

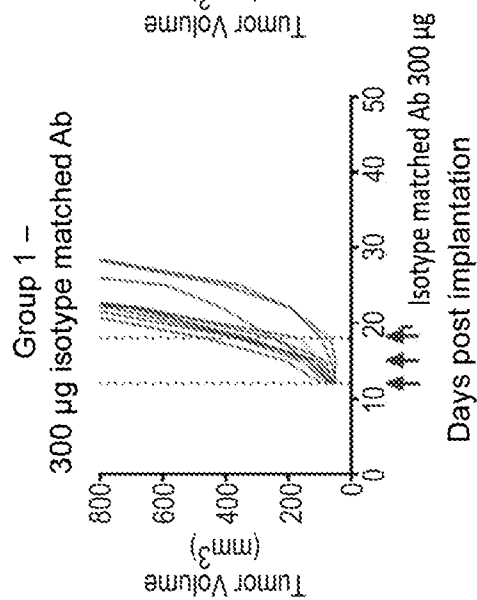
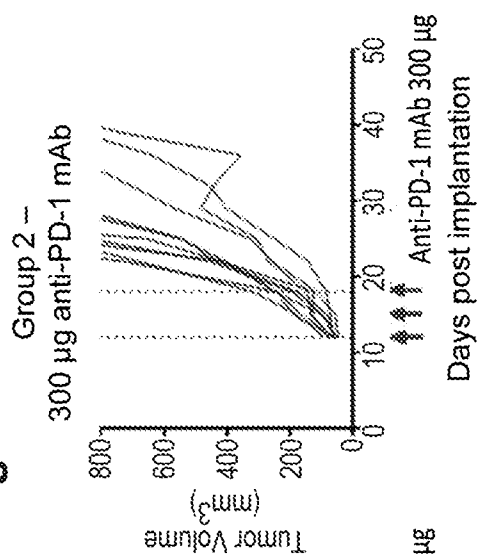
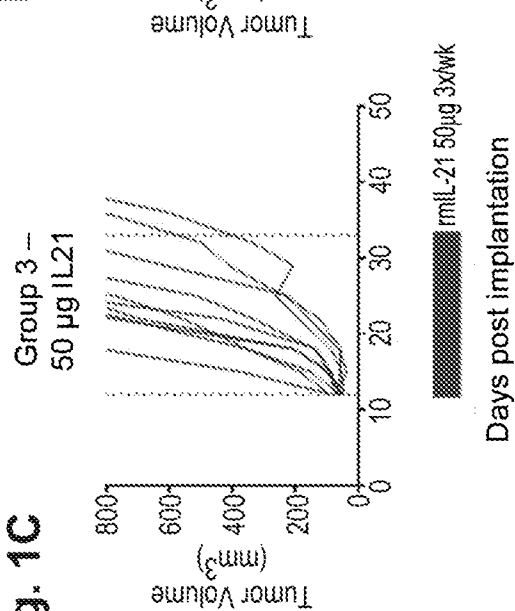
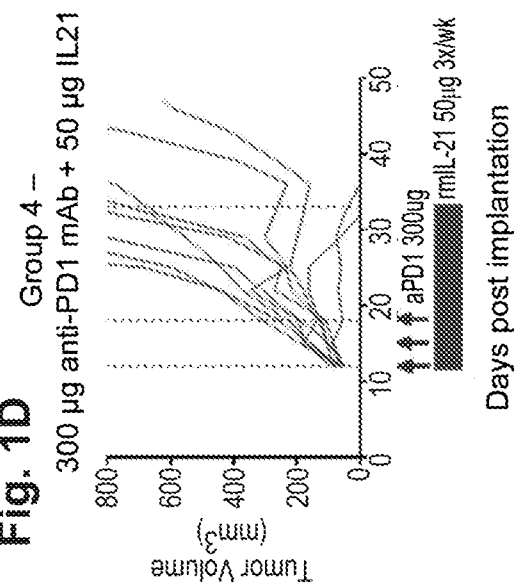
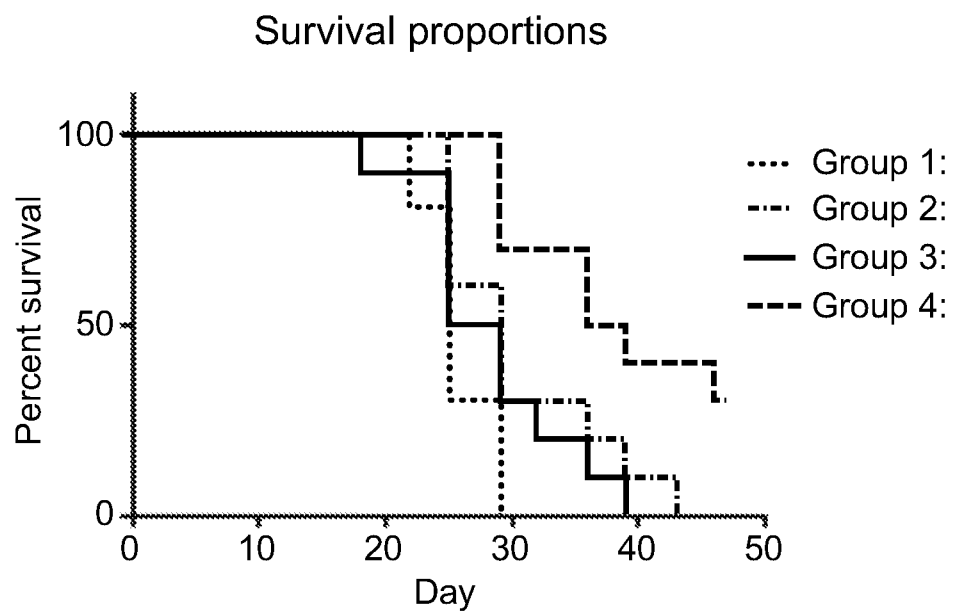
Fig. 1A**Fig. 1B****Fig. 1C****Fig. 1D**

FIG. 2

Iso vs IL-21, $p=\text{ns}$, 0.1269
Iso vs $\alpha\text{PD-1}$ mAb, $p=0.0408$
Iso combo, $p=0.0002$
 $\alpha\text{PD-1}$ mAb vs. combo, $p=0.0143$
IL-21 vs. combo, $p=0.0047$

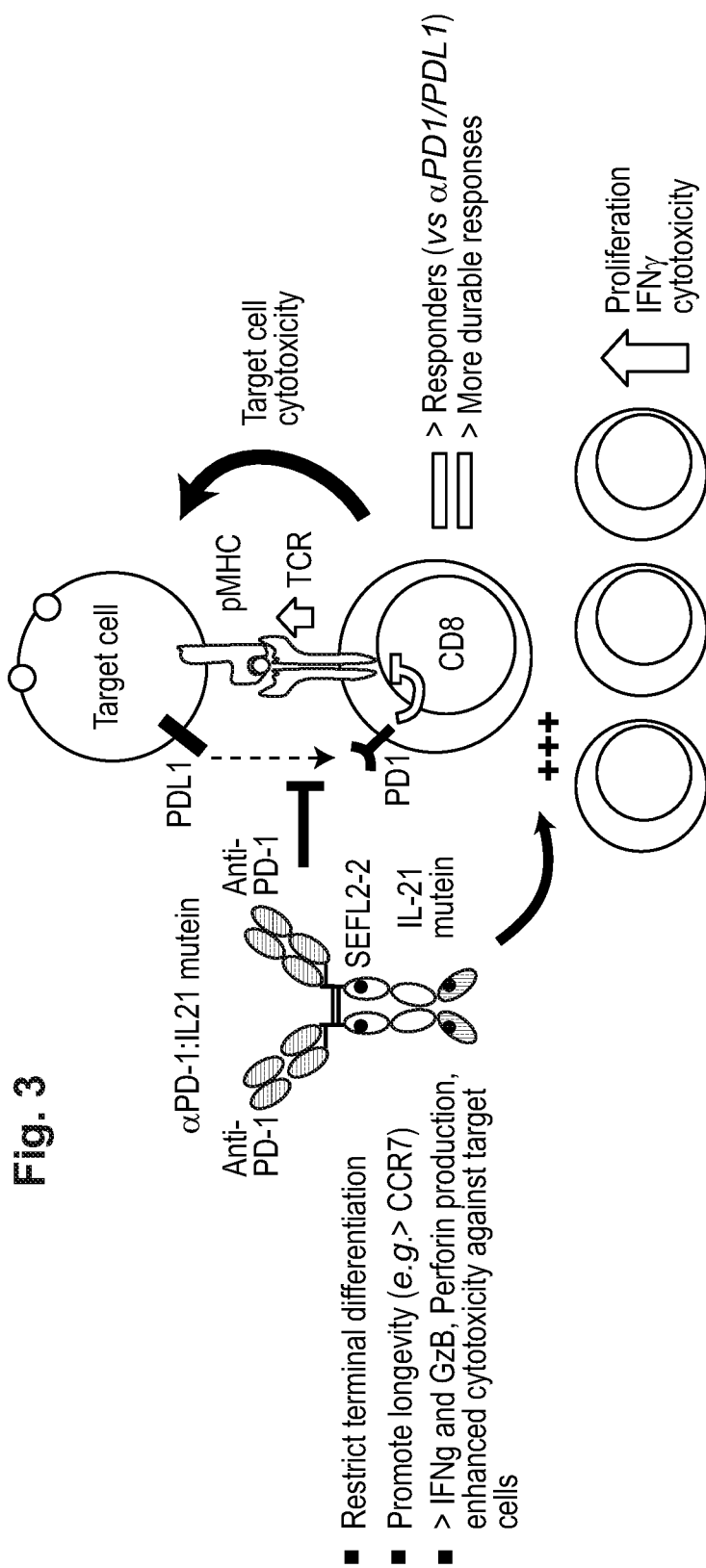


FIGURE 4C

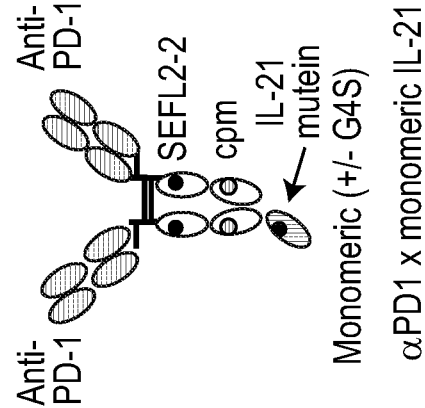


FIGURE 4B

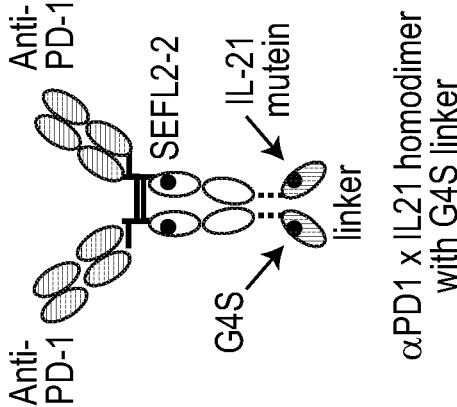


FIGURE 4A

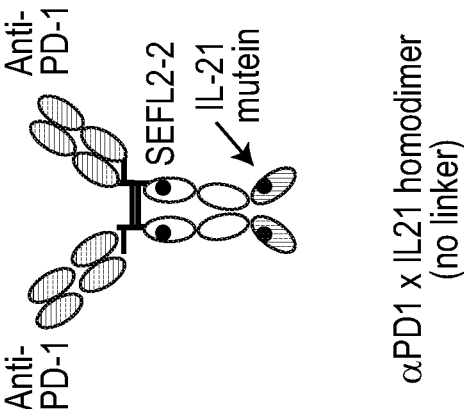


Fig. 5

FIGURE 5A:
pSTAT3 AlphaLISA Hut78 T cells:
PD-1^{-ve}

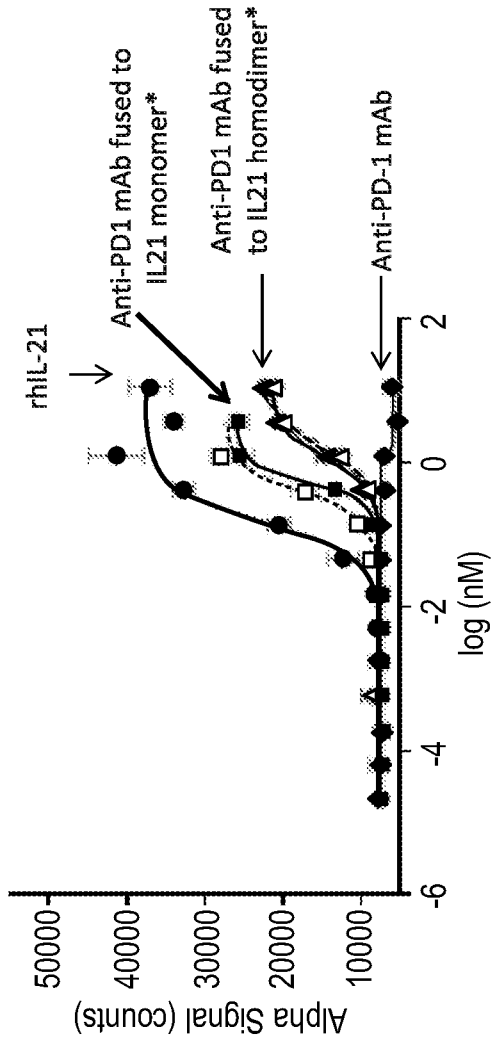
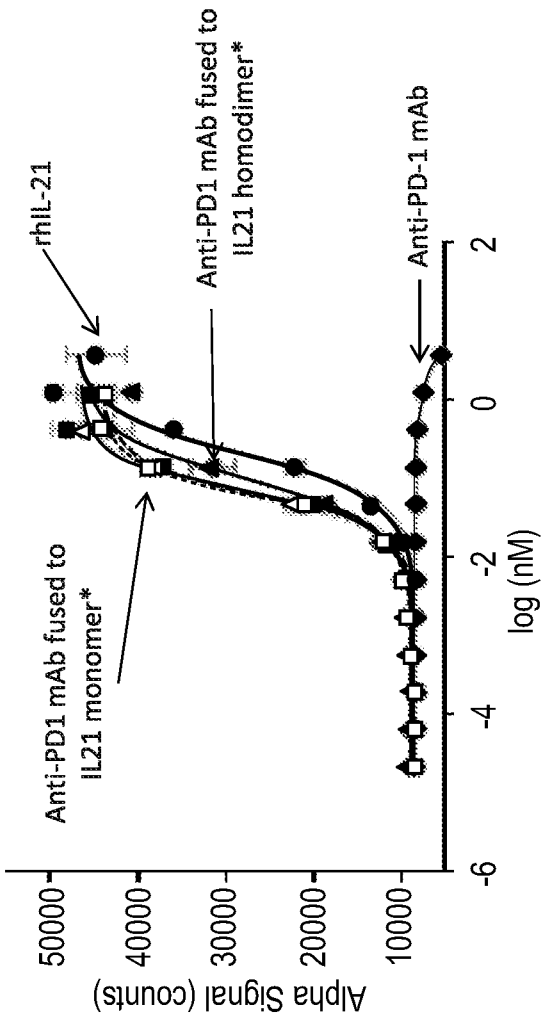


