Supplemental materials and methods

Mice and Transplantation

Mouse breeding/maintenance was performed under the supervision of Cincinnati Children’s Hospital Veterinary Services and all procedures were performed with IACUC approval (IACUC #2017-0021). $\text{Ski}^{+/\text{a}}$ or $\text{Ski}^{-/-}$ mice were backcrossed onto a C57BL/6 background for 8 generations. For fetal liver cell reconstitution analysis, lethally irradiated (1175 rad) C57BL/6-CD45.1 congenic mice were reconstituted with 1x10⁶ E-14.5 FL cells from $\text{Ski}^{+/\text{a}}$ or $\text{Ski}^{-/-}$ mice (CD45.2). For 1:1 competitive repopulation assays, 1x10⁶ E-14.5 FL cells from $\text{Ski}^{+/\text{a}}$ or $\text{Ski}^{-/-}$ mice or 1x10⁶ unfractionated bone marrow cells from 6-week old $\text{Skil}^{+/\text{a}}$ or $\text{Skil}^{-/-}$ mice (CD45.2) were injected intravenously together with 1x10⁶ wild-type E-14.5 FL cells or unfractionated bone marrow cells from 6-week old competitors (CD45.1) into lethally irradiated CD45.1 congenic recipients. For 4:1 competitive repopulation assays, 0.8x10⁶ E-14.5 FL cells from $\text{Ski}^{+/\text{a}}$ or $\text{Ski}^{-/-}$ mice (CD45.2) were injected intravenously together with 0.2x10⁶ wild-type E-14.5 FL competitors (CD45.1) into lethally irradiated CD45.1 congenic recipients. For secondary transplantation, lethally irradiated CD45.1 congenic recipients received 3x10⁶ unfractionated bone marrow cells from primary recipients. Peripheral blood chimerism (CD45.2⁺ cells) was analyzed 1, 2, 3, and 4, months following transplantation by flow cytometry. In addition, donor (CD45.2) contribution to multiple peripheral blood lineages or the HSC compartment was determined at four months post-transplant by flow cytometry.

For homing assays, E-14.5 FL cells from $\text{Ski}^{+/\text{a}}$ or $\text{Ski}^{-/-}$ mice were isolated and stained in a prewarmed solution of DPBS (Gibco, Gaithersburg, MD, USA) containing 2 µM CellTrace CFSE (Thermo Fisher, Carlsbad, CA, USA) at 10x10⁶ cells/mL for 20 minutes at 37°C, protected from light. The staining was stopped by adding 5 times the original staining volume of DPBS + 1% FBS (Atlanta Biologicals, Flowery Branch, GA, USA) then mixing well and incubating at room temperature for 5 minutes prior to centrifugation. Cells were resuspended in DPBS and split for FACS quantitation of CFSE⁺ populations and transplantation of 5x10⁶ cells into each lethally irradiated recipient. Recipients were sacrificed 16 hours later and CFSE⁺ bone marrow populations were quantified by FACS to determine the percentage of homed cells.

Flow Cytometry

Peripheral blood chimerism was monitored by staining blood cells with a cocktail of antibodies: Fc Block (Becton, Dickinson, and Company), Alexa Fluor 700-conjugated anti-CD45.2 (clone 104, BioLegend, Dedham, MA, USA), FITC-conjugated anti-CD45.1 (clone A20, BioLegend), APC-conjugated anti-CD3e (clone 145-2C11, Becton, Dickinson, and Company, Franklin Lakes, NJ, USA), PE-conjugated anti-B220 (clone RA3-6B2, Becton, Dickinson, and Company), Pacific Blue-conjugated anti-CD11b (clone M1/70, BioLegend), and PE-Cy7-conjugated anti-Gr1 (clone RB6-8C5, BioLegend) in FACS Buffer (DPBS + 1% FBS + 0.01% NaN₃). Prior to running flow cytometry, red blood cells were lysed with ACK (Gibco), cells were washed in FACS Buffer, and resuspended in FACS Buffer containing DAPI (Thermo Fisher).

