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Supplemental Information

Coordinated β-globin expression and α2-globin reduction in a multiplex lentiviral gene therapy vector for β-thalassemia

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Supplementary Information

SUPPLEMENTAL MATERIAL AND METHODS

Validation of the α-globin-specific siRNA

In order to identify and evaluate effective α-globin-specific siRNA target sequences, 5 siRNAs targeting human α-globin were electroporated into K562 cells (Supplemental Table S2). Four sequences (siα-1, siα-2 siα-3 and siα-4) generated significant reductions in human α-globin mRNA compared to negative controls (mock and siLuc electroporated K562 cells) 24 hrs and 48 hrs post electroporation as detected by real-time PCR. In brief, 5×10^6 K562 cells mixed with siRNA in 0.5ml of Opti-Mem, were transferred to 0.4 cm cuvettes and electroporated with the Gene Pulser (Bio-Rad, Hercules, CA, USA) using the following conditions: 226 Volts, 950 µF, ∞ resistance. Cells were then cultured in 10 ml DMEM containing 10% FCS and 30 µM hemin for erythroid differentiation and incubated at 37°C. Relative α-globin RNA expression levels were detected by real-time PCR 24 and 48 hours post electroporation. The most effective siRNA, siα-1 and siα-2, reduced α-globin mRNA by 95%±5% and 83%±6% relative to negative controls, while siα-3 and siα-4 generated modest, though significant, reductions of 35%±13% and 47%±14% respectively ($p<0.05$), and siα-5 had no effect. Analysis of efficacy over time demonstrated that 1 µg of siα-3 and siα-4 generated modest reductions in α-globin mRNA expression which remained significant at 48 hours relative to negative controls (Figure S3). In humans, the α-globin is encoded by two highly homologous *HBA1*(α1) and *HBA2*(α2)

23 *genes.* These genes differ significantly over the 3' UTRs. Whereas siα-1, siα-2 and siα-3
24 target both genes, siα-4 specifically targets *HBA2*.
25
26 **Lentiviral vector comparison**
27 Vesicular stomatitis virus glycoprotein (VSVG) pseudotyped lentiviral supernatants were
28 produced by transient transfection of HEK293T cells with the plasmid vector and the 3-
29 plasmid packaging system made of HPV275, ψN15 and p633.[1] Lentiviral particles were
30 produced and purified with a Mustang Q anion exchange membrane cartridge (Pall, Saint
31 Germain-en-Laye, France) and a 40K ZebaSpin desalting column (Thermo Fisher
32 Scientific, Villebon, France) before concentration by overnight precipitation with Lentivirus
33 precipitation solution (Alstem, Richmond, CA). Infectious titers were determined in
34 NIH3T3 cells. Notably, no observable difference was identified in the titres of LVβ-shRNA
35 vectors from culture supernatants harvested 24 hours post-transfection of HEK 293T,
36 before and after purification and ultracentrifugation (Figure S4). Based on these results,
37 the insertion of the miR30-shRNA expression cassette into LVβ gene therapy vector did
38 not interfere with LV vector production or transduction.
39
40 **Flow cytometry**
41 To determine knockdown efficiency of LVβ-shGFP, eGFP expression of transduced-MEL-
42 βeGFP cells were determined by flow cytometry at day 3-10 of erythroid differentiation.
43 Cells were washed and resuspended in 1X PBS. Cells were analysed using BD LSR II
44 flow cytometer (BD Biosciences). $\beta^{A\text{-}T87Q}$-globin expression of transduced cells was
45 assessed by intracellular staining. Briefly, 1×10^6 cells were fixed with 100 μl of ice-cold

46 4% paraformaldehyde at room temperature for 10 minutes. Cells were washed with 1X
47 PBS and permeabilized with 200 μl of ice-cold 0.1% Triton X-100 (v/v) in 2% FCS-PBS at
48 room temperature for 5 minutes. Cells were washed with 2% FCS-PBS. 1×10^5 cells were
49 stained with 2 μl of antibody against human β-globin and incubated on ice for 30 minutes.
50 Cells were washed 3 times with 2% FCS-PBS and subjected to analysis using BD LSR II
51 flow cytometer (BD Biosciences). Data were acquired on a LSR II cytometer (BD, San
52 Jose, CA) using FACS Diva software. For determination of erythroid differentiation
53 profiles, cells were stained with antibodies directed against CD235A, CD36, and CD71
54 and subjected to analysis using MACSQuant cytometer (Miltenyi Biotech) and analysed
55 using FlowJo software (Tree Star, OR, USA). Antibodies used are listed in Supplemental
56 Table S5.
57
58 **High performance liquid chromatography**
59 Percentage of the globin chains in human samples was determined by high-performance
60 liquid chromatography (HPLC) with a Prominence chromatograph (Shimadzu). Cells
61 lysates were injected onto an Aeris Widepore 3.6 μm C4 column 250×4.6 mm
62 (Phenomenex). Elution was achieved with a gradient of buffer A (30% acetonitrile (Carlo
63 Erba), 0.07% trifluoracetic acid (Sigma Aldrich) in Milli-Q water and buffer B (50%
64 acetonitrile, 0.05% trifluoroacetic acid), with 48% as an initial percentage of buffer B, in
65 75 minutes at a total flow rate of 0.8 mL/mn at 30°C.The detection wavelength was 220
66 nm. Data acquisition was performed with the LC Solution software (Shimadzu).
67
68

69 **Analysis of globin precipitates by western blot**

70 Cells were lysed in Milli-Q water then centrifuged to obtain water-soluble cell fractions.
71 Membrane fractions was extensively washed in 0.05% PBS and resuspended in 56 mM
72 sodium borate (pH 8.0) with 0.5% Triton X-100. After 20 minutes incubation on ice,
73 membrane fractions was centrifuged at 16,000xg, 4°C for 10 minutes. Precipitated globins
74 were dissolved in 1X sample loading buffer for Western blot analysis. [2, 3]

75

76 **Protein analysis by western blot**

77 Total cell lysate was prepared using RIPA lysis buffer system (SantaCruz Biotechnology,
78 Dallas, TX). Briefly, 5×10^6 washed-cells were gently resuspended in 200 µl of ice cold
79 RIPA lysis buffer (100 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium
80 deoxycholate, 0.1% SDS, 0.004% sodium azide), 2 mM PMSF solution, 1 mM sodium
81 orthovanadate solution and 10% (v/v) protease inhibitor cocktail solution (Sigma Aldrich).
82 Lysis reaction was incubated on ice for 30 minutes and centrifuged at 10,000xg, 4°C for
83 10 minutes. The supernatant which is total cell lysate was collected to new tube and stored
84 at -20°C.

