SUPPLEMENTARY MATERIALS for

Therapeutically relevant engraftment of a CRISPR/Cas9-edited HSC-enriched population with HbF reactivation in nonhuman primates

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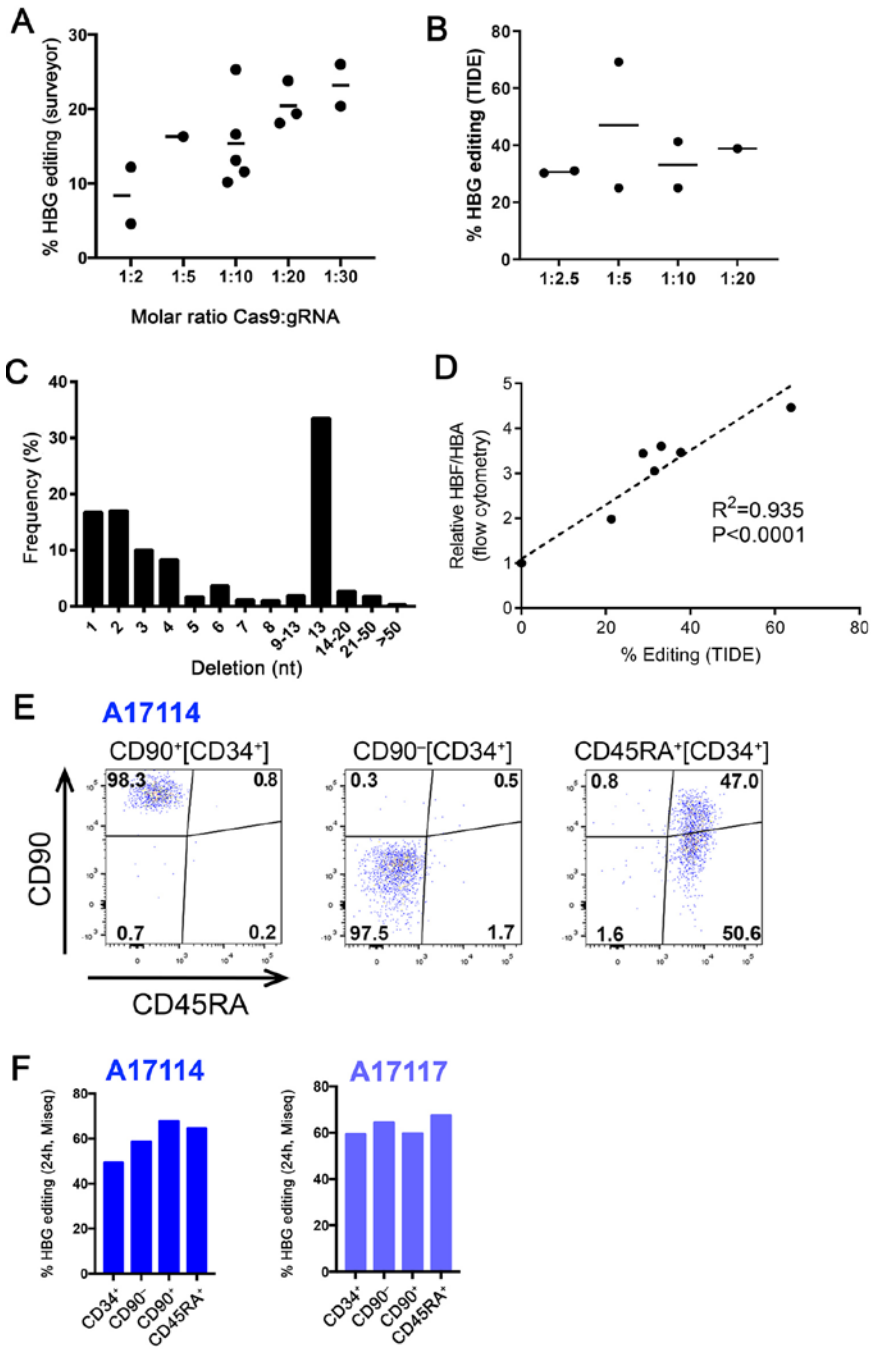


Fig. S1. Optimization of *HBG* editing in NHP HSPCs. (A), (B) Titration of molar ratios of Cas9 purified protein to gRNA for optimization of *HBG*-editing in NHP CD34$^+$ cells (A) or mobilized PB Human CD34$^+$ cells (B). Editing efficiency was determined by Surveyor assay (A) or TIDE (B) at 3-10 days after electroporation. Each circle shows a separate electroporation reaction using cells from a total of 2 (human) or 3 (NHP) donors. Lines show mean for each indicated molar ratio. **(C)** Size of deletion (in nucleotides, nt) induced at the CRISPR/Cas9 target site in edited NHP CD34$^+$ cells from one representative donor at 4 days after treatment. Results are from next-generation sequencing analysis with all deletions normalized to 100%. **(D)** Correlation by linear regression analysis of the % of in vitro differentiated CD34$^+$ cells expressing HbF over % cells expressing HbA (HbF/HbA, determined by flow cytometry) relative to editing efficiency determined by TIDE. **(E)** Flow cytometric validation of HSPC fractions sorted from enriched CD34$^+$ cells obtained from animal A17114. **(F,** *HBG* editing efficiency in HSPC-sorted subsets from the indicated animals.

