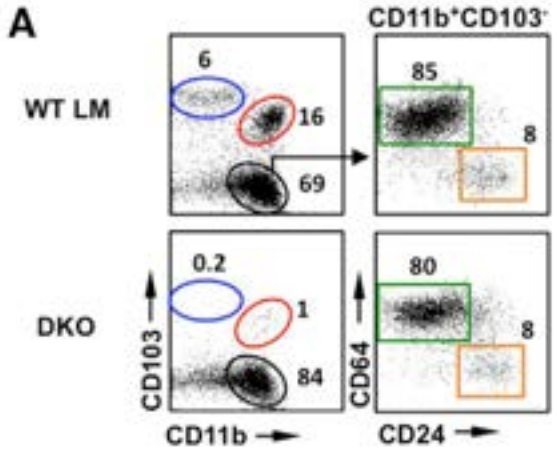
Casandra Panea,[1] Adam M. Farkas,[1] Yoshiyuki Goto,[1,4,5] Shahla Abdollahi-Roodsaz,[1,6,7] Carolyn Lee,[1] Kavitha Gowda,[2] Tobias M. Hohl,[3] Milena Bogunovic,[2] and Ivaylo I. Ivanov[1,*]

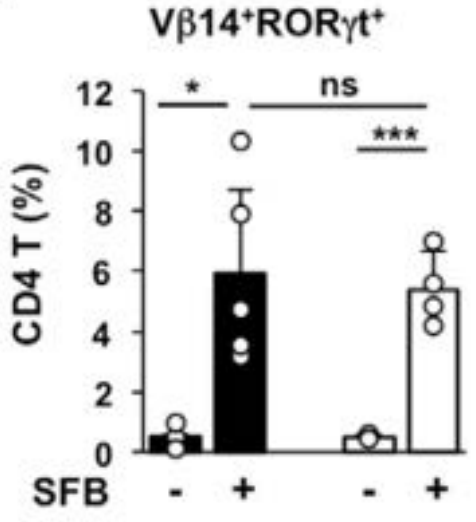
Notch2 x CD11c-Cre





Lang-DTA X Batf3 KO



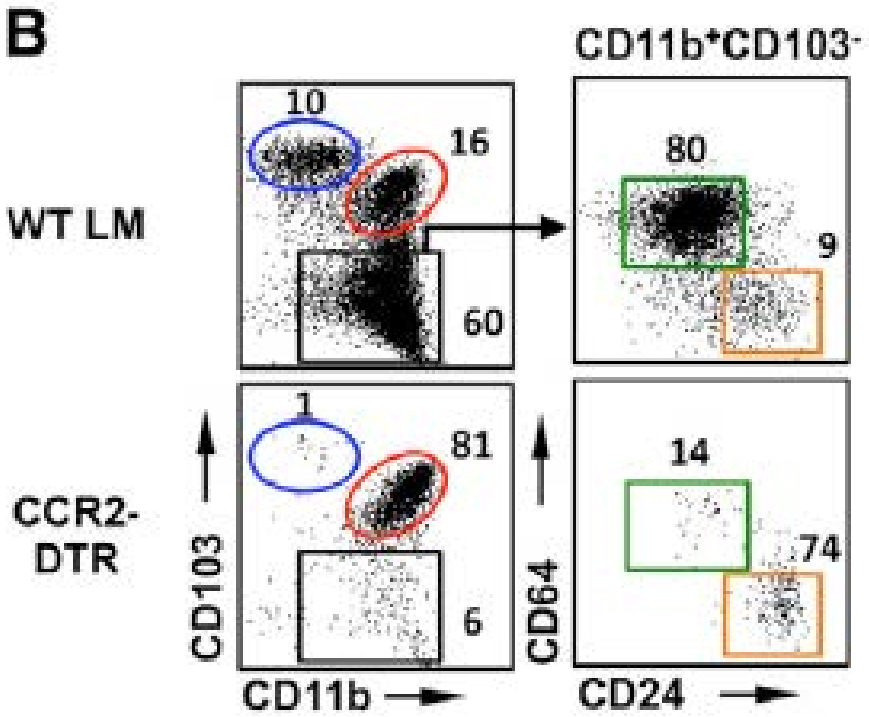


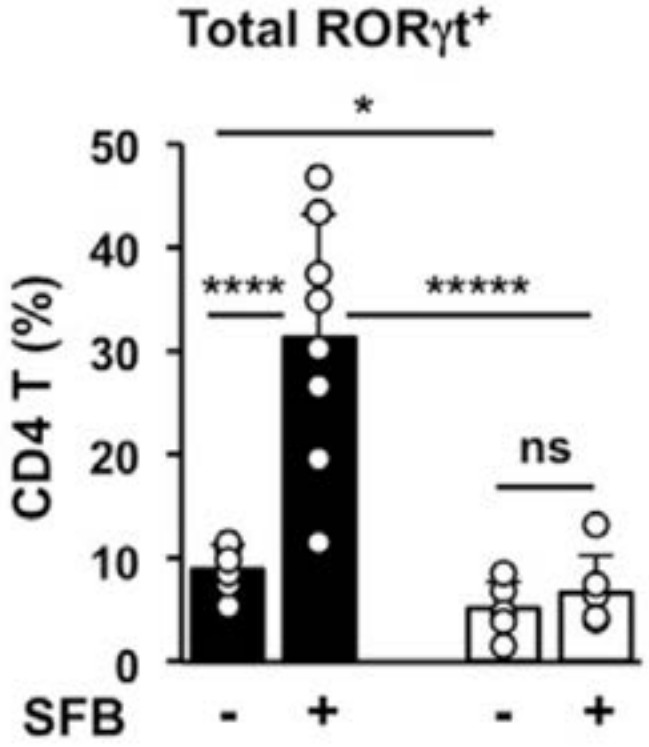
T_H17 to SFB may use MACs, not DCs.