Alternatively, for whole peripheral blood analyses, complete blood counts were calculated using the Hemavet 1700 (Drew Scientific, Miami Lakes, FL, USA) and
peripheral blood smears were stained using a Camco Stain Pak (Cambridge Diagnostic Products, Fort Lauderdale, FL, USA). For reticulocyte analysis, 5 µL of whole blood was stained with Fc Block (Becton, Dickinson, and Company) and mAbs against PE-conjugated anti-CD45.2 (clone 104, BioLegend), Brilliant Violet 605-conjugated anti-CD45.1 (clone A20, BioLegend), and APC-conjugated anti-CD71 (clone R17217, Thermo Fisher), washed with FACS buffer, then resuspended in 300 µL of Retic-Count (Becton, Dickinson, and Company) 30 minutes prior to analysis of CD45^-CD71^ FITC^ reticulocytes.

Hind limb bones were harvested and crushed in FACS Buffer to collect bone marrow cells. If used for immunoblot analysis, bone marrow cells were treated for lineage depletion using the Mouse Lineage Depletion Kit (Miltenyi Biotec, San Diego, CA, USA) according to the manufacturer and separated using an autoMACS Pro (Miltenyi Biotec). The recovered negative fraction was then lysed in laemmli buffer for subsequent immunoblot analysis. If used for FACS, bone marrow cells were washed with FACS Buffer and incubated for 30 minutes on ice with a cocktail of antibodies; Fc Block (Becton, Dickinson, and Company), biotin-conjugated anti-CD3e (clone 145-2C11, BioLegend), biotin-conjugated anti-CD4 (clone RM4-5, Thermo Fisher), biotin-conjugated anti-CD8 (clone 53-6.7), biotin-conjugated anti-CD11b (clone M1/70, Becton, Dickinson, and Company), biotin-conjugated anti-CD19 (clone 6.D5, BioLegend), biotin-conjugated anti-CD127 (clone B12-1, Becton, Dickinson, and Company), biotin-conjugated anti-B220 (clone RA3-6B2, BioLegend), biotin-conjugated anti-Gr1 (clone RB6-8C5, Becton, Dickinson, and Company), and biotin-conjugated anti-Ter119 (clone TER-119, BioLegend). Cells were then washed twice with FACS buffer and incubated on ice overnight (for analysis) or for 30 minutes (for sorting) with a cocktail of antibodies; streptavidin-APC-Cy7 (Becton, Dickinson, and Company), FITC-conjugated anti-CD45.2 (clone 104, BioLegend), APC-conjugated anti-CD117 (clone 2B8, Becton, Dickinson, and Company), PE-Cy7-conjugated anti-Sca-1 (clone D7, Becton, Dickinson, and Company), Pacific Blue-conjugated anti-CD48 (clone HM48-1, BioLegend), and PE-conjugated anti-CD150 (clone TC15-12F12.2, BioLegend). Prior to running analytical flow cytometry, red blood cells were lysed with ACK (Gibco). Cells were washed in FACS Buffer, and resuspended in FACS Buffer containing DAPI (Life Technologies). Flow cytometry analyses were conducted on a FACS LSRII or FACS LSRFortessa (Becton, Dickinson, and Company) and analyzed using FlowJo software (FlowJo, Ashland, OR, USA).

Alternatively, for FACS sorting of proerythroblasts, non-erythroid reduced whole bone marrow cells were stained incubated for 30 minutes on ice with a cocktail of antibodies; Fc Block (Becton, Dickinson, and Company), Brilliant Violet 785-conjugated anti-CD45.2 (clone 104, BioLegend), Brilliant Violet 605-conjugated anti-CD45.1 (clone A20, BioLegend), APC-conjugated anti-CD117 (clone 2B8, Becton, Dickinson, and Company), FITC-conjugated anti-Ter119 (clone TER-119, Becton, Dickinson, and Company), and PerCP-Cy5.5-conjugated anti-CD44 (clone IM7, Becton, Dickinson, and Company). Cells were washed in FACS Buffer, and resuspended in FACS Buffer for subsequent sorting.

Cell sorting was performed on a FACS Aria II (Becton, Dickenson, and Company) or SH800 (Sony Biotechnology, San Jose, CA, USA) and cells were collected in a solution of DPBS + 50% FBS (Atlanta Biologicals).
**Immunoblots**

