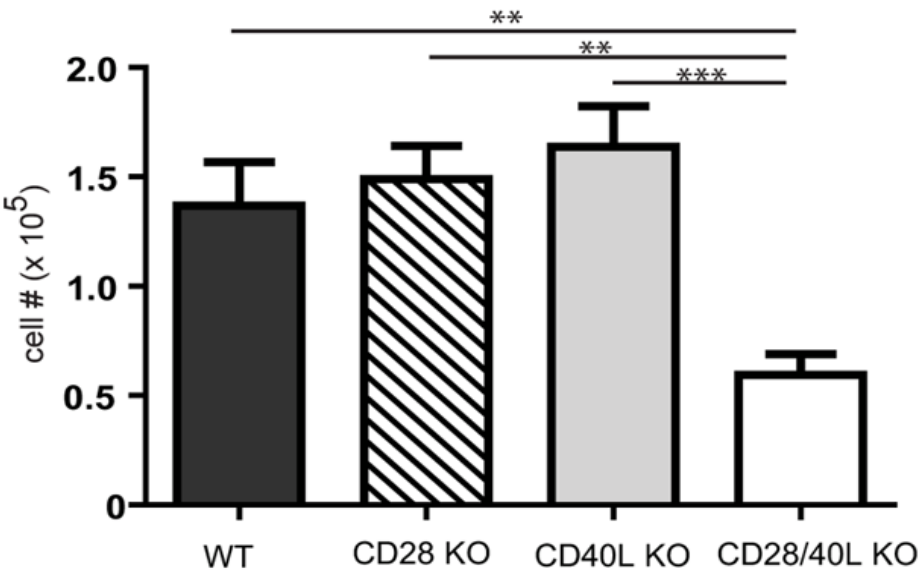

Figure S1. Reduction in mTEC number in CD28/CD40L KO mice. Thymic stromal cells from BALB WT, CD28 KO, CD40L KO and CD28/CD40L KO mice were prepared as described in the Materials and Methods section and stained with anti-CD45, UEA-1, anti-Ly51 and anti-IA/IE. Thymic stromal cells were prepared as in Figure 2. After gating on $CD45^{neg}$, IA/IE^{+} cells, numbers of mTEC (UEA^{+}) and cTEC (UEA^{neg}, $Ly51^{+}$) were calculated for each group. Data shown are averages ± SE for ≥7 mice per group All mice were between 3-5 weeks of age. (**, $p \leq 0.01$; ***, $p \leq 0.001$ ****, $p \leq 0.0001$).

