Supplementary information

Search-and-replace genome editing without double-strand breaks or donor DNA

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Supplementary Discussion

Nuclease-based methods for genome editing

By generating double-stranded DNA breaks (DSBs), nucleases can efficiently introduce mixtures of insertions and deletions (indels) at target DNA sites, and thus are ideally suited for gene or regulatory sequence disruption when mixtures of products are acceptable. In some cases, the resulting DSBs can predictably yield precise end-joining products with high efficiency[15]. Nucleases have also been used to delete target DNA regions by generating two DSBs flanking the sequence to be deleted[47,48]. In addition, DNA segments can be integrated into target sites without relying on homology-directed repair processes by making DSBs at the target site and at the ends of the DNA segments to be inserted[49,50]. These strategies are difficult to implement for achieving precise small insertions or small deletions, since it is difficult to generate closely spaced DSBs simultaneously, and small DNA inserts do not form stable double-stranded duplexes that are required for end-joining under physiological conditions.

Using nucleases to replace larger genomic DNA segments with segments containing precise edits is possible in theory by cutting 5' and 3' of the target region to excise the original DNA segment, cutting 5' and 3' of the edited segment to extract it from a larger DNA sequence such as a vector (or introducing a double-stranded edited DNA segment of precise length), and relying on cellular end-joining to incorporate the edited DNA segment at the target locus. Many byproducts such as incorporation of an inverted original DNA segment, incorporation of an inverted edited DNA segment, insertions of DNA flanking the edited DNA segment (if present), insertions of multiple edited DNA fragments, and indels at all of the above DSBs, however, can result from such an approach, however, and thus editing efficiencies and product purities are expected to be modest[51,52].

Rationale for Cas9-nicked target DNA primer extension

Upon binding to double-stranded DNA, Cas9:sgRNA complexes[53,54] displace the PAM-containing DNA strand to form a flexible single-stranded DNA R-loop[55,56]. It was previously shown *in vitro* that after cleavage of the PAM-containing strand by Cas9's RuvC nuclease domain, the PAM-distal fragment of the R-loop can be released from otherwise stable Cas9:sgRNA:DNA complexes[57]. We hypothesized that the 3' end of a nicked DNA R-loop could be sufficiently accessible to prime DNA polymerization. Moreover, guide RNA engineering efforts[58–60] and structural studies of Cas9:sgRNA:DNA complexes[27,55,61] suggest that the 5' and 3' termini of the sgRNA may be amenable to extension without disrupting Cas9:sgRNA activity. Thus, we imagined that pegRNAs could be engineered by extending sgRNAs to include two critical components: (1) a primer binding site (PBS) sequence that allows the 3' end of the nicked DNA strand to hybridize to the pegRNA and

act as a primer, and (2) a DNA synthesis template containing the desired edit that would be directly copied into the genomic DNA. Some mobile genetic elements use a related mechanism of target-primed reverse transcription during integration[62].

Complementary strand nicking in PE3

The PE3 system exploits preferential repair of nicked DNA strands to enhance prime editing efficiencies. We tested nicking the non-edited strand using the Cas9 H840A nickase already present in PE2 and a simple sgRNA to induce the preferential replacement of the non-edited strand by the cell (**Fig. 3a**). At five genomic sites in HEK293T cells, we screened sgRNAs that place a complementary strand nick 14 to 116 bases from the site of the pegRNA-induced nick, either 5' or 3' of the PAM. Nicking the non-edited strand increased the amount of prime editing products in four out of five sites tested (**Fig. 3b**). As expected, at some sites, placement of the non-edited strand nick within 40 bp of the pegRNA-induced nick led to large increases in indel formation up to 22% (**Fig. 3b**), presumably due to the formation of a double-strand break from nicking both strands close together. At other sites, however, nicking as close as 14 bp away from the pegRNA-induced nick produced only 5% indels (**Fig. 3b**), suggesting that locus-dependent factors control conversion of proximal dual nicks into double-strand DNA breaks. At one tested site (*HEK4*), complementary strand nicks either provided no benefit or led to indel levels that surpassed editing efficiency (up to 26%), even when placed at distances >70 bp from the pegRNA-induced nick (**Fig. 3b**), consistent with an unusual propensity of the edited strand at that site to be nicked by the cell, or to be ligated inefficiently.

Prime editing compared with adenine base editing

In addition to comparing prime editing with cytosine base editing (**Extended Data Fig. 6a-c**), we also compared prime editing with adenine base editing at two genomic loci using a current non-nicking adenine base editor (ABEmax[30] with dCas9 instead of a Cas9 nickase, hereafter referred to as ABEdmax) versus PE2, and by the current nicking ABEmax versus PE3. At a site with two target adenines in the base editing window (*HEK3*), ABEs were more efficient than PE2 or PE3 for conversion of A5, but PE3 was more efficient for conversion of A8, which lies at the edge of the ABEmax editing window (**Extended Data Fig. 6d**). When comparing the efficiency of precision edits with only a single converted adenine, PE3 outperformed ABEmax at both A5 and A8 (**Extended Data Fig. 6e**). At *FANCF*, in which only a single A is present within the base editing window, ABEs outperformed their prime editing counterparts in editing efficiency by 1.8- to 2.9-fold (**Extended Data Fig. 6d-e**). At both tested sites, ABEs produced far fewer indels than prime editors (**Extended Data Fig. 6f**). Taken together, these results demonstrate that base editors can result in more efficient

editing than prime editors at optimally positioned target bases, but prime editing can outperform base editing at non-optimally positioned target bases, or when precise edits at targets with multiple editable bases are needed.

Effects of prime editor expression on the cellular transcriptome

We performed RNA-seq on HEK293T cells expressing PE2, PE2-dRT, or Cas9 H840A nickase together with a *PRNP*-targeting or *HEXA*-targeting pegRNA. Compared to PE2-dRT, PE2 induced ≥2-fold upregulation of only 18 RNAs out of >14,000 quantitated RNAs in both *PRNP*-targeting and *HEXA*-targeting samples. Similarly, PE2 induced ≥2-fold downregulation of only seven RNAs out of >14,000 quantitated RNAs in both *PRNP*-targeting and *HEXA*-targeting samples. These 25 RNAs lacked evident ontological relationships. In contrast, Cas9 H840A nickase induced upregulation of several heat shock-related genes compared to PE2 or PE2-dRT (**Extended Data Fig. 8d,e,g,h**). These results suggest that active PE2 minimally perturbs the transcriptome relative to Cas9 nickase or a control lacking active RT.

Background on HBB *and* PRNP *pathogenic alleles*

Sickle cell disease is caused by an A•T-to-T•A transversion mutation in *HBB*, resulting in an E6V mutation in beta-globin[63]. Treatment of hematopoietic stem cells *ex vivo* with Cas9 nuclease and a donor DNA template for HDR, followed by enrichment of edited cells, transplantation, and engraftment is a promising potential treatment strategy[64]. This approach still generates many indel-containing byproducts, however, in addition to the correctly edited *HBB* allele, and requires considerable medical infrastructure[64,65]. While base editors generate far fewer indels and have been used *in vivo*, they cannot currently make the T•A-to-A•T transversion mutation needed to directly restore the normal sequence of *HBB.* Therefore, we explored the application of prime editing to correct the sickle cell E6V mutation in *HBB*.

PrP misfolding causes progressive and fatal neurodegenerative prion disease that can arise spontaneously, through inherited dominant mutations in the *PRNP* gene, or through exposure to misfolded PrP[66]. A naturally occurring *PRNP* G127V mutant allele confers resistance to prion disease in humans[35] and mice[36]. We used PE3 to install G127V into the human *PRNP* allele in HEK293T cells, which requires a G•C-to-T•A transversion.

Prime editing compared with HDR in non-HEK293T human cell lines

In addition to HEK293T cells, we tested prime editing at endogenous genomic sites in three additional human cell lines. In K562 cells, PE3-mediated 3-bp insertion into *HEK3* proceeded with

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25% efficiency and 2.8% indels, compared with 17% editing and 72% indels for Cas9-initiated HDR, a 40-fold editing:indel ratio difference favoring PE3 (**Extended Data Fig. 10**). In U2OS cells and HeLa, PE3 performed this 3-bp insertion with 49-fold and 210-fold higher editing:indel ratios, respectively, than Cas9-initiated HDR (**Extended Data Fig. 10**).

Supplementary Table 1. Activities of prime editors, Cas9 nuclease, Cas9 H840A nickase, and PE2-dRT at *HEK3*, *HEK4*, *EMX1*, and *FANCF* on-target and off-target sites. PE2/PE3 editing is shown as % prime editing alongside % indels (in parentheses). % indels are shown for Cas9, Cas9 H840A nickase (nCas9), and PE2-dRT at the top four previously characterized off-target sites[31,32]. sgRNA and pegRNA sequences can be found in **Supplementary Table 3** under the Figure 5 heading. All values are the average of three independent biological replicates.

PE

pegRNA / Site	HEK3 (PE3) ---	1	2	3	4	HEK4 (PE2) ---	1	2	3	4	EMX1 (PE3) ---	1	2	3	4	FANCF (PE3) ---	1	2	3	4
On-target		44.2 (11.9)	61.2 (8.8)	40.4 (16.5)	48.4 (3.3)		18.2 (0.9)	14.4 (1.8)	9.8 (2.0)	7.9 (2.2)		28.6 (3.5)	14.1 (2.4)	35.7 (3.3)	15.4 (2.9)		56.8 (9.3)	32.4 (16.7)	42.8 (13.6)	47.6 (12.0)
Off-target 1		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (<0.1)	0.4 (<0.1)	<0.1 (<0.1)	0.4 (<0.1)		<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.1)		<0.1 (0.1)	0.6 (0.1)	<0.1 (0.1)	<0.1 (0.1)
Off-target 2		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.1)		<0.1 (<0.1)	<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (0.1)	<0.1 (<0.1)
Off-target 3		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		0.2 (0.5)	6.8 (1.9)	19.2 (0.5)	7.9 (3.5)		<0.1 (0.3)	<0.1 (0.3)	<0.1 (0.3)	<0.1 (0.3)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)
Off-target 4		<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.1)	<0.1 (<0.1)		<0.1 (<0.1)	<0.1 (0.1)	<0.1 (0.2)	<0.1 (<0.1)		<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.2)	<0.1 (0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)

Cas9

pegRNA / Site	HEK3 sgRNA	1	2	3	4	HEK4 sgRNA	1	2	3	4	EMX1 sgRNA	1	2	3	4	FANCF sgRNA	1	2	3	4
On-target	91.8	87.5	89.2	89.1	86.8	71.8	68.6	72.8	72.8	70.9	85.6	79.7	70.6	76.6	76.0	78.7	55.9	58.3	51.8	52.0
Off-target 1	17.2	1.9	5.5	5.2	1.8	54.2	39.5	48.4	49.7	49.2	81.1	63.5	48.1	53.0	59.6	12.6	1.9	1.9	1.7	1.7
Off-target 2	38.0	6.5	12.6	11.8	4.7	42.5	19.5	29.4	27.3	30.3	58.3	12.0	6.0	8.2	12.9	1.1	0.2	0.2	0.2	0.1
Off-target 3	8.8	0.6	1.7	1.5	0.5	98.1	96.9	97.3	97.6	97.5	14.8	4.2	3.1	3.6	4.8	2.4	0.2	<0.1	0.2	0.2
Off-target 4	0.3	<0.1	<0.1	0.1	<0.1	45.3	16.9	28.0	27.5	29.7	39.5	1.3	0.9	0.6	1.3	1.0	0.2	0.2	0.2	0.2

nCas9

pegRNA / Site	HEK3 ---	1	2	3	4	HEK4 ---	1	2	3	4	EMX1 ---	1	2	3	4	FANCF ---	1	2	3	4
Off-target 1		<0.1	<0.1	<0.1	<0.1		<0.1	<0.1	<0.1	<0.1		0.1	0.1	<0.1	0.1		<0.1	<0.1	<0.1	<0.1
Off-target 2		<0.1	<0.1	<0.1	<0.1		<0.1	<0.1	< 0.1	<0.1		0.3	0.1	<0.1	<0.1		<0.1	<0.1	<0.1	<0.1
Off-target 3		<0.1	<0.1	<0.1	<0.1		0.3	0.5	0.7	0.7		<0.1	<0.1	<0.1	<0.1		<0.1	<0.1	<0.1	<0.1
Off-target 4		<0.1	<0.1	<0.1	<0.1		<0.1	0.1	<0.1	<0.1		1.5	0.1	0.1	0.4		<0.1	<0.1	<0.1	<0.1

PE2-dRT

pegRNA / Site	HEK3 ---	1	2	3	4	HEK4 ---	1	2	3	4	EMX1 ---	1	2	3	4	FANCF ---	1	2	3	4
Off-target 1		<0.1	<0.1	<0.1	<0.1		0.1	0.2	<0.1	<0.1		0.1	0.1	0.1	0.1		<0.1	<0.1	<0.1	<0.1
Off-target 2		<0.1	<0.1	<0.1	<0.1		<0.1	0.1	<0.1	<0.1		<0.1	0.3	<0.1	<0.1		<0.1	<0.1	<0.1	<0.1
Off-target 3		<0.1	<0.1	<0.1	<0.1		<0.1	0.1	1.4	0.9		<0.1	<0.1	<0.1	<0.1		<0.1	<0.1	<0.1	<0.1
Off-target 4		<0.1	<0.1	<0.1	<0.1		0.1	0.1	0.1	0.2		0.9	0.1	0.1	0.2		<0.1	<0.1	<0.1	<0.1

Supplementary Table 2. Sequences of DNA oligonucleotides, pegRNAs, and sgRNAs used for *in vitro* experiments.

Oligonucleotide	Sequence
AVA023	5Cy5-CCTGGGTCAATCCTTGGGGCCCAGACTGAGCACG
AVA024	5Cy5-CCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGG
AVA025	5Phos-CCTTTCCTCTGCCATCACGTGCTCAGTCTGGGCCCCAAGGATTGACCCAGG
AVA026	5Phos-TGATGGCAGAGGAAAGG
AVA037	GCAGGCTTTAAAGGAACCAATTC
AVA110	GCAGGCTTTAAAGGAACCAATTCCCTGGGTCAATCCTTGGGGC
AVA122	CTCTGGAGGATCTAGCGGAG
AVA134	CTCTGGAGGATCTAGCGGAGTTTTTTTTTTTTTTTTTTTTT
AVA135	CTCTGGAGGATCTAGCGGAGCCCCCCCCCCCCCCC

5'-extended pegRNAs

pegRNA	spacer sequence	5' extension sequence	Linker length (nt)	PBS length (nt)	RT template length (nt)
pegRNA 1	GGCCCAGACTGAGCACGTGA	GGCTAACCGTGCCATTTGATCAGGTCA	15	5	7
pegRNA 2	GGCCCAGACTGAGCACGTGA	GGCTAACCGTGCAAATTAACAAACTAA	15	5	7
pegRNA 3	GGCCCAGACTGAGCACGTGA	GGCCATCTCGTGCAAATTAACAAACTAA	15	5	8
pegRNA 4	GGCCCAGACTGAGCACGTGA	GGTCCTCTGCCATCTCGTGCAAATTAACAAACTAA	15	5	15
pegRNA 5	GGCCCAGACTGAGCACGTGA	GGCTTCCTTTCCTCTGCCATCTCGTGCAAATTAACAAACTAA	15	5	22
5'-pegRNA_RT_7_a	GGCCCAGACTGAGCACGTGA	GGCTAACCGTGCCATTTGATCAGGTCA	15	5	7
5'-pegRNA_RT_7_b	GGCCCAGACTGAGCACGTGA	GGCTAACCGTGCAAATTAACAAACTAA	15	5	7
5'-pegRNA_RT_8	GGCCCAGACTGAGCACGTGA	GGCCATCTCGTGCAAATTAACAAACTAA	15	5	8
5'-pegRNA_RT_15	GGCCCAGACTGAGCACGTGA	GGTCCTCTGCCATCTCGTGCAAATTAACAAACTAA	15	5	15
5'-pegRNA_RT_22	GGCCCAGACTGAGCACGTGA	GGCTTCCTTTCCTCTGCCATCTCGTGCAAATTAACAAACTAA	15	5	22

