**TA domain**

```
  23 gvlkkvir hkrqsgvnat lpeen  45
```

**EGFL domain**

```
 146                           cclqp atgrldtrpf csgrgnfste gcgcvcepgw
 181 kgpncsepec pgnchlrgrc idgqcicddg ftgedcsqla cpsdcndqgk cvngvcicfe
 241 gyagadcsre icpvpcseeh gtcvdglcvc hdgfagddcn kplclnncyn rgrcvenecv
 301 cdegftgedc selicpndcf drgrcingtc yceegftged cgkptcphac htqgrceegq
 361 cvcdegfagl dcsekrcpad chnrgrcvdg rcecddgftg adcgelkcpn gcsghgrcvn
 421 gqcvcdegyt gedcsqlrcp ndchsrgrcv egkcvceqgf kgydcsdmsc pndchqhgrc
 481 vngmcvcddg ytgedcrdrq cprdcsnrgl cvdgqcvced gftgpdcael scpndchgqg
 541 rcvngqcvch egfmgkdcke qrcpsdchgq grcvdgqcic hegftgldcg qhscpsdcnn
 601 lgqcvsgrci cnegysgedc s     621
```

**FNIII domain**

```
 622                        evsppkdlv vtevteetvn lawdnemrvt eylvvytpth
 661 egglemqfrv pgdqtstiiq elepgveyfi rvfailenkk sipvsarvat ylpapeglkf
 721 ksiketsvev ewdpldiafe tweiifrnmn kedegeitks lrrpetsyrq tglapgqeye
 781 islhivknnt rgpglkrvtt trldapsqie vkdvtdttal itwfkplaei dgieltygik
 841 dvpgdrttid ltedenqysi gnlkpdteye vslisrrgdm ssnpaketft tgldaprnlr
 901 rvsqtdnsit lewrngkaai dsyrikyapi sggdhaevdv pksqqattkt tltglrpgte
 961 ygigvsavke dkesnpatin aateldtpkd lqvsetaets ltllwktpla kfdryrlnys
1021 lptgqwvgvq lprnttsyvl rglepgqeyn vlltaekgrh kskparvkas teqapelenl
1081 tvtevgwdgl rlnwtaadqa yehfiiqvqe ankveaarnl tvpgslravd ipglkaatpy
1141 tvsiygviqg yrtpvlsaea stgetpnlge vvvaevgwda lklnwtapeg ayeyffiqvq
1201 eadtveaaqn ltvpgglrst dlpglkaath ytitirgvtq dfsttplsve vlteevpdmg
1261 nltvtevswd alrlnwttpd gtydqftiqv qeadqveeah nltvpgslrs meipglragt
1321 pytvtlhgev rghstrplav evvtedlpql gdlavsevgw dglrlnwtaa dnayehfviq
1381 vqevnkveaa qnltlpgslr avdipgleaa tpyrvsiygv irgyrtpvls aeastakepe
1441 ignlnvsdit pesfnlswma tdgifetfti eiidsnrlle tveynisgae rtahisglpp
1501 stdfivylsg lapsirtkti satattealp llenltisdi npygftvswm asenafdsfl
1561 vtvvdsgkll dpqeftlsgt qrklelrgli tgigyevmvs gftqghqtkp lraeivteae
1621 pevdnllvsd atpdgfrlsw tadegvfdnf vlkirdtkkq sepleitlla pertrdltgl
1681 reateyeiel ygiskgrrsq tvsaiattam gspkevifsd itensatvsw raptaqvesf
1741 rityvpitgg tpsmvtvdgt ktqtrlvkli pgveylvsii amkgfeesep vsgsfttald
1801 gpsglvtani tdsealarwq paiatvdsyv isytgekvpe itrtvsgntv eyaltdlepa
1861 teytlrifae kgpqksstit akfttdldsp rdltatevqs etalltwrpp rasvtgyllv
1921 yesvdgtvke vivgpdttsy sladlspsth ytakiqalng plrsnmiqti ftt  1973
```

**FBG domain**

```
1974                                                      igllypf
1981 pkdcsqamln gdttsglyti ylngdkaqal evfcdmtsdg ggwivflrrk ngrenfyqnw
2041 kayaagfgdr reefwlgldn lnkitaqgqy elrvdlrdhg etafavydkf svgdaktryk
2101 lkvegysgta gdsmayhngr sfstfdkdtd saitncalsy kgafwyrnch rvnlmgrygd
2161 nnhsqgvnwf hwkghehsiq faemklrpsn frnlegrrkr a   2201
```

***Figure 1***

```
   1 attacagagg aaggagctcg ctatataagc cagccaaagt tggctgcacc ggccacagcc
  61 tgcctactgt cacccgcctc tcccgcgcgc agatacacgc ccccgcctcc gtgggcacaa
 121 aggcagcgct gctggggaac tcgggggaac gcgcacgtgg gaaccgccgc agctccacac
 181 tccaggtact tcttccaagg acctaggtct ctcgcccatc ggaaagaaaa taattctttc
 241 aagaagatca gggacaactg atttgaagtc tactctgtgc ttctaaatcc ccaattctgc
 301 tgaaagtgag ataccctaga gccctagagc cccagcagca cccagccaaa cccacctcca
 361 ccatgggggc catgactcag ctgttggcag gtgtctttct tgctttcctt gccctcgcta
 421 ccgaaggtgg ggtcctcaag aaagtcatcc ggcacaagcg acagagtggg gtgaacgcca
 481 ccctgccaga agagaaccag ccagtggtgt ttaaccacgt ttacaacatc aagctgccag
 541 tgggatccca gtgttcggtg gatctggagt cagccagtgg ggagaaagac ctggcaccgc
 601 cttcagagcc cagcgaaagc tttcaggagc acacagtgga tggggaaaac cagattgtct
 661 tcacacatcg catcaacatc ccccgccggg cctgtggctg tgccgcagcc cctgatgtta
 721 aggagctgct gagcagactg gaggagctgg agaacctggt gtcttccctg agggagcaat
 781 gtactgcagg agcaggctgc tgtctccagc ctgccacagg ccgcttggac accaggccct
 841 tctgtagcgg tcggggcaac ttcagcactg aaggatgtgg ctgtgtctgc gaacctggct
 901 ggaaaggccc caactgctct gagcccgaat gtccaggcaa ctgtcacctt cgaggccggt
 961 gcattgatgg gcagtgcatc tgtgacgacg gcttcacggg cgaggactgc agccagctgg
1021 cttgccccag cgactgcaat gaccagggca agtgcgtaaa tggagtctgc atctgtttcg
1081 aaggctacgc cggggctgac tgcagccgtg aaatctgccc agtgccctgc agtgaggagc
1141 acggcacatg tgtagatggc ttgtgtgtgt gccacgatgg ctttgcaggc gatgactgca
1201 acaagcctct gtgtctcaac aattgctaca accgtggacg atgcgtggag aatgagtgcg
1261 tgtgtgatga gggtttcacg ggcgaagact gcagtgagct catctgcccc aatgactgct
1321 tcgaccgggg ccgctgcatc aatggcacct gctactgcga agaaggcttc acaggtgaag
1381 actgcgggaa acccacctgc ccacatgcct gccacaccca gggccggtgt gaggaggggc
1441 agtgtgtatg tgatgagggc tttgccggtg tggactgcag cgagaagagg tgtcctgctg
1501 actgtcacaa tcgtggccgc tgtgtagacg ggcggtgtga gtgtgatgat ggtttcactg
1561 gagctgactg tggggagctc aagtgtccca atggctgcag tggccatggc cgctgtgtca
1621 atgggcagtg tgtgtgtgat gagggctata ctggggagga ctgcagccag ctacggtgcc
1681 ccaatgactg tcacagtcgg ggccgctgtg tcgagggcaa atgtgtatgt gagcaaggct
1741 tcaagggcta tgactgcagt gacatgagct gccctaatga ctgtcaccag cacggccgct
1801 gtgtgaatgg catgtgtgtt tgtgatgacg gctacacagg ggaagactgc cgggatcgcc
1861 aatgccccag ggactgcagc aacaggggcc tctgtgtgga cggacagtgc gtctgtgagg
1921 acggcttcac cggccctgac tgtgcagaac tctcctgtcc aaatgactgc catggccagg
1981 gtcgctgtgt gaatgggcag tgcgtgtgcc atgaaggatt tatgggcaaa gactgcaagg
2041 agcaaagatg tcccagtgac tgtcatggcc agggccgctg cgtggacggc cagtgcatct
2101 gccacgaggg cttcacaggc ctggactgtg gccagcactc ctgccccagt gactgcaaca
2161 acttaggaca atgcgtctcg ggccgctgca tctgcaacga gggctacagc ggagaagact
2221 gctcagaggt gtctcctccc aaagacctcg ttgtgacaga agtgacggaa gagacggtca
2281 acctggcctg ggacaatgag atgcgggtca cagagtacct tgtcgtgtac acgcccaccc
2341 acgagggtgg tctggaaatg cagttccgtg tgcctgggga ccagacgtcc accatcatcc
2401 aggagctgga gcctggtgtg gagtacttta tccgtgtatt tgccatcctg gagaacaaga
2461 agagcattcc tgtcagcgcc agggtggcca cgtacttacc tgcacctgaa ggcctgaaat
2521 tcaagtccat caaggagaca tctgtggaag tggagtggga tcctctagac attgcttttg
2581 aaacctggga gatcatcttc cggaatatga ataaagaaga tgagggagag atcaccaaaa
2641 gcctgaggag gccagagacc tcttaccggc aaactggtct agctcctggg caagagtatg
2701 agatatctct gcacatagtg aaaaacaata cccggggccc tggcctgaag agggtgacca
2761 ccacacgctt ggatgccccc agccagatcg aggtgaaaga tgtcacagac accactgcct
2821 tgatcacctg gttcaagccc ctggctgaga tcgatggcat tgagctgacc tacggcatca
2881 aagacgtgcc aggagaccgt accaccatcg atctcacaga ggacgagaac cagtactcca
2941 tcgggaacct gaagcctgac actgagtacg aggtgtccct catctcccgc agaggtgaca
3001 tgtcaagcaa cccagccaaa gagaccttca caacaggcct cgatgctccc aggaatcttc
```

**Figure 2**

3061 gacgtgtttc ccagacagat aacagcatca ccctggaatg gaggaatggc aaggcagcta
3121 ttgacagtta cagaattaag tatgccccca tctctggagg ggaccacgct gaggttgatg
3181 ttccaaagag ccaacaagcc acaaccaaaa ccacactcac aggtctgagg ccgggaactg
3241 aatatgggat tggagtttct gctgtgaagg aagacaagga gagcaatcca gcgaccatca
3301 acgcagccac agagttggac acgcccaagg accttcaggt ttctgaaact gcagagacca
3361 gcctgaccct gctctggaag acaccgttgg ccaaatttga ccgctaccgc ctcaattaca
3421 gtctccccac aggccagtgg gtgggagtgc agcttccaag aaacaccact tcctatgtcc
3481 tgagaggcct ggaaccagga caggagtaca atgtcctcct gacagccgag aaaggcagac
3541 acaagagcaa gcccgcacgt gtgaaggcat ccactgaaca agcccctgag ctggaaaacc
3601 tcaccgtgac tgaggttggc tgggatggcc tcagactcaa ctggaccgca gctgaccagg
3661 cctatgagca ctttatcatt caggtgcagg aggccaacaa ggtggaggca gctcggaacc
3721 tcaccgtgcc tggcagcctt cgggctgtgg acataccggg cctcaaggct gctacgcctt
3781 atacagtctc catctatggg gtgatccagg gctatagaac accagtgctc tctgctgagg
3841 cctccacagg ggaaactccc aatttgggag aggtcgtggt ggccgaggtg ggctgggatg
3901 ccctcaaact caactggact gctccagaag gggcctatga gtacttttc attcaggtgc
3961 aggaggctga cacagtagag gcagcccaga acctcaccgt cccaggagga ctgaggtcca
4021 cagacctgcc tgggctcaaa gcagccactc attataccat caccatccgc ggggtcactc
4081 aggacttcag cacaacccct ctctctgttg aagtcttgac agaggaggtt ccagatatgg
4141 gaaacctcac agtgaccgag gttagctggg atgctctcag actgaactgg accacgccag
4201 atggaaccta tgaccagttt actattcagg tccaggaggc tgaccaggtg gaagaggctc
4261 acaatctcac ggttcctggc agcctgcgtt ccatggaaat cccaggcctc agggctggca
4321 ctccttacac agtcaccctg cacggcgagg tcaggggcca cagcactcga ccccttgctg
4381 tagaggtcgt cacagaggat ctcccacagc tgggagattt agccgtgtct gaggttggct
4441 gggatggcct cagactcaac tggaccgcag ctgacaatgc ctatgagcac tttgtcattc
4501 aggtgcagga ggtcaacaaa gtggaggcag cccagaacct cacgttgcct ggcagcctca
4561 gggctgtgga catcccgggc ctcgaggctg ccacgcctta tagagtctcc atctatgggg
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4681 aaattggaaa cttaaatgtt tctgacataa ctcccgagag cttcaatctc tcctggatgg
4741 ctaccgatgg gatcttcgag acctttacca ttgaaattat tgattccaat aggttgctgg
4801 agactgtgga atataatatc tctggtgctg aacgaactgc ccatatctca gggctacccc
4861 ctagtactga ttttattgtc tacctctctg gacttgctcc cagcatccgg accaaaacca
4921 tcagtgccac agccacgaca gaggccctgc cccttctgga aaacctaacc atttccgaca
4981 ttaatcccta cgggttcaca gtttcctgga tggcatcgga gaatgccttt gacagctttc
5041 tagtaacggt ggtggattct gggaagctgc tggaccccca ggaattcaca ctttcaggaa
5101 cccagaggaa gctggagctt agaggcctca taactggcat tggctatgag gttatggtct
5161 ctggcttcac ccaagggcat caaaccaagc ccttgagggc tgagattgtt acagaagccg
5221 aaccggaagt tgacaacctt ctggtttcag atgccacccc agacggtttc cgtctgtcct
5281 ggacagctga tgaaggggtc ttcgacaatt ttgttctcaa aatcagagat accaaaaagc
5341 agtctgagcc actggaaata accctacttg cccccgaacg taccagggac ataacaggtc
5401 tcagagaggc tactgaatac gaaattgaac tctatggaat aagcaaagga aggcgatccc
5461 agacagtcag tgctatagca acaacagcca tgggctcccc aaaggaagtc attttctcag
5521 acatcactga aaattcggct actgtcagct ggagggcacc cacagcccaa gtggagagct
5581 tccggattac ctatgtgccc attacaggag gtacaccctc catggtaact gtggacggaa
5641 ccaagactca gaccaggctg gtgaaactca tacctggcgt ggagtacctt gtcagcatca
5701 tcgccatgaa gggctttgag gaaagtgaac ctgtctcagg gtcattcacc acagctctgg
5761 atggcccatc tggcctggtg acagccaaca tcactgactc agaagccttg gccaggtggc
5821 agccagccat tgccactgtg gacagttatg tcatctccta cacaggcgag aaagtgccag
5881 aaattacacg cacggtgtcc gggaacacag tggagtatgc tctgaccgac ctcgagcctg
5941 ccacggaata cacactgaga atctttgcag agaaagggcc ccagaagagc tcaaccatca

## *Figure 2 (con't)*

6001 ctgccaagtt cacaacagac ctcgattctc caagagactt gactgctact gaggttcagt
6061 cggaaactgc cctccttacc tggcgacccc cccgggcatc agtcaccggt tacctgctgg
6121 tctatgaatc agtggatggc acagtcaagg aagtcattgt gggtccagat accacctcct
6181 acagcctggc agacctgagc ccatccaccc actacacagc caagatccag gcactcaatg
6241 ggcccctgag gagcaatatg atccagacca tcttcaccac aattggactc ctgtacccct
6301 tccccaagga ctgctcccaa gcaatgctga atggagacac gacctctggc ctctacacca
6361 tttatctgaa tggtgataag gctgaggcgc tggaagtctt ctgtgacatg acctctgatg
6421 ggggtggatg gattgtgttc ctgagacgca aaaacggacg cgagaacttc taccaaaact
6481 ggaaggcata tgctgctgga tttggggacc gcagagaaga attctggctt gggctggaca
6541 acctgaacaa aatcacagcc caggggcagt acgagctccg ggtggacctg cgggaccatg
6601 gggagacagc ctttgctgtc tatgacaagt tcagcgtggg agatgccaag actcgctaca
6661 agctgaaggt ggaggggtac agtgggacag caggtgactc catggcctac cacaatggca
6721 gatccttctc cacctttgac aaggacacag attcagccat caccaactgt gctctgtcct
6781 acaaaggggc tttctggtac aggaactgtc accgtgtcaa cctgatgggg agatatgggg
6841 acaataacca cagtcagggc gttaactggt tccactggaa gggccacgaa cactcaatcc
6901 agtttgctga gatgaagctg agaccaagca acttcagaaa tcttgaaggc aggcgcaaac
6961 gggcataaat tccagggacc actgggtgag agaggaataa ggcccagagc gaggaaagga
7021 ttttaccaaa gcatcaatac aaccagccca accatcggtc cacacctggg catttggtga
7081 gagtcaaagc tgaccatgga tccctggggc caacggcaac agcatgggcc tcacctcctc
7141 tgtgatttct ttctttgcac caaagacatc agtctccaac atgtttctgt tttgttgttt
7201 gattcagcaa aaatctccca gtgacaacat cgcaatagtt ttttacttct cttaggtggc
7261 tctgggaatg ggagaggggt aggatgtaca ggggtagttt gttttagaac cagccgtatt
7321 ttacatgaag ctgtataatt aattgtcatt atttttgtta gcaaagatta aatgtgtcat
7381 tggaagccat ccctttttt acatttcata caacagaaac cagaaaagca atactgtttc
7441 cattttaagg atatgattaa tattattaat ataataatga tgatgatgat gatgaaaact
7501 aaggattttt caagagatct ttctttccaa aacatttctg gacagtacct gattgtattt
7561 ttttttaaa taaaagcaca agtacttttg agtttgttaa aaaaaaaaaa aaaaaa