Intestinal Monocyte-Derived Macrophages Control Commensal-Specific Th17 Responses

Casandra Panea,[1] Adam M. Farkas,[1] Yoshiyuki Goto,[1,4,5] Shahla Abdollahi-Roodsaz,[1,6,7] Carolyn Lee,[1] Kavitha Gowda,[2] Tobias M. Hohl,[3] Milena Bogunovic,[2] and Ivaylo I. Ivanov[1,*]

CCR2-DTR system





Previous studies linked cDC2 to TH2

CD301b$^+$ Dermal Dendritic Cells Drive T Helper 2 Cell-Mediated Immunity

2013

Yosuke Kumamoto,[1] Melissa Linehan,[1] Jason S. Weinstein,[1] Brian J. Laidlaw,[1] Joseph E. Craft,[1,2] and Akiko Iwasaki[1,*]
[1]Department of Immunobiology
[2]Department of Internal Medicine
Yale University School of Medicine, New Haven, CT 06520, USA
*Correspondence: akiko.iwasaki@yale.edu
http://dx.doi.org/10.1016/j.immuni.2013.08.029

Control of T Helper 2 Responses by Transcription Factor IRF4-Dependent Dendritic Cells

2013

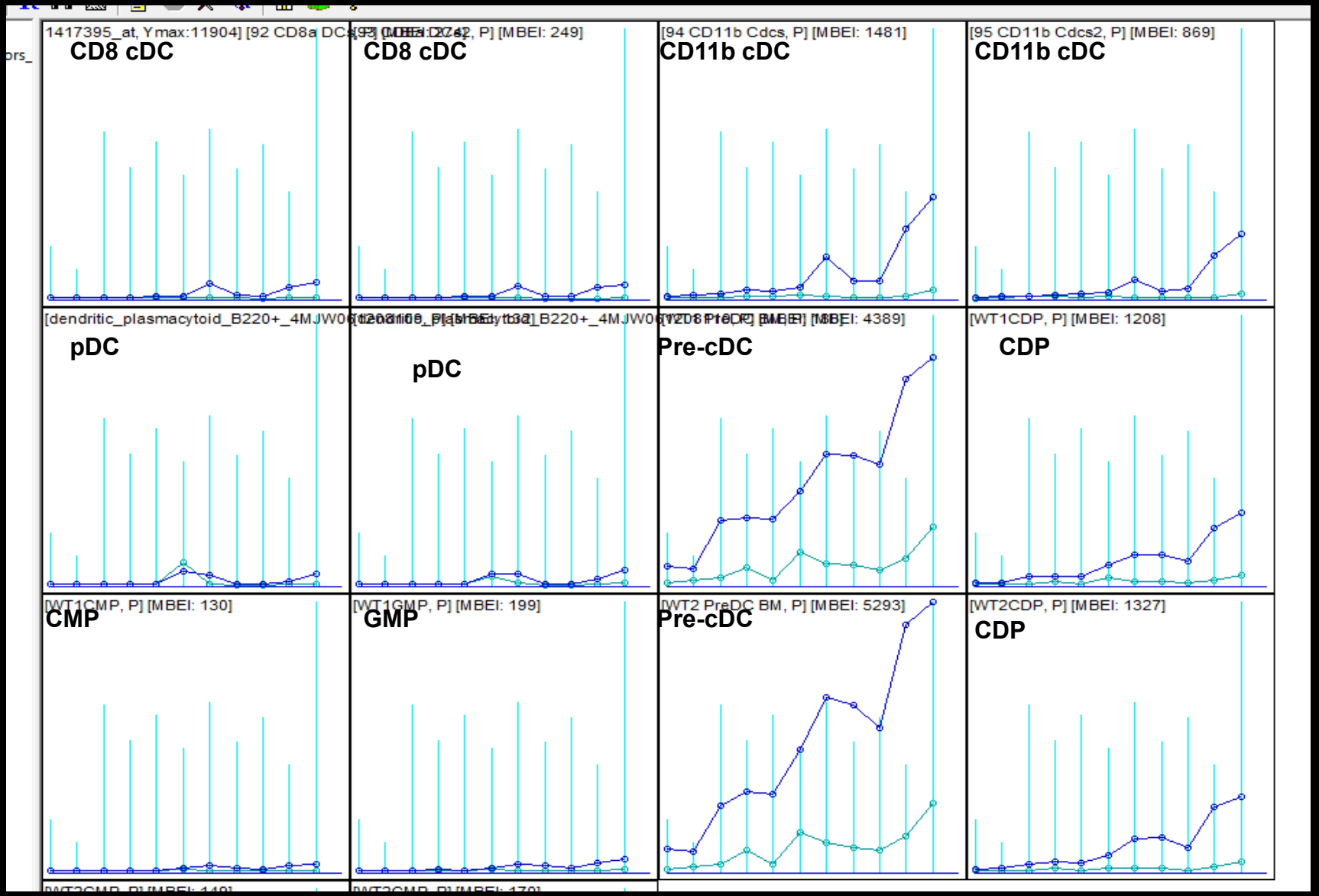
Yan Gao,[1,2,6] Simone A. Nish,[1,6] Ruoyi Jiang,[3] Lin Hou,[4] Paula Licona-Limón,[1] Jason S. Weinstein,[1] Hongyu Zhao,[4] and Ruslan Medzhitov[1,5,*]
[1]Department of Immunobiology, Yale University School of Medicine, New Haven, CT 06520, USA

Klf4 Expression in Conventional Dendritic Cells Is Required for T Helper 2 Cell Responses

