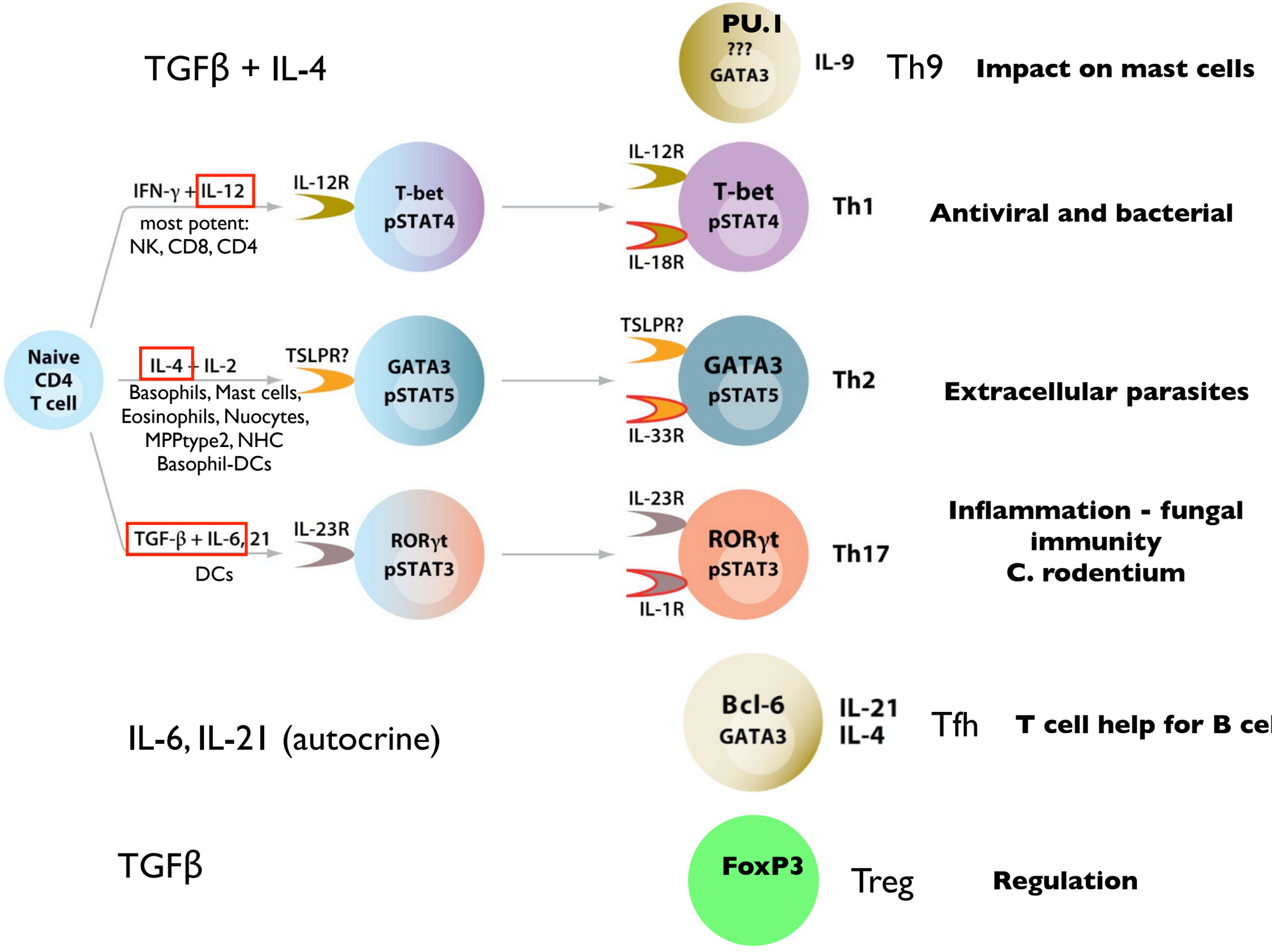
ntification of an innate T helper type 17 response to estinal bacterial pathogens

u Geddes[1,5], Stephen J Rubino[2,5], Joao G Magalhaes[1], Catherine Streutker[3], Lionel Le Bourhis[1], Ho Cho[1], Susan J Robertson[1], Connie J Kim[4], Rupert Kaul[1,4], Dana J Philpott[1] & Stephen E Girardin[2]


TGFβ + IL-4
PU.1
???
GATA3
IL-9
Th9
Impact on mast cells
IFN-γ + IL-12
most potent:
NK, CD8, CD4
IL-12R
T-bet
pSTAT4
IL-12R
T-bet
pSTAT4
IL-18R
Th1
Antiviral and bacterial
Naive
CD4
T cell
IL-4 + IL-2
Basophils, Mast cells,
Eosinophils, Nuocytes,
MPPtype2, NHC
Basophil-DCs
TSLPR?
GATA3
pSTAT5
TSLPR?
GATA3
pSTAT5
IL-33R
Th2
Extracellular parasites
TGF-β + IL-6, 21
DCs
IL-23R
RORγt
pSTAT3
IL-23R
RORγt
pSTAT3
IL-1R
Th17
Inflammation - fungal
immunity
C. rodentium
IL-6, IL-21 (autocrine)
Bcl-6
GATA3
IL-21
IL-4
Tfh
T cell help for B cells
TGFβ
FoxP3
Treg
Regulation

Models

- ***C. rodentium*–induced colitis:** robust colonic Th17 response at 2 weeks after infection

- **Streptomycin-pretreated mice infected with *S. typhimurium*** develop an acute inflammatory response in the cecum, with IL-17 produced early (24-48 h) by γδ T cells and other unidentified cells

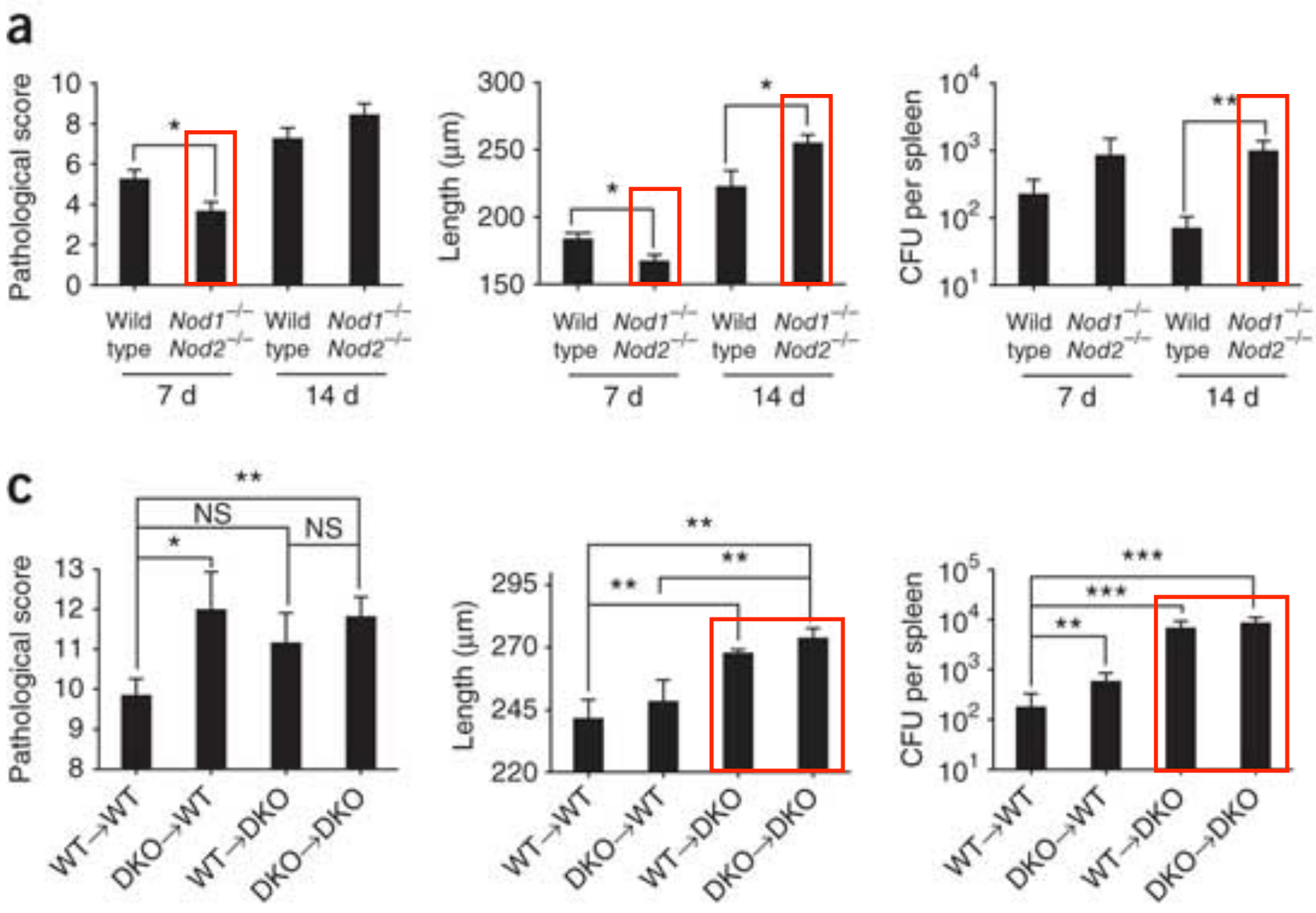
- Both early (innate) and late (adaptive) IL-17 production in intestinal infections

