# Journal Club

Mercedes GOMEZ DE AGÜERO

January 10th 2013

# Nonredundant Function of Soluble LT$\alpha_3$ Produced by Innate Lymphoid Cells in Intestinal Homeostasis

Andrey A. Kruglov,[1,2*] Sergei I. Grivennikov,[3] Dmitry V. Kuprash,[2,4] Caroline Winsauer,[1] Sandra Prepens,[1] Gitta Maria Seleznik,[5] Gerard Eberl,[6] Dan R. Littman,[7] Mathias Heikenwalder,[5,8] Alexei V. Tumanov,[9] Sergei A. Nedospasov[1,2,4*]

SCIENCE VOL 342 6 DECEMBER 2013

# TCF-1 Controls ILC2 and NKp46$^+$RORγt$^+$ Innate Lymphocyte Differentiation and Protection in Intestinal Inflammation

Lisa A. Mielke, Joanna R. Groom, Lucille C. Rankin, Cyril Seillet, Frederick Masson, Tracy Putoczki and Gabrielle T. Belz

*J Immunol* 2013; 191:4383-4391; Prepublished online 13 September 2013;

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# IgA

Contributes to host defense against intestinal pathogens and control and contains commensal microbiota

Induced by :

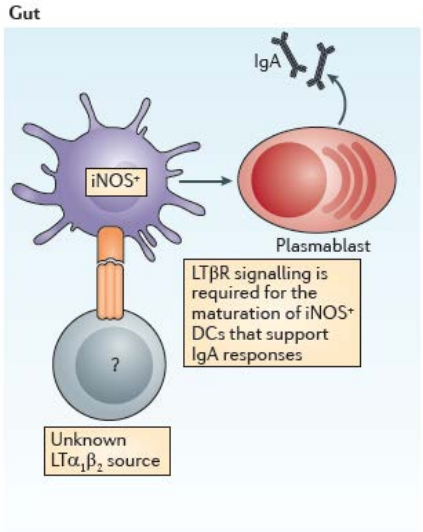
- T cell dependant pathways : takes place in PP and requires germinal centers formation

- T cell independant pathways : takes place in ILF and LP sI and is indepedant of germinal centers formation

# Lymphotoxin (LT) α & β

- Trimeric cytokines of the tumor necrosis factor superfamily
- Expressed by T, B cells, RORγt$^+$ ILC
- LTα3 is a soluble form and signal via TNFR1 and TNFR2
- LTα1β2 is a membrane-bound form signal via LTβR

Inactivation of LTα, LTβ or LtβR : block lymphoid organ development and diminish mucosal IgA → Membrane bound LT is critical for intestinal IgA




*Upadhyay Nature Rev 2013*

Which cell subtype is involved in the activation of INOS$^+$ DC?

What is the contribution of soluble LT ?

# Innate lymphoid cells (ILC)

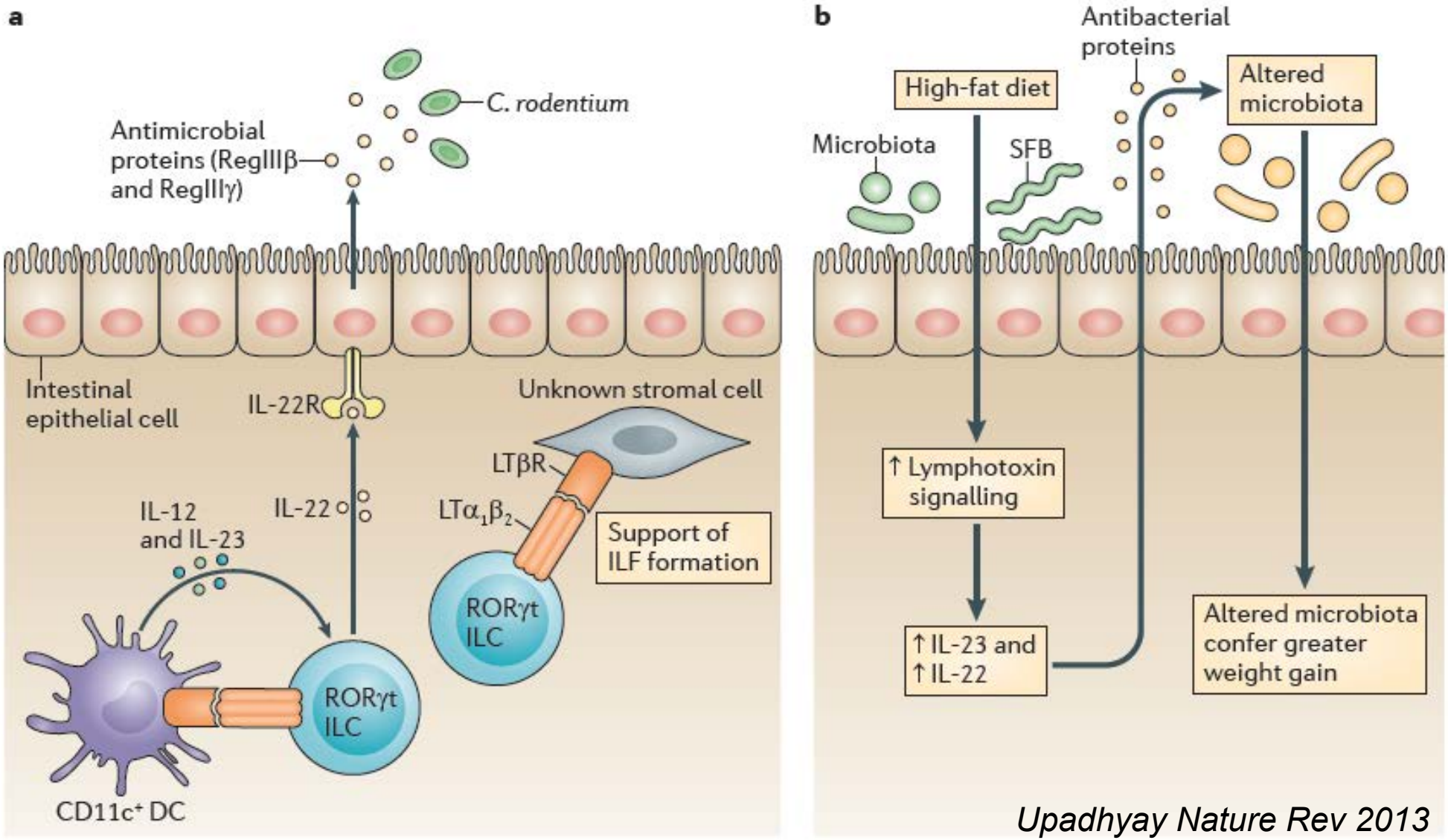
- Innate immune cells
- Lack specific Ag receptors
- Produce a large effector cytokines : IL-17, IL22, IL5, IL13
- Located in mucosal tissues

RORγt$^{+}$ ILC :

- via LT induce lymphoid tissues development : PP, LN, ILF
- Critical for protection against intestinal pathogens
- Mainteanance of epithelial barrier
- Prevention of systemic dissemination of commensal microbiota

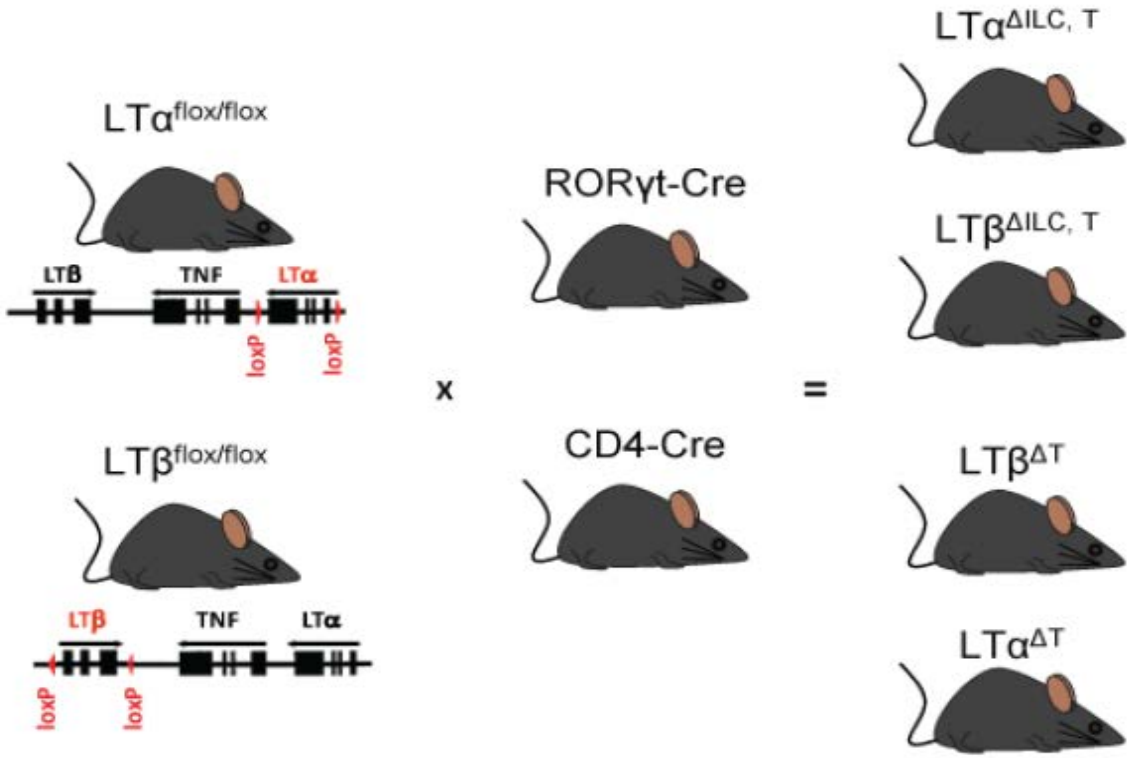
Which molecular mecanism is used by RORγt⁺ ILC to control commensals ?

## Hypothese : Could RORγt⁺ ILC use LT to control commensals ?




*Upadhyay Nature Rev 2013*

# MICE USED

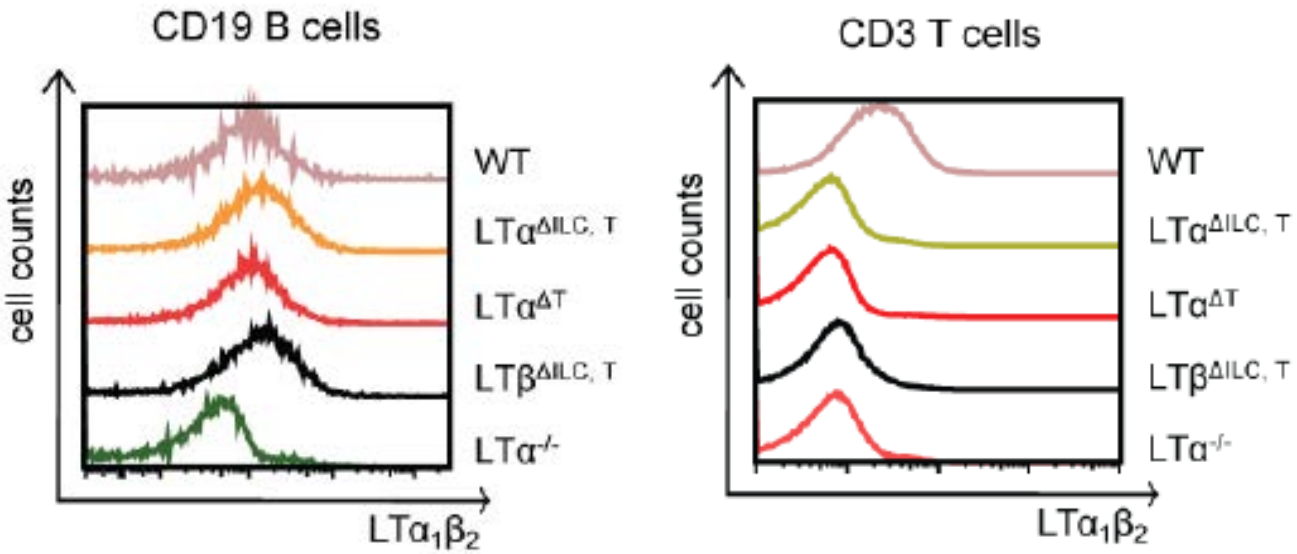



## Deficient on :

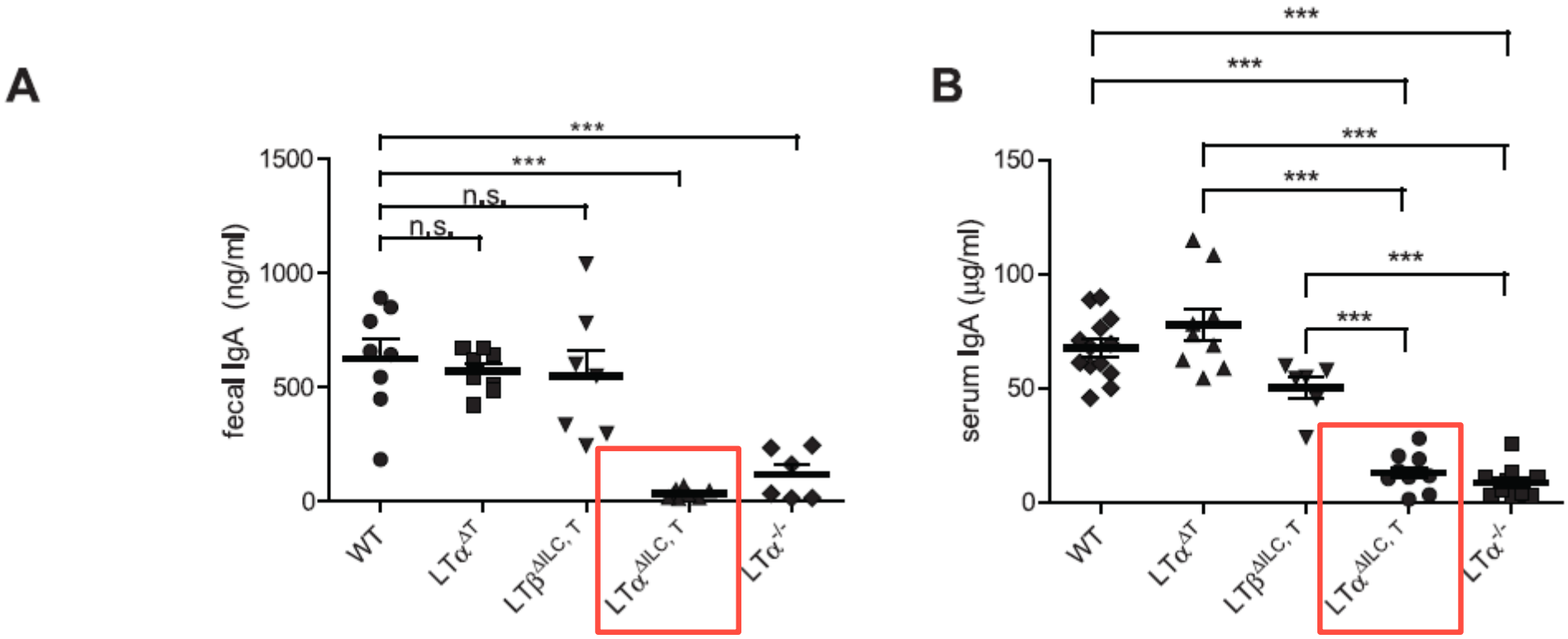
| | | | | |
|---|---|---|---|---|
| Surface LT | Soluble LT | RORγ+ ILC | T cells | PP, ILF, all pLN |
| Surface LT | - | RORγ+ ILC | T cells | PP, ILF, pLN (except MLN) |
| Surface LT | - | - | T cells | - |
| - | Soluble LT | - | T cells | - |

