Figure S1

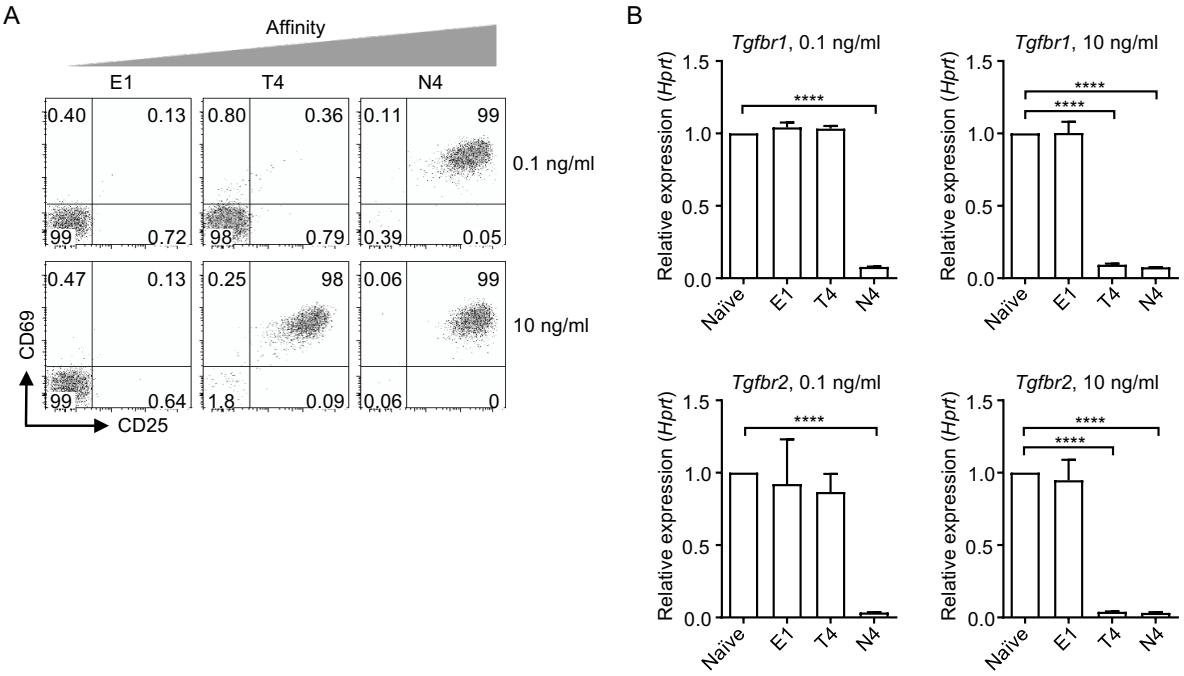

Figure S1. Antigen affinity regulates TβR expression in T cells. Related to Figure 1
Naïve OT-I T cells were cultured for 24 hr with APCs and indicated peptides at either 0.1 or 10 ng/ml. (A) Representative flow cytometric plots of CD69 and CD25 expression in stimulated OT-I T cells. (B) *Tgfbr1* and *Tgfbr2* mRNA in naïve or stimulated OT-I T cells. Data are pooled from two independent experiments. Student's *t*-test was used; bars (mean), error bars (SD), ****$P <$ 0.0001.

