Stem Cell Reports, Volume *8*

Supplemental Information

Efficient Ex Vivo Engineering and Expansion of Highly Purified Human Hematopoietic Stem and Progenitor Cell Populations for Gene Therapy

Erika Zonari, Giacomo Desantis, Carolina Petrillo, Francesco E. Boccalatte, Maria Rosa Lidonnici, Anna Kajaste-Rudnitski, Alessandro Aiuti, Giuliana Ferrari, Luigi Naldini, and Bernhard Gentner

Figure S1

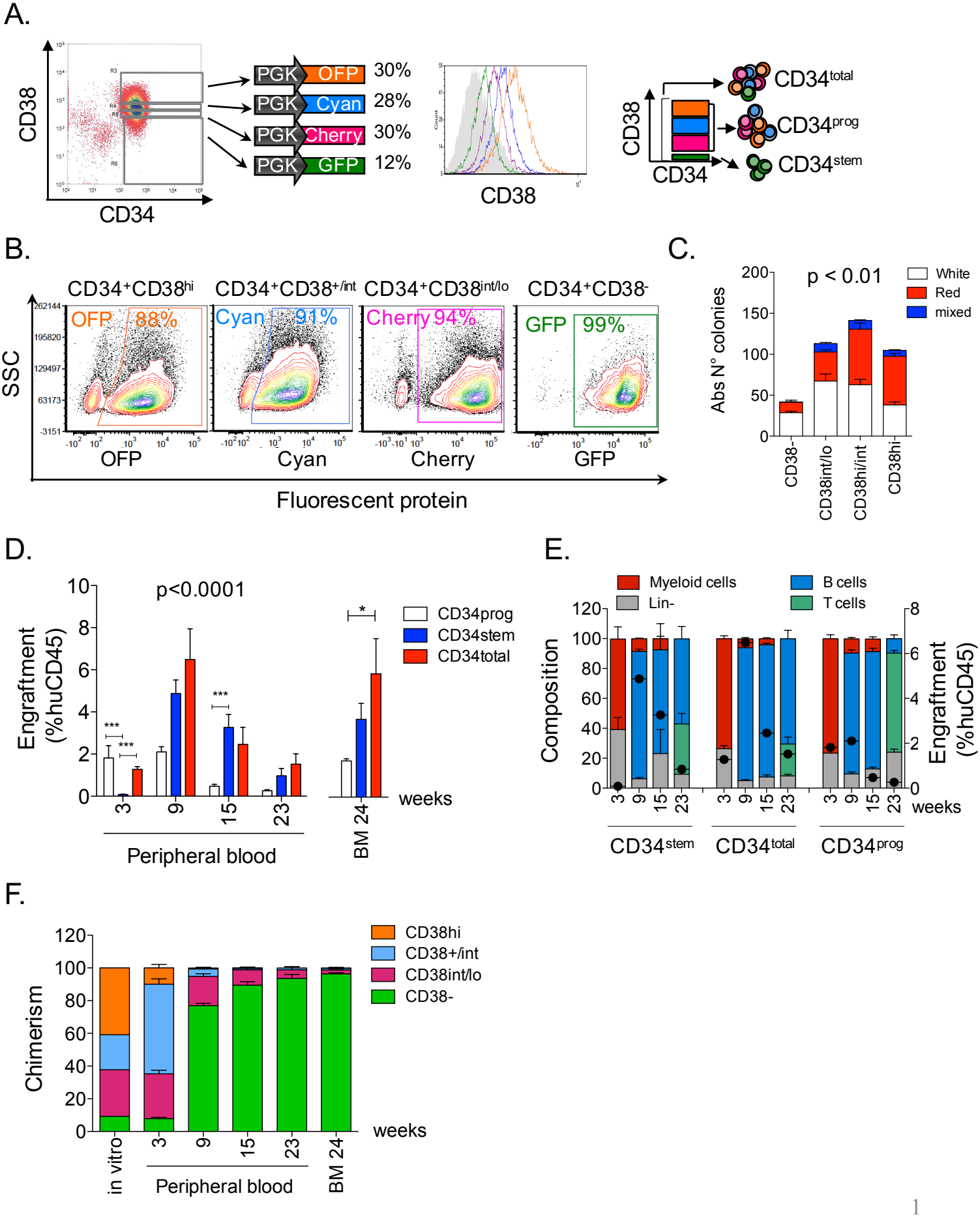

A.
CD38
CD34
PGK OFP 30%
PGK Cyan 28%
PGK Cherry 30%
PGK GFP 12%
CD38
CD34total
CD34prog
CD34stem
CD34
B.
CD34+CD38hi
CD34+CD38+/int
CD34+CD38int/lo
CD34+CD38-
OFP 88%
Cyan 91%
Cherry 94%
GFP 99%
SSC
OFP
Cyan
Cherry
GFP
Fluorescent protein
C.
p < 0.01
White
Red
mixed
Abs N° colonies
CD38-
CD38int/lo
CD38hi/int
CD38hi
D.
p<0.0001
Engraftment (%huCD45)
CD34prog
CD34stem
CD34total
weeks
Peripheral blood
BM 24
E.
Myeloid cells
Lin-
B cells
T cells
Composition
Engraftment (%huCD45)
weeks
CD34stem
CD34total
CD34prog
F.
Chimerism
CD38hi
CD38+/int
CD38int/lo
CD38-
weeks
in vitro
Peripheral blood
BM 24

Figure S1. In vivo tracking of hematopoietic reconstitution by $CD34^+$ mPB subpopulations. Replicate experiment, related to Figure 1.

