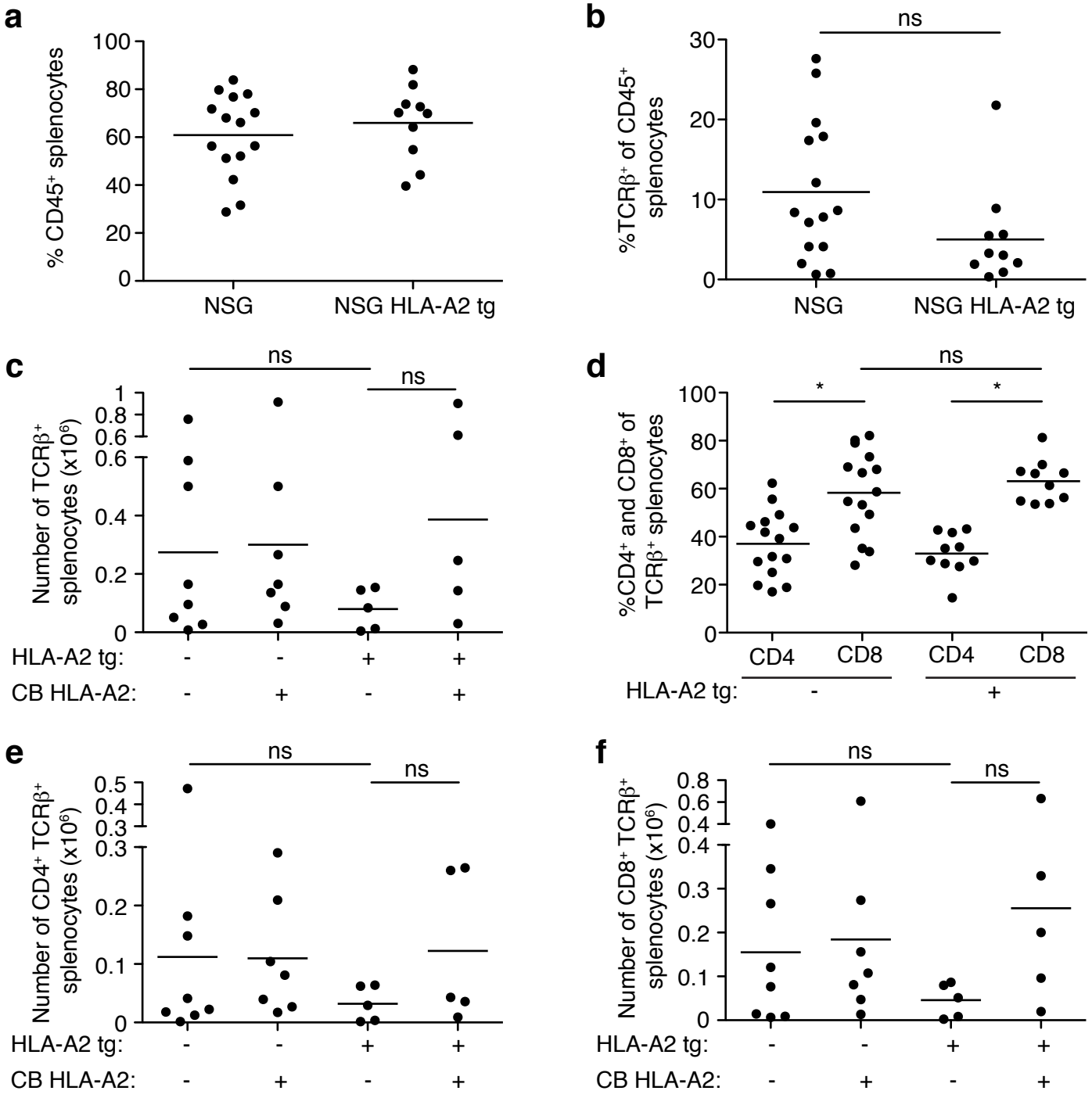
Supplementary video legends

Supplementary video 1: Cropped representative 2-photon imaging volume of human thymocytes contacting a hematopoietic-derived human DC within the thymus of a humanized mouse. Thymocytes (orange) and DC (turquoise) correlate with cells in Figure 4a.

