Single-cell gene expression analyses reveal distinct self-renewing and proliferating subsets in the leukemia stem cell compartment in acute myeloid leukemia

Supplementary Materials

Table S1. The murine LSC single-cell differentially expressed gene list. Genes differentially expressed between Group 1 and Group 3 cells.

Table S2. Ingenuity Pathway Analysis of genes differentially expressed between Group 1 and Group 3 single-cell gene expression profiles

Table S3. Clinical characteristics of human samples

Table S4. Mutational profiles of human samples

Table S5. Single-cell qPCR gene panel

Table S6. Single-cell qPCR measured, passed quality control, and enriched genes.

Table S7. Mutant genes NGS panel

Supplementary Materials and Methods

Single-Cell Capture

Primary murine *NRAS*^{G12V}*/Mll-AF9* AMLs were harvested from spleens of leukemic mice, stained with antibodies for cell surface markers, and sorted on a FACSAria (Becton Dickinson, Franklin Lakes, NJ). Cryopreserved bone marrow aspirates from healthy volunteers were purchased from Lonza (Basel, Switzerland). AML samples with >50% bone marrow blasts were obtained from the University of Minnesota Hematological Malignancies Tissue Bank in

accordance with the University of Minnesota Institutional Review Board (IRB #1506E72802 and #0611M96846).

Fresh AML bone marrow aspirates were CD34-selected using EasySep Magnet and EasySep Human CD34 antibody kit (StemCell Technologies, Vancouver, BC, Canada). Human AML and normal bone marrow samples were sorted to isolate $CD34^{+}CD38^{-}$ cells. Sorted or magnet-selected cells were stained with a Live/Dead Viability and Cytotoxicity dye (Life Technologies, Carlsbad, California, # L-3224) and loaded at 250 cells/μl for single-cell capture on the C_1 Single-Cell Auto Prep Integrated Fluidic Circuit for small cells (5-10 μm, Fluidigm, South San Francisco, California). Captured cells were imaged to identify single, live cells for cDNA preparation. SMARTer Ultra Low RNA Kit for the Fluidigm C_1 System (Takara Bio, Kusatsu, Shiga Prefecture, Japan) was used to prepare cDNA for sequencing. Fluidigm Reverse Transcription Master mix was used to prepare cDNA for PCR.

Single-cell RNA sequencing data processing

Nextera libraries were generated from the single cell cDNA using Nextera XT DNA Sample Preparation Kit and Nextera XT DNA Library Preparation Index Kit (Illumina). cDNA was quantified using a Quant-IT™ PicoGreen® dsDNA Assay Kit (Thermo Fischer Scientific). The libraries were sequenced on an Illumina HiSeq2500 (100 bp, paired-end reads, minimum 1 million reads/cell). For each cell, per base and per read quality scores were checked, and FastqQC (version 0.11.2, http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was

performed. Sequence adaptors and low-quality sequence were removed using Trimmomatic (v0.32). Trimmed reads were mapped with TOPHAT 2.0 (v2.0.10) to the mouse reference genome (UCSC GRCm38/mm10 assembly). An additional chromosome was added to the reference genome that contained the transgenes that were expressed in the mouse model (*NRAS^{G12V}, MLL-AF9 and EGFP*)[1]. Insertion size metrics were calculated for each sample using Picard software (version 1.126, http://picard.sourceforge.net.). Samtools software (version 1.0_BCFTools_HTSLib)[2] was used to sort and index the bam files. Cuffquant (Cufflinks version 2.2.1)[3] was used to generate transcript abundance files (options used include the multi-read-correct). Murine cells with less than 75% alignment to the genome were omitted from further analysis. Each cell expressed a minimum of 1,300 genes. There were 42, 33, and 51 evaluable cells in the discovery data set, validation dataset, and Ras-off dataset, respectively. Human cells were analyzed to omit outliers per SINGuLAR Analysis Toolkit (Fluidigm). This approach identifies cells whose expression of the most commonly expressed genes exceeds the 15th percentile; cells that meet this criterion are included in the analysis, the remaining cells are omitted as outliers. In the human dataset, 25 cells were included in the analysis. After mapping and abundance estimates were completed, Cuffnorm (Cufflinks version 2.2.1)[3] was used to generate fragments per kilobase of transcript per million mapped reads (FPKM) values.

Single-cell quantitative reverse transcriptase PCR

A 96-gene Delta Gene™ assay (Fluidigm, South San Francisco, California) was designed to represent genes that were differentially expressed in the murine single-cell RNA sequencing data from the validation dataset (one of the Ras-On LSC datasets) and include housekeeping genes (Supplementary Table S5). This gene list was derived from the list of genes that were differentially regulated (with a fold change of $\geq$2.0 and p value $\leq$ 0.05) between ($CD69^{High}$) Group 1 and ($CD36^{High}$) Group 3 cells in the validation dataset (Supplementary Table S1). This gene list chosen based on magnitude of the fold change difference and pattern of expression in the murine dataset and was adjusted after the first single-cell capture and qPCR experiment to remove genes that were not amplified, include new genes to replace omitted genes, and replace primers for *CD36* and *CD69* to improve performance. Delta Gene™ assay primers were designed with the following parameters: Tm 56-62°C, optimal 59°C; GC content close to 50%; last 5 bases with 2 to 4 Gs or Cs, last base with a G or C (3' end); span a lengthy intron (greater than 1000bp) or overlap an exon-exon boundary; Tm (melting temperature) within 5 degrees C, typically in the 58 to 60 degrees C range; primer length 18-21bp; and ideal amplicon length less than 200bp. Primers sequences were compared to the mouse genome to ensure there were no significant off-target products. On-chip quantitative PCR was performed using 96.96 IFCs on the BioMark HD system (Fluidigm) according to manufacturer's instructions. Raw gene expression data was provided as threshold cycle (C_t) values.

Data processing, quality control, and normalization of single-cell quantitative reverse-transcriptase PCR data

Single-cell qPCR reactions were performed on the Biomark. Calibration controls were reviewed to ensure consistent loading and amplification across well. Genes with multiple melting curves, indicating non-specific amplification, were removed from analysis for each project. The genes that were omitted after quality control varied for each patient sample. Therefore, the list of genes that passed quality control and were accurately measured varied from sample to sample, they are listed in Supplementary Table S6.

After quality control, the data was normalized to account for variations in signal magnitude between cells. To avoid the potential pitfall of over- or under-correcting expression values, we performed a rank normalization by assigning each gene a rank according to its relative expression within a cell. To avoid amplifying slight differences in expression, we used rounded values such that genes with almost identical expression were assigned the same rank. The highest rank value was set to the number of genes measured in the cell. These rank values were then z-scored by subtracting the gene's mean rank value within a sample from the gene's rank in that sample and dividing this difference by the standard deviation of that gene within the sample.

Mutational Profiling

An aliquot from each sorted and bulk human AML and healthy bone marrow aspirate was submitted for next-generation sequencing analysis using a clinically validated, hematological malignancy-focused 70-gene assay (Supplementary Table S7). DNA was extracted with Qiagen QIAamp Blood Mini Kit (Qiagen, Redwood City, California), and libraries were prepared following Nextera-based TruSight One library preparation (Illumina, San Diego, California). Libraries were enriched for genes of interest using IDT xGen Lockdown baits (Integrated DNA Technologies, Coralville, IA) designed to cover all coding regions for the 70 genes. Paired-end (2x225) sequencing was performed on a MiSeq (Illumina) to an average minimum coverage of >300x. A custom script was used to process the data and call variants. Briefly, adapter and quality trimming were performed with Cutadapt (version 1.8.1); overlapping paired-reads were merged with PandaSeq (version 2.7); alignment was performed with BWA (version 0.7.12); and variants were called using Freebayes (version v0.9.10-3-g47a713e-dirty). Variants were analyzed and annotated using wANNOVAR (wannovar.wglab.org) and manually interpreted.

Data and material availability

All gene expression datasets are available on the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/), accession number GSE140896.

