SUPPLEMENTARY INFORMATION

Small Molecule-Triggered Cas9 Protein with Improved Genome-Editing Specificity

Kevin M. Davis[1,2], Vikram Pattanayak[3], David B. Thompson[1,2], John A. Zuris[1,2], & David R. Liu[1,2]*

[1]Department of Chemistry & Chemical Biology, Harvard University, 12 Oxford St, Cambridge, MA 02138 USA

[2]Howard Hughes Medical Institute, Harvard University, 12 Oxford St, Cambridge, MA 02138 USA

[3]Department of Pathology, Massachusetts General Hospital, 55 Fruit St, Boston, MA 02114 USA

*Correspondence should be addressed to D.R.L. (drliu@fas.harvard.edu)

SUPPLEMENTARY RESULTS

Supplementary Text	Sensitivity limit of off-target cleavage assay
Supplementary Figure 1	Effect of 4-HT on cell viability
Supplementary Figure 2	Western blot analysis of intein splicing
Supplementary Figure 3	On-target and off-target indel frequencies at *EMX*, *VEGF*, and *CLTA* sites with intein-Cas9s and wild-type Cas9 in the presence of 4-HT
Supplementary Figure 4	DNA modification specificity for intein-Cas9s and wild-type Cas9 in the absence of 4-HT
Supplementary Figure 5	On-target modification at *EMX*, *VEGF*, and *CLTA* sites with intein-Cas9s and wild-type Cas9 at different transfection levels in the presence of 4-HT
Supplementary Figure 6	On-target and off-target modification at *EMX*, *VEGF*, and *CLTA* sites with intein-Cas9s and wild-type Cas9 at different transfection levels in the presence of 4-HT
Supplementary Figure 7	DNA modification specificity for intein-Cas9s and wild-type Cas9 at different transfection levels in the presence of 4-HT
Supplementary Figure 8	Genomic *EGFP* disruption activity of intein-Cas9(S219) and intein-Cas9(S219-G521R) in the presence of β-estradiol and 4-HT
Supplementary Table 1	On-target and 11 known off-target substrates of Cas9:sgRNAs that target *EMX*, *VEGF*, and *CLTA* sites
Supplementary Table 2	Raw sequence counts and modification frequencies for data plotted in Fig. 2 and Supplementary Figs. 3 and 4
Supplementary Table 3	*P*-values for comparisons between conditions in Fig. 2a
Supplementary Table 4	Raw sequence counts and modification frequencies for data plotted in Fig. 2b-d and Supplementary Figs. 3, and 5-7
Supplementary Table 5	*P*-values for comparisons between conditions in Supplementary Figure 3
Supplementary Table 6	*P*-values for comparisons between conditions in Supplementary Figs. 5 and 6
Supplementary Table 7	Raw sequence counts and modification frequencies for *EMX*, *VEGF*, and *CLTA* sites in the absence of a targeting sgRNA
Supplementary Notes	Constructs used in this study, and indel calling algorithm
Supplementary Data Set 1	Indel sequences used to calculate modification frequencies in Fig. 2 and Supplementary Figs. 3 and 4
Supplementary Data Set 2	Indel sequences used to calculate modification frequencies in Fig. 2b-e and Supplementary Figs. 5-7

SUPPLEMENTARY TEXT

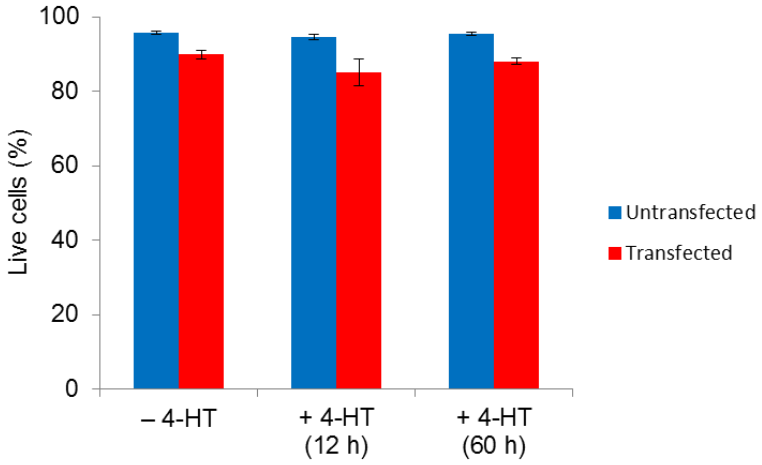
Sensitivity limit of off-target cleavage assays

We used paired end sequencing to identify indels caused by genomic on- and off-target cleavage (see Supplementary Notes). Given that published studies (see the reference below) have shown that the Illumina platform has an indel rate that is several orders of magnitude lower than the ~0.1% substitution error rate, and our requirement that all called indels occur in both paired reads, the sensitivity of the high-throughput sequencing method for detecting genomic off-target cleavage in our study 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 or 200 ng of input gDNA, which provides amplicons derived from at most 50,000 or 65,000 unique gDNA copies, respectively. Therefore, the high-throughput sequencing assay cannot detect rare genome modification events that occur at a frequency of less than approximately 1 in 50,000 (0.002%). When comparing between two conditions, such as wt Cas9 vs. intein-Cas9, this threshold becomes approximately 10 in 50,000 (0.02%) when using the Fisher exact test and a conservative multiple comparison correction (Bonferroni with 14 samples).

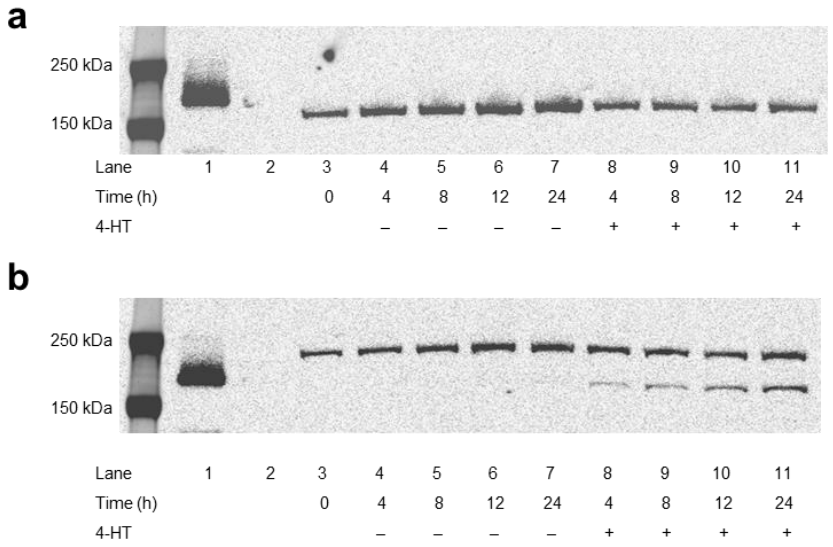
<u>Reference</u>

Minoche, A. E., Dohm, J. C., & Himmelbauer, H. Evaluation of genomic high-throughput sequencing data generated on Illumina HiSeq and Genome Analyzer systems. *Genome Biology* **12**, R112 (2011).

