D2E7(Adalimumab, HUMIRA) V_H (SEQ ID NO:2)

EVQLVESGGGLVQPGRSLRLSCAASGFTFD**DYAMH**WVRQAPGKGLEWVS**AITWNSGHIDYADSVEG**R
FTISRDNAKNSLYLQMNSLRAEDTAVYYCAK**VSYLSTASSLDY**WGQGTLVTVSS

D2E7 (Adalimumab, HUMIRA) V_L (SEQ ID NO:4)

DIQMTQSPSSLSASVGDRVTITC**RASQGIRNYLA**WYQQKPGKAPKLLIY**AASTLQS**GVPSRFSGSGSGT
DFTLTISSLQPEDVATYYC**QRYNRAPYT**FGQGTKVEIK

FIG. 1A

Antibody Chain	CDR No.	Sequence	SEQ ID NO.
Heavy	1	DYAMH	5
Heavy	2	AITWNSGHIDYADSVEG	6
Heavy	3	VSYLSTASSLDY	7
Light	1	RASQGIRNYLA	8
Light	2	AASTLQS	9
Light	3	QRYNRAPYT	10

FIG. 1B

```
GAGGTGCAGC TGGTGGAGTC TGGGGGAGGC TTGGTACAGC CCGGCAGGTC CTGAGACTC      60
TCCTGTGCGG CCTCTGGATT CACCTTTGAT GATTATGCCA TGCACTGGGT CCGGCAAGCT     120
CCAGGGAAGG GCCTGGAATG GGTCTCAGCT ATCACTTGGA ATAGTGGTCA CATAGACTAT     180
GCGGACTCTG TGGAGGGCCG ATTCACCATC TCCAGAGACA ACGCCAAGAA CTCCCTGTAT     240
CTGCAAATGA ACAGTCTGAG AGCTGAGGAT ACGGCCGTAT ATTACTGTGC GAAAGTCTCG     300
TACCTTAGCA CCGCGTCCTC CCTTGACTAT TGGGGCCAAG GTACCCTGGT CACCGTCTCG     360
AGT                                                                  363
```

Nucleotide Sequence of D2E7 Variable Heavy Chain

```
GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGGGA CAGAGTCACC      60
ATCACTTGTC GGGCAAGTCA GGGCATCAGA AATTACTTAG CCTGGTATCA GCAAAAACCA     120
GGGAAAGCCC CTAAGCTCCT GATCTATGCT GCATCCACTT TGCAATCAGG GGTCCCATCT     180
CGGTTCAGTG GCAGTGGATC TGGGACAGAT TTCACTCTCA CCATCAGCAG CCTACAGCCT     240
GAAGATGTTG CAACTTATTA CTGTCAAAGG TATAACCGTG CACCGTATAC TTTTGGCCAG     300
GGGACCAAGG TGGAAATCAA A                                               321
```

Nucleotide Sequence of D2E7 Variable Light Chain

FIG. 1C

position	kabat	WT amino acid	beneficial mutations in V_L
L1-1	24	R	
L1-2	25	A	
L1-3	26	S	
L1-4	27	Q	
L1-5	28	G	F^{ab}, I^{ab}, V^{b}, W^{bc}, Y^{ab}
L1-6	29	I	
L1-7	30	R	I^{ab}, T^{abc}, V^{abc}
L1-8	31	N	A^{abc}, D^{ac}, E^{ac}, G^{abc}, L^{abc}, M^{abc}, Q^{abc}, R^{abc}, T^{abc}
L1-9	32	Y	
L1-10	33	L	
L1-11	34	A	
L2-1	50	A	I^{bc}, T^{bc}, V^{abc}
L2-2	51	A	
L2-3	52	S	
L2-4	53	T	D^{bc}
L2-5	54	L	
L2-6	55	Q	
L2-7	56	S	
L3-1	89	Q	
L3-2	90	R	G^{a}
L3-3	91	Y	
L3-4	92	N	F^{abc}, M^{ab}, W^{abc}, Y^{c}
L3-5	93	R	L^{a}, N^{a}, W^{ab}, Y^{a}
L3-6	94	A	
L3-7	95	P	
L3-8	96	Y	
L3-9	97	T	Y^{c}

FIG. 2

position	kabat	WT amino acid	beneficial mutations in V_H
H1-1	31	D	S^d
H1-2	32	Y	A^d, C^d, K^d, M^d, R^d, S^d, V^d
H1-3	33	A	
H1-4	34	M	
H1-5	35	H	C^d, D^d, E^d, S^d, T^d
H2-1	50	A	
H2-2	51	I	
H2-3	52	T	A^d, G^d, N^d
H2-4	52a	W	A^d, F^d, H^d, L^d, M^d, V^d
H2-5	53	N	G^e
H2-6	54	S	D^e, L^e
H2-7	55	G	
H2-8	56	H	
H2-9	57	I	K^{ef}
H2-10	58	D	L^d
H2-11	59	Y	A^{ef}, C^{ef}, E^{ef}, F^{ef}, G^{ef}, H^{ef}, I^{ef}, K^{ef}, L^{ef}, M^e, N^{ef}, Q^e, R^{ef}, S^{ef}, V^{ef}, W^{ef},
H2-12	60	A	Y^e
H2-13	61	D	N^f
H2-14	62	S	
H2-15	63	V	D^{ef}, L^{ef}, M^{ef}, Q^{ef}, T^{ef}
H2-16	64	E	F^{ef}, H^e, K^{ef}, R^{ef}, T^{ef}, W^{ef}
H2-17	65	G	A^{ef}, C^{ef}, E^{ef}, H^e, I^{ef}, K^{ef}, L^{ef}, M^{ef}, N^{ef}, P^{ef}, Q^{ef}, R^{ef}, S^{ef}, T^{ef}, Y^{ef}

FIG. 3A

position	kabat	WT amino acid	beneficial mutations in V_H
H3-1	95	V	G^d, R^d, W^d
H3-2	96	S	
H3-3	97	Y	
H3-4	98	L	T^d, V^d
H3-5	99	S	
H3-6	100	T	V^d
H3-7	100a	A	
H3-8	100b	S	
H3-9	100c	S	K^d, W^e, Y^e
H3-10	100d	L	
H3-11	101	D	V^d
H3-12	102	Y	

