Supplemental material
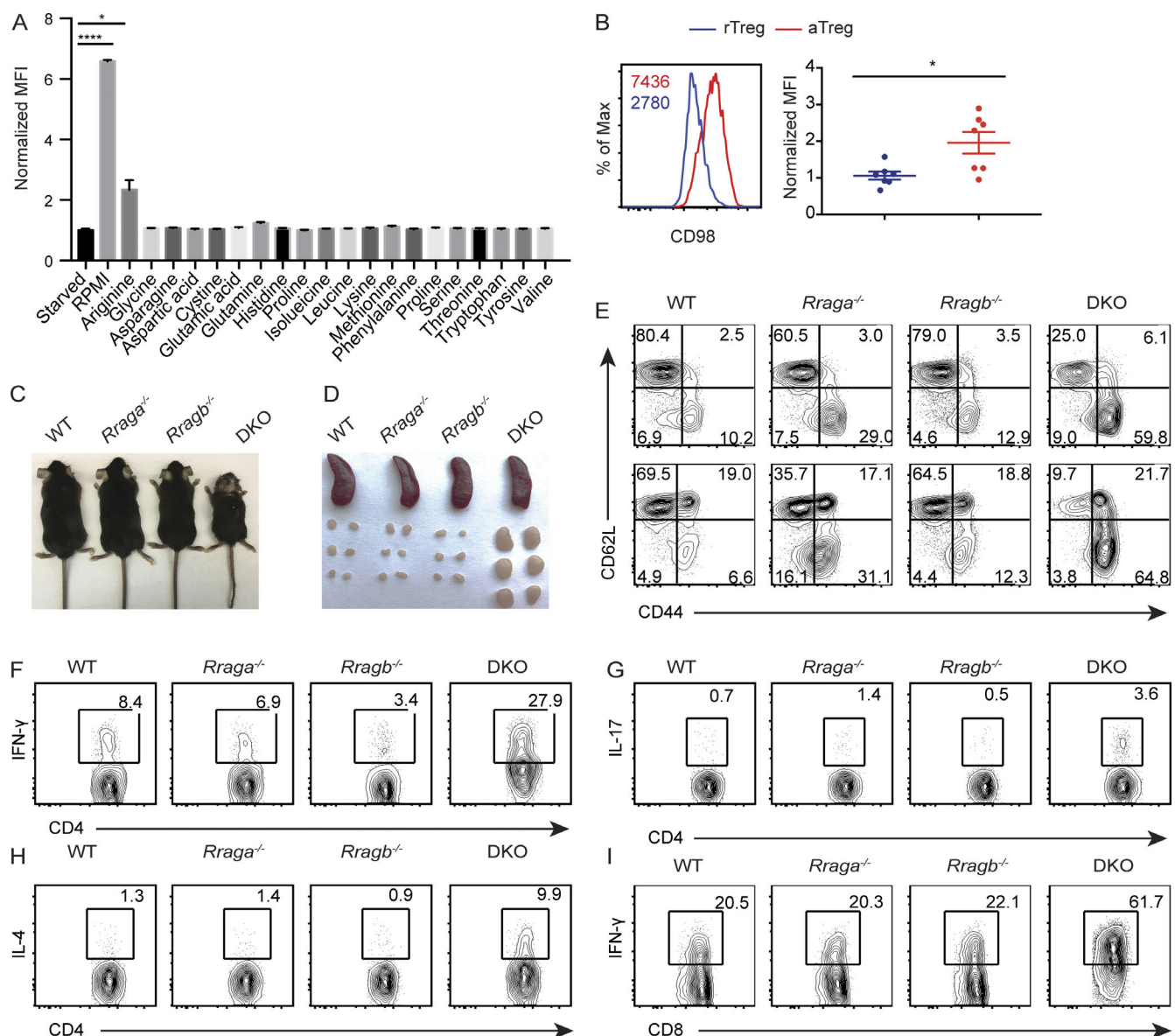
Figure S1. **Mice lacking RagA and RagB in T reg cells develop lethal autoimmune disease. (A)** Quantification of p-S6 expression in aT reg cells that were deprived of amino acids for 1 h and refed with all 20 amino acids or individual amino acid as indicated for 1 h (n = 3). Data are pooled from three independent experiments. **(B)** Flow cytometric analysis and quantification of CD98 expression in splenic aT reg and rT reg cells. Numbers in graphs indicate MFI (n = 7 mice each group). Data are pooled from three independent experiments. **(C)** Representative images of 5-wk-old WT, $Rraga^{-/-}$, $Rragb^{-/-}$, and DKO mice. Data are representative of more than five independent experiments. **(D)** Representative images of spleens and peripheral (axillary, brachial, and inguinal) lymph nodes from 5-wk-old WT, $Rraga^{-/-}$, $Rragb^{-/-}$, and DKO mice. Data are representative of more than five independent experiments. **(E)** CD44 and CD62L expression in splenic CD4+Foxp3− (top) and CD8+ (bottom) T cells from 5-wk-old WT, $Rraga^{-/-}$, $Rragb^{-/-}$, and DKO mice. Data are representative of more than five independent