- **What are the innate immune receptors involved in early IL-17 production?**

- **What are the cells producing early IL-17?**

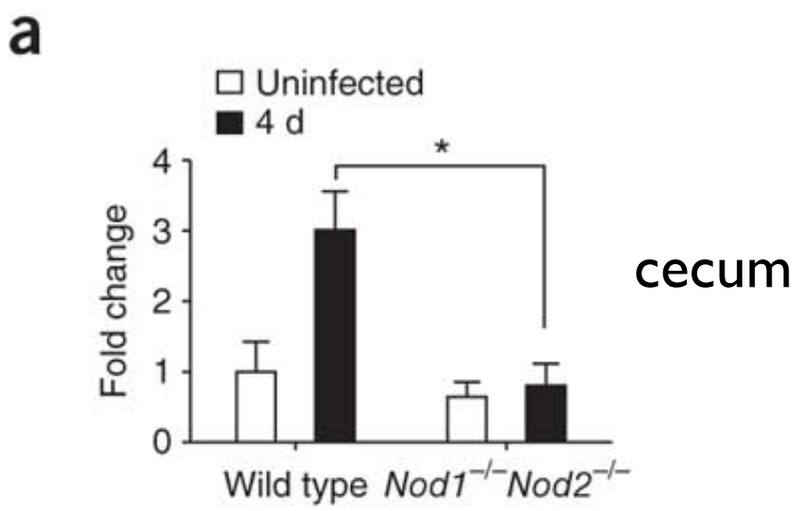
Nod1 and Nod2 are involved in early inflammation

C.rodentium infection (1×10^9 CFU)



- Nod1-/-Nod2-/- have less colonic inflammation 7d after *C.rodentium* infection
- Both Nod signaling in radio-resistant and radio-sensitive cells is required for control of infection

Early IL-17 responses are Nod1 and Nod2 dependent



cecum

colon

Nod signaling does not modulate TH17 adaptive responses

cecum

anti-microbial proteins

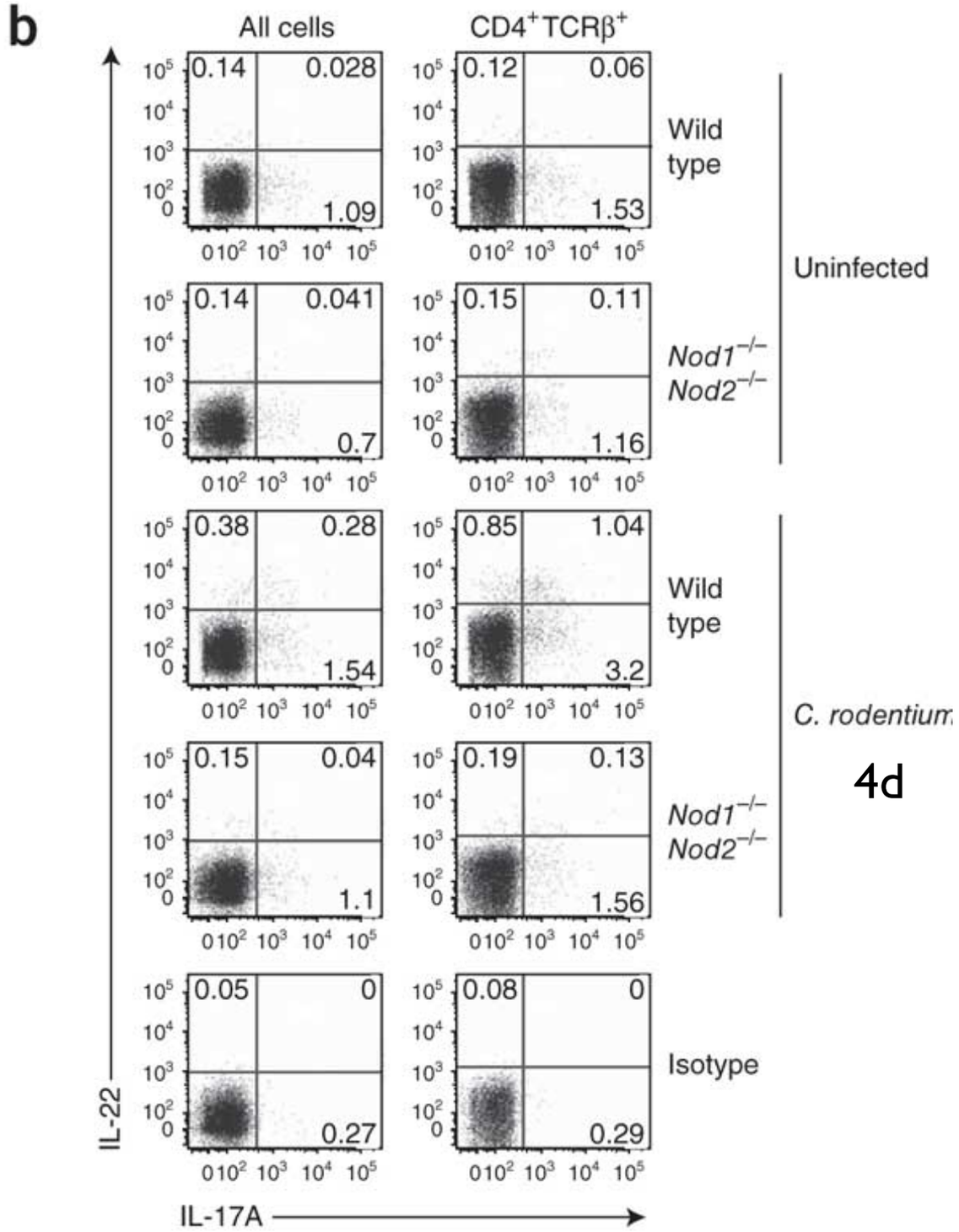
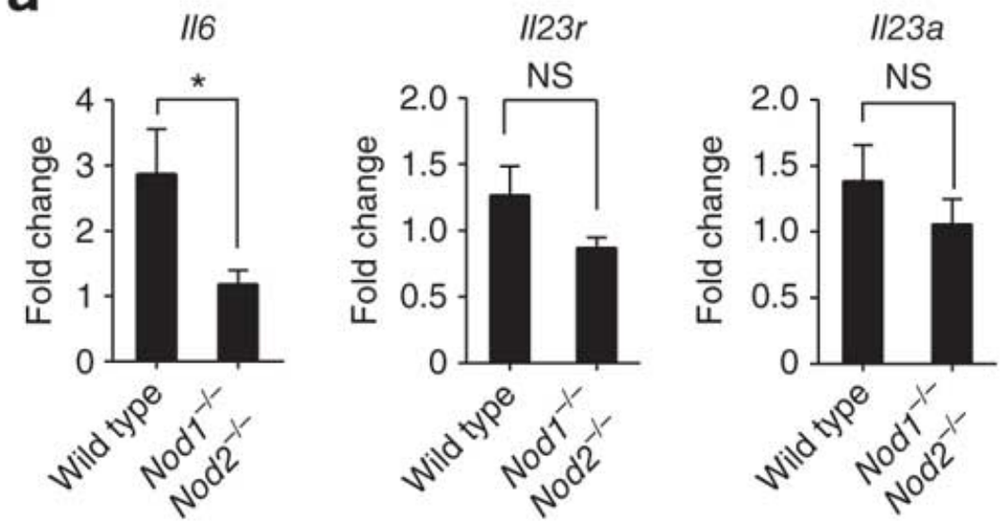