Supplementary video 2: Cropped representative 2-photon imaging volume of human thymocyte-thymocyte contacts within the thymus of a humanized mouse. Thymocytes in orange and turquoise correlate with thymocytes in Figure 4c.

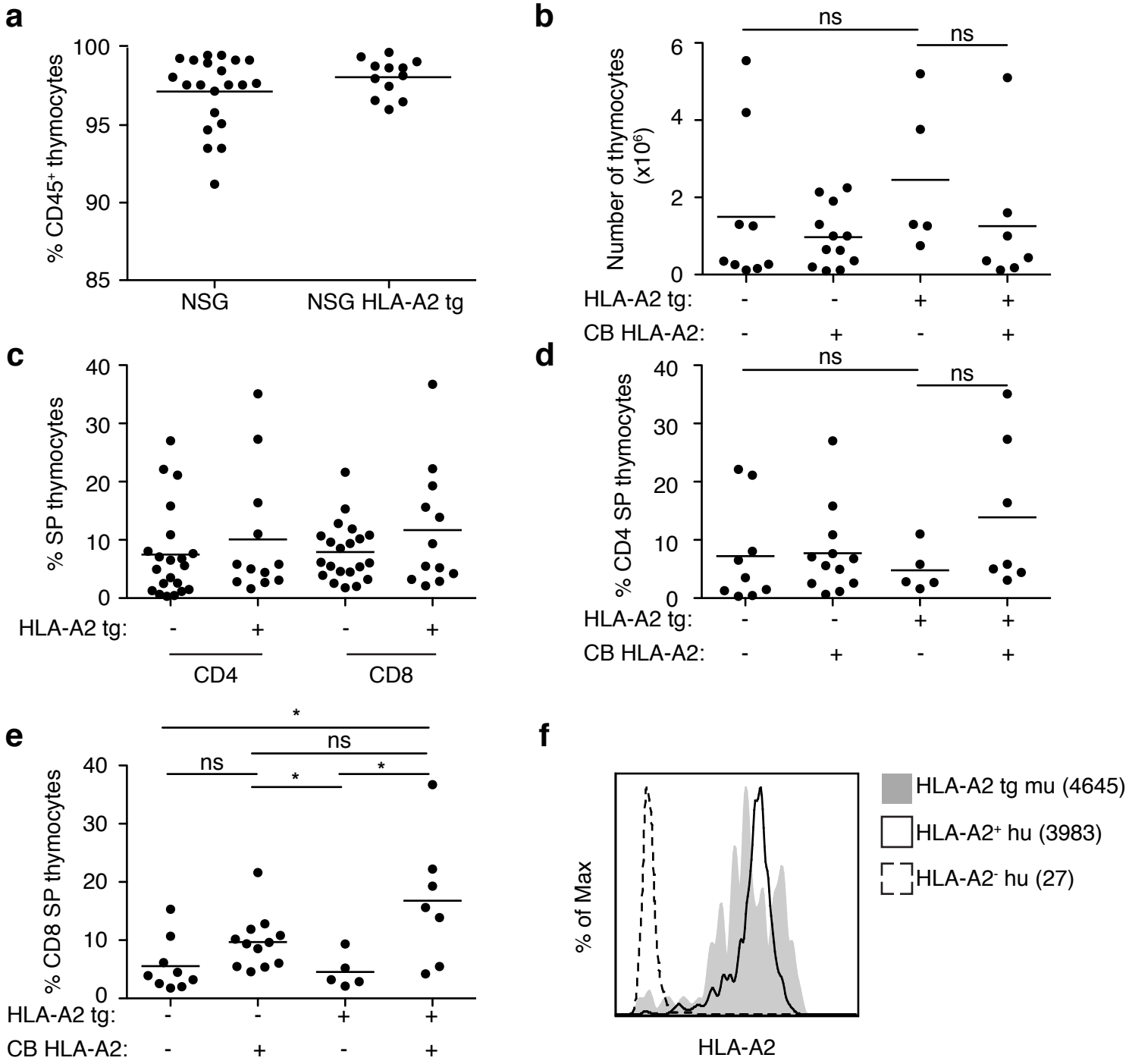
Supplementary video 3: Cropped representative 2-photon imaging volume of human thymocyte-thymocyte contacts within the thymus of a humanized mouse. Thymocytes in orange and turquoise correlate with thymocytes in Figure 4d.

