Bafilomycin A1 targets patient-derived CD34+CD19+ leukemia stem cells

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Supplementary Information

Bafilomycin A1 targets patient-derived CD34CD19 leukemia stem cells

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Reagents, human samples

Bafilomycin A1 was purchased from Sigma-Aldrich. Bone marrow (BM) samples were obtained from B-ALL patients and healthy adult donors at the First Affiliated Hospital of Soochow University (Table S1). Human B-ALL leukemia stem cells were sorted against CD34 (Biolegend Cat. 343504) and CD19 antibodies (Biolegend Cat. 302212) using flow cytometer (FACS Aria III, BD Bioscience) after isolating BM mononuclear cells with Ficoll density gradient centrifugation.

Flow cytometry, antibodies and immunohistochemistry

BM mononuclear cells were obtained by gradient centrifugation on Ficoll-hypaque medium (density 1.077; GE Healthcare). BMMNCs from patients and healthy donors were washed with phosphate-buffered saline (PBS) several times, and then resuspended in 100 μl of a binding buffer containing fluoroscein isothiocynate (FITC)-conjugated antibodies against human CD34, allophycocyanin(APC)-conjugated antibodies against human CD19 for 30 min at 4℃ according to the manufacturer's instructions, these cells were washed with PBS and then analysed by flow cytometer (Beckman Coulter Gallios).

Flow cytometry analysis of xenograft human leukemia cells in BM and PB of mice were performed with Beckman Coulter Gallios using anti-human CD45 conjugated with BV421 (Biolegend Cat. 304032) and/or anti-human CD34 conjugated with FITC (Biolegend Cat. 343504), anti-human CD19 conjugated with APC (Biolegend Cat. 302212) and anti-mouse CD45 conjugated with phycoerythrin (PE) (Biolegend Cat. 103106). Immunohistochemistry analysis was done using anti-human CD19 (Abcam Ref. 134114).

Culture of primary LSCs

The primary LSCs were cultured in serum-free medium (Stem Span^{TM} SFEM, Stem Cell Technologies Cat. #09650) in the presence of granulocyte-macrophage colony-stimulating factor (G-CSF, 20 ng/ml), stem cell factor (SCF, 100 ng/ml), IL-3 (20 ng/ml), IL-6 (20 ng/ml), IL-7 (20 ng/ml), and Flt3L (100 ng/ml). These factors were purchased from Miltenyi Biotec.

Cell Proliferation Analysis

Primary B-ALL $CD34^+CD19^+$ cells and NBM $CD34^+$ cells were sorted by flow cytometer (FACS Aria III, BD Biosciences). About 50,000 cells were plated into 96-well plate with 100 μl media for culture and then treated with either vehicle or Bafilomycin A1 (1 nM) for 24, 48, 72 and 96 h. Cell Counting Kit-8 (CCK-8; Beyotime, Shanghai, China) was used for the analysis of proliferation following manufacturer's instruction. Before assessment, 10 μl CCK-8 reagents were added into each well and the cells were incubated at 37℃ for another 4 h. The absorbance at two wavelengths (450 nm for the viable cells and 650 nm for the soluble dye) was measured with a microplate reader (SpectraMax M5; Molecular Devices, Sunnyvale, USA).

CFSE assay

Primary B-ALL $CD34^+CD19^+$ cells and NBM $CD34^+$ cells were sorted by flow cytometer (FACS Aria III, BD Biosciences), stained with 5 μM CFSE (Thermo Fisher Scientific Cat. C34554) on day 0, and then incubated at 37℃ for 20 minutes, protected from light. Termination reaction was incubated with medium containing 10% fetal bovine serum at 4℃ for 10 minutes. Next, Cells were washed twice by medium containing 10% FBS. Finally, cells were cultured with complete medium. Part of cells were fixed by 4% PFA. The rest of the sample was stimulated with or without bafilomycin A1 to allow proliferate for 3 days. Cells were analyzed by flow cytometry for cell proliferation.

Supplementary Information

Analysis of apoptosis of primary $CD34^+CD19^+$ cells

B-ALL $CD34^+CD19^+$ cells or NBM $CD34^+$ cells were labeled with annexinV-FITC and PI (BD Pharmingen™, Cat. 556547) after treatment, apoptotic cells (annexin V^+ PI^-) were detected by flow cytometer (FACS Calibur, BD Bioscience).

Engraftment of human cells in immunodeficient mice

Primary B-ALL $CD34^+CD19^+$ cells ($1\text{-}5\times10^6$ cells/mouse) were transplanted by tail vein injection into 8-week-old mail NSG mice (Beijing Biocytogen Co, Ltd). Mice were euthanized after 6-13 weeks, PB cells and BM cells were harvested. The engraftment of human cells was analyzed by flow cytometer (Beckman Coulter Gallios).

Hoechst 33342 and Pyronin Y staining

Cell cycle analysis was performed using Hoechst 33342 (Thermo Fisher Scientific Ref. H3570) and Pyronin Y (Sigma P9172-1G) according to standard techniques. Cells were harvested after Bafilomycin A1 (1 nM) treatment for 24 h. Cells (1×10^6) were collected and washed with PBS and then suspended in the remaining PBS. The cells were fixed with 70% pre-chilled ethanol and stored in freezer overnight. Cells were washed and resuspended in PBS. Followed with stained in this solution plus 1 mg/mL Hoechst 33342 at 37℃ for 30 min. Then, cells were incubated with Pyronin Y for 30 min at room temperature. After staining, washed cells were kept on ice until analysis. All gates were set, based on unstained control. The proportion of cells within the G0, G1, S-G2/M phases was analyzed by flow cytometer (FACS Aria III, BD Bioscience).

Hoechst 33342 and Ki-67 staining

Cell cycle distribution of B-ALL $CD34^+CD19^+$ cells was examined by Ki-67 (Biolegend Cat. 652417)/ Hoechst 33342 (Thermo Fisher Scientific Ref. H3570). Cells were harvested with or without bafilomycin A1 treatment and fixed with 70% cold ethanol at 4℃ overnight. Cells were washed and resuspended in PBS. Followed with stained in this solution plus 1 mg/mL Hoechst 33342 at 37℃ for 30 min. Then, cells were incubated with Ki-67 for 30 min at room temperature. After staining, washed cells were kept on ice until analysis. All gates were set, based on unstained control. These cells were analyzed by flow cytometer (FACS Aria III, BD Bioscience).

CFU assay

Human B-ALL $CD34^+CD19^+$ cells, and NBM $CD34^+$ cells were treated with or without bafilomycin A1 for 72 h, 3000 cells were plated in drug free methylcellulose medium (MethoCult H4435, Stem cell Technologies). Colonies were counted on day 14.

Analysis of cell viability

B-ALL $CD34^+CD19^+$ cells viability was detected by staining of 7-AAD (Biolegend Cat. 420403) by flow cytometer (Beckman Coulter Gallios).

Statistical analysis

All experiments were performed at least 3 times, and data were represent as mean±SD. GraphPad Prism 5.0 (GraphPad Software) was used for statistical analysis. Comparisons between 2 groups were analyzed by 2-tailed Student's test and comparisons of multiple groups by 1-way ANOVA. P value less than 0.05 was considered statistically significant.

