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Supplemental Information

Th17 Cells Express Interleukin-10 Receptor and Are Controlled by Foxp3^{-} and Foxp3^{+} Regulatory CD4^{+} T Cells in an Interleukin-10-Dependent Manner

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Inventory of Supplemental Information

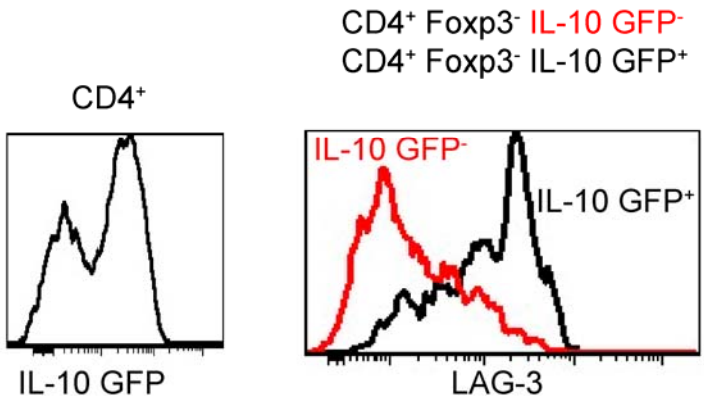

Figure S1 (related to Figure 1): IL-10 producing cells in the small intestine express LAG-3. Foxp3 RFP IL-10 eGFP double reporter mice were treated with anti-CD3. Mononuclear cells were isolated from the small intestine and stained for LAG-3 and CD4. Cells were gated as indicated.

