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Supplemental Information

Interleukin-10 Signaling in Regulatory T Cells Is Required for Suppression of Th17 Cell-Mediated Inflammation

Ashutosh Chaudhry, Robert M. Samstein, Piper Treuting, Yuqiong Liang, Marina C. Pils, Jan-Michael Heinrich, Robert S. Jack, F. Thomas Wunderlich, Jens C. Brüning, Werner Müller, and Alexander Y. Rudensky

Inventory of Supplemental Information:

Supplementary Figure 1 accompanies Figure 1 and shows efficiency of (A) IL-6Rα and (B) IL-10Rα deletion by $Foxp3^{Cre}$.

Supplementary Figure 2 accompanies Figure 2 and shows complete absence of inflammation in $Foxp3^{Cre/wt}Il10ra^{fl/fl}$ mice.

Supplementary Figure 3 accompanies Figure 3 and shows that Treg cell-specific inducible deletion of IL-10Rα does not lead to aberrant expression of Th1, Th2 and Th17 transcription factors and cytokines.

Supplementary Figure 4 and 5 accompany Figure 4 and demonstrate that lack of IL-6Rα or IL-23R on Treg cells does not result in dysregulation of immune responses.

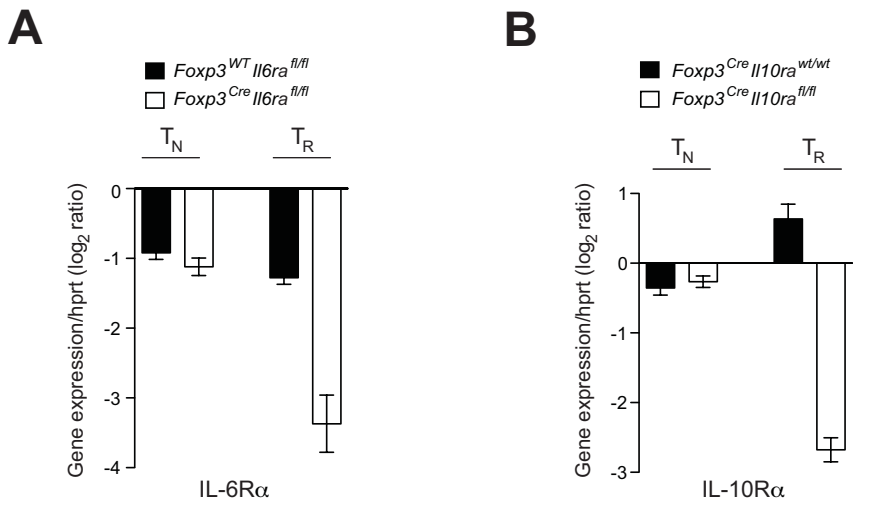

Figure S1. Foxp3^{Cre}-mediated deletion is restricted to Treg cells.
(A, B) qPCR analysis of IL-6Rα and IL-10Rα mRNA levels in RNA extracted from indicated cell populations.