Figure S2

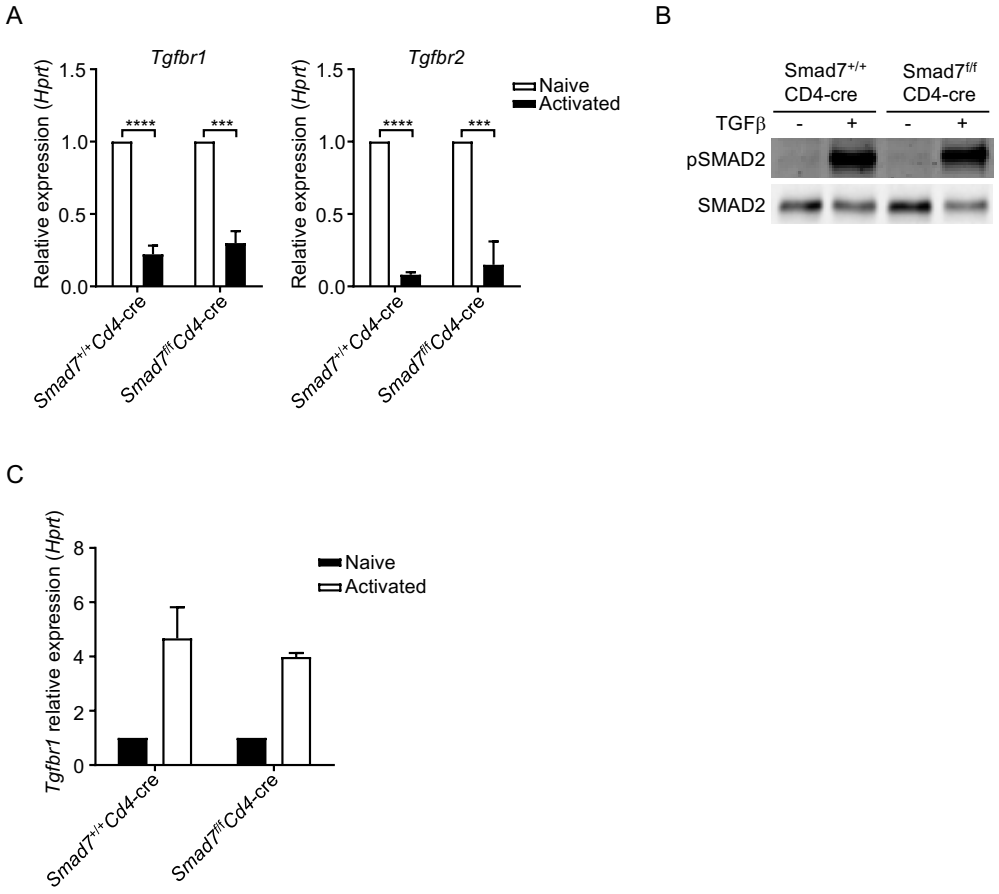

Figure S2. Downregulation of TGFβ signaling and TβR by TCR is independent of SMAD7. Related to Figure 3

(A) *Tgfbr1* and *Tgfbr2* mRNA in *Smad7*$^{+/+}$*Cd4*-cre or *Smad7*$^{f/f}$*Cd4*-cre naïve CD4$^+$ T cells or T cells stimulated for 24 hr with anti-CD3 and anti-CD28 (Activated). (B) Western blot of pSMAD2, and SMAD2 in *Smad7*$^{+/+}$*Cd4*-cre or *Smad7*$^{f/f}$*Cd4*-cre CD4$^+$ T cells stimulated for 24 hr with anti-CD3 and anti-CD28 in the presence or absence of TGFβ. (C) *Tgfbr1* mRNA in *Smad7*$^{+/+}$*Cd4*-cre or *Smad7*$^{f/f}$*Cd4*-cre CD4$^+$ T cells stimulated for 24 hours with anti-CD3 and anti-CD28 in the presence or absence of TGFβ. Data are pooled from three (A, C) independent experiments or are representative of three (B) independent experiments. Student's *t*-test was used; bars (mean), error bars (SD), ***$P < 0.001$, and ****$P < 0.0001$.

