Immunity
Article

Plasticity of Foxp3$^+$ T Cells Reflects Promiscuous Foxp3 Expression in Conventional T Cells but Not Reprogramming of Regulatory T Cells

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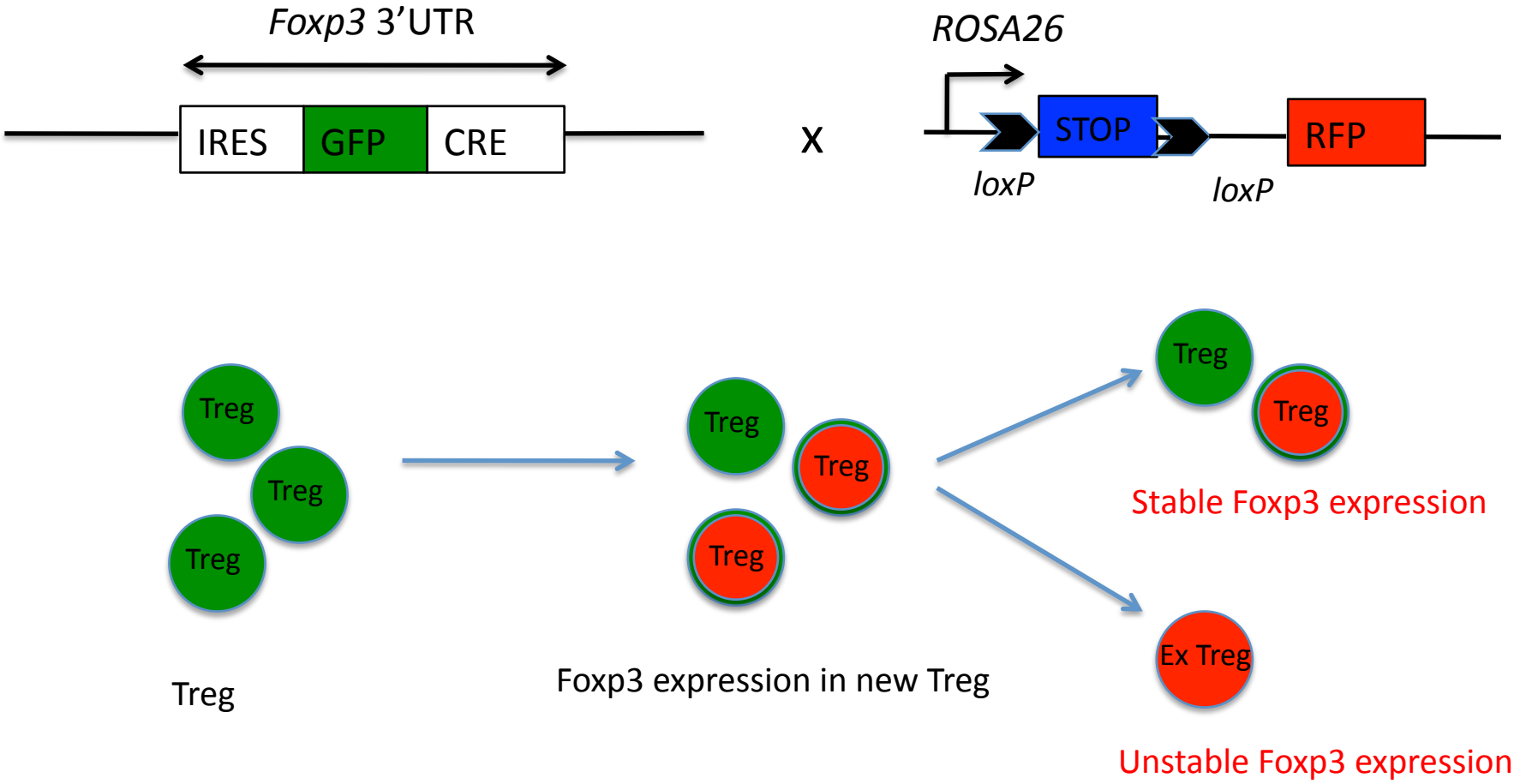
Introduction

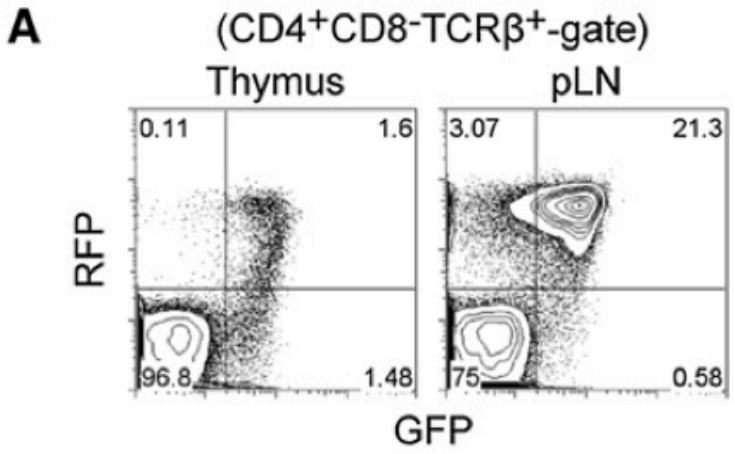
- Treg ($CD4^+CD25^+Foxp3^+$)are indispensable for immune homeostasis.
- Mutations in Foxp3 gene leads to fatal autoimmune disorder.
- Conditional deletion of Foxp3 reprograms Treg cells into pathogenic Th cells.
- Tregs are capable of maintaining stable Foxp3 expression. This is mediated by demethylation of Treg cell specific demethylation region (TSDR).
- Recent studies however suggest Treg may lose Foxp3 expression and be " reprogrammed" to effector Th cells.
- These exFoxp3 are induced in inflammatory cytokine milieus.
- Genetic fate mapping approach: 10-20 % of are Foxp3-.
- Do T cells that transiently express Foxp3 acquire Treg function ?

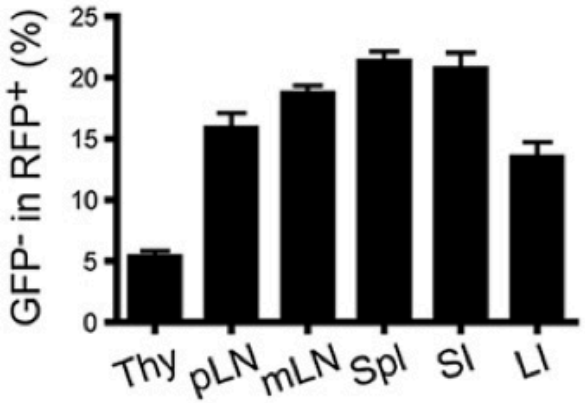
Genetic fate mapping



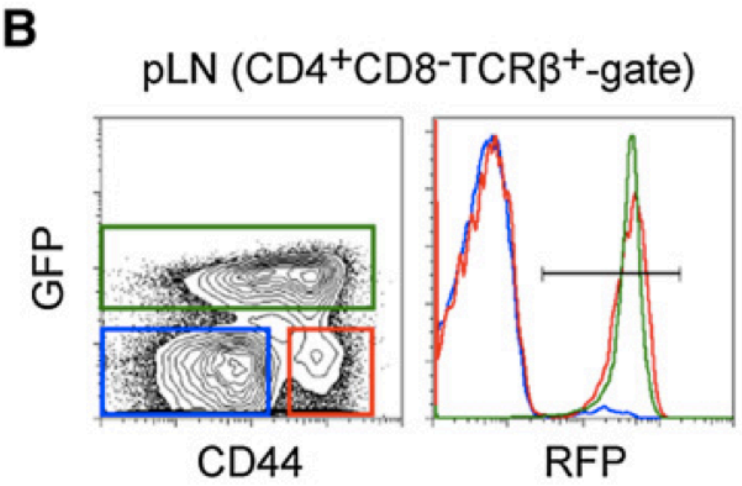
They hypothesized that exFoxp3 T cells do not reflect reprogramming of committed Tregs cells but rather a minor population of uncommitted Foxp3^{+} T cells

