

TA domain

23 gvlkkvir hkrqsgvnat lpeen 45

EGFL domain

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481 vngmcvcddg ytgedcdrdq cprdcsnrgl cvdggcvcged gftgpdcael scpndchggq
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FNIII domain

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FBG domain

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Figure 1

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Figure 2

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```

Figure 2 (con't)

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Figure 2 (con't)

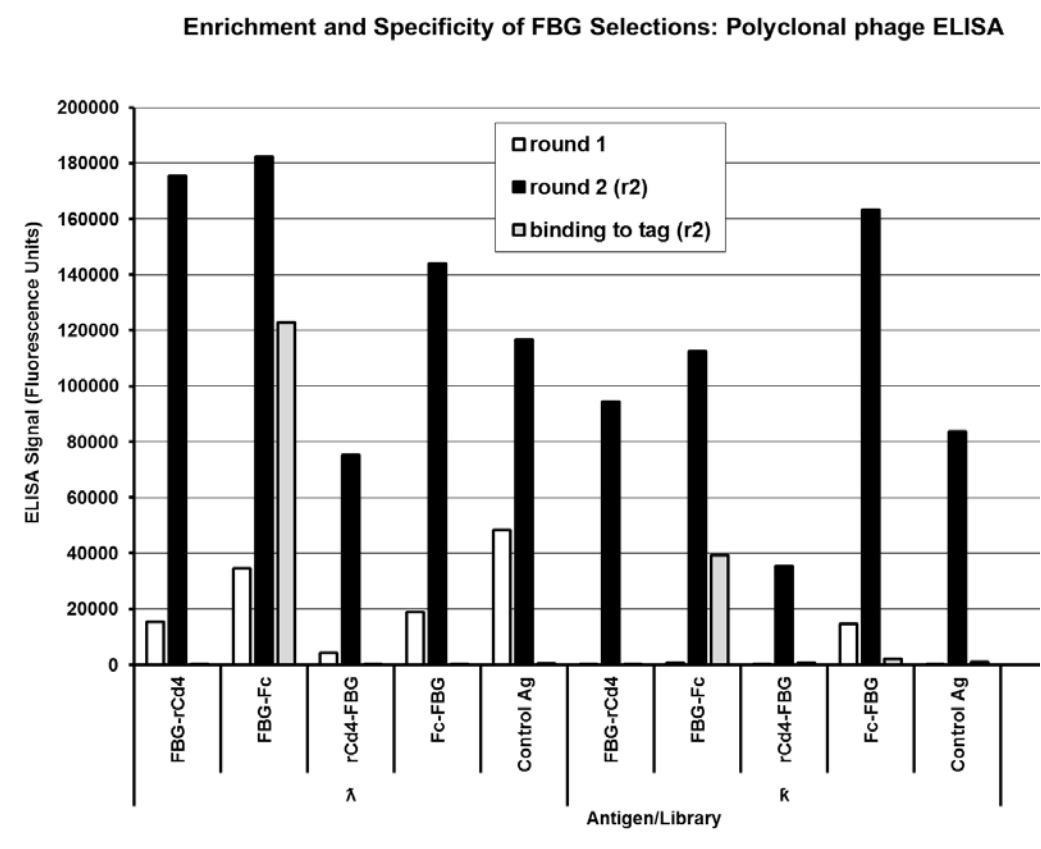


Figure 3

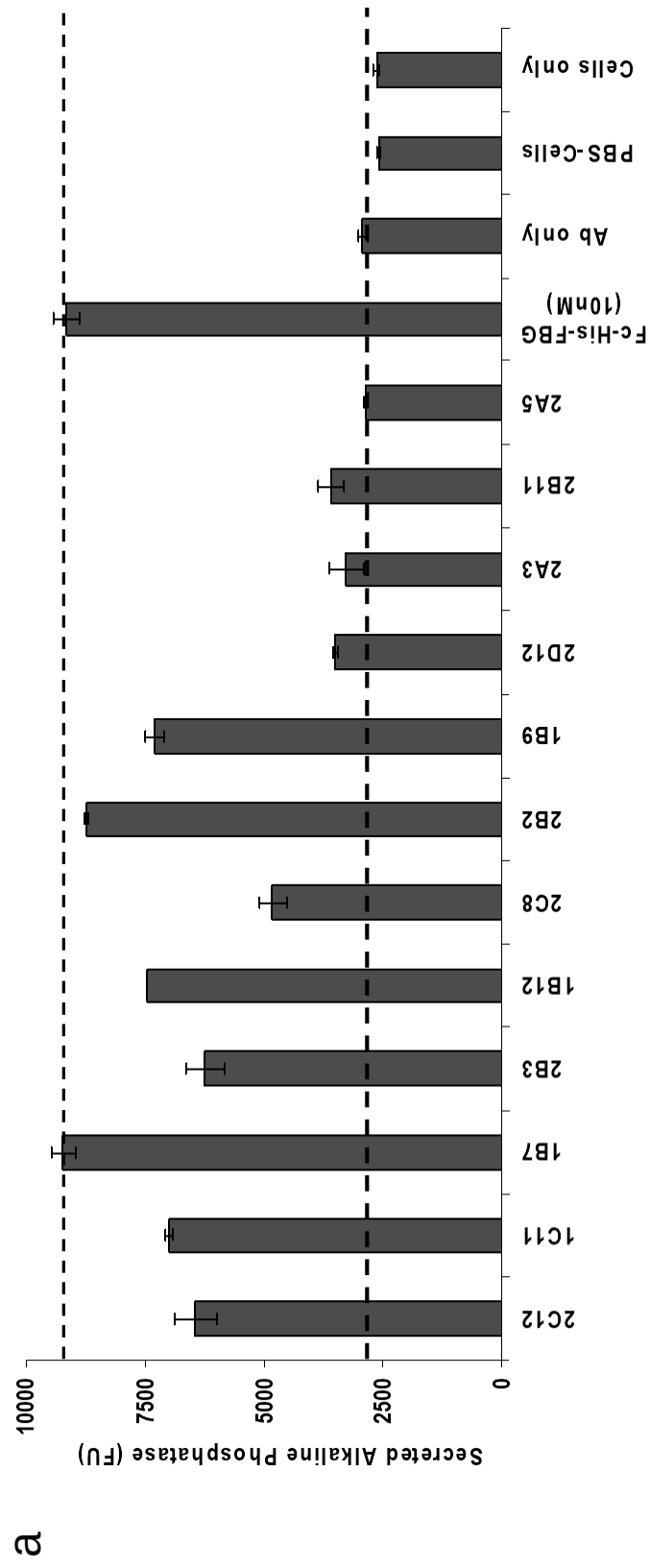
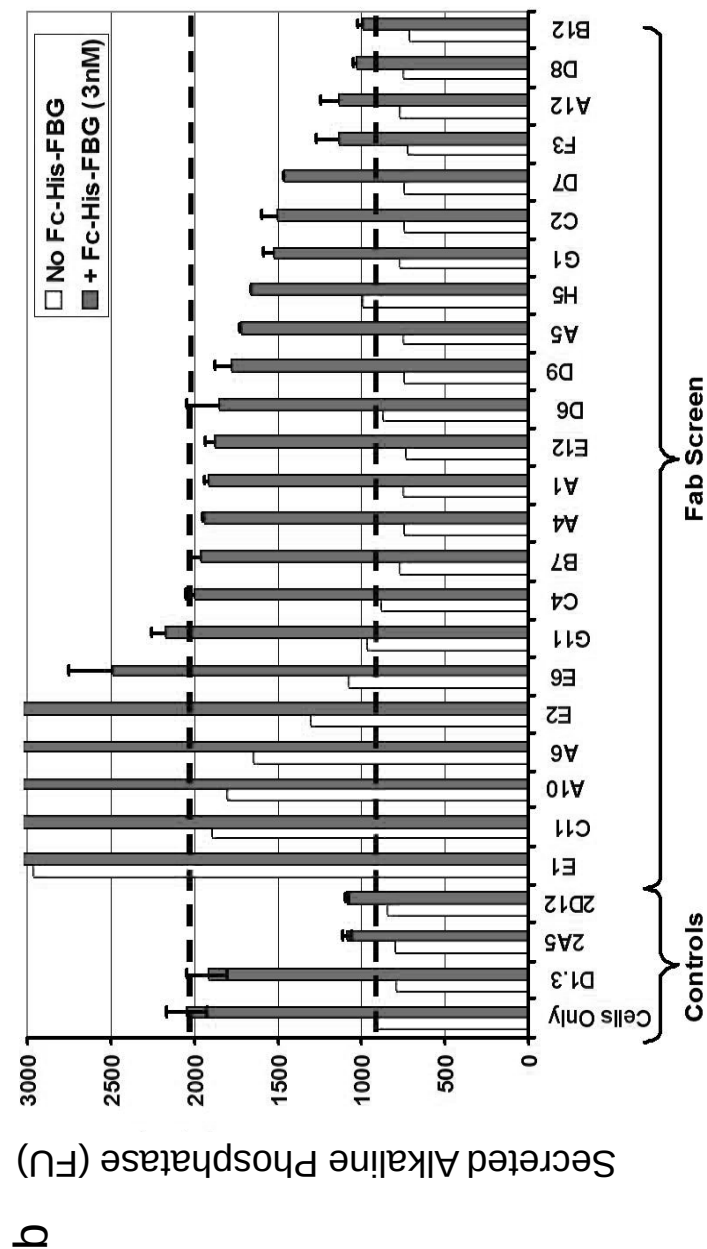


Figure 4



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

Figure 4 (con't)

