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Supplemental information

Efficient and safe correction of hemophilia A by lentiviral vector-transduced BOECs in an implantable device

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Lentiviral vector generation. Third generation self-inactivating LVs were produced as previously published.[1] Briefly, 293T cells were expanded and transiently transfected by the calcium phosphate precipitation method with four plasmids encoding for two core packaging constructs (pMDLg/pol and pRSV-Rev), the envelope construct (pMD.VSV.G), and the transfer vector construct (pVEC.hBDD-FVIII.LV or pVEC.GFP.LV). The cell supernatant was harvested, and LV particles were concentrated by ultracentrifugation. For GMP-grade production of LV, the KR2i TFF System® (Spectrum Lab) was used according to the manufacturer's protocol. The product is ISO 9001:2008 certified. The system consists of a digital peristaltic pump, man/machine interface with a graphical LCD display, digital pressure monitor, KR2i Easy-Load Pump head, Automatic Backpressure Valve, filter module stand, and a real-time data collection software. The Tangential Flow Filtration (TFF) System uses a constant turbulent flow along a porous membrane to eliminate impurities from the sample. The tangential flow along the membrane prevents the accumulation of material on the membrane surface, as opposed to the classical "dead-end filtration", and allows the maximum recovery with high purity of the product.

Analysis of lentiviral vector copy number. Genomic DNA from LV-VEC.hBDD-FVIII and LV-VEC.GFP transduced BOECs was isolated using ReliaPrep gDNA Tissue Miniprep System (Promega). Real-time qPCR was used to evaluate the integrated LV copy numbers per cell in the DNA. Primers used for the qPCR recognize the Wpre sequence: Forward TTGCTTCCCGTATGGCTTTC, Reverse AGCTGACAGGTGGTGGCAAT. Finally, TU/ml was calculated with the following formula: TU/ml = (LV copies/cell × No. of transduced cells) / LV volume (expressed in ml).

Evaluation of HIV-1 p24 in culture medium of transduced BOECs. The presence of HIV-1 p24 was evaluated in culture medium of transduced cells after several time points and passages. Samples were analyzed using Liaison® XL (Diasorin) in the Virology Laboratory of the Hospital (Ospedale Maggiore della Carità di Novara, Italy).

RNA isolation and RT-PCR. Total RNA was isolated by Isol-RNA Lysis Reagent (Invitrogen). One µg of RNA was treated with DNase I (Thermo Scientific), and cDNAs were obtained using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). All PCRs were performed with GoTaq® Flexi DNA Polymerase (Promega). Primers, annealing temperatures, and product sizes are listed below in Supplemental Table 1. PCR products were resolved in 2% agarose gels using 100 bp DNA ladder (Thermo Scientific).

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 h. A cell suspension containing 10^5 BOECs, resuspended in culture medium, was placed on top of the Matrigel. Plates were incubated at 37°C, 5% CO_2 and observed and imaged at 16 h to detect capillary-like structure formation using an inverted microscope Leica ICC50.

Flow cytometry analysis. BOECs were characterized by flow cytometric analysis using antibodies listed in Supplemental Table 2. For each sample, 1.5×10^5 live events were acquired either on the Attune NxT Acoustic Focusing Cytometer (ThermoFisher Scientific, Waltham, MA, USA) or on BD FACSCanto II. Data were analyzed by FCS Express 6 (DeNovo Software, Glendale, CA, USA) or using FlowJo Software V10.6 (FlowJo LLC).

Histopathological staining and analysis of samples from Cell Pouch™. Sernova Cell Pouches™ with transplanted FVIII-BOECs were explanted from the mice, dissected into segments, fixed in 10% neutral buffered formalin, and paraffin-embedded. Sections (5-6 µM-thick) were stained with hematoxylin and eosin (H&E) and Masson's Trichrome (Nucro-Technics, Scarborough, Ontario).

Immunostaining. For immunofluorescence (IF) staining, BOECs were cultured on plastic and fixed in PFA 4%, for nuclear staining permeabilized in 0,5% PBS-Triton X100 and then incubated with blocking buffer (BB, 5% goat serum, 1% BSA, 0.1% Triton X-100 in PBS) at room temperature (RT). Mouse tissues were fixed in 4% PFA, equilibrated in sucrose, and embedded in cryostat embedding medium (Bio-Optica). Cryostat sections of 4-µm thickness were blocked in BB, incubated with primary antibody at RT, and then incubated in the dark at RT with the secondary antibody. The Cell Pouch™ with transplanted FVIII-BOECs was explanted from each animal, dissected into segments, and immediately cryopreserved in Tissue-Tek® O.C.T. compound (VWR) using an isopentane (2-methylbutane)/dry ice slurry (-70°C), and stored at -80°C. Prior to staining, cryostat sections (5-6 µm) were air-dried and pre-treated by immersion in cold acetone (-20°C), followed by washes in tris-buffered saline (TBS). Sections were blocked in TBS containing 10% goat serum and 1% BSA. A list of the antibodies used in these experiments is provided in Supplemental Table 3.

