Corresponding amino acid numbering conventions for hinge regions hIgG1, hIgG2 and hIgG4

	Hinge															Hinge encoded by CH2 exon								
hIgG1 wt	E	P	K	S	C	D	K	T	H	T	C	P	P	C	P	A	P	E	L	L	G			
hIgG1 EU	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236			
hIgG1 Kabat	226	227	228	229[a]	230[a]	231[a]	232[a]	233[a]	234[a]	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249
hIgG1 IMGT	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	15	14	13	12	13			
hIgG2 wt	E	R	K	C	C	—	V	--	E	—	C	P	P	C	P	A	P	P	V	A	--			
hIgG2 EU	216	217	218	219[a] 221[b]	220[a] -[b]		222		224		226	227	228	229	230	231[f]	232[b]	233[a]	234[a]	235[b]	236[f]			
hIgG2 Kabat	226	227	228	232	233		235		237		239	240	241	242	243	244	245	246	247	248	249			
hIgG2 IMGT	1	2	3	4	5		6		7		8	9	10	11	12	-	-	-	-	-				
hIgG4 wt	E	S	K	Y	G	--	--		P	P	C	P	S	C	P	A	P	E	F	L	G			
hIgG4 EU	216	217	218	-[a] 219[a]	-[a] 220[a]				224	225	226	227	228	229	230	231[f]	232[b]	233[a]	234[a]	235[b]	236[f]			
hIgG4 Kabat	226	227	228	229	230				237	238	239	240	241	241	243	244	245	246	247	248	249			
hIgG4 IMGT	1	2	3	4	5				6	7	8	9	10	11	12	-	-	-	-	-	-			

wt = wild-type

- means no corresponding number reported

-- means no corresponding amino acid

[a] numbering according to the last updated IMGT Scientific Chart (IMGT®, the international ImMunoGeneTics information system®, http://www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGaber.html, created: 17 May 2001, last updated: 10 Jan 2013)

[b] numbering according to EU index as reported in Kabat, E.A. et al. Sequences of Proteins of Immunological Interest, 5th ed. US Department of Health and Human Services, NIH publication No. 91-3242 (1991)

FIG. 1

Construction of chimeric hinge

	Upper Hinge												Lower Hinge								
IgG1 EU numbering	216	217	218	219	220	221	222	223	224	225	226	227									
IgG1 Kabat numbering	226	227	228	232[a] 229[b]	233[a] 230[b]	234[a] 232[b]	235	236	237	238	239	240									
IgG1	E	P	K	S	C	D	K	T	H	T	C	P	*IgG2*	*P*	*C*	*P*	*A*	*P*	*P*	*V*	*A*
IgG4	E	S	K	Y	G	--	--	--	P	P	C	P									
IgG4 EU numbering	216	217	218	-[a] 219[b]	-[a] 220[b]				224	225	226	227	*IgG2 EU numbering*	*226*	*229*	*230*	*231*	*232*	*233*	*234*	*235*
IgG4 Kabat numbering	226	227	228	229	230				237	238	239	240	*IgG2 Kabat numbering*	*241*	*242*	*243*	*244*	*245*	*246*	*247*	*248*

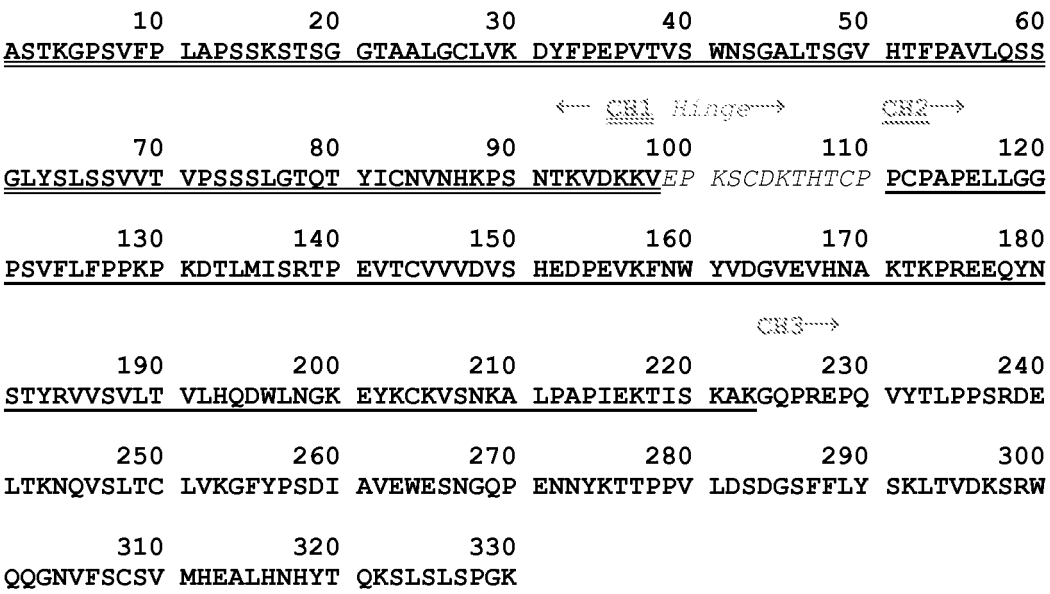
- means no corresponding number reported

