







Supplementary Figure 2

7,663 Estimated Number of Cells

45,388

Mean Reads per Cell

3,584 Median Genes per Cell

S	e	qu	lei	nci	ng	?	

Number of Reads	347,805,753
Valid Barcodes	97.3%
Valid UMIs	99.9%
Sequencing Saturation	37.1%
Q30 Bases in Barcode	94.7%
Q30 Bases in RNA Read	90.5%
Q30 Bases in UMI	93.7%

С

mitoROS High

12,688 Estimated Number of Cells

29,810 Mean Reads per Cell 1,806 Median Genes per Cell

Sequencing <a>?

378,231,706
97.5%
99.9%
32.0%
94.6%
90.3%
94.1%

mitoROS Low



Estimated Number of Cells	7,663
Fraction Reads in Cells	86.6%
Mean Reads per Cell	45,388
Median Genes per Cell	3,584
Total Genes Detected	21,832
Median UMI Counts per Cell	15,056

D

mitoROS High



Estimated Number of Cells	12,688
Fraction Reads in Cells	82.6%
Mean Reads per Cell	29,810
Median Genes per Cell	1,806
Total Genes Detected	21,350
Median UMI Counts per Cell	5,080

Supplementary Figure 3

Supplementary Figure 4





В

А



С





Supplementary Figure 6

Α









1

0

MSI2



MLLT3

В

Supplementary figure legends:

Supplementary Figure 1. The engrafting capacity of CB CD34⁺ HSCs/HPCs decreases upon ex vivo culture. (A) Strategy for analyzing the stemness of freshly isolated CB CD34⁺ cells and ex vivo cultured CB CD34⁺ cells. SRCs were determined 16 weeks after transplantation. (B, C) Representative flow plots of human CD45 cell chimerism in the bone marrow of primary recipient mice transplanted with freshly isolated CB CD34⁺ cells and ex vivo cultured CB CD34⁺ cells and ex vivo cultured CB CD34⁺ cells. Quantification data are shown as dot plots (mean±s.e.m.) in (C). n=5 mice per group. Two-tailed Student's *t*-test. ***p<0.001. (D, E) Representative flow plots of human CD45 cell chimerism in the bone marrow of secondary recipient mice transplanted with freshly isolated CB CD34⁺ cells and ex vivo cultured CB CD34⁺ cells and ex vivo flow plots of human CD45 cell chimerism in the bone marrow of secondary recipient mice transplanted with freshly isolated CB CD34⁺ cells and ex vivo cultured CB CD34⁺ cells. Quantification data are shown as dot plots (mean±s.e.m.) in (E). n=5 mice per group. Two-tailed Student's *t*-test. Shown as dot plots (mean±s.e.m.) in (E). n=5 mice per group. Two-tailed Student's *t*-test.

Supplementary Figure 2. Mitochondrial oxidative stress is induced in CB CD34⁺ HSCs/HPCs upon ex vivo culture. (A) GSEA plots showing enrichment of mitochondrion related genes in cultured CB CD34⁺ HSCs/HPCs with decreased engrafting capacity compared with fresh CB CD34⁺ HSCs/HPCs. (B) Selected GOs that are significantly enriched in cultured CB CD34⁺ HSCs/HPCs compared with fresh CB CD34⁺ HSCs/HPCs. (C) Dot plot showing OCR of freshly isolated and ex vivo cultured CB CD34⁺ HSCs/HPCs.

Data are shown as mean±s.e.m. Two-tailed Student's *t*-test. ***p<0.001. (D) Quantification data of relative mitoROS levels in freshly isolated and ex vivo cultured CB CD34⁺ HSCs/HPCs from four independent experiments. n=8. Data are shown as mean±s.d. Two-tailed Student's *t*-test. ***p<0.001. (E, F) Histogram plot showing relative mitochondrial mass level in freshly isolated and ex vivo cultured CB CD34⁺ HSCs/HPCs. Quantification data are shown as mean±s.d. in (F). n=8 from four independent experiments. Two-tailed Student's *t*-test. ***p<0.001. (G-I) The relative mitochondrial membrane potential indicated by JC1 fluorescence was measured. Representative JC-1 flow plot of freshly isolated (G) and ex vivo cultured (H) CB CD34⁺ HSCs/HPCs from four independent experiments are shown. Quantification data are shown as mean±s.d. in (I). n=8 replicates. Two-tailed Student's *t*-test. ***p<0.001. (J) Representative flow plots showing human CD45⁺ cell chimerism in the bone marrow of NSG mice 4 months after transplantation with 50,000 mitoROS low CB CD34⁺ cells, mitoROS medium CB CD34⁺ cells or mitoROS high CB CD34⁺ cells upon ex vivo culture stress.

