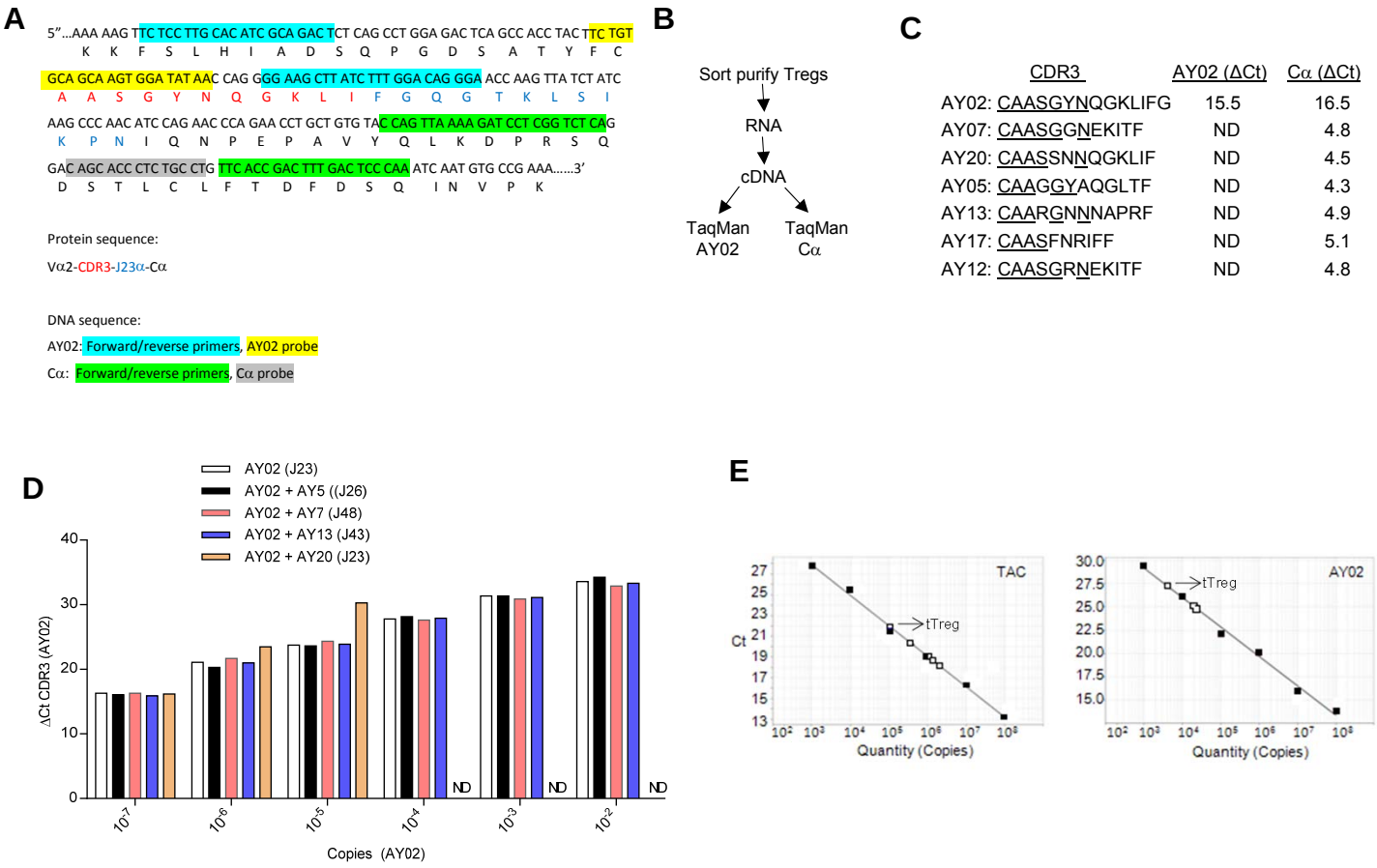
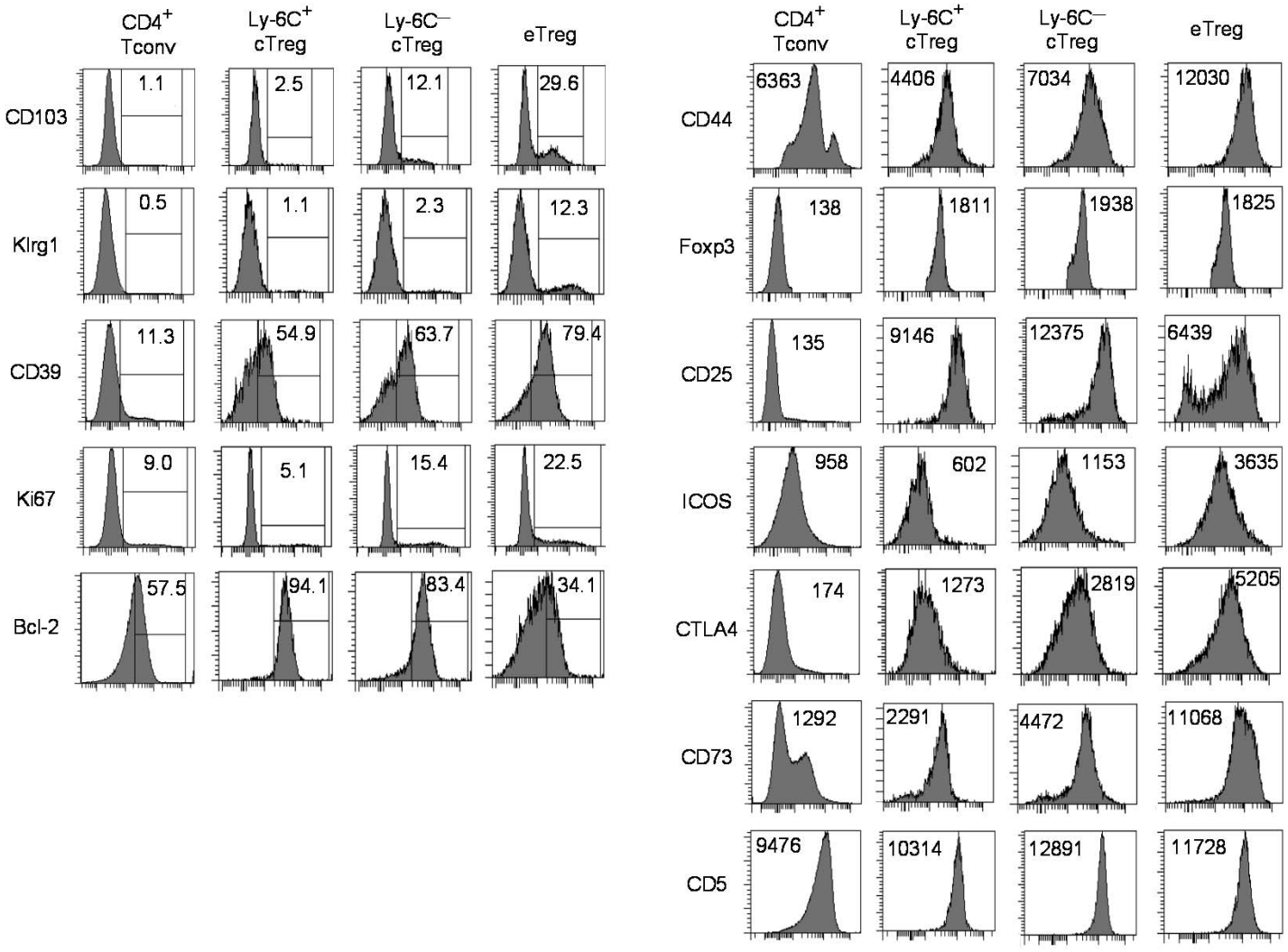
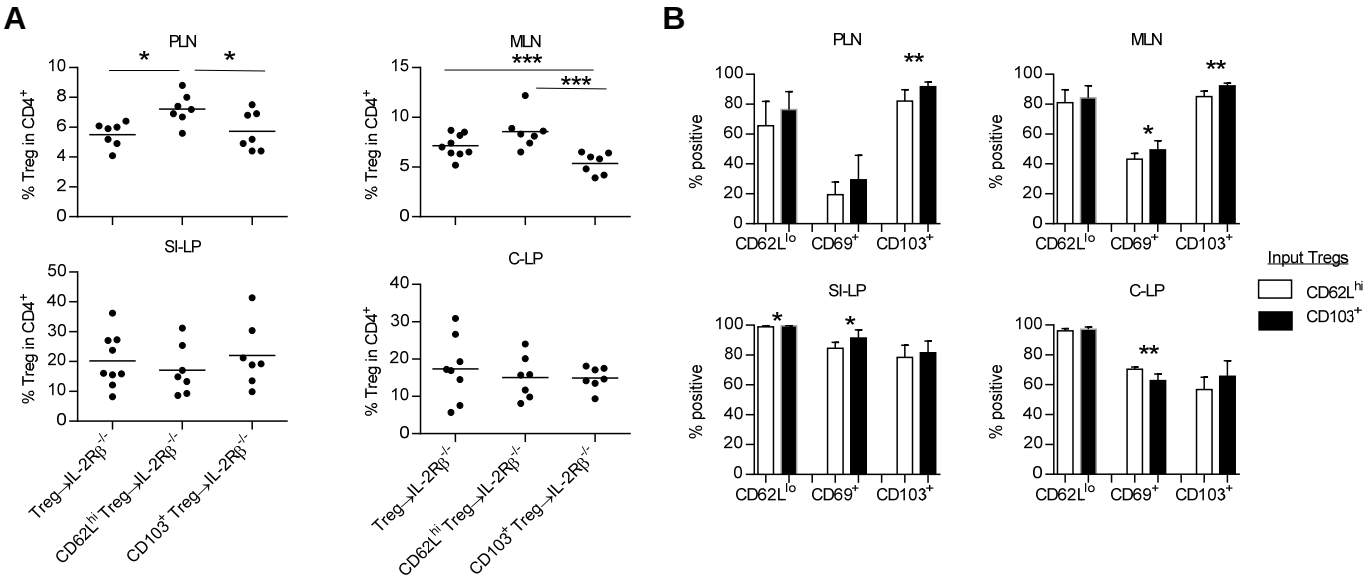


Supplemental Fig. 1. Development of a quantitative PCR assay for a dominant TCR specificity in Tregs. (**A**) Primers and probes for the Taqman RT-PCR for the AY02 clonotype. Partial DNA and protein sequences are shown for AY02. The sequences are color coded, as indicated, to highlight the primers and probes used for amplification and detection of the CDR3 and Cα regions.(**B**) The experimental scheme to detect the AY02 clonotype in Tregs from TCRβ transgenic mice. (**C**) Specificity of the TaqMan. Plasmids containing 10^7 copies of the AY02 clonotype and 10^{11} copies of 6 other Vα2 Treg clonotypes with CDR3 regions related to the probe were used to detect the AY02 clonotype. The amino acid sequence corresponding to the AY02 probe and identical amino acids in the other CDR3 are underlined. ND refers to not