Supplemental Information

Supplemental Methods

NOD/SCID xenotransplantation assays

Nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice were obtained from the Jackson Laboratory, and sub-lethally irradiated with 2.7 Gy (270 Rads) before transplantation. Equal numbers of primary AML cells (2.5×10^6 cells per mouse) were treated with 10 μM BPI for 24 h, and then injected via the tail vein in a final volume of 0.2 ml of phosphate-buffered saline (PBS) containing 0.5% fetal bovine serum (FBS). Six to eight weeks later, animals were sacrificed and the bone marrow (BM) cells were harvested. The BM cells were stained with PE-Cy5-mouse CD45 (mCD45), APC-H7-human CD45 (hCD45) and BB515-human CD33 (hCD33) antibodies at room temperature for 15 minutes. Cells were then washed, resuspended in FACS buffer containing 1 μg/ml DAPI and analyzed for the presence of viable human leukemic cells (DAPI-/mCD45-/hCD45+/hCD33+) by flow cytometry.

Statistical analysis

Statistical analyses were performed using GraphPad Prism software to evaluate significance. Test utilized is indicated in figure legends. *p<0.05, **p<0.01 and ***p<0.001.

Supplemental Figure Legend

Supplemental Figure 1 BCL6 and housekeeping gene expression in cohorts or AML cases.

The heat map illustrates the Pearson correlation coefficients between all AML specimens from Erasmus Medical Center (n= 520). A color key at lower right indicates

the Pearson coefficients. At the right shoulder of triangular plot, FAB classification, selected sets of cytogenetic or mutational status are indicated with red (positive), green (negative), or purple (not available) boxes. At the far right, expression levels based on hybridization to probesets corresponding to BCL6 (Affymetrix arrays had three independent BCL6 probesets) or housekeeping genes are shown as histograms along the diagonal axis.

Supplemental Figure 2. Comparison of BCL6 expression levels between normal hematopoietic cells

Using microarray data for expression profiles among wide variety of primary human hematopoietic cells available from human primary cell atlas database[1], expression levels of BCL6 normalized by median of 10 different housekeeping genes (the same set used in Figure 1A) were compared between various hematopoietic cells.

Supplemental Figure 3. Identification of intracellular BCL6 in normal hematopoietic and AML cells by flow cytometry

(A) Representative gating strategy showing that debris, doublets and dead cells are gated out to identify lymphocytes (CD45 positive, SSC low) to separate into B-cells (CD3 negative) and T-cells (CD3 positive), monocytes (CD45 high, CD14 high), and lineage negative cells (CD3-, CD14-) were detected and intracellular BCL6 was detected in each of the fractions. (B) BCL6 protein expression was determined by flow cytometry in five primary AML samples. Representative gating strategy to detect BCL6 expression levels in leukemia blast cells (CD45 Dim), B cells and T cells. (C) Expression levels of BCL6 obtained from flow cytometry were compared between immunophenotypically identified hematopoietic cells in either normal BMCs (from healthy donors), or primary AML specimens. Relative expression levels were calculated by MFI of PE-BCL6 normalized by that of unstained cells (FMO: fluorescence minus

one). Statistical significance was assessed within each of the specimen types by one-way ANOVA analysis.

Supplemental Figure 4. Comparison of BCL6 expression level between AML cell line cells

11 human AML cell line cells were compared for expression level of BCL6 using intracellular flowcytometry. Each of the histogram shows results from unstained (gray) and BCL6-stained (red) cells.

Supplemental Figure 5 Effects of a BCL6 inhibitor FX1 on normal blood cells.

(A) Relative viable cell counts of BM-MNCs from three individual donors upon FX1 treatment (B) Gating strategies to identify viable cells and CD34 positive fractions using the data from vehicle treated BMMNC-1 (C) Relative CD34 positive cell counts were shown in 3 BMMNCs treated with FX1. Averages were calculated from duplicates, and error bars indicate the standard deviation.

Supplemental Figure 6. Effects of a BCL6 inhibitor FX1 on colony forming capacity of normal blood cells

(**A**) Colony counts were calculated by normalizing to control DMSO treatments. Averages were calculated from duplicates and error bars indicate standard deviations. (**B**) Relative colony numbers are presented after normalization to DMSO control. (**C**) Representative entire images of colonies are demonstrated. (BMMNC-1) Microscopically detected colonies were circled for clarifications. (**D**) Quantification of the different types of colonies observed based on microscopic analysis of primary bone marrow cells treated with FX1 or vehicle. (**E**) Microscopic images showing morphology of colonies described in panel D. Images were obtained by microscopically observing BMMNC-2 treated either with DMSO (control) or FX1. Sale bars indicate 1000 mm.

Supplemental Figure 7. Effects of BCL6 Inhibitions on Normal Hematopoiesis

(A) Using the minimal EC50 dose of FX1 (30 mM) using primary AML cells, 3 replicates of normal BMMNCs were treated with FX1 for 48 hours and cell viabilities in monocytes, B cells, T cells, and lineage-negative cells were measured using FCM. Experiments were performed in duplicates and averages were calculated. Error bars indicate standard deviations. (B) Bone marrow cells from WT or BCL6-knock out mice were transplanted into lethally irradiated C57BL/6 mice (1 $x10^6$ cells/ mouse). BMCs were collected and percentages of hematopoietic stem/ progenitor cells were determined by flow cytometry. LSK: lineage negative Sca-1 positive c-Kit positive cells, CMP: common myeloid progenitors, GMP: granulocyte-monocyte progenitors, MEP: megakaryocytic-erythroid progenitors. Significances between each WT vs KO pair were assessed by ANOVA analysis.

Supplemental Figure 8. Differentially expressed genes in core FX1-regulation signatures in primary AML cells

Three primary AML cells (AML37, AML95, and AML98) were treated with FX1 using the concentration for EC50 in pilot ex vivo treatment experiments, for 12 hours. RNA was extracted from DMSO or FX1 treated cells and subjected to RNA-seq. The Heatmap represents the Z scores for expression of genes significantly up- or down-regulated by FX1 compared to DMSO in all of the three AML specimens. Examples of differentially expressed genes are listed to the left of the heatmap.

Supplemental Figure 9. Effects of inhibition of BCL6 on leukemic stem cells

(A-B) 2 primary AML cells (AML54, and AML98) were treated with or without FX1 (75 μM) for 48 hours and subjected to flow cytometry to detect LSCs (CD45 dim, CD34+,

and CD38-) and blast cells (CD45 dim, CD34+, and CD38+). Viability of those fractions were measured. Gating schemes are shown in **(A)**, using AML54. **(B)** Relative % viabilities were calculated to show activities of FX1 on each of the fractions in each of the replicates. Averages were calculated from duplicates and error bars represent standard deviations. **(C)** A primary AML cell (AML33) was transfected with siRNA for BCL6 for 48 hours and knock-down efficiency was investigated by intracellular flowcytometry. Relative inhibition of BCL6 was calculated by dividing the MFI value of negative control (NTC), or BCL6 siRNA by that of non-transfected cells. Assays were done in triplicates and averages were calculated. Error bars indicate standard deviations. Those AML cells were used for the experiments in Figure 4H-I, and Figure 6D.

