Supplemental Data

Supplemental Methods

***TCE1*-eGFP reporter plasmid constructions**

The 2.9 kb-*1b*.eGFP reporter plasmid was prepared by first linearizing *7.1-1b*.eGFP (Hosoya-Ohmura et al., 2011) with XhoI. Then, partial digestion was performed with SalI and the 9.2 kb fragment containing the *1b*.eGFP reporter, 2.9 kb of truncated *TCE-7.1* and the vector backbone was gel purified. This fragment was self-ligated to generate the 2.9 kb-*1b*.eGFP reporter plasmid. The resultant plasmid was verified by restriction enzyme digestion and sequencing. To generate transgenic mice ($Tg^{2.9}$), 2.9 kb-*1b*.eGFP was digested with PmeI and KpnI to generate the 6.5 kb fragment that was used for microinjection of fertilized eggs.

The 4.2 kb-*1b*.eGFP reporter plasmid was prepared by digesting a pGEM-4Z 7.1kb plasmid (Hosoya-Ohmura et al., 2011) with XhoI and SalI. The 4.2 kb fragment was subcloned into the SalI site of p*1b*.eGFP (Hosoya-Ohmura et al., 2011). To generate transgenic mice ($Tg^{4.2}$), the 4.2 kb-*1b*.eGFP plasmid was digested with PmeI and BsshII and the resultant 7.8 kb fragment was used for microinjection.

The 1.2 kb-*1b*.eGFP reporter plasmid was prepared by digestion of pGEM-4Z 7.1 with KpnI and XhoI. The resultant 2.9 kb fragment was gel purified and then digested with ApaLI. This digestion produced a 1.2 kb fragment, which was subsequently treated with T4 DNA polymerase to generate blunt ends. At the same time, the p*1b*.eGFP plasmid was digested with SalI and treated with T4 DNA polymerase to generate blunt ends. The 1.2 kb fragment was ligated to the p*1b*.eGFP plasmid. To generate transgenic mice ($Tg^{1.2}$), the 1.2 kb-*1b*.eGFP plasmid was digested with PmeI and PacI, and the 4.8 kb fragment was used for microinjection.

The 1.7 kb-*1b*.eGFP reporter plasmid was prepared by digesting pGEM-4Z 7.1 kb plasmid with KpnI and ApaLI; the digestion was treated with T4 DNA polymerase to

generate blunt ends, and the 1.7 kb fragment was subcloned into blunt end sites of p*1b*.eGFP plasmid. To generate transgenic mice ($Tg^{1.7}$), 1.7 kb-*1b*.eGFP plasmid was digested with PacI and PmeI to generate the 5.3 kb fragment used for microinjection.

The 7.1Δ1.2 kb-*1b*.eGFP plasmid was prepared as follows. In order to prepare a 1.3 kb fragment in which the 5′ and 3′ flanking segments of the 1.2 kb element are linked and the 1.2 kb is deleted, PCR was performed using pGEM-4Z 7.1 kb plasmid as the template. That PCR product was cloned into pGEM-5Zf (+) vector and the DNA sequence of pGEM-5Zf(+)-XhoI-1.3kb-HpaI was verified. This plasmid was digested with XhoI and HpaI, gel purified and the 1.3 kb fragment was used for subcloning. In the meantime, pGEM-4Z 7.1 kb plasmid was digested with XhoI and HpaI and the 7.5 kb fragment was recovered. To generate pGEM-4Z 7.1Δ1.2 kb, the 1.3 kb fragment and the 7.5 kb fragment were ligated. A KpnI linker was added to one end of 7.1Δ1.2 kb fragment. Next, the p*1b*.eGFP plasmid was digested with SalI, blunt ends were generated using T4 DNA polymerase, and KpnI linkers were added. The 7.1Δ1.2 kb fragment was subcloned into the KpnI site of the modified p*1b*.eGFP/KpnI plasmid. To generate transgenic mice ($Tg^{\Delta 1.2}$), 7.1Δ1.2 kb-*1b*.eGFP was digested with BssHII and PmeI and the recovered 9.5 kb fragment was used for microinjection.

The 7.1Δ1.7 kb-*1b*.eGFP plasmid was prepared by digesting pGEM-4Z 7.1 kb with KpnI and SalI followed by gel purification of the 7.1 kb fragment. This fragment was digested with ApaLI, and the gel purified 5.4 kb fragment (7.1Δ1.7 kb) was treated with T4 DNA polymerase. KpnI linkers were added to both ends, and the 7.1Δ1.7 kb fragment was subcloned into the KpnI site of p*1b*.eGFP. To generate transgenic mice ($Tg^{\Delta 1.7}$), this plasmid was digested with BssHII and PmeI and the resultant 9 kb fragment was used for microinjection.

The 7.1Δ1.5 kb-*1b*.eGFP plasmid was prepared by digesting pGEM-4Z 7.1 kb with KpnI. Then, partial digestion was performed with Bgl II, and the resultant 5.6 kb fragment (7.1Δ1.5 kb) was gel purified and subcloned into pNEB193. pNEB193-7.1Δ1.5 was digested with Sal I, blunt ends were generated and KpnI linkers were added. The 7.1Δ1.5 kb fragment which contains KpnI sites on both ends was subcloned into the KpnI site of p*1b*.eGFP. To generate transgenic mice ($Tg^{\Delta 1.5}$), the 7.1Δ1.5 kb-*1b*.eGFP plasmid was digested with BssHII and PmeI, and the resultant 9.2 kb fragment was used for microinjection.

