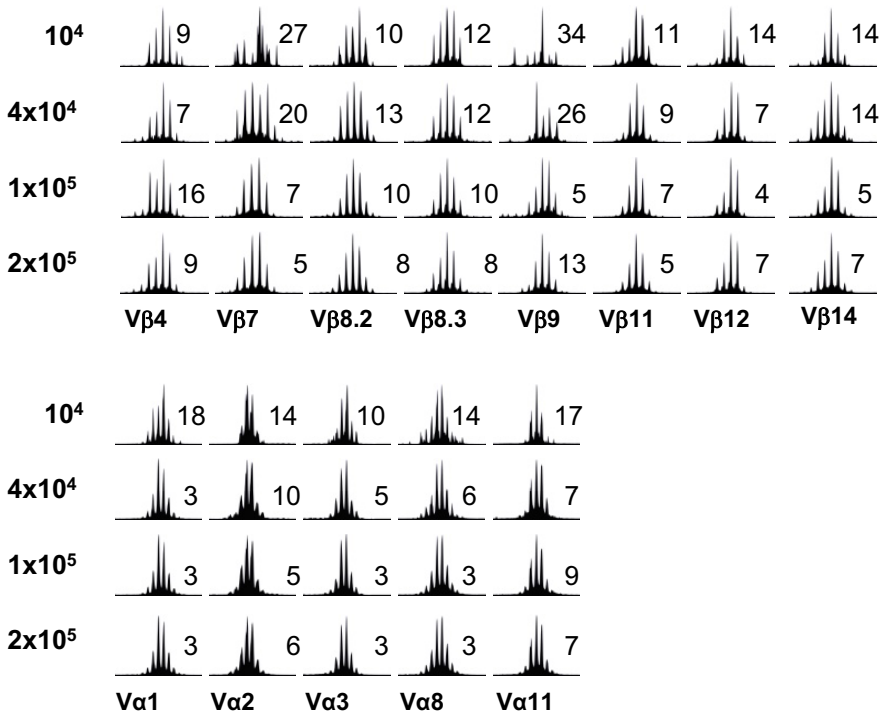
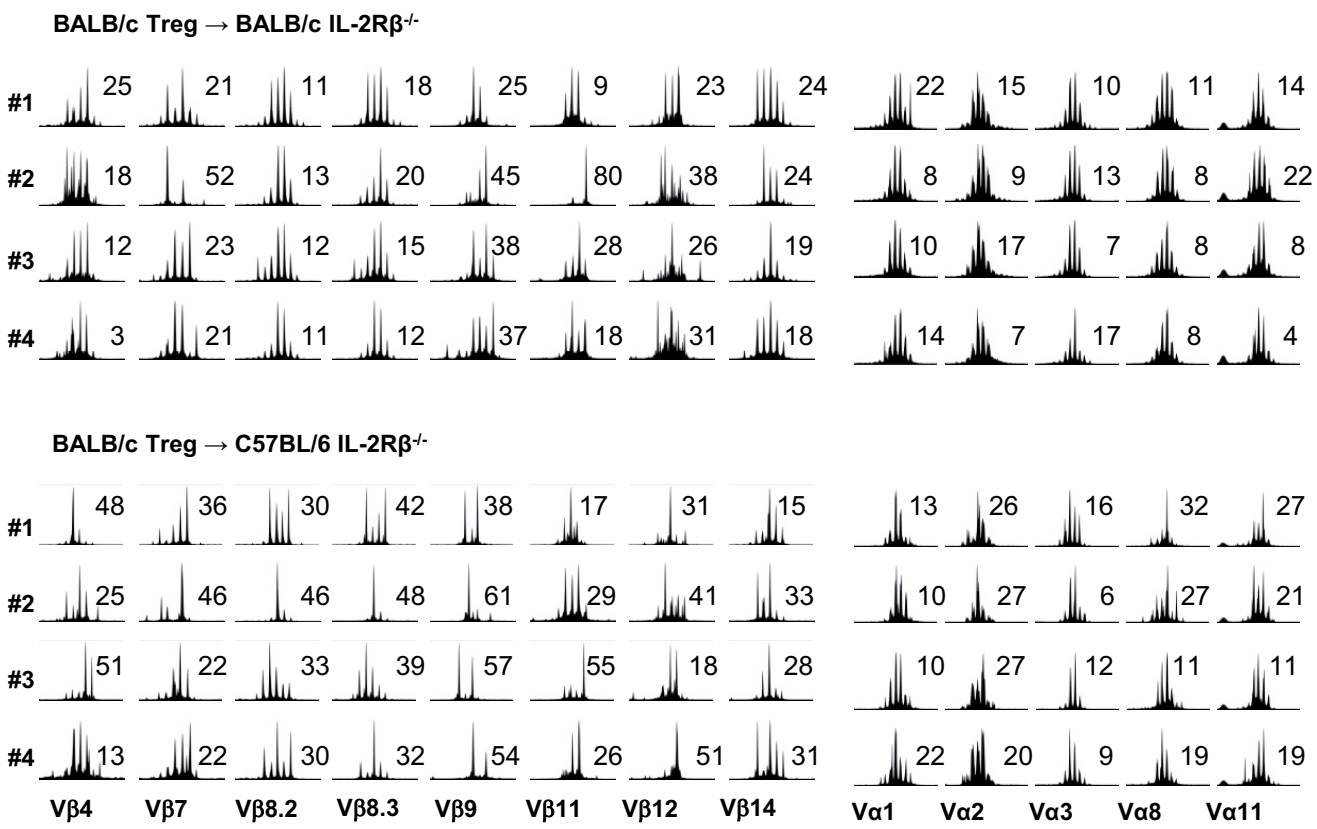
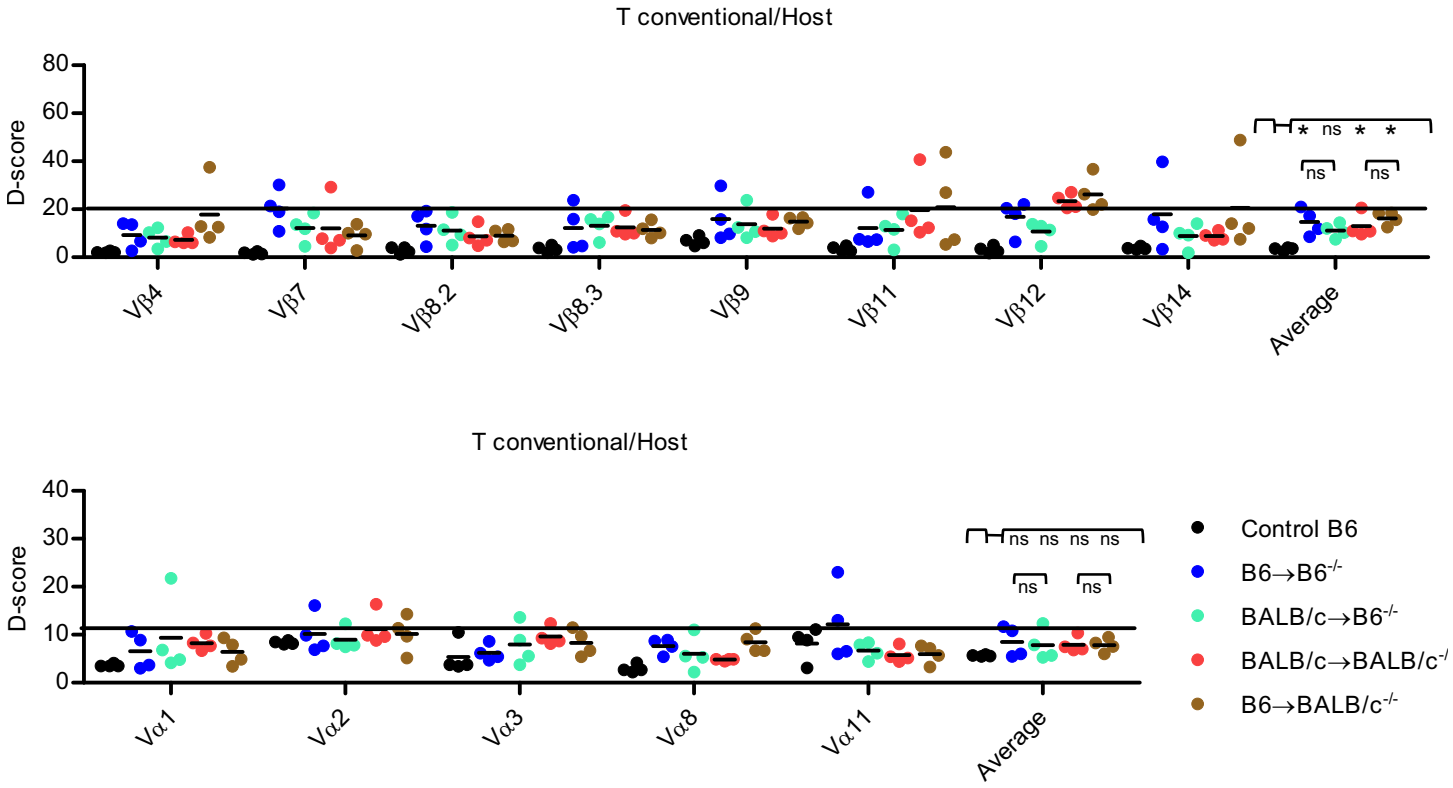


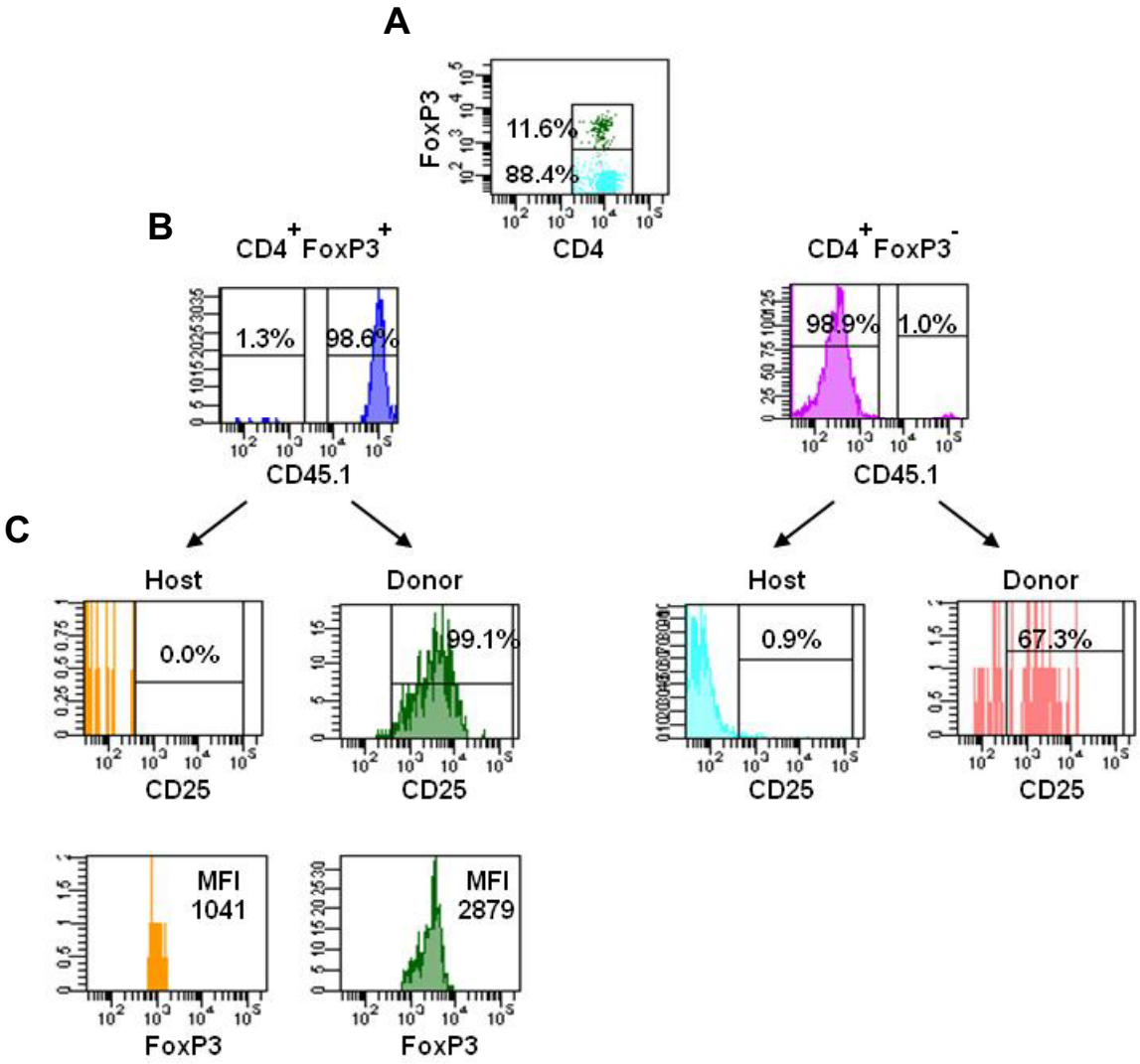
Supplemental Fig. 1. The sensitivity of CDR3 spectratype analysis. Vβ or Vα spectratype analysis were performed with serial diluted $CD4^+$ T cells a from C57BL/6 mouse. Total RNA was extracted from the indicated number of cells. One-twentieth of cDNA was used for CDR3 spectratype analysis. The D-score is shown to the right of each spectratype profile.