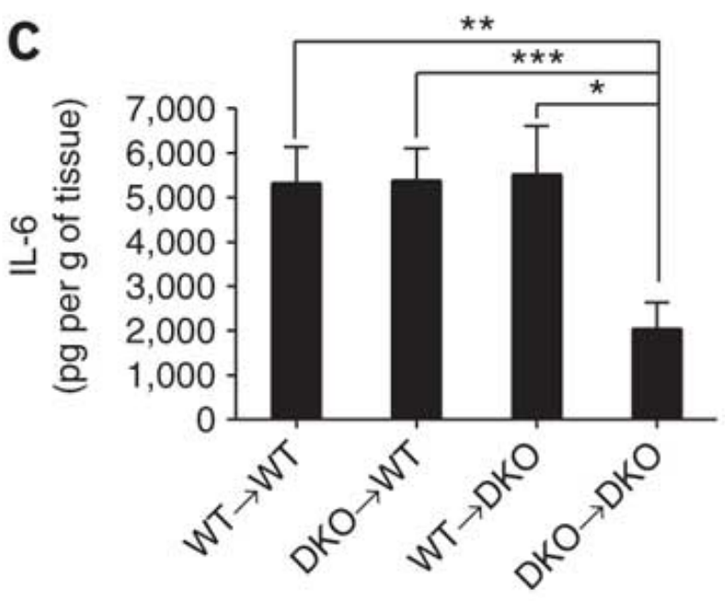
Figure 3: Similar with Salmonella infection (except that Nod signaling is also involved in γδT cell-derived IL-17)

IL-6 induction is required for early Th17 responses

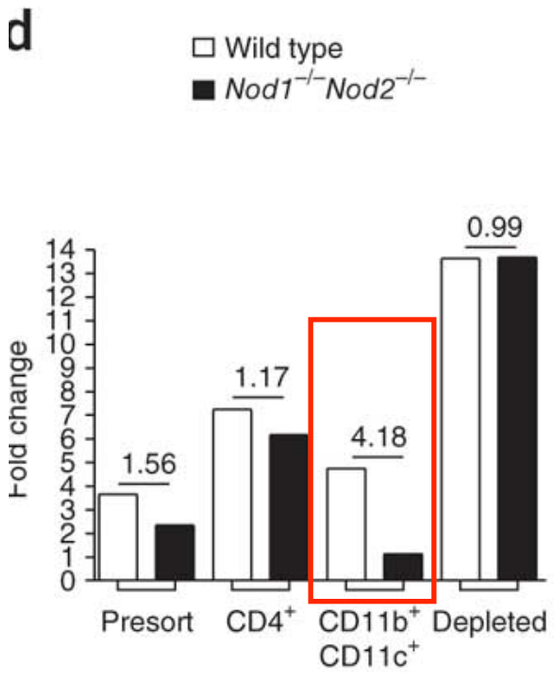
C. rodentium- cecum 4 d after infection
(same for Salmonella)



4 d

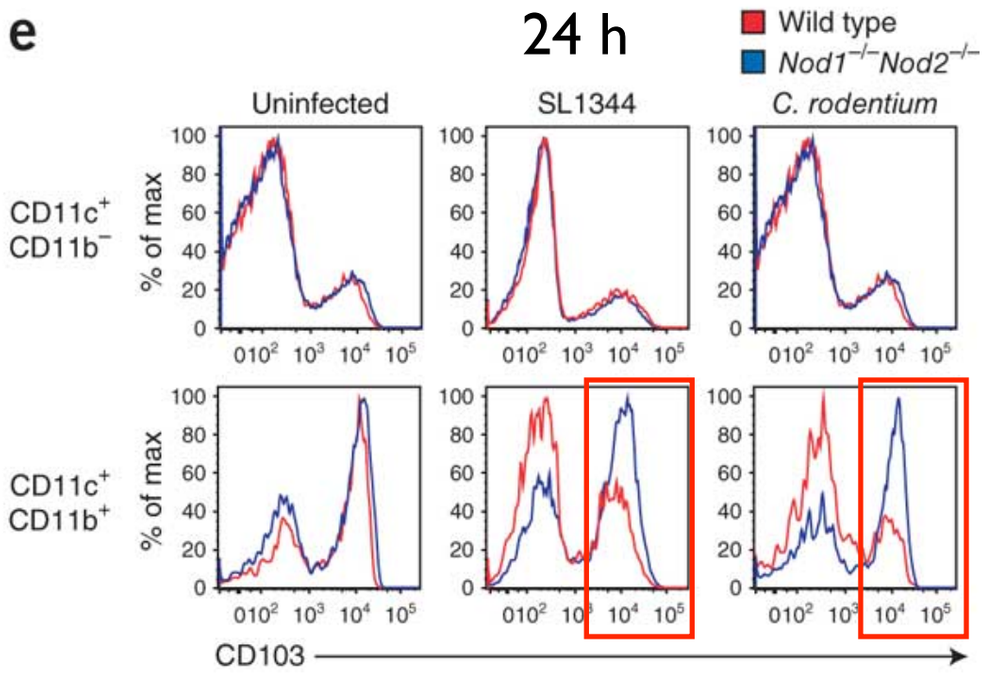


Nod signal from hematopoietic and non-hematopoietic cells regulate IL-6 induction



Fold change over pre-sort uninfected

24 h



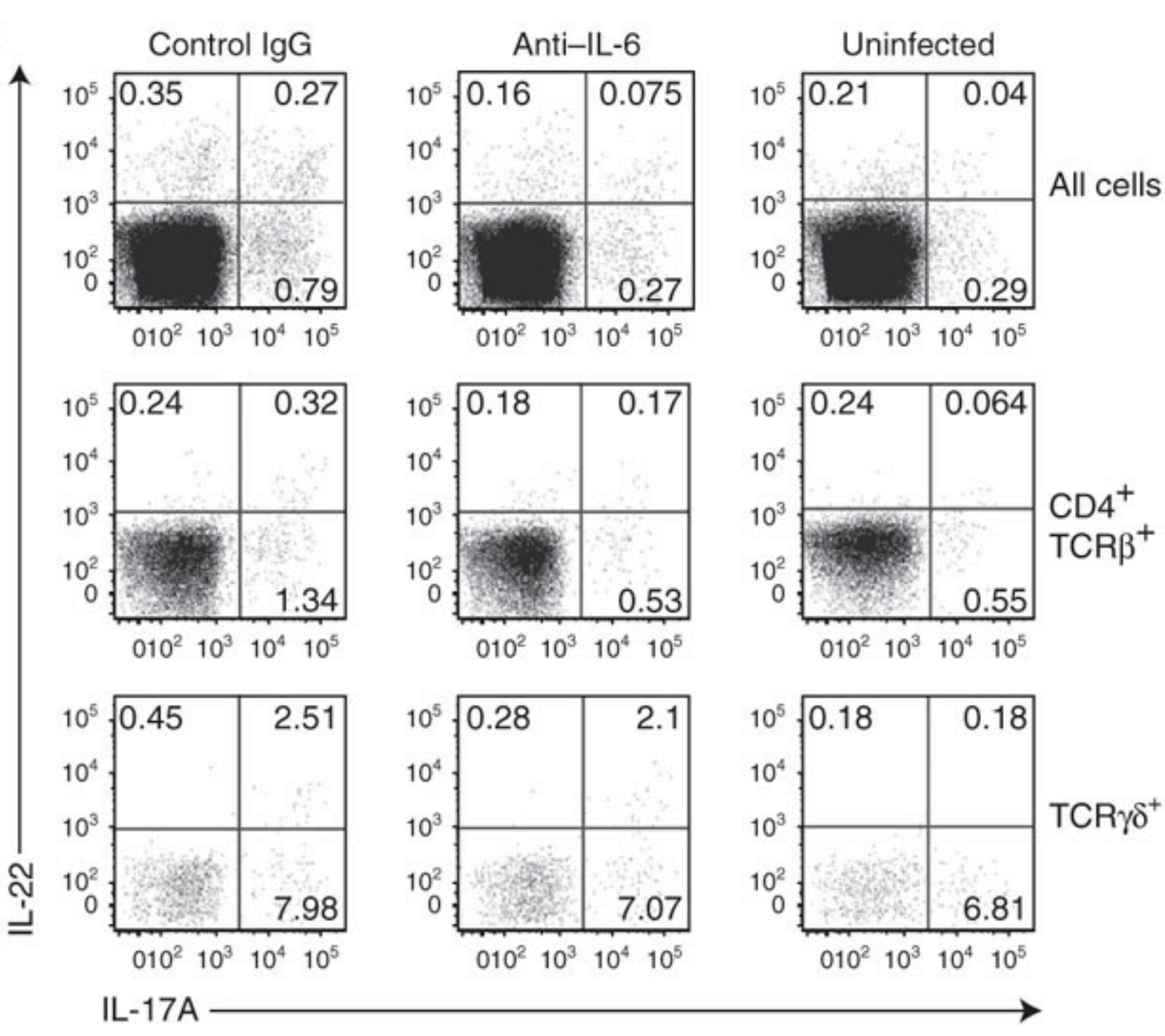
cecum
increased numbers of
$CD11c^+CD11b^+CD103^+$

