

LETTER

doi:10.1038/nature11813

A T-bet gradient controls the fate and function of CCR6⁻RORγt⁺ innate lymphoid cells

Christoph S. N. Klose¹, Elina A. Kiss¹, Vera Schwierzeck^{1,2}, Karolina Ebert^{1,3}, Thomas Hoyler¹, Yannick d'Hargues^{1,3}, Nathalie Göppert¹, Andrew L. Croxford^{4,5}, Ari Waisman⁴, Yakup Tanriver^{1,2,3*} & Andreas Diefenbach^{1,2,6*}

LETTER

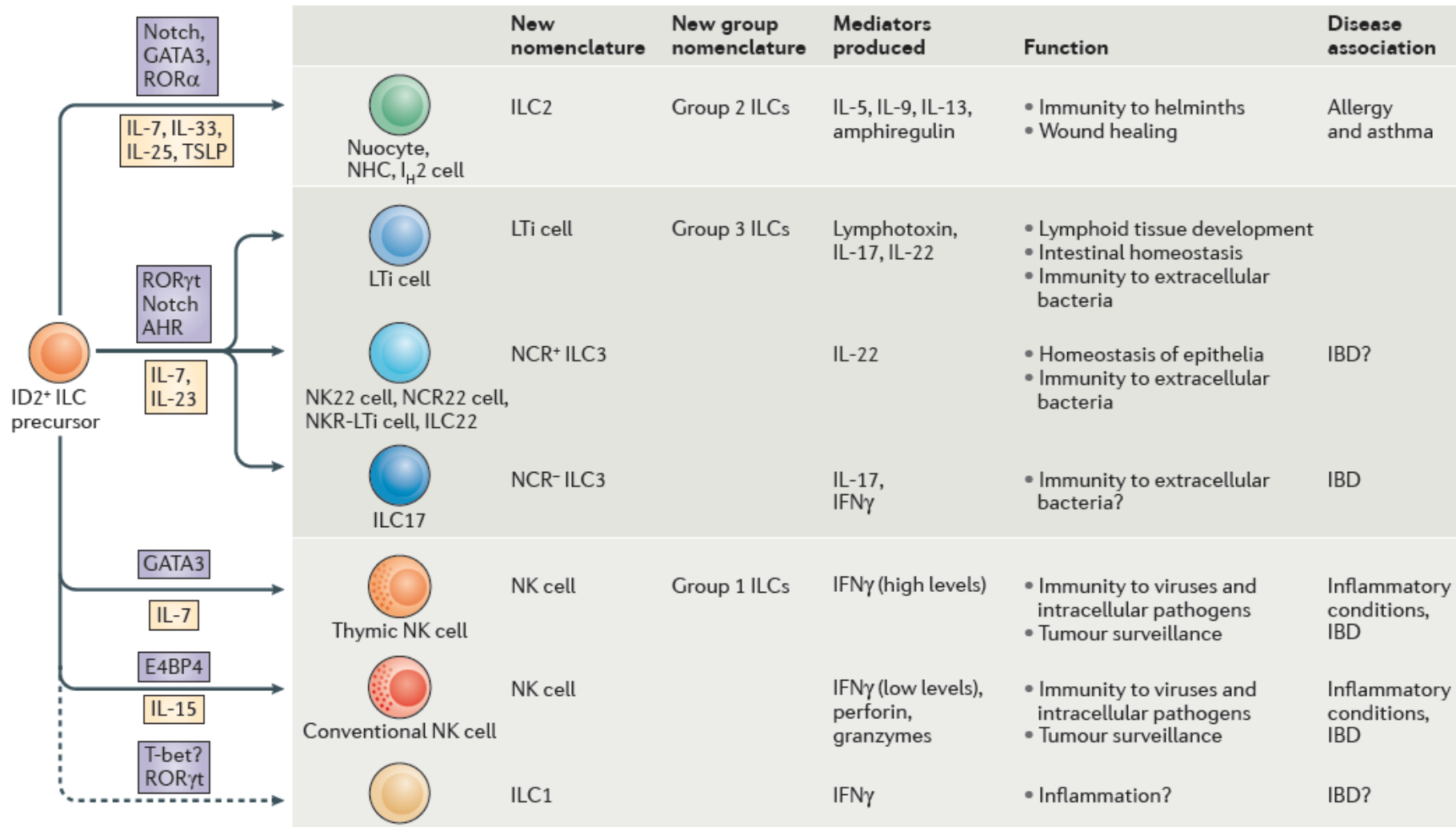
doi:10.1038/nature11809

Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX₃CR1^{hi} cells

Gretchen E. Diehl¹, Randy S. Longman^{1,2*}, Jing-Xin Zhang^{1†*}, Beatrice Breart^{1†}, Carolina Galan¹, Adolfo Cuesta^{1,3}, Susan R. Schwab¹ & Dan R. Littman^{1,3}

A T-bet gradient controls the fate and function of CCR6⁻RORγt⁺ innate lymphoid cells

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Background

- ROR γ t⁺ ILC produce IFN γ in inflammation context

Hypothesis: Different subsets or different transcription factor programs?

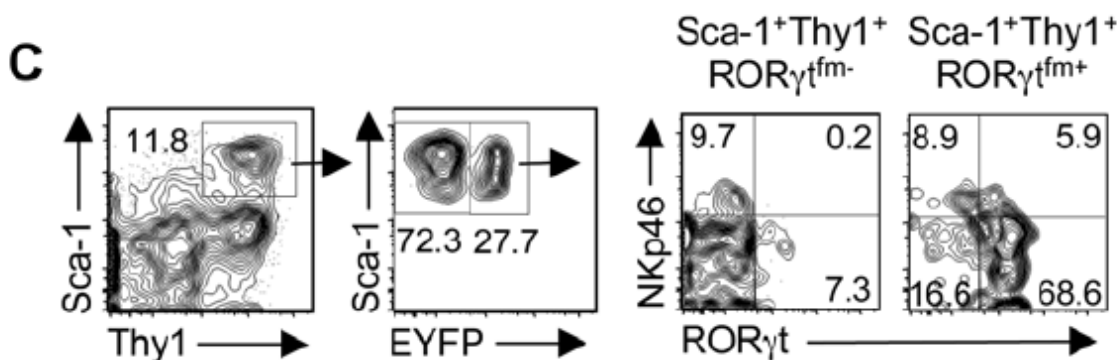
- T-Bet: central regulator of type 1 immunity controlling the expression of IFN γ

- IFN γ is required to protect during infections but inappropriate produced can drive autoimmunity and chronic inflammation

- Intestinal inflammation: T-bet RNAm detected in Sca-1⁺Thy^{high} ILC

(Buonocore et al. Nature 2010)

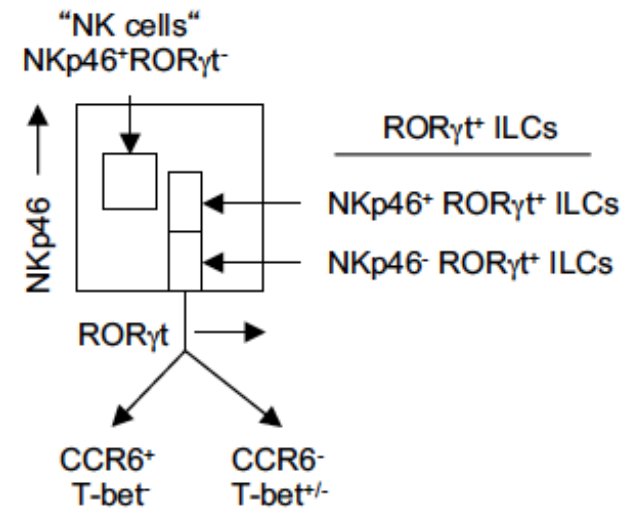
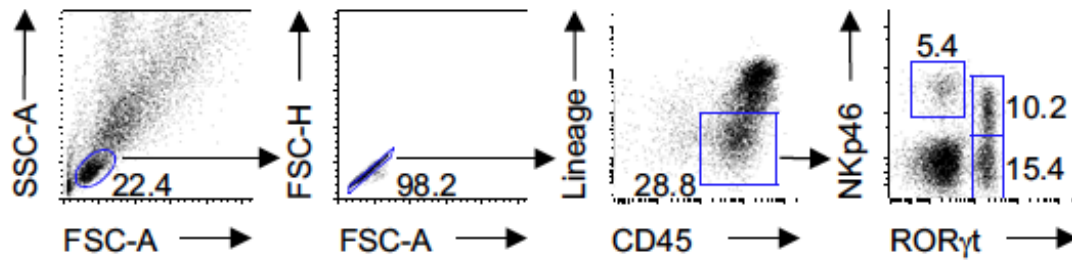
- Some of Sca-1⁺Thy^{high} ILC are ROR γ t⁺ ILC (Vonarbourg et al. Immunity 2010)



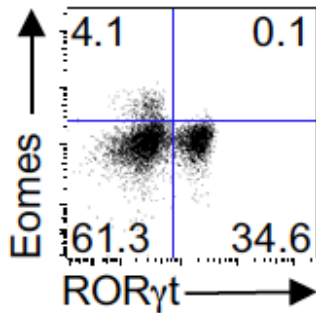
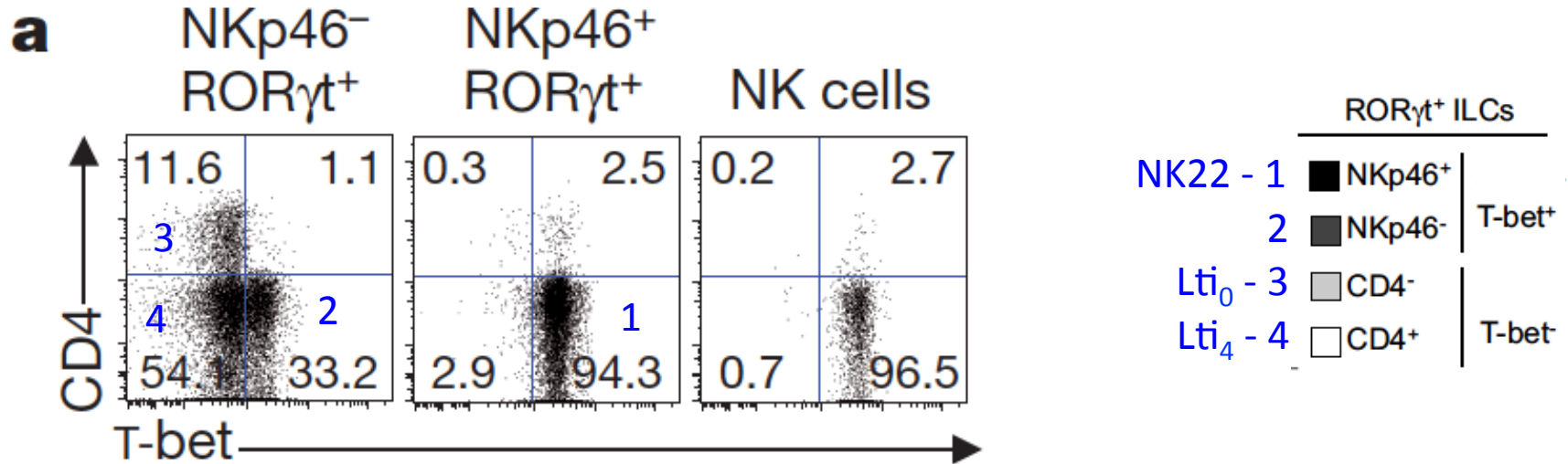
(Vonarbourg et al. Immunity 2010)

Gating strategy for intestinal ROR γ t⁺ ILC

LP small intestine



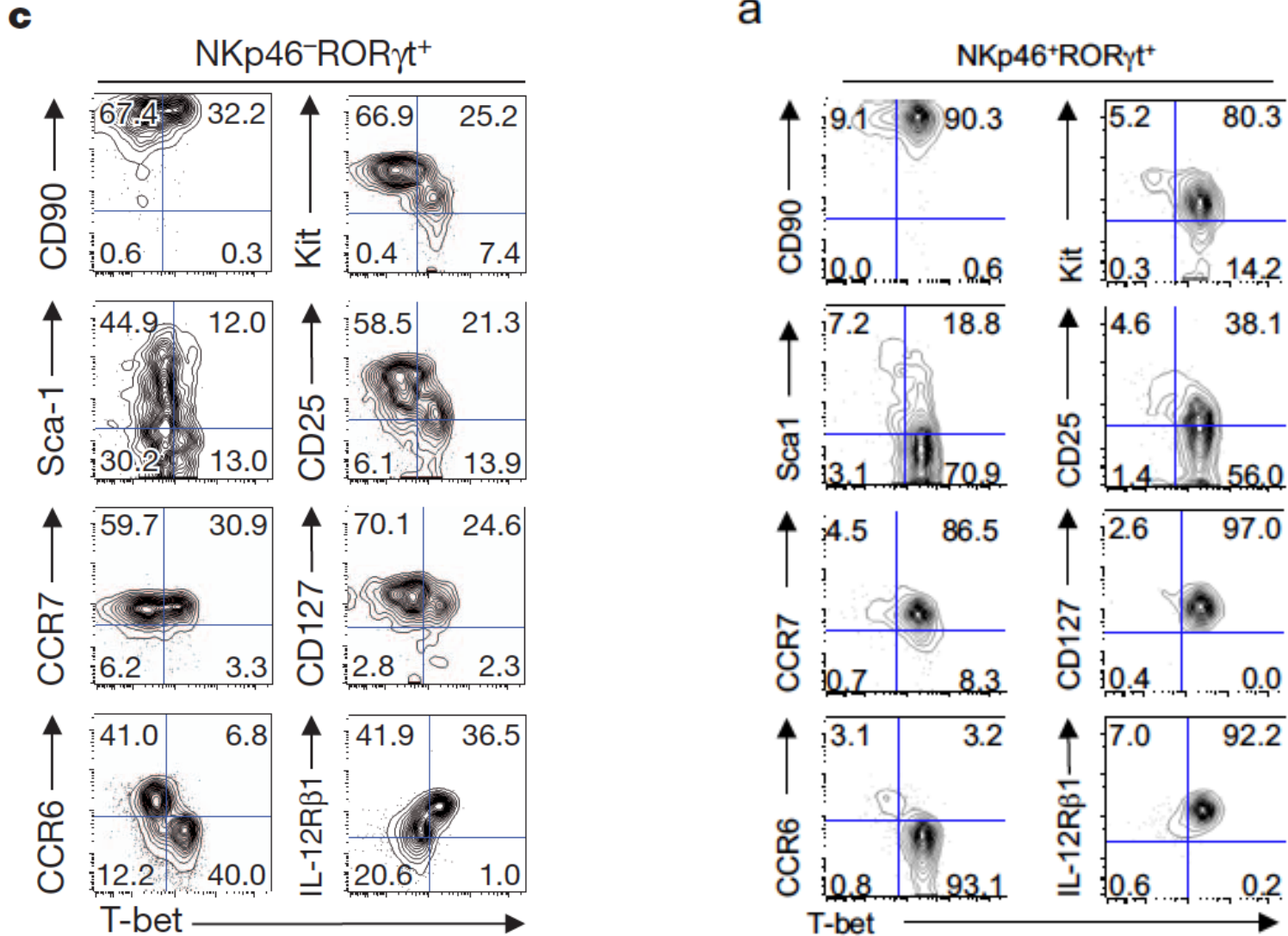
Some subsets of ROR γ t⁺ ILC express T-bet



ROR γ t⁺ ILC doesn't express Eomes

Eomesodermin: T-box transcription factor
involve in the control direct of fate of NK cells