3'-extended pegRNAs

pegRNA	spacer sequence	3' extension sequence	PBS length (nt)	RT template length (nt)
3'-pegRNA_10	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTC	7	10
3'-pegRNA_yeast_TtoA	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTC	7	10
3'-pegRNA_yeast_+1Ains	GGCCCAGACTGAGCACGTGA	TCTGCCATCATCGTGCTC	7	11
3'-pegRNA_yeast_+1Tdel	GGCCCAGACTGAGCACGTGA	TCTGCCATCCGTGCTC	7	9

Supplementary Table 3. Sequences of pegRNAs and sgRNAs used in mammalian cell experiments. All sequences are shown in 5' to 3' orientation. pegRNAs are a concatenation of the spacer sequence, the sgRNA scaffold, and the 3' extension (contains PBS and RT template). All pegRNAs used sgRNA scaffold 1 unless indicated with a * to denote the use of sgRNA scaffold 2.

sgRNA scaffold 1
GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGC
sgRNA scaffold 2 (contains two substitutions with respect to scaffold 1, inverting the second to last bp of the terminal hairpin)
GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTCGGTCC

Figure 2 and Extended Data Figure 3 sequences:

pegRNA	spacer sequence	3' extension	PBS length (nt)	RT template length (nt)
*HEK3_2a _8	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCA	8	10
*HEK3_2a_9	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAG	9	10
*HEK3_2a_10	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGT	10	10
*HEK3_2a_11	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTC	11	10
*HEK3_2a_12	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCT	12	10
*HEK3_2a_13	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTG	13	10
*HEK3_2a_14	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTGG	14	10
*HEK3_2a_15	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTGGG	15	10
*HEK3_2a_16	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTGGGC	16	10
*HEK3_2a_17	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTGGGCC	17	10
EMX1_2a_9	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGC	9	13
EMX1_2a_10	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCT	10	13
EMX1_2a_11	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTC	11	13
EMX1_2a_12	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTCG	12	13
EMX1_2a_13	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTCGG	13	13
EMX1_2a_14	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTCGGA	14	13
EMX1_2a_15	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTCGGAC	15	13
EMX1_2a_16	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTCGGACT	16	13
EMX1_2a_17	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTCGGACTC	17	13
FANCF_2a_8	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGA	8	17
FANCF_2a_9	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAA	9	17
FANCF_2a_10	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAG	10	17
FANCF_2a_11	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGG	11	17
FANCF_2a_12	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGG	12	17
FANCF_2a_13	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	17
FANCF_2a_14	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGAT	14	17
FANCF_2a_15	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGATT	15	17
FANCF_2a_16	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGATTC	16	17
FANCF_2a_17	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGATTCC	17	17
RNF2_2a_9	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACT	9	11
RNF2_2a_10	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTA	10	11
RNF2_2a_11	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAA	11	11
RNF2_2a_12	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAAG	12	11
RNF2_2a_13	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAAGA	13	11
RNF2_2a_14	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAAGAT	14	11
RNF2_2a_15	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAAGATG	15	11
RNF2_2a_16	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAAGATGA	16	11
RNF2_2a_17	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAAGATGAC	17	11
HEK4_2a_7	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAG	7	13
HEK4_2a_8	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGC	8	13
HEK4_2a_9	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCC	9	13
HEK4_2a_10	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCCG	10	13
HEK4_2a_11	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCCGC	11	13
HEK4_2a_12	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCCGCA	12	13
HEK4_2a_13	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCCGCAG	13	13
HEK4_2a_14	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCCGCAGT	14	13
HEK4_2a_15	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCCGCAGTG	15	13
*HEK3_2a_1Tdel	GGCCCAGACTGAGCACGTGA	TCTGCCATCCGTGCTCAGTCTG	13	10
*HEK3_2a_1Ains	GGCCCAGACTGAGCACGTGA	TCTGCCATCATCGTGCTCAGTCTG	13	10
*HEK3_2a_1CTTins	GGCCCAGACTGAGCACGTGA	TCTGCCATCAAAGCGTGCTCAGTCTG	13	10
*HEK3_2b_10	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTG	13	10
*HEK3_2b_11	GGCCCAGACTGAGCACGTGA	CTCTGCCATCTCGTGCTCAGTCTG	13	11
*HEK3_2b_12	GGCCCAGACTGAGCACGTGA	CCTCTGCCATCTCGTGCTCAGTCTG	13	12
*HEK3_2b_13	GGCCCAGACTGAGCACGTGA	TCCTCTGCCATCTCGTGCTCAGTCTG	13	13
*HEK3_2b_14	GGCCCAGACTGAGCACGTGA	TTCCTCTGCCATCTCGTGCTCAGTCTG	13	14
*HEK3_2b_15	GGCCCAGACTGAGCACGTGA	TTTCCTCTGCCATCTCGTGCTCAGTCTG	13	15
*HEK3_2b_16	GGCCCAGACTGAGCACGTGA	CTTTCCTCTGCCATCTCGTGCTCAGTCTG	13	16
*HEK3_2b_17	GGCCCAGACTGAGCACGTGA	CCTTTCCTCTGCCATCTCGTGCTCAGTCTG	13	17
*HEK3_2b_18	GGCCCAGACTGAGCACGTGA	TCCTTTCCTCTGCCATCTCGTGCTCAGTCTG	13	18

*HEK3_2b_19	GGCCCAGACTGAGCACGTGA	TTCCTTTCCTCTGCCATCTCGTGCTCAGTCTG	13	19
*HEK3_2b_20	GGCCCAGACTGAGCACGTGA	CTTCCTTTCCTCTGCCATCTCGTGCTCAGTCTG	13	20
EMX1_2b_10	GAGTCCGAGCAGAAGAAGAA	GGAGCCCTTGTTCTTCTGCTCGG	13	10
EMX1_2b_11	GAGTCCGAGCAGAAGAAGAA	GGGAGCCCTTGTTCTTCTGCTCGG	13	11
EMX1_2b_12	GAGTCCGAGCAGAAGAAGAA	TGGGAGCCCTTGTTCTTCTGCTCGG	13	12
EMX1_2b_13	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCCCTTGTTCTTCTGCTCGG	13	13
EMX1_2b_14	GAGTCCGAGCAGAAGAAGAA	GATGGGAGCCCTTGTTCTTCTGCTCGG	13	14
EMX1_2b_15	GAGTCCGAGCAGAAGAAGAA	TGATGGGAGCCCTTGTTCTTCTGCTCGG	13	15
EMX1_2b_16	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCTTGTTCTTCTGCTCGG	13	16
EMX1_2b_17	GAGTCCGAGCAGAAGAAGAA	TGTGATGGGAGCCCTTGTTCTTCTGCTCGG	13	17
EMX1_2b_18	GAGTCCGAGCAGAAGAAGAA	ATGTGATGGGAGCCCTTGTTCTTCTGCTCGG	13	18
EMX1_2b_19	GAGTCCGAGCAGAAGAAGAA	GATGTGATGGGAGCCCTTGTTCTTCTGCTCGG	13	19
EMX1_2b_20	GAGTCCGAGCAGAAGAAGAA	TGATGTGATGGGAGCCCTTGTTCTTCTGCTCGG	13	20
FANCF_2b_10	GGAATCCCTTCTGCAGCACC	CGATCAAGGTGCTGCAGAAGGGA	13	10
FANCF_2b_11	GGAATCCCTTCTGCAGCACC	GCGATCAAGGTGCTGCAGAAGGGA	13	11
FANCF_2b_12	GGAATCCCTTCTGCAGCACC	AGCGATCAAGGTGCTGCAGAAGGGA	13	12
FANCF_2b_13	GGAATCCCTTCTGCAGCACC	AAGCGATCAAGGTGCTGCAGAAGGGA	13	13
FANCF_2b_14	GGAATCCCTTCTGCAGCACC	AAAGCGATCAAGGTGCTGCAGAAGGGA	13	14
FANCF_2b_15	GGAATCCCTTCTGCAGCACC	AAAAGCGATCAAGGTGCTGCAGAAGGGA	13	15
FANCF_2b_16	GGAATCCCTTCTGCAGCACC	GAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	16
FANCF_2b_17	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	17
FANCF_2b_18	GGAATCCCTTCTGCAGCACC	CGGAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	18
FANCF_2b_19	GGAATCCCTTCTGCAGCACC	TCGGAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	19
FANCF_2b_20	GGAATCCCTTCTGCAGCACC	CTCGGAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	20
RNF2_2b_10	GTCATCTTAGTCATTACCTG	AACACCTCATGTAATGACTAAGATG	15	10
RNF2_2b_11	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAAGATG	15	11
RNF2_2b_12	GTCATCTTAGTCATTACCTG	CGAACACCTCATGTAATGACTAAGATG	15	12
RNF2_2b_13	GTCATCTTAGTCATTACCTG	ACGAACACCTCATGTAATGACTAAGATG	15	13
RNF2_2b_14	GTCATCTTAGTCATTACCTG	AACGAACACCTCATGTAATGACTAAGATG	15	14
RNF2_2b_15	GTCATCTTAGTCATTACCTG	CAACGAACACCTCATGTAATGACTAAGATG	15	15
RNF2_2b_16	GTCATCTTAGTCATTACCTG	ACAACGAACACCTCATGTAATGACTAAGATG	15	16
RNF2_2b_17	GTCATCTTAGTCATTACCTG	TACAACGAACACCTCATGTAATGACTAAGATG	15	17
RNF2_2b_18	GTCATCTTAGTCATTACCTG	TTACAACGAACACCTCATGTAATGACTAAGATG	15	18
RNF2_2b_19	GTCATCTTAGTCATTACCTG	GTTACAACGAACACCTCATGTAATGACTAAGATG	15	19
RNF2_2b_20	GTCATCTTAGTCATTACCTG	AGTTACAACGAACACCTCATGTAATGACTAAGATG	15	20
HEK4_2b_7	GGCACTGCGGCTGGAGGTGG	ACCCCAACCTCCAGCCGC	11	7
HEK4_2b_8	GGCACTGCGGCTGGAGGTGG	AACCCCAACCTCCAGCCGC	11	8
HEK4_2b_9	GGCACTGCGGCTGGAGGTGG	TAACCCCAACCTCCAGCCGC	11	9
HEK4_2b_10	GGCACTGCGGCTGGAGGTGG	TTAACCCCAACCTCCAGCCGC	11	10
HEK4_2b_11	GGCACTGCGGCTGGAGGTGG	TTTAACCCCAACCTCCAGCCGC	11	11
HEK4_2b_12	GGCACTGCGGCTGGAGGTGG	CTTTAACCCCAACCTCCAGCCGC	11	12
HEK4_2b_13	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCCGC	11	13
HEK4_2b_14	GGCACTGCGGCTGGAGGTGG	CGCTTTAACCCCAACCTCCAGCCGC	11	14
HEK4_2b_15	GGCACTGCGGCTGGAGGTGG	CCGCTTTAACCCCAACCTCCAGCCGC	11	15
HEK4_2b_16	GGCACTGCGGCTGGAGGTGG	TCCGCTTTAACCCCAACCTCCAGCCGC	11	16
HEK4_2b_17	GGCACTGCGGCTGGAGGTGG	CTCCGCTTTAACCCCAACCTCCAGCCGC	11	17
HEK4_2b_18	GGCACTGCGGCTGGAGGTGG	CTCCGCTTTAACCCCAACCTCCAGCCGC	11	18
HEK4_2b_19	GGCACTGCGGCTGGAGGTGG	CTCCGCTTTAACCCCAACCTCCAGCCGC	11	19

Figure 3 sequences:

pegRNA	spacer sequence	3' extension	PBS length (nt)	RT template length (nt)
RNF2_3b	GTCATCTTAGTCATTACCTG	AACGAACACCTCATGTAATGACTAAGATG	15	14
EMX1_3b	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTCGGAC	15	13
FANCF_3b	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGATT	15	17
*HEK3_3b	GGCCCAGACTGAGCACGTGA	TCTGCCATGACGTGCTCAGTCTG	13	10
HEK4_3b	GGCACTGCGGCTGGAGGTGG	TTAACCCCAACCTCCAGCC	9	10
RNF2_3c_4AtoC	GTCATCTTAGTCATTACCTG	AACGAACACCGCAGGTAATGACTAAGATG	15	14
RNF2_3c_4AtoG	GTCATCTTAGTCATTACCTG	AACGAACACCCCAGGTAATGACTAAGATG	15	14
FANCF_3c_5GtoT	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	17
FANCF_3c_7AtoC	GGAATCCCTTCTGCAGCACC	GGAAAAGCGAGCCAGGTGCTGCAGAAGGGAT	14	17

nicking sgRNA	spacer sequence
RNF2_3b_+41	GTCAACCATTAAGCAAAACAT
RNF2_3b_+67	GTCTCAGGCTGTGCAGACAAA
EMX1_3b_-116	GGGGCACAGATGAGAAACTC
EMX1_3b_-57	GCCGTTTGTACTTTGTCCTC
EMX1_3b_+14	GCGCCACCGGTTGATGTGAT
EMX1_3b_+27	GCTTCGTGGCAATGCGCCAC
EMX1_3b_+53	GACATCGATGTCCTCCCCAT
EMX1_3b_+80	GTGGTTGCCCACCCTAGTCAT
FANCF_3b_-78	GCGACTCTCTGCGTACTGAT
FANCF_3b_-50	GCCCTACTTCCGCTTTCACCT
FANCF_3b_-27	GGATTCCATGAGGTGCGCGA
FANCF_3b_-17	GCTGCAGAAGGGATTCCATG
*FANCF_3b_+21	GCTTGAGACCGCCAGAAGCT

FANCF_3b_+48	GGGGTCCCAGGTGCTGACGT			
HEK3_3b_-108	GCAGAAATAGACTAATTGCA			
HEK3_3b_-38	GGATTGACCCAGGCCAGGGC			
HEK3_3b_+26	DACGCCCTCTGGAGGAAGCA			
HEK3_3b_+37	GCTGTCCTGCGACGCCCTC			
HEK3_3b_+63	GCACATACTAGCCCCTGTCT			
HEK3_3b_+90	GTCAACCAGTATCCCGGTGC			
HEK4_3b_-95	GTCCCTTCCTTCCACCCAGCC			
HEK4_3b_-52	GCCCTGCCTGTCATCCTGCTT			
HEK4_3b_-26	GCAGTGCCACCGGGGCGCCG			
HEK4_3b_+52	GCGGGGGCTCAGAGAGGGCA			
HEK4_3b_+74	GAGACACACACACAGGCCTGG			
RNF2_3c_+41	GTCAACCATTAAGCAAAACAT			
RNF2_3c_4AtoC_+5	GTGAGTTACAACGAACACCGC			
RNF2_3c_4AtoG_+5	GTGAGTTACAACGAACACCCC			
FANCF_3c_+48	GGGGTCCCAGGTGCTGACGT			
FANCF_3c_5GtoT_+7	GAAGCTCGGAAAAGCGATCA			
FANCF_3c_7AtoC_+7	GAAGCTCGGAAAAGCGAGCC			

