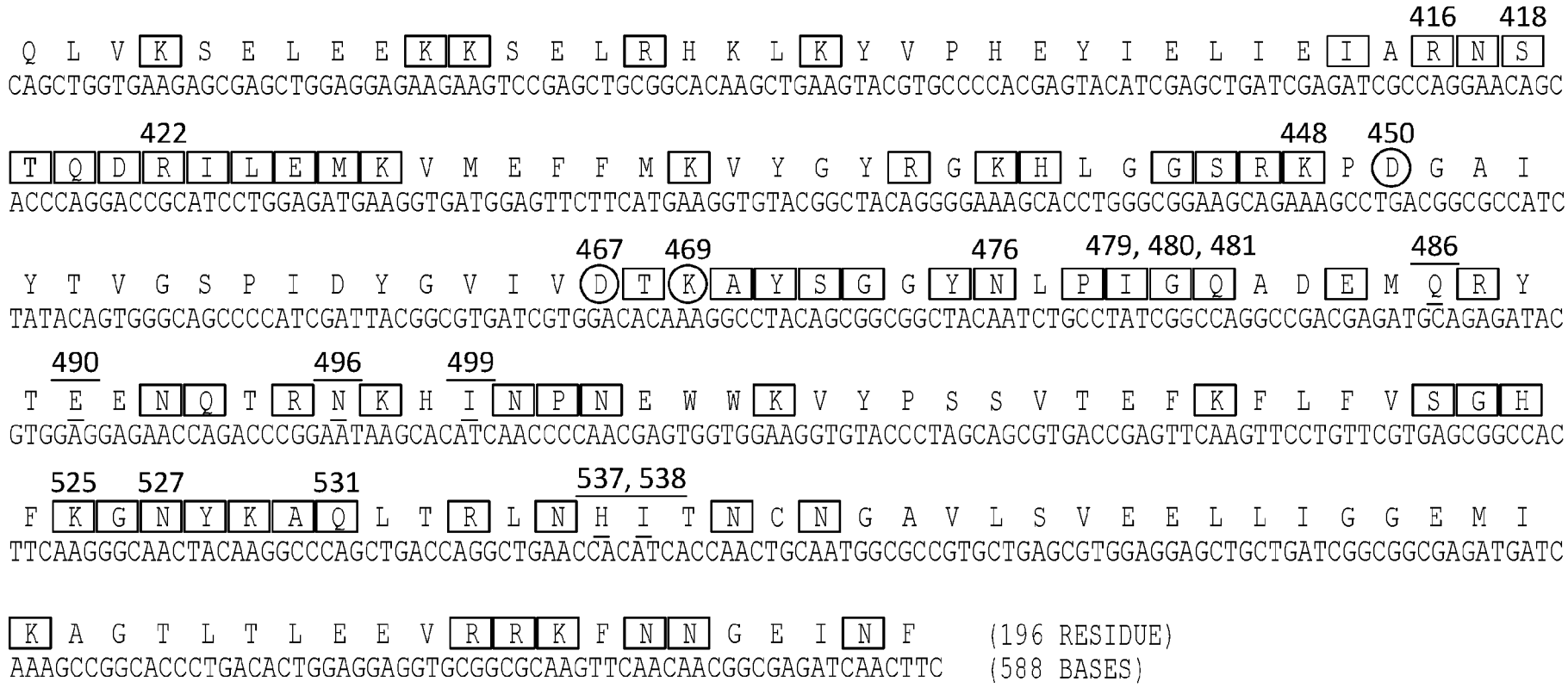


☐ POSITIONS MUTATED AND CHAREACTERIZED FOR IMPROVEMENTS IN ACTIVITY AND/OR SPECIFICITY

◯ ACTIVE SITE RESIDUE

RESIDUE MUTATED IN ELD/KKR VARIANTS

RESIDUE NUMBERS ATE INDICATED FOR ACTIVE SITE RESIDUES, ELD/KKR VARIANTS, AND HEAVILY MUTATED POSITIONS

FIG. 1

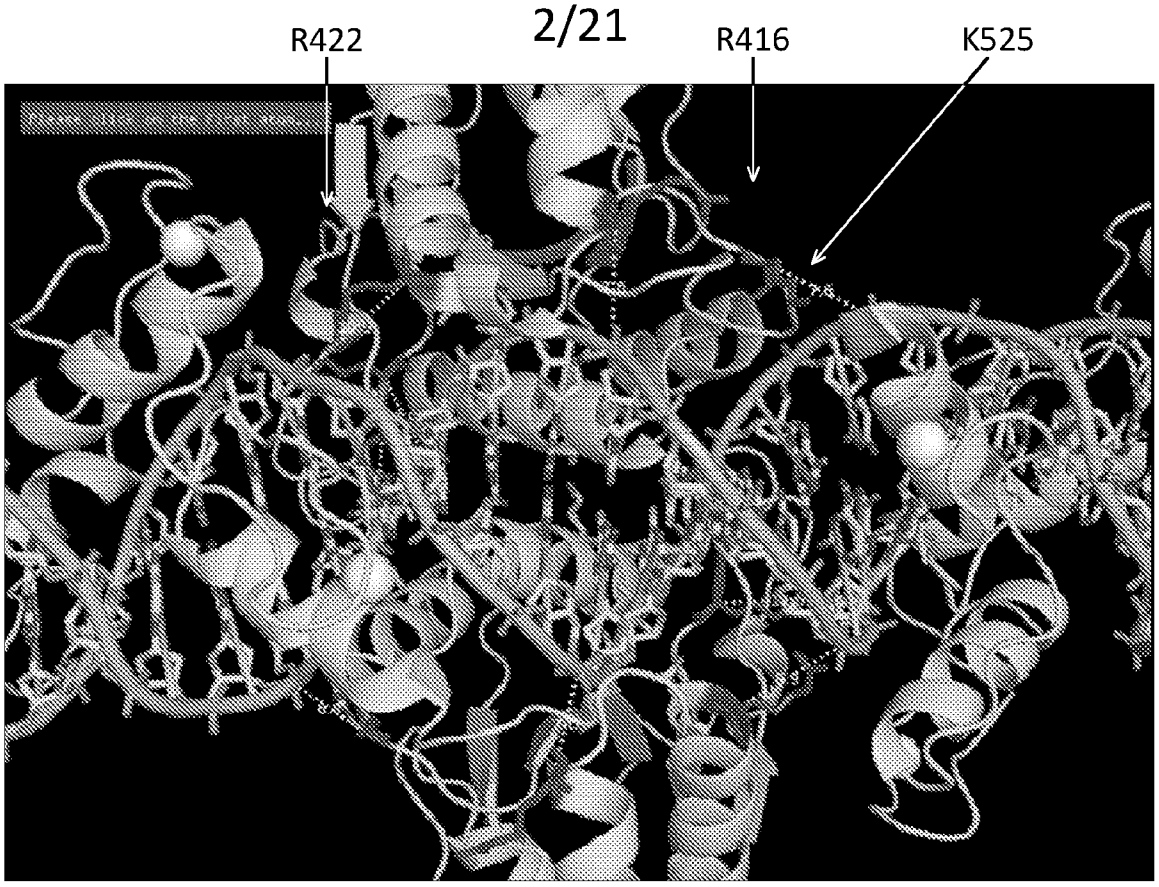


FIG. 2A



FIG. 2B

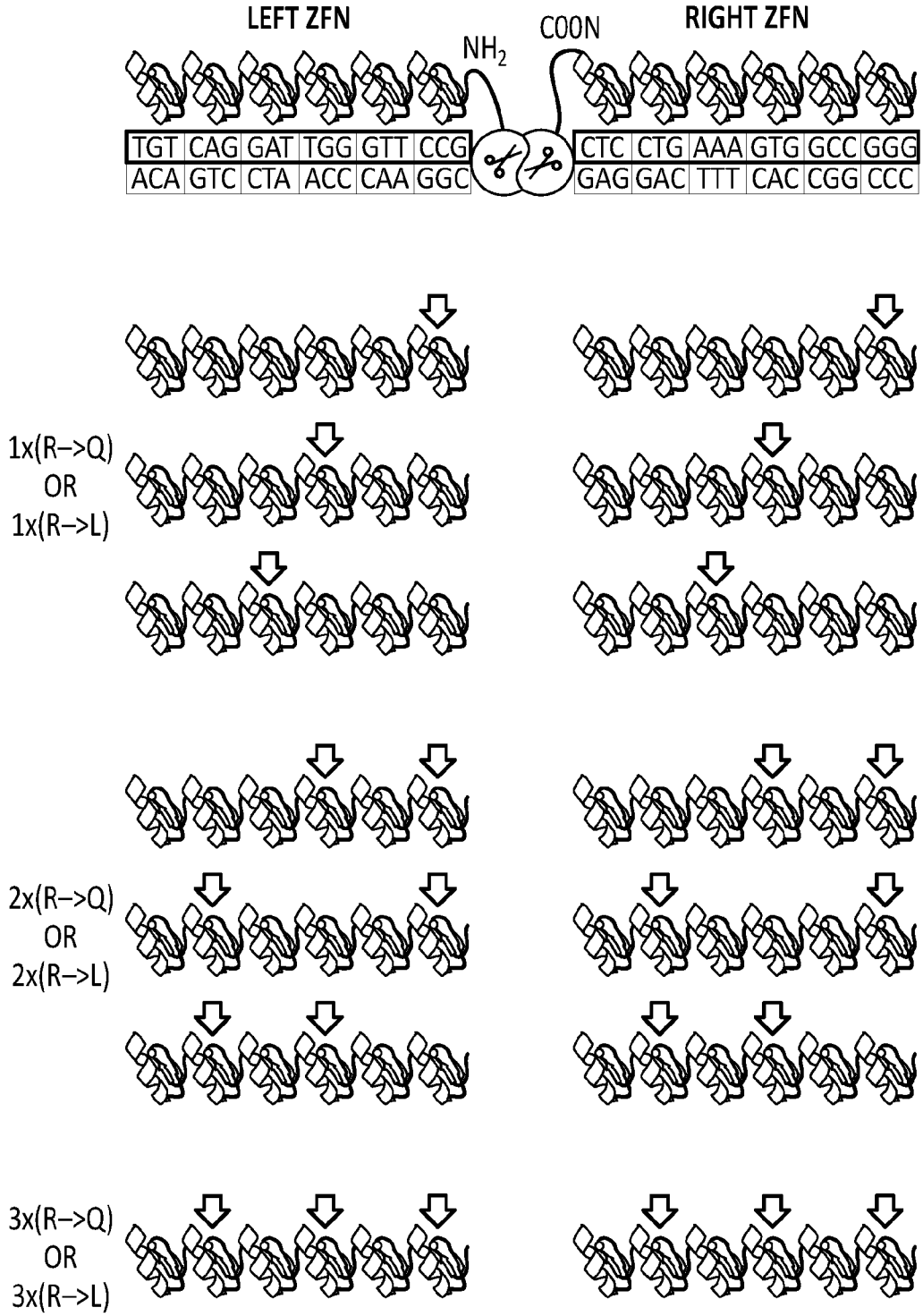

LEFT ZFN
NH2
COON
RIGHT ZFN
TGT CAG GAT TGG GTT CCG
ACA GTC CTA ACC CAA GGC
CTC CTG AAA GTG GCC GGG
GAG GAC TTT CAC CGG CCC
1x(R->Q) OR 1x(R->L)
2x(R->Q) OR 2x(R->L)
3x(R->Q) OR 3x(R->L)

