RUNX3 levels in human hematopoietic progenitors are regulated by aging and dictate erythroid-myeloid balance

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## CRITICAL REAGENTS AND RESOURCES

| REAGENT or RESOURCE | SOURCE                          | IDENTIFIER |
|---------------------|--------------------------------|------------|
| **Antibodies**      |                                 |            |
| RUNX1               | Cell Signaling Technology       | 4334S      |
| RUNX2               | Cell Signaling Technology       | 12556S     |
| RUNX3               | Cell Signaling Technology       | 9647S      |
| Tubulin             | Sigma Aldrich                   | T9026-2ML  |
| RUNX3               | R&D Systems                     | MAD3765    |
| Goat anti-mouse secondary | Thermo Fisher Scientific     | A-11003    |
| CD235a (FITC)       | Invitrogen                      | 11-9987-82 |
| CD235a (PE)         | BD Pharmingen                   | 555570     |
| CD41a (APC)         | BD Pharmingen                   | 559777     |
| CD15 (FITC)         | BioLegend                       | 301903     |
| CD36 (APC)          | BD Biosciences                  | 561822     |
| CD71 (APC)          | BD Pharmingen                   | 551374     |
| CD41a (89 Yb)       | Fluidigm                        | 3089004B   |
| CD117 (143 Nd)      | Fluidigm                        | 3143001B   |
| CD123 (151 Eu)      | Fluidigm                        | 3151001B   |
| CD45RA (153 Eu)     | Fluidigm                        | 3153001B   |
| CD36 (155 Gd)       | Fluidigm                        | 3155012B   |
| Ki-67 (162 Dy)      | Fluidigm                        | 3162012B   |
| CD235a (163 Dy)     | Fluidigm                        | 3163008B   |
| CD34 (166 Er)       | Fluidigm                        | 3166012B   |
| CD38 (172 Yb)       | Fluidigm                        | 3172007B   |
| CD71 (175 Lu)       | Fluidigm                        | 3175011B   |
| CD11b (209 Bi)      | Fluidigm                        | 3209003B   |
| RUNX3 (148 Nd)      | This study                      | R&D Systems RUNX3 antibody (MAB3765); Fluidigm Maxpar X8 antibody labeling kit (201148) |
| Cleaved Caspase-3 (173 Yb) | This study                    | BD Biosciences cleaved Caspase-3 antibody (559565); Fluidigm Maxpar X8 antibody labeling kit (201173) |
| **Biological Samples** |                             |            |
| Adult PBSC CD34 (1 million) | Fred Hutchinson Cancer Research Center | N/A        |
| Adult PBSC CD34 (5 million) | Fred Hutchinson Cancer Research Center | N/A        |
| **Chemicals, Peptides, and Recombinant Proteins** | |  |
| TPO                 | PeproTech                       | 300-18-50UG|
| SCF                 | PeproTech                       | 300-07-50UG|
| Flt3-ligand         | PeproTech                       | 300-19-50UG|
| IL-3                | PeproTech                       | 200-03-10UG|
| G-CSF               | PeproTech                       | 300-23-10UG|
| SDF1a               | PeproTech                       | 300-28A-50UG|
| IL-6                | PeproTech                       | 200-06-5UG |
| EPO                 | Procrit, Janssen                | 609-10-98-5|
| Drug                           | Manufacturer    | Code   |
|-------------------------------|-----------------|--------|
| Doxycycline                   | Sigma Aldrich   | D3447-500MG |
| Dexamethasone                 | Sigma Aldrich   | D2915  |
| Fibronectin                   | Corning         | 354008 |
| Retronectin                   | Takara          | T100B  |
| Puromycin                     | Sigma Aldrich   | P8833-10MG |
| Saponin                       | Sigma Aldrich   | S7900  |
| Sodium Azide                  | Sigma Aldrich   | S2002  |
| IC (Intracellular) Fixation Buffer | eBioscience    | 00-8222-49 |
| Paraformaldehyde, 16% Solution, EM grade | Electron Microscopy Sciences | 15710 |
| Cisplatin                     | Sigma Aldrich   | P4394  |
| Cell-ID Intercalator-Ir—125 µM | Fluidigm       | 201192A |

### Commercial Assays

| Assay                        | Manufacturer    | Code   |
|------------------------------|-----------------|--------|
| RNeasy Plus Mini Kit         | QIAGEN          | 74134  |
| PKH26 Red Fluorescent Cell Linker Kit | Sigma Aldrich | MINI26-1KT |
| CalPhos Mammalian Transfection Kit | Clontech       | 631312 |
| Zombie Violet Fixable Viability Kit | BD Biolegend   | 423113 |

### Deposited Data

| Type             | Description    | Accession Numbers |
|------------------|----------------|-------------------|
| RNA-seq          | This study     | GSE119264, GSE104406 |
| Microarray       | This study     | GSE123991 |

### Experimental Models: Cell Lines

| Cell Line               | Manufacturer | Code   |
|-------------------------|--------------|--------|
| HEK293T                 | ATCC         | CRL-3216 |
| HUDEP-2                 | Kurita, et al. 2013 | N/A |

