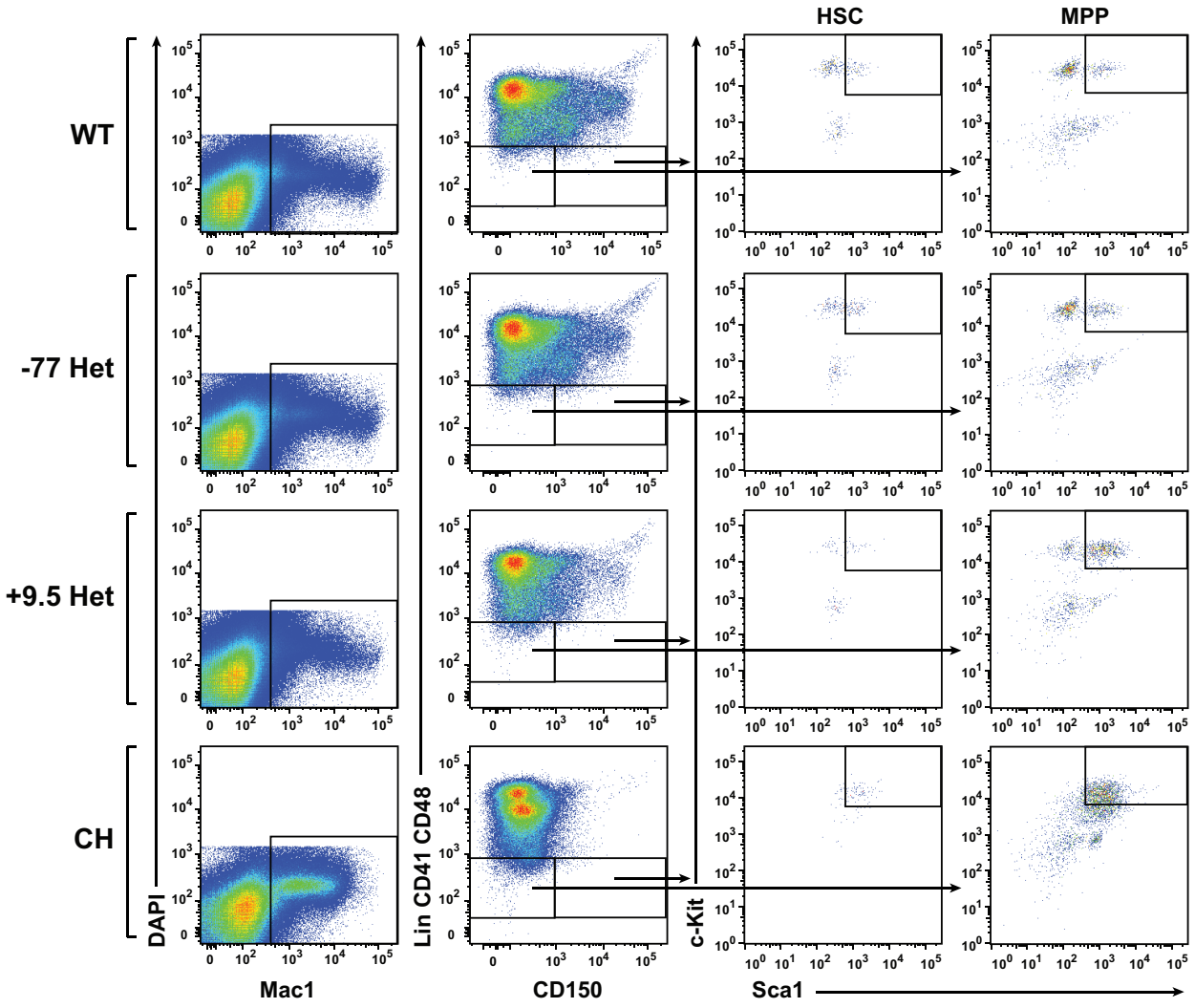

Figure S1. Flow Cytometric Analysis of E13.5 Fetal Livers for HSPCs. Related to Figure 2.
Representative flow plots for HSCs ($Lin^-Mac1^+CD41^-CD48^-CD150^+Sca1^+Kit^+$) and MPPs ($Lin^-Mac1^+CD41^-CD48^-CD150^-Sca1^+Kit^+$).

