

Supporting Information

Altered DNA repair pathway engagement by engineered CRISPR-Cas9 nucleases

Vikash P. Chauhan ^{1,2}, Phillip A. Sharp ^{1,2*}, and Robert Langer ^{1,3*}

¹ *Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.*

² *Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.*

³ *Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.*

* *Correspondence to: sharppa@mit.edu (PAS), rlanger@mit.edu (RL)*

Supporting Figures

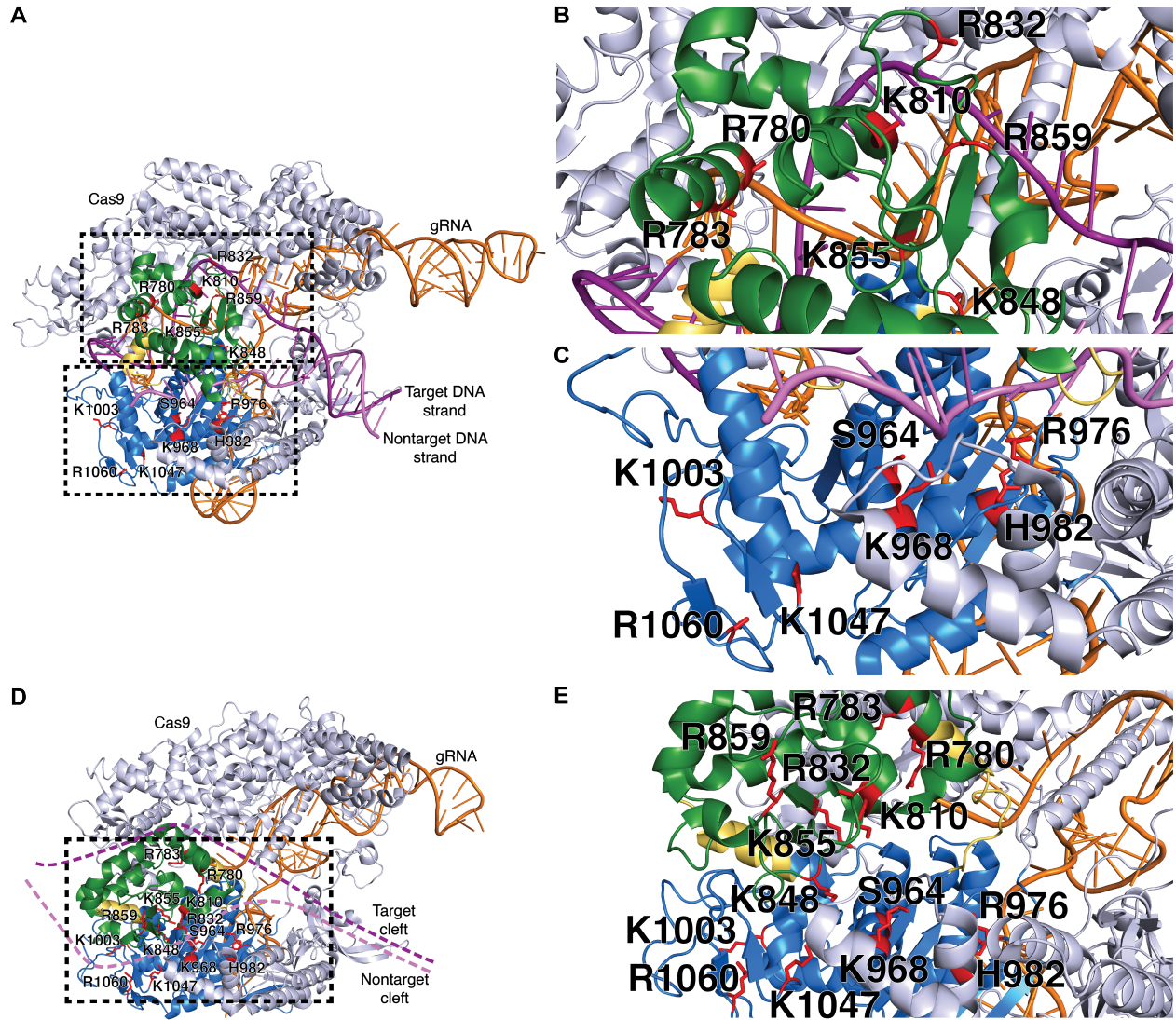


Figure S1. Detailed views of mutated Cas9 residues. (A) Wide view of Cas9 residues at the interface with the substrate DNA strands that were selected for mutation and screening. The mutated residues (red) are located in either the mobile HNH domain (green) or the immobile RuvC domain (blue), which are connected by linkers (yellow). (B) Focused view of the residues from A in the target cleft. (C) Focused view of the residues from A in the nontarget cleft. (D) Location of the same Cas9 residues in a structure of Cas9 without the DNA substrate bound. (E) Focused view of the residues from D in the nontarget cleft.

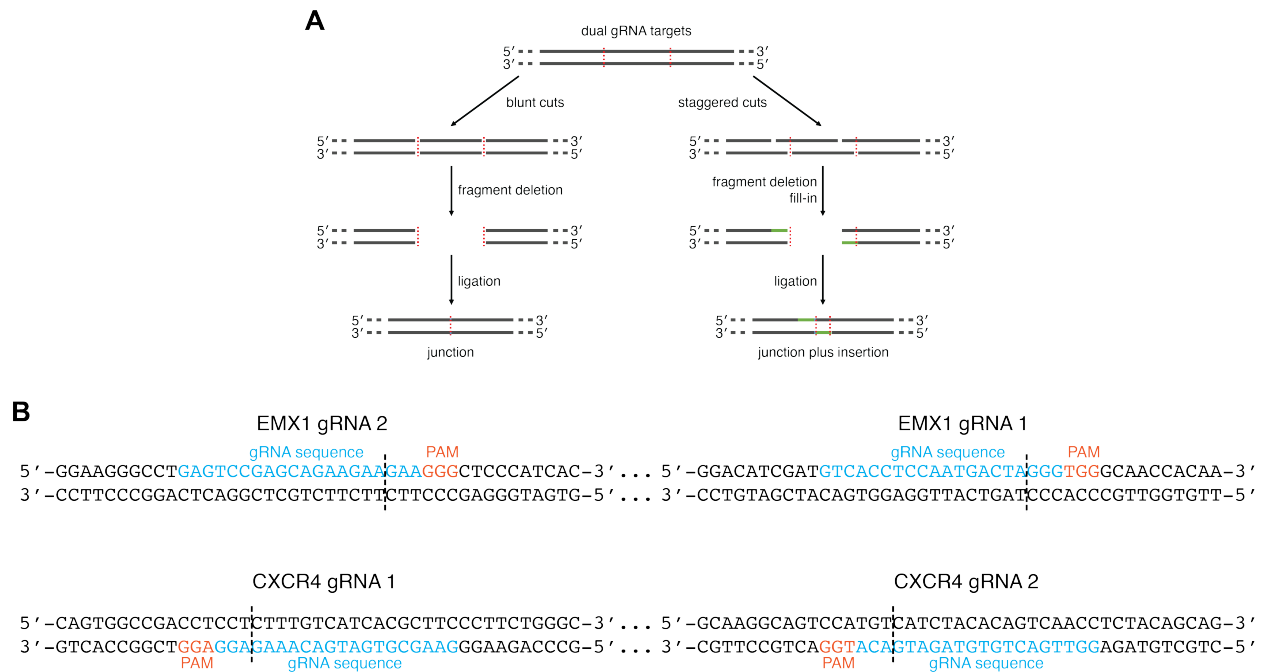


Figure S2. Assay for determining DNA break structure induced by Cas9 variants. (A) Assay for DNA break structure, where paired DSBs lead to perfect deletion junctions for blunt cuts and insertions in the deletion junctions for staggered cuts. The sequences of the insertions indicate cut positions in each strand. **(B)** Design of gRNA pairs to introduce deletion junctions.