### Recombinant DNA

| DNA                        | Manufacturer   | Code   |
|----------------------------|----------------|--------|
| TRC RUNX3 shRNA (TRCN0000013668, TRCN0000013670, TRCN0000013671, TRCN0000013672) | GE Dharmacon | RHS4533-EG864 |
| pLKO.1 plasmid             | GE Dharmacon   | RHS4080 |
| pCMV-DR8.2 dvpr            | Addgene        | 8455   |
| pMD2.G                     | Addgene        | 12259  |

### Software and Algorithms

| Tool                       | Reference                  | Website                                      |
|----------------------------|---------------------------|----------------------------------------------|
| Trimomatic                 | Bolger et al., 2014.      | usegalaxy.org                                |
| HISAT2                     | Kim et al., 2015.         | usegalaxy.org                                |
| RmDup                      | Li et al., 2009; Li et al., 2011; Li et al., 2011; Danecek et al.; Durbin et al.; Li et al. | usegalaxy.org                                |
| featureCounts              | Liao et al., 2013.        | usegalaxy.org                                |
| DESeq2                     | Love et al., 2014.        | usegalaxy.org                                |
| The Synergizer             | Roth laboratory           | http://llama.mshri.on.ca/synergizer/translate/ |
| Cufflinks                  | Cole et al., 2010.        | usegalaxy.org                                |
| FlowSOM                    | Van Gassen et al. 2017.   | bioconductor.org                             |
| flowType                   | Aghaeepour et al. 2014.   | bioconductor.org                             |
METHODS

Cell Culture

Cryopreserved primary human adult G-CSF-mobilized CD34+ cells were purchased from Fred Hutchinson Cancer Research Center. Cells were thawed and expanded 2-3 days in medium composed of IMDM (GIBCO BRL) supplemented with BIT 9500 (Stem Cell Technologies), and the following cytokines: 100 ng/ml each of rhTPO, rhSCF, and rhFlt3-l, plus 10 ng/ml rhIL-3 (all from PeproTech). After expansion, cells were moved into unilineage media, and cultured from 1-8 days. Erythroid medium is composed of IMDM supplemented with BIT 9500, and the following cytokines: 4.5 U/ml rhEPO (Procrit, Janssen) and 25 ng/ml rhSCF (PeproTech). Megakaryocyte medium is composed of IMDM supplemented with BIT 9500, and the following cytokines: 40 ng/ml rhTPO, 25 ng/ml rhSCF, and 20 ng/ml rhSDF1-alpha (PeproTech). Megakaryocyte cultures utilized fibronectin-coated plates, which were prepared by incubating plates at room temperature for one hour with IMDM containing 20 µg/ml fibronectin (Becton Dickinson). Granulocyte medium is composed of IMDM supplemented with BIT 9500, and the following cytokines: 25 ng/ml rhSCF, 10 ng/ml rhIL-3, and 20 ng/ml rhG-CSF (PeproTech). For colony formation assays, 3,000 undifferentiated CD34+ cells were seeded into MethoCult SF H4236 (Stem Cell Technologies) supplemented with the following cytokines: 50 ng/ml rhSCF, 10 ng/ml rhIL-3, 20 ng/ml rhIL-6 (PeproTech), 3 U/ml rhEPO, 20 ng/ml rhG-CSF, and 10 ng/ml rhGM-CSF. Cells were cultured for 10 days, followed by colony scoring and counting.

HUDEP-2 cells (Kurita, et al. 2013) were maintained in expansion medium composed of StemSpan (Stem Cell Technologies) supplemented with 50 ng/ml rhSCF, 3 U/ml rhEPO, 1 µM dexamethasone (Sigma-Aldrich), and 1 µg/ml doxycycline (Sigma-Aldrich). HUDEP-2 differentiation induction was achieved by culturing the cells for 48 hours in doxycycline-free medium. HEK293T cells (ATCC) were grown in DMEM (GIBCO BRL) supplemented with 2 mM L-glutamine, antibiotic-antimycotic (Thermo Fisher Scientific), and 10% FBS (GIBCO BRL).

Transfection and Transduction

For production of lentivirus, pCMV-dR8.74 (GAG POL TAT REV) and pMD2.G (VSV-G) were co-transfected into HEK293T with pLKO.1 shRNA vectors at a 3:1:4 mass ratio using the Clontech CalPhos mammalian transfection kit (Clontech 631312). pLKO.1 shRNA
plasmids were purchased from GE Dharmacon. After 12-16 hours, the transfection medium was replaced with Opti-MEM I (Thermo Fisher Scientific), and viral supernatant was collected 36 hours later, passed through a 0.45 µm filter, and stored at -80°C. HUDEP-2 cells were transduced via incubation for 24 hours in a 1:1 mixture of viral supernatant and StemSpan, supplemented with 50 ng/ml rhSCF, 3 U/ml rhEPO, 1 µM dexamethasone, and 1 µg/ml doxycycline. Transduced cells were selected by adding 2 µg/ml puromycin (Sigma Aldrich) for 72 hours. CD34+ cells, pre-expanded for 2 days, were transduced over two additional days in a 1:1 combination of viral supernatant and IMDM, supplemented with 100 ng/ml each of rhTPO, rhSCF, and rhFlt3-l, plus 10 ng/ml rhIL-3. These cultures were seeded onto retronectin-coated plates, prepared by pre-treatment with 40 µg/ml retronectin (Takara) in PBS at room temperature for 2 hours, followed by incubation with 2% Fraction V BSA (Sigma Aldrich) in PBS, and then a wash with 2.5% wt/wt HEPES in HBSS (GIBCO BRL). The transduction cultures were incubated at 37°C for 2 hours, spun at 500 rcf for 90 minutes at room temperature, then incubated at 37°C overnight. The spin procedure was repeated the following day with fresh viral medium. Cells were selected on the third day with 2 µg/ml puromycin until we observed total cell death in un-transduced control cells (a minimum of 24 hours), followed by unilineage cultures as described above.

