SUPPLEMENTAL MATERIAL

Kamanaka et al., http://www.jem.org/cgi/content/full/jem.20102149/DC1

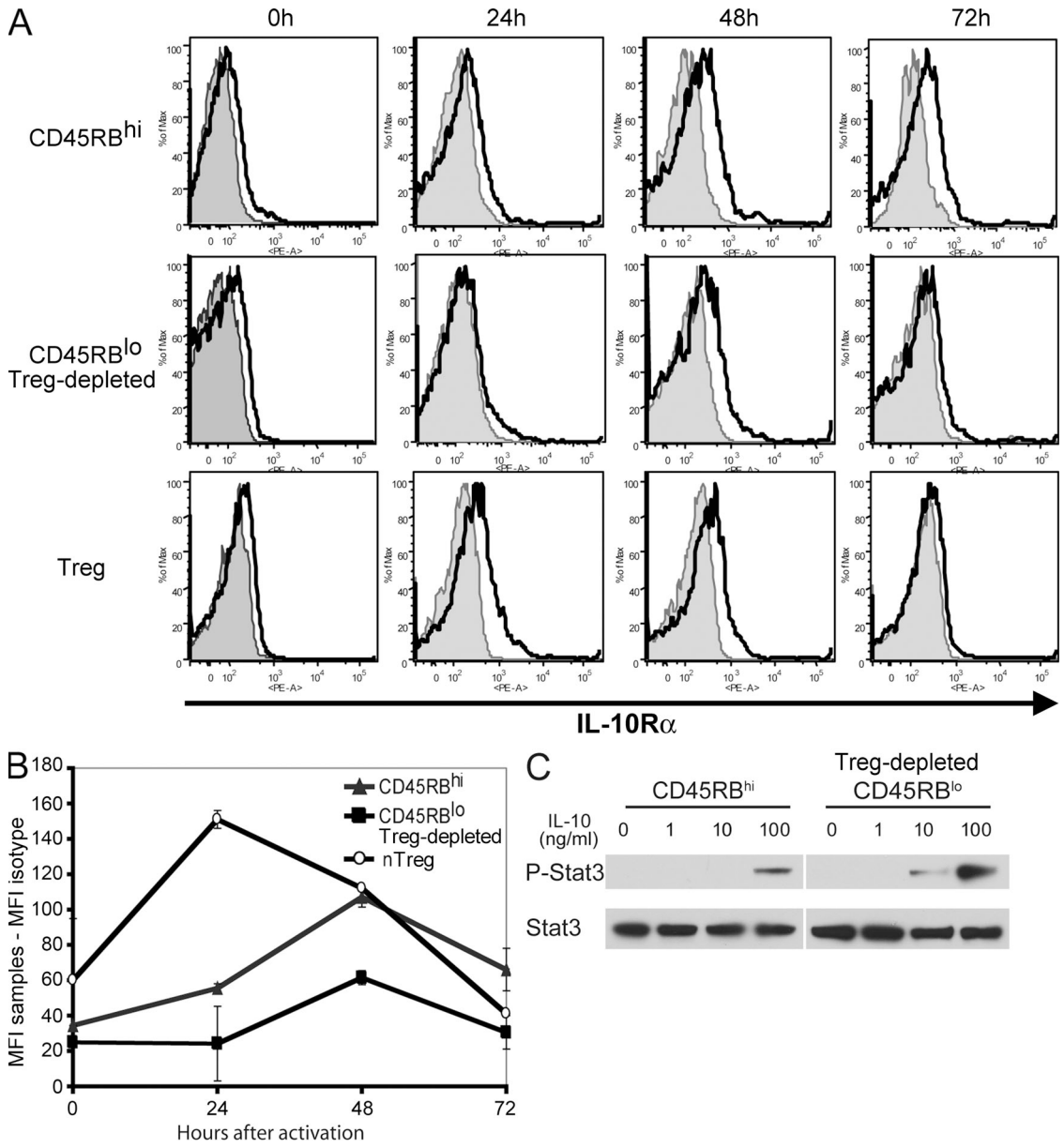

Figure S1. Expression of IL-10Rα in murine CD4 T cells. Splenocytes from Foxp3 reporter mice were sorted into CD45RB^{hi}, Foxp3^{-}CD45RB^{lo}, and Foxp3^{+} cells and activated with soluble anti-CD3 mAb and CD28 mAb in the presence of irradiated APC for the indicated time. (A) IL-10Rα expression (open histograms) was analyzed using flow cytometry. PE-conjugated rat IgG1 was used as an isotype control (closed histograms). Results shown are representative of three experiments. (B) Mean fluorescence intensity (MFI) of each staining minus isotype control shown in A. Error bars represent mean ± SEM. (C) Stat3 phosphorylation of CD45RB^{hi} and Foxp3^{-}CD45RB^{lo} CD4^{+} T cells incubated with different concentrations of IL-10. The experiments were repeated twice with similar results.