Supplementary Figure 1



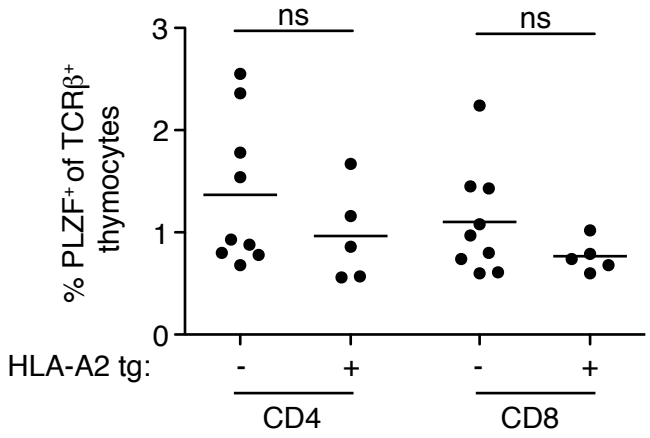
Supplementary Figure 1. Chimerism and T cell reconstitution in the spleen of humanized mice. (**a**) Proportion of human CD45$^+$ splenocytes in neonatal chimera NSG and NSG HLA-A2 tg mice. (**b**) Proportion of human TCRβ$^+$ splenocytes in neonatal chimera NSG and NSG HLA-A2 tg mice. p=0.097 (**c**) Number of human TCRβ$^+$ splenocytes in neonatal chimera NSG and NSG HLA-A2 tg mice by HLA-A2$^-$ or HLA-A2$^+$ CB donor. p=0.099 for NSG HLA-A2 tg mice reconstituted with HLA-A2$^-$ or HLA-A2$^+$ CB. (**d**) Proportion of human CD4$^+$ and CD8$^+$ TCRβ$^+$ T cells in the spleen of neonatal chimera NSG and NSG HLA-A2 tg mice. (**e**) Number of human CD4 TCRβ$^+$ splenocytes in neonatal chimera NSG and NSG HLA-A2 tg mice by HLA-A2$^-$ or HLA-A2$^+$ cord blood donor. p=0.164 between NSG HLA-A2 tg mice reconstituted with HLA-A2$^-$ or HLA-A2$^+$ CB. (**f**) Number of human CD8 TCRβ$^+$ splenocytes in neonatal chimera NSG and NSG HLA-A2 tg mice by HLA-A2$^-$ or HLA-A2$^+$ CB donor. p=0.09 for NSG HLA-A2 tg mice reconstituted with HLA-A2 positive or negative CB. Each dot represents one mouse. Line represents average. *=p<0.05. All statistical differences are marked with an *.

Supplementary Figure 2



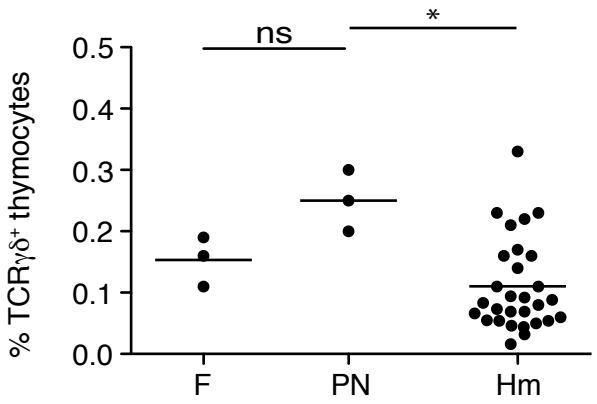
Supplementary Figure 2. Chimerism and reconstitution in the thymus of humanized mice. (**a**) Proportion of human CD45$^+$ thymocytes in neonatal chimera NSG and NSG HLA-A2 tg mice. (**b**) Number of human CD45$^+$ thymocytes in neonatal chimera NSG and NSG HLA-A2 tg mice by HLA-A2$^-$ or HLA-A2$^+$ CB donor. (**c**) Proportion of human CD4$^+$ and CD8$^+$ SP thymocytes in neonatal chimera NSG and NSG HLA-A2 tg mice. (**d**) Proportion of human CD4$^+$ SP thymocytes in neonatal chimera NSG and NSG HLA-A2 tg mice by HLA-A2$^-$ or HLA-A2$^+$ CB donor. (**e**) Proportion of human CD8$^+$ SP thymocytes in neonatal chimera NSG and NSG HLA-A2 tg mice by HLA-A2$^-$ or HLA-A2$^+$ CB donor. p=0.056 in NSG mice reconstituted with HLA-A2$^-$ or HLA-A2$^+$ CB.(**f**) Representative histogram of HLA-A2 expression on thymic epithelial cells of reconstituted NSG HLA-A2 tg mice (HLA-A2 tg mu thy), and HLA-A2$^+$ and HLA-A2$^-$ human fetal thymic samples (HLA-A2$^+$ hu thy, HLA-A2$^-$ hu thy). The numbers in parentheses indicate the mean fluorescence intensity. Each dot represents one mouse. Line represents average. ns, not statistically significant, * indicates $p< 0.05$.

