Supplemental Materials
Topfer et al, Disrupting the adult-globin promoter alleviates promoter competition and reactivates foetal-globin gene expression.

Supplementary Table 1: sgRNA nucleotide sequence information

Guide name	Oligo Number	Fwd/Rev	Nucleotide sequence
-98 guide	A9675	Fwd	CACCGATCACTTAGACCTCACCCTG
	A9676	Rev	AAACCAGGGTGAGGTCTAAGTGATC
-90 guide	A97	Fwd	CACCGATTGGCCAACCCTAGGGTG
	A98	Rev	AAACCACCCTAGGGTTGGCCAATC
-84 guide	B99	Fwd	CACCGGAGTAGATTGGCCAACCCT
	B100	Rev	AAACAGGGTTGGCCAATCTACTCC
-21 guide	A9470	Fwd	CACCGCAGGGCTGGGCATAAAAGTC
	A9471	Rev	AAACGACTTTTATGCCCAGCCCTGC
+10 guide	A9425	Fwd	CACCGAAGCAAATGTAAGCAATAGA
	A9426	Rev	AAACTCTATTGCTTACATTTGCTTC
+68 guide	A9677	Fwd	CACCGGTAACGGCAGACTTCTCCTC
	A9678	Rev	AAACGAGGAGAAGTCTGCCGTTAC
+1561 guide	A9427	Fwd	CACCGAGGCAGAATCCAGATGCTCA
	A9428	Rev	AAACTGAGCATCTGGATTCTGCCTC

Supplementary Table 2: Single stranded oligo nucleotide donor sequences

Deletion	ssOND sequence
HBB promoter deletion	GGCTGAGGGTTTGAAGTCCAACTCCTAAGCCAGTGCCAGAAGAGCC AAGGACAGGTACGGCTGTCATCACTTAGATCAAACAGACACCATGG TGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGC AAGGTGAACGT
HBB gene deletion	CAATCTACTCCCAGGAGCAGGGAGGGCAGGAGCCAGGGCTGGGCAT AAAAGTCAGGGCGAGCCATCTATTGCTTTGATGTATTTAAATTATTT CTGAATATTTTACTAAAAAGGGAATGTGGGAGGTCAGTGCATTTAAA ACATAAAGA

Supplementary Table 3: qPCR primer sequences used for determining mRNA expression of the β-like globin genes

Primer name	Primer number	Primer sequence
*18*s qPCR forward	A1560	CACGGCCGGTACAGTGAAAC
*18*s qPCR reverse	A1561	AGAGGAGCGAGCGACCAA
HBG qPCR forward	A2524	CCTGTCCTCTGCCTCTGCC
HBG qPCR forward	A2525	GGATTGCCAAAACGGTCAC
HBB qPCR forward	A4069	TGTCCACTCCTGATGCTGTTATG
HBB qPCR reverse	A4070	GGCACCGAGCACTTTCTTG

Supplementary Table 4: Primer sequences used for PCR screening and Sanger sequencing

Primer number	Primer sequence	Screening purpose
A9595	GAGGGTTTGAAGTCCAACTCCTA	Forward screening primer *HBB* promoter deletion and mutations; Forward primer *HBB* gene deletion
A9617	TCCACATGCCCAGTTTCTATTG	Reverse screening primer *HBB* promoter deletion and mutations
A9578	AGGGATGAATAAGGCATATGCAT	Reverse screening primer *HBB* gene deletion

Supplementary Table 5: Copy number qPCR primers used to determine number of copies of sections of chromosomal DNA.

