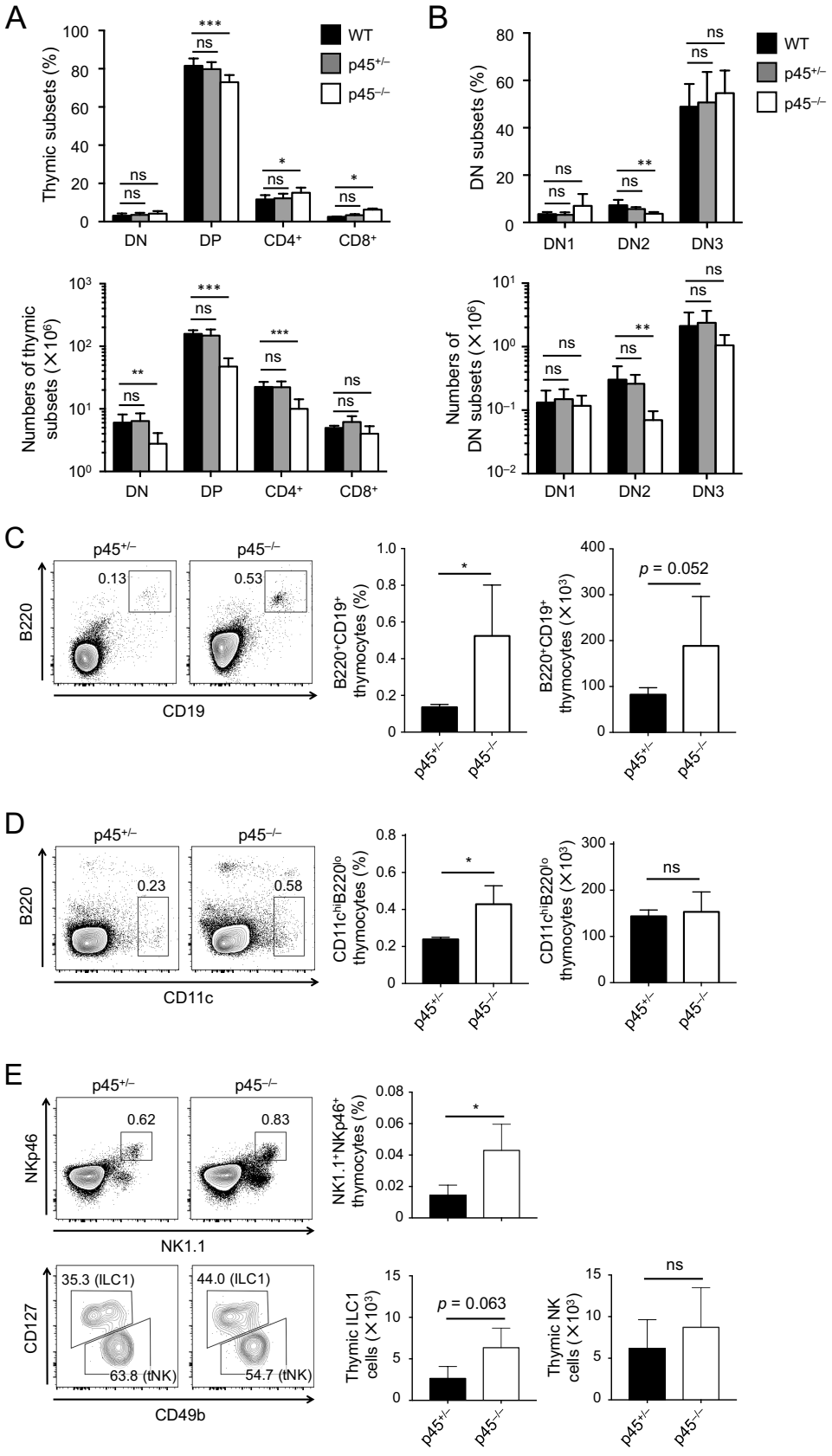
Xu et al. Supplemental Figure S1



Supplemental Figure S1. Impact of loss of Tcf1 long isoforms on T cell development and non-T lineage thymocytes.

Thymocytes were isolated from WT, $p45^{+/-}$, or $p45^{-/-}$ mice, enumerated and surface stained.

(A) Thymic maturation stages. Lin^- thymocytes were analyzed for CD4 and CD8 expression, and DN, DP, $CD4^+$ and $CD8^+$ thymic subsets were identified. The percentage (top) and numbers (bottom) of each subset are summarized as means ± s.d.