IL-6 induction is required for early Th17 responses

Cecum - Salmonella infection at 24 h

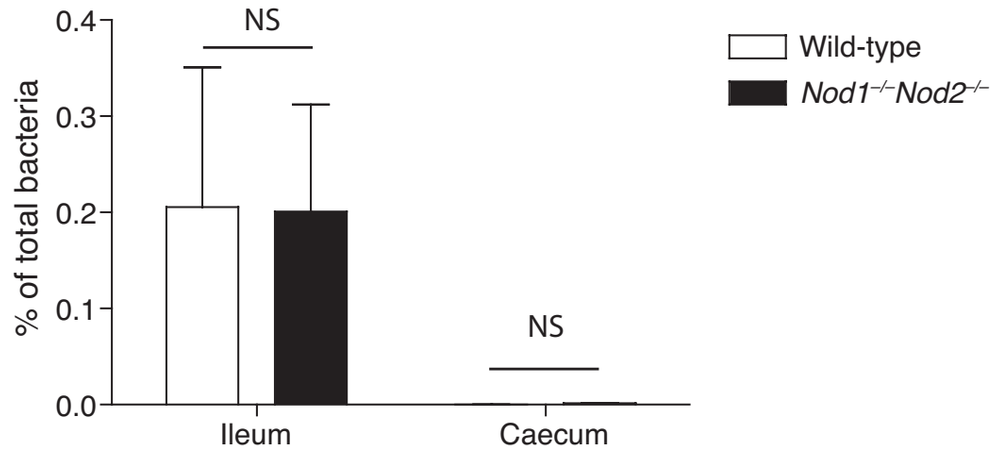
Reduction in proportion of CD4+TCRβ+ expressing IL-17A and IL-22.

Total numbers also drop in anti-IL6 and IL-6-/-.
BM chimeras: IL-6 produced by hematopoietic cells drives IL-17A - IL-22 production



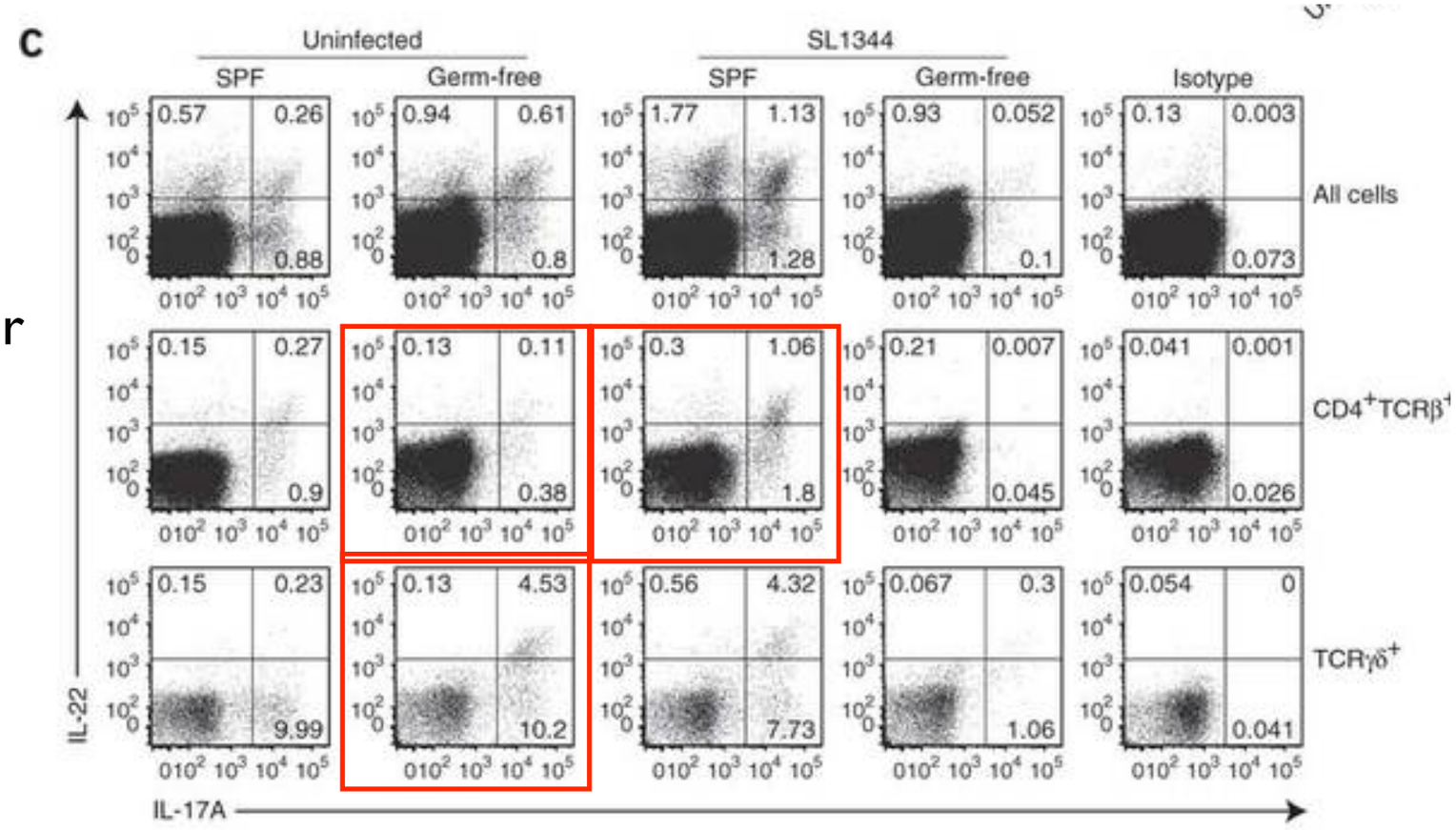
Are innate Th17 conditioned by the microbiota?

The SPF colony is SFB+ but Nod1 and Nod2 does not influence SFB colonization



cecum 24h

Swiss Webster



Compensatory mechanism?
Also observed following CD4 depletion

Discussion - Summary

- Nod1-/-Nod2-/- mice do not generate early Th17 responses in the cecum - named iTh17
- Results in delayed pathology and increased disease
- Th17 cells may have innate-like properties
- IL-23 has been shown to regulate innate IL-17 from LT1 and ϒδT cells but iTh17 require IL-6
- This iTh17 response may not happen in very clean mice?

:p3$^+$ follicular regulatory T cells control the germinal ter response

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NATURE MEDICINE | ARTICLE

Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions

Yeonseok Chung, Shinya Tanaka, Fuliang Chu, Roza I Nurieva, Gustavo J Martinez, Seema Rawal, Yi-Hong Wang, Hoyong Lim, Joseph M Reynolds, Xiao-hui Zhou, Hui-min Fan, Zhong-ming Liu, Sattva S Neelapu & Chen Dong

Affiliations | **Contributions** | **Corresponding authors**

TFH cells

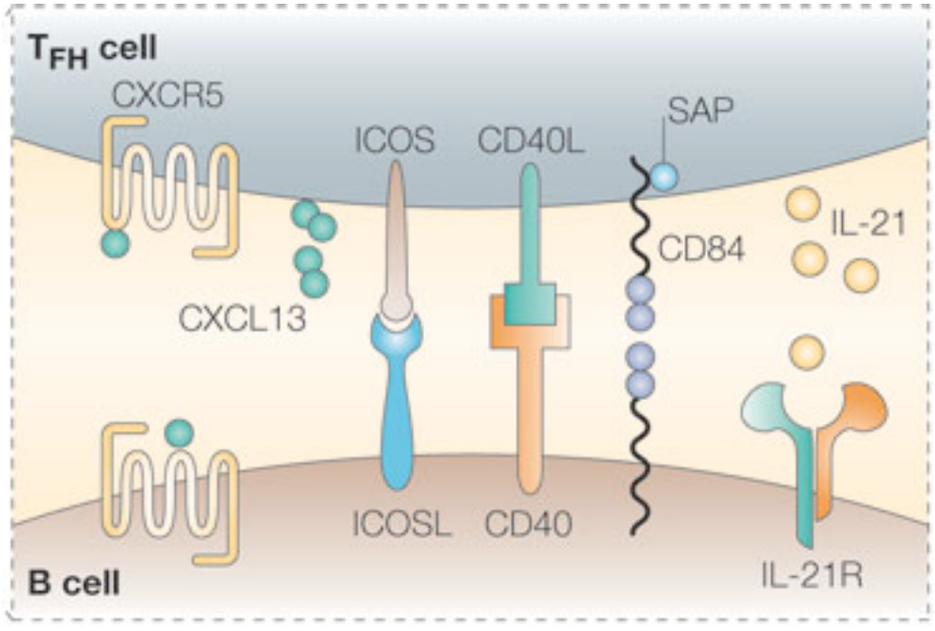
- Secreted cytokines are directing CSR and SHM: Reinhardt et al.. Nat Immunol (2009)
- Bcl6 -/-: multiple organs inflammatory disease, elevated IgE, defective GC formation
- **What cells control TFH cells?**
  - mice: Kim et al. Inhibition of follicular T-helper cells by CD8(+) regulatory T cells is essential for self tolerance. Nature (2010) vol. 467 (7313) pp. 328-32 (Qa-1 non-classical MHC)
  - humans: CD4+CD25+CD69- T cells found in GCs

