Fusion of Inactivated Cas9 to *Fok*I Nuclease Improves Genome Modification Specificity

John P. Guilinger, David B. Thompson, and David R. Liu

SUPPLEMENTARY INFORMATION

Supplementary Results	Development of the XTEN-based linker Additional NLS-*Fok*I linker variants Sensitivity limit of off-target cleavage assays
Supplementary Figure 1	Target DNA sequences in integrated genomic *GFP* gene
Supplementary Figure 2	GFP disruption assay for *Fok*I-dCas9 activity
Supplementary Figure 3	Activities of *Fok*I-dCas9 fusion variants and spacer orientations
Supplementary Figure 4	Optimization of linker sequence and length between *Fok*I and dCas9 in NLS-*Fok*I-dCas9
Supplementary Figure 5	Target DNA sequences in endogenous human *EMX*, *VEGF*, *CLTA,* and *HBB* genes
Supplementary Figure 6	Spacer length preference of genomic DNA modification by fCas9, Cas9 nickase, and wild-type Cas9
Supplementary Figure 7	Efficiency of genomic DNA modification by fCas9, Cas9 nickase, and wild-type Cas9 with varying amounts of total Cas9 and sgRNA expression plasmids
Supplementary Figure 8	Dependence of fCas9 on sgRNA pair orientation.
Supplementary Figure 9	Ability of fCas9, Cas9 nickase, and wild-type Cas9 to modify genomic DNA in the presence of a single sgRNA
Supplementary Figure 10	fCas9 indel frequency on genomic targets reflects sgRNA pair spacer length preference
Supplementary Figure 11	Modifications induced by Cas9 nuclease, Cas9 nickases, or fCas9 nucleases at endogenous loci
Supplementary Table 1	Paired sgRNA target site abundances for fCas9 and Cas9 nickase in the human genome
Supplementary Table 2	Cas9 off-target sites of target sites in *EMX*, *VEGF* and *CLTA*
Supplementary Table 3	Cellular modification induced by wild-type Cas9, Cas9 nickase and fCas9 nucleases at on-target and off-target genomic sites
Supplementary Table 4	Genomic fCas9 target sites A, B and C with highly similar off-target substrates
Supplementary Table 5	Cellular modification induced by Cas9 nickase and fCas9 at target sites A, B and C and highly similar off-target genomic sites
Supplementary Notes	Constructs, scripts, and oligonucleotides used in this study

Nature Biotechnology: doi:10.1038/nbt.2909

SUPPLEMENTARY RESULTS

Development of the XTEN-based linker

The XTEN protein was originally designed to extend the serum half-life of translationally fused biologic drugs by increasing their hydrodynamic radius, acting as protein-based functional analog to chemical PEGylation.[1] Since XTEN is chemically stable, non-cationic, non-hydrophobic, and predicted to adopt an extended, unstructured conformation, we hypothesized that an XTEN-based linker could functional as a stable, inert linker sequence for fusion proteins. The sequence of the XTEN protein tag from E-XTEN was analyzed, and repeating motifs within the amino acid sequence were aligned. The sequence used in the *Fok*I-dCas9 fusion construct *Fok*I-L8 (**Supplementary Figure 4a**) was derived from the consensus sequence of a common E-XTEN motif, and a 16-residue sequence was chosen from within this motif to test as a *Fok*I-dCas9 linker.

Additional NLS-*Fok*I linker variants

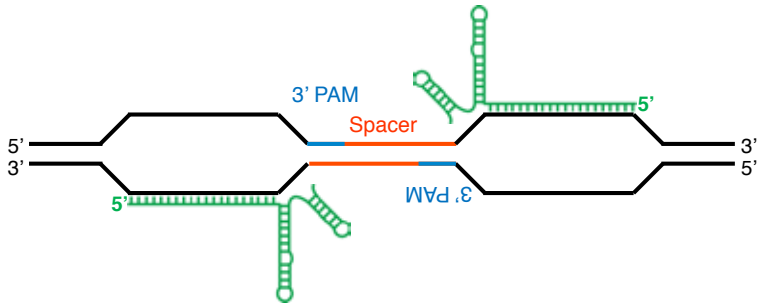
In addition to assaying linkers between the *Fok*I domain and dCas9 in the NLS-*Fok*I-dCas9 architecture, we also tested four linker variants between the N-terminal NLS and the *Fok*I domain (**Supplementary Figure 4a**). Although a NLS-GSAGSAAGSGEF-*Fok*I-dCas9 linker exhibited nearly 2-fold better GFP gene modification than the other NLS-*Fok*I linkers tested when a simple GGS linker was used between the *Fok*I and dCas9 domains (**Supplementary Figure 4b**), the GSAGSAAGSGEF linker did not perform substantially better when combined with the XTEN linker between the *Fok*I and dCas9 domains.

Sensitivity limit of off-target cleavage assays

The sensitivity of the high-throughput sequencing method for detecting genomic off-target cleavage is limited by the amount genomic DNA (gDNA) input into the PCR amplification of each genomic target site. A 1 ng sample of human gDNA represents only ~330 unique genomes, and thus only ~330 unique copies of each genomic site are present. PCR amplification for each genomic target was performed on a total of 150 ng, 300 ng, or 600 ng of input gDNA, which provides amplicons derived from at most 50,000, 100,000 or 200,000 unique gDNA copies, respectively. Therefore, the high-throughput sequencing assay cannot detect rare genome modification events that occur at a frequency of less than 1 in 50,000 (0.002%), less than 1 in 100,000 (0.001%), or less than 1 in 200,000 (0.0005%), respectively.

Nature Biotechnology: doi:10.1038/nbt.2909

Orientation B:



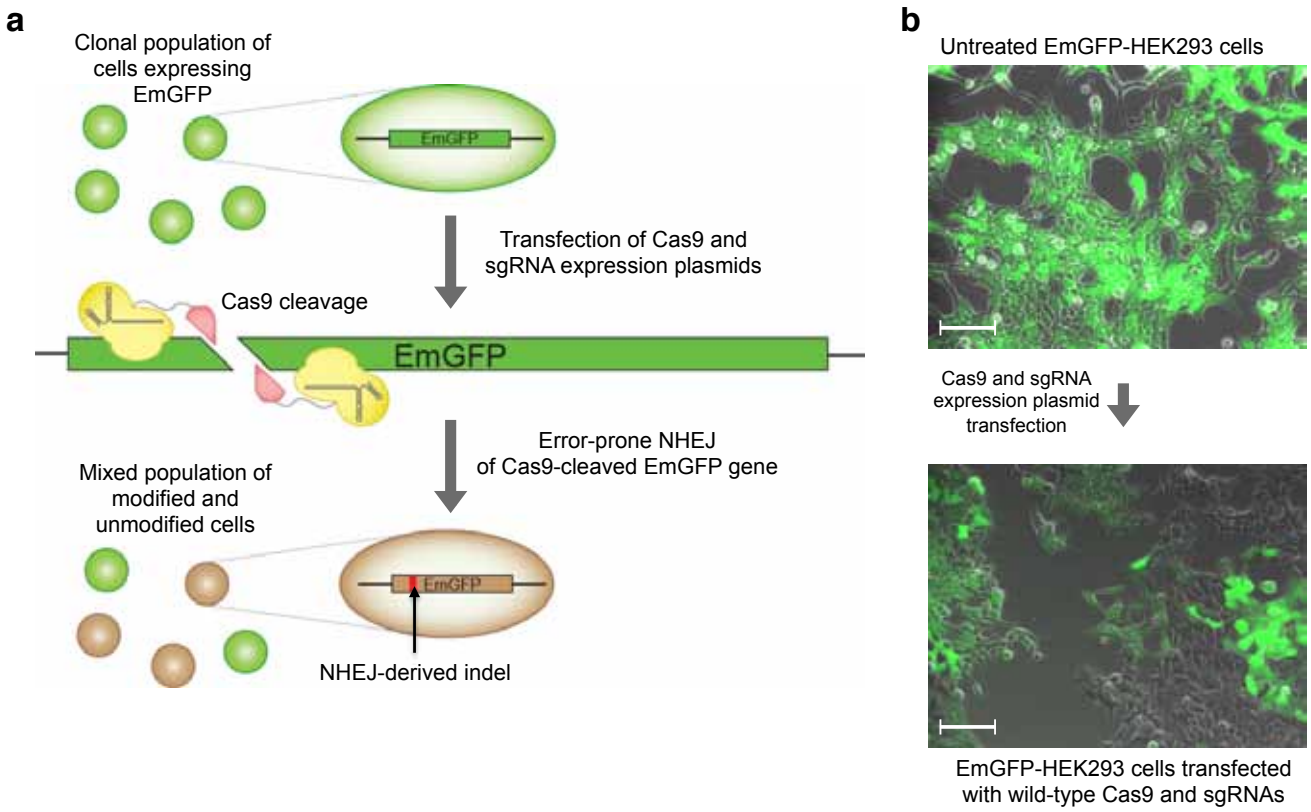
Guide RNA spacer length (bp)

	G12	G13	G14
G8	11	42	
G9		19	28
G10			16
G11			4

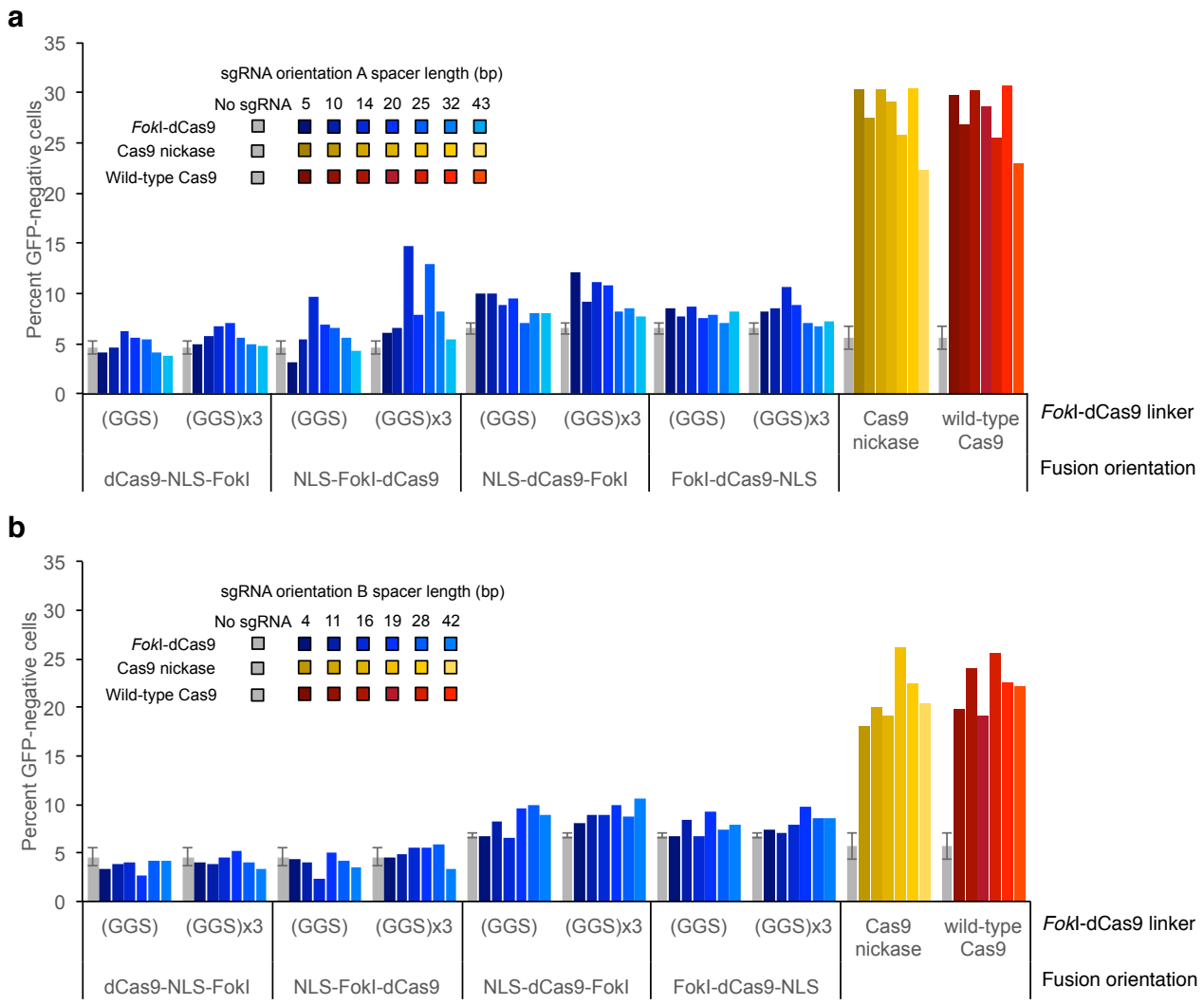
Guide RNA sequences within EmGFP (bp 297-388)

```
     5'-GGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCG-3'
G8             ACCATCTTCTTCAAGGACGACGG
G9                                    CAACTACAAGACCCGCGCCGAGG
G10                                             CCGCGCCGAGGTGAAGTTCGAGG
G11                                                      GAAGTTCGAGGGCGACACCCTGG
G12                                             CCCGCGCCGAGGTGAAGTTCGAG
G13                                                                     CCTGGTGAACCGCATCGAGCTGA
G14                                                                        CCGCATCGAGCTGAAGGGCATCG
```

Supplementary Figure 1. Target DNA sequences in a genomic *GFP* gene. Seven sgRNA target sites were chosen to test *Fok*I-dCas9 candidate activity in an orientation in which the PAM is adjacent from the cleaved spacer sequence (orientation B). Together, these seven sgRNAs enabled testing of *Fok*I-dCas9 fusion variants across six spacer lengths ranging from 4 to 42 bp.



Supplementary Figure 2. GFP disruption assay for measuring genomic DNA-modification activity. (**a**) A HEK293-derived cell line constitutively expressing a genomically integrated EmGFP gene was used to test the activity of candidate *Fok*I-dCas9 fusion constructs. Co-transfection of these cells with appropriate nuclease and sgRNA expression plasmids leads to dsDNA cleavage within the EmGFP coding sequence, stimulating error-prone NHEJ and generating indels that can disrupt the expression of GFP, leading to loss of cellular fluorescence. The fraction of cells displaying a loss of GFP fluorescence is then quantitated by flow cytometry. (**b**) Typical epifluorescence microscopy images at 200x magnification of EmGFP-HEK293 cells before and after co-transfection with wild-type Cas9 and sgRNA expression plasmids. White scale bars represent 200 μm.

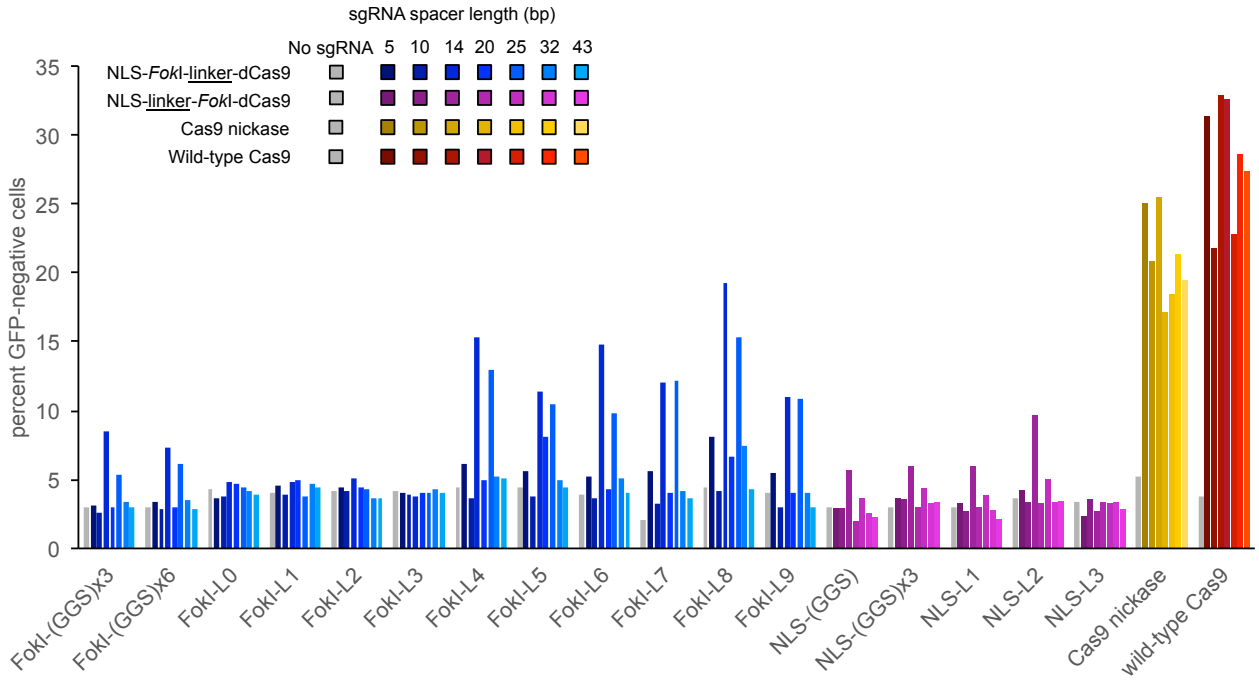


Supplementary Figure 3. Activities of *Fok*I-dCas9 fusion candidates combined with sgRNA pairs of different orientations and varying spacer lengths. Activity of *Fok*I-dCas9 fusion orientations in GFP disruption assay. The fusion architectures described in Figure 1b were tested for functionality by flow cytometry using the GFP loss-of-function reporter across all (**a**) orientation A sgRNA spacers and (**b**) orientation B sgRNA spacers (Figure 1c and Supplemental Figure 1). All *Fok*I-dCas9 fusion data shown are the results of single trials. Wild-type Cas9 and Cas9 nickase data are the average of two replicates, while the 'no treatment' negative control data is the average of 6 replicates, with error bars representing one standard deviation. The gray dotted line across the Y-axis corresponds to the average of the 'no treatment' controls performed on the same day.

a

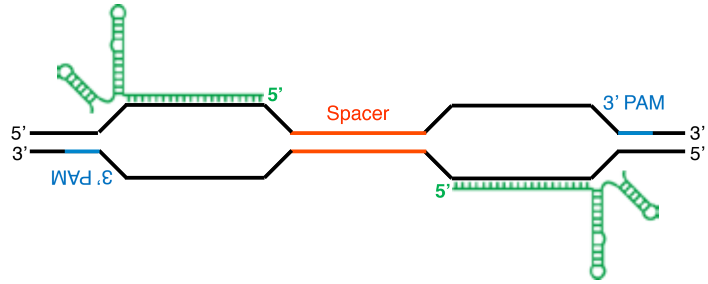
Name	NLS-**linker**-Fok1	Fok1-**linker**-dCas9
*Fok*I-(GGS)x3	GGS	GGSGGSGGS
*Fok*I-(GGS)x6	GGS	GGSGGSGGSGGSGGSGGS
*Fok*I-L0	GGS	-
*Fok*I-L1	GGS	MKIIEQLPSA
*Fok*I-L2	GGS	VRHKLKRVGS
*Fok*I-L3	GGS	VPFLLEPDNINGKTC
*Fok*I-L4	GGS	GHGTGSTGSGSS
*Fok*I-L5	GGS	MSRPDPA
*Fok*I-L6	GGS	GSAGSAAGSGEF
*Fok*I-L7	GGS	SGSETPGTSESA
*Fok*I-L8	GGS	SGSETPGTSESATPES
*Fok*I-L9	GGS	SGSETPGTSESATPEGGSGGS
NLS-(GGS)	GGS	GGSM
NLS-(GGS)x3	GGSGGSGGS	GGSM
NLS-L1	VPFLLEPDNINGKTC	GGSM
NLS-L2	GSAGSAAGSGEF	GGSM
NLS-L3	SIVAQLSRPDPA	GGSM
wild-type Cas9	N/A	N/A
Cas9 nickase	N/A	N/A

b



Supplementary Figure 4. Optimization of protein linkers in NLS-*Fok*I-dCas9. (**a**) Table of all linker variants tested. Wild-type Cas9 and Cas9 nickase were included for comparison. The initial active construct NLS-*Fok*I-dCas9 with a $(GGS)_3$ linker between *Fok*I and dCas9 was tested across a range of alternate linkers. The final choice of linkers for fCas9 is highlighted in blue. (**b**) The activity of *Fok*I-dCas9 fusions with linker variants. Each variant was tested across a range of spacer lengths from 5 to 43 bp using sgRNA pair orientation A. A control lacking sgRNA (grey) was included for each separate fusion construct. NLS-*Fok*I-dCas9 variant L8 showed the best activity, approaching the activity of Cas9 nickase. Variants L4 through L9 show peak activity with 14- and 25-bp spacer lengths, suggesting two optimal spacer lengths roughly one helical turn of dsDNA apart.