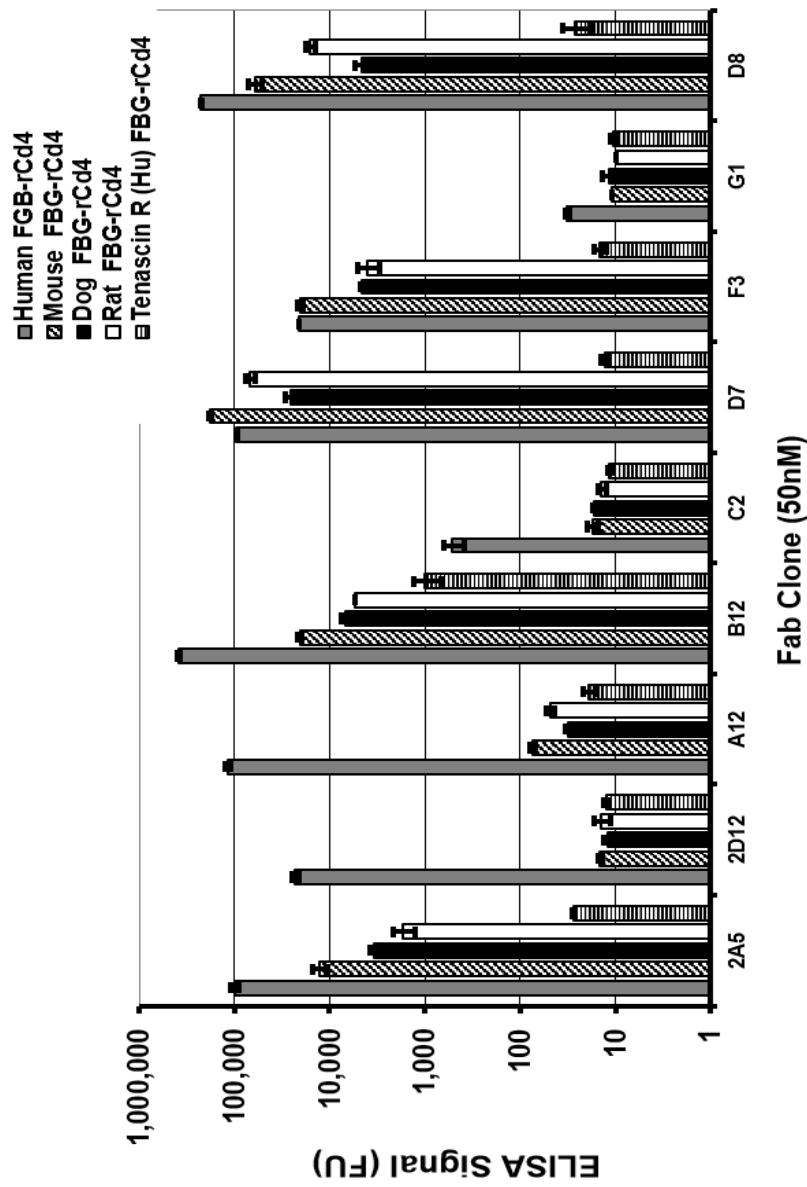


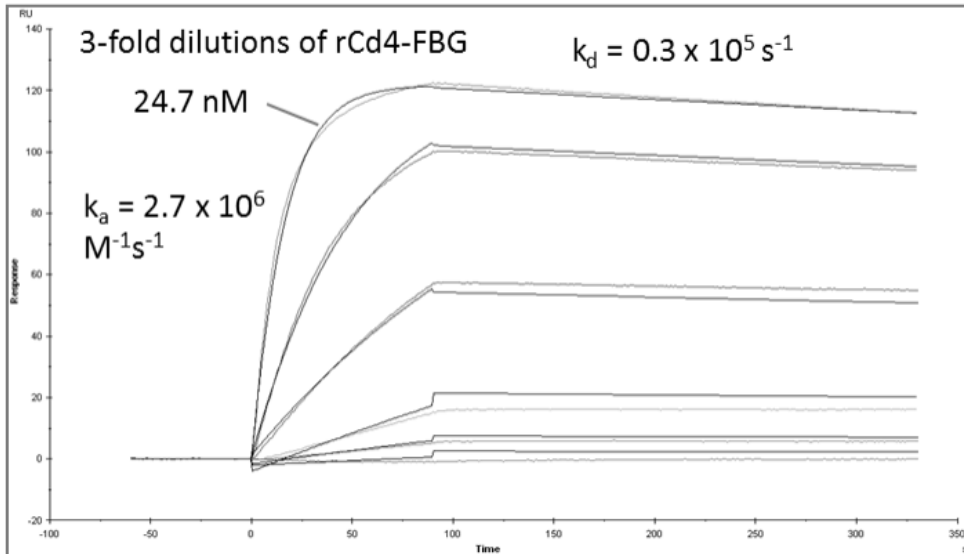
Figure 5

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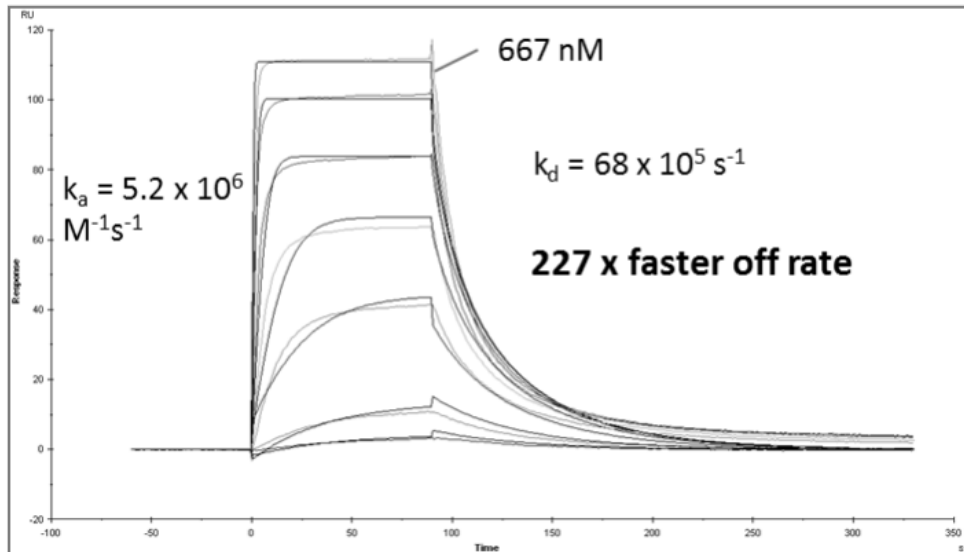
a

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- $C_L\kappa$ / anti- $C_L\lambda$ CM5 sensor chip followed by rCd4-FBG injection. Regeneration with 10 mM Glycine, pH2

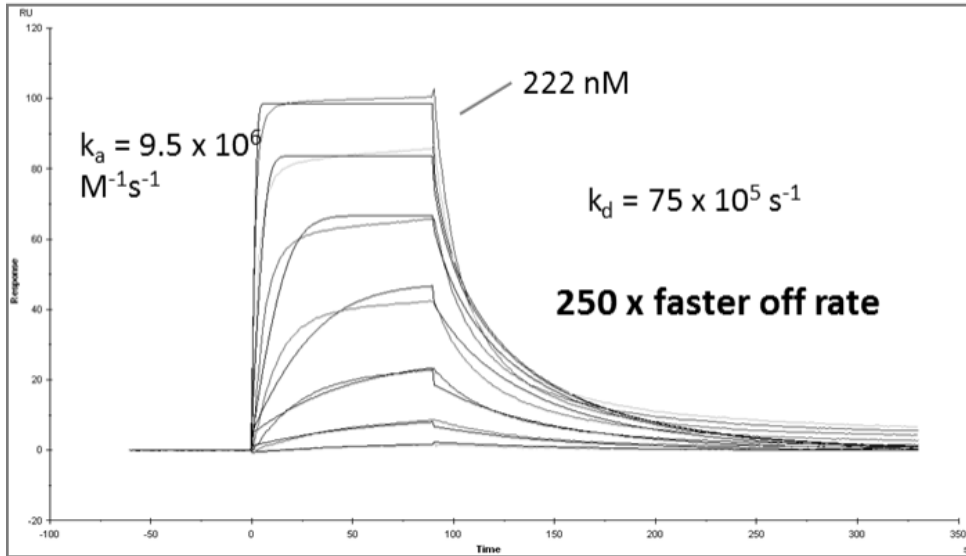
Figure 6a

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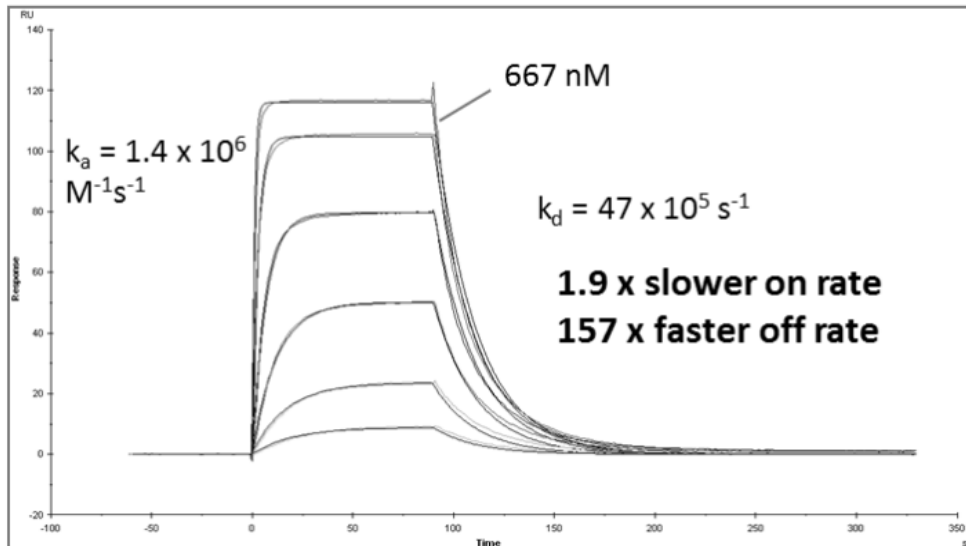
a

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- $C_L\kappa$ / anti- $C_L\lambda$ CM5 sensor chip followed by rCd4-FBG injection. Regeneration with 10 mM Glycine, pH2

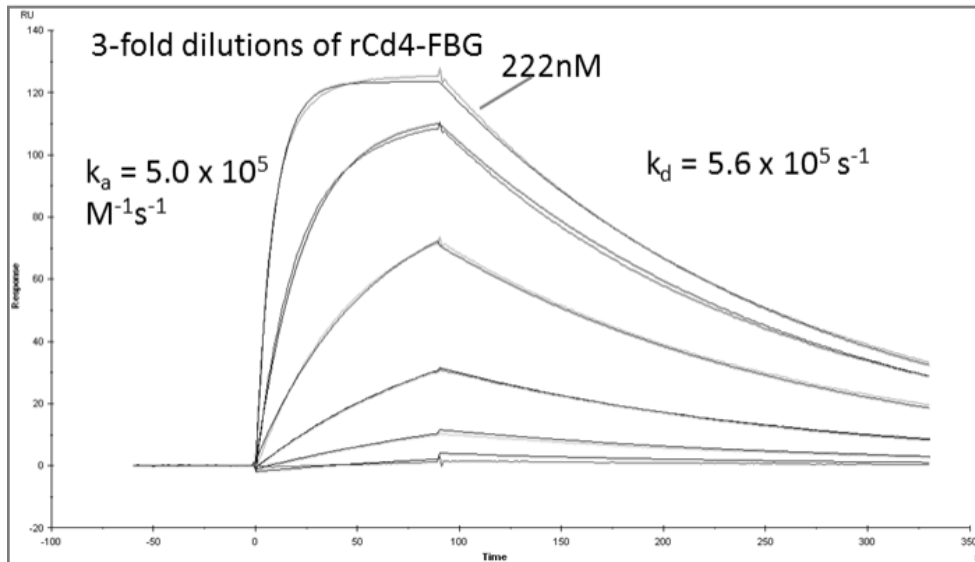
Figure 6a (Con't)

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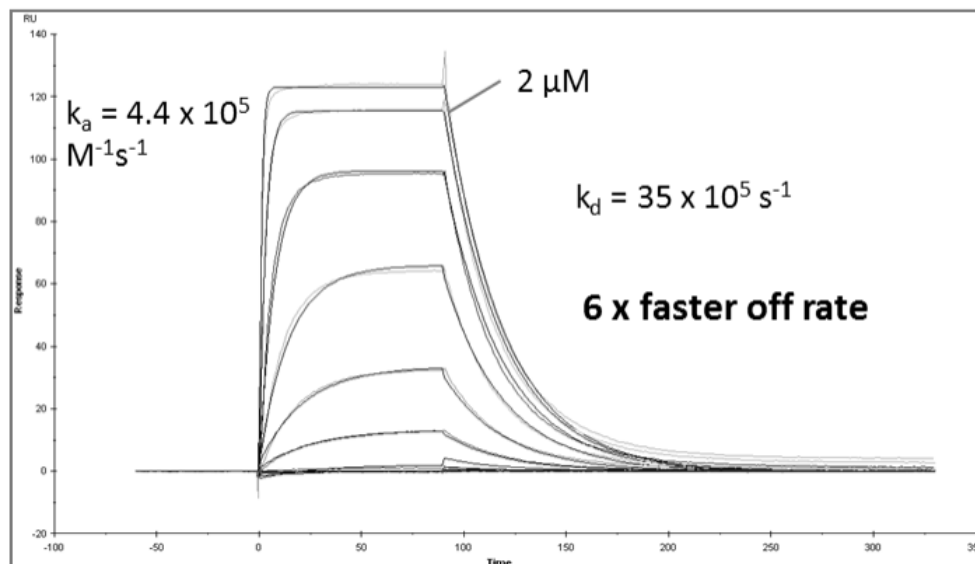
b

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- $C_L\kappa$ / anti- $C_L\lambda$ CM5 sensor chip followed by rCd4-FBG injection. Regeneration with 10 mM Glycine, pH2

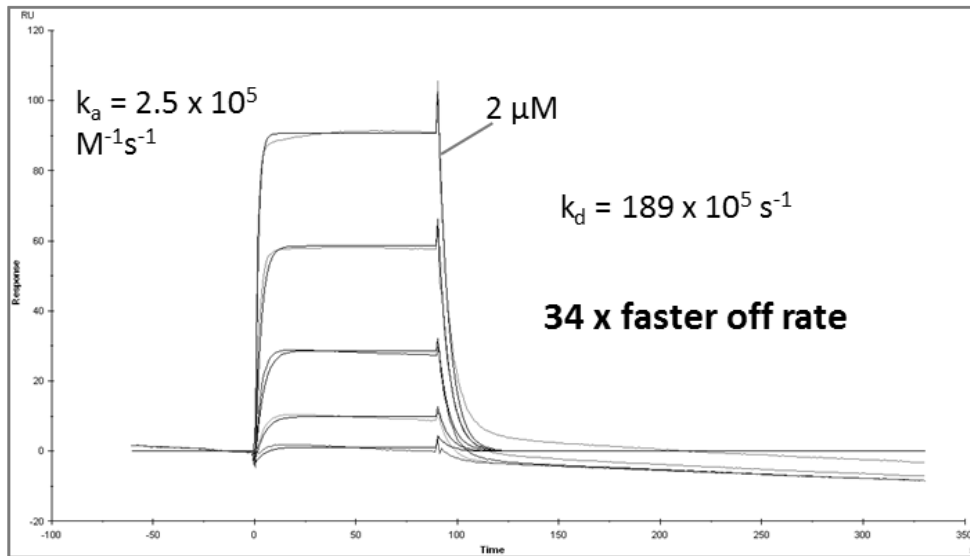
Figure 6b

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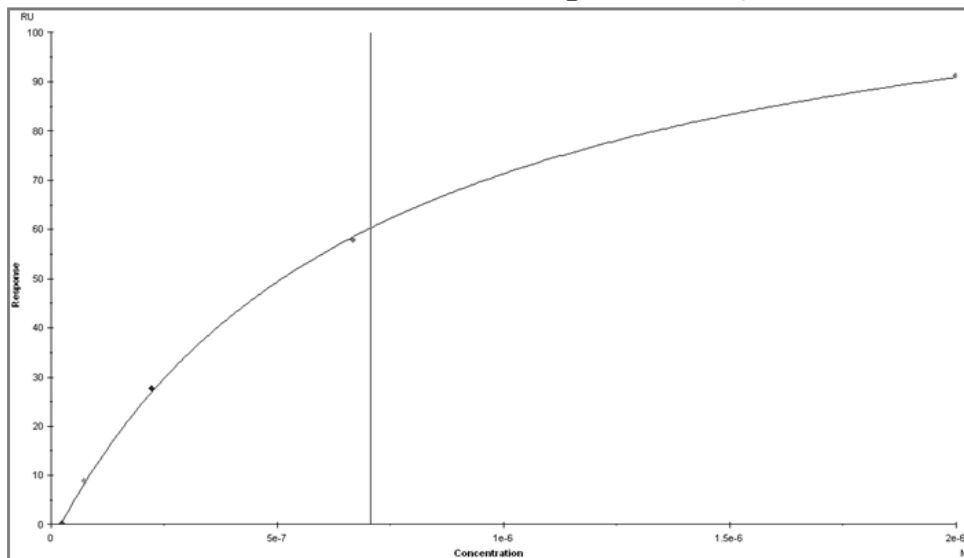
b