Supplemental Figure 10. BCL6 expression levels in AML cell line cells treated with chemotherapeutic agents

Three human AML cell line cells, MV4-11, U937, and TUR, were treated with 6 different chemotherapeutic agents, and levels of BCL6 were measured using intracellular flowcytometry. Histogram shows comparison of the results between unstained (gray), and BCL6-stained (red) samples.

Supplemental Figure 11. BCL6 expression levels affecting chemosensitivity of AML cells

(A) As a representative primacy AML cell for main Figure 6B, pictures of colonies from AML33 primary AML cells treated with RI-BPI, AraC, or combination of them for 24 hours prior to plating are demonstrated. Entire plates are shown in upper panels, and zoomed view is at lower panels. Circles indicate identified colonies in each of the representative zoomed panels to calculate the numbers for Figure 6B. (**B**) BCL6 expression and overall survival (OS) from 3 different AML cohorts (TCGA, Erasmus,

and OHSA) were merged after normalizing BCL6 expression level by house keeping genes (as mentioned in Rev Figure 3). The entire AML subjects were devided into auantiles (Q1 with the lowest level and Q4 the highest), and Kaplan-Meyer curves were cretad. Significances between each pairs are calculated by Cox Proportional Hazard model, and only significant differences are presented in a right box. A p value to calculate significance between OS and continuous values of BCL6 expression is also shown below the box.

Supplemental Tables

Supplemental Table 1. Profiles of primary AML specimens, blood cell specimens from healthy donors.

Along with the patient ID numbers, gender, mutational profiles (somatic mutations and cytogenetic changes), percentages of blast cells in bone marrow, and white blood cell counts in peripheral blood. NA : not available

Supplemental Table 2. Results of FX1 treatment on 55 primary AML cells

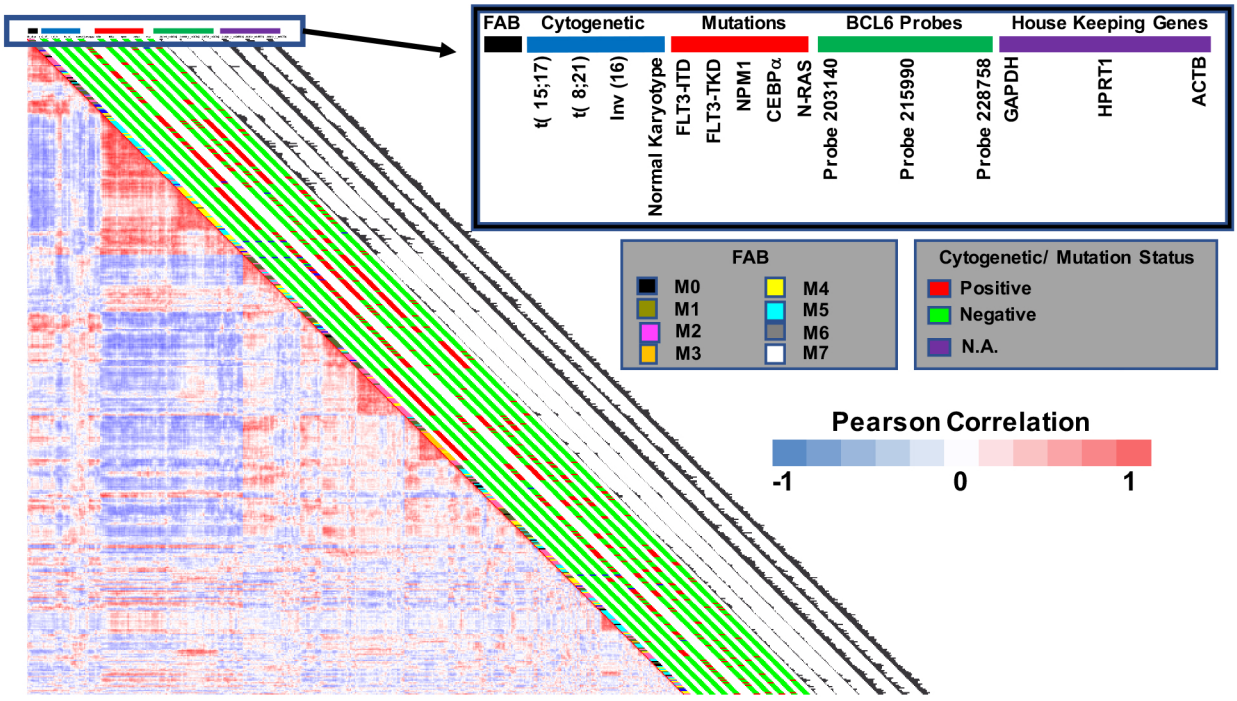
Entire results of the experiments demonstrated in Figure2A.

Supplemental Table 3. Differentially expressed genes in primary AML cells treated with FX1, compared to negative controls.

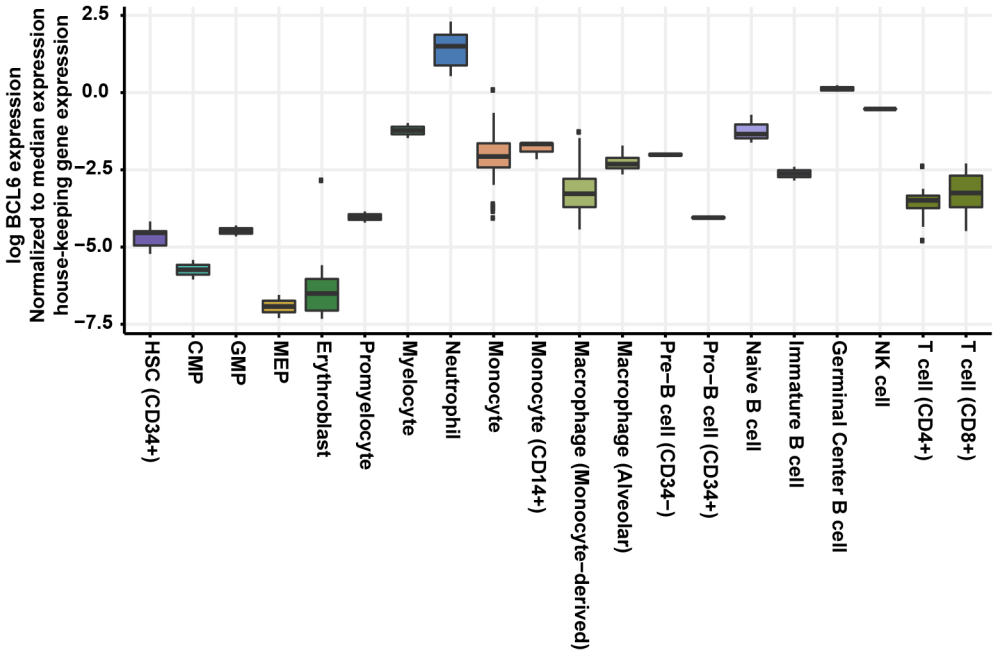
Differently expressed genes were selected by the criteria log2Fold Change > 1.5, and q < 0.01

1. Mabbott NA, Baillie JK, Brown H, Freeman TC, Hume DA. An expression atlas of human primary cells: inference of gene function from coexpression networks. *BMC Genomics*. 2013;14:632.

