Figure 1

MAPISLSWLLRLATFCHLTVLLAGQHHGVTKCNITCSKMTSKIPVALLIHYQQN
QASCGKRAIILETRQHRLFCADPKEQWVKDAMQHLDRQAAALTRNGGTFEKQI
GEVKPRTTPAAGGMDESVVLEPEATGESSSLEPTPSSQEAQRALGTSPELPTGVTG
SSGTRLPPTPKAQDGGPVGTELFRVPPVSTAATWQSSAPHQPGPSLWAEAKTSE
APSTQDPSTQASTASSPAPEENAPSEGGQRVWGQGQSPRPENSLEREEMGPVPA
HTDAFQDWGPGSMAHVSVPVSSEGTPSREPVASGSWTPKAEEPIHATMDPQR
LGVLITPVPDAQAATRRQAVGLLAFLGLLFCLGVAMFTYQSLQGCPRKMAGEMAE
GLRYIPRSCGSNSYVLVPV (SEQ ID NO: 1)

Figure 2

		1F3-1	3A5-2	3H7-6
Chemotaxis assay IC_{50} (nM)	Mouse	-	-	-
	Human	12.67 (±5.07)	4.80 (±1.33)	19.07 (±5.13)
Affinity (BIACORE) KD (nM)	Mouse	-	-	-
	Human	0.66	0.60	0.83
Reactivity to cynomolgus monkey FKN (ELISA)		Not equal to human	equal to human	equal to human

- : no activity

Figure 3

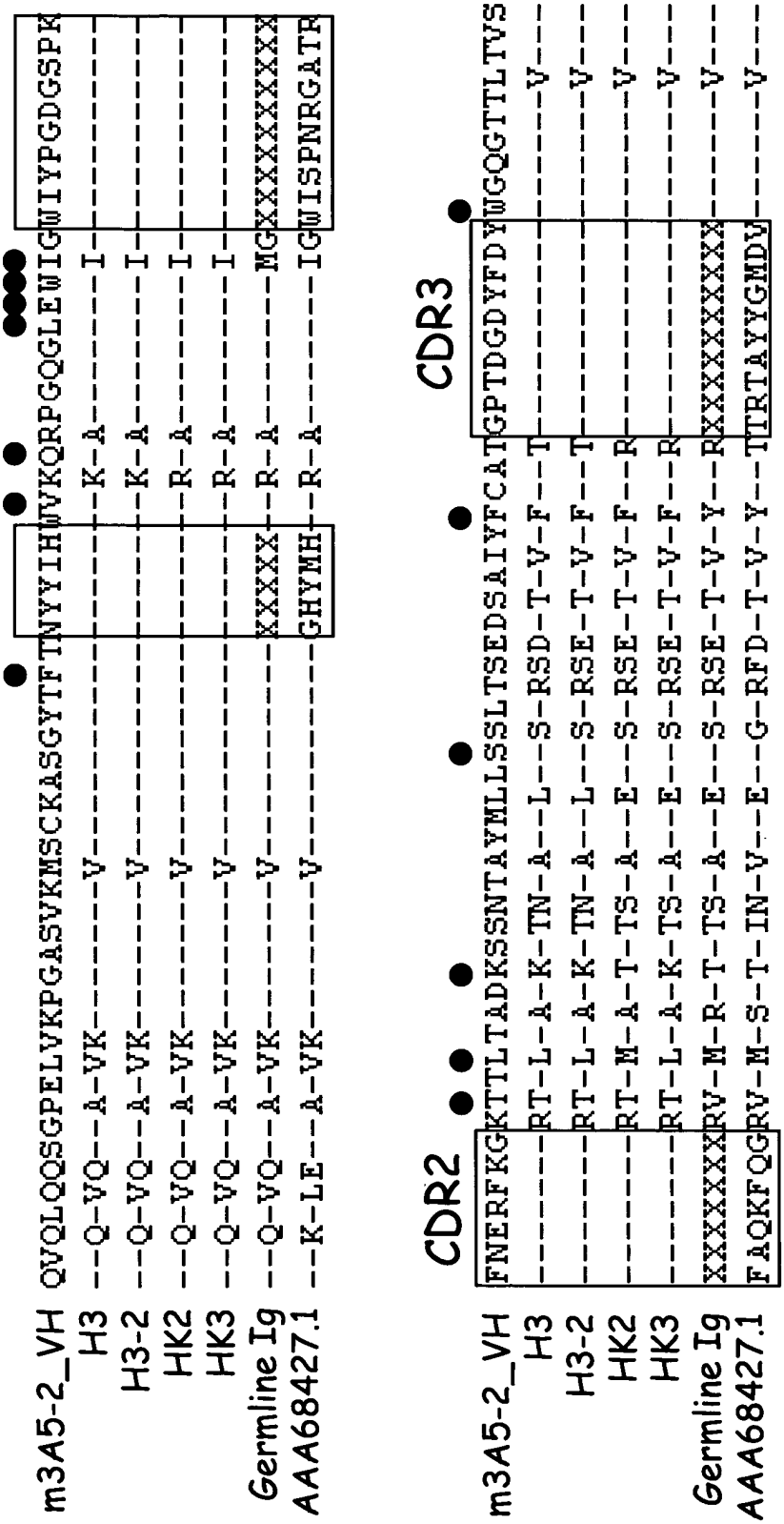

CDR1
CDR2
CDR3
m3A5-2_VH
H3
H3-2
HK2
HK3
Germline Ig
AAA68427.1
QVQLQQSGPELVKPGASVKMSCKASGYTFTNYYIHWVKQRPGQGLEWIGWIYPGDGSPK
FNERFKGKTTLTADKSSNTAYMLLSSLTSEDSAIYFCATGPTDGDYFDYWGQGTTLTVSS
Narrowed Key residues

