SUPPLEMENTAL MATERIALS AND METHODS

CCL-βAS3-FB LV vector construction

The *HBBAS3* cassette (human *HBB* gene with 3 amino acid substitutions, *HBB* promoter, 3' *HBB* enhancer, and DNAase hyper-sensitive sites HS2, HS3 and HS4) and the WPRE were amplified by PCR from the DL-βAS3 LV plasmid (1) (generously provided Tim Townes, UAB, Birmingham, AL) using AccuPrime Pfx DNA polymerase (Invitrogen, Carlsbad, CA) with the primers AS3-forward (F)-and AS3-reverse (R)-. The 6.6 Kb PCR product was purified by PureLink QuickGel Extraction Kit (Invitrogen, Carlsbad, CA) and subcloned into the plasmid pCR2.1-TOPO-TA (Invitrogen, Carlsbad, CA). To include the FB insulator in the 3'LTR of the pCCLcPPT-x- plasmid, a PCR reaction was done using pHR'-CMV-EGFP to generate a 1-LTR (SIN) plasmid, using the primers: PHR' 3'LTR-amp-ori F and PHR' 3'LTR-amp-ori R2. The 1-LTR plasmid was digested with EcoRV and PvuII, phosphatase treated and ligated with a phosphorylated oligonucleotide cassette containing the FB (77bp) insulator sequence (CCCAGGGATGTACGTCCCTAACCCGCTAGGGGGCAGCACCCAGGCCTGCACTGCCGCCT GCCGGCAGGGGTCCAGTC) (2) to obtain the 1-LTR-FB plasmid.

After verifying the 1-LTR-FB clone, PCR was performed with the 1-LTR-FB plasmid with primers 3'LTR F (*58*) and 3'LTR R (*58*); and then with the pCCL-cPPT empty backbone using the primers pCCL LTR insert F (*60*) and pCCL LTR insert R (*59*). These PCR products were used in an In-Fusion reaction (Clontech Laboratories, Inc, Mountain View CA). The two fragments overlapped at the 3'LTR, making the pCCL-cPPT-x-FB backbone. The pCCL-cPPT-x-cHS4 backbone was created by digesting the 1-LTR plasmid created from pHR', as described above, with EcoRV and PvuII. The 1.2kb cHS4 insulator was amplified using primers 1.2kb-F and 1.2kb-R. The resulting product was cloned into the linearized 1-LTR plasmid via In-Fusion (Clontech Laboratories, Inc, Mountain View CA). The full 3' LTR was transferred to pCCL-cPPT-x as described above for the FB-containing LTR. To include the βAS3-WPRE fragment into the

pCCL-cPPT-x- backbone, the PCR2.1 –TOPO-βAS3-WPRE plasmid was digested with ScaI and KpnI, the purified product was blunted and digested with XhoI. The 6.6kb band corresponding to the βAS3-WPRE fragment was isolated by gel purification and cloned into the pCCL-cPP-x- backbone, previously digested by EcoRV and XhoI. The resulting pCCL-cPPT-βAS3-WPRE (called CCL-βAS3) vector plasmid was fully sequenced to verify the construction. The same procedure was performed to develop the insulated versions CCL-βAS3-FB and CCL-βAS3-cHS4, cloning the βAS3-WPRE cassette in the previously described pCCL-cPPT-x-FB and pCCL-cPPT-x-cHS4 backbones, respectively. (Primers sequences are provided in **Supplemental Table 4**).

Production and titration of βAS3-globin LV

For small-scale production of LV for titer analysis, 293T cells ($5x10^6$) (ATCC, Manassas, VA) were seeded per 10 cm cell culture dishes coated with Poly L-Lysine (Sigma-Aldrich, St. Louis, MO) in 10 ml of D10 medium, consisting of DMEM (Mediatech, Herndon, VA) with 10% fetal bovine serum (FBS) (Gemini Bio-products, Sacramento, CA), 1X Glutamine, Penicillin and Streptomycin (Gemini Bio-Products, West Sacramento, CA), 24 hours before transfection. On the day of the transfection, 3µl of TransIT-293 (Mirus, Madison, WI) were used per 1 µg of DNA. The TransIT volume needed for each condition was mixed with 500ul of OPTI-MEM (Invitrogen, Carlsbad, CA), vortexed and incubated for 20 minutes at room temperature. The OPTI-MEM/TransIT solution was mixed with (a) 5µg of the transfer plasmid, (b) 5µg of pMDL gag-pol/pRRE, (c) 2.5 µg of pRSV-Rev (both were kind gifts of Luigi Naldini, CellGenesys, Foster City, CA), and (d) 1 µg of pMDG-VSV-G (3). In the transfections that were done with TAT, 2.5µg of pSV2-tat were used (4) (provided by the NIH AIDS Research and Reagent Program, Germantown, MD). The DNA and OPTI-MEM/TransIT solutions were incubated for 15-30 minutes at room temperature. The 293T cells were washed with 10ml of D10 before adding the transfection mixture to each plate. Approximately 18-20 hours post-transfection, the medium on

the transfected cells was changed to medium containing 10 mM sodium butyrate (Sigma-Aldrich, St. Louis, MO) and 20 mM HEPES (Invitrogen, Carlsbad, CA). After 6-8 hours, the cells were washed with DPBS (Mediatech, Herndon, VA) and 6ml of medium containing 20 mM HEPES were added. After 48 hours, the vectors were harvested, filtered (0.45 µm) and titered by qPCR as described previously (5). Large-scale viral preparations were produced and concentrated using tangential flow filtration and titered by qPCR as described previously (5).