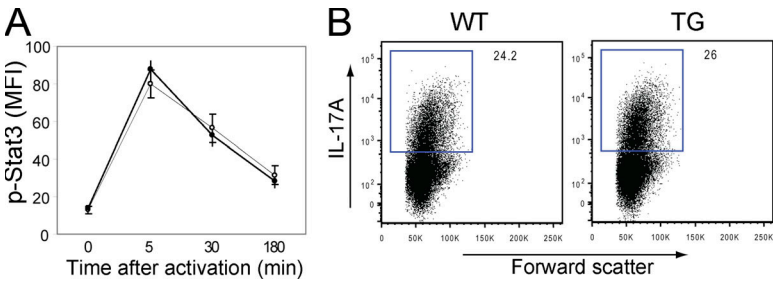

Figure S2. IL-6–mediated Stat3 activation is not abrogated in TG CD4 T cells. (A) Naive CD4 T cells from WT (closed circles) or TG (open circles) mice were incubated with IL-6, and phospho-Stat3 (p-Stat3) was detected after the indicated time using flow cytometry (mean fluorescence intensity [MFI] ± SD). (B) Naive T cells were cultured in the presence of 10 ng/ml IL-6, 20 ng/ml IL-23, and 1 ng/ml TGF-β for 4 d. IL-17A expression was analyzed using flow cytometry. Results are representative of three independent experiments.

