

Supplementary Information

CRISPR prime editing for unconstrained correction of oncogenic *KRAS* variants.

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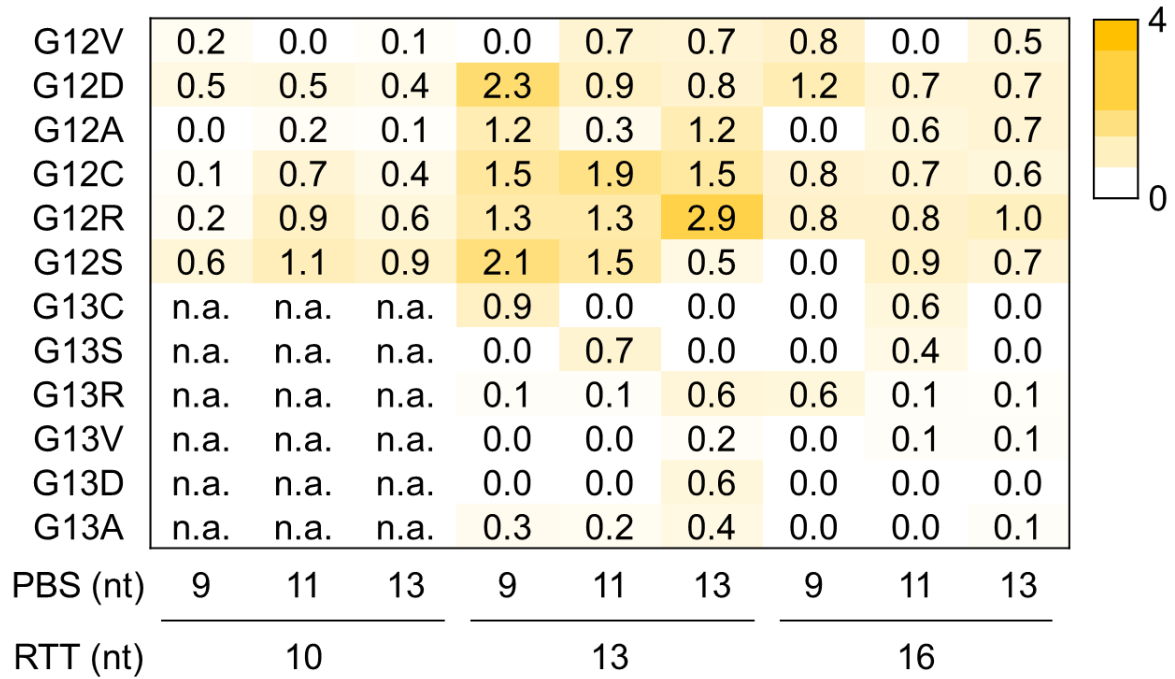
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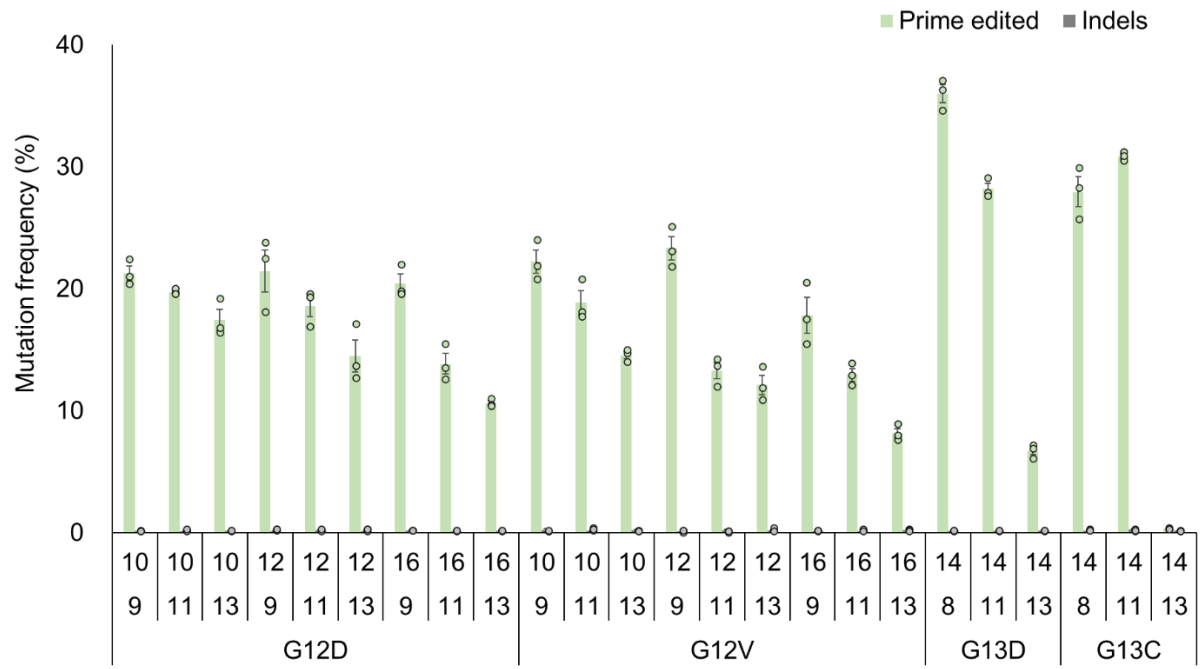
