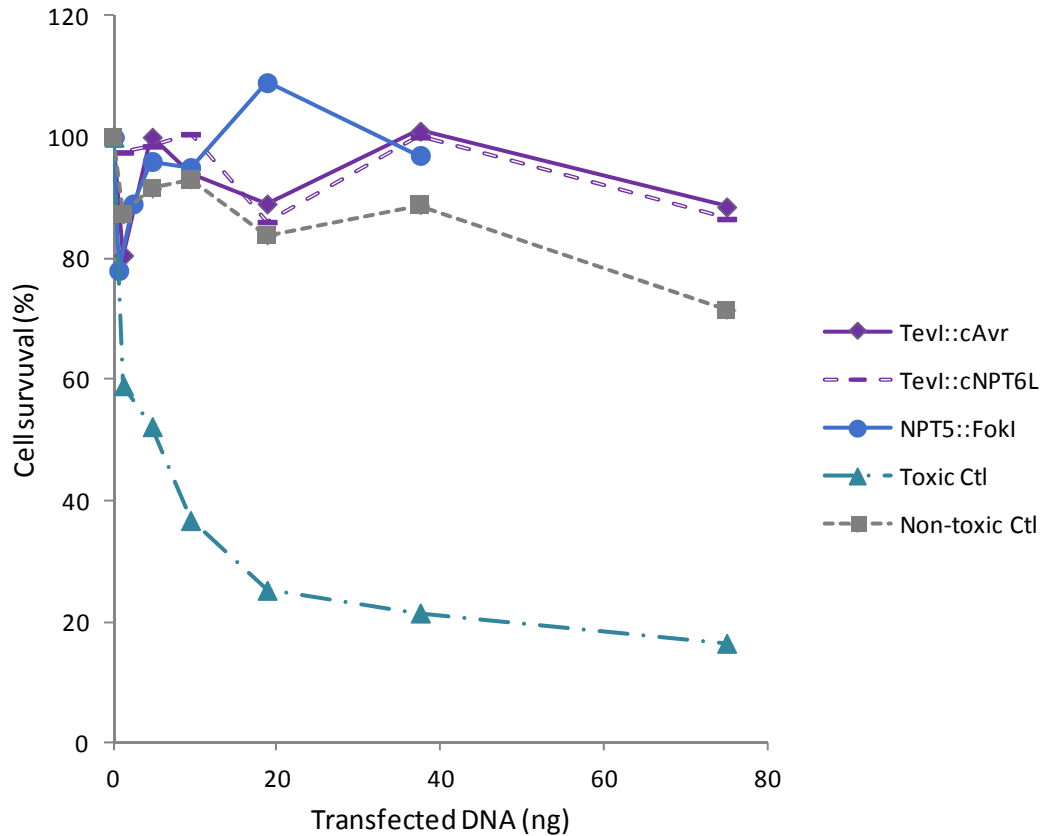
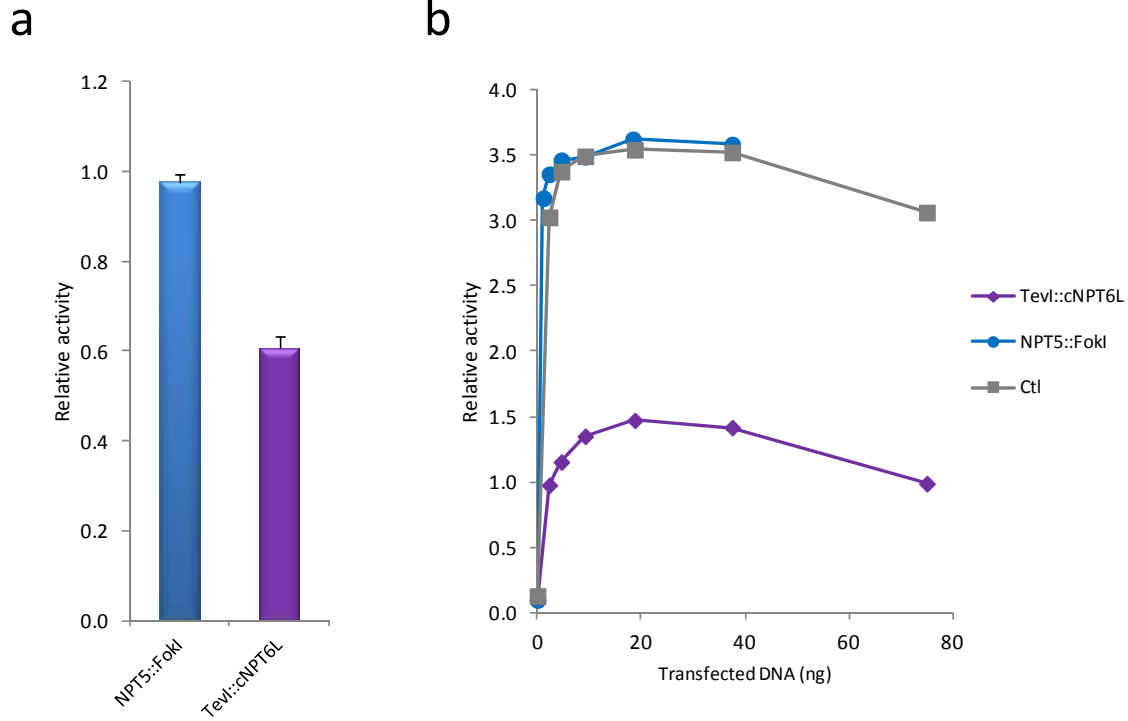