Figure 4

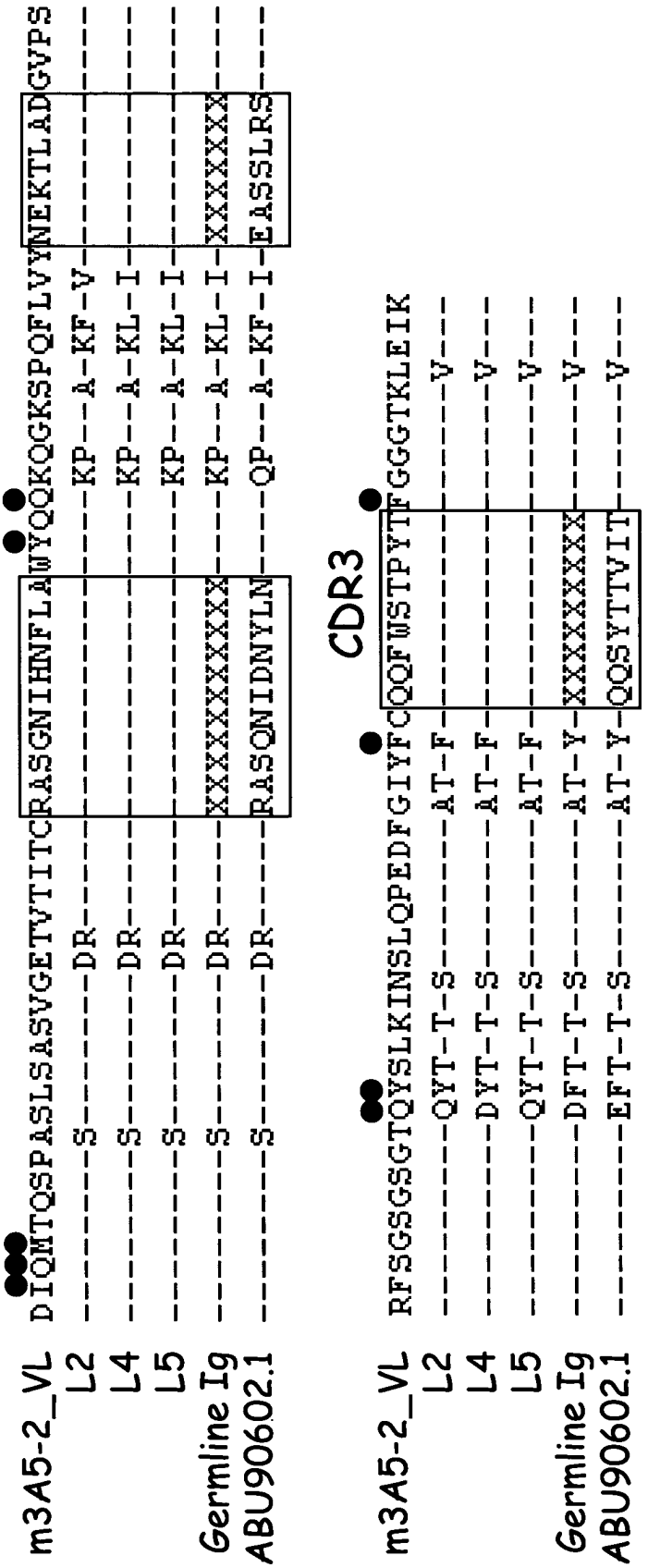

CDR1
CDR2
CDR3
m3A5-2_VL DIQMTQSPASLSASVGETVTITCRASGNIHNFLAWYQQKQGKSPQFLVYNEKTLADGVPS
L2 ----------S----------DR----------KP--A-KF-V----------
L4 ----------S----------DR----------KP--A-KL-I----------
L5 ----------S----------DR----------KP--A-KL-I----------
Germline Ig ----------S----------DR----------XXXXXXXXXX----------KP--A-KL-I-XXXXXXX---
ABU90602.1 ----------S----------DR----------RASQNIDNYLN----------QP--A-KF-I-EASSLRS---
m3A5-2_VL RFSGSGSGTQYSLKINSLQPEDFGIYFCQQFWSTPYTFGGGTKLEIK
L2 ----------QYT-T-S----------AT-F----------V---
L4 ----------DYT-T-S----------AT-F----------V---
L5 ----------QYT-T-S----------AT-F----------V---
Germline Ig ----------DFT-T-S----------AT-Y-XXXXXXXXX----------V---
ABU90602.1 ----------EFT-T-S----------AT-Y-QQSYTTVIT----------V---
● Narrowed Key residues