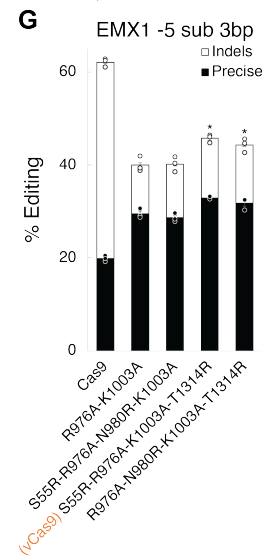
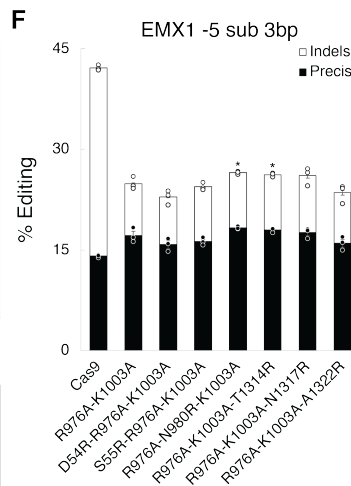
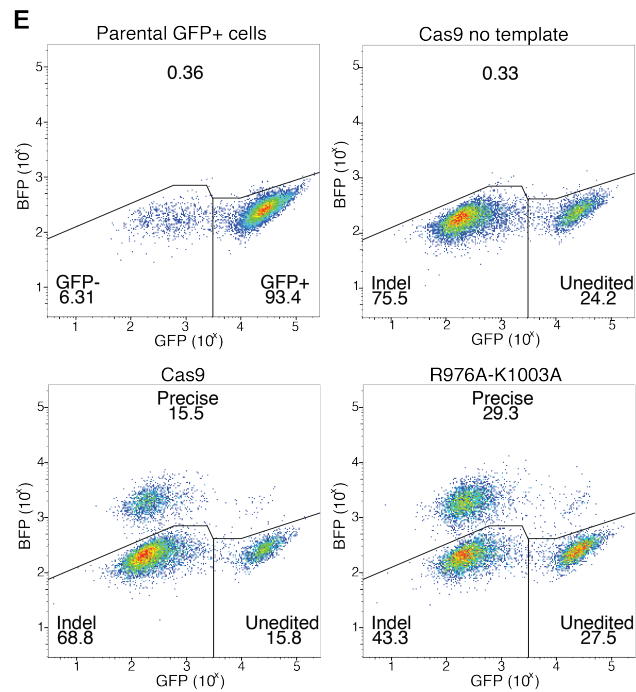
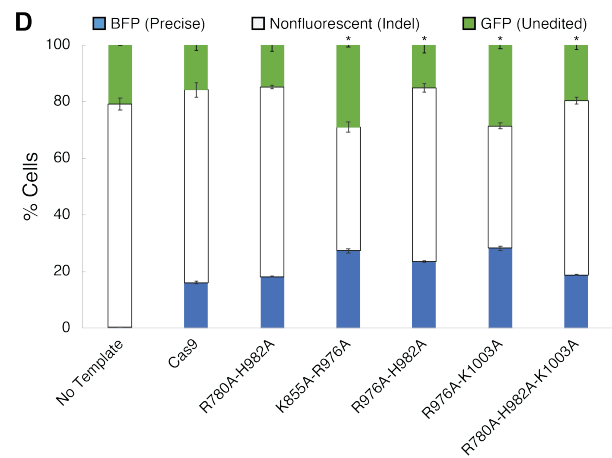
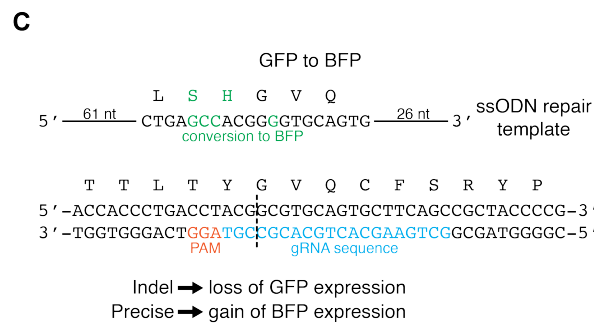
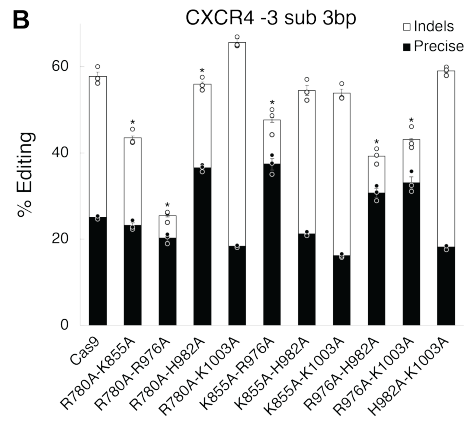
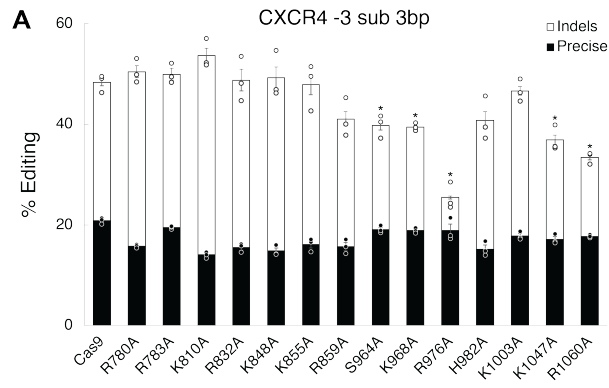


Figure S3. Rational engineering produces Cas9 variants with increased HDR frequency. (A, B) Screen of precise editing and indel frequencies for engineered Cas9 **(A)** single-mutant and **(B)** double-mutant variants using an HDR template. **(C)** Design of a gRNA and HDR template to introduce a GFP to BFP conversion. **(D)** Frequency of precise gene conversion from GFP to BFP. **(E)** Flow cytometry plots showing BFP-positive (indicating precise edits), nonfluorescent (indicating indels), and GFP-positive (indicating unedited cells) cell population fractions. **(F, G)** Screen of precise editing and indel frequencies for engineered Cas9 **(F)** triple-mutant and **(G)** quadruple-mutant variants using an HDR template. * indicates $p < 0.05$ for precise editing frequency compared to wild-type Cas9 in **A, B, D** or for total editing frequency compared to wild-type Cas9 in **F, G**. Data were analyzed by deep sequencing in **A, B, F, G** or flow cytometry in **D, E** and represent means of $n = 3$ independent replicates with standard errors.

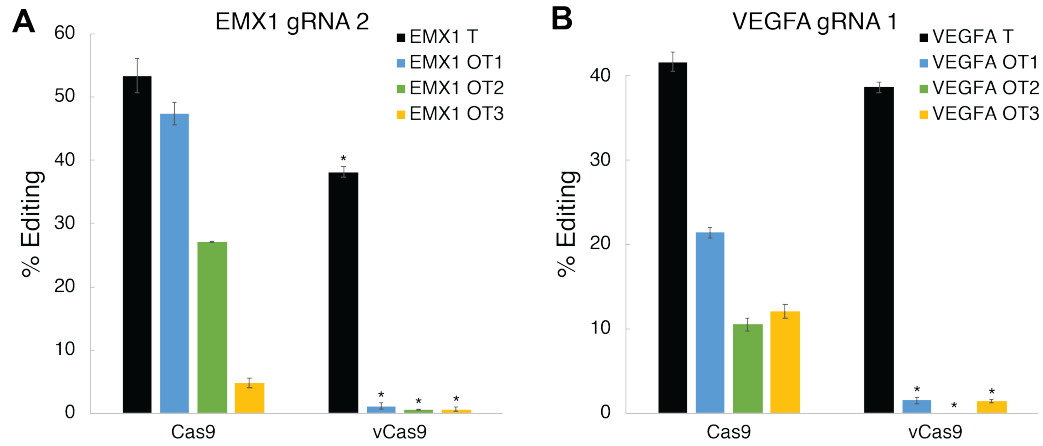


Figure S4. vCas9 improves on-target versus off-target editing specificity. (A, B) Indel frequencies at on-target (T) and off-target (OT1-OT3) loci. * indicates $p < 0.05$ for indel frequency compared to wild-type Cas9. Data were analyzed by Sanger sequencing and represent means of $n = 2-3$ independent replicates with standard errors.