85

86 **Quantification of proteins**

87 Proteins in total cell lysates were quantified using BCA protein assay kit (Thermo
88 Scientific) according to the manufacturer's protocol. Briefly, BSA standard curve was
89 generated in the range of 0-1,000 µg/ml. A working reagent (WR) solution was prepared
90 by mixing BCA Reagent A with BCA Reagent B at ratio 50:1. 1 ml of WR solution was
91 combined to 50 µl of protein sample or standard BSA and incubated at 37°C for 30

92 minutes. After incubation, the reaction was transferred to cuvette and measured using a
93 spectrophotometer at OD562. Protein quantity was calculated using linear regression.
94
95 **Western blot analysis of globin chains**
96 K562: A total 10 µg of protein cell lysate was denatured by heating at 95°C for 5 minutes
97 in 1X sample loading buffer (50 mM Tris-HCl pH 6.8, 100 mM DTT, 2% SDS, 10% glycerol,
98 0.02% bromophenol blue) and separated on 4-15% SDS-polyacrylamide Mini-
99 PROTEAN® TGX™ precast gel (Bio-Rad, Hercules, CA) in Tris-glycine running buffer (25
100 mM Tris pH 8.3, 192 mM Glycine, 0.1% SDS) at 120 volts for 40 minutes. After
101 electrophoresis, protein was transferred to 0.22 µm PVDF membrane (GE healthcare,
102 Chicago, IL) in Towbin transfer buffer (25 mM Tris, 192 mM glycine, 10% methanol) at
103 100 V for 90 minutes in cold room. After blocking the membranes in 1X Tris buffer saline
104 (TBS) with Tween-20 (20 mM Tris-Hcl pH 7.5, 154 mM NaCl, 0.1% Tween-20
105 supplemented with 3% bovine serum albumin (BSA)) at room temperature for 2 hours,
106 membranes were incubated with primary antibody either against human α-globin (at
107 dilution 1:10,000 in 3% BSA-TBS-T or human β-globin (at dilution 1:10,000 in 3% BSA-
108 TBS-T or human β-actin antibody conjugated with horseradish peroxidase (HRP) (at
109 dilution 1: 20,000 in 3% BSA-TBS-T at 4°C overnight. Membranes were washed 3 times
110 with 1X TBS-T for 10 minutes each wash before incubated with secondary antibody
111 conjugated with HRP at dilution 1:5,000 in 3% BSA-TBS-T at room temperature for 2
112 hours when required. Membranes were washed 3 times with 1X TBS-T for 10 minutes
113 each wash before protein was detected by ImageQuant LAS4000 (GE Healthcare) using
114 ECL solution (1.25 mM Luminol, 200 µM p-Coumaric acid, 0.01% H_2O_2, 100 mM Tris-HCl
115 pH 8.5) according to the manufacturer's protocol.

Cell lines

i) The MEL-βeGFP cell is derived from the MEL (murine erythroleukemia) cell line modified to carrying the 180kb BAC human genomic fragment containing the β-globin locus, where the coding regions of the β-globin has been replaced by the eGFP reporter.[4] Cells were grown and maintained in DMEM supplemented with 10% FCS, 100 U/ml penicillin and 100 g/ml streptomycin at 37°C 5% CO_2. Cells were induced to undergo erythroid differentiation by culturing in medium containing 2% DMSO at initial concentration 2×10^5 cells/ml for 7 to 10 days.

ii) Human erythroleukemia (K562) cell line, derived from chronic myeloid leukemia patient, predominantly expresses embryonic hemoglobin, Hb Glower I ($\zeta_2\varepsilon_2$), Hb Portland ($\zeta_2\gamma_2$) and fetal hemoglobin, HbF ($\alpha_2\gamma_2$).[5-7] Cells were grown and maintained in DMEM supplemented with 10% FCS, 100 U/ml penicillin and 100 g/ml streptomycin at 37°C 5% CO2. K562 cells were induced to differentiate by culturing in media containing 30 μM Hemin for 3-5 days.

iii) HUDEP-2 cell line was previously created by lentiviral transduction of human cord blood haematopoietic stem cells with doxycycline (DOX)-inducible human papilloma virus 16 E6/E7 expression system.[8] The cells express mostly adult hemoglobin (HbA). HUDEP-2 cells containing biallelic β^0-globin mutations, termed β^0-HUDEP-2 cells, were created using CRISPR-Cas9 genome editing to recapitulate a sustainable in vitro model of β^0-thalassemia. Following clonal analysis, a β^0-HUDEP-2 *cell line with biallelic* β-globin *gene*

139 *disruptions* was identified by sanger sequencing (manuscript in preparation). Cells were
140 grown at a density of 0.1-2 × 10^6 cells/ml in StemSpan SFEM (Stem Cell technologies)
141 supplemented with human stem cell factor (hSCF, 50 ng/ml), erythropoietin (EPO, 3
142 IU/ml), dexamethasone (Dex, 1 µM), and doxycycline (DOX, 1 µg/ml), 1X
143 pen/strep/glutamine (PSG). Differentiation of HUDEP-2 cells was induced by culture in
144 IMDM supplemented with EPO (10 IU/ml), mifepristone (1 µM), human AB serum (5%),
145 holotransferrin (500 µg/ml), heparin (2 IU/ml) and insulin (10 µg/ml), 1X PSG.
146 Differentiation media was replaced every two days and cell concentration was kept at 0.5-
147 1 × 10^6 cells/ml.
148
149 **Human hematopoietic cells**
150 Human cells of de-identified donors were obtained from normal cord blood (CB) from
151 Saint-Louis Hospital (Paris, France) and from non-mobilized peripheral blood of
152 thalassemia patients from Ramathibodi Hospital (Bangkok, Thailand), following patient
153 informed consent. They were enriched using the CD34 progenitor cell isolation kit (Miltenyi
154 Biotec and the autoMACSPro instrument) according to manufacturer instructions. The
155 study was approved by the ethics evaluation committees of the French Institute of Medical
156 Research and of the Faculty of Medicine Ramathibodi Hospital, under protocol numbers
157 16-315 and 06-57-07 respectively.
158
159 **Mathematical model of α to β-globin mRNA ratio decay according to vector copy**
160 **number**
161 In order to compare the level of globin transcripts between groups of cells transduced with
162 BB305 or LVβ-shRNA, α-globin to β-globin mRNA ratios were determined at several time

163 points after erythroid differentiation and eventually normalized to those measured in non-
164 transduced cells at the same time point. The resulting values were plotted as a function
165 of VCNs, and the exponential decay equation model ($Y=e^{-kx}$, were Y is the mRNA ratio, K
166 is the decay rate and X is the vector copy number) was used to fit the data. For comparison
167 of groups using this model, the transcript ratios were fixed to 0 at infinite VCNs (bottom
168 plateau) and to 1 in the absence of transduction when normalized. The influence of shRNA
169 on α/β globin mRNA ratio and the differential vector efficiencies were calculated according
170 to this model. When $Y1=e^{-k1x1}$ and $Y2=e^{-k2x2}$, the fold difference between α/β mRNA ratios
171 is equal to e^{-k1x1}/e^{-k2x2}. Therefore, the mean fold variation of α/β mRNA ratios per vector
172 copy is equal to $e^{(k2-k1)}$. When the α-globin to β-globin mRNA ratio are equal (Y1=Y2), $e^{-k1x1}= e^{-k2x2}$. Therefore, at equal α:β mRNA ratio, the VCN ratio (x1/x2) is equal to k2/k1.
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177 **SUPPLEMENTAL FIGURES**