-- means no corresponding amino acid

[a] numbering according to the last updated IMGT Scientific Chart (IMGT®, the international ImMunoGeneTics information system®, http://www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html, created: 17 May 2001, last updated:10 Jan 2013)

[b] numbering according to EU index as reported in Kabat, E.A. et al. Sequences of Proteins of Immunological Interest. 5th ed. US Department of Health and Human Services, NIH publication No. 91-3242 (1991)

FIG. 2



Human IGHG1 heavy chain constant region UniProtKB/Swiss-Prot Accn. No. P01857 (SEQ ID NO:13)

FIG. 3

```
        10         20         30         40         50         60
ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS

                                  ←CH1 Hinge→        CH2→
        70         80         90        100        110        120
GLYSLSSVVT VPSSNFGTQT YTCNVDHKPS NTKVDKTVER KCCVECPPCP APPVAGPSVF

       130        140        150        160        170        180
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTFR

                                       CH3→
       190        200        210        220        230        240
VVSVLTVVHQ DWLNGKEYKC KVSNKGLPAP IEKTISKTKG QPREPQVYTL PPSREEMTKN

       250        260        270        280        290        300
QVSLTCLVKG FYPSDISVEW ESNGQPENNY KTTPPMLDSD GSFFLYSKLT VDKSRWQQGN

       310        320
VFSCSVMHEA LHNHYTQKSL SLSPGK
```

Human IGHG2 heavy chain constant region UniProtKB/Swiss-Prot Accn. No. P01859 (SEQ ID NO:14)

FIG. 4

```
        10         20         30         40         50         60
ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS

                                  <-- CH1 Hinge-->       CH2-->
        70         80         90        100        110        120
GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPSCP APEFLGGPSV

       130        140        150        160        170        180
FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY

                                           CH3-->
       190        200        210        220        230        240
RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK

       250        260        270        280        290        300
NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG

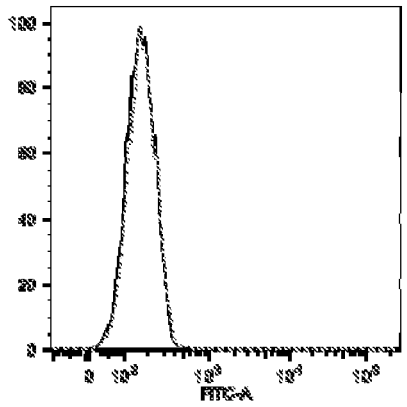
       310        320
NVFSCSVMHE ALHNHYTQKS LSLSLGK
```

Human IGHG4 heavy chain constant region UniProtKB/Swiss-Prot Accn. No. P01861 (SEQ ID NO:15)

FIG. 5

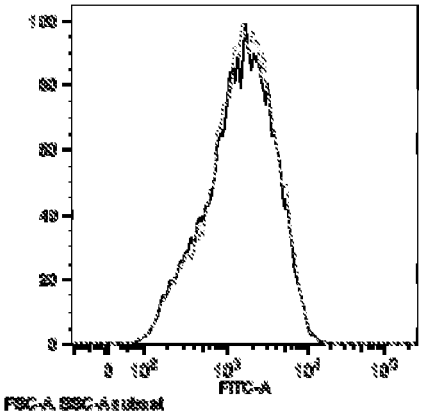
Comparison of Ab Binding on Jurkat Cells

(Chimeric Hinge Abs v. Control Abs)



