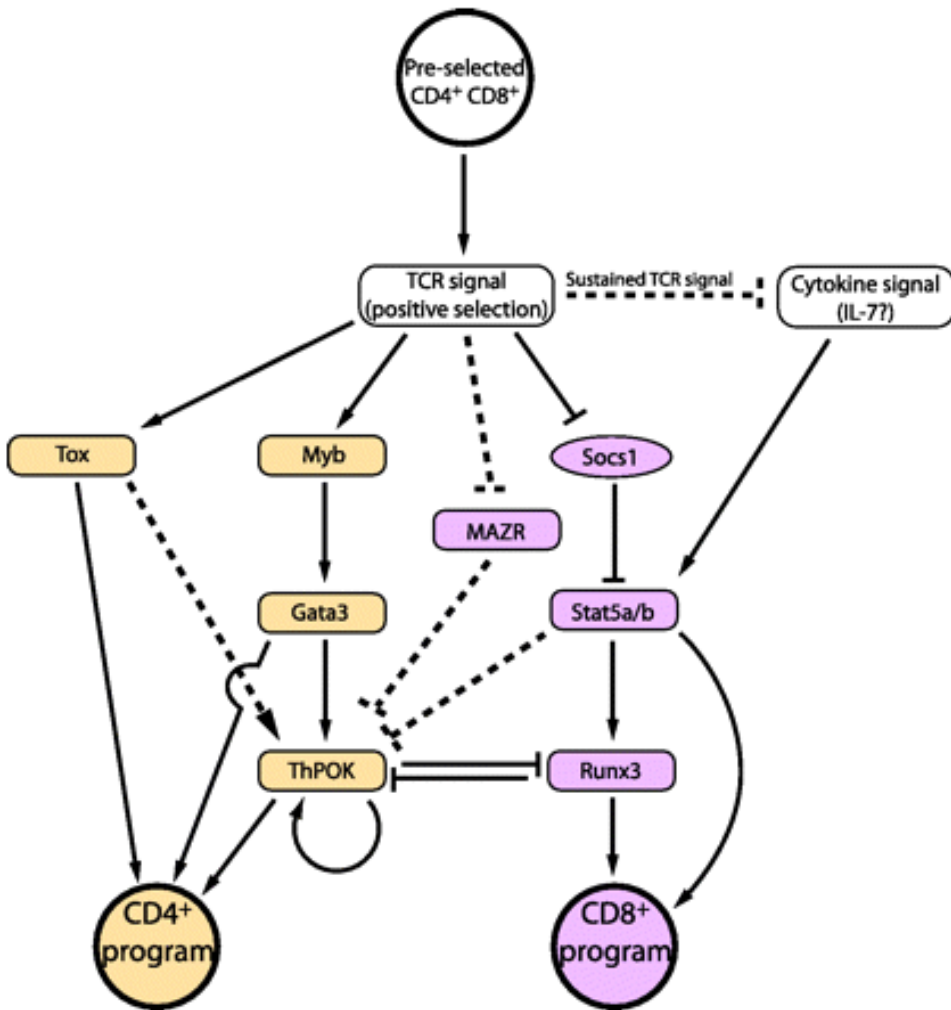


Mutual expression of the transcription factors Runx3 and ThPOK regulates intestinal CD4⁺ T cell immunity

Bernardo Sgarbi Reis¹, Aneta Rogoz¹, Frederico Azevedo Costa-Pinto^{1,2}, Ichiro Taniuchi³ & Daniel Mucida¹



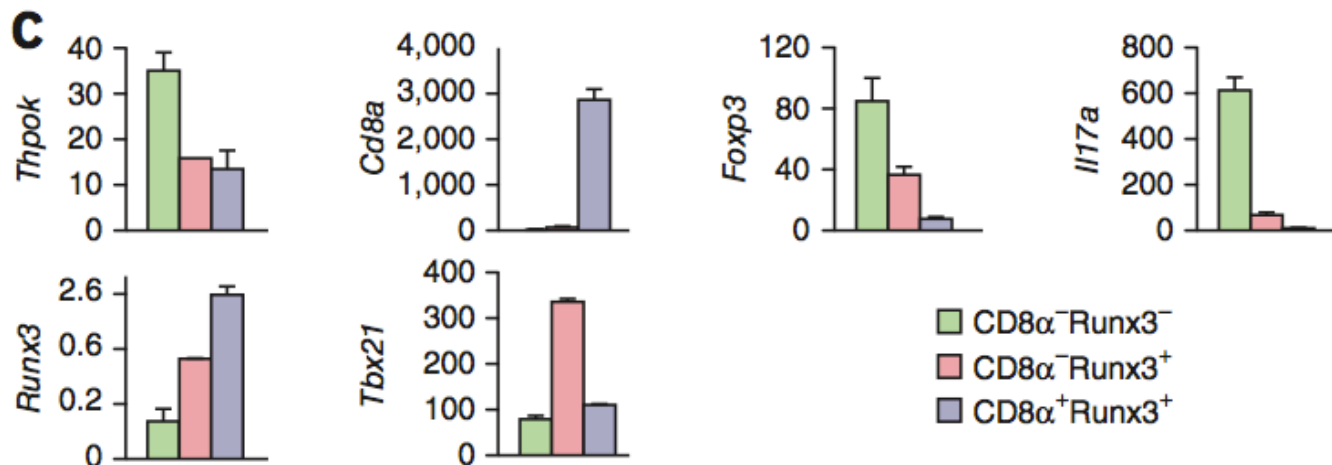
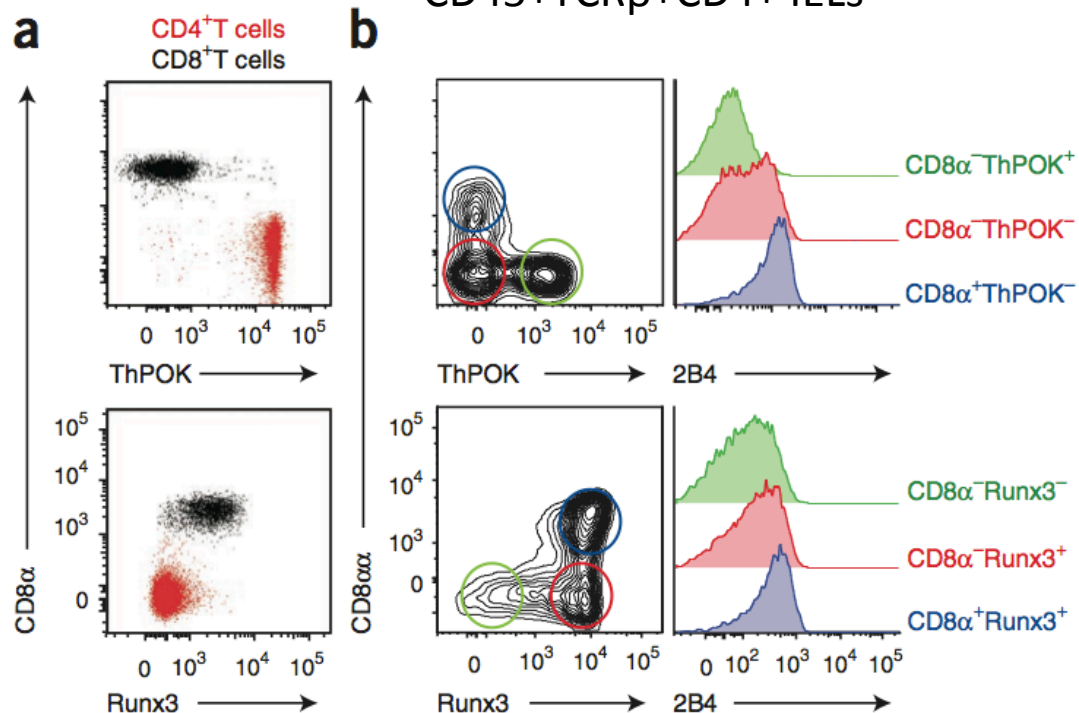
- The transcription factor ThPOK drives the development of CD4⁺ T cells from CD4⁺CD8⁺ double-positive precursors, whereas the development of CD8⁺ T cells requires mainly the transcription factor Runx3.
- ThPOK and Runx3 regulate each other's expression, and are mutually exclusive
- Peripheral mature CD4⁺ T cells and CD8⁺ T cells express ThPOK and Runx3, and are functional and stable as previously believed.
- The fate of T cells differentiating into the CD4 or CD8 lineage is typically fixed when cells leave the thymus

