SUPPORTING INFORMATION

SI MATERIALS AND METHODS

In vitro enrichment assay

Target site oligonucleotides containing a randomized 12 bp spacer in the context of a *td* target site were cloned into the pSP72 vector as previously described to create pSP72-N12 (1). MegaTev purification was performed as previously described (2). Cleavage assays were performed with 360 nM or 1100 nM purified MegaTev and 10 nM pSP72-N15 plasmid in cleavage buffer (50 mM Tris-HCl pH 7.9, 300 mM NaCl, 10 mM $MgCl_2$ and 1 mM dithiothereitol (DTT)) at 37^{o}C for 5 min. Subsequent rounds of enrichment was performed as previously described (1). Samples were prepared for Illumina sequencing at the London Regional Genomics Centre by PCR amplification of target site from both the input and round 3 enrichment samples using Phusion polymerase (New England Biolabs) with barcoded primers. Custom Perl scripts were used to separate the reads by barcode and to extract the randomized region. Enrichment was determined by using the center logarithmic transformation of the nucleotide counts (3), and by taking the difference between the enriched and input values per nucleotide per position.

Bacterial two-plasmid selection

The bacterial two-plasmid selection was performed with single MegaTev constructs or with MegaTev linker libraries as previously described (2, 4). Briefly, 50 ng of MegaTev pEndo vector was transformed into *E. coli* BW25141 cells harbouring the pTox plasmid containing the target site of choice. Cells were recovered at 37 ^{o}C for 1.5 hrs and aliquots plated on selective (LB media plus 25 µg/ml chloramphenicol and 10 mM L-(+)-arabinose) and non-selective plates (LB media plus 25 µg/ml chloramphenicol, 50 µg/ml kanamycin and 0.2% glucose). Survival was expressed as the ratio of the number of colonies on the selective plate versus the number of colonies on the non-selective plate. The MegaTev mutant linker libraries were screened either on solid media, or in liquid LB media under selective and non-selective conditions. Identified MegaTev linker variants were re-cloned into pEndo and the selection repeated on the appropriate target sites.

TevCas9 site prediction

The TevCas9 binding model included two components essential for I-TevI activity: a 5'-CNNNG-3' cleavage motif, and a DNA spacer. Putative TevCas9 binding sites were found using custom Perl scripts that windowed across human coding regions for 5'-CNNNG-3 motifs followed by a DNA spacer of 15-18 bps. The raw score of the putative binding site was calculated by: (1) the $\log_2$ value of the activity of the 5'-CNNNG-3' cleavage motif (relative to the 5'-CAACG-3' native motif); (2) the $\log_2$ score of the DNA spacer length, and given scores 18 bp = 0.6; 17 bp = 1; 16 bp = 0.6; 15 bp = 0.4; 14 bp = 0; (3) the $\log_2$ score of the fit of the DNA spacer sequence to the position weight matrix (PWM) derived from the DNA spacer *in vitro* enrichment data. The predicted binding sites were subsequently ranked by their *Z* score. The gRNA portion of highly ranked TevCas9 sites were individually examined using the CRISPR Design website (5), and TevCas9 sites that included poor gRNAs were excluded. Predicted sites are available in Dataset S2.

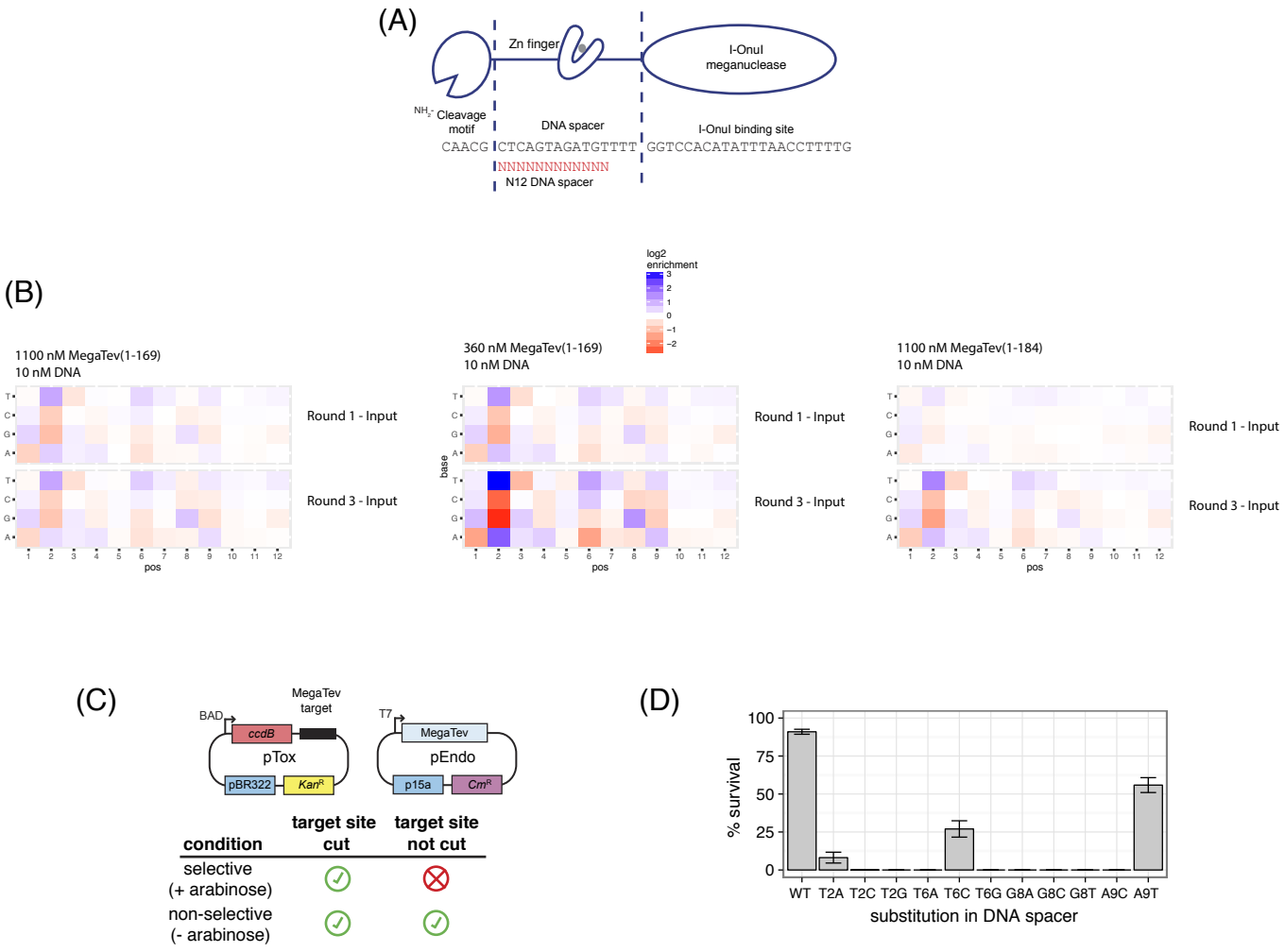
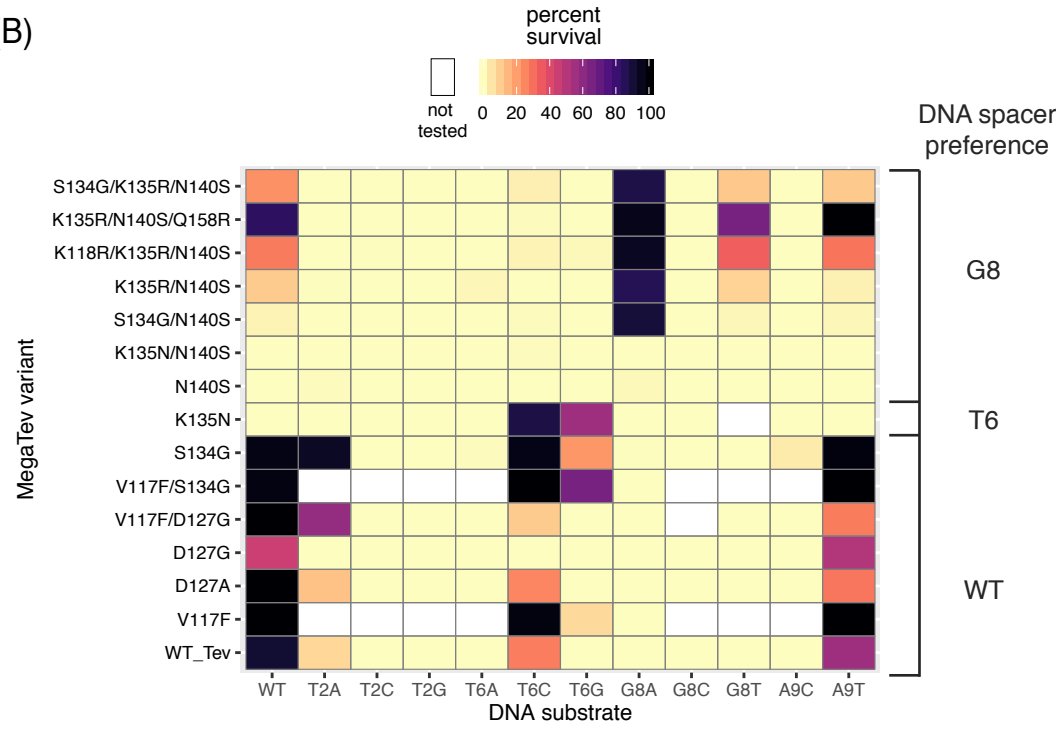