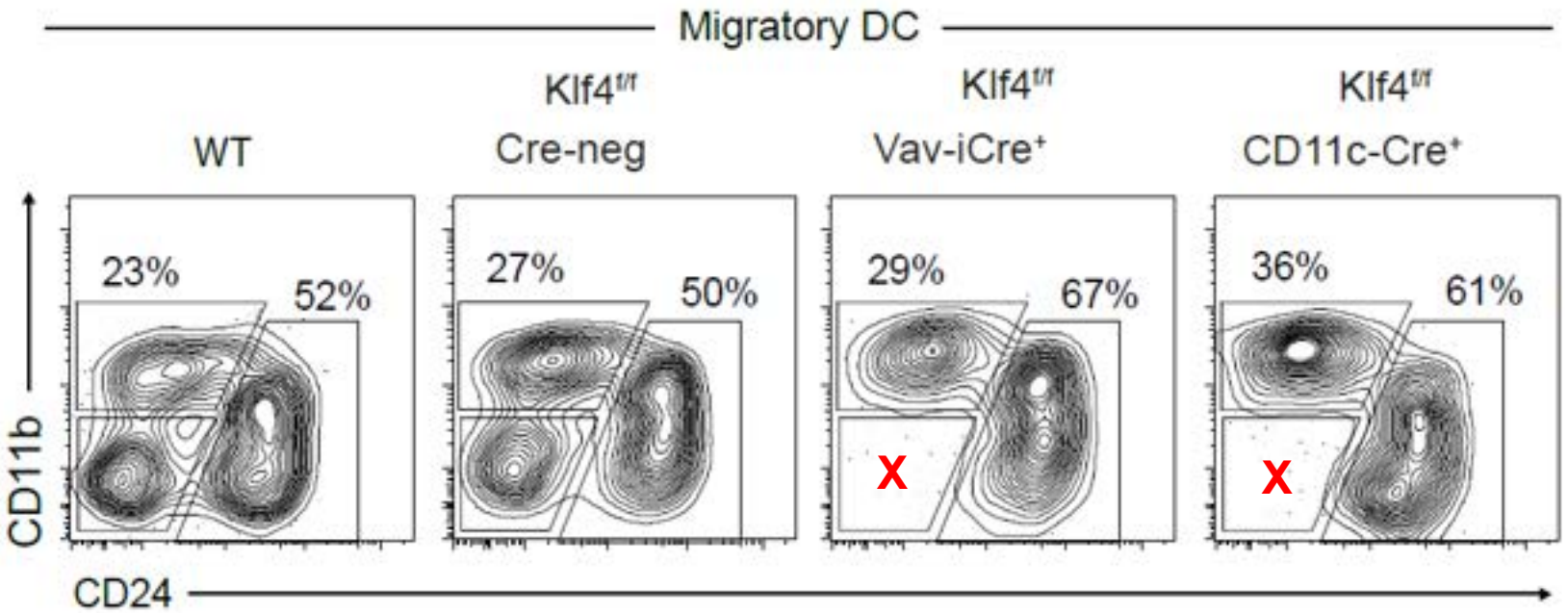
2015

Roxane Tussiwand,[1,3,*] Bart Everts,[1,4] Gary E. Grajales-Reyes,[1] Nicole M. Kretzer,[1] Arifumi Iwata,[1] Juhi Bagaitkar,[5] Xiaodi Wu,[1] Rachel Wong,[1] David A. Anderson,[1] Theresa L. Murphy,[1] Edward J. Pearce,[1] and Kenneth M. Murphy[1,2,*]

Klf4 is highly induced in pre-cDCs



KLF4 deletion in cDCs eliminates CD11b^{lo} migratory cDCs

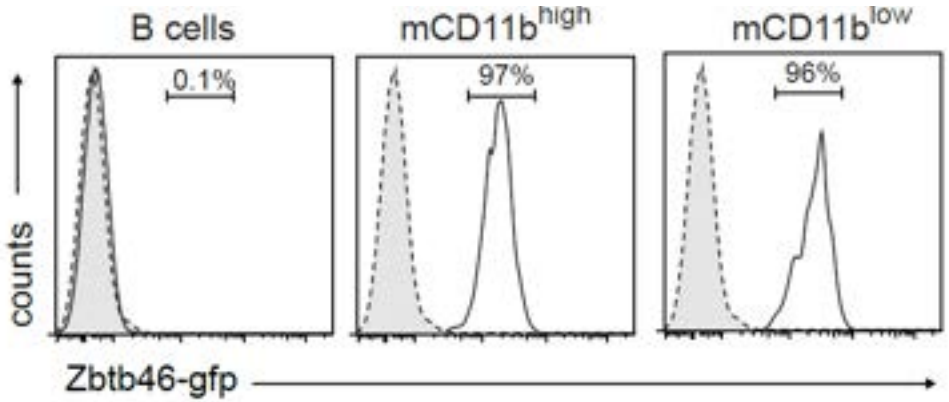


CD326^{lo}CD103^{lo}CD11b^{lo} Dermal Dendritic Cells Are Activated by Thymic Stromal Lymphopoietin during Contact Sensitization in Mice

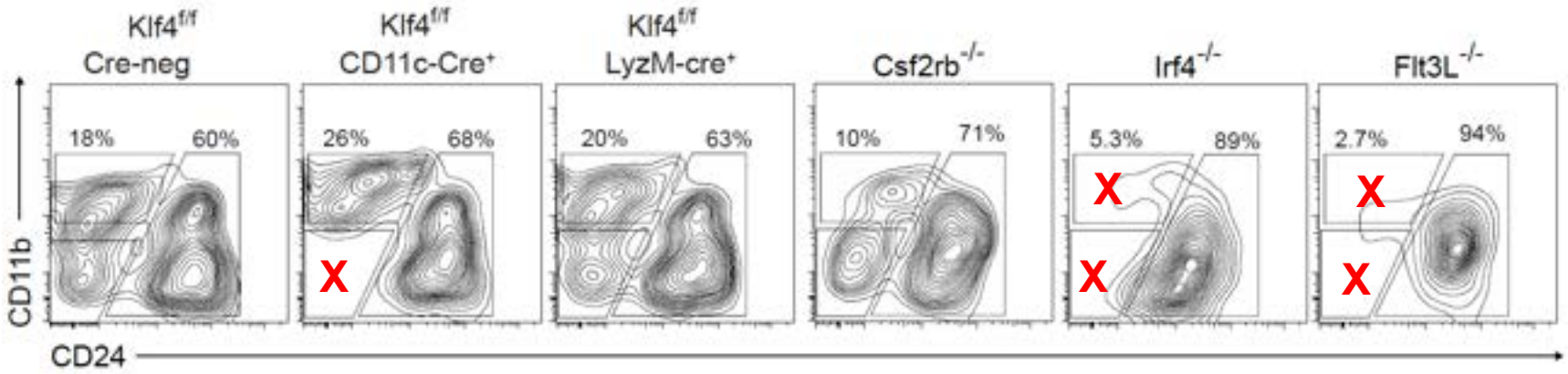
Sotaro Ochiai,*,† Ben Roediger,‡ Arby Abtin,‡ Elena Shklovskaya,‡ Barbara Fazekas de St. Groth,‡ Hidehiro Yamane,§ Wolfgang Weninger,‡,¶ Graham Le Gros,* and Franca Ronchese*