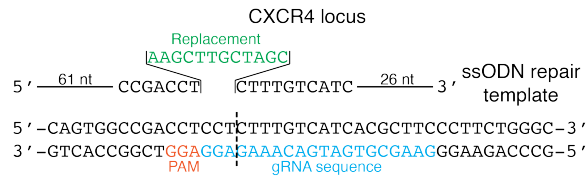
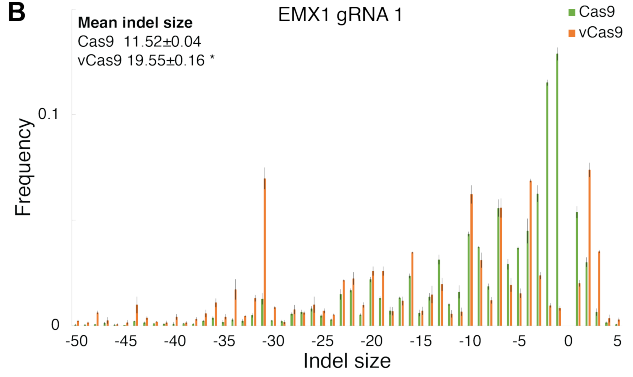
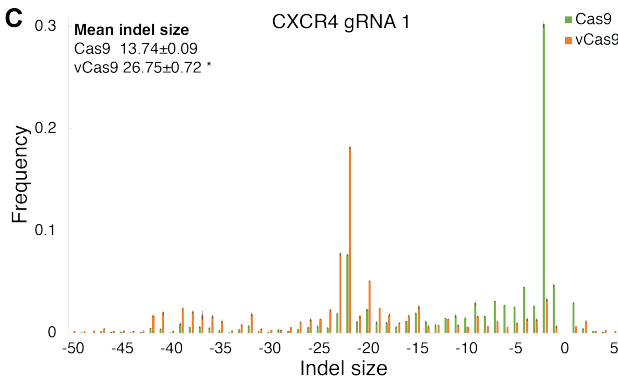
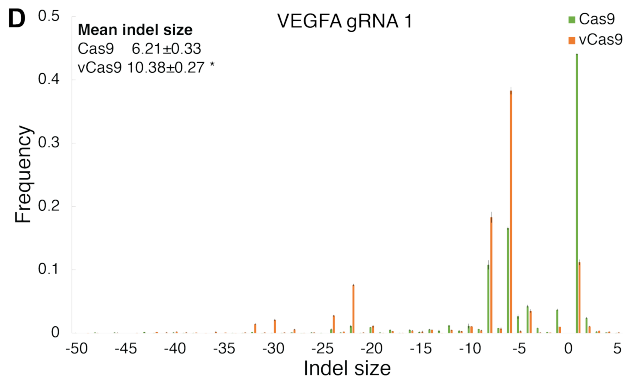
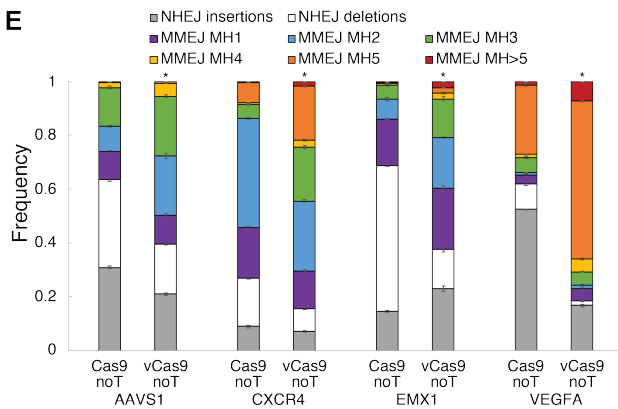
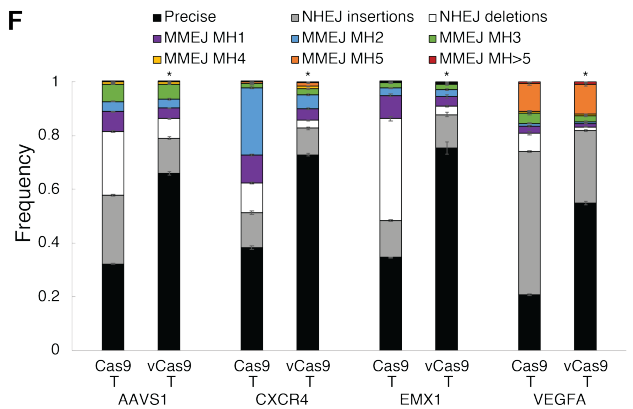
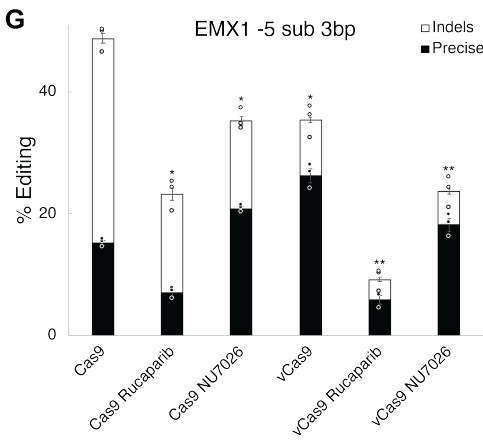
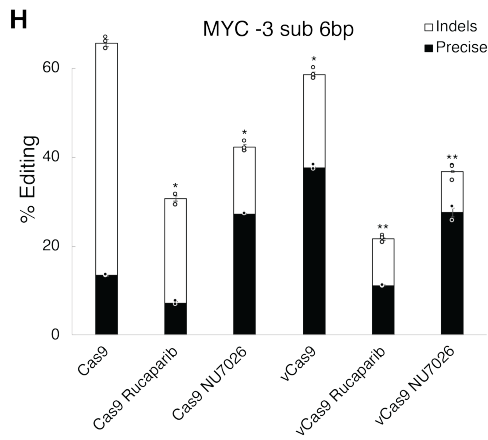
A**B****C****D****E****F****G****H**

Figure S5. Engineered Cas9 variants suppress small NHEJ indels and promote larger MMEJ indels.

(A) Design of a gRNA and HDR template to introduce precise edits. (B-D) Distributions of indel sizes induced. (E, F) Frequencies of repair pathways engaged (E) without (noT) and (F) with (T) a repair template at several loci. NHEJ mutations are subset into insertions or deletions, while MMEJ mutations are subset by microhomology length (MH1 to MH>5). (G, H) Precise editing and indel frequencies using HDR templates without or with an MMEJ inhibitor (Rucaparib) or NHEJ inhibitor (NU7026). * indicates $p < 0.05$ for indel size compared to wild-type Cas9 in B-D, for NHEJ frequency compared to wild-type Cas9 in E, F, or for precise editing frequency compared to wild-type Cas9 in G, H. ** indicates $p < 0.05$ for precise editing frequency compared to vCas9. Data were analyzed by deep sequencing and represent means of $n = 3$ independent replicates with standard errors.

Figure S6. vCas9 suppresses NHEJ and promotes MMEJ. (A, B) Rates of the top sequences resulting from editing with Cas9 variants at the (A) EMX1 and (B) AAVS1 loci. Substitutions, insertions, and deletions are depicted. Deletions at microhomologies are labeled. The most frequent fifteen sequences are displayed along with percentages out of all sequencing reads. Data were analyzed by deep sequencing and represent a single replicate.

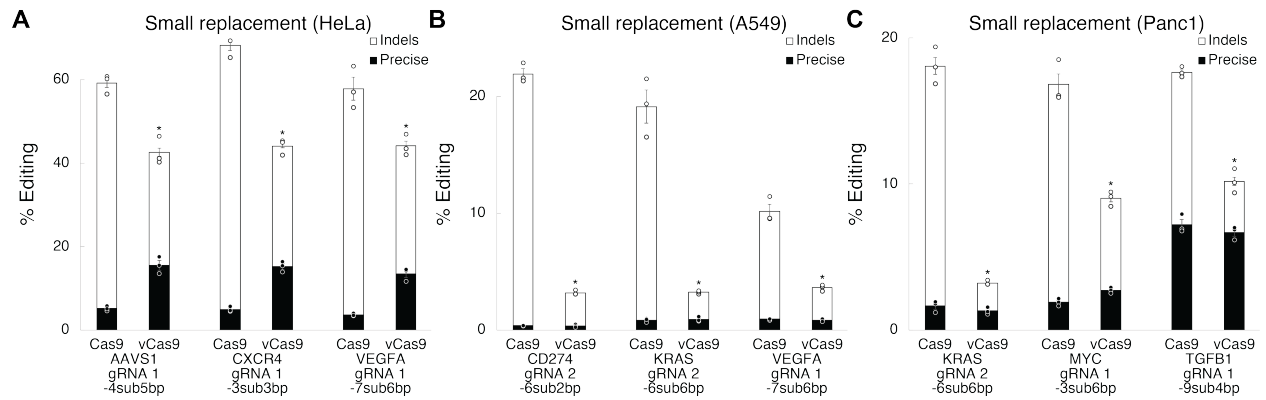


Figure S7. vCas9 enhances precise editing and suppresses indels across cell types. (A-C) Precise editing and indel frequencies using small replacement (<10 bp) HDR templates at several loci in (A) HeLa, (B) A549, and (C) Panc1 cells. * indicates $p < 0.05$ for precise editing frequency compared to wild-type Cas9. Data were analyzed by deep sequencing and represent means of $n = 3$ independent replicates with standard errors.