FB insulator integrity in the CCL- βAS3-FB provirus

The integrity of the FB insulator was analyzed by PCR from both LTRs in transduced BM CD34+ cells at day 14 of in vitro erythroid culture after genomic DNA isolation using the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA). A first set of primers was designed (5'LTR-F and 5'LTR-R) to amplify the 5' LTR flanking the FB insertion site, with an expected band of 382 bp when the FB insulator was present and intact. The second set of primers was designed (3'LTR-F and 3'LTR-R) to amplify specifically the 3' LTR; in this case the predicted band was 249bp in the presence of the FB insulator. A third PCR reaction was performed combining the 5' LTR-F and the 3' LTR-R to amplify the FB insulator by itself from both LTRs. In this case the corresponding amplicon had a length of 135bp. (All primers sequence provided in **Supplemental Table 4)**. PCR was executed using Taq DNA Polymerase, Native (Invitrogen, Carlsbad, CA) on an Eppendorf (Hamburg, Germany) thermocycler. PCR products were visualized by GelGreen on 2% agarose gels.

Southern Blot

Southern blot analysis was performed to confirm the integrity of the CCL-βAS3-FB LV provirus in the genome. 293T cells were transduced with the CCL-βAS3-FB LV and expanded over two weeks, followed by genomic DNA isolation (Invitrogen, Carlsbad, CA). 10µg of genomic DNA was digested by Afl II (New England Biolabs, Ipswich, MA), electrophoresed at 20 volts

overnight in a 0.8% agarose gel, transferred to a nylon membrane and probed with a ^{32}P-labelled-WPRE fragment overnight.

Chromatin Immunoprecipitation (ChIP)

K562 cells (ATCC # CCL-243™) were transduced with CCL-βAS3, CCL-βAS3-FB and CCL-βAS3-cHS4 LV vectors at a concentration of 2x10^{8}TU/ml for each vector. 2x10^7 transduced K562 cells were collected, washed with PBS and cross-linked by incubation in 1% formaldehyde for 5 minute at room temperature. Nuclei were isolated using the truChIP Low Cell Chromatin Shearing Kit (Covaris, Woburn, MA), and the DNA-protein complexes were sheared for 6 minutes in a COVARIS M220 sonicator per manufacturer instructions. Sheared chromatin was immuno-precipitated (in triplicate) for 12-16h at 4°C using 5 µg of anti-CTCF antibody (Abcam, Cambridge, MA) or rabbit IgG as negative control (Invitrogen, Carlsbad, CA) following the "MAGnify Chromatin Immunoprecipitation System" protocol (Invitrogen, Carlsbad, CA). After reversing the cross-linking, DNA was quantified using "Quant-IT PicoGreen dsDNA Reagent and Kits" (Molecular Probes, Invitrogen, Carlsbad, CA). The same amounts of DNA from CTCF immuno-precipitated, IgG control and input DNA samples were used to perform real-time qPCR in triplicate using the Viia7 Applied Biosystems real time PCR machine with the following conditions: hold stage: 50°C for 2 minutes, 95°C for 10 minutes; PCR stage: 95°C for 15 seconds, 60°C for 1 minute (40 cycles). (Primers sequences are described in **Supplemental Table 4**).

Data were analyzed using relative quantitation method as described in the ABI User Bulletin #2 "Relative quantitation of gene expression" (6), and Litt *et al.* (7). In brief, fold enrichment for a particular target sequence was determined using the following formula: fold enrichment= $AE^{(Ct\ input-Ct\ IP)}$. AE=amplification efficiency, input=amount of the target sequence in input DNA; IP=amount of target sequence in immune-precipitated DNA.

BM CD34+ cell isolation

Human $CD34^+$ cells were isolated from BM aspirates from HD and SCD donors ($beta^S/beta^S$ or $beta^S/betathal^0$). The mononuclear fractions obtained by density gradient centrifugation on Ficoll-Hypaque (Amersham Pharmacia Biotech Piscataway, NJ) were processed using the Human CD34 Microbead kit (Miltenyi Biotech, Bergisch Gladbach, Germany) and the $CD34^+$ cells recovered were cryopreserved.

CFU progenitor assay in methylcellulose

100, 300 and 1000 BM CD34+ cells (non-transduced or transduced) were plated per 35 mm gridded cell culture dish in duplicate, using methylcellulose medium (Stem Cell Technologies, Vancouver, BC, Canada) enriched to support optimal growth of human hematopoietic progenitors from $CD34^+$-enriched cells. After 14 days of culture at 5% CO_2, 37°C and humidified atmosphere, the different types of colonies were identified based in their morphology, and then counted and plucked for genomic DNA isolation (NucleoSpin Tissue XS, Clontech, Mountain View, CA) for determination of VC/cell by qPCR as described before (5).