FIGURE 5B:
pSTAT3 AlphaLISA Hut78 T cells:
PD-1^{+ve}



* Solid line represents fusion without linker and dotted line represents fusion with linker

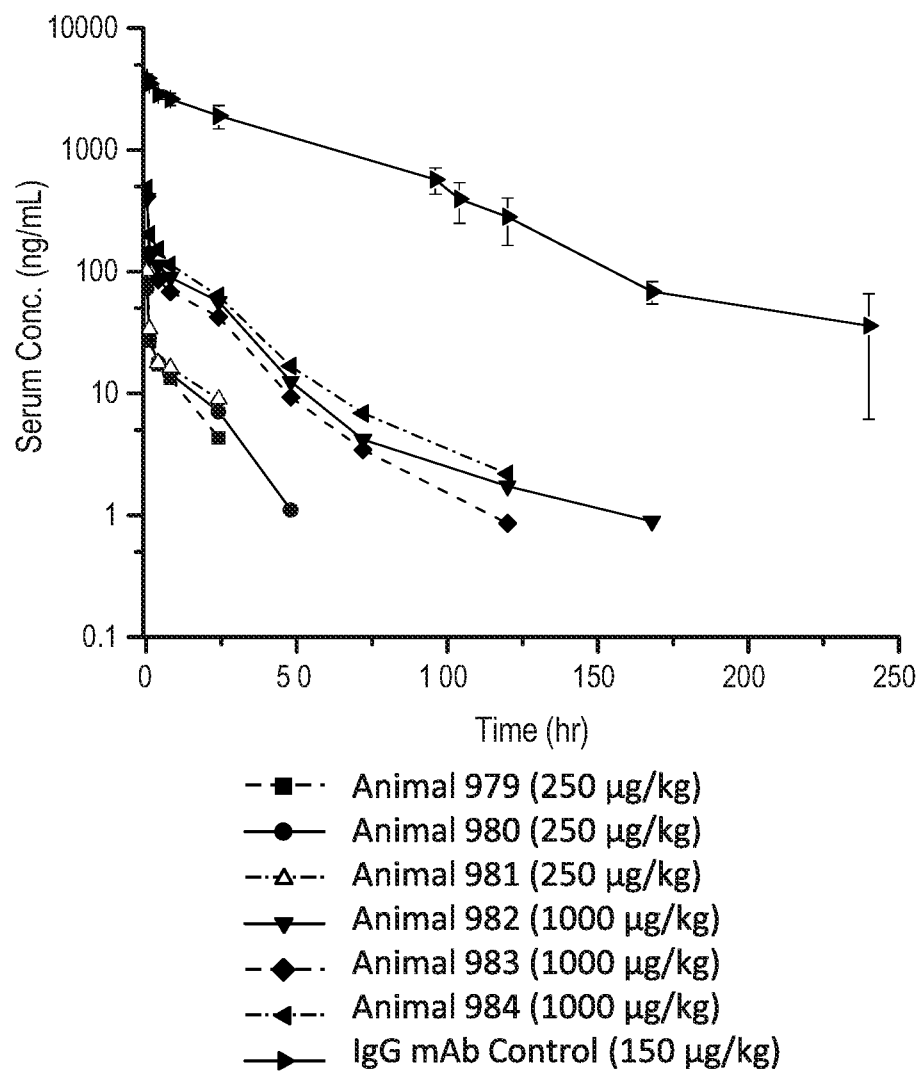
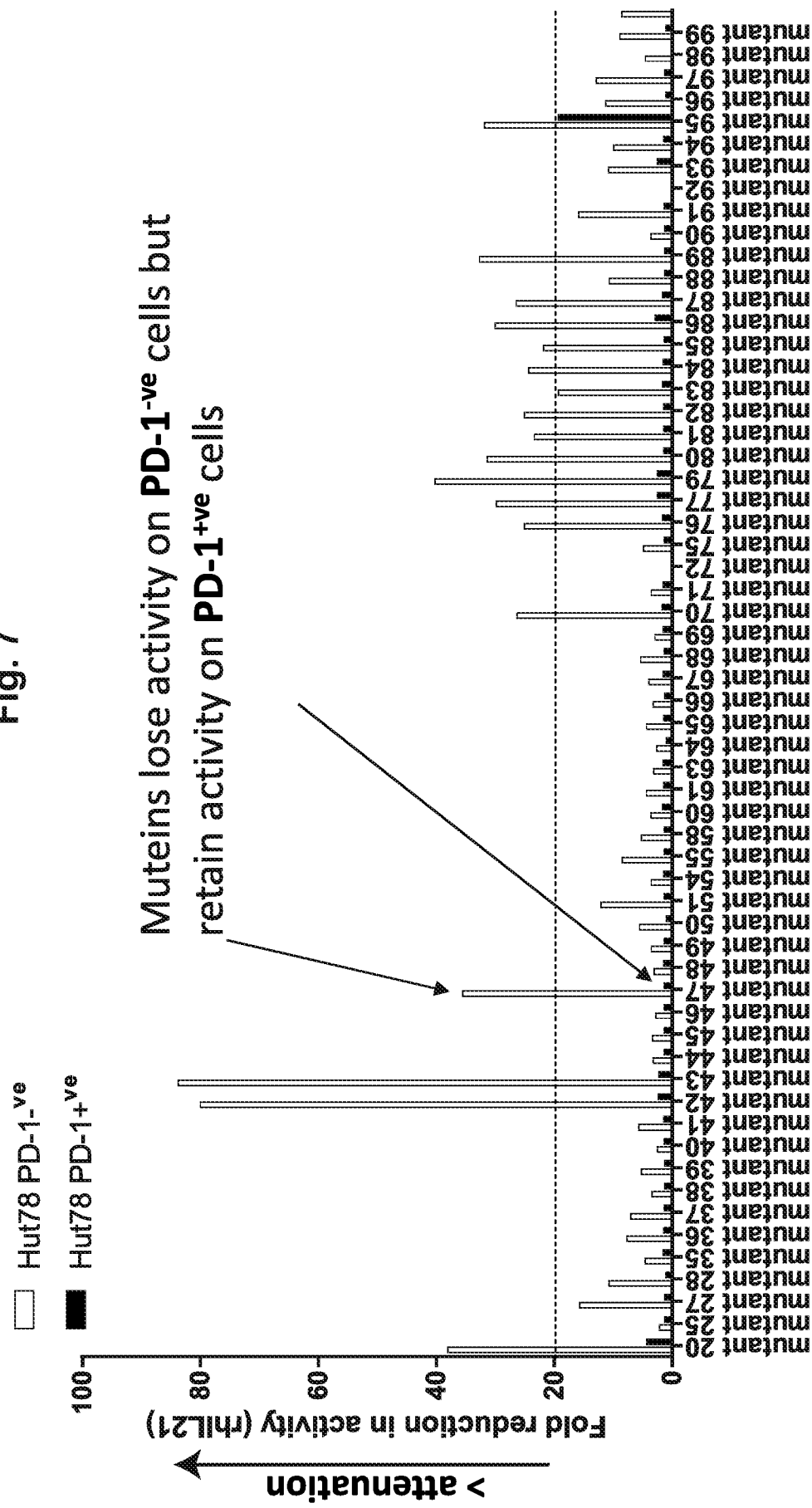
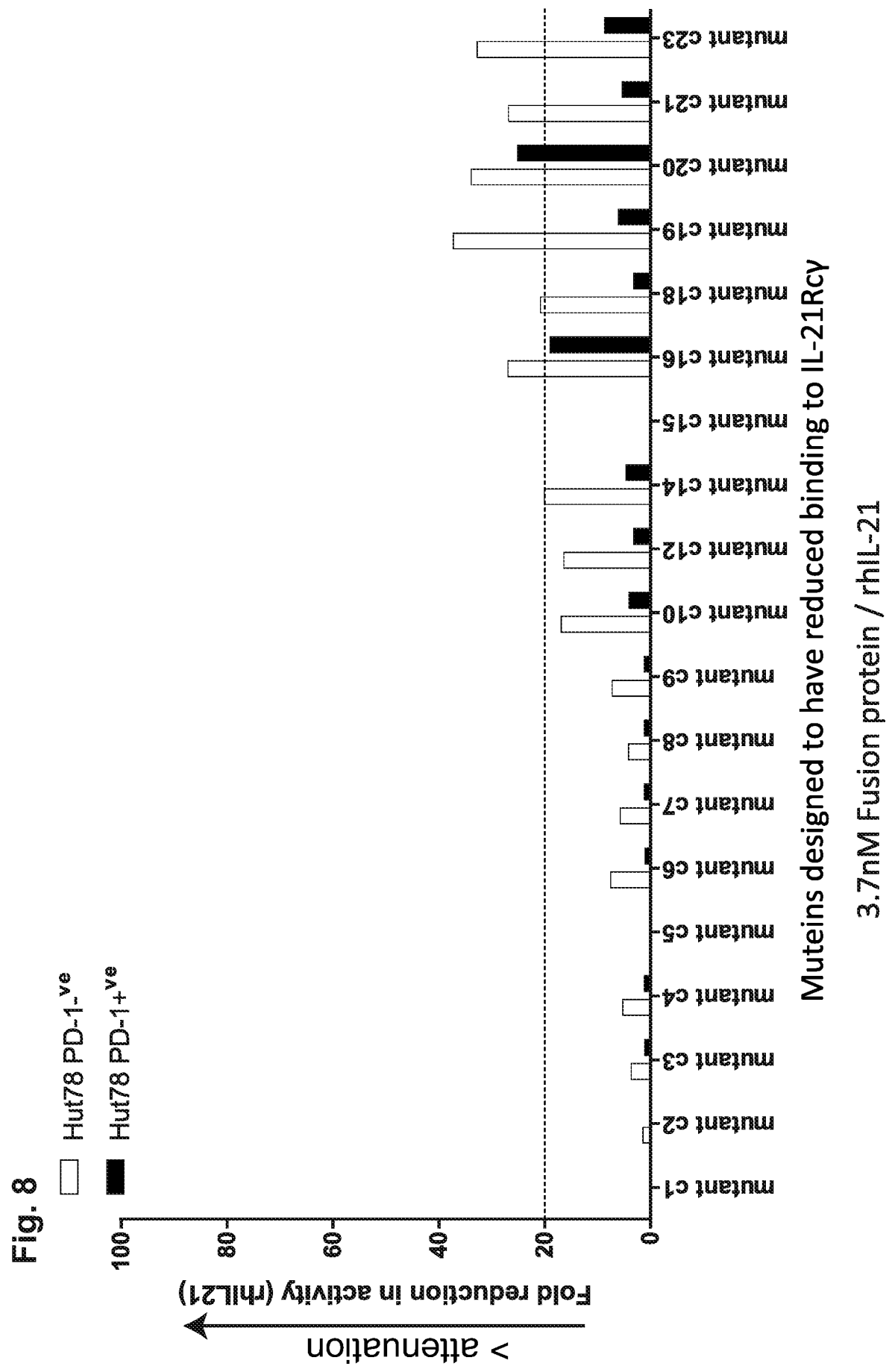
Fig. 6

Fig. 7





Identification of muteins which meet criteria (>20 fold attenuation on PD-1^{-ve} cells)
 & are dependent on PD-1 for targeting

- Human IL-21
- PD1 mAb
- PD1 mAb x WT IL-21 (mono)
-▲..... PD1mAb x IL-21 mutant 51

