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Supplemental Information

Naturally Occurring Off-Switches for CRISPR-Cas9

April Pawluk, Nadia Amrani, Yan Zhang, Bianca Garcia, Yurima Hidalgo-Reyes, Jooyoung Lee, Alireza Edraki, Megha Shah, Erik J. Sontheimer, Karen L. Maxwell, and Alan R. Davidson

Supplemental Table 1: Anti-CRISPR DNA and Protein Sequences Used in this Study. Related to Figure 1.

Anti-CRISPR	DNA sequence	Protein sequence
AcrIIC1$_{Boe}$	ATGGCCAAGGAGGTCTTCAAGCTGAAGCCGGAGCTGGTGACGT ACAAGGGCTGCGGGTGGGCCCTGGCCTGCATCAAGGATGGCGA GATCATCGACCTGACCTACGTGCGTGACCTGGGCATCGAGGAG TACGATGAAAACTTCGACGGCCTGGAGCCGGAGATCATCTATT ACGACGTCGTCGCCTCGCAGGCGTGCAAGGAAGTGGCCTACCG CTATGAAGAGATGGGCGAATTCACCTTCGGCCTCTGCAGCTGC TGGGAATTCAACGTCATGTAA	MAKEVFKLKPELVTYKGCGWALACIK DGEIIDLTYVRDLGIEEYDENFDGLE PEIIYYDVVASQACKEVAYRYEEMGE FTFGLCSCWEFNVM
AcrIIC1$_{Nme}$	ATGGCCAATAAAACTTATAAAATTGGAAAAAATGCCGGGTATG ATGGCTGCGGTCTTTGTCTTGCGGCCATTTCTGAAAATGAAGC TATCAAAGTTAAGTATTTGCGCGACATTTGTCCTGATTACGAT GGCGATGATAAAGCTGAGGATTGGCTGAGATGGGGAACGGACA GCCGCGTCAAAGCAGCCGCTCTTGAAATGGAGCAGTACGCATA TACGTCGGTTGGTATGGCCTCATGTTGGGAGTTTGTTGAACTA TGA	MANKTYKIGKNAGYDGCGLCLAAISE NEAIKVKYLRDICPDYDGDDKAEDWL RWGTDSRVKAAALEMEQYAYTSVGMA SCWEFVEL
AcrIIC2$_{Nme}$	ATGGCCAGCAAAAACAATATTTTCAACAAGTATCCAACAATTA TTCACGGCGAAGCGCGGGGGGAGAATGACGAATTTGTGGTGCA TACGCGCTACCCGCGATTCTTGGCGCGGAAATCTTTTGACGAC AATTTCACGGGCGAAATGCCTGCAAAACCTGTTAACGGGGAAT TGGGACAAATCGGCGAACCGCGCCGCCTTGCTTATGATTCACG GCTTGGTTTGTGGCTTTCTGACTTCATCATGTTGGACAACAAC AAGCCGAAAAACATGGAGGATTGGCTTGGGCAATTAAAAGCCG CCTGCGATCGAATCGCGGCGGATGATTTGATGCTGAATGAAGA TGCGGCGGATTTGGAGGGCTGGGATGATTGA	MASKNNIFNKYPTIIHGEARGENDEF VVHTRYPRFLARKSFDDNFTGEMPAK PVNGELGQIGEPRRLAYDSRLGLWLS DFIMLDNNKPKNMEDWLGQLKAACDR IAADDLMLNEDAADLEGWDD
AcrIIC3$_{Nme}$	ATGGCCTTCAAACGCGCTATTATCTTCACTTCTTTCAACGGCT TTGAAAAAGTTTCTCGAACTGAAAAACGCCGCCTTGCCAAAAT CATCAATGCTCGAGTTTCCATCATCGACGAATACTTGAGAGCC AAAGACACCAACGCATCGCTTGACGGTCAGTACCGCGCTTTCT TGTTCAACGACGAATCGCCCGCAATGACCGAATTTCTGGCAAA ACTTAAAGCCTTTGCCGAAAGTTGCACCGGAATCAGCATCGAC GCATGGGAAATTGAAGAAAGCGAATACGTCCGCCTGCCGGTGG AACGCAGGGATTTCTTAGCGGCAGCCAACGGCAAAGAGATTTT TAAAATTTAA	MAFKRAIIFTSFNGFEKVSRTEKRRL AKIINARVSIIDEYLRAKDTNASLDG QYRAFLFNDESPAMTEFLAKLKAFAE SCTGISIDAWEIEESEYVRLPVERRD FLAAANGKEIFKI
AcrE2	ATGGCCAATACCTATCTCATCGACCCCCGCAAAAACAACGACA ACTCCGGCGAGCGCTTCACGGTTGACGCTGTCGACATTACAGC CGCCGCGAAGAGCGCAGCCCAACAGATTCTTGGCGAGGAATTC GAGGGCCTCGTATACCGTGAAACCGGGGAGAGTAACGGAAGTG GCATGTTCCAGGCCTACCACCACCTGCACGGCACTAACCGCAC GGAGACGACCGTTGGCTATCCGTTTCATGTAATGGAACTCTGA	MANTYLIDPRKNNDNSGERFTVDAVD ITAAAKSAAQQILGEEFEGLVYRETG ESNGSGMFQAYHHLHGTNRTETTVGY PFHVMEL

