SUPPLEMENTAL MATERIAL

Chen et al., https://doi.org/10.1084/jem.20161784

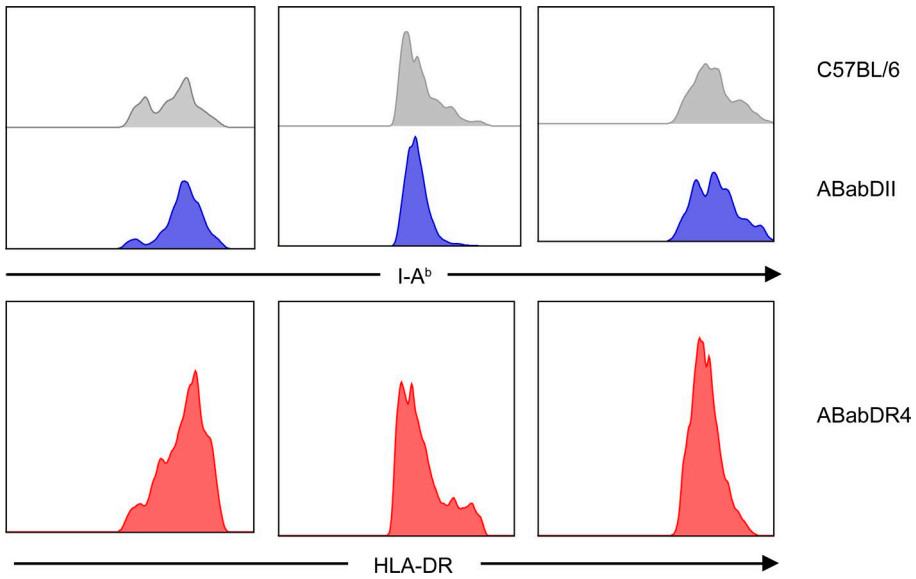


Figure S1. **Representative plots of surface MHC II staining on different thymic APC cell types.** One representative staining from two independent experiments. Each experiment includes 2–3 C57BL/6, ABabDII, and ABabDR4 mice. cTEC, cortical thymic epithelial cells; mTEC, medullary thymic epithelial cells; tDCs, thymic DCs.

