Memorial Sloan Kettering Cancer Center

New insights into the role of Notch signaling in T and B cell immunity

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Cancer Biology GSK course
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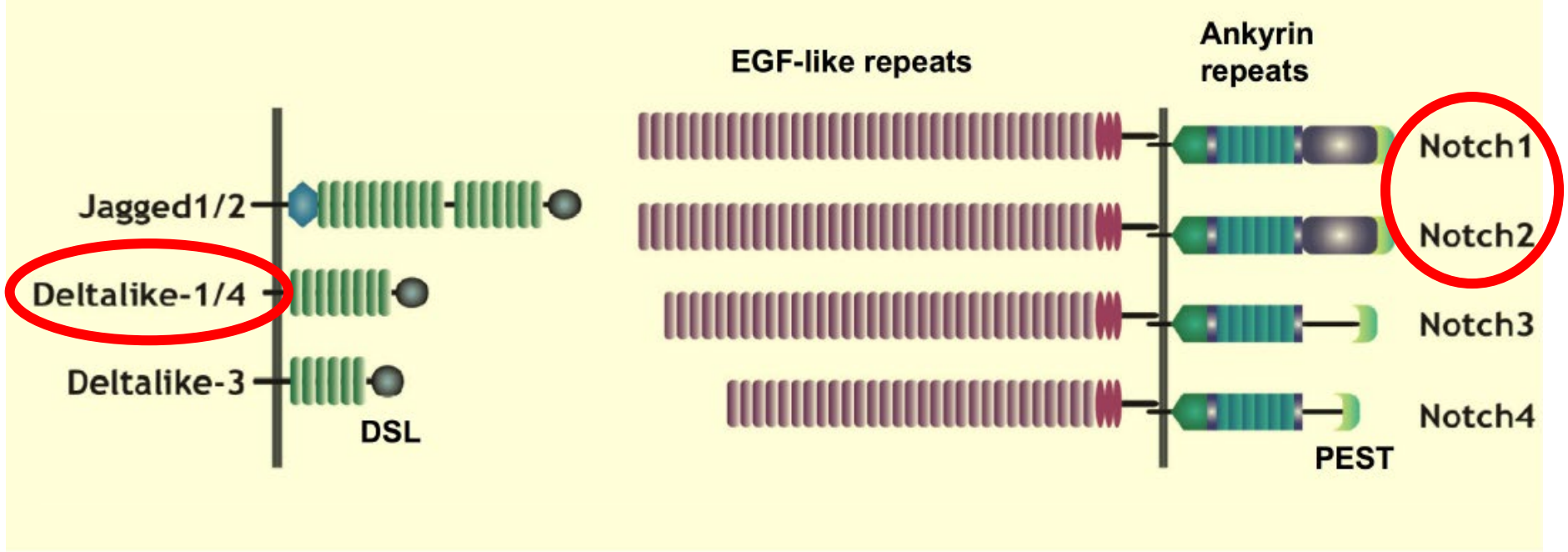
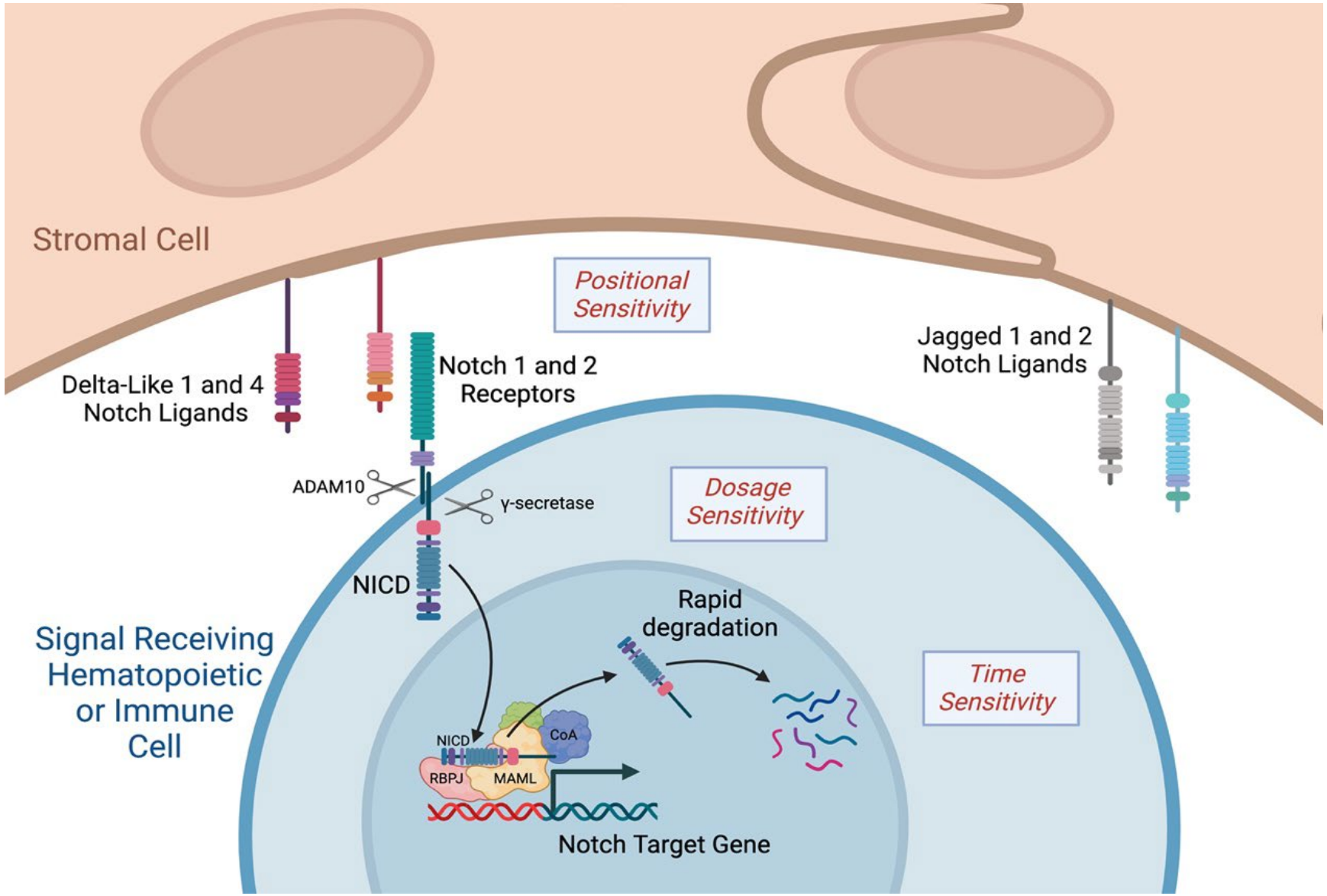
Presentation outline

- Introduction to Notch signaling in T and B cell immunity
- Stromal Notch ligands as key regulators of splenic marginal zone B cell homeostasis
  - *conserved Notch-driven B cell transcriptional program from mice to humans*
  - *hijacked in Notch-driven human B cell lymphomas*
- Conclusions and perspectives

Mammalian Notch ligands and receptors

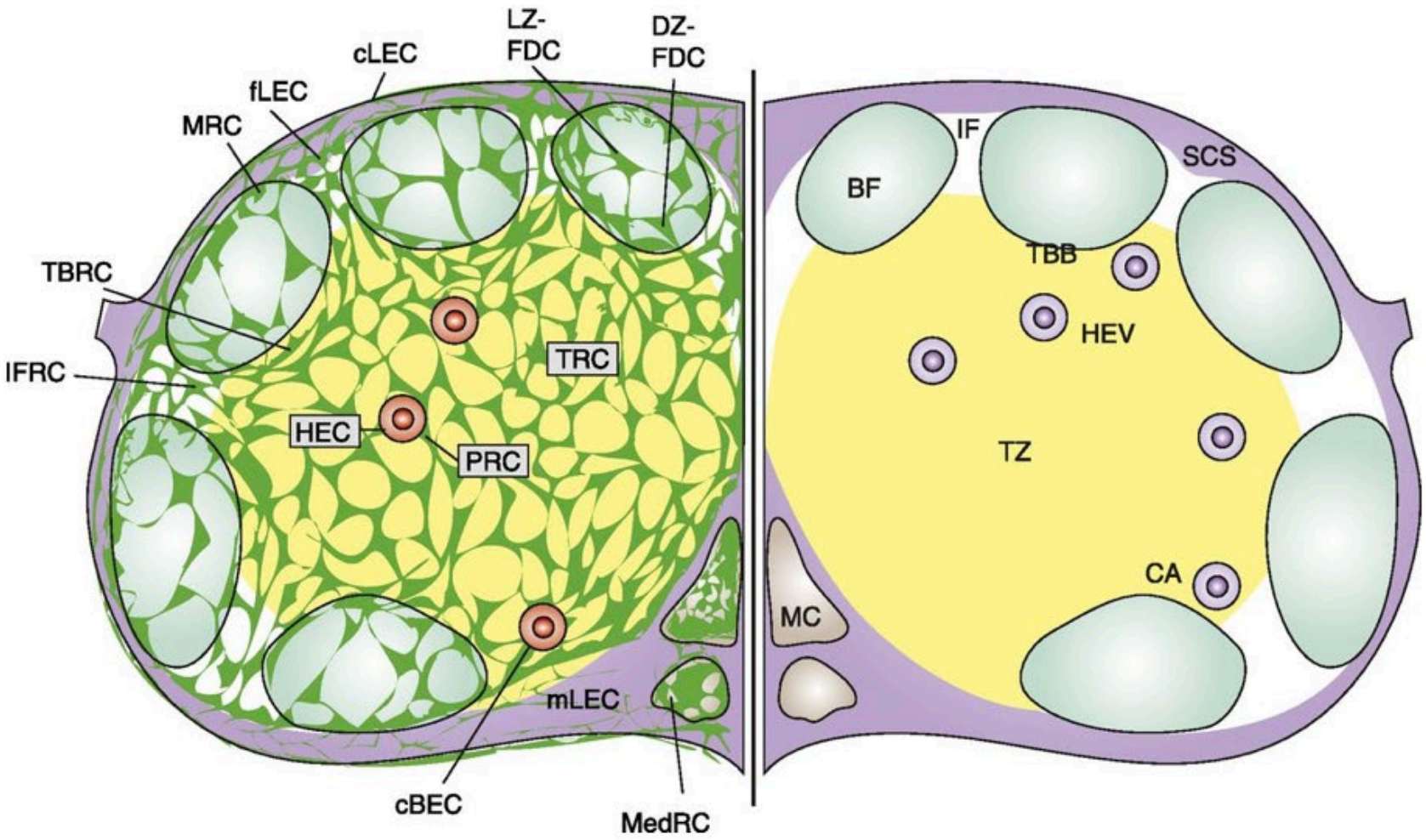


Overview of Notch signaling in the immune system



Schneider, Allman and Maillard, *Curr Opin Cell Biol* 2023

Specialized stromal cells provide structure and regulate lymph node functions



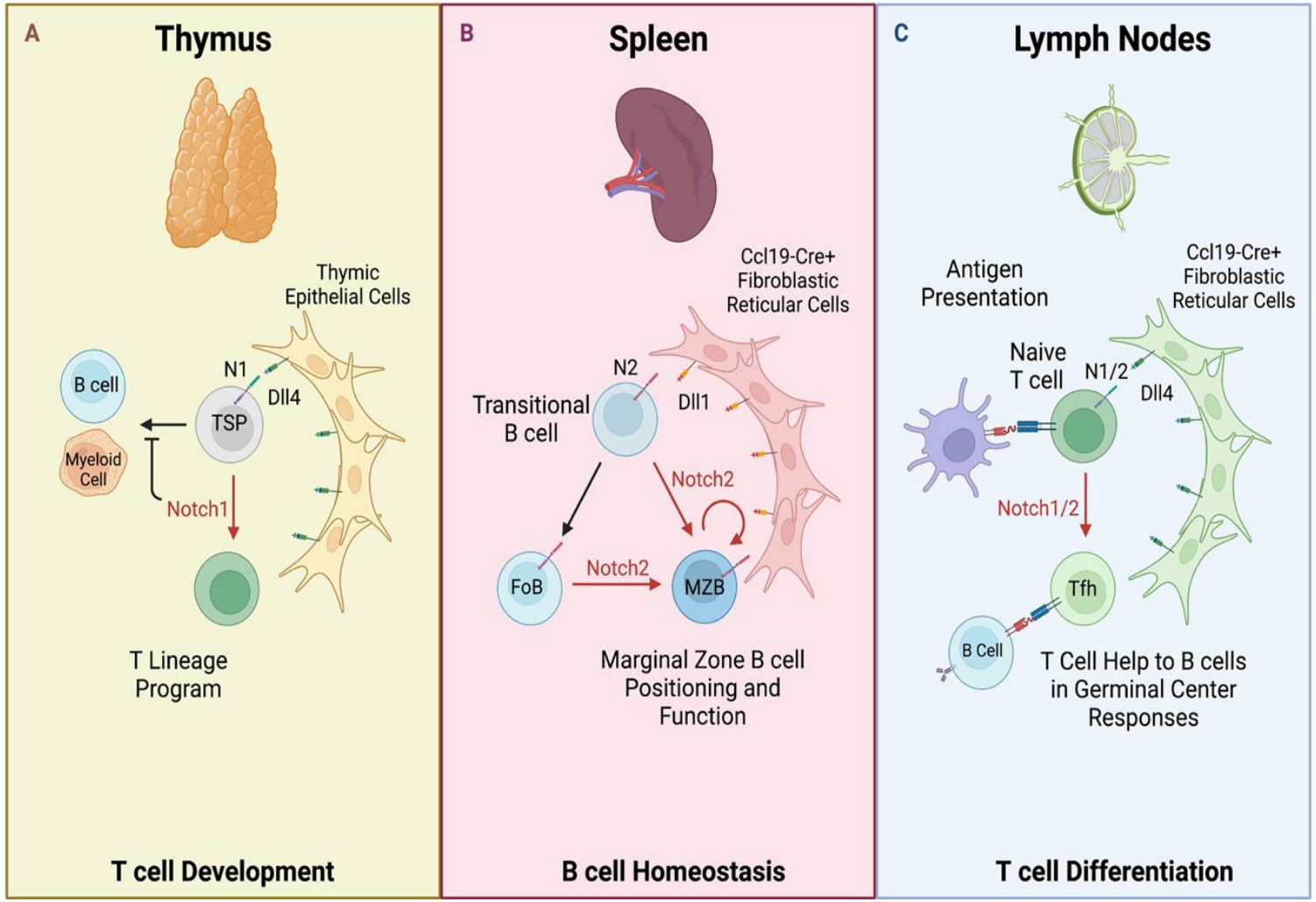
Lymph node
SCS = subcapsular sinus
BF = B cell follicles
TBB = T/B border
HEV = high endothelial venule
TZ = T cell zone
MC – medullary cords

Pikor et al., *J Immunol* 2021

Key source of Notch ligands in vivo!