Statistical Analysis

158 In addition to the statistical tests used for gene expression analysis,
159 student's t-test was performed (Prism 7, GraphPad, San Diego, California) to
160 assess significance in comparing values between two groups. Kaplan-Meier
161 survival curves (Prism, GraphPad, La Jolla, CA) were used to compare survival
162 of mouse reconstitution assays.

163

164 **Gene Set Enrichment Analysis**

165 Gene set enrichment analysis (GSEA,
166 http://www.broadinstitute.org/gsea)[4,5] was performed by comparing the
167 normalized gene expression values of Group 1 cells to those of Group 3 cells. All
168 genes that were not expressed in any of the cells analyzed were removed from
169 the dataset prior to analysis.

170

171 **Mice and leukemia reconstitution assays**

172 Mice were housed in aseptic conditions with autoclaved cages, bedding
173 material, water, bottles, and irradiated food. Primary murine *NRAS*^{G12V}*/Mll-AF9*
174 AMLs[1,6] were maintained by serial transplantation into recipient mice (they were
175 not passaged *in vitro*). In this model, human *AF9* is fused to the endogenous
176 murine *Mll*. *NRAS*^{G12V} is a human transgene, controlled by a tetracycline
177 repressible transgene under the control of the murine *Vav1* promoter [1,7]:
178 treatment with the tetracycline analog, doxycycline, causes loss of *NRAS*^{G12V}
179 expression and leads to disease remission. For leukemia reconstitution assays,
180 10^4 or 10^5 sorted primary *NRAS*^{G12V}*/Mll-AF9* leukemia cells were injected via tail

vein into SCID/Beige mice[1]. Mice were visually inspected three times per week. Leukemia development was monitored by weekly retro-orbital eye bleeds and complete blood counts. Leukemia onset was defined as WBC > 10,000 cells/μL of peripheral blood. Moribund mice were sacrificed, and their spleens and bone marrow were harvested for immunophenotypic analysis.

Colony forming assays

Cryopreserved primary *NRAS^{G12V}/Mll-AF9* leukemia cells, isolated from the spleens of leukemic mice, were thawed in a 37^{o}C water bath, washed in PBS, and plated in Methocult^{TM} M3231 (StemCell Technologies, Vancouver, BC, Canada) supplemented with 1 ng/ml mGM-CSF, 100 U/ml Penicillin G, and 100 mg/ml streptomycin (Invitrogen, Carlsbad, California). 10,000 leukemia cells were plated per well at a concentration of 5x10^{4} cells/ml. Colony formation was scored after seven days in culture.

Fluorescence-based flow cytometry, cell sorting, and cell separation

Primary murine AML cells were stained with a dead cell exclusion dye, Fixable Viability Dye eFluor^{TM}450 (eBioscience, San Diego, California), then incubated in Fc block (mouse-specific anti-CD16/CD32 antibody, BD Pharmingen, San Jose, California) diluted 1:200 in FACS buffer (PBS/2% FBS), and incubated in conjugated primary antibodies for 1 hour. Mouse-specific cell-surface protein antibodies were purchased from BD Pharmingen and were specific for: Mac-1-PerCP5.5 (1:12,800, M1/70), c-Kit-BV786 (1:200, 2B8), Sca-

1-PE (1:50, E13-161.7), CD36-BB515 (1:400, CRF-2712), and CD69-PE-Cy7 (1:400, H1.2F3).

Cryopreserved primary human bone marrow aspirates from patients with AML or healthy volunteers were thawed at 37°C. A freshly obtained human AML bone marrow specimen and thawed bone marrow specimens were subject to mononuclear cell isolation using Lymphoprep™ (Stemcell Technologies), washed, stained with Fixable Viability Dye eFluor™450 (eBioscience), incubated with Fc block (human-specific anti-CD16/CD32 antibody, BD Pharmingen) diluted 1:200 in FACS buffer (PBS/2% FBS), and incubated in conjugated primary antibodies for 1 hour. Human-specific cell-surface protein included CD34-APC (1:200, 581) and CD38-FITC (1:200, HB7) (BD Pharmingen). Cells were analyzed on a FACSCalibur or LSRII flow cytometer or sorted on a FACSAria II (Becton Dickinson, Franklin Lakes, NJ) using CellQuest Pro software. CD34+CD38- isolation of primary human AML or normal bone marrow cells was performed by sorting on a FACSAria II. CD34+ isolation was performed by EasySep Magnet and EasySep Human CD34 antibody kit and CD34-conjugated beads (StemCell Technologies).

Mass cytometry (CyTOF)

Cryopreserved primary human bone marrow aspirates obtained at diagnosis from patients with AML were thawed at 37°C. Bone marrow mononuclear cells (BMMCs) were isolated using Lymphoprep™ (Stemcell Technologies). BMMCs were incubated for 30 minutes at 37°C in a medium of RPMI 1640 (Gibco, Billings, MT) with 10% fetal bovine serum and 1% penicillin/streptomycin

(RPMI10). Cells were washed in phosphate buffered saline (PBS), treated with cisplatin dead-cell exclusion reagent for mass cytometry (Fluidigm, San Francisco, CA), blocked with Human Fc block (BD Pharmingen, San Diego, CA), and stained for cell surface markers (with metal-conjugated antibodies to human proteins CD34-148Nd (581), CD38-172Yb (HIT2), CD69-162Dy (FN50), and CD36-152Sm (5-271)). Stained cells were fixed in 1.6% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA), permeabilized in cold methanol, and stored at -80^{o}C. Permeabilized cells were stained with anti-Ki67 antibody (Ki-67, conjugated to 168Er) and intercalated with IdU (a DNA intercalator used for single-cell recognition in mass cytometry data). Samples were analyzed with a CyTOF2 mass cytometer (Fluidigm, San Francisco, CA). All antibodies and reagents for mass cytometry were purchased from Fluidigm.

In vivo proliferation assays (CellTrace)

Primary leukemia cells were labeled with CellTrace Far Red Kit (Thermo Scientific, Waltham, MA) according to the manufacturer's instructions at 1 nM of CellTrace for 20 minutes at 37^{o}C. 10^{7} CellTrace-labeled cells were transplanted via tail vein into SCID/Beige mice[1]. The spleens of leukemic mice were harvested 14 days after transplant and stained with cell surface markers. Levels of CellTrace Far Red were assessed by fluorescence-based flow cytometry on the day of harvest.

Analysis of previously published single-cell RNA sequencing data

We assessed the expression of our murine LSC single-cell gene expression profile in a recently published dataset of normal human bone marrow

single-cell transcriptional data. Single-cell RNA Sequencing data from 25 normal human bone marrow samples was obtained from Oetjen, 2018[8]. Cells expressing *CD34* (values >0) and not expressing *CD38* (values <=1) in this dataset were included for analysis. Samples with fewer than ten CD34+CD38- cells were eliminated from this analysis. All remaining CD34+CD38- cells were pooled. The expression of the murine LSC single-cell gene expression profile was assessed in this dataset. Genes expressed in fewer than 25% of the pooled cells were omitted from analysis. Expression data was transformed with the log-like hyperbolic arcsine (asinh(data/5)), normalized by dividing each cell's value by the cell mean, and genes were mean centered by subtracting the gene mean from each gene value. Pooled data were clustered using hierarchical agglomerative clustering using the clustergram function in MATLAB (Mathworks Inc) with the cosine distance function.

Significance assessment for enrichment was calculated using a hypergeometric test: in each cluster the number of Group 1 genes selected out of the total number of Group 1 genes measured in that dataset. In a total population size of all genes present in the dataset and number of trials equal to the size of the cluster. The same analysis was repeated for Group 3 genes in each cluster.

