CRISPR/Cas9 systems have off-target activity despite insertions or deletions between target DNA and guide RNA sequences

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Supplementary Information

Contents

Supplementary Figure S1

HBB gene

			20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1			
		HBB	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	-19		G	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	N	G	G
				*																					
		HBB	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	-18		G	T	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	N	G	G
				*	*																				
		HBB	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	-7/6	G	T	G	A	A	C	G	T	G	G	A	T	G	A	G	T	T	G	G	N	G	G	
																	*	*		*			*		
		HBB	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	-2/1	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	N	G	G	
																							*		

CCR5 gene

			20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1			
		CCR5	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	-19		G	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	N	G	G
				*																					
		CCR5	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	-18		G	T	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	N	G	G	
				*	*																				
		CCR5	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	-17		G	T	A	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	N	G	G	
				*	*	*																			
		CCR5	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	-16		G	T	A	G	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	N	G	G	
				*	*	*	*																		
		CCR5	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	-11	G	T	A	G	A	G	C	G	G	G	G	C	A	G	G	A	G	G	C	N	G	G	
												*		*	*	*		*	*		*				
		CCR5	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	-10/9	G	T	A	G	A	G	C	G	G	A	G	C	A	G	G	A	G	G	C	N	G	G	
														*	*	*		*	*		*				
		CCR5	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	-8	G	T	A	G	A	G	C	G	G	A	G	G	A	G	G	A	G	G	C	N	G	G	
															*	*		*	*		*				
		CCR5	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	-7	G	T	A	G	A	G	C	G	G	A	G	G	C	G	G	A	G	G	C	N	G	G	
																*		*	*		*				
		CCR5	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	-3/2	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	C	N	G	G	
																					*				
		CCR5	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	-1	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	N	G	G	

Supplementary Figure S1. Alignment of -1 nt sgRNA variants to the *HBB* and *CCR5* target loci showing mismatches instead of DNA bulge. Only the variants with detectable intracellular activities are shown. The target loci and index names of the sgRNA variants are indicated on the left of each alignment. Mismatches in the guide sequence and in the “NGG” PAM are marked with asterisks below each alignment. The alignment with the minimum number of mismatches is shown for each sgRNA variant. Nucleotide “U” in the guide RNA is replaced with “T” for the ease of comparison to the target site.

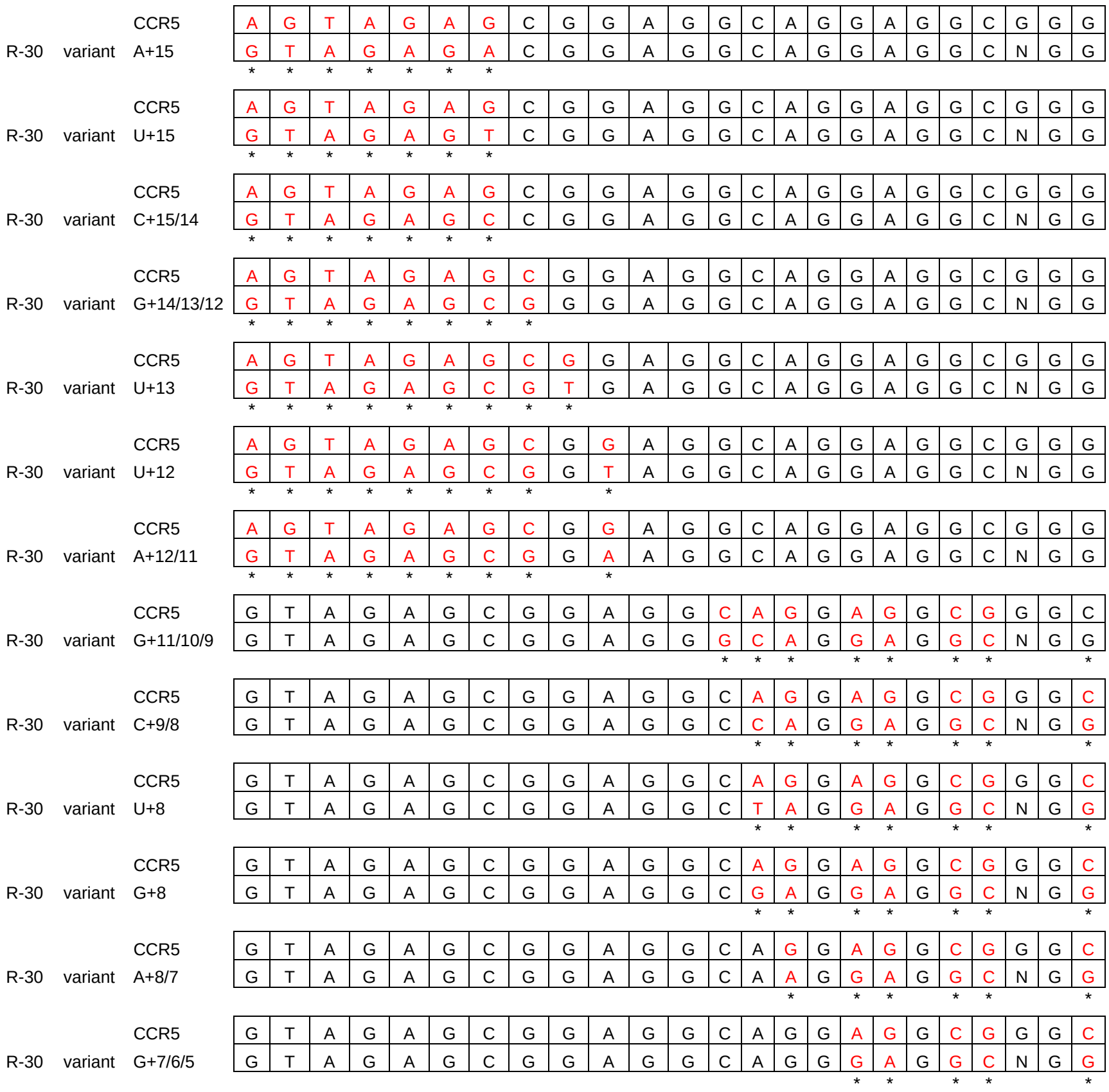
Supplementary Figure S2

HBB gene

			21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1			
		HBB	G	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	U+20/19	G	T	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	N	G	G
				*																						
		HBB	G	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	G+19/18	G	T	G	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	N	G	G
				*	*																					
		HBB	G	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	U+16	G	T	G	A	A	T	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	N	G	G
				*	*	*		*																		
		HBB	G	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	C+16/15	G	T	G	A	A	C	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	N	G	G
				*	*	*		*																		
		HBB	G	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	U+15	G	T	G	A	A	C	T	G	T	G	G	A	T	G	A	A	G	T	T	G	G	N	G	G
				*	*	*		*	*																	
		HBB	G	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	A+15	G	T	G	A	A	C	A	G	T	G	G	A	T	G	A	A	G	T	T	G	G	N	G	G
				*	*	*		*	*																	
		HBB	G	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	G+15/14	G	T	G	A	A	C	G	G	T	G	G	A	T	G	A	A	G	T	T	G	G	N	G	G
				*	*	*		*	*																	
		HBB	G	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	U+14/13	G	T	G	A	A	C	G	T	T	G	G	A	T	G	A	A	G	T	T	G	G	N	G	G
				*	*	*		*	*	*																
		HBB	G	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	C+13	G	T	G	A	A	C	G	T	C	G	G	A	T	G	A	A	G	T	T	G	G	N	G	G
				*	*	*		*	*	*	*															
		HBB	G	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	G+13/12/11	G	T	G	A	A	C	G	T	G	G	G	A	T	G	A	A	G	T	T	G	G	N	G	G
				*	*	*		*	*	*	*															
		HBB	G	G	T	G	A	A	C	G	T	G	G	A	T	G	A	A	G	T	T	G	G	T	G	G
R-01	variant	C+12	G	T	G	A	A	C	G	T	G	C	G	A	T	G	A	A	G	T	T	G	G	N	G	G
				*	*	*		*	*	*	*	*														