Fig. S1. (A) Schematic of MegaTev construct used to profile nucleotide preference in the DNA spacer region, with the N12 randomized region indicated. (B) *In vitro* enrichment of N12 DNA spacer library with the MegaTev(1-169) or MegaTev(1-184) constructs under different reaction conditions. Nucleotide preference in the DNA spacer region is displayed as proportional $\log_2$ enrichment of each nucleotide at each position relative to the abundance in the input pool. Black dots indicate the wild-type nucleotide in the *td* DNA spacer at each position. (C) Two-plasmid genetic selection system used to assay MegaTev activity on different DNA spacer substrates in *E. coli*. (D) Barplot of two-plasmid selection on different DNA spacer substrates. Data are at least three independent replicates with error bars representing s tandard deviation.

(A)

substrate	DNA spacer	substitutions identified
td-T6G	CTCAGGAGATGTTTT	K135N
td-G8A	CTCAGTAAATGTTTT	K135R/N140S, S134G/N140S K118R/K135R/N140S K135R/N140S/Q158R
td-A9T	CTCAGTAGTTGTTTT	S134G
MAO-B(A8G)	TTGGTCCGACATAGG	V117F/D127G

(B)

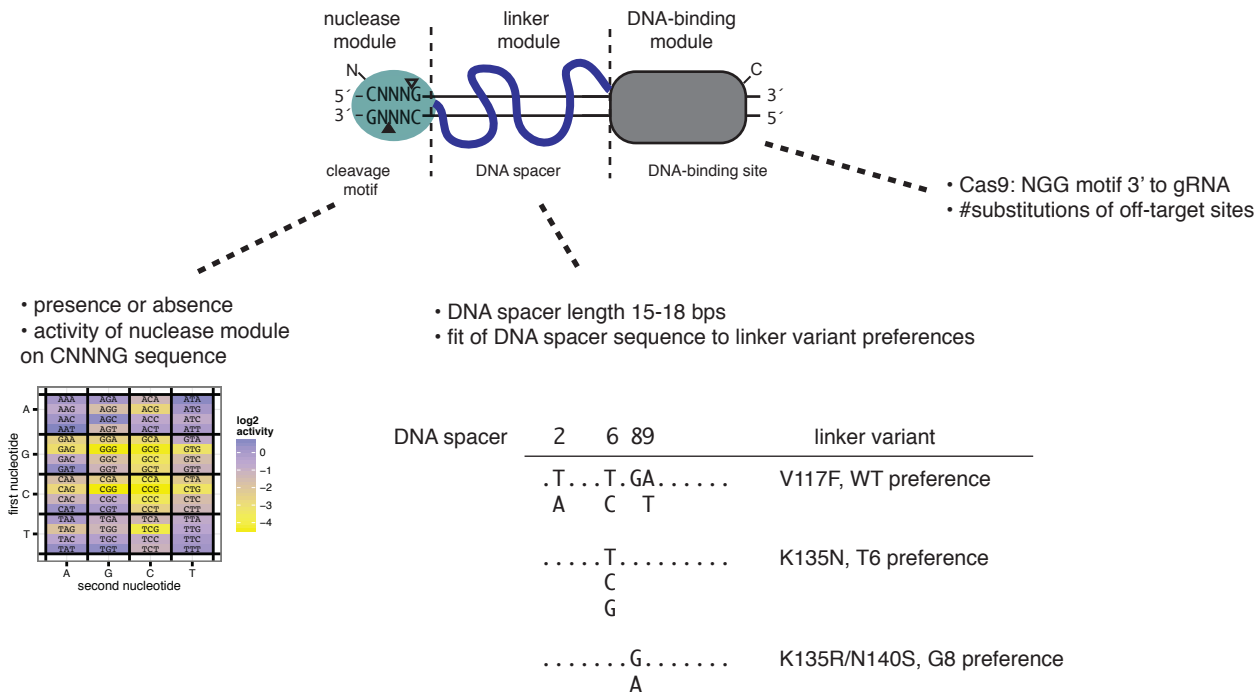


(C)

position in DNA spacer	2	6	8	9	linker variant
	.	T	G	A	V117F, WT preference
	A	C		T	
	.	T	.	.	K135N, T6 preference
		C			
		G			
	.	G	.	.	K135R/N140S, G8 preference
		A			