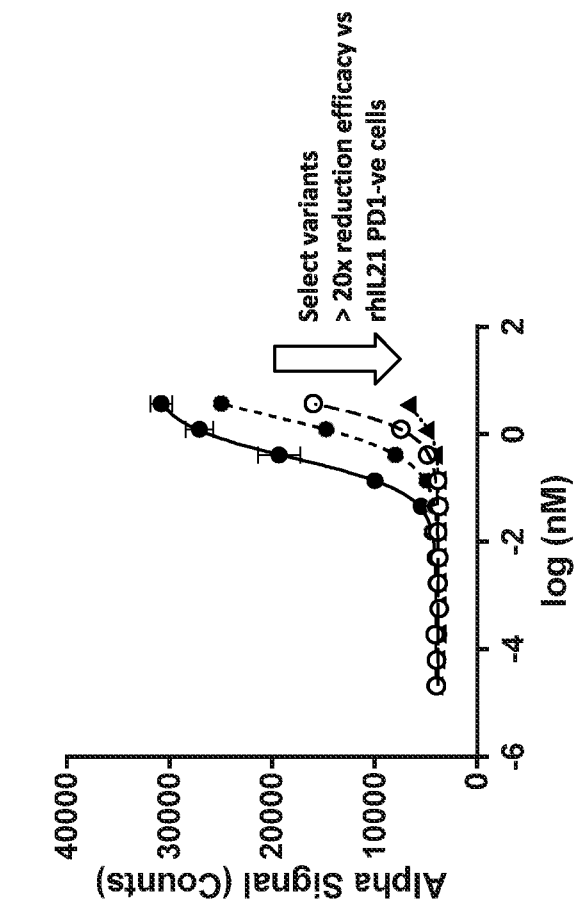


Fig. 9A
 PD1^{-ve}

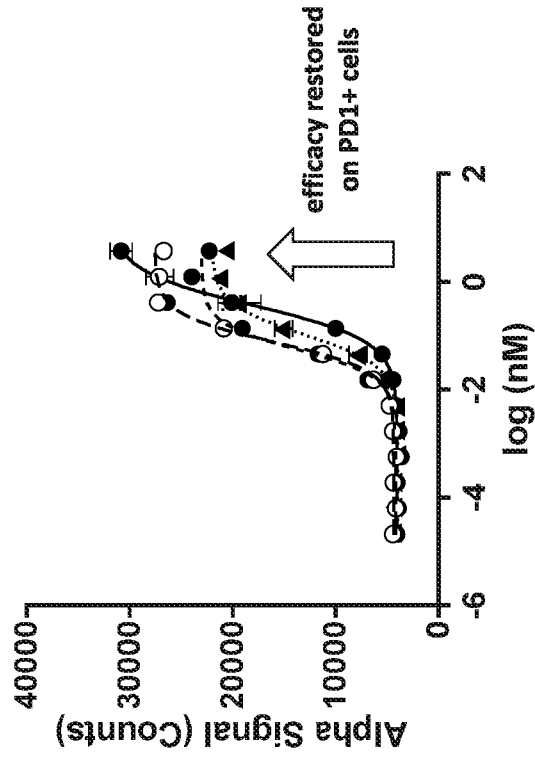
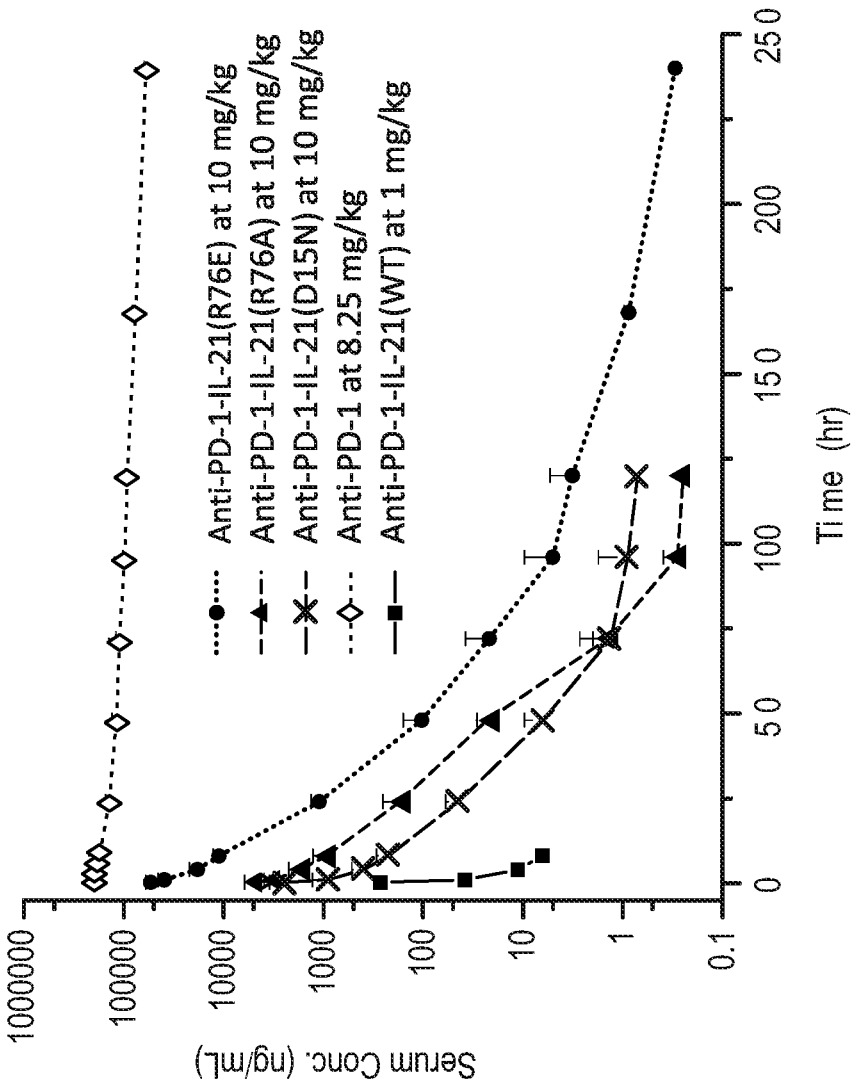


Fig. 9B
 PD1^{+ve}

Fig. 10



Anti-PD-1 mAb-IL-21 mutein fusion proteins exhibited altered distribution compared to parent anti-PD-1 mAb

Fig. 11

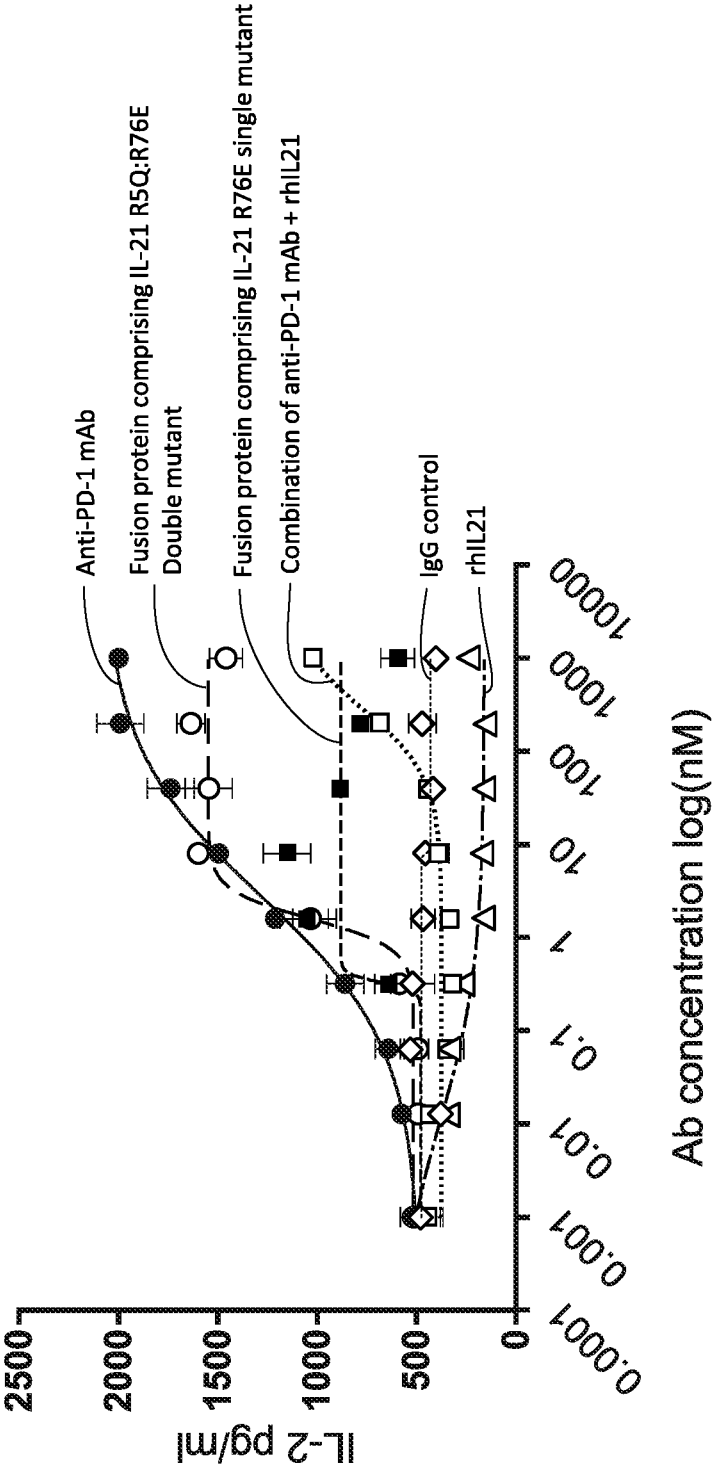


Fig. 12

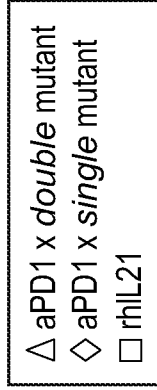
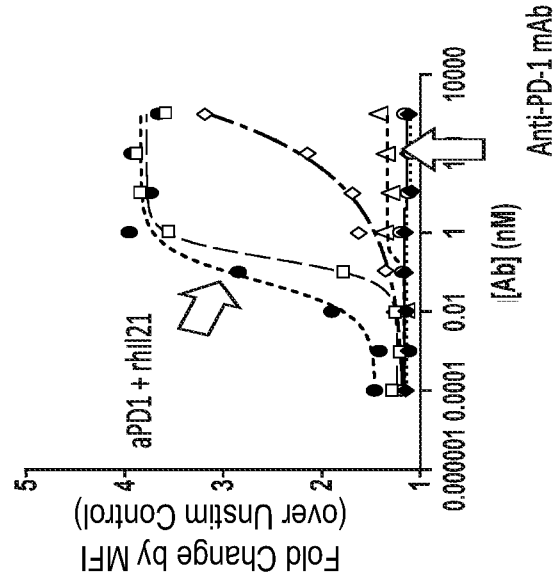


FIGURE 12A:

Solid line = Fusion protein
 comprising anti-PD-1 mAb fused to
 IL-21 R5E:R76A double mutein



pSTAT3

CTLs rested for 48h before 10min stimulation
 Basal PD1 is low after 24h resting

FIGURE 12B:

Solid line = Fusion protein
 comprising anti-PD-1 mAb fused to
 IL-21 R5Q:R76E double mutein

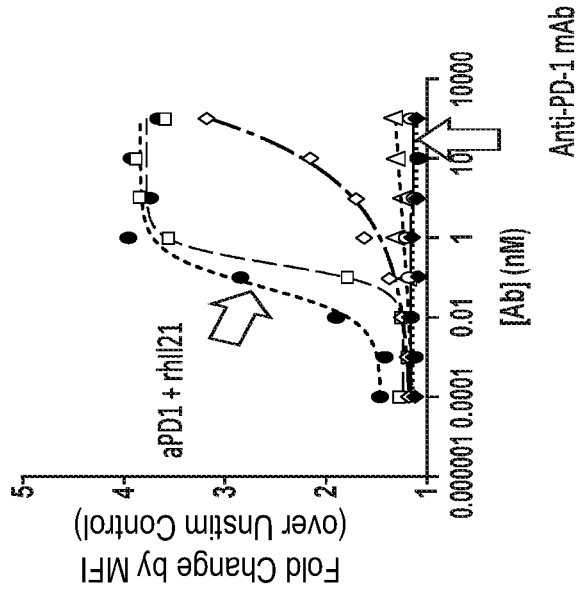
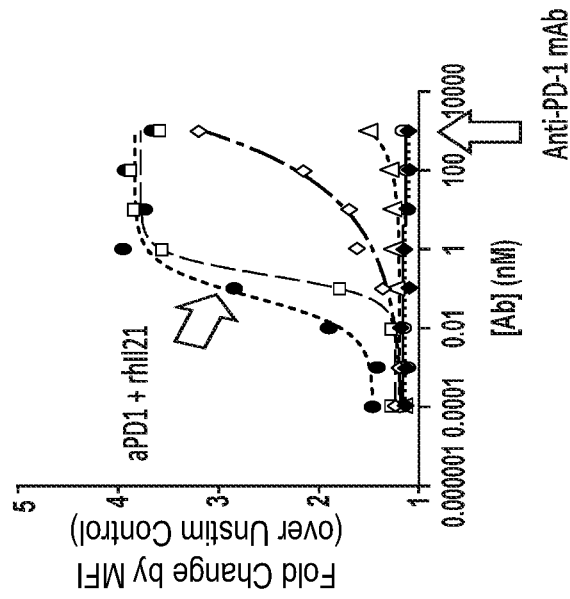


FIGURE 12C:

Solid line = Fusion protein
 comprising anti-PD-1 mAb fused to
 IL-21 R9E:R76A double mutein



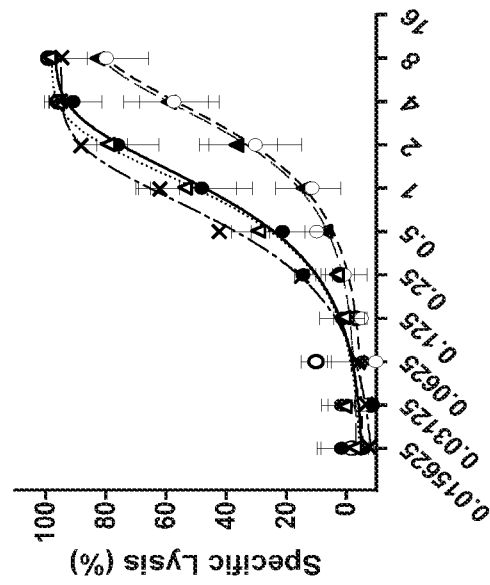
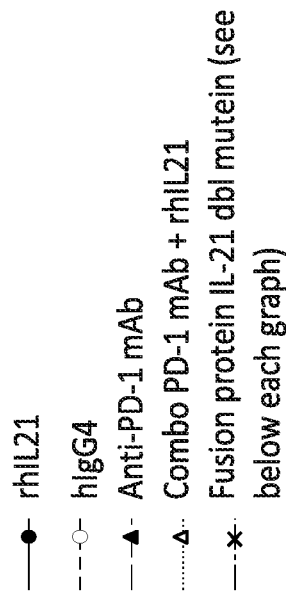