Supplementary Material References

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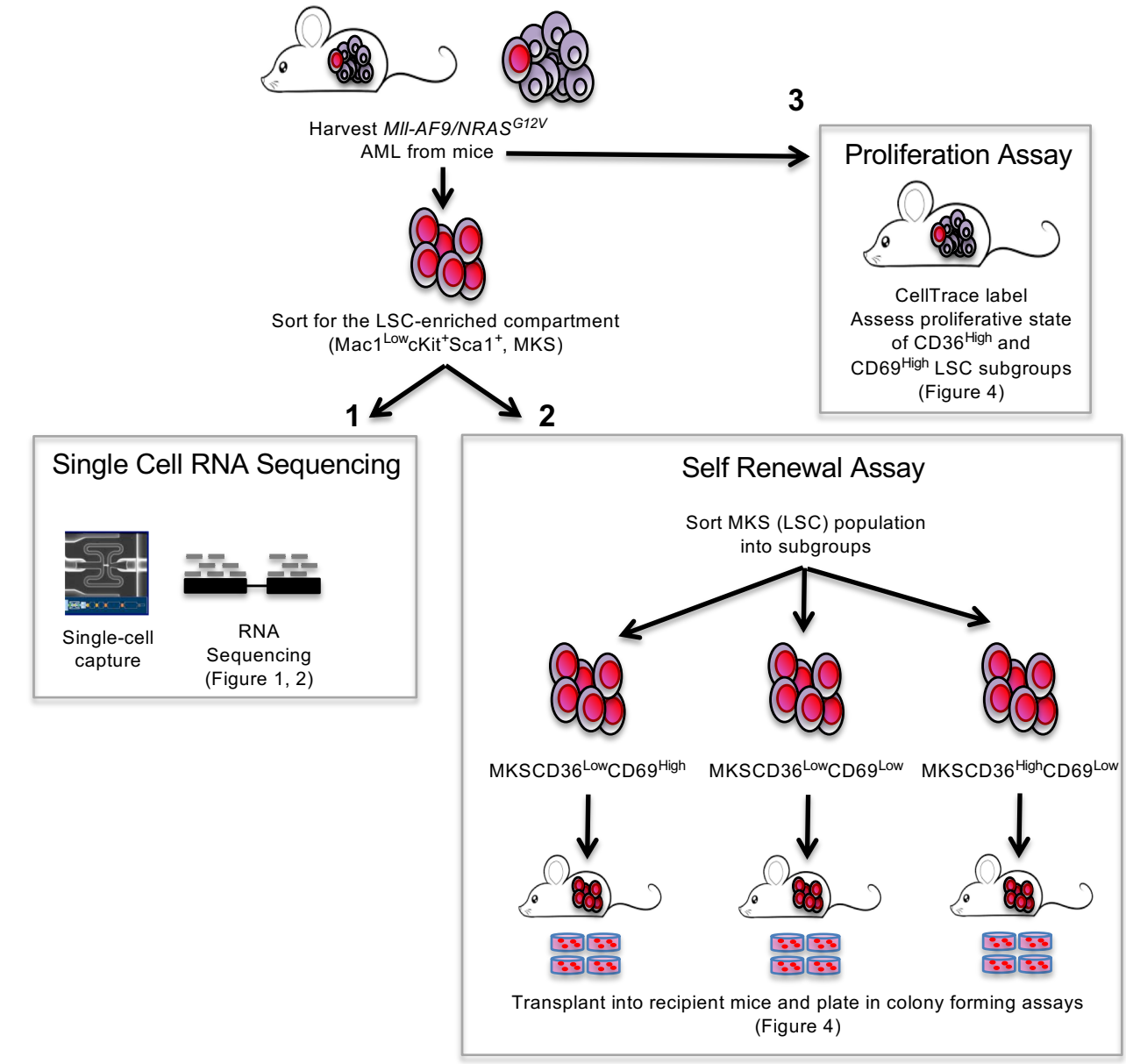
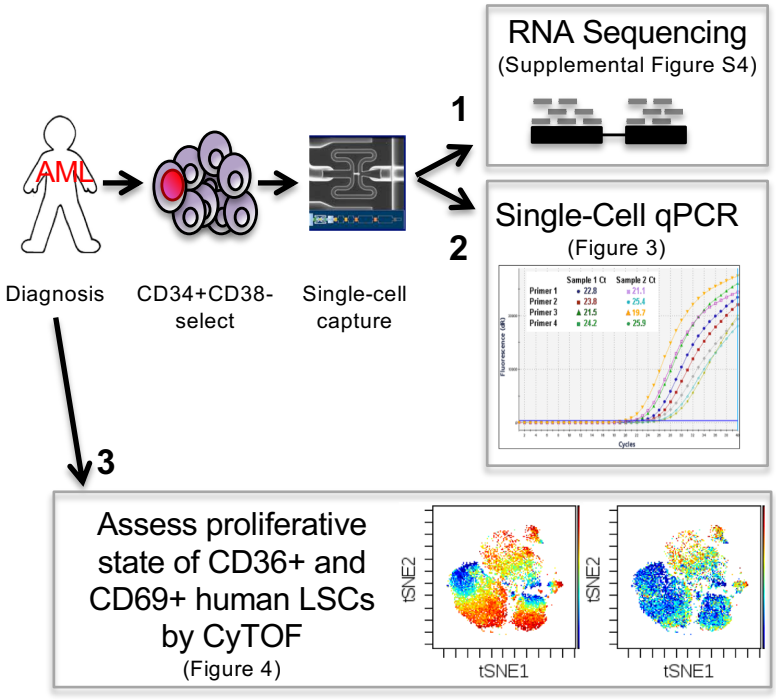
Supplementary Figure Legends

Supplementary Figure S1. Experimental Workflow. **A,** Primary murine *Mll-AF9/NRAS*^{G12V} leukemia cells were used to identify the single-cell gene expression profile of self-renewal and in functional assays to validate this profile. **1.** The LSC-enriched compartment (Mac1^{Low}Kit^{+}Sca1^{+}, "MKS") was isolated by sorting and submitted for single cell capture and RNA sequencing. **2.** The LSC-enriched compartment was further sorted based on CD36 and CD69 status and transplanted into secondary recipients and plated in colony forming assays. **3.** Leukemia cells were labeled with CellTrace and transplanted into secondary recipients. Leukemia was harvested twelve days later and CellTrace levels were assessed in LSC subgroups by flow cytometry. **B,** Primary human AML bone marrow samples were obtained at diagnosis. **1.** AMLs and normal bone marrow mononuclear cells were sorted to isolate CD34+CD38- cells (immunophenotypic LSCs) and submitted for single-cell capture and qPCR. **2.** An AML sample was CD34+ selected to enrich for LSCs and submitted for single cell capture and RNA sequencing. **3.** CyTOF was used to measure the levels of Ki67 (proliferation maker) in CD36^{High} and CD69^{High} subpopulations of AML bone marrow samples obtained at diagnosis.

