Stem Cell Reports, Volume *11*

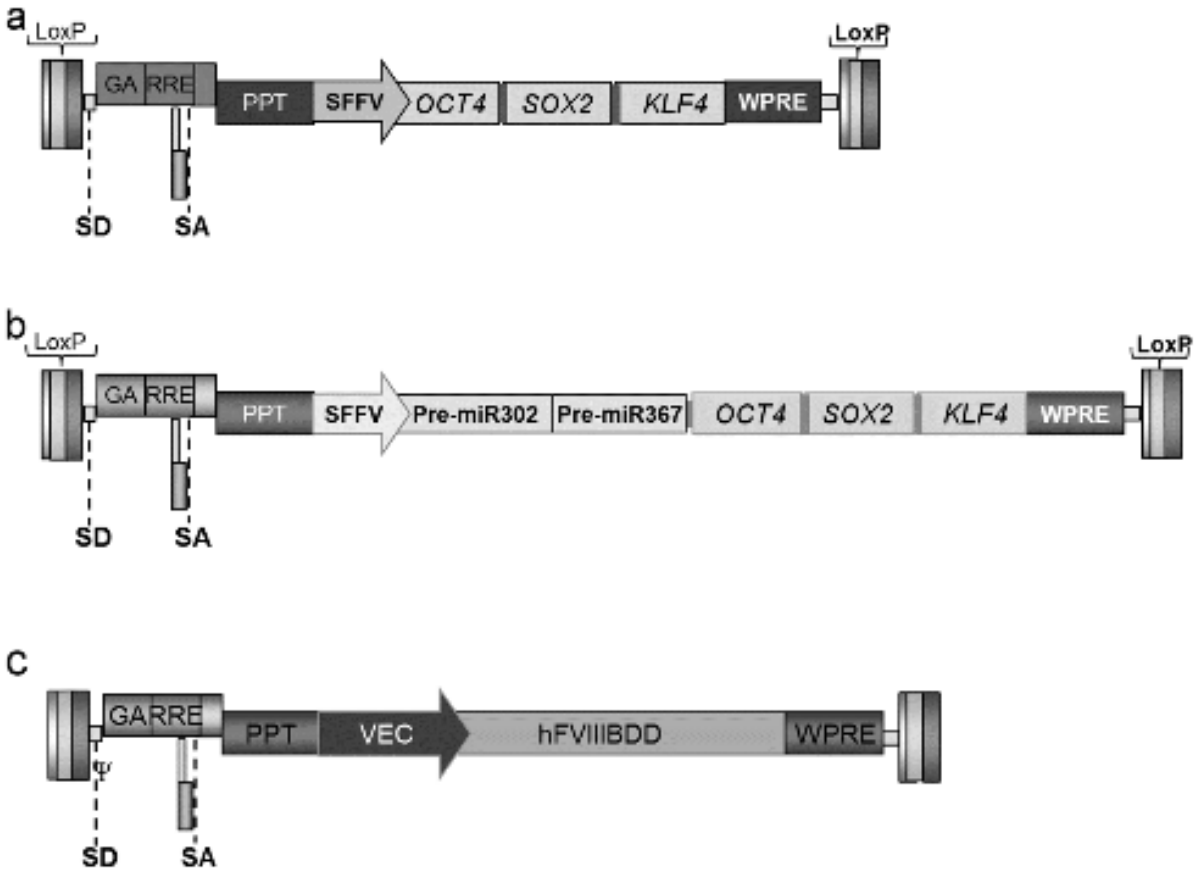
Supplemental Information

Patient-Specific iPSC-Derived Endothelial Cells Provide Long-Term Phenotypic Correction of Hemophilia A

Cristina Olgasi, Maria Talmon, Simone Merlin, Alessia Cucci, Yvonne Richaud-Patin, Gabriella Ranaldo, Donato Colangelo, Federica Di Scipio, Giovanni N. Berta, Chiara Borsotti, Federica Valeri, Francesco Faraldi, Maria Prat, Maria Messina, Piercarla Schinco, Angelo Lombardo, Angel Raya, and Antonia Follenzi