Myeloid Culture

In parallel to the erythroid culture $5x10^4$cells per condition were grown in myeloid conditions for 14 days to measure VC/cell by qPCR as described before (5). The basic myeloid medium consists of IMDM supplemented with 20% of FBS (Life Technologies, Grand Island, NY), 35% BSA (Sigma-Aldrich, St. Louis, MO), 1X Glutamine, Penicillin and Streptomycin, 5 ng/ml hIL-3, 10 ng/ml hIL-6 (both from R&D) and 25 ng/ml hSCF(StemGent, Cambridge, MA).

Flow cytometry during erythroid culture

At days 3, 14 and 21 of the in vitro erythroid differentiation, $2x10^5$ cells were collected for flow cytometry analysis. The samples were stained with the following antibodies: phycoerythrin (PE)-

conjugated anti-human CD34, V450-conjugated anti-human CD45, allophycocyanin (APC)-conjugated anti-human CD71 (all from BD Biosciences, San Jose, CA) and fluorescein isothyocyanate (FITC)-conjugated anti-GlycophorinA (GpA) (Santa Cruz Biotechnologies, Santa Cruz, CA). At day 21, the percentage of enucleated RBC produced was measured by double staining: DRAQ5 (Biostatus Limited, UK) for nuclear staining and FITC-conjugated anti-GpA; enucleated RBC were defined as being GpA+/DRAQ5-. All the flow cytometry analyses were performed on an LSR Fortessa cell analyzer (BD Biosciences, San Jose, CA).

In vitro immortalization (IVIM) assay

To obtain lineage-negative (stem cell enriched) populations from BM, untreated 7- to 12-week-old male B6.SJL-Ptprc^{a}Pepc^{b}/BoyJ ("Pep Boys") were used as donors. BM cells were collected from the long bones (2 femurs, 2 tibias and 2 humeri) of each mouse into IMDM supplemented with 10% FBS. Lineage-negative cells were isolated from single cell suspensions of whole BM cells by using the Lineage Cell Depletion Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer's instructions and cryopreserved in aliquots.

Upon thawing, lineage-negative cells were pre-stimulated in StemSpan SFEM serum-free expansion medium (STEMCELL Technologies Inc., Vancouver, Canada) containing 50ng/ml mSCF, 100ng/ml human Interleukin-11(hIL-11), 20ng/ml mIL-3 (all PeproTech Inc., Rocky Hill, NJ, USA), 100ng/ml hFlt3-L (Celldex Therapeutics, Needham, MA) and 1X Glutamine, Penicillin and Streptomycin in Retronectin (20μg/ml) coated wells of 24 well plates at a concentration of 0.5-1x10^{6} cells/ml for 2 days before exposure to vector particles.

For retroviral transduction, RSF91-GFP-WPRE viral particles were preloaded onto Retronectin coated wells of 24 well plates by centrifugation at 1000g for 30 minutes at 4^{o}C at multiplicity of infection ranging from 1 to 20. The viral supernatant was aspirated, and 1x10^{5} pre-stimulated lineage negative cells were added in 500μL StemSpan medium containing cytokines

on day 3. On day 4, cells were transferred to a new 24 well plate, freshly preloaded with retroviral particles in 1mL to account for increasing cell numbers. For LV transduction, $1x10^5$ pre-stimulated lineage-negative cells were transduced with concentrated CCL-βAS3, CCL-βAS3-FB and CCL-βAS3- cHS4 LV supernatants at $2x10^7$ TU/mL and $2x10^8$ TU/ml in 500μL StemSpan medium containing cytokines on day 3. On day 4, 500μL medium was added to account for increasing cell numbers.

Starting on day 5 (day 1 pTD), mock-, retroviral- and lentiviral-transduced samples were expanded as mass cultures for 2 weeks in IMDM supplemented with 10% FBS, 1X Glutamine, Penicillin and Streptomycin, 50ng/ml mSCF, 100ng/ml hIL-11, 20ng/ml mIL-3 and 100ng/ml hFlt3-L. During this time, cell density was adjusted to $5x10^5$/ml on days 4, 6, 8, 11, and 13 pTD. On day 15 pTD, cells were plated in a limiting dilution assay in 96 well plates at a density of 100 cells/well and 1000 cells/well, respectively, in 100μl IMDM supplemented with FBS, Glutamine, Penicillin, Streptomycin and cytokines. Two weeks later the positive wells were counted, and the frequency of replating cells was calculated based on Poisson statistics using L-Calc Software (STEMCELL Technologies Inc., Vancouver, Canada).

