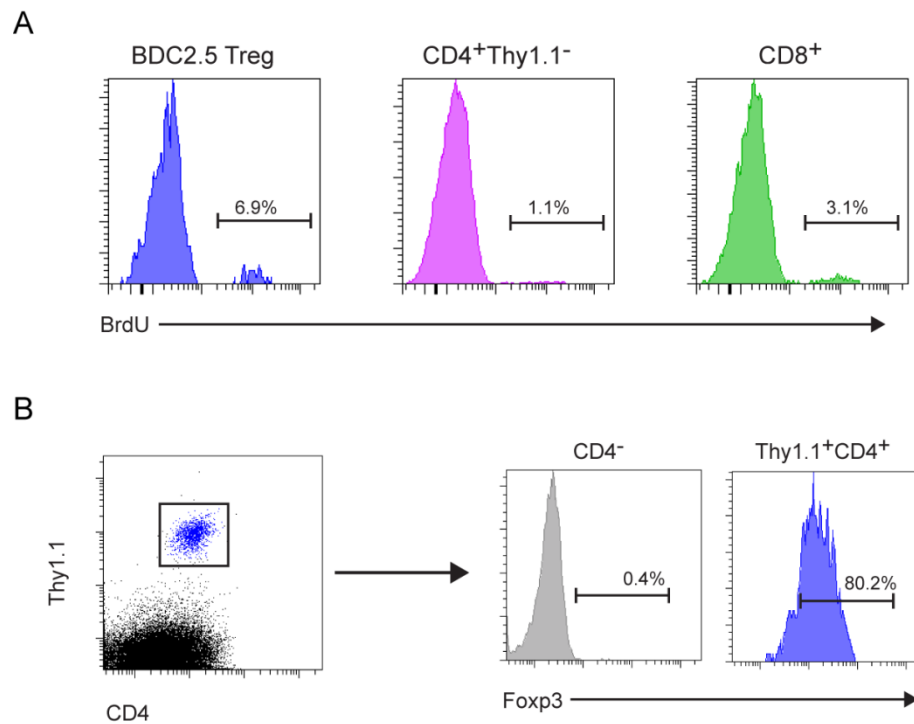
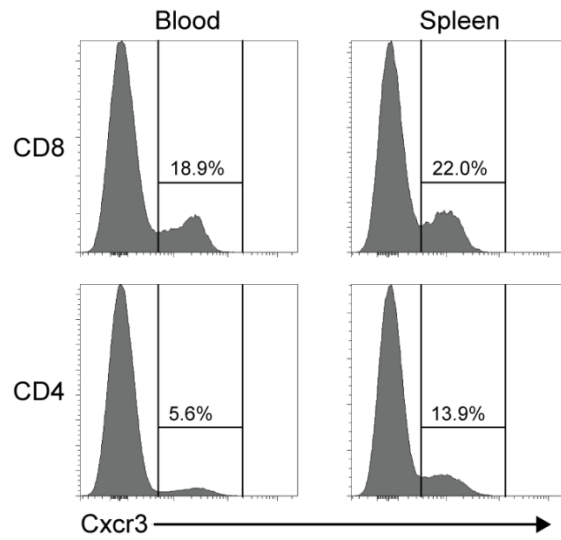