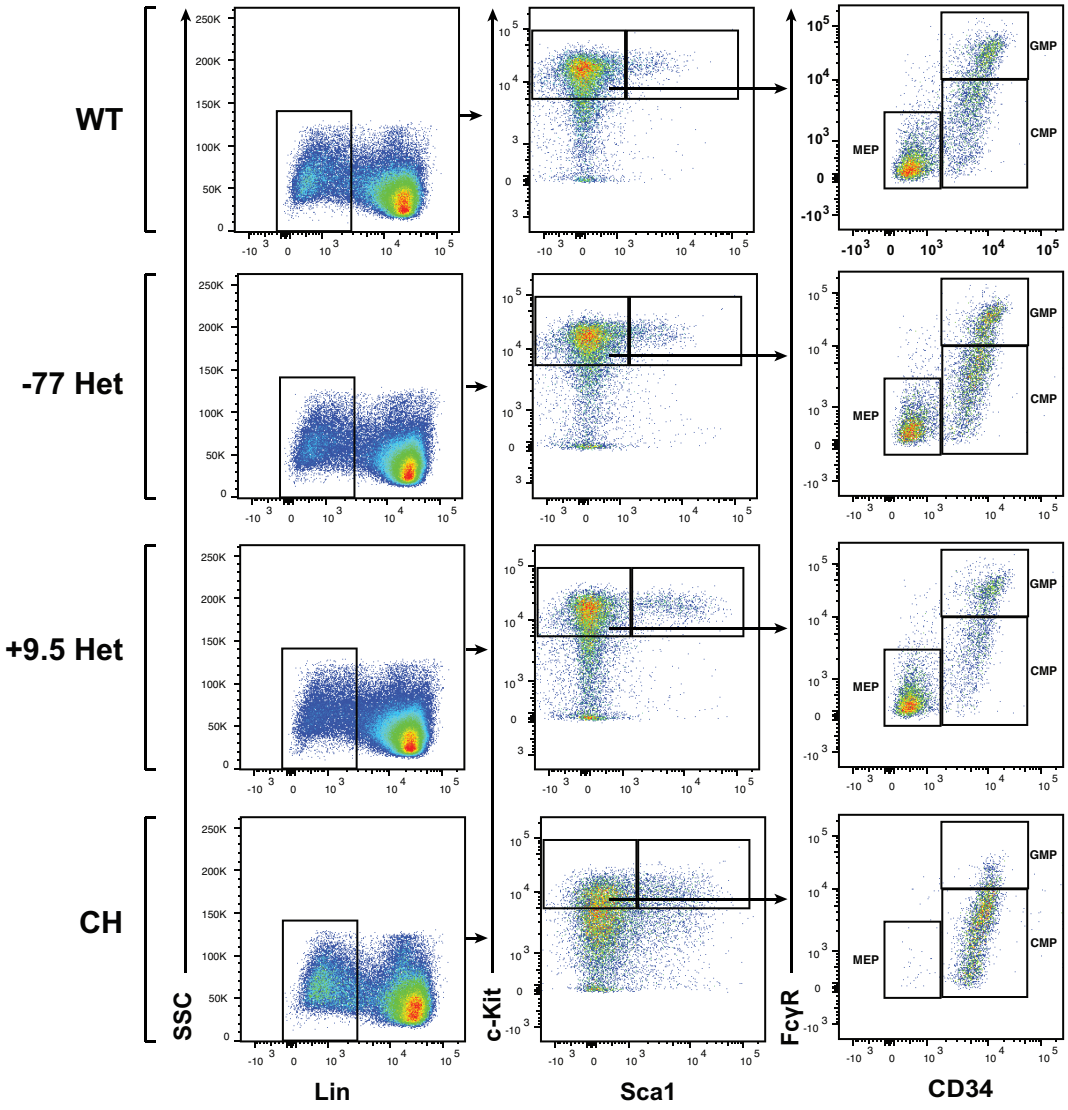

Figure S2. Flow Cytometric Analysis of E13.5 Fetal Livers for Myeloid Progenitors. Related to Figure 2.
Representative flow plots for CMPs ($Lin^-CD34^+FcgR^{low}Kit^+Sca1-$), GMPs ($Lin^-CD34^+FcgR^{high}Kit^+Sca1^-$) and MEPs ($Lin^-CD34^-FcgR^{low}Kit^+Sca1^-$).