Fab 2A5 – SPR Results

Human TNR FBG – $K_D = 0.76 \mu\text{M}$



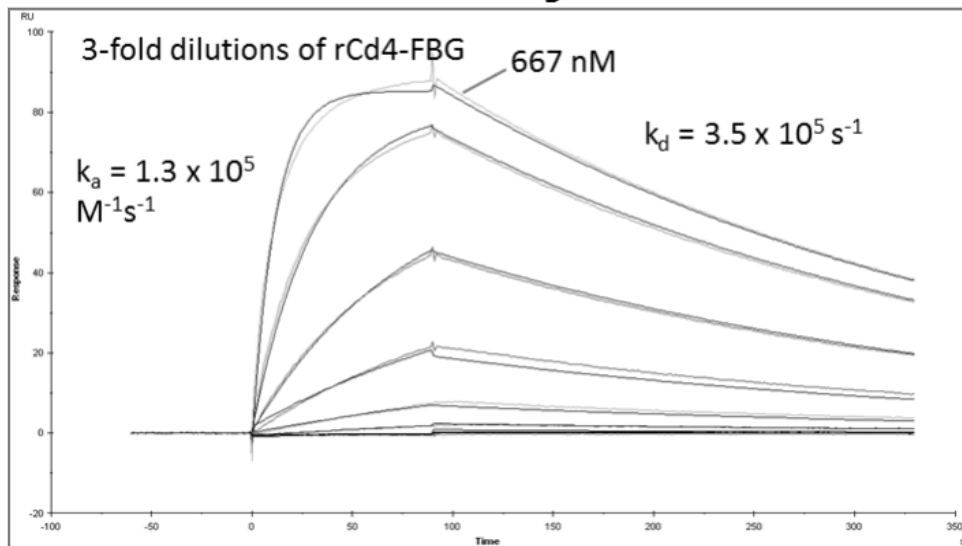
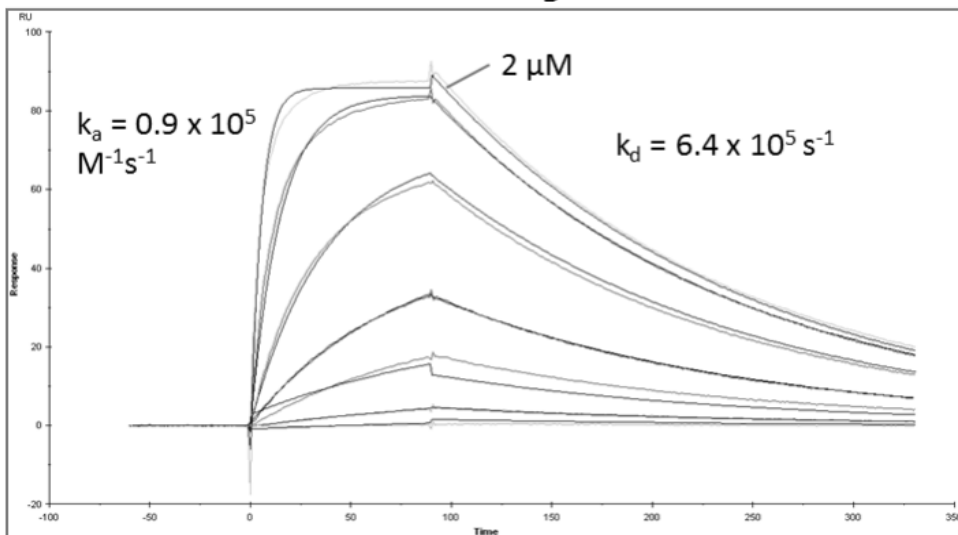
Human TNR FBG – $K_D = 0.71 \mu\text{M}$



Assay format = Fab capture (23 nM) on anti- $\text{C}_\text{L}\kappa$ / anti- $\text{C}_\text{L}\lambda$ 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- $C_L\kappa$ / anti- $C_L\lambda$ CM5 sensor chip followed by rCd4-FBG injection. Regeneration with 10 mM Glycine, pH2

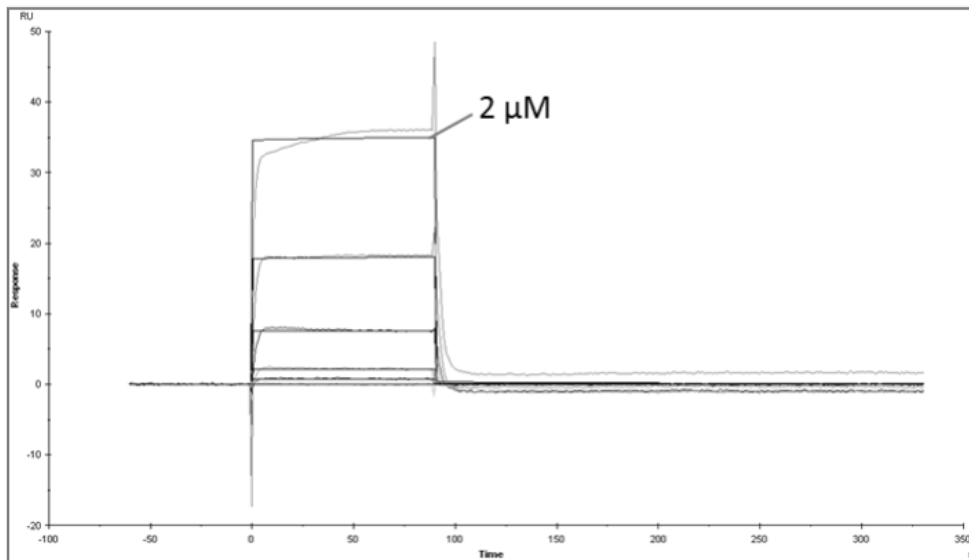
Figure 6c

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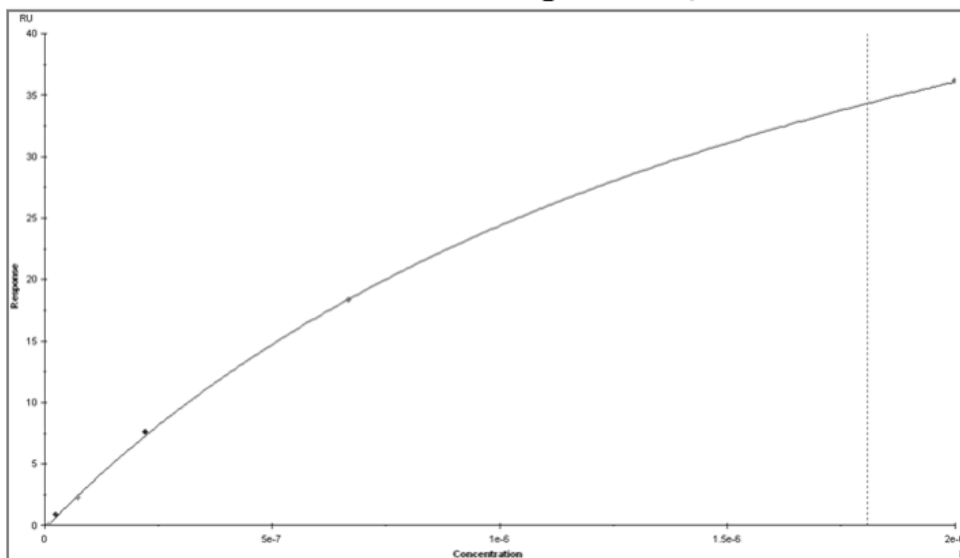
C

Fab F3 – SPR Results

Human TNR FBG – off rate too rapid



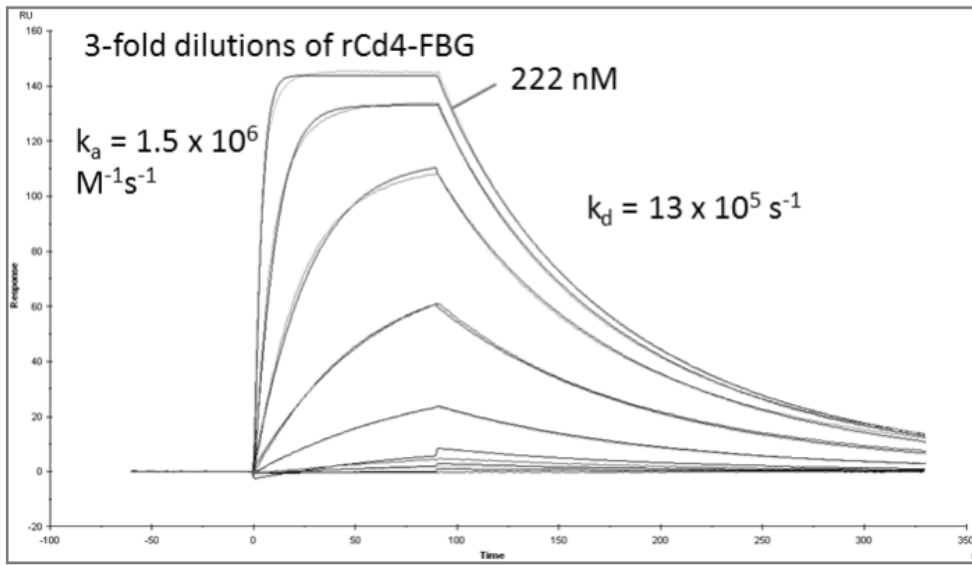
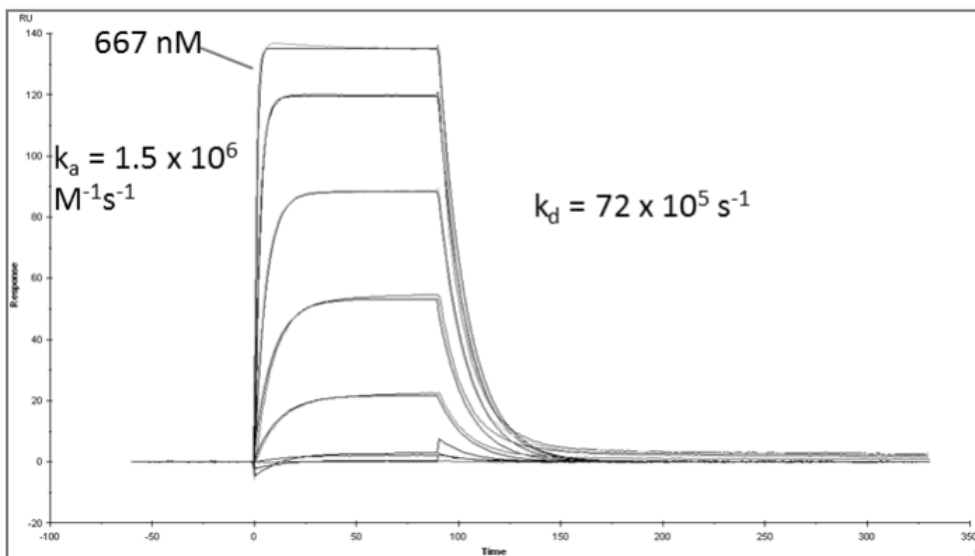
Human TNR FBG – $K_D = 1.8 \mu\text{M}$



Assay format = Fab capture (23 nM) on anti-C_Lκ / anti-C_Lλ 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- $C_L\kappa$ / anti- $C_L\lambda$ CM5 sensor chip followed by rCd4-FBG injection. Regeneration with 10 mM Glycine, pH2

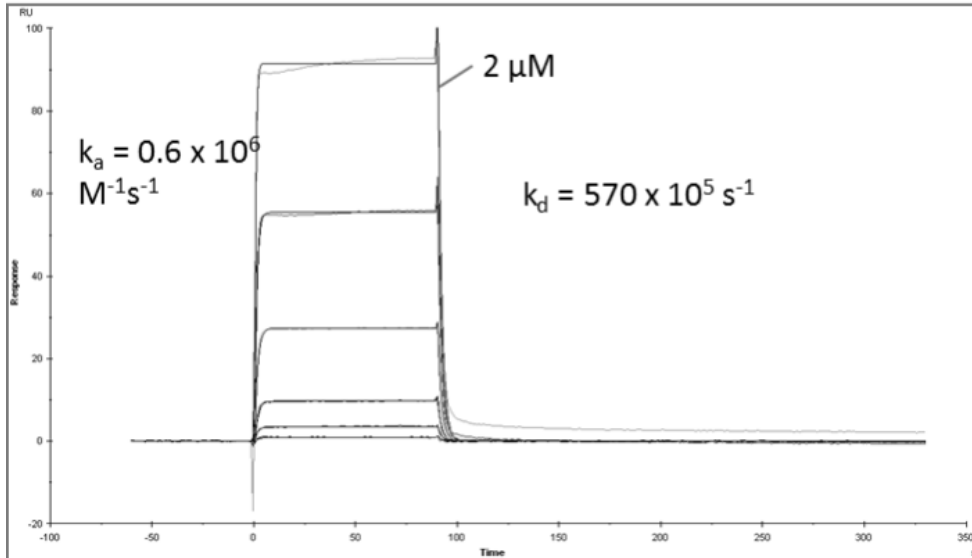
Figure 6d

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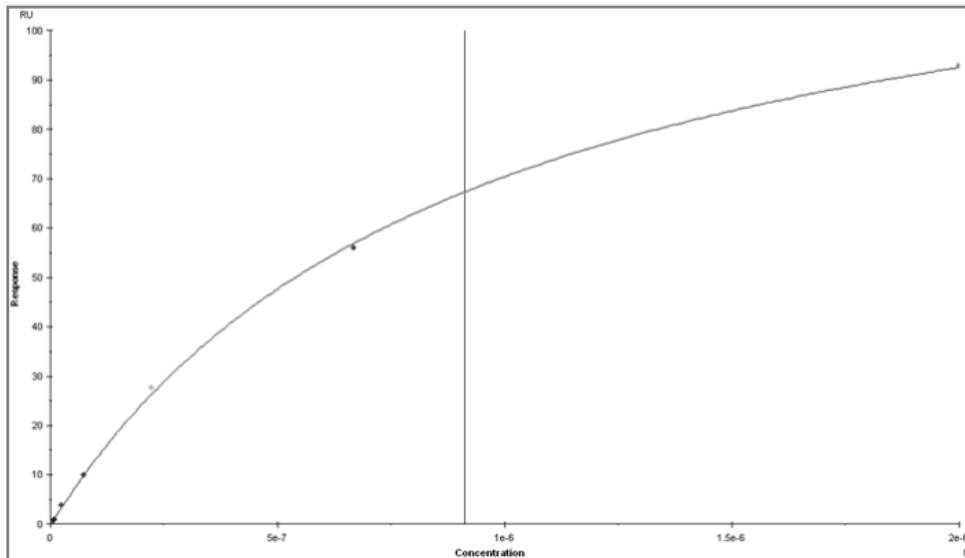
d