Critical role of LT production by RORγt⁺ ILC during embryogenesis for secondary lymphoid organ development

# Ltα $^{-/-}$ mice are deficient in all LT productors

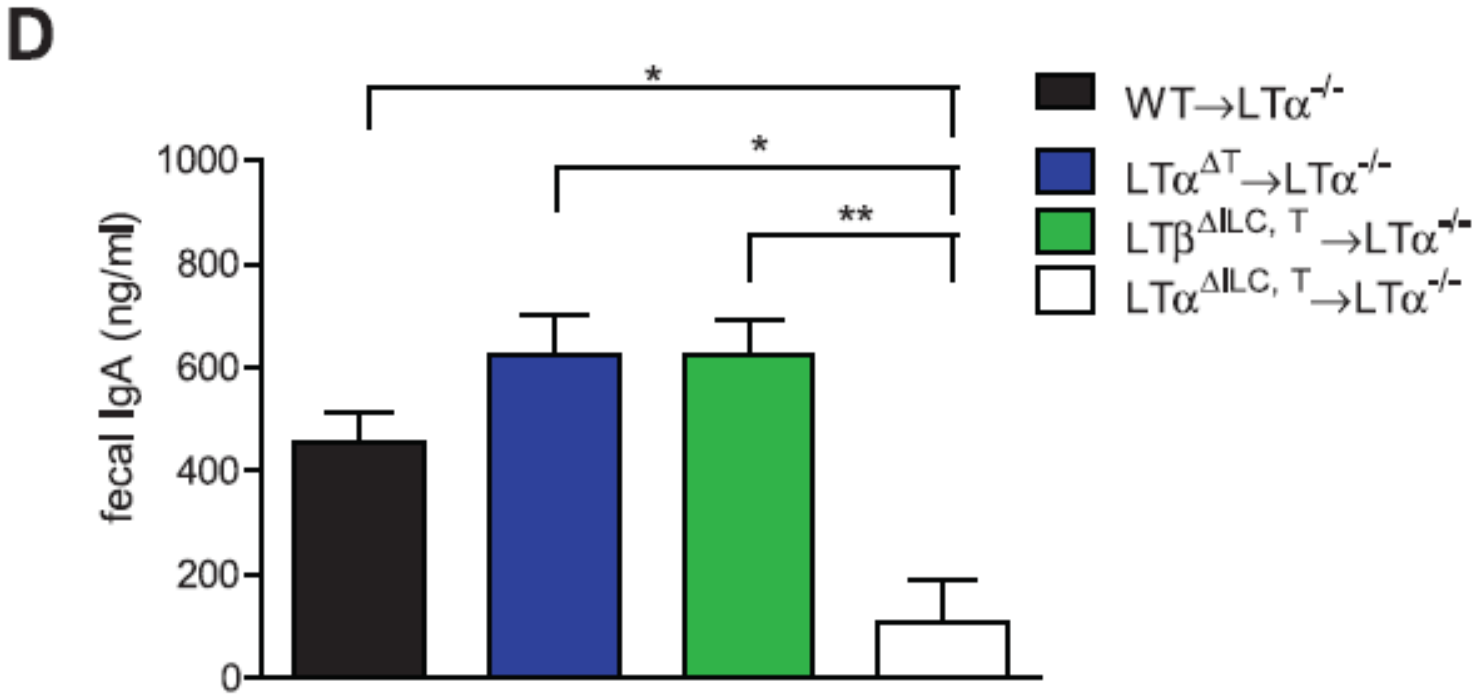


# Soluble LT produced by RORγt$^{+}$ ILC are involved in the generation of IgA



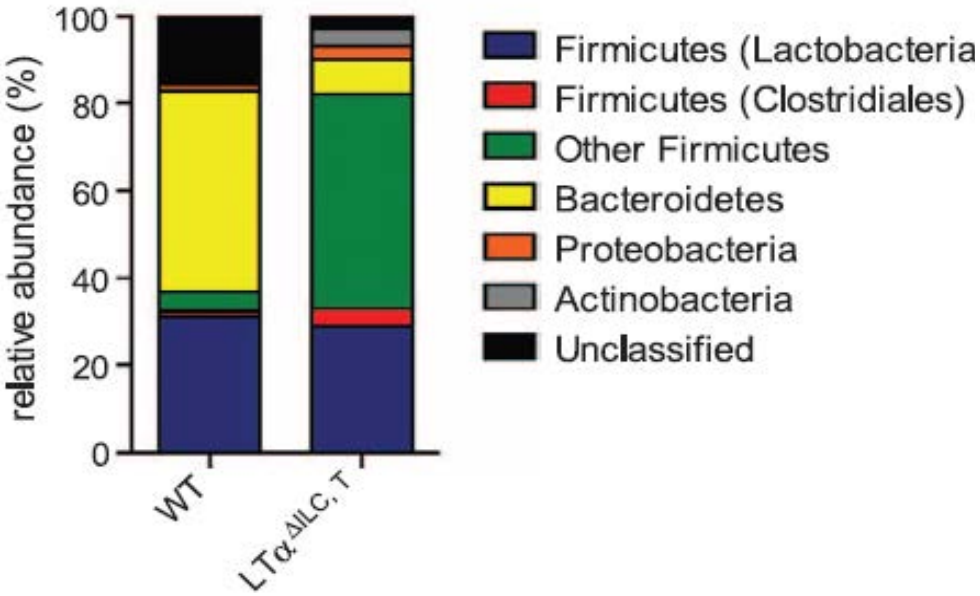
# RORγt$^{+}$ ILC drive IgA generation via LTα irrespective of MLN

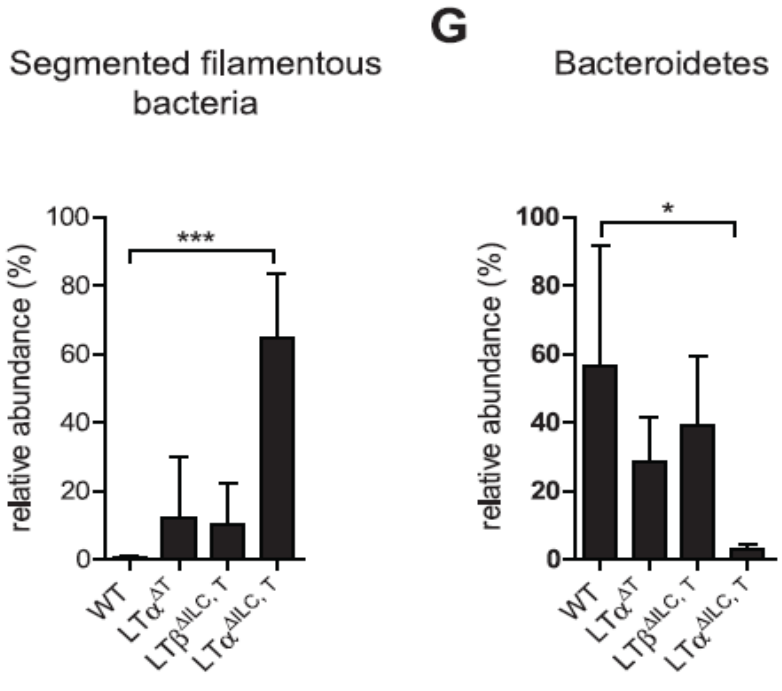
6 weeks after BM transfer



# LTα expression by RORγt$^{+}$ ILC controls gut microbiota

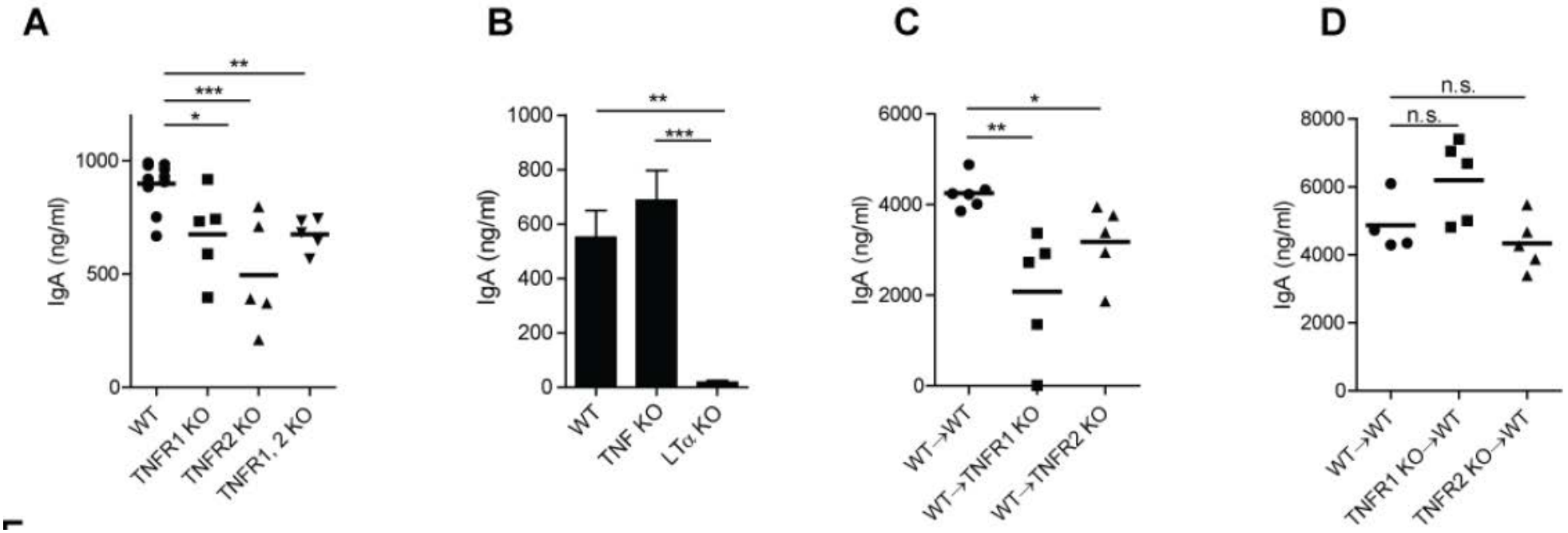
16S RNA sequencing Ileal commensal microflora



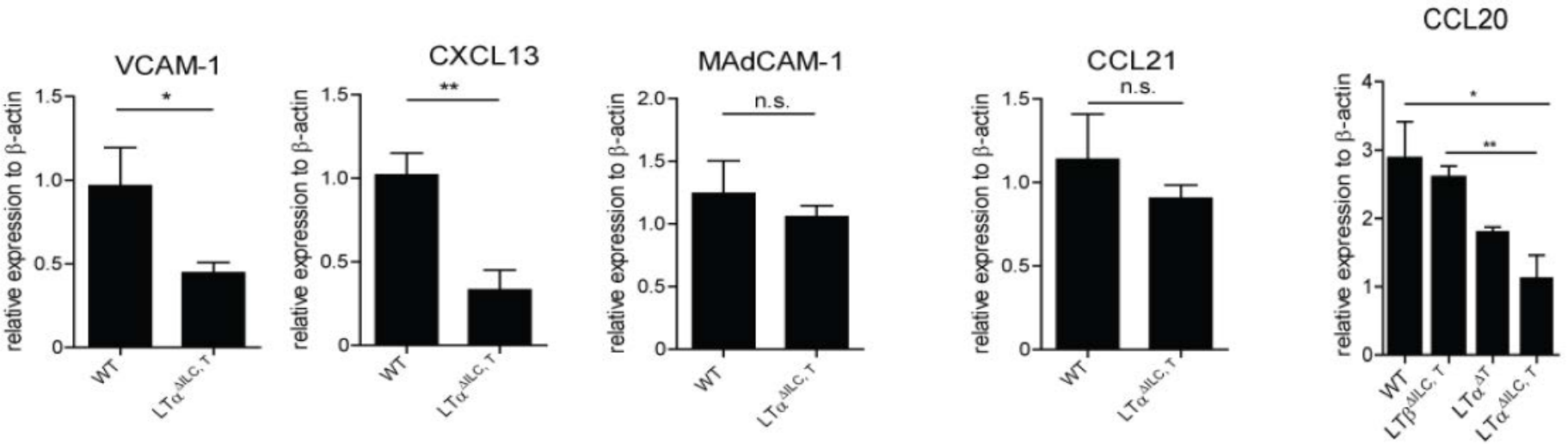
# Soluble LT acts via TNFR1 and TNFR2 expressed by LP stromal cells to promote IgA production

