Expanded View Figures

Figure EV1. Pervasive and dynamic alternative splicing occurs during human hematopoietic differentiation induced from hESCs.

A Principal component analysis (PCA) of genes (left) and transcripts (right) in highly purified cells at various differentiation stages, including ESCs, $APLNR^+$ cells, $CD31^+CD34^+$ cells, and $CD43^+$ cells during hematopoietic differentiation induction from hESCs.

B Widespread occurrence (> 85% of expressed genes) of alternative splicing in expressed genes (FPKM> 1 in at least one differentiation stage) at distinct differentiation stages during hematopoietic development.

C Average number of isoforms per gene at each differentiation stage.

D Analysis of isoform variants within each expressing gene at distinct differentiation stages.

E Number and frequency of five major splicing events at distinct differentiation stages, including mutually exclusive exon (MXE), alternative 5′ splicing (A5SS), alternative 3′ splicing (A3SS), intron retention (IR), and exon skipping (ES). The cutoff of splicing event of an expressed gene is $0.05 < PSI < 0.95$.

F Heatmap showing the genes within the minor spliceosomal machinery ($n = 52$) at the indicated stages. The heatmap was row normalized. The heatmap was scaled with Z-Score using the $\log_2$(FPKM) expression of indicated components within the minor spliceosomal machinery.

G Frequency of splicing site at different differentiation stages.

H The elbow plot assessing the potential clusters of the dynamic splicing factors expression in the heatmap of Fig 1 (C).

I Heatmap showing the genes in the hnRNP family at the indicated stages. The heatmap was row normalized. The heatmap was scaled with Z-Score using the $\log_2$(FPKM) expression of indicated genes.

Data information: Results given are mean ± SD.For all panels $n = 3$ technical replicates.