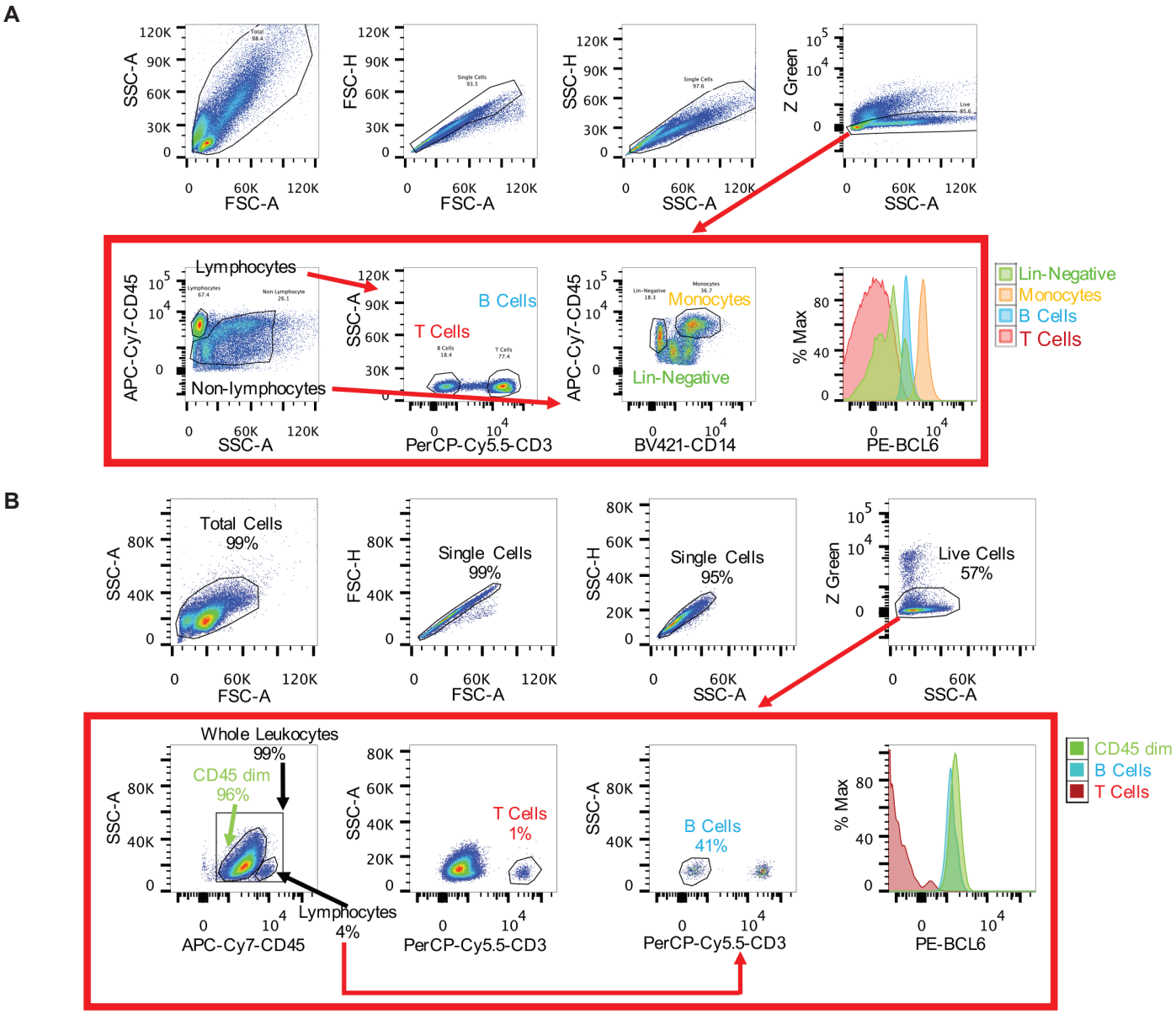
SFigure1

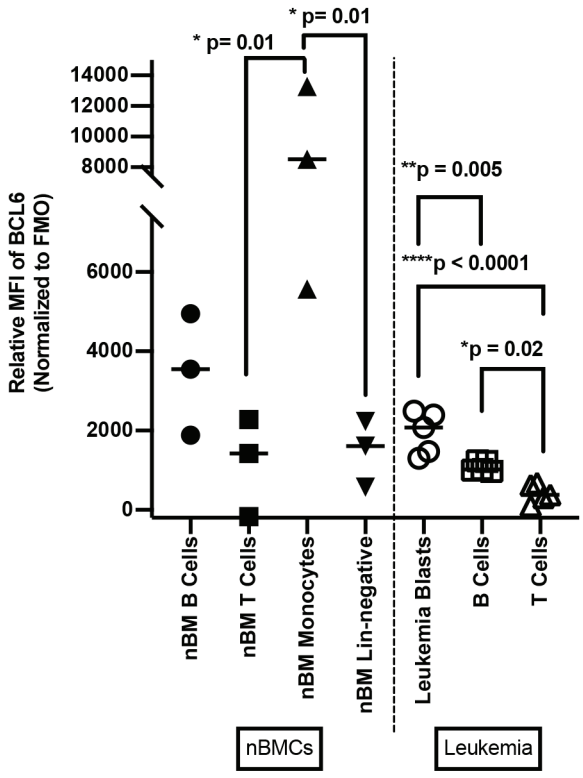

FAB
Cytogenetic
Mutations
BCL6 Probes
House Keeping Genes
t(15;17)
t(8;21)
Inv (16)
Normal Karyotype
FLT3-ITD
FLT3-TKD
NPM1
CEBPα
N-RAS
Probe 203140
Probe 215990
Probe 228758
GAPDH
HPRT1
ACTB
FAB
M0
M1
M2
M3
M4
M5
M6
M7
Cytogenetic/ Mutation Status
Positive
Negative
N.A.
Pearson Correlation
-1
0
1