Figure 4 sequences:

pegRNA	spacer sequence	3' extension	PBS length (nt)	RT template length (nt)
*HEK3_4a_1TtoA	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTG	13	10
*HEK3_4a_1TtoC	GGCCCAGACTGAGCACGTGA	TCTGCCATCGCGTGCTCAGTCTG	13	10
*HEK3_4a_1TtoG	GGCCCAGACTGAGCACGTGA	TCTGCCATCCCGTGCTCAGTCTG	13	10
*HEK3_4a_2GtoA	GGCCCAGACTGAGCACGTGA	TCTGCCATTACGTGCTCAGTCTG	13	10
*HEK3_4a_2GtoC	GGCCCAGACTGAGCACGTGA	TCTGCCATGACGTGCTCAGTCTG	13	10
*HEK3_4a_2GtoT	GGCCCAGACTGAGCACGTGA	TCTGCCATAACGTGCTCAGTCTG	13	10
*HEK3_4a_3AtoC	GGCCCAGACTGAGCACGTGA	TCTGCCAGCACGTGCTCAGTCTG	13	10
*HEK3_4a_3AtoG	GGCCCAGACTGAGCACGTGA	TCTGCCACCACGTGCTCAGTCTG	13	10
*HEK3_4a_3AtoT	GGCCCAGACTGAGCACGTGA	TCTGCCAACACGTGCTCAGTCTG	13	10
*HEK3_4a_4TtoA	GGCCCAGACTGAGCACGTGA	TCTGCCTTCACGTGCTCAGTCTG	13	10
*HEK3_4a_4TtoC	GGCCCAGACTGAGCACGTGA	TCTGCCGTCACGTGCTCAGTCTG	13	10
*HEK3_4a_4TtoG	GGCCCAGACTGAGCACGTGA	TCTGCCCTCACGTGCTCAGTCTG	13	10
*HEK3_4a_5GtoA	GGCCCAGACTGAGCACGTGA	TCTGCTATCACGTGCTCAGTCTG	13	10
*HEK3_4a_5GtoC	GGCCCAGACTGAGCACGTGA	TCTGCGATCACGTGCTCAGTCTG	13	10
*HEK3_4a_5GtoT	GGCCCAGACTGAGCACGTGA	TCTGCAATCACGTGCTCAGTCTG	13	10
*HEK3_4a_6GtoA	GGCCCAGACTGAGCACGTGA	TCTGTCATCACGTGCTCAGTCTG	13	10
*HEK3_4a_6GtoC	GGCCCAGACTGAGCACGTGA	TCTGGCATCACGTGCTCAGTCTG	13	10
*HEK3_4a_6GtoT	GGCCCAGACTGAGCACGTGA	TCTGACATCACGTGCTCAGTCTG	13	10
*HEK3_4a_7CtoA	GGCCCAGACTGAGCACGTGA	TCTTCCATCACGTGCTCAGTCTG	13	10
*HEK3_4a_7CtoG	GGCCCAGACTGAGCACGTGA	TCTCCCATCACGTGCTCAGTCTG	13	10
*HEK3_4a_7CtoT	GGCCCAGACTGAGCACGTGA	TCTACCATCACGTGCTCAGTCTG	13	10
*HEK3_4a_8AtoC	GGCCCAGACTGAGCACGTGA	TCGGCCATCACGTGCTCAGTCTG	13	10
*HEK3_4a_8AtoG	GGCCCAGACTGAGCACGTGA	TCCGCCATCACGTGCTCAGTCTG	13	10
*HEK3_4a_8AtoT	GGCCCAGACTGAGCACGTGA	TCAGCCATCACGTGCTCAGTCTG	13	10
HEK3_4b_1TtoA	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCTCGTGCTCAGTCTG	13	34
HEK3_4b_12GtoC	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTGCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4b_14AtoT	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTATCCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4b_17GtoC	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTGCTTTCCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4b_20GtoC	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGGTTCCTTTCCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4b_23CtoG	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCACGGCTTCCTTTCCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4b_24TtoA	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCTGGGCTTCCTTTCCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4b_26CtoG	GGCCCAGACTGAGCACGTGA	TGGAGGAACCAGGGCTTCCTTTCCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4b_30CtoG	GGCCCAGACTGAGCACGTGA	TGGACGAAGCAGGGCTTCCTTTCCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4b_33CtoG	GGCCCAGACTGAGCACGTGA	TCGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCACGTGCTCAGTCTG	13	34
RNF2_4c_1CtoA	GTCATCTTAGTCATTACCTG	AACGAACACCTCATGTAATGACTAAGATG	15	14
RNF2_4c_1CtoG	GTCATCTTAGTCATTACCTG	AACGAACACCTCACGTAATGACTAAGATG	15	14
RNF2_4c_1CtoT	GTCATCTTAGTCATTACCTG	AACGAACACCTCAAGTAATGACTAAGATG	15	14
RNF2_4c_2TtoA	GTCATCTTAGTCATTACCTG	AACGAACACCTCTGGTAATGACTAAGATG	15	14
RNF2_4c_2TtoG	GTCATCTTAGTCATTACCTG	AACGAACACCTCCGGTAATGACTAAGATG	15	14
RNF2_4c_3GtoC	GTCATCTTAGTCATTACCTG	AACGAACACCTGAGGTAATGACTAAGATG	15	14
RNF2_4c_4AtoC	GTCATCTTAGTCATTACCTG	AACGAACACCGCAGGTAATGACTAAGATG	15	14
RNF2_4c_4AtoT	GTCATCTTAGTCATTACCTG	AACGAACACCACAGGTAATGACTAAGATG	15	14
RNF2_4c_4AtoG	GTCATCTTAGTCATTACCTG	AACGAACACCCCAGGTAATGACTAAGATG	15	14
RNF2_4c_5GtoT	GTCATCTTAGTCATTACCTG	AACGAACACATCAGGTAATGACTAAGATG	15	14
RNF2_4c_6GtoA	GTCATCTTAGTCATTACCTG	AACGAACATCTCAGGTAATGACTAAGATG	15	14
RNF2_4c_7TtoC	GTCATCTTAGTCATTACCTG	AACGAACGCCTCAGGTAATGACTAAGATG	15	14
RUNX1_4d_1CtoA	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCATCTCTTCCTCCTGAAAAT	15	15
RUNX1_4d_1CtoG	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCATCCCTTCCTCCTGAAAAT	15	15
RUNX1_4d_1CtoT	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCATCACTTCCTCCTGAAAAT	15	15
RUNX1_4d_2GtoA	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCATTGCTTCCTCCTGAAAAT	15	15
RUNX1_4d_3AtoC	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCAGCGCTTCCTCCTGAAAAT	15	15
RUNX1_4d_3AtoG	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCACCGCTTCCTCCTGAAAAT	15	15

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RUNX1_4d_3AtoT	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCAACGCTTCCTCCTGAAAAT	15	15
RUNX1_4d_4TtoA	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCTTCGCTTCCTCCTGAAAAT	15	15
RUNX1_4d_4TtoC	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCGTCGCTTCCTCCTGAAAAT	15	15
RUNX1_4d_4TtoG	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCCTCGCTTCCTCCTGAAAAT	15	15
RUNX1_4d_5GtoT	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	15
RUNX1_4d_6GtoC	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGGCATCGCTTCCTCCTGAAAAT	15	15
VEGFA_4e_1TtoA	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCTCTTCTGGCCTGCAGA	13	22
VEGFA_4e_1TtoC	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCTCGTCTGGCCTGCAGA	13	22
VEGFA_4e_1TtoG	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCTCCTCTGGCCTGCAGA	13	22
VEGFA_4e_2GtoA	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCTTATCTGGCCTGCAGA	13	22
VEGFA_4e_3AtoC	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCGCATCTGGCCTGCAGA	13	22
VEGFA_4e_3AtoG	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCCCATCTGGCCTGCAGA	13	22
VEGFA_4e_3AtoT	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCACATCTGGCCTGCAGA	13	22
VEGFA_4e_5GtoT	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	22
VEGFA_4e_6GtoC	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGGCCTCATCTGGCCTGCAGA	13	22
VEGFA_4e_7CtoA	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGATCCCTCATCTGGCCTGCAGA	13	22
VEGFA_4e_7CtoT	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAACCCTCATCTGGCCTGCAGA	13	22
VEGFA_4e_9CtoG	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGCAGCCCTCATCTGGCCTGCAGA	13	22
*HEK3_4f_1Ains	GGCCCAGACTGAGCACGTGA	TCTGCCATCATCGTGCTCAGTCTG	13	11
*HEK3_4f_1CTTins	GGCCCAGACTGAGCACGTGA	TCTGCCATCAAAGCGTGCTCAGTCTG	13	13
*HEK3_4f_1Tdel	GGCCCAGACTGAGCACGTGA	TCTGCCATCCGTGCTCAGTCTG	13	9
HEK3_4f_1-3TGAdel	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCACGTGCTCAGTCTG	13	31
RNF2_4f_1Tins	GTCATCTTAGTCATTACCTG	AACGAACACCTCAGAGTAATGACTAAGATG	15	15
RNF2_4f_1GTAins	GTCATCTTAGTCATTACCTG	AACGAACACCTCAGTACGTAATGACTAAGATG	15	17
RNF2_4f_4Adel	GTCATCTTAGTCATTACCTG	AACGAACACCCAGGTAATGACTAAGATG	15	13
RNF2_4f_3-5GAGdel	GTCATCTTAGTCATTACCTG	AACGAACACAGGTAATGACTAAGATG	15	11
FANCF_4f_3Cins	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAGGGTGCTGCAGAAGGGAT	14	18
FANCF_4f_4GATins	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAATCGGTGCTGCAGAAGGGAT	14	20
FANCF_4f_6Gdel	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAGGTGCTGCAGAAGGGAT	14	16
FANCF_4f_5-7GGAdel	GGAATCCCTTCTGCAGCACC	GGAAAAGCGAAGGTGCTGCAGAAGGGAT	14	14
EMX1_4f_6Tins	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCACCTTCTTCTTCTGCTCGGA	14	17
EMX1_4f_1TGCins	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCTTCGCATTCTTCTGCTCGGA	14	19
EMX1_4f_5Gdel	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCTTCTTCTTCTGCTCGGA	14	15
EMX1_4f_4-6GGGdel	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGTTCTTCTTCTGCTCGGA	14	13
RUNX1_4f_1Cins	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCATCGGCTTCCTCCTGAAAAT	15	16
RUNX1_4f_1ATGins	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCATCCATGCTTCCTCCTGAAAAT	15	18
RUNX1_4f_2Gdel	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCATGCTTCCTCCTGAAAAT	15	14
RUNX1_4f_2-4GATdel	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCGCTTCCTCCTGAAAAT	15	12
VEGFA_4f_4Cins	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCGCTCATCTGGCCTGCAGA	13	23
VEGFA_4f_2ACAins	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCTTGTCATCTGGCCTGCAGA	13	25
VEGFA_4f_3Adel	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCCATCTGGCCTGCAGA	13	21
VEGFA_4f_2-4GAGdel	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCATCTGGCCTGCAGA	13	19
DNMT1_4f_4Cins	GATTCCTGGTGCCAGAAACA	TCCCGTCACCCGCTGTTTCTGGCACCAGG	13	16
DNMT1_4f_1TCAins	GATTCCTGGTGCCAGAAACA	TCCCGTCACCCCTGTGATTTCTGGCACCAGG	13	18
DNMT1_4f_3Adel	GATTCCTGGTGCCAGAAACA	TCCCGTCACCCCGTTTCTGGCACCAGG	13	14
DNMT1_4f_3-5AGGdel	GATTCCTGGTGCCAGAAACA	TCCCGTCACCGTTTCTGGCACCAGG	13	12
HEK3_4g_del1-5	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCGTGCTCAGTCTG	13	29
HEK3_4g_del1-10	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCCGTGCTCAGTCTG	13	24
HEK3_4g_del1-15	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCCGTGCTCAGTCTG	13	19
HEK3_4g_del1-25	GGCCCAGACTGAGCACGTGA	TGTCCTGCGACGCCCTCTGGAGGAAGCGTGCTCAGTCTG	13	26
HEK3_4g_del1-30	GGCCCAGACTGAGCACGTGA	TGTCCTGCGACGCCCTCTGGACGTGCTCAGTCTG	13	21
HEK3_4g_del1-80	GGCCCAGACTGAGCACGTGA	AGTATCCCGGTGCAGGAGCTCGTGCTCAGTCTG	13	20
HEK3_4h_1CTTins_5Gdel	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCATCAAAGCGTGCTCAGTCTG	13	36
HEK3_4h_1CTTins_2GtoC	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATGAAAGCGTGCTCAGTCTG	13	37
HEK3_4h_1Tdel_5GtoC	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCGATCCGTGCTCAGTCTG	13	33
HEK3_4h_2GtoC_6GtoT	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGACATGACGTGCTCAGTCTG	13	34
RNF2_4h_2AAins_3-4GAdel	GTCATCTTAGTCATTACCTG	AACGAACACCATTGGTAATGACTAAGATG	15	14
RNF2_4h_1Ains_5GtoC	GTCATCTTAGTCATTACCTG	AACGAACACGTCAGTGTAATGACTAAGATG	15	15
RNF2_4h_1-2CTdel_6GtoT	GTCATCTTAGTCATTACCTG	AACGAACAACTCGTAATGACTAAGATG	15	12
RNF2_4h_1CtoA_5GtoT	GTCATCTTAGTCATTACCTG	AACGAACACATCATGTAATGACTAAGATG	15	14
FANCF_4h_1Tins_4-5TGdel	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCGGTAGCTGCAGAAGGGAT	14	16
FANCF_4h_1Tins_6GtoA	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATTCAGGTAGCTGCAGAAGGGAT	14	18
FANCF_4h_2Cdel_5GtoT	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGTGCTGCAGAAGGGAT	14	16
FANCF_4h_1AtoC_6GtoT	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATACAGGGGCTGCAGAAGGGAT	14	17

nicking sgRNA	spacer sequence
HEK3_4a_+90	GTCAACCAGTATCCCGGTGC
HEK3_4b_+90	GTCAACCAGTATCCCGGTGC
RNF2_4c_+41	GTCAACCATTAAGCAAAACAT
FANCF_4d_+48	GGGGTCCCAGGTGCTGACGT
EMX1_4e_+53	GACATCGATGTCCTCCCCAT
RUNX1_4f_+38	GATGAAGCACTGTGGGTACGA
VEGFA_4g_+57	GATGTACAGAGAGCCCAGGGC
DNMT1_4h_+49	GCCCTTCAGCTAAAATAAAGG

Figure 5 sequences:

pegRNA	spacer sequence	3' extension	PBS length (nt)	RT template length (nt)
HBB_5a_install	GCATGGTGCACCTGACTCCTG	AGACTTCTCCACAGGAGTCAGGTGCAC	13	14
HBB_5a_correct	GCATGGTGCACCTGACTCCTG	AGACTTCTCCTCAGGAGTCAGGTGCAC	13	14
HBB_5a_correct_w_silent	GCATGGTGCACCTGACTCCTG	AGACTTCTCTTCAGGAGTCAGGTGCAC	13	14
HEXA_5b_install	GTACCTGAACCGTATATCCTA	AGTCAGGGCCATAGGATAGATATACGGTTC	12	14
HEXA_5b_correct	GATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCCTATGGCCCTGACTG	10	21
HEXA_5b_correct_w_silent	GATCCTTCCAGTCAGGGCCAT	GTACCTGAACCGTATATCTTATGGCCCTGACT	9	27
PRNP_5c_6GtoT	GCAGTGGTGGGGGGCCTTGG	ATGTAGACGCCAAGGCCCCCCACC	12	12
DNMT1_5d_5GtoT	GCGGGCTGGAGCTGTTCGCGC	AAGATGCAAGCGCGAACAGCTCCAG	13	12
*HEK3_5e_1TtoG	GGCCCAGACTGAGCACGTGA	TCTGCCATCCCGTGCTCAGTCTG	13	10
*HEK3_5e_1CTTins	GGCCCAGACTGAGCACGTGA	TCTGCCATCAAAGCGTGCTCAGTCTG	13	10
RNF2_5e_1CtoG	GTCATCTTAGTCATTACCTG	AACGAACACCTCACGTAATGACTAAGATG	15	14
PRNP_5e_6GtoT	GCAGTGGTGGGGGGCCTTGG	ATGTAGACGCCAAGGCCCCCCACC	12	12
HBB_5e_4AtoT	GCATGGTGCACCTGACTCCTG	AGACTTCTCCACAGGAGTCAGGTGCAC	13	14
HBB_5e_correct	GCATGGTGCACCTGACTCCTG	AGACTTCTCTTCAGGAGTCAGGTGCAC	13	14
HEK3_5g_1His6ins	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCAATGATGGT GATGATGGTGCGTGCTCAGTCTG	13	52
HEK3_5g_1FLAGins	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCACTTATCGT CGTCATCCTTGTAATCCGTGCTCAGTCTG	13	58
HEK3_5g_1LoxPins	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCAATAACTTC GTATAATGTATGCTATACGAAGTTATAACAATCGTGCTCAGT CTG	13	74

nicking gRNA	spacer sequence
HBB_5a,e	GCCTTGATACCAACCTGCCCA
HEXA_5b_install	GCTTTCACCTTCAAATGCCA
HEXA_5b_correct	GTACCTGAACCGTATATCCTA
HEXA_5b_correct_w_silent	GTACCTGAACCGTATATCTTA
PRNP_5c,e	GCATGTTTTCACGATAGTAA
HEK3_5e,g	GTCAACCAGTATCCCGGTGC
DNMT1_5d	GCCGCGCGCGCGAAAAAGCCG
RNF2_5e	GTCAACCATTAAGCAAAACAT

