Figure 1

QDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRG
SKPEDANFYLYLRARVDDGSADDAKKDAAKKDDAKKDDAKKDGSQSIKGNHLVKVYDYQEDGSVLLTCDAEAKNITWFKD
GKMIGFLTEDKKKWNLGSNAKDPRGMYCKGSQNKSKPLQVYYRM (SEQ ID NO: 282)

Figure 2A

	ELISA		FACS			T cell activation	
Clone	Hu-CD3ε/γ muFc	Cyno-CD3ε/γ muFc	Jurkat	Human-PBMCs	Cyno-PBMCs	T cell activation	Isotype
13A3	+	+	+	+	+	weak	IgG2b/k
19B1	+	+	+	+	+	weak	IgG2b/k
72H6	+	+	+	+	+	weak	IgG2a/k
30A1	+	+	+	+	+	weak	IgG1/k
41D9	+	+	+	+	+	weak	IgG1/λ
71H7	+	+	+	+	+	weak	IgG1/λ

Figure 2B

mu IgG	Commercial hCD3eg KD
30A1	0.4 nM
41D9	1.4 nM
13A3	0.6 nM
19B1	0.7 nM
72H6	2.5 nM

Figure 3A

	ELISA		FACS			T cell activation	
Clone	Hu-CD3e 1-27 peptide	Cyno CD3e 1-27 peptide	Jurkat	Human-PBMCs	Cyno-PBMCs	T cell activation	Isotype
39B7	+	+	+	+	+	+	IgG2b/k
27H5	+	+	+	+	+	+	IgG2b/k
38E4	+	+	+	+	+	+	IgG3/k
127B3	+	+	+	+	+	+	IgG3/k
79B7	+	+	+	+	+	+	IgG3/k
95A2	+	+	+	+	+	+	IgG3/k
40D2	+	+	+	+	+	+	IgG2b/k
118G9	+	+	+	+	+	+	IgG3/k
18F2	+	+	+	+	+	+	IgG2a/k
43H8	+	+	+	+	+	+	IgG3/k
32B8	+	+	+	+	+	+	IgG3/k
40G5	+	+	+	+	+	+	IgG2a/k
21B2	+	+	+	+	+	weak	IgG3/k
24H11	+	+	+	+	+	+	IgG2b/k
125A1	+	+	+	+	+	weak	IgG3/k
21A9	+	+	+	+	+	+	
Rab17	+	+	+	+	+	+	Rabbit IgG1

Figure 3B

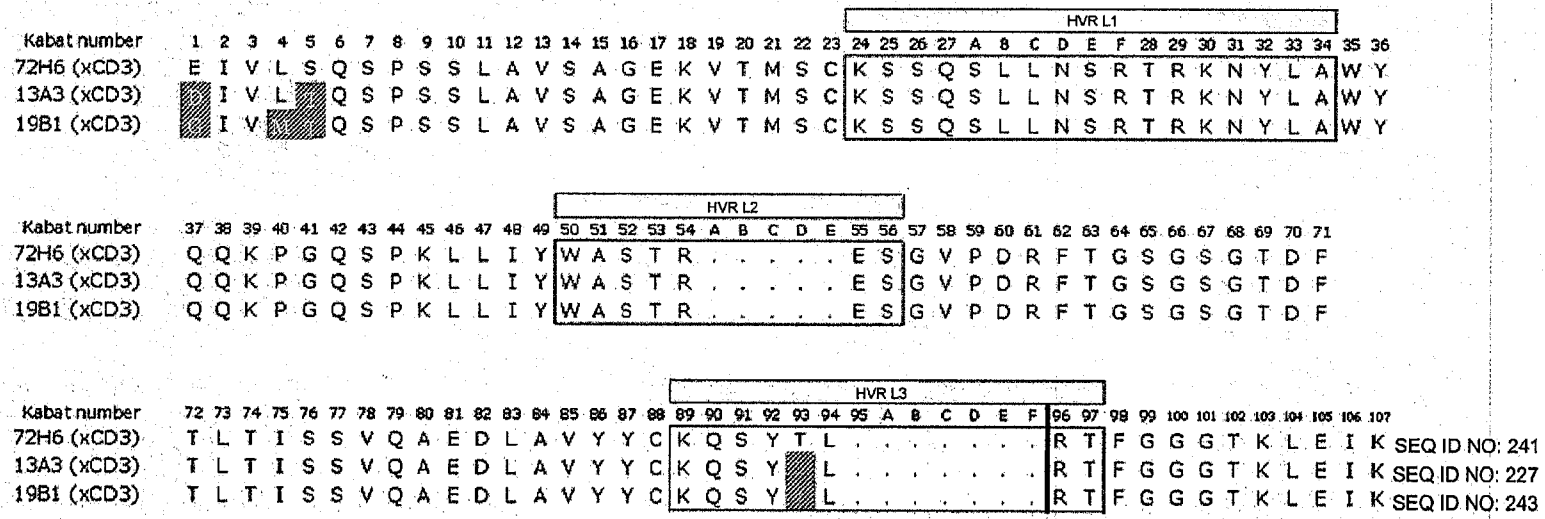
IgG	ka (1/Ms)	kd (1/s)	KD (M)
127B3	1.30E+05	9.67E-05	7.44E-10
14C7	1.66E+05	3.79E-05	2.28E-10
40D2	1.97E+05	1.11E-04	5.63E-10
79B7	1.52E+05	3.41E-05	2.24E-10
40G5	1.56E+05	2.48E-05	1.59E-10
18F12	1.34E+05	1.59E-05	1.19E-10
38E4	4.41E+05	3.45E-05	7.83E-11
95A2	2.90E+05	1.87E-05	6.44E-11
43H8	2.66E+05	1.23E-04	4.61E-10
118G9	1.79E+05	3.74E-05	2.09E-10
27H5-1	2.34E+05	1.15E-04	4.92E-10
39B7	2.20E+05	7.31E-05	3.31E-10
muSP34	1.61E+05	4.00E-05	2.49E-10

Figure 3C

IgG	ka (1/Ms)	kd (1/s)	KD (nM)
mu38E4	5.60E+04	5.32E-05	0.95
mu40G5	3.11E+04	2.69E-05	0.86
Rab17	2.86E+04	2.42E-04	8.46

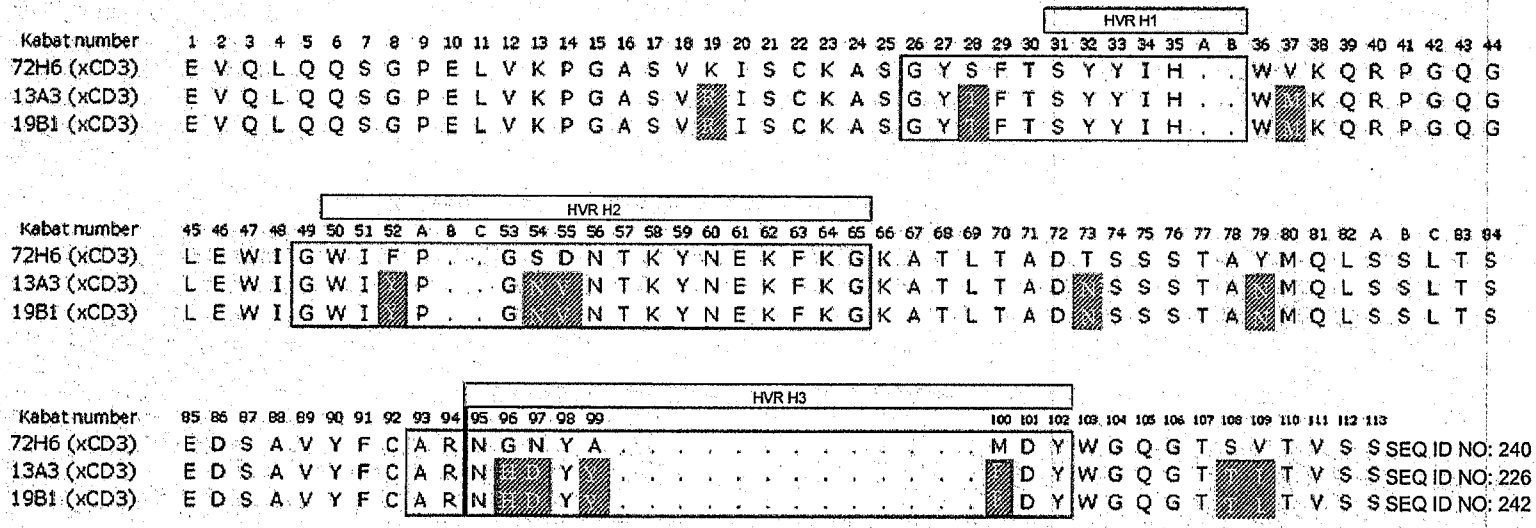
Figure 4A

Light chain, Kappa: Mouse antibody aligned to 72H6 (xCD3)



SEQ ID NO: 241
SEQ ID NO: 227
SEQ ID NO: 243

Heavy chain: Mouse antibody aligned to 72H6 (xCD3)



SEQ ID NO: 240
SEQ ID NO: 226
SEQ ID NO: 242

Figure 4B

13A3	DIVLTQSPSSLAVSAGEKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWAS TRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCKQSYILRTFGGGTKLEIK (SEQ ID NO: 227)
19B1	GIVMTQSPSSLAVSAGEKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWA STRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCKQSYILRTFGGGTKLEIK (SEQ ID NO: 243)
72H6	EIVLSQSPSSLAVSAGEKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWAS TRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCKQSYTLRTFGGGTKLEIK (SEQ ID NO: 241)
30A1	NIVMTQSPSSLAVSAGEKVTMSCKSSQSLLSGRTRKNYLAWYQQKPGQSPKLLIYWA STRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCKQSYYLLTFGAGTKLELK (SEQ ID NO: 231)
41D9a	QAVVTQESALTTSPGGTVILTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTSNRAPG VPVRFSGSLIGDKAALTITGAQTEDDAMYFCALWYSTHFVFGGGTKVTV (SEQ ID NO: 221)
71H7	QAVVTQESALTTSPGGTVILTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTSNRAPG VPVRFSGSLIGDKAALTITGAQTEDDAMYFCALWYSTHFVFGGGTKVTV (SEQ ID NO: 245)

Figure 4C

13A3	EVQLQQSGPELVKPGASVRISCKASGYTFTSYYIHWMKQRPGQGLEWIGWIYPGNVNT KYNEKFKGKATLTADNSSSTANMQLSSLTSEDSAVYFCARNHDYYFDYWGQGTTLTVSS (SEQ ID NO: 226)
19B1	EVQLQQSGPELVKPGASVRISCKASGYTFTSYYIHWMKQRPGQGLEWIGWIYPGNVNT KYNEKFKGKATLTADNSSSTANMQLSSLTSEDSAVYFCARNHDYYFDYWGQGTTLTVSS (SEQ ID NO: 242)
72H6	EVQLQQSGPELVKPGASVKISCKASGYSFTSYYIHWVKQRPGQGLEWIGWIFPGSDNT KYNEKFKGKATLTADTSSSTAYMQLSSLTSEDSAVYFCARNGNYAMDYWGQGTSVTVS S (SEQ ID NO: 240)
30A1	QVQLKESGPGLVAPSQSLSITCTVSGFSLTNYAIHWVRQPPGKGLDWLGVIWAGGNTK YNSALMSRLSFSKDNSKSQVFLEINSLQTDDTAMYYCAREDSSGYVALDYWGQGTSVT VSS (SEQ ID NO: 230)
41D9a	QIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTNTGK PTYAEEFKGRFVFSLETSASTTYLQINNLKNEDTATYFCARRGDYRYAWFLYWGQGTLVT VSA (SEQ ID NO: 220)
71H7	QIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTNTGK PTYAEDFKGRFAFSLETSASTAYLQINNLKNEDTATYFCARRGDYRYAWFTYWGQGTLVT VSA (SEQ ID NO: 244)

Figure 5A

14C7	DVVMTQTPSSLAVSTGEKVTMTCKSSQSLLNIRTRKNCLAWYQQKPGQSPKLLIFWASTRYSGVPDRFTGSGSGTDFTLTIRSVQPEDLAV YYCTQSYTLRTFGGGTKLEIK (SEQ ID NO: 247)
127B3	DIVMTQSPSSLAVSAGEKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQTEDLAV YYCCQSFILRTFGGGTKLEIK (SEQ ID NO: 249)
18F12	DIVMTQSPSSLAVSAGEKVTMTCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAV YYCKQSFILRTFGGGTKLELK (SEQ ID NO: 251)
27H5-1	DIVMTQSPSSLAVSVGEKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWASTRESGVPVRFTGSGSGTDFTLTISSVQAEDLAV YYCTQSFILRTFGGGTKLEIK (SEQ ID NO: 253)
38E4	DIVMTQSPSFLAVSAGEKVTMSCKSSQSLLNSRTRKNYLAWYQQRPGQSPKLLIYWTSTRKSGVPDRFTGSGSGTDFTLTISSVQAEDLAI YYCKQSFILRTFGGGTKLEIK (SEQ ID NO: 286)
39B7	DIVMTQSPSSLAVSAGEKVTMTCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAV YYCKQSFILRTFGGGTKLEIK (SEQ ID NO: 255)
40D2	DIVMTQSPSSLAVSAGEKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIFWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAV YYCKQSFILRTFGGGTKLELK (SEQ ID NO: 257)
40G5	DIVLTQSPSSLAVSAGEKVTMSCKSSQSLLNSRTRKNYLTWYQQKPGQSPKLLIYWASTRDSGVPDRFSGSGSGTDFTLTISSVQAEDLAV YYCTQSYTLRTFGGGTKLEIK (SEQ ID NO: 292)
79B7	DIVMTQSPSSLAVSAGEKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQTEDLAV YYCCQSFILRTFGGGTKLEIK (SEQ ID NO: 259)
95A2	DIVMTQSPSSLAVSAGEMVTLHCKSSQSLLNIRTRKNYLAWYLQKPGQSPRLLIYWASTRASGVPDRFTGSGSGTDFTLTISSVQPEDLAV YYCTQSFILRTFGGGTKLELK (SEQ ID NO: 261)
118G9	DIVMTQSPSSLAVSAGEKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGLSPKLLIYWASTRESGVPERFTGSGSGTDFTLTISSVQTEDLAV YYCTQSFILRTFGGGTRLEIK (SEQ ID NO: 263)

Figure 5B

14C7	EVQLQQSGPELVKPGTSVKISCKASGFSFTNYYIHWMKQRPGQGLEWIGWIYPGSDNTKFNDKFKGKATLTADTSSTTAYMQLSSL TSEDSAVYYCARDSITNYYFDYWGQGTTLTVSS (SEQ ID NO: 246)
127B3	QVQLQQSGSELVRPGASLKLSCKASGYTFTSYWMHWVRQRPGQGLEWIGNFYPGDLTVNYDEKFKNKVTLAVDTSSSTAFLQLSSL TSEDSAVYYCTRDAYSRYFFDYWGQGTTLTVSS (SEQ ID NO: 248)
18F12	QVQLQQSGPELVKPGASVKISCKASGYSFTNYYMHWVKQRPGQGLEWIGWISPGSGSIKYNEKFKGKATLTADTSSSTAYMQLSSL TSEDSAVYYCARDGYSLYFFDFWGQGTTLTVSS (SEQ ID NO: 250)
27H5-1	QVQLQQSGPELVKPGTSVKISCKTSGYSFINYYTHWVKQRPGQGLEWIGWIYPGNGNIKYNEKFMGKATLTADTSSTTAHMHLSSL VSEDSAVYYCARDSYGSYYFDYWGQGTTLTVSS (SEQ ID NO: 252)
38E4	QVQLQQSGPELVKPGASVTISCTASGFTFTSYYIHWVRQRPGQGLEWIGWIYPENDNTKYNEKFKDKATLTADTSSSTAYMQLSSL TSEDSAVYYCARDGYSRYYFDYWGQGTTLTVSS (SEQ ID NO: 285)
39B7	QVQLQQSGPELVKPGTSVKISCKTSGYNFINYYTHWVKQRPGQGLEWIGWIYPGNGNIKYNEKFMGKATLTADTSSTTAHMHLSSL VSEDSAVYYCARDSYGSYYFDYWGQGTTLTVSS (SEQ ID NO: 254)
40D2	QVQLQQSGPELVKPGASLKISCKASGYSFANYYTHWVRQRPGQGLEWIGWLYPGNGDTRYNEKFKDKATLTADTSSNTASMQLNSL TSEDSAVYYCTRDSYGNYFFDYWGQGTTLIVSS (SEQ ID NO: 256)
40G5	QVQLQQSGPELVKPGTSVKMSCKASGYTFTNYYIHWVRQRPGQGLEWIGWIYPGDVSTKYNEKFKGKTTLTADKSSSTAYMLLSGL TSEDSAIYFCARDGYSFYYFDYWGQGTTLTVSS (SEQ ID NO: 291)
79B7	QVQLQQSGSELVRPGASLKLSCKASGYTFTSYWMHWVRQRPGQGLEWIGNSYPGDLNVNYDEKFKNKVTLAVDTSSSTAFLQLSSL TSEDSAVYYCTRDAYSRYFFDYWGQGTTLTVSS (SEQ ID NO: 258)
95A2	QVQLQQSGPEMVKPGASVTISCKTSGFTFTSYYIHWMMQRPGQGLEWIGWLYPGDVSTRYNEKFRDKATLTADKSSTTAYLYLSSL TSEDSAVYFCARDSSASYYFDFWGQGTTLTVSS (SEQ ID NO: 260)
118G9	QVQLQQPGSELVRPGASVTLSCTASGYSFTSYWMHWVKQRPGQGLEWVGNIYPGGEIINYAEKFKTKGTLTVDISSSTAYMHLSSL TSDDSAVYYCTRDTTGNYFFDYWGQGTTLTVSS (SEQ ID NO: 262)

Figure 6A

21A9

DIQMTQSPSSLAVSLGERATINCKSSQSVLYSSNNKNYLVWYQQKLGQPPKLLIYRASTR
ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSVPWTFGQGTKVEIK
(SEQ ID NO: 235)

Rab17

DMTQTPSSKSVPVGDTVTINCQASETVYSNNYLAWFQQKPGQPPKRLIYGVSTLDSGVP
PRFSGSGSGTQFTLTISDVVCDDAATYYCAGYKTSSSYAIAFGGGTELEIL
(SEQ ID NO: 265)

Figure 6B

21A9

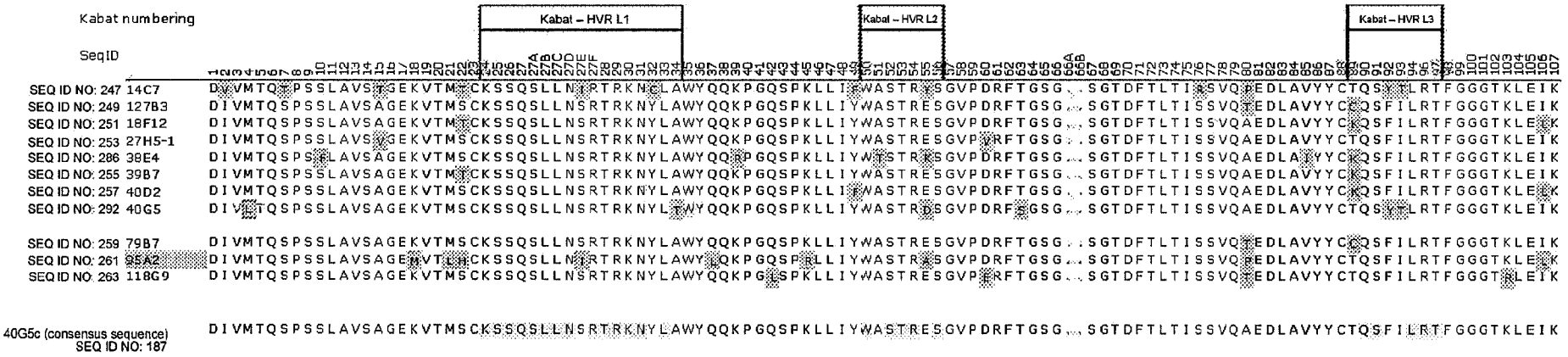
EVQLVESGGGVVQPGGSLKLSCAASGFTFSGSAMHWVRQASEKGLEWVGRIRSRANSYA
TAYAASVKDRFTISRDNSKNTAYLQMNSLKTEDTAVYYCIRDTMVRGIDYWGQGTLVTVSS
(SEQ ID NO: 234)

Rab 17

QSVKESEGGLFKPTDTLTLTCTVSGCSLSSCAISWVRQAPGKGLEWIGFMSVTGSAYYAN
WAKSRSTITRNTNENTVTLKMTRLTAADTAIYFCARVGIGSGLNIWGQGTLVTVSS
(SEQ ID NO: 264)

Figure 7

Light chain



Heavy chain

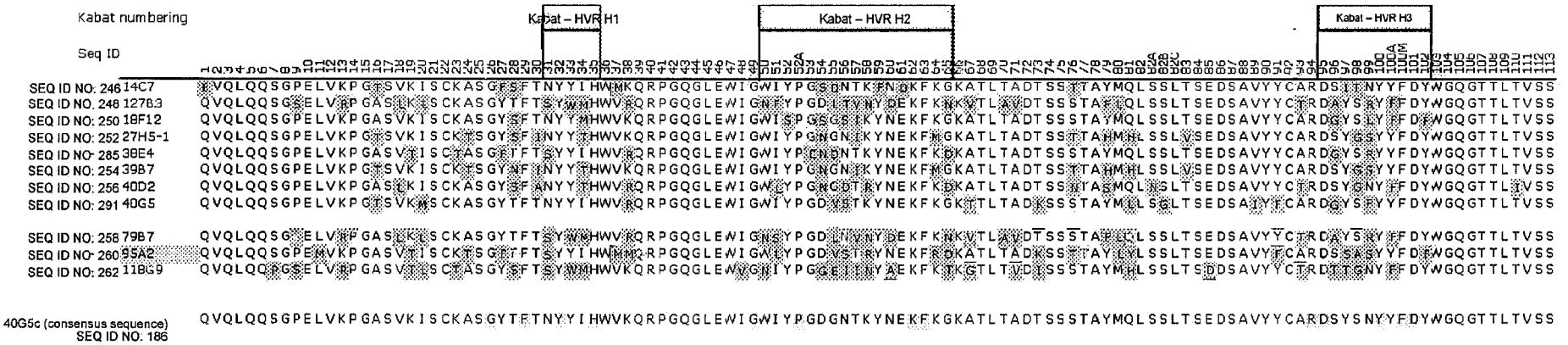
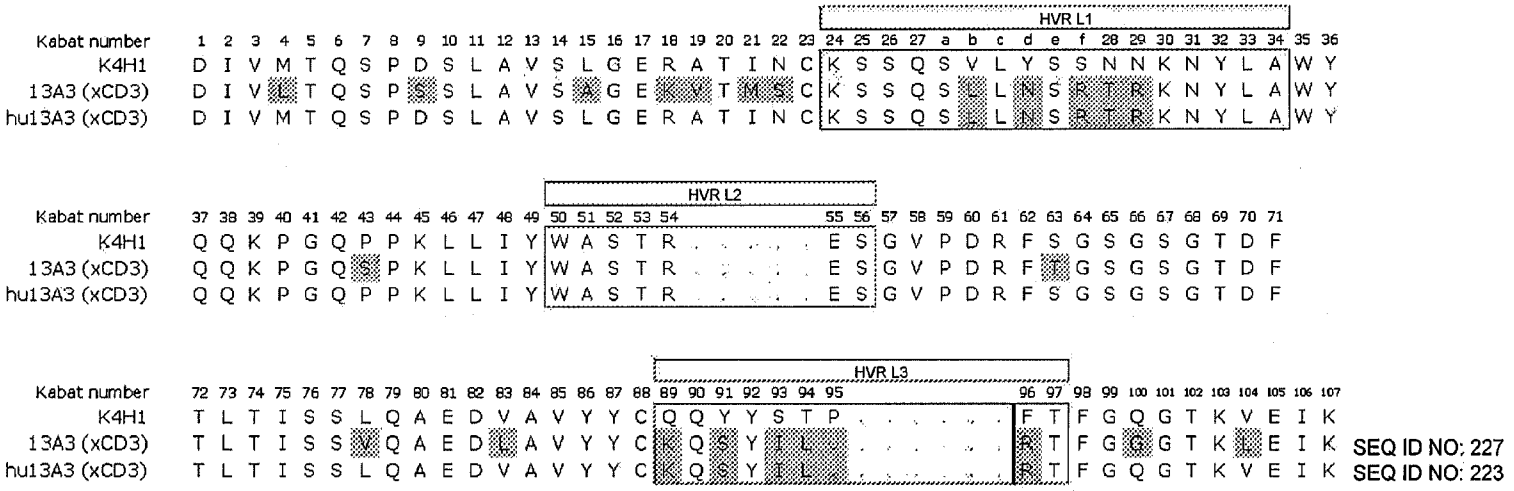


Figure 8A

Light chain, Kappa: Mouse antibody aligned to K4H1



Heavy chain: Mouse antibody aligned to K4H1

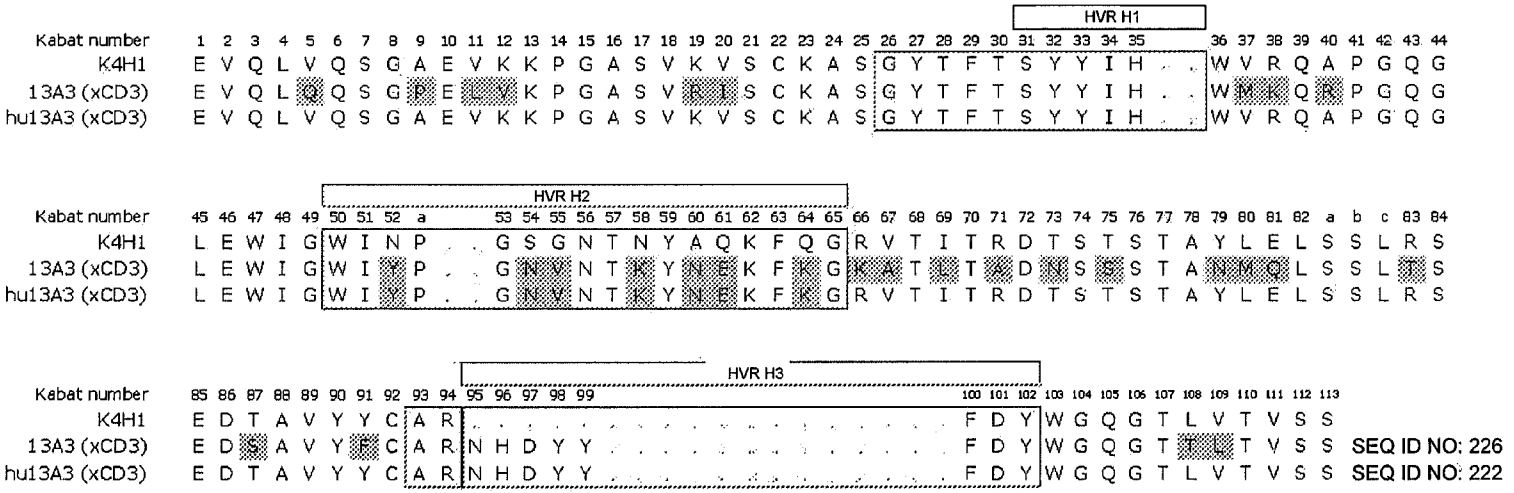
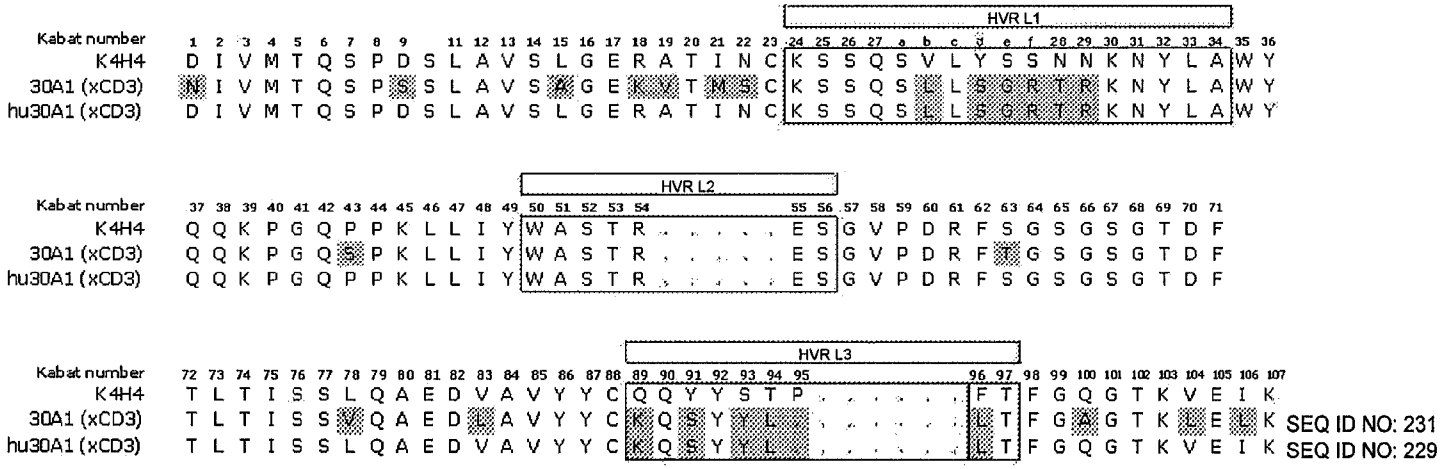


Figure 8B

Light chain, Kappa: Mouse antibody aligned to K4H4



Heavy chain: Mouse antibody aligned to K4H4

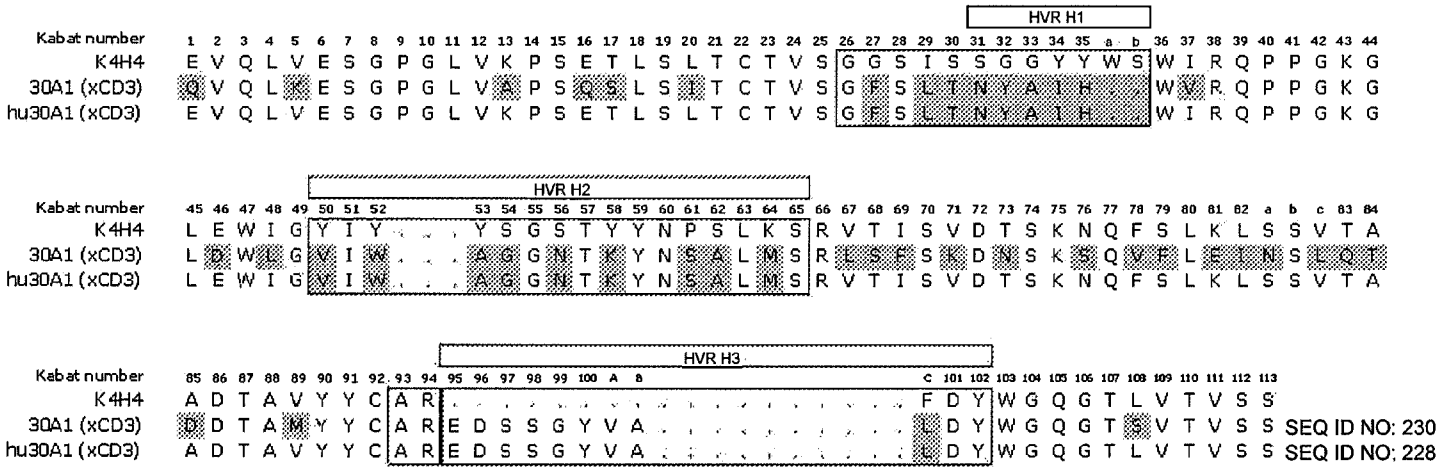
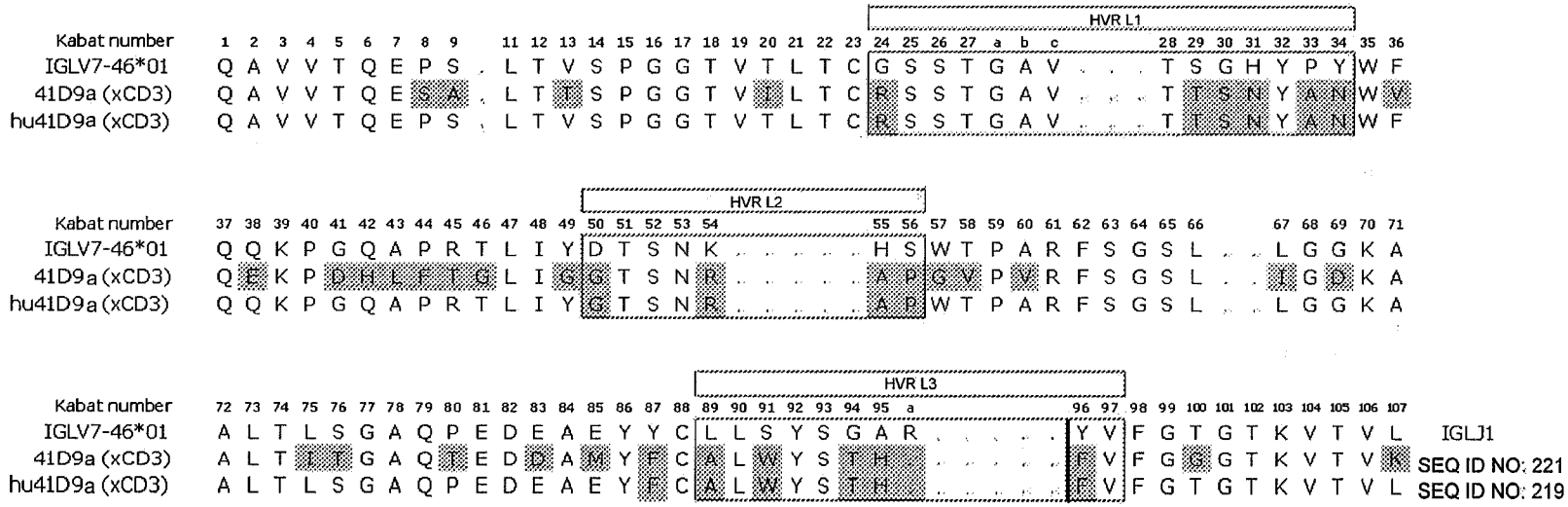


Figure 8C

Light chain, Lambda: Mouse antibody aligned to human germlines



Heavy chain: Mouse antibody aligned to human germlines

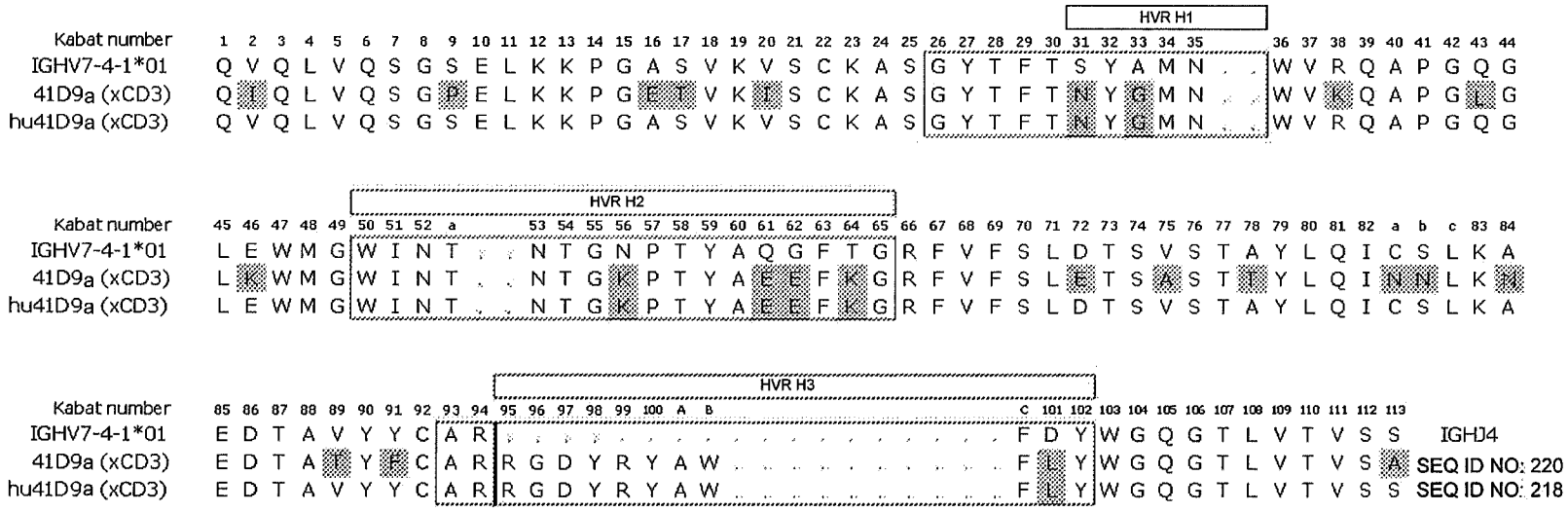
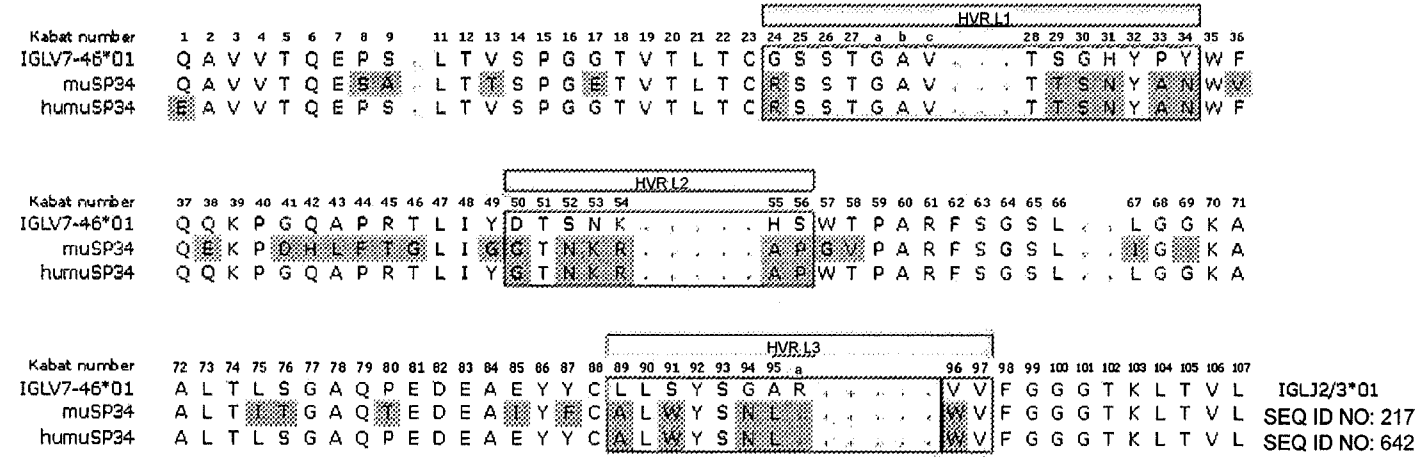


Figure 8D

Light chain, Lambda: Mouse antibody aligned to human germlines



Heavy chain: Mouse antibody aligned to human germlines

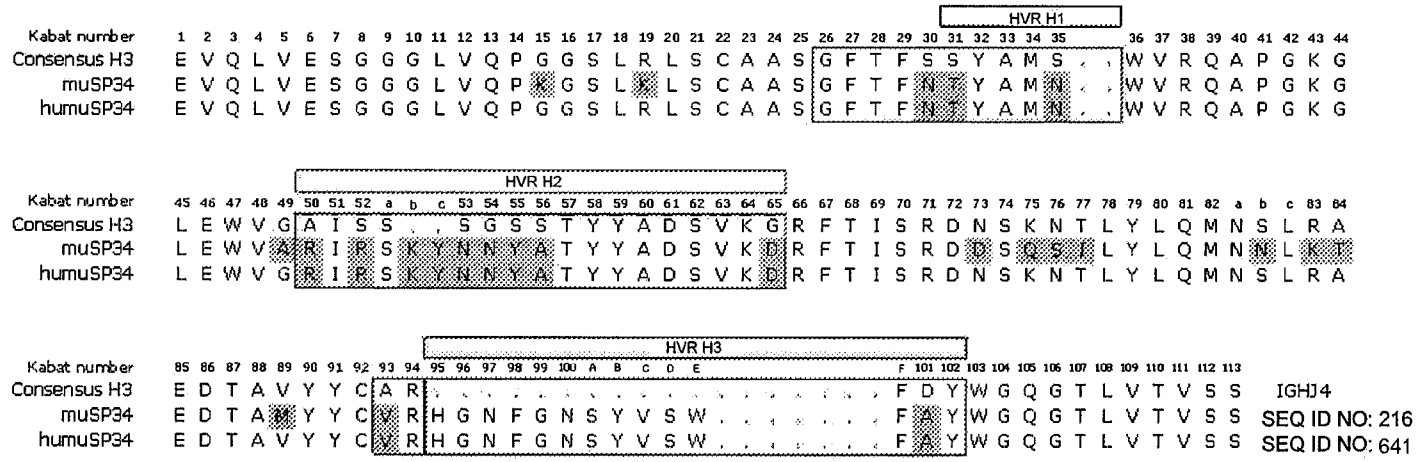
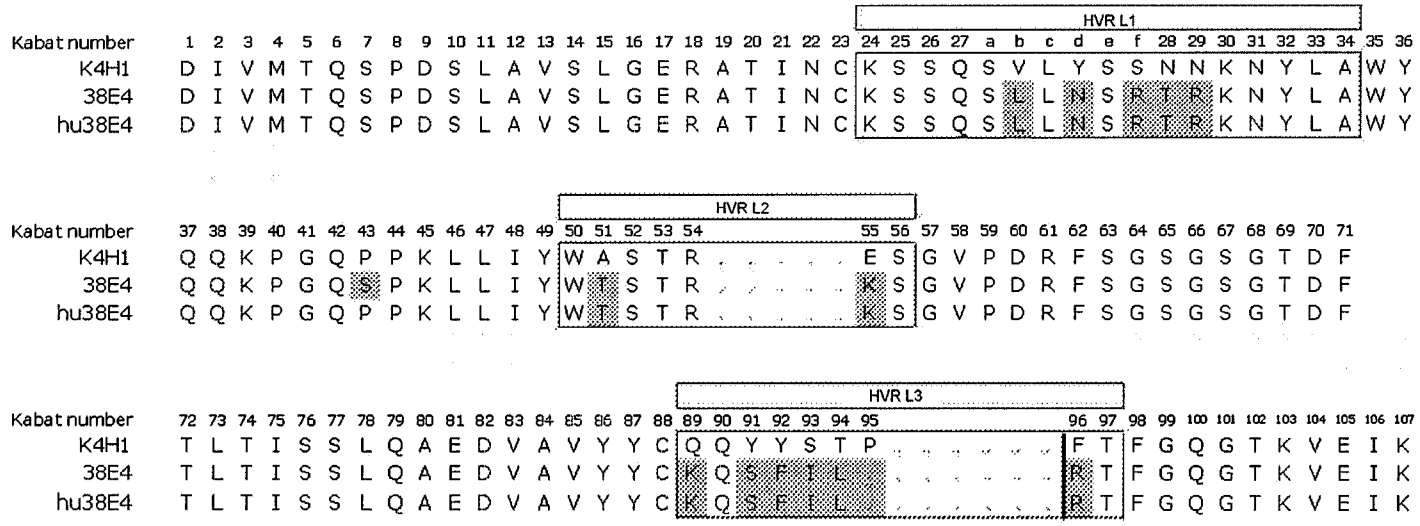


Figure 8E

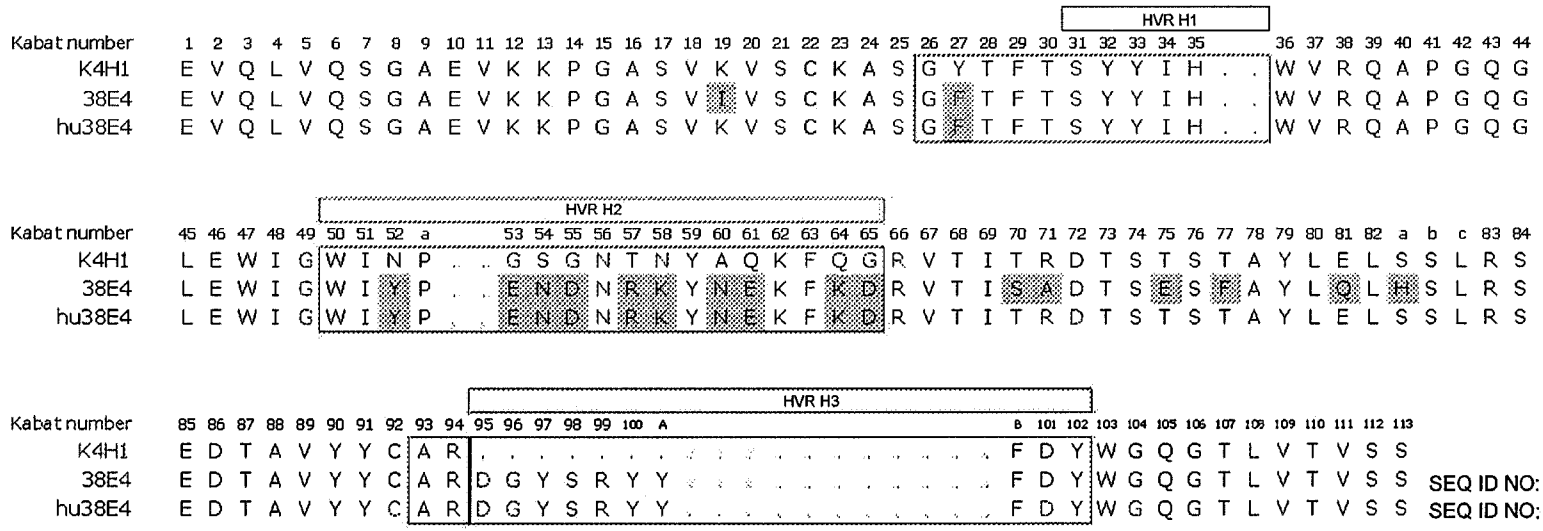
Light chain, Kappa: Humanized antibody aligned to K4H1



SEQ ID NO: 288

SEQ ID NO: 290

Heavy chain: Humanized antibody aligned to K4H1

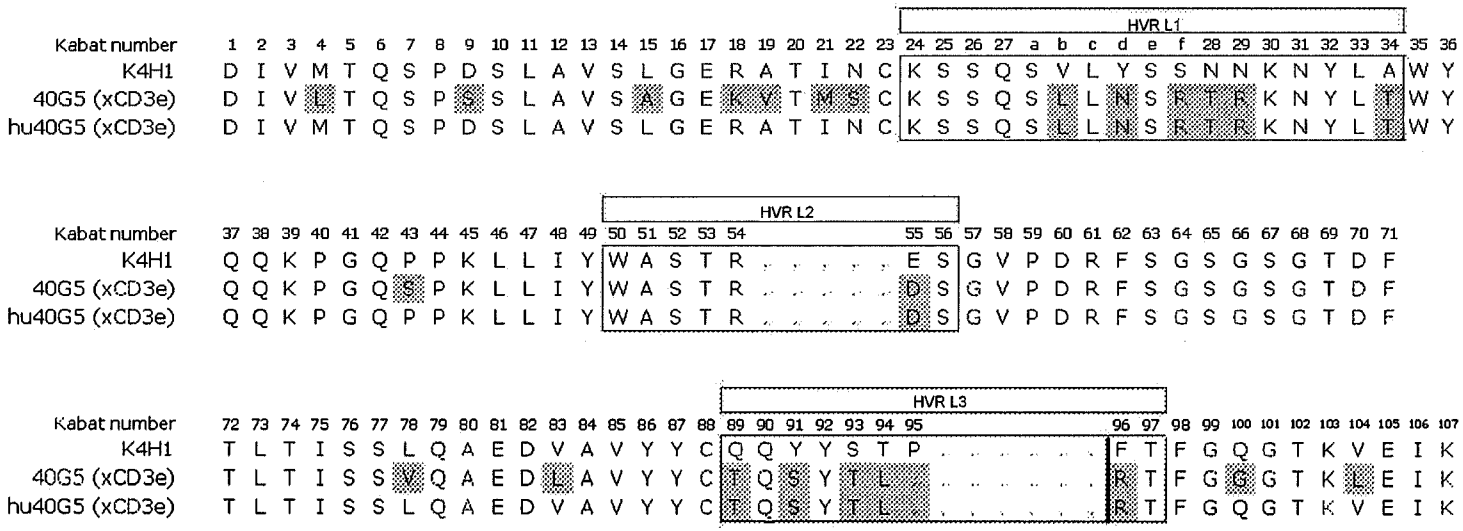


SEQ ID NO: 287

SEQ ID NO: 289

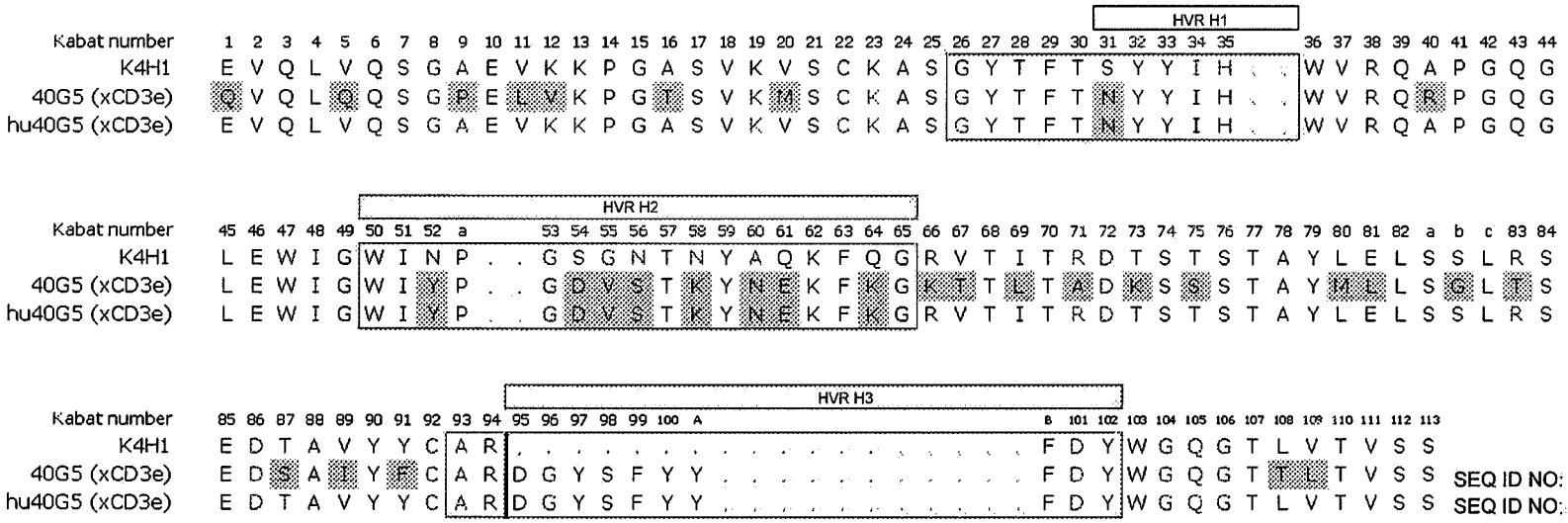
Figure 8F

Light chain, Kappa: Mouse antibody aligned to K4H1



SEQ ID NO: 292
SEQ ID NO: 640

Heavy chain: Mouse antibody aligned to K4H1



SEQ ID NO: 291
SEQ ID NO: 639

Figure 9A

murine 13A3 = 0.7 nM

Humanized 13A3 variants:

Heavy chain	K4 graft	K4 graft + S43A
VH1 graft	1.5 nM	1.8 nM
VH1 graft + R71A	1.0 nM	1.4 nM
VH1 graft + V37M + V67A + R71A + T73N	0.8 nM	ND
VH1 graft + V37M + V67A + R71A + T73N + Y91F	1.2 nM	1.4 nM

Figure 9B

murine 30A1= 0.4 nM

Humanized 30A1 variants:

Heavy chain	K4 graft	K4 graft + S43A
VH3 graft	2.9nM	1.4 nM
VH3 graft + A24V	1.7 nM	1.7 nM
VH3 graft + R71K + L78V	1.8 nM	2.2 nM
VH3 graft + A24V + R71K + L78V	2.3 nM	1.5 nM
VH3 graft + A24V + V48L + F67L + I69F + R71K + L78V	2.4 nM	1.4 nM
VH4 graft + V71K + F78V	15.4 nM	5.7 nM
VH4 graft + I37V + I48L + V67L + I69F + V71K + F78V	2.4 nM	1.7 nM

Figure 9C

murine 41D9 = 1.1 nM

Humanized 41D9 variants:

Heavy chain	λ7 graft + F36V + Q38E + A43L + P44F + T46G + Y49G + G69D + Y87F
VH7 graft + V2I + A78T + Y91F	ND

Figure 9D

murine SP34 = 7 nM

Humanized SP34 variants:

Light chain	VH3 graft +T93V	VH3 graft + G49A +T93V	VH3 graft + N73D + T93V	VH3 graft G49A + N73D + T93V
λ7 graft + Y49G			weak binding	
λ7 graft + F36V + T46G + Y49G	15.9 nM		3.5 nM	14.5 nM
λ7 graft + F36V + Q38E + A43L + P44F + T46G + Y49G + T58V + Y87F		21 nM	3.1 nM	8 nM
λ2 graft + Y49G		no binding		
λ2 graft + A43L + P44F + L46G + M47L + Y49G		weak binding	13 nM	47 nM

Figure 9E

murine 38E4 = 0.75 nM

Humanized 38E4 variants:

Heavy chain	K4 graft + P43S
VH1 graft + R71A	0.5 nM

Figure 9F

murine 40G5c CDRs were derived from consensus sequence

Humanized 40G5c variants:

Heavy chain	K4 graft
VH1 graft + V67A + R71A	37 nM
VH1 graft + I69L + R71A	36 nM
VH1 graft + V67A + I69L + R71A	24 nM

Figure 10

Variants	hCD3ε$^{1-27}$-Fc (nM)	huCD3εγ (nM)	cyCD3εγ (nM)	Commercial hCD3εγ
hu13A3.v2				1
hu30A1.v2				2
hu41D9a				ND
huSP34.v52	11	19	12	4
hu38E4.v1	1.0	0.5	0.7	
hu40G5c	13	12	14	

Figure 11

	Version	ka (1/Ms)	kd (1/s)	KD (nM)
38E4	v1	6.06E+06	0.0051	0.8
38E4	v2	2.29E+06	0.0036	1.6
38E4	v3	4.11E+06	0.0140	3.4
38E4	v4	3.25E+05	0.0034	10
40G5	**c**	**1.20E+06**	**0.01764**	**15**
38E4	v5	2.05E+06	0.0225	11
38E4	v6	5.11E+04	0.0017	34
38E4	v7	2.83E+05	0.0246	87
38E4	v8	1.12E+06	0.2018	180
38E4	v9	7.26E+04	0.0166	229

Figure 12A

	Single Cycle Kinetics				Full Cycle Kinetics			
L3 mutants	ka (1/Ms)	kd (1/s)	KD (nM)	ratio (fold)	ka (1/Ms)	kd (1/s)	KD (nM)	ratio (fold)
K89A	4.28E+05	1.02E-03	2.4	0.7				
Q90A					1.78E+05	4.22E-03	23.8	29.9
S91A	3.44E+05	1.35E-03	3.9	1.2				
F92A					1.46E+06	2.32E-02	15.9	20.0
I93A					3.08E+06	1.24E-02	4.0	5.1
L94A	2.83E+05	2.95E-04	1.0	0.3				
R96A			NB	NB				
T97A	2.96E+05	8.60E-04	2.9	0.9				
H3 mutants	ka (1/Ms)	kd (1/s)	KD (nM)	ratio (fold)	ka (1/Ms)	kd (1/s)	KD (nM)	ratio (fold)
R94A	5.55E+04	4.35E-03	78.4	23.3				
D95A	1.27E+05	3.09E-03	24.3	7.2				
D95T					51140	0.001749	34.2	43.0
D95S					1.12E+06	0.2018	179.5	225.8
G96A					1.86E+06	3.57E-03	1.9	2.4
Y97A			NB					
S98A	3.85E+05	1.47E-03	3.8	1.1				
R99A			NB					
Y100A					4.60E+04	1.91E-02	414.5	521.4
Y100aA					2.83E+05	2.46E-02	87.0	109.5
F100bA			NB					
D101A	1.42E+05	3.42E-03	24.1	7.2				
Y102A	3.22E+05	1.32E-03	4.1	1.2				
hu38E4.v1	3.20E+05	1.08E-03	3.4	1.0				
hu38E4.v1					5.44E+06	4.33E-03	0.8	1.0

Figure 12B

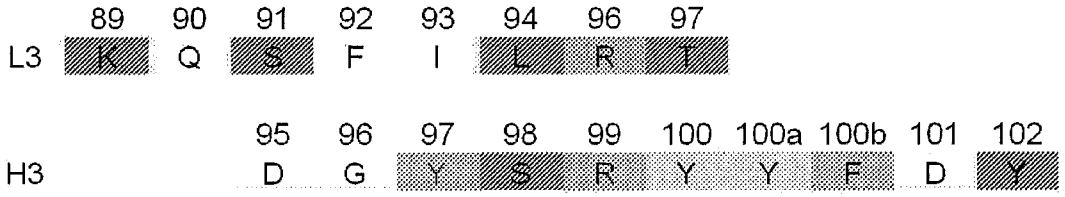
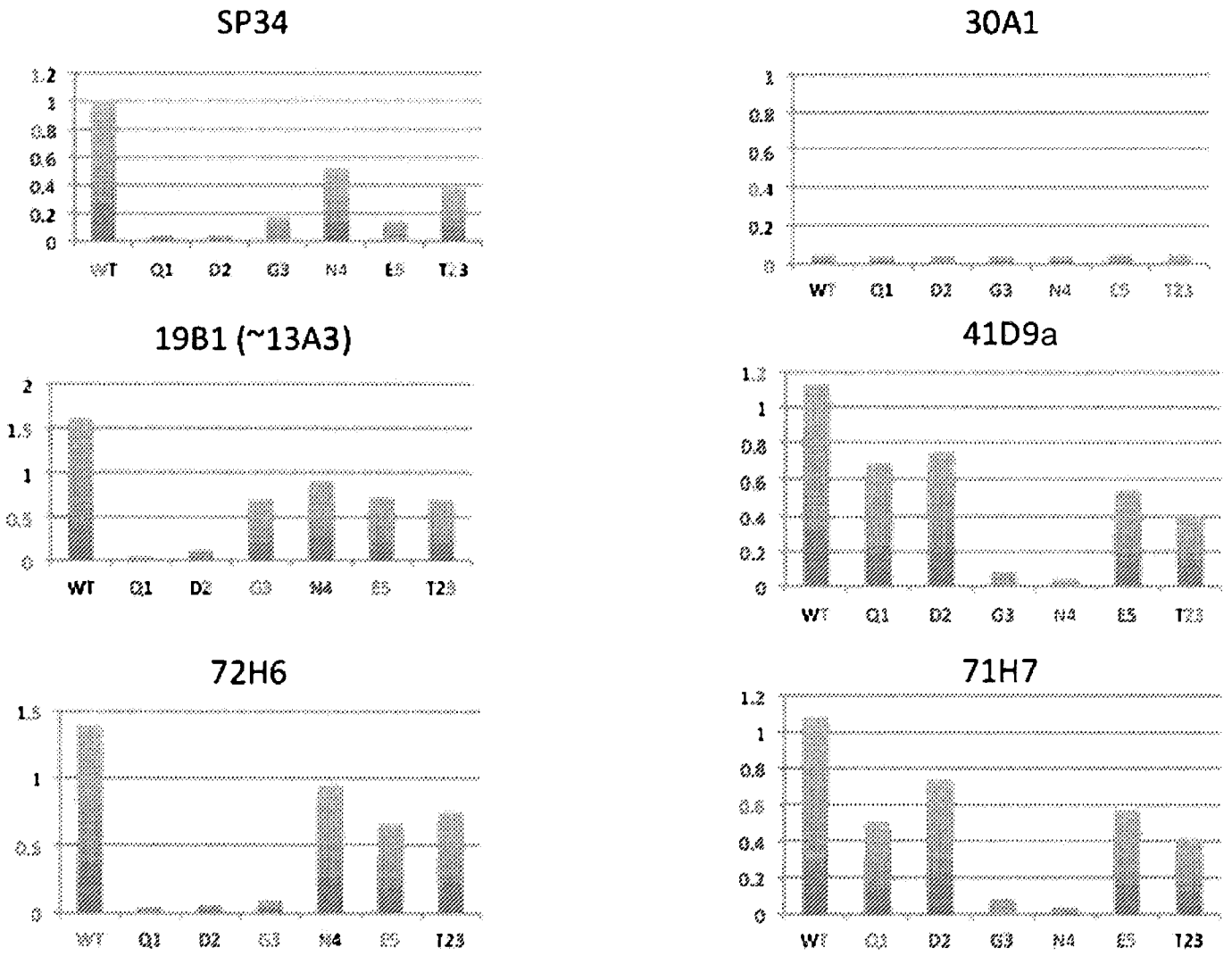

89 90 91 92 93 94 96 97
L3 K Q S F I L R T
95 96 97 98 99 100 100a 100b 101 102
H3 D G Y S R Y Y F D Y

Figure 13A



Antibody binding to peptide: each IgG at 3nM. secondary anti-mouse IgG-HRP, OD @ 450nm

Figure 13B

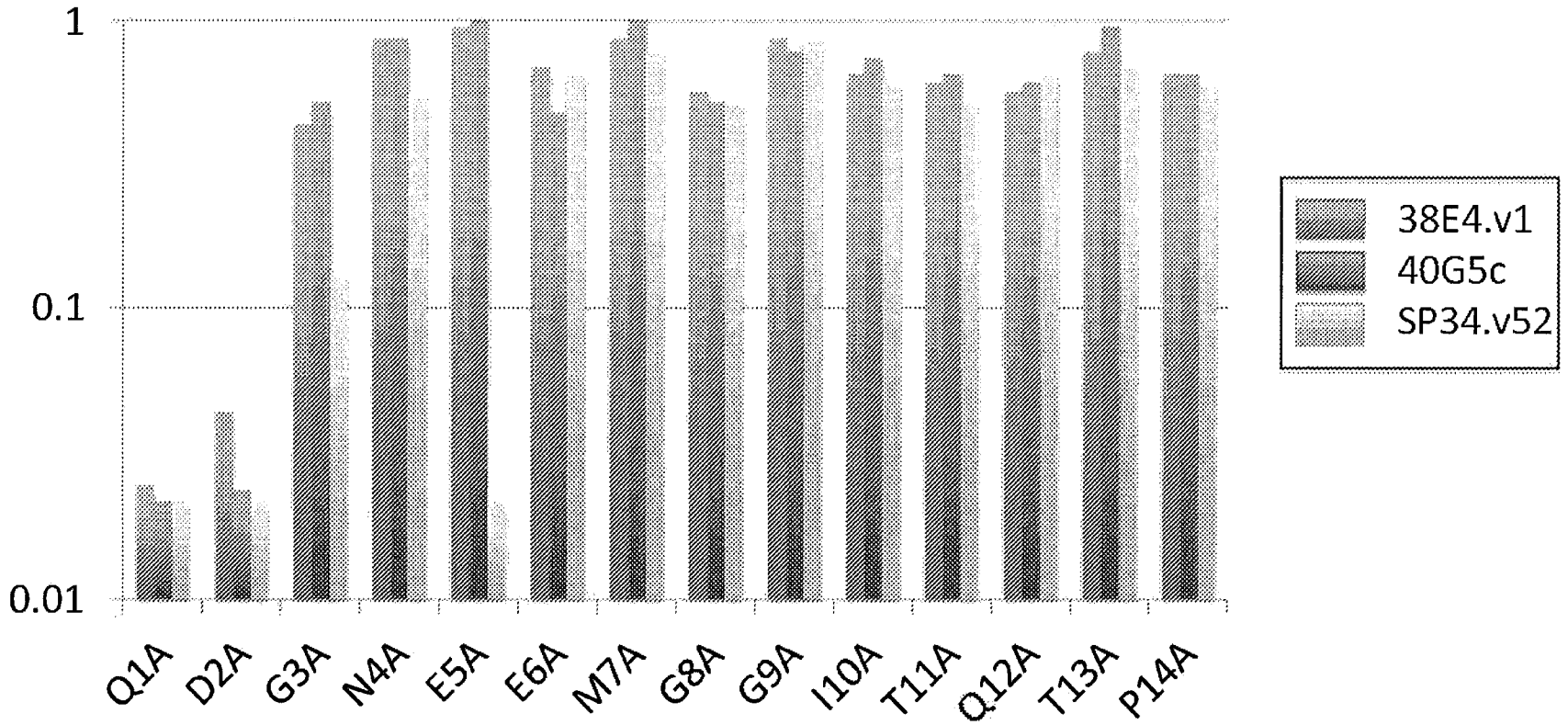

1
0.1
0.01
Q1A
D2A
G3A
N4A
E5A
E6A
M7A
G8A
G9A
I10A
T11A
Q12A
T13A
P14A
38E4.v1
40G5c
SP34.v52

Figure 13C

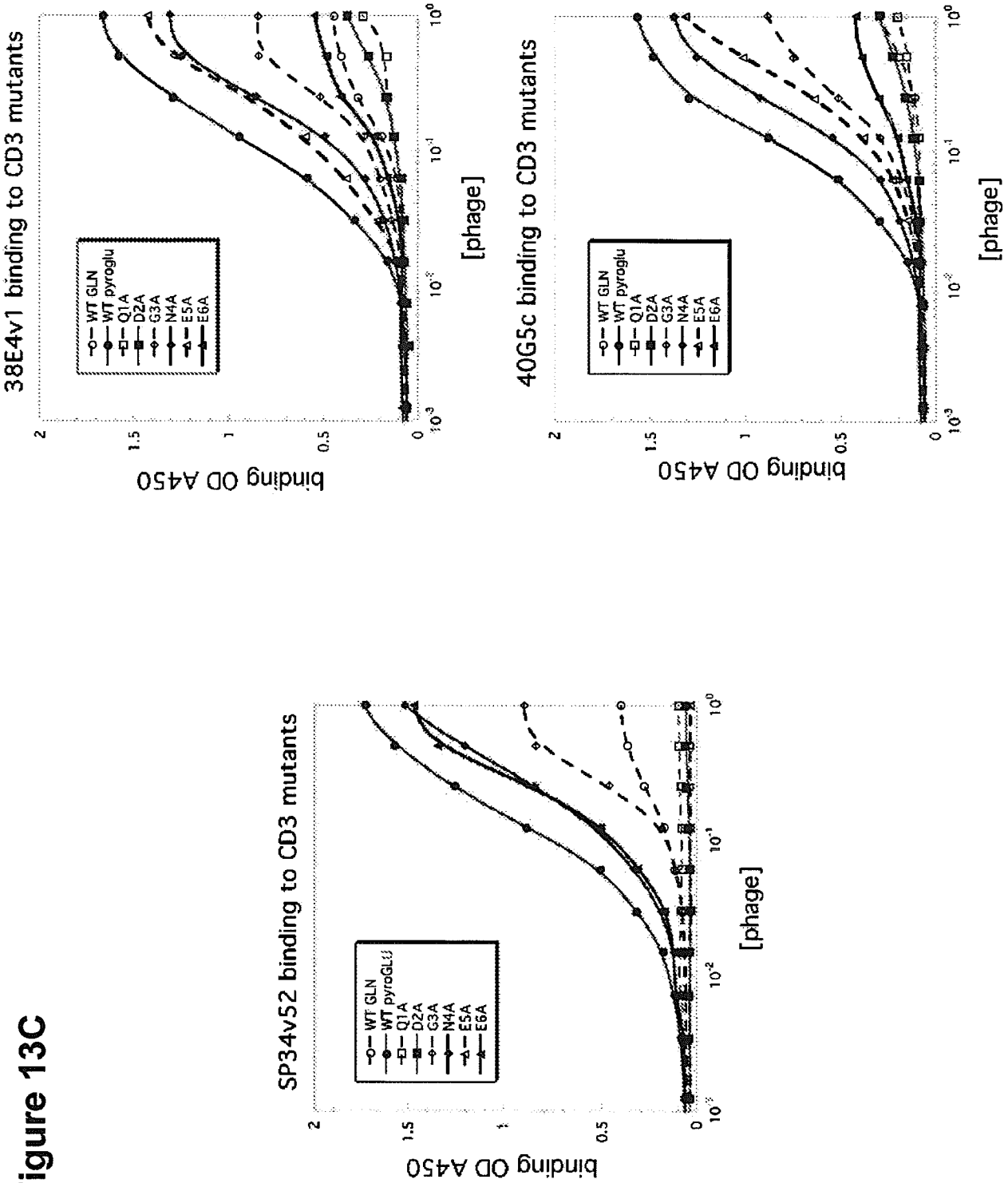

38E4v1 binding to CD3 mutants
40G5c binding to CD3 mutants
SP34v52 binding to CD3 mutants
binding OD A450
[phage]
WT GLN
WT pyroglu
WT pyroGLU
Q1A
D2A
G3A
N4A
E5A
E6A