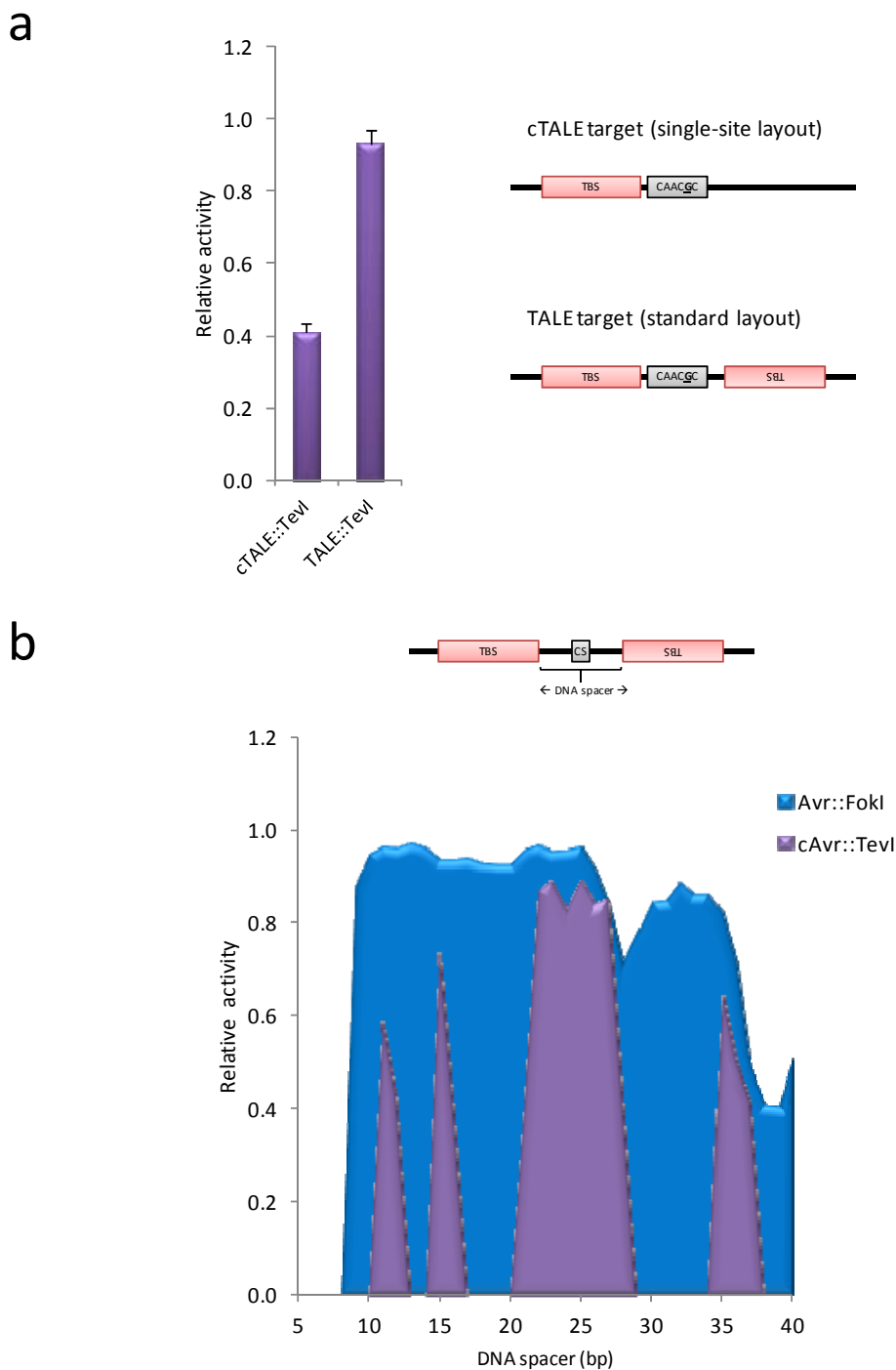
Supplementary Figures



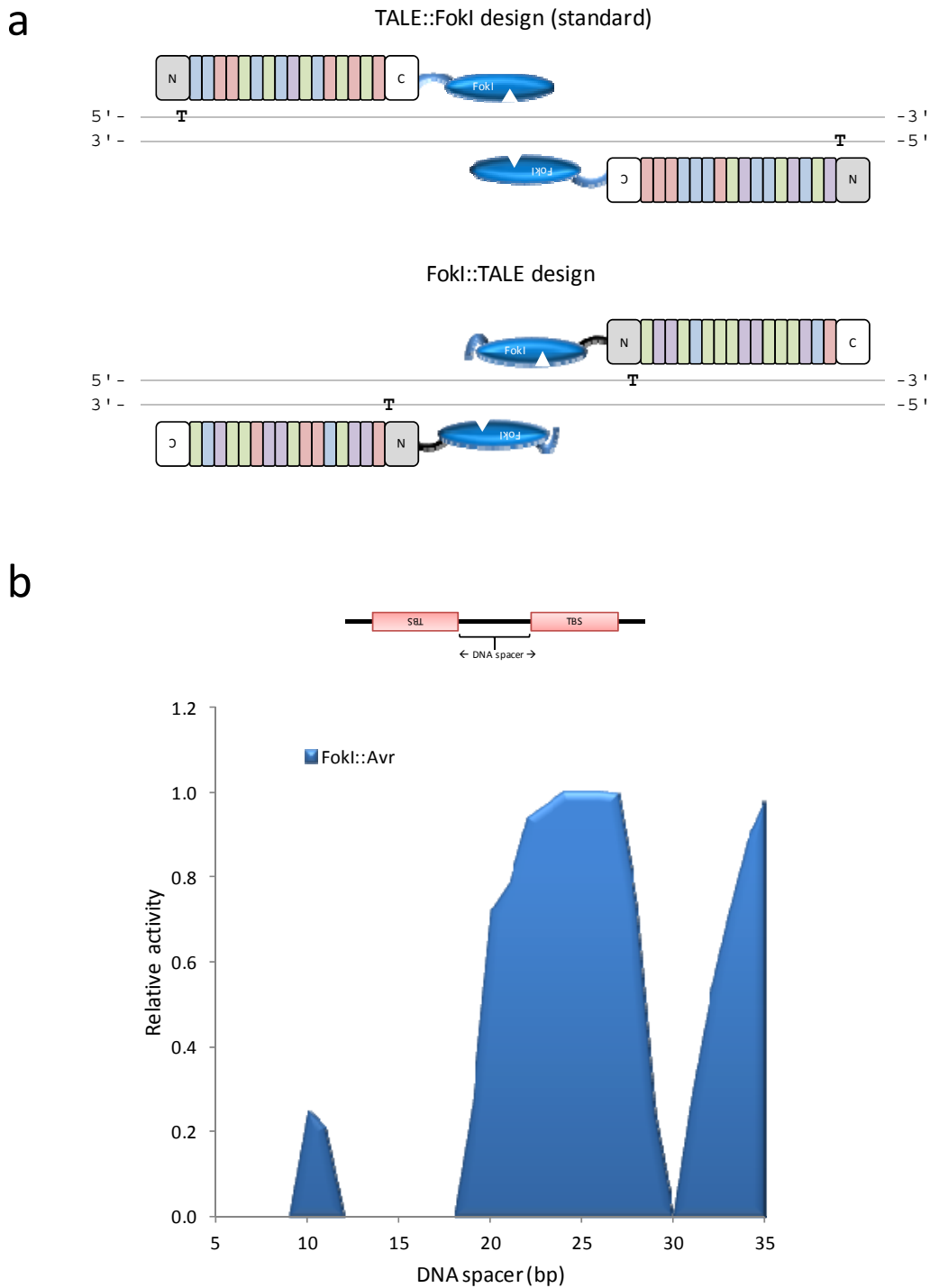
Supplementary Figure S1 | Evaluation of TevI::TALE toxicity. Various amounts (0 to 75 ng) of TALEN expression vector and a constant amount of GFP-encoding plasmid (10 ng) were used to co-transfect CHO-K1 cells (2.5×10^3 cells/well). Cell survival is expressed as the percentage of cells expressing GFP 6 days post transfection ($n = 2$). TevI::cAvr, a compact TALEN targeting the AvrBs3 TALE binding site. TevI::cNPT6L, a compact TALEN targeting a site in the neomycin phosphotransferase II (*nptII*) gene. NPT5::FokI, a standard TALEN targeting a site that overlaps with cNPT6L in the *nptII* gene. Toxic Ctl, a meganuclease known to promote cell toxicity through non-specific cleavage. Non-toxic Ctl, the sequence-specific I-SceI meganuclease.