Supplemental Table 2: Information About Anti-CRISPR Homologs. Related to Figure 1, Figure S1.

Anti-CRISPR		Species	Genome region prediction	Accession #	% ID to *	Size (aa)	Tested?
AcrIIC1	*	*Brackiella oedipodis*	putative integrated conjugative element	WP_028357638.1	100	91	Yes
		Alicycliphilus denitirificans	plasmid	ADV02121.1	26	92	
		Neisseria meningitidis	unclear	WP_049360089.1	29	85	Yes
		Bordetella hinzii	prophage	WP_032962436.1	29	87	
		Verminephrobacter eiseniae	putative integrated conjugative plasmid	WP_041950174.1	23	103	
		Alicycliphilus denitirificans	prophage	WP_013520332.1	28	133	
		Verminephrobacter eiseniae	unclear	ABM59472.1	23	147	
		Pseudoalteromonas lipolytica	unclear	WP_036972373.1	30	117	
		Tistrella mobilis	prophage	WP_014743597.1	32	85	
		Fenollaria massiliensis	putative transposable element	WP_019214717.1	28	149	
		Bordetella sp.	putative integrated conjugative plasmid	WP_019939893.1	29	87	
AcrIIC2	*	*Neisseria meningitidis*	prophage	WP_042743678.1	100	123	Yes
		Neisseria meningitidis	prophage	CWP55982.1	96	132	
		Neisseria meningitidis	prophage	WP_061725849.1	96	123	
		Neisseria meningitidis	prophage	WP_002212355.1	95	123	
		Neisseria meningitidis	prophage	WP_021439709.1	94	123	
		Neisseria meningitidis	prophage	WP_061695141.1	93	123	
		Neisseria meningitidis	prophage	WP_002238681.1	92	123	
		Neisseria meningitidis	prophage	WP_002231709.1	91	123	
		Neisseria meningitidis	prophage	WP_061693463.1	90	123	
		Neisseria meningitidis	prophage	WP_061706309.1	90	123	
		Neisseria meningitidis	prophage	WP_002255675.1	89	123	
		Neisseria meningitidis	prophage	WP_061384810.1	89	123	
		Ralstonia solanacearum	putative integrated conjugative element	WP_019718638.1	33	130	
		Ralstonia solanacearum	putative integrated conjugative element	WP_011001812.1	32	130	
		Ralstonia solanacearum	putative integrated conjugative element	AMP37321.1	32	130	
		Ralstonia syzygii	unclear	CCA86204.1	32	130	
		Ralstonia solanacearum	putative transposable element	WP_013212199.1	32	130	
		Cupriavidus basilensis	unclear	WP_017226229.1	31	128	
		Ralstonia solanacearum	putative transposable element	WP_014616771.1	31	130	
		Ralstonia solanacearum	putative integrated conjugative element	WP_003265552.1	31	130	
		Ralstonia solanacearum	putative integrated conjugative element	WP_013205753.1	31	130	
		Ralstonia mannitolilytica	unclear	WP_045787125.1	31	130	
		Ralstonia pickettii	unclear	WP_024975412.1	31	130	
		Ralstonia pickettii	putative transposable element	WP_004634308.1	31	131	