Fab D8 – SPR Results

Human TNR FBG – $K_D = 1.03 \mu\text{M}$



Human TNR FBG – $K_D = 1.11 \mu\text{M}$



Assay format = Fab capture (23 nM) on anti- $\text{C}_\text{L}\kappa$ / anti- $\text{C}_\text{L}\lambda$ CM5 sensor chip followed by rCd4-FBG injection. Regeneration with 10 mM Glycine, pH2

Figure 6d (Con't)

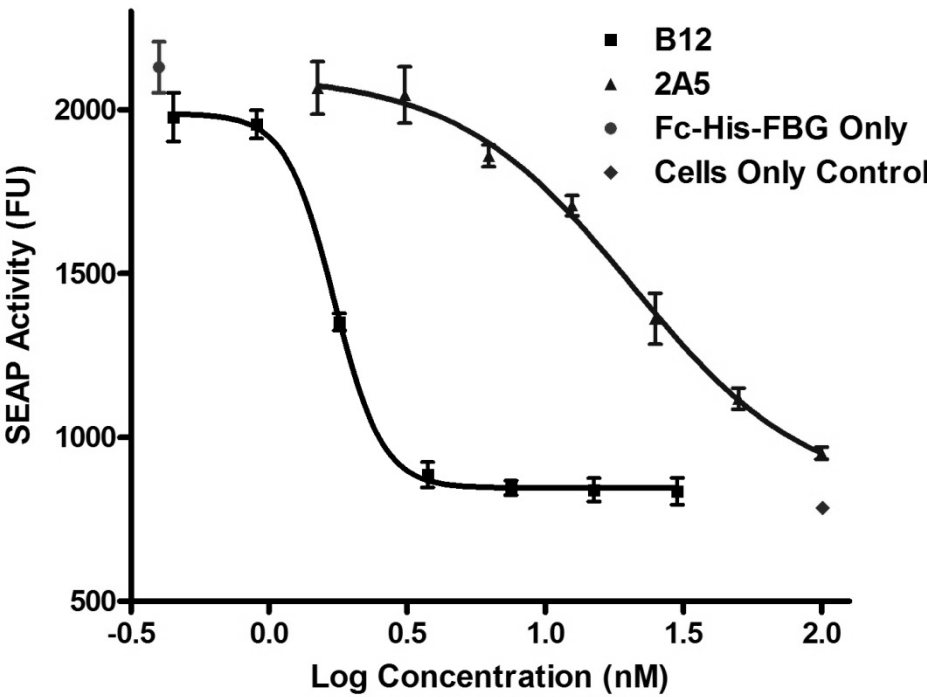
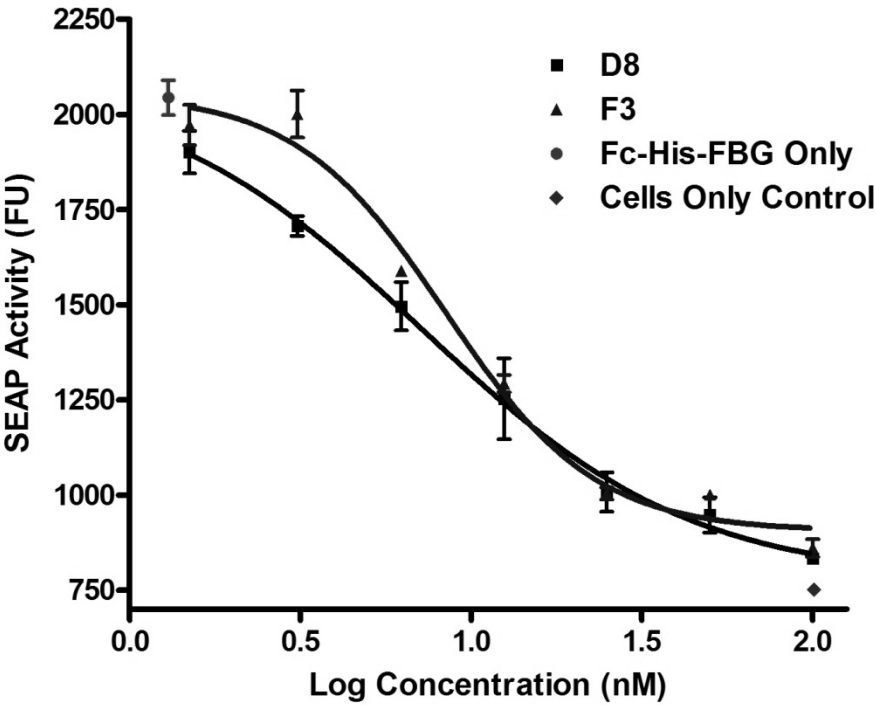


Figure 7

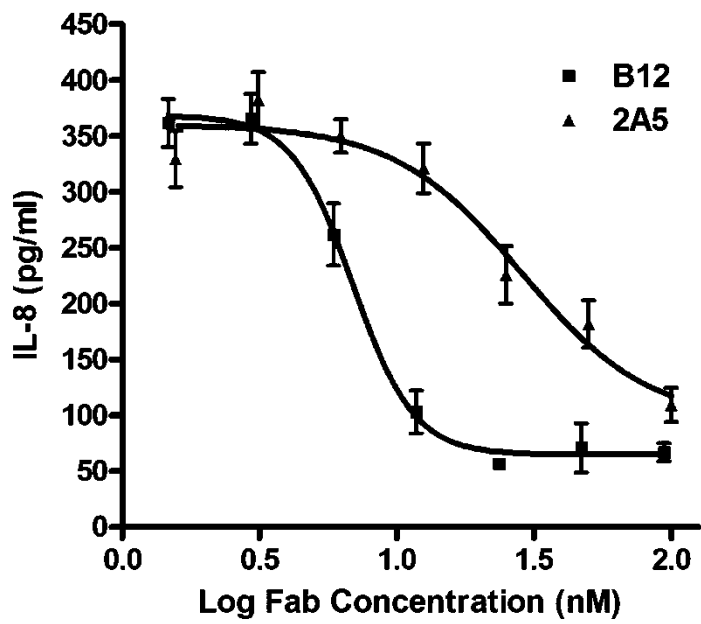
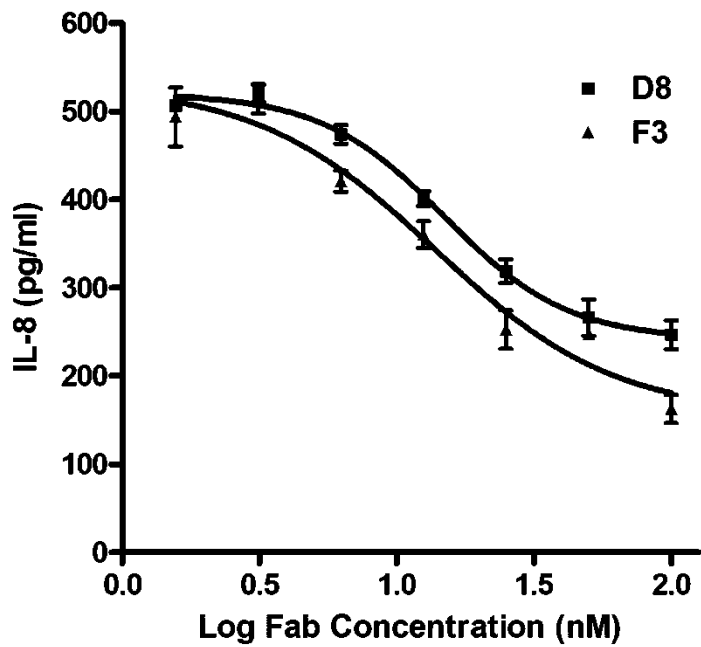
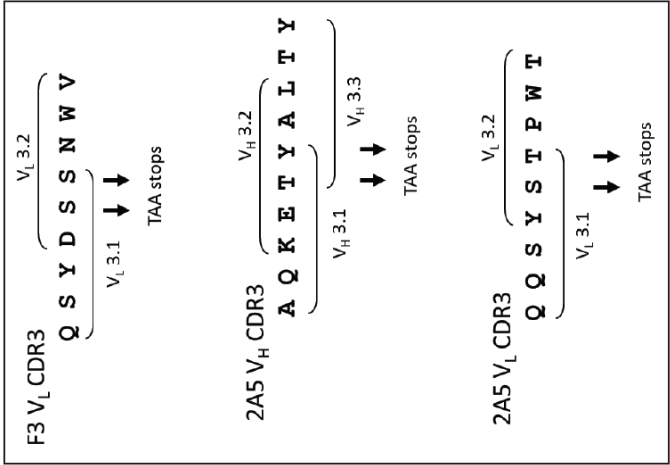


Figure 8

a

Heavy chain:



Light chain:

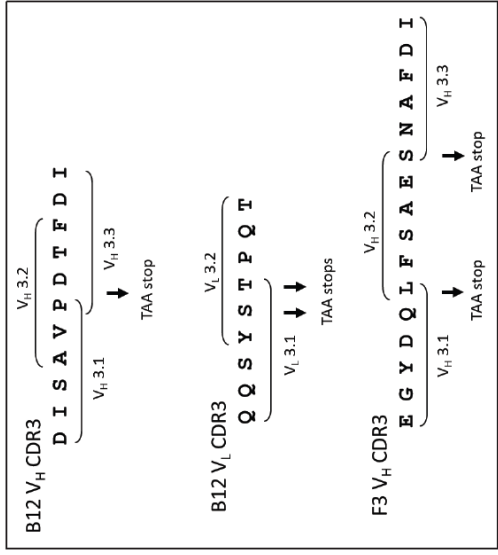


Figure 9

b

Oligonucleotides for VH and VL CDR3 mutagenesis

F3 VH 3.1	AGCACTACTCTCAGCAGAAAAA	NSNSNSNSNSNNCTCGCACAGTAATACAGCCGTGTC	SEQ ID NO: 97
F3 VH 3.2	CTGGCCCCAGATATCAAAAGCATTS	NSNSNSNSNSNNTTGGTCATAGCCTTCTCTGCACAG	SEQ ID NO: 98
F3 VH 3.3	GGTGACCAGGGTTCCCTGGCCCCAS	NSNSNSNSNNCTCAGCAGAAAAATAGTTGGTCATAGCC	SEQ ID NO: 99
F3 VL 3.1	GGTCCCTCCGCCGAAACACACCAATTS	NSNSNSNSNNACAGTAGTCAGCCTCGTCCTC	SEQ ID NO: 100
F3 VL 3.2	GGTGACCTTGGTCCCTCCGCCGAAS	NSNSNSNNATAAGACTGACAGTAGTCAGC	SEQ ID NO: 101
B12 VH 3.1	CTTGCCCCAGATATCAAAAGTATCS	NSNSNSNNNTTTTGCACAGTAATACAAGGCCGTGTC	SEQ ID NO: 102
B12 VH 3.2	CATTGTCCCTTGGCCCCAGATATCS	NSNSNSNNCGAGATATCTTTGCACAGTAATAC	SEQ ID NO: 103
B12 VH 3.3	GGTGACCAATTGTCCCTTGGCCCCAS	NSNSNSNNNGACTGCCGAGATATCTTTTGCACAG	SEQ ID NO: 104
B12 VL 3.1	GGTCCCTTGGCCGAACGCTGAGGS	NSNSNSNNACAGTAGTAAGTTGCAAAATCTTC	SEQ ID NO: 105
B12 VL 3.2	GATATCCACTTTGGTCCCTTGGCCGAAS	NSNSNNNACTCTGTTGACAGTAGTAAGTTGC	SEQ ID NO: 106
2A5 VH 3.1	GGTTCCCTGGCCCCAGTAGGTCAAGGC	NSNSNSNNNTGTTGCACAGTAATACAGGCCGT	SEQ ID NO: 107
2A5 VH 3.2	CAGGGTTCCCTGGCCCCAGTAGGTS	NSNSNSNNCTGGGCTGTTGCACAGTAATACAC	SEQ ID NO: 108
2A5 VH 3.3	GGTGACCAGGGTTCCCTGGCCCCAS	NSNSNNCTCTTTCTGGGCTGTTGCACAGTA	SEQ ID NO: 109
2A5 VL 3.1	GGTCCCTTGGCCGAACGCTCCAGGS	NSNSNNACAGTAGTAAGTTGCAAAATCTTC	SEQ ID NO: 110
2A5 VL 3.2	CTCCACCTTGGTCCCTTGGCCGAAS	NSNSNNNACTCTGTTGACAGTAGTAAGTTGC	SEQ ID NO: 111

Figure 9 (con't)