**Figure 2 (con’t)**

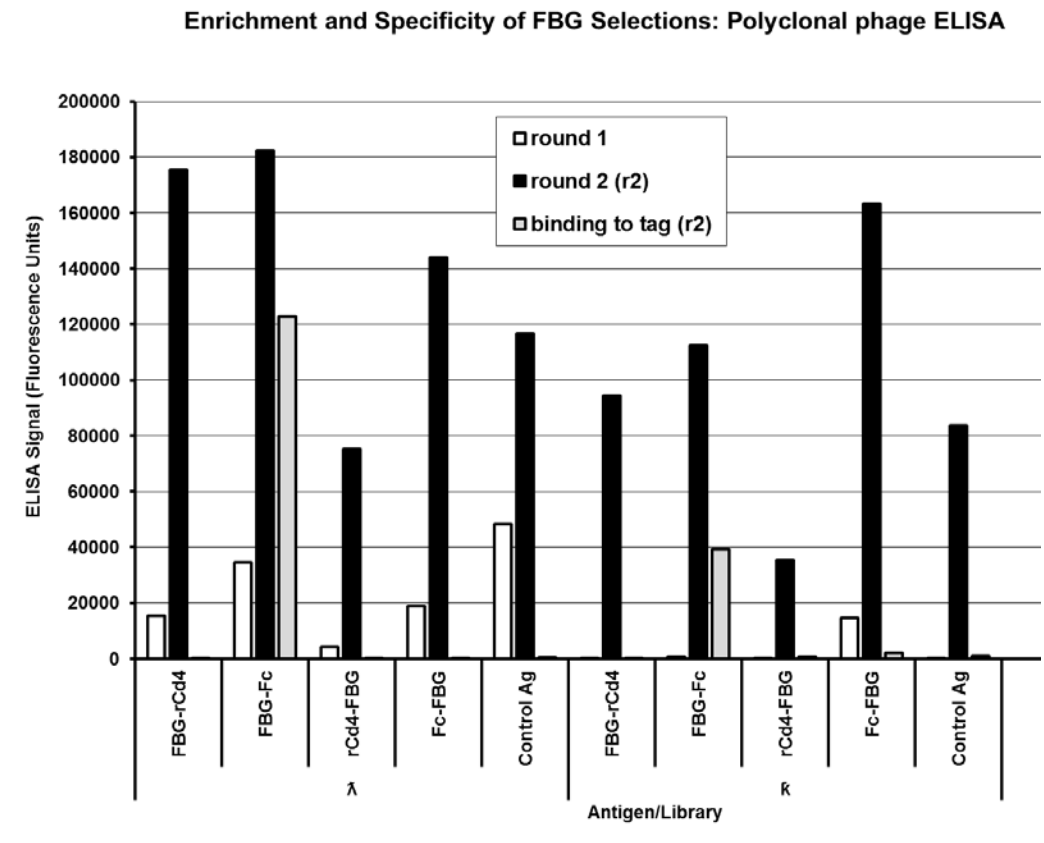

Enrichment and Specificity of FBG Selections: Polyclonal phage ELISA
round 1
round 2 (r2)
binding to tag (r2)
ELISA Signal (Fluorescence Units)
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
FBG-rCd4
FBG-Fc
rCd4-FBG
Fc-FBG
Control Ag
FBG-rCd4
FBG-Fc
rCd4-FBG
Fc-FBG
Control Ag
λ
κ
Antigen/Library

Figure 3

a

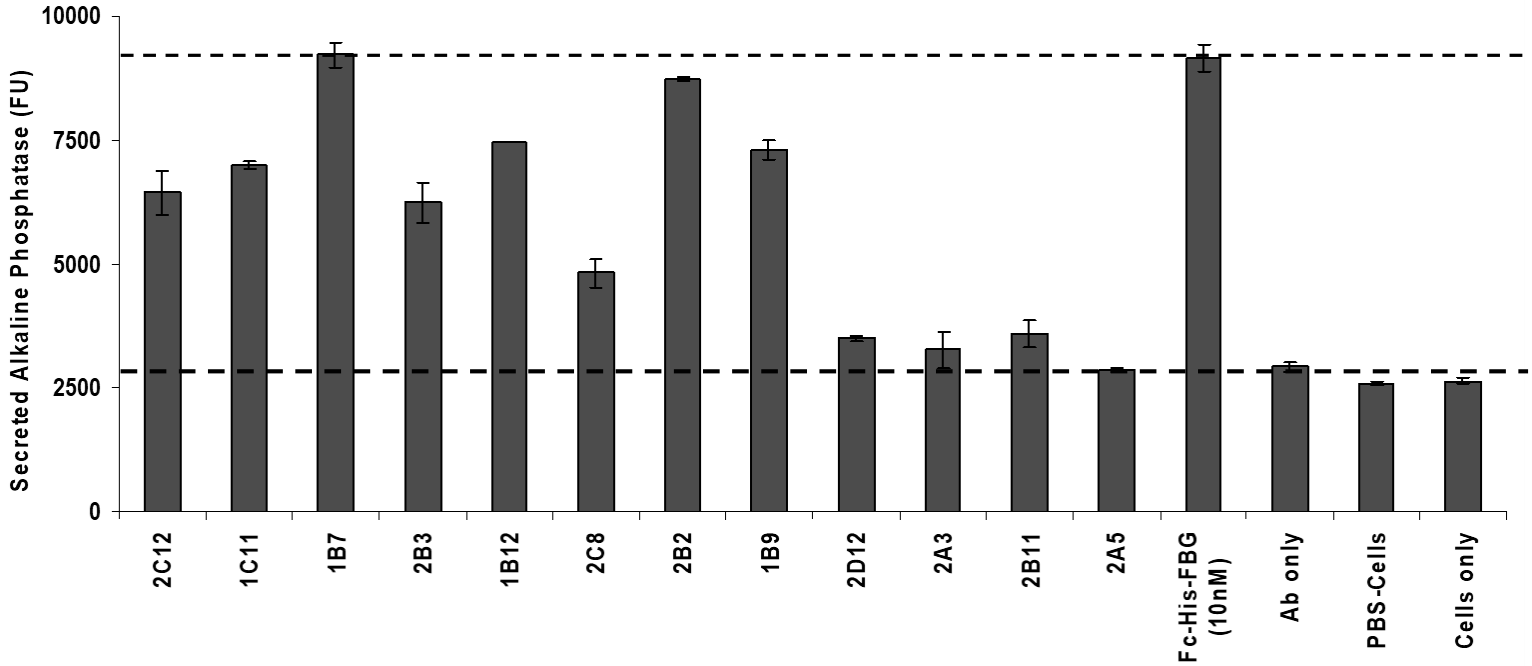
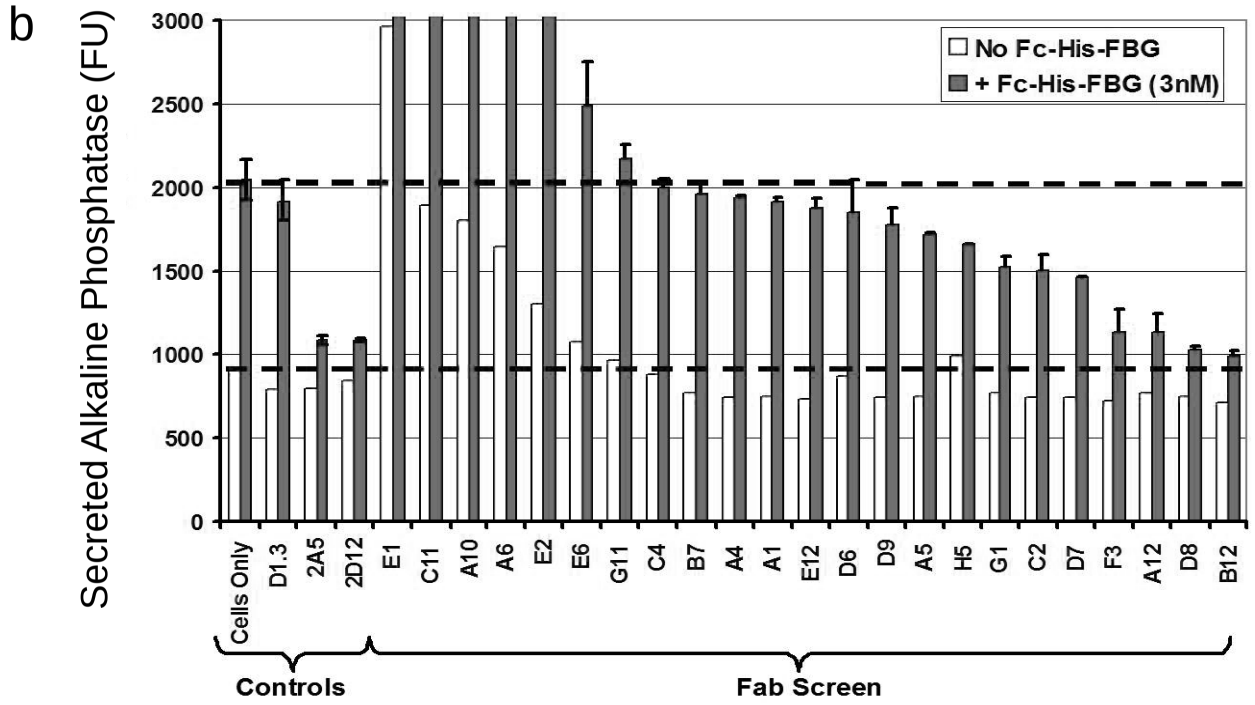

Secreted Alkaline Phosphatase (FU)
10000
7500
5000
2500
0
2C12
1C11
1B7
2B3
1B12
2C8
2B2
1B9
2D12
2A3
2B11
2A5
Fc-His-FBG (10nM)
Ab only
PBS-Cells
Cells only

Figure 4




All Fabs screened at 200 nM except B12 which was at 94 nM

***Figure 4 (con't)***

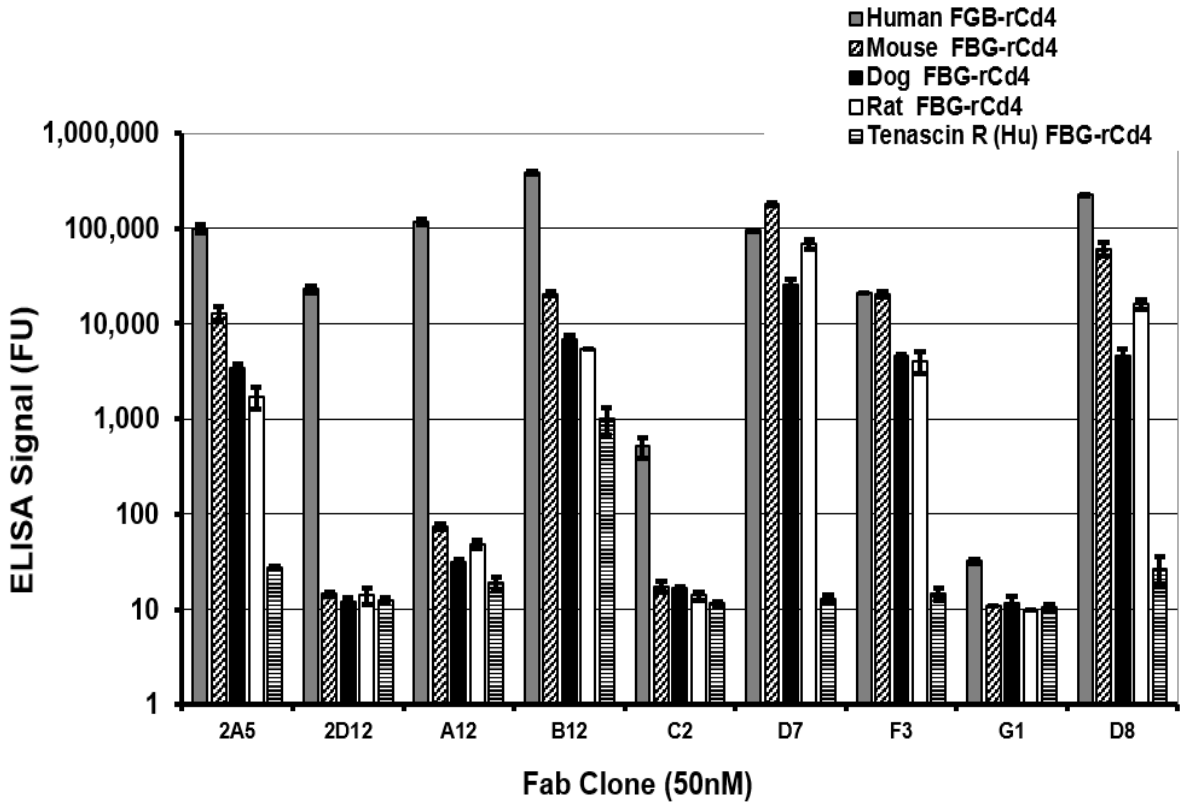
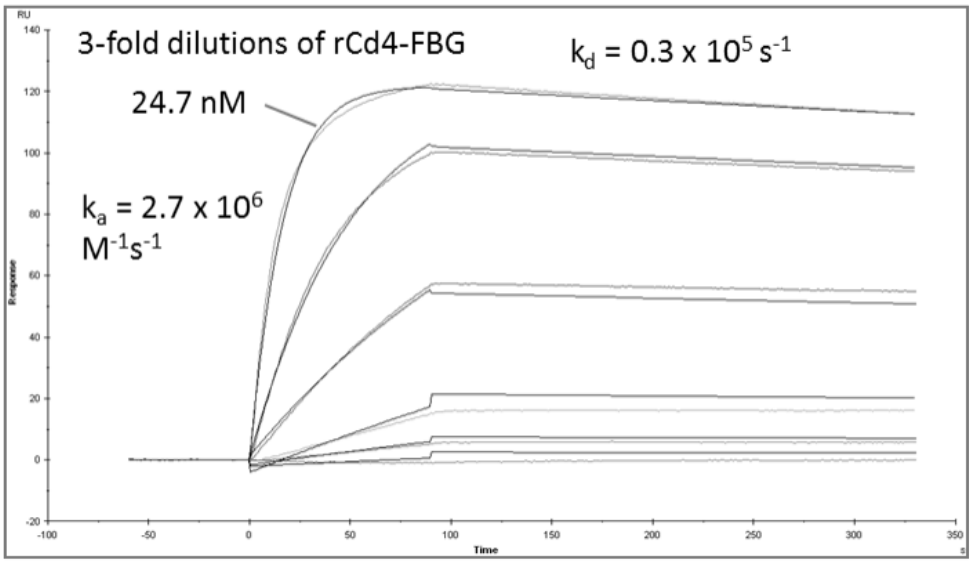

Human FGB-rCd4
Mouse FBG-rCd4
Dog FBG-rCd4
Rat FBG-rCd4
Tenascin R (Hu) FBG-rCd4
1,000,000
100,000
10,000
1,000
100
10
1
ELISA Signal (FU)
2A5
2D12
A12
B12
C2
D7
F3
G1
D8
Fab Clone (50nM)