Extended Data Figure 4 sequences:

pegRNA	spacer sequence	3' extension sequence	PBS length (nt)	RT template length (nt)
*HEK3_ED4b_1Tdel	GGCCCAGACTGAGCACGTGA	TCTGCCATCCGTGCTCAGTCTG	13	9
*HEK3_ED4b_1Ains	GGCCCAGACTGAGCACGTGA	TCTGCCATCATCGTGCTCAGTCTG	13	11
*HEK3_ED4b_1CTTins	GGCCCAGACTGAGCACGTGA	TCTGCCATCAAAGCGTGCTCAGTCTG	13	13
*HEK3_ED4c_2GtoC	GGCCCAGACTGAGCACGTGA	TCTGCCATGACGTGCTCAGTCTG	13	10
HEK3_ED4d_1FLAGins	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCACTTATCGT CGTCATCCTTGTAATCCGTGCTCAGTCTG	13	58
RNF2_ED4e_1CtoA	GTCATCTTAGTCATTACCTG	AACGAACACCTCATGTAATGACTAAGATG	15	14
EMX1_ED4f_1GtoC	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCCCTTGTTCTTCTGCTCGG	13	13
HBB_ED4g_2TtoA	GTAACGGCAGACTTCTCCTC	ATCTGACTCCTGTGGAGAAGTCTGCC	12	14
FANCF_ED4h_1GtoC	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAGGAGCTGCAGAAGGG	12	17

Extended Data Figure 5 sequences:

pegRNA	spacer sequence	3' extension sequence	PBS length (nt)	RT template length (nt)
VEGFA_ED5a_31	GATGTCTGCAGGCCAGATGA	CCCTCTGACAATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	31
VEGFA_ED5a_30	GATGTCTGCAGGCCAGATGA	CCTCTGACAATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	30
VEGFA_ED5a_29	GATGTCTGCAGGCCAGATGA	CTCTGACAATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	29
VEGFA_ED5a_28	GATGTCTGCAGGCCAGATGA	TCTGACAATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	28
VEGFA_ED5a_27	GATGTCTGCAGGCCAGATGA	CTGACAATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	27
VEGFA_ED5a_26	GATGTCTGCAGGCCAGATGA	TGACAATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	26
VEGFA_ED5a_25	GATGTCTGCAGGCCAGATGA	GACAATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	25
VEGFA_ED5a_24	GATGTCTGCAGGCCAGATGA	ACAATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	24
VEGFA_ED5a_23	GATGTCTGCAGGCCAGATGA	CAATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	23
VEGFA_ED5a_22	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	22
VEGFA_ED5a_21	GATGTCTGCAGGCCAGATGA	ATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	21
VEGFA_ED5a_20	GATGTCTGCAGGCCAGATGA	TGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	20
VEGFA_ED5a_19	GATGTCTGCAGGCCAGATGA	GTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	19
VEGFA_ED5a_18	GATGTCTGCAGGCCAGATGA	TGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	18
VEGFA_ED5a_17	GATGTCTGCAGGCCAGATGA	GCCATCTGGAGCACTCATCTGGCCTGCAGA	13	17
VEGFA_ED5a_16	GATGTCTGCAGGCCAGATGA	CCATCTGGAGCACTCATCTGGCCTGCAGA	13	16
VEGFA_ED5a_15	GATGTCTGCAGGCCAGATGA	CATCTGGAGCACTCATCTGGCCTGCAGA	13	15
VEGFA_ED5a_14	GATGTCTGCAGGCCAGATGA	ATCTGGAGCACTCATCTGGCCTGCAGA	13	14
VEGFA_ED5a_13	GATGTCTGCAGGCCAGATGA	TCTGGAGCACTCATCTGGCCTGCAGA	13	13

VEGFA_ED5a_12	GATGTCTGCAGGCCAGATGA	CTGGAGCACTCATCTGGCCTGCAGA	13	12
VEGFA_ED5a_11	GATGTCTGCAGGCCAGATGA	TGGAGCACTCATCTGGCCTGCAGA	13	11
VEGFA_ED5a_10	GATGTCTGCAGGCCAGATGA	GGAGCACTCATCTGGCCTGCAGA	13	10
VEGFA_ED5a_9	GATGTCTGCAGGCCAGATGA	GAGCACTCATCTGGCCTGCAGA	13	9
VEGFA_ED5a_8	GATGTCTGCAGGCCAGATGA	AGCACTCATCTGGCCTGCAGA	13	8
DNMT1_ED5b_31	GATTCCTGGTGCCAGAAACA	AGGACTAGTTCTGCCCTCCCGTCACCACTGTTTCTGGCACCAGG	13	31
DNMT1_ED5b_30	GATTCCTGGTGCCAGAAACA	GGACTAGTTCTGCCCTCCCGTCACCACTGTTTCTGGCACCAGG	13	30
DNMT1_ED5b_29	GATTCCTGGTGCCAGAAACA	GACTAGTTCTGCCCTCCCGTCACCACTGTTTCTGGCACCAGG	13	29
DNMT1_ED5b_28	GATTCCTGGTGCCAGAAACA	ACTAGTTCTGCCCTCCCGTCACCACTGTTTCTGGCACCAGG	13	28
DNMT1_ED5b_27	GATTCCTGGTGCCAGAAACA	CTAGTTCTGCCCTCCCGTCACCACTGTTTCTGGCACCAGG	13	27
DNMT1_ED5b_26	GATTCCTGGTGCCAGAAACA	TAGTTCTGCCCTCCCGTCACCACTGTTTCTGGCACCAGG	13	26
DNMT1_ED5b_25	GATTCCTGGTGCCAGAAACA	AGTTCTGCCCTCCCGTCACCACTGTTTCTGGCACCAGG	13	25
DNMT1_ED5b_24	GATTCCTGGTGCCAGAAACA	GTTCTGCCCTCCCGTCACCACTGTTTCTGGCACCAGG	13	24
DNMT1_ED5b_23	GATTCCTGGTGCCAGAAACA	TTCTGCCCTCCCGTCACCACTGTTTCTGGCACCAGG	13	23
DNMT1_ED5b_22	GATTCCTGGTGCCAGAAACA	TCTGCCCTCCCGTCACCACTGTTTCTGGCACCAGG	13	22
DNMT1_ED5b_21	GATTCCTGGTGCCAGAAACA	CTGCCCTCCCGTCACCACTGTTTCTGGCACCAGG	13	21
DNMT1_ED5b_20	GATTCCTGGTGCCAGAAACA	TGCCCTCCCGTCACCACTGTTTCTGGCACCAGG	13	20
DNMT1_ED5b_19	GATTCCTGGTGCCAGAAACA	GCCCTCCCGTCACCACTGTTTCTGGCACCAGG	13	19
DNMT1_ED5b_18	GATTCCTGGTGCCAGAAACA	CCCTCCCGTCACCACTGTTTCTGGCACCAGG	13	18
DNMT1_ED5b_17	GATTCCTGGTGCCAGAAACA	CCTCCCGTCACCACTGTTTCTGGCACCAGG	13	17
DNMT1_ED5b_16	GATTCCTGGTGCCAGAAACA	CTCCCGTCACCACTGTTTCTGGCACCAGG	13	16
DNMT1_ED5b_15	GATTCCTGGTGCCAGAAACA	TCCCGTCACCACTGTTTCTGGCACCAGG	13	15
DNMT1_ED5b_14	GATTCCTGGTGCCAGAAACA	CCCGTCACCACTGTTTCTGGCACCAGG	13	14
DNMT1_ED5b_13	GATTCCTGGTGCCAGAAACA	CCGTCACCACTGTTTCTGGCACCAGG	13	13
DNMT1_ED5b_12	GATTCCTGGTGCCAGAAACA	CGTCACCACTGTTTCTGGCACCAGG	13	12
DNMT1_ED5b_11	GATTCCTGGTGCCAGAAACA	GTCACCACTGTTTCTGGCACCAGG	13	11
DNMT1_ED5b_10	GATTCCTGGTGCCAGAAACA	TCACCACTGTTTCTGGCACCAGG	13	10
DNMT1_ED5b_9	GATTCCTGGTGCCAGAAACA	CACCACTGTTTCTGGCACCAGG	13	9
DNMT1_ED5b_8	GATTCCTGGTGCCAGAAACA	ACCACTGTTTCTGGCACCAGG	13	8
RUNX1_ED5c_31	GCATTTTCAGGAGGAAGCGA	AATGACTCAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	31
RUNX1_ED5c_30	GCATTTTCAGGAGGAAGCGA	ATGACTCAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	30
RUNX1_ED5c_29	GCATTTTCAGGAGGAAGCGA	TGACTCAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	29
RUNX1_ED5c_28	GCATTTTCAGGAGGAAGCGA	GACTCAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	28
RUNX1_ED5c_27	GCATTTTCAGGAGGAAGCGA	ACTCAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	27
RUNX1_ED5c_26	GCATTTTCAGGAGGAAGCGA	CTCAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	26
RUNX1_ED5c_25	GCATTTTCAGGAGGAAGCGA	TCAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	25
RUNX1_ED5c_24	GCATTTTCAGGAGGAAGCGA	CAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	24
RUNX1_ED5c_23	GCATTTTCAGGAGGAAGCGA	AAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	23
RUNX1_ED5c_22	GCATTTTCAGGAGGAAGCGA	AATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	22
RUNX1_ED5c_21	GCATTTTCAGGAGGAAGCGA	ATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	21
RUNX1_ED5c_20	GCATTTTCAGGAGGAAGCGA	TATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	20
RUNX1_ED5c_19	GCATTTTCAGGAGGAAGCGA	ATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	19
RUNX1_ED5c_18	GCATTTTCAGGAGGAAGCGA	TGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	18
RUNX1_ED5c_17	GCATTTTCAGGAGGAAGCGA	GCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	17
RUNX1_ED5c_16	GCATTTTCAGGAGGAAGCGA	CTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	16
RUNX1_ED5c_15	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	15
RUNX1_ED5c_14	GCATTTTCAGGAGGAAGCGA	GTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	14
RUNX1_ED5c_13	GCATTTTCAGGAGGAAGCGA	TCTGAAGCAATCGCTTCCTCCTGAAAAT	15	13
RUNX1_ED5c_12	GCATTTTCAGGAGGAAGCGA	CTGAAGCAATCGCTTCCTCCTGAAAAT	15	12
RUNX1_ED5c_11	GCATTTTCAGGAGGAAGCGA	TGAAGCAATCGCTTCCTCCTGAAAAT	15	11
RUNX1_ED5c_10	GCATTTTCAGGAGGAAGCGA	GAAGCAATCGCTTCCTCCTGAAAAT	15	10
RUNX1_ED5c_9	GCATTTTCAGGAGGAAGCGA	AAGCAATCGCTTCCTCCTGAAAAT	15	9
FANCF_ED5d_1AtoG	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAGGCGCTGCAGAAGGGAT	14	17
FANCF_ED5d_1AtoT	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAGGAGCTGCAGAAGGGAT	14	17
FANCF_ED5d_2CtoA	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAGTTGCTGCAGAAGGGAT	14	17
FANCF_ED5d_3CtoG	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCACGTGCTGCAGAAGGGAT	14	17
FANCF_ED5d_3CtoT	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAAGTGCTGCAGAAGGGAT	14	17
FANCF_ED5d_4TtoA	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCTGGTGCTGCAGAAGGGAT	14	17
FANCF_ED5d_4TtoG	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCCGGTGCTGCAGAAGGGAT	14	17
FANCF_ED5d_5GtoA	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCTAGGTGCTGCAGAAGGGAT	14	17
FANCF_ED5d_6GtoC	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATGCAGGTGCTGCAGAAGGGAT	14	17
FANCF_ED5d_7AtoC	GGAATCCCTTCTGCAGCACC	GGAAAAGCGAGCCAGGTGCTGCAGAAGGGAT	14	17
FANCF_ED5d_8TtoC	GGAATCCCTTCTGCAGCACC	GGAAAAGCGCTCCAGGTGCTGCAGAAGGGAT	14	17
FANCF_ED5d_10GtoT	GGAATCCCTTCTGCAGCACC	GGAAAAGAGATCCAGGTGCTGCAGAAGGGAT	14	17
EMX1_ED5e_2AtoC	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCTGCTTCTTCTGCTCGGA	14	16
EMX1_ED5e_2AtoT	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCTACTTCTTCTGCTCGGA	14	16
EMX1_ED5e_3AtoG	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCTCTTCTTCTGCTCGGA	14	16
EMX1_ED5e_4GtoC	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCGTTCTTCTTCTGCTCGGA	14	16
EMX1_ED5e_5GtoA	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCTCTTCTTCTTCTGCTCGGA	14	16
EMX1_ED5e_5GtoT	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCACTTCTTCTTCTGCTCGGA	14	16
EMX1_ED5e_7CtoA	GAGTCCGAGCAGAAGAAGAA	GTGATGGGATCCCTTCTTCTTCTGCTCGGA	14	16
EMX1_ED5e_8TtoA	GAGTCCGAGCAGAAGAAGAA	GTGATGGGTGCCCTTCTTCTTCTGCTCGGA	14	16
EMX1_ED5e_8TtoC	GAGTCCGAGCAGAAGAAGAA	GTGATGGGGGCCCTTCTTCTTCTGCTCGGA	14	16
EMX1_ED5e_8TtoG	GAGTCCGAGCAGAAGAAGAA	GTGATGGGCGCCCTTCTTCTTCTGCTCGGA	14	16
EMX1_ED5e_9CtoG	GAGTCCGAGCAGAAGAAGAA	GTGATGGCAGCCCTTCTTCTTCTGCTCGGA	14	16
EMX1_ED5e_9CtoT	GAGTCCGAGCAGAAGAAGAA	GTGATGGAAGCCCTTCTTCTTCTGCTCGGA	14	16

DNMT1_ED5f_1AtoC	GATTCCTGGTGCCAGAAACA	GTCACCCCTGGTTCTGGCACCAGG	13	11
DNMT1_ED5f_1AtoG	GATTCCTGGTGCCAGAAACA	GTCACCCCTGCTTCTGGCACCAGG	13	11
DNMT1_ED5f_2CtoA	GATTCCTGGTGCCAGAAACA	GTCACCCCTTTTTCTGGCACCAGG	13	11
DNMT1_ED5f_2CtoG	GATTCCTGGTGCCAGAAACA	GTCACCCCTCTTTCTGGCACCAGG	13	11
DNMT1_ED5f_2CtoT	GATTCCTGGTGCCAGAAACA	GTCACCCCTATTTCTGGCACCAGG	13	11
DNMT1_ED5f_3AtoT	GATTCCTGGTGCCAGAAACA	GTCACCCCAGTTTCTGGCACCAGG	13	11
DNMT1_ED5f_4GtoA	GATTCCTGGTGCCAGAAACA	GTCACCCTTGTTTCTGGCACCAGG	13	11
DNMT1_ED5f_5GtoT	GATTCCTGGTGCCAGAAACA	GTCACCACTGTTTCTGGCACCAGG	13	11
DNMT1_ED5f_6GtoC	GATTCCTGGTGCCAGAAACA	GTCACGCCTGTTTCTGGCACCAGG	13	11
DNMT1_ED5f_8TtoA	GATTCCTGGTGCCAGAAACA	GCCCTCCCGTCTCCCCTGTTTCTGGCACCAGG	13	19
DNMT1_ED5f_8TtoC	GATTCCTGGTGCCAGAAACA	GCCCTCCCGTCGCCCCTGTTTCTGGCACCAGG	13	19
DNMT1_ED5f_8TtoG	GATTCCTGGTGCCAGAAACA	GCCCTCCCGTCCCCCCTGTTTCTGGCACCAGG	13	19

nicking sgRNA	spacer sequence
FANCF_4d_+48	GGGGTCCCAGGTGCTGACGT
EMX1_4e_+53	GACATCGATGTCCTCCCCAT
DNMT1_4h_+49	GCCCTTCAGCTAAAATAAAGG