**Fluorescence Cytometry**

Cells were washed once with PBS, followed by staining for 30 minutes on ice with PBS containing 1:500 Zombie Violet viability dye (BioLegend), and 3 µl fluorochrome-conjugated antibody per sample. After staining, cells were washed once with PBS, followed by fixation for 20 minutes on ice using IC Fixation Buffer (eBioscience). After fixation, cells were washed once with PBS with 1% FBS, and re-suspended in 1% Fraction V BSA in PBS, and sorted into the serum-free expansion medium using a BD FACS Aria Fusion. The PKH26 membrane staining was performed according to the manufacturer's protocol, and cells were cultured for 3 days before analysis.

**Immunoblot**

For lysis, cells were washed once with PBS, followed by resuspension in Laemmli buffer (60 mM Tris-HCl, pH 6.8, 2% SDS, 100 µM dithiothreitol, 10% glycerol, and 0.01% bromophenol blue) supplemented with cOmplete protease inhibitor cocktail (Roche Diagnostics 1183617001) and PhosSTOP phosphatase inhibitor cocktail (Roche Diagnostics 04906845001). Samples were passed through 27G needles to shear DNA, followed by 5-10 minutes incubation at 95°C. After SDS-polyacrylamide gel electrophoresis and Western transfer, nitrocellulose or PVDF membranes were blocked with 5% non-fat dried milk in Tris-buffered saline (50 mM Tris and 150 mM NaCl) with 0.1% Tween-20. For protein detection, primary antibodies were applied overnight at a dilution of 1:1000 at 4°C with shaking. HRP-conjugated secondary antibodies were applied for one hour at a dilution of 1:10,000 at room temperature with shaking. All antibodies were diluted in TBS with Tween-20 and 1% non-fat dried milk. HRP substrates consisted of Super Signal West Pico and Super Signal West Femto (Thermo Fisher Scientific). Immunoblot signal quantitation was performed using a BioRad GS-800 scanning densitometer.
Immunofluorescence

10^5 cells were cytospun onto glass slides, fixed for 15 minutes at room temperature with 4% paraformaldehyde, and permeabilized for one hour at room temperature with PBS containing 2% FBS, 2% BSA, and 0.03% Triton X-100. Mouse anti-human RUNX3 primary antibody (R&D Systems) was applied overnight at 20 µg/ml in staining buffer (PBS with 2% FBS, 2% BSA, and 0.03% Triton X-100) at 4°C. Goat anti-mouse Alexa Fluor 546 secondary antibody (Thermo Fisher) was applied at 1:300 in staining buffer at room temperature for one hour. The slides were washed three times with staining buffer and once with PBS before mounting coverslips with Vectashield medium (Vector Laboratories H-1000). Images were obtained with a Zeiss LSM-700 confocal microscope using the 63x objective, and image processing was performed with Fiji ImageJ v2 (National Institutes of Health).

Immunohistochemistry

Immunohistochemistry was performed using a robotic platform (Ventana discover Ultra Staining Module, Ventana Co., Tucson, AZ, USA) on paraffin-embedded bone marrow clots of young (<10 years old) and aged (60-70 years old) subjects obtained according to IRB-reviewed protocols. A heat-induced antigen retrieval protocol was carried out using a TRIS–ethylenediamine tetracetic acid (EDTA)–boric acid pH 8.4 buffer (Cell Conditioner 1), with a 64 minute setting. Endogenous peroxidases were blocked with peroxidase inhibitor (CM1) for 8 min before incubating the tissues with RUNX3 antibody (Abcam, Cat # Ab 135248) at a 1:800 dilution for 60 min at room temperature. Antigen-antibody complex was then detected using DISCOVERY anti-mouse HQ HRP detection system and DISCOVERY ChromoMap DAB Kit (Ventana Co.). All the slides were counterstained with hematoxylin subsequently; they were dehydrated, cleared and mounted for the assessment. Slides were imaged using a NonoZoomer S360 (Hamamatsu Corporation) and the final images were prepared using NDP.view2 Plus Image viewing software U12388-02.

