Supplemental Materials

Methods:

Mice

Six to 8-week old SCD mice were purchased from the Jackson Laboratory (Stock number: 013071). Mice homozygous at the *Hba* locus for the human α-globin (hα) mutation (*Hba*$^{tm1(HBA)Tow}$) and homozygous at the *Hbb* locus for the human Aγ- and sickle β^S-globin (-1400 γ-β^S) mutation (*Hbb*$^{tm2(HBG1,HBB*)Tow}$) are referred to as hα/hα::β^S/β^S, which were used for all animal studies. In this Townes model, the human fetal Aγ-globin gene was placed in a position that would mimic its location and orientation between the murine embryonic and human adult β^S-globin genes. Therefore, the inserted human Aγ-globin gene is regulated as murine embryonic β-like globin genes, and the adult SCD mice exhibit low HbF background (~0.2%) in the peripheral blood. Mice were intraperitoneally injected daily with chemical inhibitors of LSD1 (**Supplemental Table 1**) for 4 consecutive weeks and blood for analysis was collected by retro-orbital bleeding. All animal experiments were approved by Boston University's Institutional Animal Care and Use Committee.

Human CD34$^+$ cell purification and two-phase culture system

Human CD34$^+$ cells were purified from peripheral blood (PB) of deidentified normal volunteers. Briefly, the blood components of 100 mL PB were separated using Ficoll-Opaque, and CD34$^+$ cells were selectively isolated from the interface cells using anti-CD34$^+$ antibody conjugated microbeads and Mini-MACS column according to the manufacturer's instructions (Miltenyi Biotech). CD34$^+$ cells were cultured in a two-phase culture system. For the first phase, we expanded CD34$^+$ cells in StemSpan^{TM} SFEM II media (StemCell Technology) supplemented

with StemSpan™ $CD34^+$ Expansion Supplement for 7 days at a density of $1x10^5$ cells/mL. For the second phase (day 7 to 15), $CD34^+$ cells were erythroid differentiation-induced by culturing in α-MEM media with 14% Gibco™ fetal bovine serum (Fisher Scientific), 14% BIT Serum Substitute (StemCell Technology), 85 μM β-mercaptoethanol (Fisher Scientific), 2 mM L-glutamine (Fisher Scientific), 1% penicillin streptomycin (Fisher Scientific), 10 ng/mL human stem cell factor (hSCF, Fisher Scientific), 10 ng/mL interleukin-6 (IL-6, Fisher Scientific), and 5 U/mL erythropoietin (EPO, Amgen).

Culture of sickle iPSC-derived erythroblasts

Sickle cell disease-specific Induced Pluripotent Stem Cells (iPSCs) were generated and differentiated to adult-type, definitive erythroblasts (SS24 cells) as described.[1,2] After thawing, SS24 cells were recovered for 3 days before the drug treatment in StemSpan™ SFEM I with 100 ng/mL hSCF, 0.5 μM dexamethasone, 0.5 U/mL EPO, 40 ng/mL human Insulin-like Growth Factor 1 (hIGF-1, Fisher Scientific), and 1% L-glutamine. All media were filtered with Corning™ Disposable Vacuum Filter/Storage System (Fisher Scientific) before used.

LSD-1 inhibition tests

$CD34^+$ derived erythroid cells and SS24 erythroid progenitor cells were treated with different concentrations of GSK-LSD1 (Tocris) or OG-L002 (ApexBio Technology) in parallel with control compounds that consisted of HU (Sigma) diluted with PBS (Fisher Scientific), TC (Tocris) and RN-1 (Tocris) diluted with DMSO (Sigma), for 3 or 5 days, after which flow cytometric assays were performed to quantify HbF positive cells or F-cells. Fresh media and compounds were supplied every 2 to 3 days. For the human primary $CD34^+$ cell culture, after 7

days of expansion (phase 1) and 3 days of differentiation (phase 2), cells were treated with 2 different doses of OG-L002 (0.05 and 0.1 μM) or GSK-LSD1 inhibitors (0.02 and 0.1 μM). Controls were dosed with 50 μM HU, 2 or 4 μM TCP, 0.05 or 0.1 μM RN-1, PBS, and DMSO for 5 days in phase 2 culture. For the SS24 cells, after 3 days recovery in expansion media, cells were treated with OG-L002 (0.1 and 0.5 μM) or GSK-LSD1 (0.1 and 0.5 μM) along with controls dosed with 50 μM HU, 4 and 8 μM TC, 0.1 and 0.5 μM RN-1, PBS, or DMSO for 3 days in the expansion media.