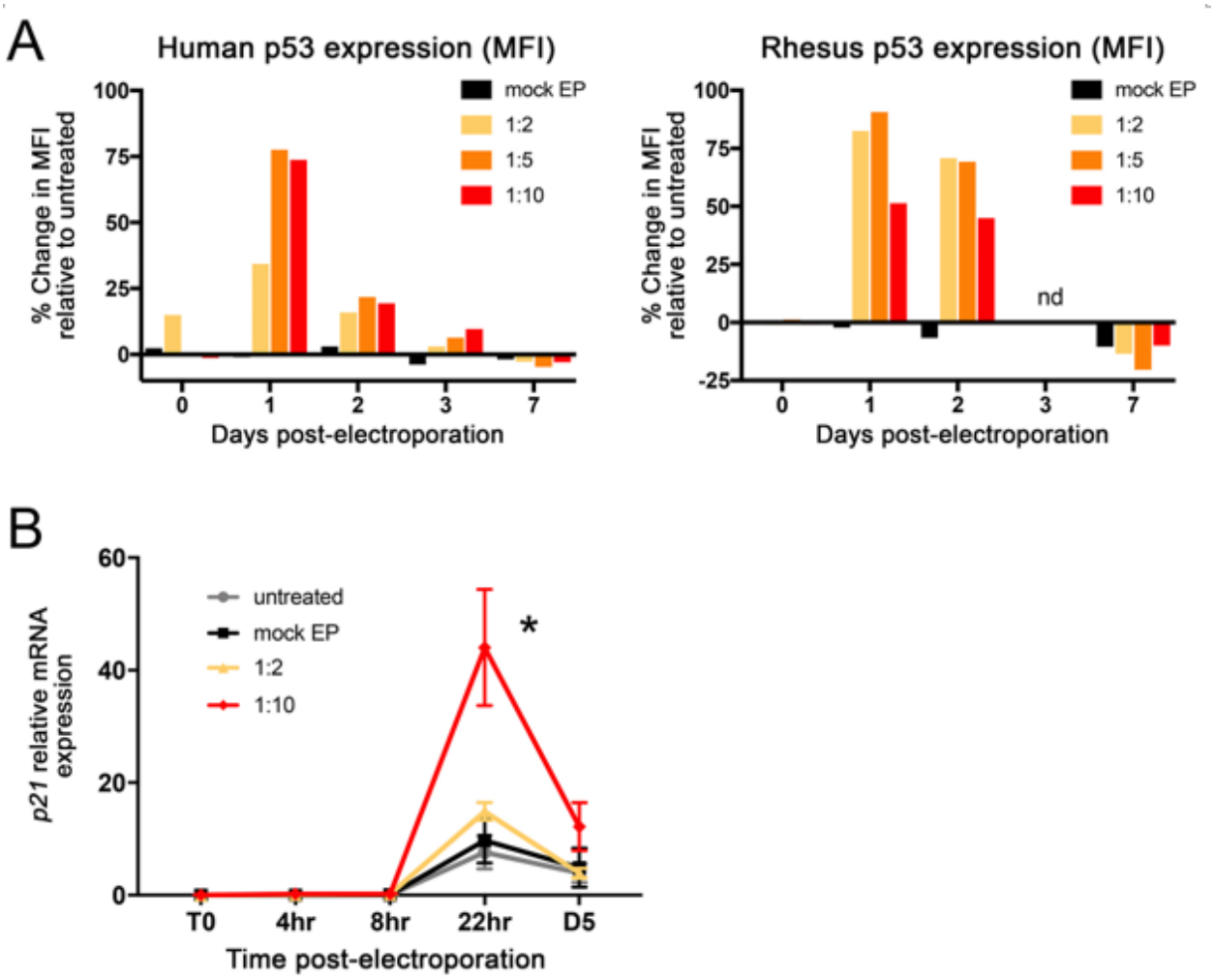


Fig. S2. Induction of the p53/p21 DNA damage response in human and rhesus CD34$^+$ HSPCs treated with CRISPR/Cas9. (A) p53 induction as measured by intracellular flow cytometric staining and quantified as mean fluorescence intensity (MFI) in mock electroporated (EP) CD34$^+$ HSPCs or in cells treated with increasing molar ratios of Cas9 protein to gRNA relative to untreated cells. Results are from human (left) and rhesus CD34$^+$ HSPCs (right). **(B)** RT-qPCR analysis of *p21* (*CDKN1A*) mRNA expression in rhesus CD34$^+$ HSPCs treated as described in (A) relative to *GAPDH*. Data are mean and SEM from triplicate reactions run from one donor. * denotes statistical significance of the difference between the 1:10 reaction and the untreated or mock EP (two-tailed unpaired t-test, $P<0.05$).

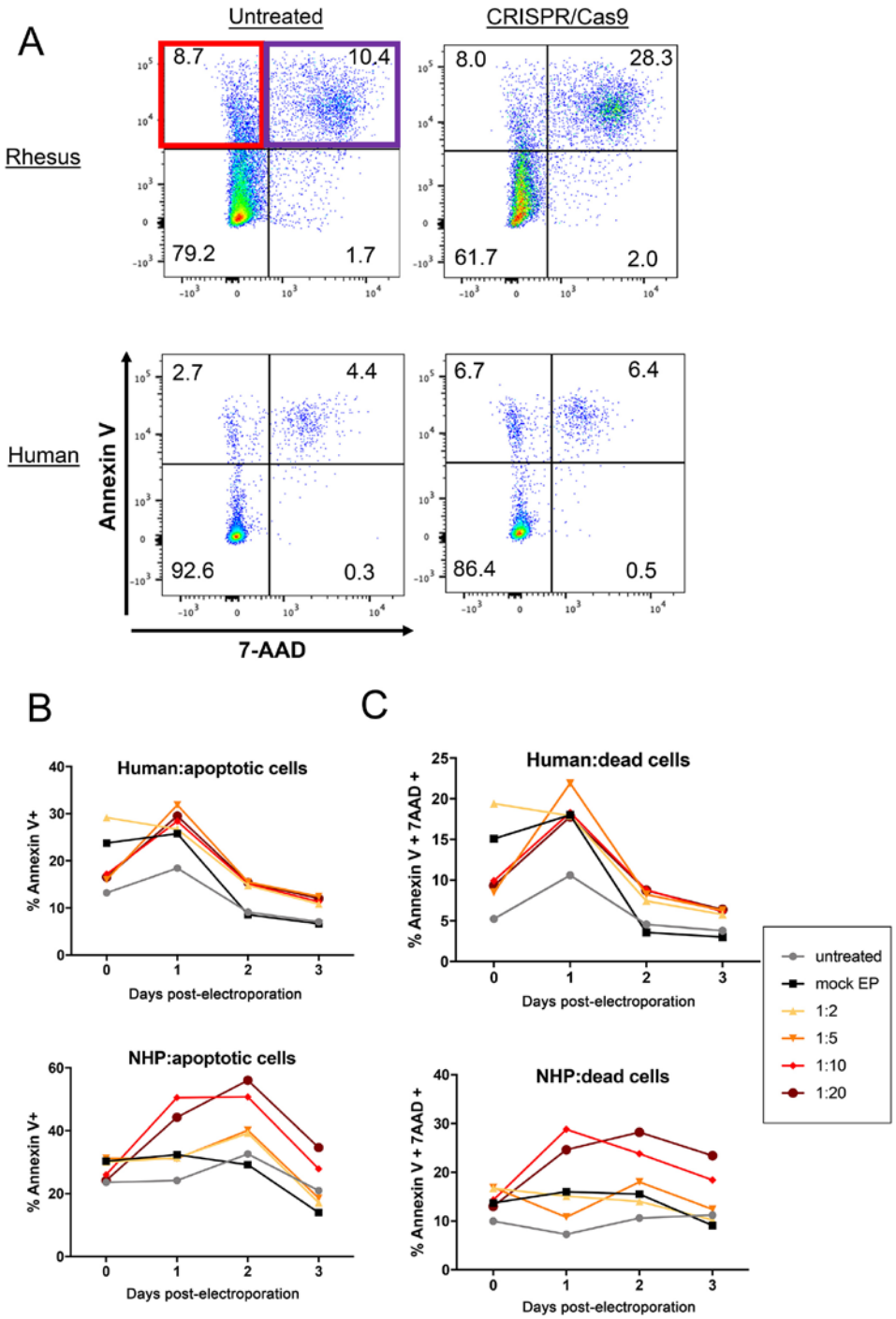


Fig. S3. CRISPR/Cas9-induced cell death and apoptosis in human and rhesus HSPCs. (A) Representative flow plots of annexin V/7-AAD staining in rhesus and human CD34$^+$ HSPCs. Annexin V staining indicates apoptotic cells (red frame) and annexin V/7-AAD dual staining (purple frame) shows dead cells. **(B), (C)** Quantification of apoptosis **(B)** and cell death **(C)** of human (top) and rhesus (bottom) CD34$^+$ HSPCs left untreated, treated by mock electroporation (EP), or treated with increasing molar ratios of Cas9 protein to gRNA. Results are from one donor and comparable results were obtained using a different donor.