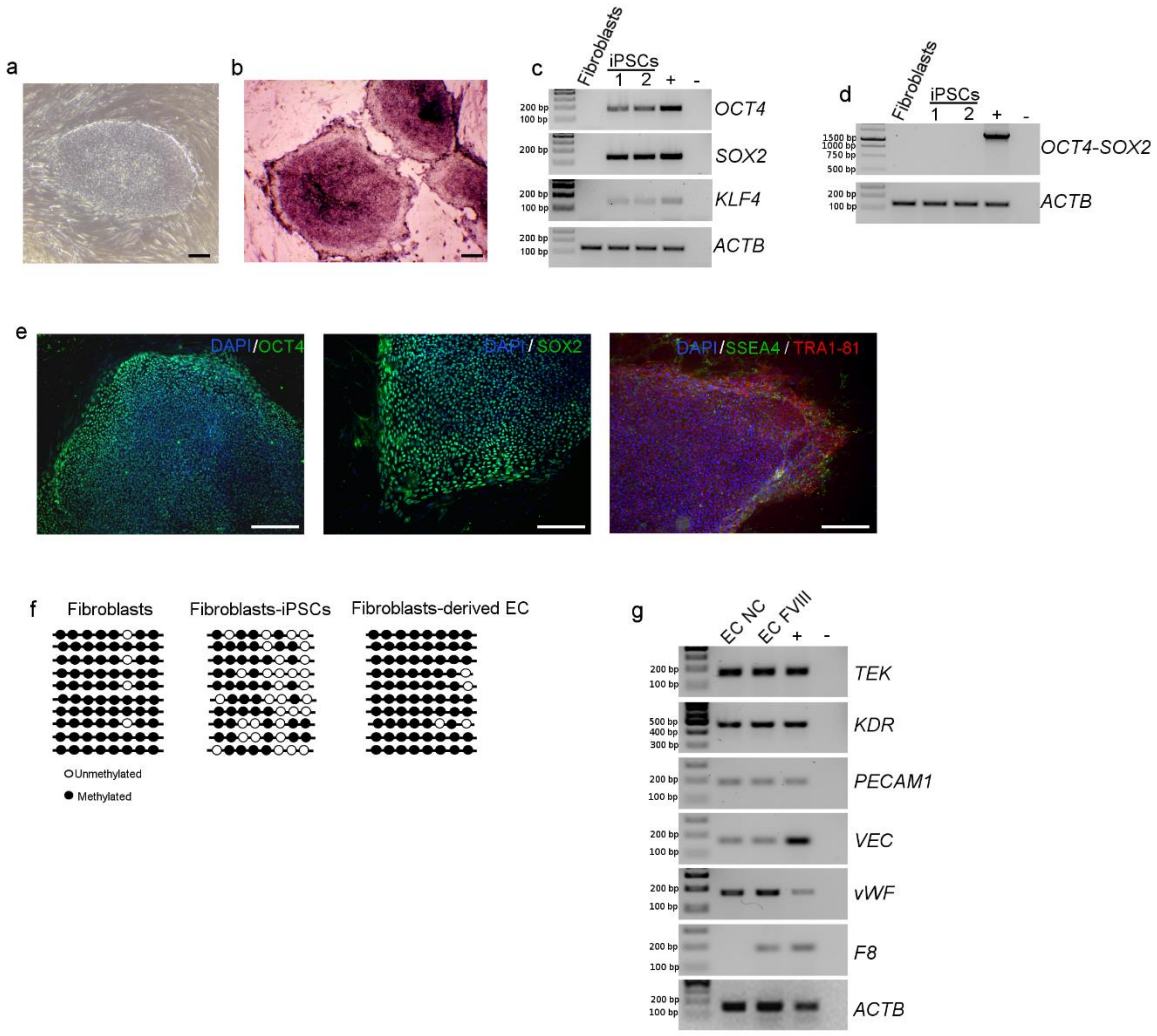
Supplemental Information

Supplemental Figures and Tables



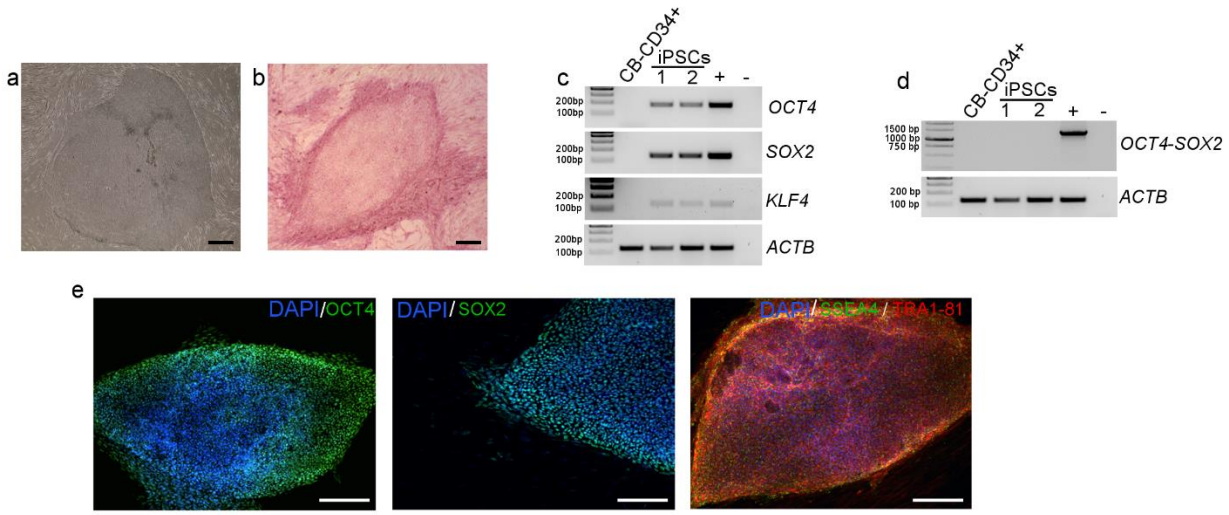
Supplemental Figure S1 (Related to figure 1, 4) – Lentiviral vector used for reprogramming and gene correction.

Healthy and hemophilic CD34+ cells were reprogrammed using third generation LVs carrying a LoxP-flanked polycystronic cassette containing *OCT4*, *SOX2* and *KLF4* (a) and a third generation LVs carrying a LoxP-flanked polycystronic cassette containing *OCT4*, *SOX2* and *KLF4* and pri-miR302/pri-miR367 (b). HA cells were corrected using a third generation LV carrying hBDD-FVIII under the control of VE-cadherin endothelial specific promoter (c).