A. Representative sorting gates applied to mPB $CD34^+$ cells. Four subpopulations differing in CD38 expression levels were randomly assigned to transduction with LVs expressing 1 of 4 fluorescent proteins (PGK: phosphoglycerate kinase promoter; OFP: orange fluorescent protein; Cyan: cyan/blue fluorescent protein; Cherry: red fluorescent protein; GFP: enhanced green fluorescent protein). Subpopulation frequency within total $CD34^+$ cells and CD38 expression upon post-sorting re-analysis are shown (histogram plot). The scheme on the right shows the subpopulations transplanted into sub-lethally irradiated NSG mice, together with the nomenclature applied to the 3 groups. When different subpopulations were mixed, their relative frequency during sorting was maintained.

B. Representative transduction efficiency of the different subpopulations ($CD34^+CD38^{hi}$, $CD34^+CD38^{+/int}$, $CD34^+CD38^{int/lo}$, $CD34^+CD38^-$), as measured in the myeloid colonies after 14 days of culture.

C. In vitro clonogenic potential assessed by the CFC assay performed on the transduced cells (1000 cells/plate) – the total number (mean ± SEM) of different types of colonies (white, red and mixed) was counted for the different subpopulations (n=4 dishes). Data were analyzed by One-way analysis of variance (ANOVA) for red, white and mixed colonies. Bonferroni's Multiple Comparison Test (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) for white colonies: $CD38^-$ vs $CD38^{int/lo}$ **, $CD38^-$ vs $CD38^{int/hi}$ **; for red colonies: $CD38^-$ vs $CD38^{int/lo}$ *, $CD38^-$ vs $CD38^{int/hi}$ ***, $CD38^-$ vs $CD38^{hi}$ ***; for mixed colonies: $CD38^-$ vs $CD38^{int/lo}$ ***, $CD38^-$ vs $CD38^{int/hi}$ ***, $CD38^-$ vs $CD38^{hi}$ **.

D. Human $CD45^+$ cell engraftment (mean ± SEM) in peripheral blood and bone marrow of NSG mice at the indicated time point (weeks post-transplant). $CD34^{total}$: n=6 mice; $CD34^{prog.}$: n=7 mice; $CD34^{stem}$: n=6 mice. Data were analyzed by Two-way analysis of variance (ANOVA) after log transformation. Significance values from Bonferroni's Multiple Comparison Test with respect to the $CD34^{stem}$ group are shown in the graph (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

E. Lineage composition (mean ± SEM; left axis) of the human $CD45^+$ cell graft (black dot; right axis) in the PB at the indicated n. of weeks post-transplantation of the different groups of mice. B cells, $CD19^+$; Myeloid cells, $CD13^+$, T cells, $CD3^+$; negative, lineage negative ($CD19^-$, $CD13^-$, $CD3^-$) cells.

F. Distribution of the 4 fluorescent proteins in the $CD34^{total}$ group before injection (in vitro) or within human $CD45^+$ cells sampled from the PB and BM at the indicated n. of weeks post-transplantation allows backtracking the origin of hematopoietic reconstitution to the originally transplanted cell subpopulations indicated in the legend.