Generation and accumulation of exFoxp3 T cells during ontogeny

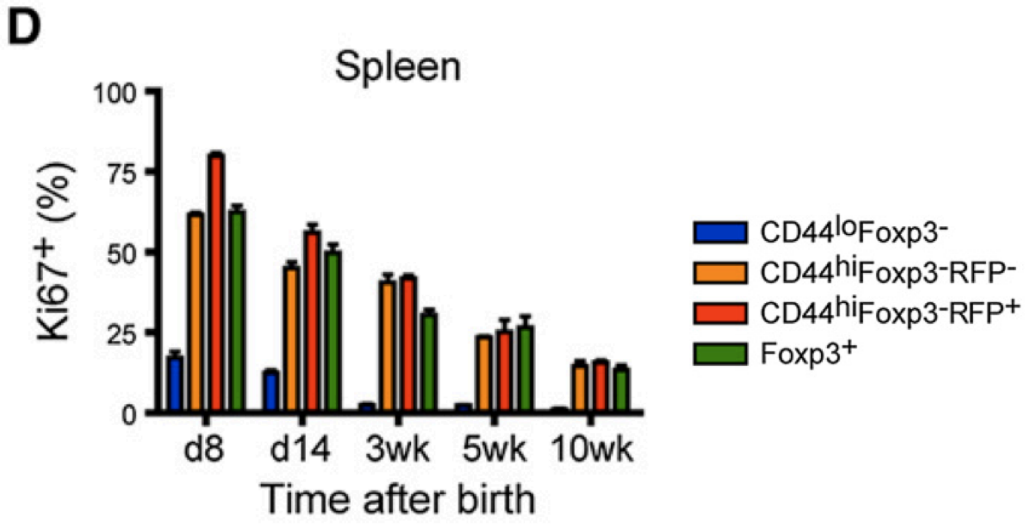




5 to 6 weeks old mice

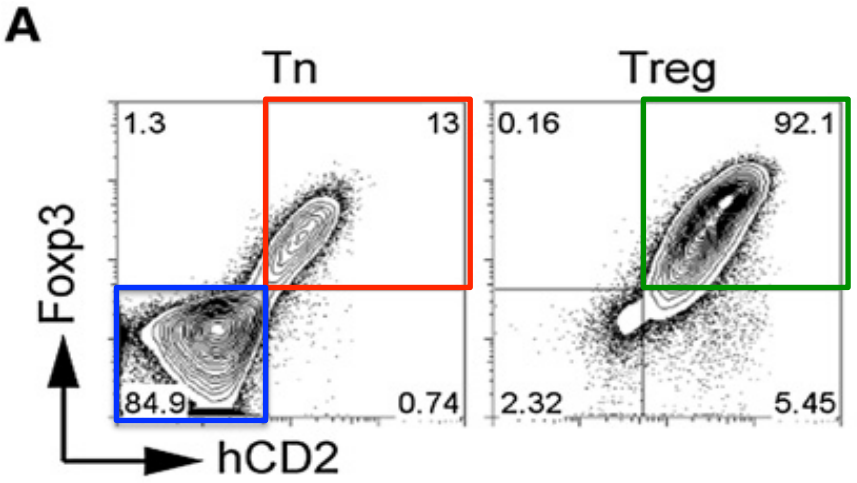


CD62L, CD69 and CCR7

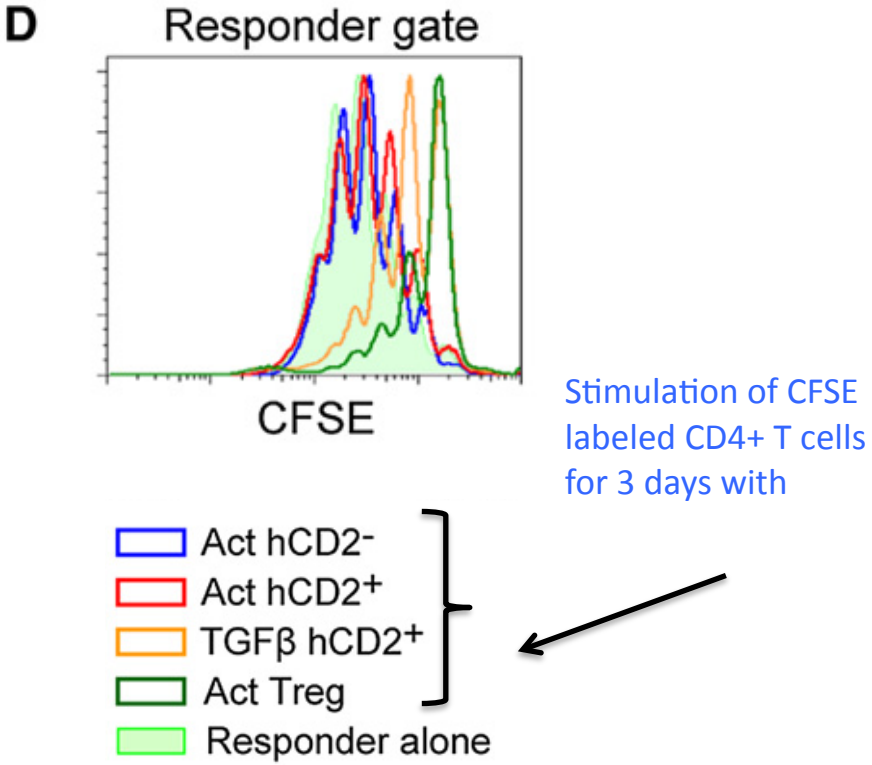


Foxp3^{-}RPF^{+} T cells results from downregulation of Foxp3 expression in Foxp3^{+} T cells and lymphopenia driven proliferation promotes accumulation of exFoxp3 T cells

Foxp3 expression in activated T cells



Naïve T cells from lymph node or spleen stimulated with αCD3, αCD28 and IL-2 for 4 days and **(with TGFβ)**