The 7.1Δ2.7 kb-*1b*.eGFP plasmid was prepared by digesting p*1b*.eGFP-7.1 with XhoI, and then partial digestion was performed with Bgl II. The resultant 10.7 kb fragment (7.1Δ2.7 kb-*1b*.eGFP fragment) was gel purified, treated with T4 DNA polymerase to generate blunt ends, and self-ligated. The resultant plasmid was verified by restriction enzyme digestion patterns and the junctions over the self-ligated ends were verified by DNA sequencing. To generate transgenic mice ($Tg^{\Delta 2.7}$), 7.1Δ2.7 kb-*1b*.eGFP was digested with PmeI and KpnI, and the resultant 8 kb fragment was used for microinjection.

ChIP Assays

Total thymocytes or each stage of sorted thymocytes from 6 to 10 weeks old of C57BL/6J mice were washed with PBS(-) and resuspended in PBS(-) (10^7 cells in 4.5 ml or 10^6 cells in 450 μl). The cells were crosslinked with either EGS (ethylene glycolbis (succinimidylsuccinate)) (Thermo Scientific, cat#21565) and formaldehyde (Sigma-Aldrich, F8775) or formaldehyde alone. For crosslinking with EGS and formaldehyde, 20 mM EGS (final [2 mM] EGS) was added to cells and incubated at room temperature for 30 min. After centrifugation and discarding the supernatant, cells were resuspended in

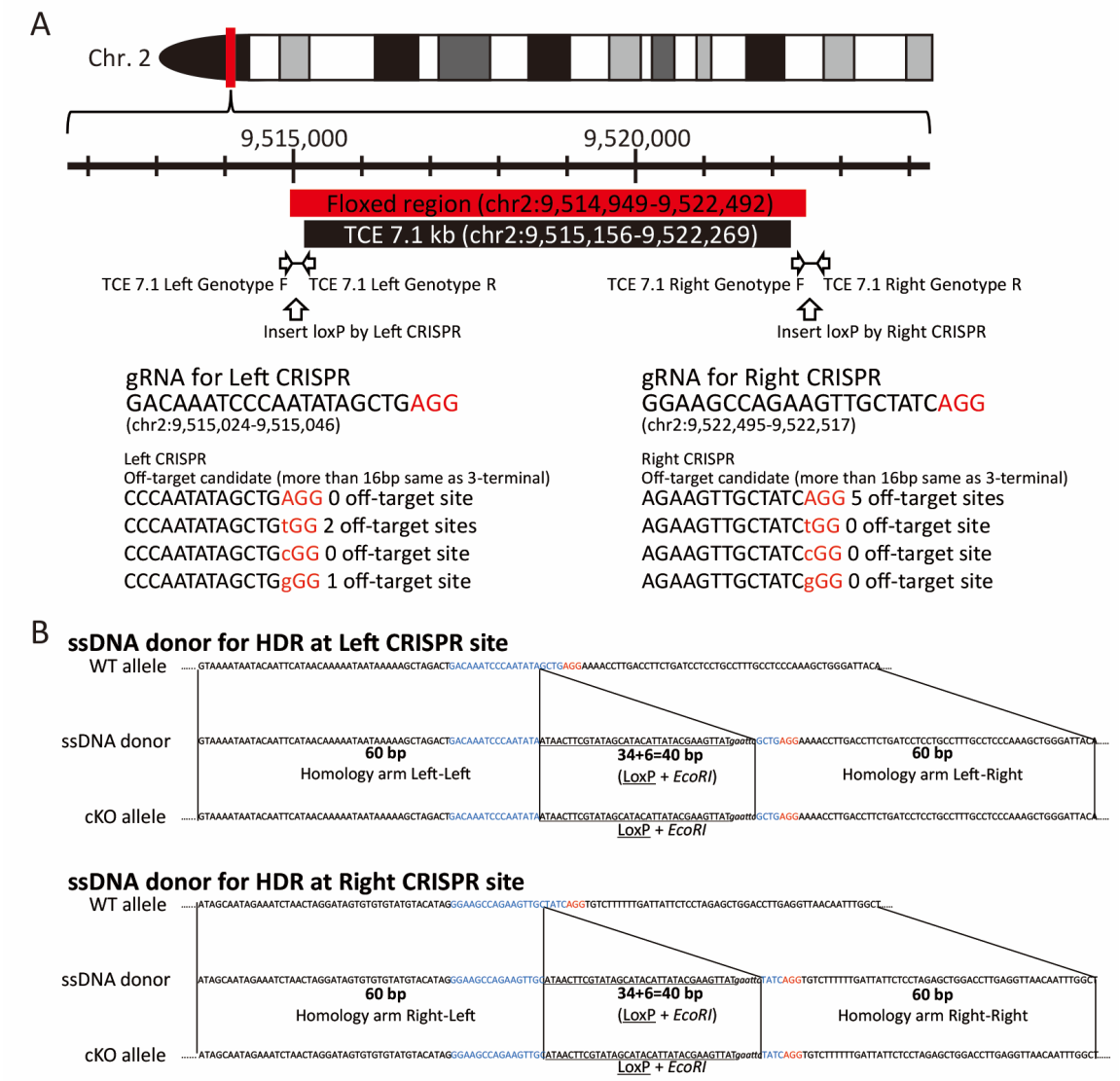
PBS(-) (10^7 cells in 4.5 ml or 10^6 cells in 450 μl) to cross-link with formaldehyde. For crosslink with formaldehyde, 500 μl (for 10^7 cells) or 50 μl (for 10^6 cells) of fixing solution (50 mM HEPES (pH7.5), 100 mM NaCl, 1mM EDTA, 0.5 mM EGTA, 10% formaldehyde) was added to the cells and incubated at room temperature for 10 min. Next, 500 μl (for 10^7 cells) or 50 μl (for 10^6 cells) of 1.5 M glycine was added to the cells and incubated at room temperature for 5 min. After washing with ice-cold PBS(-) twice, cells were frozen using dry ice-EtOH and stored at -80°C for subsequent analysis. To lyse cells, 5 ml of Lysis Buffer 1 (50 mM HEPES (pH7.5), 140 mM NaCl, 1 mM EDTA, 10% Glycerol, 0.5% NP-40, 0.25% TritonX-100, 1 mM PMSF, 1x protease inhibitor cocktail (Sigma-Aldrich, P8340)) was added to 10^7 cells, which were incubated at 4°C for 10 min. After pelleting the supernatant was discarded, 5 ml of 4°C Lysis Buffer 2 (10 mM Tris-Cl (pH8.0), 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 1mM PMSF, 1x protease inhibitor cocktail) was added; cells were resuspended and incubated at 4°C for 10 min. After pelleting again, 400 μl of 4°C Lysis Buffer 3 (10 mM Tris-Cl (pH8.0), 300 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 1% TritonX-100, 0.1% Sodium Deoxycholate, 0.5% N-lauroylsarcosine, 1 mM PMSF, 1x protease inhibitor cocktail) was added and chromatin was sonicated to an average fragment size of 200-500 bp.

