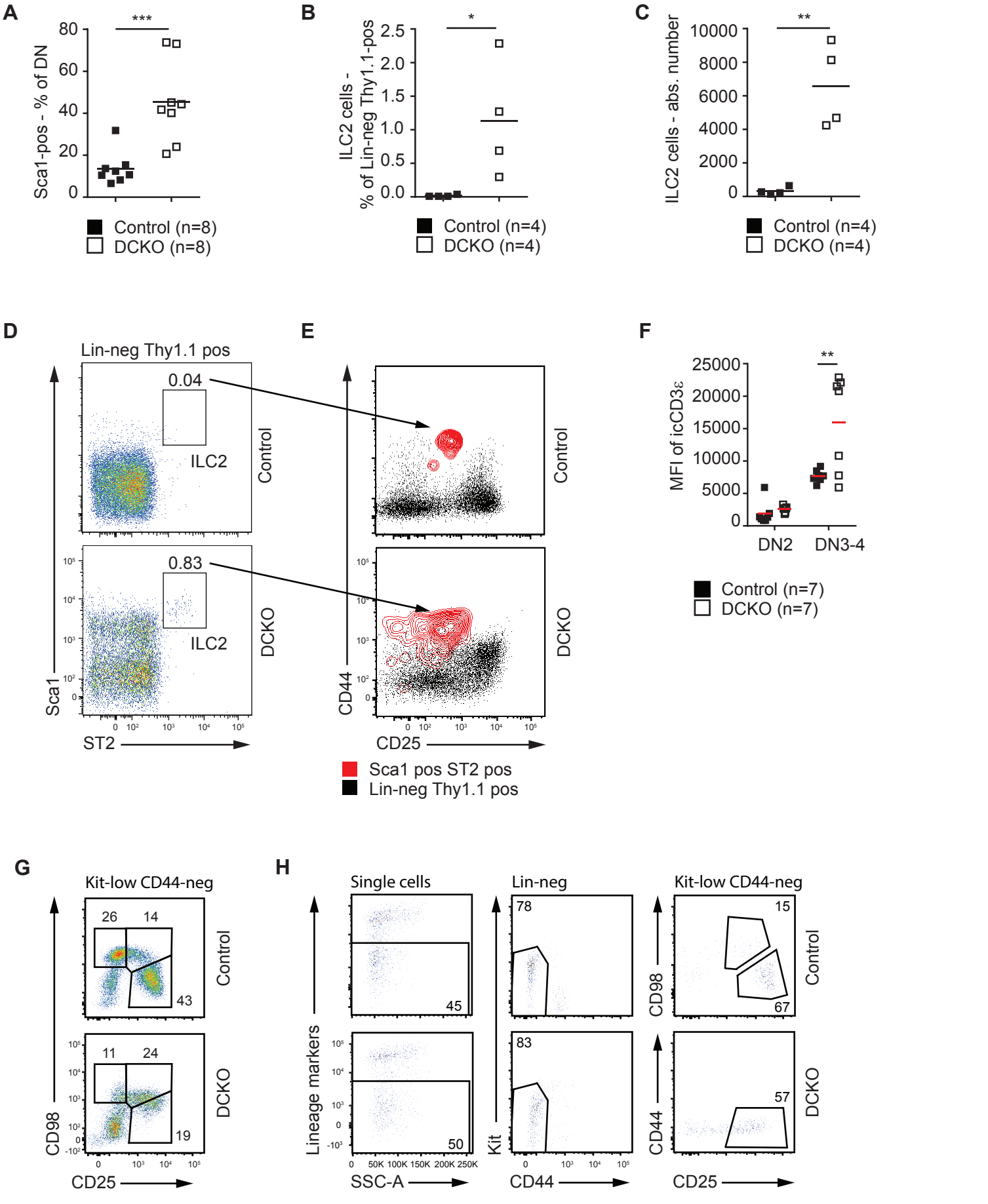


Vogel, Bell Figure S1

Figure S1: Aberrant phenotype of *Zfp36l1/l2*-deficient mice is not due to increased ILC2 numbers

(A) Percentage of Sca1^{+} DN (CD4^{-}, CD8^{-}) cells from control (*Zfp36l1/l2*$^{fl/fl}$) and *Zfp36l1/l2*$^{fl/fl}$*; CD2Cre* (DCKO) mice.

(B) Innate lymphocyte 2 (ILC2) cells depicted as percentage of Lin^{-}, Thy1^{+} cells from control and DCKO mice.

(C) Absolute numbers of ILC2 cells as in (B).

(D) Representative FACS plots showing ILC2 cells (Lin^{-}, Thy1^{+}, Sca1^{high}, ST2^{+}) from control and DCKO mice.