Name	**Primer number**	**Fwd/ Rev**	**Sequence**
HS2 copy number primer	A5581	Fwd	CATCACTCTAGGCTGAGAACATCTG
	A5582	Rev	GGCTCAAGCACAGCAATGC
HBB promoter copy number primer	B44	Fwd	CTAGGGTTGGCCAATCTACTCC
	B45	Rev	CTCTGCCCTGACTTTTATGCC
HBB gene copy number primer	A9681	Fwd	CAGAGGTTCTTTGAGTCCTTTGG
	A9684	Rev	GAAGGGGAAAGAAAACATCAAGC

Supplementary Table 6: Western blot antibodies

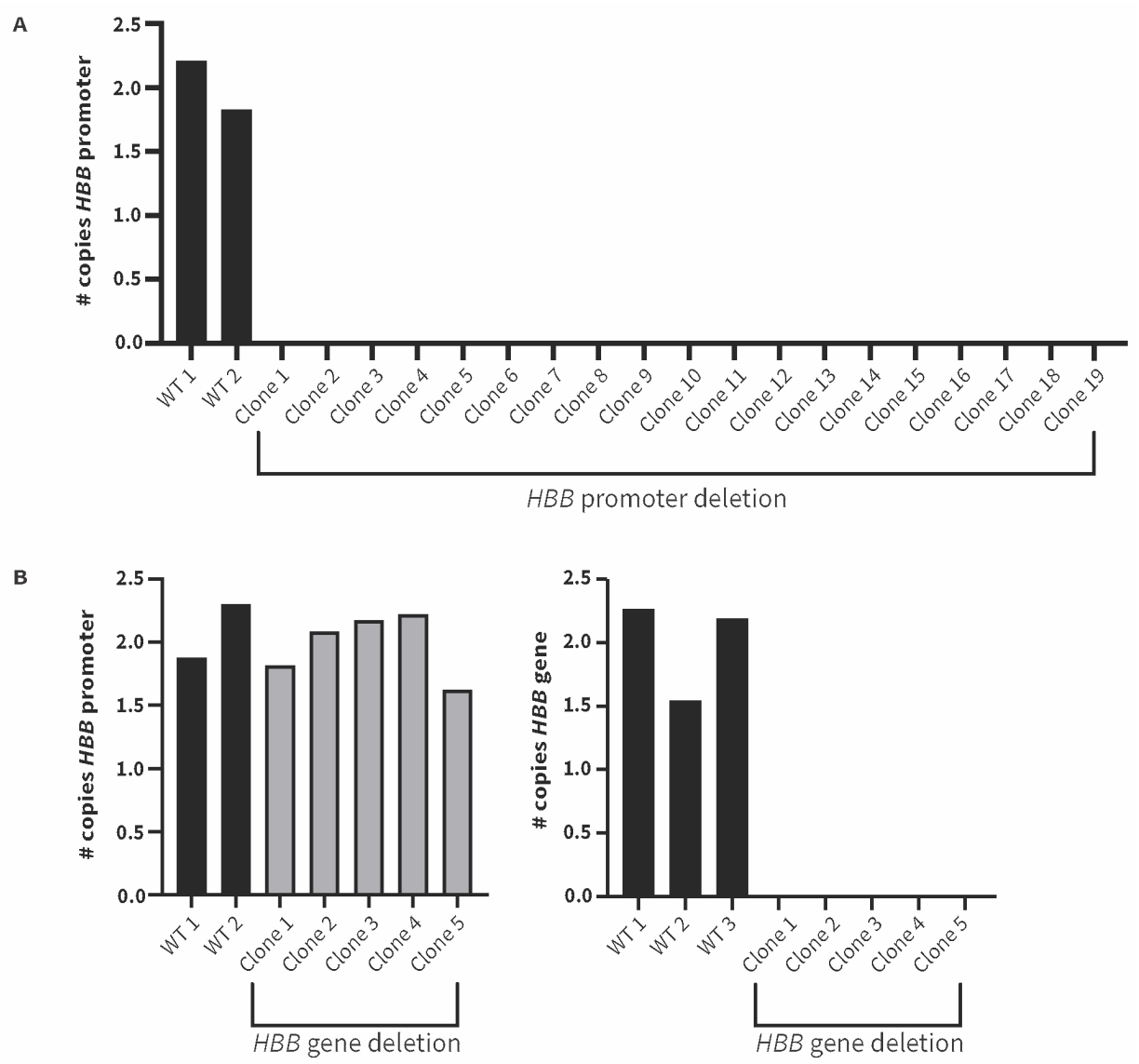
Antibody	Clone	Manufacturer	Catalog #
Haemoglobin γ antibody	51-7	*Santa Cruz*	sc-21756
Haemoglobin β antibody	37-8	*Santa Cruz*	sc-21757
HBA antibody: Rabbit Anti-Human		*Home Made*	
Monoclonal Anti-β-Actin antibody produced in mouse		*Sigma*	A2228