Extended Data Figure 6 sequences:

pegRNA	spacer sequence	3' extension	PBS length (nt)	RT template length (nt)
*HEK3_ED6a_C3	GGCCCAGACTGAGCACGTGA	TCTGTCATCACGTGCTCAGTCTG	13	10
*HEK3_ED6a_C4	GGCCCAGACTGAGCACGTGA	TCTGCTATCACGTGCTCAGTCTG	13	10
*HEK3_ED6a_C7	GGCCCAGACTGAGCACGTGA	TCTGCCATTACGTGCTCAGTCTG	13	10
FANCF_ED6a_C3	GGAATCCCTTCTGCAGCACC	GGAAAAGTGATCCAGGTGCTGCAGAAGGGAT	14	17
FANCF_ED6a_C7	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATTCAGGTGCTGCAGAAGGGAT	14	17
FANCF_ED6a_C8	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCTAGGTGCTGCAGAAGGGAT	14	17
EMX1_ED6a_C5	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGTCCTTCTTCTTCTGCTCGGA	14	16
EMX1_ED6a_C6	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCTCTTCTTCTTCTGCTCGGA	14	16
EMX1_ED6a_C7	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCTTTCTTCTTCTGCTCGGA	14	16
EMX1_ED6c_C5_6	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGTTCTTCTTCTTCTGCTCGGA	14	16
EMX1_ED6c_C5_7	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGTCTTTCTTCTTCTGCTCGGA	14	16
EMX1_ED6c_C6_7	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCTTTTCTTCTTCTGCTCGGA	14	16
EMX1_ED6c_C5_6_7	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGTTTTTCTTCTTCTGCTCGGA	14	16
*HEK3_ED6d_A5	GGCCCAGACTGAGCACGTGA	TCTGCCGTCACGTGCTCAGTCTG	13	10
*HEK3_ED6d_A8	GGCCCAGACTGAGCACGTGA	TCTGCCATCGCGTGCTCAGTCTG	13	10

nicking sgRNA	spacer sequence
HEK3_ED6a-f_+90	GTCAACCAGTATCCCGGTGC
FANCF_ED6a-f_+48	GGGGTCCCAGGTGCTGACGT
EMX1_ED6a-f_+57	GATGTACAGAGAGCCCAGGGC

base editing sgRNA	spacer sequence
HEK3_ED6a-f_BE	GTGCCATCACGTGCTCAGTCT
FANCF_ED6a-f_BE	GAGCGATCCAGGTGCTGCAGA
EMX1_ED6a-f_BE	GGAGCCCTTCTTCTTCTGCT

on-target sgRNA	spacer sequence
HEK3_ED6g	GGCCCAGACTGAGCACGTGA
HEK4_ED6g	GGCACTGCGGCTGGAGGTGG
EMX1_ED6g	GAGTCCGAGCAGAAGAAGAA
FANCF_ED6g	GGAATCCCTTCTGCAGCACC

on-target pegRNA	spacer sequence	3' extension	PBS length (nt)	RT template length (nt)
*HEK3_ED6g-h_pegRNA_1	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTG	13	10
*HEK3_ED6g-h_pegRNA_2	GGCCCAGACTGAGCACGTGA	TCTGCCATCAAAGCGTGCTCAGTCTG	13	13
*HEK3_ED6g-h_pegRNA_3	GGCCCAGACTGAGCACGTGA	TCTGCCATCCGTGCTCAGTCTG	13	9
*HEK3_ED6g-h_pegRNA_4	GGCCCAGACTGAGCACGTGA	TCTGCGATCACGTGCTCAGTCTG	13	10
HEK4_ED6g-h_pegRNA_1	GGCACTGCGGCTGGAGGTGG	TTAACGCCCACCTCCAGCC	9	10
HEK4_ED6g-h_pegRNA_2	GGCACTGCGGCTGGAGGTGG	TTAACCCCCCCCTCCAGCC	9	10
HEK4_ED6g-h_pegRNA_3	GGCACTGCGGCTGGAGGTGG	TTAACCCCTTACACCTCCAGCC	9	13
HEK4_ED6g-h_pegRNA_4	GGCACTGCGGCTGGAGGTGG	TTAACCCCCCCTCCAGCC	9	9
EMX1_ED6g-h_pegRNA_1	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCACTTCTTCTTCTGCTCGGA	14	16
EMX1_ED6g-h_pegRNA_2	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCTGCTTCTTCTGCTCGGA	14	16
EMX1_ED6g-h_pegRNA_3	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCTTCGCATTCTTCTGCTCGGA	14	19
EMX1_ED6g-h_pegRNA_4	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGTTCTTCTTCTGCTCGGA	14	13
FANCF_ED6g-h_pegRNA_1	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATGCAGGTGCTGCAGAAGGGAT	14	17
FANCF_ED6g-h_pegRNA_2	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAGGCGCTGCAGAAGGGAT	14	17
FANCF_ED6g-h_pegRNA_3	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAATCGGTGCTGCAGAAGGGAT	14	20

nicking sgRNA	spacer sequence
HEK3_ED6g-h_+90	GTCAACCAGTATCCCGGTGC
EMX1_ED6a-f_+57	GATGTACAGAGAGCCCAGGGC
FANCF_ED6a-f_+48	GGGGTCCCAGGTGCTGACGT

14

Extended Data Figure 8 sequences:

pegRNA	spacer sequence	3' extension sequence	PBS length (nt)	RT template length (nt)
*HEK3_ED8_5GtoA	GGCCCAGACTGAGCACGTGA	TCTGCTATCACGTGCTCAGTCTG	13	10
PRNP_ED8	GCAGTGGTGGGGGGCCTTGG	ATGTAGACGCCAAGGCCCCCCACC	12	12
HEXA_ED8	GTACCTGAACCGTATATCCTA	AGTCAGGGCCATAGGATAGATATACGGTTC	12	14

Extended Data Figure 9 sequences:

pegRNA	spacer	3' extension (5' to 3')	PBS length (nt)	RT template length (nt)
HBB 3.5	GTAACGGCAGACTTCTCCAC	ACCTGACTCCTGAGGAGAAGTCTGCC	12	14
HBB 3.7	GCATGGTGCACCTGACTCCTG	AGACTTCTCCTCAGGAGTCAGGTGCAC	13	14
HBB 5.2	GCATGGTGCACCTGACTCCTG	TAACGGCAGACTTCTCCTCAGGAGTCAGGTGCAC	13	19
HBB 5.3	GCATGGTGCACCTGACTCCTG	ACGGCAGACTTCTCCTCAGGAGTCAGGTGCAC	13	17
HBB 5.4	GCATGGTGCACCTGACTCCTG	GGCAGACTTCTCCTCAGGAGTCAGGTGCAC	13	16
HBB 5.5	GCATGGTGCACCTGACTCCTG	GCAGACTTCTCCTCAGGAGTCAGGTGCAC	13	13
HBB 5.6	GCATGGTGCACCTGACTCCTG	GACTTCTCCTCAGGAGTCAGGTGCAC	13	12
HBB 5.7	GCATGGTGCACCTGACTCCTG	ACTTCTCCTCAGGAGTCAGGTGCAC	13	21
HBB 5.8	GCATGGTGCACCTGACTCCTG	TAACGGCAGACTTCTCCTCAGGAGTCAGGTGCA	12	19
HBB 5.9	GCATGGTGCACCTGACTCCTG	ACGGCAGACTTCTCCTCAGGAGTCAGGTGCA	12	17
HBB 5.10	GCATGGTGCACCTGACTCCTG	GGCAGACTTCTCCTCAGGAGTCAGGTGCA	12	16
HBB 5.11	GCATGGTGCACCTGACTCCTG	GCAGACTTCTCCTCAGGAGTCAGGTGCA	12	13
HBB 5.12	GCATGGTGCACCTGACTCCTG	GACTTCTCCTCAGGAGTCAGGTGCA	12	12
HBB 5.13	GCATGGTGCACCTGACTCCTG	ACTTCTCCTCAGGAGTCAGGTGCA	12	14
HEXAs 1	GATCCTTCCAGTCAGGGCCAT	ATATCTTATGGCCCTGACTGGAA	13	14
HEXAs 2	GATCCTTCCAGTCAGGGCCAT	TATATCTTATGGCCCTGACTGGAA	13	15
HEXAs 3	GATCCTTCCAGTCAGGGCCAT	GTATATCTTATGGCCCTGACTGGAA	13	16
HEXAs 4	GATCCTTCCAGTCAGGGCCAT	ACCGTATATCTTATGGCCCTGACTGGAA	13	19
HEXAs 5	GATCCTTCCAGTCAGGGCCAT	AACCGTATATCTTATGGCCCTGACTGGAA	13	20
HEXAs 6	GATCCTTCCAGTCAGGGCCAT	GAACCGTATATCTTATGGCCCTGACTGGAA	13	21
HEXAs 7	GATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTGGAA	13	22
HEXAs 8	GATCCTTCCAGTCAGGGCCAT	ATATCTTATGGCCCTGACT	9	14
HEXAs 9	GATCCTTCCAGTCAGGGCCAT	TATATCTTATGGCCCTGACT	9	15
HEXAs 10	GATCCTTCCAGTCAGGGCCAT	GTATATCTTATGGCCCTGACT	9	16
HEXAs 11	GATCCTTCCAGTCAGGGCCAT	ACCGTATATCTTATGGCCCTGACT	9	19
HEXAs 12	GATCCTTCCAGTCAGGGCCAT	AACCGTATATCTTATGGCCCTGACT	9	20
HEXAs 13	GATCCTTCCAGTCAGGGCCAT	GAACCGTATATCTTATGGCCCTGACT	9	21
HEXAs 14	GATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACT	9	22
HEXAs 15	GATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGAC	8	22
HEXAs 16	GATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTG	10	22
HEXAs 17	GATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTGG	11	22
HEXAs 18	GATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTGGA	12	22
HEXAs 19	GATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTGGAA	13	22
HEXAs 20	GATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTGGAAG	14	22
HEXAs 21	GATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTGGAAGG	15	22
HEXAs 22	GATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCTTATGGCCCTGACT	9	25
HEXAs 23	GATCCTTCCAGTCAGGGCCAT	TACCTGAACCGTATATCTTATGGCCCTGACT	9	26
HEXAs 24	GATCCTTCCAGTCAGGGCCAT	GTACCTGAACCGTATATCTTATGGCCCTGACT	9	27
HEXAs 25	GATCCTTCCAGTCAGGGCCAT	GGTACCTGAACCGTATATCTTATGGCCCTGACT	9	28
HEXAs 26	GATCCTTCCAGTCAGGGCCAT	TGGTACCTGAACCGTATATCTTATGGCCCTGACT	9	29
HEXA 5	GATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCCTATGGCCCTGACTGGAA	13	21
HEXA 6	GATCCTTCCAGTCAGGGCCAT	ACCGTATATCCTATGGCCCTGACTGGAA	13	15
HEXA 7	GATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCCTATGGCCCTGACTGGAAGG	15	21
HEXA 8	GATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCCTATGGCCCTGACTGGAAG	14	21
HEXA 9	GATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCCTATGGCCCTGACTGGA	12	21
HEXA 10	GATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCCTATGGCCCTGACTGG	11	21
HEXA 11	GATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCCTATGGCCCTGACTG	10	21
HEXA 12	GATCCTTCCAGTCAGGGCCAT	AACCGTATATCCTATGGCCCTGACTGGAA	13	16
HEXA 13	GATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCCTATGGCCCTGACTGGAA	13	18
HEXA 14	GATCCTTCCAGTCAGGGCCAT	TACCTGAACCGTATATCCTATGGCCCTGACTGGAA	13	22
HEXA 15	GATCCTTCCAGTCAGGGCCAT	TGGTACCTGAACCGTATATCCTATGGCCCTGACTGGAA	13	25
HEXA 16	GATCCTTCCAGTCAGGGCCAT	GTACCTGAACCGTATATCCTATGGCCCTGACTGGAA	13	23
HEXA 17	GATCCTTCCAGTCAGGGCCAT	AACCGTATATCCTATGGCCCTGACTG	10	16
HEXA 18	GATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCCTATGGCCCTGACTG	10	18
HEXA 19	GATCCTTCCAGTCAGGGCCAT	TACCTGAACCGTATATCCTATGGCCCTGACTG	10	22
HEXA 20	GATCCTTCCAGTCAGGGCCAT	TGGTACCTGAACCGTATATCCTATGGCCCTGACTG	10	25
HEXA 21	GATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTGGAAGG	15	18

nicking sgRNA	spacer sequence
HBB_ED9a_+72	GCCTTGATACCAACCTGCCCA
HBB_ED9a+95 (for HBB 3.5)	GGGCTGGGCATAAAAGTCA
HEXA_ED9b_+60	GCTGGAACTGGTCACCAAGGC
HEXA_ED9b_correct_wt_PE3b	GTACCTGAACCGTATATCCTA
HEXA_ED9b_correct_silent_PE3b	GTACCTGAACCGTATATCTTA

15

Extended Data Figure 10 sequences:

pegRNA	spacer sequence	3' extension	PBS length (nt)	RT template length (nt)
HEK3_ED10_2GtoC	GGCCCAGACTGAGCACGTGA	TCTGCCATGACGTGCTCAGTCTG	13	10
EMX1_ED10_1GtoC	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCTTGTTCTTCTGCTCGG	13	16
FANCF_ED10_5GtoT	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	17
HEK3_ED10_1His6ins	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCAATGATGGT GATGATGGTGCGTGCTCAGTCTG	13	52
*HEK3_ ED10_5GtoT	GGCCCAGACTGAGCACGTGA	TCTGCAATCACGTGCTCAGTCTG	13	10
*HEK3_ED10_1CTTins	GGCCCAGACTGAGCACGTGA	TCTGCCATCAAAGCGTGCTCAGTCTG	13	10
*HEK3_ED10_1TtoG	GGCCCAGACTGAGCACGTGA	TCTGCCATCCCGTGCTCAGTCTG	13	10
*HEK3_ED10_3AtoC	GGCCCAGACTGAGCACGTGA	TCTGCCAGCACGTGCTCAGTCTG	13	10
*HEK3_ED10_3AtoT	GGCCCAGACTGAGCACGTGA	TCTGCCAACACGTGCTCAGTCTG	13	10
HEK3_ED10_3AtoT_5-6GGtoTT	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGAAAACACGTGCTCA GTCTG	13	34
RNF2_ED10_1CtoA	GTCATCTTAGTCATTACCTG	AACGAACACCTCATGTAATGACTAAGATG	15	14
RNF2_ED10_1CtoG	GTCATCTTAGTCATTACCTG	AACGAACACCTCACGTAATGACTAAGATG	15	14
RNF2_ED10_1GTAins	GTCATCTTAGTCATTACCTG	AACGAACACCTCAGTACGTAATGACTAAGATG	15	17
HBB_ED10_4AtoT	GCATGGTGCACCTGACTCCTG	AGACTTCTCCACAGGAGTCAGGTGCAC	13	14
PRNP_ED10_6GtoT	GCAGTGGTGGGGGGCCTTGG	ATGTAGACGCCAAGGCCCCCCACC	12	12

Supplementary Table 4. Sequences of primers used for mammalian cell genomic DNA amplification and HTS[30].