Figure 5

		1st	2nd	3rd	Av.	SD
mouse	IC50	0.52	0.61	0.54	0.55	0.05
	IC90	4.08	4.24	3.23	3.85	0.55
	IC95	8.61	10.84	6.66	8.70	2.09
chimeric	IC50	0.34	0.29	0.43	0.35	0.07
	IC90	3.36	2.21	1.66	2.41	0.87
	IC95	7.07	5.24	3.15	5.15	1.96
H3L2	IC50	0.47	0.26	0.29	0.34	0.12
	IC90	2.94	2.25	1.34	2.18	0.80
	IC95	5.61	4.69	2.91	4.40	1.37
H3L4	IC50	0.44	0.91	0.44	0.60	0.27
	IC90	8.34	5.11	4.43	5.96	2.09
	IC95	15.92	50.26	10.46	25.54	21.58
H3-2L2	IC50	0.25	0.23	0.27	0.25	0.02
	IC90	2.23	2.13	2.67	2.34	0.29
	IC95	4.25	5.28	5.59	5.04	0.70
H3-2L4	IC50	0.20	0.65	0.45	0.43	0.23
	IC90	2.71	3.01	2.98	2.90	0.17
	IC95	6.36	7.00	5.92	6.43	0.54
H3-2L5	IC50	0.13	0.46	0.57	0.39	0.23
	IC90	1.97	4.92	3.84	3.58	1.49
	IC95	5.81	17.47	6.68	9.99	6.50
HK2L2	IC50	16.49	27.86	ND		
	IC90	>66.67	>66.67	ND		
	IC95	>66.67	>66.67	ND		
HK2L4	IC50	16.87	19.59	ND		
	IC90	>66.67	>66.67	ND		
	IC95	>66.67	>66.67	ND		
HK3L2	IC50	1.65	8.50	9.63	6.59	4.32
	IC90	>66.67	>66.67	>66.67		
	IC95	>66.67	>66.67	>66.67		
HK3L4	IC50	0.42	25.25	>66.67	12.84	17.56
	IC90	>66.67	>66.67	>66.67		
	IC95	>66.67	>66.67	>66.67		
HK3L5	IC50	1.10	14.17	13.50	9.59	7.36
	IC90	>66.67	>66.67	>66.67		
	IC95	>66.67	>66.67	>66.67		

Figure 6

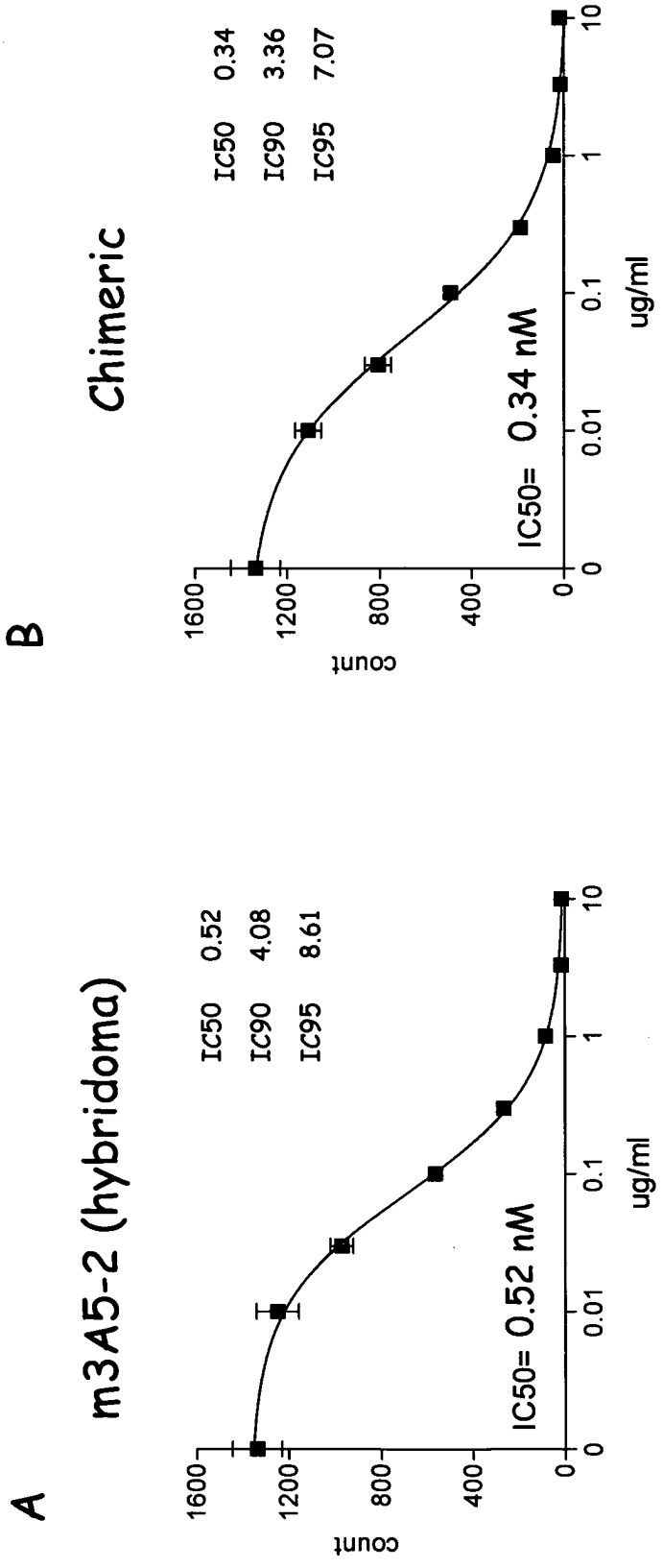

A
m3A5-2 (hybridoma)
IC50 0.52
IC90 4.08
IC95 8.61
IC50= 0.52 nM
count
ug/ml
B
Chimeric
IC50 0.34
IC90 3.36
IC95 7.07
IC50= 0.34 nM
count
ug/ml