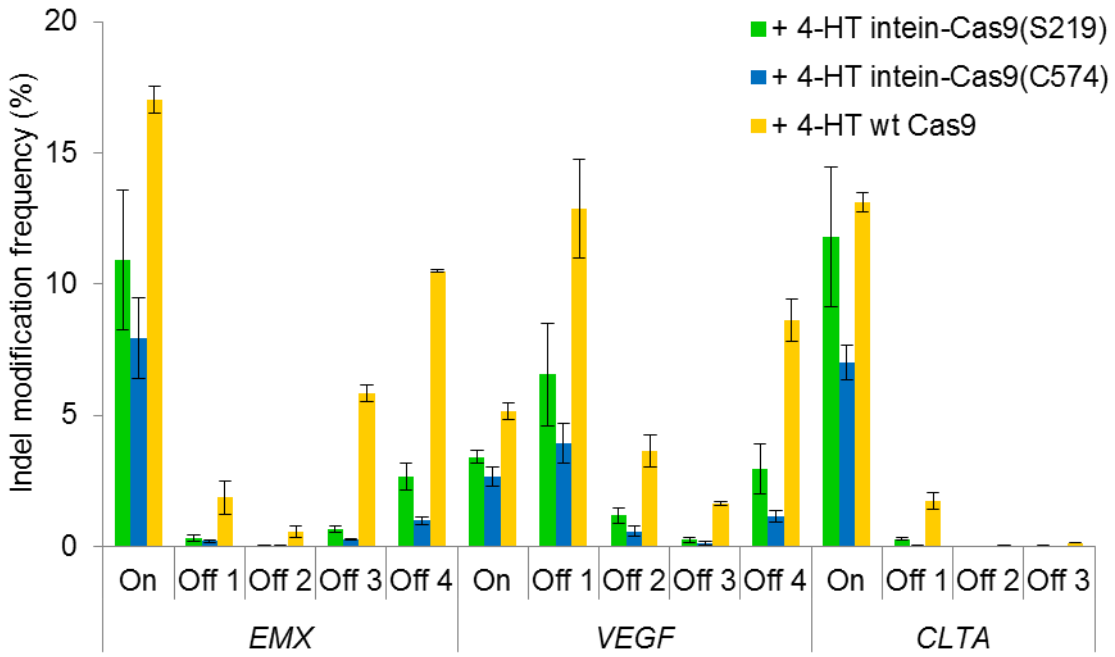
SUPPLEMENTARY FIGURES



Supplementary Figure 1. Effect of 4-HT on cellular toxicity. Untransfected HEK293-GFP stable cells, and cells transfected with intein-Cas9(S219) and sgRNA expression plasmids, were treated with or without 4-HT (1 μM). 12 h after transfection, the media was replaced with full serum media, with or without 4-HT (1 μM). Cells were thus exposed to 4-HT for 0, 12, or 60 h. The live cell population was determined by flow cytometry 60 h after transfection using TO-PRO-3 stain (Life Technologies). Error bars reflect the standard deviation of six technical replicates.



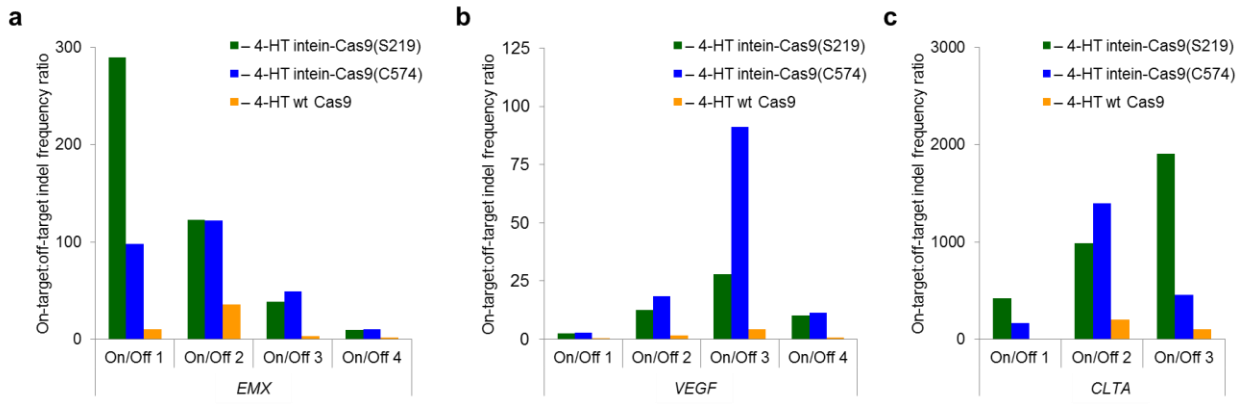
Supplementary Figure 2. Western blot analysis of HEK293-GFP stable cells transfected with (**a**) wild-type Cas9 or (**b**) intein-Cas9(S219) expression plasmid. 12 h after transfection, cells were treated with or without 4-HT (1 μM). Cells were lysed and pooled from three technical replicates 4, 8, 12, or 24 h after 4-HT treatment. An anti-FLAG antibody (Sigma-Aldrich F1804) and an anti-mouse 800CW IRDye (LI-COR) were used to visualize the gel. Lanes 1 and 2 contain purified dCas9-VP64-3×FLAG protein and lysate from untransfected HEK293 cells, respectively.



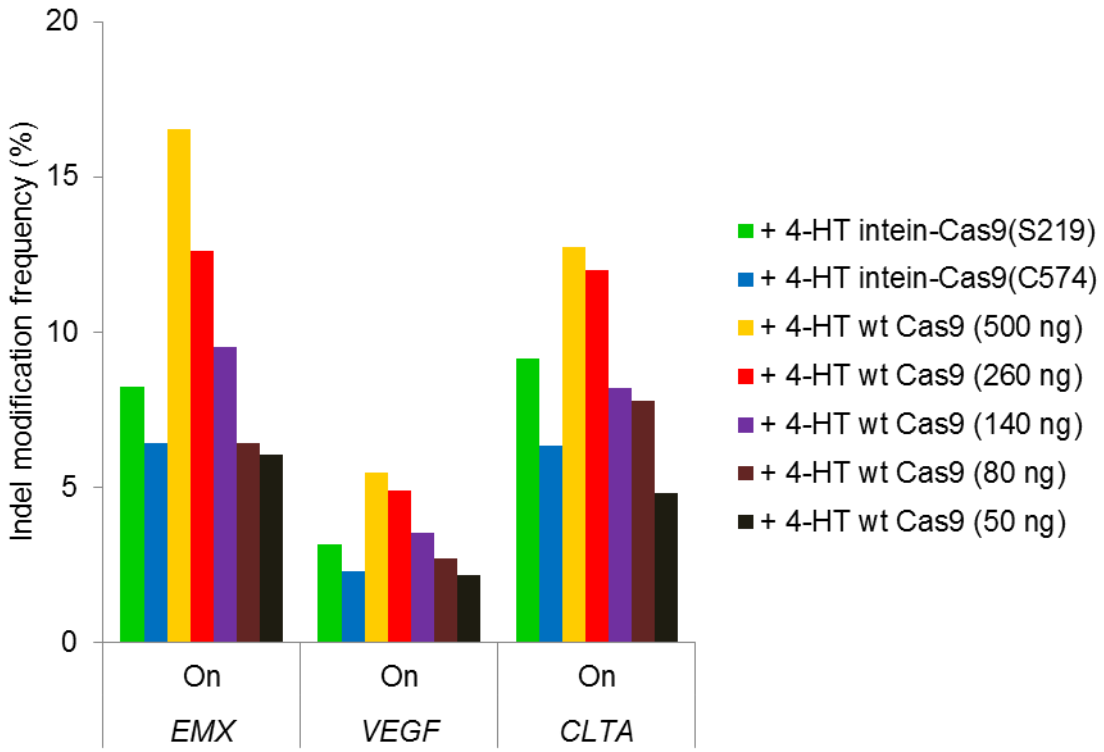
Supplementary Figure 3. Indel frequency from high-throughput DNA sequencing of amplified genomic on-target sites ("On") and off-target sites ("Off 1-Off 4") by intein-Cas9(S219), intein-Cas9(C574), and wild-type Cas9 in the presence of 4-HT. 500 ng of Cas9 expression plasmid was transfected. The higher observed efficiency of *VEGF* Off 1 modification than *VEGF* on-target modification is consistent with a previous report. *P*-values are < 0.005 for the Fisher exact test (one-sided down) on all pairwise comparisons within each independent experiment of off-target modification frequency between either intein-Cas9 variant in the presence of 4-HT versus that of wild-type Cas9 in the presence of 4-HT. *P*-values were adjusted for multiple comparisons using the Benjamini-Hochberg method, and are listed in **Supplementary Table 5**. Error bars reflect the range of two independent experiments conducted on different days.