FIG. 3B

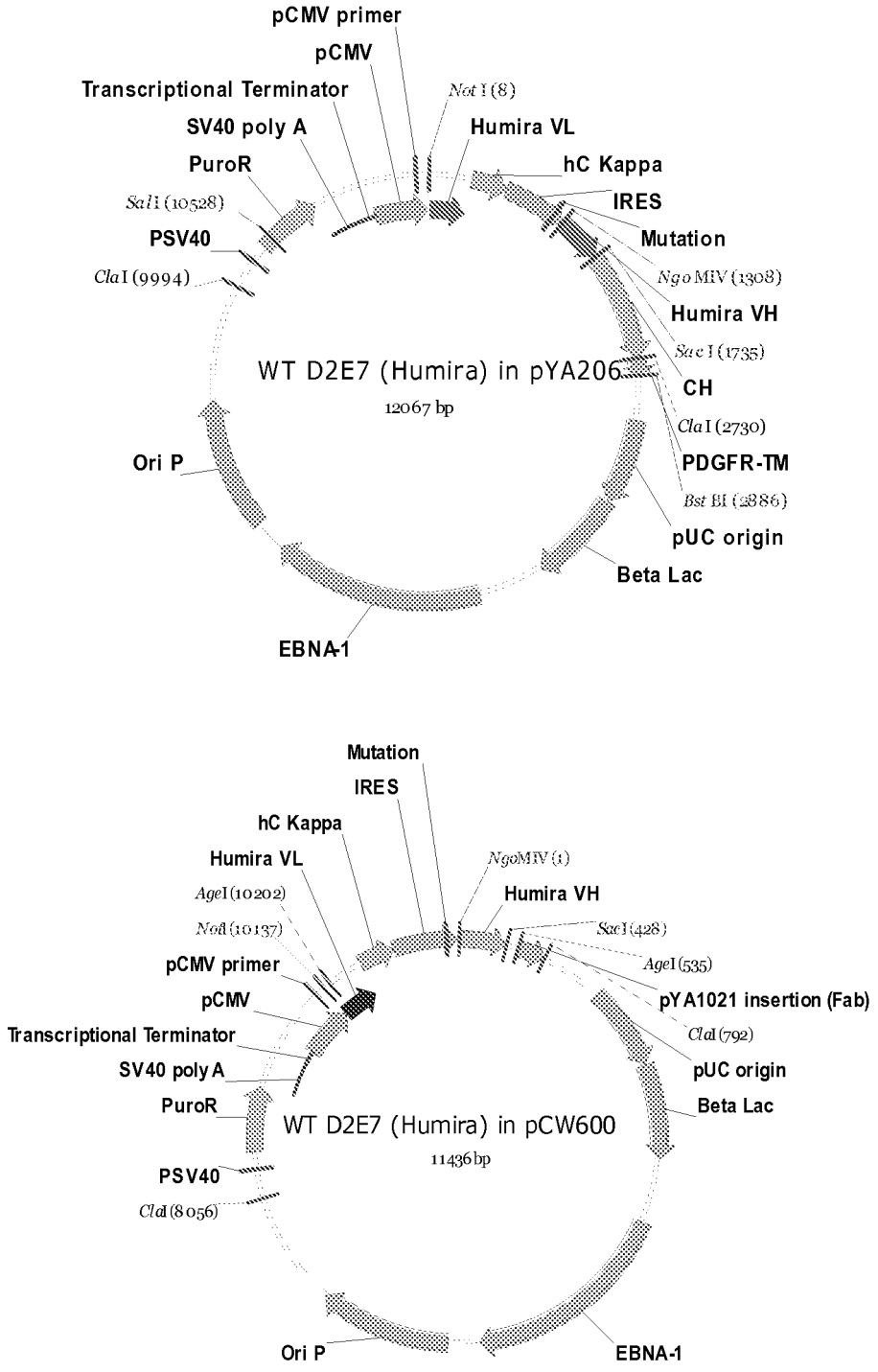

pCMV primer
pCMV
Transcriptional Terminator
SV40 poly A
PuroR
SalI (10528)
PSV40
ClaI (9994)
NotI (8)
Humira VL
hC Kappa
IRES
Mutation
NgoMIV (1308)
Humira VH
SacI (1735)
CH
ClaI (2730)
PDGFR-TM
BstBI (3886)
pUC origin
Beta Lac
EBNA-1
Ori P
WT D2E7 (Humira) in pYA206
12067 bp
Mutation
IRES
hC Kappa
Humira VL
AgeI (10202)
NotI (10137)
pCMV primer
pCMV
Transcriptional Terminator
SV40 poly A
PuroR
PSV40
ClaI (8056)
NgoMIV (1)
Humira VH
SacI (428)
AgeI (535)
pYA1021 insertion (Fab)
ClaI (792)
pUC origin
Beta Lac
EBNA-1
Ori P
WT D2E7 (Humira) in pCW600
11436 bp

FIG. 4

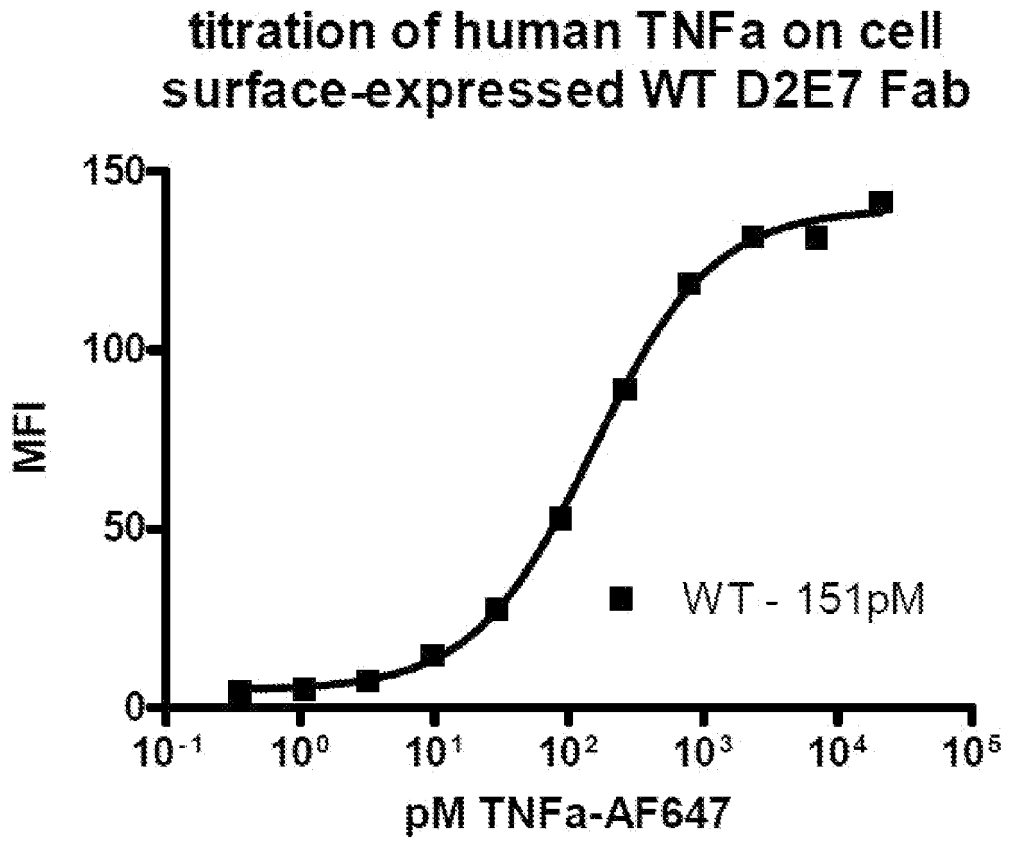

titration of human TNFa on cell surface-expressed WT D2E7 Fab
MFI
150
100
50
0
WT - 151pM
10-1
100
101
102
103
104
105
pM TNFa-AF647

FIG. 5

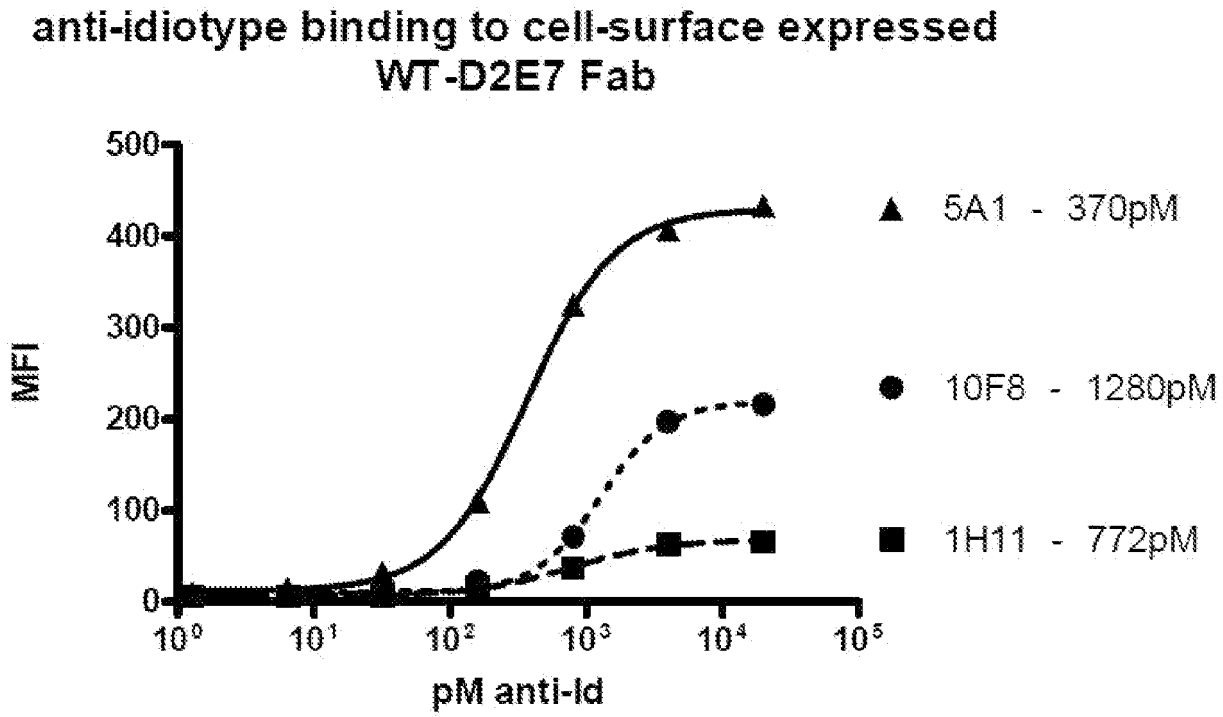

anti-idiotype binding to cell-surface expressed
WT-D2E7 Fab
MFI
500
400
300
200
100
0
10^0
10^1
10^2
10^3
10^4
10^5
pM anti-Id
5A1 - 370pM
10F8 - 1280pM
1H11 - 772pM