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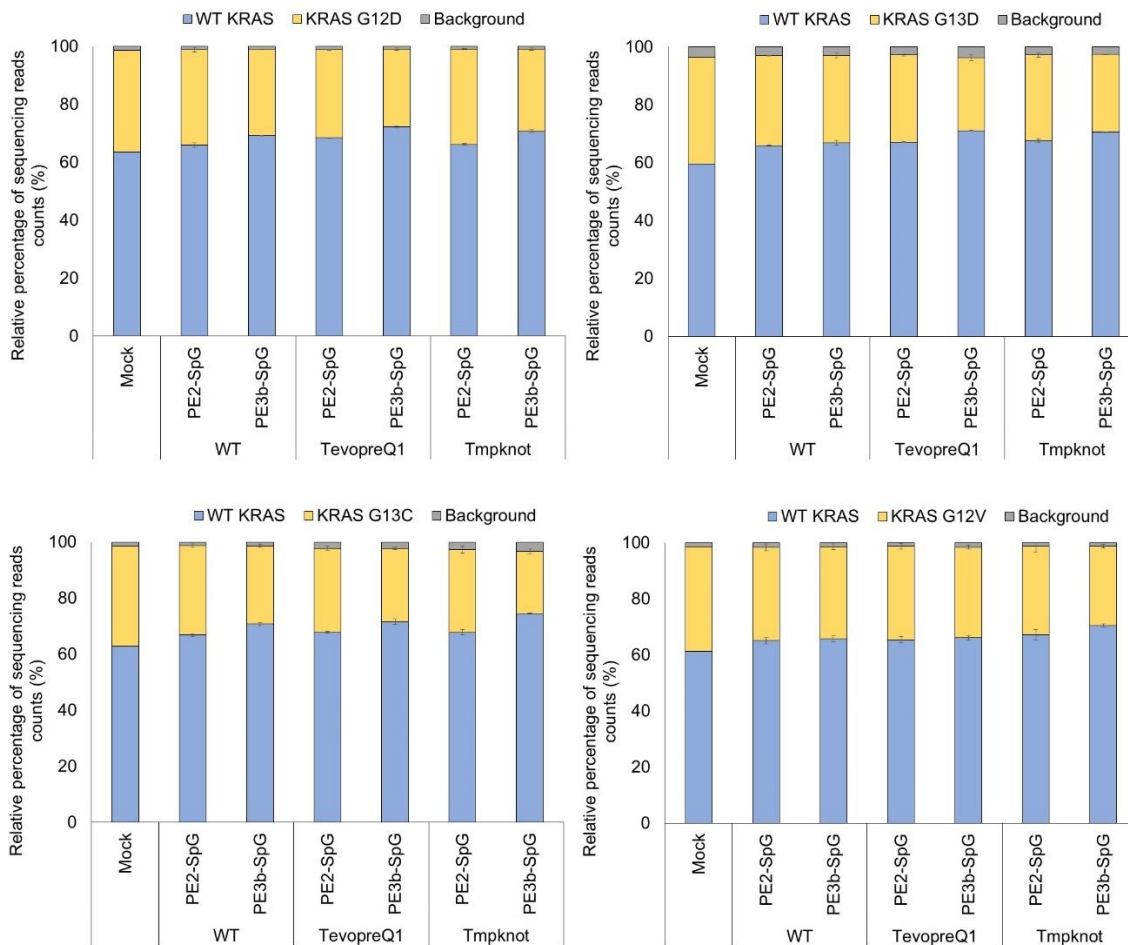
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Supplementary Figure 1. *KRAS* correction frequency of *KRAS*-#1 universal pegRNA in HEK293T/17-*KRAS* library cells.



