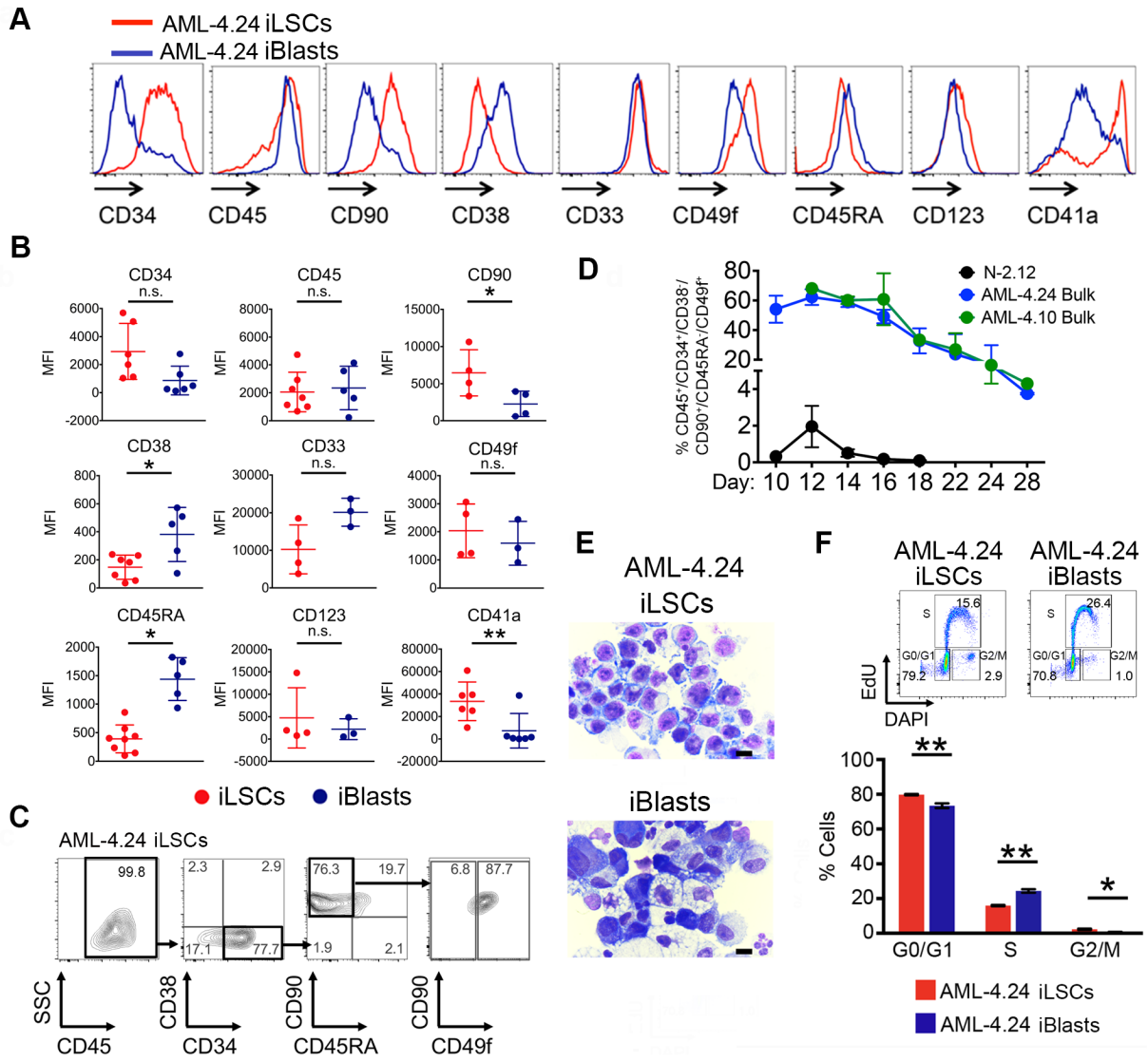
Cell Reports, Volume *31*

Supplemental Information

Acute Myeloid Leukemia iPSCs Reveal a Role for RUNX1 in the Maintenance of Human Leukemia Stem Cells

Josephine Wesely, Andriana G. Kotini, Franco Izzo, Hanzhi Luo, Han Yuan, Jun Sun, Maria Georgomanoli, Asaf Zviran, André G. Deslauriers, Neville Dusaj, Stephen D. Nimer, Christina Leslie, Dan A. Landau, Michael G. Kharas, and Eirini P. Papapetrou


A
AML-4.24 iLSCs
AML-4.24 iBlasts
CD34
CD45
CD90
CD38
CD33
CD49f
CD45RA
CD123
CD41a
B
CD34
n.s.
CD45
n.s.
CD90
*
CD38
*
CD33
n.s.
CD49f
n.s.
CD45RA
*
CD123
n.s.
CD41a
**
MFI
iLSCs
iBlasts
C
AML-4.24 iLSCs
99.8
2.3
2.9
17.1
77.7
76.3
19.7
1.9
2.1
6.8
87.7
SSC
CD45
CD38
CD34
CD90
CD45RA
CD90
CD49f
D
% CD45+/CD34+/CD38-/ CD90+/CD45RA-/CD49f+
N-2.12
AML-4.24 Bulk
AML-4.10 Bulk
Day: 10 12 14 16 18 22 24 28
E
AML-4.24 iLSCs
iBlasts
F
AML-4.24 iLSCs
AML-4.24 iBlasts
S
15.6
G0/G1
79.2
G2/M
2.9
26.4
70.8
1.0
EdU
DAPI
**
**
*
% Cells
G0/G1
S
G2/M
AML-4.24 iLSCs
AML-4.24 iBlasts

Supplemental Figure and Table Legends

Figure S1. Phenotypic heterogeneity of hematopoietic cells derived from AML-iPSCs (related to Figure 1). (A) Expression of hematopoietic markers by flow cytometry in iLSCs and iBlasts from the AML-4.24 iPSC line on day 23 of differentiation. (B) Expression of the indicated hematopoietic markers in iLSCs and iBlasts measured as mean fluorescence intensity (MFI). Mean and SEM from 3-7 different experiments including both AML-4.10 and AML-4.24 iPSC lines are shown. *$p<0.05$, **$p<0.001$, t test. (C) HSC markers in iLSCs from the AML-4.24 iPSC line on day 16 of hematopoietic differentiation. (D) Time course of the fraction of immunophenotypic HSCs (CD45+/CD34+/CD38-/CD90+/CD45RA-/CD49f+) at the indicated days of hematopoietic differentiation cultures from normal iPSCs (N-2.12 line) and from the two AML lines AML-4.24 and AML-4.10 (without separation of adherent/suspension fractions). Mean and SEM from 2-3 independent experiments are shown. (E) Wright-Giemsa-stained cytospins of manually separated cells. Scale bar, 10 μm. (F) Cell-cycle analysis of iLSCs and iBlasts from the AML-4.24 iPSC line. Mean and SEM from 3 independent experiments are shown. *$p<0.05$, **$p<0.001$, t test.