Orientation A:




```
                                                                                            Spacer (bp)
AAVS1
CCAGGATCCTCTCTGGCTCCATCGTAAGCAAACCTTAGAGGTTCTGGCAAGGAGAGAGATGG
CCAGGATCCTCTCTGGCTCCATC (A1)         GGTTCTGGCAAGGAGAGAGATGG (A2)                                16

CLTA-1
CCCCAAGTCTAGCAAGCAGGCCAAAGATGTCTCCCGCATGCGCTCAGTCCTCATCTCCCTCAAGCAGG
CCCCAAGTCTAGCAAGCAGGCCA (C1)               AGTCCTCATCTCCCTCAAGCAGG (C2)                          22

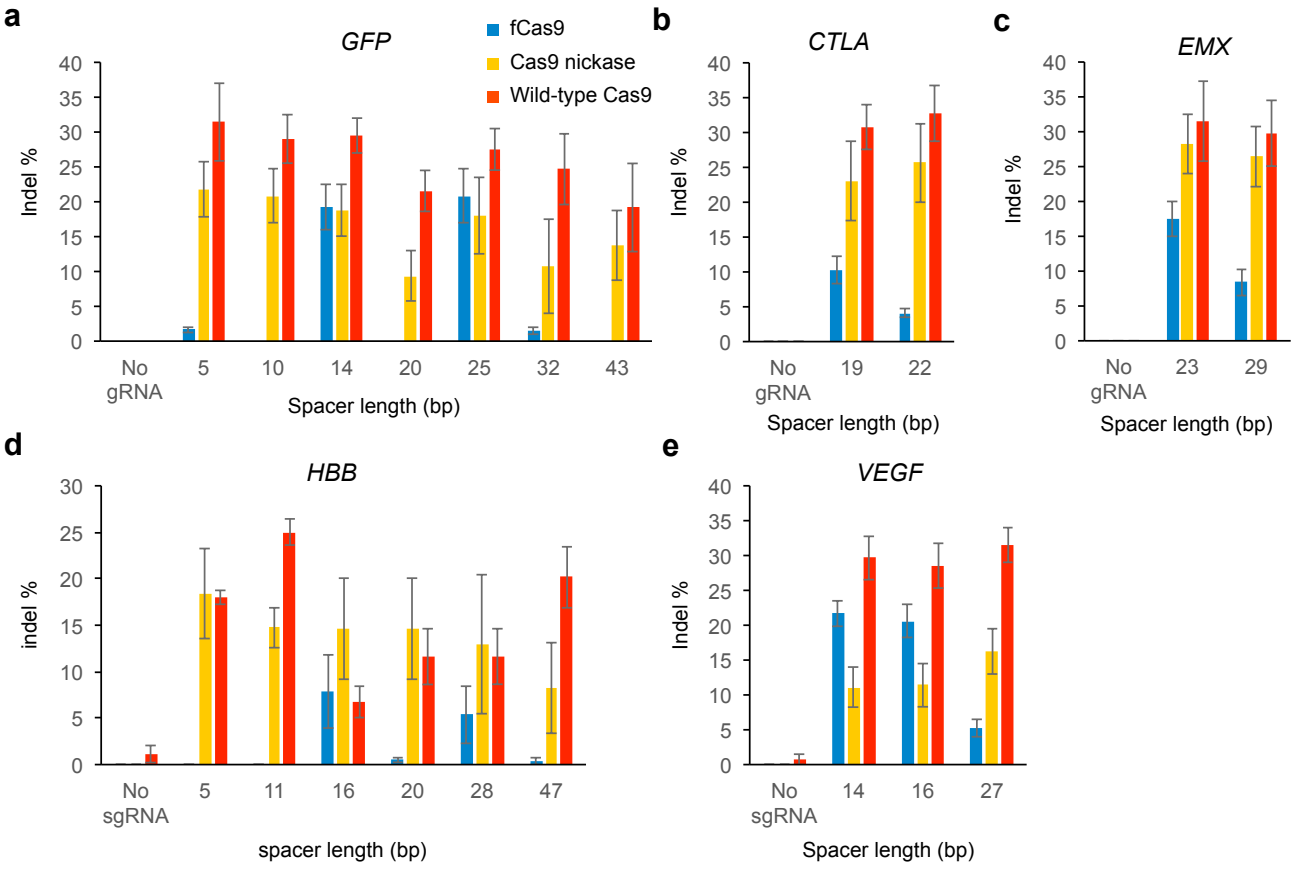
CLTA-2
CCCTGTGGAAACACTACATCTGCAATATCTTAATCCTACTCAGTGAAGCTCTTCACAGTCATTGG
CCCTGTGGAAACACTACATCTGC (C3)            GTGAAGCTCTTCACAGTCATTGG (C4)                             19

HBB
CCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACAGGTTTAAGG
CCGTTACTGCCCTGTGGGGCAAG(H1) CGTGGATGAAGTTGGTGGTGGG (H2)                                          5
                                  TGAAGTTGGTGGTGAGGCCCTGG (H3)                                   11
                                       TTGGTGGTGAGGCCCTGGGCAGG (H4)                              16
                                           TGGTGAGGCCCTGGGCAGGTTGG (H5)                          20
                                                   CCCTGGGCAGGTTGGTATCAAGG (H6)                  28
                                                                  AAGGTTACAAGACAGGTTTAAGG (H7) 47

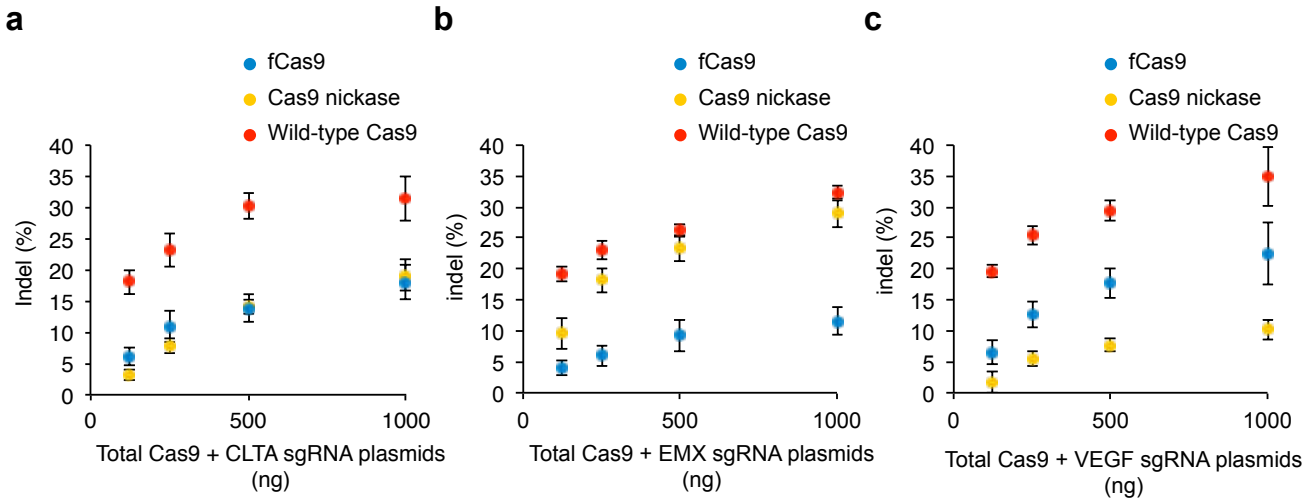
EMX
CCCTTCTTCTTCTGCTCGGACTCAGGCCCTTCCTCCTCCAGCTTCTGCCGTTTGTACTTTGTCCTCCGGTTCTGG
CCCTTCTTCTTCTGCTCGGACTC (E1)                GCCGTTTGTACTTTGTCCTCCGG (E2)                         23
                                                  TGTACTTTGTCCTCCGGTTCTGG (E3)                   29

VEGF
CCAGGAGCAAACTCCCCCCACCCCCTTTCCAAAGCCCATTCCCTCTTTAGCCAGAGCCGGGGTGTGCAGACGG
CCAGGAGCAAACTCCCCCCACCC (V1)         ATTCCCTCTTTAGCCAGAGCCGG (V2)                                14
                                       TCCCTCTTTAGCCAGAGCCGGGG (V3)                              16
                                                  CCAGAGCCGGGGTGTGCAGACGG (V4)                   27
```

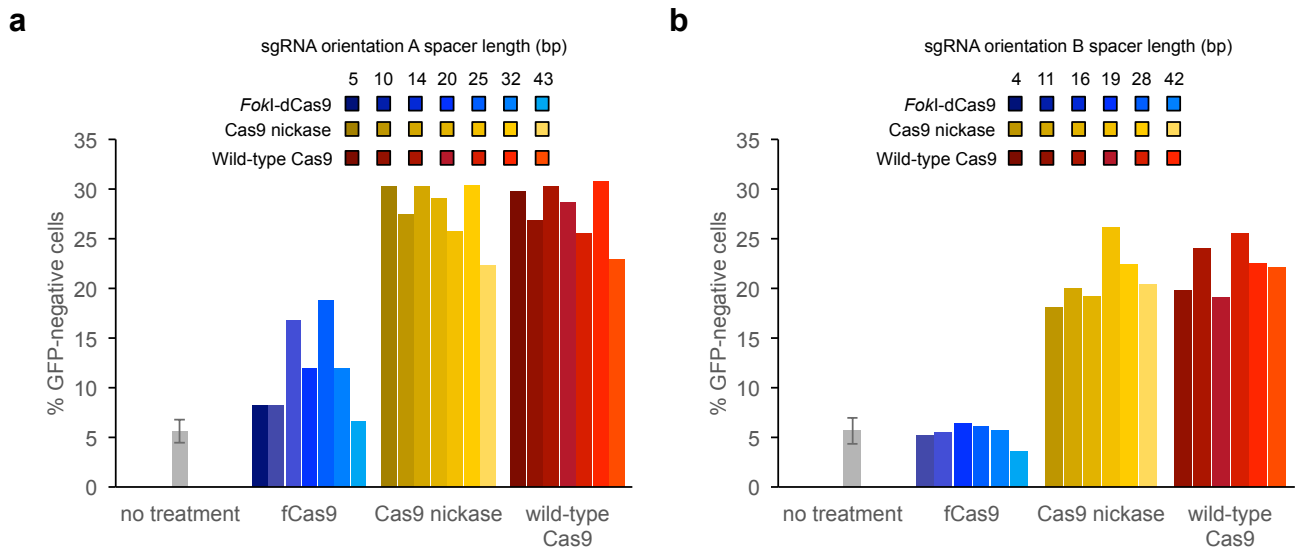
Supplementary Figure 5. Target DNA sequences in endogenous human *AAVS1, CLTA, HBB, EMX*, and *VEGF* genes. sgRNA target sites tested within endogenous human *AAVS1*, *CLTA, HBB, EMX*, and *VEGF* genes. Fourteen paired sgRNA target sites were chosen to test the activity of the optimized fCas9 fusion in an orientation in which the PAM is distal from the cleaved spacer sequence (orientation A). Together, these 14 sgRNA pairs enabled testing of fCas9 fusion variants across twelve spacer lengths ranging from 5 to 47 bp.



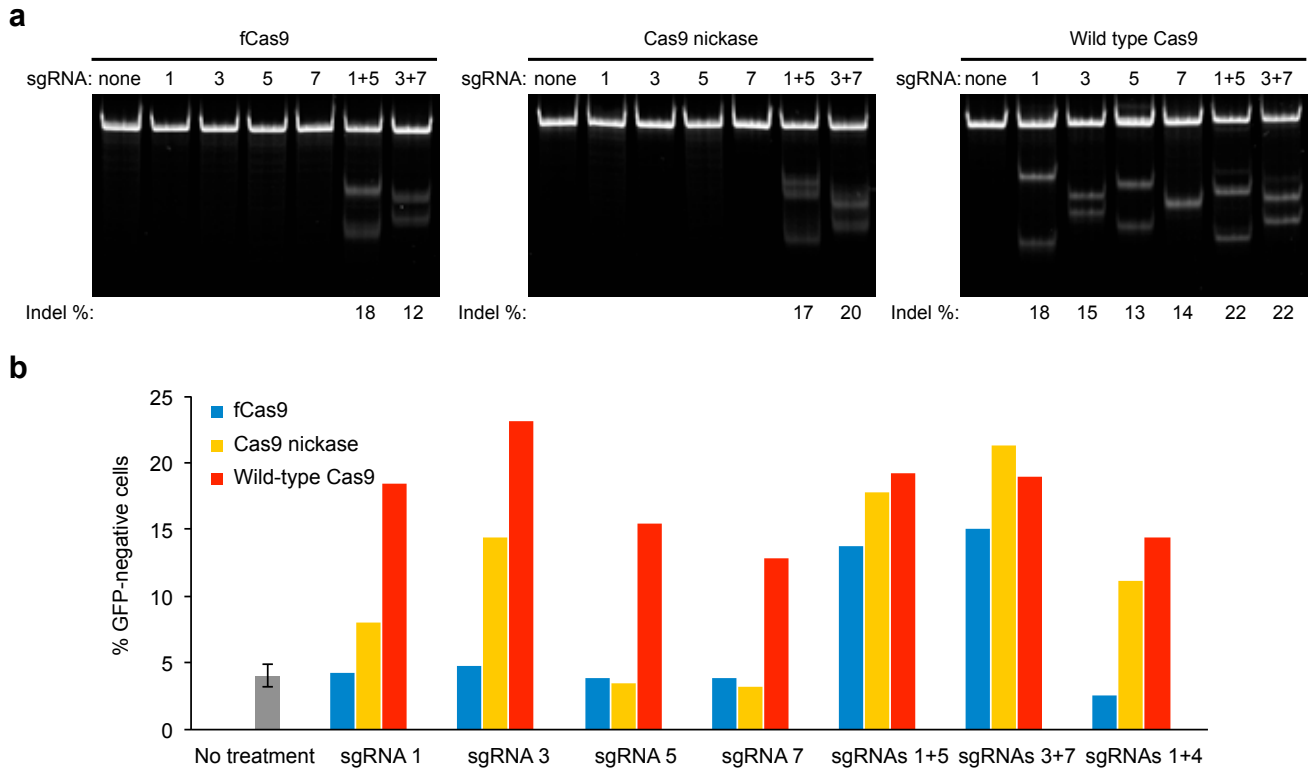
Supplementary Figure 6. Spacer length preference of genomic DNA modification by fCas9, Cas9 nickase, and wild-type Cas9. Indel modification efficiency for (**a**) pairs of sgRNAs targeting the *GFP* site, (**b**) pairs of sgRNAs targeting the *CLTA* site, (**c**) pairs of sgRNAs targeting the *EMX* site (**d**) pairs of sgRNAs targeting the *HBB* site, and (**e**) pairs of sgRNAs targeting the *VEGF* site. Error bars reflect standard error of the mean from three biological replicates performed on different days.



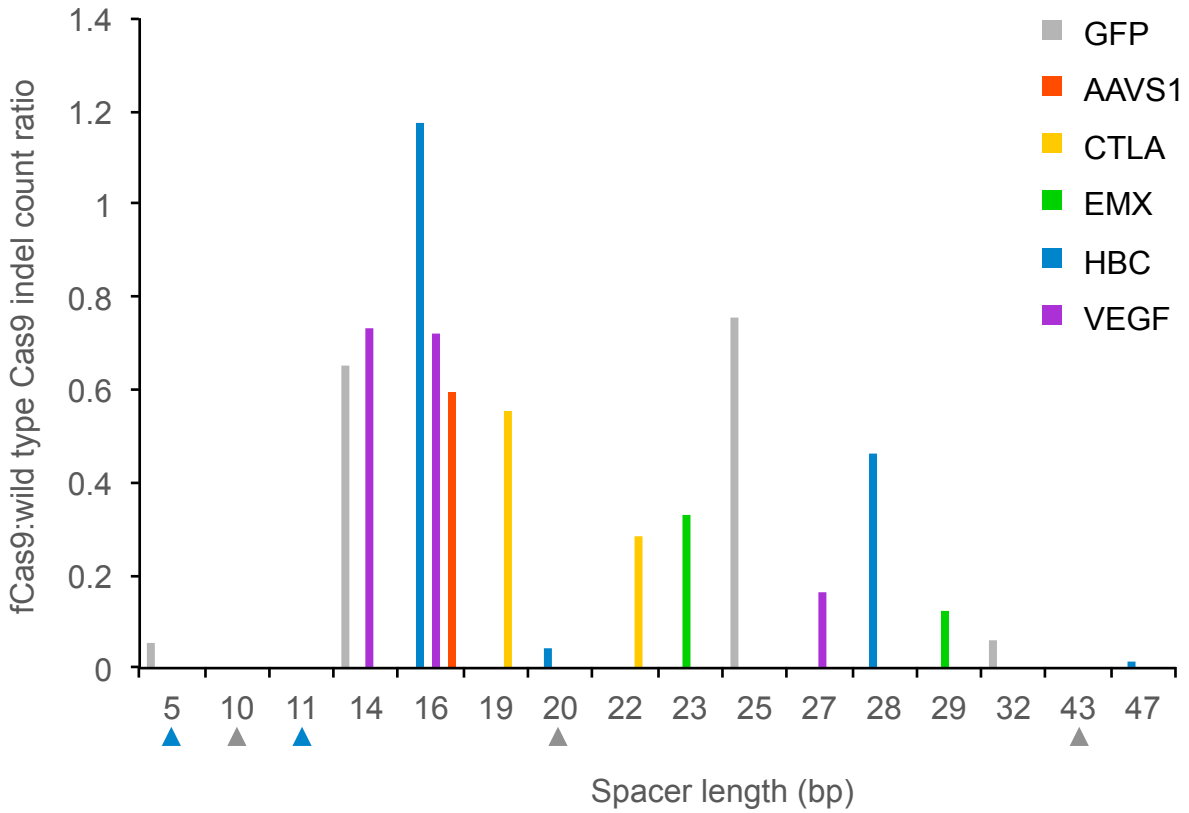
Supplementary Figure 7. Efficiency of genomic DNA modification by fCas9, Cas9 nickase, and wild-type Cas9 with varying amounts of Cas9 and sgRNA expression plasmids. Indel modification efficiency from a Surveyor assay of renatured target-site DNA amplified from a population of cells treated with fCas9, Cas9 nickase, or wild-type Cas9 and two target site sgRNAs. Either 700 ng of Cas9 expression plasmid with 250 ng of sgRNA expression plasmid (950 ng total), 350 ng of Cas9 expression plasmid with 125 ng of sgRNA expression plasmid (475 ng in total), 175 ng of Cas9 expression plasmid with 62.5 ng of sgRNA expression plasmid (238 ng in total) or 88 ng of Cas9 expression plasmid with 31 ng of sgRNA expression plasmid (119 ng in total) were transfected with an appropriate amount of inert, carrier plasmid to ensure uniform transfection of 950 ng of plasmid across all treatments. Indel modification efficiency for (**a**) sgRNAs spaced 19-bp apart targeting the *CLTA* site, (**b**) sgRNAs spaced 23 bp apart targeting the *EMX* site, and (**c**) sgRNAs spaced 14 bp apart targeting the *VEGF* site. Error bars represent the standard error of the mean from three biological replicates performed on separate days.



Supplementary Figure 8. Dependence of fCas9 on sgRNA pair orientation. (**a**) GFP gene disruption by wild-type Cas9, Cas9 nickase, fCas9 using sgRNA pairs in orientation A. High activity of fCas9 requires spacer lengths of ~15 or 25 bp. (**b**) GFP gene disruption using sgRNA pairs in orientation B. Cas9 nickase, but not fCas9, accepts either orientation of sgRNA pairs. The "no treatment" control refers to cells receiving no plasmid DNA.



Supplementary Figure 9. Ability of fCas9, Cas9 nickase, and wild-type Cas9 to modify genomic DNA in the presence of a single sgRNA. (**a**) Surveyor assay of a genomic GFP target from DNA of cells treated with the indicated combination of Cas9 protein and sgRNA(s). Single sgRNAs do not induce genome modification at a detectable level (< 2% modification) for both fCas9 and Cas9 nickase. Wild-type Cas9 effectively modifies the GFP target for all tested single and paired sgRNAs. For both fCas9 and Cas9 nickase, appropriately paired sgRNAs induce genome modification at levels comparable to those of wild-type Cas9. (**b**) GFP gene expression loss by fCas9, but not Cas9 nickase or wild-type Cas9, depends on the presence of two sgRNAs. Four single sgRNAs were tested along with three sgRNA pairs of varying spacer length. In the presence of sgRNA pairs in orientation A with spacer lengths of 14 or 25 bp (sgRNAs 1+5, and sgRNAs 3+7, respectively), fCas9 is active, but not when a sgRNA pair with a 10-bp spacer (sgRNAs 1+4) is used.



Supplementary Figure 10. fCas9 indel frequency of genomic targets reflects sgRNA pair spacer length preference. The graph shows the relationship between spacer length (number of bp between two sgRNAs) and the indel modification efficiency of fCas9 normalized to the indel modification efficiency of the same sgRNAs co-expressed with wild-type Cas9 nuclease. Colored triangles below the X-axis denote spacer lengths that were tested but which yielded no detectable indels for the indicated target gene. These results suggest that fCas9 requires ~15 bp or ~25 bp between half-sites to efficiency cleave DNA.