Figure S3

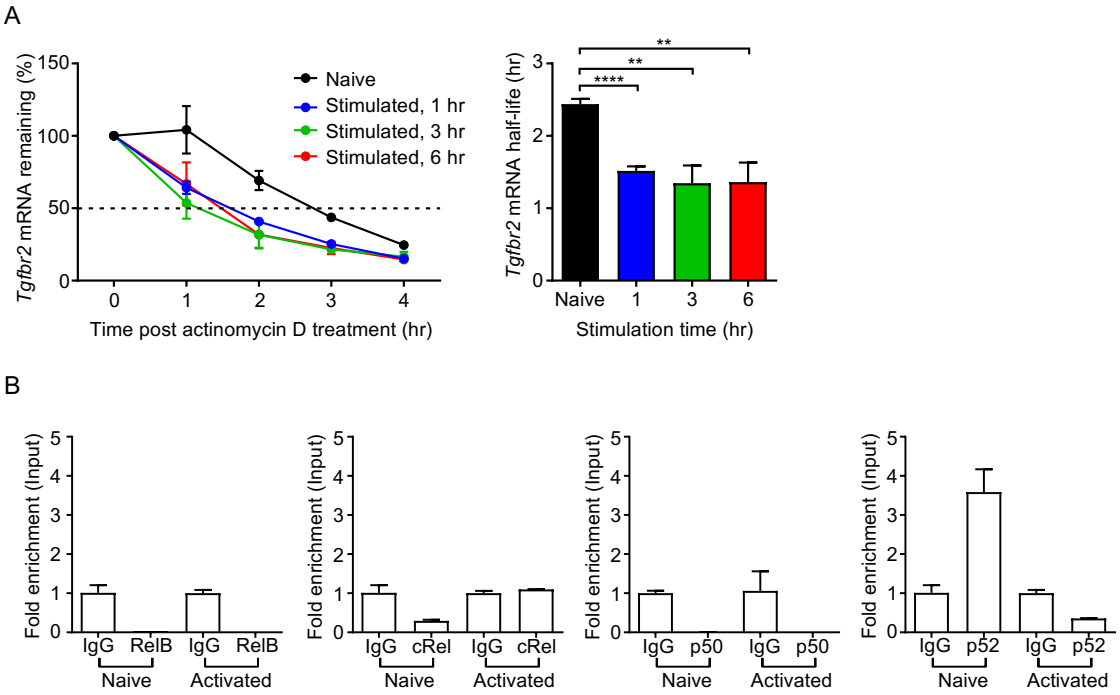

Figure S3. Increased TβRII mRNA decay and lack of p50, p52, RelB and cRel binding to TβRI promoter in activated T cells. Related to Figure 3

(A) Naïve $CD4^+$ T cells were stimulated for 1, 3 or 6 hr with anti-CD3 and anti-CD28. Actinomycin D was then added to stop transcription. *Tgfbr2* mRNA in naïve or stimulated T cells detected 1, 2, 3, and 4 hours after treatment with actinomycin D. The expression of TβRII was normalized to 18S mRNA levels. The mRNA half-life was calculated from the nonlinear regression of the exponential decay curve N0=N(t)e-λt, where the TβRII mRNA half-life t1/2=-ln(2)/λ. (B) ChIP-coupled real-time PCR analysis of p50, p52, RelB and cRel enrichments in the promoter region of *Tgfbr1* gene of naïve T cells or T cells stimulated for 24 hr with anti-CD3 and anti-CD28 (Activated). The enrichment was assessed using an antibody to p50, p52, RelB or cRel, and presented relative to input and compared with a control IgG. Data are pooled from four independent experiments (A) or are representative of two independent experiments. Student's *t*-test was used; bars (mean), error bars (SEM in A; SD in B), $^{**}P < 0.01$, and $^{****}P < 0.0001$.

