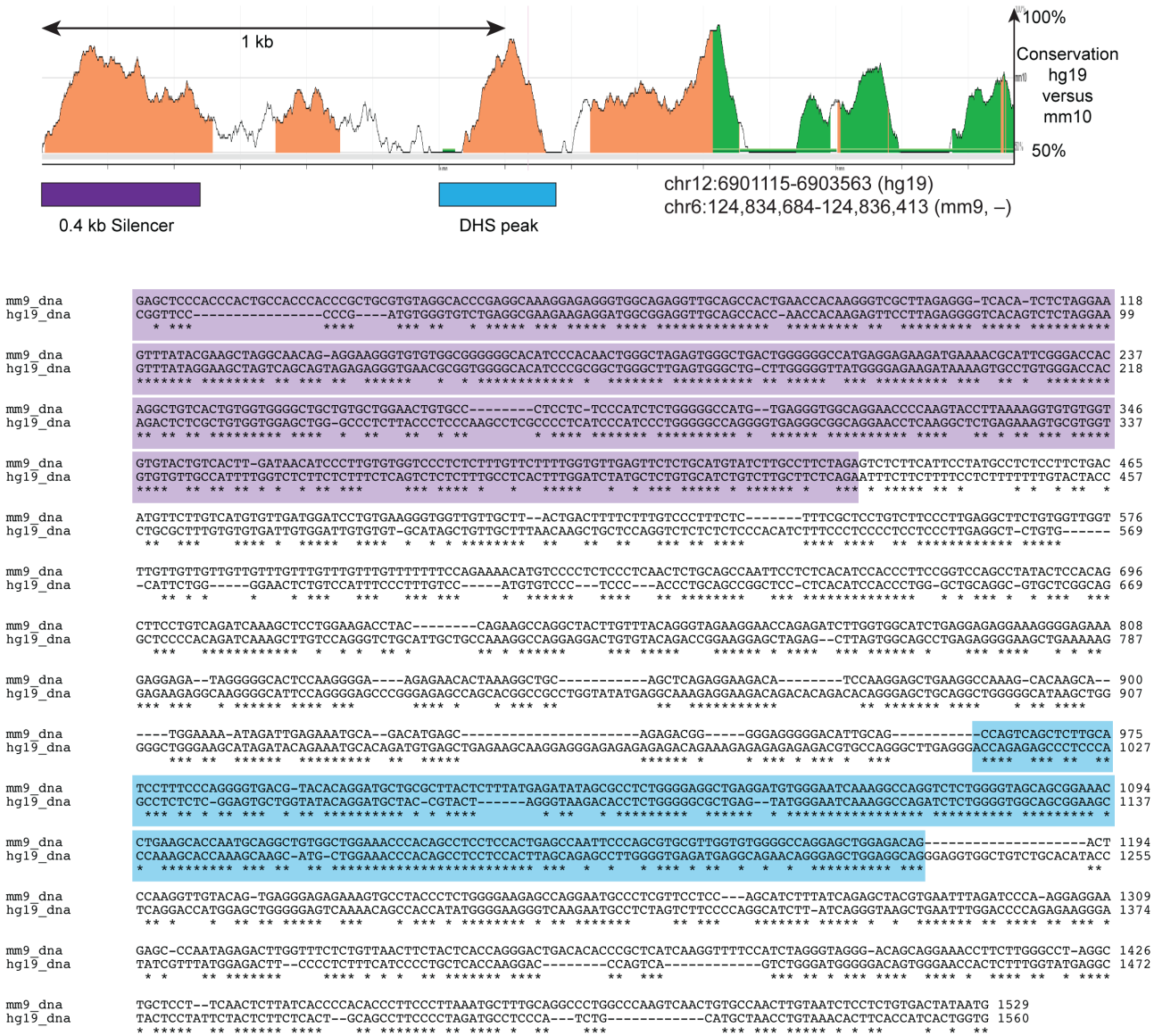
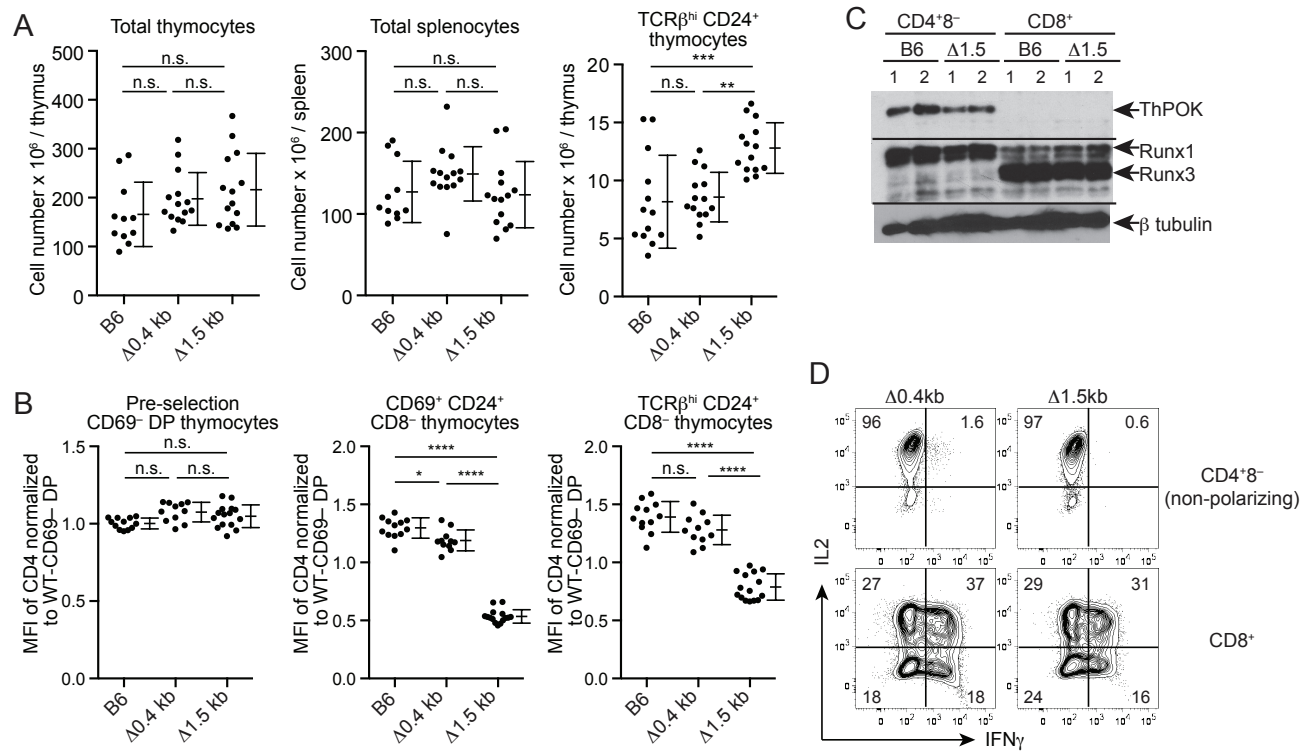
Supplementary Figure 1