Nature Biotechnology: doi:10.1038/nbt.2909

a

```
Wild-type Cas9 nuclease modifications of VEGF on-target site:
4747 gctgtttgggaggtcagaaataggggtCCAGGAGCAAACTCCCCCCACCCcctttccaaagcccATTCCCTCTTTAGCCAGAGCCGGggtgtgcagacggcagtc (ref)
4577 gctgtttgggaggtcagaaataggggtccagga-------------------------------------------------agccggggtgtgcagacggcagtc
  58 gctgtttgggaggtcagaaataggggtccaggagcaaactccccccacccccttttccaaagcccattccctctttagc------cggggtgtgcagacggcagtc
  54 gctgtttgggaggtcagaaataggggtccaggag-----------------------------------------------agccggggtgtgcagacggcagtc
  43 gctgtttgggaggtcagaaatag-------------------------------------------------------ccggggtgtgcagacggcagtc
  33 gctgtttgggaggtcagaaataggggtccaggagc-----------------------------------------------cggggtgtgcagacggcagtc
  23 gctgtttgggaggtcagaaataggggtccag---------------------------------------------ccggggtgtgcagacggcagtc
  22 gctgtttgggaggtcagaaataggggtccaggagcaaactccccccacccccttttccaaagcccattccctctttagccag------ggtgtgcagacggcagtc
  18 gctgtttgggaggtcagaaataggggtccagga-------------------t-----------------------------agccggggtgtgcagacggcagtc

Cas9 nickase modifications of VEGF on-target site:
8770 gctgtttgggaggtcagaaataggggtCCAGGAGCAAACTCCCCCCACCCcctttccaaagcccATTCCCTCTTTAGCCAGAGCCGGggtgtgcagacggcagtc (ref)
  78 gctgtttgggaggtcagaaataggggtccag-----------------------------------------------------------acggcagtc
  60 gctgtttgggaggtcagaaataggggtccaggagcaaactccccccacccccttttccaaagcc----------------------ggggtgtgcagacggcagtc
  58 gctgtttgggaggtcagaaataggggtcca----------------------------aagcccattccctctttagccagagccggggtgtgcagacggcagtc
  56 gctgtttgggaggtcagaaataggggggt-------------------------------------------------------gtgcagacggcagtc
  49 gctgtttgggaggtcagaaataggggtccag---------------------------------------------ccggggtgtgcagacggcagtc
  37 gctgtttgggaggtcagaaataggggtccagg-----------------------------------------------gtgtgcagacggcagtc
  36 gctgtttgggaggtcagaaataggggtccaggagc-----------------------------------------cggggtgtgcagacggcagtc
  27 gctgtttgggaggtcagaaatag-----------------------------------------------------ccggggtgtgcagacggcagtc

fCas9 nuclease modifications of VEGF on-target site:
8959 gctgtttgggaggtcagaaataggggtCCAGGAGCAAACTCCCCCCACCCcctttccaaagcccATTCCCTCTTTAGCCAGAGCCGGggtgtgcagacggcagtc (ref)
 125 gctgtttgggaggtcagaaataggggtccaggagcaaactccccca------------agcccattccctctttagccagagccggggtgtgcagacggcagtc
 121 gctgtttgggaggtcagaaataggggtccaggagcaaactccccccacccccttt-----------ttccctctttagccagagccggggtgtgcagacggcagtc
  77 gctgtttgggaggtcagaaataggggtccaggagcaaactccccccacccccct------------ttccctctttagccagagccggggtgtgcagacggcagtc
  73 gctgtttgggaggtcagaaataggggtccaggagcaaactccccccca-------------gcccattccctctttagccagagccggggtgtgcagacggcagtc
  48 gctgtttgggaggtcagaaataggggtccaggagcaaactcccccccacccc------------attccctctttagccagagccggggtgtgcagacggcagtc
  44 gctgtttgggaggtcagaaataggggtccaggagcaaactcccccccacccccttt--------------------agccagagccggggtgtgcagacggcagtc
  24 gctgtttgggaggtcagaaataggggtccaggagcaaactcccccccacccccttt--aaagcccattccctctttagccagagccggggtgtgcagacggcagtc
  22 gctgtttgggaggtcagaaataggggtccaggagcaaactccccc-------------aagcccattccctctttagccagagccggggtgtgcagacggcagtc
```

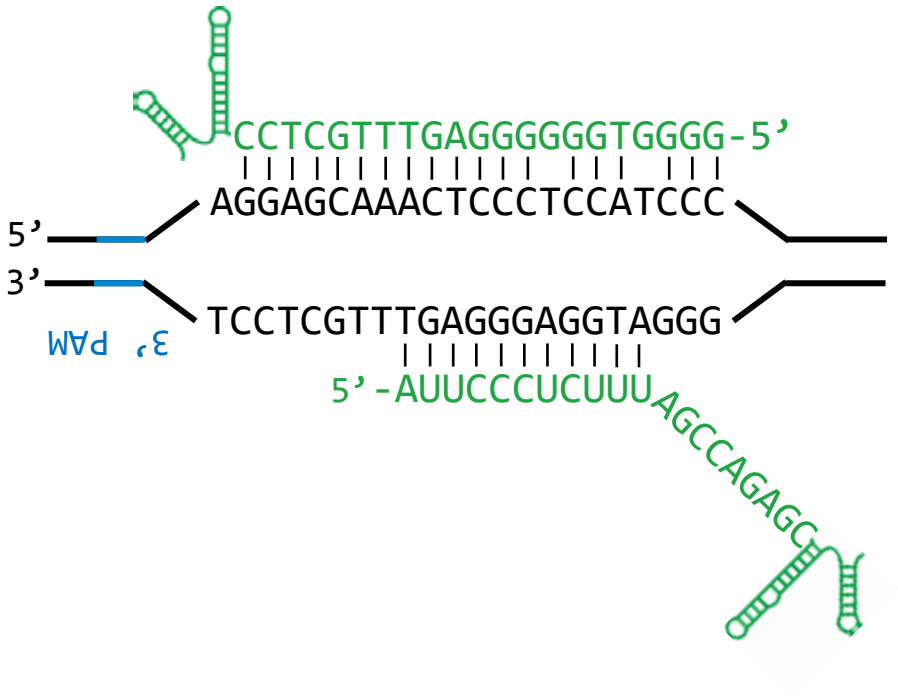
b

```
Wild-type Cas9 nuclease modifications of VEG_Off1:
 79248 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccCCAGGAGCAAACTCCCtCCAtCCCacaaatccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat (ref)
   800 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccagg--caaactccctcccatcccacaaatccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
   239 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccca----------------tcccacaaatccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
   155 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccag---caaactccctccatcccacaaatccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
    90 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacacccca----------------------caaatccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
    71 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacac-------------tccctccatcccacaaatccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
    54 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacacccc--------------tccatcccacaaatccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
    53 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccagga-----------------------------------------------------------------tgat
    47 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgac---------------tccctccatcccacaaatccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat

Cas9 nickase modifications of VEG_Off1:
302573 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccCCAGGAGCAAACTCCCtCCAtCCCacaaatccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat (ref)
    28 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccagga-----c----------------atccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
    13 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgac---------------tccctccatcccacaaatccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
    11 cattcaacagatacttactgaatgctaatgtctcagacaggacattctga-----------------------------tccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
     4 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccagg--ca---tc--tg-atg--acaaatccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
     2 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccaggagcaaactccctccatcccacaaatccgtccttagatgtgca—cccaacctcctaagaaatagaaggatgat
     1 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccaggagcaaactccctccatcccacaaatccgtccttagatgtgca—cccaacctcctaagaaatagaaggatgat
     1 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccagga----------------------------------tgtgcacacccaacctcctaagaaatagaaggatgat
     1 cattcaacagatacttactgaatgctaatgtctcagacaggacattctga-------------------------------aatccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat

fCas9 nuclease modifications of VEG_Off1:
233567  cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccCCAGGAGCAAACTCCCtCCAtCCCacaaatccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat (ref)
     6 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccaggagcaaactccctcc------------gtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
     5 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccaggagcaaactccctc-----------tccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
     4 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccaggagcaaactccctccat--------tccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
     3 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccaggagcaaactccctccatcccacaaatccgtccttagatgtgca--cccaacctcctaagaaatagaaggatgat
     3 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccaggagcaaactccctcc----------tccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
     2 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccaggagcaaactccctcc---------atccgtccttagatgtgcacacccaacctcctaagaaatagaaggatgat
     1 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccaggagcaaactccctccatcccacaaatccgtccttagatgtgcacaccc----tcctaagaaatagaaggatgat
     1 cattcaacagatacttactgaatgctaatgtctcagacaggacattctgacaccccaggagcaaactccctccatcccacaaatccgtcc--agatgtgcacacccaacctcctaagaaatagaaggatgat
```

c



d

CCAGGAGCAAACTCCCTCCATCCCACAAATCCGTCCTTAGATGTGCACACCCAACCTCCTAAGAAATAGAAGGATGATGG**CCAGGTGCA**GTGATTCATGCCTG
CCAGGAGCAAACTCCCcCCAcCC (V1) CCAGGaGCAaactccCcccaCcc

Supplementary Figure 11. Modifications induced by Cas9 nuclease, Cas9 nickases, or fCas9 nucleases at endogenous loci. (**a**) Examples of modified sequences at the *VEGF* on-target site with wild-type Cas9 nuclease, Cas9 nickases, or fCas9 nucleases and a single plasmid expressing two sgRNAs targeting the *VEGF* on-target site (sgRNA V1 and sgRNA V2). For each example shown, the unmodified genomic site is the first sequence, followed by the top eight sequences containing deletions. The numbers before each sequence indicate sequencing counts. The sgRNA target sites are bold and capitalized. (**b**) Identical analysis as in (**a**) for *VEGF* off-target site 1VEG_Off1. (**c**) Potential binding mode of two sgRNAs to *VEGF* off-target site 1. The top strand is bound in a canonical mode, while the bottom strand binds the second sgRNA, sgRNA V2, through sgRNA:DNA base pairing that includes G:U base pairs. (**d**) Alternative off-target binding mode of two sgRNAs to *VEGF* off-target site 1. Binding of V1 sgRNA with a potential seed+PAM binding site 59 bases from the VEGF off-target site 1 that is closely related to V1 sgRNA target site. PAM shown in blue, mismatched positions shown in lower case red.

Nature Biotechnology: doi:10.1038/nbt.2909

a

Spacer length (b)	Number of paired sgRNA sites in orientation A	Number of paired sgRNA sites in orientation B
-8	6874293	NC
-7	6785996	NC
-6	6984064	NC
-5	7023260	NC
-4	6487302	NC
-3	6401348	NC
-2	6981383	NC
-1	7230098	NC
0	7055143	NC
1	6598582	NC
2	6877046	NC
3	6971447	NC
4	6505614	5542549
5	6098107	5663458
6	6254974	6819289
7	6680118	6061225
8	7687598	5702252
9	6755736	7306646
10	6544849	6387485
11	6918186	6172852
12	6241723	5799496
13	6233385	7092283
14	6298717	7882433
15	6181422	7472725
16	6266909	6294684
17	6647352	6825904
18	6103603	6973590
19	5896092	6349456
20	6000683	5835825
21	5858015	6056352
22	6116108	6531913
23	5991254	6941816
24	6114969	6572849
25	6135119	5671641

b

Cas9 variant	Preferred spacer lengths (bp)	Total sites
fCas9	13 to 19, or 22 to 29, in orientation A	92354891
Cas9 nickase	-8 to 100 in orientation A 4 to 42 in orientation B	953048977

Supplementary Table 1. Paired sgRNA target site abundances for fCas9 and Cas9 nickase in the human genome. (**a**) Column 2 shows the number of sites in the human genome with paired sgRNA binding sites in orientation A allowing for a spacer length from -8 bp to 25 bp (column 1) between the

two sgRNA binding sites. sgRNA binding sites in orientation A have the NGG PAM sequences distal from the spacer sequence ($CCNN_{20}$-spacer-$N_{20}NGG$). Column 3 shows the number of sites in the human genome with paired sgRNA binding sites in orientation B allowing for a spacer length from 4 to 25 bp (column 1) between the two sgRNA binding sites. sgRNA binding sites in orientation B have the NGG PAM sequences adjacent to the spacer sequence ($N_{20}NGG$ spacer $CCNN_{20}$) . NC indicates the number of sites in the human genome was not calculated. Negative spacer lengths refer to target sgRNA binding sites that overlap by the indicated number of base pairs. (**b**) Sum of the number of paired sgRNA binding sites in orientation A with spacer lengths of 13 to 19 bp, or 22 to 29 bp, the spacer preference of fCas9 (**Supplementary Figure 8**). Sum of the number of paired sgRNA binding sites with spacer lengths of -8 bp to 100 bp in orientation A, or 4 to 42 bp in orientation B, the spacer preference of Cas9 nickases (4 to 42 bp in orientation B is based on **Figure 3b**, **c**, and -8 bp to 100 bp in orientation A is based on previous reports[2,3]).

Nature Biotechnology: doi:10.1038/nbt.2909

	Genomic target site
EMX_On	GAGTCCGAGCAGAAGAAGAAGGG
EMX_Off1	GAGgCCGAGCAGAAGAAagACGG
EMX_Off2	GAGTCCtAGCAGgAGAAGAAGaG
EMX_Off3	GAGTCtaAGCAGAAGAAGAAGaG
EMX_Off4	GAGTtaGAGCAGAAGAAGAAAGG
VEG_On	GGGTGGGGGGAGTTTGCTCCTGG
VEG_Off1	GGaTGGaGGGAGTTTGCTCCTGG
VEG_Off2	GGGaGGGtGGAGTTTGCTCCTGG
VEG_Off3	cGGgGGaGGGAGTTTGCTCCTGG
VEG_Off4	GGGgaGGGGaAGTTTGCTCCTGG
CLT2_On	GCAGATGTAGTGTTTCCACAGGG
CLT2_Off1	aCAaATGTAGTaTTTCCACAGGG
CLT2_Off2	cCAGATGTAGTaTTcCCACAGGG
CLT2_Off3	ctAGATGaAGTGcTTCCACATGG

Supplementary Table 2. Known off-target substrates of Cas9 target sites in *EMX*, *VEGF,* and *CLTA*. List of genomic on-target and off-targets sites of the EMX, VEGF, and CLTA are shown with mutations from on-target in lower case and red. PAMs are shown in blue.

Nature Biotechnology: doi:10.1038/nbt.2909

a

Nuclease type:	wt Cas9	wt Cas9	Cas9 nickase	fCas9	wt Cas9	Cas9 nickase	fCas9
sgRNA pair target:	*CLTA*	*CLTA*	*CLTA*	*CLTA*	*GFP*	*GFP*	*GFP*
Total expression plasmids (ng):	1000	125	1000	1000	1000	1000	1000
CLTA Sites							
CLT2_On							
Indels	3528	1423	3400	575	3	13	5
Total	10000	10000	10000	10000	10000	10000	10000
Modified (%)	35.280	14.230	34.000	5.750	0.030	0.130	0.050
P-value	<1.0E-300	<1.0E-300	<1.0E-300	1.4E-163			
On:off specificity	1	1		1			
CLT2_Off1							
Indels	316	44	2	2	1	3	3
Total	60620	64755	71537	63079	93883	91306	82055
Modified (%)	0.521	0.068	0.003	0.003	<0.002	0.003	0.004
P-value	1.3E-126	2.1E-16					
On:off specificity	68	209		>2850			
CLT2_Off2							
Indels	11	5	3	1	1	1	2
Total	72596	51093	59632	35541	69114	64412	39978
Modified (%)	0.015	0.010	0.005	0.003	<0.002	<0.002	0.005
P-value	6.5E-03						
On:off specificity	2328	1454		>2850			
CLT2_Off3							
Indels	11	10	0	0	1	1	1
Total	52382	44212	54072	48668	55670	58707	54341
Modified (%)	0.021	0.023	<0.002	<0.002	<0.002	<0.002	<0.002
P-value	2.7E-03	3.5E-03					
On:off specificity	1680	629		>2850			

b

Nuclease type:	wt Cas9	wt Cas9	Cas9 nickase	fCas9	wt Cas9	Cas9 nickase	fCas9
sgRNA pair:	*EMX*	*EMX*	*EMX*	*EMX*	*GFP*	*GFP*	*GFP*
Total expression plasmids (ng):	1000	125	1000	1000	1000	1000	1000
EMX Site							
EMX_On							
Indels	5111	2683	2267	522	0	0	2
Total	10000	10000	10000	10000	10000	10000	10000
Modified (%)	51.110	26.830	22.670	5.220	<0.002	<0.002	0.020
P-value	<1.0E-300	<1.0E-300	<1.0E-300	1.0E-154			
On:off specificity	1	1	1	1			
EMX_Off1							
Indels	386	122	7	1	4	9	7
Total	109787	83420	124564	88424	102817	90020	96526
Modified (%)	0.352	0.146	0.006	<0.002	0.004	0.010	0.007
P-value	1.3E-103	2.8E-37					
On:off specificity	145	183	>11222	>2584			
EMX_Off2							
Indels	74	58	3	6	3	0	4
Total	98568	94108	105747	78871	81717	79469	79193
Modified (%)	0.075	0.062	0.003	0.008	0.004	<0.002	0.005
P-value	3.2E-16	1.4E-12					
On:off specificity	681	435	>11222	>2584			
EMX_Off3							
Indels	736	178	20	14	12	11	17
Total	72888	65139	82348	59593	74341	73408	75080
Modified (%)	1.010	0.273	0.024	0.023	0.016	0.015	0.023
P-value	2.5E-202	3.1E-44					
On:off specificity	51	98	>11222	>2584			
EMX_Off4							
Indels	4149	620	3	3	6	7	5
Total	107537	91695	91368	91605	111736	119643	128088
Modified (%)	3.858	0.676	0.003	0.003	0.005	0.006	0.004
P-value	<1.0E-300	1.9E-202					
On:off specificity	13	40	>11222	>2584			

c

Nuclease type:	wt Cas9	wt Cas9	Cas9 nickase	fCas9	wt Cas9	Cas9 nickase	fCas9
sgRNA pair:	*VEGF*	*VEGF*	*VEGF*	*VEGF*	*GFP*	*GFP*	*GFP*
Total expression plasmids (ng):	1000	125	1000	1000	1000	1000	1000
VEGF Sites							
VEG_On							
Indels	5253	2454	1230	1041	8	0	1
Total	10000	10000	10000	10000	10000	10000	10000
Modified (%)	52.530	24.540	12.300	10.410	0.080	<0.002	0.010
P-value	<1.0E-300	<1.0E-300	<1.0E-300	6.6E-286			
On:off specificity	1	1	1	1			
VEG_Off1							
Indels	2950	603	22	0	0	4	1
Total	82198	71163	90434	77557	74765	79738	74109
Modified (%)	3.589	0.847	0.024	<0.002	<0.002	0.005	<0.002
P-value	<1.0E-300	3.2E-188	2.5E-06				
On:off specificity	15	29	506	>5150			
VEG_Off2							
Indels	863	72	3	3	0	2	1
Total	102501	49836	119702	65107	54247	65753	61556
Modified (%)	0.842	0.144	0.003	0.005	<0.002	0.003	<0.002
P-value	3.5E-159	9.6E-24					
On:off specificity	62	170	>6090	>5150			
VEG_Off3							
Indels	260	33	3	2	3	1	0
Total	91277	83124	90063	84385	62126	68165	69811
Modified (%)	0.285	0.040	0.003	0.002	0.005	<0.002	<0.002
P-value	6.8E-54	1.0E-05					
On:off specificity	184	618	>6090	>5150			
VEG_Off4							
Indels	1305	149	3	2	3	2	4
Total	59827	41203	65964	57828	60906	61219	62162
Modified (%)	2.181	0.362	0.005	0.003	0.005	0.003	0.006
P-value	<1.0E-300	2.7E-54					
On:off specificity	24	68	>6090	>5150			

d

Nuclease type:	Cas9 nickase	fCas9	Cas9 nickase	fCas9
sgRNA pair:	*VEGF*	*VEGF*	*GFP*	*GFP*
Total expression plasmids (ng):	1000	1000	1000	1000
VEGF Sites				
VEG_On				
Indels	2717	2122	10	13
Total	10000	10000	10000	10000
Modified (%)	27.170	21.220	0.100	0.130
P-value	<1.0E-300	<1.0E-300		
On:off specificity	1	1		
VEG_Off1				
Indels	67	30	3	2
Total	302573	233567	204454	190240
Modified (%)	0.022	0.013		
P-value	5.9E-12	2.5E-06		
On:off specificity	1227	1652		

Supplementary Table 3. Cellular modification induced by wild-type Cas9, Cas9 nickase, and fCas9 at on-target and off-target genomic sites. (**a**) Results from sequencing *CLTA* on-target and previously reported genomic off-target sites amplified from 150 ng genomic DNA isolated from human cells treated with a plasmid expressing either wild-type Cas9, Cas9 nickase, or fCas9 and a single plasmid expressing two sgRNAs targeting the *CLTA* on-target site (sgRNA C3 and sgRNA C4). As a negative control, transfection and sequencing were performed as above, but using two sgRNAs targeting the *GFP* gene on-target site (sgRNA G1, G2 or G3 and sgRNA G4, G5, G6 or G7. Indels: the number of observed sequences containing insertions or deletions consistent with any of the three Cas9 nuclease-induced cleavage. Total: total number of sequence counts while only the first 10,000 sequences were analyzed for the on-target site sequences. Modified: number of indels divided by total number of sequences as percentages. Upper limits of potential modification were calculated for sites with no observed indels by assuming there is less than one indel then dividing by the total sequence count to arrive at an upper limit modification percentage, or taking the theoretical limit of detection (1/49,500), whichever value was larger. P-values: For wild-type Cas9 nuclease, Cas9 nickase or fCas9 nuclease, P-values were calculated as previously reported[18] using a two-sided Fisher's exact test between each sample treated with two sgRNAs targeting the *CLTA* on-target site and the control sample treated with two sgRNAs targeting the *GFP* on-target site. P-values of < 0.0045 were considered significant and shown based on conservative multiple comparison correction using the Bonferroni method. On:off specificity is the ratio of on-target to off-target genomic modification frequency for each site. (**b**) Experimental and analytic methods as in (**a**) applied to *EMX* target sites using a single plasmid expressing two sgRNAs targeting the *EMX* on-target site (sgRNA E1 and sgRNA E2). (**c**) Experimental and analytic methods as in (**a**) applied to *VEGF* target sites using a single plasmid expressing two sgRNAs targeting the *VEGF* on-target site (sgRNA V1 and sgRNA v2). (d) Experimental and analytic methods as in (**a**) applied to *VEGF* on-target and *VEGF* off-target site 1 amplified from 600 ng genomic DNA to increase detection sensitivity to 1/198,000.