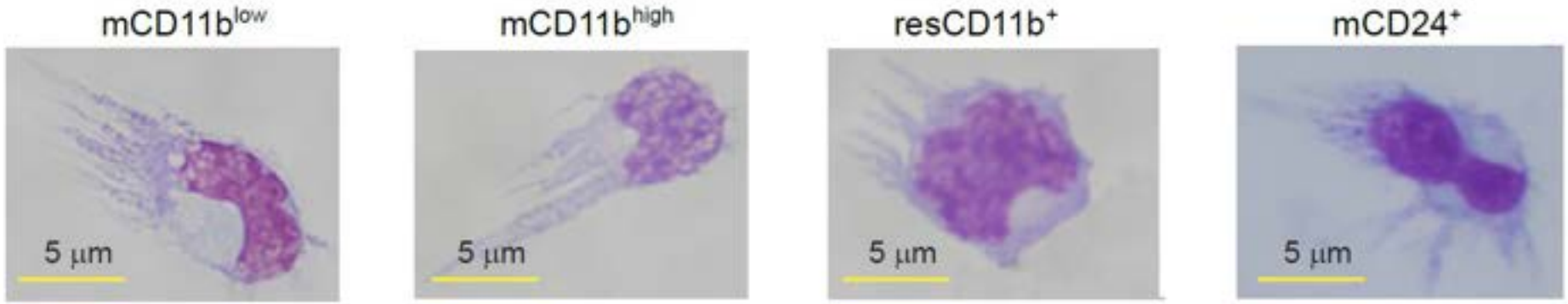
JI 2014

KLF4 cDCs express Zbtb46 and require IRF4 for migration

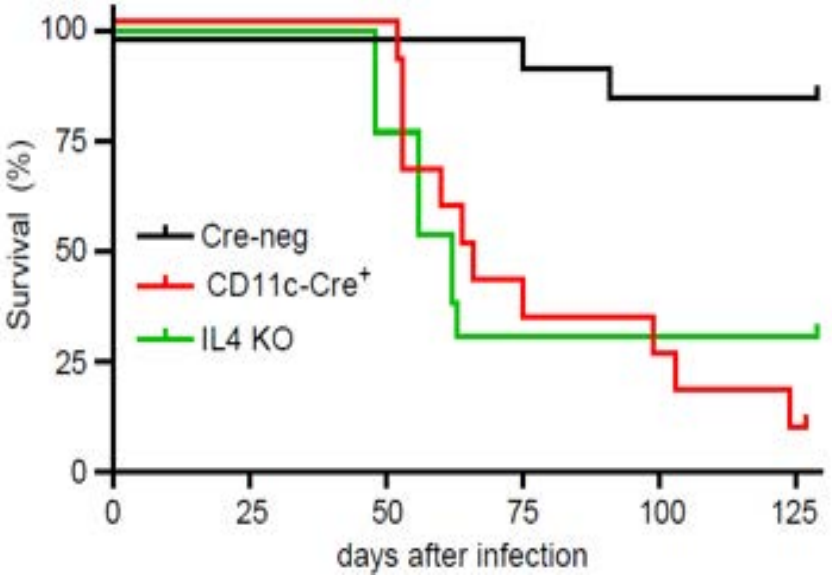


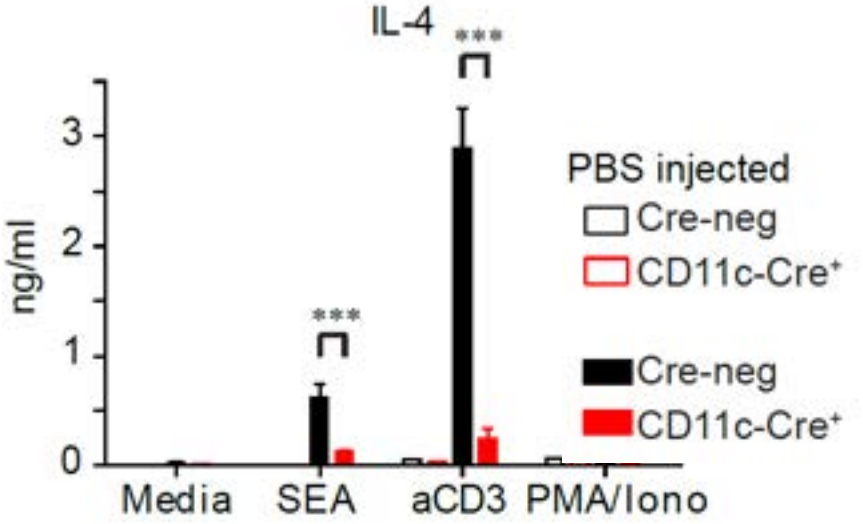
(Cell-intrinsic by chimeras)