Fecal IgA mesurements

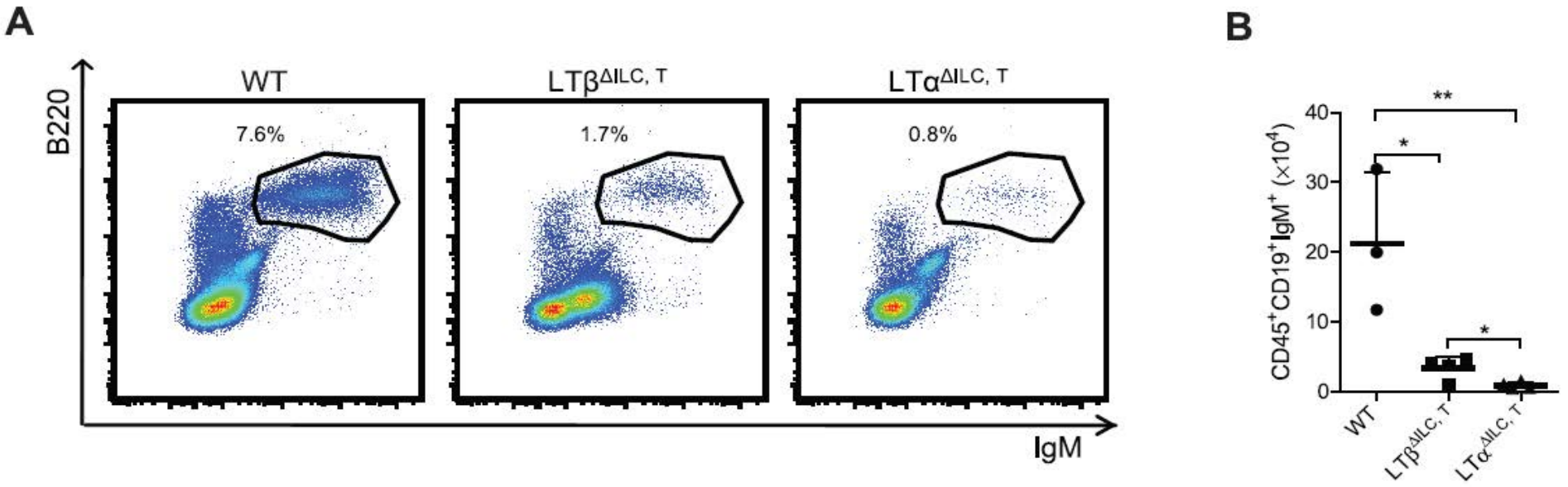



6 weeks after BM transfer

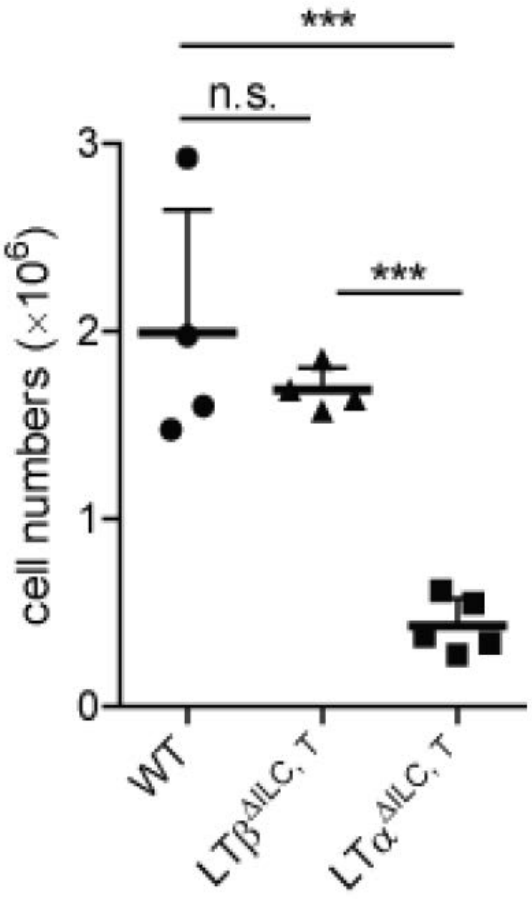
# Both soluble and membrane bound LT production facilite B cells homing to the small intestine




LTβ deficience don't affect the expression of cytokines invoved in B cells  intestinal homing

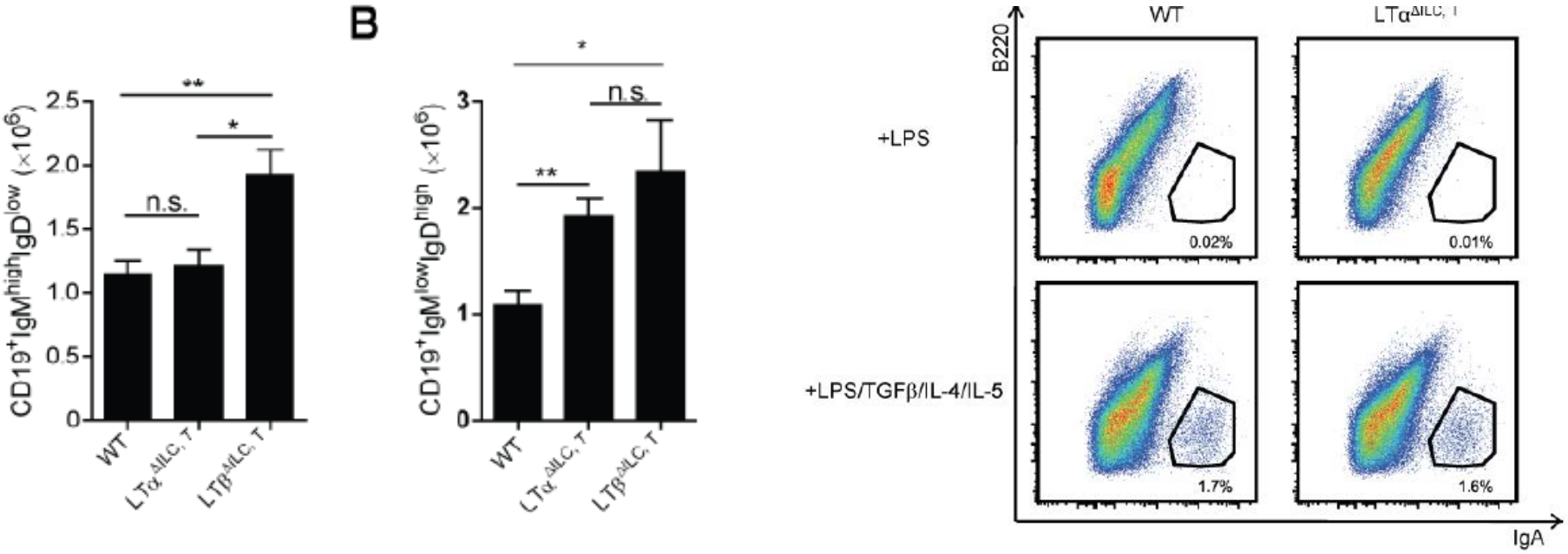


# LTα deficient mice have reduced number of lymphocyes in sI LP



Peritoneal cavity B cells are increased in LT deficient mice.

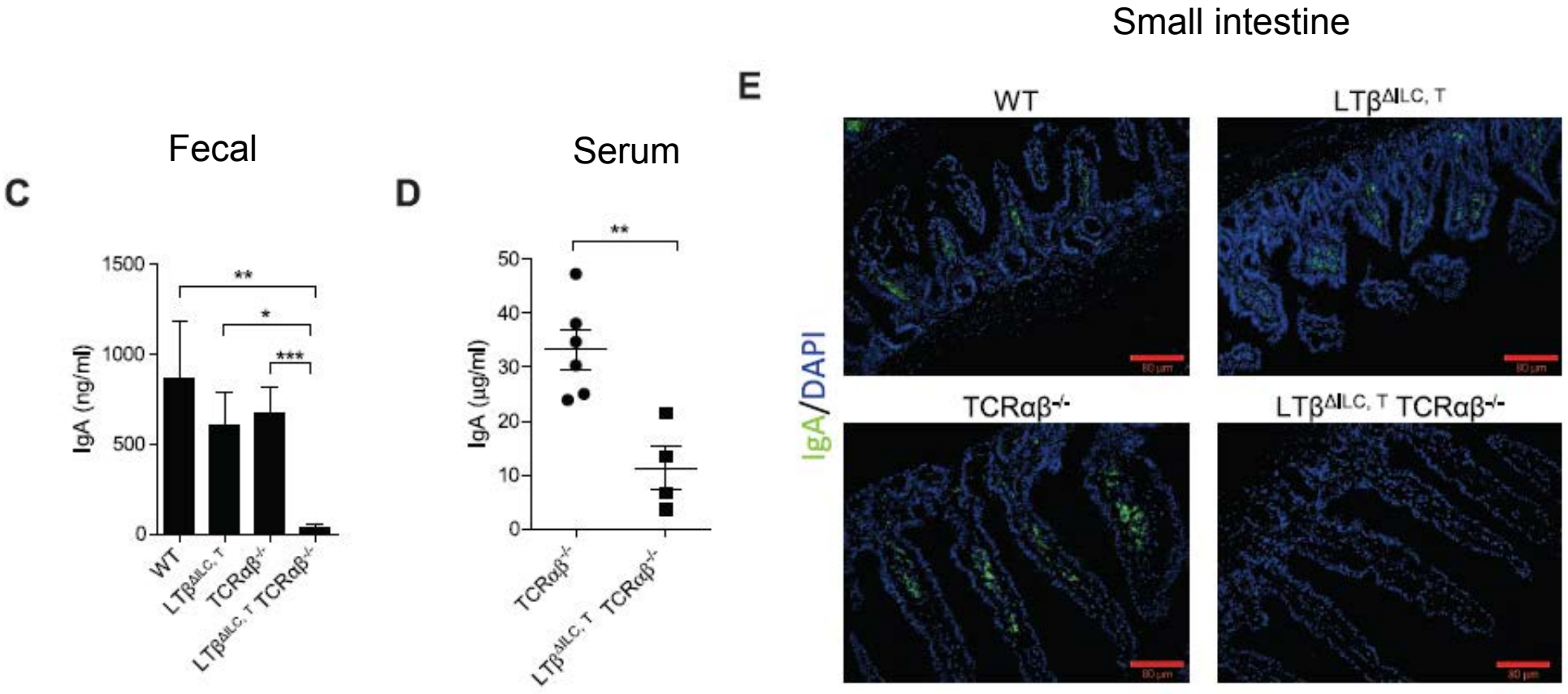
B cells-intrinsic defect in class switch recomboinant is excluded




Splenic B cells cultured for 5 days

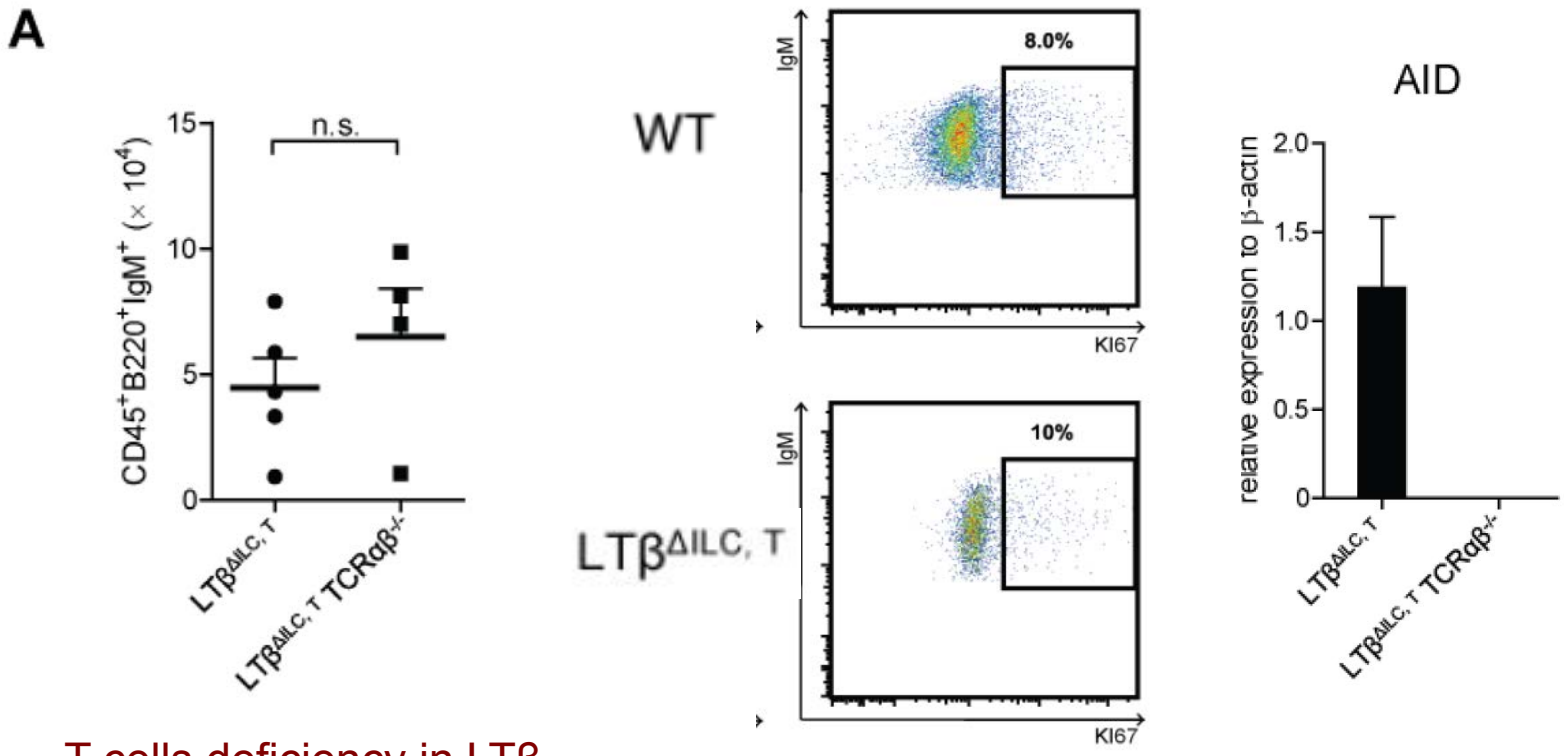
Funcional number of spleen and bone marrow B cells are normal

# IgA generationg induced by LTβ depend on TCR expression



# In absence of Ltβ by RORγt⁺ ILC, TCR depedant- IgA class switching can occurs in the LP

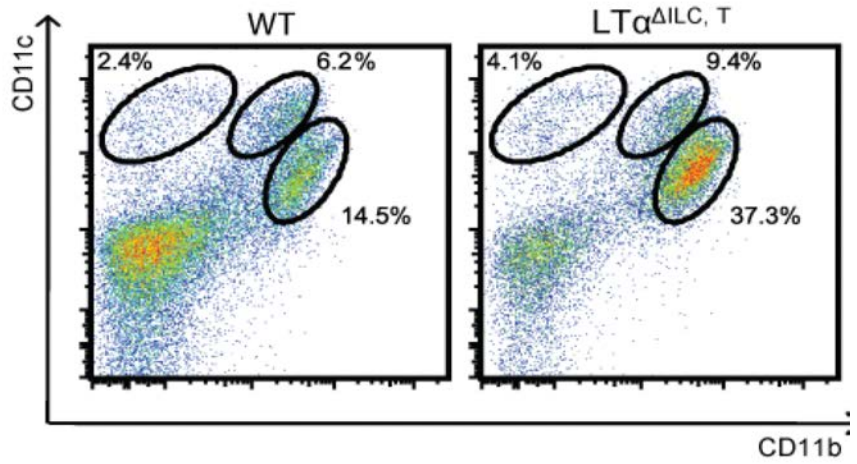
Small intestine LP




T cells deficiency in LTβ mice don't further affect B cells intestinal homing

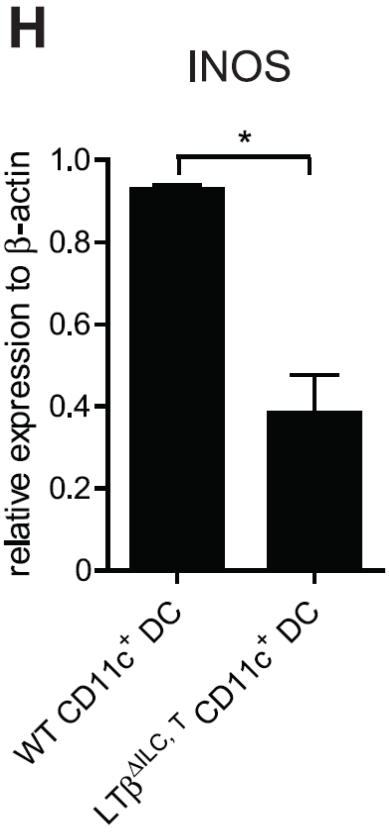
# LT may control T cells independant IgA production via regulation of iNOS expression by DC