	Genomic target site
SiteA_On	CCGCCTCTCTGAGCCTCAGTTTCCTTATCCAATTGATCTAAAGGTGGAATGGACATGCTGG
SiteA_Off1	CCGCCTCTCTGAGCCTCAGTTTCCTCATCAGCTGG---gAAAGagGGgcTGGACAaGaTGaTAT
SiteA_Off2	CCACCTCTCTGAGCCTCAGTTTCTTCACCTGTAAATTGGGAATTATAATACTTAACTTCCA
SiteA_Off3	CCACCTCTCTGAGCCTCAGTTTCTTAATCTGTAAAAAGAGATTAATAATACTTCCCTCACT
SiteB_On	CCACTGTGCCTGGCCTCTAATTTCCTAATTCTAGAATCATAAGAAGTTGCAAACATTGTACAGTGGGTGG
SiteB_Off1	CCACTGTGCCTGGCCTCTAATTcTTCATTAAAGGTGACATAGTCATTTCTGGAGCTTAAGCTGCTTCTTG
SiteB_Off2	CCACTGTGCCTGGCCTCTAATTgCTTTTTTATAAATTCCTTTGGATTTTCTTAGTCATGTTATCTTTGAA
SiteB_Off3	CCACTGTGCCTGGCCTCTAATTgTGCTGTTTTATAAGTTGGACTGGCATGGCCTTACTTAAATTTTCAGG
SiteB_Off4	CCACTGTGCCTGGCCTCTAATTTTAAGTCTTACATTTTCATCTGTCTCAAGACTGTTTGACCCAGCTTGG
SiteC_On	CCTCCGTGCCTGGCCTCAAATGCGTGCGATCTTGATTGACTGAATCCACTGCTTTCCTCACAAACTGGG
SiteC_Off1	CCTCCGTGCCTGGCCTCAAATGCTTTGTTATTGTGCCAACAGATGTTGTAATAGGTTGTGCCAGTTGGC
SiteC_Off2	CCACCGTGCCTGGCCTCAAATGCAGACCTGTTTTTGGAAAAGCTTACTGAATAAGGAAGGAAAAAGAT
SiteC_Off3	CCACCGTGCCTGGCCTCAAATGCATTTCTATACAGGTCCAGTCAGAGTAGTGGCCAGTCCCACTCATG
SiteC_Off4	CCACCGTGCCTGGCCTCAAATGgCTTTTAAACATAAGAAAATGTGCTCAGCCTCACTCATATAAAGT
SiteC_Off5	CCACCGTGCCTGGCCTCAAATGtCTTTCTATGTCAGAAAATTTCAATCCTATGAGCTGGCTATAATTA

Supplementary Table 4. Genomic fCas9 target sites A, B and C with highly similar off-target substrates. List of genomic on-target and off-targets sites are shown with mutations from on-target in lower case and red. PAMs are shown in blue. Site A corresponds to the human genomic locus chr1:21,655,401-21,655,461. Site B corresponds to the human genomic locus chr2:31,485,447-31,485,516. Site C corresponds to the human genomic locus chr3:48,747,484-48,747,552

Nature Biotechnology: doi:10.1038/nbt.2909

a

Nuclease type:	none	fCas9	Cas9 nickase
gRNA pair target:	none	SiteA	SiteA
A Sites			
SiteA_On			
Indels	0	1217	4814
Total	36079	79671	73731
Modified (%)	<0.001	1.528	6.529
P-value		1.3E-196	<1.0E-300
On:off specificity	1	1	1
SiteA _Off1			
Indels	3	4	42
Total	62539	87323	96207
Modified (%)	0.005	0.005	0.044
P-value			8.9E-07
On:off specificity		>333	150
SiteA _Off2			
Indels	3	3	147
Total	72314	80940	115745
Modified (%)	0.004	0.004	0.127
P-value			4.2E-27
On:off specificity		>412	51
SiteA _Off3			
Indels	4	4	85
Total	76285	103657	108214
Modified (%)	0.005	0.004	0.079
P-value			2.1E-15
On:off specificity		>396	83

b

Nuclease type:	none	fCas9	Cas9 nickase
gRNA pair target:	none	SiteB	SiteB
B Sites			
SiteB_On			
Indels	1	1333	1054
Total	100164	82939	10000
Modified (%)	<0.001	1.607	10.540
P-value		<1.0E-300	<1.0E-300
On:off specificity		1	1
SiteB _Off1			
Indels	0	7	8
Total	81400	75845	91087
Modified (%)	<0.001	0.009	0.009
P-value			
On:off specificity		>174	>1200
SiteB _Off2			
Indels	1	2	27
Total	92707	100170	165464
Modified (%)	0.001	0.002	0.016
P-value			9.4E-05
On:off specificity		>805	646
SiteB _Off3			
Indels	0	2	14
Total	91872	102947	117175
Modified (%)	<0.001	0.002	0.012
P-value			4.9E-04
On:off specificity		>827	882

c

Nuclease type:	none	fCas9	Cas9 nickase
gRNA pair target:	none	SiteB	SiteB
C Sites			
SiteC_On			
Indels	3	893	769
Total	85113	69013	10000
Modified (%)	0.004	1.294	7.690
P-value		<1.0E-300	<1.0E-300
On:off specificity		1	1

SiteC _Off1			
Indels	0	5	329
Total	114777	95225	117836
Modified (%)	<0.001	0.005	0.279
P-value			8.6E-98
On:off specificity		>246	28

SiteC _Off2			
Indels	1	2	20
Total	70502	73601	112343
Modified (%)	0.001	0.003	0.018
P-value			1.0E-03
On:off specificity		>476	432

SiteC _Off3			
Indels	1	3	106
Total	43908	39215	52210
Modified (%)	0.002	0.008	0.203
P-value			6.0E-27
On:off specificity		>169	38

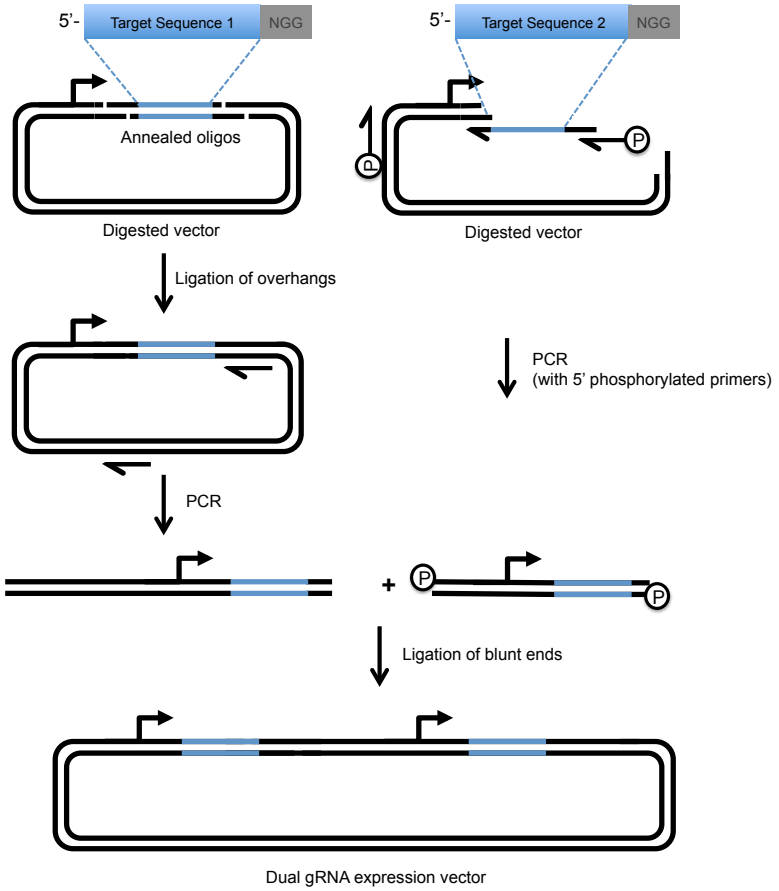
SiteC _Off4			
Indels	4	0	4
Total	143409	54192	70430
Modified (%)	0.003	<0.001	0.006
P-value			
On:off specificity		>1294	>1354

Supplementary Table 5. Cellular modification induced by Cas9 nickase and fCas9 at target sites A, B and C and highly similar off-target genomic sites. (**a**) Results from sequencing genomic site A on-target and highly similar genomic off-target sites amplified from 300 ng genomic DNA isolated from human cells treated with a plasmid expressing either Cas9 nickase, or fCas9 and two separate linear PCR fragments each expressing a distinct gRNA targeting genomic site A. As a negative control, transfection and sequencing were performed on cells with an inert plasmid. Indels: the number of observed sequences containing insertions or deletions consistent with any of the two Cas9 nuclease-induced cleavage. Total: total number of sequence counts while only the first 10,000 sequences were analyzed for some on-target site sequences. Modified: number of indels divided by total number of sequences as percentages. Upper limits of potential modification were calculated for sites with no observed indels by assuming there is less than one indel then dividing by the total sequence count to arrive at an upper limit modification percentage, or taking the theoretical limit of detection (1/99,000), whichever value was larger. P-values: For Cas9 nickase or fCas9 nuclease, P-values were calculated as previously reported[18] using a two-sided Fisher's exact test between each sample treated with two gRNAs targeting the genomic site A on-target site and the control sample treated with no Cas9 or gRNAs. P-values of < 0.005 were considered significant and shown based on conservative multiple comparison correction using the Bonferroni method. On:off specificity is the

ratio of on-target to off-target genomic modification frequency for each site. (**b**) Experimental and analytic methods as in (**a**) applied to genomic site B. PCR amplification of SiteB_Off4 was not successful. (**c**) Experimental and analytic methods as in (**a**) applied to genomic site C. PCR amplification of SiteC_Off5 was not successful.

SUPPLEMENTARY NOTES

Construction of a single plasmid expressing two separate sgRNAs. The strategy used to construct each dual sgRNA expression plasmid is shown in the diagram below. A linear DNA fragment containing a Hu6 promoter (bent arrow), followed by the target site, followed by the sgRNA constant region was constructed by PCR amplification using digested expression vector and nested primers (with 5' phosphate modifications). The phosphorylated DNA fragments were joined by blunt-end ligation with a DNA fragment containing the vector backbone and a Hu6 promoter driving expression of the second sgRNA. Ultimately, two distinct gRNA constructs are expressed from separate (but identical) Hu6 promoters on the same plasmid construct.



DNA Sequence-Processing Algorithms. All scripts were written in bash. Scripts are available upon request.

Computational Search for Potential Target Sites

1) The Patmatch program[4] was used to search the human genome (GRCh37/hg19 build) for pattern sequences corresponding to Cas9 binding sites (CCN N^{20} spacer N^{20}NGG for Orientation A and

N^{20}NGG spacer CCN N^{20} for Orientation B)

Identification of Indels in Sequences of Genomic Sites

1) Sequence reads were initially filtered removing reads of less than 50 bases and removing reads with greater than 10% of the Illumina base scores not being B-J:

Example SeqA-1st read:

TTCTGAGGGCTGCTACCTGTACATCTGCACAAGATTGCCTTTACTCCATGCCTTTCTTCTTCT
GCTCTAACTCTGACAATCTGTCTTGCCATGCCATAAGCCCCTATTCTTTCTGTAACCCCAAGATGGT
ATAAAAGCATCAATGATTGGGC

Example SeqA-2st read:

AAAACTCAAAGAAATGCCCAATCATTGATGCTTTTATACCATCTTGGGGTTACAGAAAGAA
TAGGGGCTTATGGCATGGCAAGACAGATTGTCAGAGTTAGAGCAGAAGAAGAAAGGCATGGAGT
AAAGGCAATCTTGTGCAGATGTACAGGTAA

2) Find **the first 20 bases four bases from the start** of the reverse complement of SeqA-2nd read in SeqA-1st read allowing for 1 mismatch:

Reverse complement of SeqA-2nd read:

TTAC**CTGTACATCTGCACAAGATT**GCCTTTACTCCATGCCTTTCTTCTTCTGCTCTA
ACTCTGACAATCTGTCTTGCCATGCCATAAGCCCCTATTCTTTCTGTAACCCCAAGATGGT
ATAAAAGCATCAATGATTGGGCATTTCTTTGAGTTTT

Position in SeqA-1st read

TTCTGAGGGCTGCTAC**CTGTACATCTGCACAAGATTG**CCTTTACTCCATGCCTTTCTTCTTCTGCT
CTAACTCTGACAATCTGTCTTGCCATGCCATAAGCCCCTATTCTTTCTGTAACCCCAAGATGGTATA
AAAGCATCAATGATTGGGC

3) Align and then combine sequences, removing any sequence with greater than 5% mismatches in the simple base pair alignment:

Combination of SeqA-1st read and SeqA-2nd read:

TTCTGAGGGCTGCTAC**CTGTACATCTGCACAAGATT**GCCTTTACTCCATGCCTTTCT
TCTTCTGCTCTAACTCTGACAATCTGTCTTGCCATGCCATAAGCCCCTATTCTTTCTGTAAC
CCCAAGATGGTATAAAAGCATCAATGATTGGGCATTTCTTTGAGTTTT

4) To identify the target site the flanking genomic sequences were searched for with the Patmatch program[4] allowing for varying amounts of bases from 1 to 300 between the flanking genomic sequences:

Target Site	Downstream genomic sequence	Upstream genomic sequence
EMX_On	GGCCTGCTTCGTGGCAATGC	ACCTGGGCCAGGGAGGGAGG
EMX_Off1	CTCACTTAGACTTTCTCTCC	CTCGGAGTCTAGCTCCTGCA
EMX_Off2	TGGCCCCAGTCTCTCTTCTA	CAGCCTCTGAACAGCTCCCG
EMX_Off3	TGACTTGGCCTTTGTAGGAA	GAGGCTACTGAAACATAAGT
EMX_Off4	TGCTACCTGTACATCTGCAC	CATCAATGATTGGGCATTTC
VEG_On	ACTCCAGTCCCAAATATGTA	ACTAGGGGGCGCTCGGCCAC
VEG_Off1	CTGAGTCAACTGTAAGCATT	GGCCAGGTGCAGTGATTCAT
VEG_Off2	TCGTGTCATCTTGTTTGTGC	GGCAGAGCCCAGCGGACACT
VEG_Off3	CAAGGTGAGCCTGGGTCTGT	ATCACTGCCCAAGAAGTGCA
VEG_Off4	TTGTAGGATGTTTAGCAGCA	ACTTGCTCTCTTTAGAGAAC
CLT2_On	CTCAAGCAGGCCCCGCTGGT	TTTTGGACCAAACCTTTTTG
CLT2_Off1	TGAGGTTATTTGTCCATTGT	TAAGGGGAGTATTTACACCA
CLT2_Off2	TCAAGAGCAGAAAATGTGAC	CTTGCAGGGACCTTCTGATT
CLT2_Off3	TGTGTGTAGGACTAAACTCT	GATAGCAGTATGACCTTGGG
SiteA_On	GCTCTGCCACCACAAGCTTTGGGCA	CCCTTTGCATCCATTCCCCCTACCA

SiteA_Off1	GGAGATGAACCAGCCTGCAGTCAAG	ACTGATCTATGCCTGTGCCTTTGTG
SiteA_Off2	CCCAGTCCCTATCACAAAAAAAGAT	ACATTGATCATCATGGCCACTGGAT
SiteA_Off3	TCCTGATGCCAGCACTCAGTGCCTG	AAGAGCACCAAGTACAGTCTGTGGC
SiteB_On	TTCCCAAACTGCTGGGATTACAGGC	TGCTACTGTGTACTAAGGGCATAGT
SiteB_Off1	CTCAGCCTCTCAAAGTGCTGGGATT	TATCTCCTTCCCTTTCCTTCCCTTC
SiteB_Off2	CTCCCAAAGTGCTGGGATTACAGGC	TTTGGTTTATAGAAACACCATTGAT
SiteB_Off3	CTCCCAAAGTGCTGGGATTACAAGG	GAATGTTAAGTTTGTCCAGAGGCCA
SiteB_Off4	GCCTCCCAAAGTGCTGGGATTACAG	CCAGCACTTTGGGAGGCCAAAGCGG
SiteC_On	CCTCAGCTTCCCAAACTGCTGAGAT	GTGTGACCTTTGCTTTGGAACTGTG
SiteC_Off1	TCTCGACCTCCCTAAGTGCTGGGAT	CTTGCAGAAGAGTGCCAGTTGTGGT
SiteC_Off2	AATCTGCCCACCTCGGCCTCCCAAA	TACCACTTTTAAAATTTCACTTCTC
SiteC_Off3	CTCCCAAAGTGCTGGTATTACAGGT	CTTTTGTCTTAATAATTCCTCTATT
SiteC_Off4	CTGCCTCAGCCTCCCGAAGTGCTAG	ATAATCCCAGCACTTTGAAAGGCTG

4) Any target site sequences corresponding to the same size as the reference genomic site in the human genome (GRCh37/hg19 build) were considered unmodified and any sequences not the reference size were considered potential insertions or deletions. Sequences not the reference size were aligned with ClustalW[5] to the reference genomic site. Aligned sequences with more than one insertion or one deletion in the DNA spacer sequence in or between the two half-site sequences were considered indels. Since high-throughput sequencing can result in insertions or deletions of one base pairs (mis-phasing) at a low but relevant rates - indels of two bp are more likely to arise from Cas9 induced modifications.

Oligonucleotides Used in This Study

All oligonucleotides were purchased from Integrated DNA Technologies. '/5Phos/' indicates 5' phosphorylated oligonucleotides.

dCas9-NLS-FokI primers:	
Cas9_Exp_CNF_Fok1+Plas-Fwd	CGGCGAGATAAACTTTTAA TGACCGGTCATCATCACCA
Cas9_Exp_CNF_Cas9coD10-Rev	CCAACGGAATTAGTGCCGATAGCTAAACCAATAGAATACTTTTTATC
Cas9_Exp_CNF_Cas9coD10-Fwd	GATAAAAAGTATTCTATTGGTTTAGCTATCGGCACTAATTCCGTTGG
Cas9_Exp_CNF_Cas9coH850-Rev	TTCAAAAAGGATTGGGGTACAATGGCATCGACGTCGTAATCAGATAAAC
Cas9_Exp_CNF_Cas9coH850-Fwd	GTTTATCTGATTACGACGTCGATGCCATTGTACCCCAATCCTTTTTGAA
Cas9_Exp_CNF_(Cas9)NLS+GGS-Fok-Rev	TTGGGATCCAGAACCTCCTCCTGCAGCCTTGTCATCG
Cas9_Exp_CNF_(Cas9)NLS+GGS3-Fok-Rev	TTGGGATCCAGAACCTCC GCTGCCGCCACTTCCACCTGA TCCTGCAGCCTTGTCATCG
Cas9_Exp_CNF_(Cas9)NLS+GGS-Fok-Fwd	CGATGACAAGGCTGCAGGAGGAGGTTCTGGATCCCAA
Cas9_Exp_CNF_(Cas9)NLS+GGS3-Fok-Fwd	CGATGACAAGGCTGCAGGA TCAGGTGGAAGTGGCGGCAGC GGAGGTTCTGGATCCCAA
Cas9_Exp_CNF_Fok1+Plas-Rev	TGGTGATGATGACCGGTCA TTAAAAGTTTATCTCGCCG
NLS-dCas9-FokI primers:	
Cas9_Exp_NCF_Fok1+Plas-Fwd	CGGCGAGATAAACTTTTAA TGACCGGTCATCATCACCA

Cas9_Exp_NCF_PlasS+FLAG(NLS-Fok1-Rev	TAGGGAGAGCCGCCACCATGGACTACAAAGACCATGACGG
Cas9_Exp_NCF_NLS+Cas9coD10-Rev	TAAACCAATAGAATACTTTTTATC CATAGGTACCCCGCGGTGAATG
Cas9_Exp_NCF_Cas9coD10-Fwd	GATAAAAAGTATTCTATTGGTTTAGCTATCGGCACTAATTCCGTTGG
Cas9_Exp_NCF_Cas9coH850-Rev	TTCAAAAAGGATTGGGGTACAATGGCATCGACGTCGTAATCAGATAAAC
Cas9_Exp_NCF_Cas9coH850-Fwd	GTTTATCTGATTACGACGTCGATGCCATTGTACCCCAATCCTTTTTGAA
Cas9_Exp_NCF_Cas9End+GGS-Fok-Rev	TTGGGATCCAGAACCTCCGTCACCCCCAAGCTGTG
Cas9_Exp_NCF_Cas9End+GGS3-Fok-Rev	TTGGGATCCAGAACCTCC GCTGCCGCCACTTCCACCTGA GTCACCCCCAAGCTGTG
Cas9_Exp_NCF_Cas9End+GGS-Fok-Fwd	CACAGCTTGGGGGTGACGGAGGTTCTGGATCCCAA
Cas9_Exp_NCF_Cas9End+GGS3-Fok-Fwd	CACAGCTTGGGGGTGAC TCAGGTGGAAGTGGCGGCAGC GGAGGTTCTGGATCCCAA
Cas9_Exp_NCF_Fok1+Plas-Rev	TGGTGATGATGACCGGTCA TTAAAAGTTTATCTCGCCG
FokI-dCas9-NLS primers:	
Cas9_Exp_FCN_PlasS+Fok-Fwd	TAGGGAGAGCCGCCACCATGGGATCCCAACTAGTCAAAAG
Cas9_Exp_FCN_Fok1GGS+Cas-Rev	ACCAATAGAATACTTTTTATCCATGCTGCCACCAAAGTTTATCTC
Cas9_Exp_FCN_Fok1GGS3+Cas-Rev	ACCAATAGAATACTTTTTATCCATGCTGCCGCCACTTCCACCTG
Cas9_Exp_FCN_Cas9coD10-Fwd	GATAAAAAGTATTCTATTGGTTTAGCTATCGGCACTAATTCCGTTGG
Cas9_Exp_FCN_Cas9coH850-Rev	CCAACGGAATTAGTGCCGATAGCTAAACCAATAGAATACTTTTTATC
Cas9_Exp_FCN_Cas9coH850-Fwd	GTTTATCTGATTACGACGTCGATGCCATTGTACCCCAATCCTTTTTGAA
Cas9_Exp_FCN_PlasS+Fok-Rev	CTTTTGACTAGTTGGGATCCCATGGTGGCGGCTCTCCCTA
NLS-FokI-dCas9 primers:	
Cas9_Exp_NFC_PlasS+FLAG-Fwd	**TAGGGAGAGCCGCCACCATG**GACTACAAAGACCATGACGG
Cas9_Exp_FCN_Fok1GGS+Cas-Rev	ACCAATAGAATACTTTTTATCCATGCTGCCACCAAAGTTTATCTC