Supplementary Table 7: Flow cytometry antibodies

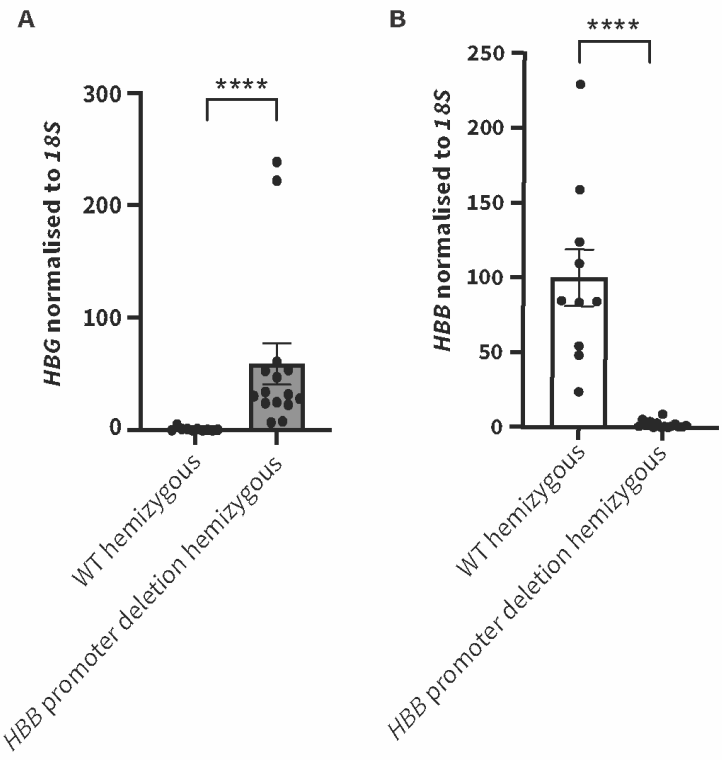
Antibody	Clone	Manufacturer	Catalog #
FITC Mouse Anti-Human CD235a	GA-R2 (HIR2) (RUO)	*BD Pharmingen™*	559943
PE Anti-Human CD49d	9F10	*BioLegend*	304304
APC Anti-Human Band3		*New York Blood*	Gift from X. An

