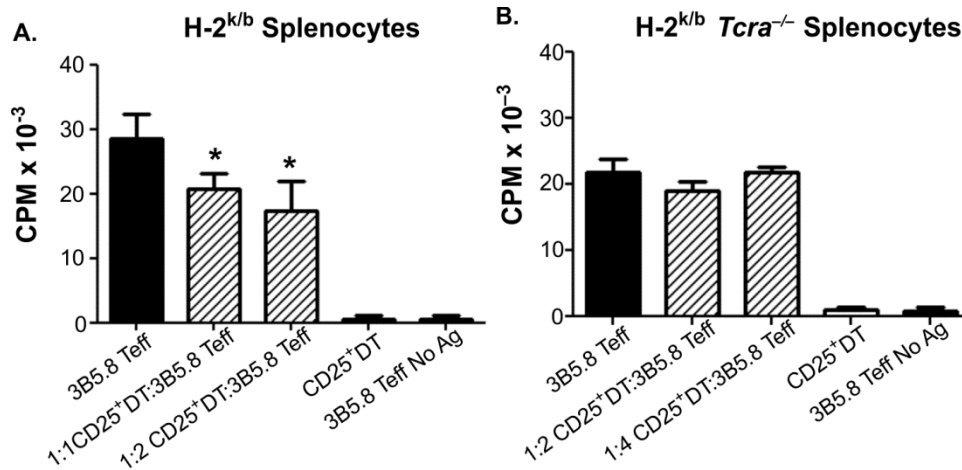
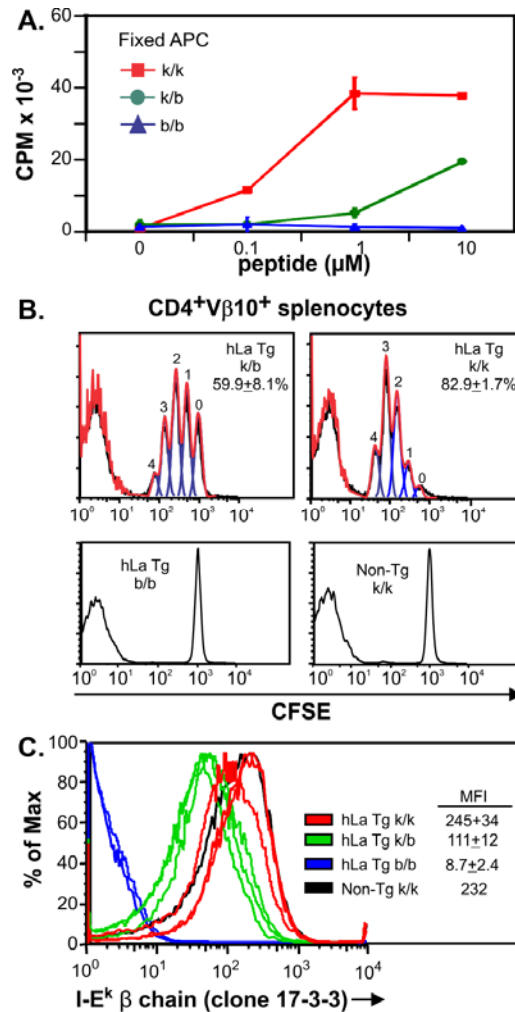


Supplemental Figure 1 Legend. A subset of endogenous Vα TCRs are expressed at low levels in H-2^{k/b} 3B5.8/hLa double Tg mice but are not included in positively selected tTreg. Vβ10⁺ tTreg from individual H-2^{k/b} 3B5.8/hLa double Tg (DT) mice (n=5) and 3B5.8 single Tg (ST) mice (n=3) were evaluated for expression of endogenous Vα chains using a cocktail of available Vα mAbs. **(A)** Gating and percentages of endogenous Vα-included cells (denoted “Vα mix”) among Vβ10⁺ CD4SP (left panel) and gating of tTreg in cells not expressing or expressing the measured Vα (denoted “Vα2/3.2/8.3/11.1/11.2⁻”, right panel). **(B)** Positive selection of tTreg occurs only in the population of cells that do not express the detected endogenous TCRα.

* p<0.05.



Supplemental Figure 2 Legend. Suppressor activity of sorted $V\beta 10^+CD4^+CD25^+$ splenocytes from $H-2^{k/b}$ 3B5.8/hLa double Tg (DT) mice is abrogated by *Tcra* deficiency. A) Proliferation of sorted 3B5.8 TCR Tg Teff cells, sorted $V\beta 10^+CD4^+CD25^+$ splenocytes from *Tcra*-intact 3B5.8/hLa double Tg (DT) mice, or the indicated combinations, with irradiated APC in the presence or absence (No Ag) of hLa61-84 peptide. * $p < 0.05$ versus 3B5.8 Teff. DT-derived $V\beta 10^+CD4^+CD25^+$ splenocytes display mild suppressor activity. Note that only 60-70% of $V\beta 10^+CD4^+CD25^+$ splenocytes expressed Foxp3 (see Figure 3A). **B)** Proliferation of sorted 3B5.8 TCR Tg Teff cells, sorted $V\beta 10^+CD4^+CD25^+$ splenocytes from *Tcra*^{-/-} 3B5.8/hLa DT mice, or the indicated combinations, with irradiated T cell deficient APC in the presence or absence (No Ag) of hLa61-84 peptide. No reduction of 3B5.8 Teff cell proliferation is detectable upon co-culture with $V\beta 10^+CD4^+CD25^+$ splenocytes from *Tcra*^{-/-} DT mice.



Supplemental Figure 3 Legend. *H-2^{k/k} APCs present hLa more efficiently than H-2^{k/b} APCs.* **A)** Responsiveness of the 3B5.8 T cell hybridoma to hLa61-84 peptide and formaldehyde-fixed splenocytes of wild type B6 mice (H-2^{b/b}) or B6 mice congenic for one or two H-2^k alleles (H-2^{k/b} or H-2^{k/k}). IL-2 in 24h culture supernatants is measured by proliferation of the IL-2-dependent cell line HT-2. **B)** CFSE-labeled 3B5.8 TCR Tg splenic T cells from H-2^{k/k} mice were transferred by retroorbital injection into three hLa Tg H-2^{k/k}, three hLa Tg H-2^{k/b}, two hLa Tg H-2^{b/b}, and one Non-Tg H-2^{k/k} mice. CFSE dilution was analyzed 48 h post-transfer in splenic $\text{CD4}^+\text{V}\beta 10^+$ lymphocyte-gated cells. Representative CFSE dilution plots are shown for each group and mean \pm SD of % divided are reported. **C)** Surface I-E^k β chain histograms of splenic B cells from the recipient mice in B). Mean \pm SEM MFI values for each group are reported.