Supplementary Information

Table S1. Patients general information

Sample	Classification	Type	Age (Year) / Sex	Clinical Status	Risk Standard	Primary Cytogenetic Abnormality	Blasts(%)	Differentiation Antigen Expression
ALL-1	B-ALL	BM	40 / F	Diagnosis	HR	45,XX,del(1)[p35],t(9;22)(q34;q31),-19,-20,+M[5]/46,XX[5]	37.50	CD34,CD10,CD19,CD20
ALL-2	B-ALL	BM	21 / M	Diagnosis	HR	45,XY,-7,t(9;22)(q34;q11)[3]/46,XY[7]	73.50	CD34,CD10,CD19,CD13,CD33,TDT,cCD79a,CD7
ALL-3	B-ALL	BM	27 / F	Diagnosis	HR	None	7.50	CD34,CD10,CD19,HLA-DR
ALL-4	B-ALL	BM	35 / F	PR	HR	None	43.20	CD34,CD10,CD19,CD33,CD79a,CD13low
ALL-5	B-ALL	BM	19 / M	PR	SR	None	32.30	CD34,CD10,CD19,CD33,HLA-DR,CD13
ALL-6	B-ALL	BM	73 / M	Diagnosis	HR	t(9;22)(q34;q11) BCR-ABL	69.00	CD34,CD10,CD19,CD33
ALL-7	B-ALL	BM	26 / M	PR	HR	None	43.10	CD34,CD10,CD19,CD33low,cCD79a,CD20
ALL-8	B-ALL	BM	32 / F	PR	SR	None	24.30	CD34,CD19,CD13,CD33,CD7,CD10,CD20,CD14,CD2
ALL-9	B-ALL	BM	62 / F	Diagnosis	HR	46,XX,t(2;3)(p12,q27),t(9;22)(q34;q11)[10]	80.00	CD34,CD19,CD33,CD79a,CD10,CD20,HLA-DR
ALL-10	B-ALL	BM	38 / M	PR	HR	None	5.00	CD22,CD3,CD10,CD19,HLA-DR
ALL-11	B-ALL	BM	65 / F	Diagnosis	HR	39,X,-X,4q-,5q+,-6,-7,78p+,12p+,-13,der(16),17q+,-18,20q-,-21,-22,ace,inc[5]/46,XX[5]	90.00	CD34,CD10,CD19,CD13,CD117,cCD79a
ALL-12	B-ALL	BM	31 / F	Diagnosis	HR	56-57,XX,+1,+3,+5,+11,+13,+14,+15,-18,+19,+21,+21,+22[CP2]/46,XX[12]	88.00	CD34,HLA-DR,CD10,CD19
ALL-13	B-ALL	BM	48 / M	Diagnosis	SR	None	75.00	CD34,HLA-DR,CD10,CD19,cCD79a
ALL-14	B-ALL	BM	37 / M	PR	HR	None	70.70	CD34,CD19,CD33,CD79a,CD10,CD20,HLA-DR
ALL-15	B-ALL	BM	21 / M	PR	HR	None	68.40	CD34,CD19,CD38,CD33,CD22,CD10,CD20,CD123,HLA-DR
ALL-16	B-ALL	BM	36 / F	Diagnosis	HR	50,XX,+5,+8,i(9q),t(9;22;16)(q34;q11;p13),+ph,+M[7]/46,XX[3] BCR/ABL	83.00	CD20,CD10,CD19,CD123,CD38,TDT,cCD79a,CD22low
ALL-17	B-ALL	BM	26 / F	Diagnosis	SR	None	76.00	CD34,CD10,CD19,CD38,CD22
ALL-18	B-ALL	BM	26 / M	Diagnosis	SR	46,XX,inv[3](q21q26),del(11)(q23q25)[8]	86.00	CD34,CD10,CD19,CD38,CD22
ALL-19	B-ALL	BM	62 / M	Diagnosis	HR	None	93.90	CD34,CD19,CD10,CD79a
ALL-20	B-ALL	BM	59 / M	Diagnosis	HR	45,XY,-7,t(9;22)(q34;q11)[9]/46,XY[1] BCR-ABL	81.50	CD34,CD10,CD19,CD13,CD33,cCD79a
ALL-21	B-ALL	BM	53 / M	PR	HR	t(9;22)(q34;q11) BCR-ABL	78.70	CD34,CD10,CD19,CD13,CD33,cCD79a
ALL-22	B-ALL	BM	55 / F	Diagnosis	HR	46,XX,t(9;22)(q34;q11)[10] BCR/ABL	80.80	CD34,CD19,CD13,CD33,cCD79a
ALL-23	B-ALL	BM	25 / M	Diagnosis	HR	None	92.00	CD7,CD34,CD10,CD20,CD19,CD33,CD22,cCD79a
ALL-24	B-ALL	BM	34 / F	PR	HR	None	65.00	CD10,CD19,CD13,CD33,cCD79a
ALL-25	B-ALL	BM	23 / M	Diagnosis	HR	t(9;22)(q34;q11) BCR-ABL	93.20	CD34,CD10,CD19,CD22
ALL-26	B-ALL	BM	15 / F	Relapse	HR	None	57.00	CD34,HLA-DR,CD10,CD19,CD25
ALL-27	B-ALL	BM	30 / F	Diagnosis	SR	None	92.00	CD34,CD10,CD19,HLA-DR,CD22low
ALL-28	B-ALL	BM	17 / M	Diagnosis	SR	46,XY,t(8;21)(q22;q22)[6]/45,idm,-Y[4]	54.60	CD34,CD19,CD13,CD33
ALL-29	B-ALL	BM	50 / F	Diagnosis	HR	46,XX,?t(9;22)(q34,q11),inc[1] BCR/ABL	50.00	CD10,CD19,CD13,CD33,cCD79a,CD25,CD22
ALL-30	B-ALL	BM	5 / M	Diagnosis	SR	None	69.20	CD34,CD19
ALL-31	B-ALL	BM	15 / F	Diagnosis	SR	47,XX,+21[4]/46,XX[6]	71.00	CD34,HLA-DR,CD10,CD19,CD13,CD2,CD22,cCD79a
ALL-32	B-ALL	BM	7 / F	Diagnosis	HR	55,XX,+X,+X,+4,+6,dic[11;12][p11;p11],+14,+14,-15,+17,+18,+21,+21,+?21[6]/56,XX,+X,+X,+4+6,+14,+14,+17,+18,+21,+21[3]/46,xx[1]	84.00	CD34,CD10,CD20,CD19,CD22
ALL-33	B-ALL	BM	23 / M	Diagnosis	HR	None	75.00	CD10,CD19,CD33,cCD79a,CD20,CD22
ALL-34	B-ALL	BM	15 / F	Diagnosis	HR	46,XY,t(4;11)(q21;q23)[5]/46,idem,i(7)(q10)[4]/46,idem,t(10;14)(p14;q12)[1]	80.50	CD34,CD19,TDT,cCD79a

Bone marrow aspirates were obtained from B-ALL patients. BM: bone marrow, M: male, F: female, PR: partial response, HR: high-risk, SR: standard risk.