Mass Cytometry

Cells were washed once with PBS, then stained for 60 seconds with 100 µM cisplatin (Sigma Aldrich P4394) in PBS. 1% FBS in PBS was promptly added to quench the staining. After washing, cells were fixed with 0.2 µm filtered 1.6% paraformaldehyde in PBS for 10 minutes at room temperature. 1% FBS in PBS was promptly added to quench fixing, and after washing, cells were stored at -80°C. Upon thawing, each sample was washed once with cell staining medium consisting of 0.5% Fraction V BSA (Sigma Aldrich), and 0.02% w/v sodium azide (Sigma Aldrich S2002) in PBS. Subsequently, cells were washed twice with 0.02% w/v saponin (Sigma Aldrich S7900) and 0.02% w/v sodium azide in PBS to transiently permeabilize them. Each sample was then separately stained with a unique barcoding combination of palladium isotopes, shaking at room temperature for 15 minutes followed by washing three times with cell staining medium. The barcoded samples were pooled and simultaneously stained for surface antigens for 30 minutes at room temperature with shaking. Antibodies to CD45RA, CD11b, CD117, CD36, and CD71 were used at a dilution of 1:800. Antibodies to CD34, CD38, CD123, CD41, and CD235a were used at 1:400. Cells were washed twice with cell staining medium, then permeabilized in -20°C methanol for 10 minutes on ice with intermittent shaking. Cells were washed once with cell staining medium, followed by intracellular staining for 1 hour at room temperature with shaking. The Ki-67 antibody was used at 1:400, and antibodies to RUNX3 and cleaved Caspase-3 were used at 0.25 µg/ml. After staining, cells were washed twice with cell staining medium, then incubated for at least 15 minutes at room temperature in intercalator buffer consisting of 0.2 µm filtered PBS/1.6%
paraformaldehyde with a 1:5,000 dilution of CellID Ir-Intercalator (Fluidigm 201192A). Cells were washed once with cell staining medium, once with 0.05% Tween-20 (Sigma Aldrich) in water, and finally re-suspended in water. The sample volume was adjusted with water and normalization beads (Fluidigm 201078) to achieve a cell concentration of approximately 0.5 x 10^6/mL. Data acquired using the Fluidigm CyTOF 2 was bead-normalized and underwent barcode deconvolution using the debarcoding tool MATLAB standalone executable from Zunder et al. Nature Protocols 2015.

Data was inverse hyperbolic sine-transformed using a cofactor of 0.25. FlowSOM (Van Gassen et al. 2017) was used to construct a self-organizing map with a number of grid points equal to the square of the number of lineage markers. Each cell was assigned a phenocode for every lineage marker using flowType (Aghaeepour et al. 2014). Each grid point was then immunophenotyped using the aggregated phenocodes of the cells assigned to the grid point. For any given grid point to be assigned an immunophenotype for a particular marker (i.e. CD45RA+), 75 percent of the cells assigned to the gridpoint had to be labelled with the same phenocode for the particular marker. For each immunophenotype observed, number of cells were tabulated to form a hierarchical count table. Every level of the hierarchy was tested for differential abundance between conditions using edgeR (Robinson et al. 2010; McCarthy et al. 2012) with a quasi-likelihood framework as specified by the cydar™ package (Lun 2017).

**RNA-sequencing**

Cells were washed with PBS, followed by RNA extraction using the QIAgen RNeasy Plus Mini Kit (QIAgen 74134), with added DNA digestion. Briefly, cells were lysed using buffer RLT supplemented with β-mercaptoethanol, and lysates were applied to the gDNA eliminator column. The flow-through was applied to the RNeasy spin column, and the column was washed once with buffer RW1. Freshly prepared DNase solution (QIAgen 79254) was applied to the column and incubated at room temperature for 10 minutes. The column was washed once with buffer RW1, and twice with buffer RPE. RNA was eluted with nuclease-free water, and the eluate was re-applied to the column for a second spin to ensure complete elution of the RNA. RNA yield was assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific). Samples were sequenced by the Genomic Services Laboratory at HudsonAlpha Institute for Biotechnology using ribosomal reduction, 100bp, paired-end, and 50 million read-depth parameters on an Illumina HiSeq 2500 machine.

Raw fastq.gz data files were merged (fastq Merger; gsl.hudsonalpha.org) and uploaded to usegalaxy.org. Trimmomatic was used to eliminate low quality sequences from the reads, followed by alignment to the hg19 human reference genome using HISAT2. RmDup was used to eliminate PCR duplicates from the resulting bam files. Differential gene expression was determined with DESeq2 applied to data converted by featureCounts. The data was also processed using Cufflinks to estimate FPKM for each sample, followed by Student’s t-test to assess statistical significance for specific transcripts. The Synergizer tool was used to convert UCSC gene identifiers into hgnc gene symbols.

**Unexplained Anemia of the Elderly Studies**

Normal young, normal elderly, and UAE human bone marrow samples were obtained according to IRB-approved protocols. Mononuclear cells were prepared using Ficoll-Paque Plus (GE Healthcare), and stained with the appropriate antibodies and analyzed and sorted using a FACSARiaII cytometer (BD Biosciences). Cell types were defined as follows: HSC, Lin-CD34+CD38-CD90+ CD45RA-; MEP, Lin-CD34+CD38+CD123-CD45RA-; CMP, Lin-
CD34+CD38+CD123+CD45RA-. Methylcellulose colony formation was assayed by sorting cells into individual wells of a 6-well plate, each containing 3 ml of complete methylcellulose (Methocult GF+ H4435, Stemcell Technologies). Plates were incubated for 12-14 days at 37°C, and colonies then scored based on morphology.