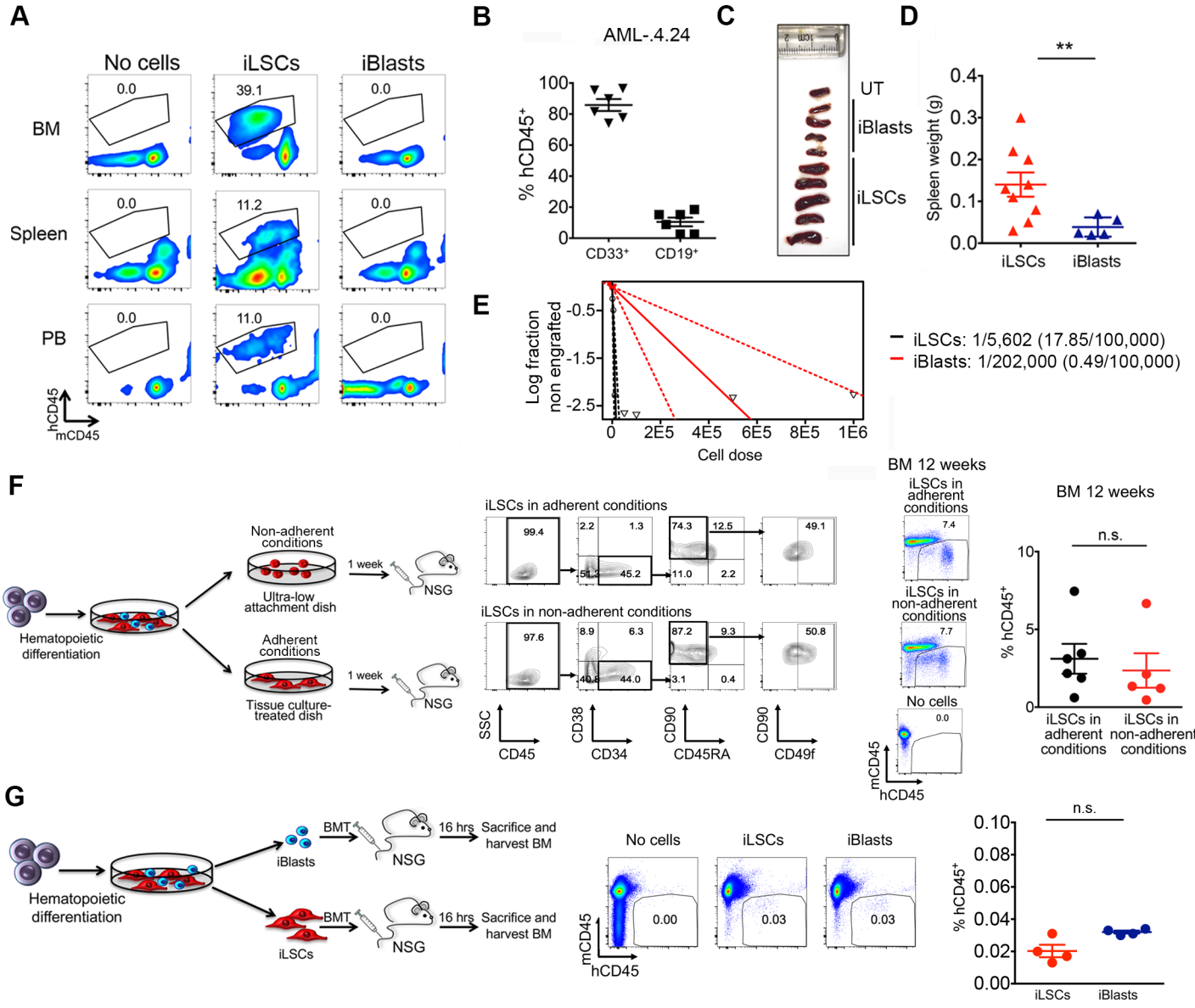

A
No cells
iLSCs
iBlasts
BM
Spleen
PB
0.0
39.1
11.2
11.0
hCD45
mCD45
B
AML-.4.24
% hCD45+
CD33+
CD19+
C
UT
iBlasts
iLSCs
D
Spleen weight (g)
iLSCs
iBlasts
E
Log fraction non engrafted
Cell dose
iLSCs: 1/5,602 (17.85/100,000)
iBlasts: 1/202,000 (0.49/100,000)
F
Hematopoietic differentiation
Non-adherent conditions
Ultra-low attachment dish
Adherent conditions
Tissue culture-treated dish
1 week
NSG
iLSCs in adherent conditions
iLSCs in non-adherent conditions
SSC
CD45
CD38
CD34
CD90
CD45RA
CD49f
BM 12 weeks
No cells
n.s.
G
Hematopoietic differentiation
iBlasts
iLSCs
BMT
16 hrs
Sacrifice and harvest BM
NSG
No cells
iLSCs
iBlasts
mCD45
hCD45
n.s.
% hCD45+

Figure S2. iLSCs serially transplant a lethal leukemia in NSG mice (related to Figure 2).

(A) Representative flow cytometry panels assessing human cell engraftment of iLSCs and iBlasts in the BM, spleen and peripheral blood (PB) of recipient mice 8-12 weeks post-transplantation. (B) Fraction of myeloid ($CD33^+$) and lymphoid ($CD19^+$) lineage cells within the $hCD45^+$ population in the BM of mice transplanted with iBlasts. (C) Representative picture of spleens from one transplantation experiment. Spleens from 4 mice injected with iBlasts, 5 mice injected with iLSCs and one untransplanted (UT) mouse are shown. (D) Spleen weight of mice transplanted with iLSCs and iBlasts 8-12 weeks post-transplantation. **$p<0.001$, t test. (E) Frequency of leukemia initiating cells in iLSCs and iBlasts calculated based on limiting dilution analysis (95% confidence interval). (F) Left panel: Experimental scheme. Middle panel: Flow cytometry analysis of HSC markers at the end of a 1 week culture in adherent vs non-adherent conditions and before transplantation into mice (one of two experiments). Right panels: BM engraftment at 12 weeks. Representative mice (left) and cumulative data (right) from two independent differentiation and transplantation experiments. (G) Left panel: Experimental scheme. Middle and right panels: Detection levels of hCD45+ cells in mouse BM 16 hours following iv injection. Representative mice (middle) and cumulative data (right) from two independent differentiation experiments (with 2 mice per group per experiment).

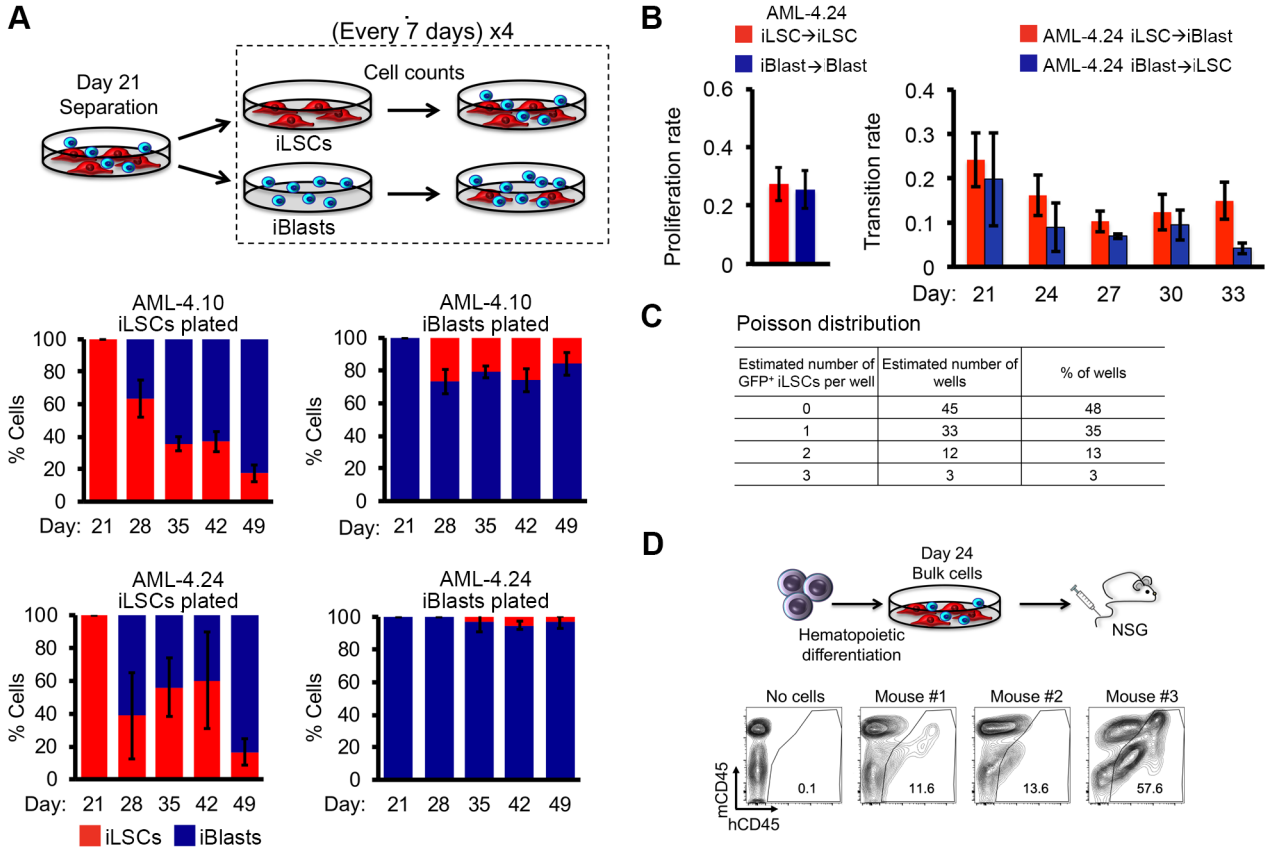

A
(Every 7 days) x4
Day 21
Separation
Cell counts
iLSCs
iBlasts
AML-4.10
iLSCs plated
AML-4.10
iBlasts plated
AML-4.24
iLSCs plated
AML-4.24
iBlasts plated
% Cells
Day: 21 28 35 42 49
iLSCs
iBlasts
B
AML-4.24
iLSC→iLSC
iBlast→Blast
AML-4.24 iLSC→iBlast
AML-4.24 iBlast→iLSC
Proliferation rate
Transition rate
Day: 21 24 27 30 33
C
Poisson distribution
Estimated number of GFP+ iLSCs per well
Estimated number of wells
% of wells
0 45 48
1 33 35
2 12 13
3 3 3
D
Day 24
Bulk cells
Hematopoietic
differentiation
NSG
No cells
Mouse #1
Mouse #2
Mouse #3
mCD45
hCD45
0.1
11.6
13.6
57.6

Figure S3. iLSCs give rise to iBlasts (related to Figure 3).

