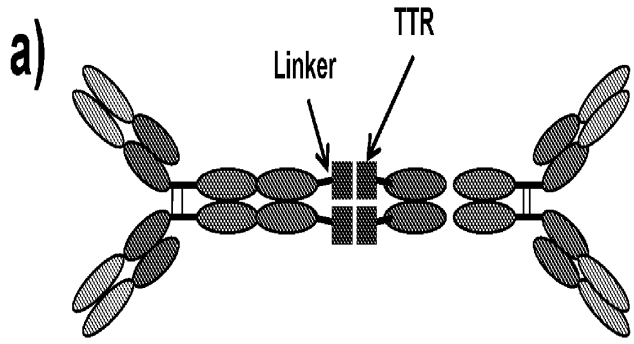
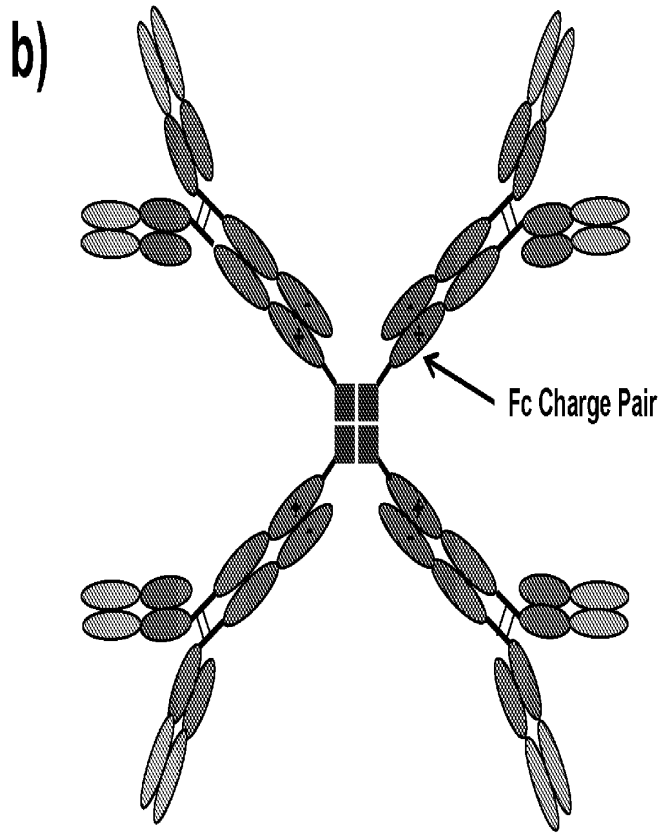
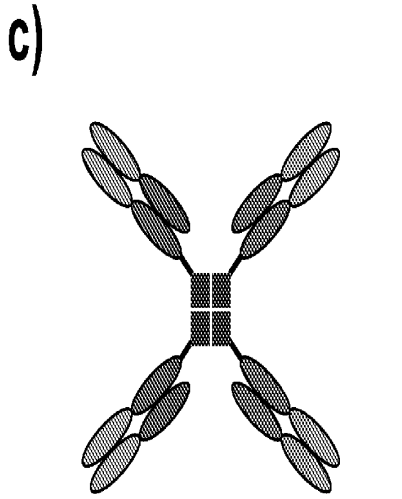
Figure 1



TTR antibody homodimer fusion protein

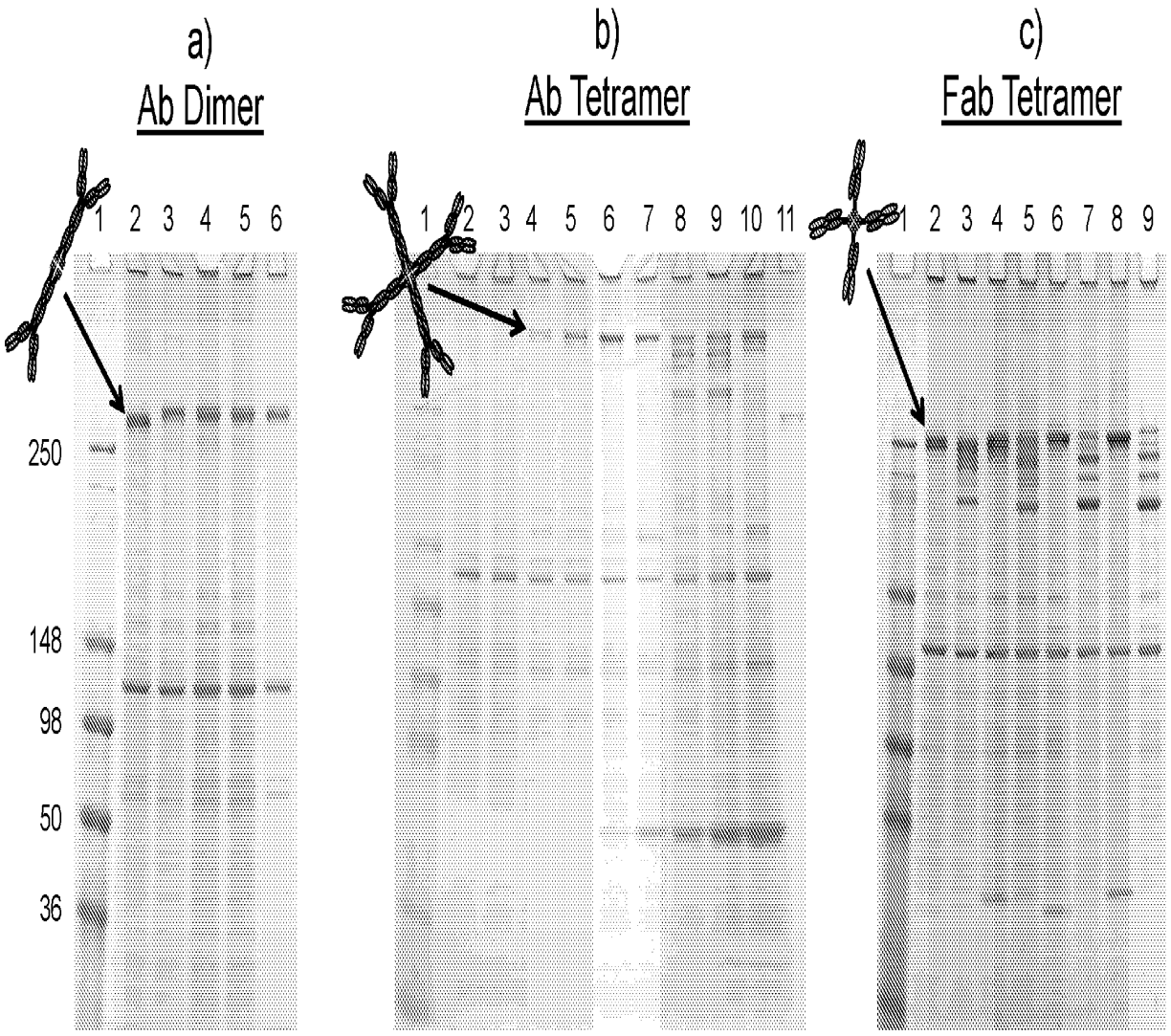


TTR antibody homotetramer fusion protein



TTR Fab homotetramer fusion protein

Figure 2



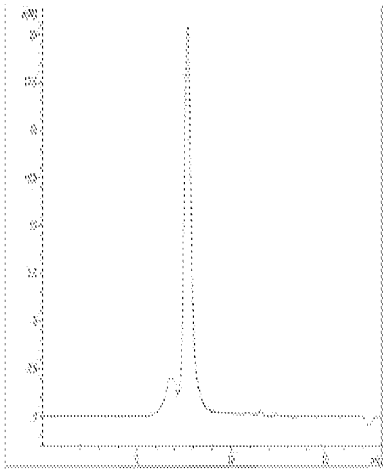
Lane 2: No linker
Lane 3: GGGGS linker
Lane 4: $(GGGGS)_2$ linker
Lane 5: $(GGGGS)_3$ linker
Lane 6: $(GGGGS)_4$ linker

DNA ratios:
Lane 2: LC:HC = 1:9
Lane 3: LC:HC = 2:8
Lane 4: LC:HC = 3:7
Lane 5: LC:HC = 4:6
Lane 6: LC:HC = 5:5
Lane 7: LC:HC = 6:4
Lane 8: LC:HC = 7:3
Lane 9: LC:HC = 8:2
Lane 10: LC:HC = 9:1
Lane 11: 100 ng anti-DNP Ab