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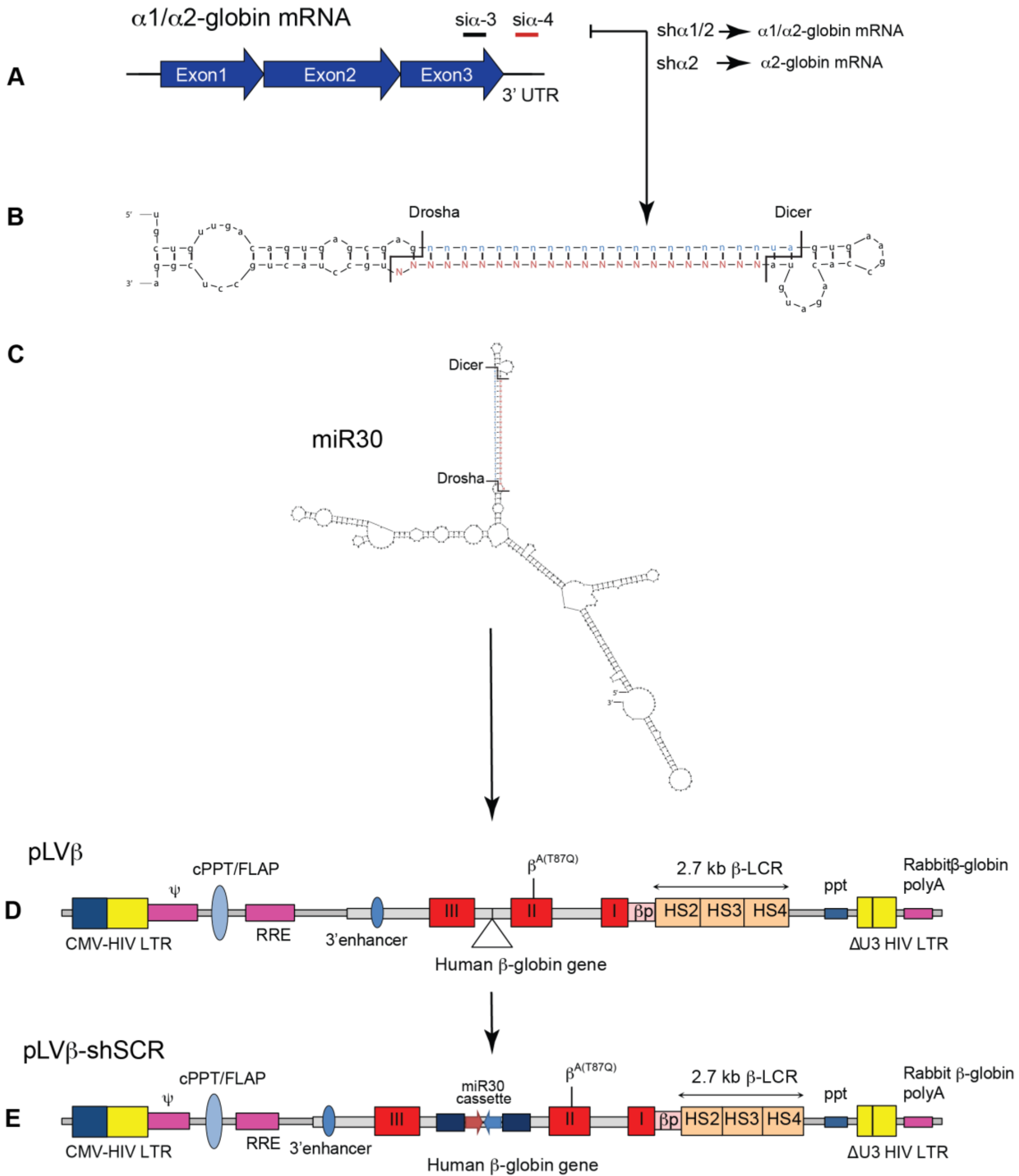

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182 **Figure S1. Schematic diagram illustrating the construction of the LVβ-shRNA**
183 **vector containing miR30-shRNA expression cassette.** **A**) Schematic diagram of
184 human α-globin mRNA showing the target sites for siα-3 and siα-4 which are adapted into
185 **B**) miR30-based shRNA; shα1/2 and shα2, respectively. **C**) Predicted secondary
186 structure of miR30-shRNA expression cassette generated by mfold. miR30-based shRNA
187 is flanked by 125-nt of miR30 flanking region on either side of the hairpin. Sequence for
188 target site (sense orientation) is shown in blue lowercase letter, while the guide strand
189 sequence is shown in red uppercase letters. Putative Drosha and Dicer cleavage sites
190 are indicated by horizontal lines.[9] miR30-shRNA expression cassette is inserted into **D**)
191 the LentiGlobin BB305 (pLVβ) gene therapy vector encoding the $\beta^{A\text{-}T87Q}$-globin gene to
192 generate **E**) pLVβ-shRNA vector containing the intronic miR30-shRNA expression
193 cassette. The $\beta^{A\text{-}T87Q}$-globin gene is placed under control of the human β-globin promoter
194 (βp) and elements from the human β-globin locus control region (LCR), 5'DNaseI
195 hypersensitive sites (HS2-4), truncated β-globin Intron 2 containing a 374bp *Rsa*I site
196 deletion (Δ), long terminal repeat (LTR), extended packaging signal (ψ), miR30-shRNA
197 expression cassette flanked by the 5'miR30 and 3'miR30 scaffold, hybrid human
198 immunodeficiency type-1 virus long terminal repeat (HIV-LTR) whereby the U3 region of
199 5' HIV LTR is replaced with cytomegalovirus (CMV) promoter (CMV-R-U5), deletion in the
200 U3 (ΔU3) region of 3' HIV-LTR, Rev-responsive element (RRE) and polypurine tract (ppt).
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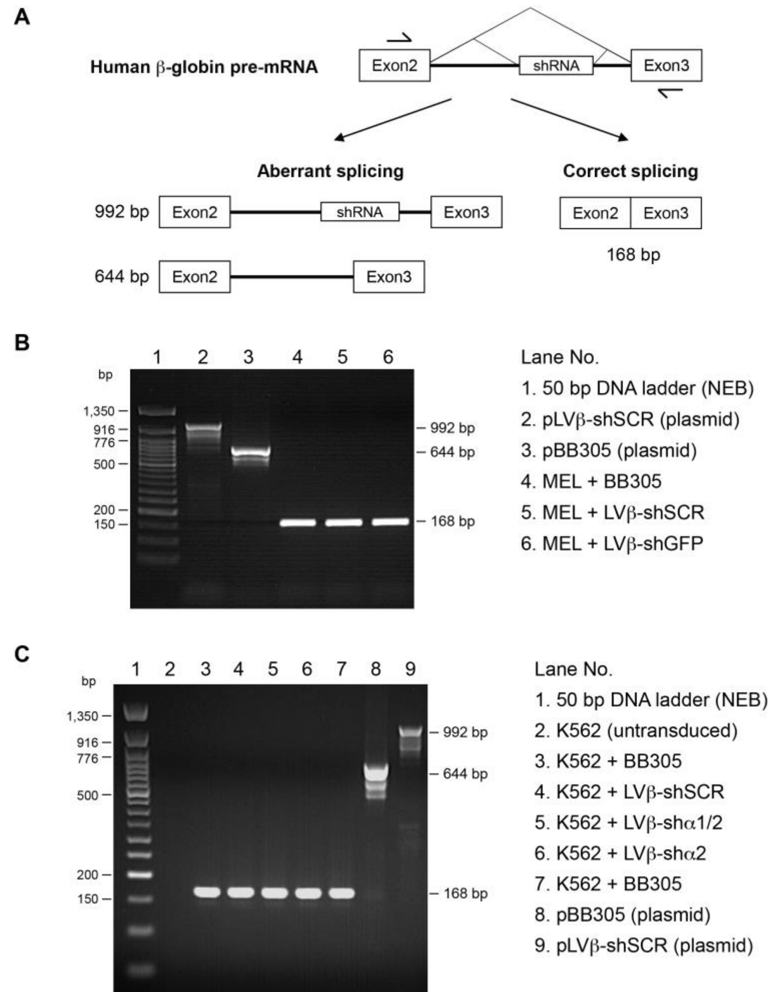

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203 **Figure S2. Analysis of human β-globin mRNA splicing following transduction of**
204 **BB305 and LVβ-shRNA vectors. A**) Diagram of human β-globin splicing and location of
205 PCR spanning β-globin exon 2 to exon 3. **B**) Analysis of human β-globin mRNA splicing
206 in MEL-βeGFP cells transduced with BB305 and LVβ-shGFP vectors **C**) Human β-globin
207 mRNA splicing in K562 cells transduced with BB305, LVβ-shα1/2 and LVβ-shα2 vectors.
208 PCR products were visualized on 1% agarose gel electrophoresis. A single PCR product
209 of the expected size (168 bp) representing the correctly spliced $\beta^{A\text{-}T87Q}$-globin mRNA was
210 identified in cells transduced with BB305 and LVβ-shRNA vectors (**b**: Lanes 4-6; **c**: Lanes
211 3-7).