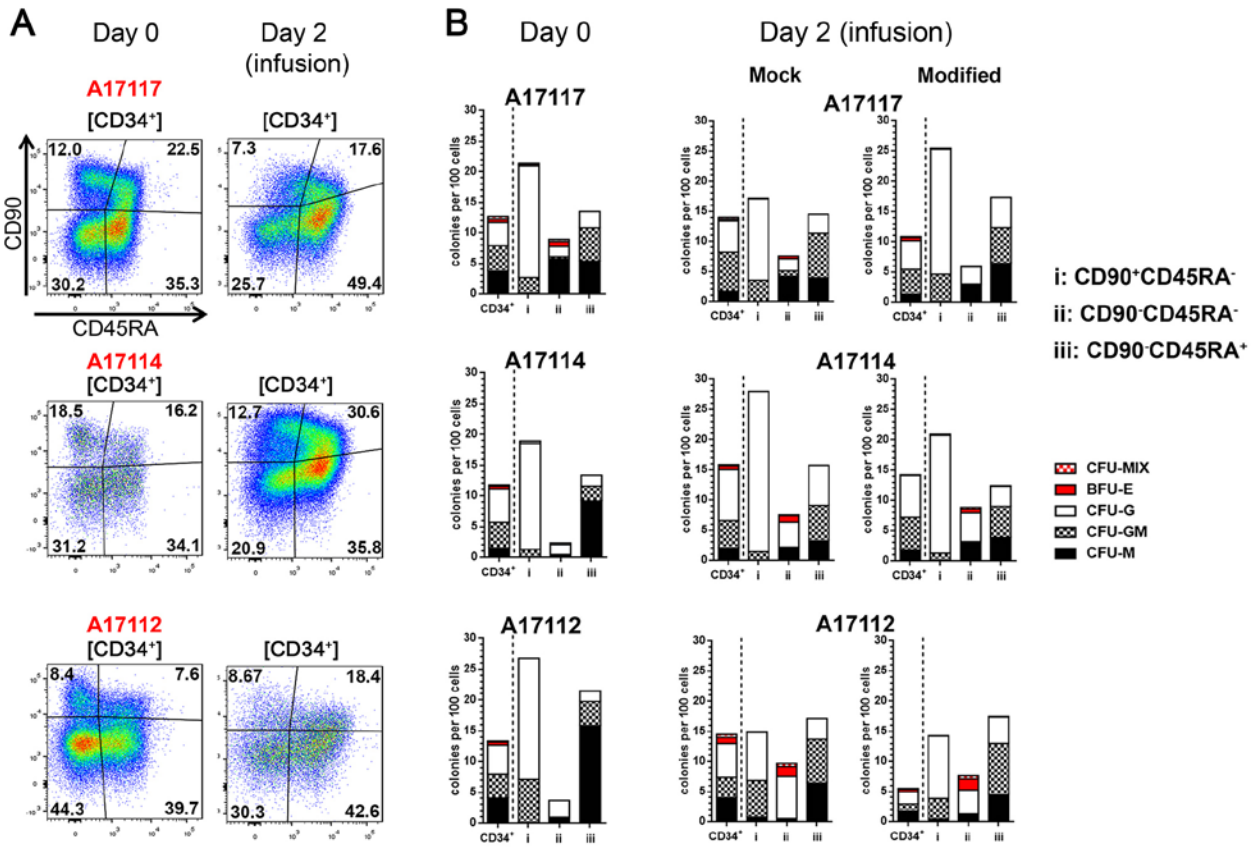


Fig. S4. Immunophenotypic and functional characterization of infusion products of CD34 transplants. (A) Flow cytometric analysis of $CD34^+$ enriched cells at time of enrichment (day 0) or after editing at time of infusion (day 2) using the markers CD90 and CD45RA in the three $CD34^+$/CRISPR transplanted animals. **(B)** CFCs obtained from bulk $CD34^+$ enriched cells or from the indicated HSPC subsets in $CD34^+$/CRISPR transplanted animals at time of enrichment (day 0) or infusion (day 2) in mock-electroporated and CRISPR/Cas9-treated cells.

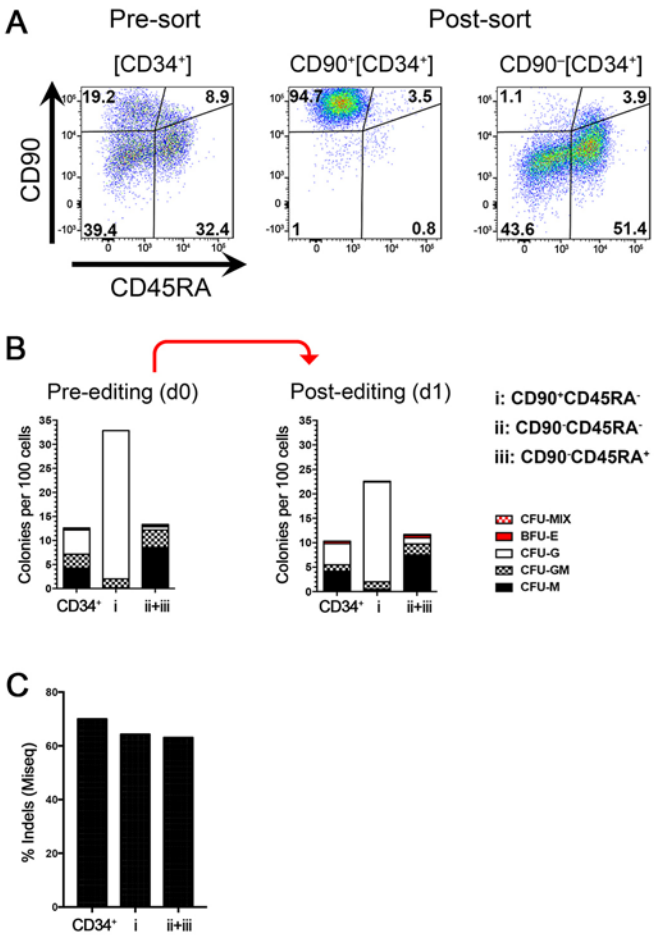


Fig. S5. In vitro validation of the CD90$^+$ sorting/editing approach. (A) Flow cytometric analysis of CD34$^+$ enriched cells before (pre-sort) and after (post-sort) sorting for the CD90$^+$ population from animal A17114. **(B)** CFC assay from the cells shown in (A) before and 24 hours after CRISPR/Cas9-editing. **(C)** Editing efficiency measured in CD34$^+$ enriched cells, and in the CD90$^+$ and CD90$^-$ fractions (obtained from (A)) by Miseq analysis at 3 days after treatment.

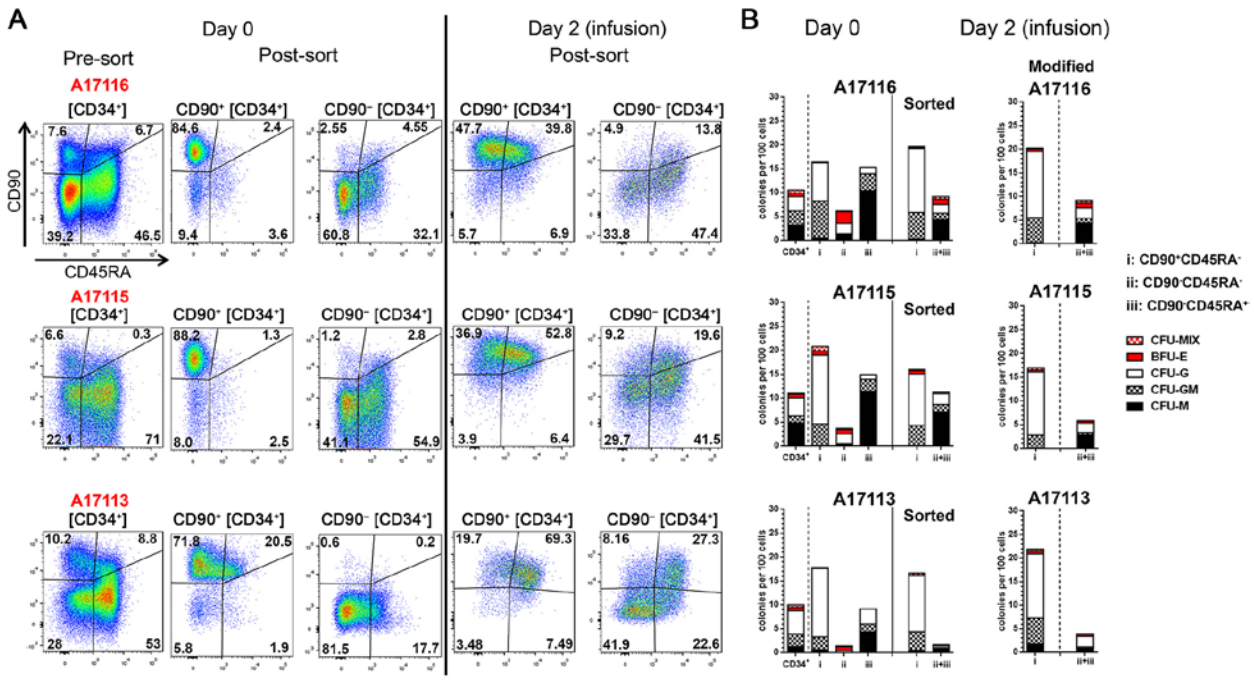


Fig. S6. Immunophenotypic and functional characterization of infusion products of CD90 transplants. (A) Flow cytometric analysis of $CD34^+$ enriched cells before (left) and after (right) CD90 sorting at time of enrichment (day 0) or at time of infusion (day 2) in the three $CD90^+$/CRISPR transplanted animals. **(B)** CFCs obtained from bulk $CD34^+$ enriched cells or from the indicated HSPC subsets in $CD90^+$/CRISPR transplanted animals at time of enrichment (day 0) or infusion (day 2).