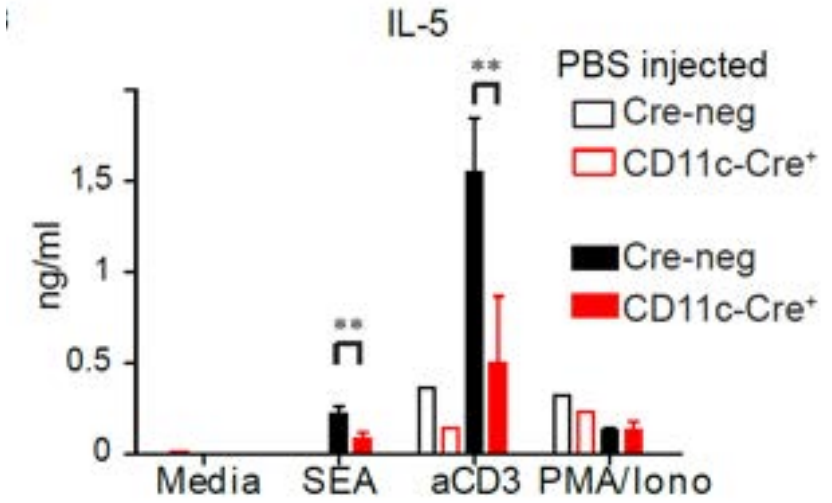




KLF4 cDCs are required for resistance to *Schistosoma mansoni*



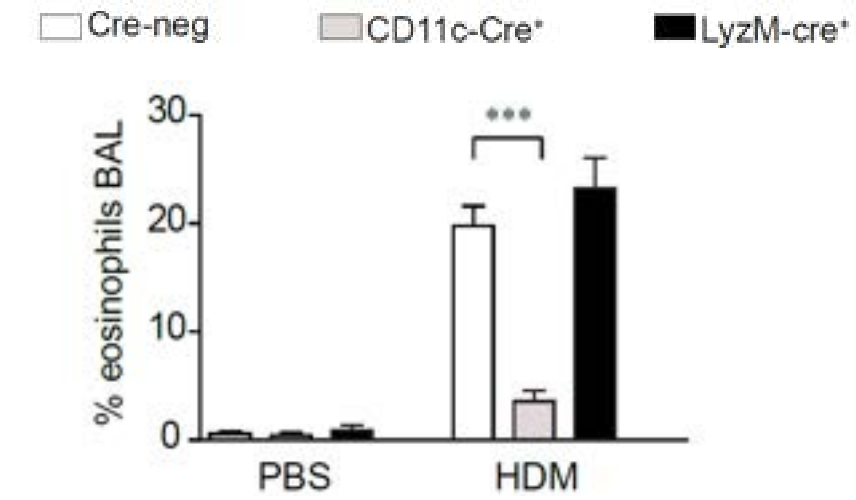


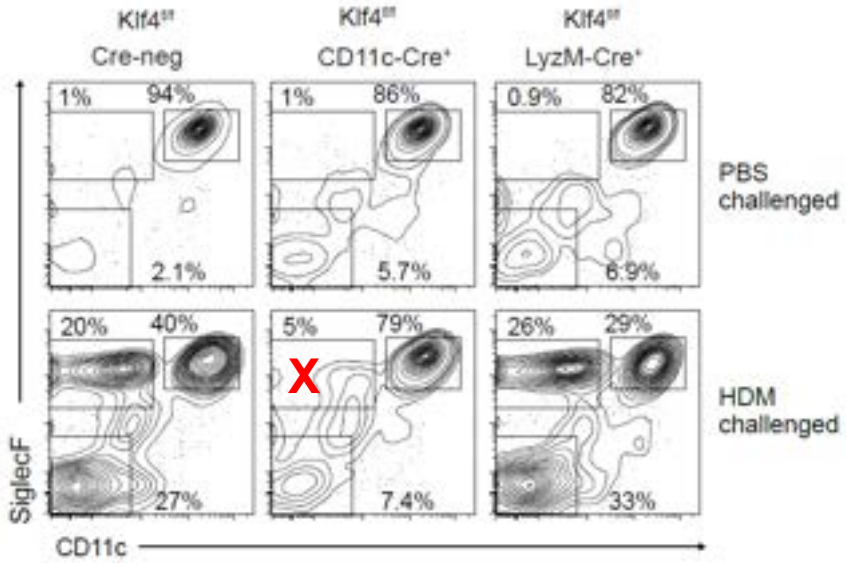


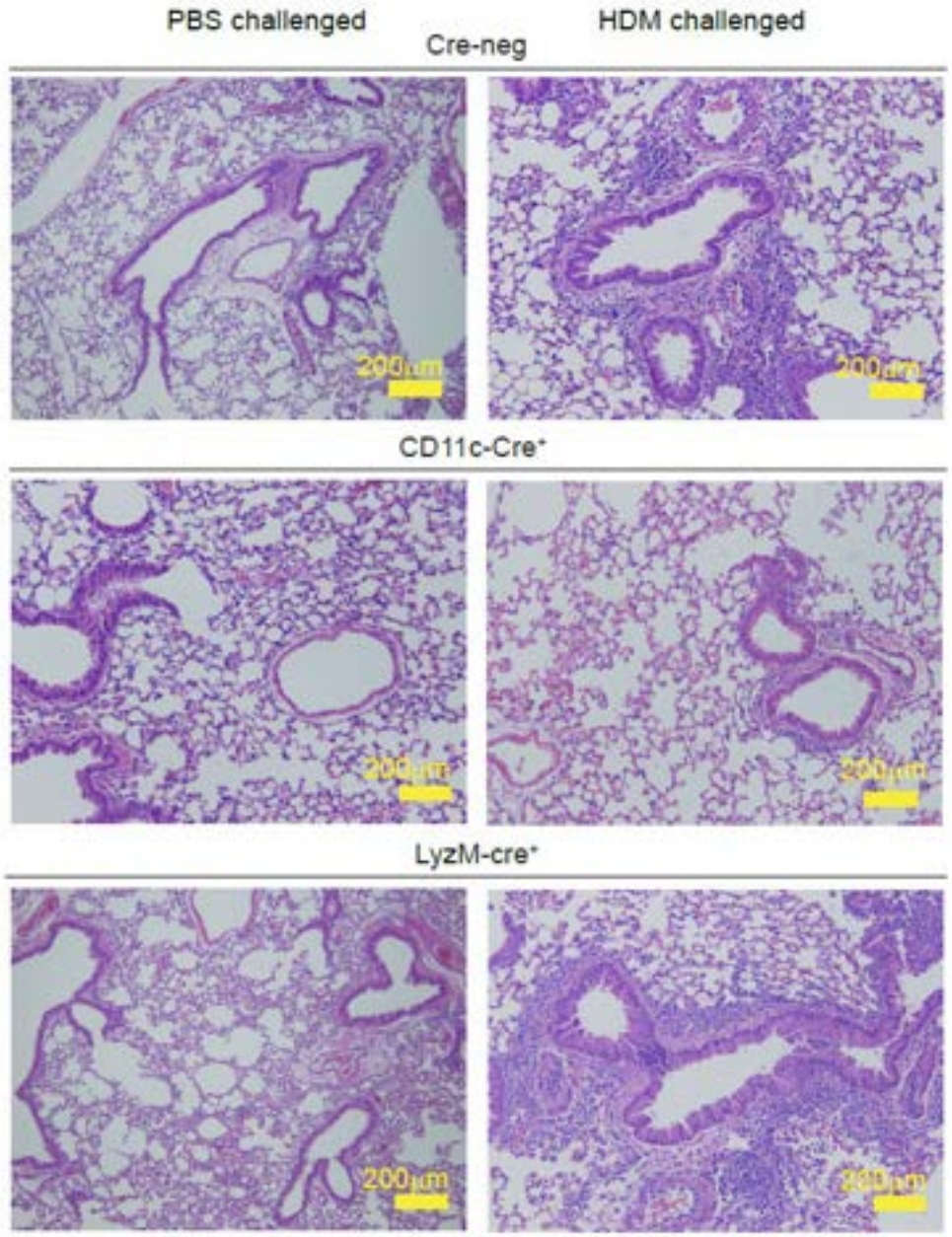
KLF4 cDC are not required for resistance to *C. rodentium* or *Toxoplasma gondii*

And KLF4 is not required in T cells for Th1, Th2 or Th17 differentiation, or for ILC2 development.

KLF4 cDCs are required for Th2 responses in HDM challenge







Mechanism of KLF4-dependent cDCs?

No *in vitro* system.

No obvious gene candidates from gene expression.

May involve other cells, such as ILC2.

May be due to lack of IL-12 or IL-23 (i.e., balance).

Challenges – lack of selective Cre deleter strains.