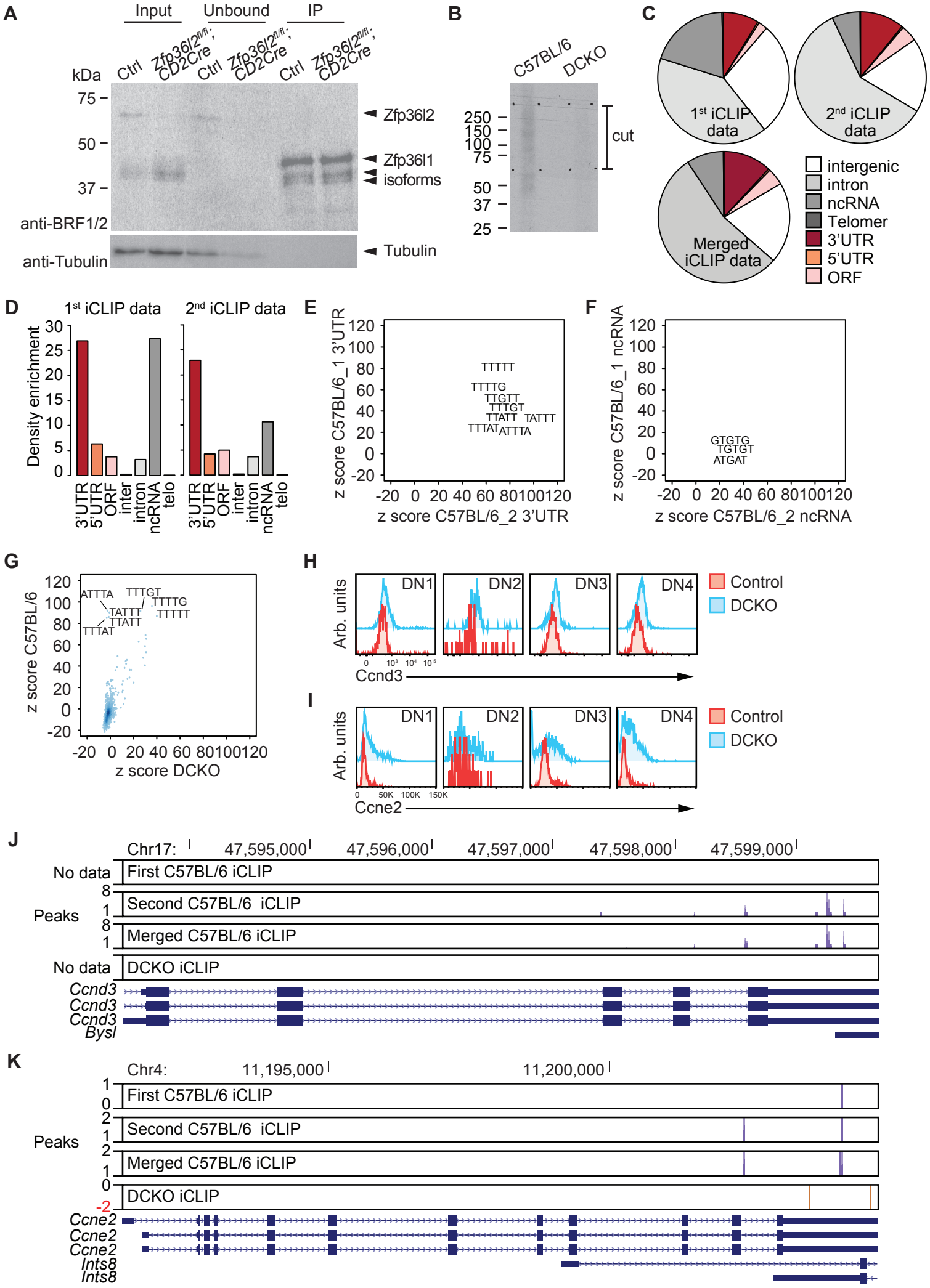
(E) Representative FACS plot overlays of Lin^{-}, Thy1^{+} cells (black) with ILC2 cells (red) from control and DCKO mice.

(F) Median fluorescence intensity (MFI) of intracellular CD3-epsilon (icCD3ε) in DN2 and DN3-4 cells as gated in **Figure 1C**.

(G) Representative FACS plots showing CD98 and CD25 expression in Kit^{low}, CD44^{-} DN thymocytes from control and DCKO mice.