Flow cytometric analysis of F-cells

F-cells were quantified by flow cytometry after drug exposure for $CD34^+$ cell derived erythroid cells and SS24 cells. Cells were harvested, fix with 0.08% glutaraldehyde (Fisher Scientific) in PBS, permeabilized with 0.2% Triton X100 (Life Technologies™) in 0.1% BSA/PBS, and then stained with 5 μL APC-conjugated HbF antibody (ThermoFisher) or APC anti-human IgG1 antibody (BioLegend) for 15 minutes in the dark at room temperature. Flow cytometric analyses were performed on a FACSCanto cytometer (BD) and data were analyzed using FlowJo software (Tree Star).

QRT-PCR

mRNA abundance of globin genes in treated SCD mice and $CD34^+$ cells was determined by quantitative real-time polymerase chain reaction (QRT-PCR). Primers for QRT-PCR as previously described.[3]

Cell viability assay

The viability of cultured $CD34^+$ cells was quantified by flow cytometry after Zombie NIR staining according to manufacture's instruction (Biolegend, 423105). Cells were harvested and washed with PBS (no Tris buffer and protein free), and then stained with 100 μL diluted Zombie NIR™ dye at 1:1000 in PBS for 15 minutes in the dark at room temperature. Flow cytometric analyses were performed on a FACSCanto cytometer (BD) and data were analyzed using FlowJo software (Tree Star).

Statistics

The data for the primary human erythroid cell derived from PB $CD34^+$ cells and SS24 cells represent the average of multiple, independently performed biological replicates (N). All statistical analyses were calculated using an unpaired 2-tailed Student *t* test on EXCEL. $P < 0.05$ was considered statistically significant. Details regarding exact *P* values and N are described in the figure legends.

Reference:

1. Park S, Gianotti-Sommer A, Molina-Estevez FJ, et al. A Comprehensive, Ethnically Diverse Library of Sickle Cell Disease-Specific Induced Pluripotent Stem Cells. *Stem Cell Reports*. 2017;8(4):1076-1085.

2. Leung A, Zulick E, Skvir N, et al. Notch and Aryl Hydrocarbon Receptor Signaling Impact Definitive Hematopoiesis from Human Pluripotent Stem Cells. *Stem Cells*. 2018;36(7):1004-1019.

3. Cui S, Lim KC, Shi L, et al. The LSD1 inhibitor RN-1 induces fetal hemoglobin synthesis and reduces disease pathology in sickle cell mice. *Blood*. 2015;126(3):386-396.

Figure legends:

Supplemental Figure 1. Induction of HbF in SCD mice treated with GSK-LSD1 and OG-L002 for 4 weeks. (A) Quantification of HbF by HPLC as a fraction of total hemoglobin in control and treated SCD mice. **(B, C)** QRT- PCR quantification of γ- and β-globin mRNA abundance in SCD mice. Statistically significant differences between GSK-LSD1 or OG-L002-treated and control DMSO-treated SCD mice are indicated (* $P < 0.05$). Bar graph data are presented as the mean ± SD, n = 3 mice per group.

Supplemental Figure 2. mRNA expression of globin genes in $CD34^+$ cells treated with GSK-LSD1 and OG-L002 for 5 days. (A, B) QRT- PCR quantification of γ- and β-globin mRNA abundance in $CD34^+$ cells. Statistically significant differences between GSK-LSD1 or OG-L002-treated and control DMSO-treated $CD34^+$ cells are indicated (* $P < 0.05$). Bar graph data are presented as the mean ± SD, n = 3 replicates.

Supplemental Figure 3. The viability of $CD34^+$ cells treated with GSK-LSD1 and OG-L002 for 3 days (A), 5 days (B), and 9 days (C). Statistically significant differences between treated and control DMSO-treated $CD34^+$ cells are indicated (* $P < 0.05$). Bar graph data are presented as the mean ± SD, n = 3 replicates.