A Experimental workflow: murine AML

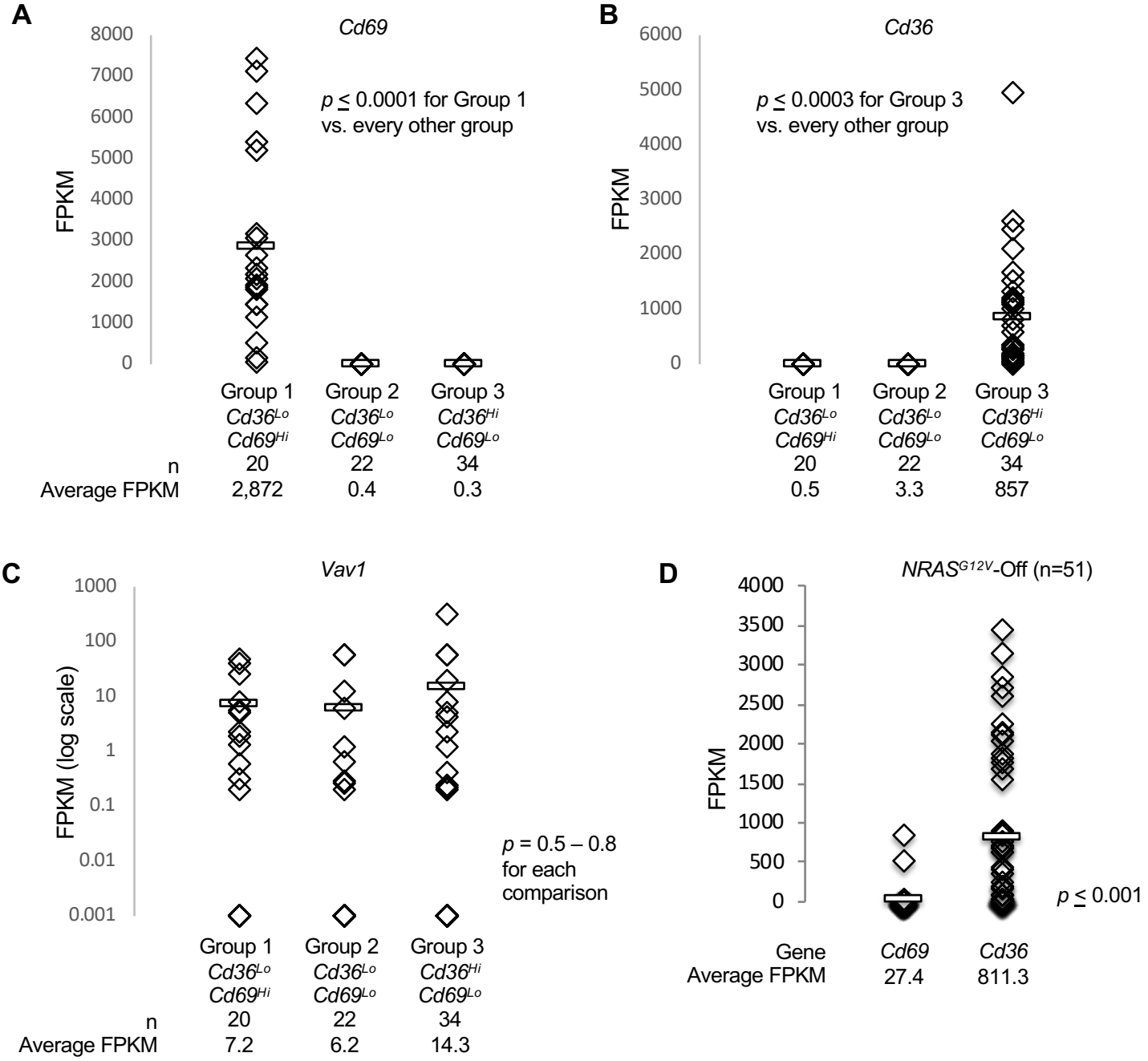


B Experimental workflow: human AML



Supplementary Figure S1

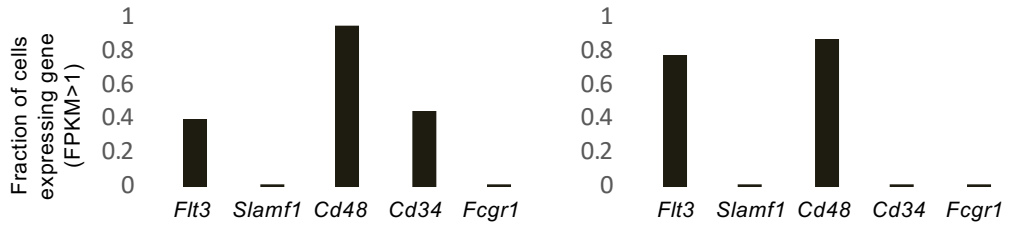
Supplementary Figure S2. Cell surface markers delineate LSC subgroups. **A-C,** Single-cell normalized expression levels (Fragments Per Kilobase of transcript per Million mapped reads, FPKM) of **A,** *Cd69*, **B,** *Cd36* **C,** *Vav1* by transcriptional subgroups in cells pooled from the discovery and validation datasets (single-cell RNA sequencing data of murine LSCs). **D,** Single-cell FPKM expression values of *Cd69* and *Cd36* in Ras-off LSCs.



Supplementary Figure S2

Supplementary Figure S3. HSPC markers in the murine data and *Cd69 and Cd36* are expressed in different stages of early hematopoiesis. **A,** Expression of gene encoding hematopoietic cell surface markers in our single-cell murine dataset (data pooled from both the validation and discovery datasets). **B-G** *Cd69* and *Cd36* expression in normal mouse LT-HSCs, HSPCs, and progenitors per the Nestorowa et al. map of mouse single-cell transcriptional profiles. **B-D,** Average normalized expression values of *Cd36* and *Cd69* in each compartment is **B,** listed and **C-D,** shown. **E-G,** Normalized expression of *Cd36* plotted against *Cd69* in the single-cell transcriptional data. **E,** Progenitors (n=798), **F,** HSPCs (n=701), **G,** LT-HSCs (n=155).

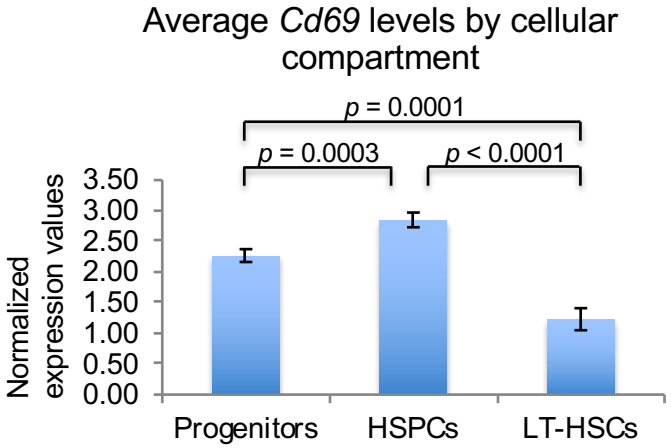
A



B

	Average Expression		Fold Change	
	Cd69	*Cd36*	*Cd69/Cd36*	
Progenitors (n=798)	2.27	0.99	2.3	$p<0.0001$
HSPCs (n=701)	2.85	0.40	7.1	$p<0.0001$
LT-HSCs (n=155)	1.23	0.57	2.2	$p=0.0023$

C



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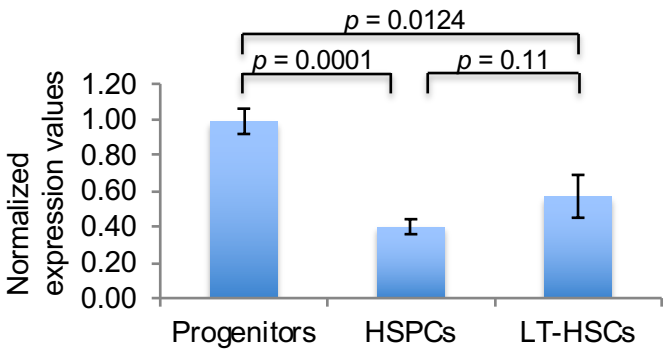

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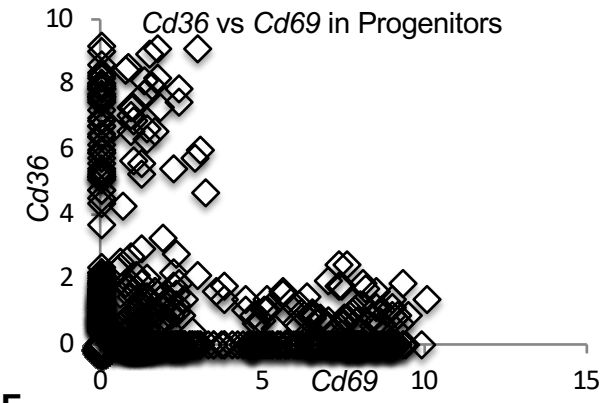

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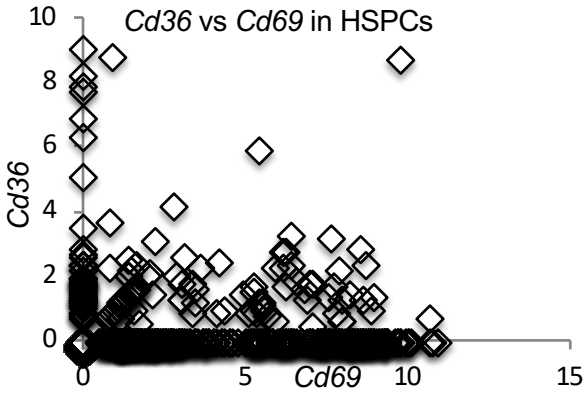