Histology analysis and preservation techniques of tissues. Following explantation, gross observations of Cell Pouches™ were made and images taken just prior to further histological processing. The 1 Plug Cell Pouches™ were then dissected into 3 segments (a – c) (see Figure 1). Segments 'a' and 'c' were processed for fixation in 10% neutral buffered formalin (Sernova Histology SOP-H900). Cell Pouches™ were washed from 10% formalin in 1X phosphate buffered saline (PBS) to 70% ethanol and subsequently processed for paraffin embedding. Segment 'b' was flash frozen for cryopreservation at the time of dissection using an isopentane/dry ice slurry and embedded in optimal cutting temperature compound (O.C.T.) (Sernova Histology SOP-H936). Cryopreserved or paraffin-embedded segments were then serially sectioned with a cryostat or microtome, respectively.

Preparation of slides and high-resolution images with description of tissue stains. Prior to staining or immunohistochemistry (IHC) analysis, sections were deparaffinized and rehydrated. Sections were stained with H&E for either formalin-fixed paraffin embedded (FFPE) or cryopreserved tissues. Masson's trichrome staining was performed on FFPE (Nucro-Technics, Scarborough, Ontario). For IHC staining, cryopreserved O.C.T. embedded tissues were dried, pre-treated with fixation and permeabilization with cold acetone (-20 ºC, 10 min) and stained to detect microvessel

formation with von Willebrand factor (vWF). For human cell detection, sections were stained with a human leukocyte antigen (HLA-ABC) antibody. High resolution images and full slide scanning of the sections were imaged with an EVOS™ FL Auto 2 Imaging System (Invitrogen™, ThermoFisher Scientific) for both light microscopy (H&E and Masson's Trichrome) and fluorescent IHC (vWF/HLA).

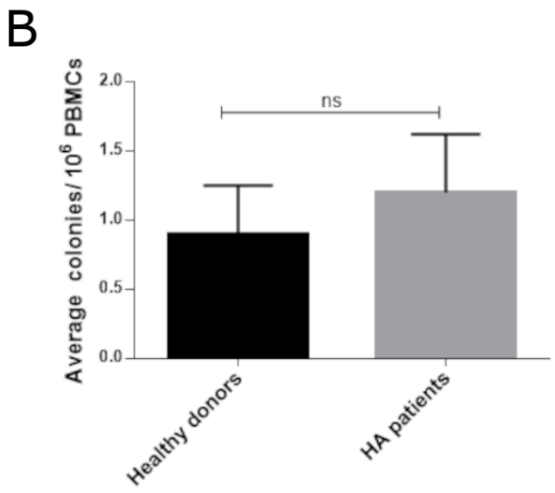
Analysis methodology. The following histological assessment was conducted on the serial sections of formalin-fixed paraffin embedded (FFPE) segments taken within the chambers of each of the Cell Pouches™ from the animals across the doses and explanation time points: 1) stromal development, including type, distribution, and maturity; 2) vascularity, including neovascularization, established vessels, and their respective relationship to the chamber area; 3) inflammation, including type and relative abundance; and 4) hemorrhage. Masson's trichrome stains were assessed for collagen deposition as a marker of stromal maturity. Histological variables were assessed in a semi-quantitative fashion: - absent; + mild; ++ moderate; and +++ marked.

A histological assessment was conducted of the serial sections of frozen embedded segments taken within the chambers of each of the Cell Pouches™ from the animals across the doses and explanation time points: 1) cell transplant survival; 2) interactions and development post-transplant; 3) interactions of the surrounding pre-vascularized tissue of the Cell Pouch™; and 4) blood vessel formation relative to transplant cells. Histological variables were assessed in a semi-quantitative fashion: - negative (no staining); +/- equivocal staining; + mild positivity; ++ moderate positivity; +++ marked positivity; n/a image not available.

For histological assessment the slides and high-resolution images were sent to a certified pathologist for analysis. The assessment was conducted in a blinded-fashion, with no knowledge of the animal time points. The assessment was unblinded for writing the final report. Pathological definitions were as follows: Fibroblastic stroma – mesenchymal tissue consisting of fibroblastic cells and the extracellular matrix, including variable collagen produced by these cells; Collagen – usually a fibrillar protein within the extracellular matrix of connective tissue that provides mechanical strength to the tissues; Neovascularization – tiny, immature capillary-like vessels arising during new blood vessel formation and growth.

A

Name of donor	Donor	Mutation	ml of peripheral blood samples	No. of BOECs colonies
pHA1	Severe HA	c.6273G>A exon 21	25	30
pA	Severe HA	intron 22 inversion	22	60
pC	Severe HA	unknown	25	40
pD	Severe HA	intron 22 inversion	26	30