Radtke, Maillard & Labrecque labs

Recurrent rules of Notch engagement in the immune system



Schneider, Allman and Maillard, *Curr Opin Cell Biol* 2023

Notch signaling in B and T cell immunity - key projects

Notch signaling in graft-versus-host disease

Positional regulation of B cell homeostasis by stromal Notch ligands

Notch signaling in organ transplant rejection (with Keith Bishop, UMich)

Recurrent rules of Notch pathway engagement

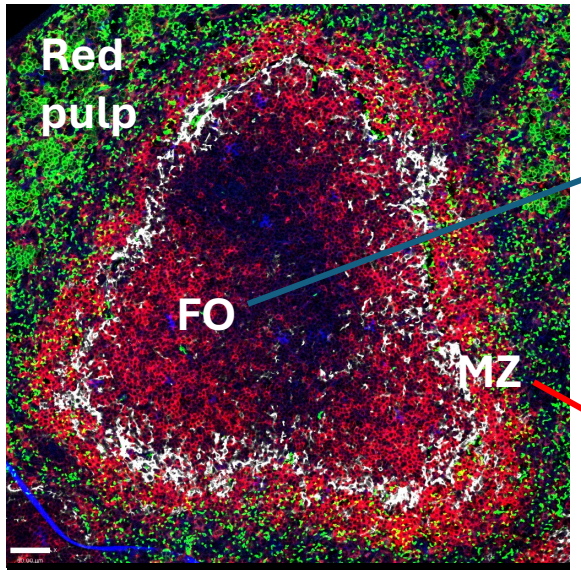
Notch signaling in the immune response to mRNA vaccination (with Michela Locci and Norbert Pardi, UPenn)

Notch signaling in $CD8^+$ T cell immunity (with Nathalie Labrecque, Montréal)

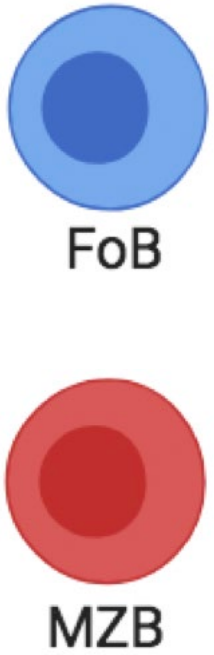
Stroma-derived Notch signaling as oncogenic drivers in T cell lymphomas (with Ryan Wilcox, UMich)

Zhang et al., 2011; Tran et al., 2013; Sandy et al., 2013; Chung et al., 2017; Radojcic et al., 2018; Chung et al., 2019; Perkey et al., 2020; Gaudette et al., 2021; Gao et al., 2022; Gómez Atria et al., 2022; Tkachev et al., 2023; Allman et al., 2025; Maurice De Sousa et al., 2025

Positional regulation of splenic B cell homeostasis by stromal Notch ligands



CD1d (MZ B cells)
CD169 (MZ macrophages)
Ter119 (Erythrocytes –red pulp)



Follicular B cells

- Circulating population
- Reside mainly in B cell follicles of spleen and lymph nodes

Marginal zone B cells

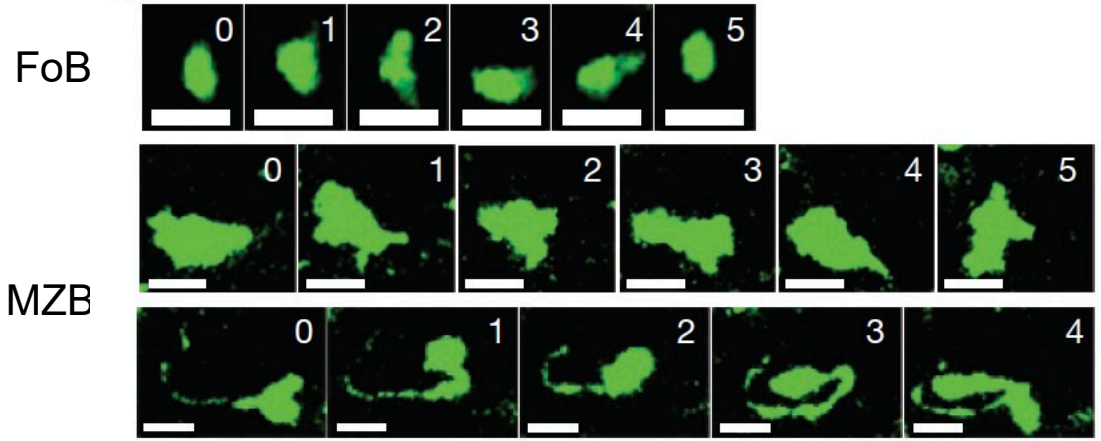
- Non-circulating cells exposed to blood flow and shear stress
- Segregate into the marginal zone of the spleen, but constantly shuttle between follicle and marginal zone
- “Innate-like B cells” that make plasma cells rapidly

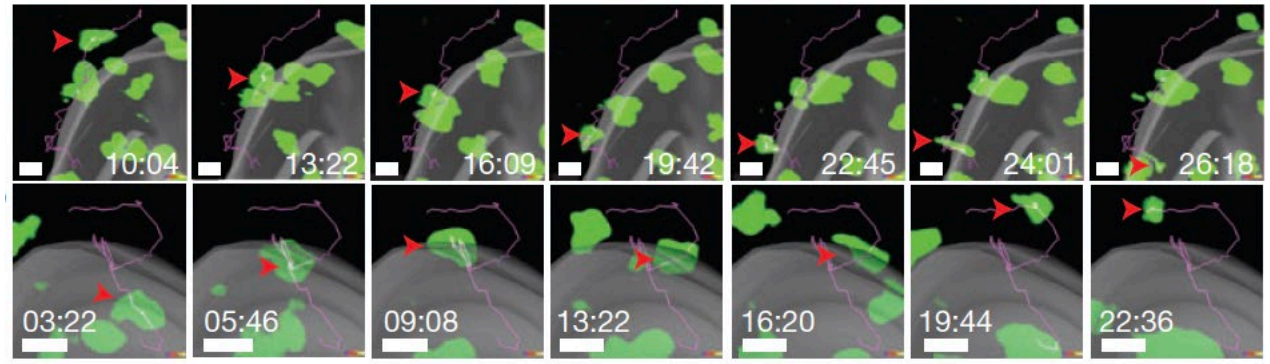
Active shuttling of marginal zone B cells across the marginal sinus

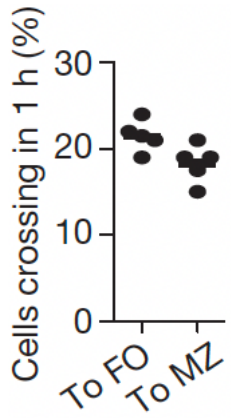
Visualization of splenic marginal zone B-cell shuttling and follicular B-cell egress

Tal I. Arnon[1], Robert M. Horton[1], Irina L. Grigorova[1]† & Jason G. Cyster[1]

Time lapse intravital imaging







Nature, 2013

Notch regulation of marginal zone B cells

- Marginal zone B cells rely on Notch signals mediated by Delta-like1, Notch2 and a Notch2/Rbpj/Maml1 transcriptional activation complex
  - *First reported by Tasuku Honjo's group (2002)*
  - *Highly dosage-sensitive with haploinsufficiency for each gene involved*

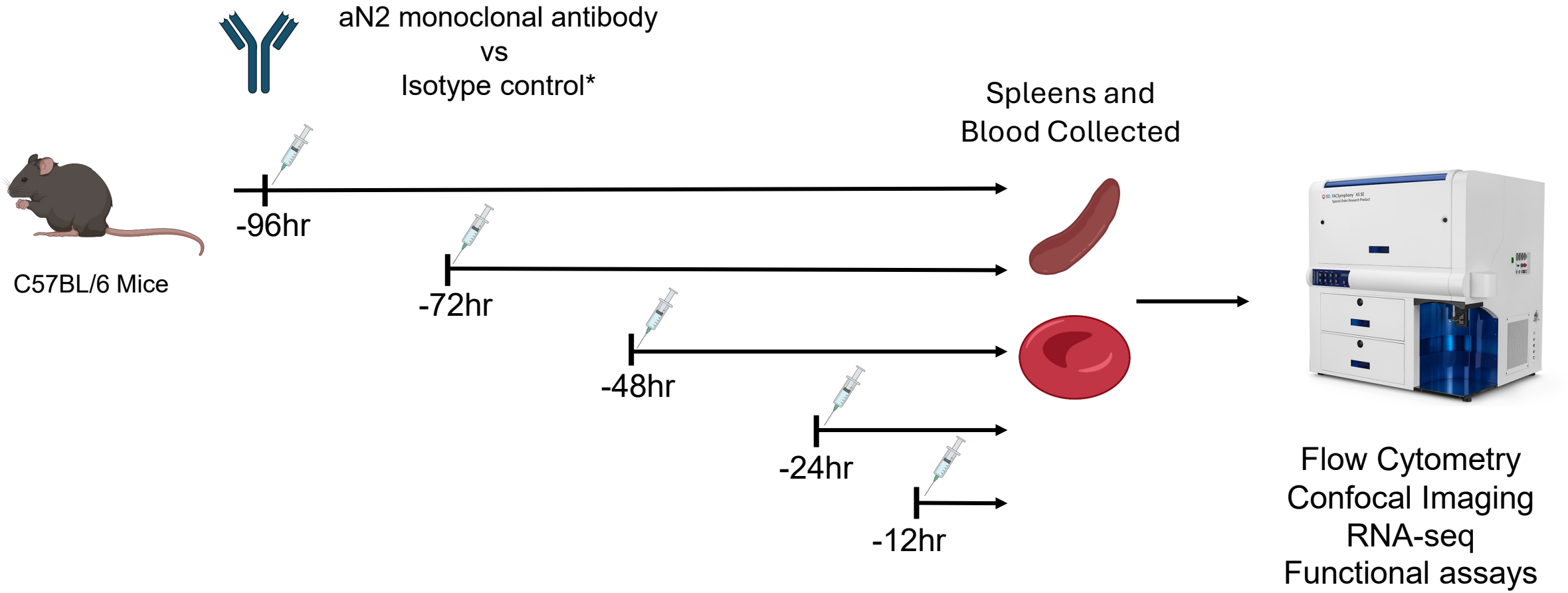
- Critical Delta-like1 Notch ligands are expressed in a radiation-resistant non-hematopoietic compartment
  - *First recognized by Cynthia Guidos's lab*
  - *Evidence of competition for stromal niches (Tan et al, Immunity 2009)*
  - *Ligand source subsequently identified among Ccl19-Cre^{+} lymphoid tissue fibroblastic reticular cells (Fasnacht et al, J Exp Med 2014)*

Key questions about stromal niches, Notch signaling and B cells

- *What are the defining features of stromal niches that control marginal zone B cell homeostasis?*
- *Where precisely do B cells encounter Notch ligands and experience Notch signaling in the spleen?*
- *What are the Notch-regulated transcriptional programs that regulate marginal zone B cell positioning, and are they conserved in humans?*

Anneka Allman, Brian Gaudette, Samantha Kelly, Nagham Alouche et al. (*Immunity*, 2025)

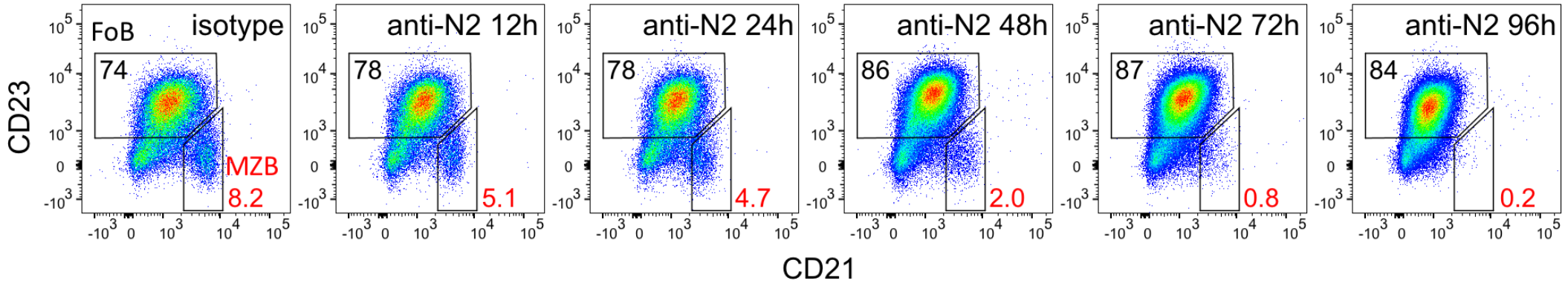
Experimental approach to identify acute effects of Notch blockade in naïve B cells

