

Additional file 2

Data S1 The complete amino acid sequences of the *HBB* targeting ZFNs.

>HBB_ZFN Left Arm

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAAMAERPFQCRICMRNFSRSDN
 LSAHIRTHTGEKPFACDICGRKFARSDNRTTHTKIHTGSQKPFQCRICMRNFSQSSDLR
 HIRTHTGEKPFACDICGRKFARRDALLMHTKIHTGSQKPFQCRICMRNFSQSGDLTRHIR
 THTGEKPFACDICGRKFARRDQLPQHTKIHLRGIPAGEERAGGEEVRAAAQAEVRAPRVH
 RADRDREQHPGPHPGDEGDGVLHEGVRLQKAPGRKQKAMKVMEFFMKVYGYRGKHLGG
 SRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTRDKHLNPNNEW
 KVPSSVTEFKFLFVSGHFKGNYKAQLTRLNHNITCNGAVLSVEELLIGGEMIKAGTLTL
 EEVRRKFNNGEINFRS*

>HBB_ZFN Right Arm

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAAMAERPFQCRICMRNFSQSSD
 LSRHIRTHTGEKPFACDICGRKFARATSCTSIPRYTRAAKSPSSVESACVTSVCATTMRN
 FSLRHHLTRHIRTHTGEKPFACDICGRKFAQSGTRKTHTKIHTGSQKPFQCRICMRNFSR
 SDHLSQHIRTHTGEKPFACDICGRKFAQKATRITHTKIHLRGSQLVKSELEEKSELRHK
 LKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPID
 YGVIVDTKAYSGGYNLPIGQADEMORYVKENQTRNKHINPNNEWKVPSSVTEFKFLFV
 SGHFKGNYKAQLTRLNRKTNCNGAVLSVEELLIGGEMIKAGTLTLLEEVRRKFNNGEINF*

Table S1: TALE FLASH IDs and RVDs targeting the promoter of *HBB* gene

Plasmids Left Arm	374	53	125	134	325	Backbone
RVDs	HD	NI NG HD NI	HD NG NG NI	NN NI HD HD	NG HD NI	HD
Plasmids Right Arm	373	144	166	6	301	Backbone
RVDs	NI	NN NI NG NG	NN NN HD HD	NI NI HD HD	HD NG NI	NN

Table S2: Details of ssODNs (sequence, symmetry and length of homology arms)

Name	Sequence (5' <i>GCTAGC</i> (<i>NheI</i> tag)..... 3')	Symmetry	Homology
1 TS	AGACTCTGGGTTTCTGATAGGCACTGACTCTCTGCCTATT GCTAGC GGTCTATTTTCCCACCCTTAGGC TGCTGGTGGTCTACCCTTGG	symmetric	43 bp + 43 bp
2 TS	GAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTGCCTATT GCTAGC GGTCTATTTTCCC ACCCTTAGGCTGCTGGTGGTCTACCCTTGGACCCAGAGGT	symmetric	53 bp + 53 bp
3 TS	TGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTGCCTATT GCTAGC GGTCT ATTTTCCCACCCTTAGGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTCTTTGAGTC	symmetric	63 bp + 63 bp
4 TS	CAATAGAAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTGCCTATT GCTAGC GGTCTATTTTCCCACCCTTAGGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTCTTTGAGTC CTTTGGGGAT	symmetric	73 bp + 73 bp
5 TS	AGACAGGTTTAAGGAGACCAATAGAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACT GACTCTCTGCCTATT GCTAGC GGTCTATTTTCCCACCCTTAGGCTGCTGGTGGTCTA	symmetric	91 bp + 36 bp
6 TS	ATAGAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTGCCTATT GCTAGC GGTCTATTTTCCCACCCTTAGGCTGCTGGTGGTCTA	asymmetric	71 bp + 36 bp
7 TS	GGTGAGGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCATGTGG AGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTGCCTATT GCTAGC GGTCTATTTTCCCAC CCTTAGGCTGCTGGTGGTCTA	asymmetric	126 bp + 36 bp
8 TS	CAGGTTGGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCATGTGGAGACAGAGAAGACT CTTGGGTTTCTGATAGGCACTGACTCTCTGCCTATT GCTAGC GGTCTATTTTCCCACCCTTAGGCTGCTG GTGGTCTACCCTTGGAC	asymmetric	112 bp + 45 bp
9 TS	AGACAGGTTTAAGGAGACCAATAGAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACT GACTCTCTGCCTATT GCCTAGCTTTGGCCGTAGG TCTATTTTCCCACCCTTAGGCTGCTGGTGGTCTA	asymmetric	93 bp + 46 bp
1 NTS	AGACTCTGGGTTTCTGATAGGCACTGACTCTCTGCCTATT GCTAGC GGTCTATTTTCCCACCCTTAGGCTGCTGGTG GTCTACCCTTGG	symmetric	43 bp + 43 bp
2 NTS	GAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTGCCTATT GCTAGC GGTCTATTTTCCCACCCTTAG GCTGCTGGTGGTCTACCCTTGGACCCAGAGGT	symmetric	53 bp + 53 bp
3 NTS	TGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTGCCTATT GCTAGC GGTCTATTTT CCACCCTTAGGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTCTTTGAGTC	symmetric	63 bp + 63 bp
4 NTS	CAATAGAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTGCCTATT GCTAGCG GTCTATTTTCCCACCCTTAGGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTCTTTGAGTCCTTTGGGGAT	symmetric	73 bp + 73 bp
5 NTS	AGACAGGTTTAAGGAGACCAATAGAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCT CTCTGCCTATT GCTAGC GGTCTATTTTCCCACCCTTAGGCTGCTGGTGGTCTA	symmetric	91 bp + 36 bp
6 NTS	ATAGAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTGCCTATT GCTAGCGGT CTATTTTCCCACCCTTAGGCTGCTGGTGGTCTA	asymmetric	71 bp + 36 bp