Cas9_Exp_FCN_Fok1GGS3+Cas-Rev	ACCAATAGAATACTTTTTATCCATGCTGCCGCCACTTCCACCTG
Cas9_Exp_FCN_Cas9coD10-Fwd	GATAAAAAGTATTCTATTGGTTTAGCTATCGGCACTAATTCCGTTGG
Cas9_Exp_NFC_Cas9coH850-Rev	CCAACGGAATTAGTGCCGATAGCTAAACCAATAGAATACTTTTTATC
Cas9_Exp_NFC_Cas9coH850-Fwd	GTTTATCTGATTACGACGTCGATGCCATTGTACCCCAATCCTTTTTGAA
Cas9_Exp_NFC_Cas9End+PlasE-Rev	TGGTGATGATGACCGGTCA GTCACCCCCAAGCTGTG
Cas9_Exp_NFC_Cas9End+PlasE-Fwd	**CACAGCTTGGGGGTGAC** TGACCGGTCATCATCACCA
Cas9_Exp_NFC_PlasS+FLAG-Rev	CCGTCATGGTCTTTGTAGTCCATGGTGGCGGCTCTCCCTA
sgRNA_G1-top	ACACCCCTCGAACTTCACCTCGGCGG
sgRNA_G2-top	ACACCGTCGCCCTCGAACTTCACCTG
sgRNA_G3-top	ACACCCAGCTCGATGCGGTTCACCAG
sgRNA_G4-top	ACACCGGTGAACCGCATCGAGCTGAG
sgRNA_G5-top	ACACCGCTGAAGGGCATCGACTTCAG
sgRNA_G6-top	ACACCGGCATCGACTTCAAGGAGGAG
sgRNA_G7-top	ACACCCAAGGAGGACGGCAACATCCG
sgRNA_G8-top	ACACCACCATCTTCTTCAAGGACGAG
sgRNA_G9-top	ACACCCAACTACAAGACCCGCGCCGG
sgRNA_G10-top	ACACCCCGCGCCGAGGTGAAGTTCGG
sgRNA_G11-top	ACACCGAAGTTCGAGGGCGACACCCG
sgRNA_G12-top	ACACCTTCGAACTTCACCTCGGCGCG
sgRNA_G13-top	ACACCTCAGCTCGATGCGGTTCACCG
sgRNA_G14-top	ACACCCGATGCCCTTCAGCTCGATGG
sgRNA_G1-bottom	AAAACCGCCGAGGTGAAGTTCGAGGG
sgRNA_G2-bottom	AAAACAGGTGAAGTTCGAGGGCGACG
sgRNA_G3-bottom	AAAACTGGTGAACCGCATCGAGCTGG
sgRNA_G4-bottom	AAAACTCAGCTCGATGCGGTTCACCG
sgRNA_G5-bottom	AAAACTGAAGTCGATGCCCTTCAGCG
sgRNA_G6-bottom	AAAACTCCTCCTTGAAGTCGATGCCG
sgRNA_G7-bottom	AAAACGGATGTTGCCGTCCTCCTTGG
sgRNA_G8-bottom	AAAACTCGTCCTTGAAGAAGATGGTG
sgRNA_G9-bottom	AAAACCGGCGCGGGTCTTGTAGTTGG
sgRNA_G10-bottom	AAAACCGAACTTCACCTCGGCGCGGG
sgRNA_G11-bottom	AAAACGGGTGTCGCCCTCGAACTTCG
sgRNA_G12-bottom	AAAACGCGCCGAGGTGAAGTTCGAAG
sgRNA_G13-bottom	AAAACGGTGAACCGCATCGAGCTGAG
sgRNA_G14-bottom	AAAACCATCGAGCTGAAGGGCATCGG

sgRNA_A1-top	AAAACGGATCCTCTCTGGCTCCATCG
sgRNA_C1-top	ACACCTGGCCTGCTTGCTAGACTTGG
sgRNA_C3-top	ACACCGCAGATGTAGTGTTTCCACAG
sgRNA_H1-top	ACACCCTTGCCCCACAGGGCAGTAAG
sgRNA_E1-top	ACACCGAGTCCGAGCAGAAGAAGAAG
sgRNA_V1-top	ACACCGGGTGGGGGGAGTTTGCTCCG
sgRNA_A1-bottom	ACACCGATGGAGCCAGAGAGGATCCG
sgRNA_C1-bottom	AAAACCAAGTCTAGCAAGCAGGCCAG
sgRNA_C3-bottom	AAAACTGTGGAAACACTACATCTGCG
sgRNA_H1-bottom	AAAACTTCTTCTTCTGCTCGGACTCG
sgRNA_E1-bottom	AAAACTTACTGCCCTGTGGGGCAAGG
sgRNA_V1-bottom	AAAACGGAGCAAACTCCCCCCACCCG
PCR_Pla-fwd	AGG AAA GAA CAT GTG AGC AAA AG
PCR_Pla-rev	CAGCGAGTCAGTGAGCGA
PCR_sgRNA-fwd1	CTGTACAAAAAAGCAGGCTTTA
PCR_sgRNA-rev1	AACGTAGGTCTCTACCGCTGTACAAAAAAGCAGGCTTTA
PCR_sgRNA-rev2	AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTAT TTTAACTTGCTATTTCTAGCTCTAAAAC
PCR_sgRNA_A2	TTGCTATTTCTAGCTCTAAAACTCTCTCTCCTTGCCAGAACCGGTGTTTCGTCCTTT CCA
PCR_sgRNA_G1	TTGCTATTTCTAGCTCTAAAACCGCCGAGGTGAAGTTCGAGGGGTGTTTCGTCCTT TCCA
PCR_sgRNA_G2	TTGCTATTTCTAGCTCTAAAACAGGTGAAGTTCGAGGGCGACGGTGTTTCGTCCTT TCCA
PCR_sgRNA_G3	TTGCTATTTCTAGCTCTAAAACTGGTGAACCGCATCGAGCTGGGTGTTTCGTCCTT TCCA
PCR_sgRNA_G4	TTGCTATTTCTAGCTCTAAAACTCAGCTCGATGCGGTTCACCGGTGTTTCGTCCTT TCCA
PCR_sgRNA_G5	TTGCTATTTCTAGCTCTAAAACTGAAGTCGATGCCCTTCAGCGGTGTTTCGTCCTT TCCA
PCR_sgRNA_G6	TTGCTATTTCTAGCTCTAAAACTCCTCCTTGAAGTCGATGCCGGTGTTTCGTCCTTT CCA
PCR_sgRNA_G7	TTGCTATTTCTAGCTCTAAAACGGATGTTGCCGTCCTCCTTGGGTGTTTCGTCCTTT CCA
PCR_sgRNA_C2	TTGCTATTTCTAGCTCTAAAACGCTTGAGGGAGATGAGGACTGGTGTTTCGTCCTT TCCA
PCR_sgRNA_C4	TTGCTATTTCTAGCTCTAAAACATGACTGTGAAGAGCTTCACGGTGTTTCGTCCTT TCCA
PCR_sgRNA_E2	TTGCTATTTCTAGCTCTAAAACGAGGACAAAGTACAAACGGCGGTGTTTCGTCCT TTCCA
PCR_sgRNA_E3	TTGCTATTTCTAGCTCTAAAACGAACCGGAGGACAAAGTACAGGTGTTTCGTCCT TTCCA
PCR_sgRNA_H2	TTGCTATTTCTAGCTCTAAAACCACCACCAACTTCATCCACGGGTGTTTCGTCCTT TCCA
PCR_sgRNA_H3	TTGCTATTTCTAGCTCTAAAACGGGCCTCACCACCAACTTCAGGTGTTTCGTCCTT TCCA
PCR_sgRNA_H4	TTGCTATTTCTAGCTCTAAAACGCCCAGGGCCTCACCACCAAGGTGTTTCGTCCTT TCCA
PCR_sgRNA_H5	TTGCTATTTCTAGCTCTAAAACACCTGCCCAGGGCCTCACCAGGTGTTTCGTCCTT TCCA
PCR_sgRNA_H6	TTGCTATTTCTAGCTCTAAAACTGATACCAACCTGCCCAGGGGGTGTTTCGTCCTT TCCA

PCR_sgRNA_H7	TTGCTATTTCTAGCTCTAAAACTAAACCTGTCTTGTAACCTTGGTGTTTCGTCCTTTCCA
PCR_sgRNA_V2	TTGCTATTTCTAGCTCTAAAACGCTCTGGCTAAAGAGGGAATGGTGTTTCGTCCTTTCCA
PCR_sgRNA_V3	TTGCTATTTCTAGCTCTAAAACCGGCTCTGGCTAAAGAGGGAGGTGTTTCGTCCTTTCCA
PCR_sgRNA_V4	TTGCTATTTCTAGCTCTAAAACTCTGCACACCCCGGCTCTGGGGTGTTTCGTCCTTTCCA
PCR_sgRNA_SA1	TTGCTATTTCTAGCTCTAAAACCCTCTCTGAGCCTCAGTTTCGGTGTTTCGTCCTTTCCA
PCR_sgRNA_SA2	TTGCTATTTCTAGCTCTAAAACGCATGTCCATTCCACCTTTAGGTGTTTCGTCCTTTCCA
PCR_sgRNA_SB1	TTGCTATTTCTAGCTCTAAAACCTGTGCCTGGCCTCTAATTTGGTGTTTCGTCCTTTCCA
PCR_sgRNA_SB2	TTGCTATTTCTAGCTCTAAAACCCCACTGTACAATGTTTGCAGGTGTTTCGTCCTTTCCA
PCR_sgRNA_SC1	TTGCTATTTCTAGCTCTAAAACCCGTGCCTGGCCTCAAATGCGGTGTTTCGTCCTTTCCA
PCR_sgRNA_SC2	TTGCTATTTCTAGCTCTAAAACAGTTTGTGAGGAAAGCAGTGGGTGTTTCGTCCTTTCCA
Survey_GFP-fwd	TACGGCAAGCTGACCCTGAA
Survey_GFP-rev	GTCCATGCCGAGAGTGATCC
Survye_CLTA-fwd	GCCAGGGGCTGTTATCTTGG
Survye_CLTA-rev	ATGCACAGAAGCACAGGTTGA
Survey_EMX-fwd	CTGTGTCCTCTTCCTGCCCT
Survey_EMX-rev	CTCTCCGAGGAGAAGGCCAA
Survey_HBB-fwd	GGTAGACCACCAGCAGCCTA
Survey_HBB-rev	CAGTGCCAGAAGAGCCAAGG
Survey_VEGF-fwd	CCACACAGCTTCCCGTTCTC
Survey_VEGF-rev	GAGAGCCGTTCCCTCTTTGC
Survey_AAVS1-fwd	ACTGCTTCTCCTCTTGGGAAGT
Survey_AAVS1-rev	TCATGGCATCTTCCAGGGGT
HTS_EXM_ON-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT CCTCCCCATTGGCCTGCTTC
HTS_EXM_Off1-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT TCGTCCTGCTCTCACTTAGAC
HTS_EXM_Off2-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT TTTTGTGGCTTGGCCCCAGT
HTS_EXM_Off3-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT TGCAGTCTCATGACTTGGCCT
HTS_EXM_Off4-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT TTCTGAGGGCTGCTACCTGT
HTS_VEFG_ON-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT ACATGAAGCAACTCCAGTCCCA
HTS_EXM_Off1-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT AGCAGACCCACTGAGTCAACTG
HTS_EXM_Off2-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT CCCGCCACAGTCGTGTCAT
HTS_EXM_Off3-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT CGCCCCGGTACAAGGTGA
HTS_EXM_Off4-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT GTACCGTACATTGTAGGATGTTT
HTS_CLTA2_ON-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT CCTCATCTCCCTCAAGCAGGC
HTS_CLTA2_Off1-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT ATTCTGCTCTTGAGGTTATTTGT
HTS_CLTA2_Off2-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT CACCTCTGCCTCAAGAGCAGAAAA
HTS_CLTA2_Off3-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT TGTGTGTGTGTGTGTGTAGGACT
HTS_EXM_ON-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT TCATCTGTGCCCCTCCCTCC

Nature Biotechnology: doi:10.1038/nbt.2909

HTS_EXM_Off-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT CGAGAAGGAGGTGCAGGAG
HTS_EXM_Off-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT CGGGAGCTGTTCAGAGGCTG
HTS_EXM_Off-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT CTCACCTGGGCGAGAAAGGT
HTS_EXM_Off-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT AAAACTCAAAGAAATGCCCAATCA
HTS_VEFG_ON-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT AGACGCTGCTCGCTCCATTC
HTS_EXM_Off1-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT ACAGGCATGAATCACTGCACCT
HTS_EXM_Off2-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT GCGGCAACTTCAGACAACCGA
HTS_EXM_Off3-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT GACCCAGGGGCACCAGTT
HTS_EXM_Off4-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT CTGCCTTCATTGCTTAAAAGTGGAT
HTS_CLTA2_ON-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT ACAGTTGAAGGAAGGAAACATGC
HTS_CLTA2_Off1-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT GCTGCATTTGCCCATTTCCA
HTS_CLTA2_Off2-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT GTTGGGGGAGGAGGAGCTTAT
HTS_CLTA2_Off3-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT CTAAGAGCTATAAGGGCAAATGACT
HTS_SiteA_On-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT AATCCCAGCTCTGCCACCAC
HTS_SiteA_Off1-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT GTGCATTGAGGAGATGAACCAGC
HTS_SiteA_Off2-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT AAGACAGATGCCCAGTCCCT
HTS_SiteA_Off3-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT CGGGTTCGAATCCTGATGCC
HTS_SiteB_On-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT CTCGGCTTCCCAAACTGCTG
HTS_SiteB_Off1-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT ATCCACCCACCTCAGCCTCT
HTS_SiteB_Off2-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT TCGACCTCCCAAAGTGCTGG
HTS_SiteB_Off3-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT GCCTCCCAAAGTGCTGGGAT
HTS_SiteB_Off4-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT ACCCGCTTCAGCCTCC
HTS_SiteC_On-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT AATCCTCCCGCCTCAGCTTC
HTS_SiteC_Off1-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT GATCCGCCTGTCTCGACCTC

HTS_SiteC_Off2-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT GAGCTCAGGCAATCTGCCCA
HTS_SiteC_Off3-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT GGCCTCCCAAAGTGCTGGTA
HTS_SiteC_Off4-fwd	CACTCTTTCCCTACACGACGCTCTTCCGATCT AGTGATCCACCTGCCTCAGC
HTS_SiteA_On-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT GAACATCGCCTGGTAGGGGG
HTS_SiteA_Off1-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT CCATCAGCACCACAAAGGCA
HTS_SiteA_Off2-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT CAGCTGCATATCCAGTGGC
HTS_SiteA_Off3-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT CCTGTTCCATGCCACAGACT
HTS_SiteB_On-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT AGCCAGACAGACTATGCCCT
HTS_SiteB_Off1-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT CGGGGGACAGAAGGGAAGG
HTS_SiteB_Off2-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT ACTCAACATACAAAAATCAATGGTG
HTS_SiteB_Off3-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT TTCTGGCATCTGGCCTCTGG
HTS_SiteB_Off4-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT ATCCACCCGCTTTGGC
HTS_SiteC_On-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT AGTTCAATTGCATTCACAGTTCCA
HTS_SiteC_Off1-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT CACAGCCACCACCACAACTG
HTS_SiteC_Off2-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT ACAAGTAGAGAACAGAGAAGTGAA
HTS_SiteC_Off3-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT GTCTAATGAAGTTGCTAATAGAGGAA
HTS_SiteC_Off4-rev	GGAGTTCAGACGTGTGCTCTTCCGATCT TCCTCCCACTTCAGCCTTTCA

*Fok*I and Cas9 Protein Domain Sequences

Cas9:
MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATR
LKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQ
TYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN
FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS
ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEK
MDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFR
IPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK
HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKI
ECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLK
TYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHD

Nature Biotechnology: doi:10.1038/nbt.2909

DSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIE
MARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMY
VDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQ
LLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK
LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYG
DYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWD
KGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSP
TVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKY
SLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHK
HYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFD
TTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

Cas9 nickase (**D10A**):
MDKKYSIGL**A**IGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATR
LKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQ
TYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN
FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS
ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEK
MDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFR
IPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK
HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKI
ECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLK
TYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHD
DSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIE
MARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMY
VDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQ
LLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK
LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYG
DYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWD
KGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSP
TVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKY
SLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHK
HYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFD
TTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

dCas9 (**D10A and H840A**):
MDKKYSIGL**A**IGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATR
LKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQ
TYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN
FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS
ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEK
MDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFR
IPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK
HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKI
ECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLK
TYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHD

DSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIE
MARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMY
VDQELDINRLSDYDVD**A**IVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQ
LLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK
LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYG
DYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWD
KGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSP
TVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKY
SLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHK
HYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFD
TTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

*Fok*I:
GSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLG
GSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWK
VYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRR
KFNNGEINF

DNA Coding Sequences of wild-type Cas9 Nuclease, Cas9 Nickase and *FCas9* fusions
Wild-type Cas9 nuclease:
ATGGATAAAAAGTATTCTATTGGTTTAGACATCGGCACTAATTCCGTTGGATGGGCTGTCA
TAACCGATGAATACAAAGTACCTTCAAAGAAATTTAAGGTGTTGGGGAACACAGACCGTC
ATTCGATTAAAAAGAATCTTATCGGTGCCCTCCTATTCGATAGTGGCGAAACGGCAGAGG
CGACTCGCCTGAAACGAACCGCTCGGAGAAGGTATACACGTCGCAAGAACCGAATATGTT
ACTTACAAGAAATTTTTAGCAATGAGATGGCCAAAGTTGACGATTCTTTCTTTCACCGTTT
GGAAGAGTCCTTCCTTGTCGAAGAGGACAAGAAACATGAACGGCACCCCATCTTTGGAAA
CATAGTAGATGAGGTGGCATATCATGAAAAGTACCCAACGATTTATCACCTCAGAAAAAA
GCTAGTTGACTCAACTGATAAAGCGGACCTGAGGTTAATCTACTTGGCTCTTGCCCATATG
ATAAAGTTCCGTGGGCACTTTCTCATTGAGGGTGATCTAAATCCGGACAACTCGGATGTC
GACAAACTGTTCATCCAGTTAGTACAAACCTATAATCAGTTGTTTGAAGAGAACCCTATA
AATGCAAGTGGCGTGGATGCGAAGGCTATTCTTAGCGCCCGCCTCTCTAAATCCCGACGG
CTAGAAAACCTGATCGCACAATTACCCGGAGAGAAGAAAAATGGGTTGTTCGGTAACCTT
ATAGCGCTCTCACTAGGCCTGACACCAAATTTTAAGTCGAACTTCGACTTAGCTGAAGAT
GCCAAATTGCAGCTTAGTAAGGACACGTACGATGACGATCTCGACAATCTACTGGCACAA
ATTGGAGATCAGTATGCGGACTTATTTTTGGCTGCCAAAAACCTTAGCGATGCAATCCTCC
TATCTGACATACTGAGAGTTAATACTGAGATTACCAAGGCGCCGTTATCCGCTTCAATGAT
CAAAAGGTACGATGAACATCACCAAGACTTGACACTTCTCAAGGCCCTAGTCCGTCAGCA
ACTGCCTGAGAAATATAAGGAAATATTCTTTGATCAGTCGAAAAACGGGTACGCAGGTTA
TATTGACGGCGGAGCGAGTCAAGAGGAATTCTACAAGTTTATCAAACCCATATTAGAGAA
GATGGATGGGACGGAAGAGTTGCTTGTAAAACTCAATCGCGAAGATCTACTGCGAAAGC
AGCGGACTTTCGACAACGGTAGCATTCCACATCAAATCCACTTAGGCGAATTGCATGCTA
TACTTAGAAGGCAGGAGGATTTTTATCCGTTCCTCAAAGACAATCGTGAAAAGATTGAGA
AAATCCTAACCTTTCGCATACCTTACTATGTGGGACCCCTGGCCCGAGGGAACTCTCGGTT
CGCATGGATGACAAGAAAGTCCGAAGAAACGATTACTCCATGGAATTTTGAGGAAGTTGT
CGATAAAGGTGCGTCAGCTCAATCGTTCATCGAGAGGATGACCAACTTTGACAAGAATTT
ACCGAACGAAAAAGTATTGCCTAAGCACAGTTTACTTTACGAGTATTTCACAGTGTACAA
TGAACTCACGAAAGTTAAGTATGTCACTGAGGGCATGCGTAAACCCGCCTTTCTAAGCGG
AGAACAGAAGAAAGCAATAGTAGATCTGTTATTCAAGACCAACCGCAAAGTGACAGTTA
AGCAATTGAAAGAGGACTACTTTAAGAAAATTGAATGCTTCGATTCTGTCGAGATCTCCG