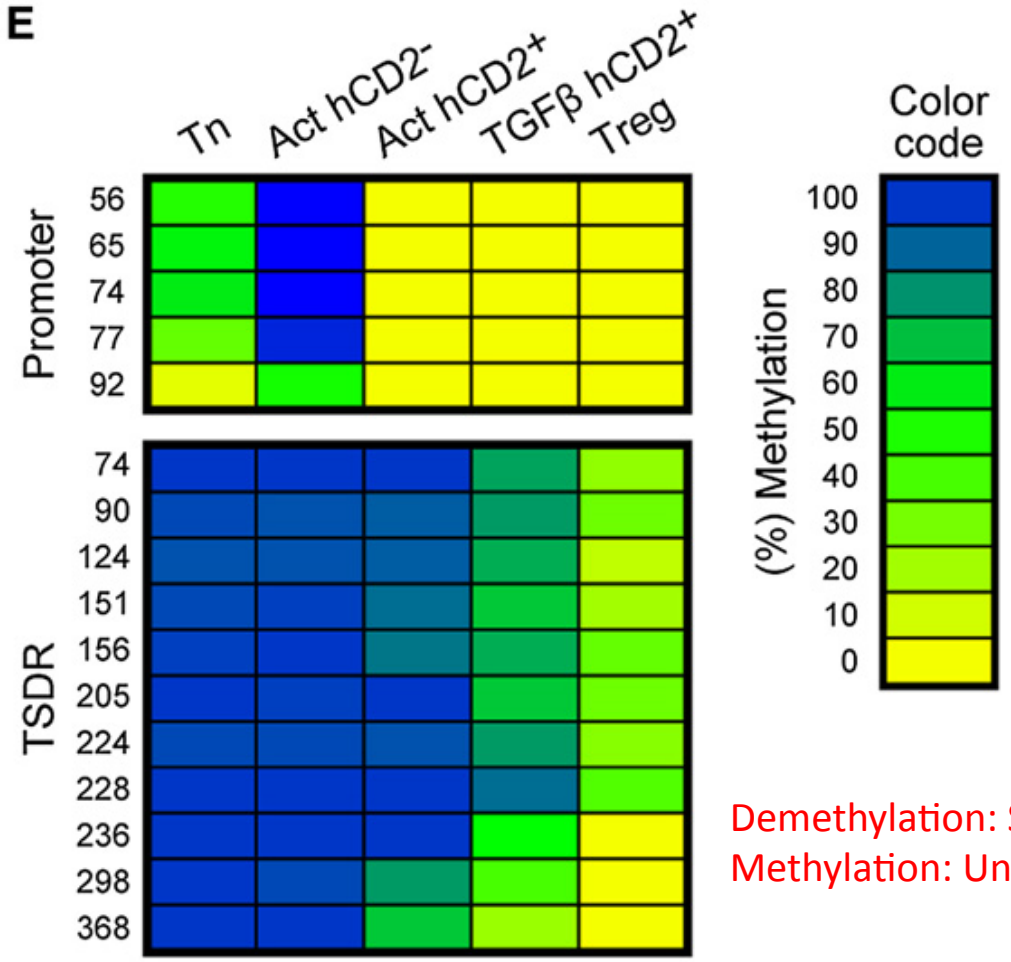


Stimulation of CFSE labeled CD4+ T cells for 3 days with

Naïve T cells can exhibit promiscuous Foxp3 expression upon activation

Methylation status in different populations

TSDR: Treg cell specific demethylation region



Demethylation: Stable
Methylation: Unstable

Genomic DNA

↓ Sodium bisulphite

Deamination of unmethylated cytosines to uracil

↓

Methylated cytosines remain unchanged

↓

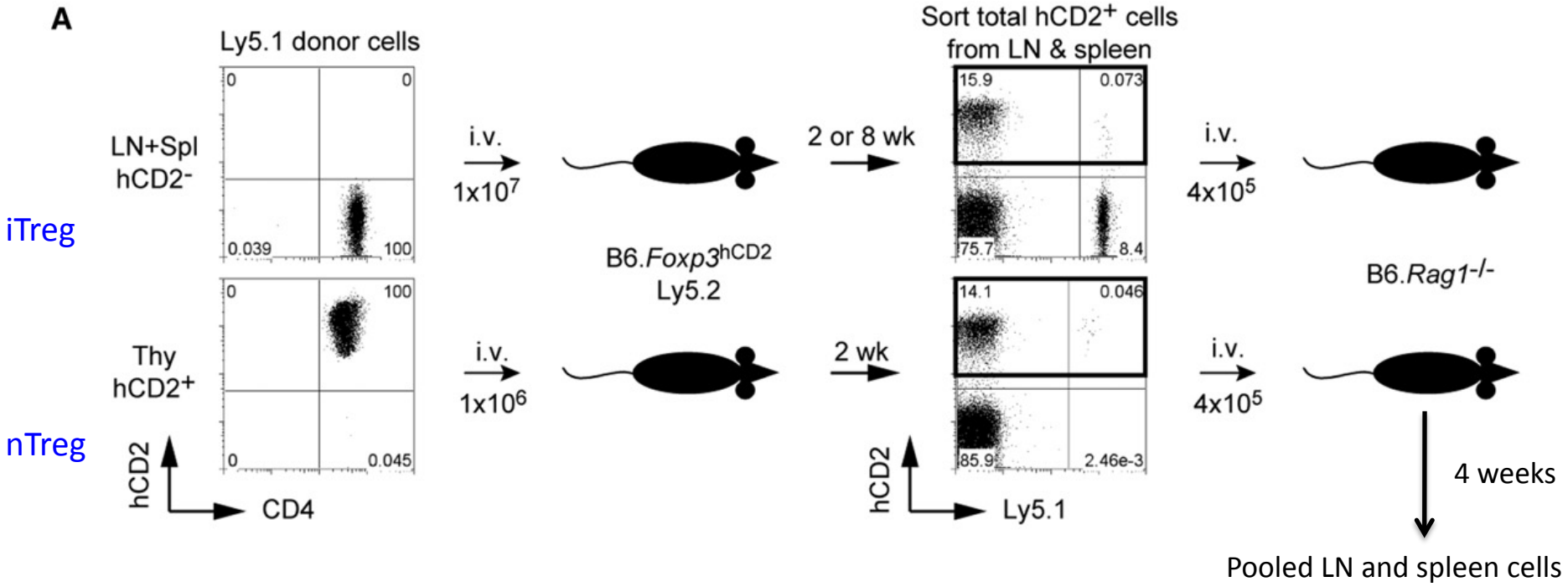
Detection of T reflects unmethylation

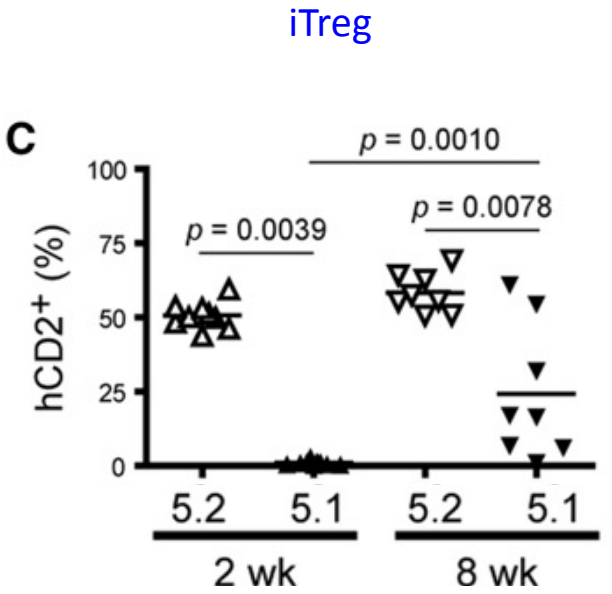
↓

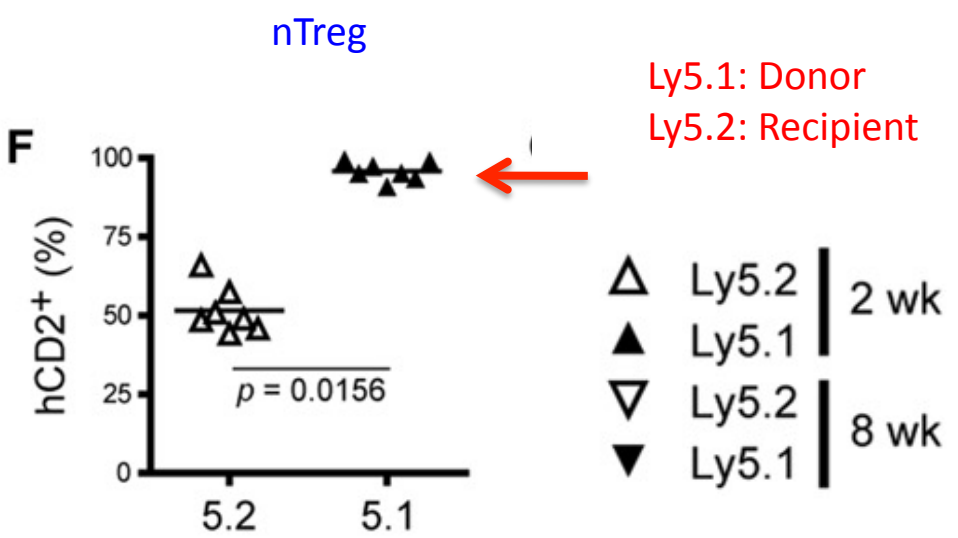
Sequencing

Activity and stability of Foxp3 transcription is differentially associated with demethylation of the TSDR

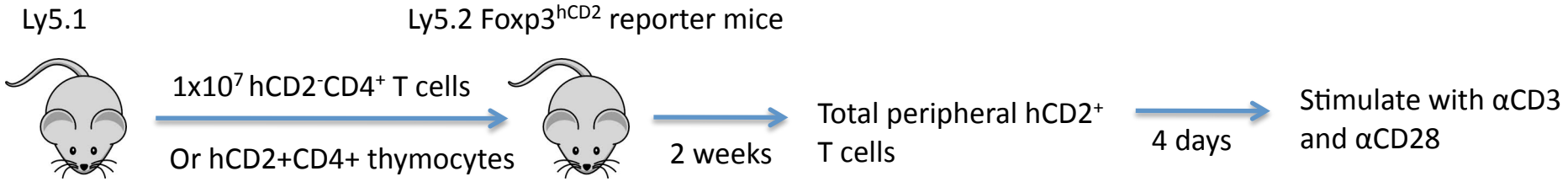
Generation of exFoxp3 T cells in lymphopenic environment