Supplementary Figure 3. CellRanger web summary of mitoROS low and mitoROS high CB CD34⁺ cells upon ex vivo culture stress. (A, B) Estimated number of mitoROS low CB CD34⁺ cells is 7,663, with 45,388 mean reads per cell and 3,584 median genes per cell. UMI count plot of mitoROS low CB CD34⁺ cells is shown in (B). Medium UMI counts per cell is 15,056. (C,

D) Estimated number of mitoROS high CB CD34⁺ cells is 12,688, with 29,810 mean reads per cell and 1806 median genes per cell. UMI count plot of mitoROS high CB CD34⁺ cells is shown in (D). Medium UMI counts per cell is 5,080.

Supplementary Figure 4. Violin plot of data quality of mitoROS low and mitoROS high CB CD34⁺ cells upon ex vivo culture stress and PPI network of signature genes. (A) Violin plots showing RNA, Feature count distribution and the percentage of mitochondria in mitoROS low CB CD34⁺ cells. (B) Violin plots showing RNA, Feature count distribution and the percentage of mitochondria in mitoROS high CB CD34⁺ cells. (C) PPI network of the differentially expressed genes in cluster 11 of mitoROS low CB CD34⁺ cells upon oxidative stress. ADGRG1(named as GPR56) is associated with CD34 and CD133 (PROM1).

Supplementary Figure 5. ADGRG1 expression is decreased in CB CD34⁺ cells upon ex vivo culture and ADGRG1⁺CD34⁺CD133⁺ cells are more quiescent than ADGRG1⁻CD34⁺CD133⁺ cells. (A) Dot plots showing the percentage of ADGRG1⁺ cells in day 0, day 4 and day 7 ex-vivo cultured CD34⁺ cells. One-way ANOVA, ***p<0.001, n.s., not significant. (B) Flow histogram plots showing expression of ADGRG1 in freshly isolated CB HSC (which was currently defined as CD34⁺CD38⁻CD45RA⁻CD90⁺CD49f⁺), MPP (CD34⁺CD38⁻CD45RA⁻CD90⁻CD49f⁻), CD34⁺CD38⁻ cells, CD34⁺CD38⁺ cells

and CD34⁻ cells. (C) Flow histogram plots showing expression of ADGRG1 in day 0, day 4 and day 7 ex-vivo cultured CD34⁺CD133⁺ CB cells. (D) Quantification data showing relative mitoROS level of CD34⁺CD133⁺ADGRG1⁺ CB cells and CD34⁺CD133⁺ADGRG1⁻ CB cells. Two-tailed Student's t-test. ***p<0.001. (E) Dot plot showing OCR of CD34⁺CD133⁺ADGRG1⁺ CB cells and CD34⁺CD133⁺ADGRG1⁻ CB cells. Data are shown as mean±s.e.m. Two-tailed Student's t-test. ***p<0.001. (F) Representative flow plots showing cell cycle of status CD34⁺CD133⁺ADGRG1⁺ CB cells and CD34⁺CD133⁺ADGRG1⁻ CB cells.

Supplementary Figure 6. The human cell chimerism was significantly higher in recipients transplanted with CD34+CD133+ADGRG1+ CB cells than CD34+CD133+ADGRG1- CB cells upon oxidative stress induced by ex vivo culture. (A, B) 50,000 CD34+CD133+ADGRG1+ CB cells or CD34+CD133+ADGRG1- CB cells were transplanted intoNSG recipient mice. The percentage of human CD45+ cells in peripheral blood (PB) was determined 4 months after transplantation. Representative pseudocolor plot is shown in (A). Quantification data are shown as dot plots (mean±s.e.m.) in (B). Data are combined from two independent experiments. n=10 mice per group. *p<0.05. (C, D) The percentage of human CD33+ myeloid cells in bone marrow was determined 4 months after transplantation. Representative pseudocolor plot is none marrow was determined 4 months after transplantation. Representative pseudocolor plot plot is none marrow was determined 4 months after transplantation. Representative pseudocolor plot plot is none marrow was determined 4 months after transplantation. Representative pseudocolor plot plot is none marrow was determined 4 months after transplantation. Representative pseudocolor plot is none marrow was determined 4 months after transplantation. Representative pseudocolor plot is none marrow was determined 4 months after transplantation. Representative pseudocolor plot is none marrow was determined 4 months after transplantation. Representative pseudocolor plot is none marrow was determined 4 months after transplantation. Representative pseudocolor plot is none marrow was determined 4 months after transplantation. Representative pseudocolor plot is shown in (C). Quantification data are shown as dot plots (mean±s.e.m.)