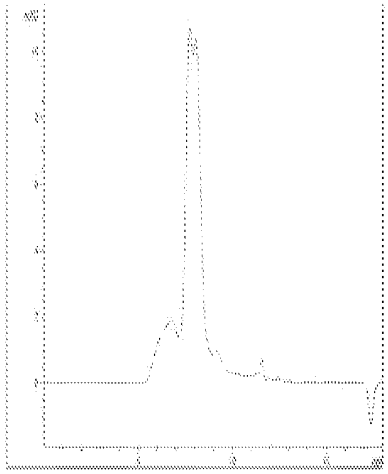
Lane 2: LC + HC-TTR-His
Lane 3: HC + LC-TTR-His
Lane 4: LC-His + HC-TTR
Lane 5: HC-His + LC-TTR
Lane 6: LC(CL+) + HC(CH1-)-TTR-His
Lane 7: HC(CH1-) + LC(CL+)-TTR-His
Lane 8: LC(CL+)-His + HC(CH1-)-TTR
Lane 9: HC(CH1-)-His + LC(CL+)-TTR

Figure 3

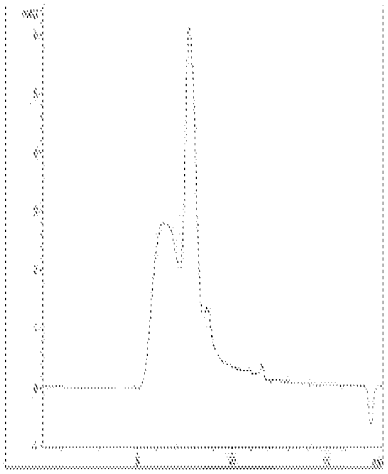
Ab-TTR



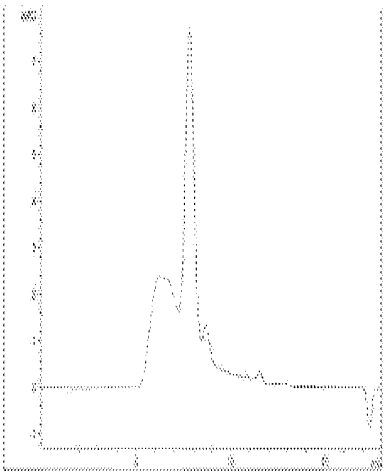
b) Ab-$(G4S)_1$-TTR



c) Ab-$(G4S)_2$-TTR



d) Ab-$(G4S)_3$-TTR



e) Ab-$(G4S)_4$-TTR

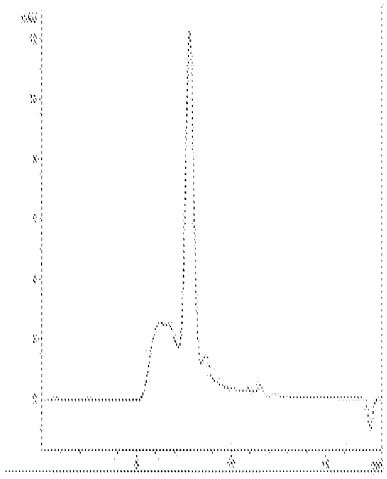


Figure 4

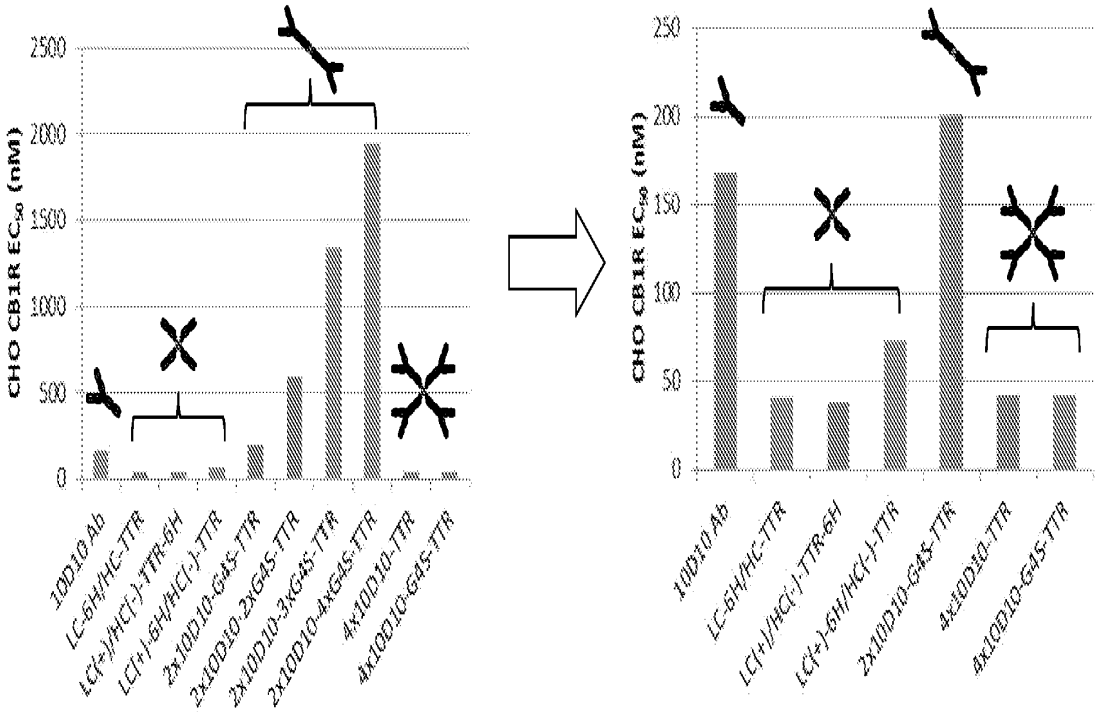
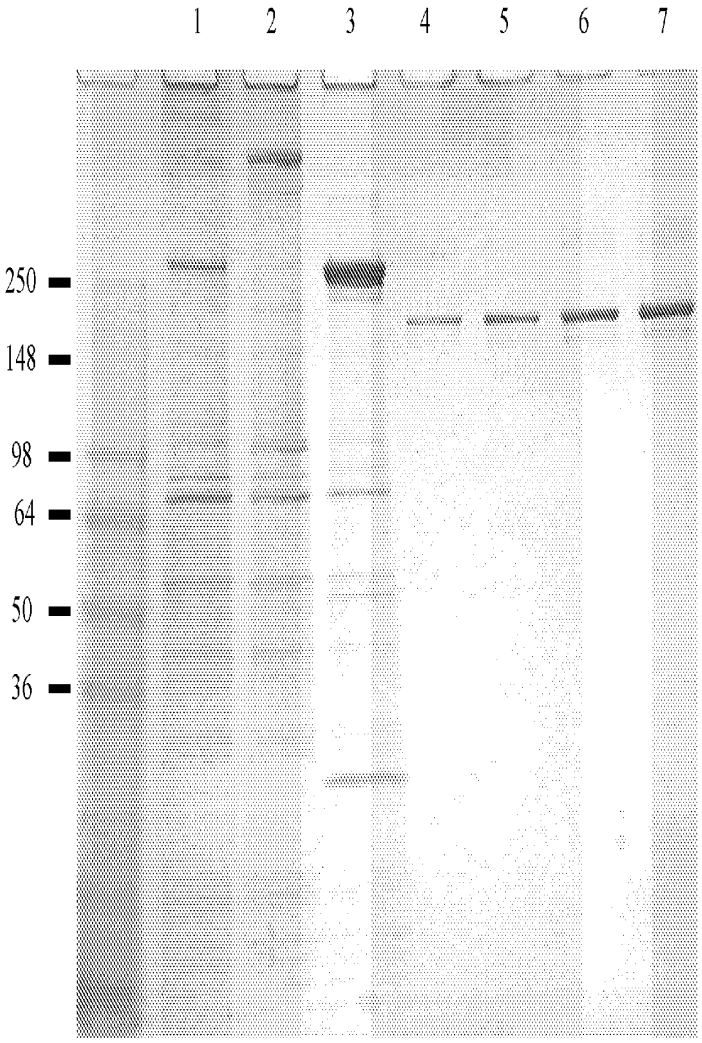
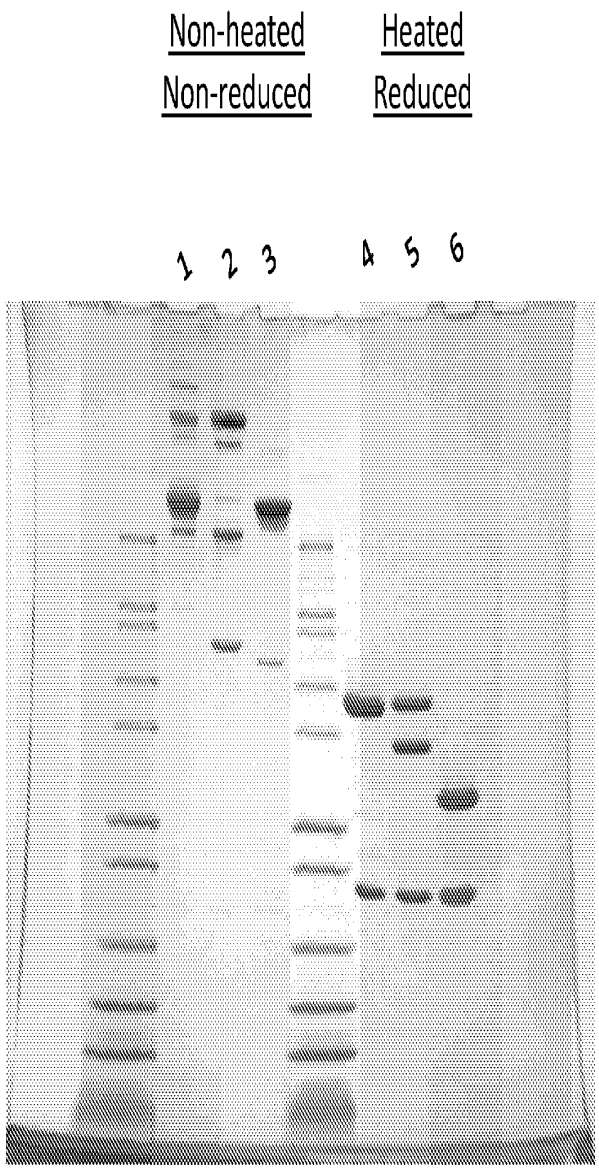