Chromatin from 5 x 10^6 cells equivalents was used for each ChIP assay as described below. For TCF-1 ChIP and HEB ChIP, Dynabeads M-280 sheep anti-rabbit IgG (Invitrogen) were washed with 0.5% BSA-PBS 4 times, antibodies [for TCF-1 ChIP, anti-TCF1 (Cell Signaling Technology, #2203); for HEB ChIP, anti-HEB (Santa Cruz Biotechnology, sc-357X)] were added to the beads and rotated at 4°C for 6 hours to overnight. Dynabeads coupled with antibodies were washed with 0.5% BSA-PBS twice at 4°C, and then washed with 4°C Lysis Buffer 3 once. Chromatin was added to the coupled Dynabeads and rotated at 4°C overnight. Immune complexes were washed with

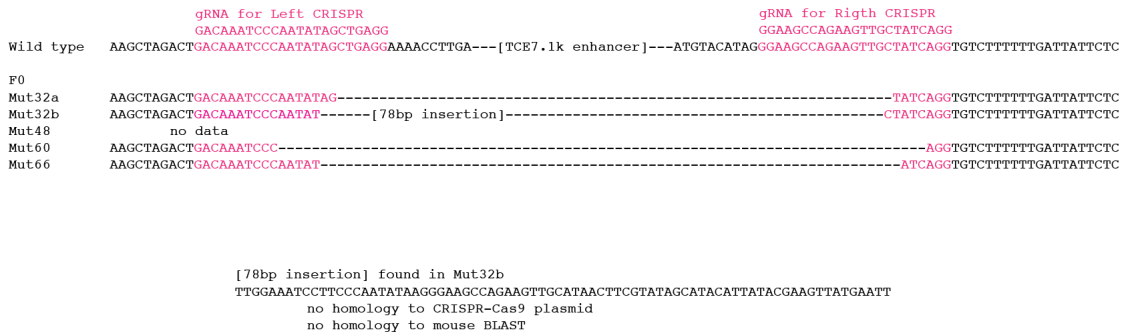
4°C low salt buffer (20 mM Tris-Cl (pH8.0), 150 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% TritonX-100) once, washed with 4°C high salt buffer (20 mM Tris-Cl (pH8.0), 400 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% TritonX-100) twice, washed with RIPA wash buffer (50 mM HEPES (pH7.6), 500 mM LiCl, 1 mM EDTA, 1% NP-40, 0.7% Sodium Deoxycholate) at 4°C 4 times, and finally washed with 4°C TE plus 50 mM NaCl once. After washing, 200 μl of Elution buffer (50 mM Tris-Cl (pH8.0), 10 mM EDTA, 1% SDS) was added to immune complexes which were then incubated at 65°C for 15 min. Supernatants were transferred to a new tube and incubated at 65°C overnight to to complete the crosslinking reversal. To purify DNA, 200 μl of TE and RNase A [0.2 μg/μl final] was added and incubated at 55°C for 1 hour, and then proteinase K [0.2 μg/μl final] was added and incubated at 55°C for 2 hours. DNA was extracted with phenol-chloroform-isoamyl alcohol twice and precipitated with EtOH. For RBP-J ChIP, antibody (anti-RBPSUH, Cell Signaling Technology, #5313) was added to sonicated chromatin and rotated at 4°C overnight. Dynabeads M-280 sheep anti-rabbit IgG were washed with 0.5% BSA-PBS twice and washed with Lysis buffer 3 once. Washed Dynabeads were added to the chromatin/antibody mixture and rotated at 4°C for 4 to 5 hours. Immune complexes were washed, eluted and DNA was purifed as described above. As negative controls, rabbit monoclonal IgG (Cell Signaling Technology, #3900, for TCF-1 ChIP and RBP-J ChIP) or normal rabbit IgG (Cell Signaling Technology, #2729, for HEB-ChIP) were used.