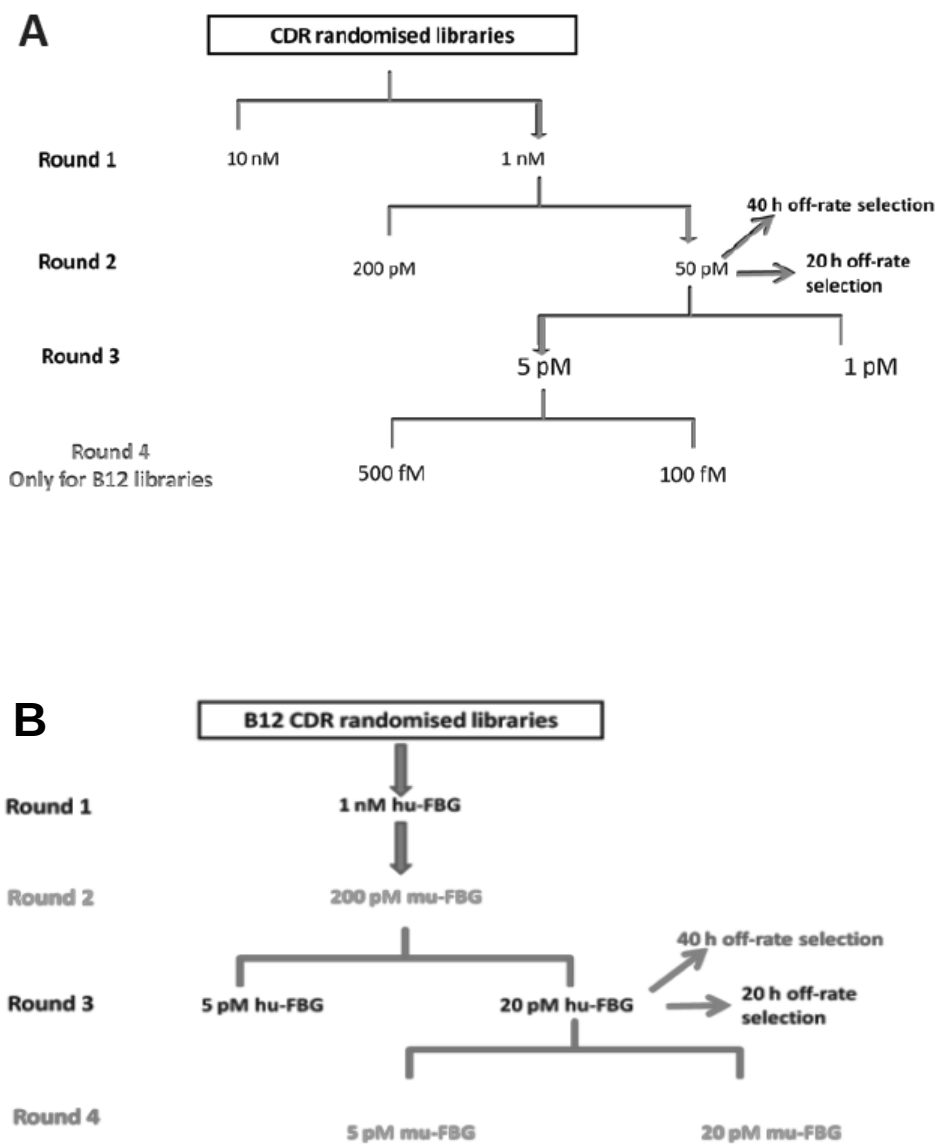


Figure 10

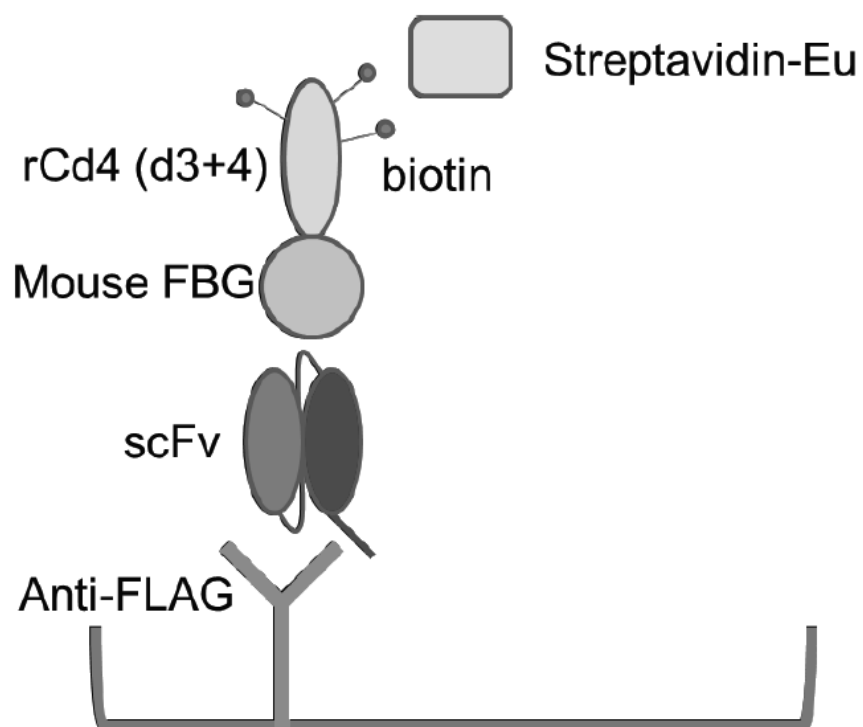


Figure 11

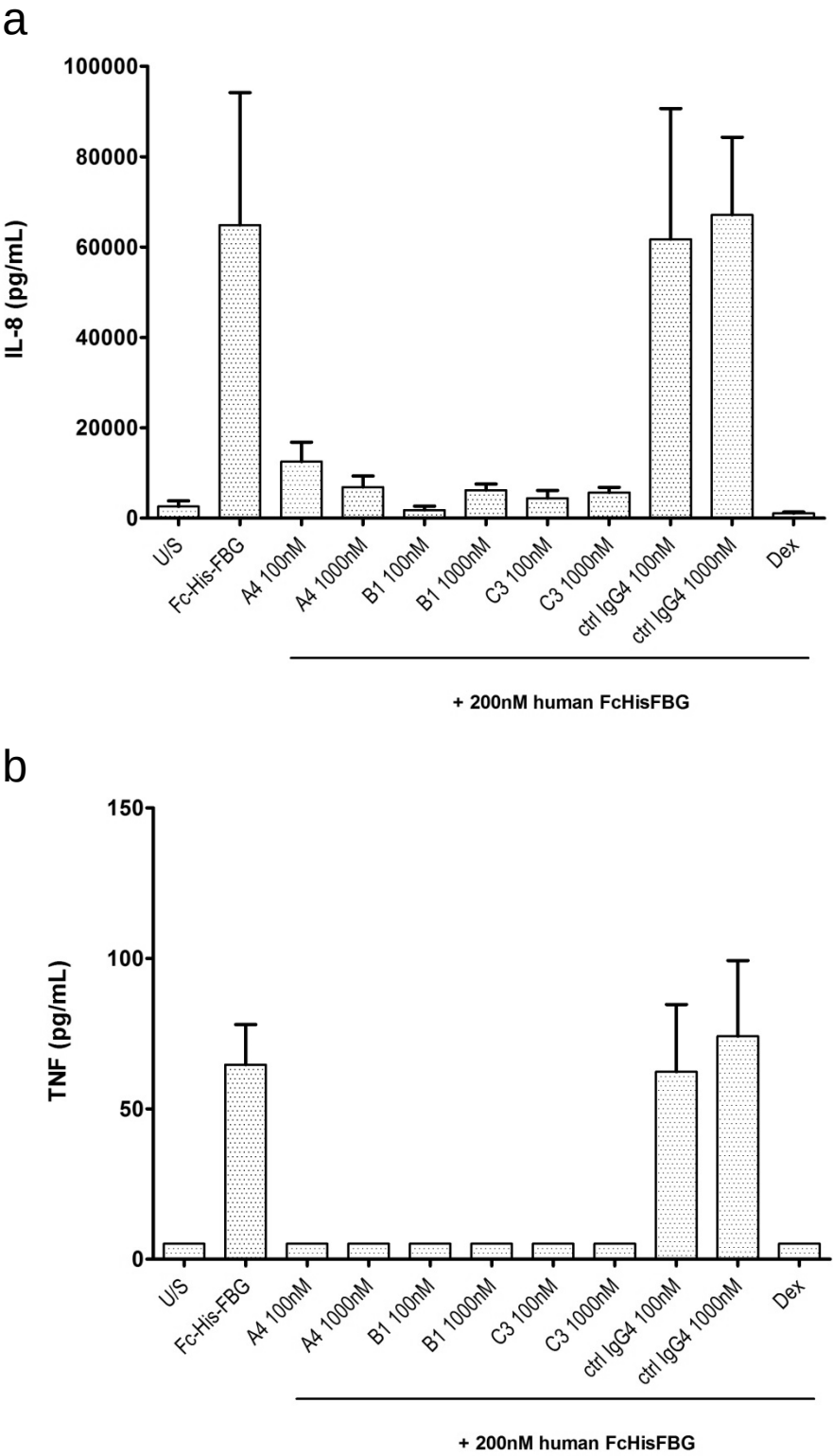


Figure 12