Supplementary Figure 2. Optimization of pegRNAs to generate *KRAS* heterozygous HEK293T/17 cells. Error bar means s.e.m. of $n=3$ biological triplicate samples.



Supplementary Figure 3. *KRAS* correction frequencies in *KRAS* heterozygous HEK293T/17 cells. Target sites were amplified and analyzed by targeted-deep sequencing. Relative counts of sequencing reads were calculated from total number of sequencing reads of wild-type *KRAS* sequences and mutant *KRAS* sequences. Error bar means s.e.m. of $n=3$ biological triplicate samples.

KRAS G12V mutant alleles of HEK293T/17 heterogenous cells

E Y K L V V V G A V G V G K S A L T
 5' -GAATATAAACTTGTGGTAGTTGGAGC **T**GTGGCGTAGGCAAGAGTGCCTTGACGA-3'
 3' -CTTATATTTGAACACCATCAACCTCGACAACCGC **A**TCCGTTCTCACGGAAGTGC**T**-5'

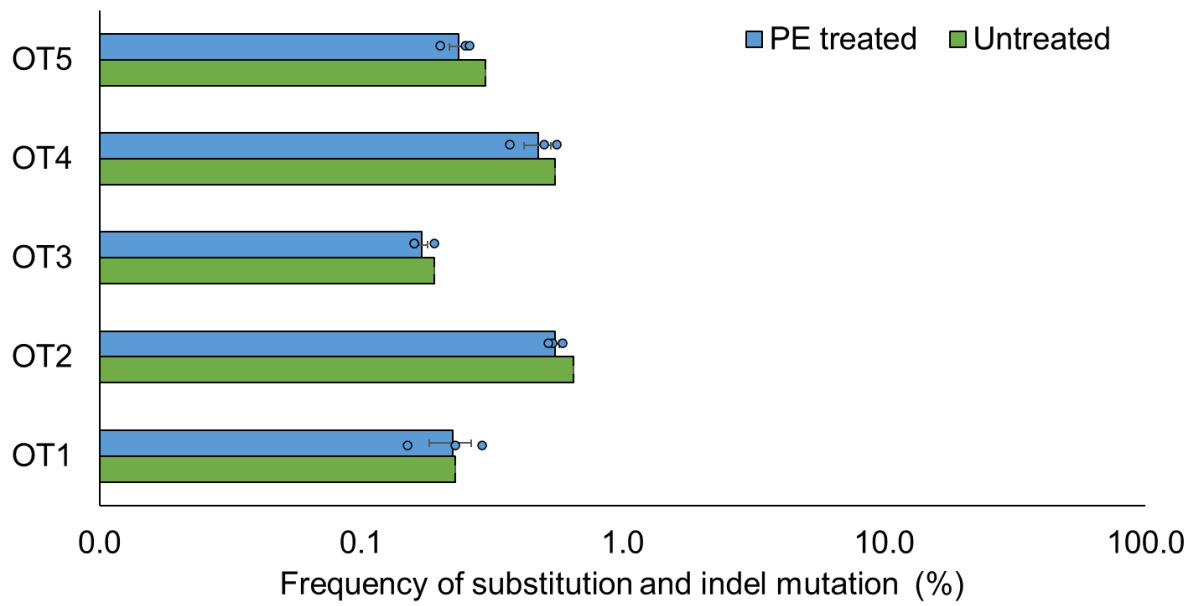
↓
 Prime editing
 using the universal pegRNA
 containing a silent mutation

E Y K L V V V G A G G V G K S A L T
 5' -GAATATAAACTTGTGGTAGTTGGAGC **A**GTGGCGTAGGCAAGAGTGCCTTGACGA-3'
 3' -CTTATATTTGAACACCATCAACCTCGTCCACCGCATCCGTTCTCACGGAAGTGC**T**-5'