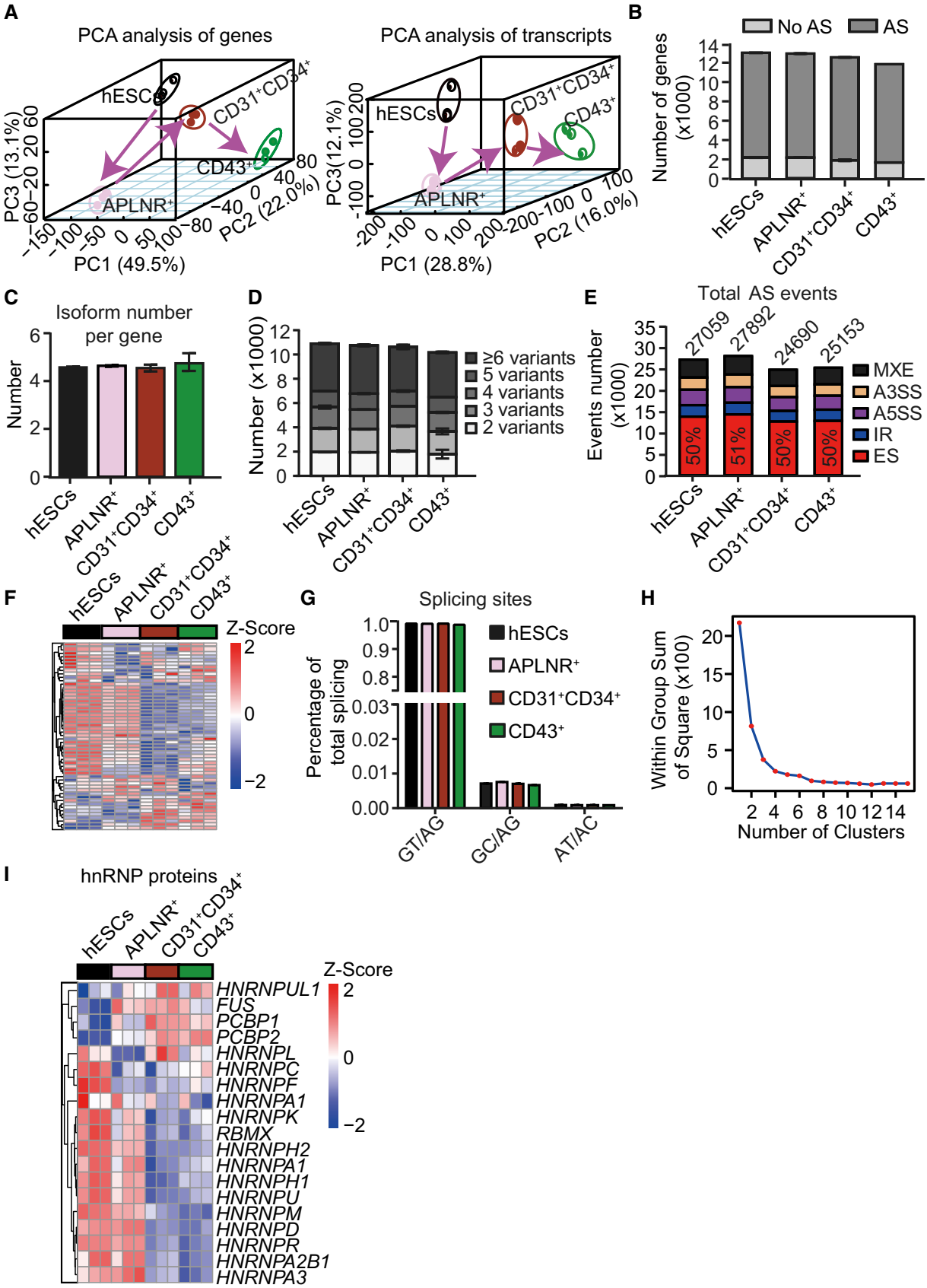

Figure EV1.

Figure EV2. Effects of long and short PLB treatment on hematopoietic differentiation.

A The morphological alterations of cells treated without (DMSO control) or with various amount of PLB throughout the hematopoietic differentiation. Scale bar = 20 μm.

B Representative FACS plots illustrating the $CD43^+$ HSPCs without or with PLB treatment at the indicated concentrations and treatment periods.

C Representative FACS plots showing the frequency of $APLNR^+$ cells on day 2 of differentiation and $CD31^+CD34^+$ EPCs on day 5 without or with PLB treatment from day 0 to 2 at the indicated concentrations.

D The bar graph showing the percentage of $APLNR^+$ cells and $CD31^+CD34^+$ EPCs of (C). Results given are mean ± SD. *P*-values were calculated by one-way ANOVA followed by Dunnett's test. ns represents no significant difference. All experiments were conducted for at least 3 biological replicates.