For microarray analysis, total RNA was extracted using TRIzol (Invitrogen) or Ambion RNA Isolation Kit (Applied Biosystems by Life Technologies) according to the manufacturer’s protocols and treated with DNase I (Qiagen). All RNA samples were quantified with the RiboGreen RNA Quantitation Kit (Molecular Probes), subjected to reverse transcription, two consecutive rounds of linear amplification, and production and fragmentation of biotinylated cRNA (Affymetrix). Fifteen micrograms of cRNA from each sample was hybridized to Affymetrix HG U133 Plus 2.0 microarrays. Hybridization and scanning were performed according to the manufacturer’s instructions (Affymetrix). Data was analyzed using the gene expression commons platform (Seita J, Sahoo D, Rossi DJ, Bhattacharya D, Serwold T, Inlay MA, Ehrlich LI, Fathman JW, Dill DL, Weissman IL. PLoS One. 2012;7(7):e40321. doi: 10.1371/journal.pone.0040321. PMID: 22815738).
Supplementary Figure 1. HSPC RUNX3 Levels Decline With Aging

(A) Human RUNX3 mRNA levels from RNA-seq analysis of Lin-CD34+CD38+ bone marrow HSC obtained from healthy young and aged individuals. (B) Immunostaining of RUNX3 protein in bone marrow clots of two young (<10 years) and two aged (60-70) subjects shown at 20X magnification. (C) Mouse Runx3 mRNA expression heat-map overlaid on a multi-lineage hematopoietic SPRING plot derived from single cell RNA-seq on Kit+ marrow cells (Ery: erythroid; MPP: multipotent progenitor; Mk: megakaryocyte; Ly: Lymphoid; Gr: granulocyte. Gene visualizer from Tusi et al. 2018 is available at https://kleintools.hms.harvard.edu/paper_websites/tusi_et_al/).
Supplementary Figure 2. RUNX3 Participates in Human Erythroid and Megakaryocyte Differentiation

(A) RUNX3 protein levels by immunoblot in CD34+ HSPC, undifferentiated and at indicated durations of erythroid culture. Representative immunoblot of whole cell lysates from 3 independent experiments. (B) Flow cytometry plots for erythroid differentiation of CD34+ cells transduced with empty vector (EV) or RUNX3-targeting (RUNX3 sh1 and sh2) lentiviral shRNA constructs and subjected to unilineage erythroid culture for 3 days. Graph summarizes the quantitation of erythroid differentiation from 3 independent experiments. (C) RUNX family protein expression by immunoblot in CD34+ cells transduced with lentiviral empty vector (EV) or shRNA expression constructs targeting RUNX3. Graph summarizes mean fold change in tubulin-normalized protein levels associated with RUNX3 knockdown. N=3. (D) Histogram overlay for erythroid differentiation of un-transduced CD34+ cells (blue), CD34+ cells transduced with a GFP-targeting lentiviral shRNA construct (red), and unstained cells (black). (E) Histogram overlay for erythroid differentiation of CD34+ cells transduced with empty vector (blue), CD34+ cells transduced with a RUNX1-targeting lentiviral shRNA construct (red), and unstained cells (black). (F) Flow cytometry plots for megakaryocyte differentiation of CD34+ cells transduced with empty vector (EV) or RUNX3-targeting (RUNX3 sh4) lentiviral shRNA constructs and subjected to unilineage megakaryocytic culture for 3 days. Graph summarizes the quantitation of megakaryocyte differentiation from 3 independent experiments. (G-I) Summary of differentiation status by flow cytometry on transduced HUDEP-2 cells -/- differentiation induction, and immunoblot of RUNX3. Graphs show mean fold changes in CD235a+ percentage or CD71 mean fluorescence intensity associated with RUNX3 knockdown. N = 3. B: one-way ANOVA with Tukey’s post hoc test. C, F, and G: Two-way ANOVA with Bonferroni’s test. E: two-tailed Student t test. *P < 0.05, **P < 0.01, ***P < 0.005. Error bars: +/-SEM.
Supplementary Figure 3. Cellular localization of RUNX3
Immunofluorescent detection of RUNX3 in untransduced, empty vector transduced, and RUNX3 sh4 transduced CD34+ HSPC and HUDEP-2 erythroblasts. HSPC were analyzed undifferentiated and at day 3 of erythroid culture, and HUDEP-2 were uninduced.
Supplementary Figure 4. RUNX3 Levels Influence Progenitor Lineage Output Balance

(A) Minimum spanning tree plots depicting cell population-nodes identified by CyTOF on indicated cultures of transduced CD34+ progenitors. Heatmap coloration of nodes reflects log2(fold changes) in their frequency associated with RUNX3 knock-down. (B) Histogram overlay comparing RUNX3 levels in cells transduced with empty vector (black) or RUNX3 sh4 construct (orange). (C-D) CyTOF histogram overlays from control EV-transduced progenitors cultured in erythroid medium, comparing expression of CD36 and CD41 between myeloid (blue) and erythro-megakaryocytic (Ery/Mk, red) compartments as depicted in Figure 3A. (E) Gating strategy used to determine CMP, MEP, and GMP population frequencies. (F) Histogram overlay comparing CD36, CD235a, CD71, and CD11b expression between control (EV, black) and RUNX3-deficient (RUNX3 sh4, orange) GMP.
SUPPLEMENTARY TABLE