Table S1. Patients general information (continued)

ALL-35	B-ALL	BM	5 / M	Diagnosis	SR	None	69.20	CD34,CD19
ALL-36	B-ALL	BM	46 / F	PR	HR	None	6.00	CD81,CD10,CD19,CD22low
ALL-37	B-ALL	BM	24 / M	PR	HR	None	7.00	CD81,CD10,CD19,CD22low
ALL-38	B-ALL	BM	23 / F	Diagnosis	HR	None	87.87	CD10,CD19
ALL-39	B-ALL	BM	1 / M	Diagnosis	HR	None	92.00	CD123,CD38,CD58,CD34,CD19,CD8
ALL-40	B-ALL	BM	18 / M	Diagnosis	SR	None	54.80	CD34,CD19,CD13,CD33
ALL-41	B-ALL	BM	30 / F	PR	HR	None	5.75	CD19,CD20,CD13
ALL-42	B-ALL	BM	52 / M	PR	HR	None	5.00	CD19,CD10,CD33
ALL-43	B-ALL	BM	44 / F	PR	HR	None	6.00	CD81,CD19,CD22low
ALL-44	B-ALL	BM	34 / F	Relapse	HR	46,XX,add(19)(p13)[3]/46,XX[17]	10.50	CD10,CD19,cCD79a,CD33,HLA-DR
ALL-45	B-ALL	BM	32 / F	Diagnosis	HR	46,XX,t(9;22)(q34;q11)[6]	46.00	CD13,CD19,cCD79aCD33,HLA-DR, CD25,CD22low
ALL-46	B-ALL	BM	23 / M	Diagnosis	SR	None	2.00	CD19,CD13,CD10
ALL-47	B-ALL	BM	62 / F	Diagnosis	SR	None	15.00	CD19,CD33
ALL-48	B-ALL	BM	29 / F	Relapse	HR	None	0.05	CD34,CD10,CD19,CD79a,CD45
ALL-49	B-ALL	BM	46 / M	Diagnosis	HR	46,XX,t(9;22)(q34;q11)[6]	83.50	CD7,CD34,CD10,CD13,CD33,CD19, CD79a,CD25,CD22low
ALL-50	B-ALL	BM	35 / M	Diagnosis	HR	None	84.00	CD34,CD10,CD33,CD19,CD79a,CD22low
ALL-51	B-ALL	BM	57 / M	Diagnosis	HR	None	47.00	CD34,HLA-DR,CD13,CD33,CD19,CD2low
ALL-52	B-ALL	BM	20 /M	Diagnosis	HR	None	17.50	CD34,CD19,CD33,CD13
ALL-53	B-ALL	BM	37 / M	Diagnosis	HR	46,XY,t(9;22)(q34;q31)[6]/45,idem,-7[1]/46, idem,-7,+8[1]/46,46,XY[2]	62.50	CD34,CD19,CD13,CD33,CD123,HLA-DR, CD38,CD10
ALL-54	B-ALL	BM	17 / M	Diagnosis	SR	45,X,-Y,del(6)(q15;q23)[2]/46,+M[5]/46, XY[16]	59.00	CD34,CD19
ALL-55	B-ALL	BM	44 / F	Diagnosis	HR	t(9;22)(q34;q11) BCR-ABL	78.00	CD34,CD19,cCD79a,CD33,CD38,CD22
ALL-56	B-ALL	BM	19 / M	Diagnosis	SR	None	50.50	CD34,HLA-DR,CD10,CD19,CD38, CRLF2,CD22
ALL-57	B-ALL	BM	57 / M	Diagnosis	SR	None	8.00	CD34,CD19,CD33,CD22
ALL-58	B-ALL	BM	27 / F	Diagnosis	SR	None	76.00	CD34,CD10,CD19,CD38,CD22
ALL-59	B-ALL	BM	14 / F	Diagnosis	HR	None	90.00	CD34,CD19,CD7,CD10,CD33,cCD3,CD5, CD99
ALL-60	B-ALL	BM	54 / F	Diagnosis	HR	t(9;22)(q34;q11) BCR-ABL	85.00	CD34,CD19,CD13,CD10,CD33,CD25, cCD79a,CD22low,CD38low
ALL-61	B-ALL	BM	22 / M	Diagnosis	HR	6,XY,t(9;22)(q34;q11)[2]/46,XY,add(2)(p25) ,-7,der(9)?dic(9;10)(p11;p11),t(9;22), +Ph,+m[5]/46,XY[3]	94.00	CD34,CD10,CD19,CD20
ALL-62	B-ALL	BM	24 / F	Diagnosis	HR	CRLF2 rearrange	89.00	CD34,CD19,CD38,CD10,CD33,cCD79a, CRLF2, CD22low
ALL-63	B-ALL	BM	22 / F	Diagnosis	HR	50-53,XX,dup(1)[q23q42],+5,+8,+10,+13, +17,+18,+20,+21[cp3]/46,XX[7]	24.23	CD34,CD19,CD10,CD13,CD33
ALL-64	B-ALL	BM	45 / M	Diagnosis	HR	None	68.93	CD34,CD19,CD10

Bone marrow aspirates were obtained from B-ALL patients. BM: bone marrow, M: male, F: female, PR: partial response, HR: high-risk, SR: standard risk.

Supplementary Information

Table S2. Comparison on the factors used for culturing primary B-ALL LSCs

Corresponding Author	Years	Magazine	Title	Factors
In this study			**Bafilomycin A1 targets patient-derived CD34CD19 leukemia stem cells**	**SCF, IL3, IL6, IL7, Flt3L, G-CSF**
Connie J.Eaves	2007	***Stem cells***	A Modified Polymerase Chain Reaction-Long Serial Analysis of Gene Expression Protocol Identifies Novel Transcripts in Human CD34+ Bone Marrow Cells	Human SF, G-CSF, IL-3, IL6
J.H. Frederik Falkenburg	2009	***Experimental Hematology***	Long–term culture of primary human lymphoblastic leukemia cells in the absence of serum or hematopoietic growth factors	SCF, IL3, IL7
Jingxuan Pan	2016	***The Journal of Clinical Investigation***	Targeting methyltransferase PRMT5 eliminates leukemia stem cells in chronic myelogenous leukemia	SCF, IL3, IL6, GM-CSF
David Vetrie	2016	***Cancer Discovery***	Epigenetic Reprogramming Sensitizes CML Stem Cells to Combined EZH2 and Tyrosine Kinase Inhibition	SCF, IL3, IL6, Flt3L, G-CSF
Sai-Juan Chen	2016	***EBioMedicine***	Genomic Profiling of Adult and Pediatric B-cell Acute Lymphoblastic Leukemia	SCF, IL3, IL6, IL7, Flt3L
H P Koeffler	2017	***Leukemia***	Targeting the vulnerability to NADt depletion in B-cell acute lymphoblastic leukemia	IL7
Junke Zheng	2018	***The Journal of Clinical Investigation***	JAM3 maintains leukemia-initiating cell self-renewal through LRP5/AKT/β-catenin/CCND1 signaling	SCF, IL3, IL6