(A) Upper panel: Schematic of experimental setup. iLSCs and iBlasts were separated on day 21 of hematopoietic differentiation and counted. Every 7 days for 4 weeks, iLSCs and iBlasts were separated, counted and plated back. Lower panels: Fraction of iLSCs and iBlasts in cultures inititated from separated iLSCs (left panels) or iBlasts (right panels) from lines AML-4.10 (middle panels) and AML-4.24 (lower panels) at the indicated time points. Mean and SEM of 3 independent experiments are shown. (B) Proliferation rate (left) and transition rate (right) of cells derived from line AML-4.24, calculated as shown in Table S1. Mean and SEM from 3 independent experiments are shown. Proliferation rate is averaged across all time points. (C) Poisson distribution of single cell plating experiment shown in Figure 3C. Based on the number of GFP^- wells (45 of a total of 93 wells assessed for GFP), the number of wells predicted to have 1, 2 or 3 GFP^+ iLSCs plated are estimated. (D) Upper panel: Schematic of experiment. Bulk cells from day 24 of hematopoietic differentiation of the AML-4.10 line were intravenously injected into sublethally irradiated or busulfan-treated NSG mice. Lower panels: Human cell engraftment in the BM of 3 recipient mice 10 weeks post-transplantation.

Figure S4. Single cell transcriptome analysis (related to Figure 4).

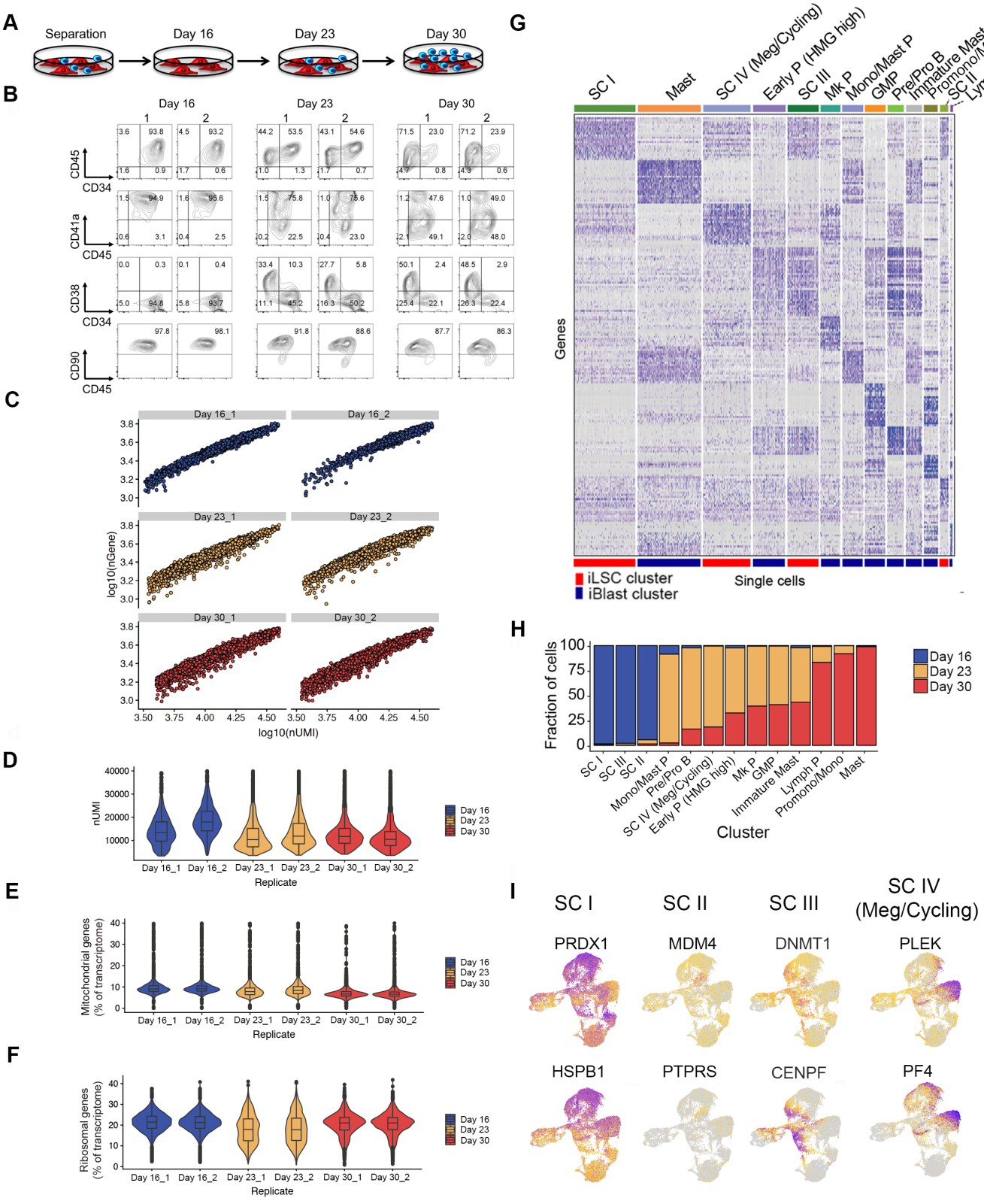


Figure S4. Single cell transcriptome analysis (related to Figure 4).

(A) Scheme of experimental setup for scRNAseq analyses. iLSCs were separated on day 16. Cells were harvested on day 16 (corresponding to isolated iLSCs), day 23 and day 30. (B) Immunophenotypes of duplicate samples analyzed by scRNA-seq at the indicated time points. (C) Number of unique molecular identifiers (nUMI) vs number of genes (nGene) detected per cell in each sample. (D) Violin plots of numbers of UMIs per cell for each sample. (E) Violin plots showing the percentage of mitochondrial genes per cell in each sample. (F) Violin plots showing the percentage of ribosomal genes per cell in each sample. (G) Heatmap of 13 clusters across all single-cell transcriptomes. Cluster annotation (top) was based on manual curation of marker genes. (H) Fraction of cells in each cluster corresponding to the 3 different sampling time points. (I) Expression of selected genes differentiating the 4 SC clusters, projected onto the UMAP plot from Figure 4B.