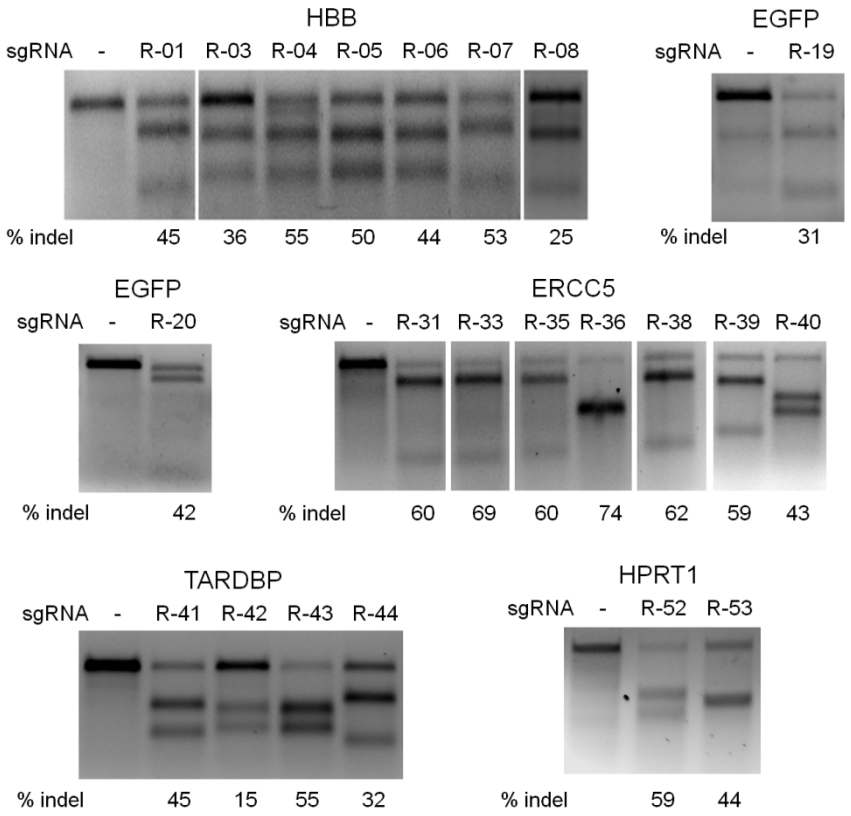
CCR5 gene

			21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1			
		CCR5	A	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	U+20/19	G	T	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	N	G	G
			*	*																						
		CCR5	A	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	A+19/18	G	T	A	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	N	G	G
			*	*	*																					
		CCR5	A	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	G+18/17	G	T	A	G	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	N	G	G
			*	*	*	*																				
		CCR5	A	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	C+17	G	T	A	G	C	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	N	G	G
			*	*	*	*	*																			
		CCR5	A	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	A+17/16	G	T	A	G	A	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	N	G	G
			*	*	*	*	*																			
		CCR5	A	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	U+16	G	T	A	G	A	T	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	N	G	G
			*	*	*	*	*	*																		
		CCR5	A	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	C+16	G	T	A	G	A	C	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	N	G	G
			*	*	*	*	*	*																		
		CCR5	A	G	T	A	G	A	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	G	G	G
R-30	variant	G+16/15	G	T	A	G	A	G	G	C	G	G	A	G	G	C	A	G	G	A	G	G	C	N	G	G
			*	*	*	*	*	*																		



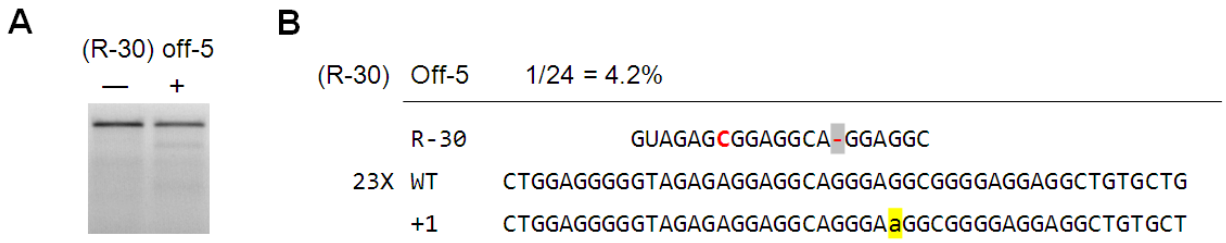
Supplementary Figure S2. Alignment of +1 nt sgRNA variants to the *HBB* and *CCR5* target loci showing mismatches instead of sgRNA bulge. Only the variants with detectable intracellular activities are shown. The target loci and index names of the sgRNA variants are indicated on the left of each alignment. Mismatches in the guide sequence and in the “NGG” PAM are marked with asterisks below each alignment. The alignment with the minimum number of mismatches is shown for each sgRNA variant. Nucleotide “U” in the guide RNA is replaced with “T” for the ease of comparison to the target site.

Supplementary Figure S3



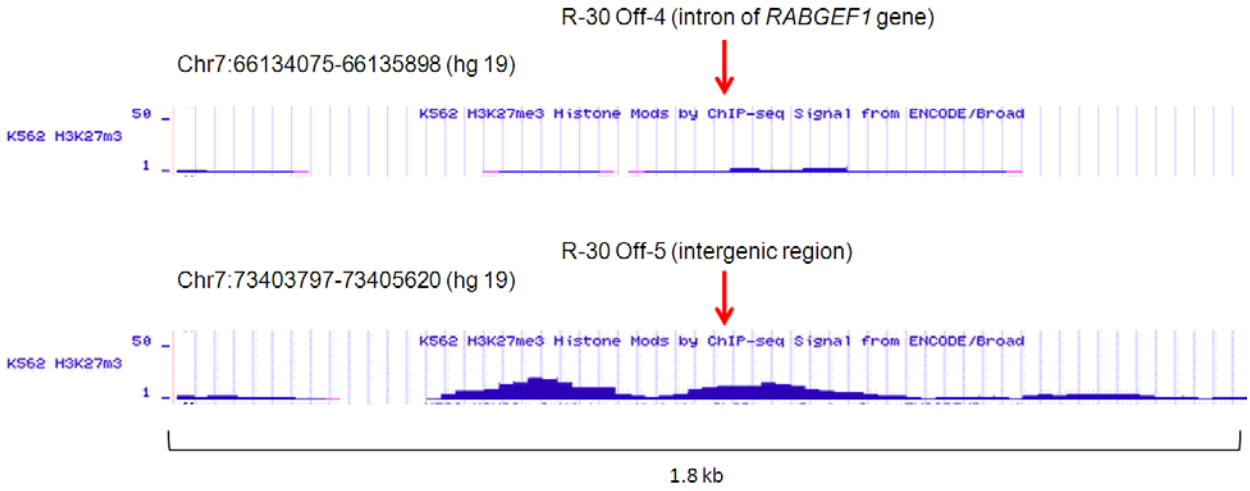
Supplementary Figure S3. T7E1 assay measuring the on-target endogenous gene modification efficiency of sgRNAs in HEK293T cells. Lane headings indicate the target genes and the sgRNA index names. "-" denotes samples transfected with a stuffer plasmid. Numbers below each lane having detectable activity show the percentage of modified alleles. Primers used for the PCR amplification are listed in **Supplementary Table S3**.

Supplementary Figure S4



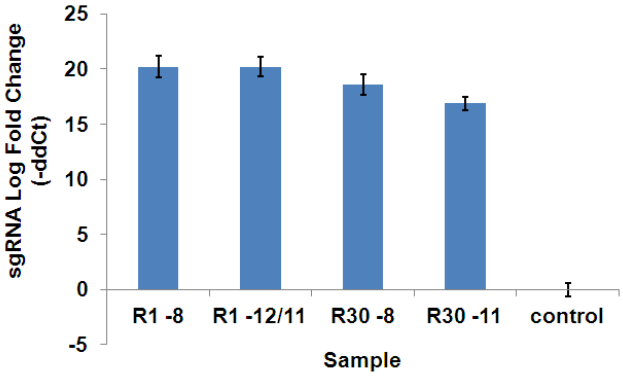
Supplementary Figure S4. Off-target cleavage of R30 Off-5 quantified by **(A)** T7E1 assay and **(B)** Sanger sequencing. "-" and "+" denote samples treated without and with nuclease, respectively. The occurrence of each sequence is indicated to the left of the alignment, if greater than one. Unmodified reads are indicated by "WT". Deletions are marked in gray, and insertions marked in yellow.

Supplementary Figure S5



Supplementary Figure S5. Histone modification status and annotation of R30 Off-4 and Off-5 loci obtained from the UCSC genome browser.