SFigure2

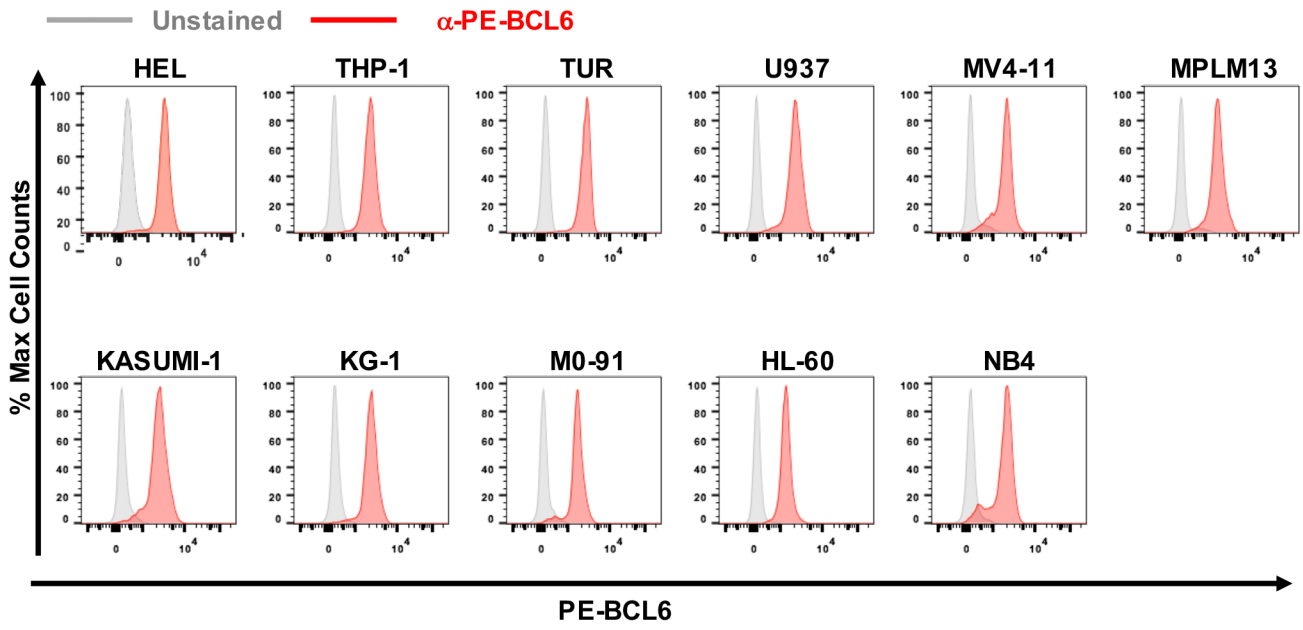

log BCL6 expression
Normalized to median expression
house-keeping gene expression
2.5
0.0
−2.5
−5.0
−7.5
HSC (CD34+)
CMP
GMP
MEP
Erythroblast
Promyelocyte
Myelocyte
Neutrophil
Monocyte
Monocyte (CD14+)
Macrophage (Monocyte−derived)
Macrophage (Alveolar)
Pre−B cell (CD34−)
Pro−B cell (CD34+)
Naive B cell
Immature B cell
Germinal Center B cell
NK cell
T cell (CD4+)
T cell (CD8+)

SFigure3

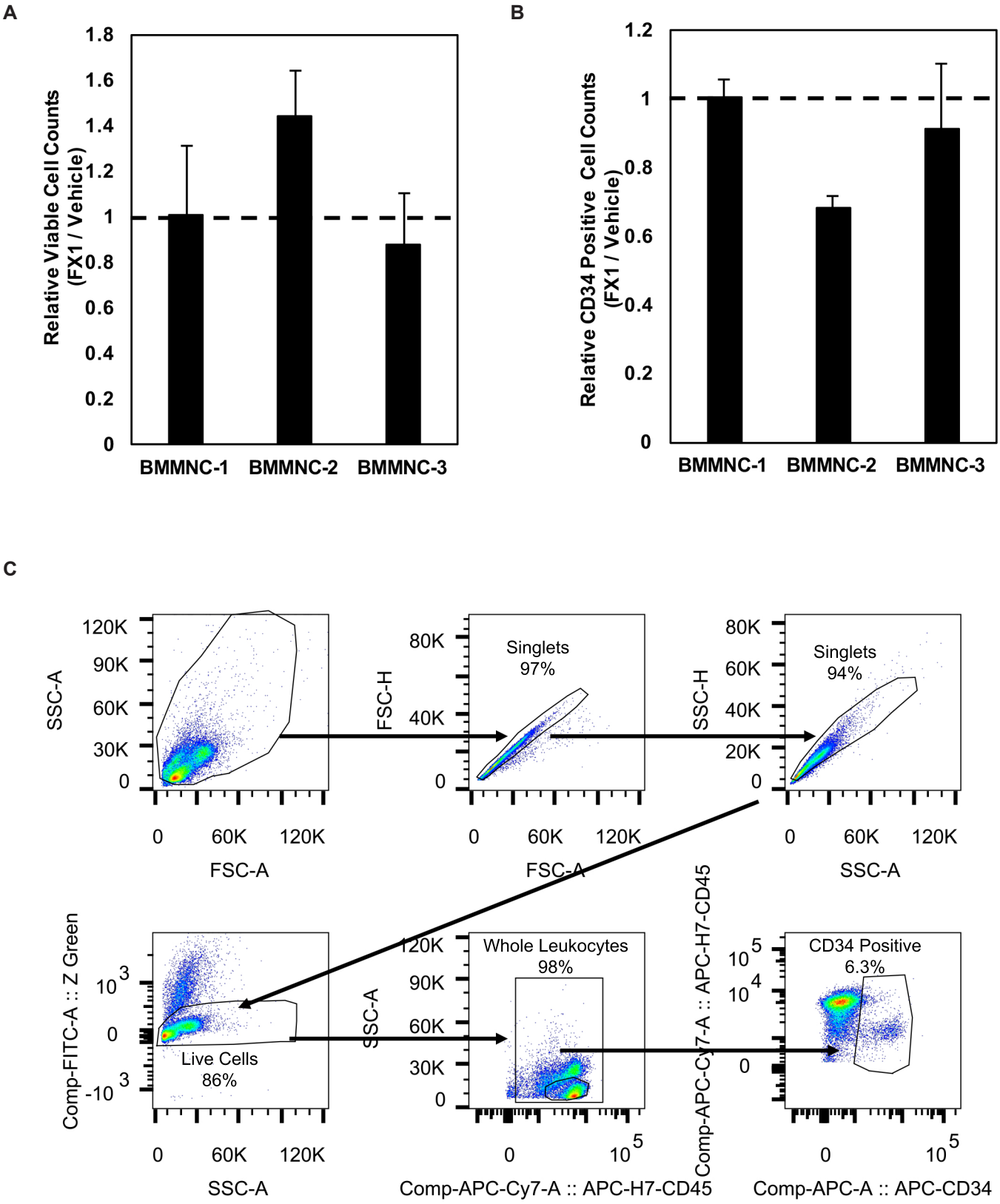

A
SSC-A
FSC-A
FSC-H
SSC-H
Z Green
Lymphocytes
Non-lymphocytes
B Cells
T Cells
Monocytes
Lin-Negative
APC-Cy7-CD45
PerCP-Cy5.5-CD3
BV421-CD14
PE-BCL6
% Max
Lin-Negative
Monocytes
B Cells
T Cells
B
Total Cells 99%
Single Cells 99%
Single Cells 95%
Live Cells 57%
Whole Leukocytes 99%
CD45 dim 96%
Lymphocytes 4%
T Cells 1%
B Cells 41%
CD45 dim
B Cells
T Cells