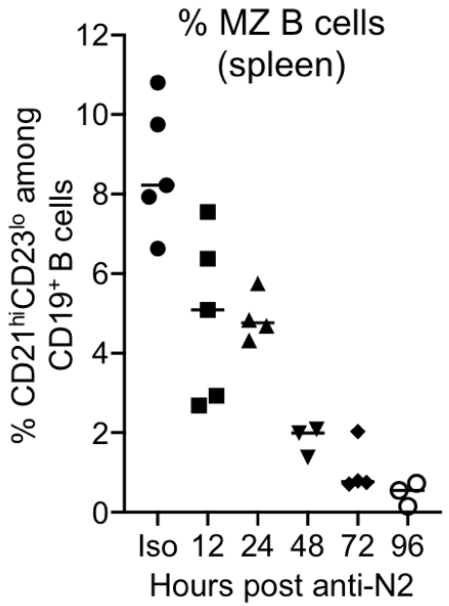


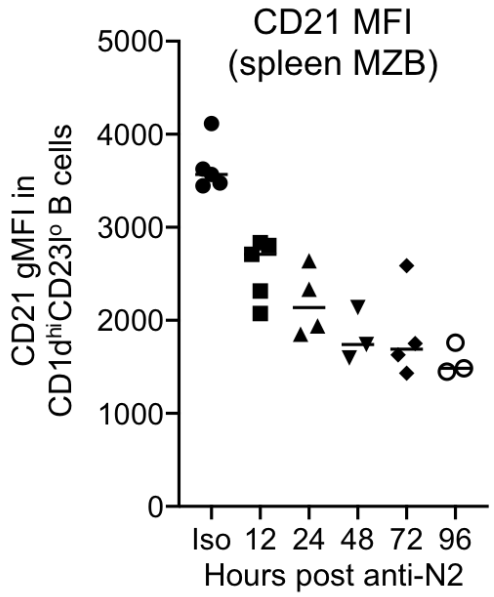
*From Chris Siebel, Genentech

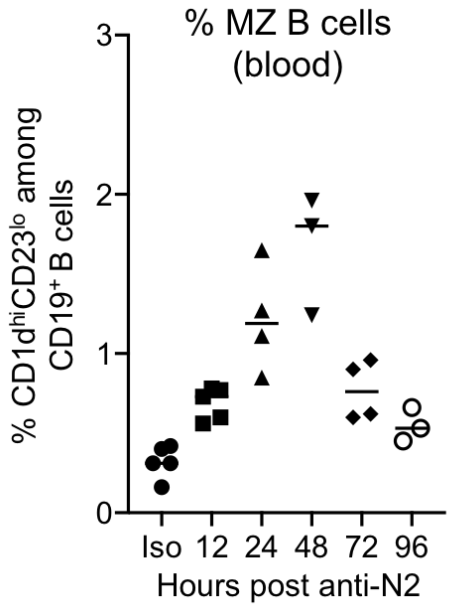
Rapid loss of splenic marginal zone B cells upon Notch2 blockade

Gated on Singlets, Live, CD19⁺, CD93⁻ naïve splenic B cells





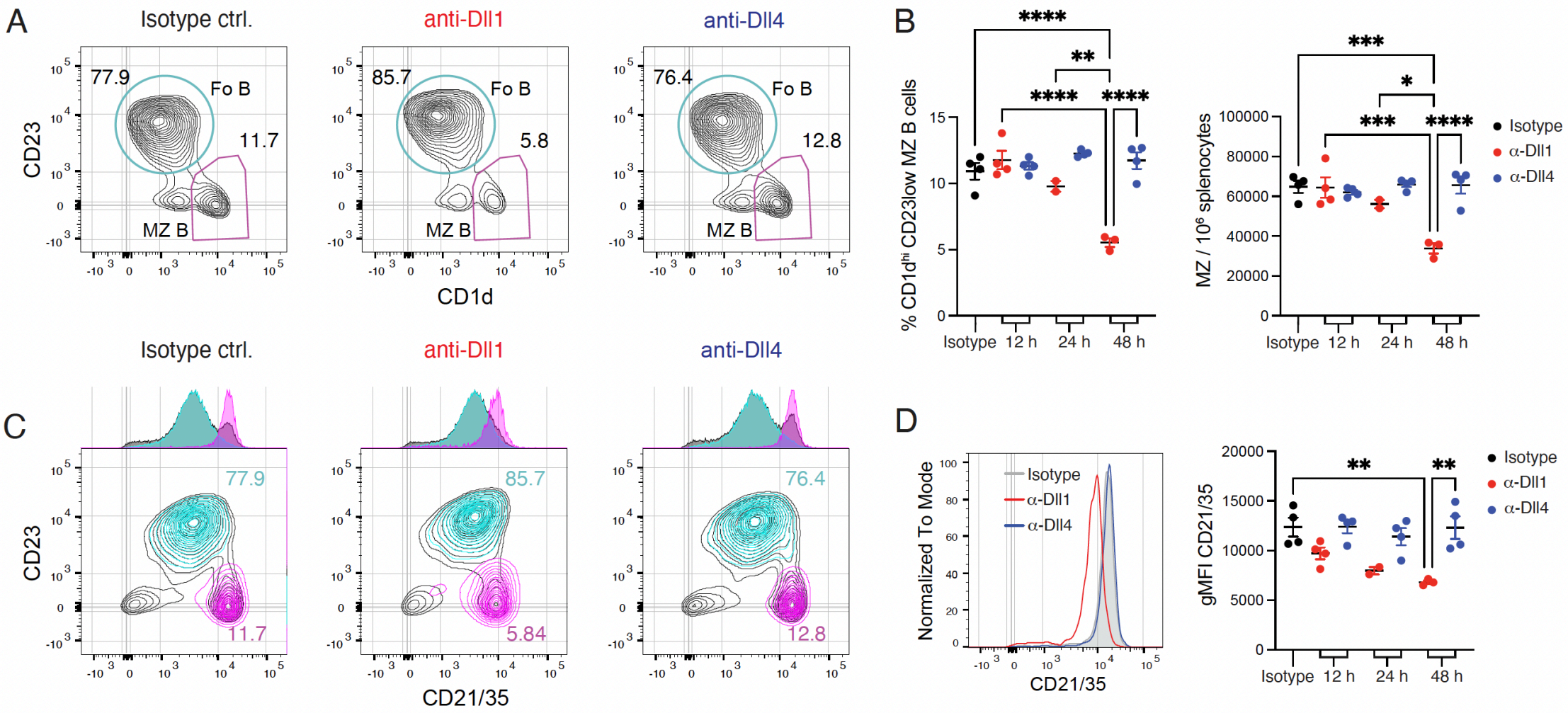




Gaudette el al.
JCI 2021

Blocking anti-Notch2 (anti-N2) antibodies from Chris Siebel/Lluc Mosteiro, Genentech

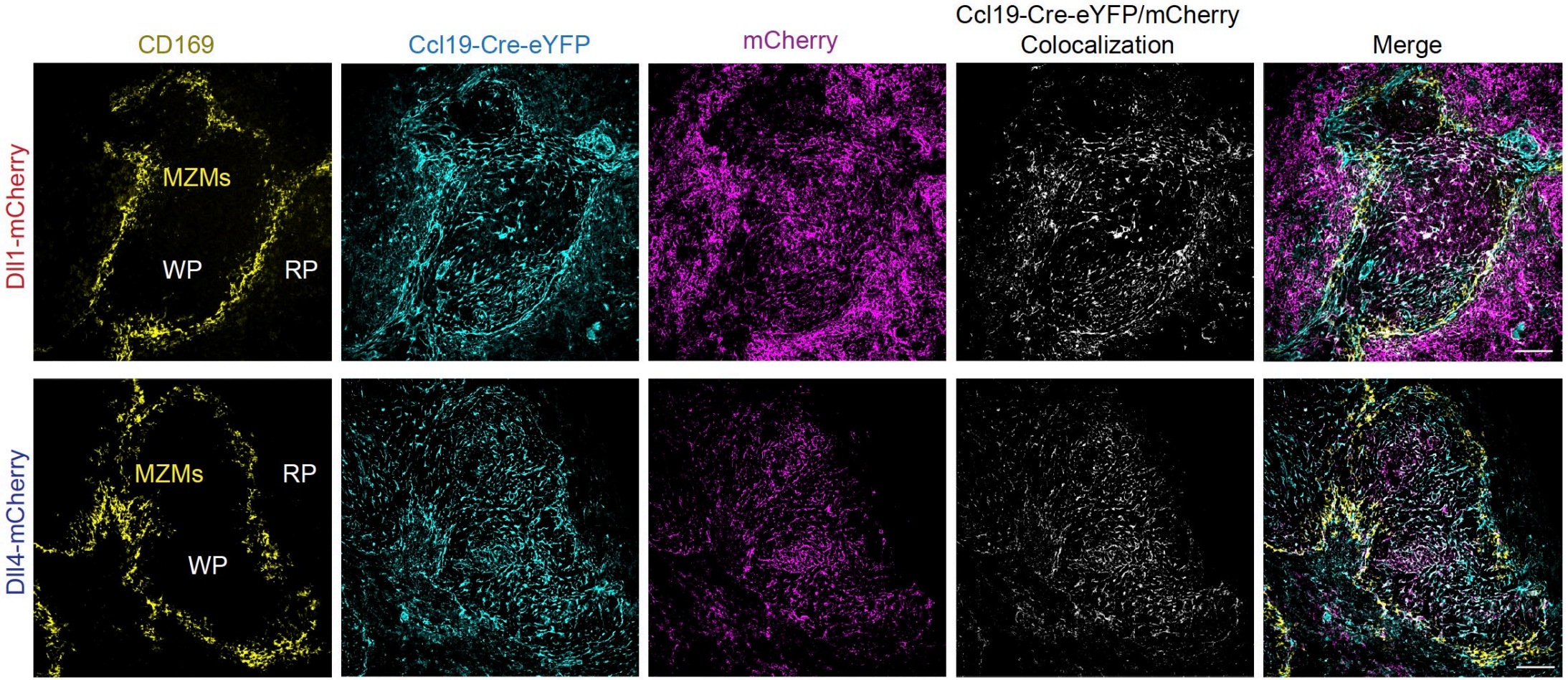
Divergent effects of Dll1 or Dll4 Notch ligand blockade on marginal zone B cells



Brian Gaudette, Anneka Allman, Daniela Gómez Atria

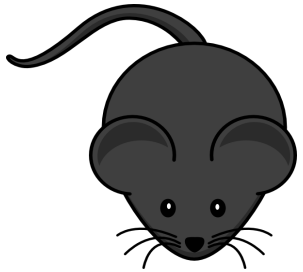
Identifying candidate stromal sources of spleen Dll1 and Dll4 Notch ligands

Ccl19-Cre;ROSA26^{YFP} from Burkhard Ludewig
Dll1-mCherry and *Dll4-mCherry* BAC transgenic reporter mice from Iannis Aifantis

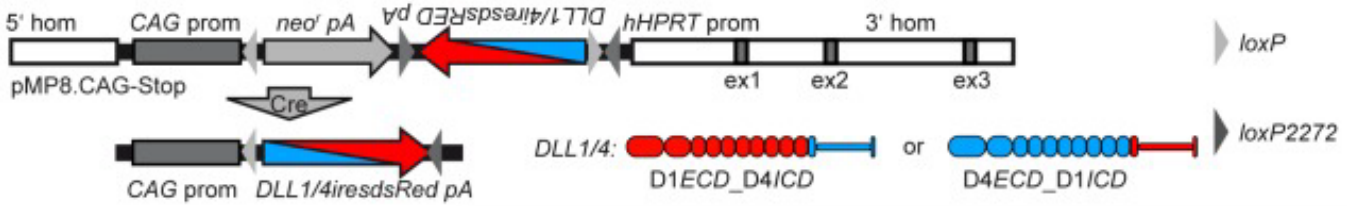


Anneka Allman

Can expression of Dll4 in the correct stromal niche support marginal zone B cell homeostasis?



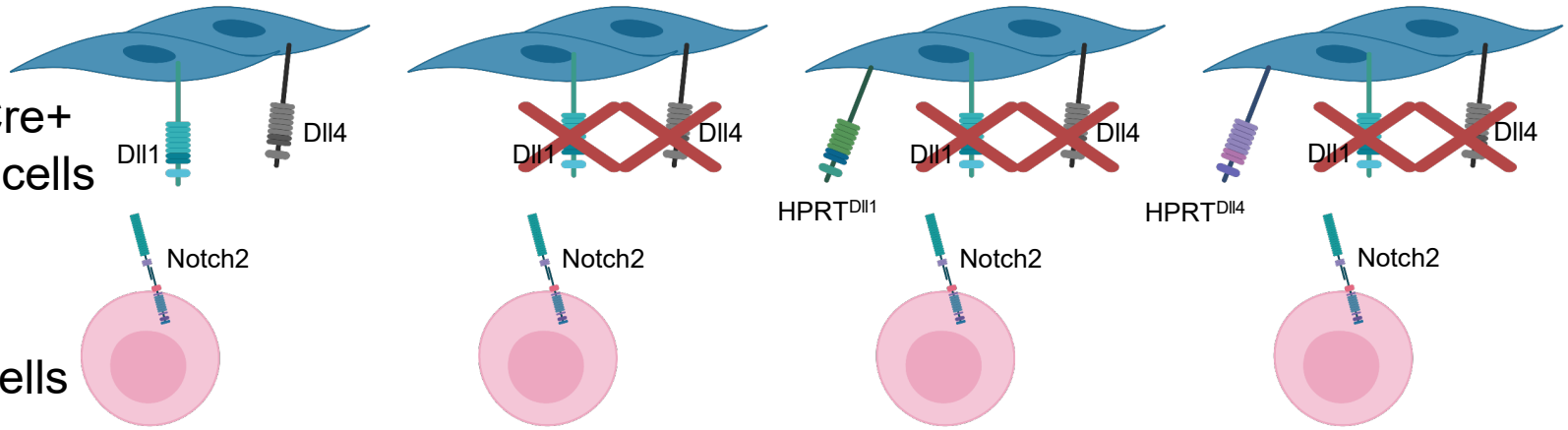
Hprt-Dll1 or *Hprt-Dll4* mice provided by the Gossler Lab