Description	Sequence
HEK3 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNATGTGGGCTGCCTAGAAAGG
HEK3 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCCAGCCAAACTTGTCAACC
RNF2 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACGTCTCATATGCCCCCTTGG
RNF2 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTACGTAGGAATTTTGGTGGGACA
HEK4 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGAACCCAGGTAGCCAGAGAC
HEK4 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTTTCAACCCGAACGGAG
EMX1 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGCTCAGCCTGAGTGTTGA
EMX1 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTCGTGGGTTTGTGGTTGC
FANCF fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCATTGCAGAGAGGCGTATCA
FANCF rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGTCCCAGGTGCTGAC
HBB fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGGGTTGGCCAATCTACTCCC
HBB rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTTCTCTGTCTCCACATGCC
PRNP fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTCAGTGGAACAAGCCGAGT
PRNP rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGGTTGGGGTAACGGTG
HEXA fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCATACAGGTGTGGCGAGAGG
HEXA rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCCTCCTTTGGTTAGCA
RUNX1 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCACAAACAAGACAGGGAACTG
RUNX1 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTAGATGTAGGGCTAGAGGGGTG
VEGFA fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACTTGGTGCCAAATTCTTCTCC
VEGFA rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTAAAGAGGGAATGGGCTTTGGA
DNMT fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCACAACAGCTTCATGTCAGCC
DNMT rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTACGTTAATGTTTCCTGATGGTCC
HEK3 off-target site 1 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCCCCTGTTGACCTGGAGAA
HEK3 off-target site 1 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCACTGTACTTGCCCTGACCA
HEK3 off-target site 2 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTGGTGTTGACAGGGAGCAA
HEK3 off-target site 2 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGATGTGGGCAGAAGGG
HEK3 off-target site 3 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGAGAGGGAACAGAAGGGCT
HEK3 off-target site 3 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCAAAGGCCCAAGAACCT
HEK3 off-target site 4 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCCTAGCACTTTGGAAGGTCG
HEK3 off-target site 4 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCTCATCTTAATCTGCTCAGCC
HEK4 off-target site 1 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGCATGGCTTCTGAGACTCA
HEK4 off-target site 1 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTCCCTTGCACTCCCTGTCTTT
HEK4 off-target site 2 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTTGGCAATGGAGGCATTGG
HEK4 off-target site 2 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGAGGCTGCCCATGAGAG
HEK4 off-target site 3 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGTCTGAGGCTCGAATCCTG
HEK4 off-target site 3 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGGCCTCCATATCCCTG
HEK4 off-target site 4 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTTCCACCAGAACTCAGCCC
HEK4 off-target site 4 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCGGTTCCTCCACAACAC
EMX1 off-target site 1 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTGGGGAGATTTGCATCTGTGGAGG
EMX1 off-target site 1 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTTTATACCATCTTGGGGTTACAG
EMX1 off-target site 2 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAATGTGCTTCAACCCATCACGGC
EMX1 off-target site 2 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCATGAATTTGTGATGGATGCAGTCTG
EMX1 off-target site 3 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGAGAAGGAGGTGCAGGAGCTAGAC
EMX1 off-target site 3 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCATCCCGACCTTCATCCCTCCTGG
EMX1 off-target site 4 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTAGTTCTGACATTCCTCCTGAGGG
EMX1 off-target site 4 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCAAACAAGGTGCAGATACAGCA
FANCF off-target site 1 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCGGGCAGTGGCGTCTTAGTCG
FANCF off-target site 1 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTGGGTTTGGTTGGCTGCTC
FANCF off-target site 2 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTCCTTGCCGCCCAGCCGGTC
FANCF off-target site 2 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCACTGGGGAAGAGGCGAGGACAC
FANCF off-target site 3 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCAGTGTTTCCCATCCCCAACAC
FANCF off-target site 3 rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGAATGGATCCCCCCCTAGAGCTC
FANCF off-target site 4 fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGGCCCACAGGTCCTTCTGGA

Supplementary Table 5. Sequences of 100-mer single-stranded DNA oligonucleotide donor templates used in HDR experiments and in the creation of the *HBB* E6V HEK293T cell line. Oligonucleotides are 100-103 nt in length with homology arms centered around the site of the edit. Oligonucleotides were from Integrated DNA Technologies, purified by PAGE.

HEK3 +3 A to T:
GCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGTGTTGGCAGAGGAAAGGAAGCC
CTGCTTCCTCCAGAGGGCGTCGCAGGAC

HEK3 +3 A to T, +5,6 GG to TT:
GCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGTGTTTTCAGAGGAAAGGAAGCC
CTGCTTCCTCCAGAGGGCGTCGCAGGAC

HEK3 +1 T to G:
GCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGGGATGGCAGAGGAAAGGAAGCC
CTGCTTCCTCCAGAGGGCGTCGCAGGAC

HEK3 +3 A to C:
GCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGTGCTGGCAGAGGAAAGGAAGCC
CTGCTTCCTCCAGAGGGCGTCGCAGGAC

HEK3 +1 CTT insertion:
GCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGCTTTGATGGCAGAGGAAAGGAA
GCCCTGCTTCCTCCAGAGGGCGTCGCAGGAC

RNF2 +1 C to A:
CCCAGTTTACACGTCTCATATGCCCCTTGGCAGTCATCTTAGTCATTACATGAGGTGTTCGTTGTAACTCATATA
AACTGAGTTCCCATGTTTTGCTTAA

RNF2 +1 C to G:
CCCAGTTTACACGTCTCATATGCCCCTTGGCAGTCATCTTAGTCATTACGTGAGGTGTTCGTTGTAACTCATATA
AACTGAGTTCCCATGTTTTGCTTAA

RNF2 +1 GTA insertion:
CAGTTTACACGTCTCATATGCCCCTTGGCAGTCATCTTAGTCATTACGTACTGAGGTGTTCGTTGTAACTCATAT
AAACTGAGTTCCCATGTTTTGCTTA

HBB E6V installation (also used for creation of the *HBB* E6V HEK293T cell line):
ACTTCATCCACGTTCACCTTGCCCCACAGGGCAGTAACGGCAGACTTCTCCACAGGAGTCAGATGCACCATGG
TGTCTGTTTGAGGTTGCTAGTGAACAC

HBB E6V correction protospacer A:
ACTTCATCCACGTTCACCTTGCCCCACAGGGCAGTAACGGCAGACTTCTCCTCAGGAGTCAGGTGCACCATGG
TGTCTGTTTGAGGTTGCTAGTGAACAC

HBB E6V correction protospacer B:
GTGTTCACTAGCAACCTCAAACAGACACCATGGTGCACCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCT
GTGGGGCAAGGTGAACGTGGATGAAGT

HBB E6V correction protospacer B, silent PAM mutation:
GTGTTCACTAGCAACCTCAAACAGACACCATGGTGCACCTGACTCCTGATGAGAAGTCTGCCGTTACTGCCCT
GTGGGGCAAGGTGAACGTGGATGAAGT

PRNP G127V:
CACATGGCTGGTGCTGCAGCAGCTGGGGCAGTGGTGGGGGGCCTTGGCG**T**CTACATGCTGGGAAGTGCCAT
GAGCAGGCCCATCATACATTTCGGCAGTG

Supplementary Sequences 1. Sequences of yeast dual fluorescent reporter plasmids used in this study

p425-GFP_stop_mCherry:

ATGTCTAAAGGTGAAGAATTATTCACTGGTGTTGTCCCAATTTTGGTTGAATTAGATGGTGATGTTAATGGTCACAAATTTTCTGTCTCCGGTGAAGGTGAAGGTGATGCTA
CTTACGGTAAATTGACCTTAAAATTTATTTGTACTACTGGTAAATTGCCAGTTCCATGGCCAACCTTAGTCACTACTTTCGGTTATGGTGTTCAATGTTTTGCTAGATACCG
AGATCATATGAAACAACATGACTTTTTCAAGTCTGCCATGCCAGAAGGTTATGTTCAAGAAGAACTATTTTTTTCAAAGATGACGGTAACTACAAGACCAGAGCTGAAGTC
AAGTTTGAAGGTGATACCTTAGTTAATAGAATCGAATTAAAAGGTATTGATTTTAAAGAAGATGGTAACATTTTAGGTCACAAATTGGAATACAACTATAACTCTCACAATG
TTTACATCATGGCTGACAAACAAAAGAATGGTATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGTTCTGTTCAATTAGCTGACCATTATCAACAAAATACTCC
AATTGGTGATGGTCCAGTCTTGTTACCAGACAACCATTACTTATCCACTCAATCTGCCTTATCCAAAGATCCAAACGAAAAGAGAGACCACATGGTCTTGTTAGAATTTGTT
ACTGCTGCTGGTATTACCCATGGTATGGATGAATTGTACAAAGCTAGCAACCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGTGA**TGG**CAGAGCACAGGAGACGTCATGG
TTTCAAAAGGTGAAGAAGATAATATGGCTATTATTAAAGAATTTATGAGATTTAAAGTTCATATGGAAGGTTCAGTTAATGGTCATGATTTGAAATTGAAGGTGAAGGTA
AGGTAGACCATATGAAGGTACTCAAACTGCTAAATTGAAAGTTACTAAAGGTGGTCCATTACCATTTGCTTGGGATATTTTGTCACCACAATTTATGTATGGTTCAAAAGCT
TATGTTAAACATCCAGCTGATATTCCAGATTATTTAAAATTGTCATTTCCAGAAGGTTTTAAATGGGAAAGAGTTATGAATTTTGAAGATGGTGGTGTTGTTACTGTTACTC
AAGATTCATCATTACAAGATGGTGAATTTATTTATAAAGTTAAATTGAGAGGTACTAATTTTCCATCAGATGGTCCAGTTATGCAAAAAAAAACTATGGGTTGGGAAGCTTC
ATCAGAAGAATGTATCCAGAAGATGGTGCTTTAAAAGGTGAAATTAAACAAAGATTGAAATTAAAAGATGGTGGTCATTATGATGCTGAAGTTAAAACTACTTATAAAGCT
AAAAAACCAGTTCAATTACCAGGTGCTTATAATGTTAATATTAAATTGGATATTACTTCACATAATGAAGATTATACTATTGTTGAACAATATGAAAGAGCTGAAGGTAGAC
ATTCAACTGGTGGTATGGATGAATTATATAAAGGTACCGCTCGAGCAGCTGTGATTGATGAGTCGACTTGGTTGAACACGTTGCCAAGGCTTAAGTGAATTTACTTTAAAT
CTTGCATTTAAATAAATTTTCTTTTTATAGCTTTATGACTTAGTTTCAATTTATATACTATTTTAATGACATTTTCGATTCGGATCCACTAGTTCTAGAGCGGCCGCCACCG
CGGTGGAGCTCCAGCTTTTGTTCCCTTTAGTGAGGGTTAATTGCGCGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACA
ACATAGGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGGTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTG
CCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGA
GCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGG
CCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTT
TCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGT
AGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACC
CGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTA
CACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTT
TTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTT
TGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATG
CTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCC
AGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCT
CCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTT
TGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGT
AAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCAT
TCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTC
GGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGA
GCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATT
GTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGAACGAAGCATCTGTGCTTCATTTTG
TAGAACAAAAATGCAACGCGAGAGCGCTAATTTTTCAAACAAAGAATCTGAGCTGCATTTTTACAGAACAGAAATGCAACGCGAAAGCGCTATTTTACCAACGAAGAATCTG
TGCTTCATTTTTGTAAAACAAAAATGCAACGCGAGAGCGCTAATTTTTCAAACAAAGAATCTGAGCTGCATTTTTACAGAACAGAAATGCAACGCGAGAGCGCTATTTTACC
AACAAAGAATCTATACTTCTTTTTTGTTCTACAAAAATGCATCCCGAGAGCGCTATTTTTCTAACAAAGCATCTTAGATTACTTTTTTTTCTCCTTTGTGCGCTCTATAATGC
AGTCTCTTGATAACTTTTTGCACTGTAGGTCCGTTAAGGTTAGAAGAAGGCTACTTTGGTGTCTATTTTCTCTTCCATAAAAAAAGCCTGACTCCACTTCCCGCGTTTACTG
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TTCTTCATTGGTCAGAAAATTATGAACGGTTTCTTCTATTTTGTCTCTATATACTACGTATAGGAAATGTTTACATTTTCGTATTGTTTTCGATTCACTCTATGAATAGTTC
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GGATATAGCACAGACTATATAGCAAAGAGATACTTTTGAGCAATGTTTGTGGAAGCGGTATTCGCAATATTTTAGTAGCTCGTTACAGTCCGGTGCGTTTTTGGTTTTTTG
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CTACCCTTTAGCTGTTCTATATGCTGCCACTCCTCAATTGGATTAGTCTCATCCTTCAATGCTATCATTTCCTTTGATATTGGATCATACTAAGAAACCATTATTATCATGA
CATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCAC
CATATCGACTACGTCGTAAGGCCGTTTCTGACAGAGTAAAATTCTTGAGGGAACTTTCACCATTATGGGAAATGCTTCAAGAAGGTATTGACTTAAACTCCATCAAATGGTC
AGGTCATTGAGTGTTTTTTATTTGTTGTATTTTTTTTTTTTAGAGAAAATCCTCCAATATCAAATTAGGAATCGTAGTTTCATGATTTTCTGTTACACCTAACTTTTTGTG
TGGTGCCCTCCTCCTTGTCAATATTAATGTTAAAGTGCAATTCTTTTTCCTTATCACGTTGAGCCATTAGTATCAATTTGCTTACCTGTATTCCTTTACTATCCTCCTTTTT
CTCCTTCTTGATAAATGTATGTAGATTGCGTATATAGTTTCGTCTACCCTATGAACATATTCCATTTTGTAATTTCGTGTCGTTTCTATTATGAATTTCATTTATAAAGTTT
ATGTACAAATATCATAAAAAAAGAGAATCTTTTTAAGCAAGGATTTTCTTAACTTCTTCGGCGACAGCATCACCGACTTCGGTGGTACTGTTGGAACCACCTAAATCACCAG
TTCTGATACCTGCATCCAAAAACCTTTTTAACTGCATCTTCAATGGCCTTACCTTCTTCAGGCAAGTTCAATGACAATTTCAACATCATTGCAGCAGACAAGATAGTGGCGAT
AGGGTCAACCTTATTCTTTGGCAAATCTGGAGCAGAACCGTGGCATGGTTCGTACAAACCAAATGCGGTGTTCTTGTCTGGCAAAGAGGCCAAGGACGCAGATGGCAACAAA
CCCAAGGAACCTGGGATAACGGAGGCTTCATCGGAGATGATATCACCAAACATGTTGCTGGTGATTATAATACCATTTAGGTGGGTTGGGTTCTTAACTAGGATCATGGCGG
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p425-GFP_+1fs_mCherry:

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p425-GFP_-1fs_mCherry:

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GFP open reading frame
Linker containing stop codon +1 frameshift, or –1 frameshift
mCherry open reading frame
Plasmid backbone (containing the GPD promoter, Leu2 marker, and AmpR)
Protospacer (underlined)
PAM (boldfaced)

Supplementary Sequences 2. DNA sequences of mammalian prime editor plasmids and example pegRNA plasmid

pCMV-PE1:

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N-terminal NLS + Cas9 H840A
Flexible linker
M-MLV reverse transcriptase + C-terminal NLS
Plasmid backbone (containing CMV promoter and AmpR)

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U6 Promoter sequence
Spacer sequence
sgRNA scaffold
3' extension (contains PBS and RT template)
Backbone (contains AmpR)

pLenti-hSyn-N-PE2-NpuN-P2A-GFP-KASH_U6-DNMT1-pegRNA:

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hSyn promoter
C-term Npu
C-term wt Cas9

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CAAACTCATCAATGTATCTTATCATGTCTGGCTCTAGCTATCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGAC
TAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGGACGTACCCAATTCGCCCTATAGT
GAGTCGTATTACGCGCGCTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGC
GTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTAC
GCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGG
CTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTT
TGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGC
CTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTA
TTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCG
TGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTAC
ATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTA
TTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGA
ATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCAT
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GCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGA
TAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATG
GATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTT
AATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATC
TTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAG
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TCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTG
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AGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGT
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CCTTACAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTGGAAGTAAGGTGGTACGATCGTGCCTTATTAGGAAGGCAACAGACGGGTCTGACATGGATTGGACGAACCAC
TGAATTGCCGCATTGCAGAGATATTGTATTTAAGTGCCTAGCTCGATACATAAACGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAAC
CCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGA
AAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGCGAAAGGGAAACCAGAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGG
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TTCGGTTAAGGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGG
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ATAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGACCACCGCACAGCAAGCGGCCGCTGATCTTCAGACCTGGAGGAGGAGATATGAGG
GACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGG
GAATAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCA
GCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAG
GATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAACTCATTTGCACCACTGCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTGGAACAGATTTGGAATCACA
CGACCTGGATGGAGTGGGACAGAGAAATTAACAATTACACAAGCTTAATACACTCCTTAATTGAAGAATCGCAAAACCAGCAAGAAAAGAATGAACAAGAATTATTGGAATT
AGATAAATGGGCAAGTTTGTGGAATTGGTTTAACA