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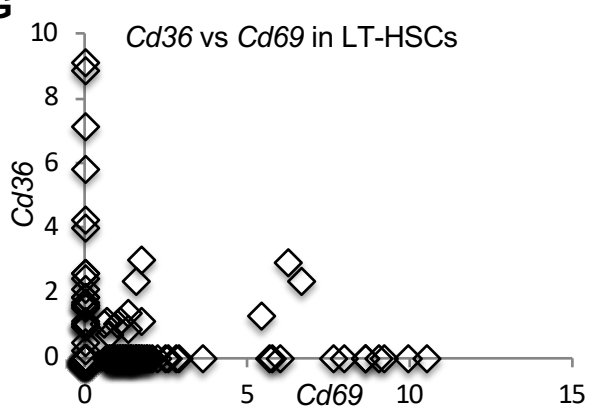


Supplementary Figure S3

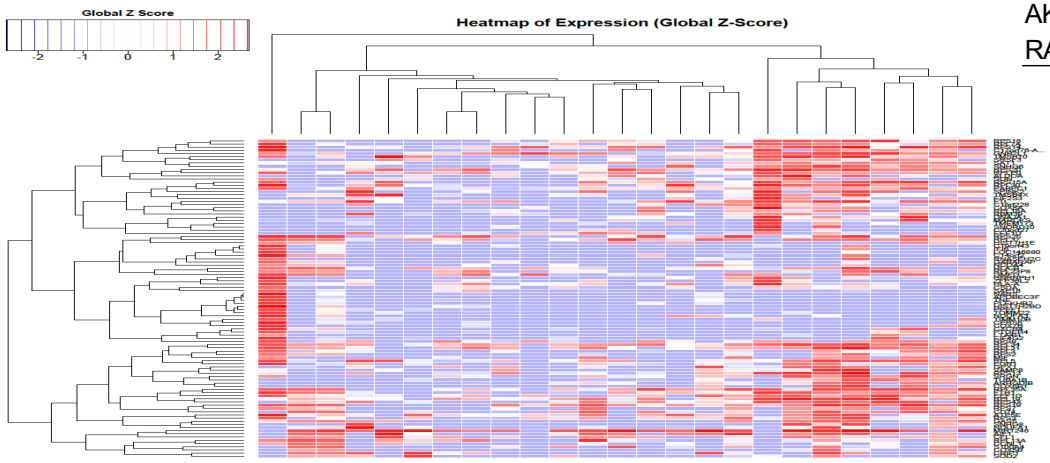
Supplementary Figure S4.

Primary human AML stem and progenitor cells recapitulate features of the single-cell gene expression patterns of murine *Mll-AF9/NRAS*^{G12V} AML. A diagnostic bone marrow aspirate from a patient with AML (sample a2, Supplementary Tables S3-4) was CD34+ selected and submitted for single-cell capture and whole transcriptome RNA sequencing. **A,** Schematic of experimental workflow. A bone aspirate sample was obtained from a patient with AML at diagnosis. CD34+ cells were isolated from the aspirate using magnetic beads and submitted for single-cell capture (C1, Fluidigm) and whole transcriptome RNA sequencing. Outlier analysis was performed per SINGuLAR (Fluidigm) to omit outliers, remaining (non-outlier) cells were used for analysis. Cells (n=25) had a mean of 911 expressed genes per cell, range of expressed genes/cell=238-2053. **B,** Heatmap shows unsupervised, two-dimensional hierarchical clustering of the differentially expressed genes within this dataset. **C,** Ingenuity Pathway Analysis and Upstream Regulators Analysis was performed on differentially expressed genes within the human single-cell RNA sequencing dataset. **D-E,** The gene expression of each CD34+CD38-AML sample (as measured by single-cell qPCR) was compared to that of the pooled CD34+CD38- normal bone marrow samples and the pooled CD34+CD38- AML samples. The Kolmogorov-Smirnoff test was used to assess the degree of divergence of each AML sample from the reference distribution (using either the pooled normal bone marrow or the pooled AML samples as the reference distribution). The pooled AML reference distribution was generated by excluding the AML sample being tested for each analysis

(such that each sample is tested against the pooled AML samples, but excluding itself). **D,** The graph represents the cumulative distribution function for a representative gene. The red line represents the cumulative distribution function of all normal bone marrow samples (pooled). The black line represents all of the AML samples (pooled). The blue lines represent individual AML samples for which this gene passed quality control. **E,** The percentage of genes whose expression deviates from the normal samples (with a $p < 0.05$) is displayed for each AML sample. The average percentage of deviation of all AML samples from the normal distribution is displayed as a bar.

A Single cell RNA sequencing of diagnostic human AML workflow

B

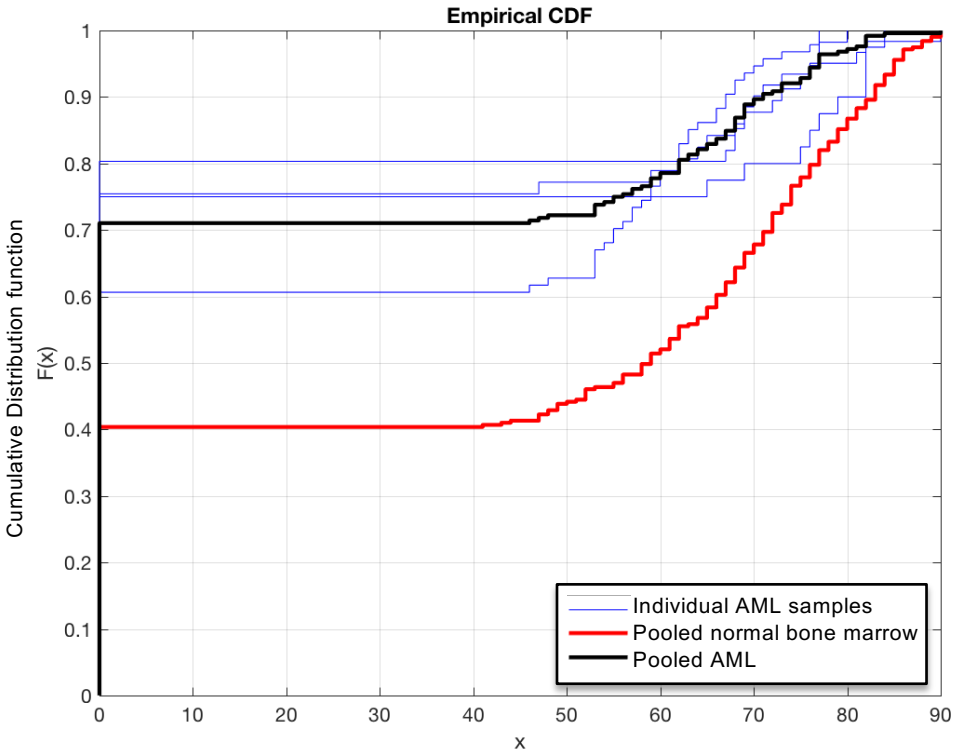


C

Ingenuity Pathway Analysis	
Top Pathways	**p value**
Hematological Development	8.8×10^{-7}
Cell Death and Survival	3.3×10^{-8}

