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Supplemental Information

Removal of innate immune barriers allows efficient transduction of quiescent human hematopoietic stem cells

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Supplemental Figures

Figure S1

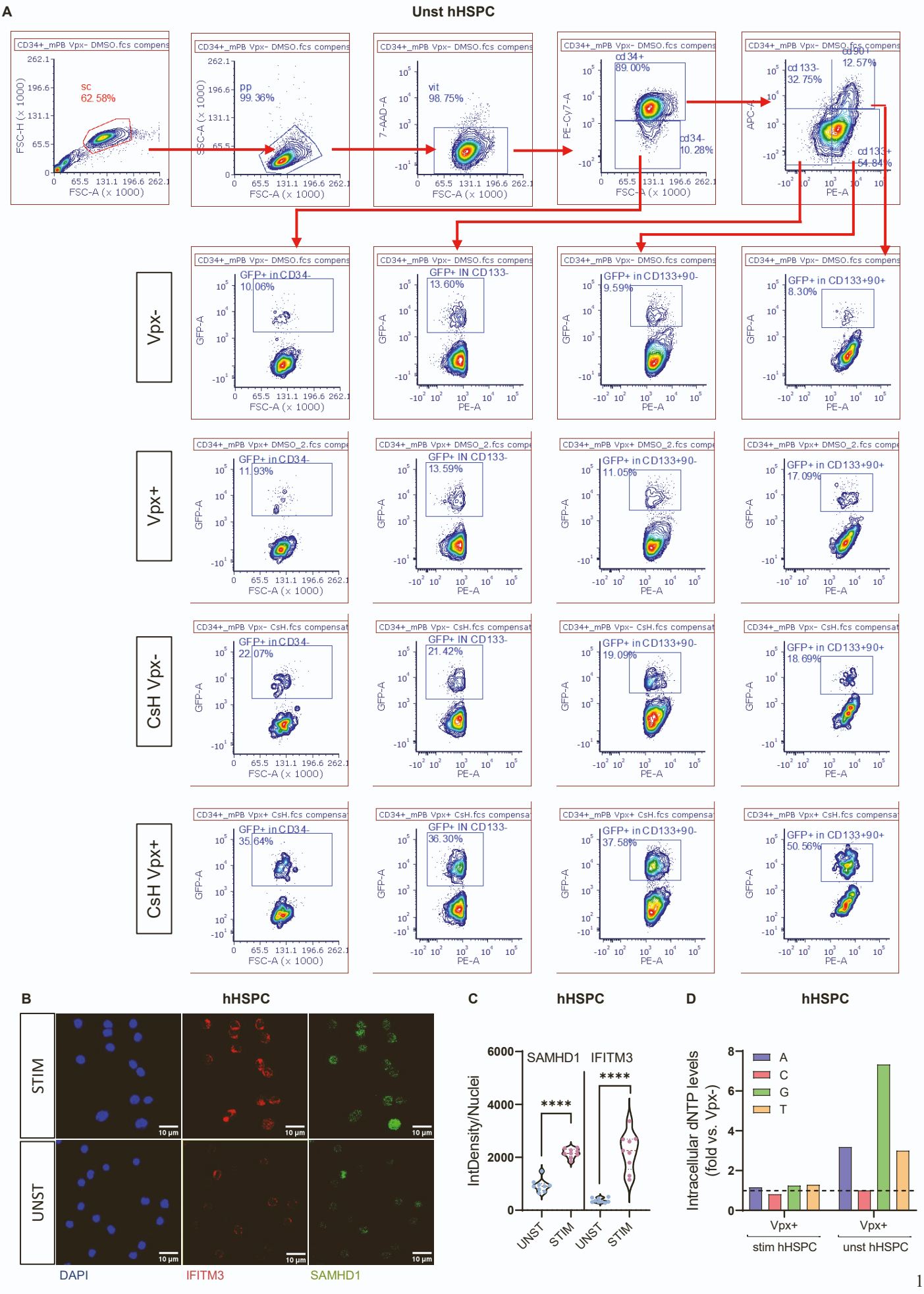

Figure S1. Impact of Vpx in human HSPC. (A) Flow cytometry data of one representative experiment from Figure 1C to show the gating strategy used to determine the GFP expression in the different hHSPC subpopulations. (B-C) Immunofluorescence (IF) of stimulated and unstimulated hHSPC from the same donors stained