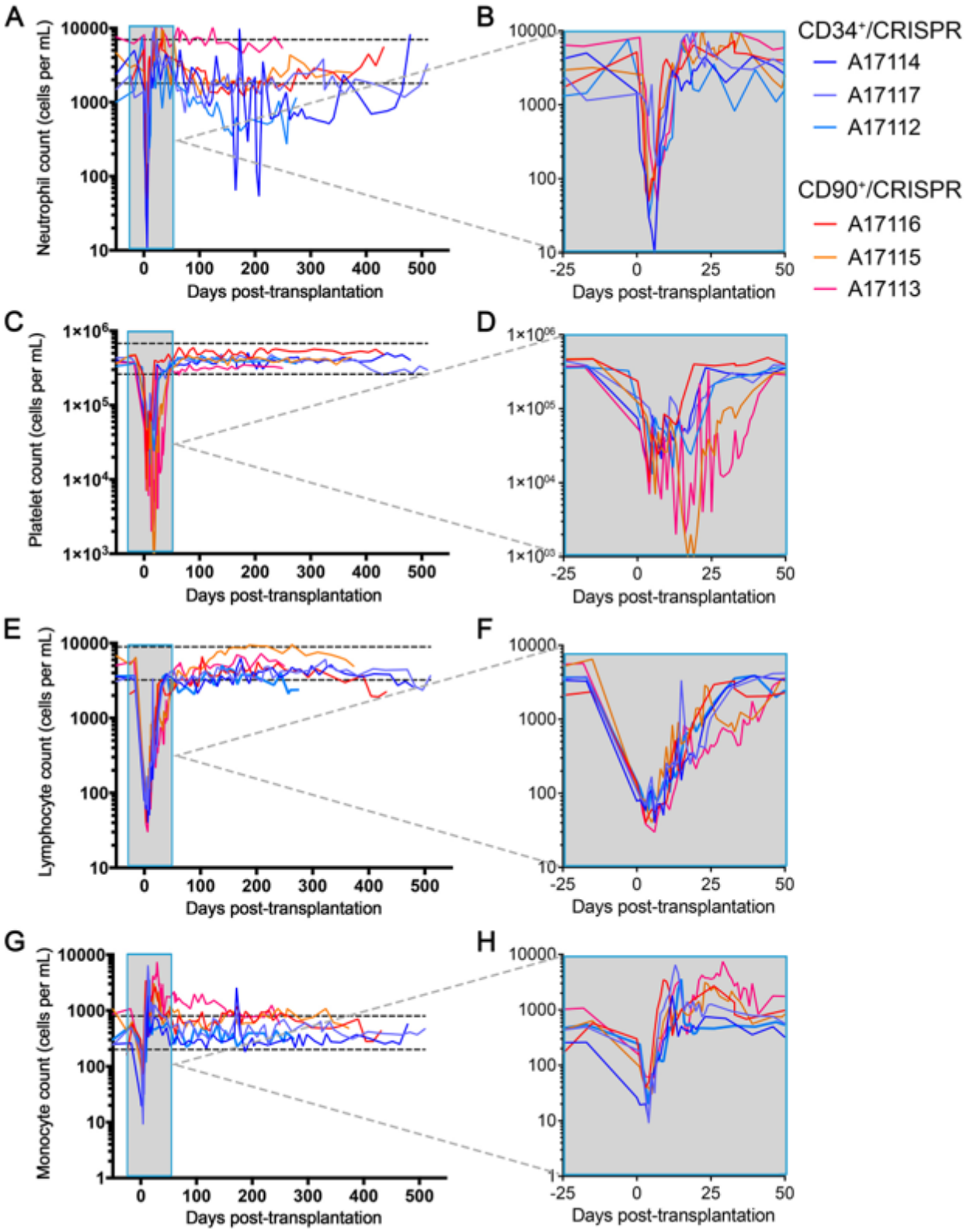


Figure S7. Complete blood cell count in transplanted animals. Day 0 shows time of transplant. Dashed lines show normal count range for each lineage. The graphs on the right magnify blood cell count shown in gray area on the left up to 50 days after transplant.

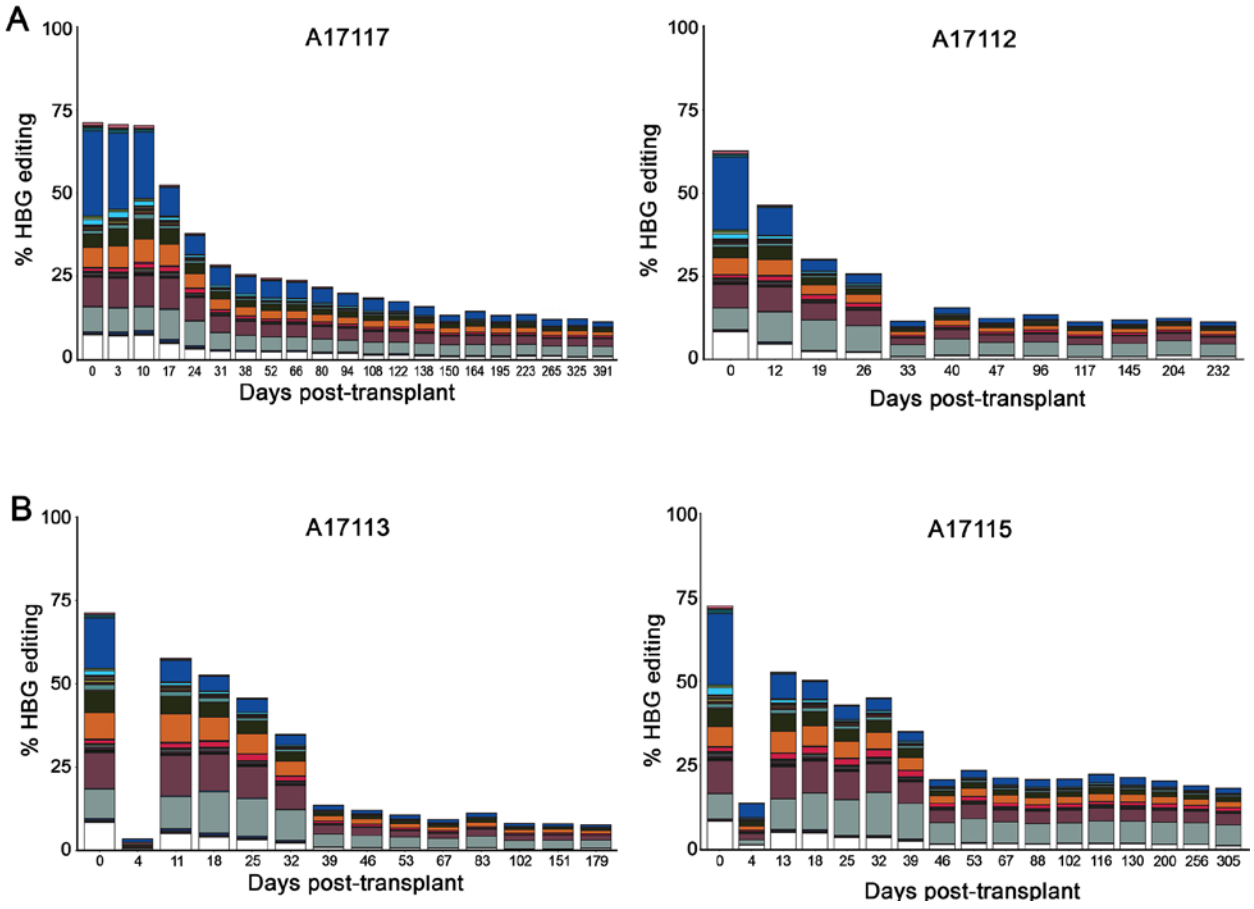


Figure S8. Tracking of deletion profiles in transplanted animals. Deletion profile was determined by next generation sequencing analysis of PB sampled at different time points after transplant in the CD34^{+}/CRISPR cohort **(A** or in the CD90^{+}/CRISPR cohort **(B)**. Each colored box denotes the individual contribution of an identified distinct deletion to the total sequencing pool and the white portion shows all combined deletions contributing less than 1% to the total pool. The 13-nt HPFH deletion is shown in the dark blue box on top.