Figure S2

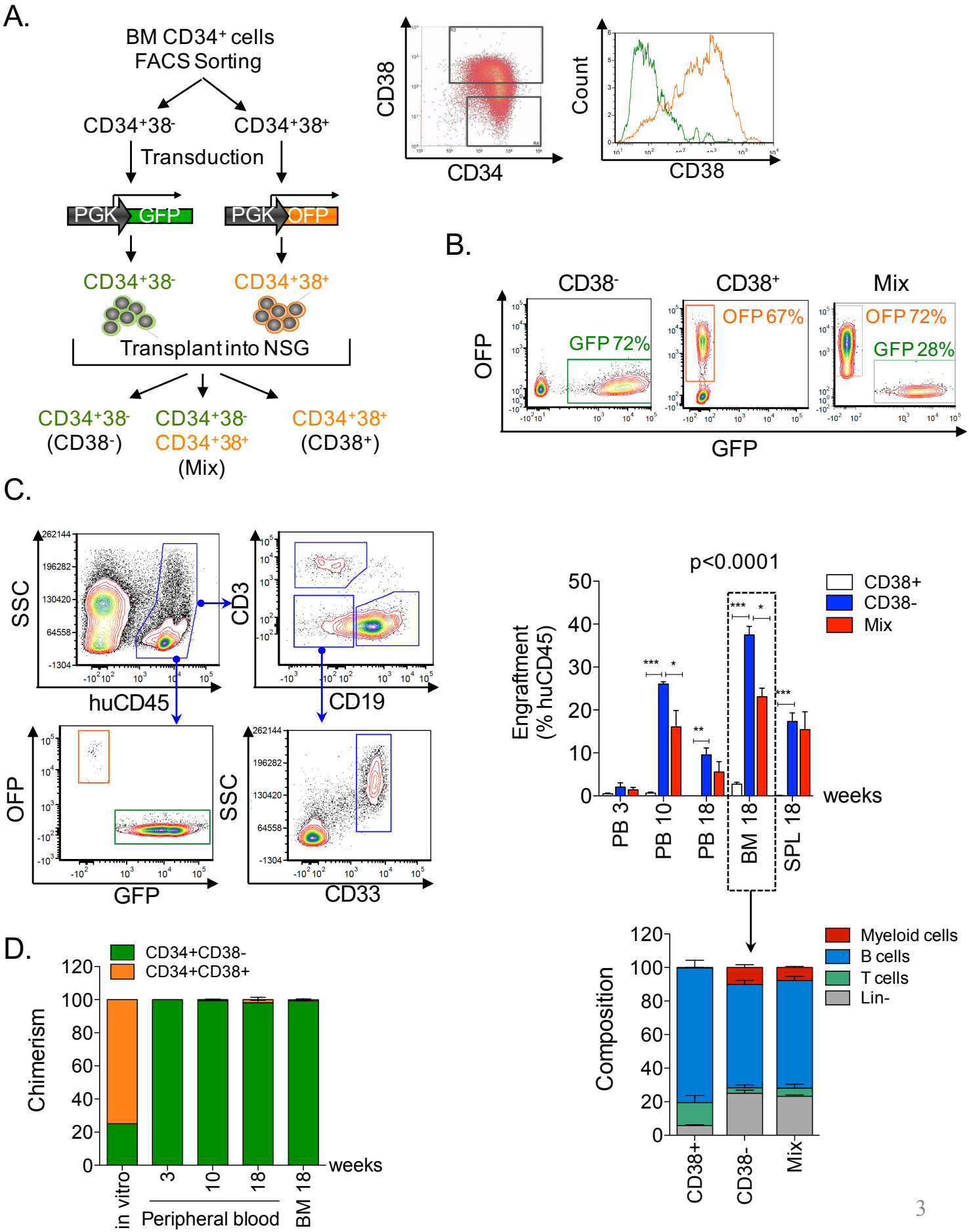

A.
BM CD34+ cells
FACS Sorting
CD34+38-
CD34+38+
Transduction
PGK GFP
PGK OFP
CD34+38-
CD34+38+
Transplant into NSG
CD34+38-
(CD38-)
CD34+38-
CD34+38+
(Mix)
CD34+38+
(CD38+)
CD38
CD34
Count
CD38
B.
CD38-
CD38+
Mix
OFP
GFP 72%
OFP 67%
OFP 72%
GFP 28%
GFP
C.
SSC
huCD45
CD3
CD19
OFP
GFP
SSC
CD33
p<0.0001
CD38+
CD38-
Mix
Engraftment
(% huCD45)
PB 3
PB 10
PB 18
BM 18
SPL 18
weeks
Composition
Myeloid cells
B cells
T cells
Lin-
CD38+
CD38-
Mix
D.
CD34+CD38-
CD34+CD38+
Chimerism
in vitro
3
10
18
BM 18
Peripheral blood
weeks

Figure S2. In vivo tracking of hematopoietic reconstitution by $CD34^+$ BM subpopulations. Related to Figure 1.

A. Experimental design and representative sorting gates applied to BM $CD34^+$ cells. $CD34^+CD38^+$ and $CD34^+CD38^-$ cells were transduced respectively with LV expressing OFP or GFP under the control of a PGK promoter. CD38 expression within transduced cells upon sorting is shown. The scheme on the left shown the subpopulations transplanted into sub-lethally irradiated NSG mice, together with the nomenclature applied to the 3 groups ($CD38^-$, n=3; $CD38^+$, n=3, Mix, n=3). When different subpopulations were mixed, their relative frequency during sorting was maintained.

B. Representative transduction efficiency of the different subpopulations ($CD38^-$, $CD38^+$ and Mix), as measured in the myeloid progeny after 2 days of culture.

C. Left panel: representative cytofluorimetric FACS plot to identify human $CD45^+$ cells in PB of NSG transplanted mice and the different lineages populations within $CD45^+$. Right panel: human $CD45^+$ cell engraftment (mean ± SEM) in PB, BM and spleen (SPL) of NSG mice at the indicated time point (weeks post-transplant). Lineage composition (mean ± SEM) of the human $CD45^+$ cell graft in the BM 18 weeks post-transplantation. B cells, $CD19^+$; Myeloid cells, $CD13^+$, T cells, $CD3^+$; negative, lineage negative ($CD19^-$, $CD13^-$, $CD3^-$) cells. Data were analyzed by Two-way analysis of variance (ANOVA) after log transformation. Significance values from Bonferroni's Multiple Comparison Test with respect to the $CD34^{stem}$ group are shown in the graph (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

D. Distribution of the 2 fluorescent proteins in the Mix group before injection (in vitro) or within human $CD45^+$ cells sampled from the PB and BM at the indicated n. of weeks post-transplantation allows back-tracing the origin of hematopoietic reconstitution to the originally transplanted cell subpopulations indicated in the legend.