GGGTAGAAGATCGATTTAATGCGTCACTTGGTACGTATCATGACCTCCTAAAGATAATTA
AAGATAAGGACTTCCTGGATAACGAAGAGAATGAAGATATCTTAGAAGATATAGTGTTGA
CTCTTACCCTCTTTGAAGATCGGGAAATGATTGAGGAAAGACTAAAAACATACGCTCACC
TGTTCGACGATAAGGTTATGAAACAGTTAAAGAGGCGTCGCTATACGGGCTGGGGACGAT
TGTCGCGGAAACTTATCAACGGGATAAGAGACAAGCAAAGTGGTAAAACTATTCTCGATT
TTCTAAAGAGCGACGGCTTCGCCAATAGGAACTTTATGCAGCTGATCCATGATGACTCTTT
AACCTTCAAAGAGGATATACAAAAGGCACAGGTTTCCGGACAAGGGGACTCATTGCACG
AACATATTGCGAATCTTGCTGGTTCGCCAGCCATCAAAAAGGGCATACTCCAGACAGTCA
AAGTAGTGGATGAGCTAGTTAAGGTCATGGGACGTCACAAACCGGAAAACATTGTAATCG
AGATGGCACGCGAAAATCAAACGACTCAGAAGGGGCAAAAAAACAGTCGAGAGCGGAT
GAAGAGAATAGAAGAGGGTATTAAAGAACTGGGCAGCCAGATCTTAAAGGAGCATCCTG
TGGAAAATACCCAATTGCAGAACGAGAAACTTTACCTCTATTACCTACAAAATGGAAGGG
ACATGTATGTTGATCAGGAACTGGACATAAACCGTTTATCTGATTACGACGTCGATCACAT
TGTACCCCAATCCTTTTTGAAGGACGATTCAATCGACAATAAAGTGCTTACACGCTCGGAT
AAGAACCGAGGGAAAAGTGACAATGTTCCAAGCGAGGAAGTCGTAAAGAAAATGAAGA
ACTATTGGCGGCAGCTCCTAAATGCGAAACTGATAACGCAAAGAAAGTTCGATAACTTAA
CTAAAGCTGAGAGGGGTGGCTTGTCTGAACTTGACAAGGCCGGATTTATTAAACGTCAGC
TCGTGGAAACCCGCCAAATCACAAAGCATGTTGCACAGATACTAGATTCCCGAATGAATA
CGAAATACGACGAGAACGATAAGCTGATTCGGGAAGTCAAAGTAATCACTTTAAAGTCA
AAATTGGTGTCGGACTTCAGAAAGGATTTTCAATTCTATAAAGTTAGGGAGATAAATAAC
TACCACCATGCGCACGACGCTTATCTTAATGCCGTCGTAGGGACCGCACTCATTAAGAAA
TACCCGAAGCTAGAAAGTGAGTTTGTGTATGGTGATTACAAAGTTTATGACGTCCGTAAG
ATGATCGCGAAAAGCGAACAGGAGATAGGCAAGGCTACAGCCAAATACTTCTTTTATTCT
AACATTATGAATTTCTTTAAGACGGAAATCACTCTGGCAAACGGAGAGATACGCAAACGA
CCTTTAATTGAAACCAATGGGGAGACAGGTGAAATCGTATGGGATAAGGGCCGGGACTTC
GCGACGGTGAGAAAAGTTTTGTCCATGCCCCAAGTCAACATAGTAAAGAAAACTGAGGTG
CAGACCGGAGGGTTTTCAAAGGAATCGATTCTTCCAAAAAGGAATAGTGATAAGCTCATC
GCTCGTAAAAAGGACTGGGACCCGAAAAAGTACGGTGGCTTCGATAGCCCTACAGTTGCC
TATTCTGTCCTAGTAGTGGCAAAAGTTGAGAAGGGAAAATCCAAGAAACTGAAGTCAGTC
AAAGAATTATTGGGGATAACGATTATGGAGCGCTCGTCTTTTGAAAAGAACCCCATCGAC
TTCCTTGAGGCGAAAGGTTACAAGGAAGTAAAAAAGGATCTCATAATTAAACTACCAAAG
TATAGTCTGTTTGAGTTAGAAAATGGCCGAAAACGGATGTTGGCTAGCGCCGGAGAGCTT
CAAAAGGGGAACGAACTCGCACTACCGTCTAAATACGTGAATTTCCTGTATTTAGCGTCC
CATTACGAGAAGTTGAAAGGTTCACCTGAAGATAACGAACAGAAGCAACTTTTTGTTGAG
CAGCACAAACATTATCTCGACGAAATCATAGAGCAAATTTCGGAATTCAGTAAGAGAGTC
ATCCTAGCTGATGCCAATCTGGACAAAGTATTAAGCGCATACAACAAGCACAGGGATAAA
CCCATACGTGAGCAGGCGGAAAATATTATCCATTTGTTTACTCTTACCAACCTCGGCGCTC
CAGCCGCATTCAAGTATTTTGACACAACGATAGATCGCAAACGATACACTTCTACCAAGG
AGGTGCTAGACGCGACACTGATTCACCAATCCATCACGGGATTATATGAAACTCGGATAG
ATTTGTCACAGCTTGGGGGTGACGGATCCCCCAAGAAGAAGAGGAAAGTCTCGAGCGACT
ACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGAC
AAGGCTGCAGGA

Cas9 nickase:
ATGGACTATAAGGACCACGACGGAGACTACAAGGATCATGATATTGATTACAAAGACGA
TGACGATAAGATGGCCCCAAAGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAG
CCGATAAAAAGTATTCTATTGGTTTAGCTATCGGCACTAATTCCGTTGGATGGGCTGTCAT
AACCGATGAATACAAAGTACCTTCAAAGAAATTTAAGGTGTTGGGGAACACAGACCGTCA
TTCGATTAAAAAGAATCTTATCGGTGCCCTCCTATTCGATAGTGGCGAAACGGCAGAGGC

GACTCGCCTGAAACGAACCGCTCGGAGAAGGTATACACGTCGCAAGAACCGAATATGTTA
CTTACAAGAAATTTTTAGCAATGAGATGGCCAAAGTTGACGATTCTTTCTTTCACCGTTTG
GAAGAGTCCTTCCTTGTCGAAGAGGACAAGAAACATGAACGGCACCCCATCTTTGGAAAC
ATAGTAGATGAGGTGGCATATCATGAAAAGTACCCAACGATTTATCACCTCAGAAAAAAG
CTAGTTGACTCAACTGATAAAGCGGACCTGAGGTTAATCTACTTGGCTCTTGCCCATATGA
TAAAGTTCCGTGGGCACTTTCTCATTGAGGGTGATCTAAATCCGGACAACTCGGATGTCG
ACAAACTGTTCATCCAGTTAGTACAAACCTATAATCAGTTGTTTGAAGAGAACCCTATAA
ATGCAAGTGGCGTGGATGCGAAGGCTATTCTTAGCGCCCGCCTCTCTAAATCCCGACGGC
TAGAAAACCTGATCGCACAATTACCCGGAGAGAAGAAAAATGGGTTGTTCGGTAACCTTA
TAGCGCTCTCACTAGGCCTGACACCAAATTTTAAGTCGAACTTCGACTTAGCTGAAGATGC
CAAATTGCAGCTTAGTAAGGACACGTACGATGACGATCTCGACAATCTACTGGCACAAAT
TGGAGATCAGTATGCGGACTTATTTTTGGCTGCCAAAAACCTTAGCGATGCAATCCTCCTA
TCTGACATACTGAGAGTTAATACTGAGATTACCAAGGCGCCGTTATCCGCTTCAATGATCA
AAAGGTACGATGAACATCACCAAGACTTGACACTTCTCAAGGCCCTAGTCCGTCAGCAAC
TGCCTGAGAAATATAAGGAAATATTCTTTGATCAGTCGAAAAACGGGTACGCAGGTTATA
TTGACGGCGGAGCGAGTCAAGAGGAATTCTACAAGTTTATCAAACCCATATTAGAGAAGA
TGGATGGGACGGAAGAGTTGCTTGTAAAACTCAATCGCGAAGATCTACTGCGAAAGCAGC
GGACTTTCGACAACGGTAGCATTCCACATCAAATCCACTTAGGCGAATTGCATGCTATACT
TAGAAGGCAGGAGGATTTTTATCCGTTCCTCAAAGACAATCGTGAAAAGATTGAGAAAAT
CCTAACCTTTCGCATACCTTACTATGTGGGACCCCTGGCCCGAGGGAACTCTCGGTTCGCA
TGGATGACAAGAAAGTCCGAAGAAACGATTACTCCATGGAATTTTGAGGAAGTTGTCGAT
AAAGGTGCGTCAGCTCAATCGTTCATCGAGAGGATGACCAACTTTGACAAGAATTTACCG
AACGAAAAAGTATTGCCTAAGCACAGTTTACTTTACGAGTATTTCACAGTGTACAATGAA
CTCACGAAAGTTAAGTATGTCACTGAGGGCATGCGTAAACCCGCCTTTCTAAGCGGAGAA
CAGAAGAAAGCAATAGTAGATCTGTTATTCAAGACCAACCGCAAAGTGACAGTTAAGCA
ATTGAAAGAGGACTACTTTAAGAAAATTGAATGCTTCGATTCTGTCGAGATCTCCGGGGT
AGAAGATCGATTTAATGCGTCACTTGGTACGTATCATGACCTCCTAAAGATAATTAAAGA
TAAGGACTTCCTGGATAACGAAGAGAATGAAGATATCTTAGAAGATATAGTGTTGACTCT
TACCCTCTTTGAAGATCGGGAAATGATTGAGGAAAGACTAAAAACATACGCTCACCTGTT
CGACGATAAGGTTATGAAACAGTTAAAGAGGCGTCGCTATACGGGCTGGGGACGATTGTC
GCGGAAACTTATCAACGGGATAAGAGACAAGCAAAGTGGTAAAACTATTCTCGATTTTCT
AAAGAGCGACGGCTTCGCCAATAGGAACTTTATGCAGCTGATCCATGATGACTCTTTAAC
CTTCAAAGAGGATATACAAAAGGCACAGGTTTCCGGACAAGGGGACTCATTGCACGAAC
ATATTGCGAATCTTGCTGGTTCGCCAGCCATCAAAAAGGGCATACTCCAGACAGTCAAAG
TAGTGGATGAGCTAGTTAAGGTCATGGGACGTCACAAACCGGAAAACATTGTAATCGAGA
TGGCACGCGAAAATCAAACGACTCAGAAGGGGCAAAAAAACAGTCGAGAGCGGATGAA
GAGAATAGAAGAGGGTATTAAAGAACTGGGCAGCCAGATCTTAAAGGAGCATCCTGTGG
AAAATACCCAATTGCAGAACGAGAAACTTTACCTCTATTACCTACAAAATGGAAGGGACA
TGTATGTTGATCAGGAACTGGACATAAACCGTTTATCTGATTACGACGTCGATCACATTGT
ACCCCAATCCTTTTTGAAGGACGATTCAATCGACAATAAAGTGCTTACACGCTCGGATAA
GAACCGAGGGAAAAGTGACAATGTTCCAAGCGAGGAAGTCGTAAAGAAAATGAAGAACT
ATTGGCGGCAGCTCCTAAATGCGAAACTGATAACGCAAAGAAAGTTCGATAACTTAACTA
AAGCTGAGAGGGGTGGCTTGTCTGAACTTGACAAGGCCGGATTTATTAAACGTCAGCTCG
TGGAAACCCGCCAAATCACAAAGCATGTTGCACAGATACTAGATTCCCGAATGAATACGA
AATACGACGAGAACGATAAGCTGATTCGGGAAGTCAAAGTAATCACTTTAAAGTCAAAAT
TGGTGTCGGACTTCAGAAAGGATTTTCAATTCTATAAAGTTAGGGAGATAAATAACTACC
ACCATGCGCACGACGCTTATCTTAATGCCGTCGTAGGGACCGCACTCATTAAGAAATACC
CGAAGCTAGAAAGTGAGTTTGTGTATGGTGATTACAAAGTTTATGACGTCCGTAAGATGA
TCGCGAAAAGCGAACAGGAGATAGGCAAGGCTACAGCCAAATACTTCTTTTATTCTAACA

TTATGAATTTCTTTAAGACGGAAATCACTCTGGCAAACGGAGAGATACGCAAACGACCTT
TAATTGAAACCAATGGGGAGACAGGTGAAATCGTATGGGATAAGGGCCGGGACTTCGCG
ACGGTGAGAAAAGTTTTGTCCATGCCCCAAGTCAACATAGTAAAGAAAACTGAGGTGCAG
ACCGGAGGGTTTTCAAAGGAATCGATTCTTCCAAAAAGGAATAGTGATAAGCTCATCGCT
CGTAAAAAGGACTGGGACCCGAAAAAGTACGGTGGCTTCGATAGCCCTACAGTTGCCTAT
TCTGTCCTAGTAGTGGCAAAAGTTGAGAAGGGAAAATCCAAGAAACTGAAGTCAGTCAA
AGAATTATTGGGGATAACGATTATGGAGCGCTCGTCTTTTGAAAAGAACCCCATCGACTT
CCTTGAGGCGAAAGGTTACAAGGAAGTAAAAAAGGATCTCATAATTAAACTACCAAAGT
ATAGTCTGTTTGAGTTAGAAAATGGCCGAAAACGGATGTTGGCTAGCGCCGGAGAGCTTC
AAAAGGGGAACGAACTCGCACTACCGTCTAAATACGTGAATTTCCTGTATTTAGCGTCCC
ATTACGAGAAGTTGAAAGGTTCACCTGAAGATAACGAACAGAAGCAACTTTTTGTTGAGC
AGCACAAACATTATCTCGACGAAATCATAGAGCAAATTTCGGAATTCAGTAAGAGAGTCA
TCCTAGCTGATGCCAATCTGGACAAAGTATTAAGCGCATACAACAAGCACAGGGATAAAC
CCATACGTGAGCAGGCGGAAAATATTATCCATTTGTTTACTCTTACCAACCTCGGCGCTCC
AGCCGCATTCAAGTATTTTGACACAACGATAGATCGCAAACGATACACTTCTACCAAGGA
GGTGCTAGACGCGACACTGATTCACCAATCCATCACGGGATTATATGAAACTCGGATAGA
TTTGTCACAGCTTGGGGGTGAC

dCas9-NLS-GGS3linker-*Fok*I:
ATGGATAAAAAGTATTCTATTGGTTTAGCTATCGGCACTAATTCCGTTGGATGGGCTGTCA
TAACCGATGAATACAAAGTACCTTCAAAGAAATTTAAGGTGTTGGGGAACACAGACCGTC
ATTCGATTAAAAAGAATCTTATCGGTGCCCTCCTATTCGATAGTGGCGAAACGGCAGAGG
CGACTCGCCTGAAACGAACCGCTCGGAGAAGGTATACACGTCGCAAGAACCGAATATGTT
ACTTACAAGAAATTTTTAGCAATGAGATGGCCAAAGTTGACGATTCTTTCTTTCACCGTTT
GGAAGAGTCCTTCCTTGTCGAAGAGGACAAGAAACATGAACGGCACCCCATCTTTGGAAA
CATAGTAGATGAGGTGGCATATCATGAAAAGTACCCAACGATTTATCACCTCAGAAAAAA
GCTAGTTGACTCAACTGATAAAGCGGACCTGAGGTTAATCTACTTGGCTCTTGCCCATATG
ATAAAGTTCCGTGGGCACTTTCTCATTGAGGGTGATCTAAATCCGGACAACTCGGATGTC
GACAAACTGTTCATCCAGTTAGTACAAACCTATAATCAGTTGTTTGAAGAGAACCCTATA
AATGCAAGTGGCGTGGATGCGAAGGCTATTCTTAGCGCCCGCCTCTCTAAATCCCGACGG
CTAGAAAACCTGATCGCACAATTACCCGGAGAGAAGAAAAATGGGTTGTTCGGTAACCTT
ATAGCGCTCTCACTAGGCCTGACACCAAATTTTAAGTCGAACTTCGACTTAGCTGAAGAT
GCCAAATTGCAGCTTAGTAAGGACACGTACGATGACGATCTCGACAATCTACTGGCACAA
ATTGGAGATCAGTATGCGGACTTATTTTTGGCTGCCAAAAACCTTAGCGATGCAATCCTCC
TATCTGACATACTGAGAGTTAATACTGAGATTACCAAGGCGCCGTTATCCGCTTCAATGAT
CAAAAGGTACGATGAACATCACCAAGACTTGACACTTCTCAAGGCCCTAGTCCGTCAGCA
ACTGCCTGAGAAATATAAGGAAATATTCTTTGATCAGTCGAAAAACGGGTACGCAGGTTA
TATTGACGGCGGAGCGAGTCAAGAGGAATTCTACAAGTTTATCAAACCCATATTAGAGAA
GATGGATGGGACGGAAGAGTTGCTTGTAAAACTCAATCGCGAAGATCTACTGCGAAAGC
AGCGGACTTTCGACAACGGTAGCATTCCACATCAAATCCACTTAGGCGAATTGCATGCTA
TACTTAGAAGGCAGGAGGATTTTTATCCGTTCCTCAAAGACAATCGTGAAAAGATTGAGA
AAATCCTAACCTTTCGCATACCTTACTATGTGGGACCCCTGGCCCGAGGGAACTCTCGGTT
CGCATGGATGACAAGAAAGTCCGAAGAAACGATTACTCCATGGAATTTTGAGGAAGTTGT
CGATAAAGGTGCGTCAGCTCAATCGTTCATCGAGAGGATGACCAACTTTGACAAGAATTT
ACCGAACGAAAAAGTATTGCCTAAGCACAGTTTACTTTACGAGTATTTCACAGTGTACAA
TGAACTCACGAAAGTTAAGTATGTCACTGAGGGCATGCGTAAACCCGCCTTTCTAAGCGG
AGAACAGAAGAAAGCAATAGTAGATCTGTTATTCAAGACCAACCGCAAAGTGACAGTTA
AGCAATTGAAAGAGGACTACTTTAAGAAAATTGAATGCTTCGATTCTGTCGAGATCTCCG
GGGTAGAAGATCGATTTAATGCGTCACTTGGTACGTATCATGACCTCCTAAAGATAATTA

AAGATAAGGACTTCCTGGATAACGAAGAGAATGAAGATATCTTAGAAGATATAGTGTTGA
CTCTTACCCTCTTTGAAGATCGGGAAATGATTGAGGAAAGACTAAAAACATACGCTCACC
TGTTCGACGATAAGGTTATGAAACAGTTAAAGAGGCGTCGCTATACGGGCTGGGGACGAT
TGTCGCGGAAACTTATCAACGGGATAAGAGACAAGCAAAGTGGTAAAACTATTCTCGATT
TTCTAAAGAGCGACGGCTTCGCCAATAGGAACTTTATGCAGCTGATCCATGATGACTCTTT
AACCTTCAAAGAGGATATACAAAAGGCACAGGTTTCCGGACAAGGGGACTCATTGCACG
AACATATTGCGAATCTTGCTGGTTCGCCAGCCATCAAAAAGGGCATACTCCAGACAGTCA
AAGTAGTGGATGAGCTAGTTAAGGTCATGGGACGTCACAAACCGGAAAACATTGTAATCG
AGATGGCACGCGAAAATCAAACGACTCAGAAGGGGCAAAAAAACAGTCGAGAGCGGAT
GAAGAGAATAGAAGAGGGTATTAAAGAACTGGGCAGCCAGATCTTAAAGGAGCATCCTG
TGGAAAATACCCAATTGCAGAACGAGAAACTTTACCTCTATTACCTACAAAATGGAAGGG
ACATGTATGTTGATCAGGAACTGGACATAAACCGTTTATCTGATTACGACGTCGATGCCAT
TGTACCCCAATCCTTTTTGAAGGACGATTCAATCGACAATAAAGTGCTTACACGCTCGGAT
AAGAACCGAGGGAAAAGTGACAATGTTCCAAGCGAGGAAGTCGTAAAGAAAATGAAGA
ACTATTGGCGGCAGCTCCTAAATGCGAAACTGATAACGCAAAGAAAGTTCGATAACTTAA
CTAAAGCTGAGAGGGGTGGCTTGTCTGAACTTGACAAGGCCGGATTTATTAAACGTCAGC
TCGTGGAAACCCGCCAAATCACAAAGCATGTTGCACAGATACTAGATTCCCGAATGAATA
CGAAATACGACGAGAACGATAAGCTGATTCGGGAAGTCAAAGTAATCACTTTAAAGTCA
AAATTGGTGTCGGACTTCAGAAAGGATTTTCAATTCTATAAAGTTAGGGAGATAAATAAC
TACCACCATGCGCACGACGCTTATCTTAATGCCGTCGTAGGGACCGCACTCATTAAGAAA
TACCCGAAGCTAGAAAGTGAGTTTGTGTATGGTGATTACAAAGTTTATGACGTCCGTAAG
ATGATCGCGAAAAGCGAACAGGAGATAGGCAAGGCTACAGCCAAATACTTCTTTTATTCT
AACATTATGAATTTCTTTAAGACGGAAATCACTCTGGCAAACGGAGAGATACGCAAACGA
CCTTTAATTGAAACCAATGGGGAGACAGGTGAAATCGTATGGGATAAGGGCCGGGACTTC
GCGACGGTGAGAAAAGTTTTGTCCATGCCCCAAGTCAACATAGTAAAGAAAACTGAGGTG
CAGACCGGAGGGTTTTCAAAGGAATCGATTCTTCCAAAAAGGAATAGTGATAAGCTCATC
GCTCGTAAAAAGGACTGGGACCCGAAAAAGTACGGTGGCTTCGATAGCCCTACAGTTGCC
TATTCTGTCCTAGTAGTGGCAAAAGTTGAGAAGGGAAAATCCAAGAAACTGAAGTCAGTC
AAAGAATTATTGGGGATAACGATTATGGAGCGCTCGTCTTTTGAAAAGAACCCCATCGAC
TTCCTTGAGGCGAAAGGTTACAAGGAAGTAAAAAAGGATCTCATAATTAAACTACCAAAG
TATAGTCTGTTTGAGTTAGAAAATGGCCGAAAACGGATGTTGGCTAGCGCCGGAGAGCTT
CAAAAGGGGAACGAACTCGCACTACCGTCTAAATACGTGAATTTCCTGTATTTAGCGTCC
CATTACGAGAAGTTGAAAGGTTCACCTGAAGATAACGAACAGAAGCAACTTTTTGTTGAG
CAGCACAAACATTATCTCGACGAAATCATAGAGCAAATTTCGGAATTCAGTAAGAGAGTC
ATCCTAGCTGATGCCAATCTGGACAAAGTATTAAGCGCATACAACAAGCACAGGGATAAA
CCCATACGTGAGCAGGCGGAAAATATTATCCATTTGTTTACTCTTACCAACCTCGGCGCTC
CAGCCGCATTCAAGTATTTTGACACAACGATAGATCGCAAACGATACACTTCTACCAAGG
AGGTGCTAGACGCGACACTGATTCACCAATCCATCACGGGATTATATGAAACTCGGATAG
ATTTGTCACAGCTTGGGGGTGACGGATCCCCCAAGAAGAAGAGGAAAGTCTCGAGCGACT
ACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGAC
AAGGCTGCAGGATCAGGTGGAAGTGGCGGCAGCGGAGGTTCTGGATCCCAACTAGTCAA
AAGTGAACTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTGAAATATGTGCCTCATGA
ATATATTGAATTAATTGAAATTGCCAGAAATTCCACTCAGGATAGAATTCTTGAAATGAA
GGTAATGGAATTTTTTATGAAAGTTTATGGATATAGAGGTAAACATTTGGGTGGATCAAG
GAAACCGGACGGAGCAATTTATACTGTCGGATCTCCTATTGATTACGGTGTGATCGTGGA
TACTAAAGCTTATAGCGGAGGTTATAATCTGCCAATTGGCCAAGCAGATGAAATGCAACG
ATATGTCGAAGAAAATCAAACACGAAACAAACATATCAACCCTAATGAATGGTGGAAAG
TCTATCCATCTTCTGTAACGGAATTTAAGTTTTTATTTGTGAGTGGTCACTTTAAAGGAAA
CTACAAAGCTCAGCTTACACGATTAAATCATATCACTAATTGTAATGGAGCTGTTCTTAGT