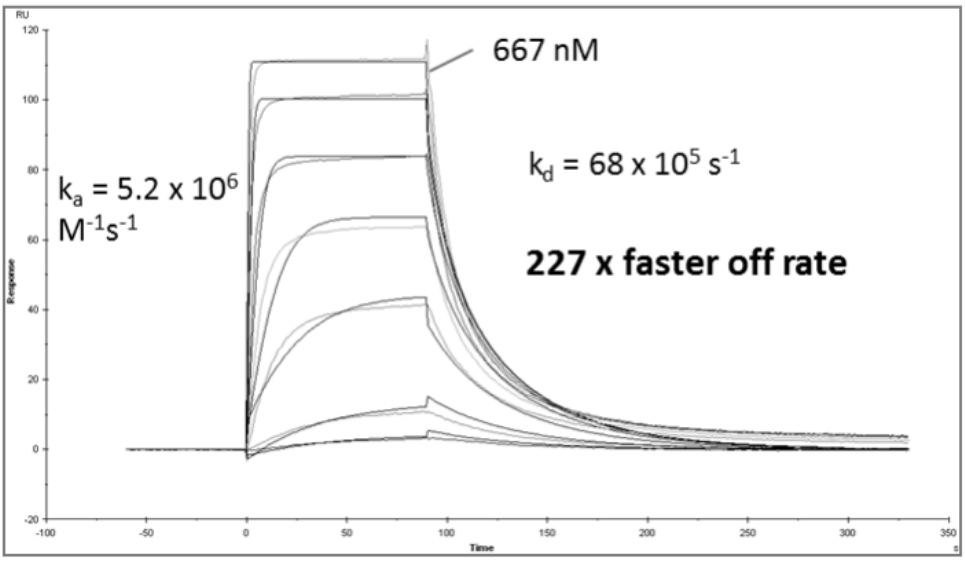
*Figure 5*

a **Fab B12 – SPR Results**

Human TNC FBG – $K_D$ = 0.11 nM




Mouse TNC FBG – $K_D$ = 13 nM

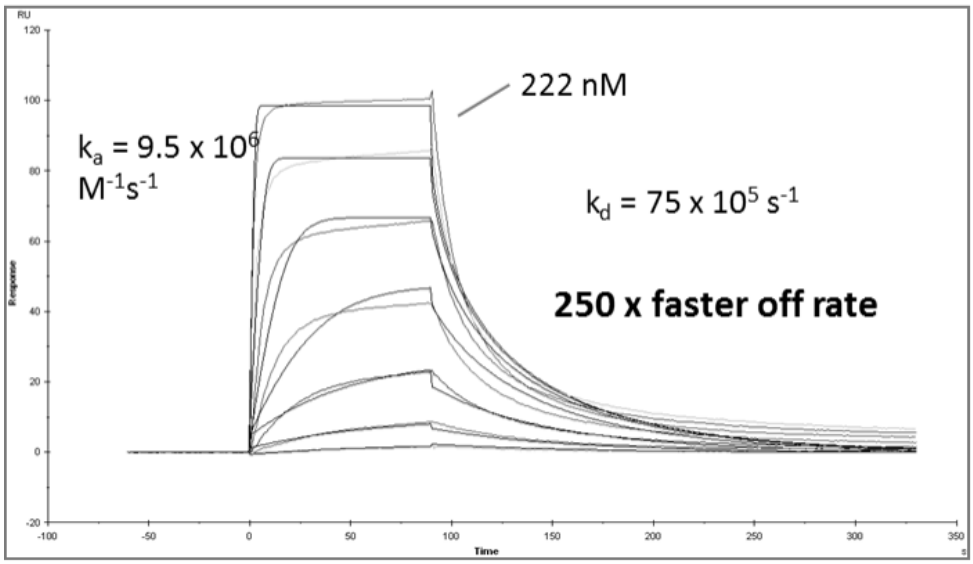



**Assay format = Fab capture (23 nM) on anti-$C_L$κ / anti-$C_L$λ CM5 sensor chip followed by rCd4-FBG injection. Regeneration with 10 mM Glycine, pH2**

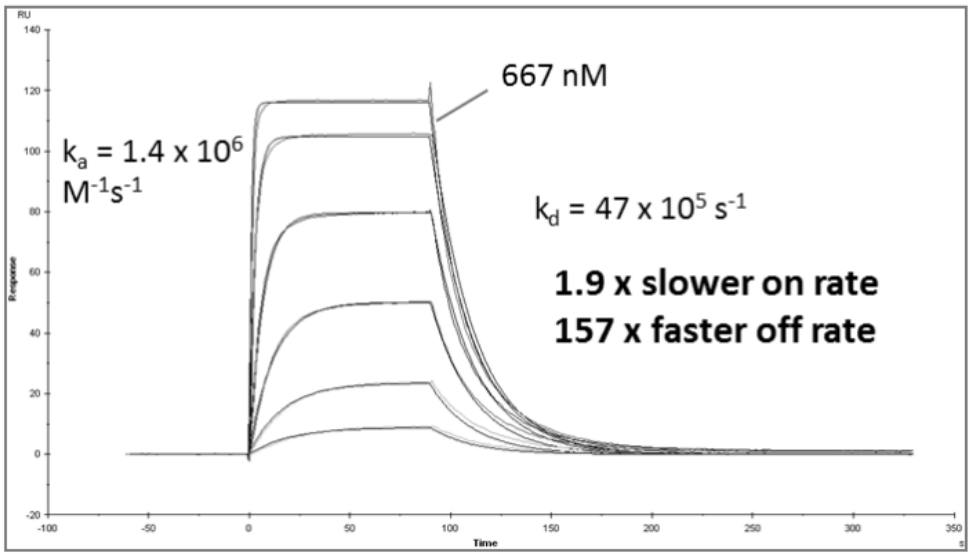
***Figure 6a***

a **Fab B12 – SPR Results**

Rat TNR FBG – $K_D$ = 7.9 nM




Human TNR FBG – $K_D$ = 33.9 nM



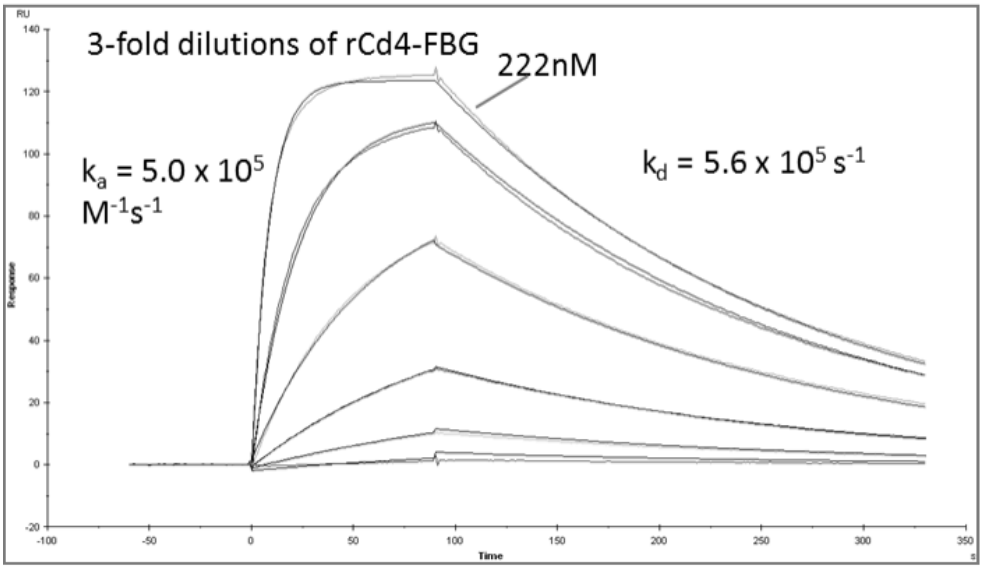

**Assay format = Fab capture (23 nM) on anti-$C_L$κ / anti-$C_L$λ CM5 sensor chip followed by rCd4-FBG injection. Regeneration with 10 mM Glycine, pH2**

***Figure 6a (Con't)***

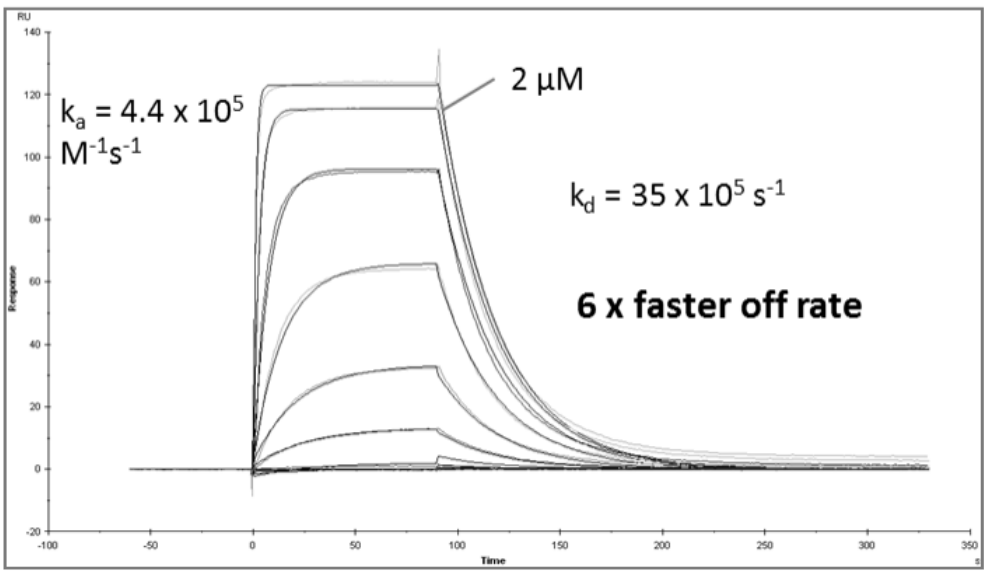
b

## Fab 2A5 – SPR Results

Human TNC FBG – $K_D$ = 11.4 nM




Mouse TNC FBG – $K_D$ = 78.6 nM

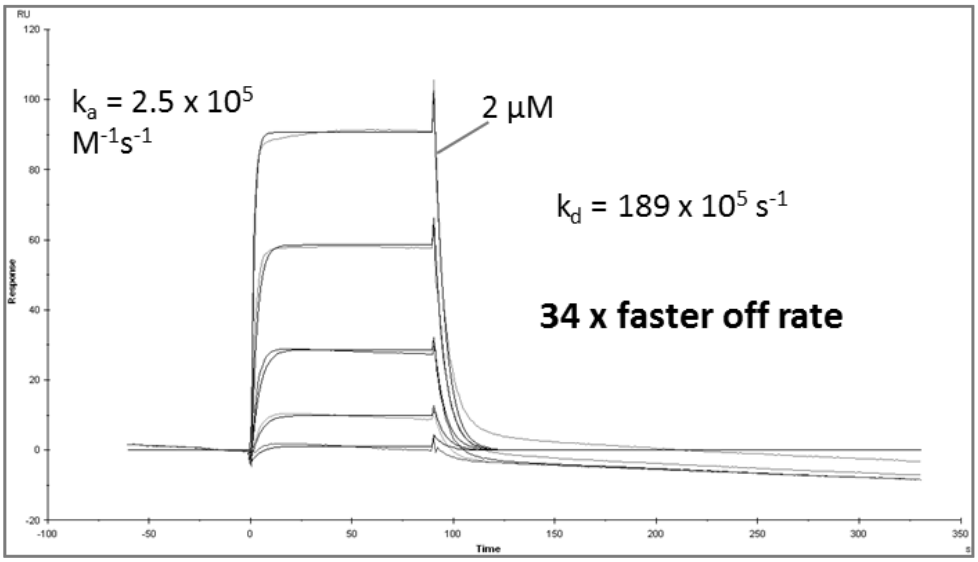



**Assay format = Fab capture (23 nM) on anti-$C_L$κ / anti-$C_L$λ CM5 sensor chip followed by rCd4-FBG injection. Regeneration with 10 mM Glycine, pH2**

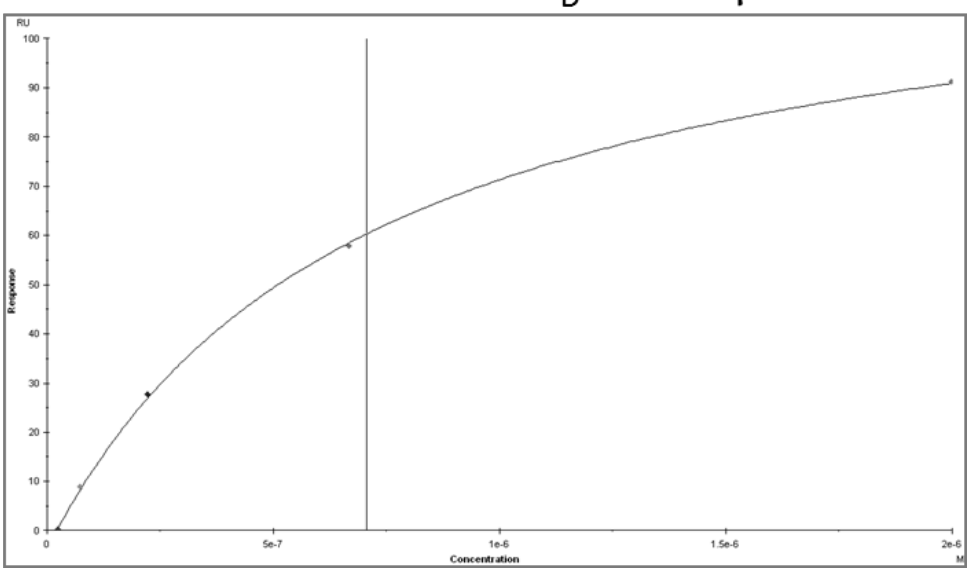
***Figure 6b***

b **Fab 2A5 – SPR Results**

Human TNR FBG – $K_D$ = 0.76 µM




Human TNR FBG – $K_D$ = 0.71 µM



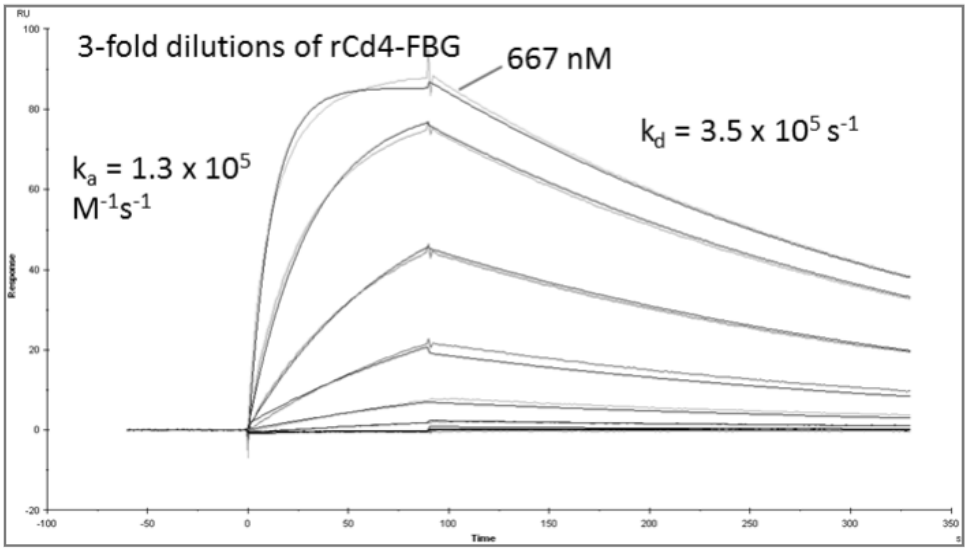

**Assay format = Fab capture (23 nM) on anti-$C_L$κ / anti-$C_L$λ CM5 sensor chip followed by rCd4-FBG injection. Regeneration with 10 mM Glycine, pH2**

***Figure 6b (Con't)***

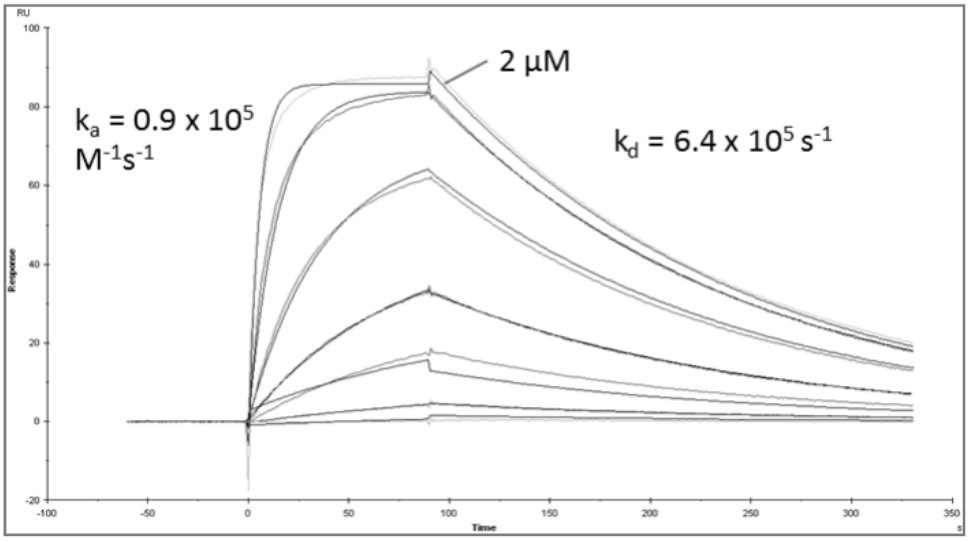
c

## Fab F3 – SPR Results

Human TNC FBG – $K_D$ = 27.4 nM




Mouse TNC FBG – $K_D$ = 70.6 nM

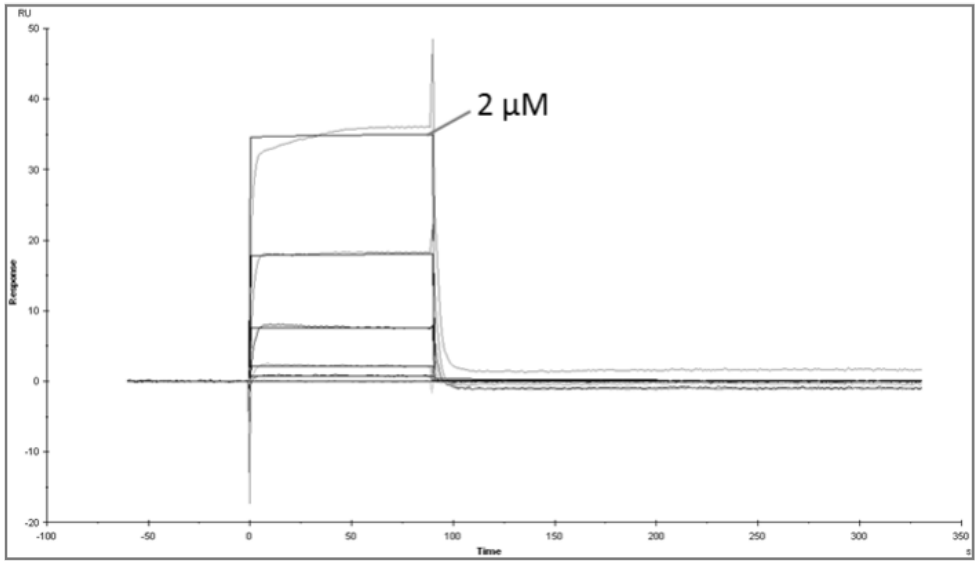



**Assay format = Fab capture (23 nM) on anti-$C_L$κ / anti-$C_L$λ CM5 sensor chip followed by rCd4-FBG injection. Regeneration with 10 mM Glycine, pH2**