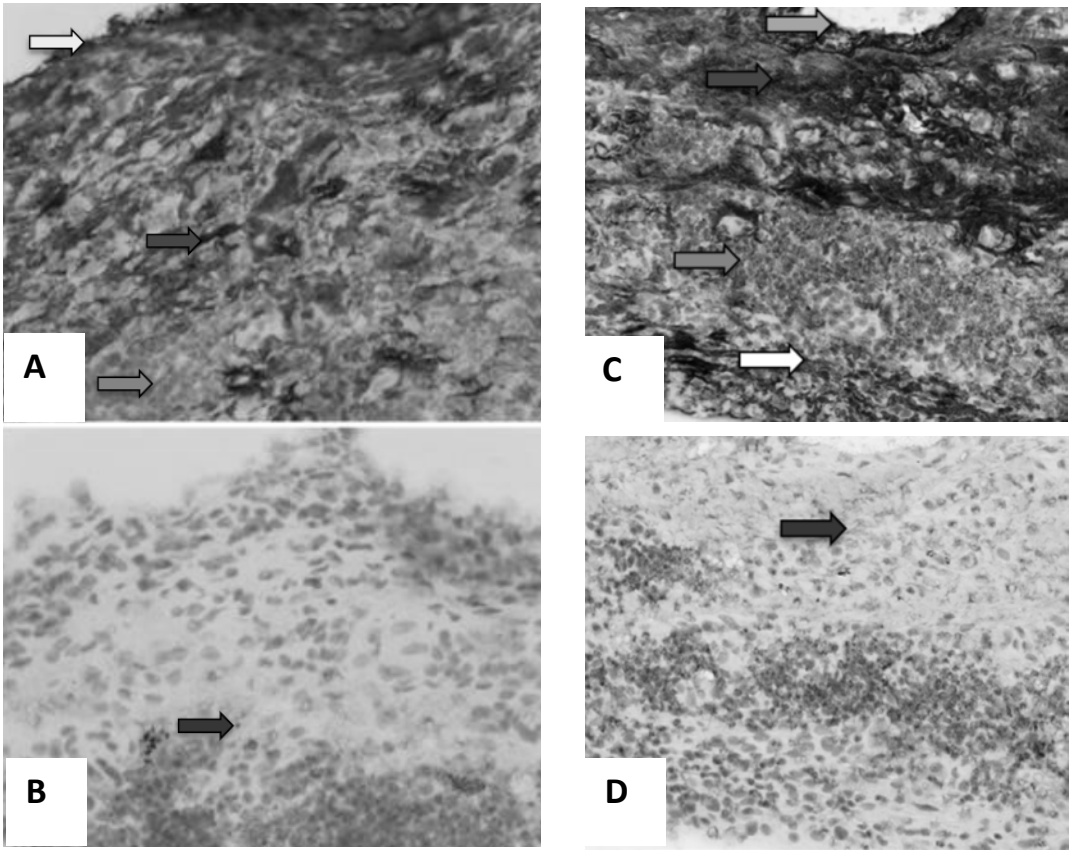


Figure 13

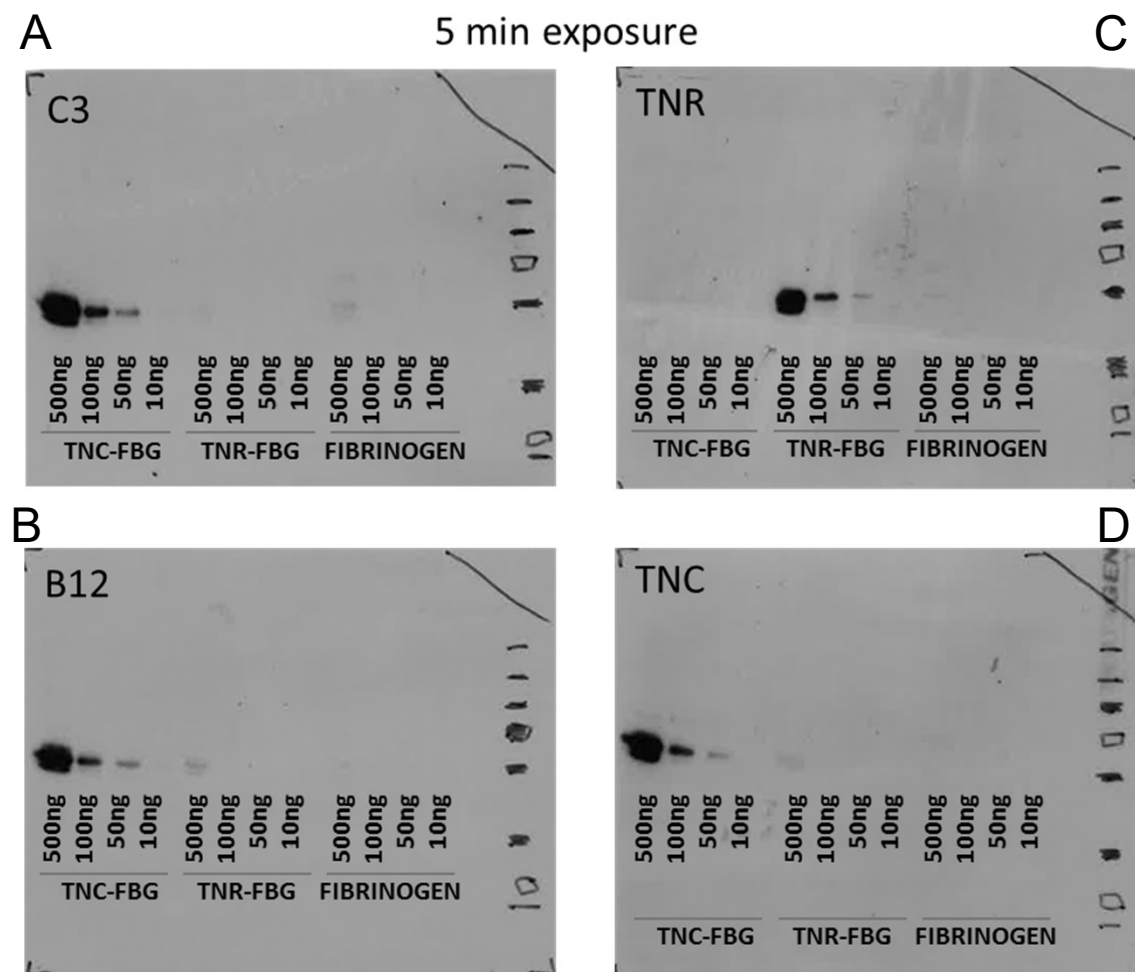


Figure 14

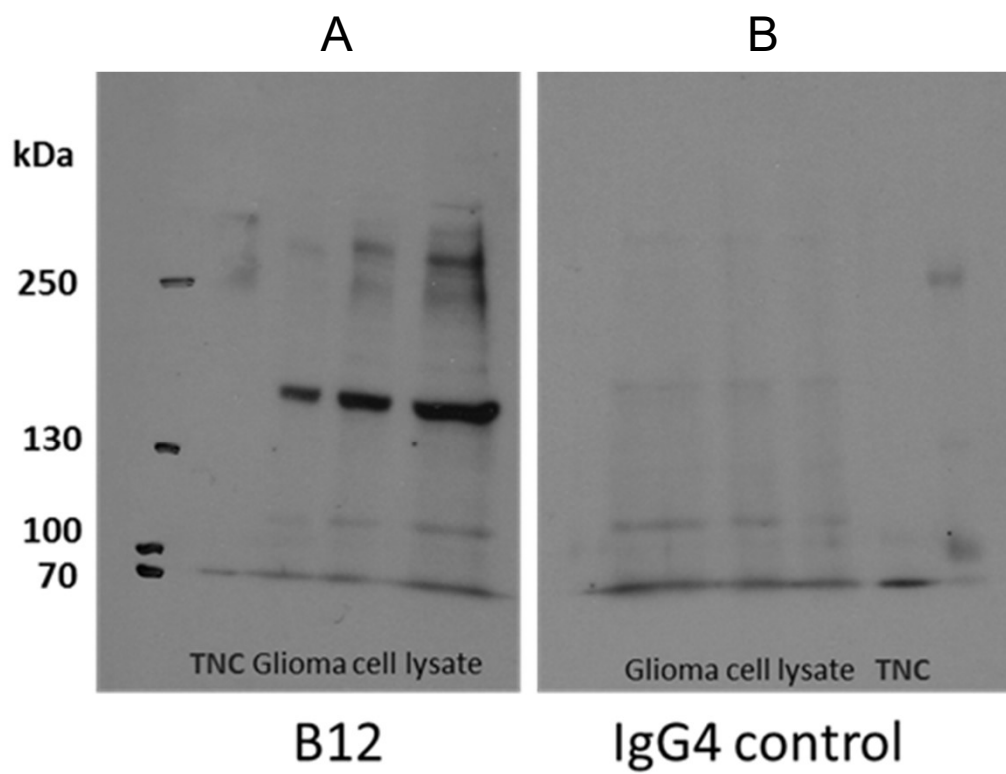
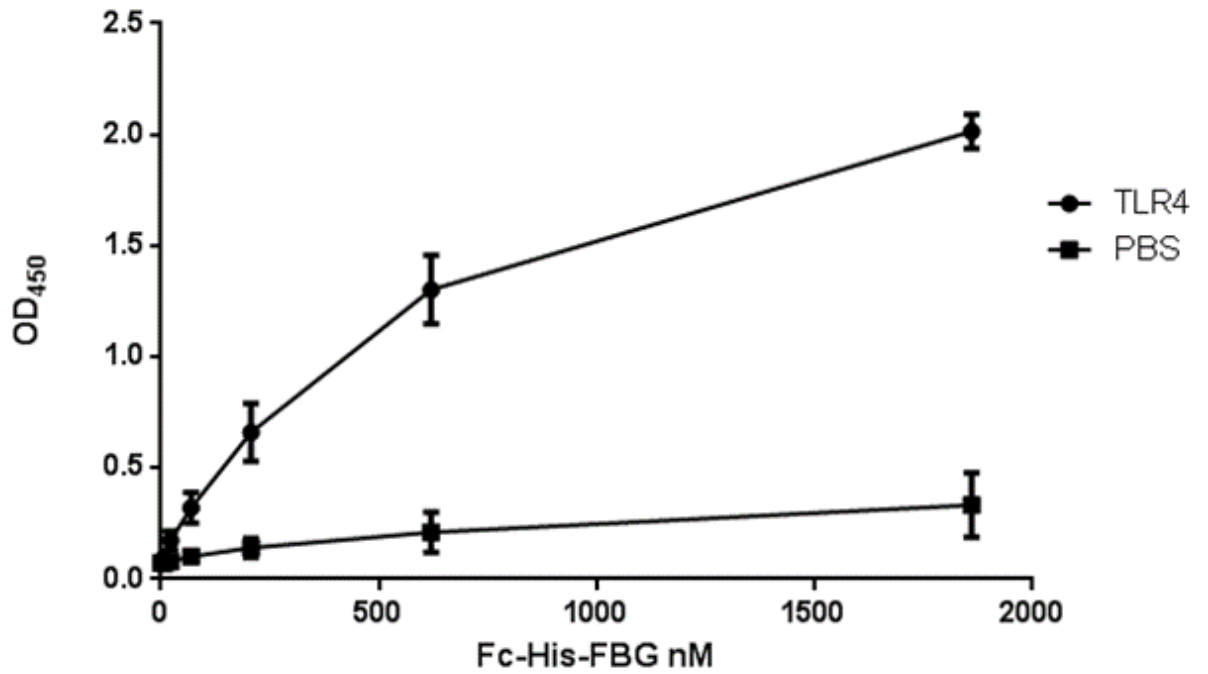