GTAGAAGAGCTTTTAATTGGTGGAGAAATGATTAAAGCCGGCACATTAACCTTAGAGGAA
GTCAGACGGAAATTTAATAACGGCGAGATAAACTTT

NLS- dCas9-GGS3linker -*Fok*I:
ATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGAC
GATGACAAGATGGCCCCCAAGAAGAAGAGGAAGGTGGGCATTCACCGCGGGGTACCTAT
GGATAAAAAGTATTCTATTGGTTTAGCTATCGGCACTAATTCCGTTGGATGGGCTGTCATA
ACCGATGAATACAAAGTACCTTCAAAGAAATTTAAGGTGTTGGGGAACACAGACCGTCAT
TCGATTAAAAAGAATCTTATCGGTGCCCTCCTATTCGATAGTGGCGAAACGGCAGAGGCG
ACTCGCCTGAAACGAACCGCTCGGAGAAGGTATACACGTCGCAAGAACCGAATATGTTAC
TTACAAGAAATTTTTAGCAATGAGATGGCCAAAGTTGACGATTCTTTCTTTCACCGTTTGG
AAGAGTCCTTCCTTGTCGAAGAGGACAAGAAACATGAACGGCACCCCATCTTTGGAAACA
TAGTAGATGAGGTGGCATATCATGAAAAGTACCCAACGATTTATCACCTCAGAAAAAAGC
TAGTTGACTCAACTGATAAAGCGGACCTGAGGTTAATCTACTTGGCTCTTGCCCATATGAT
AAAGTTCCGTGGGCACTTTCTCATTGAGGGTGATCTAAATCCGGACAACTCGGATGTCGA
CAAACTGTTCATCCAGTTAGTACAAACCTATAATCAGTTGTTTGAAGAGAACCCTATAAAT
GCAAGTGGCGTGGATGCGAAGGCTATTCTTAGCGCCCGCCTCTCTAAATCCCGACGGCTA
GAAAACCTGATCGCACAATTACCCGGAGAGAAGAAAAATGGGTTGTTCGGTAACCTTATA
GCGCTCTCACTAGGCCTGACACCAAATTTTAAGTCGAACTTCGACTTAGCTGAAGATGCC
AAATTGCAGCTTAGTAAGGACACGTACGATGACGATCTCGACAATCTACTGGCACAAATT
GGAGATCAGTATGCGGACTTATTTTTGGCTGCCAAAAACCTTAGCGATGCAATCCTCCTAT
CTGACATACTGAGAGTTAATACTGAGATTACCAAGGCGCCGTTATCCGCTTCAATGATCA
AAAGGTACGATGAACATCACCAAGACTTGACACTTCTCAAGGCCCTAGTCCGTCAGCAAC
TGCCTGAGAAATATAAGGAAATATTCTTTGATCAGTCGAAAAACGGGTACGCAGGTTATA
TTGACGGCGGAGCGAGTCAAGAGGAATTCTACAAGTTTATCAAACCCATATTAGAGAAGA
TGGATGGGACGGAAGAGTTGCTTGTAAAACTCAATCGCGAAGATCTACTGCGAAAGCAGC
GGACTTTCGACAACGGTAGCATTCCACATCAAATCCACTTAGGCGAATTGCATGCTATACT
TAGAAGGCAGGAGGATTTTTATCCGTTCCTCAAAGACAATCGTGAAAAGATTGAGAAAAT
CCTAACCTTTCGCATACCTTACTATGTGGGACCCCTGGCCCGAGGGAACTCTCGGTTCGCA
TGGATGACAAGAAAGTCCGAAGAAACGATTACTCCATGGAATTTTGAGGAAGTTGTCGAT
AAAGGTGCGTCAGCTCAATCGTTCATCGAGAGGATGACCAACTTTGACAAGAATTTACCG
AACGAAAAAGTATTGCCTAAGCACAGTTTACTTTACGAGTATTTCACAGTGTACAATGAA
CTCACGAAAGTTAAGTATGTCACTGAGGGCATGCGTAAACCCGCCTTTCTAAGCGGAGAA
CAGAAGAAAGCAATAGTAGATCTGTTATTCAAGACCAACCGCAAAGTGACAGTTAAGCA
ATTGAAAGAGGACTACTTTAAGAAAATTGAATGCTTCGATTCTGTCGAGATCTCCGGGGT
AGAAGATCGATTTAATGCGTCACTTGGTACGTATCATGACCTCCTAAAGATAATTAAAGA
TAAGGACTTCCTGGATAACGAAGAGAATGAAGATATCTTAGAAGATATAGTGTTGACTCT
TACCCTCTTTGAAGATCGGGAAATGATTGAGGAAAGACTAAAAACATACGCTCACCTGTT
CGACGATAAGGTTATGAAACAGTTAAAGAGGCGTCGCTATACGGGCTGGGGACGATTGTC
GCGGAAACTTATCAACGGGATAAGAGACAAGCAAAGTGGTAAAACTATTCTCGATTTTCT
AAAGAGCGACGGCTTCGCCAATAGGAACTTTATGCAGCTGATCCATGATGACTCTTTAAC
CTTCAAAGAGGATATACAAAAGGCACAGGTTTCCGGACAAGGGGACTCATTGCACGAAC
ATATTGCGAATCTTGCTGGTTCGCCAGCCATCAAAAAGGGCATACTCCAGACAGTCAAAG
TAGTGGATGAGCTAGTTAAGGTCATGGGACGTCACAAACCGGAAAACATTGTAATCGAGA
TGGCACGCGAAAATCAAACGACTCAGAAGGGGCAAAAAAACAGTCGAGAGCGGATGAA
GAGAATAGAAGAGGGTATTAAAGAACTGGGCAGCCAGATCTTAAAGGAGCATCCTGTGG
AAAATACCCAATTGCAGAACGAGAAACTTTACCTCTATTACCTACAAAATGGAAGGGACA
TGTATGTTGATCAGGAACTGGACATAAACCGTTTATCTGATTACGACGTCGATGCCATTGT
ACCCCAATCCTTTTTGAAGGACGATTCAATCGACAATAAAGTGCTTACACGCTCGGATAA

GAACCGAGGGAAAAGTGACAATGTTCCAAGCGAGGAAGTCGTAAAGAAAATGAAGAACT
ATTGGCGGCAGCTCCTAAATGCGAAACTGATAACGCAAAGAAAGTTCGATAACTTAACTA
AAGCTGAGAGGGGTGGCTTGTCTGAACTTGACAAGGCCGGATTTATTAAACGTCAGCTCG
TGGAAACCCGCCAAATCACAAAGCATGTTGCACAGATACTAGATTCCCGAATGAATACGA
AATACGACGAGAACGATAAGCTGATTCGGGAAGTCAAAGTAATCACTTTAAAGTCAAAAT
TGGTGTCGGACTTCAGAAAGGATTTTCAATTCTATAAAGTTAGGGAGATAAATAACTACC
ACCATGCGCACGACGCTTATCTTAATGCCGTCGTAGGGACCGCACTCATTAAGAAATACC
CGAAGCTAGAAAGTGAGTTTGTGTATGGTGATTACAAAGTTTATGACGTCCGTAAGATGA
TCGCGAAAAGCGAACAGGAGATAGGCAAGGCTACAGCCAAATACTTCTTTTATTCTAACA
TTATGAATTTCTTTAAGACGGAAATCACTCTGGCAAACGGAGAGATACGCAAACGACCTT
TAATTGAAACCAATGGGGAGACAGGTGAAATCGTATGGGATAAGGGCCGGGACTTCGCG
ACGGTGAGAAAAGTTTTGTCCATGCCCCAAGTCAACATAGTAAAGAAAACTGAGGTGCAG
ACCGGAGGGTTTTCAAAGGAATCGATTCTTCCAAAAAGGAATAGTGATAAGCTCATCGCT
CGTAAAAAGGACTGGGACCCGAAAAAGTACGGTGGCTTCGATAGCCCTACAGTTGCCTAT
TCTGTCCTAGTAGTGGCAAAAGTTGAGAAGGGAAAATCCAAGAAACTGAAGTCAGTCAA
AGAATTATTGGGGATAACGATTATGGAGCGCTCGTCTTTTGAAAAGAACCCCATCGACTT
CCTTGAGGCGAAAGGTTACAAGGAAGTAAAAAAGGATCTCATAATTAAACTACCAAAGT
ATAGTCTGTTTGAGTTAGAAAATGGCCGAAAACGGATGTTGGCTAGCGCCGGAGAGCTTC
AAAAGGGGAACGAACTCGCACTACCGTCTAAATACGTGAATTTCCTGTATTTAGCGTCCC
ATTACGAGAAGTTGAAAGGTTCACCTGAAGATAACGAACAGAAGCAACTTTTTGTTGAGC
AGCACAAACATTATCTCGACGAAATCATAGAGCAAATTTCGGAATTCAGTAAGAGAGTCA
TCCTAGCTGATGCCAATCTGGACAAAGTATTAAGCGCATACAACAAGCACAGGGATAAAC
CCATACGTGAGCAGGCGGAAAATATTATCCATTTGTTTACTCTTACCAACCTCGGCGCTCC
AGCCGCATTCAAGTATTTTGACACAACGATAGATCGCAAACGATACACTTCTACCAAGGA
GGTGCTAGACGCGACACTGATTCACCAATCCATCACGGGATTATATGAAACTCGGATAGA
TTTGTCACAGCTTGGGGGTGACTCAGGTGGAAGTGGCGGCAGCGGAGGTTCTGGATCCCA
ACTAGTCAAAAGTGAACTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTGAAATATGT
GCCTCATGAATATATTGAATTAATTGAAATTGCCAGAAATTCCACTCAGGATAGAATTCTT
GAAATGAAGGTAATGGAATTTTTTATGAAAGTTTATGGATATAGAGGTAAACATTTGGGT
GGATCAAGGAAACCGGACGGAGCAATTTATACTGTCGGATCTCCTATTGATTACGGTGTG
ATCGTGGATACTAAAGCTTATAGCGGAGGTTATAATCTGCCAATTGGCCAAGCAGATGAA
ATGCAACGATATGTCGAAGAAAATCAAACACGAAACAAACATATCAACCCTAATGAATG
GTGGAAAGTCTATCCATCTTCTGTAACGGAATTTAAGTTTTTATTTGTGAGTGGTCACTTT
AAAGGAAACTACAAAGCTCAGCTTACACGATTAAATCATATCACTAATTGTAATGGAGCT
GTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGATTAAAGCCGGCACATTAACC
TTAGAGGAAGTCAGACGGAAATTTAATAACGGCGAGATAAACTTT

*Fok*I-GGS3linker-dCas9-NLS:
ATGGGATCCCAACTAGTCAAAAGTGAACTGGAGGAGAAGAAATCTGAACTTCGTCATAA
ATTGAAATATGTGCCTCATGAATATATTGAATTAATTGAAATTGCCAGAAATTCCACTCAG
GATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATGAAAGTTTATGGATATAGAGGT
AAACATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCGGATCTCCTATT
GATTACGGTGTGATCGTGGATACTAAAGCTTATAGCGGAGGTTATAATCTGCCAATTGGC
CAAGCAGATGAAATGCAACGATATGTCGAAGAAAATCAAACACGAAACAAACATATCAA
CCCTAATGAATGGTGGAAAGTCTATCCATCTTCTGTAACGGAATTTAAGTTTTTATTTGTG
AGTGGTCACTTTAAAGGAAACTACAAAGCTCAGCTTACACGATTAAATCATATCACTAAT
TGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGATTAAAGCC
GGCACATTAACCTTAGAGGAAGTCAGACGGAAATTTAATAACGGCGAGATAAACTTTGGC
GGTAGTGGGGGATCTGGGGGAAGTATGGATAAAAAGTATTCTATTGGTTTAGCTATCGGC

ACTAATTCCGTTGGATGGGCTGTCATAACCGATGAATACAAAGTACCTTCAAAGAAATTT
AAGGTGTTGGGGAACACAGACCGTCATTCGATTAAAAAGAATCTTATCGGTGCCCTCCTA
TTCGATAGTGGCGAAACGGCAGAGGCGACTCGCCTGAAACGAACCGCTCGGAGAAGGTA
TACACGTCGCAAGAACCGAATATGTTACTTACAAGAAATTTTTAGCAATGAGATGGCCAA
AGTTGACGATTCTTTCTTTCACCGTTTGGAAGAGTCCTTCCTTGTCGAAGAGGACAAGAAA
CATGAACGGCACCCCATCTTTGGAAACATAGTAGATGAGGTGGCATATCATGAAAAGTAC
CCAACGATTTATCACCTCAGAAAAAAGCTAGTTGACTCAACTGATAAAGCGGACCTGAGG
TTAATCTACTTGGCTCTTGCCCATATGATAAAGTTCCGTGGGCACTTTCTCATTGAGGGTG
ATCTAAATCCGGACAACTCGGATGTCGACAAACTGTTCATCCAGTTAGTACAAACCTATA
ATCAGTTGTTTGAAGAGAACCCTATAAATGCAAGTGGCGTGGATGCGAAGGCTATTCTTA
GCGCCCGCCTCTCTAAATCCCGACGGCTAGAAAACCTGATCGCACAATTACCCGGAGAGA
AGAAAAATGGGTTGTTCGGTAACCTTATAGCGCTCTCACTAGGCCTGACACCAAATTTTA
AGTCGAACTTCGACTTAGCTGAAGATGCCAAATTGCAGCTTAGTAAGGACACGTACGATG
ACGATCTCGACAATCTACTGGCACAAATTGGAGATCAGTATGCGGACTTATTTTTGGCTGC
CAAAAACCTTAGCGATGCAATCCTCCTATCTGACATACTGAGAGTTAATACTGAGATTAC
CAAGGCGCCGTTATCCGCTTCAATGATCAAAAGGTACGATGAACATCACCAAGACTTGAC
ACTTCTCAAGGCCCTAGTCCGTCAGCAACTGCCTGAGAAATATAAGGAAATATTCTTTGAT
CAGTCGAAAAACGGGTACGCAGGTTATATTGACGGCGGAGCGAGTCAAGAGGAATTCTA
CAAGTTTATCAAACCCATATTAGAGAAGATGGATGGGACGGAAGAGTTGCTTGTAAAACT
CAATCGCGAAGATCTACTGCGAAAGCAGCGGACTTTCGACAACGGTAGCATTCCACATCA
AATCCACTTAGGCGAATTGCATGCTATACTTAGAAGGCAGGAGGATTTTTATCCGTTCCTC
AAAGACAATCGTGAAAAGATTGAGAAAATCCTAACCTTTCGCATACCTTACTATGTGGGA
CCCCTGGCCCGAGGGAACTCTCGGTTCGCATGGATGACAAGAAAGTCCGAAGAAACGATT
ACTCCATGGAATTTTGAGGAAGTTGTCGATAAAGGTGCGTCAGCTCAATCGTTCATCGAG
AGGATGACCAACTTTGACAAGAATTTACCGAACGAAAAAGTATTGCCTAAGCACAGTTTA
CTTTACGAGTATTTCACAGTGTACAATGAACTCACGAAAGTTAAGTATGTCACTGAGGGC
ATGCGTAAACCCGCCTTTCTAAGCGGAGAACAGAAGAAAGCAATAGTAGATCTGTTATTC
AAGACCAACCGCAAAGTGACAGTTAAGCAATTGAAAGAGGACTACTTTAAGAAAATTGA
ATGCTTCGATTCTGTCGAGATCTCCGGGGTAGAAGATCGATTTAATGCGTCACTTGGTACG
TATCATGACCTCCTAAAGATAATTAAAGATAAGGACTTCCTGGATAACGAAGAGAATGAA
GATATCTTAGAAGATATAGTGTTGACTCTTACCCTCTTTGAAGATCGGGAAATGATTGAGG
AAAGACTAAAAACATACGCTCACCTGTTCGACGATAAGGTTATGAAACAGTTAAAGAGGC
GTCGCTATACGGGCTGGGGACGATTGTCGCGGAAACTTATCAACGGGATAAGAGACAAG
CAAAGTGGTAAAACTATTCTCGATTTTCTAAAGAGCGACGGCTTCGCCAATAGGAACTTT
ATGCAGCTGATCCATGATGACTCTTTAACCTTCAAAGAGGATATACAAAAGGCACAGGTT
TCCGGACAAGGGGACTCATTGCACGAACATATTGCGAATCTTGCTGGTTCGCCAGCCATC
AAAAAGGGCATACTCCAGACAGTCAAAGTAGTGGATGAGCTAGTTAAGGTCATGGGACG
TCACAAACCGGAAAACATTGTAATCGAGATGGCACGCGAAAATCAAACGACTCAGAAGG
GGCAAAAAAACAGTCGAGAGCGGATGAAGAGAATAGAAGAGGGTATTAAAGAACTGGG
CAGCCAGATCTTAAAGGAGCATCCTGTGGAAAATACCCAATTGCAGAACGAGAAACTTTA
CCTCTATTACCTACAAAATGGAAGGGACATGTATGTTGATCAGGAACTGGACATAAACCG
TTTATCTGATTACGACGTCGATGCCATTGTACCCCAATCCTTTTTGAAGGACGATTCAATC
GACAATAAAGTGCTTACACGCTCGGATAAGAACCGAGGGAAAAGTGACAATGTTCCAAG
CGAGGAAGTCGTAAAGAAAATGAAGAACTATTGGCGGCAGCTCCTAAATGCGAAACTGA
TAACGCAAAGAAAGTTCGATAACTTAACTAAAGCTGAGAGGGGTGGCTTGTCTGAACTTG
ACAAGGCCGGATTTATTAAACGTCAGCTCGTGGAAACCCGCCAAATCACAAAGCATGTTG
CACAGATACTAGATTCCCGAATGAATACGAAATACGACGAGAACGATAAGCTGATTCGG
GAAGTCAAAGTAATCACTTTAAAGTCAAAATTGGTGTCGGACTTCAGAAAGGATTTTCAA
TTCTATAAAGTTAGGGAGATAAATAACTACCACCATGCGCACGACGCTTATCTTAATGCC

GTCGTAGGGACCGCACTCATTAAGAAATACCCGAAGCTAGAAAGTGAGTTTGTGTATGGT
GATTACAAAGTTTATGACGTCCGTAAGATGATCGCGAAAAGCGAACAGGAGATAGGCAA
GGCTACAGCCAAATACTTCTTTTATTCTAACATTATGAATTTCTTTAAGACGGAAATCACT
CTGGCAAACGGAGAGATACGCAAACGACCTTTAATTGAAACCAATGGGGAGACAGGTGA
AATCGTATGGGATAAGGGCCGGGACTTCGCGACGGTGAGAAAAGTTTTGTCCATGCCCCA
AGTCAACATAGTAAAGAAAACTGAGGTGCAGACCGGAGGGTTTTCAAAGGAATCGATTCT
TCCAAAAAGGAATAGTGATAAGCTCATCGCTCGTAAAAAGGACTGGGACCCGAAAAAGT
ACGGTGGCTTCGATAGCCCTACAGTTGCCTATTCTGTCCTAGTAGTGGCAAAAGTTGAGA
AGGGAAAATCCAAGAAACTGAAGTCAGTCAAAGAATTATTGGGGATAACGATTATGGAG
CGCTCGTCTTTTGAAAAGAACCCCATCGACTTCCTTGAGGCGAAAGGTTACAAGGAAGTA
AAAAAGGATCTCATAATTAAACTACCAAAGTATAGTCTGTTTGAGTTAGAAAATGGCCGA
AAACGGATGTTGGCTAGCGCCGGAGAGCTTCAAAAGGGGAACGAACTCGCACTACCGTCT
AAATACGTGAATTTCCTGTATTTAGCGTCCCATTACGAGAAGTTGAAAGGTTCACCTGAA
GATAACGAACAGAAGCAACTTTTTGTTGAGCAGCACAAACATTATCTCGACGAAATCATA
GAGCAAATTTCGGAATTCAGTAAGAGAGTCATCCTAGCTGATGCCAATCTGGACAAAGTA
TTAAGCGCATACAACAAGCACAGGGATAAACCCATACGTGAGCAGGCGGAAAATATTAT
CCATTTGTTTACTCTTACCAACCTCGGCGCTCCAGCCGCATTCAAGTATTTTGACACAACG
ATAGATCGCAAACGATACACTTCTACCAAGGAGGTGCTAGACGCGACACTGATTCACCAA
TCCATCACGGGATTATATGAAACTCGGATAGATTTGTCACAGCTTGGGGGTGACGGATCC
CCCAAGAAGAAGAGGAAAGTCTCGAGCGACTACAAAGACCATGACGGTGATTATAAAGA
TCATGACATCGATTACAAGGATGACGATGACAAGGCTGCAGGA