Supplementary Figure 3



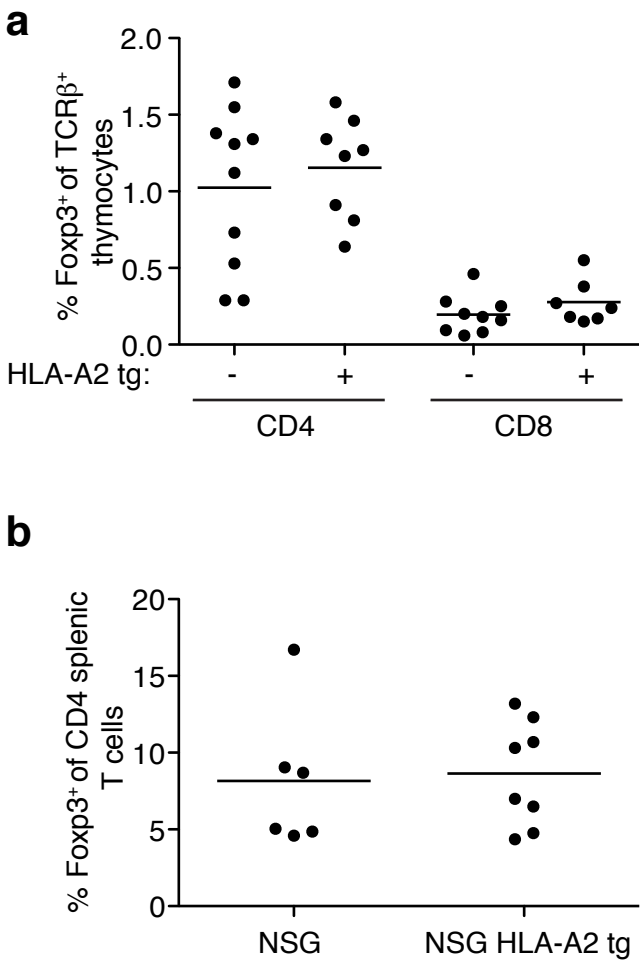
Supplementary Figure 3. An HLA-A2 tg does not appear to affect PLZF$^+$ T cell development in humanized mice. Proportion of PLZF$^+$ cells of CD4$^+$ and CD8$^+$ TCRβ$^+$ thymocytes in neonatal chimera NSG and NSG HLA-A2 tg humanized mice thymic samples. p=0.286 for CD4$^+$, and p=0.1989 for CD8$^+$ thymocytes. Each dot represents one mouse. Line represents average. ns, not statistically significant.

Supplementary Figure 4



Supplementary Figure 4. Proportion of TCRγδ+ thymocytes in human fetal (F), postnatal (PN), and neonatal chimera NSG humanized mice (Hm) thymic samples. Each dot represents an individual thymus sample. Line represents average. ns, not statistically significant, * indicates $p<0.05$.

Supplementary Figure 5



Supplementary Figure 5. An HLA-A2 transgene does not preferencially support Foxp3$^+$ T cell development in neonatal chimera NSG mice. (**a**) Proportion of Foxp3$^+$ cells among CD4$^+$ or CD8$^+$ SP thymocytes in neonatal chimera NSG and NSG HLA-A2 tg humanized mice. (**b**) Proportion of Foxp3$^+$ cells among CD4$^+$ TCRβ$^+$ splenocytes in neonatal chimera NSG and NSG HLA-A2 tg humanized mice. Each dot represents one mouse. Line represents average.