Untreated	TAGTTGGAGCT G TGGCGTAGGCAA	(2434/4029)	60.4%
	TAGTTGGAGCT T TGGCGTAGGCAA	(1519/4029)	37.7%
	TAGTTGGAGC A G TGGCGTAGGCAA	(0/4029)	0.0%
	TAGTTGGAGC A T TGGCGTAGGCAA	(0/4029)	0.0%

pegRNA treated	TAGTTGGAGCT G TGGCGTAGGCAA	(2889/4738)	61.0%
	TAGTTGGAGCT T TGGCGTAGGCAA	(1561/4738)	32.9%
	TAGTTGGAGC A G TGGCGTAGGCAA	(206/4738)	4.3%
	TAGTTGGAGC A T TGGCGTAGGCAA	(0/4738)	0.0%

Supplementary Figure 4. *KRAS* correction with pegRNA capable of inducing silent mutation in G12V heterozygous HEK293T/17 cells. The pegRNA could induce T:A to A:T silent mutation at alanine 11 residue and correct *KRAS* G12V mutation. The silent mutation and *KRAS* G12V mutation were highlighted in blue and red, respectively, and the spacer sequences of pegRNA were boxed. NGS sequencing results showed that *KRAS* correction was accompanied by the silent mutation, indicating that *KRAS* correction was induced by prime editing.



Supplementary Figure 5. Analysis of potential off-target effects of the universal epegRNA. Five potential-offtarget sites were selected *in silico* and the universal epegRNA was transfected into HEK293T/17-KRAS G12V cells with PEmax-SpG-hMLH1dn. Targeted-deep sequencing results showed that there were no significant off-target mutations in the five potential off-target sites. The potential off-target sites were listed in Supplementary Table 3. Error bar means s.e.m. of biological triplicate.

Supplementary Table 1. Substitution and indel frequencies at endogenous *KRAS* sites in HEK293T/17-*KRAS* library cells.

pegRNA names	PBS length (nt)	RTT length (nt)	Replicate 1		Replicate 2	
			Substitutions (%)	Indels (%)	Substitutions (%)	Indels (%)
Universal pegRNA #2-1	9	10	1.3%	0.1%	1.3%	0.1%
Universal pegRNA #2-2	11	10	1.3%	0.1%	1.3%	0.1%
Universal pegRNA #2-3	13	10	1.5%	0.1%	1.2%	0.0%
Universal pegRNA #2-4	9	13	1.3%	0.0%	1.2%	0.0%
Universal pegRNA #2-5	11	13	1.5%	0.1%	1.4%	0.0%
Universal pegRNA #2-6	13	13	1.4%	0.0%	1.5%	0.0%
Universal pegRNA #2-7	9	16	1.5%	0.0%	1.2%	0.0%
Universal pegRNA #2-8	11	16	1.4%	0.1%	1.6%	0.0%
Universal pegRNA #2-9	13	16	1.4%	0.1%	1.4%	0.0%
Mock			1.6%	0.1%		

PE systems	PBS length (nt)	RTT length (nt)	RNA scaffolds	Replicate 1		Replicate 2	
				Substitutions (%)	Indels (%)	Substitutions (%)	Indels (%)
PE2-SpG	13	16	Wild-type	0.0%	0.0%	0.6%	0.0%
PE3b-SpG	13	16	Wild-type	0.8%	0.0%	0.6%	0.0%
PE2-SpG	13	16	TevopreQ1	0.8%	0.0%	0.5%	0.0%
PE3b-SpG	13	16	TevopreQ1	0.5%	0.0%	0.6%	0.1%
PE2-SpG	13	16	Tmpknot	0.6%	0.2%	0.6%	0.1%
PE3b-SpG	13	16	Tmpknot	0.5%	0.0%	0.6%	0.1%
Mock				0.8%	0.1%		

Supplementary Table 2. Prime editing and indel frequencies in HEK293T/17-*KRAS* heterogenous cells.