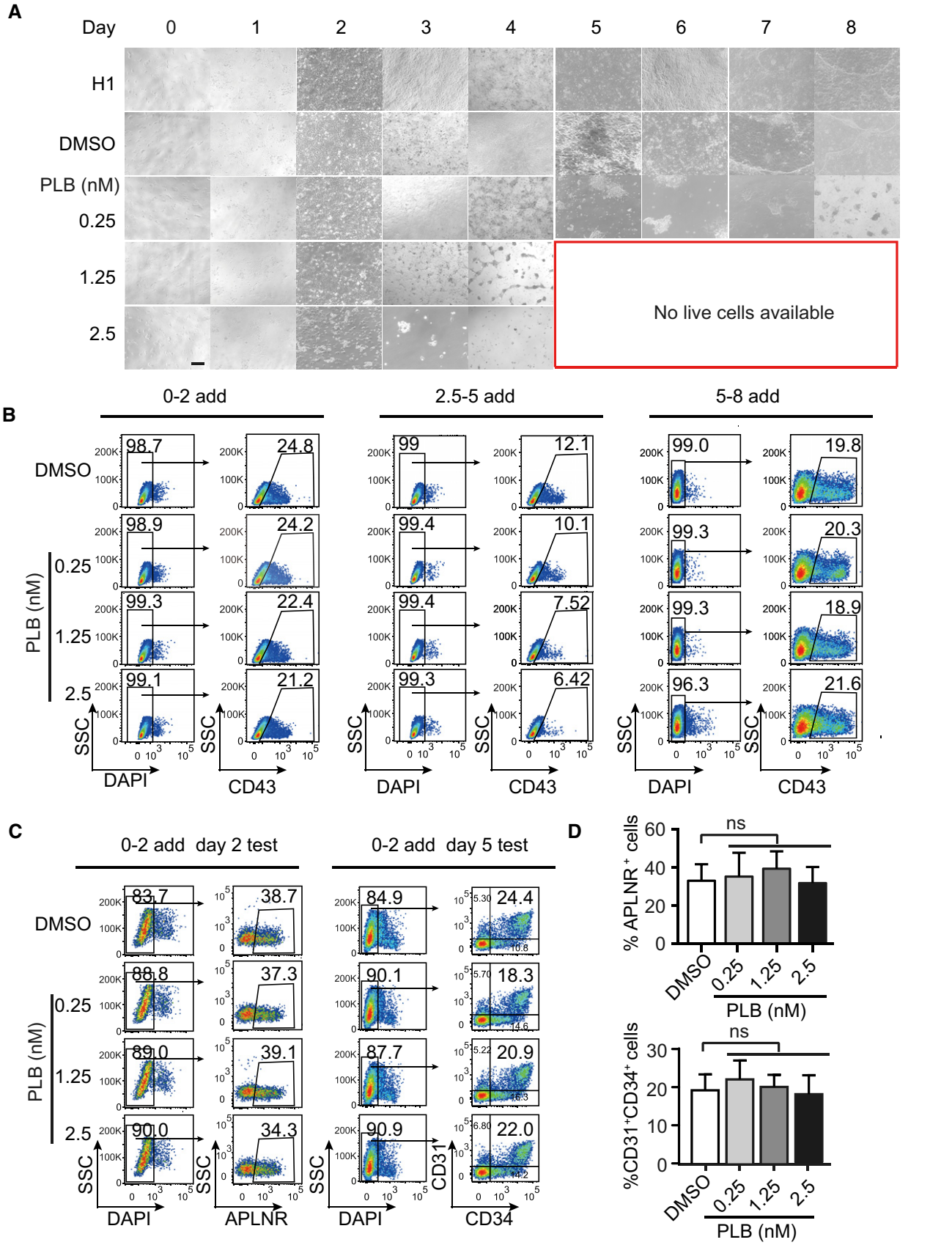
A
Day 0 1 2 3 4 5 6 7 8
H1
DMSO
PLB (nM)
0.25
1.25
2.5
No live cells available
B
0-2 add
2.5-5 add
5-8 add
DMSO
PLB (nM)
0.25
1.25
2.5
98.7
24.8
98.9
24.2
99.3
22.4
99.1
21.2
99
12.1
99.4
10.1
99.4
7.52
99.3
6.42
99.0
19.8
99.3
20.3
99.3
18.9
96.3
21.6
SSC
DAPI
CD43
C
0-2 add day 2 test
0-2 add day 5 test
DMSO
PLB (nM)
0.25
1.25
2.5
83.7
38.7
88.8
37.3
89.0
39.1
90.0
34.3
84.9
24.4
90.1
18.3
87.7
20.9
90.9
22.0
SSC
DAPI
APLNR
CD31
CD34
D
% APLNR+ cells
ns
DMSO
0.25
1.25
2.5
PLB (nM)
%CD31+CD34+ cells
ns
DMSO
0.25
1.25
2.5
PLB (nM)

Figure EV2.

Figure EV3. Short PLB treatment exhibits minor cytotoxic effects.

A Expression of *LAS1L* in day 2-differentiated APLNR$^+$ cells and day 5-differentiated CD31$^+$CD34$^+$ cells by RNA-Seq. The bottom panel showing the inclusion/exclusion of *LAS1L* exon 9 during hematopoietic differentiation by RT–PCR.

B The inclusion/exclusion of exon 9 of *ATP5F1C* and *HDAC7* detecting by RT–PCR during hematopoietic differentiation.

C The top panel is a representative RT–PCR electropherogram showing the inclusion or exclusion of exon 9 in *ATP5F1C* (left) and *HDAC7* (right) in cells at days 2 and 5 without or with PLB treatment at indicated concentrations, respectively. The quantification of PSI is presented in the bottom bar graph. *P*-values were calculated by one-way followed by Dunnett's test.

