Supplementary Figure 1



mm9_dna hg19_dna	GAGCTCCCACCCACCCACCCACCCGCTGCGTGTAGGCACCGAGGCAAAGGAGAGGGTGGCAGAGGGTGGCACAGGCCACTGAACCACAAGGGTCGCTTAGAGGG-TCACA-TCTCTAGGAA CGGTTCCCCCGATGTGGGTGTCTGAGGCGAAGAAGAGGATGGCGGAGGTTGCAGCCACCAAAGAGTTCCTTAGAGGGGTCACAGTCTCTAGGAA * *** *** *** *** **** ***** ***** **** ****	118 99
mm9_dna hg19_dna	GTTTATACGAAGCTAGGCAACAG-AGGAAGGGTGTGTGGGGGGGGGCACATCCCACAACTGGGCTAGAGTGGGCTGACTGGGGGGCCATGAGAAGAAGATGAAAACGCATTCGGGACAC GTTTATAGGAAGCTAGTCAGCAGTAGAGAGGGTGAACGCGGTGGGGGGCACATCCCGCGGCTGGGCTTGAGTGGGGTG-CTTGGGGGTTATGGGGAGAAAAAAGTGCCTGTGGGGACCAC ****** ******* ** *** *** *** *** ******	237 218
mm9_dna hg19_dna	AGGCTGTCACTGTGGGGGCTGCTGTGCTGGGACTGTGCCCTCCTC-TCCCATCCTTGGGGGCCATGTGAGGGTGGCAGGAACCCCAAGTACCTTAAAAGGTGTGGGG AGACTCTCGCTGTGGGGGGCGGGGGGGGGG	346 337
mm9_dna hg19_dna	GTGTACTGTCACTT-GATAACATCCCTTGTGTGGGGCCCCTCCTTTGGTTCTTTGGTGTTGAGTTCTCGCGATGTATCTTGCCTTCTAGAGTCTTCATTCCTATGCCTCTCCTCTGGCATCTGCCTTCTGGAGTCTTTGGTGTGCCTCTTCTCTTTTCTCTCTTTTTTGGACTACC GTGTGTGTGCCATTTGGCTCTCTCTCTCTCTCTCAGTCTCTCTC	465 457
mm9_dna hg19_dna	ATGTTCTTGTCATGTGTTGATGGATCCTGTGAAGGGTGGTTGTTGTGTTTACTGACTTTTCTTTGTCCCTTTCTCTTTCGCTCCTGTGTCTTCCCTTGAGGCTTCTGTGGTGGT CTGCGCTTTGTGTGTGTGTGTGTG	576 569
mm9_dna hg19_dna	TTGTTGTTGTTGTTGTTGTTTGTTTGTTTGTTTGTTTG	696 669
mm9_dna hg19_dna	CTTCCTGTCAGATCAAAGCTCCTGGAAGACCTACCAGAAGCCAGGCTACTGTTTACAGGGTAGAAGGAACCAGAGATCTTGGTGGCATCTGAGGGAGG	808 787
mm9_dna hg19_dna	GAGGAGATAGGGGGCACTCCAAGGGGAAGAGAACACTAAAGGCTGCAGCTCAGAGGAAGACATCCAAGGAGCTGAAGGCCAAAG-CACAAGCA- GAGAAGAGGCAAGGGGCATTCCAGGGGGACCCGGGGAGAGCCAGCACGCCGCCGCGCGGGCTATATGAGGCAAAGAGGAAGACAGAC	900 907
mm9_dna hg19_dna	TGGAAAA-ATAGATTGAGAAATGCAGACATGAGCACAGCACGGGGACACGGGGGAGGGGGACATTGCAG	975 1027
mm9_dna hg19_dna	TCCTTTCCCAGGGGTGACG-TACACAGGATGCTGCGCTTACTCTTTATGAGATATAGCGCCTCTGGGGAGGCTGAGGAATGTAGGGAATCAAAGGCCAGGGTCTCTGGGGGTGGCAGCGGAAAC GCCTCTCTC-GGAGTGCTGGTATACAGGATGCTAC-CGTACTAGGGTAAGACACCTCTGGGGGCGCTGAGTATGGGAATCAAAGGCCAGATCTCTGGGGGTGGCAGCGGAAGC *** ** ** *** *** *** *** ***********	1094 1137
mm9_dna hg19_dna	CTGAAGCACCAATGCAGGCTGTGGGCTGGAAACCCACAGCCTCCTCCCACTGAGCCAATTCCCAGCGTGGGTGG	1194 1255
mm9_dna hg19_dna	CCAAGGTTGTACAG-TGAGGGAGAAAAGTGCCTACCCTCTGGGGAAGAGCCAGGAATGCCCTCGTTCCTCCAGCATCTTTATCAGAGCTACGTGAATTTAGATCCCA-AGGAGGAA TCAGGACCATGGAGCTGGGGGGGGTCAAAACAGCCACCATATGGGGAAGGGTCAAGAATGCCTCTAGTCTTCCCCAGGCATCTT-ATCAGGGGAAGCTGAATTTGGACCCCCAGGAAAGGGA ** * * * * * * * * * * * * * * * * * *	1309 1374
mm9_dna hg19_dna	GAGC-CCAATAGAGACTTGGTTTGCTGTTAACTTCTACTCACCAGGGACTGACAACACCCGCTCATCAAGGTTTTCCATCTAGGGTAGGG-ACAGCAGGAAACCTTCTTGGGGCT-AGGC TATCGTTTATGGAGACTTCCCCTCTTTCATCCACCCAGGGACACCAGGGACACTCCTCTGGCACCAGGAAACCACCTCTTTGGTATGAGGC * * ** ******* **** *** ************	1426 1472
mm9_dna hg19_dna	TGCTCCTTCAACTCTTATCACCCCACACCCTTCCCTTAAATGCTTTGCAGGCCCAGGCCCAAGTCGACCTGTGCCAACTTGTAATCTCCTCTGTGACTATAATG 1529 TACTCCTATTCTACTCTTCTCACACTGCAGCCTTCCCCTAGATGCCTCCCATCTG	

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, $Cd4(0.4k)^{-/-}$ ($\Delta 0.4kb$) and $Cd4(1.5k)^{F/F}$;Cd4-cre ($\Delta 1.5kb$) 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 $\Delta 1.5kb$ and control B6 mice. Whole cell extract of two independent sets of CD4⁺ CD8⁻ and CD8⁺ T cells from $\Delta 1.5kb$ 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 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.