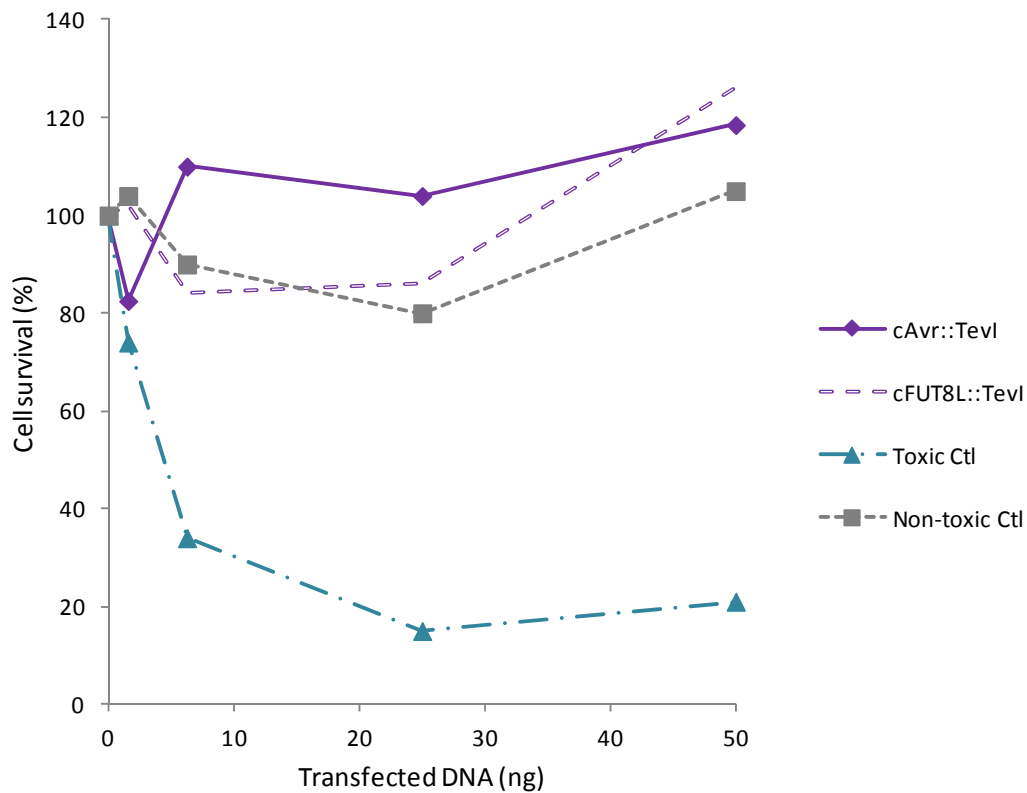
Supplementary Figure S2 | *In vivo* characterization of TevI::cNPT6L activity. (a) Yeast SSA assay comparing relative activity to a standard FokI-based TALEN (n = 3). Data are shown as the mean + s.e.m. (b) A dose-response SSA assay comparing relative activity to a standard FokI-based TALEN in CHO-K1 cells (2.5×10^3 cells/well). The NPT5 target (Fig. 1a) used in all assays (n = 2) contains the I-TevI cleavage sequence CGACGT. TevI::cNPT6L, a compact TALEN targeting a site in the neomycin phosphotransferase II (*nptII*) gene. NPT5::FokI, a standard TALEN targeting a site that overlaps with cNPT6L in the *nptII* gene. Ctl, the sequence-specific I-SceI meganuclease.