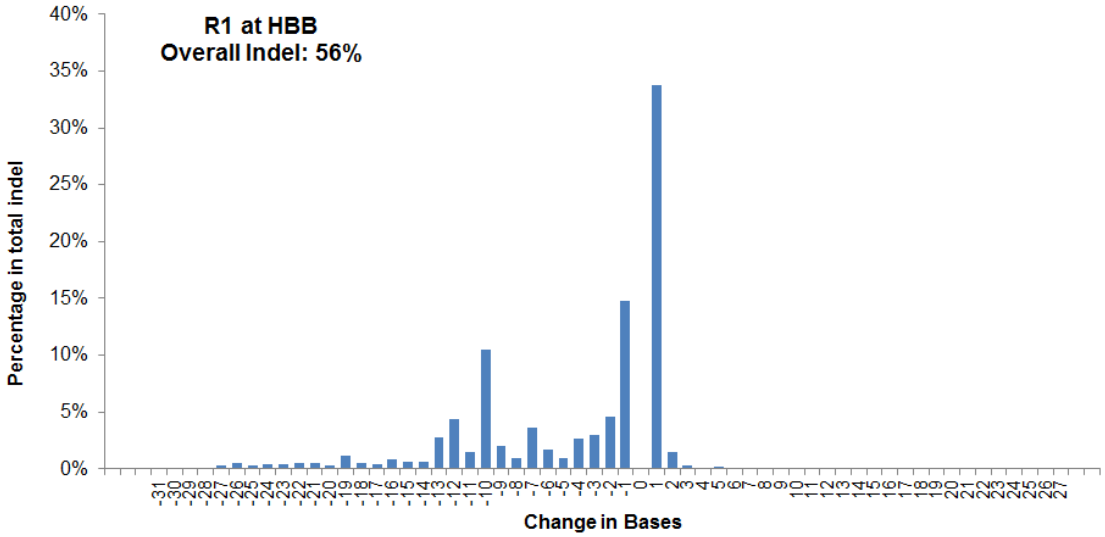
Supplementary Figure S6

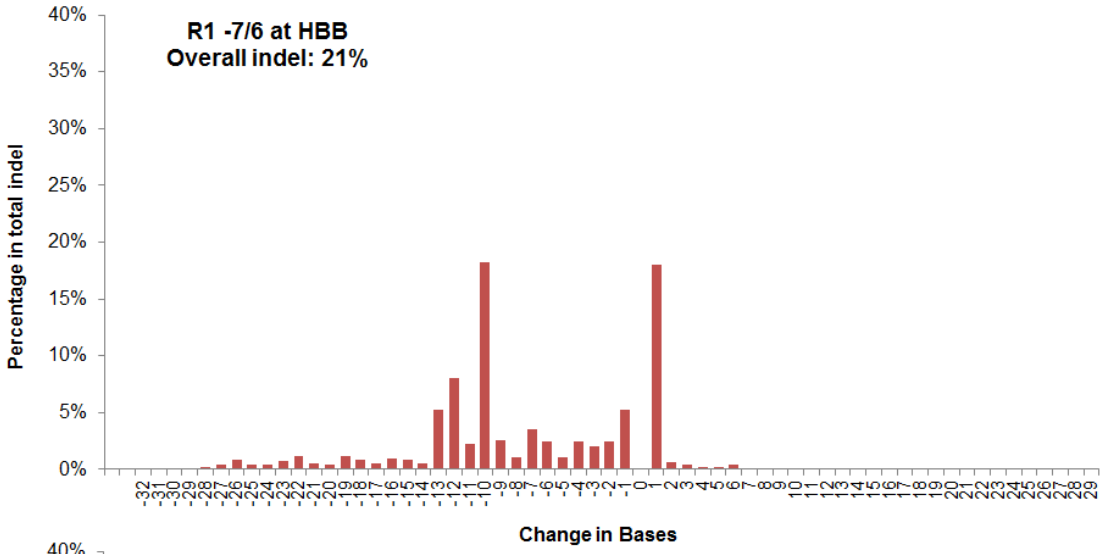


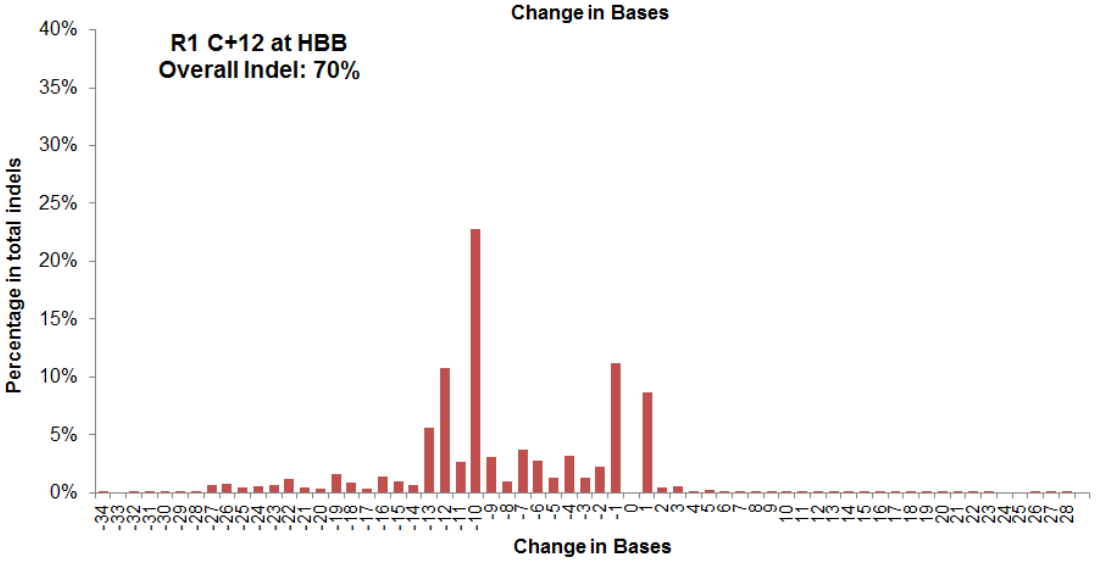
Supplementary Figure S6. Quantitative PCR of sgRNA expression levels in HEK293T cells for R-01 and R-30 variants. Relative expression of these sgRNAs was quantified using the ddCt method (see "**Material and Methods**").

Supplementary Figure S7

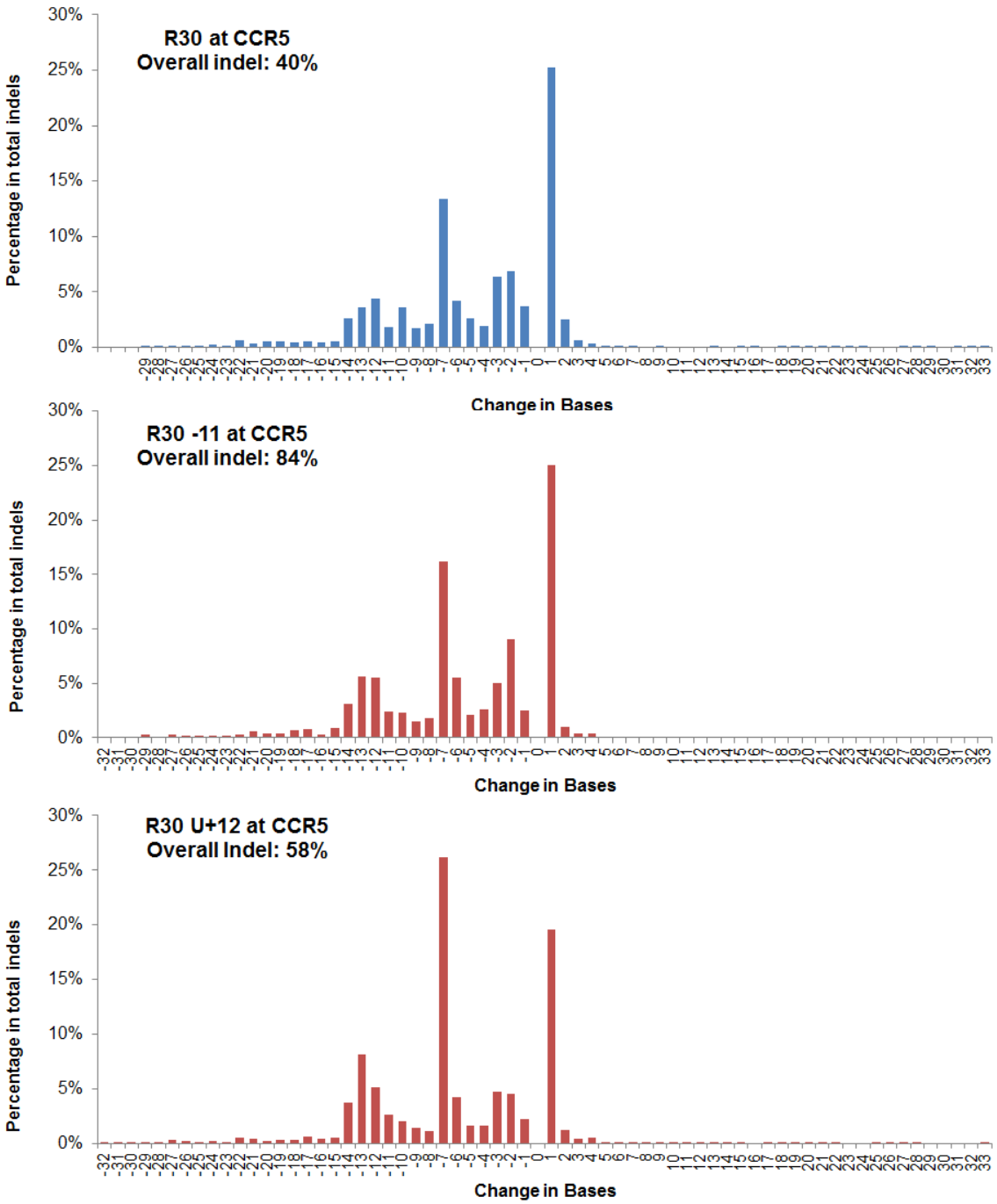
A







B



Supplementary Figure S7. Indel spectra for original sgRNAs and sgRNA variants determined using deep sequencing. (A) R-01 original sgRNA and variants for DNA bulge (R1 -7/6) and sgRNA bulge (R1 C+12). (B) R-30 original sgRNA and variants for DNA bulge (R30-11) and sgRNA bulge (R30 U+12). The change in bases at predicted cut sites resulting from indicated sgRNAs was calculated from ~10^4 reads per sample. The y-axis represents percentages in all indel-reads for that sgRNA. Overall % indel in total reads are indicated in each graph.

Supplementary Table S1. Protospacer target sites for the sgRNAs studed. Target genes, target site sequences, and PAM sequences are listed.

Gene	Storage Index	Protospacer Target (5' to 3')	PAM
HBB	R-01	GTGAACGTGGATGAAGTTGG	TGG
HBB	R-03	GACGTTCACCTTGCCCCACA	GGG
HBB	R-04	GCACGTTCACCTTGCCCCAC	AGG
HBB	R-05	GGTCTGCCGTTACTGCCCTG	TGG
HBB	R-06	GGTTACTGCCCTGTGGGGCA	AGG
HBB	R-07	GAGGTGAACGTGGATGAAGT	TGG
HBB	R-08	GCTGTGGGGCAAGGTGAACG	TGG
EGFP	R-19	GGTGGTGCAGATGAACTTCA	GGG
EGFP	R-20	GACCAGGATGGGCACCACCC	CGG
CCR5	R-25	GTGTTCATCTTTGGTTTTGT	GGG
CCR5	R-26	GCTGCCGCCCAGTGGGACTT	TGG
CCR5	R-27	GGCAGCATAGTGAGCCCAGA	AGG
CCR5	R-29	GTGAGTAGAGCGGAGGCAGG	AGG
CCR5	R-30	GTAGAGCGGAGGCAGGAGGC	GGG
ERCC5	R-31	GCCAAGCACTTAAAGGAGTC	CGG
ERCC5	R-33	GCAAGCACTTAAAGGAGTCC	GGG
ERCC5	R-35	GTGAGTTCCCATGGCGATCC	CGG
ERCC5	R-36	GCTATTGAAGAAACAGACTT	TGG
ERCC5	R-38	GATTTTCTATTGAGTTCCCA	TGG
ERCC5	R-39	GGAAACAAAGTGAGAAGATG	AGG
ERCC5	R-40	GCCTATTTTTGTGTTTGATG	GGG
TARDBP	R-41	GCAGAGCAGTTGGGGTATGA	TGG
TARDBP	R-42	GGCAGCACTACAGAGCAGTT	GGG
TARDBP	R-43	GCAGCACTACAGAGCAGTTG	GGG
TARDBP	R-44	GCCTGACTGGTTCTGCTGGC	TGG
HPRT1	R-52	GTTTGTGTCATTAGTGAAAC	TGG
HPRT1	R-53	GCAACTTGAACTCTCATCTT	AGG

Supplementary Table S2. Guide sequences of sgRNA variants used in the model systems and their cleavage activities tested in HEK 293T cells. Index names correspond to the index in Supplementary Figures S1-S2 and Figures 2-4. Red dashes indicate deleted nucleotides. Inserted nucleotides are colored in red. "nd", activity not detected in the T7E1 assay.