Fig. S2. (A) Summary of directed evolution experiments to isolate I-TevI linker variants in the MegaTev backbone. For the three *td* substrates, nucleotide substitutions relative to the wild-type sequence are in red type font. Positions 2, 6, 8, and 9 of the DNA spacer that are important for I-TevI activity are highlighted by grey rectangles. (B) De-convolution of identified amino acid substitutions on MegaTev survival against singly substituted DNA spacer substrates in the bacterial two-plasmid survival assay. Mean values from three independent biological replicates are reported in the heat map, with white boxes representing linker variant-substrate combinations that were not tested. (C) Summary of I-TevI linker variant nucleotide preferences in the DNA spacer.

(A)



DNA spacer 2 6 89	linker variant
.T...T.GA..... / A C T	V117F, WT preference
.....T..... / C / G	K135N, T6 preference
.......G..... / A	K135R/N140S, G8 preference

(B)

gene	rawscore	pos	strand	spacer_len	cnnng	cnnng_activity	spacer	crispr	PAM	z-score
TSC1	3.91	3179	R	17	CTGTG	1.29	CTAGGCTGAGTAGTTGG	GACCACGCCCTGCCACAGGC	TGG	2.6
TSC1	3.76	7467	R	17	CATAG	1.39	CTATTCTGTGTGTCAGC	ATAAGGGCTGGTGGTGACAT	CGG	2.5
TSC1	3.54	3179	R	18	CTGTG	1.29	CTAGGCTGAGTAGTTGGG	ACCACGCCCTGCCACAGGCT	GGG	2.4
TSC1	3.3	5074	F	18	CTGTG	1.29	GTCTGGTGTTCCAGCTGA	GGCAGGGGGATTTGGTAGGA	AGG	2.3
TSC1	3.21	19	F	15	CAACG	1	CTCAGTAGATGTTTT	GGCGGCGCCTGTAGGCTGGA	GGG	2.2
TSC1	3.14	3805	F	17	CAAAG	0.96	CTTTCCTGTTTCCAGGG	TCTGAGTGCAAGTTCATGTG	TGG	2.2
TSC1	2.8	6733	R	17	CTTTG	1.04	GTGTGTCAGGCCCAAGC	TTGTCCAGGGAGGAGTGTAA	AGG	2.0
TSC1	2.76	2145	F	17	CAGAG	0.96	GAAGATGGTGTGCCCTC	TACCTCCCCAATGGAAGTGC	TGG	2.0
TSC1	2.49	6804	F	17	CTGCG	0.76	GAATCTTCTTACTAAGC	TTGAAGAGTGGAGAGGCGAG	AGG	1.9
TSC1	2.33	3866	F	14	CTTTG	1.04	GACAGCTGACTGAA	TGCAGAACGGTTTTTGGATC	TGG	1.8
TSC1	2.21	19	F	14	CAACG	1	CTCAGTAGATGTTT	TGGCGGCGCCTGTAGGCTGG	AGG	1.8
TSC1	2.18	4907	F	17	CAGAG	0.96	CAGTCTAAACAGAGTCC	GGGAGAATGCTGGCAAAGGC	TGG	1.8

(C)

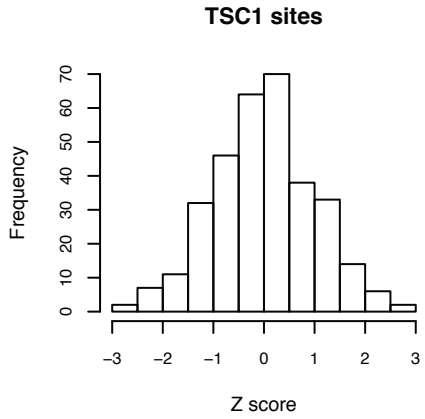
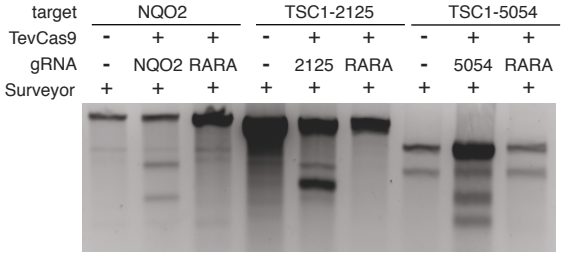
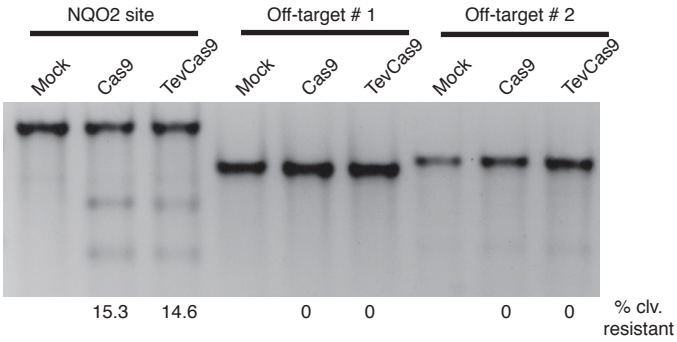


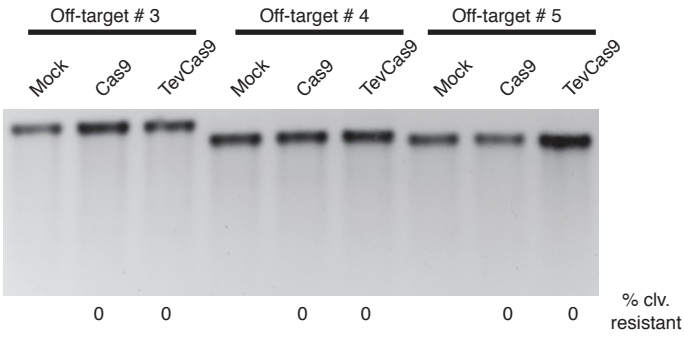
Fig. S3. Overview of TevCas9 binding model. (A) Schematic of TevCas9, with the individual components of the binding model highlighted for each domain. The heatmap of relative cleavage CNNNG activity is derived from supplemental reference 4. (B) Example of TevCas9 site predictions in the human TSC1 coding region ranked by Z-score. (C) Histogram of predicted sites binned according to the Z-score for the TSC1 gene.