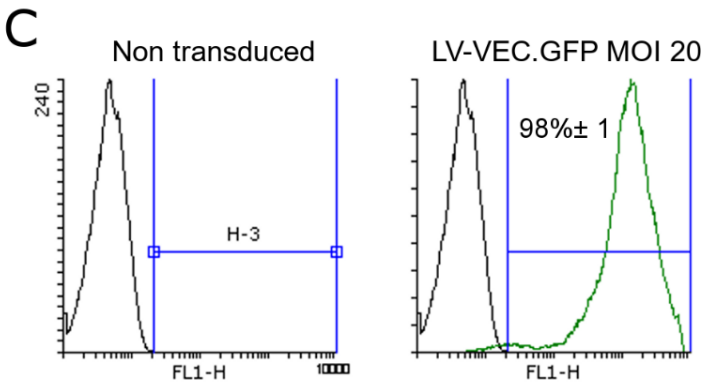
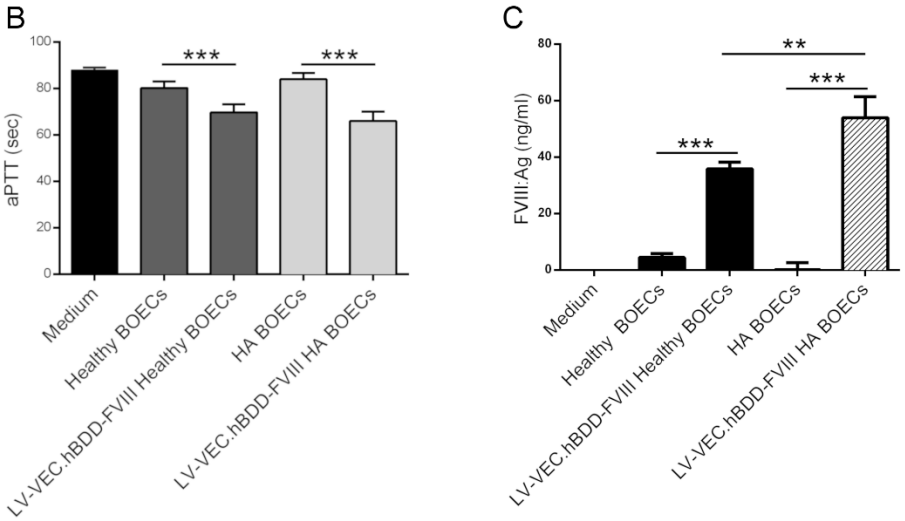


Figure S 1. (A) List of hemophilic patients from whom BOECs were isolated. (B) Average number of colonies calculated on 10^6 PBMCs cultured. Statistical analysis was performed using t-test, non-parametric, p-value 0.098. (C) Representative histograms for GFP evaluation by FACS analysis in healthy and HA BOECs transduced with an MOI of 20.

A

Sample	LV copy number/ cell
Healthy BOECs	0
LV-VEC.GFP Healthy BOECs	5.2± 0.6
LV-VEC.hBDD-FVIII Healthy BOECs	3.1± 0.5
HA BOECs	0
LV-VEC.GFP HA BOECs	6.2± 0.8
LV-VEC.hBDD-FVIII HA BOECs	3±0.2



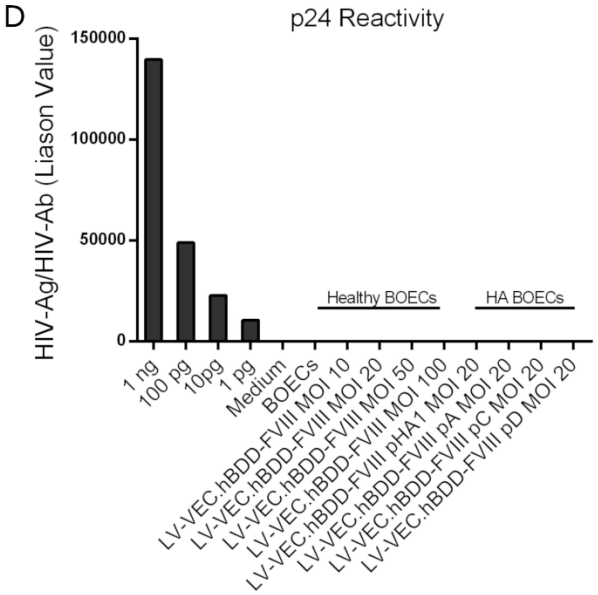


Figure S2. (A) qPCR analysis of integrated LV copy number/cell. (**B**) aPTT assay on supernatant of transduced and non-transduced healthy and HA BOECs (**C**) Antigen assay on supernatant of transduced and non-transduced healthy and HA BOECs. Data are expressed as mean ± SD and are representative of four independent experiments. (**D**) HIV-1 p24 analysis on medium of non-transduced and transduced and healthy and HA BOECs at different MOIs.

Table S1. Histological features of NSG-HA Cell Pouches™ transplanted with FVIII-BOECs (H&E and Trichrome).