Figure 7

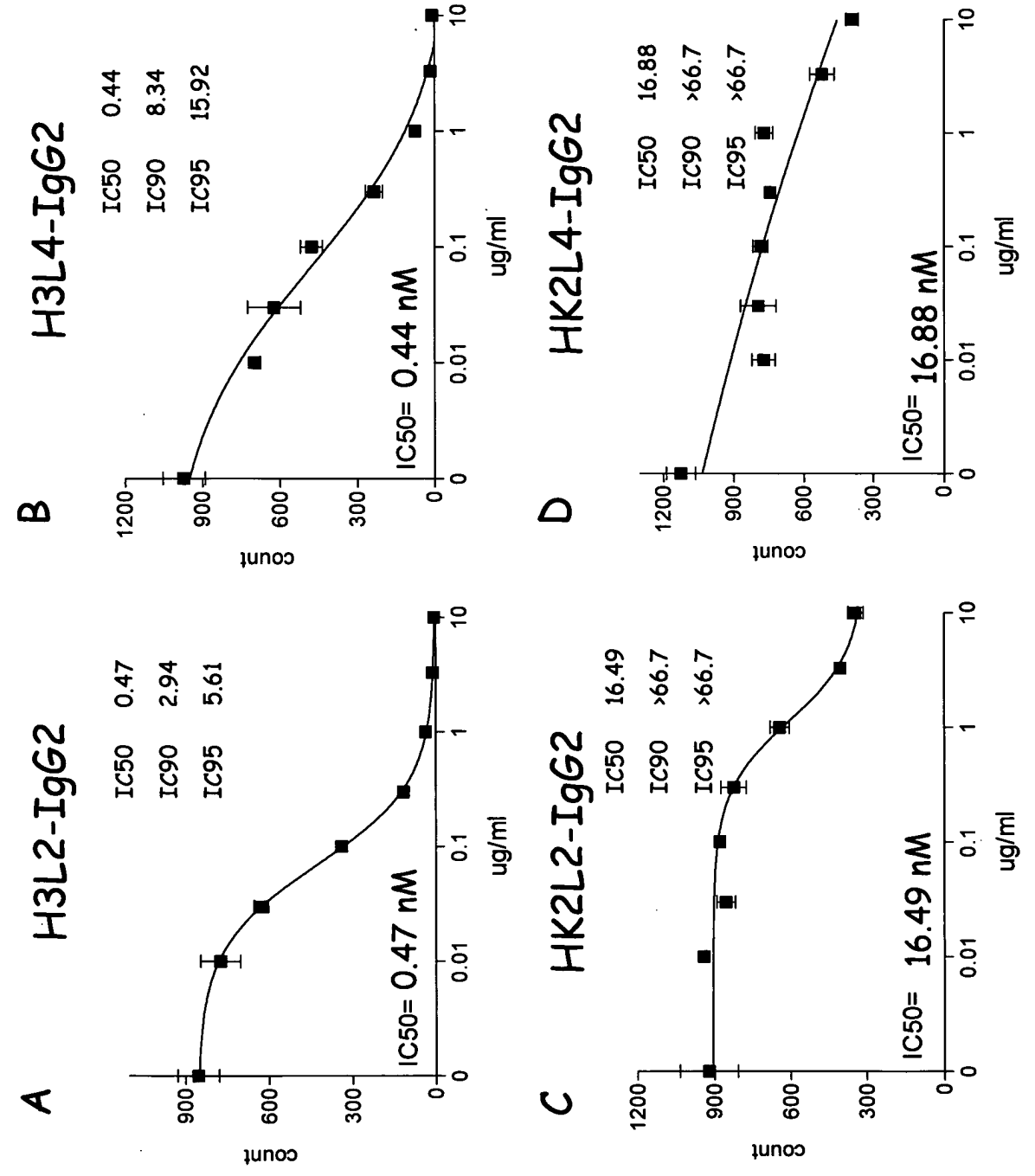

A
H3L2-IgG2
IC50 0.47
IC90 2.94
IC95 5.61
IC50= 0.47 nM
B
H3L4-IgG2
IC50 0.44
IC90 8.34
IC95 15.92
IC50= 0.44 nM
C
HK2L2-IgG2
IC50 16.49
IC90 >66.7
IC95 >66.7
IC50= 16.49 nM
D
HK2L4-IgG2
IC50 16.88
IC90 >66.7
IC95 >66.7
IC50= 16.88 nM
count
ug/ml