Supplementary Figure S3 | TALE::TevI activity as a standard TALEN. (a) Yeast SSA assay comparing the relative activity of monomeric (cAvr::TevI) vs. paired (TALE::TevI) versions of the TALE::TevI architecture ($n = 3$). Data are shown as the mean + s.e.m. Schematic right, layout of single-site vs. standard TALE targets. A TevI cleavage sequence (CAACGC) is positioned 3' to the TALE binding site (TBS) to match the anticipated overlying protein configuration. (b) Relative paired TALE::TevI activity as a function of DNA spacer. A series of pseudo palindromic targets (top schematic) were generated that contain two identical AvrBs3 recognition sequences juxtaposed with the 3' ends proximal (tail-to-tail) and separated by spacer DNA ranging from 5 to 40 bp. On these targets, both Avr::FokI and cAvr::TevI function as standard TALEN configurations targeting the AvrBs3 TALE binding site. Relative activity based on a yeast SSA assay at 37°C ($n = 3$).



Supplementary Figure S4 | Design and preliminary characterization of FokI::TALE. (a) Schematic illustrating the differences in protein layout and target site configuration for standard TALE::FokI vs. FokI::TALE constructs. Notably, the FokI catalytic domain in FokI::TALE is fused N-terminally, which is inverted relative to its natural context (C-terminal) within wild-type FokI and standard TALENs. (b) Relative FokI::TALE activity as a function of DNA spacer. A series of pseudo palindromic targets (top schematic) were generated that contain two identical AvrBs3 recognition sequences juxtaposed with the 5' ends proximal (head-to-head) and separated by spacer DNA ranging from 5 to 35 bp. FokI::Avr functions in a standard TALEN configuration. Relative activity based on a yeast SSA assay at 37°C (n = 3).



Supplementary Figure S5 | Evaluation of TALE::TevI toxicity. Various amounts (0 to 50 ng) of TALEN expression vector and a constant amount of GFP-encoding plasmid (10 ng) were used to co-transfect CHO-K1 cells (2.5×10^3 cells/well). Cell survival is expressed as the percentage of cells expressing GFP 6 days post transfection ($n = 2$). cAvr::TevI, a compact TALEN targeting the AvrBs3 TALE binding site. cFUT8L::TevI, a compact TALEN targeting a site in alpha1-6-fucosyltransferase (*fut8*) gene. Toxic Ctl, a meganuclease known to promote cell toxicity through non-specific cleavage. Non-toxic Ctl, a sequence-specific meganuclease.

> Avr::FokI

MVDLRTLGYSSQQQEQEKIKPKVRSSTVAQHHEALVGHGFTHAHI VALSQHPAALGTVAVKYQDMIAALPEAT
HEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKI AKRGGVTAVEAVHAWRNALTGAPLN
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LTPQQVVAIASNNGGGKQALETVQRLLPVLCQAHG
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LTPEQVVAIASNI GGKQALETVQALLPVLCQAHG
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LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHG
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LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHG
LTPQQVVAIASNNGGRP ALE SIVAQLSRPDPGSSGPNRGGVTKQLVKSELEEKKSELRHKLKYVPHEYIEL
TEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQ
ADEMQRVVEENQTRNKHINPNEWKVPSSVTEFKFLFVSGHFKGNKYKAQLTRLNHI TNCNGAVLSVEEL
LIGGEMIKAGTLTLEEVRKFNNGEINF GSSAD

> cAvr::TevI

MVDLRTLGYSSQQQEQEKIKPKVRSSTVAQHHEALVGHGFTHAHI VALSQHPAALGTVAVKYQDMIAALPEAT
HEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKI AKRGGVTAVEAVHAWRNALTGAPLN
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LTPEQVVAIASNGGGRPALESIVAQLSRPDPGSKSGIYQIKNTLNNKVYVGS AKDFEKRWKRHF KDLEKGC
HSSIKLQRSFNKHG N VFEC SILEEIPYEKDLIIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEE
IIKKRSETVKAKMLKLGPDGRKALYSKPGSKNGRWNPE THKFCKCGVRIQTSAYTCSKCRNRS GENNSFF
NHHKSQGPSAD

> Fokl::Avr

MASGPNRGVTKQLVKSELEEKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKH
LGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRVVEENQTRNKHINPNEWKVPSSV
TEFKFLFVSGHFKGNYKAQLTRLNHI TNCNGAVLSVEEL LIGGEMIKAGTTLTEE VRRKFNNGEINFGSS
GVDLRTLGY SQQQE KIKPKV RSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPEAT
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HSSIKLQRSFNKHG N VFEC SILEEIPYEKDLIIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEE
IIKKRSETVKAKMLKLGPDGRKALYSKPGSKNGRWNPE THKFCKCGVRIQTSAYTCSKCRNRS GENNSFF
NHHKSQGPSAD