<u>Reference</u>

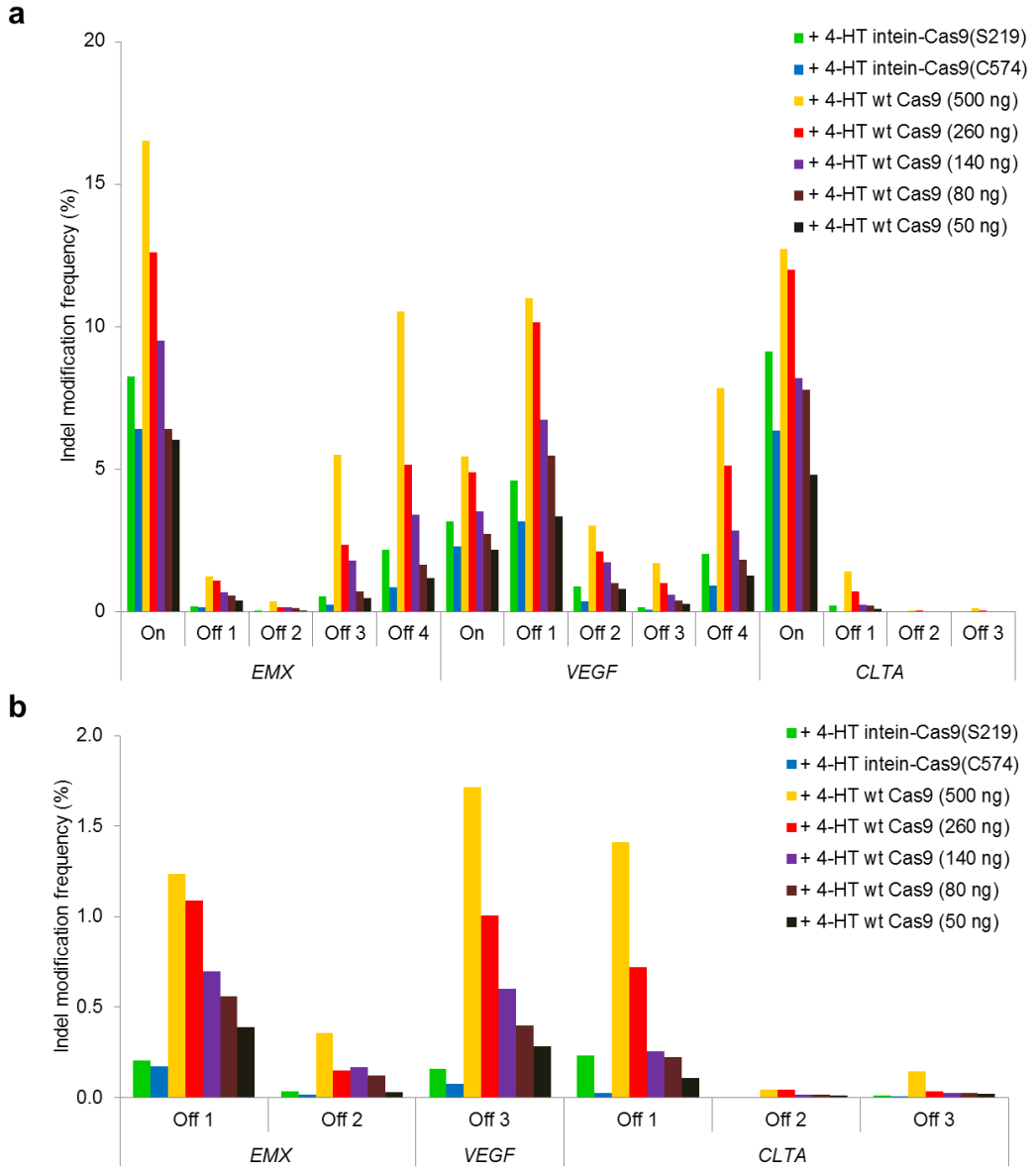
Fu, Y. *et al.* High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nature biotechnology* **31**, 822-826 (2013).



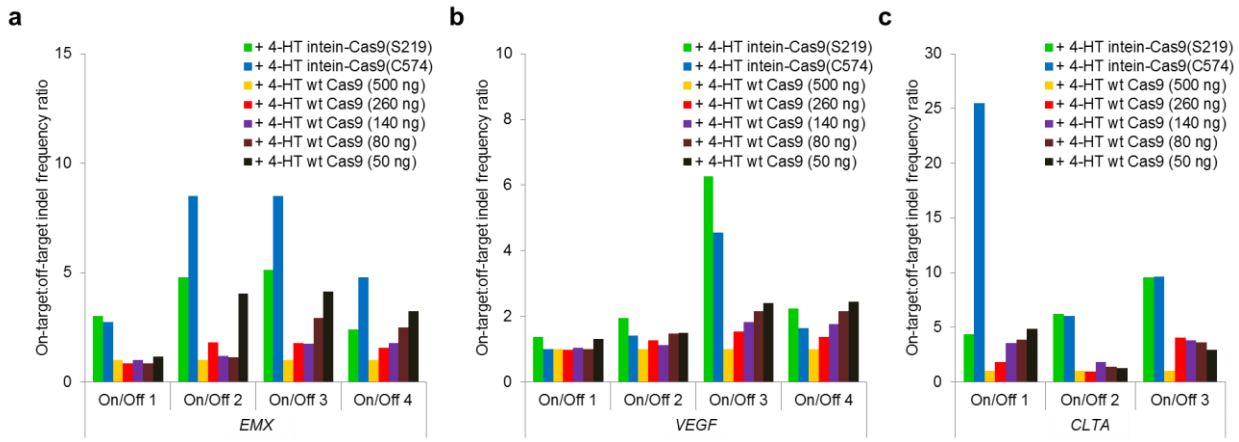
Supplementary Figure 4. DNA modification specificity of intein-Cas9(S219), intein-Cas9(C574), and wild-type Cas9 in the absence of 4-HT. (**a-c**) On-target:off-target indel frequency ratio following the transfection of 500 ng of intein-Cas9(S219), intein-Cas9(C574), or wild-type Cas9 expression plasmid.