D The cellular morphology on days 3, 4, and 5 during hematopoietic differentiation after treatment with 1.25 or 2.5 nM PLB from day 2.5 to 5. Scale bar = 40 μm.

E Cellular apoptosis assessed using Annexin V and 7-AAD at days 3 and 4 of differentiation by flow cytometry, with or without PLB treatment from day 2.5 to 5.

F The proportion of G0/G1, S, and G2/M cells at days 3 and 4 of differentiation, with or without PLB treatment from day 2.5 to 5, respectively. The cell cycle was determined using propidium iodide staining by flow cytometry.

G Immunofluorescent staining depicting low-density lipoprotein (AcLDL) uptake from FACS-sorted CD31$^+$ cells without (DMSO) or with PLB (1.25 nM) treatment. CD144, LDL, and DAPI were stained (upper) by red, green, and blue, respectively. Scale bar = 40 μm.

H The bar graph showing the quantification of the LDL fluorescent intensity by the Volocity 3D image analysis software.

I The schematic illustrating the DOX-inducible knockdown system with expression of SF3B1 shRNAs. The knockdown of SF3B1 was confirmed by RT–qPCR (left) and Western blotting (right) after inducing with DOX.

J The representative FACS plots of CD31$^+$CD34$^+$ EPCs after SF3B1 depletion at day 5 of differentiation.

K The percentage of CD31$^+$CD34$^+$ EPCs after SF3B1 depletion at day 5 of differentiation.

Data information: Results given are mean ± SD. *P*-values were determined by Student's *t*-test in (A), (E), (F), (H), (I), and (K). ns represents no significant difference. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. All experiments were conducted for at least 3 biological replicates.

Source data are available online for this figure.