HBBAS3 **mRNA expression in erythroid and myeloid conditions**

After BM-CD34+ cells transduction, samples were divided into parallel cultures under myeloid and erythroid differentiation conditions. At 14 day of culture, 1.5×10^5 cells were harvested for each group. RNA extraction and cDNA synthesis were performed as described in the Materials and Methods section. The ddHBBAS3 assay sequences are provided in **Supplemental Table 4**. The β-Actin, ACTB (Hs 99999903_m1), was purchased as a 20x-premix of primers and FAM-MGBNFQ probe (Applied Biosystems, San Francisco, California). Reaction mixtures of 20 μl volume comprising 1× ddPCR Master Mix (Bio-Rad, Hercules, California), relevant primers and probe (900nM and 250nM for ACTB primers and probe respectively; 500nM and 100nM for

ddHBB^{AS3} primers and probe), and 1ul of cDNA were prepared. Droplet generation was performed as described in Hindson *et al*., (8). The droplet emulsion was then transferred with a multichannel pipet to a 96-well propylene plate (Eppendorf, Hamburg, Germany), heat sealed with foil, and amplified in a conventional thermal cycler (T100 Thermal Cycler, Bio-Rad). Thermal cycling conditions consisted of 95°C 10 min, 94°C 30 s and 60°C 1 min (55 cycles), 98°C 10 min (1 cycle), and 12°C hold. After PCR, the 96-well plate was transferred to a droplet reader (Bio-Rad). Acquisition and analysis of the ddPCR data was performed with the QuantaSoft software (Bio-Rad), provided with the droplet reader. The relative expression of *HBBAS3*/*ACTB* was calculated by dividing the concentration (copies/ul) of *HBBAS3* by the concentration of *ACTB*, and normalized to the VC/cell.

Supplemental Table 1: Measurement of %HbAS3 by HPLC and IEF

	%HbAS3 or HbA		%HbS		%HbAS3/VC		VC/cell
	*HPLC	IEF	*HPLC	IEF	*HPLC	IEF	
SCD Mock #1	0.00	0.00	100.00	100.00	NA	NA	NA
SCD-βAS3-FB #1	24.40	26.70	75.60	73.30	24.65	26.97	0.99
SCD Mock #2	8.10	0.00	91.90	100.00	NA	NA	NA
SCD-βAS3-FB #2	15.20	9.50	84.40	90.50	15.78	21.11	0.45

NA: Not Applicable

*For this comparison, we only considered the HbA, HbAS3, and the HbS peaks. The percentage of HbAS3 was defined as %HbAS3 / (%HbA+%HbAS3+%HbS). In the case where there was expression of HbA in the mock-transduced control, this value was subtracted before normalizing to VC.

HPLC was performed by the UCLA Clinical Laboratory and Pathology Services using the Bio-Rad CDM 5.1 VII instrument.

Supplemental Table 2: IVIM assay results

Vector	Exp. No.	Titer [TU/mL]	MOI	VC d.8	No. of pos. wells (10^2 cells/well)	No. of pos. wells (10^3 cells/well)	Replating frequency	Replating frequency/VC
Non-transduced	1	-	-	-	0	0	-	-
	2	-	-	-	0	0	-	-
	3	-	-	-	0	0	-	-
	4	-	-	-	0	0	-	-
	4	-	-	-	0	0	-	-
RSF91-GFP	1	$1.9x10^6$	1	1.26	9	47	7.10E-04	5.63E-04
	1		20	12.83	0	0	-	-
	2		5	6.09	0	2	1.91E-05	3.14E-06
	2		8	9.78	81	96	1.86E-02	1.90E-03
	3		8	7.90	33	94	4.04E-03	5.12E-04
	3		8	5.02	0	0	-	-
	4		8	4.65	96	96	>4.56E-02	>9.81E-03
	4		8	4.92	0	0	-	-
	4		8	6.94	0	0	-	-
	4		8	7.10	17	87	2.26E-03	3.18E-04
	4		14	8.24	91	96	2.95E-02	3.59E-03
	4		14	8.35	96, 96	96	>5.26E-02	>6.0E-03
CCL-βAS3	1	$1.5x10^9$	1000	1.02	0	0	-	-
	2		100	1.10	0	0	-	-
	3	$5.0x10^9$	100	2.32	0	0	-	-
	3		100	3.39	0	0	-	-
CCL-βAS3-FB	1	$6.0x10^8$	1000	4.68	0	0	-	-
	1		100	4.76	0	0	-	-
	2		100	4.32	0	0	-	-
	2		100	4.52	0	0	-	-
	3	$6.0x10^8$	100	3.66	0	0	-	-
	3		100	3.31	0	0	-	-
	4		100	3.22	0	0	-	-
	4		100	3.56	0	0	-	-
	4		100	3.23	0	0	-	-
	4		100	4.23	0	0	-	-
	4		100	4.55	0	0	-	-
	4		100	3.49	0	0	-	-
	4		100	3.49	0	0	-	-
	4		100	2.56	0	0	-	-
CCL-βAS3-cHS4	2	$1.6x10^8$	100	0.30	0	0	-	-
	3		100	0.54	0	0	-	-
	3		100	0.36	0	0	-	-
	3		100	0.41	0	0	-	-

Supplemental Table 3: CCL-βAS3-FB most frequent integration sites and the genes involved

