

## Supporting Information

### Minimizing the off-target frequency of the CRISPR/Cas9 system via zwitterionic polymer conjugation and peptide fusion

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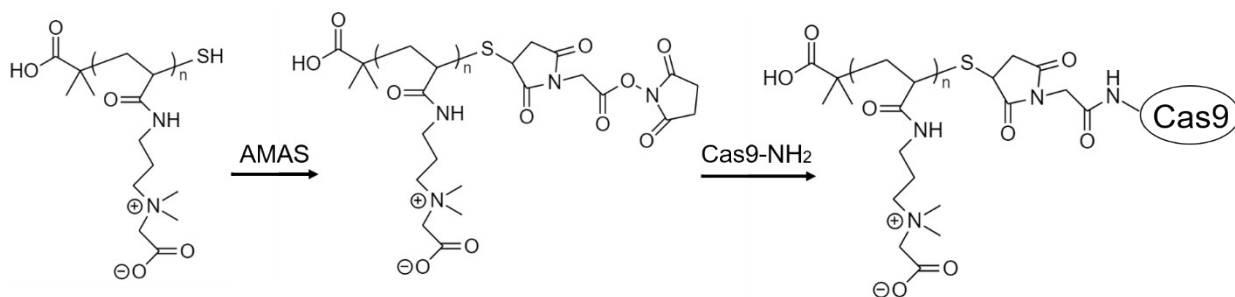
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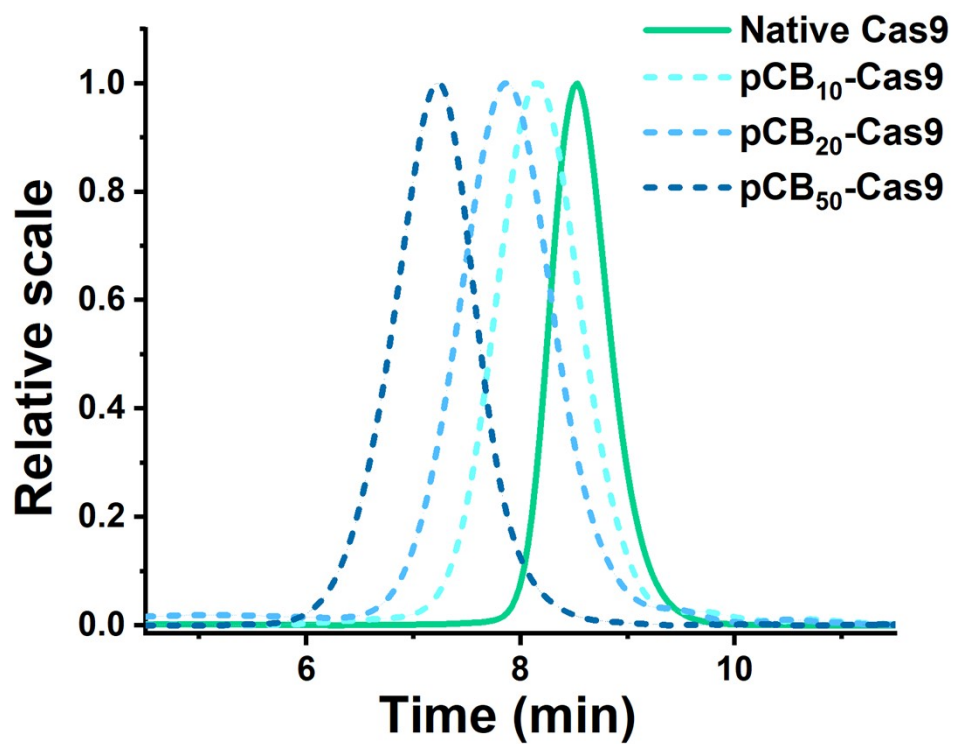
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Figures S1 to S5