Figure 13D

SP34v52 Affinity KD (nM)	
WT	12
Q1A	NB
D2A	NB
G3A	172
N4A	20
E5A	19000
E6A	17
M7A	17
G8A	27
G9A	25

38E4v1 Affinity KD (nM)	
WT	3
Q1A	1000
D2A	1500
G3A	7
N4A	3
E5A	7
E6A	65
M7A	13
G8A	8
G9A	6

Figures 14A-14F

A 16mer peptide sequence: pyroGlu-D-G-N-E-E-M-G-G-I-T-Q-T-P-Y-K

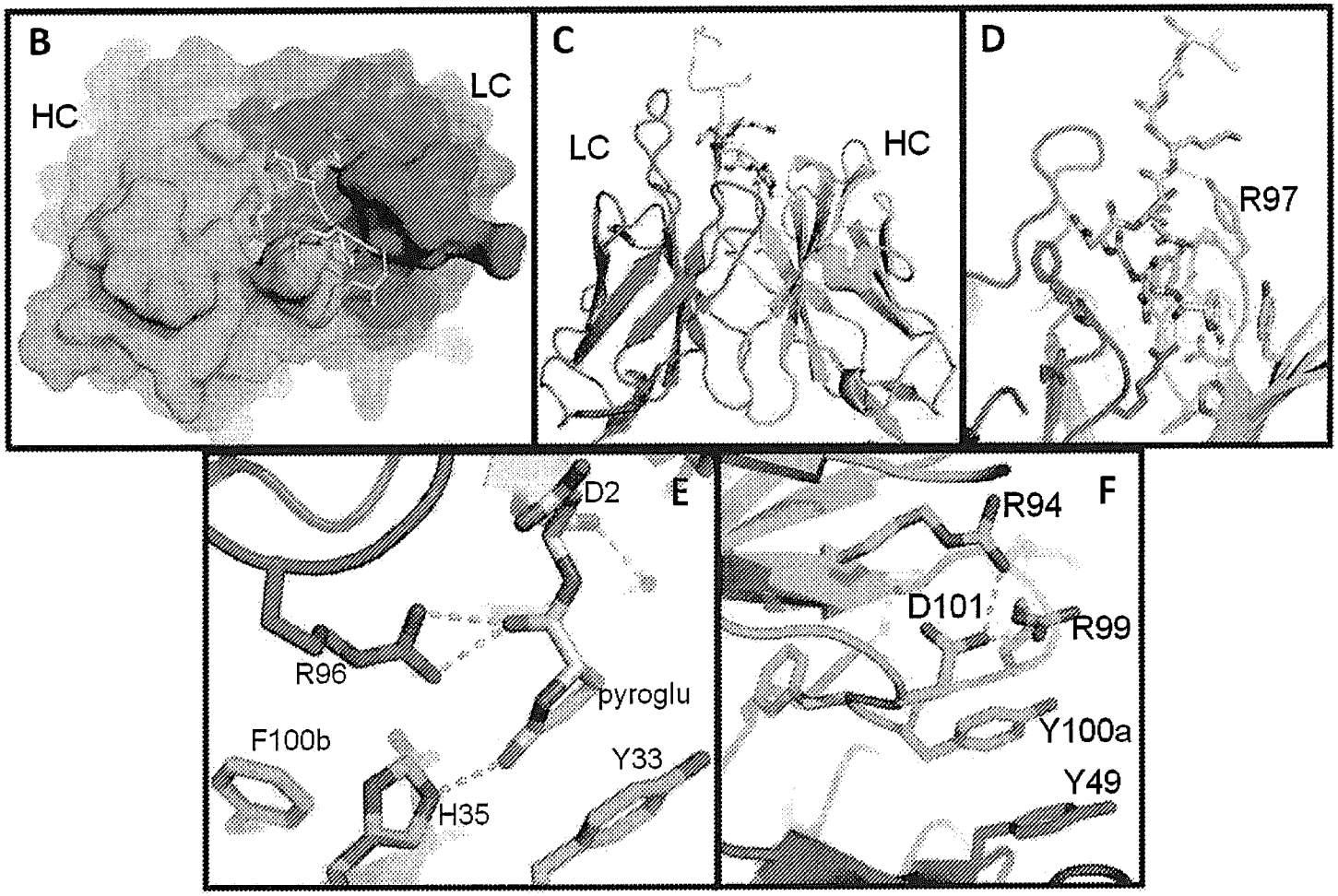
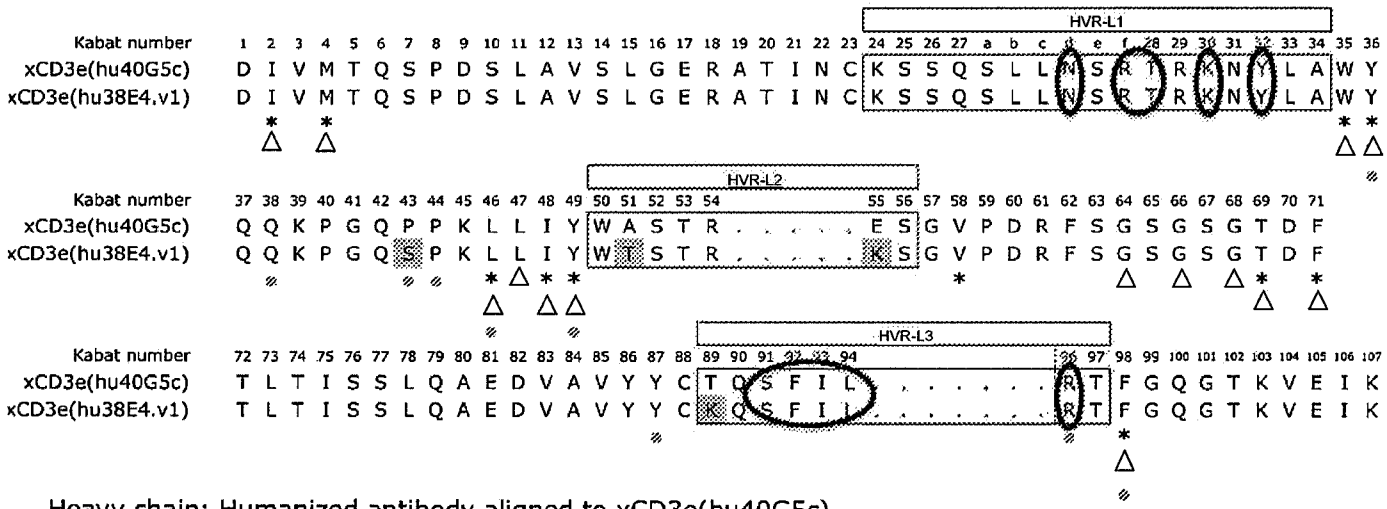
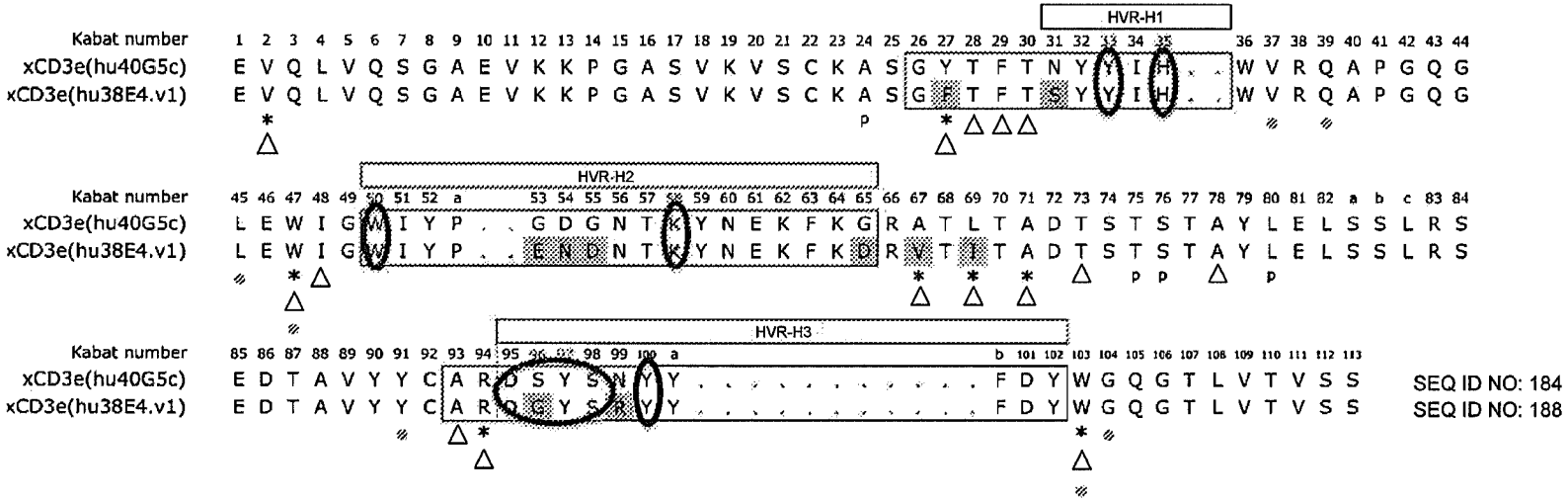


Figure 14G

Light chain, Kappa: Humanized antibody aligned to xCD3e(hu40G5c)



Heavy chain: Humanized antibody aligned to xCD3e(hu40G5c)

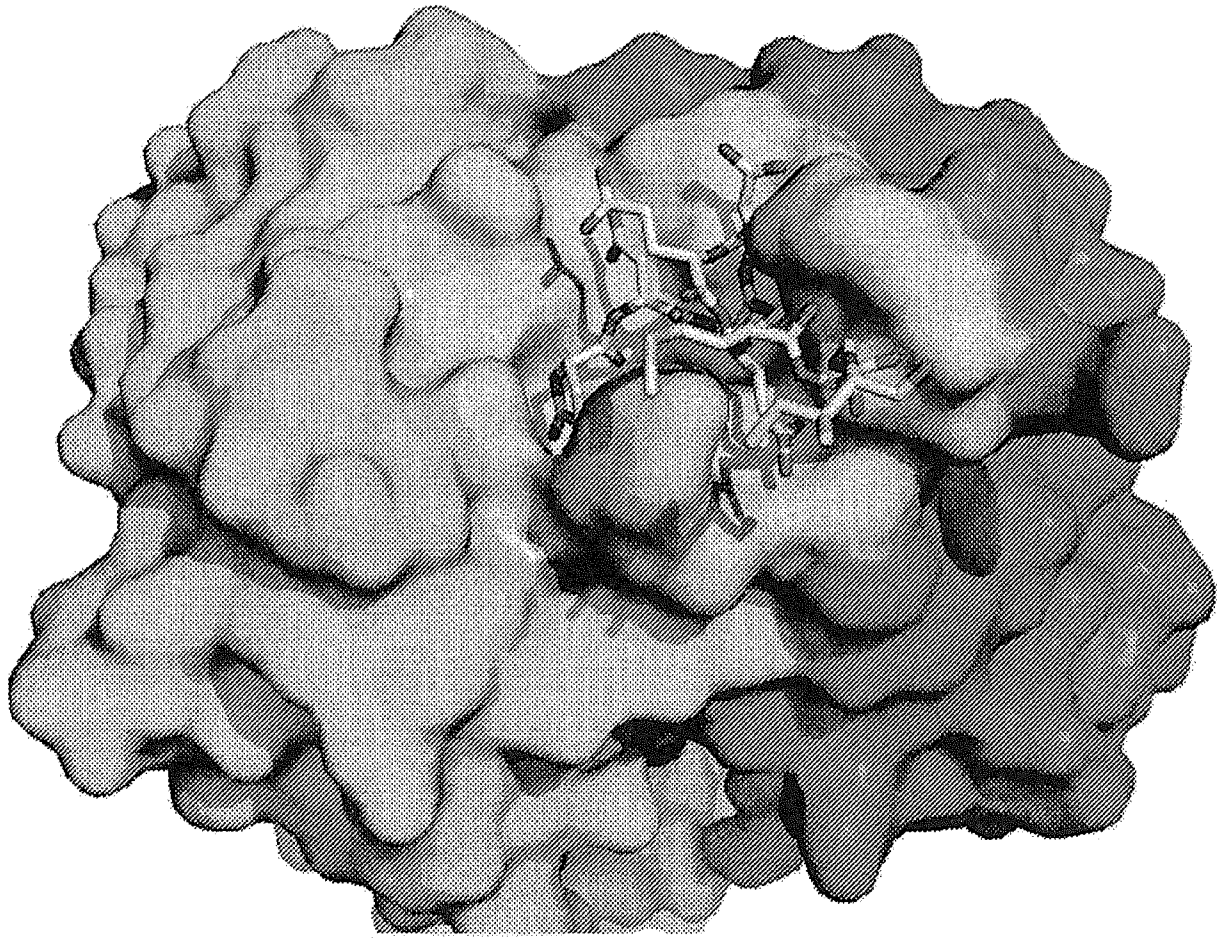


△ Vernier positions; Foote and Winter JMB (1992) 224:487

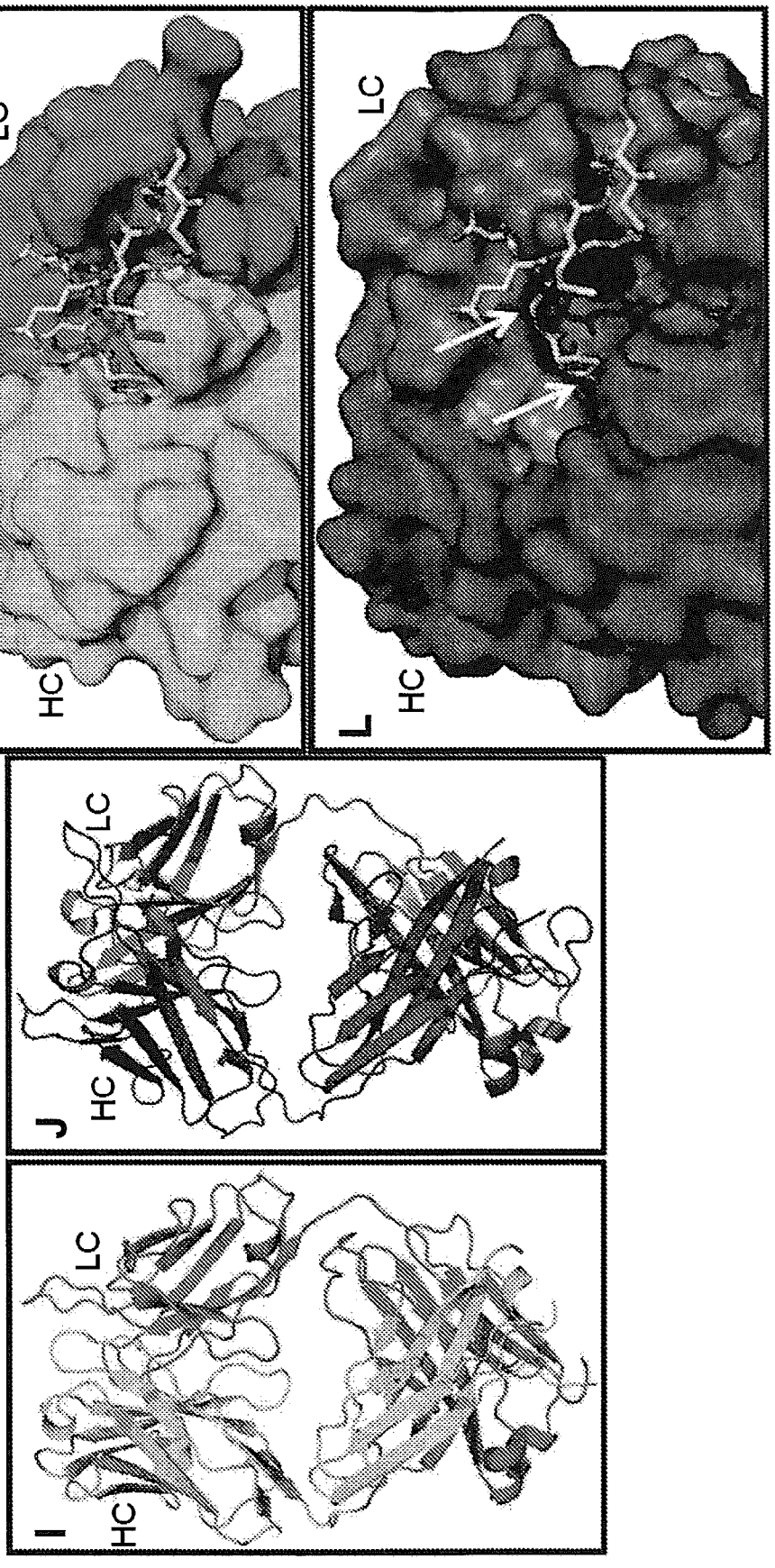
* FW-CDR interactions; Padlan Mol. Immunol. (1994) 31:169

// VH-VL interactions; Padlan Mol. Immunol. (1994) 31:169

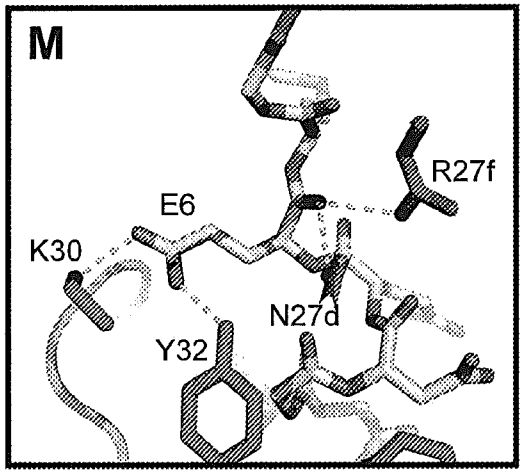
Figure 14H



Figures 14I-14L



Figures 14M-14N



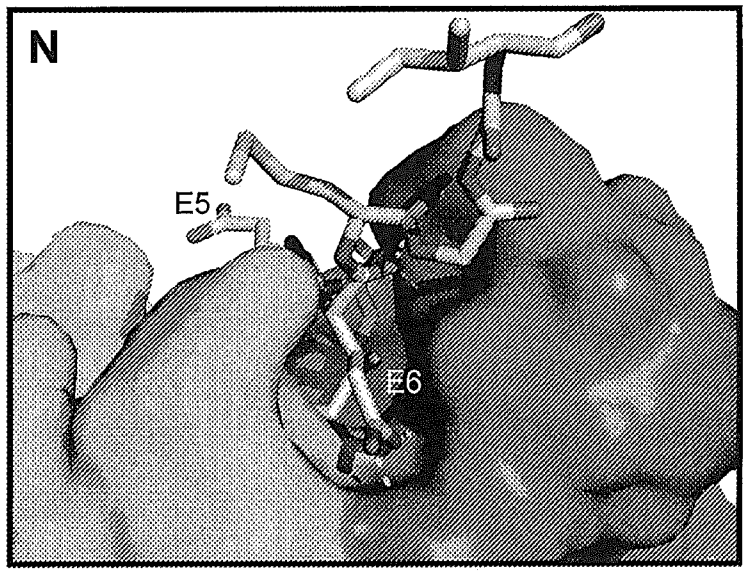


Figure 15

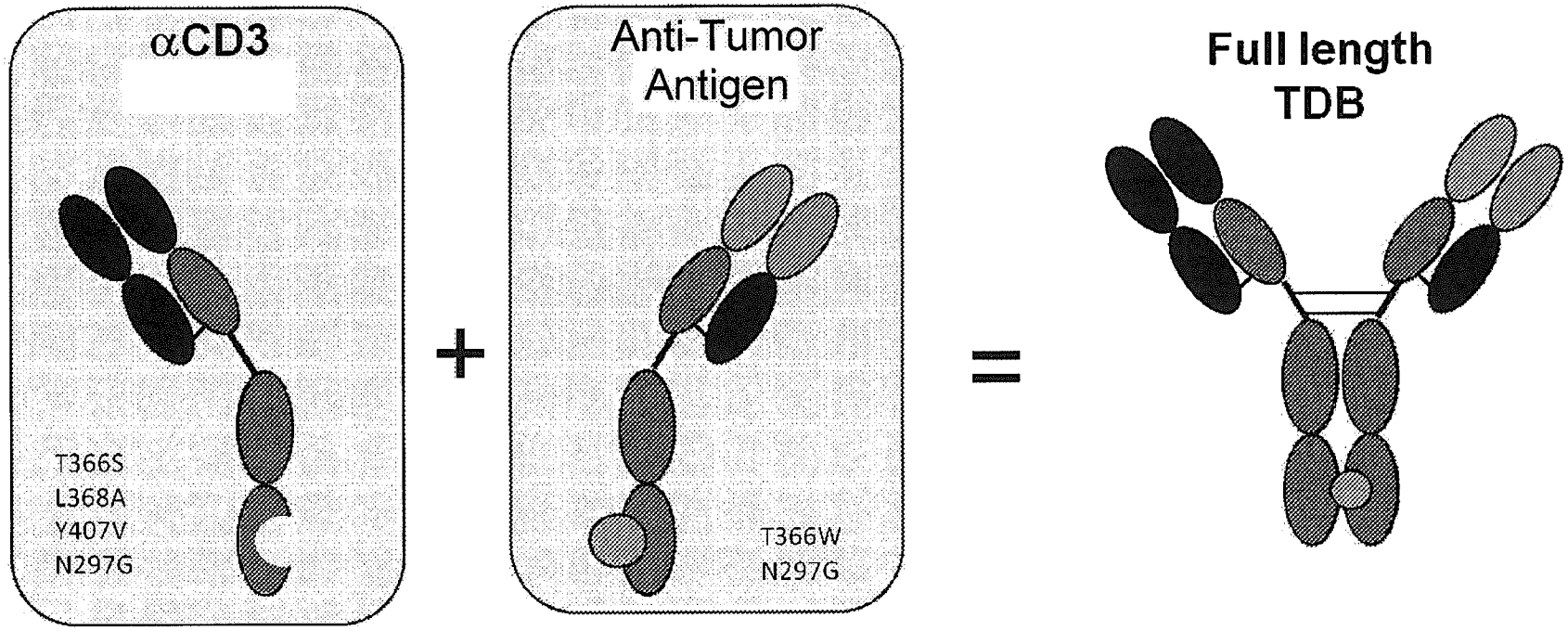

αCD3
T366S
L368A
Y407V
N297G
+
Anti-Tumor Antigen
T366W
N297G
=
Full length TDB

Figure 16

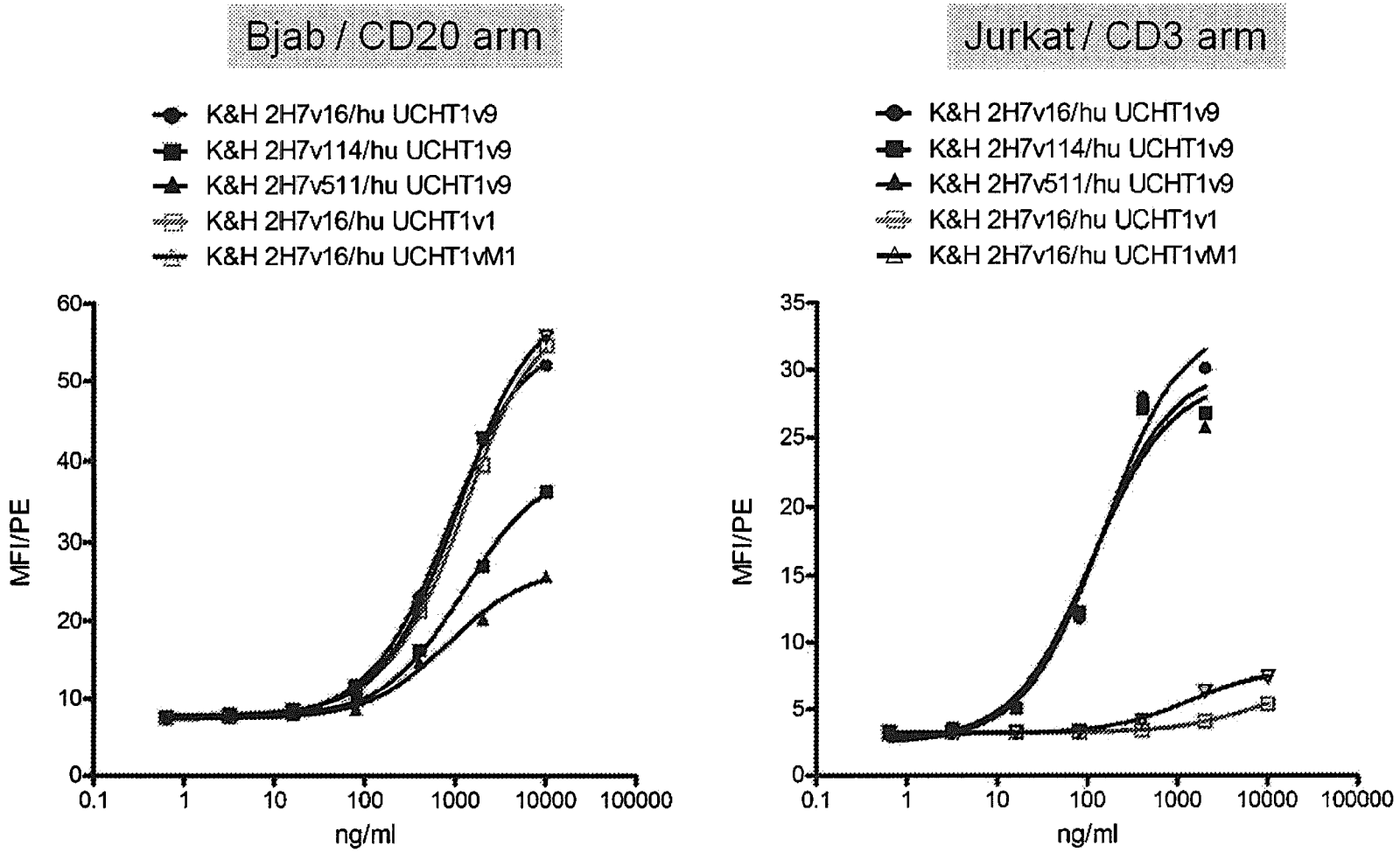

Bjab / CD20 arm
K&H 2H7v16/hu UCHT1v9
K&H 2H7v114/hu UCHT1v9
K&H 2H7v511/hu UCHT1v9
K&H 2H7v16/hu UCHT1v1
K&H 2H7v16/hu UCHT1vM1
MFI/PE
ng/ml
Jurkat / CD3 arm
K&H 2H7v16/hu UCHT1v9
K&H 2H7v114/hu UCHT1v9
K&H 2H7v511/hu UCHT1v9
K&H 2H7v16/hu UCHT1v1
K&H 2H7v16/hu UCHT1vM1
MFI/PE
ng/ml

Figure 17

Monovalent Binding Affinity

Antibody	anti-CD20/2H7			anti-CD3/UCHT1		
clone	v16	v114	v511	v9	vM1	v1
Kd (nM) *	54	44	53	4.2	318	NA

*monovalent binding Kd measured by scatchard

Bivalent Binding Affinity

Antibody	anti-CD20/2H7 w/UCHT1v9			anti-CD3/UCHT1 w/2H7v16		
clone	v16	v114	v511	v1	vM1	v9
Kd (nM) *	46	4.5	2.3	300	40	3

*monovalent binding Kd measured by scatchard

Figure 18

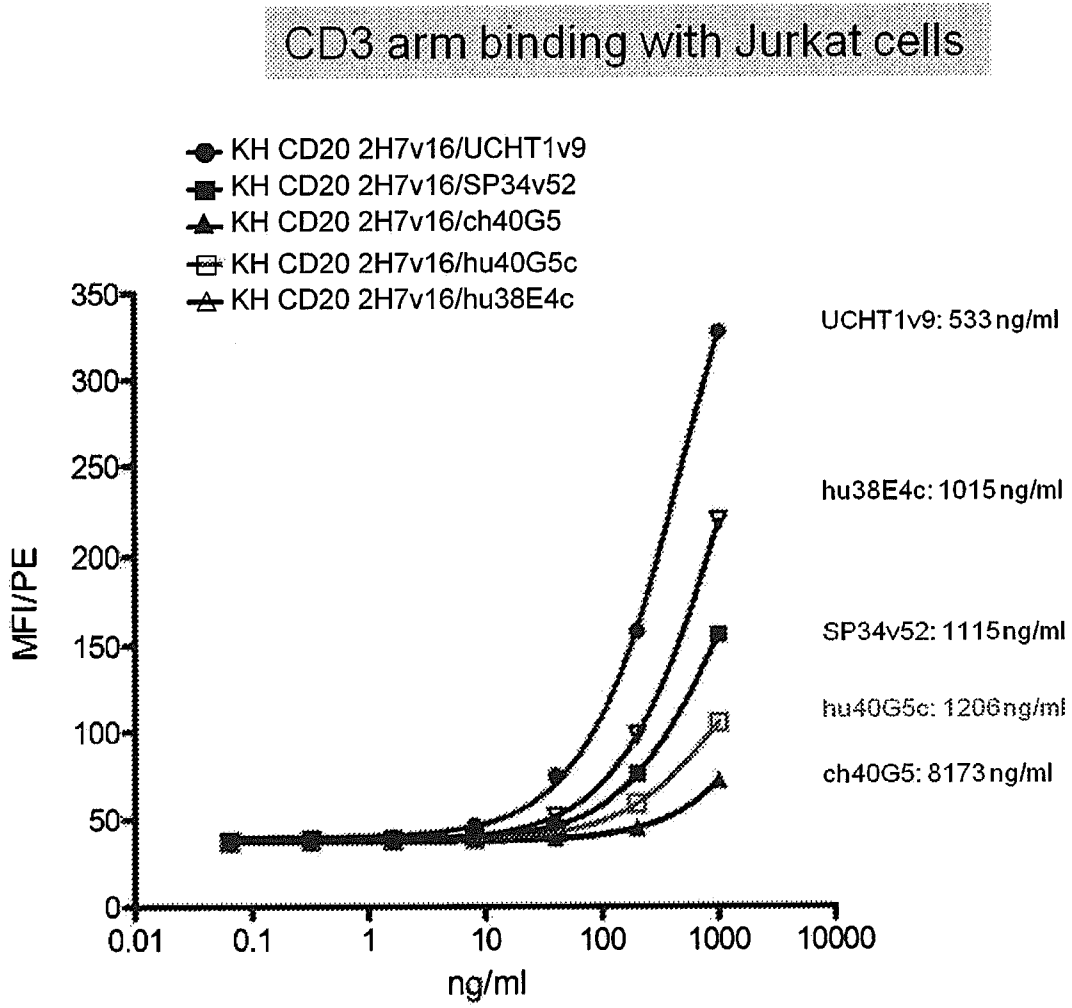

CD3 arm binding with Jurkat cells
KH CD20 2H7v16/UCHT1v9
KH CD20 2H7v16/SP34v52
KH CD20 2H7v16/ch40G5
KH CD20 2H7v16/hu40G5c
KH CD20 2H7v16/hu38E4c
MFI/PE
350
300
250
200
150
100
50
0
0.01
0.1
1
10
100
1000
10000
ng/ml
UCHT1v9: 533 ng/ml
hu38E4c: 1015 ng/ml
SP34v52: 1115 ng/ml
hu40G5c: 1206 ng/ml
ch40G5: 8173 ng/ml

Figure 19

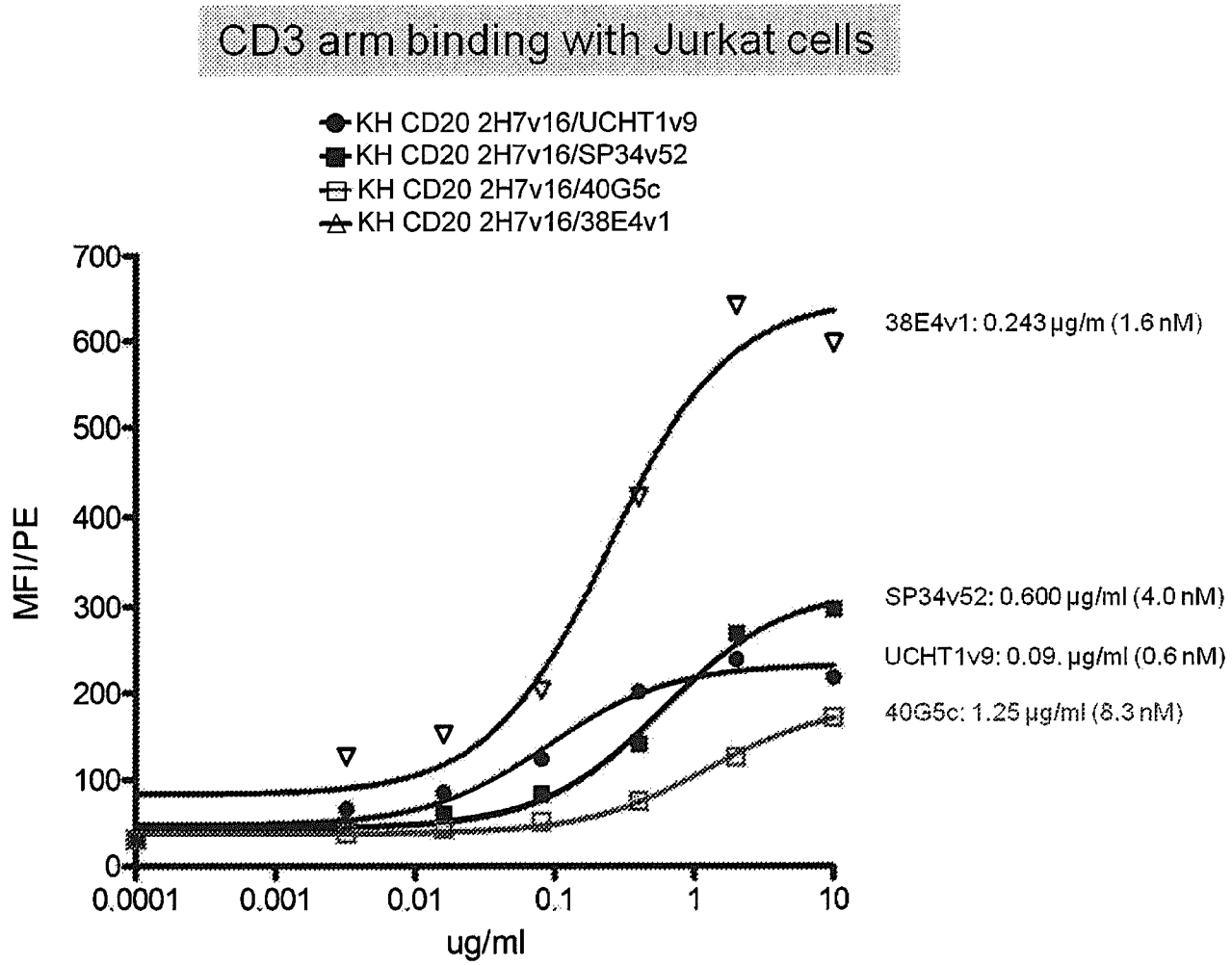
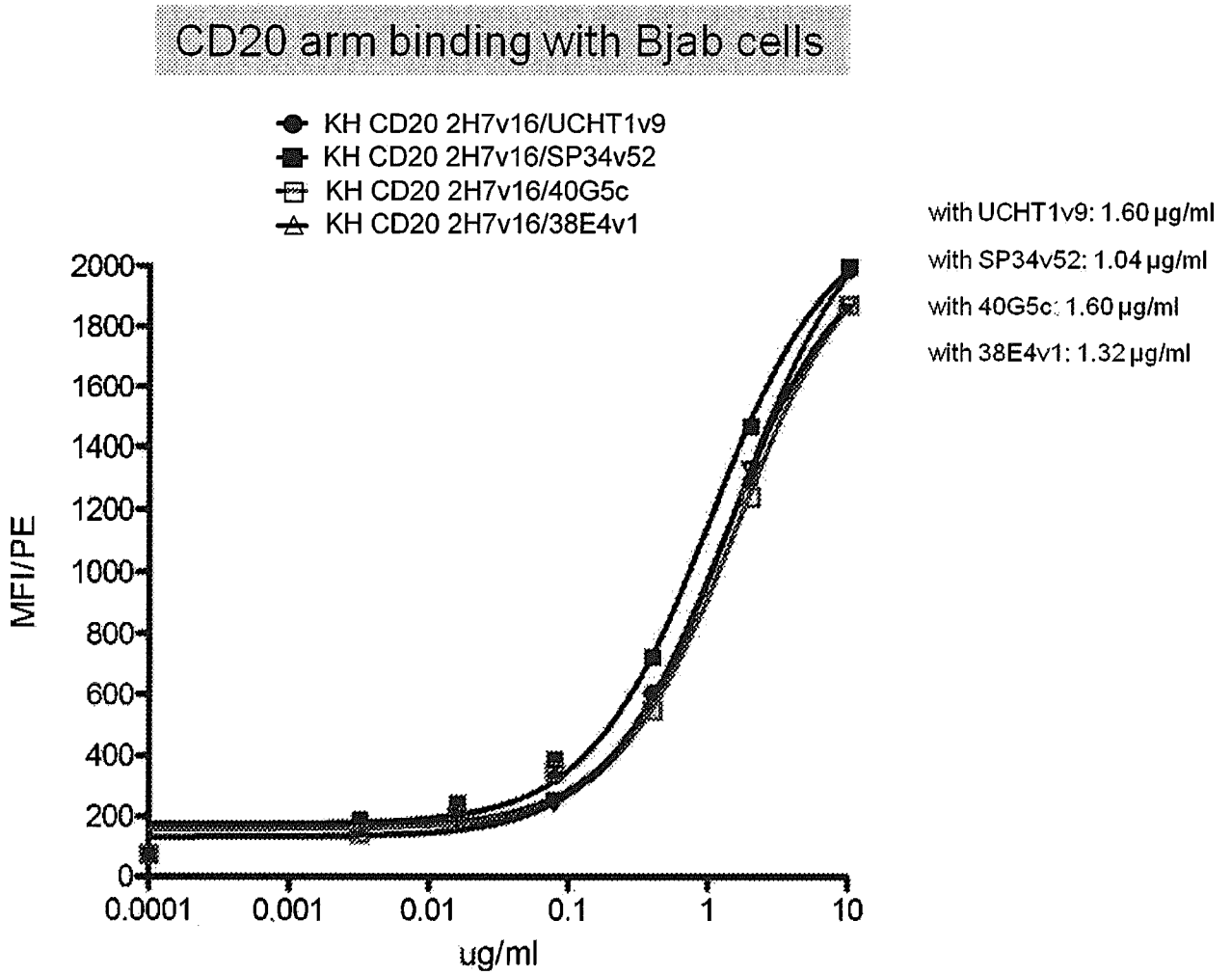
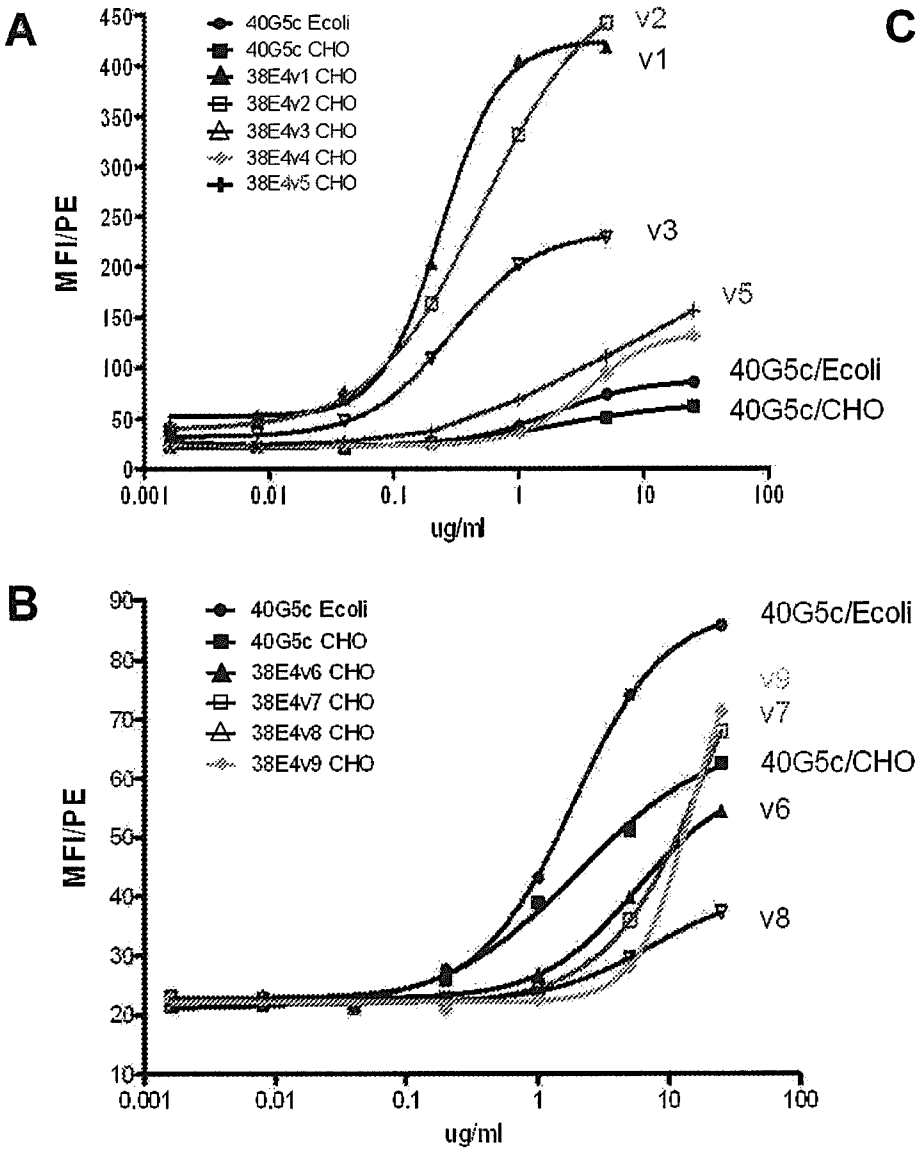

CD3 arm binding with Jurkat cells
KH CD20 2H7v16/UCHT1v9
KH CD20 2H7v16/SP34v52
KH CD20 2H7v16/40G5c
KH CD20 2H7v16/38E4v1
MFI/PE
700
600
500
400
300
200
100
0
0.0001
0.001
0.01
0.1
1
10
ug/ml
38E4v1: 0.243 µg/m (1.6 nM)
SP34v52: 0.600 µg/ml (4.0 nM)
UCHT1v9: 0.09. µg/ml (0.6 nM)
40G5c: 1.25 µg/ml (8.3 nM)

Figure 20


CD20 arm binding with Bjab cells
KH CD20 2H7v16/UCHT1v9
KH CD20 2H7v16/SP34v52
KH CD20 2H7v16/40G5c
KH CD20 2H7v16/38E4v1
with UCHT1v9: 1.60 µg/ml
with SP34v52: 1.04 µg/ml
with 40G5c: 1.60 µg/ml
with 38E4v1: 1.32 µg/ml
MFI/PE
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0.0001
0.001
0.01
0.1
1
10
ug/ml

Figures 21A-21C



C

CD3 arm	Source	EC50 / µg/ml
40G5c	E.coli	1.85 (~12nM)
40G5c	CHO	2.01 (~13nM)
38E4v1	CHO	0.24 (~1.6nM)
v2	CHO	0.52 (~3.4nM)
v3	CHO	0.29 (~1.9nM)
v4	CHO	3.51(~23nM)
v5	CHO	4.11 (~27nM)
v6	CHO	5.56 (~36nM)
v7	CHO	22.6 (~147nM)
v8	CHO	7.23 (~47nM)
v9	CHO	15.0 (~97nM)

* N297G variant for all CHO material

Figures 22A-22B

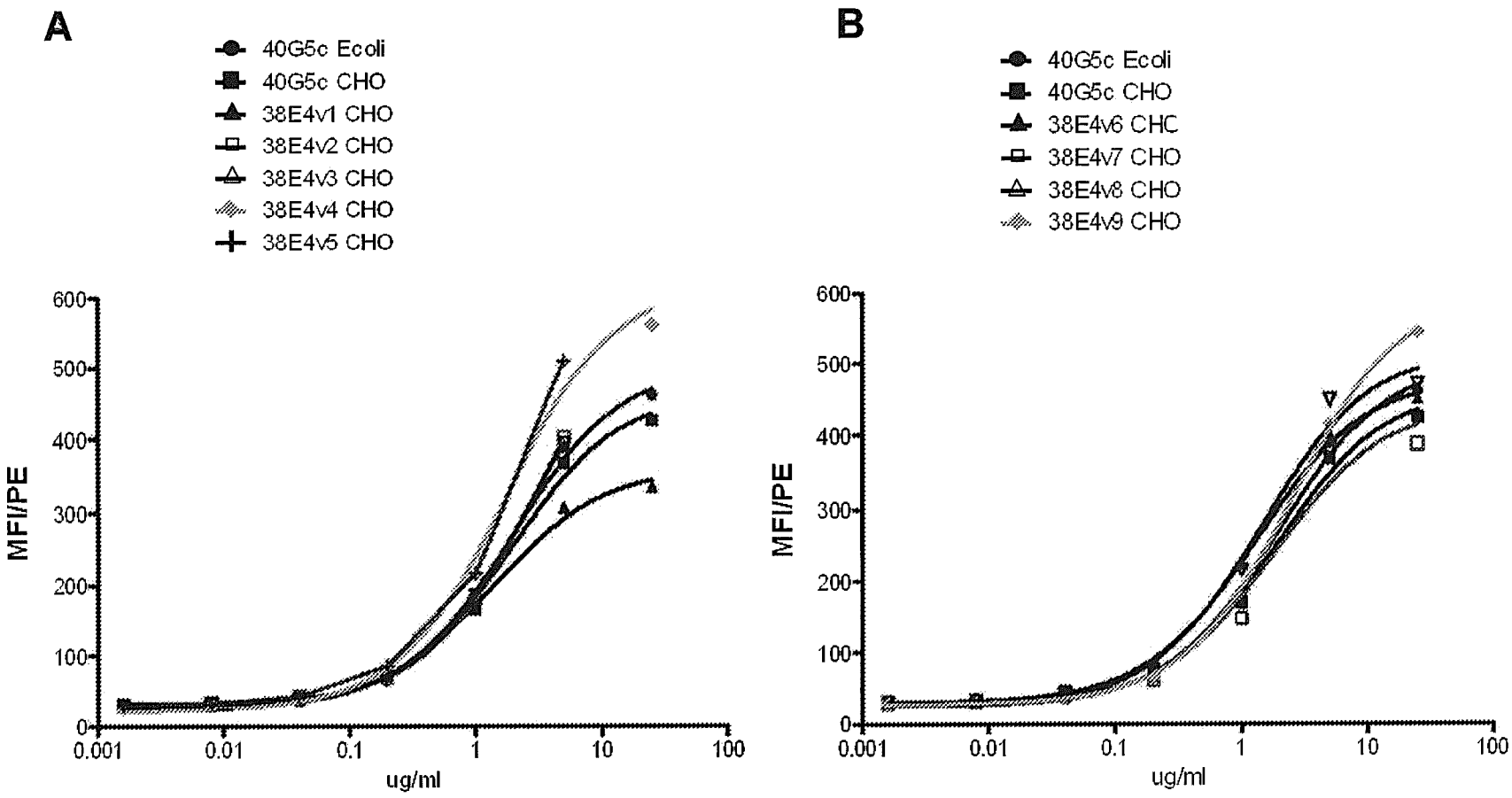


Figure 23

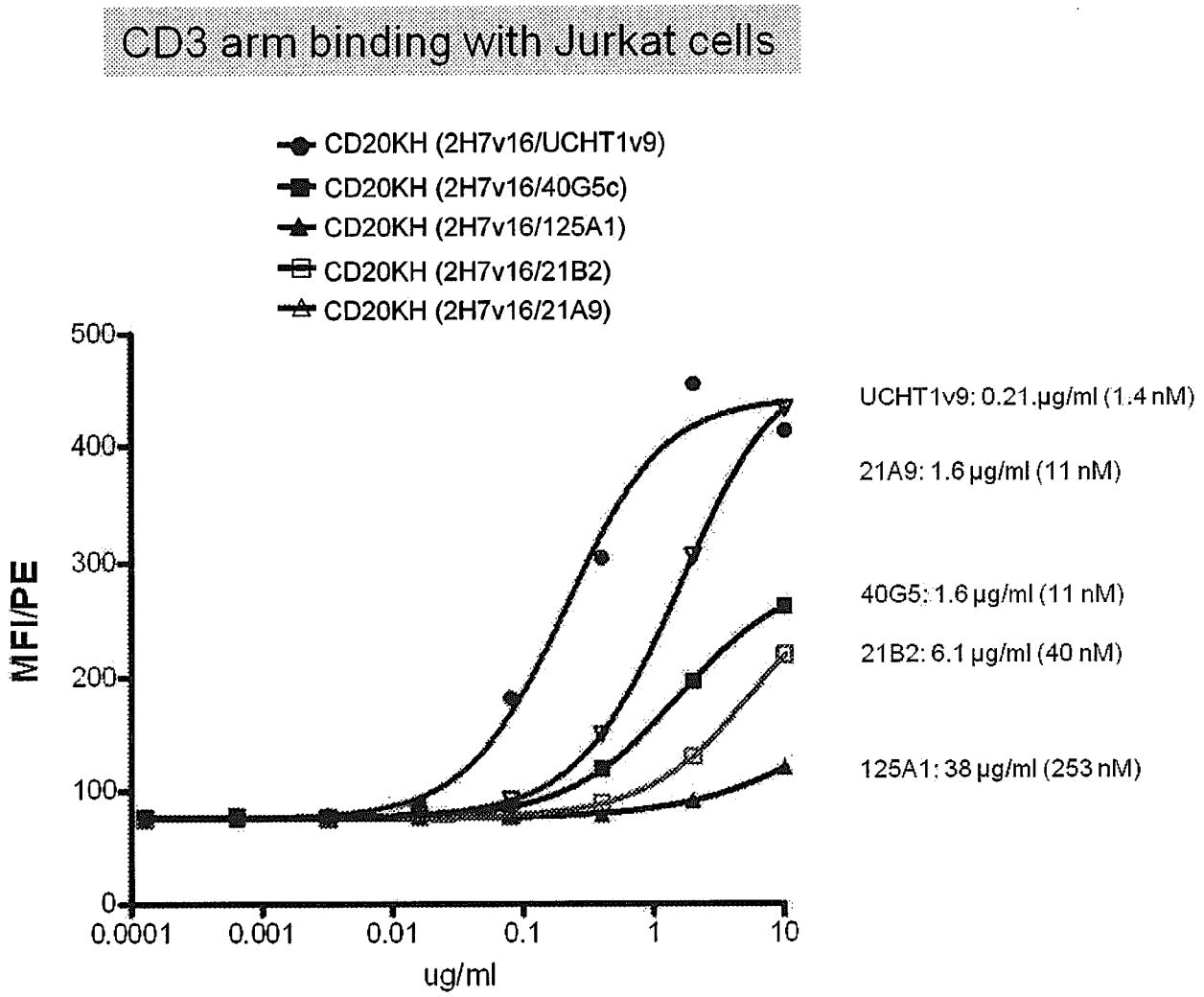
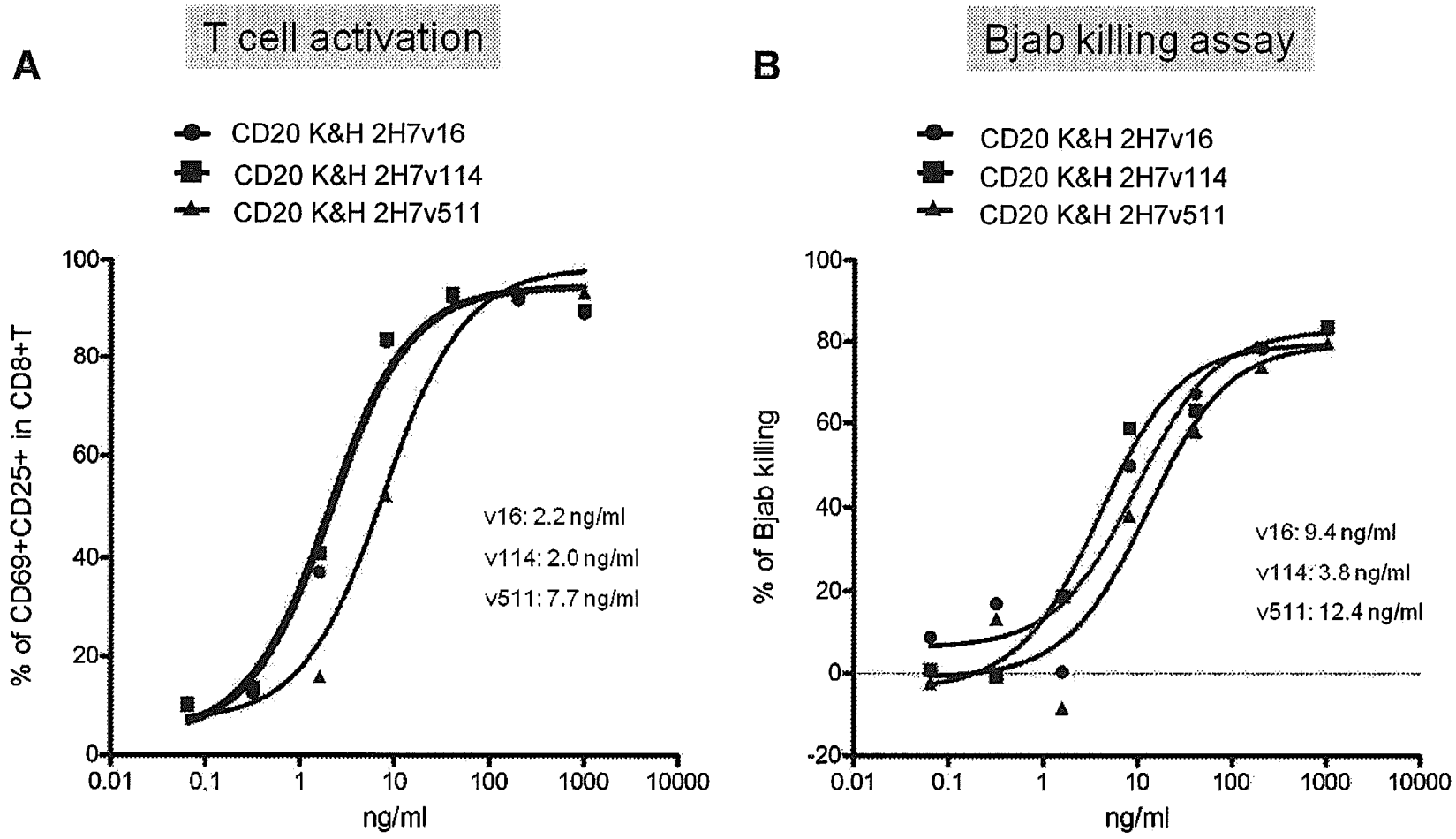

CD3 arm binding with Jurkat cells
CD20KH (2H7v16/UCHT1v9)
CD20KH (2H7v16/40G5c)
CD20KH (2H7v16/125A1)
CD20KH (2H7v16/21B2)
CD20KH (2H7v16/21A9)
MFI/PE
500
400
300
200
100
0
0.0001
0.001
0.01
0.1
1
10
ug/ml
UCHT1v9: 0.21 µg/ml (1.4 nM)
21A9: 1.6 µg/ml (11 nM)
40G5: 1.6 µg/ml (11 nM)
21B2: 6.1 µg/ml (40 nM)
125A1: 38 µg/ml (253 nM)

Figure 24

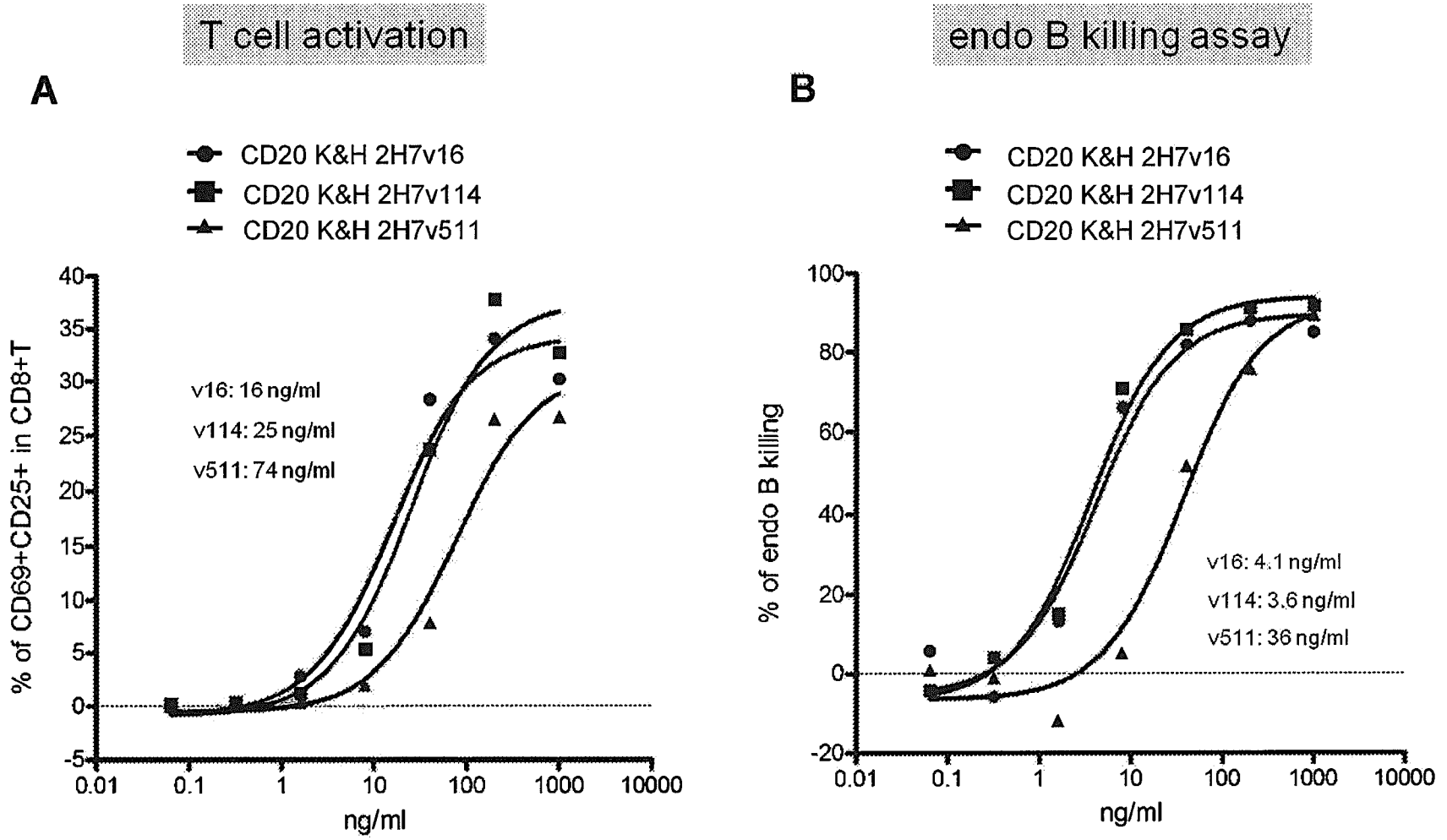
	TDBs				Fabs		
Sample name	ka (1/Ms)	kd (1/s)	KD (M)	KD (nM)	ka (1/Ms)	kd (1/s)	KD (nM)
40G5c (E. coli)	3.58E+05	0.0256	7.15E-08	71.5	4.01E+05	0.02533	63.2
40G5c (CHO)	5.97E+05	0.03069	5.15E-08	51.5			
sp34.v52	6.09E+05	0.00535	8.78E-09	8.8			
38E4v1	4.03E+06	0.00515	1.28E-09	1.3	6.06E+06	0.005074	0.8
38E4v2	2.11E+06	0.00513	2.43E-09	2.4	2.29E+06	0.003586	1.6
38E4v3	4.98E+06	0.01599	3.21E-09	3.2	4.11E+06	0.014	3.4
38E4v4	7.21E+05	0.0984	1.37E-07	136.5	3.25E+05	0.003361	10.3
38E4v5	3.22E+06	0.05154	1.60E-08	16.0	2.05E+06	0.02246	11.0
38E4v6	2.34E+04	0.8784	3.76E-05	37600.0	51140	0.001749	34.2
38E4v7	1.14E+06	0.2897	2.54E-07	254.2	2.83E+05	0.02464	87.0
38E4v8	1.13E+04	0.04426	3.93E-06	3933.0	1.12E+06	0.2018	179.5
38E4v9	1.59E+04	1.018	6.42E-05	64210.0	72610	0.0166	228.6

Figures 25A-25B

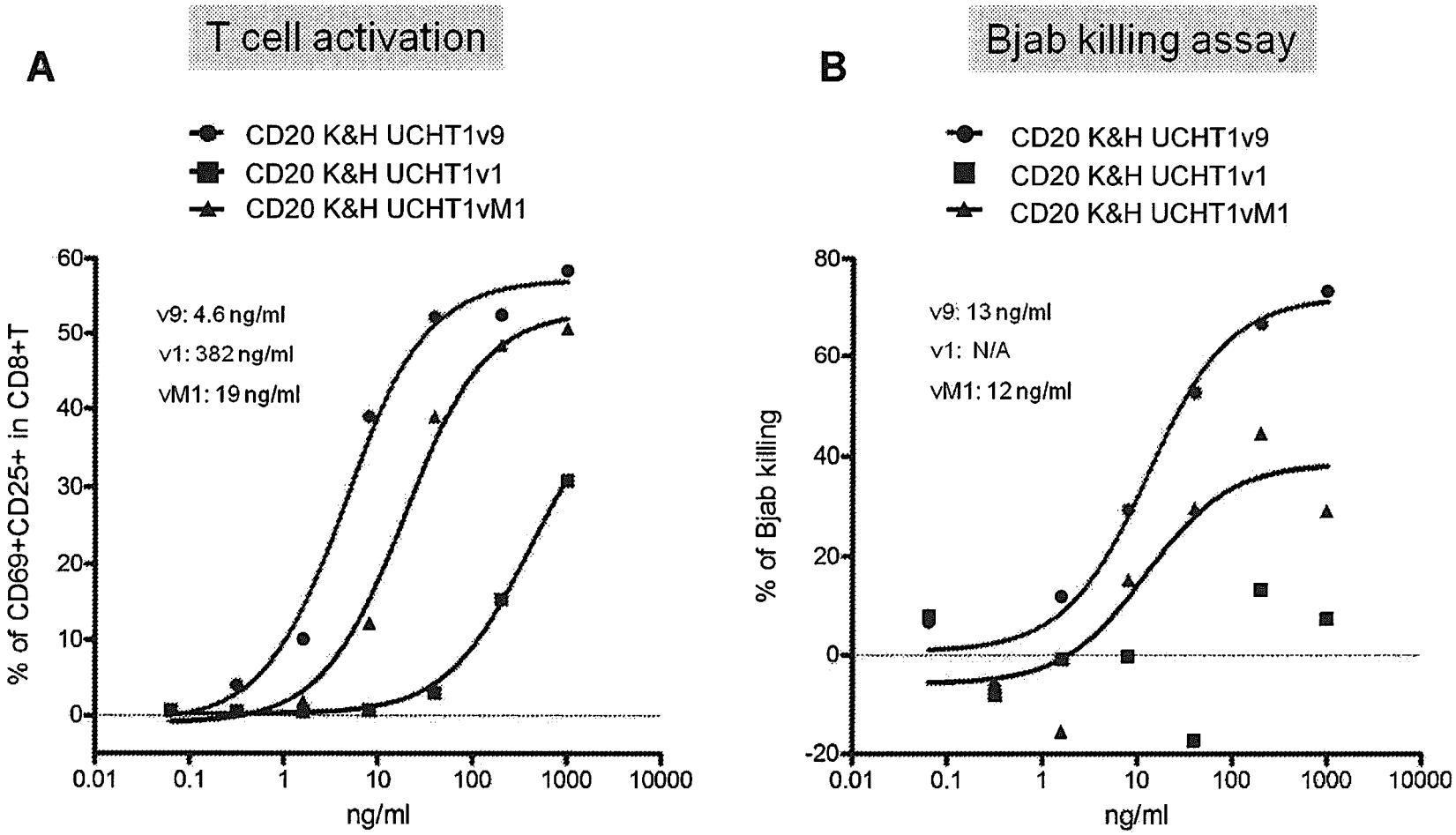


20,000 Bjab with 5× purified hu CD8+T cells, antibody 1:5 dilution from 1000ng/ml, 24 hours.