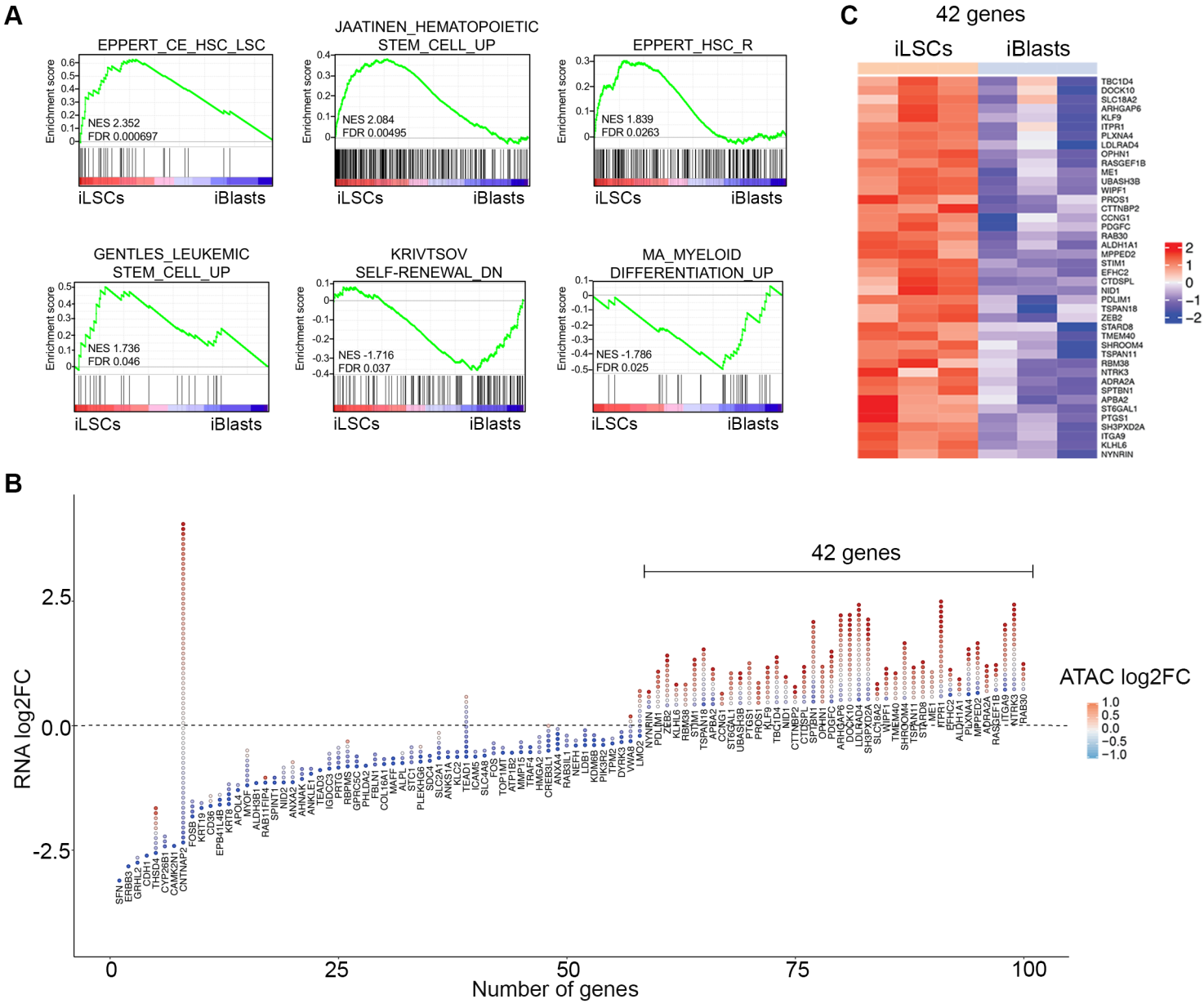

A
EPPERT_CE_HSC_LSC
NES 2.352
FDR 0.000697
JAATINEN_HEMATOPOIETIC STEM_CELL_UP
NES 2.084
FDR 0.00495
EPPERT_HSC_R
NES 1.839
FDR 0.0263
GENTLES_LEUKEMIC STEM_CELL_UP
NES 1.736
FDR 0.046
KRIVTSOV SELF-RENEWAL_DN
NES -1.716
FDR 0.037
MA_MYELOID DIFFERENTIATION_UP
NES -1.786
FDR 0.025
Enrichment score
iLSCs
iBlasts
C
42 genes
iLSCs
iBlasts
TBC1D4
DOCK10
SLC18A2
ARHGAP6
KLF9
ITPR1
PLXNA4
LDLRAD4
OPHN1
RASGEF1B
ME1
UBASH3B
WIPF1
PROS1
CTTNBP2
CCNG1
PDGFC
RAB30
ALDH1A1
MPPED2
STIM1
EFHC2
CTDSPL
NID1
PDLIM1
TSPAN18
ZEB2
STARD8
TMEM40
SHROOM4
TSPAN11
RBM38
NTRK3
ADRA2A
SPTBN1
APBA2
ST6GAL1
PTGS1
SH3PXD2A
ITGA9
KLHL6
NYNRIN
2
1
0
−1
−2
B
42 genes
RNA log2FC
2.5
0.0
-2.5
0
25
50
75
100
Number of genes
ATAC log2FC
1.0
0.5
0.0
−0.5
−1.0

Figure S5. Transcriptome and chromatin accessibility analyses (related to Figure 5).

(A) GSEA plots of the indicated gene sets against the rank list of differentially accessible genes in iLSCs and iBlasts, showing enrichment of HSC and LSC transcriptional programs in iLSCs. (B) Fold change (log2FC) of gene expression and accessibility in iLSCs vs iBlasts. 58 genes have decreased expression and decreased accessibility (left) and 42 genes have increased expression and increased accessibility (right) in iLSCs vs iBlasts. For each gene, the position of the gene symbol in the y axis indicates the expression change. All peaks belonging to a gene are shown as circles stacked on top of the gene symbol. The color of the circles indicates the accessibility log2FC of each peak. (C) Heatmap of gene expression of the 42 genes (from Figure 5G) in iLSCs and iBlasts.