Fig. 13A
Double mutein = IL-21
R5E/R76A

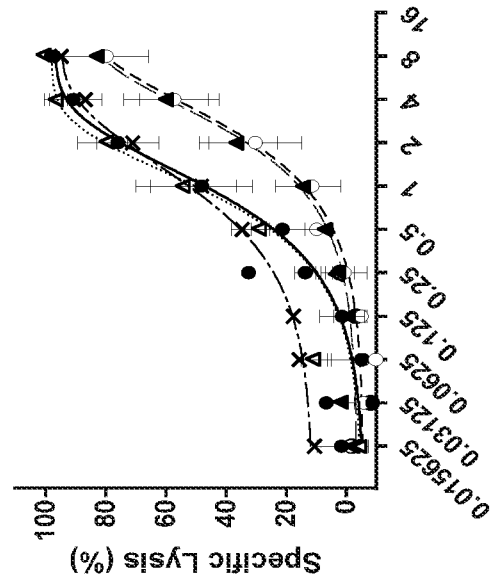


Fig. 13B
Double mutein = IL-21
R5Q/R76E

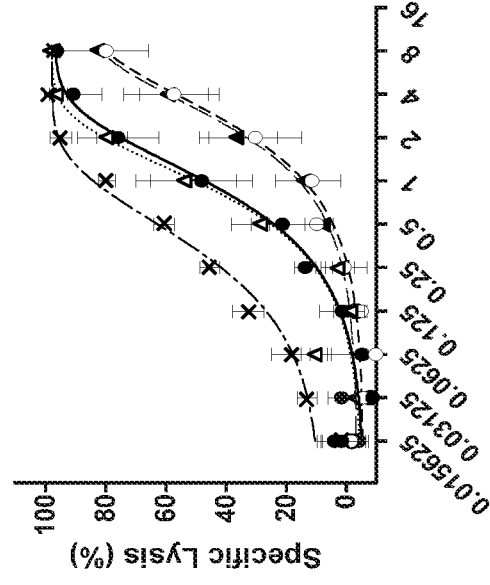
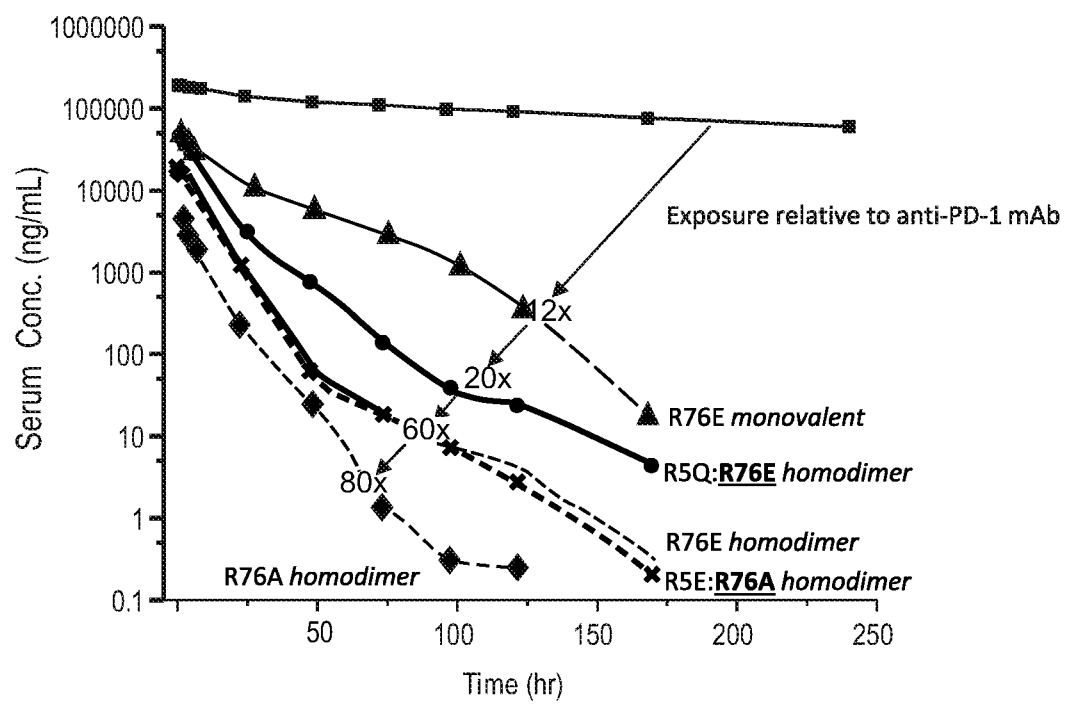
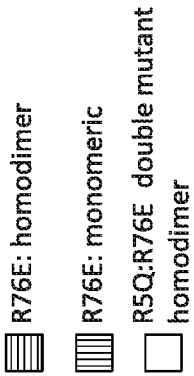
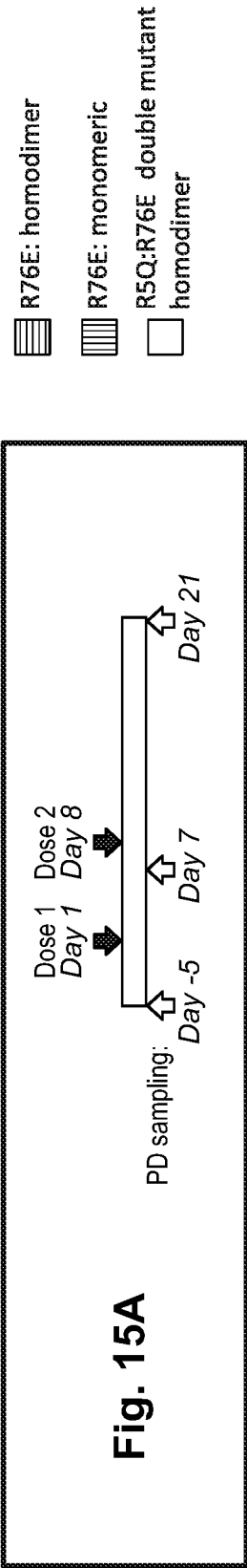


Fig. 13C
Double mutein = IL-21
R9E/R76A

Fig. 14





Day 7 (measurement post-single dose)

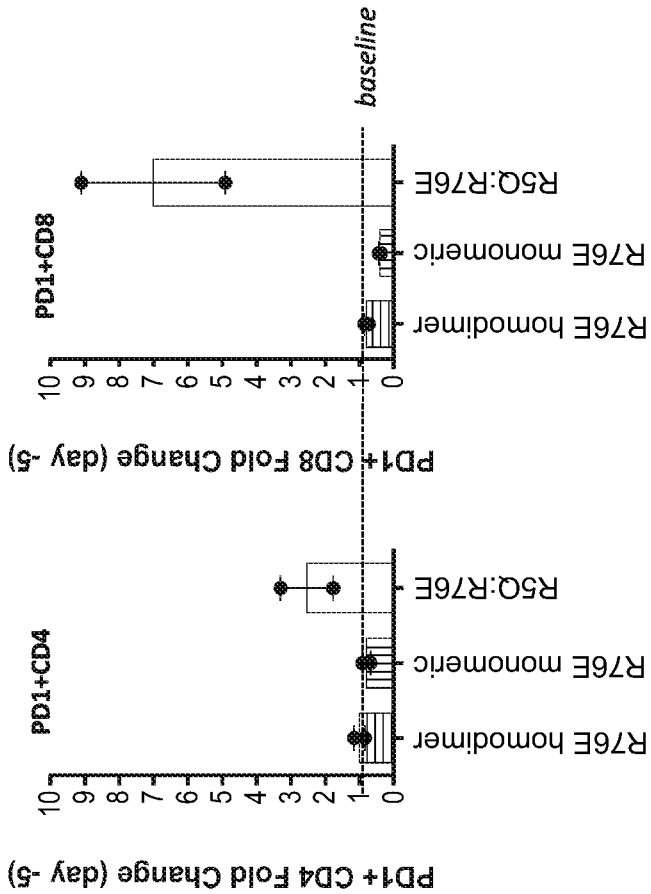


Fig. 15B

Day 21 (measurement 14 days post-repeat dose)

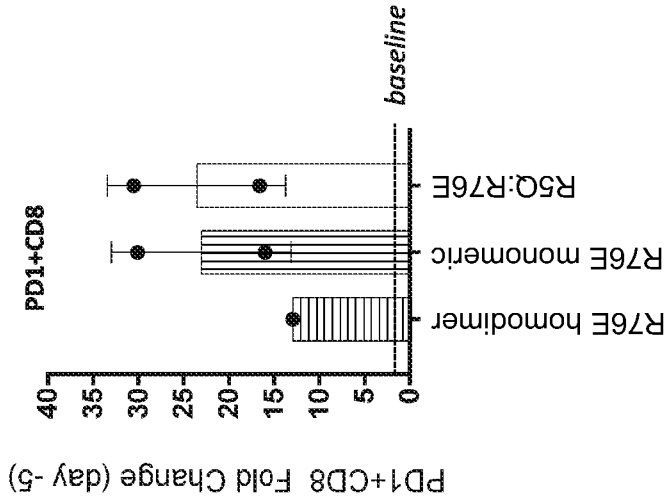
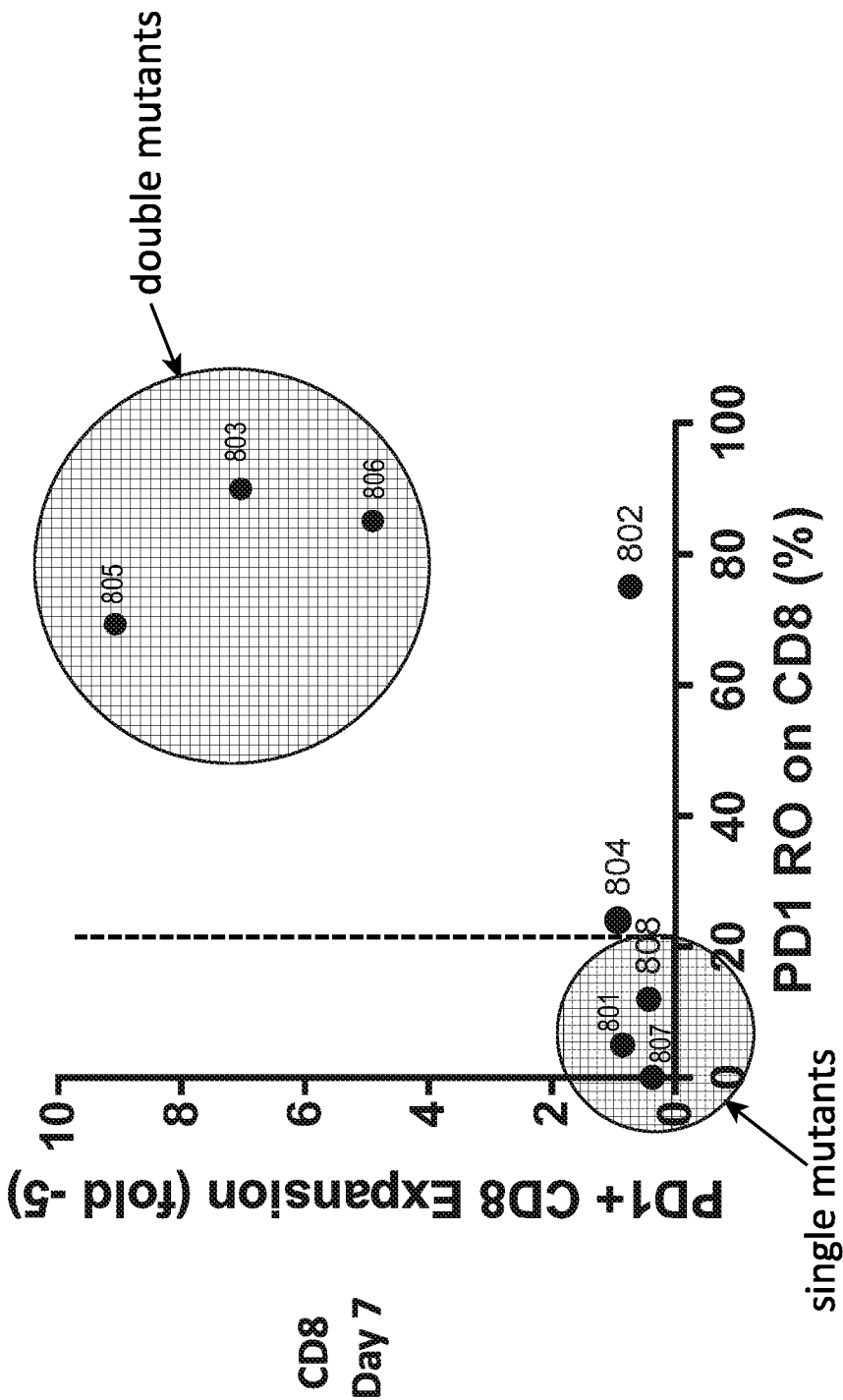
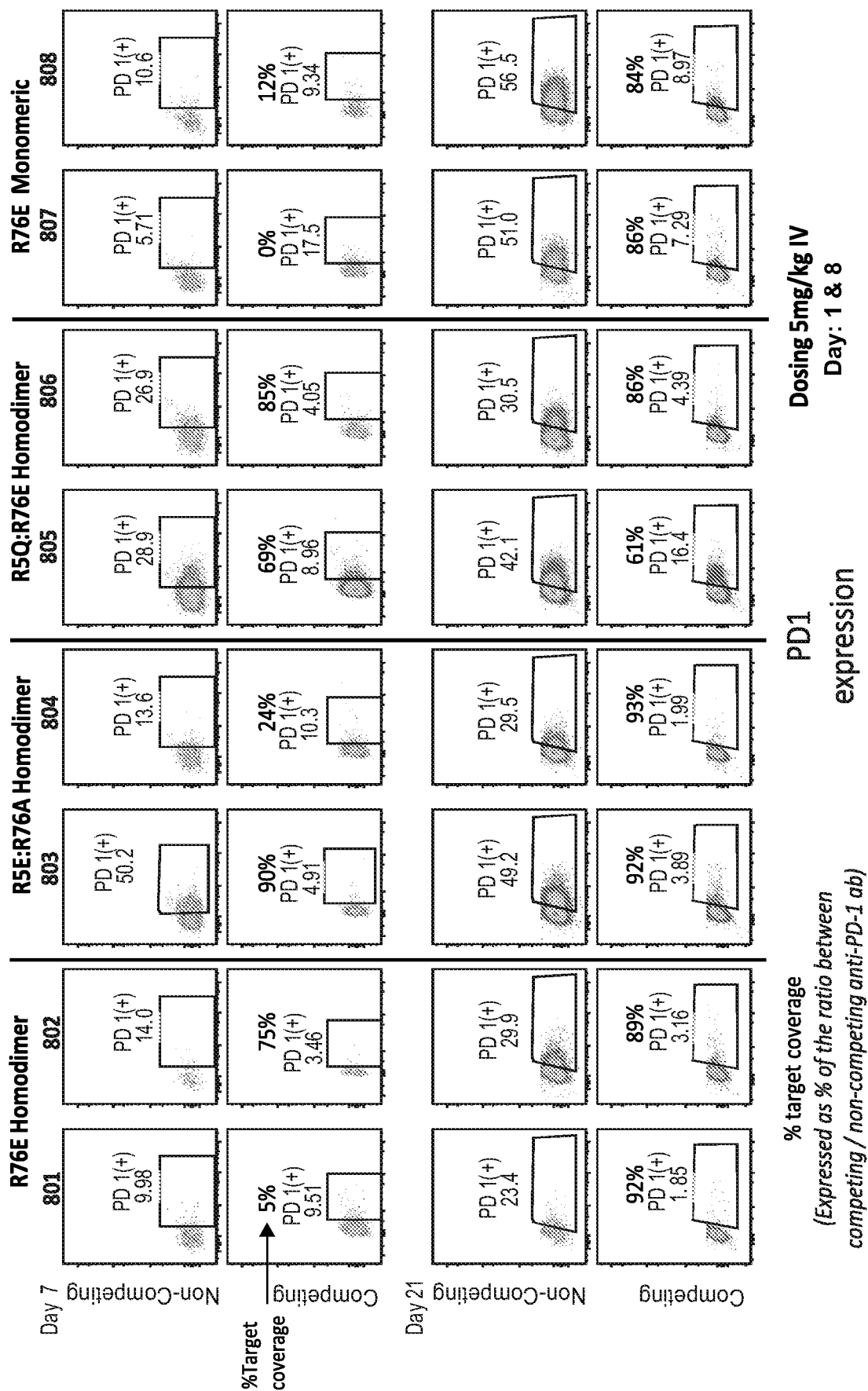


Fig. 15D

Fig. 16
Day 7 (receptor occupancy vs PD1+ T cells expansion)





PD-1 targeting needed for pSTAT3 signal, activity of mutein similar across different PD-1 mAb variants