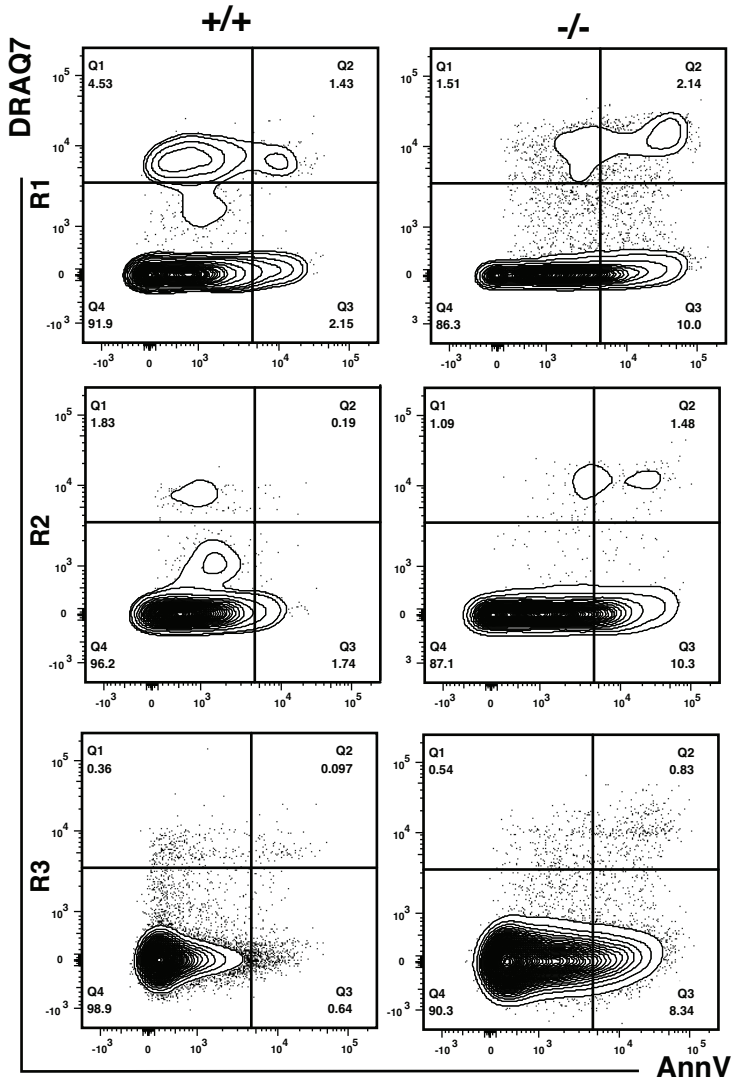

Figure S3. Flow Cytometric Analysis of Apoptosis in E14.5 Fetal Livers. Related to Figure 3.
Representative flow plots for live (AnnexinV$^-$DRAQ7$^-$), early apoptotic (AnnexinV$^+$DRAQ7$^-$), late apoptotic (AnnexinV$^+$DRAQ7$^+$) and dead (AnnexinV$^-$DRAQ7$^+$) cells.