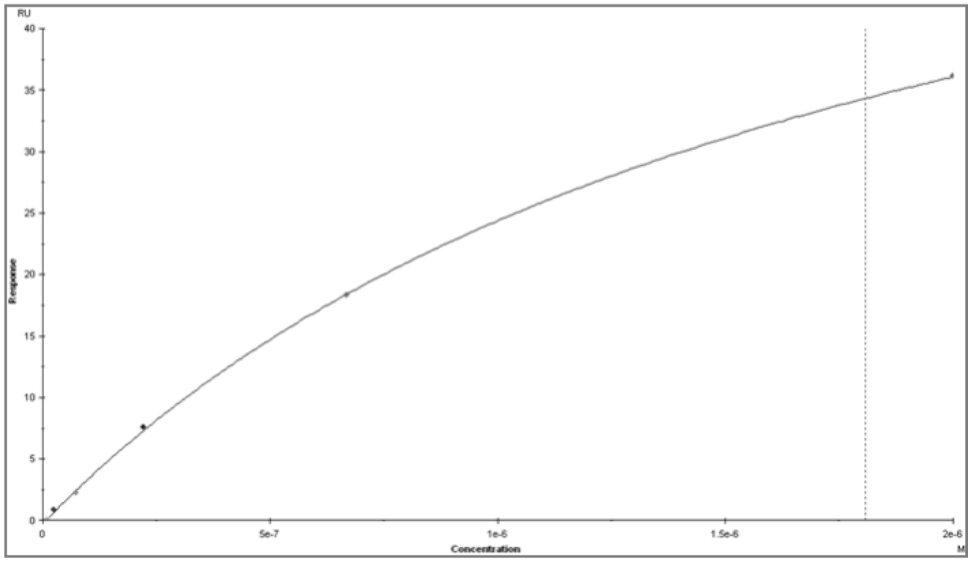
***Figure 6c***

c **Fab F3 – SPR Results**

Human TNR FBG – off rate too rapid




Human TNR FBG – $K_D$ = 1.8 µM

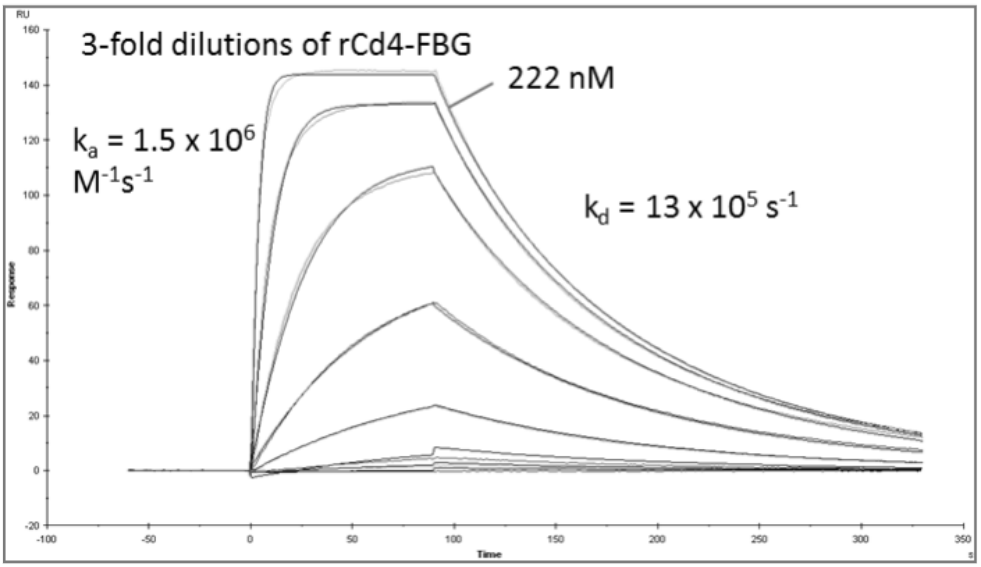



**Assay format = Fab capture (23 nM) on anti-$C_L$κ / anti-$C_L$λ CM5 sensor chip followed by rCd4-FBG injection. Regeneration with 10 mM Glycine, pH2**

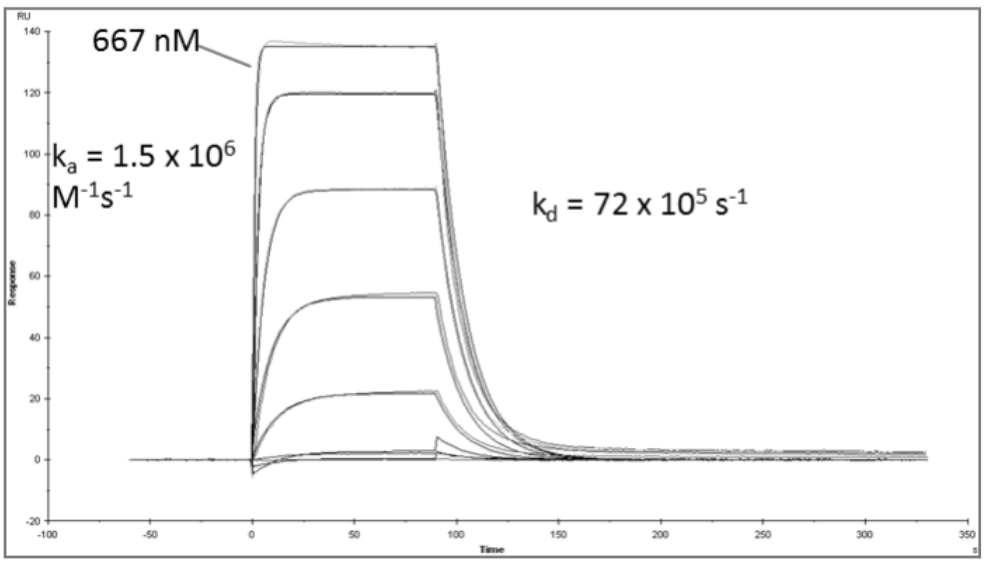
***Figure 6c (Con't)***

d **Fab D8 – SPR Results**

Human TNC FBG – $K_D$ = 8.5 nM




Mouse TNC FBG – $K_D$ = 48.4 nM

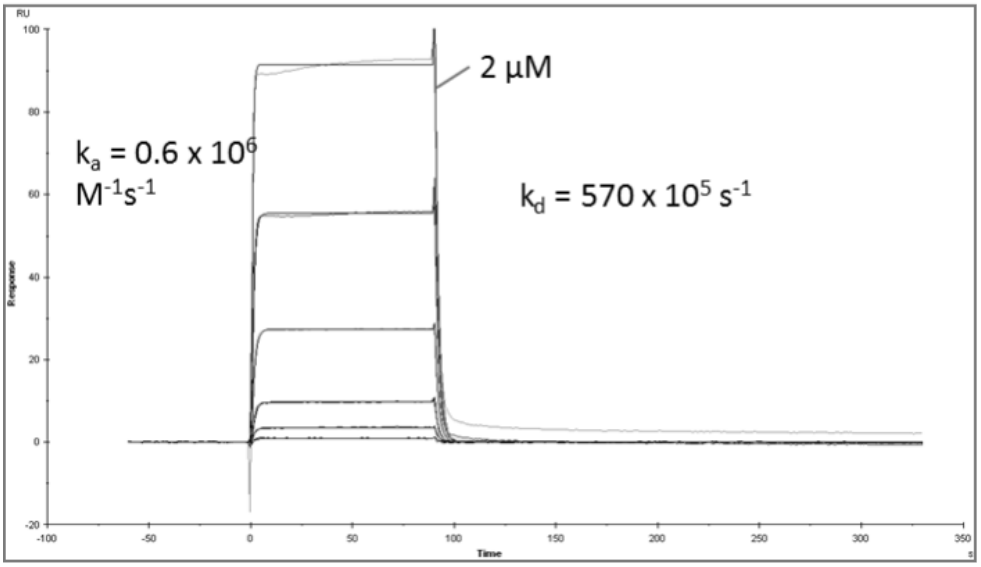



**Assay format = Fab capture (23 nM) on anti-$C_L$κ / anti-$C_L$λ CM5 sensor chip followed by rCd4-FBG injection. Regeneration with 10 mM Glycine, pH2**

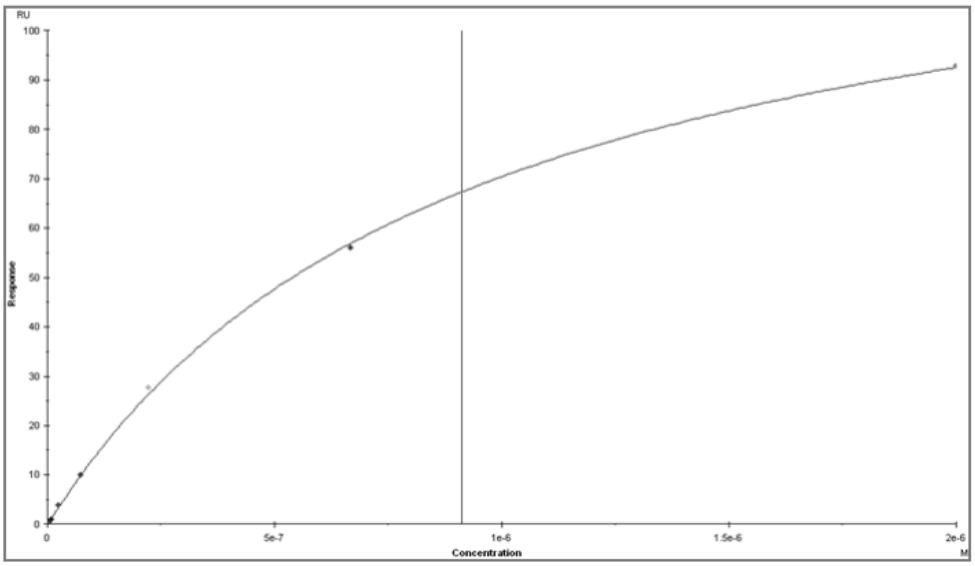
***Figure 6d***

d **Fab D8 – SPR Results**

Human TNR FBG – $K_D$ = 1.03 µM

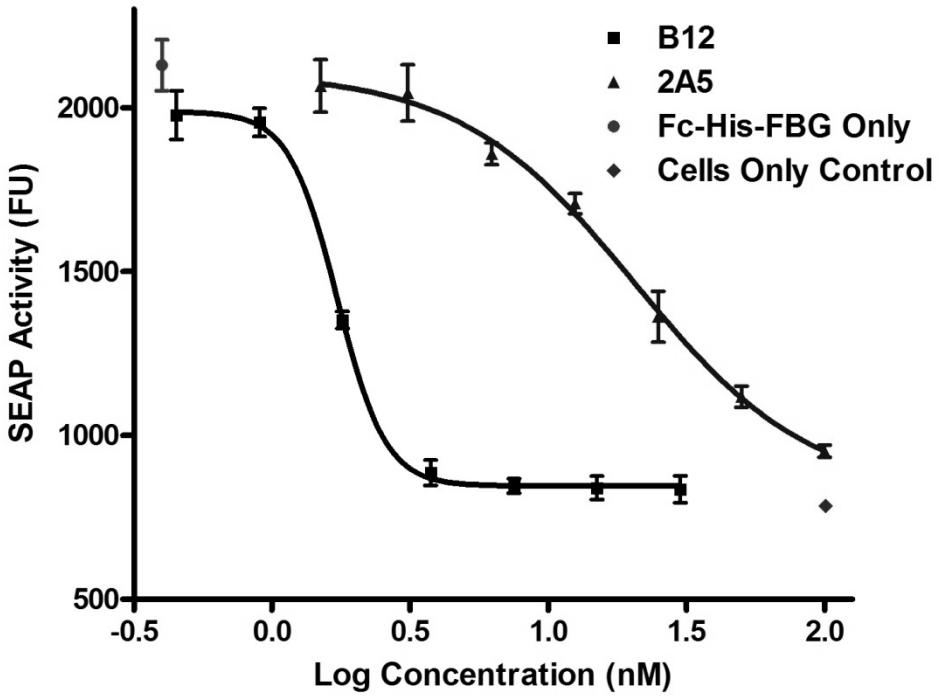



Human TNR FBG – $K_D$ = 1.11 µM




**Assay format = Fab capture (23 nM) on anti-$C_L$κ / anti-$C_L$λ CM5 sensor chip followed by rCd4-FBG injection. Regeneration with 10 mM Glycine, pH2**

***Figure 6d (Con't)***


B12
2A5
Fc-His-FBG Only
Cells Only Control
SEAP Activity (FU)
2000
1500
1000
500
-0.5
0.0
0.5
1.0
1.5
2.0
Log Concentration (nM)

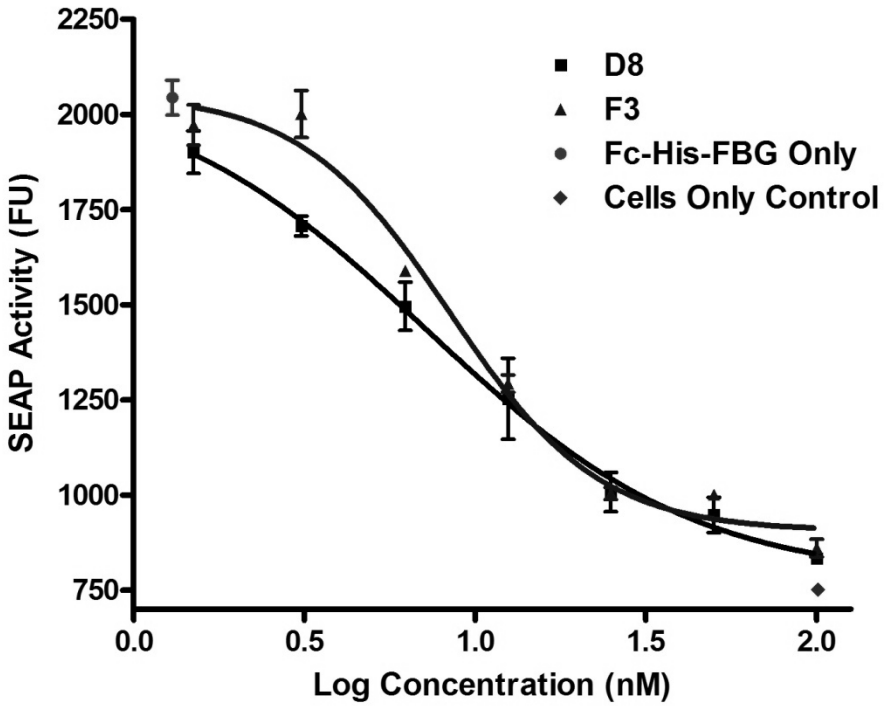
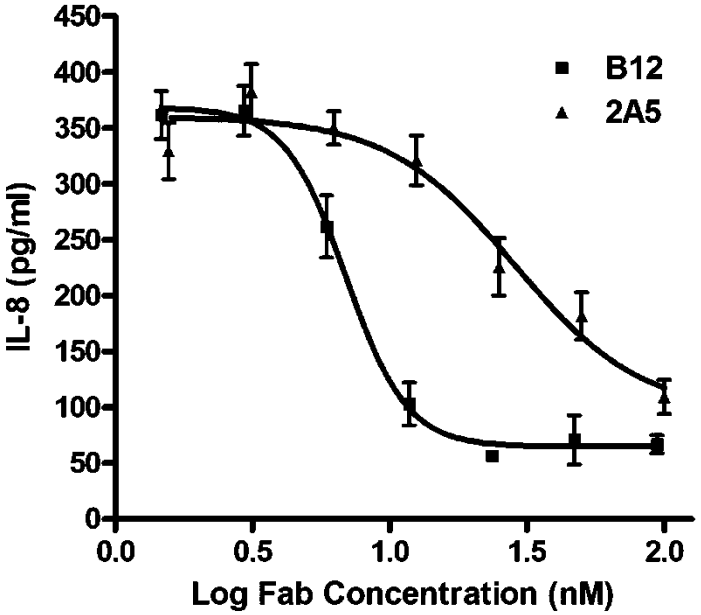

D8
F3
Fc-His-FBG Only
Cells Only Control
SEAP Activity (FU)
2250
2000
1750
1500
1250
1000
750
0.0
0.5
1.0
1.5
2.0
Log Concentration (nM)

*Figure 7*


450
400
350
300
250
200
150
100
50
0
IL-8 (pg/ml)
B12
2A5
0.0
0.5
1.0
1.5
2.0
Log Fab Concentration (nM)

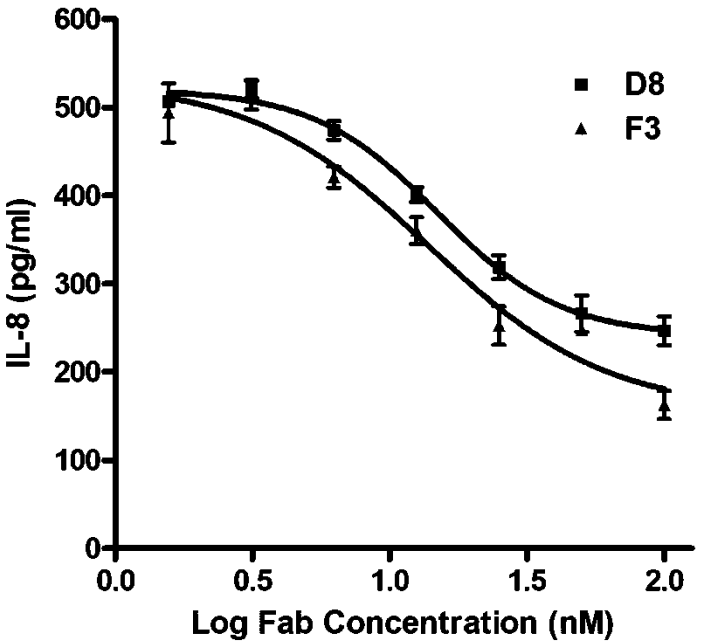
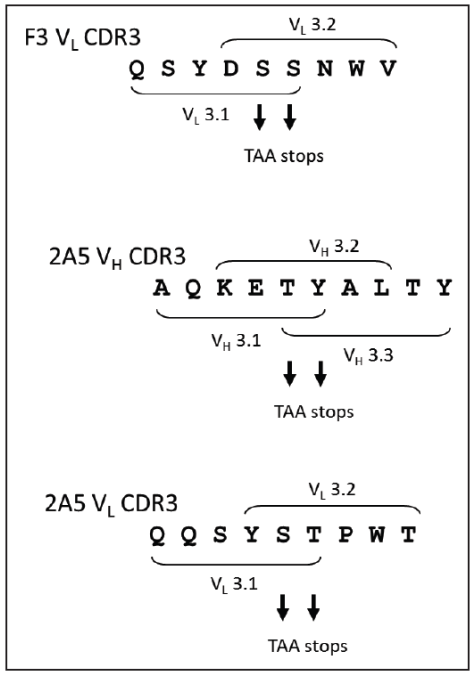

600
500
400
300
200
100
0
IL-8 (pg/ml)
D8
F3
0.0
0.5
1.0
1.5
2.0
Log Fab Concentration (nM)

*Figure 8*

a

Heavy chain:




Light chain:

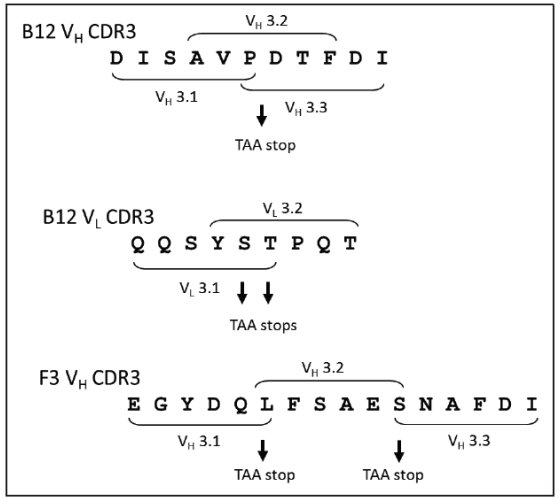



***Figure 9***

b **Oligonucleotides for VH and VL CDR3 mutagenesis**

| | | |
|---|---|---|
| F3 VH 3.1 | AGCATTACTCTCAGCAGAAAASNNSNNSNNSNNSNNSNNTCTCGCACAGTAATACACAGCCGTGTC | SEQ ID NO: 97 |
| F3 VH 3.2 | CTGGCCCCAGATATCAAAAGCATTSNNSNNSNNSNNSNNSNNTTGGTCATAGCCTTCTCTCGCACAG | SEQ ID NO: 98 |
| F3 VH 3.3 | GGTGACCAGGGTTCCCTGGCCCCASNNSNNSNNSNNSNNSNNCTCAGCAGAAAATAGTTGGTCATAGCC | SEQ ID NO: 99 |
| F3 VL 3.1 | GGTCCCTCCGCCGAACACCCAATTSNNSNNSNNSNNSNNSNNACAGTAGTAGTCAGCCTCGTCCTC | SEQ ID NO: 100 |
| F3 VL 3.2 | GGTGACCTTGGTCCCTCCGCCGAASNNSNNSNNSNNSNNSNNATAAGACTGACAGTAGTAGTCAGC | SEQ ID NO: 101 |
| B12 VH 3.1 | CTTGGCCCCAGATATCAAAAGTATCSNNSNNSNNSNNSNNSNNTTTTGCACAGTAATACAAGGCCGTGTC | SEQ ID NO: 102 |
| B12 VH 3.2 | CATTGTCCCTTGGCCCCAGATATCSNNSNNSNNSNNSNNSNNCGAGATATCTTTTGCACAGTAATAC | SEQ ID NO: 103 |
| B12 VH 3.3 | GGTGACCATTGTCCCTTGGCCCCASNNSNNSNNSNNSNNSNNGACTGCCGAGATATCTTTTGCACAG | SEQ ID NO: 104 |
| B12 VL3.1 | GGTCCCTTGGCCGAACGTCTGAGGSNNSNNSNNSNNSNNSNNACAGTAGTAAGTTGCAAAATCTTC | SEQ ID NO: 105 |
| B12 VL 3.2 | GATATCCACTTTGGTCCCTTGGCCGAASNNSNNSNNSNNSNNSNNACTCTGTTGACAGTAGTAAGTTGC | SEQ ID NO: 106 |
| 2A5 VH 3.1 | GGTTCCCTGGCCCCAGTAGGTCAAGGCSNNSNNSNNSNNSNNSNNTGTTGCACAGTAATACACGGCCGT | SEQ ID NO: 107 |
| 2A5 VH 3.2 | CAGGGTTCCCTGGCCCCAGTAGGTSNNSNNSNNSNNSNNSNNCTGGGCTGTTGCACAGTAATACAC | SEQ ID NO: 108 |
| 2A5 VH 3.3 | GGTGACCAGGGTTCCCTGGCCCCASNNSNNSNNSNNSNNSNNCTCTTTCTGGGCTGTTGCACAGTA | SEQ ID NO: 109 |
| 2A5 VL 3.1 | GGTCCCTTGGCCGAACGTCCACGGSNNSNNSNNSNNSNNSNNACAGTAGTAAGTTGCAAAATCTTC | SEQ ID NO: 110 |
| 2A5 VL 3.2 | CTCCACCTTGGTCCCTTGGCCGAASNNSNNSNNSNNSNNSNNACTCTGTTGACAGTAGTAAGTTGC | SEQ ID NO: 111 |

***Figure 9 (con't)***

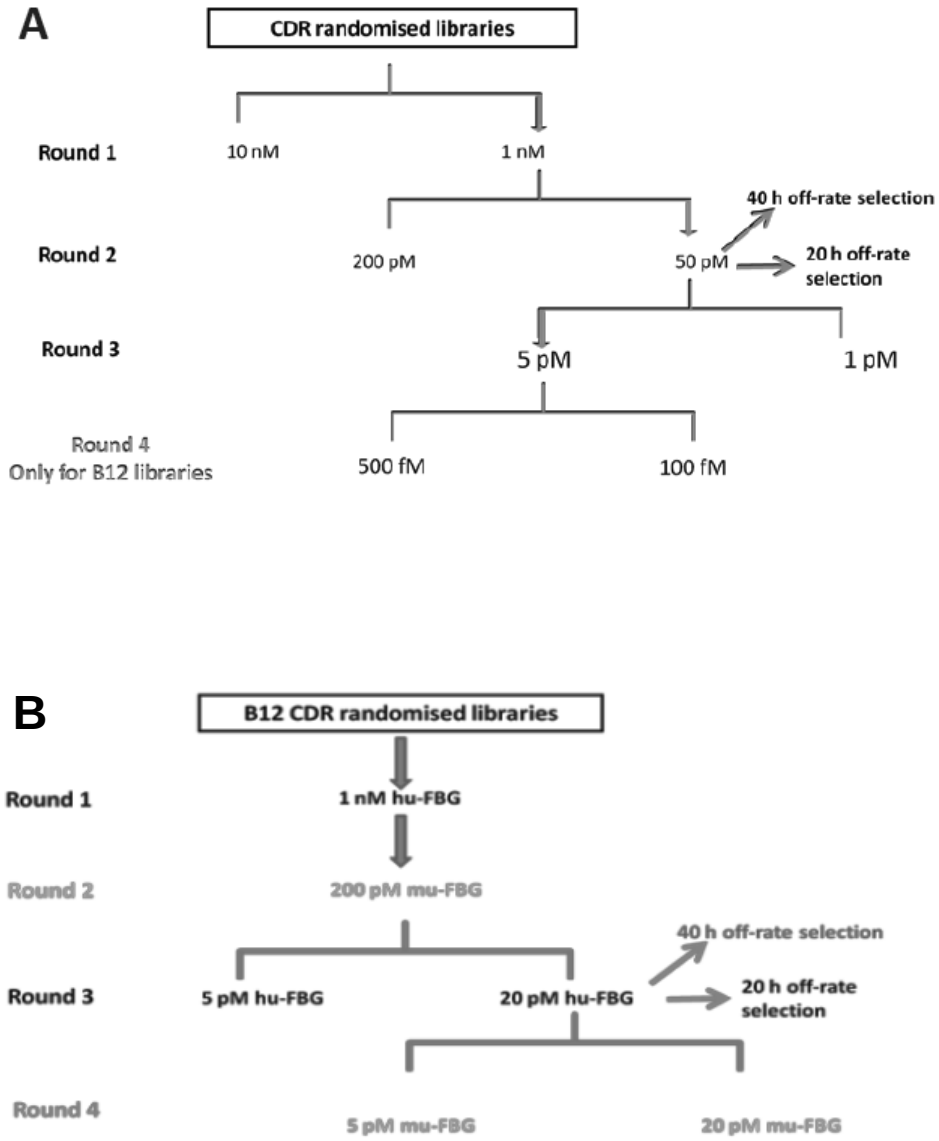

A
CDR randomised libraries
Round 1
10 nM
1 nM
40 h off-rate selection
Round 2
200 pM
50 pM
20 h off-rate selection
Round 3
5 pM
1 pM
Round 4
Only for B12 libraries
500 fM
100 fM
B
B12 CDR randomised libraries
Round 1
1 nM hu-FBG
Round 2
200 pM mu-FBG
40 h off-rate selection
Round 3
5 pM hu-FBG
20 pM hu-FBG
20 h off-rate selection
Round 4
5 pM mu-FBG
20 pM mu-FBG

Figure 10

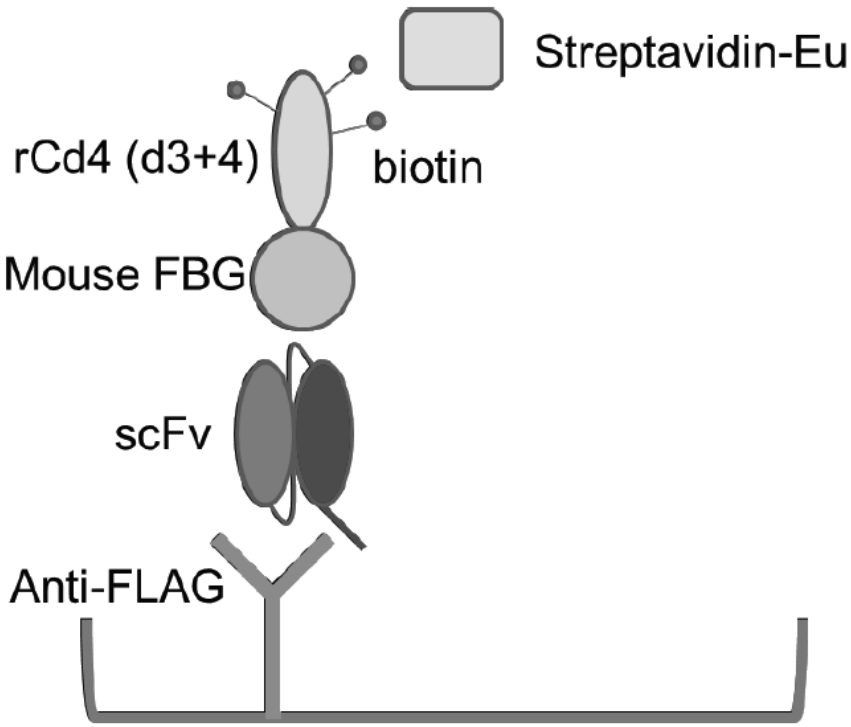



***Figure 11***

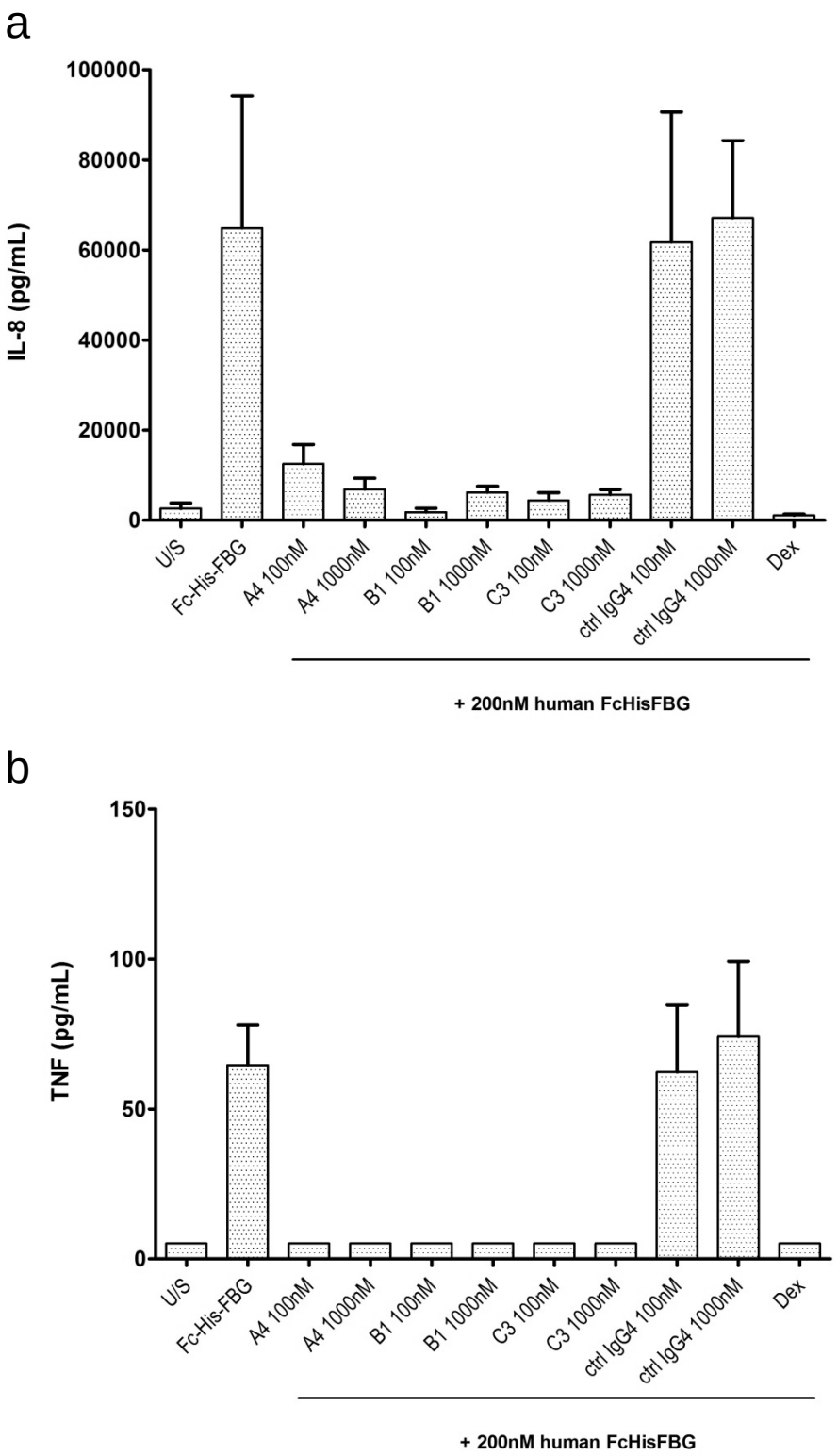

a
IL-8 (pg/mL)
100000
80000
60000
40000
20000
0
U/S
Fc-His-FBG
A4 100nM
A4 1000nM
B1 100nM
B1 1000nM
C3 100nM
C3 1000nM
ctrl IgG4 100nM
ctrl IgG4 1000nM
Dex
+ 200nM human FcHisFBG
b
TNF (pg/mL)
150
100
50
0
U/S
Fc-His-FBG
A4 100nM
A4 1000nM
B1 100nM
B1 1000nM
C3 100nM
C3 1000nM
ctrl IgG4 100nM
ctrl IgG4 1000nM
Dex
+ 200nM human FcHisFBG