PE systems	PBS length (nt)	RT length (nt)	RNA scaffolds	G12D		G12V		G13D		G13C	
				% of wild-type <i>KRAS</i> sequences	Indels (%)	% of wild-type <i>KRAS</i> sequences	Indels (%)	% of wild-type <i>KRAS</i> sequences	Indels (%)	% of wild-type <i>KRAS</i> sequences	Indels (%)
PE2-SpG	13	16	Wild-type	66.6	0.0	65.1	0.0	66.1	0.0	66.1	0.0
PE3b-SpG	13	16	Wild-type	68.8	0.0	65.8	0.0	67.4	0.0	69.2	0.0
PE2-SpG	13	16	TevopreQ1	68.6	0.0	65.5	0.0	68.3	0.0	67.1	0.0
PE3b-SpG	13	16	TevopreQ1	71.0	0.0	66.2	0.0	71.2	0.0	70.1	0.0
PE2-SpG	13	16	Tmpknot	67.2	0.0	67.2	0.0	68.8	0.0	66.5	0.0
PE3b-SpG	13	16	Tmpknot	69.9	0.0	70.6	0.0	71.2	0.2	72.1	0.0
		Mock		62		61.2		59.5		62.9	

Supplementary Table 3. List of potential off-target sites.

Target mutations		Target sequences (5' to 3')	PAM	Chr.	Mismatches (bp)
	ON	CGTCAAGGCACTCTTGCCTA	CG	chr12	0
c.35T>G (G12V)	OT1	CGTCActGCACTCTTGCCTA	GG	chr19	2
	OT2	aGaCAAGGCACTtTTGCCTA	TG	chr4	3
	OT3	CGTCAtGGgACTCTTtCCCTA	CG	chr10	3
	OT4	aGTCAAGGCACTCcTaCCCTA	TG	chr6	3
	OT5	CGTCActGCACTCTaGCCTA	GG	chr5	3

Supplementary Table 4. *KRAS*-mutant target sequences used in HEK293T/17-*KRAS* library cells.

<i>KRAS</i> mutation	<i>KRAS</i> mutant target sequence(5' to 3')	Barcode sequence
G12V	ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGtTGGCGTAGGCAAGAGTGCCTTGACG	CGAGTAAT
G12D	ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGaTGGCGTAGGCAAGAGTGCCTTGACG	TCTCCGGA
G12A	ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGcTGGCGTAGGCAAGAGTGCCTTGACG	AATGAGCG
G12C	ATGACTGAATATAAACTTGTGGTAGTTGGAGCTtGTGGCGTAGGCAAGAGTGCCTTGACG	GGAATCTC
G12R	ATGACTGAATATAAACTTGTGGTAGTTGGAGCTcGTGGCGTAGGCAAGAGTGCCTTGACG	TTCTGAAT
G12S	ATGACTGAATATAAACTTGTGGTAGTTGGAGCTaGTGGCGTAGGCAAGAGTGCCTTGACG	ACGAATTC
G13C	ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTtGCGTAGGCAAGAGTGCCTTGACG	AGCTTCAG
G13S	ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTaGCGTAGGCAAGAGTGCCTTGACG	GCGCATTa
G13R	ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTcGCGTAGGCAAGAGTGCCTTGACG	CATAGCCG
G13V	ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTgCGTAGGCAAGAGTGCCTTGACG	TTCGCGGA
G13D	ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTgAcGCGTAGGCAAGAGTGCCTTGACG	GCGCGAGA
G13A	ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTgCcGCGTAGGCAAGAGTGCCTTGACG	CTATCGCT

Supplementary Table 5. Sequences of gRNA for PE3b, pegRNAs and epegRNAs used in this study.