Splenocytes

Pre-gated on CD45+TCRβ+CD4+ IELs

Thpok-GFP knock-in

Runx3-YFP knock-in



CD45+TCRβ+CD4+ IELs
From *Runx3*-YFP
knock-in mice

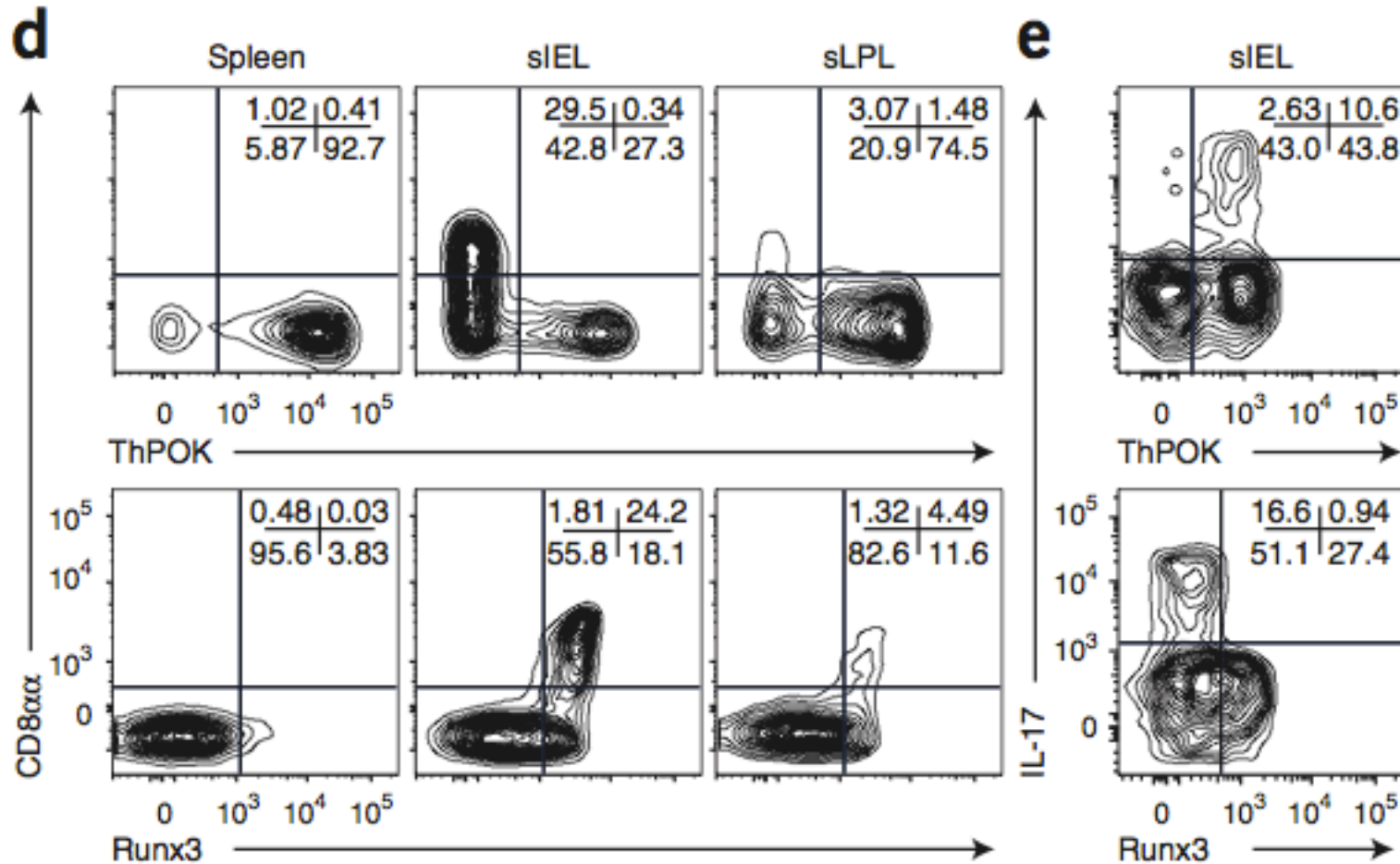
- CD4⁺ T cells in the gut are diverted into toward developing into CTLs or innate-like cytotoxic cells and lose the expression of ThPOK

T cell-transfer model of colitis

CD25⁻CD62L^{hi}CD4^{lo} from reporter mice to *Rag1*^{-/-} mice

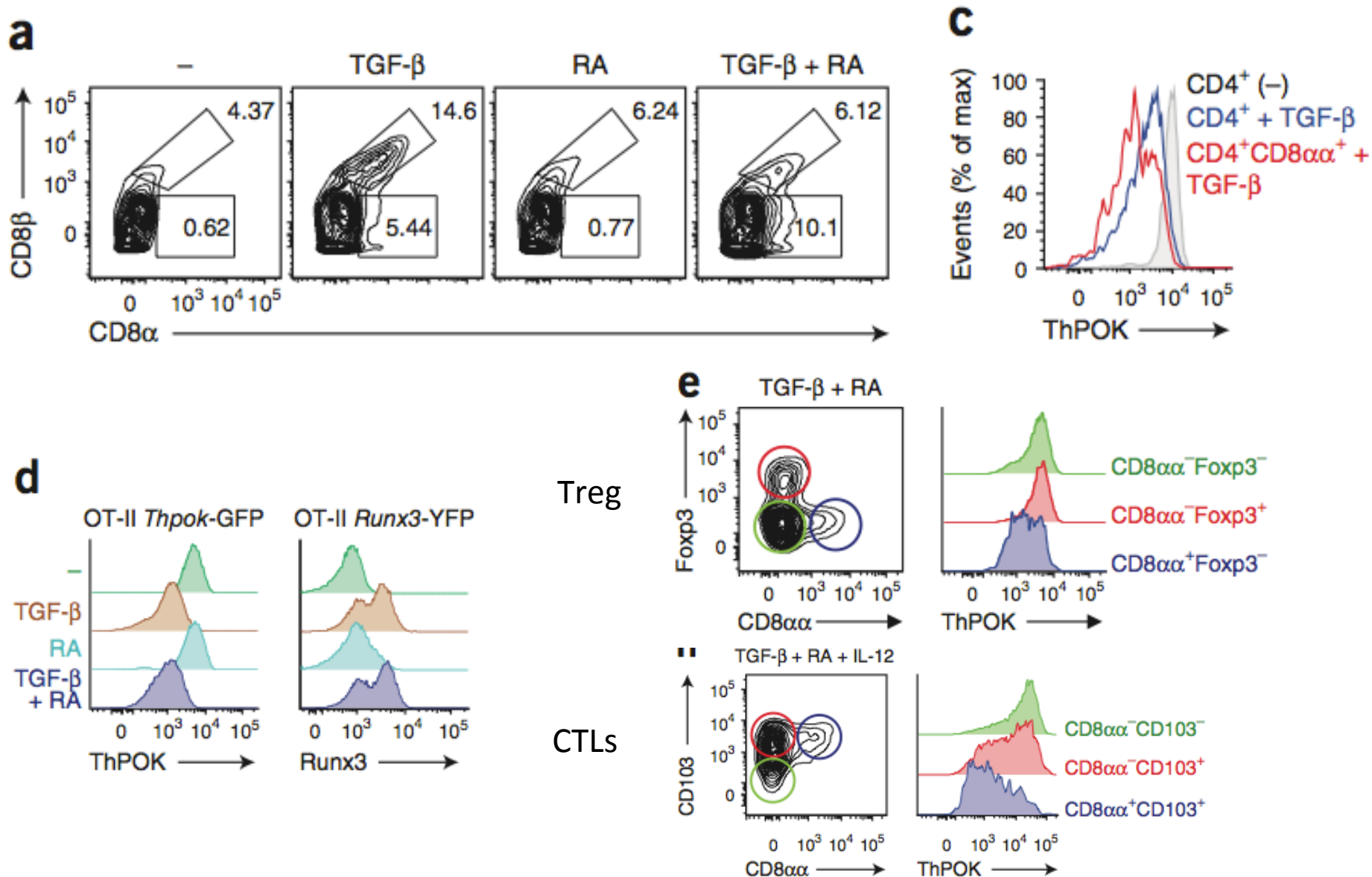
40 d after adoptive transfer

Gated on CD45 TCRβ CD4 cells



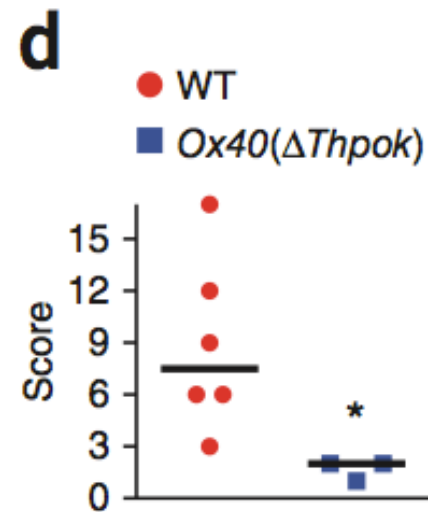
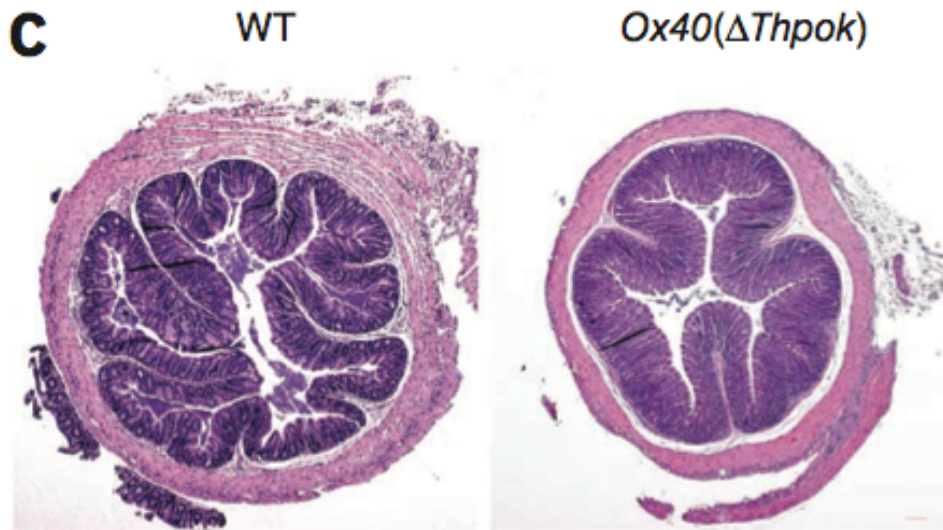
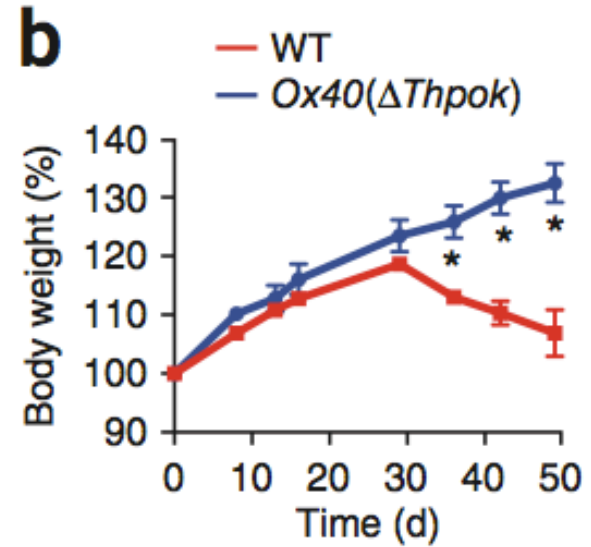
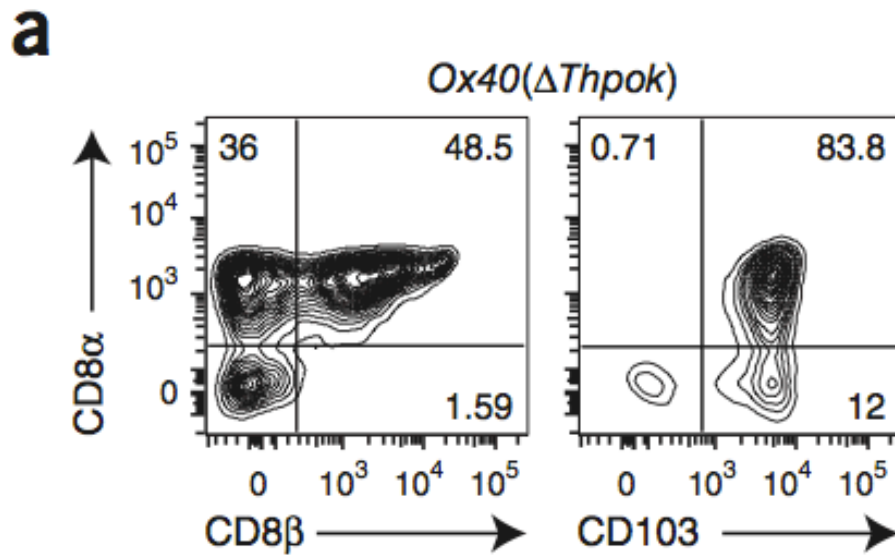
- Up-regulation of Runx3 expression in CD4⁺ T cells preferentially happens in the intraepithelial compartment via post-thymic alteration