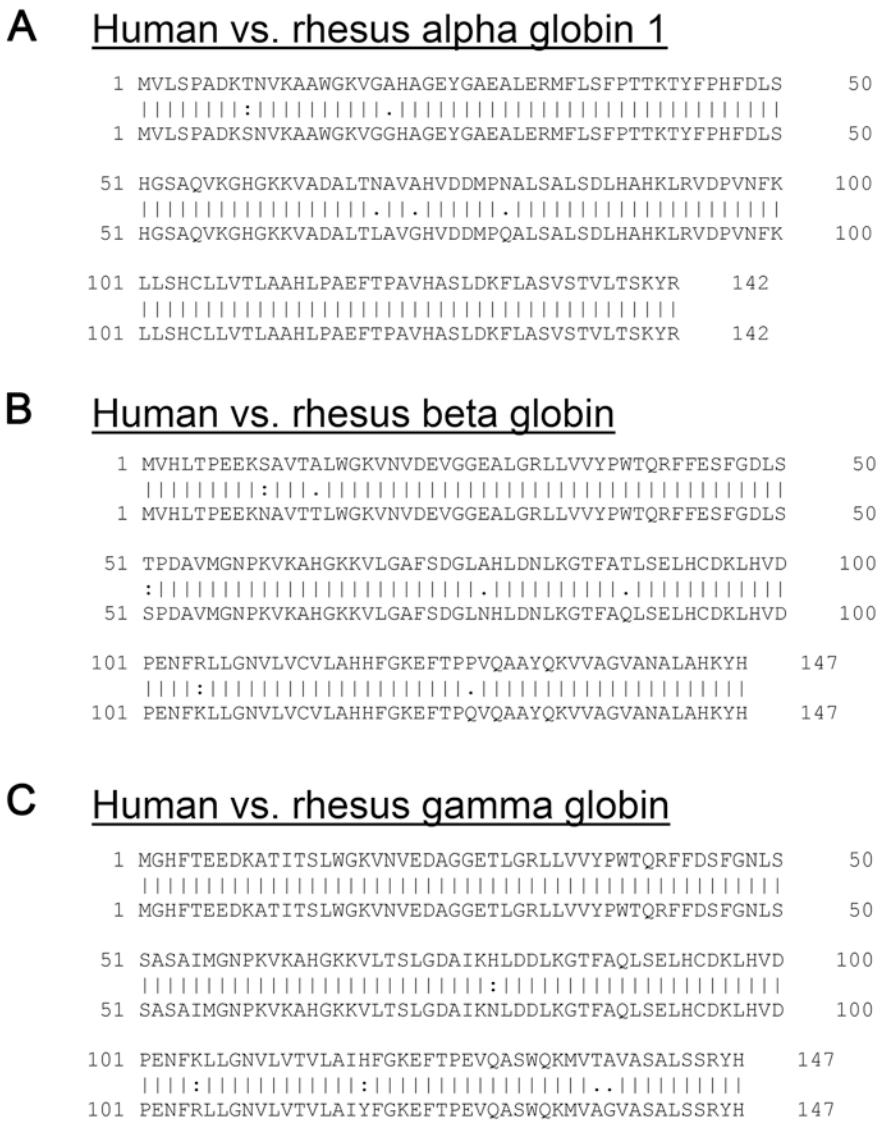
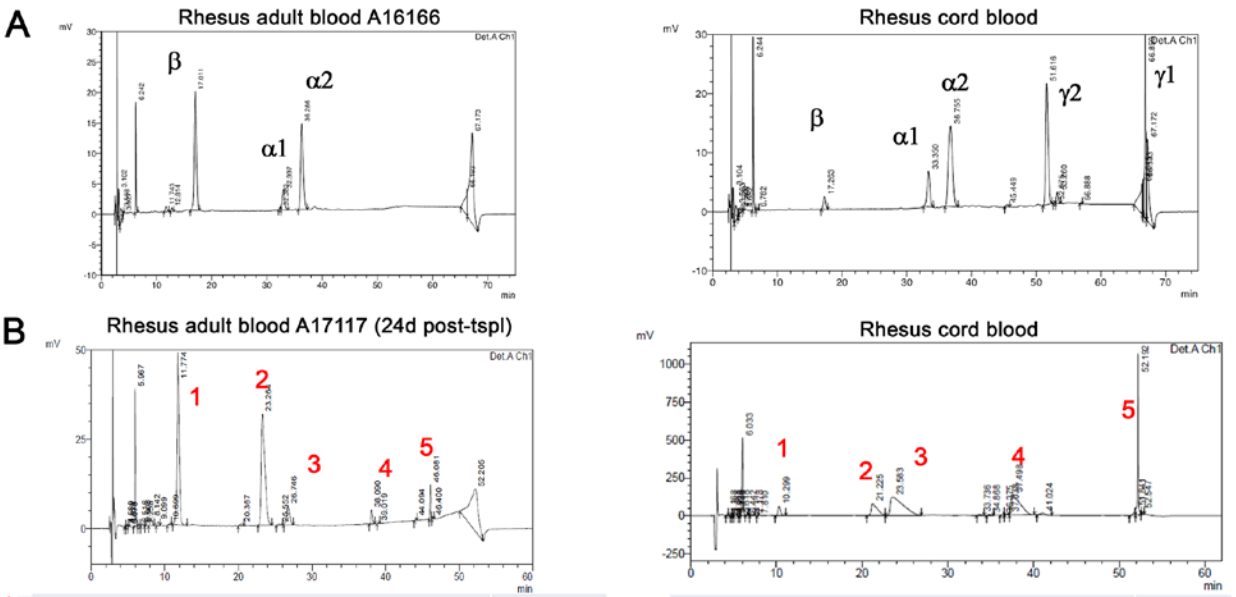


Figure S9. Conservation of globin polypeptide sequences between human and rhesus macaque. Alignment of alpha (A), beta (B) and gamma (C) globin polypeptide sequences between human (top) and rhesus macaque (bottom). Sequences are from reference genome sequences available in the UCSC Genome Browser http://genome.ucsc.edu/.

Figure S10. Conservation of globin polypeptide homologs in rhesus macaque. Alignment of the two rhesus gamma (A) and alpha (B) polypeptide sequences. Sequences are from reference genome sequences available in the UCSC Genome Browser http://genome.ucsc.edu/. Highlighted are the polymorphic sites between the two polypeptides.



	Description	# PSMs
1	Globin A1 OS=Macaca mulatta GN=HBB PE=2 SV=1	1352
	Hemoglobin subunit beta OS=Macaca mulatta GN=HBB PE=1 SV=1	1264
	Uncharacterized protein OS=Macaca mulatta GN=LOC715607 PE=3 SV=2	224
	Hemoglobin subunit epsilon OS=Macaca mulatta GN=HBE1 PE=2 SV=3	186
2	Hemoglobin alpha chain OS=Macaca mulatta GN=HBA2 PE=2 SV=1	768
	Uncharacterized protein OS=Macaca mulatta PE=3 SV=2	482
	Hemoglobin subunit beta OS=Macaca mulatta GN=HBB PE=1 SV=1	312
	Hemoglobin subunit gamma OS=Macaca mulatta GN=HBG PE=1 SV=2	135
3	Hemoglobin alpha chain OS=Macaca mulatta GN=HBA2 PE=2 SV=1	371
	Hemoglobin subunit beta OS=Macaca mulatta GN=HBB PE=1 SV=1	206
	Hemoglobin subunit gamma OS=Macaca mulatta GN=HBG PE=1 SV=2	118
4	Hemoglobin subunit gamma OS=Macaca mulatta GN=HBG PE=1 SV=2	155
	Hemoglobin subunit beta OS=Macaca mulatta GN=HBB PE=1 SV=1	68
5	Keratin 5 OS=Macaca mulatta GN=KRT5 PE=3 SV=2	87
	Gamma-globin OS=Macaca mulatta GN=LOC715607 PE=3 SV=1	86