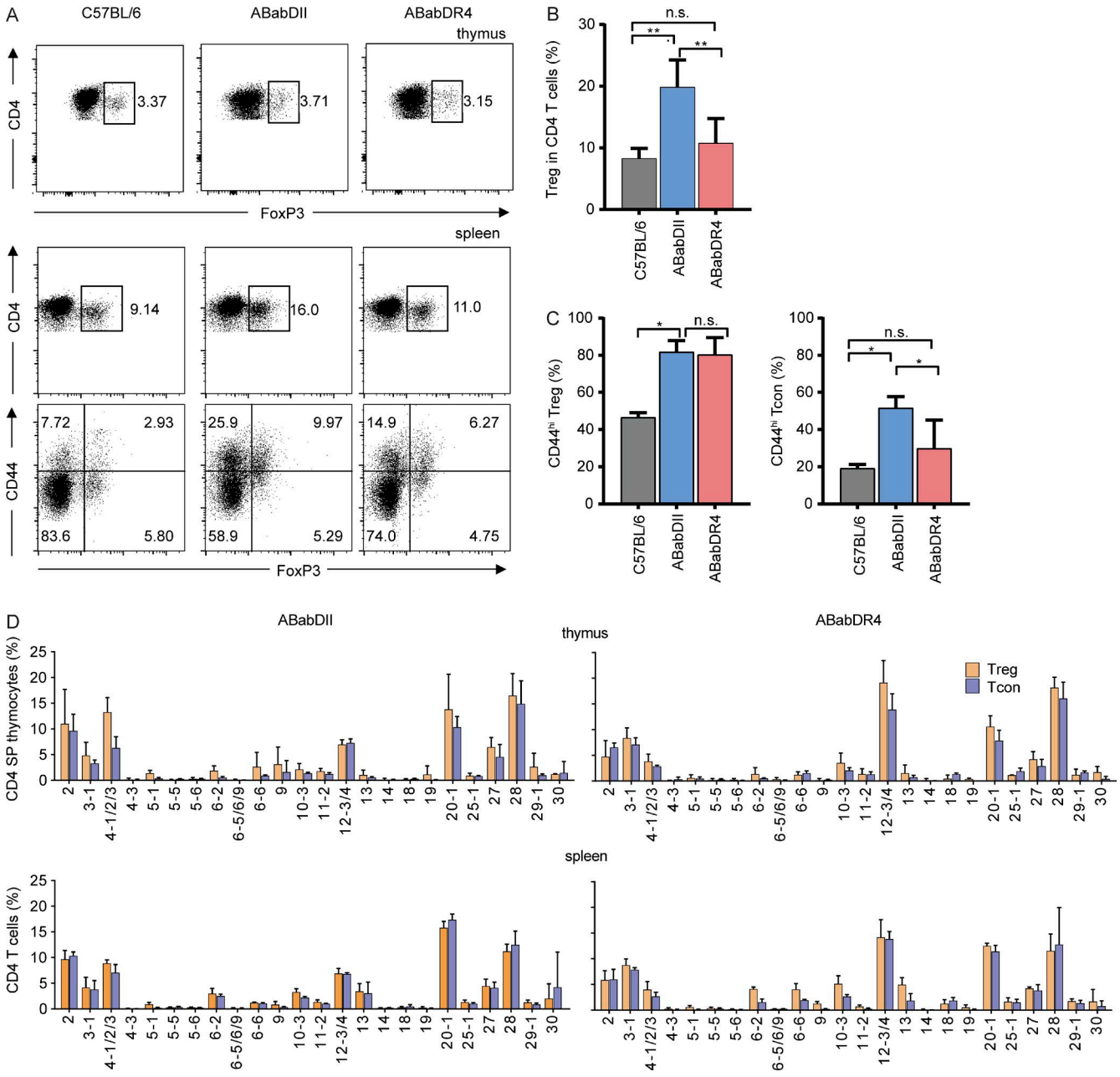


Figure S2. **FoxP3 staining, frequencies, and CD44 frequencies within Treg and Tcon populations.** (A, top) Representative plots of FoxP3 staining of thymocytes gated on CD4 single-positive cells. (Middle) FoxP3 staining of CD4 T cells from spleen. Gated on $CD3^+CD4^+$ cells. (Bottom) CD44 expression of Tcon ($FoxP3^-$) and Treg ($FoxP3^+$) from spleen; gated, $CD3^+CD4^+$. (B) Frequencies of Treg cells in CD4 T cells of C57BL/6 ($n = 4$), ABabDII ($n = 9$), and ABabDR4 ($n = 9$) mice. (C) Frequencies of $CD44^{hi}$-expressing cells within Treg (left) or Tcon (right) populations of C57BL/6 ($n = 3$), ABabDII ($n = 6$), and ABabDR4 ($n = 6$) mice. **, $0.001 \leq P < 0.05$; *, $0.05 \leq P < 0.1$; n.s., not significant (Mann–Whitney test, two-tailed). (D) The frequencies of Vβ usage of CD4 single-positive thymocytes or spleen CD4 T cells of ABabDII and ABabDR4 mice, based on staining with human Vβ-specific antibodies. Pooled samples from 3–4 mice were used for each stain. Summarized data from three independent experiments. Data are shown as means ± SD in B–D. Fig. 1 contains additional information.