Top Upstream Regulators	**p-value**
ERK	0.002
AKT	0.003
RAS	0.002

D



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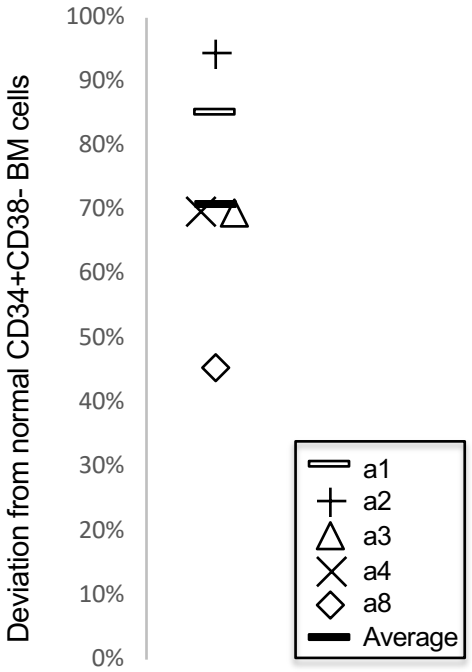
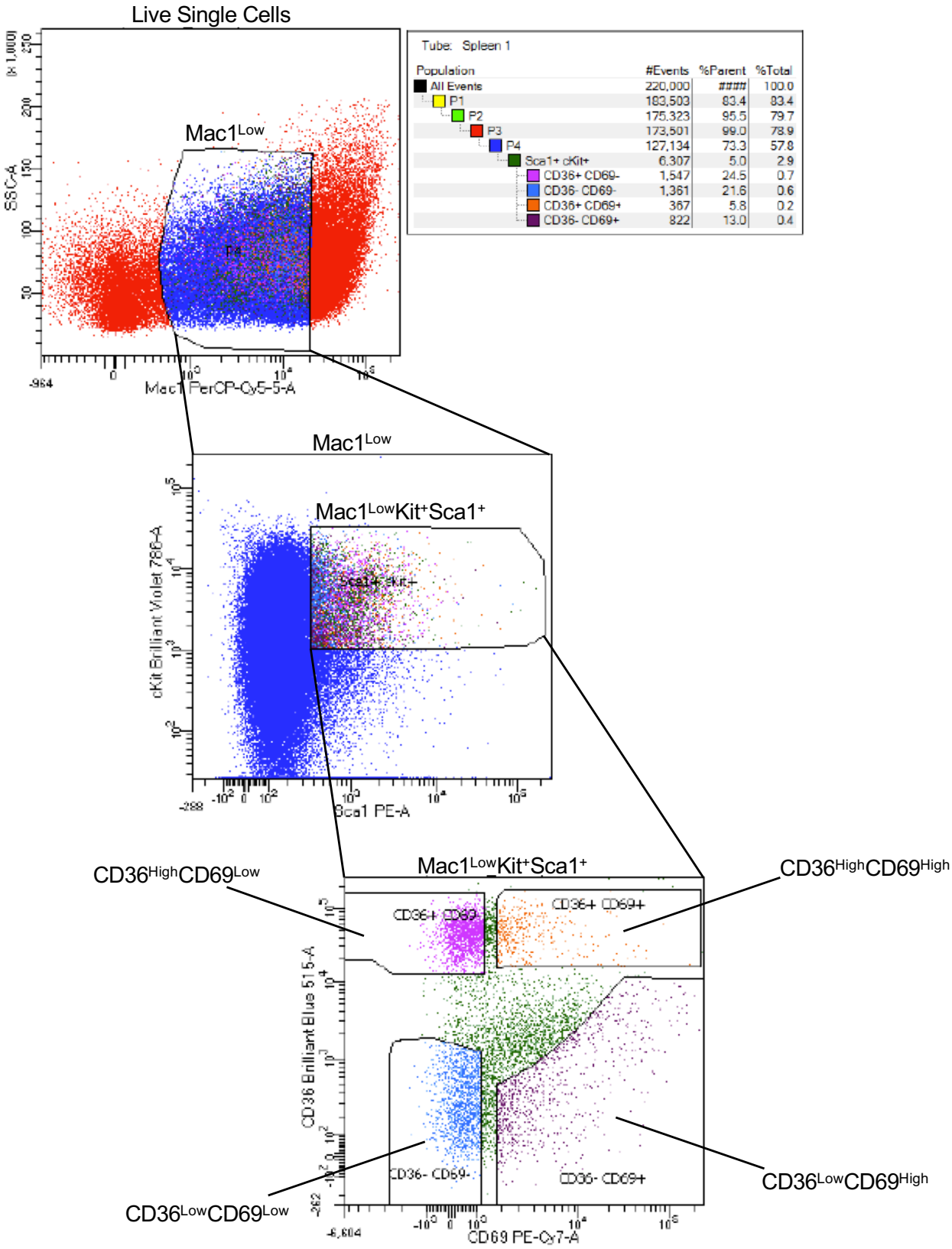


Deviation of each AML sample relative to the normal bone marrow reference distribution compared to the deviation of each AML sample relative to the AML reference distribution : ***p* < 0.0003**

Supplementary Figure S4

Supplementary Figure S5

Sorting strategy for murine AML for *in vivo* leukemia reconstitution assays and *in vitro* colony forming assays. Within the Mac1^{Low}Kit+Sca1+ LSC-enriched subpopulation, CD36^{High}CD69^{Low}, CD36^{Low}CD69^{Low}, CD36^{Low}CD69^{High}, and CD36^{High}CD69^{High} compartments were isolated.

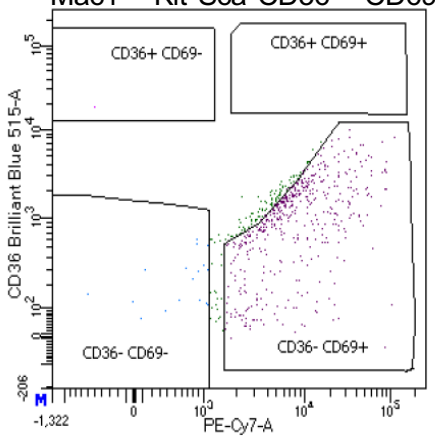


Supplementary Figure S5

Supplementary Figure S6

Post sort assessments. The purity of $CD36^{High}CD69^{Low}$, $CD36^{Low}CD69^{Low}$, $CD36^{Low}CD69^{High}$, and $CD36^{High}CD69^{High}$ compartments within the $Mac1^{Low}$Kit+Sca1+ LSC-enriched subpopulation was assessed after sorting.

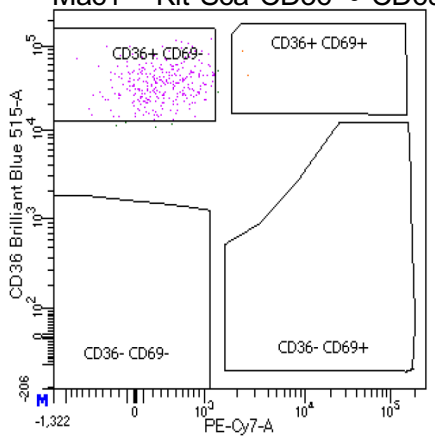
Mac1^{Low}Kit^{+}Sca^{+}CD36^{Low}CD69^{High}



Tube: CD36- CD69+

Population	#Events	%Parent	%Total
All Events	1,958	####	100.0
P1	1,528	78.0	78.0
P2	1,497	98.0	76.5
P3	1,484	99.1	75.8
P4	1,341	90.4	68.5
Sca1+ cKit+	610	45.5	31.2
CD36+ CD69-	1	0.2	0.1
CD36- CD69-	18	3.0	0.9
CD36+ CD69+	0	0.0	0.0
CD36- CD69+	478	78.4	24.4

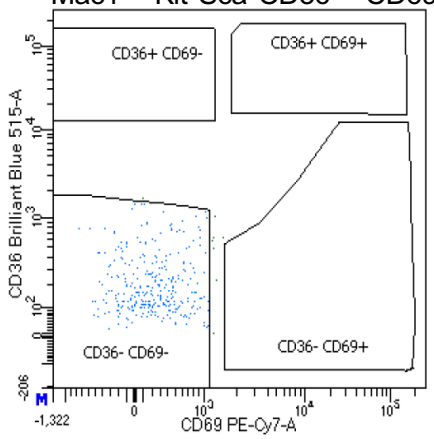
Mac1^{Low}Kit^{+}Sca^{+}CD36^{High}CD69^{Low}