Animal ID	Inflammation	Fibroblastic stroma	Collagen deposition	Neovascularization	Established vessels	Hemorrhage
Cell Lot HA1, 4 weeks, 10×10^6 (Dose 1)						
396-LEP	-	+++	+	++	*	-
398-REP	-	+++	++	++	++	-
Cell Lot HA1, 4 weeks, 5×10^6 (Dose 2)						
397-LEP	-	+	+	+	*	-
397-BEP	-	+++	+++	++	++	-
Cell Lot HA1, 4 weeks, 2×10^6 (Dose 3)						
398-BEP	-	+++	+	++	*	-
Cell Lot HA1, 8 weeks, 10×10^6 (Dose 1)						
405-NEP	-	+++	+++	++	++	-
Cell Lot HA1, 8 weeks, 5×10^6 (Dose 2)						
414-NEP	-	+++	+	++	+	-
415-BEP	-	+++	+++	++	+	-
Cell Lot HA1, 8 weeks, 2×10^6 (Dose 3)						
402-LEP	-	+++	++	++	++	-
405-REP	-	++	+	++	+	-
413-LEP	-	++	+++	++	*	-
Cell Lot HA1, 12 weeks, 10×10^6 (Dose 1)						
399-NEP	-	+++	++	++	+	-
413-BEP	-	+++	+++	++	++	-
Cell Lot HA1, 12 weeks, 5×10^6 (Dose 2)						
406-REP	-	+++	++	++	*	-
406-NEP	-	+++	++	++	+	-
411-BEP	-	+++	+++	+	++	-
Cell Lot HA1, 12 weeks, 2×10^6 (Dose 3)						
396-REP	-	+++	+++	+	+	+
398-NEP	-	+++	++	++	+	-
399-BEP	-	+++	++	++	+	-
Cell Lot HAA, 4 weeks, 10×10^6 (Dose 1)						
520-RREP	-	+++	++	++	++	-
Cell Lot HAA, 4 weeks, 5×10^6 (Dose 2)						
523-LEP	-	+++	++	++	+	-
Cell Lot HAA, 8 weeks, 10×10^6 (Dose 1)						
520-NEP	-	+++	+++	++	+	-
524-LEP	-	+++	+++	++	++	-
Cell Lot HAA, 8 weeks, 5×10^6 (Dose 2)						
523-REP	-	+++	++	++	++	-
Cell Lot HAA, 12 weeks, 10×10^6 (Dose 1)						
522-RREP	-	+++	++	++	++	-
524-BEP	-	+++	++	++	++	-
Controls (no cell transplant)						
4 weeks						
525-REP	-	++	++	++	+	-
8 weeks						
521-RREP	-	+++	++	++	++	-
525-RREP	-	+++	+	++	++	-
12 weeks						
403-NEP	-	+++	+	++	+	-
521-BEP	-	+++	++	++	++	-
525-BEP	-	+++	++	++	++	-

*present immediately adjacent to Cell Pouch™

Table S2. Immunofluorescence of NSG-HA Mouse Cell Pouches™ transplanted with FVIII-BOECs.

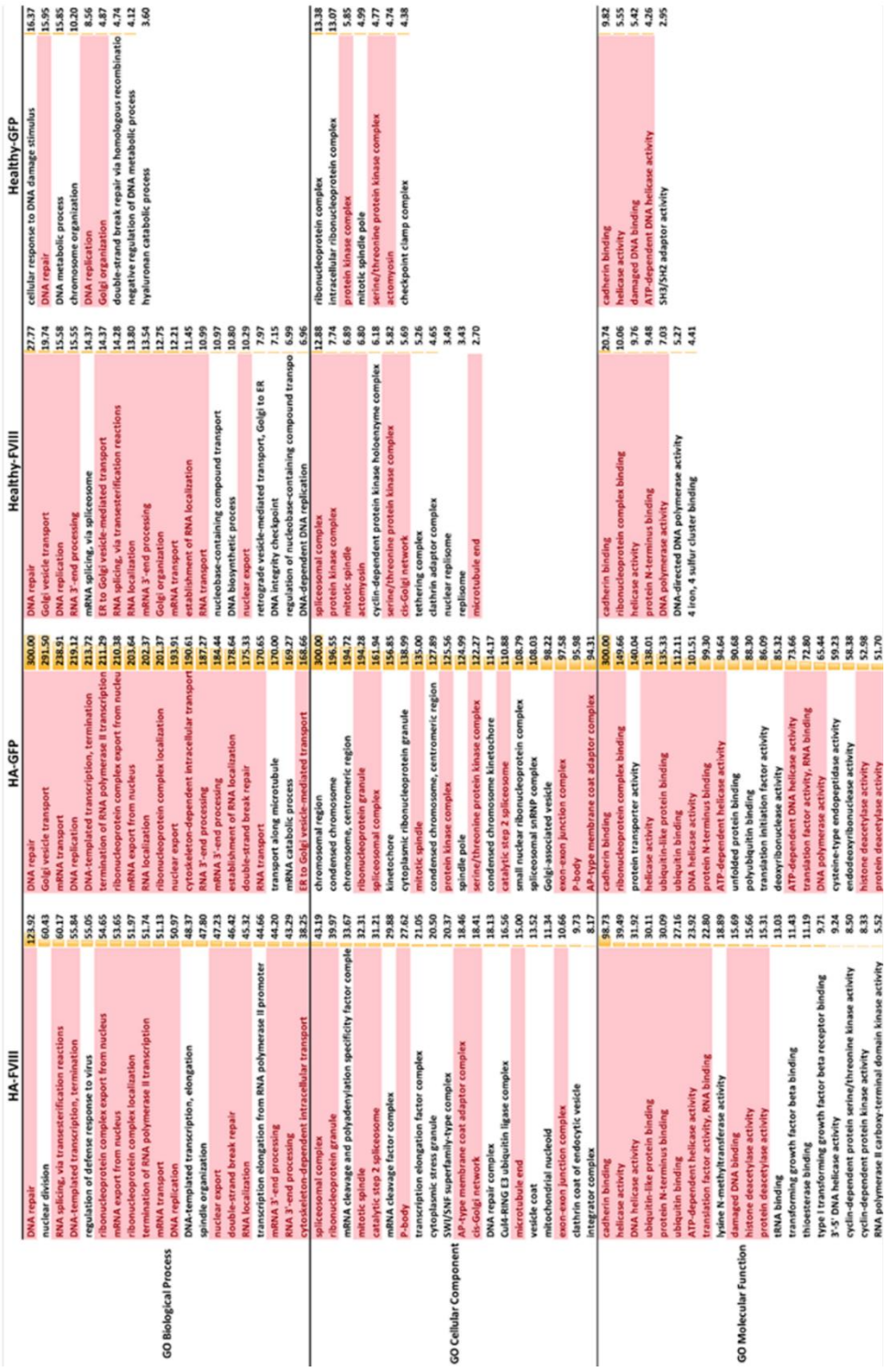
Animal ID	HLA-ABC (red)	vWF (green)
Cell Lot HA1, 4 weeks, 10×10^6 (Dose 1)		
396-LEP	+	+
398-REP	+	+
Cell Lot HA1, 4 weeks, 5×10^6 (Dose 2)		
397-REP	+	+
397-LEP	n/a	n/a
414-LEP	+/-	+
Cell Lot HA1, 4 weeks, 2×10^6 (Dose 3)		
398-BEP	-	++
Cell Lot HA1, 8 weeks, 10×10^6 (Dose 1)		
405-NEP	++	-
Cell Lot HA1, 8 weeks, 5×10^6 (Dose 2)		
414-NEP	+	n/a
415-BEP	+/-	+
Cell Lot HA1, 8 weeks, 2×10^6 (Dose 3)		
402-LEP	+/-	+
405-REP	+/-	+
413-LEP	n/a	n/a
Cell Lot HA1, 12 weeks, 10×10^6 (Dose 1)		
399-NEP	-	n/a
413-BEP	+/-	+
Cell Lot HA1, 12 weeks, 5×10^6 (Dose 2)		
406-REP	+	+
406-NEP	+/-	++
411-BEP	+/-	+
Cell Lot HA1, 12 weeks, 2×10^6 (Dose 3)		
396-REP	+/-	+
398-NEP	+	+
399-BEP	+/-	+
Cell Lot HAA, 4 weeks, 10×10^6 (Dose 1)		
520-RREP	++	++
Cell Lot HAA, 4 weeks, 5×10^6 (Dose 2)		
520-REP	++	+
523-LEP	+++	+++
Cell Lot HAA, 8 weeks, 10×10^6 (Dose 1)		
520-NEP	++	+
524-LEP	+	+
Cell Lot HAA, 8 weeks, 5×10^6 (Dose 2)		
523-REP	-	+++
Cell Lot HAA, 12 weeks, 10×10^6 (Dose 1)		
522-RREP	++	++
524-BEP	+++	+
Controls		
4 weeks		
525-REP	-	++
8 weeks		
521-RREP	-	N/A
525-RREP	-	+
12 weeks		
403-NEP	-	+
521-BEP	-	+
525-BEP	-	+

