

A universal method for sensitive and cell-free detection of CRISPR-associated nucleases

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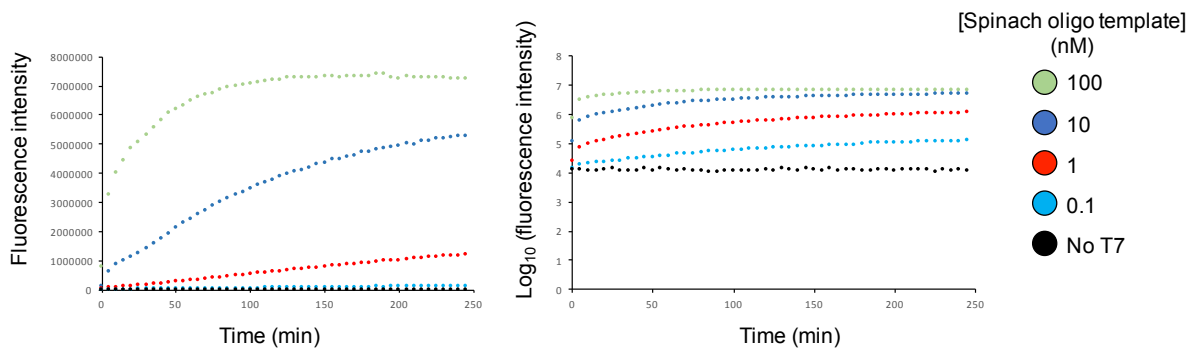
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University of California - Riverside,