(H) Representative FACS plots showing the gating strategy for sorting control DN3a (Lin^{-}, Kit^{low}, CD44^{-}, CD25^{high}, CD98^{low}), DN3b (Lin^{-}, Kit^{low}, CD44^{-}, CD25^{int}, CD98^{high}) and DN3 cells (Lin^{-}, Kit^{low}, CD44^{-}, CD25^{+}.) from DCKO mice. Samples have been partially depleted for Lin^{+} cells before sorting. Data is representative for 4 biological replicates per genotype.

Data is collated from 2 (A), 1 (B, C) and 2 (F) independent experiments with 8 (A), 4 (B, C) and 7 (F) mice per genotype. Horizontal line represents mean with statistical significances calculated by unpaired T test (A-C) or 2way ANOVA with Sidak's multiple comparisons test (F) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).



Vogel, Bell Figure S2

Figure S2: The iCLIP is specific for Zfp36l1 in thymocytes

(A) Immunoprecipitation (IP) of protein extracts from control (Ctrl) or *Zfp36l2*-deficient (*Zfp36l2*$^{fl/fl}$*; CD2Cre*) thymocytes with magnetic beads coupled to polyclonal anti-Zfp36l1/l2 antibodies (BRF1/2) followed by immunoblot analysis with BRF1/2 or Tubulin as loading control. Input, unbound fraction and IP of two biological replicates are depicted.

(B) Autoradiograph of precipitated Zfp36l1/RNA complexes in thymocytes from C57BL/6 and DCKO mice.

(C) Percentage of iCLIP hits in gene features of two individual biological C57BL/6 replicates and the merged data set.

(D) Density enrichment of iCLIP hits, normalized to gene feature length for each individual replicate. Y axis represents percentage of enrichment.

(E) K-mer analysis of the binding motifs in the 3'untranslated region (3'UTR), ranked according to z scores of two biological iCLIP replicates from C57BL/6 thymocytes.

(F) K-mer analysis of the binding motifs in non-coding RNA (ncRNA) as in (G).

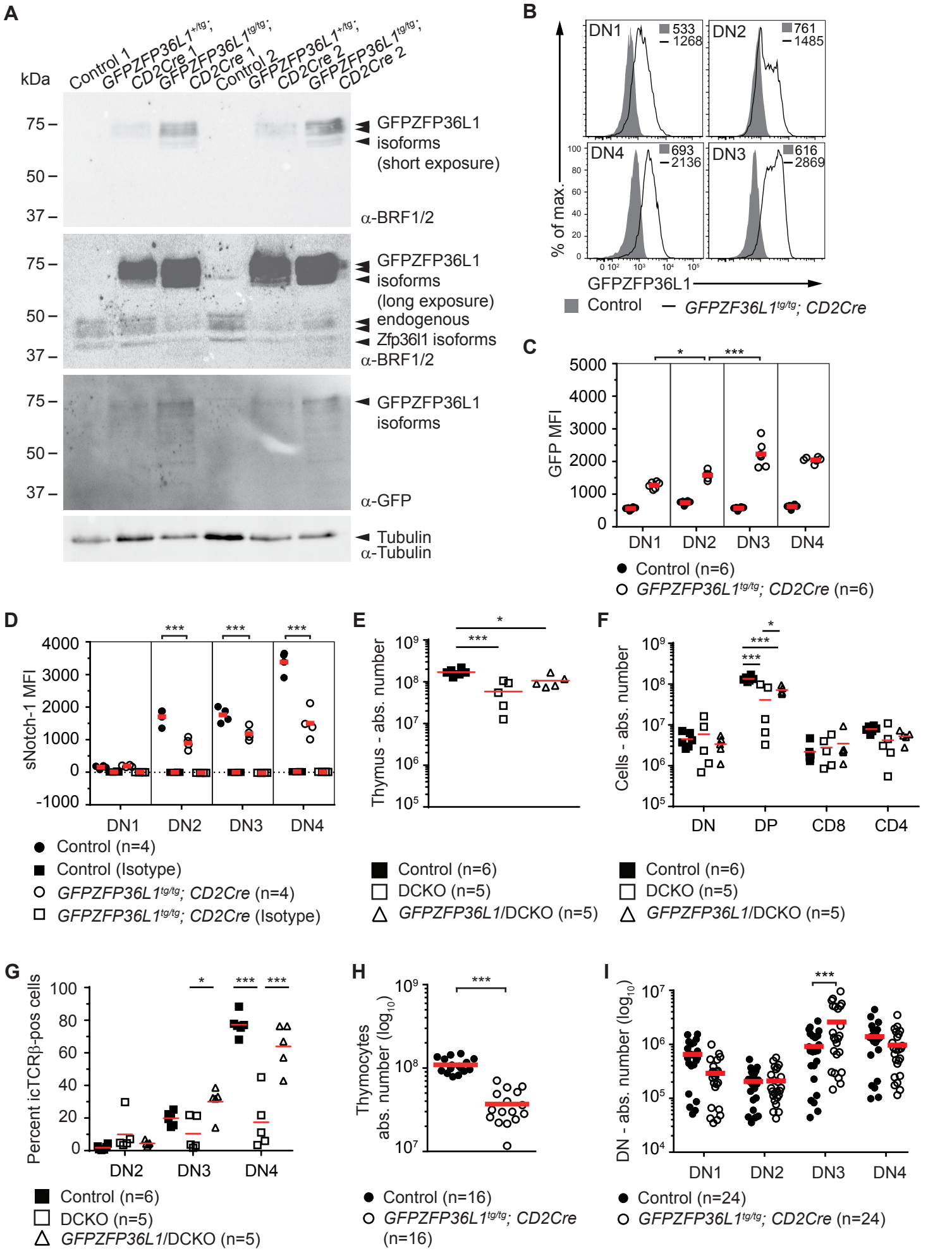
(G) K-mer analysis of the binding motifs in all gene features, ranked according to z scores of C57BL/6 and *Zfp36l1/l2*$^{fl/fl}$*; CD2Cre* (DCKO) thymocytes.