JI 2014

$CD326^{lo}CD103^{lo}CD11b^{lo}$ Dermal Dendritic Cells Are Activated by Thymic Stromal Lymphopoietin during Contact Sensitization in Mice

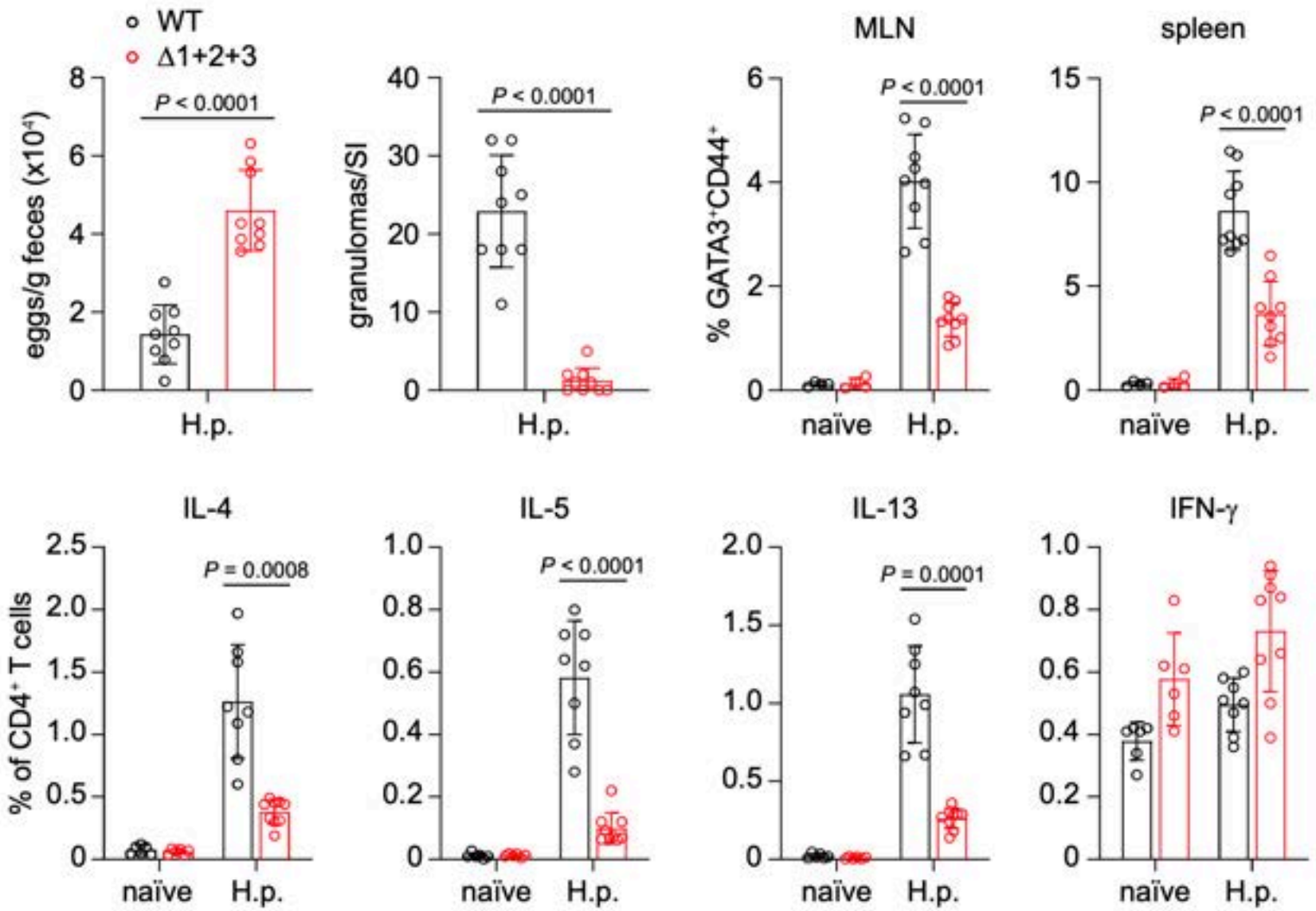
Sotaro Ochiai,*,† Ben Roediger,‡ Arby Abtin,‡ Elena Shklovskaya,‡ Barbara Fazekas de St. Groth,‡ Hidehiro Yamane,§ Wolfgang Weninger,‡,¶ Graham Le Gros,* and Franca Ronchese*

December 2021

Homeostatic IL-13 in healthy skin directs dendritic cell differentiation to promote T_H2 and inhibit T_H17 cell polarization

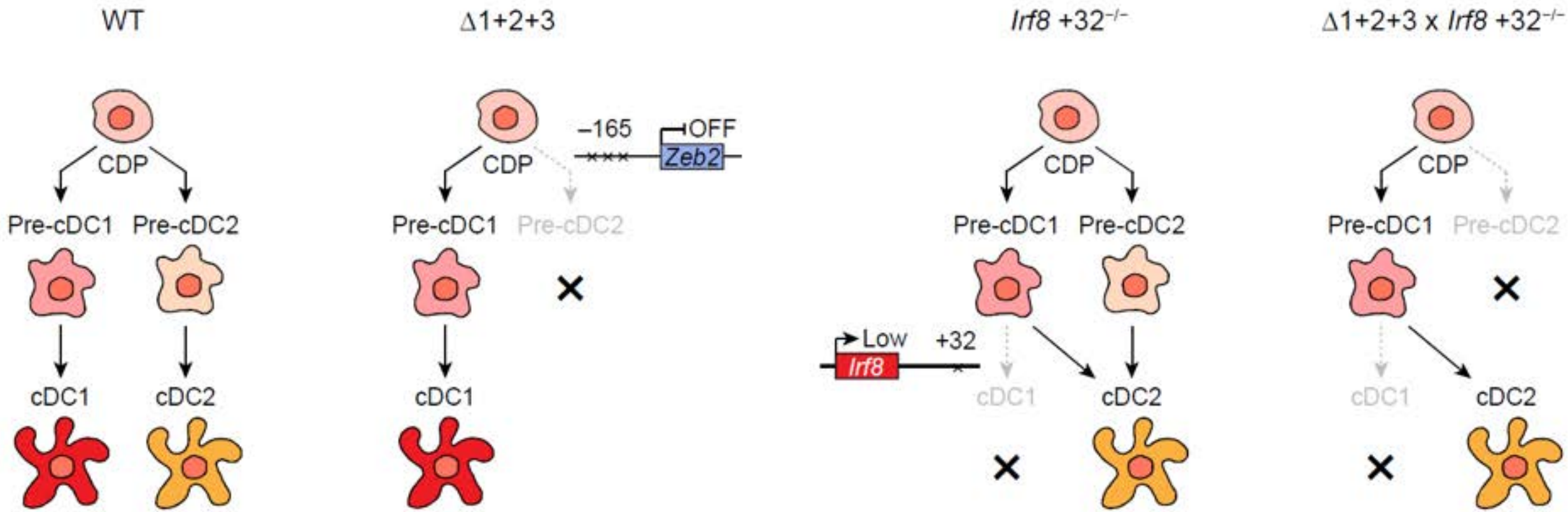
Johannes U. Mayer[1,11], Kerry L. Hilligan[1,2], Jodie S. Chandler[1], David A. Eccles[1], Samuel I. Old[1], Rita G. Domingues[3], Jianping Yang[1], Greta R. Webb[1], Luis Munoz-Erazo[1], Evelyn J. Hyde[1], Kirsty A. Wakelin[1], Shiau-Choot Tang[1], Sally C. Chappell[1], Sventja von Daake[1], Frank Brombacher[4], Charles R. Mackay[5], Alan Sher[2], Roxane Tussiwand[6,7], Lisa M. Connor[1,12], David Gallego-Ortega[8,9], Dragana Jankovic[10], Graham Le Gros[1], Matthew R. Hepworth[3], Olivier Lamiable[1,13] and Franca Ronchese[1,13]

cDC2 are required for T_H2 response against *H. polygyrus* infection



With great help from Pritesh Desai, Michael Diamond, Steven van Dyken Do-Hyun Kim