CHO CB1R EC50 (nM)
2500
2000
1500
1000
500
0
10D10 Ab
LC-6H/HC-TTR
LC(+)/HC(-)-TTR-6H
LC(+)-6H/HC(-)-TTR
2x10D10-G4S-TTR
2x10D10-2xG4S-TTR
2x10D10-3xG4S-TTR
2x10D10-4xG4S-TTR
4x10D10-TTR
4x10D10-G4S-TTR
CHO CB1R EC50 (nM)
250
200
150
100
50
0
10D10 Ab
LC-6H/HC-TTR
LC(+)/HC(-)-TTR-6H
LC(+)-6H/HC(-)-TTR
2x10D10-G4S-TTR
4x10D10-TTR
4x10D10-G4S-TTR

Figure 5



Lane 1 = anti-GITR TTR antibody homodimer
Lane 2 = anti-GITR TTR antibody homotetramer
Lane 3 = anti-GITR TTR Fab homotetramer
Lanes 4-7 = anti-DNP antibody

Figure 6



Lanes 1 and 4 = anti-GITR TTR antibody homodimer
Lanes 2 and 5 = anti-GITR TTR antibody homotetramer
Lanes 3 and 6 = anti-GITR TTR Fab homotetramer

Figure 7

SEC analysis of each of the anti-GITR TTR fusion proteins

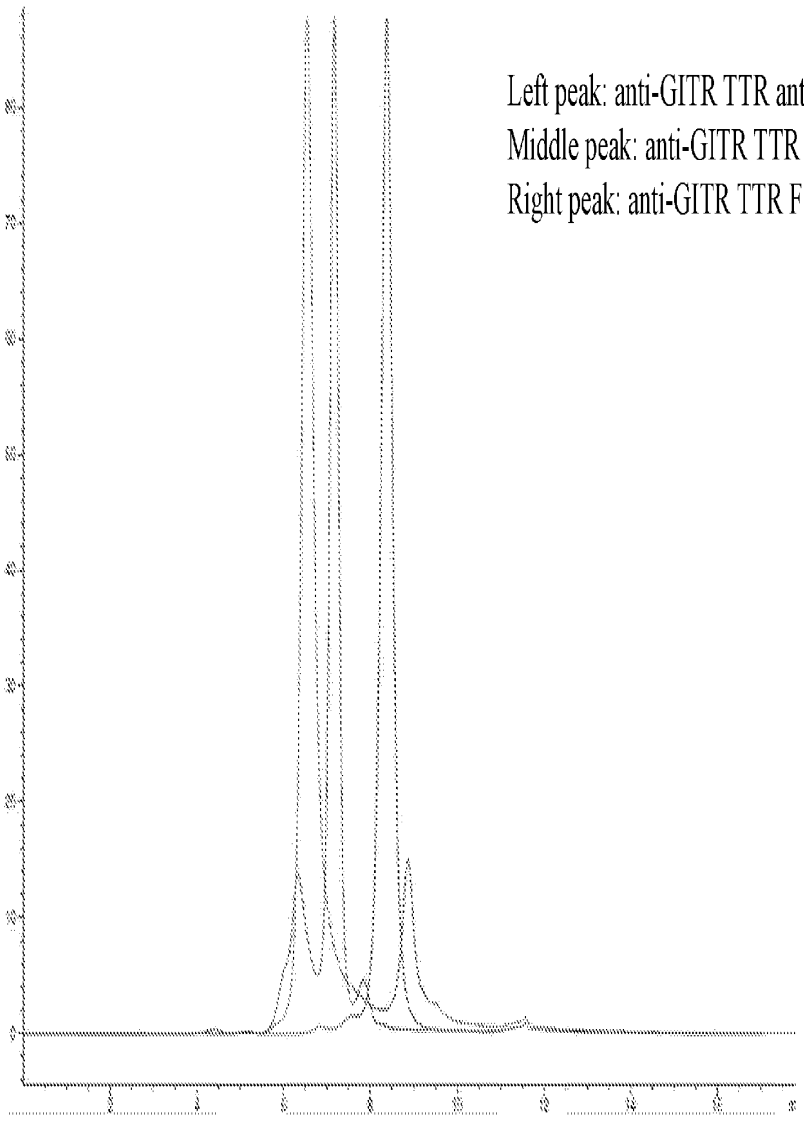


Figure 8

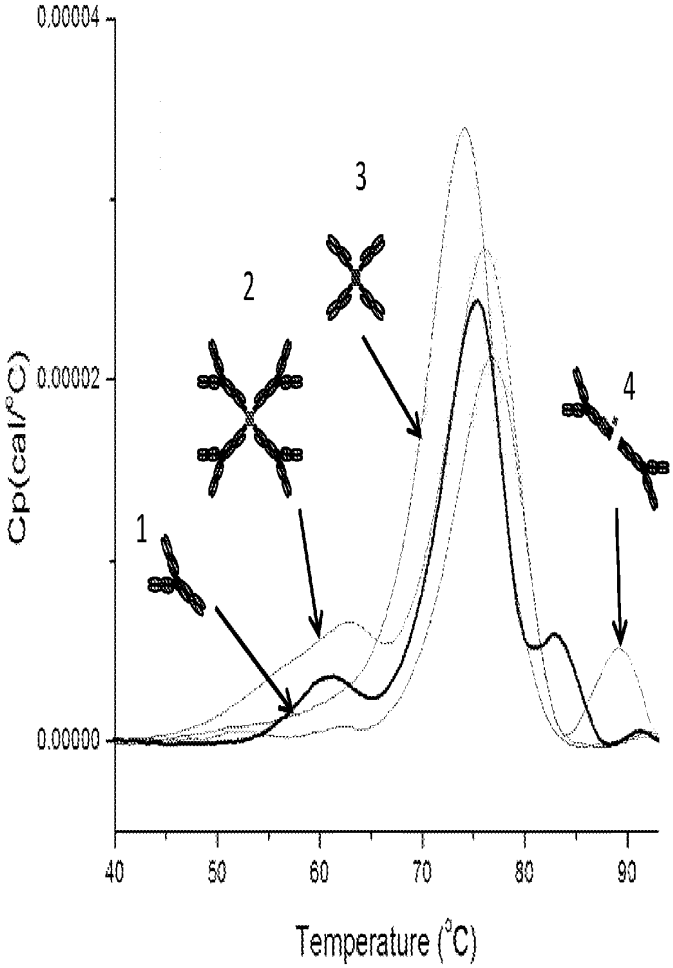
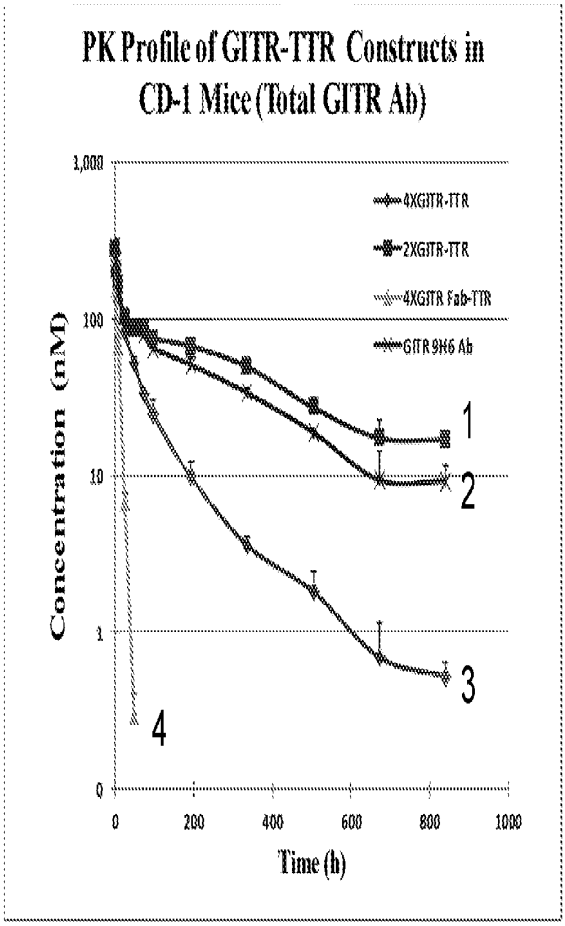

