NATURE IMMUNOLOGY | ARTICLE

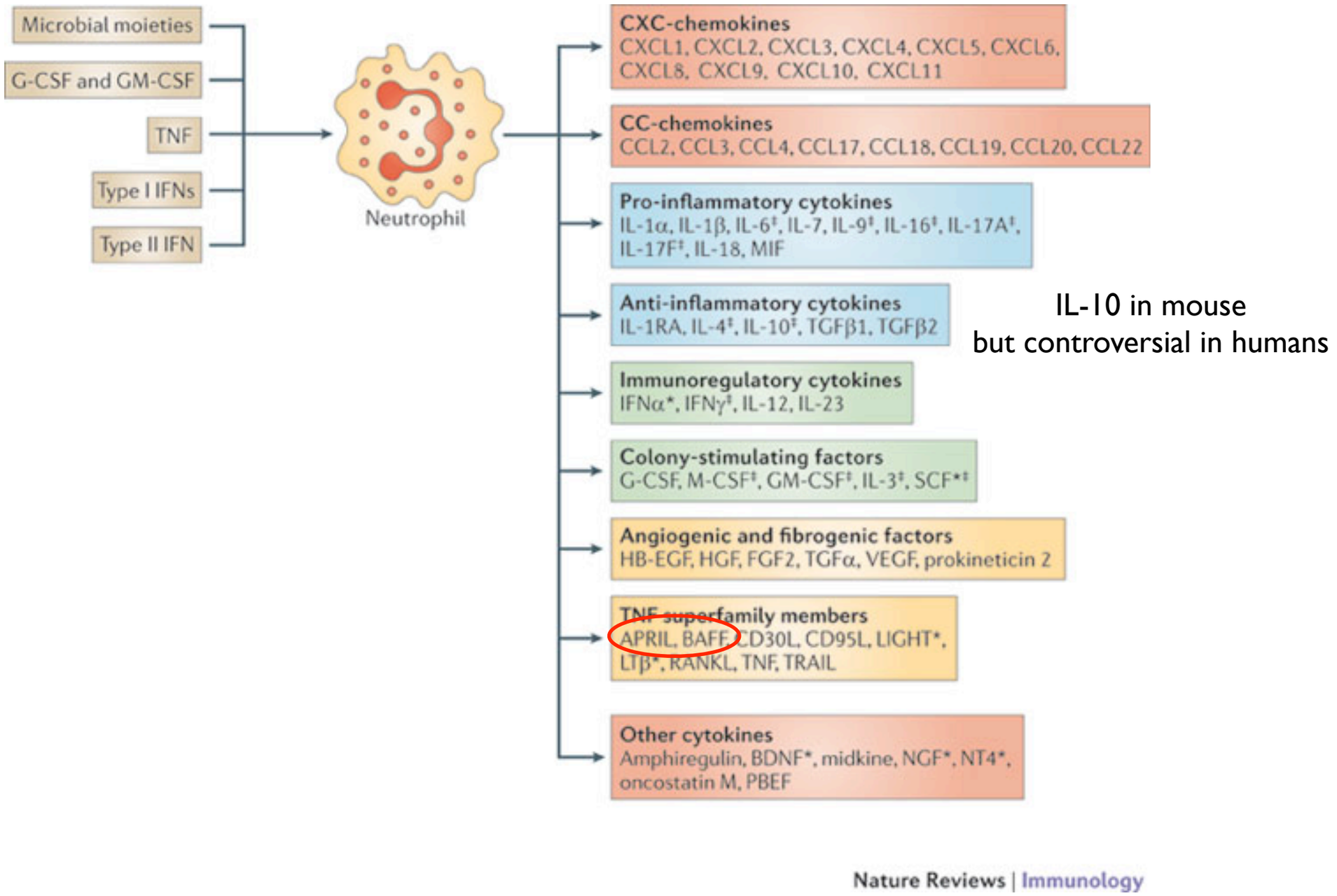
B cell–helper neutrophils stimulate the diversification and production of immunoglobulin in the marginal zone of the spleen

Irene Puga, Montserrat Cols, Carolina M Barra, Bing He, Linda Cassis, Maurizio Gentile, Laura Comerma, Alejo Chorny, Meimei Shan, Weifeng Xu, Giuliana Magri, Daniel M Knowles, Wayne Tam, April Chiu, James B Bussel, Sergi Serrano, José Antonio Lorente, Beatriz Bellosillo, Josep Lloreta, Nuria Juanpere, Francesc Alameda, Teresa Baró, Cristina Díaz de Heredia, Núria Torán, Albert Català, Montserrat Torrebadell, Claudia Fortuny, Victoria Cusí, Carmen Carreras, George A Diaz, J Magarian Blander, Claire-Michèle Farber, Guido Silvestri, Charlotte Cunningham-Rundles, Michaela Calvillo, Carlo Dufour, Lucia Dora Notarangelo, Vassilios Lougaris, Alessandro Plebani, Jean-Laurent Casanova, Stephanie C Ganal, Andreas Diefenbach, Juan Ignacio Aróstegui, Manel Juan, Jordi Yagüe, Nizar Mahlaoui, Jean Donadieu, Kang Chen & Andrea Cerutti – Show fewer authors

Neutrophil functions

- **Clearance of extracellular pathogens**
  - phagocytosis
  - intracellular degradation
  - anti-microbial factor secretion
  - secrete cellular components that form neutrophil extracellular traps (NET)
  - secrete cytokines/chemokines that recruit monocytes

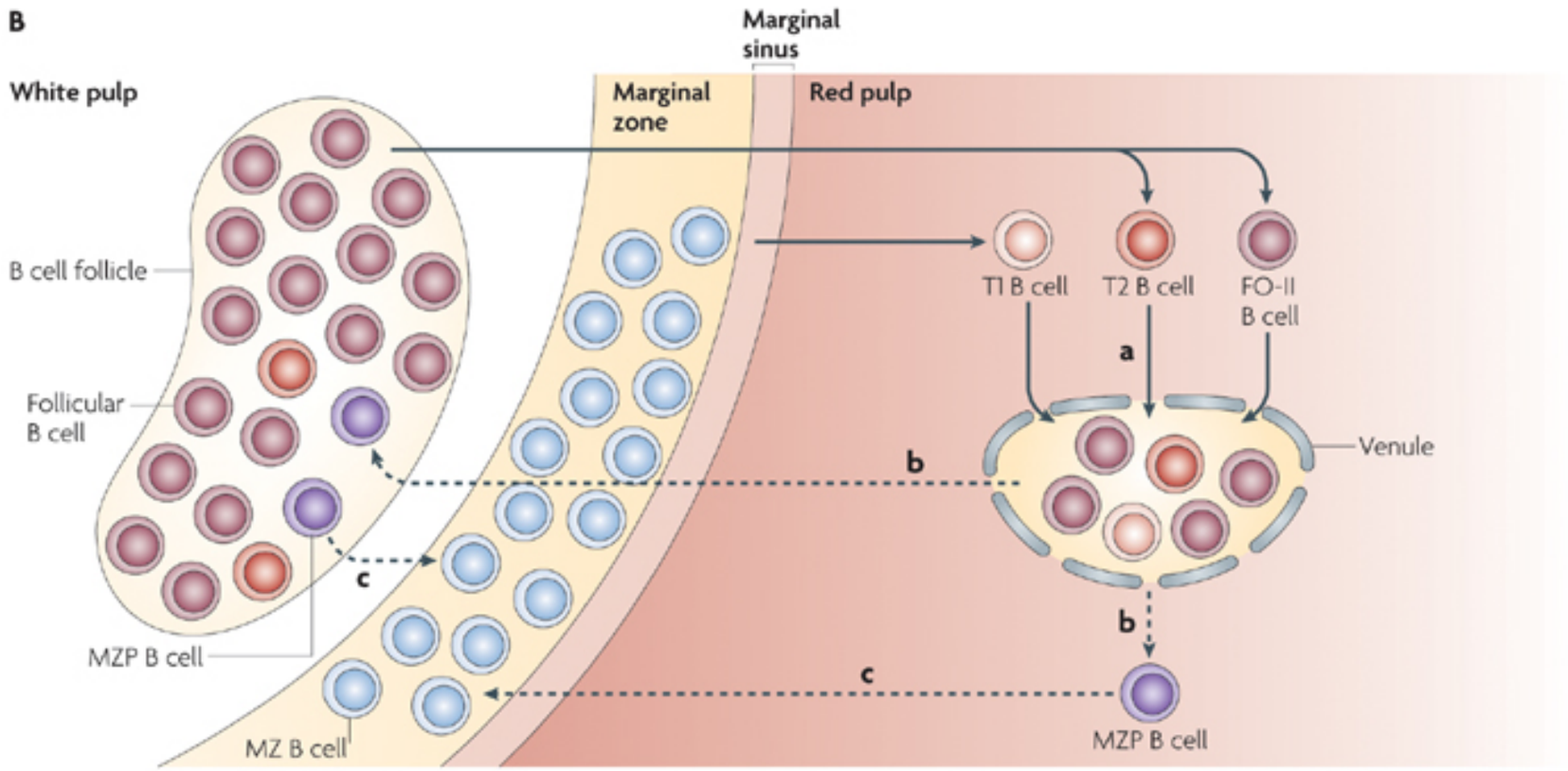
- **Interact with immune cells**
  - DCs (activation)
  - In the presence of inflammation can present antigen
  - Induction of T cell responses
  - Suppress T cell responses in pregnancy or cancer (iNOs, arginase)
  - Express FcRϒ and FcRα that interact with opsonized microbes
  - Express BAFF and APRIL (B cell survival and differentation, CSR from IgM to IgG and IgA)



IL-10 in mouse
but controversial in humans

Mantovani, A., Cassatella, M. A., Costantini, C., & Jaillon, S. (2011).
Neutrophils in the activation and regulation of innate and adaptive immunity *Nature Reviews Immunology*, *11*(8), 519–531.

