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An engineered ScCas9 with broad PAM range and high specificity and activity

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Supplementary Information

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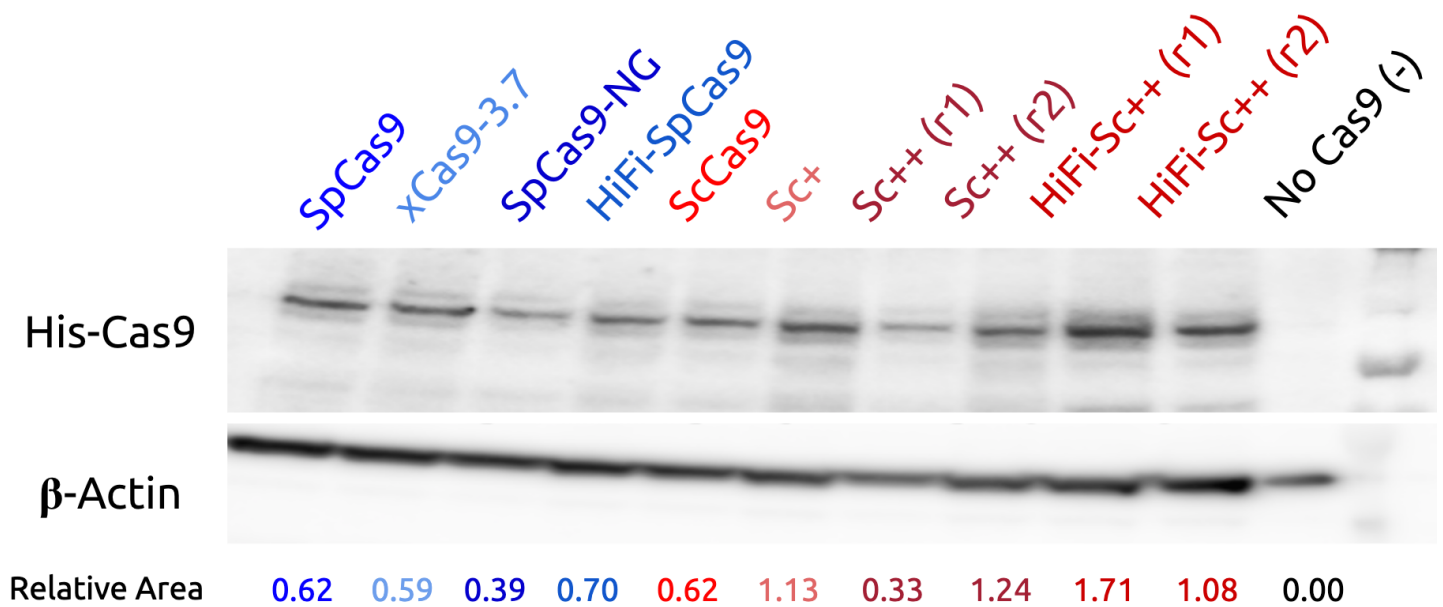
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Supplementary Figures

1. Western Blot for Cas9 expression.
2. Sequences of off-target sites identified by GUIDE-Seq.
3. GUIDE-Seq read counts at detected on- and off- target sites.



Supplementary Figure 1. Western Blot for Cas9 expression. N-terminal His-tagged Cas9 plasmids were transfected into HEK293T cells. After 5 days post-transfection, whole cell extracts were run on SDS-PAGE gels and transferred onto a PVDF membrane. Membranes were either probed with an anti- β -actin or anti-His primary antibodies, followed by treatment with HRP-conjugated goat anti-mouse IgG secondary antibody. The expression levels of the tested Cas9 variants and β -actin loading control are shown. Sc++ and HiFi-Sc++ samples were blotted in independent biological duplicates (n=2). Relative areas of the Cas9 protein level to that of β -actin was calculated given the dynamic range threshold of 182 in the unit of grayscale (0-255), utilizing the ImageJ software.