gRNA or pegRNA name	Spacer sequence (5' to 3')	PAM	3' extension sequence (5' to 3')	PBS length (nt)	RTT length (nt)
G12D mutant pegRNA #1	AAACTTGTGGTAGTTGGAGC	TGG	GCCAtCAGCTCCAAC TACC	9	10
G12D mutant pegRNA #2	AAACTTGTGGTAGTTGGAGC	TGG	GCCAtCAGCTCCAAC TACCAC	11	10
G12D mutant pegRNA #3	AAACTTGTGGTAGTTGGAGC	TGG	GCCAtCAGCTCCAAC TACCACAA	13	10
G12D mutant pegRNA #4	AAACTTGTGGTAGTTGGAGC	TGG	ACGCCAtCAGCTCCAAC TACC	9	12
G12D mutant pegRNA #5	AAACTTGTGGTAGTTGGAGC	TGG	ACGCCAtCAGCTCCAAC TACCAC	11	12
G12D mutant pegRNA #6	AAACTTGTGGTAGTTGGAGC	TGG	ACGCCAtCAGCTCCAAC TACCACAA	13	12
G12D mutant pegRNA #7	AAACTTGTGGTAGTTGGAGC	TGG	GCCTACGCCAtCAGCTCCAAC TACC	9	16
G12D mutant pegRNA #8	AAACTTGTGGTAGTTGGAGC	TGG	GCCTACGCCAtCAGCTCCAAC TACCAC	11	16
G12D mutant pegRNA #9	AAACTTGTGGTAGTTGGAGC	TGG	GCCTACGCCAtCAGCTCCAAC TACCACAA	13	16
G12V mutant pegRNA #1	AAACTTGTGGTAGTTGGAGC	TGG	GCCAAcAGCTCCAAC TACC	9	10
G12V mutant pegRNA #2	AAACTTGTGGTAGTTGGAGC	TGG	GCCAAcAGCTCCAAC TACCAC	11	10
G12V mutant pegRNA #3	AAACTTGTGGTAGTTGGAGC	TGG	GCCAAcAGCTCCAAC TACCACAA	13	10
G12V mutant pegRNA #4	AAACTTGTGGTAGTTGGAGC	TGG	ACGCCAAcAGCTCCAAC TACC	9	12
G12V mutant pegRNA #5	AAACTTGTGGTAGTTGGAGC	TGG	ACGCCAAcAGCTCCAAC TACCAC	11	12
G12V mutant pegRNA #6	AAACTTGTGGTAGTTGGAGC	TGG	ACGCCAAcAGCTCCAAC TACCACAA	13	12
G12V mutant pegRNA #7	AAACTTGTGGTAGTTGGAGC	TGG	GCCTACGCCAAcAGCTCCAAC TACC	9	16
G12V mutant pegRNA #8	AAACTTGTGGTAGTTGGAGC	TGG	GCCTACGCCAAcAGCTCCAAC TACCAC	11	16
G12V mutant pegRNA #9	AAACTTGTGGTAGTTGGAGC	TGG	GCCTACGCCAAcAGCTCCAAC TACCACAA	13	16
G13C mutant pegRNA #1	CTTGTGGTAGTTGGAGCTGG	TGG	TGCCTACGCaACCAGCTCCAAC	8	14
G13C mutant pegRNA #2	CTTGTGGTAGTTGGAGCTGG	TGG	TGCCTACGCaACCAGCTCCAAC TAC	11	14
G13C mutant pegRNA #3	CTTGTGGTAGTTGGAGCTGG	TGG	TGCCTACGCaACCAGCTCCAAC TACCA	13	14
G13D mutant pegRNA #1	CTTGTGGTAGTTGGAGCTGG	TGG	TGCCTACGtCACCAGCTCCAAC	8	14
G13D mutant pegRNA #2	CTTGTGGTAGTTGGAGCTGG	TGG	TGCCTACGtCACCAGCTCCAAC TAC	11	14
G13D mutant pegRNA #3	CTTGTGGTAGTTGGAGCTGG	TGG	TGCCTACGtCACCAGCTCCAAC TACCA	13	14
Universal pegRNA #1-1	TATAAACTTGTGGTAGTTGG	AG	AccAGCTCCAAC TACCACA	9	10
Universal pegRNA #1-2	TATAAACTTGTGGTAGTTGG	AG	AccAGCTCCAAC TACCACAAG	11	10
Universal pegRNA #1-3	TATAAACTTGTGGTAGTTGG	AG	AccAGCTCCAAC TACCACAAGTT	13	10
Universal pegRNA #1-4	TATAAACTTGTGGTAGTTGG	AG	GccAccAGCTCCAAC TACCACA	9	13
Universal pegRNA #1-5	TATAAACTTGTGGTAGTTGG	AG	GccAccAGCTCCAAC TACCACAAG	11	13
Universal pegRNA #1-6	TATAAACTTGTGGTAGTTGG	AG	GccAccAGCTCCAAC TACCACAAGTT	13	13
Universal pegRNA #1-7	TATAAACTTGTGGTAGTTGG	AG	TACGccAccAGCTCCAAC TACCACA	9	16
Universal pegRNA #1-8	TATAAACTTGTGGTAGTTGG	AG	TACGccAccAGCTCCAAC TACCACAAG	11	16
Universal pegRNA #1-9	TATAAACTTGTGGTAGTTGG	AG	TACGccAccAGCTCCAAC TACCACAAGTT	13	16
Universal pegRNA #2-1	CGTCAAGGCACTCTTGCCCTA	CG	ggTggCGTAGGCAAGAGTG	9	10
Universal pegRNA #2-2	CGTCAAGGCACTCTTGCCCTA	CG	ggTggCGTAGGCAAGAGTGCC	11	10
Universal pegRNA #2-3	CGTCAAGGCACTCTTGCCCTA	CG	ggTggCGTAGGCAAGAGTGCCCT	13	10
Universal pegRNA #2-4	CGTCAAGGCACTCTTGCCCTA	CG	GCTggTggCGTAGGCAAGAGTG	9	13
Universal pegRNA #2-5	CGTCAAGGCACTCTTGCCCTA	CG	GCTggTggCGTAGGCAAGAGTGCC	11	13
Universal pegRNA #2-6	CGTCAAGGCACTCTTGCCCTA	CG	GCTggTggCGTAGGCAAGAGTGCCCT	13	13
Universal pegRNA #2-7	CGTCAAGGCACTCTTGCCCTA	CG	GGAGCTggTggCGTAGGCAAGAGTG	9	16
Universal pegRNA #2-8	CGTCAAGGCACTCTTGCCCTA	CG	GGAGCTggTggCGTAGGCAAGAGTGCC	11	16
Universal pegRNA #2-9	CGTCAAGGCACTCTTGCCCTA	CG	GGAGCTggTggCGTAGGCAAGAGTGCCCT	13	16
PE3b Correction gRNA #1	GCACCTTTGCCTACGCCACC	AG	N/A	N/A	N/A
PE3b Correction gRNA #2	GTGGTAGTTGGAGCTGGTGG	CG	N/A	N/A	N/A
pegRNA-silent mutation	CGTCAAGGCACTCTTGCCCTA	CG	GGAGCaggTggCGTAGGCAAGAGTGCCCT	13	16