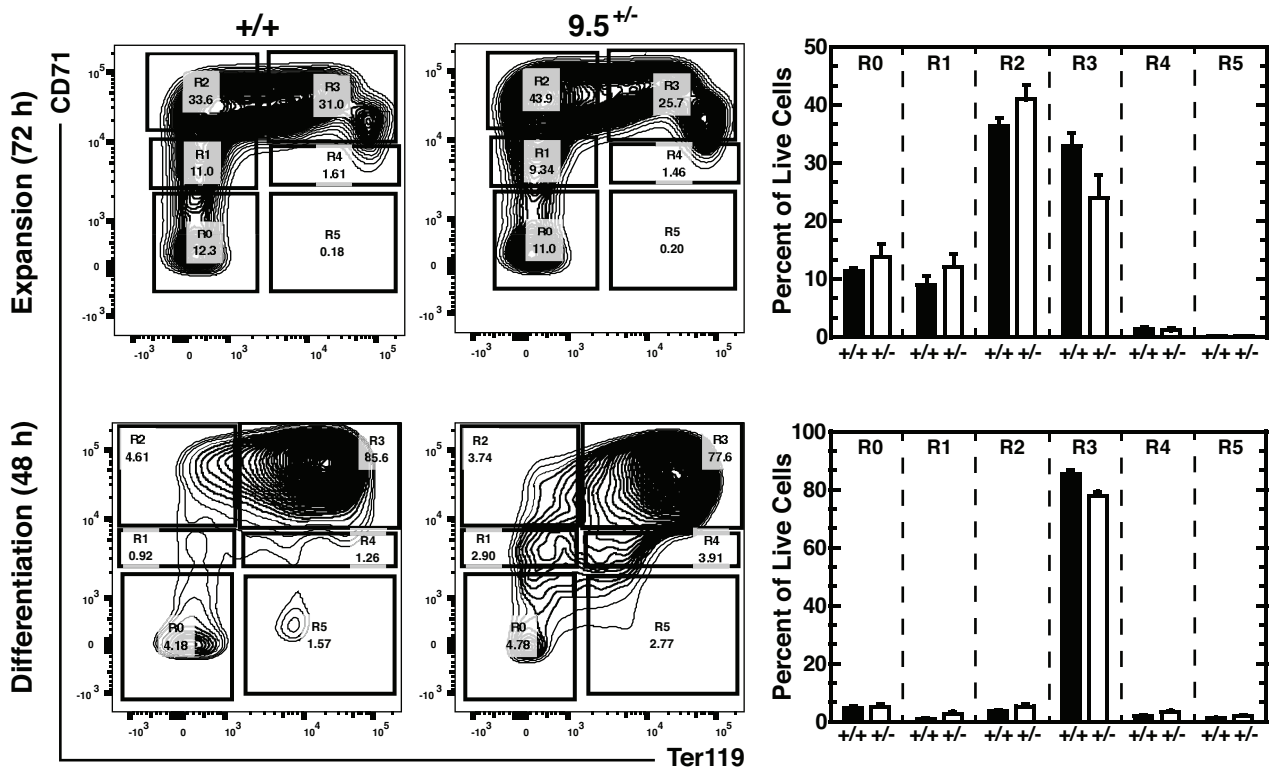

Figure S4. Flow Cytometric Analysis of +9.5$^{+/-}$ Erythroid Differentiation. Related to Figure 4.
Representative flow cytometric analysis and quantitation of R0-R5 populations from E14.5 +9.5$^{+/+}$ and +9.5$^{+/-}$ lineage-depleted fetal liver cells cultured in expansion or differentiation conditions (+9.5$^{+/+}$ [n = 3]; +9.5$^{+/-}$ [n = 7]).