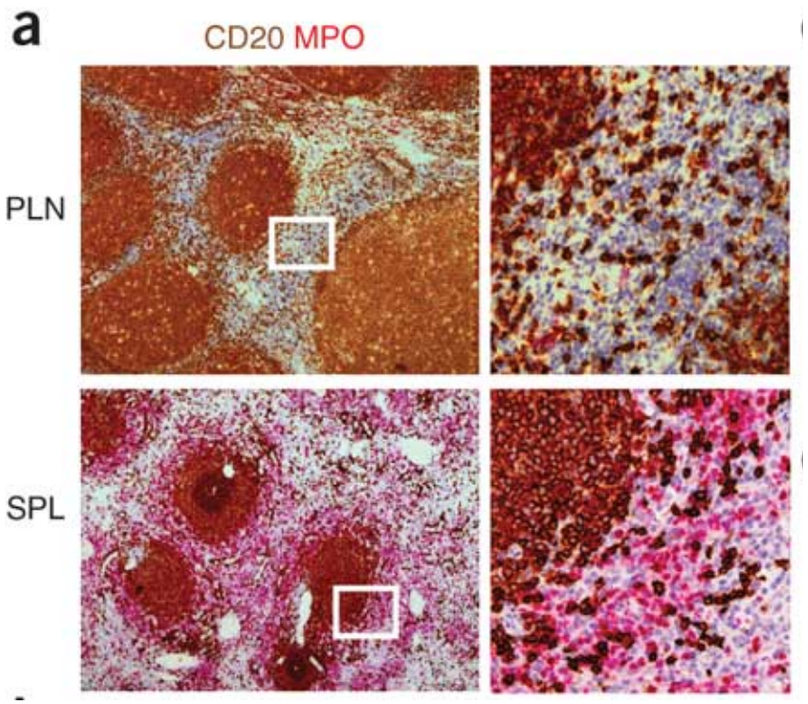
The MZ, an interface between the circulation and the immune system

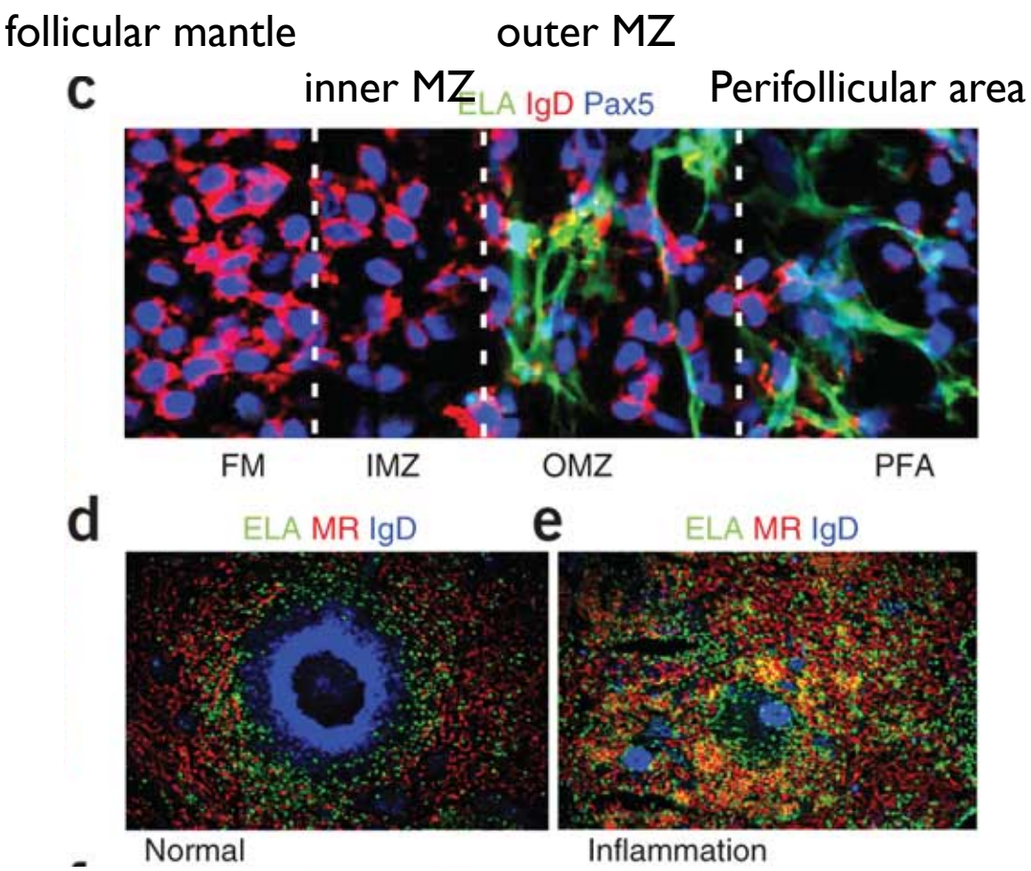


Pillai, S., & Cariappa, A. (2009). The follicular versus marginal zone B lymphocyte cell fate decision. *Nature Reviews Immunology*, *9*(11), 767–777. doi:10.1038/nri2656

MZ B cells mount rapid T-independent immunoglobulin responses
In humans MZ B cells circulate and are CD27+IgM+IgD+
Weller, S.,et al. (2004). Human blood IgM "memory" B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire *Blood*, *104*(12))
Granulocytes home to the MZ in response to blood-borne bacteria
Balázs, M., Martin, F., Zhou, T., & Kearney, J. (2002). Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses. *Immunity*, *17*(3), 341–352

Neutrophils colonize peri-MZ areas





Hyper IgD syndrome

Partial or complete loss of the MZ - aberrant location of neutrophils

Elastase
Mannose receptor expressed on sinusoidal epithelial cells

Neutrophils have MZ B cell-helper function

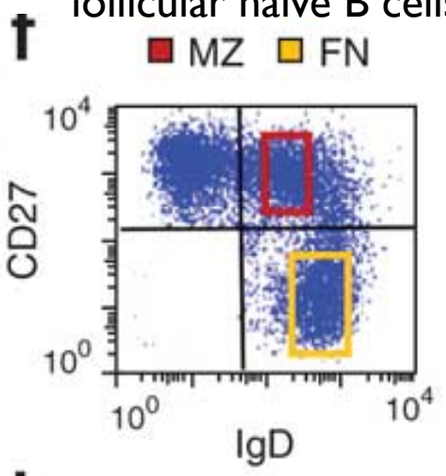
10% in spleen, 2% in MLN, 0.5% in pLN, 0.3% in tonsils (of total cells)