Inverse correlation in the expression of T-bet and CCR6 in ROR γ ⁺ ILC

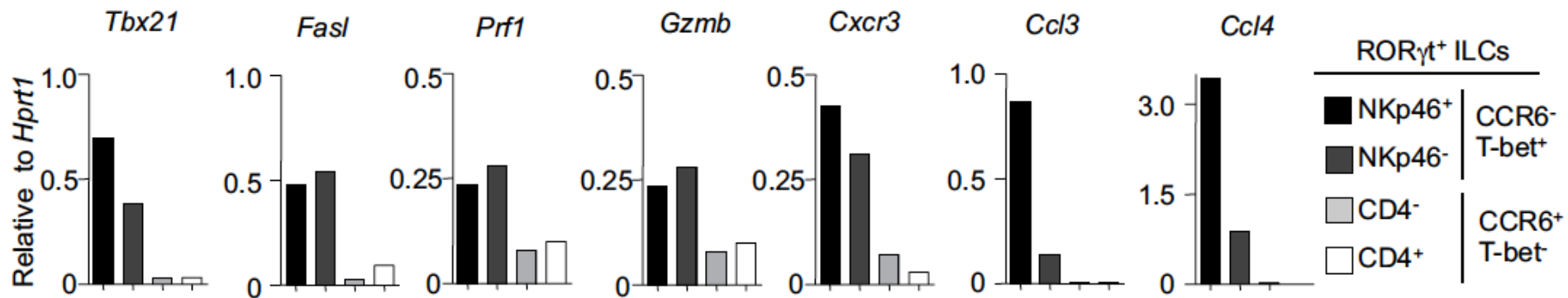


= expression levels of Thy1, Sca-1 and CCR7

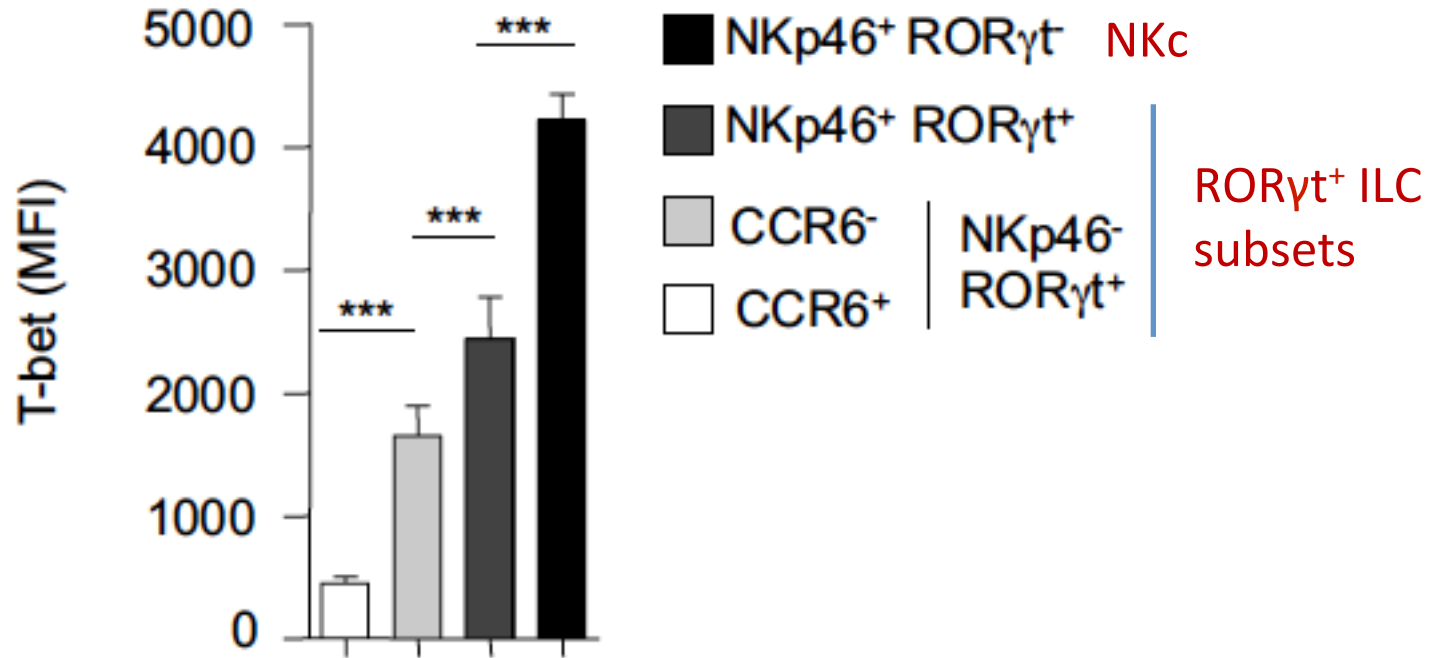
↓ expression levels of c-Kit, CD25 and CD127

↑ expression levels of IL-12R β 2 (IL-12 and IL23 R subunit)

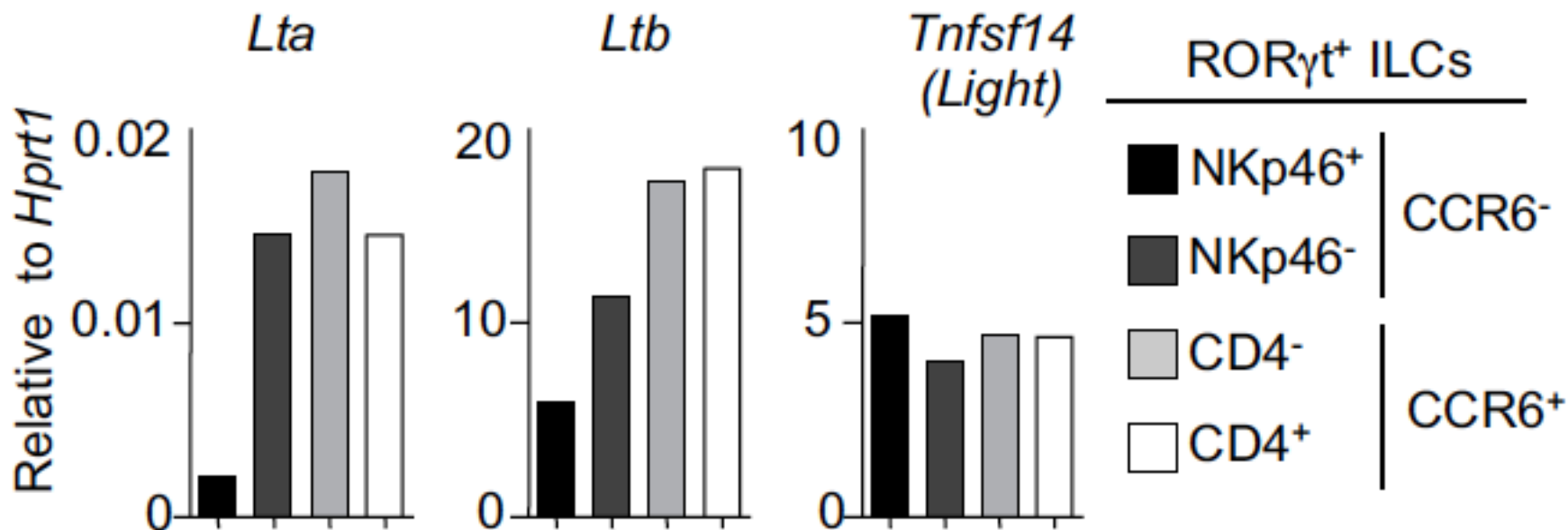
Transcriptional profile according with T-bet target genes



Intensity of T-bet expression increase with NKp46 expression

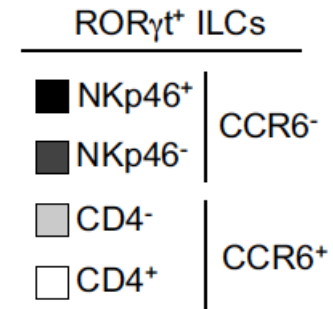
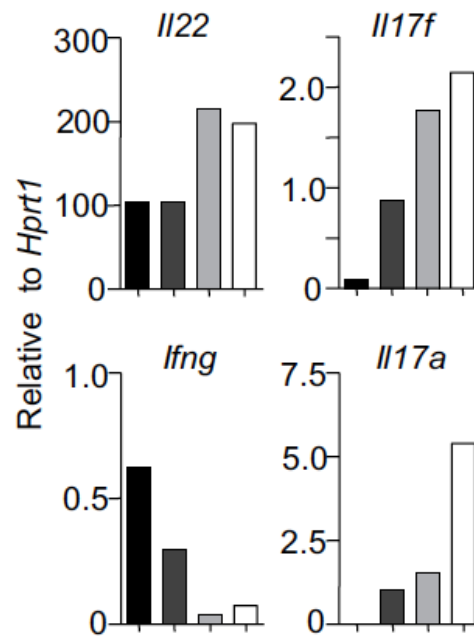
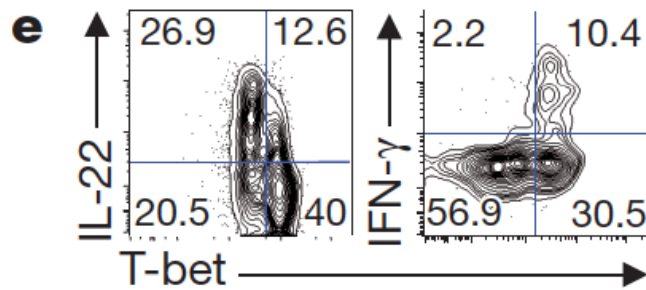


Equivalent levels of Lti genes
(TNF superfamily of genes required for lymphoid tissue induction function)

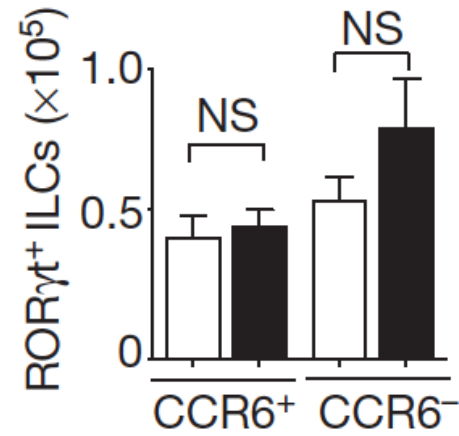
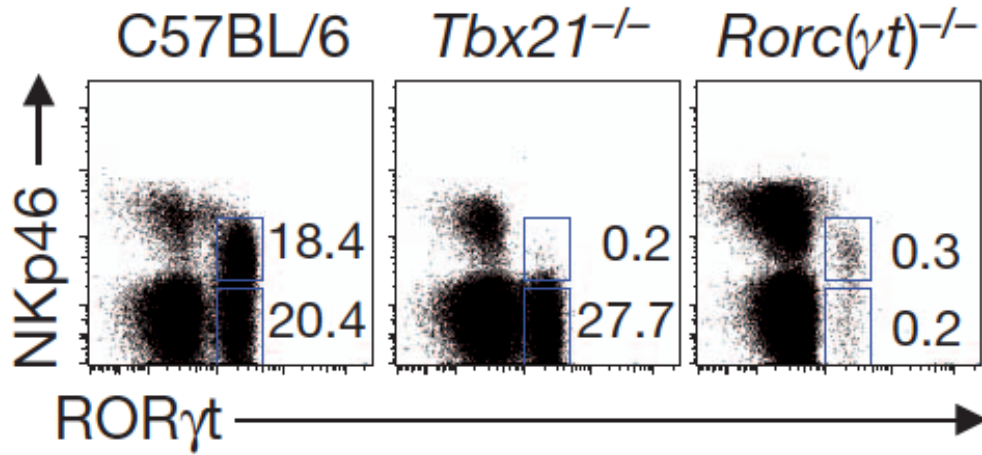


Different cytokine profil between ROR γ t⁺ ILC subsets:

- All population express IL-22,
- CCR6⁻T-bet⁺ROR γ t⁺ ILC subsets express IFN γ
- CCR6⁺T-bet⁻ROR γ t⁺ ILC subsets express Il-17



T-Bet is necessary for the development of NKp46⁺RORγt⁺ ILC

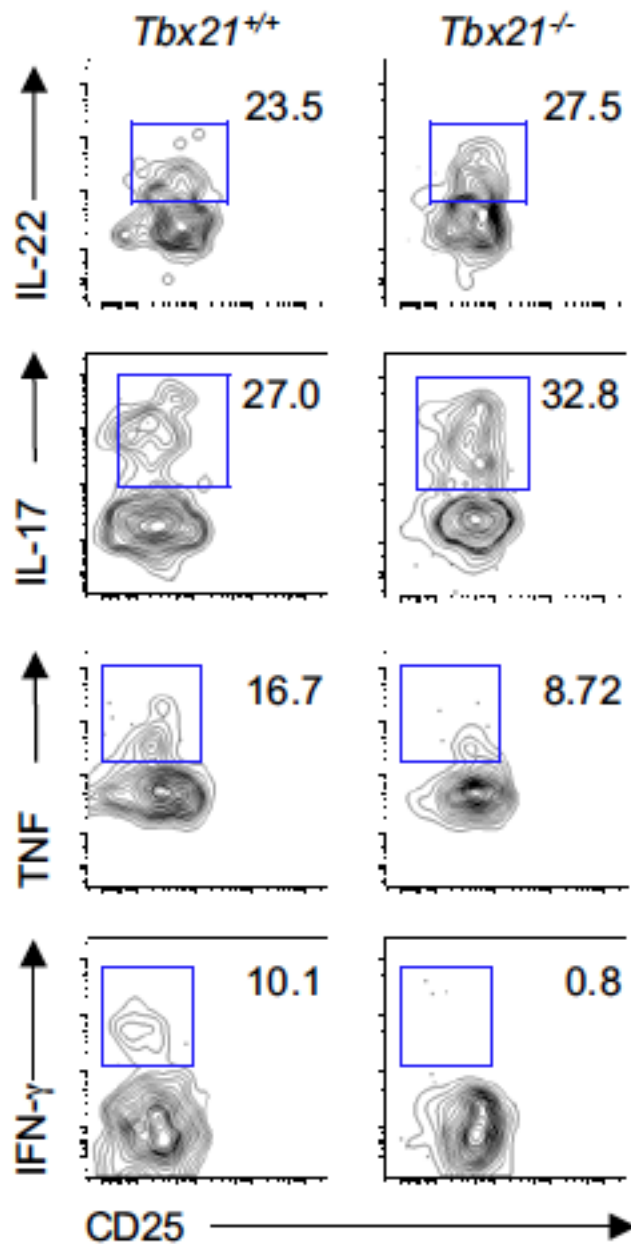


NKp46⁻RORγt⁺ ILC are not affected in *Tbx21*^{-/-} mice,
but NKp46⁺RORγt⁺ ILC are completely absent

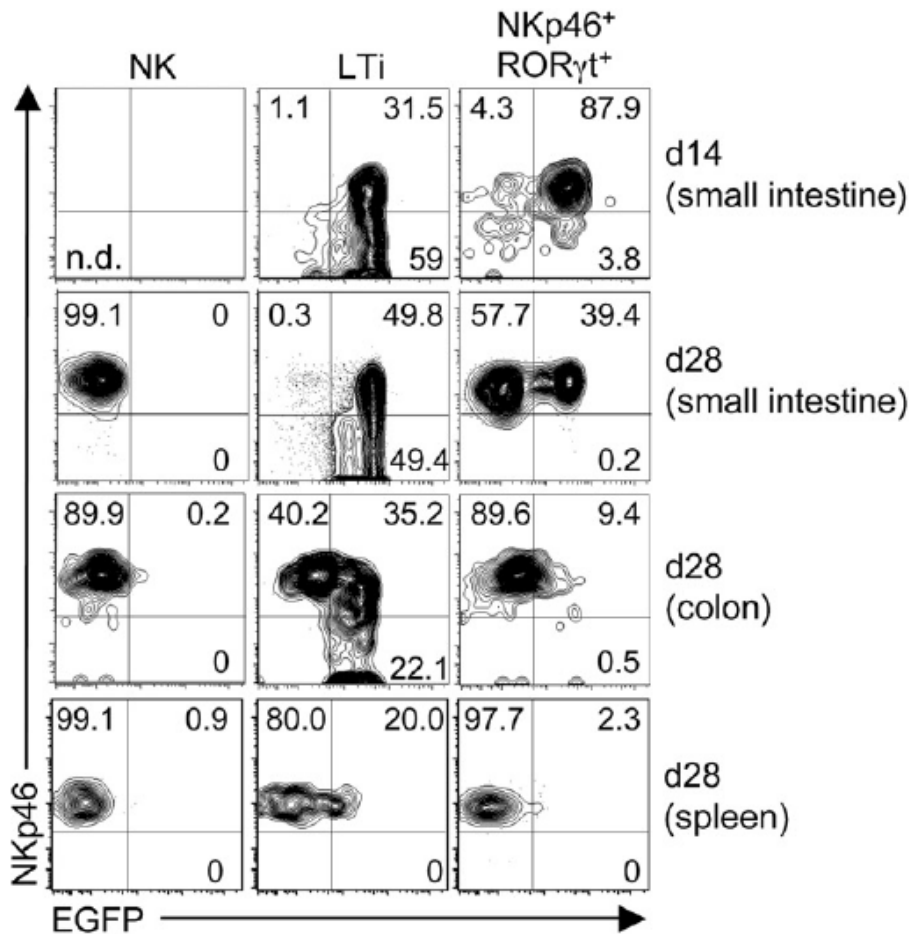
T-bet control NKp46⁻RORγt⁺ ILC fonction

e

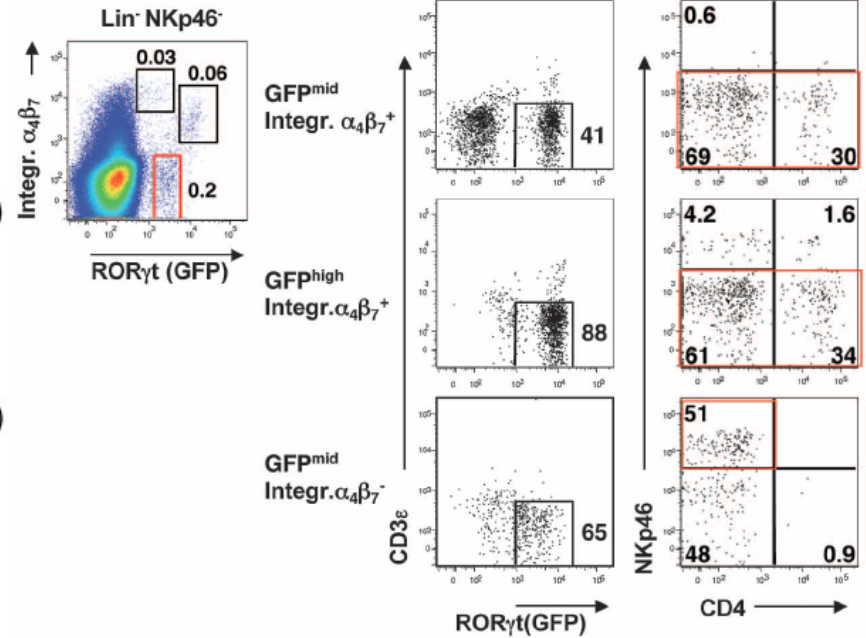
LP sl
PMA-IONO/Bref A for 4h
Gate NKp46⁻RORγt⁺