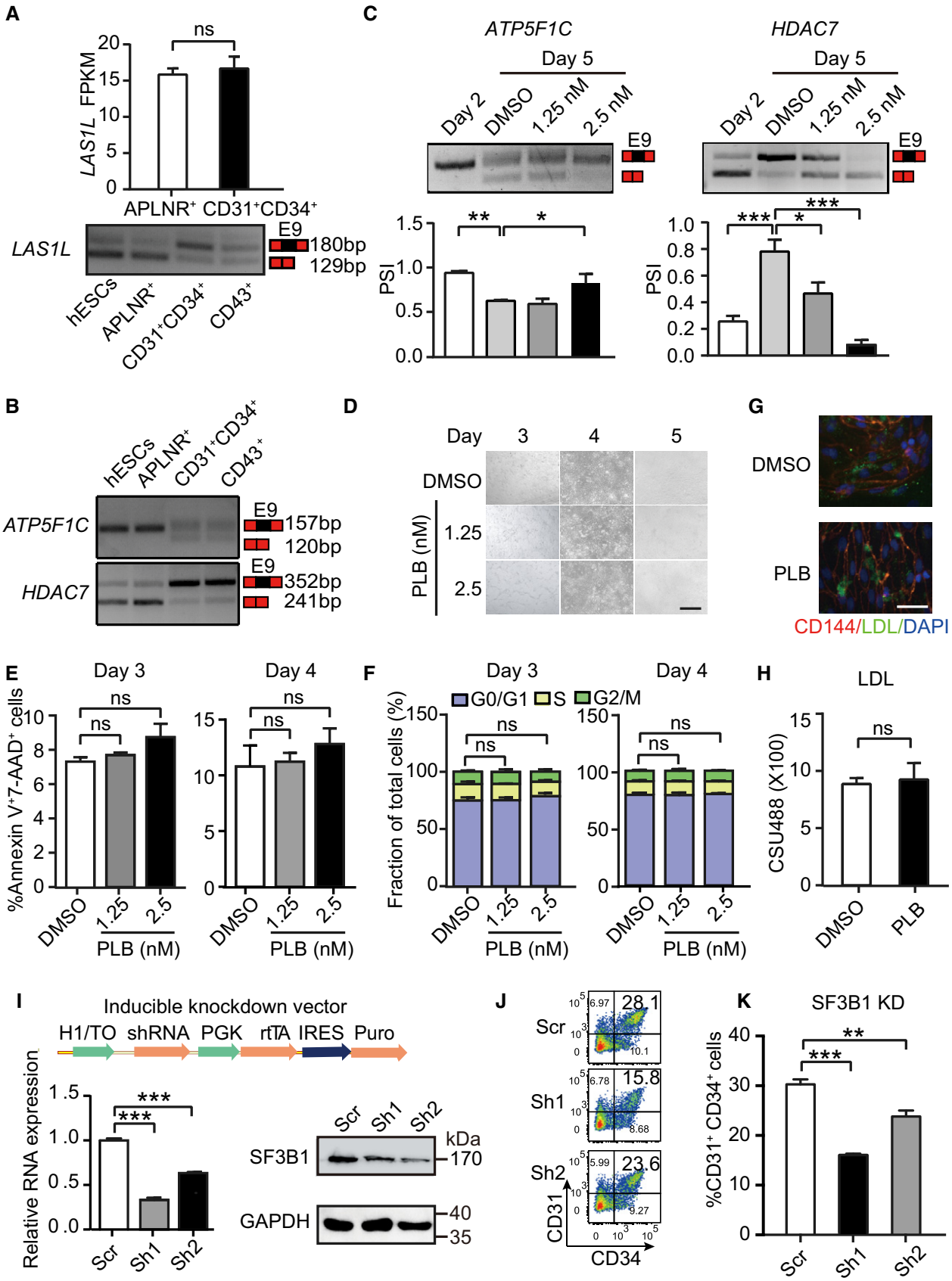
A
LAS1L FPKM
ns
APLNR+ CD31+CD34+
LAS1L
E9
180bp
129bp
hESCs
APLNR+
CD31+CD34+
CD43+
B
ATP5F1C
HDAC7
E9
157bp
120bp
352bp
241bp
C
ATP5F1C
HDAC7
Day 5
Day 2
DMSO
1.25 nM
2.5 nM
E9
PSI
D
Day 3 4 5
DMSO
PLB (nM)
1.25
2.5
G
DMSO
PLB
CD144/LDL/DAPI
E
Day 3
Day 4
%Annexin V+7-AAD+ cells
PLB (nM)
F
G0/G1 S G2/M
Fraction of total cells (%)
H
LDL
CSU488 (X100)
I
Inducible knockdown vector
H1/TO shRNA PGK rtTA IRES Puro
Relative RNA expression
SF3B1
GAPDH
kDa
170
40
35
J
Scr
Sh1
Sh2
CD31
CD34
K
SF3B1 KD
%CD31+ CD34+ cells

Figure EV3.

Figure EV4. Effects of ectopic expression of constitutive splicing factors on human hematopoietic differentiation.

A Volcano plot shows the differential splicing events regulated by PLB treated from day 2.5 to 5. The dotted line denotes FDR = 0.05.

B, C The scatter plot showing the correlation of splicing factors form Fig 3c (B) and all expressed genes (C) between day 2-APLNR$^+$ cells and day 5-PLB-treated cells. r refers to the correlation coefficient.

D The protein–protein interaction network of 38 intersected splicing factors (Fig 3D) (STRING: https://string-db.org/).

E The mRNA expression of *SF3A3*, *SNRPD1*, and *SNRPE* in day 2-APLNR$^+$ cells, day 5-DMSO–treated, and day 5-PLB-treated cells. $N = 2$ technical replicates.

F The upper panel illustrates the DOX-inducible overexpression system. Western blotting confirmed the SRSF2 overexpression upon DOX induction with anti-FLAG antibody. GAPDH was used as the loading control.

G RT–qPCR assay of the relative mRNA expression of *SF3A3*, *SNRPD1*, and *SNRPE* after overexpression upon DOX induction. The *ACTB* gene was used as a control. Data were normalized to the mRNA level of empty vector controls cells.

H Western blotting showing the protein expression of SF3A3, SNRPD1, and SNRPE after overexpression with anti-FLAG antibody. The GAPDH gene was used as a control.