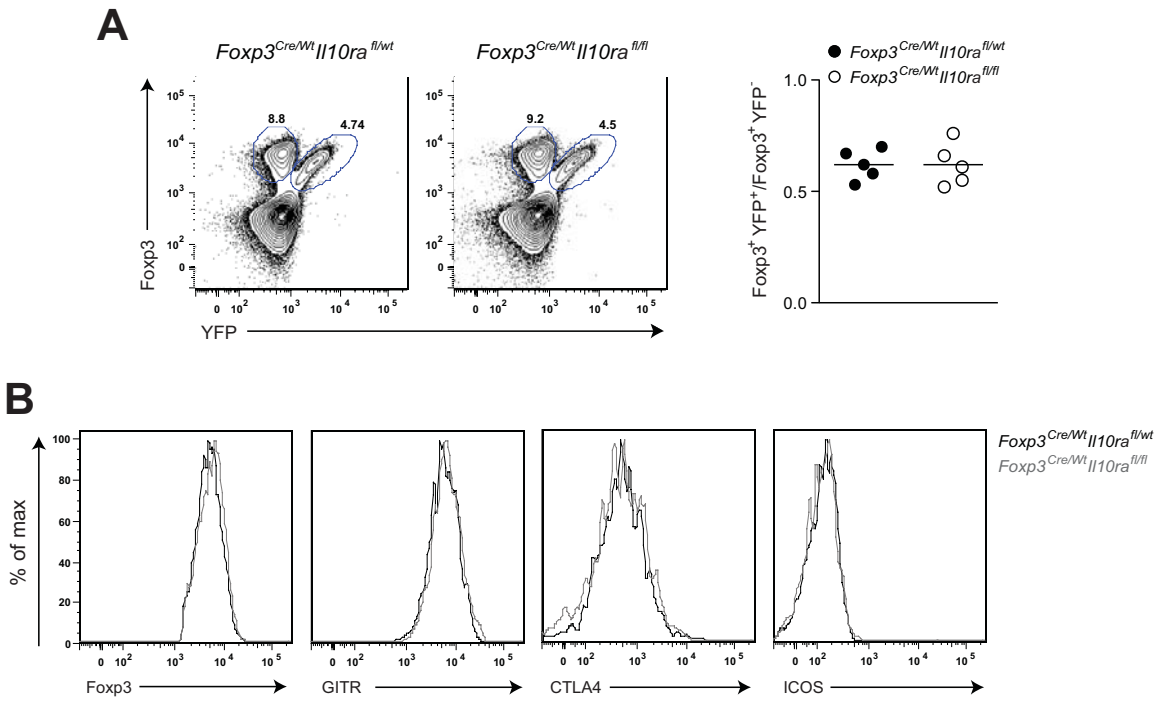

Figure S2. Absence of inflammation in heterozygous females.
(A) Relative frequencies of CD4$^+$Foxp3$^+$YFP$^+$ and CD4$^+$Foxp3$^+$YFP$^-$ Treg cells in the spleen of *Foxp3*$^{Cre/Wt}$*Il10ra*$^{fl/wt}$ and *Foxp3*$^{Cre/Wt}$*Il10ra*$^{fl/fl}$ mice. (B) Flow cytometric analysis of Foxp3 levels as well as Treg cell activation and putative effector molecules on splenic CD4$^+$YFP$^+$ Treg cells in *Foxp3*$^{Cre/Wt}$*Il10ra*$^{fl/fl}$ and control littermate mice.