Supplementary Information

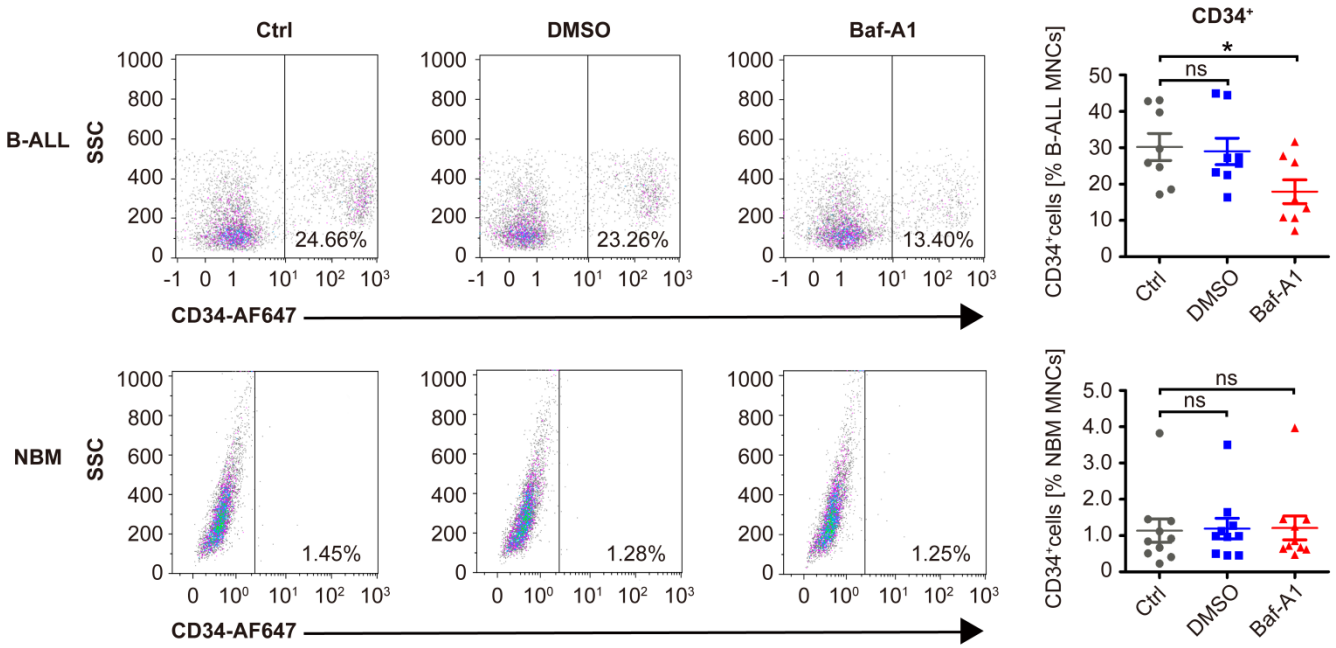

Figure S1. ***In vitro*** **treatment with bafilomycin A1 reduced the proportion of primary B-cell acute lymphocytic leukemia stem cells derived from patients.**

B-ALL CD34⁺ cells (upper panel, n=8, ALL1-8) and NBM CD34⁺ cells (lower panel, n=10) after 1 nM bafilomycin A1 treatment.

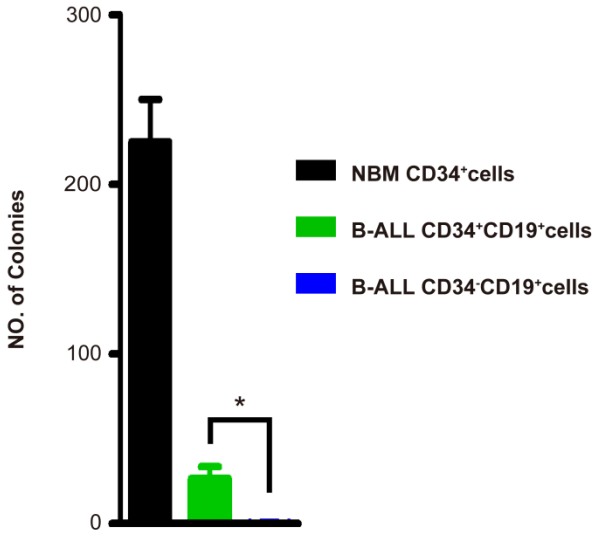

Figure S2. Colony-forming unit assay detecting the capacity of self-renewal of human B-ALL LSCs.

$CD34^+CD19^+$ cells and $CD34^-CD19^+$ cells were sorted from B-ALL patients by flow cytometry. For $CD34^+CD19^+$ cell samples, n=10, ALL18,27-35; for $CD34^-CD19^+$ cell samples, n=4, ALL36-39. NBM $CD34^+$ cells as a positive control, n=8.

