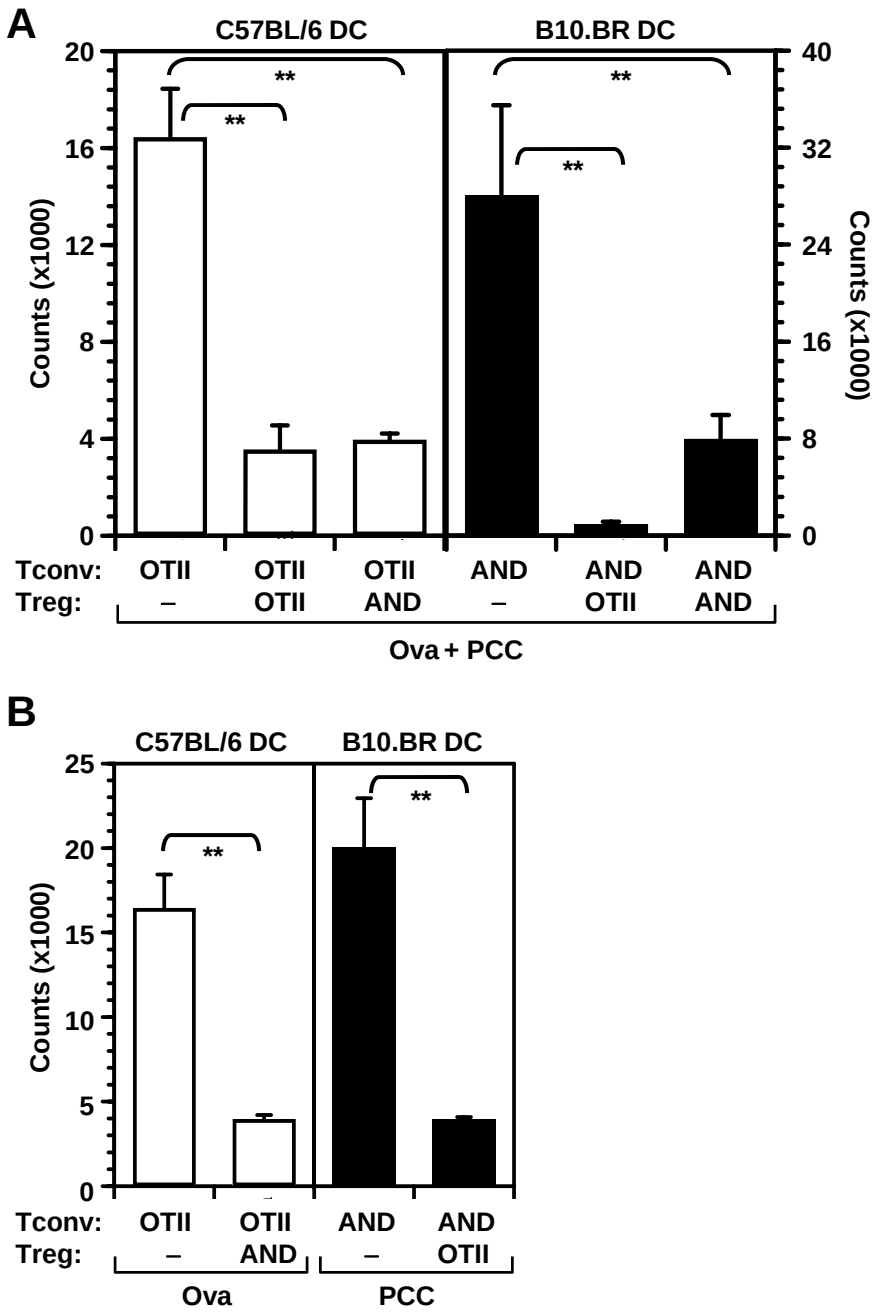
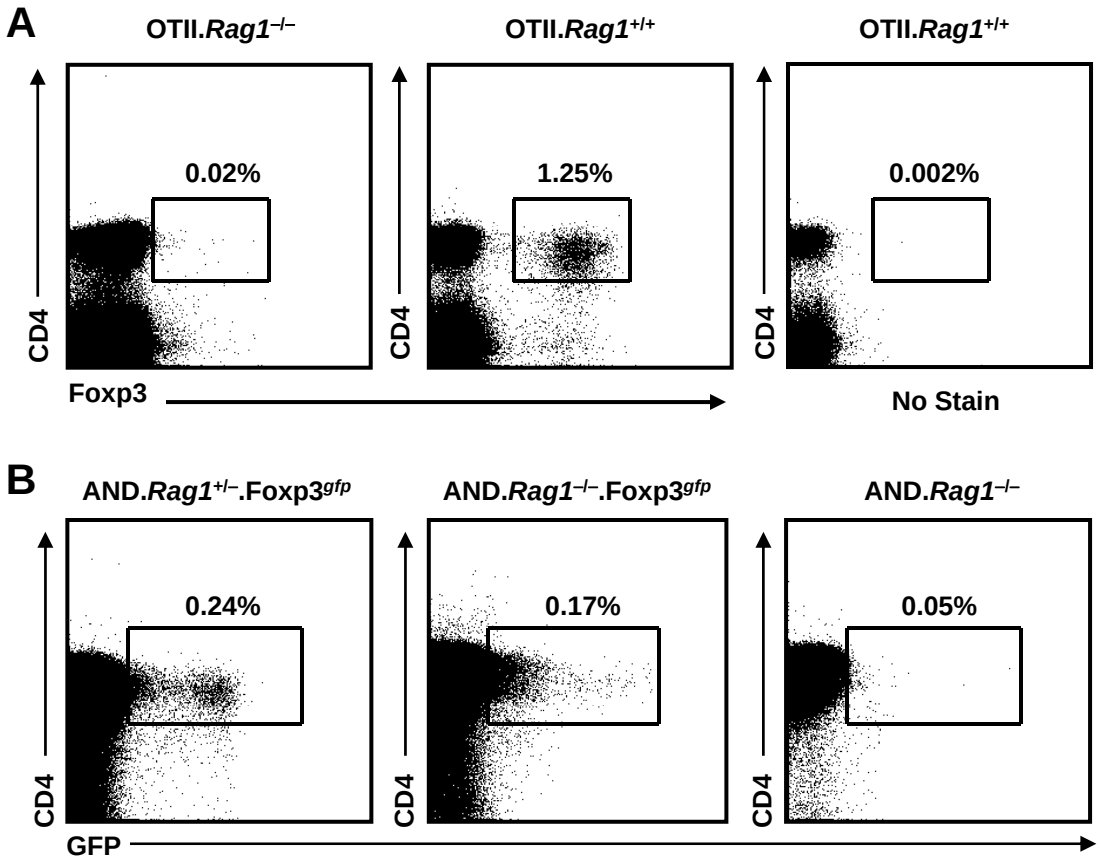
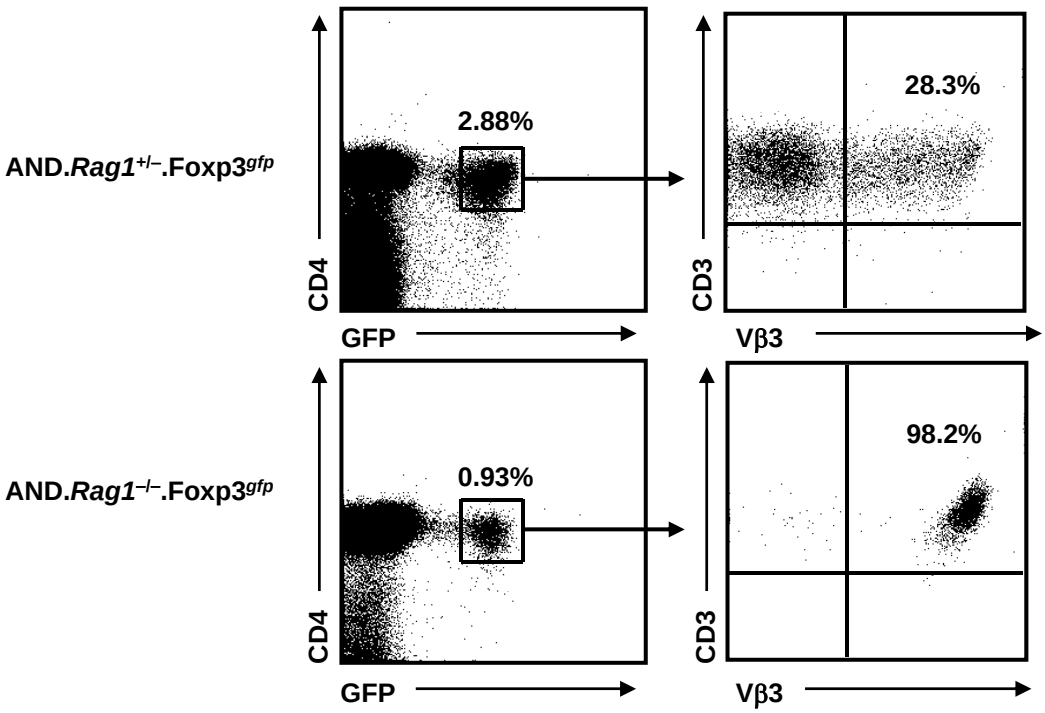


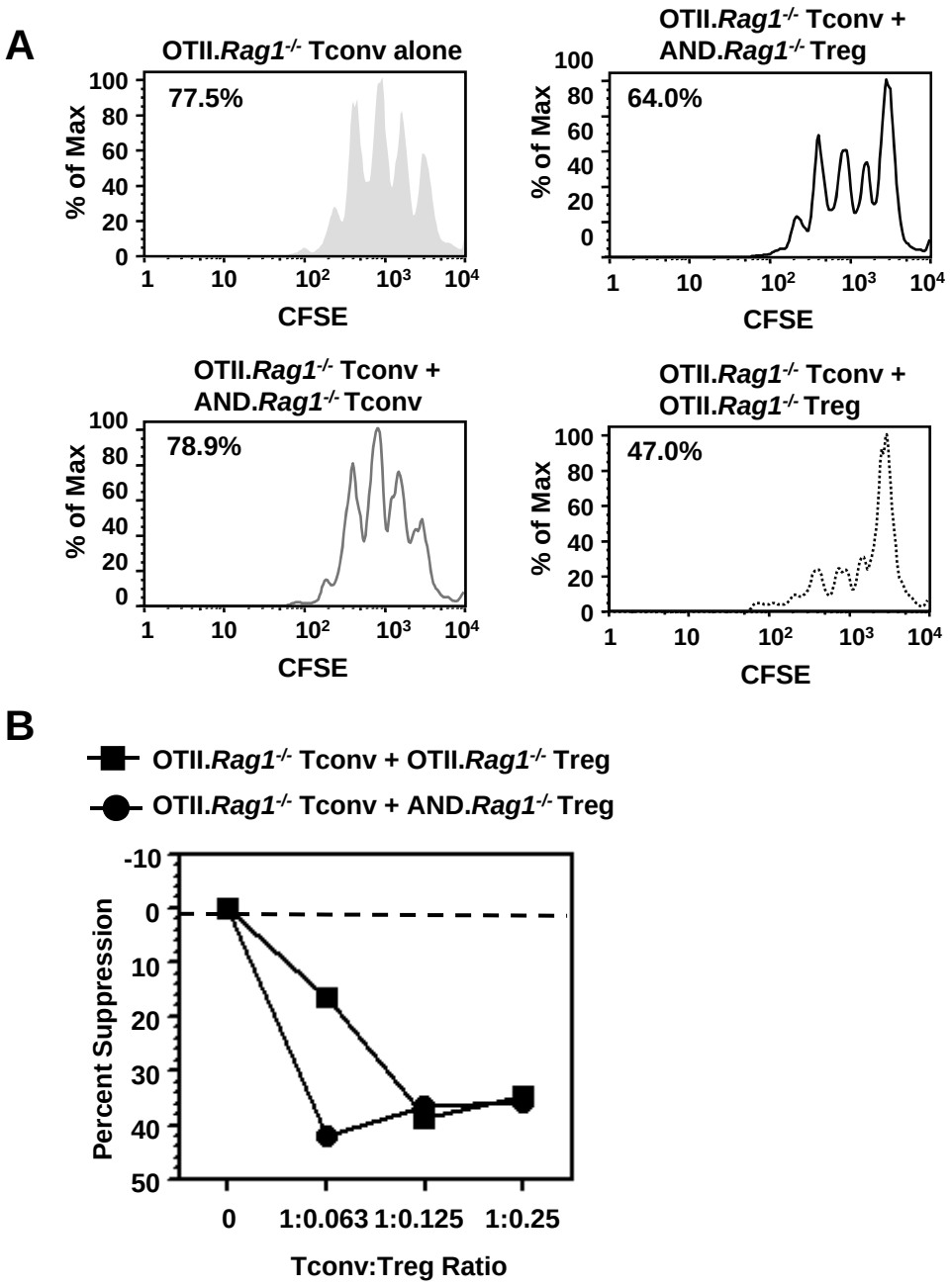
Supplemental Figure 1. Tregs so not require activation by their cognate peptide:DC combination to supress. *A,* Tconv (2.5 X 10^4) were incubated with DCs (6.25 X 10^3) and Tregs (1.25 X 10^4) in the presence of peptide ($Ova_{326-339}$ and PCC_{88-104}; 0.3μM) for 68 h, pulsed with [^{3}H]thymidine for the last 8 h of culture and Tconv proliferation measured. A representative from 3 experiments is shown. *B*, Tconv (2.5 X 10^4) were incubated with DCs (6.25 X 