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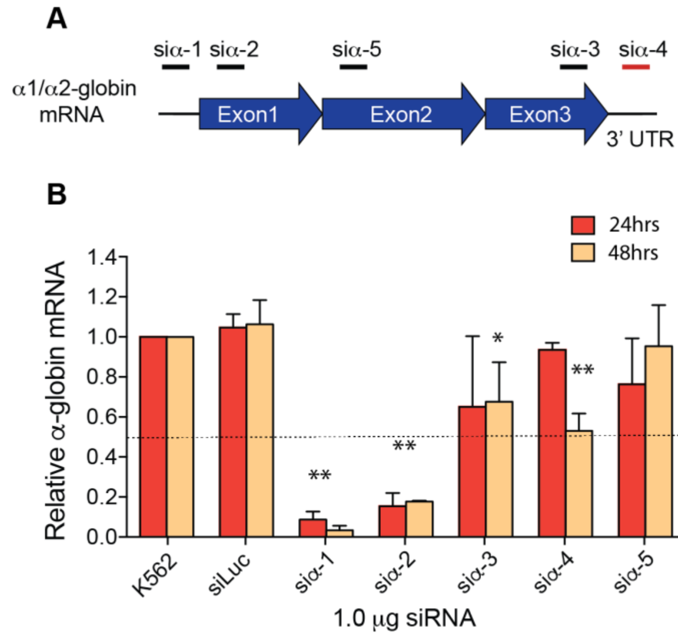

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214 **Figure S3. Relative α-globin mRNA expression in K562 cells following**
215 **electroporation of human α-globin-specific siRNAs. A**) Schematic diagram of human
216 α-globin mRNA showing the target sites for siα-1, siα-2, siα-3, siα-4 and siα-5 siRNAs. **B**)
217 Relative α-globin RNA expression post electroporation of 1 μg of siRNA. Relative
218 expression of α-globin was normalized to relative expression levels in mock
219 electroporated K562 cells using β-actin expression as an RNA loading control. An siRNA
220 sequence targeting luciferase (siLuc) was included in all experiments as an irrelevant
221 control. Values represent the mean average of at least three independent experiments
222 (mean±SD). Statistical analysis was performed using one-way ANOVA (*$p \le 0.05$,
223 **$p \le 0.01$).

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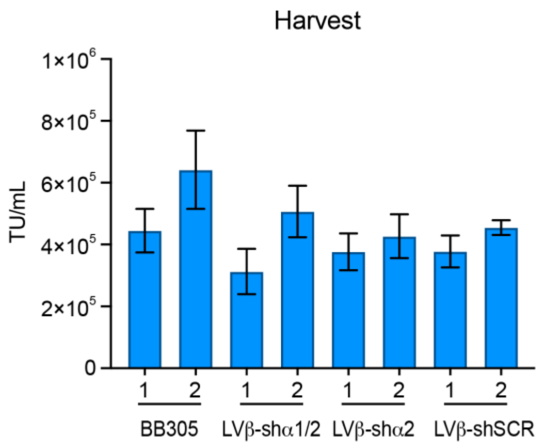

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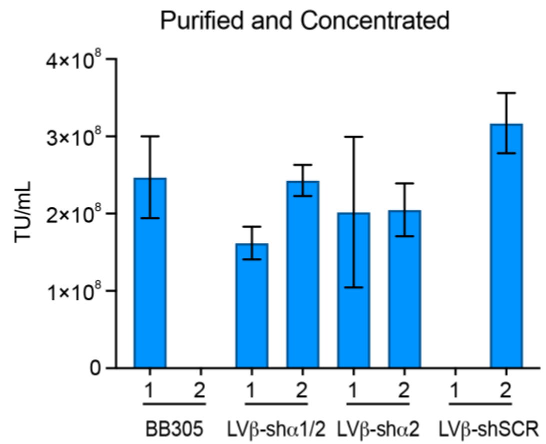

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226 **Figure S4. Comparison of lentiviral vector titres. A**) Lentiviral titre from culture
227 supernatants harvested 24 hours post-transfection of HEK 293T cells (2 independent
228 productions per vector) **B**) Lentiviral titre of harvested lentiviral vector following purification
229 by ion exchange chromatography and 1000-fold concentration.

230

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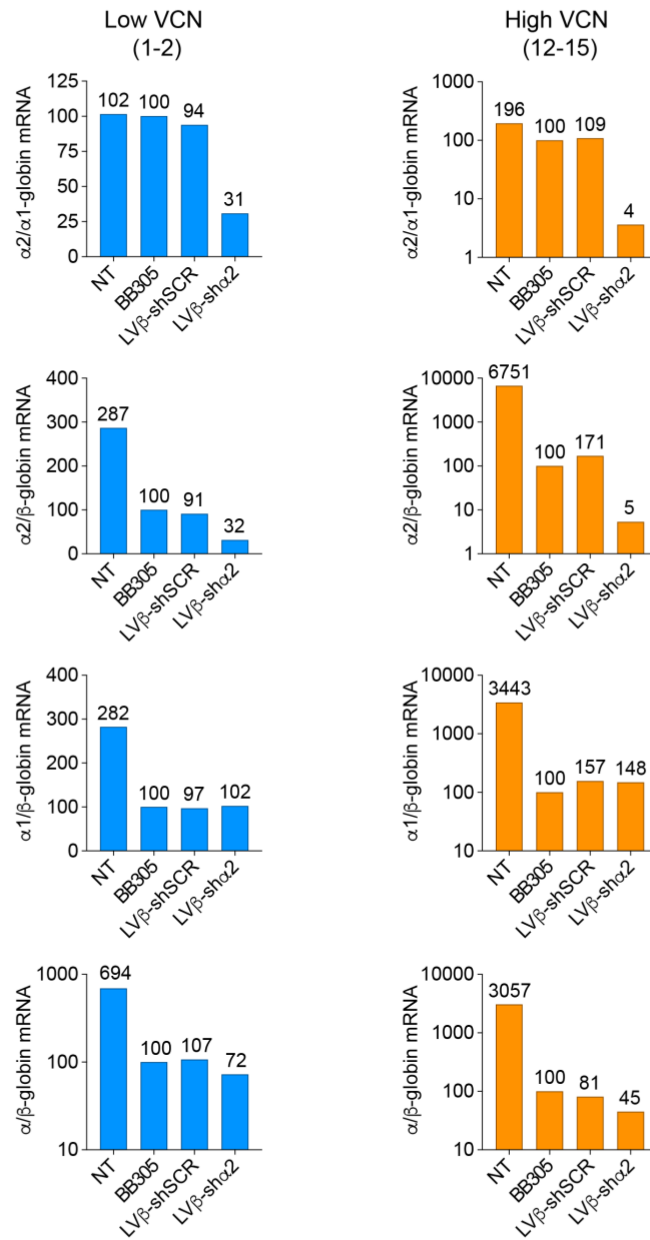

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234 **Figure S5. Analysis of globin mRNA ratio in transduced β^0-HUDEP-2 cells.** RT-qPCR

235 were made from RNA extracted in proliferating β^0-HUDEP-2 cells transduced at low VCN

236 (1-2) and high VCN (12-15).