Figure S4

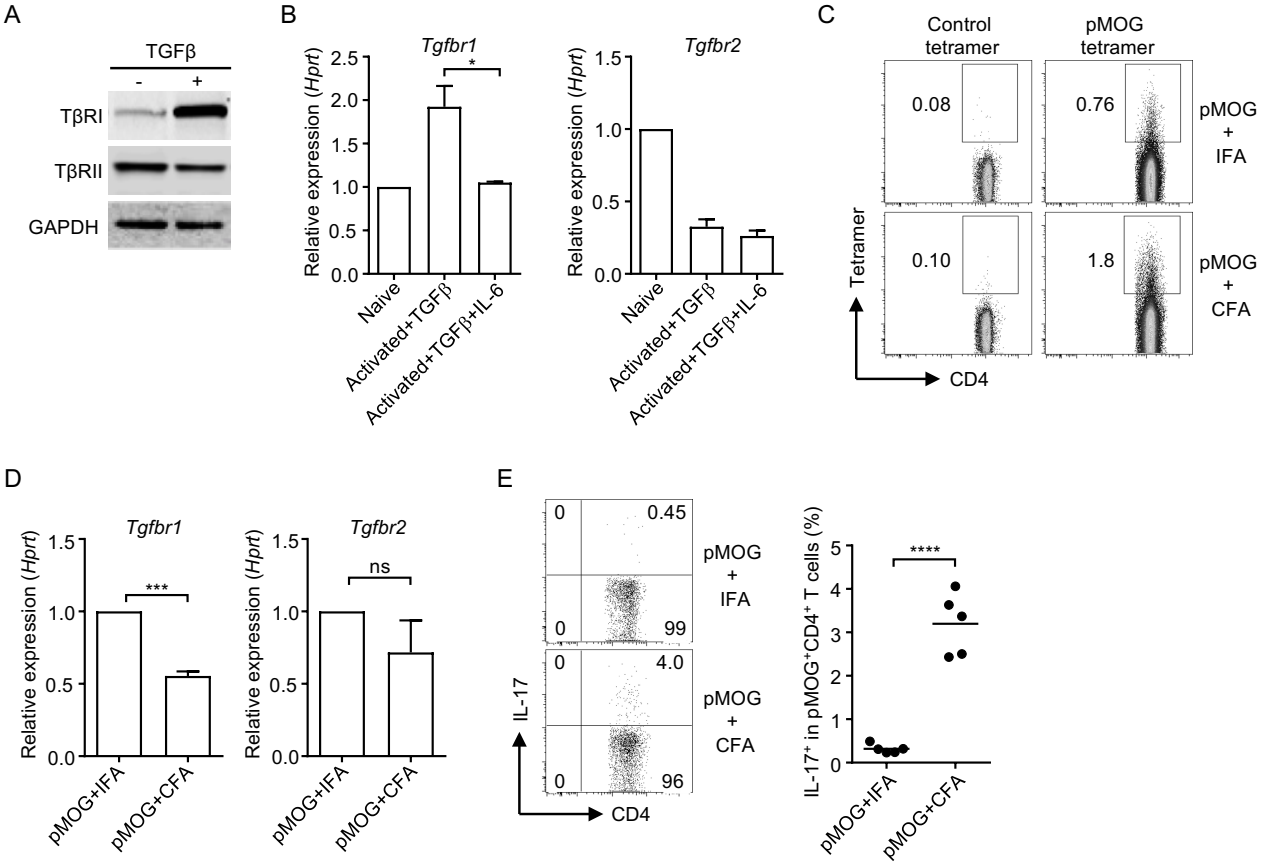

Figure S4. TGFβ upregulates TβRI and IL-6 abrogates the effect. Related to Figure 4

(A) Western blot of TβRI, TβRII and GAPDH in naïve T cells cultured for 24 hr in the presence or absence of TGFβ. (B) *Tgfbr1* and *Tgfbr2* mRNA in naïve T cells or T cells stimulated for 24 hr with anti-CD3 and anti-CD28 in the presence of TGFβ (Activated + TGFβ) or TGFβ plus IL-6 (Activated + TGFβ + IL-6). (C-E) Mice were immunized with MOG peptide (pMOG) plus either CFA or IFA at tail base. Seven days after pMOG immunization, MOG-specific T cells in the inguinal lymph nodes were identified using pMOG tetramer. (C) Representative flow cytometric plots. The values indicate the percentages of tetramer-specific $CD4^+$ T cells in the inguinal lymph nodes of the immunized mice. (D) *Tgfbr1* and *Tgfbr2* mRNA, (E) IL-17^+ in MOG-specific $CD4^+$ T cells. Data are pooled from three (D), four (B) or five (E) independent experiments or are representative of two (A) or five (C) independent experiments. In (E), each circle represents the data pooled from three mice from each experiment. Student's *t*-test was used; bars (mean), error bars (SEM), $^*P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$ and ns, not significant.