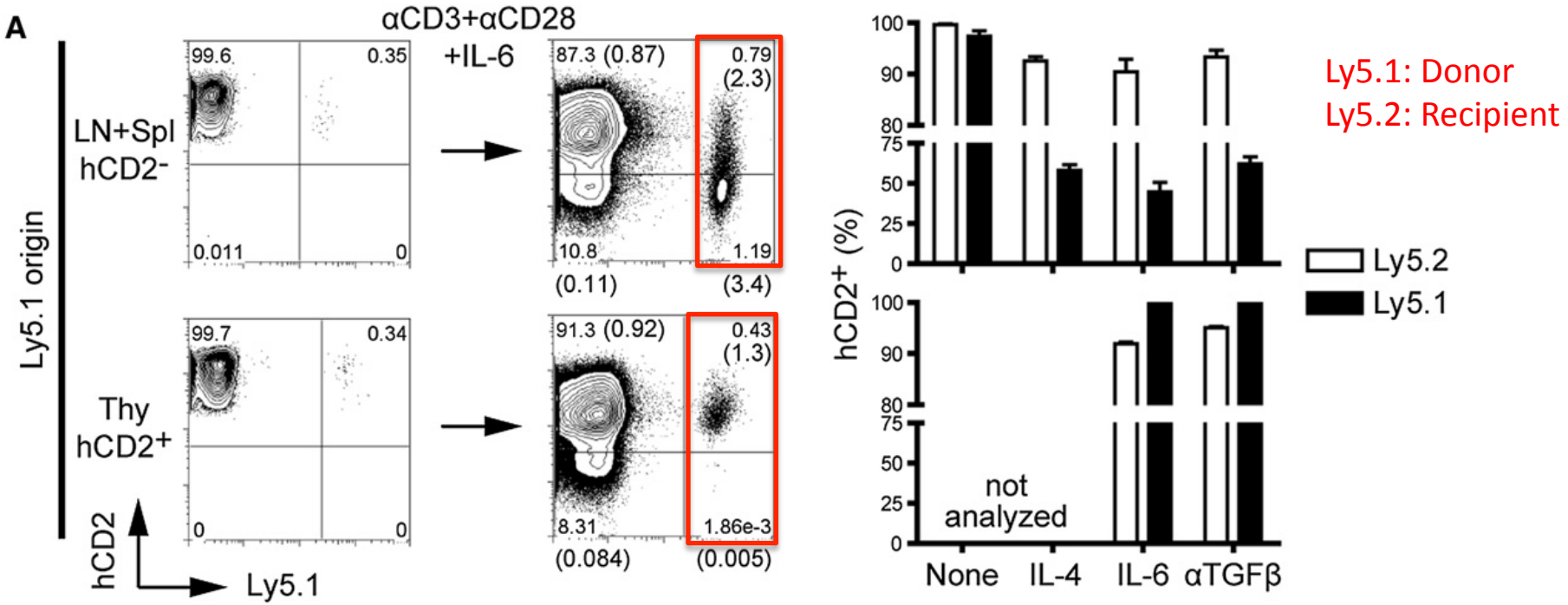






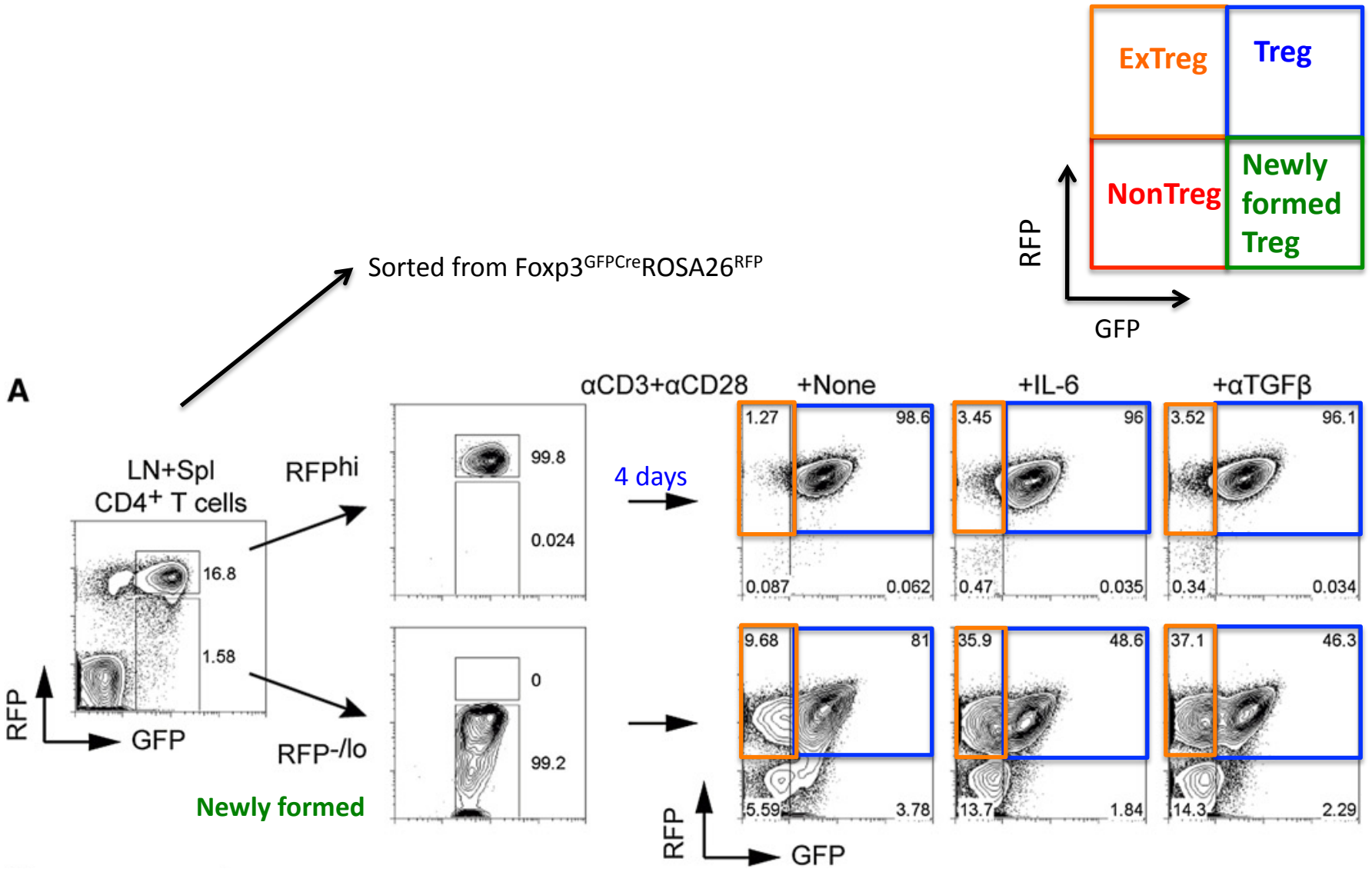
Generation of exFoxp3 in inflammatory conditions in vitro





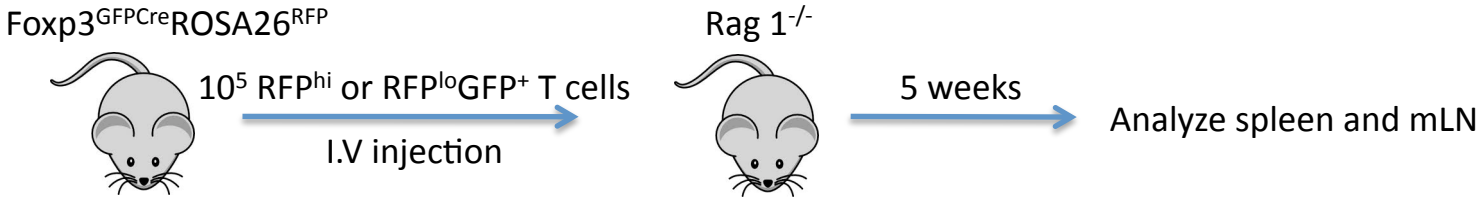
Peripherally induced Foxp3+ T cells differentiate into Foxp3- Th cells under inflammatory conditions

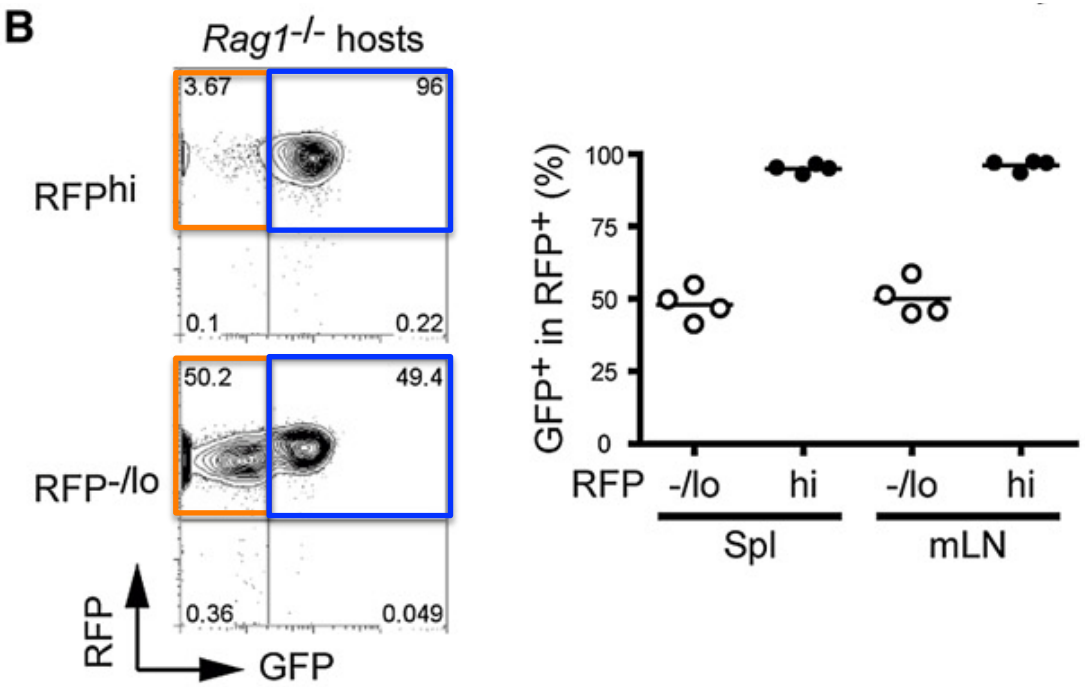
Promiscuous and unstable Foxp3 expression in normal mice