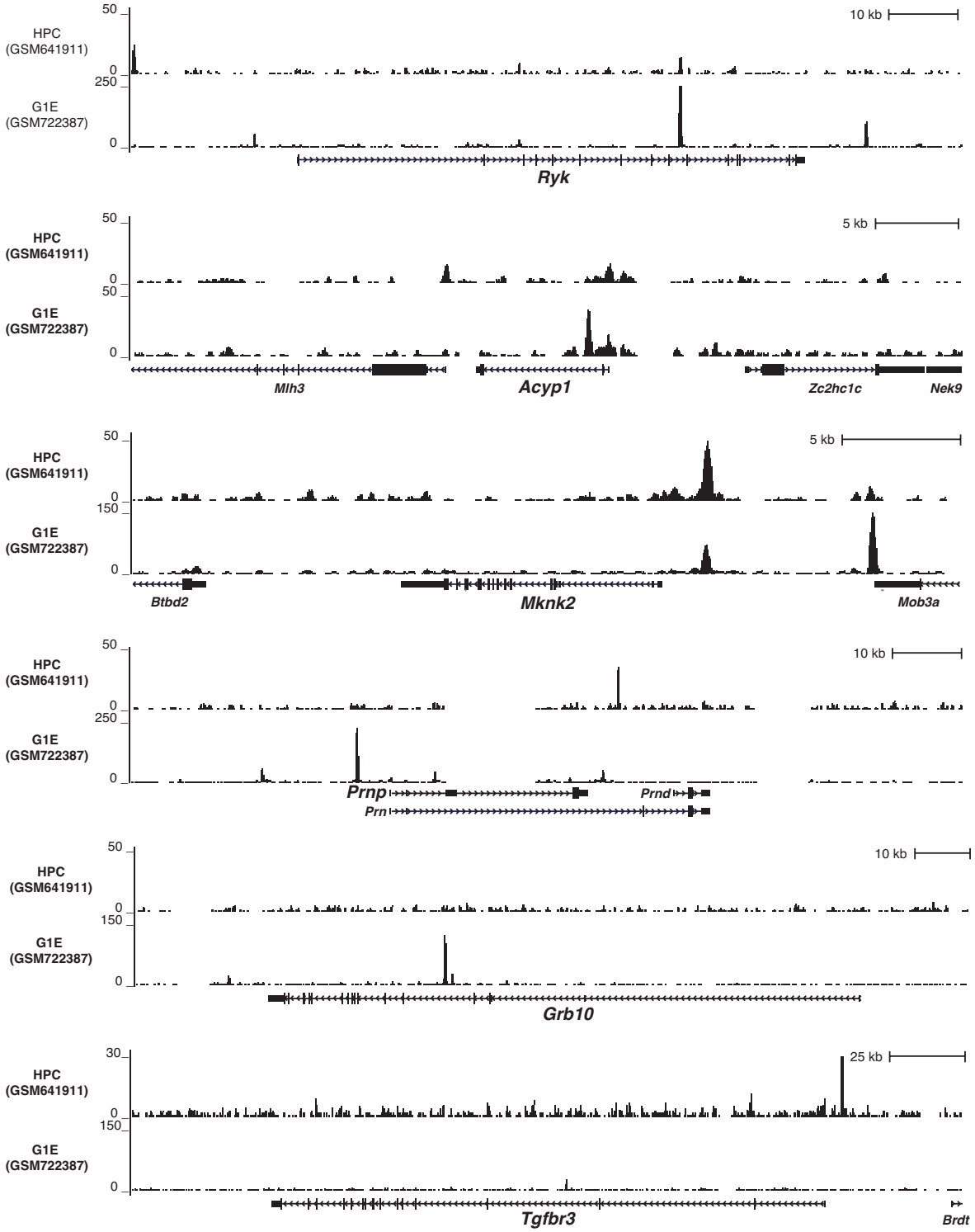

Figure S5. GATA-2 ChIP-seq Profiles for GATA-2 Target Genes. Related to Figure 6.
GATA-2 ChIP-seq profiles were mined from existing datasets (GSM641911 and GSM722387) representing GATA-2 occupancy in primary murine hematopoietic progenitor cells (HPC) and GATA-1-null G1E erythroid precursor cells.

Supplementary Table 1: Primer Sequences. Related to Experimental Procedures.