Purified ChIP samples, IgG samples and Input DNA (an aliquot of sheared chromatin before immunoprecipitation) was analyzed using PowerSYBR Green PCR Master Mix (Applied Biosystems) and either ABI Prism 7000 (Applied Biosystems) or StepOnePlus (Applied Biosystems). The data were quantified using the standard curve method and Input DNA was used for normalization of samples for the amount of

chromatin in each sample. Primer sequences used for qPCR are listed in Supplementary Table 3.

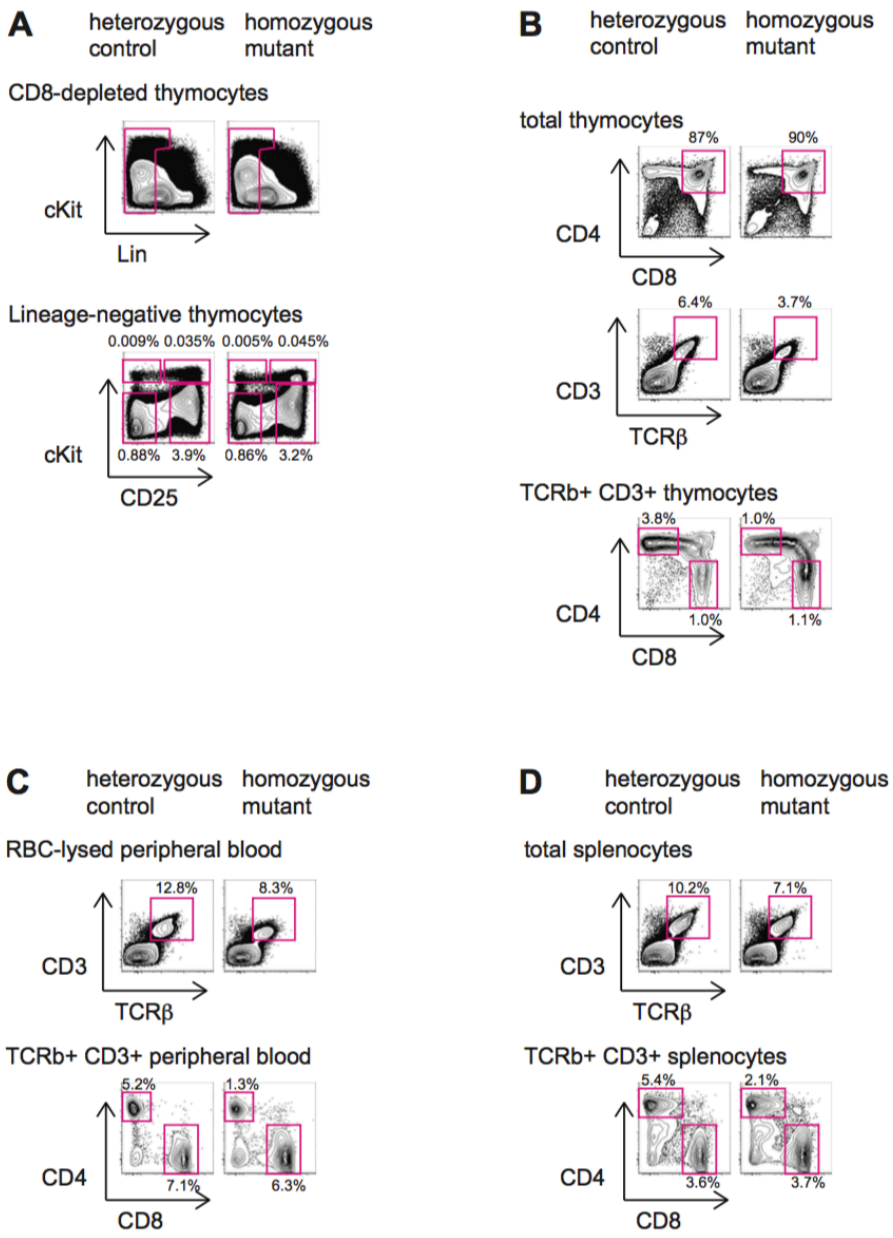


Supplemental Figure 1. Genome editing of *TCE1* using CRISPR-Cas9
(A) gRNA sequences corresponding to sequences surrounding the 7.1 kbp that define the boundaries of *TCE1*. *TCE1* left genotype F: 5'-TTTTGATGCATTTCGAACCA-3'. *TCE1* left genotype R: 5'-ATCGCATGGGAATGTTTCTC-3'. *TCE1* right genotype F: 5'-TGGTTGAGGTGGAAGAGTCC-3'. *TCE1* right genotype R: 5'-CAAACAGGGTTGGGGAAATA-3'. (B) Single-stranded oligonucleotides containing loxP sequence, EcoRI site and 60 bp homology arms.