Figure S3

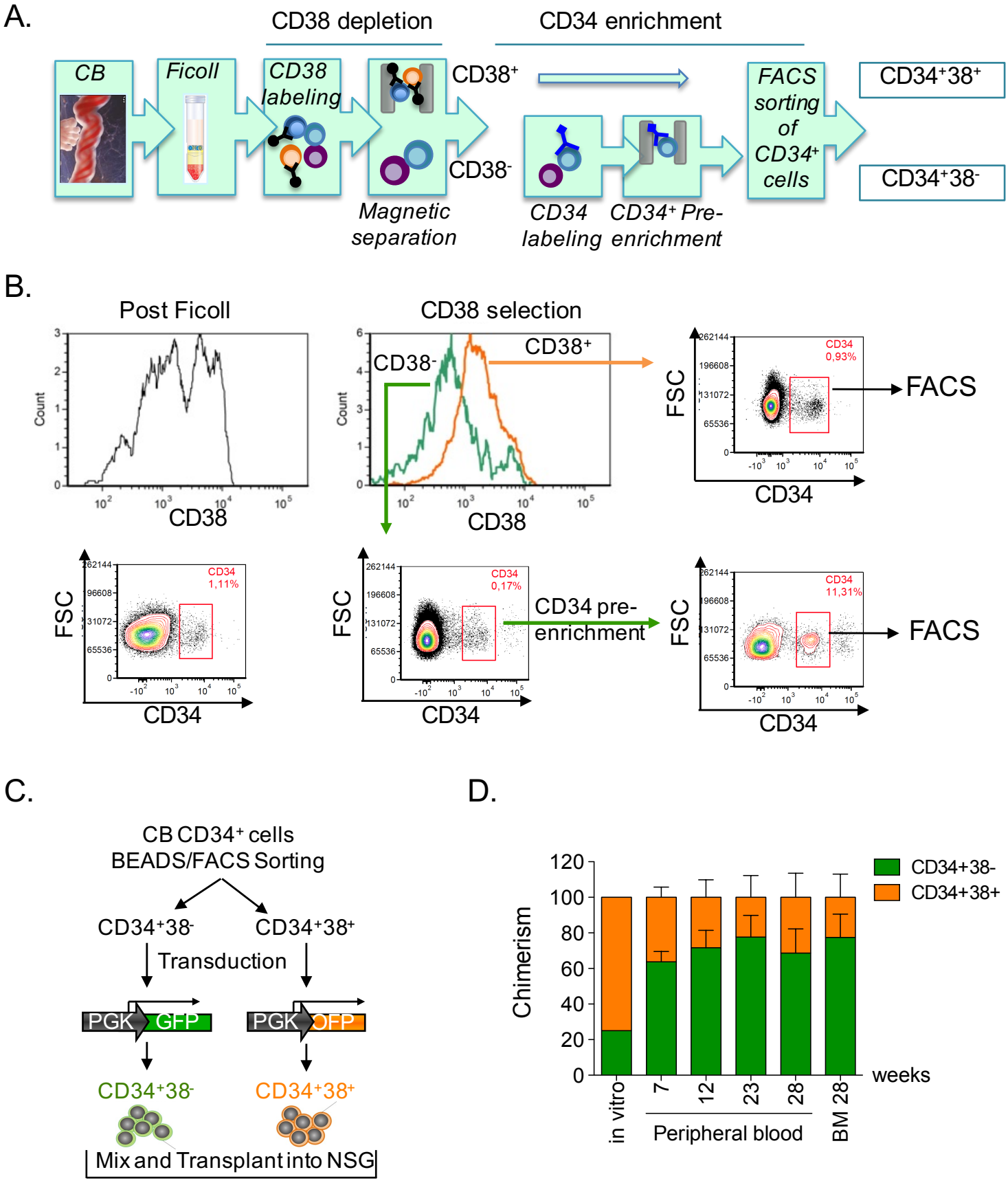

A.
CD38 depletion
CD34 enrichment
CB
Ficoll
CD38 labeling
CD38+
CD38-
Magnetic separation
CD34 labeling
CD34+ Pre-enrichment
FACS sorting of CD34+ cells
CD34+38+
CD34+38-
B.
Post Ficoll
CD38 selection
Count
CD38
CD38-
CD38+
FSC
CD34
CD34 0,93%
FACS
CD34 1,11%
CD34 0,17%
CD34 pre-enrichment
CD34 11,31%
C.
CB CD34+ cells
BEADS/FACS Sorting
CD34+38-
CD34+38+
Transduction
PGK
GFP
OFP
Mix and Transplant into NSG
D.
Chimerism
CD34+38-
CD34+38+
in vitro
7
12
23
28
BM 28
Peripheral blood
weeks

Figure S3. In vivo tracking of hematopoietic reconstitution by $CD34^+$ CB subpopulations sorted by a bead-based procedure. Related to Figure 1.

A. Schematic representation of the beads-based sorting procedure applied to separate $CD38^+$ and $CD38^-$ cells from CB.

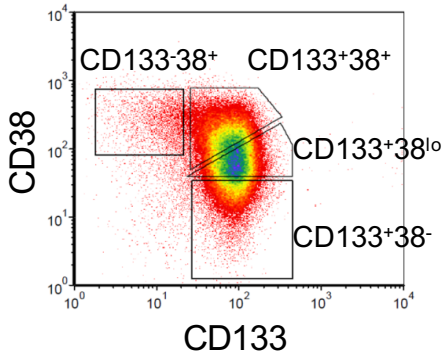
B. Representative FACS plots showing the percentage of $CD34^+$ cells during the different steps of bead-based selection procedure (post-Ficoll, CD38 selection, CD34 sorting).

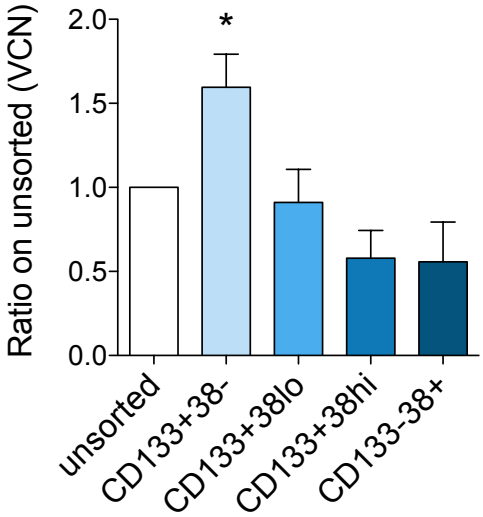
C. Experimental design applied to CB $CD34^+$ cells. $CD34^+CD38^+$ and $CD34^+CD38^-$ sorted cells were transduced respectively with LV expressing OFP or GFP under the control of a PGK promoter. The two subpopulations were mixed in a naturally ratio and transplanted into sub-lethally irradiated NSG mice (n=3).