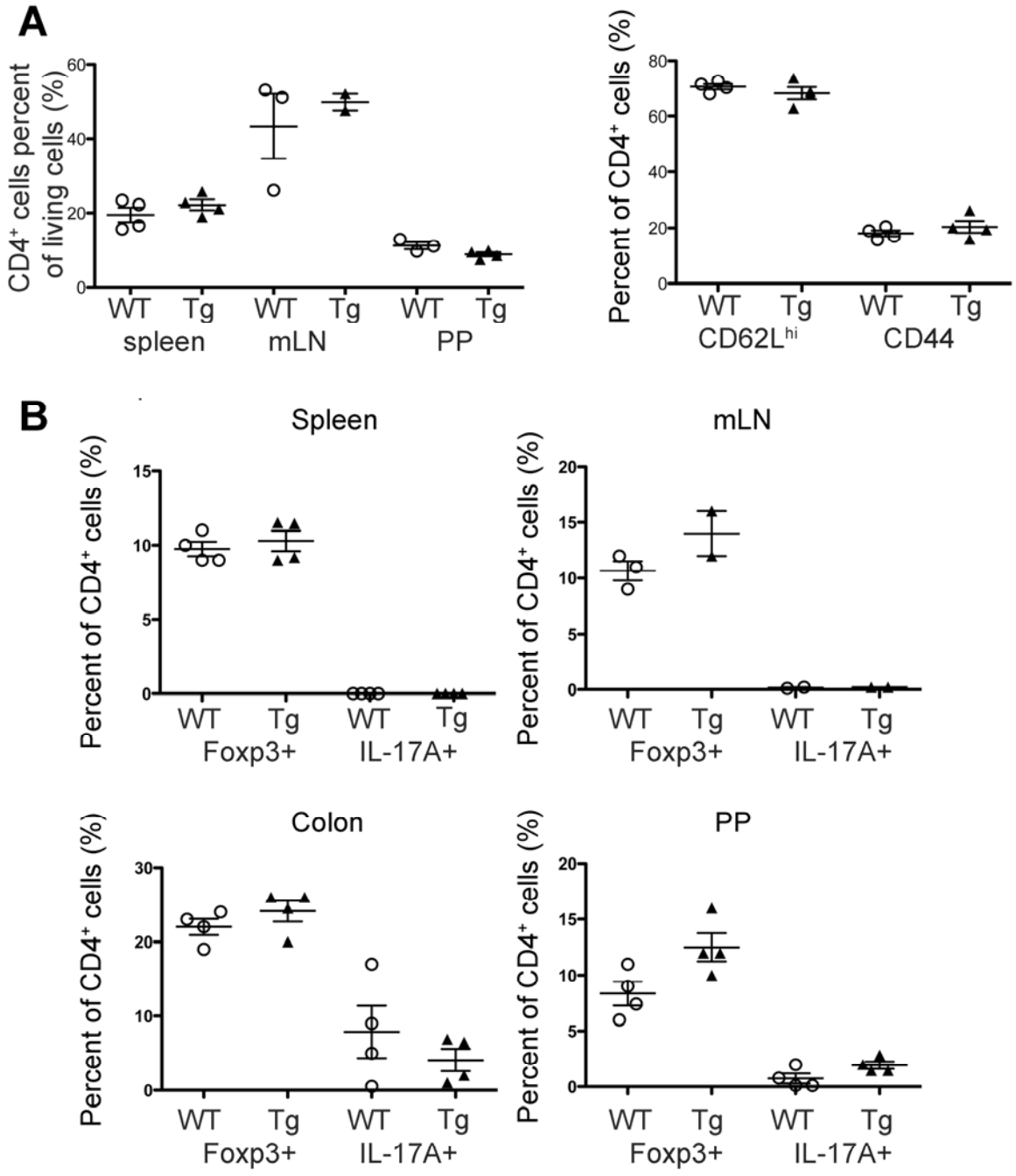

Figure S2 (related to Figure 2): *Cd4*-DNIL-10R transgenic (Tg) mice show no spontaneous CD4 T cell phenotype. A: CD4 T cell frequency in spleen, mesenteric lymph nodes (mLN) and Peyers Patches (PP). Cells are gated on living events. **B:** Frequency of Foxp3 RFP and IL-17A eGFP positive cells in spleen, MLN, colon, and PP. Cells are gated on $CD4^+$ events. 8-week-old littermates were analyzed. Results are representative of at least three independent experiments. Mean ± SEM are shown.

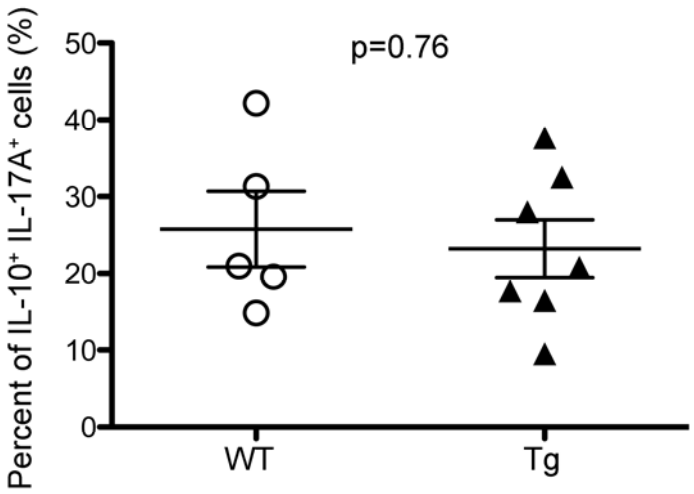

Figure S3 (related to Figure 4): IL-10 signaling in T cells is not essential for the emergence of IL-17A$^+$IL-10$^+$ double producing T cells. *Cd4*-DNIL-10R or WT Foxp3 RFP IL-10 eGFP double reporter mice were injected with anti-CD3. CD4$^+$ T cells were isolated from the small intestine. Cells were re-stimulated *in vitro* with PMA and Ionomycin, and intracellular cytokine staining for IL-17A and anti-GFP was performed. Cells are gated on CD4$^+$IL-17A$^+$ events. Results are representative of four independent experiments. Mean ± SEM are shown.