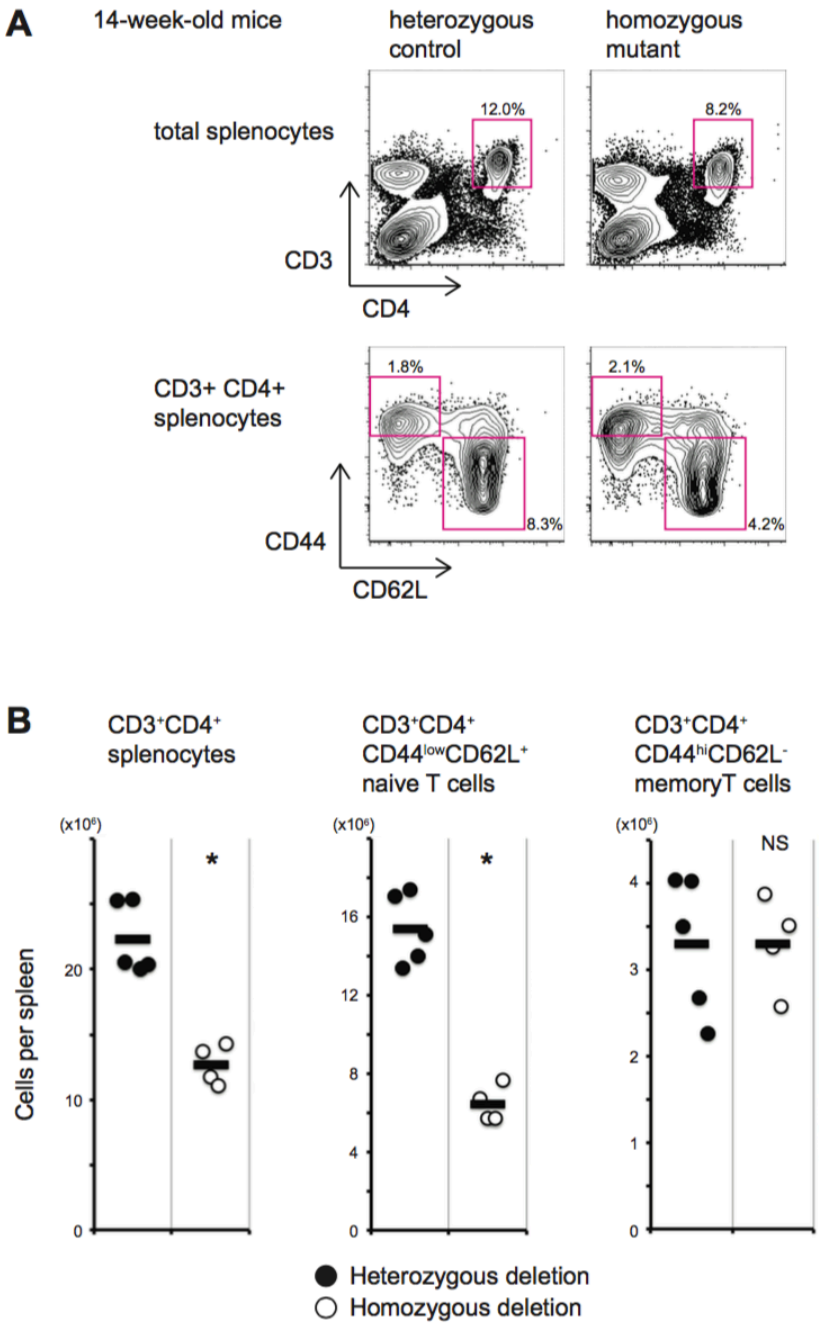


Supplemental Figure 2. *TCE1* deleted allele by CRISPR-Cas9

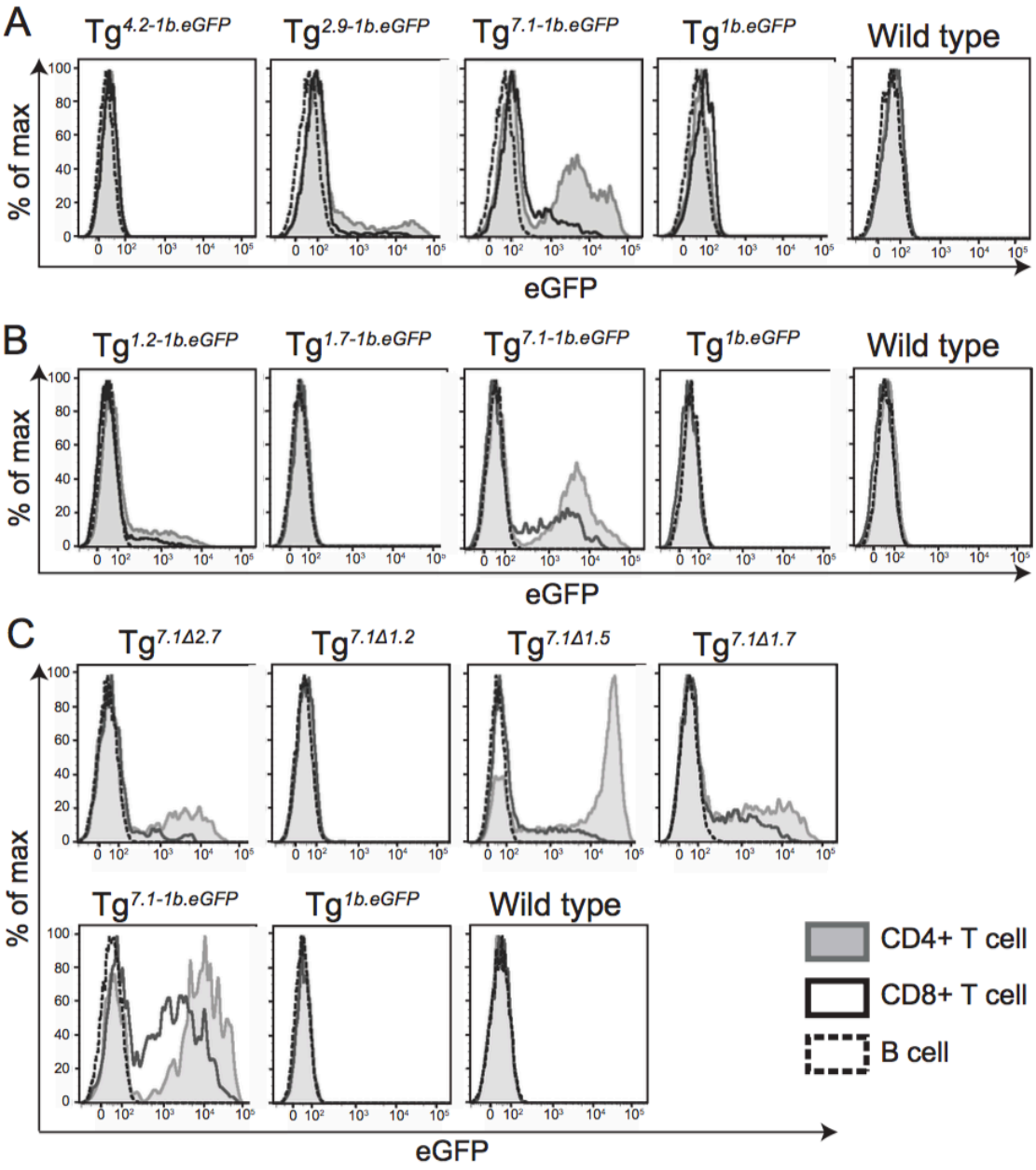
TCE1 left genotype F and *TCE1* right genotype R primers were used for detection of *TCE1* deleted allele. The PCR product was sequenced to confirm deletion.