for IFITM3 and SAMHD1. IF images were acquired using TCS SP5 Leica confocal microscope, 40× with oil. Representative zoom images are shown, scale bar 10 µM (n=9 images acquired from three independent HSPC donors; Mann Whitney test, statistical significance is for ****P< 0.0001). (D) Intracellular dNTP levels were measured in stimulated and unstimulated hHSPC 24h post transduction with LV ± Vpx, in presence of CsH (n=1).

Figure S2

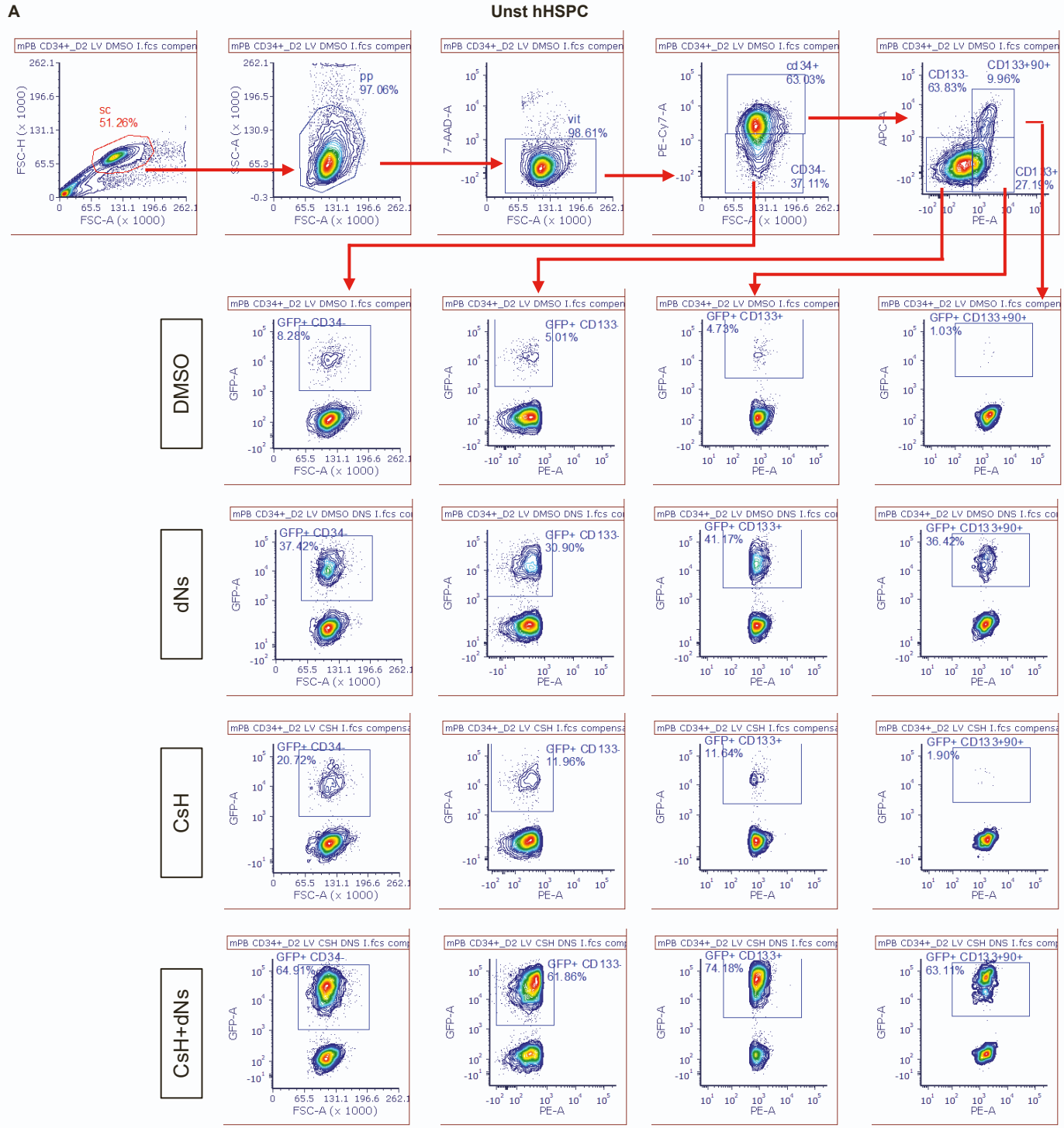
A
Unst hHSPC
mPB CD34+_D2 LV DMSO I.fcs compen
sc
51.26%
pp
97.06%
vit
98.61%
cd34+
63.03%
CD34-
37.11%
CD133-
63.83%
CD133+90+
9.96%
CD133+
27.19%
DMSO
GFP+ CD34-
8.28%
GFP+ CD133-
5.01%
GFP+ CD133+
4.73%
GFP+ CD133+90+
1.03%
dNs
mPB CD34+_D2 LV DMSO DNS I.fcs co
GFP+ CD34-
37.42%
GFP+ CD133-
30.90%
GFP+ CD133+
41.17%
GFP+ CD133+90+
36.42%
CsH
mPB CD34+_D2 LV CSH I.fcs compensa
GFP+ CD34-
20.72%
GFP+ CD133-
11.96%
GFP+ CD133+
11.64%
GFP+ CD133+90+
1.90%
CsH+dNs
mPB CD34+_D2 LV CSH DNS I.fcs comp
GFP+ CD34-
64.91%
GFP+ CD133-
61.86%
GFP+ CD133+
74.18%
GFP+ CD133+90+
63.11%
FSC-A (x 1000)
PE-A
GFP-A
B
Day 1
Day 2
Day 4
Unst hHSPC
unst mPB CD34_DMSO - DNS.fcs compensated
unst mPB CD34_DMSO.fcs compensated
DMSO -dNs
DMSO +dNs
DMSO LV -dNs
DMSO LV +dNs
Count
APC-A

