SUPPLEMENTAL MATERIAL

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Mouse models. Spi2A-KO mice were prepared as follows. To generate a KO-targeting construct, segments of the *Serpina3g* locus were amplified (by PCR) from C57BL/6 genomic DNA using the following primers: 5′ homology arm, 5′-CAAGTGCGGCCGCGGTTTTTATCTGGGGCACTAATTTGG-3′, plus 5′-CAAGATATCGTGTAGGCAATCCCCCCCATAGGTTCC-3′; 3′ homology arm, 5′-CATAAGCTTGGTGGTGGGCGGGTGAATGATA-AAGC-3′, plus 5′-CAAGCTCGAGGACTTGAGTCTCAGAGGTAAAGGC-3′; exon 4, 5′-GGCGGCCGCCTTCACTGCAACAGCCAGGACTTCC-3′, plus 5′-GGCTAGCGTCACCAGCAAACTTGAAATACTATAC-3′. A floxed Neomycin resistance (Neo^r) segment was PCR amplified with primers 5′-GGCTAGCCCGATCATATTCAATAACCC-3′ and 5′-GCCCGGGCGGTGTAGGCCGCTGGACC-3′. Homology arm segments were cloned and propagated in pBSK (Stratagene). Exon 4 and Neo^r segments were propagated in TOPO pCR2.1 (Invitrogen). An additional loxP insert was formed by annealing phosphorylated oligos 5′-ATCATAACTTCGTATAATGTATGCTATACGAAGTTATGCGGCCGCC-3′ and 5′-AGCTCCGCCGGCGTATT-GAAGCATATCGTATGTAATATGCTTCAATACTA-3′; this insert was cloned to pBSK at EcoRV and XhoI sites. The 5′ homology arm was excised (EcoRV and SpeI) and ligated into the aforementioned pBSK-loxP construct. The exon 4 segment was excised and ligated into the Neo^r-TOPO vector at KpnI and NheI sites. The combined exon 4-Neo^r segment was then excised (NotI and SmaI) and ligated into the 3′ homology arm-pBSK (at NotI and EcoRV sites). Finally, the 5′ homology arm-loxP insert was ligated into the exon 4-Neo^r-3′ homology arm-pBSK vector (at NotI). All targeting vector components were confirmed by DNA sequencing.

This completed targeting vector was then linearized (with XhoI) and electroporated into the C57BL/6 embryonic stem cell line PRX-B6tN-1 (Primogenix). C57BL/6 ES cells were cultured on mitomycin C–treated mouse embryonic fibroblasts. Successfully recombined Neo^r colonies were selected after 1 wk of culture in 125 μg/ml G418. ES cells harboring a targeted (t) *Serpina3g* allele were screened first by Southern blotting. Specifically, genomic DNA was isolated from G418-resistant embryonic stem cell clones and digested with NheI (5′) or EcoRV (3′) for Southern blot analysis. 5′ and 3′ probes were PCR amplified from C57BL/6 genomic DNA using primers 5′-AGCCCAGCACAAGTGTCACCTTTACCC-3′ plus 5′-CTTAGGAAATGTTGATGCAGATAAGC-3′, and 5′-GCCTTTACCTCTGAGACTCAAGTC-3′ plus 5′-AGGTCCTAAAAGCTGAGGTTACTTGG-3′, respectively. Probes were propagated in TOPO pCR2.1 (Invitrogen) and excised with EcoRI, and BamHI plus EcoRV. Probes (100 ng) were labeled using [α^{32}P] dCTP (GE Healthcare) and High Prime reagents (Roche). The 5′ probe was hybridized at 65°C (100 μg/ml denatured salmon sperm DNA, 10% dextran sulfate, 6x SSC, 1% SDS). The 3′ probe was hybridized at 42°C (100 μg/ml denatured salmon sperm DNA, 50% formamide, 10% dextran sulfate, 1 M NaCl, 1% SDS). Two independently targeted ES cell clones were chosen, amplified, and preserved. To remove the floxed Neo^r selection marker, targeted ES cells were transiently transfected with 30 μg of pBS185 (which expresses Cre recombinase from a cytomegalovirus promoter; Life Technologies). Clones containing the floxed (f) allele, but lacking the Neo^r selection marker were PCR-screened and verified by DNA sequencing. Successfully recombined and Cre-processed ES clones carrying the floxed *Serpina3g* allele were injected into C57BL/6 blastocysts. (University of Chicago Transgenic Mouse Facility). Chimeric mice were identified by PCR, and were bred with C57BL/6 mice for germline transmission of the floxed allele to produce mice with a $Serpina3g^{+/f}$ genotype. To delete *Spi2A*, C57BL/6 $Serpina3g^{+/f}$ mice were crossed with C57BL/6 transgenic mice constitutively expressing Cre under an adenovirus EIIa promoter. The Cre transgene was then outbred, and germline cells carrying the deleted allele (Δ) generated $Serpina3g^{+/\Delta}$ mice. Mice were genotyped using PCR from genomic DNA prepared from tail or ear biopsies (digested with XhoI). These mice were inter-crossed to generate homozygous $Serpina3g^{\Delta/\Delta}$ mice (Spi2A-KO mice). Spi2A-KO mice were further crossed with *cathepsin* $B^{-/-}$ mice, also on a C57BL/6 background. C57BL/6 mice were purchased from Charles River. Spi2A-KO mice were crossed with *cathepsin* $B^{-/-}$ mice (also on a C57BL/6 background) to generate Spi2A-KO x cathepsin B KO mice. All mice were maintained in accordance with Maine Medical Center Research Institute or University of Chicago IACUCs, and UK Home Office regulations.