Populations in erythroid culture identified by CyTOF to be significantly affected by RUNX3 deficiency.

| Population Phenotype (diminished frequency) | log(fold change abundance) | FDR         |
|---------------------------------------------|----------------------------|-------------|
| CD117- CD235a+ CD34+ CD36hi CD71+          | -3.719262064               | 0.020801921 |
| CD117- CD34+ CD36hi                        | -2.93570212                | 0.020801921 |
| CD117- CD34+                               | -2.927367589               | 0.020801921 |
| CD36hi                                     | -2.026476318               | 0.020801921 |
| CD235a+ CD36hi                            | -1.962454631               | 0.020801921 |
| CD117+ CD36hi                             | -1.94043204                | 0.020801921 |
| CD36hi CD71+                               | -1.924672676               | 0.020801921 |
| CD117+ CD235a+ CD36hi                     | -1.906223603               | 0.020801921 |
| CD235a+ CD36hi CD71+                       | -1.87934004                | 0.020801921 |
| CD34+ CD36hi CD45RA- CD71+                 | -5.527807648               | 0.021172785 |
| CD36hi CD38-                               | -3.20231726                | 0.021172785 |
| CD117+ CD123- CD235a+ CD36hi CD71+        | -2.324952873               | 0.021172785 |
| CD123- CD36hi                              | -2.29506993                | 0.021172785 |
| CD34+ CD36hi                               | -2.272313596               | 0.021172785 |
| CD34+ CD36hi CD71+                         | -2.169146303               | 0.021172785 |
| CD117+ CD235a+ CD34- CD71+                 | -2.112339189               | 0.021172785 |
| CD117+ CD235a+ CD36hi CD71+                | -1.821118771               | 0.021172785 |
| CD117+ CD235a+ CD71+                       | -1.623566908               | 0.021172785 |
| CD235a+ CD71+                              | -1.336391726               | 0.021172785 |
| CD117+ CD71+                               | -1.068179401               | 0.021172785 |
| CD117+ CD123- CD235a+ CD34- CD71+          | -4.221637154               | 0.02181571  |
| CD123+ CD235a+ CD34+ CD36hi CD45RA+ CD71+  | -2.424672903               | 0.02181571  |
| CD117+ CD34+ CD36hi                        | -2.183237624               | 0.02181571  |
| CD235a+ CD34+ CD36hi CD71+                 | -1.911194053               | 0.021826715 |
| CD117+ CD235a+ CD34-                      | -1.741000304               | 0.021826715 |
| CD117+ CD34- CD71+                         | -1.546220087               | 0.021826715 |
| CD117+ CD123- CD71+                        | -1.37695306                | 0.021826715 |
| CD117+ CD38- CD71+                         | -2.62508303                | 0.022251608 |
| CD117+ CD235a+ CD34+ CD36hi CD45RA+ CD71+  | -2.464415391               | 0.022251608 |
| CD235a+ CD34+ CD36hi CD45RA+               | -2.378683681               | 0.022251608 |
| CD235a+ CD34+ CD36hi                       | -2.154723865               | 0.022251608 |
| CD235a+ CD34+ CD36hi CD45RA+ CD71+         | -2.044416618               | 0.022251608 |
| CD117+ CD123- CD34a+ CD71+                 | -1.505742441               | 0.022251608 |
| CD117+ CD123-                              | -1.289467248               | 0.022251608 |
| CD117+ CD235a+                             | -1.064811503               | 0.022251608 |
| CD71+                                      | -0.7540356                 | 0.022251608 |
| CD34- CD36hi                               | -1.839183625               | 0.022768574 |
| CD117+ CD235a+ CD34+ CD45RA- CD71+         | -4.18452121                | 0.023049541 |
| Population Phenotype (increased frequency) | log(fold change abundance) | FDR |
|------------------------------------------|---------------------------|-----|
| CD45RA+                                  | 0.961620903               | 0.021172785 |
| CD36lo CD45RA+                           | 1.949999308               | 0.021172785 |