Plasticity of Treg cells

- **Foxp3+ are converted into Tfh in PP**: Tsuji et al. Science (2009)
- IFNg secretion: Treg cell upregulate T-bet and CXCR3
- Koch et al. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. Nat. Immunol. (2009) vol. 10 (6) pp. 595-602
- High amounts of IRF4, a transcription factor essential for Th2 effector cell differentiation, is dependent on Foxp3 expression. Ablation of a conditional Irf4 allele in Treg cells results in selective dysregulation of Th2 responses, IL4-dependent immunoglobulin isotype production,
- Zheng et al. Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T(H)2 responses. Nature (2009) vol. 458 (7236) pp. 351-6
- Suppression was lost upon Treg-specific ablation of Stat3, a TF critical for Th17 differentiation, and resulted in the development of a fatal intestinal inflammation.
- Chaudhry et al. CD4+ Regulatory T Cells Control TH17 Responses in a Stat3-Dependent Manner. Science (2009) pp.

Retain intense CXCR5 expression - migration to CXCL13-rich areas within GCs

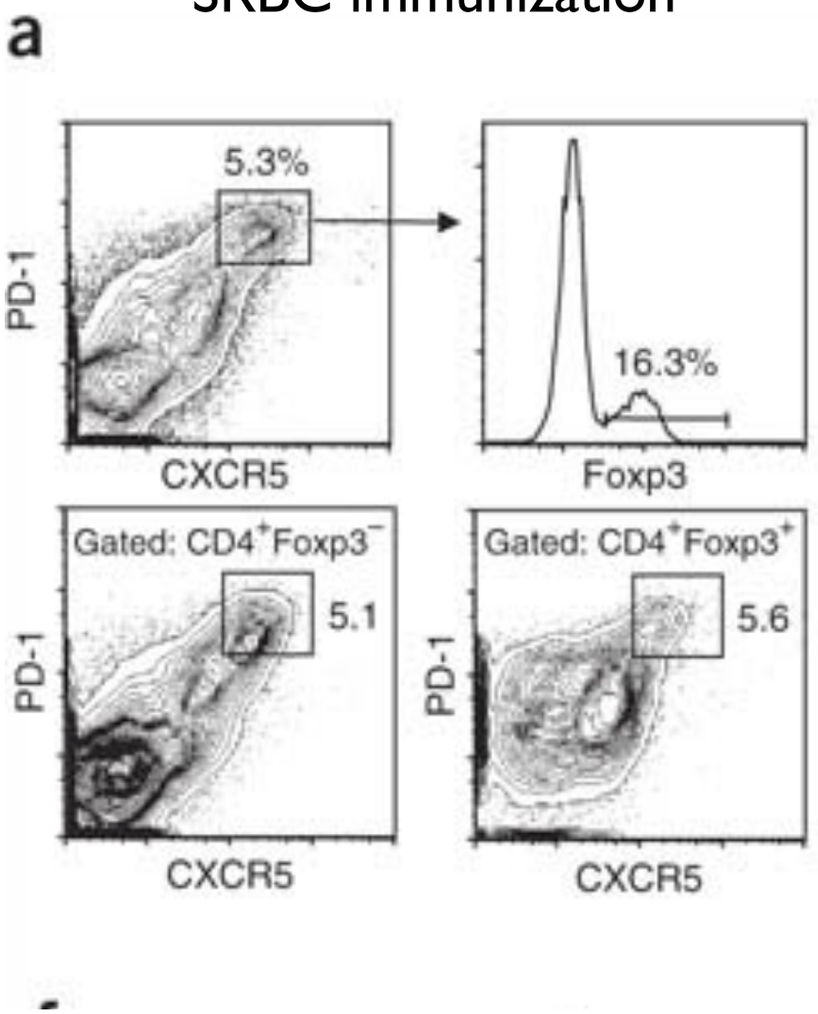
CXCR5+ ICOS+ CD28+ CD40L+ PD-1+ IL21R+ BTLA+,SLAM (CD150)lo, CD122lo CD200hi secrete IL-21



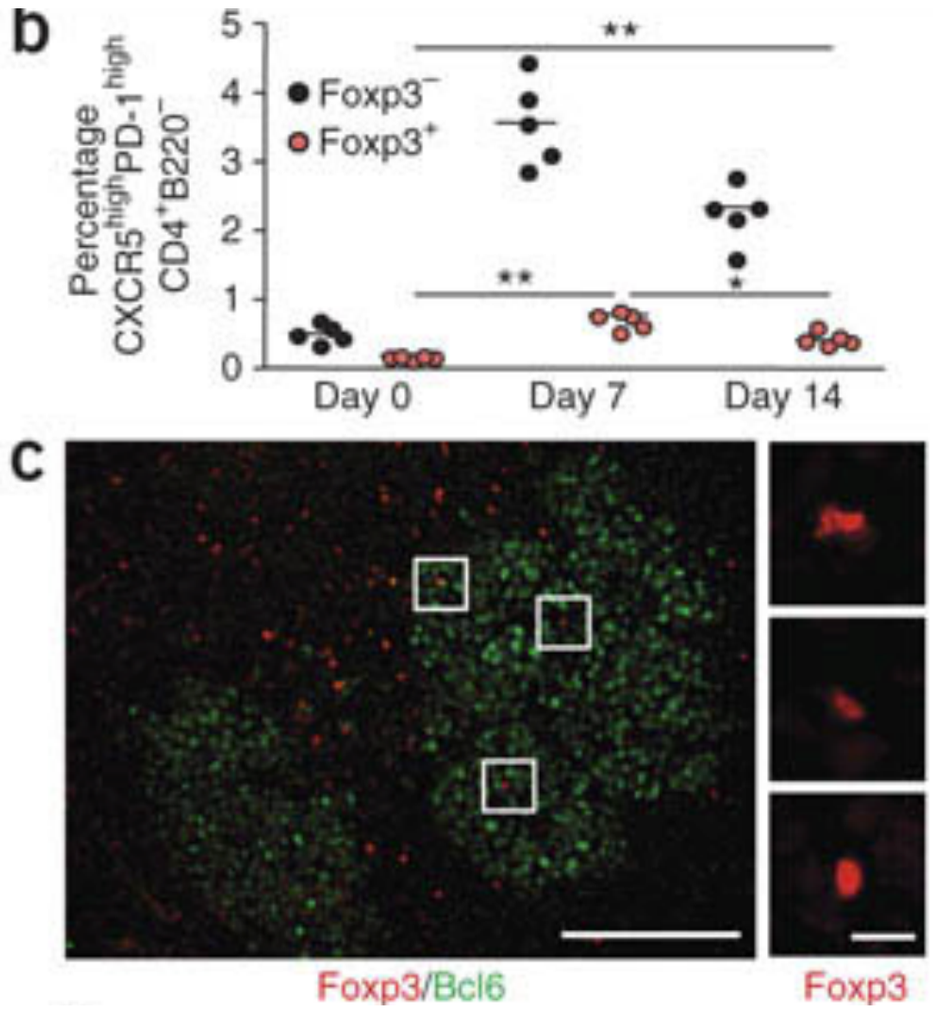
Tfh cell differentiation: ICOSL-dependent
(as well as GC formation and antibody production)

TFR are distinct but share similarities with TFH and Treg

SRBC immunization



Similar kinetics as TFH



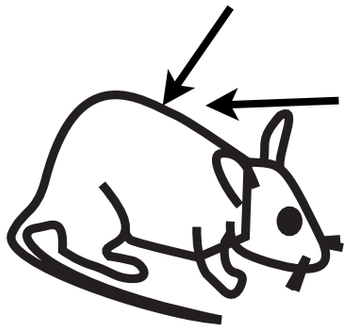
FoxP3+ cells are present within the Bcl6 area

TFR resemble Treg but also TFH gene expression
Treg: FoxP3, **Ctla4**, **Gitr**, Klrg1 and Prdm1, Il10
TFH: **Cxcr5**, Pdcd1, Bcl6, **Cxcl13**, **Icos**
No expression of IL-4 and IL-21 or CD40L

TFR and TFH colocalize: do they require similar signaling cues for their formation?