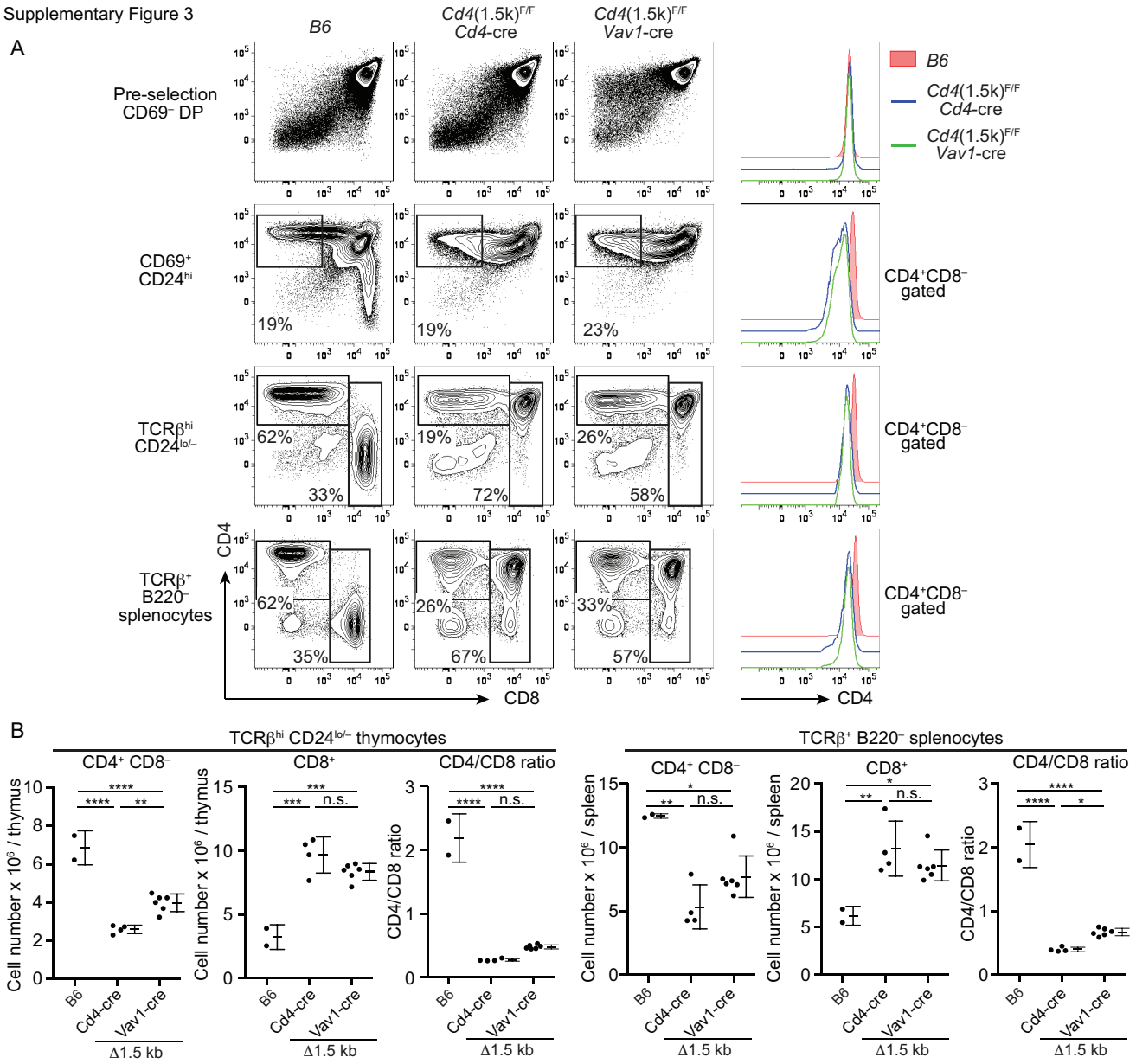
Supplementary Fig. 1. Sequence conservation of the *Cd4* silencer and DHS+3 between human and mouse genomes.