B Transferred cells (from small intestine)



C



NKp46⁺ And NKp46⁻ RORγt⁺ ILC have the same precursor

(Sawa et al. Science 2010)

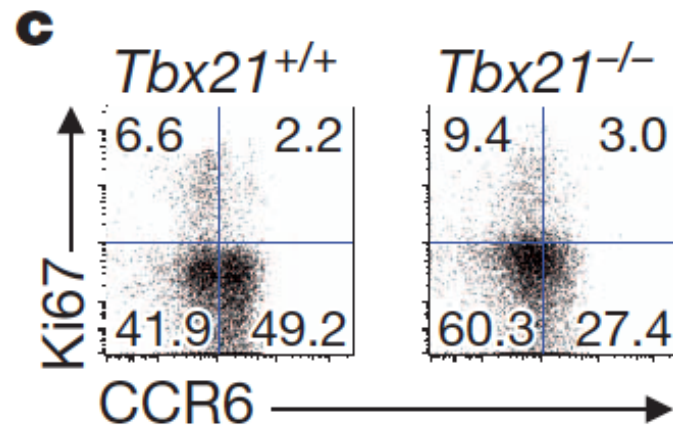
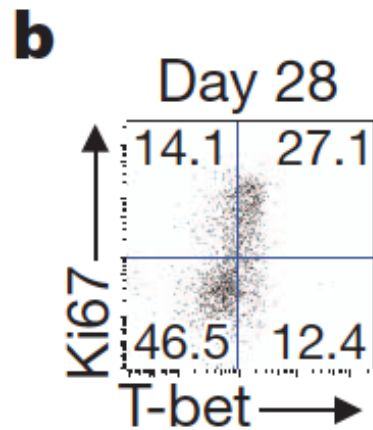
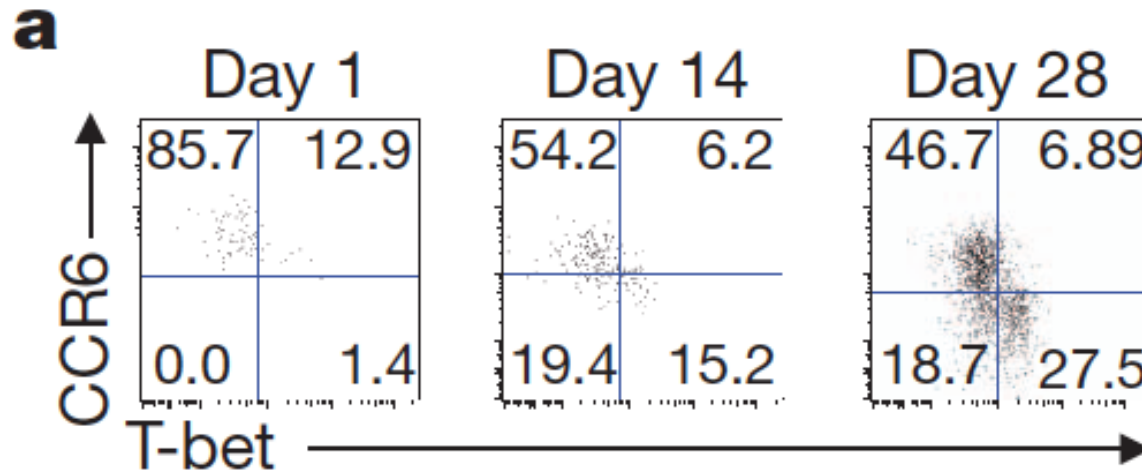
Figure 1. NKp46⁺RORγt⁺ Cells Are Derived from NKp46⁻RORγt⁺ Precursors

(Vonarbourg et al. Immunity 2010)

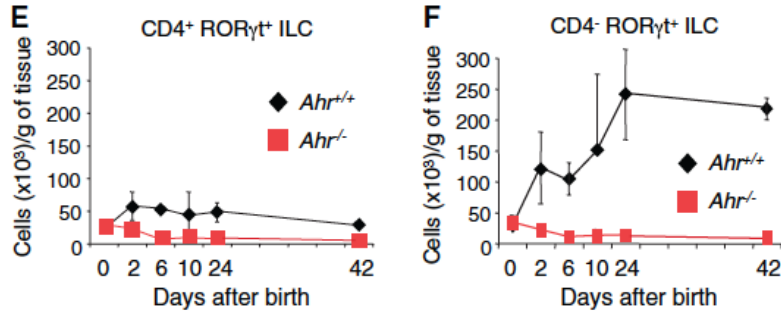
Could be T-bet required for the development of NKp46⁺RORγt⁺ ILC from NKp46⁻RORγt⁺ ILC ?

CCR6-ROR γ t⁺ increases with the age

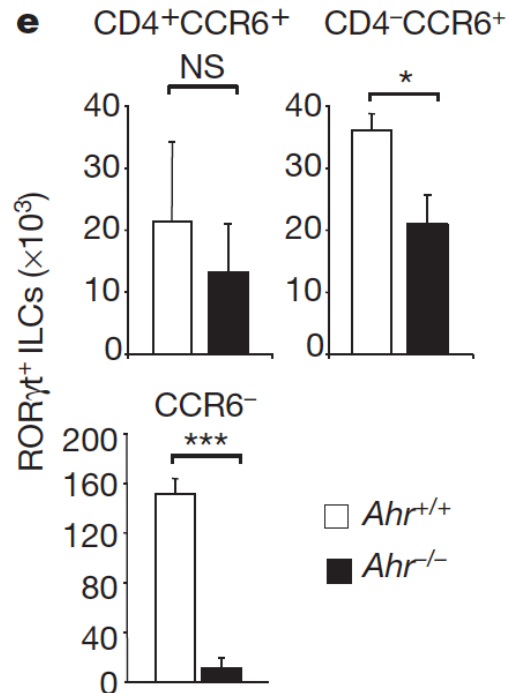
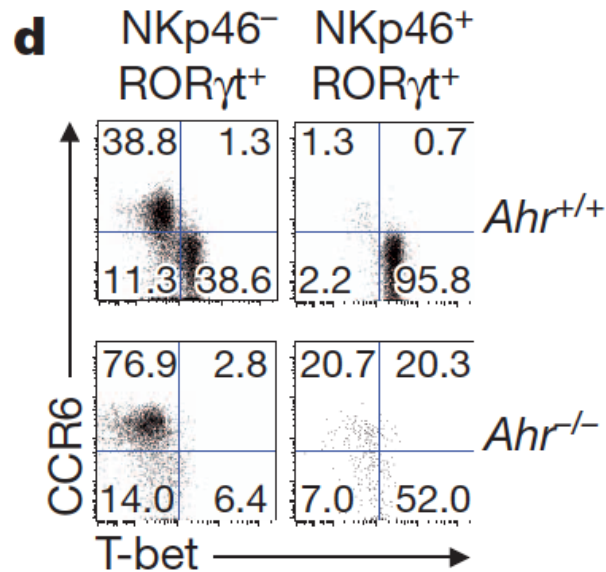
LP sl
Gate NKp46⁻ROR γ t⁺



Ahr TF is required for CCR6⁻RORγt⁺ ILC generation



(Kiss et al. Science 2011)

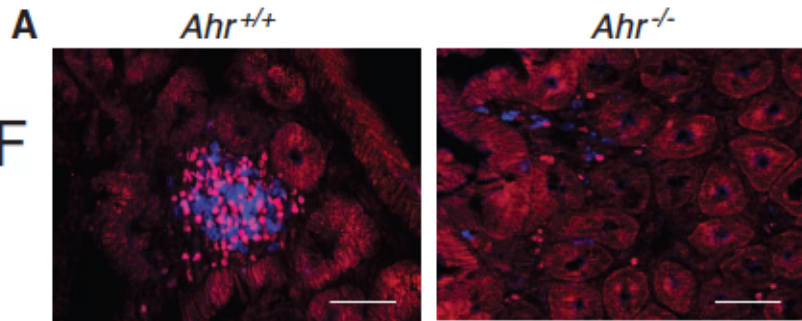


Absent of Nkp46⁺RORγt⁺ ILC and CCR6⁻RORγt⁺ ILC (both Tbet⁻ and T-Bet⁺ ?)

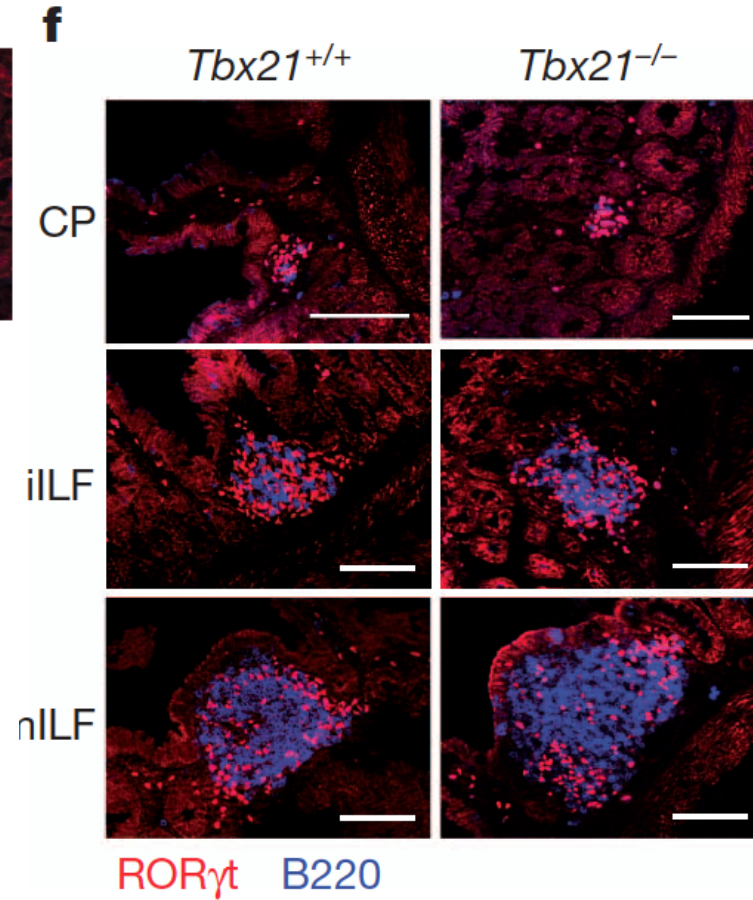
Important role of CCR6⁻RORγt⁺ on tertiary lymphoid structure formation

CCR6⁻RORγt⁺ (both Nkp46⁻ and Nkp46⁺)
absent in *Ahr*^{-/-}

CCR6⁻Nkp46⁻RORγt⁺ present in *Tbx21*^{-/-}
(Absent of Nkp46⁺RORγt⁺)

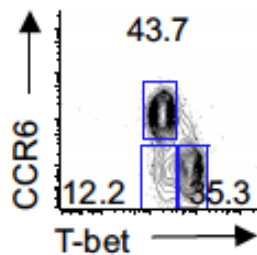
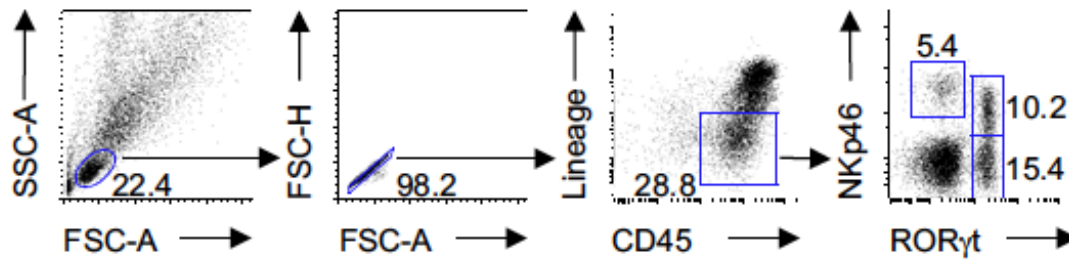


(Kiss et al. Science 2011)



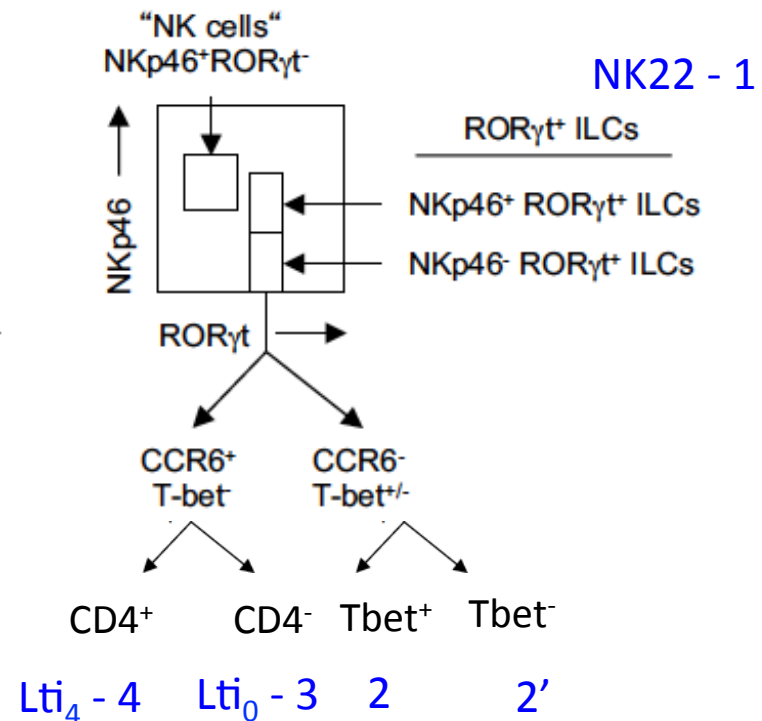
Gating strategy for intestinal ROR γ t⁺ ILC

LP small intestine



NKp46⁻ ROR γ t⁺ ILCs

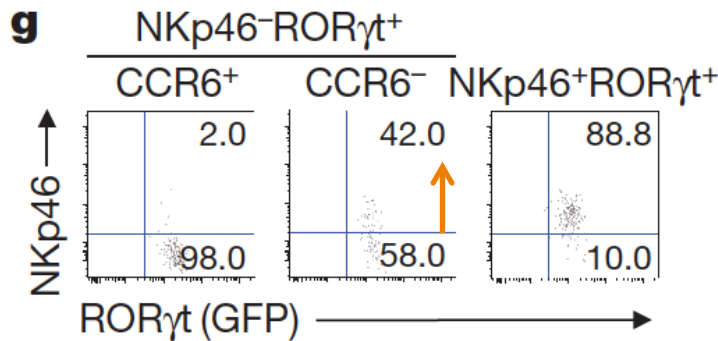
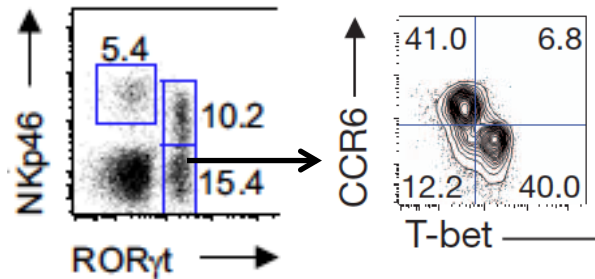
- CCR6⁻ T-bet⁺
- CCR6⁻ T-bet⁻
- CCR6⁺



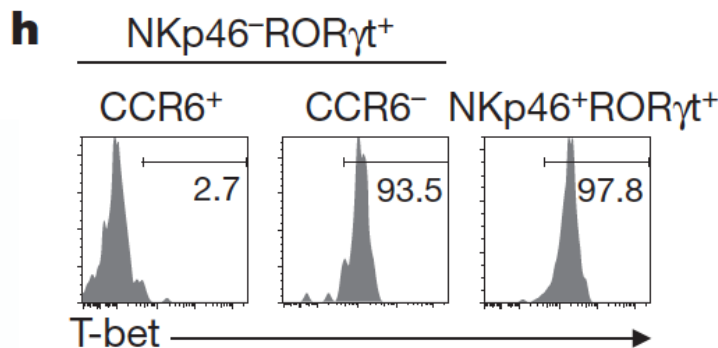
Hypothesis: CCR6⁻RORγt⁺ and CCR6⁺RORγt⁺ may constitute separate lineages