(B) DN thymocyte profile. Lin^- DN thymocytes were analyzed for CD44 and CD25 expression, and DN1-DN3 subsets were identified. The percentage (top) and numbers (bottom) of each subset are summarized as means ± s.d.

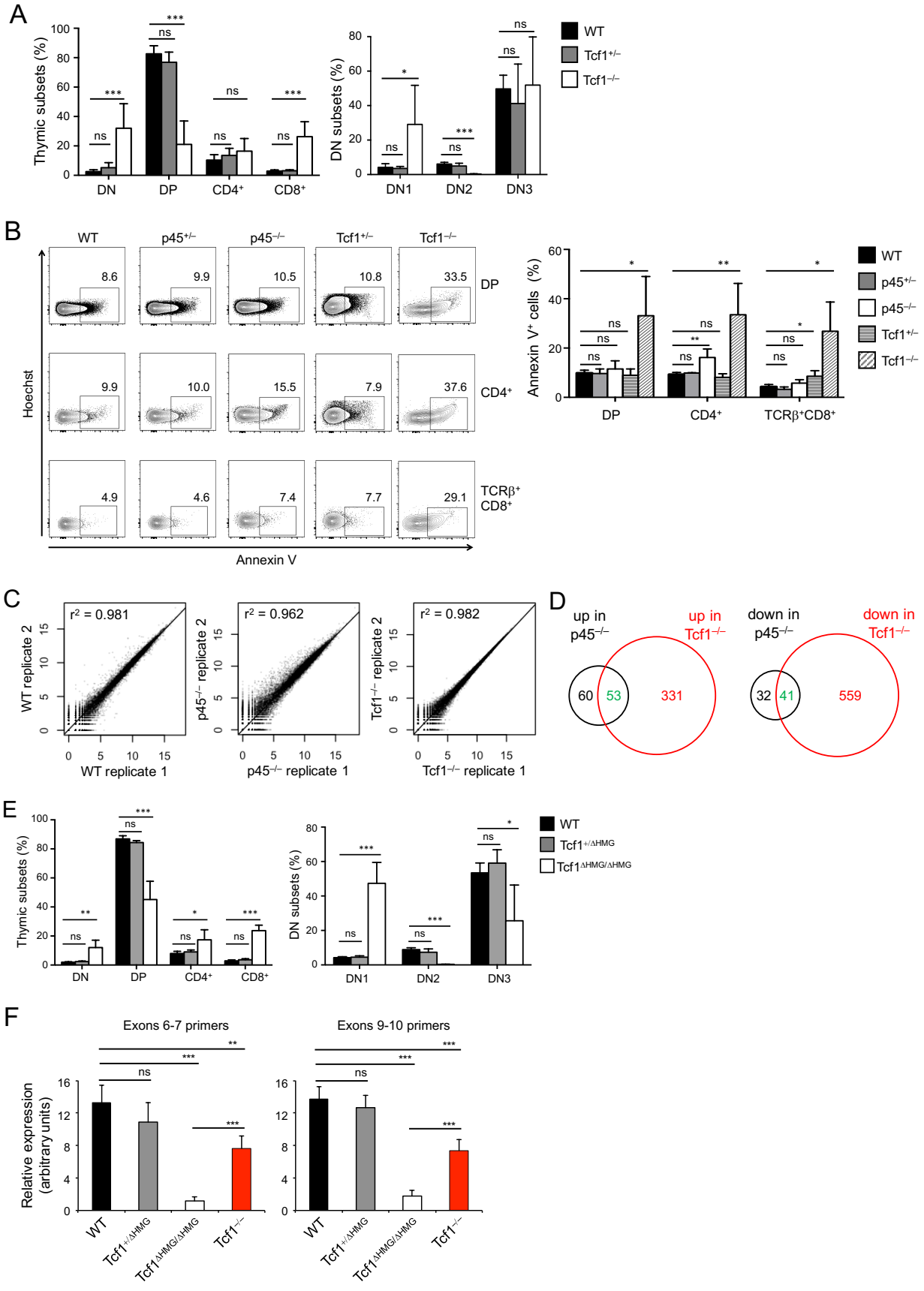
(C) Thymic B cells. $B220^+CD19^+$ cells are detected in total thymocytes, as shown in representative contour plots (left panels). The frequency and numbers of thymic B cells are summarized in bar graphs (right panels).

(D) Thymic dendritic cells (DCs). $B220^-CD11c^+$ cells are detected in total thymocytes, as shown in representative contour plots (left panels). The frequency and numbers of thymic DCs are summarized in bar graphs (right panels).