Supplementary Table 8: Probes for Capture-C

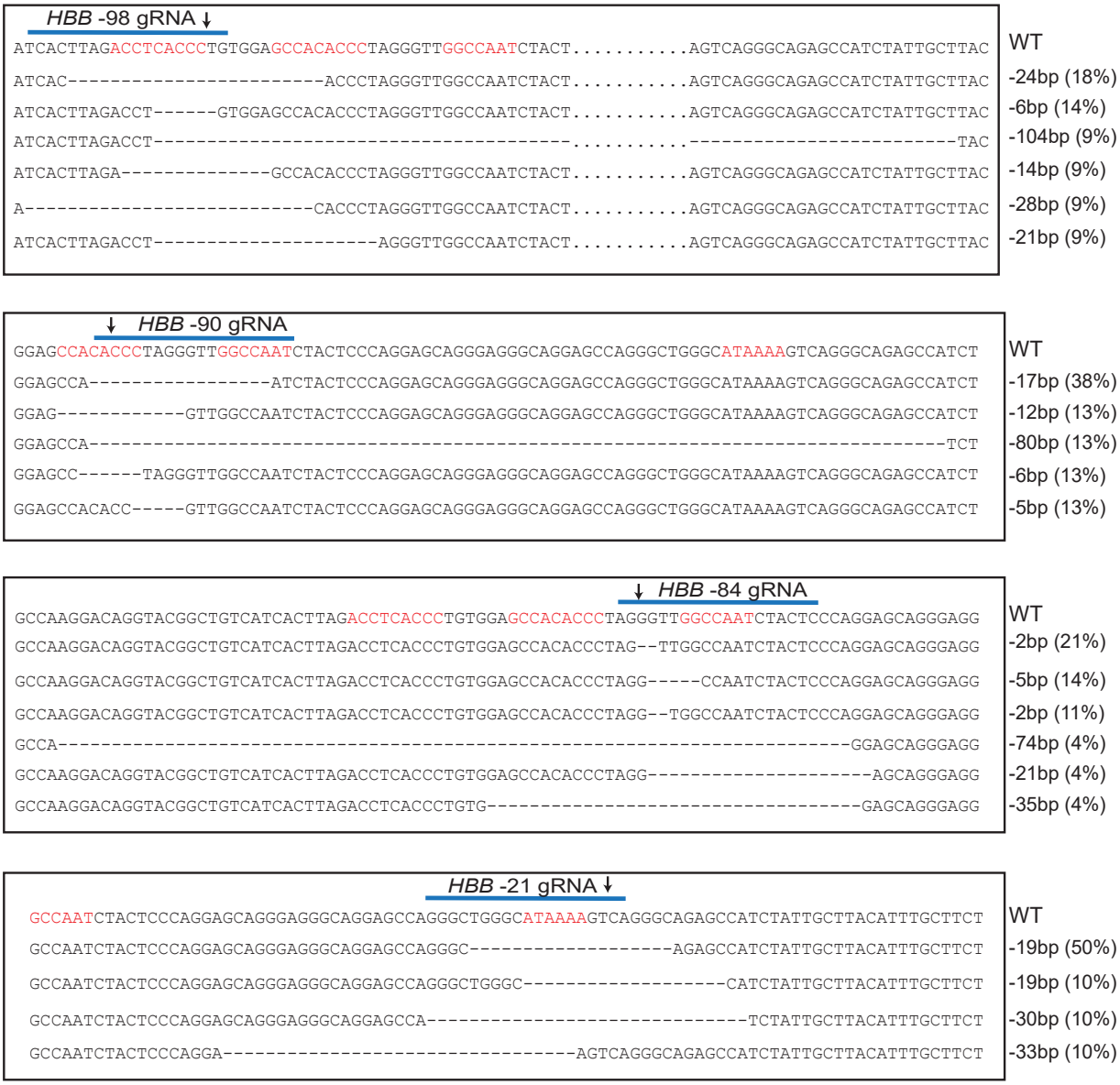
Target	Sequence
HS3 enhancer	CTGAAAACATAGGAGTCAAGGCACTTGCCCCTAGCTGGGGGTATAGGGGAGCAGTCCCATGTAGTAGTAGAATGA
	AGGAGTCAAGGCACTTGCCCCTAGCTGGGGGTATAGGGGAGCAGTCCCATGTAGTAGTAGAATGAAAAATGCTGC
	GCACTTGCCCCTAGCTGGGGGTATAGGGGAGCAGTCCCATGTAGTAGTAGAATGAAAAATGCTGCTATGCTGTGC
	CTAGCTGGGGGTATAGGGGAGCAGTCCCATGTAGTAGTAGAATGAAAAATGCTGCTATGCTGTGCCTCCCCCACC
	GTATAGGGGAGCAGTCCCATGTAGTAGTAGAATGAAAAATGCTGCTATGCTGTGCCTCCCCCACCTTTCCCATGT
	TACAGTCATAGACTTCTTCATGGCTGTCTCCTTTATCCACAGAATGATTCCTTTGCTTCATTGCCCCATCCATCT
	TCCTGCTCCCAAATTTACAGTCATAGACTTCTTCATGGCTGTCTCCTTTATCCACAGAATGATTCCTTTGCTTCATTGCC
	CAAGTCCTTAGAGACTCCTGCTCCCAAATTTACAGTCATAGACTTCTTCATGGCTGTCTCCTTTATCCACAGAATGATTCCTTTG
	CCTTGAAATCCAAGTCCTTAGAGACTCCTGCTCCCAAATTTACAGTCATAGACTTCTTCATGGCTGTCTCCTTTATCCAC
	AGTCAAAATTCCTTGAAATCCAAGTCCTTAGAGACTCCTGCTCCCAAATTTACAGTCATAGACTTCTTCATGGCTGTCTC