(A)



(B)





(C)

Site	25bp upstream sequence	gRNA sequence	Score	Mismatches (MMs)	CNNNG Motif
NQO2	tctttcaacggatccttgaagaatg	TGGCTGTAGATGAACTGAGCAGG	—	No MMs	Yes
Off-target # 1	gtttgggaagcacctcagcaggaca	GGGCTGTAGAGGAACTGAGGTGG	0.9	3MMs [1:11:20]	No
Off-target # 2	tcatctacgtgcatccttgaagaac	TGGGTGTGGGTGAACTGAGCAGG	2.3	3MMs [4:8:10]	No
Off-target # 3	ctggacgaggtggcgggccttgggg	AGGCGGTGGATGAACTGAACCAG	0.5	4MMs [1:5:8:19]	Yes
Off-target # 4	acaggttcatctcgtctctgctgct	TGGCTAAAGATGAACTGACCTGG	0.4	3MMs [6:7:19]	No
Off-target # 5	cgggatctggggttcaccatcccccc	TGGCTTTCAAGGAACTGAGCTGG	0.3	4MMs [6:8:9:11]	Yes

Fig. S4. (A) I-TevI nuclease and linker domains do not affect gRNA-mediated targeting. Show is a representative gel of T7E1 mismatch cleavage assays on the indicated target sites from HEK 293 cells transfected with different gRNAs. (B) Gel images of T7E1 mismatch cleavage assays on off-target sites predicted for the NQO2.54 gRNA. (C) Off-target predictions for the NQO2.54 gRNA from the CRISPR Design website (http:// http://crispr.mit.edu/).

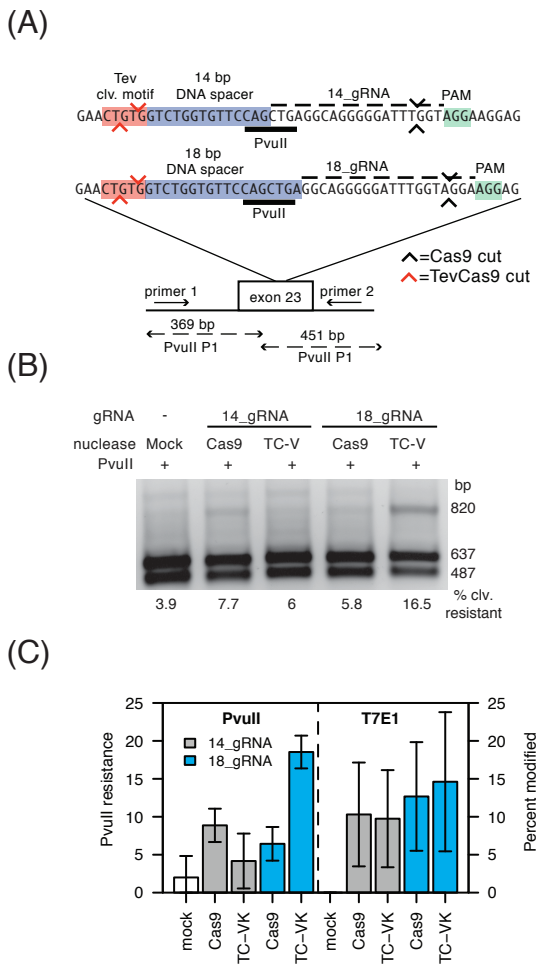
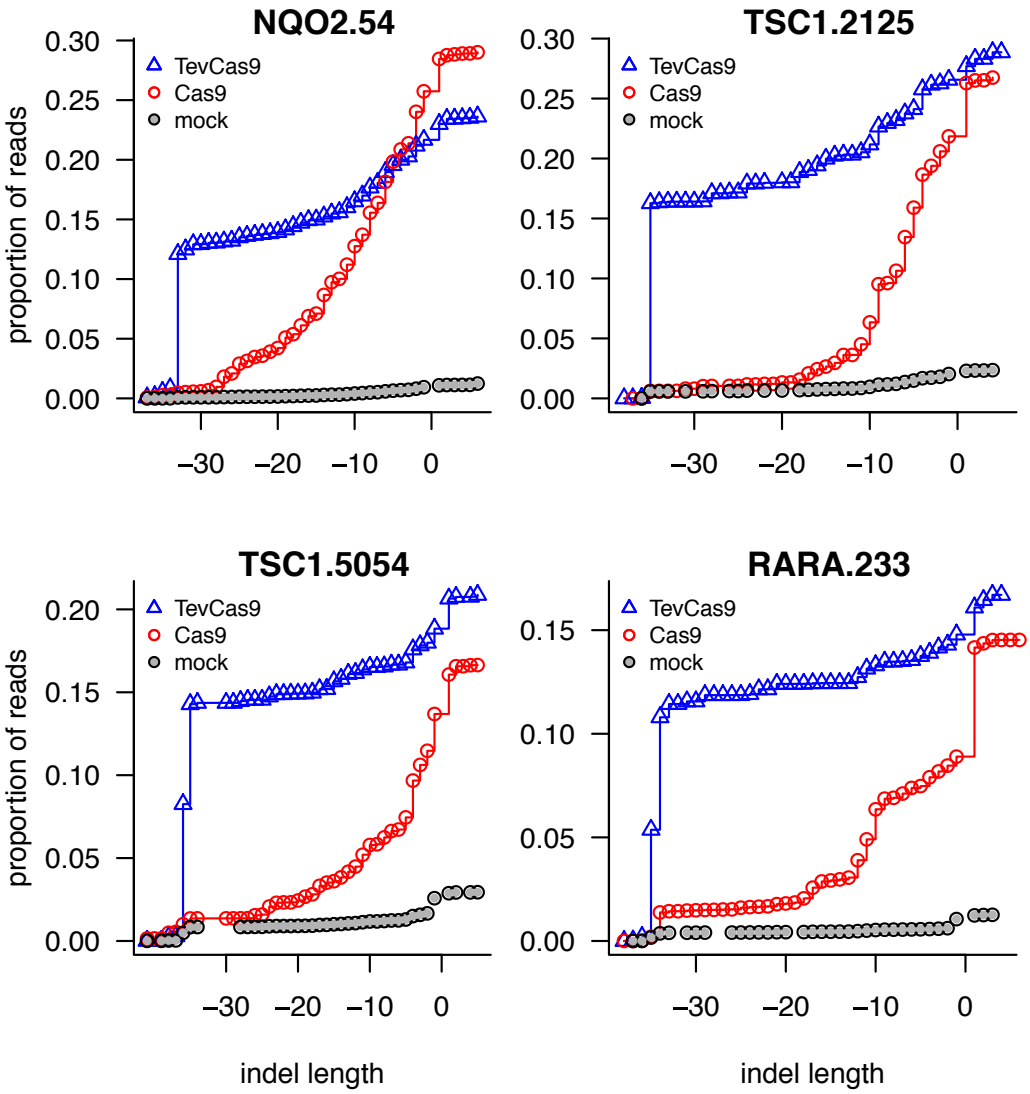