$V\alpha 2^+CD4^+$ T cells isolated from OT-II *Thpok*-GFP reporter mice *in vitro*
 Co-cultured with DCs and OVA (-), or in the presence of cytokines



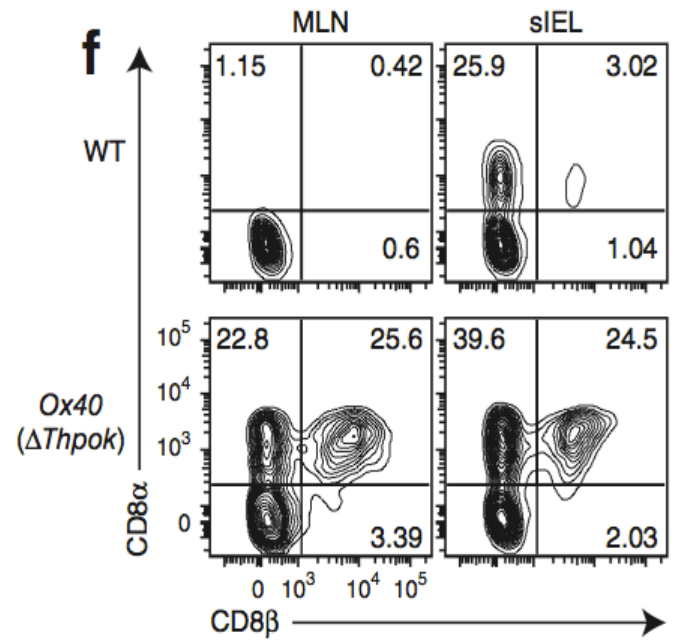
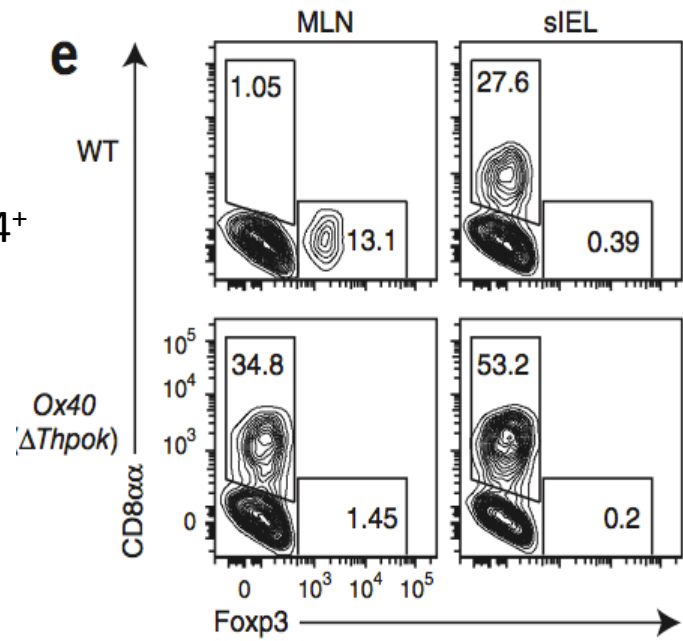
- Intestinal cues influence the terminal differentiation of $CD4^+$ T cells and the expression of ThPOK and Runx3

Rag1^{-/-} recipients transferred with naive CD4⁺ T cells

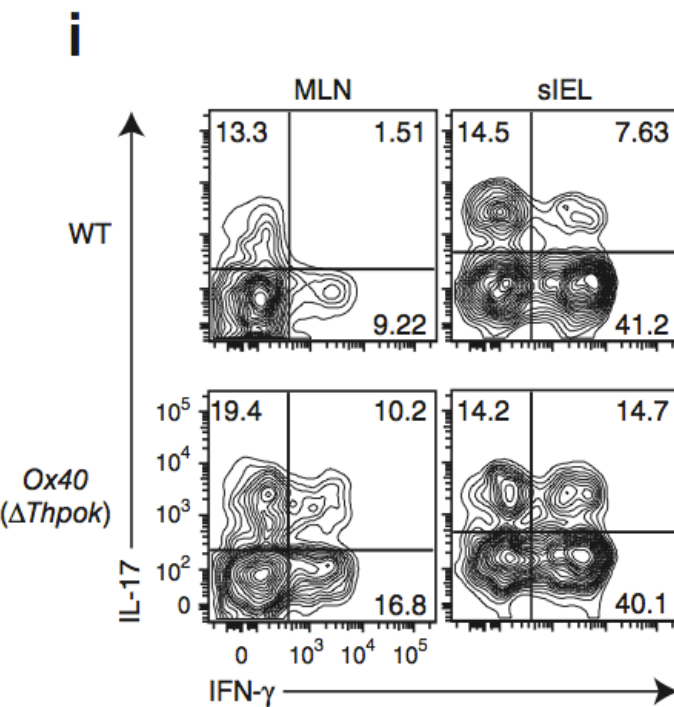
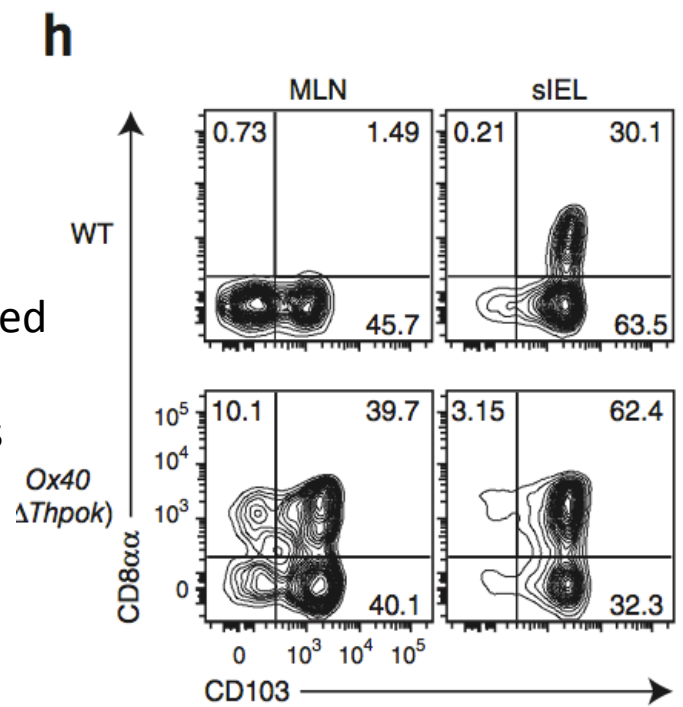


Loss of ThPOK by activated CD4⁺ T cells deminishes the development of colitis

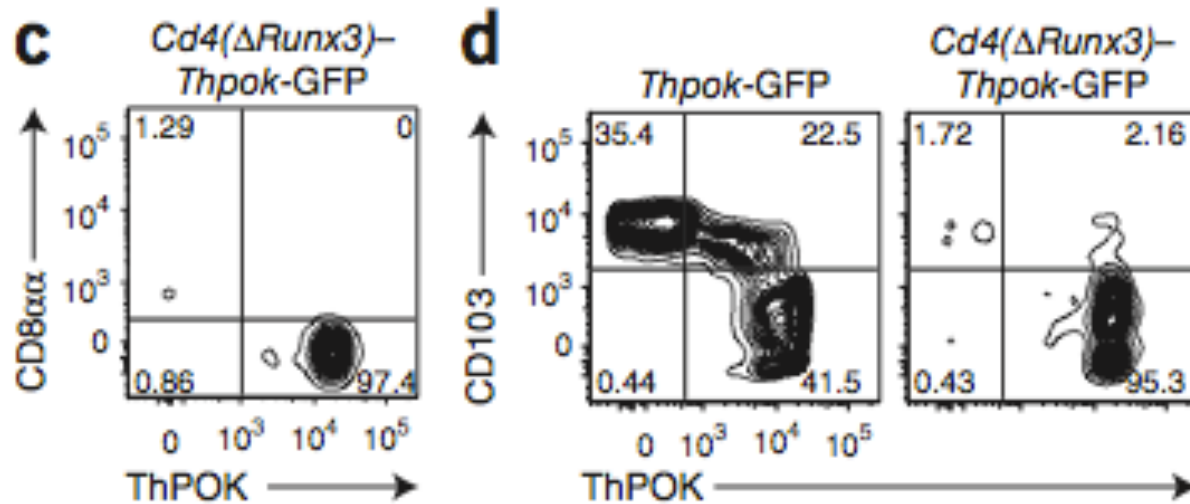
Gated on CD45⁺TCRβ⁺CD4⁺



Not because of Treg
Not because of IL-17
and IFN- γ , but the increased
proportion of CTLs or
innate-like cytotoxic cells