	Description	# PSMs
1	Globin A1 OS=Macaca mulatta GN=HBB PE=2 SV=1	265
	Hemoglobin subunit beta OS=Macaca mulatta GN=HBB PE=1 SV=1	248
	Hemoglobin alpha chain OS=Macaca mulatta GN=HBA2 PE=2 SV=1	60
	Hemoglobin subunit gamma OS=Macaca mulatta GN=HBG PE=1 SV=2	42
2	Hemoglobin alpha chain OS=Macaca mulatta GN=HBA2 PE=2 SV=1	187
	Hemoglobin subunit alpha OS=Macaca mulatta GN=HBA PE=1 SV=2	183
	Uncharacterized protein OS=Macaca mulatta PE=3 SV=2	138
3	Hemoglobin alpha chain OS=Macaca mulatta GN=HBA2 PE=2 SV=1	233
	Uncharacterized protein OS=Macaca mulatta PE=3 SV=2	139
4	Hemoglobin subunit gamma OS=Macaca mulatta GN=HBG PE=1 SV=2	310
	Globin A1 OS=Macaca mulatta GN=HBB PE=2 SV=1	31
	Hemoglobin subunit epsilon OS=Macaca mulatta GN=HBE1 PE=2 SV=3	28
	Hemoglobin alpha chain OS=Macaca mulatta GN=HBA2 PE=2 SV=1	21
5	Gamma-globin OS=Macaca mulatta GN=LOC715607 PE=3 SV=1	371
	Arachidonate 15-lipoxygenase OS=Macaca mulatta GN=ALOX15 PE=3 SV=1	37

Figure S11. Validation of reverse phase-HPLC approach for the analysis of hemoglobin from NHP blood. (A) HPLC analysis of rhesus macaque adult PB (left) and cord blood (right) showing different peaks corresponding to the different globin peptides. **(B)** Mass spectrometry analysis of individual peaks eluted from A17117 PB at 24 days after transplant (left) and from cord blood (right). The top peptide candidates based on peptide-spectrum match (PSM) scoring are given in the table and the predominant globin species are highlighted.

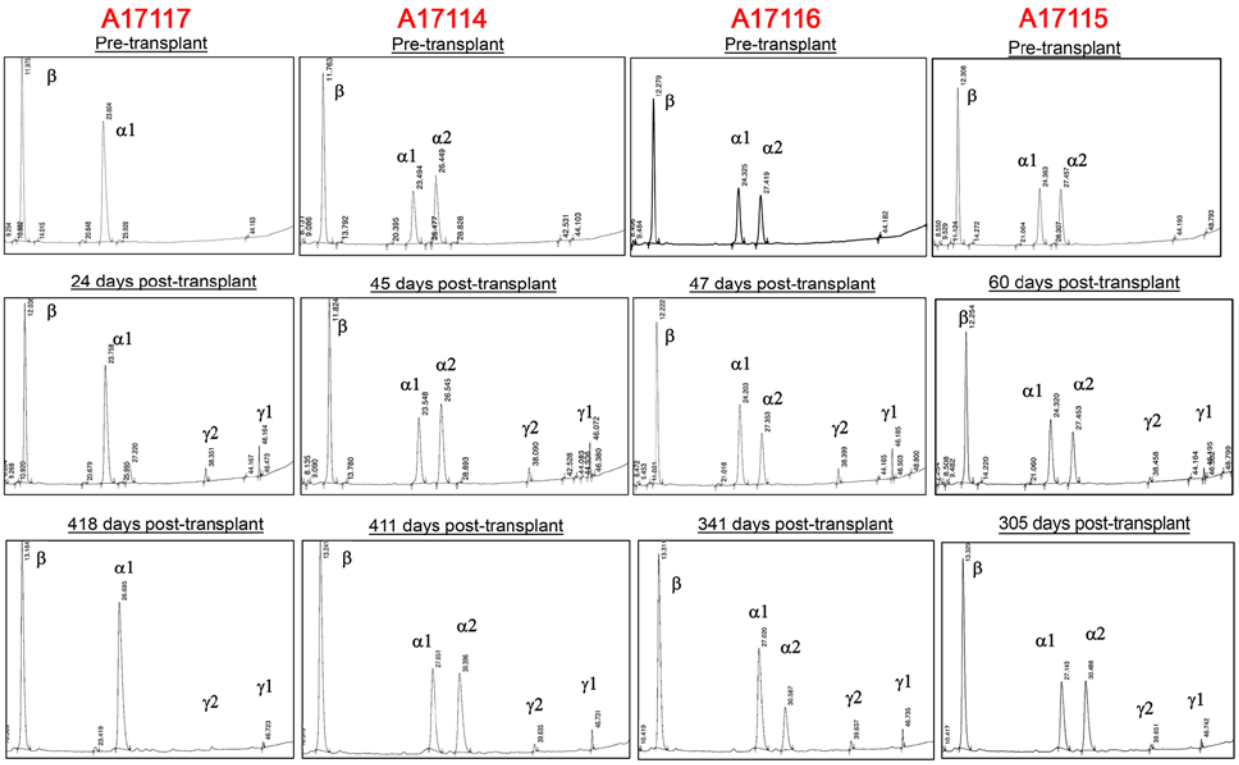


Figure S12. Representative HPLC profiles of PB from transplanted animals. HPLC analysis of PB from transplanted animals pre-treatment (top row), at 1- to 2-months after treatment (middle row) and at approximately 1-year after treatment (bottom row). The identity of the different globin peaks is given.

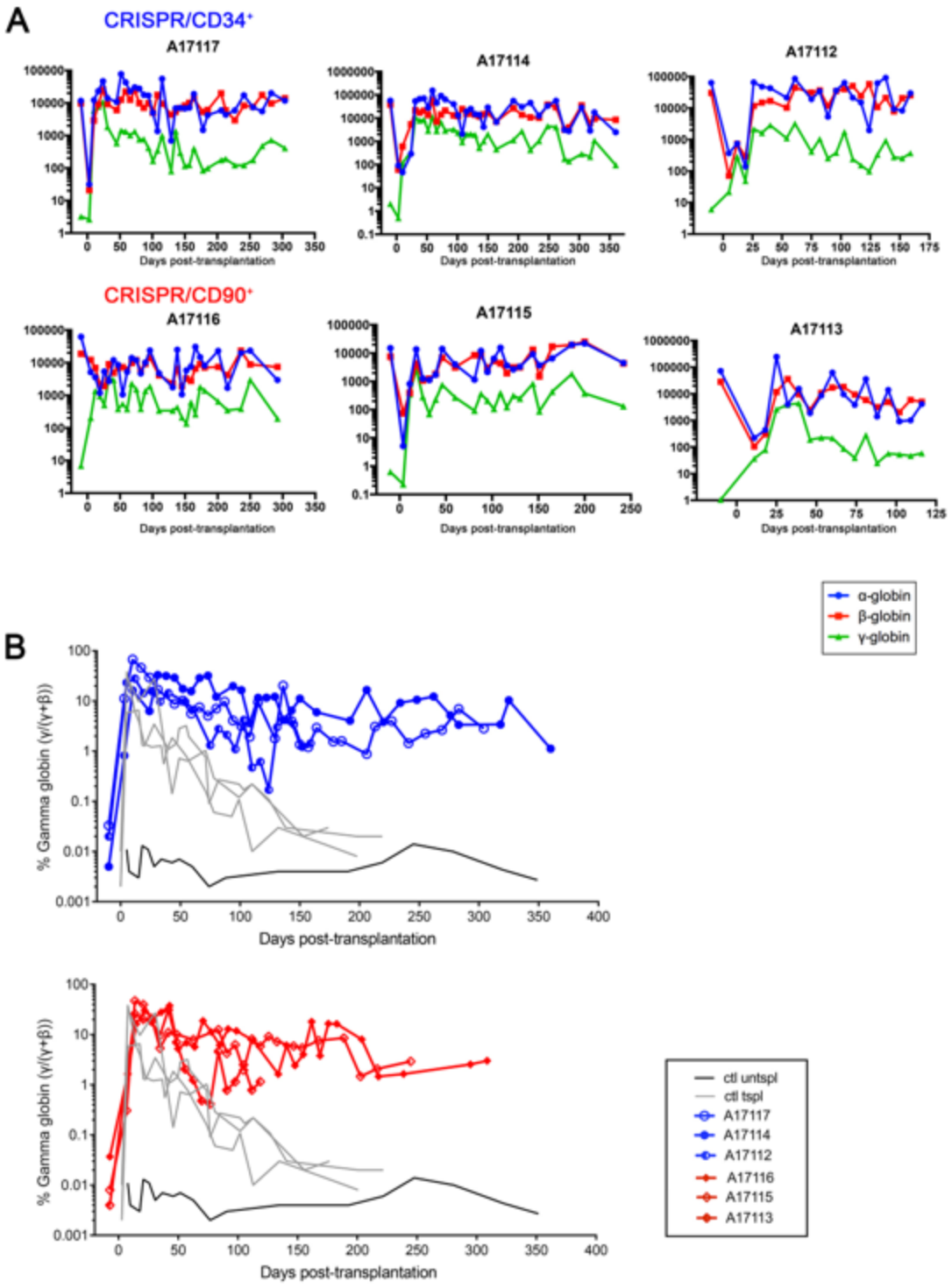


Figure S13. Quantitative PCR measurement of globin transcripts from PB of transplanted animals. (A) Time course measurement of globin transcripts from PB of transplanted animals from the $CD34^+$ (top) or $CD90^+$ (bottom) cohort. **(B)** γ-globin expression in all CRISPR/Cas9 transplanted animals calculated as γ/(γ+β) and compared to historical control transplant animals (gray) and an untransplanted control (black).