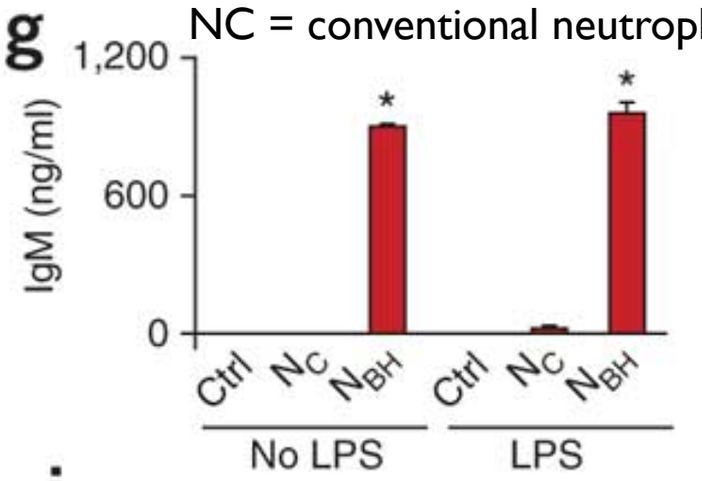
Gated on CD19+ cells

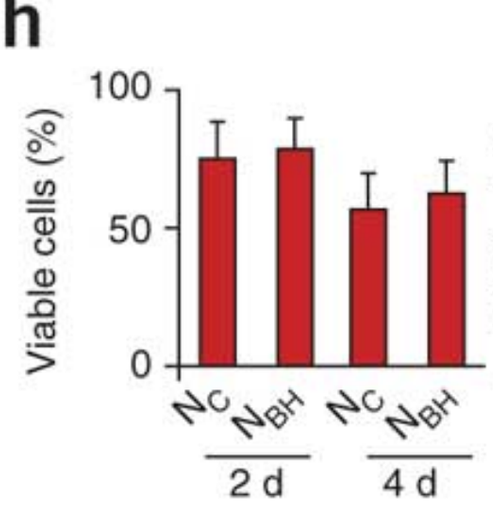
follicular naive B cells

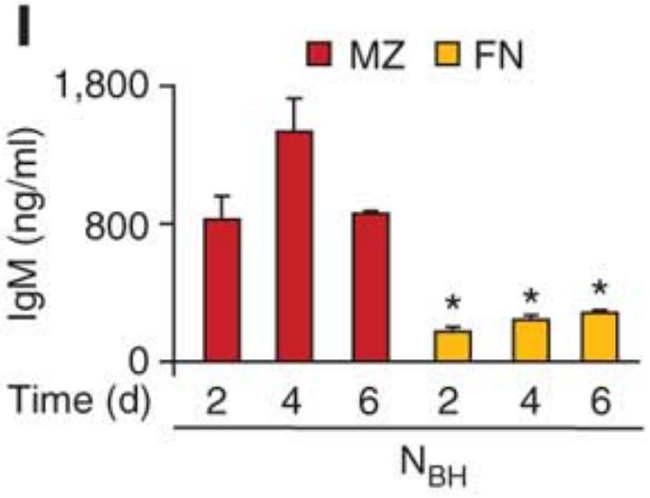
NBH = B cell helper neutrophils

NC = conventional neutrophils



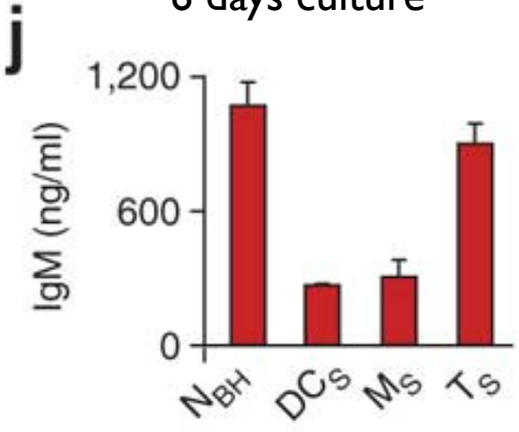




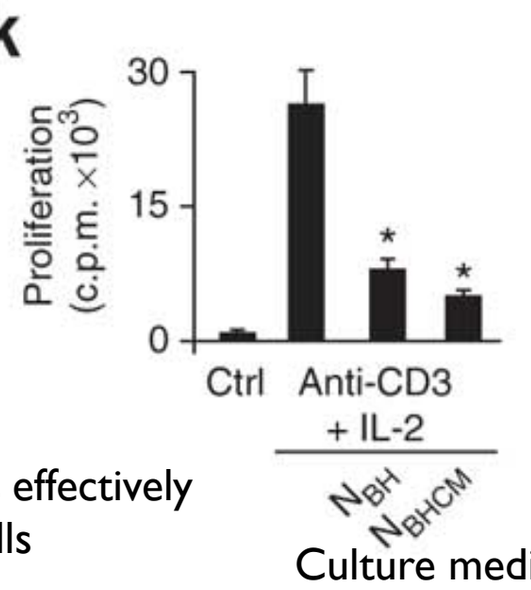


NBH and NC provide the same survival to MZ B cells

6 days culture



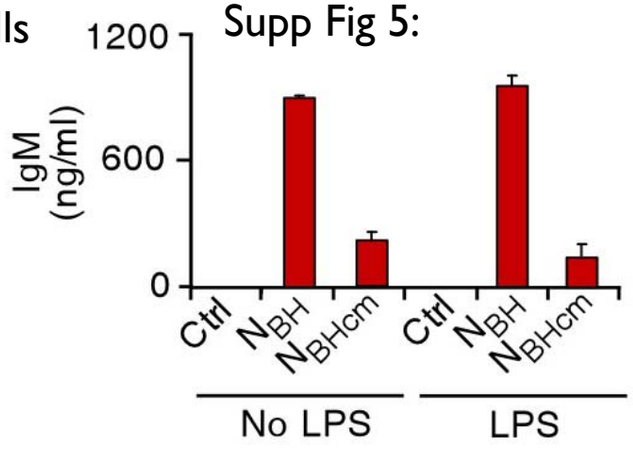
MZ B cells are activated as effectively with NBH as T cells



Culture medium

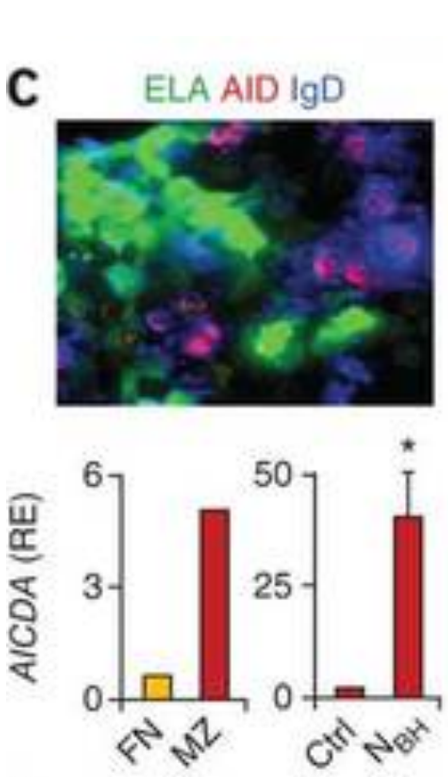
Suppress T cell responses

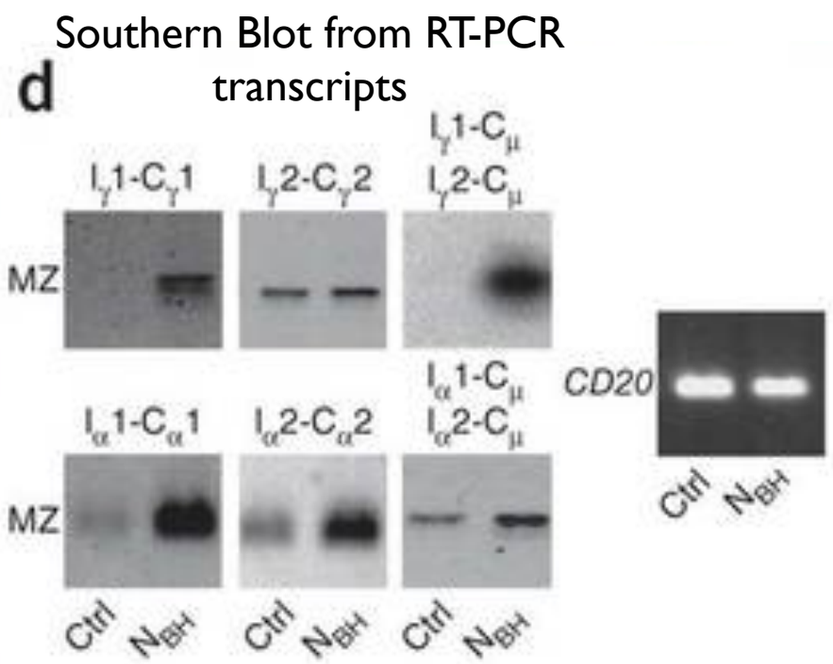
Supp Fig 5:

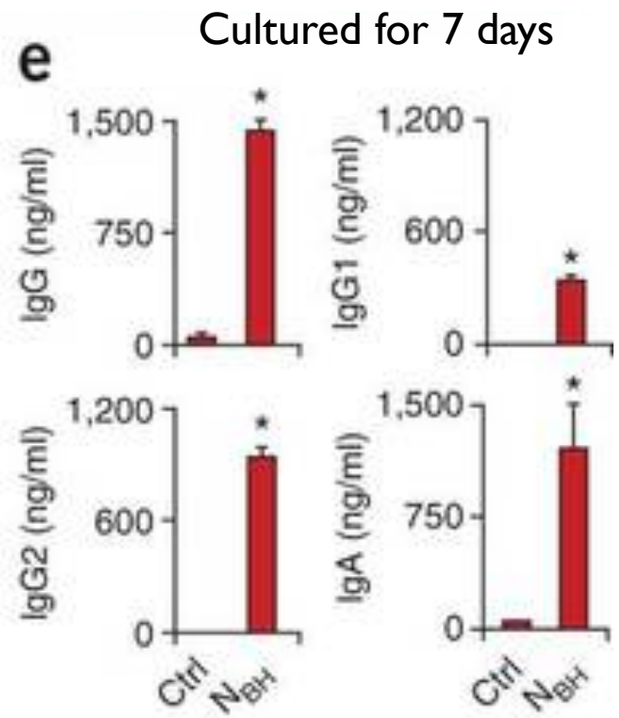


Activation of MZ B cells by contact-dependent and independent mechanisms

Neutrophils induce immunoglobulin diversification

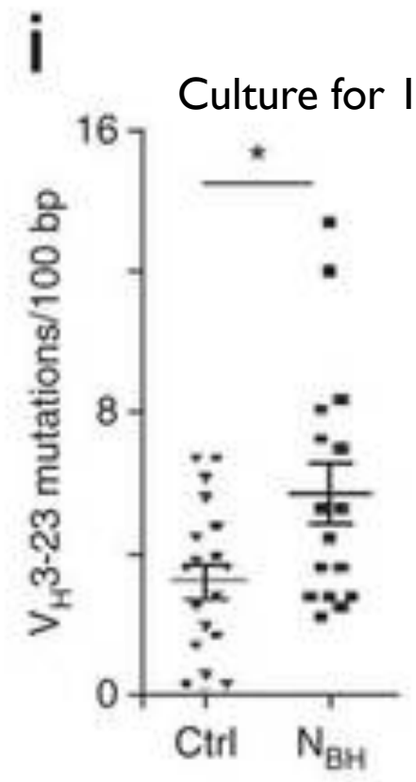






After 2d culture

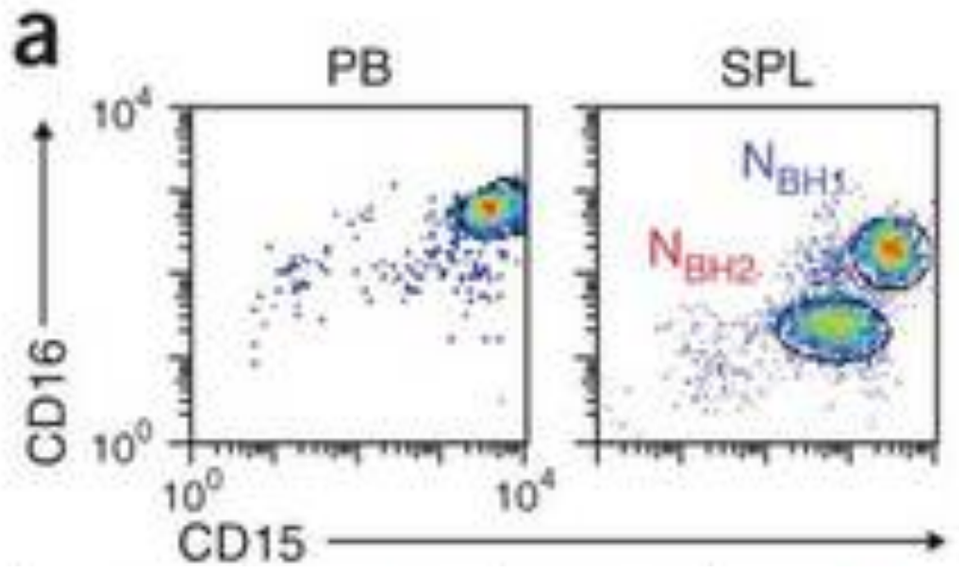
Results normalized to PAX5 mRNA, fold expression to medium alone