VEGFA	GGTGAGTGAGTGTGTGCGTGNNGG	peak_score
SpCas9		5446
	.C.....A.....	5130
	T...T...TT...T.....	4496
	A.....T.....	3524
	A...T.....	2810
	A.A.....A.....	2138
	A...G.....A.....	2088
	T...G.....	2080
	A.C.....G.....	1716
	T.....	1656
	T.....	1110
	T...A.....T.....	786
	...G.....A.A.....	654
	T...T.....A.....A	646
	T.....T.....A	604
	T...G.....A	572
	T...G.....T.....	530
	.G.A.....A.A.A	398
	C.....A.....	384
	AG.....T.....	370
	A.C.....T.....	360
	A.A.....T	334
	T.....T.....A.A	304
	T.....A.....	300
	AC.....A.T.....	294
	..A.....A.....C	288
	A.G.....A.A.....	286
	AA.....A.....C	282
	T...G.....G.....T...A	278
	T...G.....CA.....	266
	A.A...A.....T.....T	264
	A.....A.A.....	260
	T...A.A.....	248
	..T.....A.....AT	226
	..A.....A.....T	224
	A.A...A.....A	214
	T...T...T.....T.A	210
	T...T...CA...C	184
	T...T...T...C...C...T	180
	T.....A.....	178
	T...G...A.....T...AT	176
	...T...G.....T...AT	176
	T.G.T...C.....G...T	172
	A...A.....T.....	172

T...T...T.....T	168
..GA.....T.....	166
T...T.....T.....	166
...T.....A.T.....	162
A.A.....T.T.....	158
C.....A.C.....	158
T...AT.....T.....	152
..CAG...G.A.....T	152
C...T...T...T...T	144
T...T...T...T...T	140
T...T...T...T...T	132
..A.....T.....A	132
...T.....T.....	130
A...T.....T.....	128
T...TA.....A.C...CT	120
..A.....T.....A	118
A...A.....A.....A	118
A.G.....C.....	116
A.A.....T.....A	112
.....A.....A.A.....	110
T...T...T...T...AT	108
..G.G.....T.....	106
T...T...T...T...AT	100
..A.G.....T...C.....	98
A.A.....T.....A	96
A.A.....T.....T	96
A.G.....T.....A	96
..TA.....T.....	96
T...T...TA.....T...T	94
T...T...C...A.....T	92
T...T...AT.....T...C	92
..C...T...T...G.....A	90
AA.....T.....A	88
..G...A.G.....T.....	88
T...T...T...T...T	88
..A.....A.....A	82
..TA.....T.....	82
CA.G.....C.....A.....	76
T...T.....T.....T	74
T.....T.....A	70
T...T...T...T...T	70
T...T...T...T...T	66
T...T.....A.....	64
T...T...T.....T	62

A.....T.....T	60
T...AT.....T...AT	60
..A.....T.....T.....	58
T.....T.....A	58
...A.....AC.....	58
T...T...T...T...T	56
T...T...T...T...T	54
..T.TT...T...A.....CA	54
T...T...T...T...T	54
A.....T.....A	52
T...T...T...T...T	52
...T...T...T...T...T	52
TC.....A.T...CT	46
CA...T.A.T.....AT	44
...T...G.....T.....A	44
T...T...A.A...A...AT	44
T.A.....A.A.....	42
A...G.....G.....AT	42
A...G.....G.....A...AT	42
A...G.....A.T.....A	40
T...G...G.....T...T	38
..G.....T.....A	36
T.....T.....T...T	36
A.A...A.....T.....	34
T...A.....A.A...T	34
A.A...TT.....T	34
T...T...T...T...T	34
..CAT...C.....T...T	32
T...T...T...T...T	32
...T...TA.....T...A.TT	30
..T...T...A.T.....A	30
T...T...T.A...A...AT	30
..A...C.....T.....A	28
..G.T.....T.....	28
C.....GT.....	26
..T.....GT.....	26
AA.....A...A.....	24
..AA.....G...A...A.....	24
..C.....T...T...C.G...T	24
..TG.T...A.....G...T	24
T...T...T...T...T	22
...T...G.....T...A	22
T...T...AT.....T...AC	22
..A.....AT.....	22

T...T...T...T...AT	20
T...T...T...T...T	20
T...T...T...T...T	18
T...T...T...T...T	18
T...T...T...T...T	18
T...T...A.....G...T	16
...T.....A.....A	14
T...A.....A.....A	14
...A.....T.....A	14
T...T...T...T...A	14
...T.....A.T...A	14
.....C...T.G...T	12
T...T...T...A.....	12
..G...A...A...A.....	12
ACA...A.C.....A	12
..A.....A.....A	12
A...T...A...A.....	12
...T...T...T...T	12
T...T...T...T...AC	12
T...T...T...T...T	12
T...T...T...T...T	10
T...T...T...T...T	10
A...G.....T...A	10
T...C.A.T.....A...AT	10
T...T...T...T...TT	10
T...T...T...T...T	8
T...T...T...T...T	8
T...G...CAC.....	8
A.A.....T...T...T	8
T...T...T...T...T	8
..C...T...A...A...CT	8
T...T...T...T...T	8
T...T...T...T...T	6
T...T...T...T...T	6
..TG.T...T...T...CT	6
T...T...T...T...T	6
C...T...T...T...T	6
T...T...T...T...T	6
T...T...T...T...T	6

VEGFA	GGTGAGTGAGTGTGTGCGTGNNGG	peak_score
HiF1-SpCas9		12842
	A.....T.....	4387
	T.....	3763
	.C.....A.....	2394
	A.A.....A.....A	1318
	A.....A.....A	1166
	C.....A.....	737
	A.....T.....A	546
	A...A.....T.....	379
	A.....T.....A	343
	A...G.....A.....	277
	T...T...T...A	271
	T.....A.....	240
	A.....A.A.....	229
	..A.....A.A.....A	217
	A.....A.A	211
	..A.....A.....A	156
	T.....T...A.....	103
	..A.....A.....C	79
	T...G.....	40
	A.....T.....	36
	A.A.....T	35
	T...G.....A	34
	A.....A.A.....	31
	...T...G.....T...A	25
	..A.....AC.....	22
	T...T.A.....G...T	18
	T...T.....A.....A	11

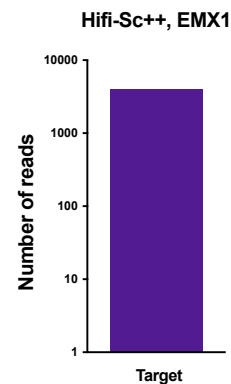
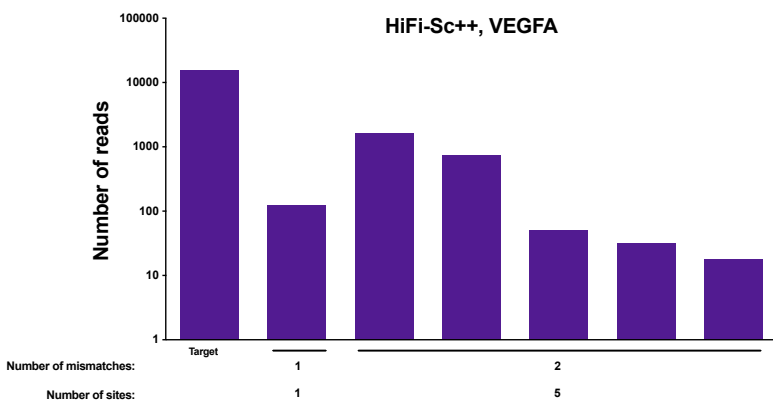
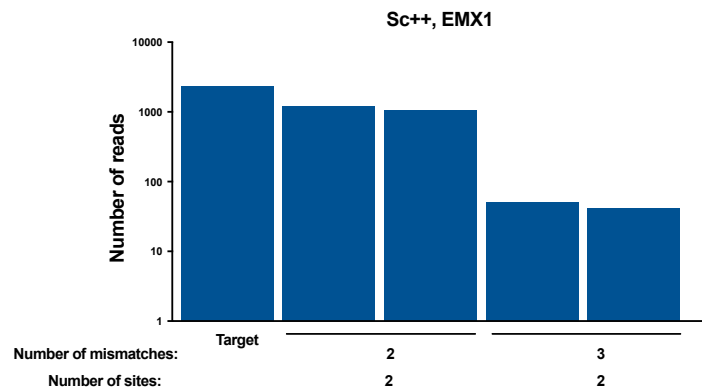
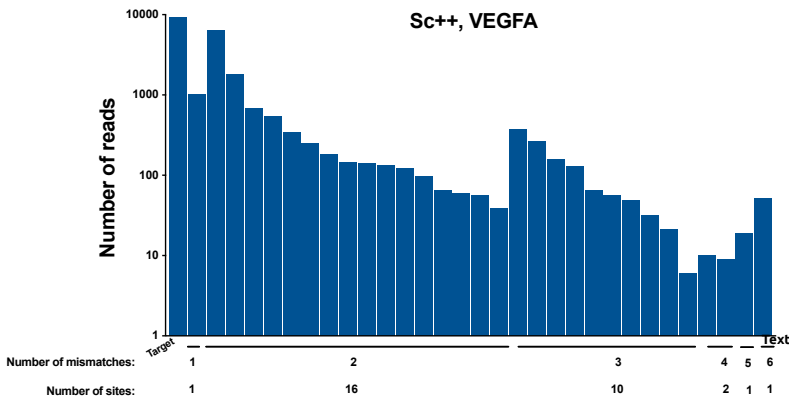
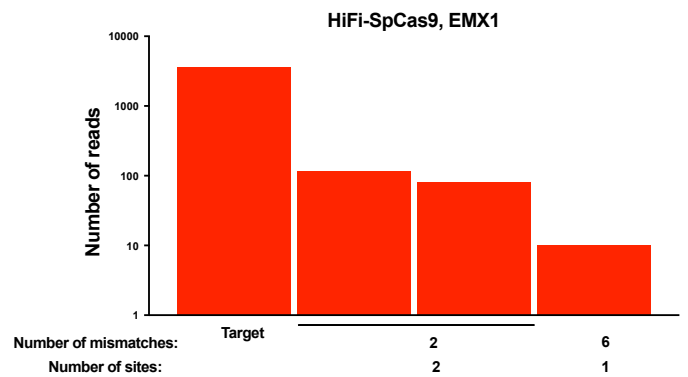
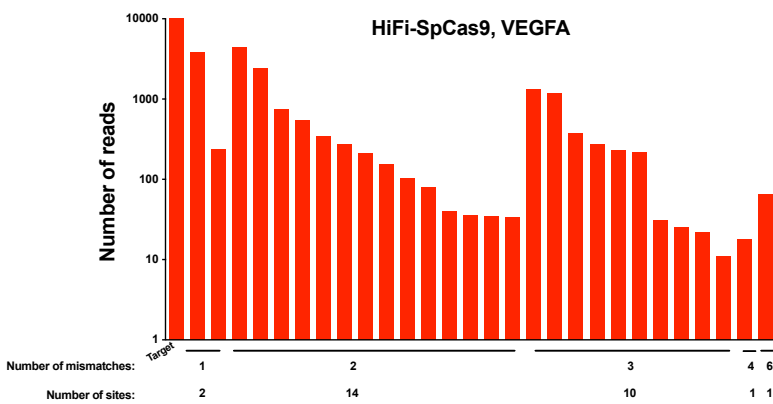
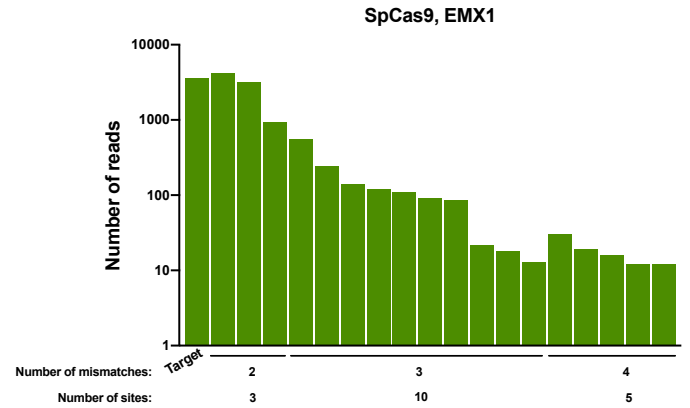
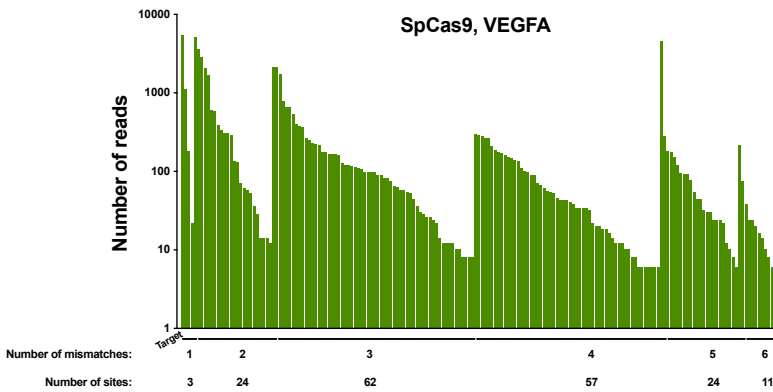
VEGFA	GGTGAGTGAGTGTGTGCGTGNNGG	peak_score
Sc++		9357
	T.....T...A.....	6454
	A.....T.....	1782
	T.....	1008
	A.....T...T.....	683
	G.....T...A.....	539
	AA.....A.....A.....	375
	..C.....A.....	346
	A...A.....T...T	263
	A.....T...A.....	249
	T.....T...A.....	182
	C.....A.C.....	159
	T.....T...T...T	145
	T.....T...A.....	143
	A.....T...AA.....	134
	T...T.....A.....A	128
	A.....T...A.....	123
	T.....T...A.....	98
	..AG.....T.....	66
	A.....A.....A.....	65
	C.....A.....	59
	A.....T...T.....	57
	..G.A.....A.A.....	57
	A...A.....T.....	49
	C...A.....T...	39
	A.....A.....A.....	32
	A...G.....A.....	21
	T...T...TA.....T...AT	19
	...T.A.G.....G.....A	10
	T...T.A.....G.....T	9
	A.....A.G.....T	6

VEGFA	GGTGAGTGAGTGTGTGCGTGNNGG	peak_score
HiF1-Sc++		15552
	A.....T.....	1626
	T.....T...A.....	744
	T.....	124
	T.....T...A.....	51
	T...A.....	32
	..C.....A.....	18

EMX1	GAGTCCGAGCAGAAGAAAGAA NGG	peak_score
SpCas9	...TA...	4215
	...	3611
	...TA...A	3209
	...T...G...A	930
	...G...AG...	552
	A...T...C...	248
	...G...A...G...	139
	...A...A...G...A	121
	...C...G...G...	109
	...AG...G...A	92
	AC...T...	87
	...G...G...G...G...	30
	A...G...G...	22
	A...G...A...G...	19
	A...A...G...	18
	A...C...A...G...	16
	...A...T...G...	13
	A...TG...G...	12
	A...A...A...G...	12
EMX1	GAGTCCGAGCAGAAGAAAGAA NGG	peak_score
HiFi-SpCas9	...	2280
	...TA...	79
	...TA...A	66
EMX1	GAGTCCGAGCAGAAGAAAGAA NGG	peak_score
Sc++	...	1774
	...T...G...A	821
	...TA...A	809
	...T...G...G...A	26
	...A...G...G...A	24
EMX1	GAGTCCGAGCAGAAGAAAGAA NGG	peak_score
HiFi-Sc++	...	2912

Supplementary Figure 2. Sequences of off-target sites identified by GUIDE-Seq.

For each Cas9, the on-target sequence is shown at the top with PAM in bold and with mismatches to the on-target site shown in color. GUIDE-Seq peak scores are shown to the right of each site. Data is shown for SpCas9, HiFi-SpCas9, Sc++, and HiFi-Sc++ Cas9 nucleases targeting VEGFA and EMX1 sites. No off-target sites were found for HiFi-Sc++ at the EMX1 site.



Supplementary Figure 3. GUIDE-Seq read counts at detected on- and off-target sites. Numbers of independent GUIDE-Seq reads for on- and off-target sites for all combinations of Cas9s (SpCas9, HiFi-SpCas9, Sc++, and HiFi-Sc++) and target sites (VEGFA and EMX1), binned by the number of mismatches (≤ 6 mismatches) with the corresponding Cas9 and target site. The total number of sites in each bin are also shown.