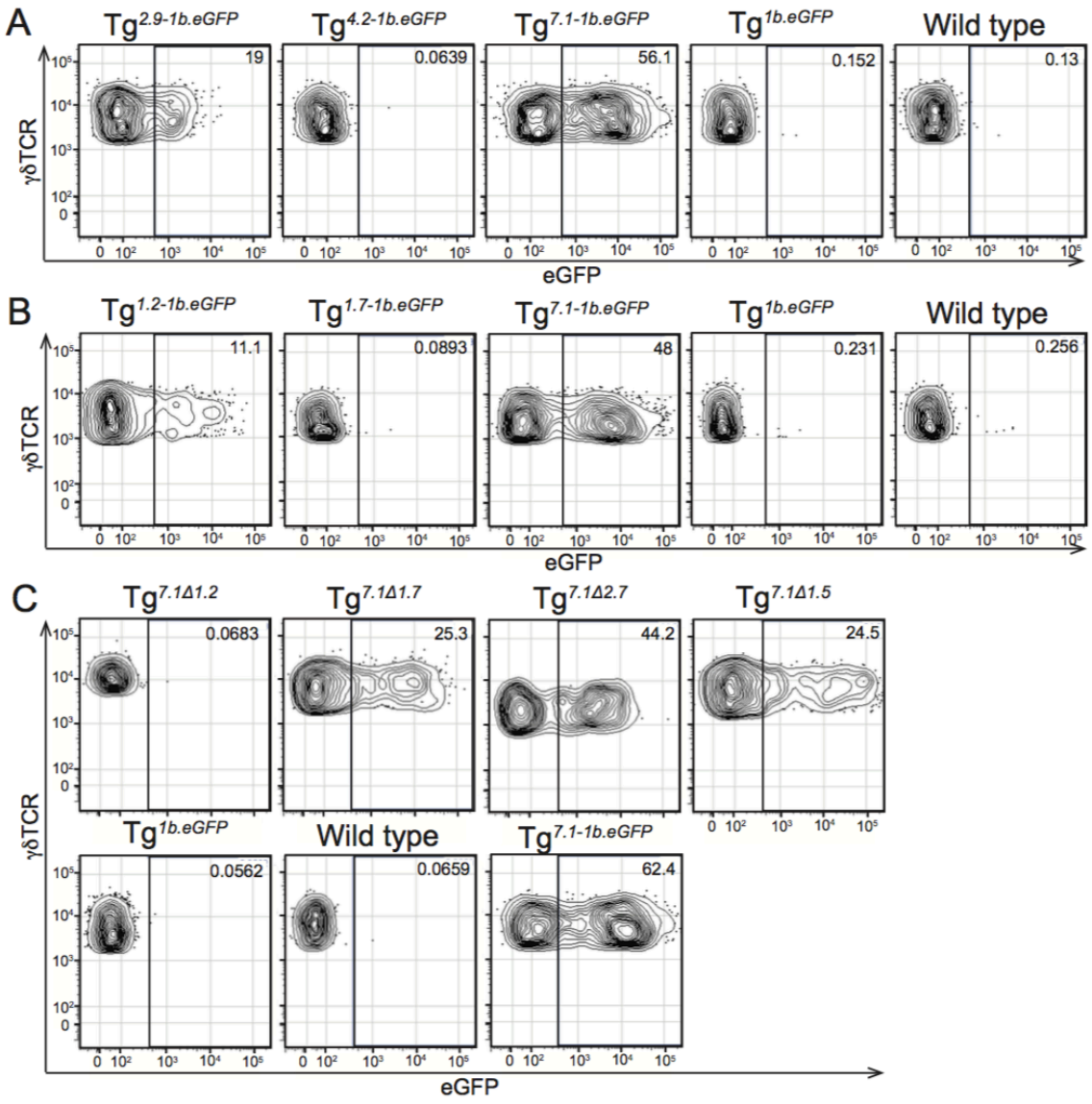
Supplemental Figure 3. T cell development in *TCE1* ablated mice
F2 animals at 5-6 weeks old bearing homozygous or heterozygous deletions of *TCE1* were analyzed for cell surface expression of T cell stage-specific markers. (A) Thymocytes were depleted for CD8-expressing cells, and then stained with antibodies against cKit (2B8), CD25 (PC61.5) and lineage mixture. (B) Thymocytes were stained with antibodies against CD3 (17A2), TCRβ (H57-597), CD4 (RM4-5) and CD8a (53-6.7). (C) Peripheral blood cells were suspended in ammonium chloride lysing solution for 5 minutes to remove erythrocytes, and then stained with antibodies shown in (B). (D) Splenocytes were stained as described in (B). Representative images of 5 control and 8 mutant mice from two independent experiments were shown. Mean percentages of cells in the boxed area per total thymocytes (A and B), per RBC-lysed peripheral blood cells (C) and pre total splenocytes (D) were shown near the box.