Figure S5

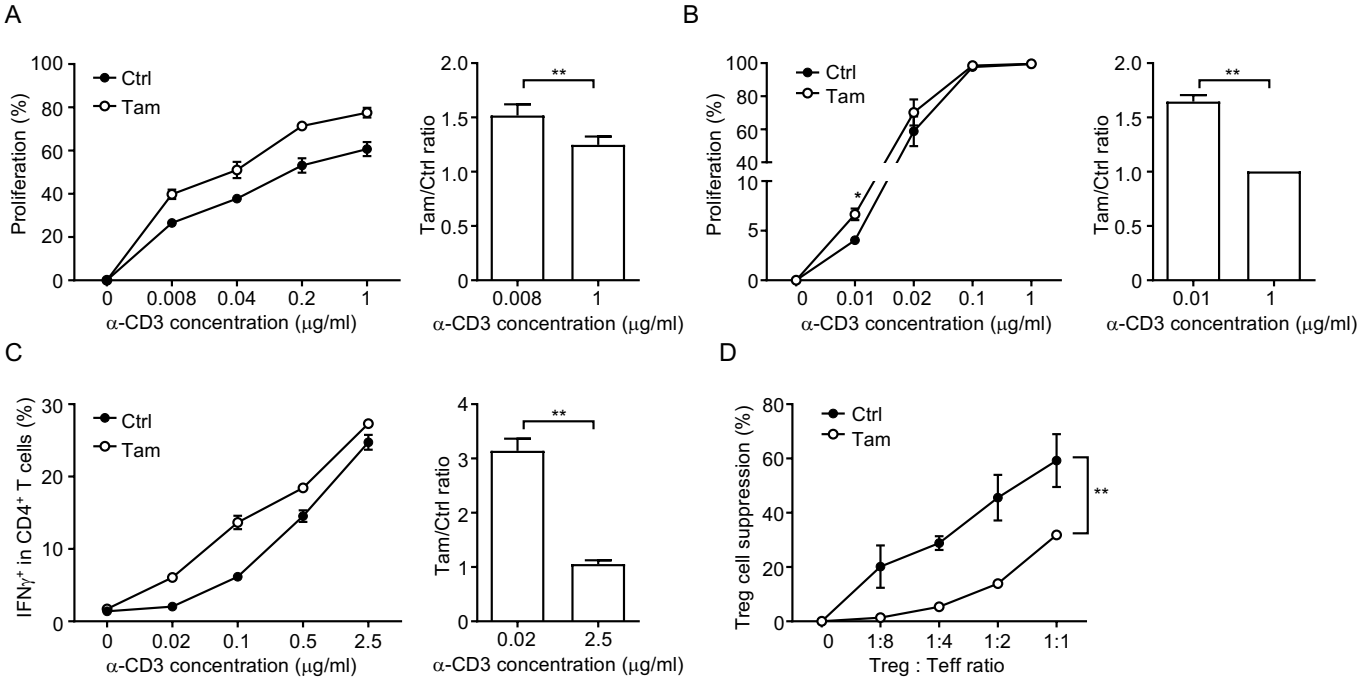

Figure S5. Lack of TβRI promotes T cell proliferation and resistance to Treg cell suppression. Related to Figure 5

Naïve CD4$^+$ T cells were isolated from untreated *Tgfbr1*$^{f/f}$*Esr1*-cre mice (Ctrl) or tamoxifen-treated *Tgfbr1*$^{f/f}$*Esr1*-cre mice (Tam). (A, B) CFSE-labeled naïve CD4$^+$ T cells were stimulated for 3 days with (A) indicated concentration of anti-CD3 and APCs or (B) indicated concentration of plate-bound anti-CD3 and anti-CD28 (1 μg/ml). Proliferation of T cells was determined by CFSE dilution. (C) Frequencies of IFNγ$^+$ in CD4$^+$ T cells stimulated for 3 days with indicated concentration of anti-CD3 and APCs. (D) CFSE-labeled naïve CD4$^+$ T cells were stimulated for 3 days with anti-CD3 and APCs in the presence of different ratios of Treg:Teff as indicated. Treg cell suppression was determined by the percentage of T cells that were inhibited by CD4$^+$CD25$^+$ Treg cells compared with T cells that were cultured without Treg cells. Data are pooled from two independent experiments (A, B, D) or are representative of two independent experiments (C). Two-way ANOVA and Student's *t*-test were used; error bars (SD), $^{**}P < 0.01$.