FIG. 6

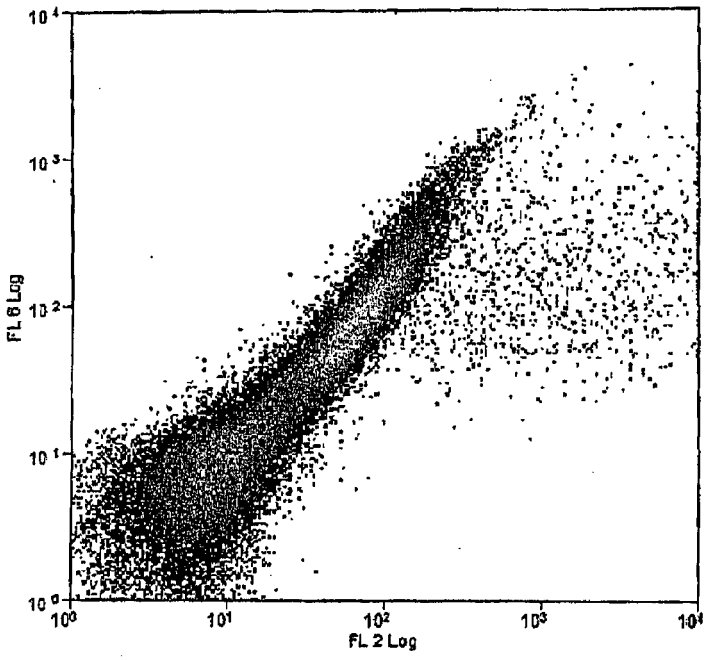


FIG. 7A

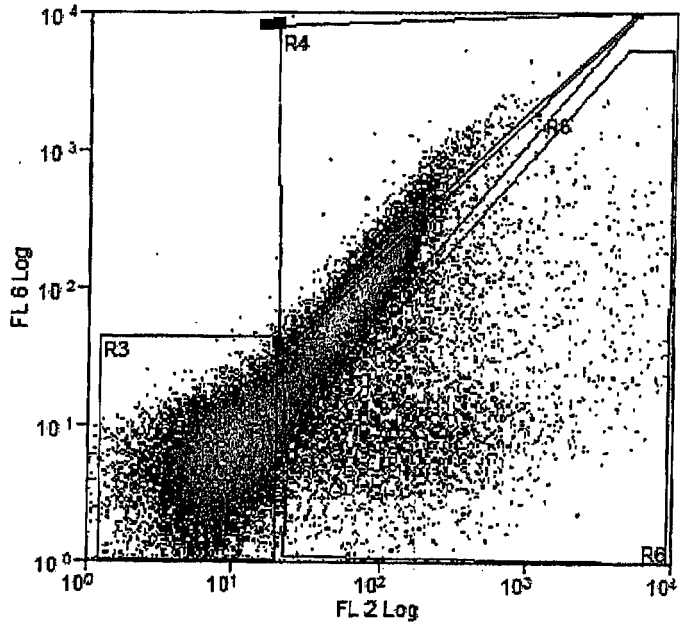


FIG. 7B

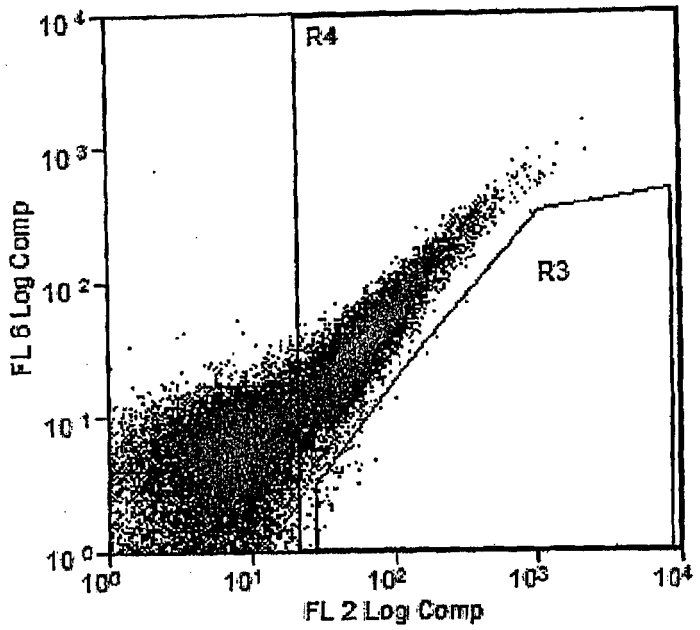

R4
R3
FL 6 Log Comp
FL 2 Log Comp
10 0
10 1
10 2
10 3
10 4

FIG. 8A

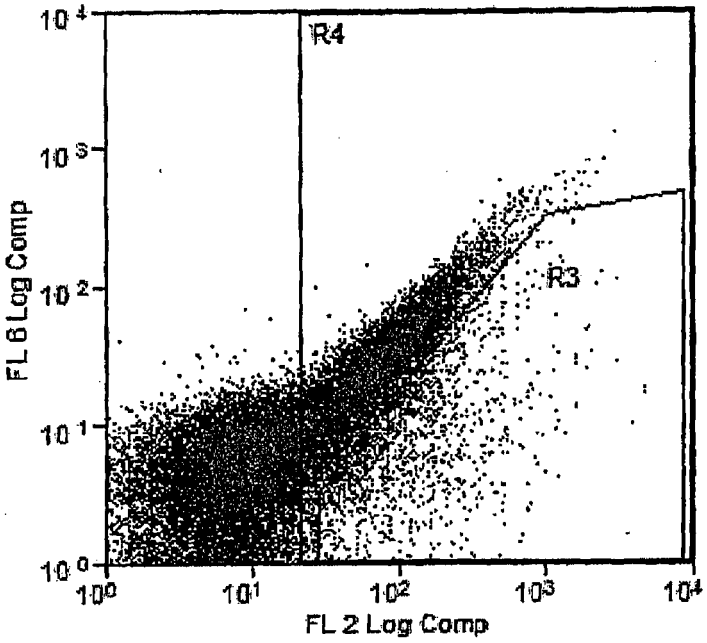

R4
R3
FL 6 Log Comp
FL 2 Log Comp
10 0
10 1
10 2
10 3
10 4

FIG. 8B

WT-AA	WT	AA	Mutant	CDR	position		1H11 ER	TNF-α-ER
D	GAC	D	GAT	CDR-H1	1		0.29	1.47
A	GCC	A	GCG	CDR-H1	3		0.06	1.64
A	GCC	A	GCT	CDR-H1	3		0.4	1.33
H	CAC	H	CAT	CDR-H1	5		0.14	1.46
A	GCG	A	GCT	CDR-H2	1		0.3	1.08
I	ATC	I	ATT	CDR-H2	2		0.08	1.23
T	ACG	T	ACT	CDR-H2	3		0.44	1.36
N	AAC	N	AAT	CDR-H2	5		0.27	1.63
G	GGC	G	GGG	CDR-H2	7		0.16	1.46
G	GGC	G	GGT	CDR-H2	7		0.07	1.27
H	CAC	H	CAT	CDR-H2	8		0.19	1.38
I	ATA	I	ATT	CDR-H2	9		0.01	1.85
A	GCA	A	GCT	CDR-H2	12		0.02	1.47
A	GCA	A	GCG	CDR-H2	12		0.02	1.11
S	AGC	S	TCT	CDR-H2	14		0.01	1.57
S	AGC	S	TCG	CDR-H2	14		0	1.58
S	AGC	S	AGT	CDR-H2	14		0.07	1.14
V	GTG	V	GTT	CDR-H2	15		0.04	1.52
E	GAA	E	GAG	CDR-H2	16		0.13	1.72
G	GGA	G	GGT	CDR-H2	17		0.02	1.29
G	GGA	G	GGG	CDR-H2	17		0.02	1.44
S	AGC	S	AGT	CDR-H3	2		0.12	1.27
L	CTC	L	CTT	CDR-H3	4		0.07	1.43
L	CTC	L	TTG	CDR-H3	4		0.02	1.43
L	CTC	L	CTG	CDR-H3	4		0.02	1.50
S	TCA	S	TCG	CDR-H3	5		0.2	1.45
S	TCA	S	TCT	CDR-H3	5		0.01	1.40
S	TCA	S	AGT	CDR-H3	5		0.04	1.39
T	ACA	T	ACT	CDR-H3	6		0.08	1.14
A	GCT	A	GCG	CDR-H3	7		0.27	1.22
S	TCC	S	AGT	CDR-H3	8		0	1.53
S	TCC	S	TCG	CDR-H3	8		0.02	1.53
S	TCC	S	TCT	CDR-H3	8		0.46	0.92
S	AGC	S	TCG	CDR-H3	9		0	1.63
S	AGC	S	AGT	CDR-H3	9		0.14	1.19
L	CTA	L	TTG	CDR-H3	10		0	1.34
Y	TAC	Y	TAT	CDR-H3	12		0.34	1.34