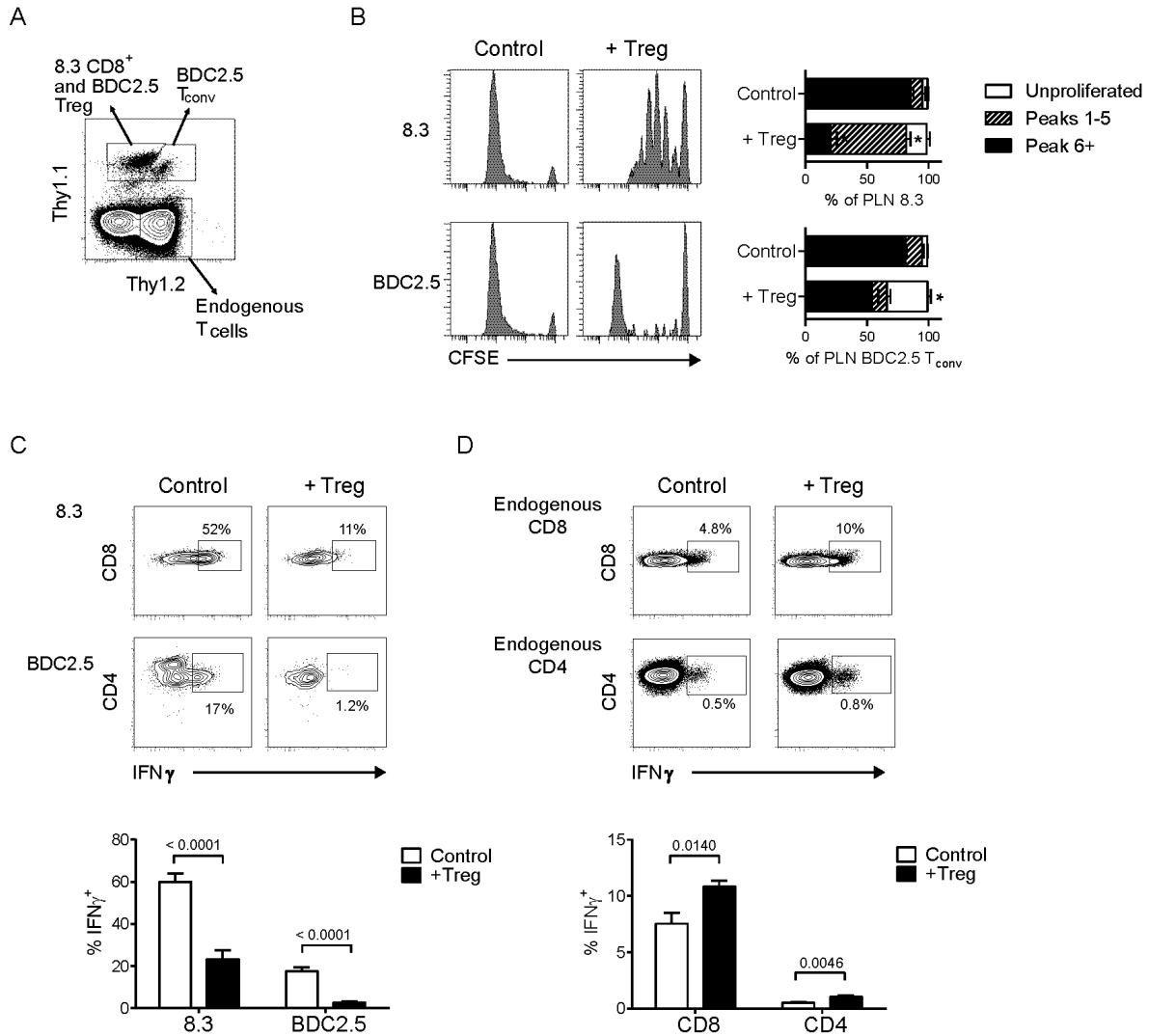
Supplemental Data



Supplemental Figure 1. (A) BDC2.5 Tregs proliferate in the islets. 2 weeks post-Treg transfer, a NOD.CD28^{-/-} mouse was i.p. injected with 2.5 mg BrdU 4 hours prior to sacrifice. Islet cell populations were distinguished as in **Fig. 1B** and assessed for BrdU incorporation by flow cytometry. (B) BDC2.5 Tregs maintain high levels of Foxp3 in the islets. 2 weeks post-transfer, CD4⁺Thy1.1⁺ BDC2.5 Tregs in the islets of a NOD.CD28^{-/-} mouse were analyzed for Foxp3 expression. Foxp3⁺ gate was set using CD4⁻ islet cells as the negative peak.



Supplemental Figure 2. Peripheral CD8⁺ T cells in NOD mice express Cxcr3. Blood cells and splenocytes from 16 week-old female NOD mice were analyzed by flow cytometry. CD4⁺ and CD8⁺ T cells were pre-gated as CD45⁺DAPI⁻B220⁻CD11c⁻.



Supplemental Figure 3. BDC2.5 Tregs suppress proliferation and priming of new effector T cells in the draining PLN. (A-D) 10^6 CFSE-labeled 8.3.Thy1.1⁺ CD8⁺ T cells and BDC2.5.Thy1.1⁺Thy1.2⁺ CD4⁺CD25⁻ T cells were transferred to NOD.CD28^{-/-} mice 3 d following BDC2.5.Thy1.1⁺ Treg treatment or to untreated littermates and analyzed 4 d later. (A) Representative flow plot depicting gating of endogenous and transferred T cells. Cells were pre-gated as CD45⁺ lymphocytes. Transferred 8.3 CD8⁺ T cells and BDC2.5 Tregs were gated as Thy1.1⁺Thy1.2⁻ and were further differentiated based on CD8 and CD4 expression. BDC2.5 conventional CD4⁺ T cells were Thy1.1⁺Thy1.2⁺. Endogenous T cells were Thy1.2⁺Thy1.1⁻.