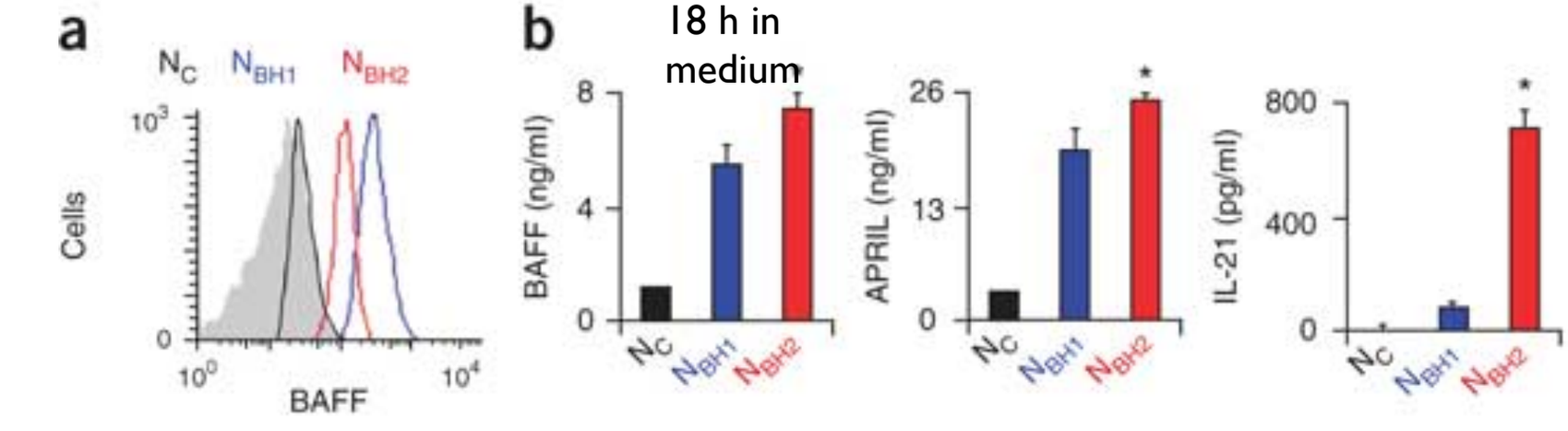
VH3-23 region often used by MZ B cells

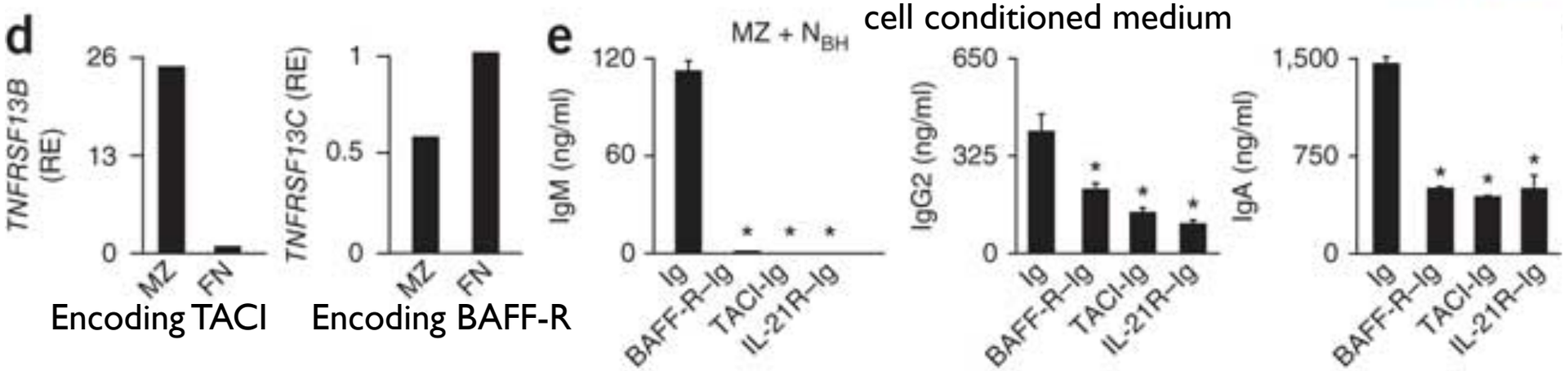
Neutrophils BH include 2 subsets distinct from Nc

CD16 = Fcg



Neutrophils activate MZ B cells via BAFF, APRIL and IL-21





Encoding TACI Encoding BAFF-R

Normalized to *ACTB* mRNA and expressed relative to naive FO B cells

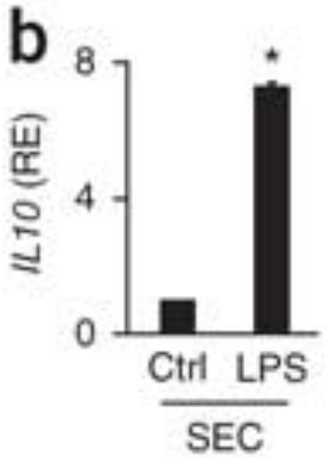
Cultured for 6 days

Deleterious mutations in TACI or STAT3 (IL-21 signaling): fewer MZ B cells with poor MZ development