Figure S2. Gating strategy in unstimulated hHSPC upon dNs delivery. (A) Flow cytometry data of one representative experiment from Figure 2A to show the gating strategy used to determine the GFP expression in the different hHSPC subpopulations. (B) Representative flow cytometry histograms from one experiment in Figure 4A showing cell proliferation dye dilution in unstimulated hHSPC at different time points after transduction and/or dNs delivery.

Figure S3

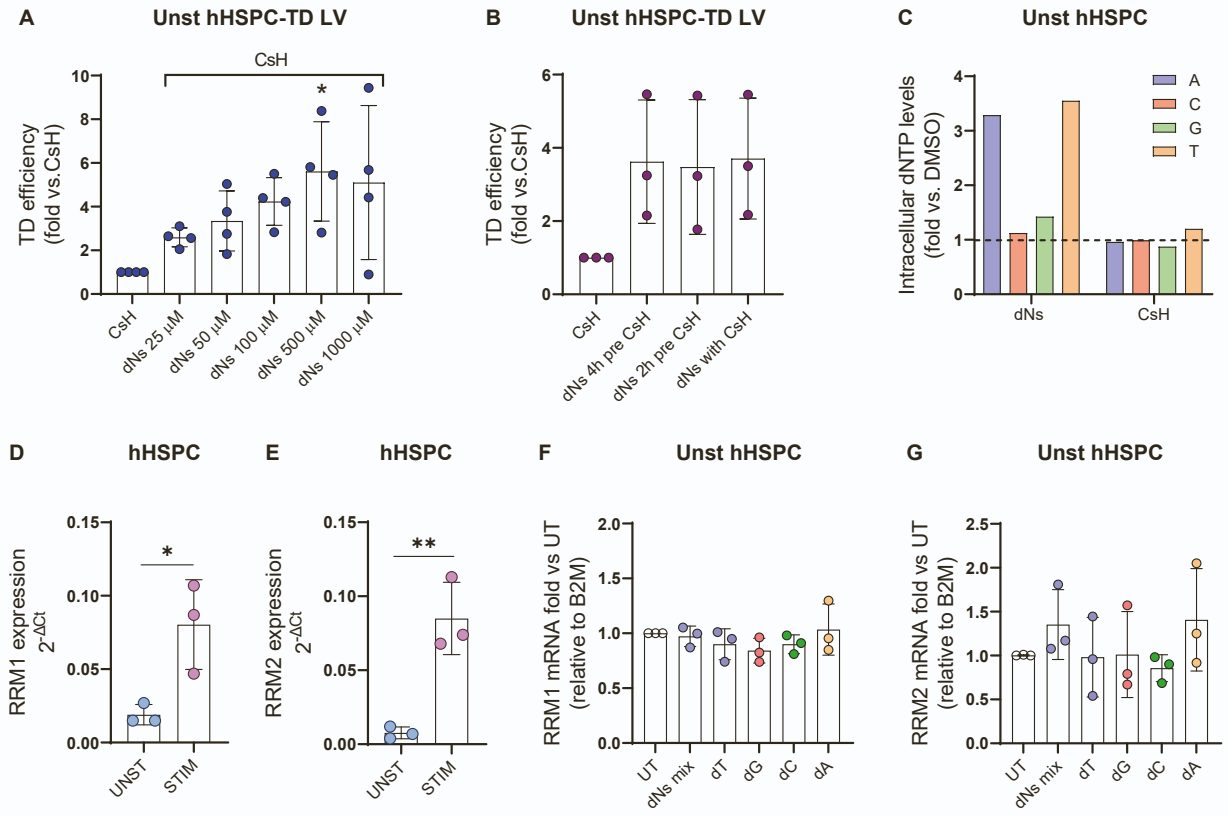

Figure S3. Impact of dNs in unstimulated hHSPC. (A) Unstimulated hHSPC were transduced in presence of CsH upon exposure to different concentrations of dNs. Percentages of transduced cells expressed as fold increase versus CsH (mean ± SD; n=4, Kruskal-Wallis test vs. CsH=1). (B) Unstimulated hHSPC were transduced in presence of dNs delivered at different time points prior to CsH/LV exposure. Percentages of transduced cells expressed as fold increase versus CsH (mean ± SD, n=3). (C) Intracellular dNTP levels were measured in unstimulated hHSPC 24h post transduction in presence of dNs alone or CsH alone (n=1). (D-E) Relative gene expression levels of RRM1 (D) and RRM2 (E) in unstimulated and stimulated hHSPC from the same donors (mean ± SD; n=3; unpaired t-test). (F-G) Gene expression levels of RRM1 (F) and RRM2 (G) in unstimulated hHSPC 16h upon delivery of single dNs or a mix of all dNs, expressed as fold vs UT control condition (mean ± SD; n=3).
Statistical significance is for *P<0.05 and **P<0.01.