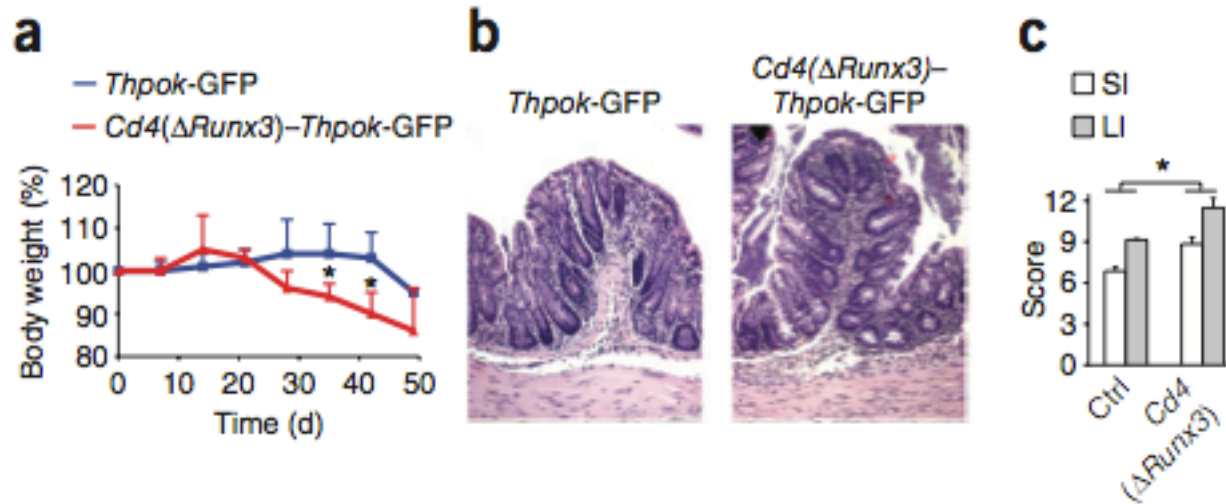


Expression of ThPOK, CD8 α and CD103 by CD45⁺TCR β ⁺CD4⁺ IELs isolated from the small intestine of a naive Thpok-GFP mouse

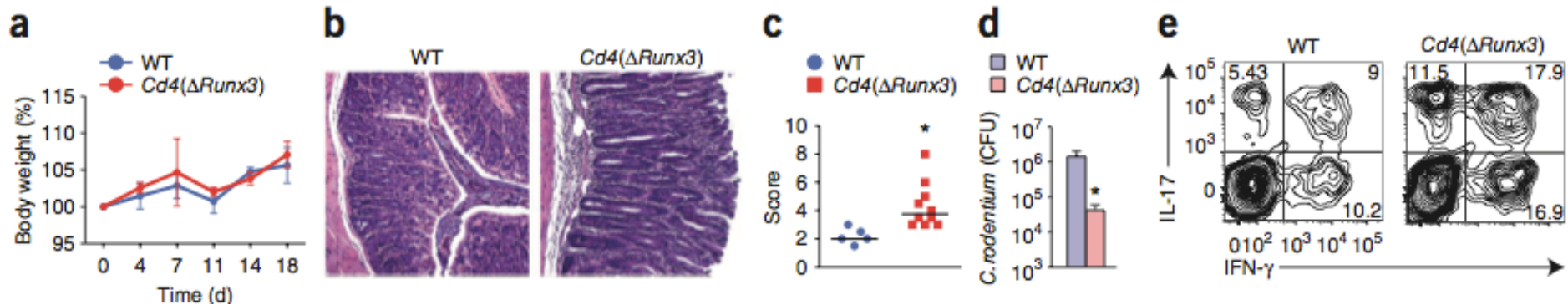


- Up-regulation of Runx3 expression precedes the down-modulation of ThPOK expression and the expression of CD8 α by intestinal CD4⁺ T cells

Naive CD4⁺ T cells isolated from spleen of *Thpok*-GFP or *Cd4(ΔRunx3)*-*Thpok*-GFP mice
 Transferred to *Rag1*^{-/-} mice



18 d after oral infection with *C. rodentium*



- Enhanced resistance of *Cd4(ΔRunx3)* mice to infection with *C. rodentium*.

Discussion

An alternative fate for CD4+ T cells that migrate to the intestinal tissue towards CTLs or innate-like lymphocytes

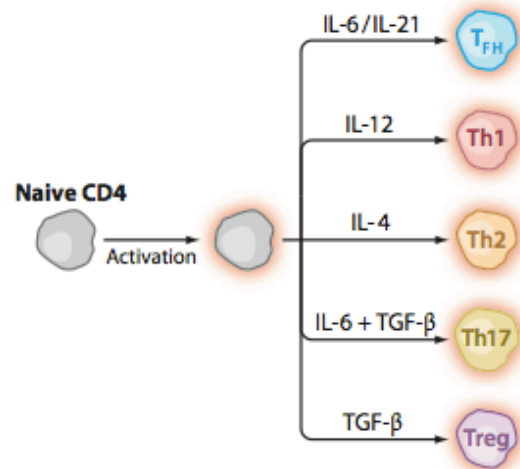
TGF- β and RA are required for a post-thymic suppression of ThPOK and upregulation of Runx3 expression

Plasticity of T_H17 cells in Peyer's patches is responsible for the induction of T cell–dependent IgA responses

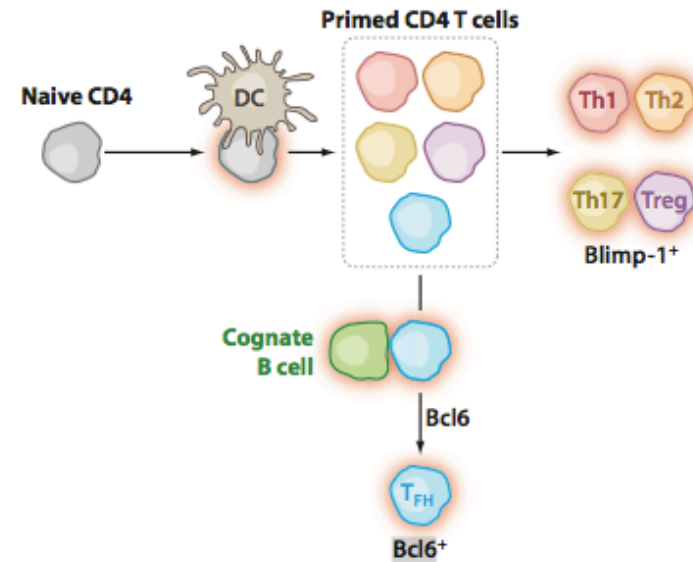
Keiji Hirota^{1,4}, Jan-Eric Turner^{1,5}, Matteo Villa^{1,5}, João H Duarte¹, Jocelyne Demengeot², Oliver M Steinmetz³ & Brigitta Stockinger¹

Three models for T_{FH} differentiation-Bcl6, CXCR5, PD-1 and ICOS

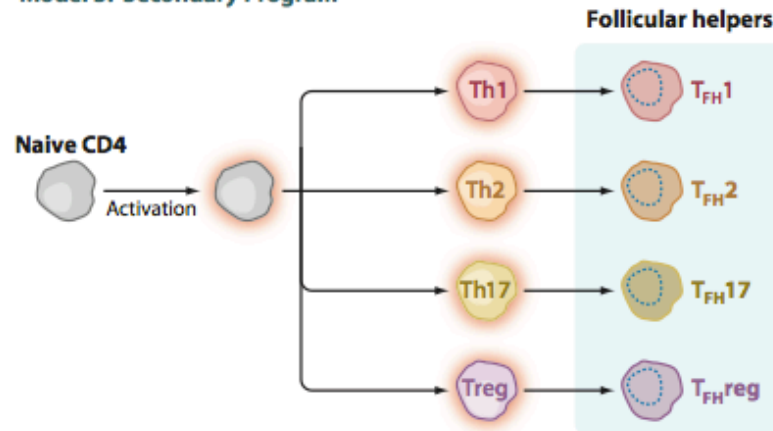
a Model 1: Direct T_{FH} differentiation via cytokine