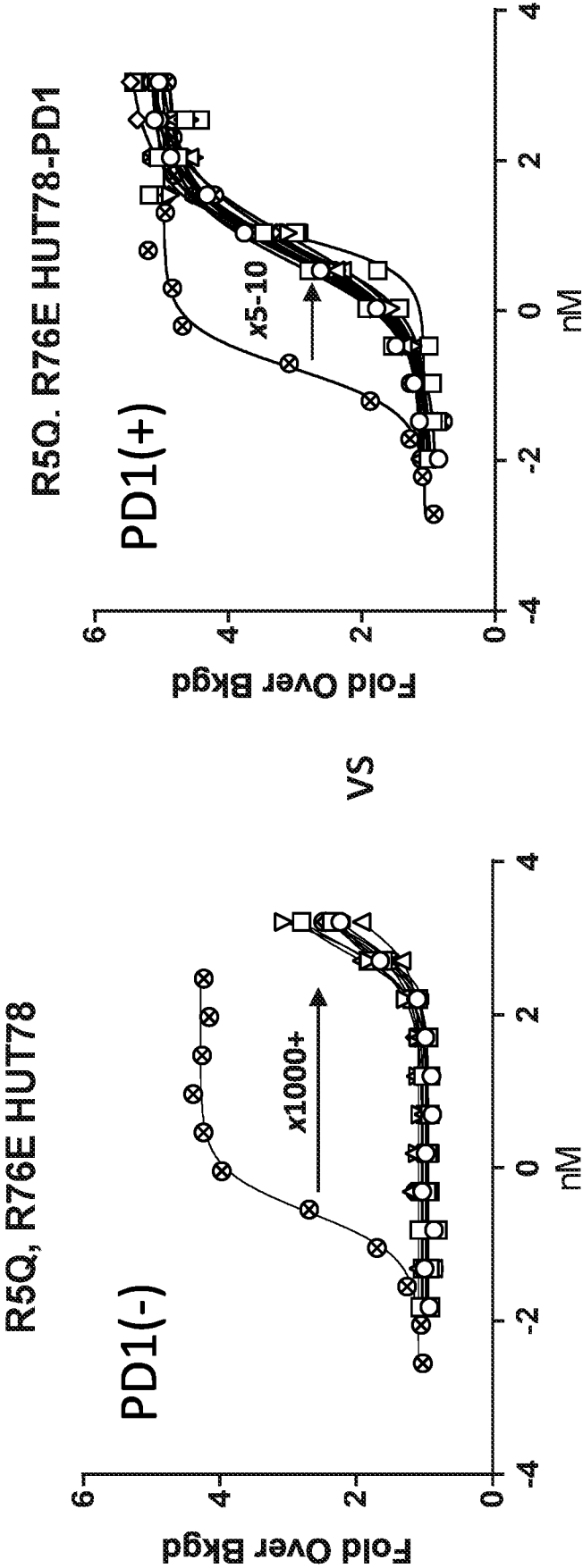


Fig. 18A

Fig. 18B

PD-1 targeting needed for pSTAT3 signal, activity of mutein similar across different PD-1 mAb variants

R9E, R76A HUT78

R9E, R76A HUT78-PD1

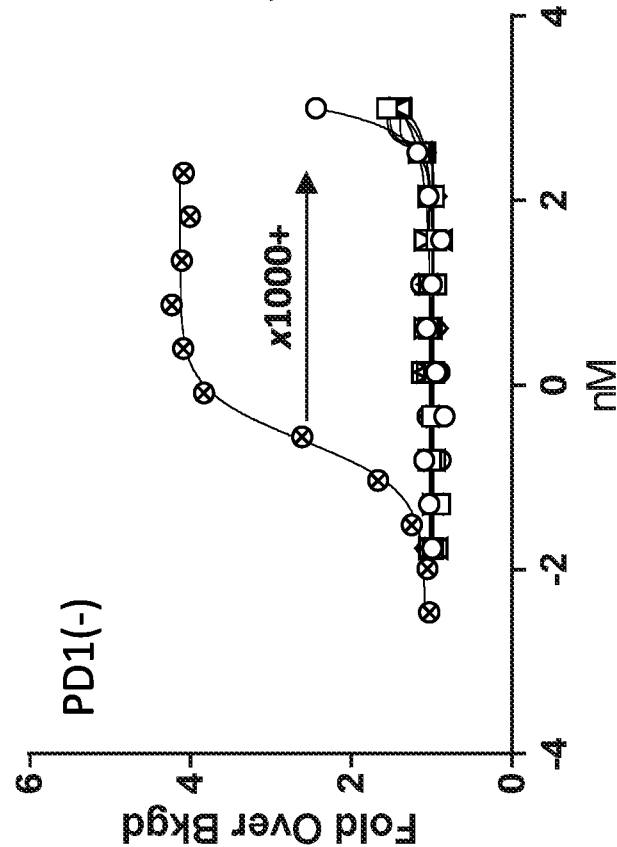


Fig. 19A

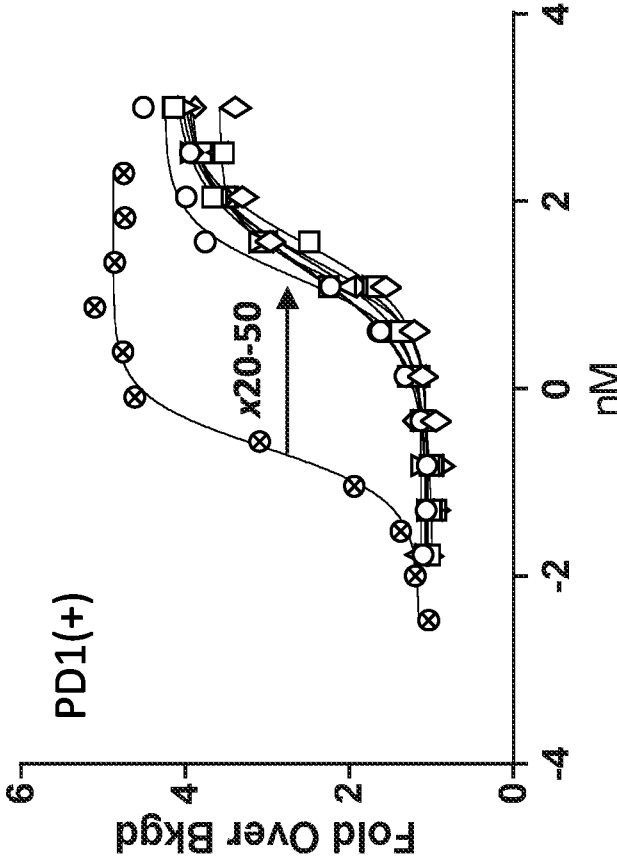


Fig. 19B

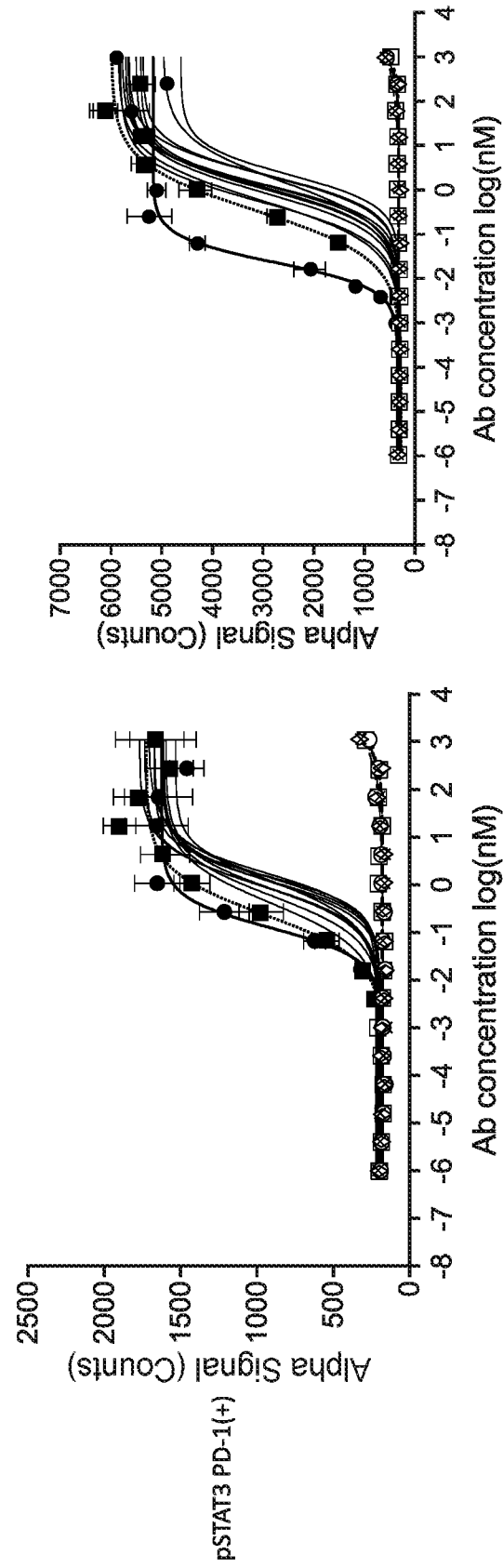
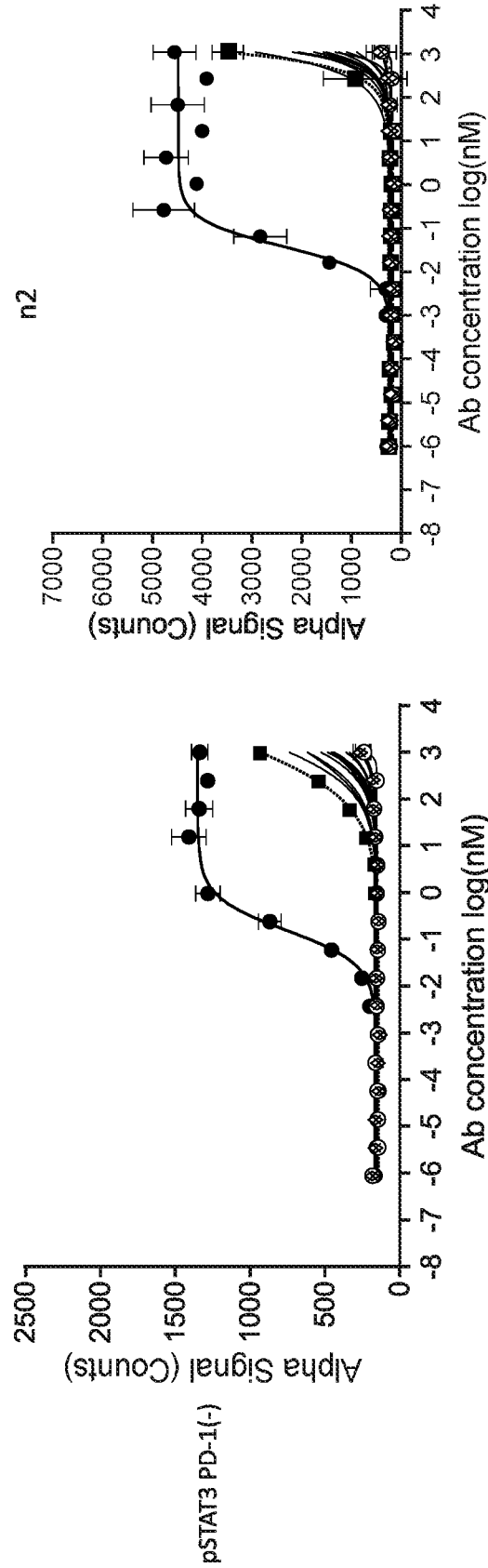


Fig. 20D

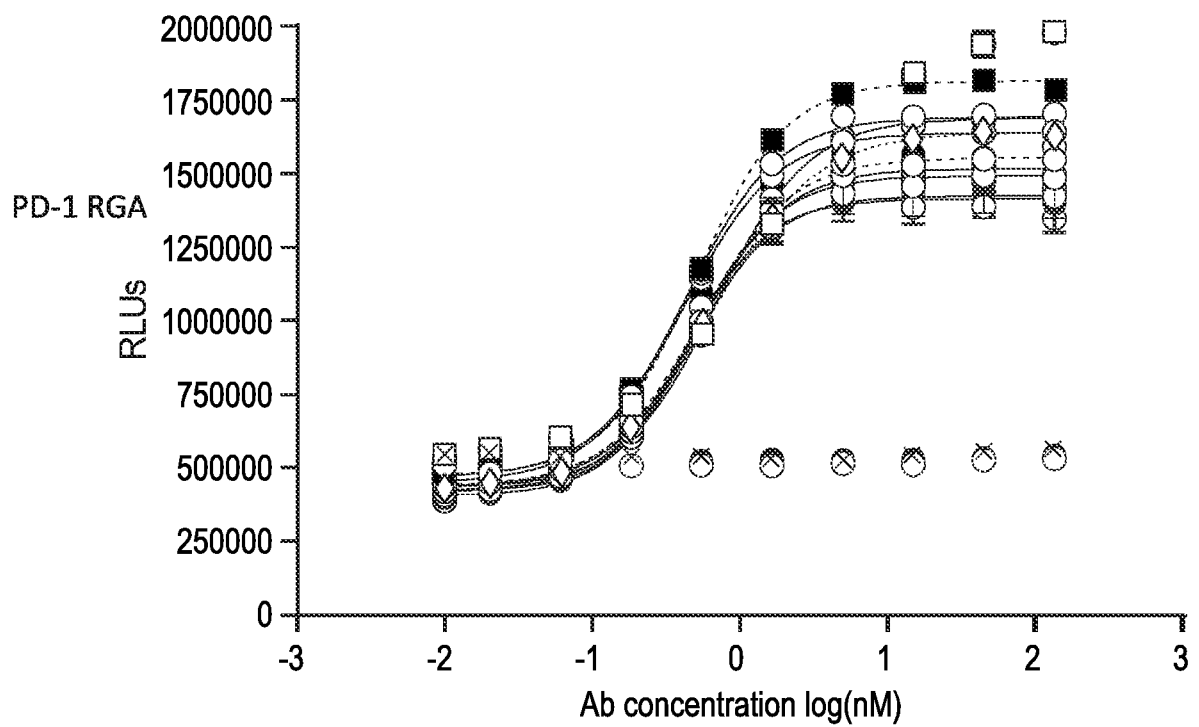
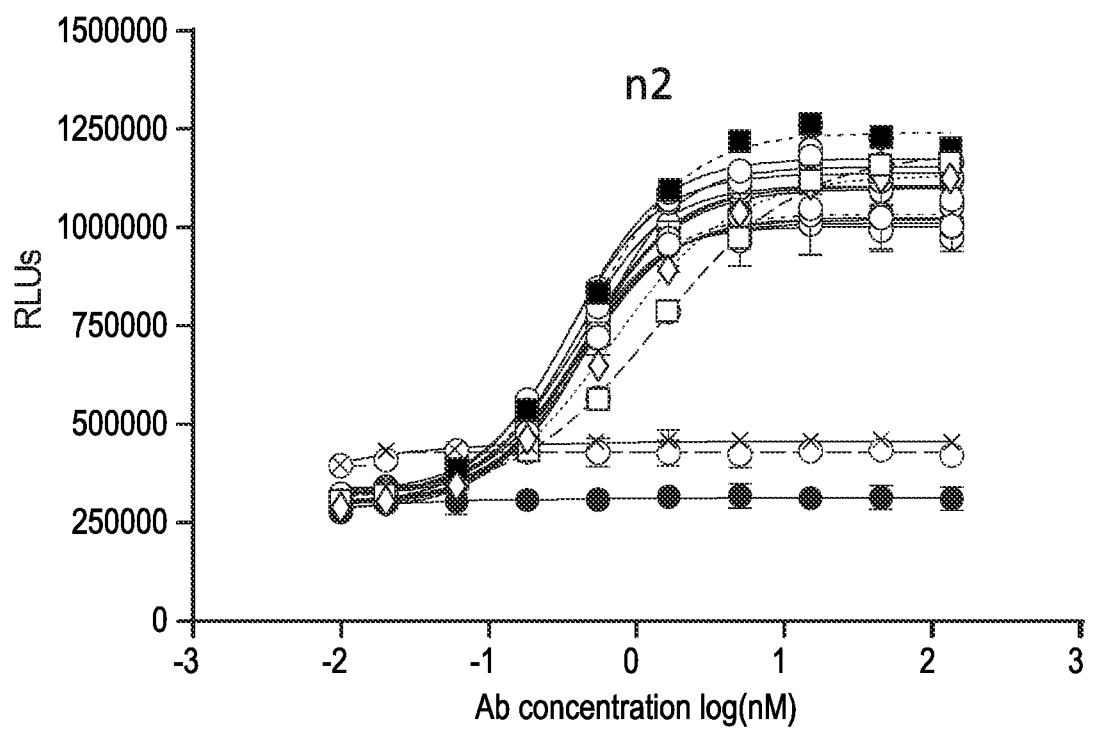
Fig. 21A**Fig. 21B**