CCR6⁻Tbet⁻RORγt⁺ could be differentiated in CCR6⁻Tbet⁺RORγt⁺ and then acquire NKP46

Adoptive transfer of RORγt⁺ ILC subsets → Rag^{-/-} IL-2rg^{-/-} mice
(Analysis 3 weeks later)



Adoptive transfer of RORγt⁺ ILC subsets

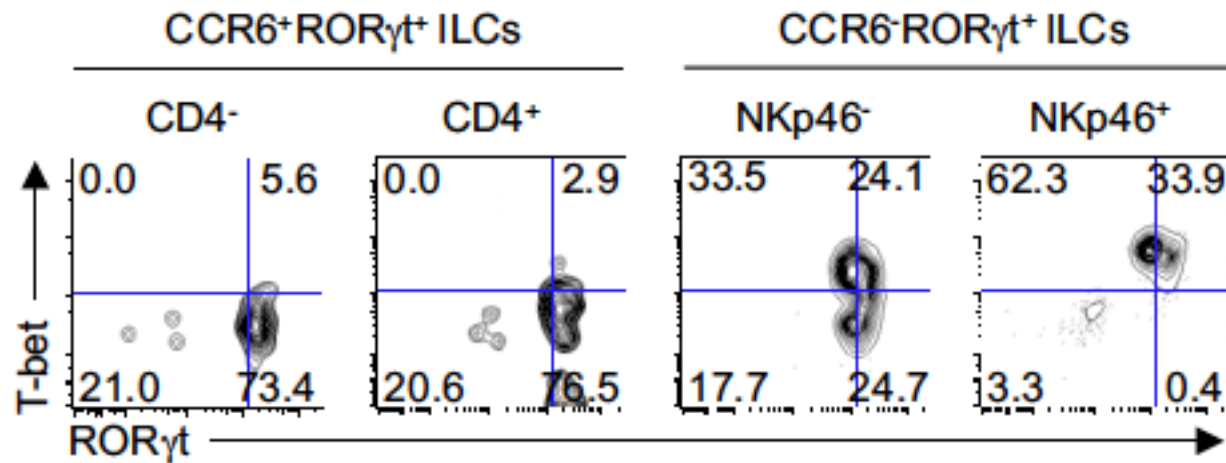


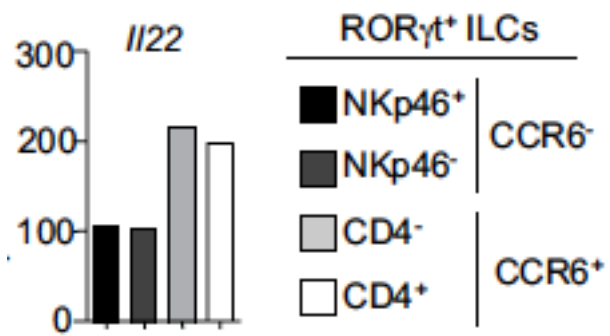
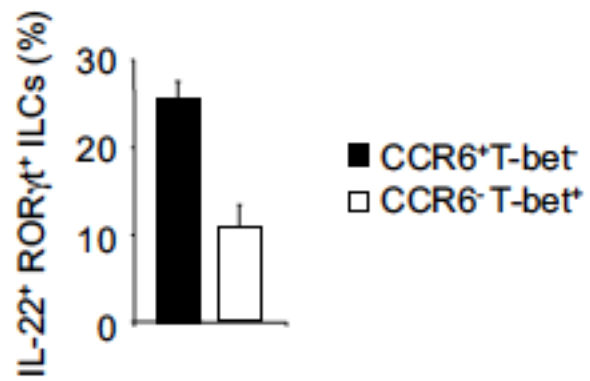
CCR6⁻RORγt⁺ (Tbet⁻ and Tbet⁺) can be differentiated in NKp46⁺RORγt⁺

CCR6⁻ROR γ t⁺ (Tbet⁻ and Tbet⁺) can be differentiated in NKp46⁺Tbet⁺ROR γ t⁺

ROR γ t⁺ ILC subsets culture in medium with IL-2, IL-7 and SCF (Analysis 2 weeks later)

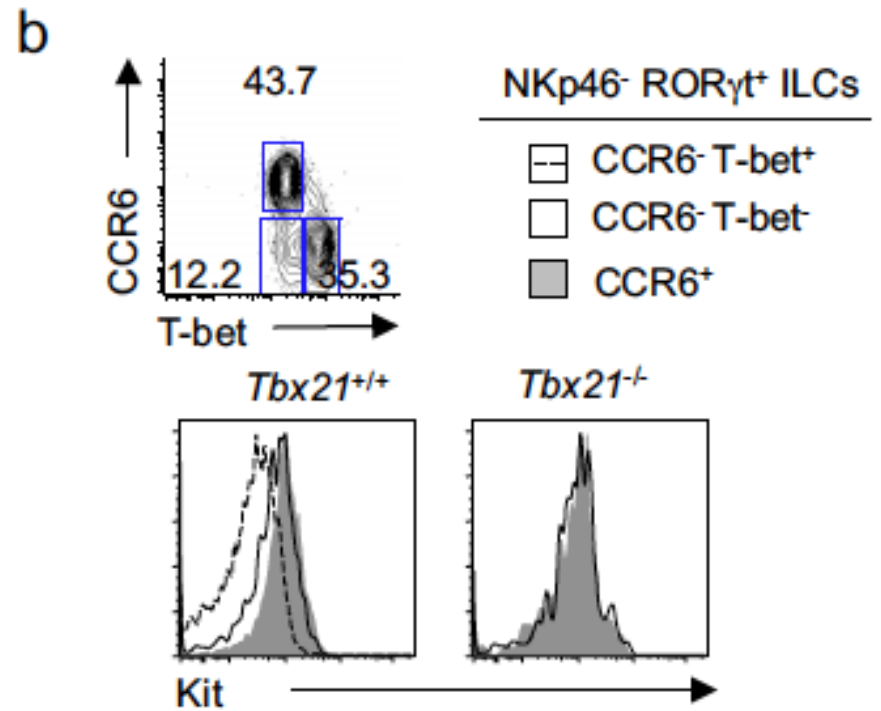
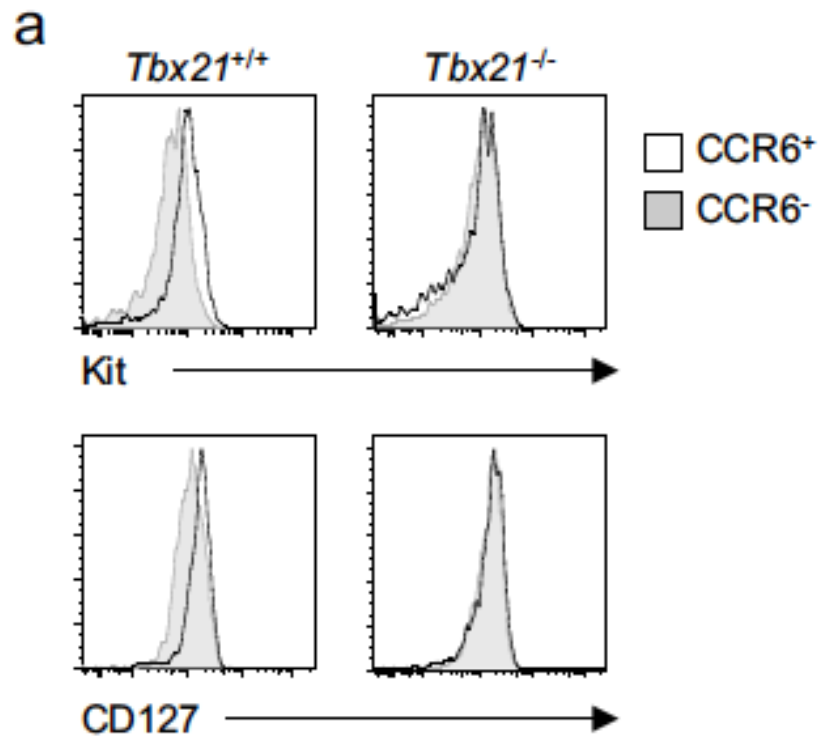
b





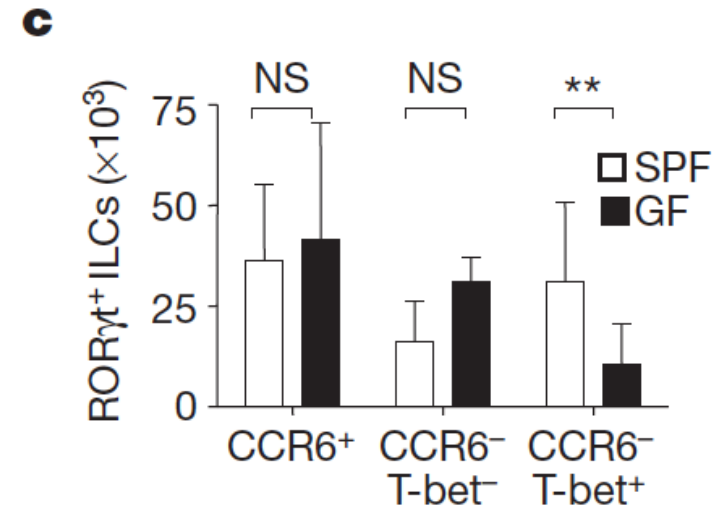
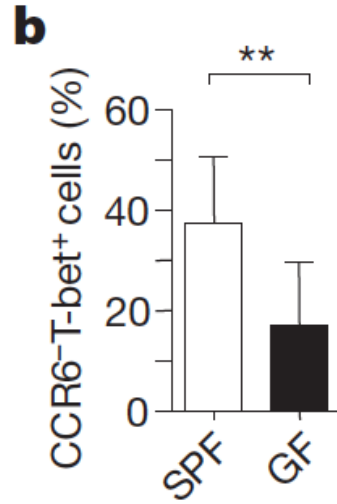
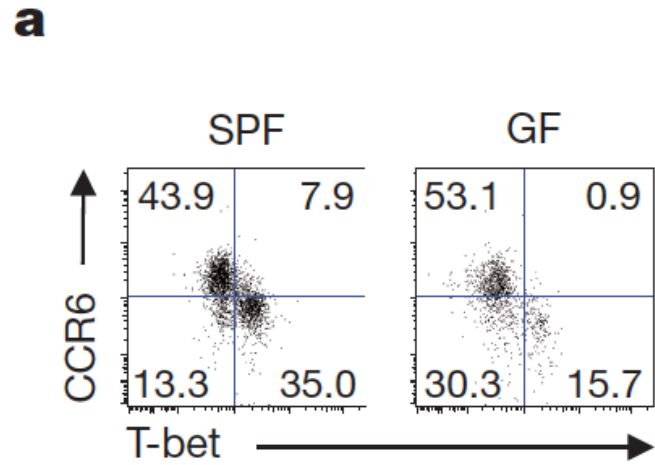
CCR6⁻Tbet⁻RORγt⁺ express c-kit but not CCR6⁻Tbet⁺RORγt⁺

LP sl



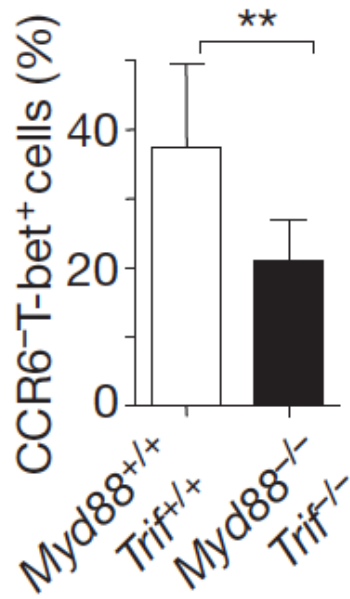
CCR6-Tbet⁺RORγt⁺ ILC are reduced in GF mice

LP sl
Gate NKp46⁻RORγt⁺

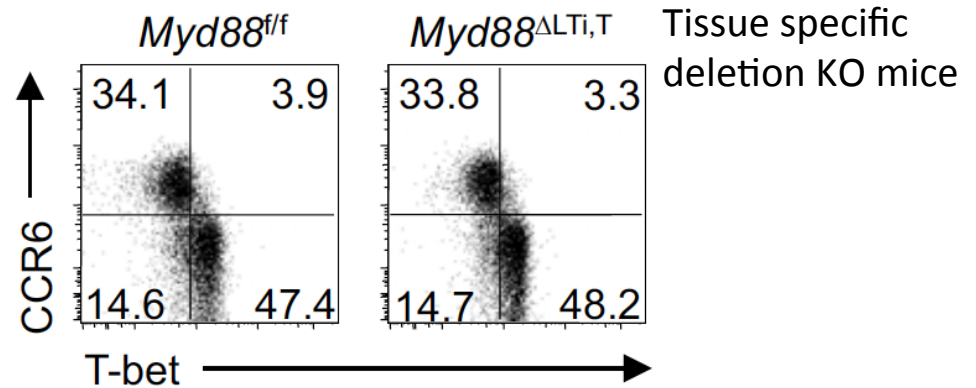


Generation of CCR6⁺Tbet⁺ROR γ ⁺ ILC depends on microbial signal

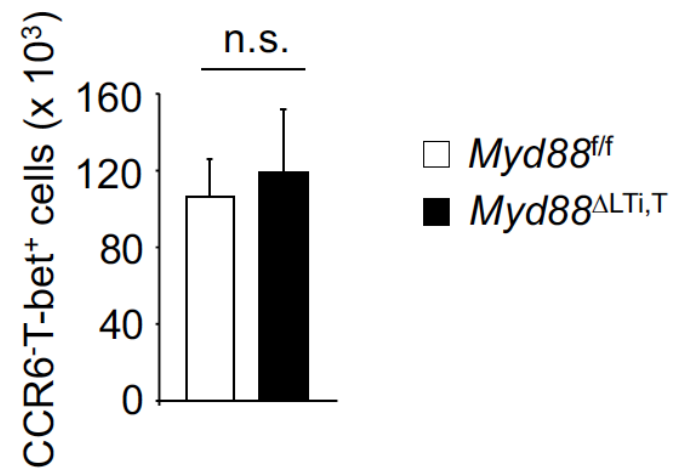
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a

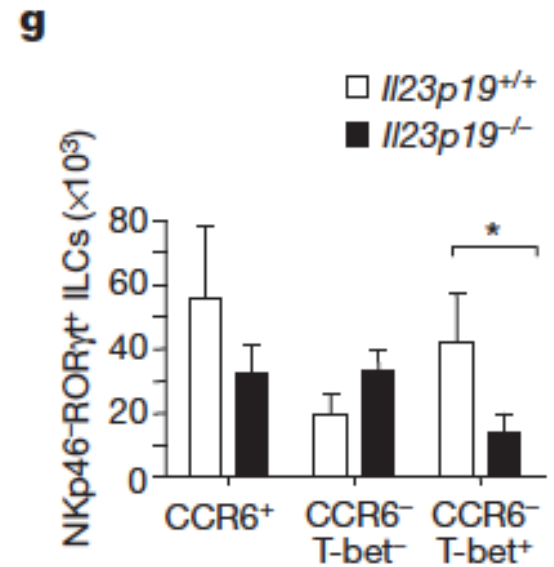
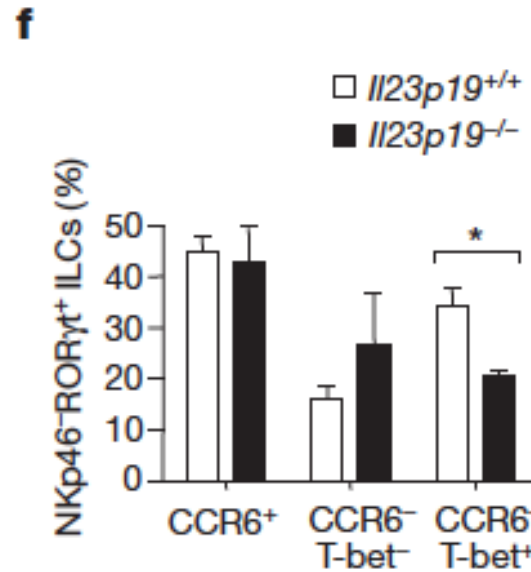
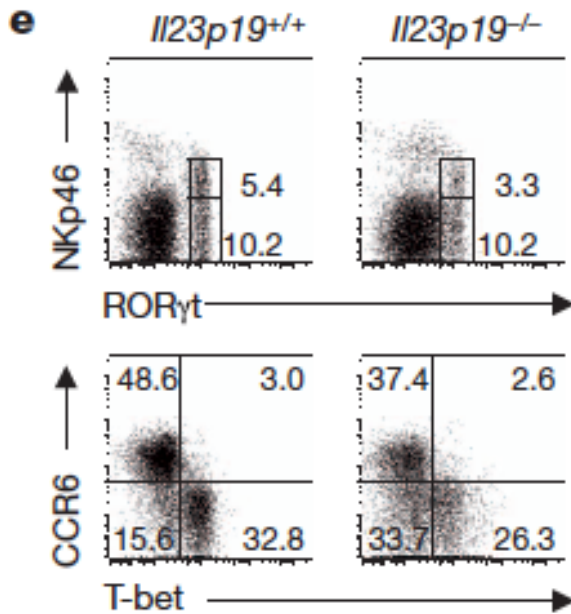


c



Generation of CCR6⁻Tbet⁺RORγt⁺ ILC depends on IL-23

IL-12 signalling (STAT4^{-/-} and IL2rg^{-/-} mice) is dispensable for CCR6⁻Tbet⁺RORγt⁺ ILC generation