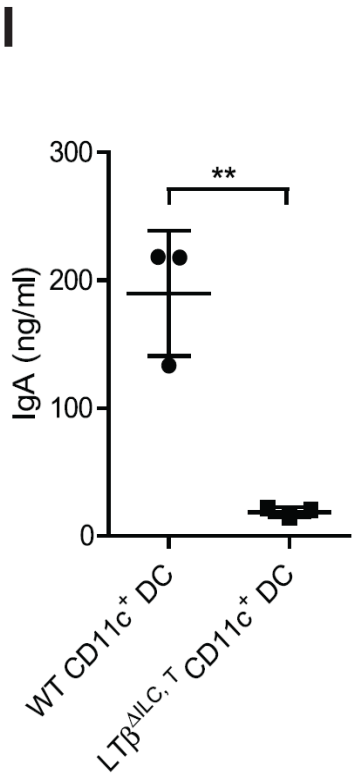
CD45$^{+}$IA-IE$^{+}$ cells
Intestinal LP




No difference in the absolute number of DC subsets in intestine LP and MLN

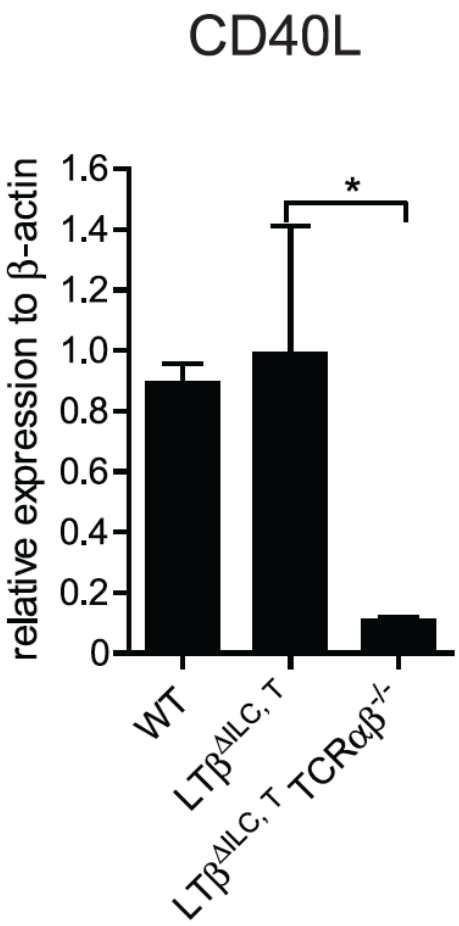
Sorted MLN DC




Culture for 5 days of sorted MLN DC and wt splenic IgM$^{+}$ B cells

# Membrane bound LT depend on TCR




Analysis in the jejunum

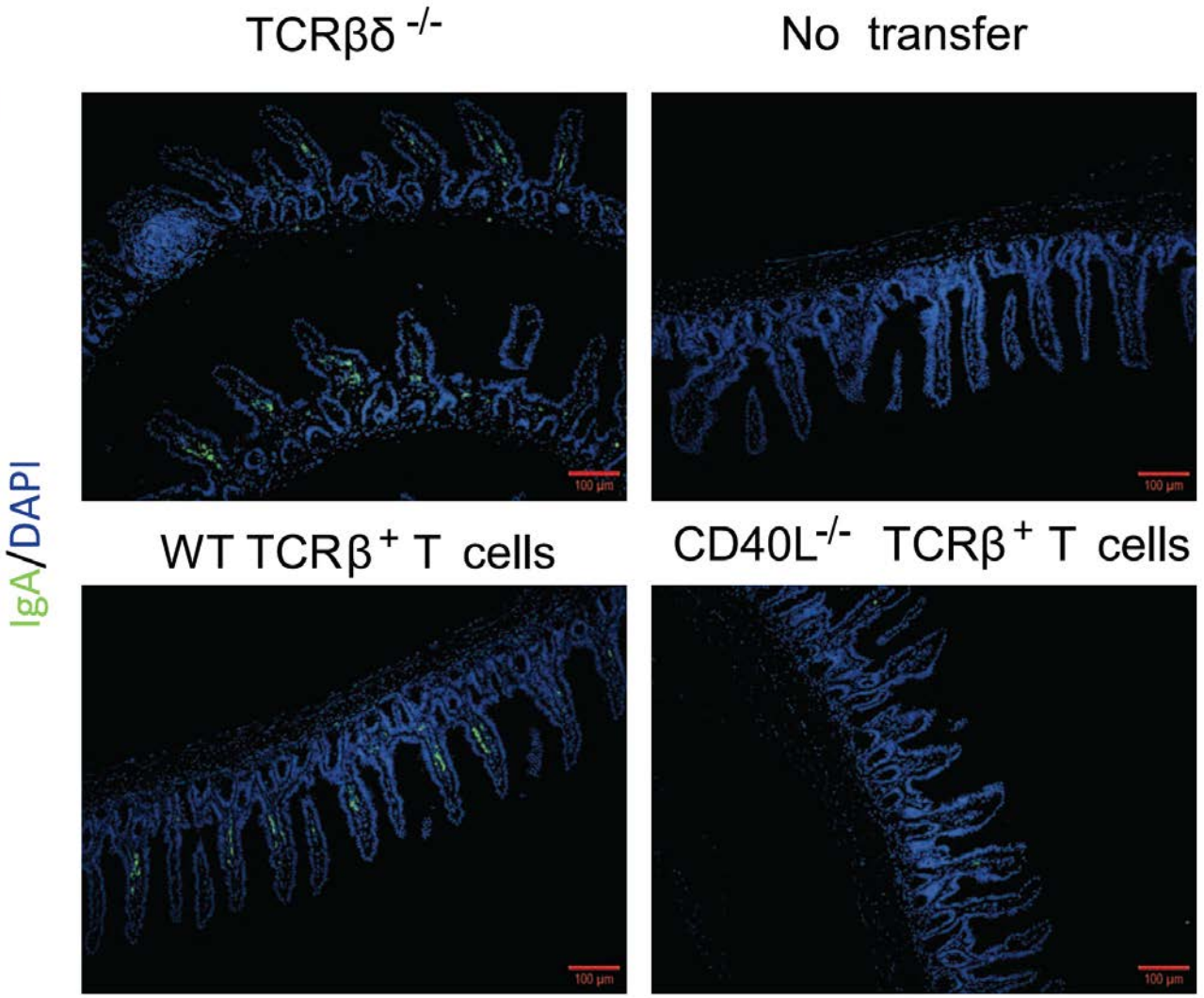
## Membrane bound LT depend on CD40L to induce IgA production

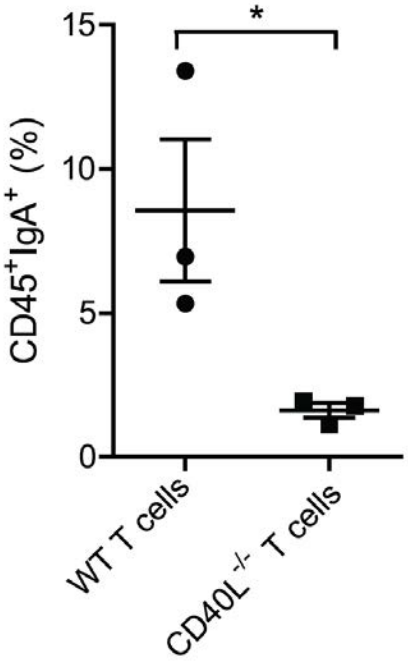
i.p injection of $3x10^6$ TCRβ$^+$ live splenocytes from wt of CD40L$^{-/-}$

LTβ$^{\Delta ILC,\ T}$ TCRβδ$^{-/-}$

Analysis 2 weeks after transfer

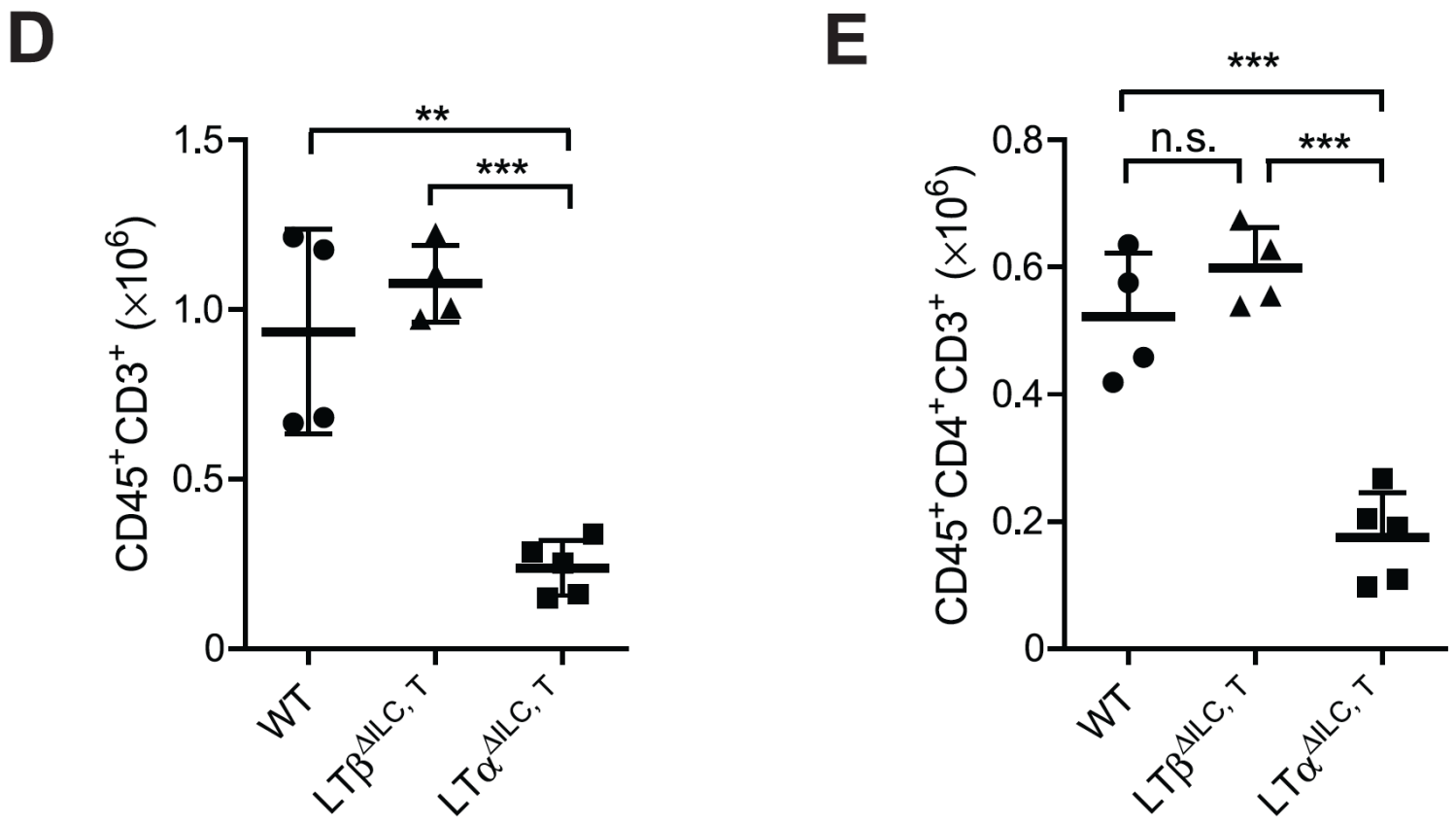
**B**



LTα derived from ILC control T cells homing to the *lamina propria*

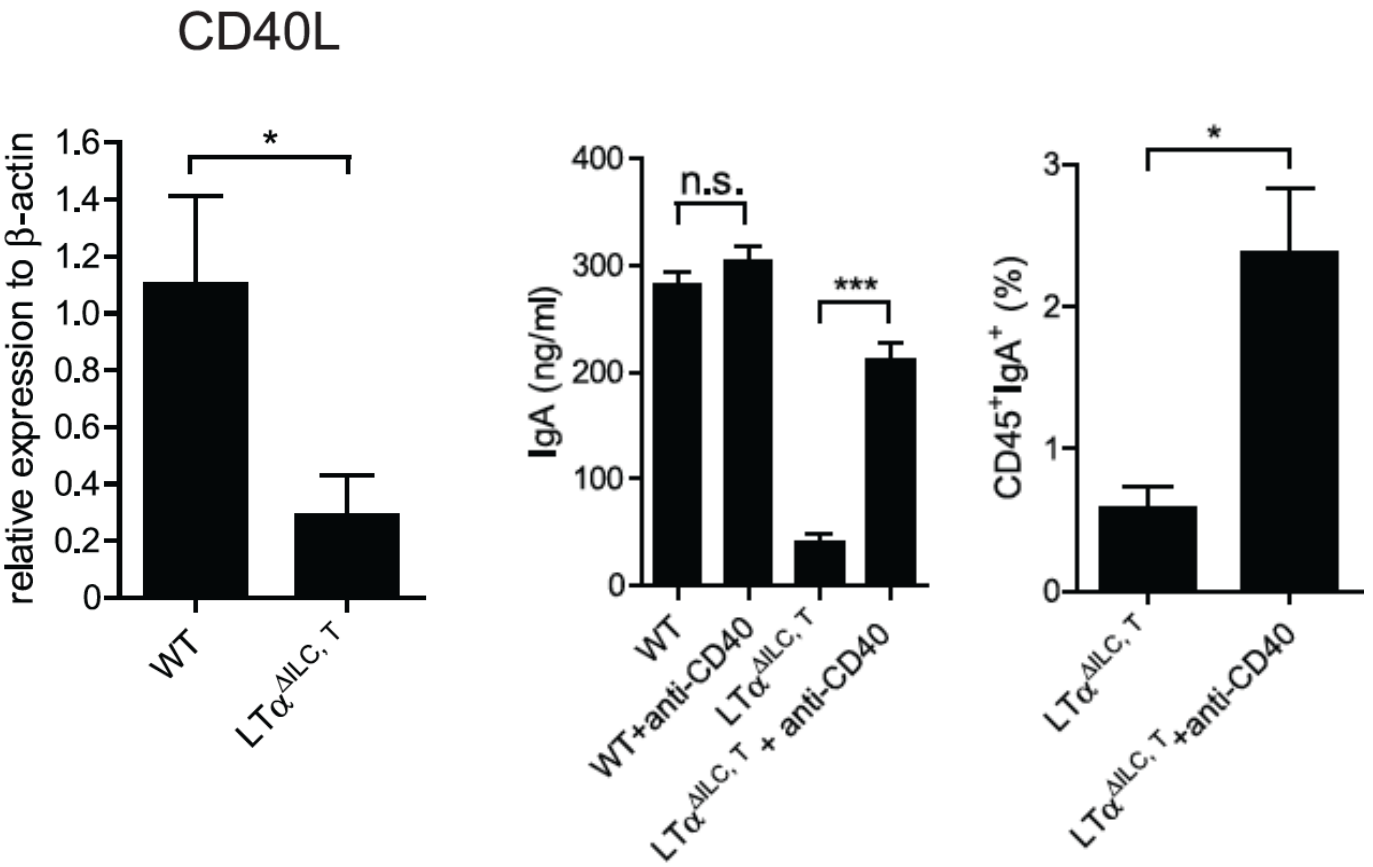
$CD45^+IA\text{-}IE^+$ cells
Intestinal LP