	1H11	TNF-α	**Neutral binding to TNF-α**
average	0.12	1.40	**1.21-1.59**
SD	0.13	0.19	

FIG. 9

	1	2	3	4	5	6	7	8
A	VL CDR1-1 R24	VL CDR1-9 Y32	VL CDR2-6 Q55	VL CDR3-7 P95	VH CDR2-1 A50	VH CDR2-9 I57	VH CDR2-17 G65	VH CDR3-8 S100b
B	VL CDR1-2 A25	VL CDR1-10 L33	VL CDR2-7 S56	VL CDR3-8 Y96	VH CDR2-2 I51	VH CDR2-10 D58	VH CDR3-1 V95	VH CDR3-9 S100c
C	VL CDR1-3 S26	VL CDR1-11 A34	VL CDR3-1 Q89	VL CDR3-9 T97	VH CDR2-3 T52	VH CDR2-11 Y59	VH CDR3-2 S96	VH CDR3-10 L100d
D	VL CDR1-4 Q27	VL CDR2-1 A50	VL CDR3-2 R90	VH CDR1-1 D31	VH CDR2-4 W52a	VH CDR2-12 A60	VH CDR3-3 Y97	VH CDR3-11 D101
E	VL CDR1-5 G28	VL CDR2-2 A51	VL CDR3-3 Y91	VH CDR1-2 Y32	VH CDR2-5 N53	VH CDR2-13 D61	VH CDR3-4 L98	VH CDR3-12 Y102
F	VL CDR1-6 I29	VL CDR2-3 S52	VL CDR3-4 N92	VH CDR1-3 A33	VH CDR2-6 S54	VH CDR2-14 S62	VH CDR3-5 S99	
G	VL CDR1-7 R30	VL CDR2-4 T53	VL CDR3-5 R93	VH CDR1-4 M34	VH CDR2-7 G55	VH CDR2-15 V63	VH CDR3-6 T100	
H	VL CDR1-8 N31	VL CDR2-5 L54	VL CDR3-6 A94	VH CDR1-5 H35	VH CDR2-8 H56	VH CDR2-16 E64	VH CDR3-7 A100a	

FIG. 10

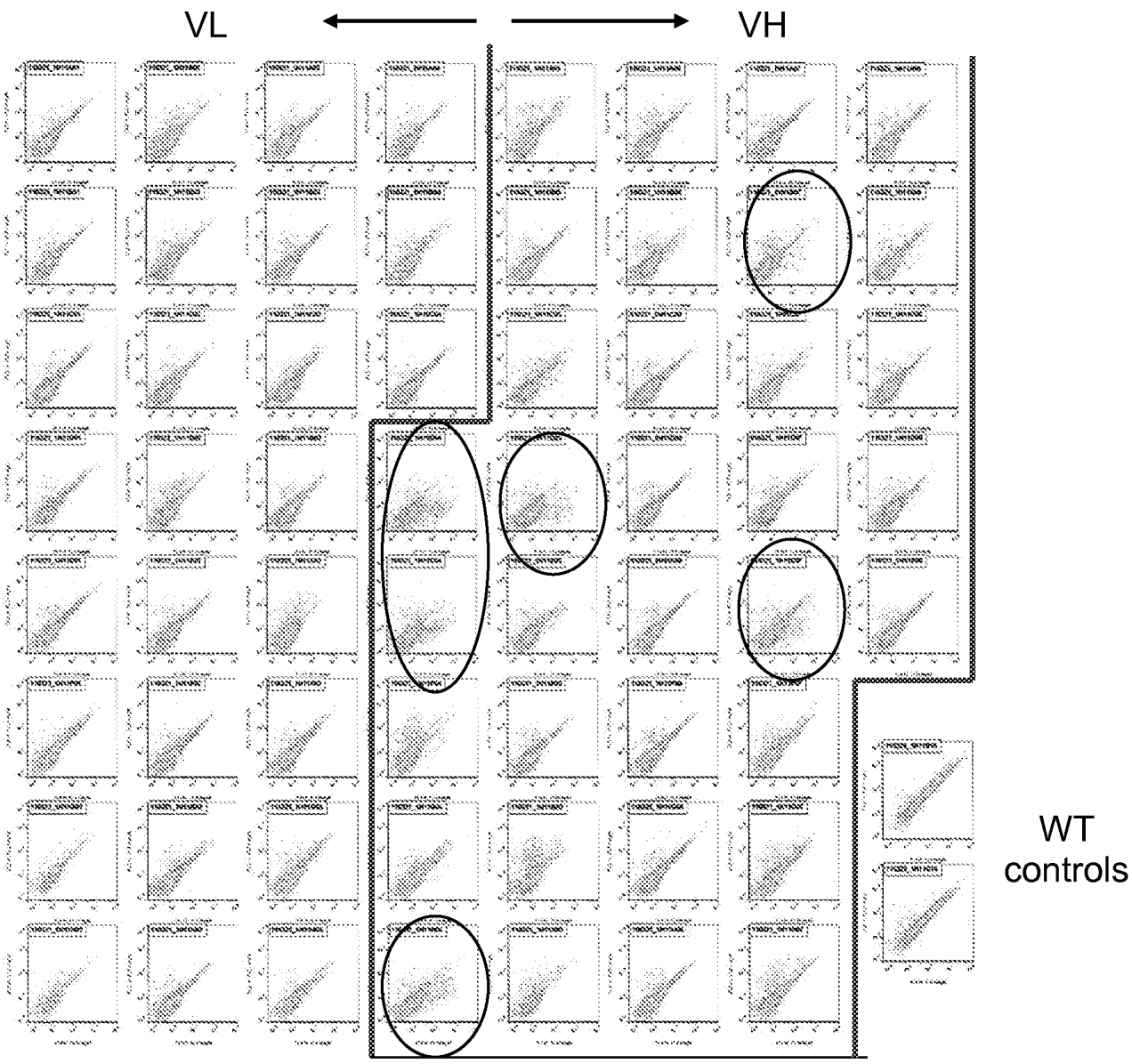
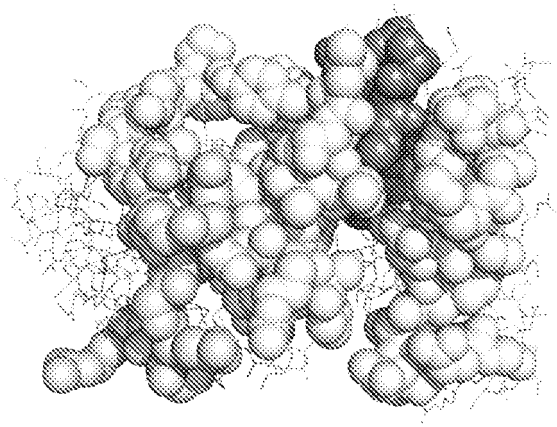