Fig. S5. TevCas9 activity at the TSC1.5054 site. (A) Schematic of the two TevCas9 target sites at nucleotide 5054 of the TSC1 gene that differ in the position of the PAM and the length of the DNA spacer (14_gRNA and 18_gRNA). (E) Representative agarose gel of PvuII cleavage assays. Sizes of the substrate (820 bp) and two PvuII cleavage resistant products (369 bp and 451 bp) are indicated. (C) Activity of TevCas9 or Cas9 programmed with the 14_gRNA and 18_gRNA at the TSC1-5054 site, measured by PvuII cleavage resistance (left) or T7E1 mismatch cleavage assay (right). Data are represented as barplots of mean values from three independent transfections with error bars indicating standard deviation.

(A)



(B)

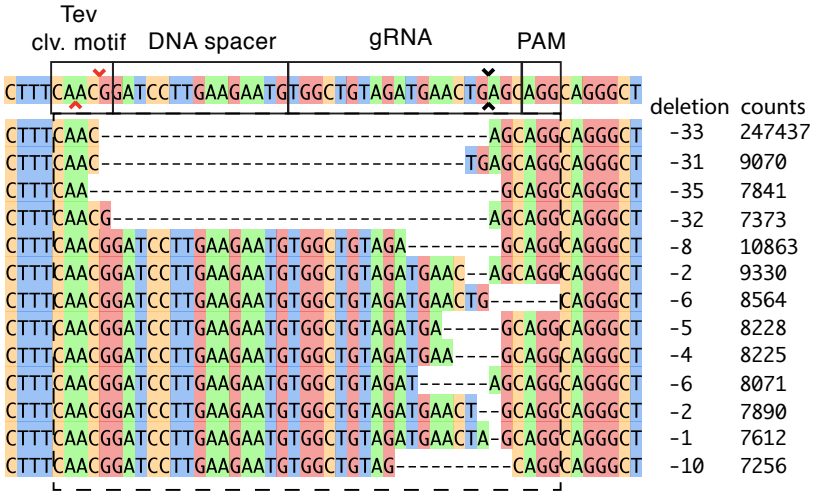


Fig. S6. (A) Cumulative proportion of Illumina reads of indicated length for TevCas9 and Cas9 at the indicated target sites in HEK 293. (B) Examples of deletion products at the NQO2.54 target site from Illumina read data. The dashed rectangle indicates the TevCas9 target site.

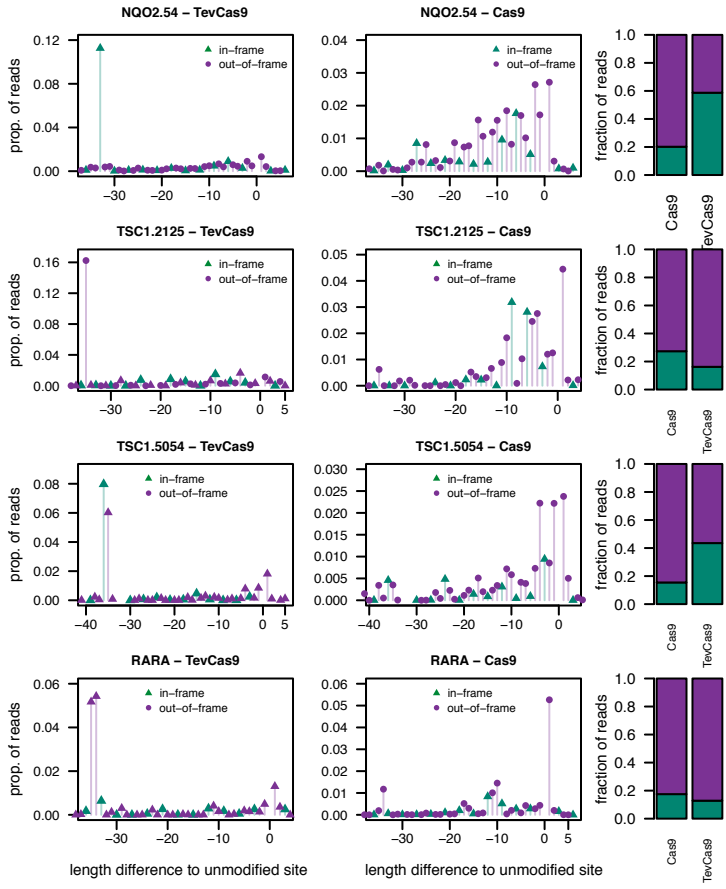


Fig. S7. Proportion of TevCas9 and Cas9 reads that are in-frame or out-of-frame with the surrounding exonic sequences for the indicated gene and target site