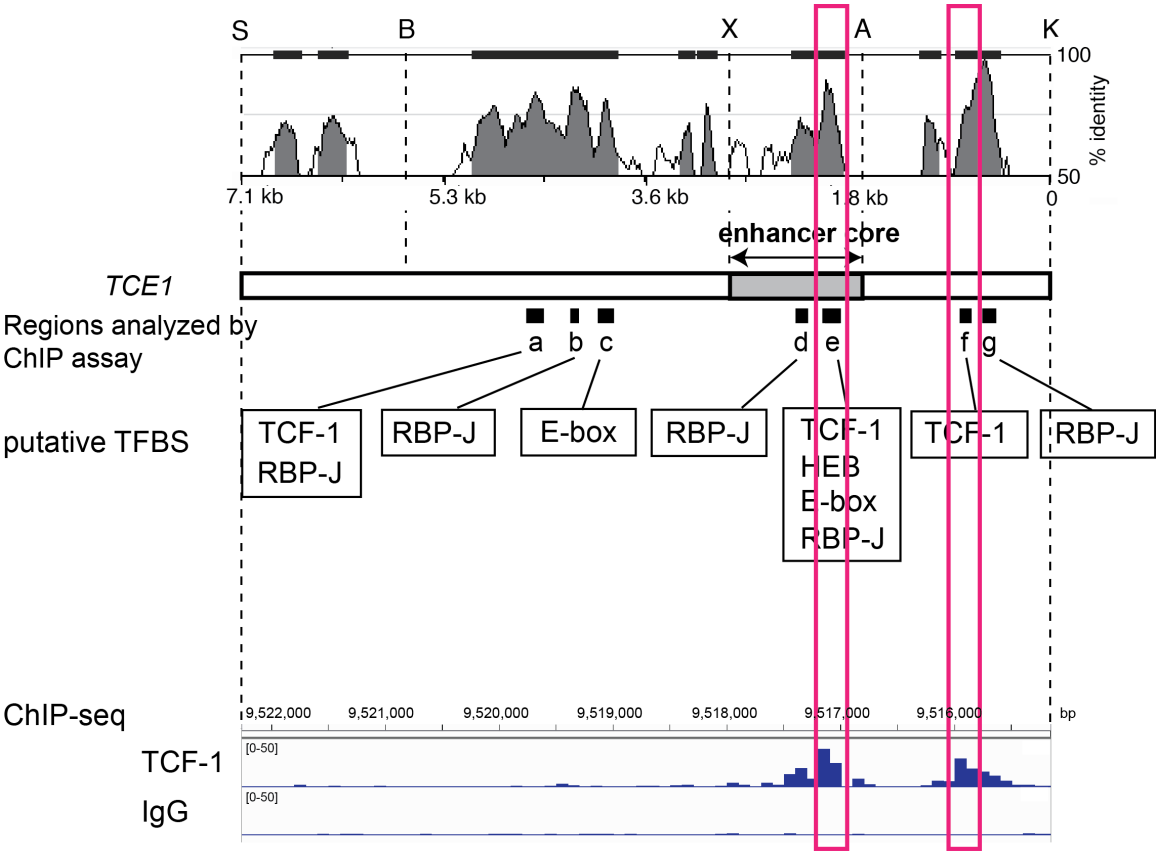
Supplemental Figure 4. T cell development in *TCE1* ablated mice
F2 animals at 14 weeks old bearing homozygous or heterozygous deletions of *TCE1* were analyzed for cell surface expression of T cell stage-specific markers. (A) Splenocytes were stained with antibodies against CD3 (145-2C11), CD4 (RM4-5), CD44 (IM7) and CD62L (MEL-14). Representative images of 5 animals for heterozygous and 4 animals for homozygous deletion were shown. Mean percentages of cells in the boxed area per total splenocytes were shown near the box. (B) Cell number in each population shown in (A) was summarized. Each circle represents individual animal. Solid bar indicates average cell number for each genotype. * indicates statistically significant, $P < 0.05$ by student's *t* test. NS: not significant, $P > 0.05$.