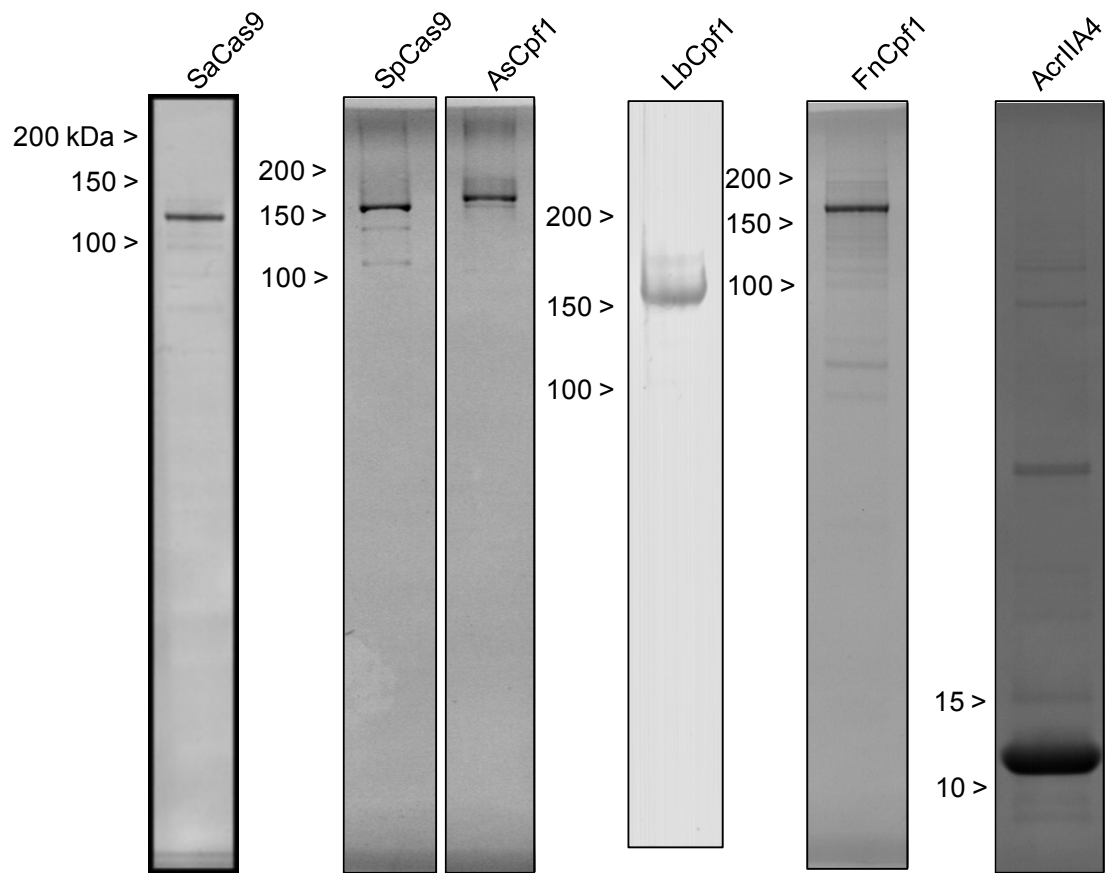
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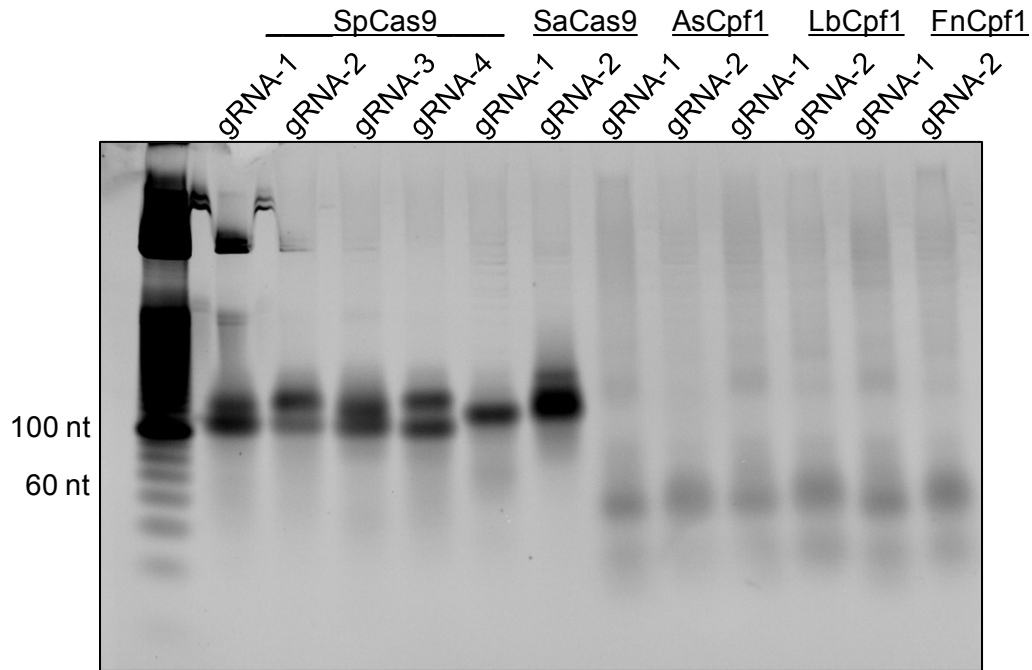
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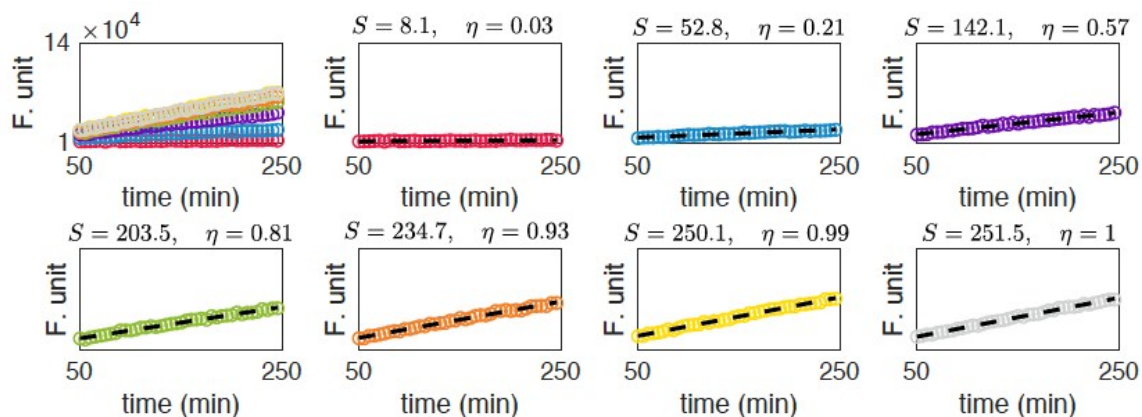
Supplementary Figure S1: Titrating the *in vitro* transcription (IVT) Spinach oligo template. Raw intensity values (left) and \log_{10} values (right) are shown. The experiment was performed to determine the workable detection range of Spinach transcription and was performed once ($n = 1$).



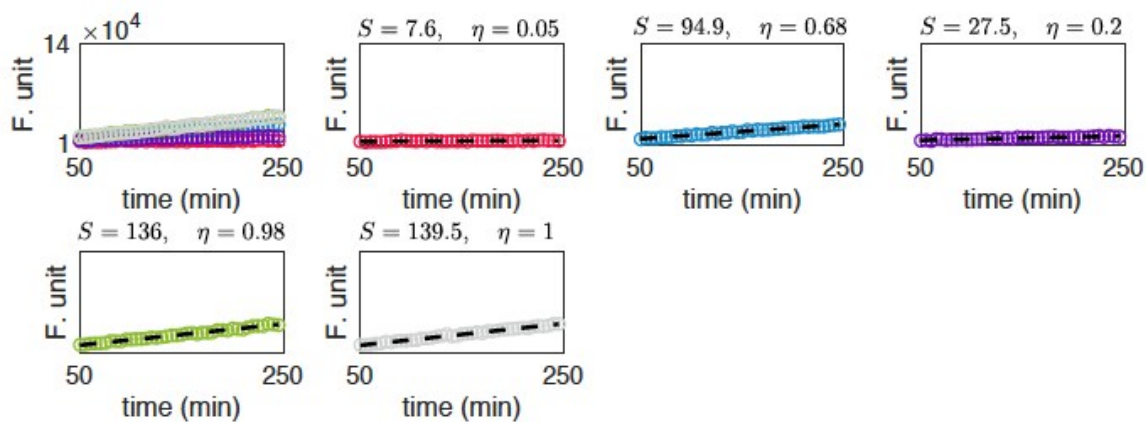
Supplementary Figure S2: 4–12% PAGE gels of all purified proteins.



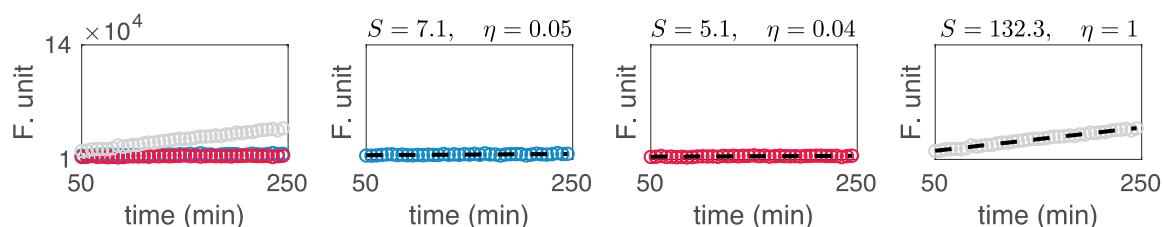
Supplementary Figure S3: 15% Urea-PAGE gel of all purified sgRNAs.



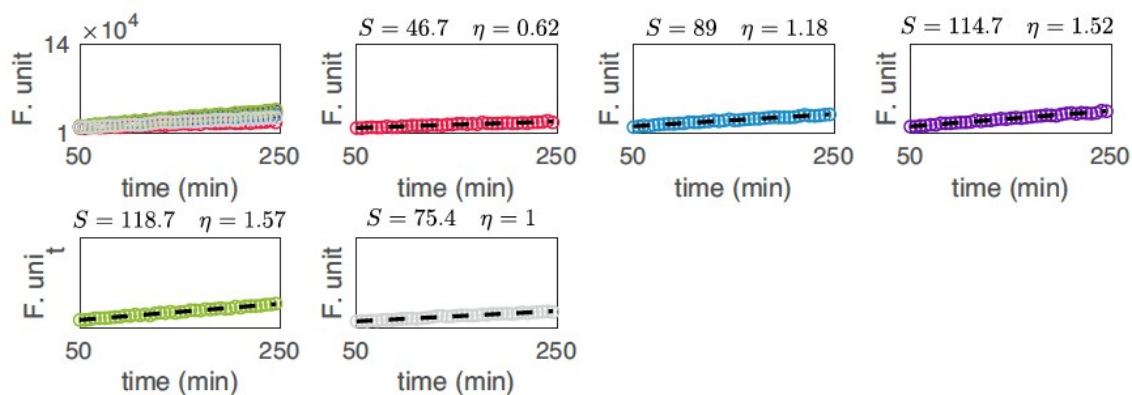
Supplementary Figure S4: Model fitting results for the traces shown in Fig. 1C (dose-dependent activity of SpCas9). The numbers above the plot indicate the absolute slope (S) and normalized slope (η). The colors correspond to those in Fig. 1C.