C
* p= 0.01
* p= 0.01
**p = 0.005
****p < 0.0001
*p = 0.02
Relative MFI of BCL6 (Normalized to FMO)
14000
12000
10000
8000
6000
4000
2000
0
nBM B Cells
nBM T Cells
nBM Monocytes
nBM Lin-negative
Leukemia Blasts
B Cells
T Cells
nBMCs
Leukemia

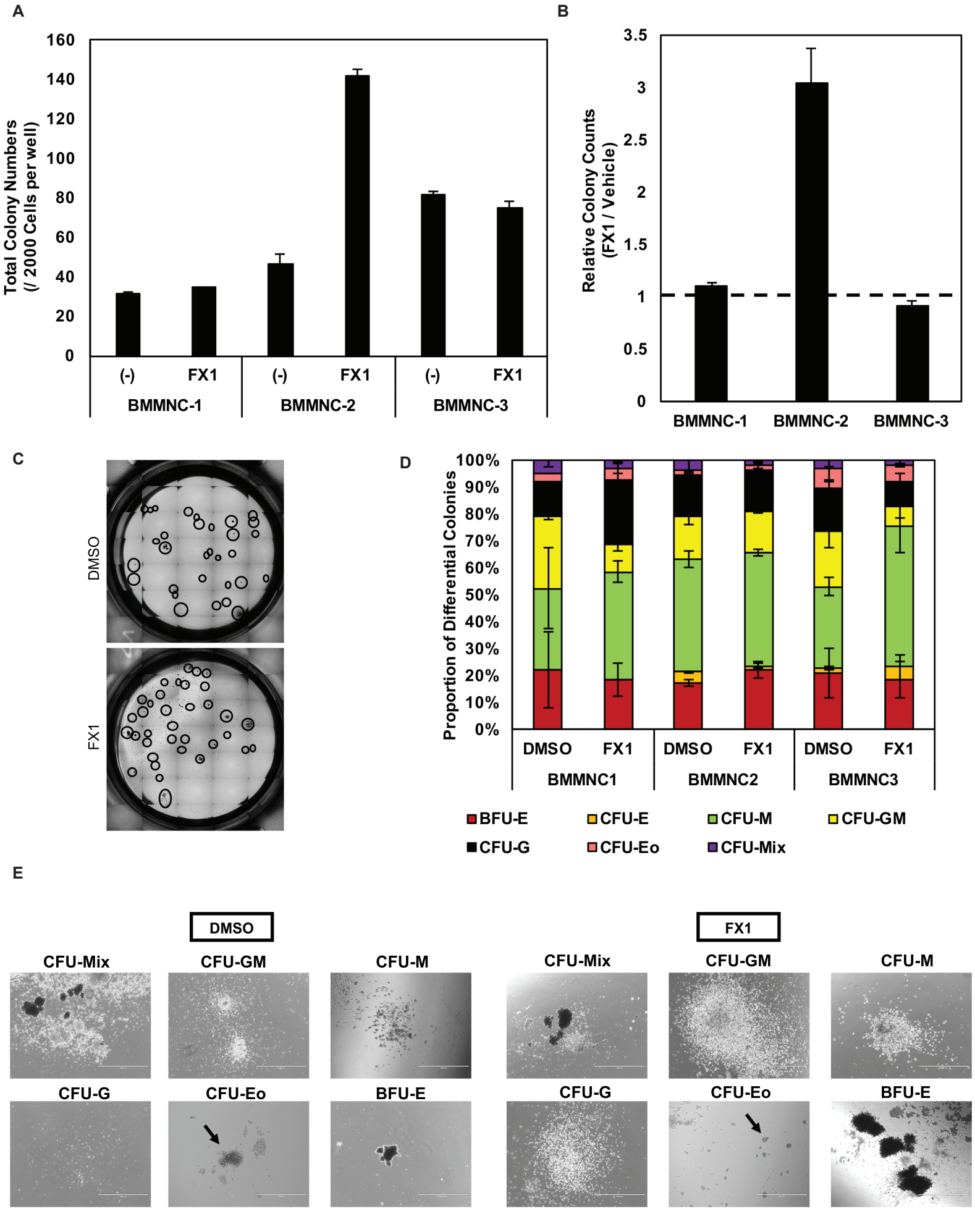
SFigure4


Unstained
α-PE-BCL6
HEL
THP-1
TUR
U937
MV4-11
MPLM13
KASUMI-1
KG-1
M0-91
HL-60
NB4
% Max Cell Counts
PE-BCL6