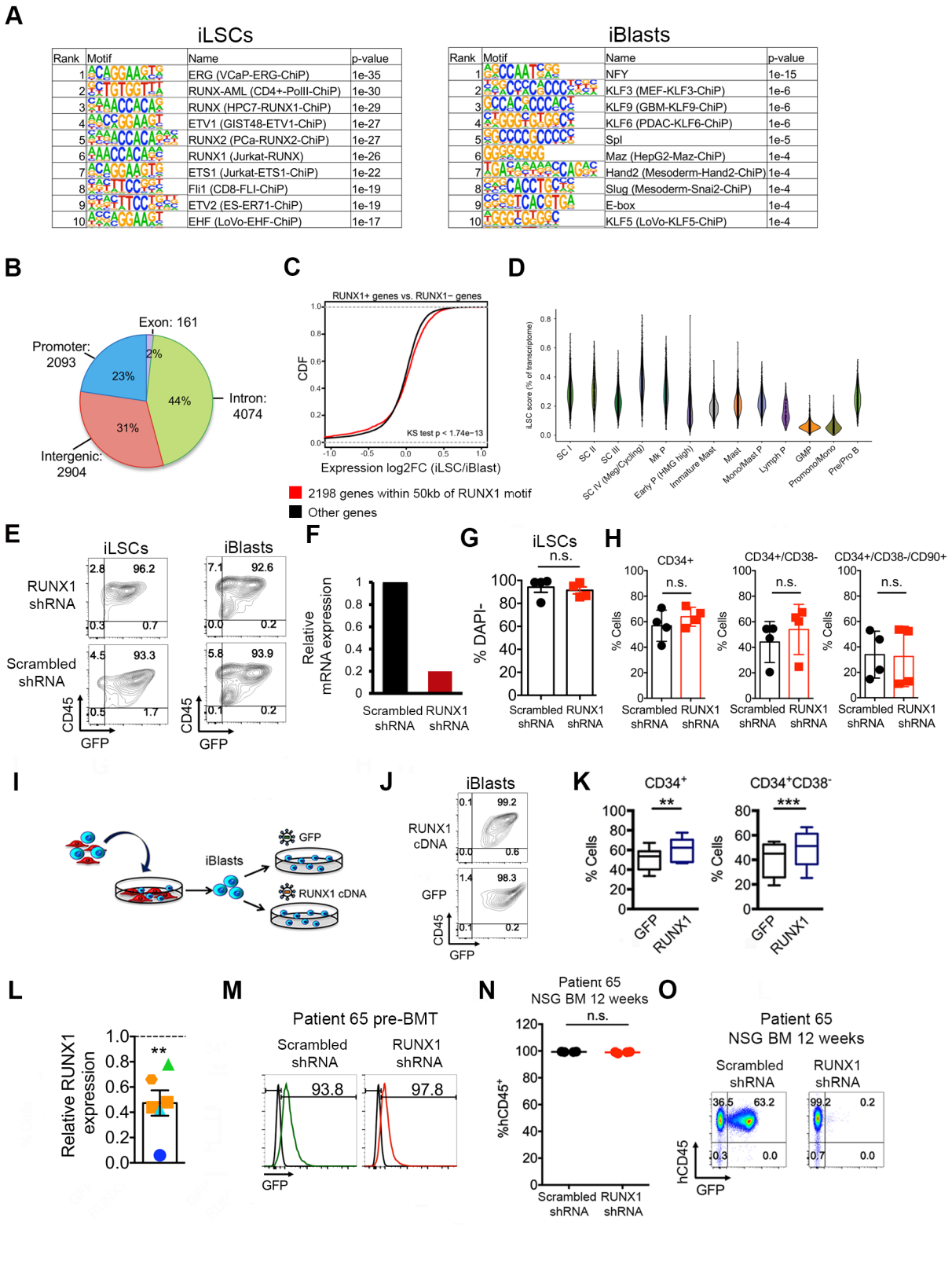

A
iLSCs
Rank Motif Name p-value
1 ERG (VCaP-ERG-ChiP) 1e-35
2 RUNX-AML (CD4+-PolII-ChiP) 1e-30
3 RUNX (HPC7-RUNX1-ChiP) 1e-29
4 ETV1 (GIST48-ETV1-ChiP) 1e-27
5 RUNX2 (PCa-RUNX2-ChiP) 1e-27
6 RUNX1 (Jurkat-RUNX) 1e-26
7 ETS1 (Jurkat-ETS1-ChiP) 1e-22
8 Fli1 (CD8-FLI-ChiP) 1e-19
9 ETV2 (ES-ER71-ChiP) 1e-19
10 EHF (LoVo-EHF-ChiP) 1e-17
iBlasts
Rank Motif Name p-value
1 NFY 1e-15
2 KLF3 (MEF-KLF3-ChiP) 1e-6
3 KLF9 (GBM-KLF9-ChiP) 1e-6
4 KLF6 (PDAC-KLF6-ChiP) 1e-6
5 SpI 1e-5
6 Maz (HepG2-Maz-ChiP) 1e-4
7 Hand2 (Mesoderm-Hand2-ChiP) 1e-4
8 Slug (Mesoderm-Snai2-ChiP) 1e-4
9 E-box 1e-4
10 KLF5 (LoVo-KLF5-ChiP) 1e-4
B
Exon: 161
Promoter: 2093
Intergenic: 2904
Intron: 4074
2%
23%
44%
31%
C
RUNX1+ genes vs. RUNX1− genes
CDF
KS test p < 1.74e−13
Expression log2FC (iLSC/iBlast)
2198 genes within 50kb of RUNX1 motif
Other genes
D
iLSC score (% of transcriptome)
SC I
SC II
SC III
SC IV (Meg/Cycling)
Mk P
Early P (HMG high)
Immature Mast
Mast
Mono/Mast P
Lymph P
GMP
Promono/Mono
Pre/Pro B
E
iLSCs
iBlasts
RUNX1 shRNA
Scrambled shRNA
2.8 96.2 0.3 0.7
4.5 93.3 0.5 1.7
7.1 92.6 0.0 0.2
5.8 93.9 0.1 0.2
CD45
GFP
F
Relative mRNA expression
Scrambled shRNA
RUNX1 shRNA
G
iLSCs
n.s.
% DAPI-
H
CD34+
CD34+/CD38-
CD34+/CD38-/CD90+
n.s.
% Cells
I
iBlasts
GFP
RUNX1 cDNA
J
iBlasts
RUNX1 cDNA
GFP
0.1 99.2 0.0 0.6
1.4 98.3 0.1 0.2
K
CD34+
CD34+CD38-
**
***
% Cells
GFP
RUNX1
L
Relative RUNX1 expression
**
M
Patient 65 pre-BMT
Scrambled shRNA
RUNX1 shRNA
93.8
97.8
GFP
N
Patient 65 NSG BM 12 weeks
n.s.
%hCD45+
Scrambled shRNA
RUNX1 shRNA
O
Patient 65 NSG BM 12 weeks
Scrambled shRNA
RUNX1 shRNA
36.5 63.2 0.3 0.0
99.2 0.2 0.7 0.0
hCD45
GFP

Figure S6. Role of RUNX1 in LSC maintenance (related to Figure 6).

(A) Top enriched motifs in peaks with significantly increased accessibility in iLSCs (left) and in peaks with significantly increased accessibility in iBlasts (right). (B) Genomic annotation of all ATAC-seq peaks containing RUNX1 motifs. (C) Cumulative distribution functions (CDF) plot of the expression change (log2FC) of genes within 50 kb of a RUNX1 motif (red) and of all other genes (black), showing higher expression of RUNX1-associated genes in iLSCs. (D) Violin plots showing the iLSC score (determined by the expression levels of the 42 genes from Figure 5G) of each scRNAseq cluster. (E) Assessment of transduction efficiency (GFP^+ cells) in iLSCs (left) and iBlasts (right) 48 hours after lentiviral transduction with a lentiviral vector encoding RUNX1 or scrambled shRNA. (F) Relative RUNX1 mRNA expression in iLSCs transduced with lentiviral vectors encoding RUNX1 or scrambled shRNA 48 hours after transduction. (G) Cell viability of iLSCs measured by DAPI staining 7 days after transduction with lentiviral vectors encoding RUNX1 or scrambled shRNA. Mean and SEM of 4 independent experiments are shown. (H) Immunophenotype of iLSCs 7 days after transduction with lentiviral vectors encoding RUNX1 or scrambled shRNA. Mean and SEM of 4 independent experiments are shown. (I) Separated iBlasts were transduced with a lentiviral vector encoding GFP or RUNX1 cDNA. (J) Assessment of transduction efficiency (GFP^+ cells) of iBlasts 48 hours after transduction with lentiviral vectors encoding GFP or RUNX1 cDNA. (K) Immunophenotype of iBlasts transduced with a lentiviral vector encoding GFP or RUNX1. Mean and SEM of 7 independent experiments are shown. $^{**}p<0.001$, $^{***}p<0.001$, t test. (L) RUNX1 mRNA expression in 6 primary AML samples after RUNX1 shRNA KD, relative to RUNX1 expression in the same samples after scrambled shRNA transduction. Mean and SEM are shown. $^{**}p< 0.01$, t test. (M) Assessment of transduction efficiency of primary AML patient cells (Patient 65) 48 hours after transduction with RUNX1 shRNA or scrambled shRNA. (N) Engraftment levels of AML Patient 65 cells transduced with RUNX1 or scrambled shRNA in the BM of mice 12 weeks after transplantation. Each data point represents a unique mouse. Mean and SEM are shown. (O) Representative flow cytometry panels showing human cell engraftment and engraftment of GFP+ cells in the BM of recipient mice 12 weeks post-transplantation with AML Patient 65 cells transduced with RUNX1 shRNA or scrambled shRNA.