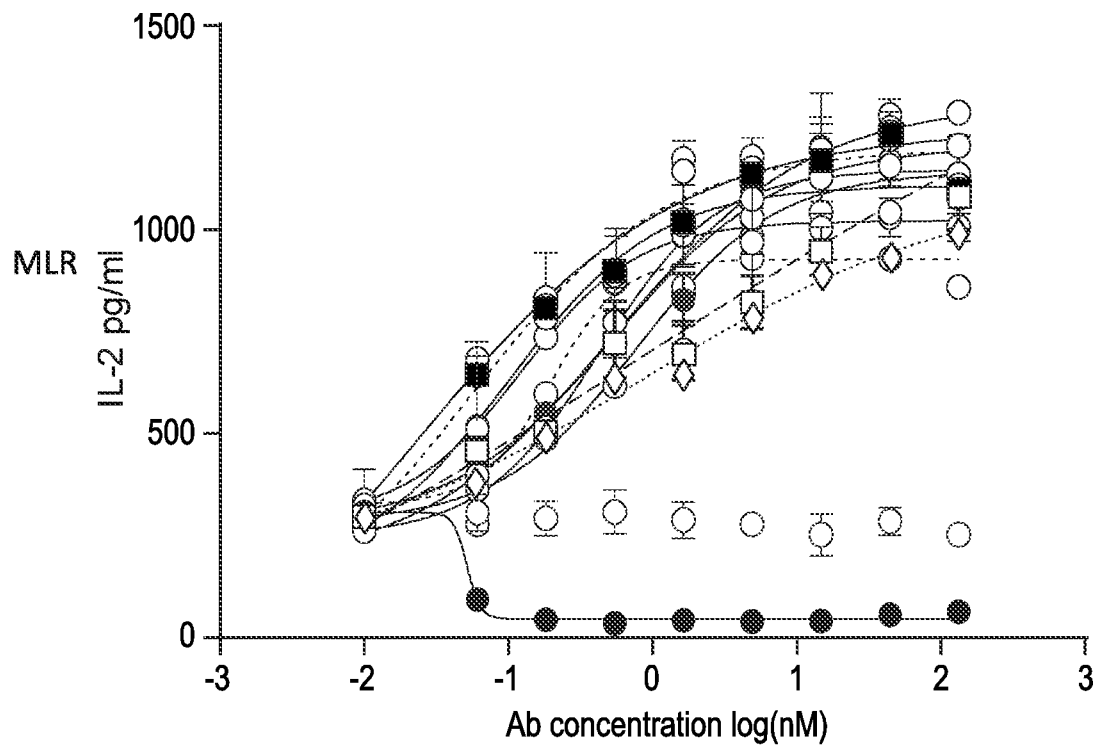
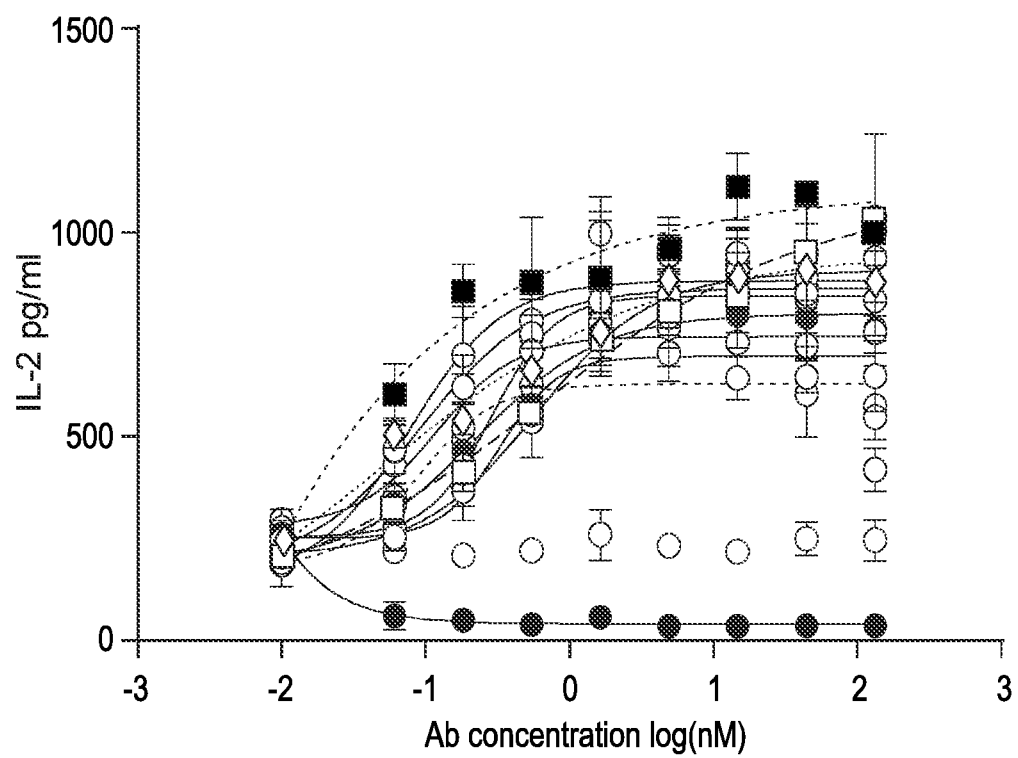
Fig. 21C**Fig. 21D**

Fig. 22A

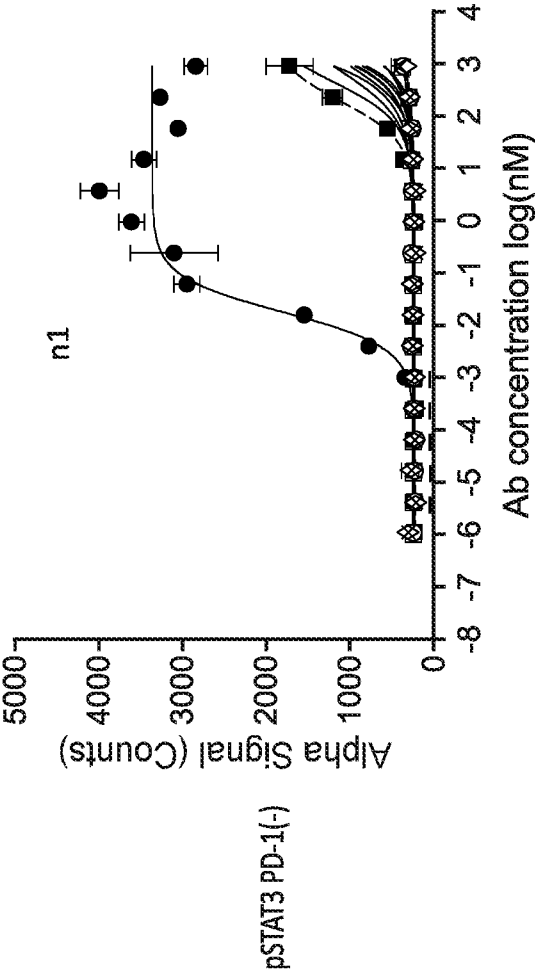


Fig. 22B

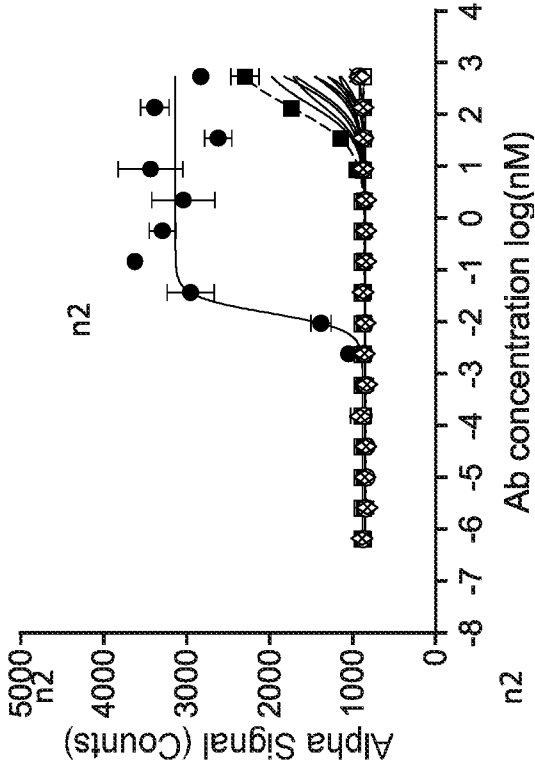


Fig. 22C

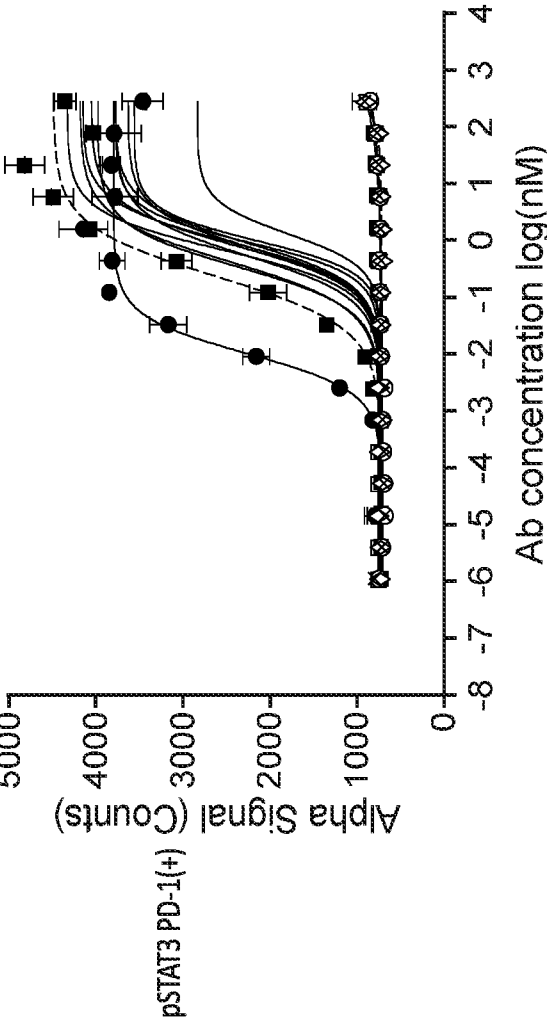
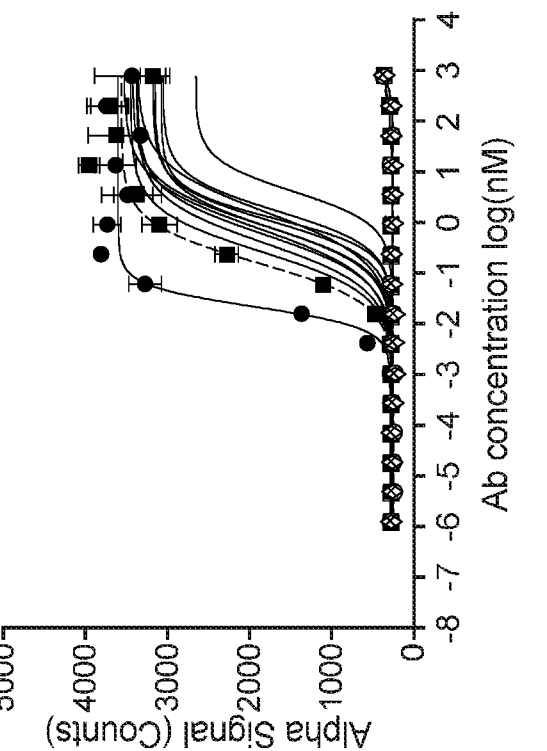