Splenic signals reprogram neutrophils

sinusoidal epithelial cells and NBH cells are in close proximity

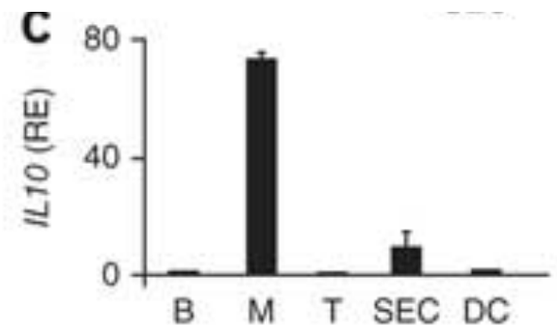
12h incubation



sinusoidal epithelial cells upregulate *IL-10* in response to LPS

fold expression to ctrl

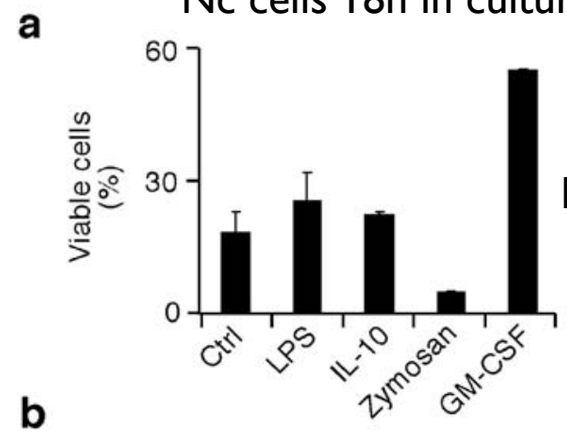
12h incubation



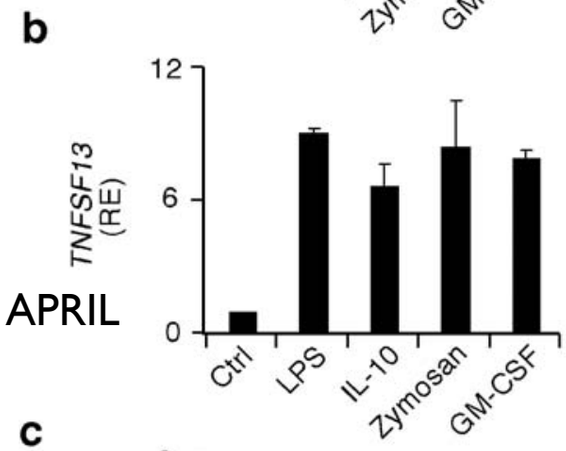
fold expression to B

relative to *ACTB* mRNA

Nc cells 18h in culture

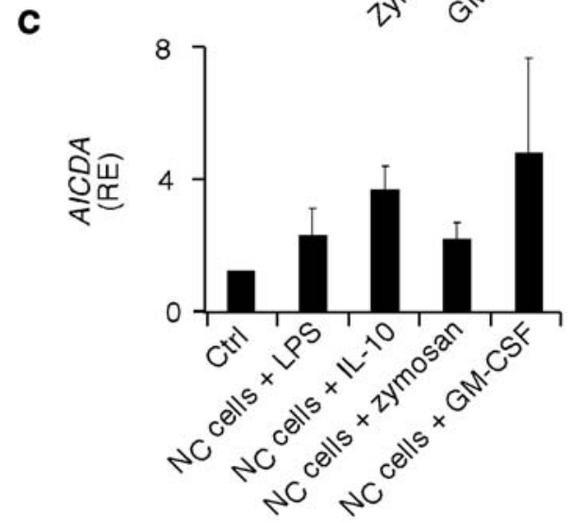


Annexin V-
Propidium iodide-



APRIL

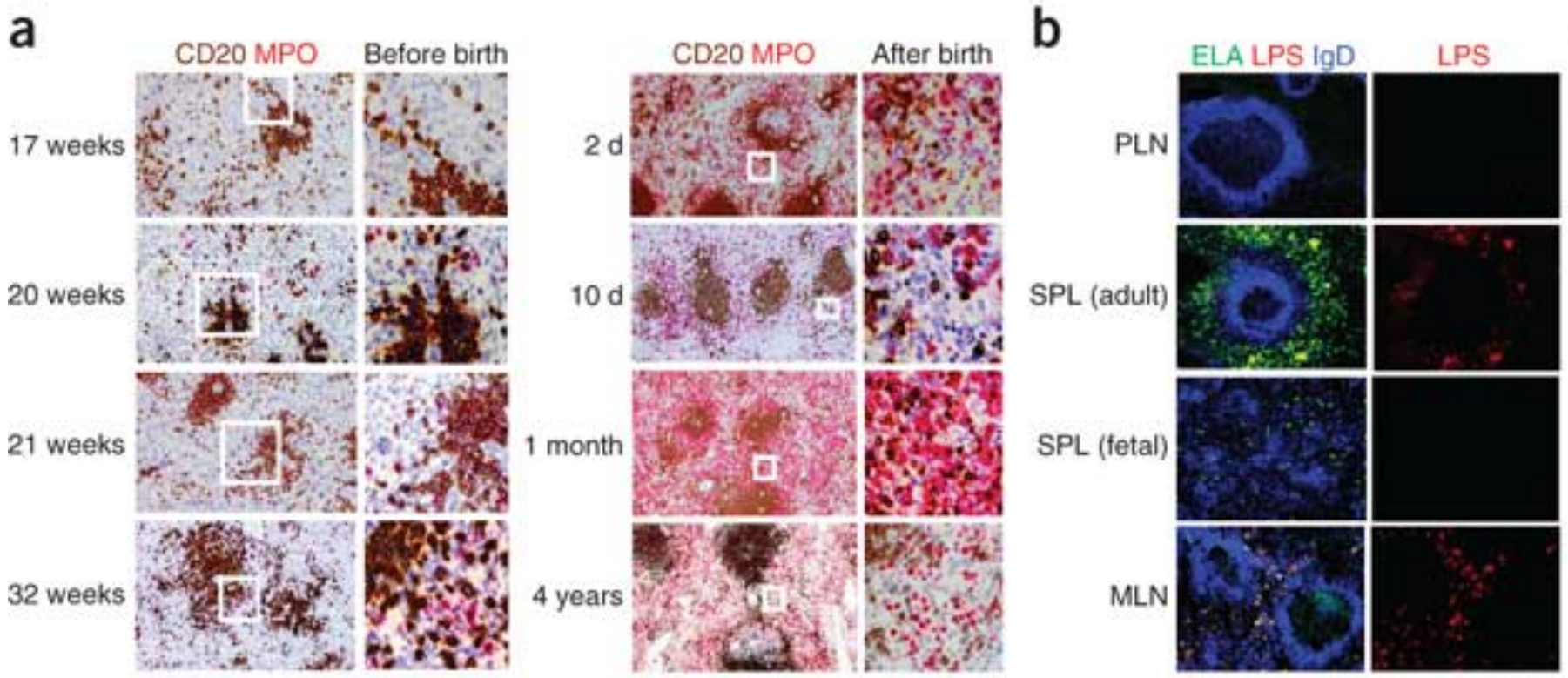
Fold increase to unstimulated cells

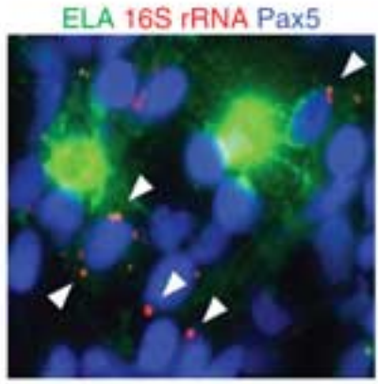


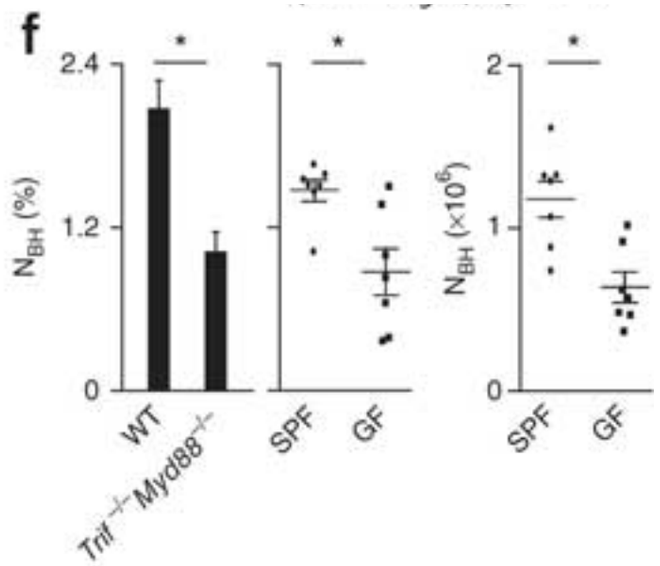
Circulating IgD+ B cells cultured for 2 d

Splenic NBH home to the spleen after intestinal colonization

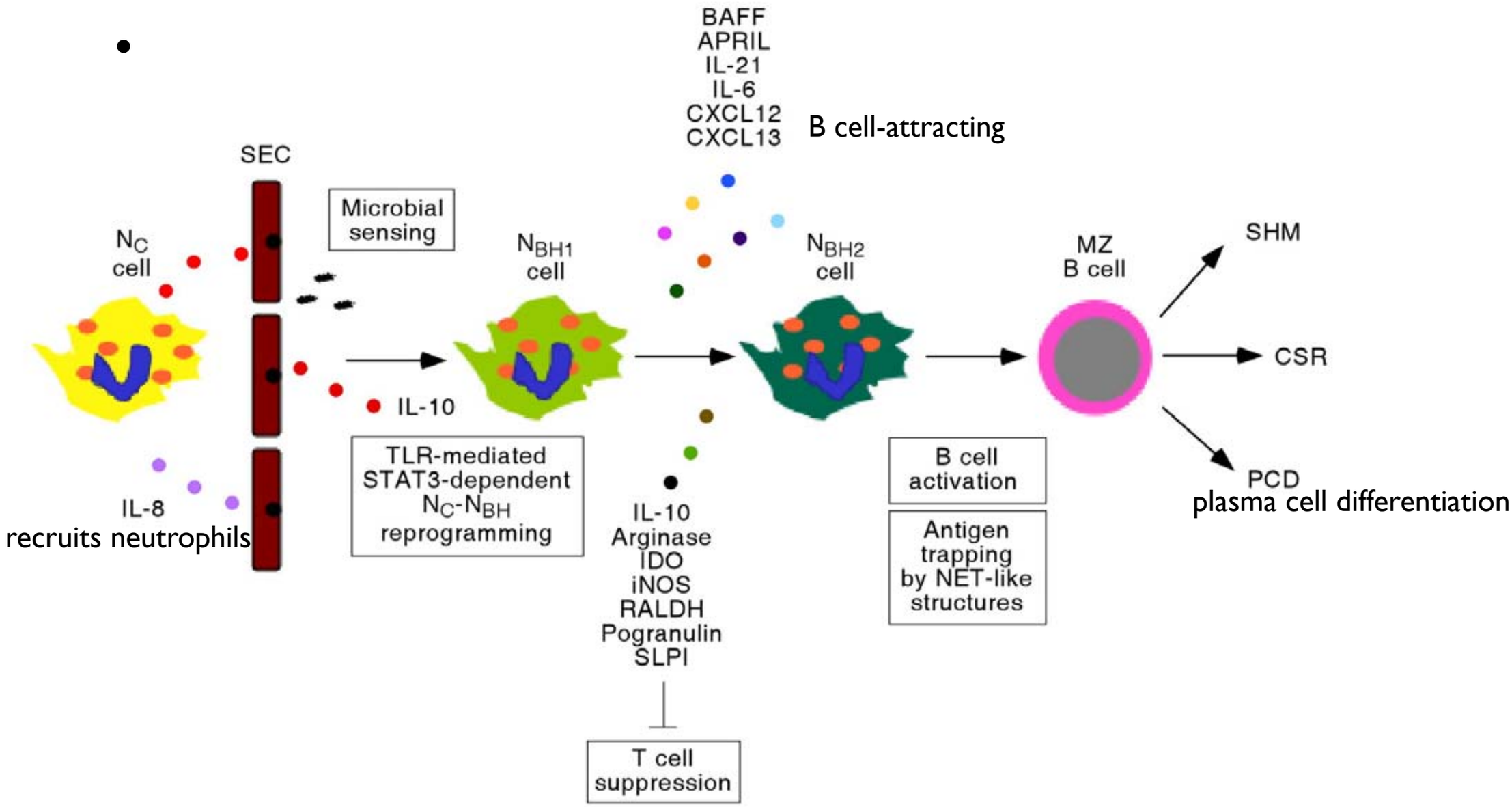
systemic translocation of microbial products from mucosal surfaces influences the function of neutrophils Clarke, T. B., Davis, K. M., Lysenko, E. S., Zhou, A. Y., Yu, Y., & Weiser, J. N. (2010). Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity *Nature Medicine*, *16*(2), 228–231.







- Neutrophils colonize splenic peri-MZ areas following colonization
- Patients with severe congenital neutropenia had decreased proportions and hypomutated MZ B cells but not naive B cells or total B cells and showed decreased T-I antibodies (LPS, LTA, PGN) - Fig 6-
- NBH cells activated MZ B cells as efficiently as CD4 T cells through contact-dependent and independent mechanisms
- Dual B cell helper and T cell suppressor function (extrafollicular B cell response to T-I)
-