- negative (no staining); +/- equivocal staining; + mild positivity; ++ moderate positivity; +++ marked positivity; N/A image not available

Table S3. Summary of sequencing reads and IS retrieved by group. Non redundant IS (column N.IS) retrieved from Healthy or HA Donors and transduced with VEC-FVIII or VEC-GFP were grouped. IS shared between different time points of the same transduction are counted once.

Group	Donor	Transduction	Vector	MOI	Timepoint	Sample ID	N.IS
Healthy.GFP	D45	4	VEC-GFP	10+10	P5	BOEC-008	1,862
Healthy.FVIII	D45	1	VEC-FVIII	20	P5	BOEC-001	5,864
					P8	BOEC-002	
					P11	BOEC-003	
					P15	BOEC-004	
					70 days HA Beads	BOEC-010	
					70 days HA Beads	BOEC-011	
					70 days HA Beads	BOEC-013	
		2	VEC-FVIII	30	P11	BOEC-005	
		3		50	P11	BOEC-006	
	D2	5	VEC-FVIII	20	P5	BOEC-014	
					P8	BOEC-043A	
	D3	6	VEC-FVIII	20	P5	BOEC-043B	
					P8	BOEC-043C	
HA.GFP	pHA1	8	VEC-GFP	20	P5	BOEC-020	106,554
					P8	BOEC-021	
					P12	BOEC-022	
	pA	10	VEC-GFP	20	P5	BOEC-032	
					P8	BOEC-033	
	pC	12	VEC-GFP	20	P5	BOEC-040	
					P8	BOEC-041	
HA.FVIII	pHA1	7	VEC-FVIII	20	P5	BOEC-016	28,069
					P8	BOEC-017	
					P10	BOEC-018	
					P15	BOEC-019	
					P11	BOEC-024	
					P11	BOEC-025	
					P14	BOEC-026	
					P11	HA1-VEC-UNILO	
					91 days HA Beads	BOEC-044A	
					91 days HA Beads	BOEC-044B	
					91 days HA Beads	BOEC-044C	
					2 weeks Cell Pouch	397-LEP	
					2 weeks Cell Pouch	398-LEP	
					4 weeks Cell Pouch	398-BEP	
					4 weeks Cell Pouch	414-LEP	
					4 weeks Cell Pouch	396-LEP	
					8 weeks Cell Pouch	402-LEP	
					8 weeks Cell Pouch	415-BEP	
					8 weeks Cell Pouch	405-NEP	
					12 weeks Cell Pouch	398-NEP	
					12 weeks Cell Pouch	406-REP	
					12 weeks Cell Pouch	399-NEP	
	pA	9	VEC-FVIII	20	P5	BOEC-027	
					P8	BOEC-028	
					P10	BOEC-029	
					P14	HA-A-p14	
					P15	BOEC-030	
					P12	BOEC-031	
					112 days HA Beads	BOEC-045A	
					112 days HA Beads	BOEC-045B	
					112 days HA Beads	BOEC-045C	
					4 weeks Cell Pouch	520-REP	
					4 weeks Cell Pouch	520-RREP	
					8 weeks Cell Pouch	520-LEP	
					8 weeks Cell Pouch	520-NEP	
					12 weeks Cell Pouch	522-RREP	
	pC	11	VEC-FVIII	20	P5	BOEC-035	
					P8	BOEC-036	
					P8	HA-C-p8	
					P10	BOEC-037	
					P11	BOEC-039	
					P14	HA-C-p14	
					P15	BOEC-038	
					P19	HA-C-p19	
					112 days HA Beads	BOEC-046A	
					112 days HA Beads	BOEC-046B	
					112 days HA Beads	BOEC-046C	
	pD	13	VEC-FVIII	20	P16	HA-D-p16	
	TOTAL						142,349