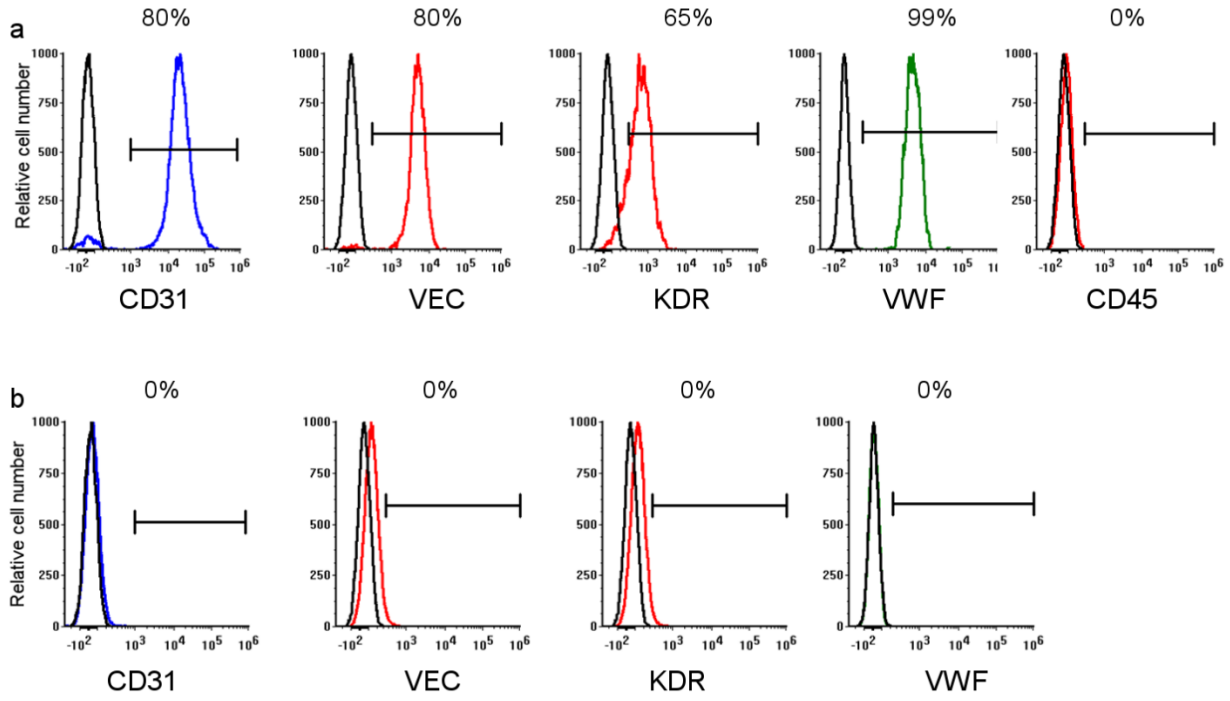
Supplemental Figure S2 (Related to figure 1) – iPSC colonies obtained by reprogramming of fibroblasts.

iPSCs were efficiently obtained by reprogramming of fibroblasts. Representative phase contrast microscopy showing ESC-like morphology of iPSCs (A) and positivity for alkaline phosphatase staining (B). RT-PCR for endogenous stem cell markers (*OCT4*, *SOX2*, *KLF4*) (C) and exogenous stem cell markers (D). Fibroblasts were used as negative control and HEK293T cells transduced with the reprogramming vector was used as positive control. Data are representative of three independent experiments. Stem cell markers expression confirmed by immunofluorescence for OCT4, SOX2, SSEA-4 and TRA1-81 (E). Markers used were: OCT4 (green), SOX2 (green), SSEA4 (green), TRA1-81 (red) and 4′,6-diamidino-2- phenylindole dihydrochloride (DAPI, blue). NANOG promoter methylation analysis showed that the 40% of CpG islands in the fibroblasts-derived iPSCs were unmethylated while the 96% in the starting fibroblasts were methylated (F). Fibroblasts were differentiated efficiently in ECs using BMP4 differentiation protocol. ECs expressed several endothelial markers (G). Data are representative of three independent experiments. Scale bars: 200 μm. Data are representative of clone HA 4.1.7.



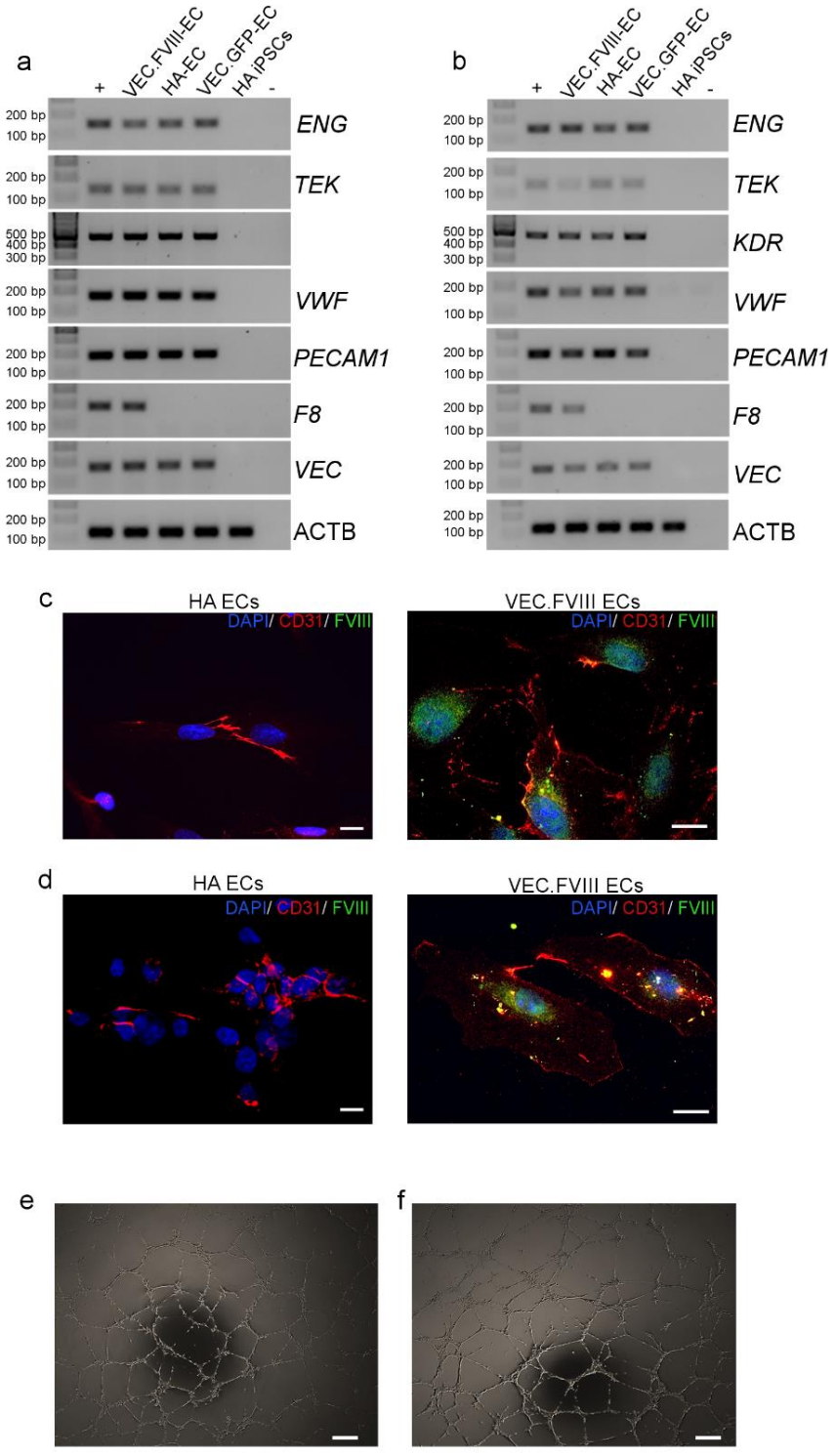
Supplemental Figure S3 (Related to figure 1) – iPSC colonies obtained by reprogramming of cord blood CD34+.