0.00004
0.00002
0.00000
Cp(cal/°C)
1
2
3
4
40
50
60
70
80
90
Temperature (°C)

Figure 9

a)


PK Profile of GITR-TTR Constructs in CD-1 Mice (Total GITR Ab)
Concentration (nM)
1,000
100
10
1
0
0
200
400
600
800
1000
Time (h)
4XGITR-TTR
2XGITR-TTR
4XGITR Fab-TTR
GITR 9H6 Ab
1
2
3
4

b)

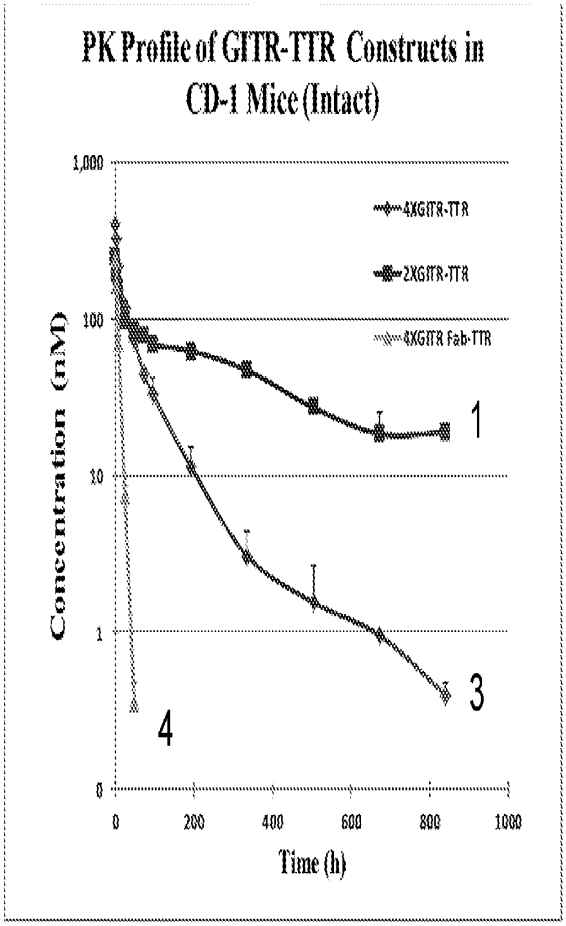
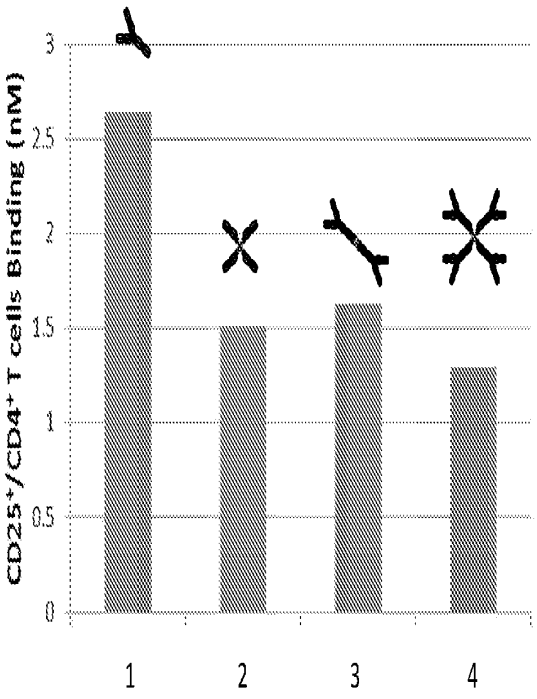

PK Profile of GITR-TTR Constructs in CD-1 Mice (Intact)
Concentration (nM)
1,000
100
10
1
0
0
200
400
600
800
1000
Time (h)
4XGITR-TTR
2XGITR-TTR
4XGITR Fab-TTR
1
3
4

Figure 10

a)


CD25+/CD4+ T cells Binding (nM)
3
2.5
2
1.5
1
0.5
0
1
2
3
4

b)

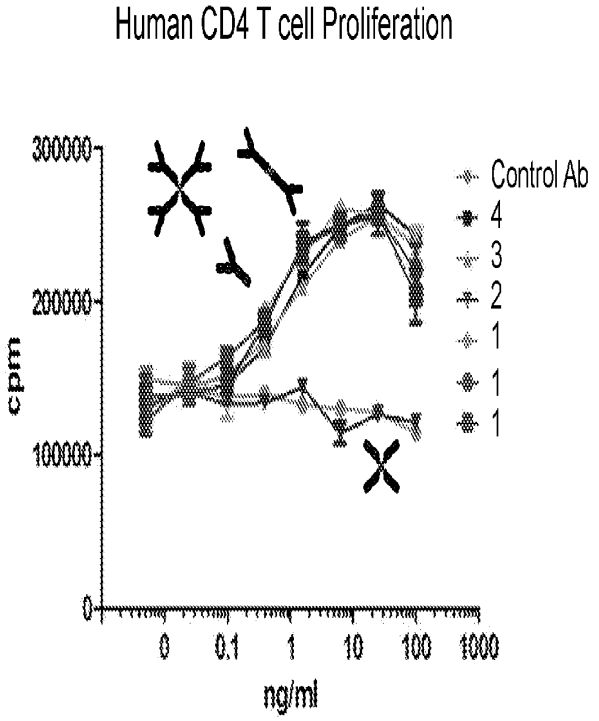

Human CD4 T cell Proliferation
cpm
300000
200000
100000
0
0
0.1
1
10
100
1000
ng/ml
Control Ab
4
3
2
1
1
1