Figure 8

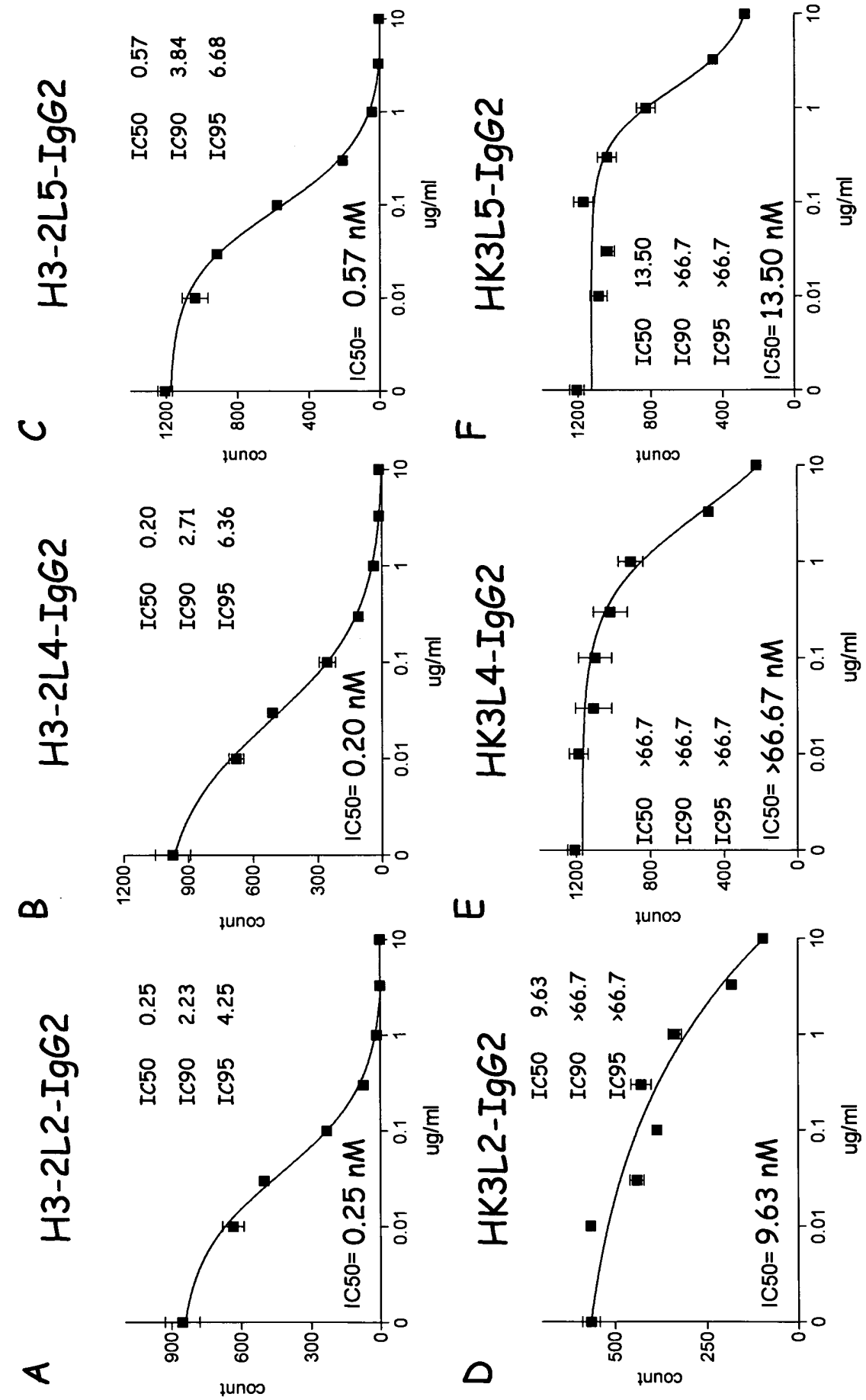

A
H3-2L2-IgG2
IC50 0.25
IC90 2.23
IC95 4.25
IC50= 0.25 nM
B
H3-2L4-IgG2
IC50 0.20
IC90 2.71
IC95 6.36
IC50= 0.20 nM
C
H3-2L5-IgG2
IC50 0.57
IC90 3.84
IC95 6.68
IC50= 0.57 nM
D
HK3L2-IgG2
IC50 9.63
IC90 >66.7
IC95 >66.7
IC50= 9.63 nM
E
HK3L4-IgG2
IC50 >66.7
IC90 >66.7
IC95 >66.7
IC50= >66.67 nM
F
HK3L5-IgG2
IC50 13.50
IC90 >66.7
IC95 >66.7
IC50= 13.50 nM
count
ug/ml

Figure 9

	human FKN-SEAP			cynomolgus FKN-SEAP		
mAbs	ka (1/Ms)	kd (1/s)	KD (M)	ka (1/Ms)	kd (1/s)	KD (M)
chimeric	1.34E+07	9.58E-05	7.13E-12	1.43E+07	9.54E-05	6.62E-12
H3L2	1.32E+07	1.16E-04	8.84E-12	1.26E+07	1.22E-04	9.62E-12
H3-2L2	1.50E+07	8.83E-05	5.92E-12	1.39E+07	9.43E-05	6.75E-12
H3L4	1.56E+07	1.05E-04	6.43E-12	1.61E+07	1.87E-04	1.20E-11
H3-2L4	1.88E+07	1.01E-04	5.34E-12	1.49E+07	1.15E-04	7.71E-12
HK2L4	1.83E+07	6.14E-04	3.40E-11	2.49E+07	6.35E-04	2.60E-11