iPSCs were efficiently obtained by reprogramming cord blood CD34+ cells. Representative images showing that all obtained iPSCs showed ESC-like morphology (A) and were positive at AP staining (B). RT-PCR showed that iPSCs expressed endogenous stem cells factors (C) but not the exogenous (D). CB-CD34+ cells were used as negative control and HEK293T cells transduced with the reprogramming vector was used as positive control. Stem cell markers expression was confirmed by immunofluorescence for OCT4, SOX2, SSEA-4 and TRA1-81 (E). Markers used were: OCT4 (green), SOX2 (green), SSSEA4 (green), TRA1-81 (red) and 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI, blue). Data are representative of three independent experiments. Scale bars: 200 μm. Data are representative of clone HD 6.11.



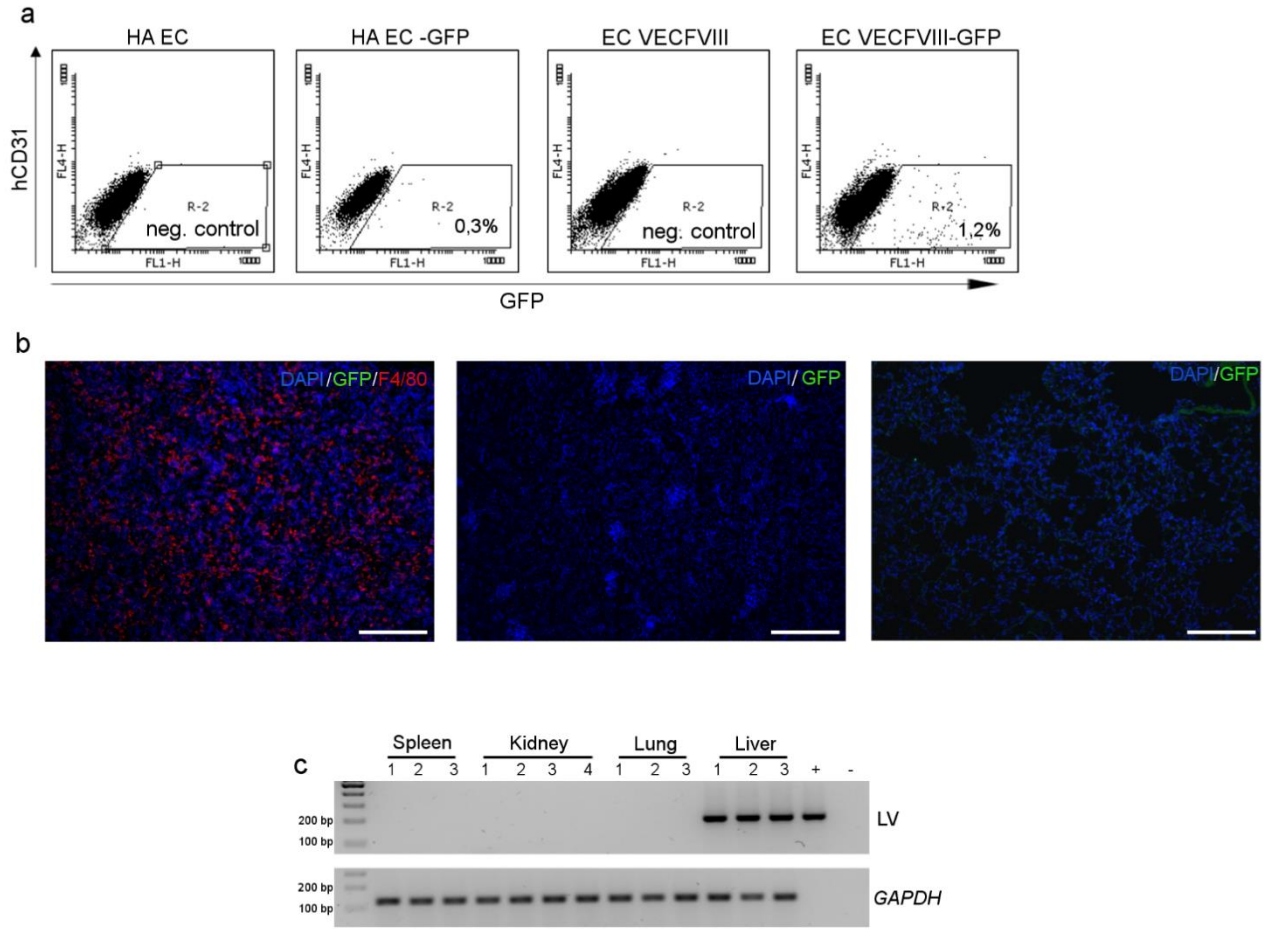
Supplemental Figure S4 (Related to figure 2, 4) – FACS analysis of human BOECs and Fibroblasts for endothelial markers.