(H) Representative FACS plot overlays of DN subsets from control and DCKO mice, showing Ccnd3 expression.

(I) Representative FACS plot overlays of DN subsets from control and DCKO mice, showing Ccne2 expression.

(J) Genome tracks of Zfp36l1 binding sites in the *Ccnd3* gene. Depicted are binding peaks of the individual C57BL/6, the merged C57BL/6 and the DCKO iCLIP. Chromosomal location is indicated on the top and genome features of *Ccnd3* isoforms and the overlapping gene *BysI* are shown on the bottom. The blue boxes depict exons connected by a line of arrows which are introns. Narrow boxes indicated 5' or 3' untranslated regions. Binding of Zfp36l1 mainly takes place in the 3'UTR.

(K) Genome tracks for Zfp26l1 binding site in the *Ccne2* gene as described in (J).



Vogel, Bell Figure S3

Figure S3: Validation of GFPZFP36L1 transgenic mice

(A) Western blot analysis showing GFPZFP36L1 expression in total thymocyte lysates of cells from two control (*GFPZFP36L1*$^{tg/tg}$), *GFPZFP36L1*$^{+/tg}$*; CD2Cre* and *GFPZFP36L1*$^{tg/tg}$*; CD2Cre* mice each. The blot has been probed with antibodies detecting endogenous and GFPZFP36L1 protein, GFP and Tubulin as a loading control.

(B) Representative FACS plot overlays showing GFPZFP36L1 expression in DN subsets from control and *GFPZFP36L1*$^{tg/tg}$*; CD2Cre* mice. Inset numbers are median fluorescence intensity (MFI) of the indicated histograms.

(C) MFI of GFPZFP36L1 in DN subsets. Data is from 1 experiment with 6 mice per genotype. Horizontal line represents the mean.

(D) MFI of surface Notch1 (sNotch1) or isotype staining in DN subsets. Data is from 1 experiment with 4 biological replicates each. Horizontal line represents the mean.

(E) Total number of thymocytes from control (*Zfp36l1/l2*$^{fl/fl}$), *Zfp36l1/l2*$^{fl/fl}$*; CD2Cre* (DCKO) or *Zfp36l1/l2*$^{fl/fl}$*; GFPZFP36L1*$^{+/tg}$*; CD2Cre* (*GFPZFP36L1*/DCKO) mice.

(F) Absolute cell number of DN, DP, CD4 and CD8 thymocyte subsets from control, DCKO or *GFPZFP36L1*/DCKO mice.

(G) Percentage of cells positive for intracellular TCRβ (icTCRβ) in DN2, DN3 and DN4 subsets from control, DCKO or *GFPZFP36L1*/DCKO mice.

(H) Total number of thymocytes from control and *GFPZFP36L1*$^{tg/tg}$*; CD2Cre* mice.

(I) Absolute cell numbers of DN subsets from control and *GFPZFP36L1*$^{tg/tg}$*; CD2Cre* mice.

Data is from 1 (E, F, G) experiment with 5-6 mice per genotype or collated from 3 (H) or 5 (I) independent experiments with 16 (H) or 24 (I) mice per genotype. Horizontal line represents the mean and statistical significances were calculated by 1way ANOVA with Tukey's multiple comparisons test (E), by 2way ANOVA with Holm-Sidak's multiple comparisons test (A, B, C, D, G, I) or unpaired, two-tailed T test (H) (*$p < 0.05$, ***$p < 0.001$).