		Ralstonia sp.	unclear	WP_048932645.1	31	129	
		Ralstonia sp.	unclear	WP_009240943.1	31	131	
		Ralstonia sp.	putative integrated conjugative element	WP_021194849.1	31	129	
		Ralstonia sp.	unclear	WP_039599631.1	31	129	
		Cupriavidus basilensis	unclear	WP_006163540.1	30	128	
		Cupriavidus basilensis	unclear	WP_043347745.1	30	128	
		Burkholderiaceae bacterium	unclear	WP_045235538.1	30	128	
		Ralstonia sp.	unclear	WP_027681138.1	30	131	
		Cupriavidus sp.	unclear	WP_039006692.1	30	126	
		Cupriavidus sp.	unclear	WP_066735805.1	29	130	
		Cupriavidus sp.	unclear	WP_035835797.1	28	130	
		Cupriavidus sp.	unclear	WP_019451860.1	28	130	
		Cupriavidus sp.	unclear	WP_020202326.1	28	128	
		Cupriavidus pauculus	unclear	WP_061960205.1	28	127	
		Cupriavidus metallidurans	unclear	WP_011516945.1	28	130	
		Cupriavidus sp.	unclear	WP_029046347.1	28	126	
		Cupriavidus nantongensis	unclear	WP_062800778.1	28	126	
		Cupriavidus oxalaticus	prophage	WP_063238209.1	28	126	
		Cupriavidus gilardii	unclear	WP_053822121.1	27	127	
		Cupriavidus sp.	unclear	WP_035882356.1	27	127	
		Cupriavidus sp.	unclear	EKZ95749.1	27	130	
		Cupriavidus sp.	unclear	WP_035818297.1	27	126	
		Cupriavidus sp.	unclear	WP_006577295.1	27	127	
		Cupriavidus necator	unclear	WP_013957324.1	27	126	
		Cupriavidus sp.	unclear	WP_012353264.1	27	126	
		Cupriavidus necator	unclear	WP_042886547.1	27	126	
		Burkholderiaceae	unclear	WP_010814353.1	27	125	
		Ralstonia pickettii	unclear	WP_022535879.1	27	126	
		Cupriavidus necator	unclear	WP_058697216.1	27	125	
		Cupriavidus sp.	unclear	ODV41125.1	27	130	
		Cupriavidus pinatubonensis	unclear	WP_011298372.1	26	130	
		Ralstonia sp.	unclear	WP_009522362.1	24	131	
AcrIIC3	*	*Neisseria meningitidis*	prophage	WP_042743676.1	100	116	Yes
		Neisseria meningitidis	prophage	WP_002231708.1	93	116	
		Neisseria meningitidis	prophage	WP_061695142.1	91	116	
		Neisseria meningitidis	prophage	WP_061384809.1	91	116	
		Neisseria meningitidis	prophage	WP_061725842.1	91	116	
		Neisseria meningitidis	prophage	WP_061725391.1	88	116	
		Neisseria meningitidis	prophage	WP_025455551.1	87	116	
		Neisseria meningitidis	prophage	WP_061706308.1	86	116	
		Neisseria meningitidis	prophage	EFM05431.1	87	138	
		Neisseria meningitidis	prophage	WP_002255674.1	85	116	
		Neisseria meningitidis	prophage	WP_002238680.1	86	117	
		Neisseria meningitidis	prophage	EQD23083.1	91	75	
		Neisseria meningitidis	prophage	EQD21340.1	88	73	
		Neisseria meningitidis	prophage	EQD23377.1	78	63	