Figure S4

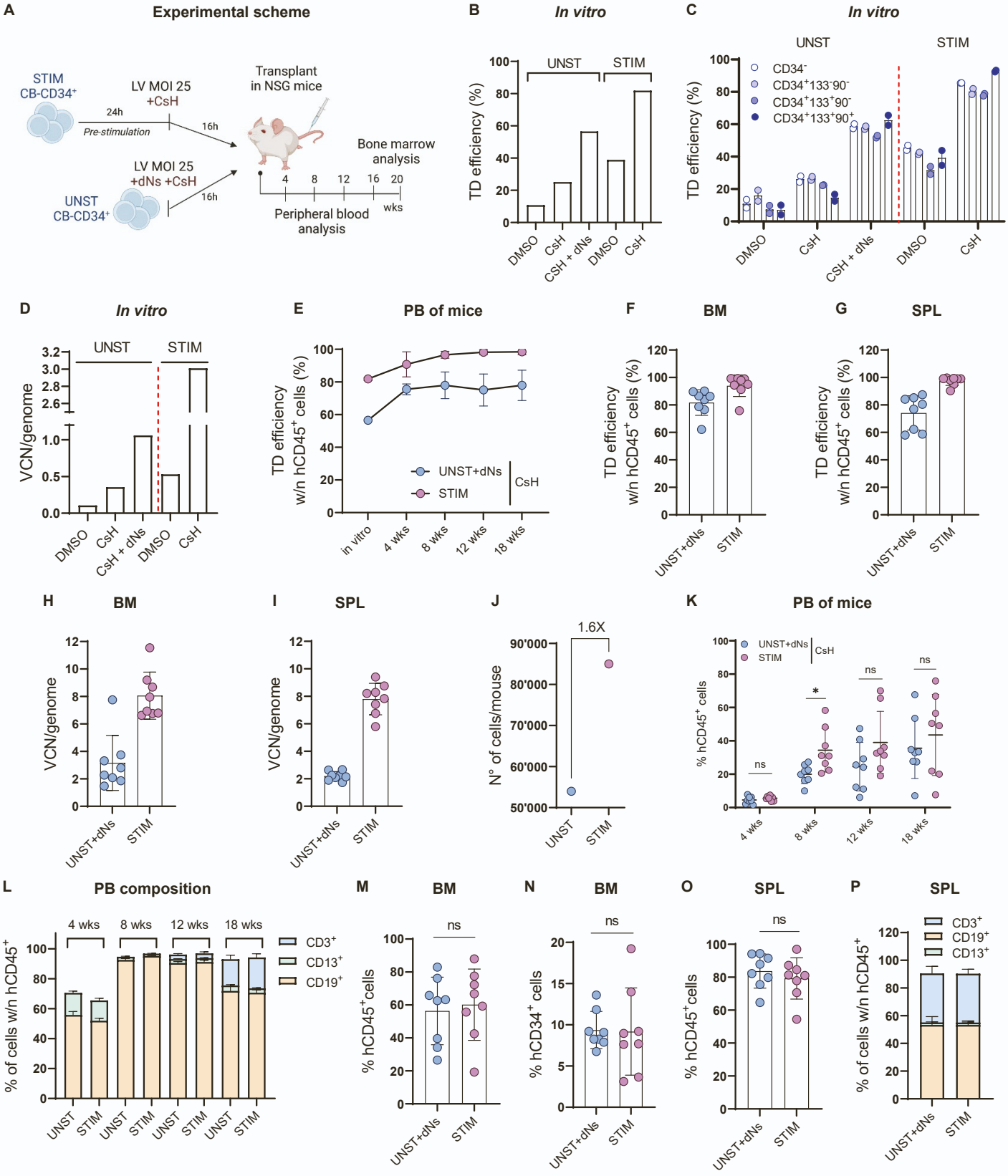
A
Experimental scheme
STIM CB-CD34+
LV MOI 25 +CsH
24h
Pre-stimulation
16h
Transplant in NSG mice
LV MOI 25 +dNs +CsH
UNST CB-CD34+
16h
Bone marrow analysis
4 8 12 16 20
wks
Peripheral blood analysis
B
In vitro
UNST STIM
TD efficiency (%)
DMSO CsH CsH + dNs DMSO CsH
C
In vitro
UNST STIM
CD34-
CD34+133-90-
CD34+133+90-
CD34+133+90+
TD efficiency (%)
DMSO CsH CsH + dNs DMSO CsH
D
In vitro
UNST STIM
VCN/genome
DMSO CsH CsH + dNs DMSO CsH
E
PB of mice
TD efficiency w/n hCD45+ cells (%)
UNST+dNs
STIM
CsH
in vitro 4 wks 8 wks 12 wks 18 wks
F
BM
TD efficiency w/n hCD45+ cells (%)
UNST+dNs STIM
G
SPL
TD efficiency w/n hCD45+ cells (%)
UNST+dNs STIM
H
BM
VCN/genome
UNST+dNs STIM
I
SPL
VCN/genome
UNST+dNs STIM
J
1.6X
N° of cells/mouse
UNST STIM
K
PB of mice
UNST+dNs
STIM
CsH
% hCD45+ cells
ns * ns ns
4 wks 8 wks 12 wks 18 wks
L
PB composition
4 wks 8 wks 12 wks 18 wks
CD3+
CD13+
CD19+
% of cells w/n hCD45+
UNST STIM UNST STIM UNST STIM UNST STIM
M
BM
ns
% hCD45+cells
UNST+dNs STIM
N
BM
ns
% hCD34+ cells
UNST+dNs STIM
O
SPL
ns
% hCD45+cells
UNST+dNs STIM
P
SPL
CD3+
CD19+
CD13+
% of cells w/n hCD45+
UNST+dNs STIM