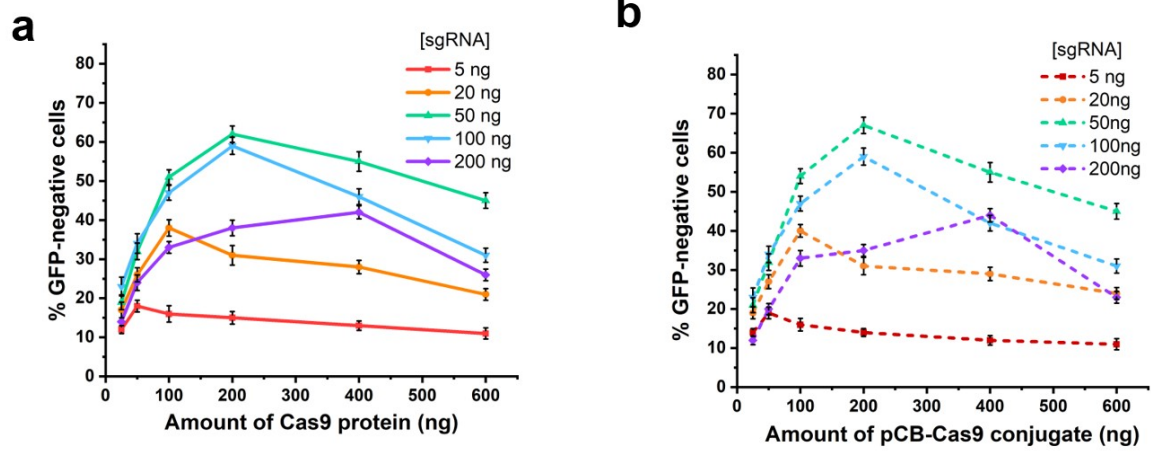
Table S1 to S3



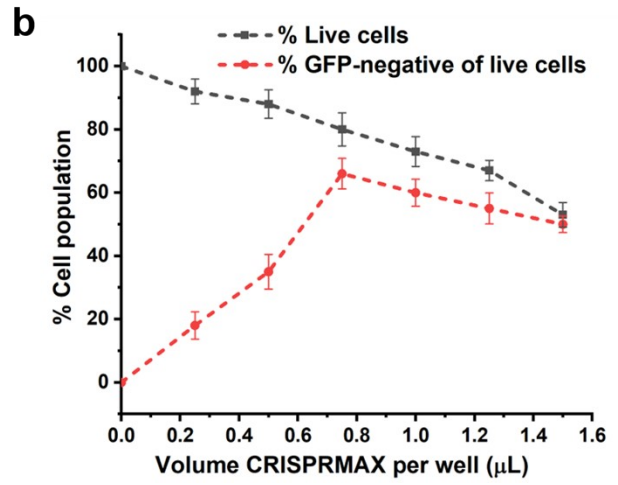
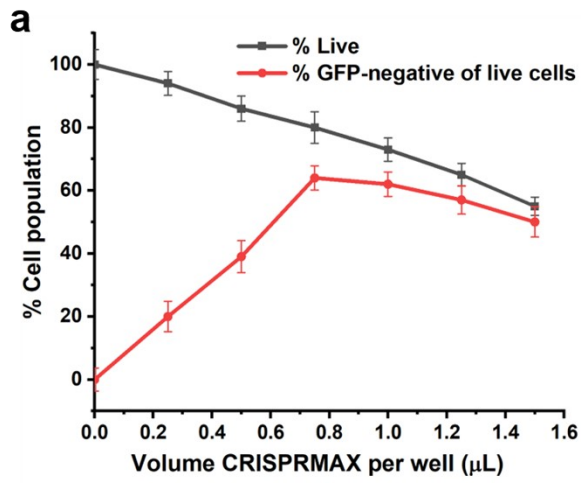
**Figure S1.** Synthetic route of pCB-Cas9 conjugates.



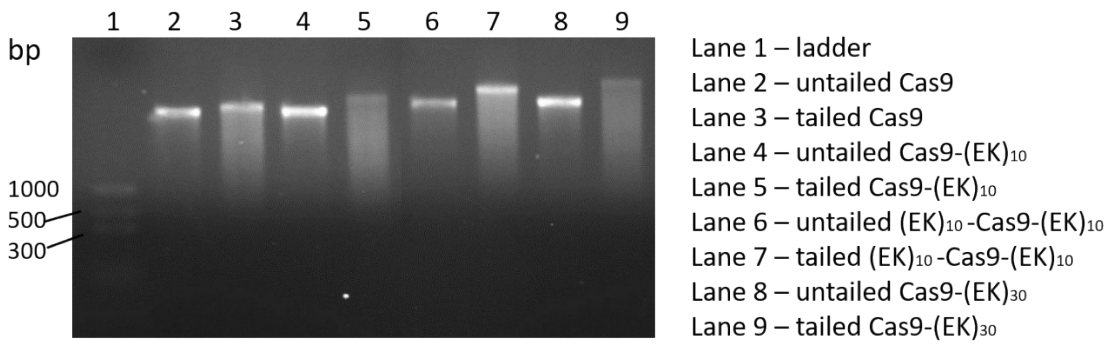
**Figure S2** Size-exclusion chromatogram of native Cas9, and pCB<sub>10</sub>-Cas9, pCB<sub>20</sub>-Cas9, pCB<sub>50</sub>-Cas9 conjugates.