SFigure5

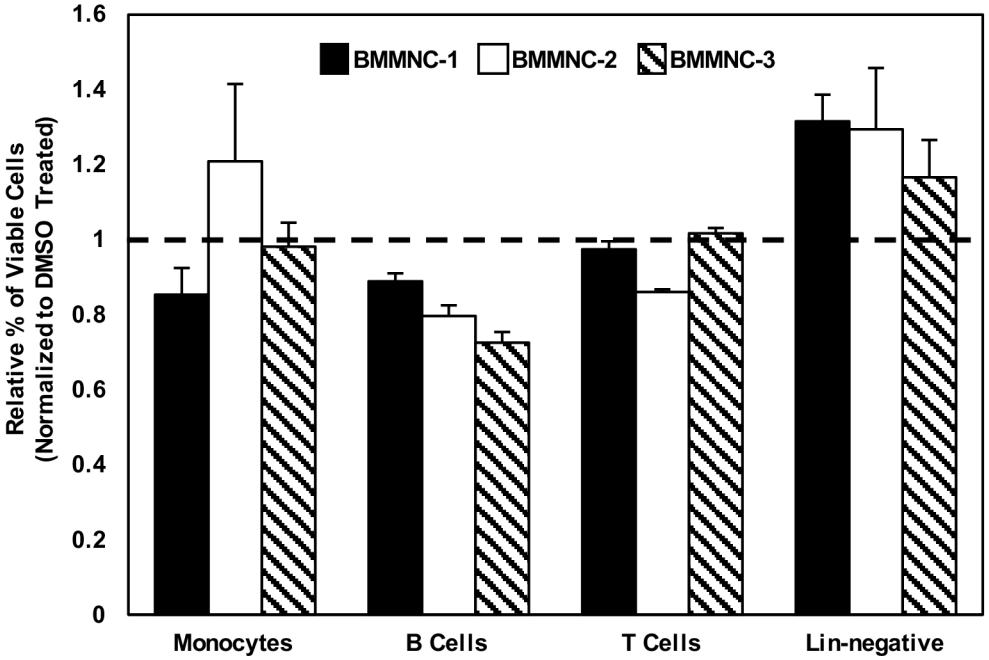

A
Relative Viable Cell Counts (FX1 / Vehicle)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
BMMNC-1
BMMNC-2
BMMNC-3
B
Relative CD34 Positive Cell Counts (FX1 / Vehicle)
1.2
1
0.8
0.6
0.4
0.2
0
BMMNC-1
BMMNC-2
BMMNC-3
C
SSC-A
120K
90K
60K
30K
0
FSC-A
FSC-H
80K
40K
Singlets
97%
SSC-H
Singlets
94%
SSC-A
Comp-FITC-A :: Z Green
Live Cells
86%
Whole Leukocytes
98%
Comp-APC-Cy7-A :: APC-H7-CD45
CD34 Positive
6.3%
Comp-APC-A :: APC-CD34

SFigure6

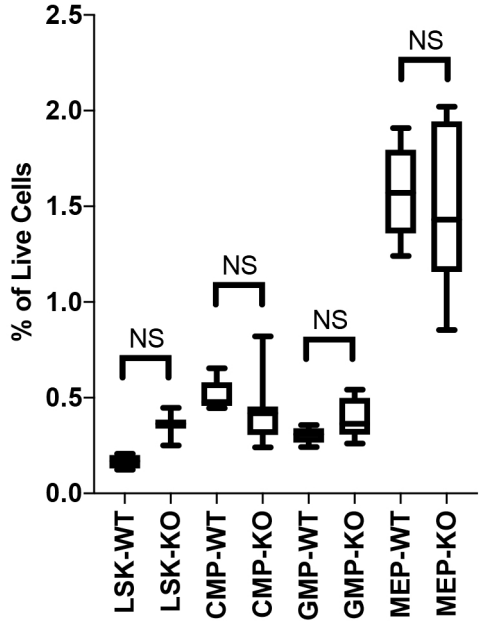

A
Total Colony Numbers (/ 2000 Cells per well)
160
140
120
100
80
60
40
20
0
(-)
FX1
(-)
FX1
(-)
FX1
BMMNC-1
BMMNC-2
BMMNC-3
B
Relative Colony Counts (FX1 / Vehicle)
3.5
3
2.5
2
1.5
1
0.5
0
BMMNC-1
BMMNC-2
BMMNC-3
C
DMSO
FX1
D
Proportion of Differential Colonies
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
DMSO
FX1
DMSO
FX1
DMSO
FX1
BMMNC1
BMMNC2
BMMNC3
BFU-E
CFU-E
CFU-M
CFU-GM
CFU-G
CFU-Eo
CFU-Mix
E
DMSO
FX1
CFU-Mix
CFU-GM
CFU-M
CFU-Mix
CFU-GM
CFU-M
CFU-G
CFU-Eo
BFU-E
CFU-G
CFU-Eo
BFU-E

SFigure7

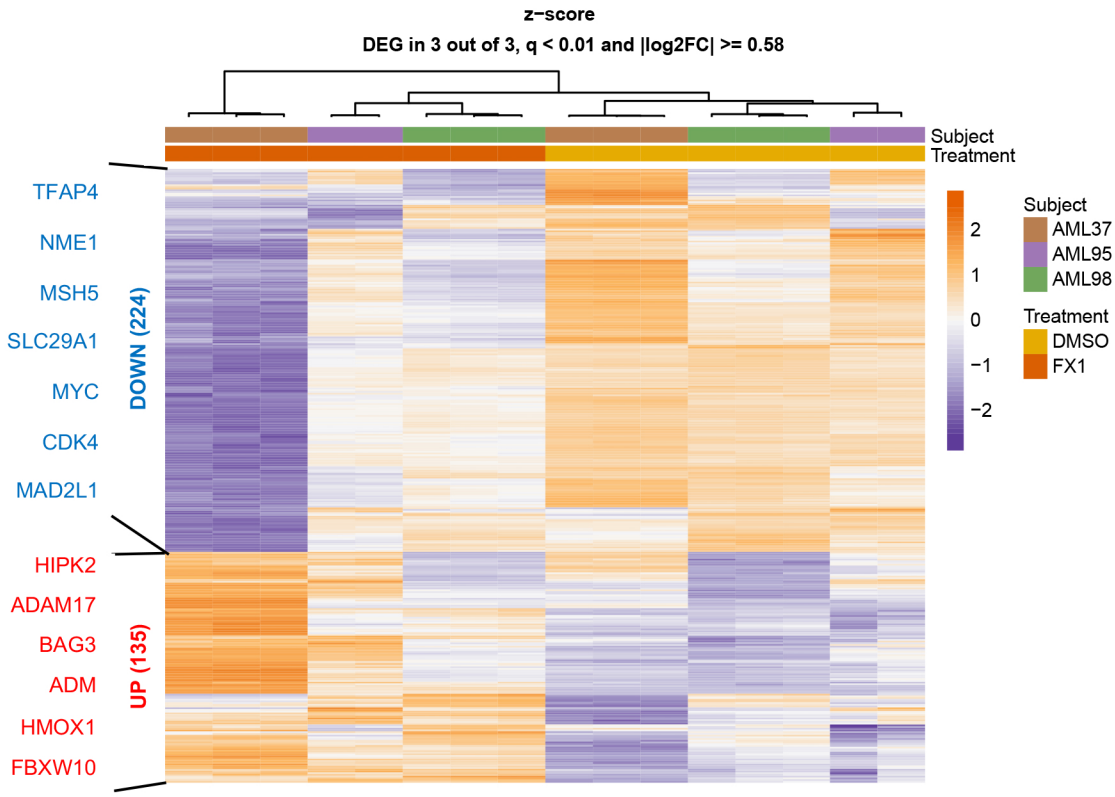
A


BMMNC-1
BMMNC-2
BMMNC-3
Relative % of Viable Cells
(Normalized to DMSO Treated)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Monocytes
B Cells
T Cells
Lin-negative