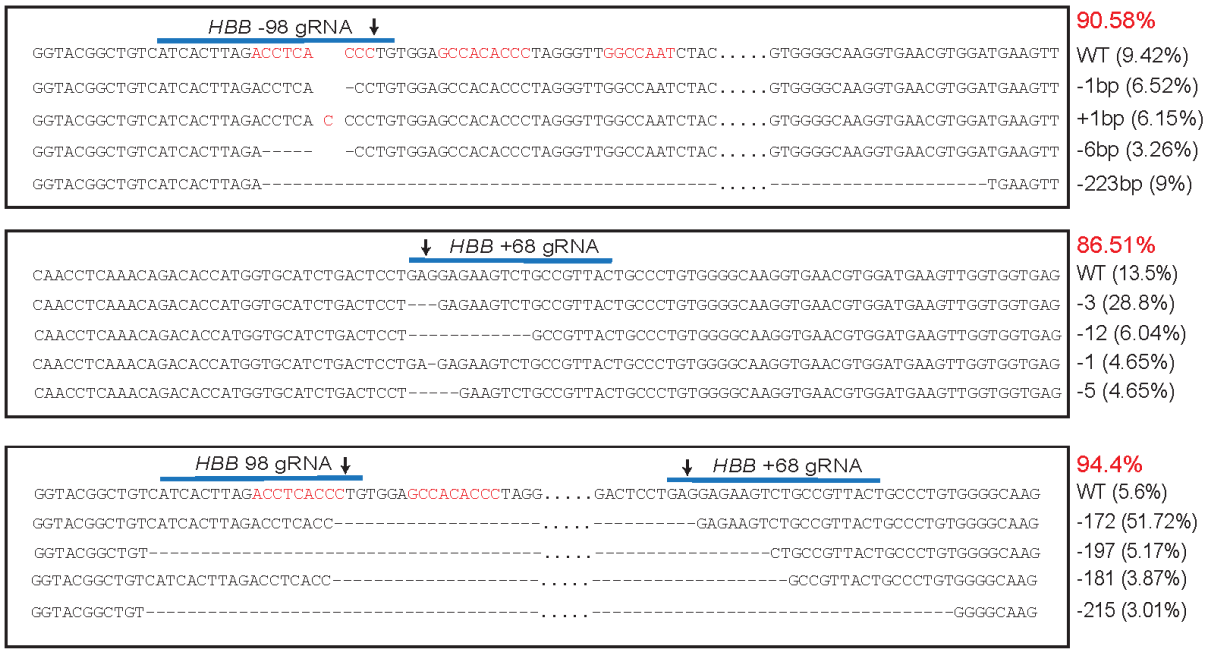
Supplementary Figure 1: Analysis of the number of copies of the *HBB* region in *HBB* promoter deletion and *HBB* gene deletion clones. The number of copies of the *HBB* promoter region or *HBB* gene region, relative to the number of copies of the hypersensitive site 2 (*HS-2*) locus control region (an upstream region on the same chromosome) was determined by qPCR from gDNA using primers described in Table S5. All data was normalised to the average number of copies of the *HBB* promoter region or *HBB* gene region relative to *HS-2* in two populations of WT clonal HUDEP-2 cells, which was set to a value of 2. (A) 19 *HBB* promoter deletion clones show no remaining copies of the *HBB* promoter region, suggesting that this region has been homozygously deleted in these clonal populations. (B) 5 *HBB* gene deletion clones show 2 copies of the *HBB* promoter region (left) and no remaining copies of the HBB gene region (right), suggesting that the *HBB* gene region has been homozygously deleted in these clonal populations but that the neighbouring *HBB* gene deletion has the expected 2 copies in each clone. This supports the hypothesis that these *HBB* gene deletion clones have had the *HBB* gene region deleted but do not contain larger deletions in either allele.