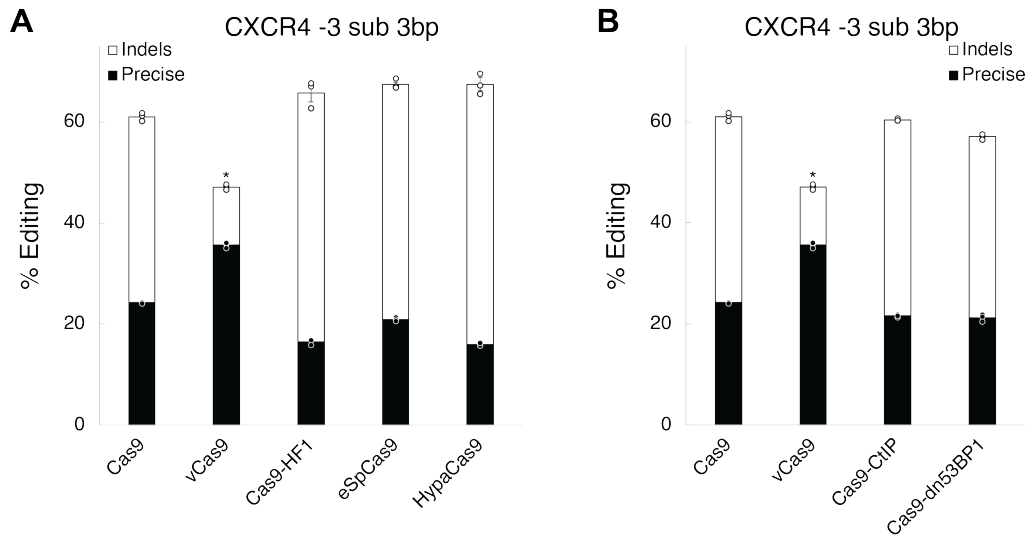


Figure S8. vCas9 enhances precise editing versus other Cas9 variants and fusions. (A, B) Precise editing and indel frequencies using an HDR template. * indicates $p < 0.05$ for precise editing frequency compared to wild-type Cas9. Data were analyzed by deep sequencing and represent means of $n = 3$ independent replicates with standard errors.

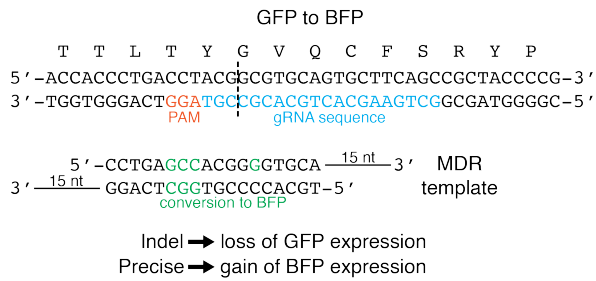
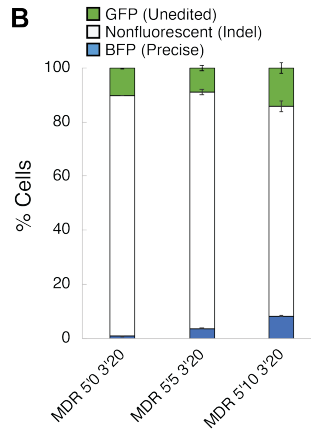
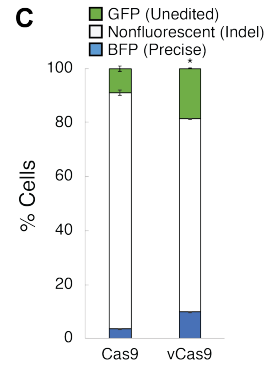
A**B****C**

Figure S9. MDR enables efficient precise editing through MMEJ. (A) Design of a gRNA and MDR template to introduce a GFP to BFP conversion. **(B)** Precise editing and indel frequencies using MDR templates with varied microhomology arm lengths (0-10 bp at the 5' ends, 20 bp at the 3' ends). **(C)** Frequency of precise gene conversion from GFP to BFP. Data were analyzed by flow cytometry and represent means of $n = 3$ independent replicates with standard errors.

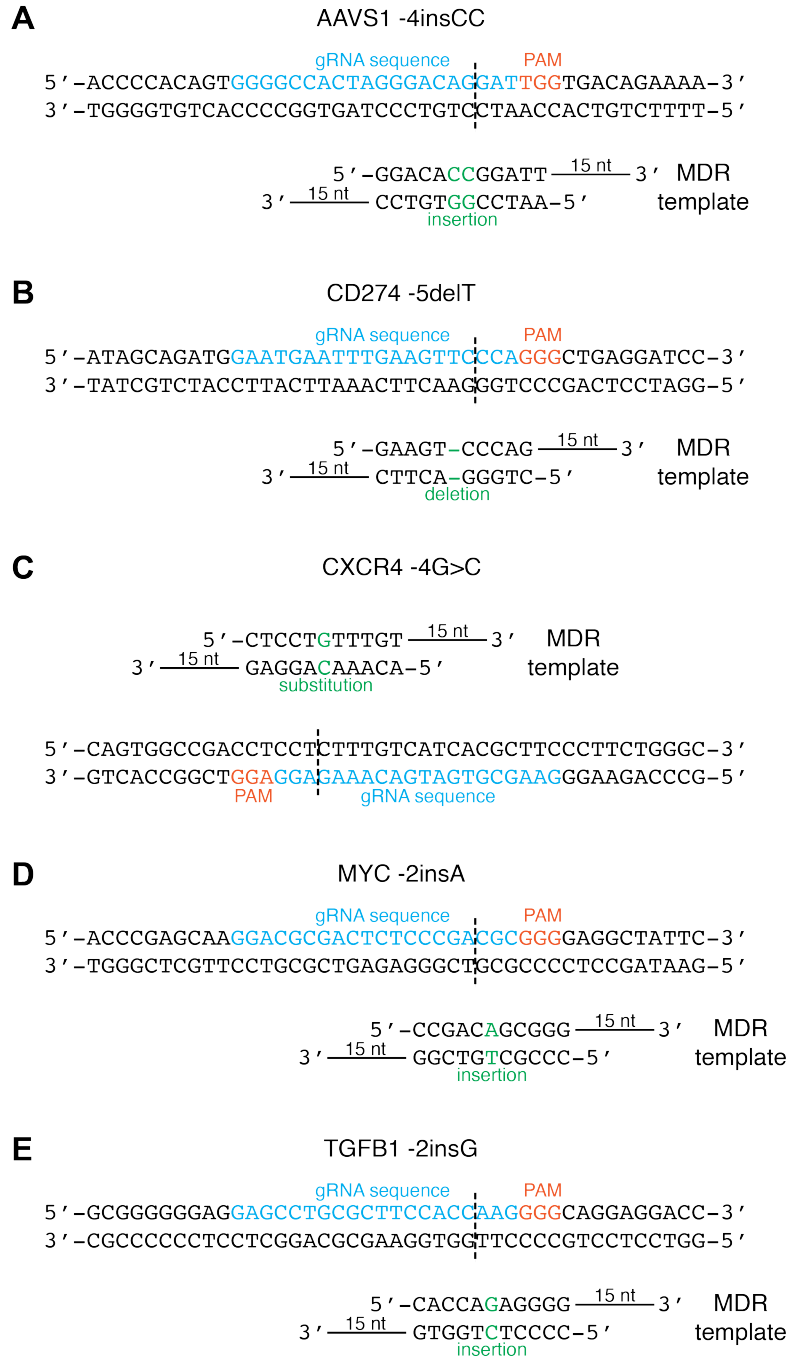


Figure S10. Additional designs for precise editing through MMEJ by MDR. (A-E) Design of gRNAs and MDR templates to introduce precise edits.