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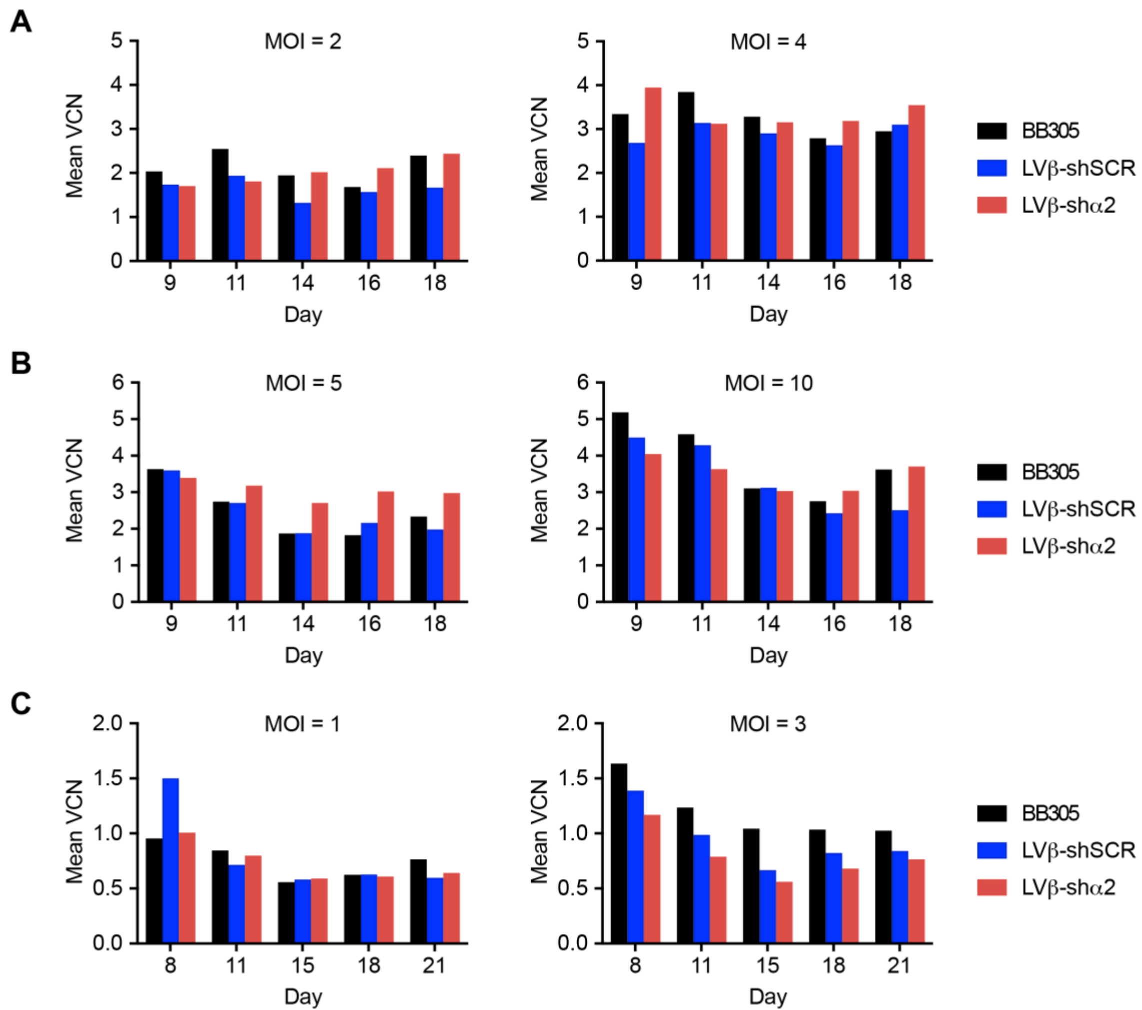


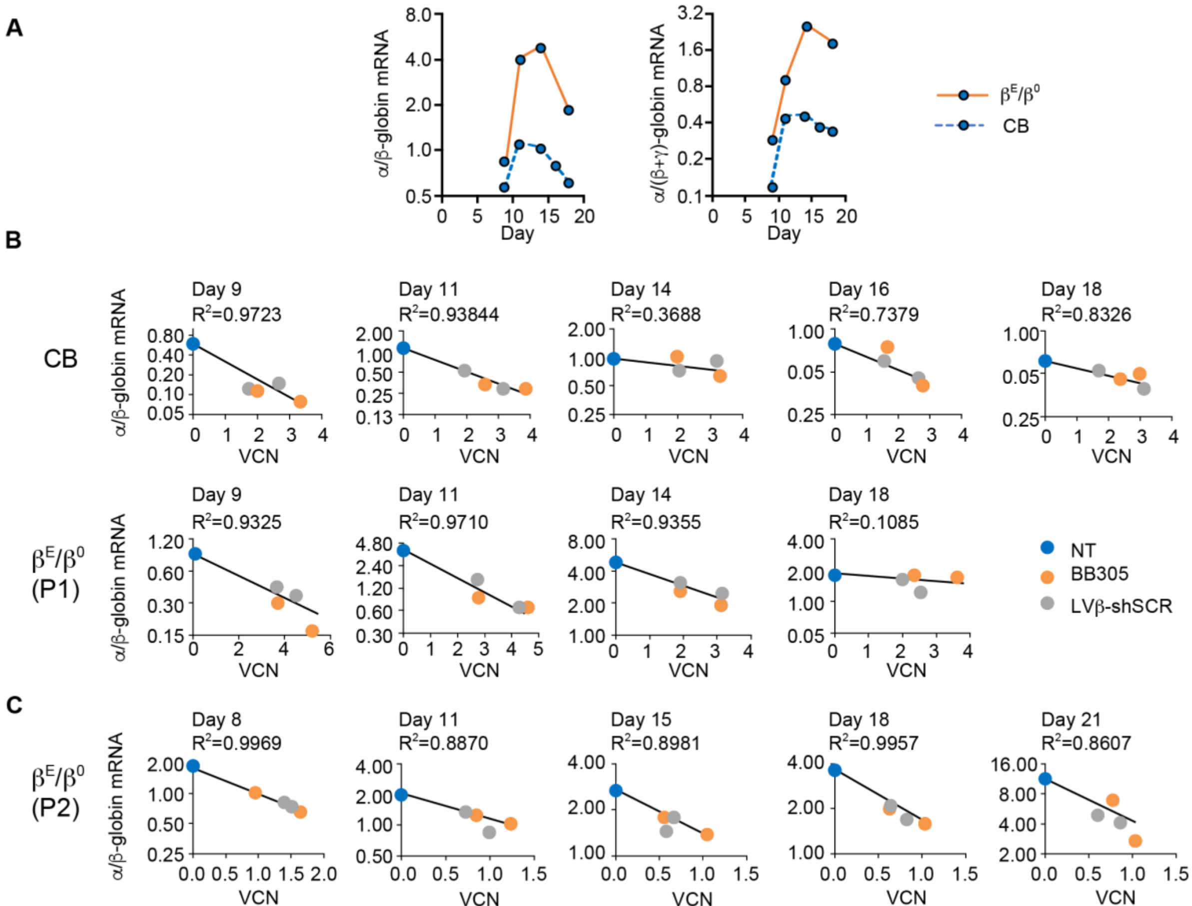
239

240 **Figure S6. Vector copy number in transduced primary cells.** Cord blood (**A**) and

241 HbE/β-thalassemia erythroid cells from patient 1 (**B**) and patient 2 (**C**) were transduced

242 with BB305, LVβ-shSCR and LVβ-shα2 vectors, and grown in differentiation medium 1

243 (**A**, **B**) or differentiation medium 2 (**C**).