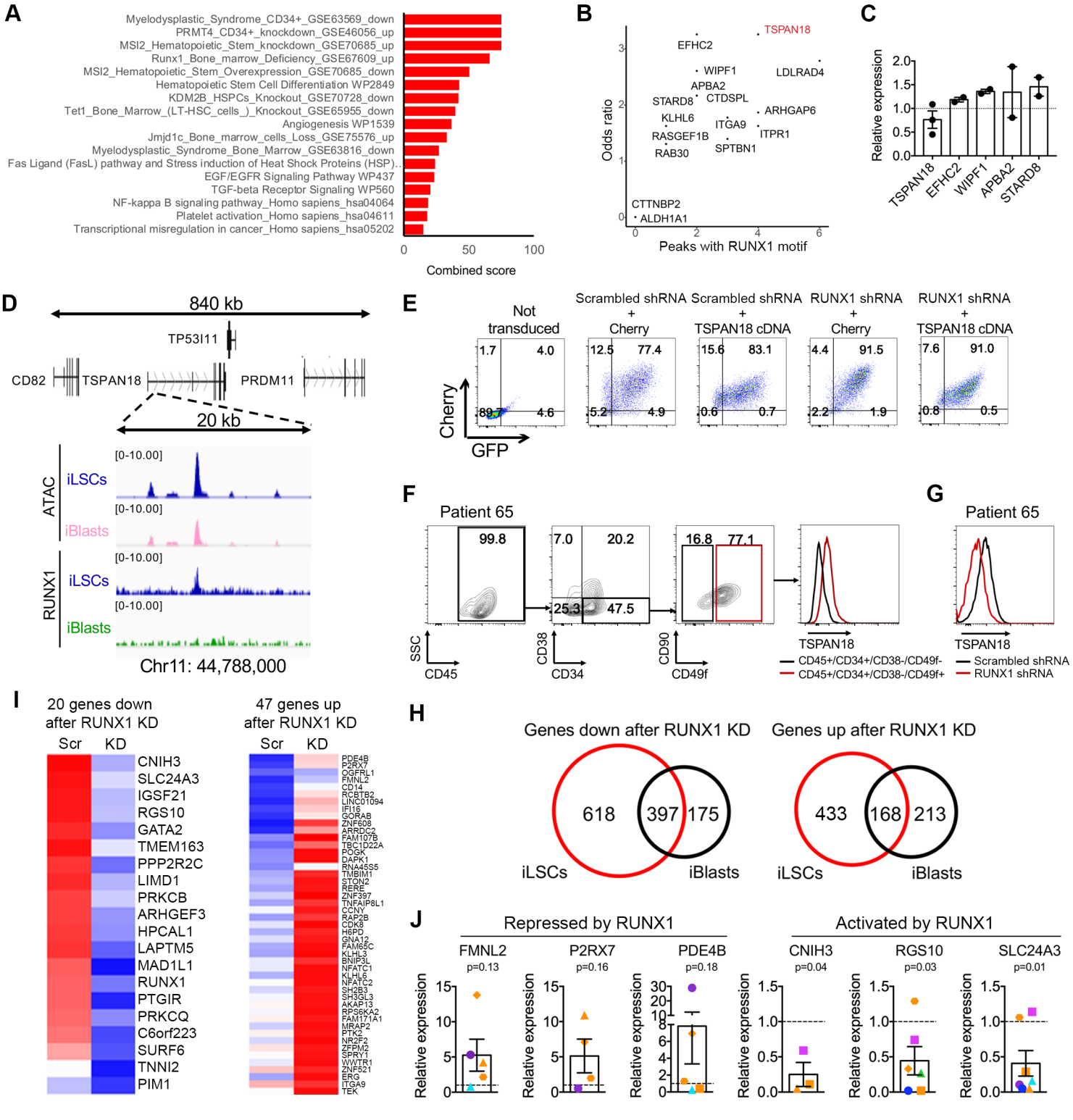

A
Myelodysplastic_Syndrome_CD34+_GSE63569_down
PRMT4_CD34+_knockdown_GSE46056_up
MSI2_Hematopoietic_Stem_knockdown_GSE70685_up
Runx1_Bone_marrow_Deficiency_GSE67609_up
MSI2_Hematopoietic_Stem_Overexpression_GSE70685_down
Hematopoietic Stem Cell Differentiation WP2849
KDM2B_HSPCs_Knockout_GSE70728_down
Tet1_Bone_Marrow_(LT-HSC_cells_)_Knockout_GSE65955_down
Angiogenesis WP1539
Jmjd1c_Bone_marrow_cells_Loss_GSE75576_up
Myelodysplastic_Syndrome_Bone_Marrow_GSE63816_down
Fas Ligand (FasL) pathway and Stress induction of Heat Shock Proteins (HSP)..
EGF/EGFR Signaling Pathway WP437
TGF-beta Receptor Signaling WP560
NF-kappa B signaling pathway_Homo sapiens_hsa04064
Platelet activation_Homo sapiens_hsa04611
Transcriptional misregulation in cancer_Homo sapiens_hsa05202
0
50
100
Combined score
B
TSPAN18
EFHC2
WIPF1
LDLRAD4
APBA2
STARD8
CTDSPL
ARHGAP6
KLHL6
ITGA9
RASGEF1B
ITPR1
RAB30
SPTBN1
CTTNBP2
ALDH1A1
Odds ratio
Peaks with RUNX1 motif
C
Relative expression
TSPAN18
EFHC2
WIPF1
APBA2
STARD8
D
840 kb
TP53I11
CD82
TSPAN18
PRDM11
20 kb
ATAC
iLSCs
iBlasts
RUNX1
[0-10.00]
Chr11: 44,788,000
E
Not transduced
Scrambled shRNA + Cherry
Scrambled shRNA + TSPAN18 cDNA
RUNX1 shRNA + Cherry
RUNX1 shRNA + TSPAN18 cDNA
Cherry
GFP
F
Patient 65
SSC
CD45
CD38
CD34
CD90
CD49f
TSPAN18
CD45+/CD34+/CD38-/CD49f-
CD45+/CD34+/CD38-/CD49f+
G
Patient 65
TSPAN18
Scrambled shRNA
RUNX1 shRNA
I
20 genes down after RUNX1 KD
47 genes up after RUNX1 KD
Scr
KD
CNIH3
SLC24A3
IGSF21
RGS10
GATA2
TMEM163
PPP2R2C
LIMD1
PRKCB
ARHGEF3
HPCAL1
LAPTM5
MAD1L1
RUNX1
PTGIR
PRKCQ
C6orf223
SURF6
TNNI2
PIM1
H
Genes down after RUNX1 KD
618
397
175
iLSCs
iBlasts
Genes up after RUNX1 KD
433
168
213
iLSCs
iBlasts
J
Repressed by RUNX1
Activated by RUNX1
FMNL2
p=0.13
P2RX7
p=0.16
PDE4B
p=0.18
CNIH3
p=0.04
RGS10
p=0.03
SLC24A3
p=0.01
Relative expression

Figure S7. RUNX1 regulation of iLSCs (related to Figure 7).

(A) Pathway enrichment analysis using genes that are preferentially bound by RUNX1 in iLSCs vs iBlasts. (B) RUNX1 motif enrichment in accessible chromatin peaks linked to the 16 genes from Figure 7C (those among the 42 gene set that were bound by RUNX1 preferentially in iLSCs than in iBlasts). The number of accessible peaks containing RUNX1 motifs for each gene is shown in the x axis. The y axis indicates RUNX1 motif enrichment for the peaks associated with the 16 genes compared to background (atlas), measured as odds ratio. (C) Expression of the indicated genes (top 5 predicted RUNX1 targets) in iLSCs transduced with RUNX1 shRNA relative to scrambled shRNA. Mean and SEM of 2-3 independent experiments are shown. (D) Genome tracks showing ATAC-seq and RUNX1 binding (CHIP-seq) peaks within intron 1 of TSPAN18 in iLSCs and iBlasts. (E) Transduction efficiency 48 hours after transduction with a GFP lentiviral vector encoding RUNX1 or scrambled shRNA and a Cherry lentiviral vector encoding mCherry or mCherry and TSPAN18 cDNA. (F) TSPAN18 expression in LSC-enriched CD45+/CD34+/CD38-/CD49f+ compared to CD45+/CD34+/CD38-/CD49f- cells from Patient 65. (G) Decrease in TSPAN18 expression after RUNX1 KD in Patient 65 cells. (Histograms are gated on GFP+ cells.) (H) Venn diagrams of DEGs after RUNX1 KD in iLSCs and iBlasts. (I) Heatmaps showing expression of the 20 and 47 genes from Figure 7E (Scr: scrambled shRNA; KD: RUNX1 KD). (J) Expression of 3 of the top RUNX1-activated and 3 of the top RUNX1-repressed genes after RUNX1 KD in primary AML patient cells relative to scrambled shRNA (each point represents one patient sample from Figure 6L). Mean and SEM are shown.

Table S1. Calculation of proliferation and transition rates (related to Figure 3).

AML-4.10					
Proliferation rate iLSCs (P)=Log[(iLSCd3/iLSCd0)/3]					
	Day 21	Day 24	Day 27	Day 30	Day 33
Experiment 1	0.31	0.33	0.28	0.26	0.30
Experiment 2	0.69	0.45	0.27	0.30	0.22
Experiment 3	0.55	0.58	0.49	0.28	0.20
Mean	0.52	0.45	0.35	0.28	0.24
SEM	0.11	0.07	0.07	0.01	0.03
Mean (all experiments)	0.37				
SEM (all experiments)	0.04				
Proliferation rate iBlasts (P)=Log[(iBlastd3/iBlastd0)/3]					
	Day 21	Day 24	Day 27	Day 30	Day 33
Experiment 1	0.29	0.10	0.41	0.37	0.28
Experiment 2	0.57	0.44	0.33	0.56	0.40
Experiment 3	0.44	0.23	0.23	0.37	0.17
Average	0.43	0.26	0.32	0.43	0.28
SEM	0.08	0.10	0.05	0.06	0.07
Mean (all experiments)	0.35				
SEM (all experiments)	0.03				

AML-4.10					
Transition rate iLSCs (T)=(iBlastd3/iLSCd3)/3					
	Day 21	Day 24	Day 27	Day 30	Day 33
Experiment 1	0.26	0.25	0.21	0.20	0.19
Experiment 2	0.12	0.15	0.13	0.10	0.11
Experiment 3	0.26	0.34	0.19	0.37	0.21
Average	0.21	0.24	0.18	0.22	0.17
SEM	0.05	0.06	0.03	0.08	0.03
Mean (all experiments)	0.21				
SEM (all experiments)	0.02				
Transition rate iBlasts (T)=(iLSCd3/iBLastd3)/3					
	Day 21	Day 24	Day 27	Day 30	Day 33
Experiment 1	0.11	0.22	0.07	0.04	0.04
Experiment 2	0.23	0.10	0.07	0.02	0.02
Experiment 3	0.14	0.09	0.09	0.06	0.03
Average	0.16	0.14	0.08	0.04	0.03
SEM	0.04	0.04	0.01	0.01	0.00
Mean (all experiments)	0.09				
SEM (all experiments)	0.02				