Figure 10

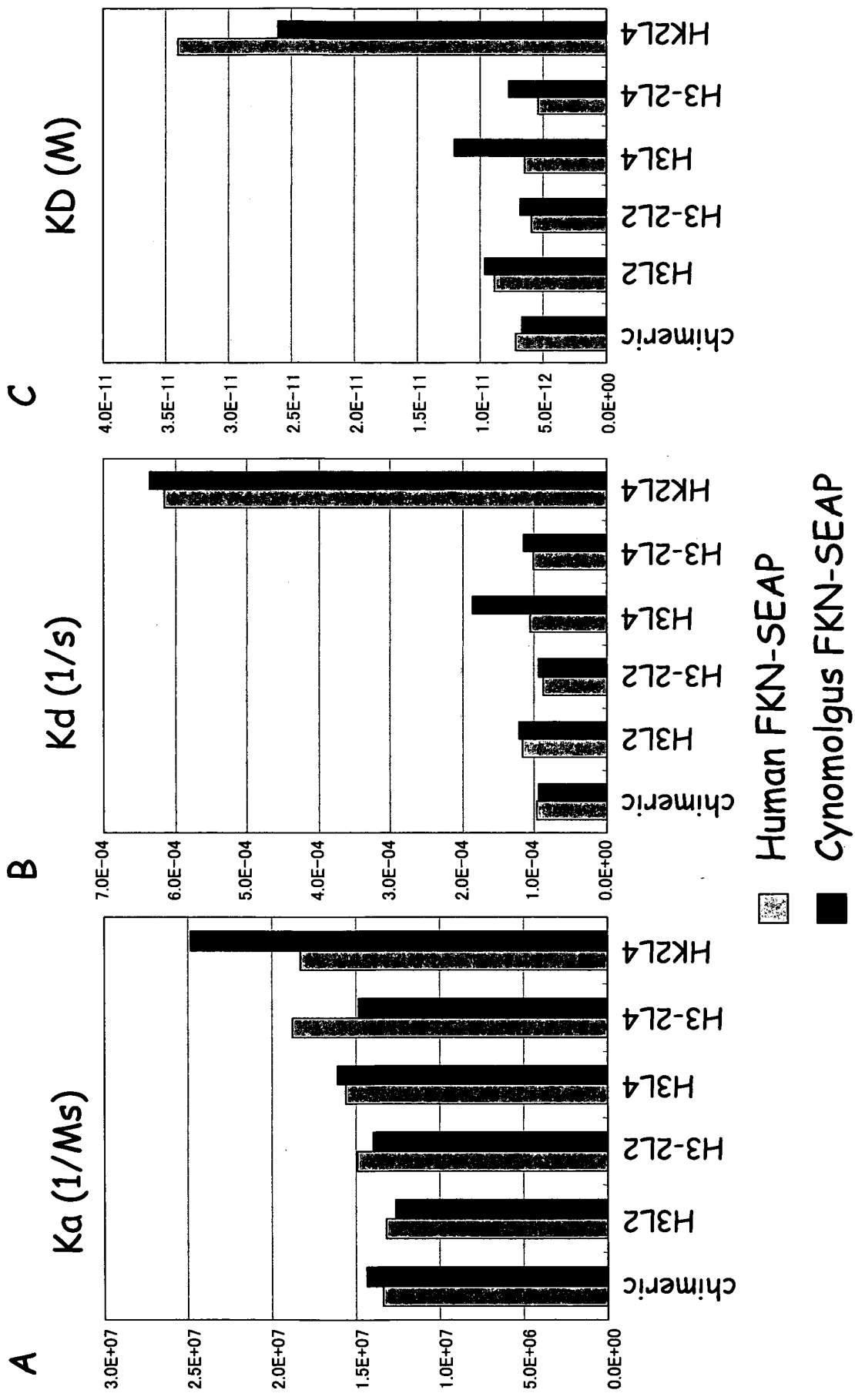

A
Ka (1/Ms)
3.0E+07
2.5E+07
2.0E+07
1.5E+07
1.0E+07
5.0E+06
0.0E+00
chimeric
H3L2
H3-2L2
H3L4
H3-2L4
HK2L4
B
Kd (1/s)
7.0E-04
6.0E-04
5.0E-04
4.0E-04
3.0E-04
2.0E-04
1.0E-04
0.0E+00
chimeric
H3L2
H3-2L2
H3L4
H3-2L4
HK2L4
C
KD (M)
4.0E-11
3.5E-11
3.0E-11
2.5E-11
2.0E-11
1.5E-11
1.0E-11
5.0E-12
0.0E+00
chimeric
H3L2
H3-2L2
H3L4
H3-2L4
HK2L4
Human FKN-SEAP
Cynomolgus FKN-SEAP