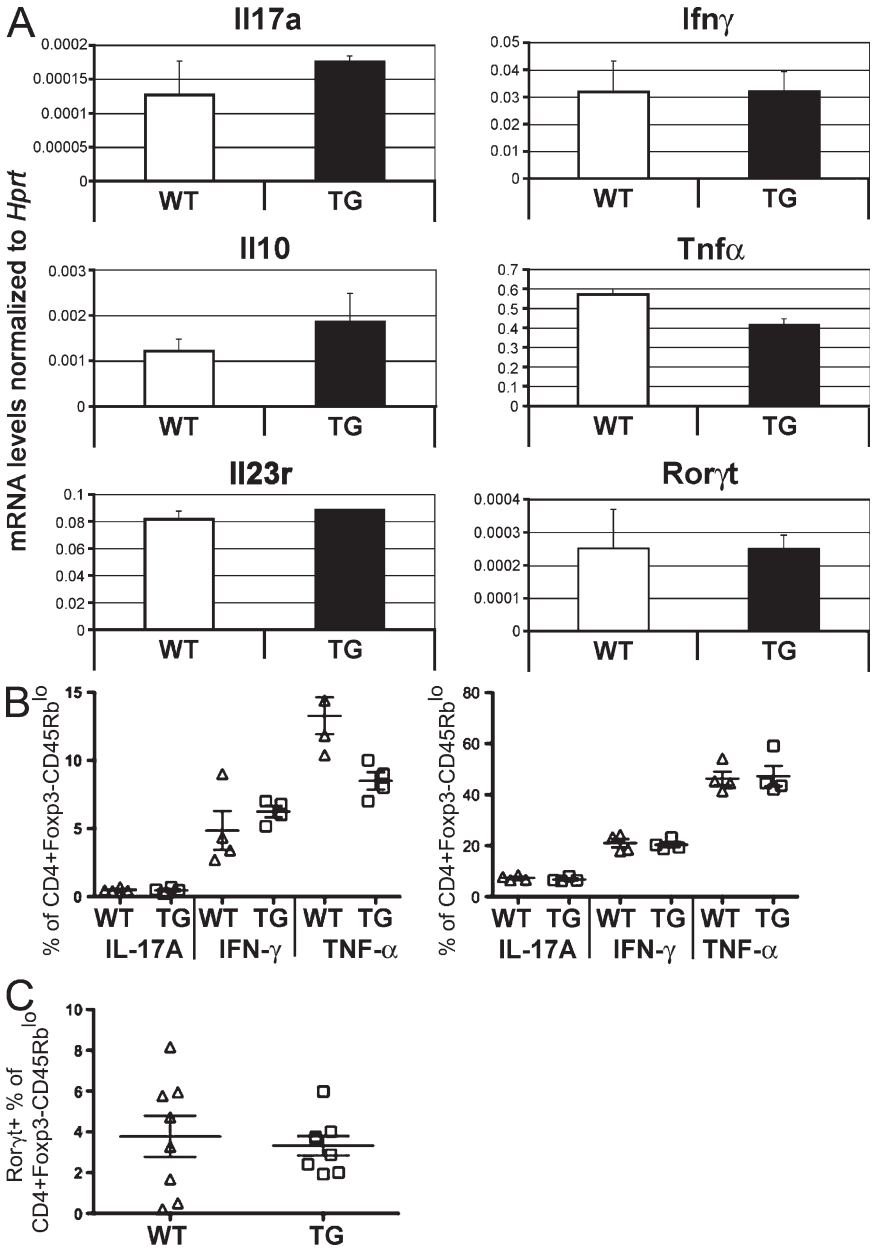

Figure S3. Characterization of TG Foxp3$^-$CD45RB^{lo} cells before the transfer. (A) No significant difference in IL-17A, IFN-γ, IL-10, TNF, IL-23R, and Ror-γt mRNA expression between WT and TG CD4$^+$Foxp3$^-$CD45RB^{lo} cells freshly isolated from the spleen. Values were normalized to HPRT. (B) Total splenocytes were activated for 3 h using PMA/ionomycin (left) or stimulated for 4 d with plate-bound anit-CD3 mAB and soluble anit-CD28 mAb for 4 d and then restimulated for 3 h with PMA/ionomycin (right). IL-17A, IFN-γ, and TNF expression were measured using intracellular cytokine staining and flow cytometry. (C) Frequency of ROR-γt–expressing cells in CD4$^+$Foxp3$^-$CD45Rb^{lo} cells was measured using flow cytometry. Each dot represents one mouse. The experiments were repeated twice with similar results. Error bars represent mean ± SEM.