Laemmli lysates were briefly sonicated and boiled at 100°C for 5 minutes before being subjected to SDS-PAGE. The resolved proteins were transferred onto nitrocellulose membrane (GE Healthcare, Chicago, IL, USA) that was subsequently blocked in 5% nonfat dried milk (Carnation, Seattle, WA, USA) in TBS-T (0.1% Tween 20, Millipore Sigma, Burlington, MA, USA) for 1 hour at room temperature with agitation. After thorough washing in TBS-T, membranes were incubated overnight at 4°C with agitation in a solution of 5% BSA Fraction V/TBS-T (Thermo Fisher) containing rabbit anti-Ski (Millipore Sigma) or goat anti-Gfi1 (R&D Systems) antibody diluted at 1:1,000. Following a wash with TBS-T, membranes were incubated for 2 hours at room temperature with agitation in a solution of 2% nonfat dried milk in TBS-T containing a 1:5,000 dilution of HRP-conjugated secondary antibodies (anti-rabbit from GE Healthcare, anti-goat from Thermo Fisher). Following three washes with TBS-T, chemiluminescent detection of blotted proteins was performed using ECL (Thermo Fisher) and detected using X-ray film (Lab Scientific, Highlands, NJ, USA). Membranes were stripped in Restore Plus (Thermo Fisher) for 10 minutes at room temperature with agitation, then washed in TBS-T, blocked, and incubated as described above with a 1:5,000 dilution of mouse anti-β-Actin (Millipore Sigma) and a 1:10,000 dilution of HRP-conjugated anti-mouse secondary antibody (GE Healthcare).

**TaqMan analysis**

RNA was isolated from whole cell lysates using TriZol (Thermo Fisher) and quantified using a Nanodrop 2000 (Thermo Fisher). cDNA was generated using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and quantitative real-time PCR was performed using a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and TaqMan assay kits for Ski (Mm00448744_m1) and Gapdh (Mm99999915_g1). Relative expression was calculated using the comparative 2^ΔΔCt method.

**Immunofluorescence staining and confocal microscopy**

Human bone marrow sections were collected after obtaining informed consent, as approved by the Institutional Review Board of Albert Einstein College of Medicine. Slides were placed on a warmer and incubated at 50°C for 1 hour to melt the paraffin. Next, slides were placed in 100% xylene three times for 10 minutes each, followed by 100% ethanol three times for 3 minutes each, then 95% ethanol three times for 3 minutes each, and 70% ethanol three times for 3 minutes each before being incubated in PBS for 5 minutes. Slides were then briefly equilibrated in antigen retrieval buffer (1.8 mM citric acid (Millipore Sigma), 8.2 mM sodium citrate (Millipore Sigma) diluted in water and adjusted to a pH of 6.0) before antigen retrieval in a microwave. After cooling to room temperature, slides were rinsed with water and incubated in PBS for 5 minutes. Slides were blocked for 2 hours at room temperature in blocking solution (4% goat serum in PBS-T). Mouse anti-SKI (clone G8, Lifespan Biosciences, Seattle, WA, USA) was diluted 1:50 in blocking buffer and slides were incubated overnight at 4°C. The next day, slides were rinsed three times in PBS-T for 5 minutes each. Goat AlexaFluor488-conjugated anti-mouse IgG1 cross-adsorbed secondary antibody (Thermo Fisher) was
diluted to 1 µg/mL and slides were incubated at room temperature for 1 hour. Slides were rinsed three times in PBS-T for 5 minutes each. After incubation with DAPI for 15 minutes at room temperature, slides were washed as before and 1 drop of Prolong Gold anti-fade mounting medium (Thermo Fisher) was added and slides were covered using Gold Seal Coverslips (Electron Microscopy Sciences, Hatfield, PA, USA). Slides were allowed to cure overnight at room temperature protected from light and imaged on an A1R Inverted microscope (Nikon, Melville, NY, USA). To quantify the results, nd2 files were imported into Nikon Elements software (Nikon) and maximum intensity projections were generated for each 20X image. An algorithm was developed to identify the centroids of DAPI signal and separately AlexaFluor488 signal above background was expanded and then trimmed to identify positive regions. The number of overlapping DAPI centroids and AlexaFluor488 regions was recorded as “SKI-expressing cells,” which was subsequently normalized to the total number of DAPI centroids to calculate the “% SKI-expressing cells” in each field.

**Generation of Ski−/− ES cells and Analysis of Chimeras**

E14 ES cells heterozygous for the Ski targeted allele were treated with 3.5 mg/ml G418 (Takara Bio, Mountain View, CA, USA); this dosage was determined by titration for a dose (between 1-5 mg/ml) that would yield a limited number of vigorously growing, discrete colonies. Colonies were picked after a week and screened for homozygosity at the targeted Ski allele by Southern blotting. Clones were injected into C57BL/6 embryos and transferred into pseudopregnant females. Chimeric offspring from each clone were analyzed at 4-6 weeks of age.