Tcell priming through CD28 is one of the first signals required for TFH cell development

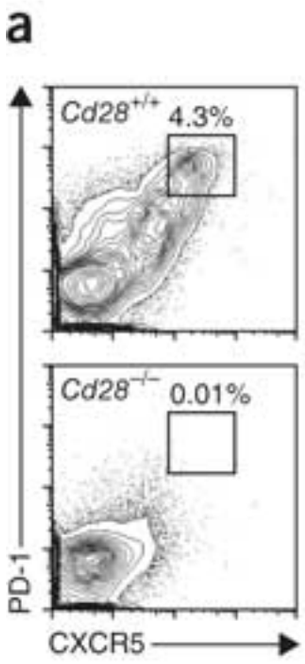
CD45.2+ CD28-/-



CD45.1+ CD28+/+

Rag2-/-

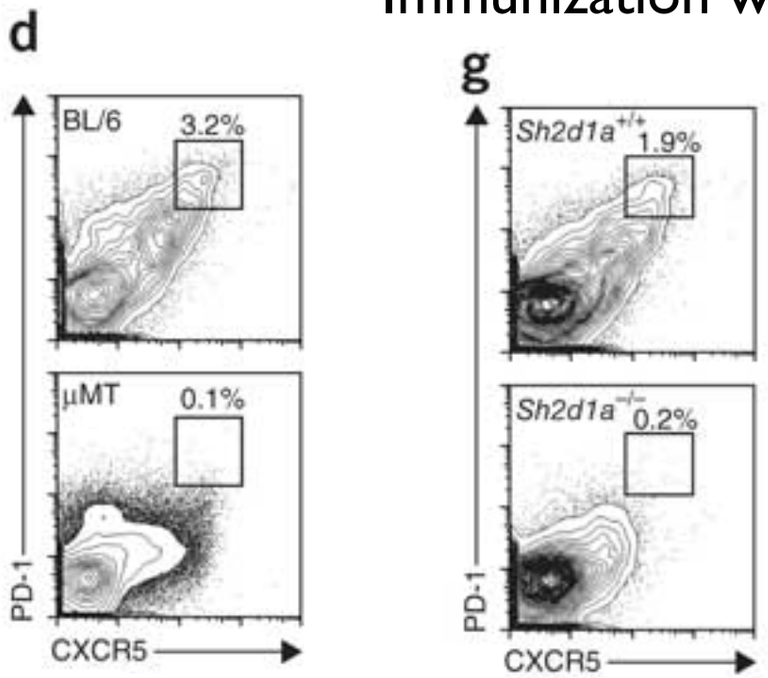
Immunization with SRBC - 7 days after analysis



TFR and TFH colocalize: do they require similar signaling cues for their formation?

SAP interaction of TFH cell precursors with B cells are required for TFH cell formation/ maintenance

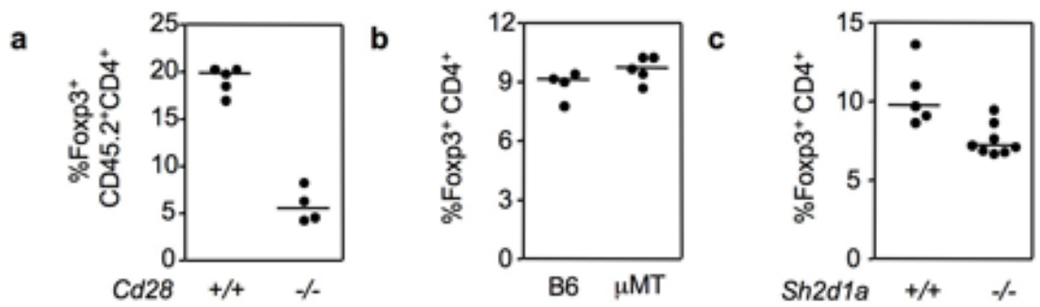
Immunization with SRBC - 7 days after analysis



In the absence of B cells

In the absence of SAP

Supp. Fig.3

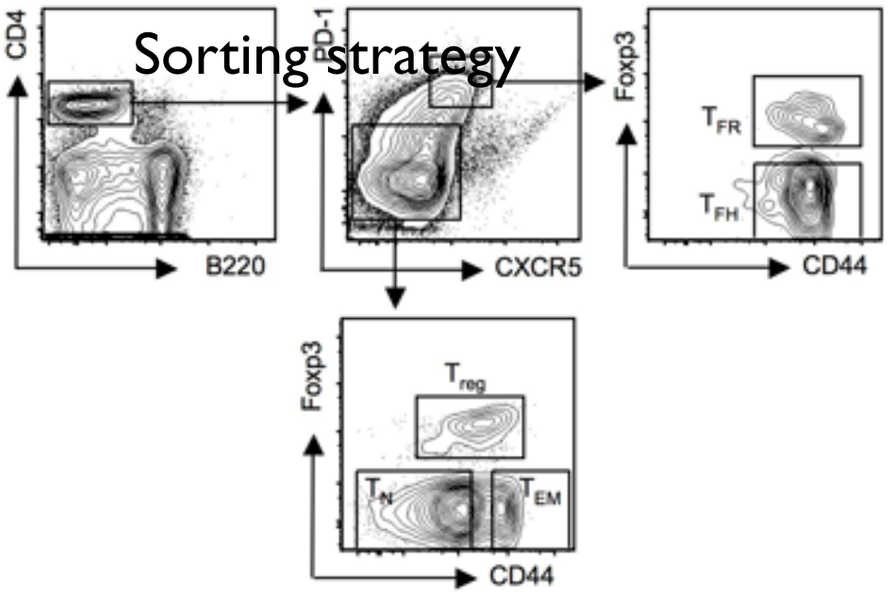


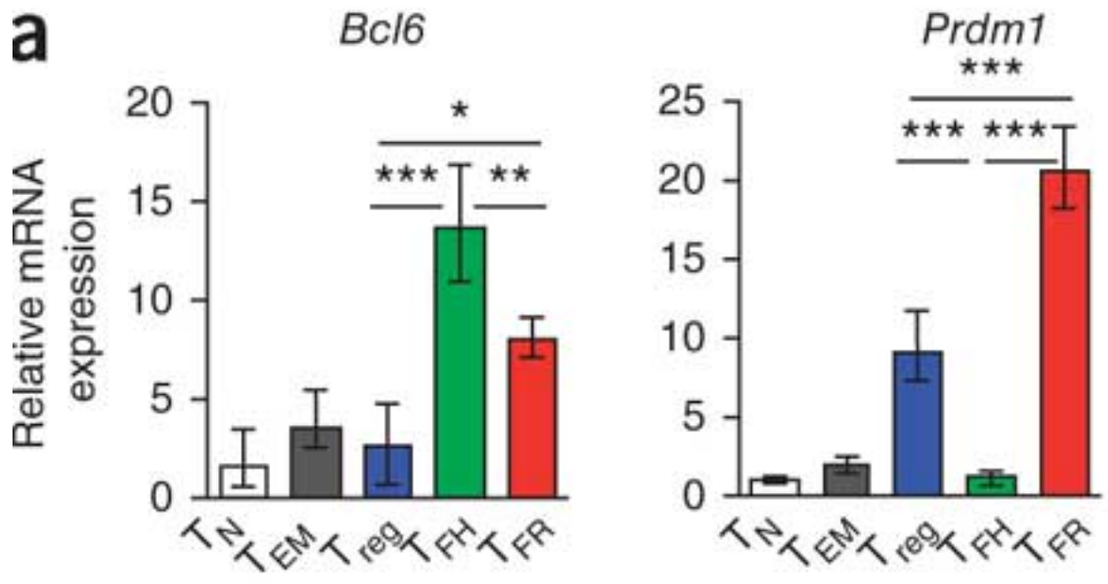
Treg cells formed independently of B cells and were only slightly reduced in the absence of SAP

Development from TFH and TFR are similar and Treg cells differentiate independently of TFH or TFR

TFR co-express Bcl6 and Blimp1

Bcl6 is the transcription factor of TFH and Blimp1 is the transcription repressor. (they mutually repress each other). Is the same true in TFR?