Fig. 22D



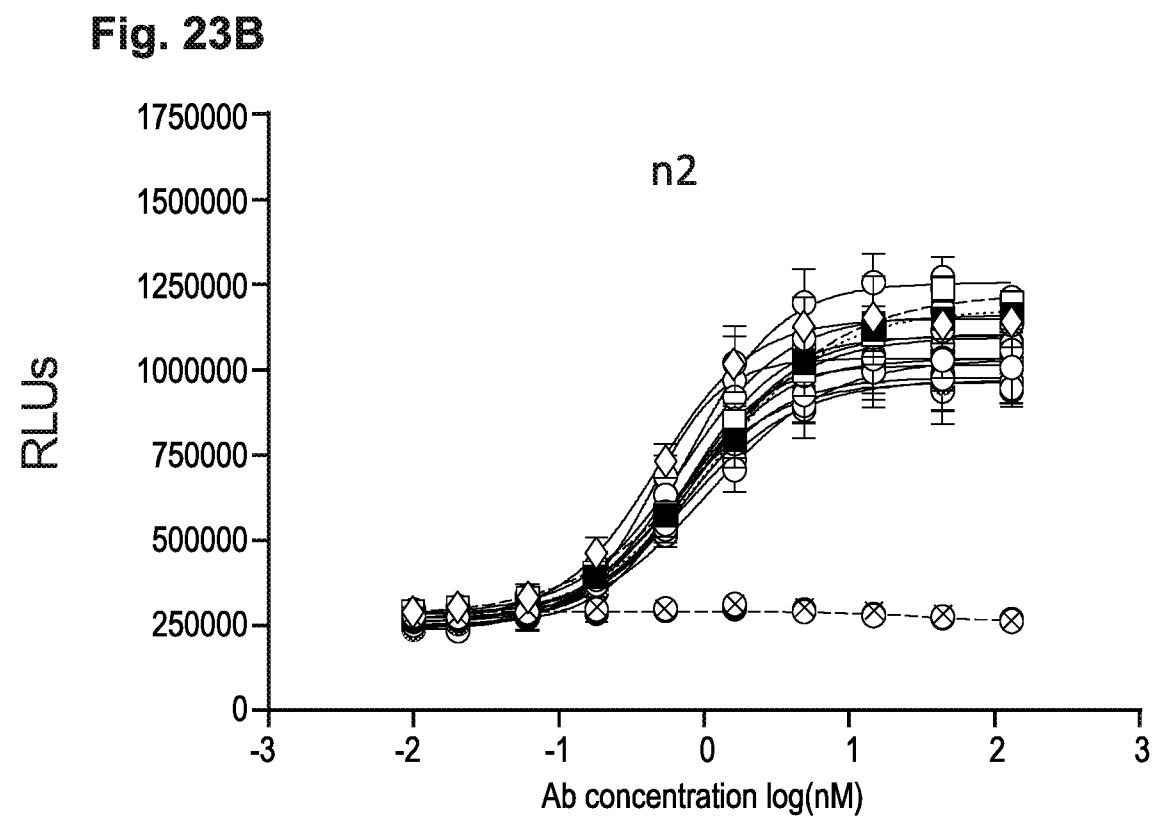
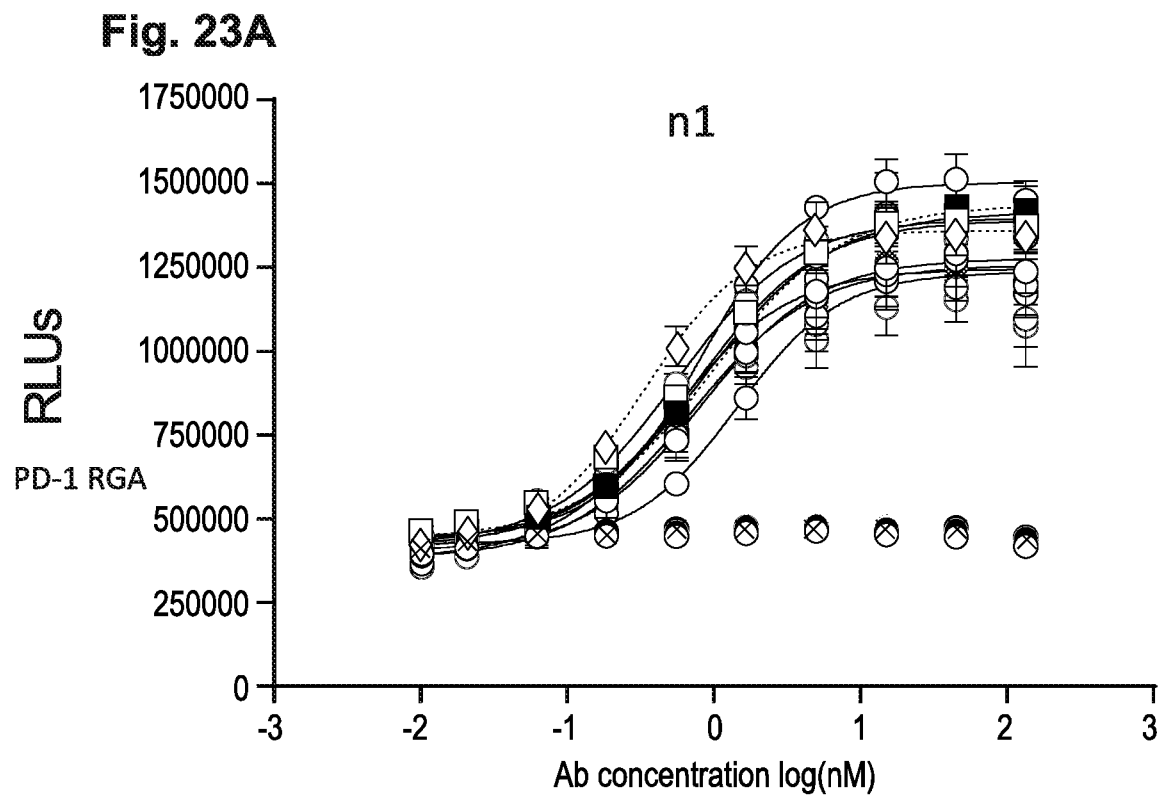


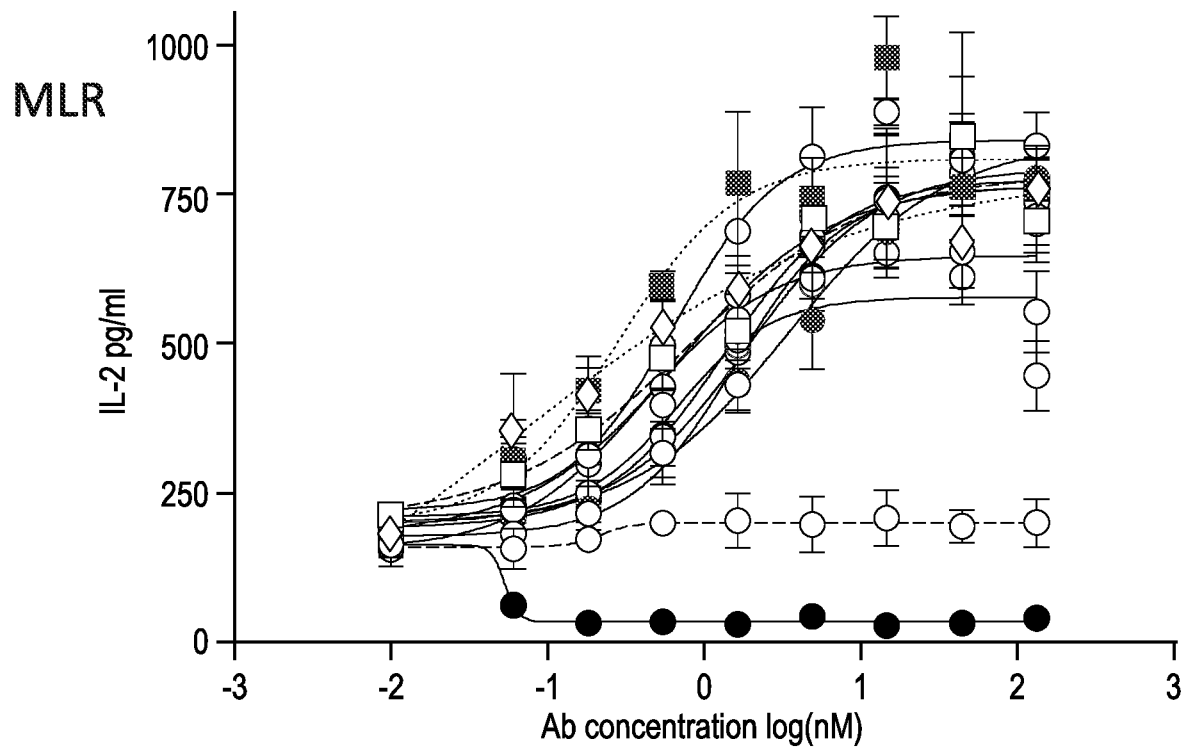
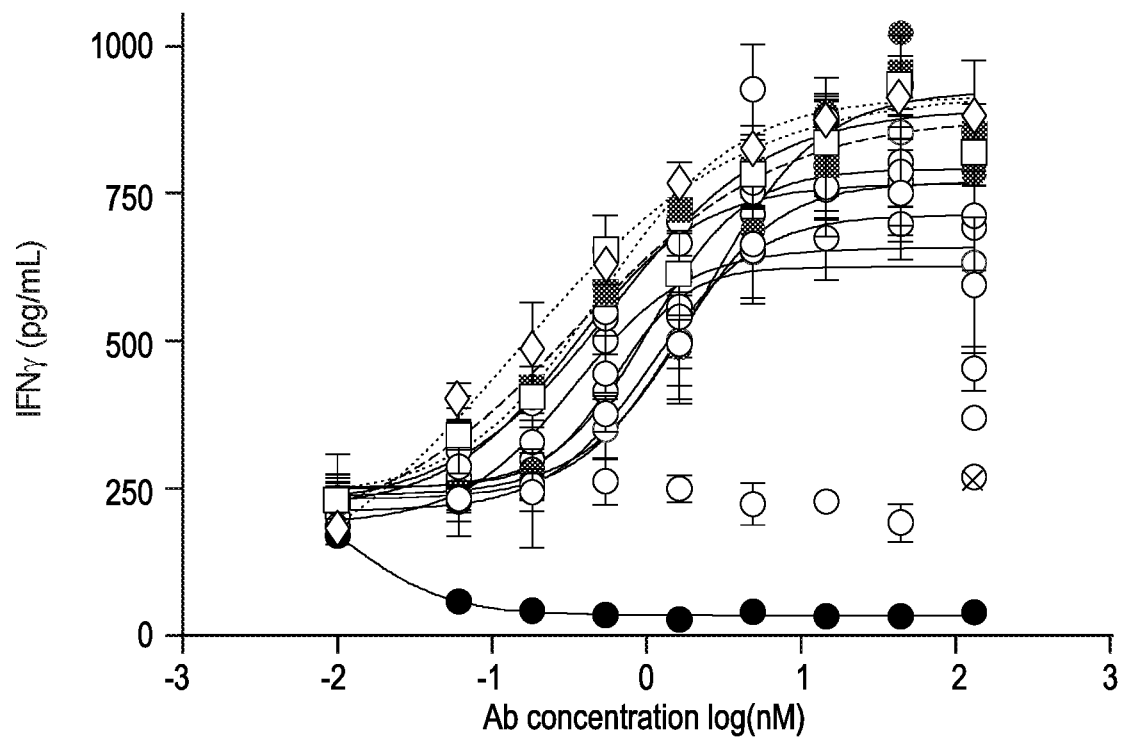
Fig. 23C**Fig. 23D**