Tveriakhina et al., 2018

Ccl19-Cre+ stromal cells

B cells



Wild Type

Ccl19-cre $Dll1^{f/f}$ $Dll4^{f/f}$

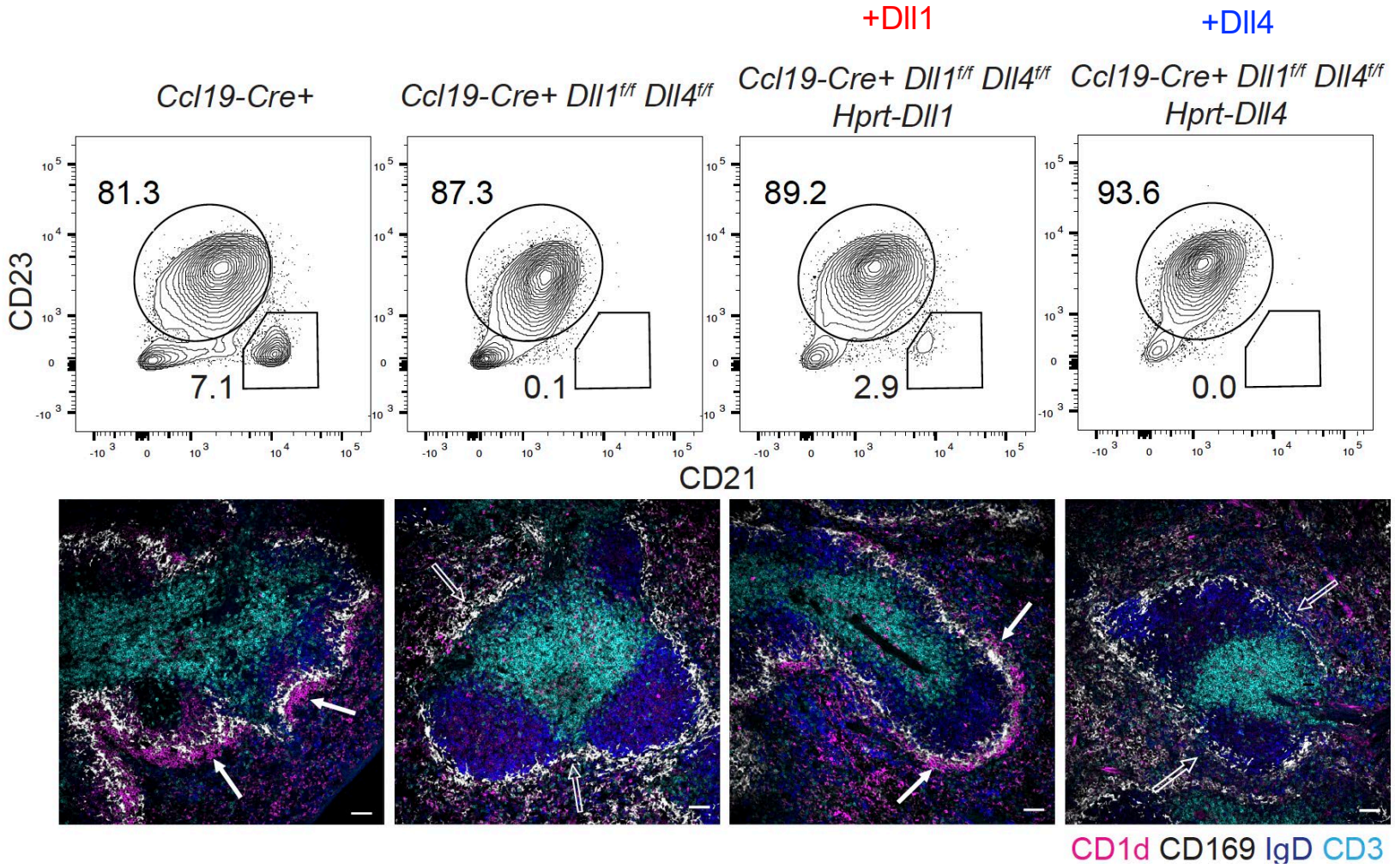
Ccl19-cre $Dll1^{f/f}$ $Dll4^{f/f}$ *Hprt-Dll1*

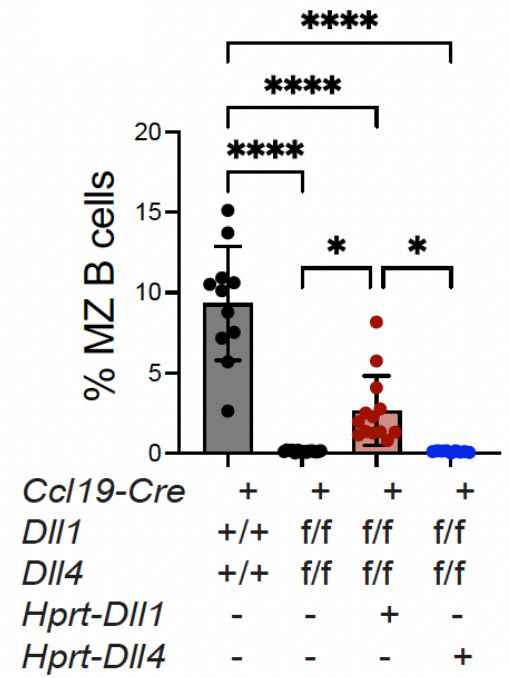
Ccl19-cre $Dll1^{f/f}$ $Dll4^{f/f}$ *Hprt-Dll4*

Hprt-Dll1 : DLL1ECD-DLL4ICD

Hprt-Dll4 : DLL4ECD-DLL1ICD

Dll4 cannot substitute for Dll1 to support marginal zone B cell homeostasis

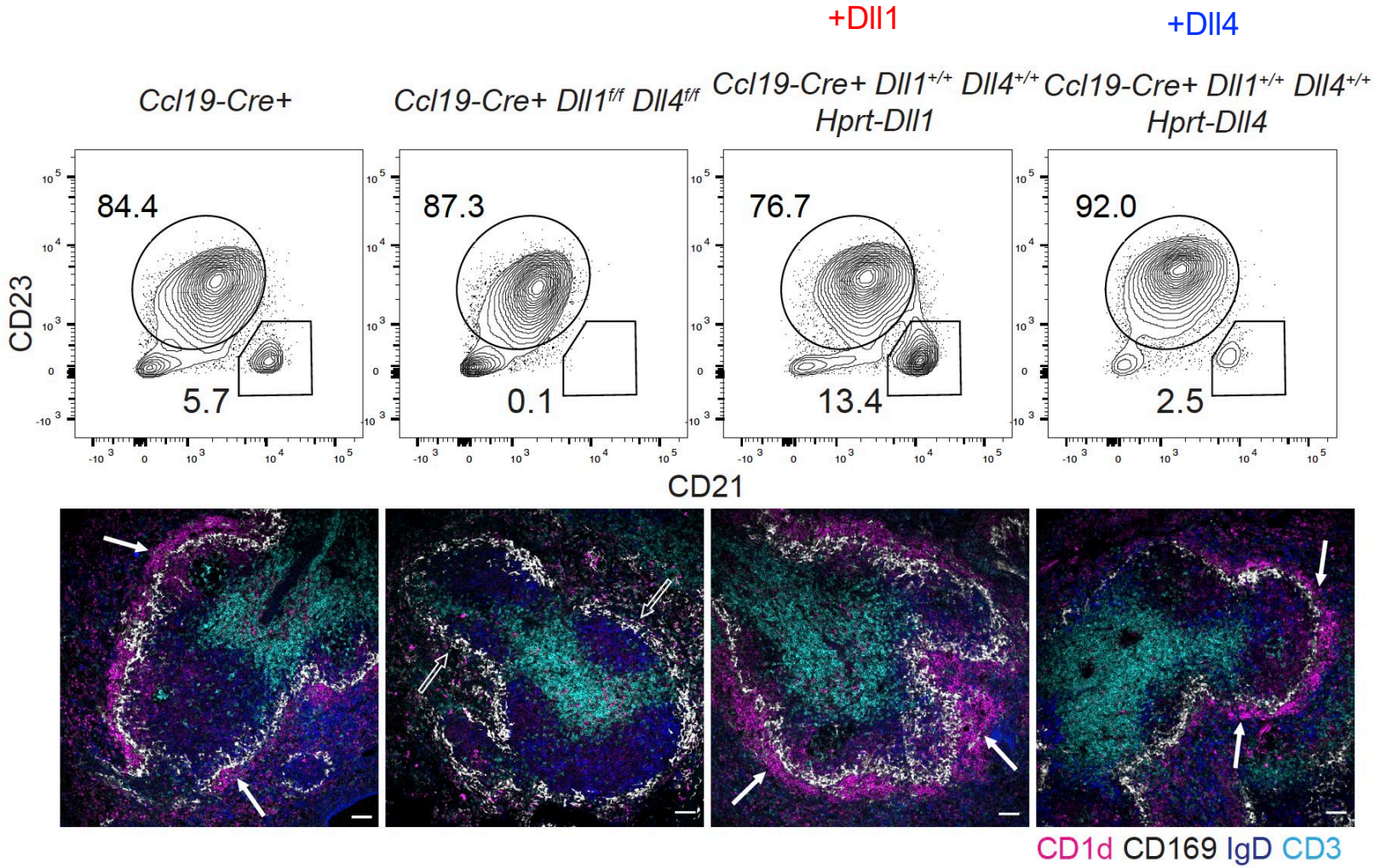


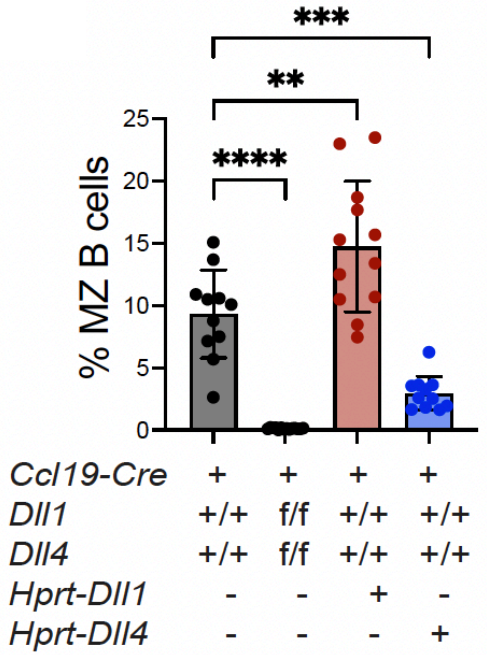


Gated on Singlets, Live, CD19+CD93- splenocytes

Anneka Allman

Dll4 competes with endogenous Dll1 in marginal zone B cell homeostasis



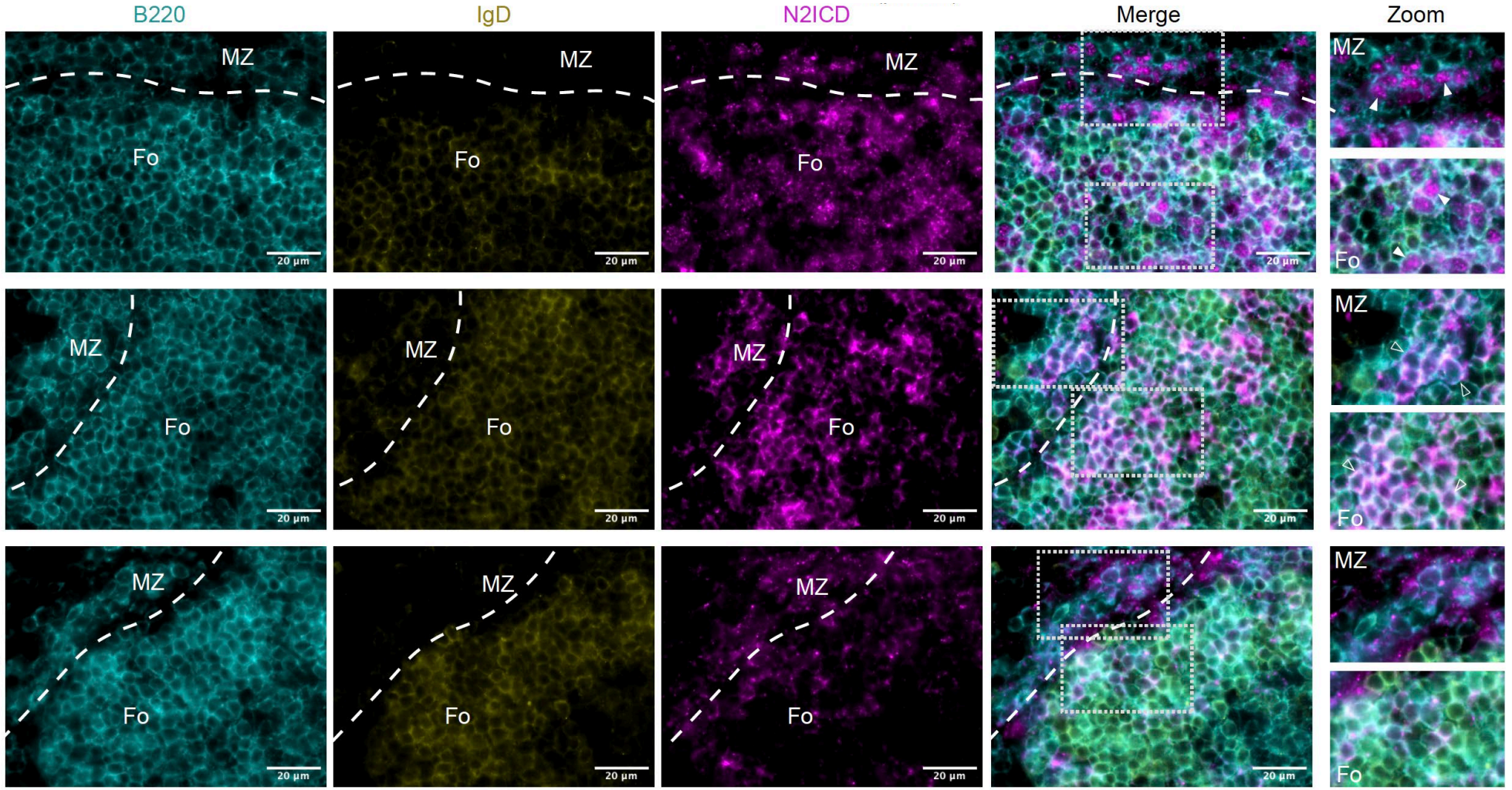


Gated on Singlets, Live, CD19+CD93- splenocytes

Anneka Allman

Where precisely do marginal zone B cells encounter Notch ligands when actively shuttling across the marginal sinus?