Chromosome	Orientation	Nucleotide Position	Genes containing IS or with IS <50kb from TSS
chr4	+	91503107	FAM190A
chr16	+	1448144	UNKL, C16orf42, GNPTG, C16orf91, CCDC154
chr17	-	76027400	TNRC6C
chr19	+	5631833	SAFB, SAFB2, C19orf70, HSD11B1L
chr9	+	140097278	LRRC26, MIR3621, ANAPC2, SSNA1, TPRN, TMEM203, NDOR1, RNF208, C9orf169, LOC643596, SLC34A3, TUBB2C, FAM166A, C9orf173
chr22	+	24782983	SPECC1L, ADORA2A
chr11	-	73279161	FAM168A
chr6	+	34599566	C6orf106
chr16	-	20839013	LOC81691, ERI2
chr13	+	28784427	PAN3
chr17	+	29584884	NF1, OMG
chr22	+	50820904	PPP6R2
chr22	+	38064948	TRIOBP, SH3BP1, PDXP, LGALS1, NOL12
chr12	-	62238951	FAM19A2
chr17	+	7158187	DLG4, ACADVL, MIR324, DVL2, PHF23, GABARAP, CTDNEP1, C17orf81, CLDN7, SLC2A4, YBX2
chr12	+	96696545	CDK17
chr5	+	88144268	MEF2C
chr22	+	38784207	LOC400927
chrX	-	153651194	FLNA, EMD, RPL10, SNORA70, DNASE1L1, TAZ, ATP6AP1, GDI1, FAM50A, PLXNA3
chr3	+	49120118	USP19, QRICH1, QARS
chr19	-	6843325	VAV1, EMR1
chr4	-	7509127	SORCS2, MIR4274
chr5	-	77481360	AP3B1
chr16	-	1437062	UNKL, C16orf42, GNPTG, C16orf91
chr10	+	70679777	DDX50, DDX21
chr8	-	125342013	TMEM65
chr15	-	75352474	PPCDC
chr11	-	96043251	MAML2, MIR1260B
chr19	-	12287594	ZNF20, ZNF625-ZNF20, ZNF625, ZNF136
chr4	+	28371	ZNF718, ZNF595
chr9	+	75760126	ANXA1
chr1	+	31467152	PUM1, SNORD103A, SNORD103B, SNORD85, PRO0611
chr19	+	54072589	ZNF331, LOC284379
chr2	+	43510437	THADA
chr9	-	140547986	ARRDC1, EHMT1, C9orf37

chr16	+	22473380	RRN3P3, LOC641298
chr17	+	4862964	PFN1, GP1BA, SLC25A11, RNF167, ENO3, SPAG7, CAMTA2, INCA1, KIF1C
chr12	+	41740589	PDZRN4
chr1	-	153864313	GATAD2B
chr12	+	1365953	ERC1
chr6	+	47720503	OPN5
chr17	+	48801784	ANKRD40, LUC7L3, C17orf73
chr9	+	131396459	WDR34, SET
chr16	-	30309972	LOC440354, LOC595101
chr13	-	30846878	KATNAL1
chr17	-	80485547	FOXK2
chr9	+	139618741	AGPAT2, FAM69B, SNHG7, SNORA43, SNORA17, LCN10, LCN6, LOC100128593, LCN8, LCN15
chr12	-	12367074	LRP6
chr4	-	151274215	LRBA
chr11	+	87034027	TMEM135
chr6	+	149696229	TAB2, SUMO4
chr1	-	150116016	VPS45, PLEKHO1
chr19	+	41886689	TGFB1, B9D2, TMEM91, EXOSC5, BCKDHA, B3GNT8
chr9	+	128680454	PBX3
chr19	+	51761097	CD33, C19orf75
chr1	-	205577716	MFSD4, ELK4
chr15	-	86147491	AKAP13
chrX	-	67562531	OPHN1
chr17	+	6497265	PITPNM3, KIAA0753, TXNDC17
chr5	+	179180503	MAML1, LTC4S, MGAT4B, MIR1229
chr17	-	7066197	ASGR1, ASGR2
chr1	-	87554536	HS2ST1, LOC339524
chr17	-	79551397	NPLOC4, C17orf70
chr11	-	64538262	NRXN2, RASGRP2, PYGM, SF1, MAP4K2, MEN1
chr19	+	19357037	NR2C2AP, NCAN, HAPLN4, TM6SF2
chr8	-	125359290	TMEM65
chr1	+	155705973	YY1AP1, DAP3, MSTO2P
chr11	+	65939541	PACS1
chr17	-	79946163	ASPSCR1, MYADML2, NOTUM, STRA13, LRRC45, RAC3, DCXR
chr16	+	89733855	LOC100128881, DPEP1, CHMP1A, C16orf55, CDK10, SPATA2L
chr17	-	79946910	ASPSCR1, MYADML2, NOTUM, STRA13, LRRC45, RAC3, DCXR
chr7	+	71448707	CALN1
chr17	-	60792253	MARCH10, MIR548W