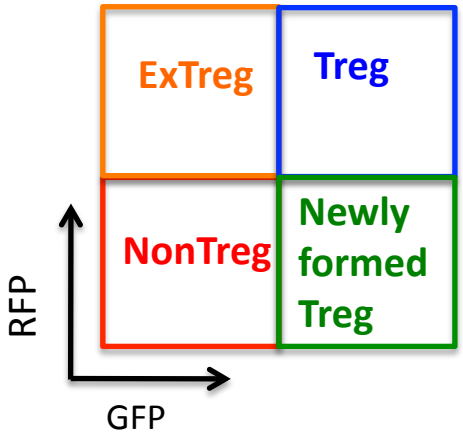


RPF-CD25-Foxp3+ T cells showed promiscuous and unstable Foxp3 expression

Promiscuous and unstable Foxp3 expression

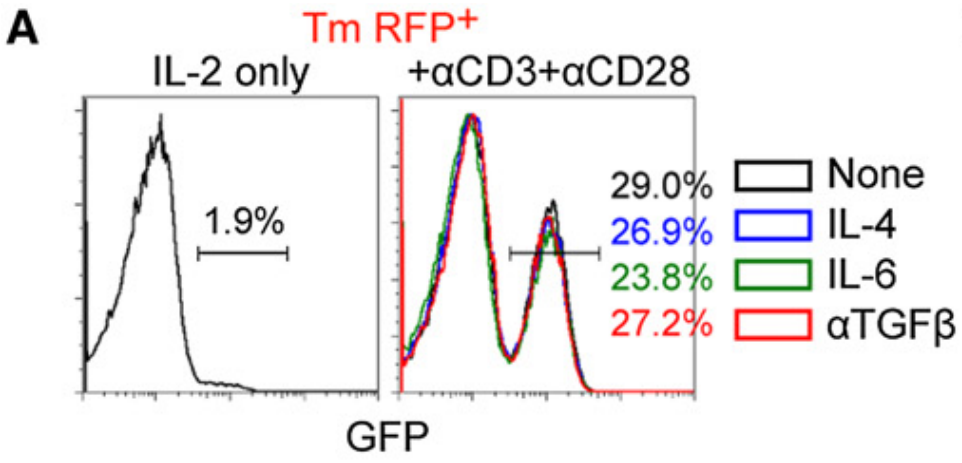






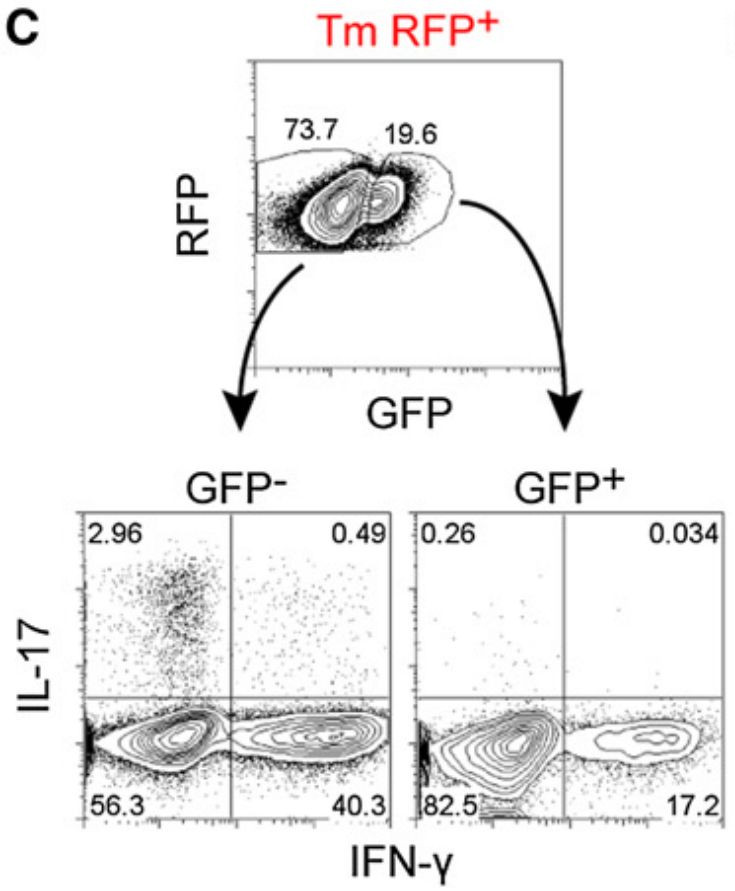
RPF-CD25-Foxp3+ T cells showed promiscuous and unstable Foxp3 expression

Re-expression of Foxp3 expression in exFoxp3 T cells following activation



CD4+ T cells from Foxp3GFPCreROSA26^{RFP} were stimulated for 4 days

Tm: memory T cells cd44^{hi}

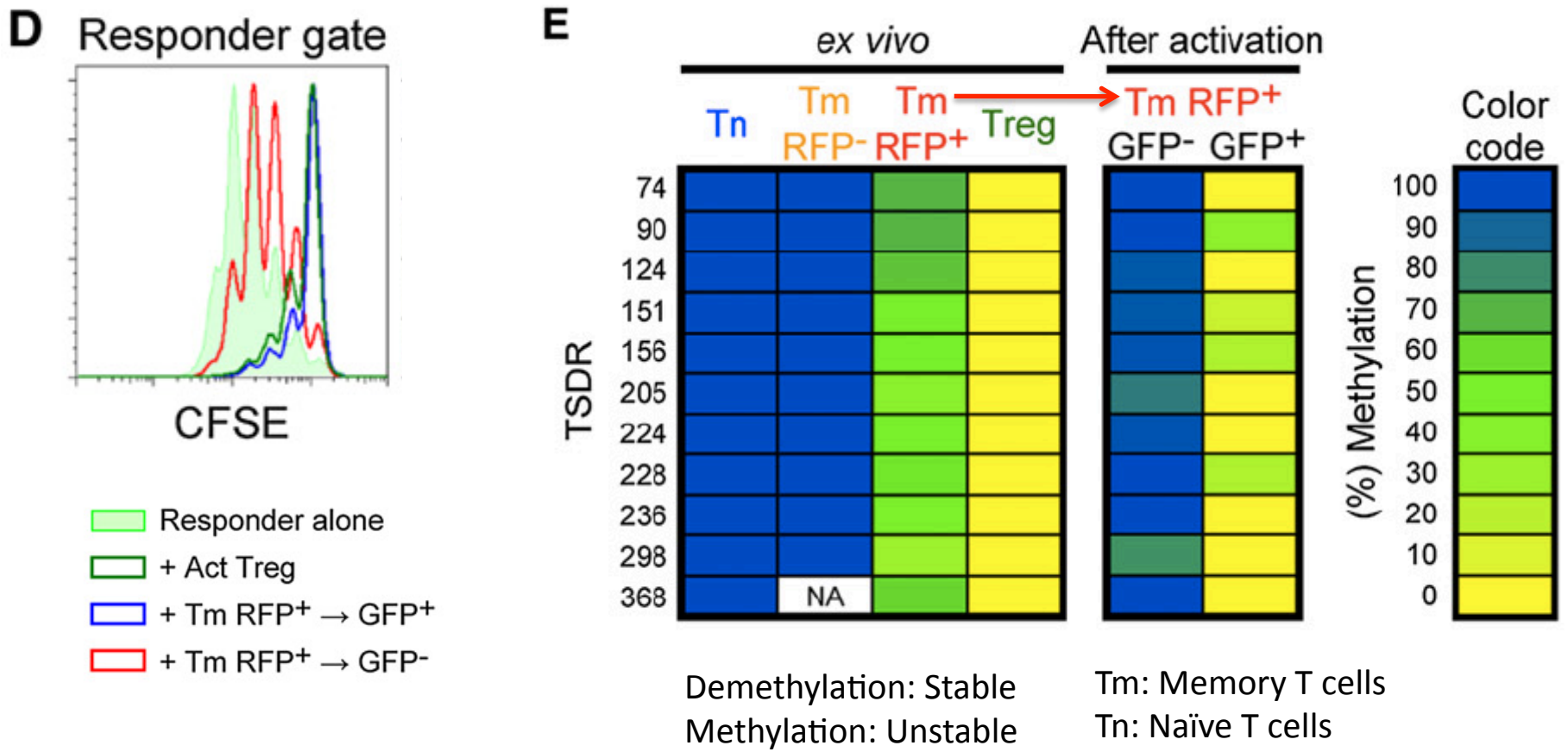


Tm cells stimulated with PMA and ionomycin for 4 hrs

exFoxp3 T cells reacquire Foxp3 expression in a TCR dependent manner

DNA methylation status of the TSDR of exFoxp3 T cells that have reacquired Foxp3 expression

TSDR: Treg cell specific demethylation region



Demethylation: Stable
Methylation: Unstable

Tm: Memory T cells
Tn: Naïve T cells

Collectively, exFoxp3 T cells also consist of “latent” Tregs that reacquire Foxp3 expression and exhibit a demethylated TSDR (stable)

Conclusions

- Minor population of Foxp3+ T cells that exhibit promiscuous Foxp3 expression and have no suppressive capacity and no Treg function.
- Confirmed previous findings that a significant portion of exFoxp3 T cells have effector or memory phenotype.
- Study refutes the notion that exTregs are generated by “reprogramming” of Tregs.
- Results suggest that Tregs are committed to Foxp3 expression under steady state or under various pertubed conditions.
- Demethylation status of TSDR identifies Foxp3+ Tregs, as well as Foxp3- latent Treg cells and therefore represents a signature for T cells committed to the Treg lineage.
- Loss of Foxp3 and subsequent re-expression of Foxp3 could be caused by the availability of TCR stimulation.
- Future studies should distinguish de novo Foxp3 induction from Foxp3 reinduction when investigating iTregs.

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JEM

Brief Definitive Report

Microbiota-induced IL-1β, but not IL-6, is critical for the development of steady-state T_H17 cells in the intestine

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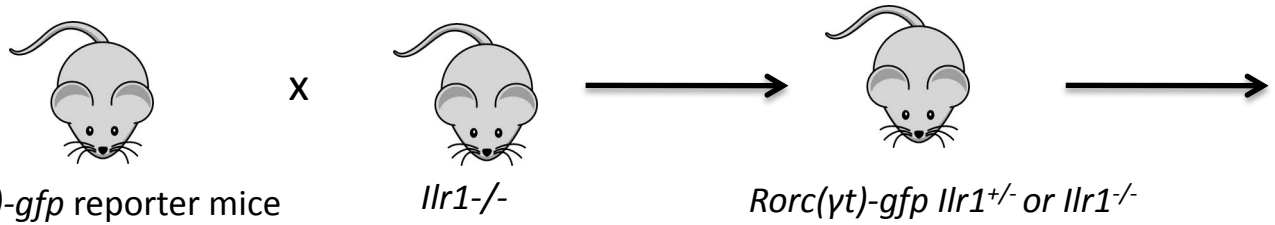
Department of Pathology and Comprehensive Cancer Center, The University of Michigan Medical School, Ann Arbor, MI 48109

Introduction

- Th17 cells are critical for host defense and autoimmunity.
- Differentiation of these cells require IL-1β, IL-6, IL-23 and TGF-β.
- Th17 cells can be found in the intestine at steady state, depending on the microbiota.
- The signaling pathway linking the microbiota to the generation of Th17 cells remains to be elucidated.
- Current evidence shows that IL-1R signaling are important for Th17 cell development.
- Intracellular staining of LP cells, after stimulation with phorbol ester and ionomycin suggest that MyD88 and IL-1β-IL-1R may not be important for the development of Th17 cells in the intestine.
- Recent findings show that stimulation with phorbol ester and ionomycin may exaggerate IL-17 expression by Th17 cells.
- What is the role of IL-1R and MyD88 signaling in the induction of Th17 cells in the intestine ?