I Representative FACS plots of CD31$^+$CD34$^+$ cells in SF3A3, SNRPD1, and SNRPE overexpressed (GFP$^+$) cells.

J Statistical analysis of the frequency of CD31$^+$CD34$^+$ in SF3A3, SNRPD1, and SNRPE overexpressed (GFP$^+$) cells.

Data information: *P*-values were calculated by one-way ANOVA followed by Dunnett's test. ns represents no significant difference. Results given are mean ± SD.. All experiments were conducted for at least 3 biological replicates unless stated otherwise
Source data are available online for this figure.

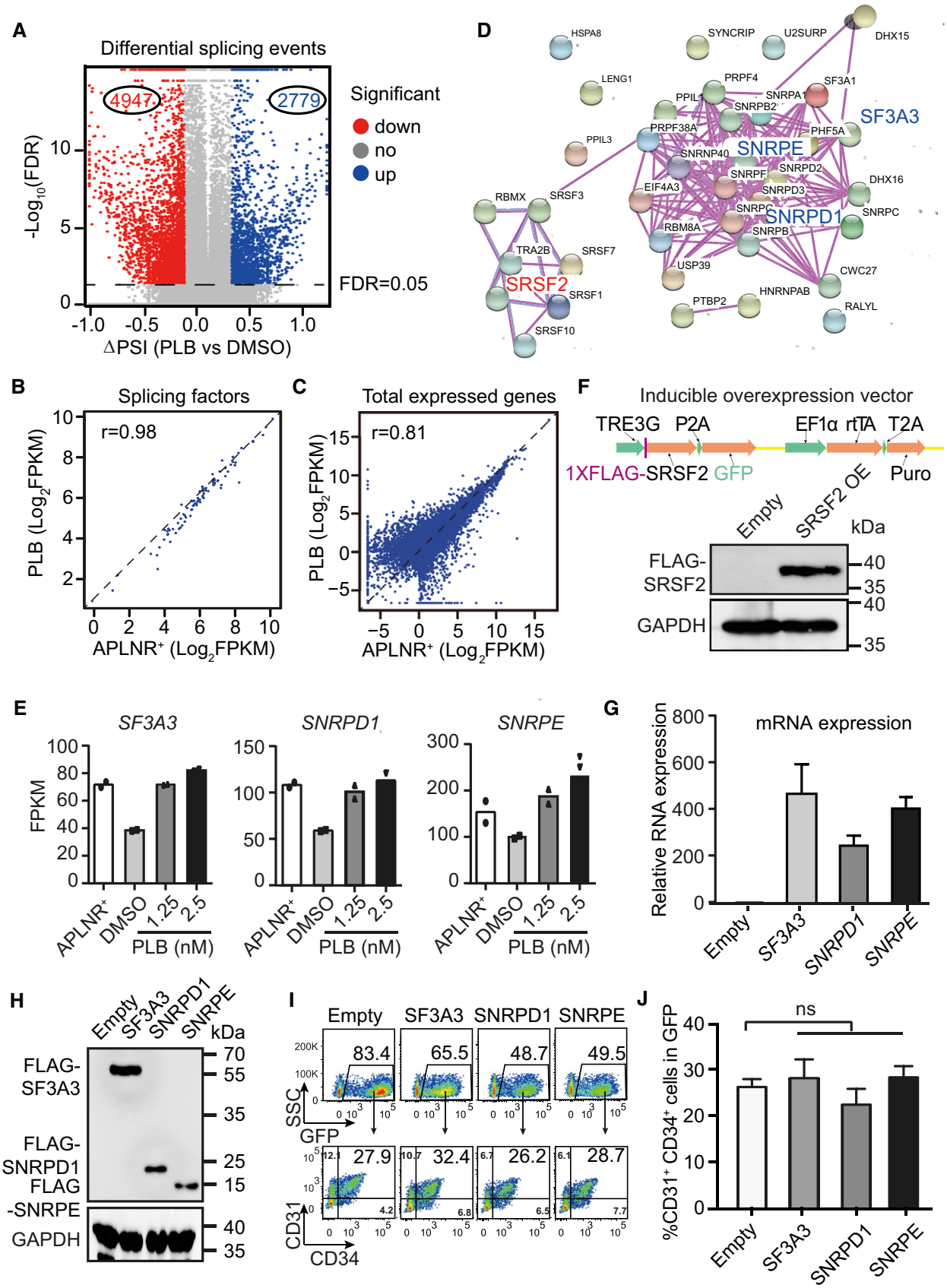
A
Differential splicing events
4947
2779
Significant
down
no
up
FDR=0.05
-Log10(FDR)
ΔPSI (PLB vs DMSO)
D
HSPA8
SYNCRIP
U2SURP
DHX15
LENG1
PRPF4
SF3A1
PPIL4
SNRPA1
SNRPB2
SF3A3
PRPF38A
PHF5A