experiments. **(F–I)** IFN-γ, IL-4, and IL-17 expression in splenic CD4+Foxp3− T cells and IFN-γ expression in splenic CD8+ T cells from 5-wk-old WT, $Rraga^{-/-}$, $Rragb^{-/-}$, and DKO mice. Data are representative of more than five independent experiments. Numbers in gates or quadrants indicate percentage of cells. Data in plots indicate means ± SEM. *, P < 0.05; ****, P < 0.0001 (unpaired t test; A and B). Max, maximum.
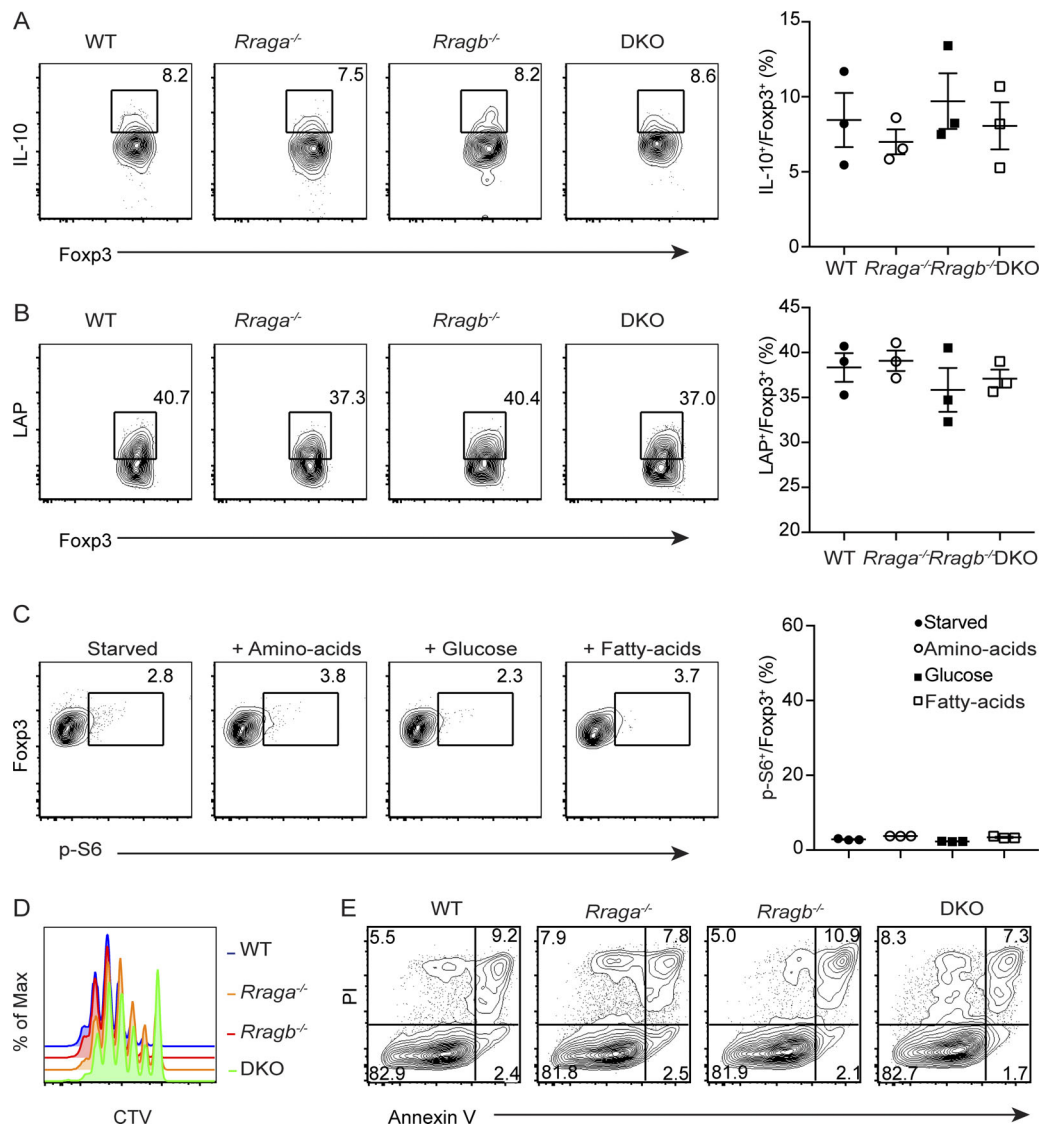
Figure S2. **RagA and RagB promote T reg cell mTORC1 signaling and proliferation. (A and B)** Flow cytometric analysis and quantification of IL-10 and TGF-β1 latency-associated peptide (LAP) expression in splenic T reg cells from WT, *Rraga*^{−/−}, *Rragb*^{−/−}, and DKO mice (*n* = 3 mice per group). Data are pooled from two independent experiments. **(C)** Flow cytometric analysis and quantification of p-S6 expression in DKO aT reg cells that were deprived of nutrient for 1 h and refed with amino acids, glucose, or fatty acids for 1 h (*n* = 3). Data are pooled from three independent experiments. **(D and E)** rT reg cells from WT, *Rraga*^{−/−}, *Rragb*^{−/−}, and DKO mice were labeled with CTV and stimulated with α-CD3/28 and IL-2 for 72 h. **(D)** T reg