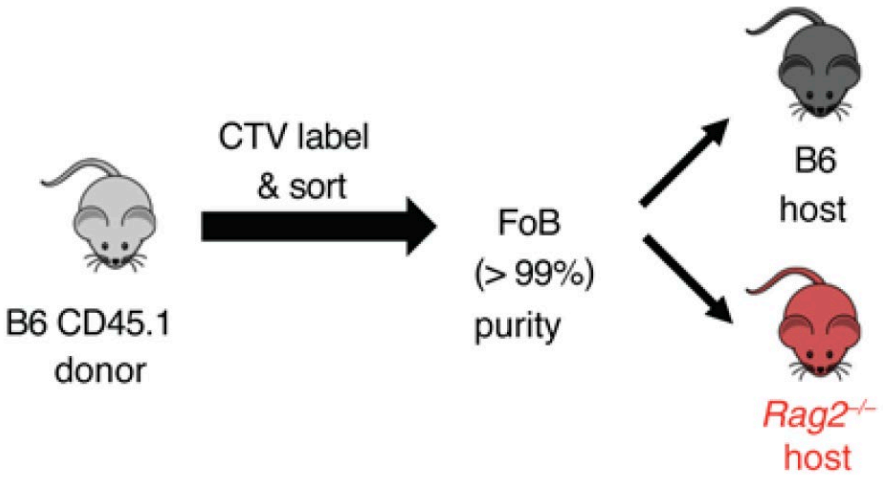
B cells receive Notch2 signals when localized in splenic follicles (and not just the marginal zone)

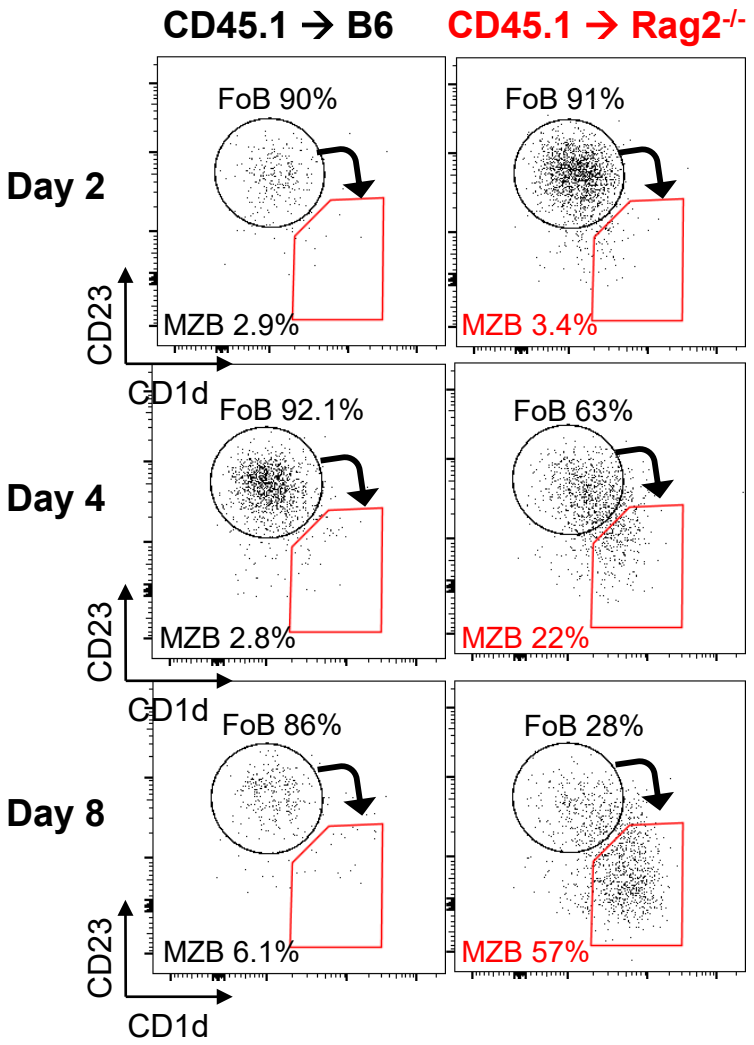


Similar conclusions with anti-Hes1 staining and Hes1-GFP detection

Nagham Alouche
Sanjiv Luther

Follicular B cells transferred into lymphopenic hosts transdifferentiate into marginal zone B cells upon Dll1/Notch2 signaling

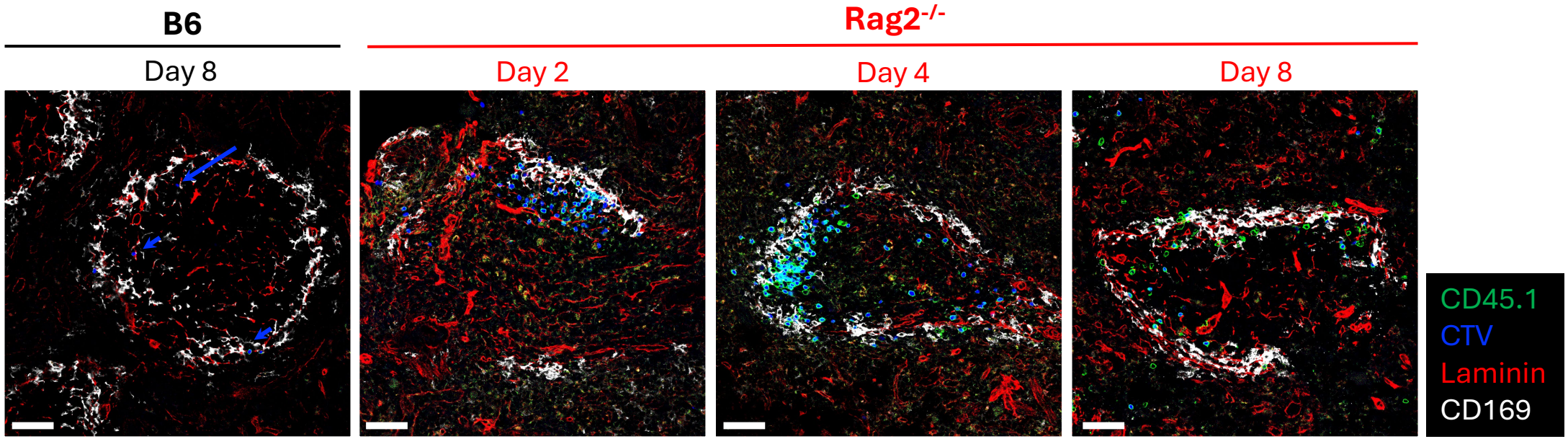




Dll1/Notch2-dependent transdifferentiation followed by proliferation!

Gómez Atria et al., *JCI* 2022

Tracking follicular B cells in the spleen after transfer into *Rag2*-deficient lymphopenic hosts



Scale Bars = 50 μm

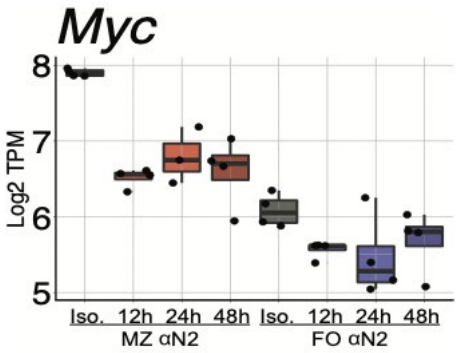
Homing/proliferation within spleen B cell follicles –
"lymphopenia sensing"

Gómez Atria et al., *JCI* 2022

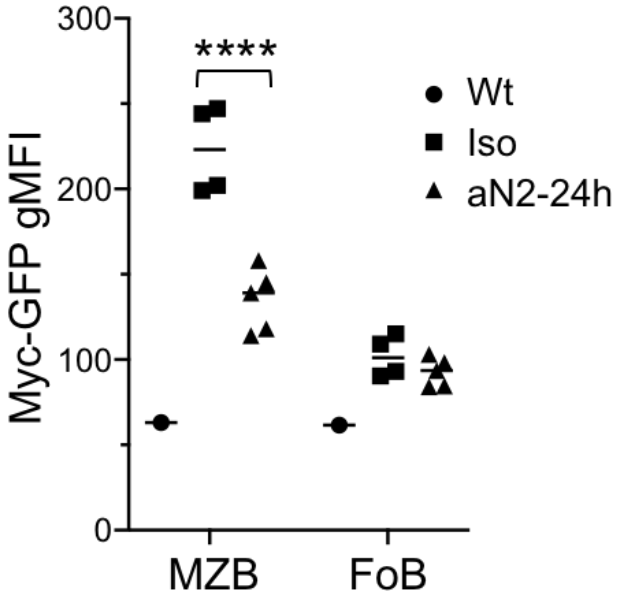
What are the transcriptional programs controlled by Notch in marginal zone B cells?

Rapid decline in *Myc* mRNA, Myc protein and and *Myc*-regulated transcripts in Notch2-deprived marginal zone B cells

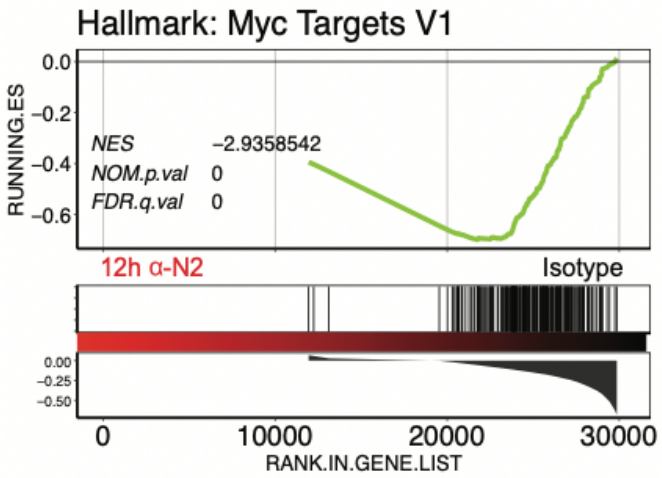
Myc mRNA



Myc protein



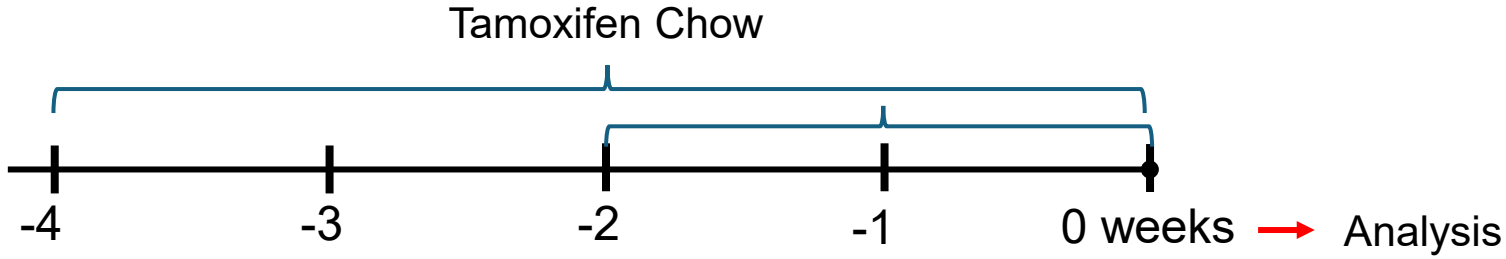
Myc program

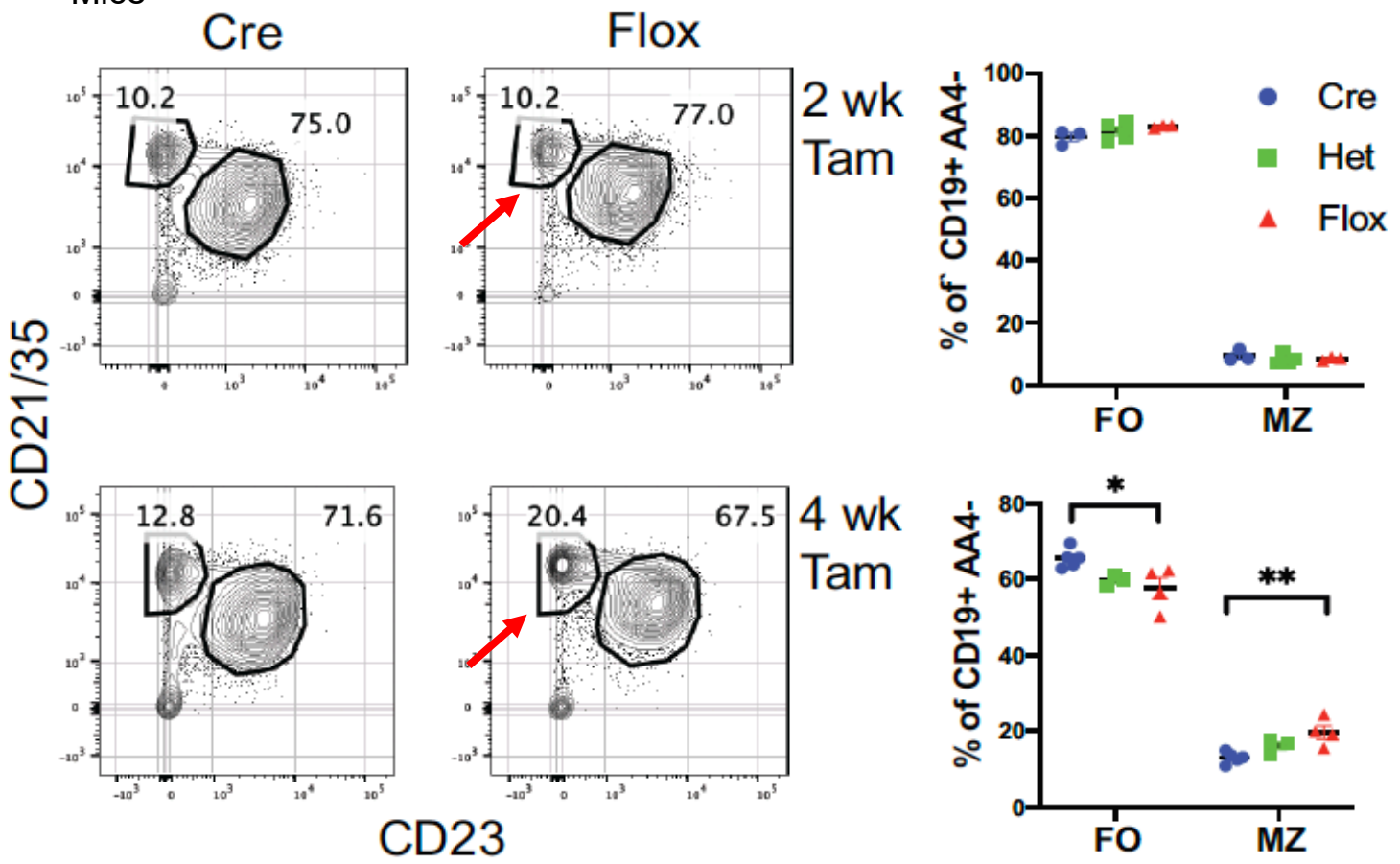


Notch-mediated Myc regulation is conserved in human Notch-driven B cell lymphomas (Ryan et al., Cell Reports 2017)