PPIL3
SNRNP40
SNRPE
SNRPF
SNRPD2
DHX16
EIF4A3
SNRPD3
RBMX
SRSF3
SNRPC
SNRPD1
SNRPC
RBM8A
SNRPB
TRA2B
SRSF7
USP39
CWC27
SRSF2
SRSF1
HNRNPAB
PTBP2
RALYL
SRSF10
B
Splicing factors
r=0.98
PLB (Log2FPKM)
APLNR+ (Log2FPKM)
C
Total expressed genes
r=0.81
PLB (Log2FPKM)
APLNR+ (Log2FPKM)
F
Inducible overexpression vector
TRE3G P2A EF1α rtTA T2A
1XFLAG-SRSF2 GFP Puro
Empty
SRSF2 OE
kDa
FLAG-SRSF2
GAPDH
40
35
40
35
E
SF3A3
SNRPD1
SNRPE
FPKM
APLNR+
DMSO
1.25
2.5
PLB (nM)
G
mRNA expression
Relative RNA expression
Empty
SF3A3
SNRPD1
SNRPE
H
Empty
SF3A3
SNRPD1
SNRPE
kDa
FLAG-SF3A3
FLAG-SNRPD1
FLAG-SNRPE
GAPDH
70
55
35
25
15
40
35
I
Empty
SF3A3
SNRPD1
SNRPE
83.4
65.5
48.7
49.5
SSC
GFP
27.9
32.4
26.2
28.7
CD31
CD34
J
ns
%CD31+ CD34+ cells in GFP
Empty
SF3A3
SNRPD1
SNRPE