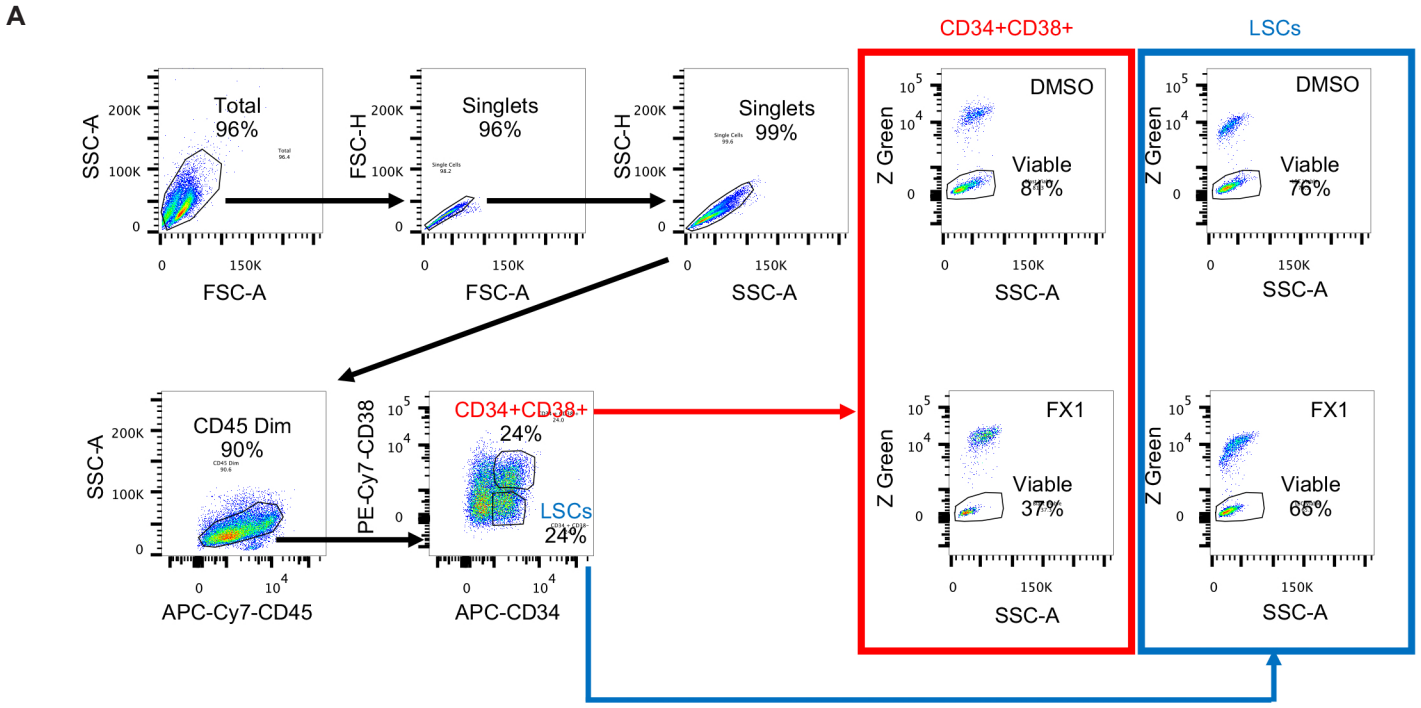
B

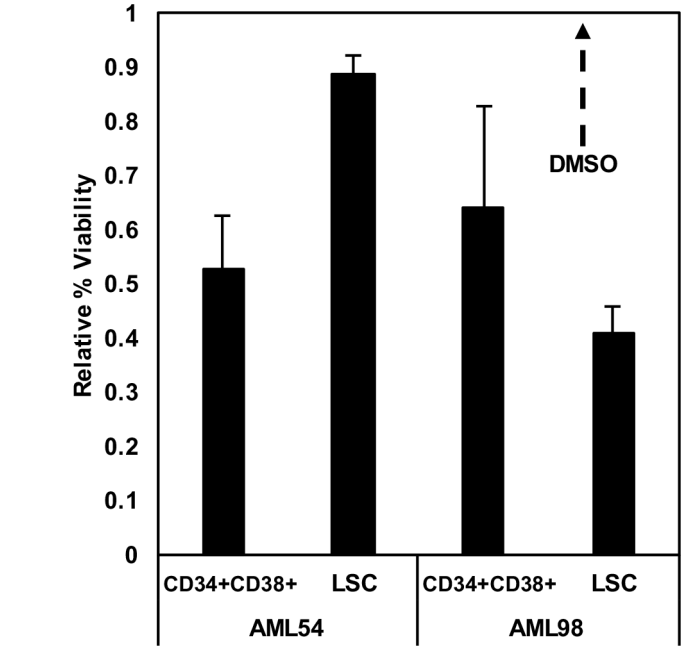

% of Live Cells
2.5
2.0
1.5
1.0
0.5
0.0
NS
NS
NS
NS
LSK-WT
LSK-KO
CMP-WT
CMP-KO
GMP-WT
GMP-KO
MEP-WT
MEP-KO

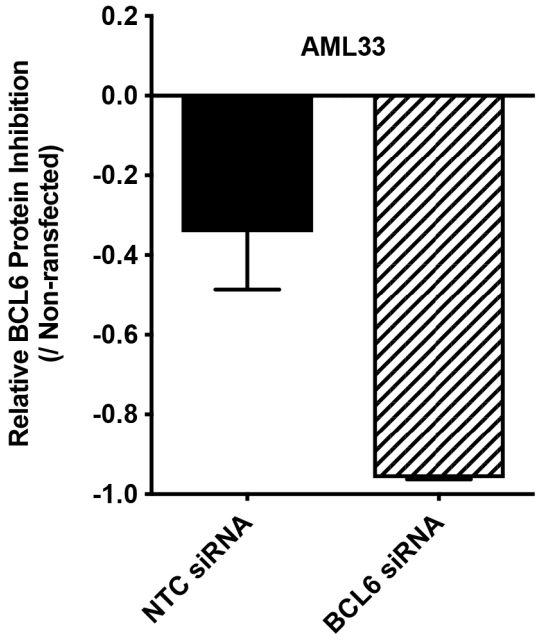
SFigure8


z−score
DEG in 3 out of 3, q < 0.01 and |log2FC| >= 0.58
Subject
Treatment
TFAP4
NME1
MSH5
SLC29A1
MYC
CDK4
MAD2L1
DOWN (224)
HIPK2
ADAM17
BAG3
ADM
HMOX1
FBXW10
UP (135)
2
1
0
−1
−2
Subject
AML37
AML95
AML98
Treatment
DMSO
FX1