Figure 11

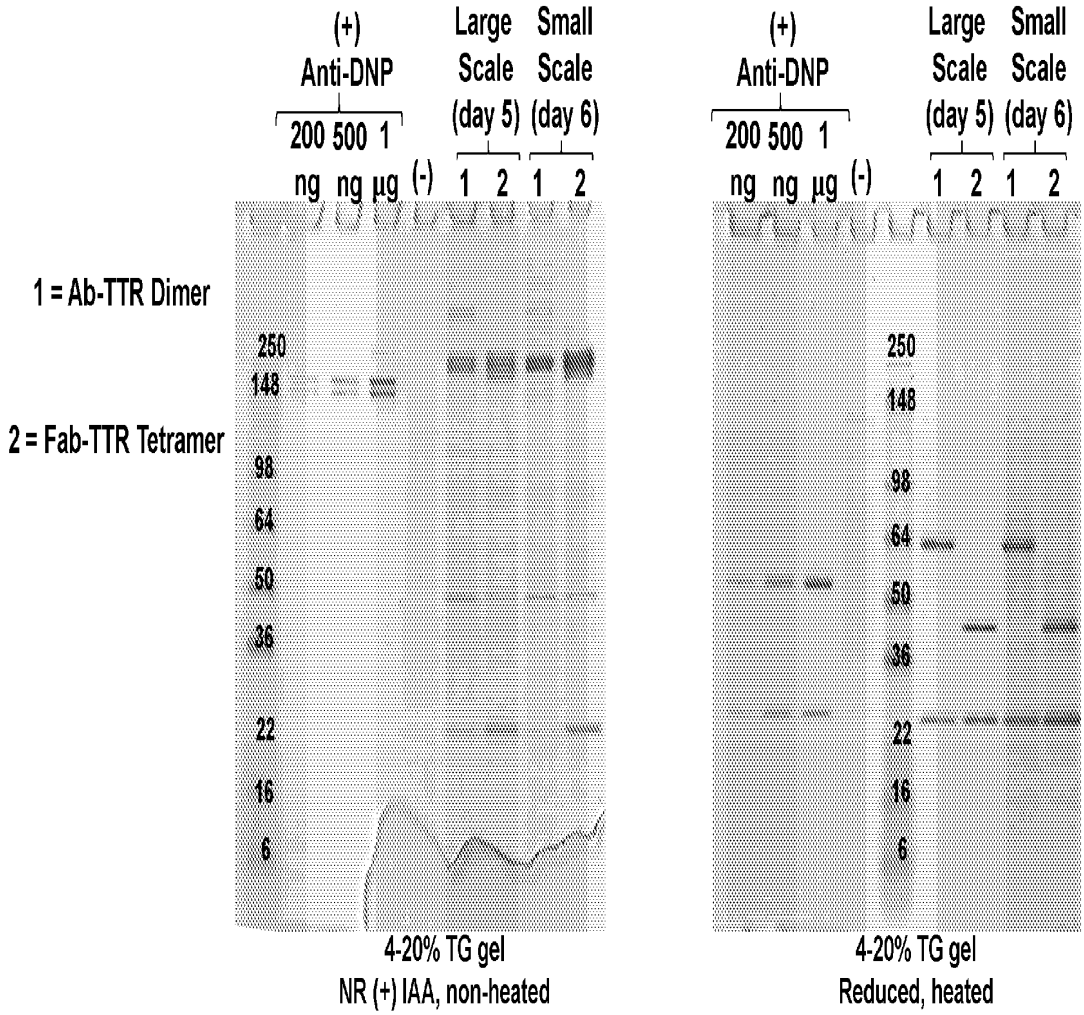
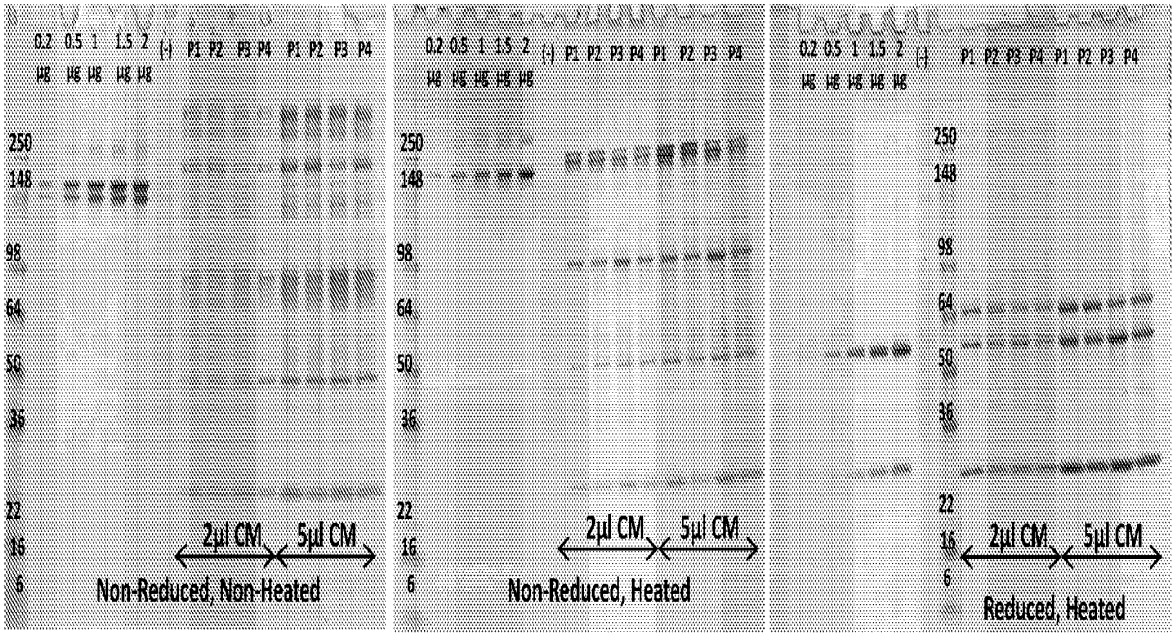

(+) Anti-DNP
200 ng
500 ng
1 µg
(-)
Large Scale (day 5)
Small Scale (day 6)
1
2
1
2
1 = Ab-TTR Dimer
2 = Fab-TTR Tetramer
250
148
98
64
50
36
22
16
6
4-20% TG gel
NR (+) IAA, non-heated
4-20% TG gel
Reduced, heated

Figure 12

Anti-TRAILR2 antibody homotetramer

a) b) c)



Lane	Content
1	SeeBlue Plus 2 Protein Ladder
2	200 ng control antibody
3	500 ng control antibody
4	1000 ng control antibody
5	1500 ng control antibody
6	2000 ng control antibody
7	CHO-K1 conditioned media no vector
8	Anti-TRAILR2 TTR antibody homotetramer Pool 1, 2 µl

Lane	Content
9	Anti-TRAILR2 TTR antibody homotetramer Pool 2, 2 µl
10	Anti-TRAILR2 TTR antibody homotetramer Pool 3, 2 µl
11	Anti-TRAILR2 TTR antibody homotetramer Pool 4, 2 µl
12	Anti-TRAILR2 TTR antibody homotetramer Pool 1, 5 µl
13	Anti-TRAILR2 TTR antibody homotetramer Pool 2, 5 µl
14	Anti-TRAILR2 TTR antibody homotetramer Pool 3, 5 µl
15	Anti-TRAILR2 TTR antibody homotetramer Pool 4, 5 µl