Supplementary Information

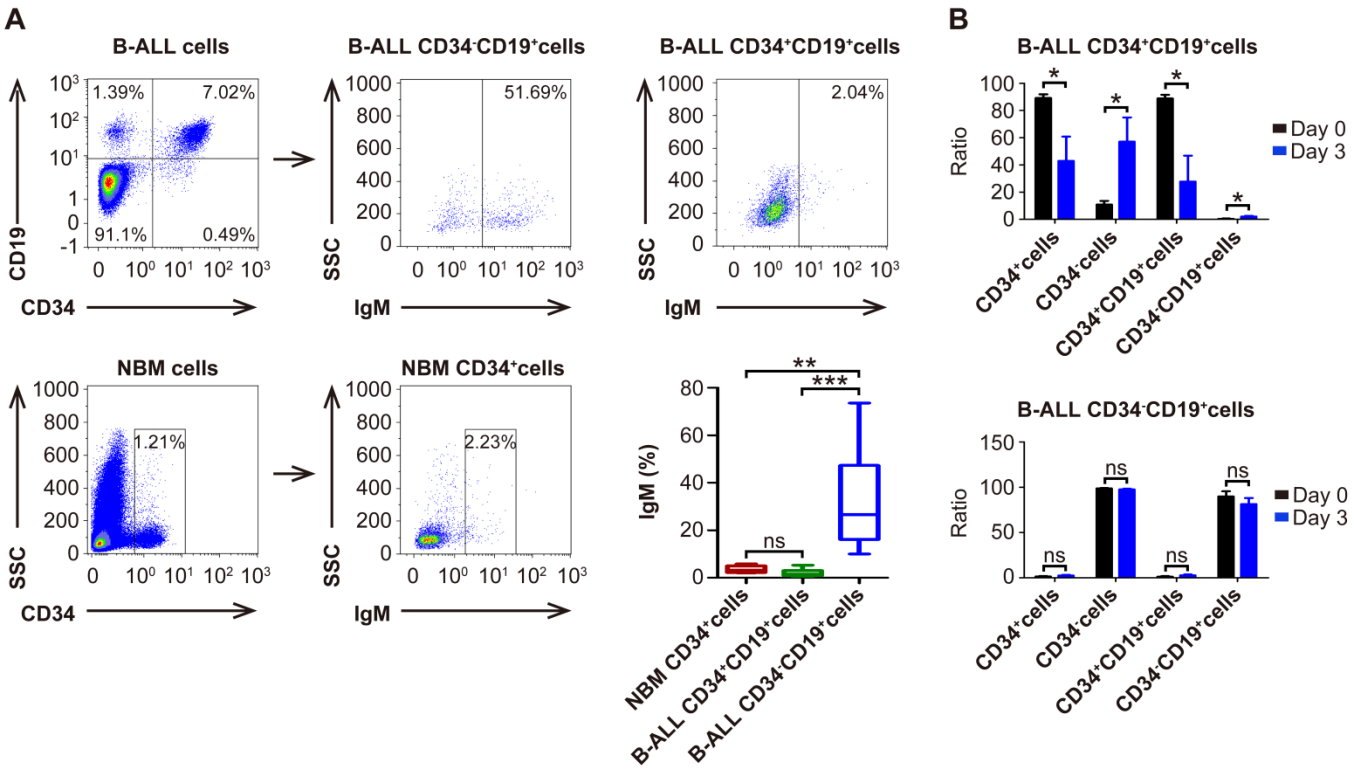

Figure S3. CD34$^+$CD19$^+$ cells are in an earlier stage than CD34$^-$CD19$^+$ cells in human B-ALL hierarchy.

A. Upper panel, human B-ALL CD34$^+$CD19$^+$ cells (n=8, ALL27,28,30,36,37,39-41) and B-ALL CD34$^-$CD19$^+$ cells (n=13, ALL28,30,36,37,39-47) were sorted from the same patient and then cultured for 72 h, and the levels of IgM protein were measured by flow cytometry. Lower panel, the levels of IgM protein of NBM CD34$^+$ cells (n=5) were detected as a negative control.

B. The levels of CD34 protein were measured in human CD34$^+$CD19$^+$ B-ALL cells (n=4, ALL27,28,30,39) and human CD34$^-$CD19$^+$ B-ALL cells (n=9, ALL28,30,37,38,40,41,43-45) by flow cytometry on day 0 and day 3 of cell culturing.

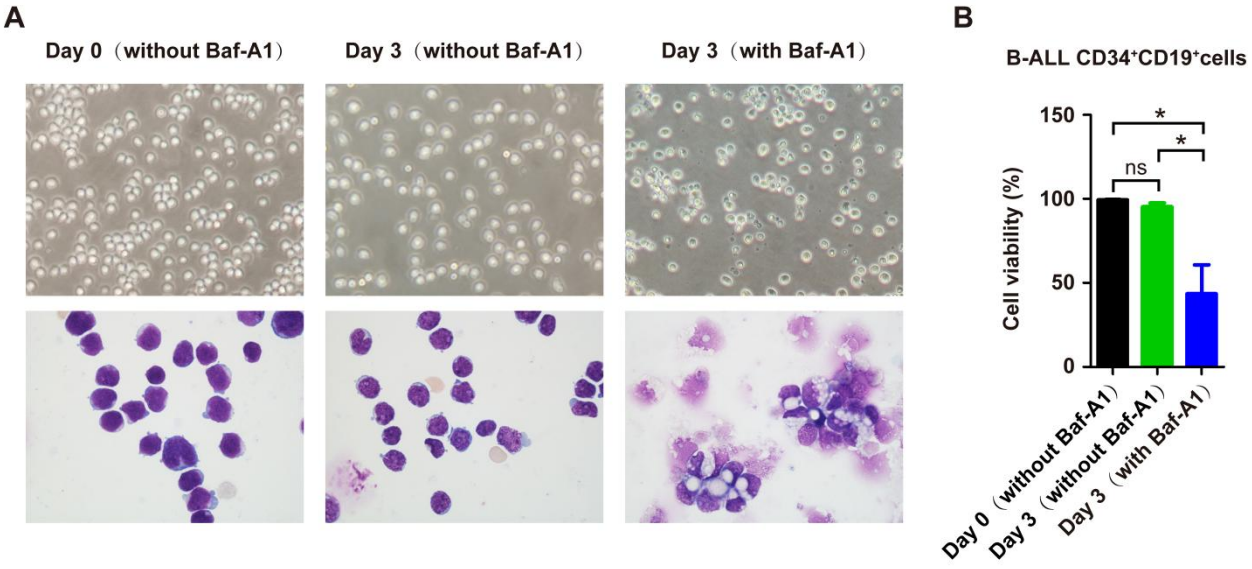

Figure S4. Validation of culture condition for human B-ALL LSCs.

A. Giemsa staining of $CD34^{+}CD19^{+}$ B-ALL cells on day 0 and day 3 of cell culturing (n=3, ALL27,30,39).

B. Cell viability of $CD34^{+}CD19^{+}$ B-ALL cells stained with 7-AAD were measured by flow cytometry on day 0 and day 3 of cell culturing (n=3, ALL27,30,39).