Table S4. Gene Ontology (GO) enrichment for Molecular Function and Biological Process. IS coordinates from IS retrieved from HA.VEC-FVIII, HA.VEC-GFP, Healthy.VEC-FVIII, and Healthy.VEC-GFP were analyzed by G.R.E.A.T. enrichment discovery algorithms. GO Biological Process, Molecular Function and Cellular Component databases highlighted significantly overrepresented gene classes. The gene classes found in at least two groups are highlighted in pink.



	HA-FVIII		HA-GFP		Healthy-FVIII		Healthy-GFP	
GO Biological Process	DNA repair	123.92	DNA repair	300.00	DNA repair	27.77	cellular response to DNA damage stimulus	16.37
	nuclear division	60.43	Golgi vesicle transport	291.50	Golgi vesicle transport	19.74	DNA repair	15.95
	RNA splicing, via transesterification reactions	60.17	mRNA transport	238.91	DNA replication	15.58	DNA metabolic process	15.85
	DNA-templated transcription, termination	55.84	DNA replication	219.12	RNA 3'-end processing	15.55	chromosome organization	10.20
	regulation of defense response to virus	55.05	DNA-templated transcription, termination	213.72	mRNA splicing, via spliceosome	14.37	DNA replication	8.56
	ribonucleoprotein complex export from nucleus	54.65	termination of RNA polymerase II transcription	211.29	ER to Golgi vesicle-mediated transport	14.37	Golgi organization	4.87
	mRNA export from nucleus	53.65	ribonucleoprotein complex export from nucleu	210.38	RNA splicing, via transesterification reactions	14.28	double-strand break repair via homologous recombinatio	4.74
	ribonucleoprotein complex localization	51.97	mRNA export from nucleus	203.64	RNA localization	13.80	negative regulation of DNA metabolic process	4.12
	termination of RNA polymerase II transcription	51.74	RNA localization	202.37	mRNA 3'-end processing	13.54	hyaluronan catabolic process	3.60
	mRNA transport	51.13	ribonucleoprotein complex localization	201.37	Golgi organization	12.75		
	DNA replication	50.97	nuclear export	193.91	mRNA transport	12.21		
	DNA-templated transcription, elongation	48.37	cytoskeleton-dependent intracellular transport	190.61	establishment of RNA localization	11.45		
	spindle organization	47.80	RNA 3'-end processing	187.27	RNA transport	10.99		
	nuclear export	47.23	mRNA 3'-end processing	184.44	nucleobase-containing compound transport	10.97		
	double-strand break repair	46.42	establishment of RNA localization	178.64	DNA biosynthetic process	10.80		
	RNA localization	45.32	double-strand break repair	175.33	nuclear export	10.29		
	transcription elongation from RNA polymerase II promoter	44.66	RNA transport	170.65	retrograde vesicle-mediated transport, Golgi to ER	7.97		
	mRNA 3'-end processing	44.20	transport along microtubule	170.00	DNA integrity checkpoint	7.15		
	RNA 3'-end processing	43.29	mRNA catabolic process	169.27	regulation of nucleobase-containing compound transpo	6.99		
	cytoskeleton-dependent intracellular transport	38.25	ER to Golgi vesicle-mediated transport	168.66	DNA-dependent DNA replication	6.96		
GO Cellular Component	spliceosomal complex	43.19	chromosomal region	300.00	spliceosomal complex	12.88	ribonucleoprotein complex	13.38
	ribonucleoprotein granule	39.97	condensed chromosome	196.55	protein kinase complex	7.74	intracellular ribonucleoprotein complex	13.07
	mRNA cleavage and polyadenylation specificity factor comple	33.67	chromosome, centromeric region	194.72	mitotic spindle	6.89	protein kinase complex	5.85
	mitotic spindle	32.31	ribonucleoprotein granule	194.28	actomyosin	6.80	mitotic spindle pole	4.99
	catalytic step 2 spliceosome	31.21	spliceosomal complex	161.94	cyclin-dependent protein kinase holoenzyme complex	6.18	serine/threonine protein kinase complex	4.77
	mRNA cleavage factor complex	29.88	kinetochore	156.85	serine/threonine protein kinase complex	5.82	actomyosin	4.74
	P-body	27.62	cytoplasmic ribonucleoprotein granule	138.99	cis-Golgi network	5.69	checkpoint clamp complex	4.38
	transcription elongation factor complex	21.05	mitotic spindle	135.00	tethering complex	5.26		
	cytoplasmic stress granule	20.50	condensed chromosome, centromeric region	127.89	clathrin adaptor complex	4.65		
	SWI/SNF superfamily-type complex	20.37	protein kinase complex	125.56	nuclear replisome	3.49		
	AP-type membrane coat adaptor complex	18.46	spindle pole	124.99	replisome	3.43		
	cis-Golgi network	18.41	serine/threonine protein kinase complex	122.27	microtubule end	2.70		
	DNA repair complex	18.13	condensed chromosome kinetochore	114.17				
	Cul4-RING E3 ubiquitin ligase complex	16.56	catalytic step 2 spliceosome	110.88				
	microtubule end	15.00	small nuclear ribonucleoprotein complex	108.79				
	vesicle coat	13.52	spliceosomal snRNP complex	108.03				
	mitochondrial nucleoid	11.34	Golgi-associated vesicle	58.22				
	exon-exon junction complex	10.66	exon-exon junction complex	97.58				
	clathrin coat of endocytic vesicle	9.73	P-body	95.98				
	integrator complex	8.17	AP-type membrane coat adaptor complex	94.31				
GO Molecular Function	cadherin binding	58.73	cadherin binding	300.00	cadherin binding	20.74	cadherin binding	9.82
	helicase activity	39.49	ribonucleoprotein complex binding	169.66	ribonucleoprotein complex binding	10.06	helicase activity	5.55
	DNA helicase activity	31.92	protein transporter activity	140.04	helicase activity	9.76	damaged DNA binding	5.42
	ubiquitin-like protein binding	30.11	helicase activity	138.01	protein N-terminus binding	9.48	ATP-dependent DNA helicase activity	4.26
	protein N-terminus binding	30.09	ubiquitin-like protein binding	135.33	DNA polymerase activity	7.03	SH3/SH2 adaptor activity	2.95
	ubiquitin binding	27.16	ubiquitin binding	112.11	DNA-directed DNA polymerase activity	5.27		
	ATP-dependent helicase activity	23.92	DNA helicase activity	101.51	4 iron, 4 sulfur cluster binding	4.41		
	translation factor activity, RNA binding	22.80	protein N-terminus binding	99.30				
	lysine N-methyltransferase activity	18.89	ATP-dependent helicase activity	94.64				
	damaged DNA binding	15.69	unfolded protein binding	90.68				
	histone deacetylase activity	15.66	polyubiquitin binding	88.30				
	protein deacetylase activity	15.31	translation initiation factor activity	86.09				
	tRNA binding	13.03	deoxyribonuclease activity	85.32				
	transforming growth factor beta binding	11.43	ATP-dependent DNA helicase activity	73.66				
	thioesterase binding	11.19	translation factor activity, RNA binding	72.80				
	type I transforming growth factor beta receptor binding	9.71	DNA polymerase activity	65.44				
	3'-5' DNA helicase activity	9.24	cysteine-type endopeptidase activity	59.23				
	cyclin-dependent protein serine/threonine kinase activity	8.50	endodeoxyribonuclease activity	58.38				
	cyclin-dependent protein kinase activity	8.33	histone deacetylase activity	52.98				
	RNA polymerase II carboxy-terminal domain kinase activity	5.52	protein deacetylase activity	51.70				