Index			Guide sequence	% indel	s.e.m.
R-01 -1 nt					
R-01	variant	-19	G–GAACGUGGAUGAAGUUGG	40.1	5.4
R-01	variant	-18	GU–AACGUGGAUGAAGUUGG	24.3	5.5
R-01	variant	-17/16	GUGA–CGUGGAUGAAGUUGG	nd	
R-01	variant	-15	GUGAA–GUGGAUGAAGUUGG	nd	
R-01	variant	-14	GUGAAC–UGGAUGAAGUUGG	nd	
R-01	variant	-13	GUGAACG–GGAUGAAGUUGG	nd	
R-01	variant	-12/11	GUGAACGUG–AUGAAGUUGG	nd	
R-01	variant	-10	GUGAACGUGG–UGAAGUUGG	nd	
R-01	variant	-9	GUGAACGUGGA–GAAGUUGG	nd	
R-01	variant	-8	GUGAACGUGGAU–AAGUUGG	nd	
R-01	variant	-7/6	GUGAACGUGGAUG–AGUUGG	14.3	1.5
R-01	variant	-5	GUGAACGUGGAUGAA–UUGG	nd	
R-01	variant	-4/3	GUGAACGUGGAUGAAG–UGG	nd	
R-01	variant	-2/1	GUGAACGUGGAUGAAGUU–G	31.9	3.7
R-01 5' truncation					
R-01	d1	(variant 19)	GGAACGUGGAUGAAGUUGG	40.1	5.4
R-01	d2		GAACGUGGAUGAAGUUGG	39.3	17.3
R-01	d3		GACGUGGAUGAAGUUGG	nd	
R-01	d4		GCGUGGAUGAAGUUGG	nd	
R-01	d5		GGUGGAUGAAGUUGG	nd	
R-01	d6		GUGGAUGAAGUUGG	nd	
R-30 -1 nt					
R-30	variant	-19	G–AGAGCGGAGGCAGGAGGC	44.0	4.5
R-30	variant	-18	GU–GAGCGGAGGCAGGAGGC	43.8	1.3
R-30	variant	-17	GUA–AGCGGAGGCAGGAGGC	5.7	2.2
R-30	variant	-16	GUAG–GCGGAGGCAGGAGGC	4.8	0.5
R-30	variant	-15	GUAGA–CGGAGGCAGGAGGC	nd	
R-30	variant	-14	GUAGAG–GGAGGCAGGAGGC	nd	
R-30	variant	-13/12	GUAGAGCG–AGGCAGGAGGC	nd	
R-30	variant	-11	GUAGAGCGG–GGCAGGAGGC	53.4	3.0
R-30	variant	-10/9	GUAGAGCGGA–GCAGGAGGC	28.4	3.9

R-30	variant	-8	GUAGAGCGGAGG–AGGAGGC	40.8	3.3
R-30	variant	-7	GUAGAGCGGAGGC–GGAGGC	22.1	11.2
R-30	variant	-6/5	GUAGAGCGGAGGCA–GAGGC	nd	
R-30	variant	-4	GUAGAGCGGAGGCAGG–GGC	nd	
R-30	variant	-3/2	GUAGAGCGGAGGCAGGA–GC	54.5	4.7
R-30	variant	-1	GUAGAGCGGAGGCAGGAGG–	32.1	10.7
R-08 -1 nt					
R-08	variant	-19	G–UGUGGGGCAAGGUGAACG	13.0	0.3
R-08	variant	-18	GC–GUGGGGCAAGGUGAACG	23.5	1.4
R-08	variant	-17	GCU–UGGGGCAAGGUGAACG	30.8	3.5
R-08	variant	-16	GCUG–GGGGCAAGGUGAACG	nd	
R-08	variant	-15/14/13/12	GCUGU–GGGCAAGGUGAACG	0.3	0.3
R-08	variant	-11	GCUGUGGGG–AAGGUGAACG	nd	
R-08	variant	-10/9	GCUGUGGGGCA–GGUGAACG	nd	
R-08	variant	-8/7	GCUGUGGGGCAA–GUGAACG	1.1	0.9
R-08	variant	-6	GCUGUGGGGCAAGG–GAACG	nd	
R-08	variant	-5	GCUGUGGGGCAAGGU–AACG	nd	
R-08	variant	-4/3	GCUGUGGGGCAAGGUG–ACG	nd	
R-08	variant	-2	GCUGUGGGGCAAGGUGAA–G	2.2	0.5
R-08	variant	-1	GCUGUGGGGCAAGGUGAAC–	1.5	0.5
R-25 -1 nt					
R-25	variant	-19	G–GUUCAUCUUUGGUUUUGU	nd	
R-25	variant	-18	GU–UUCAUCUUUGGUUUUGU	nd	
R-25	variant	-17/16	GUG–UCAUCUUUGGUUUUGU	nd	
R-25	variant	-15	GUGUU–AUCUUUGGUUUUGU	nd	
R-25	variant	-14	GUGUUC–UCUUUGGUUUUGU	nd	
R-25	variant	-13	GUGUUCA–CUUUGGUUUUGU	nd	
R-25	variant	-12	GUGUUCAU–UUUGGUUUUGU	nd	
R-25	variant	-11/10/9	GUGUUCAUC–UUGGUUUUGU	nd	
R-25	variant	-8/7	GUGUUCAUCUUU–GUUUUGU	nd	
R-25	variant	-6/5/4/3	GUGUUCAUCUUUGG–UUUGU	nd	
R-25	variant	-2	GUGUUCAUCUUUGGUUUU–U	nd	
R-25	variant	-1	GUGUUCAUCUUUGGUUUUG–	nd	
R-01 +1 nt					
R-01	variant	U+20/19	GUUGAACGUGGAUGAAGUUGG	28.2	21.4
R-01	variant	G+19/18	GUGGAACGUGGAUGAAGUUGG	30.9	4.1
R-01	variant	U+18	GUGUAACGUGGAUGAAGUUGG	nd	

R-01	variant	U+17	GUGAUACGUGGAUGAAGUUGG	nd	
R-01	variant	U+16	GUGAAUCGUGGAUGAAGUUGG	39.9	4.1
R-01	variant	A+18/17/16	GUGAAACGUGGAUGAAGUUGG	nd	
R-01	variant	C+16/15	GUGAACCGUGGAUGAAGUUGG	44.7	6.7
R-01	variant	U+15	GUGAACUGUGGAUGAAGUUGG	53.5	1.5
R-01	variant	A+15	GUGAACAGUGGAUGAAGUUGG	37.5	4.9
R-01	variant	G+15/14	GUGAACGGUGGAUGAAGUUGG	17.1	11.2
R-01	variant	C+14	GUGAACGCUGGAUGAAGUUGG	nd	
R-01	variant	A+14	GUGAACGAUGGAUGAAGUUGG	nd	
R-01	variant	U+14/13	GUGAACGUUGGAUGAAGUUGG	39.7	3.0
R-01	variant	A+13	GUGAACGUAGGAUGAAGUUGG	nd	
R-01	variant	C+13	GUGAACGUCGGAUGAAGUUGG	9.0	0.2
R-01	variant	G+13/12/11	GUGAACGUGGGAUGAAGUUGG	41.3	0.7
R-01	variant	C+12	GUGAACGUGCGAUGAAGUUGG	56.5	3.8
R-01	variant	C+11	GUGAACGUGGCAUGAAGUUGG	nd	
R-01	variant	A+11/10	GUGAACGUGGAAUGAAGUUGG	nd	
R-01	variant	U+10/9	GUGAACGUGGAUUGAAGUUGG	nd	
R-01	variant	G+9/8	GUGAACGUGGAUGGAAGUUGG	nd	
R-01	variant	A+8/7/6	GUGAACGUGGAUGAAAGUUGG	nd	
R-01	variant	G+6/5	GUGAACGUGGAUGAAGGUUGG	nd	
R-01	variant	U+5/4/3	GUGAACGUGGAUGAAGUUUGG	nd	
R-01	variant	G+3/2/1	GUGAACGUGGAUGAAGUUGGG	nd	
R-30 +1 nt					
R-30	variant	U+20/19	GUUAGAGCGGAGGCAGGAGGC	37.5	2.3
R-30	variant	A+19/18	GUAAGAGCGGAGGCAGGAGGC	15.5	6.9
R-30	variant	G+18/17	GUAGGAGCGGAGGCAGGAGGC	16.4	1.1
R-30	variant	C+17	GUAGCAGCGGAGGCAGGAGGC	2.9	1.4
R-30	variant	U+17	GUAGUAGCGGAGGCAGGAGGC	nd	
R-30	variant	A+17/16	GUAGAAGCGGAGGCAGGAGGC	23.8	3.2
R-30	variant	U+16	GUAGAUGCGGAGGCAGGAGGC	44.2	6.9
R-30	variant	C+16	GUAGACGCGGAGGCAGGAGGC	24.5	5.1
R-30	variant	G+16/15	GUAGAGGCGGAGGCAGGAGGC	23.4	0.5
R-30	variant	A+15	GUAGAGACGGAGGCAGGAGGC	35.8	3.3
R-30	variant	U+15	GUAGAGUCGGAGGCAGGAGGC	37.8	14.7
R-30	variant	C+15/14	GUAGAGCCGGAGGCAGGAGGC	23.8	7.4
R-30	variant	A+14	GUAGAGCAGGAGGCAGGAGGC	nd	
R-30	variant	U+14	GUAGAGCUGGAGGCAGGAGGC	nd	