FIG. 2C

ZFN1	ZFN2	PRJIYLFN	NIFMAEVG	PEVYOHIU
51857 ELD	51949 KKR	80.59	9.04	0.65
51857 ELD	51949 KKR_R416S	80.63	0.75	0.08
51857 ELD	51949 KKR_R422S	53.31	0.36	0.02
51857 ELD	51949 KKR_R447S	11.60	0.05	0.03
51857 ELD	51949 KKR_K448S	80.95	1.63	0.67
51857 ELD	51949 KKR_K525S	73.55	0.34	0.03
51857 ELD_R416S	51949 KKR	49.29	1.38	0.09
51857 ELD_R422S	51949 KKR	55.46	1.15	0.22
51857 ELD_R447S	51949 KKR	0.09	0.07	0.05
51857 ELD_K448S	51949 KKR	78.35	9.00	0.51
51857 ELD_K525S	51949 KKR	22.39	0.37	0.11
51857 ELD_R416S	51949 KKR_R416S	48.70	0.26	0.05
51857 ELD_R422S	51949 KKR_R422S	19.78	0.14	0.04
51857 ELD_R447S	51949 KKR_R447S	0.47	0.11	0.06
51857 ELD_K448S	51949 KKR_K448S	69.45	2.59	0.34
51857 ELD_K525S	51949 KKR_K525S	6.53	0.07	0.05

FIG. 3A

ZFN1	ZFN2	PRJIYLFN	NIFMAEVG	PEVYOHIU
51857_KKR	51949_ELD	88.28	9.26	0.87
51857_KKR	51949_ELD_R416S	81.35	1.68	0.38
51857_KKR	51949_ELD_R422S	67.78	0.12	0.12
51857_KKR	51949_ELD_K447S	70.79	0.09	0.08
51857_KKR	51949_ELD_K448S	83.02	1.00	0.33
51857_KKR	51949_ELD_K525S	76.60	0.06	0.09
51857_KKR_R416S	51949_ELD	59.43	0.76	0.12
51857_KKR_R422S	51949_ELD	71.64	3.27	0.26
51857_KKR_K447S	51949_ELD	25.01	0.07	0.06
51857_KKR_K448S	51949_ELD	83.71	15.88	0.34
51857_KKR_K525S	51949_ELD	33.14	0.21	0.11
51857_KKR_R416S	51949_ELD_R416S	68.35	0.04	0.11
51857_KKR_R422S	51949_ELD_R422S	26.25	0.07	0.11
51857_KKR_K447S	51949_ELD_K447S	1.42	0.06	0.11
51857_KKR_K448S	51949_ELD_K448S	84.31	3.06	0.71
51857_KKR_K525S	51949_ELD_K525S	6.99	0.06	0.09

FIG. 3B

EACH ZFN DELIVERED AT 2 μG MRNA

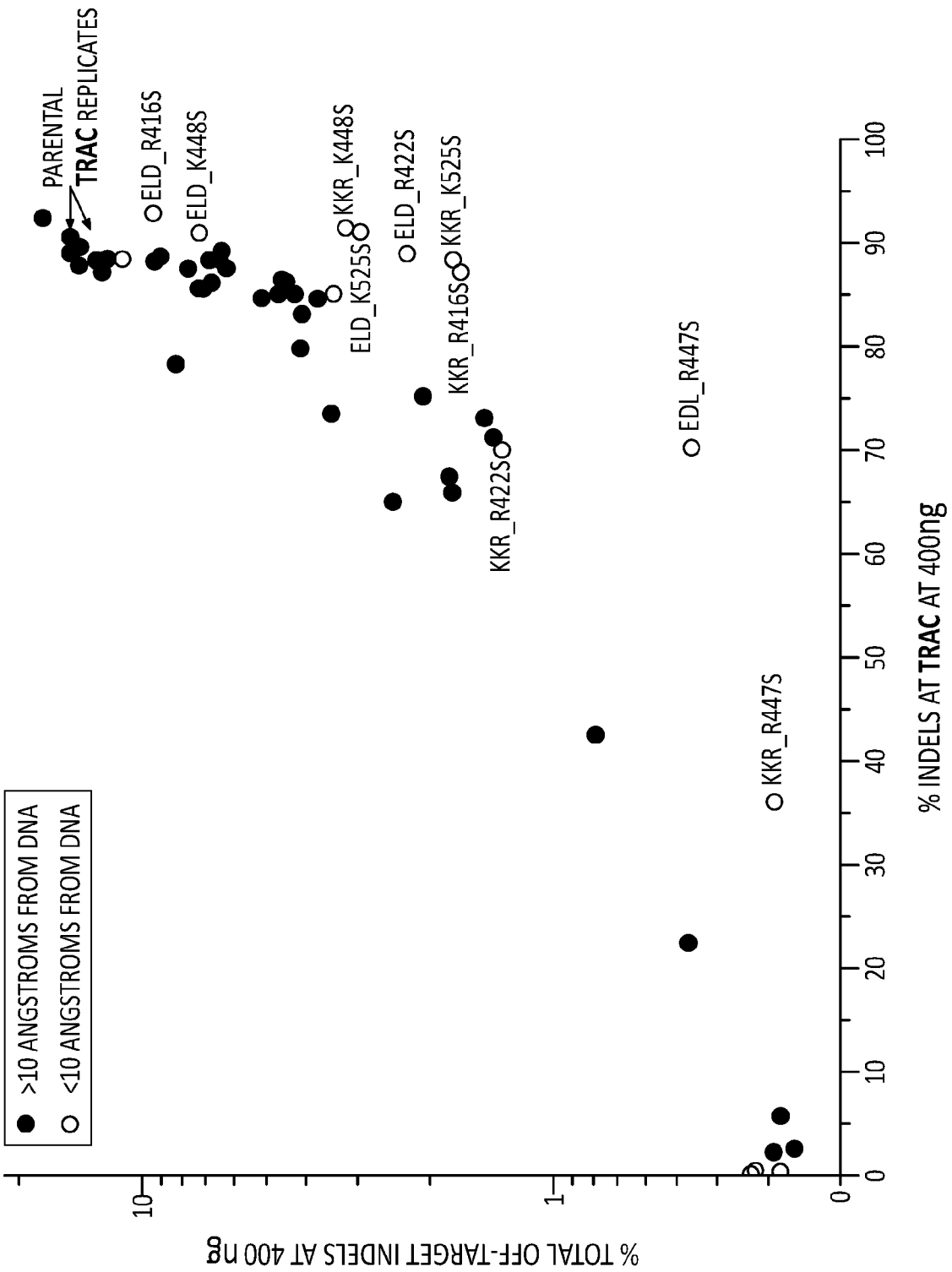

PARENTAL
TRAC REPLICATES
ELD_R416S
ELD_K448S
KKR_K448S
ELD_K525S
ELD_R422S
KKR_R416S
KKR_K525S
KKR_R422S
EDL_R447S
KKR_R447S
>10 ANGSTROMS FROM DNA
<10 ANGSTROMS FROM DNA
% TOTAL OFF-TARGET INDELS AT 400 ng
% INDELS AT TRAC AT 400ng
10
1
0
0
10
20
30
40
50
60
70
80
90
100

FIG. 4

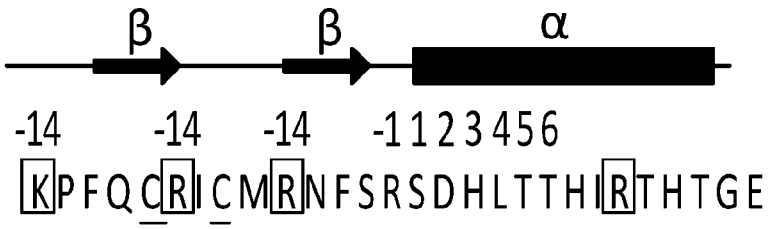

β
β
α
-14
-14
-14
-1 1 2 3 4 5 6
K P F Q C R I C M R N F S R S D H L T T H I R T H T G E

FIG. 5A

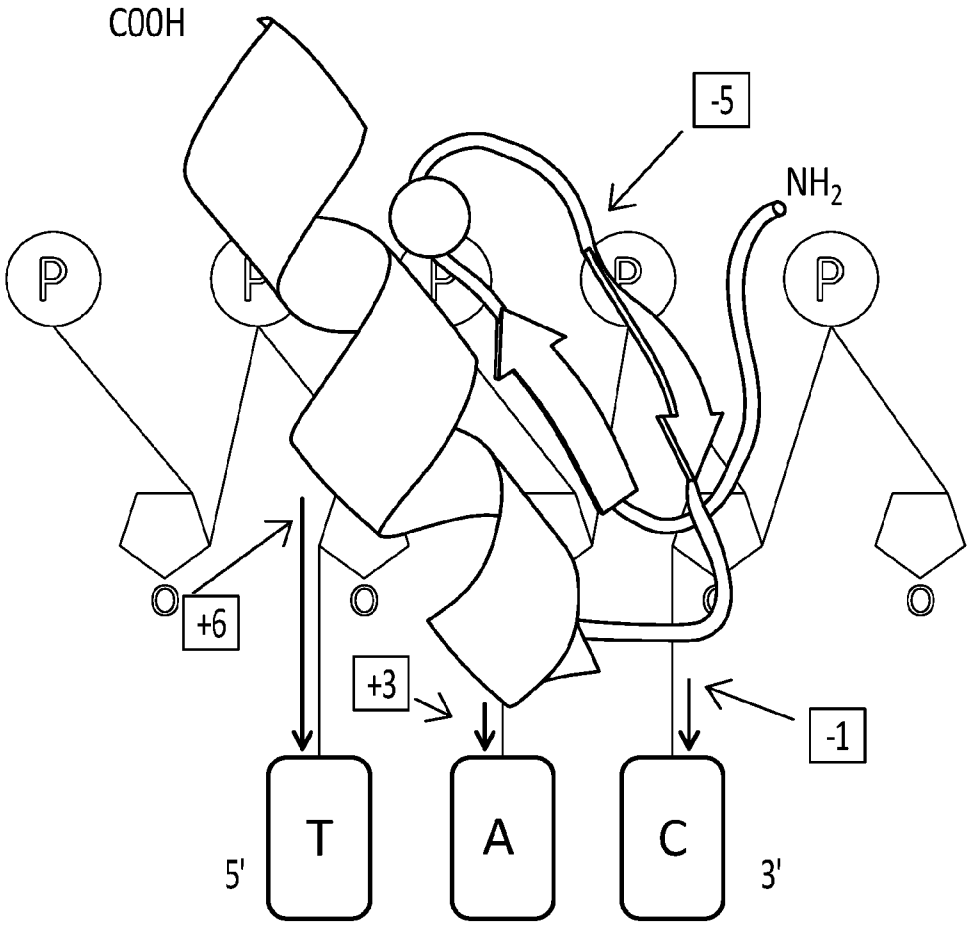

COOH
-5
NH2
P
P
P
P
P
O
O
O
O
+6
+3
-1
T
A
C
5'
3'