Tube: CD36+ CD69-

Population	#Events	%Parent	%Total
All Events	416	####	100.0
P1	393	94.5	94.5
P2	392	99.7	94.2
P3	392	100.0	94.2
P4	382	97.4	91.8
Sca1+ cKit+	312	81.7	75.0
CD36+ CD69-	302	96.8	72.6
CD36- CD69-	0	0.0	0.0
CD36+ CD69+	2	0.6	0.5
CD36- CD69+	0	0.0	0.0

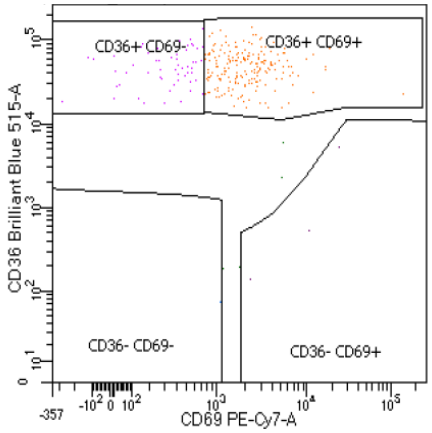
Mac1^{Low}Kit^{+}Sca^{+}CD36^{Low}CD69^{Low}



Tube: CD36- CD69-

Population	#Events	%Parent	%Total
All Events	1,051	####	100.0
P1	981	93.3	93.3
P2	976	99.5	92.9
P3	975	99.9	92.8
P4	950	97.4	90.4
Sca1+ cKit+	318	33.5	30.3
CD36+ CD69-	0	0.0	0.0
CD36- CD69-	310	97.5	29.5
CD36+ CD69+	0	0.0	0.0
CD36- CD69+	0	0.0	0.0

Mac1^{Low}Kit^{+}Sca^{+}CD36^{High}CD69^{High}



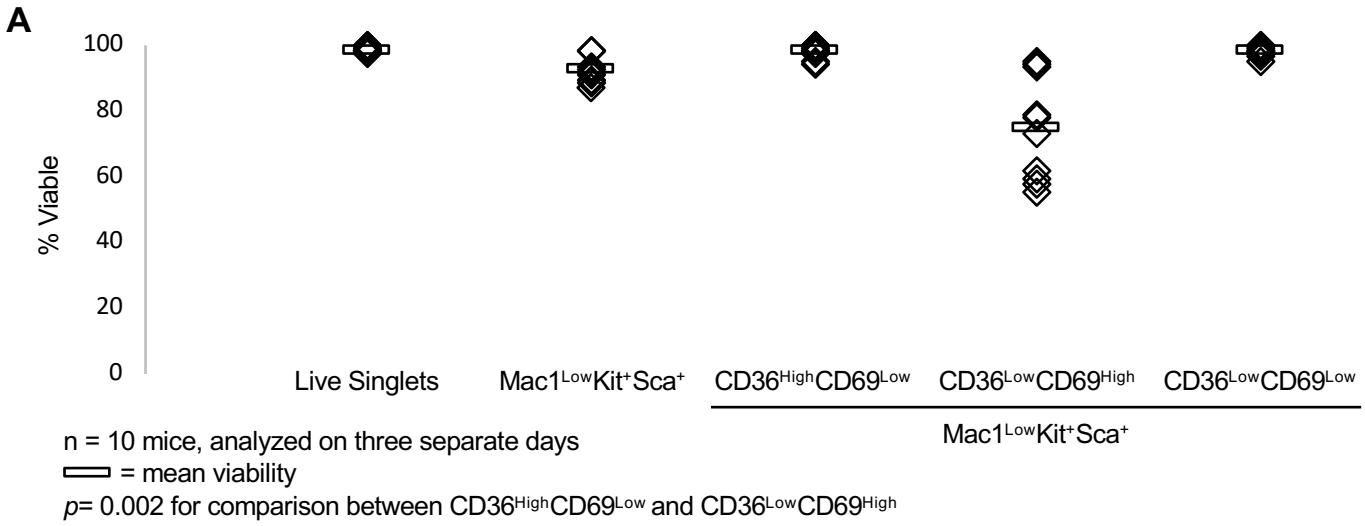
Tube: Spleen 1 CD36+ CD69+ Post-Sort

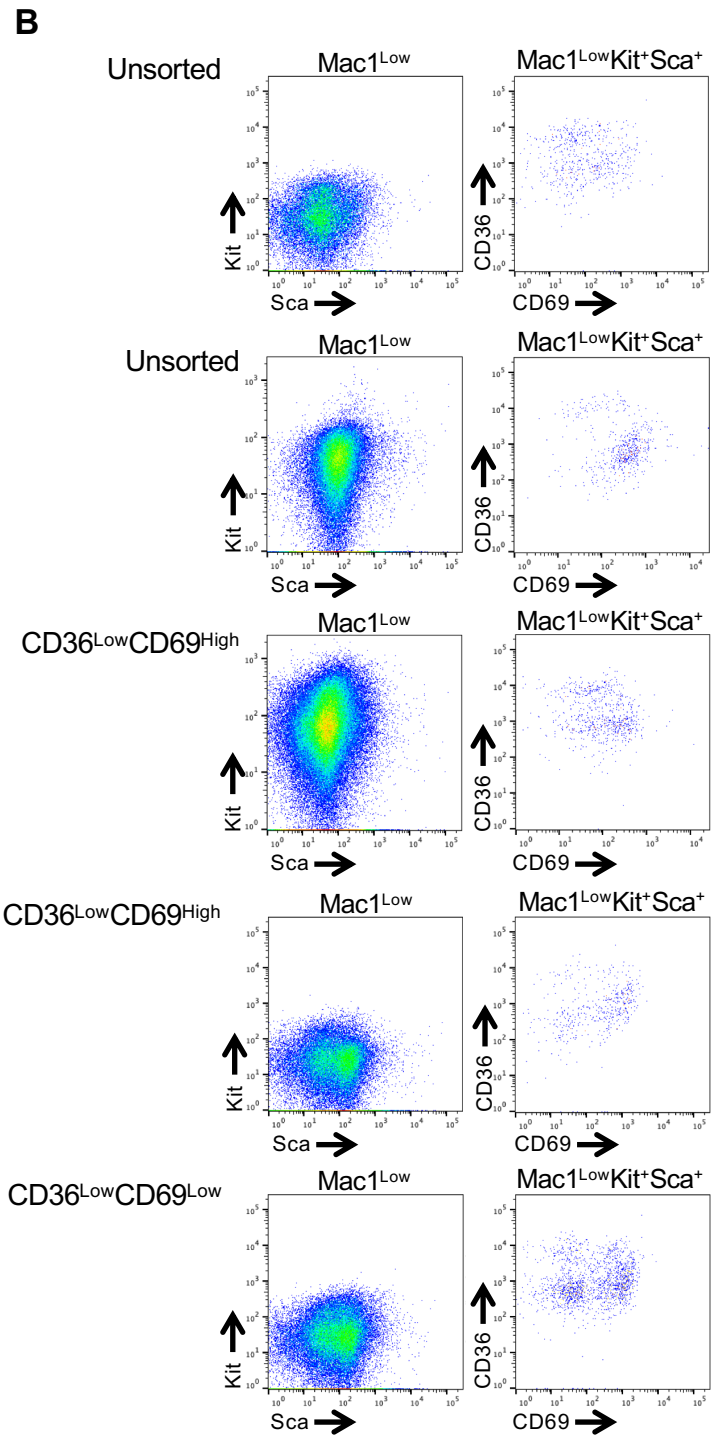
Population	#Events	%Parent	%Total
All Events	549	####	100.0
P1	358	65.2	65.2
P2	356	99.4	64.8
P3	356	100.0	64.8
P4	351	98.6	63.9
Sca1+ cKit+	269	76.6	49.0
CD36+ CD69-	60	22.3	10.9
CD36- CD69-	1	0.4	0.2
CD36+ CD69+	201	74.7	36.6
CD36- CD69+	3	1.1	0.5