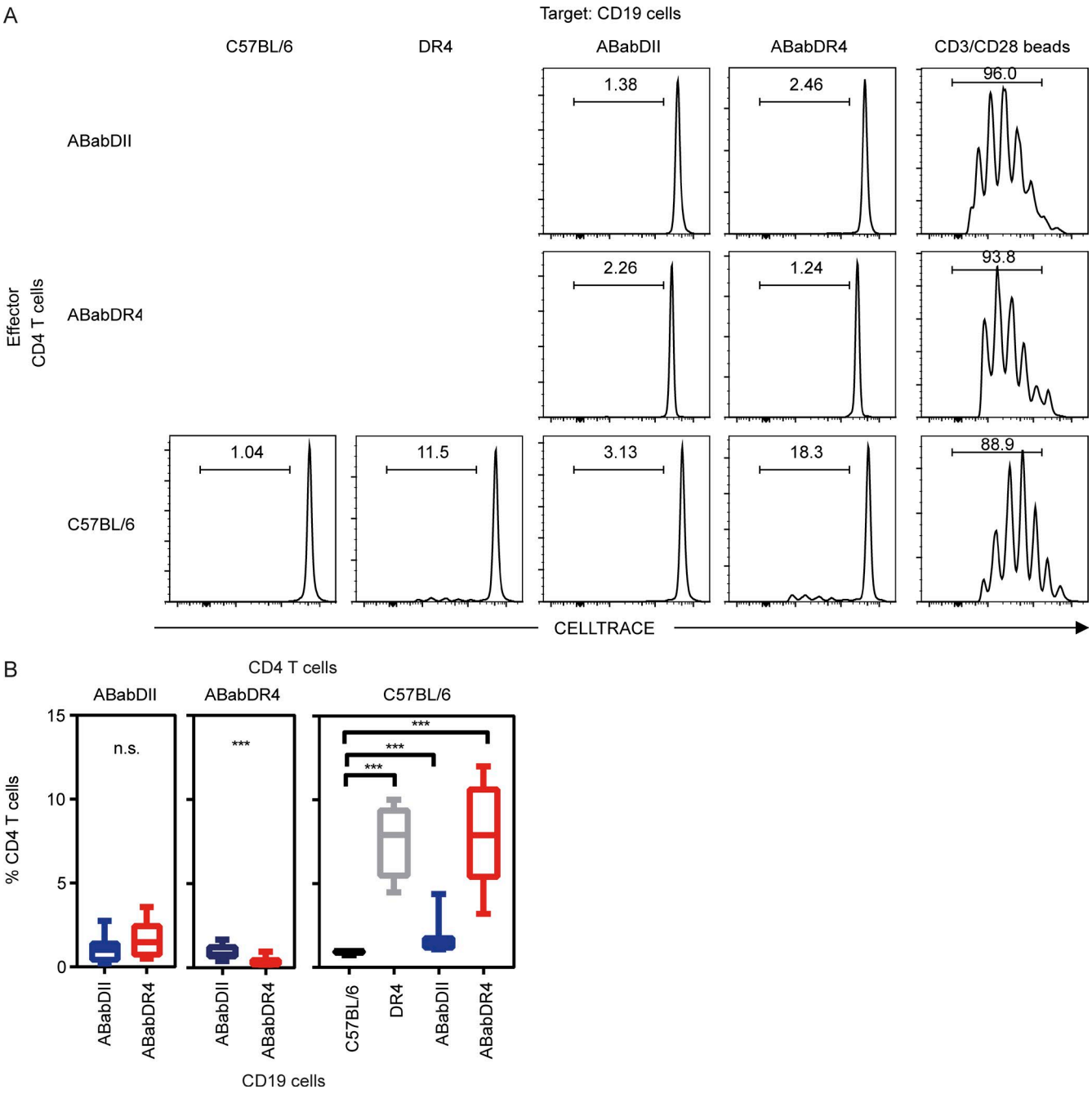


Figure S3. **CD4 T cell proliferation upon MMTV superantigen-positive CD19⁺ cell stimulation.** (A) Representative proliferation plots of CD4 T cells from ABabDII, ABabDR4, and C57BL/6 mice co-cultured for 84 h with CD19⁺ cells from indicated mouse strains or CD3/CD28 beads. (B) Summarized data of the proportions of proliferated CD4 T cells from ABabDII ($n = 6$), ABabDR4 ($n = 6$), and C57BL/6 ($n = 6$) mice co-cultured with CD19⁺ cells from the indicated mouse strains, shown as mean ± SD. n.s., not significant; ***, $P < 0.001$ (Mann–Whitney test, two-tailed).