Is Myc necessary for marginal zone B cell retention in spleen?

cd20-TamCre Myc$^{f/f}$
Mice



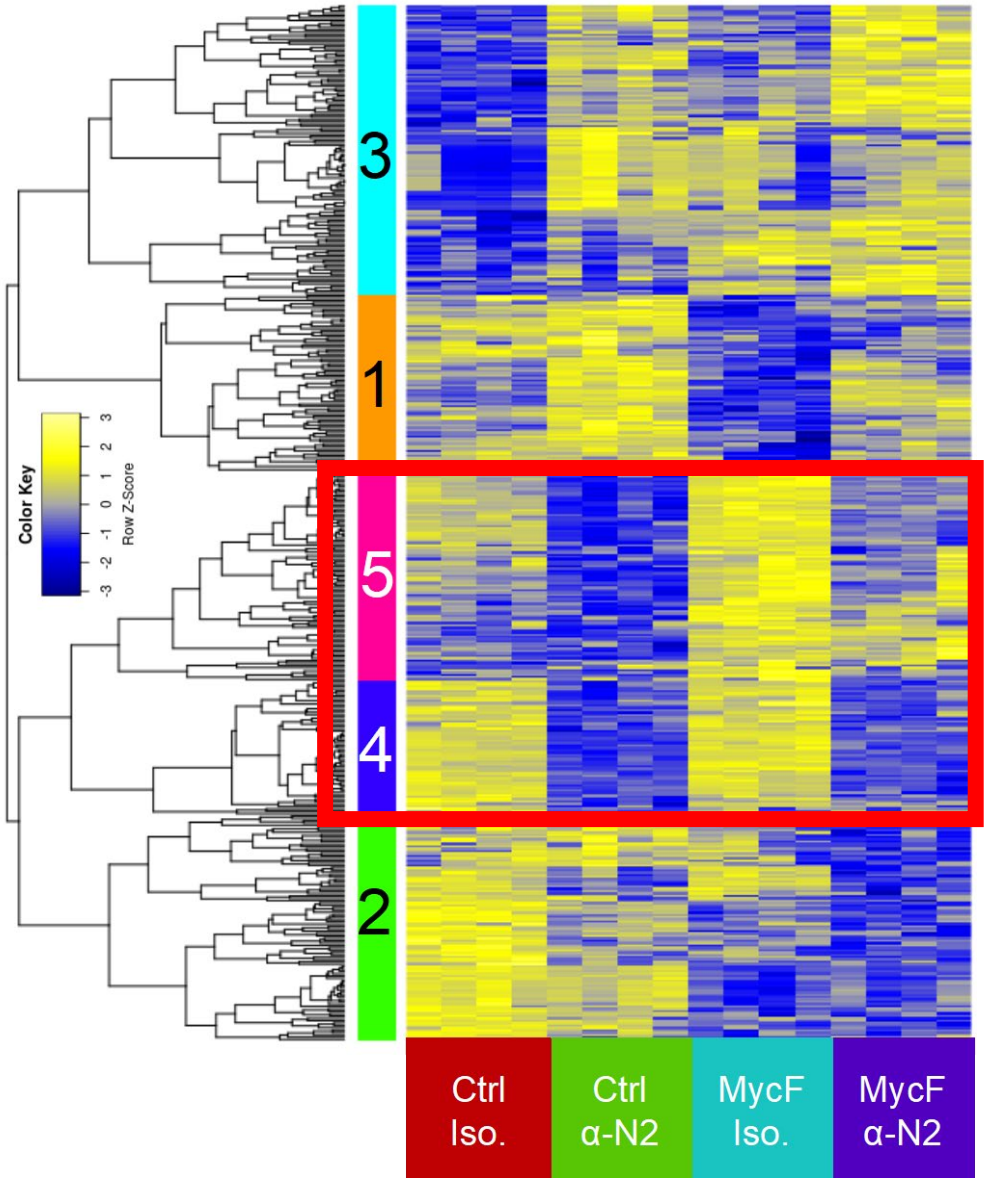


No!

Marginal zone B cell numbers are preserved after *Myc* inactivation

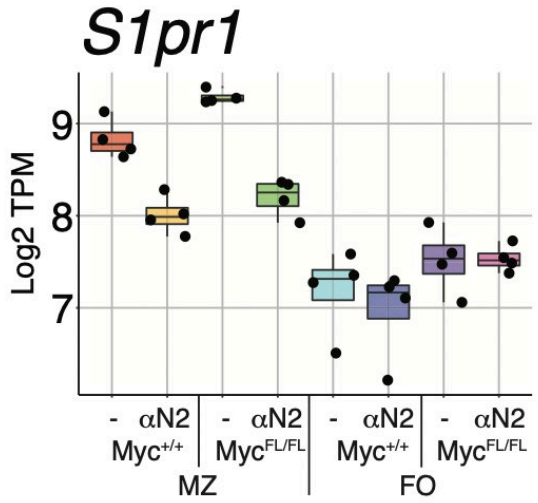
Gaudette el al, *JCI* 2021

RNA-seq analysis identifies a large cohort of Myc-independent Notch-regulated genes in marginal zone B cells



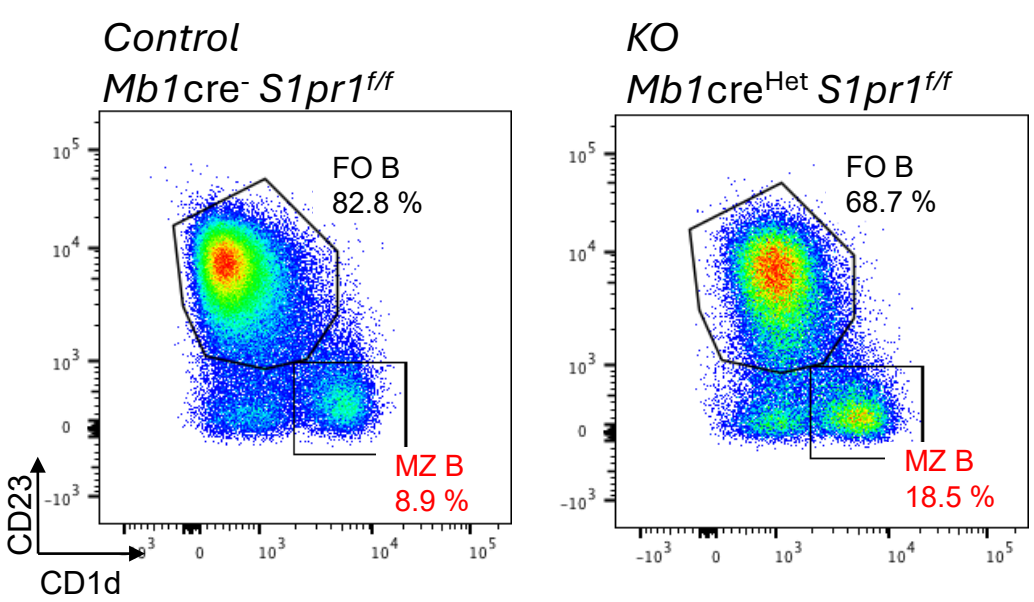
Notch-regulated
Myc-independent

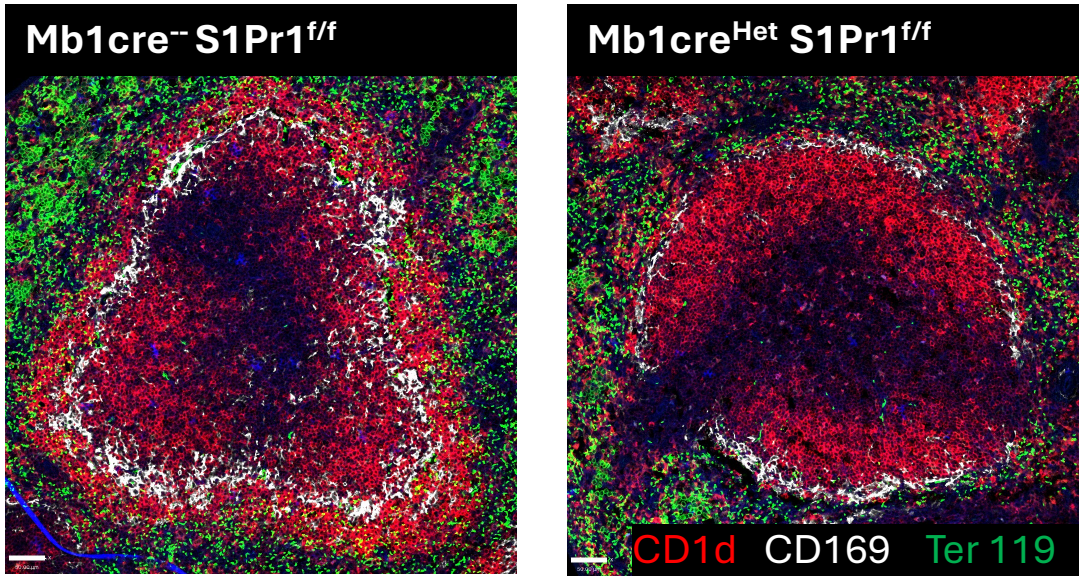
Enriched in:
Chemotactic receptors
Integrins
Signaling molecules



Brian Gaudette

*Mb1*cre^{het} *S1pr1$^{f/f}$* mice have phenotypic marginal zone B cells trapped in splenic B cell follicles