(B) Representative histograms pre-gated on Thy1.1⁺ CD8⁺ 8.3 T cells and Thy1.1⁺Thy1.2⁺CD4⁺ BDC2.5 T cells depicting CFSE dilution of transferred cells in the PLN with and without Treg treatment. Bar graphs depict the percentage of cells that did not divide (Unproliferated), underwent 1-5 rounds of division (Peaks 1-5), or underwent 6 or more rounds of division (Peak 6+). Kruskal-Wallis test followed by Dunn's post test, *, P < 0.05. **(C and D)** PLN cells were restimulated *in vitro* with PMA and ionomycin in the presence of Brefeldin A for 2-3 hours prior to intracellular staining for IFN γ . **(C)** Representative flow plots pre-gated on 8.3 CD8⁺ (top) and BDC2.5 T_{conv} (bottom) cells showing IFN γ expression by intracellular flow cytometry. Bar graph depicts summary data from 6 mice per group from 2 independent experiments. **(D)** Representative flow plots pre-gated on endogenous islet Thy1.2⁺Thy1.1⁻ CD8⁺ (top) and CD4⁺ (bottom) T cells and quantification of the percent expressing intracellular IFN γ ⁺. Bar graph depicts summary data as in C. Bar graphs depict mean \pm SEM. P values for C and D were determined by Mann-Whitney test.

Supplemental Table 1. Fold-regulation in Treg-treated versus age-matched controls

Gene	Day 3 post-Treg	Day 7 post-Treg	Gene	Day 3 post-Treg	Day 7 post-Treg
Actb	-1.2	-1.1	Cxcr5	-1.5	-1.3
Ccl1	-1.1	-1.5	Cxcr6	-2.1	-2.9
Ccl11	1.2	1.2	Foxp3	1.1	-1.5
Ccl12	-1.4	-1.7	Gapdh	1.1	-1.0
Ccl19	-1.4	-2.3	Gzma	-4.7	-7.8
Ccl2	-1.2	1.5	Gzmb	-6.1	-8.5
Ccl20	-1.3	-1.2	Hprt1	1.0	1.1
Ccl25	-1.5	1.2	Hsp90ab1	1.1	1.0
Ccl3	-1.4	1.5	Icam1	-1.4	1.1
Ccl4	-1.1	1.7	Icos	-1.5	-1.9
Ccl5	-2.0	-2.1	Ifng	-2.0	-3.5
Ccl7	-1.1	1.6	Igsf6	-1.5	-1.7
Ccl8	-2.1	-4.3	Il10	-1.0	<i>3.1</i>
Ccl9	-1.9	-1.6	Il12b	-1.2	1.5
Ccr1	-1.7	-1.5	Il15	-1.4	-1.0
Ccr10	-1.1	1.1	Il16	-1.8	-2.7
Ccr2	-1.5	-1.9	Il-17F	-1.2	1.8
Ccr3	-2.0	-1.8	Il1a	-1.1	2.1
Ccr4	-1.1	-1.2	Il1b	-1.0	1.0
Ccr5	-1.9	-2.1	Il1r1	1.5	1.9
Ccr6	-1.4	-1.6	Il1r2	1.2	1.3
Ccr7	1.2	-1.0	Il1rn	-1.7	1.0
Ccr8	1.0	-1.4	Il27ra	-1.5	-2.2
Ccr9	-2.6	-1.9	Il6	-1.5	3.3
Ccr12	-1.2	-1.2	Ins1	1.1	1.9
Cd3e	-1.3	-2.7	Ins2	1.3	2.1
Cd4	-1.4	-2.2	Itgax	-1.5	-1.4
Cd40	-1.9	-1.7	Itgb2	-1.7	-2.6
Cd40lg	-1.0	-3.1	Klrd1	-2.0	-4.5
Cd68	-1.3	-1.7	Lta	-1.2	-2.6
Cd80	-1.4	1.4	Ltb	-1.5	-2.8
Cd86	-1.5	-1.2	Ly6c1	-1.2	-1.5
Cd8a	-2.6	-5.3	Madcam1	1.0	-1.4
Csflr	-1.5	-1.5	Ppbp	-1.2	1.6
Cx3cl1	1.0	1.6	Ptprc	-1.8	-2.0
Cx3cr1	-1.3	-2.0	Reg3a	-1.6	-2.1
Cxcl1	1.5	1.9	Reg3b	-2.2	-3.8
Cxcl10	-1.9	1.0	Reg3g	-1.7	-1.5
Cxcl11	-1.6	1.0	Tnf	-1.0	1.3
Cxcl12	-1.1	1.2	Tnfrsf4	-1.3	-2.1
Cxcl13	-2.4	-2.9	Tnfsf4	-2.1	1.1
Cxcl16	-1.7	-1.8	Vcam1	-1.7	-1.4
Cxcl2	-1.1	-1.0	Xcl1	-1.9	-3.7
Cxcl9	-2.0	-3.7	Xcr1	-1.6	-1.4
Cxcr3	-1.4	-4.4			
Cxcr4	-1.2	-2.1			

Genes downregulated by Tregs > 3-fold in **bold**.

Genes upregulated by Tregs > 3-fold in *italics*.