LTα derived from ILC not affect T cells homing on the periphery

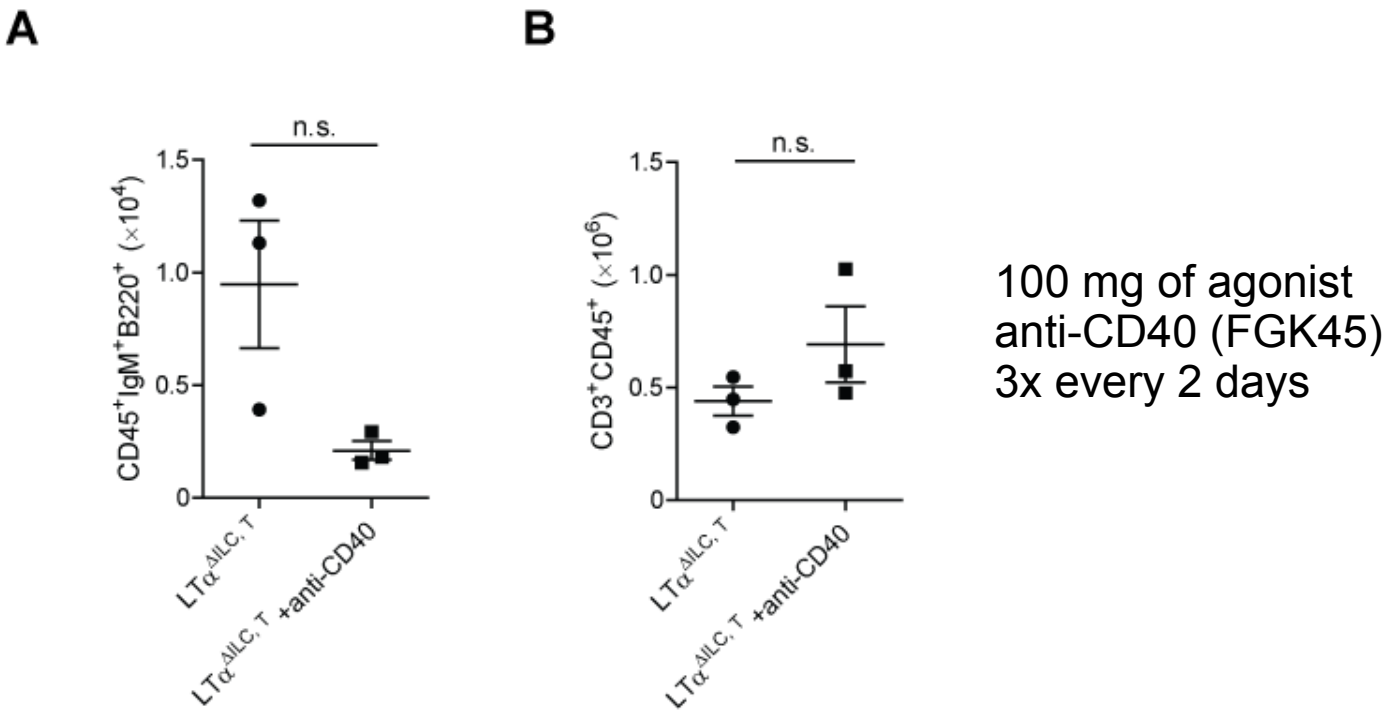
# Soluble LTα derived from ILC need CD40L-T cells pathway to induce IgA production

Analysis in the jejunum



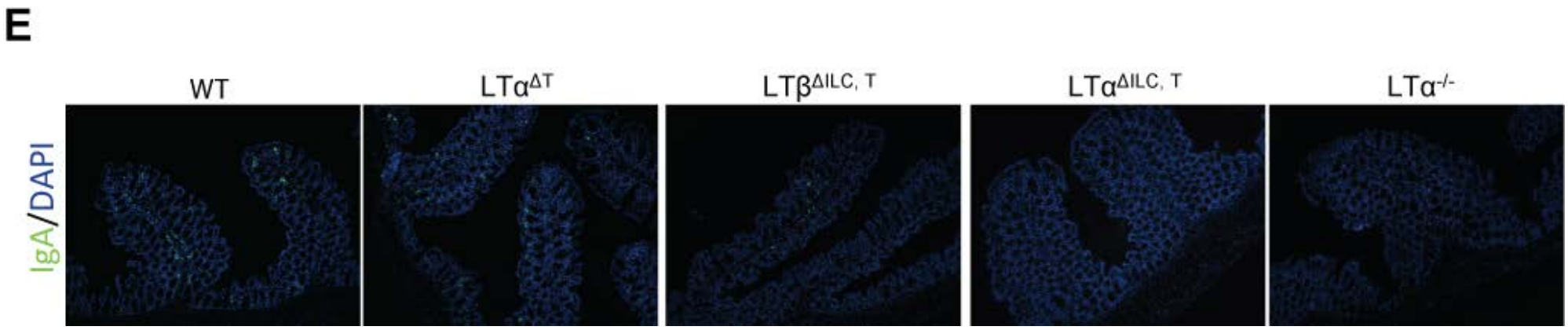

100 mg of agonist anti-CD40 (FGK45) 3x every 2 days

CD40L doesn't improve T cells homing to the *lamina propria* or IgA production after inflammation



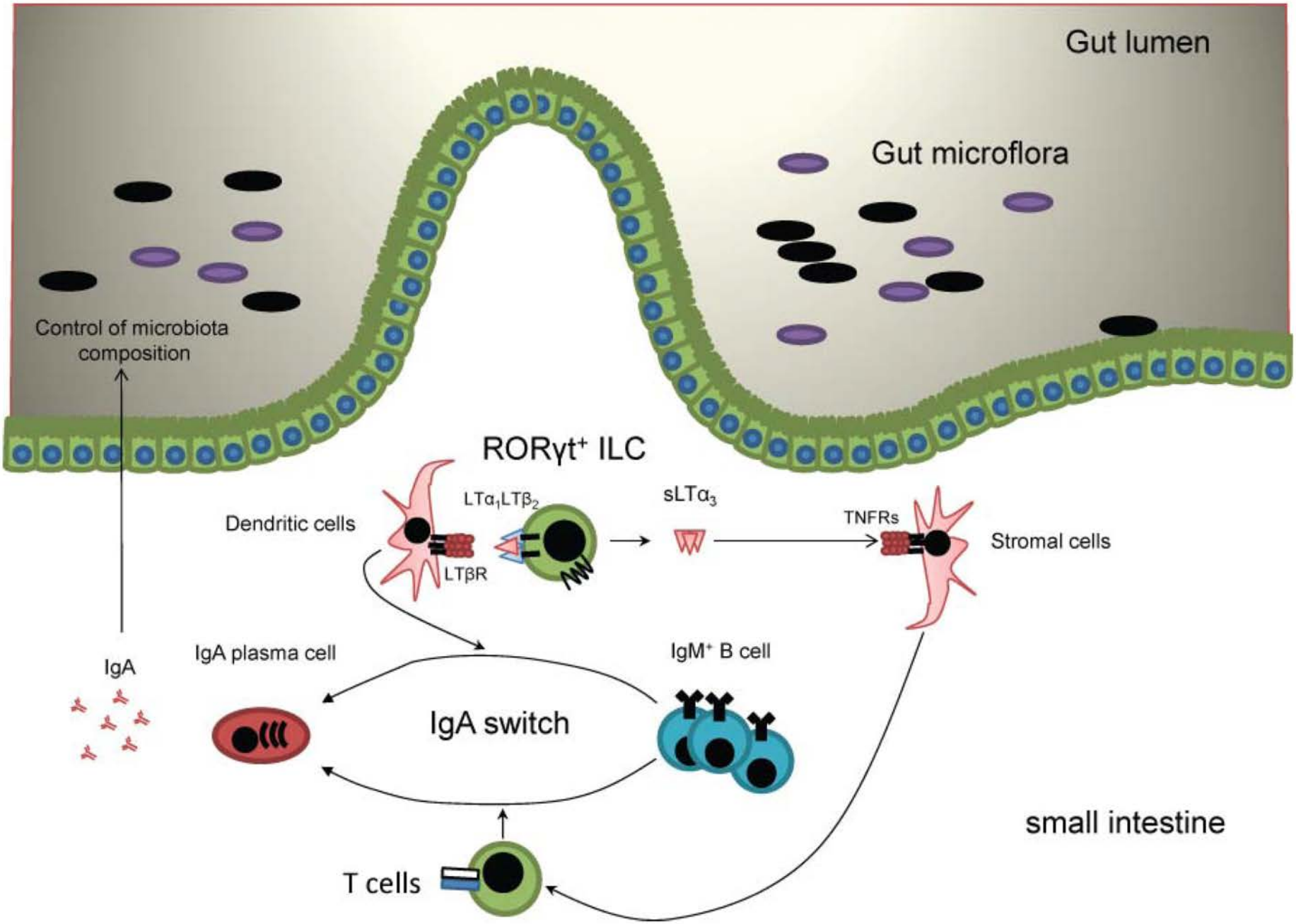

100 mg of agonist anti-CD40 (FGK45) 3x every 2 days

1.5% DSS for 5 days and 3 days of water




Soluble LTα derived from ILC may control IgA induction via regulation of T cells homing to the *lamina propria*

# Conclusion


Gut lumen
Gut microflora
Control of microbiota composition
RORγt⁺ ILC
Dendritic cells
LTα1LTβ2
LTβR
sLTα3
TNFRs
Stromal cells
IgA
IgA plasma cell
IgM⁺ B cell
IgA switch
T cells
small intestine

# TCF-1 Controls ILC2 and NKp46$^{+}$RORγt$^{+}$ Innate Lymphocyte Differentiation and Protection in Intestinal Inflammation

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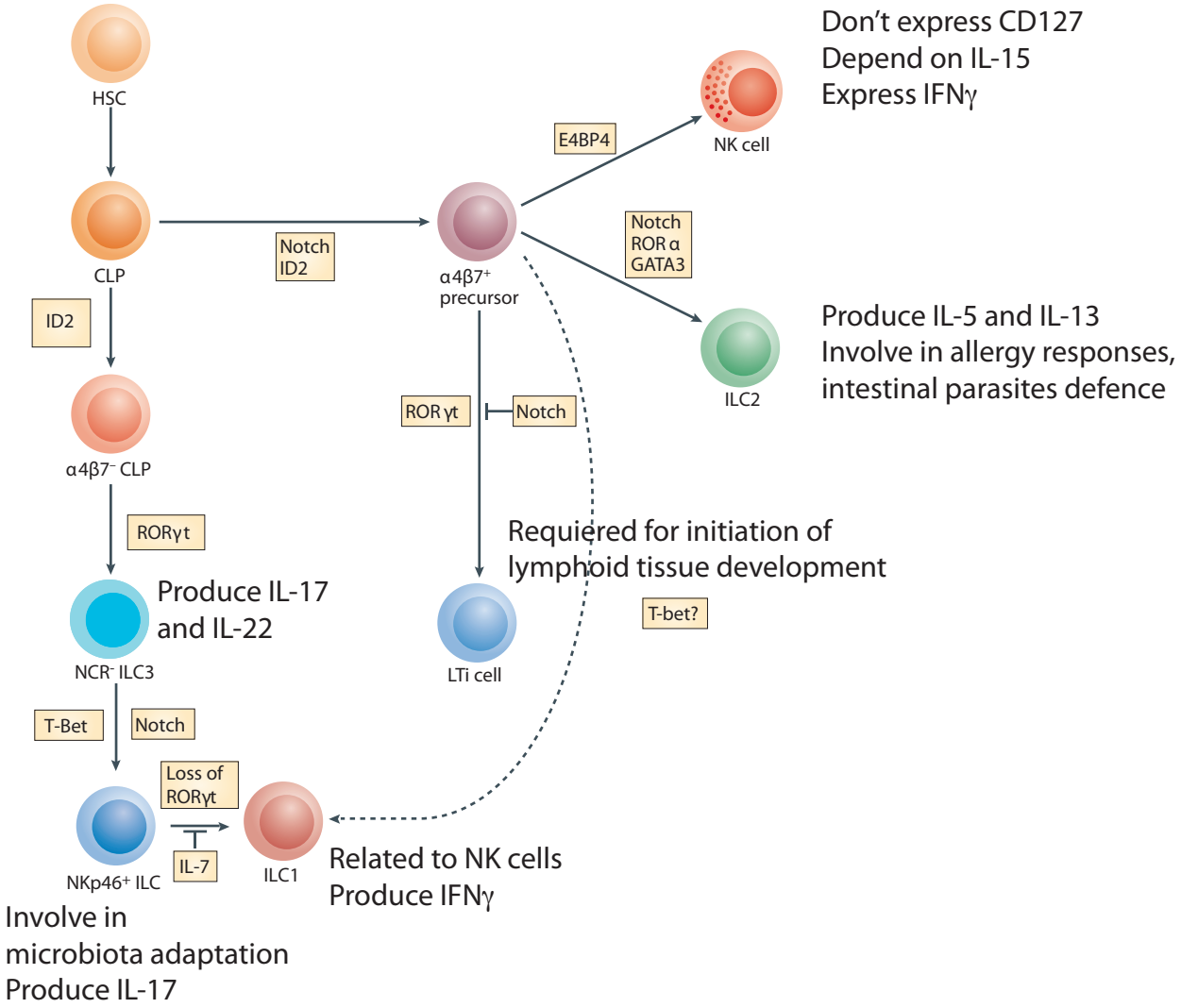
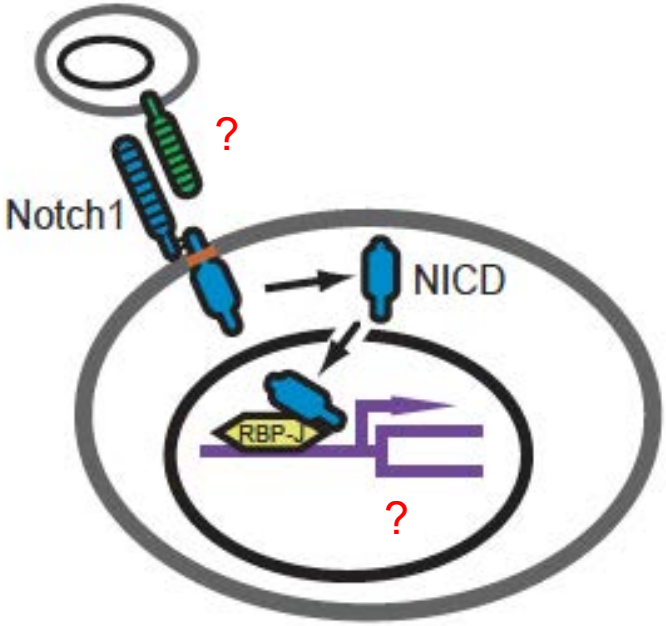