> Tevl::cAvr

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IIKKRSETVKAKMLKLGPDGRKALYSKPGSKNGRWNPE THKFCKCGVRIQTSAYTCSKCRNRS GENNSFFNHHKSQGPSGVDLRTLGY SQQQE KIKPKV RSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPEAT
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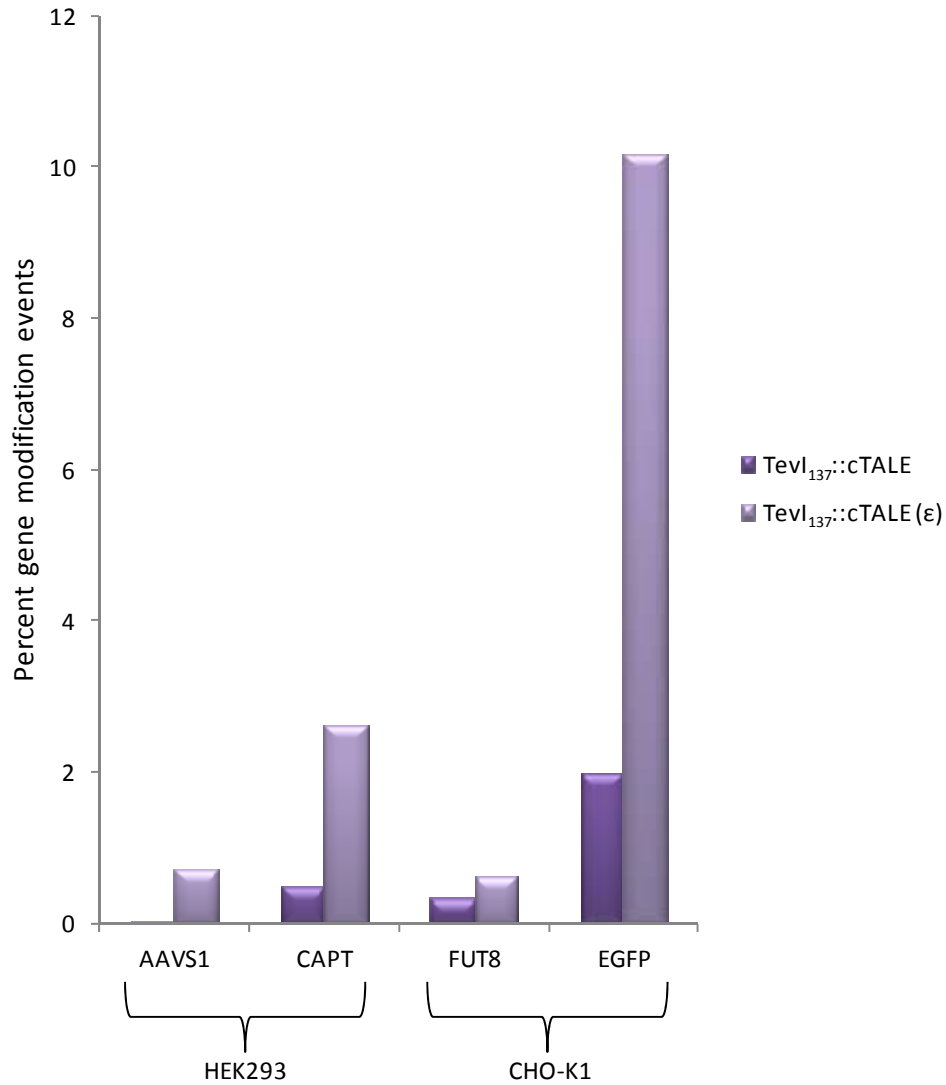
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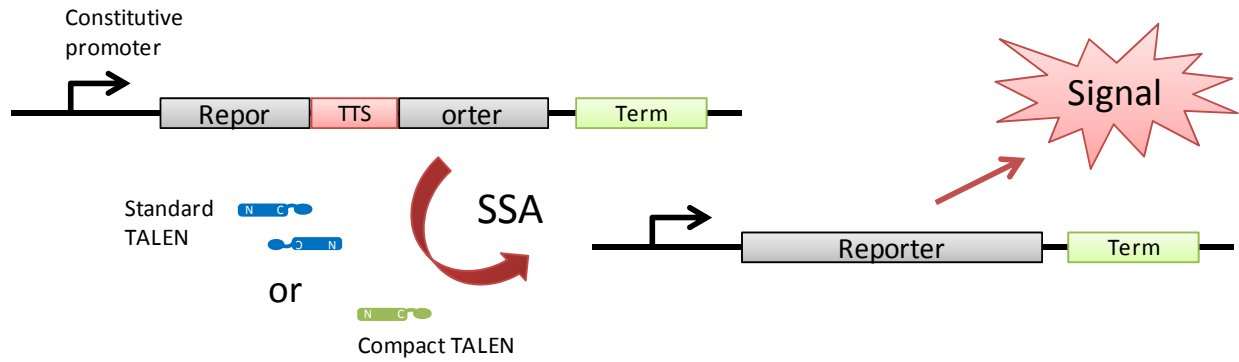
> Tevl₁₃₇::cAvr

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 EAIIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKI AKRGGVTAVEAVHAWRNAL TGAPLN
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Supplementary Figure S6 | Sequences of TALEs used in this study. Representative amino acid sequences for constructs that target the AvrBs3 TALE binding site. Differences in additional constructs arise solely from the number and RVD identities used to redirect the TALE DNA binding domain. Avr::FokI, standard TALEN half-construct. cAvr::Tevl, compact TALEN with Tevl fused C-terminally. FokI::Avr, TALEN half-construct with FokI fused N-terminally. Tevl::cAvr, compact TALEN with first 183 a.a. of I-Tevl fused N-terminally. Tevl₁₃₇::cAvr, compact TALEN with first 137 a.a. of I-Tevl fused N-terminally. Sequences are highlighted to indicate the TALE N-terminus (gray), TALE repeats (block format, RVD in yellow), TALE C-terminus (green), linkers (magenta) and catalytic domain (red).



Supplementary Figure S7 | *In vivo* characterization of Tev₁₃₇-based cTALEs. Targeted gene disruption at endogenous loci in HEK293 or CHO-K1 cells in the absence (Tev₁₃₇::cTALE) or presence (Tev₁₃₇::cTALE (ε)) of enhancer reagent TREX2. Appropriate cTALEN constructs were created to target endogenous regions as noted: AAVS1, adeno-associated virus integration site 1; CAPT, calpain small subunit 1; FUT8, alpha1-6-fucosyltransferase; EGFP, artificially integrated enhanced green fluorescent protein sequence expressed under the control of a CMV promoter. Gene modification events determined by amplicon sequencing of the target site. Negative controls consisting of transfection with empty vector or the TREX2 plasmid alone resulted in no detectable events.