FIG. 5B

PHOSPHATE - CONTACTING RESIDUES (+ CHARGE)

EXAMPLES	CYS			(+)	CYS			(+)	RECOGNITION HELIX							HIS		(+)			HIS	
ZIF-F2	C	R	I	C	M	R	N	F	S	R	S	D	H	L	T	T	H	I	R	T	H	ZIF-F2
ZIF-F3	C	D	I	C	G	R	K	F	A	R	S	D	E	R	K	K	H	T	K	I	H	ZIF-F3
SP1-F3	C	P	E	C	P	K	R	F	M	R	S	D	H	L	S	K	H	I	K	T	H	SP1-F3
A	0	124	88	0	67	(52)	2079	19	177	41	171	354	297	6	307	210	0	44	79	98	0	A
C	4867	17	8	4867	7	3	80	71	119	226	70	51	66	24	28	37	0	29	4	28	0	C
D	0	302	348	0	192	5	54	0	44	196	21	105	255	0	45	78	0	1	11	12	0	D
E	0	631	2508	0	201	97	41	0	20	113	49	68	126	0	80	329	0	425	38	14	0	E
F	0	16	65	0	13	40	89	4451	48	87	141	65	50	389	58	17	0	35	13	43	0	F
G	0	223	12	0	3785	7	424	5	198	82	165	174	159	0	19	76	0	35	51	37	0	G
H	0	110	123	0	38	22	27	3	73	424	153	70	734	14	97	61	4867	159	30	13	4867	H
I	0	89	206	0	2	14	50	13	292	27	83	53	78	48	975	386	0	95	234	1958	0	I
K	0	917	221	0	63	(3841)	71	0	270	165	883	82	143	7	314	537	0	570	686	69	0	K
L	0	75	178	0	20	(39)	76	74	75	194	215	66	115	4127	280	218	0	343	130	222	0	L
M	0	29	39	0	7	43	17	2	52	44	46	23	19	45	64	71	0	337	58	90	0	M
N	0	766	27	0	79	38	50	0	457	148	351	61	685	0	228	181	0	25	57	49	0	N
P	0	327	6	0	60	0	34	1	82	3	251	78	23	3	10	7	0	3	10	1	0	P
Q	0	143	468	0	48	(95)	26	0	38	1142	112	71	299	3	270	711	0	2011	96	24	0	Q
R	0	146	46	0	99	(452)	210	0	702	805	502	97	123	40	459	953	0	419	3079	87	0	R
S	0	634	21	0	136	26	740	43	1569	359	1404	2987	905	1	258	202	0	52	99	261	0	S
T	0	225	87	0	19	14	507	20	481	203	89	272	395	1	967	311	0	39	65	1170	0	T
V	0	57	332	0	11	12	275	5	86	122	64	53	107	86	362	398	0	138	103	678	0	V
W	0	7	5	0	17	1	2	1	12	210	18	18	1	8	5	8	0	44	10	7	0	W
Y	0	29	79	0	3	66	15	159	72	276	79	119	287	65	41	76	0	62	14	6	0	Y
		-9				-5				-1	1	2	3	4	5	6						NUMBERING