Figure S6

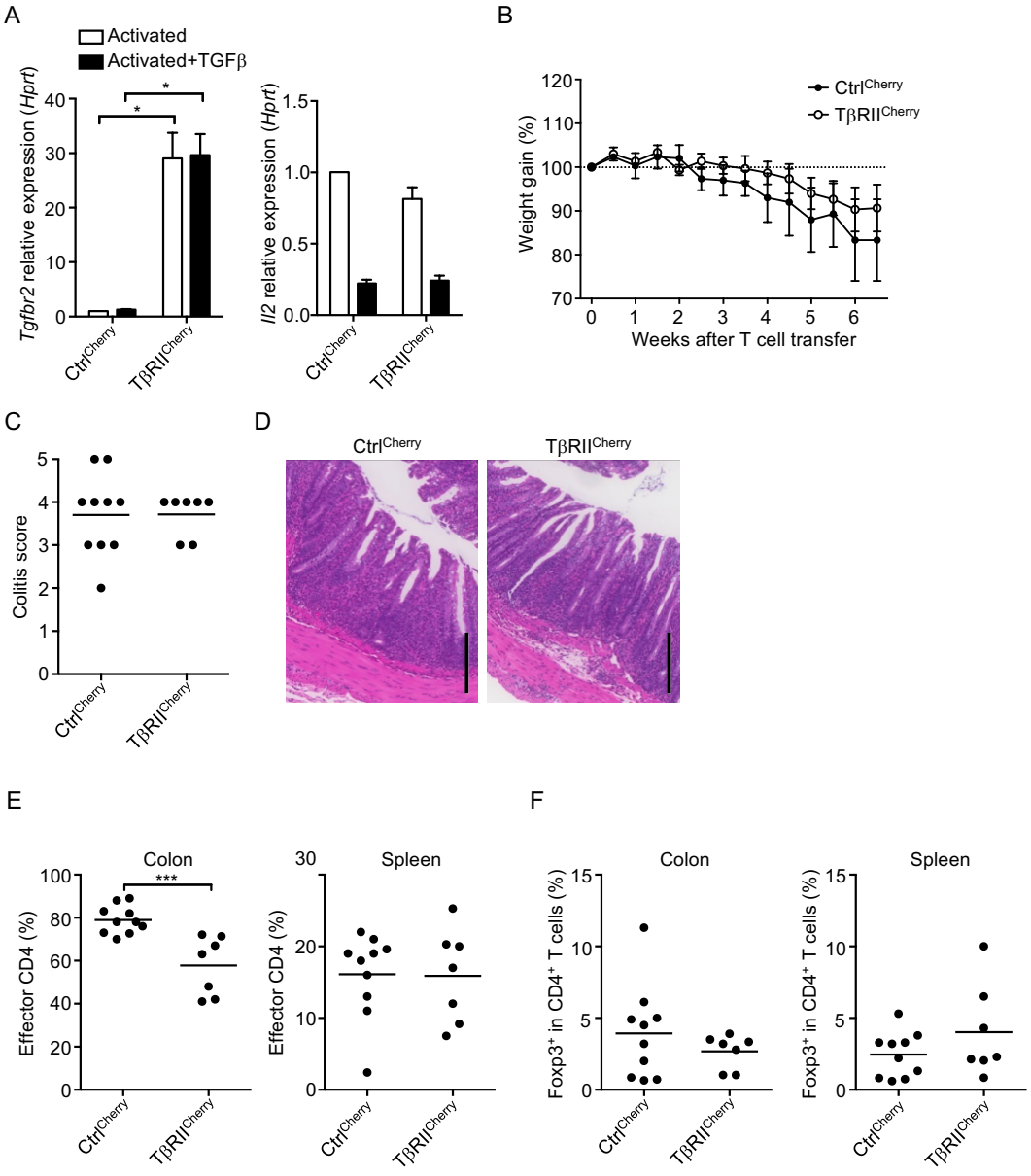

Figure S6. Overexpression of TβRII in naïve T cells does not suppress autoimmunity. Related to Figure 5

Naïve CD4$^+$ T cells were isolated from TβRII^{Cherry} and Ctrl^{Cherry} retrogenic mice. (A) *Tgfbr2* and *Il2* mRNA in T cells stimulated for 24 hr with anti-CD3 and anti-CD28 in the presence or absence of TGFβ. (B-F) Naïve CD4$^+$ T cells from retrogenic mice were transferred into *Rag1*$^{-/-}$ mice. (B) Weight gain of *Rag1*$^{-/-}$ recipient mice at indicated time after T cell transfer. (C) Quantitation of colon pathology as indicated by the colitis score. (D) Representative histology images of colon sections. Scale bars, 200 μm. (E) Frequencies of the donor TβRII$^{Cherry+}$ or Ctrl$^{Cherry+}$ CD4$^+$ T cells in the recipient mice. (F) Frequencies of Foxp3$^+$ in the donor CD4$^+$ T cell population. Data are pooled from two (A) or three (B-F) independent experiments. In (C, E, F), each circle represents the data from one mouse. Student's *t*-test was used; bars (mean), error bars (SD in A; SEM in B), **P* < 0.05 and ****P* < 0.001.