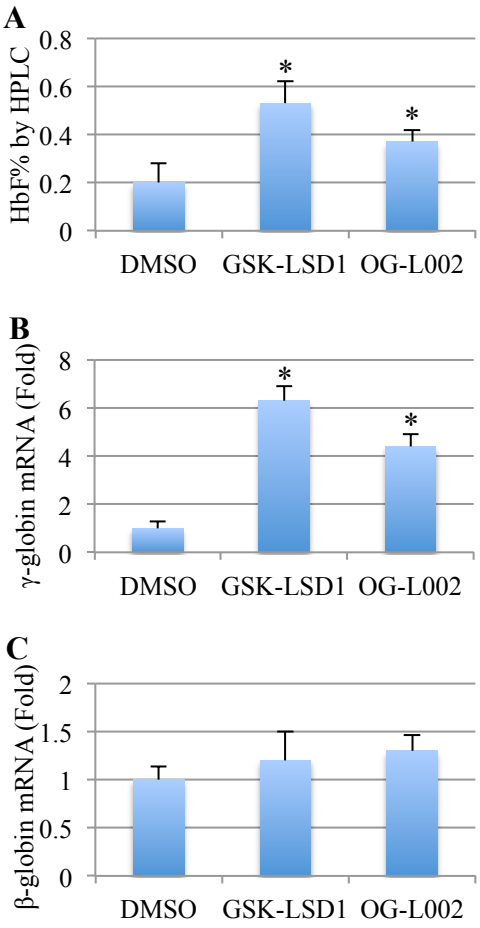
Supplemental Figure 4. Some LSD1 inhibitors increase HbF in sickle iPSC-derived erythroblasts (SS24 cells). (**A**) Representative flow cytometry plots of HbF-stained cultured cells treated with drugs or controls. SS24 cells were recovered in expansion media for 3 days, and then treated with 0.1 and 0.5 μM OG-L002 or 0.1 and 0.5 μM GSK-LSD1 along with

controls (50 μM HU, 4 and 8 μM TC, 0.1 and 0.5 μM RN-1, PBS, or DMSO) for 3 additional days in expansion media. (**B**) Representative histograms showed the fluorescence, and (**C**) mean fluorescence intensity (MFI), of the treated SS24 cells compared to controls.

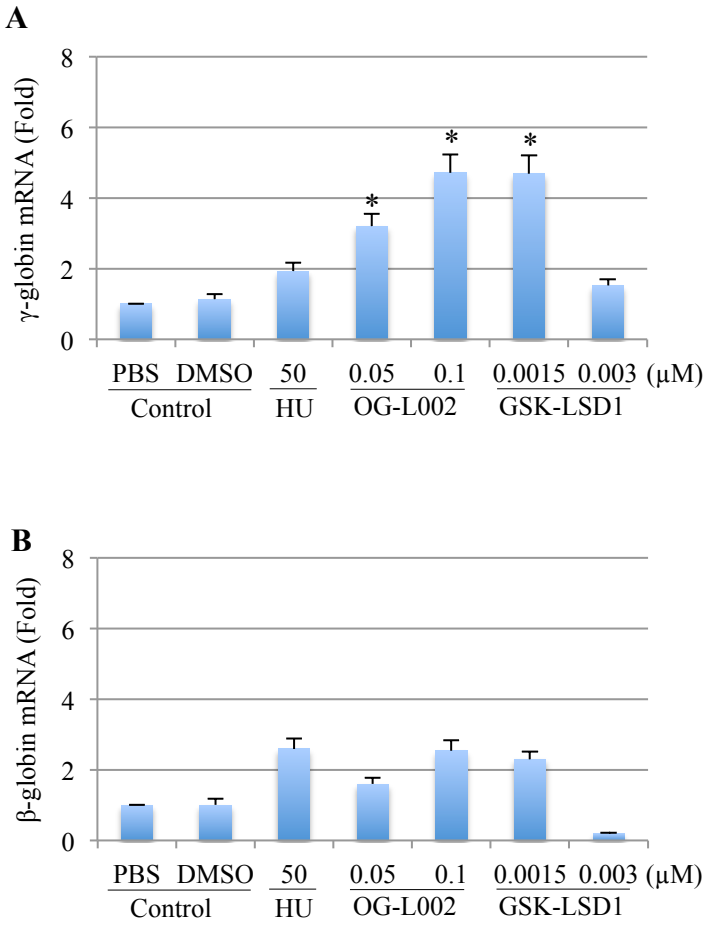
Supplemental Figure 5. $CD34^+$ cells treated with inactive LSD1 inhibitors. (**A**) Representative flow cytometry plots of HbF-stained cultured $CD34^+$ cells treated with inactive LSD1 inhibitors. After 7 days of expansion and 3 days in differentiation phase, cells were treated with 0.5 μM LSD1-C12, LSD1-C76 or S2101 along with controls (20 μM HU, 0.1 μM OG-L002, or DMSO) for a further 5 days in differentiation culture. (**B**) $CD34^+$ cells flow cytometry data shown as bar graphs. (All data represent the average of 3 independent biological replicates. All statistical analyses were calculated using an unpaired 2-tailed Student t-test. *$p < 0.005$ and **$p < 0.0005$. ns = not significant.)