Genotyping primers

-77 enhancer	5'-GGTATGTCGTGGGAGGCTGTTGA-3' 5'-GTTCTGCCCACCGCACAGCA-3'
+9.5 enhancer	5'-ATGTCCTTTCGGATCTCCTGCC-3' 5'-GGTAAACAGAGCGCTACTCCTGTGTGTT-3'

Real-time PCR primers

18S	5'-CGCCGCTAGAGGTGAAATTCT-3' 5'-CGAACCTCCGACTTTCGTTCT-3'
Gata2	5′-GCAGAGAAGCAAGGCTCGC-3′ 5′-CAGTTGACACACTCCCGGC-3′
Gata1	5'-GGCCCAAGAAGCGAATGATT-3' 5'-GGTTCACCTGATGGAGCTTGA-3’
Zfpm1	5’-CCTTGCTACCGCAGTCATCA-3’ 5’-ACCAGATCCCGCAGTCTTTG-3’
Kit	5'-AGCAATGGCCTCACGAGTTCTA-3' 5'-CCAGGAAAAGTTTGGCAGGAT-3'
Grb10	5’-CAGAGTGCTGCCCTGGAAGA-3’ 5’-TGGGCTTTGGCTGCTTAGGA-3’
Tgfbr3	5'-CCTGGTGTGGCATGTGAAGA-3' 5'-GACCACAGAACCCTCCGAAA-3'
Ryk	5'-TGCAACGCCTATCACCAGTT-3' 5'-CCTTGGCTTTGGCTTCCAGA-3'
Acyp1	5'-GGGCGGTTGATCTGAAGG-3' 5'-CGTAATCCACTGAGACCAAGG-3'
Mknk2	5'-GGGAGGTGGAGATGCTGTA-3' 5'-CTATGGATGTGGCTTAGGATGG-3'
Prnp	5'-CTTCCTCATCTTCCTGATCGTG-3' 5'-GGGACACAGAGAAGCAAGAAT-3'

Supplemental Experimental Procedures

Apoptosis

To quantify apoptosis after CD71/Ter119 staining, cells were washed in Annexin V Buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM CaCl2, pH 7.4) and stained with Annexin V-Pacific blue (ThermoFisher, A35122) (1:40) and DRAQ7 (Abcam, ab109202) (1:100) for 20 min in the dark at room temperature. To detect intracellular p-Akt, Ter119$^-$ cells were isolated from E14.5 fetal livers using magnetic beads (Biolegend, 76447). Cells were serum-starved in 1% BSA (in IMDM for 1 h at 37°C before stimulation with 10 ng/ml SCF for 10 min and fixed in 2% paraformaldehyde for 10 min at 37°C. After permeabilization overnight at -20°C in 95% methanol, cells were incubated for 1 h in HBSS (Cellgro, 21-021-CV)/4% FBS at 4°C. Cells were stained with rabbit phospho-Akt (Cell Signaling Technology, 4060S) (1:200) for 30 min before incubation in donkey anti rabbit-APC (Jackson Immunoresearch, 711-136-152) (1:200), Kit-PEcy7 (25-1171-82) (1:50) and CD71-PE (Biolegend, 113808) (1:50) for 30 min at room temperature. Cells were washed twice in PBS before analysis. DAPI (Biolegend, 422801) or DRAQ7 (Abcam, ab109202) were used for live/dead discrimination. For fixed cells, Zombie UV (Biolegend) staining discriminated dead cells. After staining, cells were washed once in PBS containing 10% FBS or PBS containing 2% FBS, 10 mM glucose and 2.5 mM EDTA. The stained cells were analyzed on a LSR II or LSR Fortessa flow cytometers (BD Biosciences) or collected on a FACSAria II cell sorter (BD Bioscience). The data were analyzed using FlowJo v10.1 software (TreeStar).

Protein Analysis

Equal numbers of sorted cells harvested and boiled for 10 min in sodium dodecyl sulfate (SDS) lysis buffer (50 mM Tris (pH 6.8), 2% β-mercaptoethanol, 2% SDS, 0.1% bromophenol blue, 10% glycerol) were resolved in 10% SDS-polyacrylamide gel electrophoresis and analyzed by semiquantitative western blotting with ECL Plus (Pierce) or with West Femto (Pierce) with rabbit polyclonal GATA-1 (Im et al., 2005) or mouse β-actin (Cell Signaling Technology, 3700S).