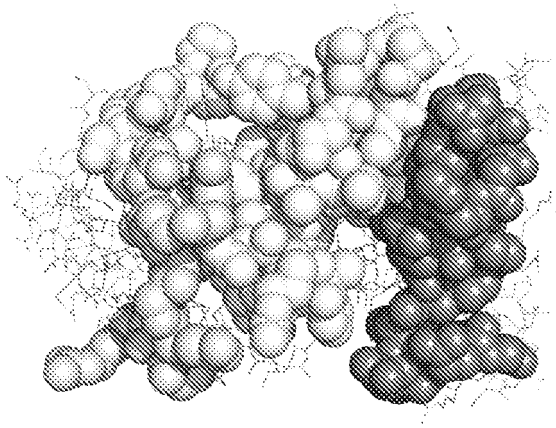
FIG. 11



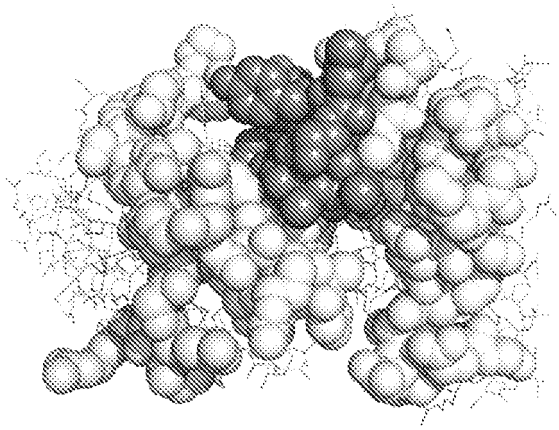
FIG. 12



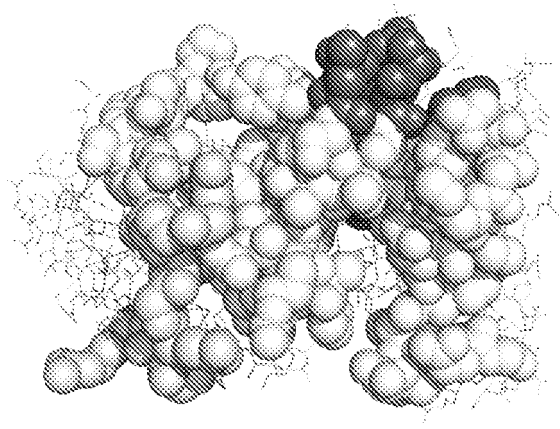
A



B



C



D

FIG. 13A-D

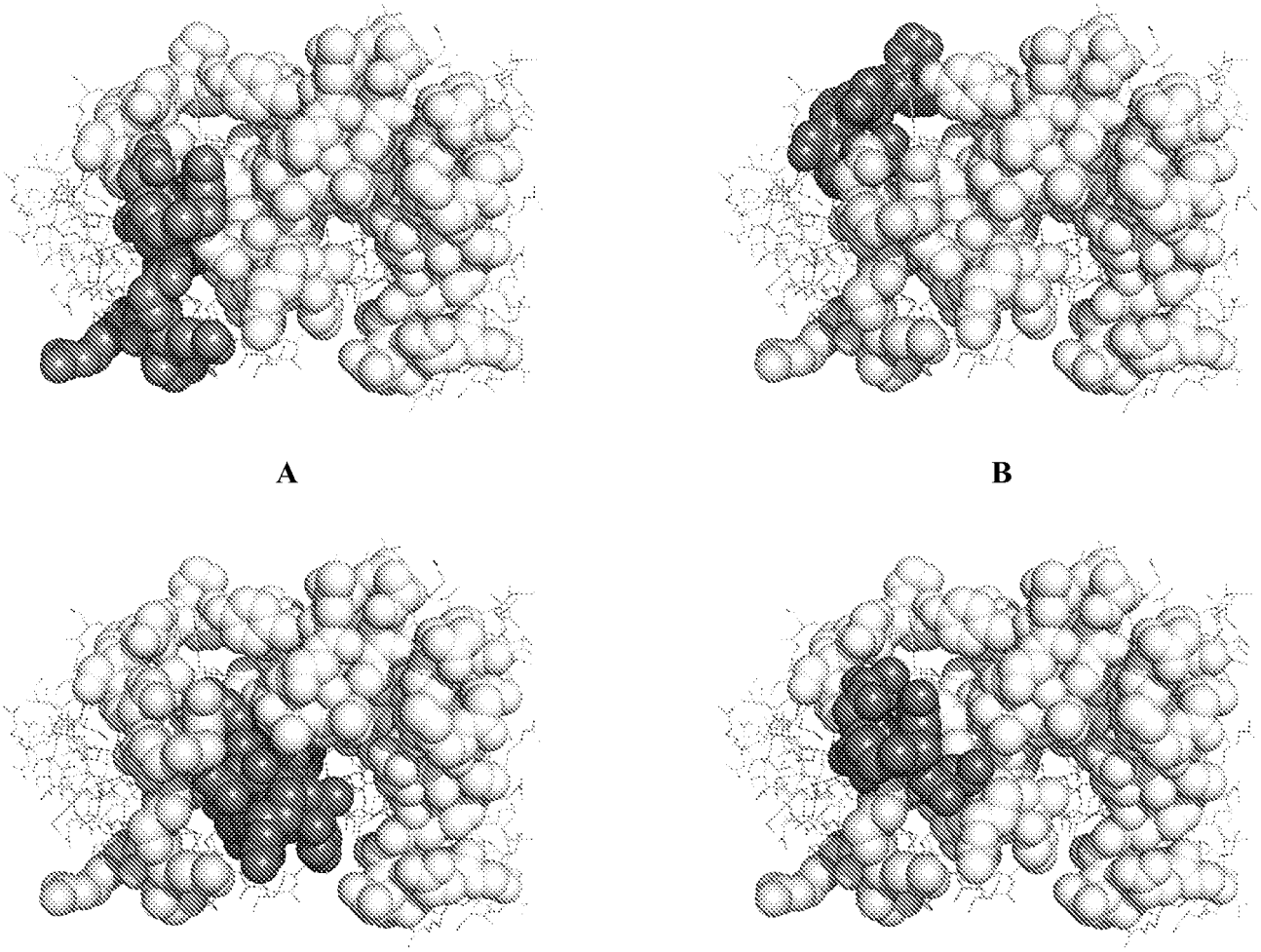


FIG. 14A-D

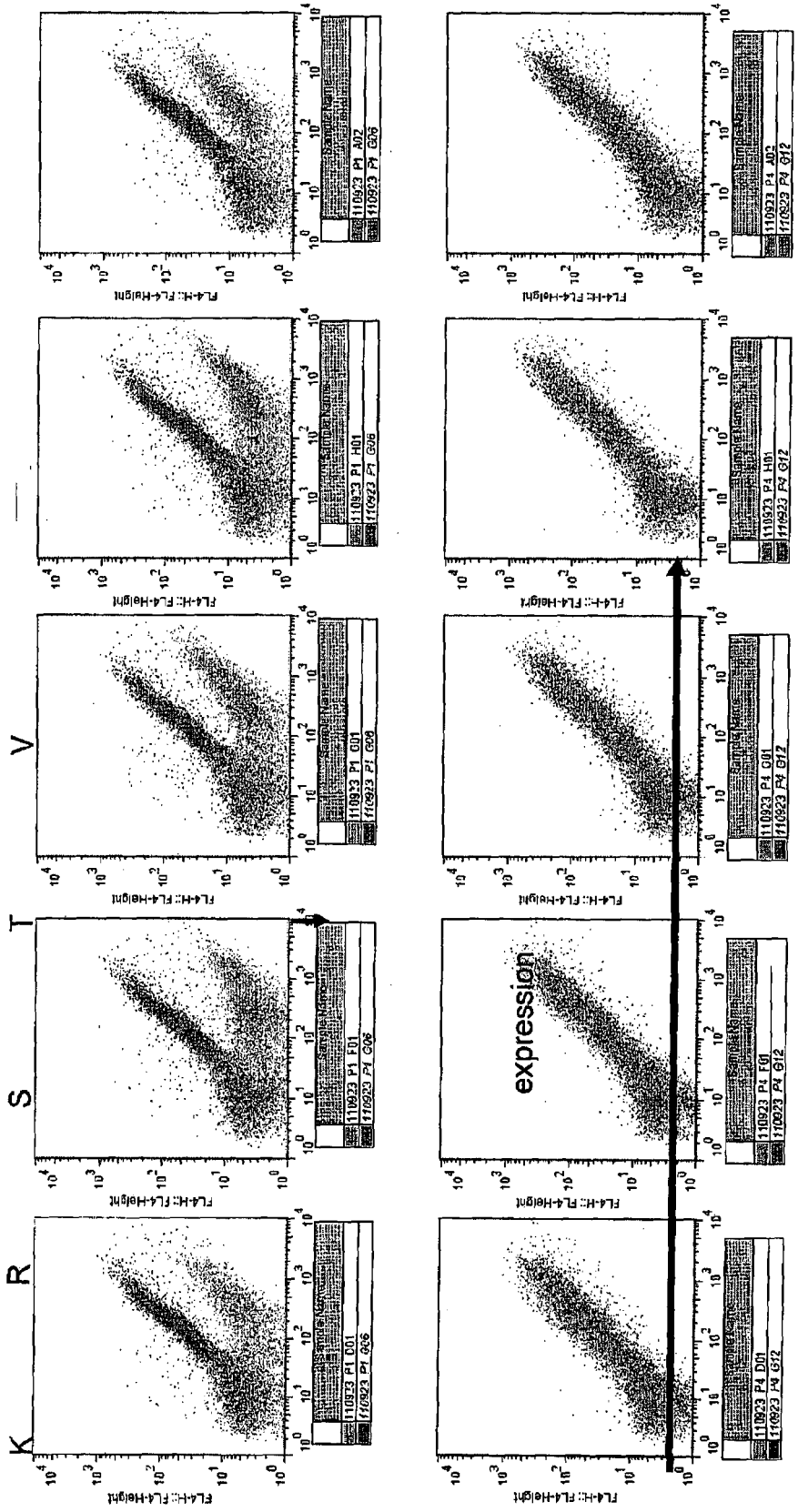


FIG. 15

AA	CDR	position	1H11 ER	Avg 1H11 ER in position	TNFα-ER
A	CDR-H1	2	5.22	5.69	1.37
A	CDR-H1	2	6.21	5.69	1.32
C	CDR-H1	2	11.17	5.69	1.22
D	CDR-H1	2	0.56	5.69	0.03
E	CDR-H1	2	0.44	5.69	0.00
F	CDR-H1	2	0.03	5.69	1.32
G	CDR-H1	2	6.95	5.69	0.39
G	CDR-H1	2	5.42	5.69	0.25
H	CDR-H1	2	3.58	5.69	1.14
I	CDR-H1	2	6.80	5.69	1.20
K	CDR-H1	2	9.58	5.69	1.47
L	CDR-H1	2	1.44	5.69	0.95
L	CDR-H1	2	1.40	5.69	0.91
L	CDR-H1	2	1.29	5.69	0.64
M	CDR-H1	2	4.37	5.69	1.25