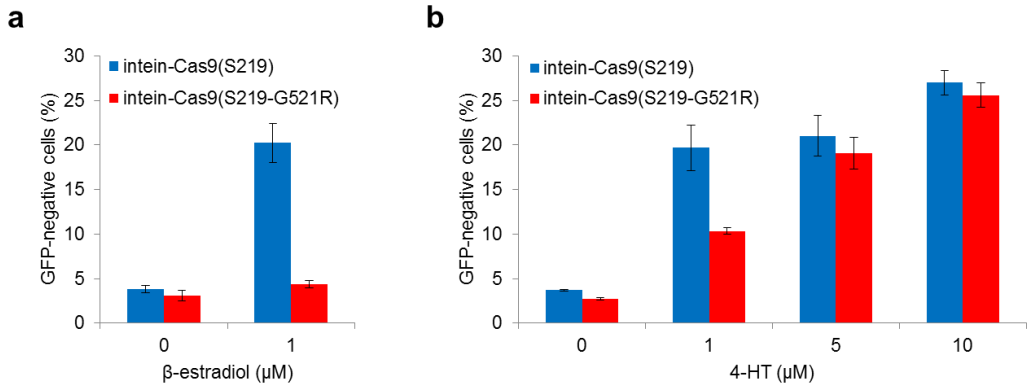
Supplementary Figure 5. Genomic on-target DNA modification by intein-Cas9(S219), intein-Cas9(C574), and wild-type Cas9 in the presence of 4-HT. Five different amounts of wild-type Cas9 expression plasmid, specified in parenthesis, were transfected. *P*-values for comparisons between conditions (**Supplementary Table 6**) were obtained using the Fisher exact test and adjusted for multiple comparisons using the Benjamini-Hochberg Method.



Supplementary Figure 6. Indel frequency from high-throughput DNA sequencing of amplified genomic on-target sites ("On") and off-target sites ("Off 1-Off 4") by intein-Cas9(S219), intein-Cas9(C574), and wild-type Cas9 in the presence of 4-HT. Five different amounts of wild-type Cas9 expression plasmid, specified in parenthesis, were transfected. Genomic sites with low modification frequencies are enlarged in (**b**). *P*-values for comparisons between conditions (**Supplementary Table 6**) were obtained using the Fisher exact test and adjusted for multiple comparisons using the Benjamini-Hochberg Method.



Supplementary Figure 7. DNA modification specificity of intein-Cas9(S219), intein-Cas9(C574), and wild-type Cas9 in the presence of 4-HT. (**a-c**) On-target:off-target indel frequency ratio normalized to wild-type Cas9 (500 ng). Five different amounts of wild-type Cas9 expression plasmid, specified in parenthesis, were transfected.



Supplementary Figure 8. Genomic *EGFP* disruption activity of intein-Cas9(S219) and intein-Cas9(S219-G521R) in the presence of (**a**) β-estradiol or (**b**) 4-HT. Error bars reflect the standard deviation of three technical replicates.

SUPPLEMENTARY TABLES

EMX On	GAGTCCGAGCAGAAGAAGAAGGG
EMX Off 1	GAGgCCGAGCAGAAGAAagACGG
EMX Off 2	GAGTCCtAGCAGgAGAAGAAGaG
EMX Off 3	GAGTCtaAGCAGAAGAAGAAGaG
EMX Off 4	GAGTtaGAGCAGAAGAAGAAAGG
VEGF On	GGGTGGGGGGAGTTTGCTCCTGG
VEGF Off 1	GGaTGGaGGGAGTTTGCTCCTGG
VEGF Off 2	GGGaGGGtGGAGTTTGCTCCTGG
VEGF Off 3	cGGgGGaGGGAGTTTGCTCCTGG
VEGF Off 4	GGGgaGGGGaAGTTTGCTCCTGG
CLTA On	GCAGATGTAGTGTTTCCACAGGG
CLTA Off 1	aCAtATGTAGTaTTTCCACAGGG
CLTA Off 2	cCAGATGTAGTaTTcCCACAGGG
CLTA Off 3	ctAGATGaAGTGcTTCCACATGG

Supplementary Table 1. On-target and 11 known off-target substrates of Cas9:sgRNAs that target sites in *EMX*, *VEGF,* and *CLTA*. List of genomic on-target and off-targets sites of the *EMX*, *VEGF*, and *CLTA* sites are shown with mutations from the on-target sequence shown in lower case and red. Protospacer-adjacent motifs (PAMs) are shown in blue.

	– 4-HT intein-Cas9(S219)			– 4-HT intein-Cas9(C574)			– 4-HT wt Cas9 (500 ng)			+ 4-HT intein-Cas9(S219)			+ 4-HT intein-Cas9(C574)			+ 4-HT wt Cas9 (500 ng)		
	Indels	Total	Modification frequency	Indels	Total	Modification frequency	Indels	Total	Modification frequency	Indels	Total	Modification frequency	Indels	Total	Modification frequency	Indels	Total	Modification frequency
EMX On	1123	59967	1.87%	561	56700	0.99%	15589	72127	21.61%	7434	54764	13.57%	5209	54997	9.47%	9820	55972	17.54%
EMX Off 1	3	46360	0.01%	4	39544	0.01%	1143	55334	2.07%	185	43554	0.42%	116	42432	0.27%	1043	41387	2.52%
EMX Off 2	8	52362	0.02%	3	36983	0.01%	540	89945	0.60%	20	56997	0.04%	22	61504	0.04%	412	52780	0.78%
EMX Off 3	32	66472	0.05%	10	49582	0.02%	5804	83231	6.97%	413	53819	0.77%	160	56140	0.29%	4149	67153	6.18%
EMX Off 4	146	76633	0.19%	57	60976	0.09%	11817	86566	13.65%	2413	76405	3.16%	574	50867	1.13%	6561	62651	10.47%
VEGF On	359	34089	1.05%	379	44841	0.85%	3815	42732	8.93%	1285	35095	3.66%	1179	38909	3.03%	1120	23157	4.84%
VEGF Off 1	214	49383	0.43%	117	40358	0.29%	14578	71764	20.31%	2951	34729	8.50%	2272	48512	4.68%	6262	42489	14.74%
VEGF Off 2	29	34582	0.08%	10	21753	0.05%	2551	43775	5.83%	288	19326	1.49%	273	35253	0.77%	1199	28117	4.26%
VEGF Off 3	18	47664	0.04%	4	43171	0.01%	1743	82128	2.12%	167	45573	0.37%	107	56967	0.19%	679	42675	1.59%
VEGF Off 4	58	56732	0.10%	33	44096	0.07%	14114	116598	12.10%	1465	37619	3.89%	1229	88062	1.40%	3159	33446	9.45%
CLTA On	2087	48566	4.30%	930	51240	1.81%	16930	88447	19.14%	5691	39290	14.48%	4348	56815	7.65%	7974	59031	13.51%
CLTA Off 1	8	79008	0.01%	8	72536	0.01%	3361	111154	3.02%	286	79836	0.36%	32	72909	0.04%	1468	72166	2.03%
CLTA Off 2	3	69103	0.00%	0	76788	0.00%	75	78021	0.10%	0	25019	0.00%	11	64317	0.02%	18	36863	0.05%
CLTA Off 3	1	44342	0.00%	2	49937	0.00%	94	51070	0.18%	13	38264	0.03%	4	42814	0.01%	78	58340	0.13%

Supplementary Table 2. Raw sequence counts and modification frequencies for data plotted in **Fig. 2** and **Supplementary Figs. 3** and **4**. Total: total number of sequence counts. Modification frequency: number of indels divided by the total number of sequences listed as percentages.

	intein-Cas9(S219) (+ 4-HT vs. – 4-HT)	**intein-Cas9(C574) (+ 4-HT vs. – 4-HT)**	**wt Cas9 (+ 4-HT vs. – 4-HT)**
EMX On	$< 3.3 \times 10^{-16}$	$< 3.3 \times 10^{-16}$	1
VEGF On	$< 3.3 \times 10^{-16}$	$< 3.3 \times 10^{-16}$	1
CLTA On	$< 3.3 \times 10^{-16}$	$< 3.3 \times 10^{-16}$	1

Supplementary Table 3. *P*-values for comparisons between conditions in **Fig. 2a**. *P*-values were obtained using the Fisher exact test and adjusted for multiple comparisons using the Benjamini-Hochberg Method.