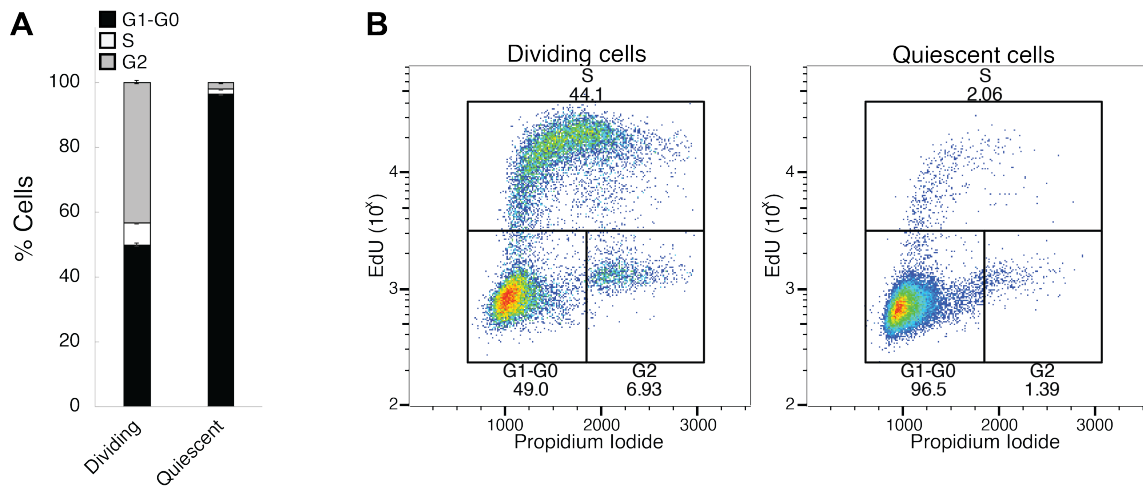


Figure S11. Quiescent primary human dermal fibroblasts are nearly all non-dividing. (A) Cell cycle profiling of dividing and quiescent primary human dermal fibroblasts. **(B)** Flow cytometry plots showing EdU-high (indicating S phase), Propidium Iodide-high (indicating G2 phase), and EdU-low Propidium Iodide-low (indicating G1 or G0 phase) cell population fractions. All data were analyzed by flow cytometry and represent means of $n = 3$ independent replicates with standard errors.

Supporting Tables

Name	Sequence (5' - 3')
cas9-mut-FWD	GGTTGGACCGGTGCCACC
cas9-mid-REV	GGCCAGAGGGCCCACGTAGTAGG
cas9-mid-FWD	CCTACTACGTGGGCCCTCTGGCC
cas9-mut-REV	CTCTAGGAATTCTTACTTTTTCTTTTTGCCTGGCC
R780A-BOT	CCCTCTTCGATCCGCTTCATGGCCTCGCGGCTGTTCTTCTGTCC
R780A-TOP	GGACAGAAGAACAGCCGCGAGGCCATGAAGCGGATCGAAGAGGG
R783A-BOT	GCTCTTTGATGCCCTCTTCGATGGCCTTCATTCTCTCGCGGCTGTTCC
R783A-TOP	GAACAGCCGCGAGAGAATGAAGGCCATCGAAGAGGGCATCAAAGAGC
K810A-BOT	TCTGCAGGTAGTACAGGTACAGGGCCTCGTTCTGCAGCTGGGTGTTT
K810A-TOP	AAACACCCAGCTGCAGAACGAGGCCCTGTACCTGTACTACCTGCAGA
R832A-BOT	CCACATCGTAGTCCGACAGGGCGTTGATGTCCAGTTCCTGGTCC
R832A-TOP	GGACCAGGAACTGGACATCAACGCCCTGTCCGACTACGATGTGG
K848A-BOT	CCTTGTTGTCGATGGAGTCGTCGGCCAGAAAGCTCTGAGGCACGATA
K848A-TOP	TATCGTGCCTCAGAGCTTTCTGGCCGACGACTCCATCGACAACAAGG
K855A-BOT	TTGTCGCTTCTGGTCAGCACGGCGTTGTCGATGGAGTCGTCCT
K855A-TOP	AGGACGACTCCATCGACAACGCCGTGCTGACCAGAAGCGACAA
S964A-BOT	AAATCCTTCCGGAATCGGGCCACCAGCTTGGACTTCAG
S964A-TOP	CTGAAGTCCAAGCTGGTGGCCGATTTCCGGAAGGATTT
K968A-BOT	CGCACTTTGTAAACTGGAAATCGGCCCGGAAATCGGACACCAGCTTGG
K968A-TOP	CCAAGCTGGTGTCCGATTTCCGGGCCGATTTCCAGTTTTACAAAGTGCG
R976A-BOT	GCGTGGTGGTAGTTGTTGATCTCGGCCACTTTGTAAACTGGAAATCCT
R976A-TOP	AGGATTTCCAGTTTTACAAAGTGGCCGAGATCAACAACCTACCACCACGC
H982A-BOT	GTAGGCGTCGTGGGCGTGGGCGTAGTTGTTGATCTCGCG
H982A-TOP	CGCGAGATCAACAACCTACGCCACGCCACGACGCCTAC
K1003A-BOT	CGTACACGAACTCGCTTTCCAGGGCAGGGTACTTTTTGATCAGGGCG
K1003A-TOP	CGCCCTGATCAAAAAGTACCCTGCCCTGGAAAGCGAGTTCGTGTACG
K1047A-BOT	GCCGTTGGCCAGGGTAATCTCGGTGGCGAAAAGTTCATGATGTTGCTG
K1047A-TOP	CAGCAACATCATGAACTTTTTCGCCACCGAGATTACCCTGGCCAACGGC
R1060A-BOT	CCGTTTGTCTCGATCAGAGGGGCTTCCGGATCTCGCCGTTGG
R1060A-TOP	CCAACGGCGAGATCCGGAAGGCCCTCTGATCGAGACAAACGG
R976A-H982A-BOT	GTAGGCGTCGTGGGCGTGGGCGTAGTTGTTGATCTCGGC
R976A-H982A-TOP	GCCGAGATCAACAACCTACGCCACGCCACGACGCCTAC
D54R-BOT	CGGCTGTTTCGCCGCTCCGGAACAGCAGGGCTCCG
D54R-TOP	CGGAGCCCTGCTGTTCCGGAGCGGCGAAACAGCCG
S55R-BOT	CTCGGCTGTTTCGCCCGGTGCAACAGCAGGGC
S55R-TOP	GCCCTGCTGTTTCGACCGGGGCGAAACAGCCGAG
N980R-BOT	CGTCGTGGGCGTGGTGGTACCGGTTGATCTCGGCCACTTTG

N980R-TOP	CAAAGTGGCCGAGATCAACCGGTACCACCACGCCACGACG
T1314R-BOT	CAGGGGCTCCCAGATTGGTCAGCCGAAACAGGTGGATGATATTCTCG
T1314R-TOP	CGAGAATATCATCCACCTGTTTCGGCTGACCAATCTGGGAGCCCCTG
N1317R-BOT	AGGCGGCAGGGGCTCCCAGCCGGTCAGGGTAAACAGGTGG
N1317R-TOP	CCACCTGTTTACCCTGACCCGGCTGGGAGCCCCTGCCGCCT
A1322R-BOT	GTGGTGTCAAAGTACTTGAAGGCCCGAGGGGCTCCCAGATTGGTCAGGG
A1322R-TOP	CCCTGACCAATCTGGGAGCCCCTCGGGCCTTCAAGTACTTTGACACCAC

Table S1. Oligodeoxynucleotide sequences used for mutagenesis and cloning.

Name	Sequence (5' - 3')
gRNA-scaffold-NheI-FWD	CTCAGCTAGCGAGGGCCTATTTCCCATGATTCCCTTCATATTTGC
gRNA-scaffold-EcoRI-REV	ATCAGAATTCAAAAAAGCACCGACTCGGTGCCACTT
AAVS1-gRNA1-BOT	AAACCTAGGGACAGGATTGGTGAC
AAVS1-gRNA1-TOP	CACCGTCACCAATCCTGTCCCTAG
AAVS1-gRNA2-BOT	AAACATCCTGTCCCTAGTGGCCCC
AAVS1-gRNA2-TOP	CACCGGGGCCACTAGGGACAGGAT
ASNS-gRNA1-BOT	AAACTGCGCCCCGCGCCAGCATCC
ASNS-gRNA1-TOP	CACCGGATGCTGGCGCGGGGCGCA
CD274-gRNA1-BOT	AAACGGGAACCTTCAAATTCATTCC
CD274-gRNA1-TOP	CACCGGAATGAATTTGAAGTTCCC
CD274-gRNA2-BOT	AAACTGGGAACCTTCAAATTCATTCC
CD274-gRNA2-TOP	CACCGAATGAATTTGAAGTTCCCA
CXCR4-gRNA1-BOT	AAACCCTCTTTGTCATCACGCTTC
CXCR4-gRNA1-TOP	CACCGAAGCGTGATGACAAAGAGG
EMX1-gRNA1-BOT	AAACCCCTAGTCATTGGAGGTGAC
EMX1-gRNA1-TOP	CACCGTCACCTCCAATGACTAGGG
EMX1-gRNA2-BOT	AAACTTCTTCTTCTGCTCGGACTC
EMX1-gRNA2-TOP	CACCGAGTCCGAGCAGAAGAAGAA
GFP-gRNA1-BOT	AAACACGGCGTGCAAGTGCTTCAGC
GFP-gRNA1-TOP	CACCGCTGAAGCACTGCACGCCGT
HOTAIR-gRNA1-BOT	AAACCACCGCAGTTCTAGGCAAGC
HOTAIR-gRNA1-TOP	CACCGCTTGCTAGAACTGCGGTG
KRAS-gRNA1-BOT	AAACCGATTATTATCAGCCTCAGC
KRAS-gRNA1-TOP	CACCGCTGAGGCTGATAATAATCG
KRAS-gRNA2-BOT	AAACCCCCGATTATTATCAGCCTC
KRAS-gRNA2-TOP	CACCGAGGCTGATAATAATCGGGG
MYC-gRNA1-BOT	AAACGCGTCGGGAGAGTCGCGTCC
MYC-gRNA1-TOP	CACCGGACGCGACTCTCCCGACGC
MYC-gRNA2-BOT	AAACCGCGTCGGGAGAGTCGCGTC
MYC-gRNA2-TOP	CACCGACGCGACTCTCCCGACGCG
MYC-gRNA3-BOT	CACCGCGACTCTCCCGACGCGGGG
MYC-gRNA3-TOP	AAACCCCCGCGTCGGGAGAGTCGC
STAT1-gRNA1-BOT	AAACCCAGCTGCAAGCATGTCATC
STAT1-gRNA1-TOP	CACCGATGACATGCTTGCAGCTGG
STAT1-gRNA2-BOT	AAACCAGCTGCAAGCATGTCATCC
STAT1-gRNA2-TOP	CACCGGATGACATGCTTGCAGCTG
TGFB1-gRNA1-BOT	AAACCTTGGTGGAAGCGCAGGCTC
TGFB1-gRNA1-TOP	CACCGAGCCTGCGCTTCCACCAAG
THORLNC-gRNA1-BOT	AAACCTTTGTTACATCATCTCAC