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246 **Figure S7. Analysis of α/(β like)-globin mRNA ratio relative to VCN A**) Analysis of

247 α/(β like)-globin mRNA ratio in unmodified primary cells from cord blood (CB) and HbE/β-

248 thalassemia patient collected at several time points after erythroid differentiation. **B**)

249 Globin mRNA ratios in transduced cells from CB and HbE/β-thalassemia patient (P1)

250 relative to VCN. **C**) Globin mRNA ratios in unmodified and transduced cells from second

251 HbE/β-thalassemia patient (P2), grown three weeks in medium 2. The values are

252 distributed in a similar way around the regression line, whether they come from samples

253 transduced by BB305 or LVβ-shSCR.

254

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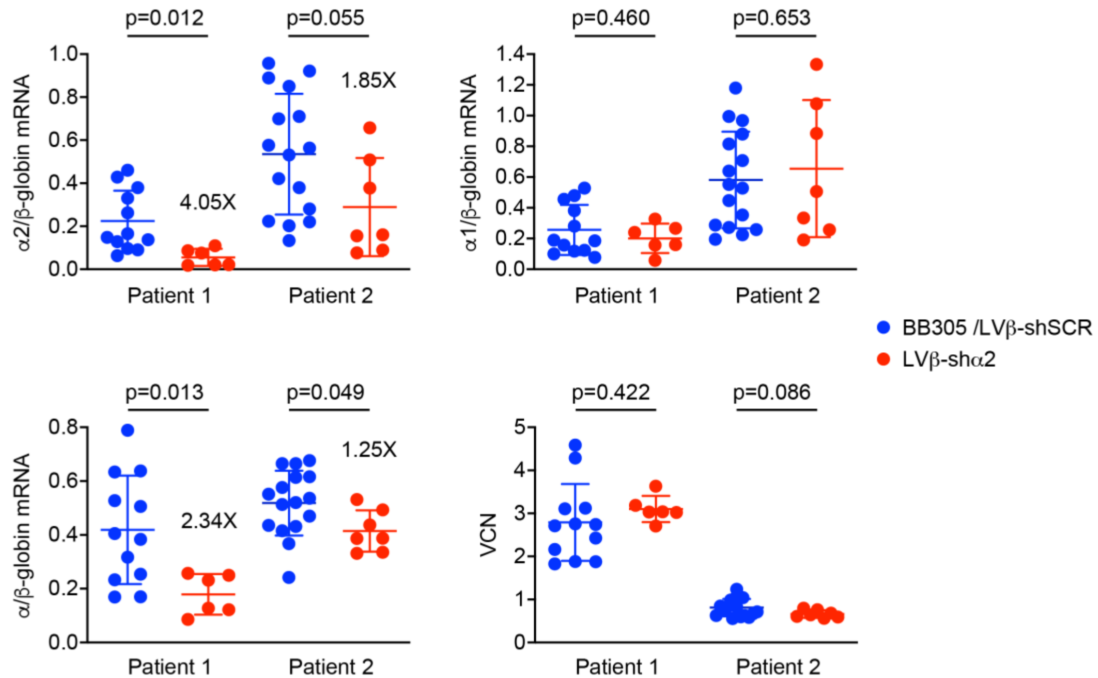

256

257 **Figure S8. Analysis of α/β-globin ratios and VCNs in transduced primary**
258 **HbE/β-thalassemia erythroid cells.** α2/β -, α1/β-, α/β-globin mRNA ratios and VCNs in
259 transduced primary cells from two patients with HbE/β-thalassemia. Cells were grown for
260 three weeks in medium 1 (P1) or 2 (P2) and transduced at MOI 5 and 10 (P1) or 1 and 3
261 (P2). mRNA ratios were normalized to non-transduced cells (set to 1).

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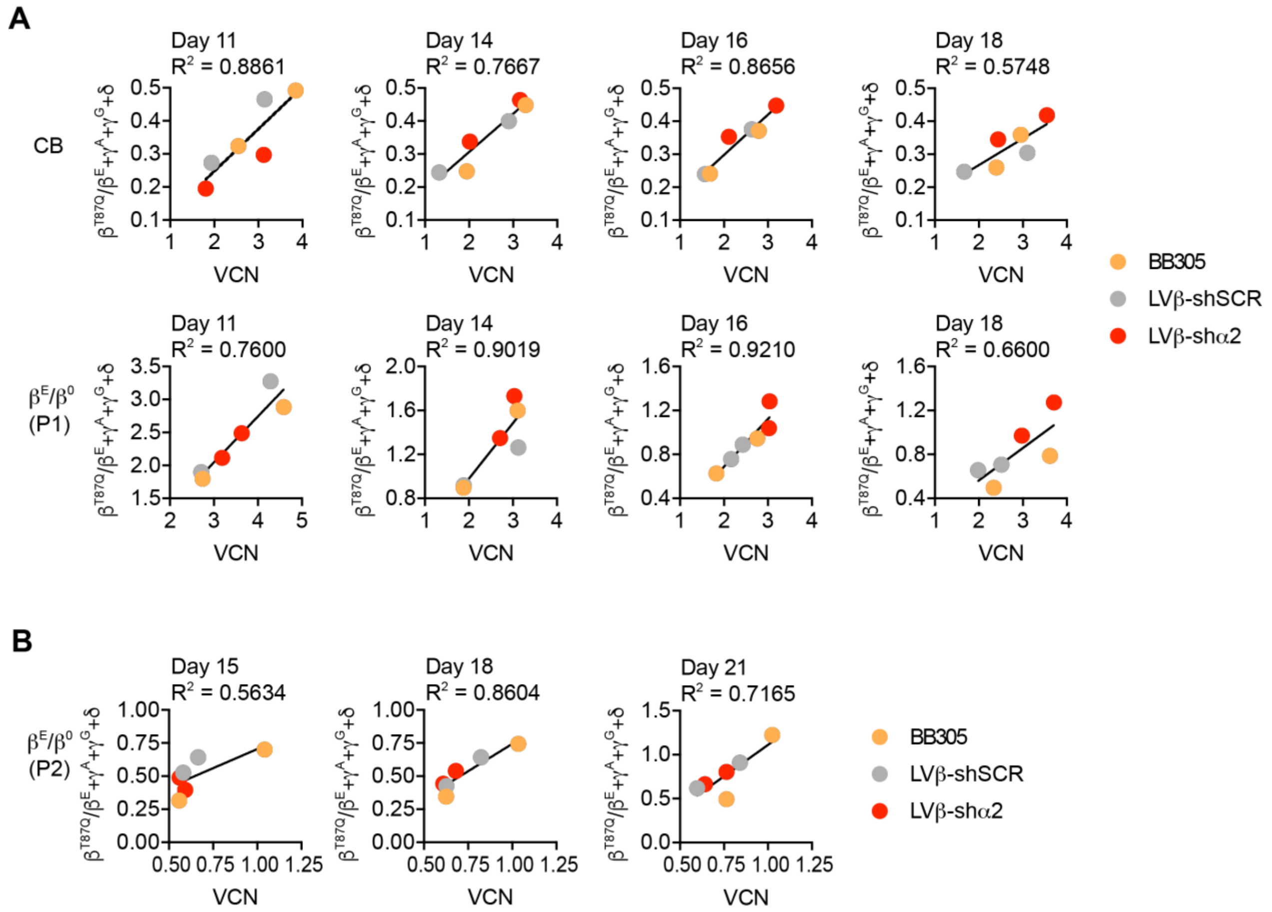