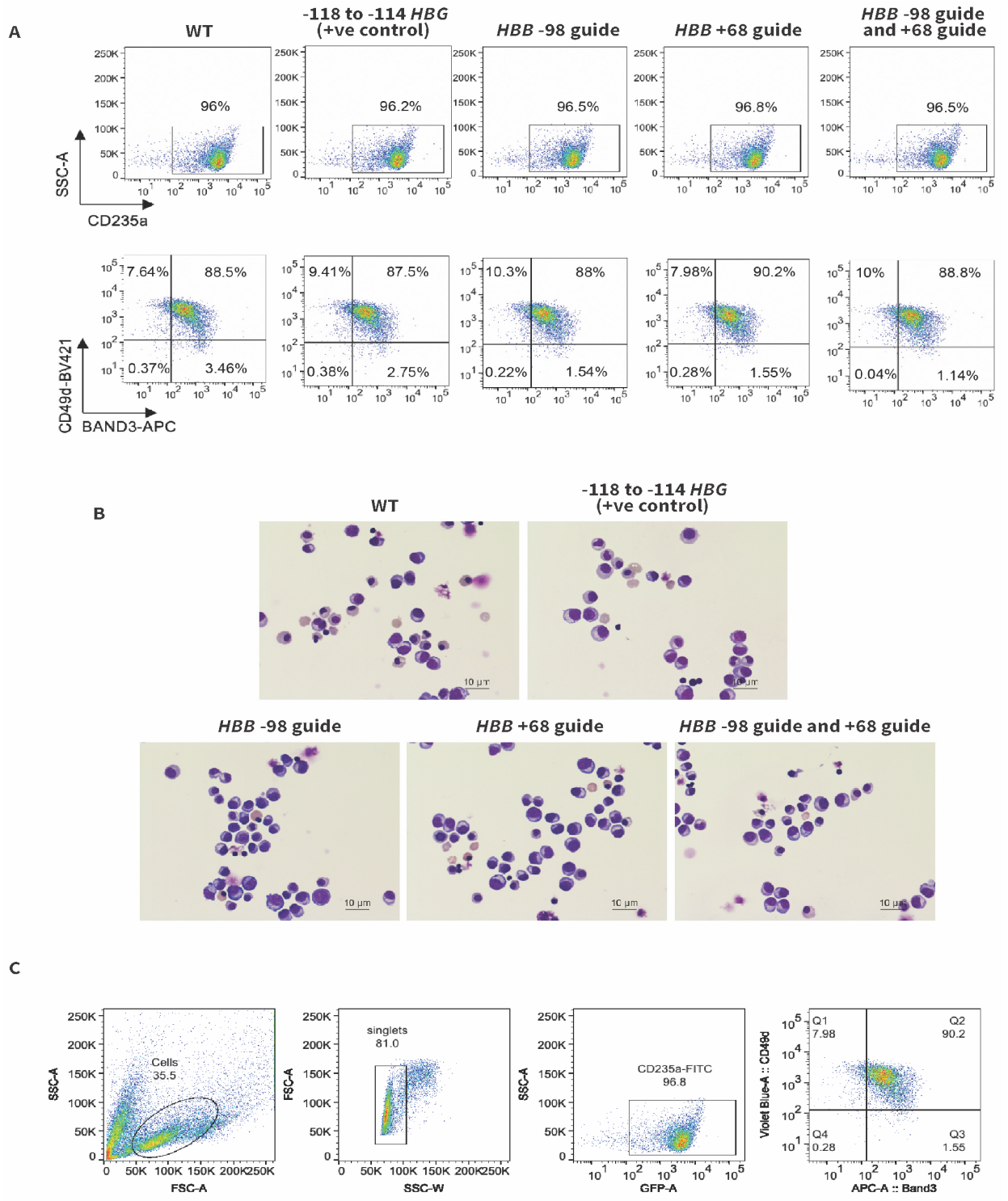
Supplementary Figure 2: Deletion of the *HBB* promoter leads to increased expression of *HBG* and decreased expression of *HBB* in 2 which lack the entire *β-like globin* gene cluster on one chromosome (Δεγδβ) and thus are hemizygous for the *β-like globin* gene cluster. Using the same strategy as that outlined in Figure 2A, the *HBB* promoter was deleted in HUDEP-2 cells which are hemizygous for the *β-like globin* gene cluster. *HBG* (A) and *HBB* (B) mRNA levels in WT hemizygous HUDEP-2 clonally sorted (n=10) and hemizygous HUDEP-2 cells with the *HBB* promoter deletion (n=15) (mean ± standard error of the mean (SEM)). Significance was determined by a Mann-Whitney U test (****P<0.0001).