Supplemental Figure 6. Sickle iPSC-derived erythroblasts (SS24 cells) treated with inactive LSD1 inhibitors. (A) Representative flow cytometry plots of HbF-stained cultured cells treated with drugs or controls. SS24 cells were recovered in expansion media for 3 days, and then treated with 0.1 and 0.5 μM LSD1-C12, LSD1-C76 or S2101 along with controls (50 μM HU, or DMSO) for 3 additional days in expansion media. (B) SS24 cells flow cytometry data of HbF-stained cultured cells treated with drugs or controls shown as bar graphs. (All data represent the average of 3 independent biological replicates. All statistical analyses were calculated using an unpaired 2-tailed Student t-test. *$p < 0.005$ and **$p < 0.0005$. ns = not significant.)

Supplemental Figure 1



Supplemental Figure 2



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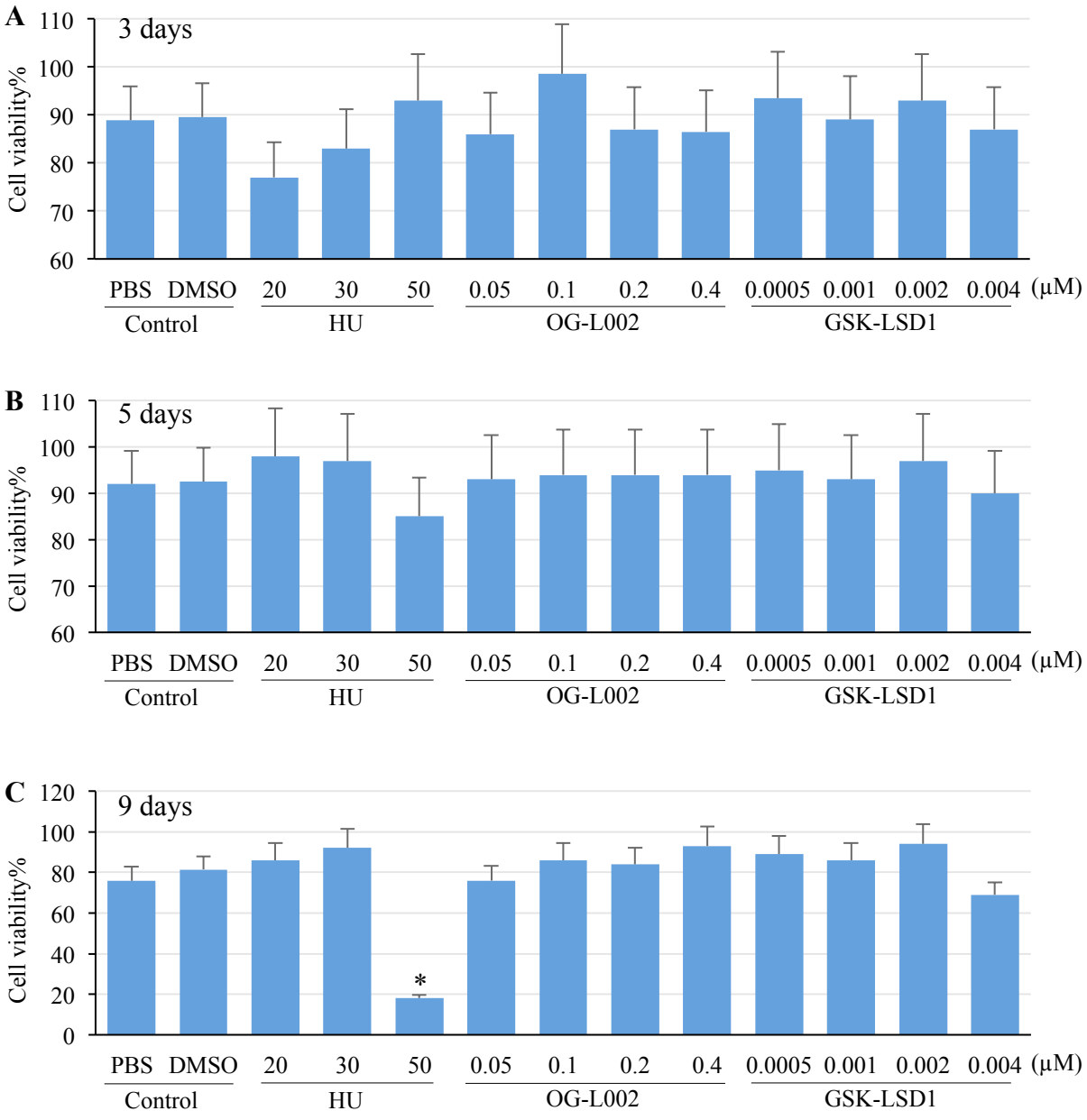