Figures 26A-26B

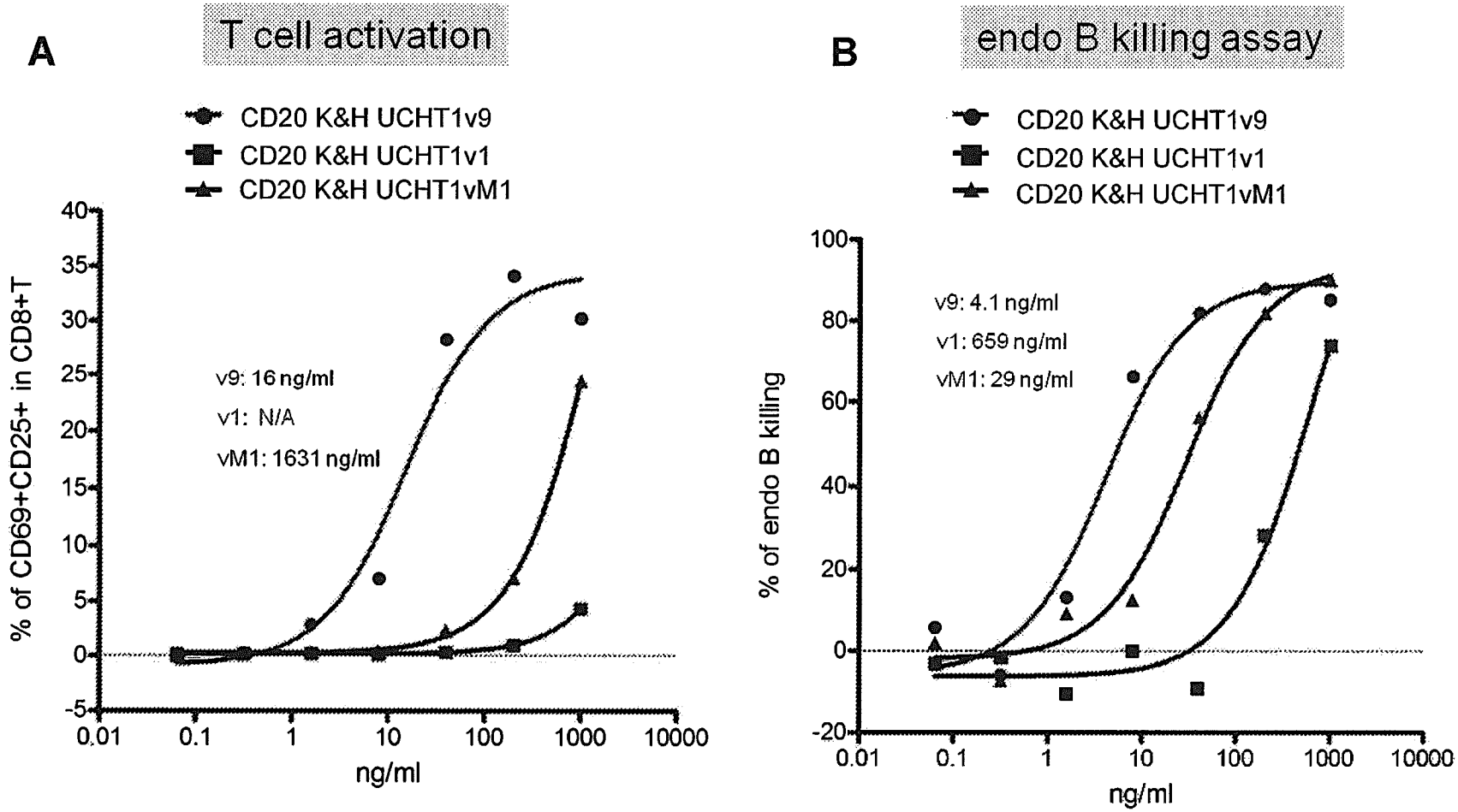


Figures 27A-27B



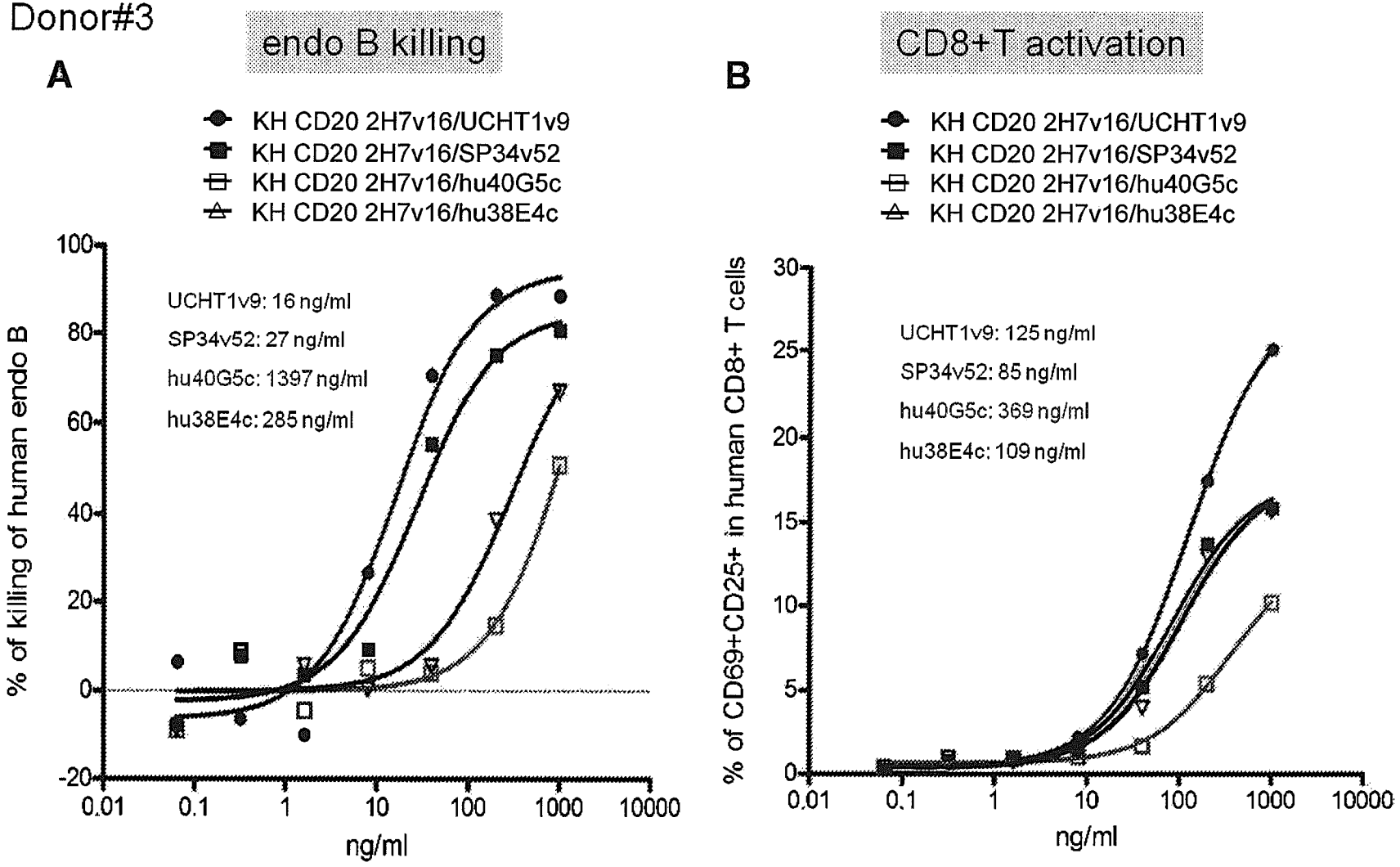
20,000 Bjab with 10× human PBMCs, antibody 1:5 dilution from 1000ng/ml, 24 hours.

Figures 28A-28B



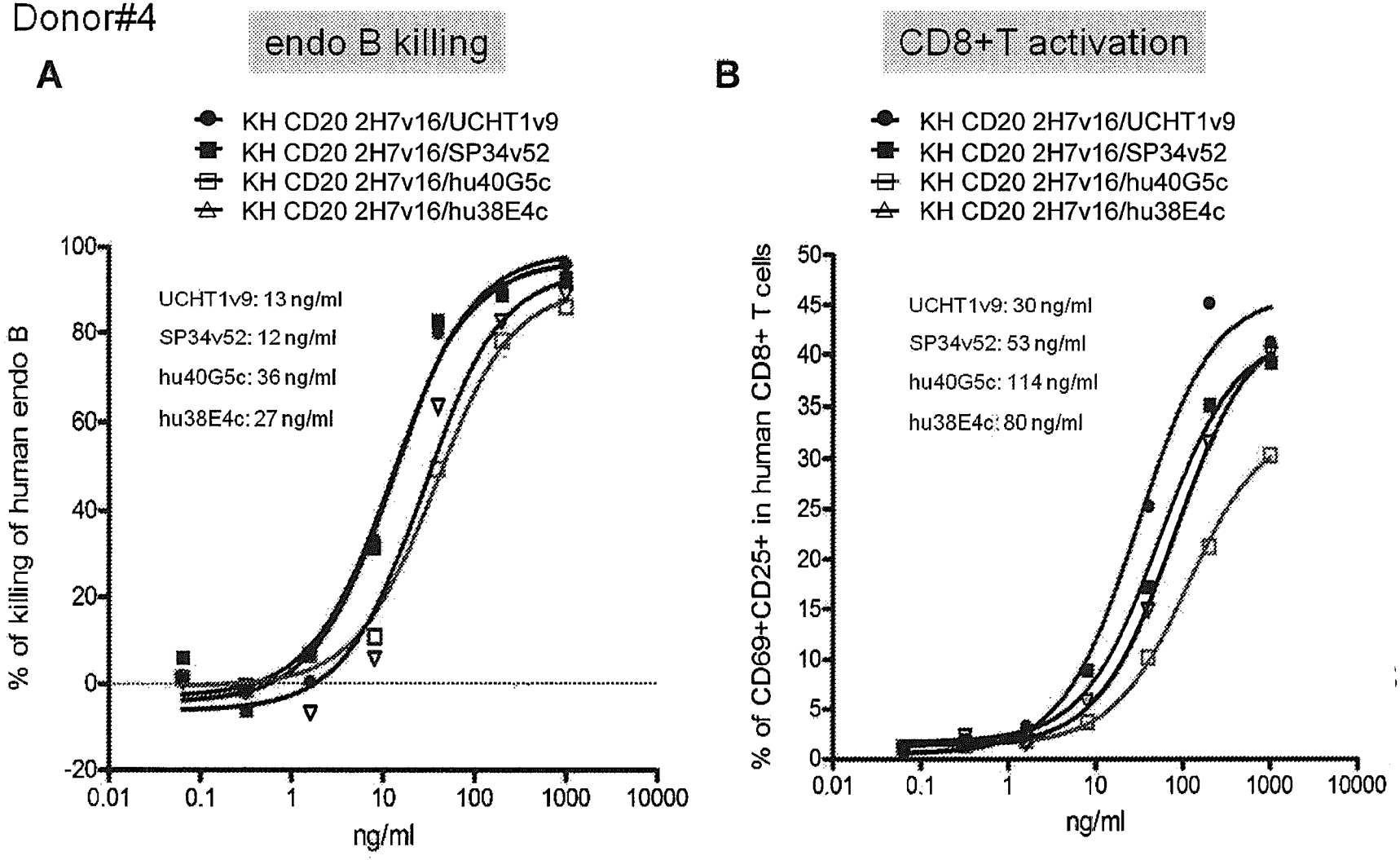
200,000 human PBMCs only, antibody 1:5 dilution from 1000ng/ml, 24 hours.

Figures 29A-29B



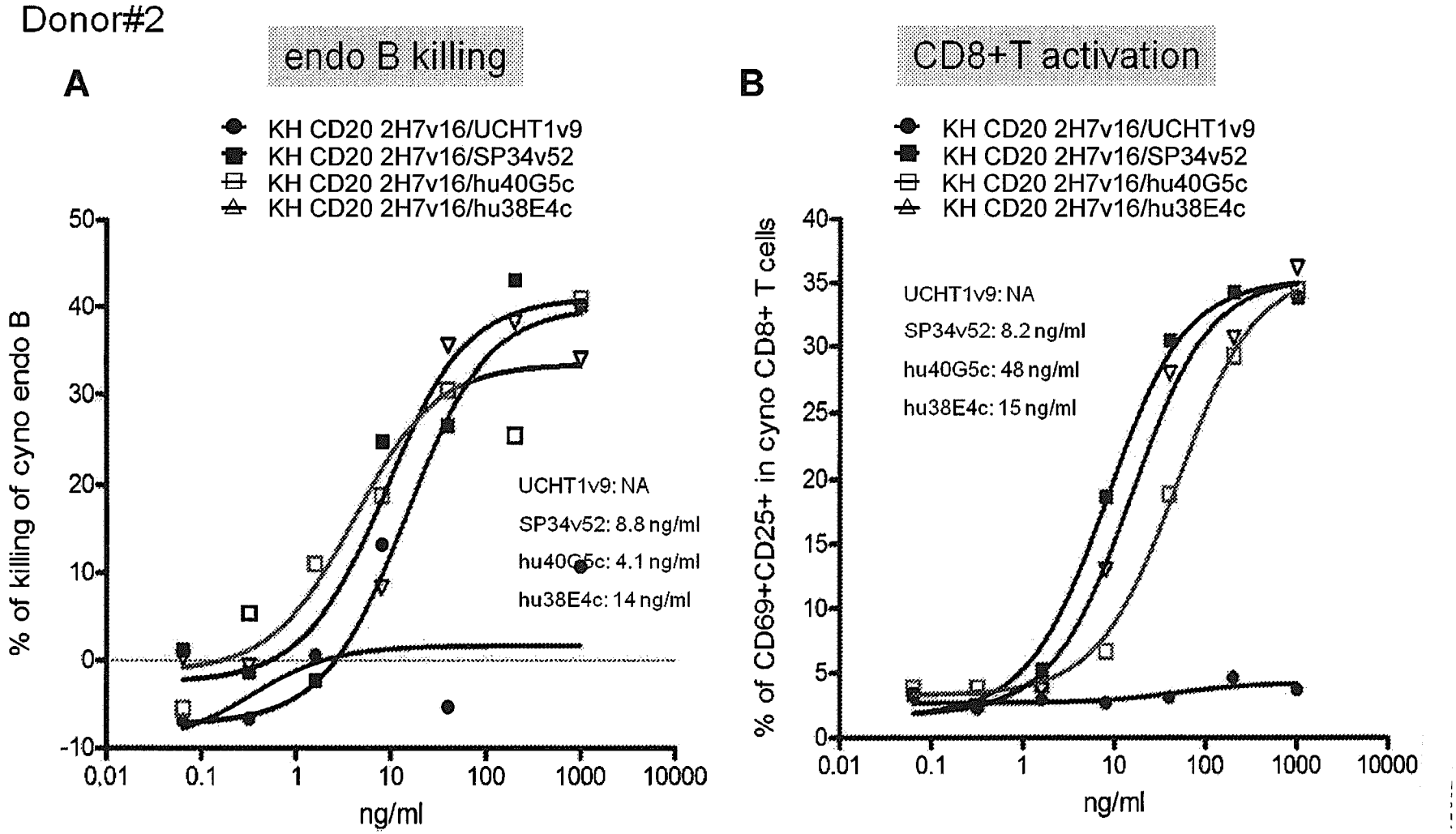
200,000 huPBMCs per well, 24 hours. Human B cells were labeled with CD19-v450 (BD).

Figures 30A-30B



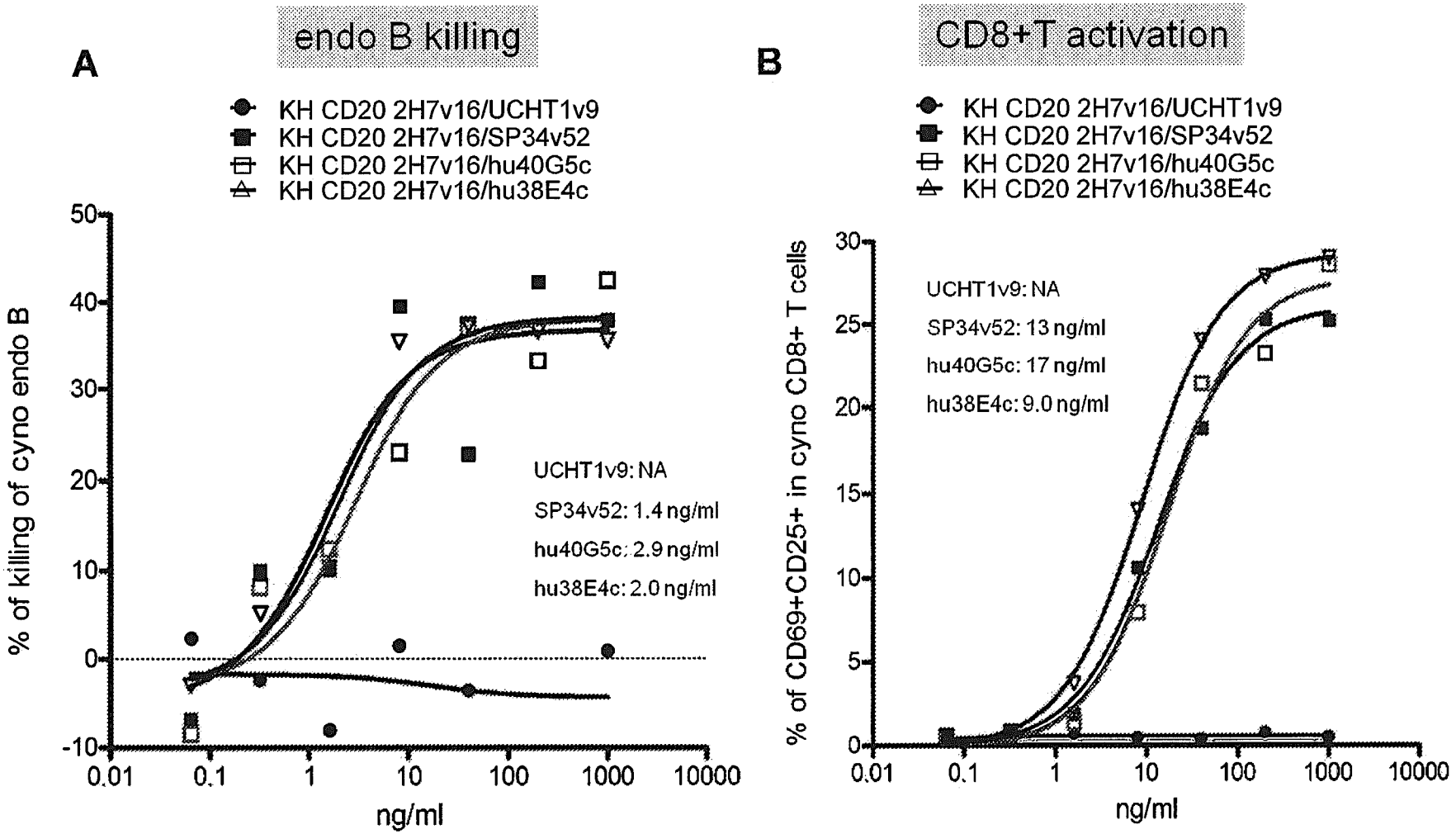
200,000 huPBMCs per well, 24 hours. Human B cells were labeled with CD19-v450 (BD).

Figures 31A-31B



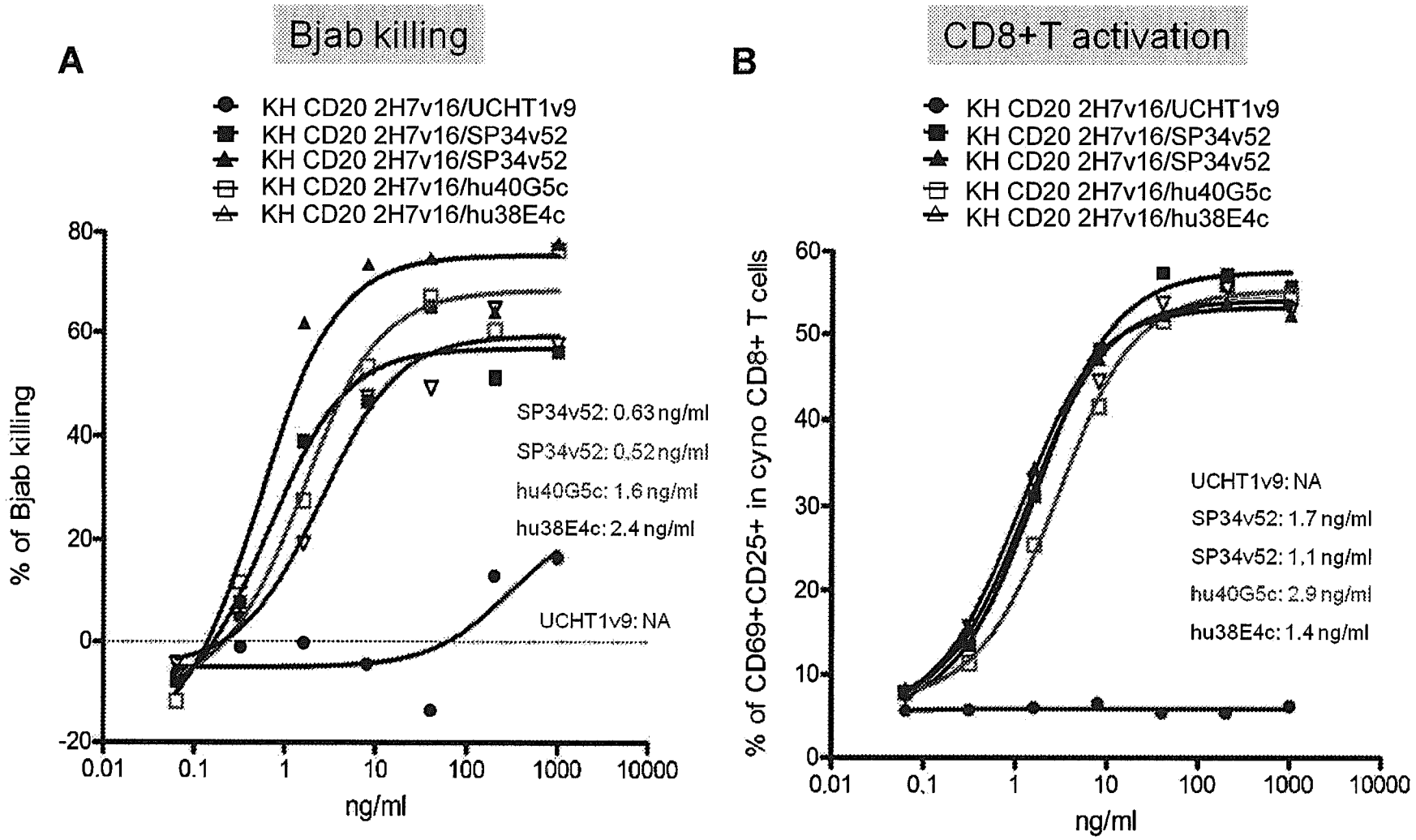
Cyno PBMCs 200,000 per well, 24 hours. Cyno B cells were labeled with cynoCD79b-PE (in house).

Figures 32A-32B



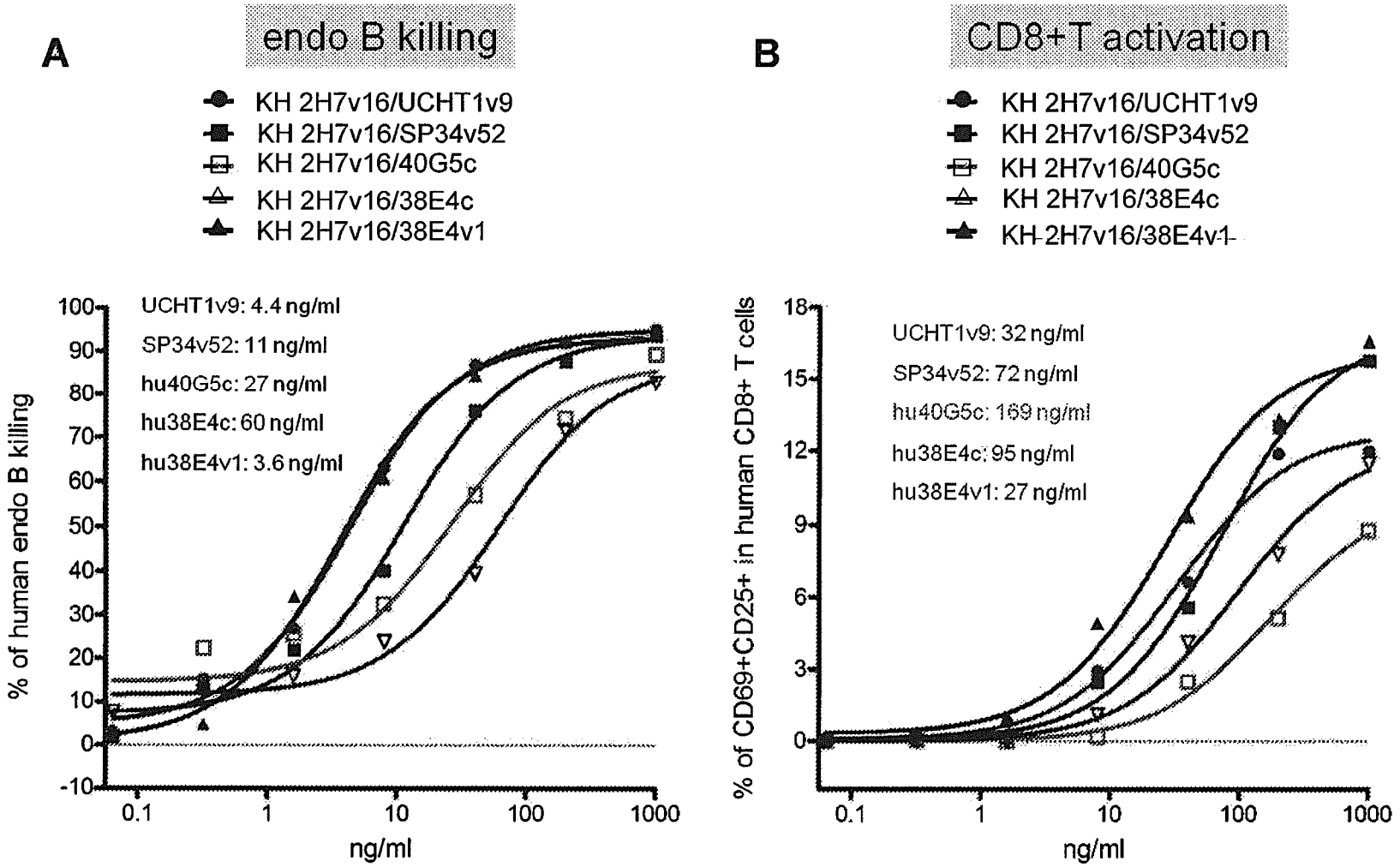
Cyno PBMCs 200,000 per well, 24 hours. Cyno B cells were labeled with cynoCD79b-PE (in house).

Figures 33A-33B



Cyno PBMCs 200,000 per well, 24 hours. Bjab cells were labeled with CD19-PE (BD).

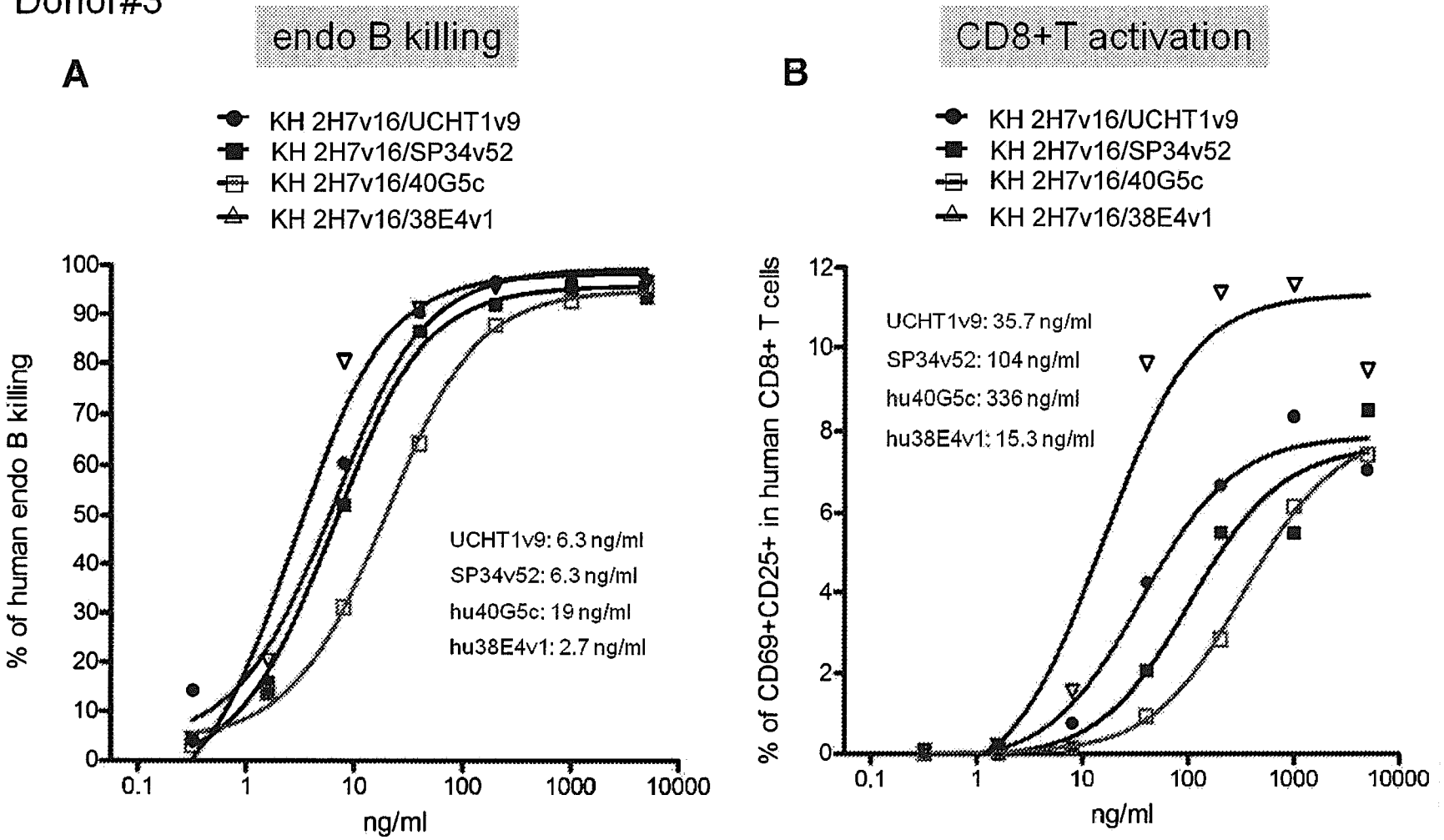
Figures 34A-34B



200,000 huPBMCs per well, 24 hours. Human B cells were labeled with CD19-v450 (BD).

Figures 35A-35B

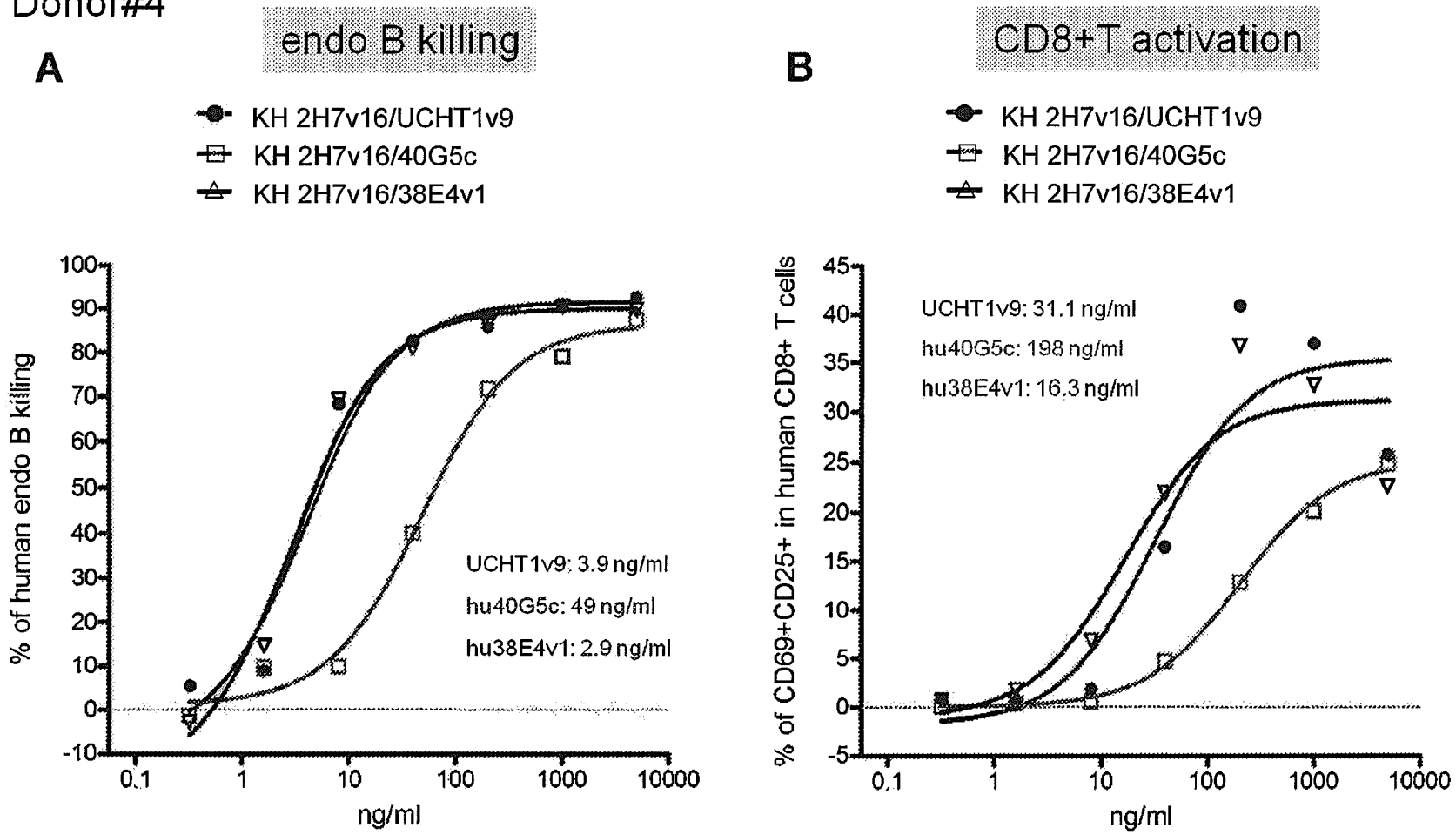
Donor#3



200,000 huPBMCs per well, 24 hours. Human B cells were labeled with CD19-v450 (BD).

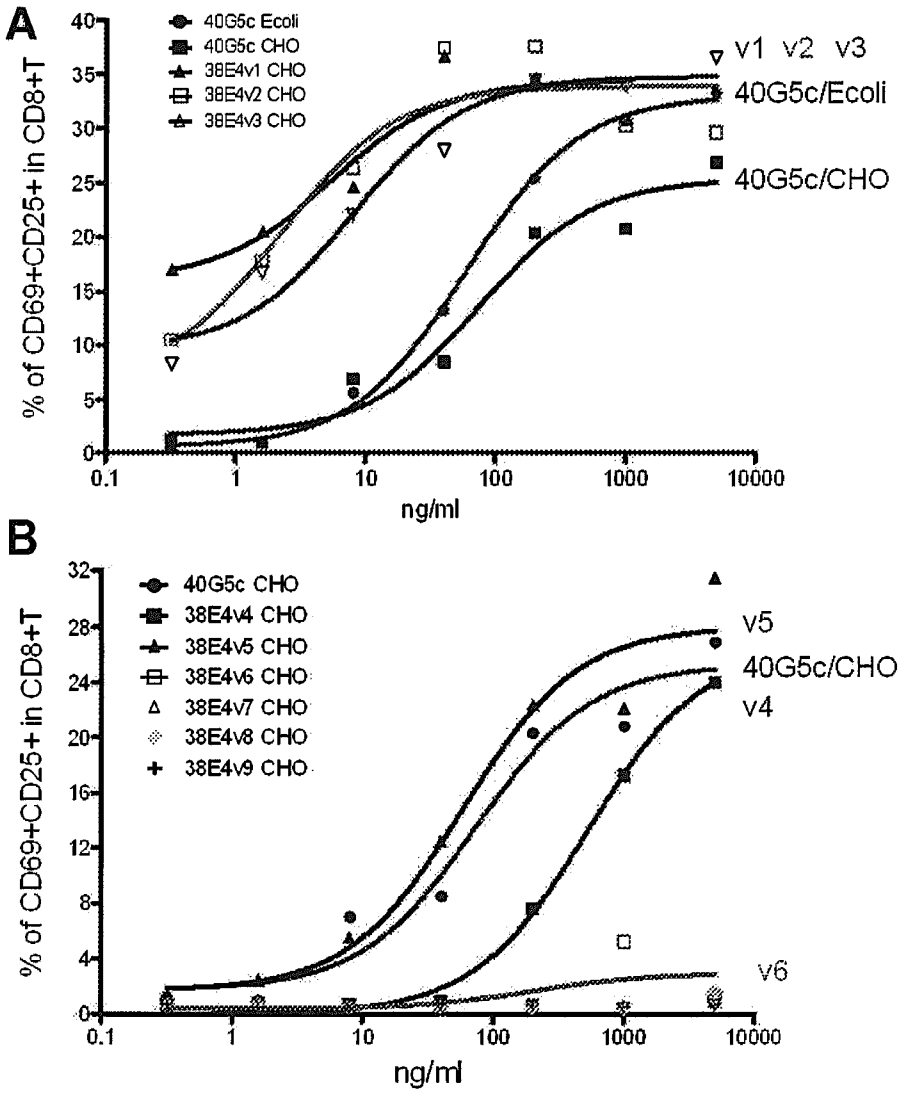
Figures 36A-36B

Donor#4



200,000 huPBMCs per well, 24 hours. Human B cells were labeled with CD19-v450 (BD).

Figures 37A-37C



200,000 huPBMC only.

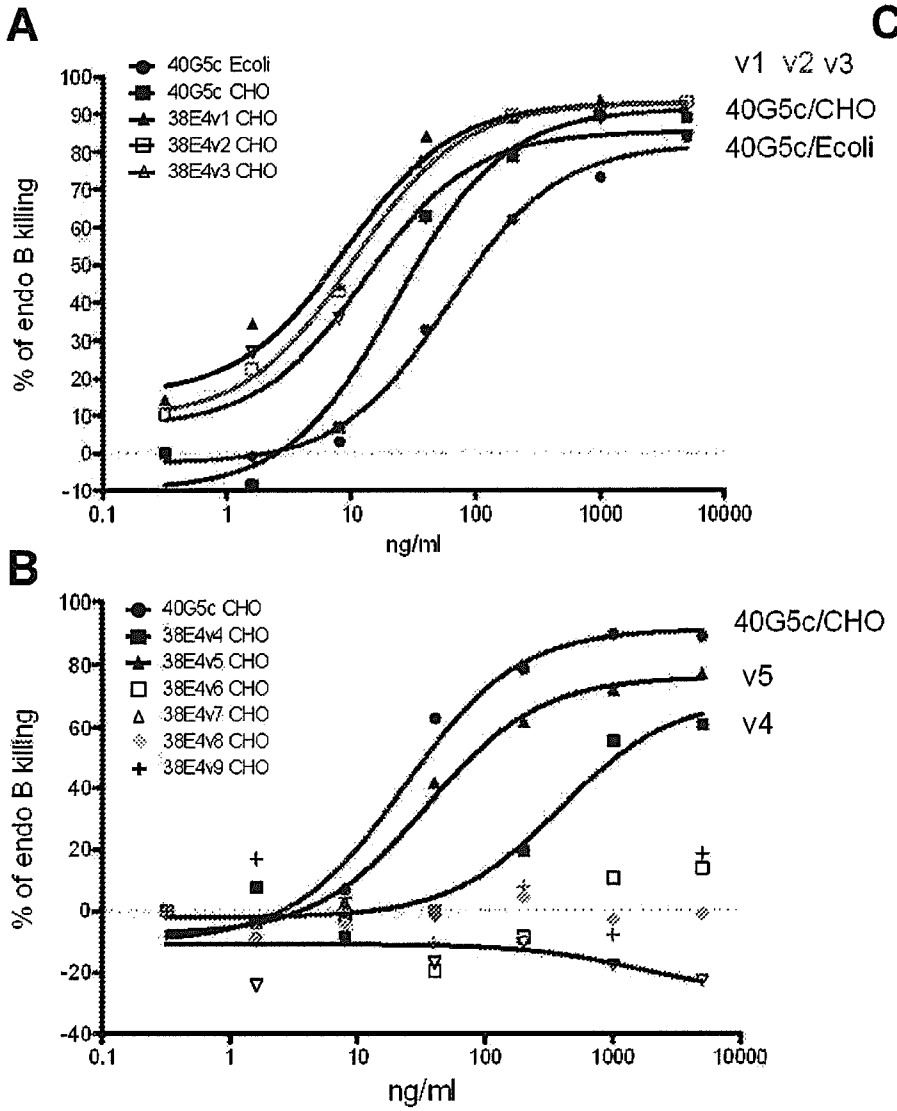
C

Donor#1 – 24h

CD3 arm	Source	CD8+T activation EC50 / ng/ml
40G5c	E.coli	59.4
40G5c	CHO	74.2
38E4v1	CHO	5.46
v2	CHO	2.27
v3	CHO	8.10
v4	CHO	530
v5	CHO	59.2
v6	CHO	NA
v7	CHO	NA
v8	CHO	NA
v9	CHO	NA

* N297G variant for all CHO material

Figures 38A-38C



C

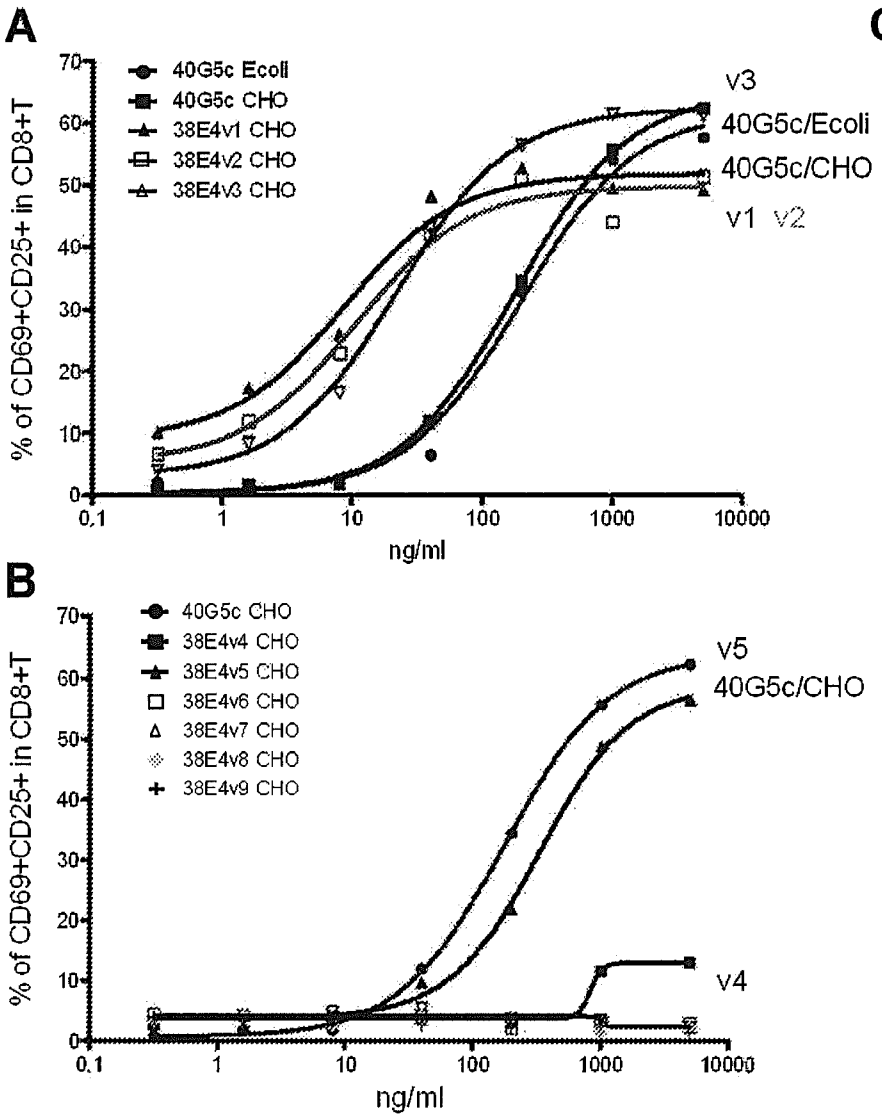
Donor#1 – 24h

CD3 arm	Source	endo B killing EC50 / ng/ml
40G5c	E.coli	61.4
40G5c	CHO	23.4
38E4v1	CHO	9.20
v2	CHO	10.5
v3	CHO	12.9
v4	CHO	391
v5	CHO	35.3
v6	CHO	NA
v7	CHO	NA
v8	CHO	NA
v9	CHO	NA

* N297G variant for all CHO material

200,000 huPBMC only.

Figures 39A-39C



C

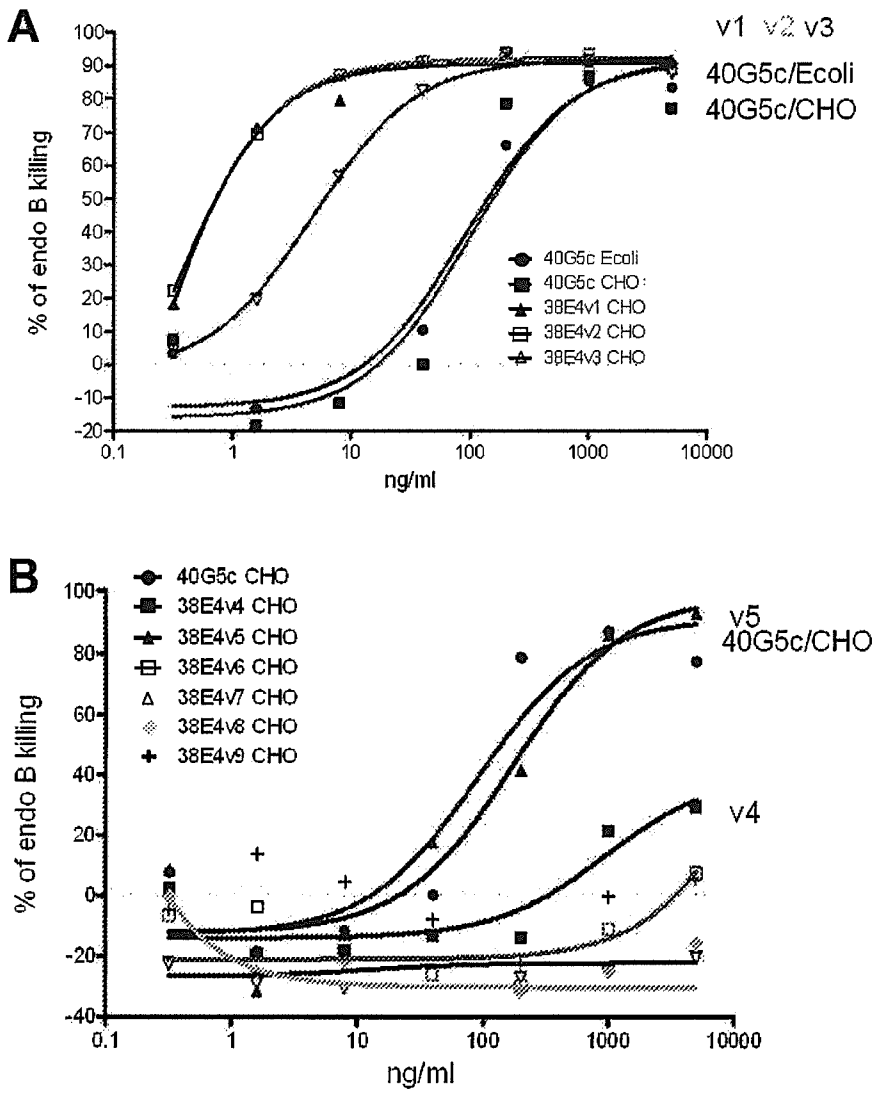
Donor#1 – 48h

CD3 arm	Source	CD8+T activation EC50 / ng/ml
40G5c	E.coli	196
40G5c	CHO	178
38E4v1	CHO	8.73
v2	CHO	10.5
v3	CHO	22.1
v4	CHO	829
v5	CHO	328
v6	CHO	NA
v7	CHO	NA
v8	CHO	NA
v9	CHO	NA

* N297G variant for all CHO material

200,000 huPBMC only.

Figures 40A-40C



C

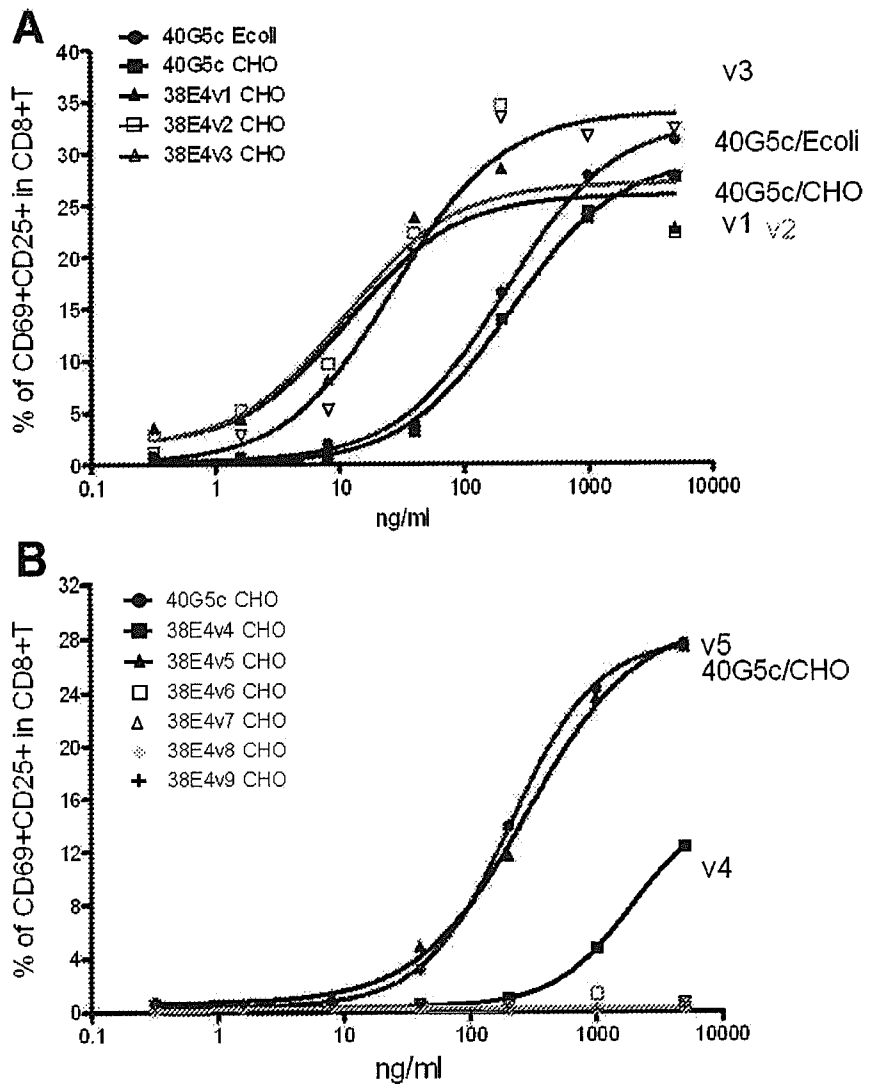
Donor#1 – 48h

CD3 arm	Source	Endo B killing EC50 / ng/ml
40G5c	E.coli	99.5
40G5c	CHO	92.0
38E4v1	CHO	0.197
v2	CHO	0.282
v3	CHO	4.84
v4	CHO	934
v5	CHO	173
v6	CHO	NA
v7	CHO	NA
v8	CHO	NA
v9	CHO	NA

* N297G variant for all CHO material

200,000 huPBMC only.

Figures 41A-41C



C

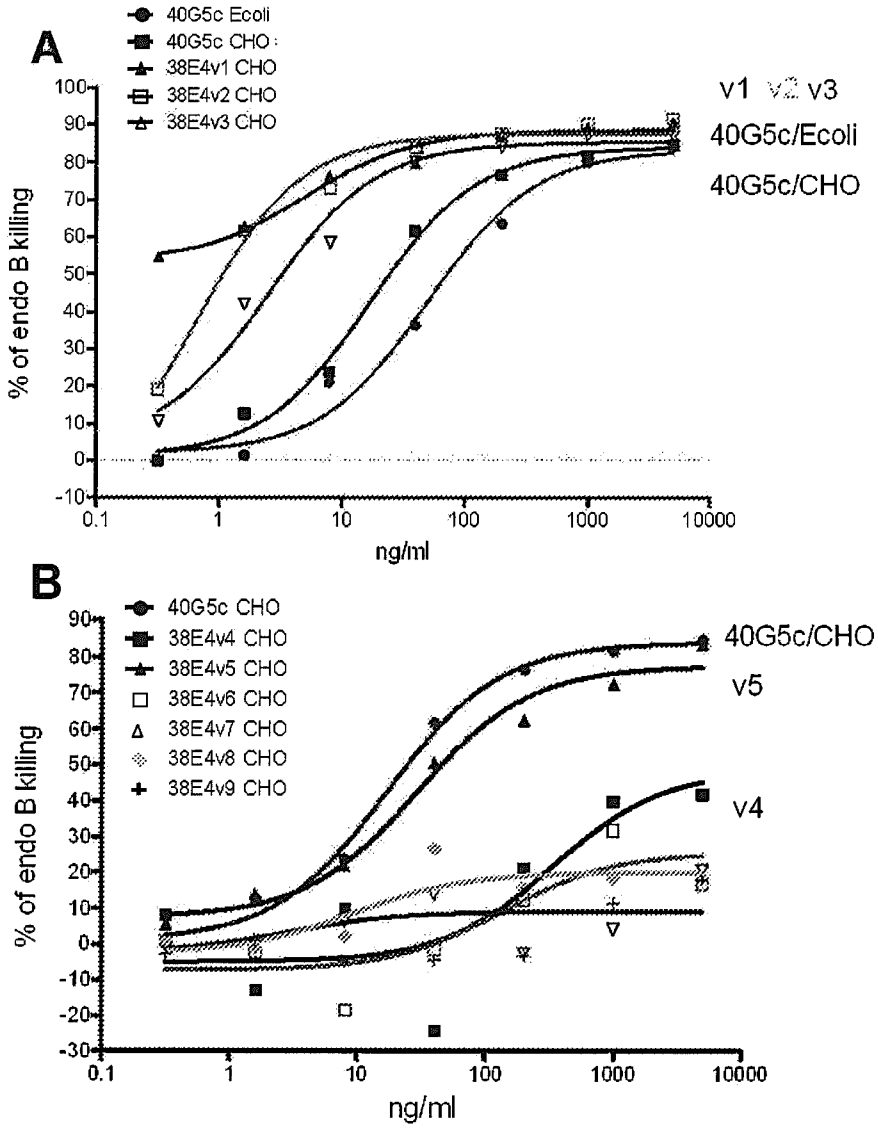
Donor#2 – 24h

CD3 arm	Source	CD8+T activation EC50 / ng/ml
40G5c	E.coli	215
40G5c	CHO	233
38E4v1	CHO	12.0
v2	CHO	11.4
v3	CHO	27.6
v4	CHO	9.51
v5	CHO	1.42
v6	CHO	NA
v7	CHO	NA
v8	CHO	NA
v9	CHO	NA

* N297G variant for all CHO material

200,000 huPBMC only.

Figures 42A-42C



C

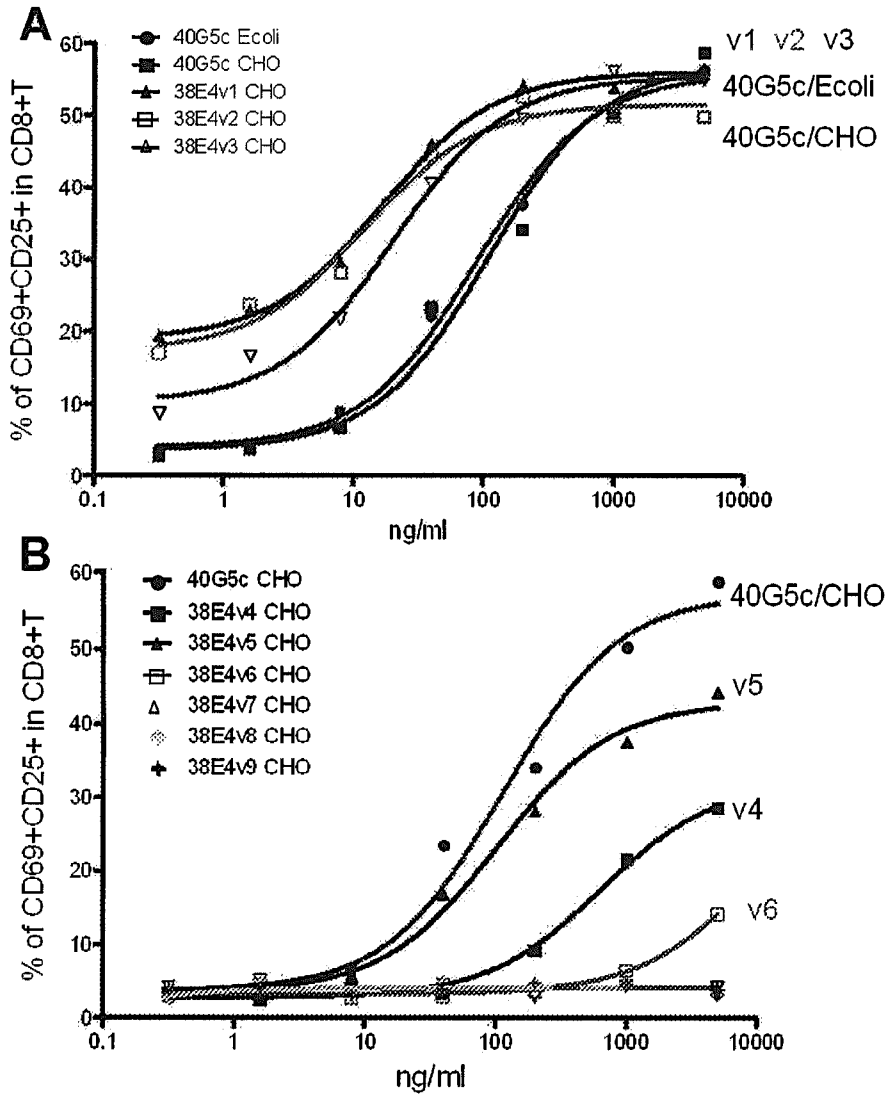
Donor#2 – 24h

CD3 arm	Source	endo B killing EC50 / ng/ml
40G5c	E.coli	50.3
40G5c	CHO	17.1
38E4v1	CHO	5.21
v2	CHO	0.624
v3	CHO	2.55
v4	CHO	343
v5	CHO	29.4
v6	CHO	NA
v7	CHO	NA
v8	CHO	NA
v9	CHO	NA

* N297G variant for all CHO material

200,000 huPBMC only.

Figures 43A-43C



C

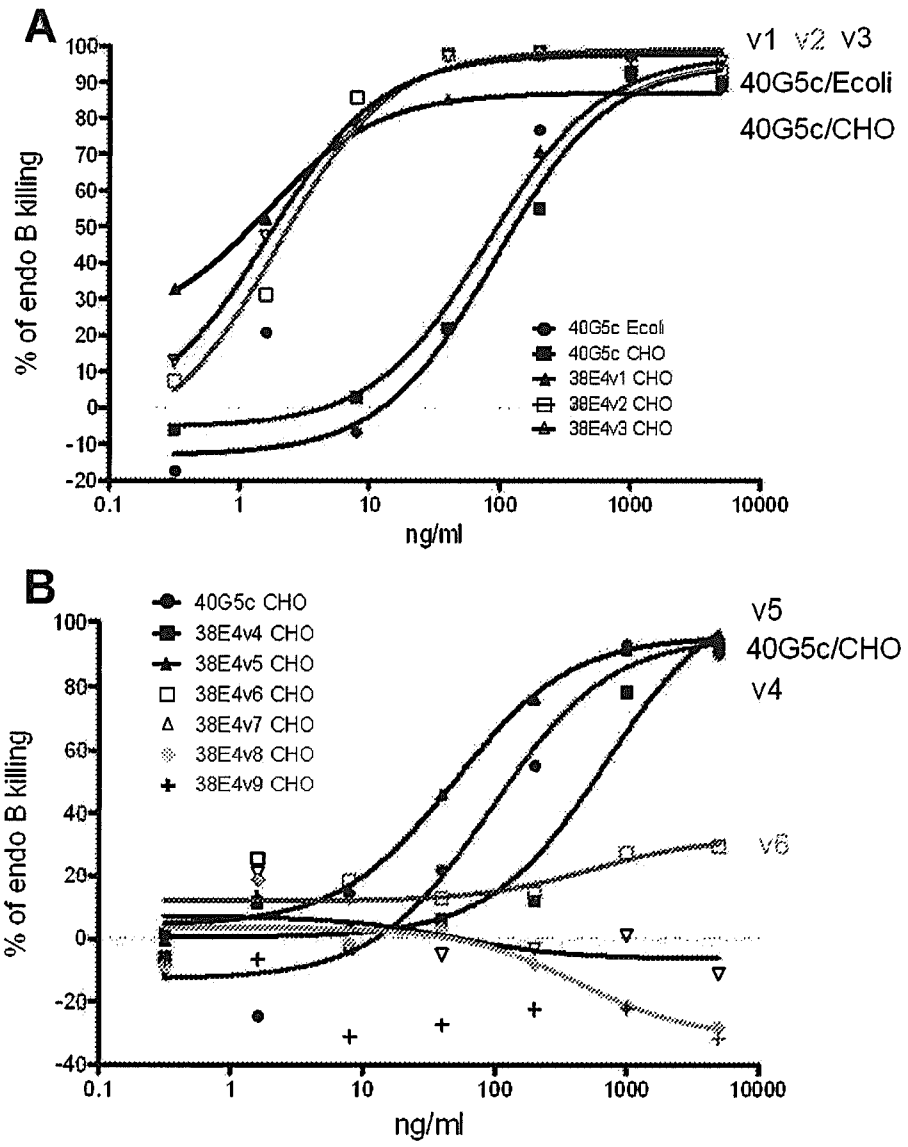
Donor#2 – 48h

CD3 arm	Source	CD8+T activation EC50 / ng/ml
40G5c	E.coli	90.4
40G5c	CHO	111
38E4v1	CHO	16.7
v2	CHO	12.6
v3	CHO	21.0
v4	CHO	631
v5	CHO	102
v6	CHO	8820
v7	CHO	NA
v8	CHO	NA
v9	CHO	NA

* N297G variant for all CHO material

200,000 huPBMC only.

Figures 44A-44C



C

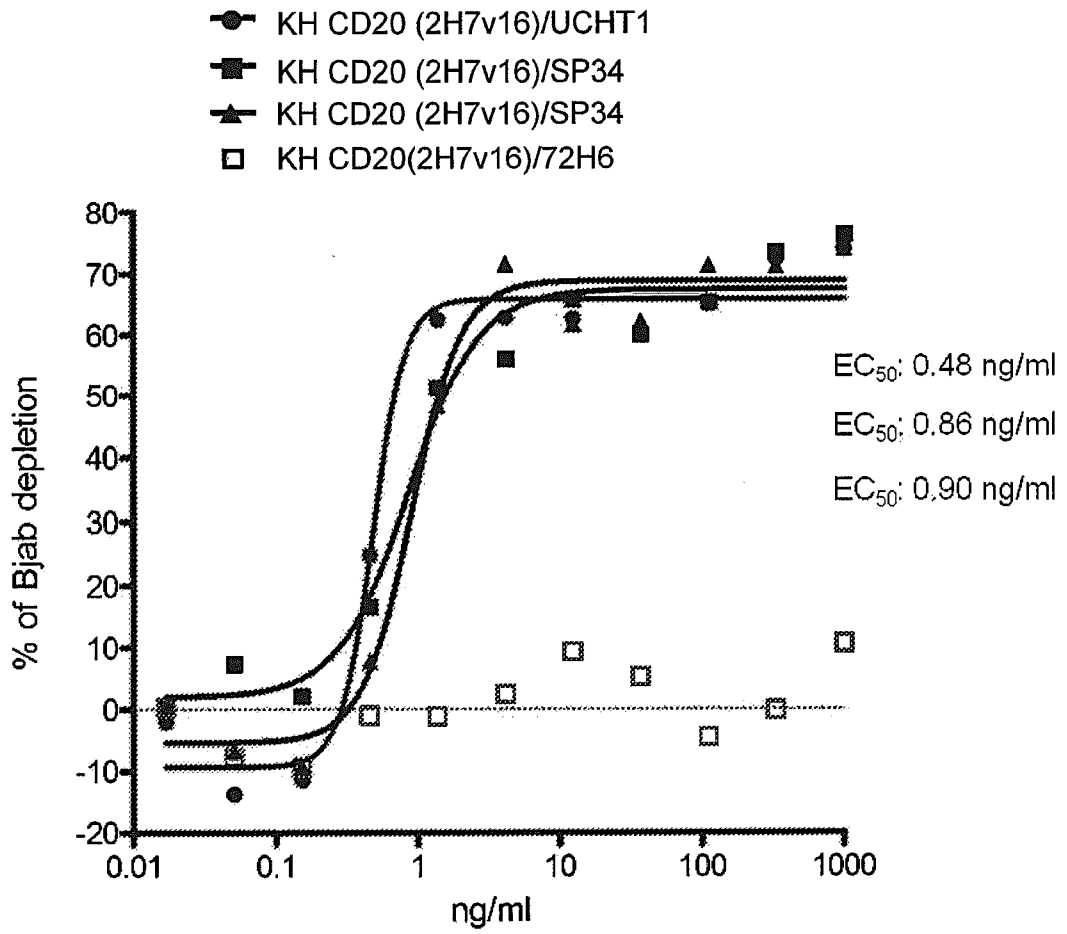
Donor#2 -48h

CD3 arm	Source	endo B killing EC50 / ng/ml
40G5c	E.coli	81.7
40G5c	CHO	92.8
38E4v1	CHO	1.59
v2	CHO	1.97
v3	CHO	1.70
v4	CHO	671
v5	CHO	49.2
v6	CHO	550
v7	CHO	NA
v8	CHO	NA
v9	CHO	NA

* N297G variant for all CHO material

200,000 huPBMC only.

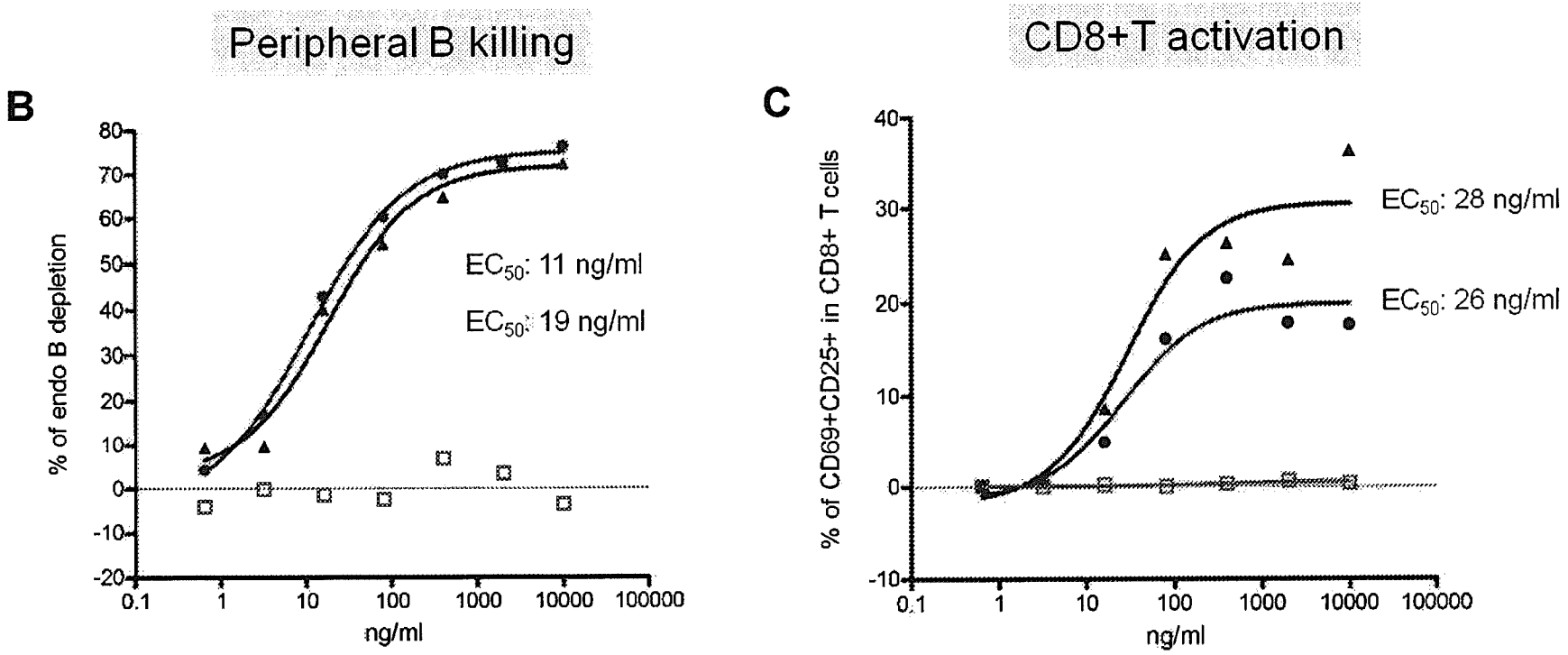
Figure 45A



20,000 Bjab-luc cells per well with 10×huPBMCs, 24 hours.

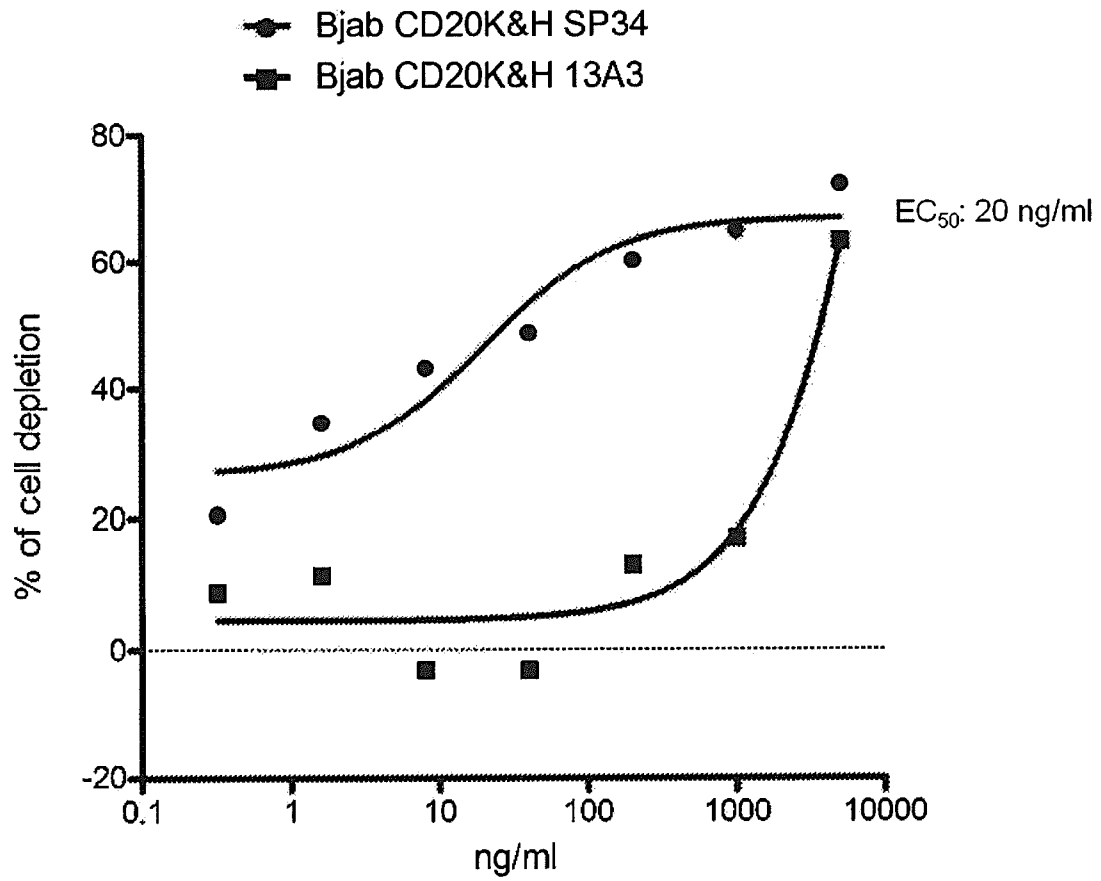
Figures 45B-45C

KH CD20 (2H7v16)/UCHT1
KH CD20 (2H7v16)/SP34
KH CD20(2H7v16)/72H6



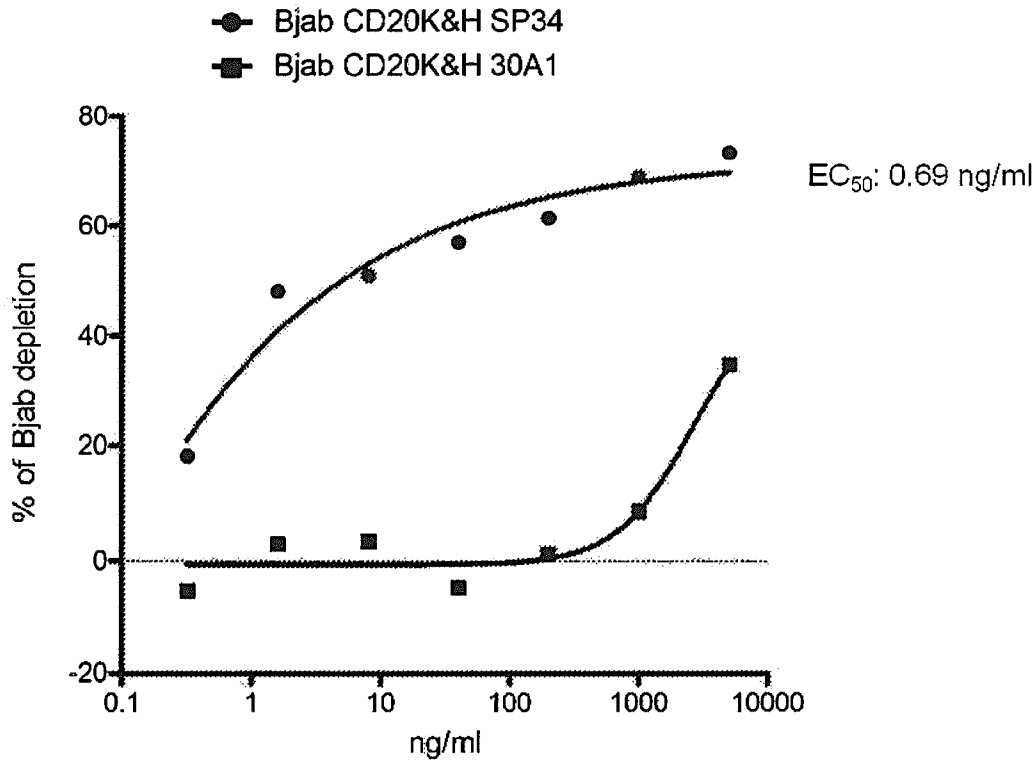
200,000 huPBMCs only, 24 hours.

Figure 46A



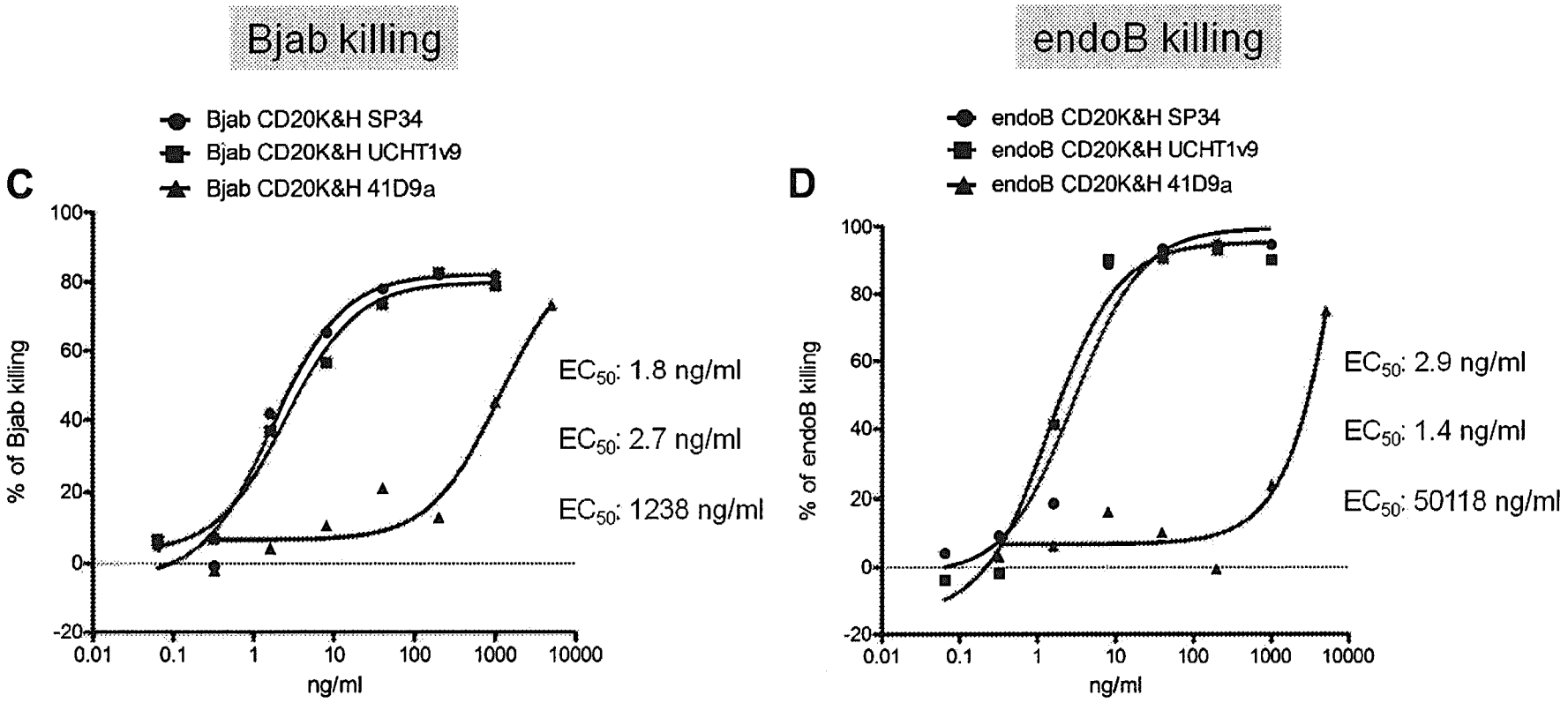
20,000 Bjab-luc cells per well with 10×huPBMCs, 24 hours.
Up to 5000ng/ml antibody.

Figure 46B



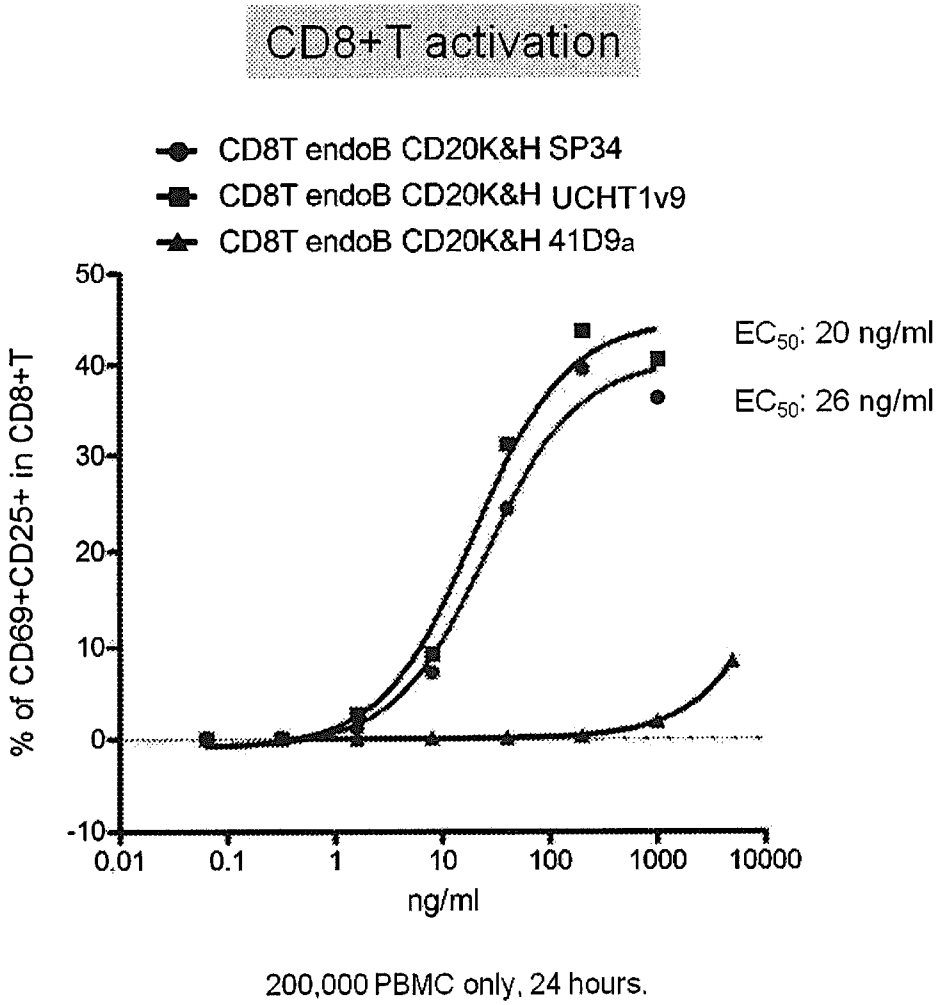
20,000 Bjab-luc cells per well with 10×huPBMCs, 24 hours.
Up to 5000ng/ml antibody.

Figures 46C-46D



20,000 Bjab-luc cells per well with 10×huPBMCs, or PBMC only, 24 hours.

Figure 46E



200,000 PBMC only, 24 hours.

Figure 47A

Donor#1

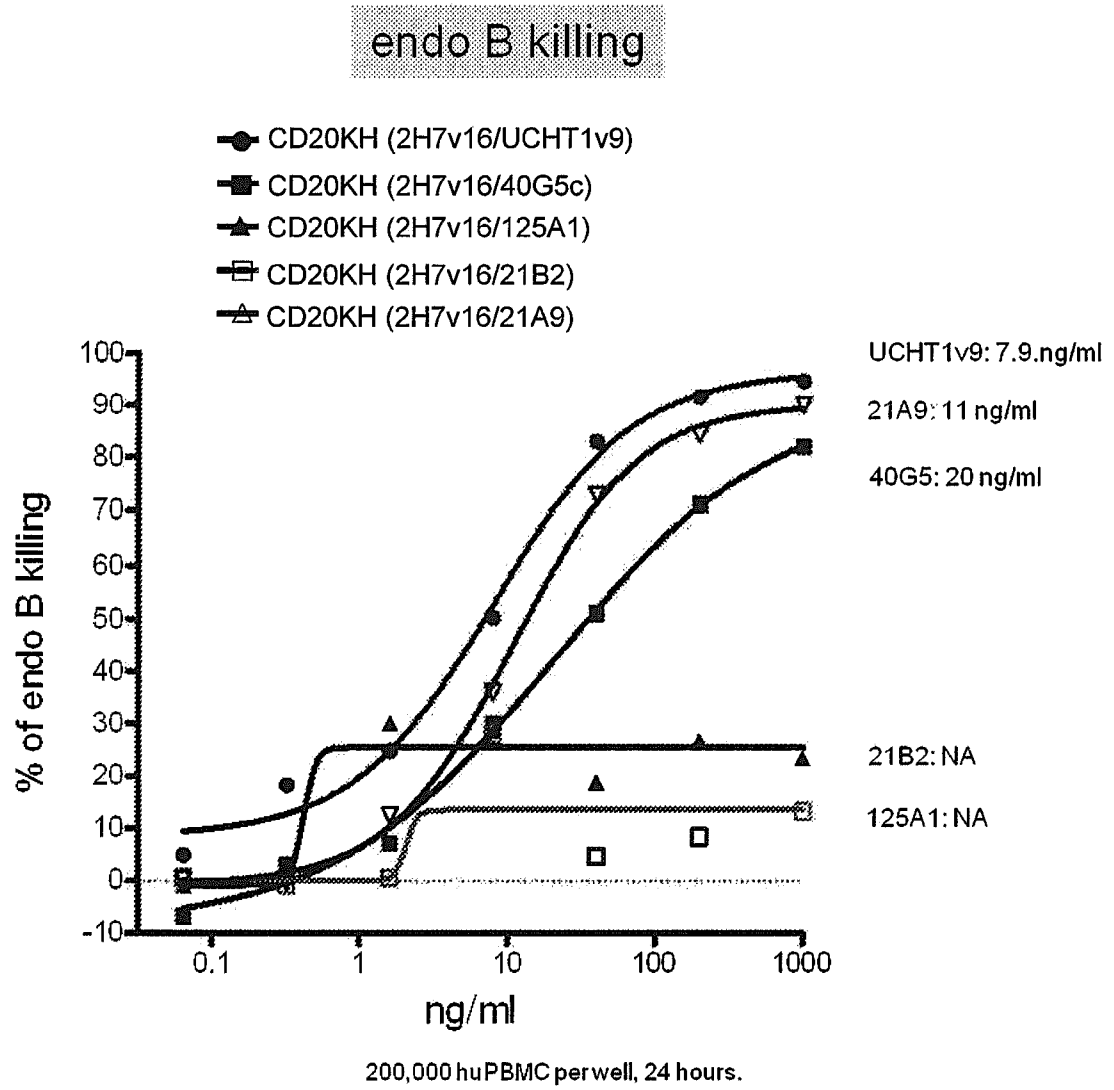


Figure 47B

Donor#1

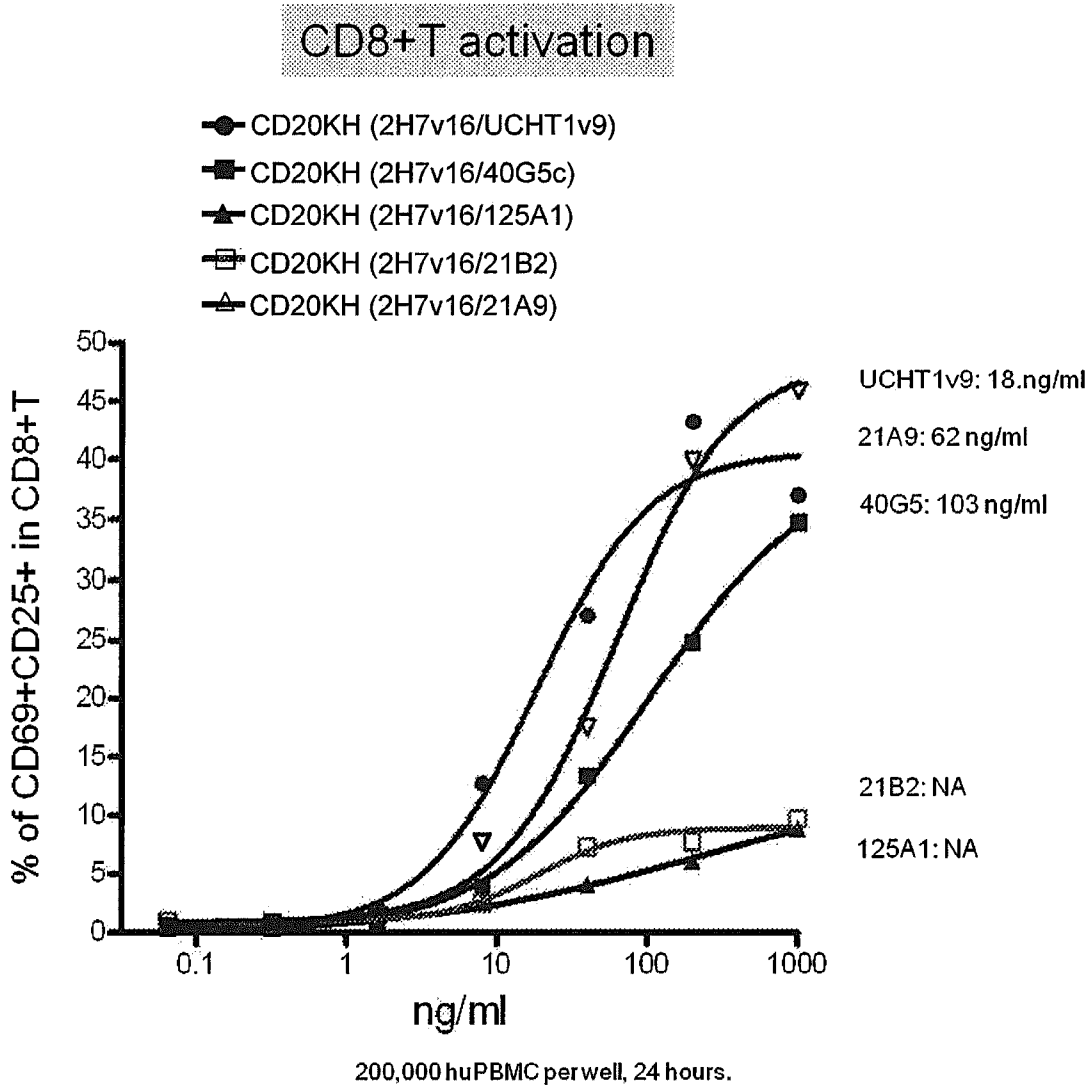
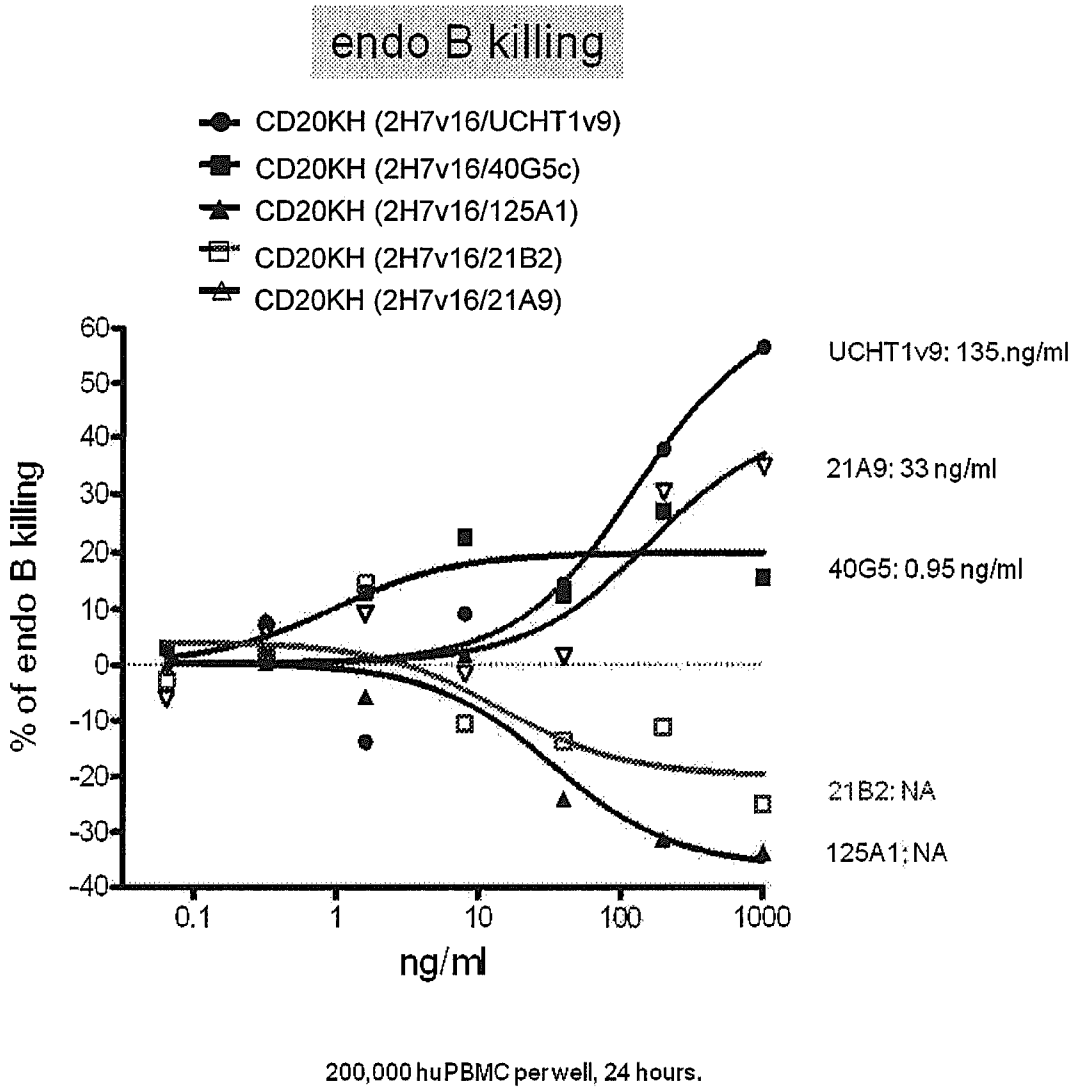


Figure 48A

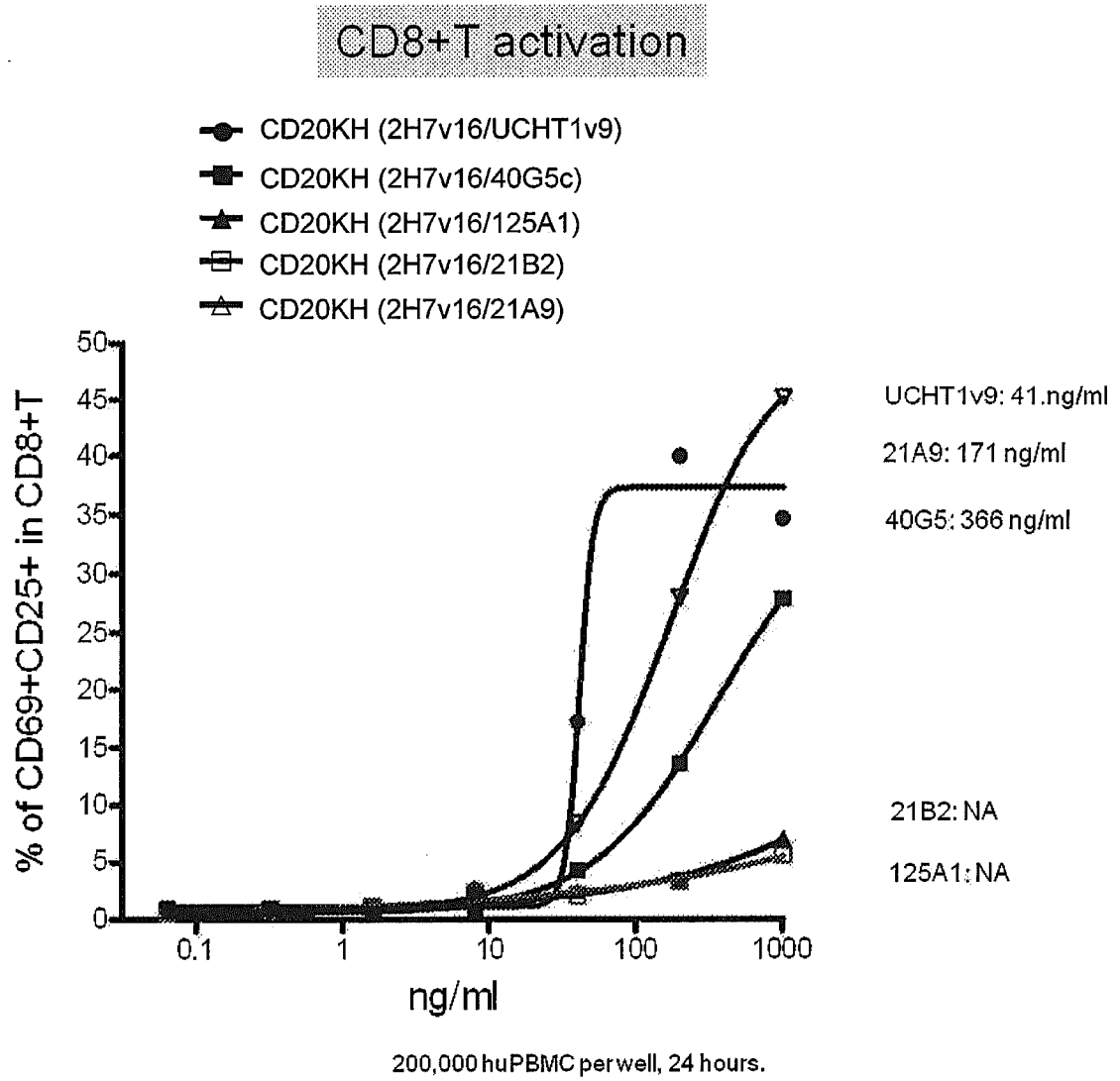
Donor#2



200,000 huPBMC per well, 24 hours.

Figure 48B

Donor#2



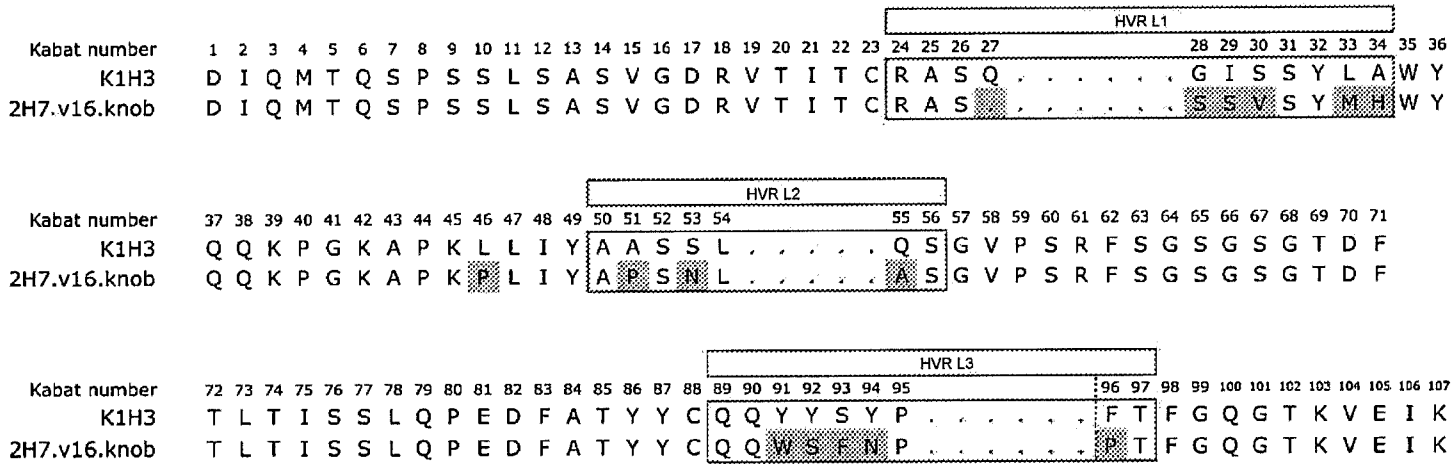
77169

Figure 49

CD20 arm	CD3 arm	Format	Potency In vitro
2H7v16	UCHT1v9	K&H	high
2H7v16	72H6	K&H	no
2H7v16	13A3	K&H	low
2H7v16	30A1	K&H	low
2H7v16	41D9a	K&H	low
2H7v16	SP34v52	K&H	high
2H7v16	40G5c	K&H	high
2H7v16	38E4c, 38E4V1-V9	K&H	high
2H7v16	21B2	K&H	very low
2H7v16	125A1	K&H	very low
2H7v16	21A9	K&H	high

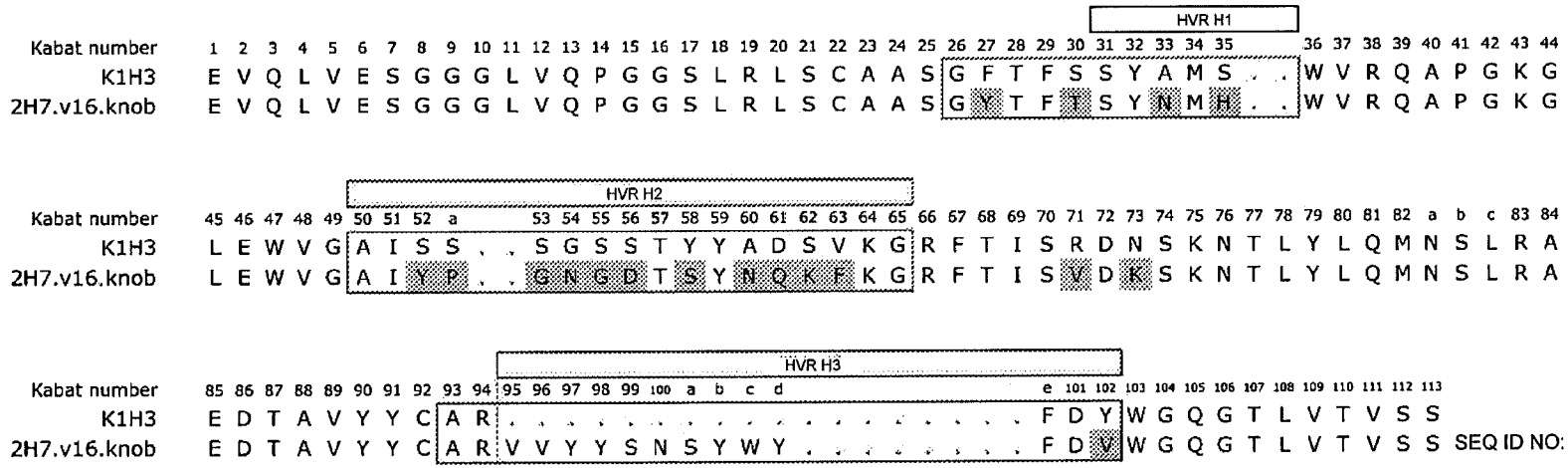
Figure 50

Light chain, Kappa: Humanized antibody aligned to K1H3



SEQ ID NO: 267

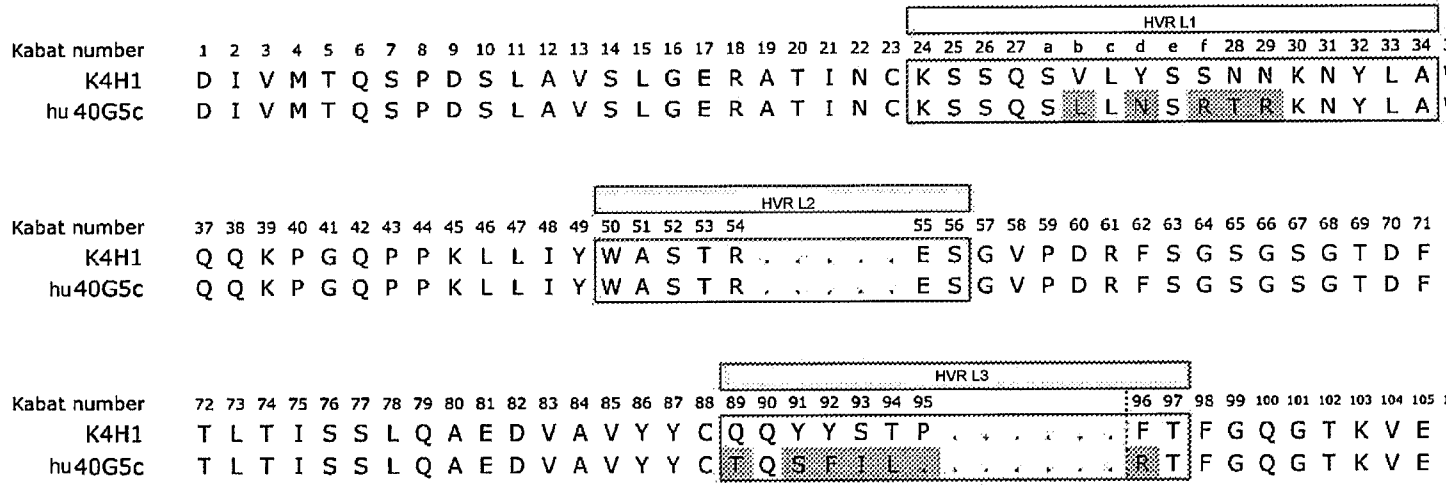
Heavy chain: Humanized antibody aligned to K1H3



SEQ ID NO: 266

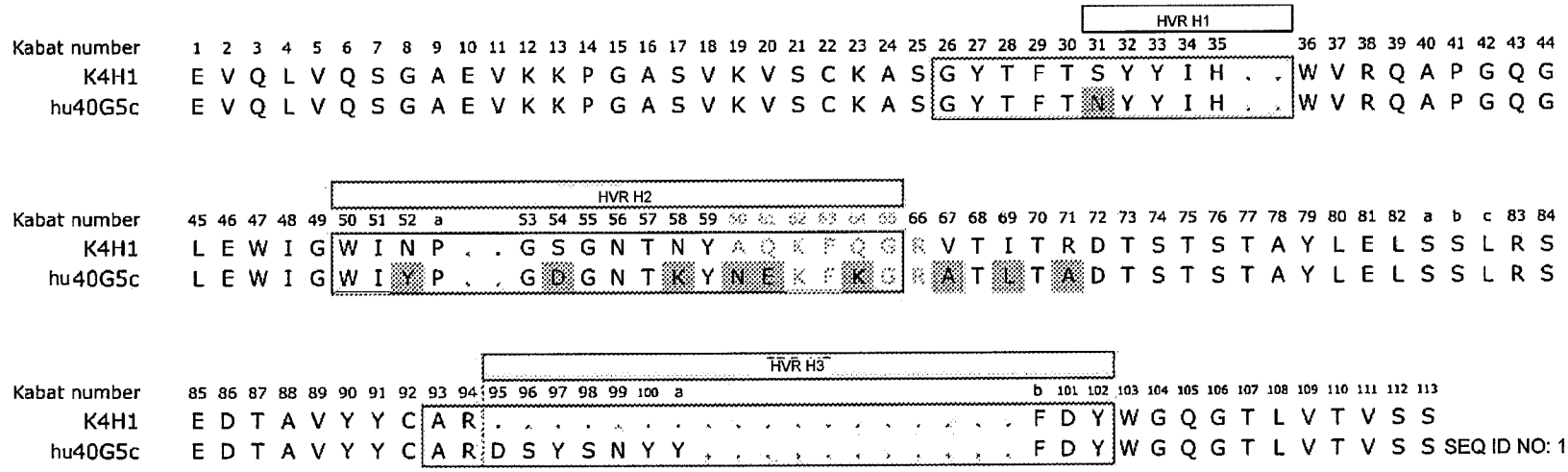
Figure 51

Light chain, Kappa: Humanized antibody aligned to K4H1



SEQ ID NO: 185

Heavy chain: Humanized antibody aligned to K4H1



SEQ ID NO: 184

Figure 52A

QC analysis for CD20 TDB

SEC analysis: No detectable aggregation

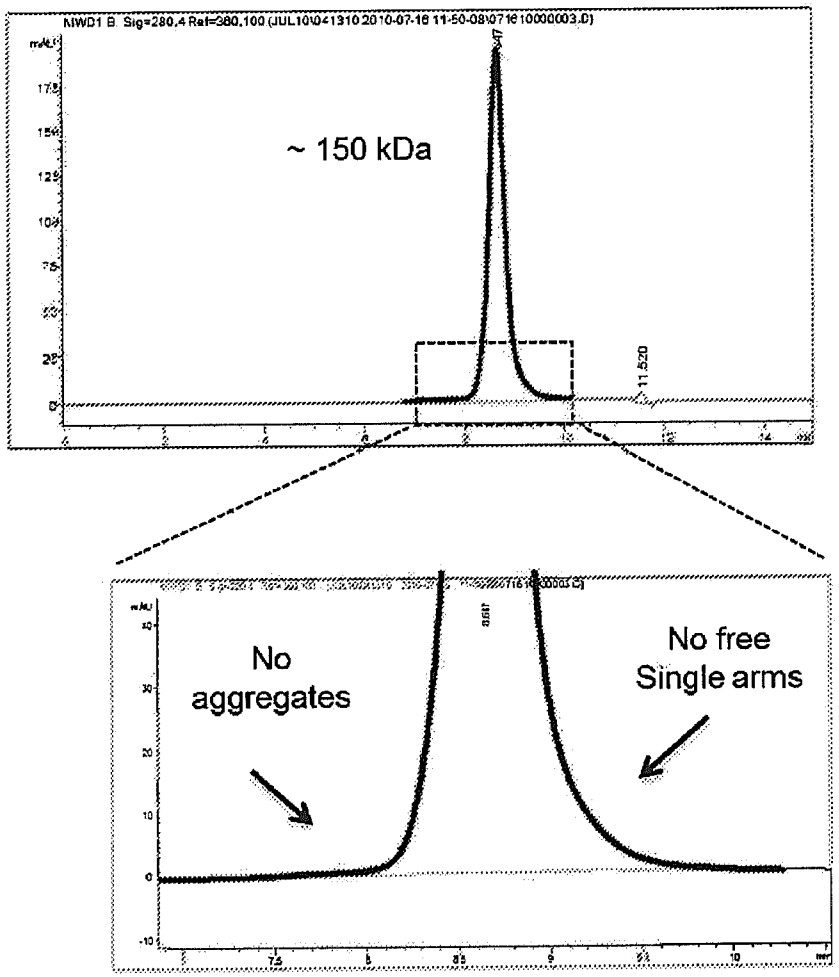
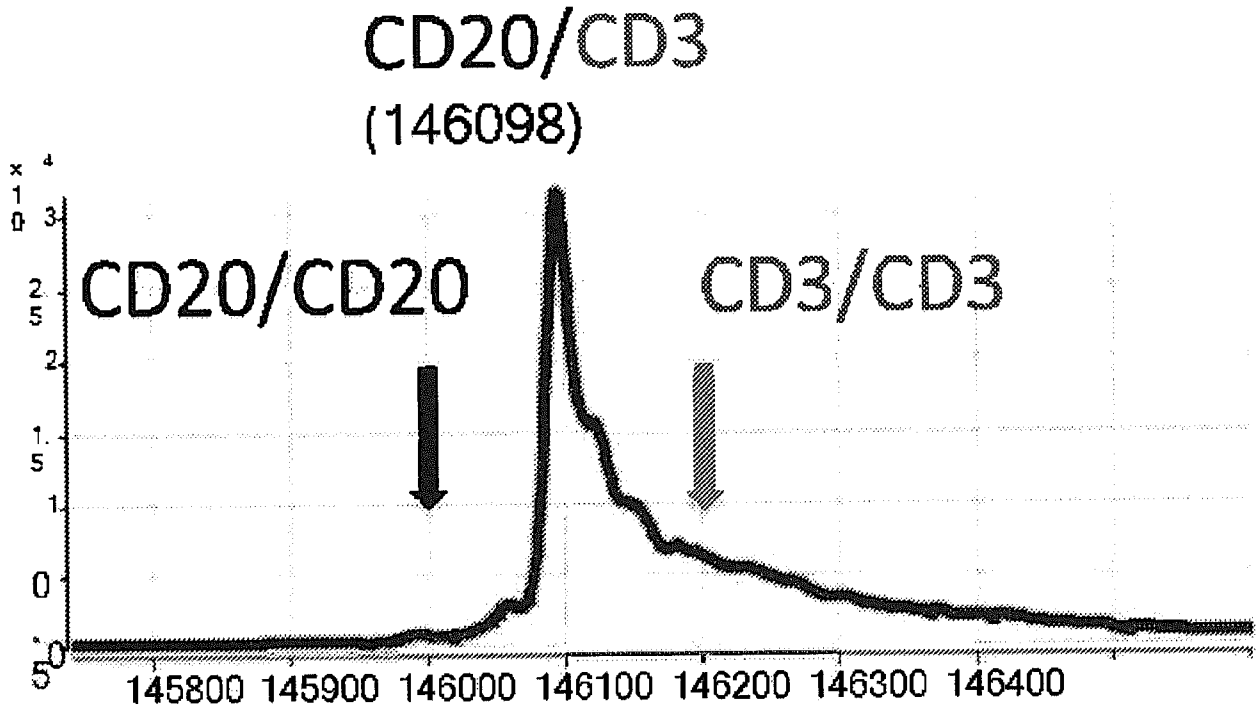


Figure 52B

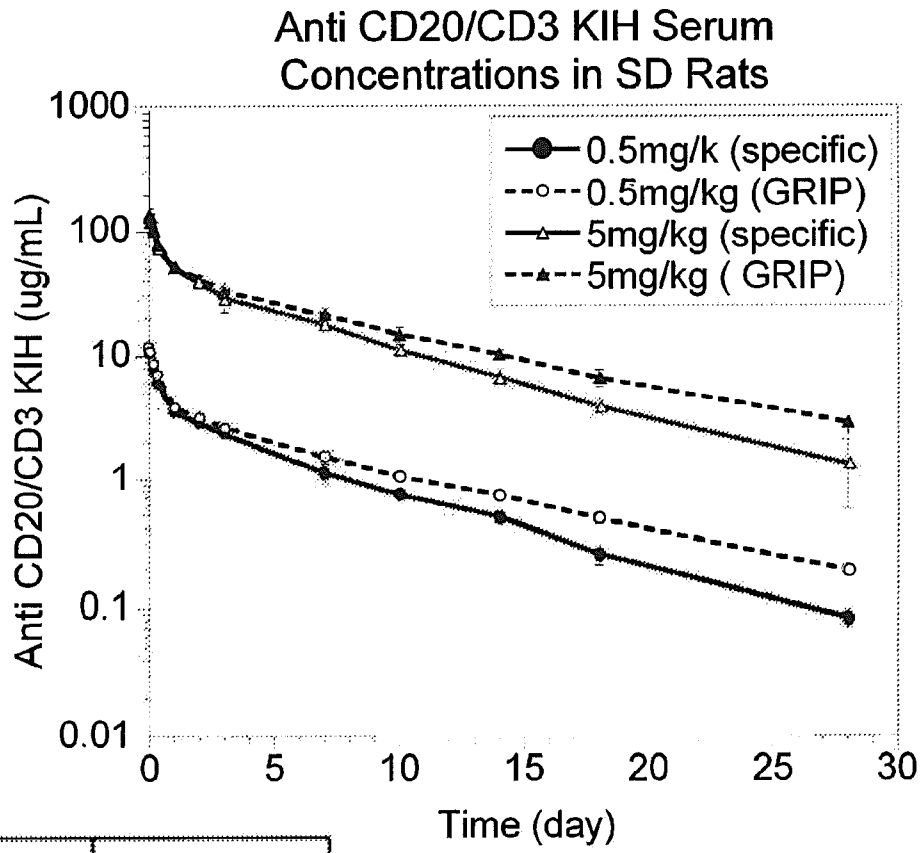
MS analysis: Only hetero-dimeric (Knob-hole) antibody detected



Figures 53A-53B

Rat PK analysis for CD20 TDB

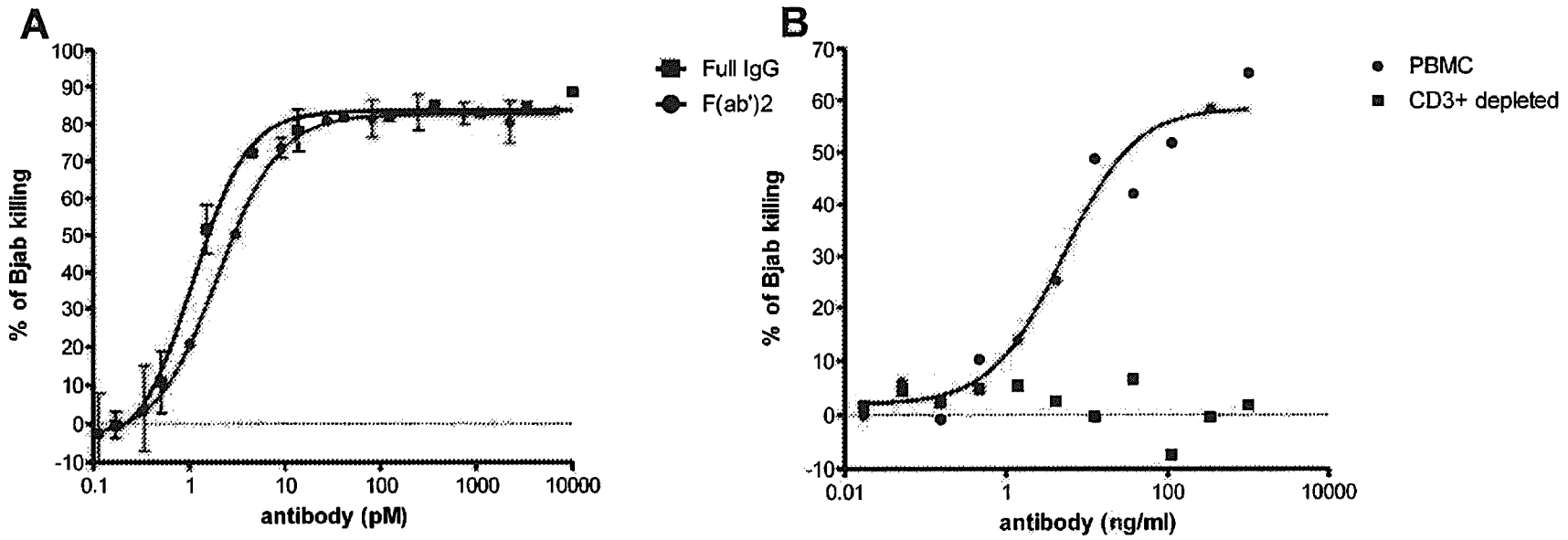
A



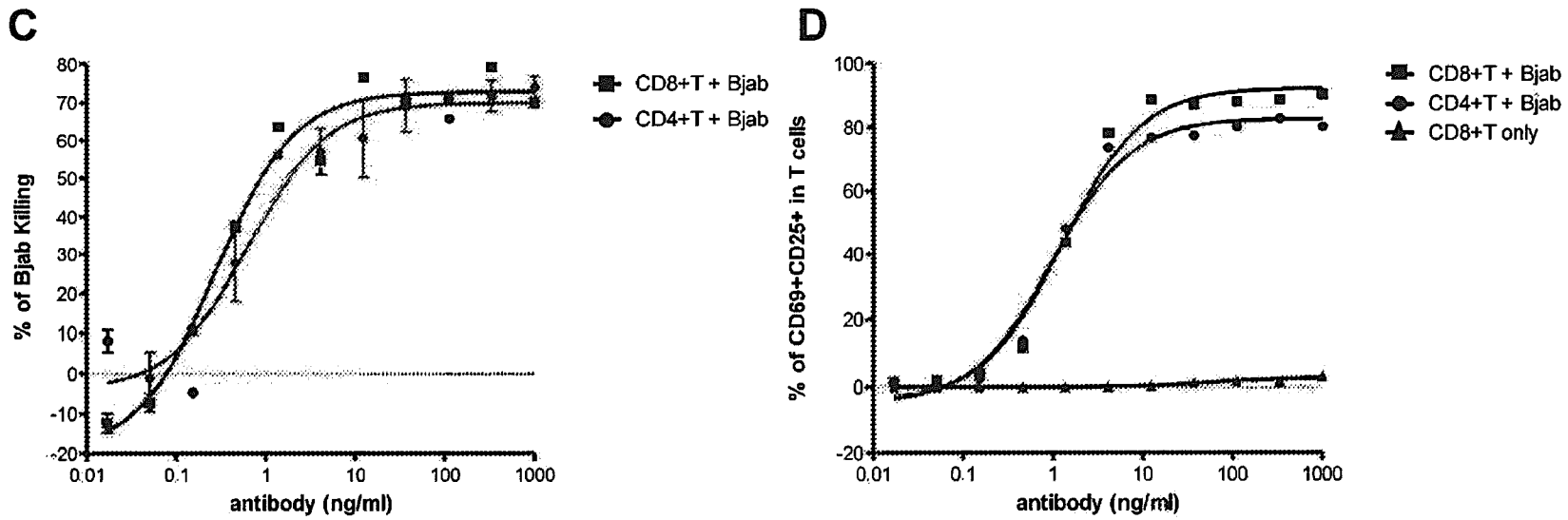
B

Dose	Assay	Cmax (ug/mL)	CL (mL/day/kg)	Beta HL (day)
0.5	GRIP	12.0 ± 0.600	14.1 ± 0.255	6.08 ± 0.116
5	GRIP	139 ± 20.6	10.5 ± 0.942	6.40 ± 0.572
0.5	Specific	11.9 ± 0.638	18.5 ± 0.529	4.62 ± 0.185
5	Specific	137 ± 18.2	13.3 ± 1.14	4.98 ± 0.400

Figures 54A-54B



Figures 54C-54D



Figures 54E-54F

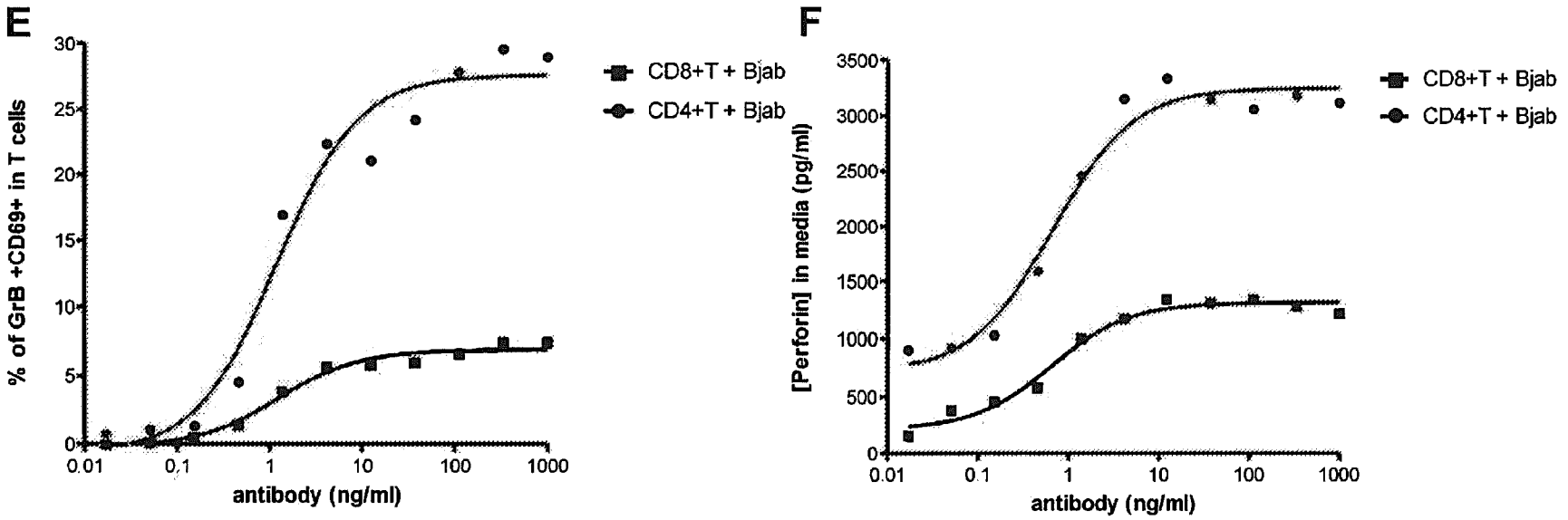


Figure 55

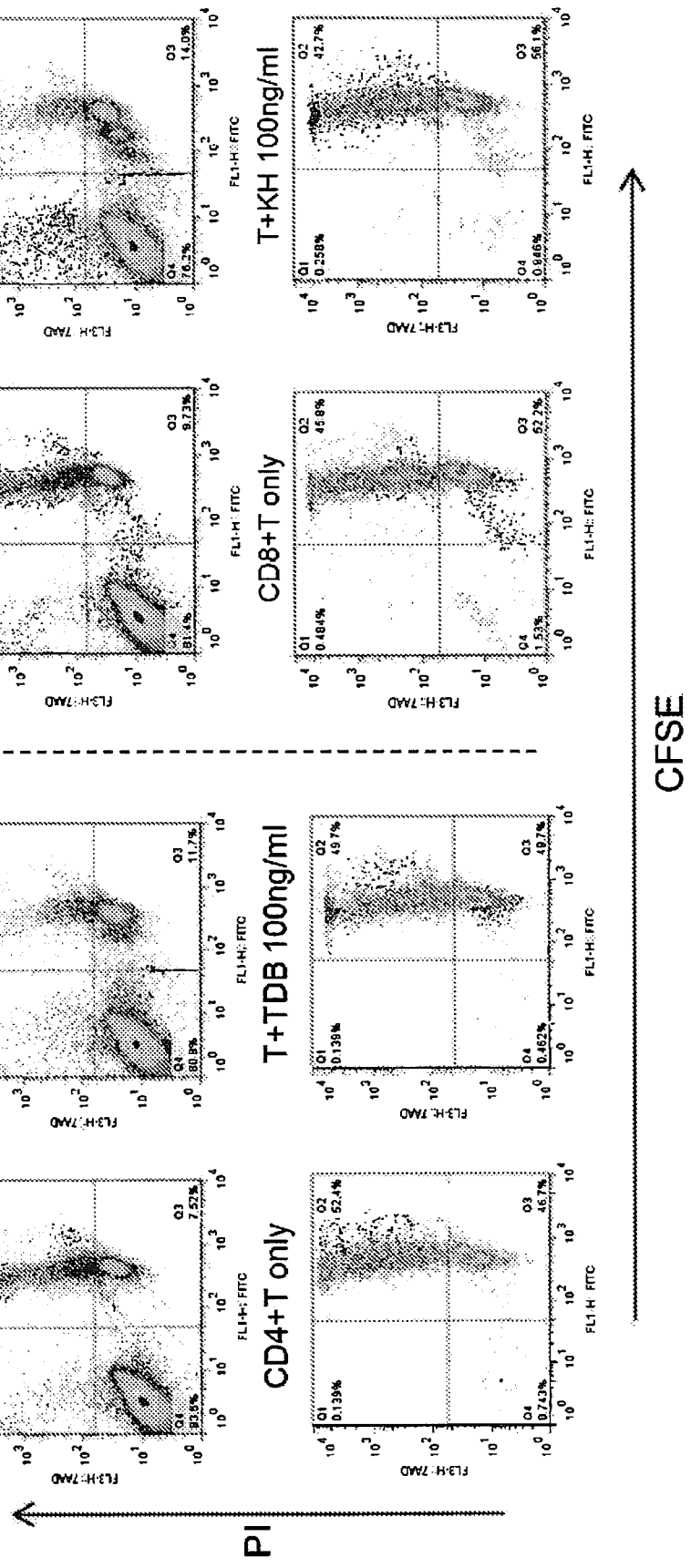
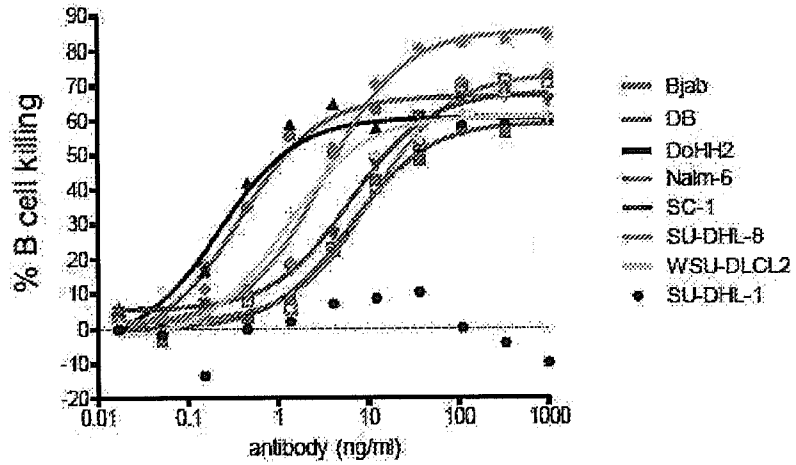

CD4+T + Bjab
T+B+TDB 100ng/ml
CD8+T + Bjab
T+B+KH 100ng/ml
CD4+T only
T+TDB 100ng/ml
CD8+T only
T+KH 100ng/ml
PI
CFSE

Figure 56A

A.