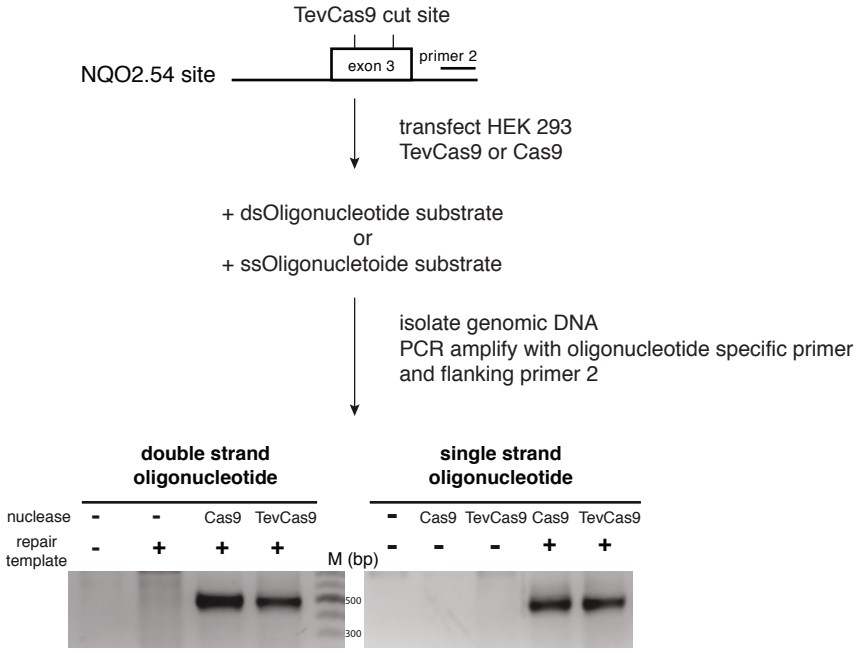


Fig. S8. TevCas9 can promote homology-directed repair with a single-stranded oligonucleotide or insertion of double-stranded oligonucleotide at the NQO2.54 site. Shown is a schematic of the experimental workflow, and agarose gel of PCR reactions to detect integration in HEK 293 cells.

Table S1. Strains used in this study.

Strains	Description	Source
DH5α	F$^-$, φ80d*lacZ*ΔM15, Δ(*lacZYA-argF*)U169, *deoR*, *recA*1, *endA*1, *hsdR*17(rk$^-$, mk$^+$), *phoA*, *supE*44, λ$^-$, *thi*-1, *gyrA*96, *relA*1	Invitrogen
ER2566	F- λ- fhuA2 [lon] ompT lacZ::T7 gene 1 gal sulA11 Δ(mcrC-mrr)114::IS10 R(mcr-73::miniTn10-TetS)2 R(zgb-210::Tn10)(TetS) endA1 [dcm]	N.E.B.
BW25141 (λDE3)	F$^-$ *lacI*^{q} *rrnB*$_{T14}$ D*lacZ*$_{WJ16}$ D*phoBR580 hsdR514* D*araBAD*$_{AH33}$ D*rhaBAD*$_{LD78}$ *galU95 endA*$_{BT333}$ *uidA*(D*Mlu*I)::*pir*+ *recA*1, λDE3 lysogen	
***S.cerevisiae* - YPH499**	MATa ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1	Dr. Adam Bogdanove
***S.cerevisiae* - YPH500**	MATα ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1	Dr. Adam Bogdanove
HEK 293	Human Embryonic kidney 293 cells	Dr. Schild-Poulter

Table S2: Oligonucleotides used in this study

Name	Sequence (5'-3')	Notes
DE-1180	CGCATGCGGCGCNNCAAAAGGTTA AATATGTGGACCNNNNNNNNNNNNN NNCGTTGGCCGAATTCCG	Bottom strand for MegaTev TO15 N15 randomized spacer target site
DE-1181	CGGAATTCGGCCAAC	Forward primer to make DE1180 double-stranded DNA for cloning
DE-1064	CTAGACAACGCTCAGTAGATGTTTT GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 (15-bp spacer) target site
DE-1065	CAAAAGGTTAAATATGTGGACCAAA ACATCTACTGAGCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 (15-bp spacer) target site
DE-1412	CTAGACAACGCTCAGTACATGTTTT GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 G8C target site
DE-1413	CAAAAGGTTAAATATGTGGACCAAA ACATGTACTGAGCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 G8C target site
DE-1414	CTAGACAACGCTCAGTAAATGTTTT GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 G8A target site
DE-1415	CAAAAGGTTAAATATGTGGACCAAA ACATTTACTGAGCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 G8A target site
DE-1416	CTAGACAACGCTCAGTATATGTTTTG GTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 G8T target site
DE-1417	CAAAAGGTTAAATATGTGGACCAAA ACATATACTGAGCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 G8T target site
DE-1418	CTAGACAACGCTCAGTAGTTGTTTT GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 A9T target site

DE-1419	CAAAAGGTTAAATATGTGGACCAAA ACAACTACTGAGCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 A9T target site
DE-1420	CTAGACAACGCTCAGTAGGTGTTTT GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 A9G target site
DE-1421	CAAAAGGTTAAATATGTGGACCAAA ACACCTACTGAGCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 A9G target site
DE-1422	CTAGACAACGCTCAGTAGCTGTTTT GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 A9C target site
DE-1423	CAAAAGGTTAAATATGTGGACCAAA ACAGCTACTGAGCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 A9C target site
DE-1209	CTAGACAACGCCCAGTAGACATAGG GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 T2C (includes 10-15 bp spacer swapped for MAO-B sequence) target site
DE-1210	CAAAAGGTTAAATATGTGGACCCCT ATGTCTACTGGGCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 T2C (includes 10-15 bp spacer swapped for MAO-B sequence) target site
DE-1497	CTAGACAACGCGCAGTAGATGTTTT GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 T2G target site
DE-1498	CAAAAGGTTAAATATGTGGACCAAA ACATCTACTGCGCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 T2G target site
DE-1499	CTAGACAACGCACAGTAGATGTTTT GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 T2A target site
DE-1500	CAAAAGGTTAAATATGTGGACCAAA ACATCTACTGTGCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 T2A target site
DE-1501	CTAGACAACGATCAGTAGATGTTTT GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 C1A target site
DE-1502	CAAAAGGTTAAATATGTGGACCAAA ACATCTACTGATCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 C1A target site
DE-1503	CTAGACAACGGTCAGTAGATGTTTT GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 C1G target site
DE-1504	CAAAAGGTTAAATATGTGGACCAAA ACATCTACTGACCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 C1G target site
DE-1505	CTAGACAACGTTCAGTAGATGTTTT GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 C1T target site
DE-1506	CAAAAGGTTAAATATGTGGACCAAA ACATCTACTGAACGTTGT	Bottom strand oligonucleotide for TevOnu TO15 C1T target site
DE-1958	CTAGACAACGCTCAGCAGATGTTTT GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 T6C target site
DE-1959	CAAAAGGTTAAATATGTGGACCAAA ACATCTGCTGAGCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 T6C target site
DE-1960	CTAGACAACGCTCAGGAGATGTTTT GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 T6G target site
DE-1961	CAAAAGGTTAAATATGTGGACCAAA ACATCTCCTGAGCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 T6G target site
DE-1962	CTAGACAACGCTCAGAAGATGTTTT GGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15 T6A target site
DE-1963	CAAAAGGTTAAATATGTGGACCAAA ACATCTTCTGAGCGTTGT	Bottom strand oligonucleotide for TevOnu TO15 T6A target site