U6 promoter
sgRNA

Supplementary Sequences 3. Amino acid sequences of Maloney murine leukemia virus reverse transcriptase (M-MLV RT) variants used in this study.
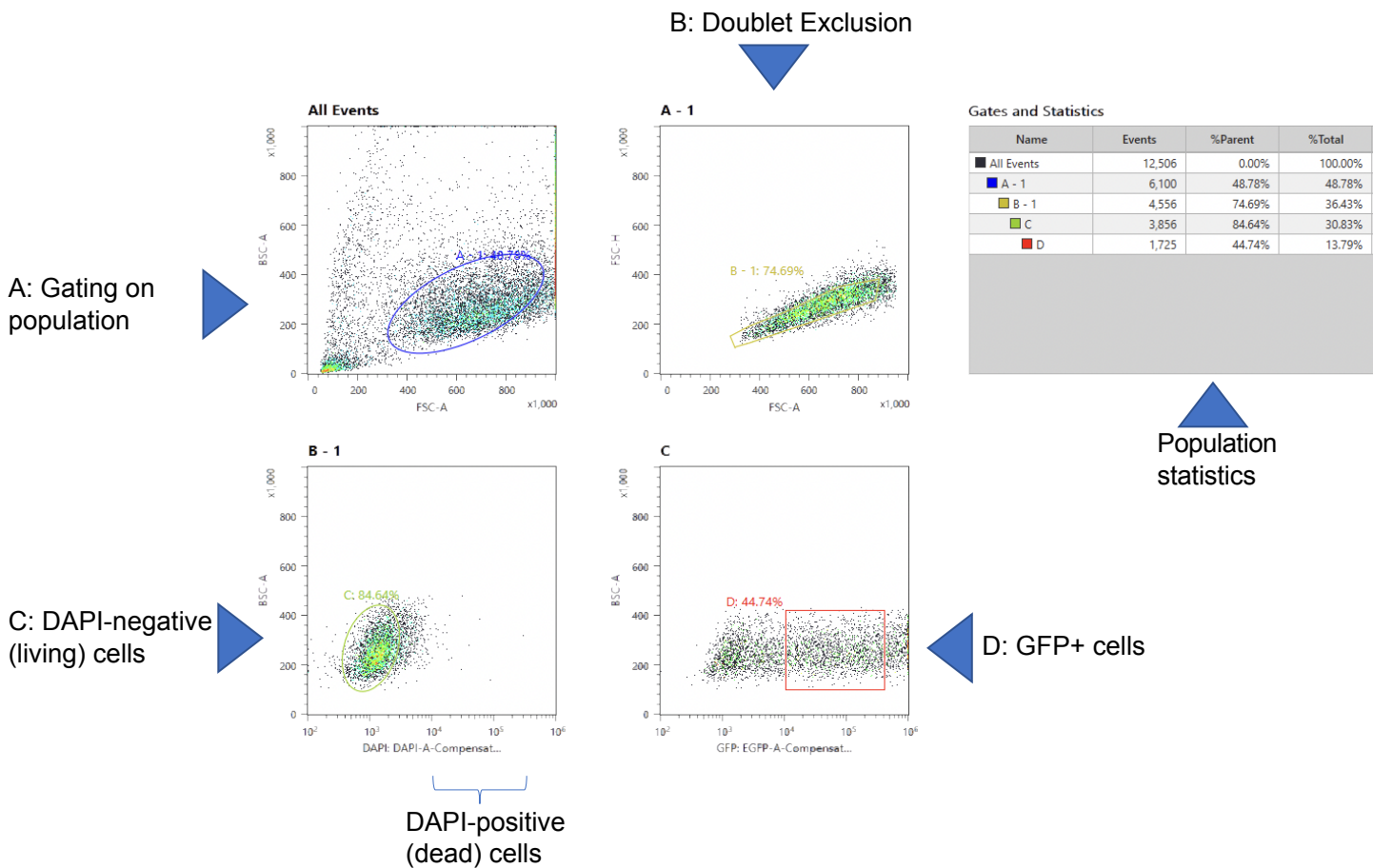
PE1 M-MLV RT:

```
TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGI
KPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTV
LDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADFRIQHPDLILLQY
VDDLLLAATSELDCQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKT
PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQALLTAPALGLPDLTKPFELFV
DEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALV
KQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDAD
HTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAH
IHGEIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPD
TSTLLIENSSP
```

M3 M-MLV RT (D200N, T330P, L603W) (see Baranauskas *et al.*[23]):

```
TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGI
KPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTV
LDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFNEALHRDLADFRIQHPDLILLQY
VDDLLLAATSELDCQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKT
PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKPGTLFNWGPDQQKAYQEIKQALLTAPALGLPDLTKPFELFV
DEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALV
KQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDAD
HTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAH
IHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPD
TSTLLIENSSP
```

PE2 M-MLV RT (D200N, T306K, W313F, T330P, L603W):

```
TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGI
KPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTV
LDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFNEALHRDLADFRIQHPDLILLQY
VDDLLLAATSELDCQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKT
PRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWGPDQQKAYQEIKQALLTAPALGLPDLTKPFELFV
DEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALV
KQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDAD
HTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAH
IHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPD
TSTLLIENSSP
```

M3-deadRT M-MLV RT:

```
TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGI
KPHIQRLLDQGILVPCQSPWNTPLLPVKLPGTNDYSPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTV
LDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFNEALHRDLADFRIQHPDLILLQY
VDDLLLAATSELDCQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKT
PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKPGTLFNWGPDQQKAYQEIKQALLTAPALGLPDLTKPFELFV
DEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALV
KQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDAD
HTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAH
IHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPD
TSTLLIENSSP
```

Supplementary Note 1. FACS gating examples for GFP-positive cell sorting. Below are examples of original batch analysis files outlining the sorting strategy used for generating *HEXA* 1278+TATC and *HBB* E6V HEK293T cell lines. The image data was generated on a Sony LE-MA900 cytometer using Cell Sorter Software v. 3.0.5. Graphic 1 shows gating plots for cells that do not express GFP. Graphic 2 shows an example sort of P2A–GFP-expressing cells used for isolating the *HBB* E6V HEK293T cell lines. HEK293T cells were initially gated on population using FSC-A/BSC-A (Gate A), then sorted for singlets using FSC-A/FSC-H (Gate B). Live cells were sorted for by gating DAPI-negative cells (Gate C). Cells with GFP fluorescence levels that were above those of the negative-control cells were sorted for using EGFP as the fluorochrome (Gate D). Graphic 3 shows allele tables for HEK293T cells homozygous for the *HEXA* 1278+TATC mutation isolated using FACS after treatment with PE3. Graphic 4 shows allele tables for HEK293T cells homozygous for the *HBB* E6V mutation isolated using FACS after treatment with PE3. Allele tables were generated using CRISPResso2[5].

1. HEK293T cells (GFP-negative):

Name	Events	%Parent	%Total
All Events	2,172	0.00%	100.00%
A	671	30.89%	30.89%
B	628	93.59%	28.91%
C	3	0.48%	0.14%

2. Representative plot of FACS gating for cells expressing PE2–P2A–GFP:

B: Doublet Exclusion

A: Gating on population

Population statistics

C: DAPI-negative (living) cells

D: GFP+ cells

DAPI-positive (dead) cells

3. Genotypes for *HEXA* 1278+TATC homozygote HEK293T cells

Line 1

Reference
92.16% (28182 reads)
1.54% (471 reads)
1.10% (335 reads)
0.94% (288 reads)
0.61% (188 reads)
0.51% (156 reads)

Line 2

Reference
91.74% (18375 reads)
1.34% (269 reads)
1.08% (217 reads)
0.85% (171 reads)
0.59% (119 reads)
0.57% (115 reads)
0.53% (106 reads)
0.23% (47 reads)

bold — Substitutions
☐ — Insertions
- — Deletions
------ — Predicted cleavage position
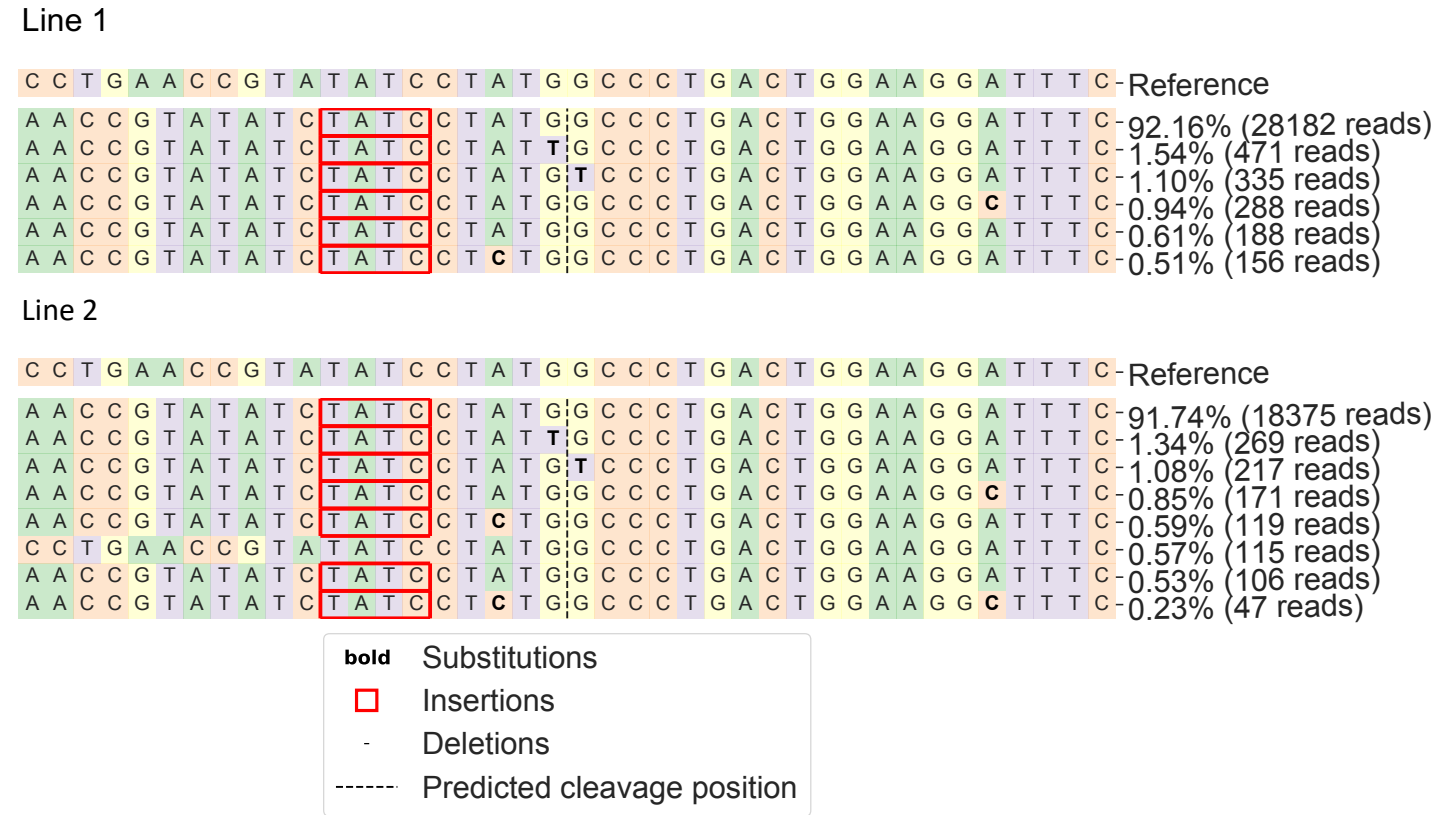
4. Allele tables for *HBB* E6V homozygote HEK293T cell lines

Line 1

C A C C A T G G T G C A C C T G A C T C C T G A G G A G A A G T C T G C C G T T	Reference
C A C C A T G G T G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G C C G T T	97.45% (87424 reads)
C A C C A T G G T G C A C C T G A C T C**i**C T G A G G A G A A G T C T G C C G T T	0.27% (240 reads)
C A C C A T G G **C** G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G C C G T T	0.24% (212 reads)

Line 2

C A C C A T G G T G C A C C T G A C T C C T G A G G A G A A G T C T G C C G T T	Reference
C A C C A T G G T G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G C C G T T	97.16% (63793 reads)
C A C C A T G G T G C A C C T G A C T C**i**C T G A G G A G A A G T C T G C C G T T	0.36% (239 reads)
C A C C A T G G **C** G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G C C G T T	0.22% (142 reads)

Line 3

C A C C A T G G T G C A C C T G A C T C C T G A G G A G A A G T C T G C C G T T	Reference
C A C C A T G G T G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G C C G T T	97.32% (77514 reads)
C A C C A T G G T G C A C C T G A C T C**i**C T G A G G A G A A G T C T G C C G T T	0.29% (228 reads)
C A C C A T G G **C** G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G C C G T T	0.22% (172 reads)
C A C C A T G G T G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G **T** C G T T	0.21% (167 reads)

Line 4

C A C C A T G G T G C A C C T G A C T C C T G A G G A G A A G T C T G C C G T T	Reference
C A C C A T G G T G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G C C G T T	96.94% (70061 reads)
C A C C A T G G T G C A C C T G A C T C**i**C T G A G G A G A A G T C T G C C G T T	0.58% (419 reads)
C A C C A T G G **C** G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G C C G T T	0.23% (165 reads)
C A C C A T G G T G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G **T** C G T T	0.21% (150 reads)

Line 5

C A C C A T G G T G C A C C T G A C T C C T G A G G A G A A G T C T G C C G T T	Reference
C A C C A T G G T G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G C C G T T	97.16% (54265 reads)
C A C C A T G G T G C A C C T G A C T C**i**C T G A G G A G A A G T C T G C C G T T	0.33% (183 reads)
C A C C A T G G **C** G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G C C G T T	0.21% (120 reads)
C A C C A T G G T G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G **T** C G T T	0.20% (113 reads)

Line 6

C A C C A T G G T G C A C C T G A C T C C T G A G G A G A A G T C T G C C G T T	Reference
C A C C A T G G T G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G C C G T T	97.35% (95642 reads)
C A C C A T G G T G C A C C T G A C T C**i**C T G A G G A G A A G T C T G C C G T T	0.27% (267 reads)
C A C C A T G G **C** G C A C C T G A C T C**i**C T G **T** G G A G A A G T C T G C C G T T	0.21% (211 reads)

bold   Substitutions
☐   Insertions
-    Deletions
------   Predicted cleavage position

Supplementary Note 2. Representative FACS gating for neuronal nuclei sorting. Nuclei were sequentially gated on the basis of DyeCycle Ruby signal, FSC/SSC ratio, SSC-Width/SSC-height ratio, and GFP/DyeCycle ratio, as shown below.
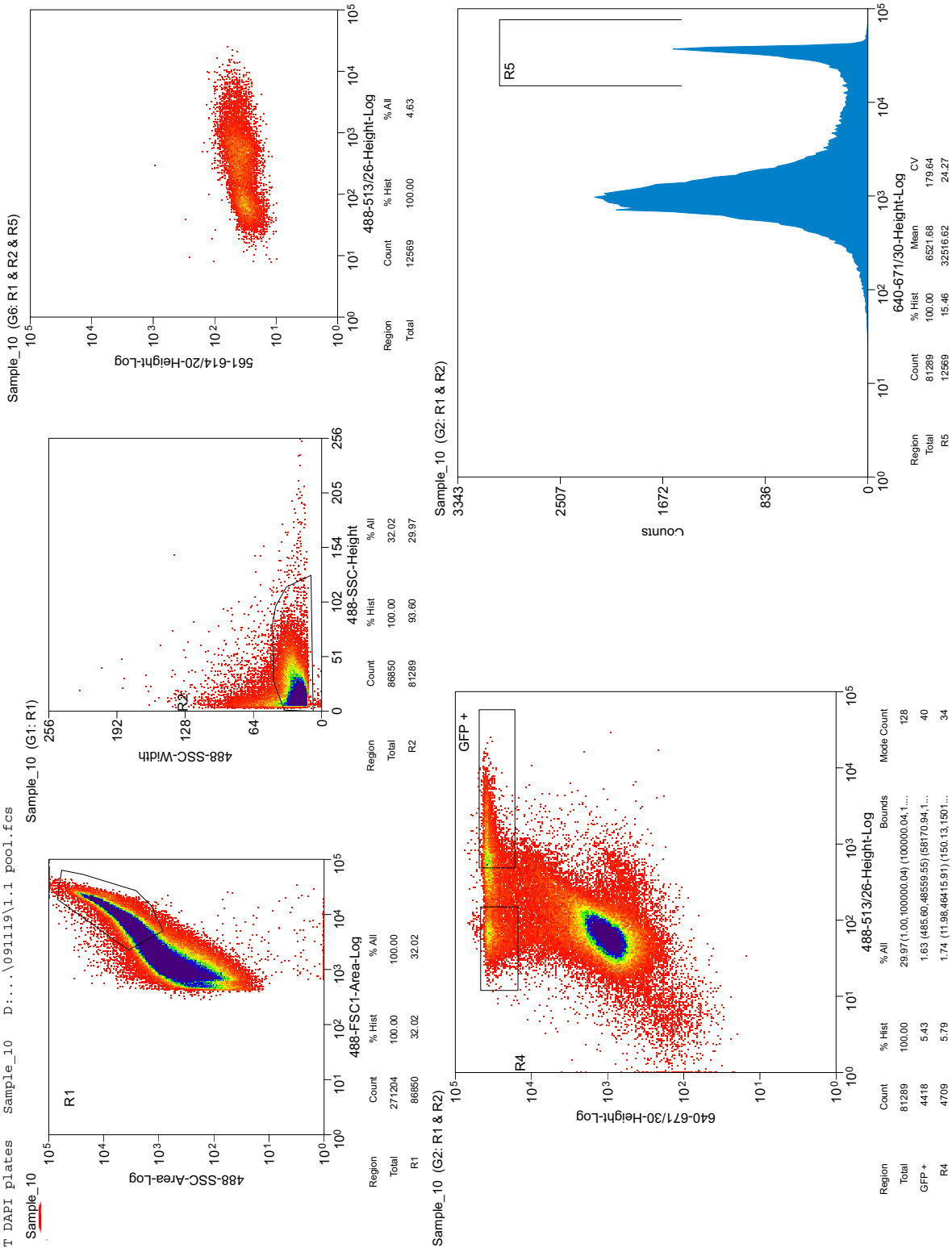
Supplementary Note 3. Protocol for cloning 3'-extended pegRNAs into mammalian U6 expression vectors by Golden Gate assembly.