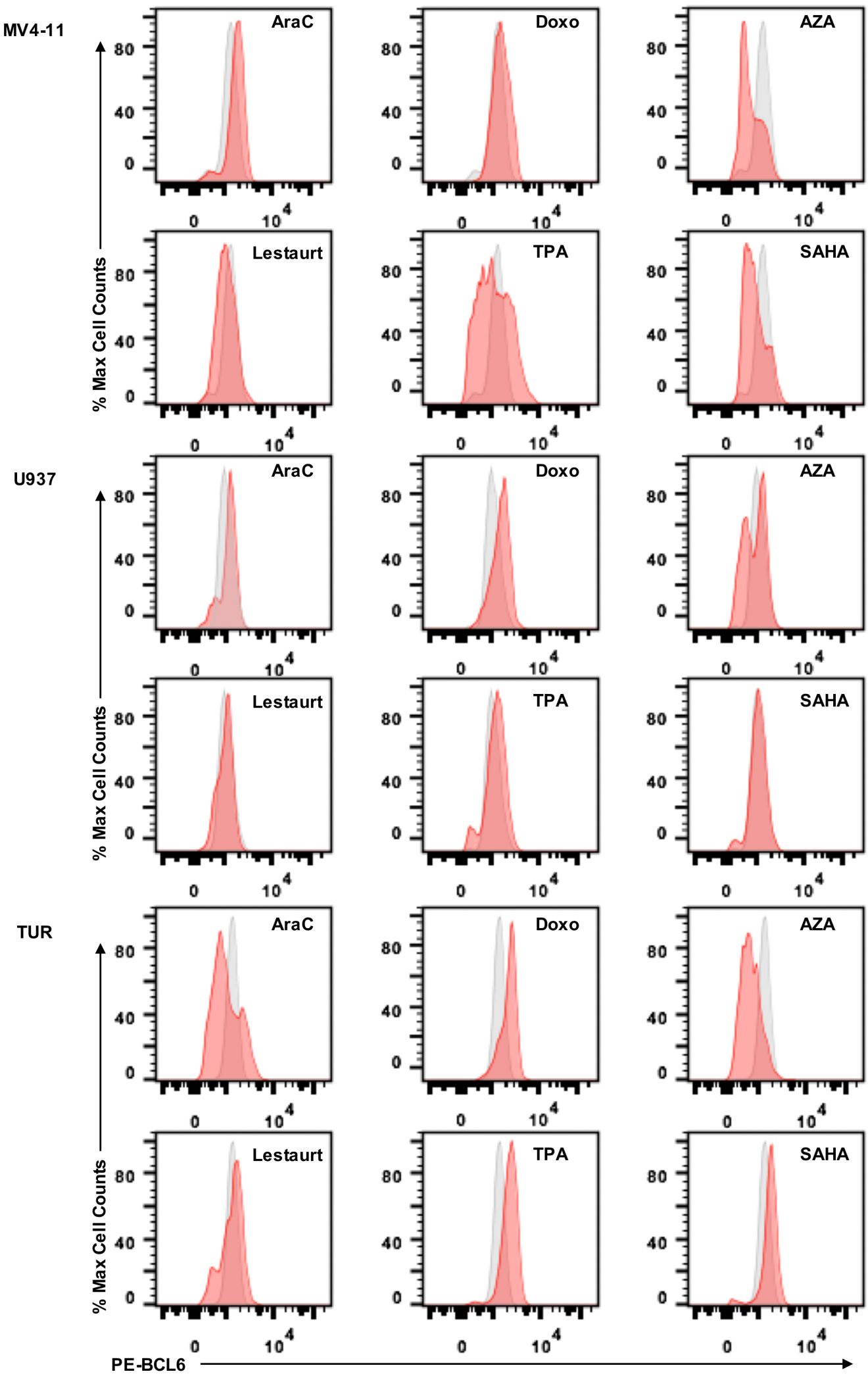
SFigure9


A
CD34+CD38+
LSCs
Total
96%
Singlets
96%
Singlets
99%
SSC-A
FSC-H
FSC-A
SSC-H
SSC-A
DMSO
Viable
81%
Viable
76%
Z Green
SSC-A
CD45 Dim
90%
PE-Cy7-CD38
CD34+CD38+
24%
LSCs
24%
APC-Cy7-CD45
APC-CD34
FX1
Viable
37%
Viable
65%


B
Relative % Viability
DMSO
CD34+CD38+
LSC
CD34+CD38+
LSC
AML54
AML98

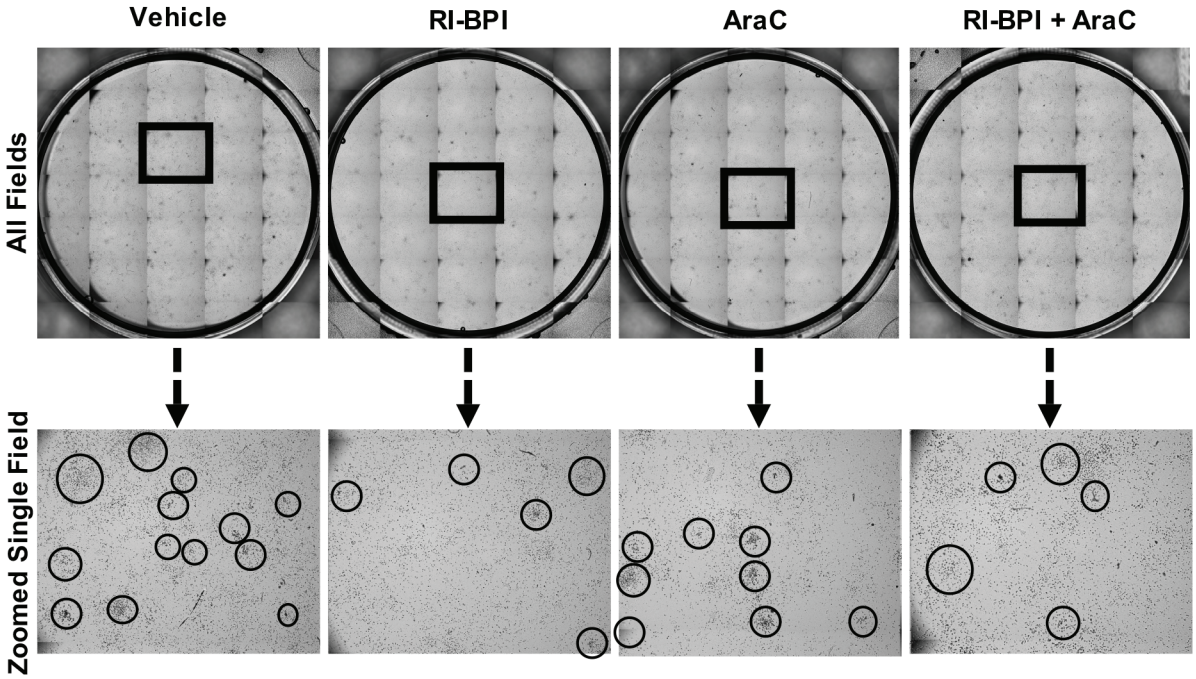

C
AML33
Relative BCL6 Protein Inhibition
(/ Non-ransfected)
NTC siRNA
BCL6 siRNA

SFigure10


MV4-11
U937
TUR
AraC
Doxo
AZA
Lestaurt
TPA
SAHA
% Max Cell Counts
PE-BCL6
0
40
80
10^4

SFigure11

A



B