Figure 15

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A



B

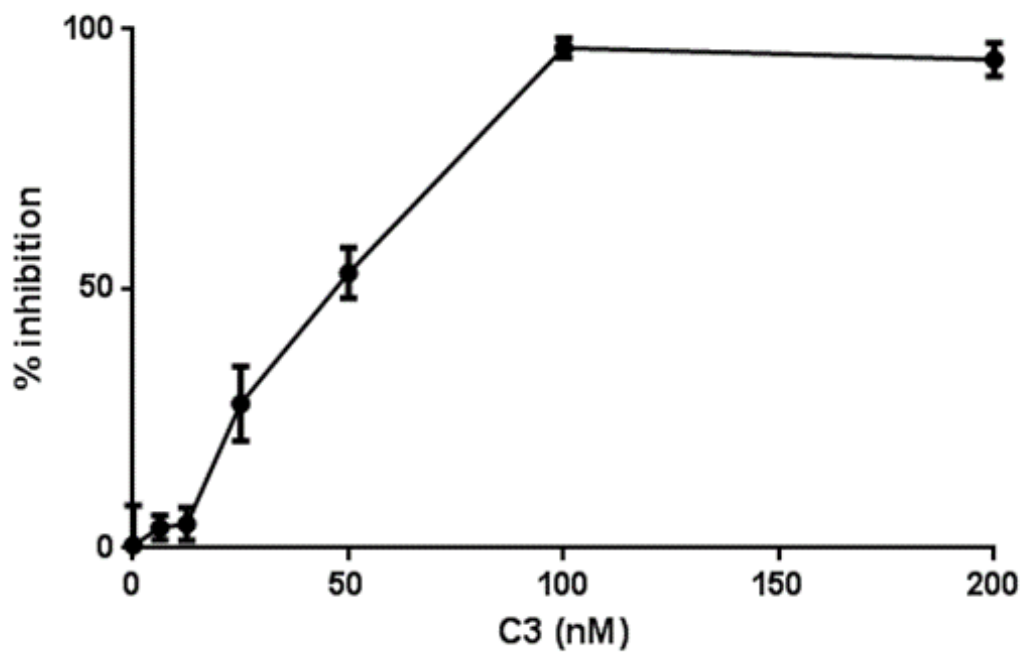


Figure 16

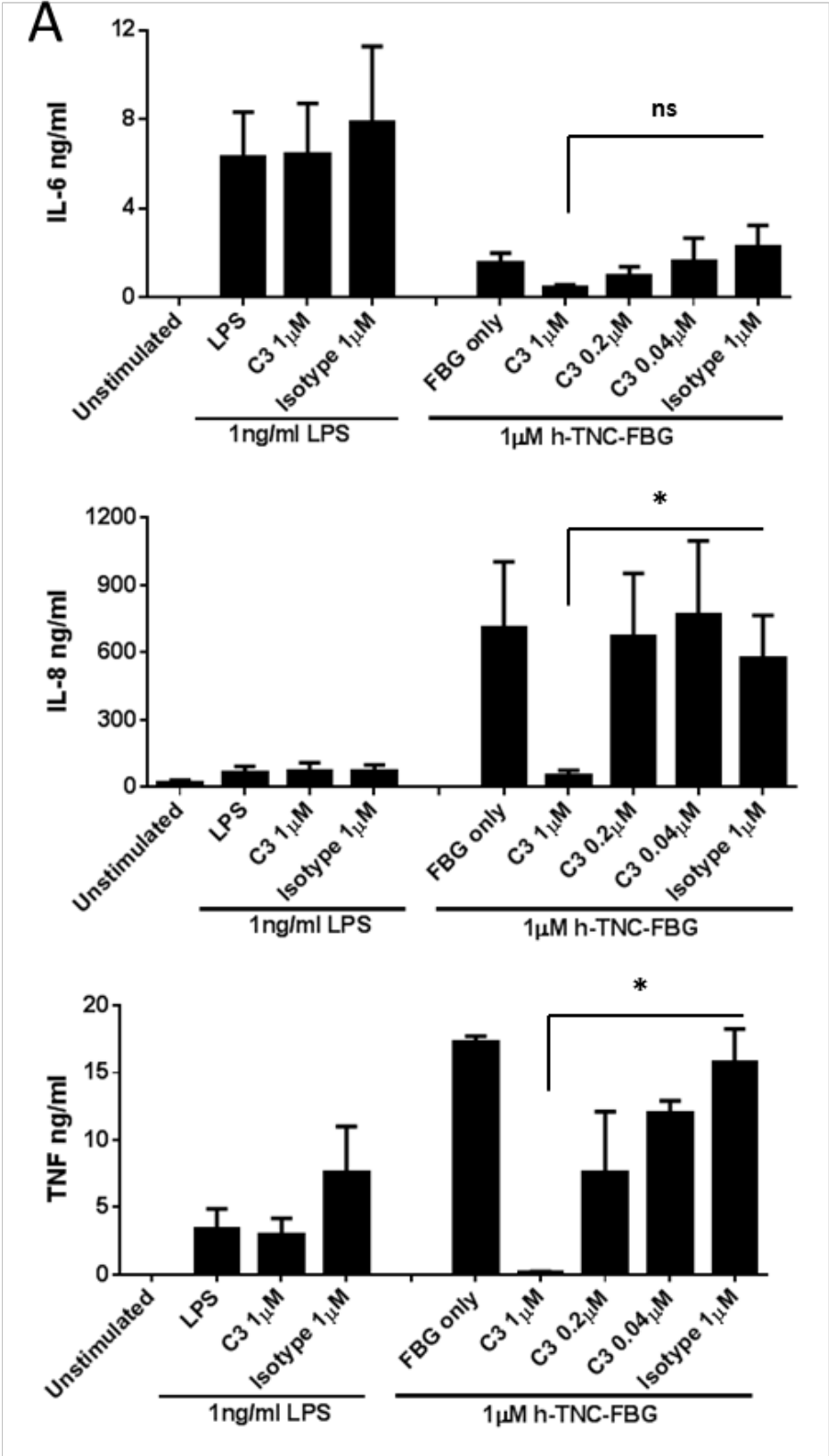


Figure 17

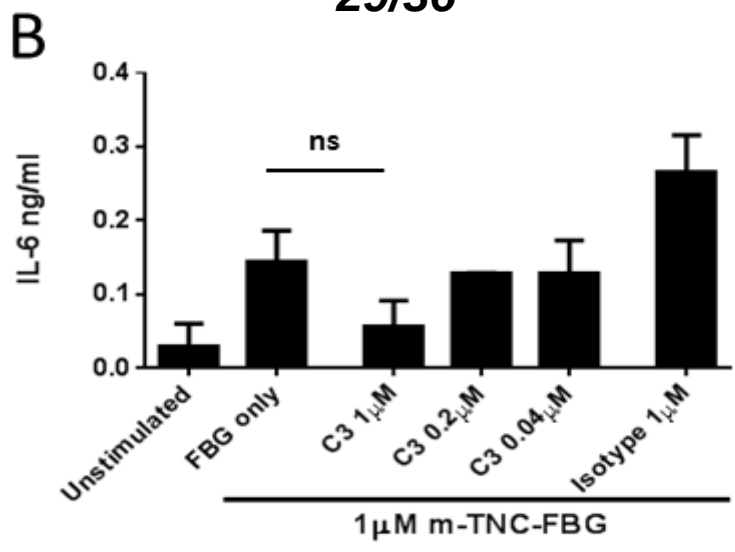
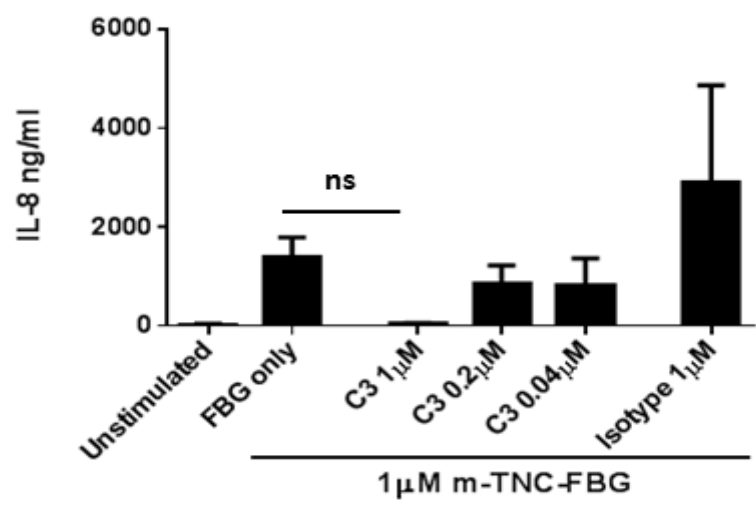
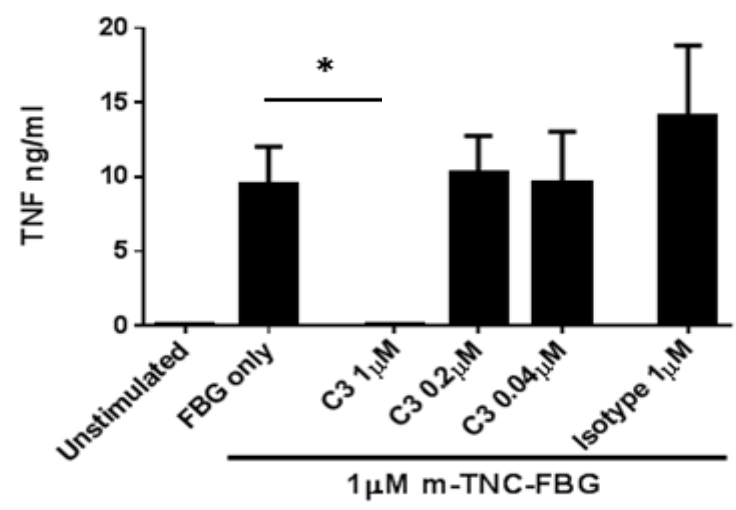


Figure 17 cont'd

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C

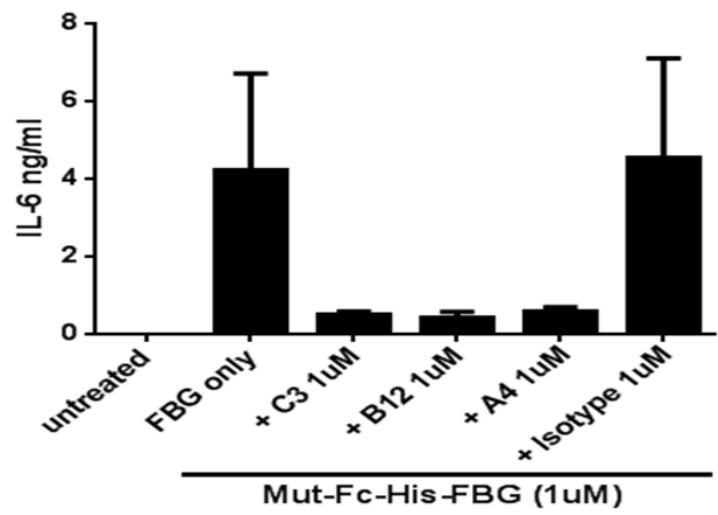
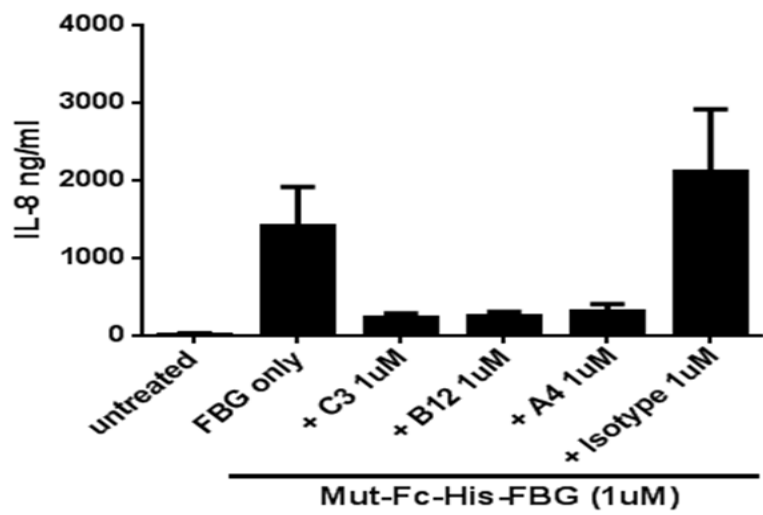
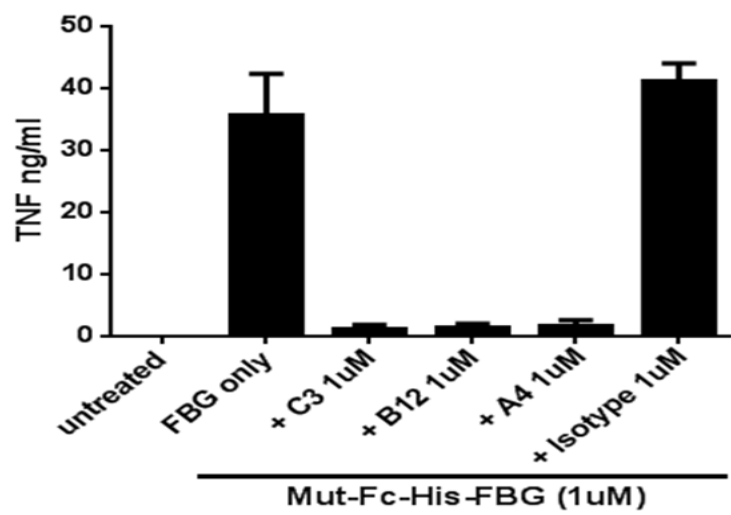


Figure 17 cont'd

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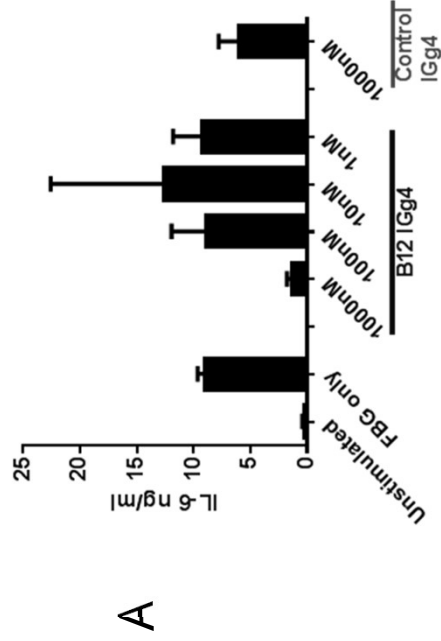
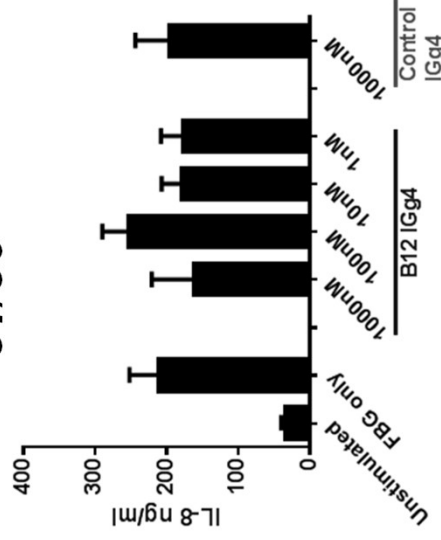
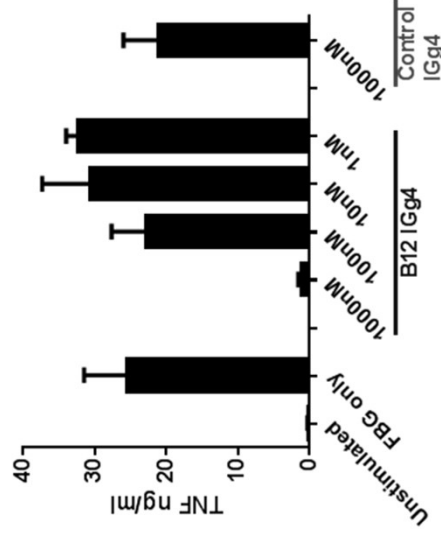
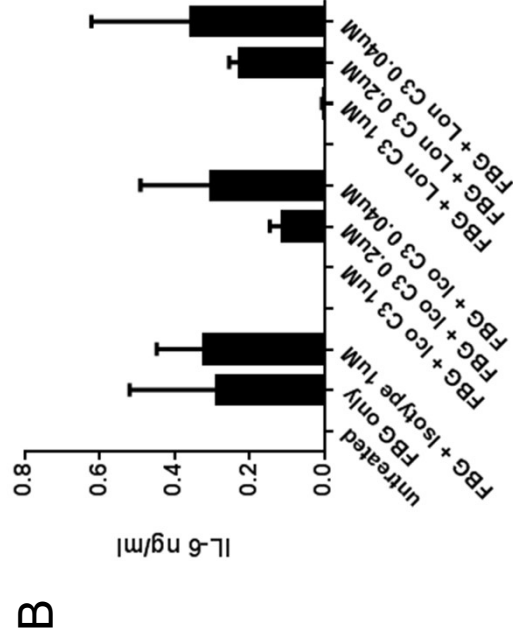
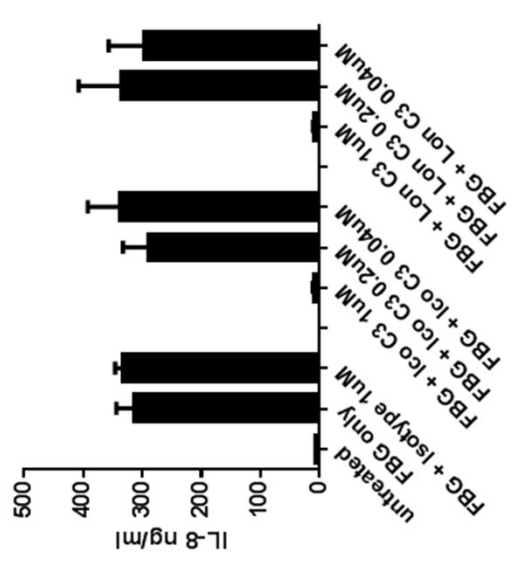
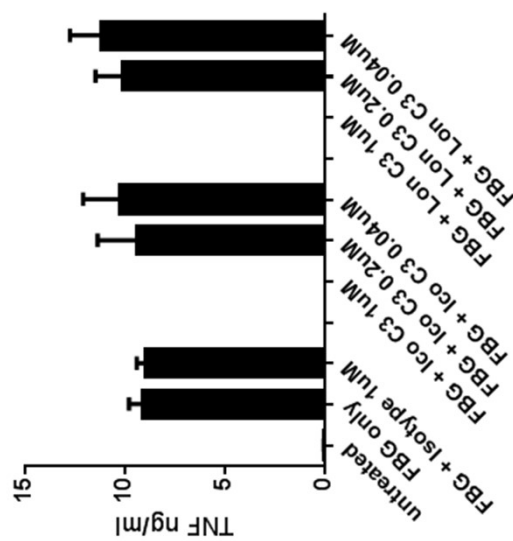