FACS analysis of BOECs (A) and Fibroblast (B). The histogram overlays show the expression of different endothelial markers (CD31, VEC, KDR, VWF) and of CD45, used as negative marker. Non-labeled cells were used as negative control in each plot (black line).



Supplemental Figure S5 (Related to figure 4) – Endothelial differentiation of hemophilic CD34+-derived iPSCs (clones HA 2.2 and HA 4.9).

HA iPSCs were corrected with a LV.VEC-hBDDFVIII. LV.VEC-GFP was used as control of transduction. Representative RT-PCR for endothelial markers on FVIII-expressing and not expressing ECs from clones HA 2.2 (a) and HA 4.9 (b). HA-CD34+-derived iPSCs were used as negative control, HUVECs as positive control. HEK293T cells transduced with VEC.FVIII were used as positive control only for F8. Immunofluorescence staining with CD31 (red), FVIII (green) and 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI, blue) on HA ECs and VEC.FVIII ECs from clones HA2.2 (c) and HA 4.9 (d). Scale bars: 25μm. VEC-FVIII-ECs from clones HA2.2 (e) and HA 4.9 (f) formed tubules network when cultured in matrigel.



Supplemental Figure S6 (Related to figure 5) – FACS analysis and integration analysis on different organs from transplanted mice.

FACS analysis for GFP and hCD31 on hepatocytes isolated from the liver of mice transplanted with GFP+-HA-ECs, VEC.FVIII ECs and GFP+-VEC.FVII ECs (A). Representative immunofluorescence staining on spleen, kidney and lung of transplanted (B) mice after 12 weeks (n=8). GFP (green), F4/80 (red) and 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI, blue). (C) PCR on gDNA for integration on recovered beads. LV.VEC.FVIII was used as positive control. Scale bars: 200 μm.

Supplemental Table S1 (Related to figure 1, 2, 4, S2, S3) – List of iPSCs lines obtained from hemophilic patients and healthy donors.

Cell Source	Donor	Clones obtained	iPSC lines	iPSC lines differentiated into EC
Healthy Donors-Peripheral blood	HD 1	30	HD 1.1-HD 1.12	HD 1.5
	HD 2	19	HD 2.1-HD 2.10	HD 2.9
	HD 3	40	HD 3.1-HD 3.15	HD 3.11
	HD 4	60	HD 4.1-HD 4.15	
	HD 5	60	HD 5.1-HD 5.15	
Healthy Donors-Cord Blood	HD 6	25	HD 6.1-HD 6.15	
	HD 7	35	HD 7.1-HD 7.20	
Heterozygous Donor	Hete 1	22	Hete1.1-1.10	
Hemophilic patients-Peripheral blood	HA 1	10	HA 1.1-HA 1.10	
	HA 2	18	HA 2.1-HA 2.10	HA 2.2
	HA 3	20	HA 3.1-HA 3.12	HA 3.1
	HA 4	25	HA 4.1-HA 4.12	HA 4.9
Hemophilic patient-Skin biopsy	HA 4.1	11	HA 4.1.1-HA 4.1.11	HA 4.1.7

Supplemental Table S2 (Related to figure 1, 2) – Reprogramming LV integrated copy number/cell pre-transduction with Cre recombinase.

Clone	Copy number
CD34+ cells	0,0014
CD34+-iPSC clone 1	0,43
CD34+-iPSC clone 2	2,3
CD34+-iPSC clone 3	2
CD34+-iPSC clone 4	0,5
CD34+-iPSC clone 5	0,3
CD34+-iPSC clone 6	2,8
CD34+-iPSC clone 7	2,1
CD34+-iPSC clone 8	1
CD34+-iPSC clone 9	0,5
Mean	**1,3**
HA-CD34+-iPSC clone 1	0,7
HA-CD34+-iPSC clone 2	1,1
HA-CD34+-iPSC clone 3	0,8
HA-CD34+-iPSC clone 4	1,8
HA-CD34+-iPSC clone 5	2,8
HA-CD34+-iPSC clone 6	2,1
Mean	**1,6**

Supplemental Table S3 (Related to figure 1, 2, 4) – Reprogramming LV integrated copy number/cell pre- and post-transduction with Cre recombinase

Sample	Copy number
EC pre-Cre transduction	2,5
EC post-Cre transduction	0,05
EC HA pre-Cre transduction	2,5
EC HA post-Cre transduction	0,07

Supplemental Experimental Procedures

Culture and irradiation of human foreskin fibroblasts
Human foreskin fibroblasts (HFF; ATCC® SCRC-1041™) were used as feeder layer for iPSCs culture. Specifically, HFF were cultured in IMDM (Sigma-Aldrich) containing 10% fetal bovine serum (FBS, Euroclone), 2mM glutamine (Sigma-Aldrich), 50 U/ml penicillin and 50 µg/ml streptomycin (Sigma-Aldrich). Before their use as feeder layer they were mitotically inactivated by gamma ray irradiation (25 Gy) and freezed in aliquots of 10^6 or $2x10^6$ cells/ml of freezing medium (90% FBS and 10% DMSO, Sigma-Aldrich). The day before iPSCs expansion, irradiated HFF were plated on a 0,1% gelatin (Sigma-Aldrich) coated plates in IMDM.

iPSCs culture
Specifically, iPSCs were cultured at 37°C with 5% CO2, consisting in KnockOut DMEM (Life Technologies) supplemented with 20% KnockOut Serum Replacement (Life Technologies), 2 mM Glutammine (Sigma-Aldrich), 50 µM 2-mercaptoethanol (Life Technologies), non-essential amino acids (Sigma), and 10 ng/ml basic fibroblast growth factor (bFGF) (Immunotools). HES medium was changed daily. Once a week, iPSCs were detached mechanically and plated onto fresh HFFs in HES medium.