Figure S4. Cord blood-derived unstimulated human HSPC transduced in presence of CsH and dNs showed high engraftment and stable gene marking levels *in vivo*. (A) Experimental scheme of the transplant experiment. Human $CD34^+$ cells from cord blood were pre-stimulated 24h with a cocktail of early active cytokines before transduction with a LV (MOI=25) in presence or not of 8 µM CsH or kept unstimulated and transduced immediately after thawing with a LV (MOI=25) in presence or not of CsH and 500 µM mix of all dNs. Cells from the stimulated + CsH and unstimulated + CsH + dNs conditions were then injected 16h post transduction by T_0 equivalent into NSG mice, while the other conditions were maintained only for the *in vitro* analysis (n=1 experiment; n=8 mice per group). (B-C) *In vitro* transduction efficiency was assessed 5 days post transduction in the bulk population of HSPC (B) and in the indicated subpopulations (C). (D) *In vitro* VCN/genome were measured 14 days post transduction. (E) Transduction efficiencies, measured as percentages of GFP^+ cells within the $hCD45^+$ cells, in the peripheral blood of mice from the two experimental groups (mean ± SD; n=8). (F-G) Transduction efficiencies, measured as % of GFP^+ cells within the $hCD45^+$cells, in bone marrow (F) and spleen (G) at 20 weeks post-transplant (mean ± SEM; n=8). (H-I) VCN/genome were measured in the bone marrow (H) and spleen (I) at 20 weeks post-transplant (mean ± SEM; n=8). (J) N° of cells injected into mice for each experimental group was counted immediately before transplantation. (K) Engraftment levels in the peripheral blood of mice from the two experimental groups was evaluated at the indicated time points (mean ± SD; n=8 mice per group). (L) The cellular composition of peripheral blood of the mice was analyzed at the indicated time points. (M) Engraftment levels in the bone marrow at 20 weeks post-transplant (mean ± SD; n=8, ''ns'' represents non statistical significance). (N) Percentages of $hCD34^+$ cells within $hCD45^+$ in the bone marrow at 20 weeks (mean ± SD; n=8, ''ns'' represents non statistical significance). (O) Engraftment levels in the spleen at 20 weeks post-transplant (mean ± SD; n=8, ''ns'' represents non statistical significance). (P) Cellular composition of the spleen was evaluated at 20 weeks post-transplant (mean ± SD; n=8).