AMINO ACID FREQUENCIES

FIG. 6

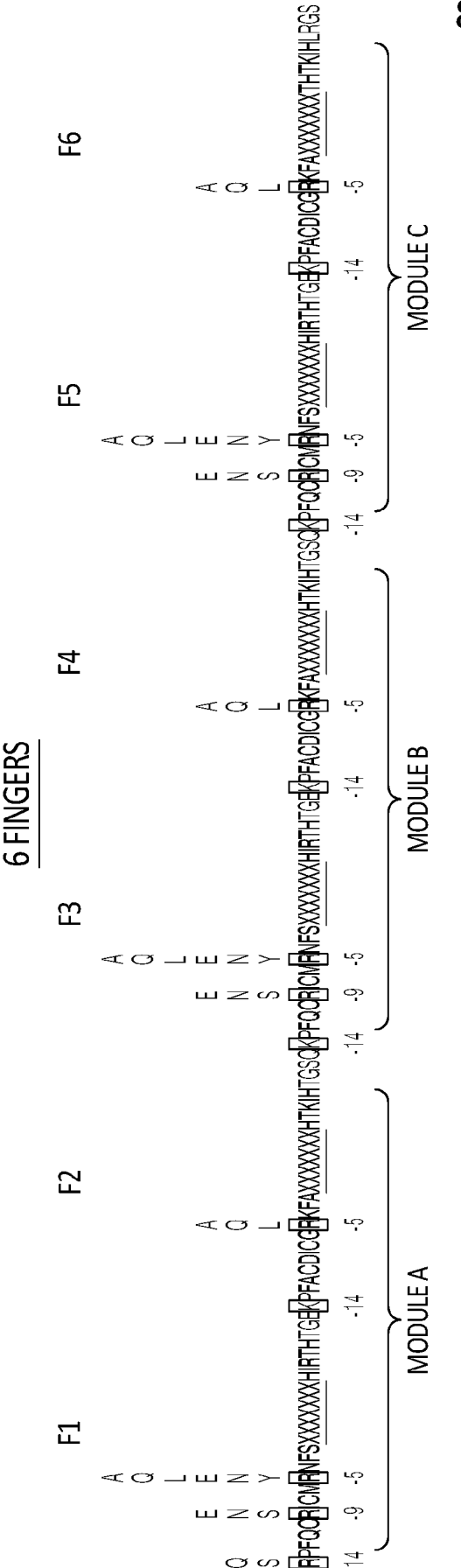

6 FINGERS
F1
F2
F3
F4
F5
F6
MODULE A
MODULE B
MODULE C

FIG. 7A

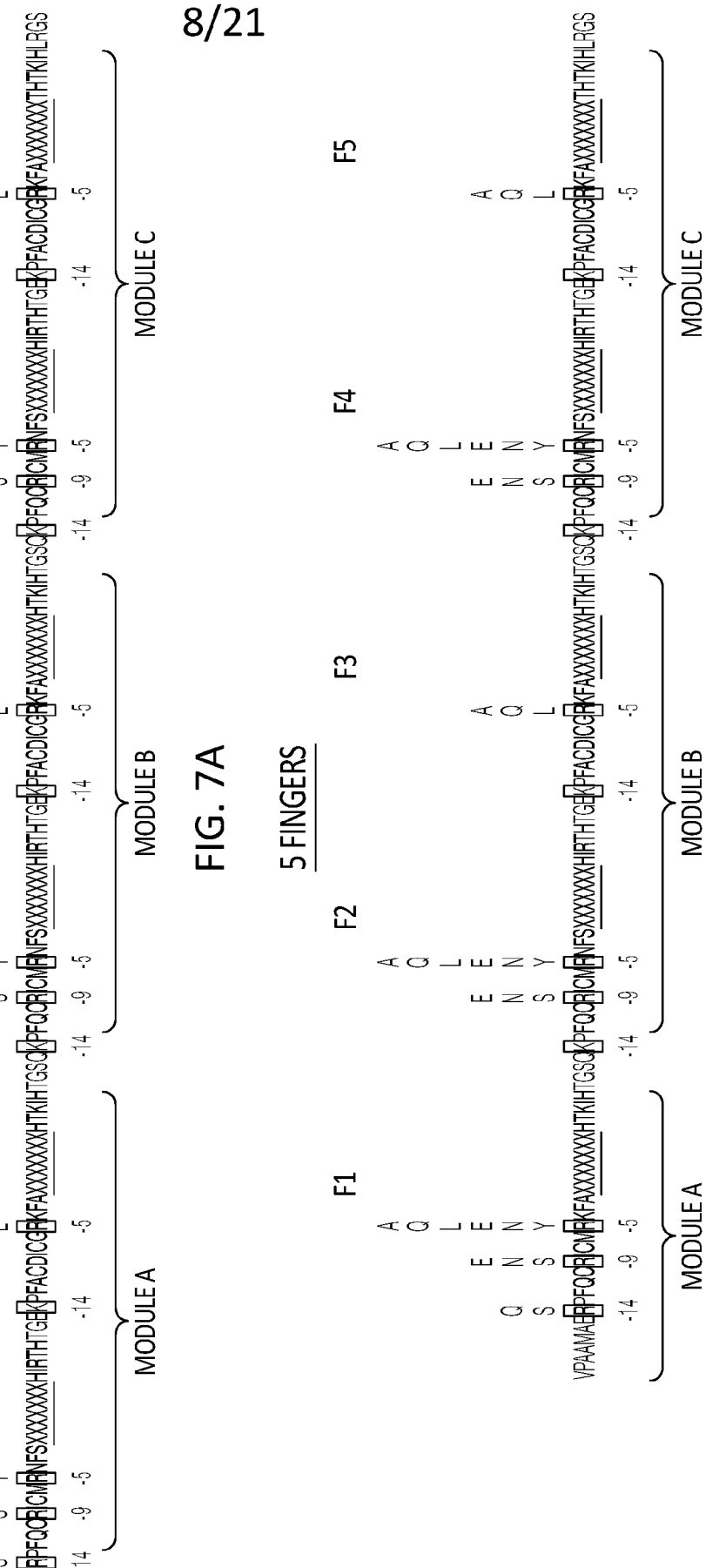

5 FINGERS
F1
F2
F3
F4
F5
MODULE A
MODULE B
MODULE C

FIG. 7B

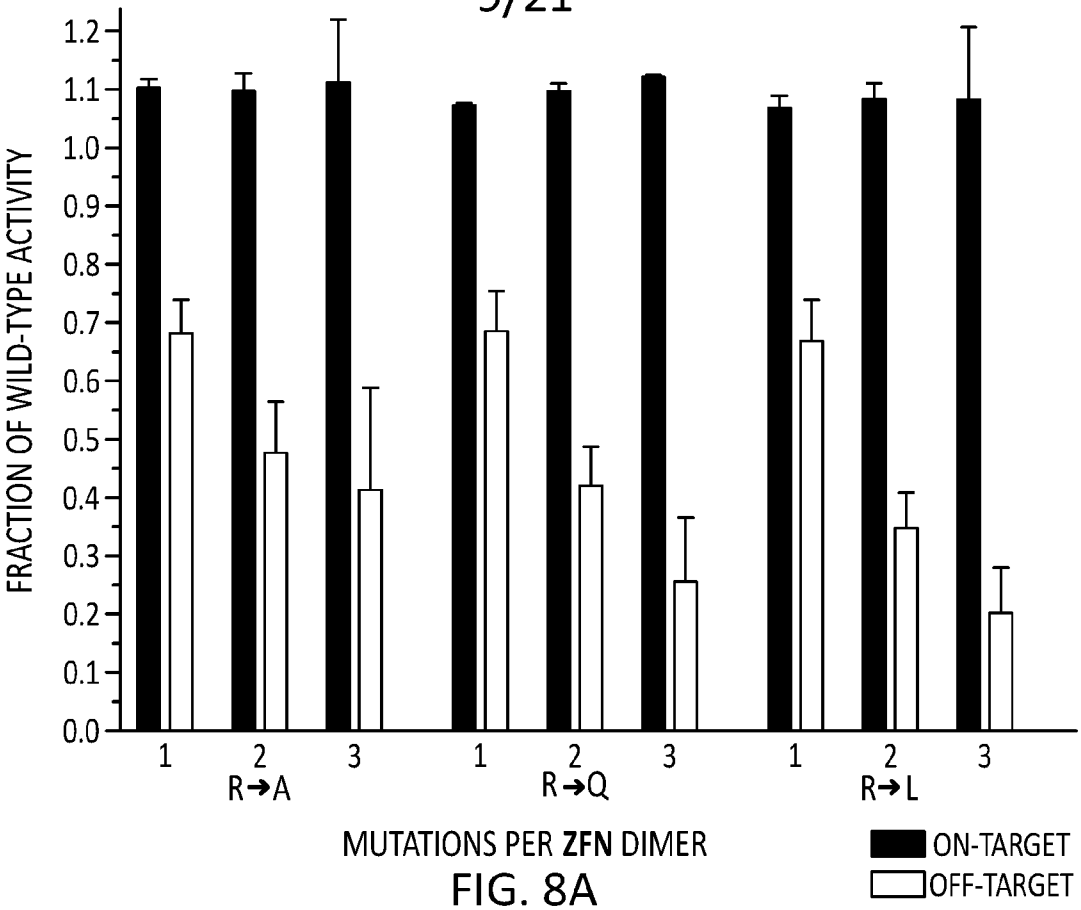

FRACTION OF WILD-TYPE ACTIVITY
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1 2 3 1 2 3 1 2 3
R→A
R→Q
R→L
MUTATIONS PER ZFN DIMER
ON-TARGET
OFF-TARGET

FIG. 8A

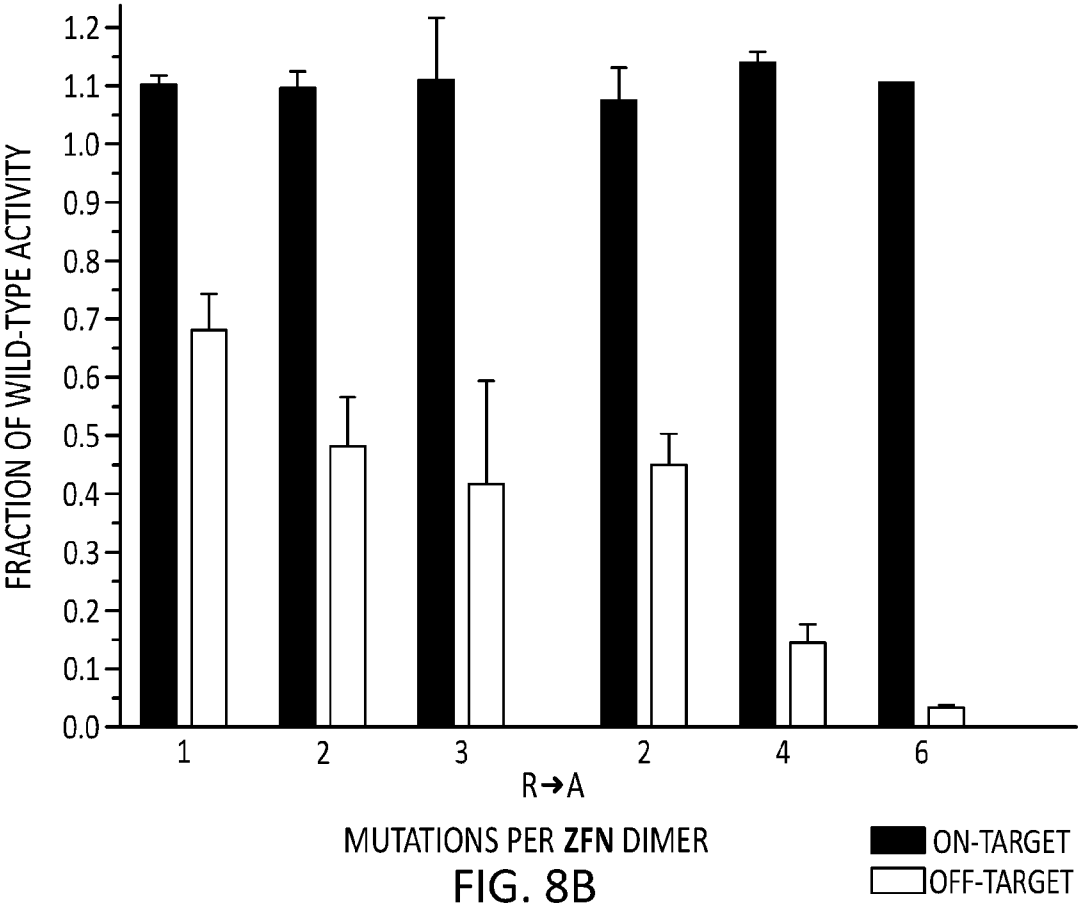

FRACTION OF WILD-TYPE ACTIVITY
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1 2 3 2 4 6
R→A
MUTATIONS PER ZFN DIMER
ON-TARGET
OFF-TARGET