10^3) and Tregs of varying concentrations in the presence of peptide ($Ova_{326-339}$ or PCC_{88-104}; 0.3μM) for 68 h, pulsed with [^{3}H]thymidine for the last 8 h of culture and Tconv proliferation measured. A representative from 2-5 experiments is shown. **, $p<0.005$, *, $p<0.05$.



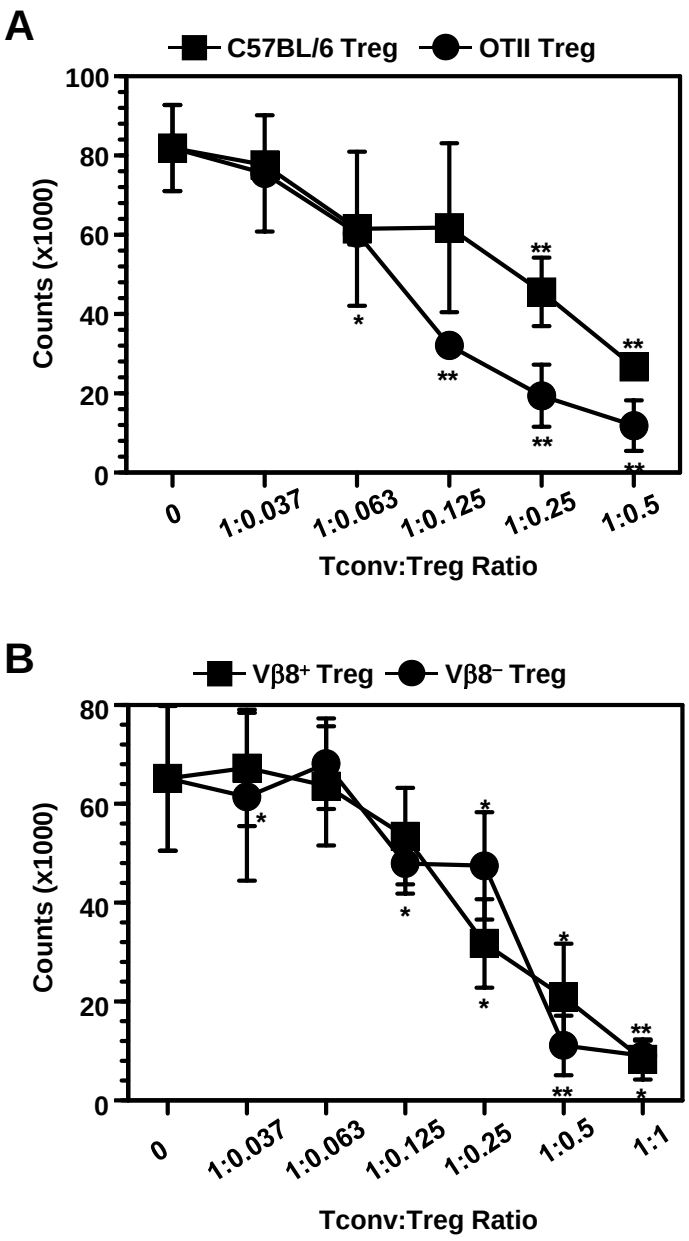
Supplementary Figure 2. Treg development in *Rag1*$^{-/-}$ AND and OTII TCR transgenic mice. *A*. Spleens and lymph nodes from indicated mice were processed, stained with antibodies against CD4 and Foxp3 and analyzed by flow cytometry. Foxp3 unstained control is shown. *B*. Thymi from AND.*Rag1*$^{+/-}$.Foxp3^{gfp}, AND.