DE-2142	CTAGACAGAGGTGGGCAATGGCGTGGGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15-PKD1 2386 target site
DE-2143	CAAAAGGTTAAATATGTGGACCCACGCCATTGCCCACCTCTGT	Bottom strand oligonucleotide for TevOnu TO15-PKD1 2386 target site
DE-2144	CTAGACTTTGGAAGGTAATTACAGTGGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15-HPRT 5740 target site
DE-2145	CCAAAAGGTTAAATATGTGGACCACTGTAATTACCTTCCAAAGT	Bottom strand oligonucleotide for TevOnu TO15- HPRT 5740 target site
DE-2146	CTAGACTGTGCTCAGGTGATCCTCCGGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15-Ku70 14915 target site
DE-2147	CAAAAGGTTAAATATGTGGACCGGAGGATCACCTGAGCACAGT	Bottom strand oligonucleotide for TevOnu TO15- Ku70 14915 target site
DE-2148	CTAGACTAAGGTGGTCCTTCCCAGAGGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15- XPC 1277 target site
DE-2149	CAAAAGGTTAAATATGTGGACCTCTGGGAAGGACCACCTTAGT	Bottom strand oligonucleotide for TevOnu TO15-XPC 1277 target site
DE-2206	CTAGACATTGCTGTGCTTTGGGGATGGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15-VEGFA 2522 target site
DE-2207	CAAAAGGTTAAATATGTGGACCCTTGCAATAATTCTGCAATGT	Bottom strand oligonucleotide for TevOnu TO15-VEGFA 2522 target site
DE-2208	CTAGACAGAGGAAGATGGTGTGCCCGGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15-TSC1-2125 target site
DE-2209	CAAAAGGTTAAATATGTGGACCGGGCACACCATCTTCCTCTGT	Bottom strand oligonucleotide for TevOnu TO15-TSC1-2125 target site
DE-2210	CTAGACTGTGGTCTGGTGTTCCAGCGGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15-TSC1-5054 target site
DE-2211	CAAAAGGTTAAATATGTGGACCGCTGGAACACCAGACCACAGT	Bottom strand oligonucleotide for TevOnu TO15-TSC1-5054 target site
DE-2212	CTAGACTGTGCAGCATGGAATTCCTGGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15-TSC1-6374 target site
DE-2213	CAAAAGGTTAAATATGTGGACCAGGAATTCCATGCTGCACAGT	Bottom strand oligonucleotide for TevOnu TO15-TSC1-6374 target site
DE-2216	CTAGACATTGCAGAATTATTGCAAGGGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15-APC 1440 target site
DE-2217	CAAAAGGTTAAATATGTGGACCCTTGCAATAATTCTGCAATGT	Bottom strand oligonucleotide for TevOnu TO15-APC 1440 site
DE-2218	CTAGACATTGGTACCTGGTACTGATGGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15-BRAC1-2541 target site
DE-2219	CAAAAGGTTAAATATGTGGACCATCAGTACCAGGTACCAATGT	Bottom strand oligonucleotide for TevOnu TO15-BRAC1-2541 target site
DE-2220	CTAGACAGAGGCGAATTTATTATCAGGTCCACATATTTAACCTTTTGCATG	Top strand oligonucleotide for TevOnu TO15-BRAC1-3999 target site
DE-2221	CAAAAGGTTAAATATGTGGACCTGATAATAAATTCGCCTCTGT	Bottom strand oligonucleotide for TevOnu TO15-BRAC1-3999 target site
DE-2645	CACCGTGGCTGTAGATGAACTGAGC	Top strand oligonucleotide for Cas9 human NQO2 gRNA with BbsI overhangs

DE-2646	AAACGCTCAGTTCATCTACAGCCAC	Bottom strand oligonucleotide for Cas9 human NQO2 gRNA with BbsI overhangs
DE-2701	CACCGTTAGCATCTTGGGGAACATG	Top strand oligonucleotide for Cas9 human RARA gRNA with BbsI overhangs
DE-2702	AAACCATGTTCCCCAAGATGCTAAC	Bottom strand oligonucleotide for Cas9 human RARA gRNA with BbsI overhangs
DE-2703	CACCGTACCTCCCCAATGGAAGTGC	Top strand oligonucleotide for Cas9 human TSC1-1 gRNA with BbsI overhangs
DE-2704	AAACGCACTTCCATTGGGGAGGTAC	Bottom strand oligonucleotide for Cas9 human TSC1-1 gRNA with BbsI overhangs
DE-2705	CACCGCTGAGGCAGGGGGATTTGGT	Top strand oligonucleotide for Cas9 human TSC1-2 14bp gRNA with BbsI overhangs
DE-2706	AAACACCAAATCCCCCTGCCTCAGC	Bottom strand oligonucleotide for Cas9 human TSC1-2 14bp gRNA with BbsI overhangs
DE-2707	CACCGGGCAGGGGGATTTGGTAGGA	Top strand oligonucleotide for Cas9 human TSC1-2 gRNA 18bp with BbsI overhangs
DE-2708	AAACTCCTACCAAATCCCCCTGCCC	Bottom strand oligonucleotide for Cas9 human TSC1-2 18bp gRNA with BbsI overhangs
DE-2510	GTTCAAACACACATGCTCTGC	Forward primer for 1st round amplification of NQ02 54 site human site
DE-2511	GCACTCCTGATGCTTCCTGTGGG	Reverse primer for 1st round amplification of NQ02 54 site human site
DE-2512	CAATCATCACAGGGTCCTGAGGC	Forward primer for 2nd round amplification of NQ02 54 site human site
DE-2513	GGAACCCCAGAAAATTGAGAAGC	Reverse primer for 2nd round amplification of NQ02 54 human site
DE-2359	TGAGAGCCGTTTTCAACCCT	Forward primer for 1st round amplification of TSC1 2125 human site
DE-2360	CCAGCCTTCTCTGTTCAGCA	Reverse primer for 1st round amplification of TSC1 2125 human site
DE-2361	GGGATGTCAGGCCATCTGAA	Forward primer for 2nd round amplification of TSC1 2125 human site
DE-2362	GGTGGAATACCGACTGCCAT	Reverse primer for 2nd round amplification of TSC1 2125 human site
DE-2779	ACGTCCATGGAGCTTCTAGC	Forward primer for 1st round amplification of TSC1 5054 human site
DE2780	CCCGAGAAGCACTTGAGCAT	Reverse primer for 1st round amplification of TSC1 5054 human site
DE2713	TTCAGTACCAACTGCCAGCC	Forward primer for 2nd round amplification of TSC1 5054 human site
DE-2714	ACCCTTCTGTCTACTGGCCT	Reverse primer for 2nd round amplification of TSC1 5054 human site
DE-2777	ACGTCCATGGAGCTTCTAGC	Forward primer for 1st round amplification of RARA 233 human site