THORLNC-gRNA1-TOP	CACCGTGAGATGATGTGAACAAAG
TP53-gRNA1-BOT	AAACTCCAGGTCCCCAGCCCAACC
TP53-gRNA1-TOP	CACCGGTTGGGCTGGGGACCTGGA
VEGFA-gRNA1-BOT	AAACCACGCACACACTCACTCACC
VEGFA-gRNA1-TOP	CACCGGTGAGTGAGTGTGTGCGTG
VEGFA-gRNA2-BOT	AAACACACGCACACACTCACTCAC
VEGFA-gRNA2-TOP	CACCGTGAGTGAGTGTGTGCGTGT

Table S2. Oligodeoxynucleotide sequences used for gRNA cloning.

Name	Sequence (5' - 3')
AAVS1-HDRtemplate	CAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCC ACCCACAGTGGGGCCGCTAGCAAGCTTGGACAGGATTGGTGACAGAAA AGCCCCATCCTTAG
CD274-HDRtemplate	TATGAAAGATAATGAAAAGCTATGGGAAAGATAACTTAGAAACAAAGAAGG CATGGATCCTCAGCCCTGGGCTAGCAAGCTTCAAATTCATTCCATCTGCTA TATAAGAAACA
CXCR4-HDRtemplate	ATGGGTACCAGAAGAACTGAGAAGCATGACGGACAAGTACAGGCTGCA CCTGTCAGTGGCCGACCTGCTAGCAAGCTTCTTTGTCATCACGCTTCCCTT CTGGGCAGTTGATGC
EMX1-HDRtemplate	CTTGGGCCACGCAGGGCCTGGCCAGCAGCAAGCAGCACTCTGCCCTC GTGGGTTTGTGGTTGCCACCCTAGCAAGCTTGTCAATTGGAGGTGACAT CGATGTCCTCCCCATTG
GFP-BFP-HDRtemplate	CCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACC CTCGTGACCACCTGAGCCACGGGGTGCAGTGCTTCAGCCGCTACCCCG ACCACATGA
KRAS-HDRtemplate	GGCGACTTCGGGGACTTAGGGAGACCGGGCGGACGATTTCCACACCGG GGCTGTCTGATCGCCGGCTAGCAAGCTTATTATCAGCCTCAGCACTTGGG CTGGGAATTTAG
MYC-HDRtemplate	AGCCTTTCAGAGAAGCGGGTCTGGCAGCGGCGGGGAAGTGTCCCCAAA TGGGCAGAATAGCCTCGCTAGCAAGCTTCGGGAGAGTGCAGTCTTGGCTC GGGTGTTGTAAGT
STAT1-HDRtemplate	GTATTTCTAATAGACTTGAAAGGACAGCCAGGAGCAAAGATGGGCAGAAG GACAACCTGTTTCCCCAAGCTTGTAGCAAGCATGTCATCCTCACATTTGG CCCCTTGGCCC
TGFB1-HDRtemplate	ACCCTGAGAGGAACTGGGACTTTGGGGTCCAGACTGCCAGCGTTTAGCG CAGCGGGTCTCCTGCCCCTTGAAGCTTGTAGCGCAGGCTCCTCCC CCCGCGCGTGGCAC
VEGFA-HDRtemplate	ACACACAGATCTATTGGAATCCTGGAGTGACCCCTGGCCTTCTCCCCGCT CCAACGCCCTCAACCCACGCTAGCAAGCTTACACTCACTCACCCACACA GACACACACGTCC
HOTAIR-INStemplate-BOT	TAGAAGTGCACACAAAAAACCAACACACAGATCTAATGAAAATAAAGAT CTTTATTGTGTGGAAGGCGCTGCCCG
HOTAIR-INStemplate-TOP	CCTCCACACAATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGTTTTT TGTGTGCGCAGTTCTAGGCAAGCACT
THORLNC-INStemplate-BOT	TTTCCCCCTTACACAAAAAACCAACACACAGATCTAATGAAAATAAAGATC TTTTATTTGTTACATCATCTCACAAA
THORLNC-INStemplate-TOP	GATGTGAACAATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGTTTTT TGTGTGAAGGGGGAAAAGTCAATCCA
AAVS1-MDRtemplate-BOT	AATCCGGTGTCCCTAGTGGCCCCACTG
AAVS1-MDRtemplate-TOP	GGACACCGGATTGGTGACAGAAAAGCC
CD274-MDRtemplate-BOT	CTGGGACTTCAAATTCATTCCATCT
CD274-MDRtemplate-TOP	GAAGTCCCAGGGCTGAGGATCCATG
CXCR4-MDRtemplate-BOT	ACAAACAGGAGGTGGCCACTGACAG
CXCR4-MDRtemplate-TOP	CTCCTGTTTGTATCACGCTTCCCTT
GFP-BFP-5'0-3'20-MDRtemplate-BOT	CCCGTGGCTCAGGGTGGTCACGAGGGTG

GFP-BFP-5'0-3'20-MDRtemplate-TOP	GCCACGGGGTGCAGTGCTTCAGCCGCTA
GFP-BFP-5'5-3'20-MDRtemplate-BOT	TGCACCCCGTGGCTCAGGGTGGTCACGAGGGTG
GFP-BFP-5'5-3'20-MDRtemplate-TOP	CCTGAGCCACGGGGTGCAGTGCTTCAGCCGCTA
GFP-BFP-5'10-3'20-MDRtemplate-BOT	AGCACTGCACCCCGTGGCTCAGGGTGGTCACGAGGGTG
GFP-BFP-5'10-3'20-MDRtemplate-TOP	ACCACCCTGAGCCACGGGGTGCAGTGCTTCAGCCGCTA
KRAS-MDRtemplate-BOT	CCGCCTAGATTATTATCAGCCTCAGCA
KRAS-MDRtemplate-TOP	TAATCTAGGCGGCGATCAGACAGCCCC
MYC-MDRtemplate-BOT	CCCGCTGTCGGGAGAGTCGCGTCCTT
MYC-MDRtemplate-TOP	CCGACAGCGGGGAGGCTATTCTGCCC
TGFB1-MDRtemplate-BOT	CCCCTCTGGTGGGAAGCGCAGGCTCCT
TGFB1-MDRtemplate-TOP	CACCAGAGGGGCAGGAGGACCCCGCT

Table S3. Oligodeoxynucleotide sequences used for HDR and MDR templates.