N	CDR-H1	2	8.40	5.69	1.60
P	CDR-H1	2	1.22	5.69	0.38
P	CDR-H1	2	1.03	5.69	0.33
Q	CDR-H1	2	9.41	5.69	1.18
R	CDR-H1	2	8.02	5.69	1.65
R	CDR-H1	2	6.88	5.69	1.46
R	CDR-H1	2	10.28	5.69	1.45
S	CDR-H1	2	7.32	5.69	1.65
S	CDR-H1	2	9.20	5.69	1.44
S	CDR-H1	2	6.28	5.69	1.28
T	CDR-H1	2	9.51	5.69	1.2
T	CDR-H1	2	6.48	5.69	1.14
V	CDR-H1	2	9.34	5.69	1.41
V	CDR-H1	2	6.44	5.69	1.28
W	CDR-H1	2	6.50	5.69	1.19

WT binding to TNFα: 1.21 – 1.60
WT binding to 1H11: 0.00 – 0.25

FIG. 16

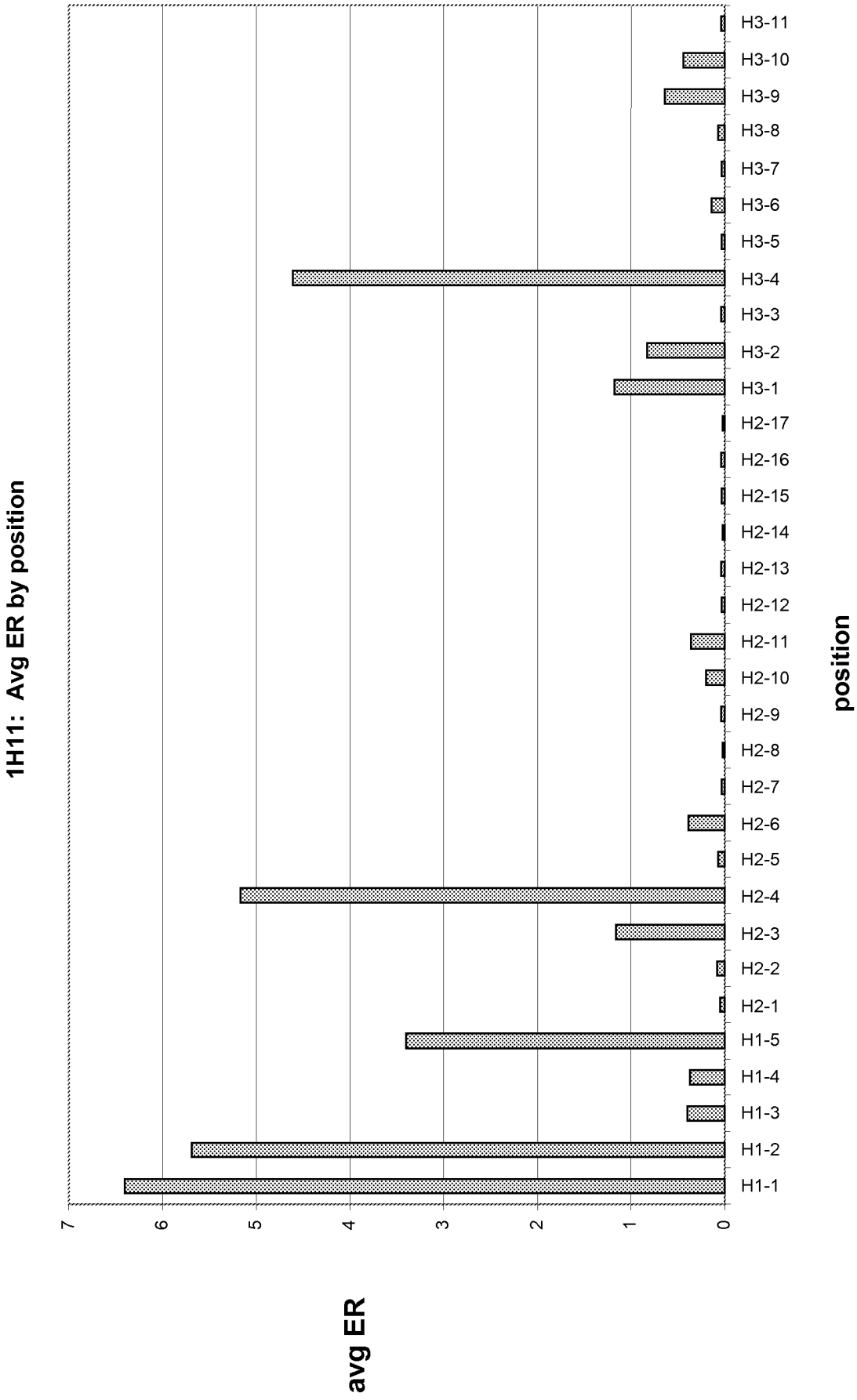

1H11: Avg ER by position
avg ER
position
0
1
2
3
4
5
6
7
H1-1
H1-2
H1-3
H1-4
H1-5
H2-1
H2-2
H2-3
H2-4
H2-5
H2-6
H2-7
H2-8
H2-9
H2-10
H2-11
H2-12
H2-13
H2-14
H2-15
H2-16
H2-17
H3-1
H3-2
H3-3
H3-4
H3-5
H3-6
H3-7
H3-8
H3-9
H3-10
H3-11