Figure S7

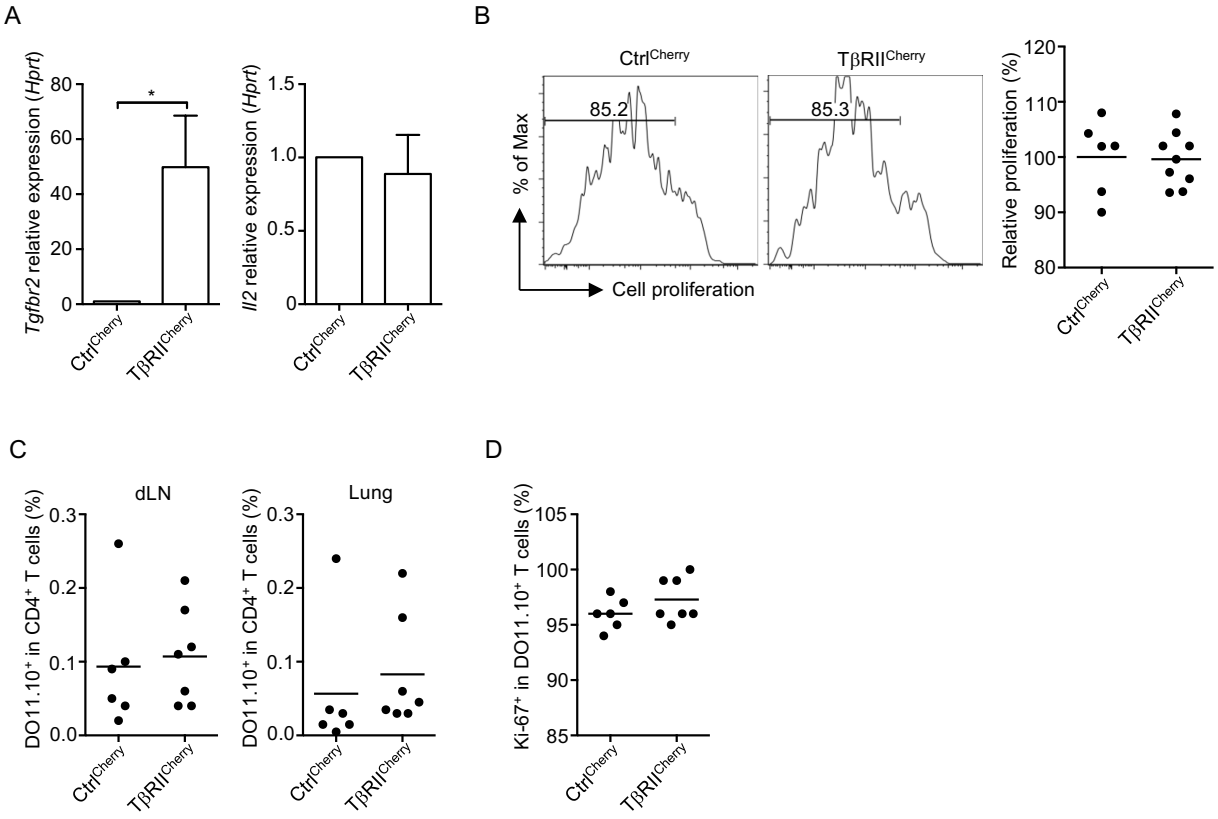

Figure S7. Overexpression of TβRII in activated T cells does not reduce T cell proliferation. Related to Figure 6

(A) Activated T cells were infected with retrovirus that carried TβRII^{Cherry} or Ctrl^{Cherry}. *Tgfbr2*, and *Il2* mRNA in TβRII$^{Cherry+}$ or Ctrl$^{Cherry+}$ activated T cells re-stimulated with anti-CD3 and anti-CD28 for 24 hr. (B) Representative flow cytometric analysis and summary of cell proliferation of TβRII$^{Cherry+}$ or Ctrl$^{Cherry+}$ activated DO11.10 T cells challenged with OVA peptide. (C) Frequency of TβRII$^{Cherry+}$ or Ctrl$^{Cherry+}$ activated DO11.10 T cells in the lungs and draining lymph nodes (dLN) of mice that were challenged with OVA protein in an airway inflammation model. (D) Frequencies of Ki-67^{+} cells in TβRII$^{Cherry+}$ or Ctrl$^{Cherry+}$ activated DO11.10 T cells in the draining lymph nodes of mice challenged with OVA protein. Data are pooled from two (A, C, D) or three (B) independent experiments. In (B-D), each circle represents the data from one mouse. Student's *t*-test was used; bars (mean), error bars (SEM), **P* < 0.05.