NLS -*Fok*I-GGS3linker-dCas9:
ATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGAC
GATGACAAGATGGCCCCCAAGAAGAAGAGGAAGGTGGGCATTCACCGCGGGGTACCTGG
AGGTTCTATGGGATCCCAACTAGTCAAAAGTGAACTGGAGGAGAAGAAATCTGAACTTCG
TCATAAATTGAAATATGTGCCTCATGAATATATTGAATTAATTGAAATTGCCAGAAATTCC
ACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATGAAAGTTTATGGATAT
AGAGGTAAACATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCGGATCT
CCTATTGATTACGGTGTGATCGTGGATACTAAAGCTTATAGCGGAGGTTATAATCTGCCAA
TTGGCCAAGCAGATGAAATGCAACGATATGTCGAAGAAAATCAAACACGAAACAAACAT
ATCAACCCTAATGAATGGTGGAAAGTCTATCCATCTTCTGTAACGGAATTTAAGTTTTTAT
TTGTGAGTGGTCACTTTAAAGGAAACTACAAAGCTCAGCTTACACGATTAAATCATATCA
CTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGATTAA
AGCCGGCACATTAACCTTAGAGGAAGTCAGACGGAAATTTAATAACGGCGAGATAAACTT
TGGCGGTAGTGGGGGATCTGGGGGAAGTATGGATAAAAAGTATTCTATTGGTTTAGCTAT
CGGCACTAATTCCGTTGGATGGGCTGTCATAACCGATGAATACAAAGTACCTTCAAAGAA
ATTTAAGGTGTTGGGGAACACAGACCGTCATTCGATTAAAAAGAATCTTATCGGTGCCCT
CCTATTCGATAGTGGCGAAACGGCAGAGGCGACTCGCCTGAAACGAACCGCTCGGAGAA
GGTATACACGTCGCAAGAACCGAATATGTTACTTACAAGAAATTTTTAGCAATGAGATGG
CCAAAGTTGACGATTCTTTCTTTCACCGTTTGGAAGAGTCCTTCCTTGTCGAAGAGGACAA
GAAACATGAACGGCACCCCATCTTTGGAAACATAGTAGATGAGGTGGCATATCATGAAAA
GTACCCAACGATTTATCACCTCAGAAAAAAGCTAGTTGACTCAACTGATAAAGCGGACCT
GAGGTTAATCTACTTGGCTCTTGCCCATATGATAAAGTTCCGTGGGCACTTTCTCATTGAG
GGTGATCTAAATCCGGACAACTCGGATGTCGACAAACTGTTCATCCAGTTAGTACAAACC
TATAATCAGTTGTTTGAAGAGAACCCTATAAATGCAAGTGGCGTGGATGCGAAGGCTATT
CTTAGCGCCCGCCTCTCTAAATCCCGACGGCTAGAAAACCTGATCGCACAATTACCCGGA
GAGAAGAAAAATGGGTTGTTCGGTAACCTTATAGCGCTCTCACTAGGCCTGACACCAAAT
TTTAAGTCGAACTTCGACTTAGCTGAAGATGCCAAATTGCAGCTTAGTAAGGACACGTAC

GATGACGATCTCGACAATCTACTGGCACAAATTGGAGATCAGTATGCGGACTTATTTTTG
GCTGCCAAAAACCTTAGCGATGCAATCCTCCTATCTGACATACTGAGAGTTAATACTGAG
ATTACCAAGGCGCCGTTATCCGCTTCAATGATCAAAAGGTACGATGAACATCACCAAGAC
TTGACACTTCTCAAGGCCCTAGTCCGTCAGCAACTGCCTGAGAAATATAAGGAAATATTC
TTTGATCAGTCGAAAAACGGGTACGCAGGTTATATTGACGGCGGAGCGAGTCAAGAGGA
ATTCTACAAGTTTATCAAACCCATATTAGAGAAGATGGATGGGACGGAAGAGTTGCTTGT
AAAACTCAATCGCGAAGATCTACTGCGAAAGCAGCGGACTTTCGACAACGGTAGCATTCC
ACATCAAATCCACTTAGGCGAATTGCATGCTATACTTAGAAGGCAGGAGGATTTTTATCC
GTTCCTCAAAGACAATCGTGAAAAGATTGAGAAAATCCTAACCTTTCGCATACCTTACTAT
GTGGGACCCCTGGCCCGAGGGAACTCTCGGTTCGCATGGATGACAAGAAAGTCCGAAGA
AACGATTACTCCATGGAATTTTGAGGAAGTTGTCGATAAAGGTGCGTCAGCTCAATCGTT
CATCGAGAGGATGACCAACTTTGACAAGAATTTACCGAACGAAAAAGTATTGCCTAAGCA
CAGTTTACTTTACGAGTATTTCACAGTGTACAATGAACTCACGAAAGTTAAGTATGTCACT
GAGGGCATGCGTAAACCCGCCTTTCTAAGCGGAGAACAGAAGAAAGCAATAGTAGATCT
GTTATTCAAGACCAACCGCAAAGTGACAGTTAAGCAATTGAAAGAGGACTACTTTAAGAA
AATTGAATGCTTCGATTCTGTCGAGATCTCCGGGGTAGAAGATCGATTTAATGCGTCACTT
GGTACGTATCATGACCTCCTAAAGATAATTAAAGATAAGGACTTCCTGGATAACGAAGAG
AATGAAGATATCTTAGAAGATATAGTGTTGACTCTTACCCTCTTTGAAGATCGGGAAATG
ATTGAGGAAAGACTAAAAACATACGCTCACCTGTTCGACGATAAGGTTATGAAACAGTTA
AAGAGGCGTCGCTATACGGGCTGGGGACGATTGTCGCGGAAACTTATCAACGGGATAAG
AGACAAGCAAAGTGGTAAAACTATTCTCGATTTTCTAAAGAGCGACGGCTTCGCCAATAG
GAACTTTATGCAGCTGATCCATGATGACTCTTTAACCTTCAAAGAGGATATACAAAAGGC
ACAGGTTTCCGGACAAGGGGACTCATTGCACGAACATATTGCGAATCTTGCTGGTTCGCC
AGCCATCAAAAAGGGCATACTCCAGACAGTCAAAGTAGTGGATGAGCTAGTTAAGGTCAT
GGGACGTCACAAACCGGAAAACATTGTAATCGAGATGGCACGCGAAAATCAAACGACTC
AGAAGGGGCAAAAAAACAGTCGAGAGCGGATGAAGAGAATAGAAGAGGGTATTAAAGA
ACTGGGCAGCCAGATCTTAAAGGAGCATCCTGTGGAAAATACCCAATTGCAGAACGAGA
AACTTTACCTCTATTACCTACAAAATGGAAGGGACATGTATGTTGATCAGGAACTGGACA
TAAACCGTTTATCTGATTACGACGTCGATGCCATTGTACCCCAATCCTTTTTGAAGGACGA
TTCAATCGACAATAAAGTGCTTACACGCTCGGATAAGAACCGAGGGAAAAGTGACAATGT
TCCAAGCGAGGAAGTCGTAAAGAAAATGAAGAACTATTGGCGGCAGCTCCTAAATGCGA
AACTGATAACGCAAAGAAAGTTCGATAACTTAACTAAAGCTGAGAGGGGTGGCTTGTCTG
AACTTGACAAGGCCGGATTTATTAAACGTCAGCTCGTGGAAACCCGCCAAATCACAAAGC
ATGTTGCACAGATACTAGATTCCCGAATGAATACGAAATACGACGAGAACGATAAGCTGA
TTCGGGAAGTCAAAGTAATCACTTTAAAGTCAAAATTGGTGTCGGACTTCAGAAAGGATT
TTCAATTCTATAAAGTTAGGGAGATAAATAACTACCACCATGCGCACGACGCTTATCTTA
ATGCCGTCGTAGGGACCGCACTCATTAAGAAATACCCGAAGCTAGAAAGTGAGTTTGTGT
ATGGTGATTACAAAGTTTATGACGTCCGTAAGATGATCGCGAAAAGCGAACAGGAGATA
GGCAAGGCTACAGCCAAATACTTCTTTTATTCTAACATTATGAATTTCTTTAAGACGGAAA
TCACTCTGGCAAACGGAGAGATACGCAAACGACCTTTAATTGAAACCAATGGGGAGACA
GGTGAAATCGTATGGGATAAGGGCCGGGACTTCGCGACGGTGAGAAAAGTTTTGTCCATG
CCCCAAGTCAACATAGTAAAGAAAACTGAGGTGCAGACCGGAGGGTTTTCAAAGGAATC
GATTCTTCCAAAAAGGAATAGTGATAAGCTCATCGCTCGTAAAAAGGACTGGGACCCGAA
AAAGTACGGTGGCTTCGATAGCCCTACAGTTGCCTATTCTGTCCTAGTAGTGGCAAAAGTT
GAGAAGGGAAAATCCAAGAAACTGAAGTCAGTCAAAGAATTATTGGGGATAACGATTAT
GGAGCGCTCGTCTTTTGAAAAGAACCCCATCGACTTCCTTGAGGCGAAAGGTTACAAGGA
AGTAAAAAAGGATCTCATAATTAAACTACCAAAGTATAGTCTGTTTGAGTTAGAAAATGG
CCGAAAACGGATGTTGGCTAGCGCCGGAGAGCTTCAAAAGGGGAACGAACTCGCACTAC
CGTCTAAATACGTGAATTTCCTGTATTTAGCGTCCCATTACGAGAAGTTGAAAGGTTCACC

TGAAGATAACGAACAGAAGCAACTTTTTGTTGAGCAGCACAAACATTATCTCGACGAAAT
CATAGAGCAAATTTCGGAATTCAGTAAGAGAGTCATCCTAGCTGATGCCAATCTGGACAA
AGTATTAAGCGCATACAACAAGCACAGGGATAAACCCATACGTGAGCAGGCGGAAAATA
TTATCCATTTGTTTACTCTTACCAACCTCGGCGCTCCAGCCGCATTCAAGTATTTTGACACA
ACGATAGATCGCAAACGATACACTTCTACCAAGGAGGTGCTAGACGCGACACTGATTCAC
CAATCCATCACGGGATTATATGAAACTCGGATAGATTTGTCACAGCTTGGGGGTGAC

fCas9

ATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGAC
GATGACAAGATGGCCCCCAAGAAGAAGAGGAAGGTGGGCATTCACCGCGGGGTACCTGG
AGGTTCTATGGGATCCCAACTAGTCAAAAGTGAACTGGAGGAGAAGAAATCTGAACTTCG
TCATAAATTGAAATATGTGCCTCATGAATATATTGAATTAATTGAAATTGCCAGAAATTCC
ACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATGAAAGTTTATGGATAT
AGAGGTAAACATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCGGATCT
CCTATTGATTACGGTGTGATCGTGGATACTAAAGCTTATAGCGGAGGTTATAATCTGCCAA
TTGGCCAAGCAGATGAAATGCAACGATATGTCGAAGAAAATCAAACACGAAACAAACAT
ATCAACCCTAATGAATGGTGGAAAGTCTATCCATCTTCTGTAACGGAATTTAAGTTTTTAT
TTGTGAGTGGTCACTTTAAAGGAAACTACAAAGCTCAGCTTACACGATTAAATCATATCA
CTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGATTAA
AGCCGGCACATTAACCTTAGAGGAAGTCAGACGGAAATTTAATAACGGCGAGATAAACTT
TAGCGGCAGCGAGACTCCCGGGACCTCAGAGTCCGCCACACCCGAAAGTATGGATAAAA
AGTATTCTATTGGTTTAGCTATCGGCACTAATTCCGTTGGATGGGCTGTCATAACCGATGA
ATACAAAGTACCTTCAAAGAAATTTAAGGTGTTGGGGAACACAGACCGTCATTCGATTAA
AAAGAATCTTATCGGTGCCCTCCTATTCGATAGTGGCGAAACGGCAGAGGCGACTCGCCT
GAAACGAACCGCTCGGAGAAGGTATACACGTCGCAAGAACCGAATATGTTACTTACAAG
AAATTTTTAGCAATGAGATGGCCAAAGTTGACGATTCTTTCTTTCACCGTTTGGAAGAGTC
CTTCCTTGTCGAAGAGGACAAGAAACATGAACGGCACCCCATCTTTGGAAACATAGTAGA
TGAGGTGGCATATCATGAAAAGTACCCAACGATTTATCACCTCAGAAAAAAGCTAGTTGA
CTCAACTGATAAAGCGGACCTGAGGTTAATCTACTTGGCTCTTGCCCATATGATAAAGTTC
CGTGGGCACTTTCTCATTGAGGGTGATCTAAATCCGGACAACTCGGATGTCGACAAACTG
TTCATCCAGTTAGTACAAACCTATAATCAGTTGTTTGAAGAGAACCCTATAAATGCAAGT
GGCGTGGATGCGAAGGCTATTCTTAGCGCCCGCCTCTCTAAATCCCGACGGCTAGAAAAC
CTGATCGCACAATTACCCGGAGAGAAGAAAAATGGGTTGTTCGGTAACCTTATAGCGCTC
TCACTAGGCCTGACACCAAATTTTAAGTCGAACTTCGACTTAGCTGAAGATGCCAAATTG
CAGCTTAGTAAGGACACGTACGATGACGATCTCGACAATCTACTGGCACAAATTGGAGAT
CAGTATGCGGACTTATTTTTGGCTGCCAAAAACCTTAGCGATGCAATCCTCCTATCTGACA
TACTGAGAGTTAATACTGAGATTACCAAGGCGCCGTTATCCGCTTCAATGATCAAAAGGT
ACGATGAACATCACCAAGACTTGACACTTCTCAAGGCCCTAGTCCGTCAGCAACTGCCTG
AGAAATATAAGGAAATATTCTTTGATCAGTCGAAAAACGGGTACGCAGGTTATATTGACG
GCGGAGCGAGTCAAGAGGAATTCTACAAGTTTATCAAACCCATATTAGAGAAGATGGATG
GGACGGAAGAGTTGCTTGTAAAACTCAATCGCGAAGATCTACTGCGAAAGCAGCGGACTT
TCGACAACGGTAGCATTCCACATCAAATCCACTTAGGCGAATTGCATGCTATACTTAGAA
GGCAGGAGGATTTTTATCCGTTCCTCAAAGACAATCGTGAAAAGATTGAGAAAATCCTAA
CCTTTCGCATACCTTACTATGTGGGACCCCTGGCCCGAGGGAACTCTCGGTTCGCATGGAT
GACAAGAAAGTCCGAAGAAACGATTACTCCATGGAATTTTGAGGAAGTTGTCGATAAAG
GTGCGTCAGCTCAATCGTTCATCGAGAGGATGACCAACTTTGACAAGAATTTACCGAACG
AAAAAGTATTGCCTAAGCACAGTTTACTTTACGAGTATTTCACAGTGTACAATGAACTCAC
GAAAGTTAAGTATGTCACTGAGGGCATGCGTAAACCCGCCTTTCTAAGCGGAGAACAGAA
GAAAGCAATAGTAGATCTGTTATTCAAGACCAACCGCAAAGTGACAGTTAAGCAATTGAA

AGAGGACTACTTTAAGAAAATTGAATGCTTCGATTCTGTCGAGATCTCCGGGGTAGAAGA
TCGATTTAATGCGTCACTTGGTACGTATCATGACCTCCTAAAGATAATTAAAGATAAGGA
CTTCCTGGATAACGAAGAGAATGAAGATATCTTAGAAGATATAGTGTTGACTCTTACCCT
CTTTGAAGATCGGGAAATGATTGAGGAAAGACTAAAAACATACGCTCACCTGTTCGACGA
TAAGGTTATGAAACAGTTAAAGAGGCGTCGCTATACGGGCTGGGGACGATTGTCGCGGAA
ACTTATCAACGGGATAAGAGACAAGCAAAGTGGTAAAACTATTCTCGATTTTCTAAAGAG
CGACGGCTTCGCCAATAGGAACTTTATGCAGCTGATCCATGATGACTCTTTAACCTTCAAA
GAGGATATACAAAAGGCACAGGTTTCCGGACAAGGGGACTCATTGCACGAACATATTGC
GAATCTTGCTGGTTCGCCAGCCATCAAAAAGGGCATACTCCAGACAGTCAAAGTAGTGGA
TGAGCTAGTTAAGGTCATGGGACGTCACAAACCGGAAAACATTGTAATCGAGATGGCACG
CGAAAATCAAACGACTCAGAAGGGGCAAAAAAACAGTCGAGAGCGGATGAAGAGAATA
GAAGAGGGTATTAAAGAACTGGGCAGCCAGATCTTAAAGGAGCATCCTGTGGAAAATAC
CCAATTGCAGAACGAGAAACTTTACCTCTATTACCTACAAAATGGAAGGGACATGTATGT
TGATCAGGAACTGGACATAAACCGTTTATCTGATTACGACGTCGATGCCATTGTACCCCA
ATCCTTTTTGAAGGACGATTCAATCGACAATAAAGTGCTTACACGCTCGGATAAGAACCG
AGGGAAAAGTGACAATGTTCCAAGCGAGGAAGTCGTAAAGAAAATGAAGAACTATTGGC
GGCAGCTCCTAAATGCGAAACTGATAACGCAAAGAAAGTTCGATAACTTAACTAAAGCTG
AGAGGGGTGGCTTGTCTGAACTTGACAAGGCCGGATTTATTAAACGTCAGCTCGTGGAAA
CCCGCCAAATCACAAAGCATGTTGCACAGATACTAGATTCCCGAATGAATACGAAATACG
ACGAGAACGATAAGCTGATTCGGGAAGTCAAAGTAATCACTTTAAAGTCAAAATTGGTGT
CGGACTTCAGAAAGGATTTTCAATTCTATAAAGTTAGGGAGATAAATAACTACCACCATG
CGCACGACGCTTATCTTAATGCCGTCGTAGGGACCGCACTCATTAAGAAATACCCGAAGC
TAGAAAGTGAGTTTGTGTATGGTGATTACAAAGTTTATGACGTCCGTAAGATGATCGCGA
AAAGCGAACAGGAGATAGGCAAGGCTACAGCCAAATACTTCTTTTATTCTAACATTATGA
ATTTCTTTAAGACGGAAATCACTCTGGCAAACGGAGAGATACGCAAACGACCTTTAATTG
AAACCAATGGGGAGACAGGTGAAATCGTATGGGATAAGGGCCGGGACTTCGCGACGGTG
AGAAAAGTTTTGTCCATGCCCCAAGTCAACATAGTAAAGAAAACTGAGGTGCAGACCGG
AGGGTTTTCAAAGGAATCGATTCTTCCAAAAAGGAATAGTGATAAGCTCATCGCTCGTAA
AAAGGACTGGGACCCGAAAAAGTACGGTGGCTTCGATAGCCCTACAGTTGCCTATTCTGT
CCTAGTAGTGGCAAAAGTTGAGAAGGGAAAATCCAAGAAACTGAAGTCAGTCAAAGAAT
TATTGGGGATAACGATTATGGAGCGCTCGTCTTTTGAAAAGAACCCCATCGACTTCCTTGA
GGCGAAAGGTTACAAGGAAGTAAAAAAGGATCTCATAATTAAACTACCAAAGTATAGTC
TGTTTGAGTTAGAAAATGGCCGAAAACGGATGTTGGCTAGCGCCGGAGAGCTTCAAAAGG
GGAACGAACTCGCACTACCGTCTAAATACGTGAATTTCCTGTATTTAGCGTCCCATTACGA
GAAGTTGAAAGGTTCACCTGAAGATAACGAACAGAAGCAACTTTTTGTTGAGCAGCACAA
ACATTATCTCGACGAAATCATAGAGCAAATTTCGGAATTCAGTAAGAGAGTCATCCTAGC
TGATGCCAATCTGGACAAAGTATTAAGCGCATACAACAAGCACAGGGATAAACCCATAC
GTGAGCAGGCGGAAAATATTATCCATTTGTTTACTCTTACCAACCTCGGCGCTCCAGCCGC
ATTCAAGTATTTTGACACAACGATAGATCGCAAACGATACACTTCTACCAAGGAGGTGCT
AGACGCGACACTGATTCACCAATCCATCACGGGATTATATGAAACTCGGATAGATTTGTC
ACAGCTTGGGGGTGAC

SUPPLEMENTARY REFERENCES

1. Schellenberger, V. *et al.* A recombinant polypeptide extends the in vivo half-life of peptides and proteins in a tunable manner. *Nat. Biotechnol.* **27,** 1186–1190 (2009).
2. Mali, P. *et al.* CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. *Nat. Biotechnol.* **31,** 833–838 (2013).
3. Ran, F. A. *et al.* Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity. *Cell* **154,** 1380–1389 (2013).
4. Yan, T. *et al.* PatMatch: a program for finding patterns in peptide and nucleotide sequences. *Nucleic Acids Res.* **33,** W262–W266 (2005).
5. Larkin, M. A. *et al.* Clustal W and Clustal X version 2.0. *Bioinformatics* **23,** 2947–2948 (2007).

Nature Biotechnology: doi:10.1038/nbt.2909