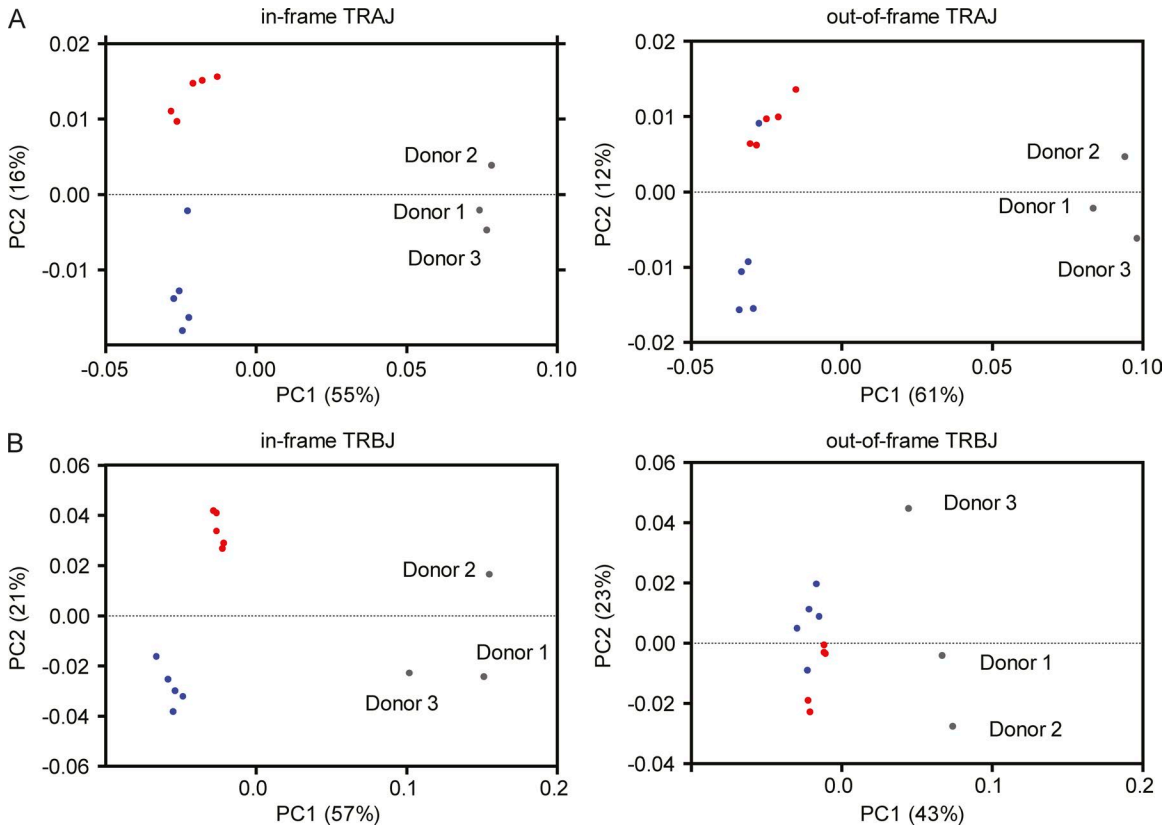


Figure S4. **PCA analysis of J gene usages correlations between mice and humans.** PCA shows the correlation of TRAJ (A) or TRBJ (B) gene usages between ABabDII (blue), ABabDR4 (red), and human donors (gray), related to Fig. 5. (Left) PCA of the in-frame J usages. (Right) PCA of out-of-frame clonotypes. Proportions of variance (PC1 and PC2) are indicated at the axis. Data are from ABabDII mice ($n$ = 5), ABabDR4 mice ($n$ = 5), and humans ($n$ = 3). Further information in Fig. 5 for PCA analysis of V gene usages and V–J pairings.