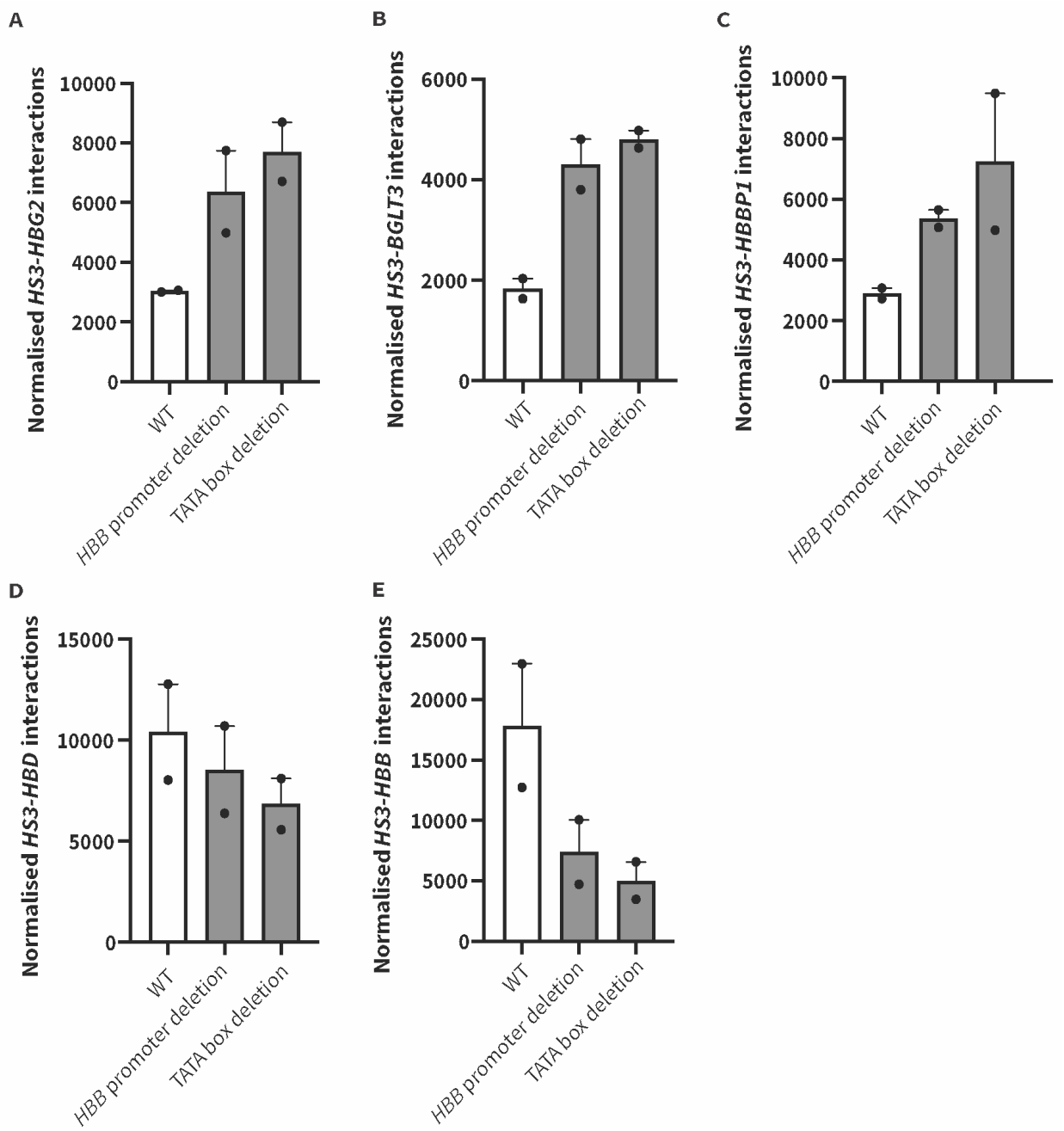
Supplementary Figure 3: Top indels made following the use of guides -98, -90, -84 and -21 for CRISPR-Cas9 genome editing in HUDEP-2 cells. The top sequence in each box represents the WT sequence prior to CRIPSR-Cas9 genome editing. The location of the sgRNA has been placed above the WT sequence with the cut site indicated by an arrow. Known transcription factors have been indicated in the WT sequence in red. The sequences below the WT sequence represent the sequence following CRIPSR-Cas9 genome editing. The number of base pairs that has been removed has been indicated to the right of each sequence followed by the percentage of cells analysed that contained this sequence.