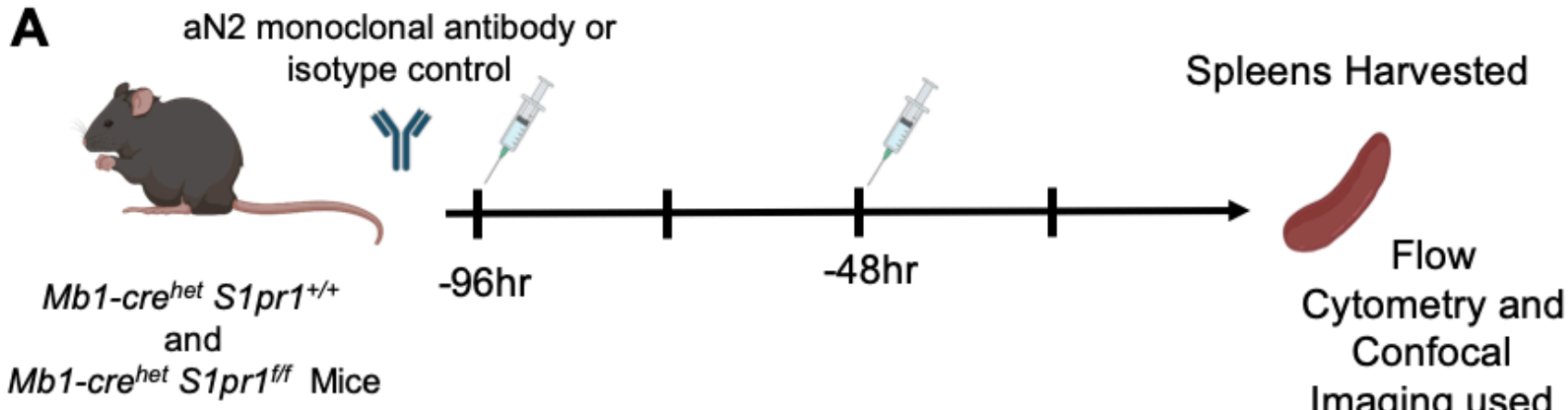


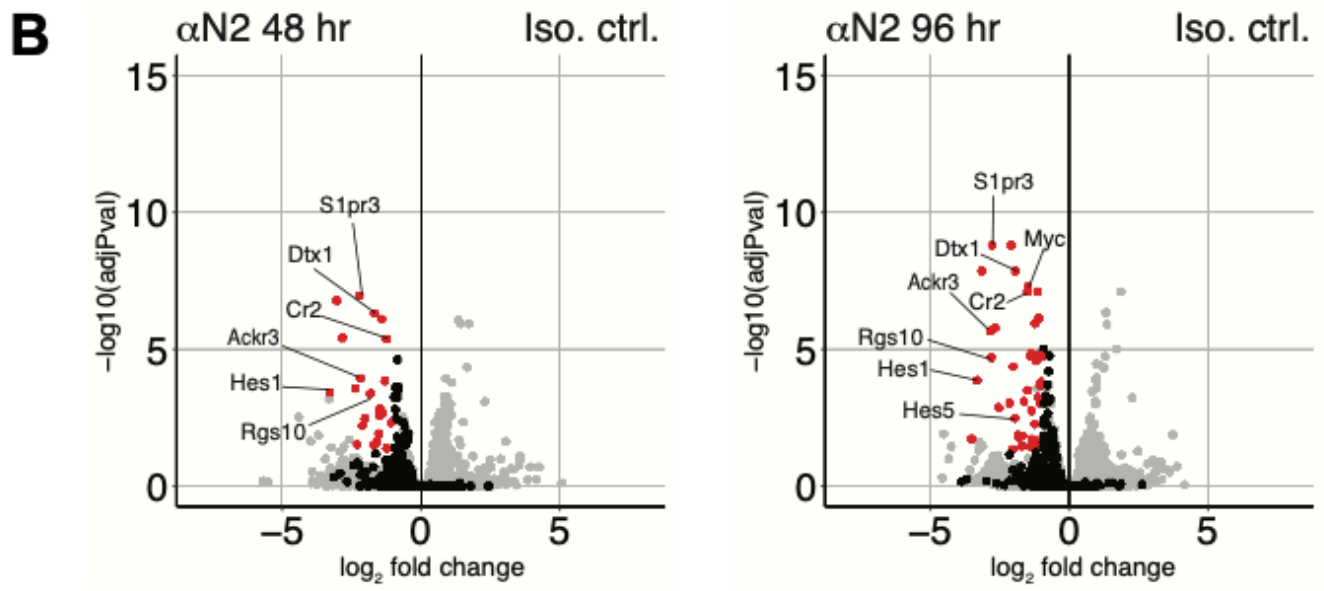


Consistent with data from the Cyster lab

Anneka Allman, Samantha Kelly

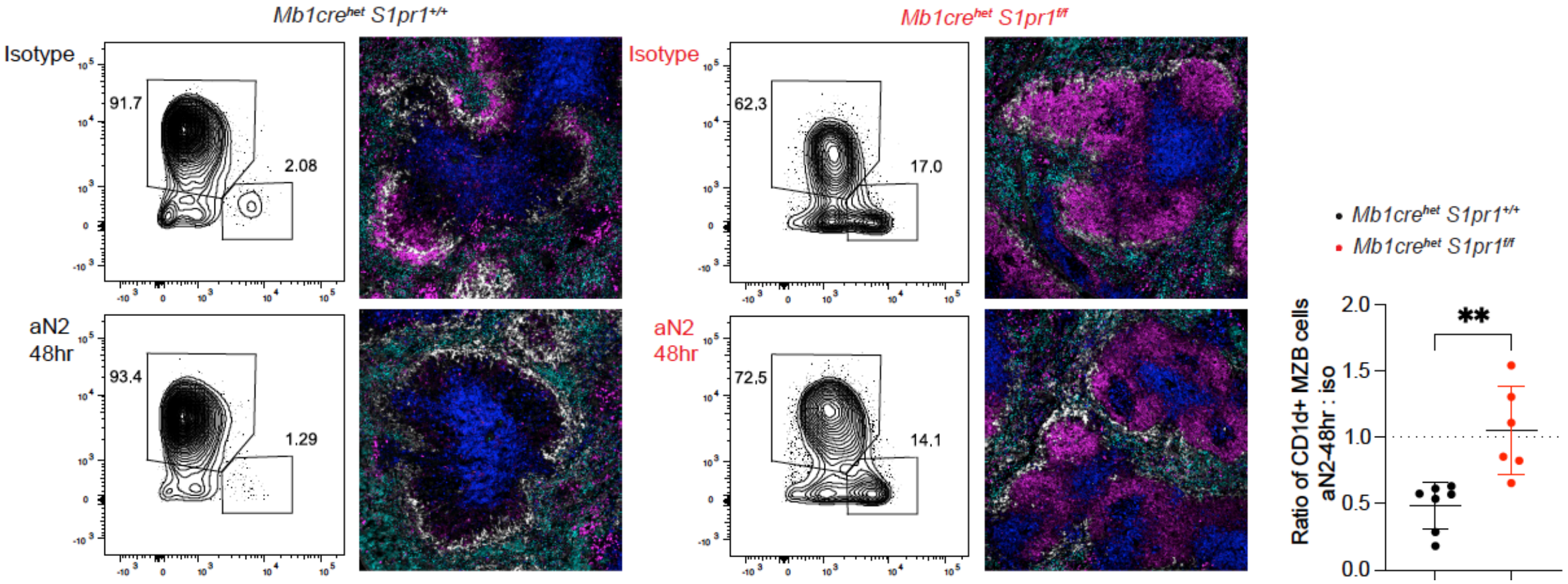
Marginal zone B cells receive Notch signals when mislocalized in the follicle





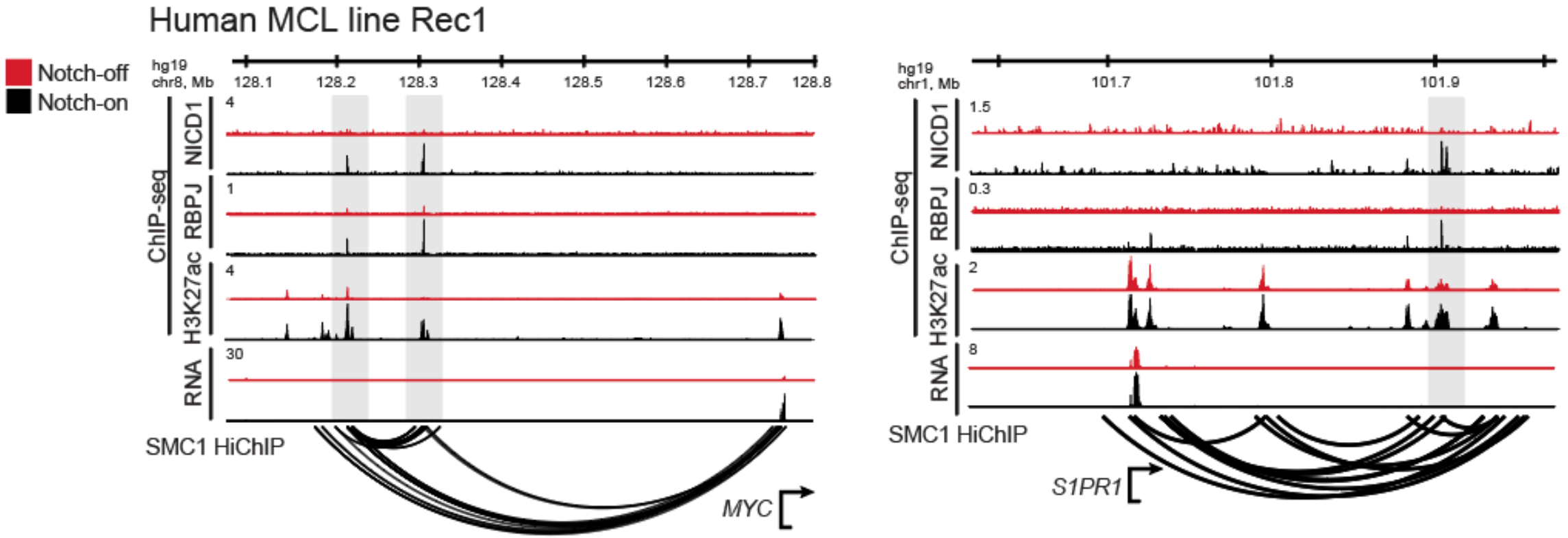
Samantha Kelly
Brian Gaudette
Anneka Allman

S1PR1-deficient MZB cells are retained in the spleen after Notch2 blockade



Samantha Kelly, Anneka Allman

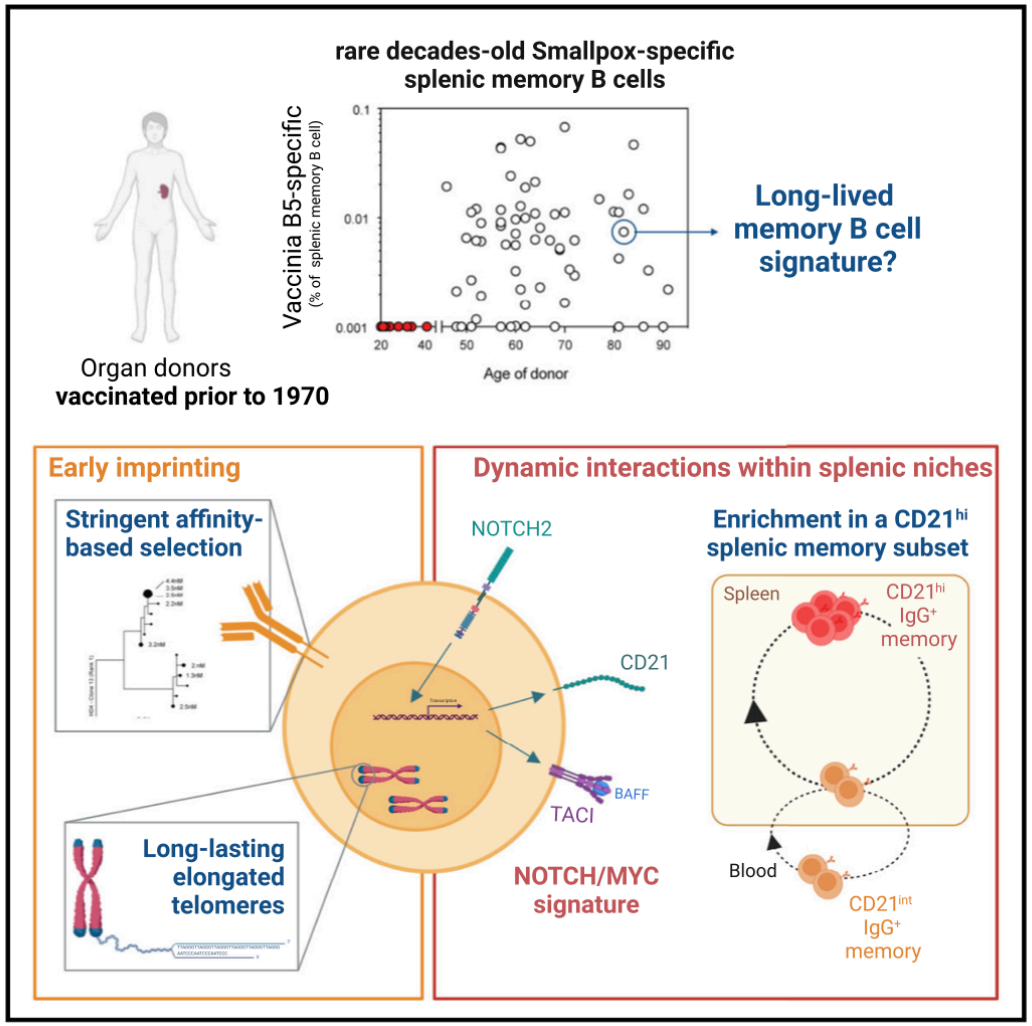
Is the Notch-regulated B cell transcriptional program conserved in humans?



Yeqiao Zhou, Babak Faryabi

Vaccinia-specific memory B cells in humans have a conserved Notch signature

Graphical abstract



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Jean-Claude Weill

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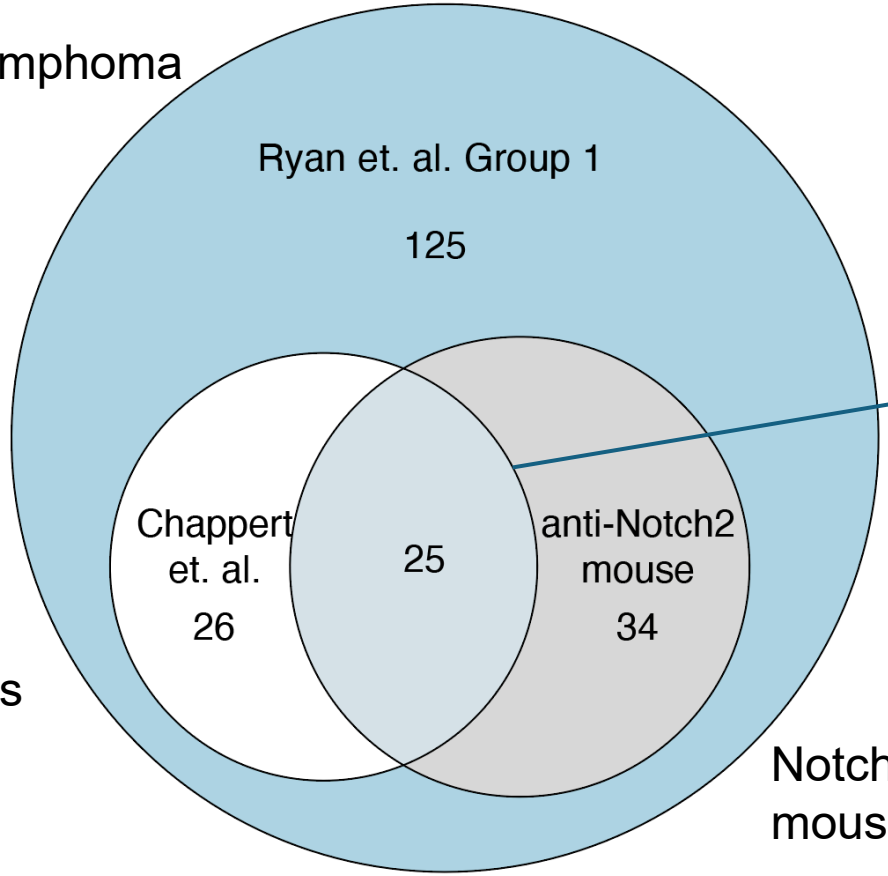
Chappert et al., *Immunity* 2022

Overlap of genes driving human B cell lymphoma, human memory B cells and mouse marginal zone B cells

Notch-driven genes in human Mantle Cell Lymphoma

Notch-driven genes in Vaccinia-specific human memory B cells



Notch-regulated genes in mouse MZB cells

Ryan+Chappert+anti-Notch2_mouse
S1pr1
Galnt6
Il21r
Xylt1
Arhgef3
Cd72
Gadd45b
Cd82
Tle3
Rilpl2
Il6ra
Itgal
Tbc1d9
Grn
Zdhhc14
Dusp16
Lgmn
Cdk5r1
Icam1
Sema7a
Fgr
Hes1
Dtx1
Cr2
Dnase1l3

Ryan et al., *Cell Rep* 2017
Chappert et al., *Immunity* 2022

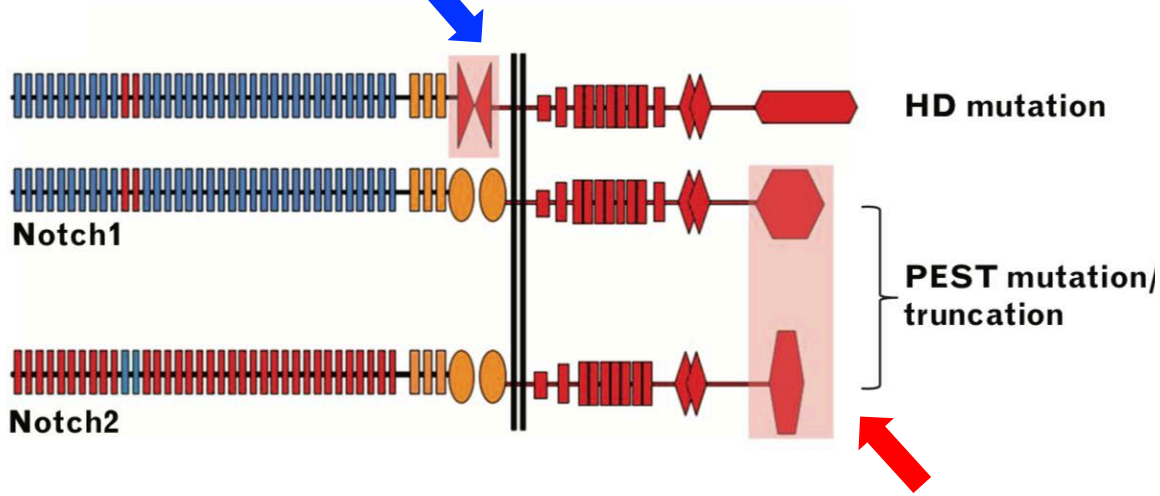
Brian Gaudette

Patterns of oncogenic *NOTCH1/2* mutations in human leukemias and lymphomas

Destabilization of Notch receptors

Ligand-independent activation!