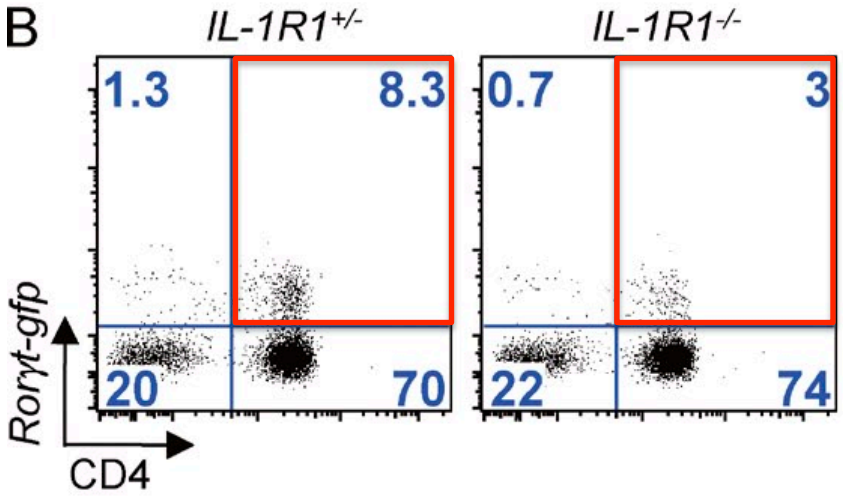
IL-1β-IL-1R signaling promotes the development of Th17 cells