R-30	variant	G+14/13/12	GUAGAGCGGGAGGCAGGAGGC	17.8	1.1
R-30	variant	U+13	GUAGAGCGUGAGGCAGGAGGC	27.2	8.5
R-30	variant	U+12	GUAGAGCGGUAGGCAGGAGGC	45.4	1.6
R-30	variant	A+12/11	GUAGAGCGGAAGGCAGGAGGC	9.4	2.9
R-30	variant	G+11/10/9	GUAGAGCGGAGGGCAGGAGGC	3.4	0.6
R-30	variant	C+9/8	GUAGAGCGGAGGCCAGGAGGC	10.6	0.6
R-30	variant	U+8	GUAGAGCGGAGGCUAGGAGGC	11.7	5.7
R-30	variant	G+8	GUAGAGCGGAGGCGAGGAGGC	13.9	7.6
R-30	variant	A+8/7	GUAGAGCGGAGGCAAGGAGGC	7.4	2.1
R-30	variant	G+7/6/5	GUAGAGCGGAGGCAGGGAGGC	1.7	0.5
R-30	variant	A+5/4	GUAGAGCGGAGGCAGGAAGGC	nd	
R-30	variant	G+4/3/2	GUAGAGCGGAGGCAGGAGGGC	nd	
R-30	variant	C+2/1	GUAGAGCGGAGGCAGGAGGCC	nd	
R-08 +1 nt					
R-08	variant	U+20	GUCUGUGGGGCAAGGUGAACG	17.0	0.7
R-08	variant	U+19/18	GCUUGUGGGGCAAGGUGAACG	13.4	2.3
R-08	variant	C+18	GCUCGUGGGGCAAGGUGAACG	27.4	0.5
R-08	variant	U+17/16	GCUGUUGGGGCAAGGUGAACG	15.5	2.7
R-08	variant	C+16	GCUGUCGGGGCAAGGUGAACG	3.2	0.2
R-08	variant	U+15	GCUGUGUGGGCAAGGUGAACG	26.3	0.3
R-08	variant	U+14	GCUGUGGUGGCAAGGUGAACG	nd	
R-08	variant	U+13	GCUGUGGGUGCAAGGUGAACG	11.0	1.4
R-08	variant	U+12	GCUGUGGGGUCAAGGUGAACG	25.2	0.8
R-08	variant	U+11	GCUGUGGGGCUAAGGUGAACG	16.5	2.6
R-08	variant	U+10	GCUGUGGGGCAUAGGUGAACG	nd	
R-08	variant	U+9	GCUGUGGGGCAAUGGUGAACG	nd	
R-08	variant	U+8	GCUGUGGGGCAAGUGUGAACG	nd	
R-08	variant	U+7/6	GCUGUGGGGCAAGGUUGAACG	nd	
R-08	variant	C+6	GCUGUGGGGCAAGGUCGAACG	nd	
R-08	variant	U+5	GCUGUGGGGCAAGGUGUAACG	nd	
R-08	variant	U+4	GCUGUGGGGCAAGGUGAUACG	nd	
R-08	variant	U+3	GCUGUGGGGCAAGGUGAAUCG	nd	
R-08	variant	U+2	GCUGUGGGGCAAGGUGAACUG	nd	
R-25 +1 nt					
R-25	variant	U+20/19	GUUGUUCAUCUUUGGUUUUGU	nd	
R-25	variant	C+19	GUCGUUCAUCUUUGGUUUUGU	nd	
R-25	variant	U+18/17/16	GUGUUUCAUCUUUGGUUUUGU	nd	

R-25	variant	C+17	GUGUCUCAUCUUUGGUUUUGU	nd	
R-25	variant	C+16/15	GUGUUCCAUCUUUGGUUUUGU	nd	
R-25	variant	U+15	GUGUUCUAUCUUUGGUUUUGU	nd	
R-25	variant	U+14/13	GUGUUCAUUCUUUGGUUUUGU	nd	
R-25	variant	C+13/12	GUGUUCAUCCUUUGGUUUUGU	nd	
R-25	variant	U+12/11/10/9	GUGUUCAUCUUUUGGUUUUGU	nd	
R-25	variant	C+11	GUGUUCAUCUCUUGGUUUUGU	nd	
R-25	variant	C+10	GUGUUCAUCUUCUGGUUUUGU	nd	
R-25	variant	C+9	GUGUUCAUCUUUCGGUUUUGU	nd	
R-25	variant	U+8	GUGUUCAUCUUUGUGUUUUGU	nd	
R-25	variant	U+7/6/5/4/3	GUGUUCAUCUUUGGUUUUUGU	nd	
R-25	variant	C+6	GUGUUCAUCUUUGGUCUUUGU	nd	
R-25	variant	C+5	GUGUUCAUCUUUGGUUCUUGU	nd	
R-25	variant	C+4	GUGUUCAUCUUUGGUUUCUGU	nd	
R-25	variant	C+3	GUGUUCAUCUUUGGUUUUCGU	nd	
R-25	variant	U+2/1	GUGUUCAUCUUUGGUUUUGUU	nd	
R-01 and R-30 +2 nt to +5 nt or -2 nt					
R-01	variant	+15+16	GUGAACuuGUGGAUGAAGUUGG	1.7	0.1
R-01	variant	+12+13	GUGAACGUGuuGAUGAAGUUGG	41.2	5.1
R-30	variant	+15+16	GUAGAGuuCGGAGGCAGGAGGC	31.7	6.5
R-30	variant	+12+13	GUAGAGCGGuuAGGCAGGAGGC	28.5	6.7
R-01	variant	-6-7	GUGAACGUGGAUG--GUUGG	nd	
R-01	variant	-1-2	GUGAACGUGGAUGAAGUU--	nd	
R-30	variant	-9-10	GUAGAGCGGA--CAGGAGGC	nd	
R-30	variant	-7-8	GUAGAGCGGAGG--GGAGGC	nd	
R-01	variant	+15+16+17	GUGAACuuuGUGGAUGAAGUUGG	nd	
R-01	variant	+12+13+14	GUGAACGUGuuuGAUGAAGUUGG	34.5	0.8
R-30	variant	+15+16+17	GUAGAGuuuCGGAGGCAGGAGGC	5.6	1.2
R-30	variant	+12+13+14	GUAGAGCGGuuuAGGCAGGAGGC	37.9	7.4
R-01	variant	+12+13+14+15	GUGAACGUGuuuuGAUGAAGUUGG	nd	
R-30	variant	+15+16+17+18	GUAGAGuuuuCGGAGGCAGGAGGC	nd	
R-30	variant	+12+13+14+15	GUAGAGCGGuuuuAGGCAGGAGGC	8.9	2.4
R-01	variant	+12+13+14+15+16	GUGAACGUGuuuuuGAUGAAGUUGG	nd	

Supplementary Table S3. Sequences of primers used to amplify endogenous loci for testing the on-target activities of sgRNAs, and primers for qPCR. Target gene, sgRNAs using the primers, special PCR conditions are listed with each pair of primers. The primer sequences are listed in the lower portion of the table.

Primers for target PCR				
Gene	**sgRNA**	**Forward primer name**	**Reverse primer name**	**special PCR condition**
HBB	R-01, R-03, R-04, R-05, R-06, R-07, R-08	B-glo-Fwd	B-glo-Rev	
EGFP	R-19, R-20	T7	SSA-Cell-R4	annealed at 50 °C
CCR5	R-25, R-26, R-27, R-29, R-30	CCR5_1_10_1_F	CCR5_1_10_1_R	
ERCC5	R-31, R-33, R-35, R-36, R-38, R-39, R-40	ERCC5-F2	ERCC5-R2	
TARDBP	R-41, R-42, R-43, R-44	TAR-F	TAR-R	
HPRT1	R-52, R-53	HPRTe9-F	HPRTe9-R	
Primers for qPCR				
Gene		**Forward primer name**	**Reverse primer name**	
sgRNA		CRI-qPCR-F	CRI-qPCR-R	

Primer name	**Primer sequence (5'to 3')**
B-glo-Fwd	CCAACTCCTAAGCCAGTGCCAGAAGAG
B-glo-Rev	AGTCAGTGCCTATCAGAAACCCAAGAG
T7	TAATACGACTCACTATAGGG
SSA-Cell-R4	TGCCGTCCTCGATGTTGTGGCG
CCR5_1_10_1_F	GCACAGGGTGGAACAAGATGG
CCR5_1_10_1_R	ACCACCCCAAAGGTGACCGT
ERCC5-F2	TGAGGATGAAGAGAAAAATCCCGGAG
ERCC5-R2	ATCATTGTACCCATGATGAACTCTCATAAAAC
TAR-F	CAATAGCAATAGACAGTTAGAAAGAAGTGGAAG
TAR-R	GCTGCACCAGAATTAGAGCCACTATAAGAG
HPRTe9-F	CAATCCGCCCAAAGGGAACTGATAG
HPRTe9-R	TGCTTTGTTTTCAAAAGATACACTCCCCA
CRI-qPCR-F	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
CRI-qPCR-R	AAAAGCACCGACTCGGTGCCAC

Supplementary Table S4. Human genomic loci tested for off-target activity using T7E1 assay. 18 off-target loci with insertions/DNA bulges and 62 off-target loci with deletions/sgRNA bulges are shown. For each site, mismatches (red letters), insertion (red "N" besides the "N" in the PAM sequence), and deletion (red "^") are indicated below the genomic target sequence. The positions of insertion and deletion relative to the PAM are listed as a separate column. The table also includes plus / minus strand and chromosomal coordinates (hg 19) which can be used in the UCSC genome browser. The last two columns are the primers used to amplify the genomic loci.