D. Distribution of the 2 fluorescent proteins in the Mix group before injection (in vitro) or within human $CD45^+$ cells sampled from the PB and BM at the indicated n. of weeks post-transplantation allows back-tracing the origin of hematopoietic reconstitution to the originally transplanted cell subpopulations indicated in the legend.

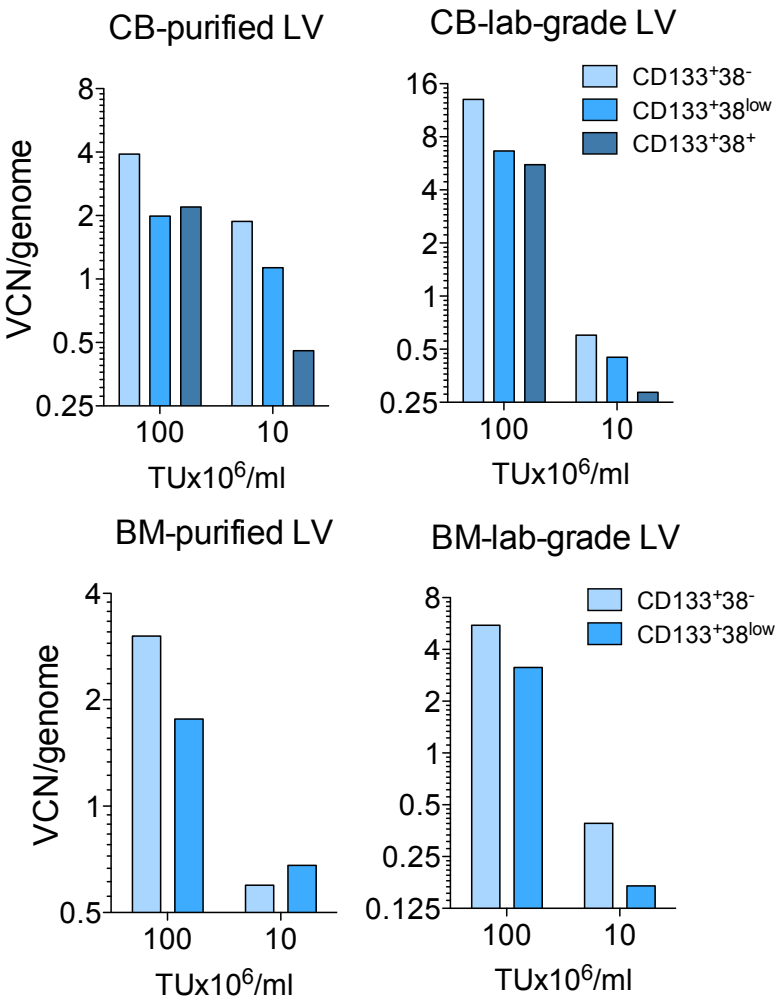
Figure S4

A.

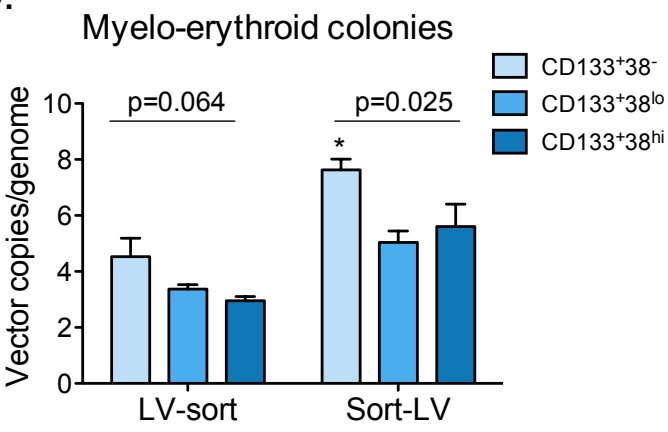




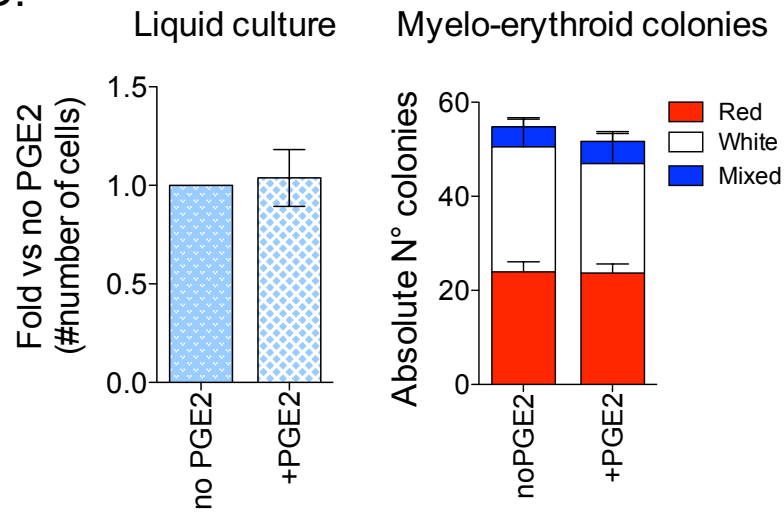
B.



C.



D.



E.