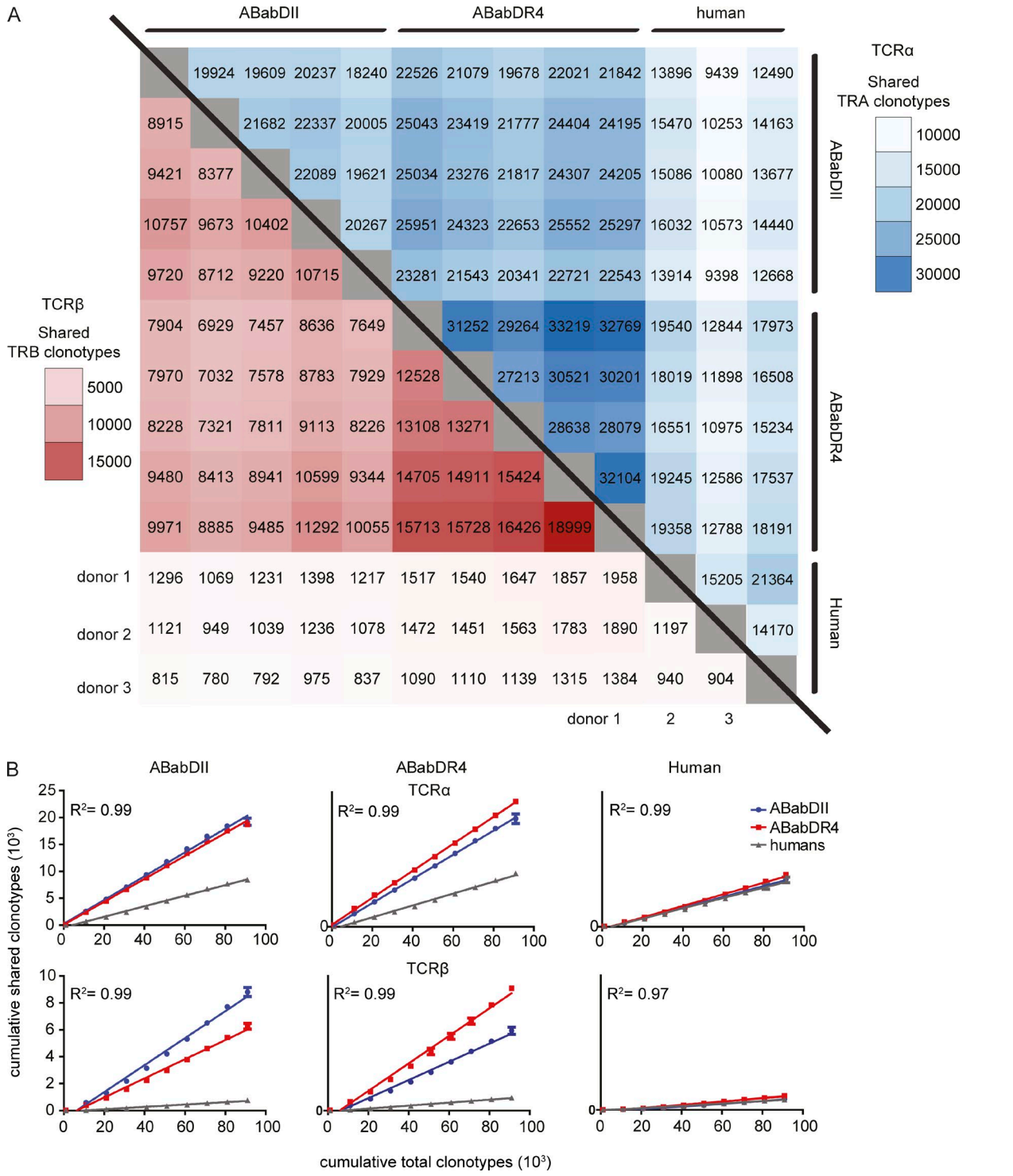


Figure S5. **Shared TCR clonotypes.** (A) Absolute numbers of shared TCR amino acid in-frame clonotypes between any two individuals from 1.2 µg genomic DNA of purified, naive CD4 T cells submitted for sequencing. (Triangle upper right, blue) TCRα. (Triangle lower left, red) TCRβ. (B) Shared TCRα (upper) and TCRβ (bottom) amino acid in-frame clonotypes. The numbers of shared clonotypes from the most- to the least-abundant clonotypes between any two individuals were calculated. Each plot shows a representative individual from each group (ABabDII mice, ABabDR4 mice, or human) and its shared clonotype analysis to other samples. Averaged cumulative shared clonotypes of the samples from the same group were used for plotting. Linear regression was assumed for all analysis, and $R^2$ is shown for each analysis. Data are from ABabDII mice ($n$ = 5); ABabDR4 mice ($n$ = 5), and humans ($n$ = 3). Additional information is available in Fig. 8.