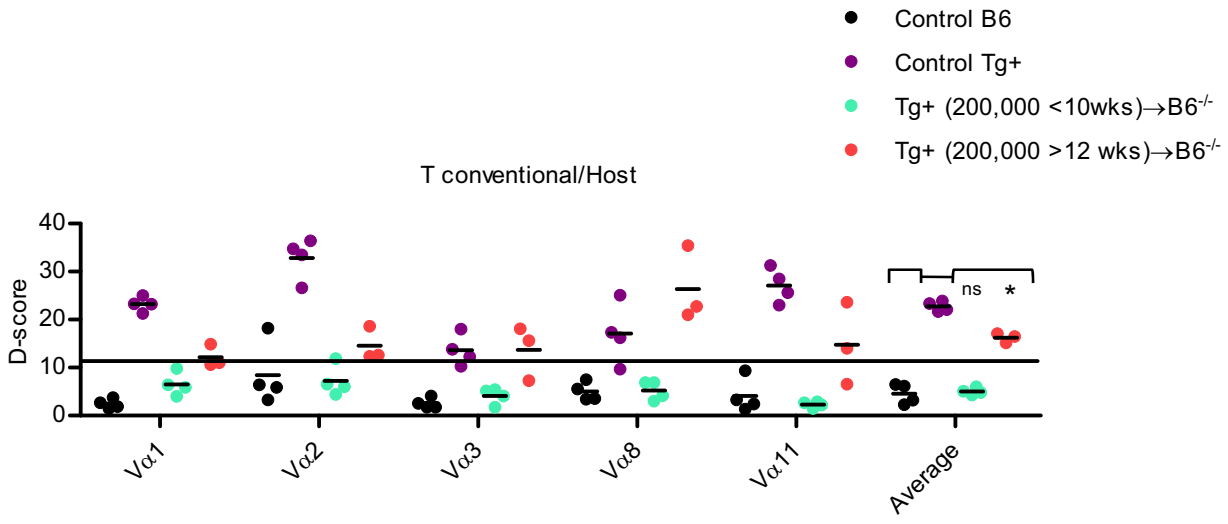
Supplemental Fig. 2. TCR spectratype analysis of donor Treg cells of adoptively treated IL-2Rβ$^{-/-}$ mice and. 2 x 10^5 Purified BALB/c CD4$^+$CD25$^+$ Treg cells were adoptively transferred into neonatal IL-2Rβ$^{-/-}$ mice and denoted as the donor Treg strain→recipient IL-2Rβ$^{-/-}$ strain. Vβ and Vα spectratype analysis for CDR3 was performed for the indicated Vβ and Jβ1.1 gene segments or indicated Vα subfamily genes and Cα gene segment for Treg cells isolated from normal or the adoptively transferred IL-2Rβ$^{-/-}$ mice. The D-score is shown to the right of each spectratype profile.