Name	Sequence (5' - 3')
AAVS1-seq-r1-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNTCACGGTTAATGTGGCTCTGGTTCTGG
AAVS1-seq-r2-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNAGTCGGTTAATGTGGCTCTGGTTCTGG
AAVS1-seq-r3-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNCAGCGGTTAATGTGGCTCTGGTTCTGG
AAVS1-seq-REV	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGTTAGACCCAATATCAGGAGACTAG
ASNS-seq-r1-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNGACTTACAGGAGCCAGGTCGGTAT
ASNS-seq-r2-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNTGATTACAGGAGCCAGGTCGGTAT
ASNS-seq-r3-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNACTTTACAGGAGCCAGGTCGGTAT
ASNS-seq-REV	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCAGGTGCGTAAACAATCGC
CD274-seq-r1-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNATCTGTATGTCTGCTGTGTACTTTGC
CD274-seq-r2-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNCGATGTATGTCTGCTGTGTACTTTGC
CD274-seq-r3-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNTAGTGTATGTCTGCTGTGTACTTTGC
CD274-seq-REV	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTAACAAATGGTGGTTGTCTAAA
CXCR4-seq-r1-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNCGTTGGTCATGGGTTACCAGAAGA
CXCR4-seq-r2-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNACGTGGTCATGGGTTACCAGAAGA
CXCR4-seq-r3-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNGTATGGTCATGGGTTACCAGAAGA
CXCR4-seq-REV	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACTGATGAAGGCCAGGATG
EMX1-seq-r1-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNGACCCTGAGTCCGAGCAGAAGAA
EMX1-seq-r2-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNTGACCTGAGTCCGAGCAGAAGAA
EMX1-seq-r3-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNACTCCTGAGTCCGAGCAGAAGAA
EMX1-seq-REV	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTGGCCAGAGTCCAGCTT
GFP-seq-r1-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNATGCGTAAACGGCCACAAGTTCAGC
GFP-seq-r2-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNGATCGTAAACGGCCACAAGTTCAGC
GFP-seq-r3-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNTGCCGTAACGGCCACAAGTTCAGC
GFP-seq-REV	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAGTTGCCGTCGTCCTTGAAGA
HOTAIR-seq-r1-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNGACCAGTCAAATCTGGCAGAGAGCAG
HOTAIR-seq-r2-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNTGACAGTCAAATCTGGCAGAGAGCAG

HOTAIR-seq-r3-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNACTCAGTCAAATCTGGC GAGAGCAG
HOTAIR-seq-REV	GACTGGAGTTCAGACGTGTGCTCTCCGATCTCAAATATGTGTTCCGCGG GTC
KRAS-seq-r1-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNGACTTGAAAGGGTCTGT CGTGTTTG
KRAS-seq-r2-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNTGATTGAAAGGGTCTGT CGTGTTTG
KRAS-seq-r3-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNACTTTGAAAGGGTCTGT CGTGTTTG
KRAS-seq-REV	GACTGGAGTTCAGACGTGTGCTCTCCGATCTAAACAAGCAGTCACCAAAA GTGG
MYC-seq-r1-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNCGTCACGAACTTTGCC CATAGCA
MYC-seq-r2-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNACGCACGAACTTTGCC CATAGCA
MYC-seq-r3-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNGTACACGAACTTTGCC CATAGCA
MYC-seq-REV	GACTGGAGTTCAGACGTGTGCTCTCCGATCTAAGTGGACTTCGGTGCTT ACC
STAT1-seq-r1-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNATGAAAGTAGTATGCGT GGGCCTC
STAT1-seq-r2-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNGATAAAGTAGTATGCGT GGGCCTC
STAT1-seq-r3-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNTGCAAAGTAGTATGCGT GGGCCTC
STAT1-seq-REV	GACTGGAGTTCAGACGTGTGCTCTCCGATCTGCTCAAAGCTGGTAAAC CTTCA
TGFB1-seq-r1-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNGACGTGACTCTACAAGA CCGAGGTG
TGFB1-seq-r2-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNTGAGTGACTCTACAAGA CCGAGGTG
TGFB1-seq-r3-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNACTGTGACTCTACAAGA CCGAGGTG
TGFB1-seq-REV	GACTGGAGTTCAGACGTGTGCTCTCCGATCTCCTGAGAGGAACTGGGAC TTTG
THORLNC-seq-r1-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNCGTTCTCCGGAGCAGAA ATAGAACAG
THORLNC-seq-r2-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNACGTCTCCGGAGCAGAA ATAGAACAG
THORLNC-seq-r3-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNGTATCTCCGGAGCAGAA ATAGAACAG
THORLNC-seq-REV	GACTGGAGTTCAGACGTGTGCTCTCCGATCTTTCATGCCGTCAAGTCTCA TTT
TP53-seq-r1-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNATGATTCCATGGGACTG ACTTTCTGC
TP53-seq-r2-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNGATATTCCATGGGACTG ACTTTCTGC
TP53-seq-r3-FWD	ACACTCTTCCCTACACGACGCTCTCCGATCTNTGCATTCCATGGGACTG ACTTTCTGC
TP53-seq-REV	GACTGGAGTTCAGACGTGTGCTCTCCGATCTCATCTGGACCTGGGTCTT CAGT
VEGFA-seq-r1-	ACACTCTTCCCTACACGACGCTCTCCGATCTNATGGCGTCTCCGAGAGT

FWD	GAGGAC
VEGFA-seq-r2-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNGATGCGTCTTCGAGAGT GAGGAC
VEGFA-seq-r3-FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNTGCGCGTCTTCGAGAGT GAGGAC
VEGFA-seq-REV	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGAGAGGGACACACA GAT

Table S4. Oligodeoxynucleotide sequences used for next-generation sequencing.

Name	Sequence (5' - 3')
EMX1-T-FWD	GGCTCCCTGGGTTCAAAGTA
EMX1-T-REV	AGAGGGGTCTGGATGTCGTAA
EMX1-T-seq	AACCCTATGTAGCCTCAGTCTTCCC
EMX1-OT1-FWD	TTATCCCCTACTCCTTCATCCCA
EMX1-OT1-REV	AAGGACAGCTTCTTATCCCTGTC
EMX1-OT1-seq	GGAGATTTGCATCTGTGGAGGC
EMX1-OT2-FWD	ACTCCTGGGACAATTATGAACGGTG
EMX1-OT2-REV	ACTATCCTTCTAGTCTTGGGCTAAATTC
EMX1-OT2-seq	GCTTCTTGTCTTTGGCTTTCTTAATGAACTG
EMX1-OT3-FWD	GCACTGATTCATTAGGAGCTGG
EMX1-OT3-REV	AGTCCTATAGATTCACCCACCCA
EMX1-OT3-seq	TCCTGGTTCTGCCACTTGCTG
VEGFA-T-FWD	GCTCCAGATGGCACATTGTCAG
VEGFA-T-REV	AGGGAGCAGGAAAGTGAGGT
VEGFA-T-seq	CAAATATGTAGCTGTTTGGGAGGTCAG
VEGF1-OT1-FWD	TCCCACCAAGGAGGGTTTCTT
VEGFA-OT1-REV	CCTCCCTCAAGGGAAGGTTGT
VEGFA-OT1-seq	CAAGTAGCTGAGATTACAGGCATGTGC
VEGFA-OT2-FWD	ATTCCTCAGGTGGGTTGATGGG
VEGFA-OT2-REV	AGAAAGGAGCCTCGACCAAGTC
VEGFA-OT2-seq	GCCTCCCTGCTGGTTCTCAGAG
VEGFA-OT3-FWD	TCCCATCCCACCTTAGTGTTCC
VEGFA-OT3-REV	TAACCCAGAACATCCAGGCAAC
VEGFA-OT3-seq	TACCATGAACGCAGCCATGC

Table S5. Oligodeoxynucleotide sequences used for Sanger sequencing.