in (D). n=10 mice per group. **p<0.01. (E, F) The percentage of human CD19+ lymphoid cells in bone marrow was determined 4 months after transplantation. Representative pseudocolor plot is shown in (E). Quantification data are shown as dot plots (mean±s.e.m.) in (F). n=10 mice per group. *p<0.05. (G, H) The percentage of human CD33⁺ myeloid cells and CD19⁺ lymphoid cells in bone marrow (BM) of secondary recipient mice was determined 4 months after transplantation. Quantification data are shown as dot plots (mean±s.e.m.). Data are combined from two independent experiments. n=8-9 mice per group. **p<0.01. Two-tailed Student's t-test for (B), (D), (F), (G) and (H). (I) The percentage of human CD45⁺ cells in peripheral blood (PB) of primary recipient mice was determined 4 weeks and 8 weeks after transplantation. Quantification data are shown as dot plots (mean±s.e.m.). n=5 mice per group. One way ANOVA. **p<0.01, n.s., not significant. (J) The percentage of human CD45⁺ cells in PB of secondary recipient mice was determined 4 weeks and 8 weeks after transplantation. Quantification data are shown as dot plots (mean±s.e.m.). n=5 mice per group. One way ANOVA. ***p<0.001.

Supplementary Figure 7. ADGRG1 enriches for functional human HSC upon ex vivo culture of CB CD34+ cells but is not involved in HSC engraftment. (A) Dot plots showing relative of ADGRG1 in *shCtrl* and *shADGRG1* transfected cells. Data are combined from two independent experiments. n=10 replicates for each group. Two-tailed Student's *t*-test. ****p<0.001. (B) Flow plots showing hCD33 and hCD19 chimerism from BM of recipients transplanted with CB CD34⁺ cells infected by Vector or *ADGRG1* OE lentivirus. (C) Quantification of hCD33 myeloid and hCD19 B cell chimerism in BM of recipients transplanted with CB CD34⁺ cells infected by Vector or *ADGRG1* OE lentivirus. One way ANOVA. n.s., not significant. (D) Relative mRNA level of *MSI2* and *MLLT3* in ADGRG1⁺CD34⁺CD133⁺ CB cells and ADGRG1⁻CD34⁺CD133⁺ CB cells upon ex vivo culture. n=6 replicates from two independent experiments. One way ANOVA. ***p<0.001. (E) Flow plot showing the percentage of CD38⁻ cells, CD45RA⁻ cells, CD90⁺ cells and CD49f⁺ cells in ADGRG1⁺CD34⁺CB cells upon ex vivo culture. (F) Quantification of the percentage of CD38⁻ cells, CD45RA⁻ cells, CD90⁺ cells and CD49f⁺ cells in ADGRG1⁺CD34⁺CB cells upon ex vivo culture. Data are combined from two independent experiments.

Supplemental Methods

Mice

NM-NSG (NOD-Prkdc^{scid}IL2rg^{em1}B2m^{em1/Smoc}) mice (6-8 weeks old) were obtained from Shanghai Model Organisms and maintained in the Laboratory Animal Center of the Shanghai Jiao Tong University School of Medicine (SJTUSM). All animal experiments followed protocols approved by The Institutional Animal Care and Use Committee of SJTUSM.

Isolation of CB CD34⁺ cells and cell culture

Mononuclear cells (MNCs) from normal human cord blood (CB) were isolated by density gradient centrifugation with Ficoll-Paque Plus (GE Healthcare, Piscataway, NJ, USA). CD34⁺ cells were enriched using an immunomagnetic selection kit (Miltenyi Biotec, Auburn, CA, USA). MNCs were resuspended in MACS buffer (0.5% BSA, 2mM EDTA in PBS, pH7.2). FcR blocking reagent (Miltenyi Biotec, #130-046-702) and CD34 microbeads (Miltenyi Biotec, #130-046-702) were added sequentially, and then the cells were incubated for 30 min at 4°C. The cells were washed with MACS buffer and centrifuged at 300 g for 10 min. The supernatant was aspirated and the cells were resuspended in 1 mL MACS buffer. Magnetic separation was performed with an appropriate MACS column (Miltenyi Biotec, #130-042-401). CB CD34⁺ cells were cultured in Stemline II Hematopoietic Stem Cell Expansion Medium (Sigma, S0912) with 100 ng/mL stem cell factor (SCF) (R&D Systems, #7466-SC-010/CF), 100 ng/mL thrombopoietin (TPO) (R&D Systems, #288-TP-200/CF), and 50 ng/mL Fms-like tyrosine kinase 3 ligand (FtI3L) (BioLegend, #710802) in the presence of with 50 IU/mL penicillin and 50 μ g/mL streptomycin. All CD34⁺ cell cultures were incubated in a 5% O₂ and 5% CO₂ humidified cell incubator.