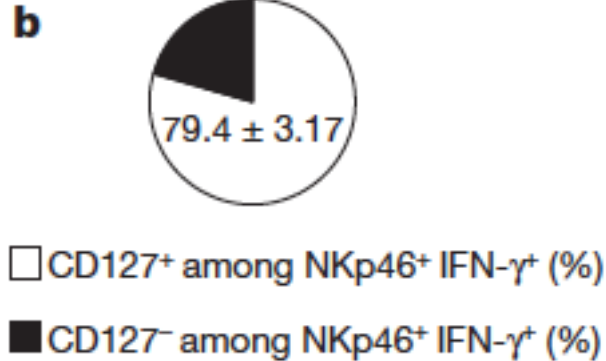
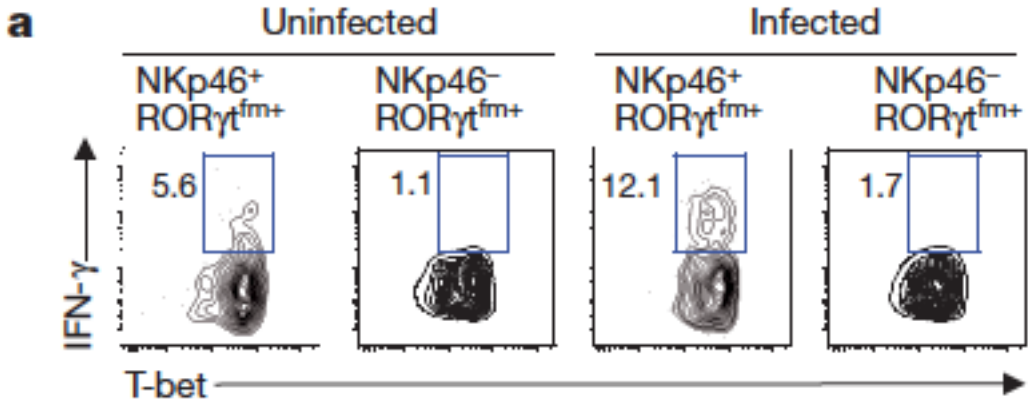


Nkp46⁺Tbet⁺RORγt^{fm+} ILC are the main source of IFNγ in *Salmonella enterica* infection

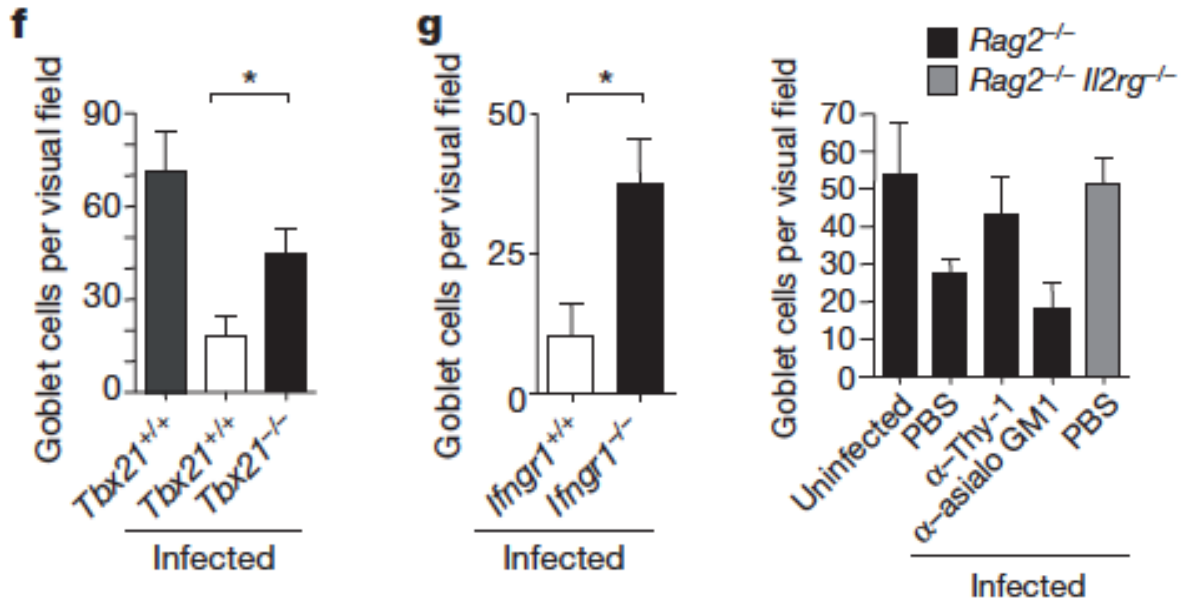
Salmonella enterica infection resistance required IFNγ (Songhet et al. Plos one 2011; Rhee et al. JI 2005)

Fasted for 4h → ig with streptomycin 24h before infection → Fasted for 4h → gavage with 10⁵-10⁷ CFU

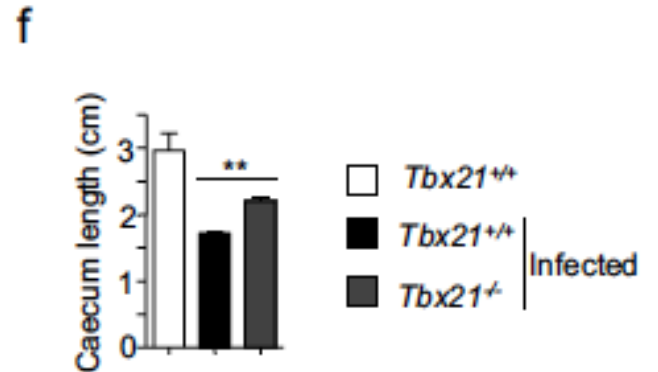
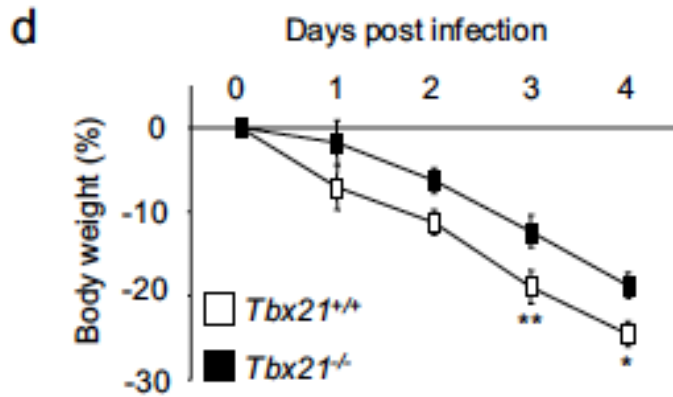
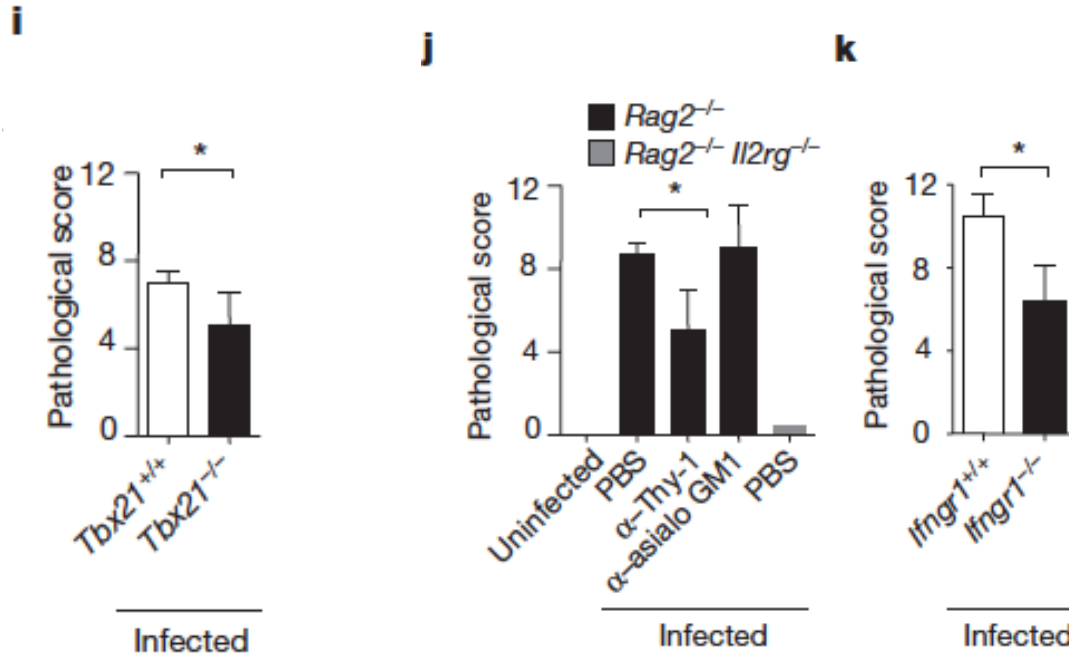
1 d after infection
LP sl



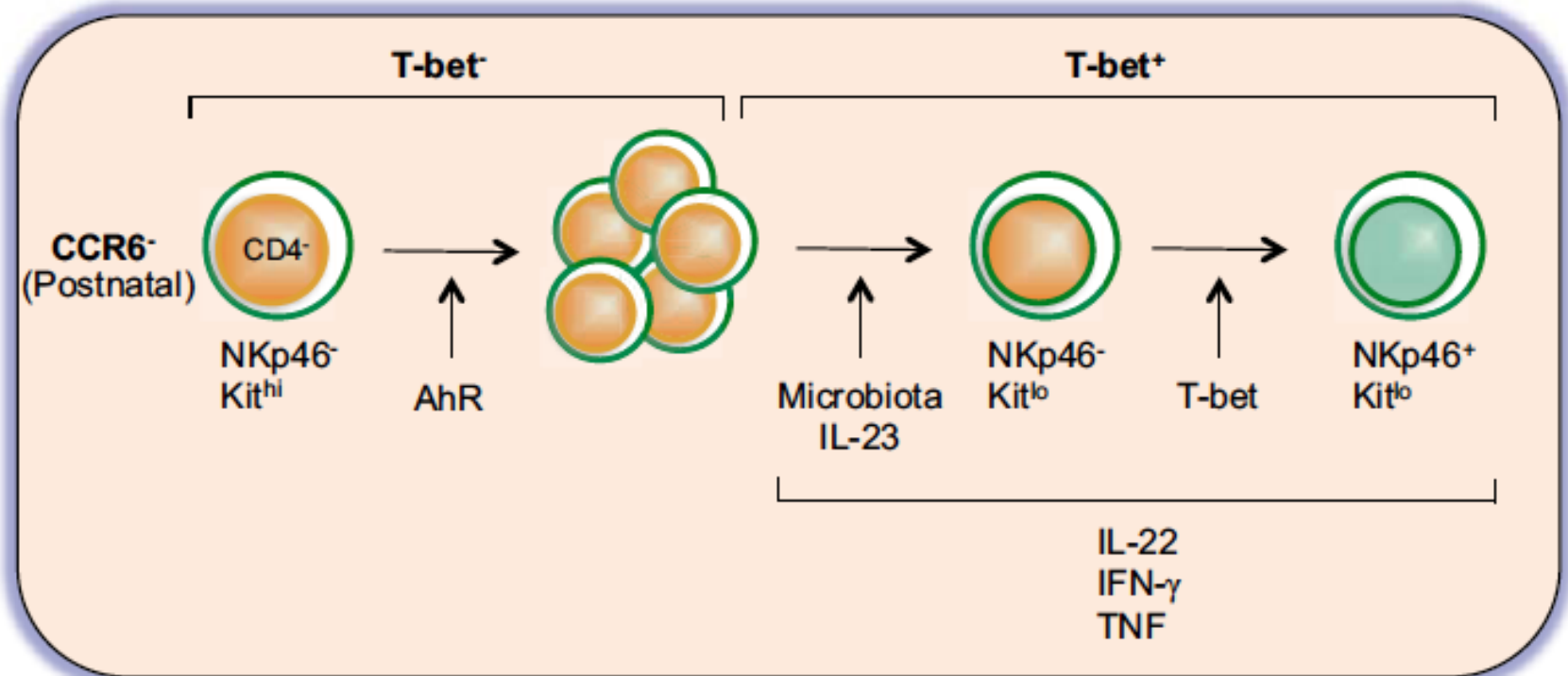
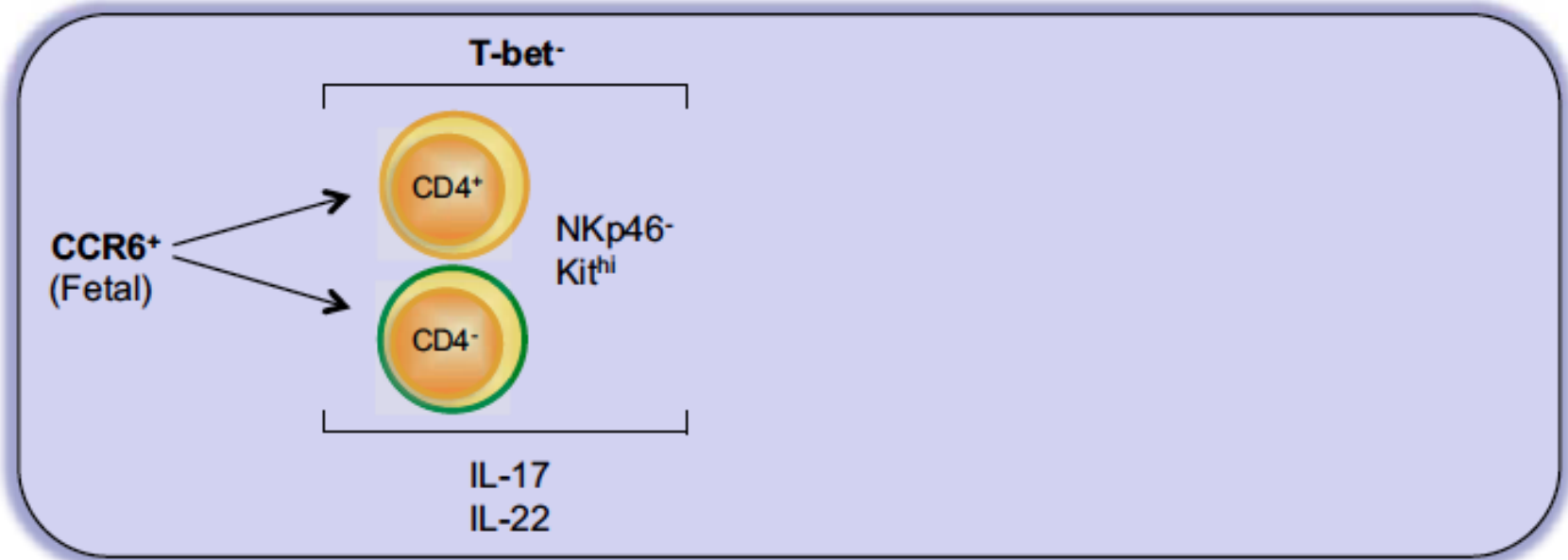
Selective depletion of Nkp46⁺Tbet⁺RORγt⁺ ILC reduce the IFNg induced mucus secretion



Nkp46⁺Tbet⁺RORγt⁺ ILC contribute to the control of *Salmonella enterica* infection



Nkp46⁺Tbet⁺RORγt⁺ ILC switch to pro-inflammatory phenotype in response to extracellular intestinal pathogens



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doi:10.1038/nature11809

Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX₃CR1^{hi} cells

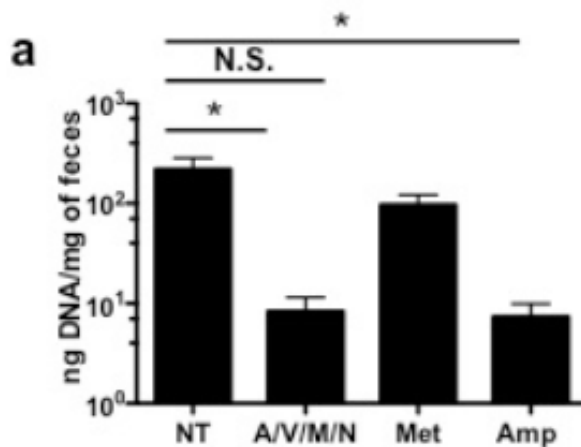
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Host sensing of commensals have been shown to be important for proper development and functionality of immune system

Hypothesis: Have commensal microbiota a role in modulating mucosal immune responses to specific microbes?