b Model 2: B cell-dependent direct T_{FH} differentiation



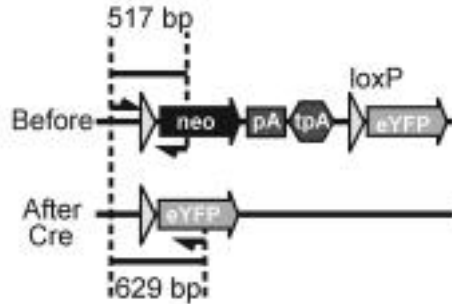
c Model 3: Secondary Program



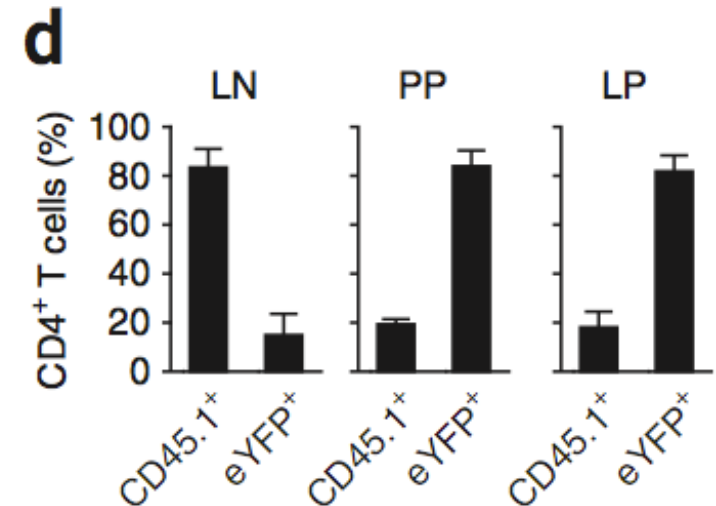
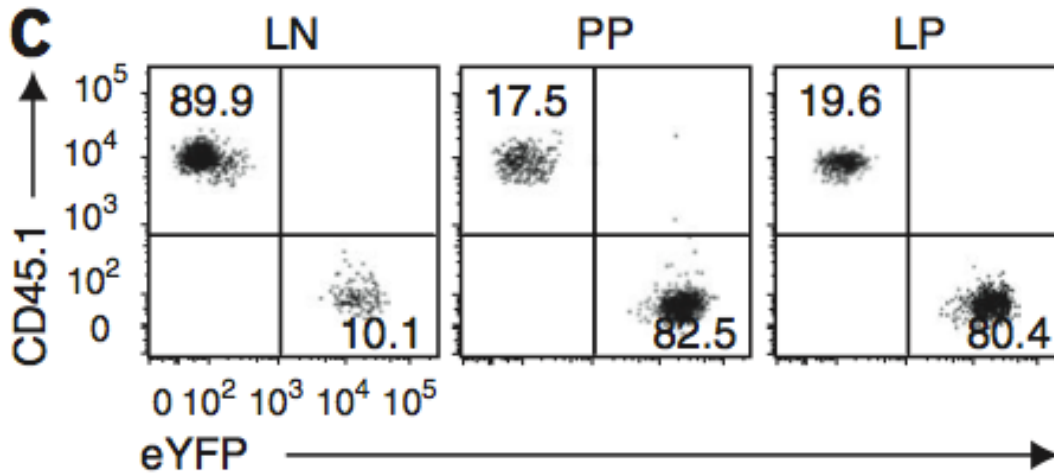
Follicular helper CD4 T cells (TFH). Crotty S et al. Annu Rev Immunol. (2011)

IL-17 fate-reporter (*Il17aCreR26ReYFP*) mice

Il17aCre knockin mice

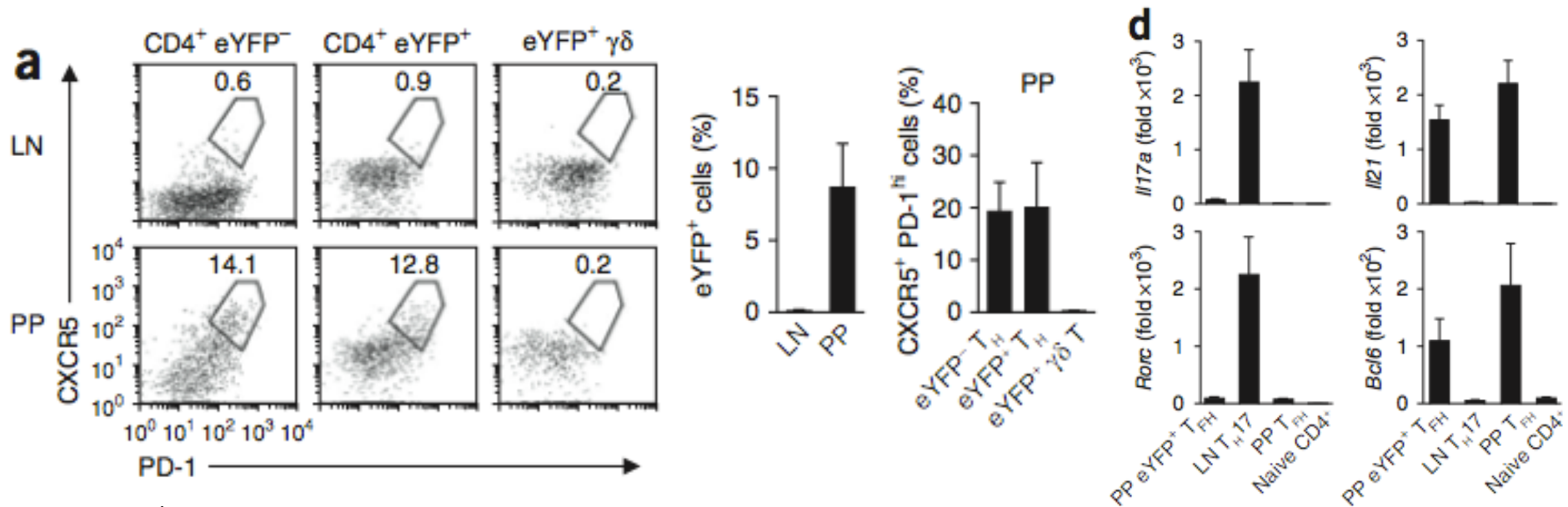


SPF *Tcra*^{-/-} mice reconstituted with CD4 eYFP TH17 cells (eYFP⁺) or CD45.1⁺eYFP⁻CD44^{hi}CD4⁺ T cells (CD45.1⁺), assessed 3 months after transfer.

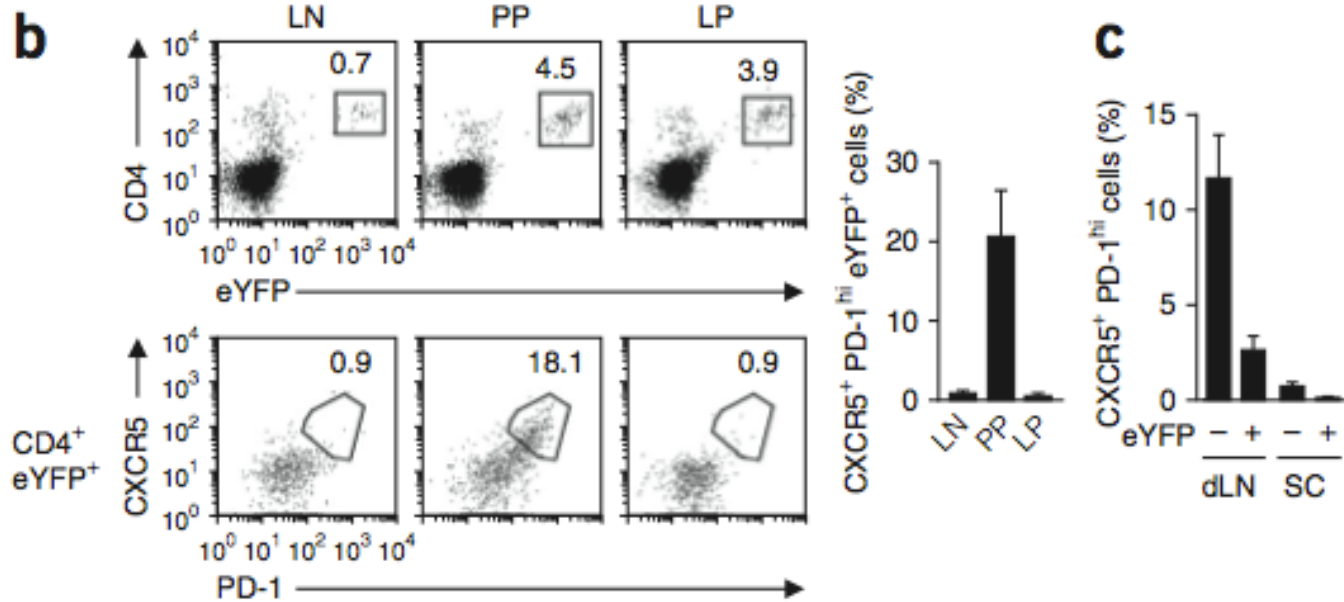


- 'Preferential' migration of eYFP⁺ TH17 cells into gut-associated tissues.

Il17aCreR26ReYFP mice

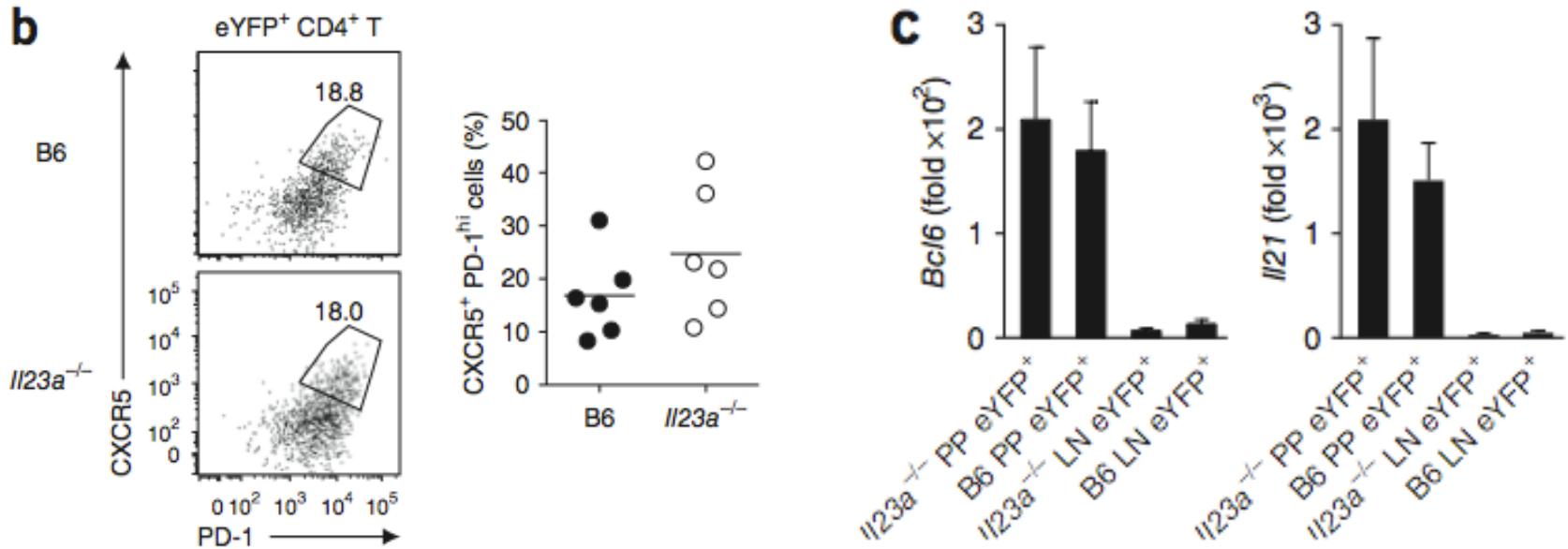


Tcra^{-/-} mice 3 months after transfer of eYFP⁺ TH17 cells.