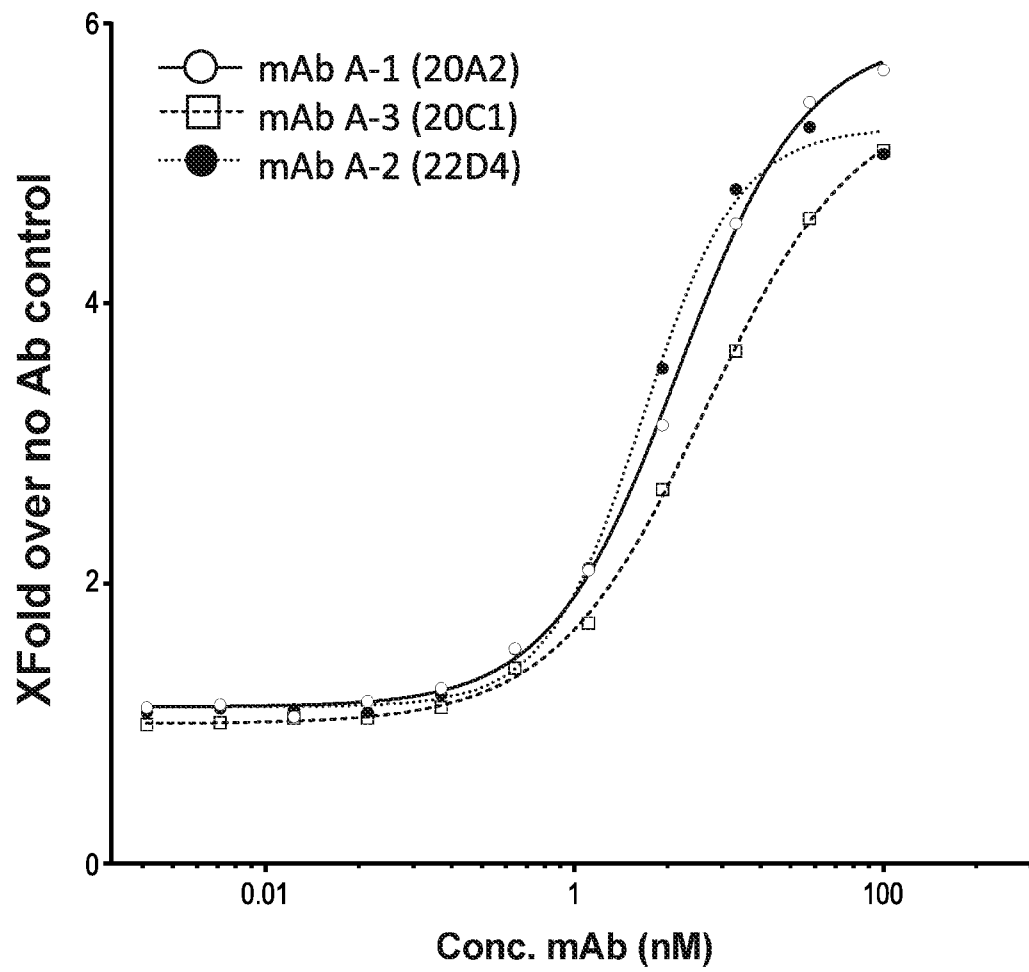
Fig. 24

Fig. 25A

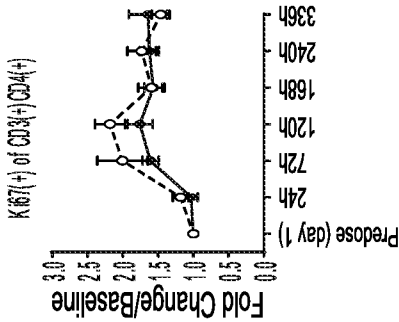


Fig. 25C

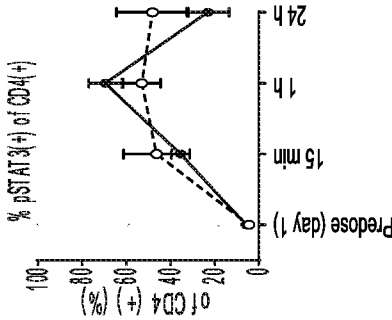


Fig. 25E

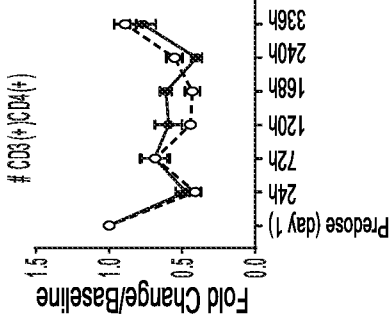


Fig. 25G

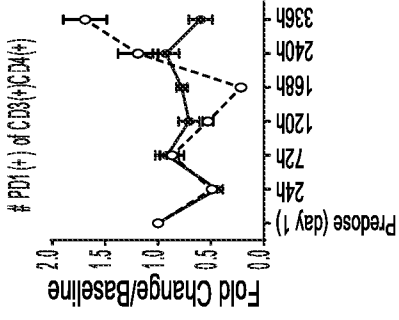


Fig. 25I

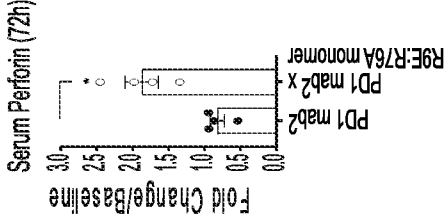


Fig. 25B

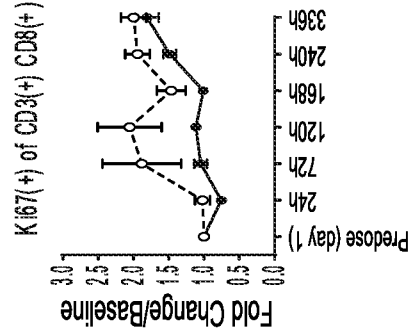


Fig. 25D

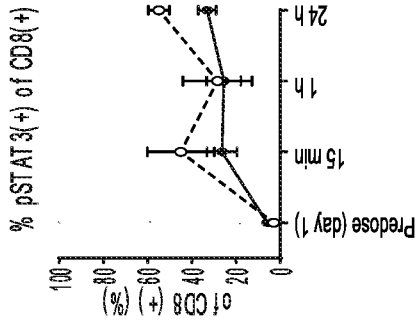


Fig. 25F

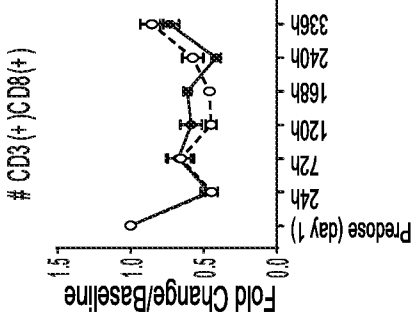


Fig. 25H

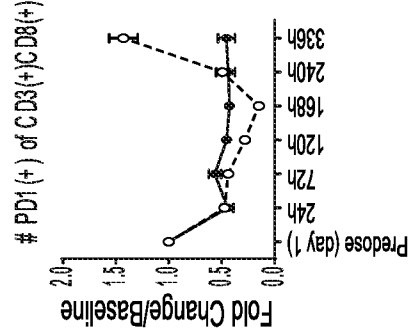
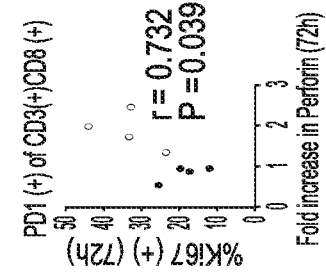


Fig. 25J



--○-- [22D4.017]-[R9E:R76A] (monomer)

—●— [22D4.017]

FIG. 26A

huPD-1 Ab 22D4.017

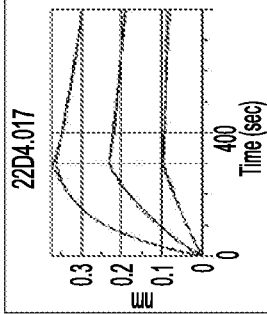


FIG. 26B

huPD-1 Ab 20C1.009

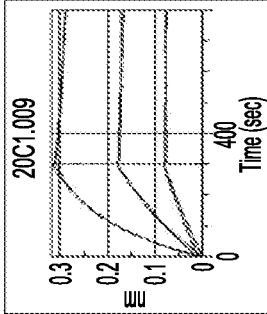


FIG. 26C

huPD1 Ab 20A2.003

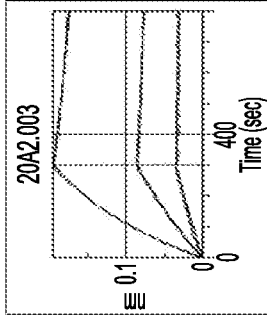


FIG. 26D

IgG1 anti-PD-1 mAb

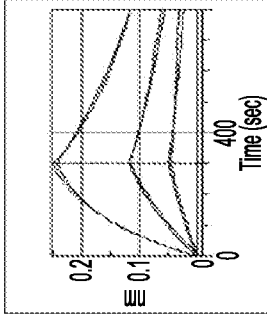


FIG. 26E

IgG4 anti-PD-1 mAb

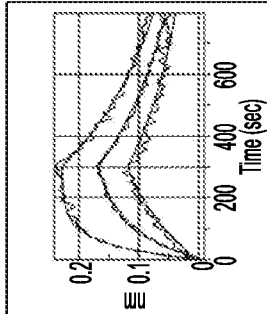


FIG. 26F

huPD-1 Ab 22D4.017

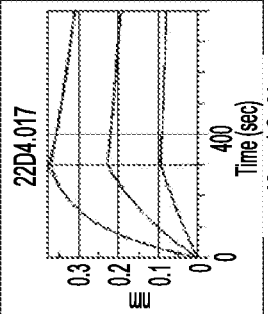


FIG. 26G

huPD-1 Ab 20C1.009

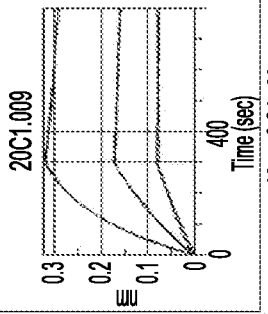


FIG. 26H

huPD1 Ab 20A2.003

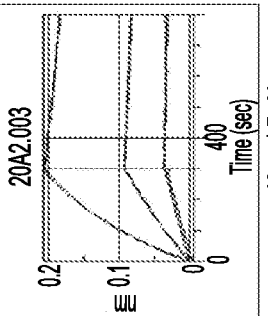


FIG. 26I

IgG1 anti-PD-1 mAb

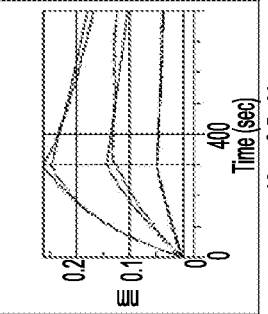
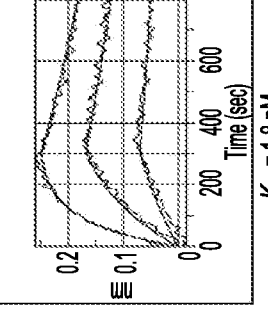


FIG. 26J

IgG4 anti-PD-1 mAb



cynoPD1(1-167)-Flag-His

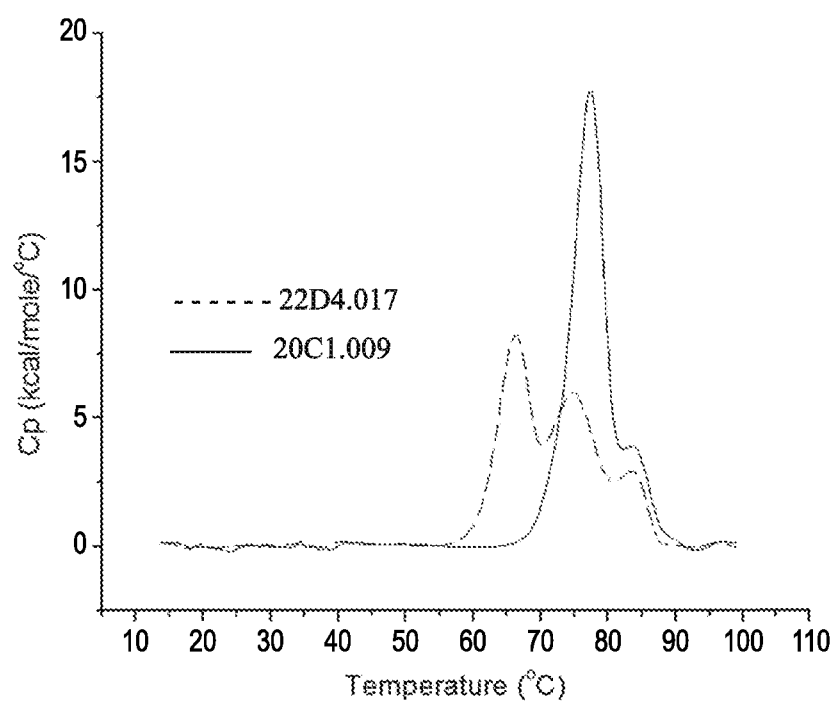
Fig. 27

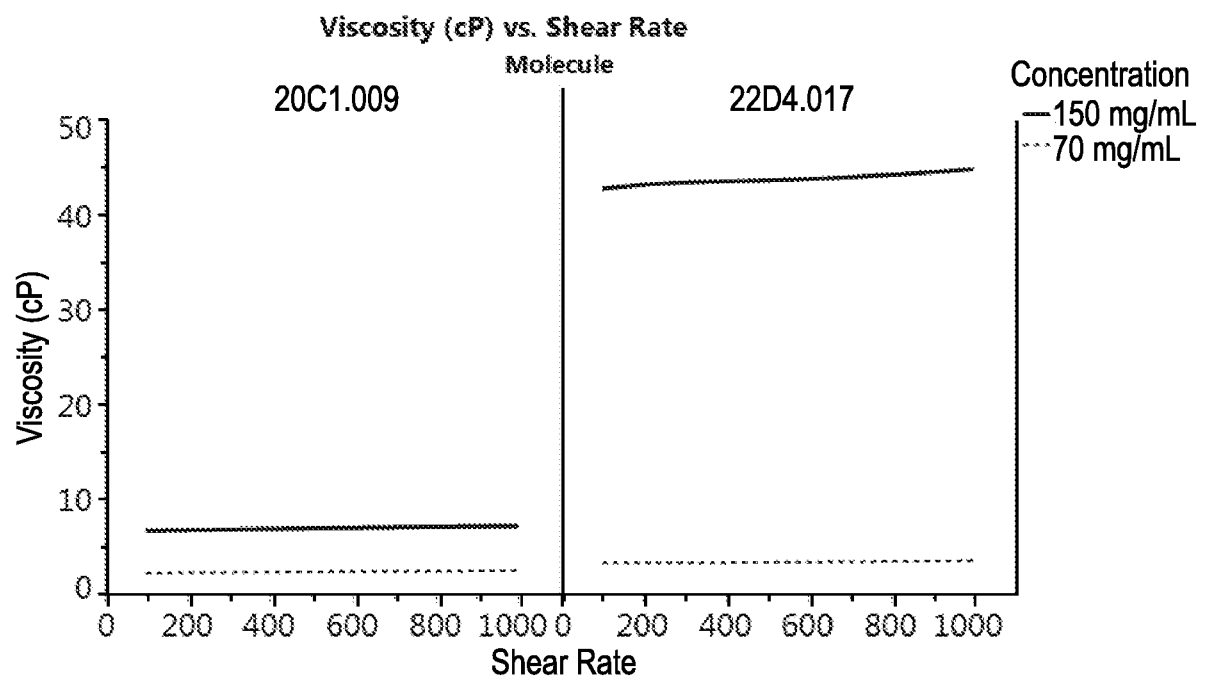
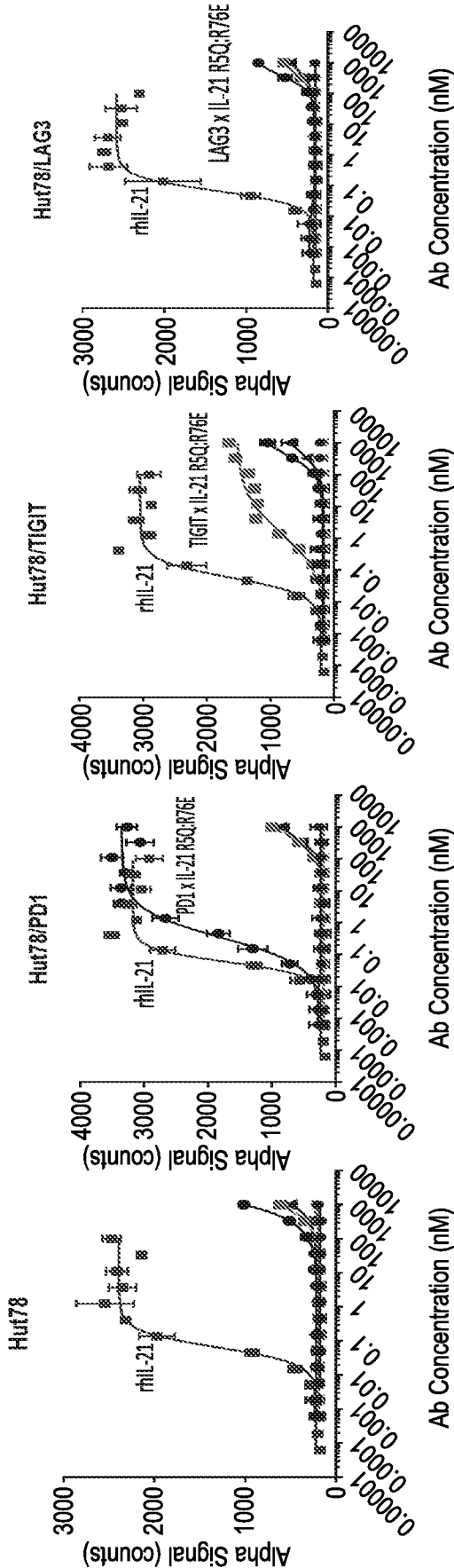
Fig. 28

Fig. 29A

Fig. 29B

Fig. 29C

Fig. 29D



Engineered Hut78 T cell lines expressing either PD-1, TIGIT or LAG3

- hulgG1
- rhIL-21
- 22D4 017
- TIGIT mAb
- LAG3 mAb
- PD1 mab x R5Q:R76E
- TIGIT mab x R5Q:R78E
- LAG3 mab x R5Q:R78E