epegRNA name	Spacer sequence (5' to 3')	PAM	3' extension sequence (5' to 3')	PBS length (nt)	RTT length (nt)	Linker
TevopreQ1 epegRNA #2-9	CGTCAAGGCACTCTTGCCCTA	CG	GGAGCTggTggCGTAGGCAAGAGTGCCCT	13	16	CTTAAGTA
Tmpknot epegRNA #2-9	CGTCAAGGCACTCTTGCCCTA	CG	GGAGCTggTggCGTAGGCAAGAGTGCCCT	13	16	TAATTATA

Supplementary Table 6. Primer sequences used in this study.

Target sites	Primer name	Forward primers	Reverse primers
Endogenous <i>KRAS</i>	1st	CTTAAGCGTCGATGGAGGAG	CCCTGACATACTCCAAGGA
	2nd	ACACTCTTCCCTACACGACGCTCTCCGATCTAGGCCTGCTGAAAATGACTG	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTTCATGAAAATGGTCAGAGAAACC
Lenti_ <i>KRAS</i> sequence (HEK293T/17- <i>KRAS</i> library cells)	1st	GTGTACGGTGGGAGGCCTAT	GTCCGCTGCGAGGGTACTA
	2nd	ACACTCTTCCCTACACGACGCTCTCCGATCTGCTCGTTTAGTGAACCGTCAG	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTACGTGAAGAATGTGCGAGA
Universal pegRNA #2 OT1	2nd	ACACTCTTCCCTACACGACGCTCTCCGATCTTGCCAGTAGTCCCAGCTA	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTTACAAAAGAACCATGGAAGG
Universal pegRNA #2 OT2	2nd	ACACTCTTCCCTACACGACGCTCTCCGATCTTGGATTTGGACTTGCATGA	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTGTCCCCAAAGCCTTAATC
Universal pegRNA #2 OT3	2nd	ACACTCTTCCCTACACGACGCTCTCCGATCTTGAGAAATGCATCGGCACAC	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTTTCAGCGCTTTCAGTGTCA
Universal pegRNA #2 OT4	2nd	ACACTCTTCCCTACACGACGCTCTCCGATCTTACACCCCTCAAATTGGCA	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTTGCCTGCTGATGGACATTA
Universal pegRNA #2 OT5	2nd	ACACTCTTCCCTACACGACGCTCTCCGATCTGCAGATACCCAGGAGAGCTT	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTAGGATCCCTTGAGCGCTG