nature biotechnology

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41587-019-0186-z

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Enhancing gene editing specificity by attenuating DNA cleavage kinetics

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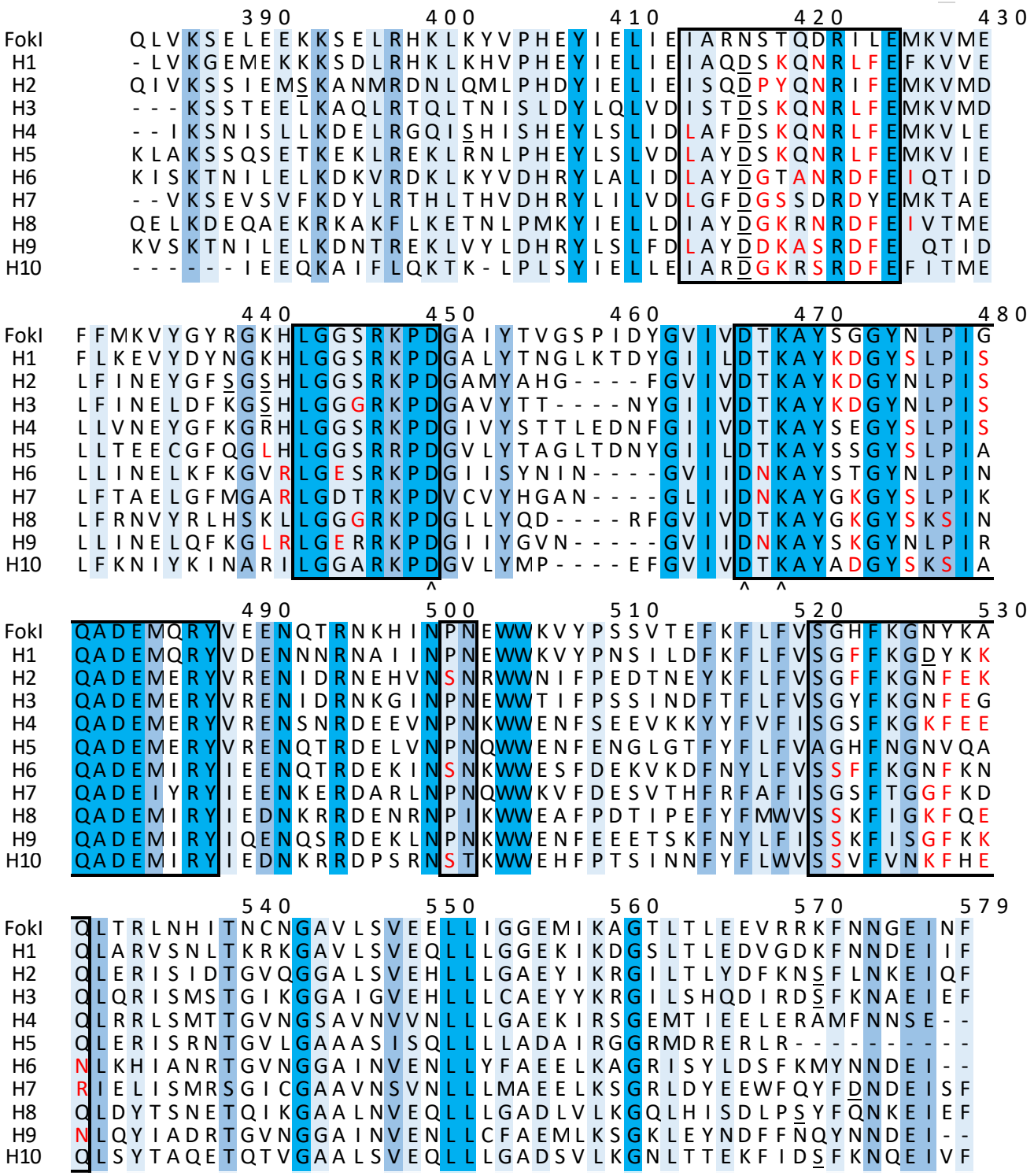
FokI domain variant	Screening results: ZFN-L context							Screening results: ZFN-R context							DNA proximity (Å)
	% indels					on:off ratio		% indels					on:off ratio		
	AAVS1	OT1	OT2	OT3	OT4			AAVS1	OT1	OT2	OT3	OT4			
1	*2*	*3*	*4*	*5*	*6*	*7*		*8*	*9*	*10*	*11*	*12*	*13*		*14*
< parent >	65.51	3.37	8.02	2.40	2.44	4.0		65.51	3.37	8.02	2.40	2.44	4.0		NA
N417D	85.45	5.52	10.52	3.64	2.84	3.8		81.91	4.31	13.15	3.08	5.24	3.2		4.3
N542D	81.21	4.47	12.76	3.15	3.12	3.5		68.21	4.56	9.61	2.90	3.58	3.3		18.8
Q481E	81.03	0.11	0.06	0.31	0.12	136.0	←	77.62	0.09	0.33	0.02	0.25	110.8		7.1
K525S	79.90	1.24	0.83	0.88	0.34	24.2	←	71.23	0.18	2.11	0.19	1.05	20.2		5.5
N476D	74.91	0.02	0.14	0.10	0.08	216.0	←	50.69	0.15	0.21	0.12	0.24	70.8		4.2
R416S	64.78	2.27	1.00	1.20	0.31	13.5	←	67.88	0.52	4.42	0.58	2.30	8.7		6.5
K448S	64.04	3.05	6.31	2.79	2.27	4.4		63.86	1.50	4.58	1.24	1.90	6.9		5.8
N527D	61.79	0.35	1.35	0.67	0.07	25.4	←	63.76	0.77	2.15	1.13	1.00	12.6		2.1
K441S	51.77	2.96	6.82	2.04	2.43	3.6		59.50	5.77	6.63	2.68	2.70	3.3		13.7
K400S	51.37	2.66	5.39	1.75	1.68	4.5		30.43	1.33	2.28	0.99	0.71	5.7		26.5
R422S	49.23	1.15	1.12	1.15	0.29	13.3	←	60.31	0.37	1.31	0.38	1.33	17.8		7.0
K497S	42.49	2.21	3.86	1.61	1.42	4.7		46.56	2.19	4.75	1.58	1.49	4.7		14.7
Q531E	40.88	0.02	0.04	0.01	0.05	348.2	←	30.74	0.30	0.47	0.74	0.06	19.5		7.0
K434S	40.77	2.66	5.19	1.75	1.75	3.6		55.30	4.26	6.55	2.87	3.35	3.2		21.3
K506S	39.60	2.13	5.25	1.62	1.70	3.7		44.66	2.24	4.57	1.48	1.51	4.6		15.6
R439S	38.71	2.64	4.70	1.57	1.68	3.7		52.25	4.09	6.93	2.30	2.76	3.2		20.0
N573D	35.83	1.75	3.42	1.24	1.09	4.8		49.18	3.72	6.36	2.16	2.45	3.3		17.6
K559S	33.44	1.88	3.51	1.38	1.09	4.3		40.49	2.00	4.12	1.25	1.29	4.7		27.8
K529S	31.39	1.21	2.51	1.23	0.87	5.4		28.09	1.07	2.42	0.84	0.75	5.5		7.3
Q420E	30.97	1.40	2.18	1.07	0.86	5.6		33.86	1.20	2.41	0.99	0.86	6.2		4.8
K571S	27.58	1.23	2.59	1.10	0.96	4.7		15.89	0.65	1.04	0.41	0.38	6.4		21.0
N578D	26.00	1.33	2.22	1.09	0.91	4.7		12.88	0.53	0.74	0.29	0.39	6.6		18.6
R570S	25.49	1.04	2.14	0.87	0.78	5.3		34.96	1.68	3.36	1.08	1.09	4.9		24.0
R398S	24.44	1.02	1.91	0.77	0.69	5.6		35.63	1.27	2.54	0.90	0.72	6.6		23.4
R447S	23.33	0.45	0.35	0.27	0.13	19.4		45.90	0.06	0.04	0.03	0.06	234.5		4.0
K402S	22.22	1.35	2.37	1.02	0.95	3.9		29.27	1.35	2.33	0.83	0.79	5.5		27.7
N540D	18.17	0.71	1.35	0.57	0.47	5.9		36.57	1.38	2.82	0.95	0.89	6.1		19.8
Q493E	16.63	0.64	1.02	0.47	0.39	6.6		22.16	0.90	1.52	0.63	0.74	5.8		17.8
K393S	13.47	0.55	0.62	0.46	0.26	7.1		17.55	0.43	1.12	0.33	0.48	7.4		19.8
K427S	12.48	0.71	1.14	0.53	0.45	4.4		17.97	0.71	1.35	0.49	0.44	6.0		12.5
K394S	12.12	0.46	0.66	0.45	0.29	6.5		20.67	0.62	1.42	0.51	0.60	6.5		19.3
N574D	10.76	0.36	0.92	0.27	0.26	6.0		6.95	0.22	0.40	0.11	0.10	8.4		14.3
N502D	9.32	0.21	0.66	0.30	0.27	6.5		12.27	0.30	0.56	0.27	0.15	9.6		9.4
K516S	6.04	0.26	0.42	0.17	0.19	5.9		9.92	0.42	0.52	0.26	0.19	7.1		18.1
N536D	5.32	0.07	0.15	0.05	0.05	16.2		7.18	0.15	0.14	0.08	0.07	16.2		15.3
R569S	5.15	0.06	0.10	0.06	0.07	17.6		10.00	0.25	0.32	0.18	0.14	11.3		23.8
N500D	2.63	0.03	0.01	0.02	0.02	36.8		3.24	0.01	0.01	0.01	0.02	72.5		11.1
R495S	2.10	0.01	0.00	0.00	0.03	42.4		3.00	0.00	0.03	0.01	0.03	42.2		16.7
N492D	0.82	0.01	0.01	0.00	0.06	10.4		0.84	0.00	0.01	0.00	0.01	26.7		14.8
R534S	ND	ND	ND	ND	ND	ND		27.26	1.61	1.76	0.82	0.83	5.4		10.8
GFP	0.03	0.01	0.00	0.00	0.02	0.9		0.03	0.01	0.00	0.00	0.02	0.9		NA

Supplementary Table 1. On and off-target activity of variants of the AAVS1 ZFN dimer bearing single-residue substitutions in the FokI cleavage domain of either the left or right ZFN (ZFN-L or ZFN-R). ZFNs were delivered to human K562 cells via mRNA nucleofection, followed by genomic DNA isolation at day 3 and deep sequencing analysis for indels at the intended target or four off-target sites. Columns 2-7 summarize results obtained for screening each variant in the ZFN-L context (i.e. each tested dimer consisted of a ZFN-L variant bearing the indicated substitution paired with an unmodified ZFN-R). Column 2 provides % indels measured at the intended target, with columns 3-6 indicating % indels at four previously known off-target sites. Each value is the average of four biological replicates (or three replicates in rare cases where a PCR reaction failed). Entries are provided in order of decreasing on-target % indels (column 2) with the exception that the parent, unmodified dimer is listed at top. Column 7 lists the on:off-target indel ratio (= % on-target indels / total off-target indels). To help highlight relative signal intensities, table values are embedded in heat maps (green – on target indels; red – off-target indels; blue – on:off ratio). Arrows highlight variants that both retain substantial on-target activity (>60% of parent) and exhibit a >3-fold increase in on:off ratio relative to parent. These

residues are underlined in figure 1b and the corresponding data points are bounded by a red box in figure 1c. Columns 8-13 summarize results obtained for screening each variant in the ZFN-R context. It can be seen by inspection of heat map intensities that results in the ZFN-L and ZFN-R contexts are broadly concordant. At far right, column 14 provides the distance in Angstroms between the alpha carbon and the nearest phosphate oxygen in the DNA backbone of a molecular model of a FokI dimer bound to DNA. Note that K469 was not mutated in this study because it is part of the active site[61] and R487 was not mutated because it is required for FokI dimerization (*Proc. Natl Acad. Sci. USA*, Bitinaite et al., 1997). Note also that variant N476D was reported as allowing enhanced cleavage activity at 30 °C but was not characterized for specificity in that study[60].



Supplementary Table 2. Alignment of FokI cleavage domain and homologous sequences (H1 through H10)-identified via a protein BLAST search of the FokI cleavage domain sequence. Black boxes highlight positions that are predicted to lie within 10 Å of the DNA backbone, while blue shading corresponds to the degree of residue conservation across the homologues. Red letters indicate alternative amino acids that were tested for activity and specificity in a large-scale mutational scan of DNA-proximal positions in a model of DNA-bound FokI[18]. Underlines highlight substitutions that were previously tested in our initial screen (i.e. in **figure 1** and **supplementary table 1**). Active site positions are indicated by a caret (^).

FokI variant		Screening results: ZFN-L context							Screening results: ZFN-R context						
Identity	Note	% indels					on:off ratio		% indels					on:off ratio	
		AAVS1	OT1	OT2	OT3	OT4			AAVS1	OT1	OT2	OT3	OT4		
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*		*9*	*10*	*11*	*12*	*13*	*14*	
< parent >		47.56	2.63	9.72	2.84	2.46	2.7		58.80	2.75	10.30	3.36	2.90	3.0	
< parent >		49.08	3.09	11.10	3.71	3.06	2.3								
Q481H	(2)	67.73	6.96	14.54	4.37	0.32	2.6		69.52	0.12	6.10	1.41	6.52	4.9	
S418P		67.64	11.36	19.29	5.53	3.85	1.7		74.02	3.16	17.76	3.61	8.51	2.2	
L424F		67.38	10.08	20.09	6.00	4.01	1.7		73.65	5.78	23.87	6.31	12.31	1.5	
N417D	(1)	67.23	5.20	17.94	5.03	3.71	2.1		73.10	2.74	16.32	3.50	5.59	2.6	
P478S		63.62	0.62	14.02	2.98	4.36	2.9		64.26	1.52	5.11	2.45	1.40	6.1	
M426I		61.70	2.74	16.65	4.59	3.84	2.2		63.92	2.71	9.92	3.45	2.36	3.5	
G480S		61.29	2.99	14.12	4.03	6.03	2.3		65.65	4.90	10.79	3.14	2.04	3.1	
G522S		60.02	2.23	12.86	3.29	3.27	2.8		63.97	3.37	12.37	3.47	2.21	3.0	
N476D	(1)	59.33	0.06	0.13	0.17	0.10	128.7		28.98	0.04	0.12	0.08	0.05	97.9	
I423L		57.80	2.48	15.54	3.29	3.51	2.3		66.91	4.37	13.71	4.47	3.16	2.6	
T419S		56.92	3.66	11.83	3.51	2.26	2.7		66.12	2.63	13.56	3.54	3.41	2.9	
N476S	(2)	55.45	2.55	10.94	3.65	2.18	2.9		59.29	1.51	8.93	2.82	2.96	3.7	
T419K		54.75	3.49	12.34	3.64	2.83	2.5		63.65	3.82	16.14	4.06	4.22	2.3	
Q481E	(1)	53.31	0.11	0.11	0.21	0.05	110.9		65.72	0.07	0.28	0.06	0.11	126.0	
Y528F		52.40	3.52	12.72	3.03	2.79	2.4		57.87	2.17	8.88	2.83	2.86	3.5	
Q531R	(2)	50.98	0.30	1.64	0.48	0.39	18.1		56.70	1.28	1.99	2.44	0.62	9.0	
D421S		50.44	1.73	16.11	2.46	6.35	1.9		51.41	7.67	10.28	3.19	2.50	2.2	
A530K		46.55	1.31	6.55	1.71	1.50	4.2		60.15	1.26	3.86	1.87	0.86	7.7	
S418D		46.39	0.14	0.29	0.34	0.16	49.7	←	58.61	0.20	0.57	0.15	0.16	54.4	
S472K		44.07	1.36	2.77	1.45	0.54	7.2		54.49	0.99	6.10	1.93	1.66	5.1	
H523F		42.63	2.12	14.68	2.27	4.62	1.8		53.03	5.28	9.32	3.38	2.74	2.6	
A530E		41.94	0.57	3.37	1.18	0.85	7.0		58.94	3.18	9.65	4.82	2.87	2.9	
N527D	(1)	41.63	0.22	1.13	0.68	0.11	19.6		52.34	0.56	1.75	1.24	0.59	12.7	
I479T		41.42	0.15	0.09	0.06	0.11	99.4	←	59.75	0.22	0.55	0.87	0.10	34.5	
T419Y		39.15	1.83	6.93	2.27	1.74	3.1		42.35	1.45	4.81	1.68	1.20	4.6	
D421N		39.03	1.77	13.33	2.41	5.30	1.7		39.41	6.27	8.14	2.59	1.84	2.1	
I414L		37.57	2.12	4.36	2.24	1.18	3.8		51.17	1.42	9.11	2.80	2.95	3.1	
E484Q		37.48	1.93	11.64	1.39	4.08	2.0		51.22	8.22	10.35	2.39	2.70	2.2	
Q420A	(2)	37.22	1.07	9.61	1.81	2.61	2.5		44.62	2.87	4.92	2.81	1.17	3.8	
S446G		35.42	1.52	3.07	1.75	1.09	4.8		48.86	1.13	4.31	1.13	1.59	6.0	
Q481N	(2)	35.13	0.59	3.37	1.56	0.35	6.0		35.21	0.10	2.64	0.83	1.15	7.5	
S418G		32.76	0.81	11.10	1.11	4.23	1.9		41.59	5.48	4.18	2.79	1.37	3.0	
K441E	(2)	32.05	2.59	9.23	2.29	2.31	2.0		45.72	5.21	8.60	3.29	2.64	2.3	
N417S	(2)	29.91	1.11	7.81	1.56	2.17	2.4		33.80	2.65	4.26	2.30	1.26	3.2	
G473D		29.64	1.15	6.26	1.52	2.12	2.7		41.00	3.40	7.64	2.26	1.52	2.8	
G473K		25.08	0.92	5.70	1.42	0.70	2.9		32.02	2.10	2.67	2.03	1.01	4.1	
N527G	(2)	23.28	0.84	2.93	1.27	1.50	3.6		28.13	0.55	2.49	1.59	0.78	5.2	
K529E	(2)	22.98	0.58	2.77	1.51	0.54	4.3		30.72	1.05	3.64	1.62	1.22	4.1	
P501S		20.02	1.60	4.32	1.47	1.17	2.3		30.29	0.79	3.83	1.57	1.02	4.2	
H442R		18.30	0.63	0.45	0.47	0.14	10.8		32.85	0.14	2.18	0.51	0.62	9.5	
Q531N	(2)	16.28	0.26	1.30	0.53	0.37	6.6		17.46	1.25	2.00	1.49	0.46	3.4	
I423D		14.05	0.39	1.84	0.75	0.57	4.0		29.67	0.93	2.84	1.08	0.66	5.4	
N527K	(2)	13.62	0.78	2.56	0.89	0.23	3.1		20.69	0.72	1.81	1.02	0.63	4.9	
K441L	(2)	13.37	0.74	2.77	0.80	0.95	2.5		23.57	1.55	2.99	1.40	0.91	3.4	
E425Q		8.67	0.10	0.07	0.05	0.07	30.3		33.35	0.05	0.09	0.08	0.08	110.0	
K441D	(2)	5.87	0.35	0.94	0.38	0.24	3.1		10.66	0.84	0.80	0.58	0.40	4.1	
G445E		2.63	0.21	0.15	0.20	0.06	4.2		5.57	0.16	0.31	0.25	0.24	5.8	
T468N		0.19	0.05	0.06	0.14	0.07	0.6		0.94	0.02	0.06	0.05	0.07	4.8	

Notes: (1) Variant previously tested in initial screen. See Supplementary Table 1.
(2) Position previously tested in initial screen, but using a different residue substitution. See Supplementary Table 1.

Supplementary Table 3. On and off-target activity of variants of the AAVS1 ZFN dimer bearing single-residue substitutions in the FokI cleavage domain of either the left or right ZFN (ZFN-L or ZFN-R). For this study, the queried positions were chosen based on proximity to the DNA backbone (generally within 10 Å) in a model of a FokI-DNA complex. Residue substitutions were biased towards variations observed in FokI homologues (see **Supplementary Table**

2). ZFNs were delivered to human K562 cells via mRNA nucleofection, followed by genomic DNA isolation at day 3 and deep sequencing analysis for indels at the intended target or four off-target sites. Values represent individual measurements. Columns 3-8 summarize results obtained for screening each variant in the ZFN-L context (i.e. each tested dimer consisted of a ZFN-L variant bearing the indicated substitution paired with an unmodified ZFN-R). Column 3 provides % indels measured at the intended target, with columns 4-7 indicating % indels at four previously known off-target sites. Entries are provided in order of decreasing on-target % indels (column 3) with the exception that the parent, unmodified dimer is listed at top. Column 8 lists the on:off-target indel ratio (= % on-target indels / total off-target indels). To help highlight relative signal intensities table values are embedded in heat maps (green – on target indels; red – off-target indels; blue – on:off ratio). Arrows highlight variants that retain substantial on-target activity (>80% of parent) and exhibit a >3-fold increase in on:off ratio relative to parent, and moreover involve new positions that were not tested in the initial screen (see column 2 notes and **Supplementary Table 1**). Columns 9-14 summarize results obtained for screening each variant in the ZFN-R context. It can be seen by inspection of heat map intensities that results in the ZFN-L and ZFN-R contexts are broadly concordant. Note that three of these variants (S418P, K441E, Q481H) were previously reported as providing enhanced cleavage activity but without any characterized specificity improvement (*J. Mol. Biol*., Guo, Gaj & Barbas, 2010).

FokI domain variant	ZFN-L$_{variant}$ + ZFN-R$_{variant}$							ZFN-L$_{variant}$							ZFN-R$_{variant}$						
	% indels					(on/∑off)		% indels					(on/∑off)		% indels					(on/∑off)	
	ON	OT1	OT2	OT3	OT4	*normalized*		ON	OT1	OT2	OT3	OT4	*normalized*		ON	OT1	OT2	OT3	OT4	*normalized*	
1	*2*	*3*	*4*	*5*	*6*	*7*		*8*	*9*	*10*	*11*	*12*	*13*		*14*	*15*	*16*	*17*	*18*	*19*	
parent - full dose	57.8	1.31	9.73	1.67	1.20	1.1		57.8	1.31	9.73	1.67	1.20	1.1		57.8	1.31	9.73	1.67	1.20	1.1	
	55.4	1.27	9.06	1.38	.98	1.2		55.4	1.27	9.06	1.38	.98	1.2		55.4	1.27	9.06	1.38	.98	1.2	
	60.6	1.81	11.83	1.87	1.39	1.0		60.6	1.81	11.83	1.87	1.39	1.0		60.6	1.81	11.83	1.87	1.39	1.0	
	55.4	2.15	13.05	2.26	2.05	.8		55.4	2.15	13.05	2.26	2.05	.8		55.4	2.15	13.05	2.26	2.05	.8	
parent - half dose	34.1	.23	2.50	.31	.12	2.9		34.1	.23	2.50	.31	.12	2.9		34.1	.23	2.50	.31	.12	2.9	
	36.7	.19	2.67	.41	.23	2.8		36.7	.19	2.67	.41	.23	2.8		36.7	.19	2.67	.41	.23	2.8	
	42.1	.32	3.72	.51	.30	2.3		42.1	.32	3.72	.51	.30	2.3		42.1	.32	3.72	.51	.30	2.3	
	40.2	.47	3.17	.57	.32	2.4		40.2	.47	3.17	.57	.32	2.4		40.2	.47	3.17	.57	.32	2.4	
R416H	94.2	3.09	18.51	3.36	2.33	.9		82.8	4.48	6.04	1.81	.31	1.8		73.8	.52	9.86	.91	2.96	1.4	
R416E	91.1	.11	.41	.19	.07	31.3	←	76.2	1.83	.49	.24	.05	7.8		68.3	.03	1.58	.16	.62	7.6	
R416N	89.3	.16	.55	.35	.12	20.4	←	74.8	1.12	.83	.59	.11	7.5		69.5	.07	2.49	.24	.75	5.2	
R416Q	88.2	1.10	7.54	1.66	.73	2.1		76.8	3.09	3.23	1.30	.25	2.6		69.0	.13	6.21	.44	2.08	2.1	
R416F	87.1	.57	2.62	.81	.38	5.3		75.2	2.09	3.87	1.24	.24	2.7		63.3	.33	3.94	.38	1.09	3.0	
R416Y	85.9	24.08	62.16	16.89	17.97	.2		81.4	7.28	20.16	3.53	1.44	.7		72.4	1.70	20.12	2.41	3.83	.7	
R416S	71.9	.22	.61	.28	.13	15.6	←	58.7	.85	.61	.72	.06	7.0		61.7	.27	4.36	.32	.94	2.8	
R416D	70.6	.04	.04	.05	.03	122.4	←	52.2	.61	.12	.16	.02	15.4		44.6	.05	.27	.04	.12	24.2	
R416M	69.3	.47	3.99	.85	.47	3.2		60.3	1.09	2.91	.99	.29	3.1		53.5	.45	5.09	.64	.93	2.0	
R416C	68.4	.34	2.75	.72	.29	4.5		61.5	1.31	2.50	1.08	.27	3.2		54.0	.35	5.09	.41	.94	2.1	
R416A	67.1	.16	1.14	.37	.12	10.0		59.2	.80	1.32	.70	.22	5.2		54.3	.13	3.73	.25	.57	3.1	
R416T	62.1	.50	3.51	.99	.43	3.1		49.5	.93	1.82	.81	.15	3.6		55.8	.31	6.64	.72	1.18	1.7	
R416G	47.9	.08	.47	.17	.12	15.3		48.3	.90	.66	.71	.15	5.3		49.0	.12	2.04	.24	.55	4.4	
R416L	44.9	.50	3.13	.74	.48	2.5		51.9	1.46	4.62	1.22	.51	1.8		46.7	.47	6.32	.78	.87	1.5	
R416W	39.2	.21	1.20	.36	.16	5.4		54.9	1.09	2.33	1.02	.36	3.1		41.2	.32	3.67	.40	.49	2.3	
R416V	34.9	1.08	4.49	1.14	.86	1.2		47.5	1.70	7.94	1.36	.94	1.1		40.1	.79	4.97	1.04	.99	1.4	
R416I	13.0	.30	1.02	.33	.23	1.9		28.5	.68	3.86	.71	.50	1.3		23.4	.38	2.91	.61	.44	1.4	
R416K	11.5	.12	.91	.23	.11	2.2		18.1	.33	.92	.37	.15	2.7		25.6	.31	1.96	.64	.30	2.1	
R416P	.1	.01	.01	.01	.03	.5		2.1	.03	.08	.02	.06	2.9		1.8	.02	.05	.02	.06	3.2	
K525T	84.7	.07	.68	.21	.21	19.3	←	74.6	.48	2.75	.94	.44	4.3		66.7	.24	5.06	.32	.45	2.9	
K525V	83.3	.04	.48	.06	.09	32.9		72.6	.36	2.82	.58	.40	4.7		58.7	.18	2.62	.18	.18	5.0	
K525C	82.1	.12	.55	.12	.19	22.2		74.3	.48	2.89	.80	.51	4.3		62.9	.13	3.74	.31	.37	3.7	
K525I	80.4	.04	.51	.13	.12	26.9		68.2	.28	2.29	.47	.61	5.0		57.3	.16	2.40	.28	.24	5.0	
K525S	77.7	.02	.11	.06	.05	84.7	←	65.8	.22	.53	.23	.15	15.6		63.2	.10	1.06	.12	.33	10.5	
K525A	73.8	.01	.07	.04	.03	132.2	←	63.3	.12	.30	.14	.11	25.3		61.2	.09	.92	.13	.21	12.1	
K525G	56.5	.04	.07	.04	.02	95.9		54.2	.25	.34	.21	.08	16.6		52.9	.04	1.09	.13	.34	8.9	
K525R	53.6	2.18	14.69	1.97	2.52	.7		51.1	1.17	10.86	1.35	1.93	.9		52.3	2.04	9.51	1.57	1.18	1.0	
K525Q	42.0	.03	.10	.07	.04	45.3		47.1	.31	.48	.34	.11	10.2		52.6	.09	1.55	.12	.26	6.9	
K525N	36.5	.04	.23	.05	.04	27.5		41.8	.14	.56	.30	.21	9.3		44.7	.15	1.61	.25	.27	5.2	
K525H	36.3	.04	.27	.06	.07	21.7		41.1	.17	.79	.24	.28	7.4		41.3	.29	2.61	.34	.24	3.2	
K525M	27.6	.02	.09	.04	.03	42.0		32.8	.08	.25	.13	.10	15.7		46.6	.15	1.29	.17	.19	7.0	
K525Y	25.8	.07	.83	.11	.11	6.2		37.8	.14	2.21	.21	.82	3.0		35.1	.70	1.42	.41	.15	3.5	
K525E	23.6	.01	.02	.03	.02	77.1		64.3	.08	.07	.10	.10	50.5		52.5	.02	.24	.06	.07	35.6	
K525L	14.3	.01	.02	.04	.04	32.0		27.1	.09	.48	.21	.20	7.5		30.3	.06	.79	.15	.07	7.5	
K525F	12.7	.07	.28	.08	.04	7.2		23.1	.04	1.13	.18	.35	3.7		25.3	.58	.74	.34	.16	3.7	
K525W	8.6	.02	.10	.03	.05	11.8		18.3	.06	.88	.11	.34	3.5		17.8	.09	.52	.21	.05	5.5	
K525D	5.0	.01	.02	.02	.04	15.2		41.8	.04	.11	.03	.07	43.8		36.2	.00	.14	.03	.04	45.2	
K525P	.0	.03	.03	.02	.02	.0		8.5	.03	.02	.02	.03	24.7		2.4	.02	.04	.01	.03	6.7	
GFP	.0	.02	.01	.03	.03	NA		.0	.02	.01	.03	.03	NA		.0	.02	.01	.03	.03	NA	
GFP	.0	.01	.04	.03	.02	NA		.0	.01	.04	.03	.02	NA		.0	.01	.04	.03	.02	NA	

Supplementary Table 4. On and off-target activity of variants of the AAVS1 ZFN dimer bearing each single-residue substitution of R416 and K525. Each variant was tested as a dimer in which both ZFN-L and ZFN-R bore the indicated substitution (columns 2-7), in addition to being tested in ZFN-L or ZFN-R alone (columns 8-13 and 14-19). ZFNs were delivered to human K562 cells via mRNA nucleofection, followed by genomic DNA isolation at day 3 and deep sequencing analysis for indels at the intended target or four off-target sites. Values represent individual measurements. Columns 2, 8 and 14 provide % indels measured at the intended target, while columns 3-6, 9-12 and 15-18 indicate % indels at four previously known off-target sites. For each tested position, entries are provided in order of decreasing on-target % indels in column 3, with the exception that full-dose and half-dose control studies of the parent, unmodified dimer are listed at top. Columns 7, 13 and 19 list the normalized on:off-target indel ratio (= % on-target indels / total off-target indels, normalized to value for the full-dose parent samples). To highlight relative signal intensities table values are embedded in heat maps (green – on target indels; red – off-target indels; blue – on:off ratio). Arrows highlight variants manifesting especially high levels of activity and specificity that were characterized in followup studies, see **Supplementary Table 9** and **Table 1**.

FokI domain variant	ZFN-L$_{variant}$ + ZFN-R$_{variant}$ % indels					(on/∑off)		ZFN-L$_{variant}$ % indels					(on/∑off)		ZFN-R$_{variant}$ % indels					(on/∑off)	
	ON	OT1	OT2	OT3	OT4	normalized		ON	OT1	OT2	OT3	OT4	normalized		ON	OT1	OT2	OT3	OT4	normalized	
1	*2*	*3*	*4*	*5*	*6*	*7*		*8*	*9*	*10*	*11*	*12*	*13*		*14*	*15*	*16*	*17*	*18*	*19*	
parent - full dose	63.6	3.50	15.40	3.29	3.11	1.0		63.6	3.50	15.40	3.29	3.11	1.0		63.6	3.50	15.40	3.29	3.11	1.0	
	63.1	3.50	18.77	3.63	3.01	.8		63.1	3.50	18.77	3.63	3.01	.8		63.1	3.50	18.77	3.63	3.01	.8	
	60.0	2.50	11.17	2.92	2.79	1.2		60.0	2.50	11.17	2.92	2.79	1.2		60.0	2.50	11.17	2.92	2.79	1.2	
	56.7	2.05	13.75	2.62	2.28	1.0		56.7	2.05	13.75	2.62	2.28	1.0		56.7	2.05	13.75	2.62	2.28	1.0	
parent - half dose	43.5	.58	6.13	.98	.58	2.0		43.5	.58	6.13	.98	.58	2.0		43.5	.58	6.13	.98	.58	2.0	
	45.1	.81	6.62	.77	.67	1.9		45.1	.81	6.62	.77	.67	1.9		45.1	.81	6.62	.77	.67	1.9	
	36.5	.31	3.47	.48	.30	3.0		36.5	.31	3.47	.48	.30	3.0		36.5	.31	3.47	.48	.30	3.0	
	34.7	.28	3.72	.68	.43	2.6		34.7	.28	3.72	.68	.43	2.6		34.7	.28	3.72	.68	.43	2.6	
S418P	91.5	9.89	35.58	6.21	8.86	.6		85.9	11.22	25.87	5.63	3.90	.7		79.2	3.90	28.77	3.93	9.53	.7	
S418E	58.2	.01	.04	.02	.05	178.5	←	80.2	.20	.52	.31	.16	25.7		72.5	.09	.34	.16	.11	39.8	
S418A	56.4	.67	3.20	.90	.57	4.0		64.9	4.13	6.75	3.56	1.13	1.6		56.8	.70	12.20	.94	1.88	1.4	
S418D	48.7	.03	.08	.05	.12	65.5		63.6	1.07	1.90	.93	.51	5.5		65.2	.16	1.15	.14	.27	14.4	
S418N	44.7	.90	8.43	1.34	.95	1.5		51.1	3.49	6.04	2.41	.62	1.5		59.2	1.08	19.05	1.70	4.67	.8	
S418H	40.2	.55	3.40	.75	.76	2.8		52.0	3.38	6.20	3.05	.97	1.5		55.6	1.14	12.98	1.42	3.65	1.1	
S418Q	39.5	.10	.85	.31	.25	10.0		49.3	1.93	1.33	1.38	.21	3.9		55.1	.22	7.54	.56	1.94	2.0	
S418K	34.8	1.33	3.16	1.29	.92	2.0		47.0	4.22	2.90	3.37	1.19	1.5		52.0	1.58	17.69	1.17	3.44	.8	
S418T	34.6	.07	.24	.14	.09	24.3		41.9	.58	.63	.56	.08	8.6		57.2	.27	6.33	.54	1.25	2.6	
S418R	29.5	3.22	5.45	1.52	2.05	.9		43.7	6.46	3.65	3.11	.68	1.2		45.8	2.31	19.09	1.60	5.19	.6	
S418G	27.0	2.45	9.45	1.64	1.93	.7		41.8	1.22	17.17	1.15	4.53	.7		44.8	7.82	8.06	3.45	1.44	.8	
S418C	24.4	.21	.74	.35	.28	5.9		48.5	2.17	6.15	3.19	1.45	1.4		42.7	1.14	6.97	1.05	1.26	1.6	
S418V	18.7	.03	.03	.05	.11	33.3		33.0	.34	.21	.27	.04	14.7		44.4	.04	.40	.10	.23	21.9	
S418M	8.9	.05	.09	.06	.10	11.4		24.0	.50	.64	.67	.13	4.7		30.9	.19	1.55	.17	.46	5.0	
S418Y	7.3	.09	.33	.06	.12	4.6		21.4	.57	1.68	.72	.34	2.5		21.7	.48	2.48	.51	.82	1.9	
S418W	6.0	.10	.12	.08	.13	5.2		19.7	1.00	1.28	.88	.15	2.3		18.3	.15	1.60	.37	.61	2.6	
S418I	6.0	.02	.02	.02	.10	14.1		18.7	.18	.11	.14	.03	15.4		29.8	.06	.28	.03	.13	23.2	
S418F	5.2	.06	.18	.09	.13	4.3		18.4	.52	6.25	.77	.25	.9		14.9	.23	1.08	.34	.42	2.7	
S418L	1.8	.01	.03	.01	.14	3.8		11.4	.16	.23	.42	.05	5.1		10.8	.03	.41	.05	.14	6.4	
R422K	78.5	2.17	23.05	3.34	3.98	.9		72.3	8.36	21.16	4.17	2.86	.8		71.2	.97	22.19	3.16	6.13	.8	
R422H	58.5	.08	.65	.39	.23	16.4	←	62.8	1.65	5.04	2.12	.40	2.6		63.4	.37	4.33	.72	1.45	3.5	
R422Q	43.1	.10	.53	.23	.14	16.4		56.2	.84	8.88	2.12	.73	1.7		60.2	.56	4.53	1.63	1.31	2.8	
R422L	37.7	.17	.71	.28	.23	10.3		53.8	.63	10.24	2.14	1.15	1.4		52.2	.94	3.79	1.36	.91	2.8	
R422Y	37.3	.08	1.31	.46	.31	6.6		53.7	2.23	4.91	3.44	.74	1.8		53.0	.37	4.80	.74	1.26	2.8	
R422T	37.2	.04	.29	.09	.17	23.8		50.9	.76	4.10	1.53	.31	2.9		54.6	.24	2.99	.81	.91	4.2	
R422S	32.7	.04	.15	.20	.20	20.8		43.7	.87	2.29	1.55	.30	3.3		52.1	.17	1.55	.63	.90	6.1	
R422E	25.1	.00	.03	.03	.14	47.2		45.7	.32	.85	.38	.07	10.7		49.8	.02	.17	.08	.14	45.8	
R422V	23.4	.02	.18	.06	.14	21.8		44.6	.43	4.71	1.50	.55	2.4		41.7	.24	1.61	.52	.30	5.9	
R422N	23.2	.03	.04	.06	.11	35.5		39.7	.80	2.03	1.24	.20	3.5		46.2	.15	1.84	.44	.81	5.4	
R422D	22.0	.02	.02	.01	.08	63.1		40.0	.50	.84	.40	.07	8.4		49.9	.03	.25	.05	.28	31.3	
R422M	20.8	.05	.21	.20	.18	12.5		40.2	.67	5.14	1.71	1.00	1.8		39.5	.56	2.40	.65	.46	3.7	
R422F	20.4	.03	.69	.30	.16	6.6		39.3	1.22	3.33	2.37	.62	2.0		40.0	.20	2.29	.32	.48	4.6	
R422G	19.8	.02	.05	.03	.12	33.9		41.3	.43	1.35	.92	.18	5.4		40.9	.20	1.64	.36	.57	5.6	
R422I	16.9	.05	.22	.14	.15	11.6		35.8	.31	3.13	1.37	.43	2.6		35.5	.31	1.97	.41	.33	4.5	
R422W	13.1	.03	.32	.10	.10	9.0		31.7	1.03	1.43	1.61	.25	2.8		33.2	.14	3.71	.47	.67	2.5	
R422C	13.0	.07	.09	.03	.11	16.3		29.9	.47	1.75	.84	.31	3.4		31.6	.13	1.22	.31	.34	6.0	
R422P	3.6	.02	.03	.01	.09	8.9		15.6	.12	.13	.11	.05	14.2		28.5	.02	.20	.03	.06	35.2	
R422A	3.4	.01	.03	.05	.15	5.4		4.8	.07	.09	.09	.04	6.3		57.4	.24	2.68	.61	1.20	4.6	
GFP	.0	.00	.02	.02	.09	NA		.0	.00	.02	.02	.09	NA		.0	.00	.02	.02	.09	NA	
GFP	.0	.01	.01	.00	.12	NA		.0	.01	.01	.00	.12	NA		.0	.01	.01	.00	.12	NA	

Supplementary Table 5. On and off-target activity of variants of the AAVS1 ZFN dimer bearing each single-residue substitution of S418 and R422. For additional detail see legend to **Supplementary Table 4**.

FokI domain variant	ZFN-L$_{variant}$ + ZFN-R$_{variant}$							ZFN-L$_{variant}$							ZFN-R$_{variant}$						
	% indels					(on/∑off)		% indels					(on/∑off)		% indels					(on/∑off)	
	ON	OT1	OT2	OT3	OT4	*normalized*		ON	OT1	OT2	OT3	OT4	*normalized*		ON	OT1	OT2	OT3	OT4	*normalized*	
1	*2*	*3*	*4*	*5*	*6*	*7*		*8*	*9*	*10*	*11*	*12*	*13*		*14*	*15*	*16*	*17*	*18*	*19*	
parent - full dose	46.5	1.46	6.60	1.41	1.26	.8		46.5	1.46	6.60	1.41	1.26	.8		46.5	1.46	6.60	1.41	1.26	.8	
	40.1	.90	3.57	1.05	.70	1.2		40.1	.90	3.57	1.05	.70	1.2		40.1	.90	3.57	1.05	.70	1.2	
	45.7	1.36	5.54	1.37	1.24	.9		45.7	1.36	5.54	1.37	1.24	.9		45.7	1.36	5.54	1.37	1.24	.9	
	41.2	1.04	3.92	.94	.86	1.1		41.2	1.04	3.92	.94	.86	1.1		41.2	1.04	3.92	.94	.86	1.1	
parent - half dose	29.2	.31	1.82	.26	.31	2.0		29.2	.31	1.82	.26	.31	2.0		29.2	.31	1.82	.26	.31	2.0	
	29.3	.27	ND	.36	.31	5.8		29.3	.27	ND	.36	.31	5.8		29.3	.27	ND	.36	.31	5.8	
	24.6	.20	1.06	.19	.17	2.8		24.6	.20	1.06	.19	.17	2.8		24.6	.20	1.06	.19	.17	2.8	
	24.6	.34	1.16	.30	.17	2.3		24.6	.34	1.16	.30	.17	2.3		24.6	.34	1.16	.30	.17	2.3	
I479Q	53.8	.04	.03	.07	.04	56.0	←	64.2	.05	.03	.03	.10	54.4		59.6	.54	1.32	.77	.05	4.1	
I479V	36.6	.04	.05	.06	.05	34.0		49.9	.24	.20	.11	.11	13.9		49.2	.18	.48	.32	.25	7.4	
I479M	34.7	.22	.22	.10	.10	9.9		41.4	.41	1.30	.41	.61	2.8		43.4	1.33	2.12	1.35	.19	1.6	
I479T	28.7	.04	.03	.03	.09	28.5		45.9	.07	.05	.04	.09	34.2		48.6	.06	.44	.50	.08	8.3	
I479L	27.7	.17	.05	.12	.08	12.4		39.3	.13	.29	.22	.96	4.6		40.1	2.96	1.91	.90	.20	1.2	
I479C	14.1	.04	.06	.03	.07	13.0		32.6	.09	.02	.05	.10	23.0		41.4	.17	.51	.27	.05	7.6	
I479F	5.1	.01	.03	.03	.03	9.2		22.5	.04	.02	.03	.11	20.7		25.1	.11	.08	.12	.02	14.1	
I479Y	1.0	.04	.01	.04	.03	1.5		23.3	.04	.02	.04	.03	32.9		21.5	.04	.04	.04	.05	22.2	
I479A	.8	.02	.01	.03	.06	1.4		41.5	.04	.03	.04	.06	45.9		40.0	.05	.06	.10	.07	26.6	
I479S	.5	.05	.01	.03	.06	.6		36.2	.03	.02	.06	.09	34.1		35.6	.06	.05	.06	.03	30.9	
I479N	.1	.06	.02	.04	.03	.2		20.2	.01	.03	.03	.06	31.0		25.0	.05	.04	.11	.08	16.9	
I479H	.1	.04	.02	.04	.04	.1		19.0	.03	.04	.02	.05	26.3		19.7	.03	.04	.04	.07	20.9	
I479G	.1	.03	.03	.03	.05	.1		11.1	.04	.02	.01	.04	16.4		18.5	.04	.03	.02	.11	17.6	
I479R	.0	.07	.02	.06	.01	.1		3.2	.01	.03	.03	.10	3.3		3.2	.04	.03	.01	.04	4.9	
I479P	.0	.02	.03	.04	.04	.1		3.8	.01	.03	.04	.04	5.9		1.3	.04	.03	.06	.07	1.2	
I479K	.0	.04	.03	.01	.03	.1		.4	.01	.02	.04	.07	.6		.8	.04	.01	.01	.06	1.2	
I479W	.0	.01	.04	.03	.04	.1		3.9	.04	.00	.04	.04	5.8		6.9	.01	.02	.06	.05	8.7	
I479E	.0	.04	.02	.06	.04	.0		1.5	.03	.02	.05	.03	2.1		13.2	.04	.02	.02	.07	15.4	
I479D	.0	.04	.04	.01	.03	.0		1.0	.01	.03	.02	.06	1.4		6.5	.03	.01	.04	.04	10.1	
GFP	.0	.06	.02	.02	.05	NA		.0	.06	.02	.02	.05	NA		.0	.06	.02	.02	.05	NA	
GFP	.0	.04	.01	.06	.03	NA		.0	.04	.01	.06	.03	NA		.0	.04	.01	.06	.03	NA	

Supplementary Table 6. On and off-target activity of variants of the AAVS1 ZFN dimer bearing each single-residue substitution of I479. For additional detail see legend to **Supplementary Table 4**.

FokI domain variant	ZFN-L$_{variant}$ + ZFN-R$_{variant}$							ZFN-L$_{variant}$							ZFN-R$_{variant}$						
	% indels					(on/∑off)		% indels					(on/∑off)		% indels					(on/∑off)	
	ON	OT1	OT2	OT3	OT4	*normalized*		ON	OT1	OT2	OT3	OT4	*normalized*		ON	OT1	OT2	OT3	OT4	*normalized*	
1	*2*	*3*	*4*	*5*	*6*	*7*		*8*	*9*	*10*	*11*	*12*	*13*		*14*	*15*	*16*	*17*	*18*	*19*	
parent - full dose	48.0	2.12	4.32	1.05	.84	.9		48.0	2.12	4.32	1.05	.84	.9		48.0	2.12	4.32	1.05	.84	.9	
	46.4	1.84	4.34	1.11	1.06	.9		46.4	1.84	4.34	1.11	1.06	.9		46.4	1.84	4.34	1.11	1.06	.9	
	45.8	1.28	3.15	.77	.92	1.2		45.8	1.28	3.15	.77	.92	1.2		45.8	1.28	3.15	.77	.92	1.2	
	47.6	1.67	4.30	.99	1.03	1.0		47.6	1.67	4.30	.99	1.03	1.0		47.6	1.67	4.30	.99	1.03	1.0	
parent - half dose	30.8	.38	1.21	.19	.18	2.5		30.8	.38	1.21	.19	.18	2.5		30.8	.38	1.21	.19	.18	2.5	
	31.6	.23	1.19	.28	.18	2.7		31.6	.23	1.19	.28	.18	2.7		31.6	.23	1.19	.28	.18	2.7	
	29.5	.35	.76	.21	.16	3.2		29.5	.35	.76	.21	.16	3.2		29.5	.35	.76	.21	.16	3.2	
	28.0	.31	.85	.20	.17	3.0		28.0	.31	.85	.20	.17	3.0		28.0	.31	.85	.20	.17	3.0	
Q481D	90.5	.05	.12	.10	.16	34.6	←	78.2	.21	.54	.87	.29	6.6		60.0	.07	1.15	.10	.30	6.0	
Q481A	86.3	.04	.02	.04	.09	74.7	←	56.1	.10	.06	.14	.05	25.9		48.3	.05	.15	.03	.09	24.3	
Q481H	84.3	.65	22.17	4.71	1.07	.5		71.3	5.48	7.24	1.76	.15	.8		55.9	.15	2.69	.51	2.74	1.5	
Q481C	66.1	.03	.05	.05	.09	48.5		61.8	.28	.05	.35	.22	11.2		53.5	.15	1.44	.04	.28	4.5	
Q481E	57.0	.03	.04	.04	.05	55.4	←	61.1	.05	.03	.12	.04	40.2		56.8	.04	.18	.03	.08	27.0	
Q481S	55.0	.04	.02	.07	.05	49.8		50.8	.07	.05	.14	.04	27.7		47.9	.07	.29	.03	.11	15.2	
Q481T	33.3	.02	.02	.00	.07	47.0		36.9	.07	.05	.11	.01	24.2		35.6	.06	.06	.04	.05	27.4	
Q481N	29.4	.05	.20	.13	.09	10.0		36.7	.50	1.29	.65	.22	2.2		38.6	.09	.50	.12	.22	6.7	
Q481G	18.1	.13	.34	.18	.12	3.8		33.5	.32	.43	.74	.24	3.1		23.6	.55	2.40	.25	.31	1.1	
Q481R	3.8	.04	.03	.03	.03	4.7		5.6	.02	.04	.04	.01	7.4		7.8	.04	.05	.05	.02	7.7	
Q481K	3.4	.05	.03	.02	.10	2.8		19.9	.06	.05	.05	.04	16.6		2.2	.03	.03	.02	.06	2.7	
Q481M	1.9	.03	.03	.09	.08	1.3		5.6	.05	.02	.04	.04	5.8		21.6	.04	.06	.06	.03	18.9	
Q481P	.4	.03	.03	.08	.10	.3		2.0	.01	.02	.07	.04	2.3		4.8	.07	.03	.02	.03	5.1	
Q481Y	.4	.01	.03	.02	.05	.6		1.8	.04	.04	.05	.02	1.9		12.7	.03	.22	.09	.11	4.6	
Q481L	.1	.01	.03	.03	.06	.2		1.2	.05	.03	.04	.03	1.3		7.0	.01	.03	.03	.03	10.8	
Q481V	.1	.01	.03	.03	.09	.1		1.5	.06	.03	.06	.02	1.3		2.3	.03	.02	.07	.03	2.4	
Q481I	.1	.01	.03	.02	.06	.1		.9	.04	.04	.07	.02	.9		3.0	.05	.03	.04	.02	3.5	
Q481F	.0	.02	.04	.08	.05	.0		.5	.05	.02	.02	.05	.6		.9	.04	.05	.04	.05	.8	
Q481W	.0	.04	.02	.04	.07	.0		.2	.02	.03	.04	.03	.3		.1	.02	.03	.04	.01	.2	
N527D	34.9	.04	.05	.07	.07	25.0		45.1	.11	.42	.21	.12	8.6		46.4	.48	.77	.61	.21	3.6	
N527G	12.8	.08	.30	.40	.34	1.8		25.3	.92	1.22	.60	.71	1.2		26.1	.46	1.25	.54	.45	1.6	
N527P	10.0	.04	.04	.06	.06	7.9		34.7	.97	2.50	.72	.63	1.2		15.1	.10	.10	.13	.05	6.4	
N527S	9.7	.14	.12	.15	.16	2.7		22.5	.67	.93	.37	.32	1.6		20.8	.44	.84	.49	.31	1.6	
N527Q	9.1	.82	.90	.33	.20	.7		25.7	1.76	2.62	1.13	.99	.6		17.4	1.19	1.69	.40	.34	.8	
N527A	8.6	.14	.25	.13	.17	2.0		20.5	.34	.76	.42	.28	1.8		18.7	.48	.77	.29	.43	1.5	
N527H	7.9	.68	.58	.31	.22	.7		20.7	1.10	1.65	.55	.13	1.0		16.7	.99	.89	.40	.31	1.0	
N527K	6.7	.29	.36	.29	.12	1.0		14.7	.66	1.31	.49	.11	.9		19.0	.77	.77	.40	.34	1.3	
N527E	4.6	.03	.03	.05	.06	4.2		18.9	.08	.06	.13	.09	8.4		24.4	.66	.65	.25	.26	2.2	
N527C	3.3	.08	.10	.04	.09	1.7		16.3	.25	.80	.25	.17	1.8		9.0	.39	.30	.17	.21	1.4	
N527V	1.4	.03	.30	.09	.10	.4		4.2	.09	.14	.10	.05	1.8		1.4	.05	.04	.07	.02	1.2	
N527M	1.3	.14	.07	.09	.09	.5		6.6	.18	.44	.23	.08	1.1		5.7	.43	.22	.20	.18	.9	
N527F	.7	.07	.03	.05	.04	.6		5.3	.16	.21	.15	.02	1.6		3.5	.21	.14	.06	.06	1.2	
N527I	.0	.03	.03	.01	.06	.0		1.6	.02	.04	.07	.01	1.8		1.0	.07	.03	.03	.02	1.1	
N527T	ND	ND	ND	ND	ND	ND		25.1	.14	.25	.12	.04	7.3		ND	ND	ND	ND	ND	ND	
N527R	ND	ND	ND	ND	ND	ND		17.4	1.07	1.70	.37	.28	.8		ND	ND	ND	ND	ND	ND	
N527Y	ND	ND	ND	ND	ND	ND		5.3	.18	.22	.12	.06	1.5		ND	ND	ND	ND	ND	ND	
N527L	ND	ND	ND	ND	ND	ND		3.4	.12	.22	.09	.06	1.1		ND	ND	ND	ND	ND	ND	
N527W	ND	ND	ND	ND	ND	ND		3.3	.05	.17	.11	.04	1.5		ND	ND	ND	ND	ND	ND	
GFP	.0	.03	.02	.02	.05	NA		.0	.03	.02	.02	.05	NA		.0	.03	.02	.02	.05	NA	
GFP	.0	.02	.03	.05	.06	NA		.0	.02	.03	.05	.06	NA		.0	.02	.03	.05	.06	NA	

Supplementary Table 7. On and off-target activity of variants of the AAVS1 ZFN dimer bearing each single-residue substitution of Q481 and N527. For additional detail see legend to **Supplementary Table 4**.

FokI domain variant	ZFN-L$_{variant}$ + ZFN-R$_{variant}$							ZFN-L$_{variant}$							ZFN-R$_{variant}$						
	% indels					(on/∑off)		% indels					(on/∑off)		% indels					(on/∑off)	
	ON	OT1	OT2	OT3	OT4	*normalized*		ON	OT1	OT2	OT3	OT4	*normalized*		ON	OT1	OT2	OT3	OT4	*normalized*	
1	*2*	*3*	*4*	*5*	*6*	*7*		*8*	*9*	*10*	*11*	*12*	*13*		*14*	*15*	*16*	*17*	*18*	*19*	
parent - full dose	42.6	3.88	7.68	2.05	2.94	.9		42.6	3.88	7.68	2.05	2.94	.9		42.6	3.88	7.68	2.05	2.94	.9	
	41.6	3.50	6.44	1.64	2.72	1.0		41.6	3.50	6.44	1.64	2.72	1.0		41.6	3.50	6.44	1.64	2.72	1.0	
	41.5	2.93	6.14	1.36	2.25	1.1		41.5	2.93	6.14	1.36	2.25	1.1		41.5	2.93	6.14	1.36	2.25	1.1	
	38.5	3.14	6.24	1.32	2.35	1.0		38.5	3.14	6.24	1.32	2.35	1.0		38.5	3.14	6.24	1.32	2.35	1.0	
parent - half dose	32.5	1.00	2.46	.46	.63	2.4		32.5	1.00	2.46	.46	.63	2.4		32.5	1.00	2.46	.46	.63	2.4	
	32.0	1.02	2.41	.53	.51	2.4		32.0	1.02	2.41	.53	.51	2.4		32.0	1.02	2.41	.53	.51	2.4	
	27.3	.45	1.29	.19	.34	4.1		27.3	.45	1.29	.19	.34	4.1		27.3	.45	1.29	.19	.34	4.1	
	30.0	.91	2.06	.36	.61	2.6		30.0	.91	2.06	.36	.61	2.6		30.0	.91	2.06	.36	.61	2.6	
N476G	58.2	.07	.10	.08	.07	63.3	←	62.7	.60	3.13	.56	.41	4.6		45.3	.59	2.34	1.16	.85	3.1	
N476S	55.3	2.32	8.64	2.13	3.28	1.2		54.4	4.38	9.71	2.67	2.74	1.0		49.9	3.00	9.00	2.07	4.76	.9	
N476T	55.0	.98	1.63	.97	1.04	4.1		53.1	2.35	5.19	1.77	1.75	1.6		48.4	2.03	4.98	1.55	2.71	1.5	
N476C	46.4	.56	.54	.43	.24	9.0		46.8	1.92	3.74	1.35	.97	2.0		44.4	1.35	3.25	.98	1.44	2.2	
N476A	38.7	.22	.88	.75	.33	6.1		43.6	1.86	3.28	1.50	1.15	1.9		41.8	1.60	3.81	1.20	1.97	1.7	
N476H	36.6	1.48	2.95	1.06	1.09	1.9		41.6	3.07	4.94	1.81	2.48	1.2		41.1	2.71	7.08	1.20	2.06	1.1	
N476R	36.3	3.04	3.33	1.67	.76	1.4		40.2	4.87	6.10	2.55	1.41	.9		44.3	3.71	6.29	1.38	3.07	1.0	
N476Q	29.7	.06	.06	.09	.12	31.1		38.6	.77	2.08	.80	.51	3.2		38.6	.42	1.03	.50	.58	5.2	
N476P	28.8	.61	1.82	.74	1.21	2.3		40.2	3.21	6.83	1.40	2.79	1.0		33.0	1.21	3.11	1.38	2.72	1.3	
N476K	25.8	.10	.31	.60	.10	8.0		32.0	1.00	3.78	1.62	.46	1.6		41.2	.87	2.04	1.11	.88	2.9	
N476V	24.4	.05	.06	.06	.07	34.8		34.8	.53	.49	.36	.22	7.5		39.9	.23	.55	.22	.46	9.3	
N476M	16.0	.12	.29	.17	.12	7.9		27.9	1.14	2.27	.73	.81	1.9		30.4	.79	2.34	.75	.77	2.2	
N476Y	14.0	.30	.80	.38	.17	2.9		26.6	1.63	2.53	.73	1.41	1.4		26.1	1.46	2.86	1.06	.50	1.5	
N476I	13.5	.03	.03	.02	.02	48.4		30.5	.55	.21	.29	.25	8.0		33.9	.30	.59	.16	.30	8.7	
N476D	8.2	.02	.02	.04	.07	19.5		67.3	.09	.20	.18	.16	36.4		31.3	.16	.10	.06	.04	30.1	
N476F	6.6	.16	.21	.05	.11	4.3		18.1	1.12	1.22	.77	.39	1.8		15.1	.60	1.21	.29	.38	2.1	
N476W	4.0	.00	.09	.08	.03	6.6		14.9	1.13	.49	.39	.22	2.3		13.6	1.64	1.05	.36	.38	1.4	
N476E	2.0	.01	.04	.03	.05	5.4		65.4	.06	.07	.08	.07	78.3		37.7	.04	.07	.04	.03	75.3	
N476L	1.7	.01	.04	.06	.04	4.1		12.5	.15	.13	.14	.10	8.1		11.8	.13	.23	.09	.12	7.1	
Q531R	43.9	.15	.46	.18	.10	16.7		48.6	.43	1.11	.26	.46	7.4		41.4	1.96	1.89	1.07	.70	2.5	
Q531H	42.7	1.12	7.88	1.19	3.73	1.0		47.2	3.04	10.36	2.12	6.48	.7		38.8	1.57	6.91	.91	2.35	1.1	
Q531K	38.5	5.20	8.02	1.54	2.03	.8		42.1	4.18	7.43	1.80	6.55	.7		38.6	6.05	9.56	1.62	1.05	.7	
Q531T	29.4	.03	.06	.07	.05	49.2		37.9	.37	.40	.30	.33	9.3		38.7	.31	2.20	.37	.43	4.0	
Q531V	19.8	.01	.05	.01	.09	43.0		33.2	.12	.11	.12	.02	30.0		33.7	.05	.81	.07	.18	10.4	
Q531A	17.7	.20	.53	.18	.22	5.4		25.9	.48	1.96	.37	.70	2.5		33.8	2.47	3.29	.89	.94	1.5	
Q531M	17.4	.04	.04	.01	.08	34.5		30.6	.40	.35	.31	.19	8.4		39.7	.57	1.07	.46	.37	5.5	
Q531S	6.7	.06	.07	.10	.06	7.7		14.9	.17	.39	.20	.33	4.7		20.1	.79	1.59	.52	.35	2.1	
Q531N	6.4	.22	.40	.08	.09	2.8		13.0	.24	.69	.28	.46	2.7		33.2	1.85	5.31	.84	1.27	1.2	
Q531E	5.4	.03	.04	.03	.04	13.1		29.5	.02	.06	.06	.05	51.6		18.2	.30	.32	.57	.13	4.7	
Q531C	3.1	.02	.03	.02	.08	7.0		12.6	.11	.16	.21	.09	7.5		13.2	.21	.33	.28	.12	4.8	
Q531I	2.6	.01	.03	.04	.05	7.2		25.7	.02	.05	.04	.06	48.3		32.4	.03	.15	.08	.07	33.3	
Q531Y	1.5	.02	.06	.02	.06	3.3		18.1	.02	.04	.04	.03	45.6		6.0	.06	.23	.08	.06	4.8	
Q531G	1.4	.11	.16	.06	.07	1.1		7.4	.20	.56	.27	.45	1.7		7.1	1.39	.63	.35	.25	.9	
Q531L	.3	.01	.03	.03	.09	.7		12.8	.04	.03	.05	.04	26.2		30.9	.02	.06	.05	.04	62.1	
Q531W	.2	.02	.05	.02	.06	.4		2.8	.02	.05	.05	.11	4.3		3.7	.06	.13	.05	.06	4.2	
Q531F	.2	.00	.03	.04	.05	.6		4.1	.02	.02	.04	.09	8.0		4.3	.04	.04	.04	.06	8.3	
Q531P	.1	.02	.03	.03	.04	.4		5.3	.03	.05	.08	.05	9.0		3.9	.09	.08	.06	.05	4.7	
Q531D	.0	.01	.02	.02	.03	.2		4.5	.01	.05	.03	.05	10.3		6.1	.02	.05	.04	.08	10.7	
GFP	.1	.01	.03	ND	.04	NA		.1	.01	.03	ND	.04	NA		.1	.01	.03	ND	.04	NA	
GFP	.1	.03	.02	.01	.02	NA		.1	.03	.02	.01	.02	NA		.1	.03	.02	.01	.02	NA	

Supplementary Table 8. On and off-target activity of variants of the AAVS1 ZFN dimer bearing each single-residue substitution of N476 and Q531. For additional detail see legend to **Supplementary Table 4**.

FokI domain variant*	RNA dose (ng)	% indels					∑ OT indels	on:off ratio	
		AAVS1	OT1	OT2	OT3	OT4			
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	
< parent >	1600	69.7	14.68	19.07	5.82	9.00	48.57	1.4	
	800	68.5	11.73	17.39	4.74	7.40	41.26	1.7	←
	400	60.2	5.00	9.50	2.49	2.80	19.78	3.0	
	200	50.1	1.78	4.33	.91	.88	7.90	6.3	
R416D	800	76.6	.08	.04	.07	.06	.25	307	←
	400	66.3	.02	.01	.02	.01	.06	1109	
	200	50.3	.03	.02	.00	.00	.06	909	
R416E	800	91.4	.80	.76	.70	.42	2.69	34	
	400	87.0	.32	.29	.19	.15	.95	92	
	200	80.9	.13	.11	.07	.03	.35	233	←
	100	61.9	.03	.03	.01	.01	.08	789	
R416N	800	88.8	.95	1.76	.89	.61	4.21	21	
	400	83.7	.37	.54	.29	.22	1.43	59	
	200	73.8	.15	.20	.06	.02	.44	168	←
R416S	800	73.8	1.22	1.89	1.27	.81	5.18	14	←
	400	65.8	.50	.71	.46	.29	1.94	34	
	200	50.8	.18	.16	.12	.08	.55	93	
S418E	800	67.8	.01	.00	.00	.02	.04	1930	←
	400	55.2	.01	.02	.01	.03	.06	869	
	200	35.7	.00	.00	.00	.02	.03	1252	
R422H	800	70.3	.66	1.00	.79	.45	2.90	24	←
	400	61.9	.30	.49	.37	.19	1.35	46	
	200	48.5	.09	.14	.06	.07	.36	136	
N476G	800	79.5	.11	.43	.34	.17	1.05	76	
	400	71.3	.05	.19	.13	.05	.43	166	←
	200	54.2	.03	.03	.05	.02	.12	437	
I479Q	800	81.0	.03	.01	.00	.01	.06	1420	
	400	73.0	.02	.00	.00	.01	.03	2191	←
	200	58.5	.01	.00	.00	.01	.02	3703	
Q481A	800	92.2	.02	.02	.02	.02	.08	1143	
	400	88.6	.01	.00	.01	.02	.05	1943	
	200	83.6	.01	.02	.00	.01	.04	2352	←
	100	67.1	.00	.00	.00	.01	.02	3244	
Q481D	800	94.6	.02	.73	.14	.45	1.34	71	
	400	92.4	.01	.24	.02	.16	.42	219	
	200	89.9	.01	.12	.03	.05	.21	423	
	100	81.7	.00	.06	.00	.03	.10	851	←
Q481E	800	80.4	.01	.00	.00	.01	.02	5058	
	400	74.1	.00	.01	.00	.03	.05	1474	←
	200	58.3	.01	.00	.00	.01	.02	2638	
K525A	800	77.6	.05	.06	.04	.05	.20	396	
	400	69.3	.01	.05	.01	.04	.11	615	←
	200	54.7	.01	.01	.00	.02	.04	1402	
K525S	800	82.2	.07	.11	.07	.11	.35	232	
	400	73.7	.06	.07	.03	.06	.21	343	←
	200	58.5	.02	.03	.01	.01	.07	877	
K525T	800	86.5	.69	2.04	.45	.94	4.12	21	
	400	81.4	.29	.67	.19	.26	1.41	58	
	200	70.3	.08	.21	.06	.06	.40	176	←
< GFP >	400	.1	.00	.00	.00	.01	.01	NA	

*Each variant was tested as a dimer in which both ZFN-L and ZFR-R bore the indicated substitution

Supplementary Table 9. Activity and specificity of selected variants identified in FokI substitution screens of the AAVS1 ZFN dimer. Each variant was tested as a dimer in which both ZFN-L and ZFN-R bore the indicated substitution. ZFNs were delivered to human K562 cells via mRNA nucleofection at the dose indicated in column 2, followed by genomic DNA isolation at 3 days and deep sequencing analysis for indels at the intended target or four off-target sites. Each % indel value represents the average from four biological replicates. Column 3 provides the % indels measured at the intended target, while columns 4-7 indicate the % indels measured at four previously known off-target sites and column 8 indicates the sum of % indels at these four off-target sites. Column 9 lists the on:off-target indel ratio (= column 3 /

column 8). To highlight relative signal intensities, table values are embedded in heat maps (green – on target indels; red – off-target indels; blue – on:off ratio). Arrows highlight samples with activities comparable to or greater than the 800 ng dose of parents and that were consequently shown in Table 1.

wt oligo capture (400 ng)			wt			I479Q			Q481A		
hg38 coordinates		integrations	%indels		pval	%indels		pval	%indels		pval
			ZFN	GFP		ZFN	GFP		ZFN	GFP	
chr19	55115768	1330	66.29	0.00	0.00	78.48	0.00	0.00	85.49	0.00	0.00
chr3	184229818	174	9.61	0.00	0.00	0.06	0.00	0.04	0.01	0.00	0.56
chr1	198172184	455	5.73	0.00	0.00	0.09	0.02	0.04	0.02	0.01	0.91
chr20	35020704	163	4.04	0.00	0.00	0.01	0.03	1.00	0.03	0.00	0.39
chr1	181141476	195	3.49	0.00	0.00	0.01	0.00	0.35	0.00	0.00	1.00
chr3	50189772	209	3.39	0.00	0.00	0.04	0.00	0.04	0.00	0.01	1.00
chr11	76300996	67	2.58	0.01	0.00	0.05	0.01	0.04	0.01	0.01	1.00
chr13	40205820	55	2.46	0.00	0.00	0.02	0.01	0.24	0.00	0.01	1.00
chr12	47782518	107	2.12	0.02	0.00	0.02	0.02	0.79	0.00	0.02	1.00
chr13	26591916	36	2.12	0.00	0.00	0.01	0.00	0.24	0.01	0.00	0.91
chr4	11364294	49	1.66	0.00	0.00	0.01	0.02	1.00	0.00	0.00	1.00
chr5	68225722	79	1.56	0.00	0.00	0.03	0.00	0.04	0.02	0.00	0.39
chr17	64036794	154	1.55	0.00	0.00	0.03	0.03	0.86	0.02	0.05	1.00
chr11	61583600	81	1.31	0.00	0.00	0.03	0.00	0.04	0.01	0.00	0.56
chr18	48835990	31	1.23	0.00	0.00	0.02	0.01	0.41	0.03	0.01	0.52
chr12	126442484	21	1.15	0.00	0.00	0.03	0.00	0.04	0.00	0.01	1.00
chr10	73488048	31	0.94	0.01	0.00	0.00	0.10	1.00	0.05	0.00	0.39
chr3	37492070	54	0.94	0.00	0.00	0.02	0.02	0.63	0.01	0.05	1.00
chr10	68842214	55	0.91	0.00	0.00	0.02	0.01	0.33	0.02	0.00	0.61
chr14	30550484	34	0.91	0.01	0.00	0.01	0.00	0.14	0.01	0.02	1.00
chr7	128456566	50	0.79	0.02	0.00	0.01	0.10	1.00	0.03	0.04	1.00
chr2	96859552	83	0.73	0.00	0.00	0.01	0.01	0.39	0.02	0.00	0.39
chr3	172056094	19	0.65	0.00	0.00	ND	ND	ND	ND	ND	ND
chr19	2306802	28	0.61	0.00	0.00	0.00	0.02	1.00	0.00	0.00	1.00
chr19	11139414	75	0.59	0.00	0.00	0.01	0.01	0.88	0.00	0.00	1.00
chr21	5065744	20	0.59	0.02	0.00	0.01	0.01	0.79	0.00	0.00	1.00
chr3	184182170	22	0.57	0.00	0.00	0.01	0.01	1.00	0.00	0.00	1.00
chr11	45538310	22	0.57	0.01	0.00	0.01	0.00	0.38	0.00	0.01	1.00
chr19	49337262	66	0.55	0.00	0.00	0.03	0.01	0.09	0.01	0.00	0.56
chr19	1224746	51	0.52	0.00	0.00	0.01	0.00	0.24	0.01	0.00	0.56
chr8	144515636	41	0.51	0.00	0.00	0.01	0.01	0.83	0.01	0.01	0.91
chr15	88834438	50	0.47	0.00	0.00	0.02	0.00	0.09	0.01	0.00	0.56
chr15	84571034	96	0.46	0.02	0.00	0.05	0.01	0.27	0.02	0.02	0.91
chr1	14112422	19	0.45	0.00	0.00	0.02	0.01	0.07	0.01	0.00	0.39
chr15	81689072	32	0.44	0.01	0.00	0.03	0.01	0.14	0.01	0.01	0.91
chrX	15856772	78	0.44	0.01	0.00	0.04	0.12	1.00	0.08	0.01	0.12
chr12	113130622	27	0.41	0.06	0.00	0.01	0.02	1.00	0.01	0.02	1.00
chr19	47470024	29	0.39	0.00	0.00	0.03	0.01	0.07	0.01	0.00	0.91
chr7	102050526	40	0.34	0.00	0.00	0.01	0.02	1.00	0.00	0.03	1.00
chr2	36967986	42	0.33	0.01	0.00	0.02	0.05	1.00	0.04	0.01	0.39
chr14	103531006	31	0.32	0.00	0.00	0.05	0.01	0.04	0.01	0.00	0.68
chr12	54823166	27	0.32	0.01	0.00	0.00	0.00	0.35	0.01	0.01	1.00
chr13	49996346	28	0.30	0.03	0.00	0.04	0.01	0.07	0.04	0.03	0.75
chr5	1217650	41	0.30	0.00	0.00	0.01	0.00	0.35	0.00	0.00	1.00
chr18	11866942	32	0.30	0.00	0.00	0.03	0.01	0.04	0.01	0.01	0.91
chr16	70428130	47	0.30	0.01	0.00	0.00	0.00	0.35	0.00	0.01	1.00
chr17	39631920	37	0.29	0.00	0.00	0.01	0.02	1.00	0.01	0.01	1.00
chr3	134287458	38	0.27	0.01	0.00	0.01	0.00	0.35	0.00	0.01	1.00
chr1	3779130	170	0.27	0.00	0.00	0.01	0.00	0.07	0.02	0.00	0.39
chr11	48138440	28	0.27	0.01	0.00	0.01	0.01	0.69	0.00	0.01	1.00

Supplementary Table 10 (continued below)

wt oligo capture- continued			wt			I479Q			Q481A		
hg38 coordinates		integrations	%indels		pval	%indels		pval	%indels		pval
			ZFN	GFP		ZFN	GFP		ZFN	GFP	
chr2	217108872	24	0.26	0.01	0.00	0.01	0.00	0.46	0.00	0.02	1.00
chr5	148910878	22	0.25	0.00	0.00	0.01	0.01	0.79	0.01	0.01	0.91
chr19	42291652	24	0.25	0.01	0.00	0.03	0.01	0.09	0.00	0.01	1.00
chr15	70478526	30	0.22	0.00	0.00	0.00	0.01	1.00	0.01	0.00	0.56
chr17	75033800	28	0.22	0.00	0.00	0.01	0.00	0.28	0.00	0.01	1.00
chr13	72639358	19	0.22	0.00	0.00	0.00	0.00	0.63	0.00	0.00	1.00
chr11	78017714	22	0.22	0.00	0.00	0.01	0.00	0.07	0.00	0.00	0.56
chr15	32555628	28	0.21	0.02	0.00	0.01	0.03	1.00	0.03	0.02	0.68
chr3	14558032	20	0.21	0.01	0.00	0.01	0.01	0.93	0.03	0.01	0.56
chr12	103878180	54	0.20	0.00	0.00	0.05	0.07	1.00	0.02	0.01	0.56
chr16	81258590	33	0.20	0.01	0.00	0.01	0.03	1.00	0.00	0.03	1.00
chr8	99656330	20	0.20	0.00	0.00	0.01	0.01	0.79	0.01	0.00	0.65
chr5	157216616	29	0.20	0.01	0.00	0.02	0.00	0.07	0.01	0.02	1.00
chr2	25362220	31	0.19	0.02	0.00	0.01	0.02	1.00	0.00	0.02	1.00
chr15	30452078	28	0.19	0.03	0.00	0.01	0.02	1.00	0.00	0.04	1.00
chr1	93699008	23	0.18	0.00	0.00	0.01	0.00	0.09	0.01	0.00	0.65
chr6	40410142	21	0.17	0.00	0.00	0.01	0.00	0.14	0.00	0.01	1.00
chr18	41955254	27	0.17	0.00	0.00	0.01	0.01	0.89	0.01	0.01	1.00
chr7	6802396	24	0.16	0.00	0.00	0.00	0.03	1.00	0.04	0.00	0.39
chr8	47662294	37	0.16	0.00	0.00	0.00	0.00	0.35	0.00	0.01	1.00
chr6	7389498	21	0.15	0.00	0.00	0.00	0.01	1.00	0.01	0.00	0.54
chr7	5922514	24	0.15	0.01	0.00	0.03	0.02	0.37	0.00	0.01	1.00
chr1	31425552	35	0.14	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00
chr22	21443968	50	0.14	0.01	0.00	0.02	0.00	0.06	0.00	0.01	1.00
chr19	14571814	20	0.14	0.02	0.00	0.03	0.00	0.04	0.00	0.04	1.00
chr3	111979030	22	0.14	0.01	0.00	0.01	0.02	1.00	0.01	0.01	1.00
chr2	105038548	39	0.13	0.00	0.00	0.01	0.02	1.00	0.01	0.01	0.91
chr9	131820192	29	0.12	0.00	0.00	0.01	0.00	0.38	0.02	0.00	0.56
chr11	78262418	22	0.11	0.00	0.00	0.01	0.00	0.07	0.00	0.00	1.00
chr8	60233954	23	0.11	0.00	0.00	0.00	0.00	0.79	0.01	0.00	0.39
chr20	45905200	21	0.10	0.01	0.00	0.01	0.01	0.74	0.00	0.00	0.91
chr7	98643926	28	0.10	0.00	0.04	0.02	0.06	1.00	0.06	0.00	0.56
chr12	53051038	26	0.09	0.01	0.00	0.02	0.03	1.00	0.02	0.01	0.83
chr11	65614374	26	0.09	0.00	0.00	0.02	0.00	0.12	0.01	0.00	0.68
chr15	40764304	29	0.09	0.01	0.00	0.01	0.00	0.14	0.01	0.01	1.00
chr22	18958826	22	0.08	0.01	0.00	0.01	0.01	0.89	0.01	0.01	1.00
chr1	31712738	29	0.08	0.00	0.00	0.01	0.00	0.35	0.02	0.00	0.50
chr20	37880952	25	0.07	0.01	0.00	0.02	0.01	0.35	0.01	0.01	0.91
chr1	39214102	23	0.05	0.02	0.02	0.02	0.03	1.00	0.00	0.02	1.00
chr22	22201860	24	0.05	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00
chr17	44086880	22	0.05	0.01	0.01	0.01	0.01	0.86	0.00	0.02	1.00
chr17	77279998	54	0.05	0.00	0.01	0.00	0.00	0.35	0.00	0.00	1.00
chr3	195857016	24	0.04	0.01	0.01	0.01	0.01	1.00	0.02	0.00	0.56
chr22	20279990	19	0.04	0.00	0.01	0.01	0.01	0.79	0.01	0.01	0.98
chr22	23187146	22	0.02	0.00	0.01	0.00	0.01	1.00	0.02	0.00	0.39
chr21	44196402	20	ND	ND	ND	ND	ND	ND	ND	ND	ND
chrX	40329304	28	ND	ND	ND	ND	ND	ND	ND	ND	ND
chr17	76685626	27	ND	ND	ND	ND	ND	ND	ND	ND	ND
chr5	180101344	20	ND	ND	ND	ND	ND	ND	ND	ND	ND
chr8	140496946	62	ND	ND	ND	ND	ND	ND	ND	ND	ND

Supplementary Table 10. Indel analysis of the top 100 ranked candidate off-target loci for the parent AAVS1 ZFNs, assessed using genomic DNA from K562 cells treated with the parent ZFNs, the I479Q variant dimer, the Q481A variant dimer, or GFP control. Loci are sorted by %indels observed with the parent AAVS1 ZFN pair. Values represent individual measurements. %indels at the intended target in AAVS1 are highlighted in green and %indels at all other loci are highlighted with a red heat map. P-values for each ZFN-GFP comparison are shown to the right of each pair of indel values (see **Supplementary Note 2 for details of the statistical test**). P-values were corrected using the Benajmini-Hochburg false discovery rate method. Statistically significant p-values are highlighted in blue. ND indicates that no data were obtained due to a failed PCR or sequencing analysis failure.

FokI domain variant	% on-taget indels	
parent - full dose	59.68	
	63.25	
	61.54	
parent - half dose	55.39	
	51.62	
	50.58	
R416E	78.02	←
R416F	81.32	←
R416N	64.13	←
S418D	56.91	
S418E	48.63	
R422H	60.19	
N476D	20.92	
N476E	14.53	
N476G	70.33	←
N476T	73.56	←
I479T	1.77	
I479Q	4.81	
Q481A	31.92	
Q481D	89.89	←
Q481E	48.98	
Q481H	86.78	←
K525A	63.12	←
K525S	71.12	←
K525T	79.44	←
K525V	77.79	←
N527D	40.64	
GFP	0.01	
GFP	0.01	
GFP	0.08	

Supplementary Table 11. On-target activity of variants of the PD1 ZFN dimer bearing single-residue substitutions within their FokI domain. Each variant was tested as a dimer in which both ZFNs bore the indicated substitution. ZFNs were delivered to human K562 cells via mRNA nucleofection (500 ng of each monomer), followed by genomic DNA isolation at day 3 and deep sequencing analysis for indels at the intended target. Values represent individual measurements. "Full dose" parent samples used the same 500ng dose as the variants and "half dose" parent samples used 250 ng RNA for delivery. To highlight relative signal intensities, table values are embedded in a green heat map. Arrows highlight variants that manifested activity at least as high as the average of the parent "full dose" replicates and that were characterized in follow-up studies.

FokI domain variant	RNA dose (ng)	% indels				∑ OT indels	on:off ratio
		PD1	OT1	OT2	OT3		
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
< parent >	1000	75.0	3.14	2.41	.85	6.41	12
	500	60.8	.28	.30	.13	.72	84
R416E	500	83.0	.01	.05	.01	.07	1234
	250	69.8	.01	.01	.00	.02	3238
R416F	250	69.5	.01	.04	.02	.07	1032
R416N	500	81.5	.04	.04	.03	.10	800
	250	66.2	.01	.01	.01	.03	2539
R422H	500	55.2	.02	.06	.03	.11	514
K448A	500	60.5	.16	.10	.07	.33	183
	250	43.3	.04	.03	.02	.08	510
N476G	500	64.7	.02	.02	.02	.05	1287
	250	51.6	.01	.01	.01	.03	2011
N476T	500	69.8	.14	.13	.14	.40	173
	250	58.8	.04	.03	.05	.12	495
Q481D	250	81.4	.01	.02	.00	.03	2882
	125	63.7	.01	.01	.00	.01	4571
Q481H	250	87.3	.94	.26	.06	1.26	69
	125	75.3	.12	.06	.02	.20	379
K525A	500	54.4	.01	.00	.00	.01	6421
K525S	500	66.3	.00	.03	.00	.03	1902
	250	46.5	.00	.00	.00	.01	5267
K525T	500	78.8	.04	.04	.02	.11	729
	250	63.6	.01	.02	.01	.04	1645
K525V	500	75.6	.01	.03	.01	.05	1477
	250	63.9	.01	.03	.01	.04	1462
GFP	500	.0	.00	.00	.00	.01	NA

Supplementary Table 12. On and off-target activity of variants of the PD1 ZFN dimer bearing single-residue substitutions within their FokI domain. Each variant was tested as a dimer in which both ZFNs bore the indicated substitution (column 1, "parent" indicates the previously reported PD1 ZFN pair[48]). ZFNs were delivered to human K562 cells via nucleofection using the indicated amount of mRNA for each ZFN monomer (column 2), followed by genomic DNA isolation at 3 days and deep sequencing analysis for indels at the intended target. Column 3 provides % indels measured at the intended target, with columns 4-6 indicating % indels at three previously known off-target sites. Values are the average of three biological replicates. Column 7 lists the sum of off-target indels, with column 8 giving on:off-target indel ratio (= column 3 / column 7). To help highlight relative signal intensities, table values are embedded in heat maps (green – on target indels; red – off-target indels; blue – on:off ratio).

sample number	Left ZFN								Right ZFN								%indels						
																	BCL11A			OT1			
	UID	F1	F2	F3	F4	F5	F6	Σ	UID	F1	F2	F3	F4	F5	Σ		100ng	200ng	400ng	100ng	200ng	400ng	
1	63007							0	63015						0		58.98	80.73	84.95	0.46	4.74	23.50	←
2	63007							0	63016	Q					1		62.36	79.09	85.79	0.51	3.29	19.21	←
3	63007							0	63017		Q				1		48.86	75.15	80.64	0.12	1.53	7.12	
4	63007							0	63018				Q		1		78.60	86.66	89.00	0.13	2.95	11.97	
5	63007							0	65765			Q			1		69.36	83.87	87.01	0.68	7.13	23.18	
6	63007							0	65766					Q	1		60.75	79.40	85.24	0.17	2.24	14.86	
7	63007							0	63019	Q	Q				2		55.87	79.97	84.86	0.16	1.21	8.03	←
8	63007							0	63020	Q			Q		2		78.13	87.03	82.04	0.56	4.16	15.45	
9	63007							0	63021		Q		Q		2		78.79	87.20	90.03	0.30	3.03	17.29	
10	63007							0	65767			Q		Q	2		70.01	86.05	89.89	0.06	0.81	6.92	
11	63007							0	63022	Q	Q		Q		3		73.04	87.42	90.72	0.12	1.20	4.48	←
12	63007							0	65768	Q	Q	Q	Q		4		72.14	88.82	90.19	0.02	0.25	1.04	←
13	63007							0	65769	Q	Q		Q	Q	4		62.17	85.31	91.68	0.03	0.27	1.41	
14	63007							0	65770	Q	Q	Q	Q	Q	5		64.47	84.45	90.46	0.01	0.05	0.38	←
15	63008	Q						1	63015						0		50.47	75.32	81.89	0.25	2.92	13.29	
16	63009			Q				1	63015						0		45.48	80.85	83.89	0.63	3.35	17.78	
17	63010					Q		1	63015						0		45.10	71.16	77.85	0.16	2.53	12.91	
18	65751		Q					1	63015						0		53.08	81.94	85.18	0.22	2.67	15.21	
19	65752				Q			1	63015						0		45.06	74.65	84.52	0.34	1.97	18.19	
20	65753						Q	1	63015						0		53.87	81.07	84.04	0.23	1.94	11.44	
21	63011	Q		Q				2	63015						0		48.79	78.31	87.88	0.20	0.95	7.49	
22	63012	Q				Q		2	63015						0		27.74	63.38	78.44	0.07	0.97	5.16	
23	63013			Q		Q		2	63015						0		33.78	67.09	82.86	0.14	0.97	6.29	
24	65754		Q		Q			2	63015						0		35.25	69.76	81.14	0.08	0.79	3.99	
25	65755		Q				Q	2	63015						0		34.35	67.85	83.35	0.13	0.68	6.95	
26	65756				Q		Q	2	63015						0		20.81	66.79	81.36	0.02	0.25	2.25	
27	63014	Q		Q		Q		3	63015						0		23.12	61.04	76.50	0.07	0.32	3.95	
28	65757		Q		Q		Q	3	63015						0		20.81	57.68	75.40	0.06	0.15	1.53	
29	65758	Q	Q	Q		Q		4	63015						0		21.13	53.08	75.41	0.04	0.29	3.02	
30	65759	Q		Q	Q	Q		4	63015						0		9.28	36.97	63.30	0.01	0.09	0.78	
31	65760	Q		Q		Q	Q	4	63015						0		10.00	35.17	61.63	0.02	0.26	0.43	
32	65761	Q	Q	Q	Q	Q		5	63015						0		6.00	31.75	61.33	0.01	0.17	0.63	
33	65762	Q	Q	Q		Q	Q	5	63015						0		5.02	25.27	57.73	0.01	0.06	0.64	
34	65763	Q		Q	Q	Q	Q	5	63015						0		1.38	14.04	39.75	0.02	0.00	0.16	
35	65764	Q	Q	Q	Q	Q	Q	6	63015						0		1.47	9.26	30.20	0.00	0.01	0.09	
36	GFP							NA	NA						NA		0.02	0.02	0.01	0.01	0.00	0.00	

Supplementary Table 13. On and off-target activity of variants of the BCL11A ZFN dimer bearing the R(-5)Q substitution in the indicated zinc fingers. ZFNs were delivered to human K562 cells via mRNA nucleofection, followed by genomic DNA isolation at day 3 and deep sequencing analysis for indels at the intended target or a known off-target site. The "Left ZFN" and "Right ZFN" panels summarize the design properties of each tested ZFN, with "Q" indicating an Arg→Gln mutation at position -5 of the indicated finger and the summation symbol (∑) indicating the total number of substituted fingers. Unique identifiers for each construct are shown in the columns labeled "UID". The "% indels" panel provides the % indels measured for each construct at the BCL11A on-target and also a known off-target (OT1) as a function of delivered mRNA dose (100ng, 200ng or 400ng). Values represent individual measurements. To help highlight relative signal intensities, table values are embedded in heat maps (green – on target indels; red – off-target indels). Arrows highlight samples that were subsequently tested in triplicate and shown in **Figure 3c**.

Right ZFP	% indels			∑ OT indels	on:off ratio
	BCL11A	OT1	OT2		
1	*2*	*3*	*4*	*5*	*6*
Parent	86.0	20.26	2.34	22.60	3.8
1x R(-5)Q	87.3	13.39	.94	14.33	6.1
2x R(-5)Q	84.5	5.39	.26	5.65	15
3x R(-5)Q	92.2	5.79	.25	6.04	15
4x R(-5)Q	91.5	1.57	.12	1.69	54
5x R(-5)Q	90.4	.26	.04	.30	301
GFP	.0	.01	.00	.01	NA

Supplementary Table 14. Tabular format of data graphed in **Figure 3c**. Each value is the average of three biological replicate 400ng transfections in human K562 cells. ZFNs tested in this study were those highlighted with an arrow in **Supplementary Table 13**.

Left ZFN		Right ZFN		% indels			∑ OT indels	on:off ratio
				BCL11A	OT1	OT2		
FokI	ZFP	FokI	ZFP					
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Parent	Parent	Parent	Parent	86.87	5.01	.31	5.33	16.3
Parent	Parent	R416S	Parent	86.79	.57	.07	.64	135.9
Parent	3x R(-5)Q	R416S	3x R(-5)Q	86.29	.07	.01	.09	1006
Parent	3x R(-5)Q	R416S	4x R(-5)Q	86.32	.02	.00	.02	4822
Parent	3x R(-5)Q	R416S	5x R(-5)Q	86.31	.00	.01	.02	5137
Parent	3x R(-5)Q	K525S	4x R(-5)Q	83.47	.01	.00	.01	5878
GFP				0.01	.01	.01	.02	NA

Supplementary Table 15. On-target and off-target activity of BCL11A ZFN variants containing different combinations of zinc finger backbone variants and FokI variants expressed in human CD34+ hematopoietic stem and progenitor cells. Values represent individual measurements. This clinically relevant cell type tends to show lower off-target activity and more tolerance for R(-5)Q mutations in the left ZFN than did the K562 cell line utilized for the initial experiments shown in **Supplementary Figure7 and Supplementary Tables 13-14**. For the left ZFN, "3xR(-5)Q" indicates substitutions within fingers 1, 3, and 5. For the right ZFN constructs, substituted fingers are: 3xR(-5)Q - fingers 1, 2, and 4; 4xR(-5)Q - fingers 1, 2, 3, and 4; 5xR(-5)Q - all fingers.

	Left ZFN		Right ZFN		TCRα on-target %indels			OT1 %indels			
ZFN Pair	FokI	ZFP	FokI	ZFP							
1	Parent	Parent	Parent	Parent	95.45	96.24	96.03	6.25	38.88	75.22	←
2	Parent	Parent	Parent	3x R(-5)Q	92.85	94.95	96.10	0.10	0.68	16.28	
3	Parent	Parent	R416E	Parent	63.01	88.89	91.66	0.06	0.89	12.39	
4	Parent	Parent	R416E	3x R(-5)Q	41.66	76.80	88.29	0.01	0.01	0.12	
5	Parent	Parent	Q481E	Parent	81.94	92.79	95.53	0.05	0.22	4.96	
6	Parent	Parent	Q481E	3x R(-5)Q	64.60	ND	94.82	0.00	0.06	0.02	
7	Parent	Parent	K525S	Parent	76.56	92.60	94.51	0.05	1.29	16.67	
8	Parent	Parent	K525S	3x R(-5)Q	58.09	88.11	93.88	0.00	0.00	0.19	
9	Parent	3x R(-5)Q	Parent	Parent	95.38	96.97	96.47	0.45	4.13	32.74	
10	Parent	3x R(-5)Q	Parent	3x R(-5)Q	92.63	ND	96.22	0.01	0.20	2.65	
11	Parent	3x R(-5)Q	R416E	Parent	94.28	ND	97.26	0.02	0.17	0.35	
12	Parent	3x R(-5)Q	R416E	3x R(-5)Q	88.98	94.24	96.65	0.01	0.03	0.01	←
13	Parent	3x R(-5)Q	Q481E	Parent	91.62	96.57	97.67	0.00	0.01	0.06	←
14	Parent	3x R(-5)Q	Q481E	3x R(-5)Q	87.87	95.39	96.74	0.00	0.01	0.02	←
15	Parent	3x R(-5)Q	K525S	Parent	94.46	95.40	95.61	0.00	0.10	0.66	
16	Parent	3x R(-5)Q	K525S	3x R(-5)Q	91.79	96.33	ND	0.00	0.01	0.04	
17	R416E	Parent	Parent	Parent	95.69	96.93	97.44	2.08	25.33	75.31	
18	R416E	Parent	Parent	3x R(-5)Q	92.58	96.06	96.80	0.02	0.26	4.87	
19	R416E	Parent	R416E	Parent	93.19	95.66	96.65	0.05	0.90	19.92	
20	R416E	Parent	R416E	3x R(-5)Q	77.24	92.52	94.97	0.00	0.06	0.22	
21	R416E	Parent	Q481E	Parent	86.79	95.41	97.49	0.01	0.11	0.65	
22	R416E	Parent	Q481E	3x R(-5)Q	71.32	93.01	96.27	0.01	0.00	0.04	
23	R416E	Parent	K525S	Parent	91.45	96.57	97.59	0.04	0.35	9.52	
24	R416E	Parent	K525S	3x R(-5)Q	82.46	94.74	96.76	0.00	0.00	0.04	←
25	R416E	3x R(-5)Q	Parent	Parent	79.74	92.67	93.38	0.06	0.32	4.49	
26	R416E	3x R(-5)Q	Parent	3x R(-5)Q	74.63	90.47	91.39	0.02	0.06	0.41	
27	R416E	3x R(-5)Q	R416E	Parent	94.26	97.58	96.69	0.03	0.06	0.26	
28	R416E	3x R(-5)Q	R416E	3x R(-5)Q	89.95	95.73	96.84	0.00	0.01	0.00	←
29	R416E	3x R(-5)Q	Q481E	Parent	69.12	93.61	95.10	0.00	0.02	0.11	
30	R416E	3x R(-5)Q	Q481E	3x R(-5)Q	71.54	91.00	93.86	0.00	0.00	0.02	
31	R416E	3x R(-5)Q	K525S	Parent	90.80	96.43	96.70	0.01	0.04	0.16	
32	R416E	3x R(-5)Q	K525S	3x R(-5)Q	90.00	95.39	97.05	0.01	0.01	0.00	←
33	Q481E	Parent	Parent	Parent	88.49	95.57	96.72	0.03	1.10	22.57	
34	Q481E	Parent	Parent	3x R(-5)Q	64.77	88.15	95.46	0.01	0.00	0.10	
35	Q481E	Parent	R416E	Parent	46.58	77.85	89.47	0.00	0.01	0.39	
36	Q481E	Parent	R416E	3x R(-5)Q	27.48	59.51	81.63	0.01	0.00	0.00	
37	Q481E	Parent	Q481E	Parent	43.36	84.24	90.98	0.00	0.01	0.03	
38	Q481E	Parent	Q481E	3x R(-5)Q	27.48	56.71	83.70	0.01	0.00	0.02	
39	Q481E	Parent	K525S	Parent	54.68	83.39	93.36	0.00	0.00	0.12	
40	Q481E	Parent	K525S	3x R(-5)Q	40.66	72.35	88.83	0.00	0.01	0.00	
41	Q481E	3x R(-5)Q	Parent	Parent	52.30	90.89	94.18	0.00	0.01	0.10	
42	Q481E	3x R(-5)Q	Parent	3x R(-5)Q	38.74	82.99	93.63	0.01	0.00	0.00	
43	Q481E	3x R(-5)Q	R416E	Parent	70.79	93.43	96.13	0.00	0.00	0.00	←
44	Q481E	3x R(-5)Q	R416E	3x R(-5)Q	49.81	86.44	91.76	0.00	0.00	0.00	
45	Q481E	3x R(-5)Q	Q481E	Parent	35.03	82.94	93.16	0.01	0.01	0.00	
46	Q481E	3x R(-5)Q	Q481E	3x R(-5)Q	21.13	72.84	ND	0.01	0.01	0.01	
47	Q481E	3x R(-5)Q	K525S	Parent	55.38	91.58	91.28	0.00	0.01	0.01	
48	Q481E	3x R(-5)Q	K525S	3x R(-5)Q	39.04	85.33	94.37	0.01	0.00	0.00	
49	K525S	Parent	Parent	Parent	94.30	96.75	94.04	0.33	6.27	55.29	
50	K525S	Parent	Parent	3x R(-5)Q	88.95	95.10	93.36	0.01	0.07	1.39	
51	K525S	Parent	R416E	Parent	69.54	89.41	93.25	0.02	0.16	1.58	
52	K525S	Parent	R416E	3x R(-5)Q	43.32	81.81	91.00	0.01	0.00	0.00	
53	K525S	Parent	Q481E	Parent	69.24	91.49	94.59	0.01	0.04	0.00	
54	K525S	Parent	Q481E	3x R(-5)Q	47.56	80.23	92.42	0.01	0.01	0.01	
55	K525S	Parent	K525S	Parent	84.24	92.86	95.10	0.00	0.07	0.34	
56	K525S	Parent	K525S	3x R(-5)Q	59.06	87.95	93.39	0.01	0.00	0.00	
57	K525S	3x R(-5)Q	Parent	Parent	85.92	94.92	92.50	0.01	0.03	2.16	
58	K525S	3x R(-5)Q	Parent	3x R(-5)Q	78.57	88.72	95.81	0.01	0.02	0.15	
59	K525S	3x R(-5)Q	R416E	Parent	92.84	97.71	97.76	0.01	0.00	0.04	←
60	K525S	3x R(-5)Q	R416E	3x R(-5)Q	87.25	91.59	96.61	0.00	0.00	0.00	←
61	K525S	3x R(-5)Q	Q481E	Parent	79.54	88.74	96.47	0.01	0.01	0.01	←
62	K525S	3x R(-5)Q	Q481E	3x R(-5)Q	62.41	91.01	94.81	0.00	0.00	0.00	←
63	K525S	3x R(-5)Q	K525S	Parent	89.91	96.22	97.42	0.00	0.01	0.06	←
64	K525S	3x R(-5)Q	K525S	3x R(-5)Q	84.83	94.76	96.63	0.01	0.00	0.00	←
GFP					0.00	0.01	0.01	0.00	0.01	0.01	

Supplementary Table 16. On and off-target activity of variants of the TCRα ZFN dimer bearing the indicated FokI domain variants and R(-5)Q substitutions in one or both ZFNs as indicated. ZFNs were delivered to human K562 cells via mRNA nucleofection, followed by genomic DNA isolation at day 3 and deep sequencing analysis for indels at the intended target or four off-target sites. The "Left ZFN" and "Right ZFN" panels summarize the design properties of each tested ZFN. For details see **Figure 4b**. Values represent individual measurements. The "% indels" panel provides the % indels measured for each construct at the TCRα on-target and also a known off-target (OT1) as a function of delivered mRNA dose (100ng, 200ng or 400ng). To help highlight relative signal intensities, table values are embedded in heat maps (green – on target indels; red – off-target indels). Dimers highlighted by arrows were further characterized by an oligonucleotide capture assay to assess their genome-wide specificity.

ZFN pair 13 oligo capture			ZFN pair 13		
hg38 coordinates		integrations	%indels ZFN	%indels GFP	pval
chr14	22550608*	3583	97.31	0.00	0.00
chr17	362912*	62	0.06	0.00	0.02
chr3	52219054*	10	0.01	0.03	1.00
chr15	44711562	6	0.01	0.02	0.97
chr19	6929724	6	0.02	0.00	0.22
chr9	128878058*	5	0.12	0.00	0.00
chr11	65980906	5	ND	ND	ND
chr12	53004488*	5	0.01	0.03	1.00
chr10	42079890	4	ND	ND	ND
chr10	42081716	4	ND	ND	ND
chr1	143197542	4	ND	ND	ND
chr1	143268830	4	ND	ND	ND
chr3	90590546	4	ND	ND	ND
chr7	158413558*	4	0.05	0.02	0.10
chr8	73445383	4	0.06	0.03	0.28

ZFN pair 14 oligo capture			ZFN pair 14		
hg38 coordinates		integrations	%indels ZFN	%indels GFP	pval
chr14	22550604*	7964	98.21	0.00	0.00
chr17	362912*	85	0.06	0.03	0.81
chr14	90113594	12	0.01	0.00	0.59
chr3	52219056*	6	0.01	0.03	1.00
chr1	109667898	6	0.00	0.01	1.00
chr1	157197150	6	ND	ND	ND
chr6	31769916*	6	0.01	0.00	0.68
chr1	143204642	6	ND	ND	ND
chr9	131231218	6	ND	ND	ND
chr17	26603966	6	0.00	0.01	1.00
chr6	43246754	5	0.01	0.01	1.00
chr19	5714676	5	ND	ND	ND
chr2	23958828	5	0.04	0.03	1.00
chr6	150872954	5	0.01	0.02	1.00
chr6	27639956	5	ND	ND	ND
chr8	91295868	5	0.00	0.00	0.68
chr7	158413560*	5	0.02	0.02	1.00
chr1	17816562	4	0.00	0.01	1.00
chr1	178369132	4	0.02	0.00	0.59
chr1	178369156	4	0.00	0.05	1.00
chr10	46389424	4	0.00	0.00	1.00
chr10	47470352	4	0.00	0.02	1.00
chr11	113374268	4	0.01	0.04	1.00
chr12	57858376	4	ND	ND	ND
chr14	63256990	4	ND	ND	ND
chr15	43609808	4	ND	ND	ND
chr15	43709280	4	ND	ND	ND
chr21	37143208	4	ND	ND	ND
chr3	64835606	4	0.00	0.00	1.00
chr7	65183196	4	0.00	0.05	1.00
chr7	65689530	4	0.02	0.00	0.59

ZFN pair 59 oligo capture			ZFN pair 59		
hg38 coordinates		integrations	%indels ZFN	%indels GFP	pval
chr14	22550604*	4178	96.33	0.00	0.00
chr9	128878058*	25	0.17	0.01	0.00
chr17	362908*	16	0.01	0.03	1.00
chr19	35309582*	6	0.02	0.02	0.57
chr17	26881268	3	ND	ND	ND
chr3	185910172	3	ND	ND	ND
chr6	170171158	3	ND	ND	ND
chr9	114733852	3	0.07	0.03	0.21

ZFN pair 60 oligo capture			ZFN pair 60		
hg38 coordinates		integrations	%indels ZFN	%indels GFP	pval
chr14	22550604*	4247	97.00	0.00	0.00
chr2	15345444	3	ND	ND	ND
chr21	8990878	3	0.12	0.07	0.52

ZFN pair 63 oligo capture			ZFN pair 63		
hg38 coordinates		integrations	%indels ZFN	%indels GFP	pval
chr14	22550604*	3379	96.65	0.00	0.00
chr9	128878054*	36	0.09	0.01	0.09
chr17	362912*	14	0.04	0.02	0.38
chr3	52219056*	6	0.06	0.00	0.05
chr1	34213558	5	0.22	0.12	0.13
chr2	218506758	5	ND	ND	ND
chr20	47319050	4	0.01	0.02	0.83
chr10	38177402	4	0.07	0.42	1.00
chr12	55494752	4	ND	ND	ND
chr17	35586862	4	0.04	0.02	0.38
chr20	39075318	4	0.05	0.05	0.83
chr20	39075358	4	0.00	0.01	1.00

Supplementary Table 17. Table view of oligonucleotide duplex capture results for variant pairs 13, 14, 59, 60 and 63 (columns 1-3 of each table), with indel levels observed at these loci in genomic DNA from T-cells treated with ZFNs or a

GFP control (columns 4-5). Integrations are the total unique integrations from four biological replicates and indel values represent an individual measurement. Loci are ranked in order of decreasing number of capture events, with the intended target locus at top. Column 6 in each table provides p-values for each corresponding ZFN-GFP comparison. See **Supplementary Note 2** for details of the statistical test.

ZFN Pair 1 oligo capture			ZFN pair 1			ZFN pair 13			ZFN pair 14			ZFN pair 59			ZFN pair 60			ZFN pair 63		
hg38 coordinates		integrations	%indels			%indels			%indels			%indels			%indels			%indels		
			ZFN	GFP	pval	ZFN	GFP	pval	ZFN	GFP	pval	ZFN	GFP	pval	ZFN	GFP	pval	ZFN	GFP	pval
chr14	22550604	3185	96.60	0.00	0.00	97.31	0.00	0.00	98.21	0.00	0.00	96.33	0.00	0.00	97.00	0.00	0.00	96.65	0.00	0.00
chr14	49621284	135	10.74	0.00	0.00	0.06	0.00	0.05	0.01	0.00	0.61	0.03	0.00	0.25	0.01	0.00	0.51	0.07	0.00	0.03
chr9	13565900	359	10.42	0.00	0.00	0.02	0.01	0.18	0.00	0.01	0.92	0.00	0.01	0.92	0.00	0.00	0.92	0.00	0.01	0.92
chr9	128878060	428	5.52	0.00	0.00	0.12	0.00	0.00	0.00	0.00	1.00	0.12	0.00	0.00	0.02	0.00	0.51	0.25	0.00	0.00
chr10	132515136	79	2.77	0.00	0.00	0.03	0.01	0.36	0.04	0.00	0.55	0.01	0.01	0.76	0.03	0.01	0.51	0.03	0.01	0.44
chr8	60701070	31	1.64	0.00	0.00	0.08	0.00	0.05	0.00	0.00	1.00	0.01	0.00	0.37	0.01	0.00	0.51	0.02	0.00	0.33
chr6	118713042	52	1.17	0.04	0.00	0.12	0.01	0.00	0.01	0.01	0.97	0.04	0.01	0.25	0.04	0.01	0.47	0.04	0.01	0.20
chr15	37345596	45	0.84	0.14	0.00	0.02	0.05	1.00	0.00	0.08	1.00	0.00	0.08	1.00	0.02	0.05	1.00	0.02	0.08	1.00
chr19	33383802	94	0.51	0.00	0.00	0.00	0.04	1.00	0.09	0.02	0.55	0.02	0.02	0.76	0.02	0.02	0.97	0.00	0.02	1.00
chr2	26018400	91	0.47	0.01	0.00	0.03	0.01	0.36	0.03	0.03	1.00	0.01	0.00	0.37	0.00	0.02	1.00	0.02	0.03	1.00
chr6	44255520	142	0.42	0.00	0.00	0.03	0.00	0.36	0.00	0.00	1.00	0.01	0.01	0.76	0.01	0.00	0.51	0.00	0.01	1.00
chr6	110569182	67	0.37	0.03	0.00	0.03	0.01	0.36	0.00	0.03	1.00	0.02	0.04	1.00	0.02	0.05	1.00	0.03	0.01	0.44
chr9	98054342	65	0.37	0.04	0.00	0.15	0.02	0.01	0.02	0.03	1.00	0.11	0.01	0.06	0.03	0.02	0.92	0.03	0.04	1.00
chr22	23280666	78	0.35	0.06	0.00	0.01	0.04	1.00	0.00	0.00	1.00	0.02	0.00	0.37	0.01	0.04	1.00	0.05	0.00	0.20
chr12	109930438	163	0.34	0.06	0.00	0.07	0.00	0.01	0.04	0.00	0.53	0.10	0.00	0.00	0.04	0.00	0.47	0.04	0.00	0.13
chr5	36839152	43	0.30	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
chr16	77191236	50	0.26	0.00	0.00	0.02	0.00	0.36	0.02	0.00	0.61	0.03	0.00	0.30	0.00	0.00	1.00	0.02	0.00	0.44
chr2	26785730	131	0.22	0.02	0.00	0.01	0.00	0.36	0.01	0.00	0.61	0.01	0.00	0.37	0.01	0.00	0.51	0.04	0.00	0.16
chr6	52934244	49	0.22	0.01	0.00	0.01	0.01	0.80	0.00	0.01	1.00	0.02	0.00	0.30	0.01	0.01	1.00	0.01	0.00	0.44
chr7	149866310	32	0.20	0.02	0.00	ND	ND	ND	0.01	0.02	1.00	0.01	0.03	1.00	0.00	0.03	1.00	0.00	0.04	1.00
chr14	60248818	40	0.20	0.00	0.00	0.00	0.00	1.00	0.01	0.00	0.61	0.02	0.00	0.30	0.02	0.00	0.47	0.06	0.00	0.16
chr9	136086174	65	0.19	0.01	0.00	0.01	0.04	1.00	0.00	0.03	1.00	0.01	0.04	1.00	0.00	0.04	1.00	0.02	0.03	1.00
chr15	45153400	33	0.18	0.01	0.00	0.03	0.01	0.36	0.00	0.04	1.00	0.00	0.04	1.00	0.04	0.01	0.47	0.01	0.01	0.90
chr20	25223350	33	0.16	0.00	0.05	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
chr11	62401684	29	0.14	0.04	0.05	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
chr20	60891662	44	0.14	0.01	0.00	0.01	0.02	1.00	0.03	0.02	0.80	0.00	0.02	1.00	0.02	0.02	0.96	0.02	0.02	0.90
chr12	21752278	41	0.12	0.00	0.00	0.01	0.01	0.68	0.02	0.04	1.00	0.02	0.01	0.53	0.01	0.03	1.00	0.02	0.02	1.00
chr17	79736684	397	0.11	0.01	0.00	0.02	0.01	0.36	0.02	0.01	0.64	0.01	0.01	0.76	0.01	0.01	1.00	0.02	0.01	0.46
chr2	171158996	28	0.11	0.01	0.00	0.01	0.00	0.36	0.03	0.00	0.55	0.02	0.00	0.30	0.02	0.00	0.47	0.00	0.00	1.00
chrX	119851942	26	0.11	0.01	0.01	0.00	0.03	1.00	0.00	0.01	1.00	0.00	0.03	1.00	0.01	0.03	1.00	0.01	0.03	1.00
chr4	3667514	50	0.11	0.01	0.00	0.01	0.00	0.36	0.02	0.00	0.55	0.00	0.02	1.00	0.00	0.02	1.00	0.00	0.01	1.00
chr8	129726442	104	0.09	0.01	0.00	0.01	0.01	0.64	0.01	0.00	0.62	0.01	0.01	0.76	0.02	0.00	0.47	0.00	0.02	1.00
chr1	241848280	31	0.09	0.02	0.05	0.02	0.01	0.36	0.01	0.02	1.00	0.02	0.02	0.53	0.01	0.01	0.96	0.01	0.02	1.00
chr19	19384988	25	0.08	0.01	0.01	0.01	0.00	0.36	0.00	0.00	1.00	0.01	0.00	0.37	0.01	0.00	0.47	0.00	0.00	0.44
chr5	169869406	32	0.08	0.00	0.02	0.01	0.04	1.00	0.01	0.11	1.00	0.04	0.00	0.25	0.01	0.08	1.00	0.02	0.07	1.00
chr19	35309590	273	0.08	0.01	0.01	0.03	0.01	0.36	0.05	0.02	0.61	0.04	0.01	0.18	0.02	0.02	1.00	0.07	0.01	0.01
chr8	28528022	77	0.08	0.00	0.01	0.00	0.00	1.00	0.01	0.00	0.80	0.01	0.00	0.37	0.01	0.00	0.65	0.00	0.00	0.94
chr12	107612674	56	0.06	0.00	0.00	0.01	0.00	0.51	0.01	0.00	0.80	0.01	0.00	0.57	0.00	0.00	1.00	0.01	0.00	0.20
chr2	3655228	45	0.06	0.00	0.05	0.00	0.03	1.00	0.01	0.02	1.00	0.02	0.02	0.76	0.00	0.04	1.00	0.00	0.02	1.00
chr3	57107802	40	0.05	0.00	0.01	0.01	0.01	0.64	0.02	0.01	0.55	0.00	0.01	1.00	0.00	0.01	1.00	0.01	0.01	1.00
chr6	35811616	39	0.05	0.00	0.03	0.05	0.04	0.64	0.02	0.04	1.00	0.02	0.03	1.00	0.03	0.05	1.00	0.01	0.06	1.00
chr6	136644276	25	0.05	0.00	0.02	0.00	0.02	1.00	0.00	0.03	1.00	0.00	0.01	1.00	0.01	0.01	1.00	0.00	0.02	1.00
chr10	45235318	67	0.11	0.04	0.08															
chr1	28878370	104	0.10	0.05	0.16															
chr17	28712496	39	0.08	0.08	0.63															
chr17	362910	246	0.07	0.07	0.61															
chr11	119024886	37	0.05	0.01	0.12															
chr15	34179254	52	0.04	0.02	0.40															
chr10	87861594	26	0.03	0.02	0.30															
chr17	81941032	36	0.03	0.00	0.14															

Supplementary Table 18 (continued below)

ZFN Pair 1 capture - continued			Pair 1 continued		
hg38 coordinates		integrations	%indels ZFN	%indels GFP	pval
chr20	46196718	28	0.03	0.01	0.33
chr7	151203002	25	0.03	0.01	0.13
chr2	240904936	25	0.03	0.01	0.25
chr3	197746150	38	0.03	0.00	0.08
chr4	186954080	48	0.02	0.01	0.44
chr3	72163900	69	0.02	0.00	0.14
chr7	158413556	345	0.02	0.03	0.80
chr22	18167878	60	0.02	0.01	0.51
chr7	101364162	37	0.02	0.01	0.51
chr11	77199762	47	0.02	0.04	0.95
chr3	195621368	37	0.02	0.04	0.94
chr1	110517930	85	0.02	0.01	0.55
chr8	139813412	29	0.01	0.04	0.99
chr6	32683930	26	0.01	0.00	0.25
chr1	234851754	79	0.01	0.01	0.48
chr22	21709082	206	0.01	0.02	0.82
chr9	35001138	39	0.01	0.02	0.94
chr6	31769912	32	0.01	0.01	0.50
chr22	19218188	31	0.01	0.05	0.99
chr21	42533054	44	0.01	0.05	0.99
chr1	247540474	28	0.01	0.00	0.25
chr8	104793608	33	0.01	0.00	0.25
chr20	57941386	62	0.01	0.00	0.25
chr15	78776288	26	0.01	0.00	0.25
chr12	53004488	136	0.00	0.02	0.96
chr19	48508592	109	0.00	0.02	0.99
chr8	66664620	96	0.00	0.00	1.00
chr1	203006352	90	0.00	0.16	0.99
chr22	20369586	67	0.00	0.02	0.99
chr8	42391528	45	0.00	0.00	1.00
chr4	79325484	36	0.00	0.03	0.96
chr1	244063132	32	0.00	0.05	0.99
chr12	56632406	93	ND	ND	ND
chr1	25146536	56	ND	ND	ND
chr16	2155772	54	ND	ND	ND
chr1	2480064	38	ND	ND	ND
chr1	14131644	32	ND	ND	ND
chr17	40100232	32	ND	ND	ND
chr3	195749386	31	ND	ND	ND
chr16	81696616	28	ND	ND	ND
chr3	56682902	28	ND	ND	ND
chr17	44520096	26	ND	ND	ND
chr5	164600842	25	ND	ND	ND

Supplementary Table 18. "Pair 1" column: Indel levels measured at the top 93 ranked candidate off-target loci for the parent TCRα ZFN dimer as assessed in genomic DNA from T-cells treated with the parent ZFNs or a GFP control. ND indicates that no data were obtained due to a failed PCR or sequencing analysis failure. Loci are sorted by %indels observed. Remaining columns: Indel levels measured at the 41 loci that yielded significant modification by the parent ZFNs, assessed in genomic DNA from T-cells treated with the indicated variant ZFN pairs or a GFP control. Integrations are the total unique integrations from four biological replicates and indel values represent an individual measurement. ND indicates that no data were obtained due to a failed PCR or sequencing analysis failure. %indels at the intended target are highlighted in green and %indels at all other loci are highlighted with a red heat map. P-values for each ZFN-GFP comparison are shown to the right of each pair of indel values. See **Supplementary Note 2** for details of the statistical test. Statistically significant p-values are highlighted in blue

Supplementary Table 19

ZFN construct information

Complete DNA and amino acid information is given for the coding sequence of each of the parent ZFN constructs. Recognition helices within each zinc finger are underlined.

Original AAVS1 ZFN pair

> 30035 (Left AAVS1 ZFN)
ATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGGCCCCCAAGAAGAAGAGGAAGGTCGGCA
TCCACGGGGTACCCGCCGCTATGGCTGAGAGGCCCTTCCAGTGTCGAATCTGCATGCGTAACTTCAGTCGCTCCGACCACCTGTCCCGCCACATCCGCAC
CCACACCGGCGAGAAGCCTTTTGCCTGTGACATTTGTGGGAGGAAATTTGCCACCTCCGGCCACCTGTCCCGCCATACCAAGATACACACGGGCAGCCAA
AAGCCCTTCCAGTGTCGAATCTGCATGCGTAACTTCAGTTACAACTGGCACCTGCAGCGCCACATCCGCACCCACACCGGCGAGAAGCCTTTTGCCTGTG
ACATTTGTGGGAGGAAATTTGCCCGCTCCGACCACCTGACCACCCATACCAAGATACACACGGGATCTCAGAAGCCCTTCCAGTGTCGAATCTGCATGCG
TAACTTCAGTCACAACTACGCCCGCGACTGTCACATCCGCACCCACACCGGCGAGAAGCCTTTTGCCTGTGACATTTGTGGGAGGAAATTTGCCCAGAAC
TCCACCCGCATCGGCCATACCAAGATACACCTGCGGGGATCCCAGCTGGTGAAGAGCGAGCTGGAGGAGAAGAAGTCCGAGCTGCGGCACAAGCTGAAGT
ACGTGCCCCACGAGTACATCGAGCTGATCGAGATCGCCAGGAACAGCACCCAGGACCGCATCCTGGAGATGAAGGTGATGGAGTTCTTCATGAAGGTGTA
CGGCTACAGGGGAAAGCACCTGGGCGGAAGCAGAAAGCCTGACGGCGCCATCTATACAGTGGGCAGCCCCATCGATTACGGCGTGATCGTGGACACAAAG
GCCTACAGCGGCGGCTACAATCTGCCTATCGGCCAGGCCGACGAGATGGAGAGATACGTGGAGGAGAACCAGACCCGGGATAAGCACCTCAACCCCAACG
AGTGGTGGAAGGTGTACCCTAGCAGCGTGACCGAGTTCAAGTTCCTGTTCGTGAGCGGCCACTTCAAGGGCAACTACAAGGCCCAGCTGACCAGGCTGAA
CCACATCACCAACTGCAATGGCGCCGTGCTGAGCGTGGAGGAGCTGCTGATCGGCGGCGAGATGATCAAAGCCGGCACCCTGACACTGGAGGAGGTGCGG
CGCAAGTTCAACAACGGCGAGATCAACTTCAGATCTTGATAA

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSRSDHLSRHIRTHTGEKPFACDICGRKFATSGHLSRHTKIHTGSQ
KPFQCRICMRNFSYNWHLQRHIRTHTGEKPFACDICGRKFARSDHLTTHTKIHTGSQKPFQCRICMRNFSHNYARDCHIRTHTGEKPFACDICGRKFAQN
STRIGHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTK
AYSGGYNLPIGQADEMERYVEENQTRDKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVR
RKFNNGEINFRS**

> 30054 (right AAVS1 ZFN)
ATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGGCCCCCAAGAAGAAGAGGAAGGTCGGCA
TTCATGGGGTACCCGCCGCTATGGCTGAGAGGCCCTTCCAGTGTCGAATCTGCATGCGTAACTTCAGTGACCGCTCCAACCTGTCCCGCCACATCCGCAC
CCACACCGGCGAGAAGCCTTTTGCCTGTGACATTTGTGGGAGGAAATTTGCCCTGAAGCAGCACCTGACCCGCCATACCAAGATACACACGCATCCCAGG
GCACCTATTCCCAAGCCCTTCCAGTGTCGAATCTGCATGCGTAACTTCAGTACCTCCGGCAACCTGACCCGCCACATCCGCACCCACACCGGCGAGAAGC
CTTTTGCCTGTGACATTTGTGGGAGGAAATTTGCCCGCCGCGACTGGCGCCGCGACCATACCAAGATACACACGGGATCTCAGAAGCCCTTCCAGTGTCG
AATCTGCATGCGTAACTTCAGTCAGTCCTCCCACCTGACCCGCCACATCCGCACCCACACCGGCGAGAAGCCTTTTGCCTGTGACATTTGTGGGAGGAAA
TTTGCCCGCCTGGACAACCGCACCGCCCATACCAAGATACACCTGCGGGGATCCCAGCTGGTGAAGAGCGAGCTGGAGGAGAAGAAGTCCGAGCTGCGGC
ACAAGCTGAAGTACGTGCCCCACGAGTACATCGAGCTGATCGAGATCGCCAGGAACAGCACCCAGGACCGCATCCTGGAGATGAAGGTGATGGAGTTCTT
CATGAAGGTGTACGGCTACAGGGGAAAGCACCTGGGCGGAAGCAGAAAGCCTGACGGCGCCATCTATACAGTGGGCAGCCCCATCGATTACGGCGTGATC
GTGGACACAAAGGCCTACAGCGGCGGCTACAATCTGCCTATCGGCCAGGCCGACGAGATGCAGAGATACGTGAAGGAGAACCAGACCCGGAATAAGCACA
TCAACCCCAACGAGTGGTGGAAGGTGTACCCTAGCAGCGTGACCGAGTTCAAGTTCCTGTTCGTGAGCGGCCACTTCAAGGGCAACTACAAGGCCCAGCT
GACCAGGCTGAACCGCAAAACCAACTGCAATGGCGCCGTGCTGAGCGTGGAGGAGCTGCTGATCGGCGGCGAGATGATCAAAGCCGGCACCCTGACACTG
GAGGAGGTGCGGCGCAAGTTCAACAACGGCGAGATCAACTTCTGATAA

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSDRSNLSRHIRTHTGEKPFACDICGRKFALKQHLTRHTKIHTHPR
APIPKPFQCRICMRNFSTSGNLTRHIRTHTGEKPFACDICGRKFARRDWRRDHTKIHTGSQKPFQCRICMRNFSQSSHLTRHIRTHTGEKPFACDICGRK
FARLDNRTAHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVI
VDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNRKTNCNGAVLSVEELLIGGEMIKAGTLTL
EEVRRKFNNGEINF**

Original PD1 ZFN pair

> 12942 (Left PD1 ZFN)
ATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGGCCCCCAAGAAGAAGAGGAAGGTGGGCA
TTCACGGGGTACCCGCCGCTATGGCTGAGAGGCCCTTCCAGTGTCGAATCTGCATGCGTAAGTTTGCCCAGTCCGGCCACCTGTCCCGCCATACCAAGAT
ACACACGGGCGAGAAGCCCTTCCAGTGTCGAATCTGCATGCGTAACTTCAGTCGTAGTGACAGCCTGAGCGTACACATCCGCACCCACACAGGCGAGAAG
CCTTTTGCCTGTGACATTTGTGGGAGGAAATTTGCCCACAACGACAGCCGCAAAAACCATACCAAGATACACACGGGATCTCAGAAGCCCTTCCAGTGTC
GAATCTGCATGCGTAACTTCAGTCGCTCCGACGACCTGACCCGCCACATCCGCACCCACACAGGCGAGAAGCCTTTTGCCTGTGACATTTGTGGGAGGAA
GTTTGCCCGCTCCGACCACCTGACCCAGCATACCAAGATACACCTGCGGGGATCCCAGCTGGTGAAGAGCGAGCTGGAGGAGAAGAAGTCCGAGCTGCGG
CACAAGCTGAAGTACGTGCCCCACGAGTACATCGAGCTGATCGAGATCGCCAGGAACAGCACCCAGGACCGCATCCTGGAGATGAAGGTGATGGAGTTCT
TCATGAAGGTGTACGGCTACAGGGGAAAGCACCTGGGCGGAAGCAGAAAGCCTGACGGCGCCATCTATACAGTGGGCAGCCCCATCGATTACGGCGTGAT
CGTGGACACAAAGGCCTACAGCGGCGGCTACAATCTGCCTATCGGCCAGGCCGACGAGATGCAGAGATACGTGGAGGAGAACCAGACCCGGAATAAGCAC
ATCAACCCCAACGAGTGGTGGAAGGTGTACCCTAGCAGCGTGACCGAGTTCAAGTTCCTGTTCGTGAGCGGCCACTTCAAGGGCAACTACAAGGCCCAGC

TGACCAGGCTGAACCACATCACCAACTGCAATGGCGCCGTGCTGAGCGTGGAGGAGCTGCTGATCGGCGGCGAGATGATCAAAGCCGGCACCCTGACACT
GGAGGAGGTGCGGCGCAAGTTCAACAACGGCGAGATCAACTTCAGATCTTGATAA

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRKFAQSGHLSRHTKIHTGEKPFQCRICMRNFSRSDSLSVHIRTHTGEK
PFACDICGRKFAHNDSRKNHTKIHTGSQKPFQCRICMRNFSRSDDLTRHIRTHTGEKPFACDICGRKFARSDHLTQHTKIHLRGSQLVKSELEEKKSELR
HKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKH
INPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 25029 (Right PD1 ZFN)
ATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGGCCCCCAAGAAGAAGAGGAAGGTGGGCA
TTCACGGGGTACCCGCCGCTATGGCTGAGAGGCCCTTCCAGTGTCGAATCTGCATGTGTAAGTTTGCCCGCAACGCCGCCCTGACCCGCCATACCAAGAT
ACACACGGGCGAGAAGCCGTTCCAGTGTCGCATCTGCATGCGTAACTTCAGTCGCTCCGACGAGCTGACCCGCCACATCCGCACCCACACAGGCGAGAAG
CCTTTTGCTTGCGACATTTGTGGGAGGAAGTTTGCCCGGCACCACCACCTGGCCGCCCATACCAAGATACACACGGGATCTCAGAAGCCCTTCCAGTGTC
GAATCTGCATGCGTAACTTCAGTACCCGCCCGGTGCTGAAGCGCCACATCCGCACCCACACAGGCGAGAAGCCTTTTGCTTGCGACATTTGTGGGAGGAA
GTTTGCCGACCGCTCCGCCCTGGCCCGCCATACCAAGATACACCTGCGGGGATCCCAGCTGGTGAAGAGCGAGCTGGAGGAGAAGAAGTCCGAGCTGCGG
CACAAGCTGAAGTACGTGCCCCACGAGTACATCGAGCTGATCGAGATCGCCAGGAACAGCACCCAGGACCGCATCCTGGAGATGAAGGTGATGGAGTTCT
TCATGAAGGTGTACGGCTACAGGGGAAAGCACCTGGGCGGAAGCAGAAAGCCTGACGGCGCCATCTATACAGTGGGCAGCCCCATCGATTACGGCGTGAT
CGTGGACACAAAGGCCTACAGCGGCGGCTACAATCTGCCTATCGGCCAGGCCGACGAGATGCAGAGATACGTGGAGGAGAACCAGACCCGGAATAAGCAC
ATCAACCCCAACGAGTGGTGGAAGGTGTACCCTAGCAGCGTGACCGAGTTCAAGTTCCTGTTCGTGAGCGGCCACTTCAAGGGCAACTACAAGGCCCAGC
TGACCAGGCTGAACCACATCACCAACTGCAATGGCGCCGTGCTGAGCGTGGAGGAGCTGCTGATCGGCGGCGAGATGATCAAAGCCGGCACCCTGACACT
GGAGGAGGTGCGGCGCAAGTTCAACAACGGCGAGATCAACTTCAGATCTTGATAA

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMCKFARNAALTRHTKIHTGEKPFQCRICMRNFSRSDELTRHIRTHTGEK
PFACDICGRKFARHHHLAAHTKIHTGSQKPFQCRICMRNFSTRPVLKRHIRTHTGEKPFACDICGRKFADRSALARHTKIHLRGSQLVKSELEEKKSELR
HKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKH
INPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

Original BCL11A ZFN pair

> 51857 (Left BCL11A ZFN)
ATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGGCCCCCAAGAAGAAGAGGAAGGTCGGCA
TCCACGGGGTACCCGCCGCTATGGCTGAGAGGCCCTTCCAGTGTCGAATCTGCATGCGTAACTTCAGTGACCAGTCCAACCTGCGCGCCCACATCCGCAC
CCACACCGGCGAGAAGCCTTTTGCCTGTGACATTTGTGGGAGGAAATTTGCCCGCAACTTCTCCCTGACCATGCATACCAAGATACACACGGGCAGCCAA
AAGCCCTTCCAGTGTCGAATCTGCATGCGTAACTTCAGTTCCACCGGCAACCTGACCAACCACATCCGCACCCACACCGGCGAGAAGCCTTTTGCCTGTG
ACATTTGTGGGAGGAAATTTGCCACCTCCGGCTCCCTGACCCGCCATACCAAGATACACACGCACCCGCGCGCCCCGATCCCGAAGCCCTTCCAGTGTCG
AATCTGCATGCGTAACTTCAGTGACCAGTCCAACCTGCGCGCCCACATCCGCACCCACACCGGCGAGAAGCCTTTTGCCTGTGACATTTGTGGGAGGAAA
TTTGCCGCCCAGTGTTGTCTGTTCCACCATACCAAGATACACCTGCGGGGATCCATCAGCAGAGCCAGACCACTGAACCCGCACCCGGAGCTGGAGGAGA
AGAAGTCCGAGCTGCGGCACAAGCTGAAGTACGTGCCCCACGAGTACATCGAGCTGATCGAGATCGCCAGGAACAGCACCCAGGACCGCATCCTGGAGAT
GAAGGTGATGGAGTTCTTCATGAAGGTGTACGGCTACAGGGGAAAGCACCTGGGCGGAAGCAGAAAGCCTGACGGCGCCATCTATACAGTGGGCAGCCCC
ATCGATTACGGCGTGATCGTGGACACAAAGGCCTACAGCGGCGGCTACAATCTGCCTATCGGCCAGGCCGACGAGATGGAGAGATACGTGGAGGAGAACC
AGACCCGGGATAAGCACCTCAACCCCAACGAGTGGTGGAAGGTGTACCCTAGCAGCGTGACCGAGTTCAAGTTCCTGTTCGTGAGCGGCCACTTCAAGGG
CAACTACAAGGCCCAGCTGACCAGGCTGAACCACATCACCAACTGCAATGGCGCCGTGCTGAGCGTGGAGGAGCTGCTGATCGGCGGCGAGATGATCAAA
GCCGGCACCCTGACACTGGAGGAGGTGCGGCGCAAGTTCAACAACGGCGAGATCAACTTCAGATCTTGATAA

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQ
KPFQCRICMRNFSSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRK
FAAQCCLFHHTKIHLRGSISRARPLNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSP
IDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTRDKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIK
AGTLTLEEVRRKFNNGEINFRS**

> 51949 (Right BCL11A ZFN)
ATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGGCCCCCAAGAAGAAGAGGAAGGTCGGCA
TTCATGGGGTACCCGCCGCTATGGCTGAGAGGCCCTTCCAGTGTCGAATCTGCATGCGTAAGTTTGCCCGCAACGACCACCGCACCACCCATACCAAGAT
ACACACGGGCGAGAAGCCCTTCCAGTGTCGAATCTGCATGCGTAACTTCAGTCAGAAGGCCCACCTGATCCGCCACATCCGCACCCACACCGGCGAGAAG
CCTTTTGCCTGTGACATTTGTGGGAGGAAATTTGCCCAGAAGGGCACCCTGGGCGAGCATACCAAGATACACACGGGATCTCAGAAGCCCTTCCAGTGTC
GAATCTGCATGCGTAACTTCAGTCGCGGCCGCGACCTGTCCCGCCACATCCGCACCCACACCGGCGAGAAGCCTTTTGCCTGTGACATTTGTGGGAGGAA
ATTTGCCCGCCGCGACAACCTGCACTCCCATACCAAGATACACCTGCGGGGATCCCAGCTGGTGAAGAGCGAGCTGGAGGAGAAGAAGTCCGAGCTGCGG
CACAAGCTGAAGTACGTGCCCCACGAGTACATCGAGCTGATCGAGATCGCCAGGAACAGCACCCAGGACCGCATCCTGGAGATGAAGGTGATGGAGTTCT
TCATGAAGGTGTACGGCTACAGGGGAAAGCACCTGGGCGGAAGCAGAAAGCCTGACGGCGCCATCTATACAGTGGGCAGCCCCATCGATTACGGCGTGAT
CGTGGACACAAAGGCCTACAGCGGCGGCTACAATCTGCCTATCGGCCAGGCCGACGAGATGCAGAGATACGTGAAGGAGAACCAGACCCGGAATAAGCAC
ATCAACCCCAACGAGTGGTGGAAGGTGTACCCTAGCAGCGTGACCGAGTTCAAGTTCCTGTTCGTGAGCGGCCACTTCAAGGGCAACTACAAGGCCCAGC
TGACCAGGCTGAACCGCAAAACCAACTGCAATGGCGCCGTGCTGAGCGTGGAGGAGCTGCTGATCGGCGGCGAGATGATCAAAGCCGGCACCCTGACACT
GGAGGAGGTGCGGCGCAAGTTCAACAACGGCGAGATCAACTTCTGATAA

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRKFARNDHRTTHTKIHTGEKPFQCRICMRNFSQKAHLIRHIRTHTGEK
PFACDICGRKFAQKGTLGEHTKIHTGSQKPFQCRICMRNFSRGRDLSRHIRTHTGEKPFACDICGRKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELR

HKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKH
INPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

Original TRAC ZFNs

> 55248 (Left TRAC ZFN)
ATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGGCCCCCAAGAAGAAGAGGAAGGTCGGCA
TTCATGGGGTACCCGCCGCTATGGCTGAGAGGCCCTTCCAGTGTCGAATCTGCATGCGTAACTTCAGTGACCAGTCCAACCTGCGCGCCCACATCCGCAC
CCACACCGGCGAGAAGCCTTTTGCCTGTGACATTTGTGGGAGGAAATTTGCCACCTCCTCCAACCGCAAGACCCATACCAAGATACACACGGGCAGCCAA
AAGCCCTTCCAGTGTCGAATCTGCATGCGTAACTTCAGTCTGCAGCAGACCCTGGCCGACCACATCCGCACCCACACCGGCGAGAAGCCTTTTGCCTGTG
ACATTTGTGGGAGGAAATTTGCCCAGTCCGGCAACCTGGCCCGCCATACCAAGATACACACGGGATCTCAGAAGCCCTTCCAGTGTCGAATCTGCATGCG
TAACTTCAGTCGCCGCGAGGACCTGATCACCCACATCCGCACCCACACCGGCGAGAAGCCTTTTGCCTGTGACATTTGTGGGAGGAAATTTGCCACCTCC
TCCAACCTGTCCCGCCATACCAAGATACACCTGCGGGGATCCCAGCTGGTGAAGAGCGAGCTGGAGGAGAAGAAGTCCGAGCTGCGGCACAAGCTGAAGT
ACGTGCCCCACGAGTACATCGAGCTGATCGAGATCGCCAGGAACAGCACCCAGGACCGCATCCTGGAGATGAAGGTGATGGAGTTCTTCATGAAGGTGTA
CGGCTACAGGGGAAAGCACCTGGGCGGAAGCAGAAAGCCTGACGGCGCCATCTATACAGTGGGCAGCCCCATCGATTACGGCGTGATCGTGGACACAAAG
GCCTACAGCGGCGGCTACAATCTGCCTATCGGCCAGGCCGACGAGATGCAGAGATACGTGAAGGAGAACCAGACCCGGAATAAGCACATCAACCCCAACG
AGTGGTGGAAGGTGTACCCTAGCAGCGTGACCGAGTTCAAGTTCCTGTTCGTGAGCGGCCACTTCAAGGGCAACTACAAGGCCCAGCTGACCAGGCTGAA
CCGCAAAACCAACTGCAATGGCGCCGTGCTGAGCGTGGAGGAGCTGCTGATCGGCGGCGAGATGATCAAAGCCGGCACCCTGACACTGGAGGAGGTGCGG
CGCAAGTTCAACAACGGCGAGATCAACTTCTGATAA

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFATSSNRKTHTKIHTGSQ
KPFQCRICMRNFSLQQTLADHIRTHTGEKPFACDICGRKFAQSGNLARHTKIHTGSQKPFQCRICMRNFSRREDLITHIRTHTGEKPFACDICGRKFATS
SNLSRHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTK
AYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVR
RKFNNGEINF**

> 55254 (Right TRAC ZFN)
ATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGGCCCCCAAGAAGAAGAGGAAGGTCGGCA
TCCACGGGGTACCCGCCGCTATGGCTGAGAGGCCCTTCCAGTGTCGAATCTGCATGCGTAACTTCAGTCGCTCCGACCACCTGTCCACCCACATCCGCAC
CCACACCGGCGAGAAGCCTTTTGCCTGTGACATTTGTGGGAGGAAATTTGCCGACCGCTCCCACCTGGCCCGCCATACCAAGATACACACGGGCAGCCAA
AAGCCCTTCCAGTGTCGAATCTGCATGCGTAAGTTTGCCCTGAAGCAGCACCTGAACGAGCATACCAAGATACACACGGGCGAGAAGCCCTTCCAGTGTC
GAATCTGCATGCGTAACTTCAGTCAGTCCGGCAACCTGGCCCGCCACATCCGCACCCACACCGGCGAGAAGCCTTTTGCCTGTGACATTTGTGGGAGGAA
ATTTGCCCACAACTCCTCCCTGAAGGACCATACCAAGATACACCTGCGGGGATCCCAGCTGGTGAAGAGCGAGCTGGAGGAGAAGAAGTCCGAGCTGCGG
CACAAGCTGAAGTACGTGCCCCACGAGTACATCGAGCTGATCGAGATCGCCAGGAACAGCACCCAGGACCGCATCCTGGAGATGAAGGTGATGGAGTTCT
TCATGAAGGTGTACGGCTACAGGGGAAAGCACCTGGGCGGAAGCAGAAAGCCTGACGGCGCCATCTATACAGTGGGCAGCCCCATCGATTACGGCGTGAT
CGTGGACACAAAGGCCTACAGCGGCGGCTACAATCTGCCTATCGGCCAGGCCGACGAGATGGAGAGATACGTGGAGGAGAACCAGACCCGGGATAAGCAC
CTCAACCCCAACGAGTGGTGGAAGGTGTACCCTAGCAGCGTGACCGAGTTCAAGTTCCTGTTCGTGAGCGGCCACTTCAAGGGCAACTACAAGGCCCAGC
TGACCAGGCTGAACCACATCACCAACTGCAATGGCGCCGTGCTGAGCGTGGAGGAGCTGCTGATCGGCGGCGAGATGATCAAAGCCGGCACCCTGACACT
GGAGGAGGTGCGGCGCAAGTTCAACAACGGCGAGATCAACTTCAGATCTTGATAA

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSRSDHLSTHIRTHTGEKPFACDICGRKFADRSHLARHTKIHTGSQ
KPFQCRICMRKFALKQHLNEHTKIHTGEKPFQCRICMRNFSQSGNLARHIRTHTGEKPFACDICGRKFAHNSSLKDHTKIHLRGSQLVKSELEEKKSELR
HKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTRDKH
LNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

BCL11A ZFP Backbone variants

To create the BCL11A ZFP backbone variants, the original BCL11A ZFNs were cloned into a vector containing the woodchuck hepatitis virus posttranslational regulatory element (WPRE). Arginine to glutamine mutations were created by changing the desired codon to CAG. The resulting mutations in the protein sequences below are highlighted in red. The mutations are denoted by a string of characters that indicate the total number of gluatamine (Q) mutations as well as which zinc fingers contain these mutations. A total of two mutations in fingers 1 and 3 would be denoted as "Q2-F13".

> 63007 Left ZFN
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQKPFQCRICMRNF
SSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFAAQCCLFHHTKIHLRGSSISRAR
PLNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQT
RDKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 63008 Left ZFN Q1-F1
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQKPFQCRICMRNF
SSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFAAQCCLFHHTKIHLRGSSISRAR
PLNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQT
RDKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 63009 Left ZFN Q1-F3
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQKPFQCRICMQNF
SSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFAAQCCLFHHTKIHLRGSSISRAR

PLNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQT
RDKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 63010 Left ZFN Q1-F5
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQKPFQCRICMRNF
SSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGRKFAAQCCLFHHTKIHLRGSSISRAR
PLNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQT
RDKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 65751 Left ZFN Q1-F2
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGQKFARNFSLTMHTKIHTGSQKPFQCRICMRNF
SSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFAAQCCLFHHTKIHLRGSISRARP
LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 65752 Left ZFN Q1-F4
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQKPFQCRICMRNF
SSTGNLTNHIRTHTGEKPFACDICGQKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFAAQCCLFHHTKIHLRGSISRARP
LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 65753 Left ZFN Q1-F6
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQKPFQCRICMRNF
SSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGQKFAAQCCLFHHTKIHLRGSISRARP
LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 63011 Left ZFN Q2-F13
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQKPFQCRICMQNF
SSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFAAQCCLFHHTKIHLRGSSISRAR
PLNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQT
RDKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 63012 Left ZFN Q2-F15
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQKPFQCRICMRNF
SSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGRKFAAQCCLFHHTKIHLRGSSISRAR
PLNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQT
RDKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 63013 Left ZFN Q2-F35
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQKPFQCRICMQNF
SSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGRKFAAQCCLFHHTKIHLRGSSISRAR
PLNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQT
RDKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 65754 Left ZFN Q2-F24
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGQKFARNFSLTMHTKIHTGSQKPFQCRICMRNF
SSTGNLTNHIRTHTGEKPFACDICGQKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFAAQCCLFHHTKIHLRGSISRARP
LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 65755 Left ZFN Q2-F26
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGQKFARNFSLTMHTKIHTGSQKPFQCRICMRNF
SSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGQKFAAQCCLFHHTKIHLRGSISRARP
LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 65756 Left ZFN Q2-F46
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQKPFQCRICMRNF
SSTGNLTNHIRTHTGEKPFACDICGQKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGQKFAAQCCLFHHTKIHLRGSISRARP
LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 63014 Left ZFN Q3-F135
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQKPFQCRICMQNF
SSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGRKFAAQCCLFHHTKIHLRGSISRARP
LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 65757 Left ZFN Q3-F246
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGQKFARNFSLTMHTKIHTGSQKPFQCRICMRNF
SSTGNLTNHIRTHTGEKPFACDICGQKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMRNFSDQSNLRAHIRTHTGEKPFACDICGQKFAAQCCLFHHTKIHLRGSISRARP
LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 65758 Left ZFN Q4-F1235
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGQKFARNFSLTMHTKIHTGSQKPFQCRICMQNF
SSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGRKFAAQCCLFHHTKIHLRGSISRARP

LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 65759 Left ZFN Q4-F1345
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQKPFQCRICMQNF
SSTGNLTNHIRTHTGEKPFACDICGQKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGRKFAAQCCLFHHTKIHLRGSISRARP
LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 65760 Left ZFN Q4-F1356
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQKPFQCRICMQNF
SSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGQKFAAQCCLFHHTKIHLRGSISRARP
LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 65761 Left ZFN Q5-F12345
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGQKFARNFSLTMHTKIHTGSQKPFQCRICMQNF
SSTGNLTNHIRTHTGEKPFACDICGQKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGRKFAAQCCLFHHTKIHLRGSISRARP
LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 65762 Left ZFN Q5-F12356
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGQKFARNFSLTMHTKIHTGSQKPFQCRICMQNF
SSTGNLTNHIRTHTGEKPFACDICGRKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGQKFAAQCCLFHHTKIHLRGSISRARP
LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 65763 Left ZFN Q5-F13456
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGRKFARNFSLTMHTKIHTGSQKPFQCRICMQNF
SSTGNLTNHIRTHTGEKPFACDICGQKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGQKFAAQCCLFHHTKIHLRGSISRARP
LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 65764 Left ZFN Q6-F123456
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGQKFARNFSLTMHTKIHTGSQKPFQCRICMQNF
SSTGNLTNHIRTHTGEKPFACDICGQKFATSGSLTRHTKIHTHPRAPIPKPFQCRICMQNFSDQSNLRAHIRTHTGEKPFACDICGQKFAAQCCLFHHTKIHLRGSISRARP
LNPHPELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTR
DKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFRS**

> 63015 Right ZFN
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRKFARNDHRTTHTKIHTGEKPFQCRICMRNFSQKAHLIRHIRTHTGEKPFACDICGRKFA
QKGTLGEHTKIHTGSQKPFQCRICMRNFSRGRDLSRHIRTHTGEKPFACDICGRKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDR
ILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

> 63016 Right ZFN Q1-F1
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQKFARNDHRTTHTKIHTGEKPFQCRICMRNFSQKAHLIRHIRTHTGEKPFACDICGRKFA
QKGTLGEHTKIHTGSQKPFQCRICMRNFSRGRDLSRHIRTHTGEKPFACDICGRKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDR
ILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

> 63017 Right ZFN Q1-F2
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRKFARNDHRTTHTKIHTGEKPFQCRICMQNFSQKAHLIRHIRTHTGEKPFACDICGRKFA
QKGTLGEHTKIHTGSQKPFQCRICMRNFSRGRDLSRHIRTHTGEKPFACDICGRKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDR
ILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

> 63018 Right ZFN Q1-F4
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRKFARNDHRTTHTKIHTGEKPFQCRICMRNFSQKAHLIRHIRTHTGEKPFACDICGRKFA
QKGTLGEHTKIHTGSQKPFQCRICMQNFSRGRDLSRHIRTHTGEKPFACDICGRKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDR
ILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

> 65765 Right ZFN Q1-F3
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRKFARNDHRTTHTKIHTGEKPFQCRICMRNFSQKAHLIRHIRTHTGEKPFACDICGQKFA
QKGTLGEHTKIHTGSQKPFQCRICMRNFSRGRDLSRHIRTHTGEKPFACDICGRKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDR
ILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

> 65766 Right ZFN Q1-F5
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRKFARNDHRTTHTKIHTGEKPFQCRICMRNFSQKAHLIRHIRTHTGEKPFACDICGRKFA
QKGTLGEHTKIHTGSQKPFQCRICMRNFSRGRDLSRHIRTHTGEKPFACDICGQKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDR
ILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

> 63019 Right ZFN Q2-F12
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQKFARNDHRTTHTKIHTGEKPFQCRICMQNFSQKAHLIRHIRTHTGEKPFACDICGRKFA
QKGTLGEHTKIHTGSQKPFQCRICMRNFSRGRDLSRHIRTHTGEKPFACDICGRKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDR

ILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

> 63020 Right ZFN Q2-F14
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQKFARNDHRTTHTKIHTGEKPFQCRICMRNFSQKAHLIRHIRTHTGEKPFACDICGRKFA
QKGTLGEHTKIHTGSQKPFQCRICMQNFSRGRDLSRHIRTHTGEKPFACDICGRKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDR
ILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

> 63021 Right ZFN Q2-F24
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRKFARNDHRTTHTKIHTGEKPFQCRICMQNFSQKAHLIRHIRTHTGEKPFACDICGRKFA
QKGTLGEHTKIHTGSQKPFQCRICMQNFSRGRDLSRHIRTHTGEKPFACDICGRKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDR
ILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

> 65767 Right ZFN Q2-F35
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMRKFARNDHRTTHTKIHTGEKPFQCRICMRNFSQKAHLIRHIRTHTGEKPFACDICGQKFA
QKGTLGEHTKIHTGSQKPFQCRICMRNFSRGRDLSRHIRTHTGEKPFACDICGQKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDR
ILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

> 63022 Right ZFN Q3-124
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQKFARNDHRTTHTKIHTGEKPFQCRICMQNFSQKAHLIRHIRTHTGEKPFACDICGRKFA
QKGTLGEHTKIHTGSQKPFQCRICMQNFSRGRDLSRHIRTHTGEKPFACDICGRKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDR
ILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

> 65768 Right ZFN Q4-1234
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQKFARNDHRTTHTKIHTGEKPFQCRICMQNFSQKAHLIRHIRTHTGEKPFACDICGQKFA
QKGTLGEHTKIHTGSQKPFQCRICMQNFSRGRDLSRHIRTHTGEKPFACDICGRKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDR
ILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

> 65769 Right ZFN Q4-F1245
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQKFARNDHRTTHTKIHTGEKPFQCRICMQNFSQKAHLIRHIRTHTGEKPFACDICGRKFA
QKGTLGEHTKIHTGSQKPFQCRICMQNFSRGRDLSRHIRTHTGEKPFACDICGQKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDR
ILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

> 65770 Right ZFN Q5-F12345
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICMQKFARNDHRTTHTKIHTGEKPFQCRICMQNFSQKAHLIRHIRTHTGEKPFACDICGQKFA
QKGTLGEHTKIHTGSQKPFQCRICMQNFSRGRDLSRHIRTHTGEKPFACDICGQKFARRDNLHSHTKIHLRGSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDR
ILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTR
LNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF**

Supplementary Table 20

mRNA PCR template primers:
N80pt: GCAGAGCTCTCTGGCTAACTAGAG
R560: TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTGGCAACTAGAAGGCACAG

Oligos used for DNA binding and off-rate experiments (target duplexes have 5' biotin on bottom strand)

N80pt	GCAGAGCTCTCTGGCTAACTAGAG
C-HATAG-Fok	GCGTAAAGCTTATCAGGCGTAGTCGGGCACGTCGTAGGGGTAGCCGGATCCGTTGATCTCGCCGTTGTTG
AV-ELISA-onR-t	TAATGGGGCCACTAGGGACAGGATTGGTGACAGTTCACAGTCAGTCCACACGTC
AV-ELISA-onR-b	GACGTGTGGACTGACTGTGAACTGTCACCAATCCTGTCCCTAGTGGCCCCATTA
AV-ELISA-onL-t	TAATTAGTGGCCCCACTGTGGGGTGGAGGGGATTCACAGTCAGTCCACACGTC
AV-ELISA-onL-b	GACGTGTGGACTGACTGTGAATCCCCTCCACCCCACAGTGGGGCCACTAATTA
AV-ELISA-OT1R-t	TAATGTTTTGGCTGGGGTGTGGATTGGTGCTCTTTCACAGTCAGTCCACACGTC
AV-ELISA-OT1R-b	GACGTGTGGACTGACTGTGAAAGAGCACCAATCCACACCCCAGCCAAAACATTA
AV-ELISA-OT1L-t	TAATCAGCCAAAACACTGTGGGCTAACCTAGATTCACAGTCAGTCCACACGTC
AV-ELISA-OT1L-b	GACGTGTGGACTGACTGTGAATCTAGGTTAGCCCACAGTGTTTTGGCTGATTA

PCR primers for indel assays. UIDs that required nested PCR are indicated with an asterisk.

AAVS1 ZFN locus specific primer info

hg38 coordinates		UID	Forward primer (5' to 3')	Reverse primer (5' to 3')
chr19	55115768	AAVS1-on	ACACGACGCTCTTCCGATCTNNNNGTGTGTCACCAGATAAGGAAT	GACGTGTGCTCTTCCGATCTGGCTCTGGTTCTGGGTACTTTTA
chr1	198172184	AAVS1-OT1	ACACGACGCTCTTCCGATCTNNNNGGTAGACATATCAGTAATGCT	GACGTGTGCTCTTCCGATCTTTCAGAGAGAAGGGGAGGG
chr3	184229818	AAVS1-OT2	ACACGACGCTCTTCCGATCTNNNNGATCCCCAGAGCCAGCCCG	GACGTGTGCTCTTCCGATCTTGGGAAAGCGAAAAGAGGGG
chr3	50189772	AAVS1-OT3	ACACGACGCTCTTCCGATCTNNNNCACCTGAACAGAGCACCAG	GACGTGTGCTCTTCCGATCTGAGAAATTGCATCCCAGAGCAG
chr20	35020704	AAVS1-OT4	ACACGACGCTCTTCCGATCTNNNNCATGCTTGGTTTCCTCCCTAGA	GACGTGTGCTCTTCCGATCTCTCCAGATGTGCAAGGTTCCC

AAVS1 parental (sorted by capture events)

chr1	198172184	SKEPBNNE	ACACGACGCTCTTCCGATCTNNNNCGAGTTAATGAATGAGCTGGAGA	GACGTGTGCTCTTCCGATCTGGAGAAAAGAGTGCTGGTATGG
chr3	50189772	ZXIDAYVB	ACACGACGCTCTTCCGATCTNNNNCACCTGAACAGAGCACCAG	GACGTGTGCTCTTCCGATCTGAGAAATTGCATCCCAGAGCAG
chr1	181141476	NJKWJSVI	ACACGACGCTCTTCCGATCTNNNNCTACCAGGGATGCTTGCATTTC	GACGTGTGCTCTTCCGATCTTTGCAAAGAGACAGACATCAGC
chr3	184229818	MDQZVDQD	ACACGACGCTCTTCCGATCTNNNNGATCCCCAGAGCCAGCCCG	GACGTGTGCTCTTCCGATCTGGAGAGGTGTCGGGCCAGG
chr1	3779130	MXMIJAII	ACACGACGCTCTTCCGATCTNNNNCATCTGACACACCCACGGCAG	GACGTGTGCTCTTCCGATCTCTTGAGACCTGGACCCACTT
chr20	35020704	TWCVQKCB	ACACGACGCTCTTCCGATCTNNNNGGTCTGCAGGAAAAGGCTACT	GACGTGTGCTCTTCCGATCTCTGAGCCTTGAAGTAGTTCCAAG
chr17	64036794	YLASZVZQ	ACACGACGCTCTTCCGATCTNNNNCTTGCTCAGAGGCCAAGTCTAC	GACGTGTGCTCTTCCGATCTGAAGCATTCCCAGAACTAGGGT
chr12	47782518	ADCBEPET	ACACGACGCTCTTCCGATCTNNNNATATCAGGGCTGGTCTCTGTGG	GACGTGTGCTCTTCCGATCTCCCTTTAACAATCTGCCCTTCC
chr15	84571034	KKDXEDBN	ACACGACGCTCTTCCGATCTNNNNCAGCTTAAAAGGACAACAGCAC	GACGTGTGCTCTTCCGATCTTGCTCTGGACTCGTGCACC
chr2	96859552	FJNBJJRY	ACACGACGCTCTTCCGATCTNNNNTGACTTCTCCTTCTGGCCAG	GACGTGTGCTCTTCCGATCTTTTGTGGTCCCTGCCTGCCT
chr11	61583600	MDRBAFMZ	ACACGACGCTCTTCCGATCTNNNNGGGGAGCTGGTGAGAGAAC	GACGTGTGCTCTTCCGATCTCAGATGGGAGCAAGCACTGG
chr5	68225722	VIJLIIJS	ACACGACGCTCTTCCGATCTNNNNTTGATATTCCTGGAGTGCAGCT	GACGTGTGCTCTTCCGATCTTTTAGGTAGGGGCTGTAGGACA
chrX	15856772	NBKTCEXS	ACACGACGCTCTTCCGATCTNNNNGACGGGAGACTTGCTGATGAC	GACGTGTGCTCTTCCGATCTAGCCAAGATTATGTAATAGCTGCT
chr19	11139414	KJKNKMQY	ACACGACGCTCTTCCGATCTNNNNGGGACAAATGACACCTCC	GACGTGTGCTCTTCCGATCTCTTTAATCTGAGCTGAGTGTGTTCA
chr11	76300996	NTXEDNEC*	ACACGACGCTCTTCCGATCTNNNNGCCTCACTACCATCTTTCAAAC	GACGTGTGCTCTTCCGATCTCCAAAGTGCAGGGATTACAGG
chr19	49337262	MECBHTDY	ACACGACGCTCTTCCGATCTNNNNCTGGGAATCCTCATCTCCACTC	GACGTGTGCTCTTCCGATCTCTGCCCTTGTTTCGGTCTCTC
chr8	140496946	QYLTTQVE	ACACGACGCTCTTCCGATCTNNNNCCTCACTCTCTAACATGGGGTAC	GACGTGTGCTCTTCCGATCTGGCAACAAGAATGAGACTCCG
chr10	68842214	DXZFWQVT	ACACGACGCTCTTCCGATCTNNNNAATGCCCTATTTGAAGATGTTA	GACGTGTGCTCTTCCGATCTAAGCCCTCTGATCTATGAAACCT
chr13	40205820	VSVXAKZK	ACACGACGCTCTTCCGATCTNNNNACAAGAACACGAGTCATACAGTC	GACGTGTGCTCTTCCGATCTCCTTTGGCCTTGTTCATCTCTT
chr12	103878180	JKMTFMQJ	ACACGACGCTCTTCCGATCTNNNNCAAACTTCACGGTCCAGAGGG	GACGTGTGCTCTTCCGATCTGCCTCAGTTTCATTAGTTGGCT
chr17	77279998	FHXTXDNR	ACACGACGCTCTTCCGATCTNNNNATAAGGAAAGGGGTGGACTCAG	GACGTGTGCTCTTCCGATCTTTGAGTAATCTGTCCAGCCACA
chr3	37492070	XSYSLVBY	ACACGACGCTCTTCCGATCTNNNNACACCCACTAACTTTCCTAGACT	GACGTGTGCTCTTCCGATCTCCTTCAAGGTAGAGTAGAATCCAGT
chr19	1224746	SPEYREZB	ACACGACGCTCTTCCGATCTNNNNCTGTGTGAGACCTGGGCTG	GACGTGTGCTCTTCCGATCTGAGTGGTCTGGCGGCCCTT
chr15	88834438	QQLZCQAN	ACACGACGCTCTTCCGATCTNNNNGAAGAAAGGGTCACTCGGAGTC	GACGTGTGCTCTTCCGATCTCTCTTGGCTCAGTCACCGC
chr7	128456566	MQDNQLMZ	ACACGACGCTCTTCCGATCTNNNNAGAGACTTCTAGGCAGGTTCC	GACGTGTGCTCTTCCGATCTGGCACAATACACAACCCCTG
chr22	21443968	QJIWJSRD	ACACGACGCTCTTCCGATCTNNNNCCCGGCGTCTATCTGGTG	GACGTGTGCTCTTCCGATCTCTTCCCATCCCCACTGAGCAG
chr4	11364294	XRNANFTP	ACACGACGCTCTTCCGATCTNNNNTGTTGCAGGATAGAAGGAGTC	GACGTGTGCTCTTCCGATCTACTTGTAATGCGAATCAATGTCAC
chr16	70428130	VDDWEPHN	ACACGACGCTCTTCCGATCTNNNNGGATAACCCTGACACCAAAATG	GACGTGTGCTCTTCCGATCTCCTGGCAAGACTGAGCTCATTC
chr2	36967986	BZCTHMJW	ACACGACGCTCTTCCGATCTNNNNTAAAGATCTAGGCTGCTGAGTGA	GACGTGTGCTCTTCCGATCTCAACCAGAGCAGACACAATGT
chr5	1217650	DHIKJQPW*	ACACGACGCTCTTCCGATCTNNNNGAGCCATTTCCGCACCAG	GACGTGTGCTCTTCCGATCTATGGTGCATAATTATAACTCACT
chr8	144515636	WDRFAKAK	ACACGACGCTCTTCCGATCTNNNNTTTCCAAAGCAGAAAAGAGTGACA	GACGTGTGCTCTTCCGATCTCACTTATGTTCCAAGCAACAGG
chr7	102050526	LYCKVKHE	ACACGACGCTCTTCCGATCTNNNNGTAACCTCATCTGGGCCGTTT	GACGTGTGCTCTTCCGATCTCTGGTTACCTCTCCCGAAATG
chr2	105038548	MRRWYPER	ACACGACGCTCTTCCGATCTNNNNAGCGACCTCCTCCTTCATTTG	GACGTGTGCTCTTCCGATCTAATTCCCATCATTCTTCTCTGGC
chr3	134287458	SABXIKVE	ACACGACGCTCTTCCGATCTNNNNGAAGTCCACGGTTCAGGG	GACGTGTGCTCTTCCGATCTGGGCTTGTTGTAAAAGCAGGTT
chr17	39631920	FYNZVZHV	ACACGACGCTCTTCCGATCTNNNNCAGAGTGTCATCTTCTCAGGAG	GACGTGTGCTCTTCCGATCTATCTTCATCCTGGCCTGTTCGC
chr8	47662294	WSIPFRJC	ACACGACGCTCTTCCGATCTNNNNAGCATCTGAGGACTGGGACCA	GACGTGTGCTCTTCCGATCTATCTTGAGAAACTTGCAACCCC
chr13	26591916	BHSAIQNX	ACACGACGCTCTTCCGATCTNNNNGGATGCTTTAGGAACGGCTG	GACGTGTGCTCTTCCGATCTCTTTCCAGTTAGACATCCATTCTGA
chr1	31425552	QNYSREDF	ACACGACGCTCTTCCGATCTNNNNGTTGGAGGAACGTGGTGGG	GACGTGTGCTCTTCCGATCTTACGCACAGAGGGCAGGATATG
chr14	30550484	XJTSWSTX	ACACGACGCTCTTCCGATCTNNNNCACAGTGTTTTGAATGTAGAAAGC	GACGTGTGCTCTTCCGATCTAGTTGACTCACTTCTTAGGTAT
chr16	81258590	KDEJKCMH	ACACGACGCTCTTCCGATCTNNNNGAAAACCACCCCACATATGCAG	GACGTGTGCTCTTCCGATCTCATCTGAGAGTGCTGACCAGAA
chr15	81689072	SVYIYHHM	ACACGACGCTCTTCCGATCTNNNNGTGCAGAGGATTGGATGGAGTC	GACGTGTGCTCTTCCGATCTCATGGAGTGGCTACCTGTCAG
chr18	11866942	ZRIPZCKP	ACACGACGCTCTTCCGATCTNNNNTTATTAAGGACCTACTGTGGGAC	GACGTGTGCTCTTCCGATCTGTGTGATCCTGGACTGCAAGT
chr10	73488048	TBZMKTDB*	ACACGACGCTCTTCCGATCTNNNNAGGCTGGTCTCGAACATATGAC	GACGTGTGCTCTTCCGATCTGGAGAAGCAACTTAATGTCTCTGA
chr14	103531006	EZMKEHSV	ACACGACGCTCTTCCGATCTNNNNTAGGGTCCAGGTTCACGAAGAG	GACGTGTGCTCTTCCGATCTCAGCTGATGGAGTTGGCTAAG
chr18	48835990	VSRTQNNE	ACACGACGCTCTTCCGATCTNNNNAGCTCCCTGTTTGCATTTGT	GACGTGTGCTCTTCCGATCTGCAAATGTTCATGGGCTAGA
chr2	25362220	VVQSNHIL	ACACGACGCTCTTCCGATCTNNNNAAATGCTGACTGTTGATTGGCT	GACGTGTGCTCTTCCGATCTCTCTGTCCCATTCCCACACAC
chr15	70478526	YPHQYDEL	ACACGACGCTCTTCCGATCTNNNNACACATCATGAAAACTTATTGCTCA	GACGTGTGCTCTTCCGATCTGAGGGTGGCCTGGGGAGAA
chr1	31712738	JJNQKMDV	ACACGACGCTCTTCCGATCTNNNNCTTCGAATCCTGGAATCTCTGG	GACGTGTGCTCTTCCGATCTTCCCTTAGCCTTCTTTCTAGCC
chr15	40764304	DBLKXVKK	ACACGACGCTCTTCCGATCTNNNNCCGGGACCAGGCCCTAGG	GACGTGTGCTCTTCCGATCTCTGAGCCCGCTTCTCCACTC
chr19	47470024	TKYAICMM	ACACGACGCTCTTCCGATCTNNNNTGATCAGAAGAACAGGACTGCA	GACGTGTGCTCTTCCGATCTCTGAGTCCCCAGCTCGGTC
chr5	157216616	YAYLTBWW	ACACGACGCTCTTCCGATCTNNNNCCACACCCAACCCATAGTAACA	GACGTGTGCTCTTCCGATCTGACTGGTCTCTCTTTCTTCGTC
chr9	131820192	CZCNILBV	ACACGACGCTCTTCCGATCTNNNNCCATGCTTCGCCTTGTGAG	GACGTGTGCTCTTCCGATCTAAACGCCACAGTAATTAGGACAG
chr11	48138440	QFSLQVNV	ACACGACGCTCTTCCGATCTNNNNTATCTGAGCATTTATGACTGGGT	GACGTGTGCTCTTCCGATCTCAGGAGAAGCTCTACCAAGGC
chr13	49996346	JCCAEFMJ	ACACGACGCTCTTCCGATCTNNNNGCGCTCCCAACGCGAATC	GACGTGTGCTCTTCCGATCTACTTCATCCGCATCTCAAGACC
chr15	30452078	FWVQARHY*	ACACGACGCTCTTCCGATCTNNNNGTAACTGGTGCGTCAATGACT	GACGTGTGCTCTTCCGATCTTCTCCTGACCTCGTGATCC

chr15	32555628	HZHSHMKN*	ACACGACGCTCTTCCGATCTNNNNTCTCCTGACCTCGTGATCC	GACGTGTGCTCTTCCGATCTGTAACTGGTGCGTCAATGACT
chr17	75033800	PEMMYWRN	ACACGACGCTCTTCCGATCTNNNNTGCTCGCTAAGTAAGGATGGGG	GACGTGTGCTCTTCCGATCTAGTGTCCCCTTTTCCTAGAGAA
chr19	2306802	RERRZMVD	ACACGACGCTCTTCCGATCTNNNNGCTGAGTTGAACCCTCCTAAGC	GACGTGTGCTCTTCCGATCTAGGATCTGAGTGGGAAGAAC
chr7	98643926	ARJTTBAY	ACACGACGCTCTTCCGATCTNNNNGTCAGTCAATGGCCAGGAATC	GACGTGTGCTCTTCCGATCTTCCAAGGACAAGAGCACCCA
chrX	40329304	FKPLWCDM	ACACGACGCTCTTCCGATCTNNNNCTACAGGCAGAGCACAAGAGG	GACGTGTGCTCTTCCGATCTCGCCCAGCCAATTATTAAGAGAT
chr12	113130622	IACDAHRA	ACACGACGCTCTTCCGATCTNNNNCAGGCCCGGAACCAACCAG	GACGTGTGCTCTTCCGATCTCTCTGGACCTAATGCTGTCCC
chr12	54823166	SERBMJRZ	ACACGACGCTCTTCCGATCTNNNNATCTTTGGGCCCTACATAAATC	GACGTGTGCTCTTCCGATCTTAGCTCTCTCTCTTCTGCCAG
chr17	76685626	FRHPJXMZ	ACACGACGCTCTTCCGATCTNNNNCCCTGTGGCTATTGGAAATCTG	GACGTGTGCTCTTCCGATCTCTTCAGCGGTACACACGTCAAA
chr18	41955254	HQAHXRQD	ACACGACGCTCTTCCGATCTNNNNGAAGTTCCGTGTTGTGGGGC	GACGTGTGCTCTTCCGATCTTCCAGGTCACAACTATAGATGT
chr11	65614374	NYDAMIMK	ACACGACGCTCTTCCGATCTNNNNTTTCCGCTCTTCTCCCTCCTG	GACGTGTGCTCTTCCGATCTGGTTCTGCCCTTTCCCTCT
chr12	53051038	ICXBPLCL	ACACGACGCTCTTCCGATCTNNNNAAACAGAGAGATGGCAAGGAGA	GACGTGTGCTCTTCCGATCTACCCAGAGATGCAGTGAGGC
chr20	37880952	QPPTLWLN	ACACGACGCTCTTCCGATCTNNNNCATTAAGCAGCCGTGACAGGAC	GACGTGTGCTCTTCCGATCTCCCCATGACAGAGAGACTTC
chr19	42291652	WQCIKXCR	ACACGACGCTCTTCCGATCTNNNNTTTCTTGCCTCTTAACTTCCAGC	GACGTGTGCTCTTCCGATCTGGTACTGAGAAGGGCGGG
chr2	217108872	SXWDQNIV	ACACGACGCTCTTCCGATCTNNNNCCTGAGCAGATAAGGGCCC	GACGTGTGCTCTTCCGATCTGGACTTCTCTACTTCCAGACCCT
chr22	22201860	YATNKBTF	ACACGACGCTCTTCCGATCTNNNNCCTGCTCCTCACTGTGCTG	GACGTGTGCTCTTCCGATCTCAACACTGAGGTCACTGCTCAG
chr3	195857016	RYJYDEME	ACACGACGCTCTTCCGATCTNNNNCAAGAAGGGAAGGGAGTAGCG	GACGTGTGCTCTTCCGATCTTGGCAAATCACTGGGTCCC
chr7	5922514	WLMMPNKS*	ACACGACGCTCTTCCGATCTNNNNTGAAGCAAGCAGAGGAGGC	GACGTGTGCTCTTCCGATCTTAGTGGGAAAAGCTGGGCC
chr7	6802396	SLHFRKHX*	ACACGACGCTCTTCCGATCTNNNNTAGTGGGAAAAGCTGGGCC	GACGTGTGCTCTTCCGATCTTGAAGCAAGCAGAGGAGGC
chr1	39214102	HVLXPDVJ	ACACGACGCTCTTCCGATCTNNNNTGTAGTCGTTTGGGCTAATCTCT	GACGTGTGCTCTTCCGATCTAGCTCCCTAAATAGAACGACTTC
chr1	93699008	RBFHVEHX	ACACGACGCTCTTCCGATCTNNNNCACACAACACAAGGGAATCGTG	GACGTGTGCTCTTCCGATCTAGAAAGCAGGGGTTCAGAAAAG
chr8	60233954	VCPBCJZX	ACACGACGCTCTTCCGATCTNNNNAACATCATCACTCACAGCTGC	GACGTGTGCTCTTCCGATCTTTTTATCCCTGTTCCATACTTTAC
chr11	45538310	KLASFDQI	ACACGACGCTCTTCCGATCTNNNNGATCAGAGTAGGACTAGGGAGC	GACGTGTGCTCTTCCGATCTCAAAGTCCCCAGCCCCAAAG
chr11	78017714	CFFFMFZP*	ACACGACGCTCTTCCGATCTNNNNTGCTAGTCTGGTCTCAAACTCC	GACGTGTGCTCTTCCGATCTAATTAAGATGCTTGAATGGGTA
chr11	78262418	FDLEFDJL	ACACGACGCTCTTCCGATCTNNNNTAAGAAATGACATAGTCCCTGCAC	GACGTGTGCTCTTCCGATCTTTTTGTACCCTCTGCGACAG
chr17	44086880	SLBEIFPM	ACACGACGCTCTTCCGATCTNNNNCCCCAATCCAGCCCTTTTCTT	GACGTGTGCTCTTCCGATCTAATGCTACTCTTTGATGTGACTCA
chr22	18958826	VWMCAWWM	ACACGACGCTCTTCCGATCTNNNNCCAGGGTCATCAGGCTCC	GACGTGTGCTCTTCCGATCTGATTAGTCATCCCCGTGCAG
chr3	111979030	ERXCSXJY	ACACGACGCTCTTCCGATCTNNNNAAGTTCCGCCTTCCAGCCTGAA	GACGTGTGCTCTTCCGATCTGTACCCAGGCCGCCACAG
chr3	184182170	XHXZNQQA	ACACGACGCTCTTCCGATCTNNNNGGAATAAAGGTAGCTGATGTCACA	GACGTGTGCTCTTCCGATCTCTTGTACTTGGCCTTCCCCTTC
chr5	148910878	LYHFMVJQ	ACACGACGCTCTTCCGATCTNNNNAGCAATCCCTGACCCACT	GACGTGTGCTCTTCCGATCTTCATAGTTAGAGAATGGGTGGGAG
chr22	23187146	IENDICDD	ACACGACGCTCTTCCGATCTNNNNCCTGGTTCTGAGCTTTACACATAG	GACGTGTGCTCTTCCGATCTTGATAAGTCACTTTCCAATGAT
chr12	126442484	PKHAHNNT	ACACGACGCTCTTCCGATCTNNNNTCTCTCCAGGCATTGTTCTGTA	GACGTGTGCTCTTCCGATCTTCTGGGTGGCATTTGAAGTCAA
chr20	45905200	YRKJZYQW	ACACGACGCTCTTCCGATCTNNNNGCATTCACAGTCAGGGTCAAG	GACGTGTGCTCTTCCGATCTTTGTGAATCCTCCAAGAACAGC
chr6	40410142	YZXHNKZD	ACACGACGCTCTTCCGATCTNNNNGAAGCCCCACTCTGTCCAG	GACGTGTGCTCTTCCGATCTGTTAGAGCCTCATCCTCCAGC
chr6	7389498	VEBDINQI	ACACGACGCTCTTCCGATCTNNNNGAGTGCTGGAACGCCCCTG	GACGTGTGCTCTTCCGATCTCGTACCTTCCTAACTTCACCAC
chr19	14571814	XWPJWAHS	ACACGACGCTCTTCCGATCTNNNNAGTCTAGACCCAAACCCAGATAC	GACGTGTGCTCTTCCGATCTCCAGACTACGGCTTCCCC
chr21	44196402	DFWCALML*	ACACGACGCTCTTCCGATCTNNNNGGATGTGCGTGCTTCTCTG	GACGTGTGCTCTTCCGATCTGGACTGAAAAGAGGAAGCAAGC
chr21	5065744	QYWSLAVY*	ACACGACGCTCTTCCGATCTNNNNACTGAAAAGAGGAAGCAAGCC	GACGTGTGCTCTTCCGATCTCGTGCTTCTCTGCTGTTGAG
chr5	180101344	LIBTXVYV	ACACGACGCTCTTCCGATCTNNNNGCACGTCTGGAGGCCCTG	GACGTGTGCTCTTCCGATCTTCCACAGCCCTGCCTCAGTG
chr8	99656330	VASSABCM	ACACGACGCTCTTCCGATCTNNNNCTGACACATTTCCTACCTTCTG	GACGTGTGCTCTTCCGATCTTCAAGTCATAGGAATCTCTCAAA
chr3	14558032	CTZKNSSC	ACACGACGCTCTTCCGATCTNNNNGGTGCTAGATAAATAATATGTAACC	GACGTGTGCTCTTCCGATCTTTCATGGAACGCATCAGATTCC
chr1	14112422	WJTKDHJS	ACACGACGCTCTTCCGATCTNNNNGACAAACCCACCACCGTTACTT	GACGTGTGCTCTTCCGATCTAAAGCCAGGACATCACTTCTTG

AAVS1-I479Q

chr1	3779132	KJVPDZHF	ACACGACGCTCTTCCGATCTNNNNTCATCTGACACACCCACGG	GACGTGTGCTCTTCCGATCTCTGCCTTGAGACCTGGACCC
chr1	227672558	QVDFYKLF	ACACGACGCTCTTCCGATCTNNNNTAAATTTAGGGCATGTTGGAGCT	GACGTGTGCTCTTCCGATCTCTAGCTGAGAGGAGTAGGGAAG
chr17	26601400	YQBXIBPD	ACACGACGCTCTTCCGATCTNNNNACGGGTATATCTTCACATAACATC	GACGTGTGCTCTTCCGATCTTCCCCTTGCAGATTCCACAGA
chr10	39568572	IYCWPMRD	ACACGACGCTCTTCCGATCTNNNNACAGCAGTTTGGAAAGACTCTG	GACGTGTGCTCTTCCGATCTCACAACACAAAGGATTTACTGA
chr10	39569918	CAFSMHXH	ACACGACGCTCTTCCGATCTNNNNTCATAGAGCAGTTTGGAAAGACTC	GACGTGTGCTCTTCCGATCTCACATAACACAAAGGATTTACTGAG
chr2	183113068	NTFRFWMT	ACACGACGCTCTTCCGATCTNNNNGGCATTTTCCCCAGATAAAT	GACGTGTGCTCTTCCGATCTTCACACTTTAGACAGCCATTCAC
chr2	183113122	IYCWHNVV*	ACACGACGCTCTTCCGATCTNNNNGGCATTTTCCCCAGATAAA	GACGTGTGCTCTTCCGATCTTCCTCTTTATTACTGAATGGTAT
chr22	19520532	ZTPMRKJZ	ACACGACGCTCTTCCGATCTNNNNGGCACATCAACATCGTTTGAAAC	GACGTGTGCTCTTCCGATCTGGACGGAGCCTAAAATAAGCAT
chr3	147172268	QXFSZLDT	ACACGACGCTCTTCCGATCTNNNNCCTGTATTCACGGTGATGATATTT	GACGTGTGCTCTTCCGATCTGATTGTGTCTAGAAATGTCAGA
chr5	117958136	ANXCBYPC	ACACGACGCTCTTCCGATCTNNNNGAAATTTAACCTTGCAAAGTCAAGT	GACGTGTGCTCTTCCGATCTTTATGTCACCCAGGGAGAGAAG

AAVS1-Q481A

chr3	50189776	ZZFPFRJV	ACACGACGCTCTTCCGATCTNNNNCAGGCCCTGGAGATAATCT	GACGTGTGCTCTTCCGATCTGAGCAGGTGTAGTGTTCCCTG
chr7	62438920	RRHRMHDA	ACACGACGCTCTTCCGATCTNNNNTCGATGCCAATTCTATTCGATT	GACGTGTGCTCTTCCGATCTTCATCATAGAACAGAATAGAATGG
chr14	92269876	KBWNPZYP*	ACACGACGCTCTTCCGATCTNNNNTAGTATTCCATTACACACACAC	GACGTGTGCTCTTCCGATCTAAACACCTAGGTACATCATATT
chr2	14095318	IBPIPYFW	ACACGACGCTCTTCCGATCTNNNNTGACTAACAATACGATATAATGCTC	GACGTGTGCTCTTCCGATCTATGTCATGAAAATGTTGGGCTTT
chr1	98870749	YFRQFTMX	ACACGACGCTCTTCCGATCTNNNNCAGGATTCAGCATATTTAACTACCT	GACGTGTGCTCTTCCGATCTCCTTAGCACACCTTAGACATTT
chr1	63096930	WZYJICWT	ACACGACGCTCTTCCGATCTNNNNACAGAGAAGGTCAGGGTTCAAG	GACGTGTGCTCTTCCGATCTGCTTCACCTTTCTTTCCACTCT
chr2	193181226	PWJQBHMJ	ACACGACGCTCTTCCGATCTNNNNAGCACTGTCAGAATATAGATTTGGA	GACGTGTGCTCTTCCGATCTATTGAAGAATTGCAAAGAGGC
chr20	31534128	ICATDKLF	ACACGACGCTCTTCCGATCTNNNNGTCTTGCCATGTTGCCTATG	GACGTGTGCTCTTCCGATCTGTGTGCGAGTGATCCTTAAAGAC
chr20	31534188	VBVADNEB	ACACGACGCTCTTCCGATCTNNNNCTCATGCAATCCTCCCGCCTT	GACGTGTGCTCTTCCGATCTATTTCATAAGCCTCTCTGAGCA

chr20	33775072	FHYSBFJH	ACACGACGCTCTTCCGATCTNNNNTGCCTCATTCTTTAAACAGCTAC	GACGTGTGCTCTTCCGATCTCCGAAGTTGTTCCCTGTATAATTGT
chr20	62859688	XWLFRJHE	ACACGACGCTCTTCCGATCTNNNNACACGTAATTTGAAGAACTTTAG	GACGTGTGCTCTTCCGATCTGCGACAGAGTGAGAGCCT

PD1 locus specific primers

chr2	241858860	PD1-on	ACACGACGCTCTTCCGATCTNNNNGACCCCACCTACCTAAGAACC	GACGTGTGCTCTTCCGATCTGAGAAGGCGGCACTCTGGT
chr19	6002846	PD1-OT1	ACACGACGCTCTTCCGATCTNNNNTCGCTGAGCATCTGGTTGA	GACGTGTGCTCTTCCGATCTCTTACAGGTGGGCGTCGTCAG
chr13	19058833	PD1-OT2	ACACGACGCTCTTCCGATCTNNNNTGAAGAGGCCAAGGAAAAGGAG	GACGTGTGCTCTTCCGATCTACATAGTAACCATGCAGACAGGT
chr12	121794802	PD1-OT3	ACACGACGCTCTTCCGATCTNNNNCCTCTTACTCCTCACCCTGGG	GACGTGTGCTCTTCCGATCTCTGGTAGGATCCCACTCTCG

BCL11A locus specific primers

chr2	60495266	BCL11A-on	ACACGACGCTCTTCCGATCTNNNNGTCCTCTTCTACCCCACCC	GACGTGTGCTCTTCCGATCTCACCAGGGTCAATACAACTTTGA
chr8	119856442	BCL11A-OT1	ACACGACGCTCTTCCGATCTNNNNGACTCCAGCCTAGCCGACT	GACGTGTGCTCTTCCGATCTCGCCGTTGTCTCCAGATATGAT
chr2	62164814	BCL11A-OT2	ACACGACGCTCTTCCGATCTNNNNTCATGCTCTAGTCCTGCCTCTC	GACGTGTGCTCTTCCGATCTGCCATTCTGATAGCTGTGTTCAT

TCRa locus specifici primers

55254_55248 (TCRa parent TOP 96 primers)

chr14	22550604	TCRa-on	ACACGACGCTCTTCCGATCTNNNNCCTCTTGGTTTTACAGATACGAAC	GACGTGTGCTCTTCCGATCTCTCACCTCAGCTGGACCACA
chr9	128878060	XWJQLHRN	ACACGACGCTCTTCCGATCTNNNNCACCATAAACTAGCTATGGGACC	GACGTGTGCTCTTCCGATCTTCATTGACAAGTGGCTGTGAG
chr17	79736684	XCTFZDRM	ACACGACGCTCTTCCGATCTNNNNAAGGGTGACATGGAAGAGGTAG	GACGTGTGCTCTTCCGATCTCTCCCATCGGTCACTTCCTTTC
chr9	13565900	KKWBKPIE	ACACGACGCTCTTCCGATCTNNNNTTTCTACTTACATTTCCTGGCCAT	GACGTGTGCTCTTCCGATCTTGGAGGAAACAGTATTACAC
chr7	158413556	XPLMZTEA	ACACGACGCTCTTCCGATCTNNNNCACTCTGGGCCTGTGACTAA	GACGTGTGCTCTTCCGATCTCTGCTGAGGTCGCCATGGAG
chr19	35309590	LKZTVIEC	ACACGACGCTCTTCCGATCTNNNNGATCCACTCACTCTTCGGCCT	GACGTGTGCTCTTCCGATCTCCTTGAACATTCCCAGGCAC
chr17	362910	SHMJMRFD	ACACGACGCTCTTCCGATCTNNNNCATCAACGACCTTCCTTCCCTC	GACGTGTGCTCTTCCGATCTCTTAGGTGCTCCCCTGGAAGAG
chr22	21709082	BTPWRKTT	ACACGACGCTCTTCCGATCTNNNNAGAAGCATCTCAAATTCCTTTT	GACGTGTGCTCTTCCGATCTTGTGTGTTGGCCATGACCTAA
chr12	109930438	HTCRKDWA	ACACGACGCTCTTCCGATCTNNNNGGGATCATTGAGAATACAGGGTG	GACGTGTGCTCTTCCGATCTTCTCTGCTCCTGTTGGCAAATC
chr6	44255520	SEDDFKHA	ACACGACGCTCTTCCGATCTNNNNAGAGCTTCGTATTGGCACCAG	GACGTGTGCTCTTCCGATCTACCGGGTGAGCGCTTTAC
chr12	53004488	XMKVWIYL	ACACGACGCTCTTCCGATCTNNNNTCTTTGTGACCTATAACTTGTGT	GACGTGTGCTCTTCCGATCTAGGCTACTGAATGGAGAGT
chr14	49621284	TPKLEYIE	ACACGACGCTCTTCCGATCTNNNNCAGCTGCTCCTTTCCGCGC	GACGTGTGCTCTTCCGATCTTGCTCCAGAGGACGAAGCC
chr2	26785730	WKVSEDEK	ACACGACGCTCTTCCGATCTNNNNAAAATGTTTGAGGAAAGCGACTT	GACGTGTGCTCTTCCGATCTCAATCTGGTTGCTGGTCTTTGC
chr19	48508592	EQPJAEWI	ACACGACGCTCTTCCGATCTNNNNCTCTCAGCTGCCCTCTTCATG	GACGTGTGCTCTTCCGATCTGAGAGGCAGGATGGTGGAG
chr8	129726442	ETARWWEE	ACACGACGCTCTTCCGATCTNNNNGGAGAAAGACATGGAAGAGGGG	GACGTGTGCTCTTCCGATCTCAGCATTGTCAAACAGCAGGAT
chr1	28878370	IESFEYSF	ACACGACGCTCTTCCGATCTNNNNAAAAGAGGAAATGGGCTG	GACGTGTGCTCTTCCGATCTCATACCTGGCCAACTTTTAAAT
chr8	66664620	NRRTZWSN	ACACGACGCTCTTCCGATCTNNNNGAATTCTCTGGCTATACTTTCCTGA	GACGTGTGCTCTTCCGATCTGGCTCCCTATTCACCGAC
chr19	33383802	ZNYIZZDP	ACACGACGCTCTTCCGATCTNNNNCTCTTGTCCCTCCCTGTAAAGA	GACGTGTGCTCTTCCGATCTCGAAGAGGATGGCTCTGCATG
chr12	56632406	VKDNLVRB	ACACGACGCTCTTCCGATCTNNNNTCTTCCCTGCCCTTTTCCAGAG	GACGTGTGCTCTTCCGATCTGGGAGGGGCGTGGCTACA
chr2	26018400	YABSYBZM	ACACGACGCTCTTCCGATCTNNNNTCAGGTGACAGCCAAGGACA	GACGTGTGCTCTTCCGATCTATACAGAAGATGCCCTCCTTTT
chr1	203006352	DEVTPIPJ	ACACGACGCTCTTCCGATCTNNNNGCCAGCAGACTTCACTTATCAG	GACGTGTGCTCTTCCGATCTTGTGACGTTTATTACAGGAGGG
chr1	110517930	TFPDLHII	ACACGACGCTCTTCCGATCTNNNNTGGAGATGATGTCAATGATGTTCA	GACGTGTGCTCTTCCGATCTGTTCACCGACCCTTTCTTCATG
chr1	234851754	EDYTHBWA	ACACGACGCTCTTCCGATCTNNNNCACAATCAGAAGCAGTGGACA	GACGTGTGCTCTTCCGATCTGGCTGGAGTTTCTAACCCCTCTT
chr10	132515136	XYPSNFMN	ACACGACGCTCTTCCGATCTNNNNCTGGAGACGCTTTCAGGCAG	GACGTGTGCTCTTCCGATCTTCTGCCATTTCCCCAGGTG
chr22	23280666	TSLFAJIL	ACACGACGCTCTTCCGATCTNNNNTAAGGAAACAGAGGCTCCAAGA	GACGTGTGCTCTTCCGATCTAGAAAGCAAGGGTGGCCAG
chr8	28528022	LARFTWME	ACACGACGCTCTTCCGATCTNNNNCCCTAAACAGAGTTCGAATTGAGA	GACGTGTGCTCTTCCGATCTAGCATCCAATTACAACCAAGAGT
chr3	72163900	MTLPNNCS	ACACGACGCTCTTCCGATCTNNNNCCACAGCTTCCAGTTAACTAGC	GACGTGTGCTCTTCCGATCTACACACCCTGGTTGCATCATAT
chr10	45235318	IAXIAMEL	ACACGACGCTCTTCCGATCTNNNNAATCCTGATTCAATAGGTTTCAGTG	GACGTGTGCTCTTCCGATCTATCTGGTGGGCATTGCATTAAA
chr22	20369586	DEPNWLCQ	ACACGACGCTCTTCCGATCTNNNNTCCTTCATCCGGATCATAT	GACGTGTGCTCTTCCGATCTGTGAGGAGCTTCTGTCTCTTG
chr6	110569182	NZABWQBQ	ACACGACGCTCTTCCGATCTNNNNGGACATCCTTCACGTTATTGTTC	GACGTGTGCTCTTCCGATCTATGGCTGACTTCATACACAAGG
chr9	136086174	LZZMEWTN	ACACGACGCTCTTCCGATCTNNNNGAGAAAACCAAGTCACCCCTGG	GACGTGTGCTCTTCCGATCTGTTCTCTCACAGGAAGGCAG
chr9	98054342	YIJMQTIS	ACACGACGCTCTTCCGATCTNNNNCCTTCCAAACCAAGATCCCTAAC	GACGTGTGCTCTTCCGATCTAGGAAGAGTCTTGGTTCTGGAG
chr20	57941386	IZQDXKLY	ACACGACGCTCTTCCGATCTNNNNCAAAGCAAATGTGGACCAAGGG	GACGTGTGCTCTTCCGATCTCTGGCCTGGATCCCTCTC
chr22	18167878	DMMQVQFS	ACACGACGCTCTTCCGATCTNNNNGTGAGGAGCTTCTGTCTCTTGG	GACGTGTGCTCTTCCGATCTTCCTTCACCCGGATCGTATAC
chr1	25146536	FZAIISEJ	ACACGACGCTCTTCCGATCTNNNNCTCTCCCTCCCTTGGCTCTG	GACGTGTGCTCTTCCGATCTGTGAAGTTAACTGGCTCCGGA
chr12	107612674	IBREIYLH	ACACGACGCTCTTCCGATCTNNNNTGACCTTACGAGTCACAGG	GACGTGTGCTCTTCCGATCTAAACGTTTACAAGGTGGCTGTG
chr16	2155772	LLDTMKII	ACACGACGCTCTTCCGATCTNNNNGGAGAGGCGGGGAACTACAC	GACGTGTGCTCTTCCGATCTCAGCCCGCTTCATGATGC
chr15	34179254	AXAAXBYZ	ACACGACGCTCTTCCGATCTNNNNTTTTGTTTAGTTCTAGAGGCTTGG	GACGTGTGCTCTTCCGATCTAAGTAACTTTGGCCCTTTTGCT
chr6	118713042	YTDVMDWE	ACACGACGCTCTTCCGATCTNNNNGGAAACTTCATGACAAAGGGCA	GACGTGTGCTCTTCCGATCTGTTCCATCCTTTAACCACATCCA
chr16	77191236	FBXTXZZD	ACACGACGCTCTTCCGATCTNNNNACCCGGAAGTGTGATGCCA	GACGTGTGCTCTTCCGATCTCCCTCCAACACTTTCCGAAA
chr4	3667514	BZRHCSWA	ACACGACGCTCTTCCGATCTNNNNATGAGAGGCCTAGTGAAGAACC	GACGTGTGCTCTTCCGATCTCCTGTTCTCTCTCAGTCAGCTAT
chr6	52934244	SDDEHTTT	ACACGACGCTCTTCCGATCTNNNNGACCAGCTAACACACTCTCAAC	GACGTGTGCTCTTCCGATCTGGCCCCGAGTTTATCATTTCAT
chr4	186954080	LKPHBNKF	ACACGACGCTCTTCCGATCTNNNNAAGAATGCTGCTCACGTTGATC	GACGTGTGCTCTTCCGATCTGAGTGGCTGGTGGTTTTCAAAA
chr11	77199762	RYHYNRJT	ACACGACGCTCTTCCGATCTNNNNATACACCTTCACCTCCAGCAAT	GACGTGTGCTCTTCCGATCTGTCCATACCAGCAGCCACTC
chr15	37345596	EHBHWKVD	ACACGACGCTCTTCCGATCTNNNNTCCACTTAGTCTTGTAGGAATTAA	GACGTGTGCTCTTCCGATCTGGTCAAACAAATTAAATTACTGCAG
chr2	3655228	WSRSQWAH	ACACGACGCTCTTCCGATCTNNNNCTTCAGGGCAATAAAGGAGAAAAG	GACGTGTGCTCTTCCGATCTCATCCACGCCCCATTCTTC
chr8	42391528	XDDAAMTI	ACACGACGCTCTTCCGATCTNNNNAGGAGGTCGAGGCTCCAG	GACGTGTGCTCTTCCGATCTGCGAGGTTGCTAGTTAACTGTT

chr21	42533054	DBZYIBDP	ACACGACGCTCTTCCGATCTNNNNCGTGACCGTGGAGCAGCTG	GACGTGTGCTCTTCCGATCTCAGCAGCCGGAAGTCCTG
chr20	60891662	IBXSAVDX	ACACGACGCTCTTCCGATCTNNNNAGAAGGAAGGATCTTGGACTCC	GACGTGTGCTCTTCCGATCTCTGCATTTCATAGTCAACAACACA
chr5	36839152	WLRXLDJS	ACACGACGCTCTTCCGATCTNNNNTTGAAGAGACAGTTGGAAGCTG	GACGTGTGCTCTTCCGATCTGCCCCATCTGAAAACTAGTGTAT
chr12	21752278	DDZYEDJV	ACACGACGCTCTTCCGATCTNNNNAAAGCAGTGTACTTTGAGTAAAGC	GACGTGTGCTCTTCCGATCTTGCTAAGTCATTTCTCACCTTGTA
chr14	60248818	YIKLWLHI	ACACGACGCTCTTCCGATCTNNNNGAGTGGGGTTTGTATCTGCAGG	GACGTGTGCTCTTCCGATCTGGCTATTAGGTGCCCAGTAGAT
chr3	57107802	QXWFBSRZ	ACACGACGCTCTTCCGATCTNNNNAGACTTCTGGGGTTCAAATCCAA	GACGTGTGCTCTTCCGATCTAAATACCCTCTGTGACAACCCT
chr17	28712496	CMNMXNVB	ACACGACGCTCTTCCGATCTNNNNCTCTCCGAAGACCTAACTCCG	GACGTGTGCTCTTCCGATCTCAGCCTGCCCTGTTCTCCA
chr6	35811616	BVTRYWIS	ACACGACGCTCTTCCGATCTNNNNGGTGGATGGGTAGAGTGCTC	GACGTGTGCTCTTCCGATCTTCTCCATCACTTCCTCCCGTG
chr9	35001138	RDBAZJJQ	ACACGACGCTCTTCCGATCTNNNNAGTATTCCTTAGTCACCGGAGC	GACGTGTGCTCTTCCGATCTCTTTAAGAGCCGAAGAAGACAGG
chr1	2480064	ZCDTHNMR	ACACGACGCTCTTCCGATCTNNNNCAATGCAGACCCAGGGACC	GACGTGTGCTCTTCCGATCTCCTGACACCTCCACCCTCTG
chr3	197746150	BPYXAYVJ	ACACGACGCTCTTCCGATCTNNNNATAAAAGACTCCCAGTTAAACCTG	GACGTGTGCTCTTCCGATCTCGATGGCTATCCTCAAGCTTTG
chr11	119024886	MZPXCNTX	ACACGACGCTCTTCCGATCTNNNNCCCCTCCTTTTCTCCCCG	GACGTGTGCTCTTCCGATCTTCTTCCTCCTACGAAACATCCG
chr3	195621368	INFQRBBA	ACACGACGCTCTTCCGATCTNNNNCAAGGGATGGTGCTTCCTATG	GACGTGTGCTCTTCCGATCTGTGAAGGAATGTTCCCTGAATTAC
chr7	101364162	PMFYXADV	ACACGACGCTCTTCCGATCTNNNNCCAGGCGTCTCCTTCCTCTC	GACGTGTGCTCTTCCGATCTCGAGTTATGCAAACGAGGCG
chr17	81941032	ARQLSMVE	ACACGACGCTCTTCCGATCTNNNNTTGGGATCGAAACAGAAGACT	GACGTGTGCTCTTCCGATCTTGGTGGCCCTGAGTGTGAT
chr4	79325484	YNIQWQZK	ACACGACGCTCTTCCGATCTNNNNCGAGAGATCCCGCTTCATAG	GACGTGTGCTCTTCCGATCTTGCACGTCAGGAAGAGTAACC
chr15	45153400	TNVTBJZB	ACACGACGCTCTTCCGATCTNNNNATCTTGGGCTGAATGAGTGAGC	GACGTGTGCTCTTCCGATCTAAGAGAGGACGCTGAGGGG
chr20	25223350	VWRKYHXL	ACACGACGCTCTTCCGATCTNNNNCCCTACCCTGCAGCCCTG	GACGTGTGCTCTTCCGATCTCTAATCTGGCTTCCTGGGCTG
chr8	104793608	LTHVFMCV	ACACGACGCTCTTCCGATCTNNNNGGCCTTCAAACACTTCTGGG	GACGTGTGCTCTTCCGATCTGGCATAATTATTATCAGTCCAAATG
chr1	14131644	JKQKHBIV	ACACGACGCTCTTCCGATCTNNNNAACCTTATTGACTGTGAGGCG	GACGTGTGCTCTTCCGATCTATTCCTCTTTTAGCTCAATTAAC
chr1	244063132	TXDHHBXE	ACACGACGCTCTTCCGATCTNNNNAGTGATTGATTGAAAGTCACAA	GACGTGTGCTCTTCCGATCTGGGGAAGGTATGAAATGTTTGT
chr17	40100232	QJQRDRQN	ACACGACGCTCTTCCGATCTNNNNCTAAGGGCGGGGAGTGGAC	GACGTGTGCTCTTCCGATCTCCTCAACGTCCAACCCTTCT
chr5	169869406	DHYFMKBL	ACACGACGCTCTTCCGATCTNNNNTTTAAAGGGGATTGAAAGTAACT	GACGTGTGCTCTTCCGATCTAGTGGCAATTTTCCTTTATGTT
chr6	31769912	QIATWCZQ	ACACGACGCTCTTCCGATCTNNNNGATGAAGCAGAAGGGAATATGGC	GACGTGTGCTCTTCCGATCTTTCTTCCCCGTTTCCTGGC
chr7	149866310	ZKNPDXEK	ACACGACGCTCTTCCGATCTNNNNTCTGGGAGGTGTTACTATCTGC	GACGTGTGCTCTTCCGATCTCTTTCCACGAGTTCACATCCAC
chr1	241848280	SPWFQCMN	ACACGACGCTCTTCCGATCTNNNNAAGTTACCCGTGTTCTGCGTTG	GACGTGTGCTCTTCCGATCTGCTCCTCTCACCTTCCGAG
chr22	19218188	DTQHDLLK	ACACGACGCTCTTCCGATCTNNNNAGAGATAATGAAAGACCAGCAGC	GACGTGTGCTCTTCCGATCTGTGACTTCATGTTGGTACTGGG
chr3	195749386	CFYRZEWN	ACACGACGCTCTTCCGATCTNNNNCTTACCAAGGGATGGTGCT	GACGTGTGCTCTTCCGATCTGAAGCTGAAAGAGTGAAGGAATG
chr8	60701070	HHNQPQLD	ACACGACGCTCTTCCGATCTNNNNACGTTATGTTTGAGTTGGAAAGTG	GACGTGTGCTCTTCCGATCTCTGCTGCTCACGAAGATGTATC
chr11	62401684	NVHPZDCI	ACACGACGCTCTTCCGATCTNNNNGTCCCCTTGCCCTCTCTC	GACGTGTGCTCTTCCGATCTCTTGTTCTCATGGAGCCCTGC
chr8	139813412	SMDYTEVI	ACACGACGCTCTTCCGATCTNNNNGACTCCTCCTGTCCCACAAG	GACGTGTGCTCTTCCGATCTATCTGCCATCGTTCTAGAGGAC
chr1	247540474	MAFCJREV	ACACGACGCTCTTCCGATCTNNNNCCCTGATGGTGTTACTTCGAATT	GACGTGTGCTCTTCCGATCTTTCTGACAGAACAAGACACATAAGT
chr16	81696616	HAPFIVTW	ACACGACGCTCTTCCGATCTNNNNCTTCCCTTGTCTGGCAGGTC	GACGTGTGCTCTTCCGATCTGGTCCCACTGCGTACCATG
chr2	171158996	VRJHWIQW	ACACGACGCTCTTCCGATCTNNNNGCTTTCCGTGAATTTTCTAGAGCA	GACGTGTGCTCTTCCGATCTAAGTGTTTTGTGCCCTGAATCC
chr20	46196718	ZCWJDJMW	ACACGACGCTCTTCCGATCTNNNNCAGGCTGGGGTTGGGAAC	GACGTGTGCTCTTCCGATCTGGCCCTTTCTTGTGATGAGCTC
chr3	56682902	VRTAHJRX	ACACGACGCTCTTCCGATCTNNNNTGGGCTCAGCGATGTTGA	GACGTGTGCTCTTCCGATCTCGGATGCGAGTTGGGAAAGTG
chr10	87861594	KXSACIKD	ACACGACGCTCTTCCGATCTNNNNCTTTGTAGATCCTTGACGGGTG	GACGTGTGCTCTTCCGATCTCAAACCTGTCAAGTCTGTTTACA
chr17	44520096	QXCWCVHH	ACACGACGCTCTTCCGATCTNNNNGGTCAAGGCTGCAGTGAG	GACGTGTGCTCTTCCGATCTAGTAAGTCTCTTGAGGTGAT
chr6	32683930	EIRDKXYC	ACACGACGCTCTTCCGATCTNNNNACATGAGCCTCAAAGATGGGATT	GACGTGTGCTCTTCCGATCTGCATCTAGTAAGGGCCATCTTG
chrX	119851942	PSKDVCFX	ACACGACGCTCTTCCGATCTNNNNATCTTCGAATTACCACCTTAAGA	GACGTGTGCTCTTCCGATCTGTTTGACTAATTCTGAGCATTGGG
chr15	78776288	RTRPJABA	ACACGACGCTCTTCCGATCTNNNNCTCCGTGAGCAGATGGACC	GACGTGTGCTCTTCCGATCTTCTCAGTAGTGGTGTCTCAGTG
chr19	19384988	XBKCTIRP	ACACGACGCTCTTCCGATCTNNNNGATTCTCCAAATGCTCCTTAGGG	GACGTGTGCTCTTCCGATCTTGCCCTGCCCGGTTTTCTG
chr2	240904936	IRTINMPX	ACACGACGCTCTTCCGATCTNNNNGAGTGGGTGTCCTCAGTCTG	GACGTGTGCTCTTCCGATCTGCTGGGCTTCACTGGACAG
chr5	164600842	JMMKRZLT	ACACGACGCTCTTCCGATCTNNNNCATGGTGTATTCAAATTTGCATCAG	GACGTGTGCTCTTCCGATCTCCAGTAGTATGCATATCAATAATAC
chr6	136644276	KPZJSQJW	ACACGACGCTCTTCCGATCTNNNNGTGCTGCCAGGGAACGTTC	GACGTGTGCTCTTCCGATCTCTCATCAGCGCCTCCTTGTG
chr7	151203002	BLTENBAQ	ACACGACGCTCTTCCGATCTNNNNTGAACTTGTGCCATCCATCCTC	GACGTGTGCTCTTCCGATCTGACTGAGGCCATAATTCTAGTGC
chr11	66419980	HXIMTHJQ	ACACGACGCTCTTCCGATCTNNNNGTAGGTGCAGGGGTCATGGAG	GACGTGTGCTCTTCCGATCTGGAATCCCAGCTCCATATTAGG
chr15	20638152	BAICRBQY	ACACGACGCTCTTCCGATCTNNNNAACCCTGAGAGCTCCTTCCA	GACGTGTGCTCTTCCGATCTCAAAACACTATAAGCTCTTGGGG
chr15	22362236	HWLNITFL	ACACGACGCTCTTCCGATCTNNNNAAAACACTATAAGCTCTTGGGG	GACGTGTGCTCTTCCGATCTCAACCCTGAGAGCTCCTTCC

68796_68853 (Pair13)

chr17	362912	ZQSLATFH	ACACGACGCTCTTCCGATCTNNNNCATCAACGACCTTCCTTCCCTC	GACGTGTGCTCTTCCGATCTCTTAGGTGCTCCCCTGGAAGAG
chr3	52219054	XADRXSHT	ACACGACGCTCTTCCGATCTNNNNCCACATGCAAAATATGGTTCCC	GACGTGTGCTCTTCCGATCTCTGGCTGGAGTCTAGGGAAGT
chr15	44711562	TJFLQKIC	ACACGACGCTCTTCCGATCTNNNNTTAATATAAGTGGAGGCGTCGCG	GACGTGTGCTCTTCCGATCTGAGAGACTCACGCTGGATAG
chr19	6929724	IKDPDZBT	ACACGACGCTCTTCCGATCTNNNNCCTGGGTTCAAGCAATTATT	GACGTGTGCTCTTCCGATCTCGAGGCAGGCAAACTACG
chr9	128878058	DTHBTWWR	ACACGACGCTCTTCCGATCTNNNNAAATAGTCCCAGCTCCACCATA	GACGTGTGCTCTTCCGATCTGTGAGCCAGGACCCACAGTATA
chr11	65980906	ZEMRVLJZ	ACACGACGCTCTTCCGATCTNNNNGTGAACCTGGGAAGCGGA	GACGTGTGCTCTTCCGATCTCTGAGTAGGTGGGATTACAGG
chr12	53004488	ABPQKADN	ACACGACGCTCTTCCGATCTNNNNTCTTTGTGACCTATAACTTGTGT	GACGTGTGCTCTTCCGATCTAGGCTACTGAATGGAGAGT
chr10	42079890	LRRIEHEA	ACACGACGCTCTTCCGATCTNNNNTGGTCTCGAATGGAATCATTATCA	GACGTGTGCTCTTCCGATCTTTCGAGTCCATTCGATGATTCTAT
chr10	42081716	NYNCXWIF	ACACGACGCTCTTCCGATCTNNNNGGAATCATCGAATGGTCACGAA	GACGTGTGCTCTTCCGATCTttcgagtccattcgatgattctat
chr1	143197542	TFAQLAVL	ACACGACGCTCTTCCGATCTNNNNTCGAATGGACTCAAATGGAATT	GACGTGTGCTCTTCCGATCTATTCCATTCCATTCCGTTCGAT

68796_68861 (Pair 14)

chr17	362912	TSCRDDZC	ACACGACGCTCTTCCGATCTNNNNCATCAACGACCTTCCTTCCCTC	GACGTGTGCTCTTCCGATCTCTTAGGTGCTCCCCTGGAAGAG

chr14	90113594	NEXDVQHN	ACACGACGCTCTTCCGATCTNNNNGGTGAAATCCAAATGAAATCTGGAG	GACGTGTGCTCTTCCGATCTCGGCCTCTCTCAGTTTCTTCTAA
chr3	52219056	SILYJMAM	ACACGACGCTCTTCCGATCTNNNNCCACATGCAAAATATGGTTCCC	GACGTGTGCTCTTCCGATCTCTGGCTGGAGTCTAGGGAAGT
chr1	109667898	TAXEHLLI	ACACGACGCTCTTCCGATCTNNNNGCGATCATAGCTCAGTTCAGTC	GACGTGTGCTCTTCCGATCTCATGGGGCTAGGAGTAGGATCT
chr1	157197150	LCMEQNHV	ACACGACGCTCTTCCGATCTNNNNGCGACATTGCCCGACTAT	GACGTGTGCTCTTCCGATCTGTTGCTGGGAGGACTAAATAGA
chr6	31769916	EDKICXHS	ACACGACGCTCTTCCGATCTNNNNGATGAAGCAGAAGGGAATATGGC	GACGTGTGCTCTTCCGATCTTTCTTCCCCGTTTCCTGGC
chr1	143204642	KYPASNKZ	ACACGACGCTCTTCCGATCTNNNNCAATTCCGTTCAATGATTCCA	GACGTGTGCTCTTCCGATCTTCAGATGGAAACGAATGGA
chr9	131231218	HBIQHWHY	ACACGACGCTCTTCCGATCTNNNNATATGTAACATACTGAAAGTGGT	GACGTGTGCTCTTCCGATCTGGGAGGCGGAGTTTATAG
chr17	26603966	WMBIJIVA	ACACGACGCTCTTCCGATCTNNNNGTTGGAAACGGAATCATCTT	GACGTGTGCTCTTCCGATCTAAACCACAAAGCCCTCCAATC
chr6	43246754	NXNXEYWF	ACACGACGCTCTTCCGATCTNNNNTAAGGACGAAACCAACATGAGTG	GACGTGTGCTCTTCCGATCTCTACCCTCCCTCTGTCCCG

68876_68805 (Pair 59)

chr9	128878058	TNINAEYK	ACACGACGCTCTTCCGATCTNNNNCACCATAAACTAGCTATGGGACC	GACGTGTGCTCTTCCGATCTTCATTGACAAGTGGCTGTGAG
chr17	362908	HMRXZIVC	ACACGACGCTCTTCCGATCTNNNNCATCAACGACCTTCCTTCCCTC	GACGTGTGCTCTTCCGATCTCTTAGGTGCTCCCCTGGAAGAG
chr19	35309582	XIRXVSFJ	ACACGACGCTCTTCCGATCTNNNNGATCCACTCACTCTTCGGCCT	GACGTGTGCTCTTCCGATCTCCTTGAACATTCCCAGGCAC
chr17	26881268	ZBCDHJLV	ACACGACGCTCTTCCGATCTNNNNGTGCATTTATACCGTTTCCAAAGAA	GACGTGTGCTCTTCCGATCTACTTTGTGATGATTGAGTTTAAC
chr3	185910172	HNHHIKNR	ACACGACGCTCTTCCGATCTNNNNTGCATAGTATTCCATGGTGTATAT	GACGTGTGCTCTTCCGATCTGCTGCTATAAAGACACATGTACA
chr6	170171158	HAMTZAQF	ACACGACGCTCTTCCGATCTNNNNTTATTACTCTCTGTTCGGGGT	GACGTGTGCTCTTCCGATCTAGACAGTAACAACCCAGA
chr9	114733852	XTEYQKJP	ACACGACGCTCTTCCGATCTNNNNAGCCGAGATCACGCCACT	GACGTGTGCTCTTCCGATCTTTCCATTCAATATTTTCAGACTGC

68876_68813 (Pair 60)

chr2	15345444	SMVWCLLS	ACACGACGCTCTTCCGATCTNNNNGCCTACAACAGATAGAGAGCCAA	GACGTGTGCTCTTCCGATCTTTTCCTTGAGCAGTGGTTTGTA
chr21	8990878	IJJLFVSJ	ACACGACGCTCTTCCGATCTNNNNGCTCTCCCATGTCATCCTC	GACGTGTGCTCTTCCGATCTGTTTGGAGAGTAGCTGGGAGA
chr1	143199522	CFKENZDM	ACACGACGCTCTTCCGATCTNNNNTGGACTCGAATGGAATAATCATTGT	GACGTGTGCTCTTCCGATCTTGTTGATTCCATTAGCTTCCGT
chr1	143199578	FFKLMLMT	ACACGACGCTCTTCCGATCTNNNNCATCGGATGGGAACTGAATGG	GACGTGTGCTCTTCCGATCTTGATTCCATTAGCTTCCGTTGG
chr10	93837534	JZYQQEMI	ACACGACGCTCTTCCGATCTNNNNCACTAGCTCCTCCATCCAAAAT	GACGTGTGCTCTTCCGATCTTTAAACAGGGTGGTCAGTTGAGA
chr11	93673350	HQAFCQIL	ACACGACGCTCTTCCGATCTNNNNGACCCTGTCTCAAATATATATATGT	GACGTGTGCTCTTCCGATCTATCCAGATATGTACAATAGAGG
chr12	55375448	QYWYTYSC	ACACGACGCTCTTCCGATCTNNNNTTCCCTGATAATAGCTTCATAGGAT	GACGTGTGCTCTTCCGATCTTGCTAGTGTAAATGAAAGGAGCC
chr14	50686876	AJWVIEXB	ACACGACGCTCTTCCGATCTNNNNGCCTGTATTCAAGCTTATTAAT	GACGTGTGCTCTTCCGATCTCAAAGTGCTGGGATTACA
chr14	50686966	CFSTYECJ	ACACGACGCTCTTCCGATCTNNNNTGCAATTAAAGGACTCCAGGCT	GACGTGTGCTCTTCCGATCTCACTACCACACCCAGCTAACTT
chr14	63848848	MHNJISJL	ACACGACGCTCTTCCGATCTNNNNAATGATACTCTTCCAAGCCCCG	GACGTGTGCTCTTCCGATCTCCATAGTCCAACATCCTAGAGA

68876_68869 (Pair 63)

chr9	128878054	PJTZBMRZ	ACACGACGCTCTTCCGATCTNNNNAATAGTCCCAGCTCCACCATAAA	GACGTGTGCTCTTCCGATCTAGCCAGGACCCACAGTATATTC
chr17	362912	NXENAPNN	ACACGACGCTCTTCCGATCTNNNNCATCAACGACCTTCCTTCCCTC	GACGTGTGCTCTTCCGATCTCTTAGGTGCTCCCCTGGAAGAG
chr3	52219056	ZLDYBYXW	ACACGACGCTCTTCCGATCTNNNNCCTCCCACATGCAAAATATGGT	GACGTGTGCTCTTCCGATCTCTGGCTGGAGTCTAGGGAAGT
chr1	34213558	BYPXMJSX	ACACGACGCTCTTCCGATCTNNNNTTTAAACATGAAATCCACACCCT	GACGTGTGCTCTTCCGATCTCGGAAGGCTGAGGTGAGATAAT
chr2	218506758	RRTWNYDS	ACACGACGCTCTTCCGATCTNNNNAAGCCCATTTCCATTTGAA	GACGTGTGCTCTTCCGATCTGCAAAACTCCGTCTCAAA
chr20	47319050	WWMIQLKZ	ACACGACGCTCTTCCGATCTNNNNGCCTAGCCTCTCTTGTTCAAAA	GACGTGTGCTCTTCCGATCTCTGGCCTGGTAAGTGGTGACTC
chr10	38177402	VSQLVDSS	ACACGACGCTCTTCCGATCTNNNNAGGCCTATATGTATGTTCTCCCT	GACGTGTGCTCTTCCGATCTTCTCTTCCATGTCATTCTGAGTACA
chr12	55494752	EQVZDSWC	ACACGACGCTCTTCCGATCTNNNNCGCCTATAATCCCAGCACTTTG	GACGTGTGCTCTTCCGATCTCACCACCTCGCCCAGCTAATTTT
chr17	35586862	BQJREFEE	ACACGACGCTCTTCCGATCTNNNNCACATGCCCGAGAGAGATACTAT	GACGTGTGCTCTTCCGATCTGTGCTGCACGTAAAAGAGGTT
chr20	39075318	YRJHEDZM	ACACGACGCTCTTCCGATCTNNNNAGACCAACAGATTTAGCTTGCA	GACGTGTGCTCTTCCGATCTCAATCCTCCTGCCTTGGCCT

*Nested Primers

UID	Forward primer (5' to 3')	Reverse primer (5' to 3')
NTXEDNEC	TGATAAAACCAGTGGCTGTGAG	GGAGTGGAAGGAGGTAAGAGAG
DHIKJQPW	CAGGTTTTATTTTGGACTGGCG	ACTCAAGGACCCATCTGAAGAC
TBZMKTDB	CCTACATGATTCCCAGGGACAT	ATACTTCTACTCCTGGCAAGCA
SLHFRKHX	CATGTTCATGTGCTGTCTGTCT	CTGTAAGTGAGCCTCCTACCTC
CFFFMFZP	GTTCAGCTCCACAACAGTAGAC	AAACTGAGGCTGTAAGAGGGAA
DFWCALML	CGCGCTAAAATCCCATTCAAAA	CATTAGGGGCATCTCACCTGA
QYWSLAVY	TATGGCTTCAAACCAACCCTGA	TCAACCTTAGATTCGACGGTGA
IYCWHNVV	TGGAAGAACATATTTGCAAACCG	TGTTGCCCTTTTATAACCCCAC
BDXYHYTB	CTATACAACAGCCCCATCAGC	CACGGATCACCCCAGTCATATAA
KBWNPZYP	GTGTTTCTCCTCCTCTTTGCAT	TGAACATCAGTCAGTAGAGGCT

Oligos used for the DNA cleavage experiments

AAVS1_target_F	TCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGATTGGTGACAGA
AAVS1_target_R	CTGTCACCAATCCTGTCCCTAGTGGCCCCACTGTGGGGTGGAGGGGAA

GUIDE-seq oligos with various overhangs

The IDT codes for the oligos with different length randomized overhangs used in **Supplementary Figure 32** are below. Note that 34bp_4N-OH_2 is the GUIDE-seq oligo with randomized 4 bp 5' overhangs used in the majority of this work. 34bp_blunt_2 corresponds to the oligo used in the original GUIDE-seq publication.

34bp_-5N-OH_2

/5Phos/G*T*TTAATTGAGTTGTCATATGTTAATAACGGTAT(25252525)(25252525)(25252525)*(25252525)*(25252525)

/5Phos/A*T*ACCGTTATTAACATATGACAACTCAATTAAAC(25252525)(25252525)(25252525)*(25252525)*(25252525)

34bp_-4N-OH_2

/5Phos/G*T*TTAATTGAGTTGTCATATGTTAATAACGGTAT(25252525)(25252525)*(25252525)*(25252525)

/5Phos/A*T*ACCGTTATTAACATATGACAACTCAATTAAAC(25252525)(25252525)*(25252525)*(25252525)

34bp_-3N-OH_2

/5Phos/G*T*TTAATTGAGTTGTCATATGTTAATAACGGTAT(25252525)*(25252525)*(25252525)

/5Phos/A*T*ACCGTTATTAACATATGACAACTCAATTAAAC(25252525)*(25252525)*(25252525)

34bp_-2N-OH_2

/5Phos/G*T*TTAATTGAGTTGTCATATGTTAATAACGGTAT*(25252525)*(25252525)

/5Phos/A*T*ACCGTTATTAACATATGACAACTCAATTAAAC*(25252525)*(25252525)

34bp_-1N-OH_2

/5Phos/G*T*TTAATTGAGTTGTCATATGTTAATAACGGTA*T*(25252525)

/5Phos/A*T*ACCGTTATTAACATATGACAACTCAATTAAA*C*(25252525)

34bp_blunt_2

/5Phos/G*T*TTAATTGAGTTGTCATATGTTAATAACGGT*A*T

/5Phos/A*T*ACCGTTATTAACATATGACAACTCAATTAA*A*C

34bp_1N-OH_2

/5Phos/(25252525)*G*TTTAATTGAGTTGTCATATGTTAATAACGGT*A*T

/5Phos/(25252525)*A*TACCGTTATTAACATATGACAACTCAATTAA*A*C

34bp_2N-OH_2

/5Phos/(25252525)*(25252525)*GTTTAATTGAGTTGTCATATGTTAATAACGGT*A*T

/5Phos/(25252525)*(25252525)*ATACCGTTATTAACATATGACAACTCAATTAA*A*C

34bp_3N-OH_2

/5Phos/(25252525)*(25252525)*(25252525)GTTTAATTGAGTTGTCATATGTTAATAACGGT*A*T

/5Phos/(25252525)*(25252525)*(25252525)ATACCGTTATTAACATATGACAACTCAATTAA*A*C

34bp_4N-OH_2

/5Phos/(25252525)*(25252525)*(25252525)(25252525)GTTTAATTGAGTTGTCATATGTTAATAACGGT*A*T

/5Phos/(25252525)*(25252525)*(25252525)(25252525)ATACCGTTATTAACATATGACAACTCAATTAA*A*C

34bp_5N-OH_2

/5Phos/(25252525)*(25252525)*(25252525)(25252525)(25252525)GTTTAATTGAGTTGTCATATGTTAATAACGGT*A*T

/5Phos/(25252525)*(25252525)*(25252525)(25252525)(25252525)ATACCGTTATTAACATATGACAACTCAATTAA*A*C

Supplementary Note 1

Cleavage rate studies

Our biochemical studies of cleavage rate were performed under conditions of excess enzyme to enforce single-turnover kinetics, with data analyzed as described in Sassa (*J. Vis. Exp.*, Sassa et al., 2013) to determine the first-order rate constant (k_{obs}) of product formation. Under such conditions, k_{obs} will approximate k_2 as long as this value exceeds the deadtime for mixing and assembly of enzyme-target complex. As hosts for this study, four-finger derivatives of our AAVS1 ZFNs were used, which were truncated to remove the two FokI-distal fingers. These hosts were chosen because they exhibited faster complex formation, relative to their six-finger counterparts, which enables discernment of a wider range of apparent k_{obs} values. Given the structural isolation of the removed fingers (separated from the FokI domains by four intervening fingers and approximately 35 Å in the DNA-bound complex) their absence should not affect FokI function.

As shown in **Supplementary Figure 6**, the cleavage time courses are well-fit via an exponential model of target decay, and the plots indicate a substantial slowing of catalysis by the Q481A FokI variant. While the parent FokI dimer yields a k_{obs} of 2.0 x $10^{-3}s^{-1}$, this value is >20-fold lower (9.1 x $10^{-5}s^{-1}$) for the dimer bearing two variant FokI domains. Mixed dimers (bearing one parent FokI domain and one Q481A variant) exhibit intermediate values. Critically, the half dose controls yield k_{obs} values that are almost identical those yielded by the full dose counterparts. This supports our assumption of limiting substrate, and also provides further evidence (along with the Western blot results) that the apparent k_{obs} reductions are not due to lower expression levels or specific activity of the variant ZFNs.

We note that while the reductions in observed cleavage rate revealed by these studies are both substantial and directionally consistent with our proposed mechanism, the effects aren't large enough to explain the full magnitude of specificity improvement (>1000-fold) seen in our cell studies. One possible explanation for this is that k_2 for the parent FokI dimer is too fast to measure in this assay, due to the confounding effects of mixing and complex formation deadtime, and that the real value for this term is substantially higher than the k_{obs} we observe. Consistent with this, a prior study that examined the reaction rate of native FokI enzyme preloaded onto its target yielded a substantially faster k_{obs} value[58]. Other possible explanations are that our reaction buffer may not fully recapitulate conditions in mammalian nucleus, or that other mechanisms act in concert with a reduced k_2 to achieve the large effects we observe in cells.

Supplementary Note 2

Data analysis for indel assay

All standard indel data displayed in Table 1, Figures 1-4, and Supplementary Figures 2, 10, 11, 16-22, 24, and 25 were processed using a combination of two separate strategies for separating *bona fide* indels from experimental artifacts. The first strategy is to disregard indels that are more than a specified distance from the expected nuclease cleavage site. Indels that do not overlap a specific region or "window" of the amplicon are excluded. For intended ZFN targets and well characterized off-target such as AAVS1 OT1, OT2, OT3, and OT4 this window is defined as the gap between the ZFP binding sites (generally 5-7 bp) plus an additional base on either side for total window sizes of 7-9 bp. For less well characterized off-target sites, the window is defined as 6 bp on either side of the peak of the distribution of oligo capture locations. For indels with multiple equivalent alignments (e.g. a 1 bp deletion within a string of 7 G's), an indel is only excluded if all of the equivalent alignments fail to place the indel within or overlapping the sequence window. After this procedure, a further step is then employed to subtract out identical indels that occur in both the ZFN treated sample and the matched control sample. This step is agnostic to which sample is the ZFN treated sample and which is the control. For indels that occur with a non-zero frequency in both samples being compared, the lower of the two frequencies is subtracted from both samples and the corrected frequencies are then rounded to the nearest integral number of sequence reads. The resulting number of sequence reads scored as indels were then used to perform the statistical test described in **ref. 50**. The set of p-values from a logically related set of measurements (e.g. genomic DNA from cells treated with the AAVS1 Q481A FokI variants and tested at the 12 candidate off-target loci for this variant as derived from the oligo capture assay) were then corrected using the false discovery rate procedure of Banjamini and Hochberg.

Standard indel data displayed in Supplementary Figures 4-9 used an older data processing method that only excluded indels that occurred at similar frequencies in the ZFN treated sample and matched control. This older method is agnostic to the location of the indel within the amplicon and uses a different method for the subtraction. This older method excluded all occurrences of a given indel from both samples if they occur at frequencies that are not statistically different from each other as gauged by Fisher's exact test with a significance level of 0.05.

The more sensitive version of the indel assay reported in Figure 2d and Supplementary figures 12 and 13 requires a different set of criteria to separate out experimental artifacts. Like the newer data processing method for standard indel data, indels that fall outside of a sequence "window" around the expected cleavage site are excluded. But this more sensitive version of the assay differs by using greater sequencing depth so that each input allele is sequenced multiple times (i.e. oversampling) and uses multiple technical replicates for each sample. The sensitivity of the assay can be adjusted by adjusting the number of technical replicates. Oversampling enables discrimination of rare technical artifacts from *bona fide* indels by virtue of their individual frequencies lying below the threshold expected from a multiply-sequenced input mutation and technical replicates allowed common background artifacts to be more accurately quantified and then subtracted via use of a negative control. Any indels that occurred at a frequency of less than 20% of the expected frequency (total sequence reads divided by the number of input genomes) were considered artifacts rather than *bona fide* indels. For each type of indel, the background samples were used to establish a mean background frequency and to determine the standard deviation (σ) of background frequencies. For test samples, if the frequency of a particular indel is less than 3σ above the mean frequency in the background samples then the indel is excluded as an artifact. If the frequency of a particular indel is more than 3σ above the mean then the indel is considered real and its frequency is corrected by subtracting the mean frequency found in the background samples. In order to make a valid comparison between ZFN treated samples and GFP treated samples for the experiment shown in Figure 2e and supplementary Figure 13, the data was processed in two stages. The first stage defined the first 24 GFP replicates as background samples and used these to process the ZFN treated replicates and the second 24 GFP replicates. The second stage defined the second 24 GFP replicates as background and used these to process the first 24 GFP replicates. In this way each replicate was processed using a set of 24 GFP replicates to define background frequencies for each indel and no replicate used itself in the set of background replicates used to define background frequencies. We found both the cluster density and the amount of PhiX DNA added to the library prior to loading the NextSeq were critical to obtaining optimal results. The experiment shown in Figure 2d had a cluster density of 113 K/mm^2 and 17.05% of the reads aligned to PhiX. Note that this method is intended to measure samples where indel frequencies do not exceed 10% and where the control sequences are free from even the slightest contamination from sequences containing real indels. Excessive

indels in the control samples could cause real indels to be incorrectly filtered out and high overall indel levels can lead to chimeric PCR products containing combinations of indels that aren't present in the genomic DNA sample.

All Illumina data was first run through a custom data processing pipeline that utilizes several open source software packages. MiSeq data was first demultiplexed using a custom script after applying a q20 quality filter to the I1 and I2 index reads that contain the barcode sequences. NextSeq data was demultiplexed using Illumina bcl2fastq software. Demultiplexed paired reads were quality filtered using fastq_quality_filter from the FASTX toolkit (q15 for MiSeq and q13 for NextSeq). Paired reads were then combined and adapter trimmed using SeqPrep. Combined reads were then required to match the intended amplicon at the outer 23 bp of each edge of the intended amplicon to exclude mispriming. Combined reads were then mapped to hg38 using Bowtie2 and sequences that map better to something other than the intended locus were excluded. Finally, reads are mapped to the intended amplicon using the Biopython pairwise2 global aligner. The alignments are used to assign an "indelcode" to each sequence read where the indelcode only describes insertions or deletions and ignores base mismatches. These indelcodes are used to perform the various operations described above. Here is a description of the indelcode:

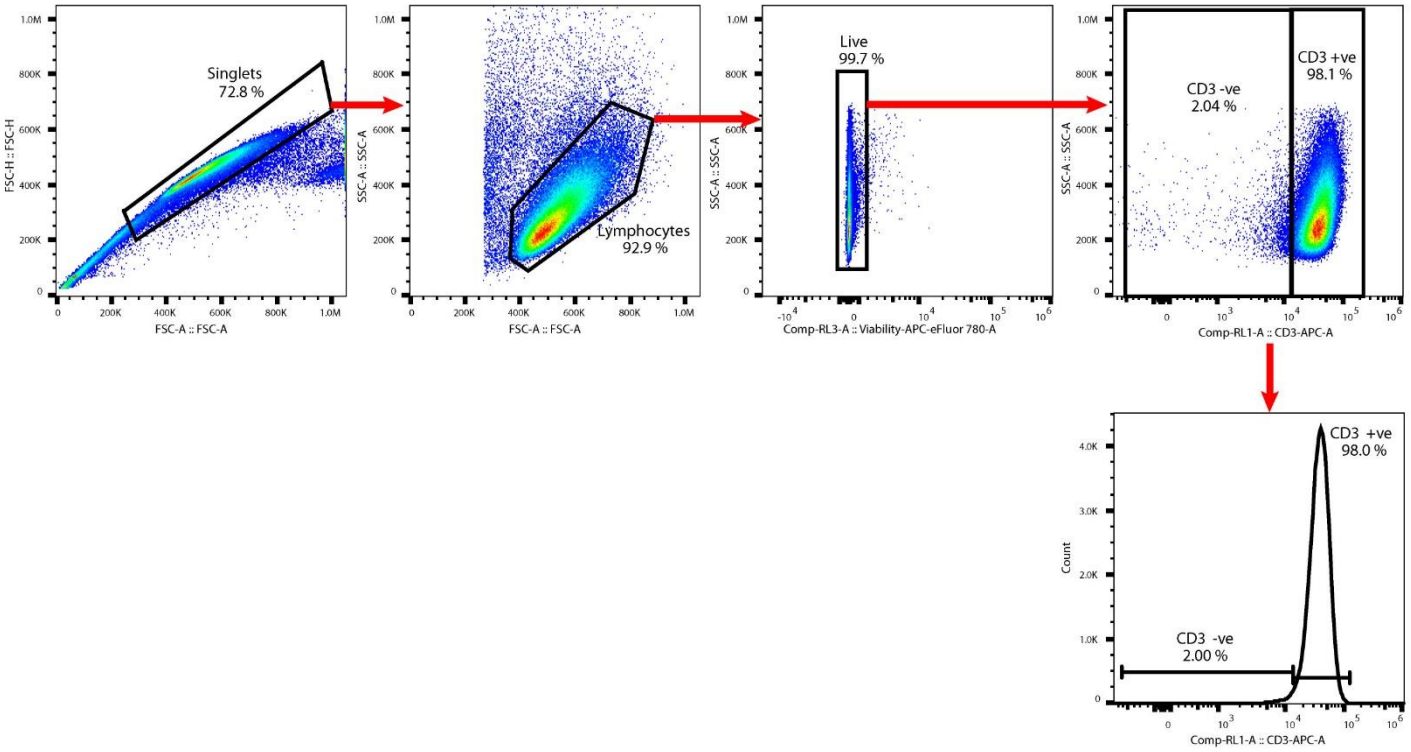
An example of the code is '113:D:43:x:113:155. The components of the code are:

- 113: starting location of the indel.
    - An asterisk is added if the indel has been merged with overlapping indels of the same type and size. More info about this below.
- D: indel type, 'D' for deletion and 'I' for insertion
- 43: indel size
- x: flankmatch flag indicating if the indel has flanking repeats.
    - x: no repeat
    - r: repeat was found
    - n: indel created a new duplication.
- 113: start of indel including flanking repeat and merges
- 155: end of indel including flanking repeat and merges

Other example indel codes are 109:D:4:x:109:112 (4bp deletion starting at position 109) and 109*:I:4:n:109:116 (4bp insertion that creates a new 4bp duplication with ambiguity about which copy of the duplication comprises the insertion). 4bp insertions that duplicate existing sequence and do not expand an existing duplication are highly diagnostic of ZFN induced indel sequences.

Flow Cytometry Gating Strategy

The gating strategy used for flow cytometry experiments is shown graphically below.



Competitive DNA binding assay and dissociation rate assay

Both experiments utilized a previously described ELISA-based DNA binding assay[14], except that DNA duplexes were created by annealing two site-specific oligos. Target duplexes were created by incorporating a 5' biotin on the bottom strand oligo and annealing this oligo with a 1.5-fold excess of the unlabeled top strand oligo. Competitor duplexes were created in the same manner except the bottom strand oligo did not contain a biotin.

This assay detects DNA binding by incubating a biotinylated DNA target site with an epitope-tagged DNA-binding protein and an antibody-peroxidase fusion, capturing protein-DNA complexes on a streptavidin coated plate, washing away unbound protein, and then reading out the peroxidase activity in each well of the plate. For the competitive DNA binding assay, non-biotinylated competitor DNA with the sequence of the ZFP binding site from either the intended target or OT1 was added to the reaction at various concentrations and data points were fit to the equation:

$$y = Max - \frac{(Max - Min)}{(1 + \frac{IC50}{X})}$$

Where y is the measured signal, x is the concentration of competitor DNA, Max is the signal with no competitor DNA, and Min is the background signal. The IC50 values determined by the curve fitting software (Microcal origin) were then used to determine the relative affinity values for different competitor DNA sequences.

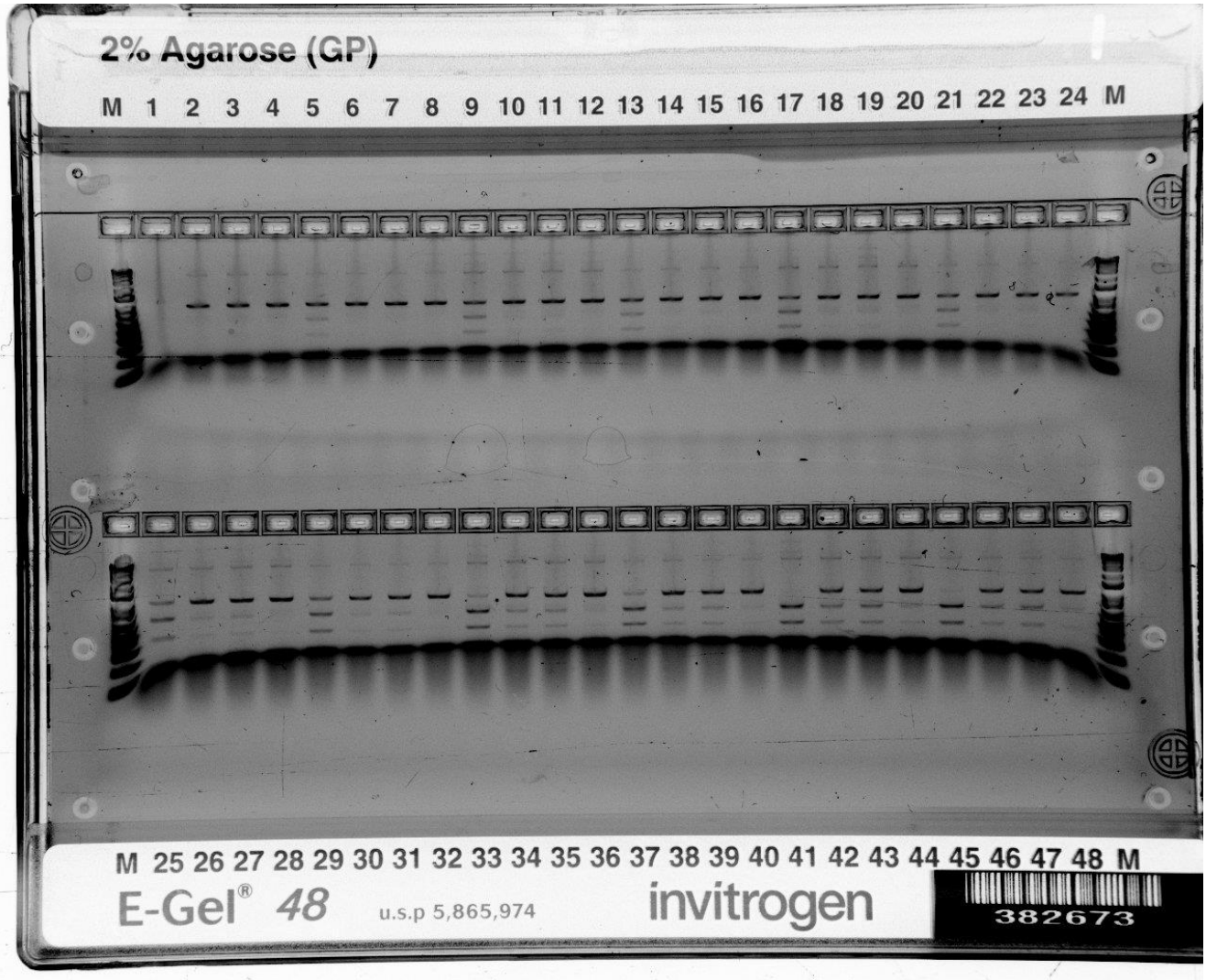
For the dissociation experiments, the protein and biotinylated target sequence was incubated for 45 minutes and then a 100-fold excess of non-biotinylated target site was added to the reaction and the protein-DNA complexes were captured on the streptavidin plate at various time points. Data points were fit to the equation:

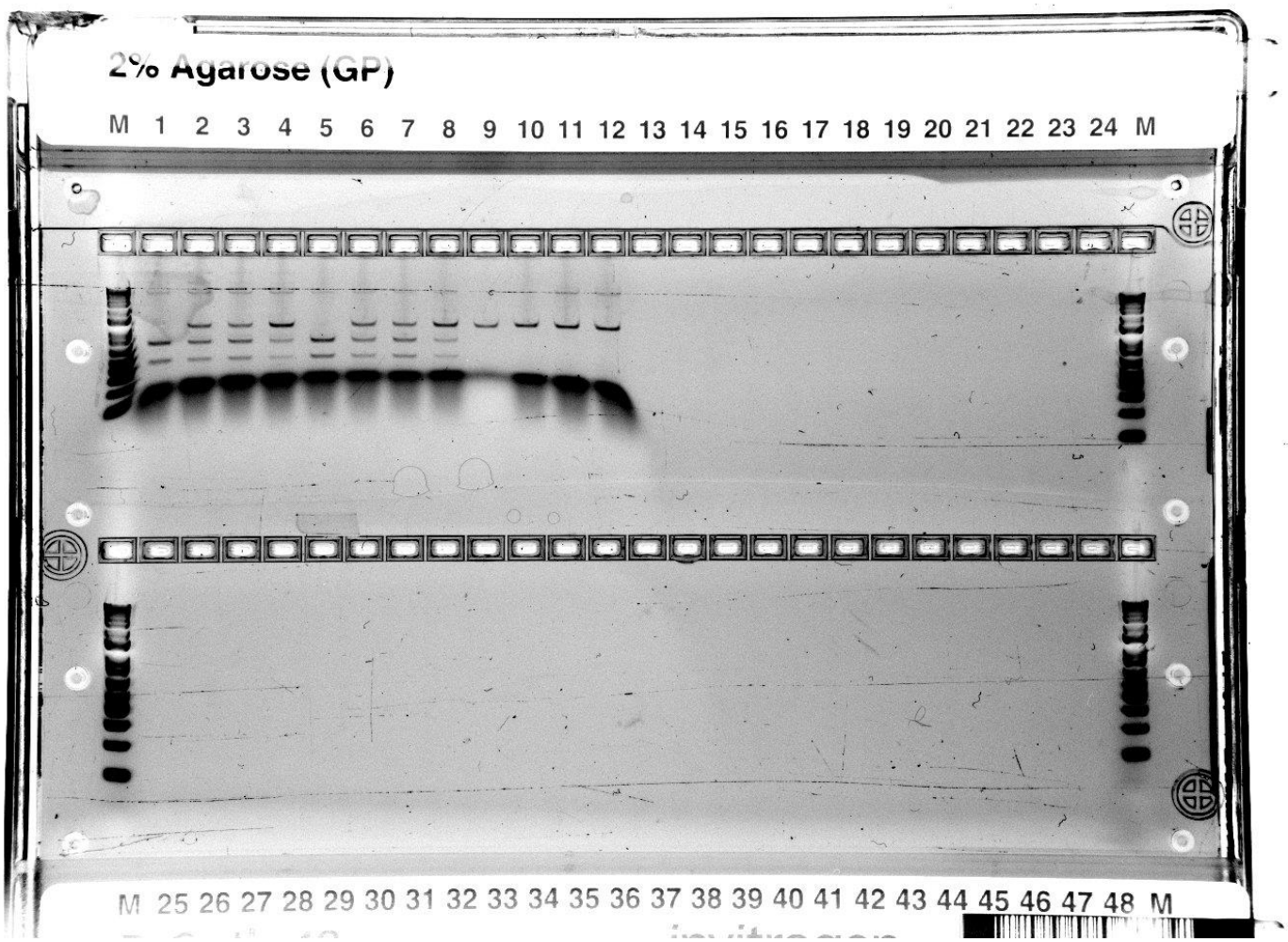
$$y = y_0 + Ae^{-\frac{x}{t}}$$

Where y is the measured signal, x is the time the measurement was taken, y0 is the assay background, A is the amplitude of the initial signal over background and t is the decay time constant.

DNA cleavage rate experiments

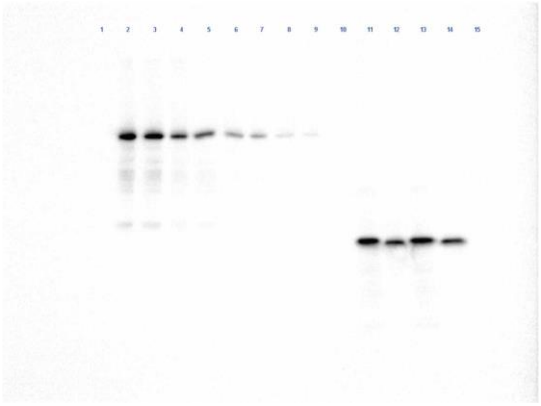
DNA targets containing the target site for the AAVS1 ZFN pair were created as described[11]. Briefly, the target site was created by annealing two complementary oligos with 1 bp 3' adenine overhangs and then cloned into PCR2.1-TOPO (Invitrogen). The resulting clones were sequence-confirmed with Sanger sequencing. PCR primers were used to amplify a ~1kb region of the PCR2.1-TOPO plasmid surrounding the target sequence insertion site and were named 2.1TGT-F (5'-AGGGGGAAACGCCTGGTATC-3') and 2.1TGT-R (5'TAGGGCGCTGGCAAGTGTAG-3'). The resulting PCR product was purified using a PCR cleanup kit (Qiagen) and quantified using a Nanodrop spectrophotometer (Thermo Scientific). ZFNs were expressed using the TnT quick coupled transcription-translation system (Promega). 100 μl of TnT extract was mixed with 2μl of 1mM Methionine, 3.3 μl of 2 mM $ZnCl_2$ and 5.6 μl of the appropriate ZFN encoding plasmid at a concentration of 450 ng/μl. The reactions were then incubated at 30 °C for 90 minutes and a portion was frozen for later expression quantification by western blot. DNA cleavage reactions were performed at room temperature (approximately 23 °C) in a buffer containing 100 mM NaCl, 50 mM Tris-HCl Ph 7.5, 10 mM $MgCl_2$, and 1 mM DTT. 225 μl cleavage reactions were initiated by mixing the target DNA diluted in buffer with the appropriate combinations of ZFN expressing TnT lysate. Target DNA was at a final concentration of 1 ng / μl and each reaction contained 12% by volume of the appropriate TnT lysate mixtures. For 1X concentration reactions, this consisted of equal parts of lysate expressing the appropriate left ZFN and lysate expressing the appropriate right ZFN. For 1/2 X reactions, the ZFN lysate mixtures were diluted with an equal volume of blank lysate (TnT lysate containing methionine and $ZnCl_2$, but lacking a template plasmid). Controls C3 and C4 contained 12% by volume of TnT lysate expressing only the left ZFN or the right ZFN respectively. At each timepoint 25 μl of the remaining reaction was removed and added to pre-warmed tubes containing 5 μl of 100 mM EDTA and incubated at 65 °C for 20 minutes in order to stop the cleavage reactions. Reactions were then treated with 0.5 μl RNAase A (2.5μg/ul - epicentre) and incubated at 37 °C for 30 minutes. Reactions were further treated with 0.4 μl Proteinase K (50 μg/ml- epicenter), incubated at 37 °C for 60 minutes, centrifuged at 4400 rpm for 20 minutes and the 15 μl of the supernatant was run on a 2% agarose e-gel (Thermo Scientific). Gels were visualized using a ChemiDoc XRS+ (Bio-Rad) and band intensities were quantified using Image J software (NIH). Calculated percent DNA cleavage vs. time was fit to a single order exponential allowing a time offset to account for mixing time and non-instantaneous reaction quenching. Microcal origin (originlab.com) was used for the non-linear curve fitting. Uncropped images of the gels from **Supplementary Figure 6** are shown below.


2% Agarose (GP)
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 M
M 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 M
E-Gel® 48
u.s.p 5,865,974
invitrogen
382673



Estimation of AAVS1 ZFN protein concentration in TNT lysates

The protein concentration of FLAG-tagged AAVS1 ZFNs expressed in TNT lysates was determined using anti-FLAG western blot and band density analysis. Known amounts of purified FLAG-tagged AAVS1 ZFN (20, 15, 10, 7.5, 5, 3.75, 2.5, or 1.25 ng respectively) were electrophoresed with diluted TnT lysates on 4-12% NuPAGE Bis-Tris Protein gels (Thermo Fisher, cat. # NP0323PK2) in 1X NuPAGE MOPS SDS buffer (Thermo Fisher). Proteins were transferred onto nitrocellulose membrane by wet transfer. The membrane was then blocked with 5% fat-free milk in TBS-T and incubated with 1 to 1000 dilution of an anti-FLAG-HRP conjugate (Sigma-Aldrich, cat. # A8592). After washing with TBS-T, the membrane was probed with ECL detection reagent (GE Lifesciences), and the images were captured and analyzed on Bio-rad's Chemidoc imager using Image Lab software (Bio-rad, v6.0.0). Band intensities were calculated, and a standard curve was plotted for the known amounts of the protein standard. Concentrations of the FLAG-tagged proteins from TNT lysates were obtained from the standard curves. Only images containing non-saturated pixels were used for this analysis. The uncropped image of the blot shown in **Supplementary Figure 6** is below.



GUIDE-seq oligo incorporation frequency analysis

Cells treated with ZFNs and the appropriate GUIDE-seq oligo were processed and sequenced with the same protocol used for the amplicon-based indel assay. GUIDE-seq oligos were always transfected at a concentration of 1 μM. For the experiment shown in **Supplementary Figure 14**, 300 ng mRNA encoding the left AAVS1 ZFN and 300 ng mRNA encoding the right AAVS1 ZFN were used. For the experiment shown in **Supplementary Figure 15**, 400 ng mRNA encoding the appropriate left ZFN and 400 ng mRNA encoding the appropriate right ZFN was used. The percentage of integrated GUIDE-seq oligos was determined with a simple shell script that processed the fastq files for amplicons of the appropriate locus (either the AAVS1 ZFN target or the TCRα ZFN target) to count the total number of sequence reads in each file, count the number of sequence reads containing the sequence GTTTAATTGAGTTGTCATATGTTAATAACGGTAT on either DNA strand, and then calculate the percentage of sequence reads containing the full 34 bp oligo sequence.

Custom computer scripts for indel analysis

Script to process indel data for the standard indel assay:

```
MiSeq_hybrid_indel_analysis.py
import string
import sys

indel_table_filename = sys.argv[1]
sample_name = indel_table_filename[:-20]
sample_info_filename = sample_name + 'sample_info.txt'
output_filename = sample_name + 'indel_analysis.txt'

indel_dict = {}

#this function takes and indel code and returns 1 if any of the individual indels in the code overlap the window
defined by window_start and window_end
def in_window(indel_code, window_start, window_end):
    is_in_window = 0
    indel_list = string.split(indel_code, ',')
    for indel in indel_list:
        data = string.split(indel, ':')
        indel_start = string.atoi(data[4])
        indel_end = string.atoi(data[5])
        if indel_start <= window_start and indel_end >= window_end:
            is_in_window = 1
        if indel_start >= window_start and indel_start <= window_end:
            is_in_window = 1
        if indel_end >= window_start and indel_end <= window_end:
            is_in_window = 1
    return is_in_window


data_file = open(indel_table_filename, 'r')
```

```
#reads in the appropriate all_indels.table to extract the indel information for each individual MiSeq sample
#note thet the indel frequencies in this file are truncated floating point numbers and are ignored and
recalculated at the required precision later
for line in data_file:
    line = string.strip(line)
    if line[:6] != 'sample' and line[0] != '#':
        sample_name, simple_indelcode, ascii_count, ascii_frequency, flag, verbose_indelcode =
string.split(line, '\t')
        indelcode = string.strip(verbose_indelcode, '"')
        indelcode = string.strip(indelcode, '()')
        indelcode = string.strip(indelcode, ',')
        count = string.atoi(ascii_count)
        if indelcode not in indel_dict:
            indel_dict[indelcode] = []
        indel_dict[indelcode].append( (sample_name, count) )

output_file = open(output_filename, 'w')
output_line =
'name\ttreated\t%indels\treads\tindels\twindowed\tcorrected\thybrid\tcontrol\t%indels\treads\tindels\twindowed\t
corrected\thybrid\twindow_start\twindow_end\n'
output_file.write(output_line)

#reads in the sample file that includes a name, the filename containing the sequence reads for the nuclease
treated sample,
#the filename containing the sequence reads for the control sample, and the start and end of the indel window
with respect to the start of the amplicon
#the indel counts are calculated and output for all indels, windowed indels, background corrected indels, and
windowed + background corrected indels
sample_info_file = open(sample_info_filename, 'r')
for line in sample_info_file:
    line = string.strip(line)
    if line:
        print line
        pair_name, filename1, filename2, ascii_window_start, ascii_window_end = string.split(line)
        window_start = string.atoi(ascii_window_start)
        window_end = string.atoi(ascii_window_end)
        file1_total = 0
        file1_all_indels = 0
        file1_windowed_indels = 0
        file1_windowed_corrected_indels = 0
        file1_unwindowed_corrected_indels = 0
        file1_indel_dict = {}
        file2_total = 0
        file2_all_indels = 0
        file2_windowed_indels = 0
        file2_windowed_corrected_indels = 0
        file2_unwindowed_corrected_indels = 0
        file2_indel_dict = {}
        #this loop identifies and stores all indel data for the two samples being compared
        for indelcode in indel_dict.keys():
            for entry in indel_dict[indelcode]:
                if entry[0] == filename1:
                    file1_indel_dict[indelcode] = entry[1]
                    file1_total += entry[1]
                    if indelcode != 'wt': #wt indelcode indicates the lack of indels since the wild-type
amplicon is defined as containing no indels
                        file1_all_indels += entry[1]
                        if in_window(indelcode, window_start, window_end):
                            file1_windowed_indels += entry[1]
                if entry[0] == filename2:
                    file2_indel_dict[indelcode] = entry[1]
                    file2_total += entry[1]
                    if indelcode != 'wt':
                        file2_all_indels += entry[1]
                        if in_window(indelcode, window_start, window_end):
                            file2_windowed_indels += entry[1]
        #this loop compares indels, calculated indel frequencies, and performs the background correction
        for indelcode in indel_dict.keys():
            if indelcode != 'wt':
                if indelcode in file1_indel_dict:
                    file1_indelcode_count = file1_indel_dict[indelcode]
                    file1_indelcode_frequency = float(file1_indel_dict[indelcode]) / float(file1_total)
                else:
                    file1_indelcode_count = 0
                    file1_indelcode_frequency = 0.0

                if indelcode in file2_indel_dict:
                    file2_indelcode_count = file2_indel_dict[indelcode]
                    file2_indelcode_frequency = float(file2_indel_dict[indelcode]) / float(file2_total)
```

```
                else:
                    file2_indelcode_count = 0
                    file2_indelcode_frequency = 0.0
                #the indel value for the sample that has the lower frequency is assumed to be background and
discarded
                #the indel value for the sample that has the higher frequency is corrected by subtracting off
the frequency of that particular indel in the other sample
                #this corrected frequency is then turned back into an integer value and subtracted from the
observed count of that particular indel
                if file1_indelcode_frequency > file2_indelcode_frequency:
                    correction = int(round(file2_indelcode_frequency * float(file1_total)))
                    corrected = file1_indelcode_count - correction
                    file1_unwindowed_corrected_indels += corrected
                    if in_window(indelcode, window_start, window_end):
                        file1_windowed_corrected_indels += corrected
                elif file2_indelcode_frequency > file1_indelcode_frequency:
                    correction = int(round(file1_indelcode_frequency * float(file2_total)))
                    corrected = file2_indelcode_count - correction
                    file2_unwindowed_corrected_indels += corrected
                    if in_window(indelcode, window_start, window_end):
                        file2_windowed_corrected_indels += corrected
        if file1_total > 0 and file2_total > 0: #this condition catches situations where there is no data to
avoid a division by zero
            percent_indels1 = 100.0 * float(file1_windowed_corrected_indels) / float(file1_total)
            percent_indels2 = 100.0 * float(file2_windowed_corrected_indels) / float(file2_total)
            output_line = '%s\t%s\t%8.4f\t%d\t%d\t%d\t%d\t%d\t%s\t%8.4f\t%d\t%d\t%d\t%d\t%d\t%d\t%d\n'
%(pair_name,\
                    filename1, percent_indels1, file1_total, file1_all_indels, file1_windowed_indels,
file1_unwindowed_corrected_indels, file1_windowed_corrected_indels,\
                    filename2, percent_indels2, file2_total, file2_all_indels, file2_windowed_indels,
file2_unwindowed_corrected_indels, file2_windowed_corrected_indels,\
                                                                                    window_start,
window_end)
        else:
            output_line = '%s\t%s\tND\tND\tND\tND\tND\tND\t%s\tND\tND\tND\tND\tND\tND\t%s\t%s\n' %(pair_name,
filename1, filename2, window_start, window_end)
        output_file.write(output_line)
output_file.close()
data_file.close()
sample_info_file.close()
```

Script to generate p-values as per Pattanayak et al. and apply multiple testing correction. Requires various external modules and indelstats.py. The relevant portion of indelstats.py is included.

hybrid_indel_pval_fdr.py

```
import math
import scipy.stats as stats
import statsmodels.api as sm
import pandas as pd
import numpy as np
import string
import os,sys
import indelstats

sample_num = 1
data_filename = sys.argv[1]
data_file = open(data_filename ,'r')
pvals = []
output_list = []
output_file_name = data_filename[:-4] + '_fdr_pvals.txt'
output_file = open(output_file_name, 'w')
for line in data_file:
    line = string.strip(line)
    if line:
        data = string.split(line)
        sample_num = data[0]
        sample_name = data[1]
        treated_hits = string.atoi(data[7])
        control_hits = string.atoi(data[14])
        treated_total = string.atoi(data[3])
        control_total = string.atoi(data[10])
        p_val = indelstats.binom_pval(treated_hits, control_hits, treated_total, control_total)
        if p_val is None:
            p_val = 1.0 #None value indicates no indels in either sample; None value will cause issues with
       multipletests correction
        pvals.append(p_val)
```

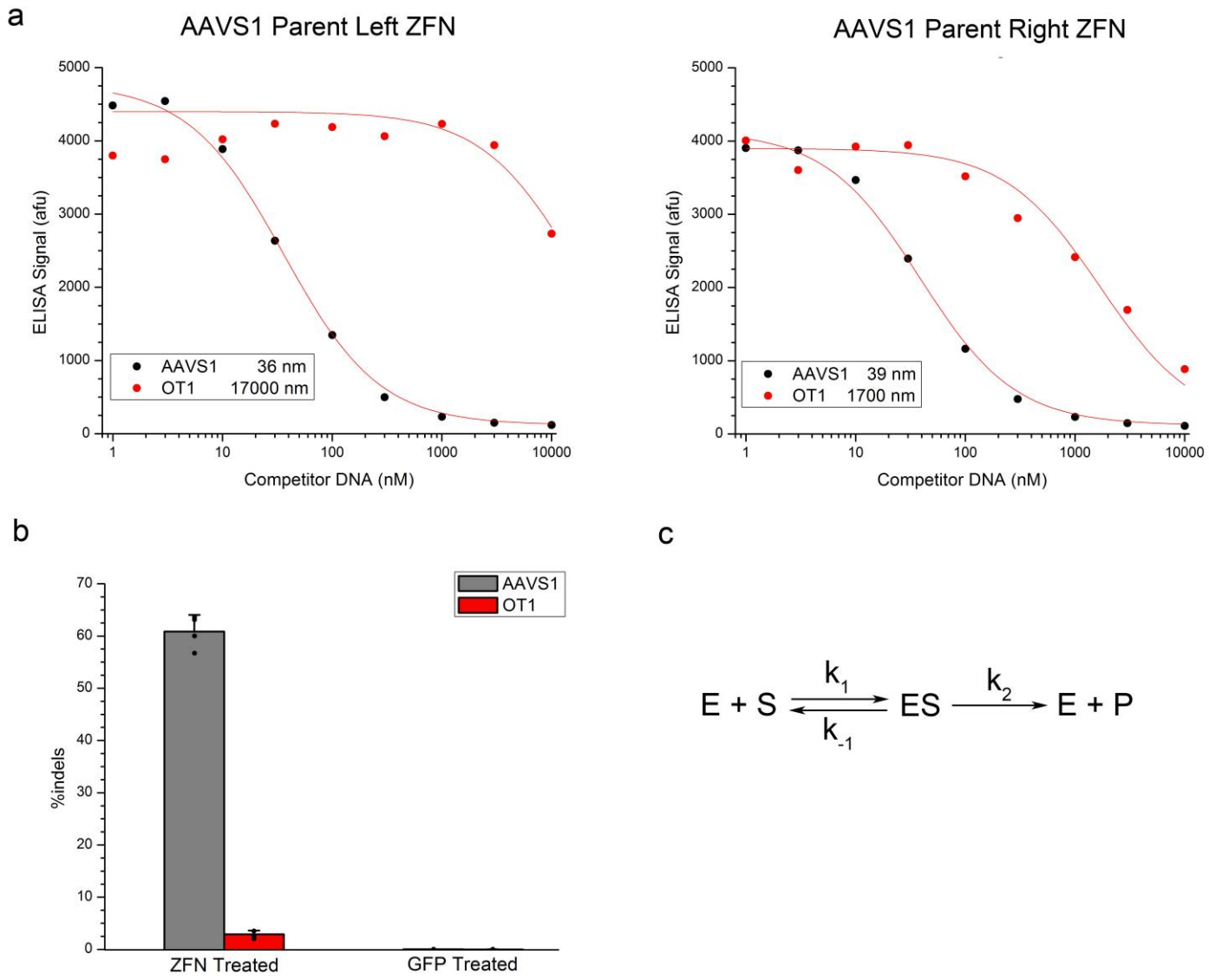
```
        output_line = '%s\t%s\t%d\t%d\t%d\t%d\t%10.8f' %(sample_num, sample_name, treated_hits, control_hits,
       treated_total, control_total, p_val)
        output_list.append(output_line)
results = sm.stats.multipletests(pvals, alpha=0.05, method='fdr_bh')
corrected_pvals = results[1]
output_line =  'sample\ttreated_indels\tcontrol_indels\ttreated_total\tcontrol_total\tpval\tfdr_pval\n'
output_file.write(output_line)
for pos in range(len(output_list)):
    output_line = '%s\t%10.8f\n' %(output_list[pos], corrected_pvals[pos])
    output_file.write(output_line)
output_file.close()
data_file.close()


indelstats.py (relevant portion only)
import math
import scipy.stats as stats
import statsmodels.api as sm
import pandas as pd
import numpy as np
'''
Test for binomial distribution differences as described in
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3164905/#SD1
Nat Methods. 2011 Aug 7;8(9):765-70. doi: 10.1038/nmeth.1670.
Revealing off-target cleavage specificities of zinc-finger nucleases by in
vitro selection.
Pattanayak V1, Ramirez CL, Joung JK, Liu DR

"In Supplementary Tables S3 and S6, P-values were calculated for a one-sided
test of the difference in the proportions of sequences with insertions or
deletions from the active ZFN sample and the empty vector control samples. The
t-statistic was calculated as t = (p_hat1 - p_hat2)/sqrt((p_hat1 (1 -
p_hat1)/n1)+ (p_hat2 (1 - p_hat2)/n2)), where p_hat1 and n1 are the proportion
and total number, respectively, of sequences from the active sample and p_hat2
and n2 are the proportion and total number, respectively, of sequences from the
empty vector control sample."

'''

def binom_pval(tr_hits,con_hits,tr_total,con_total):
    ''' Note this is a one-sided test, you cannot switch the treated and
    control order.  It is testing whether the treated is higher than the
    control. '''
    assert isinstance(tr_hits, int)
    assert isinstance(con_hits, int)
    if tr_hits == 0 and con_hits == 0:
        return None
    n1 = float(tr_total)
    n2 = float(con_total)
    total = n1 + n2
    p_hat1 = tr_hits / n1
    p_hat2 = con_hits / n2
    t = (p_hat1 - p_hat2)/math.sqrt((p_hat1 * (1 - p_hat1)/n1) + (p_hat2 * (1 - p_hat2)/n2))
#    pval = 1-stats.t.pdf(t,df=total)
    pval = 1- stats.t.cdf(t,total)
    return pval
```

Script to perform high sensitivity indel assay using oversampling and technical replicates

```
import string
import sys

def _ss(data):
    """Return sum of square deviations of sequence data."""
    c = mean(data)
    ss = sum((x-c)**2 for x in data)
    return ss

def stddev(data, ddof=0): #code from online example of calculating standard deviation in python
    """Calculates the population standard deviation
    by default; specify ddof=1 to compute the sample
    standard deviation."""
    n = len(data)
    if n < 2:
        raise ValueError('variance requires at least two data points')
    ss = _ss(data)
    pvar = ss/(n-ddof)
    return pvar**0.5
```

```
def mean(input_list):
    total = 0.0
    for item in input_list:
        total += item
    if len(input_list):
        return float(total) / float(len(input_list))
    else:
        return 0.0

def in_window(indel_code, window_start, window_end):
    is_in_window = 0
    indel_list = string.split(indel_code, ',')
    for indel in indel_list:
        data = string.split(indel, ':')
        indel_start = string.atoi(data[4])
        indel_end = string.atoi(data[5])
        if indel_start <= window_start and indel_end >= window_end:
            is_in_window = 1
        if indel_start >= window_start and indel_start <= window_end:
            is_in_window = 1
        if indel_end >= window_start and indel_end <= window_end:
            is_in_window = 1
    return is_in_window

input_filename = sys.argv[1]
output_filename = input_filename[:-4] + '.indel_analysis.txt'
input_file = open(input_filename, 'r')
output_file = open(output_filename, 'w')
total_replicates = 0
indel_dict = {}
total_counts = {}
ZFN_percent_indel_list = []
GFP_percent_indel_list = []
ZFN_unique_events = 0
GFP_unique_events = 0
sample_list = []
background_sample_list = []
sample_dict = {}
sample_num_dict = {}
sample_dict_file = open(input_filename, 'r')

#reads in config / sample list file
for line in sample_dict_file:
    line = string.strip(line)
    if line:
        if line[0] == '#':
            parameter, value = string.split(line)
            if parameter == '#all_indels_table':
                indel_table_filename = value
            if parameter == '#sigma_threshold':
                background_sigma_threshold = string.atof(value)
            if parameter == '#expected_fraction_threshold':
                read_threshold_expected_fraction = string.atof(value)
            if parameter == '#window_start':
                window_start = string.atoi(value)
            if parameter == '#window_end':
                window_end = string.atoi(value)
            if parameter == '#genomes_sampled':
                genomes_sampled = string.atoi(value)
        else:
            total_replicates += 1
            ascii_sample_num, sample_name, sample_status = string.split(line)
            sample_num = string.atoi(ascii_sample_num)
            sample_dict[sample_name] = sample_num
            sample_num_dict[sample_num] = sample_name
            sample_list.append(sample_num)
            if sample_status == 'background':
                background_sample_list.append(sample_num)
            total_counts[sample_num] = 0


data_file = open(indel_table_filename, 'r')

#reads info from all indels table
for line in data_file:
    line = string.strip(line)
    if line[:6] != 'sample' and line[0] != '#':
```

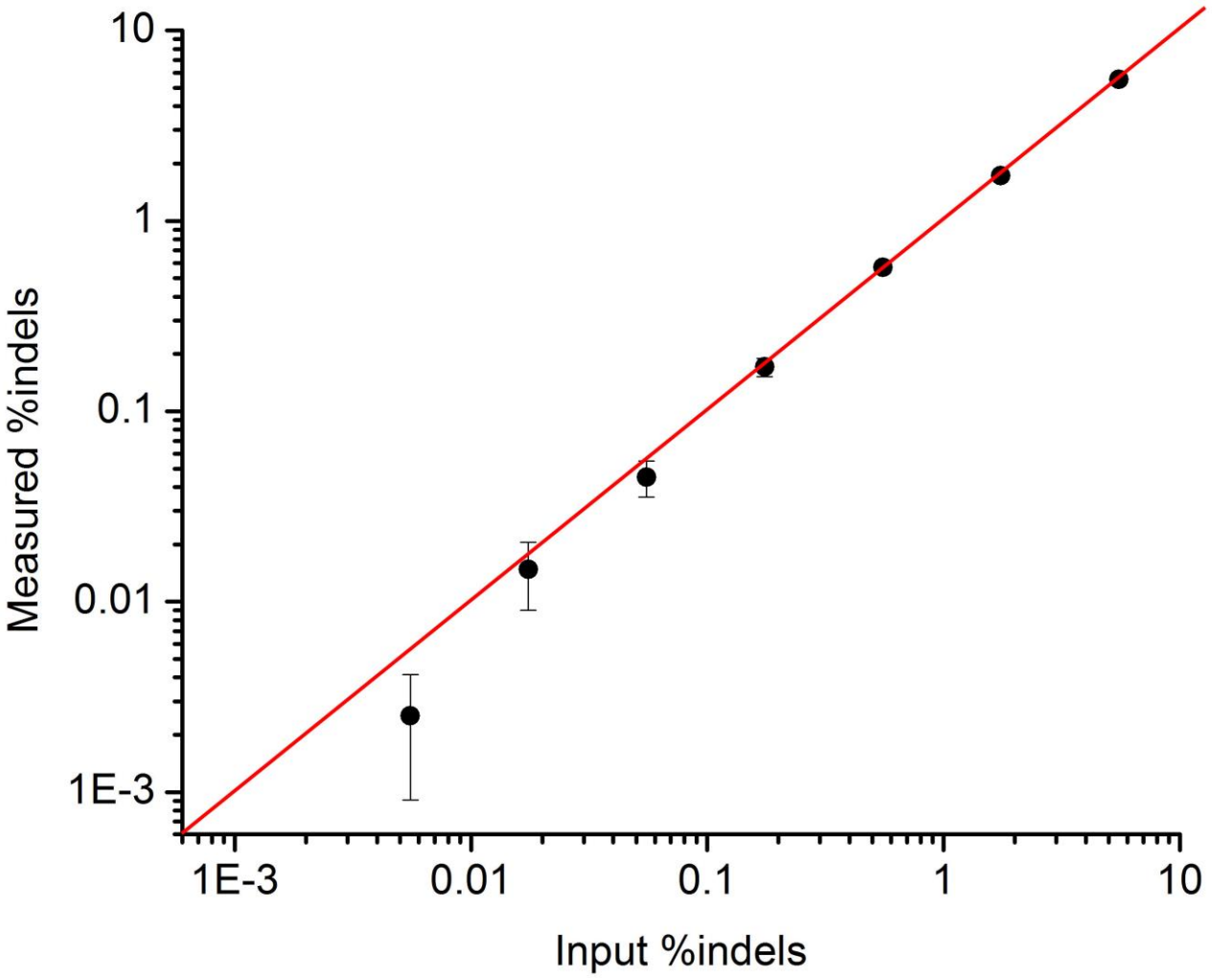
```
        sample_name, simple_indelcode, ascii_count, ascii_frequency, flag, verbose_indelcode =
       string.split(line, '\t')
        indelcode = string.strip(verbose_indelcode, '"')
        indelcode = string.strip(indelcode, '()')
        indelcode = string.strip(indelcode, ',')
        if sample_name in sample_dict:
            sample_num = sample_dict[sample_name]
            if sample_num in sample_list:
                count = string.atoi(ascii_count)
                total_counts[sample_num] += count
                frequency = string.atof(ascii_frequency)
                if indelcode not in indel_dict:
                    indel_dict[indelcode] = []
                indel_dict[indelcode].append( (sample_num, count, frequency) )

background_frequency_list = {}
for indelcode in indel_dict.keys():
    background_frequency_list[indelcode] = []
    for sample_num in background_sample_list:
        for entry in indel_dict[indelcode]:
            if entry[0] == sample_num:
                count = entry[1]
                frequency = (float(count + 1) / float(total_counts[sample_num]))
                background_frequency_list[indelcode].append(frequency)

for sample_num in sample_list:
    total = 0
    indels = 0
    indeltype_count = 0
    for indelcode in indel_dict.keys():
        for entry in indel_dict[indelcode]:
            if entry[0] == sample_num:
                total += entry[1]
    read_threshold = read_threshold_expected_fraction * float(total) / float(genomes_sampled)
    indel_string = ''
    for indelcode in indel_dict.keys():
        for entry in indel_dict[indelcode]:
            if entry[0] == sample_num:
                if indelcode != 'wt':
                    if in_window(indelcode, window_start, window_end):
                        if entry[1] >= read_threshold:
                            average_background_frequency = mean(background_frequency_list[indelcode])
                            if len(background_frequency_list[indelcode]) >= 2:
                                stdev_background_frequency = stddev(background_frequency_list[indelcode], 1)
                            else:
                                stdev_background_frequency = 0.0
                            if (float(entry[1]) / float(total_counts[sample_num])) >
       (average_background_frequency + stdev_background_frequency * background_sigma_threshold):
                                correction = int(average_background_frequency * float(total_counts[sample_num]))
                                indels += (entry[1] - correction)
                                indeltype_count += 1
                                indel_string += '%s %d %d; ' %(string.strip(indelcode), entry[1], correction)

    if total > 0:
        percent_indels = 100.0 * float(indels) / float(total)
    else:
        percent_indels = 0.0
    if sample_num not in background_sample_list:
        output_line = '%d\t%8.5f\t%d\t%d\t%d\t%s\t%s\n' %(sample_num, percent_indels, indels, total,
       indeltype_count, sample_num_dict[sample_num], indel_string)
        summary_line = '%d\t%8.5f\t%d\t%d\t%d\t%s' %(sample_num, percent_indels, indels, total, indeltype_count,
       sample_num_dict[sample_num])
        print summary_line
        output_file.write(output_line)
```



Supplementary Figure 1

Discordance between ZFN specificity as gauged via binding analysis and cellular cleavage studies

(**a**) Relative selectivity for on-target vs. off-target sites for the AAVS1 ZFNs[42] as gauged by ELISA-based competitive DNA binding assay. Data points are the average of two replicates. Insert values indicate target concentrations yielding 50% inhibition of binding to the intended site. The left ZFN shows a 472-fold preference for the intended target site vs. the OT1 off-target site while the right ZFN shows a 43.6-fold preference for the intended target site vs. the OT1 off-target site. Since both ZFNs must bind DNA in order for cleavage to occur, the total preference for complex formation at the intended target vs OT1 will be approximately 20,000-fold under non-saturating conditions. (**b**) Activity at the intended AAVS1 target and the OT1 off-target measured in human K562 cells. The mean of four independent measurements is shown and error bars represent standard deviations. Individual data points are the "parent full-dose" data points of the "ON" and "OT1" targets at the top of **Supplementary Table 5**. The cellular cleavage preference for the AAVS1 on-target vs. OT1 as gauged via indel analysis is approximately 21-fold. **(c)** Michaelis Menten framework for enzyme function (*Biochemische Zeitschrift*, Michaelis & Menten, 1913). k_1 is the rate constant for binding of enzyme (E) to substrate (S), k_{-1} is the rate at which enzyme-substrate complex (ES) dissociates into unbound enzyme and substrate, and k_2 is the rate at which the enzyme-substrate complex is converted into unbound enzyme and product (P). In this case the substrate is uncleaved genomic DNA, the product is the cleaved genomic DNA, and the enzyme is the pair of zinc finger nucleases.



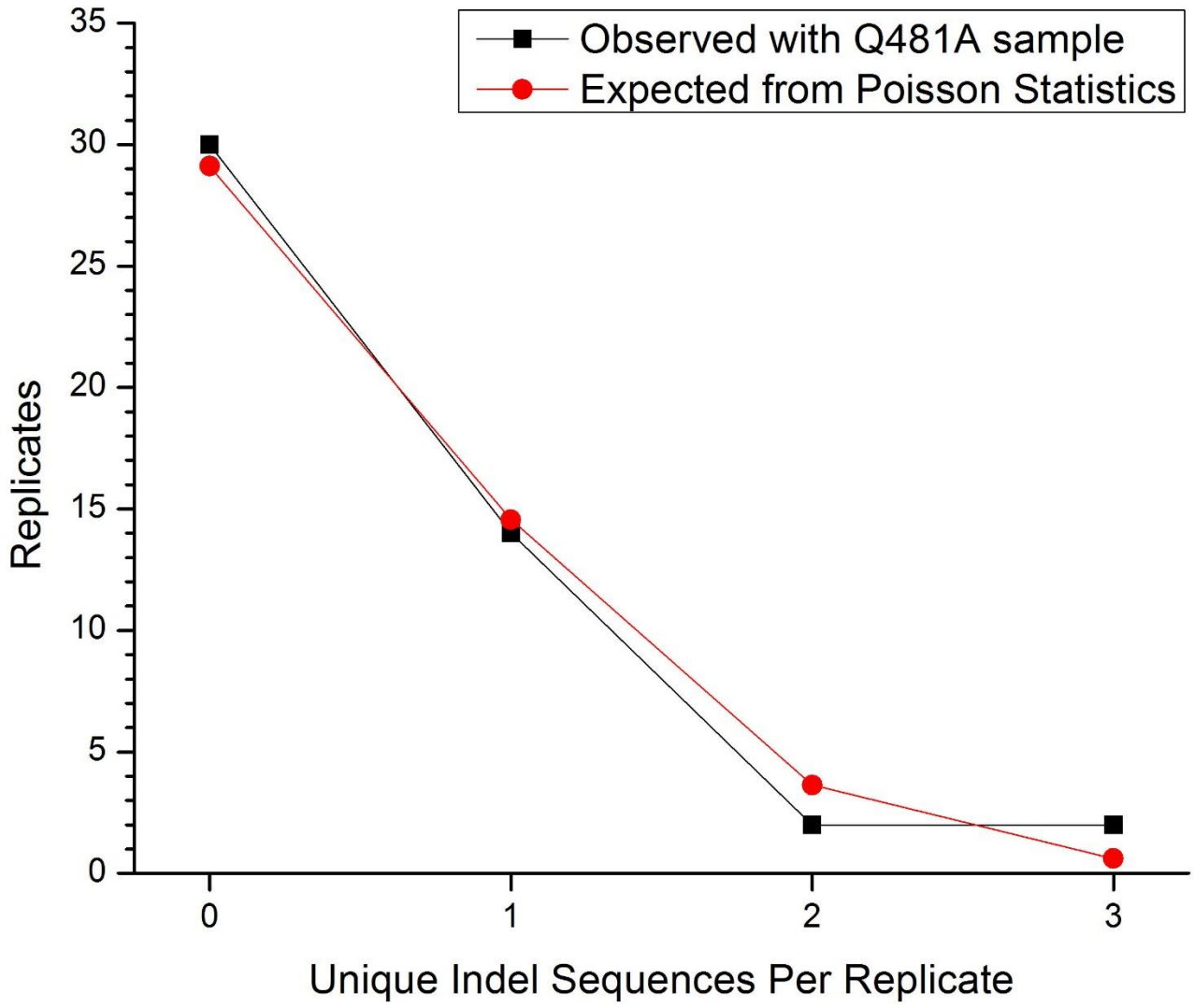
Supplementary Figure 2

Linearity of the oversampling-based indel assay

The %indels at OT1 measured in a 3-fold dilution series is shown. The highest data point was derived from genomic DNA from K562 cells treated with the AAVS1 parent ZFNs and yields an average signal of 5.53% indels; the same sample quantified via standard deep-sequencing analysis yields a value of 6.14% indels. Subsequent samples were generated by serial dilution of the ZFN treated genomic DNA with genomic DNA from control K562 cells. Each value represents the average of 12 technical replicates, with an average of 529,000 sequence reads per replicate. Error bars indicate the 95% confidence interval. Values predicted from perfect linearity are shown by the red line.

%indel values from each Q481A replicate					
0.0000	0.0199	0.0000	0.0000	0.0063	0.0012
0.0025	0.0015	0.0000	0.0000	0.0000	0.0065
0.0094	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	0.0000	0.0000	0.0039	0.0000	0.0064
0.0069	0.0000	0.0000	0.0000	0.0000	0.0000
0.0050	0.0133	0.0079	0.0111	0.0063	0.0000
0.0000	0.0000	0.0038	0.0000	0.0000	0.0000
0.0000	0.0008	0.0000	0.0012	0.0000	0.0000

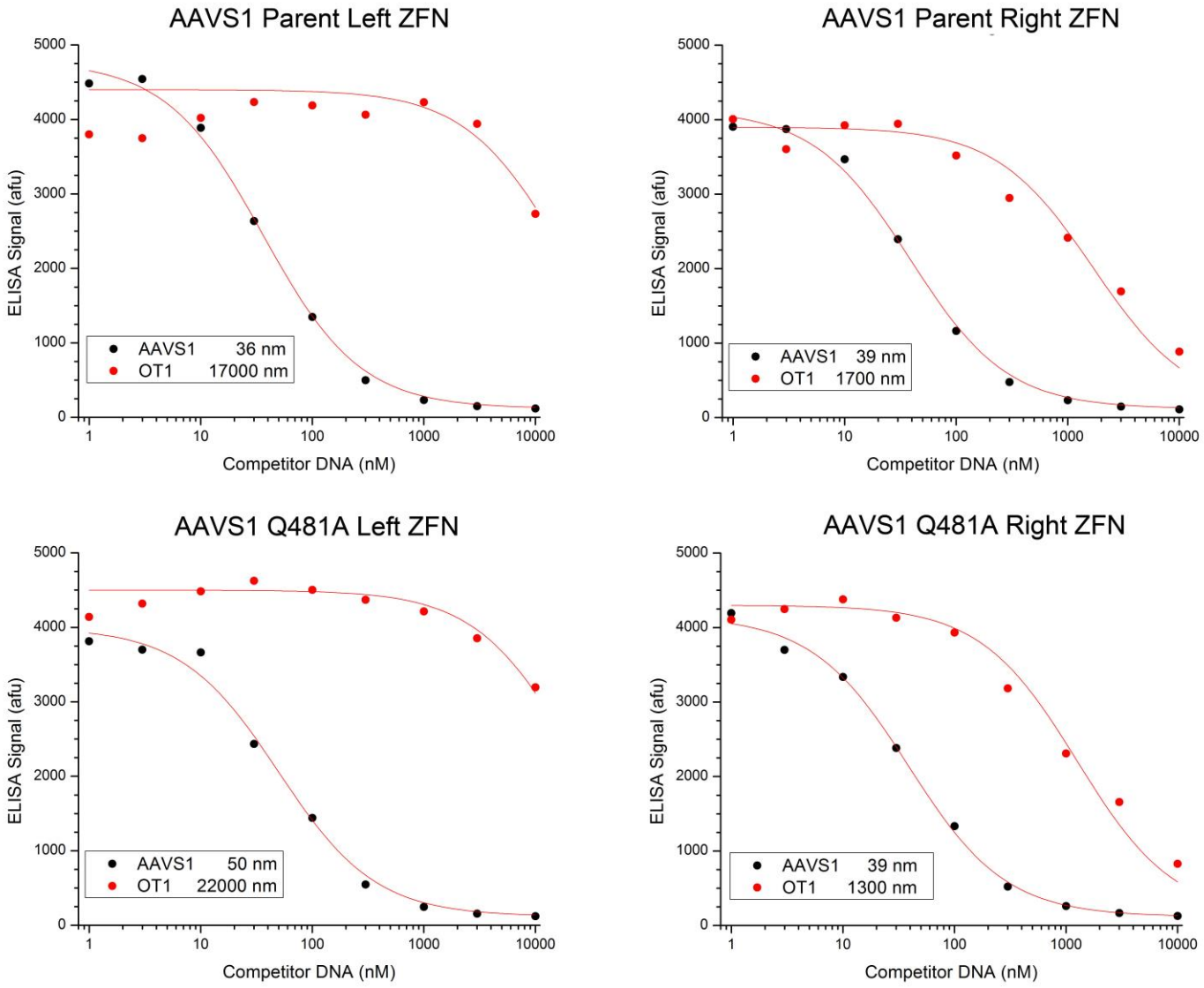
%indel values from each GFP replicate					
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	0.0000	0.0021	0.0000	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000



Supplementary Figure 3

Data underlying OT1 activity for Q481A and GFP shown in Figure 2d

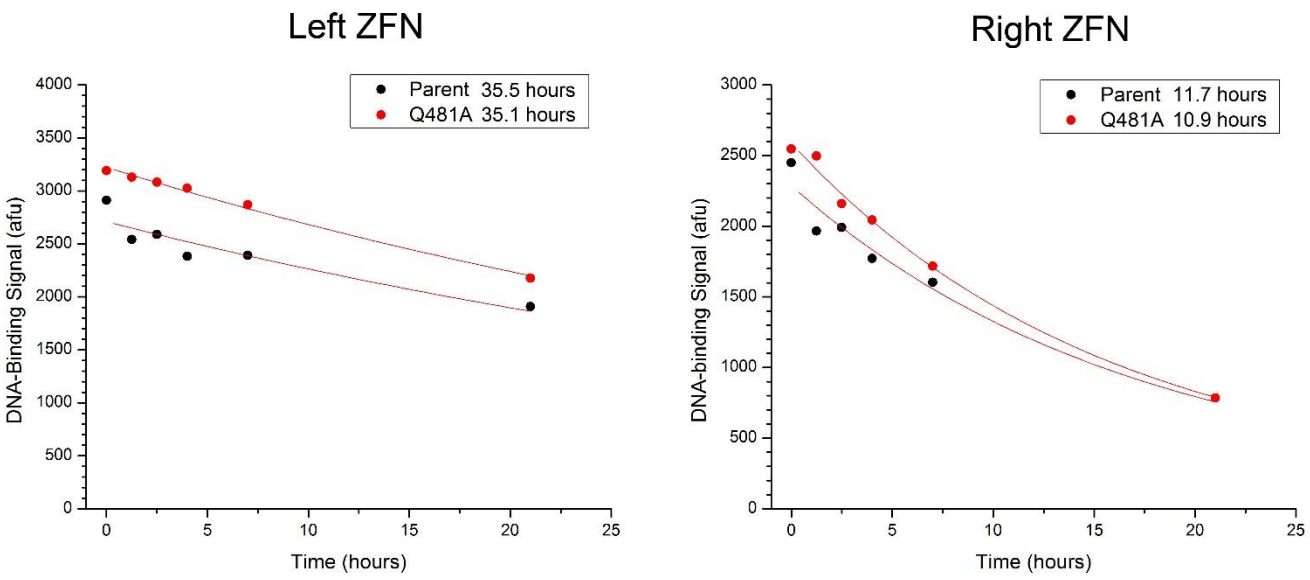
%indel values for 48 individual technical replicates are shown in the upper panel. The large number of technical replicates were performed in order to sample a sufficient number of genomes for each sample The Q481A data is significantly different than the GFP data (P=0.00014, one-tailed T-test). The average %indels for the Q481A sample were 0.0024%; simulations with this data indicated %indel values above 0.0005% would likely be significantly different from the GFP samples implying a sensitivity of less than 0.001% indels. For the Q481A sample, 30 of the replicates were negative for indel activity, 14 replicates had one unique indel sequence, 2 replicates had two unique indel sequences, and 2 replicates had 3 unique indel sequences. This distribution is a good match for the distribution predicted by Poisson statistics with a frequency of 0.5 (lower panel) and implies that the assay is accurately measuring indels in individual input alleles. 24 unique events observed in 1.44 million input alleles yields an expected %indels of 0.0017% which is within the 95% confidence interval of the observed 0.0024% indels. More detailed information for these samples can be found in **Supplementary Table 21**.



Supplementary Figure 4

Relative selectivity for on-target vs. off-target sites for the AAVS1 parent ZFNs and their Q481A variants

For an experimental overview see **Supplementary Figure 1a**. The top two panels are also shown in **Supplementary Figure 1a**. Data points are the average of two replicates. This experiment was performed twice and a representative result is shown.

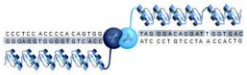


Supplementary Figure 5

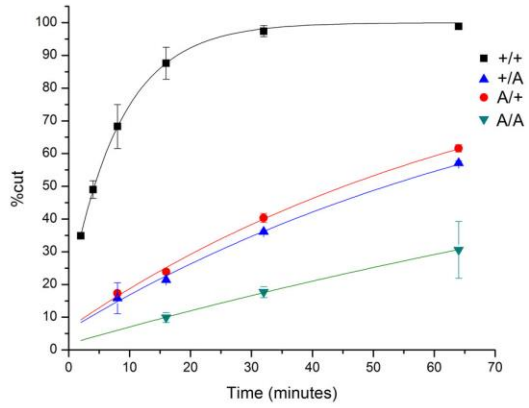
Dissociation rate measurements for interaction of the parent and Q481A ZFNs with the on-target site

Data points represent the average of two replicates. Half-lives for each construct bound to its intended target are shown. Assay was performed by pre-binding of ZFNs to a biotinylated target and then chasing with a 100-fold excess of non-biotinylated target DNA. This assay was performed essentially as described for the study in **Supplementary Figure 1a,** except for the timing of addition of competitor DNA and the timing of the assay readouts. This experiment was performed three times and a representative study is shown.