**Figure 12**

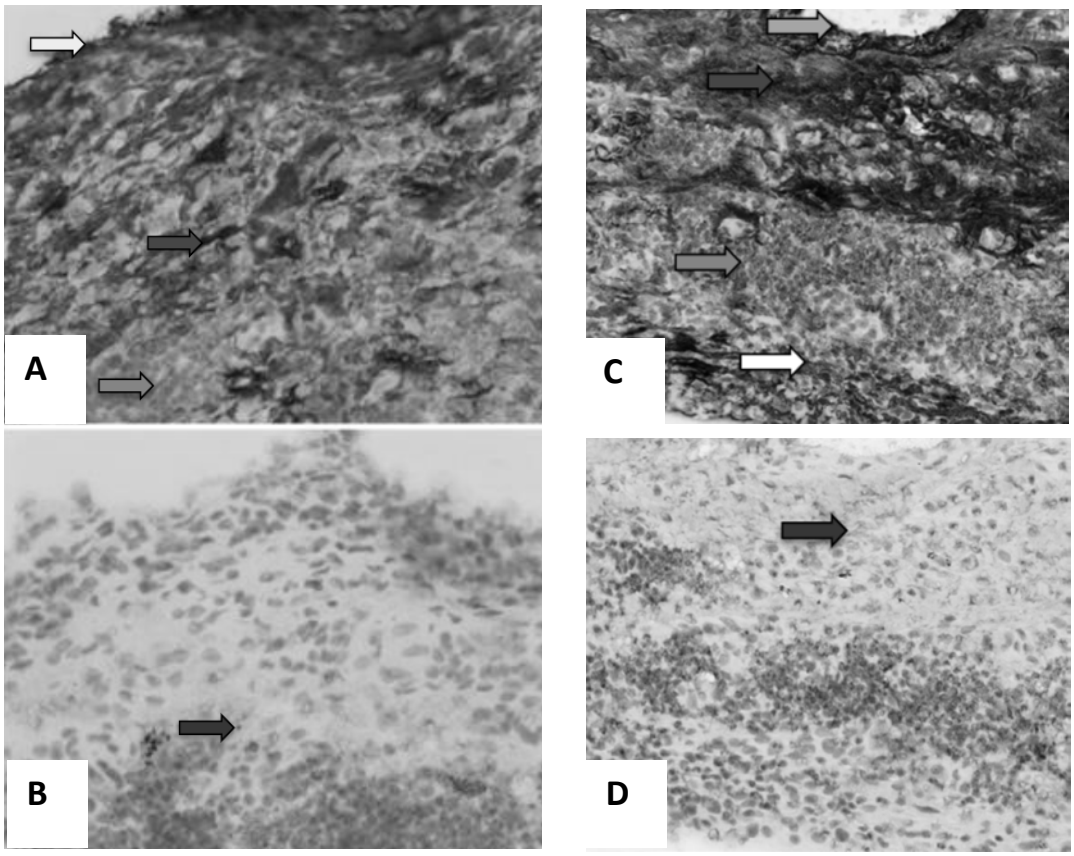



Figure 13

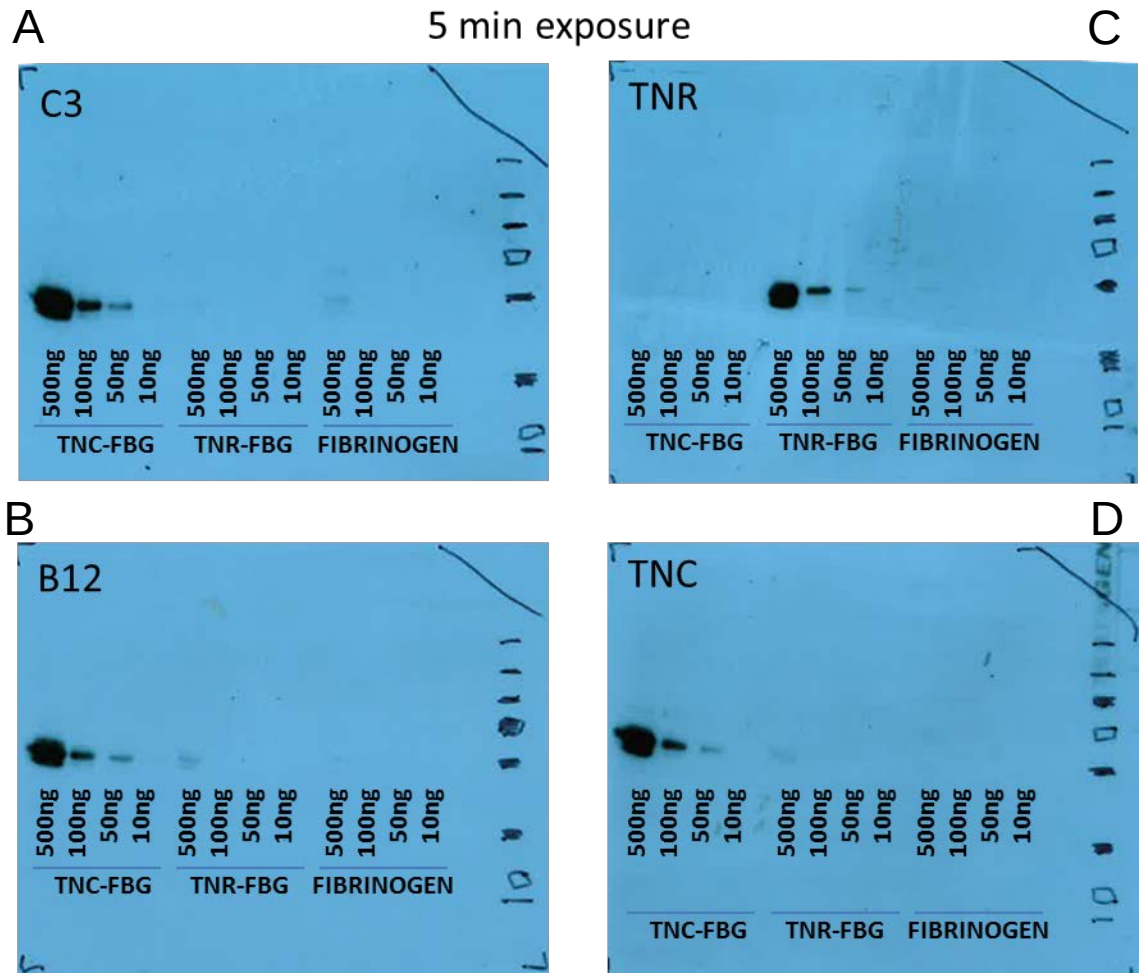

A
5 min exposure
C
C3
TNR
B
D
B12
TNC
500ng
100ng
50ng
10ng
TNC-FBG
TNR-FBG
FIBRINOGEN

***Figure 14***

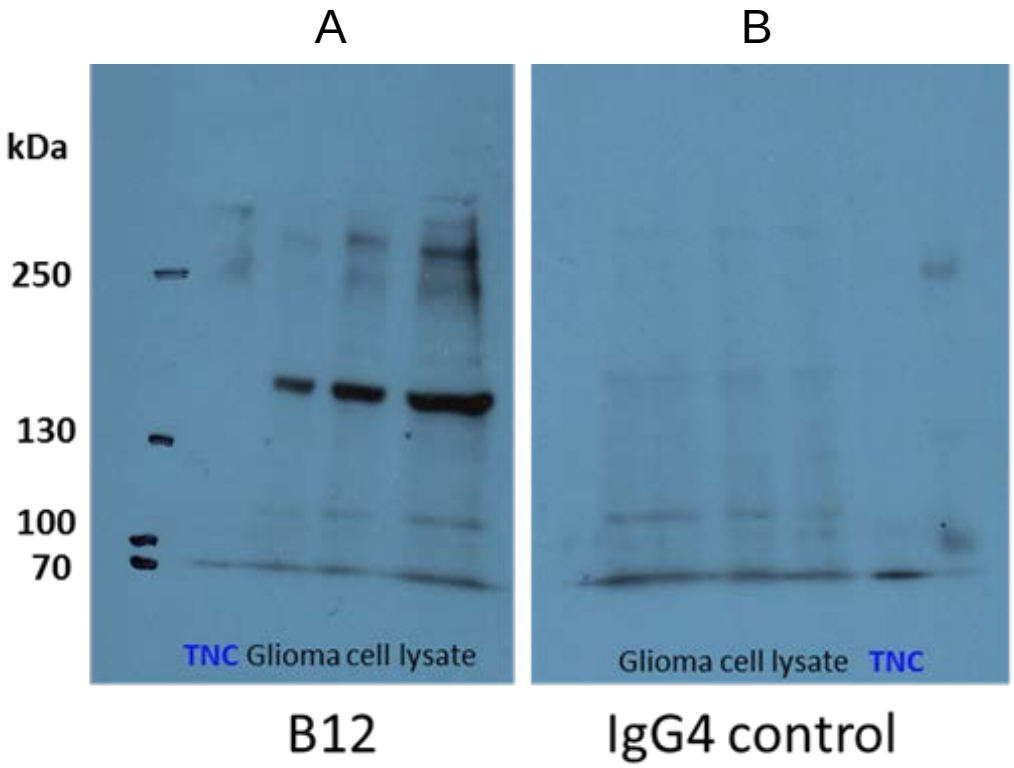
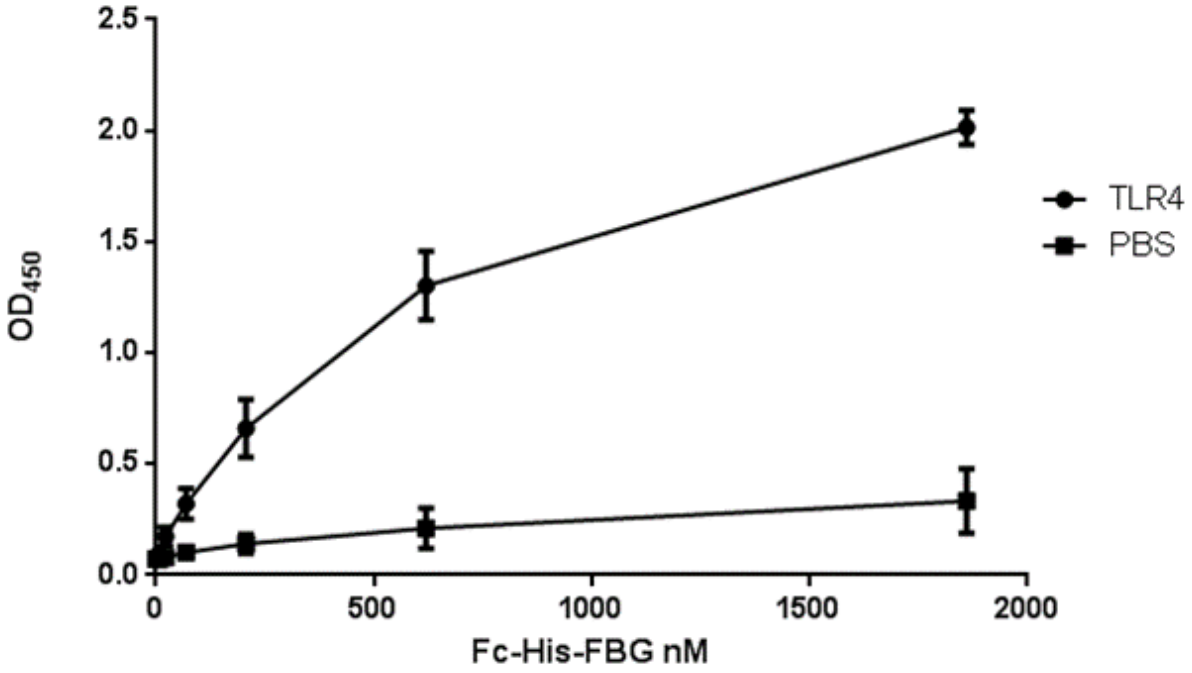

A
B
kDa
250
130
100
70
TNC Glioma cell lysate
Glioma cell lysate TNC
B12
IgG4 control

***Figure 15***

A


2.5
2.0
1.5
1.0
0.5
0.0
OD450
0
500
1000
1500
2000
Fc-His-FBG nM
TLR4
PBS

B

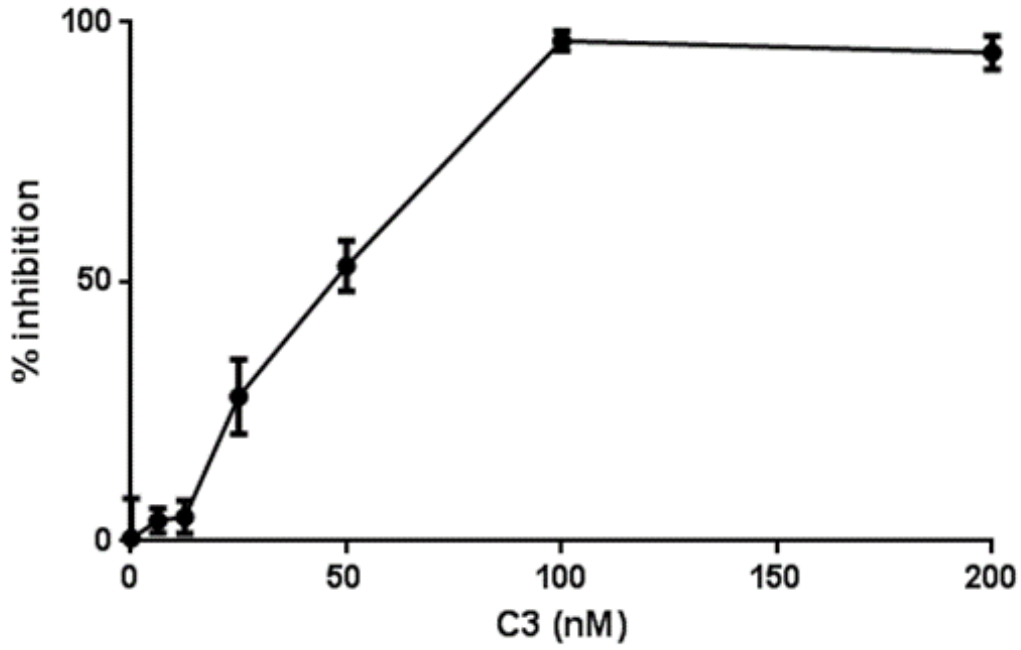

100
50
0
% inhibition
0
50
100
150
200
C3 (nM)