(E) Thymic natural killer (NK) and group 1 innate lymphoid (ILC1) cells. $NK1.1^+NKp46^+$ cells are detected in $TCR\beta^-CD8^-$ thymocytes (top), and further fractionated into $CD127^+CD49b^{lo}$ ILC1 and $CD127^-CD49b^{hi}$ NK cells (bottom), as shown in representative contour plots (left panels). The frequency of $NK1.1^+NKp46^+$ cells in total thymocytes is summarized in top bar graph, and the numbers of ILC1 and thymic NK cells were in bottom bar graphs (right panels). The gating strategy of ILC1 and thymic NK cells was based on *Sojka et al. Tissue-resident natural killer (NK) cells are cell lineages distinct from thymic and conventional splenic NK cells. Elife* ***3****, e01659, 2014.*

Data in (A)-(B) are from 4 experiments ($n \geq 4$ for each genotype), and data in (C)-(E) are from 2 independent experiments ($n = 3$ for $p45^{+/-}$ mice, $n= 4$ for $p45^{-/-}$ mice) ns, not statistically significant; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ by Student's t test, unless specified otherwise.

Xu et al. Supplemental Figure S2



Supplemental Figure S2. The differential requirements for Tcf1 long isoforms and all isoforms in T cell development and thymocyte transcriptomes.

(A) Impact of loss of all Tcf1 isoforms on T cell development. Thymocytes were isolated from WT, $Tcf1^{+/-}$, or $Tcf1^{-/-}$ mice, enumerated and surface stained. Lin^- thymocytes were analyzed for CD4 and CD8 expression, and the frequency of DN, DP, $CD4^+$ and $CD8^+$ thymic subsets was determined (left). Lin^- DN thymocytes were then analyzed for CD44 and CD25 expression, and the frequency of DN1-DN3 subsets was determined (right). Data are from 5 experiments ($n \geq 5$ for each genotype).

(B) Detection of thymocyte survival. Lin^- thymocytes from mice of indicated genotypes were surface-stained to identify DP, $CD4^+$ and $TCR\beta^+CD8^+$ single positive cells followed by Hoechst 33258 and Annexin V staining. After excluding $Hoechst^+$ dead cells, the viable cells were analyzed for Annexin V expression. The percentages of Annexin V^+ cells are shown in representative contour plots (left), and cumulative data are in bar graphs (right). Data are from 3 experiments ($n \geq 3$ for each genotype).

(C) Reproducibility of RNA-Seq replicates. Shown are scatterplots of log2-transformed counts per millions of two replicates for each genotype. Pearson coefficient (r^2) values are marked.

(D) Venn diagrams showing upregulated (left) and downregulated (right) genes in $p45^{-/-}$ and $Tcf1^{-/-}$ DN3 thymocytes identified using more stringent criteria (*i.e.*, ≥ 2 fold expression changes, and $p < 0.01$). Compared with Fig. 3B-C, *Tox*, *Fyn*, *Gzma* and *Rag1* genes are not identified as differentially expressed genes under the stringent criteria.

(E) Impact of truncating the HMG domain in Tcf1 on T cell development. Thymocytes were isolated from WT, $Tcf1^{+/\Delta HMG}$, and $Tcf1^{\Delta HMG/\Delta HMG}$ mice, enumerated and surface stained. Lin^- thymocytes were analyzed for CD4 and CD8 expression, and the frequency of DN, DP, $CD4^+$ and $CD8^+$ thymic subsets was determined (left). Lin^- DN thymocytes were analyzed for CD44 and CD25 expression, and the frequency of DN1-DN3 subsets was determined (right). Data are means ± s.d. from 4 independent experiments ($n \geq 4$).

(F) Detection of Tcf1 transcripts. RNA was extracted from total thymocytes of WT, $Tcf1^{+/\Delta HMG}$, $Tcf1^{\Delta HMG/\Delta HMG}$, and $Tcf1^{-/-}$ mice and reverse-transcribed. A stop codon was inserted in exon 8 in $Tcf1^{\Delta HMG}$ mutant mice. Tcf1 transcripts were detected with quantitative PCR using either primers spanning exons 6 and 7 (left, 5'-cccagtcctcctctctaccc and 5'-acaccagatcccagcatcaa) or those spanning exons 9 and 10 (right, 5'-agagcaggccaagtactatga and 5'-cgcctcttcttctttccgtag). The relative levels of Tcf1 transcripts were determined by normalizing to *Hprt* (detected with 5'-gcgtcgtgattagcgatgatg and 5'-ctcgagcaagtctttcagtcc primers). At least 2 mice of each genotype were analyzed and each samples measured in duplicates.

ns, not statistically significant; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ by Student's *t* test.