Immunostaining and flow cytometry

Cells were collected by centrifuging at 300 g for 10 min, washed twice with cold PBS, resuspended in 500 µL PBS, and stained with fluorescence conjugated antibodies at 4 °C for 30 min. The cells were washed with cold PBS and the samples were fixed with 1% formaldehyde. The samples were analyzed on a LSRFortessa flow cytometer (BD Biosciences). The following antibodies from were used for cell surface staining: CD34-APC (581, BD Bioscience), CD133-BV421 (293C3, BD), ADGRG1-PE (4C3, BioLegend), CD19-PE (HIB19, BD), CD33-PEcy7 (WM53, BD), CD45-APC (HI30, BD), CD38-FITC (HIT2, BD), CD45RA-PE-CF594 (HI100, BD), CD90-PEcy7 (5E10, BD) and CD49f-PerCPcy5.5 (GoHA3, BD).

Mito SOX, Mito Tracker and JC-1 staining

Live CB CD34⁺ cells were stained with MitoSOX[™] Red Mitochondrial Superoxide Indicator, MitoTracker Green FM or JC-1 following the manufacturer's protocols (Thermofisher). Briefly, live CB CD34⁺ cells were stained with cell surface markers at first. After washing, cells were incubated with Mito SOX, MitoTracker or JC-1 for 15 minutes at 37°C in the incubator. Cells were washed with ice cold PBS by spinning at 300 g, 4°C, for 10 mins. Remove the supernatant, vortex and resuspend cells in 500 PBS. Run FACS

immediately to collect the data on a LSRFortessa flow cytometer (BD Biosciences).

Cell Cycle analysis

CB CD34⁺ cells were subjected to cell surface staining of CD34, CD133 and ADGRG1. After that, cells were fixed and permeabilized with BD Cytoperm[™] Permeabilization Buffer Plus (#554714, BD). Wash cells twice with cold PBS containing 1% FBS. Cells were resuspended in 200 µLPBS and incubated with anti-Ki67-FITC (#556026, BD) at room temperature (RT) for 20-30 minutes in the dark. Cells were wash with cold PBS containing 1% FBS and stained with 0.1µg/mL DAPI (#564907, BD).

Seahorse extracellular flux assay

The oxygen consumption rate (OCR) of human CB CD34⁺ cells was determined by using Seahorse XF Extracellular Flux Analyzer (Agilent Technologies). 200 μ L XF Calibrant buffer (Seahorse Bioscience, #100840-000) was added into Seahorse Bioscience 96-well utility plate, and then the plate was incubated at 37°C overnight. The cell culture plate (Seahorse Bioscience, # 101085-004) was coated with Cell-Tak solution (CORNING, # 354241) at room temperature for one hour. 100, 000 purified CD34⁺ cells per well were plated into cell culture plates. The plates were centrifuged for 10 min, at 1, 000 g. During this process, oligomycin (Sigma, #75351), FCCP (Sigma, C2920), rotenone (Sigma, R8875) medium was sequentially (A, B, C) added into the utility plate for OCAR analysis. The utility plate with the loaded sensor cartridge was placed on the instrument tray for calibration. When prompted, replace the calibration plate with the cell culture microplate then click "Start" to evaluate OCAR of CB CD34⁺ cells.

Limiting dilution analysis

The frequency of human SCID repopulating cells (SRCs) was analyzed by LDA as previously reported (1). Increasing doses of vehicle or glucocorticoid treated CD34⁺ cells were intravenously injected into NSG recipient mice that had been sub-lethally irradiated. 16 weeks after transplantation, the mice were sacrificed and the percentage of human CD45⁺ cell, myeloid cell and lymphoid cell chimerism was determined by immunostaining and flow cytometry. Recipient mice with more than 1% human CD45⁺ cells were defined as successful transplantation. The HSC frequency was calculated using L-Calc software (Stem Cell Technologies Inc, Vancouver, BC, Canada) and plotted using ELDA software (bioinf.wehi.edu.au/software/elda/).

