Supplemental Methods

Haematology. Automated cell counts were performed on blood collected from the retro-orbital plexus into Microtainer tubes containing EDTA (Sarstedt), using an Advia 2120 haematological analyzer (Siemens). Megakaryocytes were counted manually in sections of sternum stained with haematoxylin and eosin (H&E) with a minimum of 5 fields of view analysed. Acute thrombocytopenia was induced by antiplatelet serum (Cerdalane) and assessed as previously described [1].

Flow cytometry antibodies. Antibodies for flow cytometry and cell sorting were labelled with FITC, PE, PE-Cy7, APC, Alexa Fluor 700, Pacific Blue and biotin. B220 (RA3-6B2), CD4 (GK1.5), CD8a (53.6.7), CD21 (7E9), CD44 (IM7), CD45.1 (A20), CD45.2 (104), CD11b (M170), CD23 (B3B4), CD41 (MWReg30), CD62L (MEL14), CD71 (RI7217), CD150 (TC15-12F12.2), Gr1 (RB6-8C5), Ter119 (TER-119) and Sca1 (D7) were purchased from Biolegend. CD19 (1D3), IgM (II/41), CD25 (7D4) and CD16/32 (2.4G2) were purchased from BD Biosciences. CD34 (RAM34), CD93 (AA4.1) and Kit (2B8) were purchased from eBioscience. As secondary reagent, streptavidin-Alexa700 (Molecular probes) was used.

Platelet lifespan. Mice were injected i.v. with 0.15 µg per g. of body weight of X488 (Emfret, Germany), a labelled antibody directed against the GPIbβ receptor. Briefly, 1µl of blood was obtained by tail vein bleed and was resuspended into 125µl of Aster Jandl citrate-based anticoagulant (85mM sodium citrate, 69mM citric acid, 20 mg/mL glucose, pH 4.6) [2] diluted 1:5 in resuspension buffer (10mM HEPES, 140mM NaCl, 3mM KCl, 0.5mM $MgCl_2$, 10mM glucose, and 0.5mM $NaHCO_3$, pH 7.4). Mouse platelet-rich plasma was obtained by centrifugation of the diluted blood at 125g for 5 minutes and platelets were identified from the combination of forward/side scatter profile and CD41 staining. The proportion of $X488^+$ platelet was determined by flow cytometry every 24 hours. For adoptive transfer of platelets, cells were as previously described[3] and injected intravenously into ubiquitin-GFP Tg C57BL/6 recipients, which were subsequently tail vein bled.

Erythrocyte lifespan. Animals were injected with 30mg/kg of (+) Biotin N-hydroxysuccinimide ester (Sigma Aldrich) and the proportion of biotinylated RBCs was determined by flow cytometry every 7 days. To do so, 1µl of blood was obtained by tail vein

bleed and resuspended into 100µl of Aster Jandl citrate-based anticoagulant diluted 1:20 in PBS containing anti-Ter119-APC antibody and streptavidin-PE. RBCs were identified from the combination of forward/side scatter profile and Ter119 staining.

RNA preparation and sequencing. Only males were used for isolating cells for transcriptome analyses. Platelets were purified as previously described [3] from individual males. The purity of each platelet suspension was assessed by flow cytometry and suspensions for which more than 98% of total events were $CD41^+$ platelets were pooled together. RNA extraction and purification was performed with a Norgen RNA purification kit. For naïve CD8 T cell isolation, splenocyte suspensions were submitted to red blood cell lysis, stained first with biotinylated anti-CD8 antibodies and then labelled with anti-Biotin microbeads. $CD8^+$ T cells were magnetically enriched by positive selection on MACS separation columns, stained with fluorescently-labelled antibodies before being FACS-sorted as $CD8^+$ $CD44^{low}$ $CD62L^+$. Sorted naïve T cells were fractionated with Norgen cytoplasmic and nuclear fractionation RNA purification kit. Only cytoplasmic fractions were used for subsequent RNAseq. Red blood cells were obtained from cardiac puncture (500µl), mixed with 100µl Aster Jandl citrate-based anticoagulant and subsequently diluted in 2 ml of phosphate buffer saline (PBS) with 2mM EDTA. This suspension was layered with 1.5 ml Ficoll-paque PLUS (GE Healthcare) and centrifuged at 400g for 30 minutes at room temperature with minimal acceleration and brake. The red blood cell containing pellet was washed twice with PBS 2mM EDTA and lysed for RNA extraction with Qiagen RNA extraction kit. The purity of each sample was checked by flow cytometry to assess the relative contamination of $Ter199^+$ RBC with $CD41^+$ platelets, $CD45^+$ lymphocytes and $DRAQ5^+$ (Thermo Fisher Scientific) nucleated cells. RBC suspensions used for RNAseq were > 99.8% pure. RNA integrity was evaluated on an Agilent Bioanalyser.