chr14	+	23279541	OXA1L, SLC7A7, MRPL52, MMP14
chr5	-	80777267	SSBP2
chr19	+	19355711	NR2C2AP, NCAN, HAPLN4, TM6SF2
chr17	-	80721443	TBCD, FN3KRP, FN3K
chr10	-	104904280	NT5C2
chr17	+	43292854	FMNL1, LOC100133991, C17orf46
chr16	+	28826388	ATXN2L, TUFM, SH2B1
chr9	-	134282246	PRRC2B
chr2	+	28448731	BRE
chr19	+	10724641	SLC44A2, KRI1, CDKN2D, AP1M2, LOC147727, ILF3
chr2	+	38527752	ATL2
chr1	-	228375017	OBSCN, GUK1, GJC2, IBA57
chr11	-	65908400	PACS1
chr6	-	4033876	PRPF4B, C6orf201, C6orf146
chr17	+	78797539	RPTOR
chr17	+	7128036	ASGR1, DLG4, ACADVL, MIR324, DVL2, PHF23, GABARAP, CTDNEP1, C17orf81, CLDN7
chr15	-	86198240	AKAP13
chr11	-	66924001	KDM2A
chr12	+	123810408	SBNO1
chr1	+	150643770	ENSA, GOLPH3L, HORMAD1
chr8	+	90965062	NBN, DECR1
chr4	-	215542	ZNF876P
chr6	-	34627686	C6orf106
chr12	-	2975517	TULP3, NRIP2, FOXM1, C12orf32
chr19	-	42067550	CEACAM21
chr13	+	96512078	UGGT2
chr17	-	75129947	SEC14L1, C17orf86, SCARNA16
chr7	-	99030455	PDAP1, BUD31, PTCD1, ATP5J2-PTCD1, CPSF4, ATP5J2, ZNF789
chr17	-	3974136	ZZEF1
chr6	-	2881463	SERPINB1, MGC39372, SERPINB9
chr2	-	58890146	FLJ30838
chr11	+	62577565	SLC3A2, POLR2G, TAF6L, TMEM179B, TMEM223, NXF1, STX5, WDR74, SNHG1, SNORD22, SNORD31, SNORD30, SNORD29, SNORD28, SNORD27, SNORD26, SNORD25
chrX	-	135236461	FHL1
chr17	-	15451767	FAM18B2-CDRT4, FAM18B2

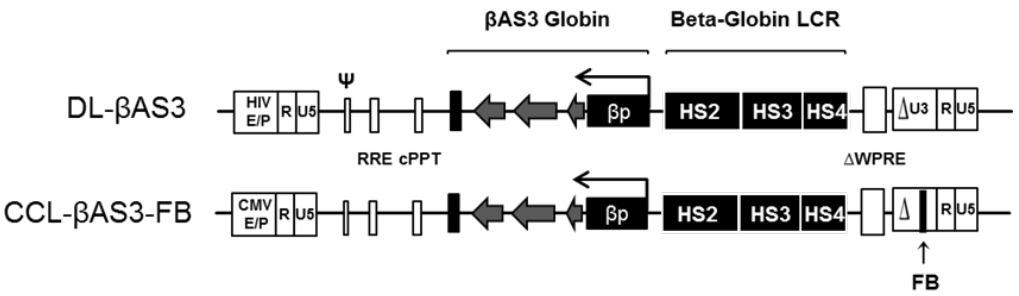
Supplemental Table 4: Oligonucleotides sequences

Primers Name	Sequence (5'-3')
AS3-F	CTACTAGTGGAGATCCC
AS3-R	GAAGCTTGAGCGAATTC
PHR' 3'LTR-amp-ori F	GGGACTGGAAGGGCTAATTCACTC
PHR' 3'LTR-amp-ori R2	CCAGCAAAAGGCCAGGAACC
3'LTR F (*58*)	GGGACTGGAAGGGCTAATTC
3'LTR R (*58*)	CCTCTCACTCTCTGATATTCATTTCTT
pCCL LTR insert F (*60*)	AGCCCTTCCAGTCCCCC
pCCL LTR insert R (*59*)	TCAGAGAGTGAGAGGAACTTGTTTATT
5'LTR-F	GGCTAATTCACTCCCAACGAAGACAAG
5'LTR-R	CTT CAG CAA GCC GAG TCC TGC
3'LTR-F	ACC TCG AGA CCT AGA AAA ACA TGG C
3'LTR-R	CAGAGAGACCCAGTACAAGCAAAAAG
HBB^{AS3} F	TGTGGGACAAGGTGAACGTGGATGCC
HBB^{AS3} R	CAAGGGTAGACCACCAGCAGCCTG
HBB^{A} /HBB^{S} F	TGTGGGGCAAGGTGAACGTGGATGAA
HBB^{A} /HBB^{S} R	CAAGGGTAGACCACCAGCAGCCTG
FB-F	ACTCCCAACGAAGACAAGATCCCA
FB-R	ACCAGAGAGACCCAGTACAAGCAA
cHS4-F	GTAATTACGTCCCTCCCCCG
cHS4-R	AAGCGTTCAGAGGAAAGCGA
U3-F	ACTCCCAACGAAGACAAGATCTGC
U3-R	ATTGAGGCTTAAGCAGTGGGTTCC
H19-F	AGAATCGGCTGTACGTGTGG