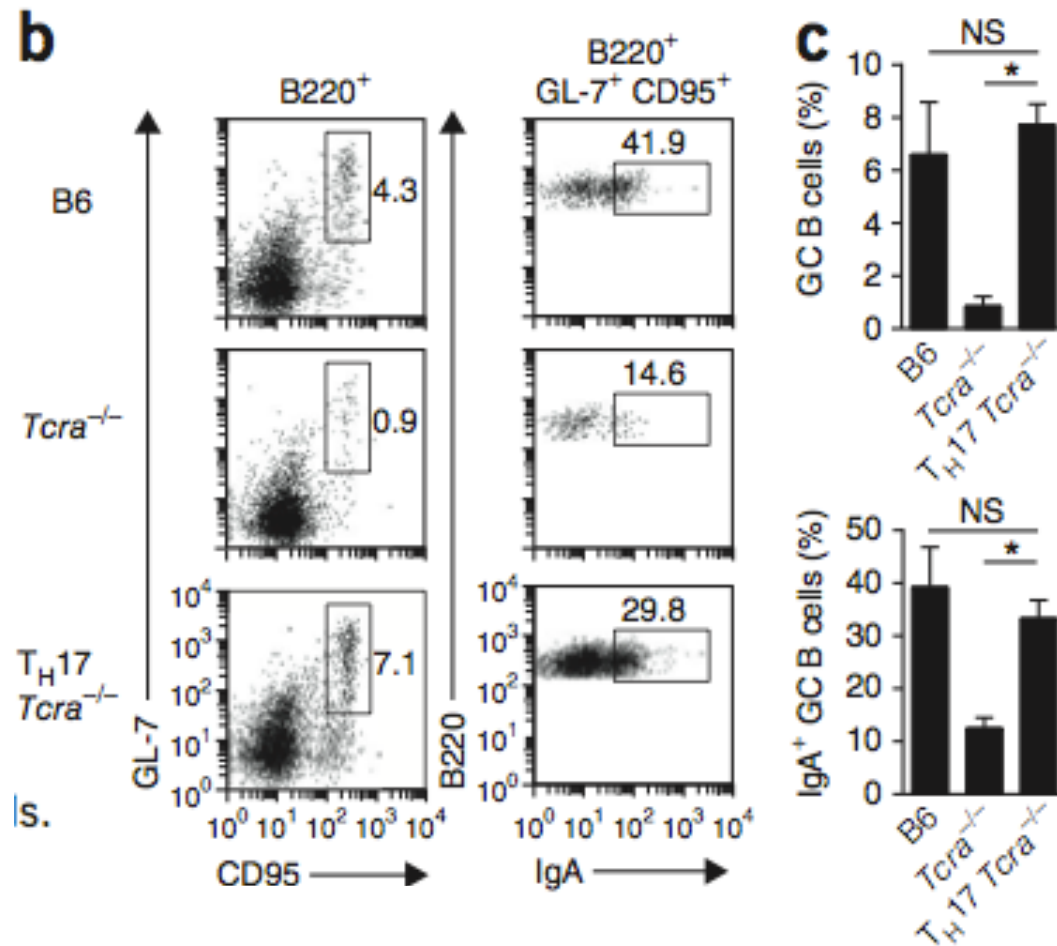


- Reprogramming of TH17 cell profiles to a TFH cell phenotype in PP.

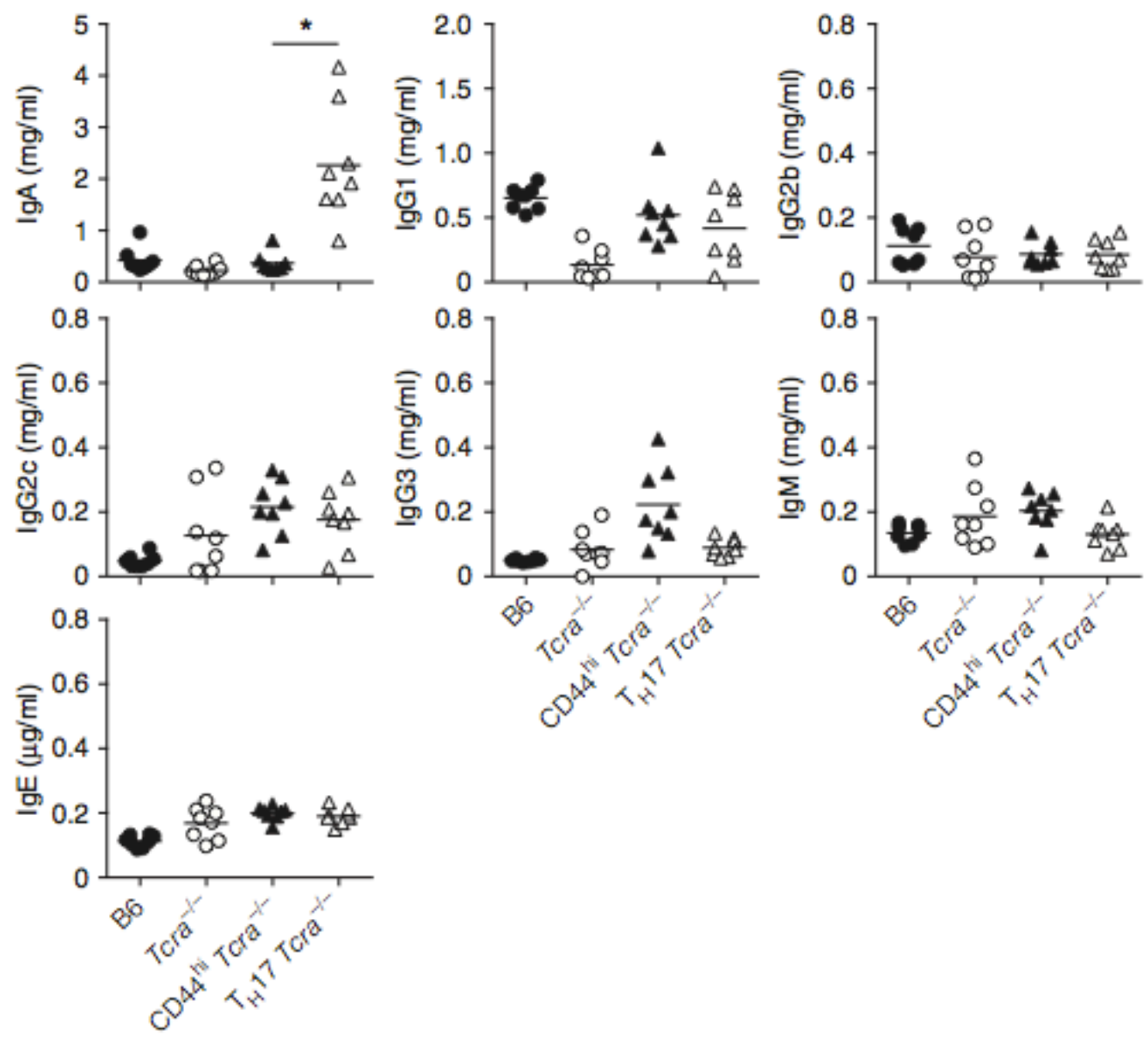
IL23a^{+/+} and *IL23a*^{-/-} *IL17aCreR26ReYFP* mice (*IL23a*^{-/-}).



- IL-23 is dispensable for the homeostatic maintenance and plasticity of intestinal TH17 cells.