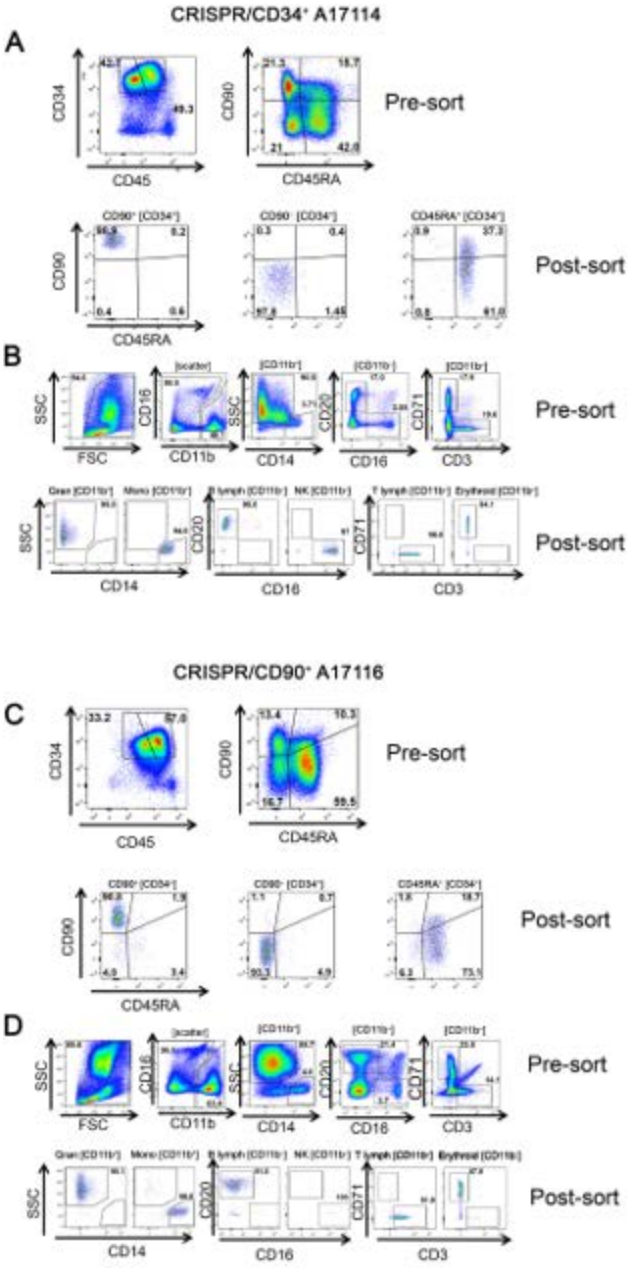


Figure S14. Flow cytometric validation of sorting approach of HPSC subsets and cell lineages from 6-month BM of transplanted animals. Flow cytometric analysis of CD34$^+$ enriched cells before (top) and after (bottom) sorting for HSPCs subsets **(A)** or for cell lineages (B) from BM of animal A17114. Flow cytometric analysis of CD34$^+$ enriched cells before (top) and after (bottom) sorting for HSPCs subsets **(C)** or for cell lineages (D) from BM of animal A17116

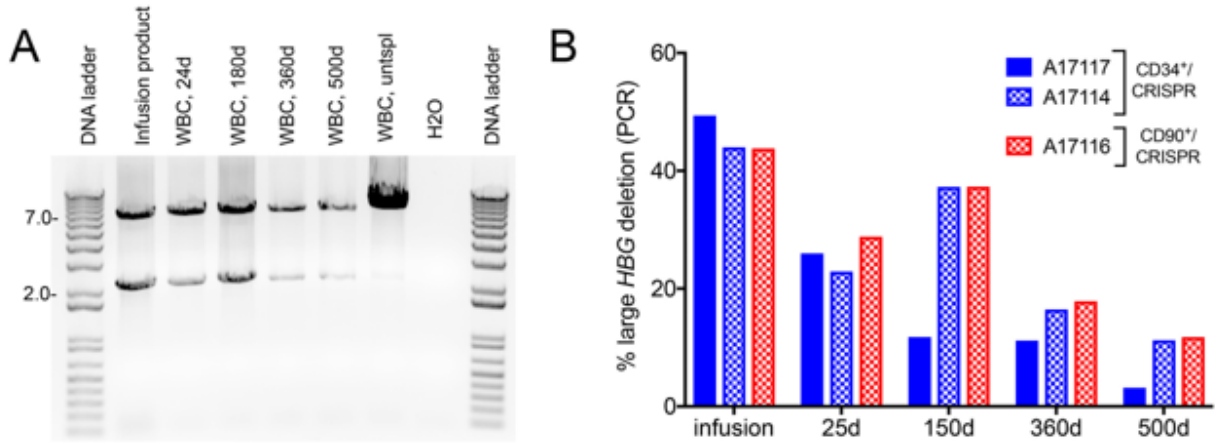


Figure S15. Long-range PCR analysis of large *HBG* deletion. (A) Representative agarose gel electrophoresis for long-range PCR performed on the infusion product and PB white blood cells (WBCs) sampled at different times after transplant from animal A17114. WBCs from an untransplanted (untspl) animal were used as control. Top band is indicative of intact allele and bottom band shows large deletion. **(B)** Semi-quantitative measurement of *HBG* large deletion by densitometry analysis of top and bottom bands in the indicated animals.

Table S1. Quantitative PCR analysis of cytomegalovirus (CMV) DNA in PB of transplanted animals

CD34+ transplant			
Animal ID	**Date**	**Days after transplant**	**CMV copies/ml**
A17117	6/15/2017	*pre	665.5
	7/12/2017	pre	0
	7/18/2017	4	0
	7/25/2017	11	0
	8/2/2017	19	0
	8/7/2017	24	0
	8/8/2017	25	0
	8/15/2017	32	0
A17114	7/18/2017	pre	0
	8/7/2017	3	0
	1/23/2018	172	925
	2/20/2018	200	0
	2/23/2018	203	0
	3/13/2018	228	0
	3/27/2018	242	0
	4/10/2018	256	0
	4/24/2018	270	0
	5/1/2018	277	0
	5/8/2018	284	0
	5/15/2018	291	0
	5/22/2018	298	0
	6/5/2018	304	0
A17112	10/11/2017	pre	0
	10/18/2017	pre	1470.5
	10/31/2017	pre	0
	1/23/2018	pre	0
	2/12/2018	4	0
	2/20/2018	12	0
	2/22/2018	14	0
	3/1/2018	21	0
	3/13/2018	33	0
	3/20/2018	40	0
	3/26/2018	46	0
	4/10/2018	61	0

CD90+ transplant			
Animal ID	**Date**	**Days after transplant**	**CMV copies/ml**
A17116	9/12/2017	pre	0
	11/3/2017	33	792.5
	12/1/2017	64	0
	12/5/2017	68	0
	12/19/2017	82	0
A17115	10/18/2017	pre	2364
	10/31/2017	pre	0
	12/7/2017	20	740.5
	12/11/2017	24	901.5
	12/18/2017	31	1073.5
	12/26/2017	39	990
	1/2/2018	46	0
	1/5/2018	49	0
	1/9/2018	53	0
	1/23/2018	67	0
	1/30/2018	74	0
A17113	3/7/2018	pre	0
	3/26/2018	3	0
	4/2/2018	10	0
	4/9/2018	17	0
	4/16/2018	24	0
	4/23/2018	31	0
	4/30/2018	38	580.5
	5/8/2018	39	0
	5/15/2018	53	0
	5/29/2018	67	0

* pre=prior to transplantation

Table S2. Sequence and chromosomal location of predicted off target (OT) sites for the given *HBG* CRISPR/Cas9.