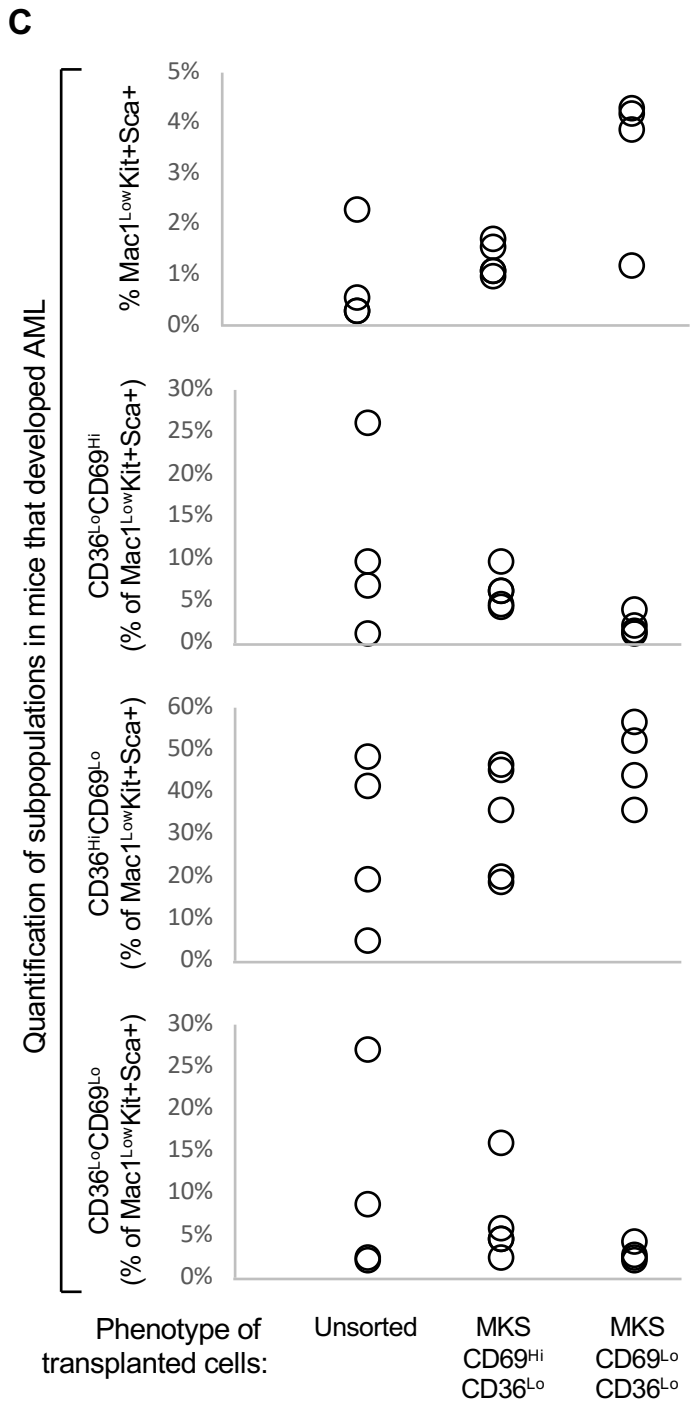
Supplementary Figure S6

Supplementary Figure S7

The viability of leukemic subpopulations and immunophenotype of transplanted leukemias. **A,** The viability of murine AML subpopulations was assessed by staining cells with immunophenotypic markers and a dead cell exclusion dye and analyzing by flow cytometry. **B-C,** The immunophenotype of leukemias that developed in mice transplanted with MKSCD36^{Low}CD69^{High} cells, MKSCD36^{Low}CD69^{Low} cells, or unsorted leukemia cells is **B,** displayed in representative plots and **C,** quantitated.



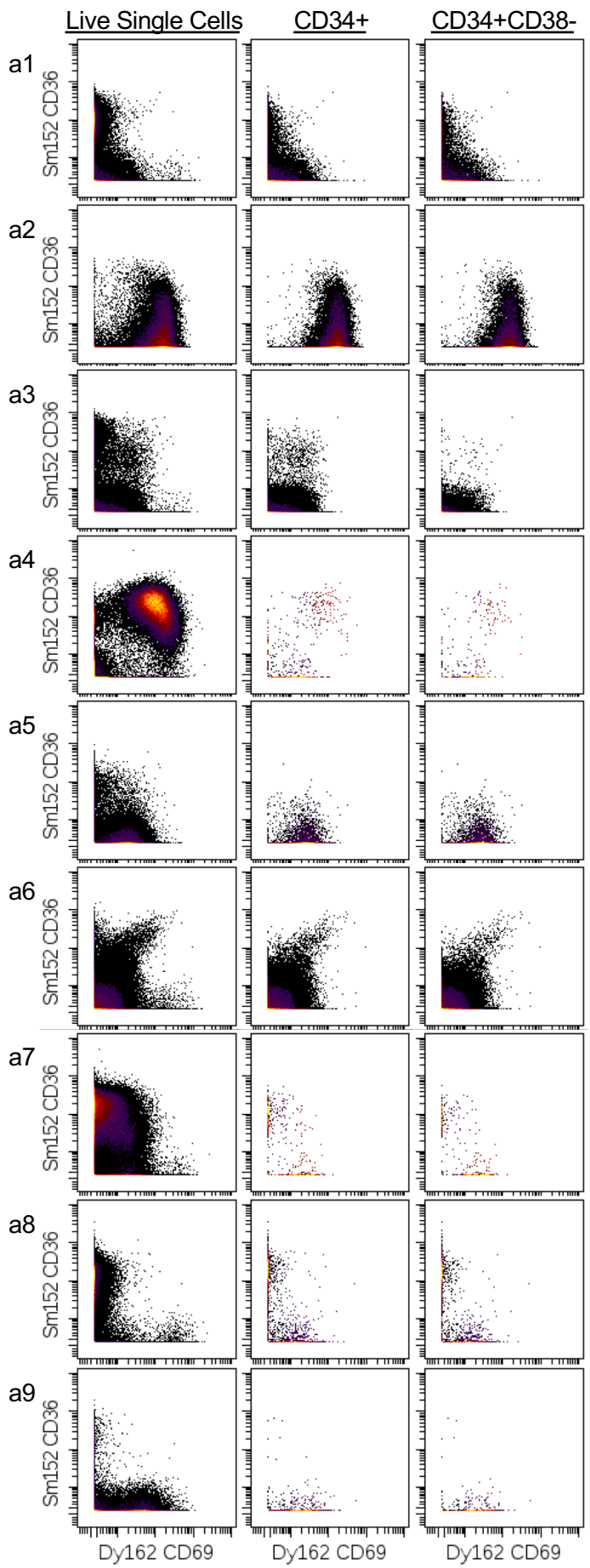




Supplementary Figure S7

Supplementary Figure S8

CD36 and CD69 expression in human AML subpopulations. Mass cytometry (CyTOF) was used to assess the expression of CD36 and CD69 in CD34+CD38- and unselected cells in a panel of nine primary human AML samples. The percentage of cells expressing Ki67% in $CD36^{High}CD69^{Low}$ or $CD36^{Low}CD69^{High}$ cells within unselected leukemia cells or within the CD34+CD38- population is displayed. Some samples had insufficient CD36+ or CD69+ cells within the CD34+CD38- compartment to assess Ki67%.



Unselected			CD34+CD38-		
% Ki67+ in $CD36^{Low}$ $CD69^{High}$ cells	Ki67% in $CD36^{High}$ $CD69^{Low}$ cells	Ratio Ki67 in $\frac{CD36^{High}}{CD69^{High}}$	% Ki67+ in $CD36^{Low}$ $CD69^{High}$ cells	Ki67% in $CD36^{High}$ $CD69^{Low}$ cells	Ratio Ki67 in $\frac{CD36^{High}}{CD69^{High}}$
26	32	1.2	14	28	2.0
54	68	1.3	*	*	*
30	50	1.7	20	19	1.0
40	79	2.0	*	*	*
41	36	0.9	*	*	*
13	28	2.2	11	9	0.8
35	42	1.2	20	38	1.9
20	80	4.0	45	74	1.6
41	62	1.5	*	*	*

*insufficient CD36+CD69- or CD36-CD69+ cells within the population to assess

Supplementary Figure S8