Isozymes of glucose phosphate isomerase (Gpi) which distinguish between C57BL/6 (Gpi-BB) or 129P3 (Gpi-AA) were analyzed as previously described. Briefly, tissue or cell lysates were prepared by freeze-thawing in water, but splenocytes and thymocytes were first separated from the stroma by gentle trituration and collection in PBS. Lysates were electrophoresed and isozyme bands visualized. Contribution of the ES cells was estimated by densitometry in comparison to standard mixtures of tissues from the 2 parental strains. In some cases, contribution was also quantified by Southern blotting of tissue samples that were lysed according to standard protocols, probed, and quantified on a phosphorimager, as previously described.

**Luciferase Sensor Assay**

The Ski 3’UTR segment from the stop codon to +500 3’ UTR which contains the miR-21 target site was amplified using the primers 5’-CACAGCACAACGTTACCG-3’ and 5’-CTGAAGAAAAACCCCTGCTG-3’ and cloned into the TA-TOPO vector (Thermo Fisher). An XbaI digested fragment was then inserted into the pGL4 Firefly vector (Promega, Madison WI, USA) immediately downstream from the luciferase stop codon. Mutation was accomplished with the Quick change II site directed mutagenesis kit (Stratagene, La Jolla, CA, USA) and confirmed by Sanger sequencing. Cells grown in 6 well plates were co-transfected with 0.5 µg of firefly luciferase reporter vector and 0.5 µg of the pRL-TK control vector containing Renilla luciferase (Promega) using Lipofectamine 2000 (Thermo Fisher) according to manufacturer’s specifications. One day later, Firefly and Renilla luciferase activities were measured consecutively using the
Muench et al.

dual luciferase assay (Promega) using Lumat LB 9507 (Berthold Technologies, Oak Ridge, TN, USA) to calculate normalized luciferase activities.

**Viral Vectors**

For virus production, the retroviral packaging and envelope plasmid pCL-Eco was used in combination with the viral vectors of interest. The retroviral mir21 expression vector was described previously.

**Cell Culture and viral transduction**

The MCF7 human breast cancer cell line was grown in DMEM (Gibco) supplemented with 10% FBS (Atlanta Biologicals). Freshly isolated murine lineage negative bone marrow cells were prestimulated in IMDM (Thermo Fisher) supplemented with 100 U/mL Penicillin-Streptomycin (Thermo Fisher), 10% BIT (Stemcell Technologies, Vancouver, BC, CA), 20 ng/mL IL-3, 20 ng/mL IL-6, and 50 ng/mL SCF (Miltenyi Biotec) at 37°C, 5% CO2 for 48 hours.

Retroviral particles were generated as described previously. Briefly, Lenti-X 293T cells (Takara Bio) were seeded one day prior to transfection with vectors and packaging/envelope plasmids using Transit-LT1 (Mirus Bio, Madison, WI) according to manufacturer instructions. Growth medium was changed the following day. Virus-containing supernatant was collected 48 and 72 hours post-transfection and immediately concentrated with a Vivaspin 20 column (MWCO 100,000, Sartorius, Bohemia, NY, USA) by centrifugation at 3000 g at 4°C. Well plates were previously coated with 20 µg/mL retronectin (Takara Bio) overnight at 4°C, blocked with a solution of 5% BSA Fraction V (Thermo Fisher) in sterile DPBS for 1 hour at room temperature, then washed 3 times with DPBS. Viral solution was centrifuged in well plates at 1000 g for 2 hours at 4°C. Supernatant was discarded and the prestimulated cells were added in fresh media to the well plate then centrifuged at 400 g for 30 minutes at room temperature before being transferred to the incubator for overnight culture. This process was repeated the next day, and cells were selected in medium containing 1.5 µg/mL puromycin (Invivogen, San Diego, CA, USA) beginning at 48 hours after the second transduction.