***Figure 16***

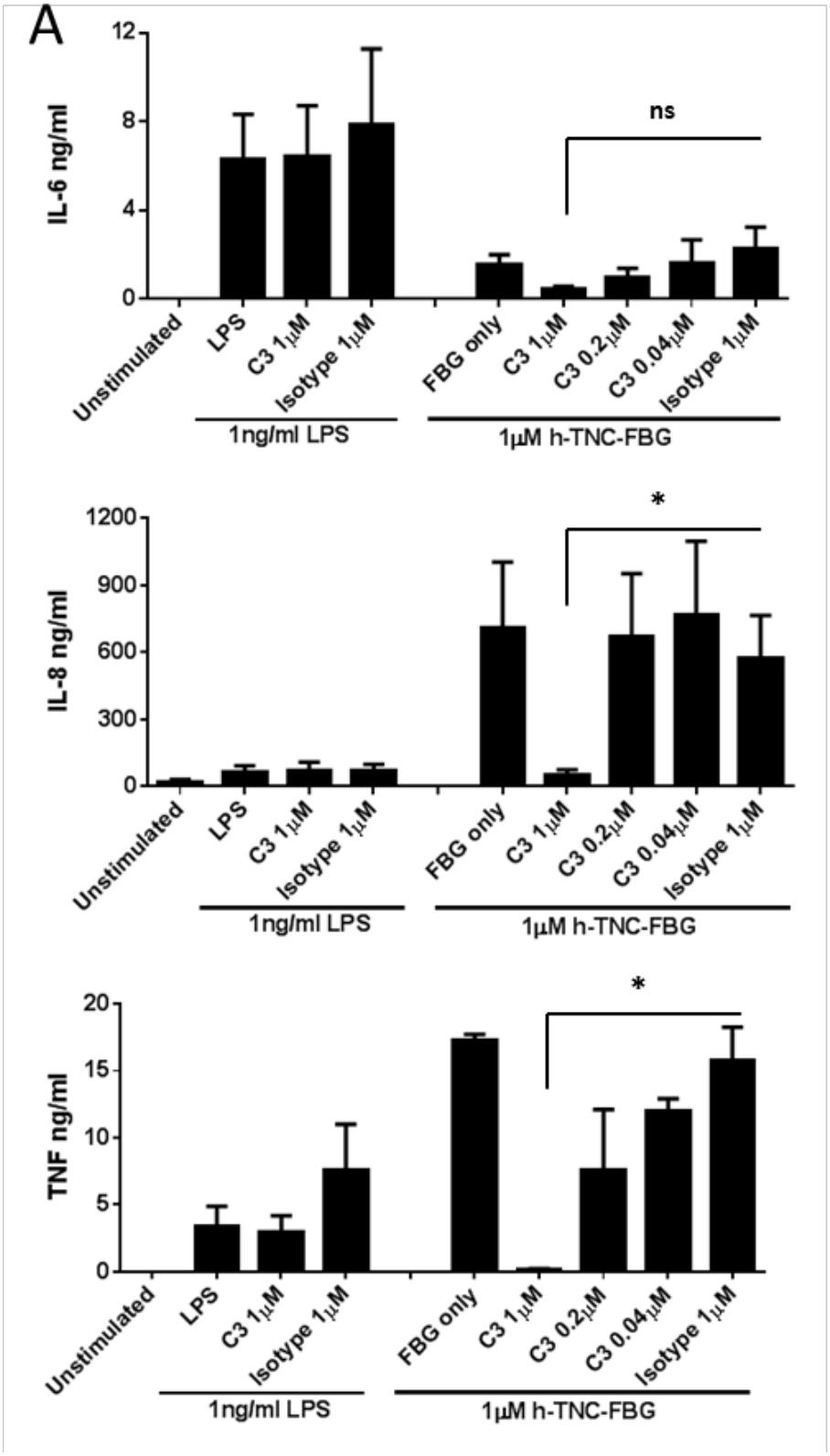

A
IL-6 ng/ml
12
8
4
0
ns
Unstimulated
LPS
C3 1μM
Isotype 1μM
FBG only
C3 1μM
C3 0.2μM
C3 0.04μM
Isotype 1μM
1ng/ml LPS
1μM h-TNC-FBG
IL-8 ng/ml
1200
900
600
300
0
*
Unstimulated
LPS
C3 1μM
Isotype 1μM
FBG only
C3 1μM
C3 0.2μM
C3 0.04μM
Isotype 1μM
1ng/ml LPS
1μM h-TNC-FBG
TNF ng/ml
20
15
10
5
0
*
Unstimulated
LPS
C3 1μM
Isotype 1μM
FBG only
C3 1μM
C3 0.2μM
C3 0.04μM
Isotype 1μM
1ng/ml LPS
1μM h-TNC-FBG

***Figure 17***

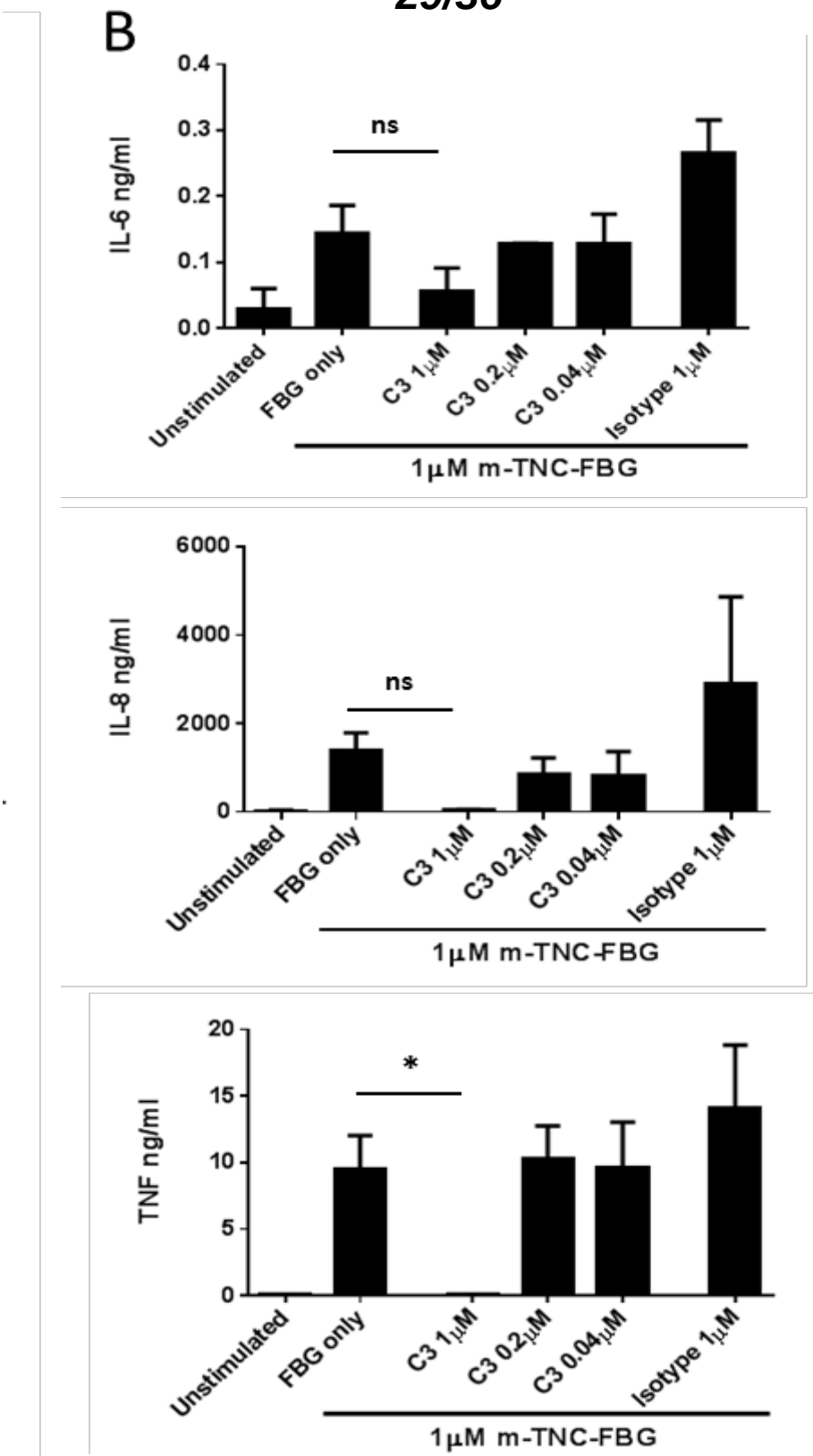



***Figure 17 cont'd***

C

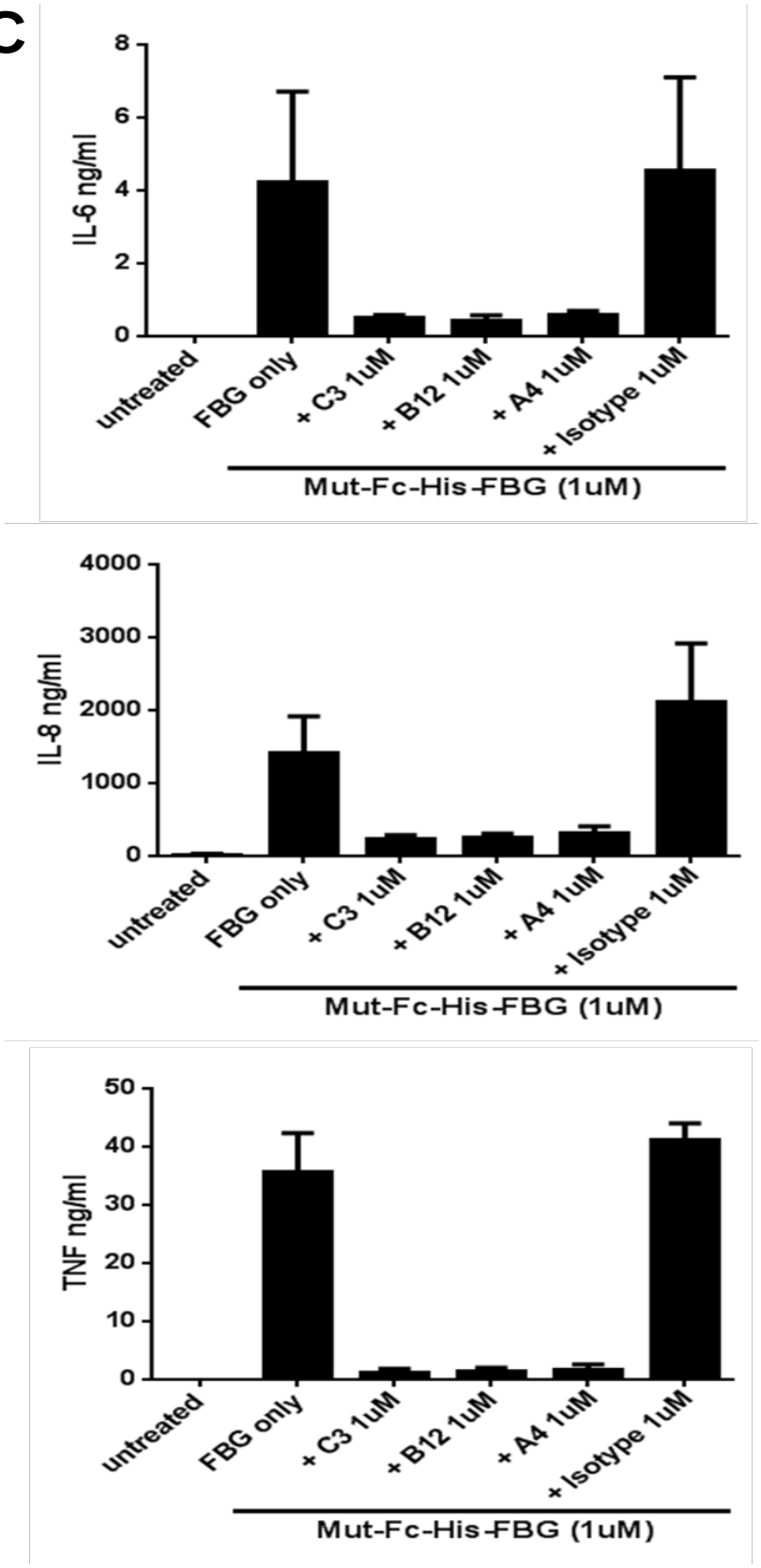
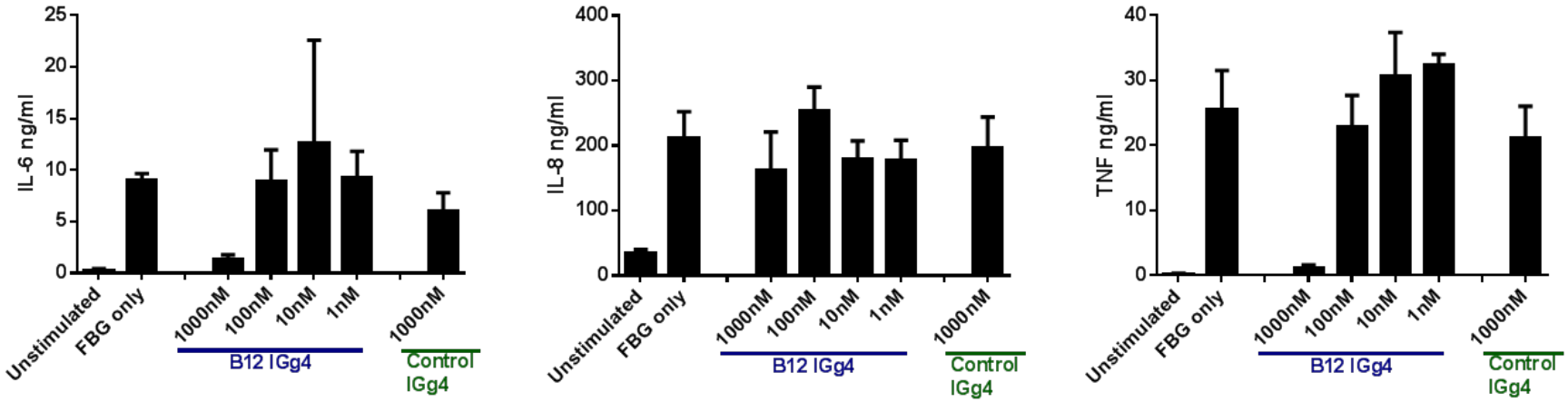



***Figure 17 cont'd***

A


IL-6 ng/ml
25
20
15
10
5
0
Unstimulated
FBG only
1000nM
100nM
10nM
1nM
B12 IGg4
1000nM
Control IGg4
IL-8 ng/ml
400
300
200
100
0
Unstimulated
FBG only
1000nM
100nM
10nM
1nM
B12 IGg4
1000nM
Control IGg4
TNF ng/ml
40
30
20
10
0
Unstimulated
FBG only
1000nM
100nM
10nM
1nM
B12 IGg4
1000nM
Control IGg4

B

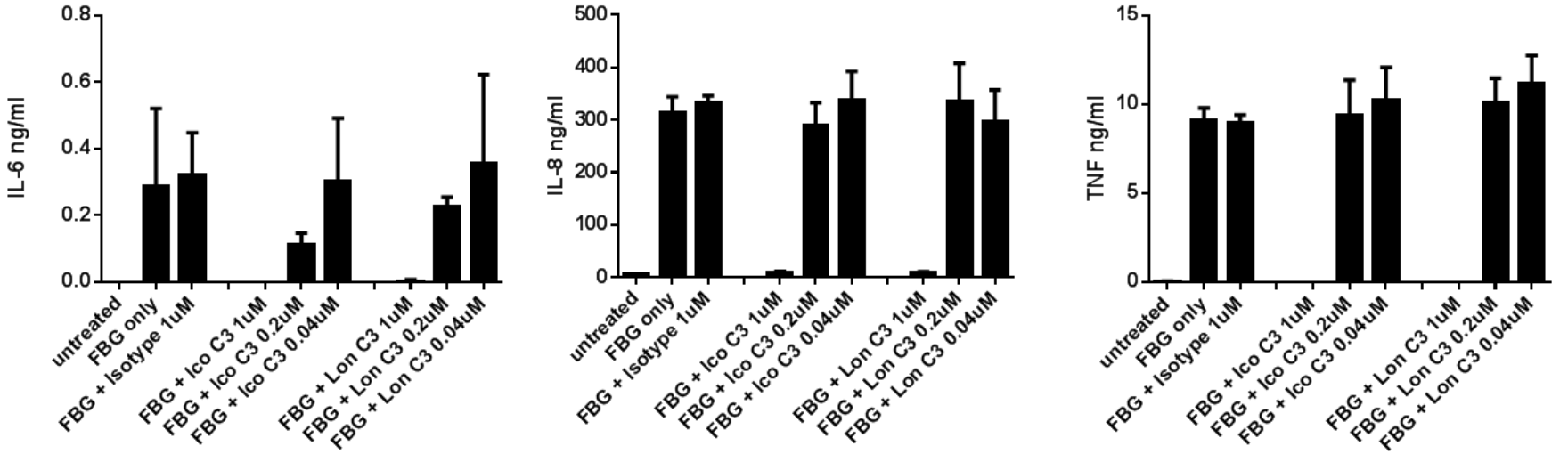

IL-6 ng/ml
0.8
0.6
0.4
0.2
0.0
untreated
FBG only
FBG + Isotype 1uM
FBG + Ico C3 1uM
FBG + Ico C3 0.2uM
FBG + Ico C3 0.04uM
FBG + Lon C3 1uM
FBG + Lon C3 0.2uM
FBG + Lon C3 0.04uM
IL-8 ng/ml
500
400
300
200
100
0
untreated
FBG only
FBG + Isotype 1uM
FBG + Ico C3 1uM
FBG + Ico C3 0.2uM
FBG + Ico C3 0.04uM
FBG + Lon C3 1uM
FBG + Lon C3 0.2uM
FBG + Lon C3 0.04uM
TNF ng/ml
15
10
5
0
untreated
FBG only
FBG + Isotype 1uM
FBG + Ico C3 1uM
FBG + Ico C3 0.2uM
FBG + Ico C3 0.04uM
FBG + Lon C3 1uM
FBG + Lon C3 0.2uM
FBG + Lon C3 0.04uM