RNA-seq analysis (naïve CD8 T cells). Poly (A) tailed RNA from three independent suspensions were used for 100 bp single-end sequencing on an Illumina HiSeq by the Australian Genome Research Facility. Reads were mapped to the mm10 mouse genome using Subread v1.2.2 [4] and reads were assigned to Entrez Gene Ids using featureCounts v1.3.5-p5 [5]. Statistical analysis was undertaken using the limma software package [6]. Gene set enrichment analysis of canonical pathways was undertaken using the CAMERA method [7] with a mouse ortholog version of the Molecular Signatures Database[8] available from http://bioinf.wehi.edu.au/MSigDB. Barcode enrichment plots were created using the

barcodeplot function.

RNA-seq analysis (platelets and erythrocytes). Poly (A) tailed RNA from three independent suspensions were used for 100 bp paired-end sequencing on an Illumina HiSeq by the Australian Genome Research Facility. Reads were mapped to the mm10 genome using Rsubread 1.8.0 [9]. Reads were assigned to Entrez Gene Ids using featureCounts and Rsubread's in-built mm10 annotation and annotated using the org.Mm.eg.db package. Genes with counts per million above 0.5 in at least 3 samples were retained for further analysis and data were TMM normalized [10] using edgeR v3.11.5 [11] and transformed into log2 counts per million. Gene-wise linear models [12] with effects for genotype (and batch for the platelet experiment) with observational level weights [13] were fitted to summarise the values from replicate samples for each dataset separately using limma v3.24.15 [6]. Moderated *t*-statistics were used to assess differential expression between Nxf1 mutant and wild-type samples, with genes ranked according to their false discovery rate [14]. Gene set enrichment was performed using limma functions goana [15] for Gene Ontology 'Biological Process' terms and roast [16] for MSigDB [17] C2 gene signatures converted to mouse as described above for all 3 datasets.

Real-time quantitative PCR. Naïve CD4 T cells were FACS-sorted as $CD4^{+}$ $CD44^{low}$ cells from spleen of mixed BM chimera and unmanipulated animals. Cells were lysed in TRIzol reagent (Thermo Fisher Scientific) and RNA purification was performed by chloroform extraction and isopropropanol precipitation. Reverse transcription was performed using 60 ng of total RNA for each sample, oligo(dT) primer and SuperScript III reverse transcriptase (Life Technologies). Real-time qPCR was carried out on a Light-Cycler 480 (Roche) using the LightCycler 480 SYBR Green I Master mix (Roche) under the following conditions: 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, 20 s at 60°C, and 20 s at 72°C. Tbp, Stat1 and Gbp2 primer pairs had identical efficiency and their sequences were as follows: Tbp FWD GGGGAGCTGTGATGTGAAGT, Tbp RVS CCAGGAAATAATTCTGGCTCA, Stat1 FWD GCAGGTGTTGTCAGATCGAA, Stat1 RVS CTACCACGAAGGAGCTCTGAA, Gbp2 FWD TGTAGACCAAAAGTTCCAGACAGA and Gbp2 RVS GATAAAGGCATCTCGCTTGG. Relative gene expression was determined using the ΔΔCT method.

Cell death assays. Lymphocytes were FACS-sorted from spleen of mutant and control animals. For each genotype, identical numbers of cells were plated in 96 well plate U bottom (Falcon) in the high-glucose version of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 250 μM L-asparagine (Sigma), 50 μM β-mercaptoethanol (Sigma) and 10% foetal calf serum (Sigma). For growth factor deprivation assays and for cells treated with Leptomycin B (Sigma) and Cyclohexamide (Sigma), quantification of viable cells was performed with APC-conjugated CaliBRITE beads and PI.