What are the consequences of antibiotic-mediated depletion of intestinal microbiota on the response to non-invasive strain of *Salmonella enterica serovar Typhimurium* ?

Tools



Antibiotic treatment:
Ampicillum, vancomycin,
neomycin, metronidazole in
drinking water for 4 weeks

Antibiotic treatment reduce commensal microbiota

Salmonella enterica serovar Typhimurium :

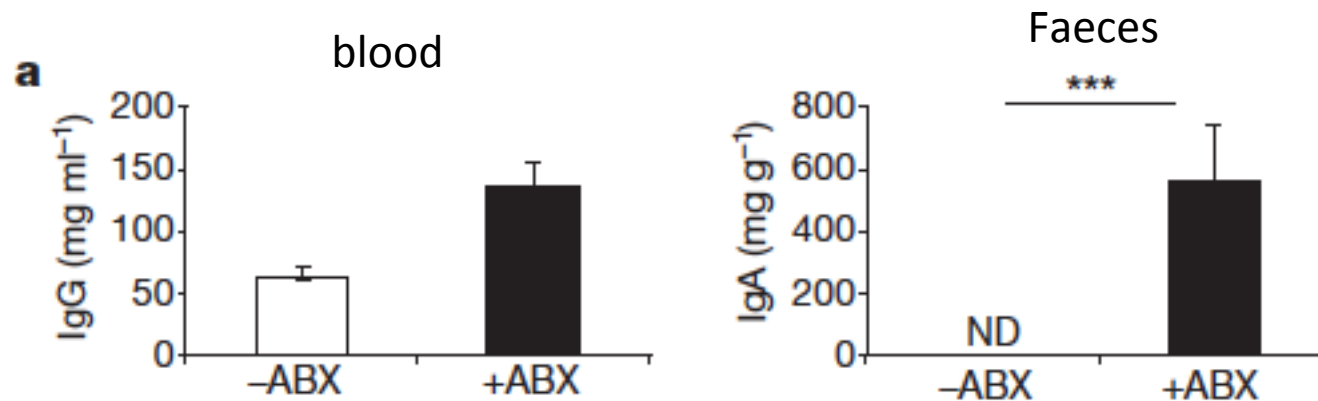
- has limited CD18-dependant access to the blood and spleen.
- Cannot cross the epithelium overling intesitnal lymphoid tissue
- Does not reach PP

Mucosal Ab responses to *Salmonella* infection is only observed after antibiotic treatment

Antibiotic treatment in drinking water for 4 weeks and 2 days with water before ig with 10^9

CFU of *Salmonella Ainv*

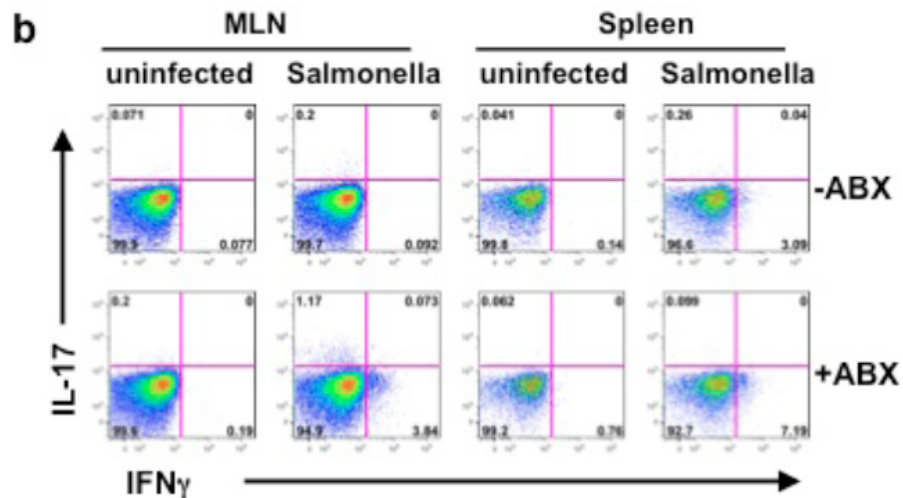
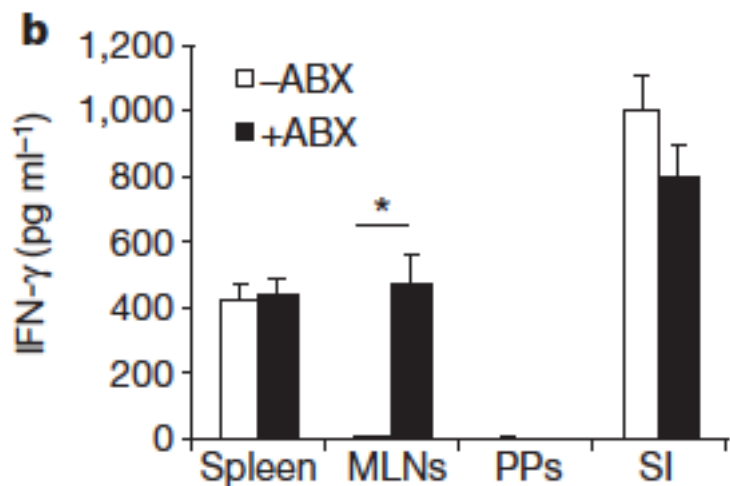
Analysis of specific Ab 2 days after infection



MLN specific immune responses to *Salmonella* infection only detected after antibiotic treatment

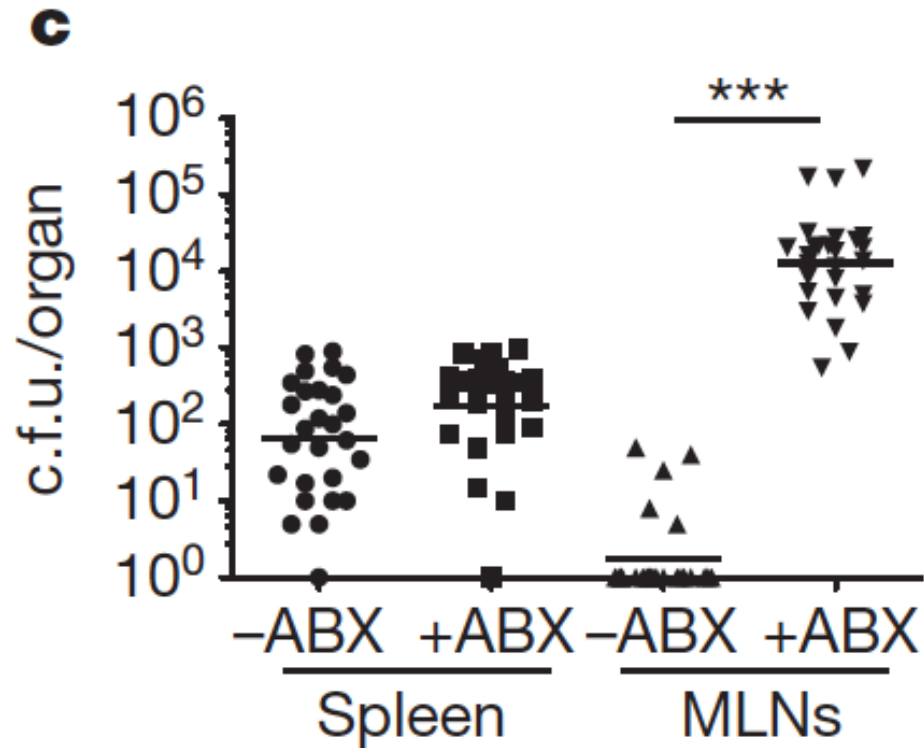
Analysis of 2 days after infection (co-culture of T cells and Ag loaded APC for 3 days)

Analysis of 9 days after infection (6h after injection of bref and Ag)



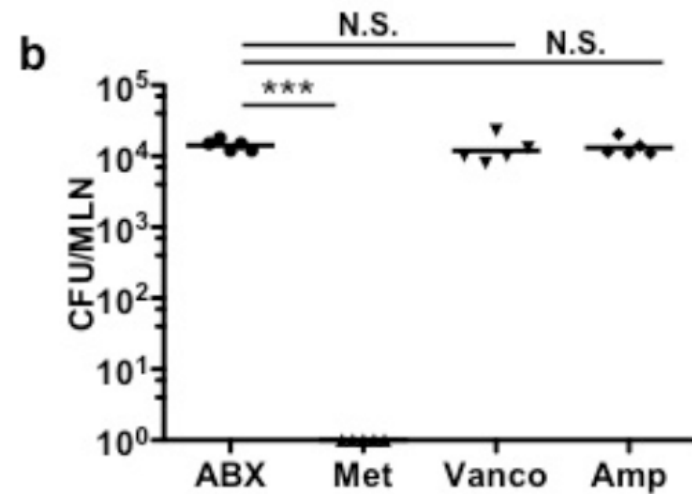
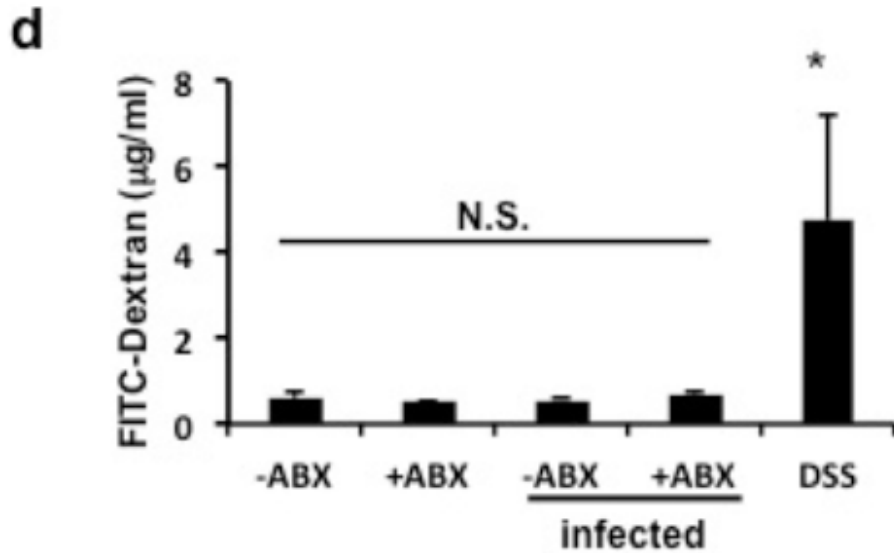
Commensal microbiota influence the amount of Ag that reaches the MLN

Analysis of 2 days after infection



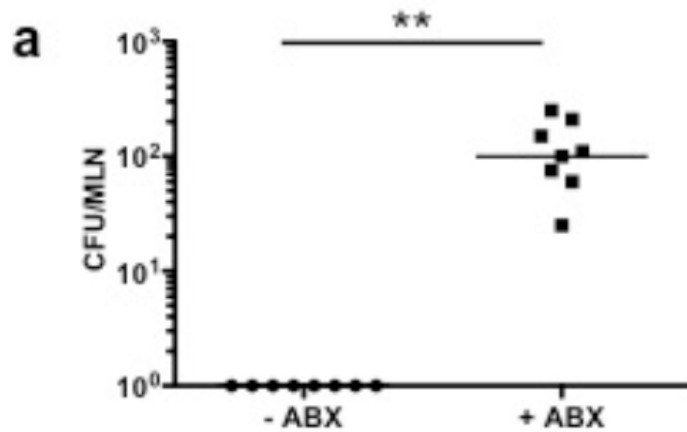
The bacterial that reach MLN aren't consequence of increase on epithelium permeability, neither impaired on colonization resistance

o.n. fasted and analyses 3h after gavage with FITC-Dextran

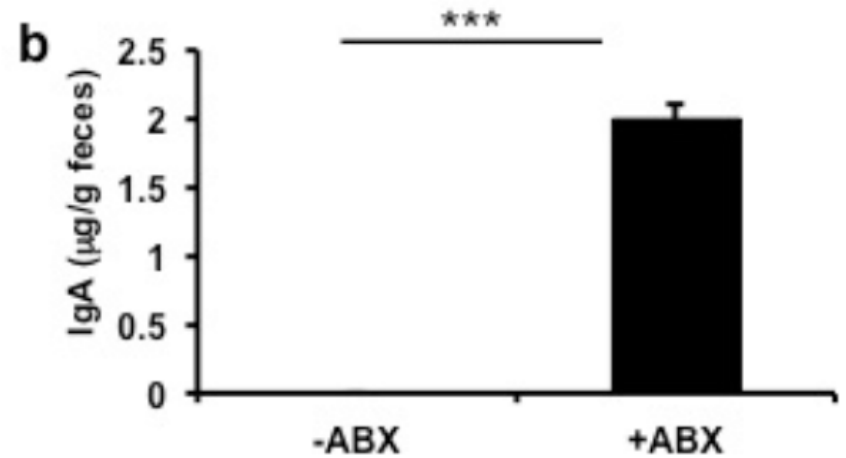


Commensal bacterial could reach MLN as well as *Salmonella*, only after depletion of commensal flora

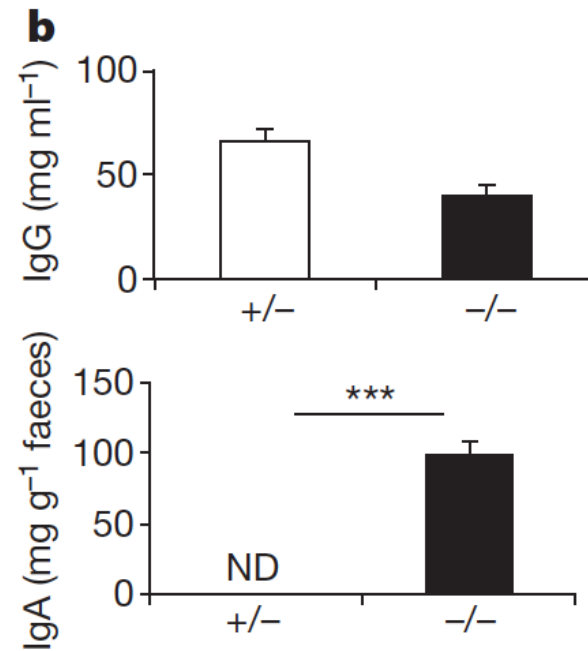
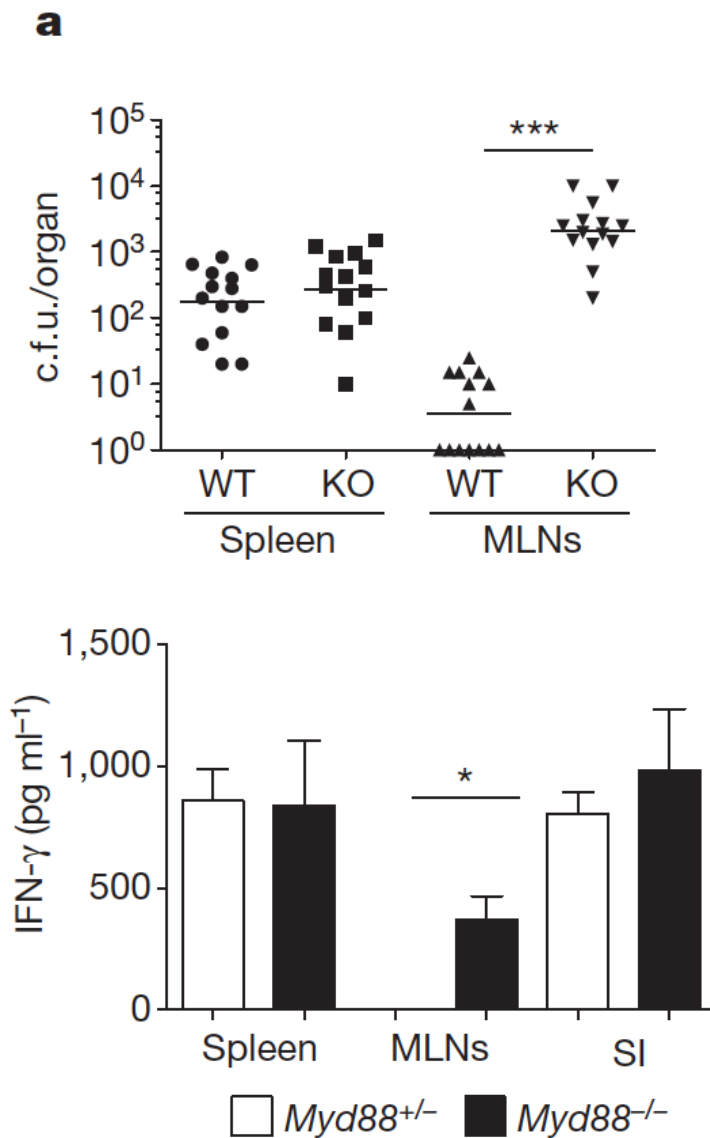
Antibiotic treatment in drinking water for 4 weeks and 2 days with water before ig with 10^9 CFU of *E. Coli*
Analysis of specific Ab 2 days after colonization