Anemia models. As a hemolytic anemia model, mice were challenged with 75 mg/kg phenylhydrazine i.p. In bone marrow transplantations, B6.Ptprca recipients were irradiated (475 Rad) at 4 h, and 1 h before transplantation. Donor bone marrow cells (5×10^5) were transplanted via retroorbital injection. EPO challenge experiments involved dosing (1,250 U/kg) at 1 and 24 h, unless otherwise indicated. In 5-fluorouracil challenge experiments, dosing was 150 mg/kg (i.p.). In sublethal irradiation experiments, mice received 7.5 Gy and blood counts were determined at the indicated subsequent intervals. For the latter experiments, erythrocyte counts were performed on a flow cytometer for 30 s using a constant flow rate. Erythrocytes were identified by forward and side scatter parameters, and cell counts were calculated based on the flow rate (as determined using AccuCheck Counting Beads; Invitrogen). For peripheral blood leukocyte counts, samples were diluted in 3% acetic acid containing gentian violet (Sigma-Aldrich) and scored (hemocytometer). Hematocrits and reticulocyte levels were determined by capillary microcentrifugation, and thiazole-orange staining (via flow cytometry), or via hematology analyzer determinations (Siemens Advia 2120). BFU-e and CFU-e assays included 100 ng/ml SCF plus 4 U/ml EPO. All procedures used were approved by the IACUC of the Maine Medical Center Research Institute, University of Chicago, and/or UK Home Office.

Primary hematopoietic cells. For ex vivo analyses, erythroid progenitors were prepared from murine bone marrow (or spleen where indicated) and expanded in SP34ex medium as recently described. Purification of developmentally staged erythroid progenitors was accomplished using optimized MACS-based depletion and selection procedures. For experiments involving peroxide exposure, SP34ex erythroid progenitor cultures were exposed at day 3 of expansion to H_2O_2 at the concentrations and times indicated. Frequencies of damaged $Ter119^{pos}$ erythroblasts were then determined by staining with YoPro3. When applied, desferrioxamine dosing lasted for 12 h at 5 μM, with DFO addition at day 3 of EPC culture. For direct analyses by flow cytometry, bone marrow (or spleen) preparations were mechanically dispersed in IMDM (Invitrogen), 2% FBS; gently passed thrice through a 20-gauge needle; sieved (40-μm strainer); washed in PBS; and analyzed without exposure to ammonium chloride (but with gating of red blood cells and reticulocytes).