Homing assay

ADGRG1⁺CD34⁺CD133⁺ cells or ADGRG1⁻CD34⁺CD133⁺ cells were intravenously injected into sub-lethally irradiated NSG mice. 500,000 cells were transplanted for each mouse. 24 hours after injection, human CD45 cell chimerism in the BM of the recipients was analyzed by FACS.

RNA sequencing

For bulk RNA-seq, CB CD34⁺ cells were lysed and RNA was extracted using RNeasy Mini Kit (QIAGEN, Valencia, CA, USA). RNA sequencing analysis was performed at SeqWright Genomic Services (GE Healthcare). RNA samples

were subjected to poly-A selection TruSeq RNA Sample Prep, library construction, and cluster generation. RNA libraries were sequenced on an Illumina HiSeq 2500 machine with 2×100 bp read lengths for a total of 2×20 M reads per sample.

single cell RNA-seq experiment was performed by NovelBio Bio-Pharm Technology Co., Ltd. For single cell RNA-seq, the libraries were generated using the 10X Genomics Chromium Controller Instrument and Chromium Single Cell 3' V3 Reagent Kits (10X Genomics, Pleasanton, CA). Two samples, mitoROS low and mitoROS high CB CD34⁺ cells were concentrated to 1000 cells/uL and approximately 10,000 cells were loaded into each channel to generate single-cell Gel Bead-In-Emulsions (GEMs), which results into expected mRNA barcoding of 8,000 single-cells for each sample. After the reverse transcription, GEMs were broken and barcoded-cDNA was purified and amplified. The amplified barcoded cDNA was fragmented, A-tailed, ligated with adaptors and index PCR amplified. The final libraries were quantified using the Qubit High Sensitivity DNA assay (Thermo Fisher Scientific) and the size distribution of the libraries were determined using a High Sensitivity DNA chip on a Bioanalyzer 2200 (Agilent). All libraries were sequenced by HiSeq Xten (Illumina, San Diego, CA) on a 150 bp paired-end run.

Bulk RNA-seq Data Analysis

The RNA-seq reads were mapped to the human genome hg38 using RNA-seq aligner STAR (v2.5) with the parameter: "--outSAMmapqUnique 60". The

feature Counts (v1.6.2) was adopted to summarize uniquely mapped sequencing reads on genes (GENCODE 25) with the parameters: "-s 2 –p –Q 10". After excluding low expressed genes with read count per million (CPM) less than 0.5 in more than two third of total samples, we normalized the gene expression using TMM (trimmed mean of M values) method, followed by differential expression analysis with the software edgeR (v3.20.8). The differentially expressed genes (DEGs) were determined by FDR-adjusted p-value less than 0.001 and amplitude of fold change (FC) larger than linear 2-fold. Functional analysis was conducted on DEGs using DAVID. Gene set enrichment analysis (GSEA) was also performed using pre-ranked gene list based on fold changes of all genes.

Single Cell RNA-seq Data Analysis

The adaptor sequences were trimmed and low-quality reads were removed before further analysis. We generated single-cell feature counts using the count function of CellRanger (version 3.1.0) with pre-built GRCh38 (human) reference genome and GENCODE V28 annotation. The samples of mitoROS low CD34⁺ cells and mitoROS high CD34⁺ cells were processed, respectively. Genes detected in less than 0.1% of total cells and cells with less than 200 detected genes were filtered out. Then the Seurat R package was adopted for following analysis. Given the distributions of numbers of unique genes and mitochondrial counts, cells with unique genes between 200 and 6000 as well as mitochondrial counts less than 10% were chosen to be normalized with the "LogNormalized"

method using 10,000 as the scaling factor. The linear transformation was performed on data integration of mitoROS low and high CD34⁺ cells. After principal component analysis (PCA) on all cells, the first 19 principal components (PC) were selected to obtain 19 clusters of cells by an algorithm based on shared nearest neighbor (SNN) modularity optimization.

To determine genes highly expressed in cluster 11, "FindMarkers" function of Seurat package with Wilcoxon rank sum test was used to compare gene expression in cluster 11 to other clusters. The cutoffs of Bonferroni adjusted pvalue less than 0.05 and average FC larger than linear 1.2-fold were selected to identify the marker genes in the cluster 11. We used STRING database to build the protein-protein interaction (PPI) network among cluster-11 marker genes.