	+ 4-HT intein-Cas9(S219)			+ 4-HT intein-Cas9(C574)			+ 4-HT wt Cas9 (500 ng)			+ 4-HT wt Cas9 (260 ng)			+ 4-HT wt Cas9 (140 ng)			+ 4-HT wt Cas9 (80 ng)			+ 4-HT wt Cas9 (50 ng)		
	Indels	Total	Modification frequency	Indels	Total	Modification frequency	Indels	Total	Modification frequency	Indels	Total	Modification frequency	Indels	Total	Modification frequency	Indels	Total	Modification frequency	Indels	Total	Modification frequency
EMX On	5446	66039	8.25%	4125	64260	6.42%	10453	63225	16.53%	6836	54232	12.61%	6215	65222	9.53%	3674	57146	6.43%	3551	58687	6.05%
EMX Off 1	134	65439	0.20%	115	65758	0.17%	466	37687	1.24%	488	44817	1.09%	373	53270	0.70%	280	49924	0.56%	196	50343	0.39%
EMX Off 2	26	69924	0.04%	10	61188	0.02%	236	65936	0.36%	94	62522	0.15%	115	67406	0.17%	76	61509	0.12%	19	58791	0.03%
EMX Off 3	438	81696	0.54%	173	68783	0.25%	4890	88690	5.51%	1760	74807	2.35%	1400	77420	1.81%	507	69582	0.73%	312	63812	0.49%
EMX Off 4	1907	87678	2.18%	708	82863	0.85%	6997	66384	10.54%	4131	80091	5.16%	3065	89659	3.42%	1372	83194	1.65%	933	78093	1.19%
VEGF On	1633	51546	3.17%	1330	57690	2.31%	2072	37912	5.47%	1873	38181	4.91%	1651	46948	3.52%	1209	44425	2.72%	994	45715	2.17%
VEGF Off 1	3132	67908	4.61%	1978	62133	3.18%	8471	76941	11.01%	5893	58006	10.16%	4073	60556	6.73%	2884	52518	5.49%	1792	53739	3.33%
VEGF Off 2	347	38567	0.90%	189	49925	0.38%	1008	33299	3.03%	668	31470	2.12%	522	30231	1.73%	388	37944	1.02%	282	34935	0.81%
VEGF Off 3	84	52871	0.16%	44	58976	0.07%	1088	63365	1.72%	490	48793	1.00%	293	48740	0.60%	183	46144	0.40%	143	50504	0.28%
VEGF Off 4	1067	52667	2.03%	845	92592	0.91%	3712	47327	7.84%	3838	74876	5.13%	1892	66578	2.84%	1224	67397	1.82%	693	54510	1.27%
CLTA On	4230	46334	9.13%	3097	48752	6.35%	7586	59582	12.73%	5747	47919	11.99%	4593	56068	8.19%	3864	49534	7.80%	2243	46510	4.82%
CLTA Off 1	169	72881	0.23%	20	72486	0.03%	1247	88428	1.41%	576	79763	0.72%	223	87021	0.26%	177	78827	0.22%	75	68762	0.11%
CLTA Off 2	2	40883	0.00%	2	56739	0.00%	27	63439	0.04%	27	64354	0.04%	11	74571	0.01%	7	38162	0.02%	6	46931	0.01%
CLTA Off 3	5	45599	0.01%	3	39745	0.01%	72	49309	0.15%	16	47504	0.03%	12	49085	0.02%	12	48776	0.02%	8	42490	0.02%

Supplementary Table 4. Raw sequence counts and modification frequencies for data plotted in **Fig. 2b-d** and **Supplementary Figs. 5-7**. Total: total number of sequence counts. Modification frequency: number of indels divided by the total number of sequences listed as percentages.

	Independent Experiment 1		Independent Experiment 2	
	+ 4-HT intein-Cas9(S219) vs. + 4-HT wt Cas9 (500 ng)	**+ 4-HT intein-Cas9(C574) vs. + 4-HT wt Cas9 (500 ng)**	**+ 4-HT intein-Cas9(S219) vs. + 4-HT wt Cas9 (500 ng)**	**+ 4-HT intein-Cas9(C574) vs. + 4-HT wt Cas9 (500 ng)**
EMX On	$< 2.4 \times 10^{-16}$	$< 2.4 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$
EMX Off 1	$< 2.4 \times 10^{-16}$	$< 2.4 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$
EMX Off 2	$< 2.4 \times 10^{-16}$	$< 2.4 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$
EMX Off 3	$< 2.4 \times 10^{-16}$	$< 2.4 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$
EMX Off 4	$< 2.4 \times 10^{-16}$	$< 2.4 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$
VEGF On	2.8×10^{-12}	$< 2.4 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$
VEGF Off 1	$< 2.4 \times 10^{-16}$	$< 2.4 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$
VEGF Off 2	$< 2.4 \times 10^{-16}$	$< 2.4 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$
VEGF Off 3	$< 2.4 \times 10^{-16}$	$< 2.4 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$
VEGF Off 4	$< 2.4 \times 10^{-16}$	$< 2.4 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$
CLTA On	1	$< 2.4 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$
CLTA Off 1	$< 2.4 \times 10^{-16}$	$< 2.4 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$
CLTA Off 2	9.1×10^{-5}	4.4×10^{-3}	1.4×10^{-4}	4.6×10^{-6}
CLTA Off 3	1.3×10^{-7}	1.5×10^{-14}	3.1×10^{-15}	3.5×10^{-15}

Supplementary Table 5. *P*-values for comparisons between conditions in **Supplementary Fig. 3**. *P*-values were obtained using the Fisher exact test and adjusted for multiple comparisons using the Benjamini-Hochberg Method.

	intein-Cas9(S219) vs. wt Cas9 (500 ng)	intein-Cas9(S219) vs. wt Cas9 (260 ng)	intein-Cas9(S219) vs. wt Cas9 (140 ng)	intein-Cas9(S219) vs. wt Cas9 (80 ng)	intein-Cas9(S219) vs. wt Cas9 (50 ng)	intein-Cas9(C574) vs. wt Cas9 (500 ng)	intein-Cas9(C574) vs. wt Cas9 (260 ng)	intein-Cas9(C574) vs. wt Cas9 (140 ng)	intein-Cas9(C574) vs. wt Cas9 (80 ng)	intein-Cas9(C574) vs. wt Cas9 (50 ng)
EMX On	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	1	1	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	0.56	1
EMX Off 1	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	6.7×10^{-9}	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	3.7×10^{-12}
EMX Off 2	$< 3.9 \times 10^{-16}$	4.3×10^{-12}	2.4×10^{-15}	1.7×10^{-8}	0.84	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	1.0×10^{-13}	7.1×10^{-2}
EMX Off 3	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	2.0×10^{-6}	1	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	7.5×10^{-13}
EMX Off 4	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	1	1	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	1.0×10^{-11}
VEGF On	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	1.7×10^{-3}	1	1	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	1.9×10^{-5}	1
VEGF Off 1	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	3.8×10^{-12}	1	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	9.6×10^{-2}
VEGF Off 2	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	5.7×10^{-2}	1	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$
VEGF Off 3	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	5.9×10^{-13}	1.8×10^{-5}	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$
VEGF Off 4	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	1	1	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	9.9×10^{-11}
CLTA On	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	1	1	1	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	1
CLTA Off 1	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	0.21	0.74	1	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	$< 3.9 \times 10^{-16}$	2.0×10^{-9}
CLTA Off 2	1.4×10^{-4}	1.6×10^{-4}	0.13	9.4×10^{-2}	0.23	4.6×10^{-6}	5.4×10^{-6}	4.8×10^{-2}	3.5×10^{-2}	0.11
CLTA Off 3	3.1×10^{-15}	2.3×10^{-2}	0.12	0.11	0.29	3.5×10^{-15}	9.3×10^{-3}	5.7×10^{-2}	5.7×10^{-2}	0.16

Supplementary Table 6. *P*-values for comparisons between conditions in **Supplementary Figs. 5** and **6**. All conditions were treated with 4-HT. *P*-values were obtained using the Fisher exact test and adjusted for multiple comparisons using the Benjamini-Hochberg Method.