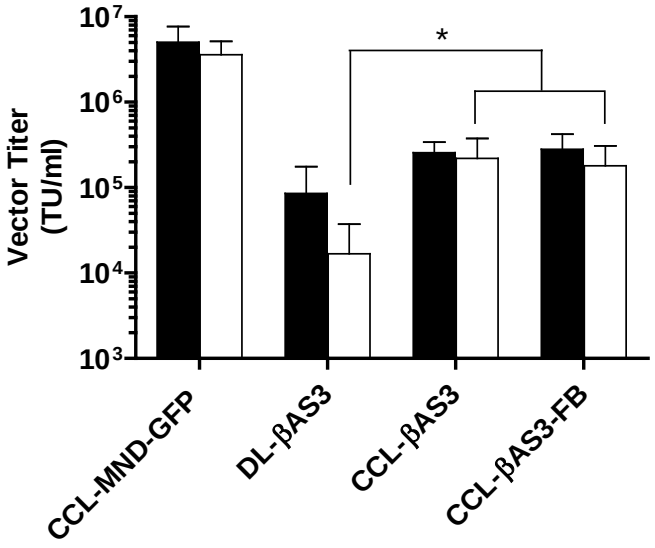
H19-R	GGGACGTTTCTGTGGGTGAA
Myc-F	GCCATTACCGGTTCTCCATA
Myc-R	CAGGCGGTTCCTTAAAACAA
ddHBB^{AS3}-F	GGAGAAGTCTGCCGTTACTG
ddHBB^{AS3}-R	CACTAAAGGCACCGAGCACT
ddHBB^{AS3} Probe	FAM-ACAAGGTGA-ZEN-ACGTGGATGCCGTTG-31ABFQ

Supplemental Figure 1. βAS3 LVs plasmid maps and production in the presence or absence of TAT protein

A

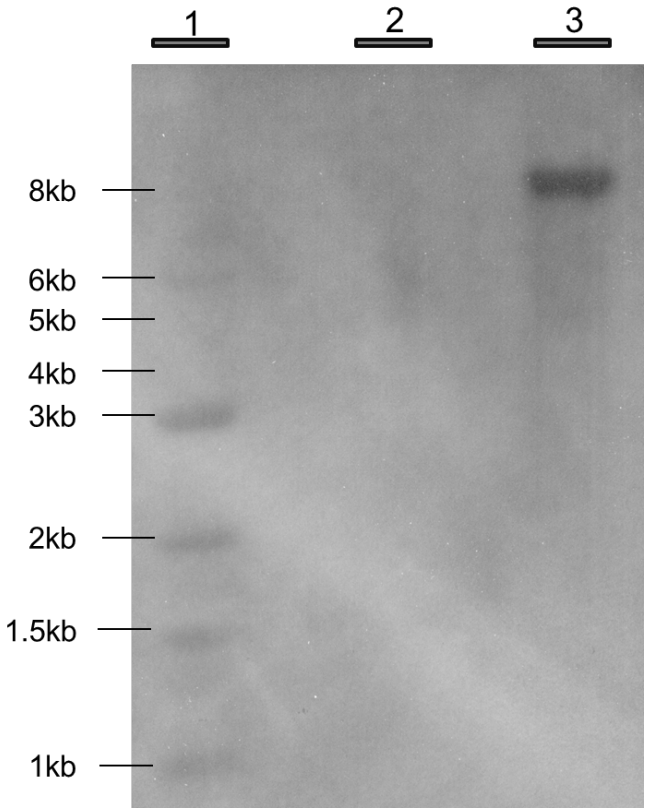


B



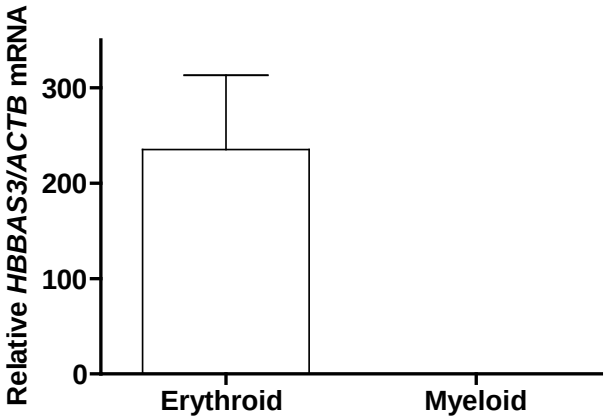
(**A**) Vector plasmid forms of the parental DL-βAS3 (top) in which transcription driven by the HIV-1 enhancer and promoter is dependent upon TAT and the CCL-βAS3-FB (bottom) in which the CMV enhancer/promoter is substituted in the 5' LTR, eliminating the need for TAT. In both cases, the HIV-1 packaging sequence (Ψ), rev responsive element (RRE), central polypurine tract (cPPT), and the Woodchuck Hepatitis Virus post-transcriptional regulatory element (WPRE) are shown. (**B**) The DL-βAS3, CCL-βAS3, CCL-βAS3-FB and the positive control CCL-MND-GFP LV vectors were packaged in the presence (black bars) or absence (white bars) of an HIV-1 TAT expression plasmid. Averages of three experiments and SD are shown.