**Single cell RNA-Sequencing**

FACS-sorted CD45.2+ SLAM HSC purified from the bone marrow of lethally irradiated recipients (CD45.1+) at 6 months post-transplant were captured using the C1 Single-Cell Auto Prep System (Fluidigm, San Francisco, CA, USA) according to the manufacturer’s instructions. Briefly, cells were counted and diluted in DPBS to 333 cells/µL then loaded onto a primed C1 Single-Cell Auto Prep Integrated Fluidic Chip for mRNA-seq (5-10 µm). Separated cells were visually scored to confirm successful capture of single cells. Cells were lysed on chip and reverse transcription was performed using the SMARTer mRNA-Seq Kit (Takara Bio) according to manufacturer’s instructions. Next, cDNAs were transferred to a 96-well plate and diluted with 5 µL C1 DNA Dilution Reagent (Fluidigm) before quantitation using the Quant-it PicoGreen dsDNA Assay Kit (Thermo Fisher) and the Agilent High Sensitivity DNA Kit (Agilent Technologies, Santa Clara, CA, USA). Samples with an initial cDNA concentration of greater than 180 pg/µL were diluted to 100 pg/µL and a total of 125 pg of cDNA was
tagmented at 55°C for 20 min using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA). The resulting libraries were pooled and purified using the AMPure bead-based magnetic separation then quantified using the Qubit dsDNA HS Assay Kit (Life Technologies) and Agilent High Sensitivity DNA Kit (Agilent Technologies) prior to sequencing.

RNA-Seq Analyses
Gene-quantified bulk MDS RNA-Seq data was obtained from the GEO database (GSE111085) in addition to associated FASTQ files for alternative splicing analyses. FASTQs were aligned to the human hg19 reference genome and transcriptome using the STAR software. Alternative splicing was quantified from STAR aligned BAM files using the MultiPath-PSI algorithm in AltAnalyze version 2.1.1. For the differential splicing analysis, events were filtered for event detection in 75% of the samples in each sample group (SKI-high, SKI-low, healthy) along with a change in the Percent Spliced-In (PSI) values between the two groups greater than 10% (dPSI > 0.1, p<0.05, two-tailed, following FDR adjustment). Assembled ENCODE splicing factor knockdown (PRJNA30709), Leucegene (GSE67040, GSE49642, GSE52656) and progenitor cell-type (GSE74246) associated splicing signatures were evaluated using the same algorithms and statistical thresholds. Concordant splicing events were assessed by comparison of unique splicing-event clusters in the reference datasets compared to MDS SKI-comparisons, regulated in the same or opposite directions (dPSI).

For scRNA-Seq analysis, we aligned bone marrow and fetal liver HSC single-cell libraries to the mouse transcriptome using the software RSEM, to obtain transcript per million estimates, and excluded outlier libraries as previously described. Prior to supervised cell-cycle clustering analyses, the bone marrow HSC single-cell gene expression data was filtered for expressed and variable genes using the ICGS workflow. In parallel, alternative splicing analyses were performed using the MultiPath-PSI workflow for single-cell data, requiring detection of splicing in at least 15% of the single-cell libraries of the k-nearest neighbor assigned cell populations (dPSI > 0.1, p<0.05, two-tailed) or a dPSI >0.1 for the pseudo-bulk library (merged BAM files) comparisons of these classified cell populations. Human and mouse orthologous splicing events from MultiPath-PSI were identified using the UCSC genome batch lift-over software and custom python code to match liftover exon-exon or exon-intron junction genome positions.

Gene-set enrichment analyses were performed using the software GO-Elite in AltAnalyze using the default options. Principal component and heatmap analyses were additionally performed in the AltAnalyze toolkit. A conservative set of TGFβ signaling pathway genes were determined by comparing pathway membership among TGFβ associated gene-set categories between the Gene Ontology, KEGG, WikiPathways and PathwayCommons, where a gene had to be reported in at least two of these sources.

CLIP-Seq and Binding-Site Enrichment Analyses
To evaluate splicing factor regulation, we examined CLIP-Seq data from the ENCODE project using a modification of a recently published method for intersecting genomic loci with ChIP-seq datasets. We first identified alternatively spliced exons plus flanking intron sequences (+500nt and -500nt) in the SKI-high MDS signature genes and stored them in BED file format. We also assembled 318 K562 and HepG2 cell line ENCODE
CLIP-Seq datasets in the form of processed BED files. Using the RELI algorithm, the genomic coordinates of the MDS signature gene alternative exons plus flanking intron sequences were systematically intersected with the genomic coordinates of the peaks contained within each of the CLIP-seq datasets, and the number of intersections for each dataset was tabulated. To determine the likelihood that the number of observed CLIP-Seq peak intersections for each dataset is likely to occur by chance alone, RELI performs an enrichment analysis for each CLIP-Seq dataset by randomly selecting RefSeq exons plus flanking intron sequences and repeating the intersection procedure. A total of 2,000 permutations were performed for each CLIP-Seq dataset, and the observed intersection counts were compared to the resulting distribution using a standard Z-score transformation. Z-scores were then converted to P-values and corrected for multiple testing using Bonferroni’s method.