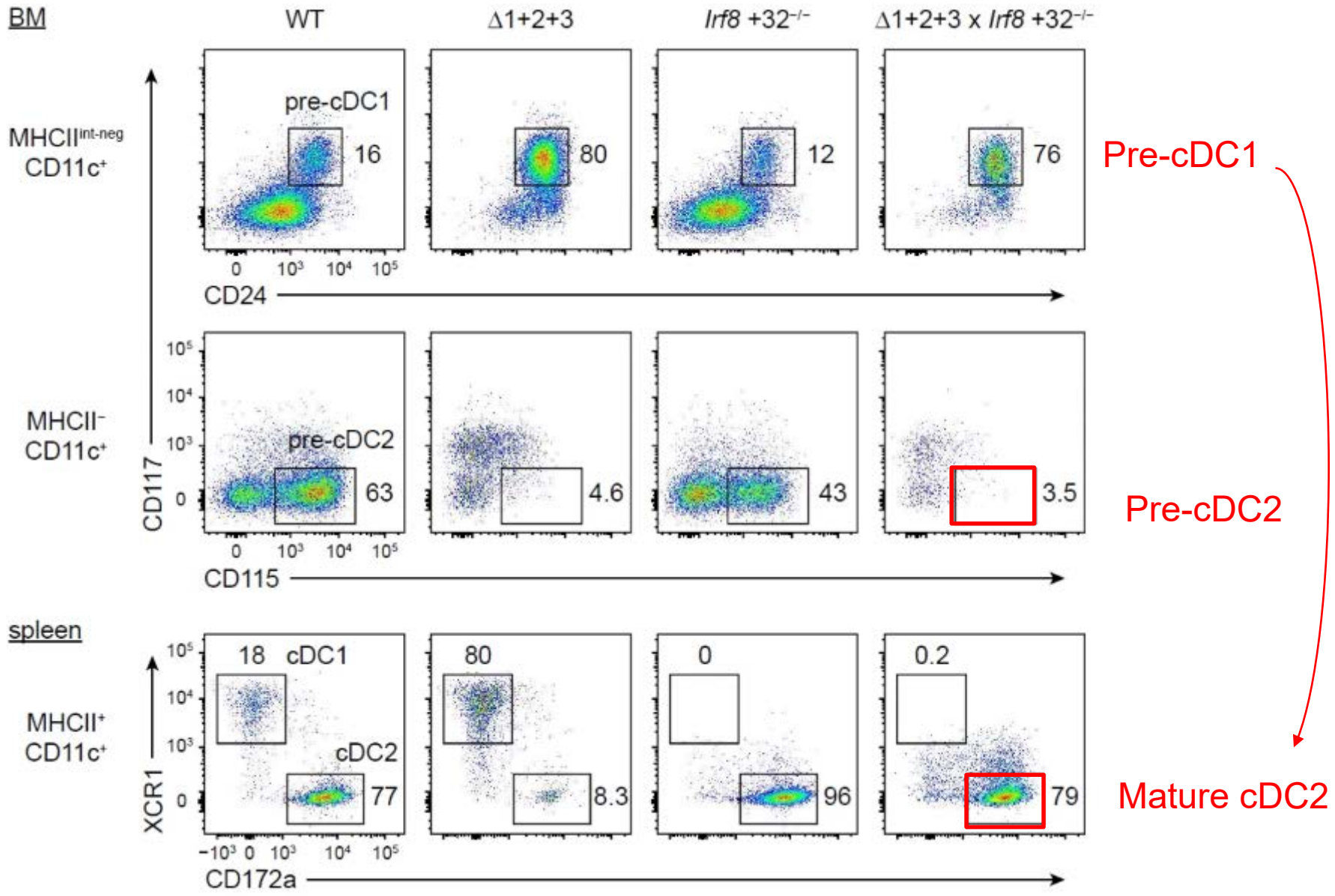
Δ1+2+3 and *Irf8* 32$^{-/-}$ mice restores cDC2s but not monocytes



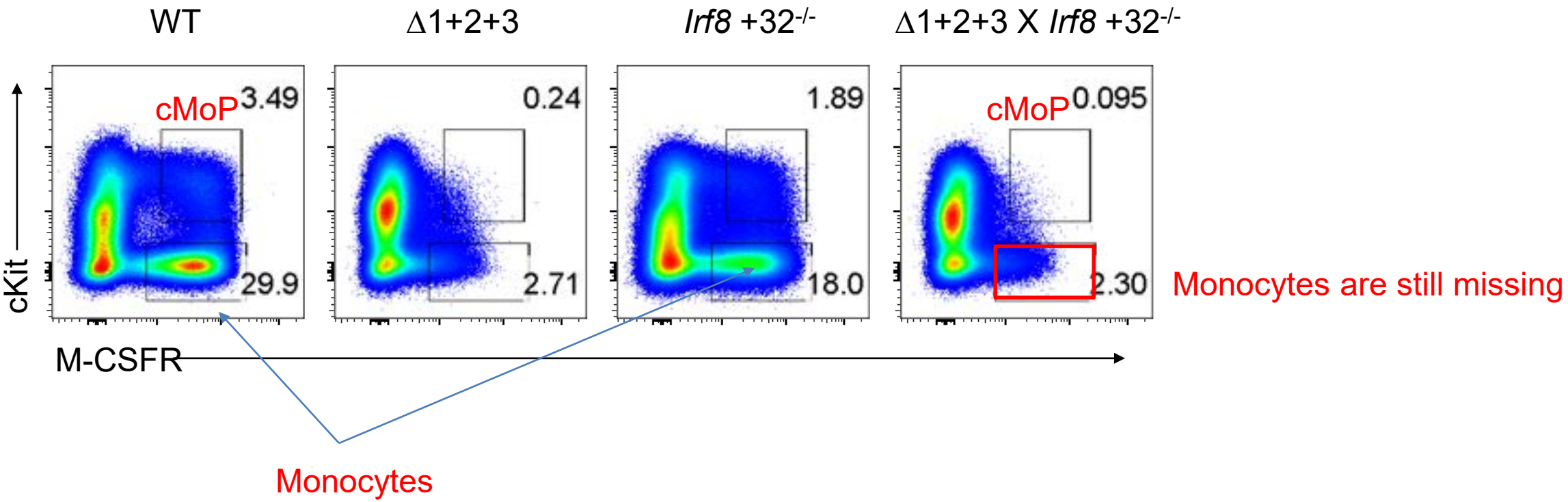
Cryptic activation of an *Irf8* enhancer governs cDC1 fate specification