Supplementary Figure S8 | Overview of the *in vivo* single-strand annealing (SSA) assay. Episomal plasmid constructs containing a reporter gene, which is interrupted by a TALE target site (TTS) flanked by sequence repeats, were generated for yeast, plant and mammalian systems. Cleavage at the TTS sequence stimulates repair via SSA to create an intact gene with a detectable signal. A high-throughput yeast-based SSA assay (LacZ reporter) was adapted and used for initial screening and characterizations. Analogous systems in plant protoplasts (YFP reporter) and mammalian cells (LacZ reporter) were also developed. Where possible, a standard FokI-based TALEN targeting an identical or overlapping TTS was included to monitor comparative baseline activity.

Supplementary Tables

Supplementary Table S1 | Relative activity* of Tevl::TALE constructs on the 256 CNNNGN targets†

#	CNNNGN	Standard (37°C)		Stringent (30°C)	
		Tevl::cAvr	Tevl ₁₃₇ ::cAvr	Tevl::cAvr	Tevl ₁₃₇ ::cAvr
1	CAAAGA	0.53	0.68	0.00	0.16
2	CAAAGC	0.70	0.77	0.38	0.45
3	CAAAGG	0.90	0.93	0.59	0.70
4	CAAAGT	0.41	0.65	0.18	0.20
5	CAACGA	0.54	0.68	0.00	0.18
6	CAACGC	0.85	0.91	0.42	0.53
7	CAACGG	0.96	0.94	0.63	0.70
8	CAACGT	0.81	0.92	0.34	0.44
9	CAAGGA	0.36	0.42	0.00	0.00
10	CAAGGC	0.44	0.52	0.00	0.00
11	CAAGGG	0.80	0.85	0.41	0.44
12	CAAGGT	0.31	0.38	0.00	0.00
13	CAATGA	0.70	0.80	0.34	0.40
14	CAATGC	0.85	0.90	0.48	0.63
15	CAATGG	0.87	0.89	0.58	0.69
16	CAATGT	0.78	0.90	0.40	0.58
17	CACAGA	0.17	0.00	0.00	0.00
18	CACAGC	0.62	0.62	0.32	0.35
19	CACAGG	0.90	0.93	0.49	0.60
20	CACAGT	0.43	0.64	0.00	0.16
21	CACCGA	0.40	0.56	0.00	0.00
22	CACCGC	0.62	0.73	0.14	0.34
23	CACCGG	0.88	0.91	0.41	0.50
24	CACCGT	0.53	0.70	0.00	0.16
25	CACGGA	0.34	0.37	0.00	0.00
26	CACGGC	0.19	0.20	0.00	0.00
27	CACGGG	0.73	0.76	0.34	0.36
28	CACGGT	0.37	0.42	0.00	0.00
29	CACTGA	0.37	0.48	0.00	0.00
30	CACTGC	0.53	0.67	0.15	0.17
31	CACTGG	0.82	0.88	0.36	0.41
32	CACTGT	0.51	0.70	0.00	0.33
33	CAGAGA	0.60	0.71	0.16	0.17
34	CAGAGC	0.76	0.83	0.34	0.43
35	CAGAGG	0.92	0.93	0.57	0.65
36	CAGAGT	0.58	0.74	0.15	0.34

37	CAGCGA	0.63	0.76	0.16	0.35
38	CAGCGC	0.85	0.88	0.38	0.50
39	CAGCGG	0.95	0.95	0.57	0.67
40	CAGCGT	0.51	0.77	0.00	0.16
41	CAGGGA	0.00	0.17	0.00	0.00
42	CAGGGC	0.37	0.47	0.00	0.00
43	CAGGGG	0.72	0.78	0.33	0.39
44	CAGGGT	0.33	0.37	0.00	0.00
45	CAGTGA	0.64	0.79	0.30	0.36
46	CAGTGC	0.65	0.79	0.38	0.55
47	CAGTGG	0.95	0.95	0.65	0.71
48	CAGTGT	0.73	0.88	0.33	0.45
49	CATAGA	0.73	0.83	0.33	0.39
50	CATAGC	0.85	0.91	0.41	0.50
51	CATAGG	0.98	0.97	0.68	0.74
52	CATAGT	0.75	0.90	0.32	0.45
53	CATCGA	0.56	0.68	0.14	0.33
54	CATCGC	0.73	0.80	0.33	0.40
55	CATCGG	0.91	0.92	0.52	0.61
56	CATCGT	0.52	0.82	0.00	0.35
57	CATGGA	0.44	0.51	0.00	0.17
58	CATGGC	0.56	0.63	0.16	0.17
59	CATGGG	0.89	0.90	0.48	0.56
60	CATGGT	0.43	0.54	0.00	0.00
61	CATTGA	0.59	0.75	0.16	0.18
62	CATTGC	0.78	0.85	0.35	0.41
63	CATTGG	0.86	0.89	0.49	0.61
64	CATTGT	0.67	0.81	0.30	0.60
65	CCAAGA	0.31	0.32	0.00	0.00
66	CCAAGC	0.39	0.43	0.00	0.00
67	CCAAGG	0.82	0.89	0.37	0.44
68	CCAAGT	0.33	0.37	0.00	0.00
69	CCACGA	0.41	0.55	0.00	0.00
70	CCACGC	0.63	0.73	0.16	0.35
71	CCACGG	0.93	0.96	0.51	0.67
72	CCACGT	0.57	0.78	0.16	0.17
73	CCAGGA	0.00	0.00	0.00	0.00
74	CCAGGC	0.00	0.00	0.00	0.00
75	CCAGGG	0.44	0.59	0.13	0.16
76	CCAGGT	0.00	0.00	0.00	0.00
77	CCATGA	0.37	0.44	0.00	0.00
78	CCATGC	0.45	0.60	0.00	0.16
79	CCATGG	0.87	0.93	0.40	0.52