Alkaline phosphatase staining
For Alkaline Phosphatase (AP) staining, iPSCs were fixed and stained using the Alkaline Phosphatase (AP) detection kit (Millipore) according to the manufacturer's protocol.

RNA isolation and RT-PCR
RNA was isolated by Isol-RNA Lysis Reagent (Invitrogen). 1µg of total RNA was reverse-transcribed with RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) and PCRs were performed on cDNA.
All the PCRs were performed with GoTaq® Flexi DNA Polymerase (Promega). PCR protocol were as follow: initial denaturation at 95°C for 5 min followed by 30 cycles (25 cycles for β-actin) of denaturation at 94°C for 30 seconds, annealing at 50-62°C for 30-45 seconds, extension at 72°C for 60 seconds, and final extension at 72°C for 7 minutes. Primers, annealing temperatures and product sizes are listed below. PCR products were resolved in 2% agarose gels.

Vector integration, copy number analysis and Cre/LoxP excision
LV-SFFV-miR-302\367-OSK integration in iPSCs was quantified using genomic DNA purified from cells and from tissues using ReliaPrep gDNA Tissue Miniprep System (Promega) and diluted to 25ng/mL. Primers used are listened in Appendix Supplementary Methods. qPCR for copy number was performed using the GoTaq® qPCR Master Mix (Promega). qPCR protocol was: denaturation at 95°C for 2 min followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 60 seconds according to the manufacturer's protocol. To excise the LoxP-flanked reprogramming vector cassette in ECs, cells were transduced with the integrase defective lentiviral vector (ID-LV) carrying Cre recombinase at MOI 30. Excision efficiency was assessed by qPCR as described above.

Telomere length analysis
Genomic DNA was purified from freshly isolated CD34+, iPSCs and ECs after 5, 10, 15, 20 passages in cultures using ReliaPrep gDNA Tissue Miniprep System (Promega). Telomere length was assed using qPCR Multiplex on genomic DNA in collaboration with Dr. Donato Colangelo from our Department as previously described (Zamperone et al., 2013).

NANOG promoter methylation analysis
Genomic DNA was purified from CD34+ cells, iPSCs and ECs using ReliaPrep gDNA Tissue Miniprep System (Promega). 1 µg genomic DNA was bisulfite-converted using EpiTect Kit (Qiagen). A total of 150 ng of converted gDNA was used for PCR using primer amplifying 8 CpG-islands in the Nanog promoter. Primers used are listed below. Amplified products were subcloned into pCR2.1 vectors using the Topo TA cloning Kit (Invitrogen). Individual

colonies were picked, plasmid DNA was purified using the NucleoSpin® Plasmid (Machery-Nagel), and DNA was sequenced using M13 Reverse and M13 (-20) Forward primers.

Chromosomal analysis

Chromosomal analysis of healthy and HA iPSCs and of iPSCs-derived ECs was carried out at passage 50 and 15 respectively as previously described (Sprio et al., 2012) adopting a conventional G-banding karyotype protocol. Unmounted slides were examined using Nikon Eclipse 1000 light microscopy and photographed with Genicon (San Diego, CA, USA) software. At least twenty-five high-quality G-banded metaphases were selected each time. The chromosomes were classified according to International System for Human Cytogenetic Nomenclature (Stevens-Kroef et al., 2017).

Adipogenic, osteogenic and chondrogenic differentiation

EBs were formed, plated on 0,1% gelatin (Sigma-Aldrich) coated plates and cultured in Mesenchymal Stem Cell Adipogenic Differentiation Medium (MSC, LONZA) or osteogenic medium consisting in α Minimum Essential Medium (αMEM, Euroclone), FBS 10% (Euroclone), 0,4 mM ascorbic acid, 1 mMβ-glicerophosphate, and 10 nM dexamethasone (all Sigma-Aldrich). Media were changed every 3 days. After 14–20 days, cells were washed in PBS, fixed with 4% PAF and stained with Oil Red O (ORO, Sigma-Aldrich) for adipogenic and with Alizarin Red (ARS, Sigma-Aldrich) 40mM pH 4.1 for osteogenic differentiation. The presence of lipid vacuoles and the production of calcium deposits was examined by light microscopy (Leica ICC50HD, 200x, 400x magnification).
For chondrogenic differentiation, iPSCs were cultured for 30 days in 15mL centrifuge tubes in Chondrogenic Medium (LONZA). The medium was changed every 2/3 days. Cells were then washed, fixed in 4% PAF, included in OCT (Fisher), and frozen at -80°C. Four µm sections were cut, stained using the primary goat antibody against collagen II (Santa Cruz Biotechnology, Inc.;1:200), and secondary AlexaFluor® 546 donkey anti-goat IgG antibody (Invitrogen; 1:500) following standard protocol. Nuclei were stained with DAPI (SIGMA; 1:1000) and observed under fluorescence microscope (LEICA DM5500B).

Flow cytometry analysis

ECs were characterized by flow cytometric analysis. Cells were detached with Acutase (Lonza), re-suspended in staining buffer (PBS, BSA 0,5% and NaN_3 0,1%) and incubated with the antibody of interest for 30 min on ice. Antibodies used are listed below. For each sample, $1.5x10^5$ live events were acquired on the Attune NxT Acoustic Focusing Cytometer (ThermoFisher Scientific, Waltham, MA, USA). Data were analyzed by FCS Express 6 (DeNovo Software, Glendale, CA, USA). Unstained cells were used as negative control. Human BOECs and human foreskin fibroblasts were used as positive and negative control for endothelial markers.

In vitro tubulogenesis assay

Pure Matrigel (BD Bioscience) was added to each well of a 24-well tissue culture plate and allowed to solidify at 37°C for 1 hour. Then 0.3 ml of a cell suspension containing 105 endothelial cells in EB medium was placed on top of the Matrigel. Plates were incubated at 37°C, 5% CO2, and observed at 16, 18 and 20 hours for cellular formation into capillary-like structures.