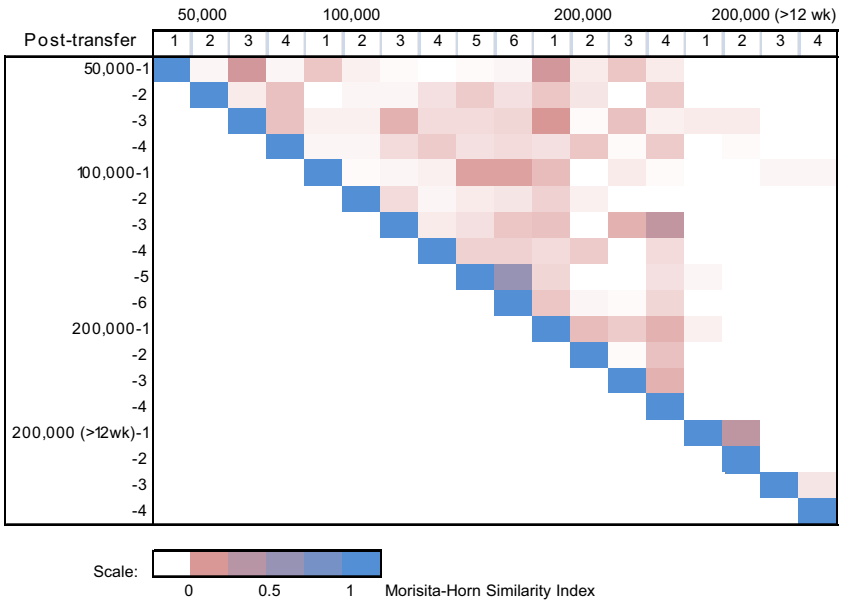
Supplemental Fig. 3. TCR diversity of recipient CD4⁺ T cells from individual IL-2Rβ$^{-/-}$ mice adoptively transferred with syngeneic and allogeneic Treg cells. Spectratyping for the indicated Vβ and Jβ1.1 gene segments or Vα subfamily genes and Cα gene segment was performed for CD4^{+}CD25^{-} T cells isolated from normal C57BL/6 (control B6) or the indicated adoptively transferred IL-2Rβ$^{-/-}$ mice. D-scores for Vβ and Vα spectratype distribution profiles for all mice (n=4 mice/group). The line in each graph represents the averaged D-score for untreated IL-2Rβ$^{-/-}$ mice. Data for the averaged D-scores were compared by 1-way ANOVA.



Supplemental Fig. 4. Donor cell engraft of autoimmune-free IL-2Rβ-deficient recipients that received congenic-marked CD45.1 syngeneic WT Treg cells. A) Spleen cells were analyzed for expression of CD4 and Foxp3. B) The indicated gated populations were assessed for host ($CD45.1^{neg}$) and donor ($CD45.1^{+}$) T cells. C) The host and donor cells were then assessed for expression of CD25 and Foxp3, as indicated. The % positive cells within the gated region or the mean fluorescent intensity (MFI) are shown for the dot plot and histograms.



Supplemental Fig. 5. TCR diversity of recipient CD4$^+$ T cells from individual IL-2Rβ$^{-/-}$ mice adoptively transferred with TCRβ Tg$^+$ Treg cells. Spectratyping for the indicated Vα subfamily genes and Cα gene segment was performed for CD4$^+$CD25$^-$ T cells isolated from normal C57BL/6 (control B6), normal TCRβ Tg$^+$ (control Tg+) or the indicated adoptively transferred IL-2Rβ$^{-/-}$ mice. D-scores for all Vα spectratype distribution profiles for all mice (n=4-6 mice/group). The line in each graph represents the averaged D-score for untreated IL-2Rβ$^{-/-}$ mice. Data for the averaged D-scores were compared by 1-way ANOVA.



Supplemental Fig. 6. The similarity of post-transferred CDR3 sequences. Individual post-transferred Treg cells CDR3 sequences were compared to each other by calculation of Morisita-Horn similarity values and represented by a heat map.

Supplemental Table 1. Primers used in this study

Primer	Specificity	Sequence
VA1-1	AV1S1, AV1S2/1S8, AV1S3/1S6, AV1S4, AV1S7	AGACTCCCAGCCCAGTGACT
VA2-1	AV2S1/2S9, AV2S2/2S7, AV2S4	TGCAGTTATGAGGACAGCACTT
VA3-1	AV3S1, AV3S2/3S5, AV3S3, AV3S6, AV3S7	CTGCAGCTGAGATGCAAGTATT
VA8-1	AV8S1/8S8/8S12/8S15, AV8S2/8S10, AV8S3/8S5, AV8S4/8S7/8S9/8S11, AV8S6/8S14	ACGCCACTCTCCATAAGAGCA
VA14-1	AV14S1 and AV14S2	CAGGCAAAGGTCTTGTGTCC
MCA2	AC	GTTTTCGGCACATTGATTTG
MCA3	AC	6-FAM/PET-AGAGGGTGCTGTCCTGAGAC
MCB2	BC	TTGTAGGCCTGAGGGTCC
MCB3	BC	6-FAM/PET-GAGACCTTGGGTGGAGTCAC
Vα2-Xho	AV2	GCCGCTCGAGTCAGTCTTGCAGACCTCAACT
Vα2-Eco	AV2	GCCGGAATTCATGGACAAGATCCTGACAGCA
pMI sequencing primer		TGGAAAGGACCTTACACAGT