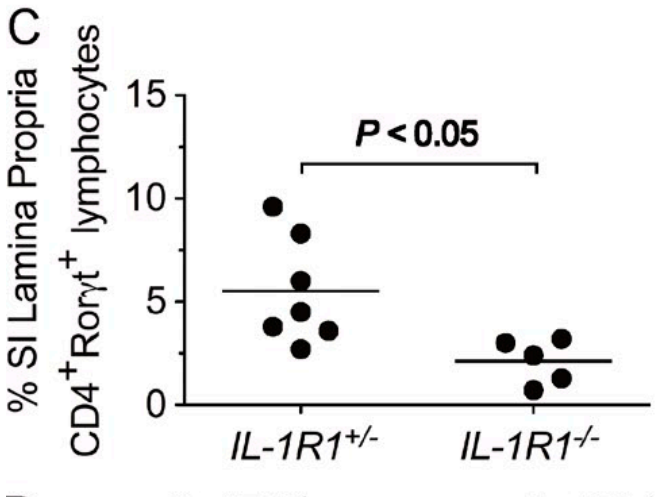


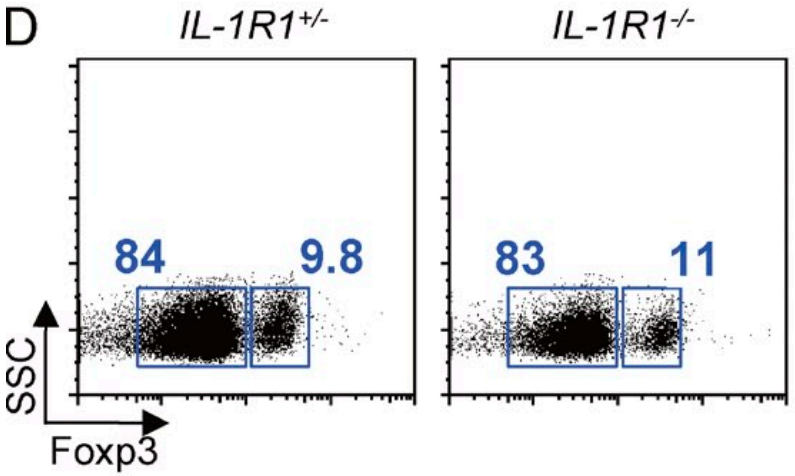
Rorc(γt)-gfp reporter mice

Il1r-/-

Rorc(γt)-gfp Il1r$^{+/-}$ *or Il1r*$^{-/-}$





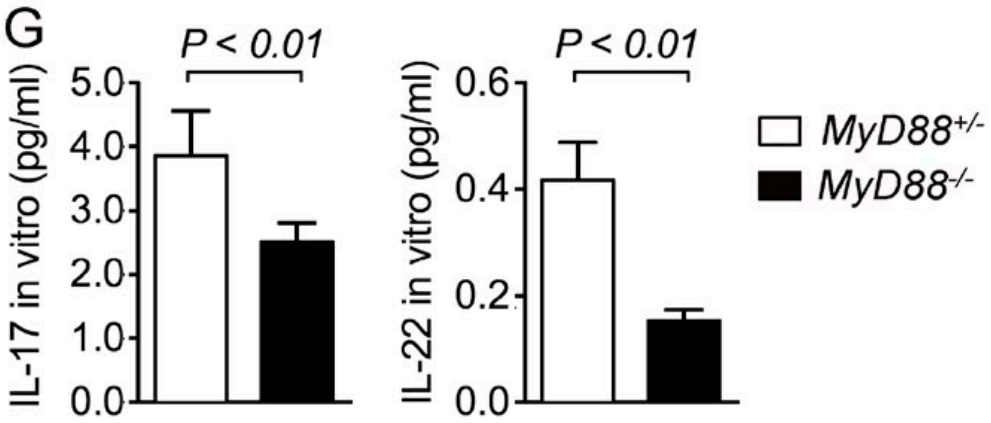


Development of intestinal Th17 responses requires MyD88 but not IL-6

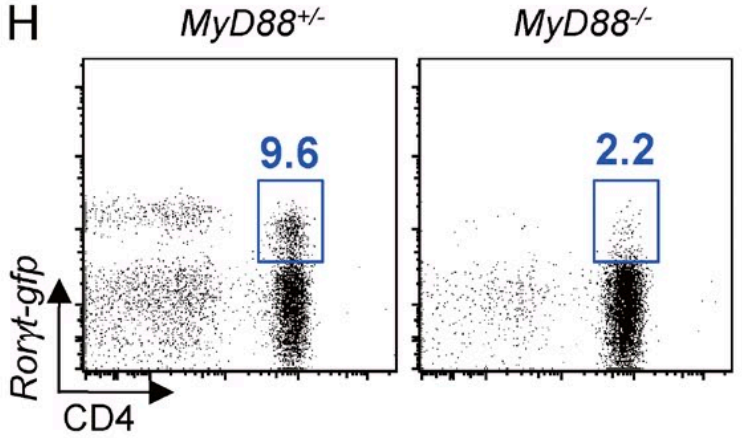

Rorc(γt)-gfp reporter mice

x

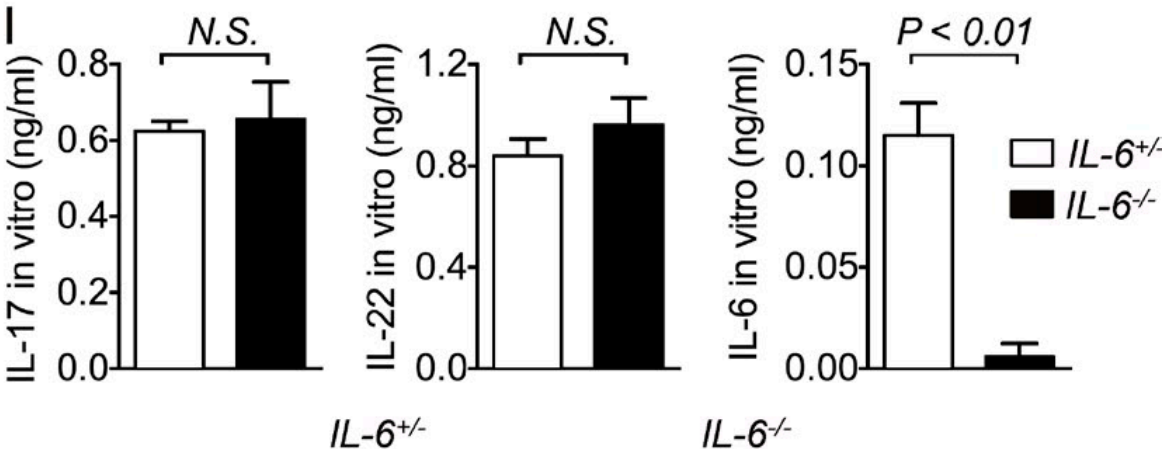

MyD88-/-



FACS sorted CD4+ T cells stimulated with αCD3 overnight

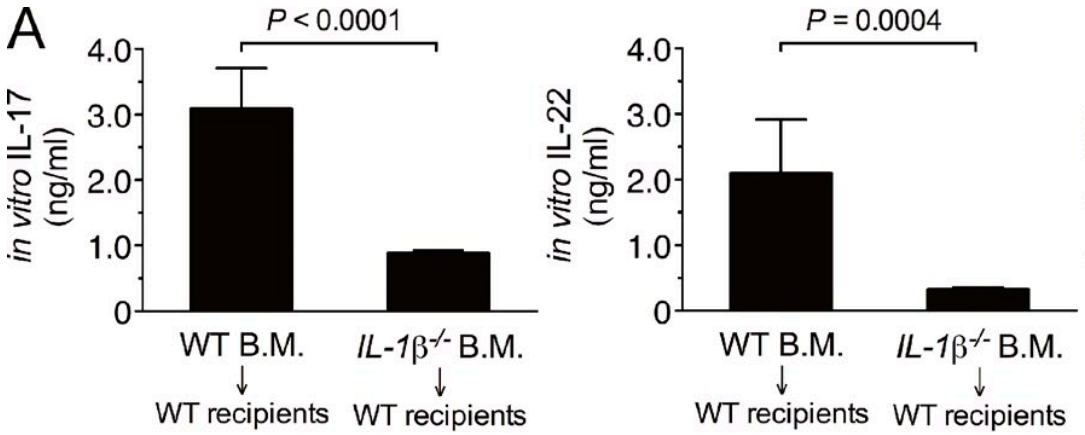


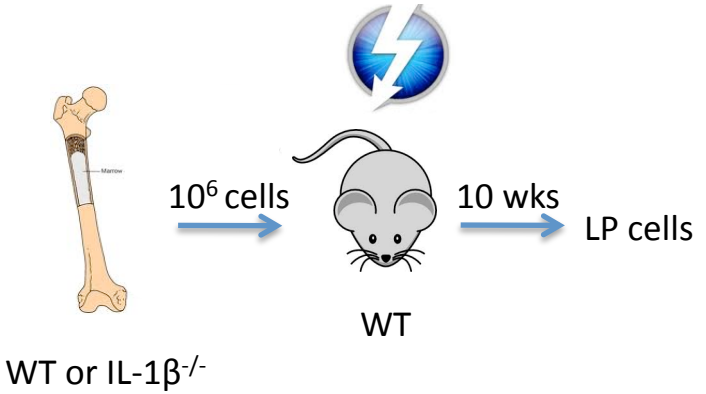
Small LP CD3⁺ T cells stimulated with αCD3 overnight



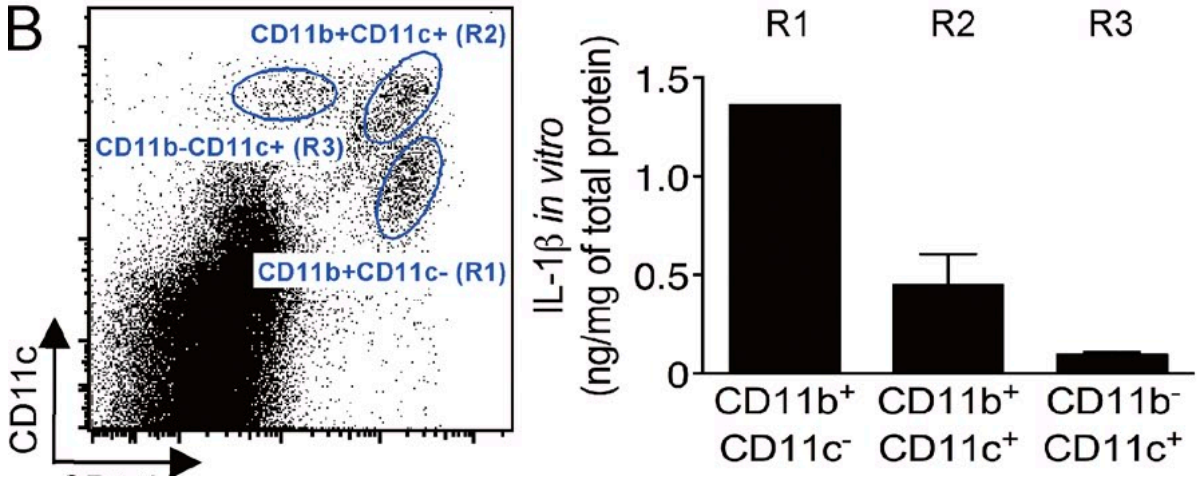
FACS sorted CD4+ T cells stimulated with αCD3 overnight

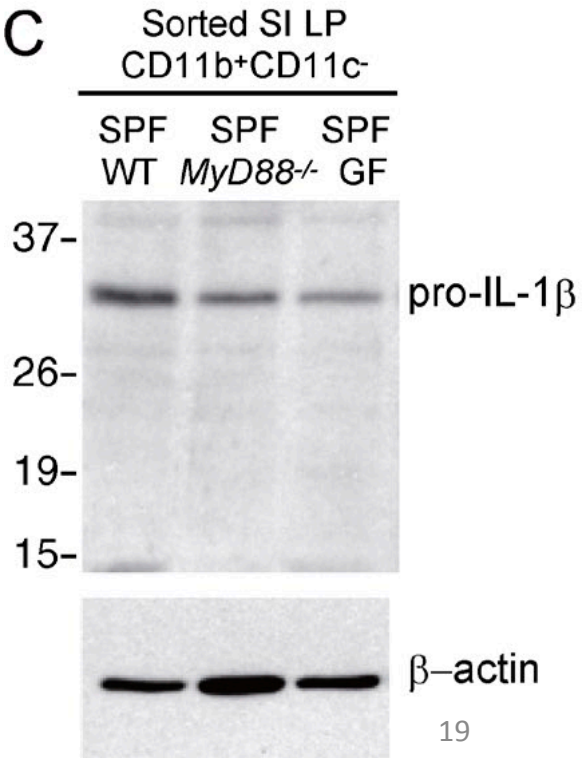
CD11b^{+}F4/80^{+}CD11c^{-} macrophages are the main source of IL-1β in the intestine