DE-2778	CCCGAGAAGCACTTGAGCAT	Reverse primer for 1st round amplification of RARA 233 human site
DE-2711	TTCAGTACCAACTGCCAGCC	Forward primer for 2nd round amplification of RARA 233 human site
DE-2712	ACCCTTCTGTCTACTGGCCT	Reverse primer for 2nd round amplification of RARA 233 human site
DE-2783	TTCTCTCCCACAGCTGTCCA	Forward primer for 1st round amplification of NQ02 54 site human site
DE-2784	CACTAGGTGCAGACTCAGGC	Reverse primer for 1st round amplification of NQ02 54 site human site
DE-2719	CAAGGCCCCTCTCTCTTTCG	Forward primer for 2nd round amplification of NQ02 54 site human site
DE-2720	ATGGGTAGAAGCAGATGCCG	Reverse primer for 2nd round amplification of NQ02 54 human site
DE-2721	CCACTGCCAGATTTCTCCCC	Forward primer for 1st round amplification of NQ02 54 site human site
DE-2722	GGGCATTCATTTGTCTGCACTT	Reverse primer for 1st round amplification of NQ02 54 site human site
DE-2723	TCCTGCCAGGGAGTGATACA	Forward primer for 1st round amplification of NQ02 54 site human site
DE-2724	CCAGGGTCGCGAACTAATGA	Reverse primer for 1st round amplification of NQ02 54 site human site
DE-2785	TGCAACACCCTCTTTAATACTGA	Forward primer for 1st round amplification of NQ02 54 site human site
DE-2786	AAAACGACCTCCGGTTTGTG	Reverse primer for 1st round amplification of NQ02 54 site human site
DE-2725	AGCTGAATCCAGATGCCAGT	Forward primer for 2nd round amplification of NQ02 54 site human site
DE-2726	GTGAAACTGGGTTTGCCCCT	Reverse primer for 2nd round amplification of NQ02 54 human site
DE-2727	GCCCCATTTTCCTGATGGGA	Forward primer for 1st round amplification of NQ02 54 site human site
DE-2728	ATCCAGAGTGGTTCCATGCG	Reverse primer for 1st round amplification of NQ02 54 site human site
DE-2787	CTTGGTGCTGTTGCACTCAT	Forward primer for 1st round amplification of NQ02 54 site human site
DE-2788	GCCGCCTACTCCTCTTTTCTT	Reverse primer for 1st round amplification of NQ02 54 site human site
DE-2729	GTGCCTCCTCAATGGTGACT	Forward primer for 2nd round amplification of NQ02 54 site human site
DE-2730	GGTCAGGTGTGAGGGACTCT	Reverse primer for 2nd round amplification of NQ02 54 human site
DE-2835	ACACTCTTTCCCTACACGACGCTCT TCCGATCTNNNNccaaggccTCTATGC ACACCAGG	Forward primer to amplify NQO2 54 for illumina sequencing
DE-2836	ACACTCTTTCCCTACACGACGCTCT TCCGATCTNNNNcattaaggTCTATGCA CACCAGG	Forward primer to amplify NQO2 54 for illumina sequencing

DE-2837	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNataacgaaTCTATGCACACCAGG	Forward primer to amplify NQO2 54 for illumina sequencing
DE-2843	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTGGCTCAAGGTTCATGGC	Reverse primer to amplify NQO2 54 for illumina sequencing
DE-2844	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNagttaaccGAGTGCCCCAGTCCC	Forward primer to amplify TSC1 2125 for illumina sequencing
DE-2845	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNcaggcttaGAGTGCCCCAGTCCC	Forward primer to amplify TSC1 2125 for illumina sequencing
DE-2846	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNtggcggctGAGTGCCCCAGTCCC	Forward primer to amplify TSC1 2125 for illumina sequencing
DE-2847	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGCCAAAGACAGCCC	Reverse primer to amplify TSC1 2125 for illumina sequencing
DE-2848	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNcttcctggTTCGTACCGTGACAG	Forward primer to amplify TSC1 5054 for illumina sequencing
DE-2849	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNgtactgcgTTCGTACCGTGACAG	Forward primer to amplify TSC1 5054 for illumina sequencing
DE-2850	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNgctcattgTTCGTACCGTGACAG	Forward primer to amplify TSC1 5054 for illumina sequencing
DE-2851	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTGGCAGTGGCAGAG	Reverse primer to amplify TSC1 5054 for illumina sequencing
DE-2852	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNgacactcgCGCTGCTGGAGGCGC	Forward primer to amplify RARA 233 for illumina sequencing
DE-2853	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNatccactaCGCTGCTGGAGGCGC	Forward primer to amplify RARA 233 for illumina sequencing
DE-2854	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNttatacagCGCTGCTGGAGGCGC	Forward primer to amplify RARA 233 for illumina sequencing

DE-2855	CGGTCTCGGCATTCCTGCTGAACCG CTCTTCCGATCTGGGCCAGGTGTCG GG	Reverse primer to amplify RARA 233 for illumina sequencing

Table S3. Summary of Illumina sequencing for target sites PCR amplified from HEK 293 cells.

Target	Experiment	Reads	Mapped reads
NQO2	mock	530661	491211
NQO2	TevCas9	2842357	2582707
NQO2	Cas9	2660524	2399872
TSC1.2125	mock	365918	337397
TSC1.2125	TevCas9	1956085	1798789
TSC1.2125	Cas9	1390504	1262671
TSC1.5054	mock	535409	481287
TSC1.5054	TevCas9	1667988	1481161
TSC1.5054	Cas9	1887131	1673952
RARA	mock	398412	360461
RARA	TevCas9	1207917	1086409
RARA	Cas9	1137422	1021858

SUPPLEMENTAL REFERENCES

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