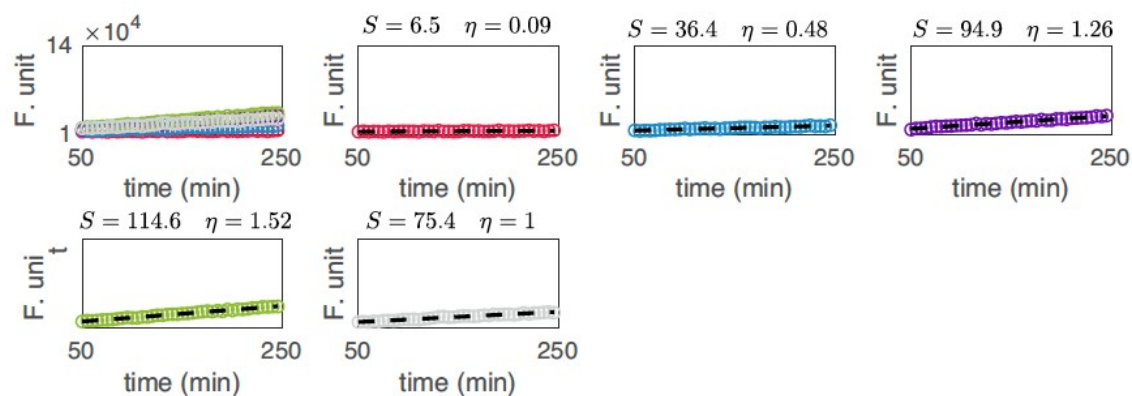
Supplementary Figure S5: Model fitting results for the traces shown in Fig. 1D (SpCas9 gRNA target-site scan). The numbers above the plot indicate the absolute slope (S) and normalized slope (η). The colors correspond to those in Fig. 1D.



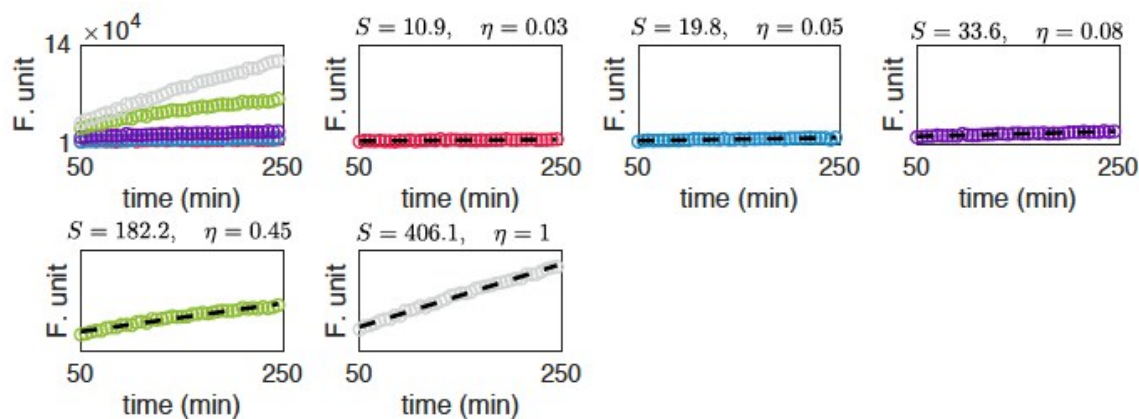
Supplementary Figure S6: Model fitting results for the traces shown in Fig. 1E (SaCas9 gRNA target-site scan). The numbers above the plot indicate the absolute slope (S) and normalized slope (η). The colors correspond to those in Fig. 1E.