% B cell killing
90
80
70
60
50
40
30
20
10
0
-10
-20
0.01
0.1
1
10
100
1000
antibody (ng/ml)
Bjab
DB
DoHH2
Nalm-6
SC-1
SU-DHL-8
WSU-DLCL2
SU-DHL-1

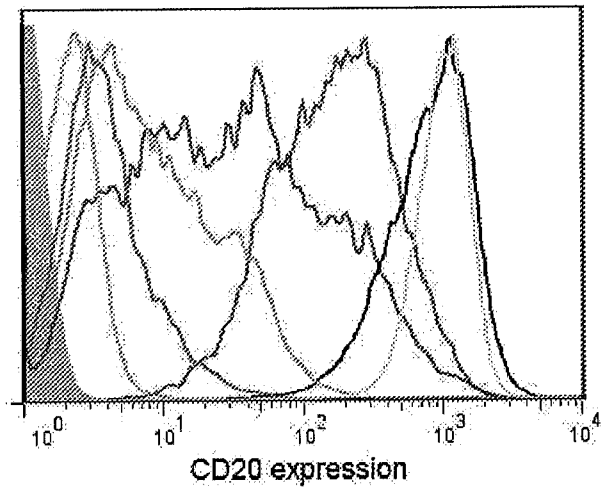
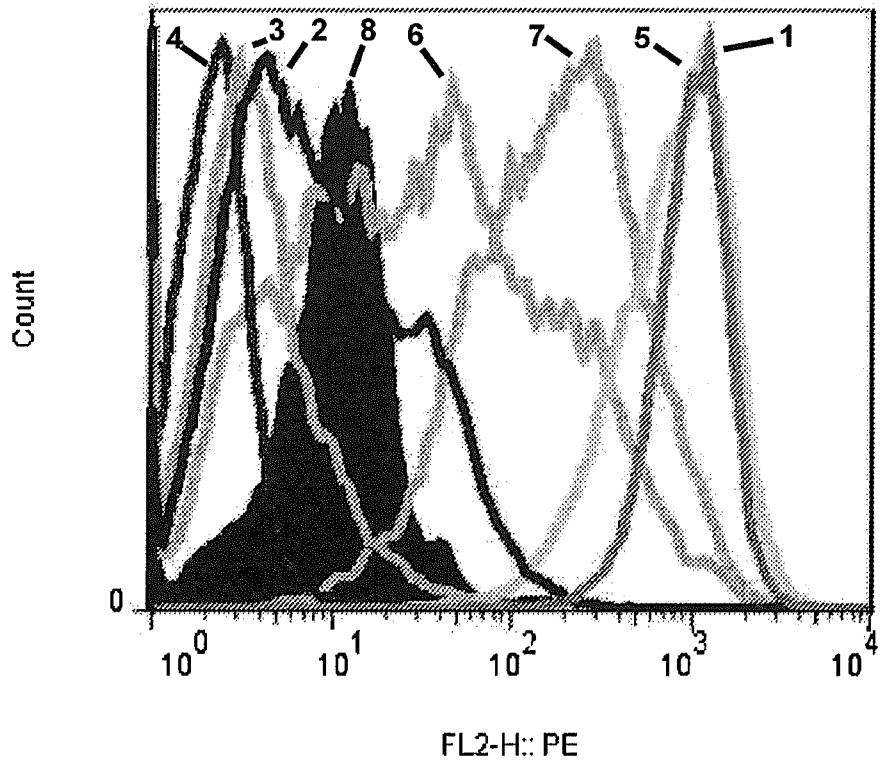
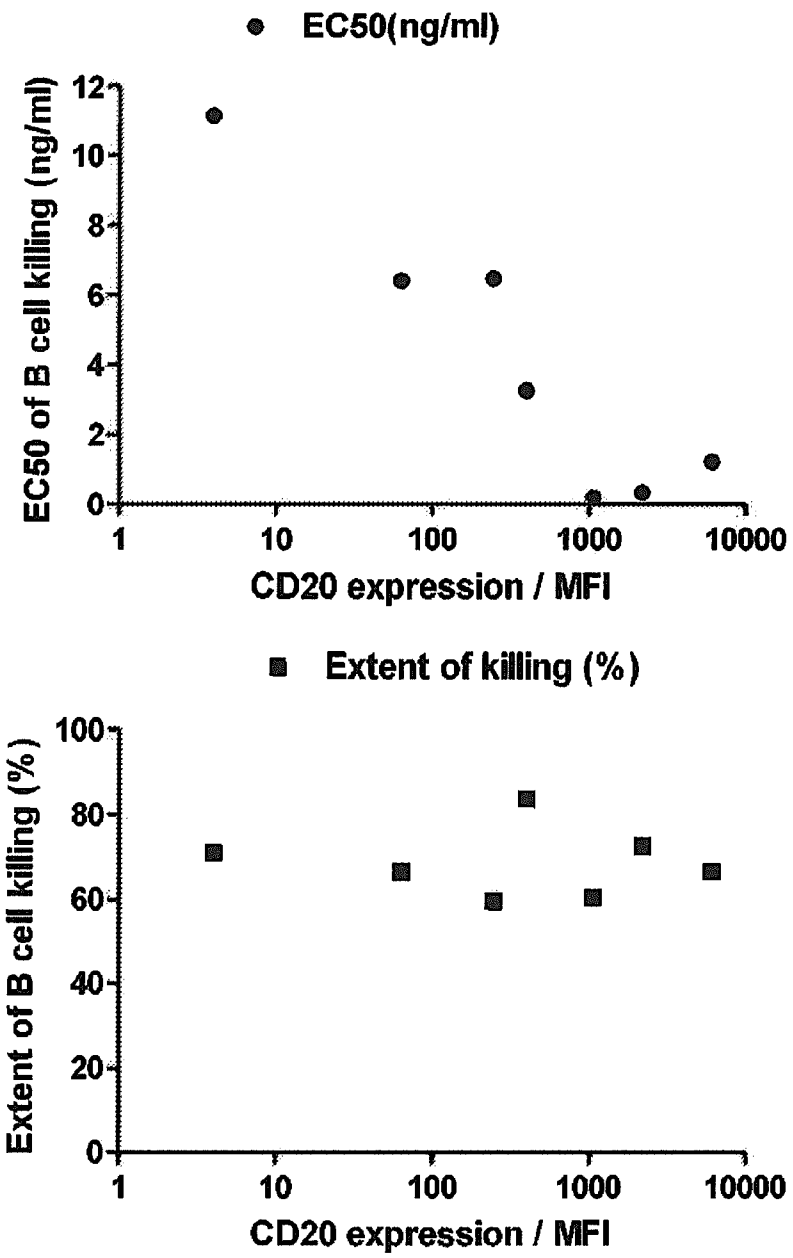

10^0
10^1
10^2
10^3
10^4
CD20 expression

Figure 56B

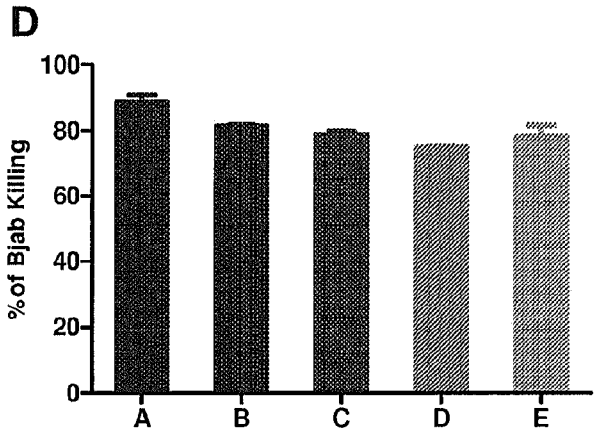


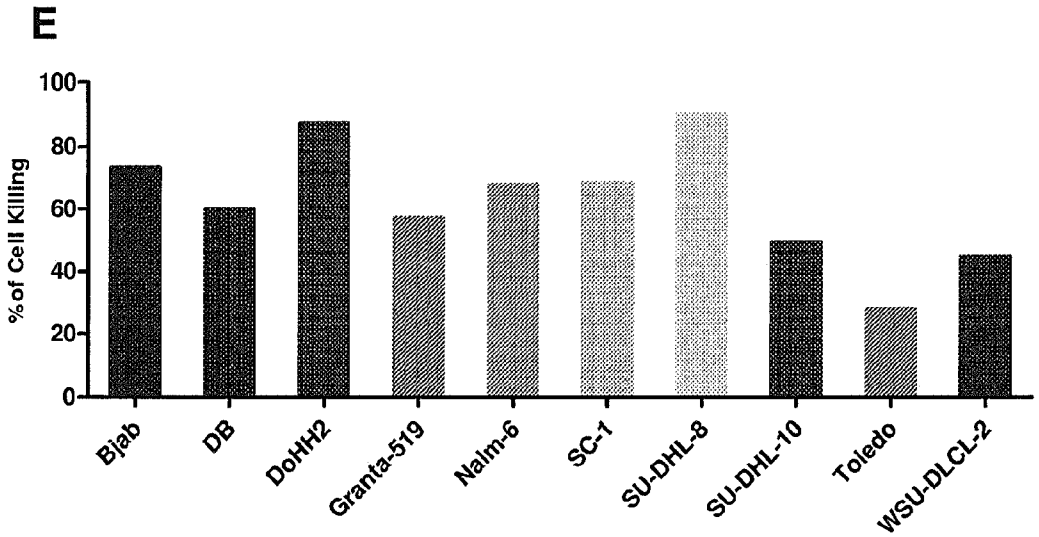
		Sample Name	Mean , FL2-H
1		LS20110307-WSU-DLCL2-CD20PE.001	1081
2		LS20110307-SU-DHL-8-CD20PE.001	17.4
3		LS20110307-SC-1-CD20PE.001	6.03
4		LS20110307-Nalm-6-CD20PE.001	2.73
5		LS20110307-DoHH2-CD20PE.001	942
6		LS20110307-DB-CD20PE.001	107
7		LS20110307-Bjab-CD20PE.001	284
8		LS20110307-PBMC-CD20PE.001	16.9

Figure 56C


EC50(ng/ml)
EC50 of B cell killing (ng/ml)
12
10
8
6
4
2
0
1
10
100
1000
10000
CD20 expression / MFI
Extent of killing (%)
Extent of B cell killing (%)
100
80
60
40
20
0
1
10
100
1000
10000
CD20 expression / MFI

Figures 56D-56E





B-cells with purified CD8+T cells

Figure 56F

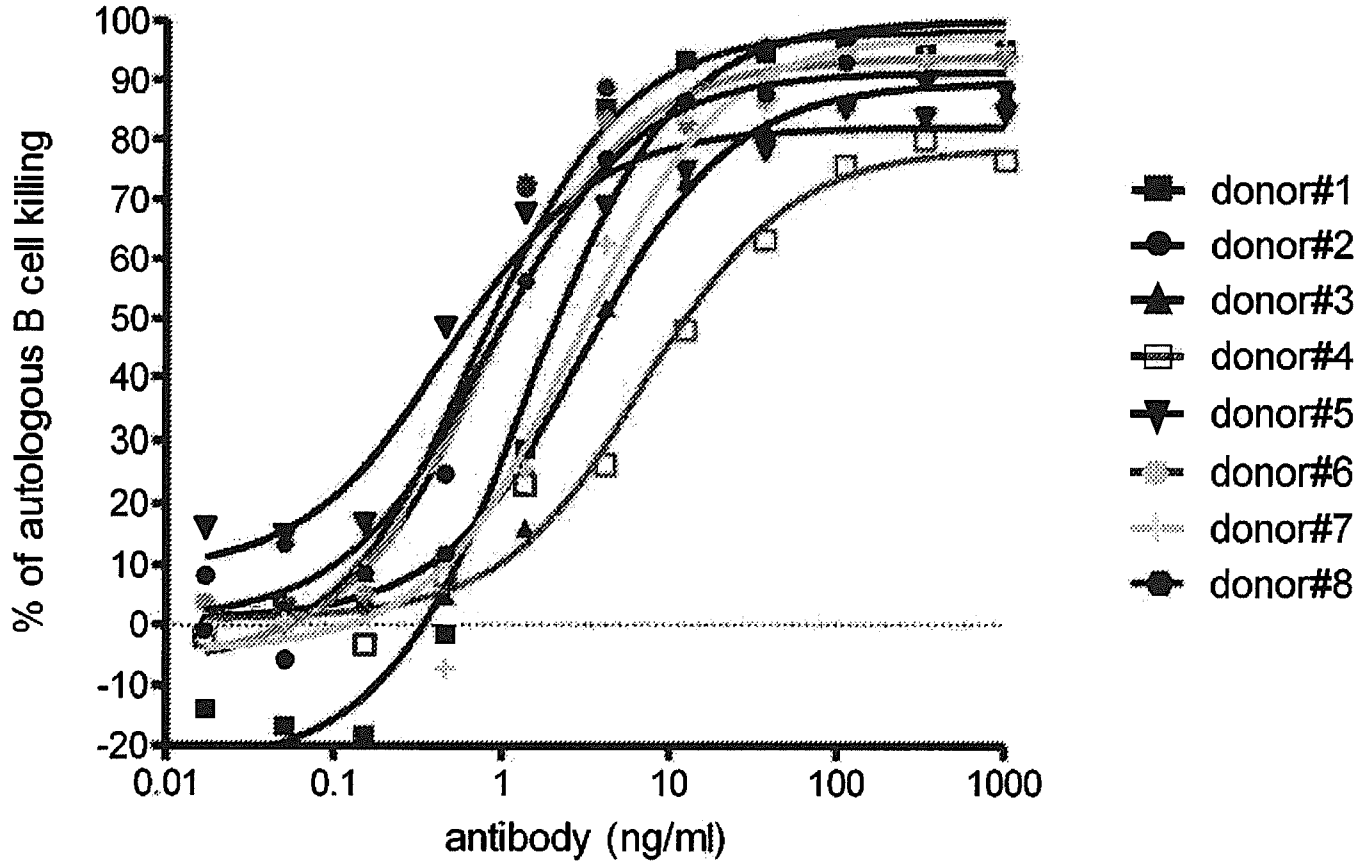

% of autologous B cell killing
100
90
80
70
60
50
40
30
20
10
0
-10
-20
0.01
0.1
1
10
100
1000
antibody (ng/ml)
donor#1
donor#2
donor#3
donor#4
donor#5
donor#6
donor#7
donor#8

Figure 56G

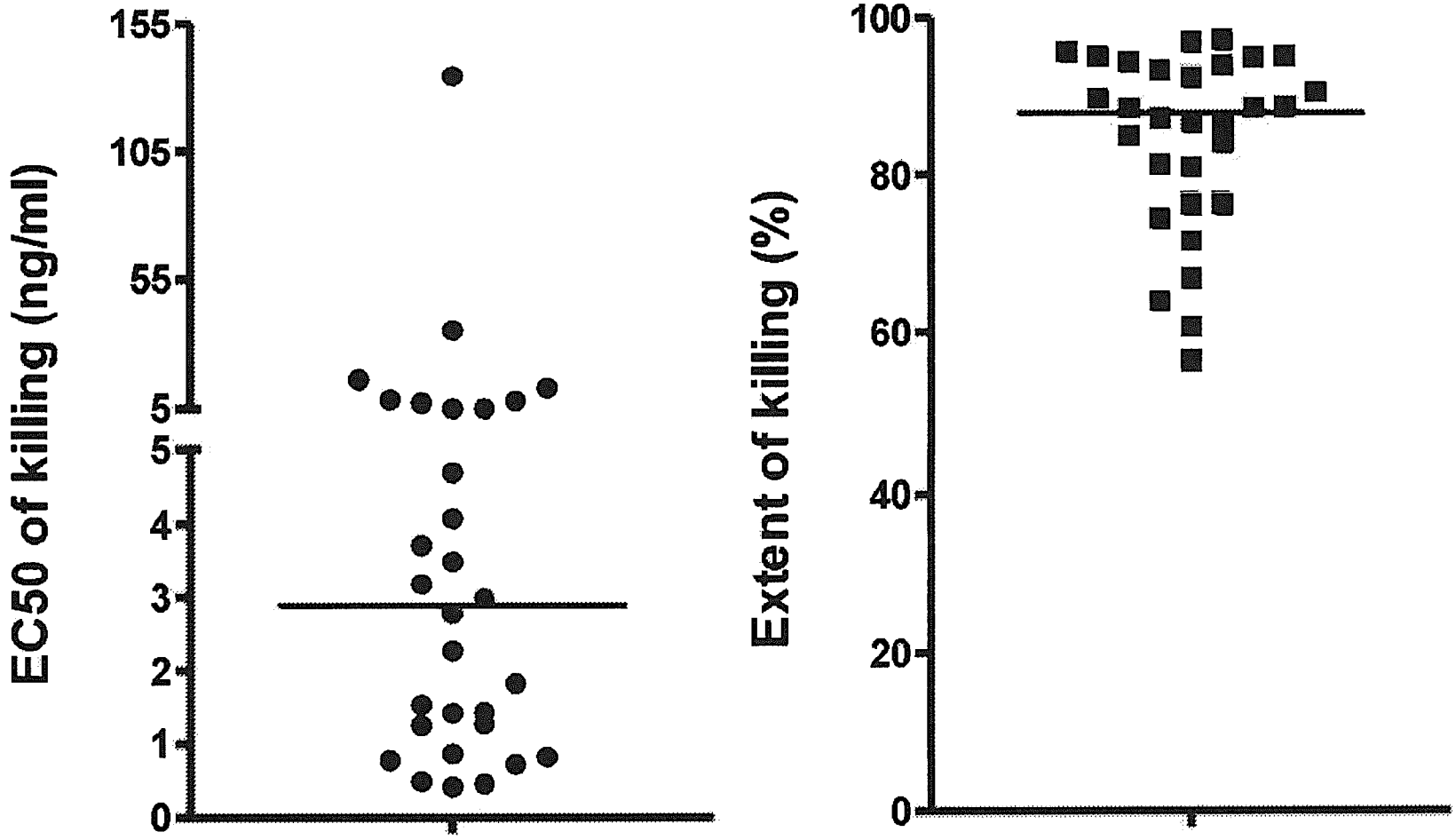

EC50 of killing (ng/ml)
155
105
55
5
5
4
3
2
1
0
Extent of killing (%)
100
80
60
40
20
0

Figure 56H

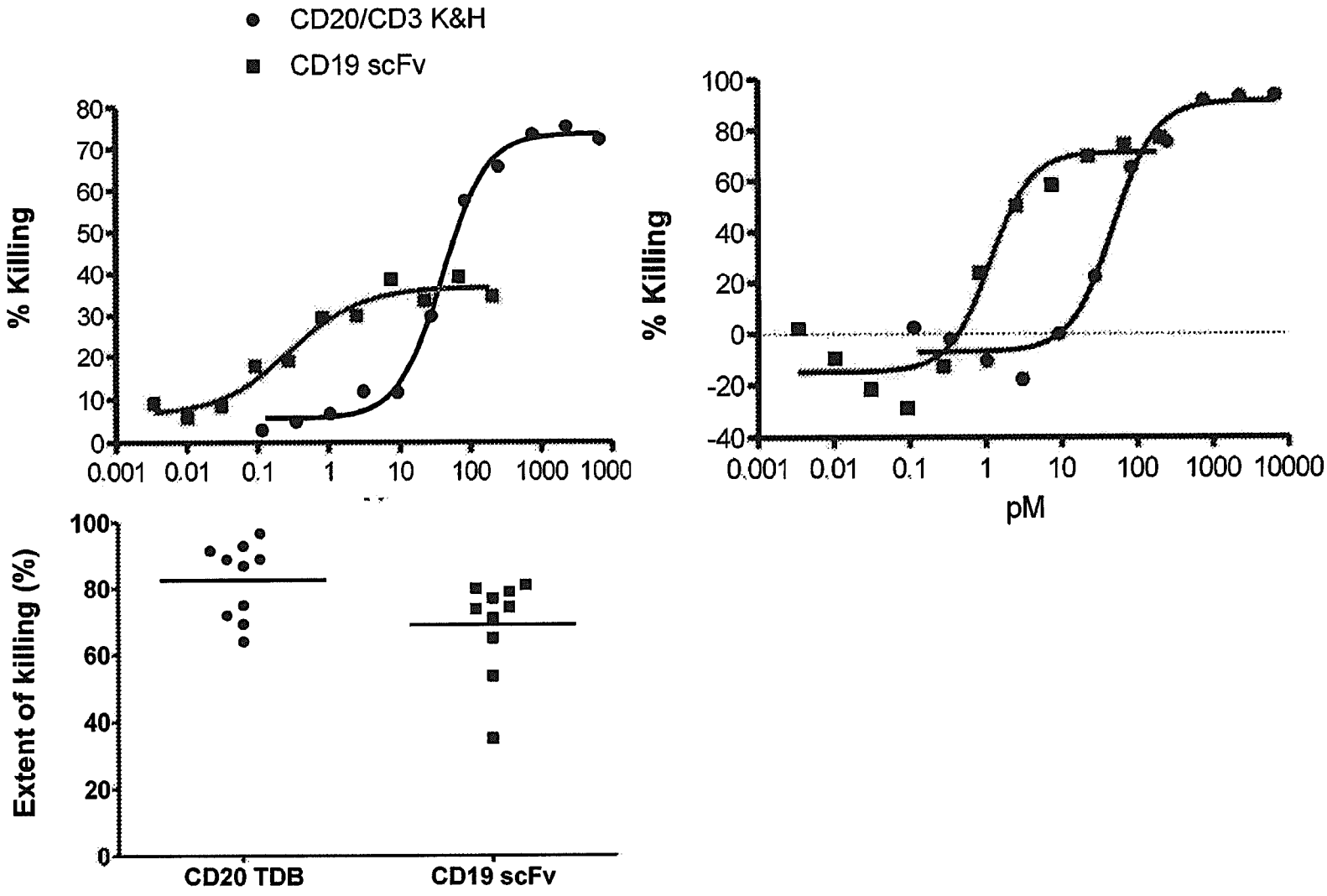

CD20/CD3 K&H
CD19 scFv
% Killing
80
70
60
50
40
30
20
10
0
0.001
0.01
0.1
1
10
100
1000
1000
% Killing
100
80
60
40
20
0
-20
-40
0.001
0.01
0.1
1
10
100
1000
10000
pM
Extent of killing (%)
100
80
60
40
20
0
CD20 TDB
CD19 scFv

Figure 56I

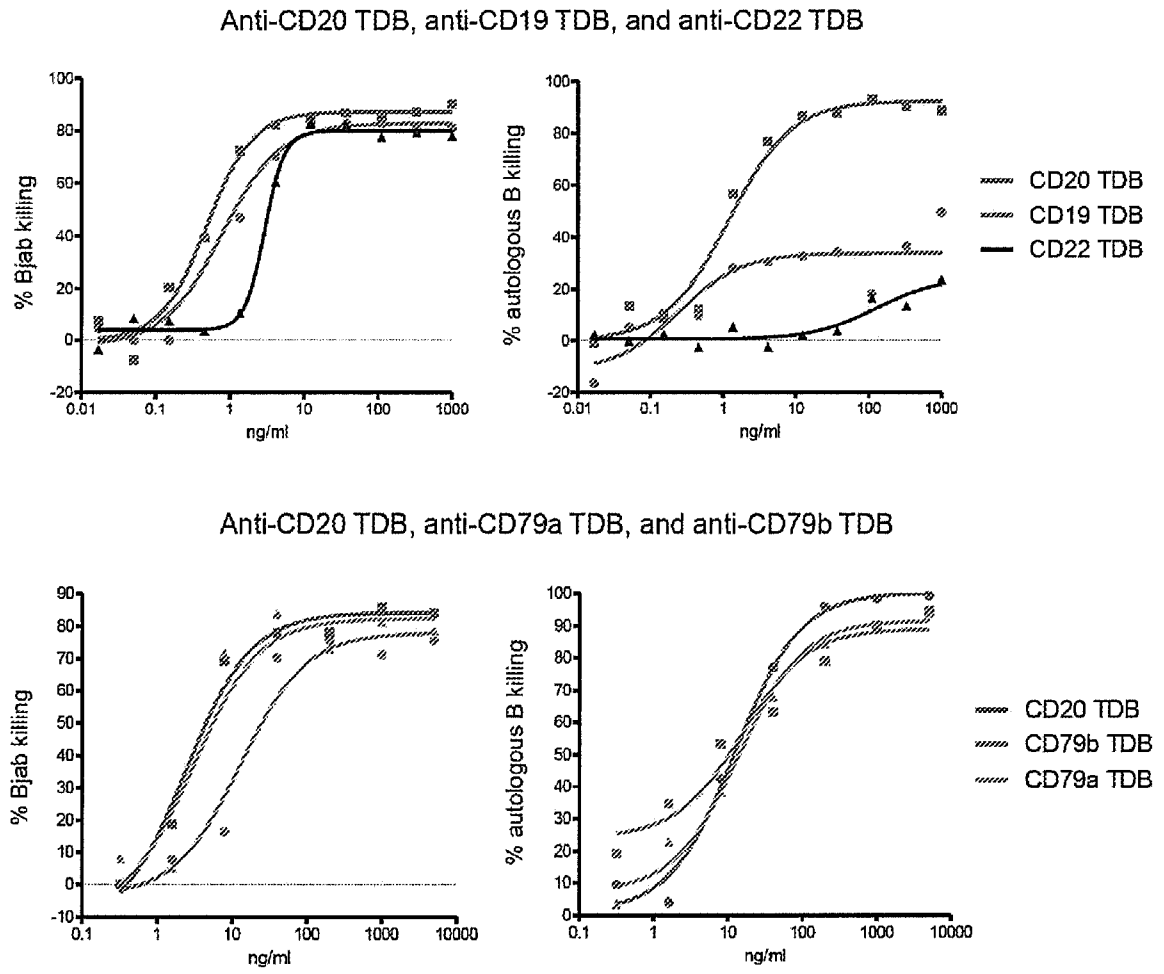
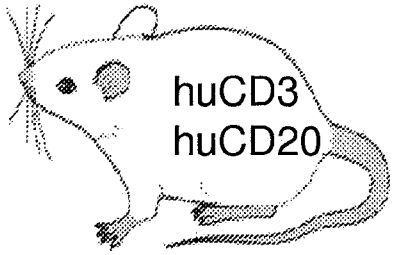
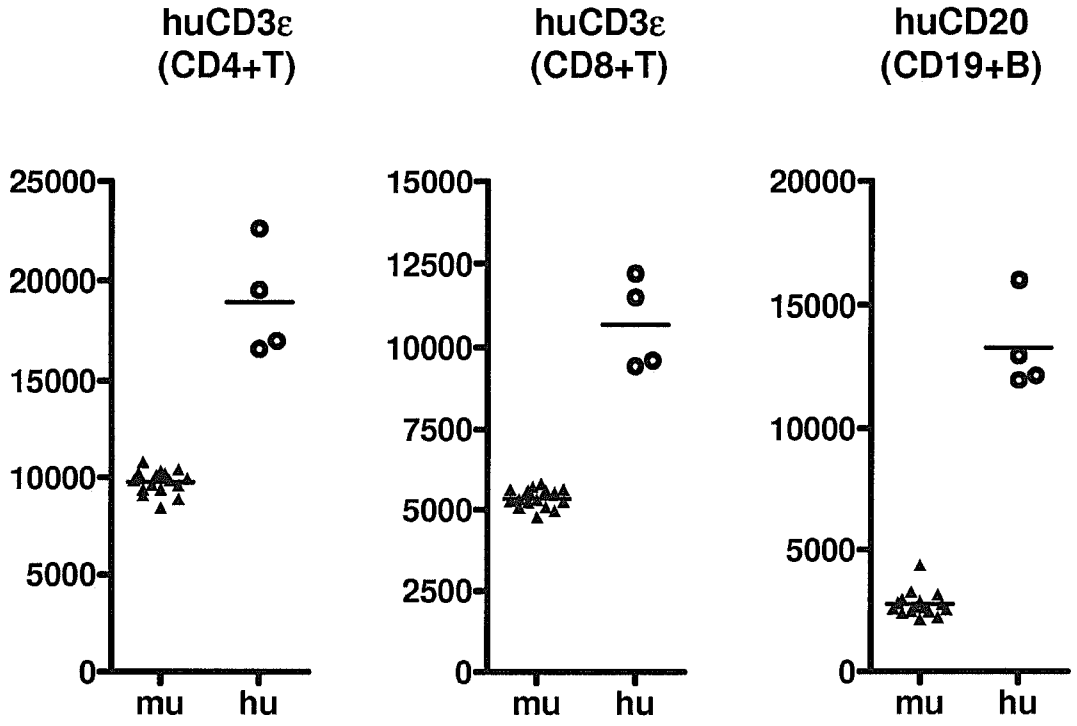

Anti-CD20 TDB, anti-CD19 TDB, and anti-CD22 TDB
% Bjab killing
% autologous B killing
ng/ml
CD20 TDB
CD19 TDB
CD22 TDB
Anti-CD20 TDB, anti-CD79a TDB, and anti-CD79b TDB
% Bjab killing
% autologous B killing
ng/ml
CD20 TDB
CD79b TDB
CD79a TDB

Figure 57A


huCD3
huCD20


huCD3ε
(CD4+T)
25000
20000
15000
10000
5000
0
mu
hu
huCD3ε
(CD8+T)
15000
12500
10000
7500
5000
2500
0
mu
hu
huCD20
(CD19+B)
20000
15000
10000
5000
0
mu
hu

Figures 57B-57C

D7 spleen
Sd, iv
0.5mg/kg TDB
10mg/kg rituximab

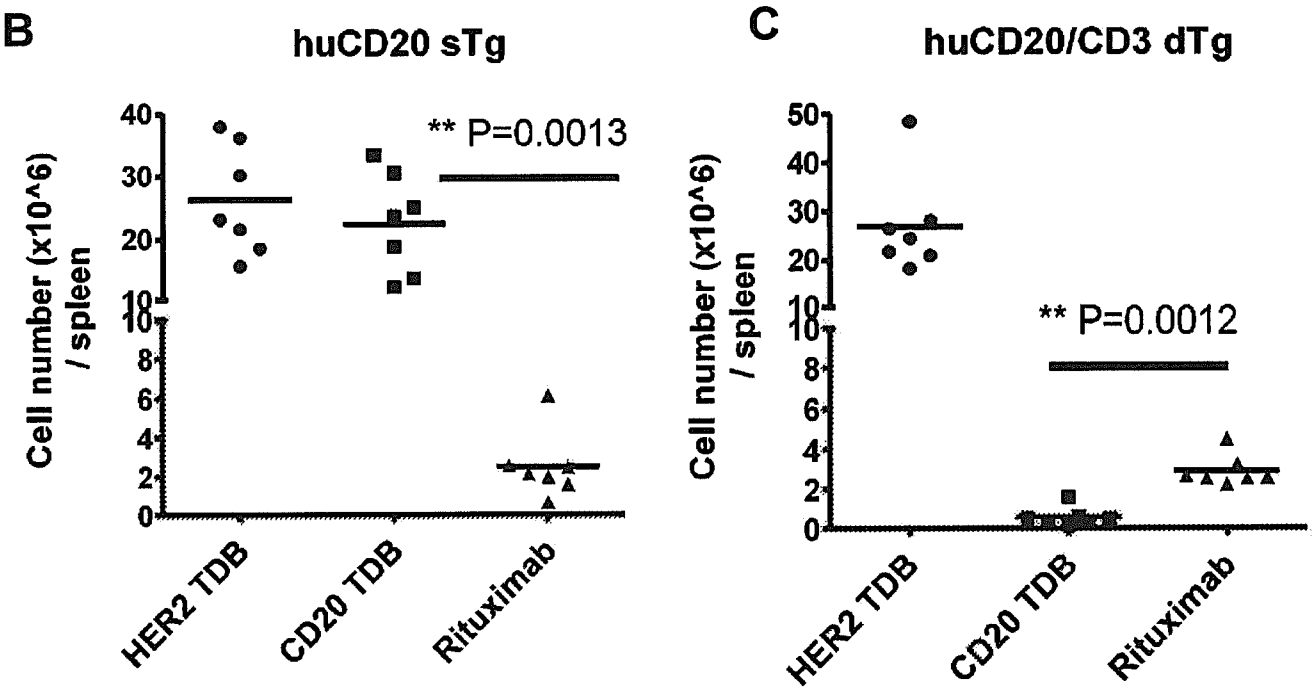


Figure 58A

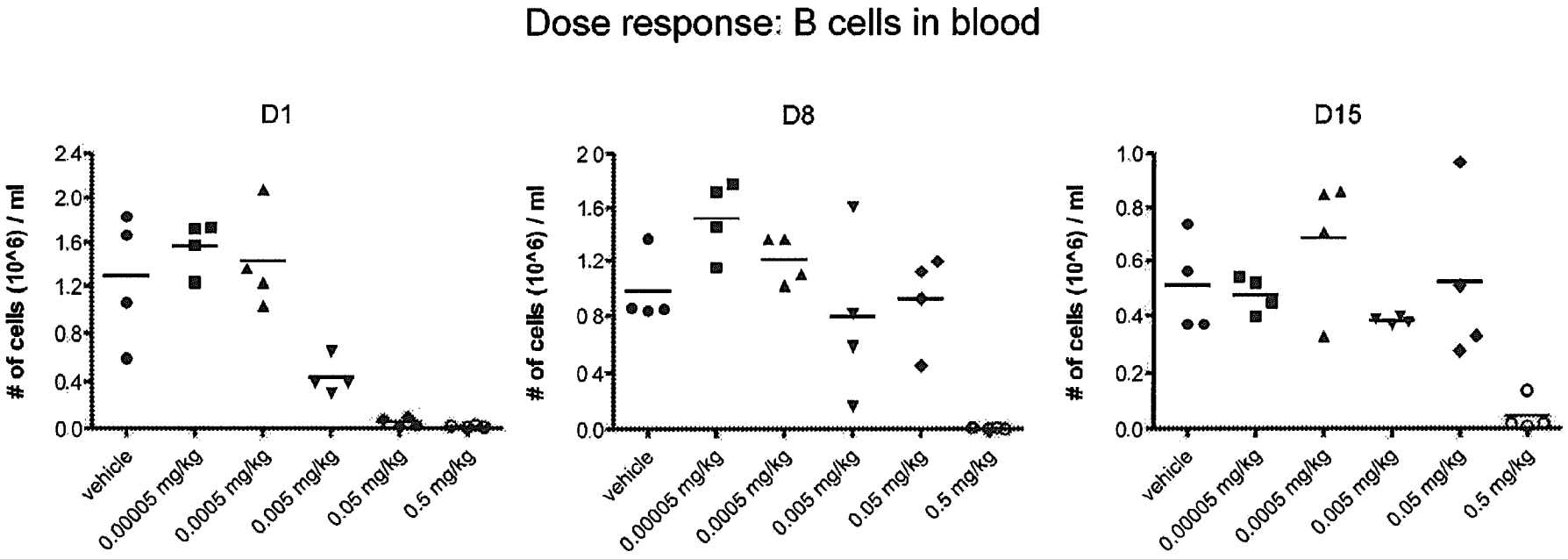
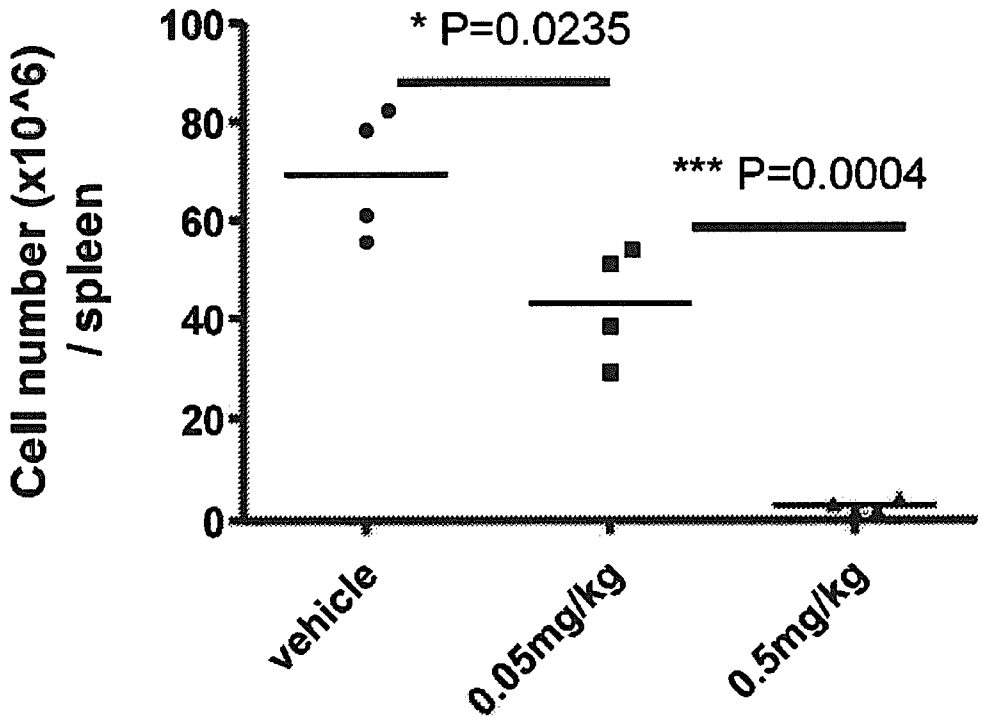
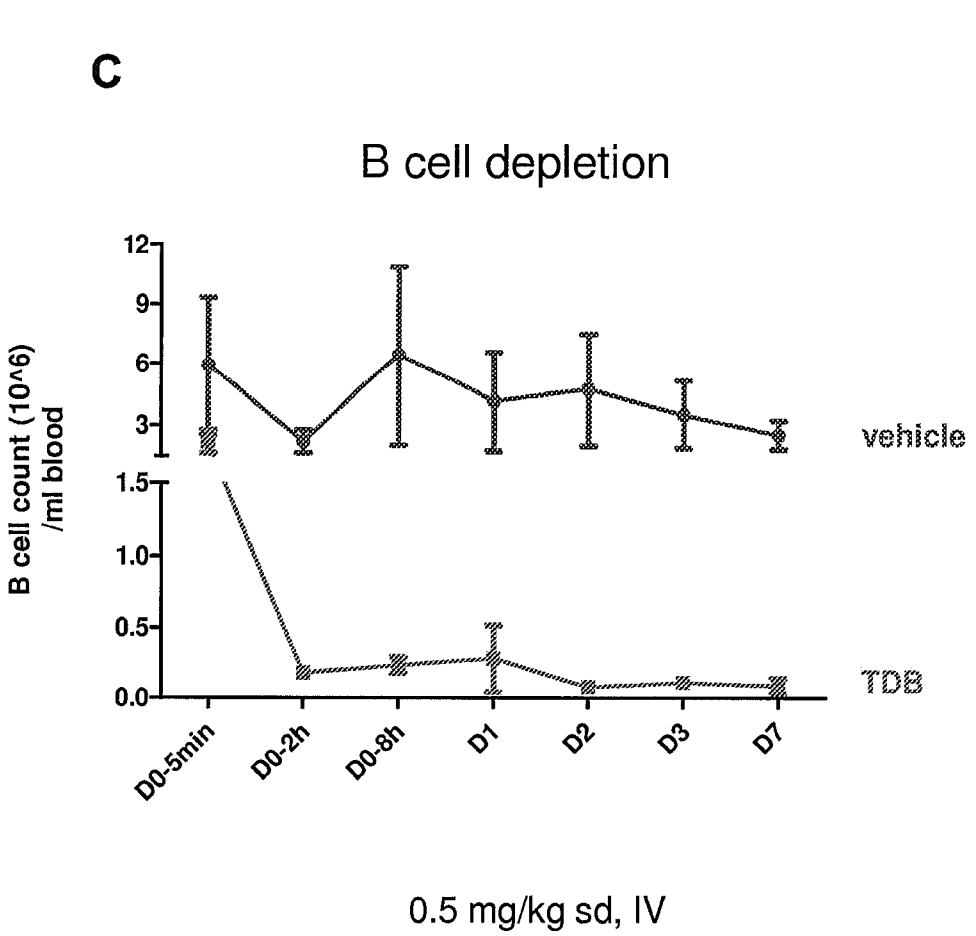

Dose response: B cells in blood
D1
D8
D15
# of cells (10^6) / ml
vehicle
0.00005 mg/kg
0.0005 mg/kg
0.005 mg/kg
0.05 mg/kg
0.5 mg/kg

Figure 58B


Cell number (x10^6) / spleen
100
80
60
40
20
0
* P=0.0235
*** P=0.0004
vehicle
0.05mg/kg
0.5mg/kg

Figures 58C-58D



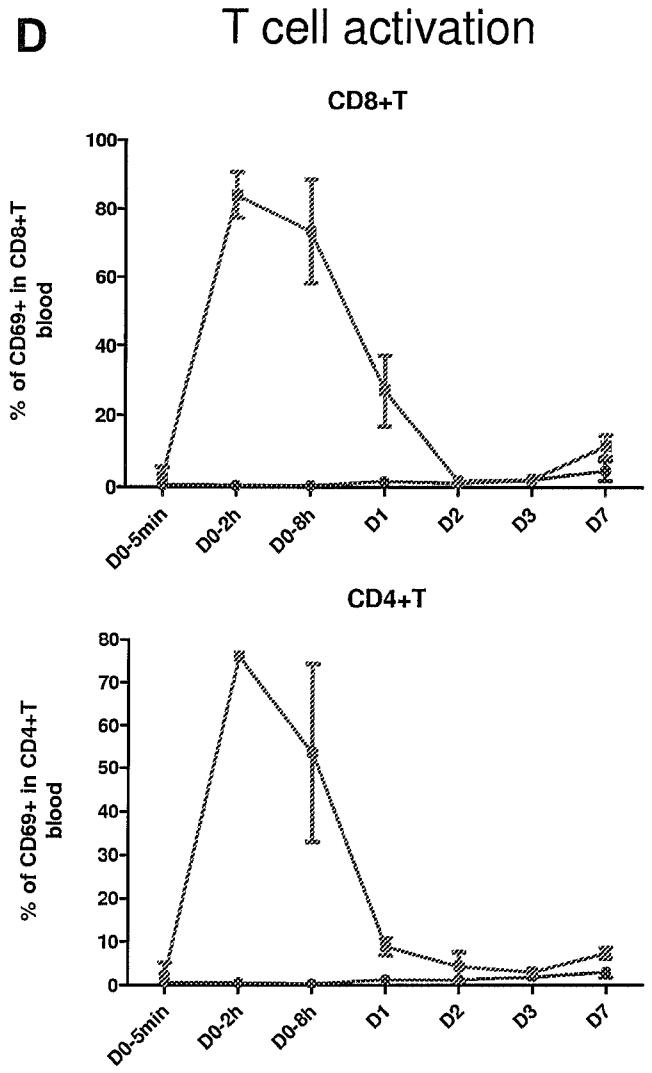
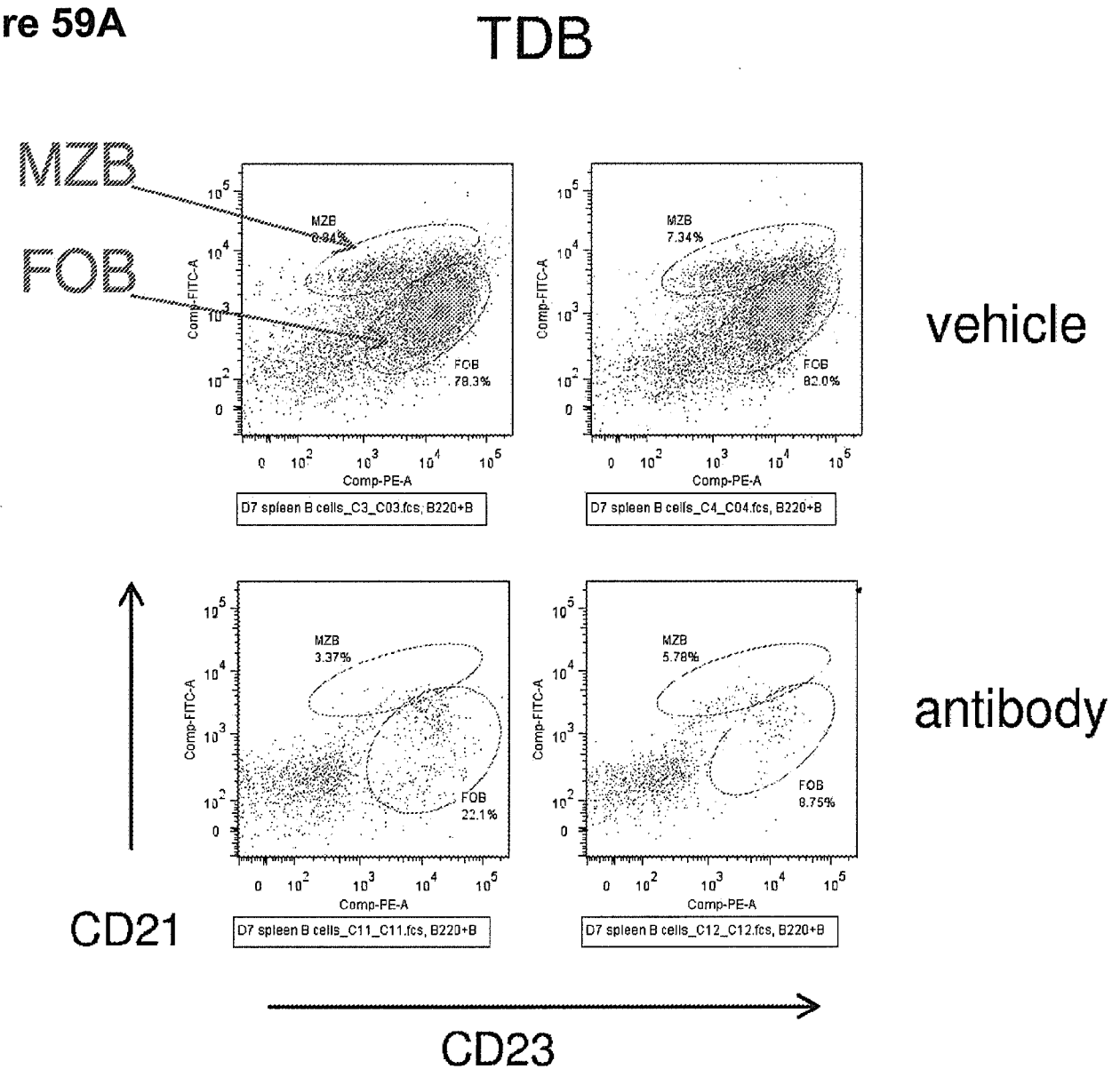


Figure 59A


TDB
MZB
FOB
MZB
0.94%
FOB
78.3%
MZB
7.34%
FOB
82.0%
vehicle
MZB
3.37%
FOB
22.1%
MZB
5.78%
FOB
8.75%
antibody
Comp-FITC-A
Comp-PE-A
D7 spleen B cells_C3_C03.fcs, B220+B
D7 spleen B cells_C4_C04.fcs, B220+B
D7 spleen B cells_C11_C11.fcs, B220+B
D7 spleen B cells_C12_C12.fcs, B220+B
CD21
CD23

Figures 59B-59E

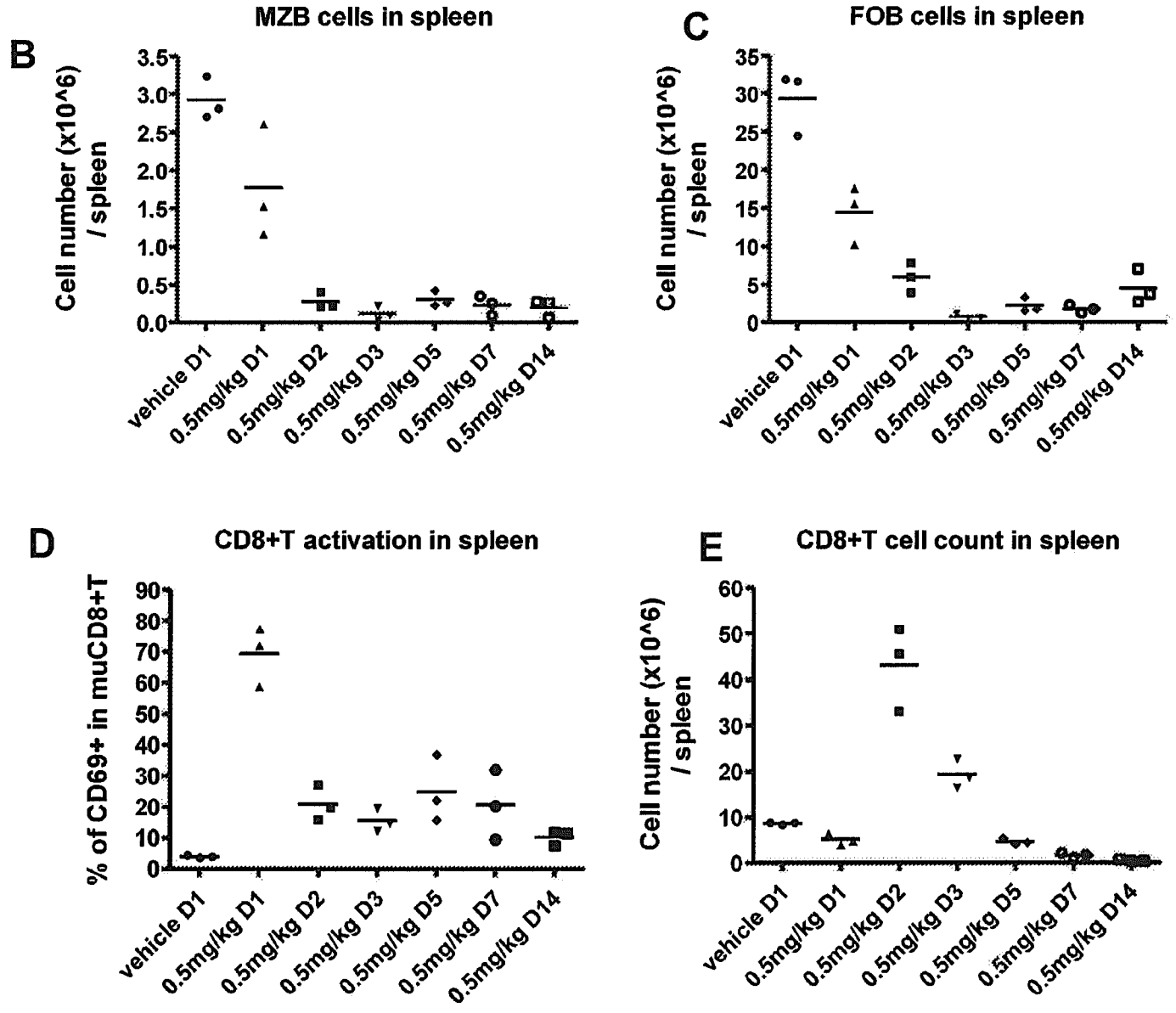


Figure 60A

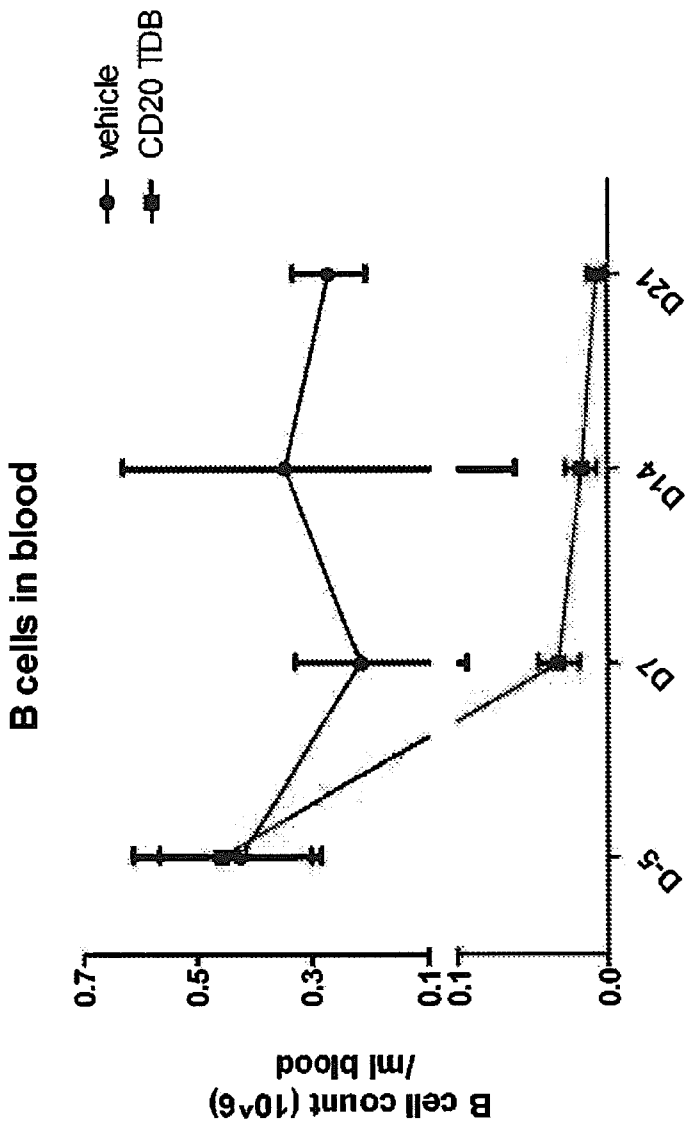

B cells in blood
vehicle
CD20 TDB
B cell count (10^6)
/ml blood
0.7
0.5
0.3
0.1
0.1
0.0
D-5
D7
D14
D21

Figure 60B

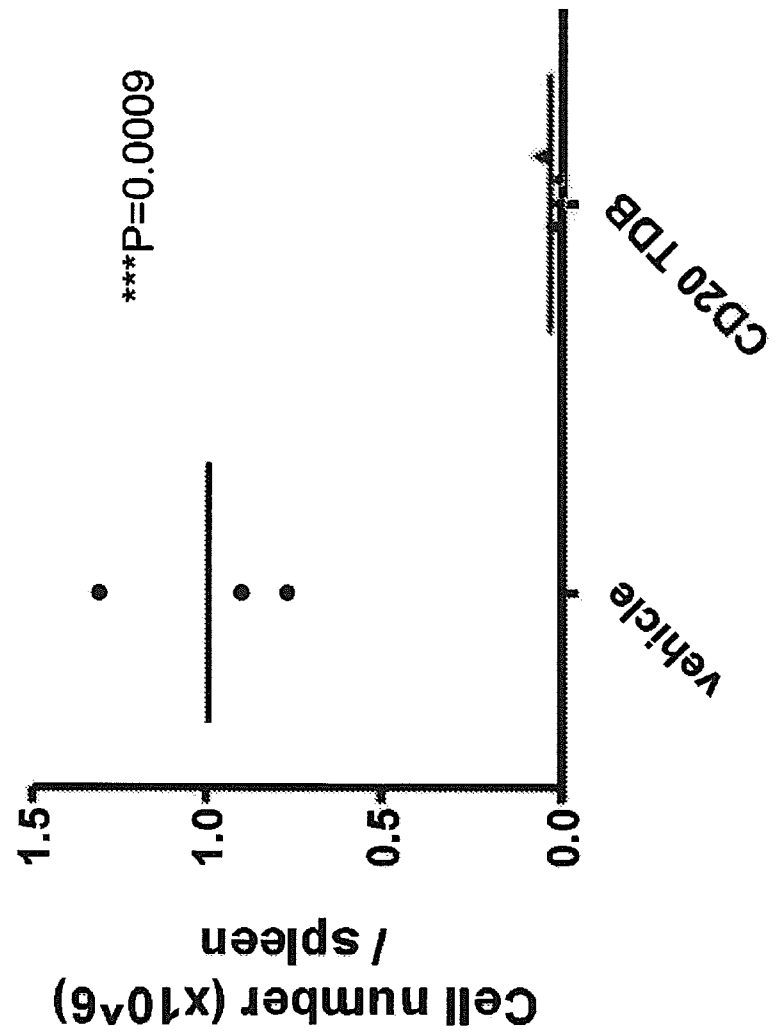
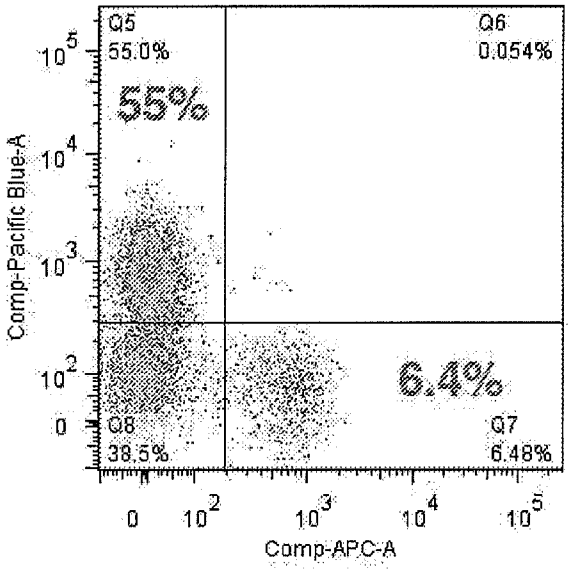

B cells in spleen
***P=0.0009
Cell number (x10^6) / spleen
1.5
1.0
0.5
0.0
Vehicle
CD20 TDB

Figure 60C

Control

low T:B ratio

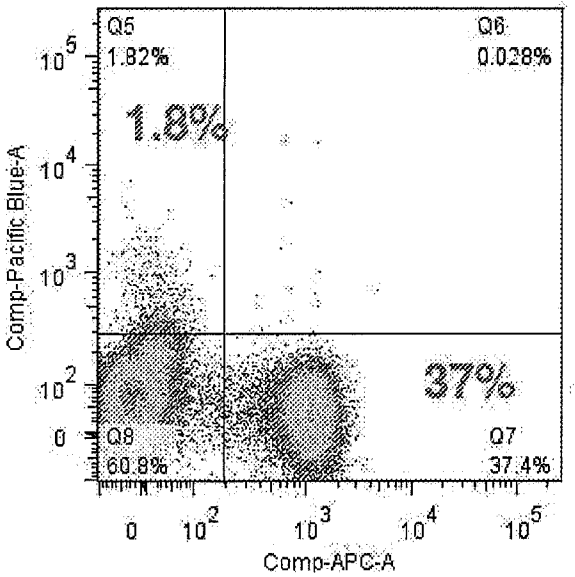


T-cell

Proliferation

upon treatment

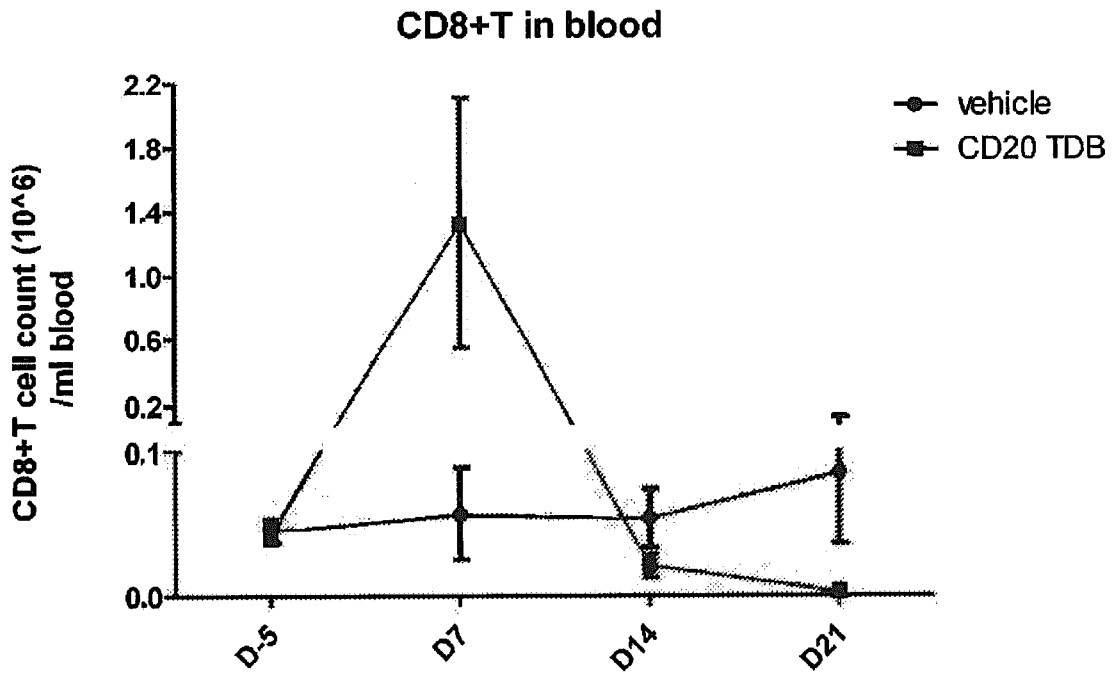
(D7, blood)



hu CD19

hu CD8

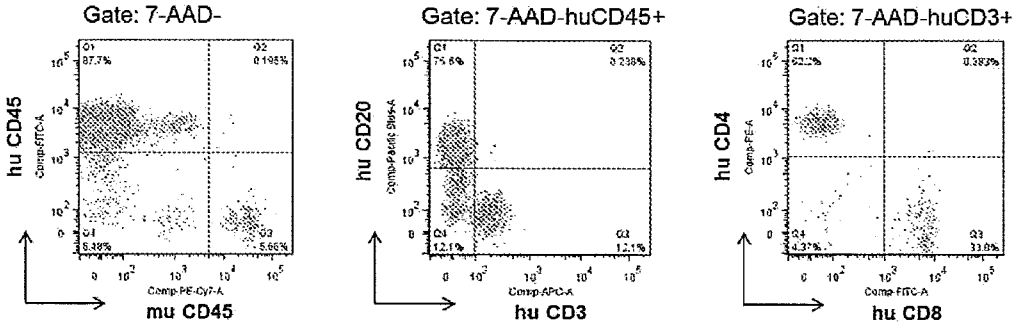
Figure 60D


CD8+T in blood
CD8+T cell count (10^6) /ml blood
2.2
1.8
1.4
1.0
0.6
0.2
0.1
0.0
D-5
D7
D14
D21
vehicle
CD20 TDB

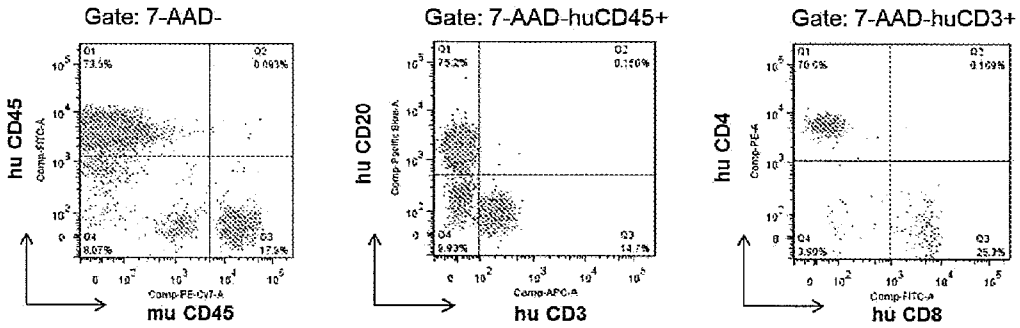
Figures 60E-60F

E

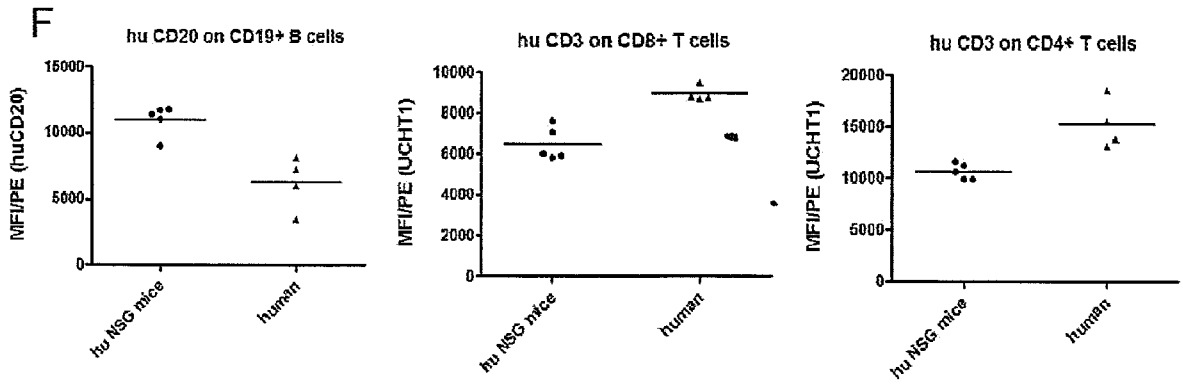
#43: 76% huCD20+B, 4.0% huCD8+T, and 7.5% huCD4+T of huCD45+ cells.



#46: 75% huCD20+B, 3.7% huCD8+T, and 10% huCD4+T of huCD45+ cells.