AML-4.24					
Proliferation rate iLSCs (P)=Log[(iLSCd3/iLSCd0)/3]					
	Day 21	Day 24	Day 27	Day 30	Day 33
Experiment 1	0.69	0.55	0.18	0.21	0.18
Experiment 2	0.25	0.34	0.25	0.14	0.09
Experiment 3	0.35	0.30	0.18	0.21	0.18
Average	0.43	0.40	0.21	0.19	0.15
SEM	0.13	0.08	0.02	0.02	0.03
Mean (all experiments)	0.27				
SEM (all experiments)	0.06				
Proliferation rate iBlasts (P)=Log[(iBlastd3/iBlastd0)/3]					
	Day 21	Day 24	Day 27	Day 30	Day 33
Experiment 1	0.35	0.42	0.52	0.14	0.18
Experiment 2	0.09	0.14	0.23	0.18	0.17
Experiment 3	0.50	0.32	0.37	0.11	0.12
Average	0.31	0.30	0.37	0.14	0.15
SEM	0.12	0.06	0.05	0.02	0.02
Mean (all experiments)	0.26				
SEM (all experiments)	0.04				

AML-4.24					
Transition rate iLSCs (T)=(iBlastd3/iLSCd3)/3					
	Day 21	Day 24	Day 27	Day 30	Day 33
Experiment 1	0.14	0.19	0.07	0.07	0.07
Experiment 2	0.35	0.22	0.15	0.20	0.21
Experiment 3	0.24	0.07	0.09	0.10	0.16
Average	0.24	0.16	0.10	0.12	0.15
SEM	0.06	0.05	0.02	0.04	0.04
Mean (all experiments)	0.16				
SEM (all experiments)	0.02				
Transition rate iBlasts (T)=(iLSCd3/iBLastd3)/3					
	Day 21	Day 24	Day 27	Day 30	Day 33
Experiment 1	0.12	0.20	0.06	0.11	0.04
Experiment 2	0.06	0.02	0.06	0.08	0.02
Experiment 3	0.40	0.05	0.05	0.06	0.06
Average	0.20	0.09	0.06	0.08	0.04
SEM	0.10	0.02	0.00	0.01	0.01
Mean (all experiments)	0.09				
SEM (all experiments)	0.03				

Table S2. Results of single GFP+ iLSC plating experiment (related to Figure 3).

Well	Population	Total cell number	%GFP$^+$	Calculated number of GFP$^+$ cells	iBlast/iLSC ratio
1	iLSC	16875	4.4	743	4.90
	iBlast	27563	13.2	3638	
2	iLSC	29250	0.51	149	0.68
	iBlast	42188	0.24	101	
3	iLSC	30750	0.073	22	N/A
	iBlast	103500	0	0	
4	iLSC	17250	0.15	26	8.24
	iBlast	193875	0.11	213	
5	iLSC	58500	0.93	544	0.06
	iBlast	25875	0.12	31	
6	iLSC	17500	0.49	86	N/A
	iBlast	36000	0	0	
7	iLSC	21000	0	0	N/A
	iBlast	27000	0.37	100	
21	iLSC	10875	3.28	357	0.39
	iBlast	140600	0.1	141	
22	iLSC	27750	0.84	233	0.08
	iBlast	3938	0.45	18	
23	iLSC	17625	0.43	76	1.12
	iBlast	8438	1.01	85	
24	iLSC	79500	0.47	374	0.15
	iBlast	111000	0.051	57	
25	iLSC	11875	0.56	67	0.90
	iBlast	45850	0.13	60	
26	iLSC	20000	0.53	106	2.71
	iBlast	220750	0.13	287	
50	iLSC	6275	0.61	38	3.57
	iBlast	85500	0.16	137	
51	iLSC	10625	1.06	113	0.31
	iBlast	31625	0.11	35	
52	iLSC	11250	0.76	86	1.04
	iBlast	24750	0.36	89	
57	iLSC	20250	3.41	691	1.21
	iBlast	20250	4.11	832	
58	iLSC	9000	3.03	273	34.34
	iBlast	211380	4.43	9364	
60	iLSC	20000	0.33	66	2.94
	iBlast	176500	0.11	194	
63	iLSC	9000	2.19	197	0.88
	iBlast	14063	1.23	173	
85	iLSC	6000	3.71	223	2.42
	iBlast	19263	2.8	539	
87	iLSC	19875	0.59	117	0.73
	iBlast	25875	0.33	85	
88	iLSC	5250	0.72	38	0.44
	iBlast	27063	0.062	17	
90	iLSC	9750	0.48	47	2.56
	iBlast	29250	0.41	120	
91	iLSC	20475	1.16	238	4.88
	iBlast	144875	0.8	1159	
94	iLSC	19875	0.59	117	0.87
	iBlast	23063	0.44	101	
Average					3.28

Table S3. The 42 genes ranked by the p value of their expression difference between LSC+ and LSC- primary AML cell fractions (related to Figure 5).