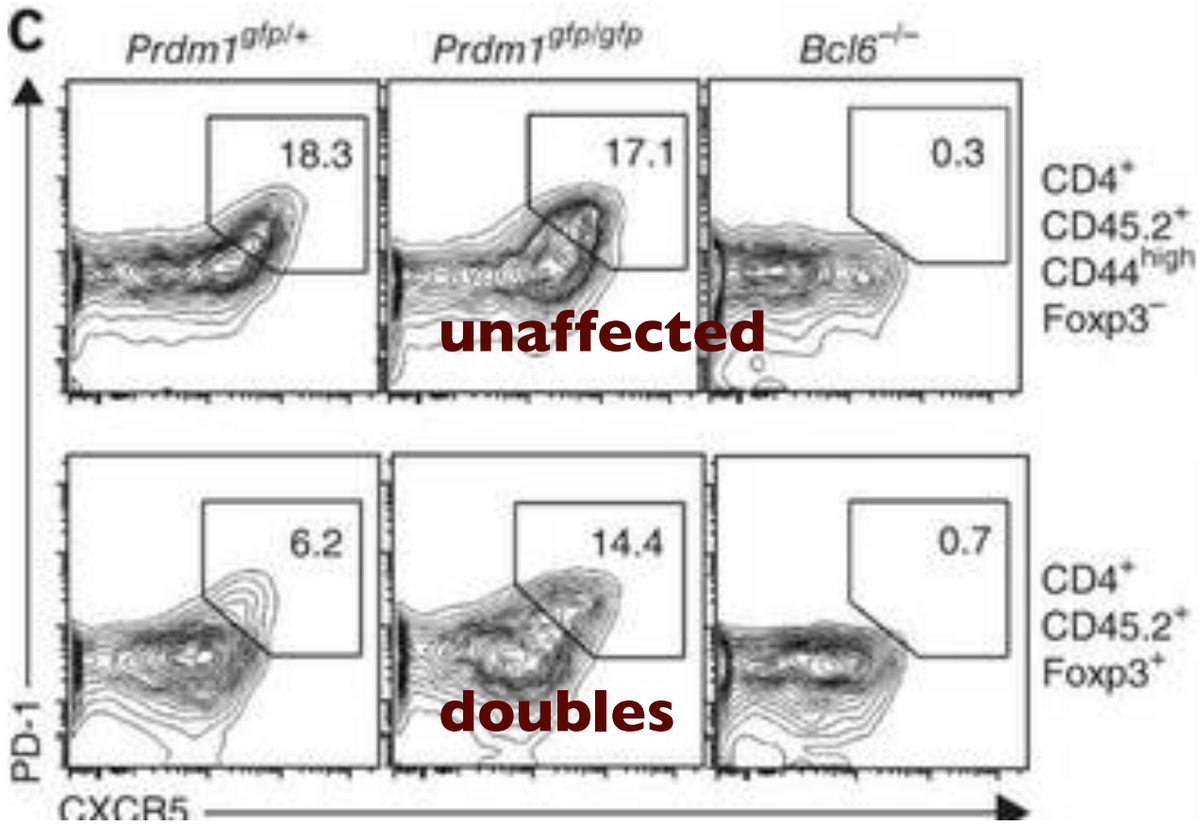


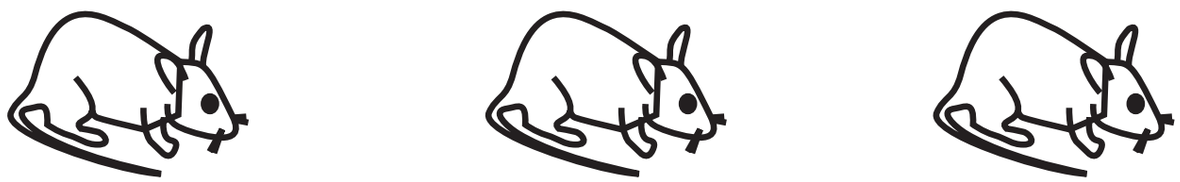
Bcl6 and Blimp1 control TFR formation and homeostasis

i.n influenza infection- 10d after in Mediastinal lymph node

TFH

TFR





embryonic 1:1 reconstitution

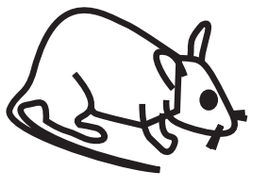
CD45.2 Prdm1gfp/+
CD45.1 Prdm1+/+

CD45.2 Prdm1gfp/gfp:
CD45.1 Prdm1+/+

CD45.2 Bcl6-/-
CD45.1Bcl6+/+

Precursors of TFH?

1x10^5 naive CD4+CD25-CD44low TCRHEL
CD45.1+

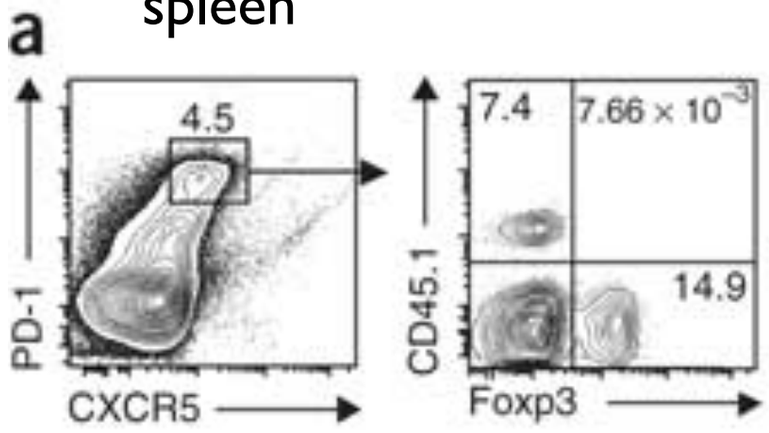


CD45.2+ B10.BR (I-Ak)

HEL in alum

7d

spleen



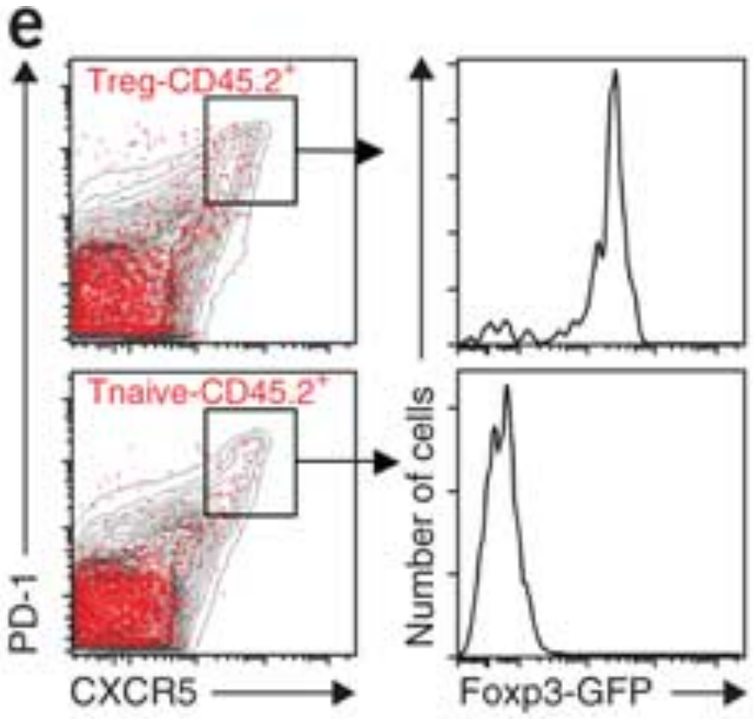
all TFR were derived from endogenous T cells

same with OTII-OVA model

Precursors of TFR?

1x10^6 naive CD4+FoxP3-CD44low or CD4+FoxP3+CD44int (CD45.2 FoxP3gfp)

KLH in Ribi



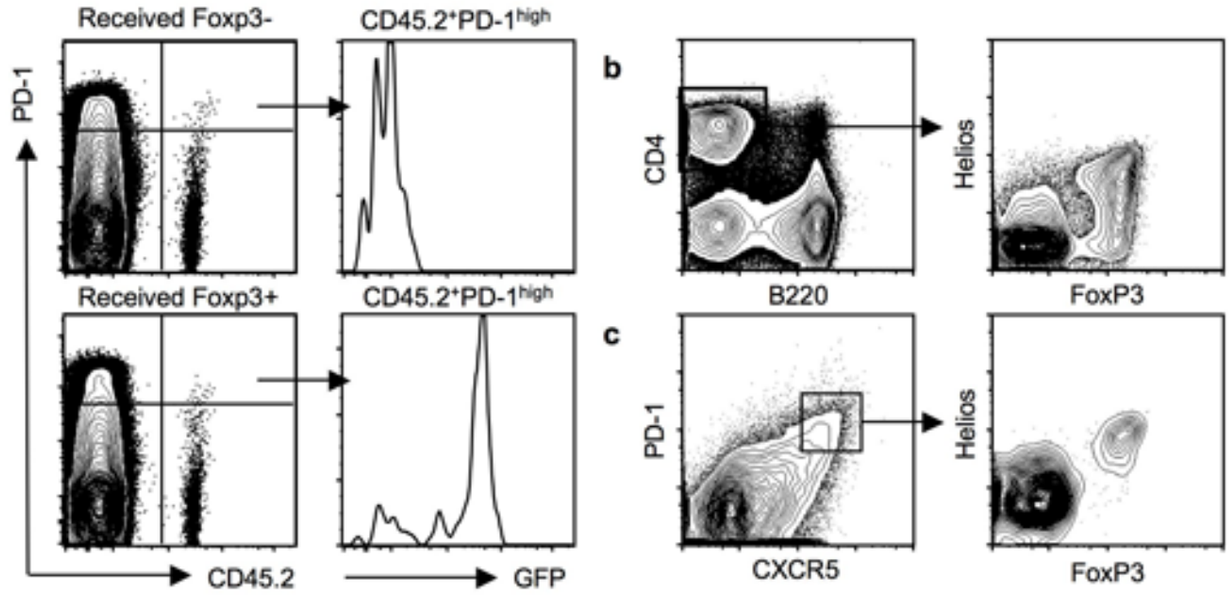
Both donor Treg and naive developed into CCR5+PD-1+ but only Treg cells maintained FoxP3 expression