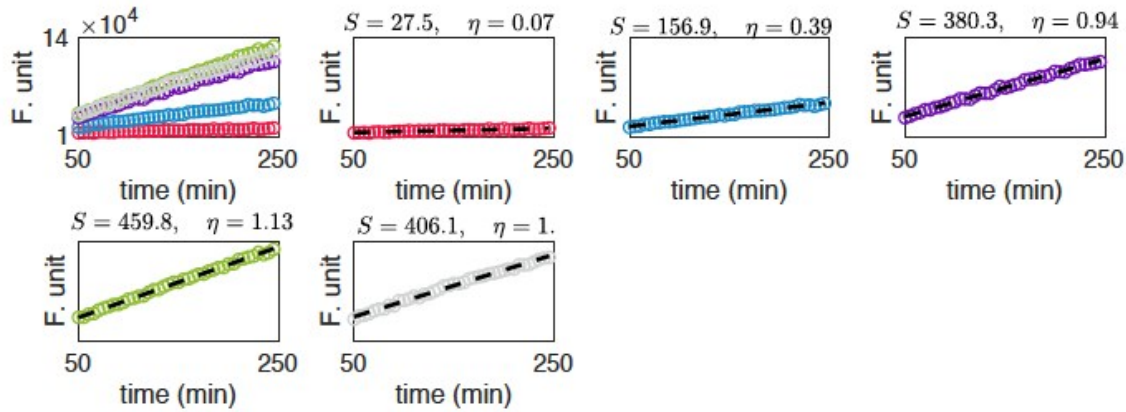
Supplementary Figure S7: Model fitting results for the traces shown in Fig. 2A-top (AsCpf1, distal PAM). The numbers above the plot indicate the absolute slope (S) and normalized slope (η). The colors correspond to those in Fig. 2A.



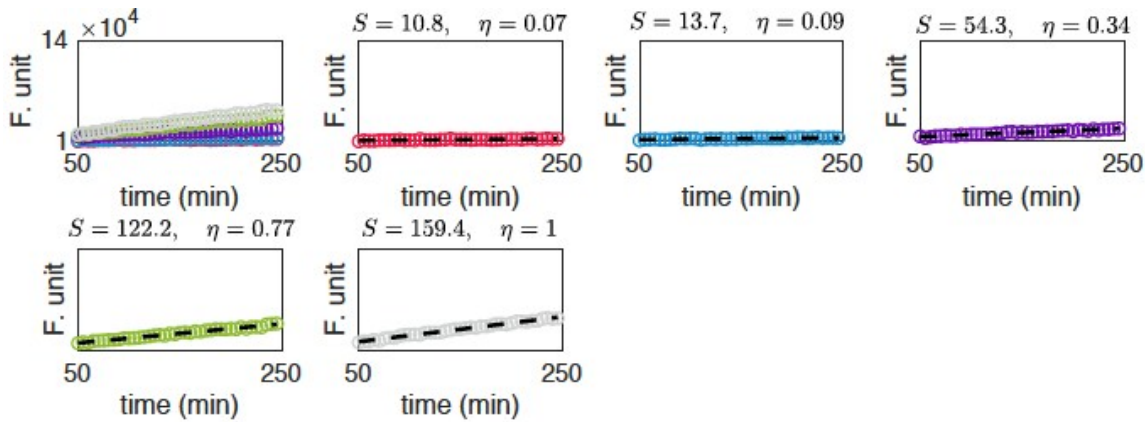
Supplementary Figure S8: Model fitting results for the traces shown in Fig. 2A-bottom (AsCpf1, internal PAM). The numbers above the plot indicate the absolute slope (S) and normalized slope (η). The colors correspond to those in Fig. 2A.