Data Sharing Statement

Dr Stephane Chappaz (stephane.chappaz@monash.edu) can be contacted for data that are not publicly accessible. Transcriptome profiling of blood cells are available at GEO using the token: oryzaaewxfadjgh

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE141161

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Supplemental Figure legends

Supplemental Figure 1. **Automated analysis of peripheral blood of pedigrees carrying Nxf1 mutation.** Automated analysis of platelet, lymphocyte and RBC counts in Nxf1 mutants and littermate controls (A) Plt42, (B) Plt43, (C) Plt64 and (D) Plt69. (E) Alignment of Nxf1 protein sequences. *Caenorhabditis elegans* [562-597], *Drosophila melanogaster* [623-658], *Mus Musculus* [569-604], *Homo sapiens* [570-605]. The red letter C represents the conserved Cysteine that is replaced in *Plt54* mice by an Arginine (position 587 in the mouse protein). The blue letter S represents the conserved Serine that is replaced in *Plt43* mice by a Proline (position 574).

Supplemental Figure 2. Nxf1 expression pattern across blood lineages and automated analysis of peripheral blood from *C587R/+* mutants and littermate controls. (A) Nxf1 expression pattern in murine hematopoietic cells extracted from the Haemosphere database [18]. Automated haematology analysis on peripheral blood. Parameters for platelets (B) and red blood cells (C). (D) Neutrophil, Eosinophil and Monocyte counts in peripheral blood. Data show mean with SD. (*) indicates significance < 0.05, (**) indicates significance < 0.01, (***) indicates significance < 0.001, (****) indicates significance < 0.0001 and (NS) indicates non-significant.

Supplemental Figure 3. Bone marrow progenitors and myeloid cells are not affected by the *C587R* mutation (A) Gating strategy for the identification of Thiazole Orange-positive platelets (B) Representative FACS plots of lineage$^-$ Kit$^+$ Sca1$^+$ populations (LSK) in the BM of *Nxf1*$^{C587R/+}$ and WT control animals. Gates indicate Kit$^+$ Sca1$^+$ cells amongst lineage$^-$ cells (C) Absolute LSK numbers in *C587R/+* mutant and control animals. (D) Representative plots of Lin$^-$Kit$^+$Sca1$^-$CD34$^-$CD16/32^{low} MEP, Lin$^-$Kit$^+$Sca1$^-$CD34$^+$CD16/32^{low} CMP and Lin$^-$Kit$^+$Sca1$^-$CD34$^+$CD16/32^{high} GMP populations. (E) Absolute numbers in *C587R/+* mutant and control animals. Representative plots (F) and absolute numbers (G) of Lin$^-$Kit$^+$Sca1$^-$CD41$^+$ CD150$^+$ megakaryocyte progenitor (MkP). Representative plots (H) and absolute numbers (I) of myeloid Gr1$^+$ CD11b$^+$ and erythroid Ter119$^+$ CD71$^+$ cells in the bone marrow of *C587R/+* mutant and control animals. Data show mean with SD. (NS) indicates non-significant.

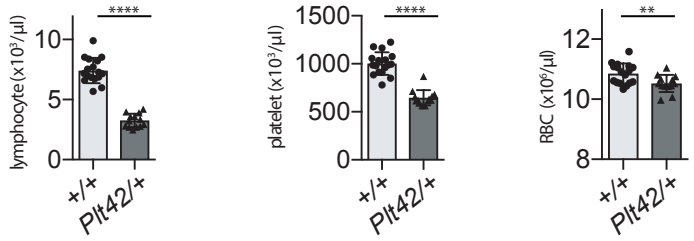
Supplemental Figure 4. Progenitors, myeloid cells and developing lymphocyte populations are normally competitive. BM competitive transplant assay were performed as shown in Figure 4C. 12 weeks-post transplant, the representation of test and competitor cells amongst hematopoietic populations in the bone marrow (A) and thymus (B) was assessed. Data show mean with SD. (*) indicates significance < 0.05.

Supplemental Figure 5. The red blood cell lineage is not affected by the *C587R/+* mutation. (A) Gating strategy for the identification of reticulated Thiazole Orange-positive red blood cells (B) Reticulated red blood cell number as measured by automated Advia analyser. (C) Red blood cell lifespan is unchanged in *C587R* mutant mice. Red blood cells were labelled via iv injection of NHS-Biotin. Data represent mean +/- SD, n=5-6 mice per group.