Adapted from *Walker et al. Nature Rev 2013*

T-Bet➔Notch

NCR$^{-}$ ILC3 ⟶ NKp46$^{+}$ ILC

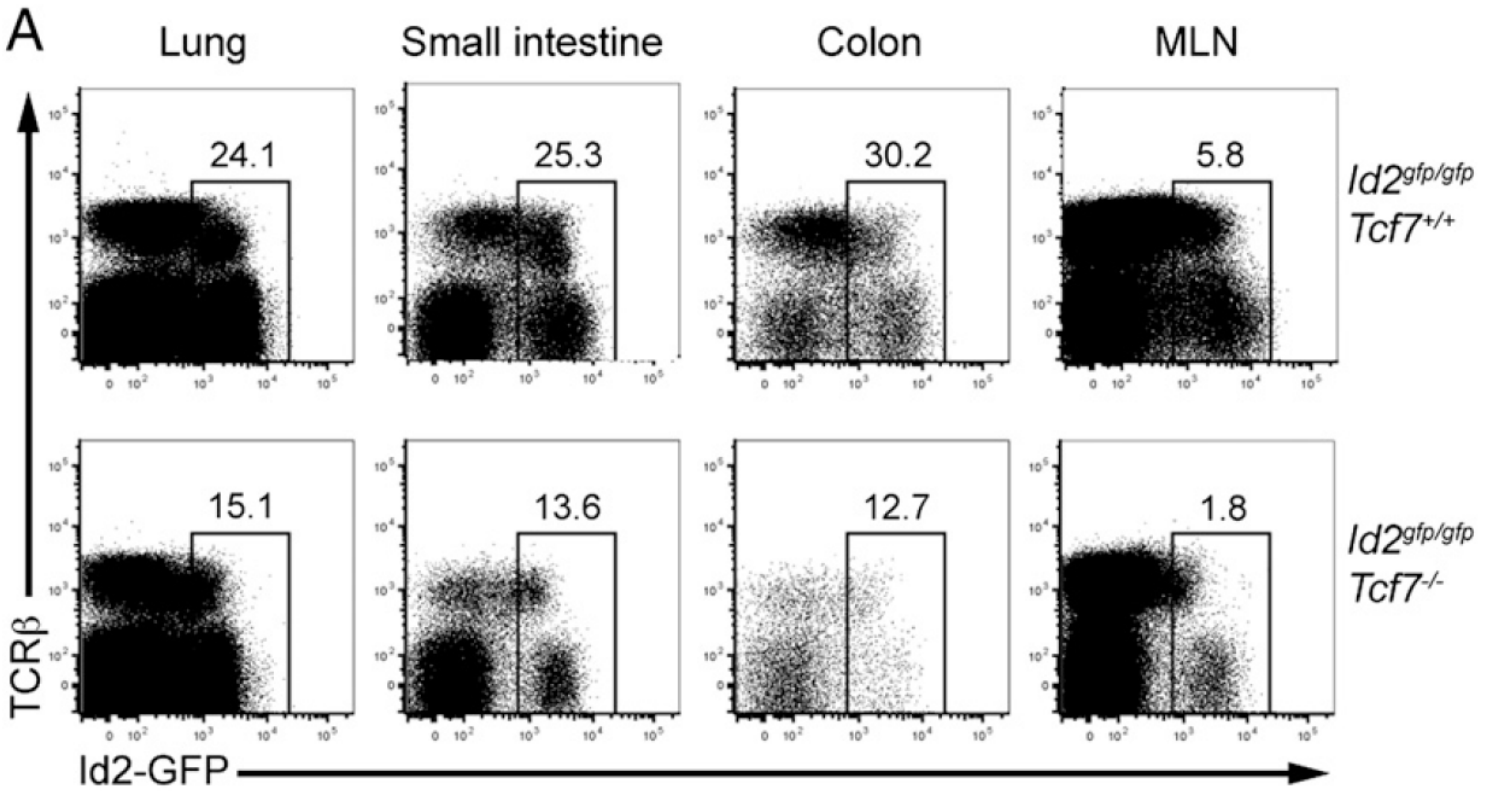



T cell factor (TCF)-1 :

- Transcription factor encoded by *Tcf7*
- Identified as a direct target gene of Notch in T cells to promote T cell development in the thymus (*Germar et al. 2011 PNAS*)

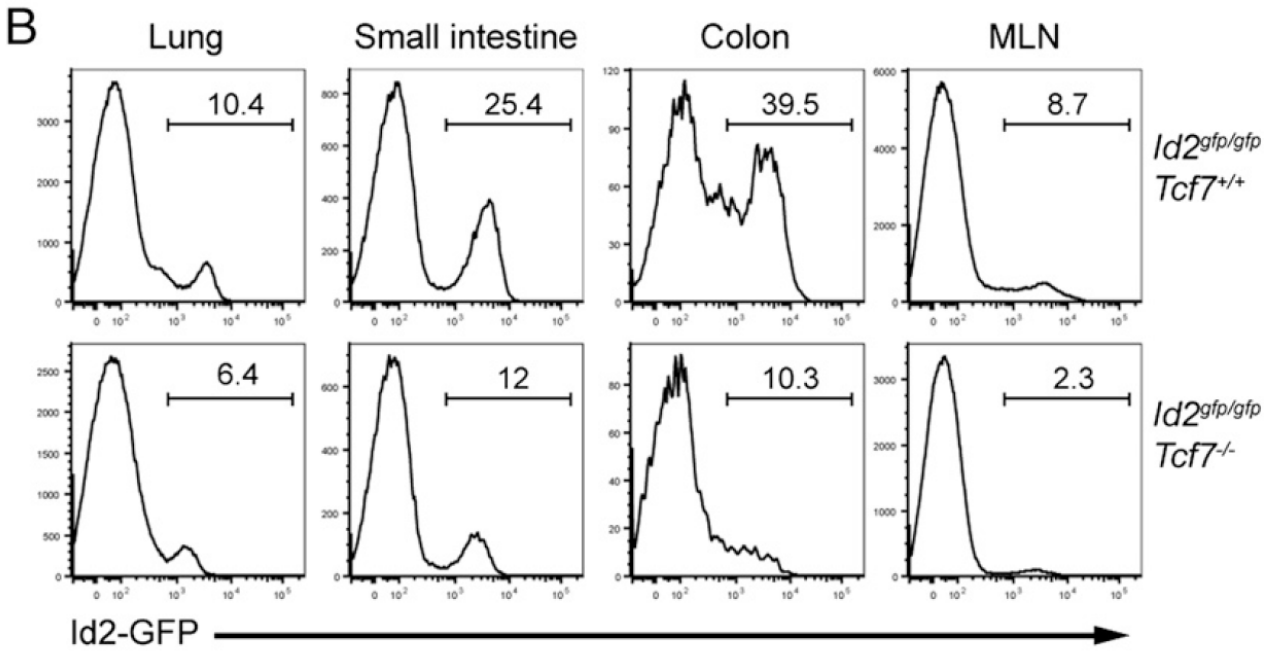
# Overall expression of Id2-GFP$^{+}$ lymphocytes is reduced in the absence of TCF-1

8-12 weeks
CD45$^{+}$ gated



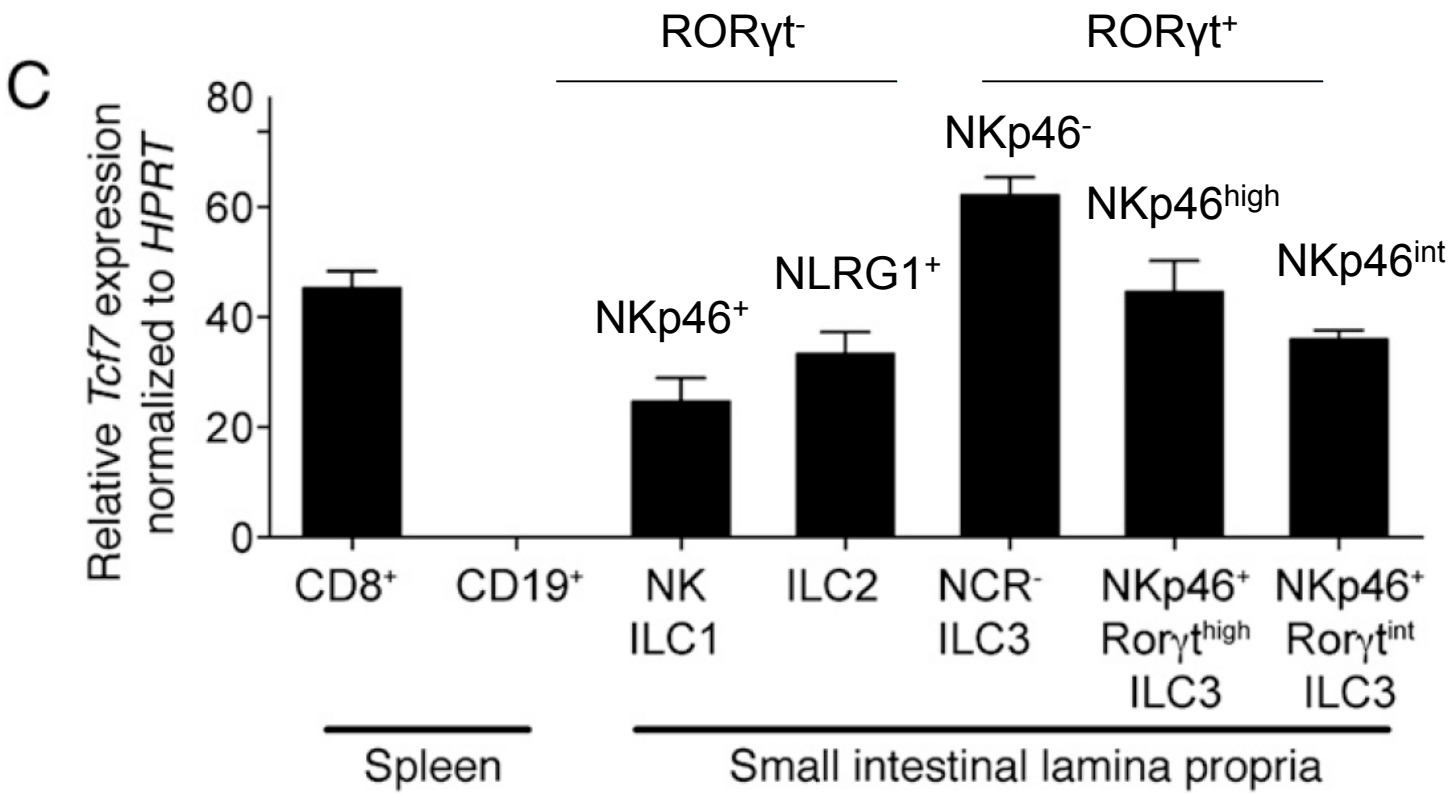
# ILC are reduced in the absence of TCF-1

8-12 weeks
TCRβ$^{-}$ gated



# Tcf7 highly expressed by ILC subsets

RT-PCR from
isolated sI LP cells
$CD3^{-}CD19^{-}$ gated

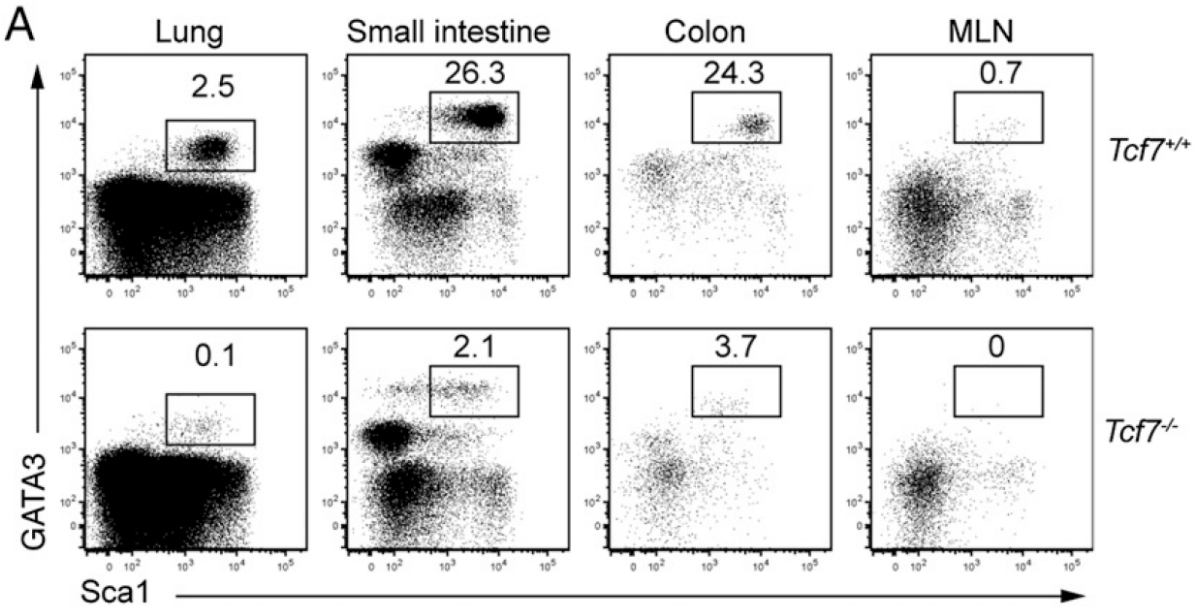



- CD8 T cells use LEF1 as TF for maturation
- NK cells aren't affected in Tcf7 deficient mice

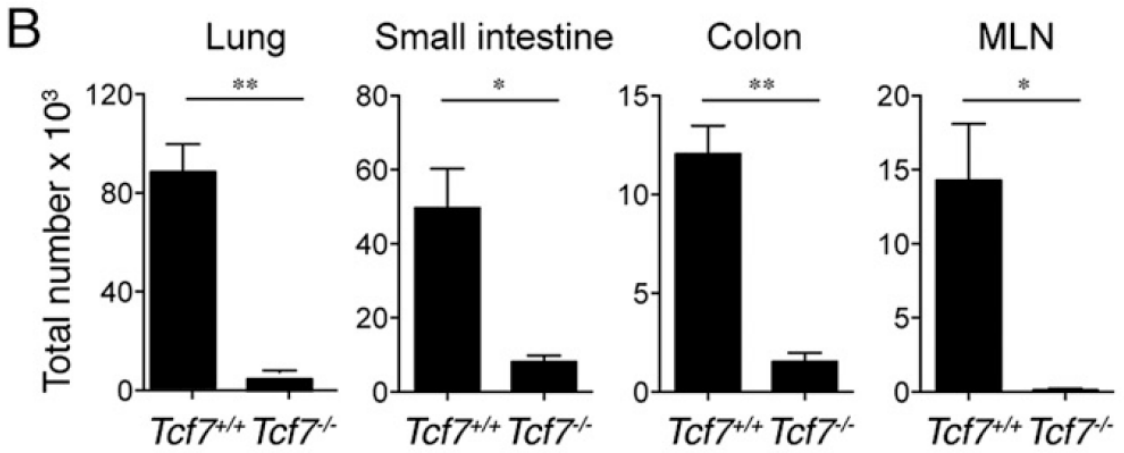
**TCF may influence the development of ILC2 and/or ILC3 subsets**

