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SUPPLEMENTARY INFORMATION

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EBF1-deficient bone marrow stroma elicits persistent changes in HSC potential

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Methods

Single-cell RNA-seq (scRNA-seq)

Single cell sort into 384 well plates. The cells were sorted on a BD FACSAria™ Fusion cell sorter using single cell mode. Cells were sorted into 384-well plates containing 240 nl lysis buffer (see below) and 1.2 µl of hydrophobic encapsulation barrier (Vapor-Lock, Qiagen, 981611). After the single cell sort, the plates were centrifuged at 2200 g at 4˚C for 10 min, snap-frozen thereafter with liquid nitrogen and stored at -80˚C until they were processed.

Amplified RNA preparation from single cells. The Volumes used in the original protocol were reduced by 5-fold for the single cell sort of cells and all the reagent ratios were kept the same. The cells were sorted into 384-well plates (Corning, PCR-384-RGD-C). Every well was filled with 240 nl lysis buffer. The lysis buffer was prepared using the following reagents (with final ratio in parentheses): 10 mM dNTP (1/12), 1:100000 ERCC mix 1 or 2 (2/12), water with 0.35% Triton® X-100 (7/12) and 25ng/µl of 1 out of 192 uniquely barcoded polydT primers with UMI (2/12). 1.2 µl of hydrophobic encapsulation barrier (Vapor-Lock, Qiagen, 981611) were added onto the lysis buffer in every well. The sorted plates were incubated at 90˚C for 3 min and then cooled to 4˚C. For cDNA first strand synthesis, 160 nl of First Strand Reaction Mix containing SuperScript™ II Reverse Transcriptase and First Strand Synthesis Buffer (Invitrogen, 18064014) and RnaseOUT™ (Invitrogen, 10777019) was added and the plates were incubated at 42˚C for 1 hour followed by heat-inactivation at 70˚C for 10 min. Second strand synthesis was performed at 16˚C for 2 h by adding 2196 nl of Second Strand Reaction Mix containing E. coli DNA Polymerase I (Invitrogen, 18010025), E. coli DNA Ligase (Invitrogen, 18052019), RNaseH (Invitrogen, 18021071) and Second Strand Buffer (Invitrogen, 10812014). For cDNA purification, 96 or 192 wells containing uniquely barcoded single cell samples were pooled and 0.8 volumes of Agencourt® AMPure XP beads (Beckman Coulter, A63881) were mixed with the pooled samples and then incubated for 15 min at RT. The samples were put on a magnetic stand (96 well format) and incubated for 5min, the supernatant was removed and the beads were washed twice by adding 180 µl of 80% ethanol and by incubating for 40 s. The beads were dried for 10 min and resuspended in 7 µl nuclease-free water (Invitrogen™, AM9937). For the generation of amplified RNA (aRNA) 1.6 µl from each nucleotide solution (ATP, CTP, GTP, UTP), 1.6 µl of 10x Reaction Buffer and 1.6 µl of T7 Enzyme from the MEGAscript® T7 Transcription Kit (Invitrogen, AM1334) were added to the eluted cDNA and incubated at 37˚C for 13 h. After *in vitro* transcription, 6 µl of Exo-SAP-IT™ Express PCR Product Cleanup Reagent (Applied Biosystems™, 75001.1.ML) was

added and the samples were incubated for another 15 min at 37˚C. Next, aRNA was fragmented by adding 2.44 µl 10x RNA Fragmentation Buffer and incubating at 94 ˚C for 3 min, and fragmentation was stopped by adding 2.44 µl RNA Fragmentation Stop Solution (NEB, E6150S). For aRNA purification, 0.8 volumes of Agencourt® RNAclean XP beads (Beckman Coulter, A63987) were added to the sample and incubated for 15 min at RT. After that, samples were placed on a magnetic stand and incubated for 5 min, the supernatant was removed and the beads were washed three times by adding 180 µl 70% ethanol and incubating for 40 s. Ethanol was removed, the beads were air-dried for 10 min and resuspended in 7 µl of nuclease-free water.

Single cell library preparation from aRNA. The aRNA was reversely transcribed in order to generate libraries for sequencing, as previously described [54]. 1 µl of custom random hexamer RT primer (GCCTTGGCACCCGAGAATTCCANNNNNN) and 0.5 µl of 10mM dNTP were added to the aRNA. The samples were incubated at 65˚C for 5 min and cooled to 4˚C afterwards. Then, 4 µl of First Strand Reaction Mix containing SuperScript™ II Reverse Transcriptase and Buffer (Invitrogen, 18064014) and RnaseOUT™ (Invitrogen, 10777019) was added, and the samples were first incubated at 25˚C for 10 min followed by incubation at 42˚C for 1 hour. 2 µl of one uniquely indexed RPI index primer and 2 µl of RP1 primer (TruSeq Library Prep, sequences available from Illumina), 25 µl of Phusion® High-Fidelity PCR Master Mix with HF Buffer (NEB, M0531) and 11 µl of nuclease-free water were added to every sample. PCR was performed using 98˚C for 30 s as initial step, followed by 11 cycles of amplification (98˚C for 10 s, 60˚C for 30 s, 72˚C for 30 s) and a final extension at 72˚C for 10 min. 1 volume (50 µl) of Agencourt® AMPure XP beads (Beckman Coulter, A63881) was added to each sample, followed by incubation at 25˚C for 15 min. The samples were then incubated for 5 min on a magnetic stand (96 well format), the supernatant was removed and the beads were washed twice by adding 180 µl of 80 % ethanol and by incubating for 40 s. Ethanol was removed and beads were dried for 10 min at room temperature. DNA was eluted with 25 µl nuclease-free water and the purification was repeated with an adjusted volume of magnetic beads (25 µl). After the final step, the samples were eluted in 10 µl nuclease-free water. DNA concentration and fragment size were determined using the Qubit® dsDNA HS Assay Kit (Invitrogen, Q32854) and the Agilent High Sensitivity DNA Kit for Bioanalyzer (Agilent Technologies, 5067-4627), respectively. Libraries were sequenced on an Illumina HiSeq 2500 System in high output run mode at a depth of ~200,000 reads per cell.

Integrated analysis using Seurat

Datasets published in Baryawno et al. and Tikhonova et al. were integrated using the standard data integration workflow of Seurat v3. The Baryawno dataset was first analyzed separaetly by

clustering with Seurat and clusters of cells expressing hematopoietic marker genes e.g. *Mpo*, *Cd19, Il7r, Gata1 and Gata3* were removed from the dataset. The reduced dataset was then integrated with our dataset using the standard data integration workflow of Seurat v3. The Tikhonova dataset was integrated using the same standard data integration workflow of Seurat v3.

Pathway enrichment analysis for scRNA-seq data of differentially expressed genes

For pathway enrichment the R package “clusterProfiler” was used for comparing biological themes among gene clusters.

Gene Set Enrichment Analysis (GSEA)

GSEAs were performed using $\log_2$+1 HSC WT and KO cufflinks quantifications as the expression dataset, and as gene sets the GSEA “GO_POSITIVE_REGULATION_OF_MYELOID_CELL_DIFFERENTIATION” ontology or the nearest gene annotated from the down- or up- regulated ATAC clusters via bedtools annotate. Analyses were run with the weighted enrichment statistic, Diff of classes gene ranking, gene set permutation type, and median of probes for multiple transcripts.