Supplemental Figure 6. GO analysis (biological process) on transcripts overrepresented in mutant CD8 naïve T cells showed that apoptosis (A) and IFN-signature (B) gene sets were highly enriched.

Suppl. Figure 1

A



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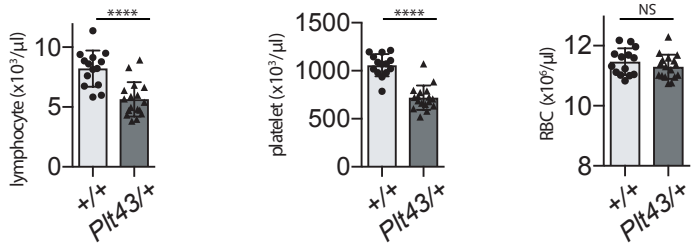

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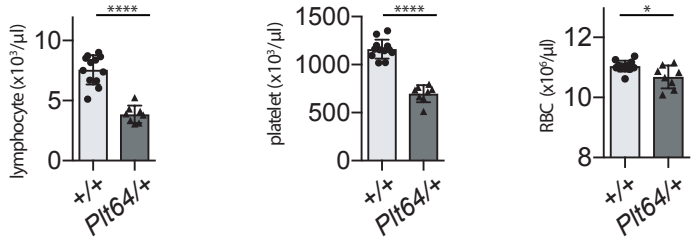

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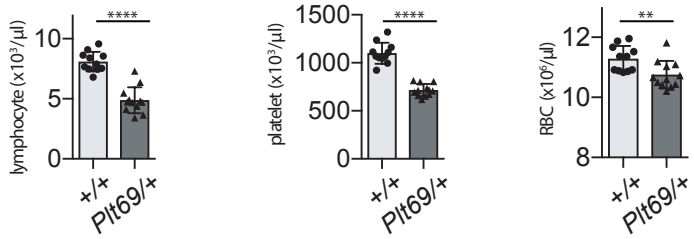