			RNA-seq		ATAC-seq info for most significant nearby peak					Gene level accessibility test		Association with LSC+ fraction of Ng et al.
	SYMBOL	GENE NAME	log2FC iLSC/iBlast	padj	Region	Annotation	Distance to nearest peak	log2FC iLSC/iBlast	padj	KS.statistic	KS.p-value	p-value
1	NYNRIN	NYN domain and retroviral integrase containing	0.269837091	0.047722148	chr14:24858365-24858779	intergenic	9212	1.071981227	0.003944795	0.336788625	0.754690637	9.66E-13
2	ITPR1	inositol 1,4,5-trisphosphate receptor type 1	0.485121506	0.002690283	chr3:4778189-4778618	intron	0	1.27779772	0.000148325	0.54201995	1.58E-05	8.06E-09
3	EFHC2	EF-hand domain containing 2	0.514754463	4.87E-05	chrX:44053980-44054613	intron	0	1.679600523	1.42E-05	0.225263955	0.92104745	8.66E-08
4	PDLIM1	PDZ and LIM domain 1	0.275524586	0.02829767	chr10:97057039-97057726	intergenic	6133	0.950032778	2.08E-05	0.530353221	0.022224124	9.52E-06
5	KLHL6	kelch like family member 6	0.313918314	0.008791974	chr3:183230965-183231436	intron	0	1.27219586	0.025894609	0.362117095	0.528475257	1.40E-05
6	UBASH3B	ubiquitin associated and SH3 domain containing B	0.346093337	0.002144685	chr11:122581722-122582354	intron	0	1.341690612	0.053323908	0.306134982	0.528115579	6.23E-05
7	CTDSPL	CTD small phosphatase like	0.371042753	0.001669566	chr3:37941421-37941893	intron	0	1.117506895	0.012421706	0.523346485	0.02501031	0.000492908
8	ALDH1A1	aldehyde dehydrogenase 1 family member A1	0.522411131	0.000106981	chr9:75612172-75613057	intergenic	43938	1.032313231	4.56E-06	0.625162839	0.08774388	0.001052108
9	SPTBN1	spectrin beta, non-erythrocytic 1	0.374561255	0.001669566	chr2:54656904-54657770	intergenic	25683	1.163796474	1.48E-07	0.289430068	0.115982128	0.001375011
10	PDGFC	platelet derived growth factor C	0.380170761	0.018811387	chr4:157936273-157936855	intergenic	43726	1.312182605	1.12E-06	0.393502336	0.066353281	0.001557831
11	ITGA9	integrin subunit alpha 9	0.617715046	5.14E-07	chr3:37495558-37495808	promoter	0	1.158265039	0.033300616	0.379190616	0.035728574	0.004015694
12	STARD8	StAR related lipid transfer domain containing 8	0.469910775	0.005215844	chrX:67920012-67920619	intron	0	1.208356949	0.000276246	0.621357055	0.004155649	0.012483754
13	CCNG1	cyclin G1	0.333401453	0.016386577	chr5:162853036-162853696	intergenic	10880	0.862620139	0.000283237	0.798898277	0.043452798	0.027691092
14	SH3PXD2A	SH3 and PX domains 2A	0.426644825	0.000190083	chr10:105456141-105456790	intron	0	1.541326307	0.000618239	0.271158145	0.164223658	0.040395172
15	LDLRAD4	low density lipoprotein receptor class A domain containing 4	0.418492757	0.011488487	chr18:13394798-13395445	intron	0	1.039248607	0.004507902	0.295209737	0.061326931	0.041302378
16	NTRK3	neurotrophic receptor tyrosine kinase 3	0.624503373	0.000781351	chr15:88554627-88555354	intron	0	1.203962862	1.50E-07	0.325138646	0.044541478	0.048749139
17	ME1	malic enzyme 1	0.470338532	0.000574712	chr6:84127694-84127914	intron	0	0.399032509	0.728661701	0.560613392	0.04605622	0.071093919
18	SLC18A2	solute carrier family 18 member A2	0.429672631	0.037421185	chr10:118986252-118986823	intergenic	13760	1.371486668	0.003792098	0.635230952	0.079275851	0.124447209
19	TSPAN18	tetraspanin 18	0.324168801	0.0332328	chr11:44938174-44938803	intron	0	1.18503913	0.000740681	0.18099031	0.826791879	0.169178825
20	PTGS1	prostaglandin-endoperoxide synthase 1	0.347381922	0.005375141	chr9:125117086-125117383	intergenic	15425	0.641227224	0.486779913	0.49905088	0.022617404	0.207064401
21	MPPED2	metallophosphoesterase domain containing 2	0.550514783	0.018058601	chr11:30413810-30415294	intron	0	1.098894028	1.41E-08	0.296484027	0.288442927	0.284585056
22	APBA2	amyloid beta precursor protein binding family A member 2	0.327914091	0.047564337	chr15:29247988-29248485	intron	0	1.341572602	0.004067932	0.281911825	0.548529089	0.47895072
23	TSPAN11	tetraspanin 11	0.459375358	0.011300692	chr12:31035623-31035948	intergenic	43889	1.163671721	0.089404314	0.657628317	0.00469755	0.512798407
24	ADRA2A	adrenoceptor alpha 2A	0.588097229	5.31E-07	chr10:112886735-112887580	intergenic	46072	0.995977458	5.88E-06	0.701343656	0.005468292	0.569429703
25	CTTNBP2	cortactin binding protein 2	0.368548889	0.003687469	chr7:117371397-117372015	intron	0	1.240097254	1.66E-05	0.570597759	0.147826242	0.654103061
26	PROS1	protein S	0.350339943	0.008295636	chr3:93688973-93689187	intron	0	0.732685285	0.092206562	0.853035322	0.001383874	0.710124506
27	PLXNA4	plexin A4	0.526968714	0.000277715	chr7:132174468-132174980	intron	0	1.047955065	0.000726688	0.234510738	0.64145773	0.725855323
28	TMEM40	transmembrane protein 40	0.44410652	0.01675798	chr3:12747907-12748284	intergenic	26744	0.974009086	0.068353044	0.639278669	0.014839952	0.810232133
29	RASGEF1B	RasGEF domain family member 1B	0.611687448	0.000510453	chr4:82425916-82426851	intergenic	32833	1.027608696	6.18E-05	0.399914393	0.292614249	0.8135817
30	ST6GAL1	ST6 beta-galactoside alpha-2,6-sialyltransferase 1	0.344246093	0.00885572	chr3:186670735-186671280	intron	0	0.666112724	0.347024083	0.543473406	0.032019662	0.84894966
31	OPHN1	oligophrenin 1	0.375041542	0.016341173	chrX:67472414-67472875	intron	0	0.78622469	0.031993949	0.773960621	0.000137806	0.849917342
32	TBC1D4	TBC1 domain family member 4	0.365831283	0.04401761	chr13:75932122-75932445	intron	0	1.245617357	0.019839369	0.311422935	0.286774179	0.930440229
33	STIM1	stromal interaction molecule 1	0.318437002	0.004157092	chr11:3887520-3887785	intron	0	0.965467944	0.199381326	0.458551383	0.029853265	0.947032772
34	ARHGAP6	Rho GTPase activating protein 6	0.410007023	0.001553048	chrX:11441491-11441965	intron	0	1.216119477	8.69E-05	0.303236519	0.073088881	0.959153288
35	SHROOM4	shroom family member 4	0.450906313	0.002884119	chrX:50386273-50387140	intron	0	1.004902976	1.21E-05	0.468983015	0.010210323	0.98354686
36	NID1	nidogen 1	0.368437132	0.002252456	chr1:236100235-236101143	intergenic	37988	1.029248426	9.64E-06	0.317378196	0.581358517	0.98563046
37	WIPF1	WAS/WASL interacting protein family member 1	0.436801481	6.37E-05	chr2:175462344-175462653	intron	0	1.50721629	0.038186494	0.558840107	0.025260407	0.996223788
38	KLF9	Kruppel like factor 9	0.362089599	0.013373328	chr9:73053217-73054038	intergenic	23643	0.962987877	0.000230972	0.597484833	0.006618989	0.999650241
39	ZEB2	zinc finger E-box binding homeobox 2	0.301616132	0.016971491	chr2:145192386-145192615	intron	0	1.882309563	0.151941563	0.48115126	0.012290184	0.999948734
40	RAB30	RAB30, member RAS oncogene family	0.629322369	9.90E-07	chr11:82765005-82765578	intron	0	1.162852947	3.74E-06	0.491935584	0.109623799	0.999993516
41	RBM38	RNA binding motif protein 38	0.31630118	0.022632928	chr20:56025539-56025843	intergenic	41152	0.718961073	0.163348167	0.649049019	0.029625778	0.999999399
42	DOCK10	dedicator of cytokinesis 10	0.418400944	0.018909882	chr2:225813418-225814067	promoter	0	1.27950519	2.01E-07	0.455033643	0.001161133	0.99999992

Table S4. TF “regulons” that correlate with the iLSC score (related to Figure 6).

Regulon	Variance	Number of genes	Variance by cluster	iLSC score pearson correlation	Pearson_pval	iLSC score spearman correlation	Spearman_pval	Slope
TAL1	0.04331244	76	31.2880242	0.716213884	0	0.726970406	0	128.21012
LMO2	0.03361308	51	24.0524975	0.692222163	0	0.685683366	0	109.162354
NFIB	0.03791132	21	27.2589038	0.667153363	0	0.672631622	0	111.733505
GATA5	0.02890615	23	20.5412291	0.663859067	0	0.663715993	0	97.0832512
MEIS1	0.04627962	16	33.5014837	0.662696897	0	0.651339137	0	122.626151
NFIB extended	0.02909622	30	20.6830136	0.661565145	0	0.66797875	0	97.0653349
MAFG	0.02269999	97	15.9115576	0.657937609	0	0.637922924	0	85.2650057
NFE2	0.03331666	57	23.8313782	0.614905916	0	0.603613637	0	96.5411953
NFATC4	0.01979853	69	13.7471294	0.56950047	0	0.590883932	0	68.9261269
MAX	0.02544935	20	17.9625269	0.540462883	0	0.529925322	0	74.161333
RUNX1	0.02505451	11	17.6679799	0.524717466	0	0.518738809	0	71.4400473
FOSB	0.02456624	20	17.3037445	0.485245648	0	0.453751773	0	65.4190599
SOX18 extended	0.01835729	21	12.6719869	0.485045421	0	0.52092829	0	56.5275168
FOSB extended	0.01765359	25	12.1470441	0.471721116	0	0.439246647	0	53.9107161
SOX18	0.02376554	13	16.7064383	0.418107011	0	0.456639522	0	55.4414521
MAZ	0.02440078	192	17.1803151	0.409785291	0	0.460090891	0	55.0594071
JUNB	0.03694968	24	26.5415378	0.285053173	0	0.260712389	0	47.1307655
AHR	0.01628705	10	11.1276295	0.277212232	0	0.263919037	0	30.4303402
JUN	0.02505944	11	17.6716556	0.234544146	1.58E-277	0.253025464	0	31.93622
ARID3A	0.01709701	10	11.7318487	-0.043773082	5.61E-11	-0.03972222	2.74E-09	-4.923121
CTCF	0.07150696	13	52.3205701	-0.173759075	2.51E-151	-0.204422814	6.74E-210	-39.96639
HMGA1	0.13989533	14	103.336913	-0.275355943	0	-0.231425345	4.75E-270	-88.586866
ASCL2	0.0197481	15	13.7095057	-0.364582767	0	-0.304554882	0	-44.068884
IKZF1	0.01668427	27	11.4239548	-0.407287905	0	-0.459792033	0	-45.251028
CEBPG	0.02301372	29	16.1455963	-0.56688495	0	-0.516712541	0	-73.971027

Table S1. Calculation of proliferation and transition rates (related to Figure 3).

Table S2. Results of single GFP^+ iLSC plating experiment (related to Figure 3).

Table S3. The 42 genes ranked by the p value of their expression difference between LSC^+ and LSC^- primary AML cell fractions (related to Figure 5).

Table S4. TF "regulons" that correlate with the iLSC score (related to Figure 6).