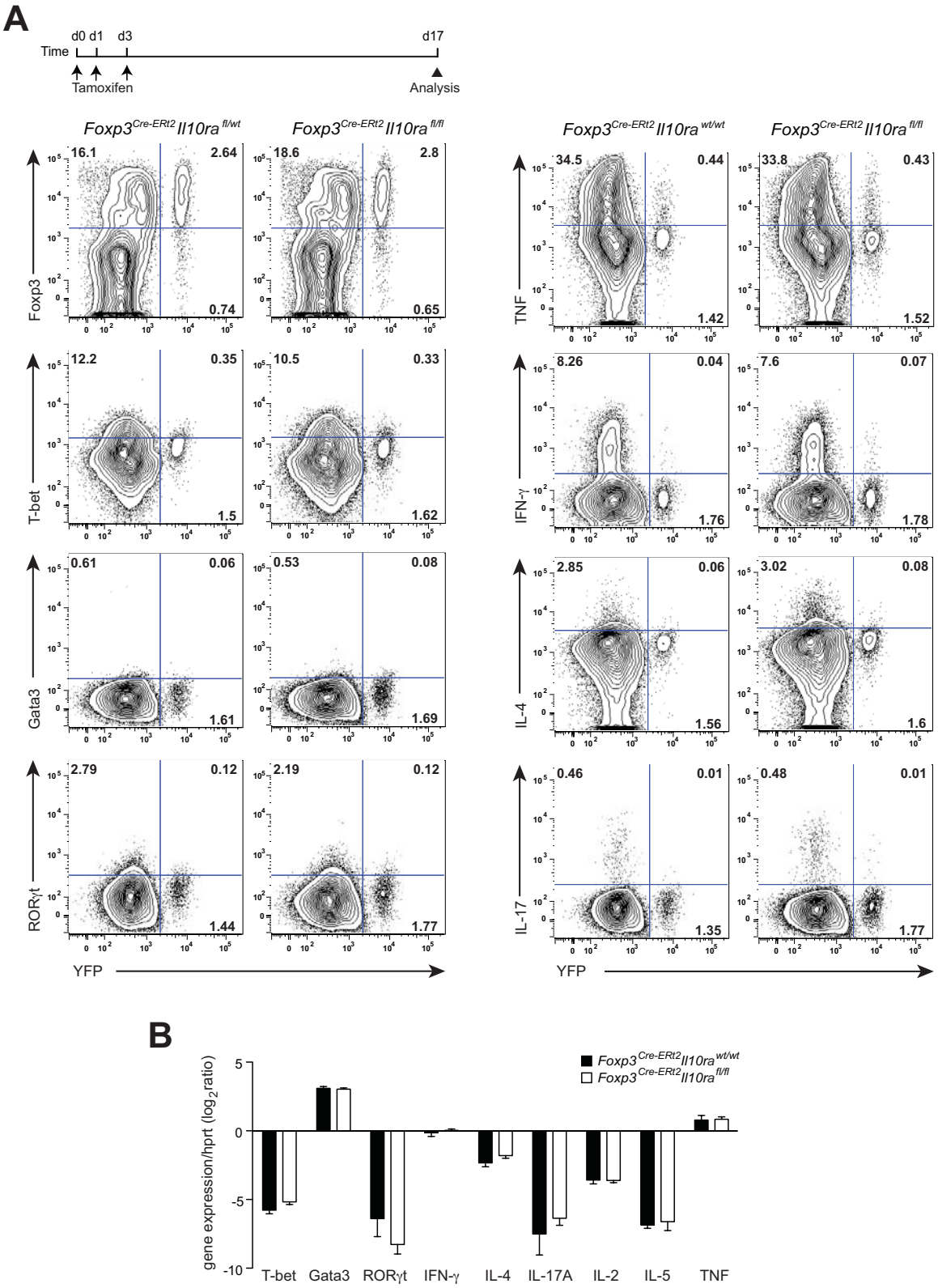

Figure S3. Treg-specific IL-10R deletion does not result in aberrant expression of Th1, Th2, and Th17 transcription factors and cytokines. (A) Flow cytometric analysis of cytokine production and transcription factor expression from splenic $CD4^+$ T cells

isolated from tamoxifen-fed *Foxp3*$^{Cre\text{-}Ert2}$*Il10ra*$^{wt/wt}$ and *Foxp3*$^{Cre\text{-}Ert2}$*Il10ra*$^{fl/fl}$ mice stimulated in presence of PMA and Ionomycin for 4-6h. (B) qPCR analysis of indicated mRNA expression in FACS-sorted CD4^{+}YFP^{+} T cells from *Foxp3*$^{Cre\text{-}Ert2}$*Il10ra*$^{wt/wt}$ and *Foxp3*$^{Cre\text{-}Ert2}$*Il10ra*$^{fl/fl}$ mice.