Figure EV4.

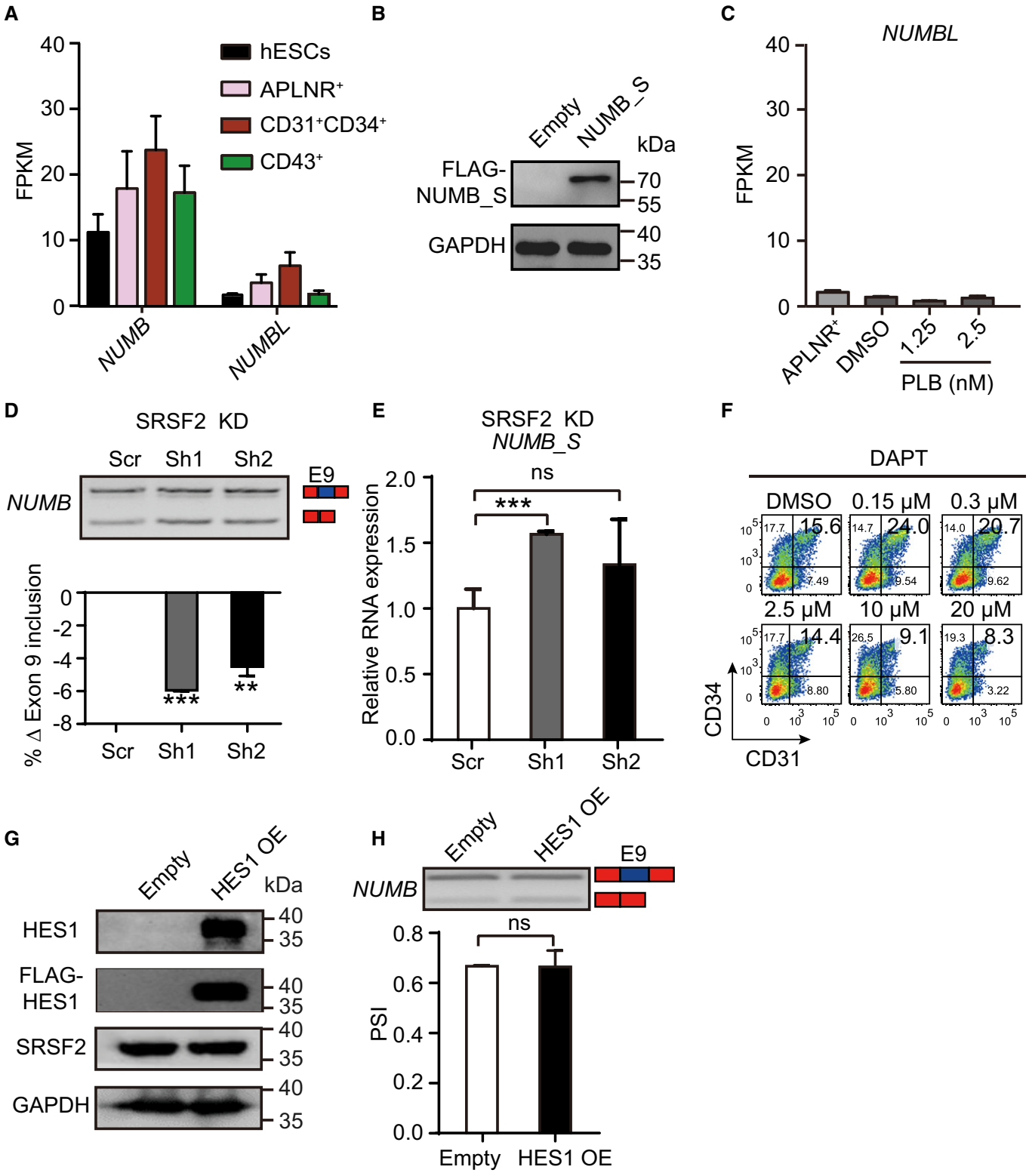

Figure EV5.

Figure EV5. NUMB Expression and its splicing regulation.

A Expression of *NUMB* and its family member *NUMBLIKE* (*NUMBL*) during human hematopoietic development by RNA-Seq.
B Western blotting showing NUMB-S overexpression upon DOX induction with anti-FLAG antibody. The GAPDH gene was used as a loading control.
C Expression of *NUMBL* in day 2-APLNR$^+$ cells, day 5-DMSO–treated, and day 5-PLB-treated cells.
D The upper panel is a representative RT–PCR electropherogram showing the inclusion or exclusion of *NUMB* exon 9 in SRSF2 depleted cells on day 5 of differentiation. The bar graph showing the changes of exon 9 inclusion obtained from the RT–PCR electropherogram.
E RT–qPCR measuring the expression of *NUMB-S* in SRSF2 depleted cells on day 5 of differentiation.
F The representative FACS plots of CD31$^+$CD34$^+$ cells at day 5 of differentiation after treatment with DMSO and NOTCH inhibitor DAPT at various concentrations from day 2.5 to 5.
G Western blotting showing the expression of HES1 (detected by endogenous HES1 antibody as well as anti-FLAG antibody) and SRSF2 in 293T cells. GADPH acts as a loading control.
H The splicing of *NUMB* exon 9 after HES1 overexpression. The top panel is a representative RT–PCR electropherogram showing the inclusion or exclusion of *NUMB* exon 9 without or with HES1 overexpression. The quantification is presented in the bottom bar graph.

Data information: Results given are mean ± SD. *P*-values were determined by unpaired two-tailed Student's *t*-test in (D), (E), and (H). ns represents no significant difference. $^{**}P < 0.01$, $^{***}P < 0.001$. All experiments were conducted for at least 3 biological replicates.
Source data are available online for this figure.