Figure 11

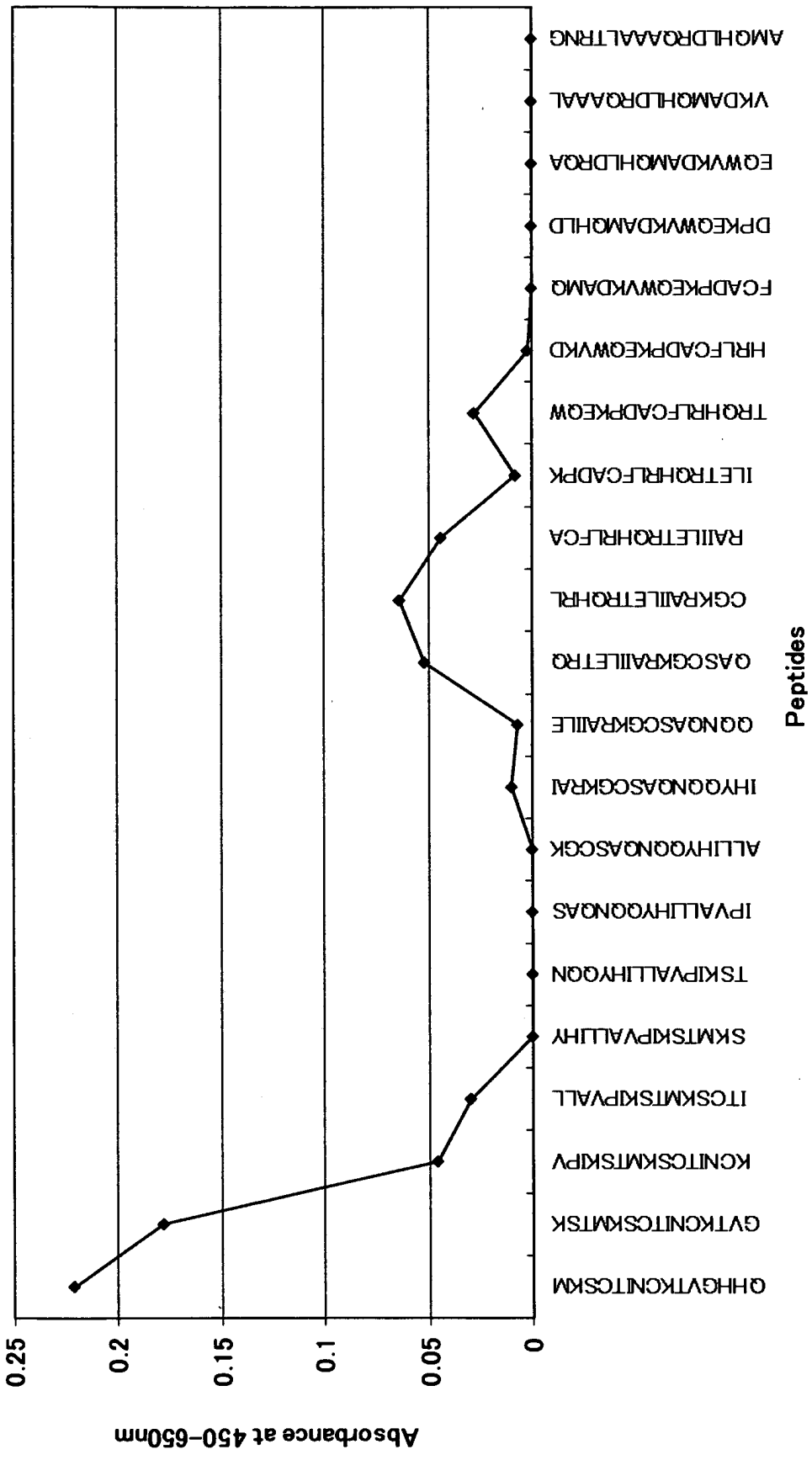

Epitope Mapping (Peptide ELISA)
Absorbance at 450–650nm
0.25
0.2
0.15
0.1
0.05
0
Peptides
QHHGVTKCNITCSKM
GVTKCNITCSKMTSK
KCNITCSKMTSKIPV
ITCSKMTSKIPVALL
SKMTSKIPVALLIHY
TSKIPVALLIHYGQN
IPVALLIHYGQNQAS
ALLIHYGQNQASCGK
IHYGQNQASCGKRAI
QQNQASCGKRAILE
QASCGKRAILETRQ
CGKRAILETRQHRL
RAILETRQHRLFCA
ILETRQHRLFCADPK
TRQHRLFCADPKEQW
HRLFCADPKEQWVKD
FCADPKEQWVKDAMQ
DPKEQWVKDAMQHLD
EQWVKDAMQHLDRQA
VKDAMQHLDRQAAAL
AMQHLDRQAAALTRNG