| CD117+ CD36lo CD45RA+                    | 2.0199171                 | 0.021172785 |
| CD123+ CD36lo CD45RA+                    | 2.079819864               | 0.021172785 |
| CD123+ CD38+ CD45RA+                     | 2.09917809                | 0.021172785 |
| CD117+ CD123+ CD36lo CD45RA+             | 2.170353906               | 0.021172785 |
| CD117+ CD38+ CD45RA+                     | 2.35114823                | 0.021172785 |
| CD117+ CD123+ CD38+ CD45RA+              | 2.370327689               | 0.021172785 |
| CD123+ CD36lo CD38+ CD45RA+              | 2.52321586                | 0.021172785 |
| CD117+ CD123+ CD36lo CD38+ CD45RA+       | 3.168493405               | 0.021172785 |
| CD117+ CD123+ CD36lo CD38+ CD45RA+ CD71-| 3.486666794               | 0.021172785 |
| CD123+ CD45RA+                           | 1.690158574               | 0.021172785 |
| CD36lo CD38+ CD45RA+                     | 2.272647679               | 0.021172785 |
| CD117+ CD123+ CD34+ CD36lo CD45RA+       | 2.283017298               | 0.021172785 |
| CD117+ CD123+ CD38+ CD45RA+              | 2.353692155               | 0.021172785 |
| CD117+ CD36lo CD38+ CD45RA+ CD71+        | 2.535769836               | 0.021172785 |
| CD117+ CD36lo CD38+ CD45RA+              | 2.600259996               | 0.021172785 |
| CD117+ CD123+ CD36lo CD45RA+ CD71-       | 2.680126994               | 0.021172785 |
| Combination                                      | Mean   | SD    |
|-------------------------------------------------|--------|-------|
| CD117+ CD123+ CD235a- CD45RA+ CD71+             | 2.687459827 | 0.021176596 |
| CD45RA+ CD71-                                   | 2.016784542 | 0.021268162 |
| CD117+ CD36- CD45RA+ CD71+                      | 2.299899769 | 0.021268162 |
| CD117+ CD123+ CD34+ CD45RA+                    | 1.704345953 | 0.02181571 |
| CD117+ CD45RA+ CD71-                            | 1.986693782 | 0.02181571 |
| CD117+ CD36lo                                   | 1.620452606 | 0.021826715 |
| CD38+ CD45RA+                                   | 1.644879664 | 0.021826715 |
| CD117+ CD123+ CD45RA+                           | 1.671525396 | 0.021826715 |
| CD123+ CD34+ CD45RA+                            | 1.684655878 | 0.021826715 |
| CD117+ CD123+ CD36lo CD71-                       | 1.966847719 | 0.021826715 |
| CD117+ CD123+ CD45RA+ CD71+                     | 1.9700123    | 0.021826715 |
| CD36lo CD38+ CD45RA+ CD71+                       | 1.975230993 | 0.021826715 |
| CD117+ CD36- CD38+ CD45RA+ CD71+                | 2.13898349   | 0.021826715 |
| CD117+ CD123+ CD34+ CD36- CD45RA+ CD71+         | 2.186756531  | 0.021826715 |
| CD117+ CD123+ CD45RA+ CD71-                     | 2.192298028  | 0.021826715 |
| CD117+ CD123+ CD34+ CD45RA+ CD71-               | 2.27775625   | 0.021826715 |
| CD117+ CD123+ CD235a- CD36lo CD38+ CD45RA+ CD71+| 2.649605266  | 0.021826715 |
| CD117+ CD123+ CD235a- CD34+ CD36- CD45RA+ CD71+ | 2.734785211  | 0.021826715 |
| CD117+ CD123+ CD34+ CD36lo CD45RA+ CD71-        | 2.839704884  | 0.021826715 |
| CD117+ CD45RA+                                   | 0.820382787  | 0.022251608 |
| CD117+ CD123+ CD34+ CD36lo                      | 1.57550775   | 0.022251608 |
| CD117+ CD123+ CD36lo                            | 1.578013625  | 0.022251608 |
| CD117+ CD71-                                    | 1.601521438  | 0.022251608 |
| CD123+ CD45RA+ CD71+                            | 1.70375139   | 0.022251608 |
| CD117+ CD123+ CD71-                             | 1.753618176  | 0.022251608 |
| CD123+ CD38+ CD45RA+ CD71+                       | 1.780140967  | 0.022251608 |
| CD123+ CD36lo CD38+ CD45RA+ CD71+               | 1.827312586  | 0.022251608 |
| CD235a- CD45RA+                                 | 1.905278528  | 0.022251608 |
| CD117+ CD235a- CD45RA+                          | 1.92979801   | 0.022251608 |
| CD117+ CD123+ CD38+                             | 2.058814479  | 0.022251608 |