Table S5. Common Insertion site (CIS) analysis in 2 healthy BOECs, and 4 HA BOECs.

Subject ID	Transduction	Vector	MOI	Gene Name	Chr.	Integration locus	N IS per Gene	average transcript length	integration frequency with tolerance	tdist fdr
dD3	6	VEC-FVIII	20	SPG7	16	89,585,048	5	39,358	0.036	0.078
dD45	1	VEC-FVIII	20	SZT2	1	43,952,080	13	64,363	0.079	0.006
				MROH1	8	145,269,797	10	89,530	0.053	0.054
				TRAF2	9	139,825,912	6	40,103	0.043	0.114
				CPSF1	8	145,629,805	5	16,288	0.043	0.114
dD45	4	VEC-GFP	10+10	NPLOC4	17	79,591,992	10	36,856	0.073	0.005
				TRAF2	9	139,825,791	8	40,103	0.057	0.020
pA	10	VEC-GFP	20	SZT2	1	43,955,802	117	64,363	0.712	0.510
				NPLOC4	17	79,602,479	106	36,856	0.775	0.510
pA	9	VEC-FVIII	20	NPLOC4	17	79,600,522	7	36,856	0.051	0.067
				PMPCA	9	139,315,510	5	13,189	0.044	0.108
pC	11	VEC-FVIII	20	SZT2	1	43,955,797	49	64,363	0.298	0.039
				MROH1	8	145,299,016	46	89,530	0.243	0.097
				NPLOC4	17	79,600,496	45	36,856	0.329	0.032
pD	13	VEC-FVIII	20	NSD1	5	176,707,259	10	166,759	0.037	0.100
				ZGPAT	20	62,354,996	6	28,232	0.047	0.047
				NONO	X	70,517,315	5	17,977	0.042	0.074
				MAN1B1	9	139,997,714	4	22,261	0.033	0.139
				PTBP1	19	808,672	4	14,936	0.035	0.122
pHA1	7	VEC-FVIII	20	PHRF1	11	599,765	8	35,777	0.059	0.077
				ZNF251	8	145,983,504	8	34,677	0.059	0.077
pHA1	8	VEC-GFP	20	MROH1	8	145,292,887	121	89,530	0.638	0.337
				SZT2	1	43,954,990	112	64,363	0.681	0.337
				NPLOC4	17	79,599,815	94	36,856	0.687	0.337
				ZNF251	8	145,987,938	77	34,677	0.572	0.361

Table S6. Primers used in RT-PCR and Real Time.