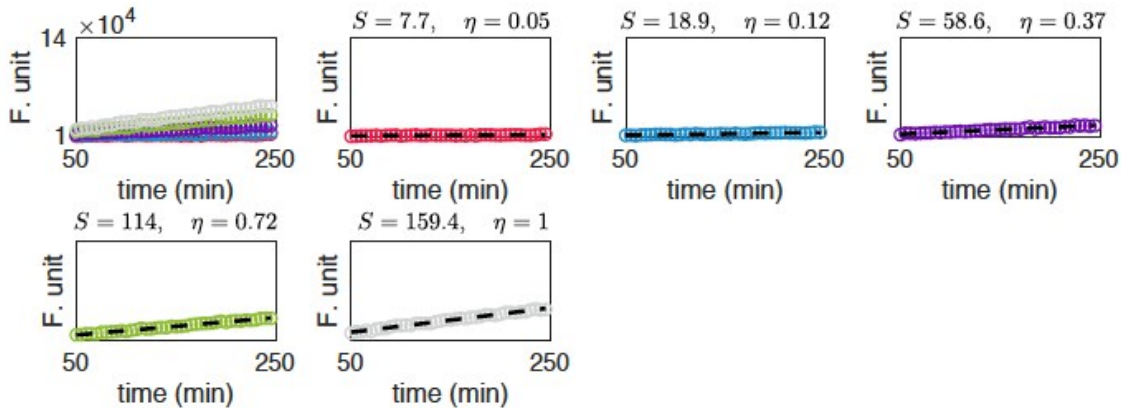
Supplementary Figure S9: Model fitting results for the traces shown in Fig. 2B-top (LbCpf1, distal PAM). The numbers above the plot indicate the absolute slope (S) and normalized slope (η). The colors correspond to those in Fig. 2B.



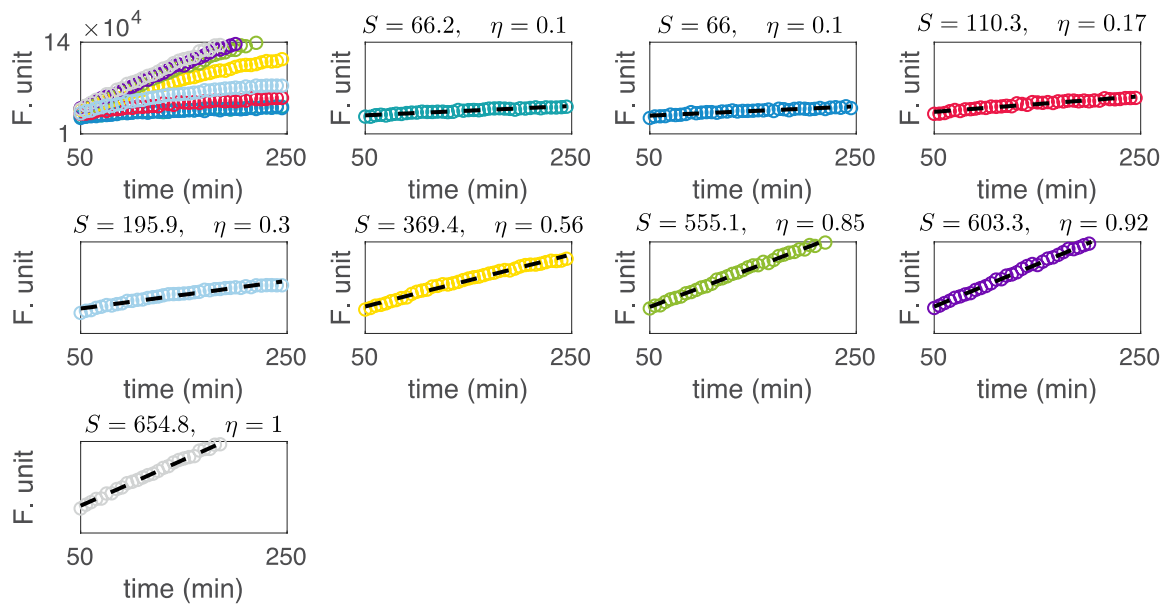
Supplementary Figure S10: Model fitting results for the traces shown in Fig. 2B-bottom (LbCpf1, internal PAM). The numbers above the plot indicate the absolute slope (S) and normalized slope (η). The colors correspond to those in Fig. 2B.



Supplementary Figure S11: Model fitting results for the traces shown in Fig. 2C-top (FnCpf1, distal PAM). The numbers above the plot indicate the absolute slope (S) and normalized slope (η). The colors correspond to those in Fig. 2C.