Supplemental Figure 5. eGFP expression in the peripheral blood of F_0 Tg mice
eGFP expression in CD4+ T cells, CD8+ T cells and B cells in peripheral blood from (**A**) $Tg^{2.9}$ and $Tg^{4.2}$ mice, (**B**) $Tg^{1.2}$ and $Tg^{1.7}$ mice, and (**C**) $Tg^{\Delta 1.2}$, $Tg^{\Delta 2.7}$, $Tg^{\Delta 1.5}$, $Tg^{\Delta 1.7}$ mice. Data are representative of multiple experiments. Gray shaded histograms, CD4+ T cells; Black solid lines, CD8+ T cells; Black dashed lines, B cells.



Supplemental Figure 6. eGFP expression in γδTCR+ thymocytes of F_0 Tg mice
eGFP expression in γδTCR+ thymocytes of (**A**) $Tg^{2.9}$ and $Tg^{4.2}$ mice, (**B**) $Tg^{1.2}$ and $Tg^{1.7}$ mice, and (**C**) $Tg^{\Delta 1.2}$, $Tg^{\Delta 2.7}$, $Tg^{\Delta 1.5}$, $Tg^{\Delta 1.7}$ mice. Data are representative of multiple experiments.



Supplemental Figure 7. Comparison of T cell developmental stage-specific ChIP-qPCR to published ChIP-seq in total thymocytes

TCF-1 ChIP-seq data in total thymocytes (GSE46662) was displayed using Integrative Genomics Viewer (IGV, https://www.broadinstitute.org/igv/). ChIP-seq data with anti-TCF-1 within 7.1kb *TCE1* sequence was shown with IgG control (bottom).

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Supplemental Table 1. eGFP expression in γδTCR+ thymocytes of F_0 Tg mice

Transgene	No. of Tg mice with γδTCR+ eGFP+ cells/ No. of Tg mice (PCR+)
$Tg^{2.9}$	8/8[a]
$Tg^{4.2}$	0/6
$Tg^{1.2}$	4/7
$Tg^{1.7}$	2/4
$Tg\Delta^{1.2}$	3/7
$Tg\Delta^{2.7}$	5/7
$Tg\Delta^{1.5}$	9/9[b]
$Tg\Delta^{1.7}$	8/9[c]

[a]Eight out of 9 $Tg^{2.9}$ mice, [b]9 out of 20 $Tg\Delta^{1.5}$ mice and [c]9 out of 10 $Tg\Delta^{1.7}$ mice whose eGFP expression in peripheral T cells were positive were analyzed.

Supplemental Table 2. eGFP expression in non-T lineage hematopoietic cells of F0 Tg mice

	No. of Tg mice with eGFP+ cells/ No. of Tg mice (PCR+)		
Transgene	Erythroid cell	B cell	Myeloid cell
$Tg^{2.9}$	0/8	0/8	0/8
$Tg^{4.2}$	0/6	0/6	0/6
$Tg^{1.2}$	0/7	0/7	0/7
$Tg^{1.7}$	0/4	0/4	0/4
$Tg\Delta^{1.2}$	0/7	0/7	1*/7
$Tg\Delta^{2.7}$	0/7	0/7	0/7
$Tg\Delta^{1.5}$	0/9	0/9	0/9
$Tg\Delta^{1.7}$	0/9	0/9	0/9

Erythroid cells ($TER119^+$), B cells ($B220^+CD19^+$) and myeloid cells ($Gr1^+Mac1^+$) in bone marrow were analyzed.
*We concluded that it was ectopic expression because *TCE1* does not drive transcription in myeloid cells.

Supplemental Table 3. Primers used for ChIP assay

qPCR primers	sequences	Location on mm9 chr2
site a-forward	5′- GTG CAG GAC AAT GGA TAC AGA AGT-3′	9,519,641
site a-reverse	5′- GCC AGG AAT ACT GCC AGC G -3′	9,519,795
site b-forward	5′- GGT TGG CAG GAG GCA GAG T -3′	9,519,403
site b-reverse	5′- CTT ACC CAA AAA GTC ACA CAG CAA -3′	9,519,329
site c-forward	5′- TGA AGC TAA AAA TAA CCA ACC TGA CAT -3′	9,519,016
site c-reverse	5′- GCA GAG TGG AAG ACG GAA AGA A -3′	9,519,157
site d-forward	5′- GGT GGC ATG CAT CAA AGT TAA G -3′	9,517,419
site d-reverse	5′- GCA GAC ATT TTC CAC AAC ACG TA -3′	9,517,310
site e-forward	5′- TCC CAA CTG AAG CTC ACA GAC A -3′ (used for TCF-1 ChIP, RBP-J ChIP)	9,517,167
site e-reverse	5′- AGC GTT GCC CTG ACT CTC A -3′ (used for TCF-1 ChIP, RBP-J ChIP)	9,517,028
site e-forward2	5′- TTG CCC TGA CTC TCA CCT GC -3′ (used for HEB ChIP)	9,517,032
site e-reverse2	5′- CTA AAT GCT GCC TGC TGT TCC -3′ (used for HEB ChIP)	9,517,185
site f-forward	5′- CAC CCT TCA CCT TCG CCA -3′	9,515,887
site f-reverse	5′- CCC ATT GCT GTC ATA CAC TCT TG -3′	9,515,991
site g-forward	5′- GAG TGC AGA GCT CAG CAC GTT -3′	9,515,675
site g-reverse	5′- GCT GAA AAG CCA CTG GAG CTA -3′	9,515,796