Figure 13

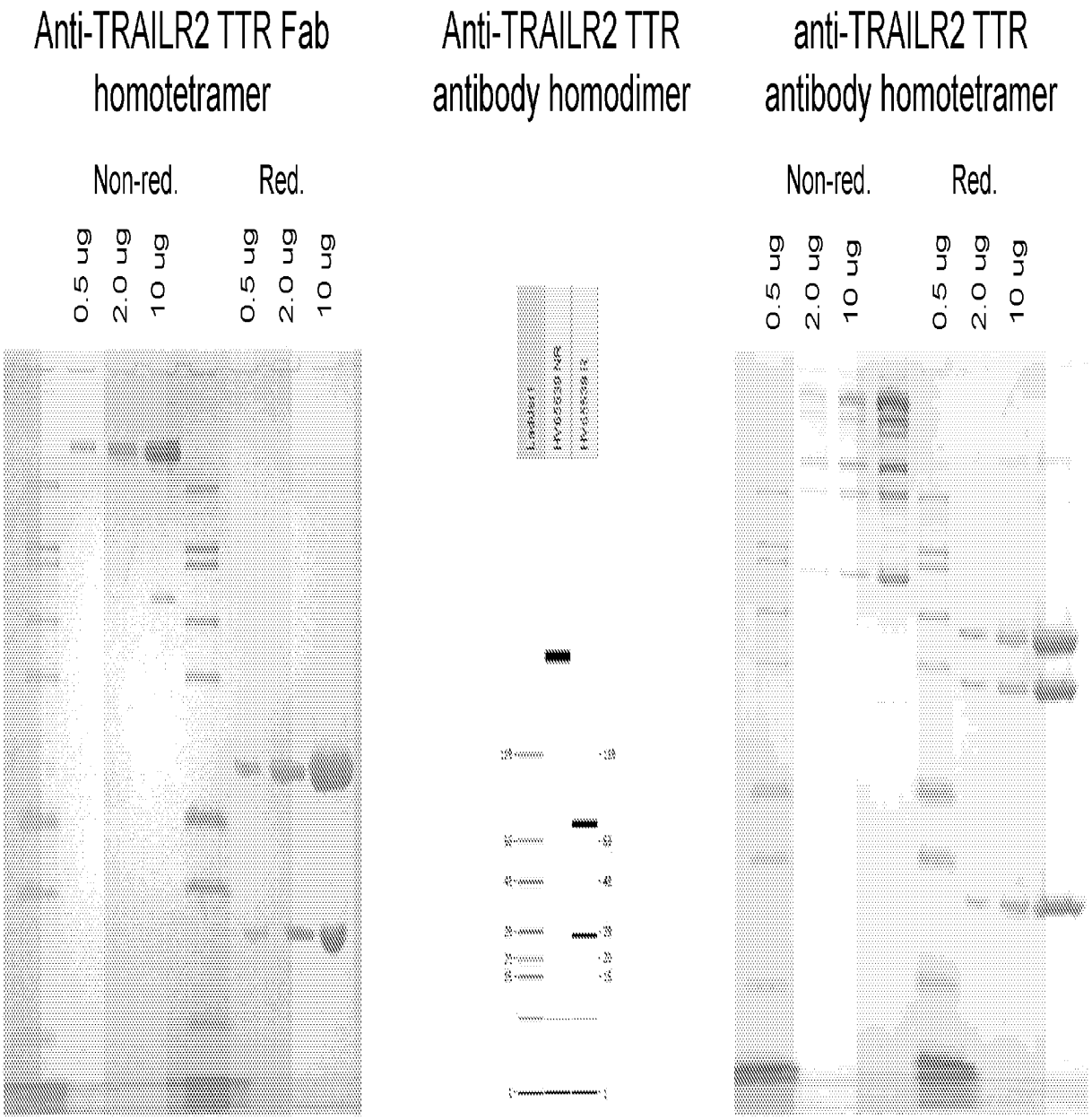

Anti-TRAILR2 TTR Fab homotetramer
Non-red.
Red.
0.5 ug
2.0 ug
10 ug
0.5 ug
2.0 ug
10 ug
Anti-TRAILR2 TTR antibody homodimer
anti-TRAILR2 TTR antibody homotetramer
Non-red.
Red.
0.5 ug
2.0 ug
10 ug
0.5 ug
2.0 ug
10 ug

Figure 14

SEC Purified anti-TRAILR2 TTR Fusions

anti-TRAILR2 TTR Fab homotetramer

anti-TRAILR2 antibody homodimer

anti-TRAILR2 antibody homotetramer

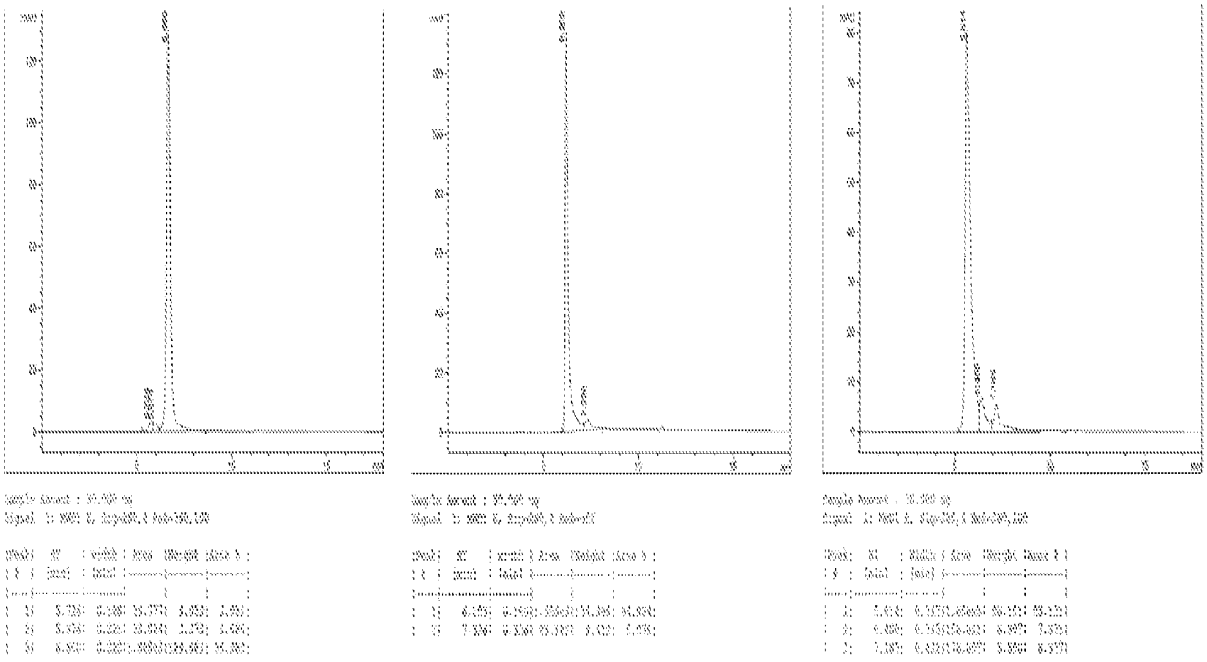
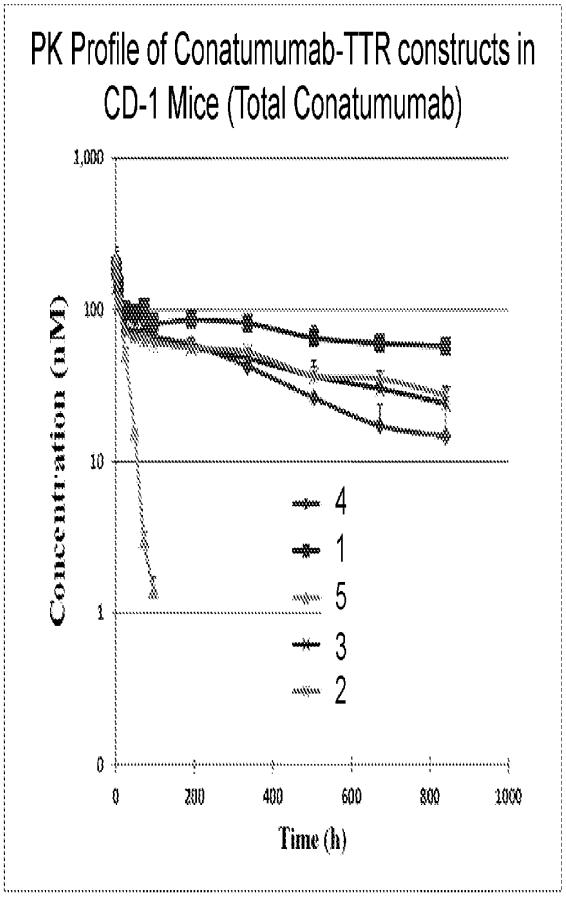


Figure 15

Murine PK of anti-TRAILR2-TTR Conjugates

a)



b)

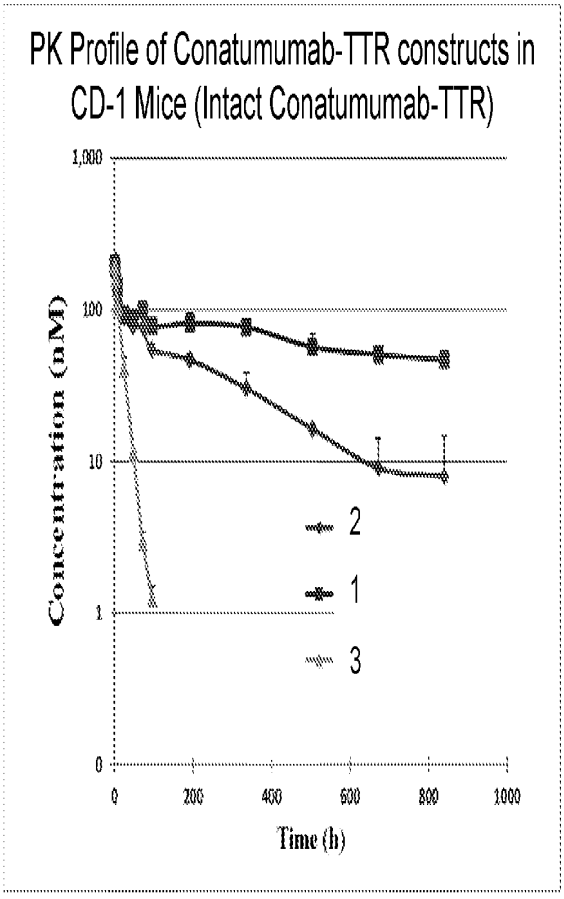


Figure 16

Tetramerization of Conatumumab Substantially Improves Potency in Cell Based Assay