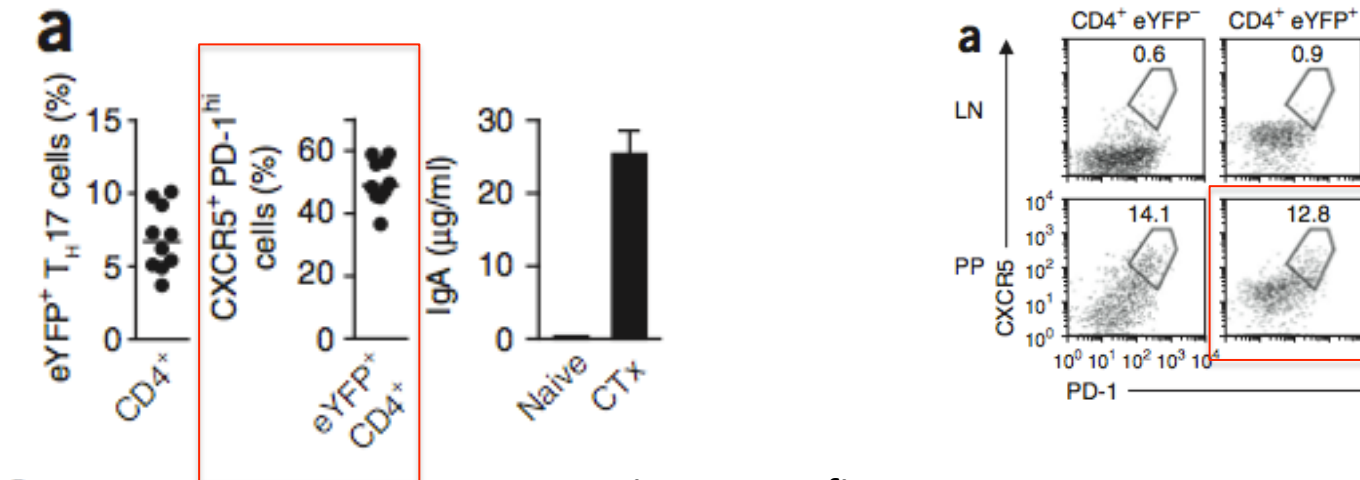


- 'Ex-TH17' cells in PP induce IgA production by GC B cells

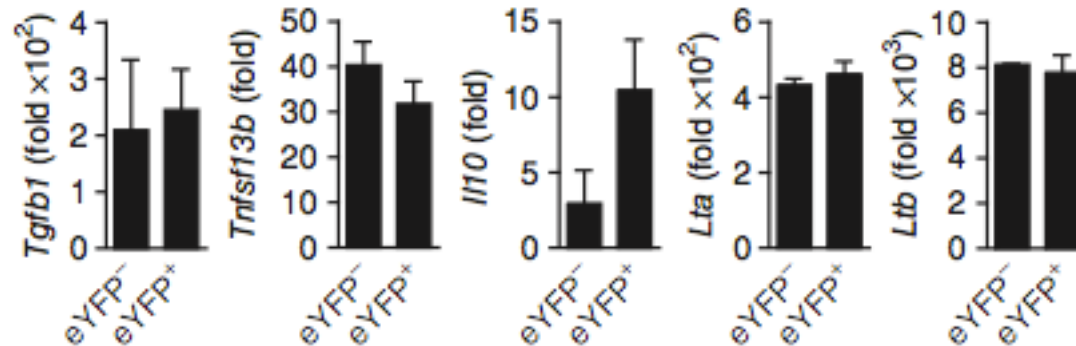
d

I17aCreR26ReYFP mice

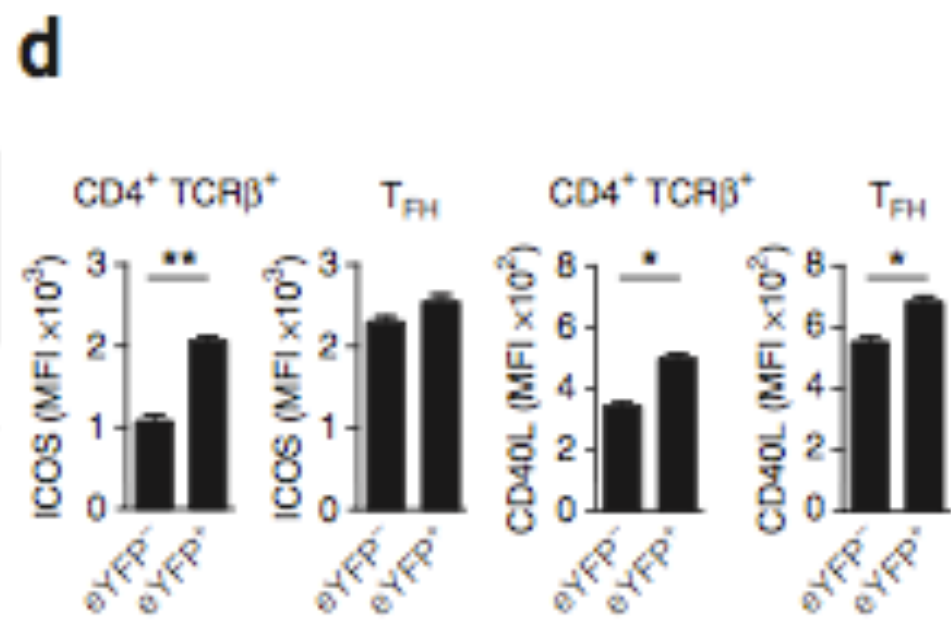
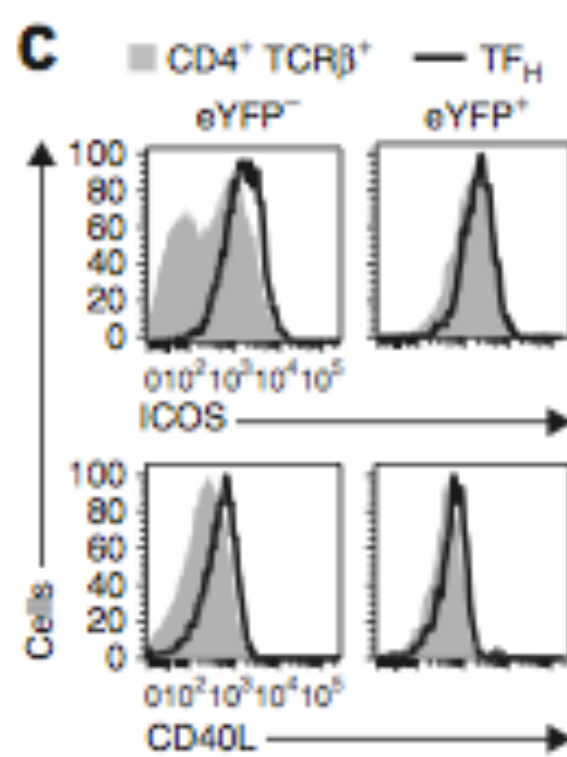
7–10 d after immunization with cholera toxin (CTx).



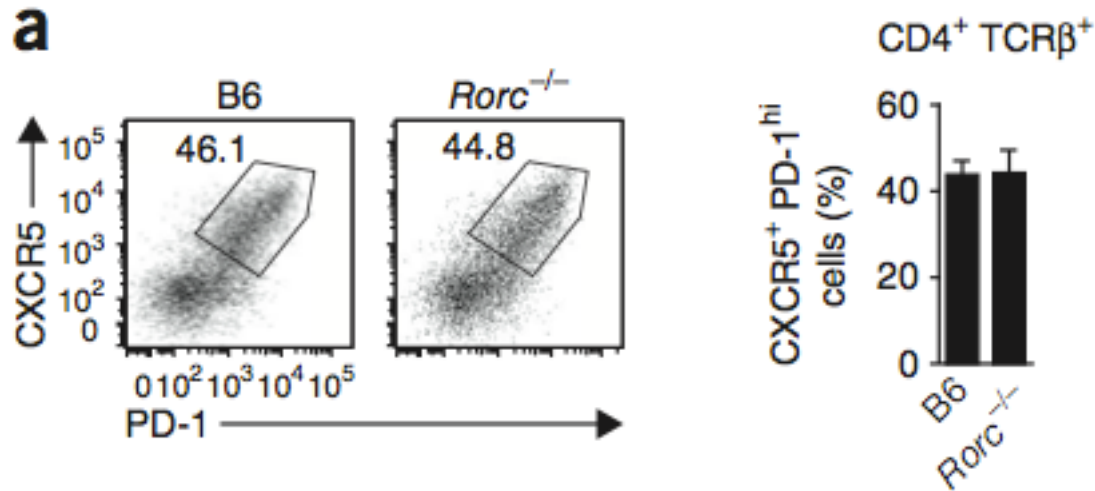
b CTx antigen specific ELISA



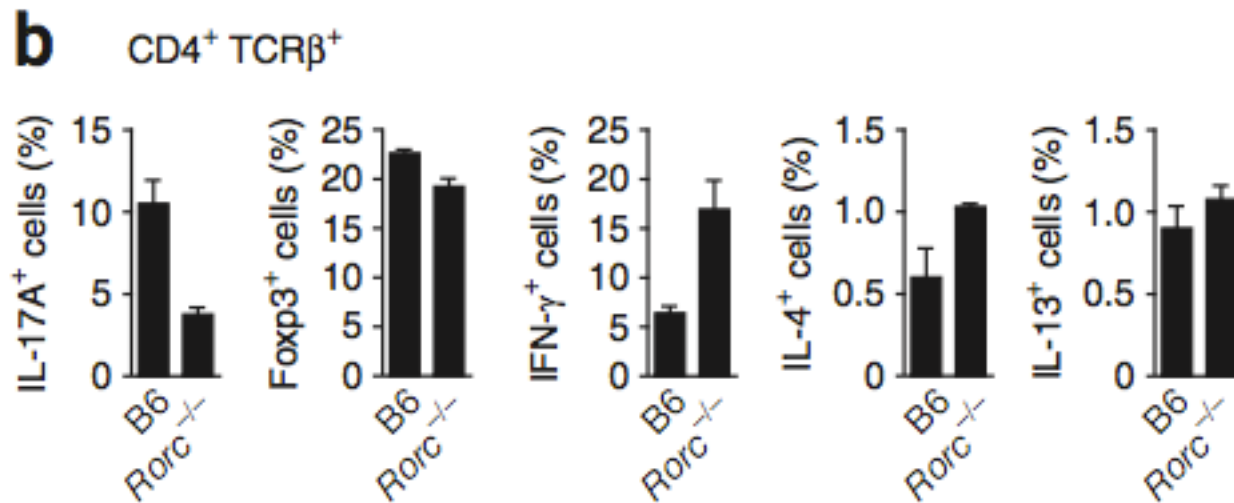
- Markers associated with class switching to IgA in eYFP⁻ and eYFP⁺ T cells from PP of cholera toxin-immunized mice were not different

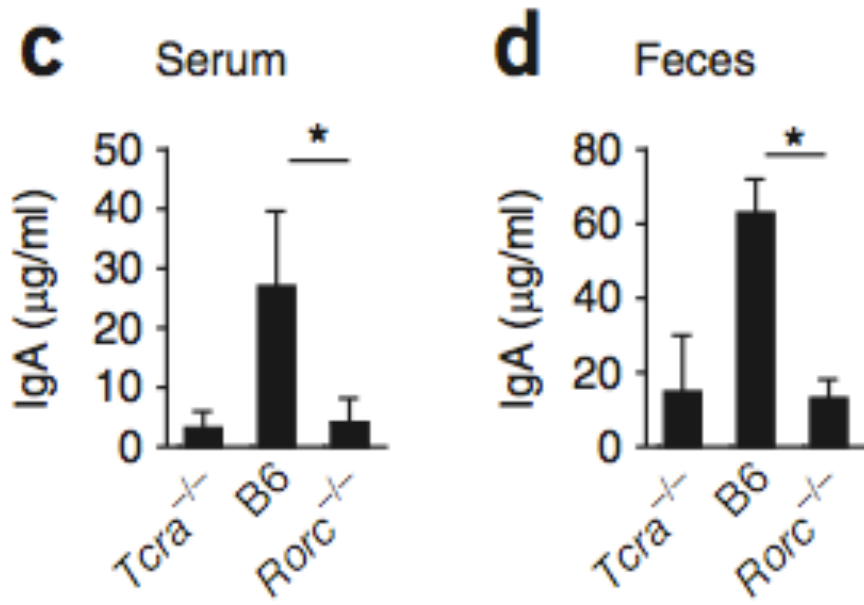


Tcr α ^{-/-} hosts reconstituted with whole bone marrow from ROR γ t-deficient (*Rorc*^{-/-}) or B6 donor mice