18 off-target sites with target-site insertions (DNA bulges)

Index	Potential Off-target Sites	Inserted (DNA bulge) Position	Chromosomal Coordinates [start..end] (hg 19)	Strand	Primer	Primer Sequence
R-01 Off-1	TTGTAACATGGATGAAGTTGGAGG G N G N	Ins 18	Chr2:186524309-186524332	+	R1off-F1	TCAGTCTTTTACTCGGGGATACCAA
					R1off-R1	TTCATCTATCGTAACGCTTGGCAAT
R-01 Off-2	CTGCAACGTGGATGAAGCTGGAGG G N T N	Ins 18	Chr21:16223748-16223771	-	R1off-F2	GAACAGAATGATGAGGAAGGGAAGA
					R1off-R2	AACCTAGATGCCCATCAATAGTGGA
R-05 Off-1	GCTCTGCCGTTTACTGCCCTGTGG G N N	Ins 10, 11, or 12	Chr1:162859322-162859345	+	R5off-F1	TTGAGATGCCGTTGTTTCATGCCAA
					R5off-R1	ATTGCTCACACCACATCAGAAAGCC
R-07 Off-1	AAGATGAACGTGGAGTGAAGTGGG G G N N	Ins 7	Chr9:116503487-116503510	+	R7off-F1	CCAGGCATCCTGCTGATCTTTTGTT
					R7off-R2	TTAGGGGTTAAAGGGCTTGCTGGTG
R-20 Off-1	CGCCAAGATGGGCAGCCACCCCGG GA G N N	Ins 7	Chr20:21687581-21687604	+	R20off-F1	GACGGCGTCTGTGACAAGTACAATG
					R20off-R1	GAGGTCTCTTACAAAAGGCCCAGGA
R-20 Off-2	ATCCAGGATGGGCACCACACCCGG GA N N	Ins 3	Chr16:57067704-57067727	-	R20off-F2	GGTACCTTGGAGGGATCTATTGCCT
					R20off-R2	CTGACACTTCTGCAGCCTTGGGTAG
R-25 Off-1	ATGTTCTTCTTTGGCTTTTGTTGG G A N N	Ins 7	Chr10:59053283-59053306	-	R25off-F1	TGACCAATGAGCAAAGAAATTATCCACA
					R25off-R1	ACATCCCAAAGAATGAAGTTGGAGA
R-25 Off-2	TATTTCATCTTTGGTTTTAGTGGG GTG N N	Ins 3	Chr13:23183816-23183839	+	R25off-F2	GCACACTAGTGGACTACTCAGGGTAT
					R25off-R2	ACAGGCATATCATATTGTATGTCAGAGTG
R-25 Off-3	AGGTTCAACTTTGGTTTTGGTGGG GT T N N	Ins 2 or 3	Chr15:37967958-37967981	-	R25off-F3	AAGAAACAGGGATCCGTGCATAAAT
					R25off-R3	AATTTCTTTGTTGGAAAACCCTGGA
R-25 Off-4	ATGTTCATATTTGGTTTTGTGTGG G C NN	Ins 1	Chr2:22543732-22543755	+	R25off-F4	CATTGATTGTTTCATCCCGACAGTT
					R25off-R4	GGCTAAGGTGAAAAACAAAGCCAAT
R-26 Off-1	TTTGCCCCCAGTGGGACATTTGG GC G N N	Ins 3	Chr3:52496409-52496432	-	R26off-F1	GCTACATCTGGTTCTGGTTTGAGGC
					R26off-R1	TCCACCCTATCCAATGTCAGCAACA
R-30 Off-1	GTGTGAGCGGAGGCAGGAGGCAGG NA N	Ins 19	Chr2:241904712-241904735	+	R30off-F1	AGGAATGCTTTAGCGAGGAGGAAG
					R30off-R1	CTCTCCACTCCTCCTCTGGTTCTC
R-30 Off-2	GTAGGAGAGGAGGCAGGAGGCAGG N C N	Ins 17 or 18	Chr19:35843790-35843813	+	R30off-F2	TGATGCACTTGAGGACAGCTACTCT
					R30off-R2	TGTGCCTGGCTTCAAATATGTCTTA
R-30 Off-3	CCAGAAGCGGAGGCAGGAGGCTGG GT N N	Ins 16 or 17	Chr9:139753254-139753277	-	R30off-F3	CCACTTTGCCTTCTTTGAAACTGG
					R30off-R3	AACACGATCTGATGGAGAAGGAAAG
R-30 Off-4	GTAGAGAGGAGGCAGGGAGGCGGG C N N	Ins 5, 6, or 7	Chr7:66134975-66134998	-	R30off-F4	CTCGGGAAATGGCACCATCATCATC
					R30off-R4	CAGGTCATGGTGAACCTCAGAGCTA

R-30 Off-5	GTAGAGAGGAGGCAGGGAGGCGGG C N N	Ins 5, 6, or 7	Chr7:73404697-73404720	-	R30off-F5	TTCTGTAATTCTGAGGCCCACGGAG
					R30off-R5	TGATGAACCTCAGAGCCATTTGGGG
R-31 Off-1	ACCAAGCACTTAAAGGAGTGCTGG G N N	Ins 2	Chr9:86698731-86698754	-	R31off-F1	ACCTCCCACATGTACCTTGCTTTTT
					R31off-R1	GCCTTTCATGTCTGGAACATTTTTG
R-42 Off-1	TCCAGCACTACAGAGCAGATTTGG GG N N	Ins 3	Chr10:48593036-48593059	-	R42off-F1	CCAACCTCAAAAGGACCTTGCTGTC
					R42off-R1	TTCACTTTCCAGAGAAGAGTCCTCC

62 off-target sites with target-site deletions (sgRNA bulges)

Index	Potential Off-target Sites	Deleted (sgRNA bulge) Position	Chromosomal Coordinates [start..end] (hg 19)	Strand	Primer	Primer Sequence
R-01 Del-1	GGGAA^TTGGATGAAGTTGGGGG T ^G N	Del 15	Chr7:85607600-85607621	+	R1_del_1_F	GAATGCAGTAAATTTAAAAGCCCAAGG
					R1_del_1_R	CATCACAGAACACCAGAAAGACAGC
R-01 Del-2	CTGAA^GTGGATAAAGTTGGTGG G ^ G N	Del 15	Chr4:44622064-44622085	+	R1_del_2_F	GCAAATCTGGGTGGATGTACTGTTG
					R1_del_2_R	CCTGCACGATCTCACTATGTCTTGC
R-01 Del-6	GGGAACG^GGATGAAGGTGGTGG T ^ T N	Del 13	Chr8:37261849-37261870	-	R1_del_6_F	TTTACATGGTGGAGGACAGGACTTC
					R1_del_6_R	CCAATGATGATTATCTCCGTGACTG
R-03 Del-1	CACCTTCACC^TGCCCCACACGG G G ^ N	Del 9 or 10	Chr7:134252717-134252738	-	R5_del_1_F	AATTCACTTTCCTTCCTTTCTTTTG
					R5_del_1_R	CTCACACTCCCAGGTTCAAACAATC
R-04 Del-1	TCACCTTC^CCTTGCCCCACAGG G G ^ N	Del 12	Chr4:57092889-57092910	-	R7_del_1_F	GTTGAAATTTGATCCCCAGCATTG
					R7_del_1_R	AGAGAGGTGTGAAGGAGAGGGAAAG
R-06 Del-1	GGCTACTGCCCTG^GGGGCAGGG GCTACTGCCCTG^GGGGCAGGG	Del 7	Chr14:100616656-100616677	-	R11_del_1_F	TTGATGCCGTCTGTGTACTCAAGCA
					R11_del_1_R	GTTTGGTCTCTTTCCAAGGGGAAGC
R-07 Del-1	GCAGTG^ACGTGGATGAAGTTGG AG ^ N	Del 13 or 14	Chr3:161230477-161230498	-	R13_del_1_F	GTTCCCATTGTTGTTTGGTTTTCTG
					R13_del_1_R	TGCTACTATAAAGACGCATGCACAC
R-07 Del-2	GTGGGG^ACGTGGATGAAGTTGG A T ^ N	Del 13 or 14	Chr8:106659900-106659921	-	R13_del_2_F	GTGAGTGAGAACATGTGGTGTTCA
					R13_del_2_R	TGGTGCTATTCACAACAGCAAAGAG
R-07 Del-3	GAGG^GAACGTGGATGAAGCTGG GAGG^GAACGTGGATGAAGTNGG	Del 16	Chr2:116826850-116826871	+	R13_del_3_F	AGACGTGGAATCAACACAAATGCCC
					R13_del_3_R	ACAGATGTGCGATGTCAAGATCACC
R-08 Del-1	GATGAGGGG^AAGGTGAACGTGG C T ^ N	Del 11	Chr23:6739538-6739559	-	R15_del_1_F	ATAGAGACTGCTTGGAAAGCGTGTG
					R15_del_1_R	AGCCTTACCGAGGACTCCTTTTACC
R-08 Del-2	GCTG^GGGGCAACGTGAACGTGG GCTG^GGGGCAAGTGAACGNGG	Del 16	Chr17:38953488-38953509	-	R15_del_2_F	CTGAGTCGTGGGAGATCTGTTGCTG
					R15_del_2_R	ATACACCTGACCGCAAACTTTGAGAC
R-19 Del-1	GGTGGT^CAGATCAACTTCAGGG GGTGGT^CAGATGAACTTCANGG	Del 14	Chr10:79211096-79211117	-	R19_del_1_F	CCCTGAGATACAAGAGGAGCCTGAC
					R19_del_1_R	CGTCCTCTGAACTTCAATTGCCCTG
R-20 Del-1	GACCAGGA^GGGCAGCACCCAGG GACCAGGA^GGGCACCACCCNGG	Del 12	Chr14:24535619-24535640	+	R20_del_1_F	GAATGACATGGAGATGCTAGAGCAGA
					R20_del_1_R	AGAGGCTTTCCATACCTATGTGCCA
R-25 Del-1	GTCTTC^TCTTTGGTTTTGTAGG G ^ N	Del 14	Chr7:121693943-121693964	+	R25_del_1_F	TGCCCAGTAAGCATTGGCTATAATAATC
					R25_del_1_R	GTCCCATATCATCCTCCAGAAATCC