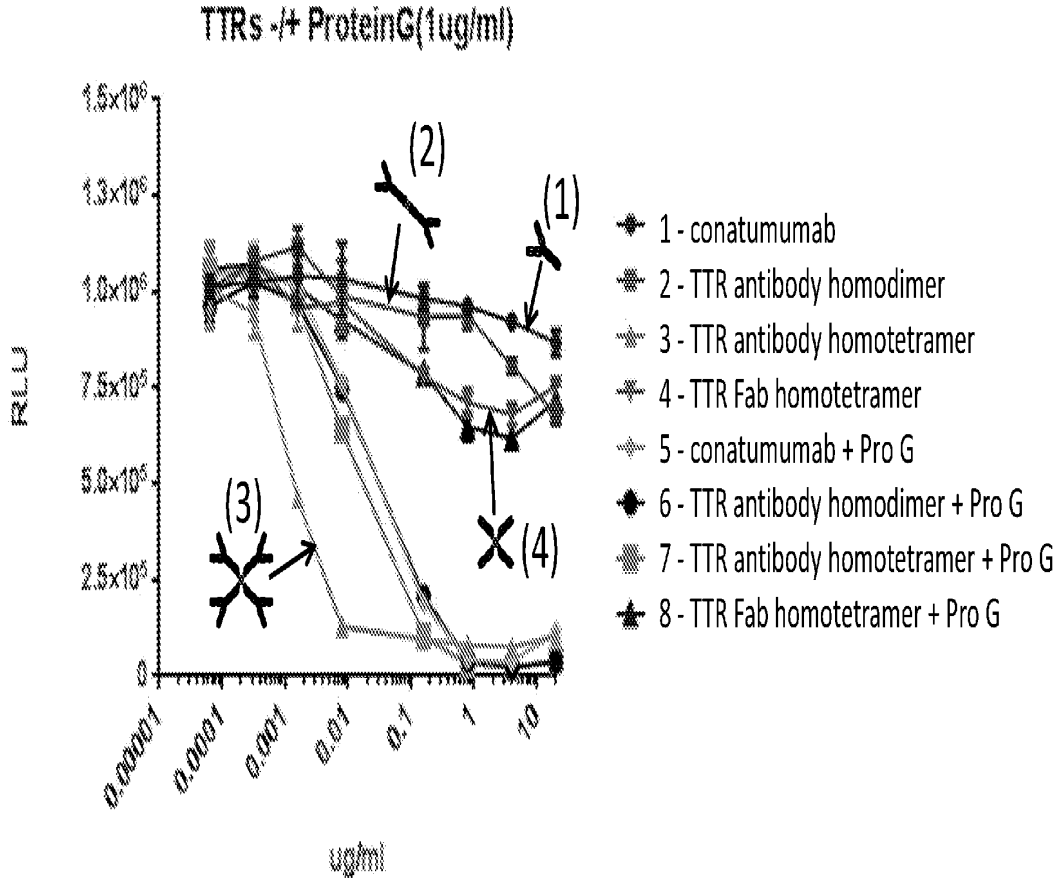


Figure 17

Tetramerization of Conatumumab Improves Potency on Primary Human Keratinocytes

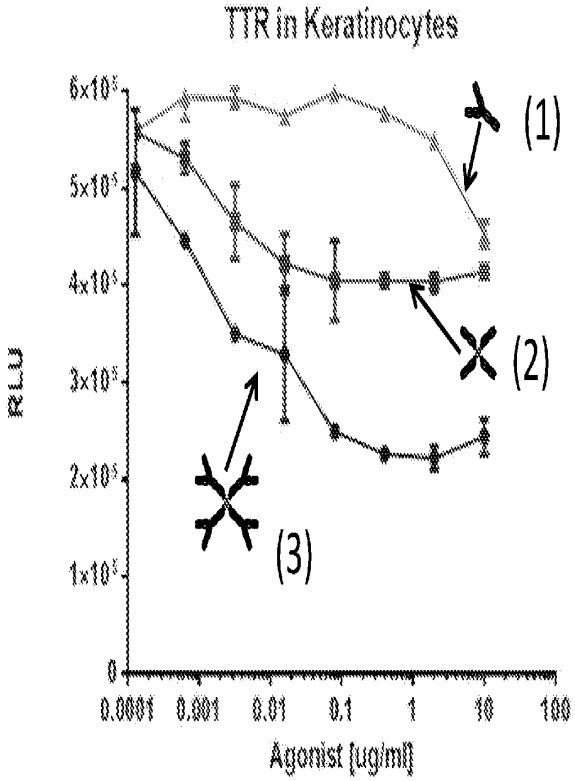


Figure 18

Tetramerized Conatumumab Reduces Tumors in Murine Colo205 Model