Immunostaining

For immunofluorescence staining iPSCs were cultured into slide flasks (NUNC) on irradiated HFF in HES medium, ECs were plated on 12 mm Ø dish glass pre-coated with 0,1% gelatin (Sigma-Aldrich) at concentration of $2x10^4$. Cells were fixed in PAF 4% for 5min, for nuclear staining permeabilized in 0,5% PBS-TritonX100 for 7 min and then incubated with blocking buffer (5% goat serum, 1% BSA, 0.1% Triton X-100 in PBS) for 1h at room temperature (RT). Mouse tissues were fixed in 4% PFA for 2h at 4°C, equilibrated in sucrose, and embedded in cryostat embedding medium (Bio-Optica). Cryostat sections of 4µm thickness were blocked in buffer containing 5% goat serum, 1% BSA, and 0.1% Triton X-100 in PBS, incubated with primary antibody for 2 hours at RT and then incubated for 45 min at RT in the dark with the secondary antibody. Nuclei were stained with DAPI (Sigma). Primary and secondary antibodies used and dilutions are detailed below.

Bleeding assay

Bleeding assay was performed on anesthetized mice by cutting the distal portion of the tail at a diameter of 2,5-3 mm. Tails were then placed in a conical tube containing 14 ml of saline at 37 °C and blood was collected for 10 min.. Tubes were centrifuged to collect erythrocytes, resuspended in red blood lysis buffer (155 mM NH4Cl, 10 mM KHCO3, and 0.1 mM EDTA), and the absorbance of the sample was measured at wavelength 575 nm. Results were analyzed by comparing the amount of blood loss obtained from treated HA mice with WT and untreated HA mice serving as controls.

Supplemental experimental procedures tables

Supplemental Experimental procedure table S2: Primers used in RT-PCR and Real Time (Related to figure 1-4, 6, S2, S3, S5)

Gene	Synthetic oligonucleotide	Attempted band
ACTB	S: 5'-GAGAAAATCTGGCACCACACC-3'	120 bp
	A: 5'-CGACGTAGCACAGCTTCTC-3'	
OCT4	S: 5'-CGTAAGCAGAAGAGGATCACC-3'	179 bp
	A: 5'-GCTTCCTCCACCCACTTCTGC-3'	
SOX2	S: 5'-GCAGCTACAGCATGATGCAGG-3'	134 bp
	A: 5'-AGCTGGTCATGGAGTTGTACTGC-3'	
KLF4	S: 5'-CCAGAGGAGCCCAAGCCAA-3'	130 bp
	A: 5'-CGCAGGTGTGCCTTGAGATG-3'	
NCAM	S: 5'-ATGGAAACTCTATTAAAGTGAACCTG-3'	178 bp
	A: 5'-TAGACCTCATACTCAGCATTCCAGT-3'	
NES	S: 5'-CAGCGTTGGAACAGAGGTTGG-3'	388 bp
	A: 5'-TGGCACAGGTGTCTCAAGGGTAG-3'	
ACTA2	S: 5'-CTGTTCCAGCCATCCTTCAT-3'	316 bp
	A: 5'-CGGCTTCATCGTATTCCTGT-3'	
TBXT	S: 5'-CGGAACAATTCTCCAACCTATT-3'	357 bp
	A: 5'-GTACTGGCTGTCCACGATGTCT -3'	
AFP	S: 5'-ACTCCAGTAAACCCTGGTGTTG-3'	255 bp
	A: 5'-GAAATCTGCAATGACAGCCTCA-3'	
FOXA2	S: 5'-ATGCACTCGGCTTCCAGTAT-3'	577 bp
	A: 5'-GGTAGATCTCGCTCAGCGTC-3'	
KDR	S: 5'- TGCAAGGACCAAGGAGACTATGT -3'	458 bp
	A: 5'- TAGGATGATGACAAGAAGTAGCC -3'	
TEK-2	S: 5'-AGACCAGCACGTTGATGTGA-3'	127 bp
	A: 5'-TGGGTTGCTTGACCCTATGT-3'	
VEC	S: 5'-CAGCCCAAAGTGTGTGAGAA-3'	162 bp
	A: 5'-TGTGATGTTGGCCGTGTTAT-3'	
ENG	S: 5'-GCCAGCATTGTCTCACTTCA-3'	135 bp
	A: 5'-GGCACACTTTGTCTGGATCA-3'	
PECAM1	S: 5'-AGGTCAGCAGCATCGTGGTCAACAT-3'	187 bp
	A: 5'-GTGGGGTTGTCTTTGAATACCGCAG-3'	
VWF	S: 5'- GTTCGTCCTGGAAGGATCGG -3'	168 bp
	A: 5'- CACTGACACCGTAGTGAGAC -3'	
hF8 A2-A3 domain	S: 5'- TGCCACAACTCAGACTTTCG-3'	184 bp
	A: 5'- GATGGCGTTTCAAGACTGGT -3'	
F8	S: 5'- GGAGAGTAAAGCAATATCAGATGC -3'	398 bp
	A: 5'- GGTGAATTCGAAGGTAGCGAC -3'	
IFI27	S: 5'- TCTGGCTCTGCCGTAGTTTT-3'	243 bp
	A: 5'- GAACTTGGTCAATCCGGAGA -3'	
CDH11	S: 5'- TGGCAGCAAGTATCCAATGG-3'	200 bp
	A: 5'- TTTGGTTACGTGGTAGGCAC-3'	
NRCAM	S: 5'- TCCAGAAGGCAATGCAAGTA-3'	117 bp
	A: 5'- AGCATTCCATCTTCCTTTGC -3'	
COL4A1	S: 5'- GGCCTATGAGTCCTGGGTAC -3'	146 bp
	A: 5'- TGGATTTCAGGGGATGCCAG -3'	
GATA2	S: 5'- GCTAACCCTTCAACCCCCTC-3'	200 bp
	A: 5'- AATTGCAAAGCTCCCAACCT-3'	
GATA3	S: 5'- GAACCGGCCCCTCATTAAG-3'	216 bp
	A: 5'- ATTTTTCGGTTTCTGGTCTGGAT-3'	
SEMA3A	S: 5'- GGCATATAATCAGACTCACTTGTACGC-3'	445 bp
	A: 5'- CTTGCATATCTGACCTATTCTAGCGTG-3'	