80	CCATGT	0.46	0.70	0.00	0.31
81	CCCAGA	0.00	0.00	0.00	0.00
82	CCCAGC	0.00	0.00	0.00	0.00
83	CCCAGG	0.51	0.64	0.16	0.32
84	CCCAGT	0.00	0.00	0.00	0.00
85	CCCCGA	0.00	0.00	0.00	0.00
86	CCCCGC	0.00	0.00	0.00	0.00
87	CCCCGG	0.52	0.62	0.16	0.16
88	CCCCGT	0.00	0.00	0.00	0.00
89	CCCGGA	0.00	0.00	0.00	0.00
90	CCCGGC	0.00	0.00	0.00	0.00
91	CCCGGG	0.00	0.35	0.00	0.00
92	CCCGGT	0.00	0.00	0.00	0.00
93	CCCTGA	0.00	0.00	0.00	0.00
94	CCCTGC	0.00	0.00	0.00	0.00
95	CCCTGG	0.46	0.62	0.00	0.00
96	CCCTGT	0.00	0.00	0.00	0.00
97	CCGAGA	0.00	0.00	0.00	0.00
98	CCGAGC	0.33	0.34	0.00	0.00
99	CCGAGG	0.62	0.73	0.28	0.32
100	CCGAGT	0.00	0.00	0.00	0.00
101	CCGCGA	0.00	0.35	0.00	0.00
102	CCGCGC	0.38	0.49	0.00	0.00
103	CCGCGG	0.73	0.84	0.31	0.35
104	CCGCGT	0.34	0.46	0.00	0.00
105	CCGGGA	0.00	0.00	0.00	0.00
106	CCGGGC	0.00	0.00	0.00	0.00
107	CCGGGG	0.36	0.38	0.00	0.00
108	CCGGGT	0.00	0.00	0.00	0.00
109	CCGTGA	0.31	0.34	0.00	0.00
110	CCGTGC	0.36	0.43	0.00	0.00
111	CCGTGG	0.75	0.84	0.31	0.37
112	CCGTGT	0.33	0.44	0.00	0.00
113	CCTAGA	0.00	0.18	0.00	0.00
114	CCTAGC	0.42	0.46	0.00	0.00
115	CCTAGG	0.79	0.87	0.32	0.37
116	CCTAGT	0.17	0.41	0.00	0.00
117	CCTCGA	0.31	0.36	0.00	0.00
118	CCTCGC	0.37	0.48	0.00	0.00
119	CCTCGG	0.74	0.83	0.31	0.38
120	CCTCGT	0.36	0.52	0.00	0.00
121	CCTGGA	0.00	0.00	0.00	0.00
122	CCTGGC	0.00	0.00	0.00	0.00

123	CCTGGG	0.48	0.60	0.00	0.00
124	CCTGGT	0.00	0.00	0.00	0.00
125	CCTTGA	0.18	0.37	0.00	0.00
126	CCTTGC	0.37	0.47	0.00	0.00
127	CCTTGG	0.73	0.86	0.31	0.36
128	CCTTGT	0.34	0.49	0.00	0.00
129	CGAAGA	0.32	0.35	0.00	0.00
130	CGAAGC	0.36	0.43	0.00	0.00
131	CGAAGG	0.78	0.85	0.34	0.42
132	CGAAGT	0.00	0.00	0.00	0.00
133	CGACGA	0.40	0.53	0.00	0.00
134	CGACGC	0.59	0.71	0.29	0.31
135	CGACGG	0.92	0.94	0.43	0.54
136	CGACGT	0.48	0.71	0.00	0.16
137	CGAGGA	0.00	0.00	0.00	0.00
138	CGAGGC	0.00	0.00	0.00	0.00
139	CGAGGG	0.56	0.62	0.00	0.00
140	CGAGGT	0.00	0.00	0.00	0.00
141	CGATGA	0.39	0.51	0.00	0.00
142	CGATGC	0.50	0.60	0.00	0.00
143	CGATGG	0.79	0.83	0.33	0.41
144	CGATGT	0.43	0.65	0.00	0.00
145	CGCAGA	0.00	0.00	0.00	0.00
146	CGCAGC	0.16	0.00	0.00	0.00
147	CGCAGG	0.60	0.67	0.16	0.16
148	CGCAGT	0.00	0.00	0.00	0.00
149	CGCCGA	0.00	0.00	0.00	0.00
150	CGCCGC	0.00	0.00	0.00	0.00
151	CGCCGG	0.59	0.68	0.00	0.17
152	CGCCGT	0.00	0.00	0.00	0.00
153	CGCGGA	0.00	0.00	0.00	0.00
154	CGCGGC	0.00	0.00	0.00	0.00
155	CGCGGG	0.00	0.00	0.00	0.00
156	CGCGGT	0.00	0.00	0.00	0.00
157	CGCTGA	0.00	0.00	0.00	0.00
158	CGCTGC	0.18	0.00	0.00	0.00
159	CGCTGG	0.44	0.55	0.00	0.00
160	CGCTGT	0.00	0.18	0.00	0.00
161	CGGAGA	0.37	0.00	0.00	0.00
162	CGGAGC	0.38	0.37	0.00	0.00
163	CGGAGG	0.75	0.79	0.32	0.33
164	CGGAGT	0.00	0.00	0.00	0.00
165	CGGCGA	0.00	0.00	0.00	0.00