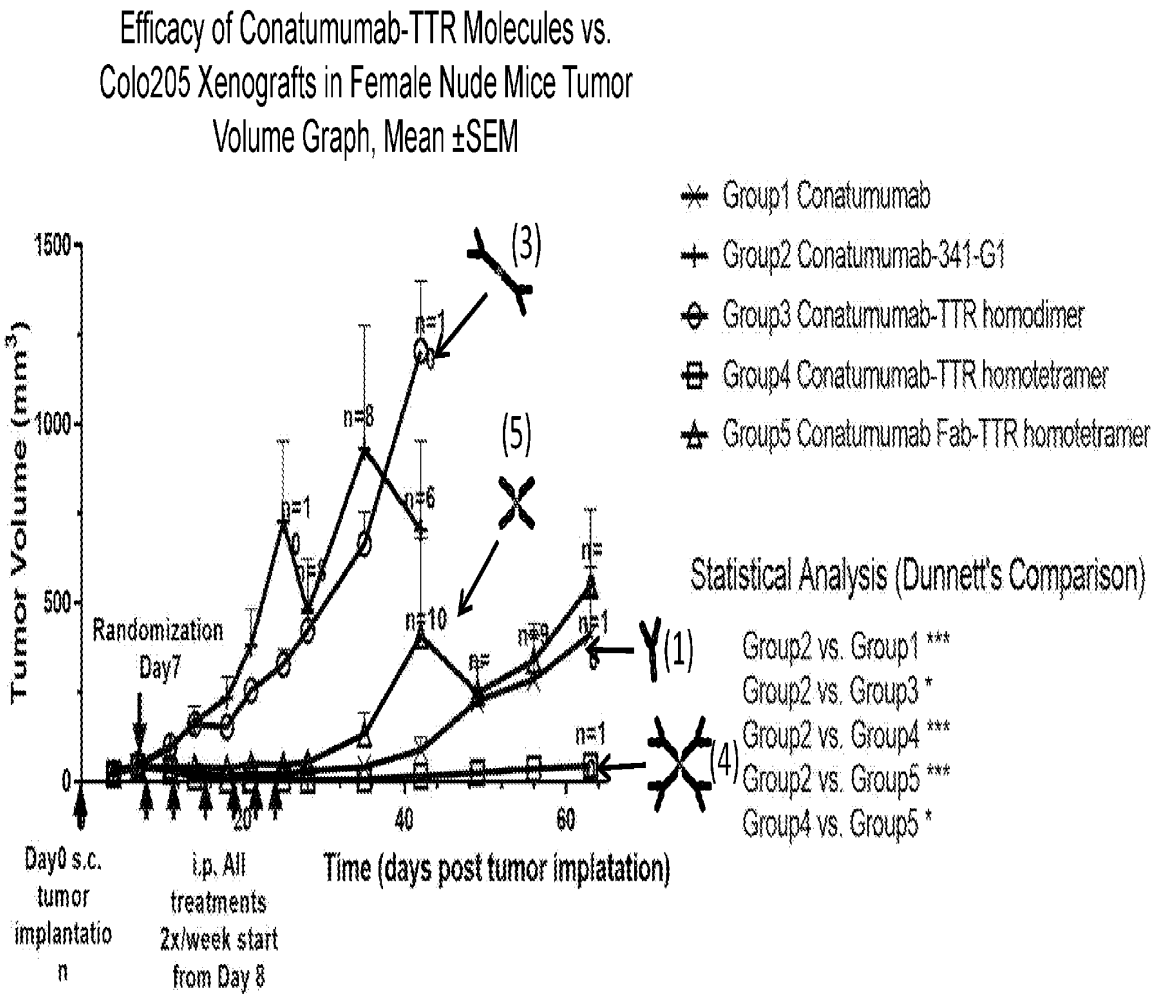


Figure 19

Tetramerized Conatumumab Reduces Tumors in Murine SW403 Model

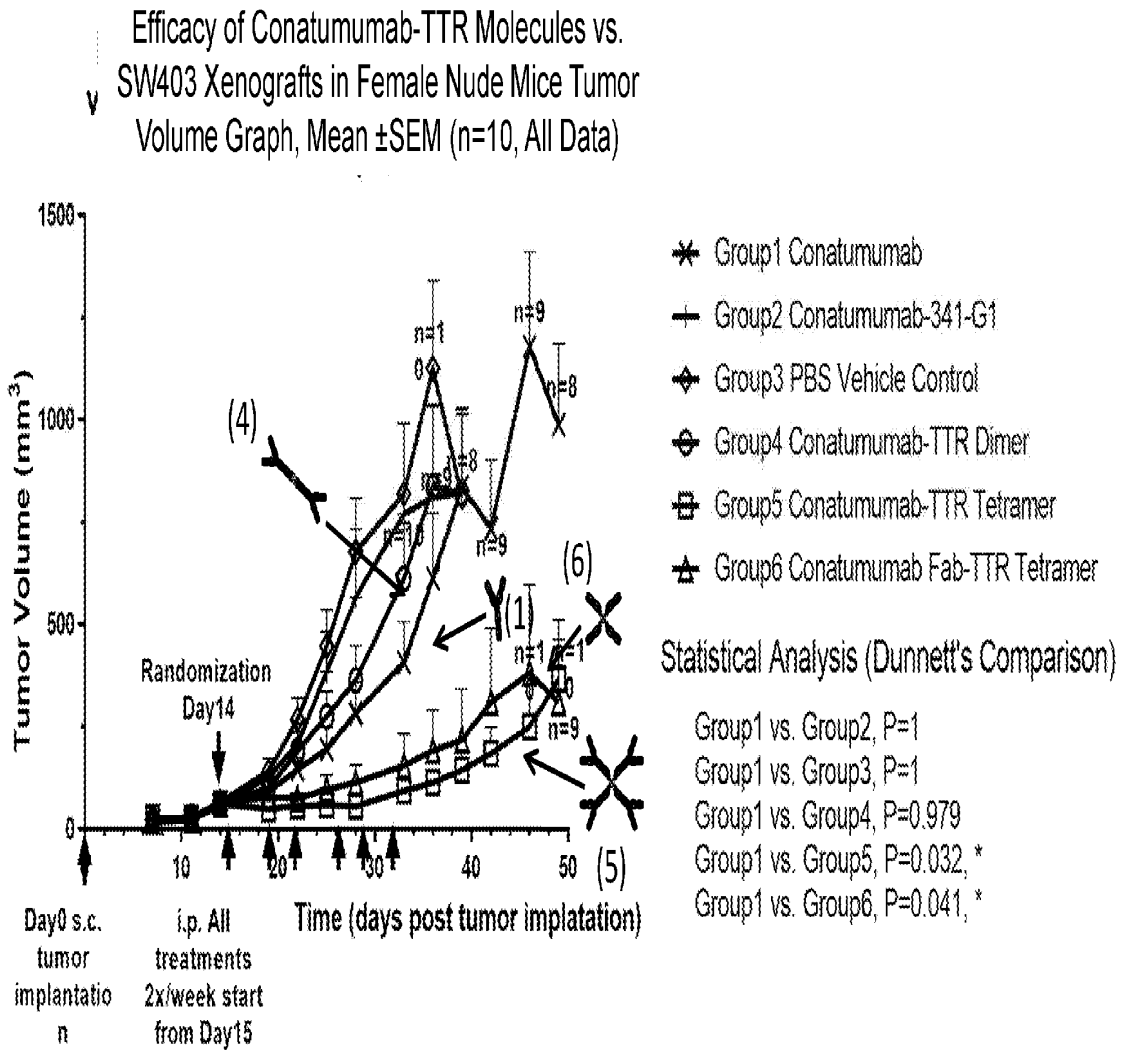
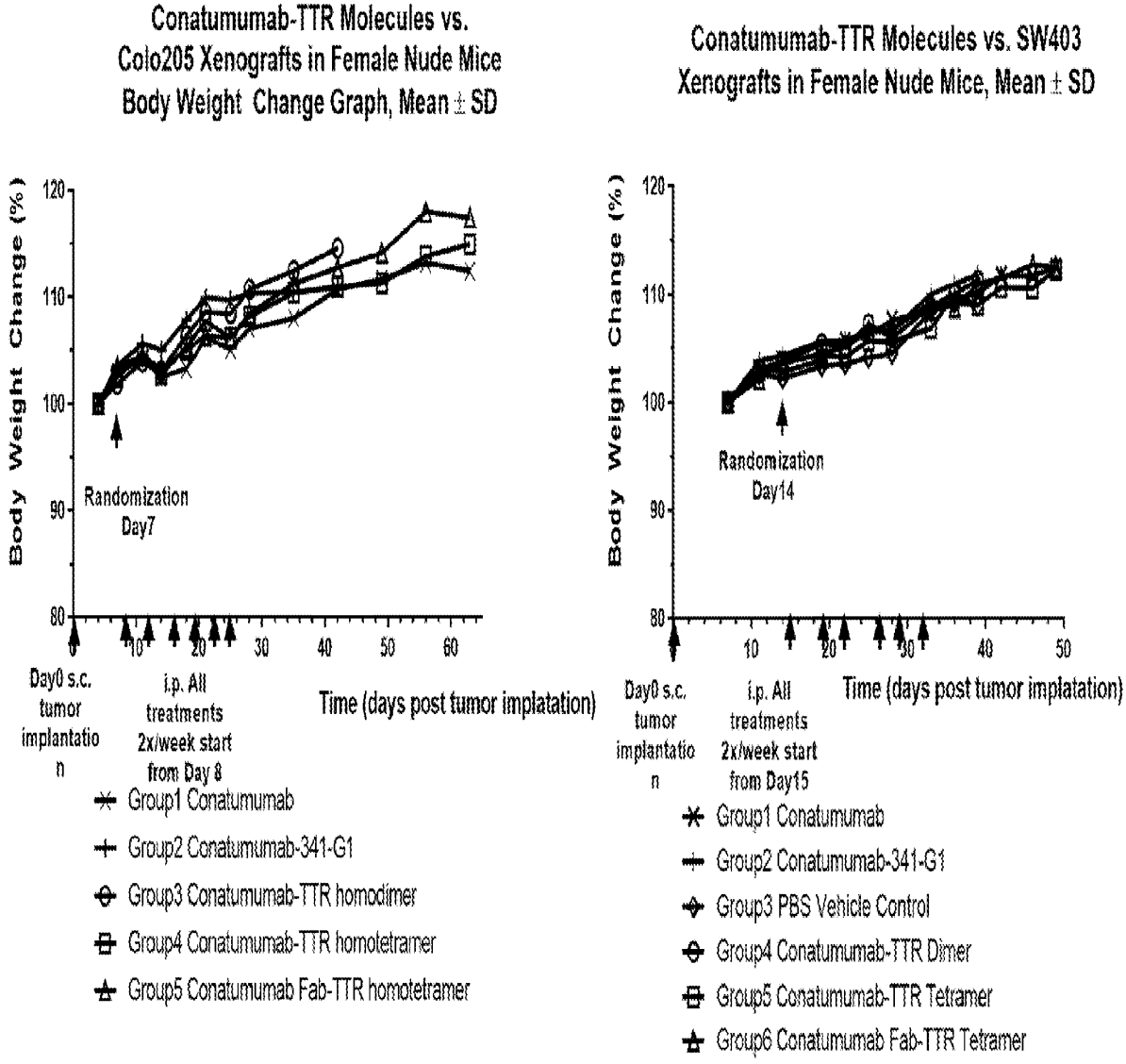


Figure 20

Treatment of Mice with Conatumumab TTR Fusions Does Not Affect Body Weight