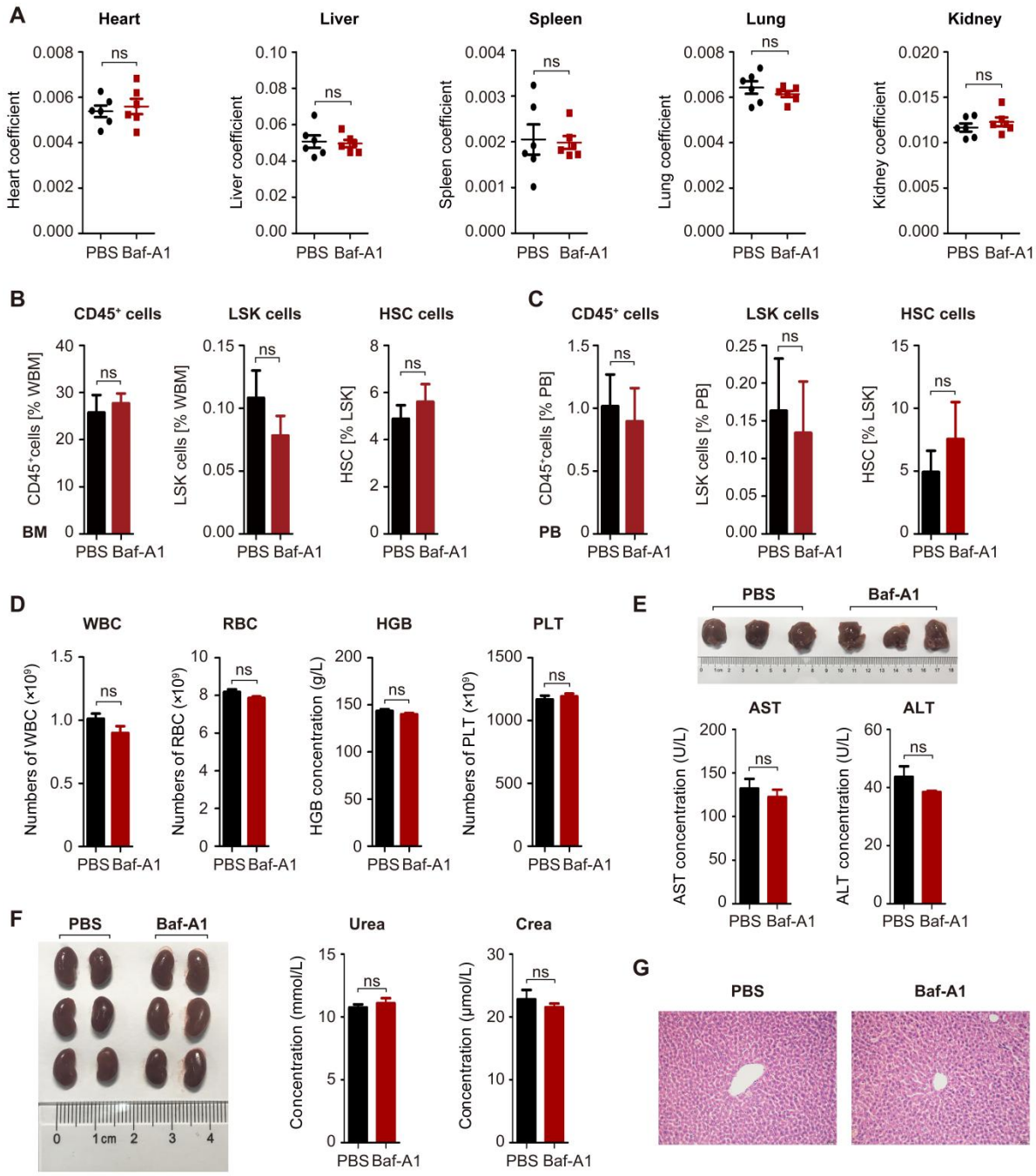

Figure S5. Bafilomycin A1 of dose 0.1mg/kg had no toxicity to NSG mice.

NSG mice (n=6 per group) were treated with 0.1 mg/kg bafilomycin A1 or vehicle in the ctrl group. **A.** Effect of 0.1 mg/kg bafilomycin A1 on organ weight after treatment. **B.** Flow cytometric analysis of effect of bafilomycin A1 (0.1 mg/kg) on hematopoietic stem and progenitor cells in NSG mice bone marrow. **C.** Flow cytometric analysis of effect of bafilomycin A1(0.1 mg/kg) on hematopoietic stem and progenitor cells in NSG mice peripheral blood. **D.** Effect of bafilomycin A1 on the peripheral blood count of NSG mice. WBC: white blood cells; RBC: red blood cells; HGB: hemoglobin; PLT: platelets. **E.** Upper panel, photographs of liver from NSG mice after 0.1 mg/kg bafilomycin A1 treatment; Lower panel, the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ratio after treatment with bafilomycin A1 (0.1 mg/kg) are shown. **F.** Left, photographs of kidney recovered from NSG mice after 0.1 mg/kg bafilomycin A1 treatment; right, the Urea and creatinine (Crea) ratio after treatment with bafilomycin A1 (0.1 mg/kg) are shown. **G.** Hematoxylin and eosin staining of liver injuries after treatment with 0.1 mg/kg bafilomycin A1. ***P<0.001, **P<0.01, *P<0.05.

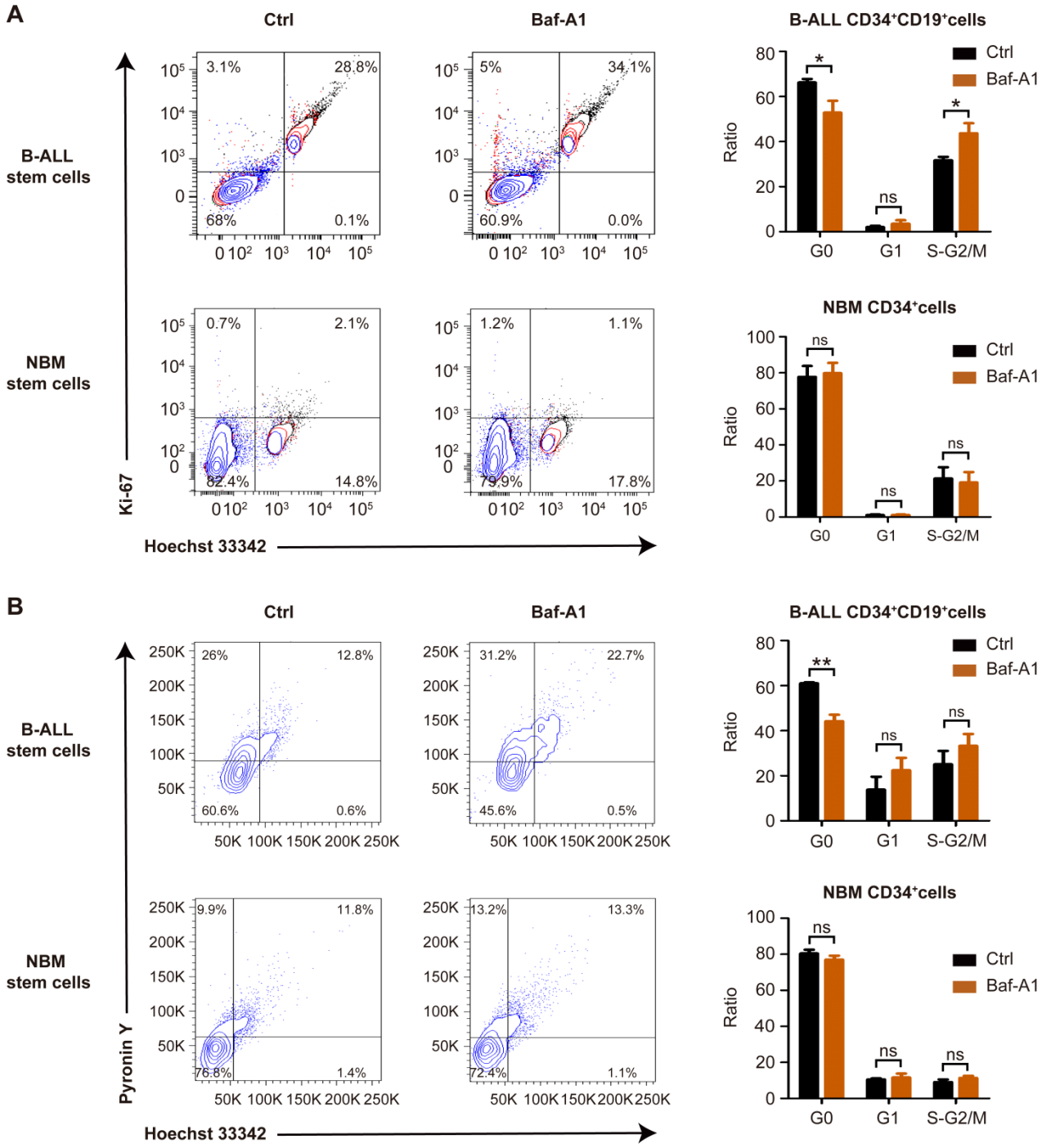

Figure S6. Bafilomycin A1 induced human B-ALL LSCs out of G0 phase.

A. Human B-ALL CD34$^+$CD19$^+$ cells (n=4, ALL59-62) and NBM CD34$^+$ cells (n=11) were sorted by flow cytometer, and then treated with 1 nM bafilomycin A1 for 72 h. The cell cycle for B-ALL stem cells and bone marrow normal stem cells were analyzed by Hoechst 33342 and Ki-67 staining.