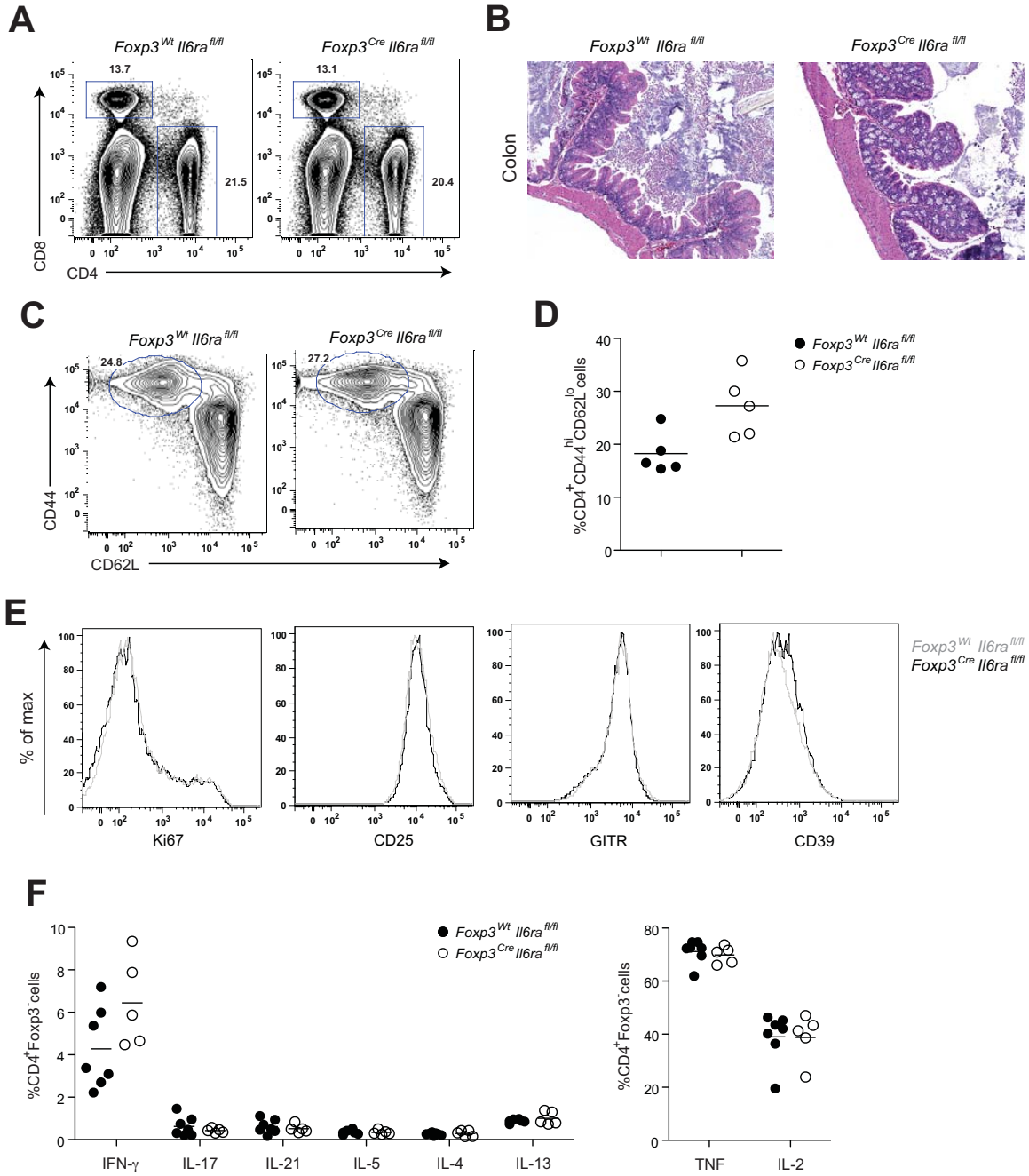

Figure S4. Treg-specific IL-6R deletion does not result in dysregulation of immune responses.

(A) Relative frequencies of CD4 and CD8 T cells in the spleen of *Foxp3*^{Wt}*Il6ra*$^{fl/fl}$ and *Foxp3*^{Cre}*Il6ra*$^{fl/fl}$ mice. (B) Representative H&E stained sections of colon tissues from *Foxp3*^{Cre}*Il6ra*$^{fl/fl}$ mice and littermate controls. (C,D) Similar frequenies of activated cells

in *Foxp3*^{Cre}*Il6ra*$^{fl/fl}$ mice (E) Flow cytometric analysis of Treg activation markers and putative effector molecules on splenic CD4^{+}Foxp3^{+} T cells in *Foxp3*^{Cre}*Il6ra*$^{fl/fl}$ and control littermate mice. (F) Flow cytometric analysis of cytokine production by splenic CD4^{+}Foxp3^{+} T cells in *Foxp3*^{Wt}*Il6ra*$^{fl/fl}$ and *Foxp3*^{Cre}*Il6ra*$^{fl/fl}$ mice.