GSEA was conducted on different gene sets by comparing the gene expression levels in cluster 11 and all other 18 clusters. The gene sets of our interest include HSC-specific genes as previously defined (18,21), genes marked as MYC target from Hall mark version 1 sets (HALLMARK_MYC_TARGETS_V1) in Molecular Signature Database (MsigDB), etc.

Knockdown and Overexpression of ADGRG1

For knocking down *ADGRG1*, 5'-GACTTCTTGCTGAGTGACAAACTCGAG TTTGTCACTCAGCAAGAAGTC-3' hairpin sequence targeting *ADGRG1* was cloned into PLKO.3G. 5'-GTTCTCCGAACGTGTCACGTCTCGAG ACGTGACACGTTCGGAGAAC-3' was used as the negative control. For overexpression of *ADGRG1* in human CB CD34⁺ cells, human *ADGRG1* cDNA was cloned into LV165 vector from GeneCopoeia. Lentivirus was generated by co-transfecting the above plasmids with psPAX2 (Addgene#12260) and pMD2.G (Addgene #12259) into Lenti-X 293T cells (Takara, #632180). psPAX2 and pMD2.G were provided as gifts by Didier Trono. Lentivirus supernatant was concentrated by spinning at 50,000 g for 2.5 h at 4°C using a Beckman SW-28 swinging bucket rotor. CB CD34⁺ cells were infected by concentrated lentivirus in the presence of 8 µg/ml polybrene (EMD Millipore, #TR-1003-G).

Statistics

Data are shown as mean values \pm standard deviation (SD), unless stated to show standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism 5.0. Two-tailed Student's *t*-tests were performed for statistical analysis between two groups. One-way ANOVA was used to compare differences in means between more than two groups. P-value less than 0.05 was considered as statistically significant (*p<0.05; **p<0.01; ***p<0.001).

Culture conditions	Cell number transplanted	Number of mice with >1% human cell
		chimerism/total number of
		mice
Fresh	500	0/5
	2500	2/4
	10000	5/5
Cultured	500	0/5
	2500	0/5
	10000	2/5

Supplementary Table 1. Determination of SRC frequency in Fresh or Cultured CB CD34⁺ HSCs and HPCs

Supplementary Table 2 SRC frequency in Fresh or Cultured CD34⁺ cells

Culture conditions	SRC frequency	95% confidence interval	Number of SRCs in 1×10 ⁶ CD34 ⁺ cells
Fresh	1/3424	1/8163 to 1/1437	292
Cultured	1/27194	1/106862 to 1/6920	37

Culture conditions	Cell number transplanted	Number of mice with >1% human cell chimerism/total number of mice
mitoROS low	10000	10/15
	50000	9/9
	100000	4/4
mitoROS high	10000	0/13
	50000	3/4
	100000	3/6

Supplementary Table 3. Determination of SRC frequency in mitoROS low or mitoROS high CB CD34⁺ cells upon oxidative stress induced by ex vivo culture

Supplementary Table 4. SRC frequency in mitoROS Low and mitoROS High CD34 $^{+}$ cells

Culture conditions	SRC frequency	95% confidence interval	<i>Number of SRCs in</i> 1×10 ⁶ CD34 ⁺ cells
mitoROS Low	1/8918	1/16491 to 1/4823	112
mitoROS High	1/112939	1/254643 to 1/50090	9

Cell population	Cell number transplanted	Number of mice with >1% human cell chimerism/total number of mice
ADGRG1 ⁺ CD34 ⁺ CD133 ⁺	5000	4/10
	10000	6/10
	50000	10/10
ADGRG1-CD34 ⁺ CD133 ⁺	5000	0/10
	10000	0/10
	50000	4/10

Supplementary Table 5. Determination of SRC frequency in ADGRG1⁺ CD34⁺CD133⁺ or ADGRG1⁻CD34⁺CD133⁺ CB cells

Supplementary Table 6. SRC frequency in ADGRG1⁺CD34⁺CD133⁺ and ADGRG1⁻CD34⁺CD133⁺ CB cells

Culture	SRC frequency	95% confidence interval	Number of SRCs in 1×10 ⁶
conditions			CD34 ⁺ CD133 ⁺ cells
ADGRG1+	1/10077	1/18021 to 1/5635	99
ADGRG1-	1/135972	1/357438 to 1/51725	7