Figure 12

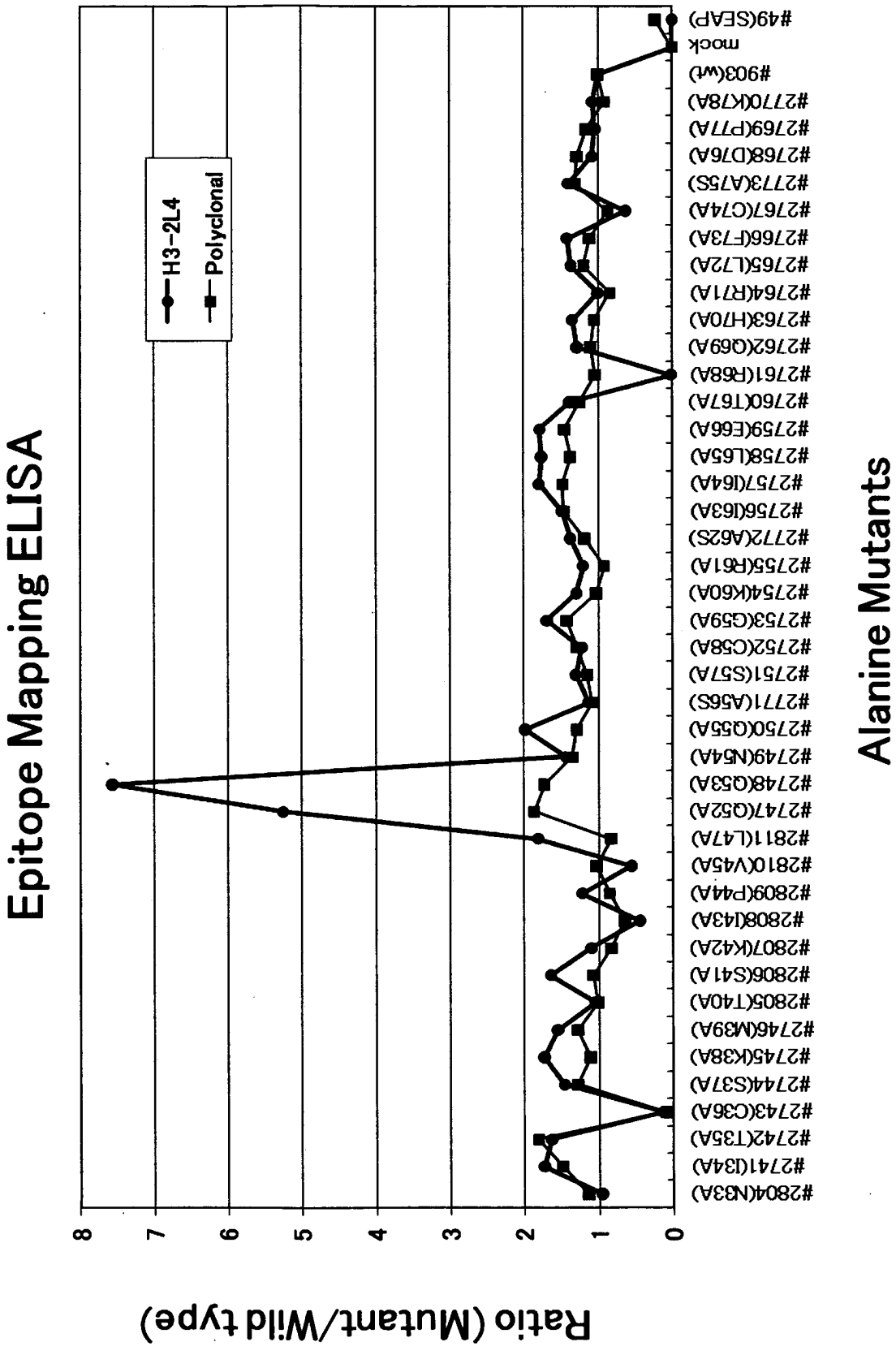

Epitope Mapping ELISA
H3-2L4
Polyclonal
Ratio (Mutant/Wild type)
0
1
2
3
4
5
6
7
8
Alanine Mutants
#2804(N33A)
#2741(I34A)
#2742(T35A)
#2743(C36A)
#2744(S37A)
#2745(K38A)
#2746(M39A)
#2805(T40A)
#2806(S41A)
#2807(K42A)
#2808(I43A)
#2809(P44A)
#2810(V45A)
#2811(L47A)
#2747(Q52A)
#2748(Q53A)
#2749(N54A)
#2750(Q55A)
#2771(A56S)
#2751(S57A)
#2752(C58A)
#2753(G59A)
#2754(K60A)
#2755(R61A)
#2772(A62S)
#2756(I63A)
#2757(I64A)
#2758(L65A)
#2759(E66A)
#2760(T67A)
#2761(R68A)
#2762(Q69A)
#2763(H70A)
#2764(R71A)
#2765(L72A)
#2766(F73A)
#2767(C74A)
#2773(A75S)
#2768(D76A)
#2769(P77A)
#2770(K78A)
#903(wt)
mock
#49(SEAP)

Figure 13

Epitope Mapping BIACORE

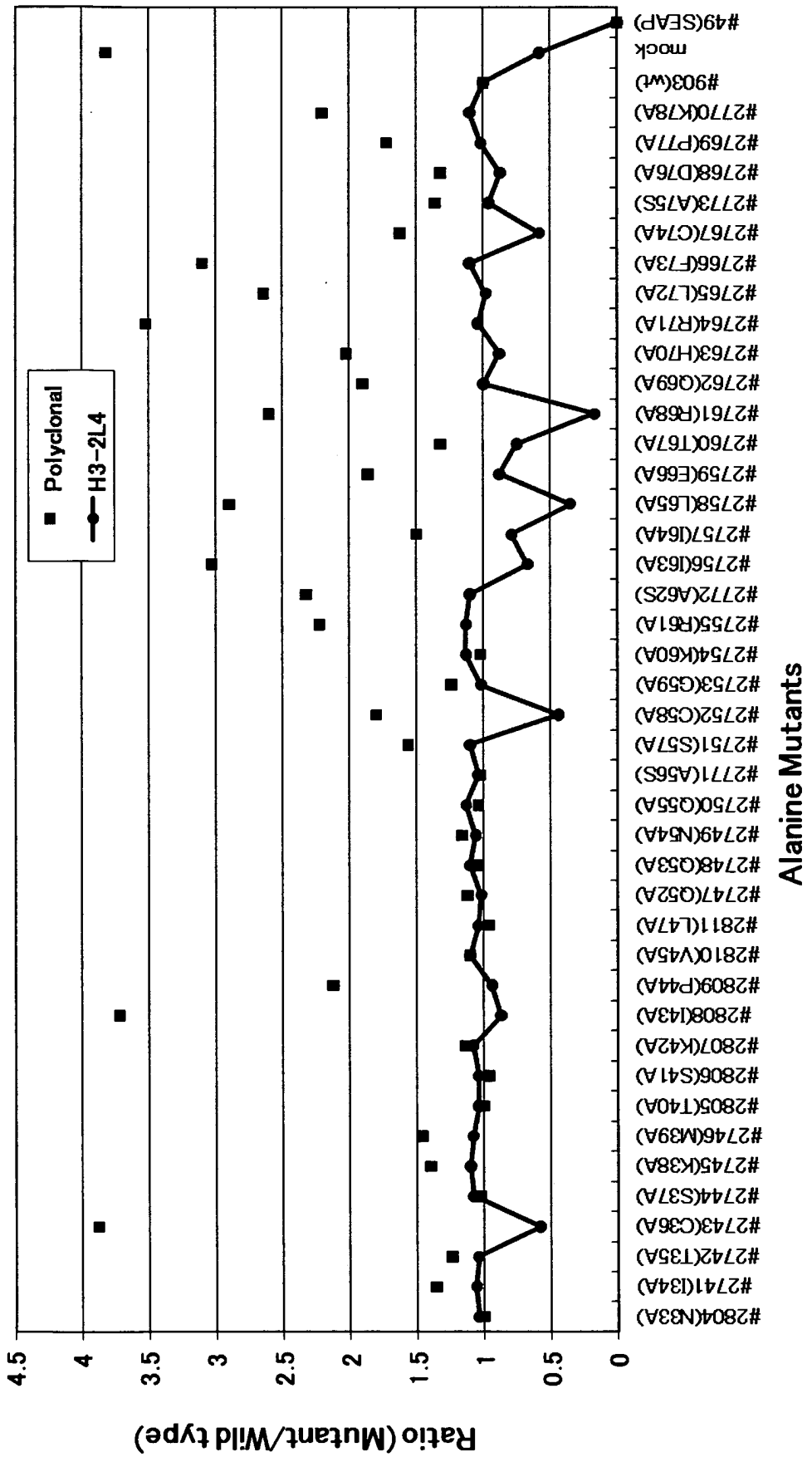


Figure 14

Gray: Cross saturation
Black: Chemical shift mapping