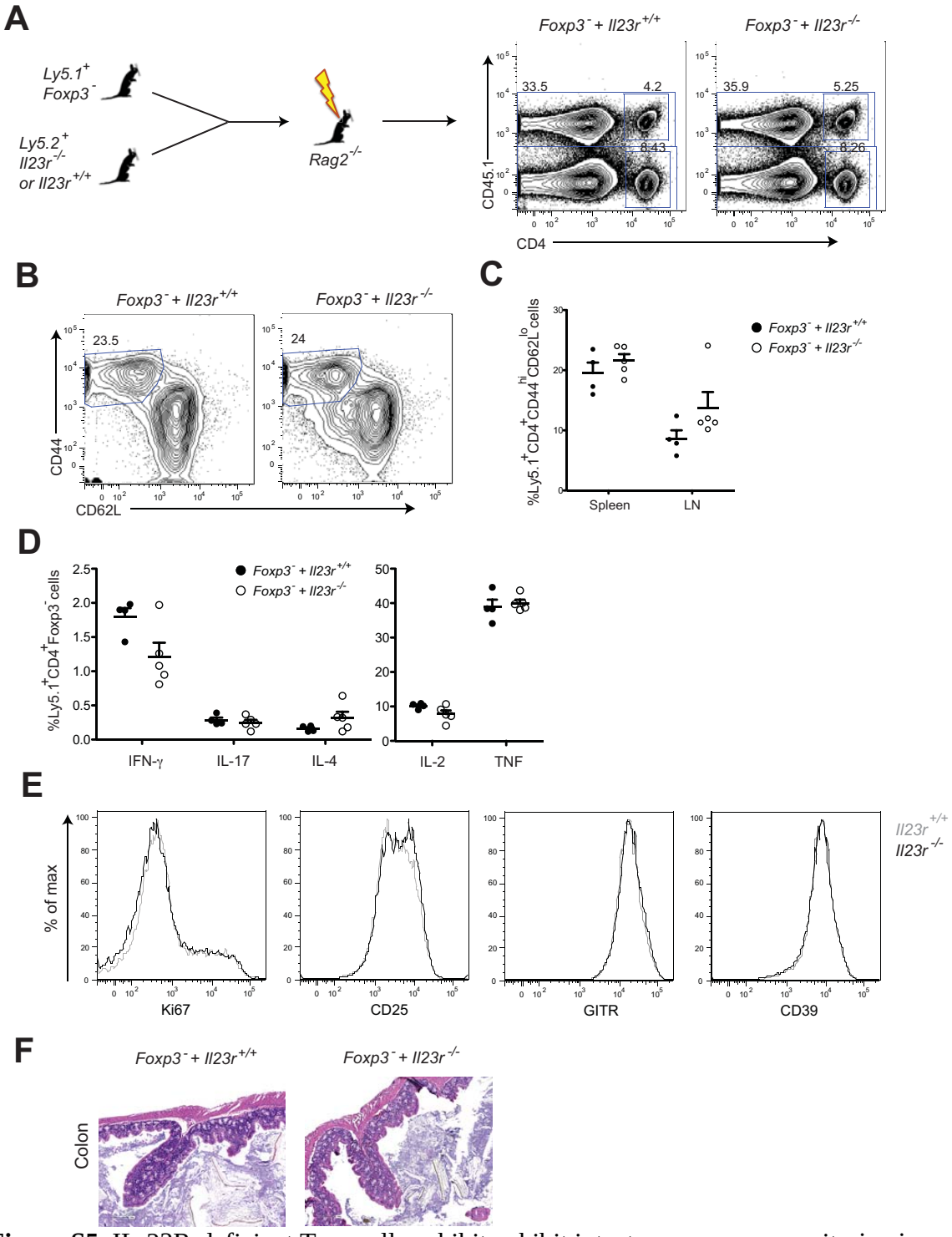

Figure S5. IL-23R-deficient Treg cells exhibit exhibit intact suppressor capacity *in vivo*. (A) Schematic representation of BM chimeras and relative reconstitution of CD4 T cells. (B,C) Similar frequenies of activated cells in chimeras harboring WT and IL-23R-deficient Treg cells. (D,E) Flow cytometric analysis of cytokine production by splenic $CD4^{+}Foxp3^{+}$ T cells in the presence of WT or IL-23R-deficient Treg cells. (F) Flow cytometric analysis of Treg activation markers and putative effector molecules on splenic

$CD4^+Foxp3^+$ T cells in BM chimeras. (G) Representative H&E stained sections of colon tissues from BM chimeras harboring WT or IL-23R deficient Treg cells.