T-ALL



B cell lymphomas
e.g. CLL/SLL, MZL

Decreased degradation of cleaved active Notch molecules

Ligand-dependent activation!

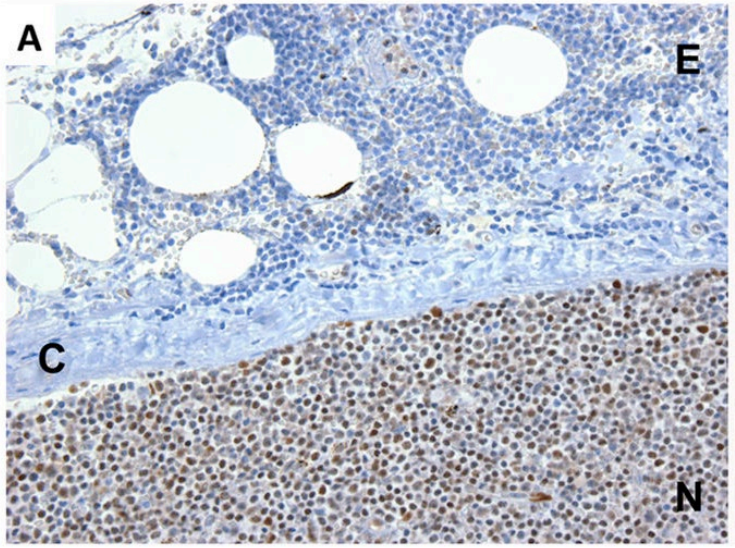
A role for lymph node stromal cell-derived Notch ligands in human lymphomas?

OPEN ACCESS Freely available online

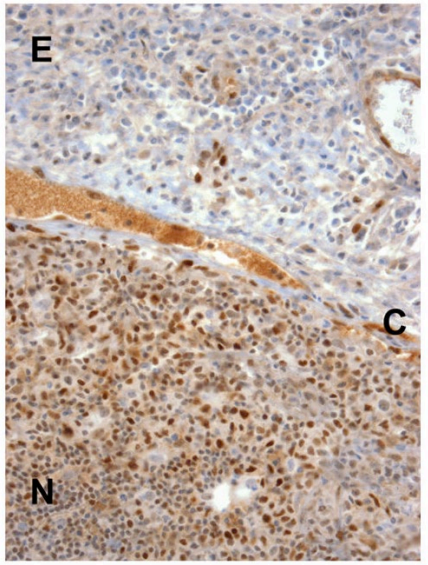
PLOS One 2013

Gauging NOTCH1 Activation in Cancer Using Immunohistochemistry

Michael J. Kluk[1], Todd Ashworth[1], Hongfang Wang[1], Birgit Knoechel[2], Emily F. Mason[1], Elizabeth A. Morgan[1], David Dorfman[1], Geraldine Pinkus[1], Oliver Weigert[2], Jason L. Hornick[1], Lucian R. Chirieac[1], Michelle Hirsch[1], David J. Oh[1], Andrew P. South[3], Irene M. Leigh[3], Celine Pourreyron[3], Andrew J. Cassidy[3], Daniel J. DeAngelo[2], David M. Weinstock[2], Ian E. Krop[2], Deborah Dillon[1], Jane E. Brock[1], Alexander J. F. Lazar[4], Myron Peto[5], Raymond J. Cho[6], Alexander Stoeck[7], Brian B. Haines[7], Sriram Sathayanrayanan[7], Scott Rodig[1], Jon C. Aster[1*]



CLL (*NOTCH1* PEST mutation)



AITL (no mutation)

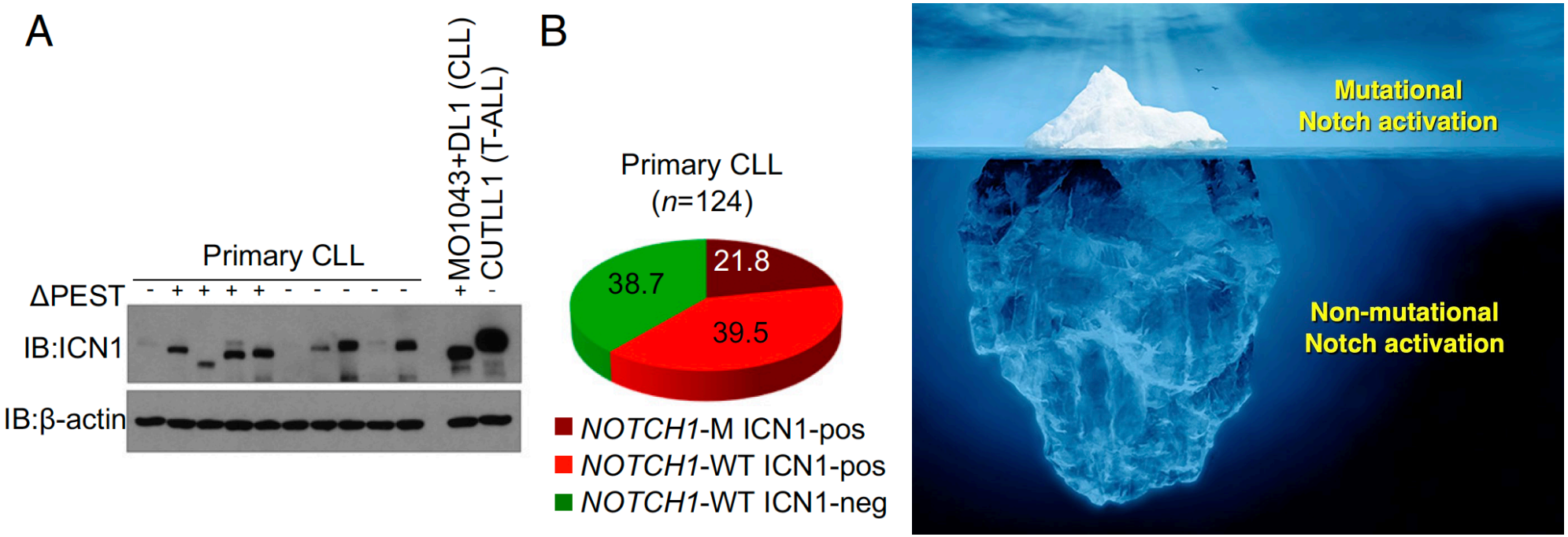
Little active Notch outside of lymph node

Cleaved active Notch within lymph node!

Common nonmutational *NOTCH1* activation in chronic lymphocytic leukemia

Giulia Fabbri[a], Antony B. Holmes[a], Mara Viganotti[a], Claudio Scuoppo[a], Laura Belver[a], Daniel Herranz[a], Xiao-Jie Yan[b], Yasmine Kieso[b], Davide Rossi[c,d], Gianluca Gaidano[e], Nicholas Chiorazzi[b], Adolfo A. Ferrando[a,f,g], and Riccardo Dalla-Favera[a,f,h,i,1]

Fabbri et al., *PNAS* 2017



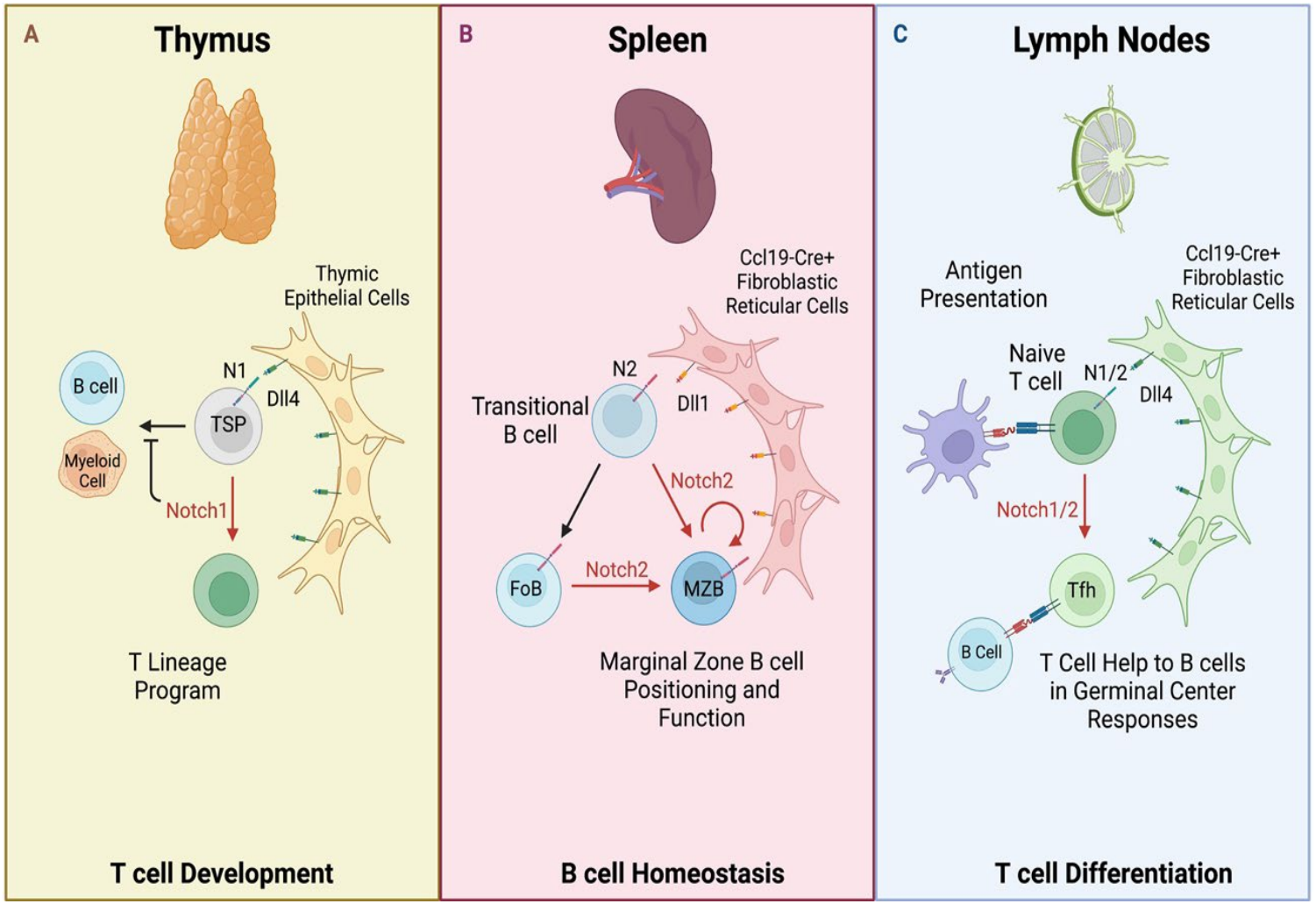
Similar data for Notch2 activation in human splenic marginal zone lymphomas (Shanmugam et al., Blood Advances 2021)

Summary

- Stromal Dll1-mediated Notch2 signals are uniquely required for marginal zone B cell homeostasis and cannot be substituted by Dll4/Notch2
- Access to a limiting pool of stromal Dll1 Notch ligands provides a mechanism for “lymphopenia sensing” by mature B cells
- The stroma-dependent transcriptional effects of Notch signaling in B cells are conserved in subsets of human memory B cells and hijacked in human B cell lymphomas (e.g. CLL/SLL, marginal zone lymphomas)

Allman et al., *Immunity 2025*

Recurrent rules of Notch engagement in the immune system



- *Stromal niches function as a critical source of Notch ligands in multiple lymphoid organs*
- *Notch ligands + chemokines define functional niches*
- *Stroma-driven Notch signals provide spatial, temporal and quantitative information*

Schneider, Allman and Maillard, *Curr Opin Cell Biol* 2023

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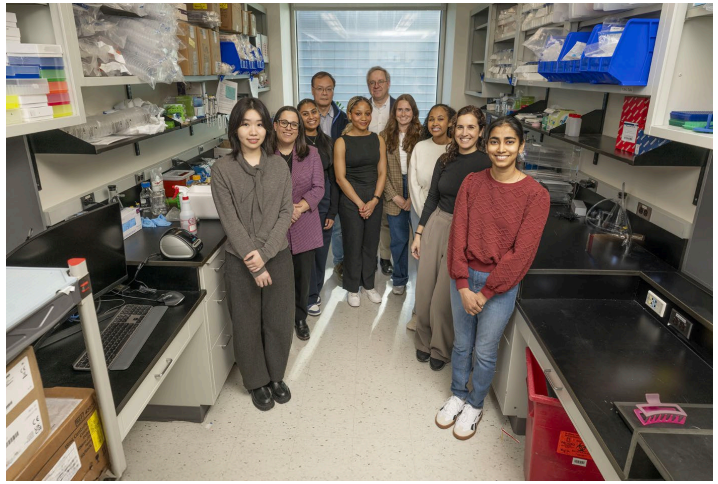

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