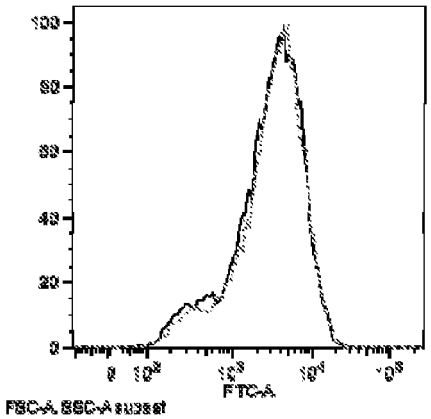
IgG type	Cell name	Geom. Mean:FITC-A
Sec	Jurkats	164
Unstained	Jurkats	155

FIG. 6A



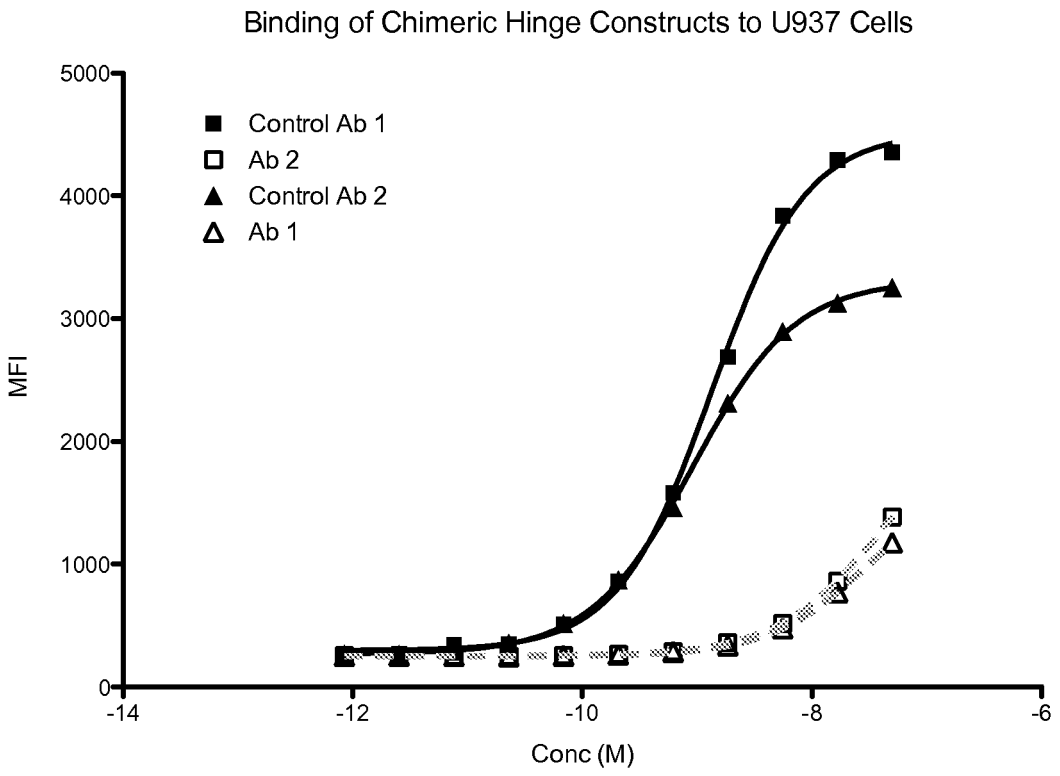
IgG type	Cell name	Geom. Mean:FITC-A
Ab 1	Jurkats	1387
Control Ab 2	Jurkats	1331