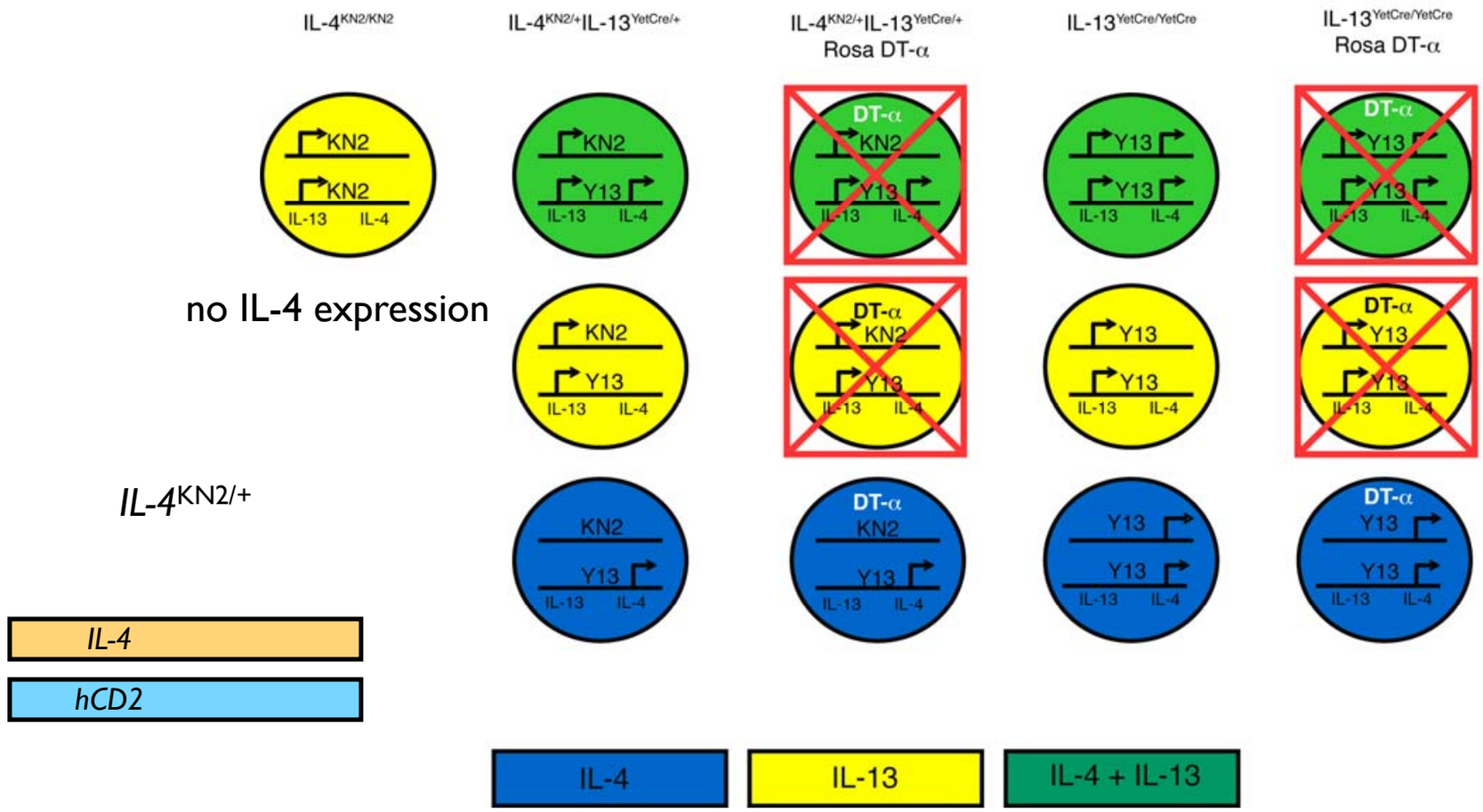
ARTICLES

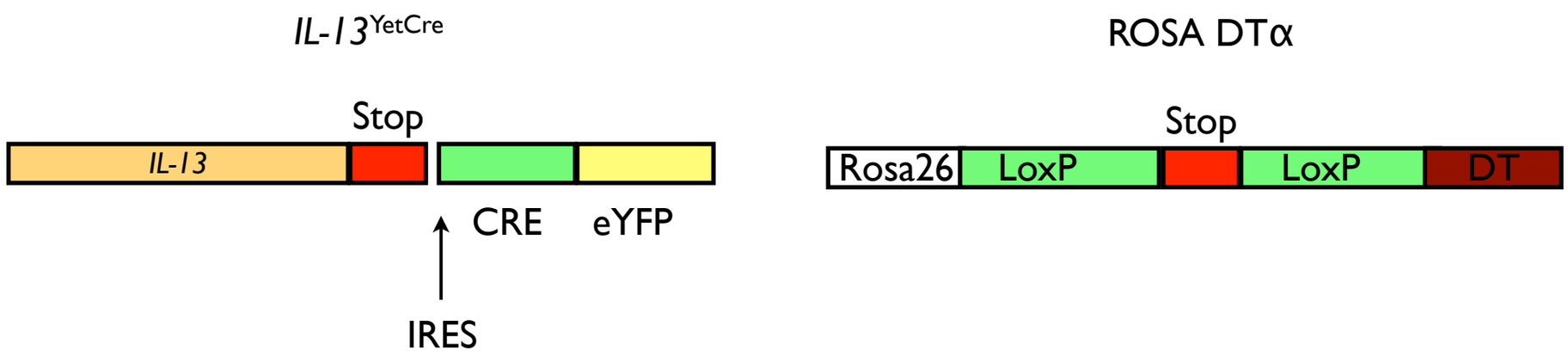
nature immunology

Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity

Hong-Erh Liang[1–3,6], R Lee Reinhardt[1–3,5,6], Jennifer K Bando[1–3], Brandon M Sullivan[1–3], I-Cheng Ho[4] & Richard M Locksley[1–3]

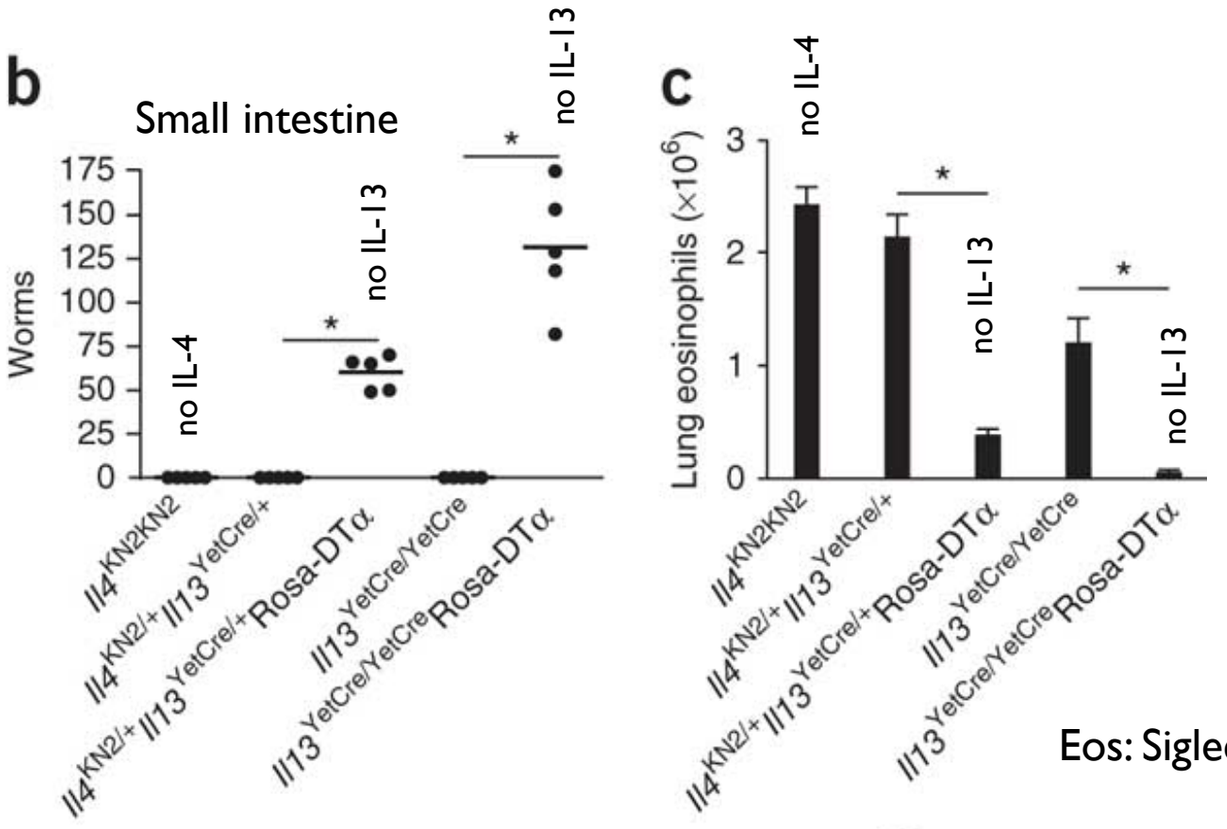
What are the cellular sources of IL-4 and IL-13 *in vivo*





Tissue responses are dependent on IL-13-expressing cells

9 d after infection with *N.brasiliensis*



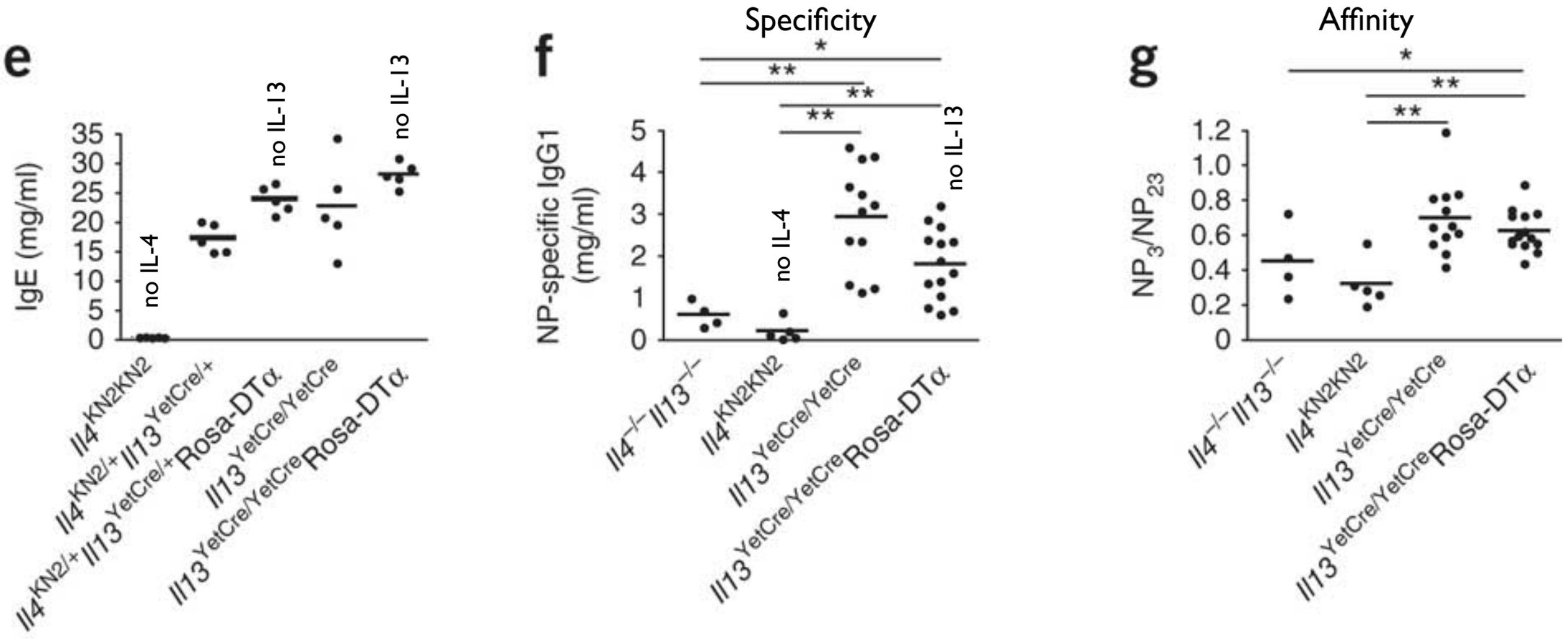
Because IL-13 and IL-5 are co-expressed: ablation of both IL-13 and IL-5 expressing cells in IL-13$^{YetCre/YetCre}$ROSA-DTa