166	CGGCGC	0.37	0.42	0.00	0.00
167	CGGCGG	0.67	0.73	0.31	0.31
168	CGGCGT	0.34	0.40	0.00	0.00
169	CGGGGA	0.00	0.00	0.00	0.00
170	CGGGGC	0.00	0.00	0.00	0.00
171	CGGGGG	0.00	0.00	0.00	0.00
172	CGGGGT	0.00	0.00	0.00	0.00
173	CGGTGA	0.42	0.38	0.00	0.00
174	CGGTGC	0.44	0.45	0.00	0.00
175	CGGTGG	0.65	0.71	0.30	0.36
176	CGGTGT	0.37	0.40	0.00	0.00
177	CGTAGA	0.37	0.41	0.00	0.00
178	CGTAGC	0.46	0.52	0.00	0.00
179	CGTAGG	0.84	0.89	0.36	0.42
180	CGTAGT	0.37	0.50	0.00	0.00
181	CGTCGA	0.40	0.39	0.00	0.00
182	CGTCGC	0.41	0.51	0.00	0.00
183	CGTCGG	0.76	0.83	0.34	0.36
184	CGTCGT	0.40	0.54	0.00	0.00
185	CGTGGA	0.00	0.00	0.00	0.00
186	CGTGGC	0.00	0.00	0.00	0.00
187	CGTGGG	0.52	0.57	0.00	0.00
188	CGTGGT	0.00	0.00	0.00	0.00
189	CGTTGA	0.00	0.37	0.00	0.00
190	CGTTGC	0.43	0.50	0.00	0.00
191	CGTTGG	0.69	0.78	0.31	0.34
192	CGTTGT	0.39	0.58	0.00	0.00
193	CTAAGA	0.43	0.51	0.00	0.00
194	CTAAGC	0.59	0.67	0.00	0.00
195	CTAAGG	0.81	0.86	0.46	0.52
196	CTAAGT	0.37	0.49	0.00	0.00
197	CTACGA	0.64	0.75	0.30	0.35
198	CTACGC	0.80	0.85	0.36	0.43
199	CTACGG	0.92	0.93	0.64	0.74
200	CTACGT	0.74	0.82	0.32	0.44
201	CTAGGA	0.00	0.00	0.00	0.00
202	CTAGGC	0.17	0.18	0.00	0.00
203	CTAGGG	0.62	0.67	0.16	0.17
204	CTAGGT	0.00	0.00	0.00	0.00
205	CTATGA	0.58	0.70	0.16	0.17
206	CTATGC	0.77	0.86	0.35	0.40
207	CTATGG	0.93	0.94	0.54	0.65
208	CTATGT	0.68	0.87	0.32	0.40

209	CTCAGA	0.00	0.00	0.00	0.00
210	CTCAGC	0.38	0.41	0.00	0.00
211	CTCAGG	0.76	0.81	0.33	0.37
212	CTCAGT	0.00	0.38	0.00	0.00
213	CTCCGA	0.00	0.00	0.00	0.00
214	CTCCGC	0.49	0.50	0.00	0.00
215	CTCCGG	0.69	0.79	0.34	0.36
216	CTCCGT	0.42	0.49	0.00	0.00
217	CTCGGA	0.00	0.00	0.00	0.00
218	CTCGGC	0.00	0.00	0.00	0.00
219	CTCGGG	0.47	0.49	0.16	0.17
220	CTCGGT	0.00	0.00	0.00	0.00
221	CTCTGA	0.00	0.00	0.00	0.00
222	CTCTGC	0.39	0.48	0.00	0.00
223	CTCTGG	0.65	0.75	0.16	0.35
224	CTCTGT	0.00	0.44	0.00	0.00
225	CTGAGA	0.40	0.46	0.00	0.00
226	CTGAGC	0.54	0.61	0.16	0.17
227	CTGAGG	0.87	0.92	0.41	0.47
228	CTGAGT	0.38	0.46	0.00	0.00
229	CTGCGA	0.46	0.59	0.00	0.00
230	CTGCGC	0.67	0.74	0.32	0.35
231	CTGCGG	0.85	0.89	0.45	0.53
232	CTGCGT	0.56	0.70	0.00	0.35
233	CTGGGA	0.00	0.00	0.00	0.00
234	CTGGGC	0.00	0.00	0.00	0.00
235	CTGGGG	0.53	0.60	0.00	0.00
236	CTGGGT	0.00	0.00	0.00	0.00
237	CTGTGA	0.42	0.56	0.00	0.00
238	CTGTGC	0.56	0.70	0.31	0.34
239	CTGTGG	0.86	0.90	0.44	0.55
240	CTGTGT	0.58	0.78	0.00	0.37
241	CTTAGA	0.41	0.50	0.00	0.00
242	CTTAGC	0.59	0.61	0.00	0.16
243	CTTAGG	0.84	0.86	0.40	0.50
244	CTTAGT	0.50	0.68	0.00	0.17
245	CTTCGA	0.39	0.53	0.00	0.00
246	CTTCGC	0.60	0.70	0.30	0.33
247	CTTCGG	0.83	0.87	0.39	0.50
248	CTTCGT	0.55	0.77	0.00	0.33
249	CTTGGA	0.00	0.00	0.00	0.00
250	CTTGGC	0.00	0.00	0.00	0.00
251	CTTGGG	0.66	0.71	0.31	0.36

252	CTTGGT	0.00	0.00	0.00	0.00
253	CTTTGA	0.35	0.44	0.00	0.00
254	CTTTGC	0.48	0.56	0.00	0.00
255	CTTTGG	0.79	0.87	0.33	0.38
256	CTTTGT	0.46	0.68	0.00	0.17

*Values represent the average of experiments performed in duplicate at either 37°C or 30°C.

†Base target sequence: acttatCNNNGNataccgatatTATATAAACCTAACCTCT

with the degenerated *NotI* target site (red) and TALE DNA binding site (green) indicated in uppercase.