FIG. 6B



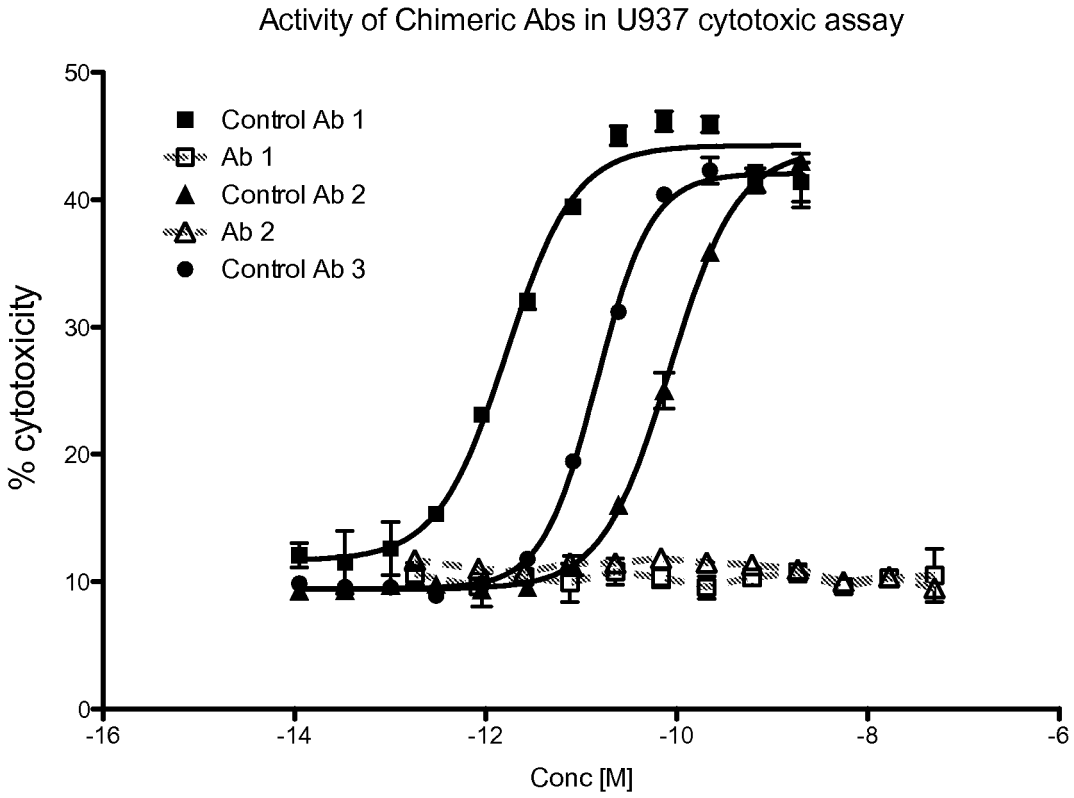
IgG type	Cell name	Geom. Mean:FITC-A
Ab 2	Jurkats	2661
Control Ab 3	Jurkats	2698

FIG. 6C



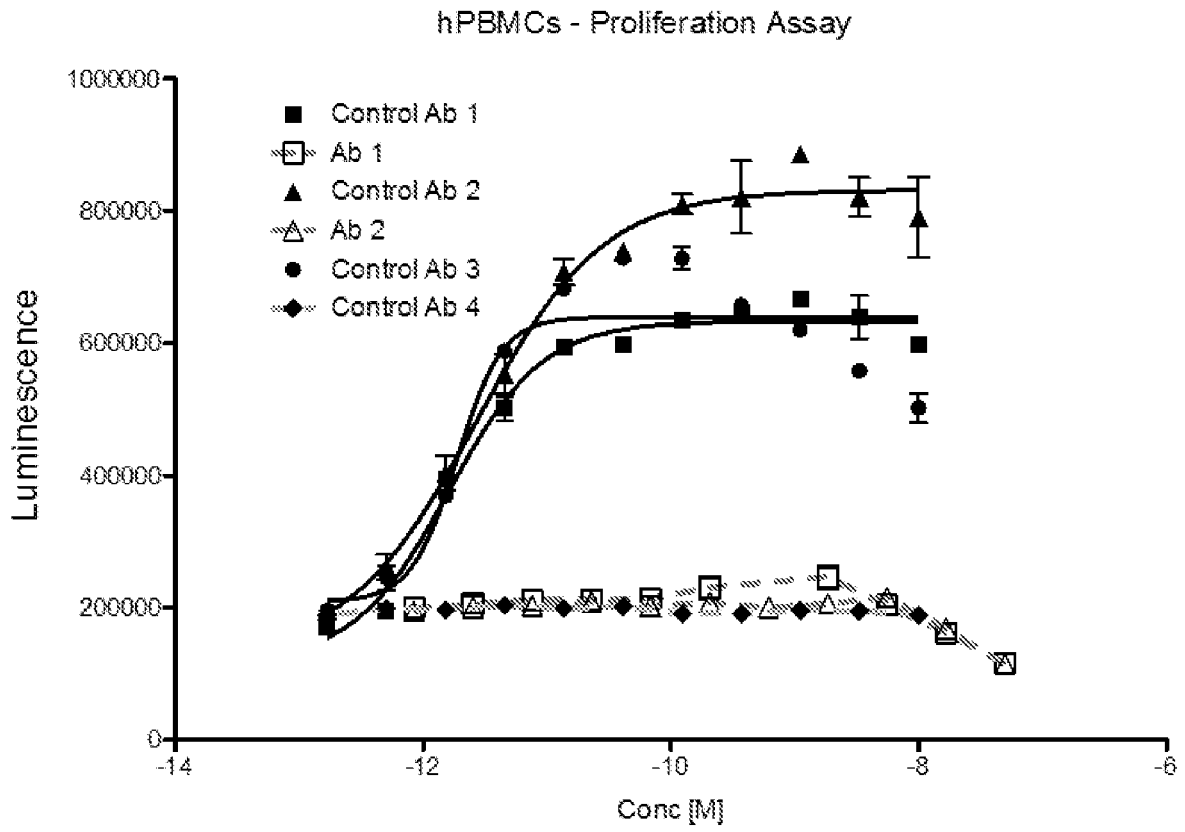
	EC50
Control Ab 1	1.338e-009
Ab 2	4.542e-008
Control Ab 2	9.138e-010
Ab 1	3.351e-008