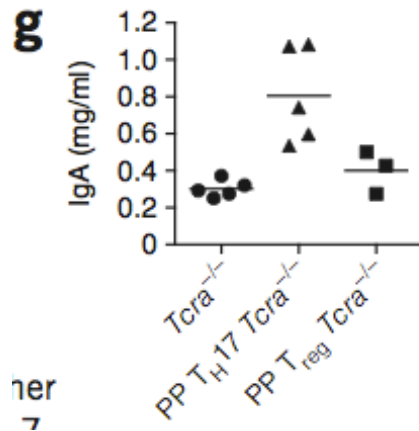
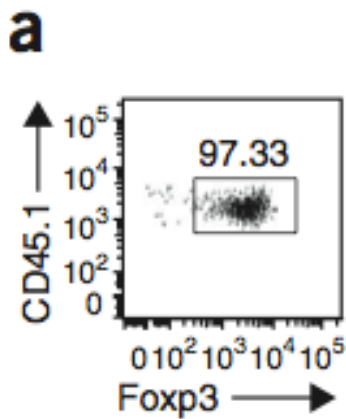


PP CD4⁺ T cells

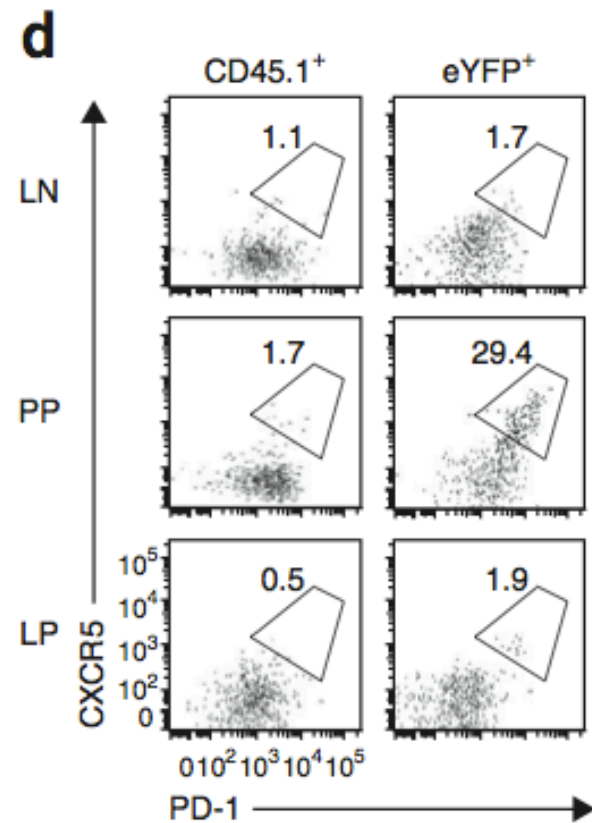
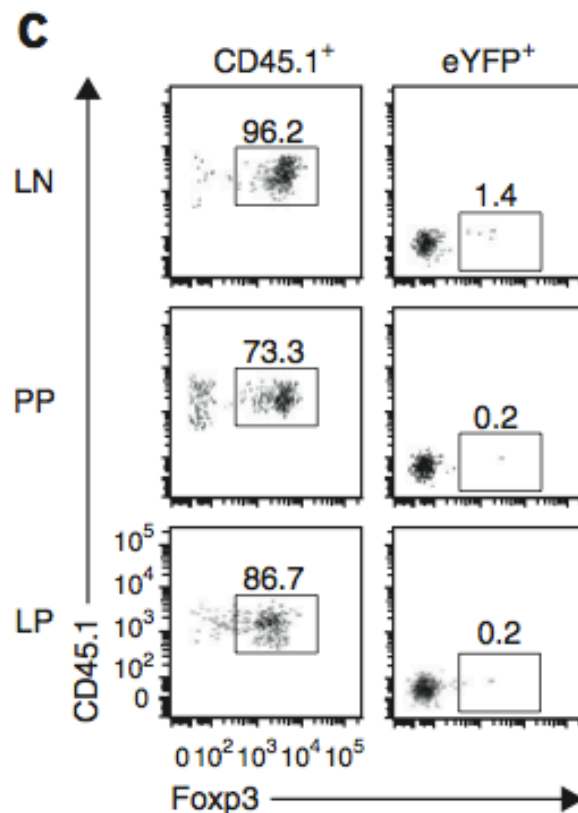
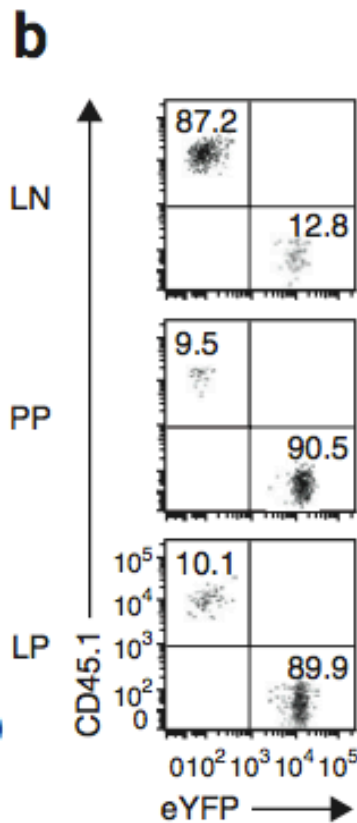




- The cholera toxin-specific IgA response requires TH17 cells.



Tsuji, M. *et al.* Preferential generation of follicular B helper T cells from Foxp3⁺ T cells in gut Peyer's patches. *Science* **323**, 1488–1492 (2009).



- Regulatory T cells are not associated with induction of IgA

Discussion

The plasticity of TH17 cells in developing toward a TFH cell program in the environment of the small intestine PP

Antigen-specific IgA responses to challenged cholera toxin depended on the presence of TH17 cells

Luminal Bacteria Recruit CD103⁺ Dendritic Cells into the Intestinal Epithelium to Sample Bacterial Antigens for Presentation

Julia Farache,¹ Idan Koren,¹ Idan Milo,¹ Irina Gurevich,¹ Ki-Wook Kim,^{1,3} Ehud Zigmond,¹ Glaucia C. Furtado,² Sergio A. Lira,² and Guy Shakhar^{1,*}

¹Department of Immunology, Weizmann Institute of Science, Rehovot 76100, Israel

²Immunology Institute, Mount Sinai School of Medicine, New York, NY 10029, USA

³Present address: Department of Microbial Pathogenesis, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, CT 06510, USA

*Correspondence: shakhar@weizmann.ac.il

<http://dx.doi.org/10.1016/j.immuni.2013.01.009>

Parents
Cd11c-YFP × *Cx3cr1*^{gfp/gfp}

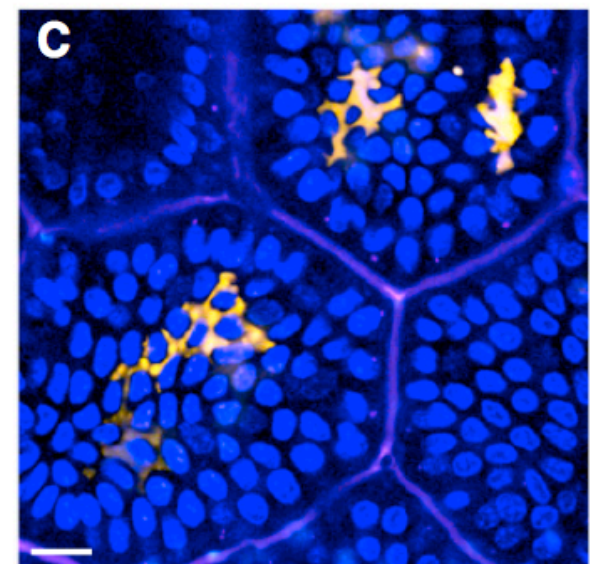
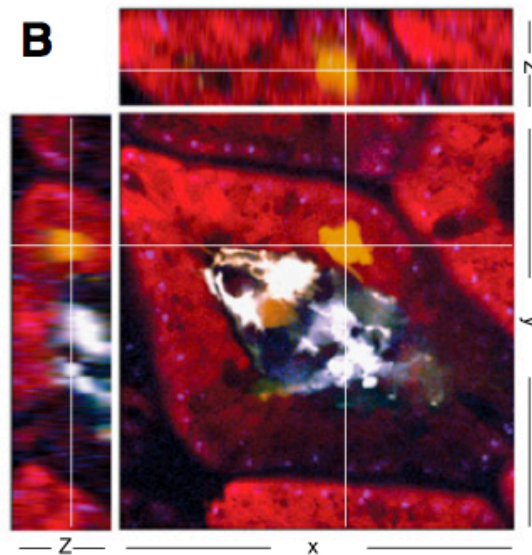
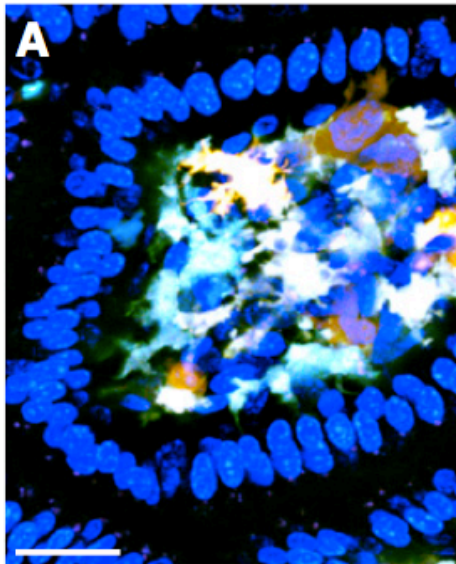
Cx3cr1^{+gfp}/*Cd11c*^{-YFP}

Transgenic mice DCs expressed enhanced yellow fluorescence
 GFP knock-in mice

Cx3cr1^{gfp/+}

CD11c-YFP *Cx3cr1*^{gfp/+}
 CD11c-YFP *Cx3cr1*^{+/+}

YFP+GFP-	CD103+ DCs
YFP+GFP+	CX3CR1+ macrophages
GFP+YFP-	Not discussed



■ Nuclei (Hoechst)
 ■ YFP⁺ cells
 ■ YFP⁺ GFP⁺ cells
 ■ Epithelial cells (CMTMR)