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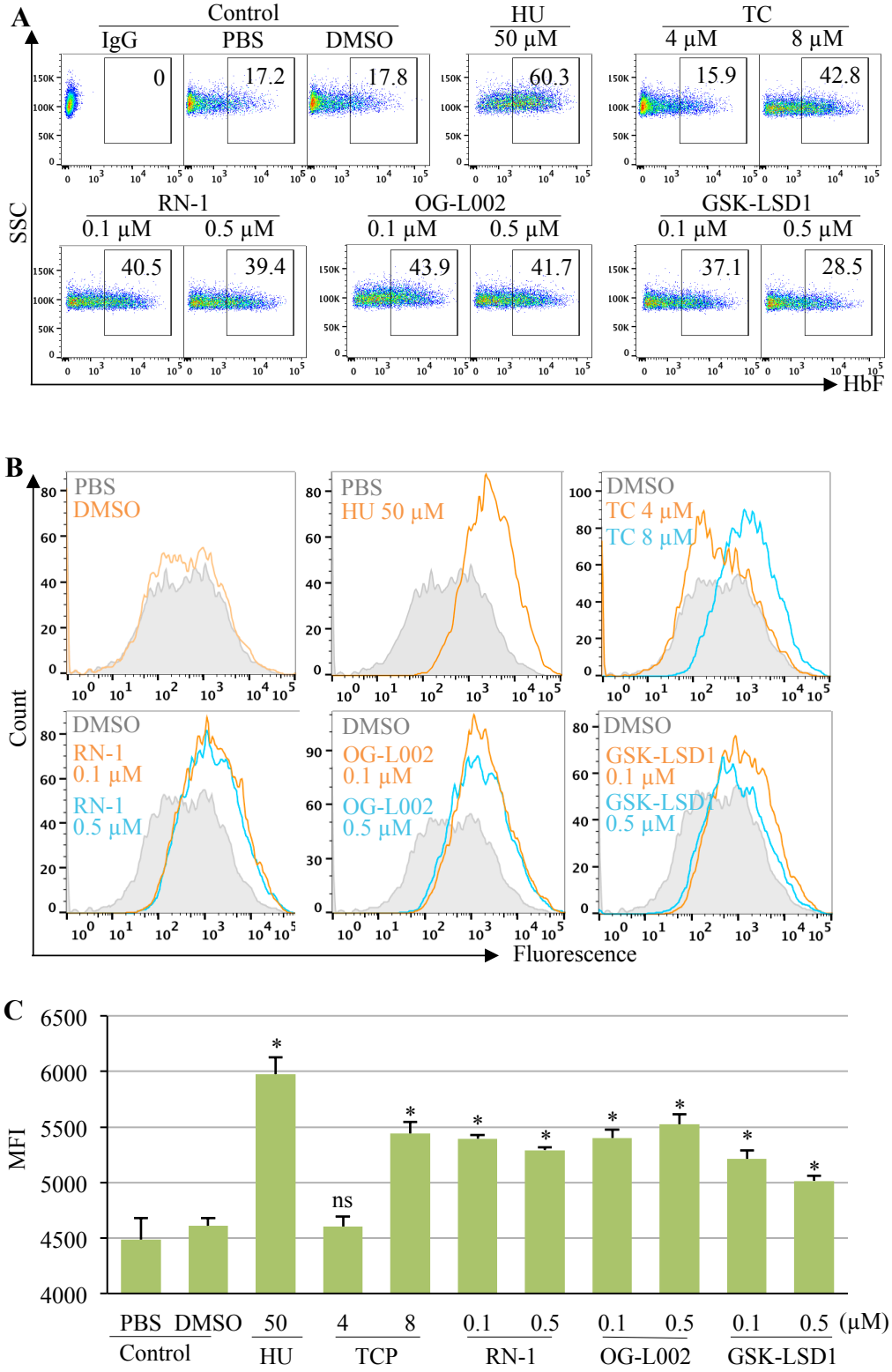