Eos: SiglecF-βc^{hi}(IL-3Rβ^{hi}) CD49b^{-}

Eosinophils do not express IL-13 (FACS)
Eosinophils were not deleted from blood, bone marrow or tissues = impaired recruitment to the lungs (but basoph and CD4+ T cells unaffected)

Ig responses are dependent on IL-4-expressing cells

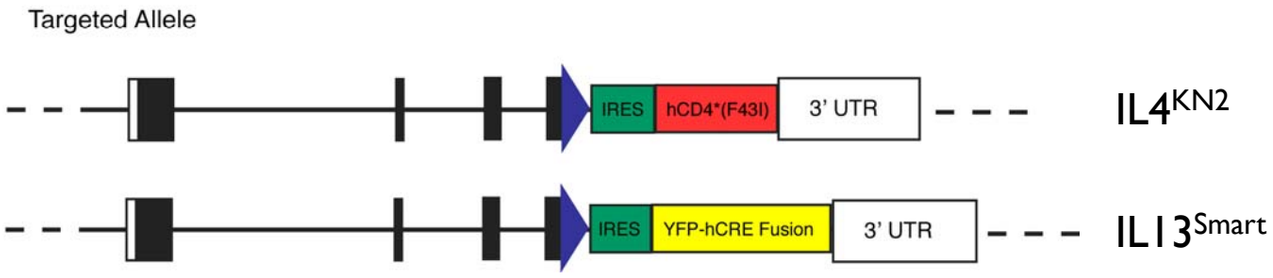
Immunized with NP-OVA in alum (footpad), day 28



Absence of IL-13-expressing TFH in germinal center reactions leading to CSR and high affinity?

Different compartmentalization (and function) of IL-4 and IL-13

Ig responses are dependent on IL-4-expressing cells

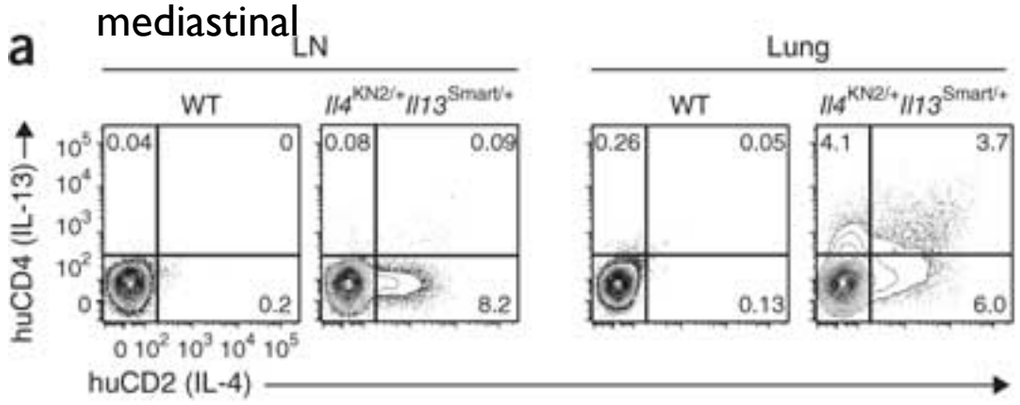


$IL4^{KN2}$

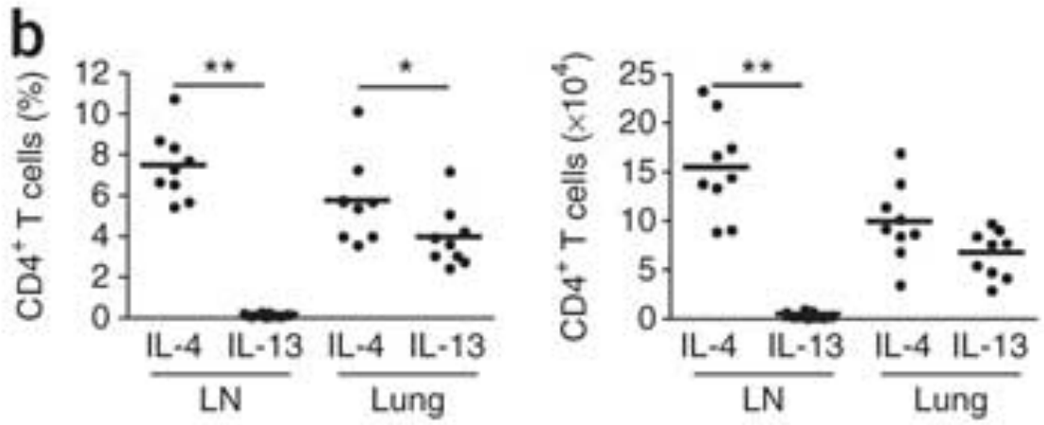
$IL13^{Smart}$

Supplementary Figure 4
Schematic for Smart13 (IL-13^{Smart}) and YetCre13 (IL-13^{YetCre}) alleles.

$IL4^{KN2/+}$x$IL13^{Smart/+}$



gated on CD4 T cells

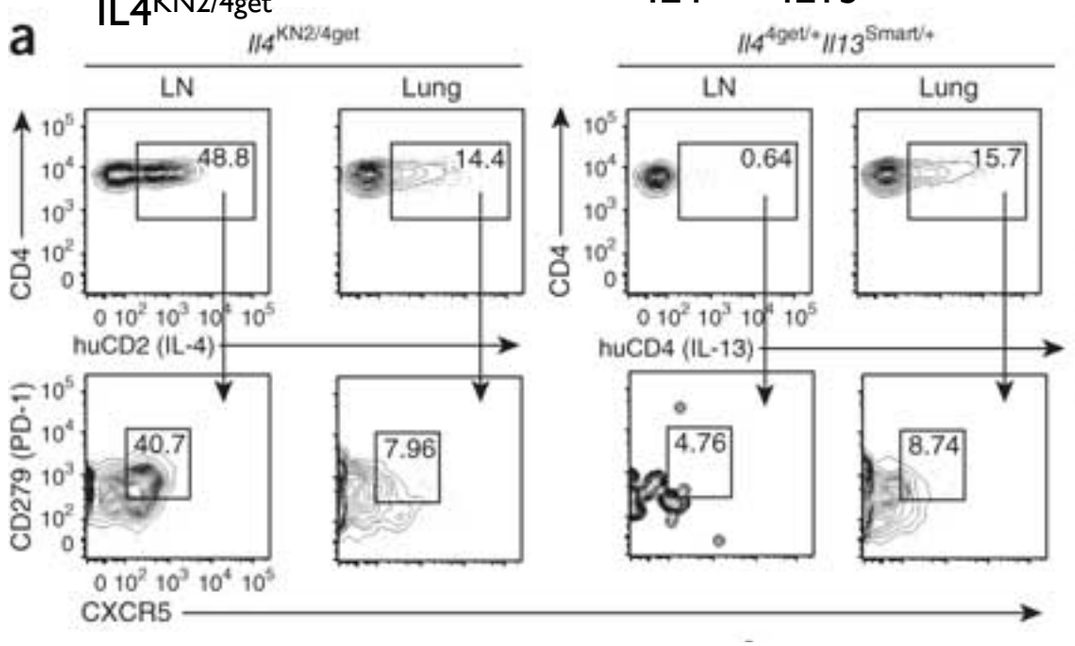


IL-4-producing CD4+ T cells are confined within the follicular and germinal center areas
as shown previously: Reinhardt, R., Liang, H., & Locksley, R. (2009). Cytokine-secreting follicular T cells shape the antibody repertoire. *Nature Immunology*)

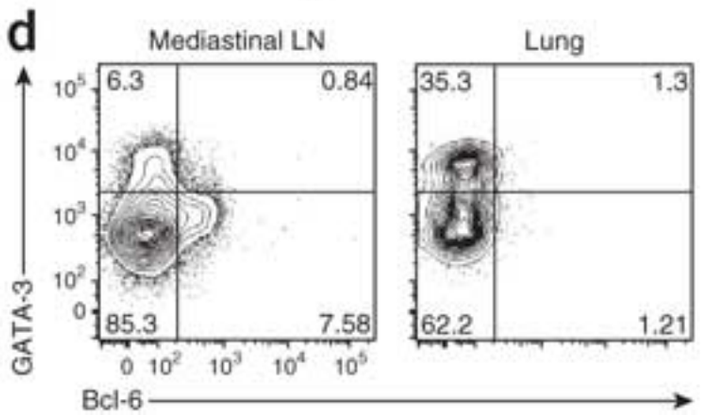
IL-4 and IL-13-expressing TFH and TH2 cells have distinct transcription factors and compartmentalization