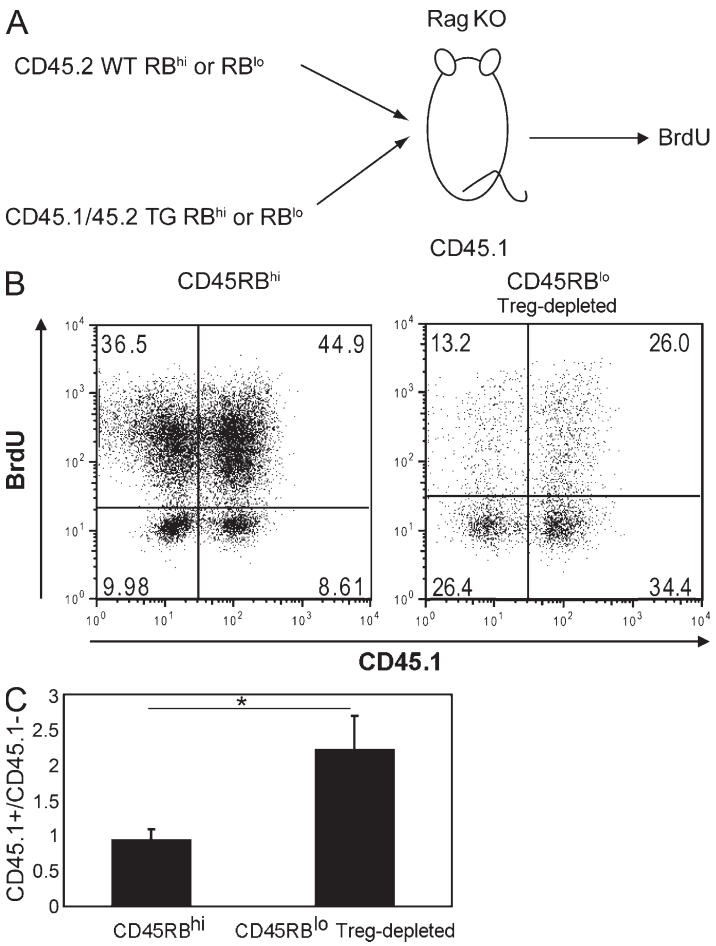

Figure S4. Competitive adoptive transfer of WT and TG CD45RB^{hi} or CD25^{-}CD45RB^{lo} cells into Rag1 KO mice. (A) Equal number (3×10^5) of WT (CD45.2 homozygote) and TG (CD45.2$^+$/CD45.1$^+$) CD45RB^{hi} or CD25$^-$CD45RB^{lo} cells were adoptively transferred into CD45.1 homozygote Rag1 KO mice. 1 mo later, BrdU was fed in the drinking water. BrdU uptake was measured in CD45.2$^+$ WT and CD45.1$^+$/CD45.2$^+$ TG cells isolated from the spleen and mesenteric lymphocytes of the Rag1 KO recipients using flow cytometry. (B) Representative dot blots are shown (left, cotransfer of WT [CD45.2$^+$/CD45.1$^-$] and TG [CD45.2$^+$/CD45.1$^+$] CD45RB^{hi} cells; right, cotransfer of CD45RB^{lo} cells). Cells were gated on CD45.2-positive donor cells. (C) Ratio of TG (CD45.2$^+$/CD45.1$^+$) and WT (CD45.2$^+$/CD45.1$^-$) cells in the Rag1 KO recipients are shown. The experiment was performed twice with similar results. Error bars represent mean ± SEM. *, $P < 0.05$.

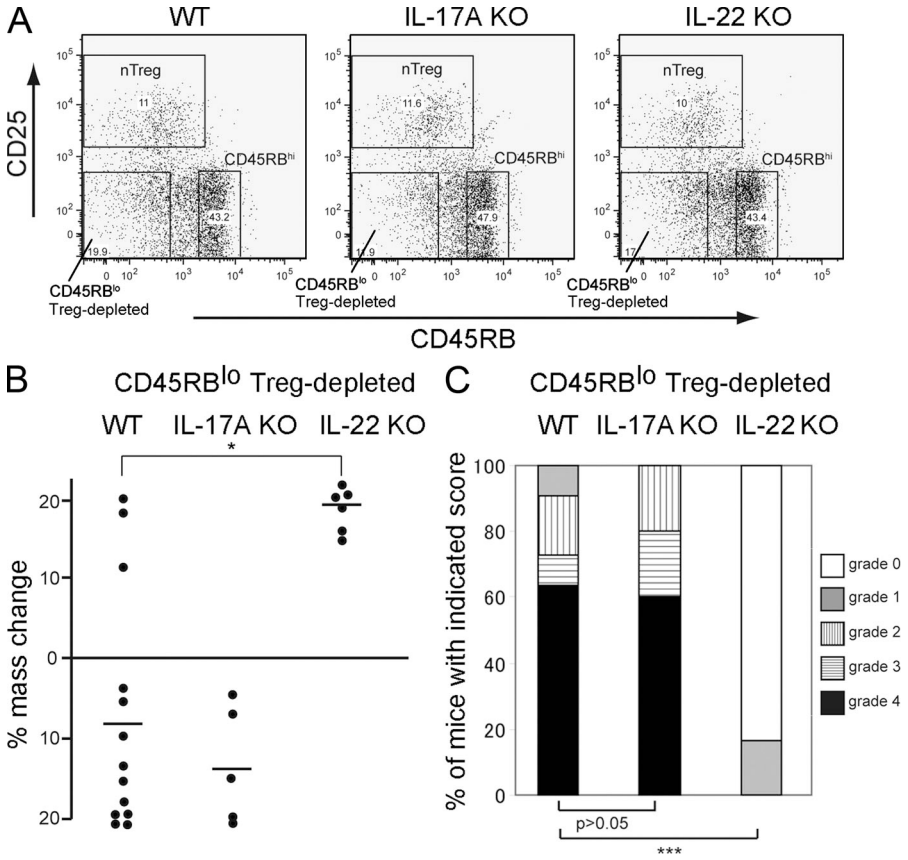

Figure S5. Induction of colitis by CD25$^-$CD45RB^{lo} cells is dependent on T cell–derived IL-22 but not IL-17A. (A) Representative dot blot showing the sorting gates for CD25$^-$CD45RB^{lo} cells obtained from WT, IL-17A KO, and IL-22 KO mice. Cells were gated on CD4$^+$ T cells. The gates show the nT$_{reg}$ cell, T$_{reg}$ cell–depleted CD45RB^{lo}, and CD45RB^{hi} populations. (B and C) CD4$^+$CD25$^-$CD45RB^{lo} cells from WT, IL-17A KO, and IL-22 KO mice were adoptively transferred into the Rag1 KO mice. Weight changes (B) and colitis score (C) 12 wk after the transfer are shown. Each dot represents one mouse; horizontal bars indicate the mean. Results are representative of two experiments. *, $P < 0.05$; ***, $P < 0.001$.