Vivek Durai[1], Prachi Bagadia[1], Jeffrey M. Granja[2,3,4], Ansuman T. Satpathy[2,5], Devesha H. Kulkarni[6], Jesse T. Davidson IV[3], Renee Wu[1], Swapneel J. Patel[7], Arifumi Iwata[1], Tian-Tian Liu[1,8], Xiao Huang[1], Carlos G. Briseño[1], Gary E. Grajales-Reyes[1], Miriam Wöhner[9], Hiromi Tagoh[9], Barbara L. Kee[10], Rodney D. Newberry[6], Meinrad Busslinger[9], Howard Y. Chang[2,11], Theresa L. Murphy[1] and Kenneth M. Murphy[1,8*]

Δ1+2+3 and *Irf8* 32$^{-/-}$ mice restores cDC2s but not monocytes

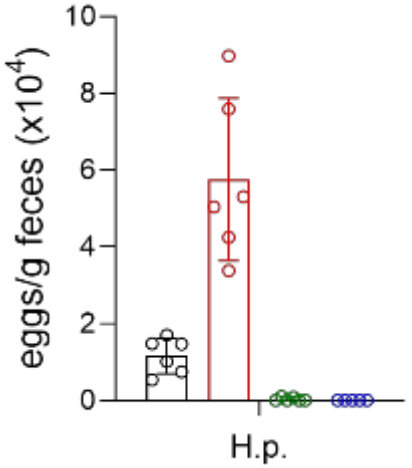


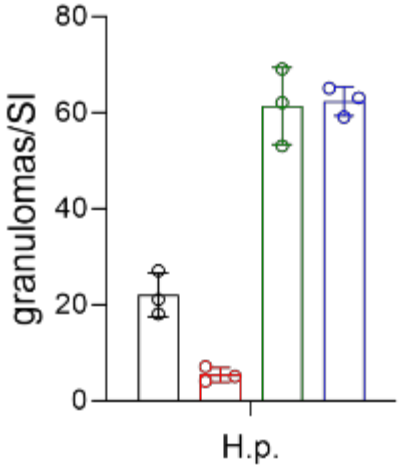
Δ1+2+3 x *Irf8* 32$^{-/-}$ mice restore cDC2, but not monocytes



cDC2, not monocytes, drive T_H2 responses to H. polygyrus

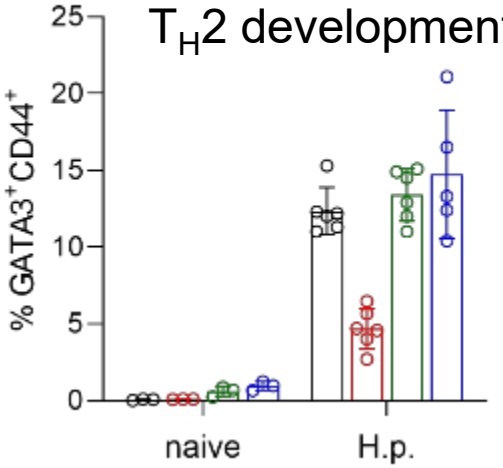
Feces egg counts and Intestinal granulomas





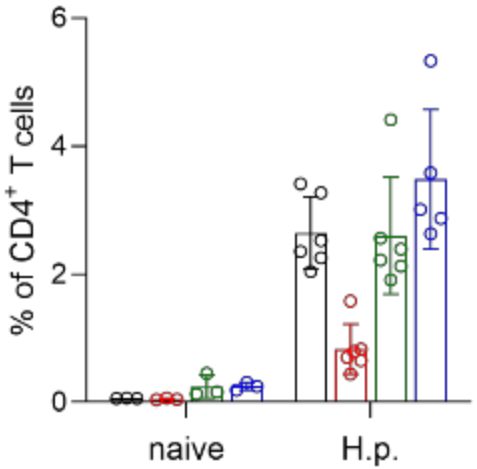
spleen

T_H2 development

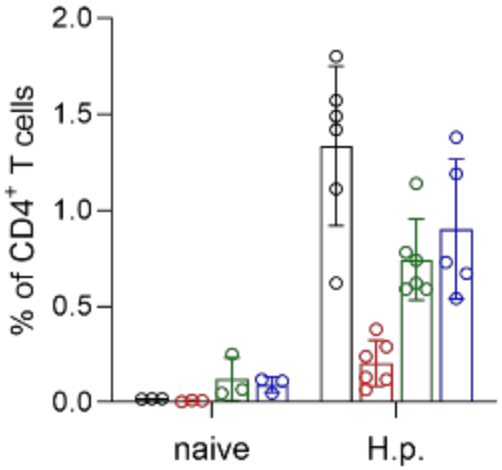


- WT
- Δ1+2+3
- Δ32
- Δ1+2+3 X Δ32

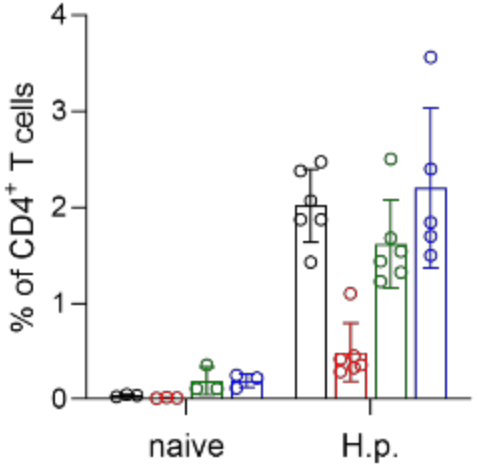
IL-4



IL-5



IL-13



Summary

What we know.
cDC2 are required for some T_H17 and T_H2 responses.
cDC2 protection against *Citrobacter rodentium* relies on IL-23 production.

What we don’t know.

Are there distinct subsets of cDC2? If so, by what molecular mechanism?
How does cDC2 support T_H2 responses? Antigen capture vs. cytokine bias?