RNA recognition element enrichment analysis was performed using the exonic queried regions as input. These sequences were scanned using a large database of RNA recognition element motif models in the form of position frequency matrices (PFMs) taken from the CisBP-RNA database. These models were used for motif enrichment analysis using the HOMER software package, which performs enrichment for both known and de novo RBP RNA recognition elements.

Supplemental References


**Supplemental Figure Legends**

**Figure S1.** A *SKI*-correlated gene signature effectively segregates a major subset of MDS patients from healthy controls in separate datasets. (A) Heatmap of gene expression in bulk RNA-Seq data of CD34+ bone marrow cells (n = 23 Healthy donors, 44 MDS patients). Each column represents one sample and each row represents one gene. Gene expression clusters (top) were generated in AltAnalyze, the clinical characterizations are noted (bottom), and key TGFβ pathway genes are indicated (right). (B) Heatmap of gene expression in microarray data of CD34+ bone marrow cells (n = 183 MDS patients). Each column represents one sample and each row represents one gene. The clinical characterizations are noted (bottom), and key genes are indicated (right). (C) GSEA enrichment plot using the *SKI*-high gene signature derived from RNA-Seq data in Figure 1B for the microarray dataset in Figure S1B (nominal P = 0.0097, FDR P = 0.0109).

**Figure S2.** *Ski* is expressed in LT-HSC and regulates long-term HSC fitness but not homing to the bone marrow. (A) Log2 expression values of *Ski* or *Skil* in murine hematopoietic cell populations. (B) Total cell counts of E-14.5 whole FLs. (C) Total cell counts of E-14.5 FL populations. LSK; Lineage negative, Sca-1+ c-Kit+. (D) The percentage of SLAM HSC within the whole FL. (E) Representative FACS plots of murine peripheral blood and quantitation of donor (CD45.2+) contribution to each peripheral blood lineage (n = 5-6 mice/group). (F) Percentage of donor derived cells in the indicated peripheral blood populations from noncompetitive transplants. (See Figure 3B and Figure S2E. (G) Representative FACS plots of murine bone marrow populations. SSC-A scale is linear and all fluorescent scales are log10. SSC-A; Side Scatter Area, LSK; Lineage-, Sca-1+, c-Kit+. (H) Whole bone marrow cells (from Figure 1F) were transplanted into secondary recipients (n = 6 mice per group) and subsequently analyzed for the percentage of donor contribution to peripheral blood chimerism and total number of donor SLAM HSC. (I) CFSE-labeled E-14.5 FL cells were assessed by FACS at 16 hours post-transplant for homing efficiency to the bone marrow of recipients to quantify the homing percentage of whole FL, homing percentage of SLAM HSC, or the total number of homed SLAM HSC (n = 5 donors and 1 recipient per donor). Data in (A) are displayed as mean ± s.e.m and were obtained from the Bloodspot Database. Data in (B-D) are displayed as mean ± s.e.m and are representative of 5 independent biological replicates (n = 5 mice per group). ** P < 0.01. Data in (F, H, and I) are displayed as mean ± s.e.m. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

**Figure S3.** Classification of *Ski−/−* SLAM HSC subpopulations. (A) Heatmap of gene expression in scRNA-Seq data of SLAM HSC from wild type 4 week old mice (n = 106 cells). Each column represents a single cell and each row represents one gene. Clusters (top), groups (bottom), and key genes (right) are indicated. (B) Heatmap of gene expression in scRNA-Seq data of SLAM HSC (n = 200 cells). Each column represents a single cell and each row represents one gene. Clusters (top), groups
(bottom), and key genes (right) are indicated. (C-D) Scatter plots displaying the FDR versus Z-score of individual biological processes enriched for the differentially expressed genes in $Ski^{-/-}$ HSC compared to $Ski^{+/+}$ HSC. Each dot represents a biological process and the dotted line indicates an FDR $P$ value of 0.05. (E) Representative cytospins of FACS-sorted proerythroblasts (CD45^- Ter119^+ CD44^high) from the bone marrow of recipients of $Ski^{+/+}$ or $Ski^{-/-}$ FL cells at 10 months post-transplant. The scale bars represent 10 $\mu$m.