FIG. 17

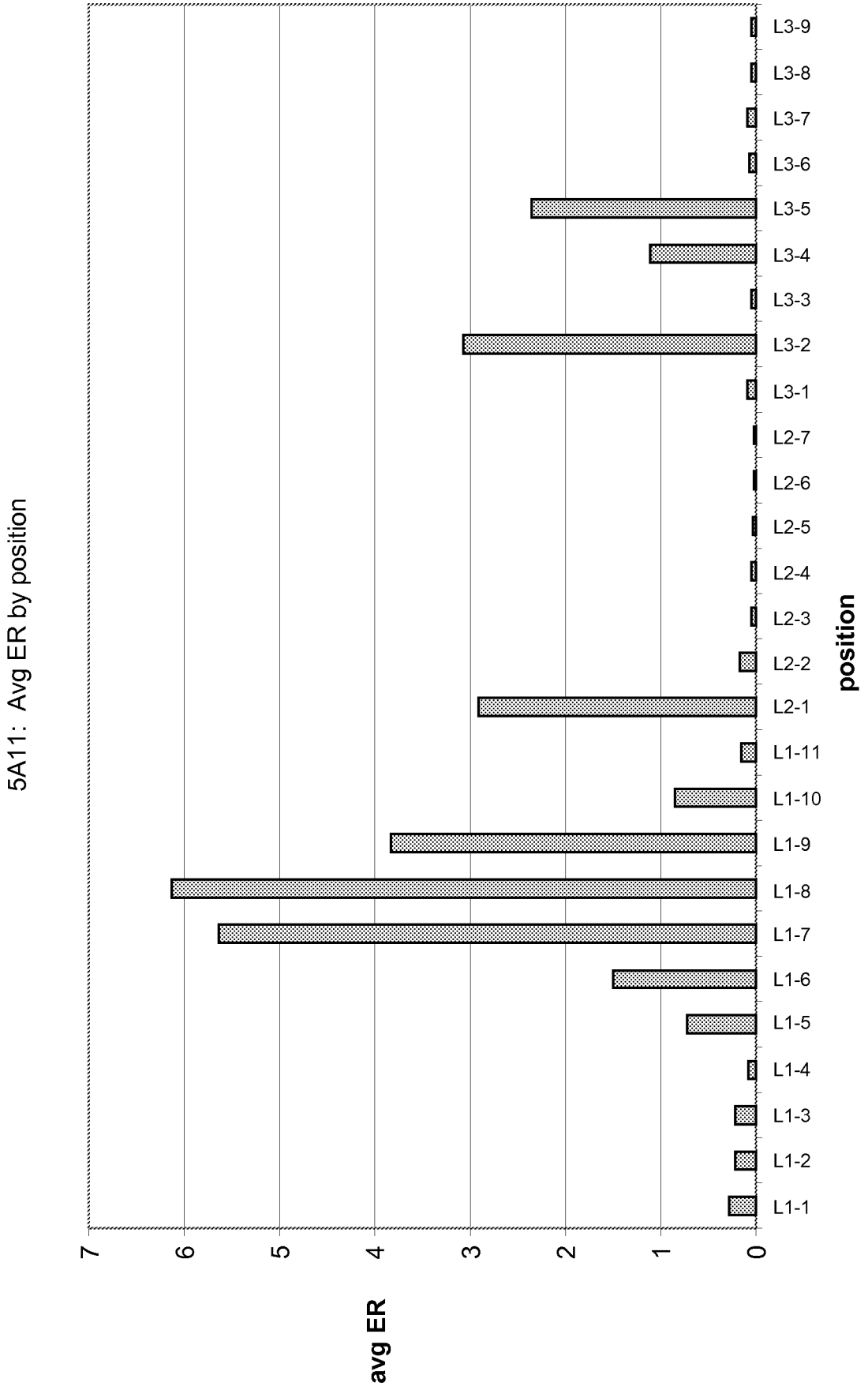

5A11: Avg ER by position
avg ER
0
1
2
3
4
5
6
7
position
L3-9
L3-8
L3-7
L3-6
L3-5
L3-4
L3-3
L3-2
L3-1
L2-7
L2-6
L2-5
L2-4
L2-3
L2-2
L2-1
L1-11
L1-10
L1-9
L1-8
L1-7
L1-6
L1-5
L1-4
L1-3
L1-2
L1-1

FIG. 18

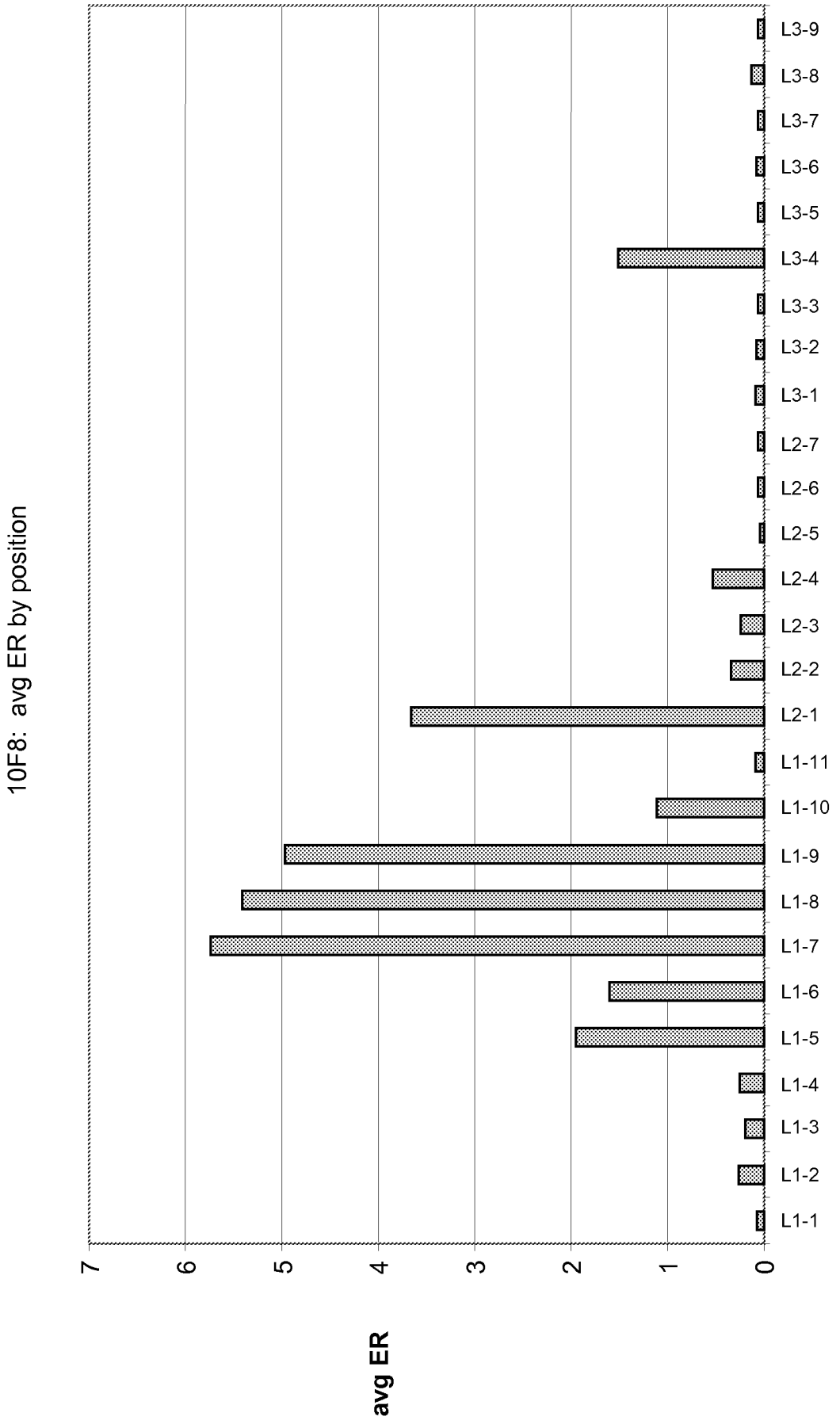

10F8: avg ER by position
avg ER
0
1
2
3
4
5
6
7
L1-1
L1-2
L1-3
L1-4
L1-5
L1-6
L1-7
L1-8
L1-9
L1-10
L1-11
L2-1
L2-2
L2-3
L2-4
L2-5
L2-6
L2-7
L3-1
L3-2
L3-3
L3-4
L3-5
L3-6
L3-7
L3-8
L3-9
position

FIG. 19

Variant	Notation	37440	23709	90814	84111	Average	SD
WT	WT	1.67	-1.16	0.00	-0.44	**0.02**	1.20
VL-SS	VL-SS	-2.90	3.79	0.35	-0.33	**0.23**	2.76
VL R30T	VL4	8.44	26.36	18.92	11.19	**16.23**	8.08
VH Y59I + VL-SS	VH16-SS	0.13	1.60	-0.66	-0.46	**0.15**	1.02
VH Y59R + VL-SS	VH20-SS	-1.23	2.82	-1.08	1.24	**0.44**	1.95
VH V63D + VL-SS	VH24-SS	-0.77	-0.37	-0.69	-0.54	**-0.59**	0.18
VL G28F	VL1	3.15	15.50	0.69	0.22	**4.89**	7.19
VL G28V	VL2	-5.29	10.13	-0.71	-0.07	**1.01**	6.51
VL G28Y	VL3	-1.46	8.33	-0.16	0.00	**1.68**	4.48
VL N31D	VL5	-0.46	13.13	0.56	1.32	**3.64**	6.37
VL N31L	VL6	3.77	10.88	1.19	1.68	**4.38**	4.48
VL N31T	VL7	3.43	8.70	1.04	0.88	**3.51**	3.65
VL A50T	VL9	1.77	15.45	0.01	-0.19	**4.26**	7.51
VL T53D	VL10	0.00	3.77	-1.25	-0.50	**0.51**	2.24
VL N92F	VL11	5.96	24.50	4.50	4.41	**9.85**	9.80
VL N92Y	VL12	7.62	22.66	6.17	6.19	**10.66**	8.03
VL R93L	VL13	2.14	17.92	1.19	1.92	**5.79**	8.09
VL R93N	VL14	1.79	15.93	1.22	1.48	**5.10**	7.22
VH D31S + VL-SS	VH1-SS	3.20	25.37	2.20	1.63	**8.10**	11.53
VH Y32A + VL-SS	VH2-SS	8.88	31.91	4.01	2.69	**11.87**	13.62
VH Y32R + VL-SS	VH3-SS	11.81	52.68	5.85	3.80	**18.54**	23.02
VH Y32S + VL-SS	VH4-SS	7.34	25.95	4.08	2.21	**9.90**	10.91
VH H35E + VL-SS	VH6-SS	0.99	20.58	0.07	0.23	**5.47**	10.08
VH H35T + VL-SS	VH7-SS	14.67	33.27	2.20	10.15	**15.07**	13.18
VH T52A + VL-SS	VH8-SS	-1.98	18.61	0.64	0.86	**4.53**	9.48
VH T52G + VL-SS	VH9-SS	2.32	24.96	1.61	1.36	**7.56**	11.60
VH I57K + VL-SS	VH10-SS	-2.69	8.43	-1.23	-0.42	**1.02**	5.03
VH Y59A + VL-SS	VH12-SS	-2.32	4.80	-1.31	-1.09	**0.02**	3.23
VH Y59C + VL-SS	VH13-SS	-4.39	4.37	-1.01	-0.49	**-0.38**	3.61
VH Y59F + VL-SS	VH14-SS	-1.51	7.70	-0.01	-0.23	**1.49**	4.19
VH Y59H + VL-SS	VH15-SS	-0.38	6.28	-0.49	-0.04	**1.34**	3.30
VH Y59L + VL-SS	VH18-SS	-0.74	5.37	-1.39	-0.48	**0.69**	3.14
VH Y59M + VL-SS	VH19-SS	0.58	2.92	-1.25	-0.53	**0.43**	1.82
VH Y59S + VL-SS	VH21-SS	-2.89	6.58	-1.41	-1.08	**0.30**	4.26
VH Y59V + VL-SS	VH22-SS	-1.52	4.05	-1.24	0.20	**0.37**	2.56
VH Y59W + VL-SS	VH23-SS	-1.16	8.76	1.03	-0.24	**2.10**	4.53
VH V63L + VL-SS	VH25-SS	-0.41	7.11	-0.45	-0.10	**1.54**	3.72
VH G65A + VL-SS	VH26-SS	-2.74	6.44	-1.32	-0.31	**0.52**	4.07
VH G65E + VL-SS	VH27-SS	-2.55	8.87	-0.38	-0.46	**1.37**	5.10
VH G65L + VL-SS	VH28-SS	-2.94	1.80	-1.25	-1.10	**-0.88**	1.97
VH G65M + VL-SS	VH29-SS	-2.30	3.73	-0.30	-1.12	**0.00**	2.62
VH G65Q + VL-SS	VH30-SS	-1.54	5.57	1.01	-0.46	**1.14**	3.13
VH G65R + VL-SS	VH31-SS	3.27	7.88	-0.47	-0.14	**2.63**	3.88