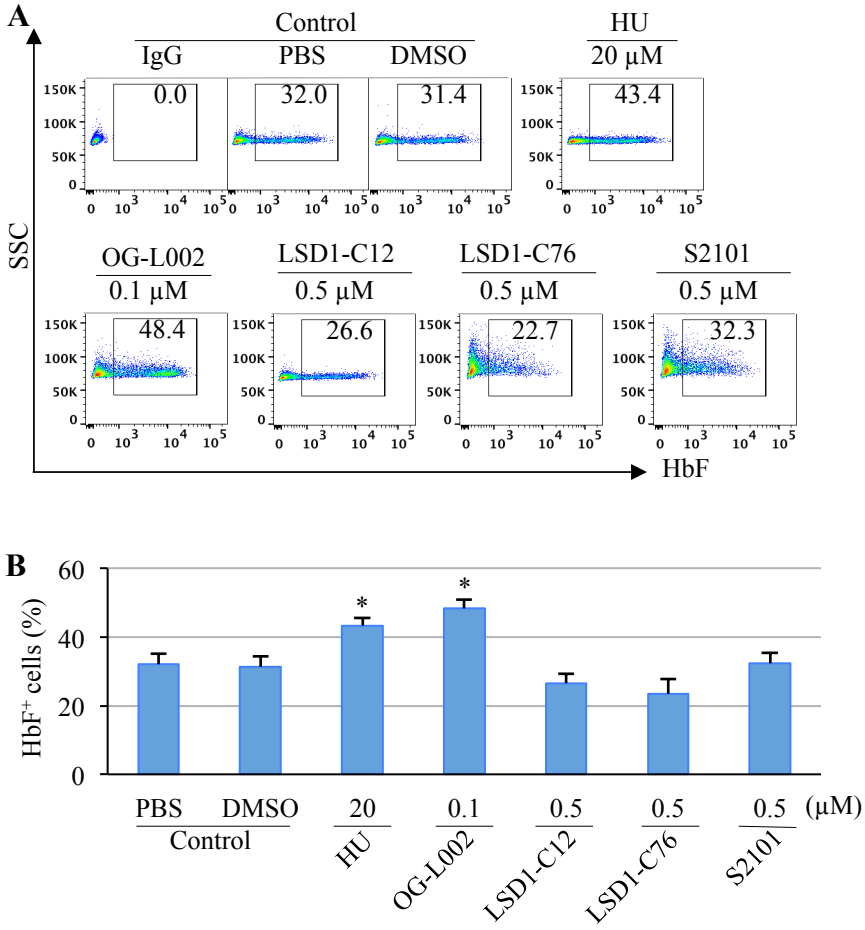
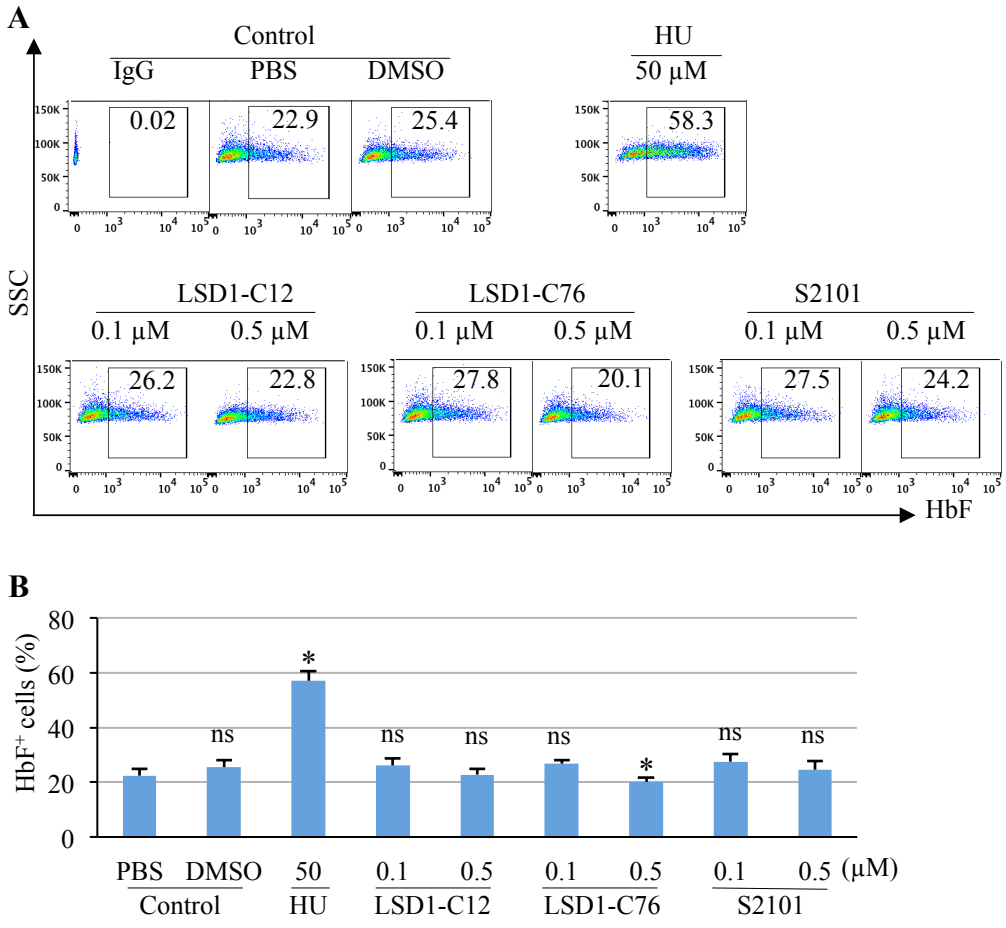