*Rag1*$^{-/-}$.Foxp3^{gfp} AND.*Rag1*$^{-/-}$ mice were processed, stained with anti-CD4 antibody and analyzed by flow cytometry.



Supplementary Figure 3. Foxp3^{+} Tregs from AND transgenic mice on a *Rag1*$^{-/-}$ background only express the clonotypic, AND-specific TCR-Vβ3. Lymph nodes from AND.*Rag1*$^{+/-}$.Foxp3^{gfp} (control) and AND.*Rag1*$^{-/-}$.Foxp3^{gfp} mice were processed, stained with antibodies against CD4, CD3 and TCR Vβ3 and analyzed by flow cytometry.



Supplemental Figure 4. TCR-ligation-independent Treg suppression is not due to alloreactivity/crossreactivity. CFSE-labeled OTII.*Rag1*$^{-/-}$ Tconv (2.5 X 10^4) were incubated with C57BL/6 DCs (6.25 X 10^3) and varying concentrations of Tregs or control Tconv in the presence of peptide ($Ova_{326-339}$; 0.3μM) for 68 h and analyzed by flow cytometry. *A,* CFSE dilution of OTII.*Rag1*$^{-/-}$ Tconv incubated with $CD25^+CD45RB^{lo}$ Tregs or AND.*Rag1*$^{-/-}$ Tconv (0.5 to 1 Treg:Tconv ratio) is shown in separate histograms with the percentage of Tconv with greater than 1 cell division indicated. B, Cells were incubated as in (*A*) for 68 h, pulsed with [^{3}H]thymidine for the last 8 h of culture and Tconv proliferation measured.



Supplementary Figure 5. Tregs do not require stimulation through their TCR to suppress Tconv proliferation. *A,* OTII Tconv (2.5 X 10^4) were incubated with irradiated splenocytes (C57BL/6; 5 X 10^4) and Tregs (1.25 X 10^4) in the presence of peptide ($Ova_{326-339}$; 2.0μM) for 68 h, pulsed with [^{3}H]thymidine for the last 8 h of culture and Tconv proliferation measured. *B*, Vβ8$^+$ Tconv (2.5 X 10^4) were incubated with anti-Vβ8 coated microbeads and Tregs of varying concentrations for 68 h, pulsed with [^{3}H]thymidine for the last 8 h of culture and Tconv proliferation measured. Data are representative of 2-3 experiments. **, p<0/005, *, p<0.5.