detected. (**D**) Accuracy of the TaqMan assay for the AY02 clonotype. Varied copies of the AY02 plasmid were mixed with a fixed amount (10^6 copies) of plasmids with a distinct Vα2 clonotype, as indicated. The ΔCt was determined for the AY02 clonotype. The Jα region is shown for all clonotypes. ND refers to not detected. The AY02 clonotype was readily detected at similar ΔCt values in all mixtures when compared to reactions containing only the AY02 plasmid, except for the mixture of AY02 + AY20, where the AY02 clonotype was not detected at low copies.(**E**) Quantitation of the AY02 clonotype. Tregs were FACS purified from Foxp3/RFP reporter mice and quantitative RT-PCR was performed using the TaqMan assay. Standard curves for the AY02 clonotype (dark squares) and Cα (TAC) were generated by performing the TaqMan assay with varied copies of a plasmid containing AY02 cDNA. Examples of experimental samples are shown with open squares. Frequency of a test sample = (copies of AY02/copies of Cα) x100.



Supplemental Fig. 2. Representative FACS analysis of the indicated molecules on Ly-6C^{+} and Ly-6C^{-} CD62L^{hi} cTregs and Ly-6C^{-} CD62L^{lo} eTregs from the spleen. As a reference, CD4^{+} Foxp3^{-} T cells (Tconv) were also examined. Gated regions (left) represent the %$^{+}$ cells. Numbers within the histograms (right) represent the MFI.



Supplemental Fig. 3. Engraftment of donor Treg subsets in IL-2Rβ$^{-/-}$ mice. (**A**) The proportion of Tregs within total CD4^{+} T cells was determined for the indicated tissues 10-12 weeks post-transfer. Data were analyzed by one-way ANOVA, using Tukey's multiple comparison test (*p<0.05). (**B**) The phenotype of the donor Tregs from the indicated tissue, derived from IL-2Rβ$^{-/-}$ mice that were adoptively transferred with CD62L^{hi} or CD103^{+} Tregs. Data were analyzed by a two-sided unpaired t-test (*p<0.05; **p<0.01).