Supplemental Table 3: Templates for Transcription of sgRNAs that Target Several Human Genome Sites. Related to Figure 4, Figure S3, and STAR Methods.

PLASMID NAME/CONSTRUCT	TARGET SITE	T7E1 F- OLIGO	T7E1 R- OLIGO	SPACER CLONING F-OLIGO	SPACER CLONING R-OLIGO	SPACER SEQUENCE	LOCUS NAME	CHROMOSOME NUMBER
pEJS161: pSimpleII-NmeCas9-sgRNA/NTS1C	N-TS1C	GCACTTATTCTGGCCCCTGACTGC	GAGAACCATGGTCTGGGGAAGAAGACC	CACCGTGGTCTGGGGTACAGCCTTGGCA	CAACTGCCAAGGCTGTACCCCAGACCAC	GTGGTCTGGGGTACAGCCTTGGCA	SLC9A9	3
pEJS173: pSimpleII-NmeCas9-sgRNA/NTS4B	N-TS4B	AGAGGAGCCTTCTGACTGCTGCAGA	AGGTCCTGGCCTTGCCTTCGA	CACCGGACAGGAGTCGCCAGAGGCCGGT	CAACACCGGCCTCTGGCGACTCCTGTCC	GGACAGGAGTCGCCAGAGGCCGGT	FLJ00328	14
pEJS174: pSimpleII-NmeCas9-sgRNA/NTS4C	N-TS4C	AGAGGAGCCTTCTGACTGCTGCAGA	AGGTCCTGGCCTTGCCTTCGA	CACCGGGGCTGGCTCCACGTCGCGCCGC	CAACGCGGCGCGACCGTGGAGCCAGCCCC	GGGGCTGGCTCCACGTCGCGCCGC	FLJ00328	14
pEJS212: pSimpleII-NmeCas9-sgRNA/NTS7	N-TS7	GGACAGAAGAGAGTAGGGAGACGAG	GCATTCTGTCATCTGCATATCCCTCTG	CACCGAGGGAGAGAGGTGAGCGGATGAA	CAACTTCATCCGCTCACCTCTCTCCCTC	GAGGGAGAGAGGTGAGCGGATGAA	LOC100505797	18
pEJS224: pSimpleII-NmeCas9-sgRNA/NTS8	N-TS8	TGCCTCACGTAACAGTTGAGACCC	TGCCCTCCCCGCTGGAACCT	CACCGGACGCAATTCCAGAGGTGATGG	CAACCCCATCACCTCTGGAATTGCGTCC	GGACGCAATTCCAGAGGTGATGGG	ESPN	1
pEJS236: pSimpleII-NmeCas9-sgRNA/NTS11	N-TS11	ACAGGCAACTCCATCCATGAGCC	CTTCACAGCACTTAGGACTGTCTG	CACCGTTCCAGTTGGGAAGGGCCAGTGC	CAACGCACTGGCCCTTCCCAACTGGAAC	GTTCCAGTTGGGAAGGGCCAGTGC	SMARCB1	22
pEJS323: pSimpleII-NmeCas9-sgRNA/NTS25	N-TS25	GCAATCCACCCAATGCTAACTGG	TGAACACAAAGGCCTCCAGATCC	CACCGGTTTCTCATCCTGTCTTCTGCCT	CAACAGGCAGAAGACAGGATGAGAAACC	GGTTTCTCATCCTGTCTTCTGCCT	AC193513	7
pEJS337: pLK.O1-NmeSgRNA/DTS3	D-TS3 (Nme)	GGACAAAAGCAGCCCATTAG	GGACTCCTAAAATGGCCACA	CACCGACTGAAGGCGAGGTCCGGGGCGG	CAACCCGCCCCGGACCTCGCCTTCAGTC	GACTGAAGGCGAGGTCCGGGGCGG	ARHGEF9	X
pEJS399: pLK.O1-SpySgRNA/DTS3	D-TS3 (Spy)	GGACAAAAGCAGCCCATTAG	GGACTCCTAAAATGGCCACA	ACCGGAAGGCGAGGTCCGGGGCGG	AAACCCGCCCCGGACCTCGCCTTC	GAAGGCGAGGTCCGGGGCGG	ARHGEF9	X
pEJS341: pLK.O1-NmeSgRNA/DTS7	D-TS7 (Nme)	AGGACTGCTCTCAGCTACCG	AAGGGCAGAGAGGCTAAAGG	CACCGGCTGGCACCCTCCATGTACCCAG	CAACCTGGGTACATGGAGGGTGCCAGCC	GGCTGGCACCCTCCATGTACCCAG	LSP1	11
pEJS400: pLK.O1-SpySgRNA/DTS7	D-TS7 (Spy)	AGGACTGCTCTCAGCTACCG	AAGGGCAGAGAGGCTAAAGG	ACCGGGCACCCTCCATGTACCCAG	AAACCTGGGTACATGGAGGGTGCC	GGCACCCTCCATGTACCCAG	LSP1	11
pEJS468: pLK.O1-NmeSgRNA/DTS13-Telomere	D-TS13 (Nme-telomere)	N/A	N/A	ACCGTTAGGGTTAGGGTTAGGGTTAGG	CAACCCCTAACCCTAACCCTAACCCTAA	TTAGGGTTAGGGTTAGGGTTAGGG	N/A	N/A
pEJS469: pLK.O1-SpySgRNA/DTS13-Telomere	D-TS13 (Spy-telomere)	N/A	N/A	ACCGTTAGGGTTAGGGTTAGGGTT	AAACAACCCTAACCCTAACCCTAA	TTAGGGTTAGGGTTAGGGTT	N/A	N/A