Whole-embryo Confocal Microscopy

Embryos were fixed, stained and analyzed as described (Gao et al., 2013; Yokomizo et al., 2012). Embryos were stained with biotinylated anti–PECAM-1 (BD Biosciences, 553371) and anti–c-Kit (BD Biosciences, 553352) antibodies. Samples were mounted in a 1:2 mix of benzyl alcohol (Sigma-Aldrich, 402834) and benzyl benzoate (Acros Organics, 105862500) (BABB) to increase tissue transparency and visualized with a Nikon A1RS Confocal Microscope. Three-dimensional reconstructions were generated from Z-stacks (80–200 optical sections) using Fiji software.

Primary Cell Isolation and Culture

Erythroid precursors were enriched from E14.5 fetal livers with EasySep negative selection Mouse Hematopoietic Progenitor Enrichment Kit (StemCell Technologies). Fetal liver cells were resuspended (5 x 10^6 cells/ml) in phosphate-buffered saline

(PBS) containing 2% FBS, 2.5 mM ethylenediamine tetraacetic acid (EDTA) and 10 mM glucose. EasySep Mouse Hematopoietic Progenitor Enrichment Cocktail was added at 50 µl/ml supplemented with 2.5 µg/mL biotin-conjugated CD71 antibody (eBioscience, 130711). After 15 min on ice, cells were washed by centrifugation for 5 min at 1200 rpm at 4°C and resuspended at 5 x 10^6 cells/ml in PBS containing 2% FBS, 2.5 mM EDTA and 10 mM glucose. EasySep Biotin Selection Cocktail was added at 100 µl/ml. After 15 min at 4°C, EasySep Mouse Progenitor Magnetic Microparticles were added at 50 µl/ml. After 10 min at 4°C, cells were resuspended to 2.5 ml and incubated for 3 min with a magnet. Unbound cells were cultured for 72 h. For expansion, erythroid precursors were maintained at a density of 2.5-3.5 x 10^5 cells/ml in StemPro-34 (Gibco, 10639-011) containing 10% nutrient supplement (Gibco, 10641-025), 2 mM L-glutamine (Cellgro), 1% penicillin-streptomycin (Cellgro, 30-002-CI), 100 µM monothioglycerol (Sigma), 1 µM dexamethasone (Sigma), 0.5 U/ml of Epo and 1% conditioned medium from a Kit ligand-producing CHO cell line.

For differentiation, cells were switched into ES IMDM (glutamine-free) (Hyclone) containing 10% FBS (Gemini), 10% PDS (Animal Technologies), 5% PFHM II (Gibco), 2 mM L-glutamine (Cellgro), 1% penicillin- streptomycin (Cellgro), 100 µM monothioglycerol (Sigma), and 5 U/ml of Epo at 1 x 10^6 cells/ml. Cells were grown in a humidified incubator at 37°C with 5% carbon dioxide. All percentages are v/v unless noted.

To downregulate *Ryk* mRNA, MiR-30-context shRyk 927 TGCTGTTGACAGTGAGCGAACCCAGTATCTGAGAGCTGACTAGTGAAGCCACAGATGTAGTCAGCTCTCAGATACTGGGTCTGCCTACTGCCTCGGA and shRyk 1617 TGCTGTTGACAGTGAGCGCCTGGAAAGTCTGGTTAATAATTAGTGAAGCCACAGATGTAATTATTAACCAGACTTTCCAGATGCCTACTGCCTCGGA shRNAs were cloned into MSCV-PIG vector (from Dr Mitchell Weiss). shRNA-expressing retrovirus were assembled in 293T cells as described (DeVilbiss et al., 2015). Lin^- E14.5 fetal liver cells were spinfected with 100 µl of retrovirus supernatant and 8 µg/ml polybrene in 400 µl of expansion media at 1315 *x* g for 90 min at 30°. After spinfection, 0.5 ml expansion media was added and incubated at 37°in 5% CO_2. After 24 h, equal numbers of cells/condition were plated in M3434 media for colony enumeration.