Sequence conservation analysis of the 1.5 kb sequence encompassing the *Cd4* silencer and DHS+3 between human and mouse genomes is shown as histogram and an alignment at the nucleotide level. Regions conserved >60% are shown in orange and repetitive sequences are shown in green as default in the ECR browser (http://ecrbrowser.dcode.org/). Sequence alignment at the nucleotide level is shown in the lower panel. The 0.4 kb silencer and the peak of DHS+3 shown in Fig. 1 are marked in purple and blue, respectively.

Supplementary Figure 2



Supplementary Fig. 2. Analysis of T cell development in *Cd4*(0.4k)$^{-/-}$ and *Cd4*(1.5k)$^{F/F}$;Cd4-cre mice. (A) Statistical analysis of numbers of total thymocytes, total splenocytes, and TCRβ^{hi} CD24$^{lo/-}$ mature thymocytes in B6, *Cd4*(0.4k)$^{-/-}$ (Δ0.4kb) and *Cd4*(1.5k)$^{F/F}$;*Cd4*-cre (Δ1.5kb) mice. (B) Statistical analysis of mean fluorescence intensities (MFI) of CD4 expression in pre-selection CD69^{-} DP thymocytes, CD69^{+} CD24^{+} CD8^{-} post-selection thymocytes, and TCRβ^{hi} CD24$^{lo/-}$ CD8^{-} mature thymocytes. Data are shown as means and SD and statistical differences were tested by one-way ANOVA. N=9-15. (C) Expression of ThPOK, Runx1 and Runx3 proteins in CD4^{+} CD8^{-} and CD8^{+} T cells from Δ1.5kb and control B6 mice. Whole cell extract of two independent sets of CD4^{+} CD8^{-} and CD8^{+} T cells from Δ1.5kb and control B6 mice was separated by SDS-PAGE and subjected to immunoblotting. Anti-β tubulin antibody was used to measure loading. (D) IL-2 and IFNγ expression in CD4^{+} CD8^{-} and CD8^{+} T cells cultured under non-polarizing conditions. Percentages of IL-2^{+} and IFNγ^{+} cells are shown. Data represent three experiments with similar results.



Supplementary Fig.3. Comparison of CD4 expression levels and T cell development in *Cd4*(1.5k)$^{F/F}$;*Cd4*-cre mice and *Cd4*(1.5k)$^{F/F}$;*Vav1*-cre mice.

(A) CD4 and CD8 expression in developing T cells from age-matched B6, *Cd4*(1.5k)$^{F/F}$;*Cd4*-cre mice and *Cd4*(1.5k)$^{F/F}$;*Vav1*-cre mice was analyzed as in Fig. 2. Percentages of gated populations in representative samples are shown. (B) Statistical analysis of numbers of CD4^{+} CD8$^{–}$ and CD8^{+} mature thymocytes, and CD4^{+} CD8$^{–}$ and CD8^{+} T cells in the spleen and CD4, and CD4/CD8 ratios. N=2 (B6), 4 (*Cd4*-cre), and 6 (*Vav1*-cre). Statistical differences were tested by one-way ANOVA.