Site	Sequence	Mismatch	Chr Position	Feature	Closest Gene	Distance
HBG2	CTTGTCAAGGCTATTGGTCAAGG	0	Chr14:68146744-68146766	Intergenic	HBG2	5027
HBG1	CTTGTCAAGGCTATTGGTCAAGG	0	Chr14:68151619-68151641	Intergenic	HBG1	152
HPFH_OT1	ACTGCCAAGGTTATTGGTCAGGG	3	Chr3:119048921-119048943	Intergenic	MIOS	246097
HPFH_OT2	TTTGTCACCTCTATTGGTCAGGG	3	Chr6:74872900-74872922	Intergenic	LHFPL2	129513
HPFH_OT3	CTTGTCAAAGCTCTTGGTCACGG	2	Chr13:135158651-135158673	Intergenic	POLR2D	835527
HPFH_OT4	GTTTTCCAGGCTGTTGGTCAGGG	3	Chr6:37873275-37873297	Intergenic	SLC1A3	1456991
HPFH_OT5	TTTGTCCAGTCCATTGGTCATGG	3	Chr13:128967907-128967929	Intergenic	TSN	964448
HPFH_OT6	CTTGACAAGGAAATTGGTCAGGG	3	Chr9:116331574-116331596	Intergenic	C9H10orf82	39765
HPFH_OT7	TTTGTCAAAGATGTTGGTCAAGG	3	Chr12:54796260-54796282	Intergenic	STAT1	93898
HPFH_OT8	GTGGTCAAGGGTAGTGGTCAAGG	3	Chr9:111226641-111226663	Intergenic	SHOC2	512613
HPFH_OT9	ACTGTCAGGGCTATTAGTCAAGG	3	Chr18:69276951-69276973	Intergenic	CNDP2	1272868
HPFH_OT10	ATTGCAAAGGCTATTTGTCAGGG	3	Chr5:132482924-132482946	Intergenic	MGST2	310884
HPFH_OT11	CTGGTCAAGGCAATTAGTCAAGG	3	Chr1:216816699-216816721	Intergenic	GREM2	277643
HPFH_OT12	ATTGTCATGGCTGTTAGTCATGG	3	Chr4:131521060-131521082	Intron	MOXD1	n.a.
HPFH_OT13	GTTGTCAAGGCCACAGGTCAGGG	3	Chr4:15460184-15460206	Intergenic	MYLIP	643672
HPFH_OT14	GTTGTCAAGGCTGAAGGTCAAGG	3	Chr16:70782012-70782034	Intron	TMEM94	n.a.
HPFH_OT15	GTCATCAAGGCTATTGGCCAAGG	3	Chr6:142055751-142055773	Intergenic	RBM27	600053
HPFH_OT16	GATGTGAAGGCTATTGGACACGG	3	ChrX:145053434-145053456	Intergenic	MIR513b-2	271816
HPFH_OT17	ATTGACCAGGCTATTGGCCAAGG	3	Chr11:38329402-38329424	Intergenic	CNTN1	356141
HPFH_OT18	TTTGCCAAGGCTAATGTTCAGGG	3	Chr11:29411539-29411561	Intergenic	FAR2	192521
HPFH_OT19	CTTCTCATGGCTATTGGTCTTGG	3	Chr8:102881659-102881681	Intergenic	ANKRD46	148033
HPFH_OT20	GTTGTCAAGGATATGGGGCAAGG	3	Chr7:112755032-112755054	Intergenic	RPS29	114615
HPFH_OT21	CTTGTCAGGGCTGTTGGTCGAGG	3	Chr6:133895388-133895410	Intron	SPOCK1	n.a.
HPFH_OT22	CTTGTCAAGGCCATGGGTGATGG	3	Chr5:139683522-139683544	Intron	EDNRA	n.a.
HPFH_OT23	GTTGTCAAGGATATTAGTCTGGG	3	Chr16:24240380-24240402	Intergenic	RPL35A	4122

Table S2. Sequence and chromosomal location of predicted off target (OT) sites for the given *HBG* CRISPR/Cas9.

Site	Sequence	Mismatch	Chr Position	Feature	Closest Gene	Distance
HPFH_OT24	ATTGTCG-GGCTATTGGTCAGGG	1	Chr7:140456710-140456731	Intron	POMT2	n.a.
HPFH_OT25	TTTCTCAAGGC-ATTGGTCAAGG	1	Chr11:14866139-14866160	Intergenic	H2AFJ	273174
HPFH_OT26	TTTCTCAAGGC-ATTGGTCAAGG	1	Chr11:14872421-14872442	Intergenic	H2AFJ	266892
HPFH_OT27	TTTCTCAAGGC-ATTGGTCAAGG	1	Chr16:26114543-26114564	Intergenic	CCL4L1	6785
HPFH_OT28	TTTCTCAAGGC-ATTGGTCAAGG	1	Chr16:26359001-26359022	Intergenic	OMG	109267
HPFH_OT29	TTTCTCAAGGC-ATTGGTCAAGG	1	ChrX:153382980-153383001	Intergenic	CLIC2	94545
HPFH_OT30	TTTCTCAAGGC-ATTGGTCAAGG	1	ChrX:63088144-63088165	Intergenic	ZC4H2	431263
HPFH_OT31	TTTCTCAAGGC-ATTGGTCAAGG	1	ChrX:76890131-76890152	Intergenic	PGK1	51959
HPFH_OT32	TTTGGCAAGGC-ATTGGTCAAGG	1	Chr7:120344414-120344435	Intergenic	AP5M1	41475
HPFH_OT33	TTTCTCAAGGCT-TTGGTCAAGG	1	Chr6:157394190-157394211	Intergenic	GABRB2	293307
HPFH_OT34	TTTGT-AAGGCTATTGTTCATGG	1	Chr6:140863923-140863944	Intron	KCTD16	n.a.
HPFH_OT35	CTTTTCCAGGCTATTGGTCAAAG	2	Chr3:107679707-107679729	Intergenic	FERD3L	676025

Table S3. List of antibodies used for flow cytometric analysis.

Epitope	Clone	Company
CD3	SP34-2	BD Biosciences
CD4	L200	BD Biosciences
CD8a	RPA-T8	BD Biosciences
CD11b	ICRF44	BioLegend
CD14	M5E2	BD Biosciences
CD16	3G8	BD Biosciences
CD20	2H7	BD Biosciences
CD34	563	BD Biosciences
CD45	D058-1283	BD Biosciences
CD45RA	5H9	BD Biosciences
CD90	5E10	BD Biosciences
p53	7F5	Cell Signaling

Table 4 List of primers.

Assay	Name	Sequence (5'-3')
sequencing/TIDE	HbG F4	CTATGCCTAAAACACGTGTCAC
	HbG R1	CTCCCAAGGAAGTCAGCAC
Next generation sequencing (Miseq)	Miseq HbG F1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCCACACTATCTCAATGC
	Miseq HbG R3	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTTCCAGAAGCGAGTGTGTG
ddPCR deletion	HbG-F	GTCTTAGAATAAGATTCCCTGAGA
	HbG-R	TATTGATAATCTCAGCCGTTCC
	HbG1 FAM probe	ACCCTGGTCATCAGCCAGCACA
	HbG2 HEX probe	ACCTGGGTCATCAGCCAGCACA
Large HBG1/HBG2 deletion	HbG F4	CTATGCCTAAAACACATTTCA
	HbG R1	CTCCCAAGGAAGTCAGCAC