FIG. 7



	EC50
Control Ab 1	1.656e-012
Control Ab 2	8.448e-011
Control Ab 3	1.465e-011

FIG. 8



	EC50
Control Ab 1	1.613e-012
Control Ab 2	2.691e-012
Control Ab 3	1.881e-012

FIG. 9

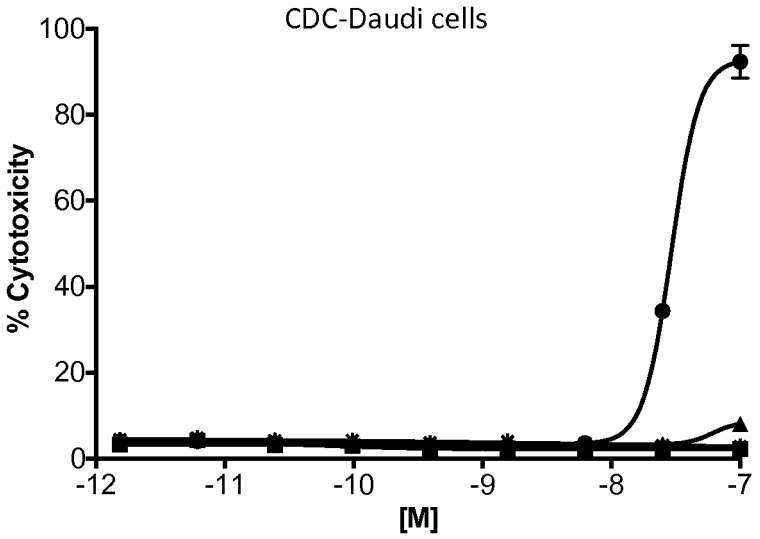