Supplemental Figure 2. Integrity of the βAS3-FB provirus



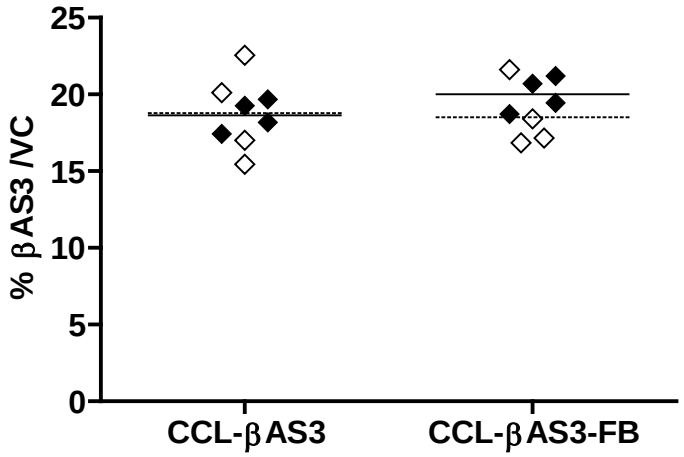
Southern Blot analysis was performed to confirm full length integrity of the provirus. Genomic DNA of 293T cells, mock-transduced or transduced with the CCL-βAS3-FB LV (with an average VC/cell of 10 analyzed by qPCR) was digested by AflII, which cuts in each LTR of the provirus and should release a nearly full-length genome fragment (8.6 Kb). The DNA ladder is shown in the lane 1, followed by the mock-transduced cells in lane 2 and the CCL-βAS3-FB-transduced cells in the lane 3, where a unique band representing the intact provirus of the right size is present.

Supplemental Figure 3. Erythroid specific expression of the *HBBAS3* cassette



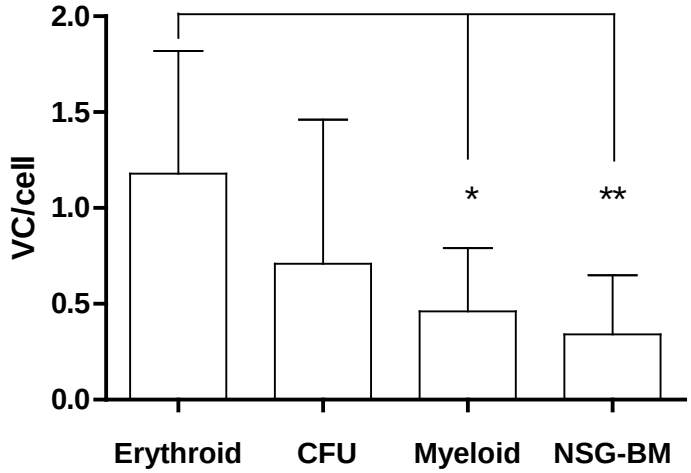
HBBAS3 mRNA expression at day 14 of erythroid or myeloid cultures was analyzed relative to the endogenous control gene *ACTB*. In three separate experiments, no mRNA expression by the *HBBAS3* transgene was detected in myeloid conditions (0.04 ±0.01) relative expression compared to ACTB. In contrast, the same cells grown under erytrhoid conditions, showed high expression of *HBBAS3* mRNA (235.35± 77.77). The mRNA expression in each condition was normalized to the VC/cell obtained from the erythroid and myeloid samples, respectively. Values shown are average ± SD.

Supplemental Figure 4. Expression of the *HBBAS3* transgene in the presence or absence of the FB insulator



Expression of the *HBBAS3* cassette from erythroid cells produced by BM-CD34+ cells from SCD donors, transduced with the CCL-βAS3 or the CCL-βAS3-FB LV, was analyzed by RT-qPCR to determine the percentage of *HBBAS3* mRNA per VC/cell (solid rhombus); or by IEF to determine the percentage of HbAS3 protein per VC/cell (empty rhombus).No differences were found in the percentage of *HBBAS3* mRNA of the total beta-globin-like mRNA (p=0.12, two-tailed t-test); or in the percentage of HbAS3 of the total Hb (p=0.89, two-tailed t-test) in erythroid cells transduced with the CCL-βAS3 or the CCL-βAS3-FB LV. Therefore, these results indicate that the FB insulator did not provide barrier activity to improve position-independent expression; since the addition of the FB insulator did not alter the expression of the *HBBAS3* cassette when compared to the non-insulated LV. Error bars represent mean values.

Supplemental Figure 5. VC/cell analysis in different assay conditions



VC/cell was determined by qPCR in CCL-βAS3-FB-transduced BM CD34+ cells grown in erythroid conditions, methylcellulose medium (CFU), myeloid conditions and expanded from engrafted NSG BM. VC/cell measurements from cells grown in erythroid culture assay were significantly higher than those measured in cells grown in myeloid culture (*p=0.0003) or from NSG BM (**p<0.0001). Values shown are average ± SD.

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