Protocol of Transduction	Hits	VCN
38h	1	0.81
24h +PGE_2	1	3.5
38h +PGE_2	1	2.25

Protocol of Transduction	Hits	VCN
24h	1	0.45
38h	1	0.34
24h +PGE_2	1	0.98
38h +PGE_2	1	0.83

Figure S4. Increased transduction of HSPC by lentiviral vectors. Related to Figure 3.

A. Human cord blood-derived $CD34^+$ cells were FACS-sorted according to CD133 and CD38 expression as shown. The sorted subpopulations were transduced with LV-GFP and transduction efficiency was measured. Quantitative Real-Time PCR (qPCR) analysis of DNA extracted after 2 weeks in culture was used to determine vector copy number (VCN). Results obtained from 3 independent CB donors using 4 different LVs. Data were analyzed by Wilcoxon signed-rank test (*$p<0.05$).

B. Human CB or BM $CD34^+$ cells were FACS-sorted as described in panel A. Sorted populations were transduced with lab-grade or GMP-grade, purified LV at different TU/ml and transduction efficiency was measured by determining the VCN by qPCR. $100x10^6$ TU/ml corresponds to an moi of 100, while $10x10^6$ TU/ml corresponds to an moi of 10.

C. In order to test whether this increased permissiveness of $CD38^-$ cells towards LV transduction is a cell-intrinsic property of more primitive HSPC or due to external factors such as cell competition for vector particles, we wondered whether the CD38- subset is also more targeted by LV when transducing bulk $CD34^+$ cultures. We thus made a side-by-side comparison of two protocols: in the first protocol, cells are sorted first, and purified populations are exposed to the LV (protocol named "sort-LV"); in the second protocol, bulk $CD34^+$ cells are LV-transduced, and CD38 subpopulations are sorted 24hr later and cultured separately in order to assess transduction levels in the purified populations (protocol named "LV-sort"). Importantly, pre-stimulation times before and cell density at the moment of LV exposure were kept constant in both protocols to minimize any potential bias impacting on transducibility. VCN measured in the colony outgrowth after 14 days in methylcellulose is shown for 3 independent CB donors transduced with LV-GFP at 10^8 TU/ml. For each group (LV-sort or Sort-LV) data were analyzed by One-way analysis of variance (ANOVA). *, $p<0.05$ with respect to $CD38^{low}$ cells by Bonferroni's Multiple Comparison Test.

D. Left plot: Ex vivo proliferation of mPB-derived HSPC transduced in the presence of $dmPGE_2$ relative to the respective control group transduced without PGE_2 (n=5 donors). Data were analyzed by Wilcoxon signed-rank test. Right plot: In vitro clonogenic potential assessed by CFC assay performed on $CD34^+$ mPB transduced with or without $dmPGE_2$. The total number (mean ± SEM) of different types of colonies (white, red and mixed) was counted for the 2 conditions (n=3 donors). Data were analyzed by One-way analysis of variance (ANOVA) for red, white and mixed colonies, and no statistically significant differences emerged.

E. Vector copy number (VCN) in the myeloid outgrowth of $CD34^+CD38^-$ cells (n=2 donors) transduced with GMP-grade beta-globin expressing lentiviral vector according to the indicated transduction protocol. These $CD34^+CD38^-$ cells were xenotransplanted (see Figure 3I-K).

Supplemental Experimental Procedures

Lentiviral vectors used in this study

Third generation self-inactivating (SIN) LV (PGK.GFP, PGK.OFP, PGK.Cyan, PGK.BFP, PGK.mCherry, SP146/gp91^{phox}P.gp91^{phox}±126T), integrase-defective LV (IDLV) and SIN-retroviral vector (SIN-RV) expressing GFP under the control of an internal PGK promoter stocks were prepared, concentrated and titered as previously described (Follenzi et al., 2000; Lombardo et al., 2007; Montini et al., 2006). SIN-LV P90A and N74D capsid mutants as well as Baboon (BaEV-LVs) and feline RD114-TR envelope pseudotyped LVs were produced as described (Girard-Gagnepain et al., 2014; Marin et al., 2016; Petrillo et al., 2015). GMP-grade LV (PGK.GFP or beta-globin expressing LV) was manufactured by MolMed.

HSPC culture, transduction and expansion protocols

HSPC pre-stimulation and transduction was performed in serum-free medium, at a cell density of 1x10^6 cells/ml. In case cells went into ex vivo expansion cultures, cell density was reduced to 1x10^5 cells/ml to avoid accumulation of paracrine factors jeopardizing HSC maintenance. Culture media and cytokines varied according to cell source and application, in order to be in line with our standards validated over the years.

Cord blood HSPC was cultured in StemSpan medium (Stem Cell Technologies) containing the following cocktail of cytokines: 20 ng/ml IL-6, 50 ng/ml TPO, 100 ng/ml SCF, and 100 ng/ml FLT-3L (all from Peprotech). During ex vivo expansion (±SR1), cytokine concentrations were adapted to the ones used in pertinent publications (Boitano et al., 2010).

Bone marrow and mobilized peripheral blood HSPC were cultured, as in our clinical trials, in CellGro medium (Cell Genix) containing the following cocktail of cytokines: 60 ng/ml IL-3, 100 ng/ml TPO, 300 ng/ml SCF, and 300 ng/ml FLT-3L (all from Cell Genix). As indicated in the text, IL-3 was removed from the updated transduction protocol of CD34$^+$CD38$^-$ mPB cells (Figures 2F-G, 3I-K, 4), without any notable differences in transduction efficiency. Expansion of mPB CD34$^+$CD38$^-$ cells (cell density: 10^5 cells/ml) was carried out in StemMacs human HSC expansion medium (Miltenyi) using the following cytokines: 100ng/ml SCF, 100ng/ml FLT3L, 50ng/ml TPO, 50ng/ml IL-6 ± UM171 ± SR1.