ITGA5	S: 5'- AATCTTCCAATTGAGGATATCAC -3'	140 bp
	A: 5'- AAAACAGCCAGTAGCAACAAT -3'	
ETS-1	S: 5'- CATATCAAGTTAATGGAGTC-3'	268 bp
	A: 5'- TGTTTGATAGCAAAGTAGTC -3'	
ETS-2	S: 5'- GTGGAGTGAGCAACAGGTAT-3'	282 bp
	A: 5'- CCAAAACCTAATGTATTGCTG -3'	
PROX1	A: 5'- GGCTCTCCTTGTCGCTCATA -3'	155 bp
	A: 5'- GGAGCTGGGATAACGGGTAT -3'	
CXCL12	A: 5'- TCAGCCTGAGCTACAGATGC -3'	161 bp
	A: 5'- CTTTAGCTTCGGGTCAATGC -3'	
COLEC12	A: 5'- AGCAGTGGAAAGTGACCTGA -3'	117 bp
	A: 5'- CGAAGTTGCTGACGGAGATC-3'	
FLT4	A: 5'- AACATCACGGAGGAGTCACA-3'	135 bp
	A: 5'- GTCCTCGCTGTCCTTGTCT-3'	
CDLN7	A: 5'- TATACGGGCCTTCTTCACTTTG-3'	253 bp
	A: 5'- CTATGCGGGTGACAACATCATC-3'	
HAPLN1	A: 5'- AGTCTACTTCTTCTGGTGCTGATTT-3'	111 bp
	A: 5'- TAGATGGGGGCCATTTTCT-3'	
PDPN	A: 5'- CCAGGAGAGCAACAACTCAA-3'	268 bp
	A: 5'- GATGCGAATGCCTGTTACAC-3'	
MRC1	A: 5'- GGGCAGTGAAAGCTTATGGA-3'	162 bp
	A: 5'- CCTGTCAGGTATGTTTGCTCA-3'	
LYVE1	A: 5'- GCCTGTAGGCTGCTGGGACTAAG-3'	519 bp
	A: 5'- CCCAGCAGCTTCATTCTTGAATG-3'	
Wpre/ dNEF	S: 5'- TCTGGCTCTGCCGTAGTTTT-3'	200 bp
	A: 5'- GGCTAAGATCTACAGCTGCCTTG-3'	
mGAPDH	S: 5'- AAACAGGGGAGCTGAGATCA-3'	133 bp
	A: 5'- TGTGGTACGTGCATAGCTGA-3'	
NANOG Met	S: 5'- TGGTTAGGTTGGTTTTAAATTTTTG-3'	336 bp
	A: 5'- ACCCACCCTTATAAATTCTCAATTA-3'	

Supplemental experimental procedures table S3: Antibodies used for FACS staining (Related to figure 2, 4, 5, S4, S5)

Antibody	Reactivity	Manufacturer	Format	Catalog #
CD45	human	MiltenyiBiotec	PE	130-110-632
CD34	human	Immunotools	PE	21270344
VWF	human	Sigma Aldrich	/	HPA001815
Goat anti-rabbit	rabbit	Life Technologies	AlexaFluor 546	A-11010
KDR	human	MiltenyiBiotec	PE	130-098-905
TIE-2	human	MiltenyiBiotec	PE	130-101-606
CD31	human	Immunotools	APC	21270316
VEC	human	MiltenyiBiotec	PE	130-100-716

Supplemental experimental procedures Table S4: Antibodies used for immunofluorescence staining (Related to figure 1, 4-6, S2, S3, S5)

Primary antibodies	**Host**	**Reactivity**	**Manufacturer**	**Dilution**	**Catalog #**
OCT4	Rabbit	Human	Abcam	1:100	ab18976
SOX2	Rabbit	Human	Abcam	1:100	ab97959
TRA1-81	Mouse	Human	Abcam	1:100	ab16289
SSEA4	Mouse	Human	Millipore	1:100	MAB4304
FVIII	Mouse	Human	Green Mountain	1:100	GMA-8015
CD31	Mouse	Human	BD Bioscience	1:100	550389
GFP	Rabbit		Life Technologies	1:300	A-11122
Secondary antibodies	**Fluorophores**		**Manufacturer**	**Dilution**	**Catalog #**
Goat anti-Rabbit	AlexaFluor 488 or 546		Life Technologies	1:500	A-11034 / A-11010
Goat anti-Mouse	AlexaFluor 488 or 546		Life Technologies	1:500	A32723 / A-11003

Supplemental references

Sprio, A.E., Di Scipio, F., Raimondo, S., Salamone, P., Pagliari, F., Pagliari, S., Folino, A., Forte, G., Geuna, S., Di Nardo, P., *et al.* (2012). Self-renewal and multipotency coexist in a long-term cultured adult rat dental pulp stem cell line: an exception to the rule? Stem cells and development *21*, 3278-3288.

Stevens-Kroef, M., Simons, A., Rack, K., and Hastings, R.J. (2017). Cytogenetic Nomenclature and Reporting. Methods Mol Biol *1541*, 303-309.

Zamperone, A., Pietronave, S., Merlin, S., Colangelo, D., Ranaldo, G., Medico, E., Di Scipio, F., Berta, G.N., Follenzi, A., and Prat, M. (2013). Isolation and characterization of a spontaneously immortalized multipotent mesenchymal cell line derived from mouse subcutaneous adipose tissue. Stem cells and development *22*, 2873-2884.