264

265 **Figure S9. Expression of $\beta^{A\text{-}T87Q}$-globin is not affected by shRNA.** $\beta^{A\text{-}T87Q}$-globin
266 expression relative to other β-like globin chains, determined by RP-HPLC in erythroid cell
267 derived from cord blood (CB) and HbE/β-thalassemia samples (patient 1; P1 and patient
268 2; P2) grown in medium 1 (**A**) or 2 (**B**), after transduction with BB305, LVβ-shSCR, or
269 LVβ-shα2.

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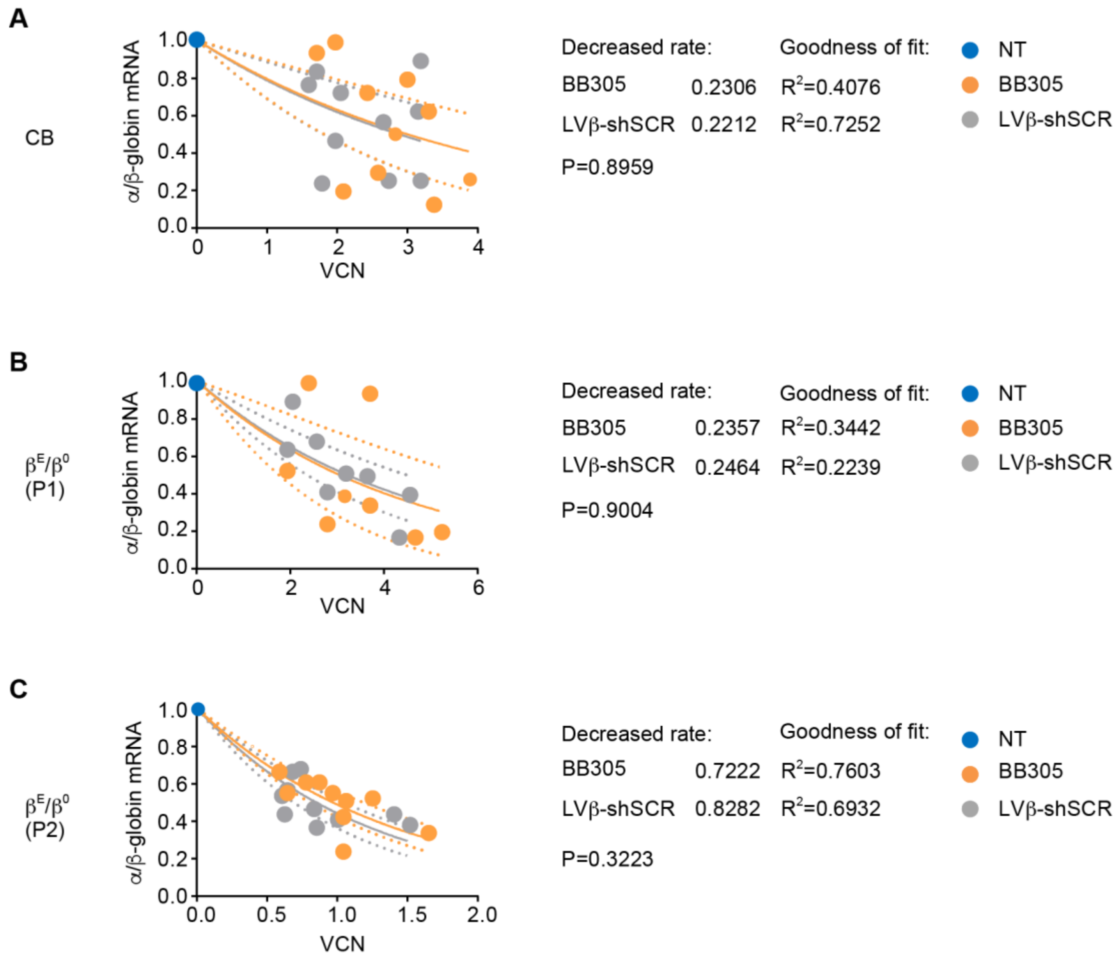

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274 **Figure S10. Analysis of α/β-globin mRNA ratio relative to VCN.** **A**) Analysis of α/β-
275 globin mRNA ratio in transduced cord blood (CB), **B**) HbE/β-thalassemia (P1) and **C**)
276 HbE/β-thalassemia (P2). Globin mRNA ratios are calculated from transduced samples
277 after normalization to values measured in non-transduced cells. Best fit curves and 95%
278 confidence intervals are shown. Determination of P values for comparison of decreased
279 rates in samples transduced with BB305 and LVβ-shSCR vectors were performed
280 assuming ratios of 1 in unmodified cells and 0 at infinite VCN.
281

SUPPLEMENTAL TABLES

Table S1: Oligonucleotides and primers use for construction of the LVβ-shRNA vectors

Assay	Primers	Sequence (5'-3')
Generation of shGFP	shGFP Fwd	ACTCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGA**GCACAAGCTGGAGTACAACTA**_TAGTGAAGCCACAG_ *
	shGFP Rev	ATTGAATTCCGAGGCAGTAGGCA**CACAAGCTGGAGTACAACTA**_TACATCTGTGGCTTC_ *
Generation of shα1/2	shα1/2 Fwd	ACTCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGA**TGAGCACCGTGCTGACCTCCA**_TAGTGAAGCCACAG_ *
	shα1/2 Rev	ATTGAATTCCGAGGCAGTAGGCA**GTGAGCACCGTGCTGACCTCCA**_TACATCTGTGGCTTC_ *
Generation of shα2	shα2 Fwd	ACTCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGA**CGGCCCTTCCTGGTCTTTGAA**_TAGTGAAGCCACAG_ *
	shα2 Rev	ATTGAATTCCGAGGCAGTAGGCAC**CGGCCCTTCCTGGTCTTTGAA**_TACATCTGTGGCTTC_ *
Screening of pLVβ-shRNA	BG-Ex1	CTGACACAACTGTGTTCACT
	shRNA Rev	GTGCAGGGTCCGAGGT
Screening of pIntron2 shuttle vector	T7	GATATACGACTCACTATAGGGC
	BG-Int2	CTGTGGCAGCAAGATAAGAG

* Underline: restriction sites for shRNA cloning, bold: anti-sense and sense sequences, italic: loop sequences