cell proliferation was assessed by flow cytometric analysis of CTV dilution. Data are representative of three independent experiments. **(E)** T reg cell death was measured by propidium iodide (PI) and Annexin V staining. Data are representative of three independent experiments. Numbers in gates or quadrants indicate percentage of cells. Data in plots indicate mean ± SEM (one-way ANOVA with the Tukey post hoc test; A–C).
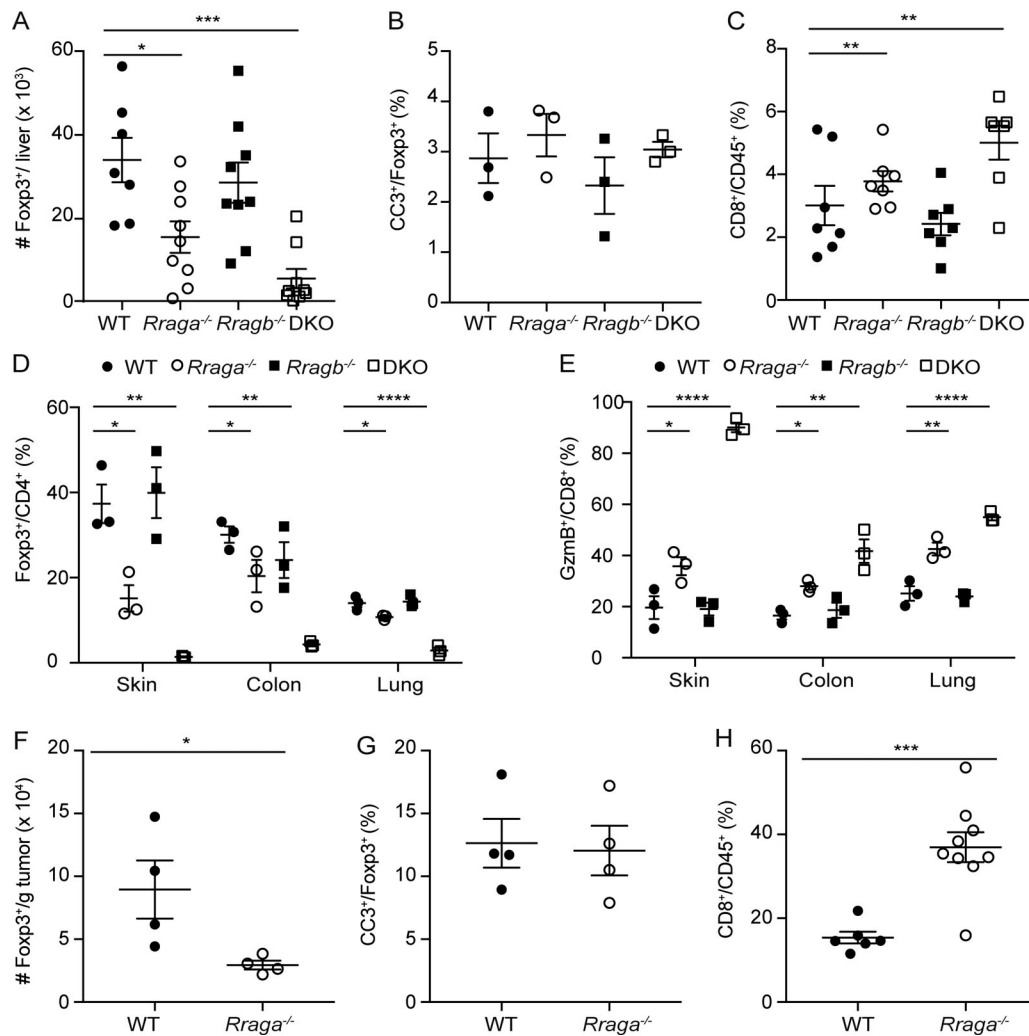
Figure S3. **Rag GTPase depletion results in CD8+ T cell expansion in nonlymphoid tissues and tumor. (A)** Quantification of absolute numbers of liver T reg cells from 9–12-d-old WT, *Rraga^−/−^*, *Rragb^−/−^*, and DKO mice (n = 7–9 mice per group). Data are pooled from more than five independent experiments. **(B)** Quantification of cleaved caspase 3 (CC3) expression in T reg cells from 9–12-d-old WT, *Rraga^−/−^*, *Rragb^−/−^*, and DKO mice (n = 3 mice per group). Data are pooled from two independent experiments. **(C)** Percentage of CD8+ T cells among total CD45+ immune cells in the liver of 9–12-d-old WT, *Rraga^−/−^*, *Rragb^−/−^*, and DKO mice (n = 5–7 mice per group). Data are pooled from more than five independent experiments. **(D)** Flow cytometric analysis and quantification of Foxp3 expression in skin, colon, and lung CD4+ T cells from 9–12-d-old WT, *Rraga^−/−^*, *Rragb^−/−^*, and DKO mice (n = 3 mice per group). Data are pooled from two independent experiments. **(E)** Flow cytometric analysis and quantification of GzmB expression in skin, colon, and lung CD8+ T cells from 9–12-d-old WT, *Rraga^−/−^*, *Rragb^−/−^*, and DKO mice (n = 3 mice per group). Data are pooled from two independent experiments. **(F)** Quantification of absolute numbers of tumor T reg cells from WT and *Rraga^−/−^* mice (n = 4 mice per group). Data are pooled from two independent experiments. **(G)** Quantification of CC3 expression in T reg cells from tumor T reg cells from WT and *Rraga^−/−^* mice (n = 4 mice per group). Data are pooled from two independent experiments. **(H)** Percentage of CD8+ T cells among total tumor-infiltrating CD45+ immune cells in B16-tumor-bearing WT and *Rraga^−/−^* mice (n = 6–9 mice per group). Data are pooled from more than five independent experiments. Data in plots indicate mean ± SEM. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001 (one-way ANOVA with the Tukey post hoc test [A–E] and unpaired t test [B]).