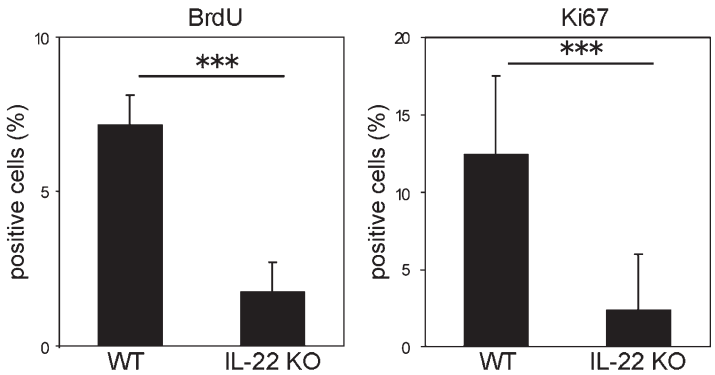

Figure S6. Increased epithelial cell proliferation in Rag1 KO mice receiving IL-22 KO CD25$^-$CD45RB^{lo} cells using flow cytometry. BrdU uptake and Ki67 expression were analyzed by intracellular staining of epithelial cells harvested from the colons of Rag1 KO mice after the adoptive transfer with IL-22 KO or WT CD25$^-$CD45RB^{lo} cells. BrdU was injected 4 h before euthanization. Results are representative of two independent experiments. Error bars represent mean ± SEM. ***, $P < 0.001$.