FIG. 8B

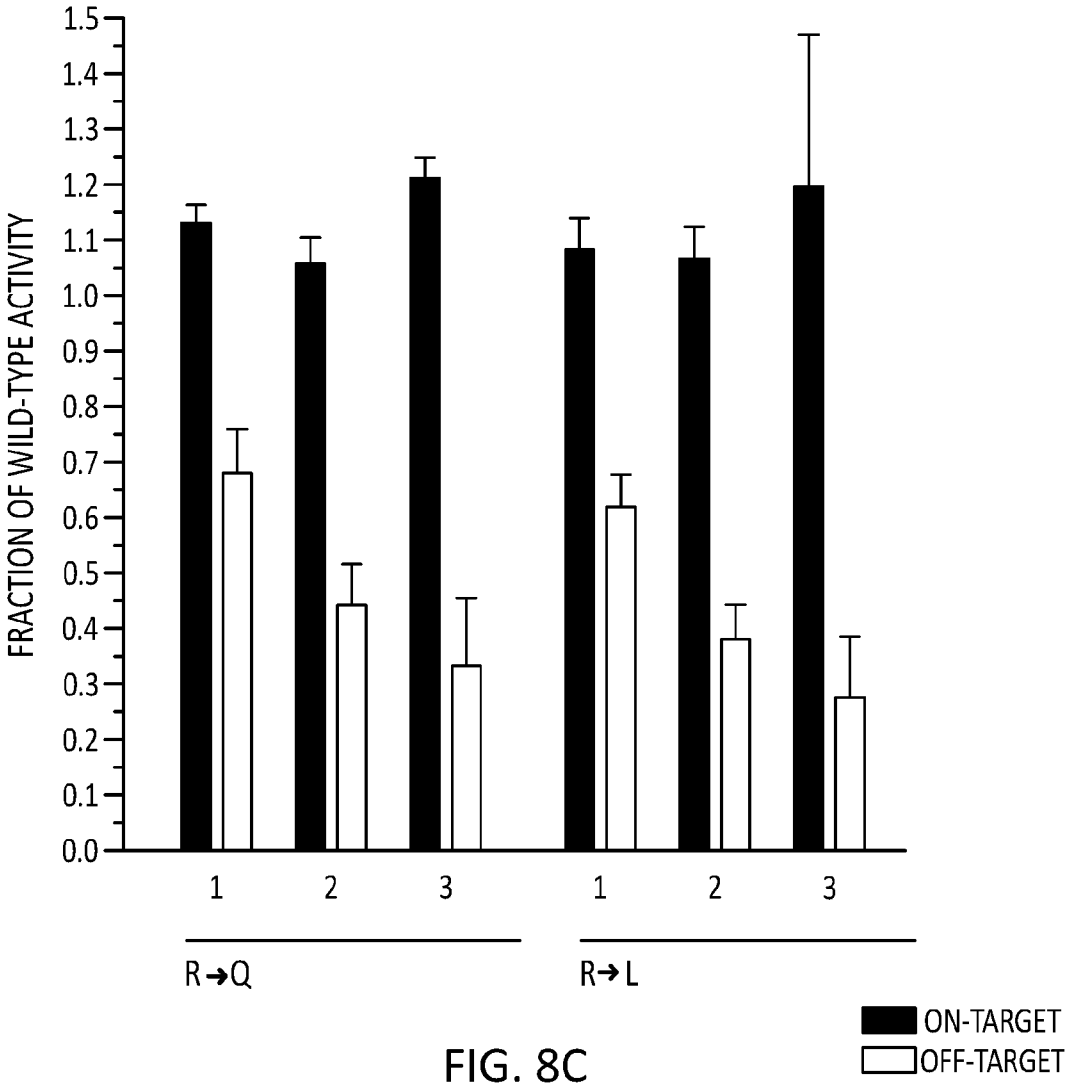

FRACTION OF WILD-TYPE ACTIVITY
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
1
2
3
R→Q
R→L
ON-TARGET
OFF-TARGET

FIG. 8C

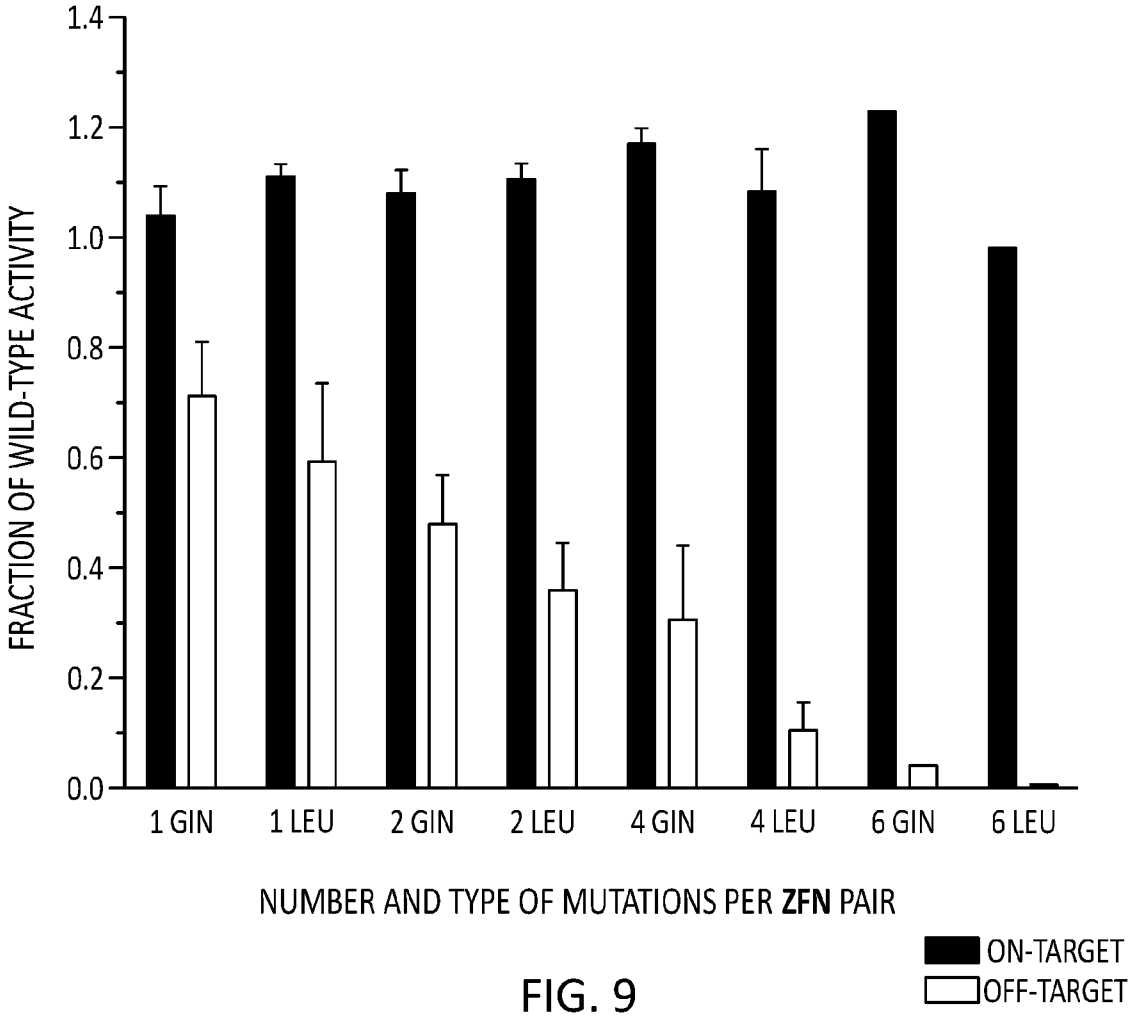

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
FRACTION OF WILD-TYPE ACTIVITY
1 GIN
1 LEU
2 GIN
2 LEU
4 GIN
4 LEU
6 GIN
6 LEU
NUMBER AND TYPE OF MUTATIONS PER ZFN PAIR
ON-TARGET
OFF-TARGET

FIG. 9

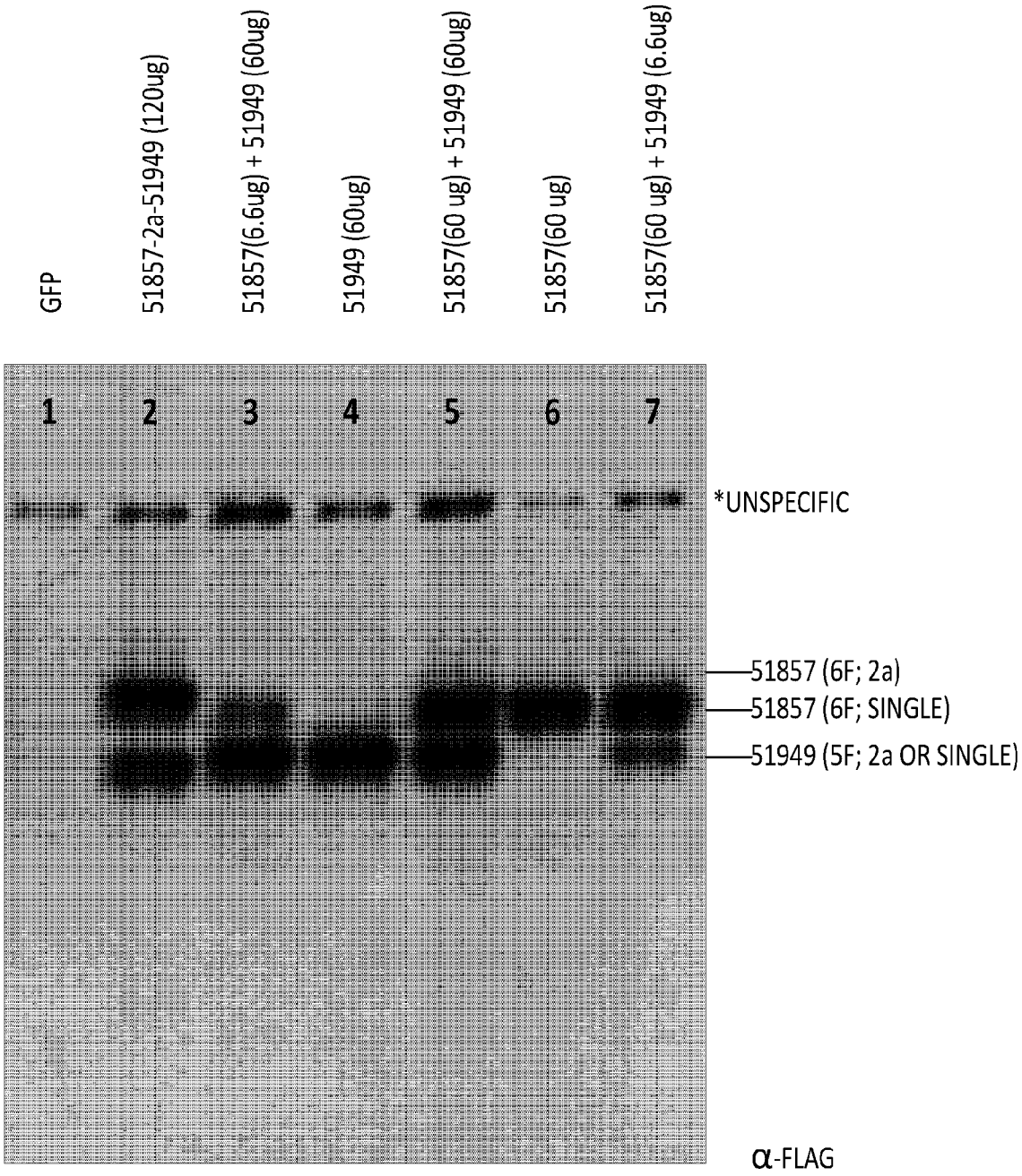

GFP
51857-2a-51949 (120ug)
51857(6.6ug) + 51949 (60ug)
51949 (60ug)
51857(60 ug) + 51949 (60ug)
51857(60 ug)
51857(60 ug) + 51949 (6.6ug)
1
2
3
4
5
6
7
*UNSPECIFIC
51857 (6F; 2a)
51857 (6F; SINGLE)
51949 (5F; 2a OR SINGLE)
α-FLAG

FIG. 10

BCL11A
51857

ug/ml	0	6.6	13	20	30	40	60	90
0	0.43							
6.6							71.59	
13						80.40		
20							83.00	
30								81.32
40			87.22			86.58		
60		86.42		85.50			85.92	
90					86.96			82.27

51949

NIFMAEVG
51857

	0	6.6	13	20	30	40	60	90
0	0.07							
6.6							5.63	
13						9.92		
20							16.97	
30								25.33
40			7.81			22.16		
60		4.21		12.12			27.34	
90					19.64			36.86

51949

CONCENTRATION 51949

CONCENTRATION 51857

CLEAVAGE ACTIVITY (%NHEJ)