Analysis of specific Ab 14 days after colonization

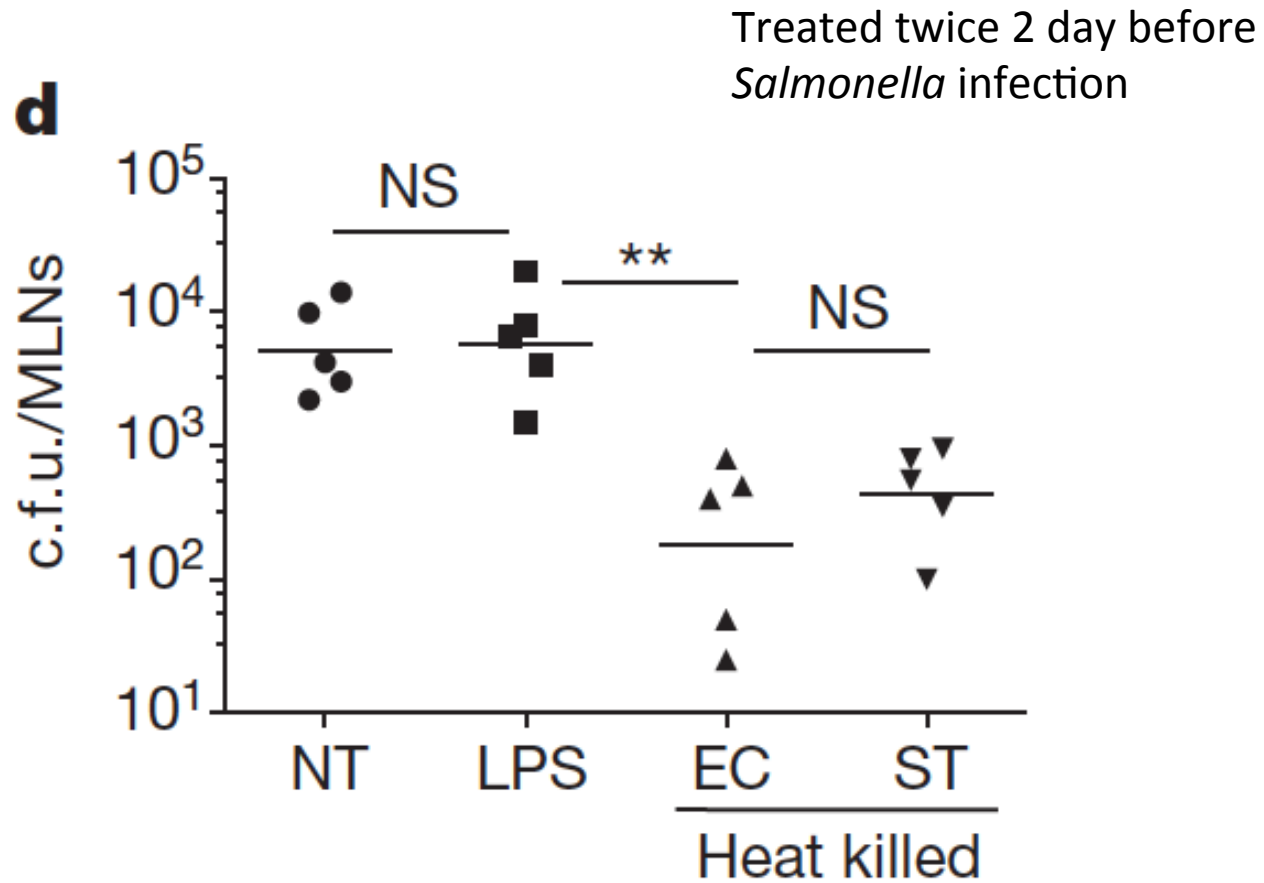


MyD88 pathway prevents *Salmonella* reach MLN



Not effect on *Nod-2* or *NALP3* deficient mice

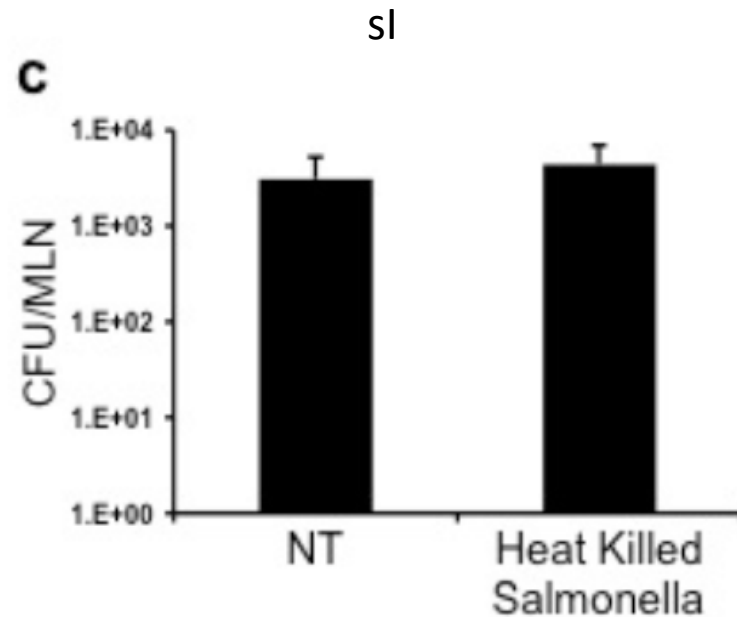
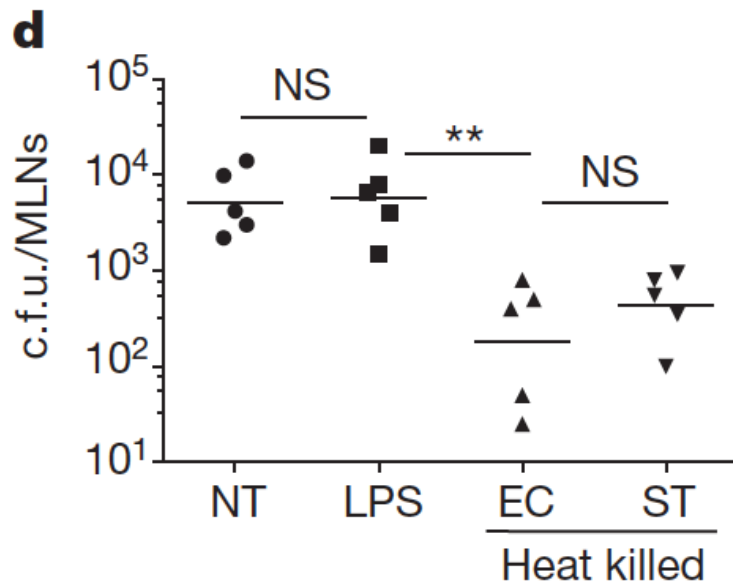
Microbial product cannot prevent Salmonella reaching MLN



Not effect on Nod-2 or NALP3 deficient mice

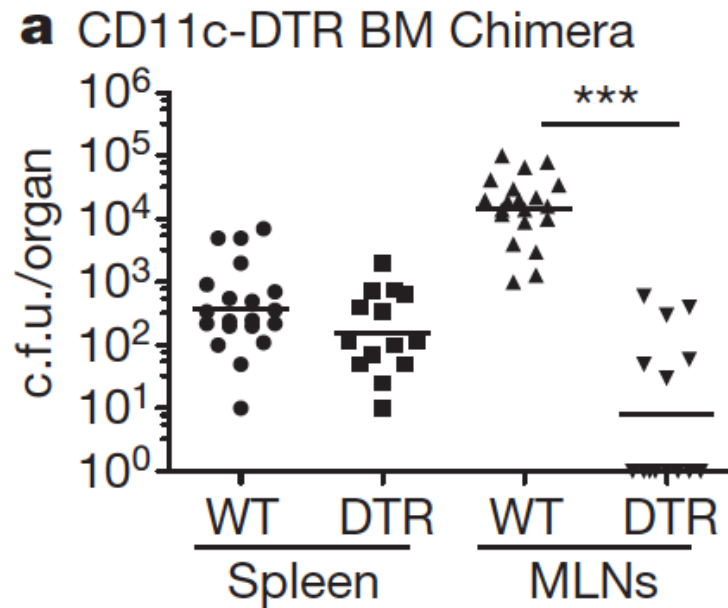
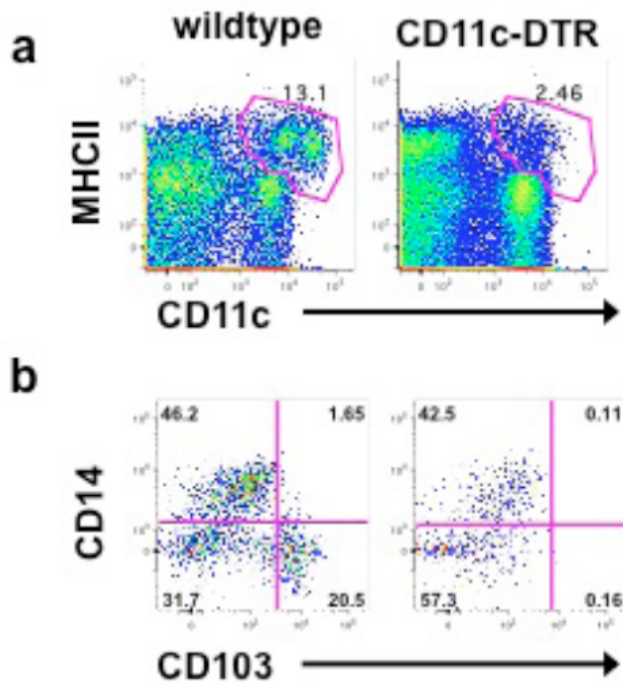
Inactivated bacteria but not commensal residues can prevent *Salmonella* from reaching MLN

Treated twice after antibiotic treatment and 2 days before *Salmonella* infection



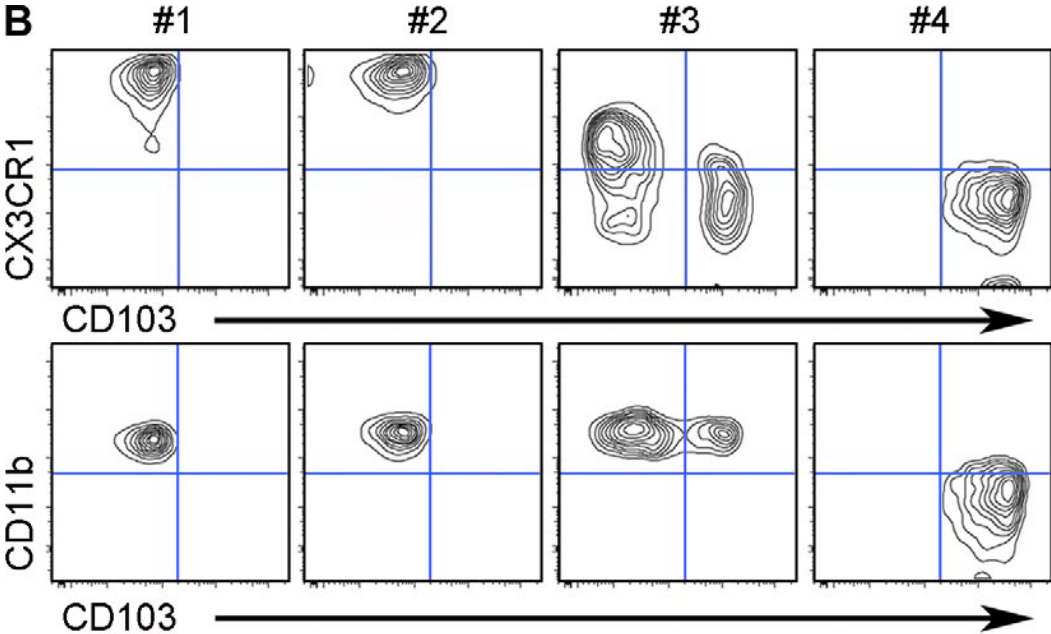
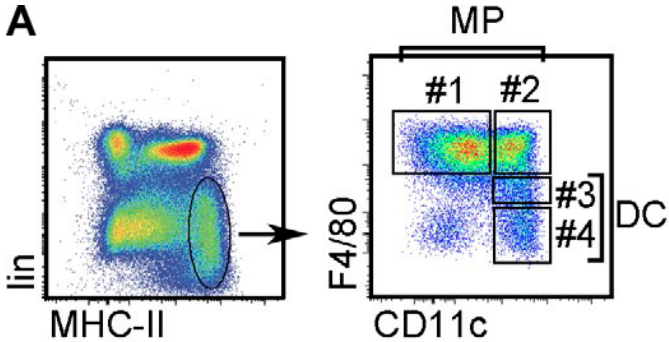
Not effect on *Nod-2* or *NALP3* deficient mice

Colonization of MLN by Salmonella requires CD11c DC

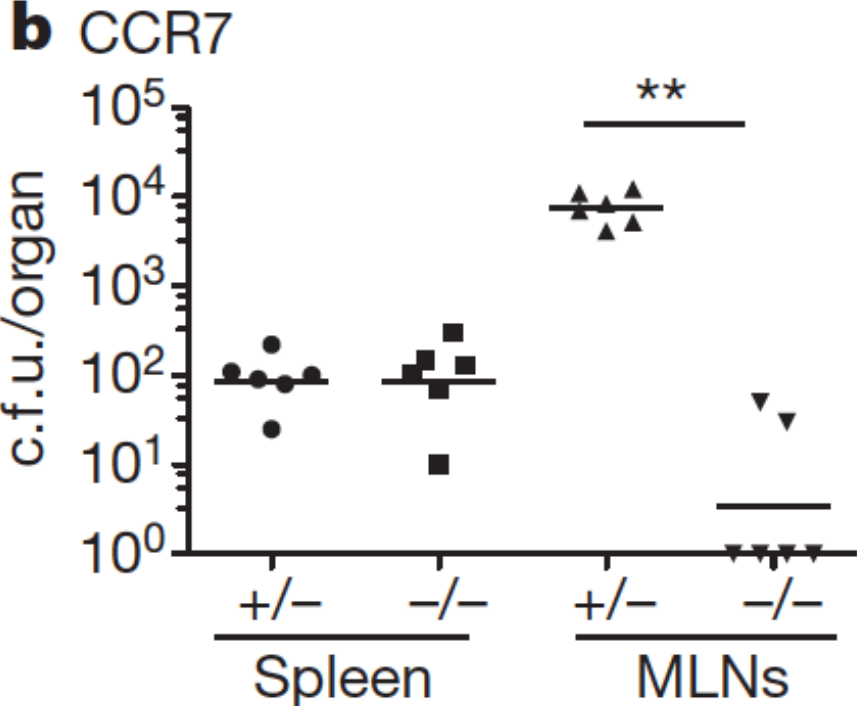


DT injected twice 2 days before infection

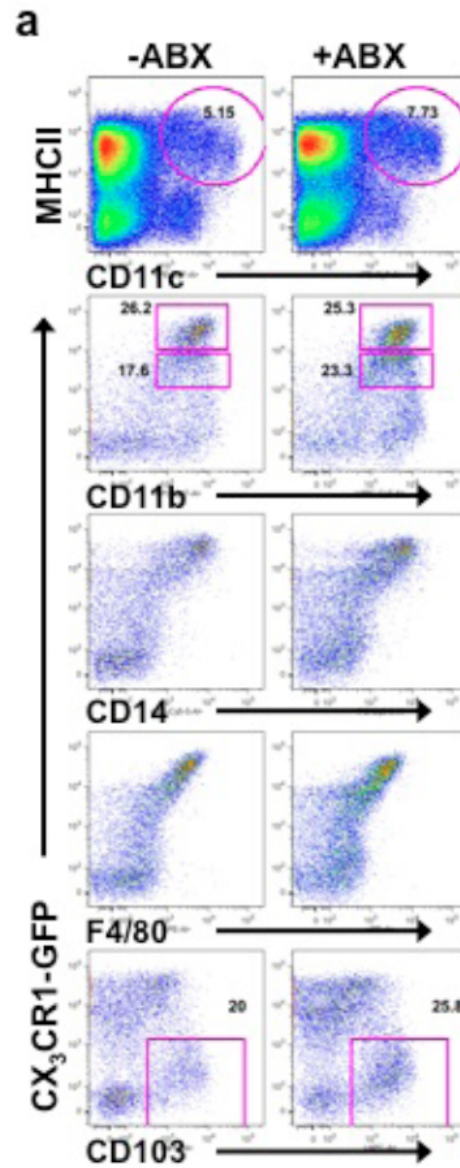
Intestinal mononuclear cells (iMNC)