Sequences

Cas9

ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGGCTGGGCCGTGATCACC
GACGAGTACAAGGTGCCAGCAAGAAATTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAG
AAGAACCTGATCGGAGCCCTGCTGTTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAAGAG
AACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGCTATCTGCAAGAGATCTTCAGCAA
CGAGATGGCCAAGGTGGACGACAGCTTCTCCACAGACTGGAAGAGTCCTTCCTGGTGGAAAGAGGA
TAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTA
CCCCACCATCTACCACCTGAGAAAGAACTGGTGGACAGCACCGACAAGGCCGACCTGCGGCTGAT
CTATCTGGCCCTGGCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAACCC
CGACAACAGCGACGTGGACAAGCTGTTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTTCGAGGA
AAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCA
GACGGCTGGAAAATCTGATCGCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTTCGAAAACCTGA
TTGCCCTGAGCCTGGGCCTGACCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGATGCCAAAC
TGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAG
TACGCCGACCTGTTTCTGGCCGCAAGAACCTGTCCGACGCCATCCTGCTGAGCGACATCCTGAGA
GTGAACACCGAGATACCAAGGCCCCCTGAGCGCCTCTATGATCAAGAGATACGACGAGCACCAC
CAGGACCTGACCCTGCTGAAAGCTCTCGTGCGGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTC
TTCGACCAGAGCAAGAACGGCTACGCCGGCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTAC
AAGTTCATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGA
GAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCACCAGATCCACCTGGG
AGAGCTGCACGCCATTCTGCGGCGGCAGGAAGATTTTTACCCATTCTGAAGGACAACCGGGAAAA
GATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCTCTGGCCAGGGGAAACAGCAG
ATTCGCCTGGATGACCAGAAAGAGCGAGGAAACCATCACCCCTGGAACCTCGAGGAAGTGGTGG
CAAGGGCGCTTCCGCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAAGAACCTGCCAACGA
GAAGGTGCTGCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAACGAGCTGACCAAAGT
GAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAGAAAAAGGCCATCGT
GGACCTGCTGTTCAAGACCAACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAA
AATCGAGTGCTTCGACTCCGTGGAAATCTCCGGCGTGGAAAGATCGGTTCAACGCCTCCCTGGGCAC
ATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACAATGAGGAAAACGAGGACATT
CTGGAAGATATCGTGCTGACCCTGACACTGTTTGGAGACAGAGAGATGATCGAGGAACGGCTGAAA
ACCTATGCCACCTGTTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACCGGCTGG
GGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTGGA

TTTCCTGAAGTCCGACGGCTTCGCCAACAGAACTTCATGCAGCTGATCCACGACGACAGCCTGACC
TTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTGCACGAGCACATTGCC
AATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCT
CGTGAAAGTGATGGGCCGGCACAAGCCCCGAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGA
CCACCCAGAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAG
CTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAGCTGTAC
CTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCAGGAACTGGACATCAACCGGCTGTCC
GACTACGATGTGGACCATATCGTGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGC
TGACCAGAAGCGACAAGAACCAGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTCGTGAAGAAG
ATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATTACCCAGAGAAAGTTCGACAATCTGA
CCAAGGCCGAGAGAGGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGTG
GAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAACACTAAGTACGAC
GAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCTGAAGTCCAAGCTGGTGTCCGATTTCC
CGGAAGGATTTCCAGTTTTACAAAGTGCGCGAGATCAACAACCTACCACCACGCCACGACGCCTACC
TGAACGCCGTCTGTGGGAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACG
GCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCT
ACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGATTACCCTGGCCAACG
GCGAGATCCGGAAGCGGCCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAG
GGCCGGGATTTTGGCACCGTGCAGAAAGTGCTGAGCATGCCCAAGTGAATATCGTGAAAAAGACC
GAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCCAAGAGGAACAGCGATAAGCTGATC
GCCAGAAAGAAGGACTGGGACCCTAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCT
GTGCTGGTGGTGGCCAAAGTGAAAAAGGGCAAGTCCAAGAACTGAAGAGTGTGAAAGAGCTGCTG
GGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCGACTTTCTGGAAGCCAAGGGC
TACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGCCTAAGTACTCCCTGTTTCGAGCTGGAAAACG
GCCGGAAGAGAATGCTGGCCTCTGCCGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCC
AAATATGTGAACTTCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGCTCCCCGAGGATAATG
AGCAGAAACAGCTGTTTGTGGAACAGCACAAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCG
AGTTCTCCAAGAGAGTGATCCTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCA
CCGGGATAAGCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAATCTGGG
AGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAAGA
GGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTC
TCAGCTGGGAGGCGAC

Cas9^{S55R-R976A-K1003A-T1314R} (vCas9), with mutations in red:

ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGGCTGGGCCGTGATCACC
GACGAGTACAAGGTGCCAGCAAGAAATTC AAGGTGCTGGGCAACACCGACCGGCACAGCATCAAG
AAGAACCTGATCGGAGCCCTGCTGTTTCGACCGGGCGAAACAGCCGAGGCCACCCGGCTGAAGAG
AACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGCTATCTGCAAGAGATCTTCAGCAA
CGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAAGAGTCCTTCTGGTGGAAAGAGGA
TAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTA
CCCCACCATCTACCACCTGAGAAAGAACTGGTGGACAGCACCGACAAGGCCGACCTGCGGCTGAT
CTATCTGGCCCTGGCCACATGATCAAGTTCGGGGGCCACTTCTGATCGAGGGCGACCTGAACCC
CGACAACAGCGACGTGGACAAGCTGTTCCATCCAGCTGGTGCAGACCTACAACCAGCTGTTTCGAGGA
AAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCA
GACGGCTGGAAAATCTGATCGCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTTCGAAAACCTGA
TTGCCCTGAGCCTGGGCCTGACCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGATGCCAAAC
TGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCAGATCGGCGACCAG
TACGCCGACCTGTTTCTGGCCGCAAGAACCTGTCCGACGCCATCCTGCTGAGCGACATCCTGAGA
GTGAACACCGAGATCACCAAGGCCCCCTGAGCGCCTCTATGATCAAGAGATACGACGAGCACAC
CAGGACCTGACCCTGCTGAAAGCTCTCGTGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTC
TTCGACCAGAGCAAGAACGGCTACGCCGGCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTAC
AAGTTCATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGA
GAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCACCAGATCCACCTGGG
AGAGCTGCACGCCATTCTGCGGCGGAGGAAGATTTTTACCCATTCTGAAGGACAACCGGGAAAA
GATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCTCTGGCCAGGGGAAACAGCAG
ATTGCCTGGATGACCAGAAAGAGCGAGGAAACCATCACCCCTGGAACCTTCGAGGAAGTGGTGG
CAAGGGCGCTTCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCAACGA
GAAGGTGCTGCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAACGAGCTGACCAAAGT
GAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTCTGAGCGGCGAGCAGAAAAAGGCCATCGT
GGACCTGCTGTTCAAGACCAACCGGAAAGTACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAA
AATCGAGTGCTTCGACTCCGTGGAATCTCCGGCGTGAAGATCGGTTCAACGCCTCCCTGGGCAC
ATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCTGGACAATGAGGAAAACGAGGACATT
CTGGAAGATATCGTGCTGACCCTGACACTGTTTGGAGACAGAGAGATGATCGAGGAACGGCTGAAA
ACCTATGCCACCTGTTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACCGGCTGG
GGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTGGA
TTTCTGAAGTCCGACGGCTTCGCCAACAGAACTTCATGCAGCTGATCCACGACGACAGCCTGACC
TTTAAAGAGGACATCCAGAAAGCCAGGTGTCCGGCCAGGGCGATAGCCTGCACGAGCACATTGCC
AATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCT

CGTGAAAGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGA
CCACCCAGAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAG
CTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAGCTGTAC
CTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCAGGAACTGGACATCAACCGGCTGTCC
GACTACGATGTGGACCATATCGTGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGC
TGACCAGAAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTCGTGAAGAAG
ATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATTACCCAGAGAAAGTTCGACAATCTGA
CCAAGGCCGAGAGAGGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGTG
GAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAACACTAAGTACGAC
GAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCTGAAGTCCAAGCTGGTGTCCGATTC
CGGAAGGATTTCCAGTTTTACAAAGTGGCCGAGATCAACAACCTACCACCACGCCACGACGCCTACC
TGAACGCCGTCGTGGGAACCGCCCTGATCAAAAAGTACCCTGCCCTGGAAAGCGAGTTCGTGTACG
GCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCT
ACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGATTACCCTGGCCAACG
GCGAGATCCGGAAGCGGCCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAG
GGCCGGGATTTTGCACCGTGCGGAAAGTGCTGAGCATGCCCAAGTGAATATCGTGAAAAAGACC
GAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCAAGAGGAACAGCGATAAGCTGATC
GCCAGAAAGAAGGACTGGGACCCTAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCT
GTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTGAAGAGTGTGAAAGAGCTGCTG
GGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCGACTTTCTGGAAGCCAAGGGC
TACAAAGAAGTGA AAAAGGACCTGATCATCAAGCTGCCTAAGTACTCCCTGTTTCGAGCTGGAAAACG
GCCGGAAGAGAATGCTGGCCTCTGCCGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCC
AAATATGTGAACTTCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGCTCCCCGAGGATAATG
AGCAGAAACAGCTGTTTGTGGAACAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCG
AGTTCTCCAAGAGAGTGATCCTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCA
CCGGGATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTCCGCTGACCAATCTGGG
AGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAGA
GGTGTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTC
TCAGCTGGGAGGCGAC

gRNA cloning backbone, with U6 promoter in green and scaffold in blue:

GAGGGCCTATTTCCCATGATTCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGG
AATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGG
GTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTG

GATTTCCTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGGGTCTTCGAGAAGACCTGTTTTAGA
GCTAGAAATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG
CTTTTTTT