E

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Dm   MIEAMSAQSQMNVIWSRKCLEETNWDFNHAAFVFEK
Mm   MLQAFSTQSGMNLEWSQKCLQDNNWDYTRSAQAFTL
Hs   MLQAFSTQSGMNLEWSQKCLQDNNWDYTRSAQAFTH
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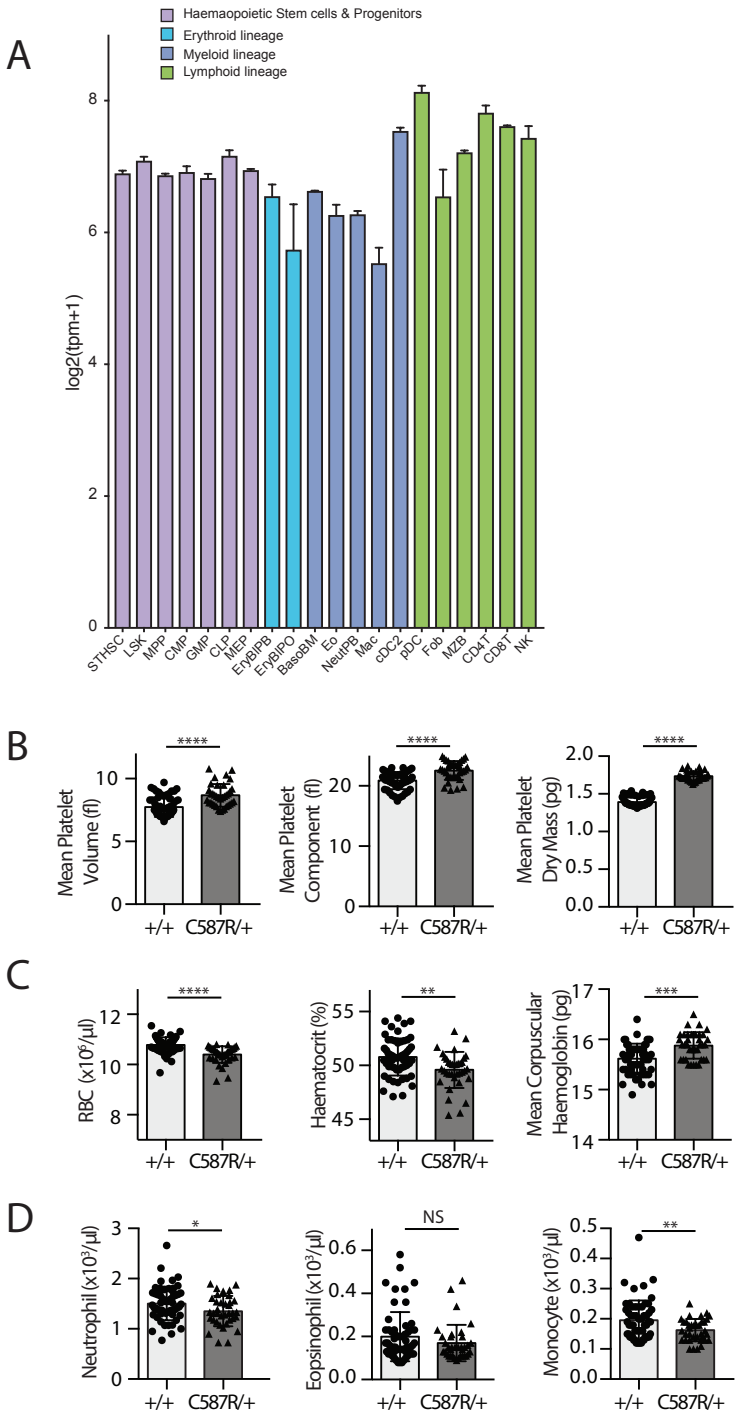
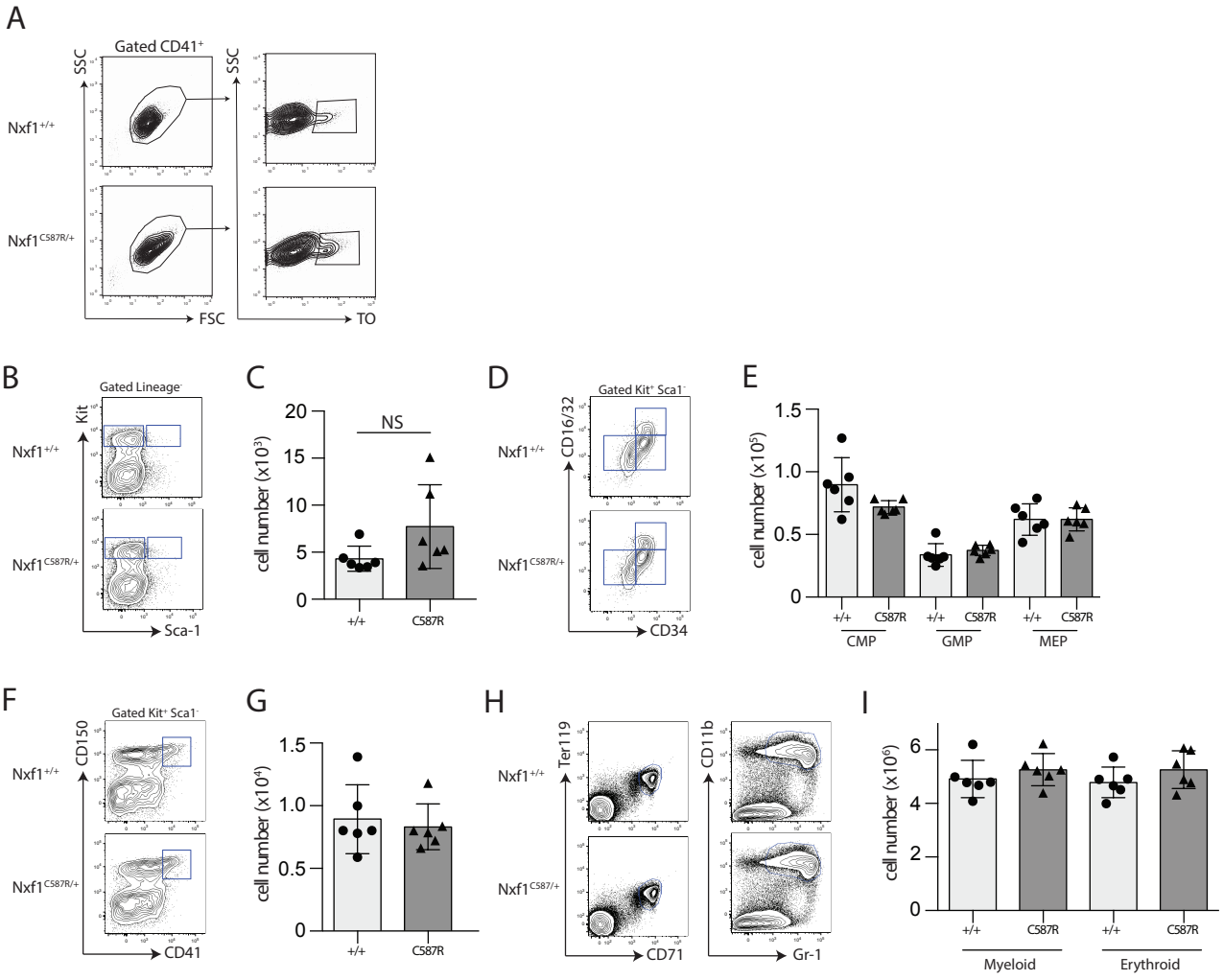
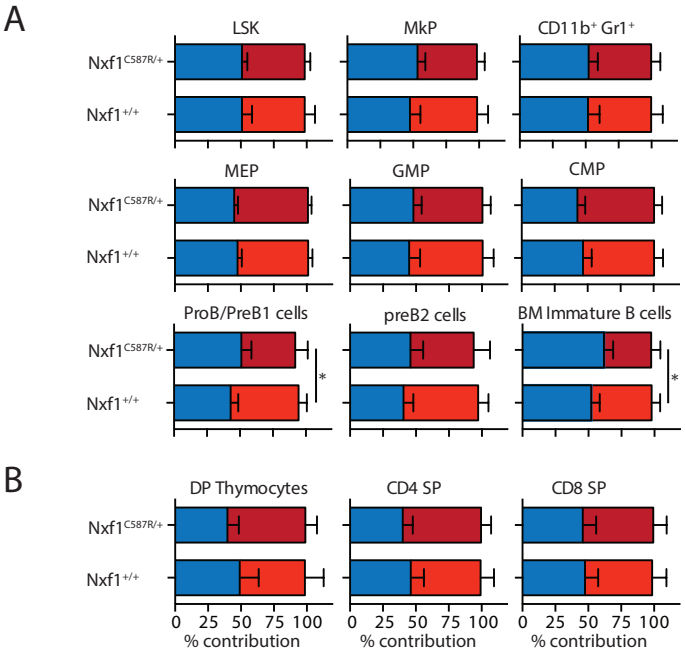
Suppl. Figure 2