R-25 Del-10	GTGTTCATCTT^GGTTTTGTACG GTGTTCATCTT^GGTTTTGTNG	Del 9, 10, or 11	Chr4:70483200-70483221	-	R25_del_10_F	GCTTTAGGATCTGCTGCCCTCCTAT
					R25_del_10_R	CGTCTTAATGGACCCTGTATGTTGCT
R-25 Del-2	ATGCTC^TCTTTGGTTTTGTTGG G T ^ N	Del 14	Chr2:230663047-230663068	-	R25_del_2_F	GACCCGGCTGCTTAAATTACAAATG
					R25_del_2_R	TTGTTCCAGACAAGGAAAAGCTGAC
R-25 Del-3	ATGGTC^TCTTTGGTTTTGTAGG G T ^ N	Del 14	Chr17:59233856-59233877	+	R25_del_3_F	TGTTTCTTTTGGGGGAAACTTAGAG
					R25_del_3_R	TTTCTTACCAAATGATGAAACTCGAC
R-25 Del-4	ATGTTCAT^TTTGGTTTTGTTGG G ^ N	Del 12	Chr21:27369860-27369881	-	R25_del_4_F	GAGAACATAACTAAAAACAAAAAGAGAAAC
					R25_del_4_R	GCAAGAAATCCTCTTCTGTTAAGAAACC
R-25 Del-5	TTATTCAT^TTTGGTTTTGTGGG G G ^ N	Del 12	Chr6:131504701-131504722	-	R25_del_5_F	ACAAAAAGGGGATTTTGGAGGTAGG
					R25_del_5_R	CAGTGCTCTCCAGGCTCACTCTC
R-25 Del-6	GGTTTCAT^TTTGGTTTTGTTGG TG ^ N	Del 12	Chr18:8673547-8673568	-	R25_del_6_F	CAGAAGATGTTCAGAAACAAGCAAGG
					R25_del_6_R	ATTCTGTCTGTGAGGCGTGTCTTTC
R-25 Del-8	GTGTTCA^CTTTGGTTGTGTAGG GTGTTCA^CTTTGGTTTTGTNG	Del 13	Chr5:74921783-74921804	+	R25_del_8_F	CTCACCATTGCAGGAGAGAGGAAGT
					R25_del_8_R	GAATGGGAAGAAGGAATCTGGCTGC
R-25 Del-9	GTGTTCTTCTT^GGTTTTGTTGG GTGTTCATCTT^GGTTTTGTNG	Del 9, 10, or 11	Chr8:114654423-114654444	-	R25_del_9_F	AAGTTACTCACCTGTCCCCTAGAGTG
					R25_del_9_R	ATTTTGCCTGAGGCTGGCCTTCATA
R-27 Del-1	GGAAGCA^AGTGAGCCCAGAAGG C ^ N	Del 13	Chr13:95847651-95847672	+	R27_del_1_F	GAACACGGGAGTTGGTTGGAAAT
					R27_del_1_R	ATAGGTGATTGTGAAAAGAAGC
R-27 Del-3	GAAAGCATAGTGA^CCCAGAGGG GC ^ N	Del 7	Chr7:51205518-51205539	-	R27_del_3_F	AATTATCACTGATTTTTACTGAGAACTG
					R27_del_3_R	ACTGGGCTATTGTTTAATATGATGG
R-27 Del-4	GGCA^CATAGTGAGCCAAGATGG GGCA^CATAGTGAGCCCAGANGG	Del 16	Chr5:99191677-99191698	+	R27_del_4_F	GACCCAGCCATCCCATTACTTGGTA
					R27_del_4_R	TCTGAAAAGCGCAATATTCGGGTGG
R-27 Del-5	GGCAGC^TAGTGAGCCCAGAGGA GGCAGC^TAGTGAGCCCAGANNG	Del 14	Chr1:164837564-164837585	+	R27_del_5_F	CATCCGTGCACAATACCAGGCTAAG
					R27_del_5_R	GCTGCTTGCAAATCAACCAGGTTTC
R-27 Del-6	GGCAGCA^AGTGAGGCCAGAAGG GGCAGCA^AGTGAGCCCAGANGG	Del 13	Chr13:19571247-19571268	+	R27_del_6_F	AGTCCAAGTCAGATGGTCAGAAAGCA
					R27_del_6_R	TCCTTGCATGCCAAGAGCAGAGATT
R-29 Del-1	GAGTGT^GAGCGGAGGCAGGAGG TA ^ N	Del 14	Chr2:238918342-238918363	-	R29_del_1_F	CAATAGCTGTCATTGTGCCTTTGTC
					R29_del_1_R	CCTGGAAGTGACATCCTATGCAAAC
R-29 Del-2	CTGAGGAG^GCGGAGGCAGGAGG G T ^ N	Del 12	Chr7:8334655-8334676	-	R29_del_2_F	CGAGCCAGAAGTATATTCCTACGTG
					R29_del_2_R	CCTGGGCAACAAAGTGAGACC
R-29 Del-5	GTGAGTAGAG^GGAGGGAGGAGG GTGAGTAGAG^GGAGGCAGGNGG	Del 10	Chr8:83327062-83327083	-	R29_del_5_F	ATATACCAGCCAACTTGGGATGCCT
					R29_del_5_R	ACAAGTTTTCAGTGAGGGGAGGGAA
R-30 Del-11	GAAGGGCGGAGG^AGGAGGCAGG T A ^ N	Del 8	Chr16:30382121-30382142	+	R30_del_11_F	AGGGCTGTAAGACCAATCAGAGGAC
					R30_del_11_R	ACCTGCTCCCCTTTTCATTGG
R-30 Del-12	GGAGAGAGGAGG^AGGAGGCTGG T C ^ N	Del 8	Chr3:194821292-194821313	-	R30_del_12_F	CAGAGTCTTCTGCCCTGGCATC
					R30_del_12_R	AGAAGGGCACCACAGCCTCAG
R-30 Del-14	GCAAAGCGGAGGC^GGAGGCAGG T G ^ N	Del 7	Chr6:105436556-105436577	-	R30_del_14_F	AGCCACTTGGCCTGTAGTTTTTCTT
					R30_del_14_R	GAGGTCAGGAGTTTGAGAACAGCCT
R-30 Del-15	GCAGCGCGGAGGC^GGAGGCGGG T A ^ N	Del 7	Chr9:132372864-132372885	+	R30_del_15_F	CCTAGCAATTTTGGGCTGAACAAC
					R30_del_15_R	AAACTTCTCAGCCTCTCGCTCCAG