Gated on eGFP+CD4+

IL4$^{KN2/4get}$ IL4$^{4get/+}$IL13$^{Smart/+}$



IL-13 expressing T cells do not express TFH cell markers

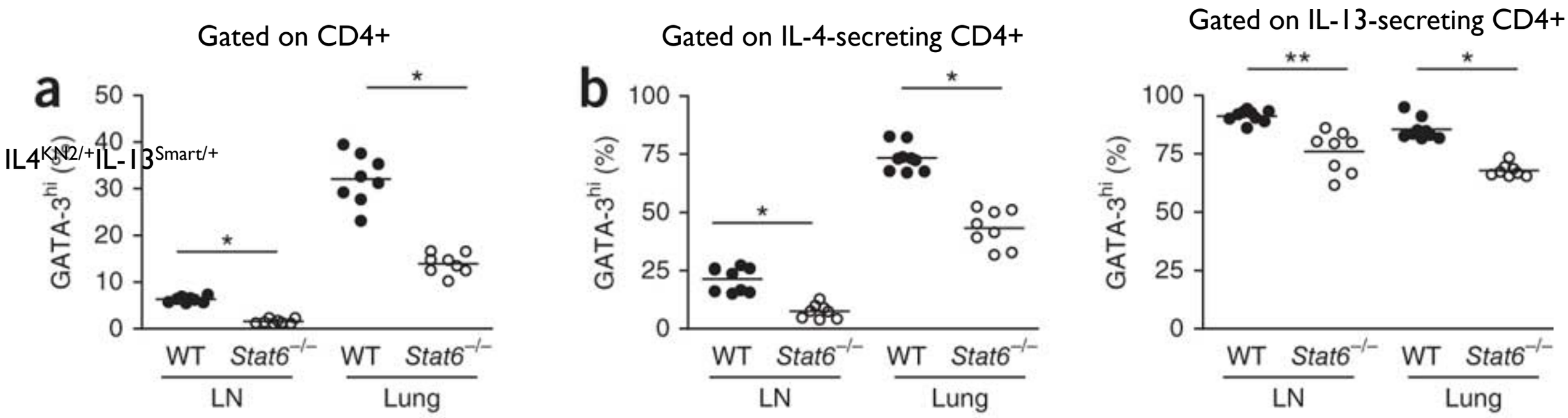


IL-4 expressing LN CD4 T cells are Bcl6+ and GATA-3 low (also confirmed by qPCR)

Impact of STAT6 deficiency on GATA-3 and cytokine expression?

Is cytokine signaling required for higher GATA3 expression and IL-13 production? STAT6-/- $IL4^{KN2/4get}$

N.Brasiliensis, d8



$IL4^{KN2/+}IL\text{-}13^{Smart/+}$

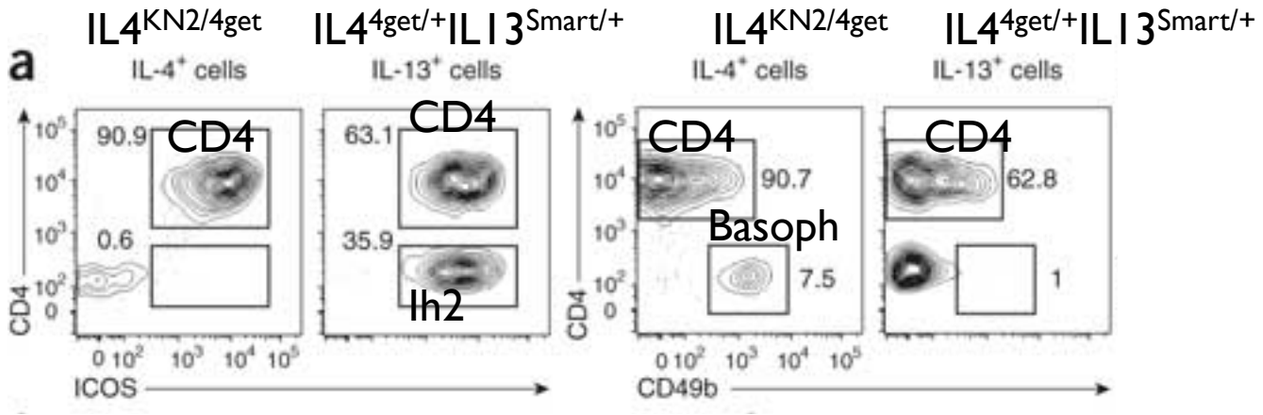
No difference in total numbers cytokine-producing CD4 T cells

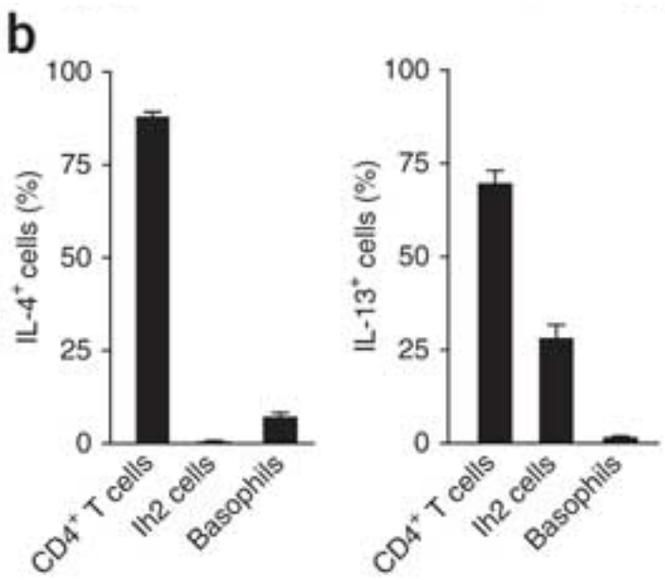
GATA-3 expression is optimized but not required by IL-4 and IL-13 signaling

Innate Type 2 cells

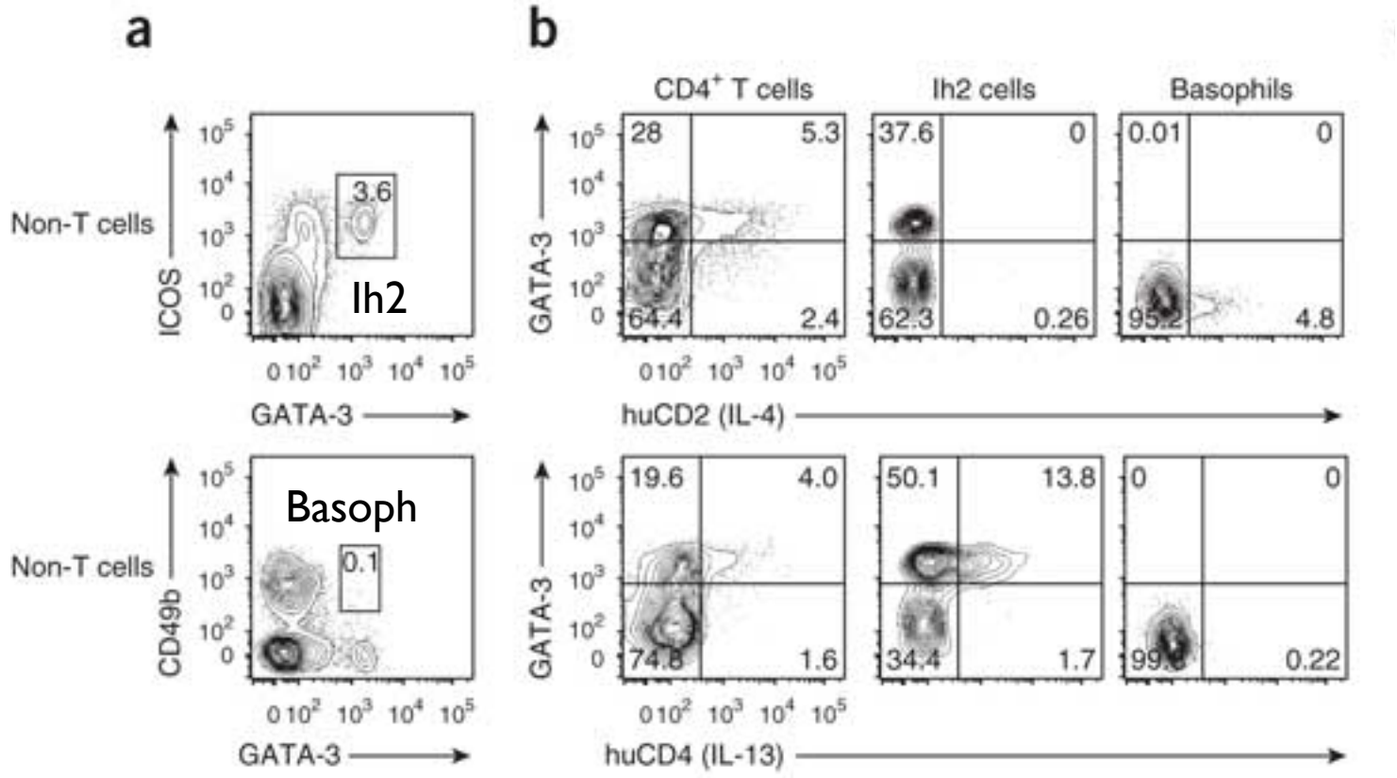
IL-4 competent cells: TH2, TFH, NKT, mast cells, basoph, eos, Ih2

9 d after N.brasiliensis, lung





IL-13-producing Ih2 cells express GATA-3



Ih2 cells express GATA-3

Using STAT6 ko
GATA-3 production and IL-13 production occur independently of STAT6 (but required for optimal numbers of Ih2)

Discussion/Summary

- New knock-in with surface surrogate markers to study IL-4 and IL-13 cytokine production
- High expression of Bcl6 in TFH may restrict GATA-3 expression and silence IL-13 production
- As TFH cells migrate to tissues, Bcl6 may decrease (and Blimp1 increased) which may favor the development of canonical Th2

	TFH, Basophils	TH2, Ih2
Cytokines	IL-4	IL-13 and/or IL-4 (Ih2 only IL-13)
Location	Germinal center, B cell follicles	Tissues
GATA-3 expression	do not require large amounts	high (STAT-6 independent)
Biological function	IgE production	peripheral immunity: worm expulsion, eos infiltration (deletion of IL-13 expressing cells and of GATA-3-expressing IL-13 cells)