<u>Cloning overview</u>
1. **Digest pU6-pegRNA-GG-Vector plasmid (component 1)** with *Bsa*I and isolate the plasmid fragment (~2.2kb) containing the origin of replication, U6 promoter, U6 poly-T termination sequence, and Amp^R gene

2. **Order oligonucleotides for:**
   a. The desired spacer (target) sequence flanked by indicated overhangs (**component 2**)
      i. Use the desired target's 5'-3' sequence for the top strand oligonucleotide (inclduing the 5' CACC and 3' GTTTT overhangs) and use the reverse complement of the target sequence for the bottom strand oligonucleotide (including the 5' CTCTAAAAC overhang). Spacer sequences must begin with a G nucleotide for efficient transcription initiation.
   b. The desired pegRNA 3' extension template flanked by the indicated overhangs (**component 3**)
      i. Use the RNA sense sequence as the top strand oligonucleotide (featuring the 5' GTGC overhang) and use the reverse complement of this sequence for the bottom strand oligonucleotide (featuring the 5' AAAA overhang).
   c. SpCas9 sgRNA scaffold sequence featuring compatible golden gate overhangs (**component 4**)
      i. These oligonucleotides are not the complete scaffold sequence, as overhangs from the remaining components contribute several missing nucleotides
      ii. Note: these oligonucleotides <u>must be 5' phosphorylated.</u> Oligonucleotides can be 5' phosphorylated by the manufacturer or 5' phosphorylated enzymatically using T4 PNK (see protocol below)
3. **Anneal top and bottom oligonucleotides** for components 2, 3, and 4 in separate annealing reactions according to the protocol below. If the SpCas9 sgRNA scaffold sequence (component 4) was not phosphorylated, phosphorylate with T4 PNK.
4. **Golden Gate assembly of isolated 2.2-kb fragment from component 1 with components 2, 3, and 4**
5. **Transform the ligation product into *E. coli.*** The antibiotic resistance conferred by component 1 from the pU6-pegRNA-GG-vector plasmid is ampicillin and carbenicillin resistance.
6. **Isolate and sequence** plasmids from the resulting clonal transformants

<u>pegRNA cloning protocol</u>
Step 1: Digest pU6-pegRNA-GG-Vector plasmid (component 1)

Combine the following in a PCR tube:

2000 ng pU6-pegRNA-GG-Vector (component 1)	X µL
Bsa1-HFv2 (NEB)	1.0 µL
10x Cutsmart Buffer	3.0 µL
H$_2$O	to 30.0 µL
_____	_____
Total reaction volume	30.0 µL

Incubate at 37 °C for 4-16 hours
Isolate ~2.2-kb fragment from cut plasmid.

Steps 2 and 3: Order and anneal oligonucleotide parts (components 2, 3, and 4)

<u>Materials</u>
      Annealing buffer: H_2O supplemented with 10 mM Tris-Cl pH 8.5 and 50 mM NaCl
      Complementary oligonucleotide pairs

<u>Protocol</u>
Combine the following in a PCR tube:

Top oligonucleotide, 100 µM	1.0 µL
Bottom oligonucleotide, 100 µM	1.0 µL
Annealing buffer (components 2, 3, and 4)	23.0 µL
_____	
Total reaction volume	25.0 µL

In thermocycler, heat at 95 °C for 3 minutes, then cool gradually (0.1 °C/s) to 22 °C

Dilute annealed oligonucleotides 1:4 by adding 75 µL H_2O. The final concentration of each oligonucleotide will be 1 µM after this dilution. Do not dilute the sgRNA scaffold (component 4) if phosphorylating by PNK in step 2.5.

Step 2.b.ii.: sgRNA scaffold phosphorylation (unnecessary if oligonucleotides were purchased phosphorylated)

<u>Protocol</u>
Combine the following in a PCR tube:

4 µM oligonucleotide duplex from step 1	6.25 µL
10x T4 DNA ligase buffer (NEB)	2.50 µL
T4 PNK (NEB)	0.50 µL
H_2O	15.75 µL
_____	
Total reaction volume	20.0 µL

In thermocycler, incubate at 37 °C for 60 minutes
Following this phosphorylation, annealed scaffold oligonucleotides are now at a concentration of 1 µM. Proceed to step 3.

Step 4: pegRNA assembly
pegRNA Golden Gate assembly reaction

Digested pU6-pegRNA-GG plasmid-vector	1.00 µL @ 30 ng/µL
- Pre-cut, isolated 2.2-kb fragment	
Annealed protospacer oligonucleotides (component 2)	1.00 µL @ 1 µM
Annealed pegRNA 3'-extension oligonucleotides (component 4)	1.00 µL @ 1 µM
sgRNA scaffold annealed oligonucleotides (component 3)	1.00 µL @ 1 µM
- Oligonucleotides *must be phosphorylated*	
*Bsa*I-HFv2 (NEB)	0.25 µL
T4 DNA ligase (NEB)	0.50 µL
10x T4 DNA ligase buffer (NEB)	1.00 µL
H_2O	4.25 µL
Total reaction volume:	10.0 µL

Incubate at room temperature for 10 min. Alternatively, cycle between 5 min at 16 ˚C and 5 min at 37 ˚C for 8 cycles. Following incubation, perform the following program in a thermocycler:
15 min at 37 °C, then 15 min at 80 °C, then hold at 12 °C

Steps 5 and 6: Transformation of assembled plasmids
Transform 1 µL of the 10 µL assembly reaction into 10 µL of competent cells. The desired transformants will be resistant to ampicillin and carbenicillin.

The following diagram summarizes the pegRNA cloning protocol.

pegRNA Cloning by Golden Gate Assembly

Assembled pegRNA vector

Supplementary Note 4. Python script for quantifying pegRNA scaffold integration.

A custom python script was generated to characterize and quantify pegRNA insertions at target genomic loci.

The script iteratively matches text strings of increasing length taken from a reference sequence (guide RNA scaffold sequence) to the sequencing reads within fastq files, and counts the number of sequencing reads that match the search query. Each successive text string corresponds to an additional nucleotide of the guide RNA scaffold sequence. Exact length integrations and cumulative integrations up to a specified length were calculated in this manner. At the start of the reference sequence, 5 to 6 bases of the 3' end of the new DNA strand synthesized by the reverse transcriptase are included to ensure alignment and accurate counting of short slices of the sgRNA.

sgRNA scaffold sequence search

```python
import pandas as pd
import Bio as bio
from Bio import SeqIO
import glob

#generates list of fastq files to analyze
sources = glob.glob('*.fastq')

#reads the fastq files into a dictionary with the file names as keys
fastqdict = {}
for i in range(len(sources)):
    temp = list(SeqIO.parse(sources[i],"fastq"))
    fastqdict[sources[i]]= [str(temp[k].seq) for k in range(len(temp))]

#the referenced sequence to be searched for is entered into the following dictionary with
#an appropriate key
scaffdict =
{'HEK3':'CAGAGGACCGACTCGGTCCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTT
GCTATTTCTAGCTCTAAAACTCACGTGCTCAGTCTGGGCCGGTG',
'EMX1':'ATCACGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACT
TGCTATTTCTAGCTCTAAAACTTCTTCTTCTGCTCGGACTCGGTG',
'FANCF':'TTTCCGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAAC
TTGCTATTTCTAGCTCTAAAACGGTGCTGCAGAAGGGATTCCGGTG',
'RNF2':'TCGTTGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTT
GCTATTTCTAGCTCTAAAACCAGGTAATGACTAAGATGACGGTG'}

#matches and counts iterative slices of the reference string to the appropriate fastq files
#reference key must be contained in the name of the fastq file
#generated values represent cumulative counts for a minimum degree of sgRNA integration
#i.e. a given value x means x reads contain y or more bases of the scaffold
resultdict = dict.fromkeys(sources)
for key in fastqdict:
    for scaffold in scaffdict:
        if scaffold in str(key):
            resultlist = []
            for j in range(len(scaffdict[scaffold])):
```

```
            extent = scaffdict[scaffold][0:(j+1)]
            counter = 0
            for i in range(len(fastqdict[key])):
                if extent in fastqdict[key][i]:
                    counter = counter + 1
            resultlist.append(counter)
        resultdict[key]=resultlist


#writes the results into a dataframe indexed from 1
resultdf = pd.DataFrame.from_dict(resultdict)
resultdf = resultdf.reindex(sorted(resultdf.columns), axis=1)
resultdf.index = range(1,len(resultdf)+1)

#converts the cumulative count values into specific counts
#i.e. a given value x means x reads contain exactly y bases of the scaffold
resultdf2=resultdf.copy()
for entry in resultdf:
    for i in range(1,len(resultdf[entry])+1):
        try:
            resultdf2[entry][i] = resultdf[entry][i]-resultdf[entry][i+1]
        except:
            resultdf2[entry][i] = resultdf[entry][i]

#converts the specific counts values into frequencies
resultdf3=resultdf2.copy()
for entry in resultdf3:
        resultdf3[entry]=resultdf2[entry].div(resultdf[entry][1])*100

#reads the results into excel files
resultdf.to_excel('cumulativecounts.xlsx')
resultdf2.to_excel('specificcounts.xlsx')
resultdf3.to_excel('specificfrequencies.xlsx')
```

Figure 1d
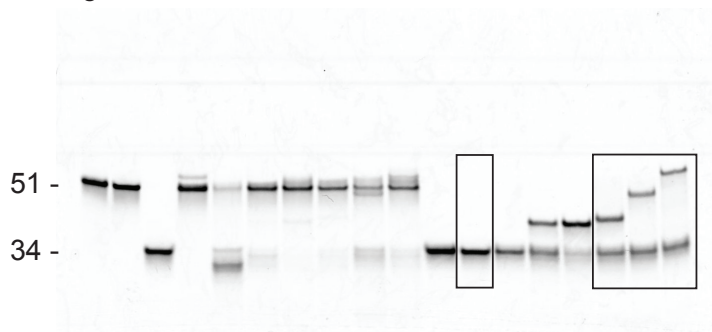
Figure 1d
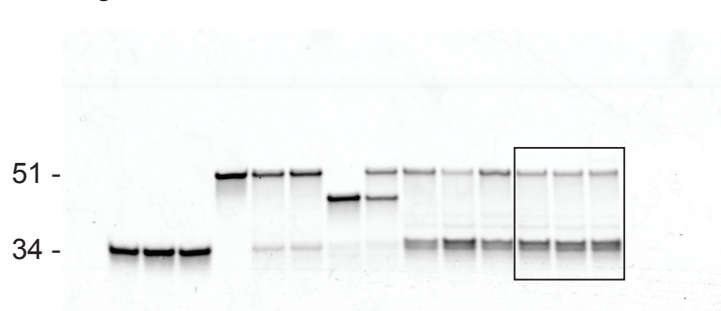
Figure 1e
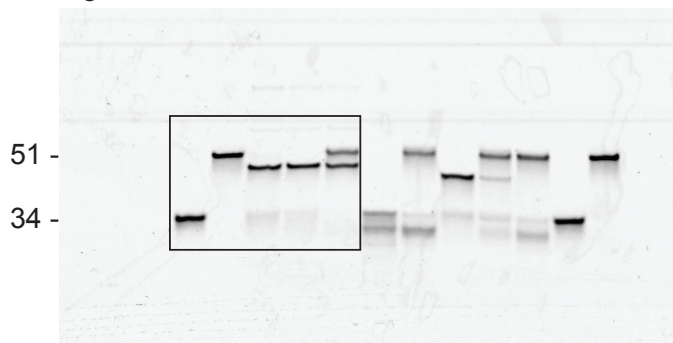
Extended Data Figure 1b
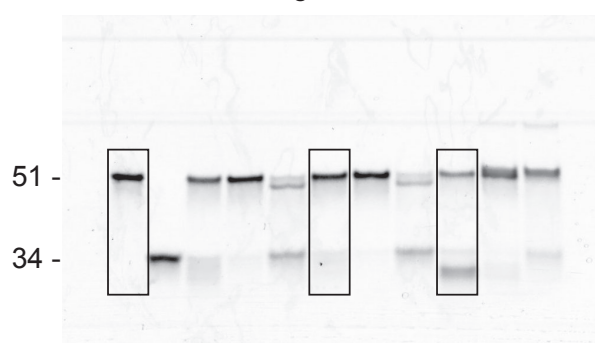
Extended Data Figure 1e
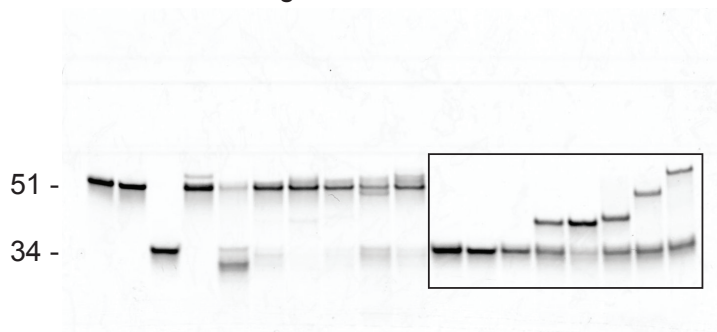
Extended Data Figure 1f
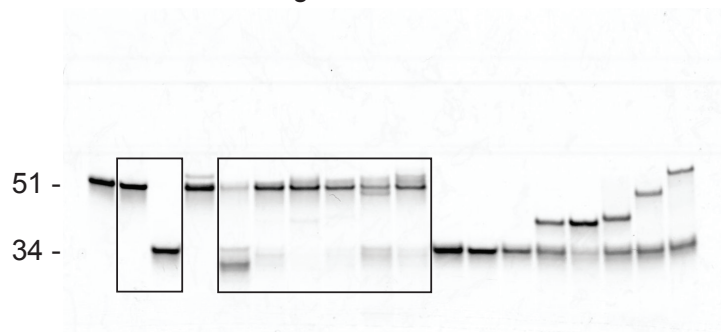
Extended Data Figure 1g
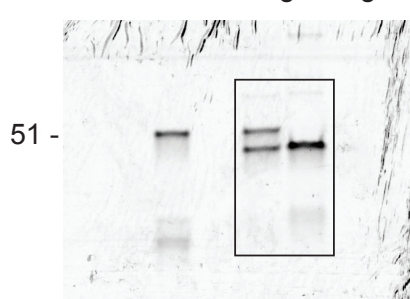
Supplementary Figure 1. Uncropped original gel electrophoresis data. Synthetic 5'-Cy5-labeled single-stranded DNA oligonucleotides were used as standards, labeled as 51 nt and 34 nt in the above denaturing PAGE images. The main-text figure or Extended Data figure that includes each image is shown above each gel, with a black outline to show the excerpted portion.

Supplementary References

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