289
290 **Table S2: siRNA and shRNA sequences**

shRNAs	Sequence (5'-3')	Target
siα-1 sense	r(CAGACUCAGAGAGAACCCA)dTdT	5'UTR
siα-1 anti-sense	r(UGGGUUCUCUCUGAGUCUG)dTdG	5'UTR
siα-2 sense	r(CCGACAAGACCAACGUCAA)dTdT	Exon 1
siα-2 antisense	r(UUGACGUUGGUCUUGUCGG)dCdA	Exon 1
siα-3 sense	r(CCGUGCUGACCUCCAAAUA)dTdT	Exon 3
siα-3 antisense	r(UAUUUGGAGGUCAGCACGG)dTdG	Exon 3
siα-4 sense	r(GGCCCUUCCUGGUCUUUGA)dTdT	3'UTR of α2-globin mRNA
siα-4 antisense	r(UCAAAGACCAGGAAGGGCC)dGdG	3'UTR of α2-globin mRNA
siα-5 sense	r(GACCUACUUCCCGCACUUC)dTdT	Exon 2
siα-5 antisense	r(GAAGUGCGGGAAGUAGGUC)dTdT	Exon2
shα1/2	tgctgttgacagtgagcgaGAGCACCGTGCTGACCTCCAATA*gtgaagccacagatgta***TTGGAGGTCAGCACGGTGCTCG**tgcctactgcctcgga	Exon 3 of α1 and α2-globin mRNAs
shα2	tgctgttgacagtgagcgaCGGCCCTTCCTGGTCTTTGAATA*gtgaagccacagatgta***TTCAAAGACCAGGAAGGGCCGG**tgcctactgcctcgga	3' UTR of α2-globin mRNA
shGFP	tgctgttgacagtgagcgaGCACAAGCTGGAGTACAACTATA*gtgaagccacagatgta***TAGTTGTACTCCAGCTTGTGCC**tgcctactgcctcgga	eGFP mRNA
shSCR	tgctgttgacagtgagcgaTCTCGCTTGGGCGAGAGTAAGTA*gtgaagccacagatgta***CTTACTCTCGCCCAAGCGAGCG**tgcctactgcctcgga	Non-targeting control

291 Bold uppercase: antisense sequences, regular uppercase: sense sequences, italic: loop sequence.
292
293

294

295 **Table S3: Primers and probes used for vector copy number determination** [10]

296

Assay	Amplicon	Primer and probe	Sequence (5'-3') or TaqMan gene expression assay number*	Modification	Conc.
Vector copy number	Lentiviral vector	GAG F	GGAGCTAGAACGATTCGC AGTTA	-	720nM
		GAG R	GGTTGTAGCTGTCCCAGT ATTTGTC	-	720nM
		GAG P	ACAGCCTTCTGATGTCTC TAAAAGGCCAGG	5'FAM 3'TAMRA	140nM
	Human β-actin	hbAct F	TCCGTGTGGATCGGCGG CTCCA	-	900nM
		hbAct R	CTGCTTGCTGATCCACAT CTG	-	900nM
		hbAct P	CCTGGCCTCGCTGTCCAC CTTCCA	5'FAM 3'TAMRA	250nM
NIH3T3 titer	Mouse β-actin	mbAct assay	Mm00607939_S1*	5'FAM 3'NFQ-MGB	1x

297 FAM, 6-carboxyfluorescein ester; TAMRA, tetramethyl-6-carboxyrhodamine; NFQ, nonfluorescent
298 quencher; MGB, minor groove binder; Conc., concentration

299 * Applied Biosystems.

300

301 **Table S4: Primers and Probes used for RT-qPCR**
302

Assay	Amplicon	Primer and probe	Sequence (5'-3') or TaqMan gene expression assay number*	Modification	Conc.
eGFP expression (SYBR Green)	EGFP	eGFP F	AACGTCTATATCATGGCCGA	-	250nM
		eGFP R	TGCACGCTGCCGTCCTCGAT	-	250nM
	Mouse β-actin	mmACTB F	CAACGAGCGGTTCCGATG	-	250nM
		mmACTB R	TACCCAAGAAGGAAGGCTGG A	-	250nM
shGFP expression (SYBR Green)	shGFP	shGFP F	GCGTATAGTTGTACTCCAGC	-	250nM
		shRNA R	GTGCAGGGTCCGAGGT	-	250nM
		shGFP-RT	GTCGTATCCAGTGCAGGGTC CGAGGTATTCGCACTGGATA CGACGCACAA	Reverse transcription	
		shGFP-3p	TAGTTGTACTCCAGCTTGTGC	Oligo control	
α/(β+γ) ratio (TaqMan)	α1/α2 -globin	HBA1/ HBA2	Hs00361191_g1*	5'FAM 3'NFQ-MGB	1X
	β-globin	HBB	Hs00747223_g1*	5'FAM 3'NFQ-MGB	1X
	Aγ/Gγ -globin	HBG1/ HBG2	Hs00361131_g1*	5'FAM 3'NFQ-MGB	1X
α1-globin expression (SYBR Green)	α1-globin	HBA1 F	CTCGGTGGCCATGCTTCT	-	200nM
		HBA1 R	GGGTACGGGTGCAGGA	-	200nM
α2-globin expression (SYBR Green)	α2-globin	HBA2 F	CTCGGTAGCCGTTCCTCC	-	200nM
		HBA2 R	GAAGGGCCGGTGCAAGG	-	200nM
β-globin expression (SYBR Green)	β-globin	HBB F	GCACGTGGATCCTGAGAACT	-	250nM
		HBB R	ACCAGCCACCACTTTCTGAT	-	250nM
Reference gene	Human β-actin	hsACTB F	AGGCACCAGGGCGTGAT	-	250nM
		hsACTB R	TCGCCCACATAGGAATCCTT	-	250nM
βA-T87Q-globin mRNA splicing	βA-T87Q -globin	HBBT87Q F	TCAAGGGCACCTTTGCCCAG	-	500nM
		HBB R	ACCAGCCACCACTTTCTGAT	-	500nM

303 FAM: 6-carboxyfluorescein ester, NFQ: non-fluorescent quencher, MGB: minor groove binder, Conc:
304 Concentration, *ThermoFisher Scientific
305

306
307 **Table S5: Antibodies**
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Antibodies	Assay	Supplier (Cat. No.)
Fixable Viability Dye eFluorTM 780	Flow cytometry	eBioscience (65-0865-14)
Propidium Iodide	Flow cytometry	Sigma Aldrich (P4864)
Hemoglobin α (D4) Alexa Fluor® 647	Flow cytometry	Santa Cruz Biotechnology (sc-514378 AF647)
Hemoglobin β (37-8) PE	Flow cytometry	Santa Cruz Biotechnology (sc-21757 PE)
PE mouse anti-human CD36 (Ac106)	Flow cytometry	Miltenyi (130-095-472)
APC mouse anti-human CD71 (Ac102)	Flow cytometry	Miltenyi (130-091-727)
FITC mouse anti-human CD235a (HIR2)	Flow cytometry	eBiosciences (11-9987)
Hemoglobin α (D-4);	Western blot	Santa Cruz Biotechnology (sc-514378)
Hemoglobin β (37-8)	Western blot (K562)	Santa Cruz Biotechnology (sc-21757)
β-actin (C4)-HRP	Western blot (K562)	Santa Cruz Biotechnology (sc-47778)
Goat anti-mouse IgG-HRP:	Western blot (K562)	Santa Cruz Biotechnology (sc-2005)
HBB monoclonal antibody (M02) clone 7B12	Western blot (HUDEP2)	Abnova (H00003043-M02)
HBA monoclonal antibody (MO2) clone 4F9	Western blot (HUDEP2)	Abnova (H00003039-M02)
Goat anti-mouse IgG-HRP	Western blot	Jackson ImmunoResearch (115-035-006)

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