Fig. 20A

Variant	Notation	37440	23709	90814	84111	Average	SD
VH G65S + VL-SS	VH32-SS	-1.77	5.59	-1.30	-0.46	**0.51**	3.43
VH V95R + VL-SS	VH33-SS	-1.47	9.95	-0.32	0.35	**2.13**	5.27
VH V95W + VL-SS	VH34-SS	26.86	59.72	63.70	37.73	**47.00**	17.63
VH L98T + VL-SS	VH35-SS	22.01	43.07	13.08	8.65	**21.70**	15.29
VH L98V + VL-SS	VH36-SS	9.75	45.57	6.23	3.32	**16.22**	19.75
VH T100V + VL-SS	VH37-SS	47.11	61.75	60.87	42.04	**52.94**	9.89
VH D101V + VL-SS	VH38-SS	0.93	13.57	-0.32	0.55	**3.68**	6.61
VH Y32K + VL-SS	VH39-SS	13.28	35.32	6.92	4.34	**14.97**	14.08
VH Y32V + VL-SS	VH40-SS	7.07	19.62	2.17	1.36	**7.56**	8.43
VH H35C + VL-SS	VH41-SS	2.04	24.36	0.94	0.77	**7.03**	11.57
VH H35S + VL-SS	VH42-SS	3.09	30.19	4.08	1.16	**9.63**	13.76
VH V95G + VL-SS	VH43-SS	29.10	53.62	18.01	13.00	**28.43**	18.09
G28I		5.05	15.84		-0.10	**6.93**	8.14
G28W		3.54	14.89		-0.05	**6.13**	7.80
R30I		18.99	44.28		8.46	**23.91**	18.41
R30V		19.04	40.09		10.16	**23.10**	15.37
N31E		10.69	29.20		3.37	**14.42**	13.31
N31G		6.16	19.35		1.03	**8.85**	9.45
V31R		5.80	13.38		0.78	**6.65**	6.35
A50V		2.15	15.98		-0.01	**6.04**	8.68
R93W		6.25	28.88		2.34	**12.49**	14.33
VH-Y32K		36.31	24.20			**30.26**	8.56
VH-Y32V		21.20	13.50			**17.35**	5.44
VH-H35C		12.70	19.40			**16.05**	4.74
VH-H35S		13.99	22.56			**18.28**	6.06
VH-V95G		34.70	38.58			**36.64**	2.75
DP10		99.82	84.18	95.33	71.54	**87.72**	12.63

Fig. 20B

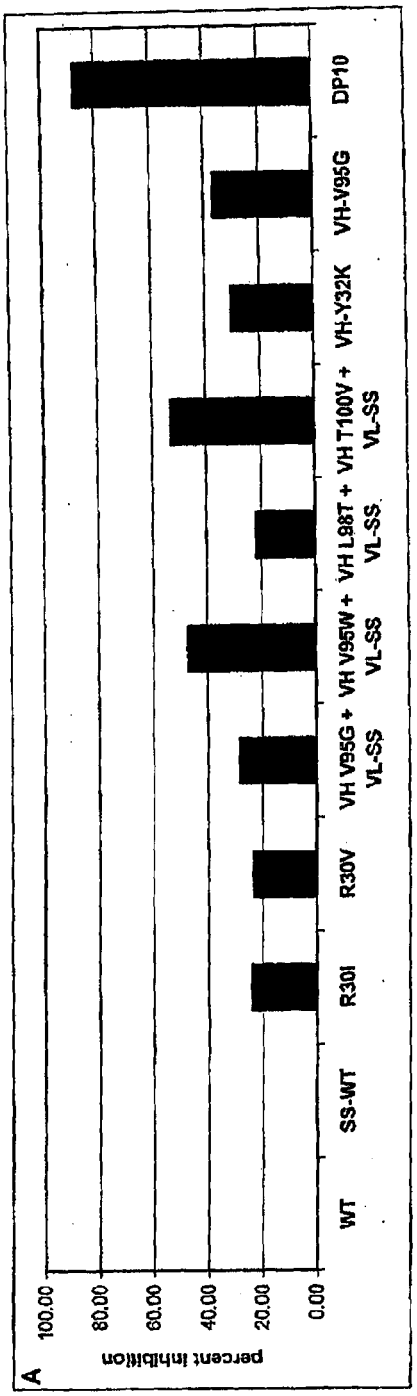


FIG. 21A

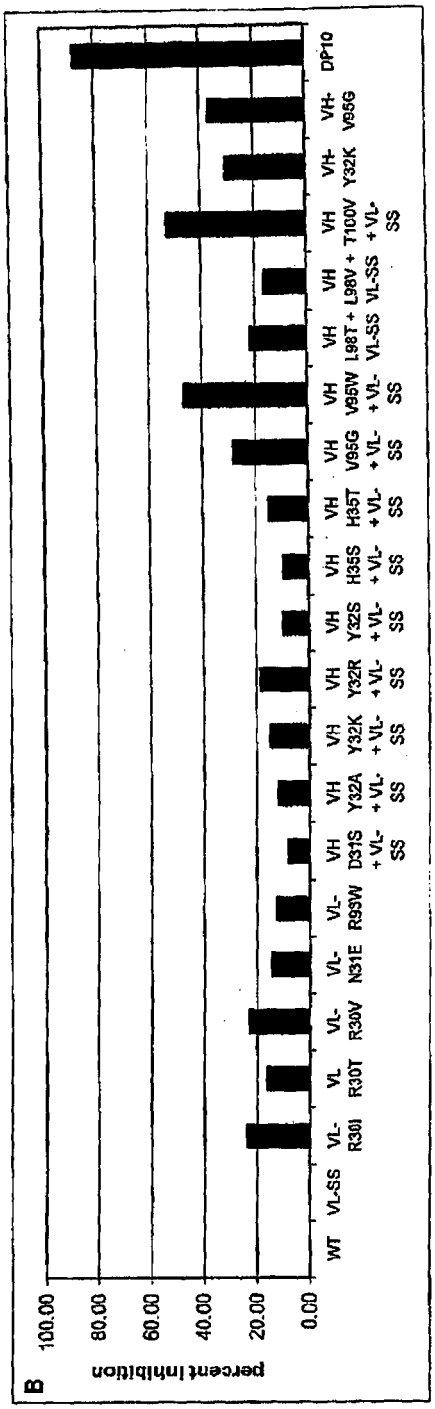


FIG. 21B

VH	VL	ADAb bridging	fold affinity	affinity pM
WT	WT	5	100	83 +/- 10
WT	SS	0.4	82	150
Y32K/T100V	SS	28	142	61
Y32K/V95G/T100V	SS	24	122	71
Y32K	SS/R30T	45	55	130
Y32K	SS/R30I	48	50	149
V95G/T100V	SS	15	25	335
Y32M	SS	0	21	411
V95W	R30I	95	23	379
V95W	R30T	97	0	>10,000

FIG. 22