# TCF-1 impairs ILC2 development

8-12 weeks
CD3$^-$CD19$^-$ gated

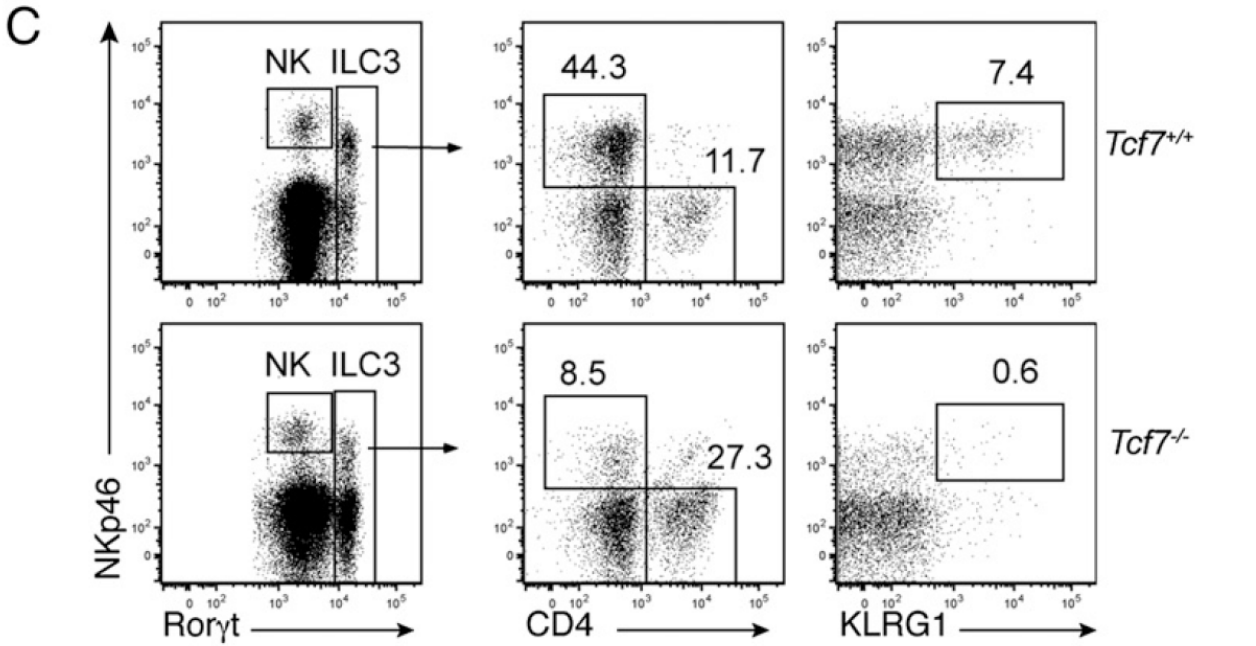



8-12 weeks
CD3$^-$CD19$^-$ GATA3$^+$ Sca1$^+$ gated

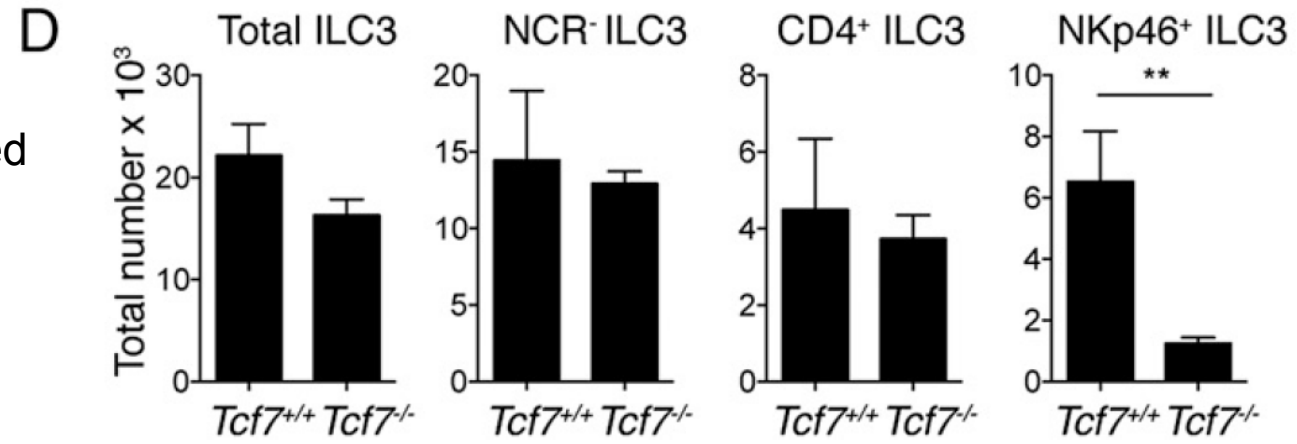


# TCF-1 impairs NKp46$^{+}$ ILC3 development

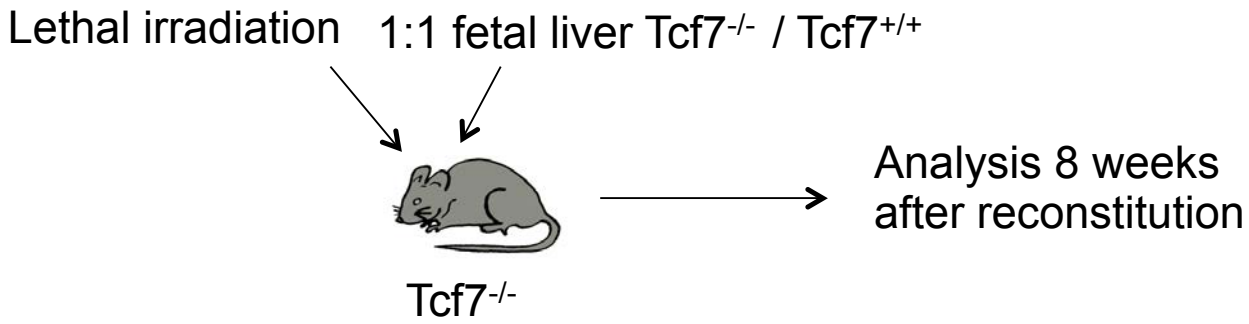
siLP
CD3$^{-}$CD19$^{-}$ gated



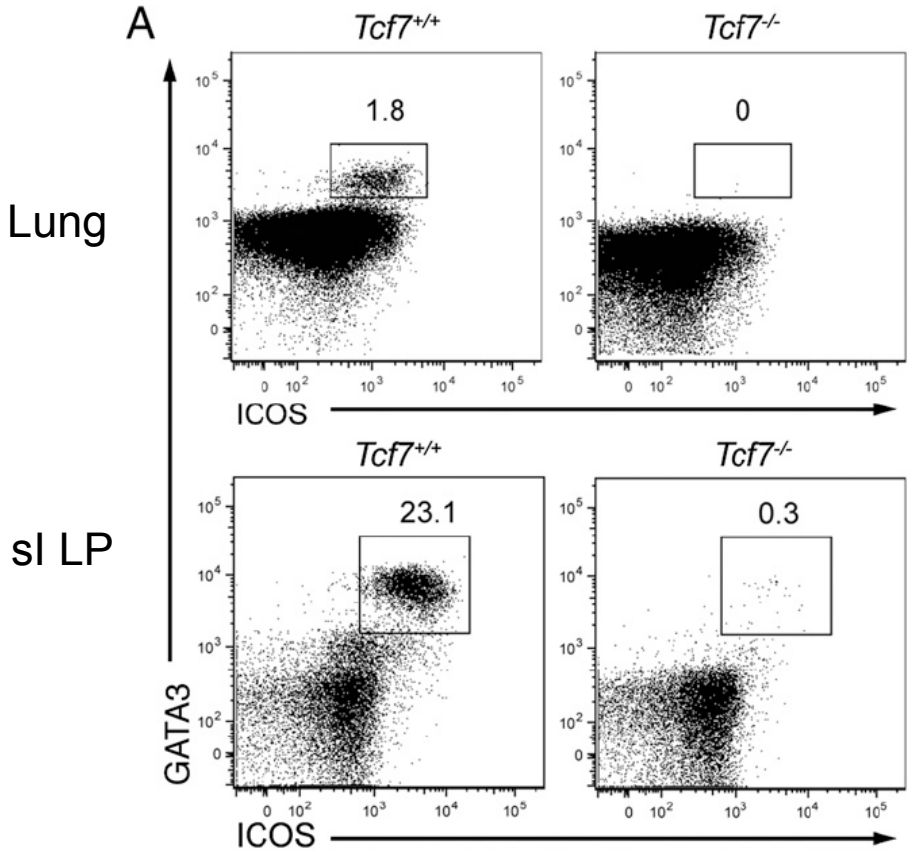

CD3$^{-}$CD19$^{-}$RORγt$^{+}$ gated

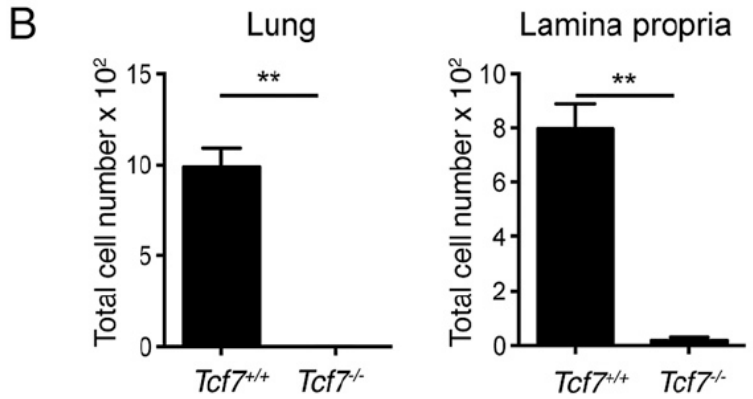


# TCF-1 control ILC2 development by a cell-intrinsic mechanism




CD3-CD19- gated

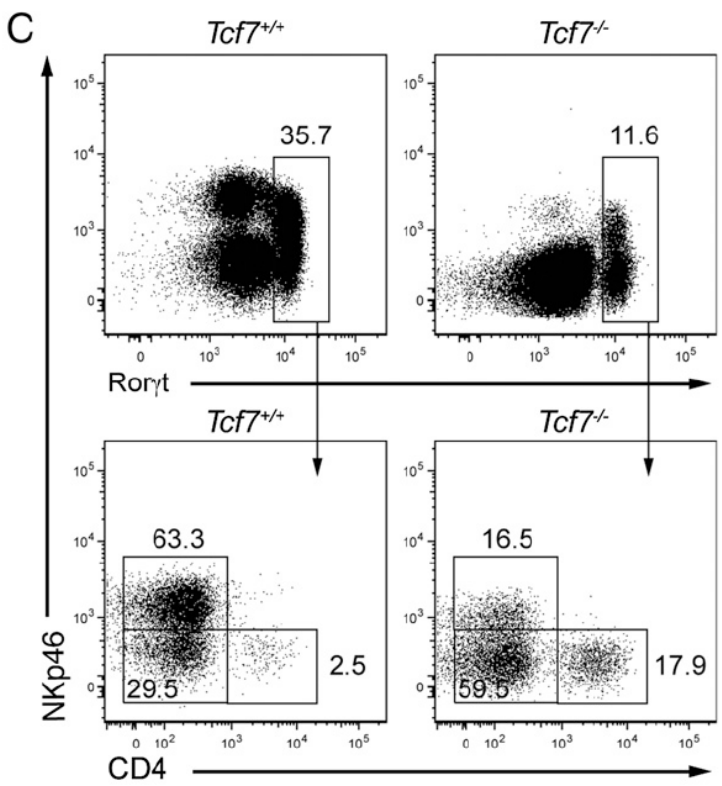


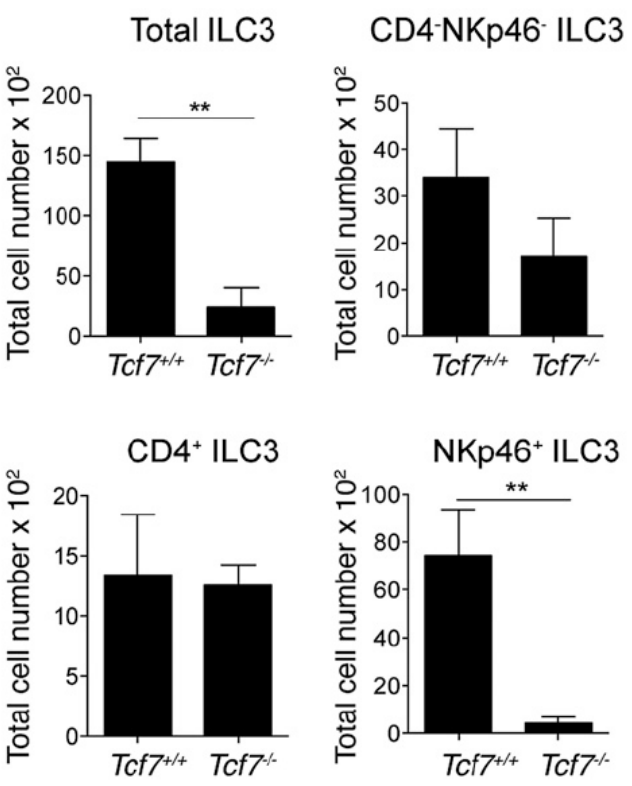



# TCF-1 drive ILC3 development by a cell-intrinsic mechanism

sI LP

CD3$^-$CD19$^-$ gated

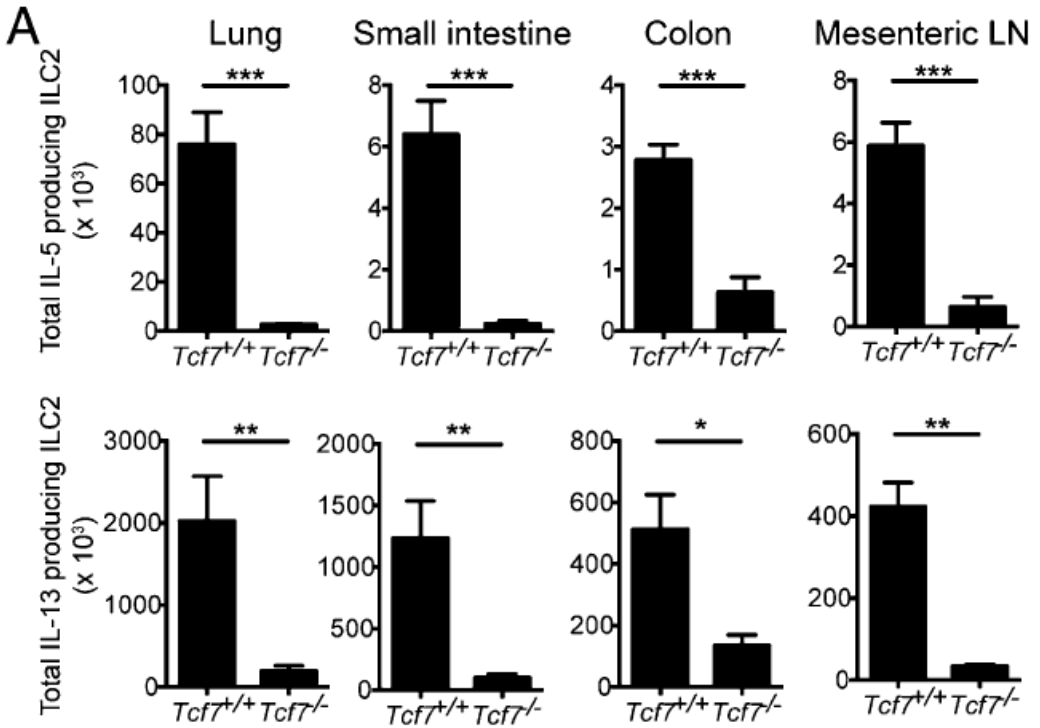


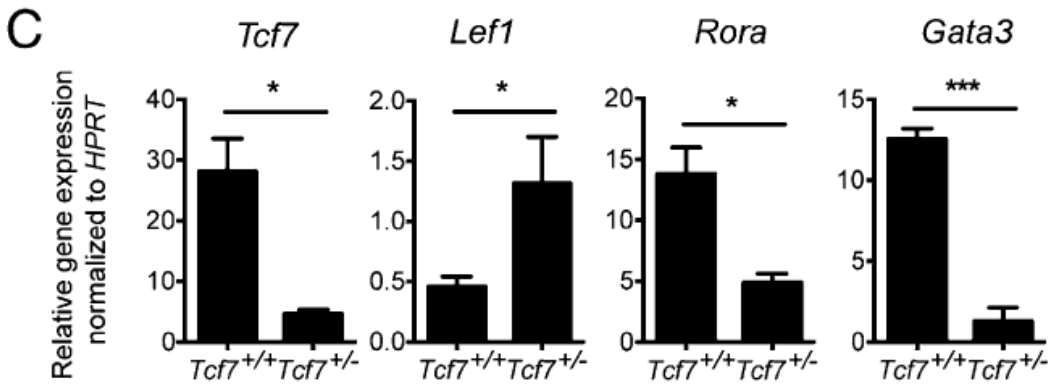



# TCF-1 control critical factors for functional ILC2

Lin$^-$ lymphocytes stimulated for 4h with PMA/IONO + Bref A