F



Figures 61A-61B

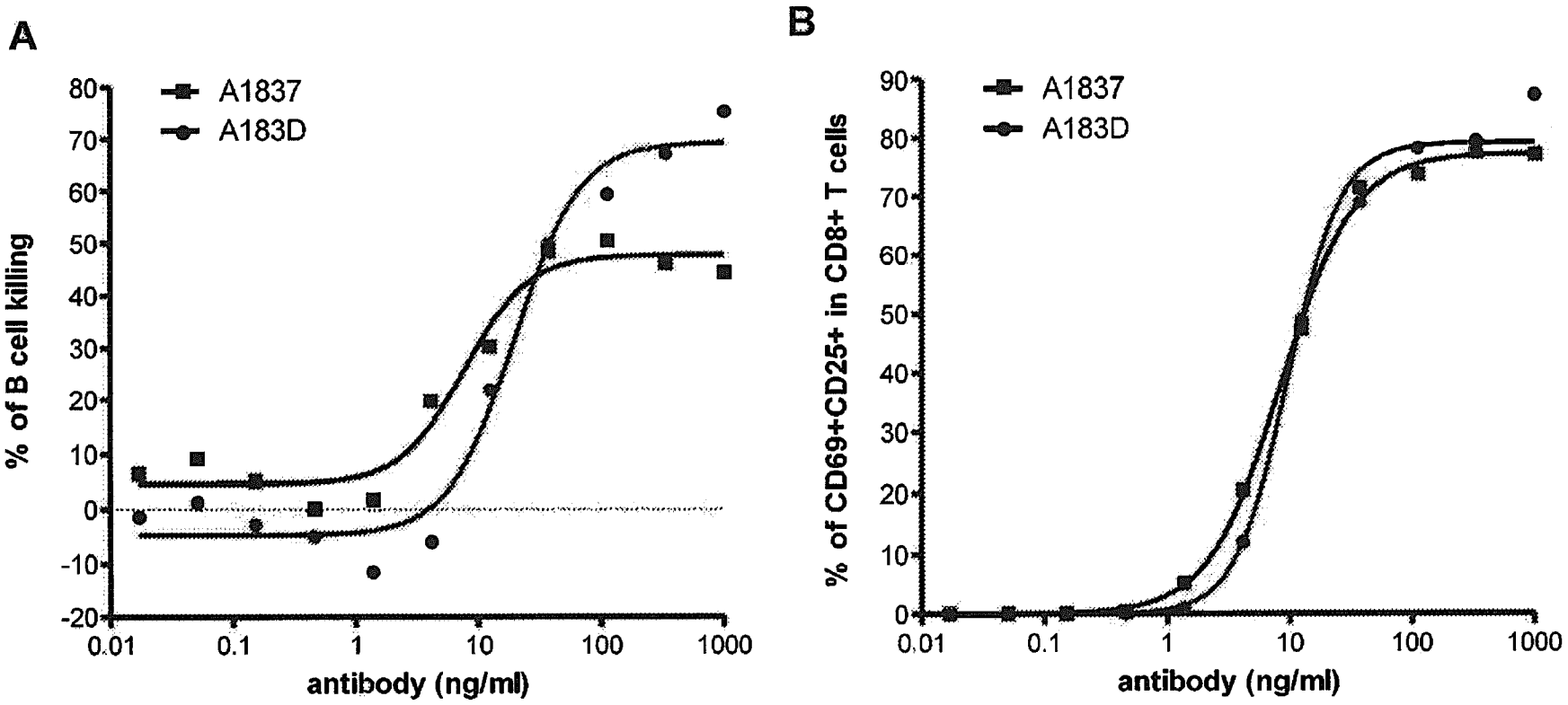


Figure 61C

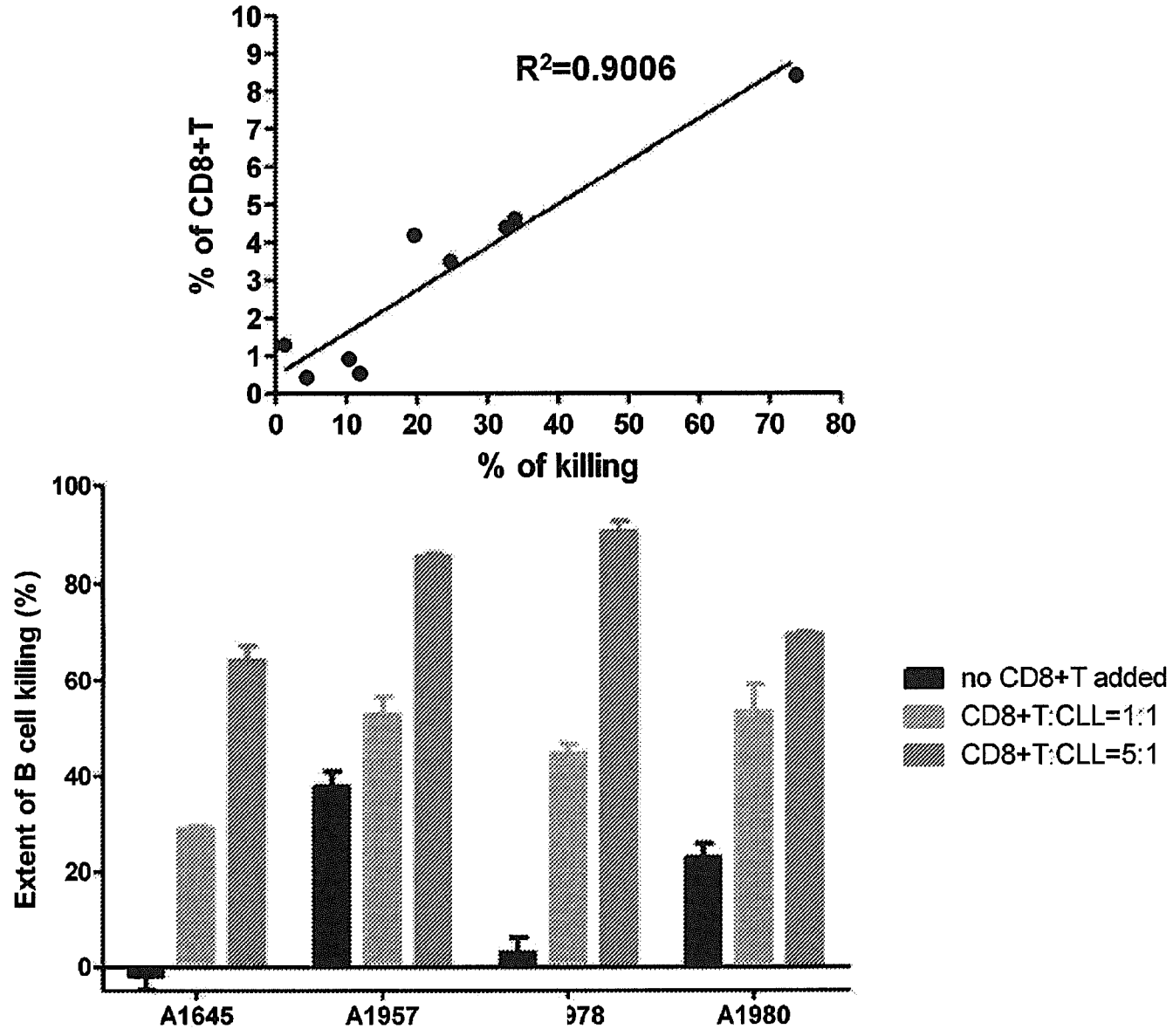

R²=0.9006
% of CD8+T
% of killing
0
1
2
3
4
5
6
7
8
9
10
0
10
20
30
40
50
60
70
80
Extent of B cell killing (%)
0
20
40
60
80
100
A1645
A1957
978
A1980
no CD8+T added
CD8+T:CLL=1:1
CD8+T:CLL=5:1

Figure 62A

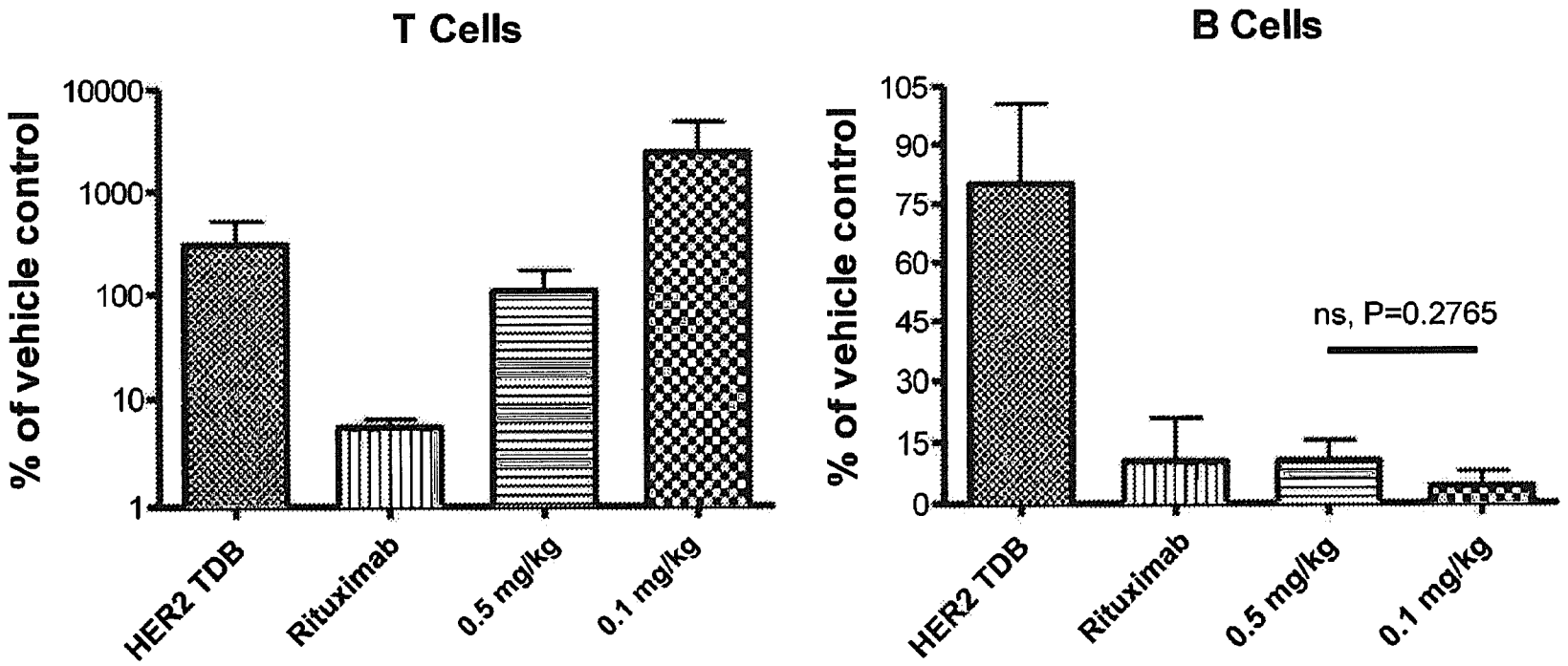

T Cells
% of vehicle control
10000
1000
100
10
1
HER2 TDB
Rituximab
0.5 mg/kg
0.1 mg/kg
B Cells
% of vehicle control
105
90
75
60
45
30
15
0
ns, P=0.2765
HER2 TDB
Rituximab
0.5 mg/kg
0.1 mg/kg

Figure 62B

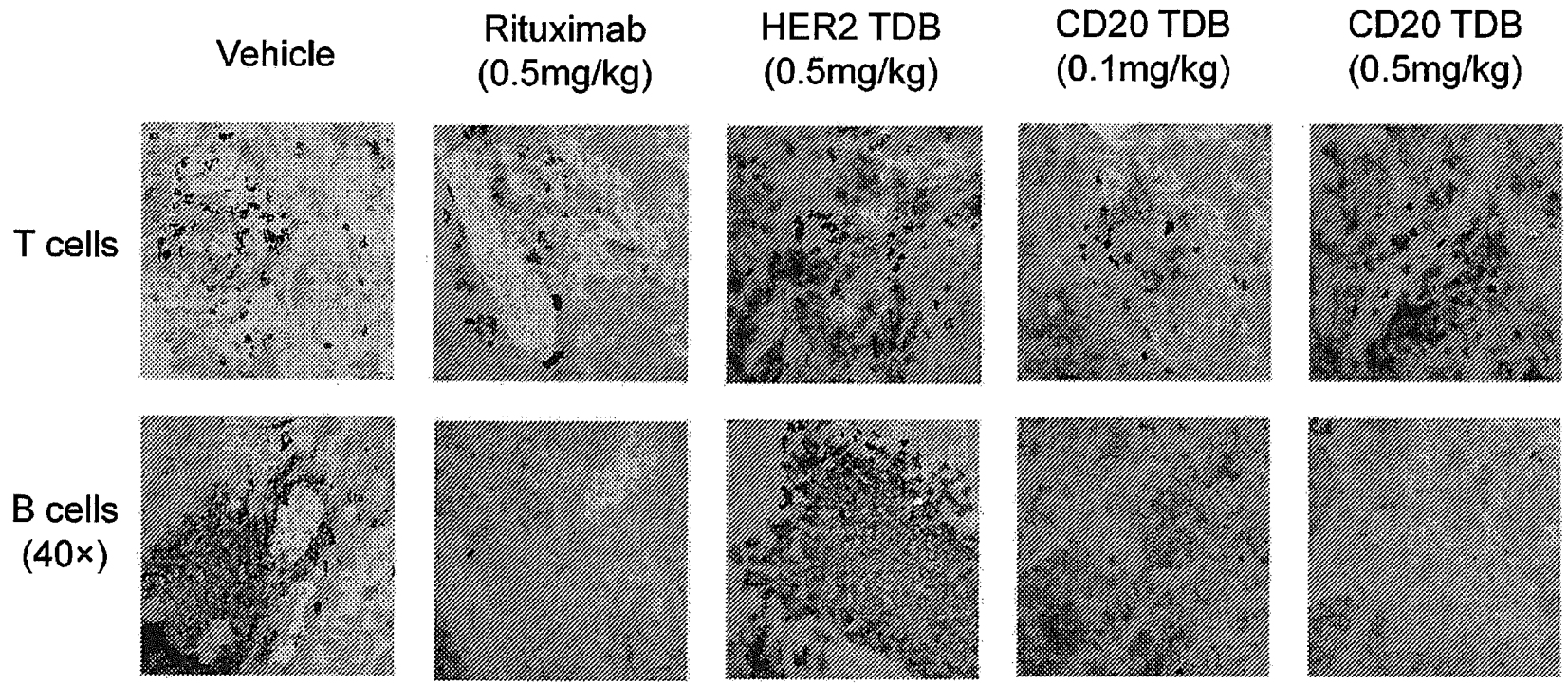
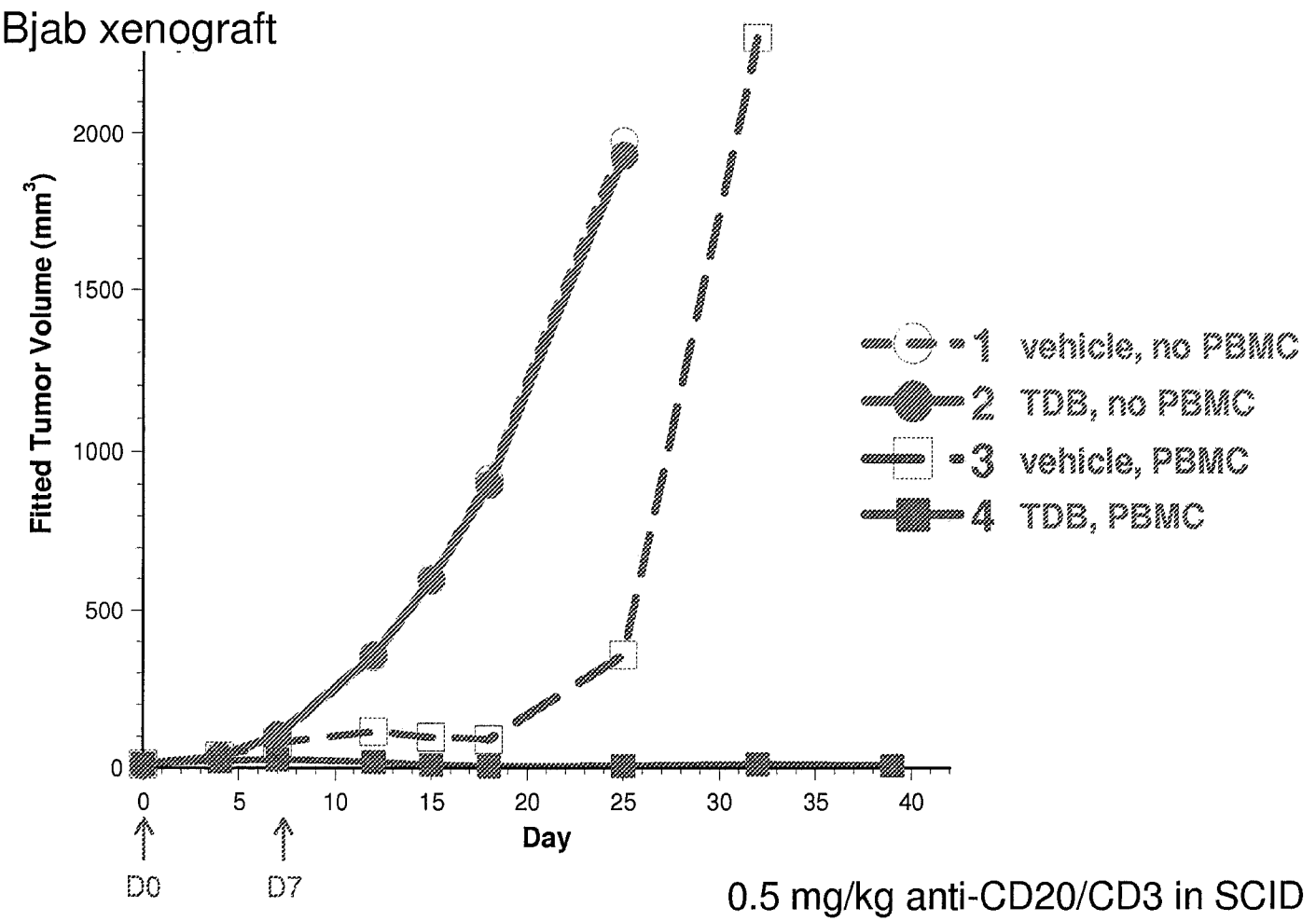

Vehicle
Rituximab (0.5mg/kg)
HER2 TDB (0.5mg/kg)
CD20 TDB (0.1mg/kg)
CD20 TDB (0.5mg/kg)
T cells
B cells (40×)

Figure 63



0.5 mg/kg anti-CD20/CD3 in SCID mice

Figure 64A

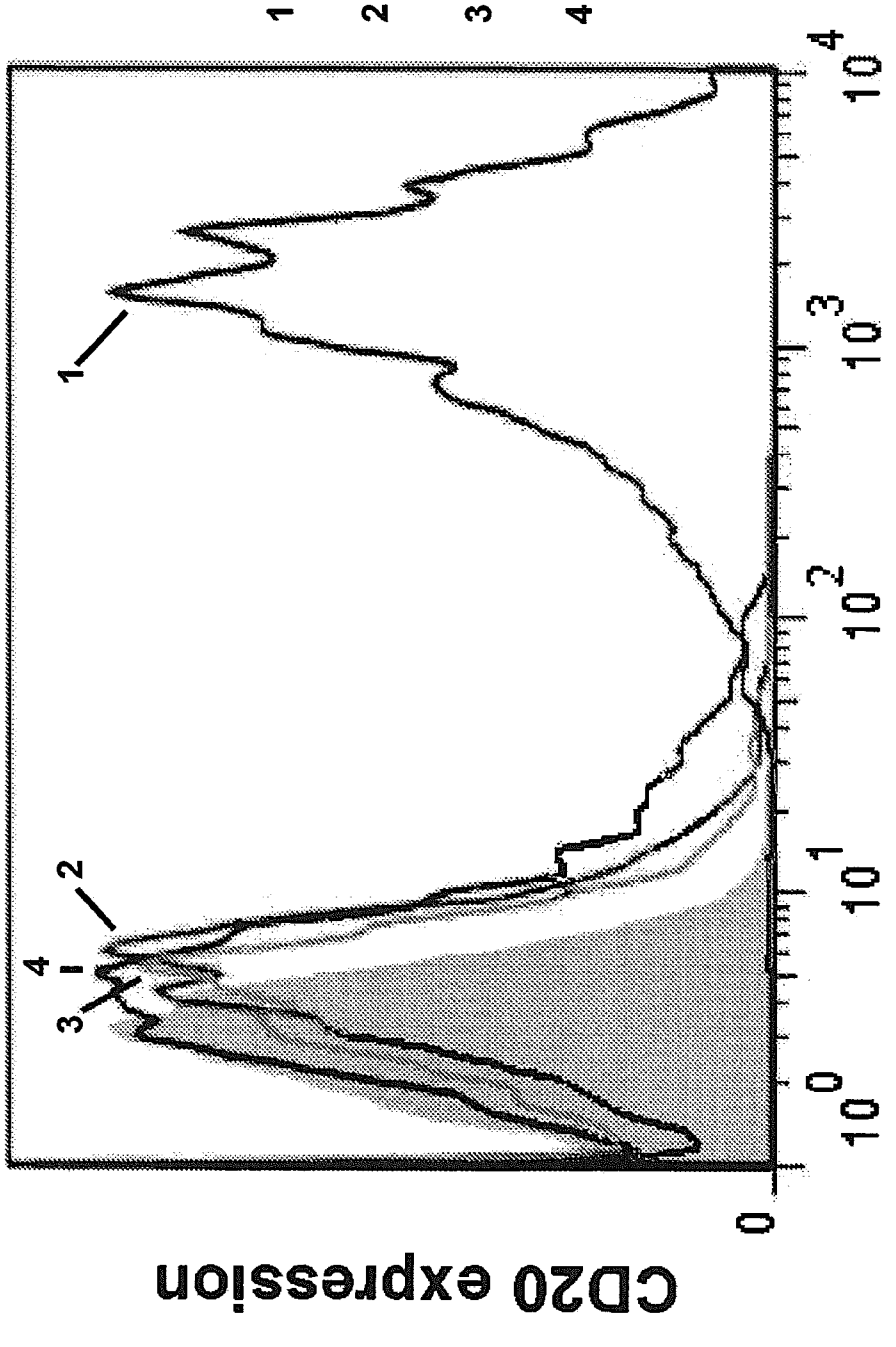

1 Bjab
2 NALM-6
3 SC-1
4 OCI-LY19
CD20 expression
0
10^0
10^1
10^2
10^3
10^4

Figure 64B

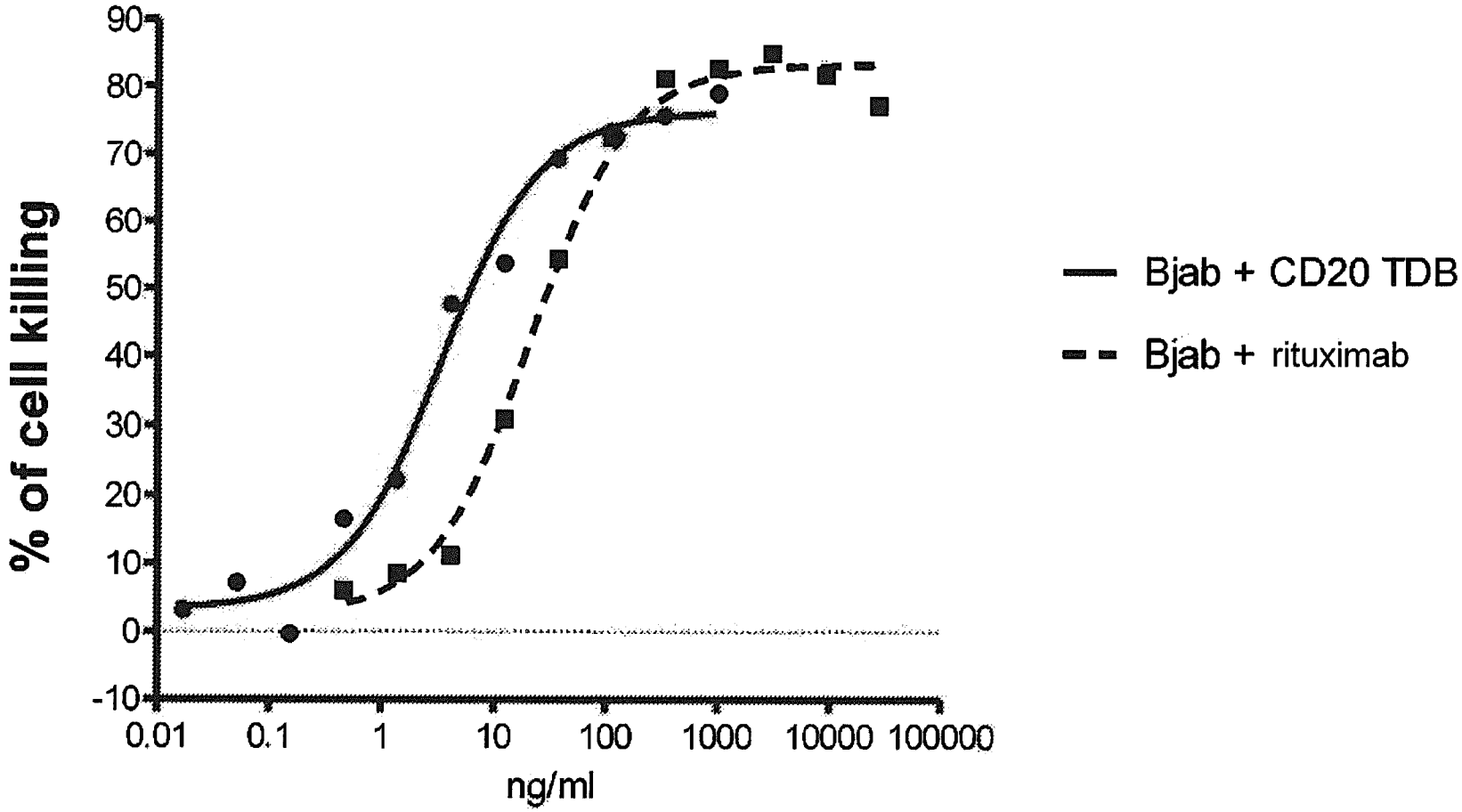

90
80
70
60
50
40
30
20
10
0
-10
% of cell killing
0.01
0.1
1
10
100
1000
10000
100000
ng/ml
Bjab + CD20 TDB
Bjab + rituximab

Figure 64C

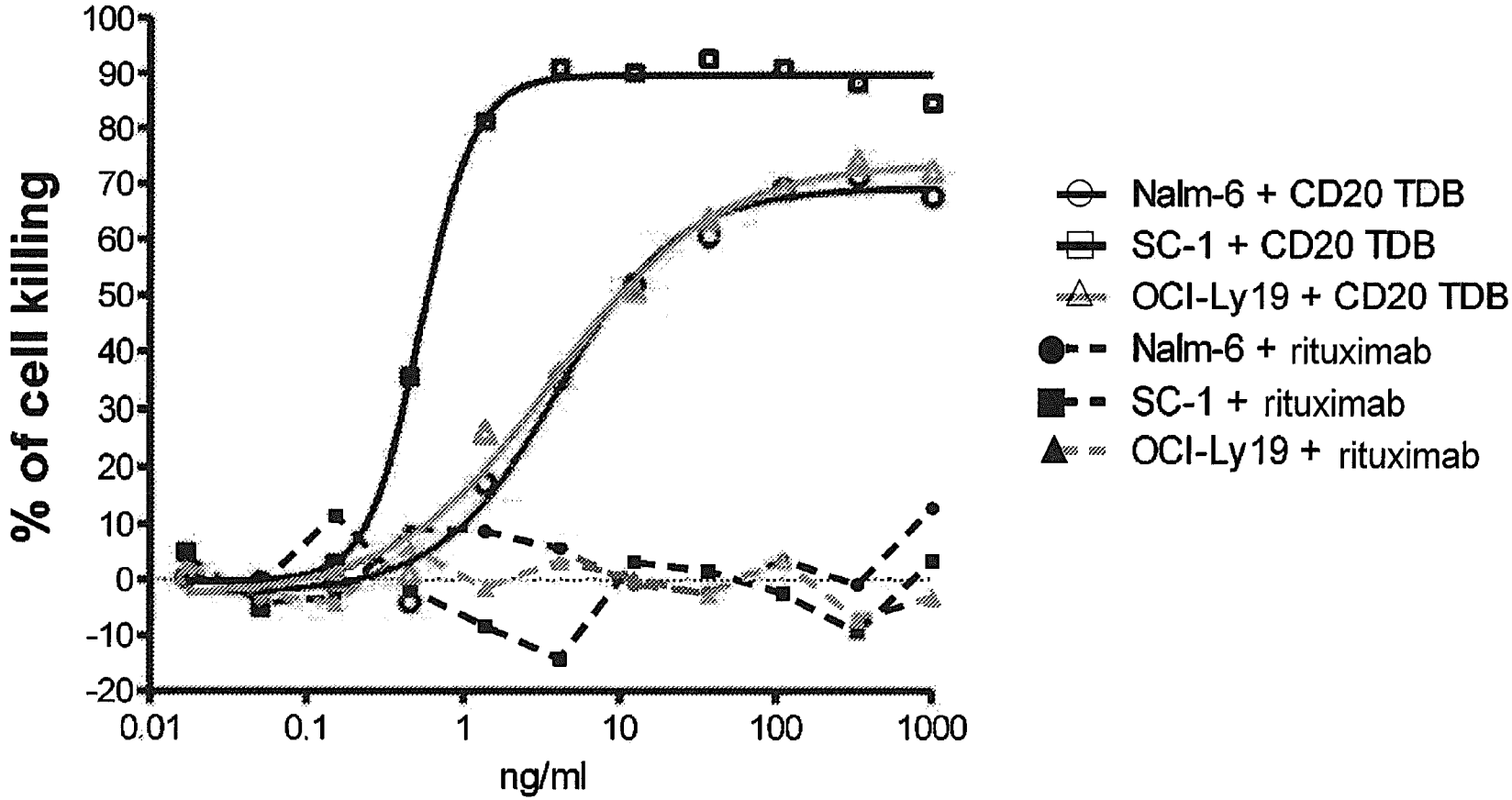

% of cell killing
100
90
80
70
60
50
40
30
20
10
0
-10
-20
0.01
0.1
1
10
100
1000
ng/ml
Nalm-6 + CD20 TDB
SC-1 + CD20 TDB
OCI-Ly19 + CD20 TDB
Nalm-6 + rituximab
SC-1 + rituximab
OCI-Ly19 + rituximab

Figure 64D

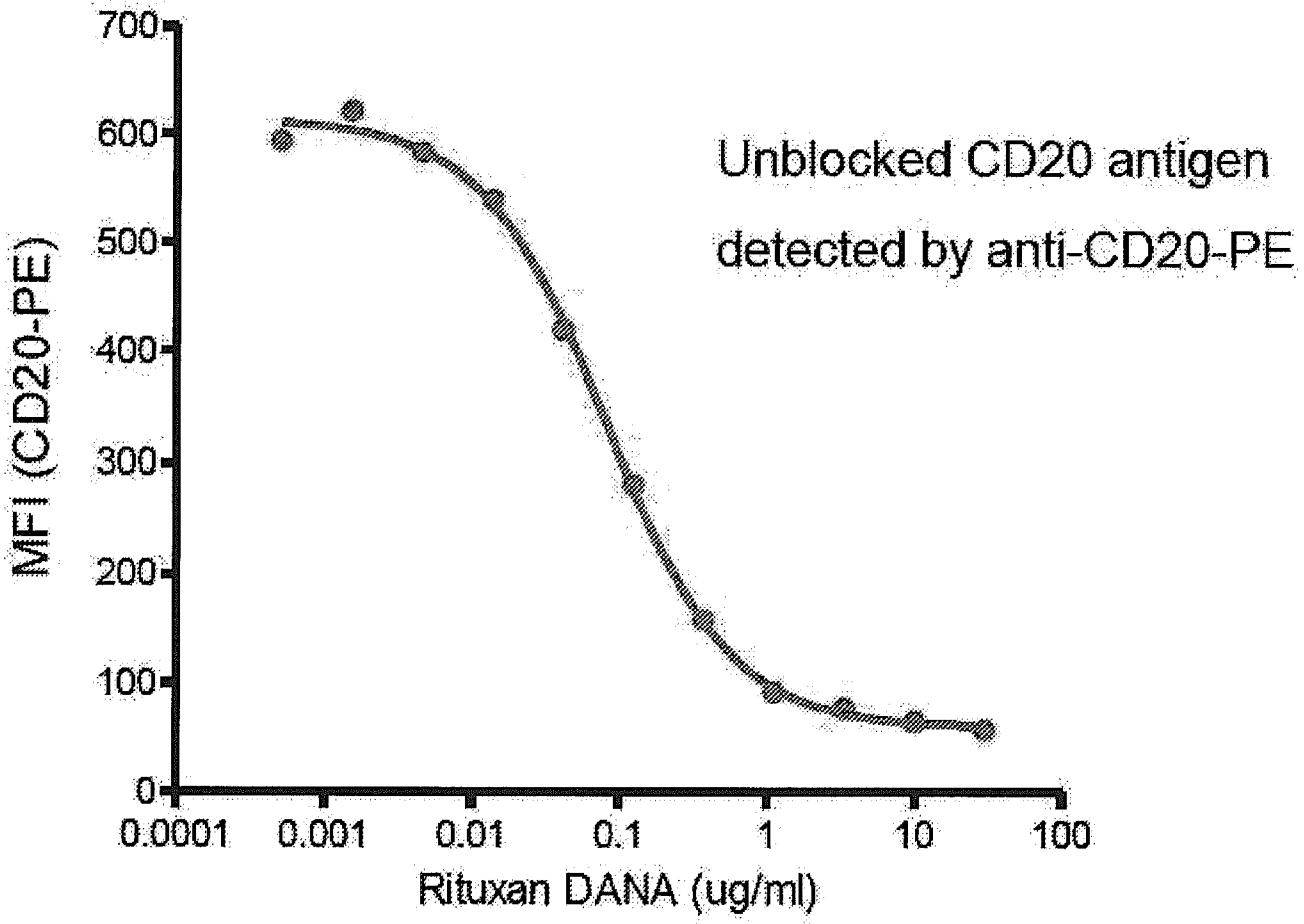

700
600
500
400
300
200
100
0
MFI (CD20-PE)
Unblocked CD20 antigen
detected by anti-CD20-PE
0.0001
0.001
0.01
0.1
1
10
100
Rituxan DANA (ug/ml)

Figure 65A

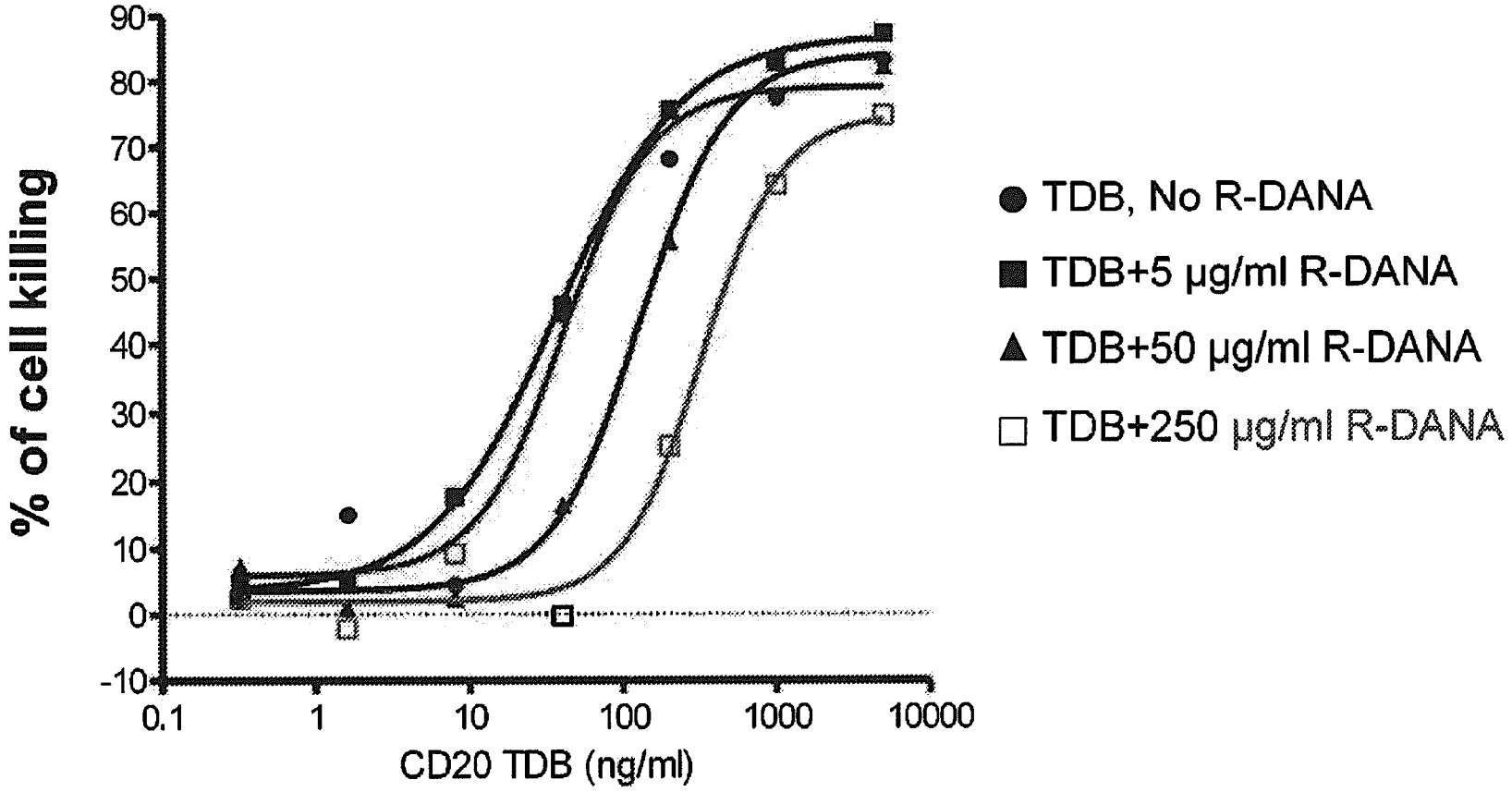

% of cell killing
90
80
70
60
50
40
30
20
10
0
-10
0.1
1
10
100
1000
10000
CD20 TDB (ng/ml)
TDB, No R-DANA
TDB+5 µg/ml R-DANA
TDB+50 µg/ml R-DANA
TDB+250 µg/ml R-DANA

Figure 65B

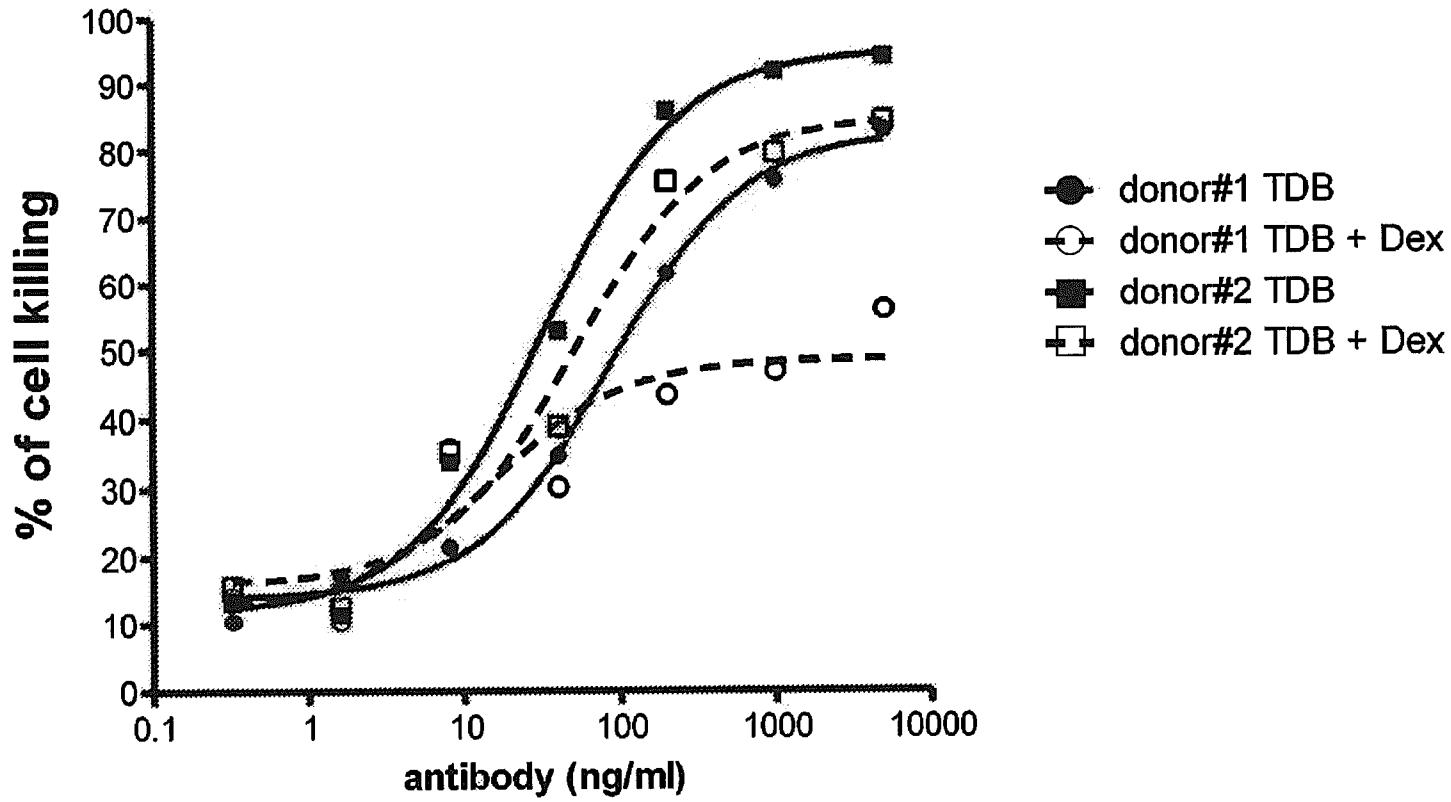

100
90
80
70
60
50
40
30
20
10
0
% of cell killing
0.1
1
10
100
1000
10000
antibody (ng/ml)
donor#1 TDB
donor#1 TDB + Dex
donor#2 TDB
donor#2 TDB + Dex

Figure 66

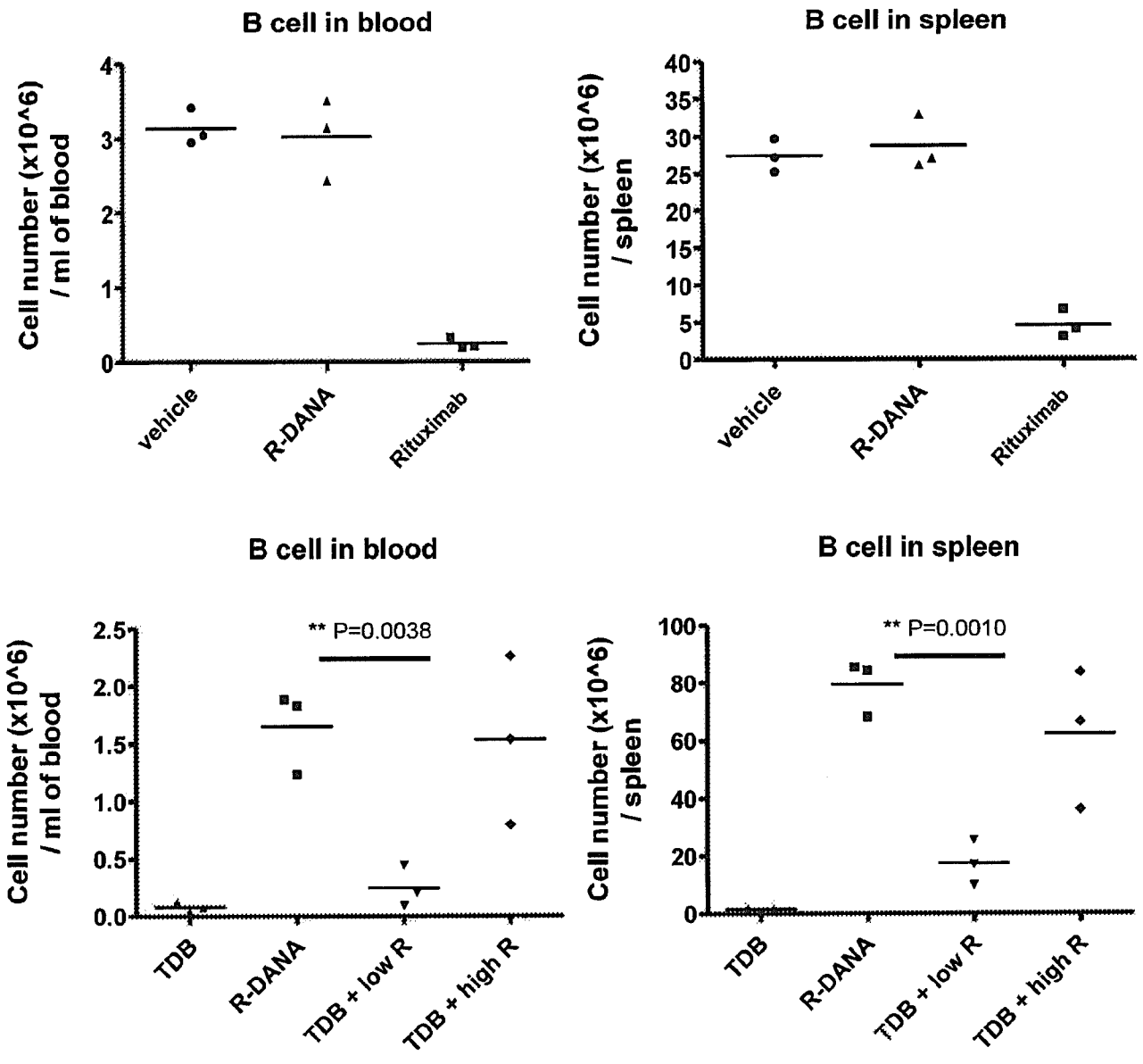

B cell in blood
Cell number (x10^6) / ml of blood
vehicle
R-DANA
Rituximab
B cell in spleen
Cell number (x10^6) / spleen
vehicle
R-DANA
Rituximab
B cell in blood
** P=0.0038
Cell number (x10^6) / ml of blood
TDB
R-DANA
TDB + low R
TDB + high R
B cell in spleen
** P=0.0010
Cell number (x10^6) / spleen
TDB
R-DANA
TDB + low R
TDB + high R

Figure 67A

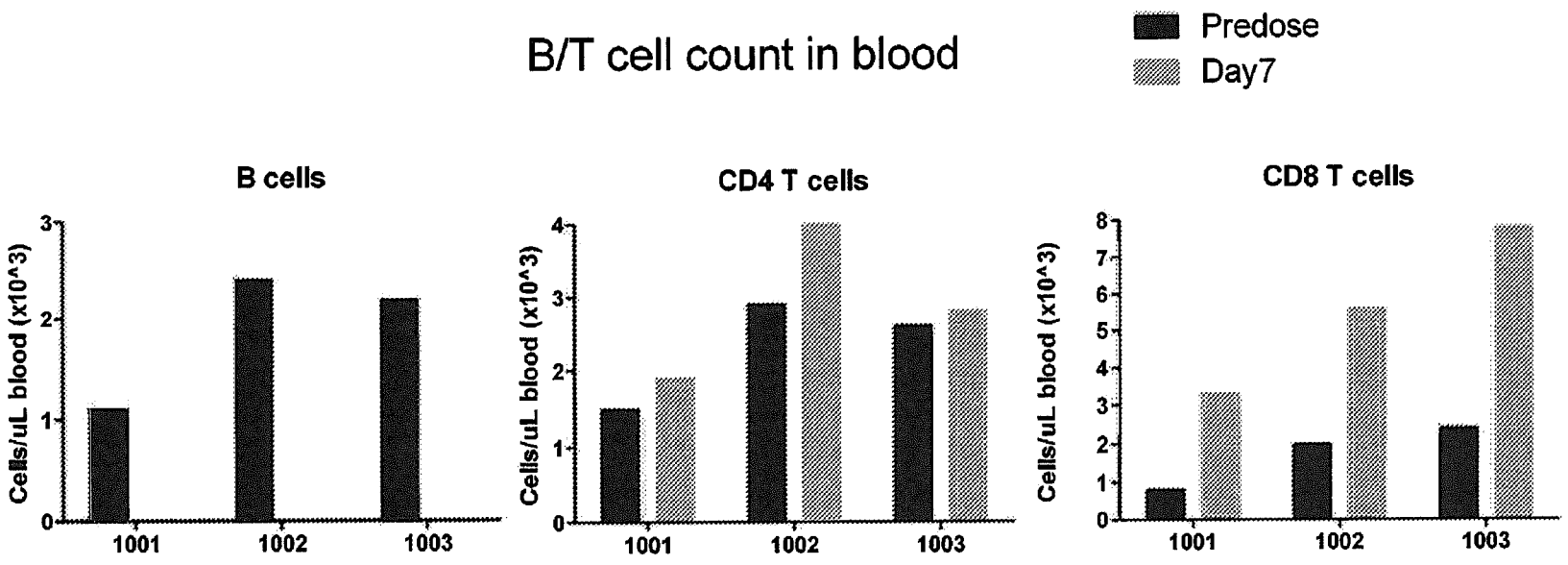

B/T cell count in blood
Predose
Day7
B cells
CD4 T cells
CD8 T cells
Cells/uL blood (x10^3)
0
1
2
3
4
5
6
7
8
1001
1002
1003

Figure 67B

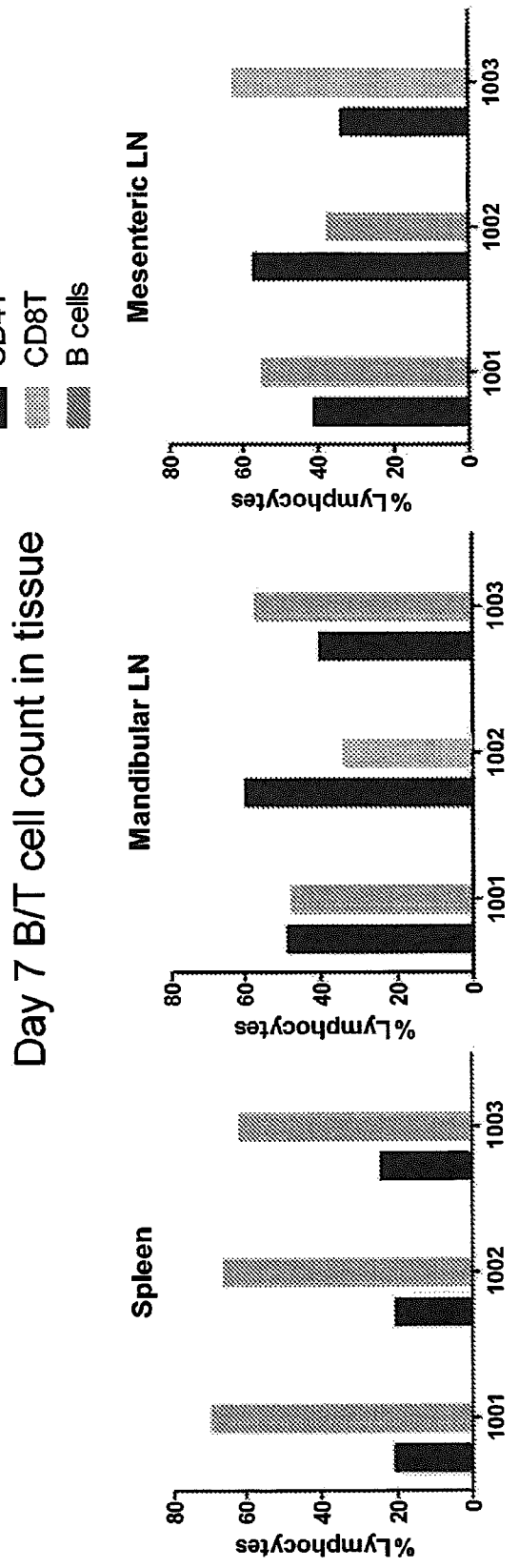

Day 7 B/T cell count in tissue
CD4T
CD8T
B cells
Spleen
Mandibular LN
Mesenteric LN
% Lymphocytes
0
20
40
60
80
1001
1002
1003

Figure 67C

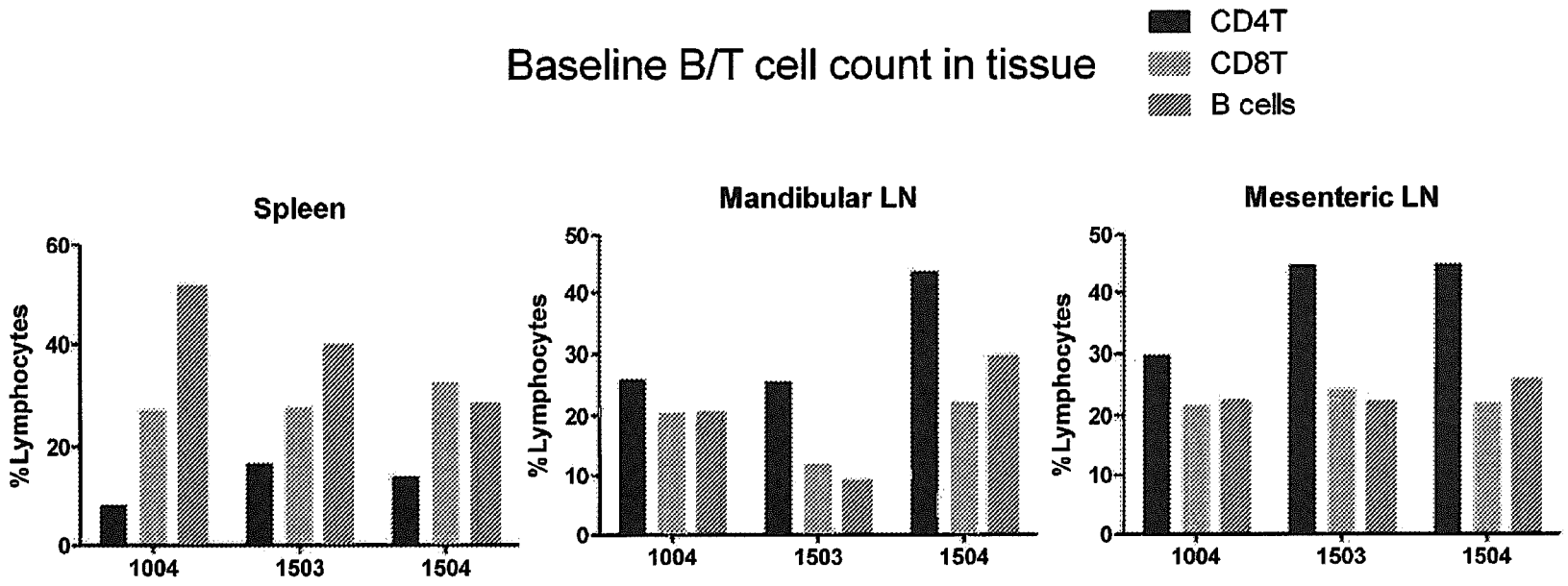

Baseline B/T cell count in tissue
CD4T
CD8T
B cells
Spleen
Mandibular LN
Mesenteric LN
% Lymphocytes
60
40
20
0
50
30
10
1004
1503
1504

Figure 67D

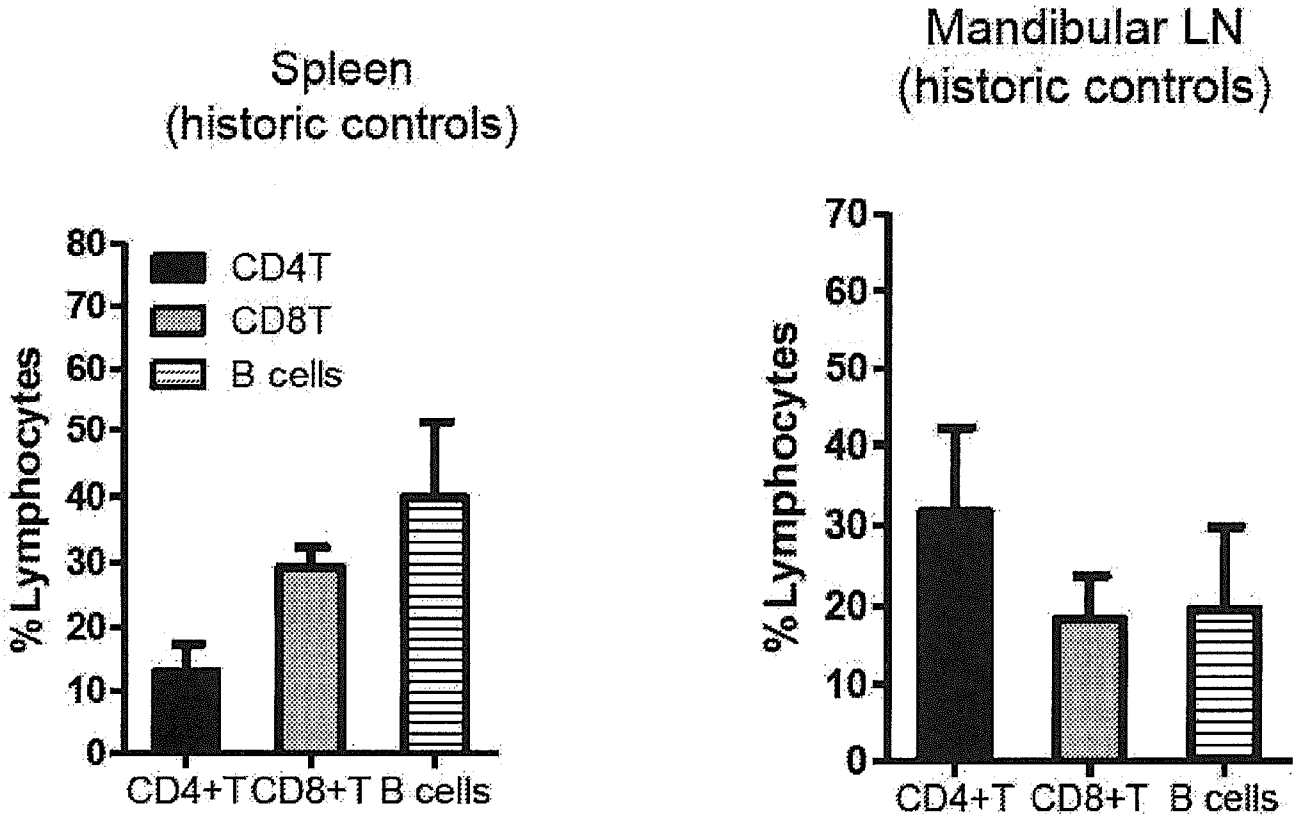
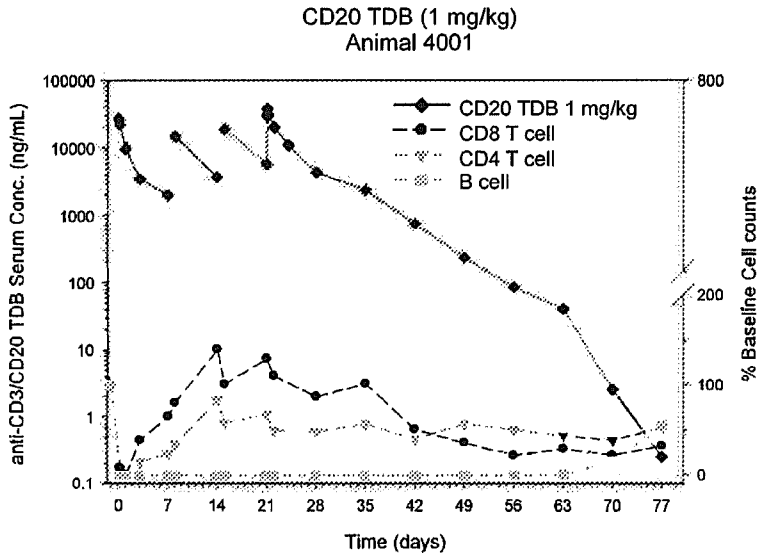
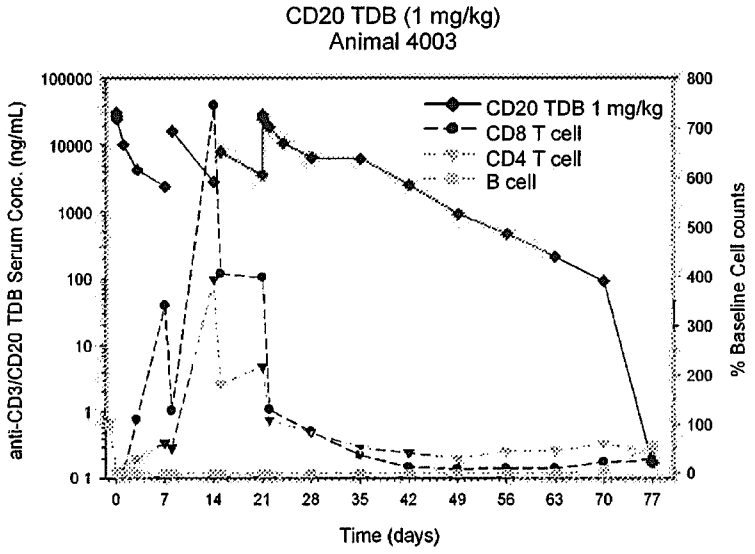
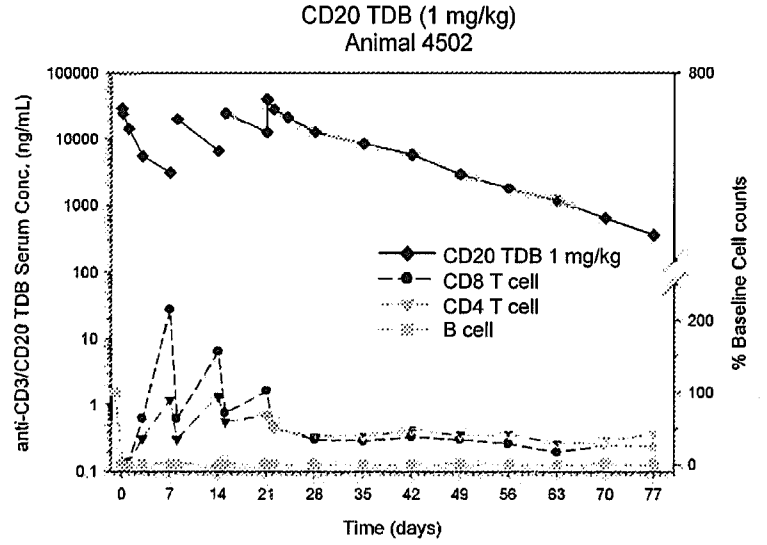

Spleen
(historic controls)
% Lymphocytes
80
70
60
50
40
30
20
10
0
CD4T
CD8T
B cells
CD4+T CD8+T B cells
Mandibular LN
(historic controls)
% Lymphocytes
70
60
50
40
30
20
10
0
CD4+T CD8+T B cells

Figure 68A


CD20 TDB (1 mg/kg)
Animal 4001
anti-CD3/CD20 TDB Serum Conc. (ng/mL)
% Baseline Cell counts
Time (days)
CD20 TDB 1 mg/kg
CD8 T cell
CD4 T cell
B cell


CD20 TDB (1 mg/kg)
Animal 4003
anti-CD3/CD20 TDB Serum Conc. (ng/mL)
% Baseline Cell counts
Time (days)
CD20 TDB 1 mg/kg
CD8 T cell
CD4 T cell
B cell


CD20 TDB (1 mg/kg)
Animal 4502
anti-CD3/CD20 TDB Serum Conc. (ng/mL)
% Baseline Cell counts
Time (days)
CD20 TDB 1 mg/kg
CD8 T cell
CD4 T cell
B cell

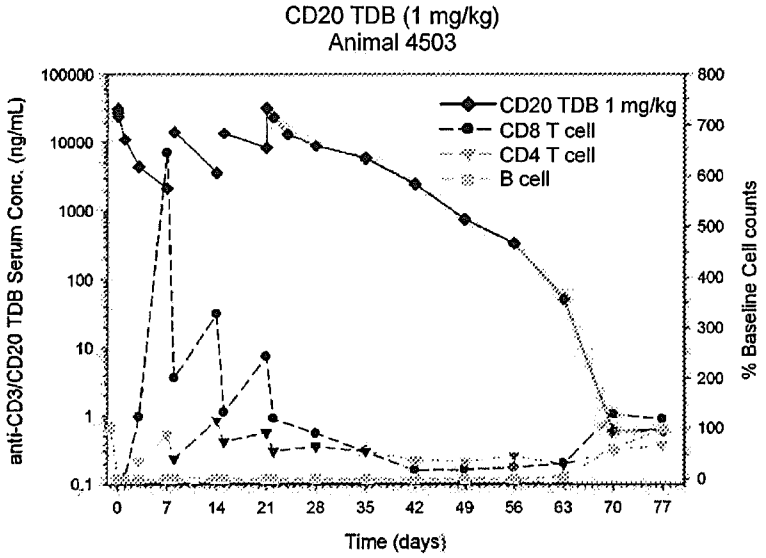
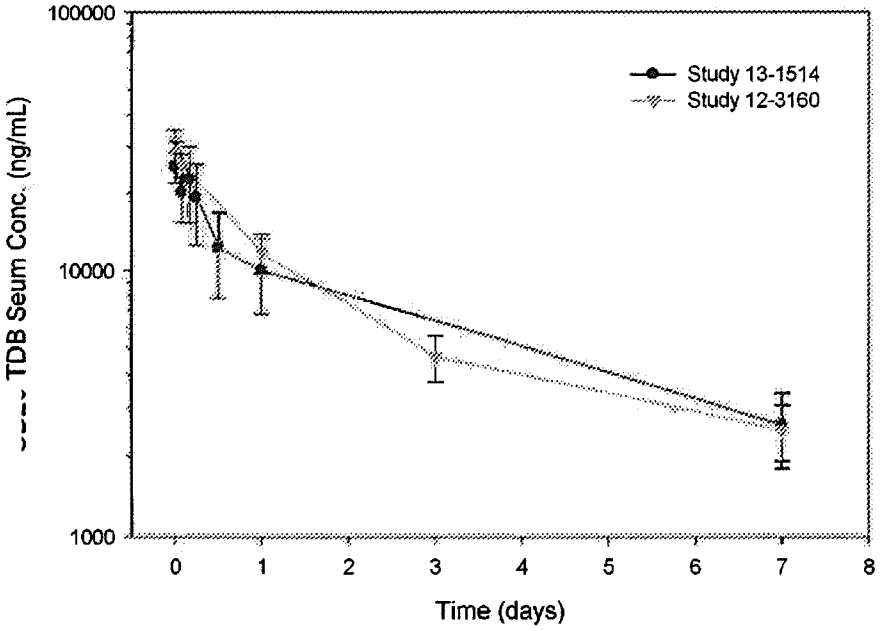
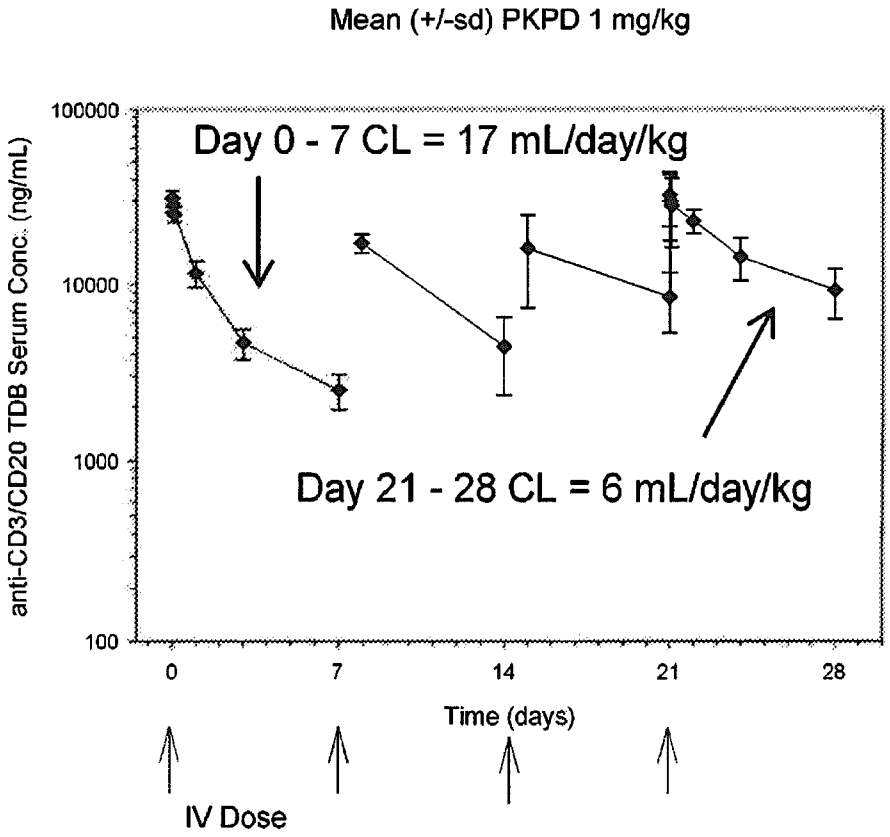