Supplementary Figure S12: Model fitting results for the traces shown in Fig. 2C-top (FnCpf1, internal PAM). The numbers above the plot indicate the absolute slope (S) and normalized slope (η). The colors correspond to those in Fig. 2C.



Supplementary Figure S13: Model fitting results for the traces shown in Fig. 4B (AcrIIA4 titration of SpCas9). The numbers above the plot indicate the absolute slope (S) and normalized slope (η). The colors correspond to those in Fig. 4B.

Table S2: Summary results for SI Fig. S4.

Concentration	$5nM$	$2nM$	$1nM$	$0.5nM$	$0.2nM$	$0.1nM$	Apo ($5nM$)
Slope (S)	8.1	52.8	142.1	203.5	234.7	250.1	251.5
Normalized Slope (η)	0.03	0.21	0.57	0.81	0.93	0.99	1
Correlation (R)	0.7381	0.9798	0.9954	0.9956	0.9965	0.9977	0.9986

Table S3: Summary results for SI Fig. S5.

guide RNA	Sp gRNA-1	Sp gRNA-2	Sp gRNA-3	Sp gRNA-4	Apo ($2nM$)
Slope (S)	7.6	94.9	27.5	136	139.5
Normalized Slope (η)	0.05	0.68	0.2	0.98	1
Correlation (R)	0.6361	0.9945	0.9346	0.9947	0.9964

Table S4: Summary results for SI Fig. S6.

guide RNA	Sp gRNA-1	Sp gRNA-2	Apo ($5nM$)
Slope (S)	7.1	5.1	132.3
Normalized Slope (η)	0.05	0.04	1
Correlation (R)	0.6068	0.5403	0.9961

Table S5: Summary results for SI Fig. S7.

Concentration	$50nM$	$20nM$	$5nM$	$2nM$	Apo ($50nM$)
Slope (S)	46.7	89	114.7	118.7	75.4
Normalized Slope (η)	0.62	1.18	1.52	1.57	1
Correlation (R)	0.9659	0.9915	0.9935	0.9940	0.9886

Table S6: Summary results for SI Fig. S8.

Concentration	$50nM$	$20nM$	$5nM$	$2nM$	Apo ($50nM$)
Slope (S)	6.5	36.4	94.9	114.6	75.4
Normalized Slope (η)	0.09	0.48	1.26	1.52	1
Correlation (R)	0.6187	0.9615	0.9939	0.9966	0.9886

Table S7: Summary results for SI Fig. S9.

Concentration	$50nM$	$20nM$	$5nM$	$2nM$	Apo ($50nM$)
Slope (S)	10.9	19.8	33.6	182.2	406.1
Normalized Slope (η)	0.03	0.05	0.08	0.45	1
Correlation (R)	0.7564	0.8854	0.9542	0.9843	0.9974

Table S8: Summary results for SI Fig. S10.

Concentration	$50nM$	$20nM$	$5nM$	$2nM$	Apo ($50nM$)
Slope (S)	27.5	156.9	380.3	459.8	406.1
Normalized Slope (η)	0.07	0.39	0.94	1.13	1
Correlation (R)	0.9427	0.9962	0.9981	0.9989	0.9974

Table S9: Summary results for SI Fig. S11.

Concentration	50nM	20nM	5nM	2nM	Apo (50nM)
Slope (S)	10.8	13.7	54.3	122.2	159.4
Normalized Slope (η)	0.07	0.09	0.34	0.77	1
Correlation (R)	0.7902	0.8675	0.9774	0.9936	0.9957

Table S10: Summary results for SI Fig. S12.

Concentration	50nM	20nM	5nM	2nM	Apo (50nM)
Slope (S)	7.7	18.9	58.6	114	159.4
Normalized Slope (η)	0.05	0.12	0.37	0.72	1
Correlation (R)	0.7057	0.9339	0.9840	0.9960	0.9957

Table S11: Summary results for SI Fig. S13.

Concentration	0nM	1nM	2.5nM	5nM	10nM	25nM	50nM	Apo (5nM)
Slope (S)	66.2	66	110.3	195.9	369.4	555.1	603.3	654.8
Normalized Slope (η)	0.1	0.1	0.17	0.3	0.56	0.85	0.92	1
Correlation (R)	0.9694	0.9523	0.9714	0.9773	0.9929	0.9979	0.9985	0.9987