7 NTS	GGTGAGGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCATGTGGAGACAG AGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTCTGCCTATT GCTAGCGG TCTATTTTCCCACCCTTAGGCTGCTG GTGGTCTA	asymmetric	126 bp + 36 bp
8 NTS	CAGGTTGGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCATGTGGAGACAGAGAAGACTCTTGGG TTTCTGATAGGCACTGACTCTCTCTGCCTATT GCTAGCGG TCTATTTTCCCACCCTTAGGCTGCTGGTGGTCTACCCTTG GAC	asymmetric	112 bp + 45 bp
9 NTS	AGACAGGTTTAAGGAGACCAATAGAAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCT CTCTGCCTATTGC GCTAGC TTTGGCCGTAGGCTCTATTTTCCCACCCTTAGGCTGCTGGTGGTCTA	asymmetric	93 bp + 46 bp
10 NTS	AGACAGGTTTAAGGAGACCAATAGAAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCT CTCTG AGA ATT GCTAGC GGTCTATTTTCCCACCCTTAGGCTGCTGGTGGTCTA	asymmetric	91 bp + 36 bp PAM-depleted
11 NTS	AGACTCTTGGGTTTCTGATAGGCACTGACTCTCTCT AGA AATT GCTAGC GGTCTATTTTCCCACCCTTAGGCTGCTGGTG GTCTACCCTTGG	symmetric	43 bp + 43 bp PAM-depleted
mod. ssODN	(*T) (*G) (*G)GCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTCTGCCTATT GCTAGCGG TCTATTTTCCCACCCTTAGGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGA (*G) (*T) (*C)	symmetric	63 bp + 63 bp chemically modified.

Table S3 Barcode and sample details of next-generation sequencing

S. No.	Name	<i>NheI</i>	Barcode
1.	Control	No	Not present
2	5 TS	Yes	ATCACG/ CGTGAT
3	10 NTS	Yes	CTTGTA/ TACAAG
4	11 NTS	Yes	TGACCA/ TGGTCA
5	mod. ssODN	Yes	ACATGT/ ACATGT

>Reference sequence of NGS (167 bp)

AGAAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTCTGCCTATT**|**GGTCTATTTT
CCCACCCTTAGGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGC
TGTTATGG

| Cut site

Table S4: Details of off-target position, primer details and Indels frequency.

Sites	Sequence	Primers (5'...3')	Intron/Exon (gene)	Locus	Indel Frequency (T7E1 Assay)
HBB IVS-I 110	GGGTGGGAAAATAGACCAATAGG	For: GGGTTTGAAGTCCAACCTCCTAA Rev: AGAAAACATCAAGCGTCCCATA	Intron	chr11:+5226803	44±18%
OT-1	TAGTGGAAAAATAGACCAATGGG	For: CTACGTCTGCTGAAACCACA Rev: AATTCAGGGCATCAGGACA	Intron	chr1:+81738787	0%
OT-2	ATGTGGGAAAATCGACCAATCAG	For: AAGCCAAGCAGAAAACCACAG Rev: CTGGGCTAATGGGAAGGGAT	Intron	chr3:-6513388	0%
OT-3	AGATGGGAAAATAGACTAATGGG	For: CTCTCACCTGAACCACACT Rev: AGCAAAGATGAGCCATGCAG	Intron	chr13:-78904261	0%
OT-4	TGGAAGGAAAACAGACCAAGAAG	For: GAGGTCCTTGGAGATTGGCT Rev: AGCCCCTGTCTAATGGTCAC	Exon (NM_024776)	chr15:-77425989	0%
OT-5	GGGAGGGATCAAGACCAATAAG	For: GGTGGCAAAATGGAGCTGAT Rev: GCCATGCTCAACTACTGCTC	Exon (NM_030581)	chr16:+74976589	0%
OT-6	GAGTGGGAAAACAGACCAGTAGG	For: TTCCTTCTGTGGGGTCTTC Rev: TCAGAACTCAGGGTGCAGAG	Exon (NM_032878)	chr19:+36503885	0%