**Figure S3** Optimal sgRNA to protein ratio for native Cas9 (a) and pCB-Cas9(b). All experiments were performed in a 96-well plate using a volume of 110  $\mu$ l.



**Figure S4** Effect of CRISPRMAX dose on the delivery efficiency and cellular toxicity of Cas9/sgRNA (a) and pCB-Cas9/sgRNA (b).



**Figure S5** Electrophoresis of in vitro transcribed Cas9 and Cas9-EK mRNA pre- and post-polyadenylation.

**Table S1** On-target and known off-target substrates of Cas9:sgRNAs that target sites in *GFP*, *EMX*, *VEGF*, and *CLTA*. List of genomic on-target and off-targets sites for *GFP*, *EMX*, *VEGF*, and *CLTA* are shown with mutations from the on-target sequence shown in lower case and red.

Target	Site name	Sequence
Target 1 (GFP)	GFP-On	GGGCACGGGCAGCTTGCCGG
Target 2 (VEGF)	VEGF-On	GGGTGGGGGGAGTTTGCTCC
	VEGF-Off1	GGaTGGaGGGAGTTTGCTCC
	VEGF-Off2	GGGaGGGtGGAGTTTGCTCC
	VEGF-Off3	cGGgGGaGGGAGTTTGCTCC
	VEGF-Off4	GGGgaGGGGaAGTTTGCTCC
Target 3 (EMX)	EMX-On	GAGTCCGAGCAGAAGAAGAA
	EMX-Off1	GAGgCCGAGCAGAAGAAgaA
	EMX-Off2	GAGTCctAGCAGgAGAAGAA
	EMX-Off3	GAGTctaAGCAGAAGAAGAA
	EMX-Off4	GAGTtaGAGCAGAAGAAGAA
Target 4 (CLTA)	CLTA-On	GCAGATGTAGTGTTCACACA
	CLTA-Off1	aCAtATGTAGT aTTTCACACA
	CLTA-Off2	cCAGATGTAGT aTTcCCACA
	CLTA-Off3	ctAGATGaAGTGcTTCCACA
	CLTA-Off4	ctAGATGaAGTGcTTCCACA

**Table S2** *P* values of Cas9-(EK)<sub>10</sub>, (EK)<sub>10</sub>-Cas9-(EK)<sub>10</sub>, and Cas9-(EK)<sub>30</sub> for target sites (VEGFA and EMX) in three different cell lines (HEK293, U2OS, and K562).

	HEK293			U2OS			K562		
	Cas9-(EK) <sub>10</sub>	(EK) <sub>10</sub> -Cas9-(EK) <sub>10</sub>	Cas9-(EK) <sub>30</sub>	Cas9-(EK) <sub>10</sub>	(EK) <sub>10</sub> -Cas9-(EK) <sub>10</sub>	Cas9-(EK) <sub>30</sub>	Cas9-(EK) <sub>10</sub>	(EK) <sub>10</sub> -Cas9-(EK) <sub>10</sub>	Cas9-(EK) <sub>30</sub>
VEGFA-OT1	0.764	0.037	0.017	0.427	0.007	0.005	0.897	0.013	0.004
VEGFA-OT2	0.507	0.031	0.045	0.687	0.004	0.007	0.813	0.026	0.024
VEGFA-OT3	0.121	0.005	0.009	0.441	0.002	0.007	0.557	0.021	0.024
VEGFA-OT4	0.851	0.045	0.047	0.292	0.025	0.027	0.61	0.025	0.029
EMX-OT1	0.649	0.001	0.003	0.793	0.045	0.05	0.344	0.006	0.005
EMX-OT2	0.077	<0.001	<0.001	0.749	0.035	0.043	0.932	0.007	0.007

**Table S3** Purity of Cas9 mRNA and EK-Cas9 mRNA measured by UV spectroscopy.

	Cas9 mRNA	Cas9-(EK) <sub>10</sub> mRNA	(EK) <sub>10</sub> -Cas9-(EK) <sub>10</sub> mRNA	Cas9-(EK) <sub>30</sub> mRNA
$A_{260}/A_{280}$ ratio	2.04 ± 0.04	1.98 ± 0.03	2.02 ± 0.04	2.01 ± 0.03