R-30 Del-16	CTAGGGCGGAGGC^GGAGGCGGG G A ^ N	Del 7	Chr9:96108620-96108641	-	R30_del_16_F	GCTGGGCTGGAGAGAAGGTG
					R30_del_16_R	GTCCTTGCAAACTCCCGTTCC
R-30 Del-17	GTGGAG^GGAGGCAGGAGGCAGG A ^ N	Del 14	Chr3:128063055-128063076	-	R30_del_17_F	TGTGTGCAGAGGTGAGATCCTATGAG
					R30_del_17_R	GGACCTGGGTTCGTAGGAAGAAAAC
R-30 Del-2	GAAG^GAGGAGGCAGGAGGCTGG T ^ C N	Del 16	Chr8:74322905-74322926	+	R30_del_2_F	AGGCTGCTGACCACAGTGCCTAC
					R30_del_2_R	GGAGTTTATTTCCCTCCTCTTGAAGC
R-30 Del-4	GGAG^GTGGAGGCAGGAGGCTGG T ^ C N	Del 16	Chr20:55620115-55620136	-	R30_del_4_F	AACTGTGAGTGCGGTGACTCTGAAG
					R30_del_4_R	AGCACACCTCTGCTCTCATGGAC
R-30 Del-6	GGTGAG^GGAGGCAGGAGGCAGG TA ^ N	Del 14	Chr7:132937943-132937964	+	R30_del_6_F	TTGGCTTCCTTGGAGCCTAGC
					R30_del_6_R	CAAGGAGGAAAGGGGAGAGCAG
R-30 Del-9	GCAGGGCGG^GGCAGGAGGCTGG T A ^ N	Del 11	Chr10:70883851-70883872	+	R30_del_9_F	GTAATTTGCCCGCCCCTCTC
					R30_del_9_R	CCCTACTCCACTCCTCTTCCCTCAG
R-31 Del-1	GGCTAGCA^TTAAAGGAGTCAGG C A ^ N	Del 12	Chr23:8280850-8280871	+	R31_del_1_F	TGTGTAACAAATTGCCACAAATTTAGC
					R31_del_1_R	GATGTTGATAGCTGCAAGAAACTGG
R-33 Del-1	GCAAG^ACTTAAAGCAGTCCGGG G N	Del 15	Chr15:70302028-70302049	-	R33_del_1_F	CTCATGGGGCAAATGGTCTTCAACC
					R33_del_1_R	CCCCATCACATGAGAGAATGTGGGT
R-35 Del-1	GTGAGGTCCCA^GGCGATCCTGG T N	Del 9	Chr1:47674820-47674841	+	R35_del_1_F	GACGCTGGAGACACATAGAATCCCT
					R35_del_1_R	GTGTTCAATGGGCTATCAGGCTTCC
R-36 Del-2	GCTAGT^AAGAAACAGACTTAGG T ^ N	Del 14	Chr2:34787318-34787339	+	R36_del_2_F	TCTCATTGATCCTCATTGCACTCTG
					R36_del_2_R	AAAGCAAATGTCTTTGGCCACATTG
R-38 Del-5	GATTTTCT^TTGAGGTCCCAAGG T N	Del 12	Chr15:57922643-57922664	+	R38_del_5_F	GGCTTCTCCATAAATGCCCCCATTG
					R38_del_5_R	CACCGGGTAGGAAGTCTATCCACAG
R-39 Del-2	GGAATCAAA^TGAGAAGATGTGG A ^ N	Del 11	Chr5:171805659-171805680	-	R39_del_2_F	AATGCACACCAATGCCAATACTACC
					R39_del_2_R	GGCCTATAGGAGCCACTTTCAAGC
R-39 Del-3	GAAGACAAAG^GAGAAGATGAGG G A ^ N	Del 10	Chr19:8322816-8322837	-	R39_del_3_F	TGGTCCCATCCTATAGCACCTTCTC
					R39_del_3_R	AGGCAGTCCTGGAATCTCAGACAC
R-39 Del-4	AGAATCAAAG^GAGAAGATGAGG G A ^ N	Del 10	Chr20:17602724-17602745	+	R39_del_4_F	GAAGGTGTTCAGCTGTGGAGGTG
					R39_del_4_R	TGACCCAGTATGCTCCTTTCATCAG
R-39 Del-5	GGAAA^AAAGTGAGAACATGTGG G N	Del 15	ChrX:71641287-71641308	+	R39_del_5_F	GTAAACGTCTGCCATGCTGGTCTG
					R39_del_5_R	AGCAGTGGAACTGAATAATAGCAGAGT
R-39 Del-6	GGAAACAAAG^GAGAAGATGTGC N G	Del 10	Chr2:96791029-96791050	-	R39_del_6_F	CCCACTTCAGATCACTCCCACCTAC
					R39_del_6_R	TATCAAGATGGTGAGCATGGGAGCA
R-39 Del-7	GGAAACAAAGT^AGAAGAAGAGG T N	Del 9	Chr20:16523350-16523371	+	R39_del_7_F	ATATGAACAAACACCTGAACGGGGC
					R39_del_7_R	GGATGCATCTCCATTCCTGTACCCT
R-39 Del-8	GGAAACAAAATGA^AAGATGAGG G N	Del 7	Chr10:21061648-21061669	+	R39_del_8_F	AACGCACAGCAATTGTATATGGAGA
					R39_del_8_R	TGGCAAGATTAACCAATTTAGCTACCCAC
R-40 Del-1	GCCTGTTTTT^TGTTTGATGTGG A N	Del 10	Chr8:32701225-32701246	+	R40_del_1_F	TAGTCACTGTTGGTAAGCACATTTCT
					R40_del_1_R	AGCCCAAACTCCAATGGTAAAGCA
R-40 Del-2	GCCTATTTTTG^GTTTGAAGGGG T N	Del 9	Chr3:104520703-104520724	+	R40_del_2_F	AACACGTCTAGGGTCATACCATGTCA
					R40_del_2_R	TCGTTGGTTGAACATCTTTCTCAGTCT

R-41 Del-1	GCAGA^CAGTTGGGGTGTGATGG GCAGA^CAGTTGGGGTA TGATGG N	Del 15	Chr11:11580913-11580934	-	R41_del_1_F	AATAACAGCACCTCCTTCACAGGCT
					R41_del_1_R	CATGAGATTGTAGATGGTGTCAGGTCC
R-42 Del-1	TCCAGC^CTACAGAGCAGTTTGG GG ^ N	Del 14	Chr9:31067668-31067689	-	R42_del_1_F	ATGAGACCACTCCCAAACGAATTG
					R42_del_1_R	TGACCAAATTCTATCAGGTTTATACCAC
R-42 Del-2	GGCAGCAC^ACAGAGCAGATTGG GGCAGCAC^ACAGAGCAGT TGG N	Del 12	Chr4:69223620-69223641	-	R42_del_2_F	TACCACAGAATGCAGCCTTGAATCC
					R42_del_2_R	ACAAAAATTAGCCAGGCATGGTGGT
R-42 Del-3	GGCAGCACTA^AGAGCAGTCGGG GGCAGCACTA^AGAGCAGT TGGG TN	Del 10	Chr20:17812309-17812330	-	R42_del_3_F	GGTCTCGGGAAAGGAGCATTTTGAC
					R42_del_3_R	AAGTCCCAGTCTGCAGGTAACAAGT
R-43 Del-1	CCAGAA^TACAGAGCAGTTGGGG G C ^ N	Del 14	Chr22:37278975-37278996	+	R43_del_1_F	CAGCTAGGACACAGGCTTTGAGG
					R43_del_1_R	ATCACCTCAGCTCTCACATCTAGGG
R-44 Del-1	GCCTG^CTGGTGCTGCTGGCAGG GCCTG^CTGGT TGCTGGCAGG T N	Del 15	Chr17:72942981-72943002	+	R44_del_1_F	ACTGAGTACTGCCTCATCTGCTGTG
					R44_del_1_R	CAATGGCCACGATGGAGAAATAGGC
R-52 Del-1	GTTT^TGTCATTAGTGAAATGGG GTTT^TGTCATTAGTGAAA GGG CN	Del 16	Chr13:31515245-31515266	+	R52_del_1_F	ATTGAAAAGTGGAGTATTGGTAAGACCAT
					R52_del_1_R	CCCAGTTACGGACTCACTGGGATAG
R-53 Del-2	ACAAGTTG^ACTCTCATCTTGGG G C ^ N	Del 11 or 12	Chr14:78919187-78919208	-	R53_del_2_F	TGGGCTTATTAATCAATGGCATCAG
					R53_del_2_R	ACACATGAGGCATTATTGGACTTGG

Supplementary Table S5. Primers used in PCRs for deep sequencing by an Illumina Miseq 2X250 paired-end read. Primers for reaction 1 contains adapter sequences shown (same adapter sequences also present in reaction-2 primers), in addition to gene-specific sequences. Full sequences for primers in reaction 1 can be found in **Supplementary Table S6**. Primers for reaction 2 contain barcodes in the reverse primers, as indicated in red. In the final pooled sample containing all the amplicons, each barcode has similar occurance to insure diversity required by Illumina sequencing. Customer sequencing primers for read 1 (forward), read 2 (reverse), and index read (read barcodes) are used in place of standard Illumina sequencing primers.

Primers for Illumina reaction 1	
Forward	TCTACAGTCCGACGATCA-gene specific sequence
Reverse	GACGTGTGCTCTTCCGATC-gene specific sequence

Primers for Illumina reaction 2	
Forward primer	
Rxn2For	AATGATACGGCGACCACCGAGATCTACACGTTCAGAGTTCTACAGTCCGACGATCA
Reverse primers with 12 different barcodes	
Kozich_bar_1	CAAGCAGAAGACGGCATACGAGatAAGTCGAG ATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
Kozich_bar_2	CAAGCAGAAGACGGCATACGAGatATACTTCG ATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
Kozich_bar_3	CAAGCAGAAGACGGCATACGAGatAGCTGCTA ATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
Kozich_bar_4	CAAGCAGAAGACGGCATACGAGatCATAGAGA ATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
Kozich_bar_5	CAAGCAGAAGACGGCATACGAGatCGTAGATC ATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
Kozich_bar_6	CAAGCAGAAGACGGCATACGAGatCTCGTTAC ATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
Kozich_bar_7	CAAGCAGAAGACGGCATACGAGatGCGCACGT ATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
Kozich_bar_8	CAAGCAGAAGACGGCATACGAGatGGTACTAT ATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
Kozich_bar_9	CAAGCAGAAGACGGCATACGAGatGTATACGC ATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
Kozich_bar_10	CAAGCAGAAGACGGCATACGAGatTACGAGCA ATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
Kozich_bar_11	CAAGCAGAAGACGGCATACGAGatTCAGCGTT ATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
Kozich_bar_12	CAAGCAGAAGACGGCATACGAGatTCGCTACG ATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC

Custom sequencing primer	
NewIndex_Read	GATCGGAAGAGCACACGTCTGAACTCCAGTCACAT
NewRead_1	TCTACACGTTCAGAGTTCTACAGTCCGACGATCA
NewRead_2	TGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC

Supplementary Table S6 (see the attached excel files).

Human genomic loci tested for off-target activity using deep sequencing (Illumina Miseq 2X250 paired-end). On-target loci (_tar), Off-target loci with insertions/DNA bulges (_ins), and deletions/sgRNA bulges (_del), are shown. Also shown are amplicon sequence, primers used for locus-specific PCR, expected sizes of left and right cleavage products (left_prod_len and right_prod_len), mock indel percentages, treated sample indel percentages, fold change of treated to mock %indel, and 2-sided P-values determined by Fisher's exact test comparing treated and mock.