FIG. 11

NAME	SEQ ID No.		51857/51949 2a ZFN	51857/51949 2a CONTROL	51857/51949 R416S ZFN	51857/51949 R416S CONTROL	RATIO
PRJIYLFN	13	PRJIYLFN	53.24	0.17	61.54	0.22	0.87
NIFMAEVG	14	NIFMAEVG	18.20	0.06	0.58	0.04	31.21
GJZEIYTO	15	GJZEIYTO	1.87	0.06	0.15	0.07	12.76
PEVYOHIU	20	PEVYOHIU	1.43	0.07	0.10	0.10	14.68
QYYPVRQWMN	51	QYYPVRQWMN	1.18	0.10	0.09	0.08	12.51
ZJCRPAXW	23	ZJCRPAXW	0.93	0.06	0.20	0.05	4.53
RFGIYSHZ	16	RFGIYSHZ	0.90	0.10	0.03	0.13	27.39
XCVJFHOB	18	XCVJFHOB	0.83	0.08	0.13	0.08	6.58
ZMIYSTJN	19	ZMIYSTJN	0.81	0.05	0.11	0.04	7.27
WOEIMTLA	36	WOEIMTLA	0.65	0.09	0.07	0.09	8.88
QRMXFJNY	21	QRMXFJNY	0.60	0.08	0.08	0.09	7.76
EMQNZDWX	25	EMQNZDWX	0.59	0.32	0.13	0.27	4.54
DYNFTSUP	46	DYNFTSUP	0.57	0.03	0.06	0.05	9.41
IRSUAEVF	40	IRSUAEVF	0.55	0.11	0.10	0.11	5.24
RFTBCWPJ	17	RFTBCWPJ	0.52	0.07	0.09	0.09	6.02
TXHULGPN	45	TXHULGPN	0.47	0.08	0.08	0.06	5.96
ZTIFQRKB	48	ZTIFQRKB	0.46	0.11	0.11	0.10	4.02
XFDECBQY	47	XFDECBQY	0.37	0.09	0.08	0.08	4.86
WBEAFQRU	43	WBEAFQRU	0.28	0.07	0.04	0.08	6.39
EKYJXRAHKV	52	EKYJXRAHKV	0.24	0.09	0.04	0.06	5.52
TBUYJIVX	37	TBUYJIVX	0.19	0.07	0.04	0.10	5.12
SIJYTMVG	31	SIJYTMVG	0.19	0.11	0.07	0.12	2.77
AKRHSGPT	39	AKRHSGPT	0.15	0.07	0.03	0.06	4.90
RSYQNPLG	30	RSYQNPLG	0.14	0.27	0.14	0.25	0.99
HBXGRVYT	27	HBXGRVYT	0.12	0.06	0.05	0.07	2.60
ATWDXHSC	41	ATWDXHSC	0.11	0.05	0.08	0.05	1.48
ZLRCYHDF	28	ZLRCYHDF	0.11	0.08	0.04	0.13	3.01
RXDOKCGI	44	RXDOKCGI	0.11	0.05	0.04	0.07	2.46
OTHMRBJL	29	OTHMRBJL	0.10	0.05	0.04	0.05	2.48
QDMBZRWI	42	QDMBZRWI	0.09	0.07	0.03	0.08	3.47
RDEWOSIT	33	RDEWOSIT	0.09	0.06	0.03	0.06	3.15
BCGJKHUV	32	BCGJKHUV	0.09	0.08	0.06	0.08	1.37
LXAFJWRI	24	LXAFJWRI	0.09	0.07	0.10	0.09	0.89
YPLXMQCB	34	YPLXMQCB	0.09	0.06	0.06	0.03	1.44
ZFUQTLMTFC	49	ZFUQTLMTFC	0.07	0.03	0.03	0.04	2.45
QBFUYVGW	22	QBFUYVGW	0.07	0.10	0.04	0.11	1.80
LPRCNGHU	38	LPRCNGHU	0.06	0.08	0.03	0.06	1.99
YJRBCUNZ	26	YJRBCUNZ	0.05	0.05	0.04	0.06	1.38
TPHSLVEM	35	TPHSLVEM	0.05	0.04	0.02	0.03	2.69
ZCNLWTPH	50	ZCNLWTPH	0.04	0.08	0.03	0.07	1.31
ADWZQXZI	53	ADWZQXZI	0.02	0.04	ND	ND	ND

51857/51949 2a: 120 µg mRNA

51857/51949 R416S: 60 µg 51949-R416S, 6.6 µg 51857

FIG. 12

FIG. 13

PARENTAL ZFN PAIR (SBS51857/SBS51949)

LOCUS				% INDELS, CD34 CELLS	
CHROMO-SOME	BASE	#CAPTURE EVENTS	ug left ZFN ug right ZFN	60 60	6.6 60
2	60495268	2616		82.23	82.16
8	119856442	2202		11.74	1.26
4	7829724	214		0.41	NS
12	132241502	207		ND	ND
2	62164812	179		2.17	0.31
1	54506812	162		0.83	0.19
2	23702832	133		0.97	0.08
10	132654830	124		1.04	0.25
14	67422056	116		0.86	0.06
17	29624230	101		0.48	0.05
10	69571178	72		1.54	0.31
13	75549350	72		0.72	0.13
16	15141542	69		NS	NS
16	67184174	69		ND	ND
16	2122340	68		NS	NS
14	73740924	66		0.18	NS
X	134914730	66		0.39	0.06
6	53805954	65		4.04	0.49
1	29182166	59		0.20	NS
20	37707466	53		0.62	0.07
10	101133378	50		1.12	0.07
12	124995298	49		0.08	NS
7	131503656	46		0.13	NS
8	29495582	44		0.05	0.04
21	33308802	41		0.19	NS
8	94988046	40		0.25	NS
19	45406684	39		ND	ND
OFF TARGET LOCI W/SIGNIFICANT INDELS:				21	13
AGGREGATE % OFF TARGET INDELS:				28.0%	3.4%

VARIANT ZFN PAIR (SBS63014/SBS65721)

LOCUS				% INDELS, CD34 CELLS	
CHROMO-SOME	BASE	#CAPTURE EVENTS	ug left ZFN ug right ZFN	60 60	20 60
2	60495270	7372		82.87	80.21
8	119856444	157		0.06	NS
16	67184174	71		ND	ND
2	23702834	65		0.04	NS
17	29624236	60		NS	0.03
10	69789196	38		NS	NS
2	60494824	36		NS	NS
X	66004392	34		0.08	NS
10	132654830	33		0.07	NS
8	29495582	33		NS	NS
4	7829724	29		NS	0.05
21	36901820	27		NS	NS
21	46423666	20		ND	ND
16	88559110	19		NS	NS
19	51327818	19		NS	NS
6	169090708	18		NS	NS
1	134914730	17		NS	NS
5	54506812	17		NS	NS
9	91430050	17		NS	NS
1	612400	17		ND	ND
1	612500	17		ND	ND
1	107771554	16		NS	NS
11	70518296	15		NS	NS
20	48636010	15		NS	NS
8	94988046	15		NS	NS
1	26938300	15		NS	NS
8	66165666	15		NS	NS
				4	2
				0.26%	0.08%

FIG. 14

CHROMO-SOME	BASE #	INTEGRANT COUNT	% INDELS		PVAL IF <0.05
			ZFN	CONTROL	
2	60495266	1893	79.54	0.11	<0.00001
8	119856440	85	0.15	0.1	NS
3	184689238	33	0.07	0.07	NS
10	132654832	16	0.07	0.06	NS
11	108224088	13	0.03	0.02	NS
16	67184174	12	ND1	ND1	
1	223123858	12	0.02	0.05	NS
2	23702834	12	0.07	0.05	NS
1	29182168	10	ND1	ND1	
20	48636010	9	0.04	0.05	NS
1	204975770	9	0.02	0.05	NS
2	62164816	9	0.0	0.04	NS
1	116494398	9	0.06	0.01	NS
16	34590356	9	ND1	ND1	
8	94988044	8	0.11	0.03	NS
1	21635648	7	0.06	0.02	NS
19	51327822	7	ND1	ND1	
4	7829724	7	0.04	0.06	NS
X	66004390	7	0.12	0.04	NS
10	91591624	7	0.07	0.02	NS
16	15220090	7	0.13	0.11	NS
6	28540486	7	0.02	0.05	NS
9	98755464	7	0.06	0.05	NS
14	73740924	6	ND1	ND1	
10	69789200	6	0.0	0.02	NS
16	18131716	6	ND1	ND1	
18	45431502	6	0.05	0.06	NS
4	59602300	6	0.24	0.21	NS
18	2920000	6	0.07	0.02	NS
2	96873180	5	0.09	0.06	NS
17	721818	5	0.04	0.04	NS
2	112583538	5	0.03	0.07	NS
2	169268666	5	ND1	ND1	NS
8	26449836	5	ND1	ND1	
8	29495584	5	0.03	0.12	
11	10961236	5	0.04	0.04	NS
16	46386624	5	0.22	0.24	NS
16	88559110	5	0.08	0.05	NS
2	164741410	5	0.03	0.01	NS
5	176402584	5	0.08	0.06	NS
5	95714316	5	0.03	0.03	NS
6	84224380	5	0.0	0.13	NS
7	106878380	5	0.03	0.06	NS
9	37906562	5	0.19	0.18	NS
1	211655464	4	0.02	0.07	NS
13	75549352	4	0.04	0.05	NS
14	49586826	4	0.02	0.06	NS
16	46399565	4	0.09	0.08	NS
1	154649476	4	0.1	0.09	NS
10	21001996	4	0.08	0.18	NS
13	79775980	4	ND1	ND1	
15	82322852	4	0.0	0.05	NS
15	82322918	4	0.02	0.28	NS
15	85267112	4	0.09	0.12	NS
15	85267176	4	0.05	0.03	NS
16	34586904	4	ND2	ND2	
17	21016474	4	0.03	0.04	NS
19	14243018	4	ND1	ND1	
2	173593302	4	0.04	0.1	NS

NS: PVALUE > 0.05 | ND1: NO DATA – LOCUS DID NOT AMPLIFY OR DID NOT SEQUENCE | ND2: NO DATA – BACKGROUND HIGH (>1%) OR SEQUENCING DEPTH INSUFFICIENT (<1000 READS)

SUBSTITUTE SHEET (RULE 26)

	FULL DOSE			HALF DOSE		
ON	29.64	30.09	29.29	20.23	19.34	21.01
RATIO	4.49	3.88	4.23	7.96	9.25	9.62

PARENTALS

FIG. 15A

	R416E	S418E	N476D	I479T	Q481A	Q481E	K525S
ON	46.99	ND	44.56	30.54	41.21	40.57	39.15
RATIO	15.03	ND	137.79	88.36	133.38	139.98	44.95

	R416E	S418E	N476D	I479T	Q481A	Q481E	K525S
ON	41.76	ND	20.04	36.62	36.52	37.26	35.00
RATIO	35.99	ND	78.85	45.83	113.98	137.34	22.10

	R416E	S418E	N476D	I479T	Q481A	Q481E	K525S
ON	61.25	ND	4.22	26.01	70.60	38.76	42.82
RATIO	138.11	ND	36.30	153.83	383.30	221.20	299.99