CD20 TDB (1 mg/kg)
Animal 4503
anti-CD3/CD20 TDB Serum Conc. (ng/mL)
% Baseline Cell counts
Time (days)
CD20 TDB 1 mg/kg
CD8 T cell
CD4 T cell
B cell

Figure 68B

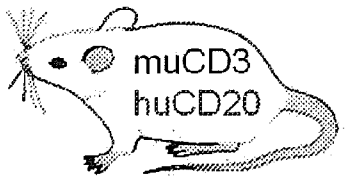

100000
10000
1000
TDB Seum Conc. (ng/mL)
Study 13-1514
Study 12-3160
0
1
2
3
4
5
6
7
8
Time (days)


Mean (+/-sd) PKPD 1 mg/kg
100000
10000
1000
100
anti-CD3/CD20 TDB Serum Conc. (ng/mL)
Day 0 - 7 CL = 17 mL/day/kg
Day 21 - 28 CL = 6 mL/day/kg
0
7
14
21
28
Time (days)
IV Dose

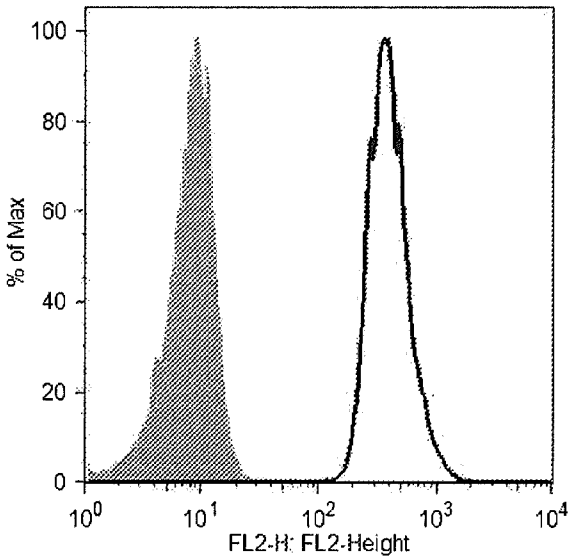
Figures 69A-69B

A

A20-huCD20 syngeneic in Balb/C mice



PD-L1 expression on A20-huCD20 cells



B

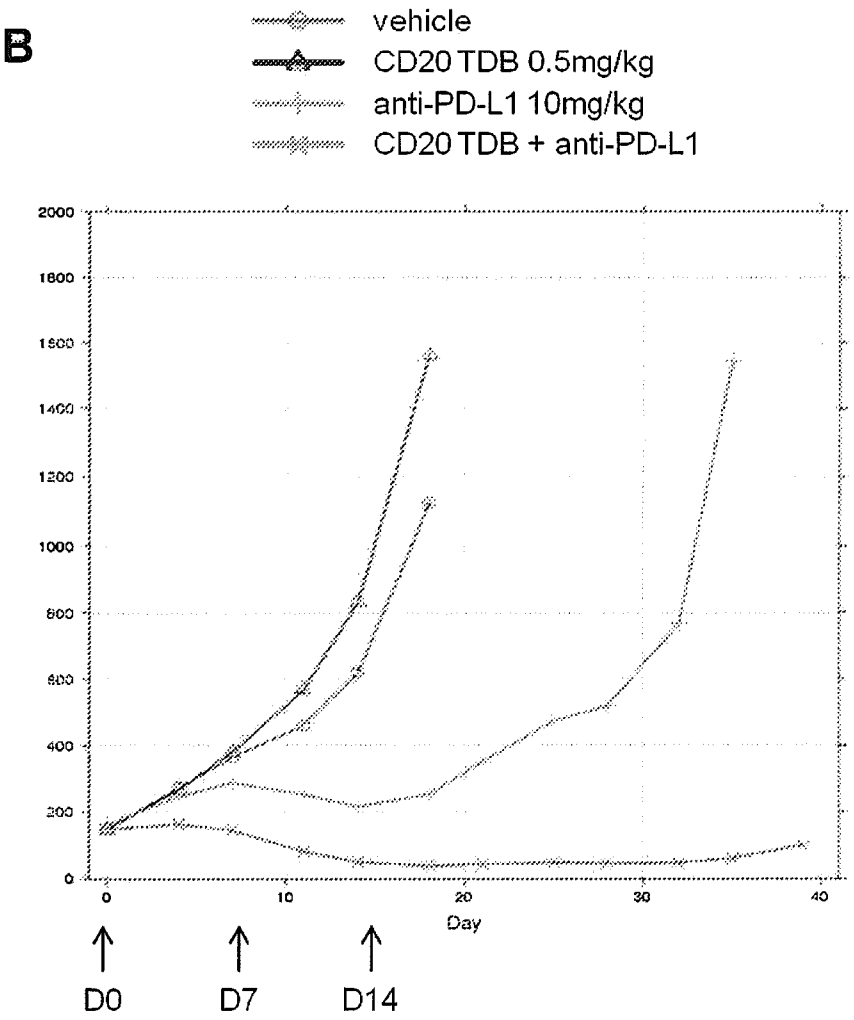
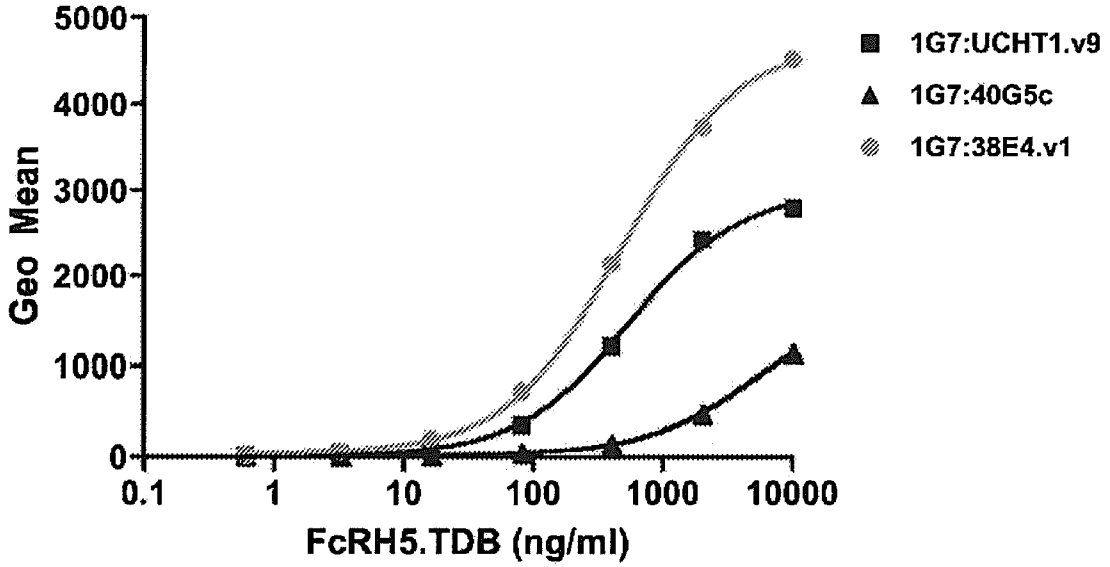


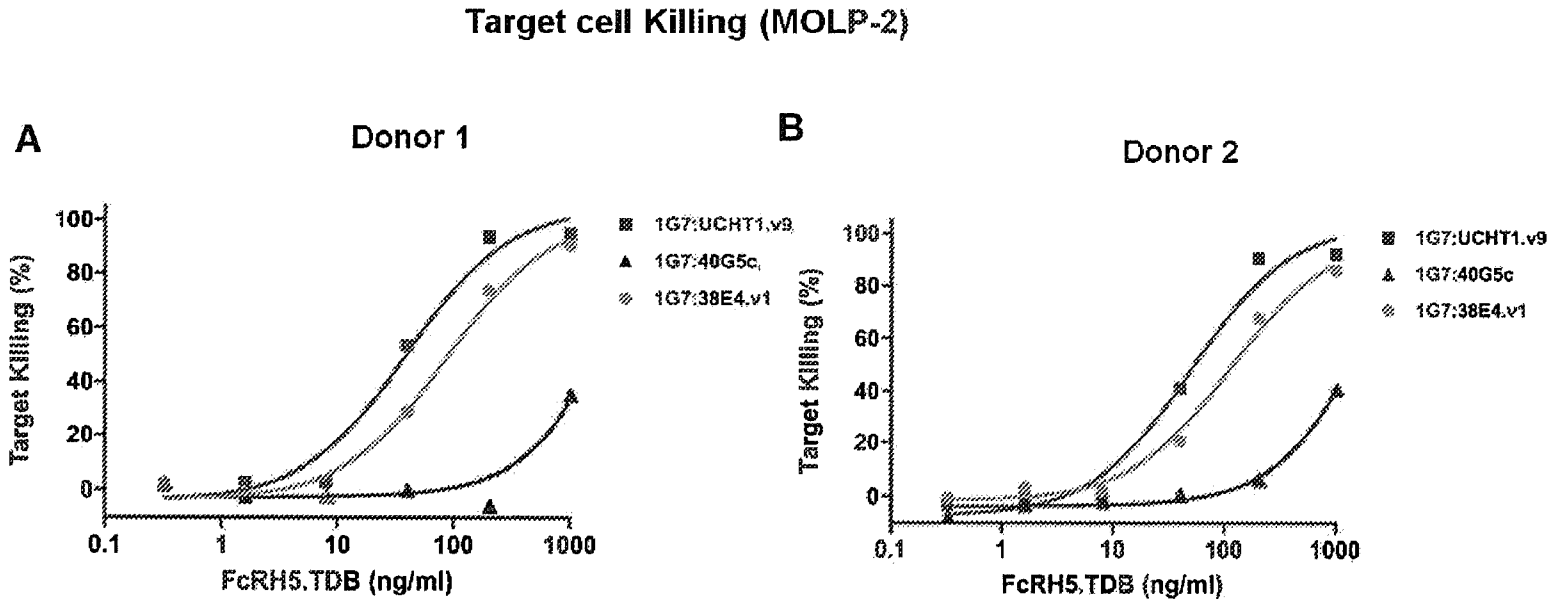
Figure 70

Binding to CD8+ Cells



Binding:
38E4.v1 > UCHT1.v9 > 40G5c

Figure 71A-71B



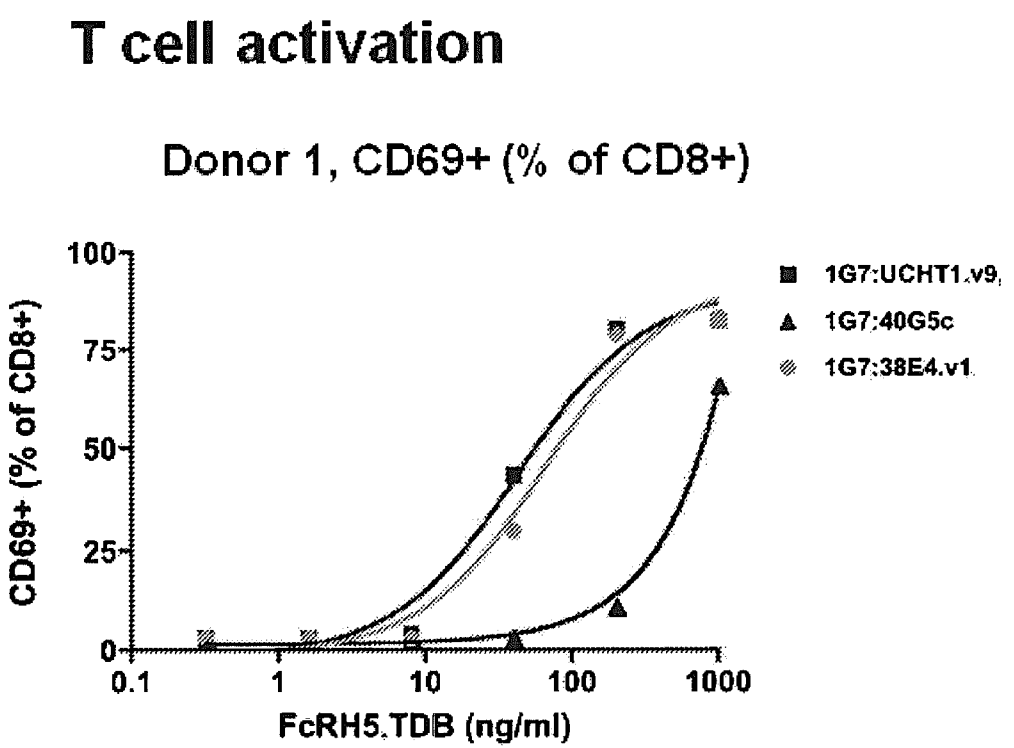
EC50
1G7.UCHT1.v9: 262 pM
1G7.40G5c: na
1G7:38E4.v1: 576 pM

Target Killing:
UCHT1.v9 > 38E4.v1 > 40G5c

EC50
1G7.UCHT1.v9: 333 pM
1G7.40G5c: na
1G7:38E4.v1: 734 pM

Effector Cells: purified human CD8+
Target cells: MOLP-2
E:T = 3:1
Time: 48 hours

Figure 72A

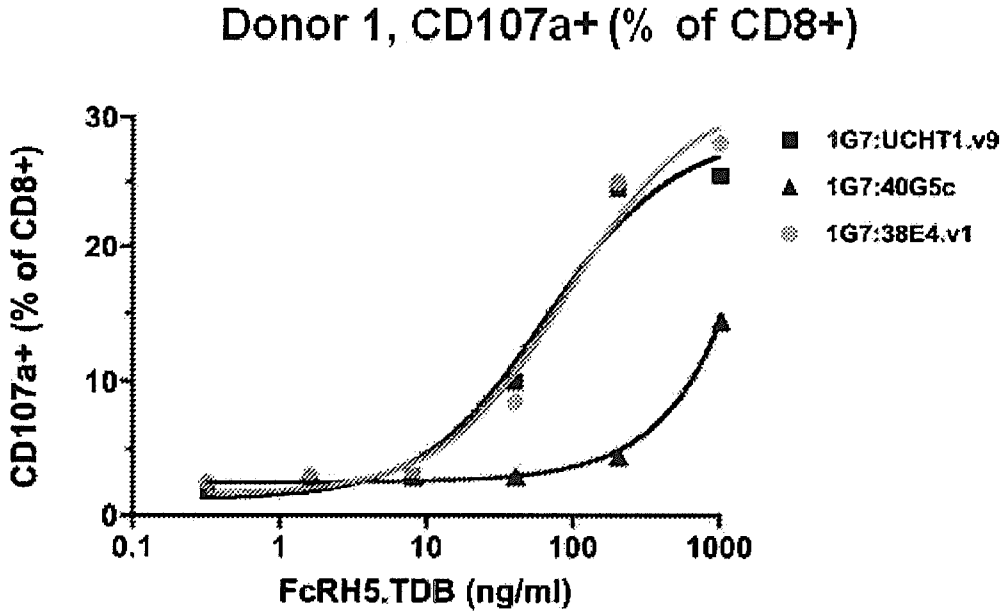


T activation:
UCHT1.v9 >= 38E4.v1 > 40G5c

Effector Cells: purified human CD8+
Target cells: MOLP-2
E:T = 3:1
Time: 24 hours

Figure 72B

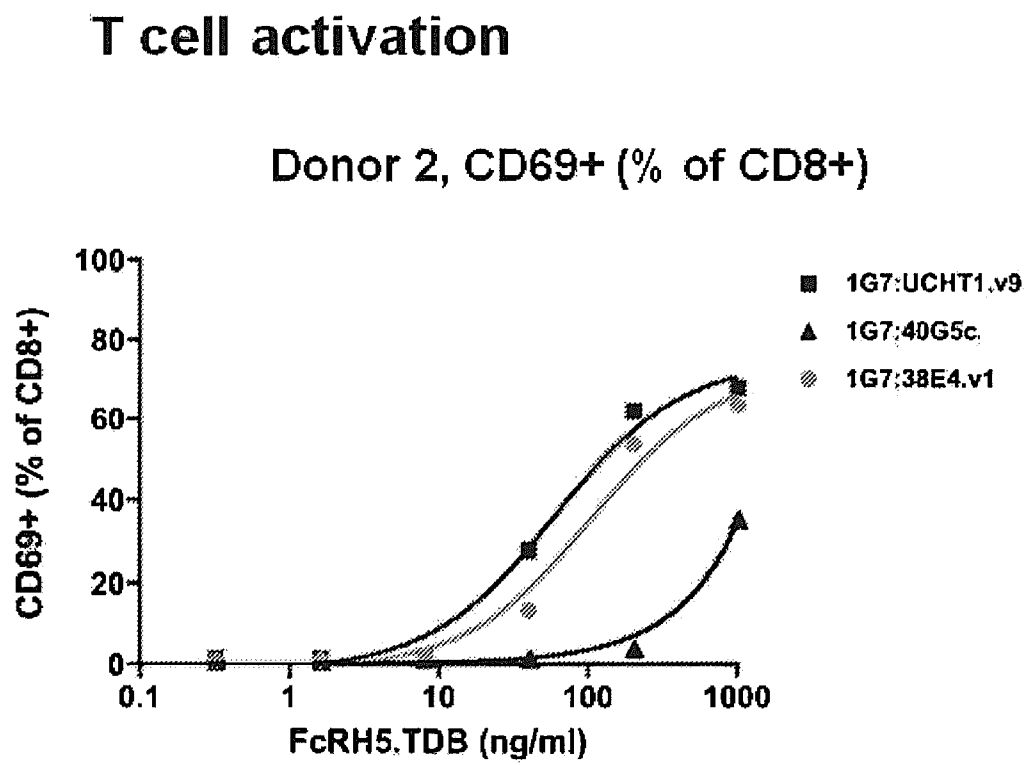
T cell activation



T activation:
UCHT1.v9 >= 38E4.v1 > 40G5c

Effector Cells: purified human CD8+
Target cells: MOLP-2
E:T = 3:1
Time: 24 hours

Figure 72C

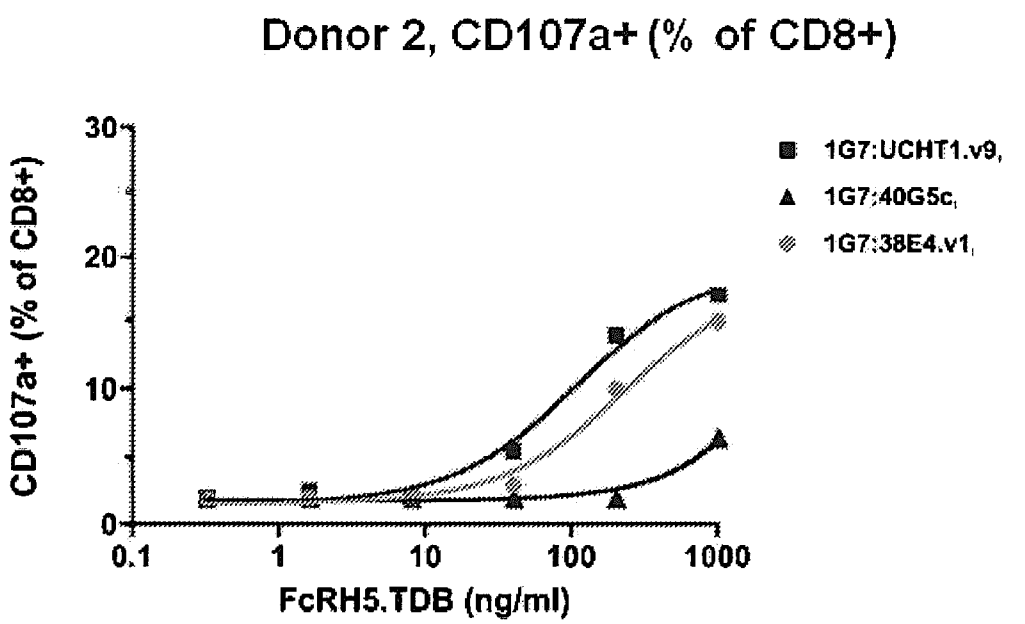


T activation:
UCHT1.v9 >= 38E4.v1 > 40G5c

Effector Cells: purified human CD8+
Target cells: MOLP-2
E:T = 3:1
Time: 24 hours

Figure 72D

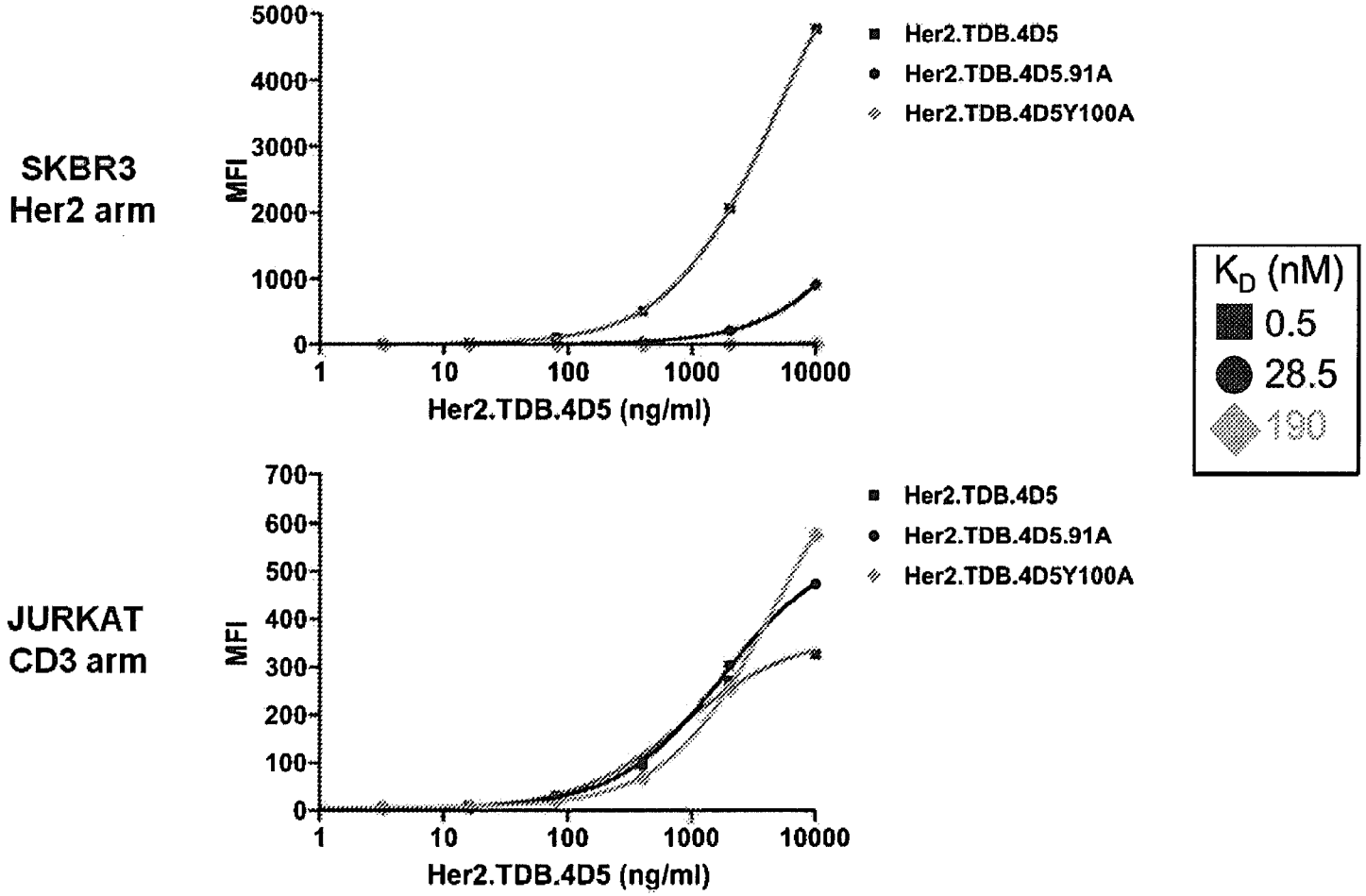
T cell activation



T activation:
UCHT1.v9 >= 38E4.v1 > 40G5c

Effector Cells: purified human CD8+
Target cells: MOLP-2
E:T = 3:1
Time: 24 hours

Figure 73


SKBR3
Her2 arm
MFI
5000
4000
3000
2000
1000
0
1
10
100
1000
10000
Her2.TDB.4D5 (ng/ml)
Her2.TDB.4D5
Her2.TDB.4D5.91A
Her2.TDB.4D5Y100A
JURKAT
CD3 arm
MFI
700
600
500
400
300
200
100
0
1
10
100
1000
10000
Her2.TDB.4D5 (ng/ml)
Her2.TDB.4D5
Her2.TDB.4D5.91A
Her2.TDB.4D5Y100A
K_D (nM)
0.5
28.5
190

Figures 74A-74C

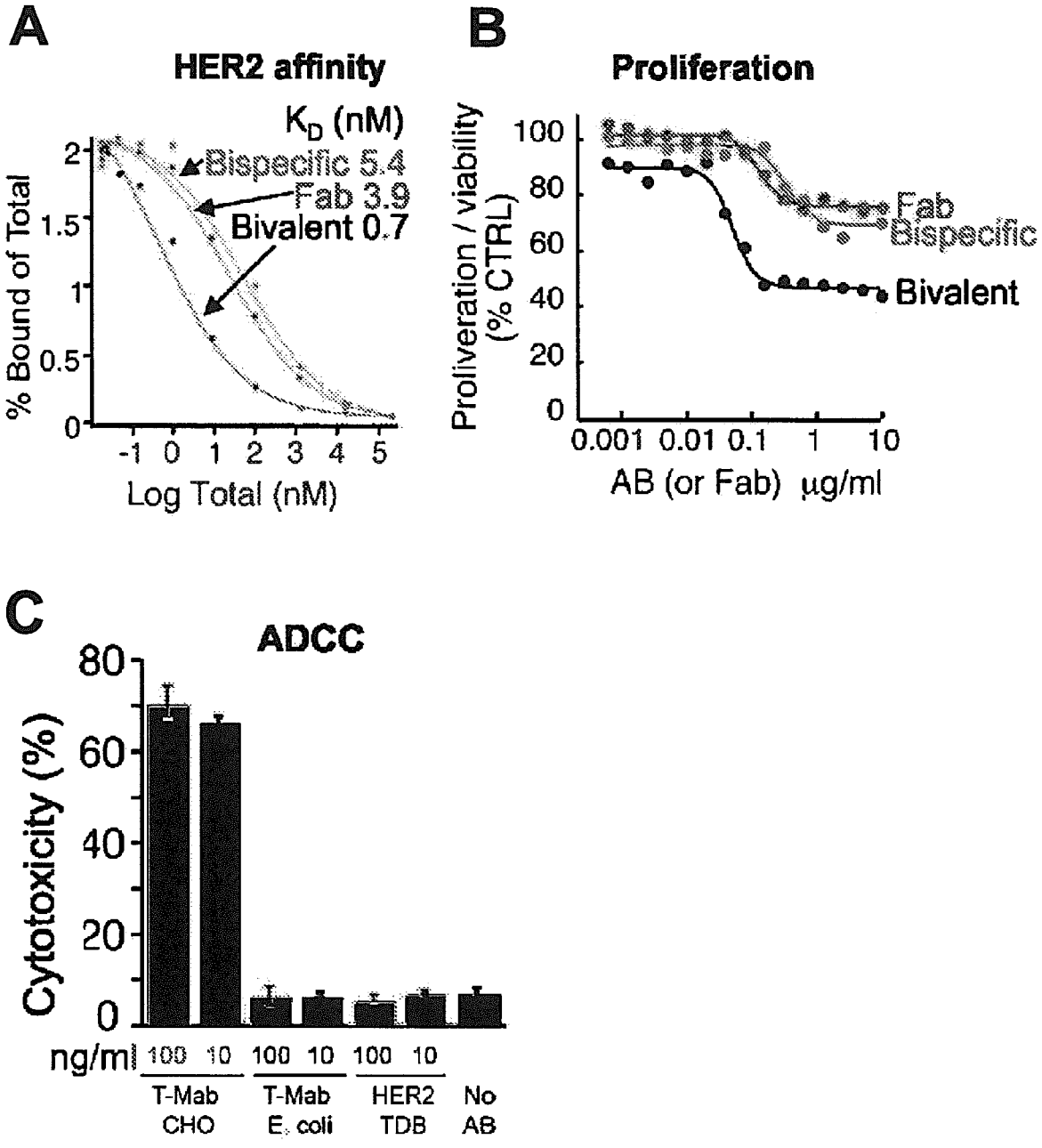
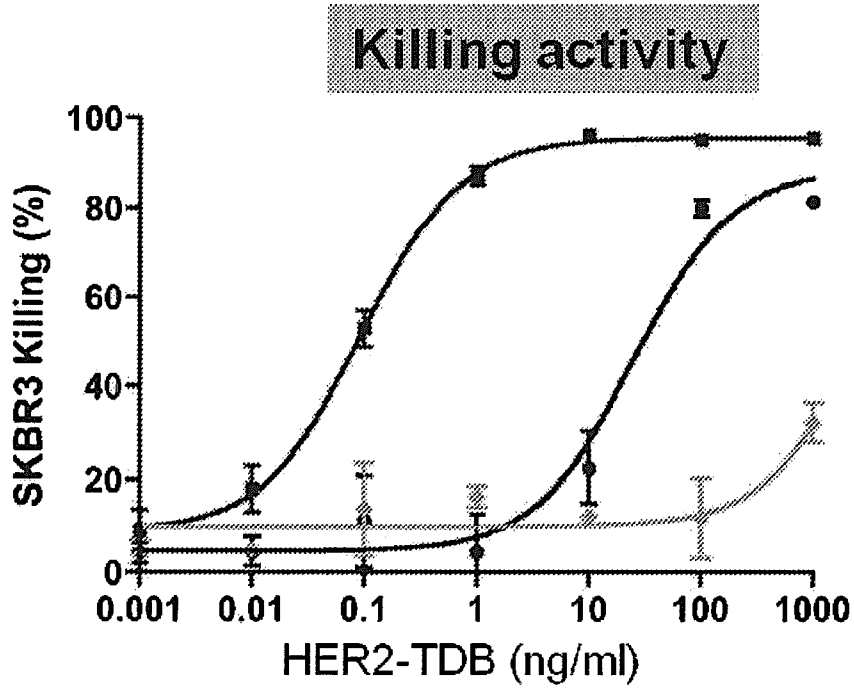


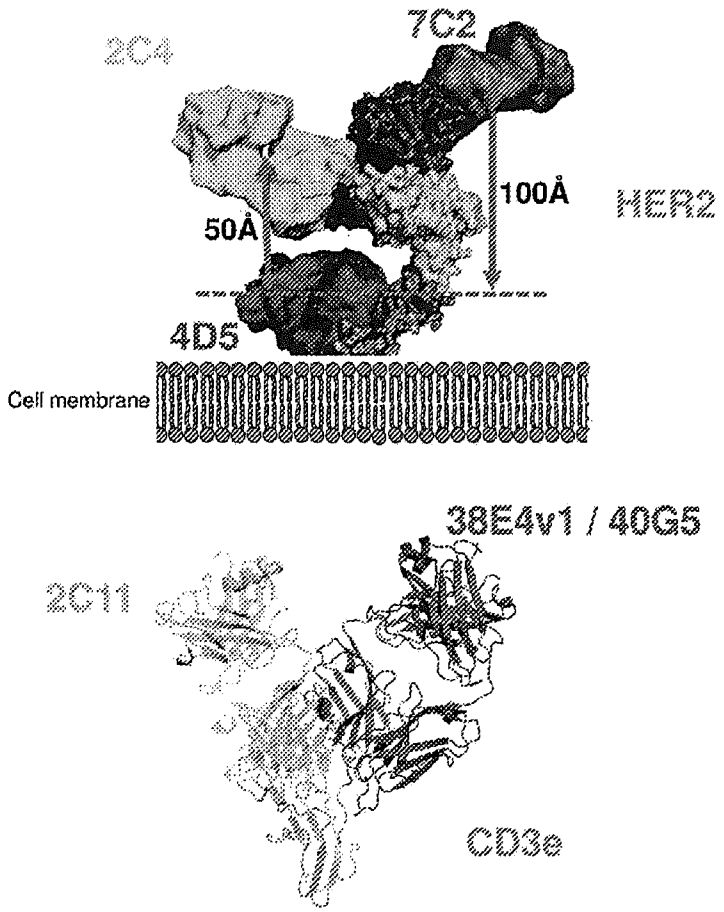
Figure 75



EC50:
Her2.TDB.4D5: 0.1 ng/ml = 0.7 pM
Her2.TDB.4D5.91A: 25.5 ng/ml = 170 pM
Her2.TDB.4D5.Y100A: --

Figures 76A-76B

A



B

HER2 arm	Monov. K_D (biacore)
4D5	0.4 nM
2C4	2.0 nM
7C2	1.7 nM

CD3 arm	Monov. K_D (biacore)
38E4v1	1.3 nM
40G5c	51 nM
2C11*	22 nM

*mouse specific, used as in vivo efficacy surrogate

Figure 76C

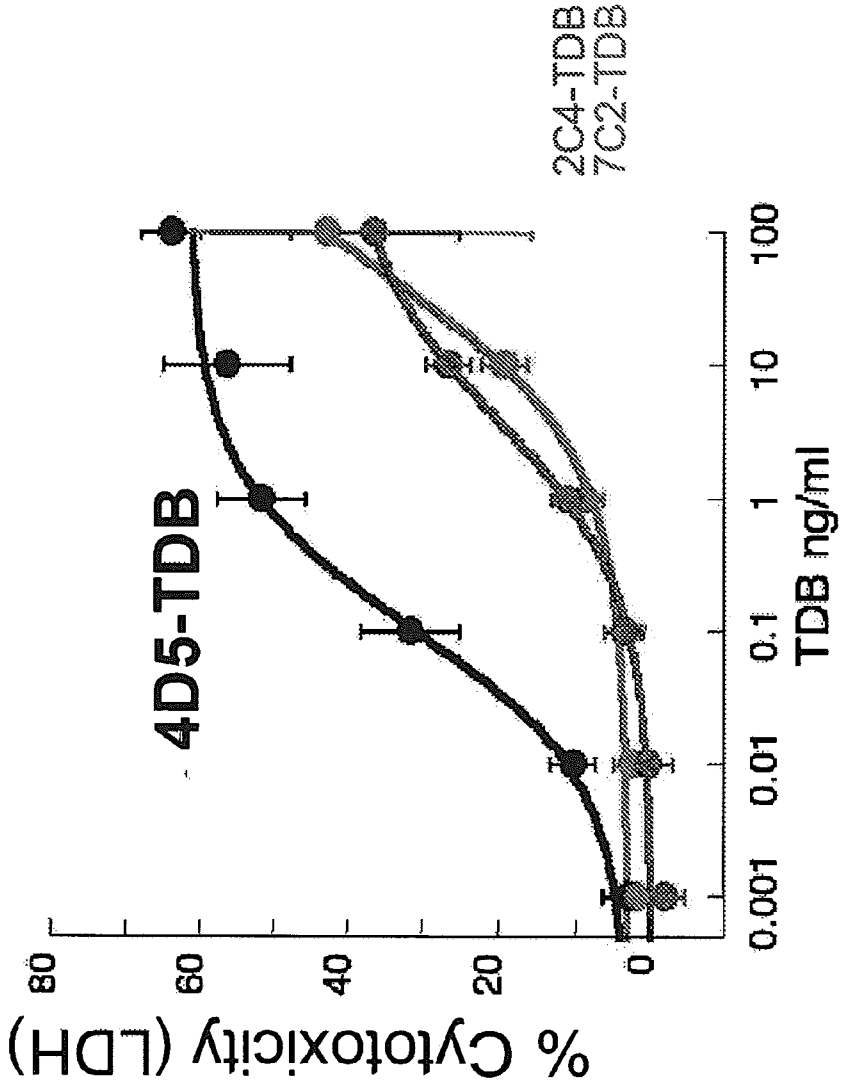

% Cytotoxicity (LDH)
80
60
40
20
0
4D5-TDB
2C4-TDB
7C2-TDB
0.001
0.01
0.1
1
10
100
TDB ng/ml

Figure 77

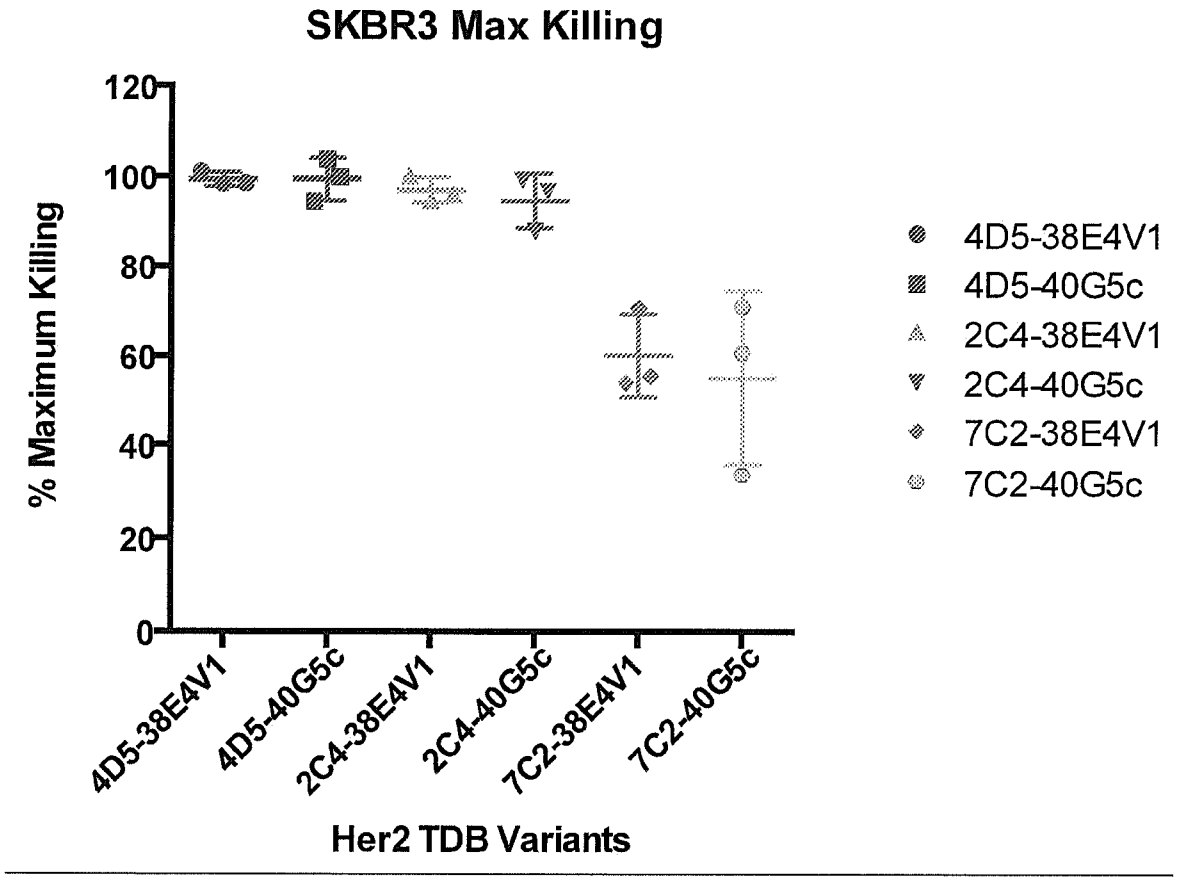

SKBR3 Max Killing
% Maximum Killing
120
100
80
60
40
20
0
4D5-38E4V1
4D5-40G5c
2C4-38E4V1
2C4-40G5c
7C2-38E4V1
7C2-40G5c
Her2 TDB Variants

Figure 78

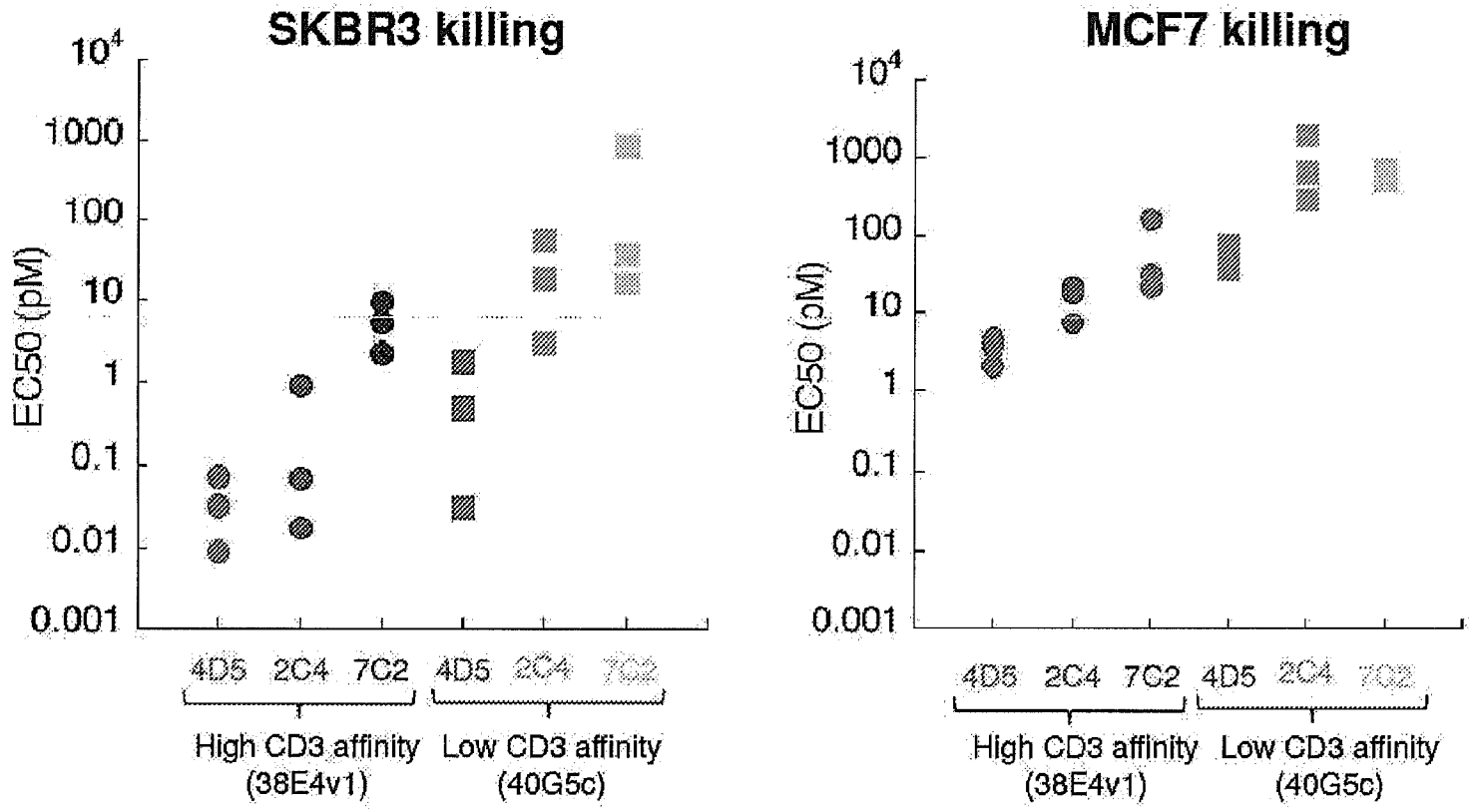

SKBR3 killing
EC50 (pM)
10^4
1000
100
10
1
0.1
0.01
0.001
4D5 2C4 7C2 4D5 2C4 7C2
High CD3 affinity (38E4v1)
Low CD3 affinity (40G5c)
MCF7 killing
EC50 (pM)
10^4
1000
100
10
1
0.1
0.01
0.001
4D5 2C4 7C2 4D5 2C4 7C2
High CD3 affinity (38E4v1)
Low CD3 affinity (40G5c)

Figure 79

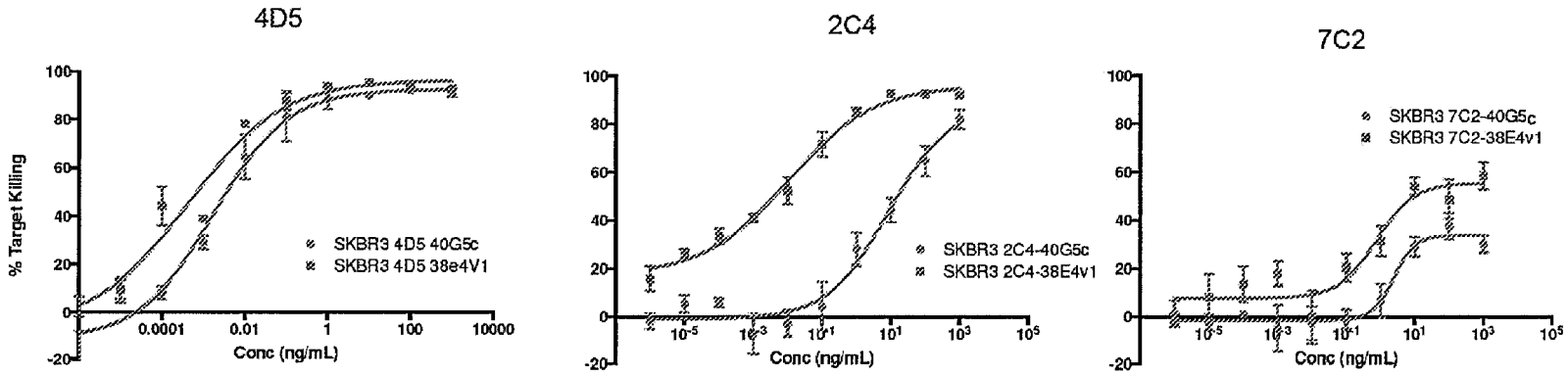
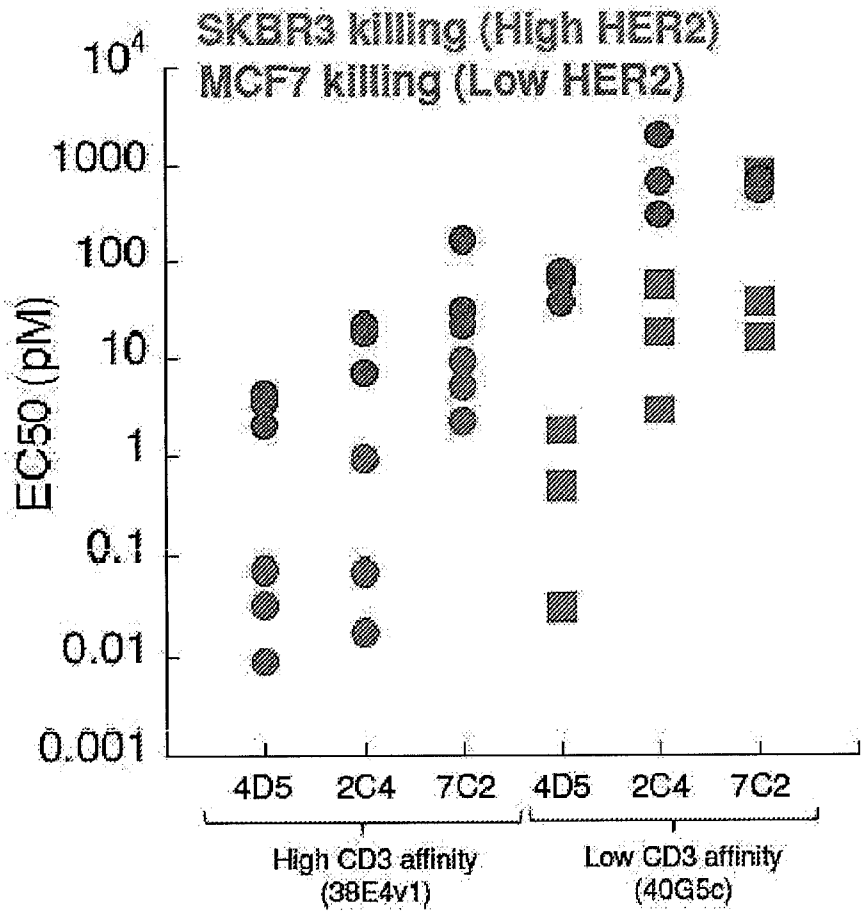

4D5
% Target Killing
100
80
60
40
20
0
-20
0.0001
0.01
1
100
10000
Conc (ng/mL)
SKBR3 4D5 40G5c
SKBR3 4D5 38e4V1
2C4
100
80
60
40
20
0
-20
10^{-5}
10^{-3}
10^{-1}
10^{1}
10^{3}
10^{5}
Conc (ng/mL)
SKBR3 2C4-40G5c
SKBR3 2C4-38E4v1
7C2
100
80
60
40
20
0
-20
10^{-5}
10^{-3}
10^{-1}
10^{1}
10^{3}
10^{5}
Conc (ng/mL)
SKBR3 7C2-40G5c
SKBR3 7C2-38E4v1

Figure 80



EC_{50} MCF7 / EC_{50} SKBR3

xHER2	xCD3	Expt1	Expt2	Expt3
4D5	38E4v1	46	225	128
2C4	38E4v1	406	311	20
7C2	38E4v1	10	6	17
4D5	40G5c	1154	126	42
2C4	40G5c	100	11	110
7C2	40G5c	26	42	0

Figure 81

Variant	SKBR3 EC50 ng/mL	HER2 KD (nM)	KD fold	SKBR3 EC50 fold
4D5:40G5c	0.007577	0.5	1	1
hu4D5.v7	0.8575	2.7	5.4	113
hu4D5.v5	75.01	15.6	31.2	9900
hu4D5.v10	127.4	9.4	18.8	16814
hu4D5.v31	3202	16	32.0	422595
hu4D5.Y100A	>10K	60	120.0	

Fold difference vs. WT 4D5

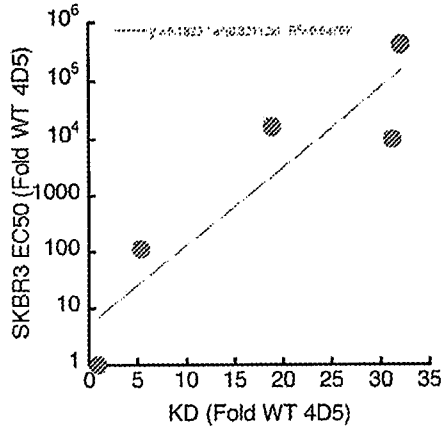


Figure 82

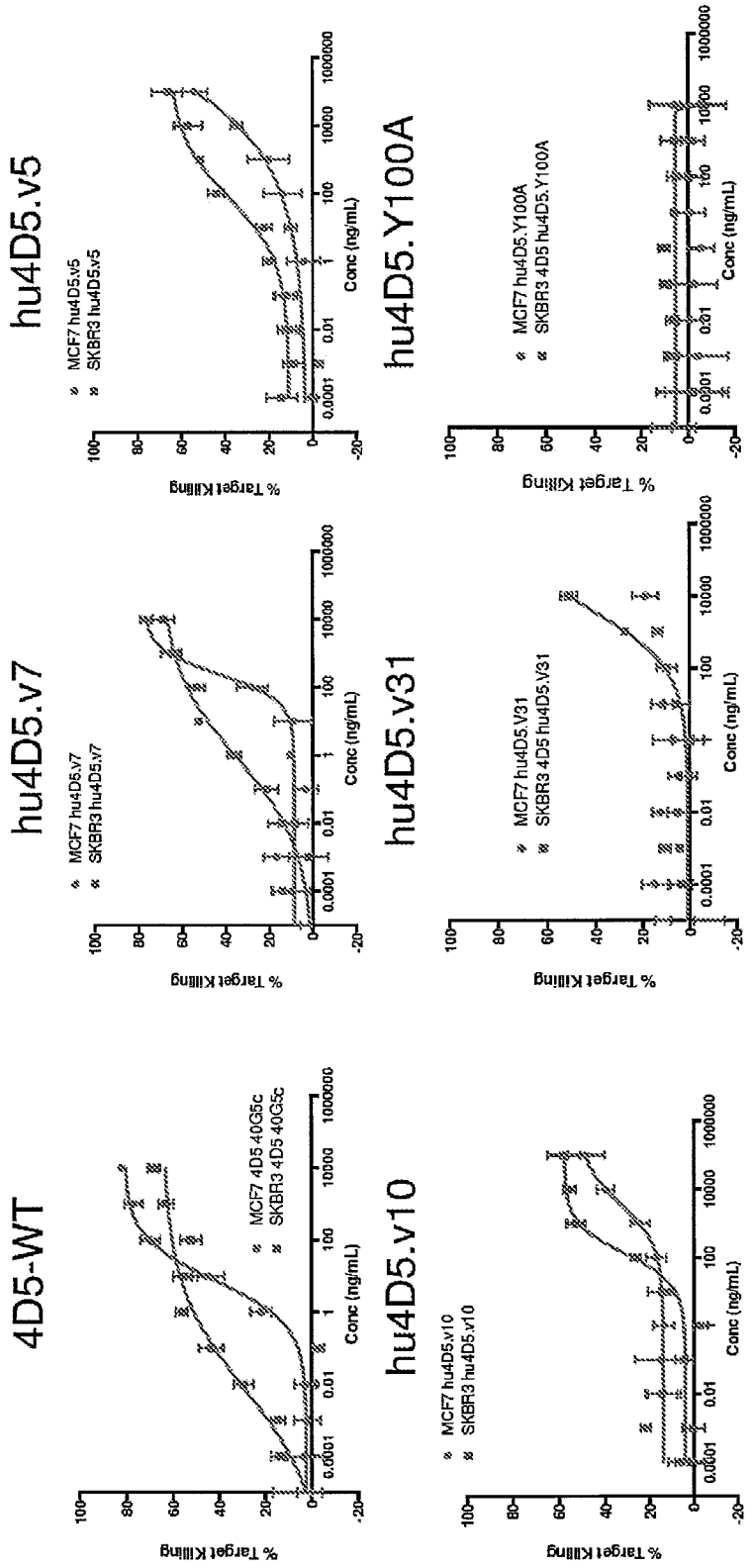
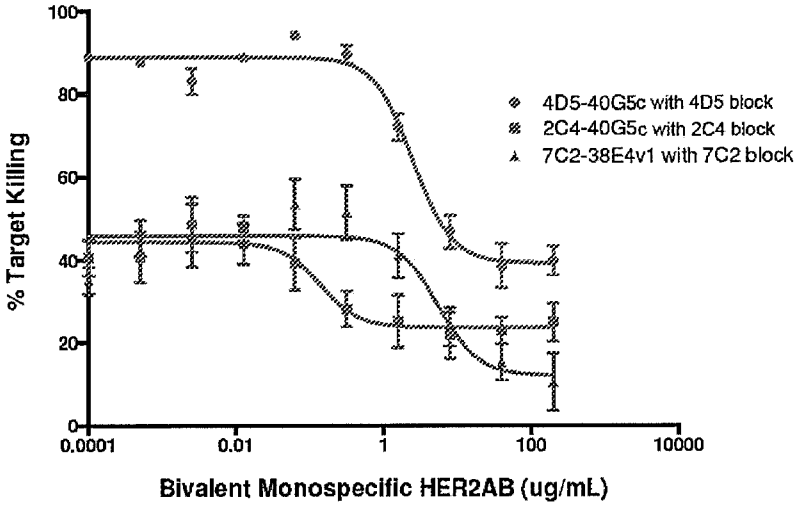

4D5-WT
MCF7 4D5 40G5c
SKBR3 4D5 40G5c
% Target Killing
Conc (ng/mL)
hu4D5.v5
MCF7 hu4D5.v5
SKBR3 hu4D5.v5
hu4D5.v7
MCF7 hu4D5.v7
SKBR3 hu4D5.v7
hu4D5.v10
MCF7 hu4D5.v10
SKBR3 hu4D5.v10
hu4D5.v31
MCF7 hu4D5.V31
SKBR3 4D5 hu4D5.V31
hu4D5.Y100A
MCF7 hu4D5.Y100A
SKBR3 4D5 hu4D5.Y100A

Figure 83


100
80
60
40
20
0
% Target Killing
0.0001
0.01
1
100
10000
Bivalent Monospecific HER2AB (ug/mL)
4D5-40G5c with 4D5 block
2C4-40G5c with 2C4 block
7C2-38E4v1 with 7C2 block

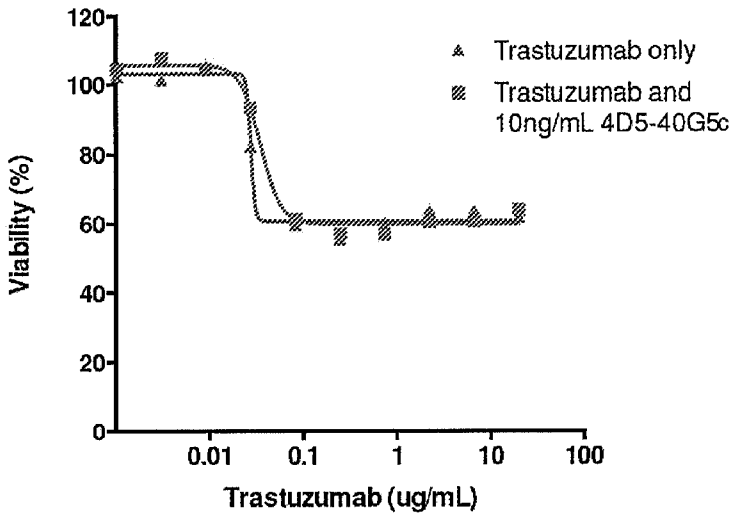

120
100
80
60
40
20
0
Viability (%)
0.01
0.1
1
10
100
Trastuzumab (ug/mL)
Trastuzumab only
Trastuzumab and
10ng/mL 4D5-40G5c

Figure 84

CLONE	ELISA*	IP*	FACS*	DOMAIN	Epitope**	Tested as bispecific	Blocked by 4D5	Note
2C4	+++	++	+++	2	22-584	Y	N	Medium activity
2H11	+	++	++	4	529-645	Y	Y	Low activity
3E8	+	+++	+++	4	512-625	N	Y	
3H4	+	+	+	4	541-599	Y	Y	High activity
4D5	++	++	+++	4	561-625	Y	Y	High activity
5B8	+	++	+	?		Y	N	Low activity
6E9	++	+	NEG	?		N		FACS neg.
7C2	++	++	+++	1	22-53	Y	N	Low activity
7F3	+++	+++	+++	1	22-53	N	N	Domain1

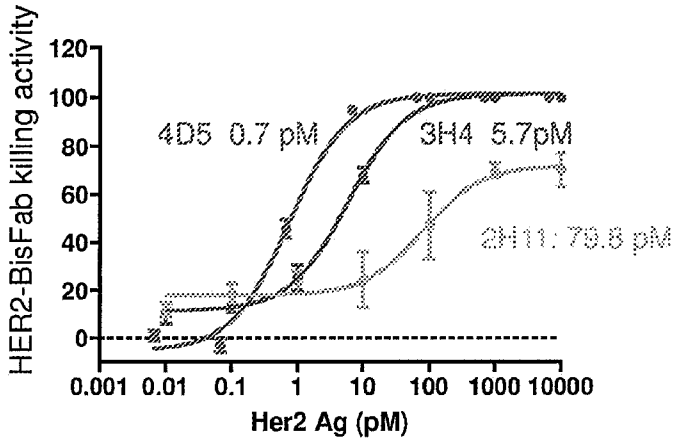
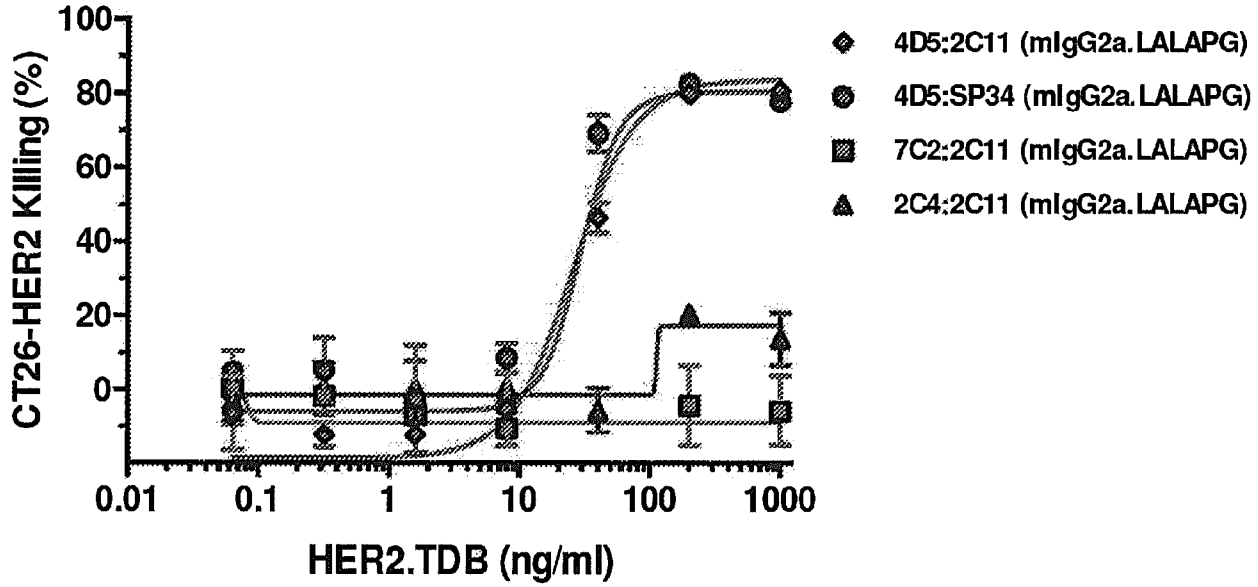


Figure 85

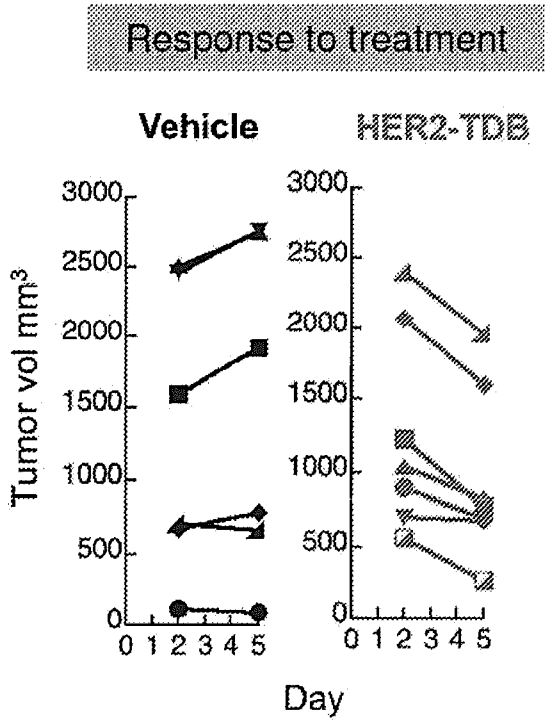
CD3 arm	Monov. K_D (nM, biacore)	Reactivity
humanized 38E4v1	1.3	hu, cyno
murine 38E4	0.75	hu, cyno
murine SP34	1.9	hu, cyno
humanized 40G5c	51	hu, cyno
2C11	22	mouse

Figure 86

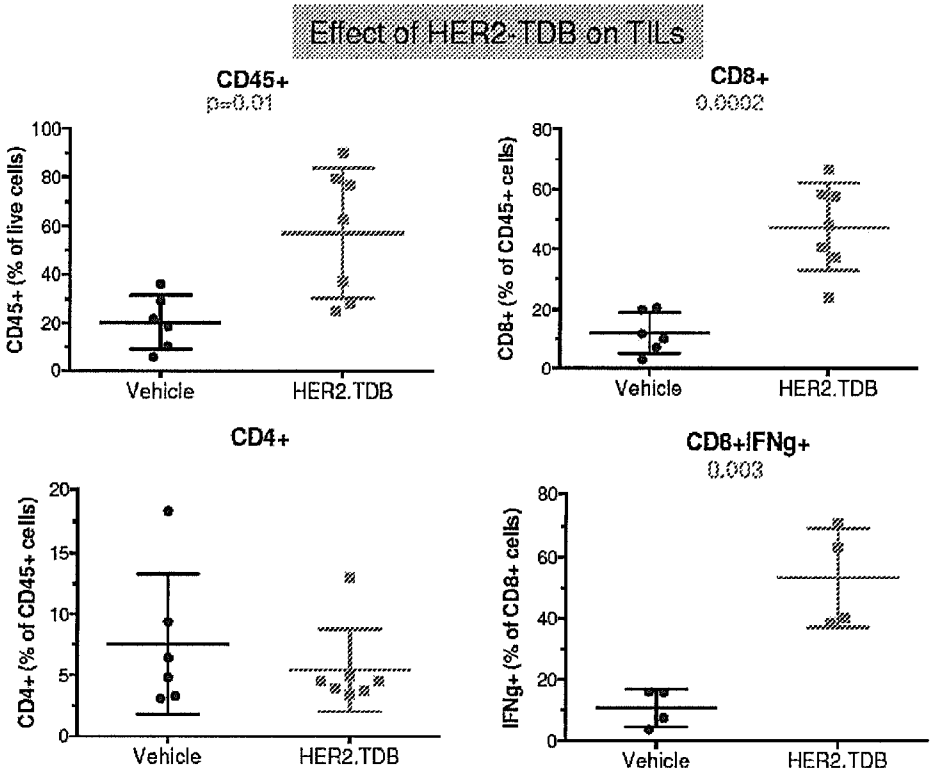

CT26-HER2 Killing (%)
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
HER2.TDB (ng/ml)
4D5:2C11 (mIgG2a.LALAPG)
4D5:SP34 (mIgG2a.LALAPG)
7C2:2C11 (mIgG2a.LALAPG)
2C4:2C11 (mIgG2a.LALAPG)