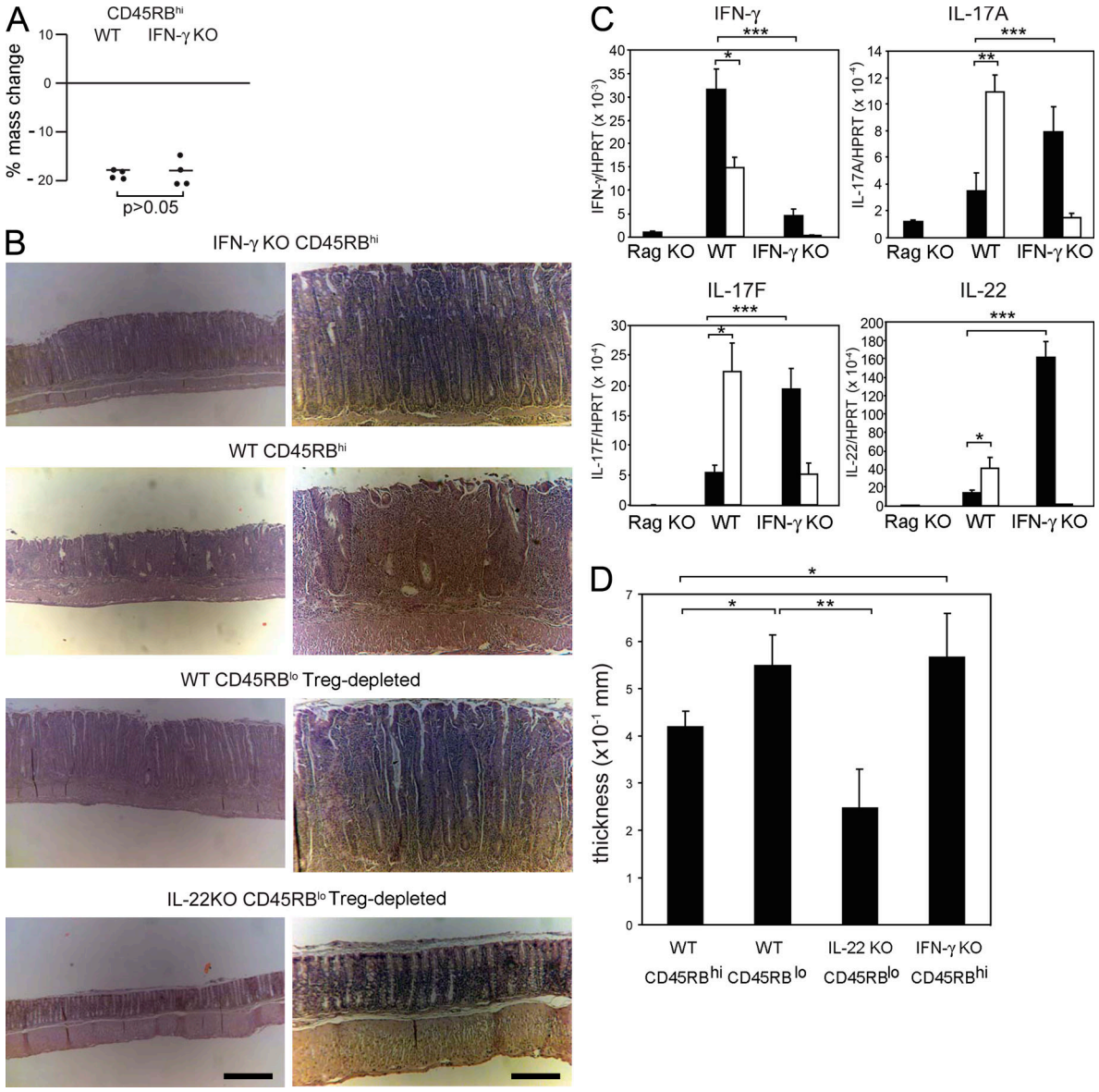

Figure S7. Characterization of colitis induced by the transfer of IFN-γ KO CD45RB^{hi} T cells into Rag1 KO mice. Rag1 KO mice were adoptively transferred with CD45RB^{hi} cells from WT or IFN-γ KO mice. (A) Mass loss 8 wk after the transfer. Each dot represents one mouse; horizontal bars indicate the mean. (B) Histological findings of the colons from mice adoptively transferred with CD45RB^{hi} WT or IFN-γ KO cells compared with T_{reg} cell–depleted CD45RB^{lo} WT or IL-22 KO cells. The left panels are of low power and the right panels of high power magnification. Bars: (left) 1,000 µm; (right) 400 µm. (C) Cytokine mRNA profiles of the colons adoptively transferred with WT and IFN-γ KO CD45RB^{hi} (closed bars) or T_{reg} cell–depleted CD45RB^{lo} (open bars) using RT-PCR. Mean of four to eight mice per group are shown. (D) Thickness of the colons of the Rag1 KO recipients of indicated cell types. Four to six colons per group were analyzed for the thickness of the colons using light microscopy (mean ± SD). Results are representative of three independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

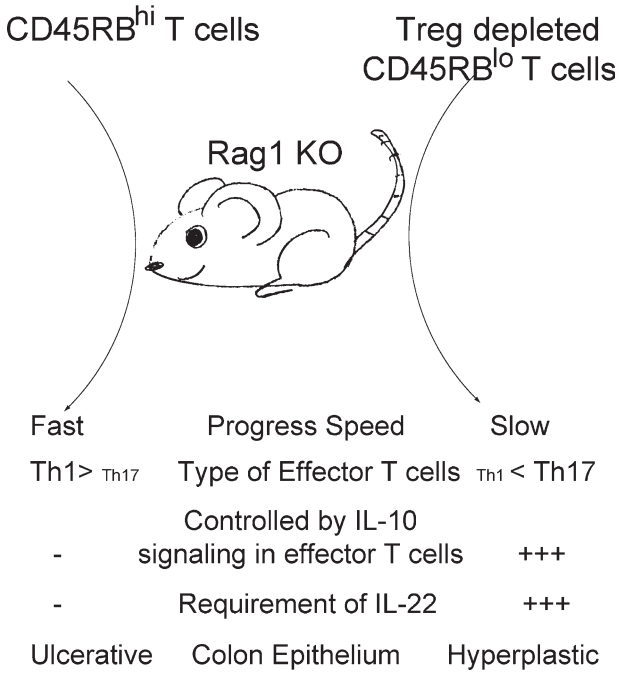

Figure S8. Summary of the colitis developed by CD45RB^{hi} and T_{reg} cell–depleted CD45RB^{lo} T cells. The characteristics and differences between the colitis developed by CD45RB^{hi} and T_{reg} cell–depleted CD45RB^{lo} T cells are summarized.