SINGLE MUTANTS

FIG. 15B

	R416E	S418E	N476D	I479T	Q481A	Q481E	K525S
R416E	NA	20.74	40.36	21.55	2.83	41.39	34.38
S418E	20.74	NA	31.37	11.14	3.20	38.73	24.79
N476D	40.36	31.37	NA	20.03	12.76	50.65	42.41
I479T	21.55	11.14	20.03	NA	8.92	35.78	25.90
Q481A	2.83	3.20	12.76	8.92	NA	ND	15.18
Q481E	41.39	38.73	50.65	35.78	ND	NA	48.15
K525S	34.38	24.79	42.41	25.90	15.18	48.15	NA

	R416E	S418E	N476D	I479T	Q481A	Q481E	K525S
R416E	NA	20.09	20.88	42.32	0.05	31.75	30.56
S418E	20.09	NA	2.68	35.49	1.55	17.90	10.28
N476D	20.88	2.68	NA	5.09	4.44	3.08	8.06
I479T	42.32	35.49	5.09	NA	25.89	35.07	37.19
Q481A	0.05	1.55	4.44	25.89	NA	ND	4.99
Q481E	31.75	17.90	3.08	35.07	ND	NA	24.26
K525S	30.56	10.28	8.06	37.19	4.99	24.26	NA

	R416E	S418E	N476D	I479T	Q481A	Q481E	K525S
R416E	NA	1.12	0.08	9.58	0.04	15.79	18.64
S418E	1.12	NA	0.03	0.07	0.06	0.25	0.04
N476D	0.08	0.03	NA	0.01	0.04	0.04	0.03
I479T	9.58	0.07	0.01	NA	1.92	1.71	0.87
Q481A	0.04	0.06	0.04	1.92	NA	ND	2.27
Q481E	15.79	0.25	0.04	1.71	ND	NA	1.58
K525S	18.64	0.04	0.03	0.87	2.27	1.58	NA

DOUBLE MUTANT GRID ON-TARGET

FIG. 15C

	R416E	S418E	N476D	I479T	Q481A	Q481E	K525S
R416E	NA	94.13	213.54	61.74	19.59	225.41	166.24
S418E	94.13	NA	221.16	62.37	16.08	400.31	160.35
N476D	213.54	221.16	NA	180.94	88.57	242.53	270.45
I479T	61.74	62.37	180.94	NA	72.43	196.60	139.72
Q481A	19.59	16.08	88.57	72.43	NA	ND	67.80
Q481E	225.41	400.31	242.53	196.60	ND	NA	274.23
K525S	166.24	160.35	270.45	139.72	67.80	274.23	NA

	R416E	S418E	N476D	I479T	Q481A	Q481E	K525S
R416E	NA	151.13	121.14	220.35	0.28	163.74	146.79
S418E	151.13	NA	15.27	234.26	9.60	135.16	55.61
N476D	121.14	15.27	NA	26.81	22.35	17.28	52.10
I479T	220.35	234.26	26.81	NA	204.61	218.88	151.36
Q481A	0.28	9.60	22.35	204.61	NA	ND	36.01
Q481E	163.74	135.16	17.28	218.88	ND	NA	142.63
K525S	146.79	55.61	52.10	151.36	36.01	142.63	NA

	R416E	S418E	N476D	I479T	Q481A	Q481E	K525S
R416E	NA	5.75	0.90	93.64	0.34	146.87	110.61
S418E	5.75	NA	0.19	0.39	0.39	1.60	0.28
N476D	0.90	0.19	NA	0.06	0.60	0.22	0.19
I479T	93.64	0.39	0.06	NA	14.25	11.29	6.00
Q481A	0.34	0.39	0.60	14.25	NA	ND	12.73
Q481E	146.87	1.60	0.22	11.29	ND	NA	10.24
K525S	110.61	0.28	0.19	6.00	12.73	10.24	NA

DOUBLE MUTANT GRID ON-OFF RATIO

FIG. 15D

KKR

R416D	K448A	R422H	I479Q	K525A	N527D
ND	46.84	36.93	16.28	25.31	29.19
ND	45.64	ND	11.10	18.22	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
0.98	35.37	15.51	4.15	8.65	15.47
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
NA	33.83	ND	4.00	5.58	ND
33.83	NA	ND	51.54	43.95	32.41
ND	ND	NA	31.90	31.01	ND
4.00	51.54	31.90	NA	27.87	ND
5.58	43.95	31.01	27.87	NA	23.46
ND	32.41	ND	ND	23.46	NA

R416D	K448A	R422H	I479Q	K525A	N527D
ND	22.04	56.30	129.05	171.87	103.79
ND	125.00	ND	60.83	132.51	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
6.23	173.76	84.46	28.92	65.76	72.82
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
NA	54.89	ND	24.24	26.88	ND
54.89	NA	ND	421.61	65.65	32.41
ND	ND	NA	232.64	152.49	ND
24.24	421.61	232.64	NA	252.87	ND
26.88	65.65	152.49	252.87	NA	116.95
ND	32.34	ND	ND	116.95	NA

ELD

R416D	K448A	R422H	I479Q	K525A	N527D
ND	46.84	36.93	16.28	25.31	29.19
ND	45.64	ND	11.10	18.22	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
0.98	35.37	15.51	4.15	8.65	15.47
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
NA	33.83	ND	4.00	5.58	ND
33.83	NA	ND	51.54	43.95	32.41
ND	ND	NA	31.90	31.01	ND
4.00	51.54	31.90	NA	27.87	ND
5.58	43.95	31.01	27.87	NA	23.46
ND	32.41	ND	ND	23.46	NA

R416D	K448A	R422H	I479Q	K525A	N527D
ND	22.04	56.30	129.05	171.87	103.79
ND	125.00	ND	60.83	132.51	ND
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
6.23	173.76	84.46	28.92	65.76	72.82
ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND
NA	54.89	ND	24.24	26.88	ND
54.89	NA	ND	421.61	65.65	32.34
ND	ND	NA	232.64	152.49	ND
24.24	421.61	232.64	NA	252.87	ND
26.88	65.65	152.49	252.87	NA	116.95
ND	32.34	ND	ND	116.95	NA

FIG. 16A

	R416E	S418E	N476D	I479T	Q481A	Q481E	K525S	R416D	K448A	R422H	I479Q	K525A	N527D
R416E	NA	20.09	20.88	42.32	0.05	31.75	30.56	ND	43.43	39.63	40.55	23.98	40.12
S418E	20.09	NA	2.68	35.49	1.55	17.90	10.28	ND	36.67	ND	36.46	6.28	ND
N476D	20.88	2.68	NA	5.09	4.44	3.08	8.06	ND	ND	ND	ND	ND	ND
I479T	42.32	35.49	5.09	NA	25.89	35.07	37.19	ND	ND	ND	ND	ND	ND
Q481A	0.05	1.55	4.44	25.89	NA	ND	4.99	1.51	19.81	12.81	31.65	3.09	ND
Q481E	31.75	17.90	3.08	35.07	ND	NA	24.26	ND	ND	ND	ND	ND	ND
K525S	30.56	10.28	8.06	37.19	4.99	24.26	NA	ND	ND	ND	ND	ND	ND
R416D	ND	ND	ND	ND	1.51	ND	ND	NA	34.82	ND	37.53	8.83	ND
K448A	43.43	36.67	ND	ND	19.81	ND	ND	34.82	NA	ND	35.23	36.25	29.74
R422H	39.63	ND	ND	ND	12.81	ND	ND	ND	ND	NA	35.05	31.03	ND
I479Q	40.55	36.46	ND	ND	31.65	ND	ND	37.53	35.23	35.05	NA	37.06	ND
K525A	23.98	6.28	ND	ND	3.09	ND	ND	8.83	36.25	31.03	37.06	NA	ND
N527D	40.12	ND	ND	ND	ND	ND	ND	ND	29.74	ND	ND	ND	NA

	R416E	S418E	N476D	I479T	Q481A	Q481E	K525S	R416D	K448A	R422H	I479Q	K525A	N527D
R416E	NA	151.13	121.14	220.35	0.28	163.74	146.79	ND	106.38	122.95	140.11	112.67	141.70
S418E	151.13	NA	15.27	234.26	9.60	135.16	55.61	ND	158.02	ND	173.06	36.37	ND
N476D	121.14	15.27	NA	26.81	22.35	17.28	52.10	ND	ND	ND	ND	ND	ND
I479T	220.35	234.26	26.81	NA	204.61	218.88	151.36	ND	ND	ND	ND	ND	ND
Q481A	0.28	9.60	22.35	204.61	NA	ND	36.01	10.67	116.04	64.84	128.89	21.23	ND
Q481E	163.74	135.16	17.28	218.88	ND	NA	142.63	ND	ND	ND	ND	ND	ND
K525S	146.79	55.61	52.10	151.36	36.01	142.63	NA	ND	ND	ND	ND	ND	ND
R416D	ND	ND	ND	ND	10.67	ND	ND	NA	160.49	ND	229.85	40.27	ND
K448A	106.38	158.02	ND	ND	116.04	ND	ND	160.49	NA	ND	74.29	183.47	55.29
R422H	122.95	ND	ND	ND	64.84	ND	ND	ND	ND	NA	66.28	126.26	ND
I479Q	140.11	173.06	ND	ND	128.89	ND	ND	229.85	74.29	66.28	NA	196.46	ND
K525A	112.67	36.37	ND	ND	21.23	ND	ND	40.27	183.47	126.26	196.46	NA	ND
N527D	141.70	ND	ND	ND	ND	ND	ND	ND	55.29	ND	ND	ND	NA

	R416E	S418E	N476D	I479T	Q481A	Q481E	K525S	R416D	K448A	R422H	I479Q	K525A	N527D	ON-TARGET	R416D
R416E	NA	1.12	0.08	9.58	0.04	15.79	18.64	ND	65.65	51.26	9.45	8.87	8.77		ND
S418E	1.12	NA	0.03	0.07	0.06	0.25	0.04	ND	12.39	ND	0.17	0.07	ND		ND
N476D	0.08	0.03	NA	0.01	0.04	0.04	0.03	ND	ND	ND	ND	ND	ND		ND
I479T	9.58	0.07	0.01	NA	1.92	1.71	0.87	ND	ND	ND	ND	ND	ND		ND
Q481A	0.04	0.06	0.04	1.92	NA	ND	2.27	0.72	52.80	17.21	1.71	0.52	ND		11.44
Q481E	15.79	0.25	0.04	1.71	ND	NA	1.58	ND	ND	ND	ND	ND	ND		ND
K525S	18.64	0.04	0.03	0.87	2.27	1.58	NA	ND	ND	ND	ND	ND	ND		ND
R416D	ND	ND	ND	ND	0.72	ND	ND	NA	34.69	ND	0.64	0.44	ND		NA
K448A	65.65	12.39	ND	ND	52.80	ND	ND	34.69	NA	ND	33.17	31.94	12.12		34.83
R422H	51.26	ND	ND	ND	17.21	ND	ND	ND	ND	NA	6.81	9.57	ND		ND
I479Q	9.45	0.17	ND	ND	1.71	ND	ND	0.64	33.17	6.81	NA	1.27	ND		57.05
K525A	8.87	0.07	ND	ND	0.52	ND	ND	0.44	31.94	9.57	1.27	NA	ND		40.59
N527D	8.77	ND	ND	ND	ND	ND	ND	ND	12.12	ND	ND	ND	NA		ND