1x10^6 naive CD4spFoxP3+ thymic Treg or CD4spFoxP3- (FoxP3gfp CD45.2)

CD45.1

SRBC 7 days after

pplementary fig. 5



In vivo selective depletion of TFR

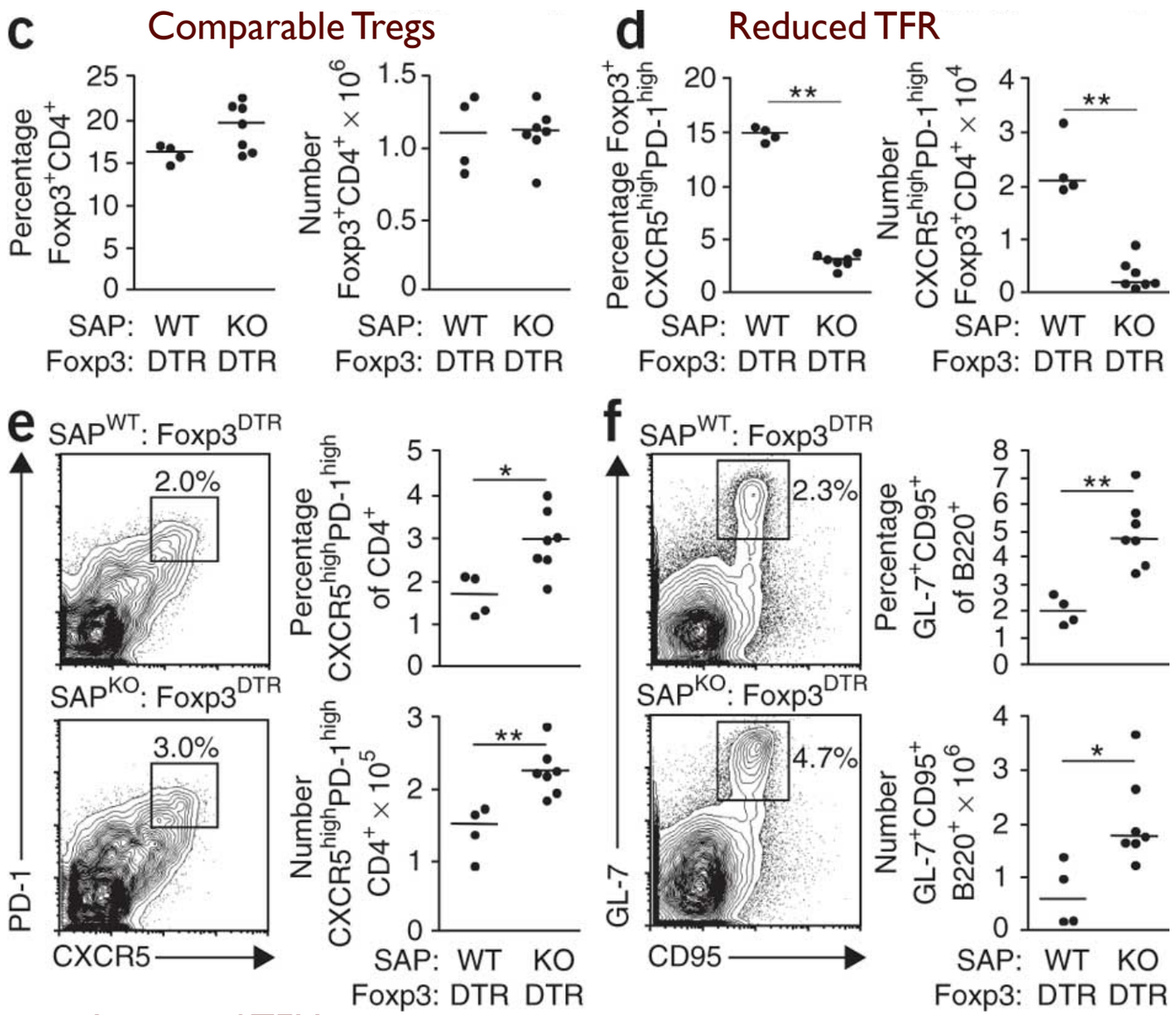
nera	Genotype	Allotype	Tfh*	Treg*	Tfr*
ıtrol	*Sh2d1a*$^{+/+}$	CD45.2	50	100	100
:t Tfr)	*FoxP3*^{DTR}	CD45.1	50	0	0
mental	*Sh2d1a*$^{-/-}$	CD45.2	0	100	0
ıg Tfr)	*FoxP3*^{DTR}	CD45.1	100	0	0

se numbers represent the percentage of cells expected to derive from the bone w cells in each of the chimeras after immunisation and DT treatment.

8 weeks after reconstitution: DT treatment one day before SRBC immunization and 2 and 5 days after

TFR suppress TFH cell numbers and B cell numbers

day 8



Increased TFH

Germinal center B cells increase

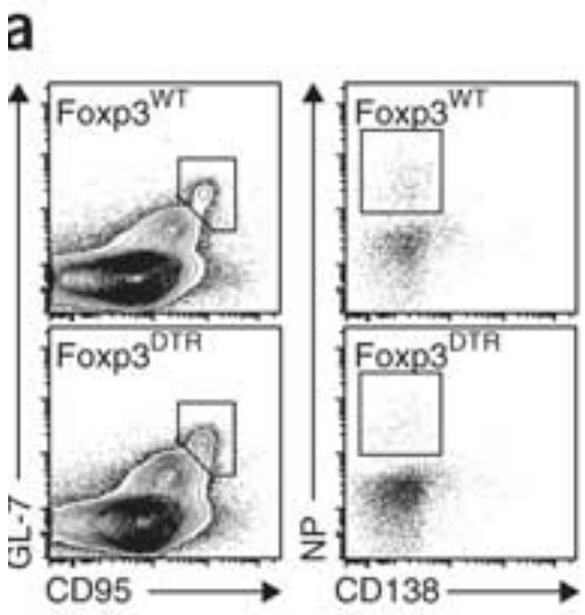
Do TFR control germinal center B cell selection?

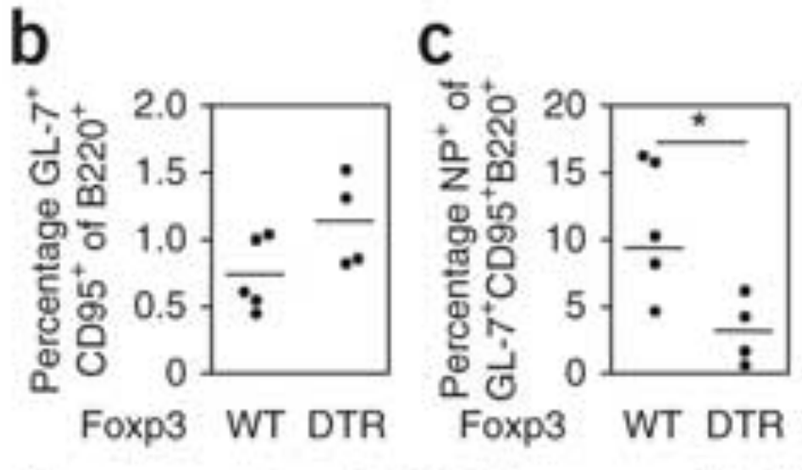
NP-KLH in alum

FoxP3DTR

FoxP3WT

DT 6 days later → analysis 10 days later





no differences in germinal center B cell percentages
NP-sp B cells were reduced in the germinal center
With BM chimeras (Sh2d1a:FoxP3) where TFR are specifically depleted they suggest that antigen-specific B cells are reduced whereas non-antigen specific B cells are increased

Discussion - Summary

- In response to T-D antigens, Treg cells adopt a TFH differentiation program (Bcl6, CXCR5...)
- They suppress TFH cells and the B cell germinal center response
- Thus: Treg cell can mold their differentiation program to the environment even for TFH cells in the germinal center response.