Supplemental Figure 5



Supplemental Figure 6



Supplemental Table 1. Kinetic parameters of LSD1 and MAO inhibitors

Inhibitors	LSD1		MAO-A		MAO-B	
	IC_{50} (μM)	K_i (μM)	IC_{50} (μM)	K_i (μM)	IC_{50} (μM)	K_i (μM)
TC	2	242.7	2.3	101.9	0.95	16
RN-1	0.07	-	0.51	-	2.79	-
OG-L002	0.02	-	1.38	-	0.72	-
GSK-LSD1	0.016	-	> 16	-	> 16	-
LSD1-C12	0.013	-	> 300	-	> 300	-
LSD1-C76	0.005	-	> 5	-	> 5	-
S2101	0.99	0.61	-	110	-	17

Supplemental Table 2. Hematologic parameters of SCD mice treated with LSD1 inhibitors

	DMSO	GSK-LSD1		OG-L002	
	Mean ± SD	Mean ± SD	*P-value*	Mean ± SD	*P-value*
RBC (x 10^6 cells/µl)	4.53 ± 0.54	6.70 ± 0.57	0.009	5.77 ± 0.31	0.025
HGB (g/dL)	2.67 ± 0.47	5.67 ± 1.25	0.017	4.33 ± 0.47	0.012
HCT (%)	25.00 ± 1.63	36.00 ± 4.32	0.014	27.67 ± 0.47	0.045
MCV (fL)	45.60 ± 1.27	53.23 ± 2.04	0.005	52.17 ± 1.16	0.003
MCH (pg)	6.40 ± 1.99	8.50 ± 0.57	0.113	7.50 ± 0.22	0.241
MCHC (g/dL)	12.80 ± 3.58	16.57 ± 0.70	0.109	15.60 ± 0.80	0.171
RDW (%)	32.33 ± 5.10	26.83 ± 2.21	0.117	27.43 ± 2.49	0.145
PLT (x 10^3 cells/µl)	1116.67 ± 181.90	640.00 ± 264.20	0.052	763.33 ± 98.43	0.037
WBC (x 10^3 cells/µl)	27.13 ± 4.24	26.27 ± 5.91	0.437	20.93 ± 2.03	0.068

HCT, hematocrit; HGB, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PLT, platelet; RBC, red blood cell; RDW, red blood cell distribution width; WBC, white blood cell. *P-value* (treated vs control).