	R416E	S418E	N476D	I479T	Q481A	Q481E	K525S	R416D	K448A	R422H	I479Q	K525A	N527D	ON-OFF RATIO	R416D
R416E	NA	5.75	0.90	93.64	0.34	146.87	110.61	ND	296.99	241.20	69.73	50.16	65.60		ND
S418E	5.75	NA	0.19	0.39	0.39	1.60	0.28	ND	105.55	ND	0.68	0.57	ND		ND
N476D	0.90	0.19	NA	0.06	0.60	0.22	0.19	ND	ND	ND	ND	ND	ND		ND
I479T	93.64	0.39	0.06	NA	14.25	11.29	6.00	ND	ND	ND	ND	ND	ND		ND
Q481A	0.34	0.39	0.60	14.25	NA	ND	12.73	3.74	351.52	97.54	10.33	5.01	ND		67.33
Q481E	146.87	1.60	0.22	11.29	ND	NA	10.24	ND	ND	ND	ND	ND	ND		ND
K525S	110.61	0.28	0.19	6.00	12.73	10.24	NA	ND	ND	ND	ND	ND	ND		ND
R416D	ND	ND	ND	ND	3.74	ND	ND	NA	171.10	ND	2.97	4.38	ND		NA
K448A	296.99	105.55	ND	ND	351.52	ND	ND	171.10	NA	ND	209.50	164.22	70.27		55.89
R422H	241.20	ND	ND	ND	97.54	ND	ND	ND	ND	NA	39.89	68.82	ND		ND
I479Q	69.73	0.68	ND	ND	10.33	ND	ND	2.97	209.50	39.89	NA	5.70	ND		434.56
K525A	50.16	0.57	ND	ND	5.01	ND	ND	4.38	164.22	68.82	5.70	NA	ND		315.67
N527D	65.60	ND	ND	ND	ND	ND	ND	ND	70.27	ND	ND	ND	NA		ND

FIG. 16B

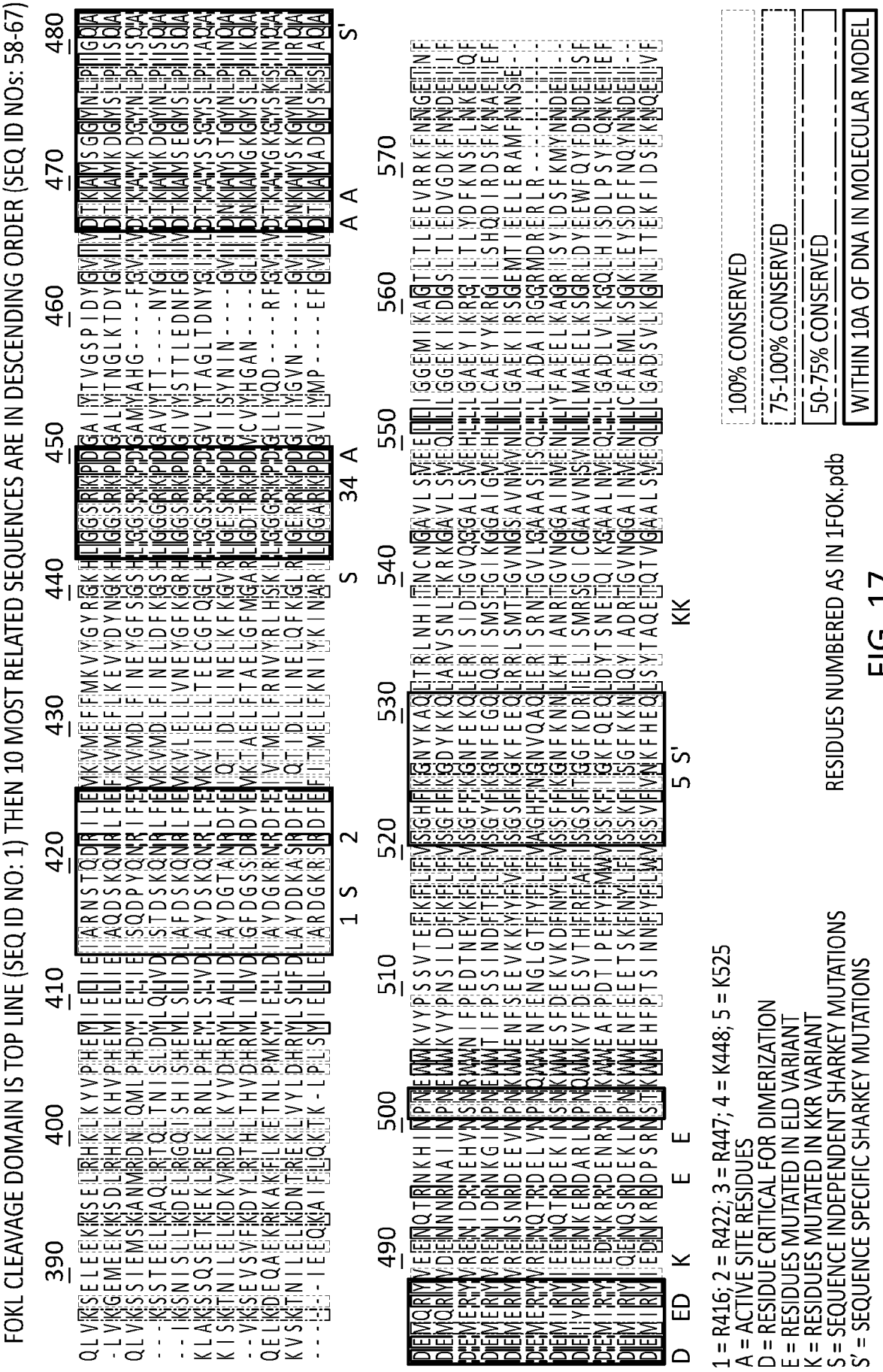

FOKL CLEAVAGE DOMAIN ALIGNED WITH HOMOLOGS
FOKL CLEAVAGE DOMAIN IS TOP LINE (SEQ ID NO: 1) THEN 10 MOST RELATED SEQUENCES ARE IN DESCENDING ORDER (SEQ ID NOs: 58-67)
1 = R416; 2 = R422; 3 = R447; 4 = K448; 5 = K525
A = ACTIVE SITE RESIDUES
D = RESIDUE CRITICAL FOR DIMERIZATION
E = RESIDUES MUTATED IN ELD VARIANT
K = RESIDUES MUTATED IN KKR VARIANT
S = SEQUENCE INDEPENDENT SHARKEY MUTATIONS
S' = SEQUENCE SPECIFIC SHARKEY MUTATIONS
RESIDUES NUMBERED AS IN 1FOK.pdb
100% CONSERVED
75-100% CONSERVED
50-75% CONSERVED
WITHIN 10A OF DNA IN MOLECULAR MODEL

FIG. 17

POSITION	MUTATION
393	K393S
394	K394S
398	R398S
416	R416A
416	R416C
416	R416D
416	R416E
416	R416F
416	R416G
416	R416H
416	R416M
416	R416N
416	R416Q
416	R416S
416	R416T
416	R416W
417	N417D
418	S418A
418	S418D
418	S418E
418	S418I
418	S418Q
418	S418T
418	S418V
419	T419D
419	T419S
421	D421S

POSITION	MUTATION
422	R422A
422	R422D
422	R422E
422	R422G
422	R422H
422	R422L
422	R422N
422	R422Q
422	R422S
422	R422T
422	R422V
422	R416W
423	I423L
425	E425Q
426	M426I
442	H442R
446	S446D
446	S446G
446	S446R
447	R447A
447	R447Q
447	R447S
448	K448A
448	K448C
448	K448E
448	K448Q
448	K448R

POSITION	MUTATION
448	K448S
471	Y471F
472	S472D
472	S472K
473	G473K
476	N476A
476	N476C
476	N476D
476	N476E
476	N476G
476	N476I
476	N476K
476	N476Q
476	N476S
476	N476T
476	N476V
478	P478D
478	P478S
479	I479A
479	I479C
479	I479F
479	I479G
479	I479H
479	I479L
479	I479M
479	I479N
479	I479Q

POSITION	MUTATION
479	I479S
479	I479T
479	I479V
479	I479Y
480	G480A
480	G480C
480	G480D
480	G480E
480	G480F
480	G480K
480	G480L
480	G480P
480	G480Q
480	G480S
480	G480W
481	Q481A
481	Q481C
481	Q481D
481	Q481E
481	Q481H
481	Q481M
481	Q481N
481	Q481S
481	Q481T
522	G522S
523	H523E
523	H523F

POSITION	MUTATION
523	H523K
525	K525A
525	K525C
525	K525D
525	K525E
525	K525G
525	K525H
525	K525I
525	K525M
525	K525N
525	K525Q
525	K525S
525	K525T
525	K525V
527	N527T
528	Y528F
530	A530E
530	A530K
531	Q531E
531	Q531I
531	Q531L
531	Q531M
531	Q531T
531	Q531V
531	Q531Y

MUTATION1	MUTATION2
R416D	K448A
R416D	I479Q
R416E	S418E
R416E	R422H
R416E	K448A
R416E	N476D
R416E	I479Q
R416E	I479T
R416E	Q481E
R416E	K525A
R416E	K525S
R416S	K525S
S418E	K448A
S418E	N476D
S418E	I479Q
S418E	I479T
S418E	Q481E
S418E	K525A
S418E	K525S
R422H	I479Q
R422H	Q481A
R422H	K525A
R422S	K525S
K448A	I479Q
K448A	Q481A
K448A	K525A
K448S	K525S
N476D	I479T
N476D	Q481A
N476D	Q481E
N476D	K525S
I479Q	Q481A
I479Q	K525A
I479T	Q481A
I479T	Q481E
I479T	K525S
Q481A	K525S
Q481E	K525S

FIG. 18