	+ 4-HT wt Cas9 (500 ng) -sgRNA		
	Indels	Total	Modification frequency
EMX On	8	78943	0.01%
EMX Off 1	1	42232	0.00%
EMX Off 2	4	79008	0.01%
EMX Off 3	60	113629	0.05%
EMX Off 4	5	104159	0.00%
VEGF On	0	60667	0.00%
VEGF Off 1	2	111409	0.00%
VEGF Off 2	0	52048	0.00%
VEGF Off 3	4	88105	0.00%
VEGF Off 4	2	123559	0.00%
CLTA On	491	68600	0.72%
CLTA Off 1	10	116033	0.01%
CLTA Off 2	6	75723	0.01%
CLTA Off 3	4	53885	0.01%

Supplementary Table 7. Raw sequence counts and modification frequencies (for cells transfected with wild-type Cas9 (500 ng) but without a targeting sgRNA, in the presence of 4-HT). Total: total number of sequence counts. Modification frequency: number of indels divided by the total number of sequences listed as percentages.

SUPPLEMENTARY NOTES

DNA sequence of intein 37R3-2.

Intein 37R32:
TGCCTTGCCGAGGGTACCCGAATCTTCGATCCGGTCACTGGTACAACGCATCGCATC
GAGGATGTTGTCGATGGGCGCAAGCCTATTCATGTCGTGGCTGCTGCCAAGGACGG
AACGCTGCTCGCGCGGCCCGTGGTGTCCTGGTTCGACCAGGGAACGCGGGATGTGA
TCGGGTTGCGGATCGCCGGTGGCGCCATCGTGTGGGCGACACCCGATCACAAGGTG
CTGACAGAGTACGGCTGGCGTGCCGCCGGGGAACTCCGCAAGGGAGACAGGGTGGC
CGGACCGGGTGGTTCTGGTAACAGCCTGGCCTTGTCCCTGACGGCCGACCAGATGGT
CAGTGCCTTGTTGGATGCTGAGCCCCCCATACTCTATTCCGAGTATGATCCTACCAGT
CCCTTCAGTGAAGCTTCGATGATGGGCTTACTGACCAACCTGGCAGACAGGGAGCTG
GTTCACATGATCAACTGGGCGAAGAGGGTGCCAGGCTTTGTGGATTTGACCCTCCAT
GATCAGGCCCACCTTCTAGAACGTGCCTGGCTAGAGATCCTGATGATTGGTCTCGTC
TGGCGCTCCATGGAGCACCCAGGGAAGCTACTGTTTGCTCCTAACTTGCTCTTGGAC
AGGAACCAGGGAAAATGTGTAGAGGGCATGGTGGAGATCTTCGACATGCTGCTGGC
TACATCATCTCGGTTCCGCATGATGAATCTGCAGGGAGAGGAGTTTGTGTGCCTCAA
ATCTATTATTTTGCTTAATTCTGGAGTGTACACATTTCTGTCCAGCACCCTGAAGTCT
CTGGAAGAGAAGGACCATATCCACCGAGCCCTGGACAAGATCACGGACACTTTGAT
CCACCTGATGGCCAAGGCAGGCCTGACCCTGCAGCAGCAGCACCAGCGGCTGGCCC
AGCTCCTCCTCATCCTCTCCCACATCAGGCACATGAGTAACAAAGGAATGGAGCATC
TGTACAGCATGAAGTACAAGAACGTGGTGCCCCTCTATGACCTGCTGCTGGAGATGC
TGGACGCCCACCGCCTACATGCGGGTGGTTCTGGTGCTAGCCGCGTGCAGGCGTTCG
CGGATGCCCTGGATGACAAATTCCTGCACGACATGCTGGCGGAAGGACTCCGCTATT
CCGTGATCCGAGAAGTGCTGCCAACGCGGCGGGCACGAACGTTCGACCTCGAGGTC
GAGGAACTGCACACCCTCGTCGCCGAAGGGGTTGTCGTGCACAACTGC

Amino acid sequences of proteins used in this study.

Cas9-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG
NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGY
AGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE
VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA
FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL
KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTG
WGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQG

DSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKN
SRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSD
YDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLIT
QRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIRE
VKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYG
DYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEI
VWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKK
YGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEV
KKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSP
EDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENII
HLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGSP
KKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(C80)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGT
LLARPVVSWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGDRVAGP
GGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEASMMGLLTNLADRELVHMI
NWAKRVPGFVDLTLHDQAHLLERAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQG
KCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHI
HRALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEHLYSMKYKNV
VPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDKFLHDMLAEGLRYSVIREVLPTR
RARTFDLEVEELHTLVAEGVVVHNCYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHE
RHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKK
NGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAA
KNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQI
HLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITP
WNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEG
MRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT
YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKR
RRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQV
SGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQK
GQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
RLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNA
KLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK
LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEF
VYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE
TGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGY
KEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLK
GSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQA
ENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD
GSPKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(A127)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVCLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVVSWFDQG
TRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGDRVAGPGGSGNSLALSLTAD
QMVSALLDAEPPILYSEYDPTSPFSEASMMGLLTNLADRELVHMINWAKRVPGFVDLTL
HDQAHLLERAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLA
TSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRALDKITDTLIHLMA
KAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEHLYSMKYKNVVPLYDLLLEMLDAHR
LHAGGSGASRVQAFADALDDKFLHDMLAEGLRYSVIREVLPTRRARTFDLEVEELHTLV
AEGVVVHNCYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPD
NSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGL
FGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNL
SDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN
GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLG
ELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNF
EEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRK
PAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHD
LLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRY
TGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQ
GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQK
NSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLS
DYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLI
TQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIR
EVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVY
GDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETG
EIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK
KYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKE
VKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS
PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENI
IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGS
PKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(T146)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSCLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAA
AKDGTLLARPVVSWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGD
RVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEASMMGLLTNLADRE
LVHMINWAKRVPGFVDLTLHDQAHLLERAWLEILMIGLVWRSMEHPGKLLFAPNLLLD
RNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEE
KDHIHRALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEHLYSMKY
KNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDKFLHDMLAEGLRYSVIREV
LPTRRARTFDLEVEELHTLVAEGVVVHNCDKADLRLIYLALAHMIKFRGHFLIEGDLNP
DNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNG

LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSK
NGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHL
GELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWN
FEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMR
KPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYH
DLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRR
YTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL
SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKL
ITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIR
EVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVY
GDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETG
EIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK
KYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKE
VKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS
PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENI
IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGS
PKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(S219)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKCLAEGTRIFDPVTGTTHRIEDV
VDGRKPIHVVAAAKDGTLLARPVVSWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYG
WRAAGELRKGDRVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEASM
MGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWLEILMIGLVWRSMEHP
GKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGV
YTFLSSTLKSLEEKDHIHRALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMS
NKGMEHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDKFLHDM
LAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVHNCRRLENLIAQLPGEKKNG
LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSK
NGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHL
GELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWN
FEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMR
KPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYH
DLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRR
YTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL
SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKL
ITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIR
EVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVY

GDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETG
EIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK
KYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKE
VKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS
PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENI
IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGS
PKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(T333)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG
NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLCLAEGTRIFDPVTGTTHRIEDVVDGRK
PIHVVAAAKDGTLLARPVVSWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAG
ELRKGDRVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEASMMGLLT
NLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWLEILMIGLVWRSMEHPGKLLF
APNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSS
TLKSLEEKDHIHRALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGME
HLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDKFLHDMLAEGL
RYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVHNCLLKALVRQQLPEKYKEIFFDQSK
NGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHL
GELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWN
FEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMR
KPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYH
DLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRR
YTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL
SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKL
ITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIR
EVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVY
GDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETG
EIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK
KYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKE
VKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS
PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENI
IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGS
PKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(T519)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG

NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGY
AGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE
VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFCLAEGTRIFDPVTGTTHRIEDV
VDGRKPIHVVAAAKDGTLLARPVVSWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYG
WRAAGELRKGDRVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEASM
MGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWLEILMIGLVWRSMEHP
GKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGV
YTFLSSTLKSLEEKDHIHRALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMS
NKGMEHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDKFLHDM
LAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVHNCVYNELTKVKYVTEGMR
KPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYH
DLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRR
YTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL
SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKL
ITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIR
EVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVY
GDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETG
EIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK
KYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKE
VKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS
PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENI
IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGS
PKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(C574)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG
NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGY
AGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE
VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA
FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECLAEGTRIFDPVTGTTHRIEDVVDG
RKPIHVVAAAKDGTLLARPVVSWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRA
AGELRKGDRVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEASMMGL
LTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWLEILMIGLVWRSMEHPGKLL
FAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLS
STLKSLEEKDHIHRALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGME
HLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDKFLHDMLAEGL
RYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVHNCFDSVEISGVEDRFNASLGTYHDL

LKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYT
GWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQ
GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQK
NSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLS
DYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLI
TQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIR
EVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVY
GDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETG
EIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK
KYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKE
VKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS
PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENI
IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGS
PKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(T622)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG
NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGY
AGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE
VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA
FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL
KIIKDKDFLDNEENEDILEDIVLCLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDG
TLLARPVVSWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGDRVAGP
GGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEASMMGLLTNLADRELVHMI
NWAKRVPGFVDLTLHDQAHLLERAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQG
KCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHI
HRALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEHLYSMKYKNV
VPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDKFLHDMLAEGLRYSVIREVLPTR
RARTFDLEVEELHTLVAEGVVVHNCLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRR
YTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL
SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKL
ITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIR
EVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVY
GDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETG
EIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK
KYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKE
VKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS
PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENI

IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGS
PKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(S701)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG
NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGY
AGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE
VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA
FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL
KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTG
WGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDCLAEGTRIFDPVTGTTHR
IEDVVDGRKPIHVVAAAKDGTLLARPVVSWFDQGTRDVIGLRIAGGAIVWATPDHKVL
TEYGWRAAGELRKGDRVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFS
EASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWLEILMIGLVWRS
MEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILL
NSGVYTFLSSTLKSLEEKDHIHRALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIR
HMSNKGMEHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDKFL
HDMLAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVHNCLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL
SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKL
ITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIR
EVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVY
GDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETG
EIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK
KYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKE
VKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS
PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENI
IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGS
PKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(A728)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG
NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGY
AGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE
VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA

FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL
KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTG
WGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQG
DSLHEHIANLCLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVVSWF
DQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGDRVAGPGGSGNSLALSL
TADQMVSALLDAEPPILYSEYDPTSPFSEASMMGLLTNLADRELVHMINWAKRVPGFV
DLTLHDQAHLLERAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFD
MLLATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRALDKITDTLI
HLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEHLYSMKYKNVVPLYDLLLEML
DAHRLHAGGSGASRVQAFADALDDKFLHDMLAEGLRYSVIREVLPTRRARTFDLEVEE
LHTLVAEGVVVHNCGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQK
GQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
RLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNA
KLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK
LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEF
VYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE
TGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGY
KEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLK
GSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQA
ENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD
GSPKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(T995)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG
NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGY
AGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE
VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA
FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL
KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTG
WGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQG
DSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKN
SRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSD
YDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLIT
QRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIRE
VKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGCLAEGTRIFDPVTGTTH
RIEDVVDGRKPIHVVAAAKDGTLLARPVVSWFDQGTRDVIGLRIAGGAIVWATPDHKV
LTEYGWRAAGELRKGDRVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPF
SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWLEILMIGLVWR
SMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIIL
LNSGVYTFLSSTLKSLEEKDHIHRALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHI

RHMSNKGMEHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDKF
LHDMLAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVHNCALIKKYPKLESEF
VYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE
TGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGY
KEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLK
GSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQA
ENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD
GSPKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(S1006)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG
NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGY
AGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE
VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA
FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL
KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTG
WGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQG
DSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKN
SRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSD
YDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLIT
QRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIRE
VKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLECLAEGT
RIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVVSWFDQGTRDVIGLRIAGGAI
VWATPDHKVLTEYGWRAAGELRKGDRVAGPGGSGNSLALSLTADQMVSALLDAEPPI
LYSEYDPTSPFSEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWL
EILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQG
EEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRALDKITDTLIHLMAKAGLTLQQQHQR
LAQLLLILSHIRHMSNKGMEHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQ
AFADALDDKFLHDMLAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVHNCEF
VYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE
TGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGY
KEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLK
GSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQA
ENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD
GSPKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(S1154)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF

GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG
NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGY
AGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE
VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA
FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL
KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTG
WGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQG
DSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKN
SRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSD
YDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLIT
QRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIRE
VKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYG
DYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEI
VWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKK
YGGFDSPTVAYSVLVVAKVEKGKCLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAK
DGTLLARPVVSWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGDRV
AGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEASMMGLLTNLADRELV
HMINWAKRVPGFVDLTLHDQAHLLERAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRN
QGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKD
HIHRALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEHLYSMKYKN
VVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDKFLHDMLAEGLRYSVIREVLPT
RRARTFDLEVEELHTLVAEGVVVHNCKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYK
EVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKG
SPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAE
NIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDG
SPKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(S1159)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG
NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGY
AGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE
VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA
FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL
KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTG
WGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQG
DSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKN
SRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSD
YDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLIT

QRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIRE
VKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYG
DYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEI
VWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKK
YGGFDSPTVAYSVLVVAKVEKGKSKKLKCLAEGTRIFDPVTGTTHRIEDVVDGRKPIHV
VAAAKDGTLLARPVVSWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELR
KGDRVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEASMMGLLTNLA
DRELVHMINWAKRVPGFVDLTLHDQAHLLERAWLEILMIGLVWRSMEHPGKLLFAPNL
LLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKS
LEEKDHIHRALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEHLYS
MKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDKFLHDMLAEGLRYSVI
REVLPTRRARTFDLEVEELHTLVAEGVVVHNCVKELLGITIMERSSFEKNPIDFLEAKGY
KEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLK
GSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQA
ENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD
GSPKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(S1274)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG
NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGY
AGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE
VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA
FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL
KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTG
WGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQG
DSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKN
SRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSD
YDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLIT
QRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIRE
VKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYG
DYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEI
VWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKK
YGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEV
KKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSP
EDNEQKQLFVEQHKHYLDEIIEQICLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAK
DGTLLARPVVSWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGDRV
AGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEASMMGLLTNLADRELV
HMINWAKRVPGFVDLTLHDQAHLLERAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRN
QGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKD
HIHRALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEHLYSMKYKN
VVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDKFLHDMLAEGLRYSVIREVLPT

RRARTFDLEVEELHTLVAEGVVVHNCEFSKRVILADANLDKVLSAYNKHRDKPIREQAE
NIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDG
SPKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Intein-Cas9(S219-G521R)-NLS-3×FLAG:
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKCLAEGTRIFDPVTGTTHRIEDV
VDGRKPIHVVAAAKDGTLLARPVVSWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYG
WRAAGELRKGDRVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEASM
MGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWLEILMIGLVWRSMEHP
GKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGV
YTFLSSTLKSLEEKDHIHRALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMS
NKRMEHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDKFLHDM
LAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVHNCRRLENLIAQLPGEKKNG
LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSK
NGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHL
GELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWN
FEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMR
KPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYH
DLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRR
YTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL
SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKL
ITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIR
EVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVY
GDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETG
EIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK
KYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKE
VKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS
PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENI
IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGS
PKKKRKVSSDYKDHDGDYKDHDIDYKDDDDKAAG

Indel Calling Algorithm

```
Read 1:
@M00265:68:000000000-AA85W:1:1101:14923:1642 1:N:0:1
TGCAGTCTCATGACTTGGCCTTTGTAGGAAAACACCATTAGAAGAGTAGATGGTTGGGTAGTGGCTCTCTTCTGCTTAGACTCTTG
TCTACTATGAATAAAGGGCTCTATTTGCAAAGGCCGTGATGGGTTGAAGCACATTGAGAAAGAGGCT
+
3>>A?FFFFFFFFGGGGBGGGGHHGHHHGHHGHHHGHHHHHGHHHGHHHHHHHHHGGGGHGHFHGGHHHHHHGHHHHHHHHHHHHH
GHFHHGHHHHHHHHHHFGGHHHHHHHHHHHGHHHHG@EEHHHHHGGHHHHHHHHHHHHHHHHHHGG

Read 2:
@M00265:68:000000000-AA85W:1:1101:14923:1642 2:N:0:1
CTCACCTGGGCGAGAAAGGTAACTTATGTTTCAGTAGCCTCTTTCTCAATGTGCTTCAACCCATCACGGCCTTTGCAAATAGAGCC
CTTTATTCATAGTAGACAAGAGTCTAAGCAGAAGAGAGCCACTACCCAACCATCTACTCTTCTAATGGT
+
3>>AAFCFFBBBGGGGGGGGGGHGHHHHHHHHHHHHHHHHHHHHHHHHHHHHHGHHHHHHHHHGGHHFHDFGGGHHHHHHHHGHFHHH
GGGHHHHHHHHHHFHHHHHHHHHHHHGHHHHHGHHHGHGHHHHHHHHHHHHGGGHHHGHHGHHHHHHHHH@
```

Step 1: Search for sequences (or reverse complements) flanking the on/off target sites in both Illumina reads from the following set:

	target site	5' flanking sequence	3' flanking sequence
EMX_On	GAGTCCGAGCAGAAGAAGAAGGG	AGCTGGAGGAGGAAGGGCCT	CTCCCATCACATCAACCGGT
EMX_Off1	GAGGCCGAGCAGAAGAAAGACGG	CCCCTTCTTCTGCAAATGAG	CGACAGATGTTGGGGGGAGG
EMX_Off2	GAGTCCTAGCAGGAGAAGAAGAG	GGCTGGGGCCAGCATGACCT	GCAGCCTAGAGTCTTCTGTG
EMX_Off3	GAGTCTAAGCAGAAGAAGAAGAG	CCTTTATTCATAGTAGACAA	AGCCACTACCCAACCATCTA
EMX_Off4	GAGTTAGAGCAGAAGAAGAAAGG	CATGGCAAGACAGATTGTCA	CATGGAGTAAAGGCAATCTT
VEGF_On	GGGTGGGGGGAGTTTGCTCCTGG	GGGAATGGGCTTTGGAAAGG	ACCCCCTATTTCTGACCTCC
VEGF_Off1	GGATGGAGGGAGTTTGCTCCTGG	CATCTAAGGACGGATTTGTG	GGTGTCAGAATGTCCTGTCT
VEGF_Off2	GGGAGGGTGGAGTTTGCTCCTGG	CTGGTCAGCCCATTATGATA	GGATGGAAGGGCCGGCTCCG
VEGF_Off3	CGGGGGAGGGAGTTTGCTCCTGG	CTGGAGAGAGGCTCCCATCA	GGAACCTGTGATCCCCACAG
VEGF_Off4	GGGGAGGGGAAGTTTGCTCCTGG	CATTTTTGCTGTCACAACTC	CATTCAGTGGGTAGAGTCCA
CLTA_On	GCAGATGTAGTGTTTCCACAGGG	CTGAGTAGGATTAAGATATT	TGGCTCTTCAGTGCACCAGC
CLTA_Off1	ACATATGTAGTATTTCCACAGGG	GTTGGGAAGAGATGCATACA	AATACAATGGACAAATAACC
CLTA_Off2	CCAGATGTAGTATTCCCACAGGG	GCCTCCTTGATTGAGGTGTC	GTCTGGCAGGCCCCTCCTGT
CLTA_Off3	CTAGATGAAGTGCTTCCACATGG	CTCATCTAGAGTTCTTTCCA	CTTTCATTAGAGTTTAGTCC

Step 2: Extract the sequence between the target sites in both reads and ensure that it is identical (reverse complementary) in read 1 and read 2 and all positions within read 1 and read 2 have a quality score >= '?' (Phred score >= 30)

In above reads, CTCTTCTGCTTAGACTC is reverse complement of GAGTCTAAGCAGAAGAG

Step 3: Align extracted sequence to the reference sequence for the relevant on/off target sequence

```
GAGTCTAAGCAGAAGAAGAAGAG reference sequence
GAGTCTAAGC------AGAAGAG sequence read
```

Step 4: For deletions, count only if deletion occurred in close proximity to expected cleavage site (within 8 bp of 3' end of reference sequence)

SUPPLEMENTARY DATA SETS

[See the accompanying Excel file.]

Supplementary Data Set 1. Indel sequences used to calculate modification frequencies in **Fig. 2** and **Supplementary Figs. 3** and **4**. Indel sequences for each genomic site are tabulated in a separate sheet of the Excel file. Deletions are represented by dashes and insertions by lower case letters. Indel sequences for each on/off-target site are represented from the most abundant to least abundant, combined across all experimental conditions.

[See the accompanying Excel file.]

Supplementary Data Set 2. Indel sequences used to calculate modification frequencies in **Fig. 2b-d** and **Supplementary Figs. 5-7**. Indel sequences for each genomic site are tabulated in a separate sheet of the Excel file. Deletions are represented by dashes and insertions by lower case letters. Indel sequences for each on/off-target site are represented from the most abundant to least abundant, combined across all experimental conditions.