FIG. 10A

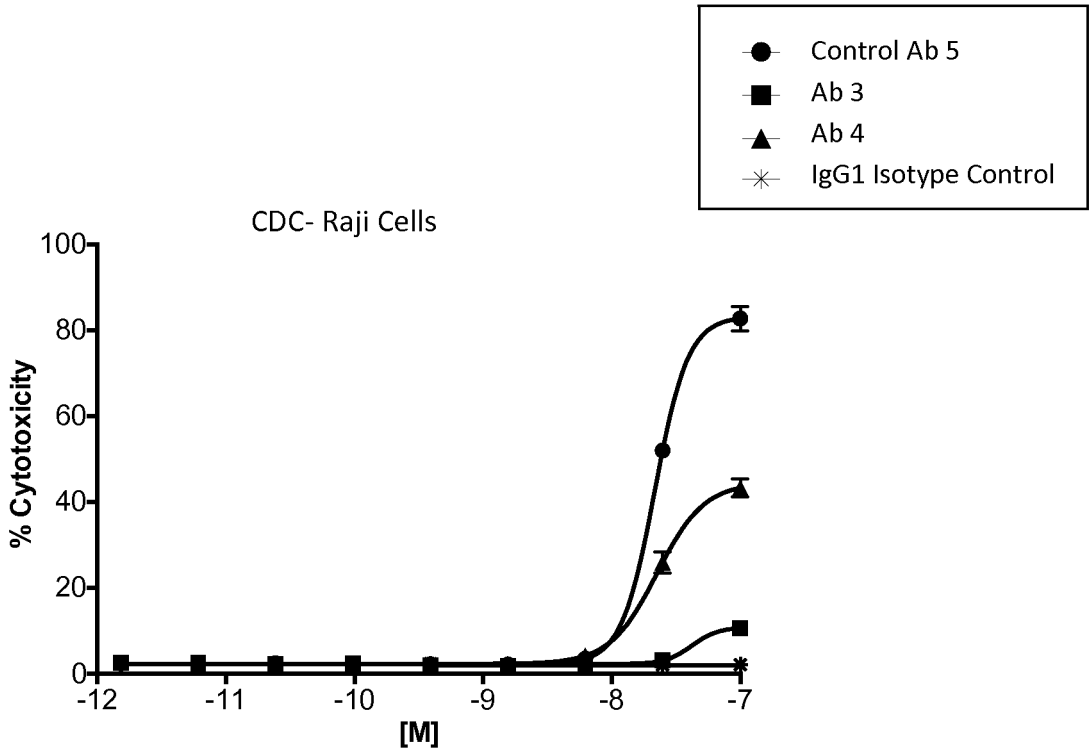


FIG. 10B

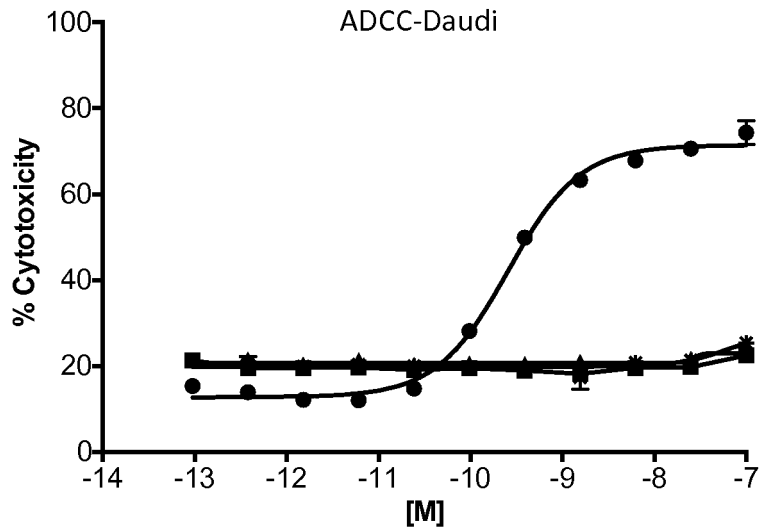
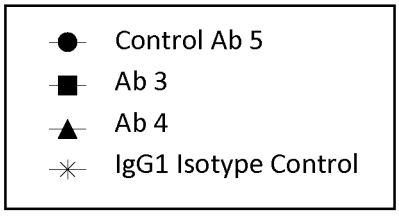


FIG. 11A



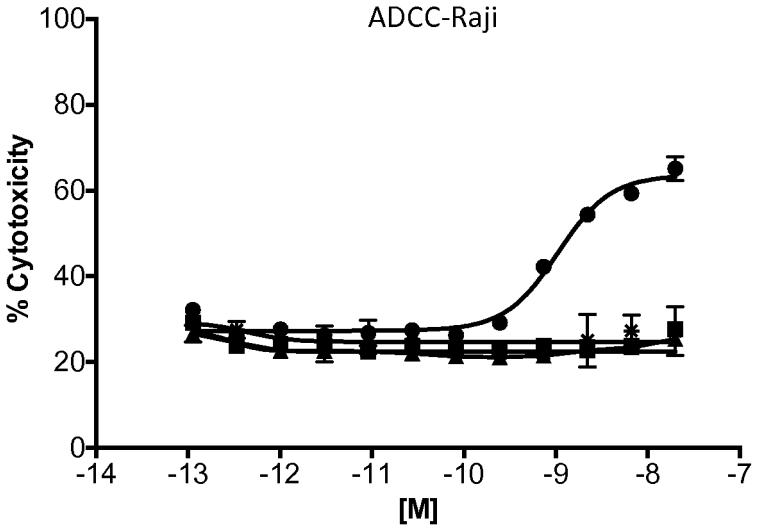


FIG. 11B