B. Human B-ALL CD34$^+$CD19$^+$ cells (n=4, ALL48,49,51,52) and NBM CD34$^+$ cells (n=4) were sorted by flow cytometer, and then treated with 1 nM bafilomycin A1 for 24 h. The cell cycle for B-ALL stem cells and bone marrow normal stem cells were analyzed by Hoechst 33342 and Pyronin Y staining.

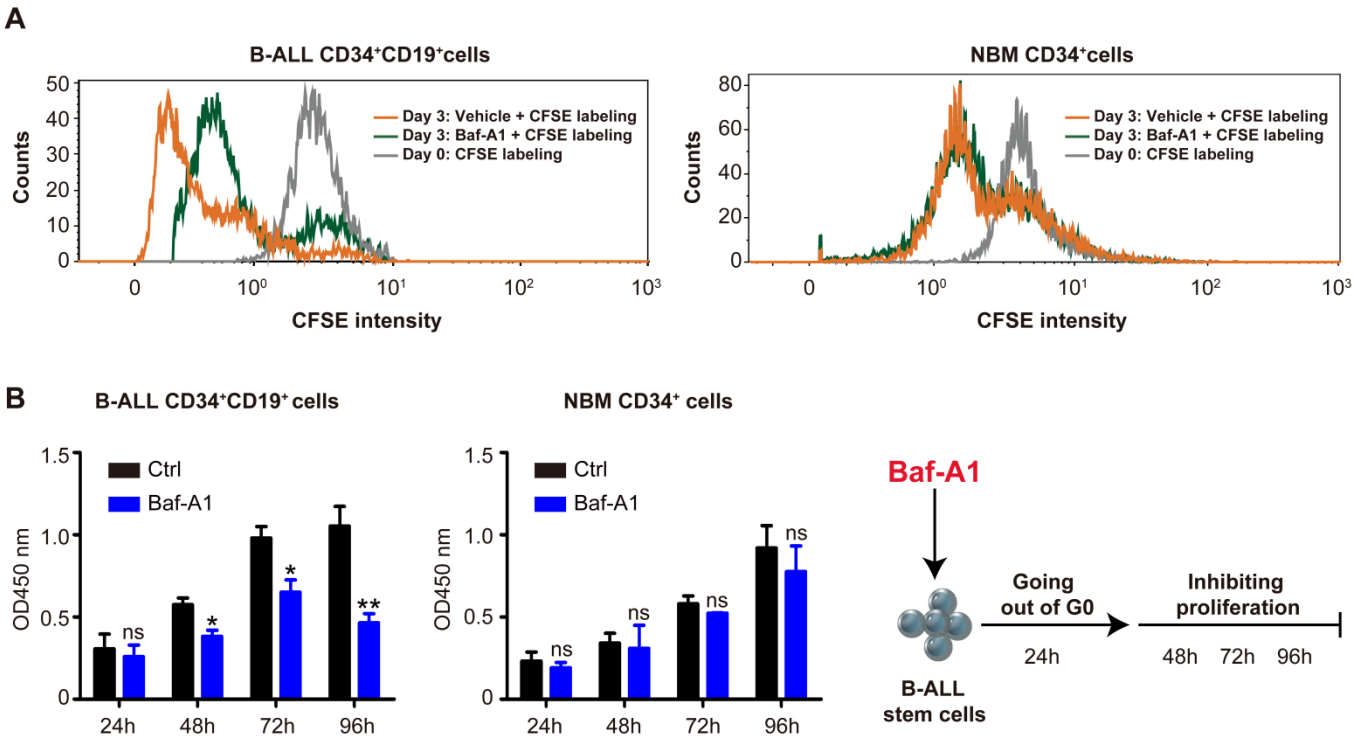

Figure S7. ***In vitro*** **bafilomycin A1 treatment inhibited the proliferation of human B-ALL LSCs but not normal hematopoietic stem cells.**

A. The fluorescence histograms on day 0 indicate the initial level of CFSE fluorescence as a positive control (gray curves); The fluorescence intensity of B-ALL $CD34^+CD19^+$ cells and NBM $CD34^+$ cells (n=4) were measured after cultured for 72 h with (green curves) or without 1 nM bafilomycin A1 (orange curves) treatment (n=3, ALL27,63-64).

B. CCK-8 was used to examine B-ALL $CD34^+CD19^+$ cells (n=4, ALL27,48-50) and NBM $CD34^+$ cells (n=3) proliferation at the indicated time points. CCK-8: Cell Counting Kit-8. ***P<0.001, **P<0.01, *P<0.05. The right panel reflects a putative model of bafilomycin A1 action on B-ALL LSCs.