Figures 87A-87B

A

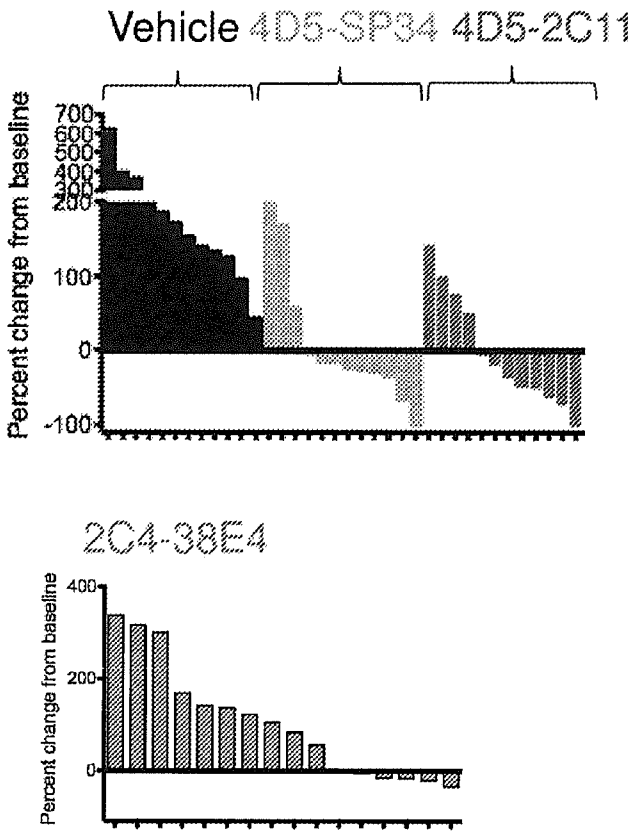


B



Figures 88A-88B

A



B

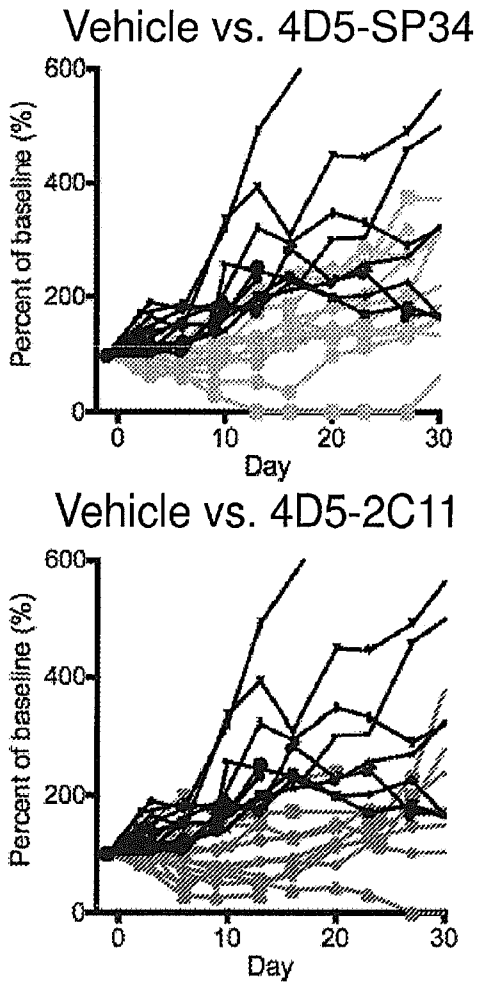
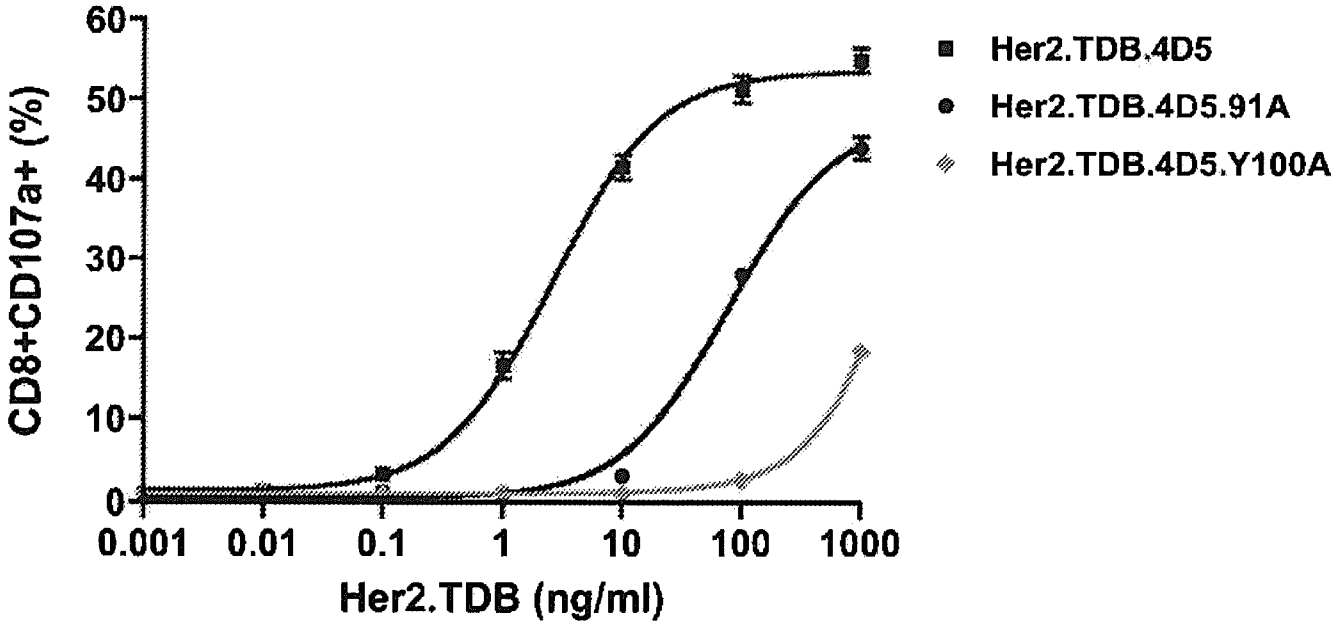
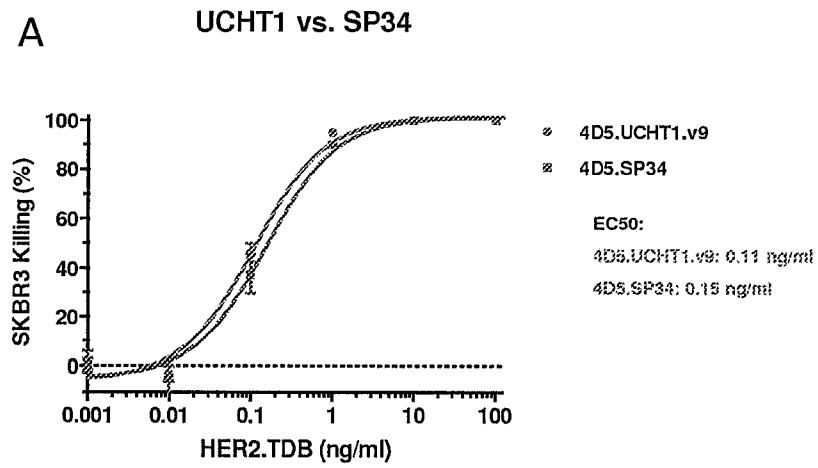


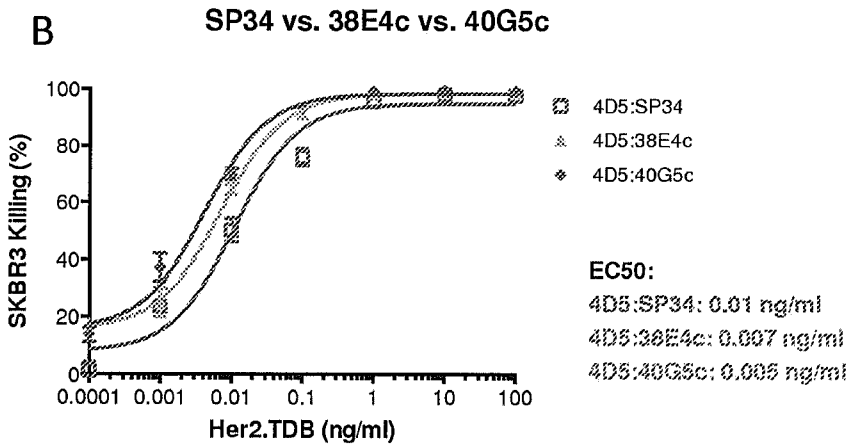
Figure 89

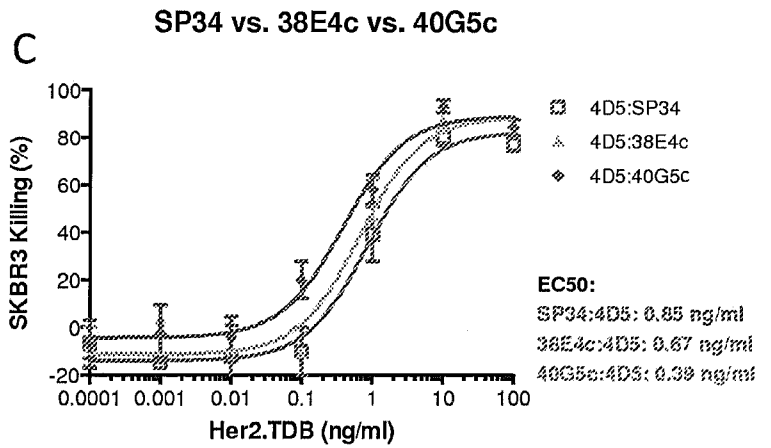


Effector Cells: CD8+ T cells
Target Cells: SKBR3
E:T ratio = 3:1
Time: 24 hours

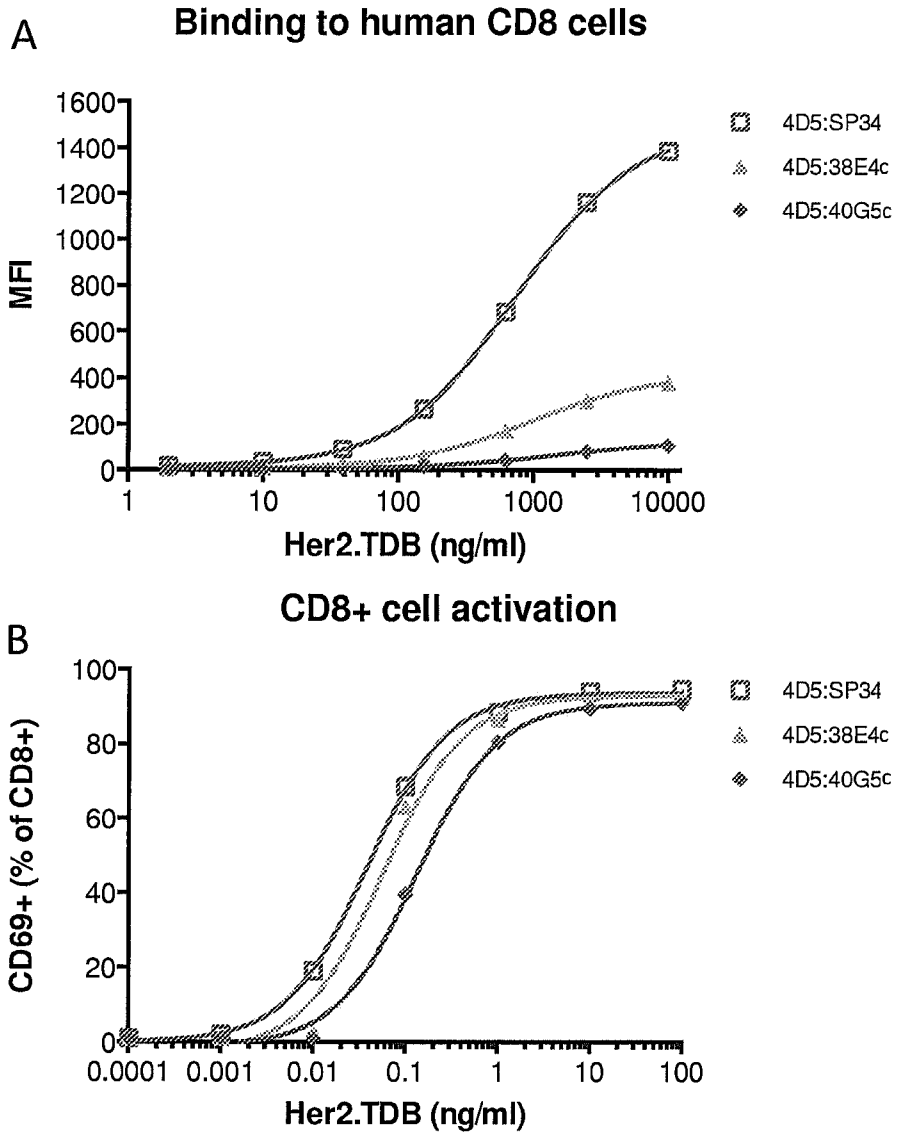
Figures 90A-90C



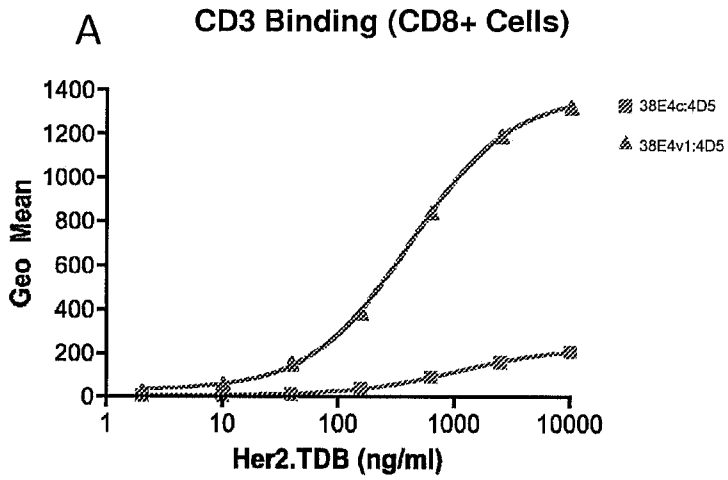


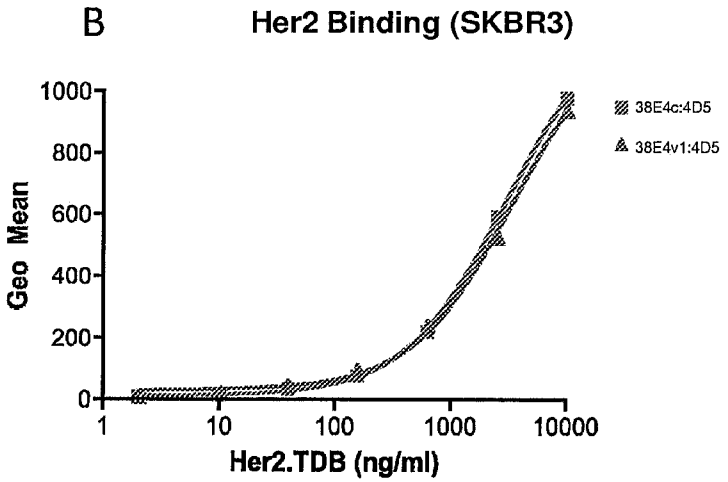


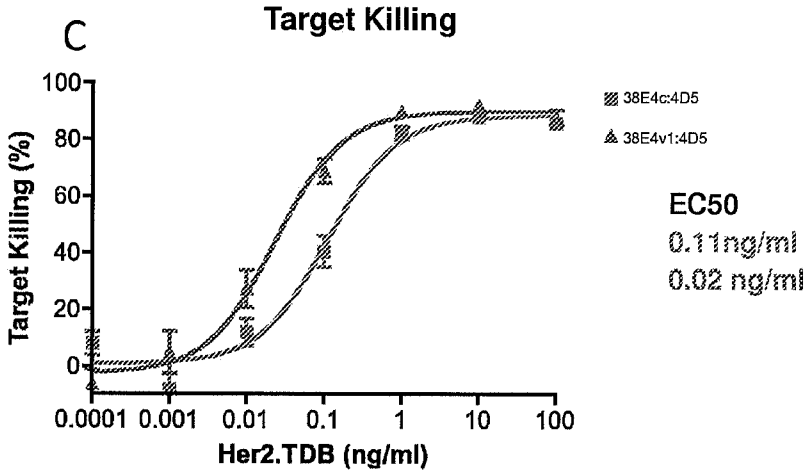
Figures 91A-91B

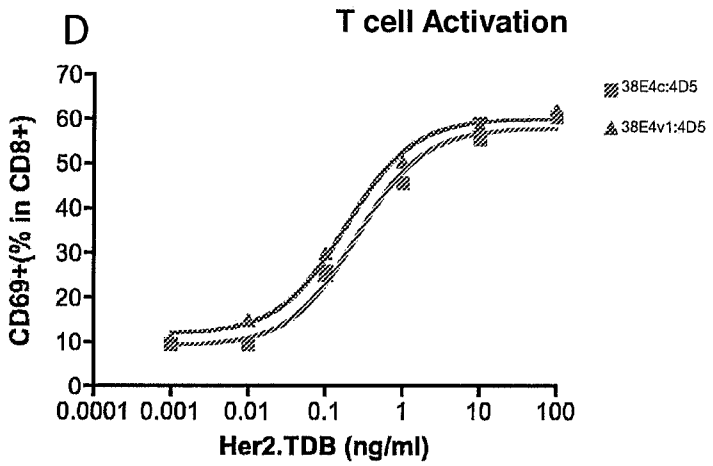


Figures 92A-92D

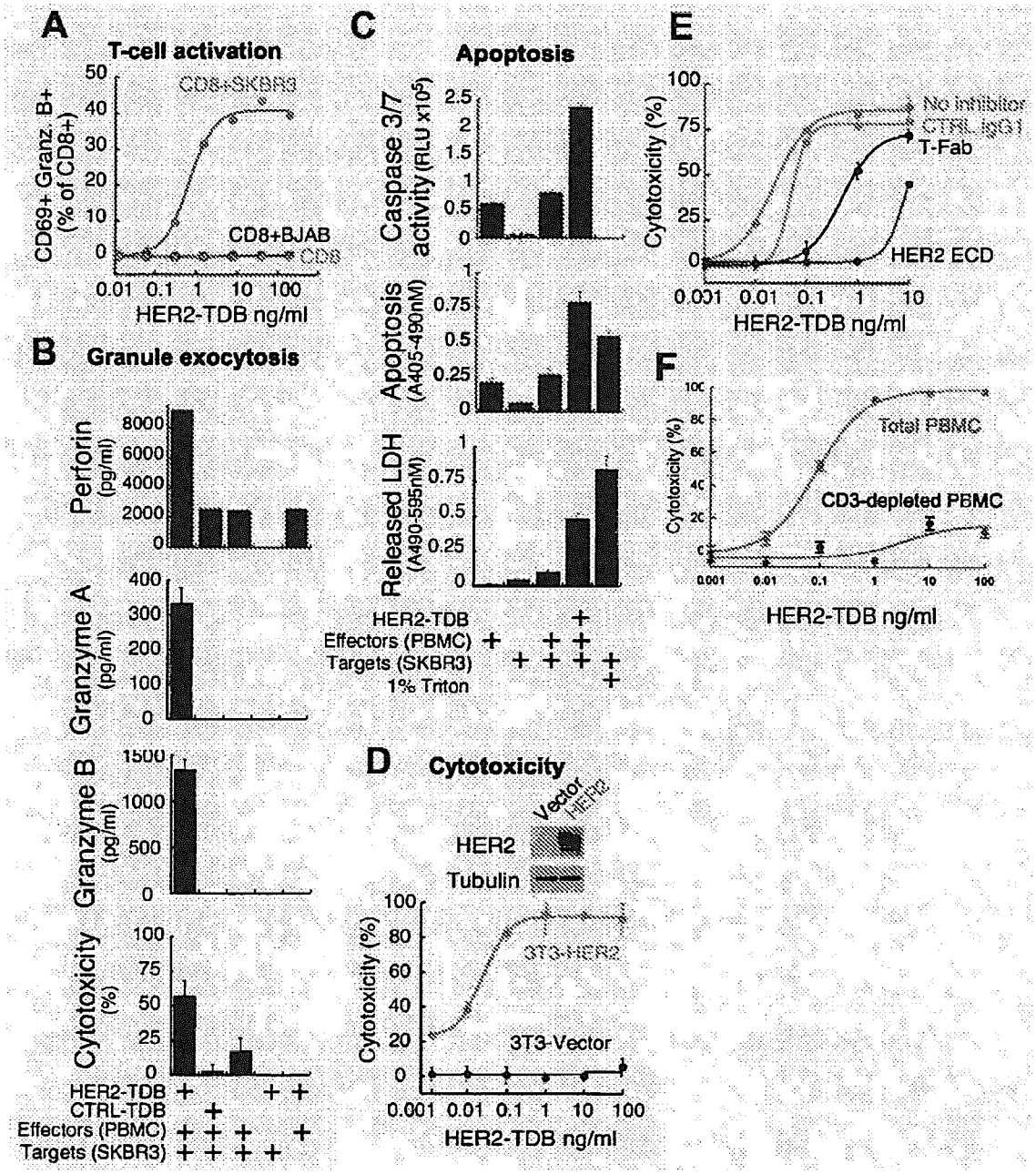




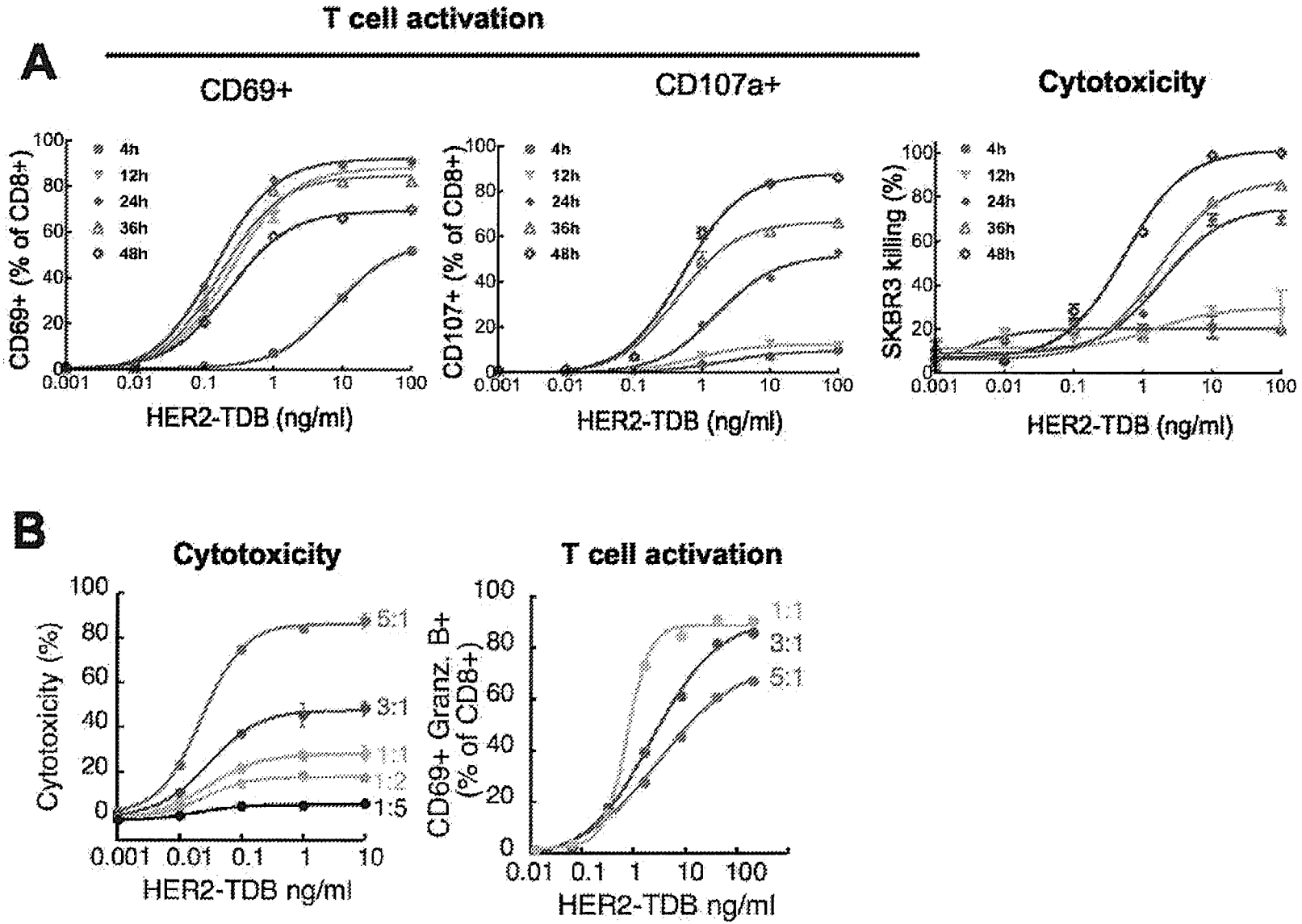




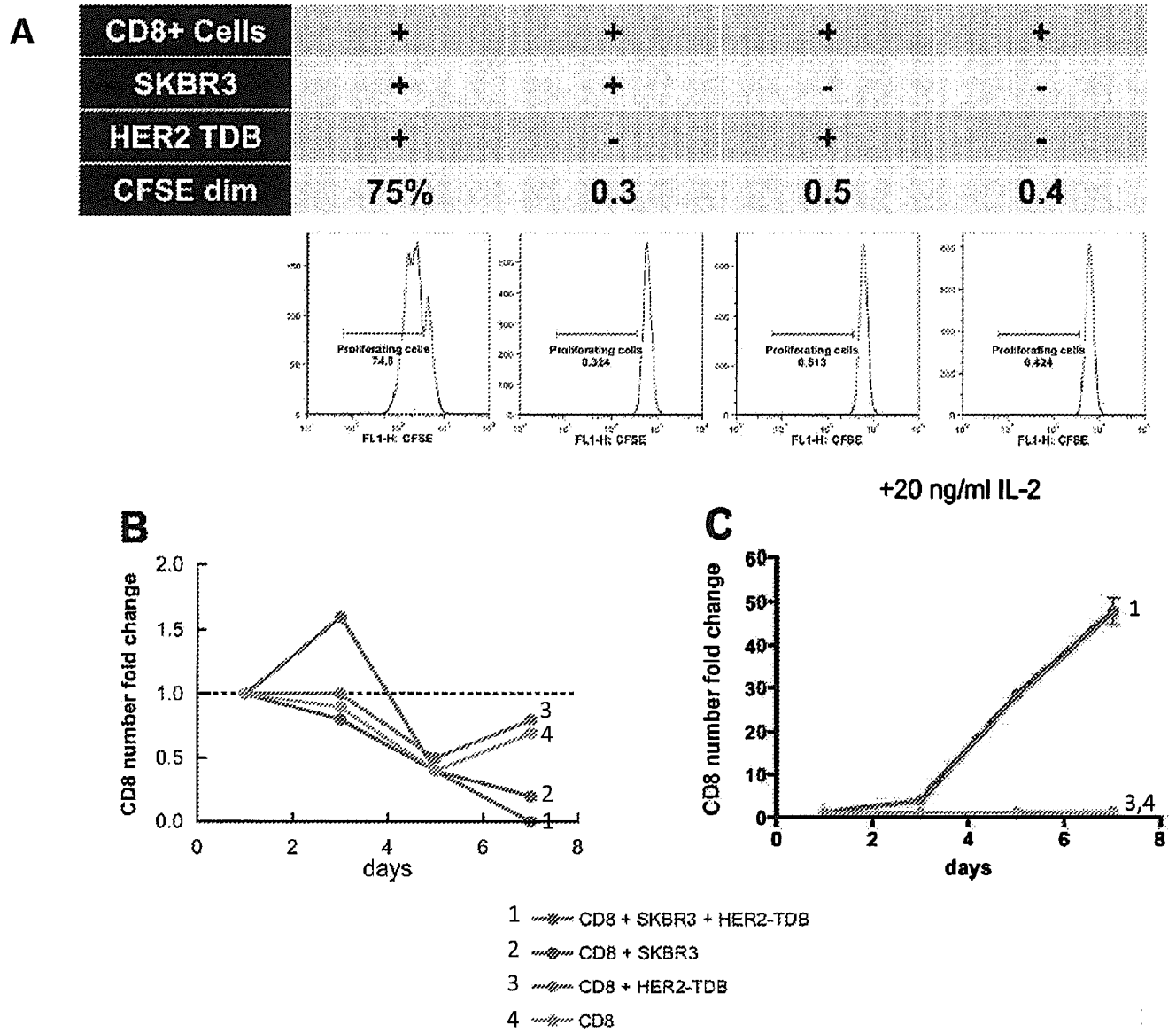
Figures 93A-93F



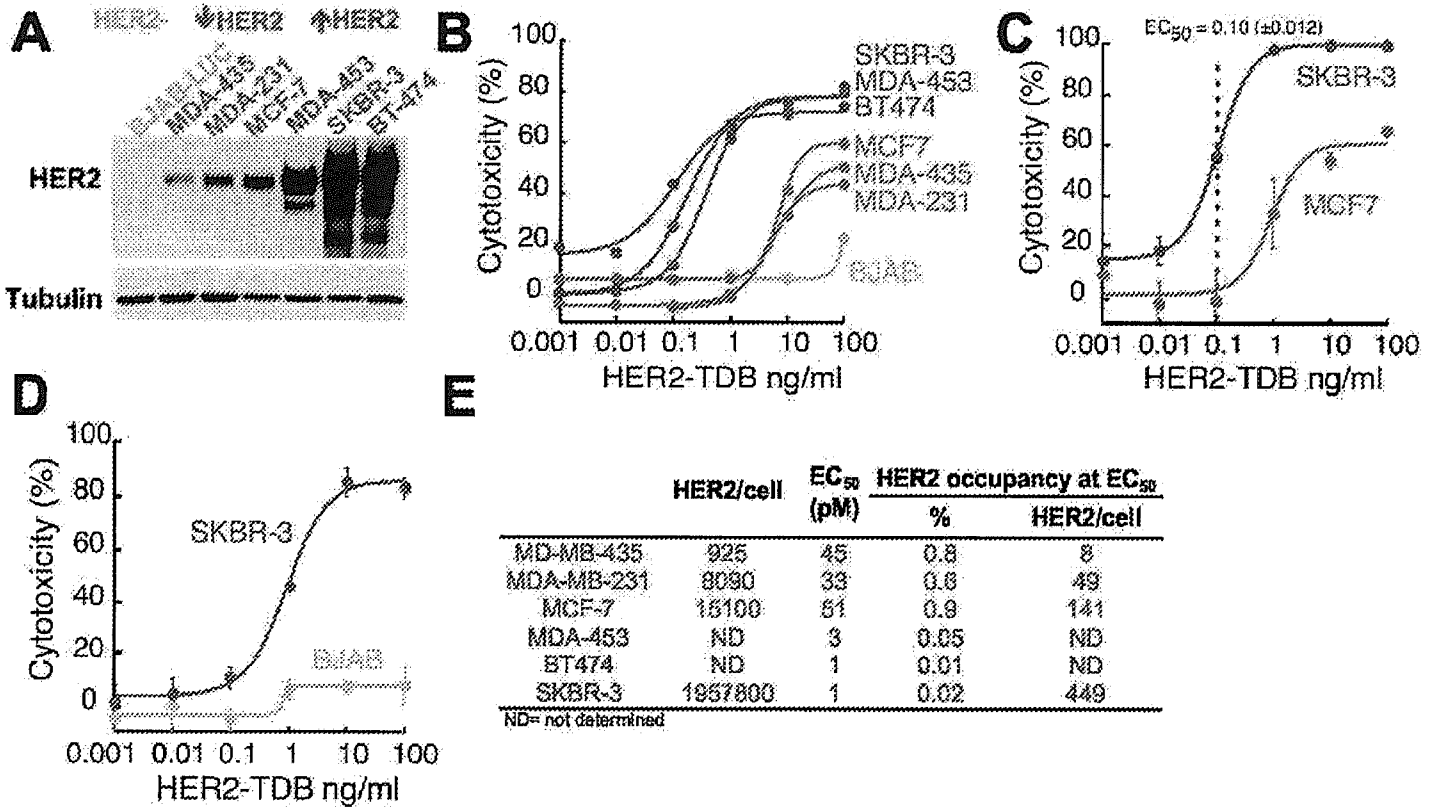
Figures 94A-94B



Figures 95A-95C



Figures 96A-96E



	HER2/cell	EC_{50} (pM)	HER2 occupancy at EC_{50}	
			%	HER2/cell
MD-MB-435	925	45	0.8	8
MDA-MB-231	8090	33	0.6	49
MCF-7	15100	51	0.9	141
MDA-453	ND	3	0.05	ND
BT474	ND	1	0.01	ND
SKBR-3	1957800	1	0.02	449

ND= not determined

Figures 97A-97C

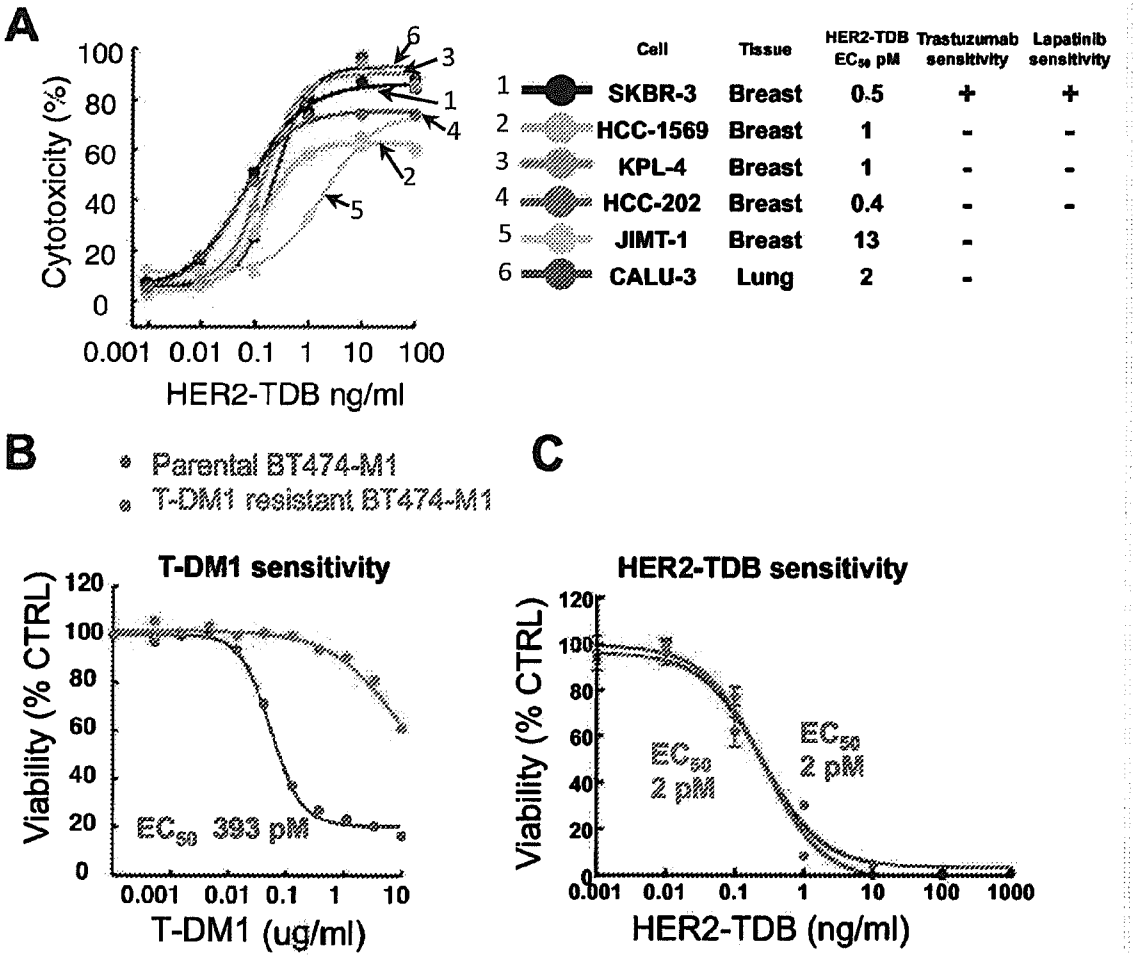
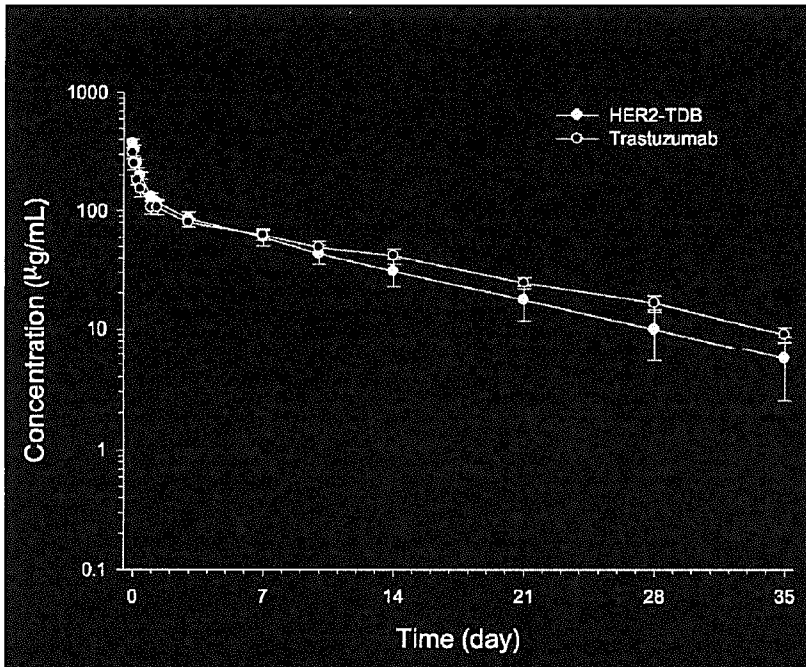
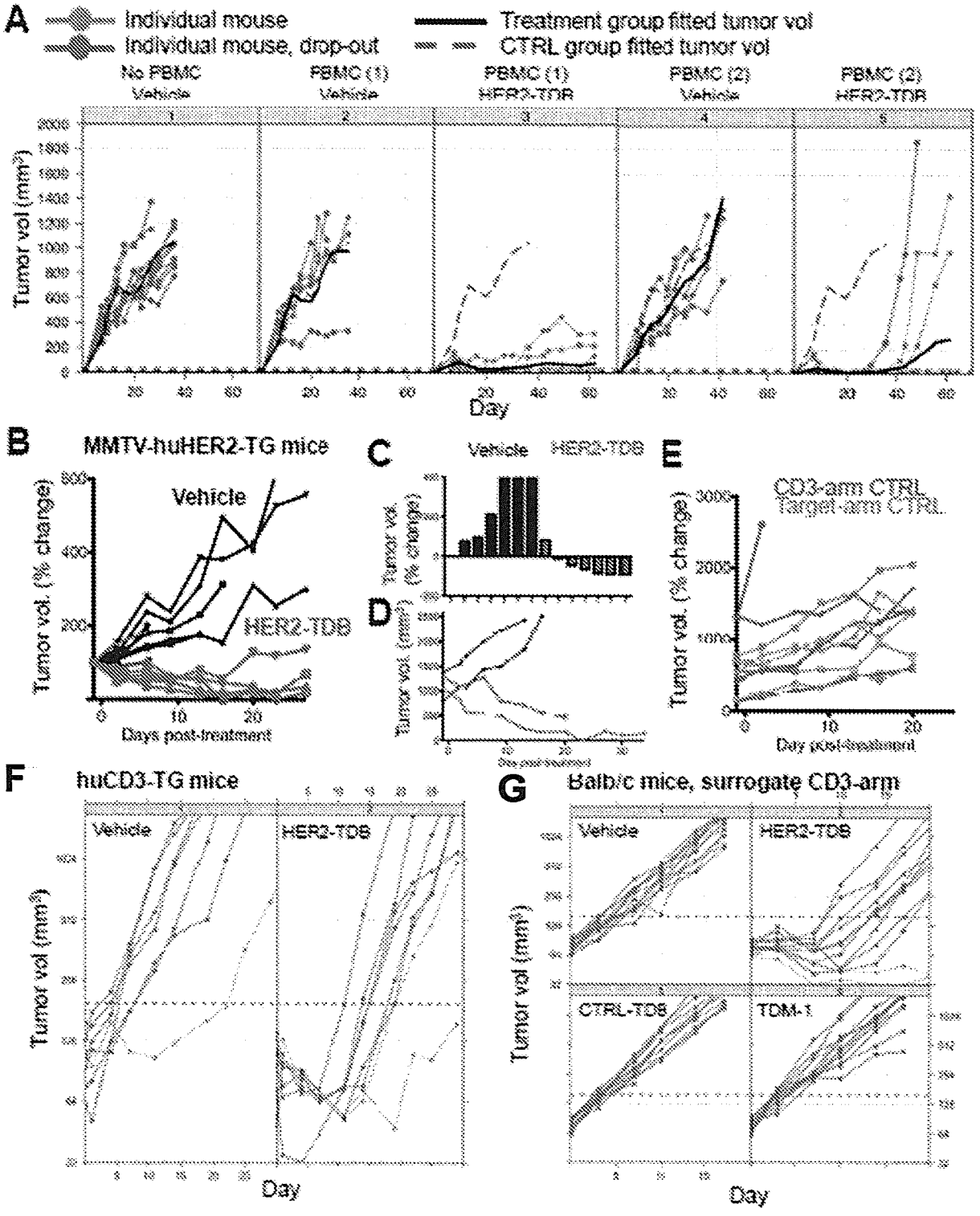


Figure 98

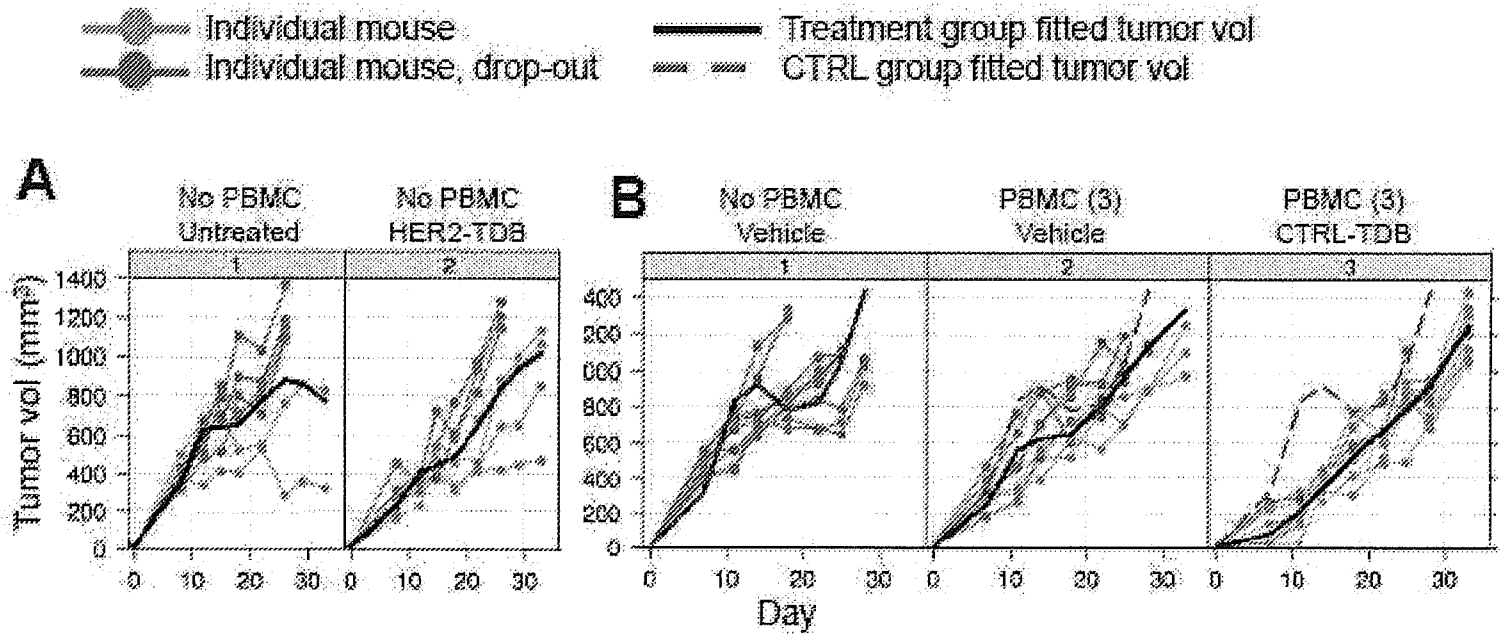


	HER2-TDB	Trastuzumab
CL (mL/day/kg)	10 ± 2	8 ± 1
AUC (day*ug/mL)	1328 ± 247	1553 ± 176
C_{max} (ug/mL)	377 ± 31	314 ± 41
V_1 (mL/kg)	33 ± 3	40 ± 5
HL_{beta} (Day)	7 ± 1	10 ± 1

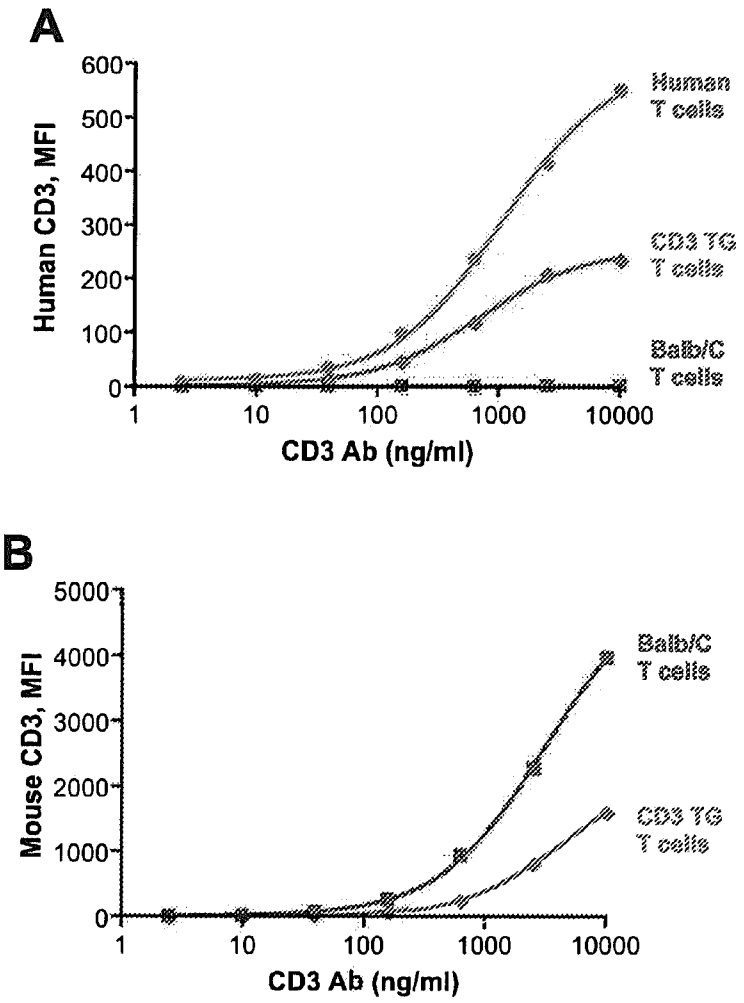
Figures 99A-99G



Figures 100A-100B



Figures 101A-101B



Figures 102A-102B

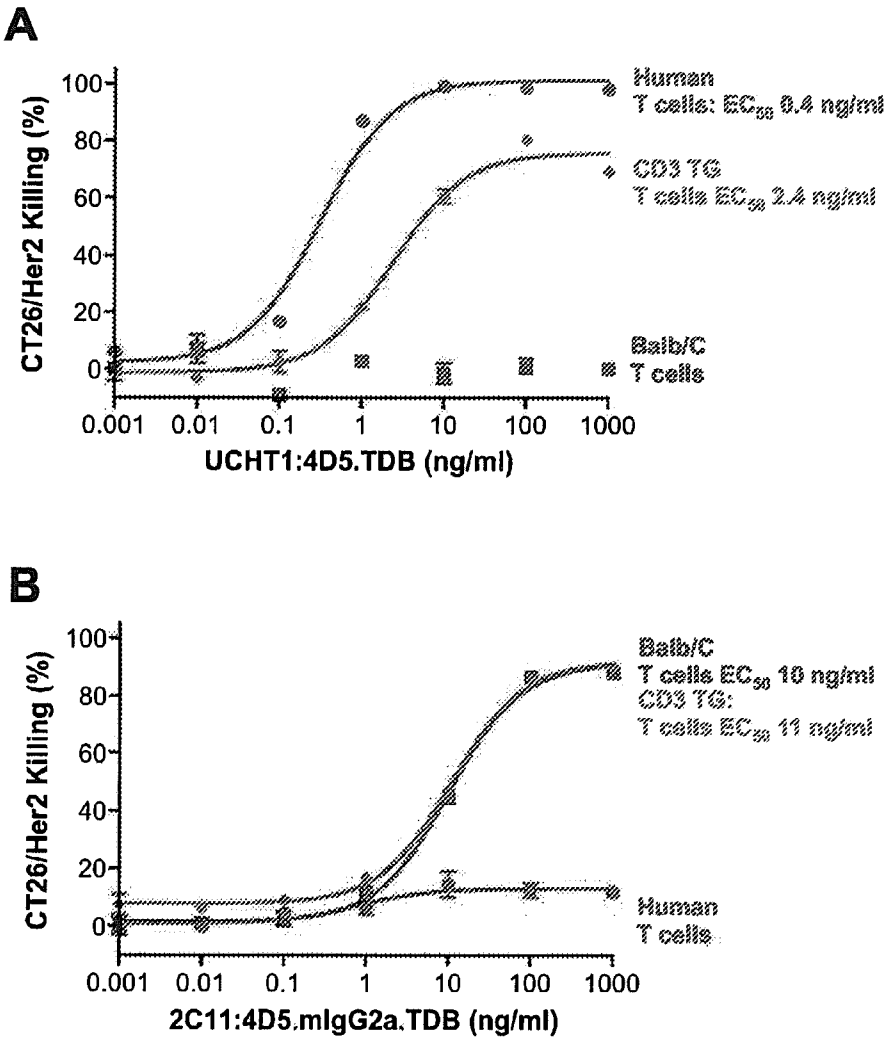


Figure 103

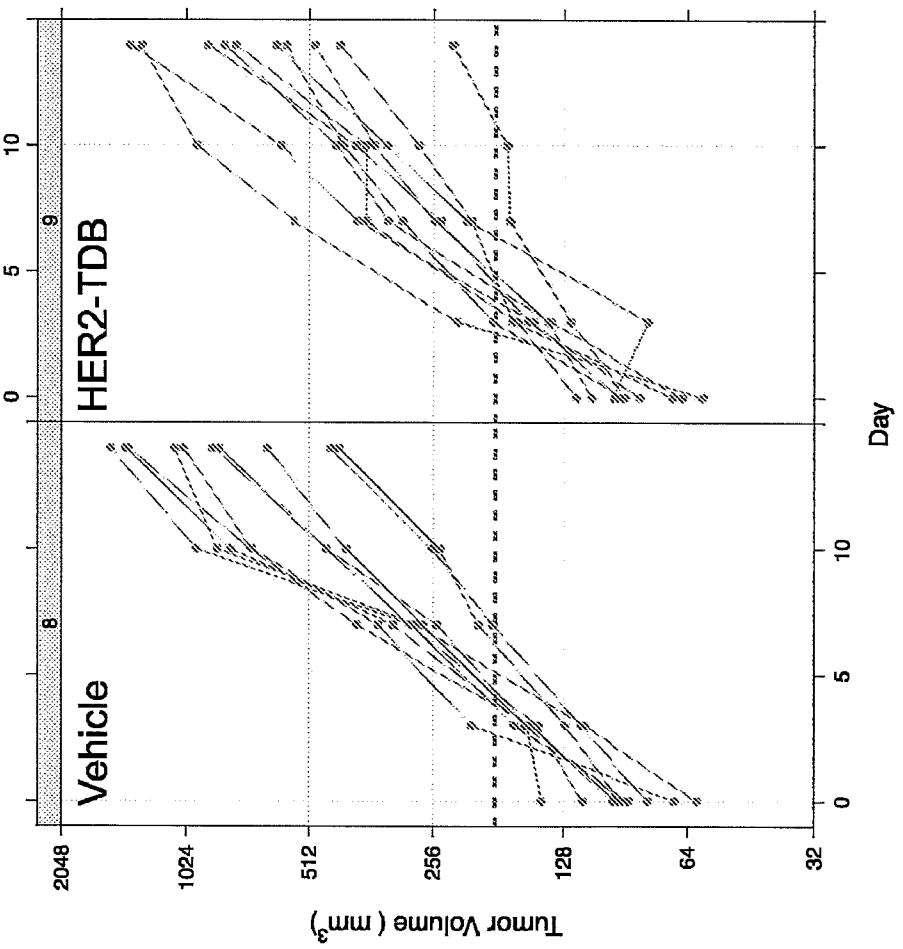

Vehicle
HER2-TDB
Tumor Volume (mm3)
2048
1024
512
256
128
64
32
0
5
10
Day

Figure 104

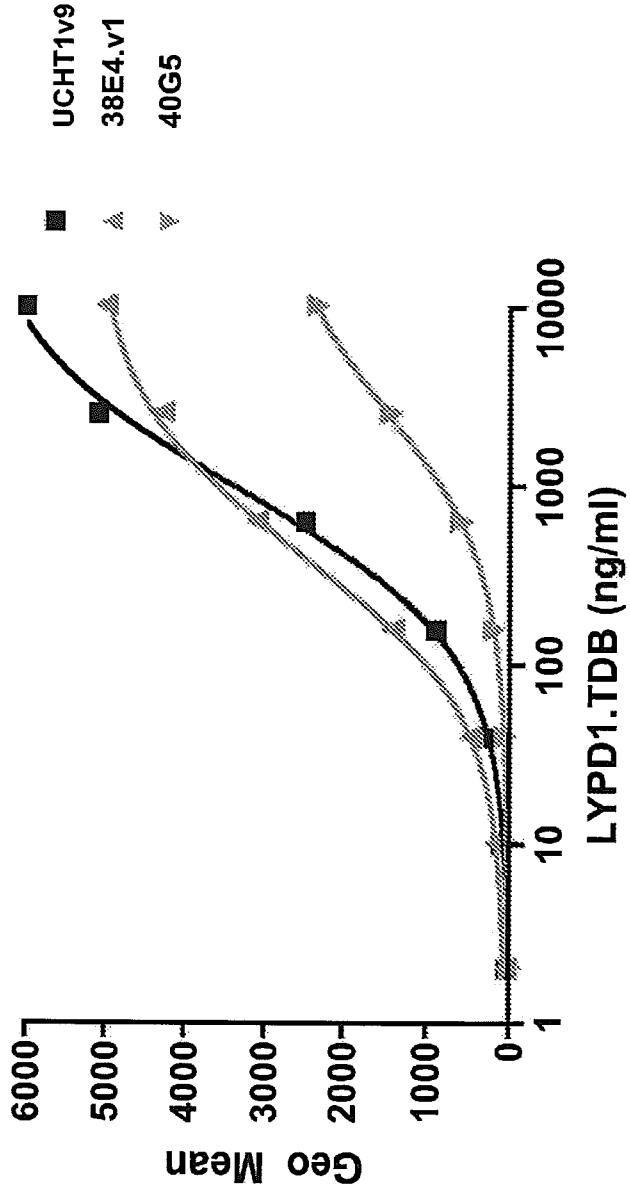
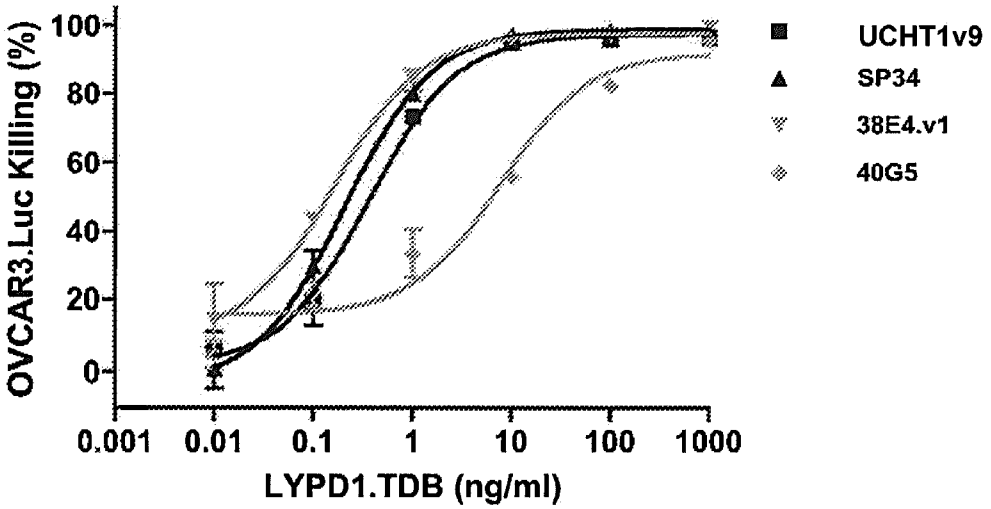

UCHT1v9
38E4.v1
40G5
Geo Mean
6000
5000
4000
3000
2000
1000
0
1
10
100
1000
10000
LYPD1.TDB (ng/ml)

Figure 105



EC50:

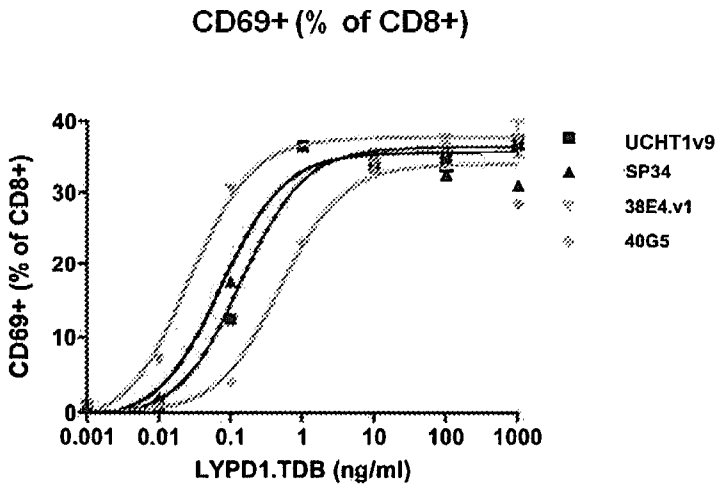
H6:UCHT1.v9: 2.5 pM

H6:SP34: 1.4 pM

H6:38E4 v1: 1.1 pM

H6:40G5: 51.6 pM

Figure 106


CD69+ (% of CD8+)
CD69+ (% of CD8+)
40
30
20
10
0
0.001
0.01
0.1
1
10
100
1000
LYPD1.TDB (ng/ml)
UCHT1v9
SP34
38E4.v1
40G5

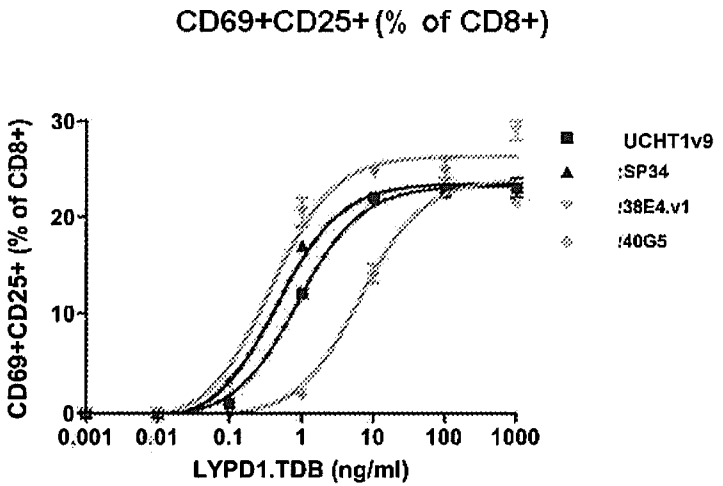

CD69+CD25+ (% of CD8+)
CD69+CD25+ (% of CD8+)
30
20
10
0
0.001
0.01
0.1
1
10
100
1000
LYPD1.TDB (ng/ml)
UCHT1v9
SP34
38E4.v1
40G5

Figure 107

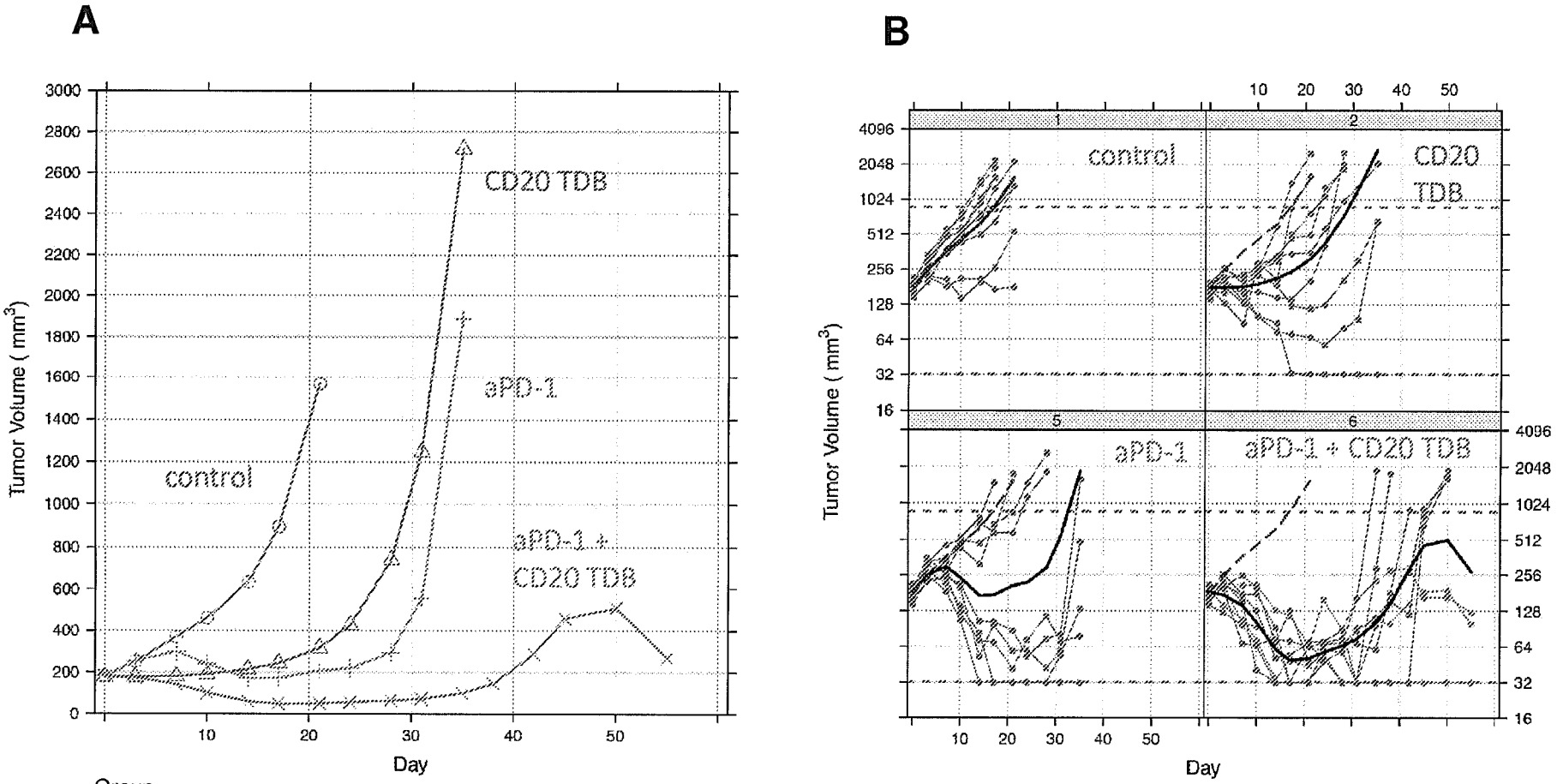
anti-RET41205.v6 Light Chain Variable Region

DIQMTQSPSSLSASVGDRVTITC**RASKSISKYLA**WYQQKPGKANKLLIS**SGSTLQS**GVPSRFSGSGSGTDFTLTISSLQPEDFAT
YYC**QQHNEYPWT**FGQGTKVEIK (SEQ ID NO:620)

anti-RET41205.v6 Heavy Chain Variable Region

EVQLVESGPGLVKPSETLSLTCTVSGYSIT**SDYVWN**WIRQPPGKGLEWIG**YIHYSGGTSYNPSLKS**RVTISRDTSKNQFSLKLS
SVTAADTAVYYCAR**GNYDWAFAY**WGQGTLVTVSS (SEQ ID NO:619)

Figures 108A-108B



Group No.

01 – Vehicle, qwx3, IV

02 – anti-CD20xCD3 TDB (2H7-2C11 murine IgG2), 0.5 mg/kg, qwx3, IV

03 – aPD1 (8F11 DANA mIgG2a), 10 mg/kg, tiwx3, IP

04 – aPD1 (8F11 DANA mIgG2a), 10 mg/kg, tiwx3, IP + anti-CD20xCD3 TDB (2H7-2C11 murine IgG2), 0.5 mg/kg, qwx3, IV