Gene	Synthetic oligonucleotide	Expected band
ACTB	F: 5'-GAGAAAATCTGGCACCACACC-3'	412 bp
	R: 5'-CGACGTAGCACAGCTTCTC-3'	
KDR	F: 5'- TGCAAGGACCAAGGAGACTATGT -3'	459 bp
	R: 5'- TAGGATGATGACAAGAAGTAGCC -3'	
TEK	F: 5'-AGACCAGCACGTTGATGTGA-3'	127 bp
	R: 5'-TGGGTTGCTTGACCCTATGT-3'	
CDH5	F: 5'-CAGCCCAAAGTGTGTGAGAA-3'	162 bp
	R: 5'-TGTGATGTTGGCCGTGTTAT-3'	
PECAM1	F: 5'-AGGTCAGCAGCATCGTGGTCAACAT-3'	469 bp
	R: 5'-GTGGGGTTGTCTTTGAATACCGCAG-3'	
VWF	F: 5'- TGGAGTACCCCTTCAGCGAG -3'	263 bp
	R: 5'- GTTGGCATTAGGGCCCACTC -3'	
F8 A2-A3 domain	F: 5'- TGCCACAACTCAGACTTTCG-3'	184 bp
	R: 5'- GATGGCGTTTCAAGACTGGT -3'	
IFI27	F: 5'- TCTGGCTCTGCCGTAGTTTT-3'	243 bp
	R: 5'- GAACTTGGTCAATCCGGAGA -3'	
CDH11	F: 5'- TGGCAGCAAGTATCCAATGG-3'	200 bp
	R: 5'- TTTGGTTACGTGGTAGGCAC-3'	
NRCAM	F: 5'- TCCAGAAGGCAATGCAAGTA-3'	117 bp
	R: 5'- AGCATTCCATCTTCCTTTGC -3'	
COL4A1	F: 5'- GGCCTATGAGTCCTGGGTAC -3'	146 bp
	R: 5'- TGGATTTCAGGGGATGCCAG -3'	
ENG	F: 5'- CCACTGCACTTGGCCTACA -3'	107 bp
	R: 5'- GCCCACTCAAGGATCTGG -3'	
GATA3	F: 5'- GAACCGGCCCCTCATTAAG-3'	216 bp
	R: 5'- CTTGCATATCTGACCTATTCTAGCGTG-3'	
ITGA5	F: 5'- TGCAGTGTGAGGCTGTGTACA -3'	88 bp
	R: 5'- GTGGCCACCTGACGCTCT -3'	
ETS-1	F: 5'- CATATCAAGTTAATGGAGTC-3'	268 bp
	R: 5'- TGTTTGATAGCAAAGTAGTC -3'	
ETS-2	F: 5'- GTGGAGTGAGCAACAGGTAT-3'	282 bp
	R: 5'- CCAAAACCTAATGTATTGCTG -3'	
Wpre/ dNEF	F: 5'- TCTGGCTCTGCCGTAGTTTT-3'	200 bp
	R: 5'- GGCTAAGATCTACAGCTGCCTTG-3'	

Table S7. Antibodies used for FACS staining.

Antibody	Reactivity	Manufacturer	Format
CD45	Human	clone 32D12, Miltenyi Biotec	PE
Isotype mouse IgG1		ThermoFisher Scientific	PE
CD34	Human	clone 4H11[APG], Invitrogen	PE
Isotype mouse IgG1		ThermoFisher Scientific	PE
Anti -mouse	Mouse	Thermo Scientific	488
FVIII	Human	Clone GMA-8015, Green Mountain	Not conjugated

KDR	Human	clone ES8-20E6, Miltenyi Biotec	PE
Isotype mouse IgG1		ThermoFisher Scientific	PE
Tie-2	Human	clone REA198, Miltenyi Biotec	PE
REA Control Antibody, human IgG1, REAfinity (REA293)		Miltenyi Biotec	PE
CD31	Human	clone MEM-05; Invitrogen	APC
Isotype mouse IgG1		ThermoFisher Scientific	APC
VE-cadherin	Human	clone REA199, Miltenyi Biotec	PE
REA Control Antibody, human IgG1, REAfinity (REA293)		Miltenyi Biotec	PE

Table S8. Antibodies used for immunofluorescence staining.

Primary antibodies	**Host**	**Reactivity**	**Manufacturer**	**Dilution**
FVIII	Mouse	Human	Clone GMA-8015, Green Mountain	1:100
CD31	Mouse	Human	BD Bioscience	1:100
HLA-ABC	Rat	Human	Novus Biologicals, clone YTH862.2	1:150
von Willebrand Factor	Rabbit	H, M, R	Millipore	1:100
GFP	Rabbit		Life Technologies	1:300
Secondary antibodies	**Fluorophores**		**Manufacturer**	**Dilution**
Goat anti-Rabbit	AlexaFluor 488 or 546		Life Technologies	1:500/1:1000
Goat anti-Rat	AlexaFluor 594			1:500
Goat anti-Mouse	AlexaFluor 488 or 546		Life Technologies	1:500

Supplemental References

1. Follenzi, A., and Naldini, L. (2002). Generation of HIV- 1 derived lentiviral vectors. Methods Enzymol *346*, 454–465.