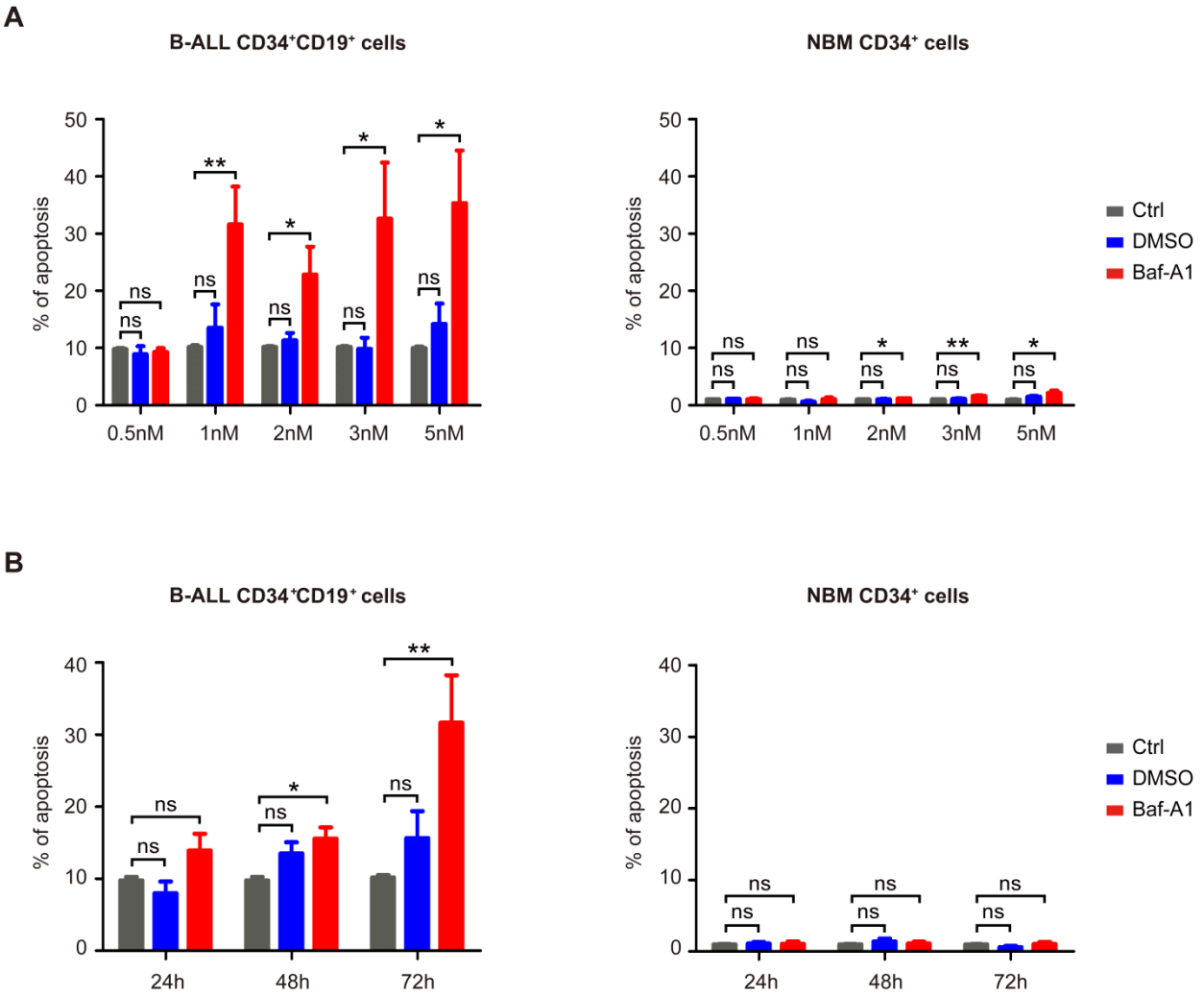

Figure S8. Bafilomycin A1 induced apoptotic death in the primary human B-ALL LSCs.

A. Bafilomycin A1 at various doses induced apoptosis of the patients-derived B-ALL stem cells (n=6, ALL9,11,12,14,21,53) but not hematopoietic stem cells (n=5) from healthy donors, analyzed by flow cytometry. Apoptosis was defined as the percentage of Annexin V-positive and PI-negative cells.

B. Flow cytometric detection of apoptosis of 1 nM bafilomycin A1-treated primary B-ALL $CD34^+CD19^+$ cells (24 h & 48 h n=4, ALL15,16,21,54; 72 h n=6, ALL9,11,12,14,21,53) and NBM $CD34^+$ cells (24 h & 48 h n=3; 72h n=5) by annexin V–FITC and PI. Apoptosis was defined as the percentage of annexin V-positive and PI-negative cells. ***P<0.001, **P<0.01, *P<0.05.

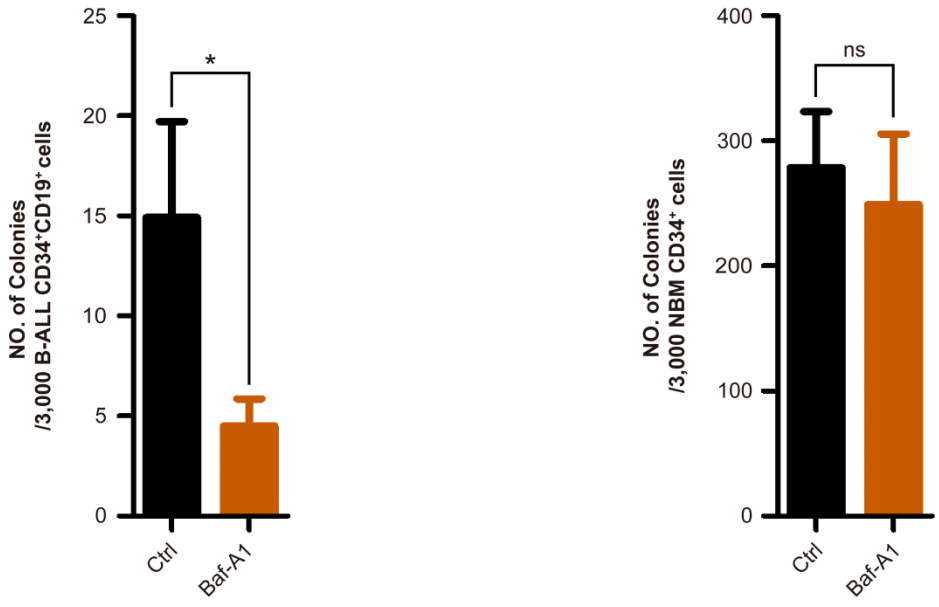

Figure S9. Treatment with 1 nM Bafilomycin A1 reduced colony formation in primary human B-ALL LSCs.

1 nM bafilomycin A1 suppressed the self-renewal of human B-ALL $CD34^+CD19^+$ cells (left, n=6, ALL18,29,31-34), whereas colony formation in NBM $CD34^+$ cells (right, n=4) has no significant difference with or without bafilomycin A1 treatment. B-ALL $CD34^+CD19^+$ cells and NBM $CD34^+$ cells were treated with 1 nM bafilomycin A1 for 72 h, washed with PBS and counted; 3,000 cells were plated in methylcellulose medium. Colonies were counted on day 14 of cell culturing.

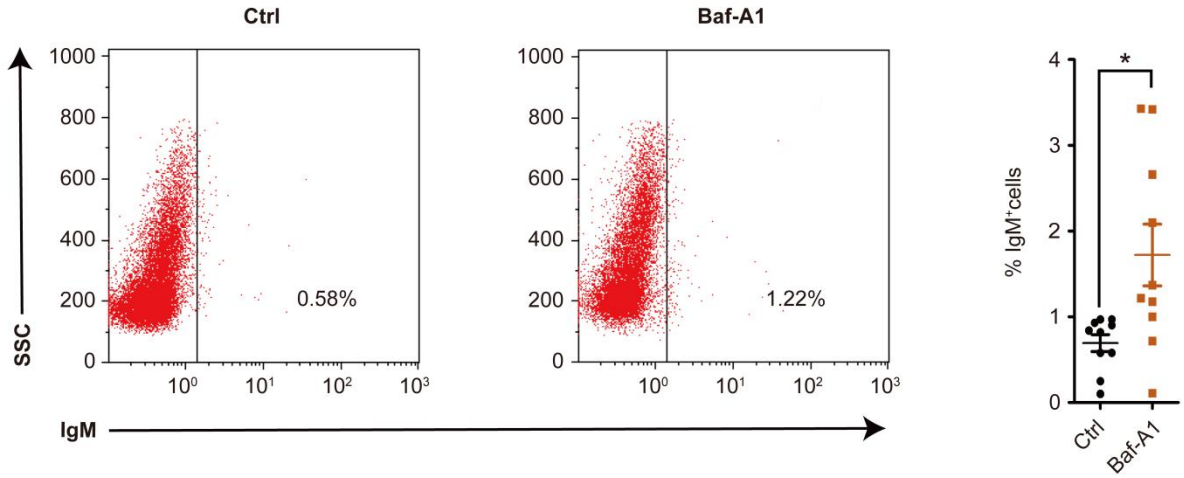

Figure S10. Bafilomycin A1 treatment induced marginal cell differentiation of primary human B-ALL LSCs.

Levels of IgM protein in primary human B-ALL $CD34^+CD19^+$ cells treated with 1 nM bafilomycin A1 were detected by flow cytometry (n=10, ALL17,18,29,31,33,34,55-58).