Supplementary Figure 4: Summary of the most frequent indels by deep sequencing following Cas9 RNP *HBB* promoter editing of CD34+ HSPCs as differentiated for 9 days. The top sequence in each box represents the WT sequence. The location of the sgRNA marked with blue line above the WT sequence with the cut site indicated by an arrow. Known transcription factors have been indicated in the WT sequence in red. The types of indels and percentage have been indicated to the right of each sequence.



Supplementary Figure 5: Flow cytometry and cell morphology of CD34+ cells after differentiation. (A) Erythroid maturation kinetics at CD34+ erythroid culture 15 days after each type of editing as indicated on top, as determined by immune-flow cytometry measurement of CD235a positive ratio and CD49d and Band3 expression on erythroid cells. (B) Morphology of day 15 CD34+ erythroid culture with RNP-edited groups indicated on top of each image. The images were acquired with a Nikon Eclipse Ni microscope with a 603 objective and Nikon NIS-Elements software (scale bars, 10 mm). **(**C) Gating strategy used for flow cytometry analysis.



Supplementary Figure 6: Deletion of the *HBB* promoter or disruption of the TATA box increases association of the Locus Control Region with *HBG2*, *BGLT3* and *HBBP1* and decreases association of the Locus Control Region with *HBB*. Capture-C interactions of HS3 with (A) *HBG2* (chr11:5254441-5254919, hg38), (B) *BGLT3* (chr11:5244554-5245546, hg38), (C) *HBBP1* (chr11:5241954-5243592, hg38), (D) *HBD* (chr11:5233668-5235096, hg38) and (E) *HBB* (chr11:5226520-5228365, hg38), in clonal populations of WT HUDEP-2 cells, *HBB* promoter deletion cells and TATA box mutation cells (n=2). Interaction values have been normalised relative to total counts. Histograms represent mean data (+ range of values as the error bar) with individual datapoints shown as filled circles.