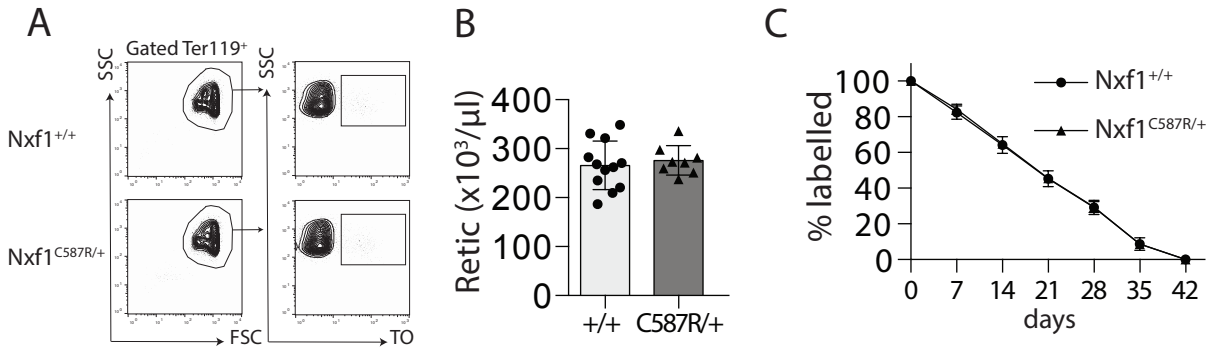
Suppl. Figure 3



Suppl. Figure 4



Suppl. Figure 5



Suppl. Figure 6