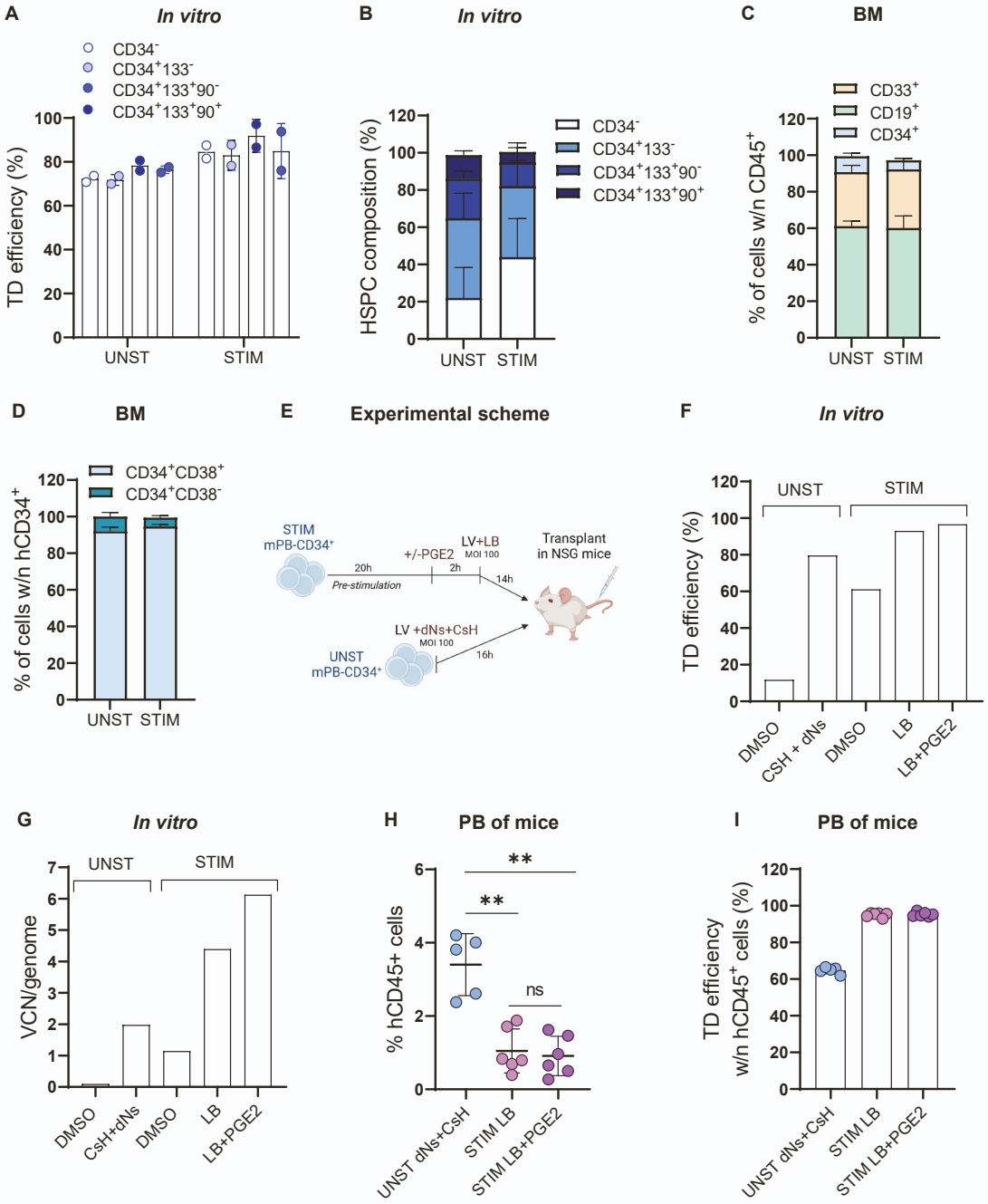

Figure S5. CsH+dNs-transduced unstimulated HSPC show superior long-term engraftment. Related to figure 5. (A) *In vitro* transduction efficiency was assessed 5 days post transduction in the indicated HSPC subpopulations (mean ± SD, n=2). (B) The composition of *in vitro* cultured HSPC was evaluated 5 days post transduction (mean ± SD, n=2). (C) Cell composition of the bone marrow at 13 weeks (mean ± SEM; n=13-16). (D) Cell composition of the hCD34$^+$ fraction in the bone marrow (mean ± SEM; n=6-7 from one experiment). (E) Experimental scheme of the transplant experiment. Human CD34$^+$ cells from mobilized peripheral blood were pre-stimulated 20 hours with a cocktail of cytokines. PGE2 was then added to one group of cells. After 2 hours from PGE2 exposure all cells were transduced with a LV (MOI=100) in presence of LentiBOOST (LB). After 14 hours 500'000 cells were injected into NSG mice. In parallel, cells were kept unstimulated and transduced immediately after thawing with a LV (MOI=100) in presence of CsH and 500 µM mix of all dNs and 500'000 cells were injected 16h post transduction into NSG mice (n=1 experiment; n=5-6 mice per group). (F) *In vitro* transduction efficiency was assessed 5 days post transduction in the bulk population of HSPC. (G) *In vitro* VCN/genome were measured 14 days post transduction. (H) Engraftment levels in the peripheral blood of mice from the two experimental groups was evaluated at 4 weeks after transplant (mean ± SD; n=5-6 mice per group; Mann Whitney test, statistical significance is for **P<0.01). (I) Transduction efficiencies, measured as percentages of GFP$^+$ cells within the hCD45$^+$ cells, in the peripheral blood of mice from the two experimental groups (mean ± SD; n=5-6 mice per group).