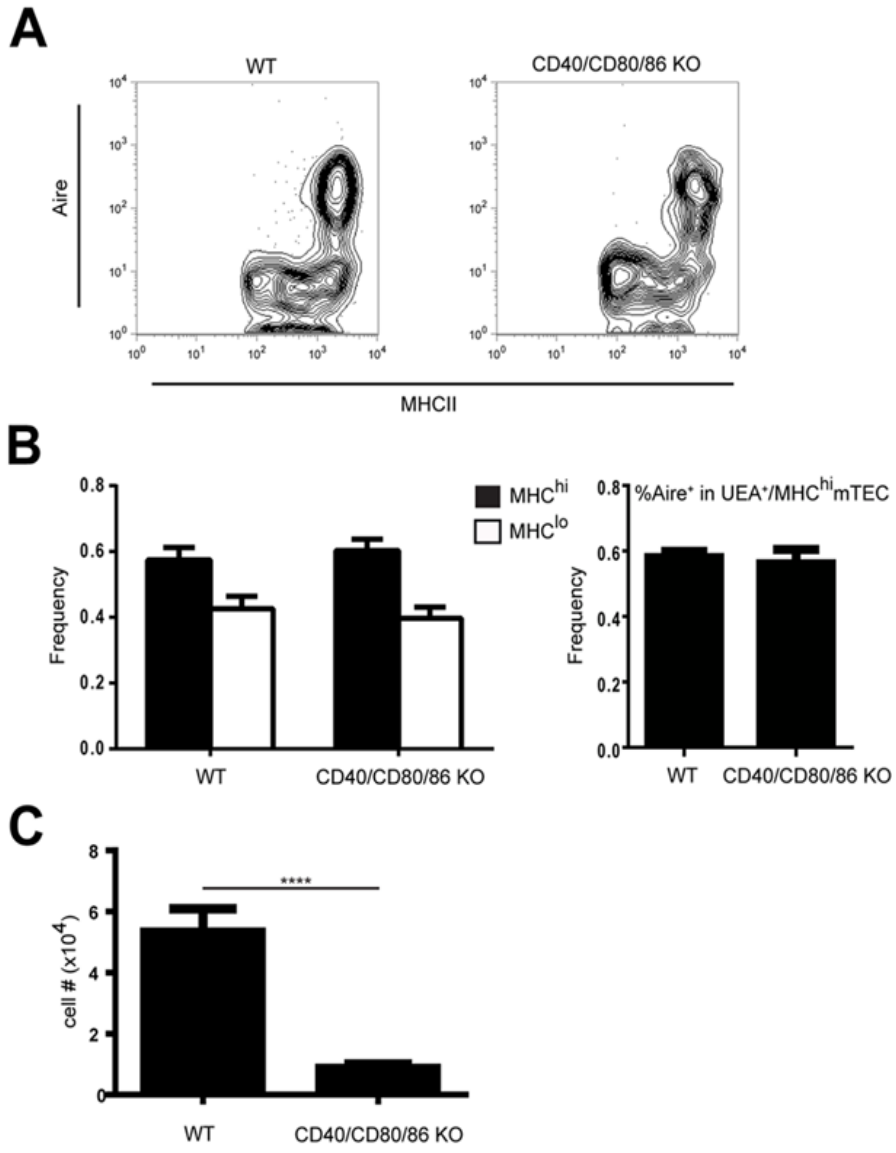

Figure S2. Frequencies of and numbers of AIRE^{+}MHC^{hi} mTECs in WT and CD40/80/86 KO mice. Thymic stromal cells from BALB WT and CD40/80/86 KO mice were prepared from 4-5 week old mice and stained with anti-CD45, UEA-1, anti-IA/IE, and anti-AIRE. (**A**) Representative flow cytometry dot plots are shown after first gating on CD45^{neg}, IA/IE^{+}, UEA^{+} mTECs. (B) Frequencies (shown as mean ± SE for n=9 mice) of MHC^{hi} and MHC^{lo} mTEC, and frequencies of AIRE^{+} cells within the MHC^{hi} mTECs are shown in the accompanying bar graphs. (C) Total number of AIRE^{+} mTECs in WT and CD40/80/86 KO mice. Numbers of AIRE^{+} mTECs are the mean ± SE for 9-10 mice per strain. (**** $p \leq 0.0001$).

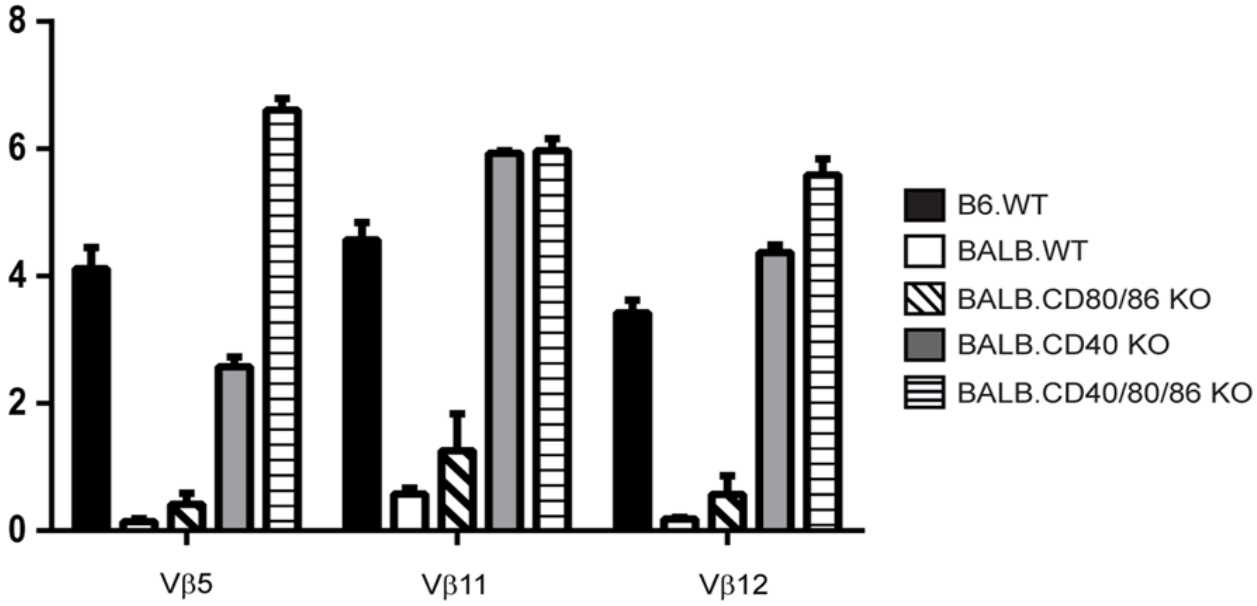

Figure S3. Negative selection is defective in BALB CD40 KO and BALB CD40/80/86 mice. Thymocytes from 8-12 week old C57BL/6 (n=5), BALB CD80/86 KO (n=5), BALB CD40 WT (n=5) and BALB CD40/80/86 KO (n=3) mice were stained with FITC anti-CD4, PE anti-CD8 and biotin-conjugated anti-TCR Vβ Abs. The percent Vβ positive CD4 SP in each strain is shown.a