***Figure 18***

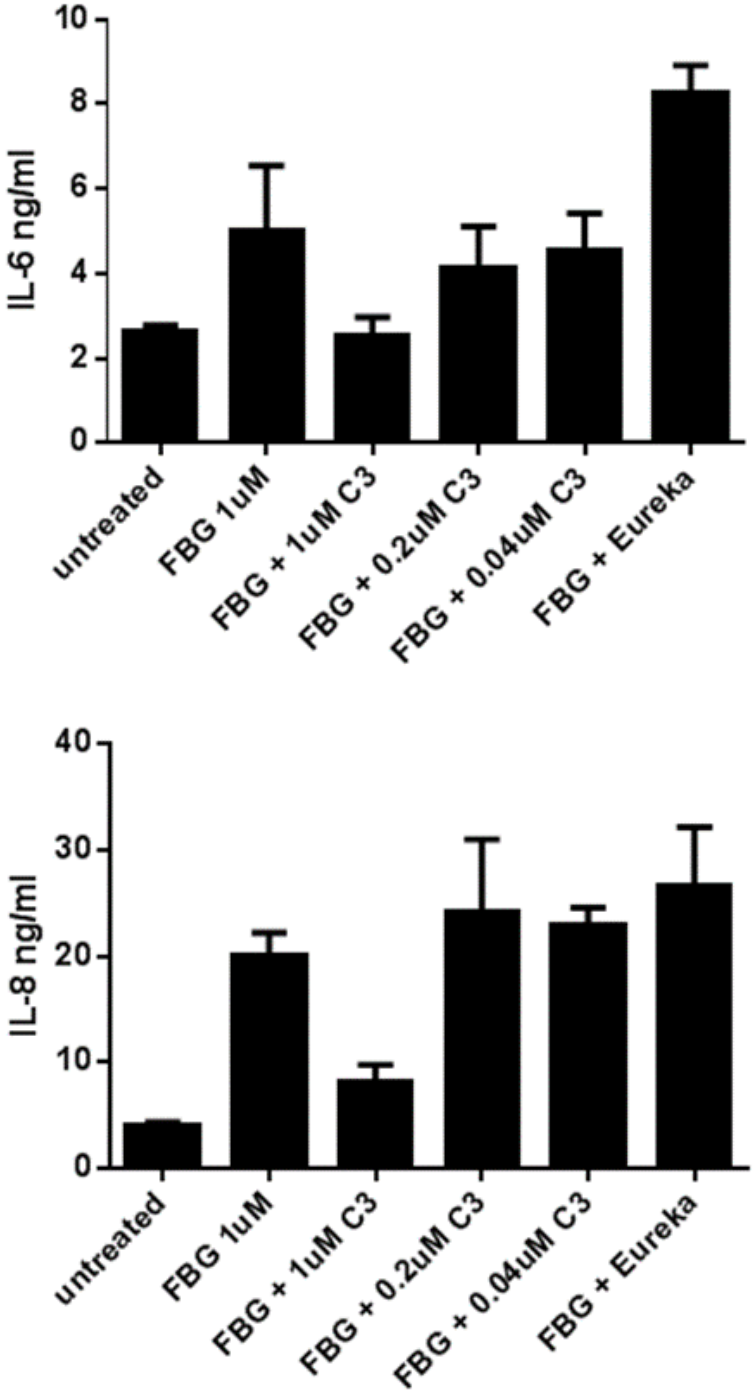

IL-6 ng/ml
10
8
6
4
2
0
untreated
FBG 1uM
FBG + 1uM C3
FBG + 0.2uM C3
FBG + 0.04uM C3
FBG + Eureka
IL-8 ng/ml
40
30
20
10
0
untreated
FBG 1uM
FBG + 1uM C3
FBG + 0.2uM C3
FBG + 0.04uM C3
FBG + Eureka

***Figure 19***

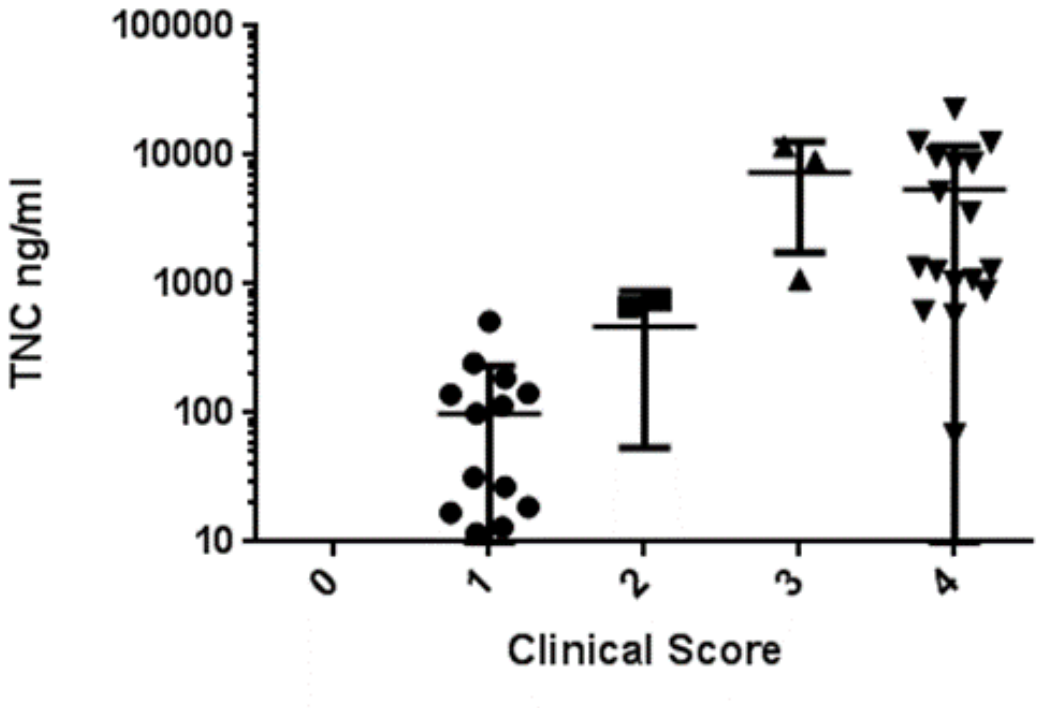



Figure 20

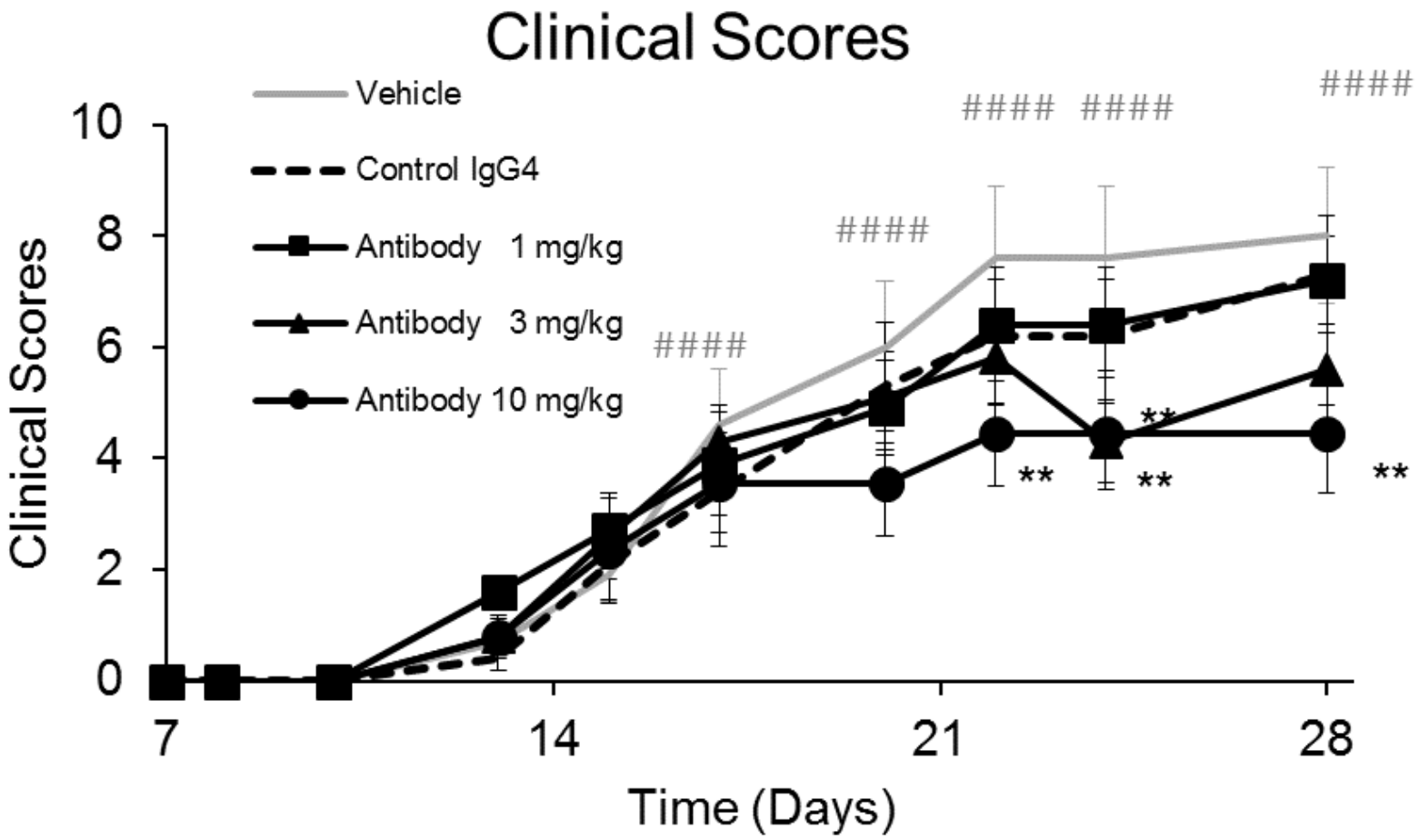

Clinical Scores
Vehicle
Control IgG4
Antibody 1 mg/kg
Antibody 3 mg/kg
Antibody 10 mg/kg
Clinical Scores
10
8
6
4
2
0
7
14
21
28
Time (Days)
####
####
####
####
####
**
**
**
**

***Figure 21***

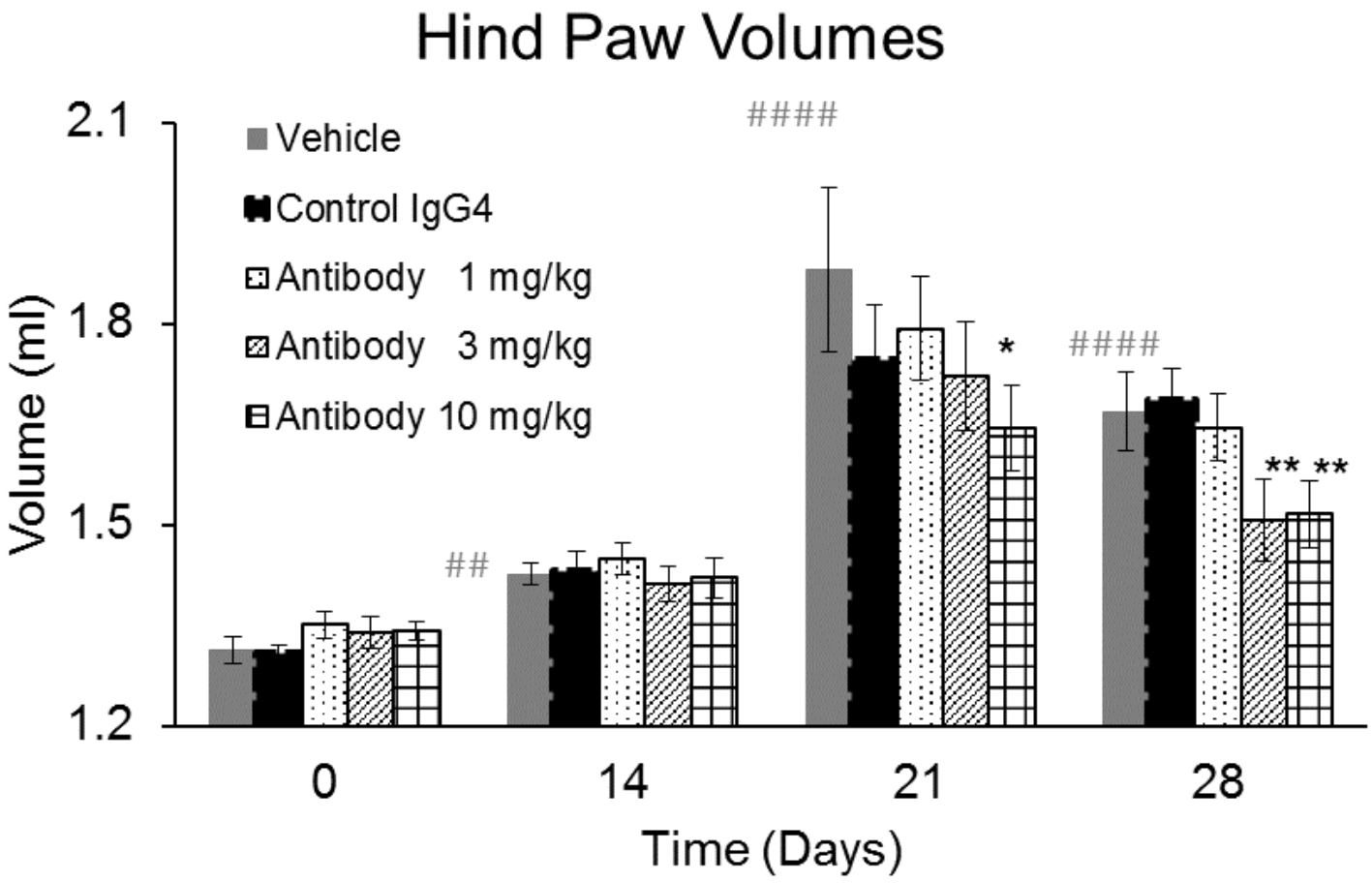

Hind Paw Volumes
Vehicle
Control IgG4
Antibody 1 mg/kg
Antibody 3 mg/kg
Antibody 10 mg/kg
Volume (ml)
2.1
1.8
1.5
1.2
0
14
21
28
Time (Days)
##
####
*
####
** **

***Figure 22***

| Construct | N-term | Species | Primer name (F) | Primer sequence (F) | Primer name (R) | Primer sequence (R) |
|---|---|---|---|---|---|---|
| FBG-X | minus | human | 2561 | GGTACCTCGCGAATGCATCTAG | 2562 | CATGCAGGCCTCTGCAGTCG |
| FBG-X | plus | human | 2565 | TTTTTTCCATGGCCCAGATTGGACTCCTGTACCCCTTCCCCAAAGATTGCTCTCAGGC | 2562 | CATGCAGGCCTCTGCAGTCG |
| FBG-X | minus | mouse | 2561 | GGTACCTCGCGAATGCATCTAG | 2562 | CATGCAGGCCTCTGCAGTCG |
| FBG-X | plus | mouse | 2566 | TTTTTTCCATGGCCCAGATTGGACTCCTGTACCCCTTCCCTCGCGACTGCTCACAG | 2562 | CATGCAGGCCTCTGCAGTCG |
| X-FBG | minus | human | 2567C | TTTTTTGGATCCCATCATCATCACCATCACTTCCCCAAAGATTGCTCTCAGGC | 2570 | TTTTTTAAGCTTTTATTACGCCCGTTTACGCCGACCCTC |
| X-FBG | plus | human | 2567 | TTTTTTGGATCCCATCATCATCACCATCACATTGGACTCCTGTACCCCTTCCCCAAAGATTGCTCTCAGGC | 2570 | TTTTTTAAGCTTTTATTACGCCCGTTTACGCCGACCCTC |
| X-FBG | minus | mouse | 2569C | TTTTTTGGATCCCATCATCATCACCATCACTTCCCTCGCGACTGCTCACAG | 2571 | TTTTTTAAGCTTTTATTACGCCCGTTTCCGCCGACCTTC |
| X-FBG | plus | mouse | 2568C | TTTTTTGGATCCCATCATCATCACCATCACATTGGACTCCTGTACCCCTTCCCTCGCGACTGCTCACAG | 2571 | TTTTTTAAGCTTTTATTACGCCCGTTTCCGCCGACCTTC |
| BamHI-His6-HindIII | N/A | N/A | 2574 | TTTTTTGGATCCCATCATCATCACCATCACTAATAAAAG | 2575 | TTTTTTAAGCTTTTATTAGTGATGGTGATGATGATGGG |
| His-FBG | plus | human | 2580 | TTTTTTCTCGAGCATCATCATCACCATCACATTGGACTCC | 2570 | TTTTTTAAGCTTTTATTACGCCCGTTTACGCCGACCCTC |
| His-FBG | plus | mouse | 2580 | TTTTTTCTCGAGCATCATCATCACCATCACATTGGACTCC | 2571 | TTTTTTAAGCTTTTATTACGCCCGTTTCCGCCGACCTTC |

Figure 23