Flow Cytometry

Fetal liver cells from E13.5 or E14.5 embryos were dissociated and resuspended in PBS with 2% FBS and passed through 25 µm cell strainers to obtain single-cell suspensions prior to antibody staining. Early hematopoietic populations analyzed were fetal liver HSCs ($Lin^-CD41^-CD48^-Mac1^+CD150^+Kit^+Sca1^+$) and MPPs ($Lin^-CD41^-CD48^-Mac1^+CD150^-Kit^+Sca1^+$). All antibodies were purchased from eBioscience unless stated. Lineage markers for the populations were stained with FITC-conjugated antibodies B220 (11-0452), CD3 (11-0031), CD4 (11-0041), CD5 (11-0051), CD8 (11-0081), CD41 (11-0411), CD48 (11-0481), Gr-1 (11-5931), and TER-119 (11-5921). Other surface proteins were detected with PE-conjugated CD150

(Biolegend, 115904; PE-Cy7–conjugated Mac1 (25-0112); PerCP-Cy5.5–conjugated Sca1 (45-5981); and allophycocyanin-conjugated (APC-conjugated) c-Kit (2B8) antibodies. Analysis of fetal liver $Lin^-Kit^+Sca1^+$, $Lin^-Kit^+Sca1^-$, CMPs ($Lin^-CD34^+FcR^{low}Kit^+Sca1^-$), GMPs ($Lin^-CD34^+FcR^{high}Kit^+Sca1^-$), and MEPs ($Lin^-CD34^-FcR^{low}Kit^+Sca1^-$) was conducted as described (Johnson et al., 2015). Lineage markers were stained with FITC-conjugated B220, CD3, CD4, CD5, CD8, CD19 (11-0193), IgM (11-5890), Il7Ra (11-1271), AA4.1 (11-5892) and TER-119 antibodies. Other surface proteins were detected with PE-conjugated FcγR (12-0161); eFluor 660-conjugated CD34 (50-0341), PerCP-Cy5.5–conjugated Sca1 (45-5981), and PE Cy7-conjugated c-Kit (Biolegend, 105814). For erythroid precursor analysis, 1 x 10^7 cells were stained with Ter119-APC (Biolegend, 116212), CD71-PE (Biolegend, 113808) at 4°C for 30 min. Contaminating myeloid and lymphoid cell types were quantitated using Mac1-APCe780 (47-0112-82), Gr1-PE-Cy7 (Biolegend, 108416), B220-FITC (11-0452) and CD4-FITC (11-0041) and CD8-FITC (11-0081). After staining, cells were washed with 2% FBS, 10 mM glucose and 2.5 mM EDTA in PBS and analyzed on a LSR II flow cytometer (BD Biosciences) or collected on a FACSAria II cell sorter (BD Biosciences).

Gene Expression Analysis

Total RNA was purified from fetal livers or sorted cells using TRIzol (Invitrogen). cDNA was synthesized by Moloney murine leukemia virus reverse transcription (RT). Real-time PCR was conducted with SYBR green master mix using a Viia7 instrument (Applied Biosystems). Control reactions lacking RT yielded little to no signal. Relative expression was determined from a standard curve of serial dilutions of cDNA samples, and values were normalized to 18S RNA expression. Primer sequences are provided in Table S1. For RNA-seq, R1 cells ($CD71^{med}Ter119^-$) were isolated by flow cytometry. RNA was harvested with Trizol and purified using RNAeasy MiniElute Spin column (Qiagen, Cat. 1026497). RNA libraries were prepared for sequencing using standard NuGEN Ovation protocols. Library sequencing was conducted by the U. Wisconsin Biotech. Center using Illumina Hiseq2500 (1 x 50). The data was deposited in the Gene Expression Omnibus database (GSE96059). Sequencing reads were adapter and quality trimmed using Skewer (Jiang et al., 2014). Quality reads were aligned to the annotated reference genome using STAR (Dobin et al., 2013). Quantification of expression was calculated by RSEM (Li and Dewey, 2011). RSEM-derived expected read counts were used to compare differential expression (DE) between the mutant and wild-type samples using DESeq2 (Love et al., 2014). The DE gene list was obtained by filtering results by FDR value (<0.05) and relative fold changes.

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