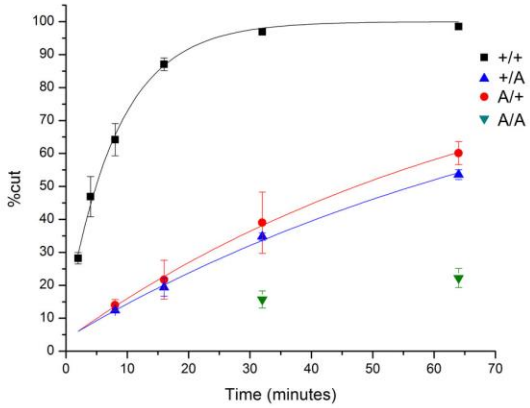
a)

Dimer Type	Dimer Sketch	Dimer Name	$t_{1/2}$(min)	k_{obs}(s^{-1})	$t_{1/2}$(min) half dose	k_{obs}(s^{-1}) half dose
parent dimer		+/+	6	2.0×10^{-3}	6	2.0×10^{-3}
mixed dimers		+/A	57	2.0×10^{-4}	60	1.9×10^{-4}
		A/+	50	2.3×10^{-4}	50	2.3×10^{-4}
Q481A / Q481A variant dimer		A/A	128	9.1×10^{-5}	ND	ND

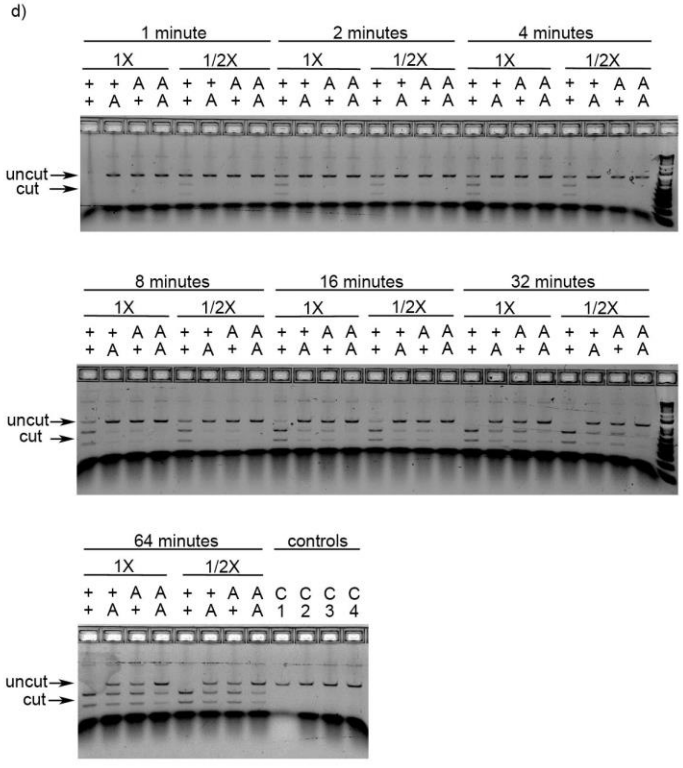
b)



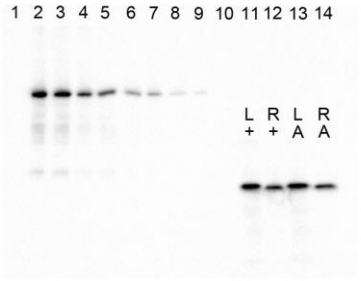
c)



d)



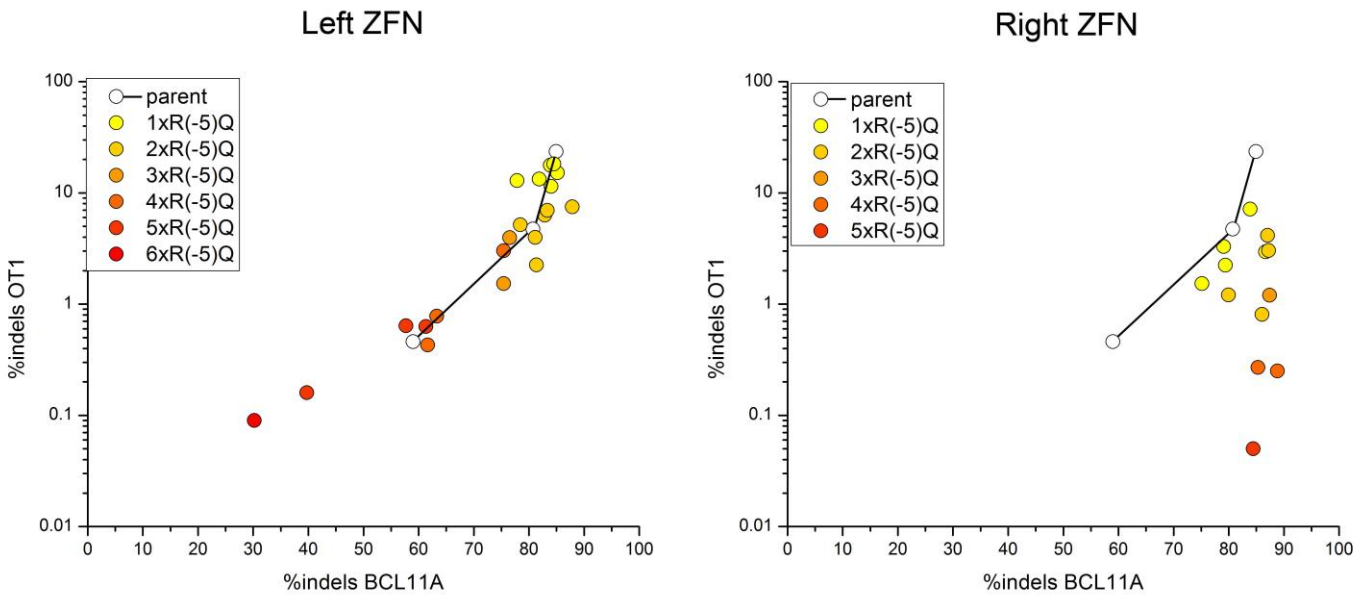
e)



Supplementary Figure 6

Comparison of DNA cleavage rates for ZFNs bearing either the parent FokI domain or the Q481A variants

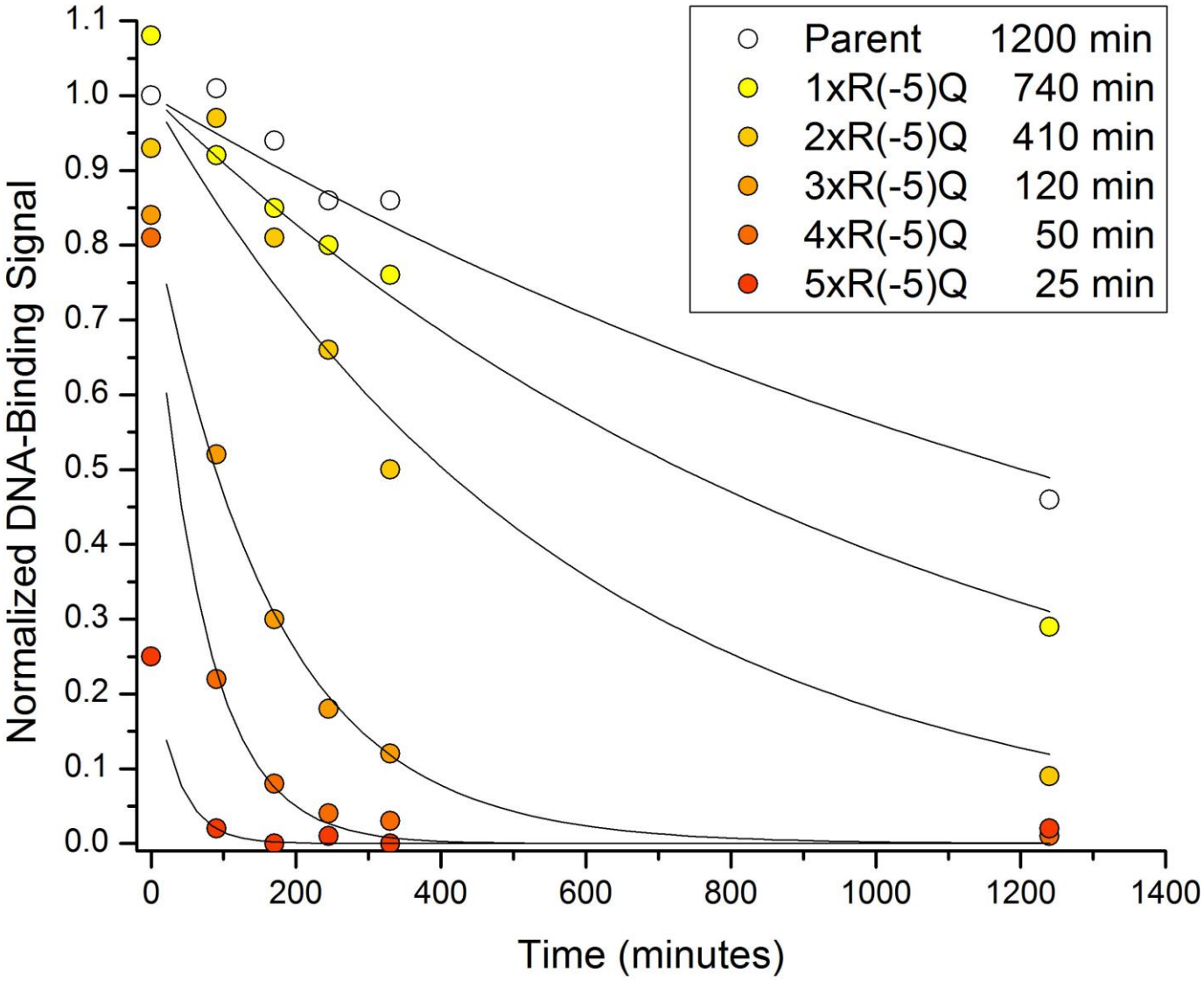
a) Assay overview: parental ZFNs or Q481A variants were expressed from plasmid templates using a coupled transcription-translation kit (Promega) and then combined to form all four possible dimer species. Dimers were then mixed with a 1 kb cleavage substrate bearing a single ZFN dimer target and incubated at room temperature for the indicated reaction times, followed by stopping of reactions and analysis by gel electrophoresis. The experiment was performed with two different doses of ZFN dimers and the entire experiment was repeated twice and the results from each replicate were averaged. Half-lives ($t_{1/2}$) and apparent rate constants (k_{obs}) determined for all four dimers at both doses are shown. Note that as hosts for this study, four-finger derivatives of our AAVS1 ZFNs were used, which were truncated to remove the two FokI-distal fingers. These hosts were chosen because they exhibited faster complex formation, relative to their six-finger counterparts, which enables discernment of a wider range of apparent k_{obs} values. For additional detail see **Supplementary Discussion**. **b**) Plot of percent cleavage of target DNA at each timepoint for the samples treated with the full dose of ZFNs. Data points are the average of two independent replicates and error bars represent standard deviations. Time points for each sample were fit with the indicated single order exponentials **c)** Plot of percent cleavage of target DNA at each timepoint for the samples treated with the half dose of ZFNs and are also the average of two independent replicates. Plots are formatted as in panel **b** and error bars represent standard deviations. **d**) Gel image. Arrows indicate the position of the full-length target DNA (labeled "uncut") and the position of the two cleaved products (labeled 'cut'). DNA cleavage was monitored at the indicated timepoints (note that the first sample for the first timepoint failed). Samples labeled "1/2X" contain half the ZFN extract dose of samples labeled "1X". Dimer combinations are labeled as in panel **a**. Negative controls C1, C2, C3, and C4 are as follows: C1 contains target site only, C2 contains blank transcription-translation lysate and target site, C3 contains lysate expressing only the left parental ZFN plus target site, and C4 contains lysate expressing only the right parental ZFN plus target site. Plasmid template from the coupled-transcription translation reaction is visible as a faint band above the uncut target DNA. The dark smear below the lower cut target DNA band is predominantly material from the coupled transcription-translation lysate as indicated by its reduction in control C1. This experiment was performed twice with one repetition shown **e)** Expression analysis of ZFNs by anti-FLAG western blot. Lanes 2-9 contain different dilutions of a purified standard while lanes 11-14 contain TnT lysate expressing the indicated ZFN variant (L+ and R+ are left and right parental ZFNs while LA and RA are left and right Q481A ZFN variants). This analysis was performed once for each of the two independent replicates and similar results were obtained for both independent replicates. Expression levels for replicate #1 L+, R+, LA, and RA are 634 nM, 448 nM, 618 nM, and 380 nM respectively while expression levels for replicate #2 L+, R+, LA, and RA are 578 nM, 412 nM, 562 nM, and 400 nM respectively. Overall there is an approximately 50% higher expression of the left ZFNs vs. the right ZFNs (an average of 598 nM vs. 410 nM in undiluted lysate). Expression levels of parental vs. Q481 versions of the same nuclease are more similar with an average concentration of parental ZFNs of 518 nM and an average concentration of Q481A ZFNs of 490 nM. The concentration of the lower expressing right ZFNs was approximately 15-fold more concentrated than the target DNA in the full dose samples.



Supplementary Figure 7

Comparison of behaviors of ZFP backbone variants in the context of the left and right BCL11A ZFNs

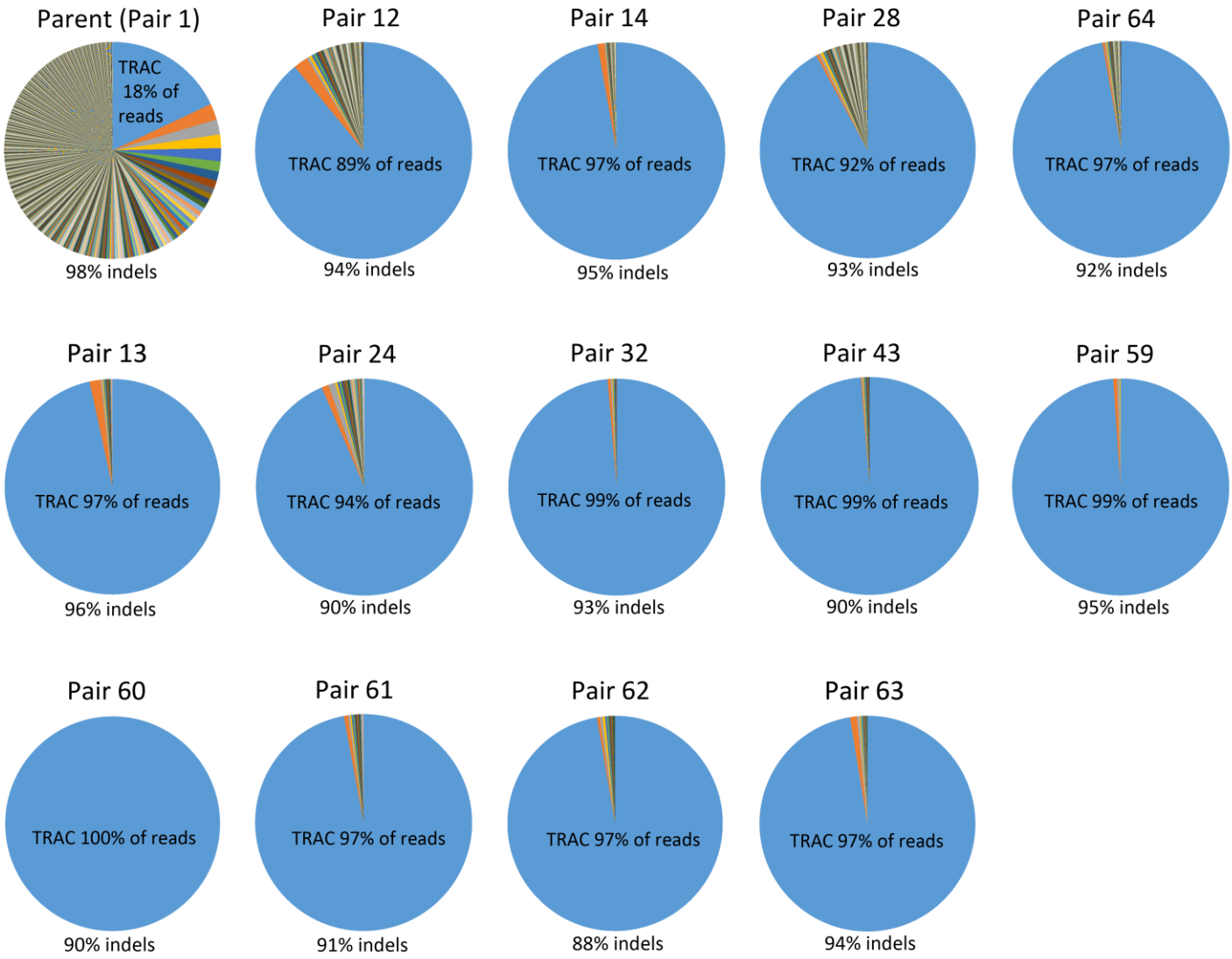
Left panel: plot of % indels at the on-target vs. off-target for all substitution variants of the left ZFN at the 400ng dose. Colored circles indicate substitution levels of the ZFN variant associated with each data point (values are from **Supplementary Table 13**). White circles connected by a black line indicate results obtained with a dilution series of the parent, unmodified ZFN (sample 1 in **Supplementary Table 13**). It can be seen that increasing levels of R(-5)Q substitutions yield very similar behavior in the ratio of on:off-target cleavage as dilution of the parent dimer. Right panel: plot of % indels at the on-target vs. off-target for all substitution variants of the right ZFN at the 200ng dose. For this ZFN, increasing levels of R(-5)Q substitutions yield a selective reduction in off-target cleavage without loss of on-target activity.



Supplementary Figure 8

Dissociation rate measurements for interaction of the BCL11A right ZFN bearing the indicated number of R(-5)Q mutations with the on-target site

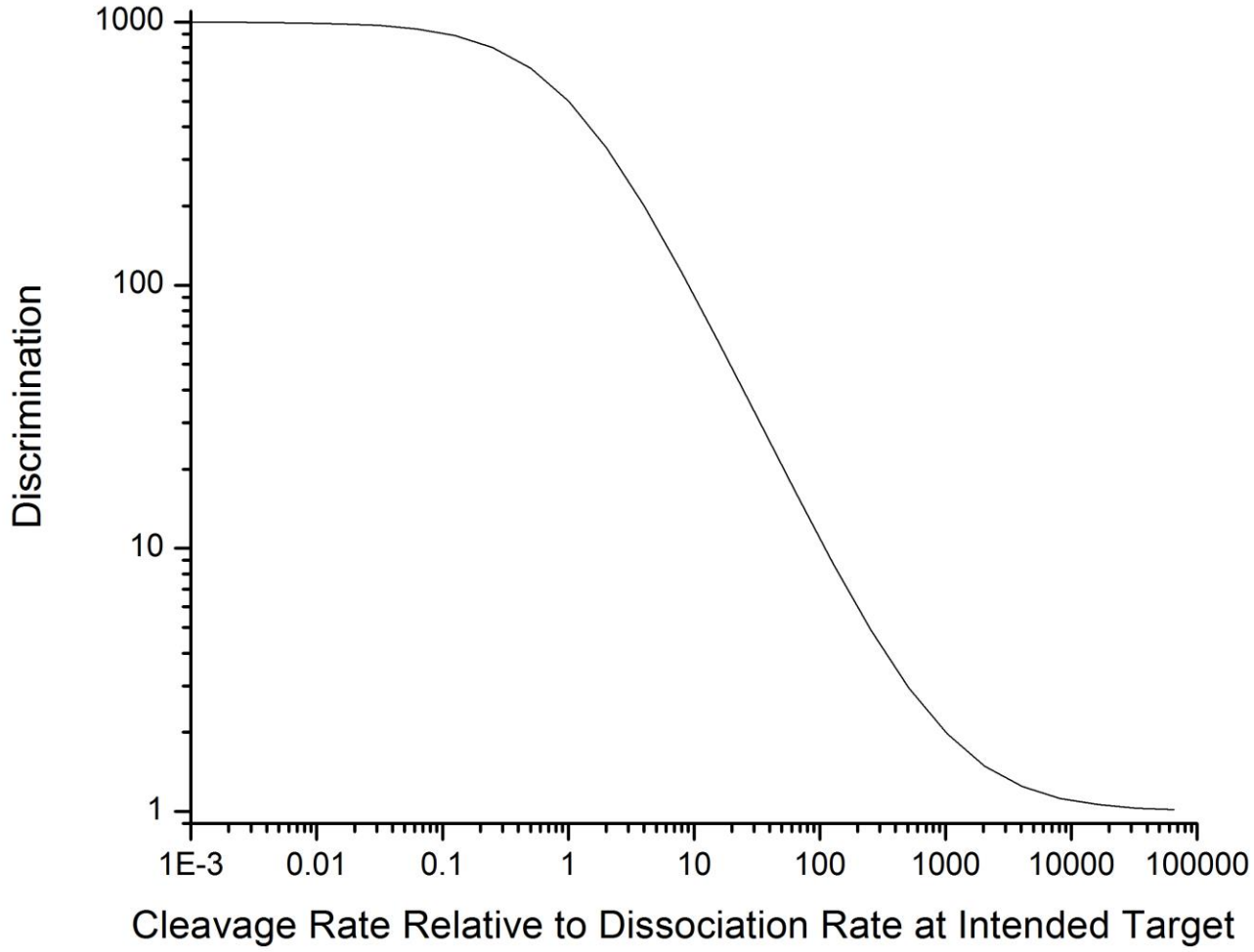
Assay was performed by pre-binding of ZFNs to a biotinylated target and then chasing with a 100-fold excess of non-biotinylated target DNA. Signal was background subtracted and normalized to the value obtained without the unlabeled chase. Constructs are described as in the right panel of **Supplementary Figure 7**. Half-lives for each construct are shown in the figure legend. A second study of R(-5)Q substitutions of the BCL11A left ZFN yielded similar results. Values are single replicates from a single study. A preliminary experiment that tested the parents and the fully substituted variants of both the left and right ZFNs yielded similar results.



Supplementary Figure 9

Summary of oligonucleotide duplex capture results for the indicated TCRα ZFN pairs

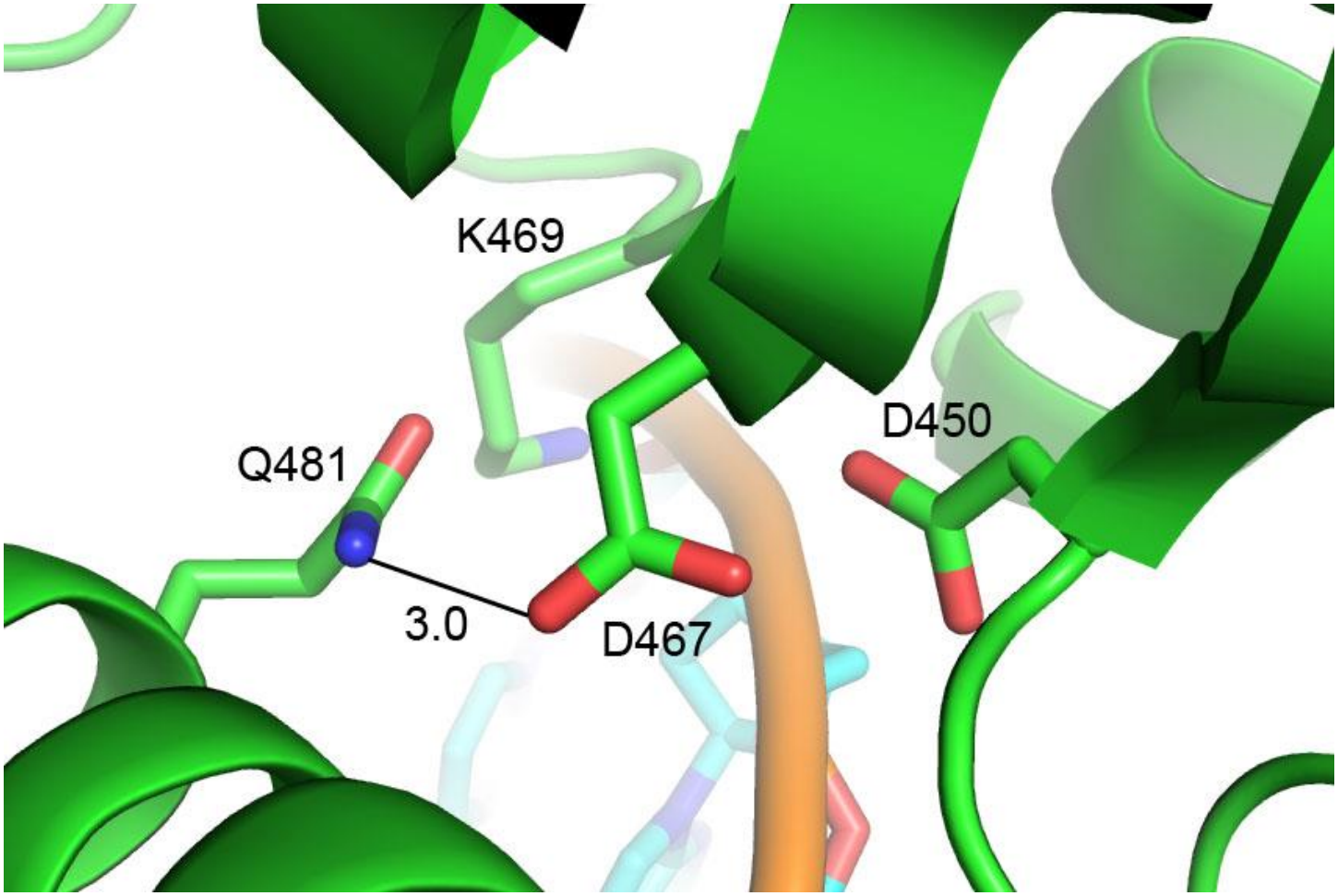
Each experiment is the combination of 4 biological replicates. Data are displayed as in **Figure 2a**, except that mean on-target % indels are listed beneath the chart for each ZFN. Pairs 1, 12, 14, 28, and 64 were characterized in an initial side-by-side experiment and the remaining pairs were characterized in a second side-by-side experiment which appeared to exhibit a lower background of candidate off-targets. Pairs that performed well within their experimental batch and that yielded high activity in an intial T-cell experiment (data not shown) were characterized further (see **figure 4c** and **4d**).



Supplementary Figure 10

Predicted results of varying k_2 on the discrimination between binding sites with k_{-1} (dissociation) rates that vary by a factor of 1000

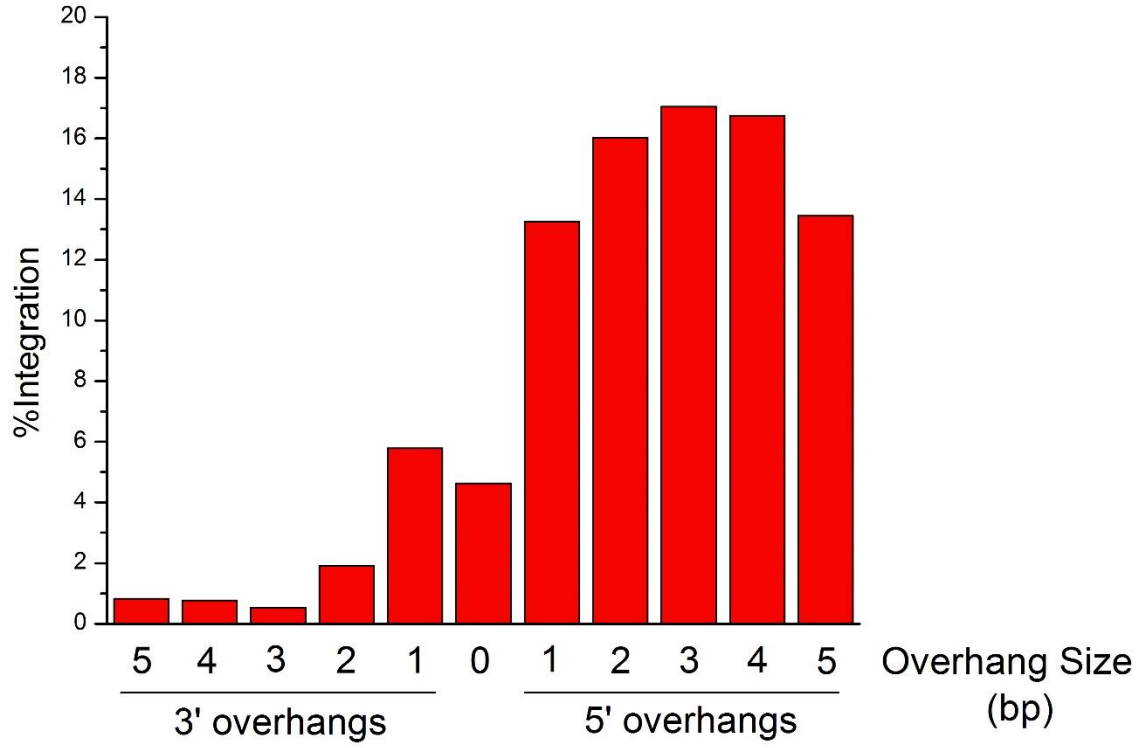
In this case, the dissociation rate for the intended target is 1 and the dissociation for the lower affinity off-target site is much faster at 1000. It is assumed that k_2 and k_1 are identical for both sites since the FokI domain is assumed to be nonspecific and association rates for DNA proteins are often identical with the discrimination between sites reflected by differences in dissociation rates[47]. As described by Hopfield (*Proc. Natl Acad. Sci. USA*, Hopfield, 1974), when k_2 is slow relative to k_{-1} for the higher affinity site, the ratio of DNA cleavage rates (discrimination) at these two targets approaches the ratio of dissociation rates (i.e. 1000). When k_2 is very fast relative to k_{-1} for the higher affinity site, the discrimination approaches 1. The plot uses the relationship described by Ninio (*Biochimie*, Ninio, 1975). In terms of the equation in Supplementary Figure 1 and using k_{-1A} and k_{-1B} to represent the dissociation rates for the ZFN and the two binding sites, the cleavage discrimination between targets sites is $(k_2 + k_{-1B}) / (k_2 + k_{-1A})$.



Supplementary Figure 11

Structural arrangement of Q481, active site residues, and the DNA backbone in a structural model of the FokI dimer from 2FOK.pdb docked with DNA[18]

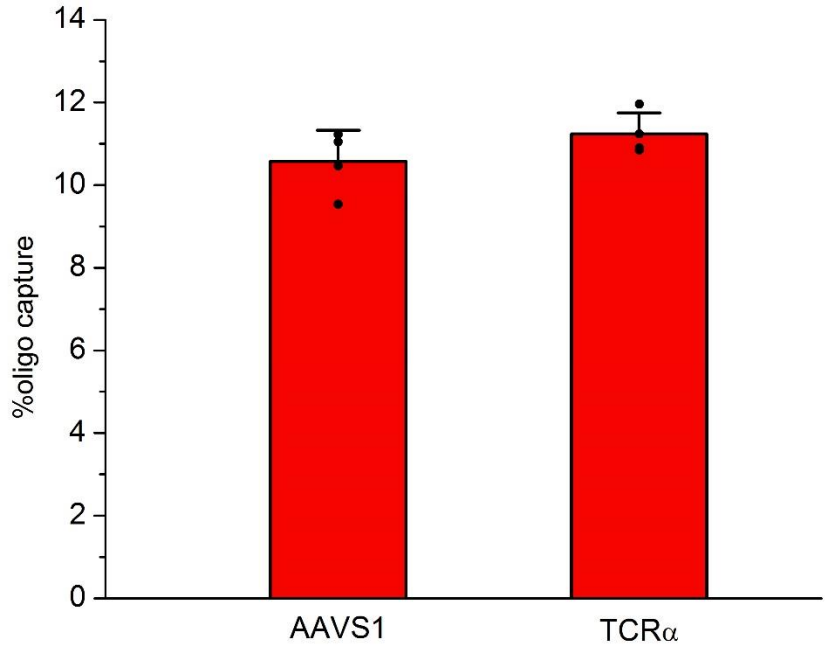
The distance in Angstroms between Q481 and active site residue D467 is shown. The DNA backbone is rendered in orange and does not contact Q481. Pymol was used to create the image and measure distances between atoms.



Supplementary Figure 12

Effect of overhang size and polarity on oligonucleotide integration into ZFN-induced double-strand breaks

Double-stranded oligonucleotides with randomized single-stranded overhangs of indicated lengths and polarities were co-transfected with mRNA encoding the AAVS1 ZFNs into human K562 cells. The locus containing the intended target for the AAVS1 ZFNs was amplified by PCR and then sequenced. The plot shows the percentage of sequence reads containing the entire 34-bp sequence of the double-stranded portion of the oligo.



Supplementary Figure 13

Comparison of oligonucleotide integration frequency at the intended targets of the parental AAVS1 and TCRα ZFNs

Bars represent mean values of the percentage of amplicon sequence reads containing the full 34 bp sequence of the GUIDE-seq oligonucleotide from samples used to perform the GUIDE-seq experiment in **Figure 2** (AAVS1) and in **Figure 4** (TCRα). Error bars indicate standard deviations and values for individual replicates (N=4) are plotted. The target sites for the two AAVS1 ZFN monomers are separated by 6 bp and yield predominantly 4 bp single-stranded overhangs (**Supplementary Figure 14**) while the target sites for the two TCRα ZFN monomers are separated by 5 bp and yield predominantly 5 bp single-stranded overhangs (**Supplementary Figure 15**).

a)

```
                                        TAGGGACAGGATtGGTGAC
5'-TCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGATTGGTGACAG
   AGGGGAGGTGGGGTGTCACCCCGGTGATCCCTGTCCTAACCACTGTC
     GGGAGGTGGGGTGTCACC
```

b)

unmodified TCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGATTGGTGACAG

	%of sequences	Insertions
Parental	11.05	TCCCCTCCACCCCACAGTGGG{GCCA}GCCACTAGGGACAGGATTGGTGACAG
	1.19	TCCCCTCCACCCCACAGT{G}GGGGCCACTAGGGACAGGATTGGTGACAG
	0.54	TCCCCTCCACCCCACAGTGGGGCC{A}ACTAGGGACAGGATTGGTGACAG
	0.44	TCCCCTCCACCCCACAGTGGG{GCC}GCCACTAGGGACAGGATTGGTGACAG
	0.28	TCCCCTCCACCCCACAGTGGGGC{CAC}CACTAGGGACAGGATTGGTGACAG
	0.19	TCCCCTCCACCCCACAGTGGGG{C}CCACTAGGGACAGGATTGGTGACAG
	0.17	TCCCCTCCACCCCACAGTGGGGCC{AC}ACTAGGGACAGGATTGGTGACAG
	0.07	TCCCCTCCACCCCACAGTGGG{CCA}GCCACTAGGGACAGGATTGGTGACAG
	0.06	TCCCCTCCACCCCACAGTGGG{GCCA}GCCACTAGGGACAGGATTGGTGACAG
	0.05	TCCCCTCCACCCCACAGTGGG{GCCA}GCCACTAGGGACAGGATTGGTGACGG
I479Q	8.29	TCCCCTCCACCCCACAGTGGG{GCCA}GCCACTAGGGACAGGATTGGTGACAG
	1.07	TCCCCTCCACCCCACAGT{G}GGGGCCACTAGGGACAGGATTGGTGACAG
	0.46	TCCCCTCCACCCCACAGTGGGGCC{A}ACTAGGGACAGGATTGGTGACAG
	0.35	TCCCCTCCACCCCACAGTGGG{GCC}GCCACTAGGGACAGGATTGGTGACAG
	0.14	TCCCCTCCACCCCACAGTGGGGC{CAC}CACTAGGGACAGGATTGGTGACAG
	0.11	TCCCCTCCACCCCACAGTGGGG{C}CCACTAGGGACAGGATTGGTGACAG
	0.06	TCCCCTCCACCCCACAGTGGG{CCA}GCCACTAGGGACAGGATTGGTGACAG
	0.06	TCCCCTCCACCCCACAGTGGGGCC{AC}ACTAGGGACAGGATTGGTGACAG
	0.05	TCCCCTCCACCCCACAGTGGG{GC}GCCACTAGGGACAGGATTGGTGACAG
	0.03	TCCCCTCCACCCCACAGTGGGGCCA{T}CTAGGGACAGGATTGGTGACAG
Q481A	11.46	TCCCCTCCACCCCACAGTGGG{GCCA}GCCACTAGGGACAGGATTGGTGACAG
	1.54	TCCCCTCCACCCCACAGT{G}GGGGCCACTAGGGACAGGATTGGTGACAG
	0.70	TCCCCTCCACCCCACAGTGGGGCC{A}ACTAGGGACAGGATTGGTGACAG
	0.55	TCCCCTCCACCCCACAGTGGG{GCC}GCCACTAGGGACAGGATTGGTGACAG
	0.17	TCCCCTCCACCCCACAGTGGGGCC{AC}ACTAGGGACAGGATTGGTGACAG
	0.16	TCCCCTCCACCCCACAGTGGG{CCA}GCCACTAGGGACAGGATTGGTGACAG
	0.16	TCCCCTCCACCCCACAGTGGGGC{CAC}CACTAGGGACAGGATTGGTGACAG
	0.10	TCCCCTCCACCCCACAGTGGGG{C}CCACTAGGGACAGGATTGGTGACAG
	0.08	TCCCCTCCACCCCACAGTGGG{GC}GCCACTAGGGACAGGATTGGTGACAG
	0.07	TCCCCTCCACCCCACAGTGGGGCCA{C}CTAGGGACAGGATTGGTGACAG

Supplementary Figure 14

Assessment of indel data for overhangs yielded by cleavage with AAVS1 ZFNs

During NHEJ-mediated repair, overhang fill-in followed by blunt-end ligation comprises a frequent outcome of ZFN-induced double strand breaks, which effectively converts overhangs into duplicated sequence (e.g. see Morton (*Proc. Natl Acad. Sci. USA*, Morton et al., 2006), figure 4). By examining indel data for minor duplications it is possible to infer the predominant overhang type(s) generated during cleavage. **a**) Arrangement of the binding sites for the left and right AAVS1 ZFNs at the intended target. **b**) The ten most common insertions identified via deep sequencing of a PCR amplicon of this region from cells treated with the parent AAVS1 ZFNs, the FokI I479Q variants, or the FokI Q481A FokI variants. Inserted bases are highlighted in red and flanked by brackets; in cases where the exact position of the insertion is ambiguous, it is shown in the leftmost possible position. Note that only the portion of the amplicon flanking the intended target site is shown. Numerical values indicate the percentage of sequence reads corresponding to each sequence from the first replicate of the relevant samples from **Table 1**. For each ZFN dimer, it can be seen that the large majority of insertions (top entry in each alignment) comprise the same GCCA duplication indicating that the I479Q and Q481A substitutions do not alter overhang lengths yielded by cleavage. Note that each entry represents a distinct amplicon sequence; multiple listings of the same insertion in a given alignment indicates the presence of additional mutations elsewhere in the amplicon.

a)

```
                                        CCTGAAAGTGGCCGG
          5'-CAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGT
             GTCACTAACCCAAGGCTTAGGAGGAGGACTTTCACCGGCCCA-'5
               CACTAACCCAAGGCTTAG
```

b)

	unmodified	CAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGT
	%of sequences	Insertions
Parental	13.15	CAGTGATTGGGTTCCGAATC{CTCCT}CTCCTCCTGAAAGTGGCCGGGT
	7.33	CAGTGATTGGGTTCCGAA{TCC}TCCTCCTCCTGAAAGTGGCCGGGT
	1.20	CAGTGATTGGGTTCCGAAT{CCTC}CCTCCTCCTGAAAGTGGCCGGGT
	0.52	CAGTGATTGGGTTCCGAATC{CT}CTCCTCCTGAAAGTGGCCGGGT
	0.42	CAGTGATTGGGTTCCGAATCC{TCCT}TCCTCCTGAAAGTGGCCGGGT
	0.12	CAGTGATTGGGTTCCGAATCCTC{CT}CTCCTGAAAGTGGCCGGGT
	0.06	CAGTGATTGGGTTCCGAA{TCCTCC}TCCTCCTCCTGAAAGTGGCCGGGT
	0.05	CAGTGATTGGGTTCCGAATCCTCC{T}TCCTGAAAGTGGCCGGGT
	0.04	CAGTGATTGGGTTCCGAATC{CTCCT}CTCCTCCTGAAAGTGGCCGGGT
	0.04	CAGTGATTGGGTTCCGAATCCTCCT{CTCCC}CCTGAAAGTGGCCGGGT
Pair14	13.81	CAGTGATTGGGTTCCGAATC{CTCCT}CTCCTCCTGAAAGTGGCCGGGT
	7.21	CAGTGATTGGGTTCCGAA{TCC}TCCTCCTCCTGAAAGTGGCCGGGT
	1.12	CAGTGATTGGGTTCCGAAT{CCTC}CCTCCTCCTGAAAGTGGCCGGGT
	0.34	CAGTGATTGGGTTCCGAATC{CT}CTCCTCCTGAAAGTGGCCGGGT
	0.33	CAGTGATTGGGTTCCGAATCC{TCCT}TCCTCCTGAAAGTGGCCGGGT
	0.08	CAGTGATTGGGTTCCGAATCCTC{CT}CTCCTGAAAGTGGCCGGGT
	0.07	CAGTGATTGGGTTCCGAATCCT{CC}CCTCCTGAAAGTGGCCGGGT
	0.06	CAGTGATTGGGTTCCGAAT{CCTCC}CCTCCTCCTGAAAGTGGCCGGGT
	0.05	CAGTGATTGGGTTCCGAATC{CTCCT}CTCCTCCTGAAAGTGGCCGGGT
	0.05	CAGTGATTGGGTTCCGAATCCTCC{T}TCCTGAAAGTGGCCGGGT
Pair60	13.52	CAGTGATTGGGTTCCGAATC{CTCCT}CTCCTCCTGAAAGTGGCCGGGT
	8.03	CAGTGATTGGGTTCCGAA{TCC}TCCTCCTCCTGAAAGTGGCCGGGT
	1.23	CAGTGATTGGGTTCCGAAT{CCTC}CCTCCTCCTGAAAGTGGCCGGGT
	0.40	CAGTGATTGGGTTCCGAATCC{TCCT}TCCTCCTGAAAGTGGCCGGGT
	0.30	CAGTGATTGGGTTCCGAATC{CT}CTCCTCCTGAAAGTGGCCGGGT
	0.11	CAGTGATTGGGTTCCGAAT{C}CCTCCTCCTGAAAGTGGCCGGGT
	0.06	CAGTGATTGGGTTCCGAATC{CTCCT}CTCCTCCTGAAGGTGGCCGGGT
	0.06	CAGTGATTGGGTTCCGAATCCTC{CT}CTCCTGAAAGTGGCCGGGT
	0.05	CAGTGATTGGGCTCCGAATC{CTCCT}CTCCTCCTGAAAGTGGCCGGGT
	0.05	CAGTGATTGGGTTCCGAATCCT{CC}CCTCCTGAAAGTGGCCGGGT

Supplementary Figure 15

Assessment of indel data for overhangs yielded by cleavage with TCRα ZFNs.

a) Arrangement of the binding sites for the left and right TCR ZFNs at the intended target. **b**) The ten most common insertions identified via deep sequencing of a PCR amplicon of this region from cells treated with the parent TCR ZFNs, or with the Pair14 or Pair60 variants. Alignments are formatted as in **Supplementary Figure 14**. Numerical values indicate the percentage of sequence reads corresponding to each sequence from the 400 ng dose underlying **Supplementary Table 16**. For each ZFN dimer, it can be seen that the large majority of insertions (top entry in each alignment) comprise the same CTCCT duplication indicating that DNA cleavage by the parent and variants yield comparable overhang lengths. For additional detail see the legend for **Supplementary Figure 14**.