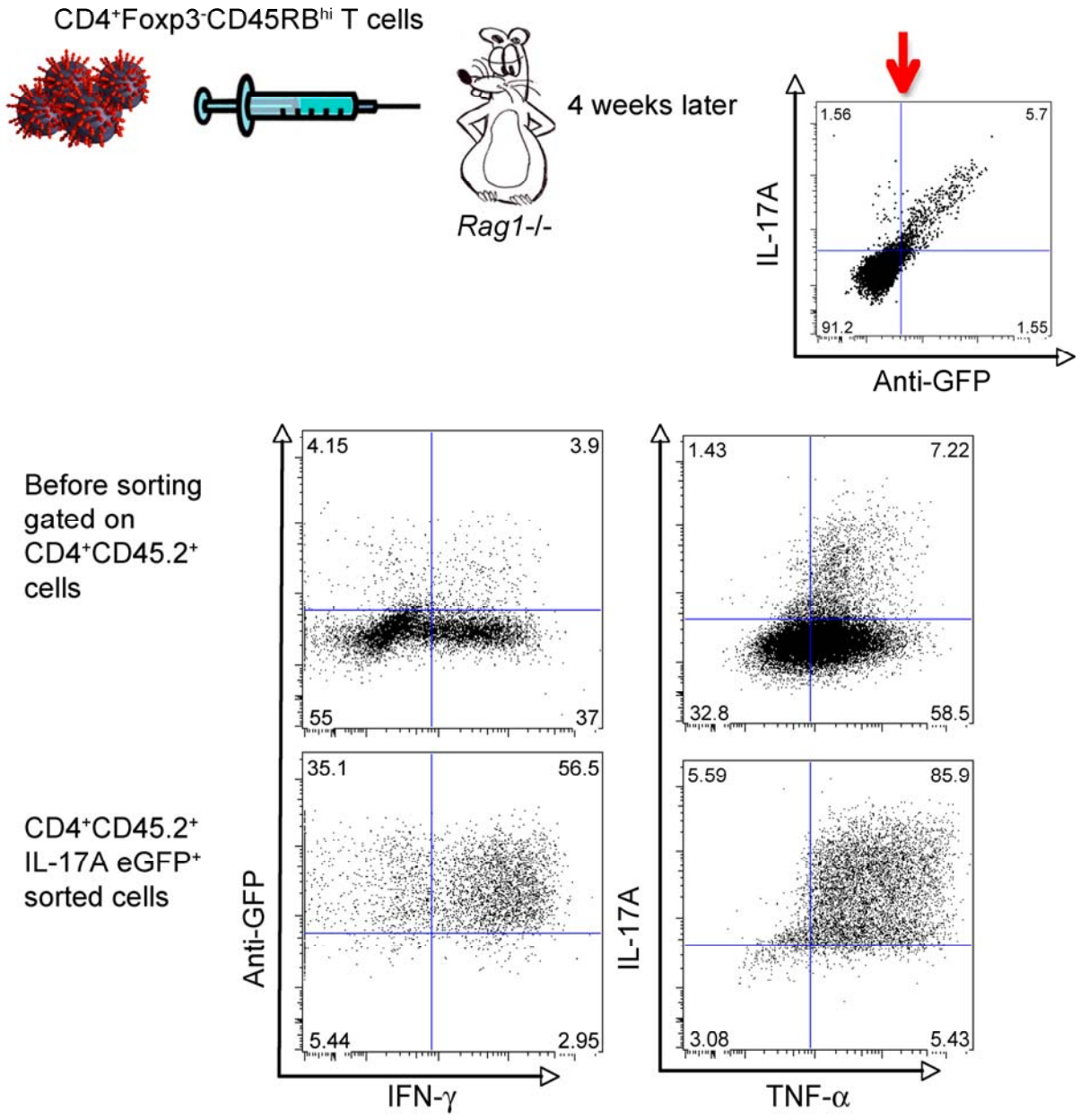

Figure S4 (related to Figure 5 + 6): Experimental design to isolate *in vivo* differentiated Th17 cells. $CD4^+Foxp3^-CD45RB^{hi}$ T cells were isolated from the spleen and lymph nodes of Foxp3 RFP IL-17A eGFP double reporter mice (CD45.2), and transferred i.p. into *Rag1*$^{-/-}$ (CD45.1) mice (250-300 x10^3 cells/mouse). Based on the endoscopic colitis score (between 9-13) mice were sacrificed about 4 weeks after the transfer, and cells isolated from the colon and mesenteric lymph nodes. (Upper left) Correlation of IL-17A protein levels with IL-17A eGFP expression. (Bottom)

$CD4^+CD45.2^+$IL-17A $eGFP^+$ Foxp RFP^- cells were sorted using FACS. Part of the samples before and after the sorting was re-stimulated using PMA and Ionomycin. TNF-α, IFN-γ, IL-17A and IL-17A eGFP expression was analyzed using intracellular cytokine staining. Data are representative of 2 independent experiments.