Figure 18

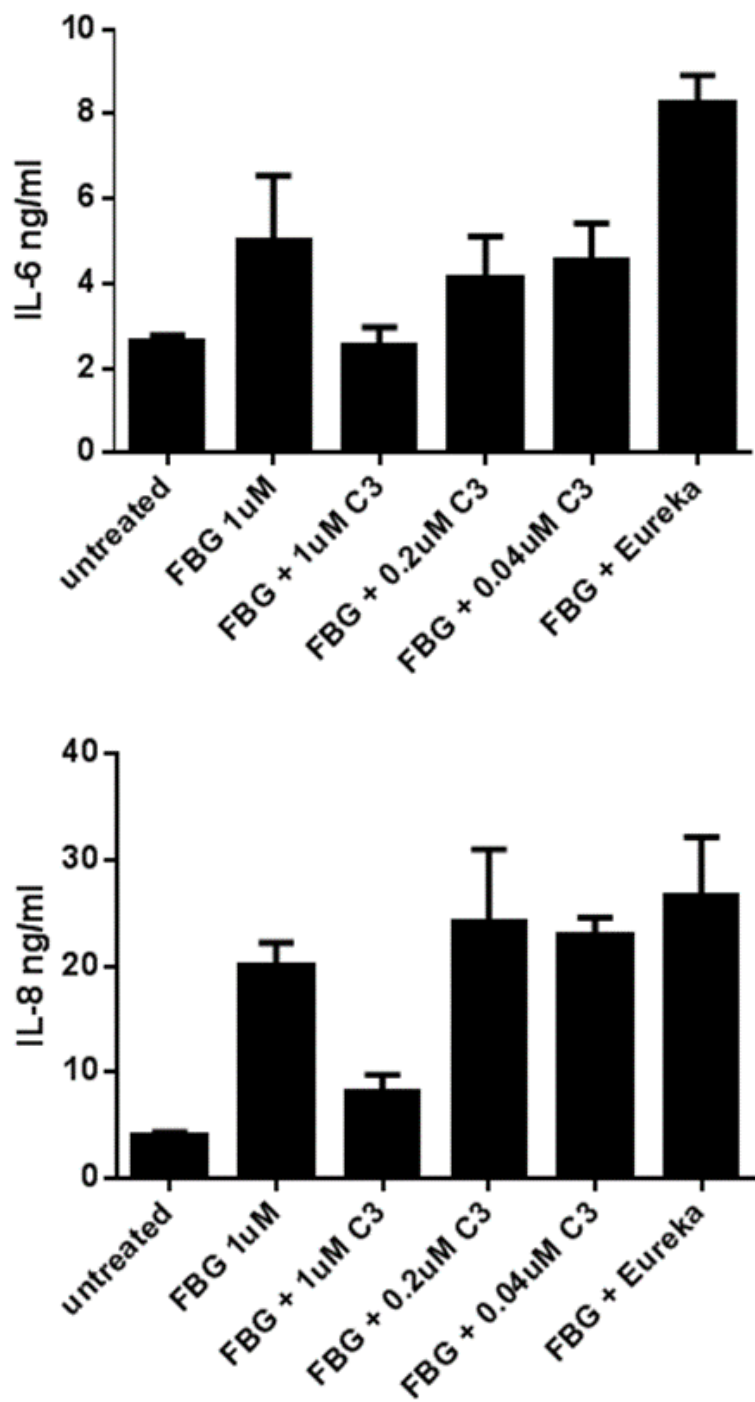


Figure 19

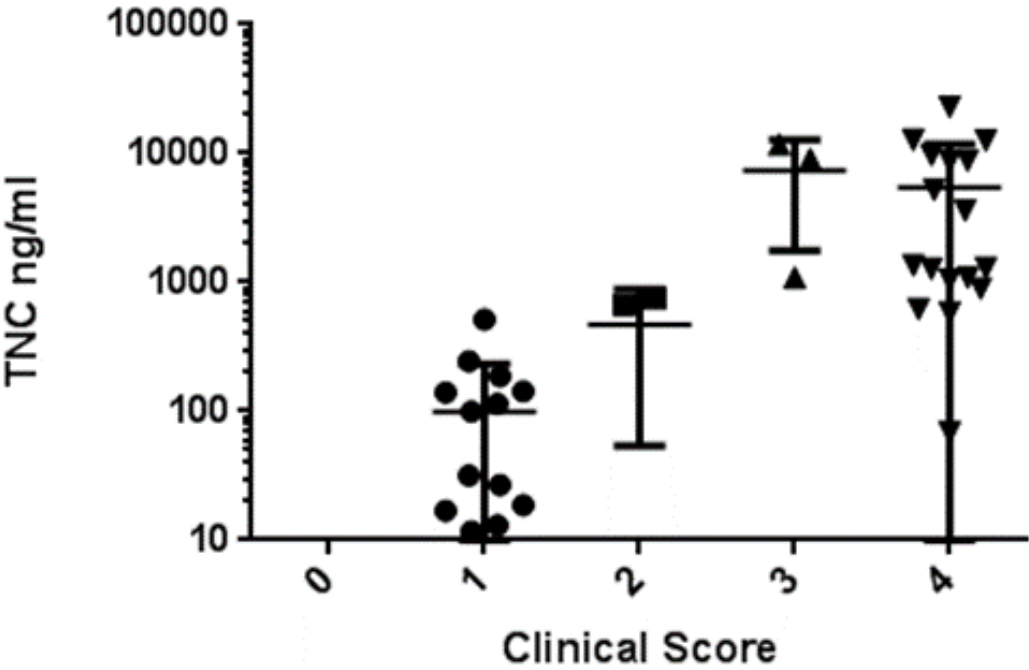


Figure 20

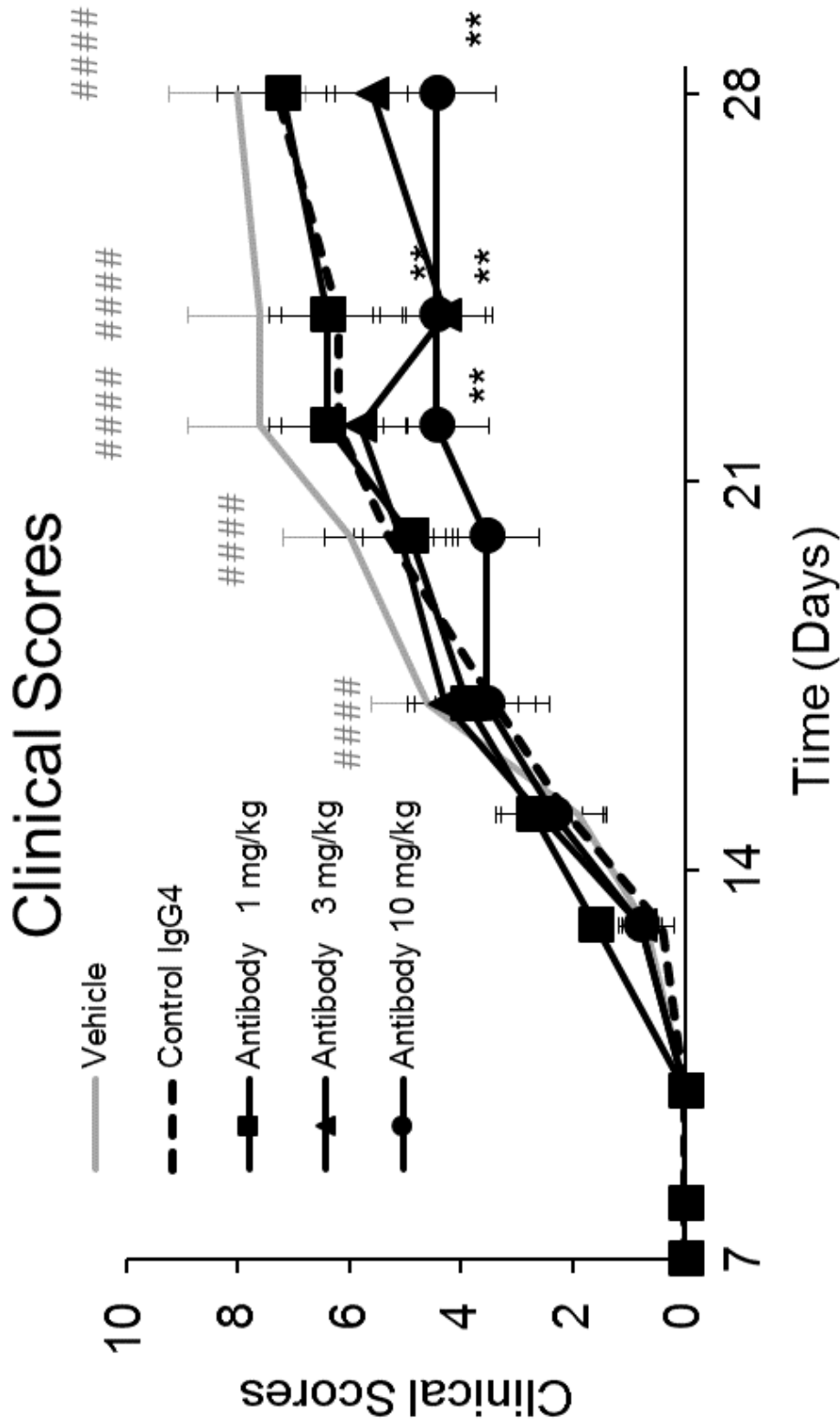


Figure 21

Hind Paw Volumes

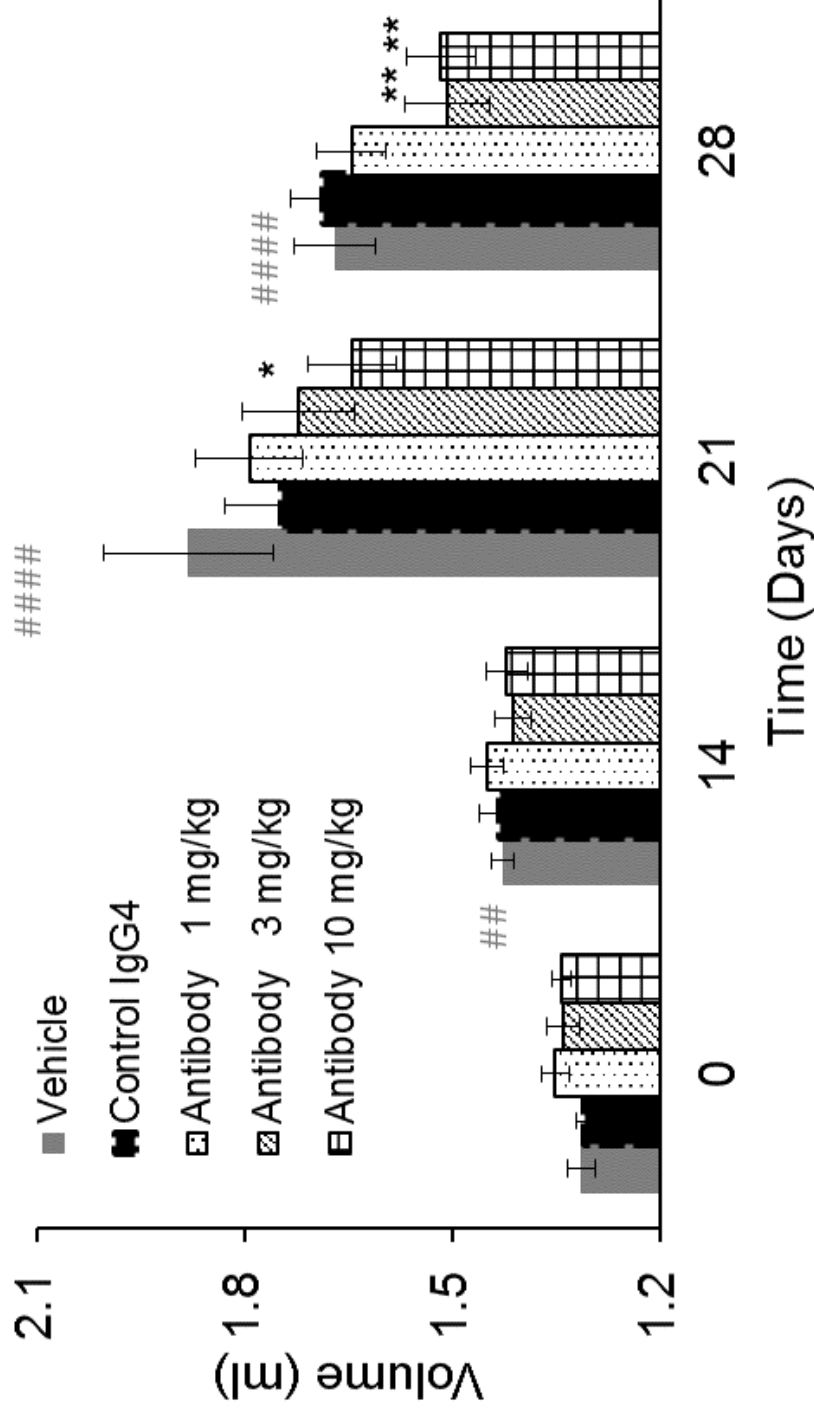


Figure 22

Construct	N-term	Species	Primer name (F)	Primer sequence (F)	Primer name (R)	Primer sequence (R)
FBG-X	minus	human	2561	GGTACCTCGCGAATGCATCTAG	2562	CATGCAGGCCCTCTGCAGTCG
FBG-X	plus	human	2565	TTTTTCCATGGCCCGAGATTGGACTCCTGTACCCCTT CCCCAAAGATTGCTCTCAGGC	2562	CATGCAGGCCCTCTGCAGTCG
FBG-X	minus	mouse	2561	GGTACCTCGCGAATGCATCTAG	2562	CATGCAGGCCCTCTGCAGTCG
FBG-X	plus	mouse	2566	TTTTTCCATGGCCCGAGATTGGACTCCTGTACCCCTT CCCTCGCGACTGCTCACAG	2562	CATGCAGGCCCTCTGCAGTCG
X-FBG	minus	human	2567C	TTTTTGGATGCCCATCATCATCACCATCAGTTCCCCA AAGATTGCTCTCAGGC	2570	TTTTTTAAGCTTTTATTACGCCCGT TTACGCCGACCCCTC
X-FBG	plus	human	2567	TTTTTGGATGCCCATCATCATCACCATCAGATTGGAC TCCTGTACCCCTTCCCAAGATTGCTCTCAGGC	2570	TTTTTTAAGCTTTTATTACGCCCGT TTACGCCGACCCCTC
X-FBG	minus	mouse	2569C	TTTTTGGATGCCCATCATCATCACCATCAGTTCCCTC GCGACTGCTCACAG	2571	TTTTTTAAGCTTTTATTACGCCCGT TTCCGCCGACCTTC
X-FBG	plus	mouse	2568C	TTTTTGGATGCCCATCATCATCACCATCAGATTGGAC TCCTGTACCCCTTCCCTCGCGACTGCTCACAG	2571	TTTTTTAAGCTTTTATTACGCCCGT TTCCGCCGACCTTC
BamHI- His6-HindIII	N/A	N/A	2574	TTTTTGGATGCCCATCATCATCACCATCAGTAATAAAA G	2575	TTTTTTAAGCTTTTATTAGTGATGG TGATGATGATGGG
His-FBG	plus	human	2580	TTTTTCTCGAGCATCATCATCACCATCAGATTGGAC TCC	2570	TTTTTTAAGCTTTTATTACGCCCGT TTACGCCGACCCCTC
His-FBG	plus	mouse	2580	TTTTTCTCGAGCATCATCATCACCATCAGATTGGAC TCC	2571	TTTTTTAAGCTTTTATTACGCCCGT TTCCGCCGACCTTC

Figure 23