LV transduction protocols were as follows:

Short protocol name	Applied to	Pre-stimulation	First round of LV transduction	Recovery	Second round of LV transduction
60-62hr/2 Hit	clinical standard	22±2 hr	14±2 hr	10±2 hr	14±2 hr
46-48hr/1 Hit	CB, BM, mPB unless otherwise indicated	22±2 hr	24±2 hr	-	-
36-38hr/1Hit	mPB	22±2 hr	14±2 hr	-	-
24hr/1Hit	mPB	14±1 hr	10±1 hr	-	-

A round of LV transduction consisted in adding 10^8 transducing units/ml (multiplicity of infection: 100, unless otherwise indicated) to the cell cultures. Wells were pre-coated with RetroNectin (Takara) in experiments performed with purified LVs.

Prostaglandin E_2

16,16-dimethyl Prostaglandin E_2 ($dmPGE_2$), formulated in methyl acetate solution, was purchased from Cayman Chemical. Upon arrival, the solvent was evaporated using a Speed Vac system, resuspended in DMSO at a 10mM concentration (1000x), aliquoted and cryopreserved at -80°C for no longer than 12 months. Upon use, the required number of 1000x $dmPGE_2$ aliquots were thawed on ice, pre-diluted in culture medium and added to the HSPC culture (10µM final concentration), either at the beginning of culture or 120 minutes before LV transduction. Cells were washed no earlier than 14 hours after adding the LV.

In vitro readouts

Myeloid differentiation liquid cultures were carried out in IMDM medium containing 10% FBS and early acting cytokines such as SCF and IL-3).

Clonogenic assays were plated at the end of LV transduction. Cells were washed, counted and resuspended in complete human Methocult medium (Stem Cell Technologies) at a concentration of 800-1000 cells/ml. Fifteen days later, colonies were scored by light microscopy for number and morphology. CFU-E and BFU-E were scored as erythroid colonies, while CFU-G, CFU-M and CFU-GM as myeloid and CFU-GEMM as mixed colonies. Human differentiated cells from CFC were harvested from a single plate (pool of colonies) and mixed into a single cell suspension. Cells were washed and resuspended in PBS containing 2% FBS. For immunostaining, cells were incubated with anti-human receptor blocking antibodies for 15 min at 4 °C and stained for 20 min at 4 °C with anti-human CD235a and CD33 antibodies (for antibodies see Table S1). To exclude dead cells from the analysis, cells were washed and resuspended in PBS containing 10 ng/ml 7-aminoactinomycin D (7-AAD).

Peripheral blood analysis

Mice were bled via the tail vein following analgesia. For each mouse, 250µl of peripheral blood was added to 10µL of PBS containing 45mg/mL EDTA. For immunostaining a known volume of whole blood (100µl) was incubated with anti-human Fc receptor blocking antibodies for 10 min at room temperature then incubated in the presence of monoclonal antibodies (for a list of antibodies, see Table S1) for 15 min at room temperature. Erythrocytes were removed by lysis with the TQ-Prep workstation (Beckman-Coulter) in the presence of an equal volume of FBS (100µl).

Bone marrow analysis

BM aspirates were obtained under deep anesthesia, by inserting a 26G needle into the distal femur shaft and aspirating the content into a 1ml syringe containing 100-200µl PBS. At the experimental endpoint, mice were humanely euthanized, and BM cells were obtained by flushing the femurs in PBS 2% FBS solution. Cells ($1x10^6$ cells) were washed, resuspended in 100µl of PBS containing 2% FBS, and incubated with anti-human and/or anti-mouse FcγIII/II receptor (Cd16/Cd32) blocking antibodies for 15 min at 4°C. Staining was performed with monoclonal antibodies (Table S1) for 20 min at 4°C.

Spleen analysis

Spleens were dissociated into single-cell suspensions, passed through 40µm nylon filter, and washed in cold phosphate buffered saline (PBS) containing 2mM EDTA

and 0.5% bovine serum albumine (BSA). Cells were incubated with anti-mouse FcγIII/II receptor (Cd16/Cd32) blocking antibodies for 15 min at 4°C and stained with anti-mouse monoclonal antibodies (Table S1) for 20 min at 4°C. To exclude dead cells from the analysis, cells were washed and resuspended in PBS containing 10ng/ml 7-aminoactinomycin D (7-AAD).

Quantitative PCR

Genomic (g)DNA was extracted using QIAmp DNA micro kit (Qiagen) or QIAmp DNA mini kit according to the starting number of cells (as suggested by manufacturer). DNA was quantified and assessed for purity. Vector copies per diploid genome (vector copy number, VCN) of the integrated lentiviral vectors were quantified by quantitative TaqMan PCR (qPCR) starting from 10-100 ng of template gDNA using the following primers (HIV sense: 5'-TACTGACGCTCTCGCACC-3'; HIV antisense: 5'-TCTCGACGCAGGACTCG-3') and probe (FAM 5'-ATCTCTCTCCTTCTAGCCTC-3') against the primer binding site region of LVs. Analysis was performed no earlier than 7-14 days post-transduction, to give enough time for non-integrated genomes to disappear during cell division. Endogenous DNA amount was quantified by a primer/probe set against the human telomerase gene (Telo sense: 5'-GGCACACGTGGCTTTTCG-3'; Telo antisense: 5'-GGTGAACCTCGTAAGTTTATGCAA-3'; Telo probe: VIC 5'-TCAGGACGTCGAGTGGACACGGTG-3' TAMRA). Copies per genome were calculated by the formula = [ng LV/ng endogenous DNA] - [number of LV integrations in the standard curve]. The standard curve for qPCR was generated by using the CEMA301 cell line stably carrying four vector integrants, which were previously determined by Southern blot analysis. All reactions were carried out in duplicate or triplicate on an Viia7 Real time PCR system (Applied Biosystems). As an alternative, VCN was determined by digital droplet PCR (ddPCR) on a QX200 Droplet Digital PCR System (Biorad).