| CD117+ CD235a- CD38+ CD45RA+                   | 2.109723532  | 0.022251608 |
| CD235a- CD36lo CD45RA+                          | 2.141941418  | 0.022251608 |
| CD117+ CD123+ CD235a- CD34+ CD36- CD45RA+       | 2.184052312  | 0.022251608 |
| CD117+ CD36lo CD38+                             | 2.24701988   | 0.022251608 |
| CD117+ CD235a- CD36lo CD45RA+                   | 2.270590293  | 0.022251608 |
| CD117+ CD235a- CD36lo CD38+ CD45RA+ CD71+       | 2.36368958   | 0.022251608 |
| CD117+ CD123+ CD36lo CD38+                      | 2.517235551  | 0.022251608 |
| CD117+ CD34- CD36- CD38+ CD45RA+ CD71+          | 2.74466792   | 0.022251608 |
| CD117+ CD123+                                   | 1.395585034  | 0.022509853 |
| CD117+ CD123+ CD34+                             | 1.425052707  | 0.022694075 |
| CD235a- CD36lo                                  | 1.940196737  | 0.022768574 |
| CD117+ CD38+ CD45RA+                            | 1.503418954  | 0.025427647 |
| CD117+ CD123+ CD38+ CD45RA+ CD71+ | 1.744456412 | 0.025427647 |
| CD117- CD123- CD36lo CD71- | 2.374625197 | 0.025456838 |
| CD34- CD45RA+ CD71- | 2.235289254 | 0.025725544 |
| CD34- CD36- CD71+ | 2.557835468 | 0.025956549 |
| CD36- CD45RA+ CD71+ | 1.931527814 | 0.026118862 |
| CD123+ CD34+ CD36lo | 1.477841918 | 0.026152719 |
| CD34+ CD36lo | 1.415031769 | 0.027394542 |
| CD34- CD45RA+ | 1.971837004 | 0.027394542 |
| CD117+ CD123+ CD34+ CD45RA+ CD71+ | 1.710120145 | 0.027678568 |
| CD34- CD36- CD45RA+ | 2.515739011 | 0.027678568 |
| CD123+ CD235a+ CD38+ CD45RA+ CD71+ | 1.610692758 | 0.028659103 |
| CD117+ CD36- CD38+ CD45RA+ | 1.747189368 | 0.028659103 |
| CD117- CD34- CD36lo | 2.228242427 | 0.028659103 |
| CD38+ CD45RA+ CD71+ | 1.439757948 | 0.028790802 |
| CD123+ CD123+ CD34+ CD71- | 1.644404536 | 0.028790802 |
| CD117- CD38+ | 1.684508468 | 0.028790802 |
| CD123+ CD235a+ CD34+ CD38+ CD45RA+ CD71+ | 1.741804463 | 0.028790802 |
| CD123+ CD235a- CD36lo CD71+ | 1.737054092 | 0.031330165 |
| CD235a+ CD36lo CD38+ CD45RA+ CD71+ | 1.632398539 | 0.03159593 |
| CD123+ CD38+ | 1.46944081 | 0.031910071 |
| CD117+ CD235a- | 1.446718275 | 0.032742922 |
| CD117- CD123+ CD34+ | 1.667976719 | 0.03293287 |
| CD117+ CD123+ CD235a- CD36lo CD45RA+ | 1.942988443 | 0.034318267 |
| CD117+ CD123+ CD34+ CD71+ | 1.506950682 | 0.034606909 |
| CD117+ CD38+ | 1.367833291 | 0.03624554 |
| CD34- CD36- CD38+ | 2.217660354 | 0.03624554 |
| CD117+ CD36lo CD38+ CD71+ | 1.882729378 | 0.03694803 |
| CD123+ CD36lo CD71- | 1.306104029 | 0.038056128 |
| CD117+ CD235a- CD36- CD45RA+ | 1.727856868 | 0.038056128 |
| CD123+ | 1.01407299 | 0.038141191 |
| CD36lo | 1.088420266 | 0.039628627 |
| CD117+ CD38+ CD45RA+ CD71+ | 1.296671901 | 0.041382437 |
| CD34+ CD71- | 1.2931619 | 0.041382437 |
| CD123+ CD235a+ CD45RA+ CD71+ | 1.368995023 | 0.041382437 |
| CD117+ CD123+ CD71+ | 1.468879417 | 0.041382437 |
| CD36- CD38+ CD45RA+ CD71+ | 1.731075573 | 0.041382437 |
| CD117+ CD34- CD45RA+ | 1.855595825 | 0.041382437 |
| CD123+ CD71- | 1.249028359 | 0.041539258 |
| CD71- | 1.130962136 | 0.042454287 |
| CD117+ CD123+ CD235a+ CD34+ CD36+ CD38+ CD45RA+ CD71+ | 1.578437851 | 0.042454287 |
| CD34- CD38+ CD71+ | 2.490096029 | 0.043667741 |
| CD38+ | 1.277308649 | 0.043784112 |
| CD123+ CD36lo CD38+ | 1.578960511 | 0.043823071 |
| CD235a- | 1.219792524 | 0.044528442 |
| CD117+ CD123+ CD235a- CD45RA+ | 1.654594197 | 0.044528442 |
| CD123+ CD235a+ CD34+ CD45RA+ CD71+ | 1.428613685 | 0.044983013 |
| CD123+ CD235a+ CD34+ CD45RA+ | 1.232751363 | 0.045322654 |
| CD117+ CD235a+ CD36- CD38+ CD45RA+ CD71+ | 1.592963585 | 0.045572809 |
| CD123+ CD34+ CD36- CD45RA+ CD71+ | 1.669095408 | 0.045572809 |
| CD117- CD235a+ CD36- CD38+ CD45RA+ CD71+ | 1.836492088 | 0.045572809 |
| CD123+ CD36lo | 0.977073229 | 0.045705551 |
| CD36lo CD71- | 1.230438919 | 0.045705551 |
| CD123+ CD235a- CD71+ | 1.624765909 | 0.045705551 |
| CD123+ CD235a+ CD45RA+ | 1.137180858 | 0.047381762 |
| CD117+ CD36- CD45RA+ | 1.286272205 | 0.047381762 |
| CD34- CD38+ | 2.047977524 | 0.049929175 |