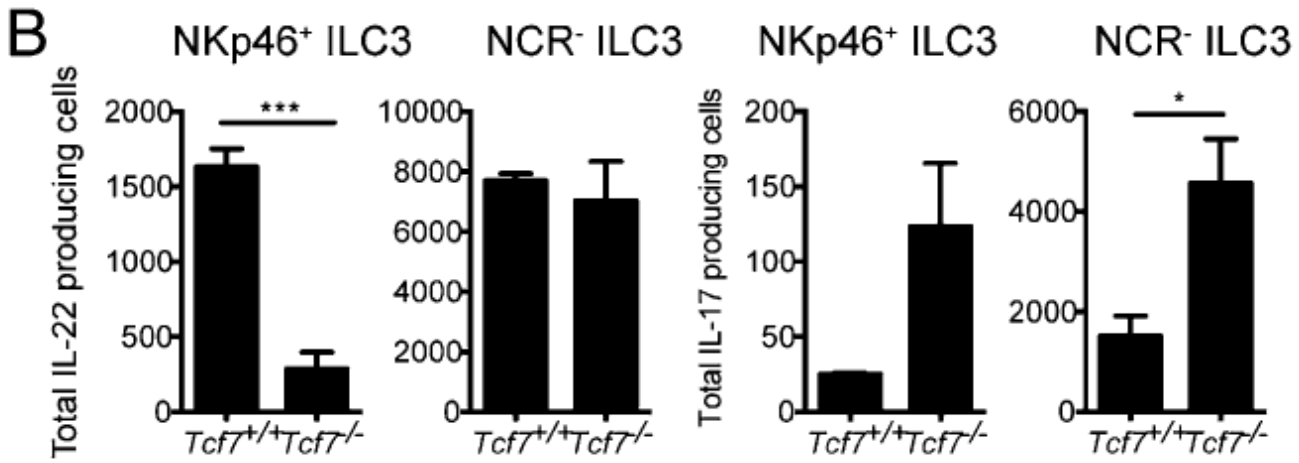
ILC2 cells from sI LP cells



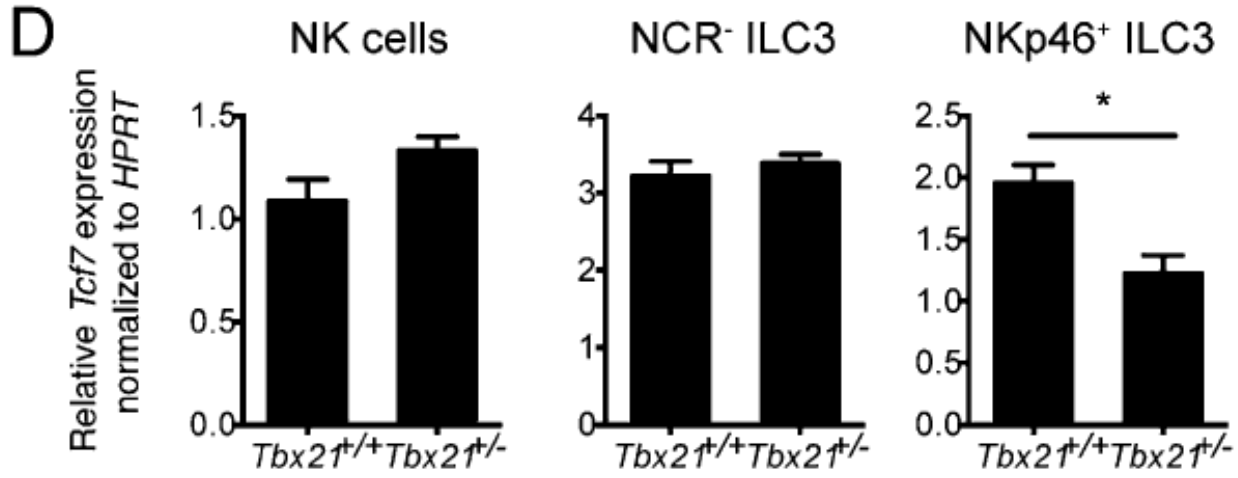
# The absence of TCF-1 convert IL22 producers NKp46$^{+}$ ILC3 into IL17 producers

ILC subsets from sI LP cells stimulated for 4h with
IL23 (10 ng/ml) + IL1β (10ng/ml) + Bref A



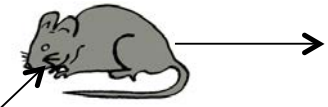
# TCF-1 control NKp46$^{+}$ ILC3 via T-Bet/Notch-signaling pathways

ILC subsets from sI LP cells

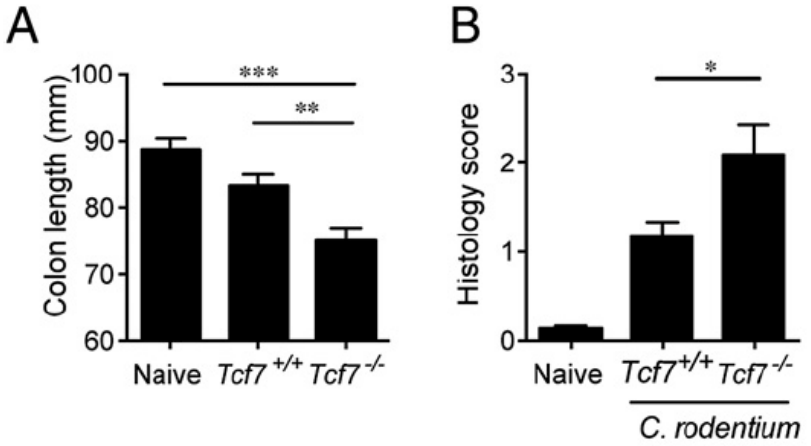


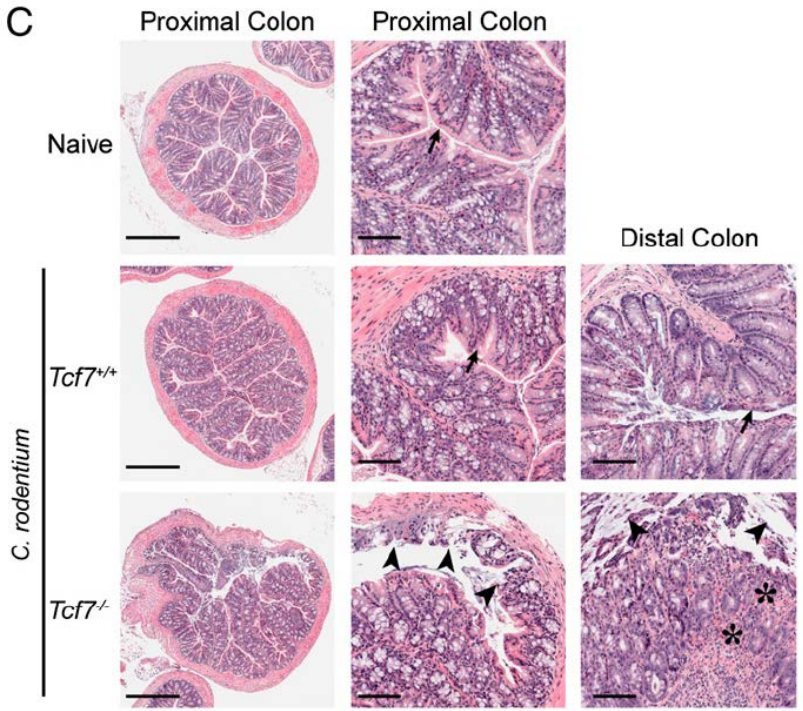
# TCF-1 deficient mice impairs to response against *C. rodentium* infection

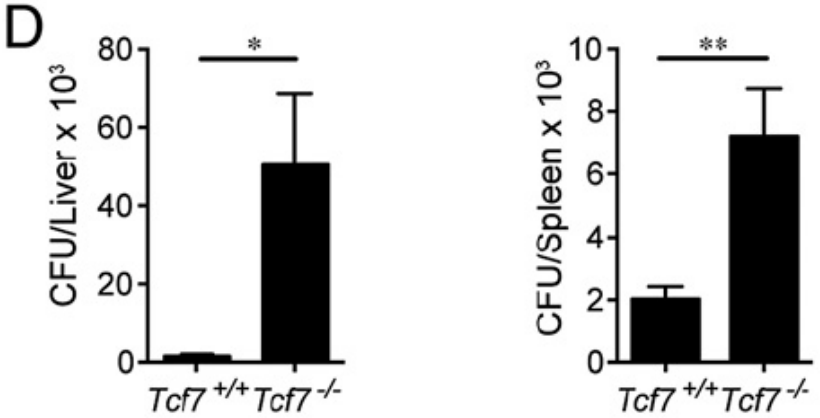
i.g. 2 $10^9$ CFU of *C. rodentium*



Analysis 8 days after infection

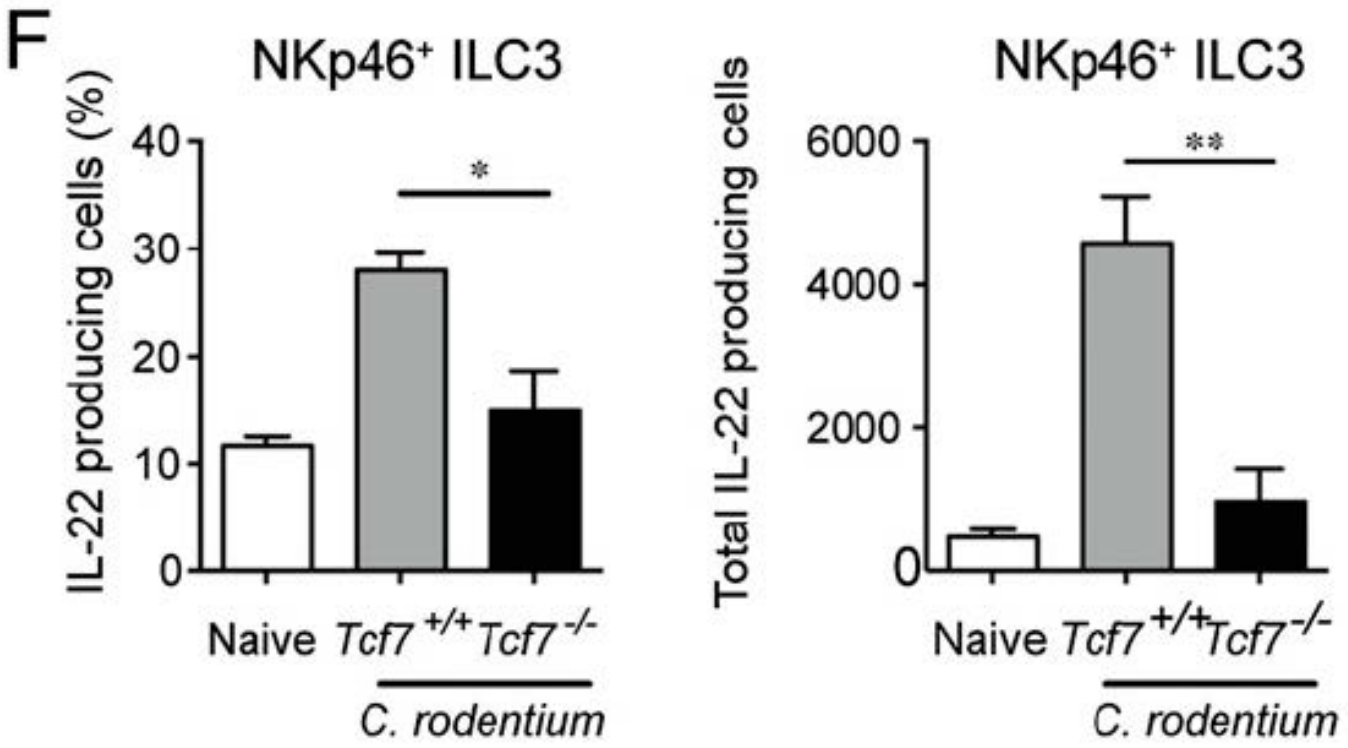


# TCF-1 control the production of IL-22 by NKp46$^{+}$ ILC3 in response to *C. rodentium* infection

sI LP



## Conclusion

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