Transduction efficiency by vector variants and quantification of replication intermediates was assessed by the following molecular analyses: IDLV: quantification of total lentiviral DNA (integrated and non-integrated) was performed as previously described (Mátrai et al., 2011), at three days post-transduction. Copy number of the reverse transcribed retroviral vector genome (both integrated and non-integrated) was performed by quantitative droplet digital PCR (ddPCR) discriminating it from plasmid carried over from the transient transfection using the following primers: RT-RV; ΔU3 sense: 5'-CGAGCTCAATAAAAGAGCCCAC-3', U3 antisense: 5'-GAGTCCTGCGTCGGAGAGAG-3'. For DNA replication intermediates analysis, cells were lysed and processed 6 and 24 hours post-transduction in Monini lysis buffer as previously described (Petrillo et al., 2015) followed by ddPCR. Primers and probes used to quantify late-RT and 2LTR replication intermediates were described (Petrillo et al., 2015).

Table S1. List of anti-human antibodies used for flow cytometry.

Antibody	Fluorochrome	Clone	Company	Code
hCD45	APC-eFluor780	HI30	eBioscience	47-0459-42
hCD45	PercP Cy5.5	H130	BioLegend	304028
hCD19	PE-Cy7	HIB19	BioLegend	302216
hCD3	PE	SK7	BD	345765
hCD3	PB	UCHT1	BioLegend	300431
hCD13	APC	WM15	BD	557454
hCD33	APC	AC104.3E3	Miltenyi Biotec	130-091-731
hCD33	PE-Cy7	P67.6	BD	333952
hCD235a	APC	REA175	Miltenyi Biotec	130-100-270
hCD34	VioBlue	AC136	Miltenyi Biotec	130-095-393
hCD38	PE-Vio770	IB6	Miltenyi Biotec	130-099-151
hCD90	APC	5E10	BD Bioscience	559869
hCD45RA	PE	T6D11	Miltenyi Biotec	130-092-248
hCD133	PE	293C3	Miltenyi Biotec	130-090-853
FcR Blocking Reagent			Miltenyi Biotec	130-059-901

Supplemental References:

Follenzi, A., Ailles, L.E., Bakovic, S., Geuna, M., Naldini, L., 2000. Gene transfer by lentiviral vectors is limited by nuclear translocation and rescued by HIV-1 pol sequences. Nat Genet 25, 217–222.

Girard-Gagnepain, A., Amirache, F., Costa, C., Levy, C., Frecha, C., Fusil, F., Nègre, D., Lavillette, D., Cosset, F.L., Verhoeyen, E., 2014. Baboon envelope pseudotyped LVs outperform VSV-G-LVs for gene transfer into early-cytokine-stimulated and resting HSCs. Blood 124, 1221–1231.

Lombardo, A., Genovese, P., Beausejour, C.M., Colleoni, S., Lee, Y.-L., Kim, K. a, Ando, D., Urnov, F.D., Galli, C., Gregory, P.D., Holmes, M.C., Naldini, L., 2007. Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. Nat. Biotechnol. 25, 1298–1306.

Marin, V., Stornaiuolo, A., Piovan, C., Corna, S., Bossi, S., Pema, M., Giuliani, E., Scavullo, C., Zucchelli, E., Bordignon, C., Rizzardi, G.P., Bovolenta, C., 2016. RD-MolPack technology for the constitutive production of self-inactivating lentiviral vectors pseudotyped with the nontoxic RD114-TR envelope. Mol. Ther. Methods Clin. Dev. 3, 16033.

Mátrai, J., Cantore, A., Bartholomae, C.C., Annoni, A., Wang, W., Acosta-Sanchez, A., Samara-Kuko, E., De Waele, L., Ma, L., Genovese, P., Damo, M., Arens, A., Goudy, K., Nichols, T.C., von Kalle, C., Marinee, M.K., Roncarolo, M.G., Schmidt, M., Vandendriessche, T., Naldini, L., 2011. Hepatocyte-targeted expression by integrase-defective lentiviral vectors induces antigen-specific tolerance in mice with low genotoxic risk. Hepatology 53, 1696–1707.

Montini, E., Cesana, D., Schmidt, M., Sanvito, F., Ponzoni, M., Bartholomae, C., Sergi Sergi, L., Benedicenti, F., Ambrosi, A., Di Serio, C., Doglioni, C., von Kalle, C., Naldini, L., 2006. Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration. Nat Biotechnol 24, 687–696. doi:nbt1216 [pii]\r10.1038/nbt1216

Petrillo, C., Cesana, D., Piras, F., Bartolaccini, S., Naldini, L., Montini, E., Kajaste-Rudnitski, A., 2015. Cyclosporin A and Rapamycin Relieve Distinct Lentiviral Restriction Blocks in Hematopoietic Stem and Progenitor Cells. Mol. Ther. 23, 352–362.