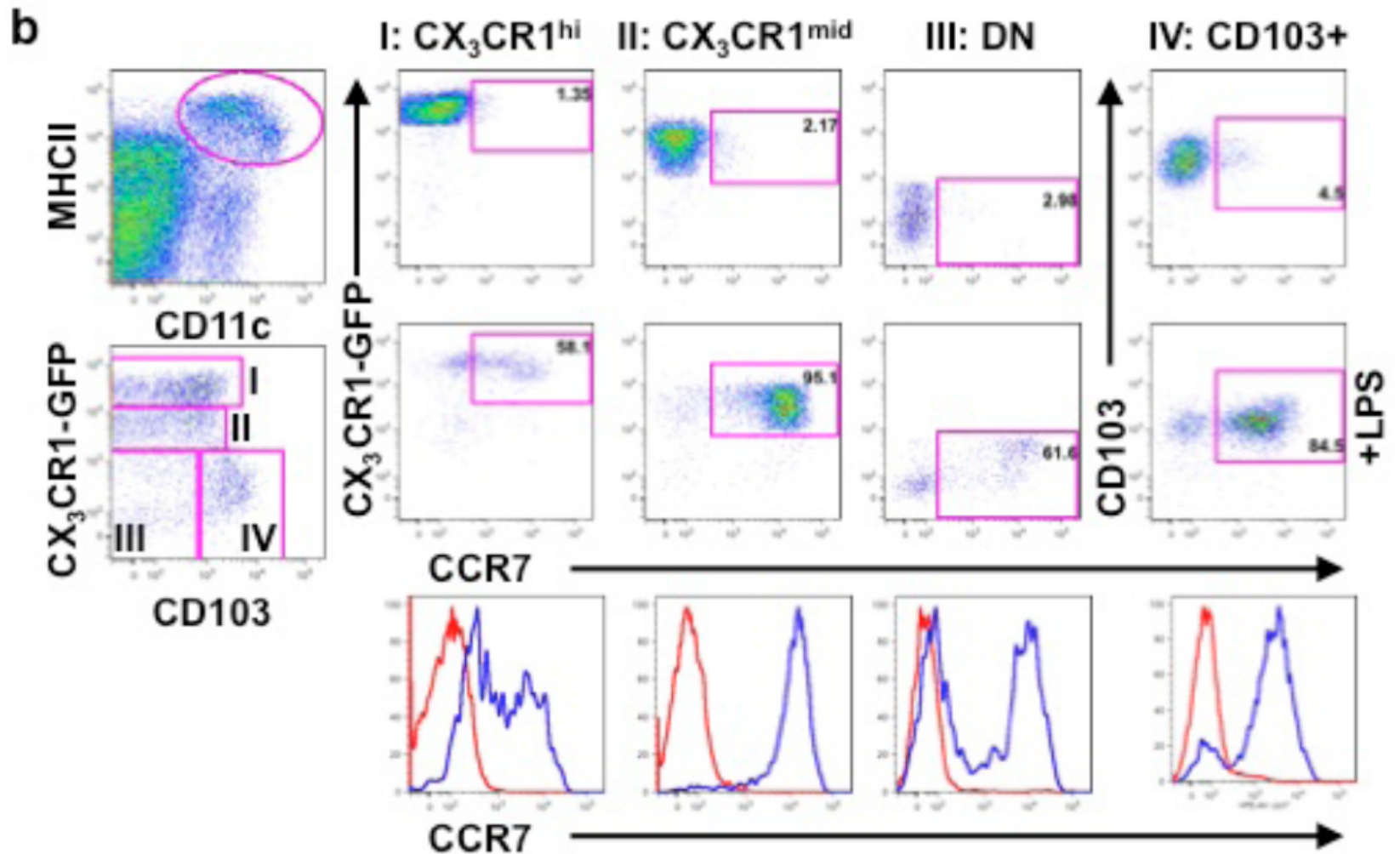
Colonization of MLN by Salmonella requires the chemokine receptor CCR7



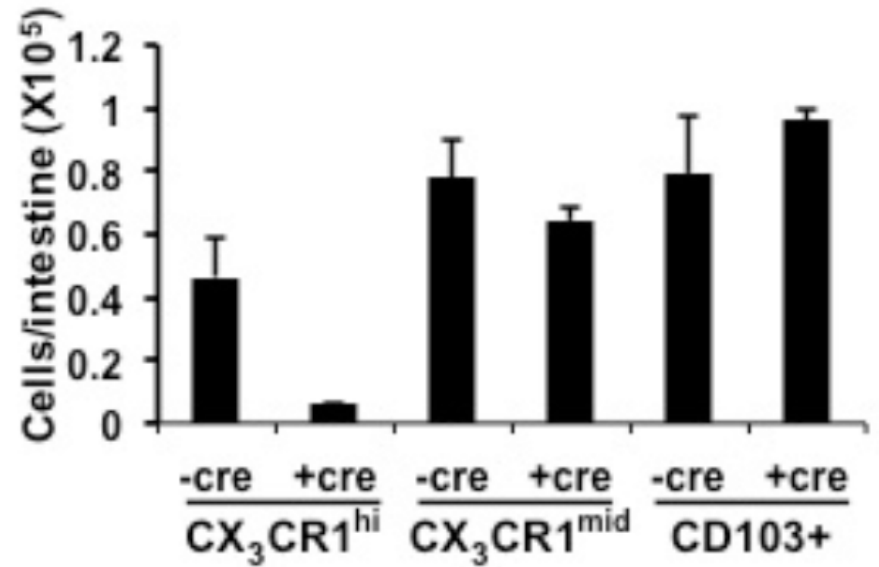
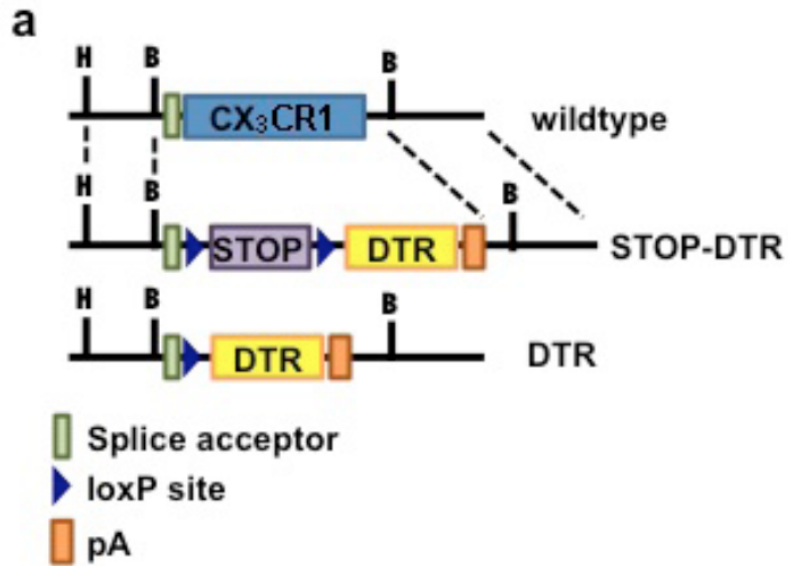
Intestinal monocular cells aren't affected by abx treatment



LPS induce CCR7 expression on both intestinal macrophage and DC

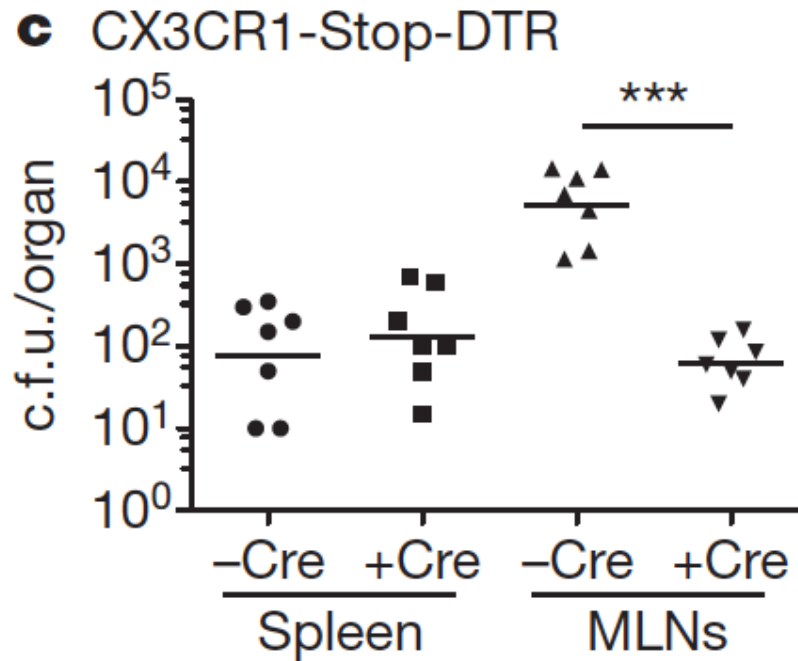


Specific depletion of CX3CR1^{hi} cells DC



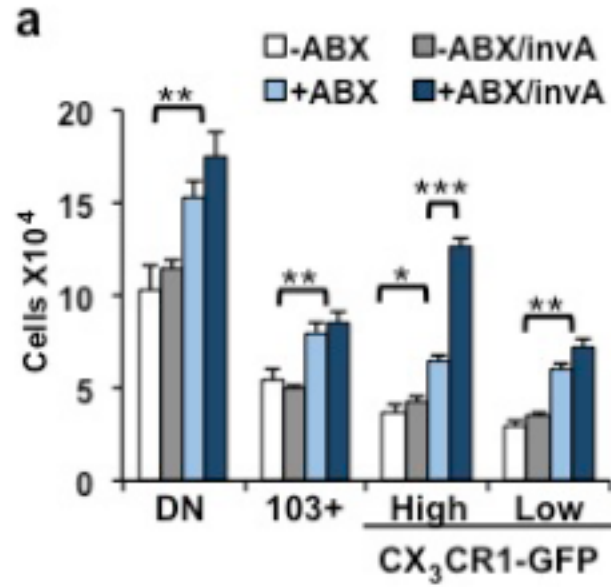
DT injected three times with DT

CX3CR1^{hi} cells DC could responsible of trafficking of *Salmonella* to MLN

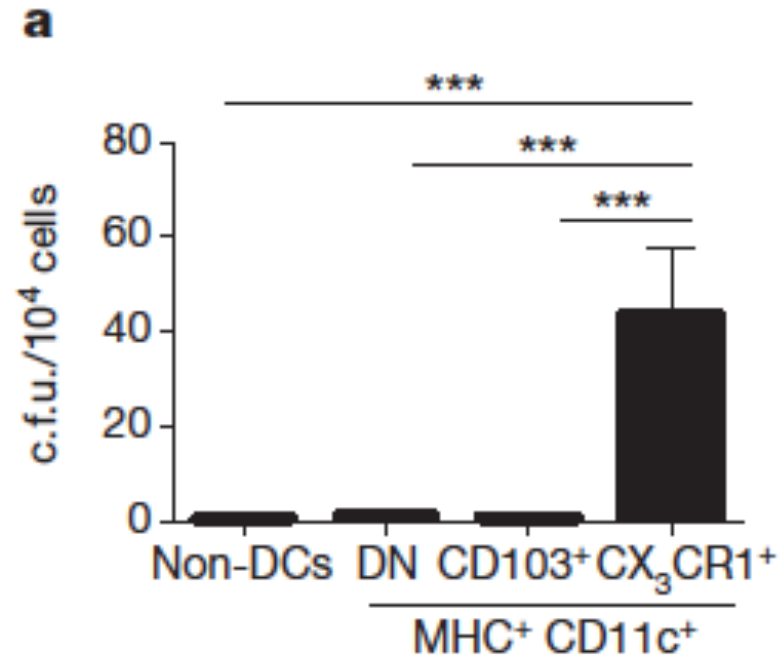
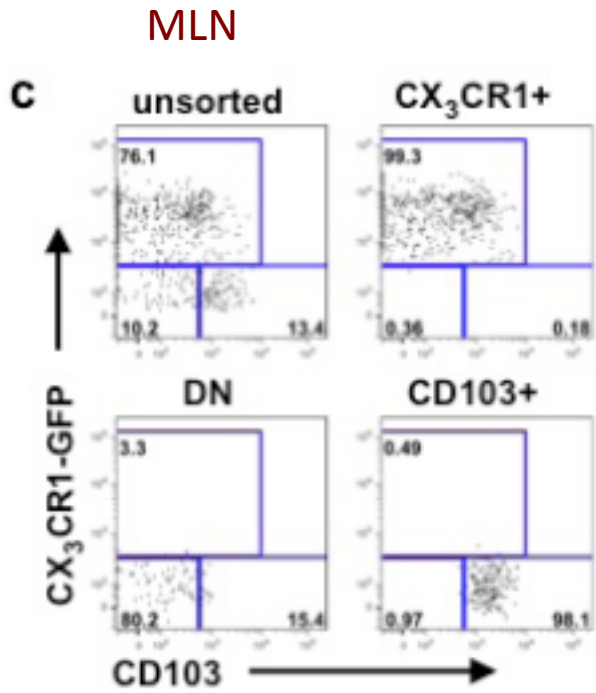


DT injected twice 2 days before infection

Salmonella colonization increase the number of DC in MLN



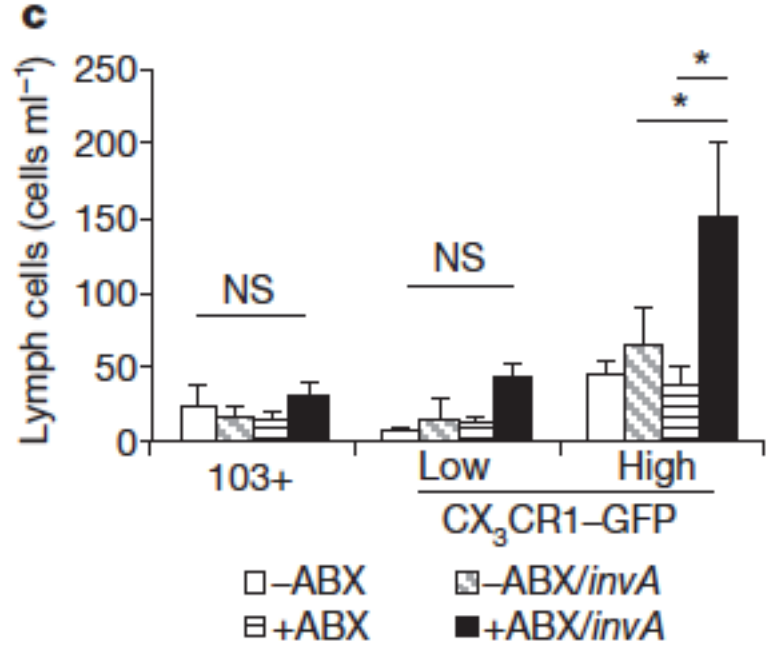
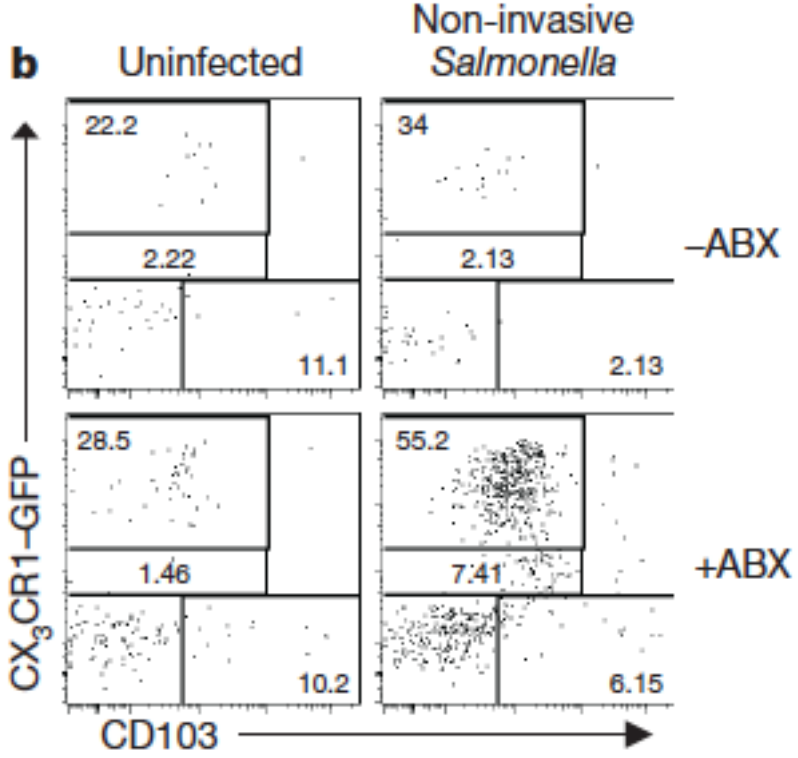
CX3CR1^{hi} cells DC are the main MNC subset loaded with the bacteria



2d after infection

CX3CR1^{hi} cells DC are the main MNC subset in the afferent lymph after *Salmonella* infection

Afferent lymph



2d after infection