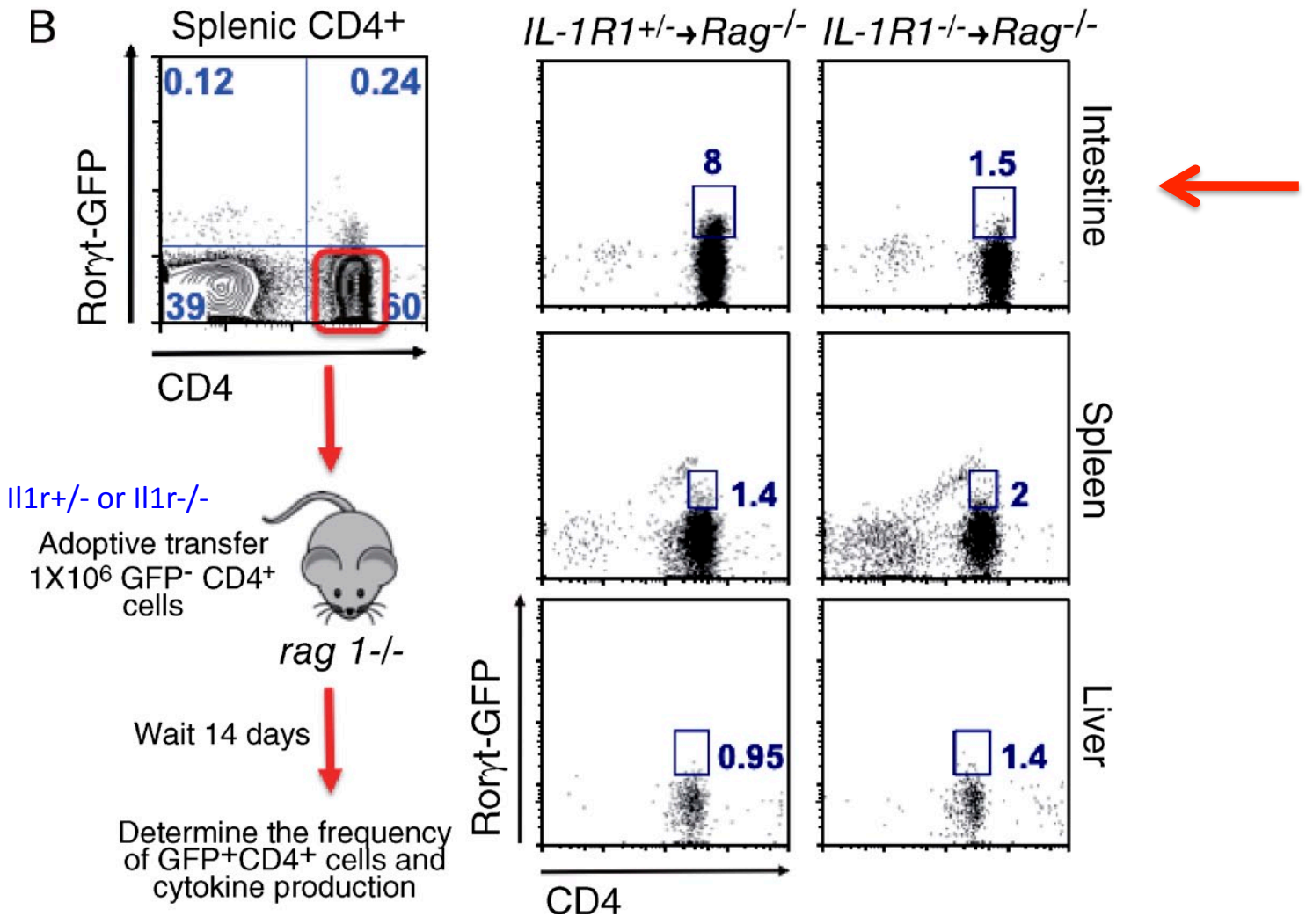


Stimulation with αCD3 overnight

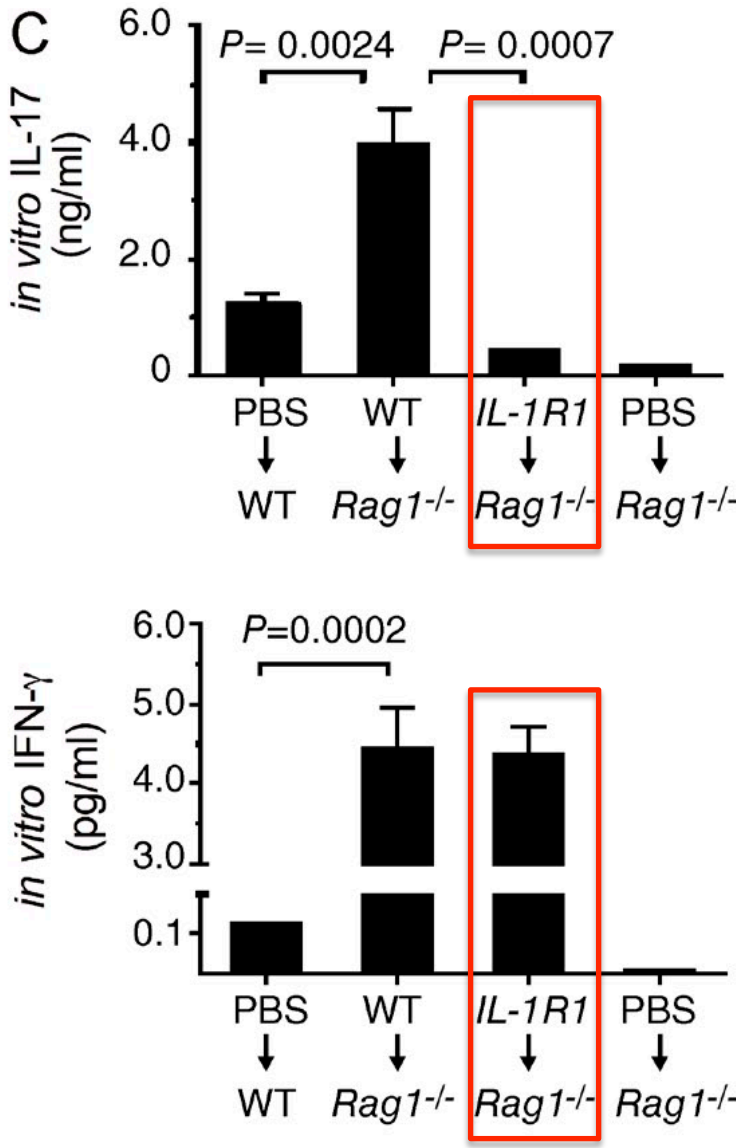




IL-1R signaling on T cells is required for the generation of intestinal Th17 responses



IL-1R signaling on T cells is required for the generation of IL-17 responses



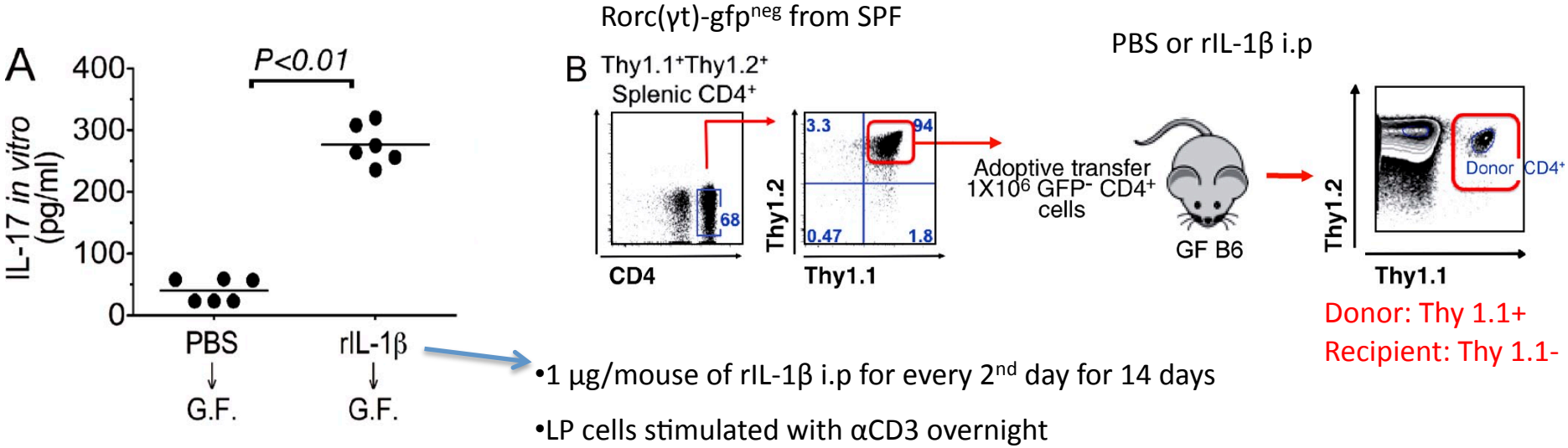
Il1r$^{+/-}$ or Il1r$^{-/-}$GFP^{-} CD4^{+} T cells

Rag 1-/-

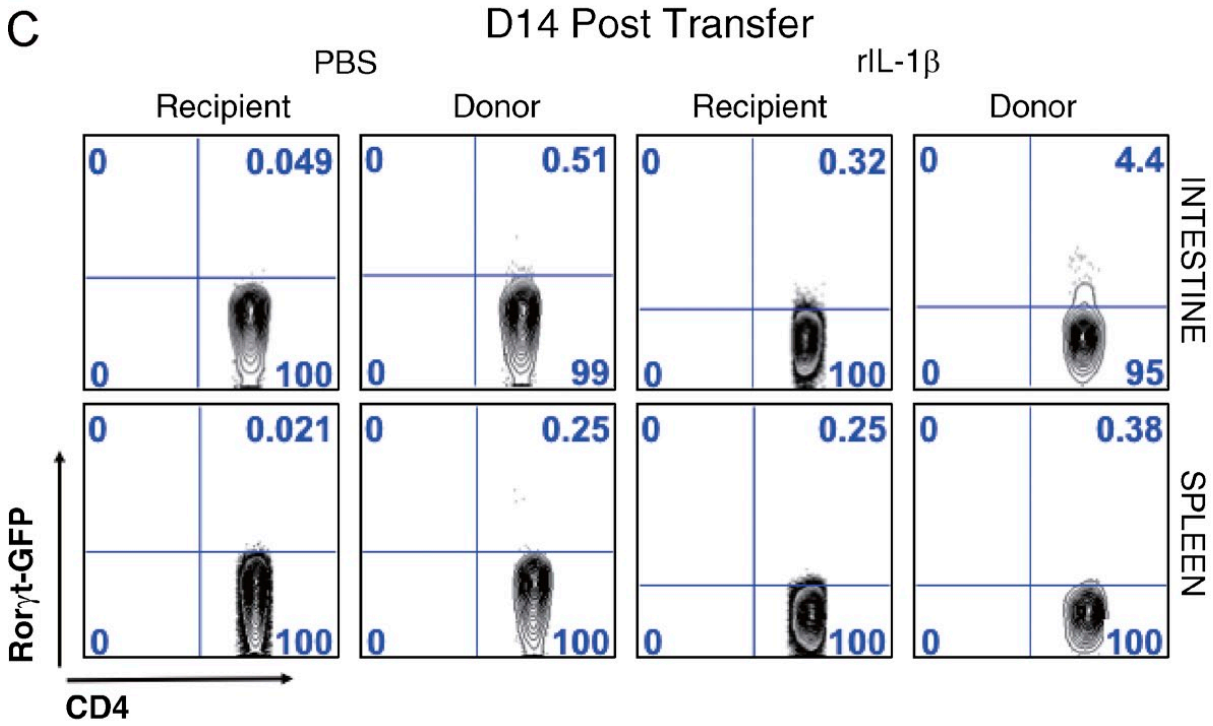
14 days

Total LP cells were isolated and stimulated ex vivo with αCD3

Administration of IL-1β is sufficient for Rorγt expressing Th17 cells in GF mice



- 1 µg/mouse of rIL-1β i.p for every 2nd day for 14 days
- LP cells stimulated with αCD3 overnight



Discussion

- IL-1β/IL-1R axis is not only required for, but also sufficient to drive development of Th17 cells in the intestine
- IL-6 unexpectedly was not required for the development of intestinal Th17 cells.
- This may suggest differential regulation for the development of Th17 cells which is dependent on the local tissue environment
- MyD88 is critical for the induction of IL-1β in intestinal macrophages
- Two steps for the regulation of Th17 cells:

I. MyD88 links the microbiota to pro-IL-1β induction in intestinal macrophages via TLR-MyD88 signaling pathway.

II. IL-1β/IL-R1/MyD88 signaling in CD4 T cells to drive development of intestinal Th17 cells.

Commensal bacteria ➔TLR signaling (via MyD88) ➔ IL-1β production in macrophages ➔ Th17 differentiation in the intestine