

Fig. 1A

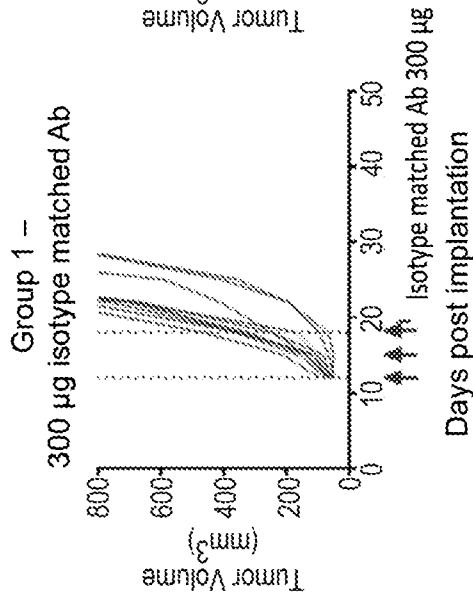


Fig. 1B

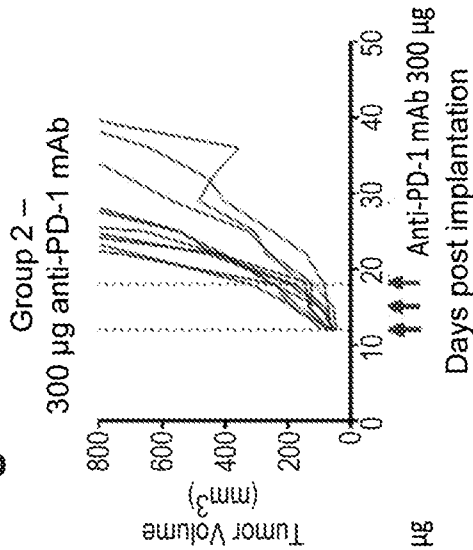


Fig. 1C

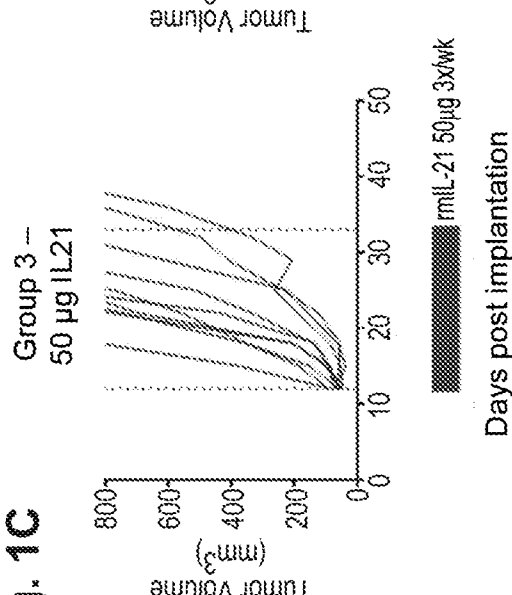


Fig. 1D

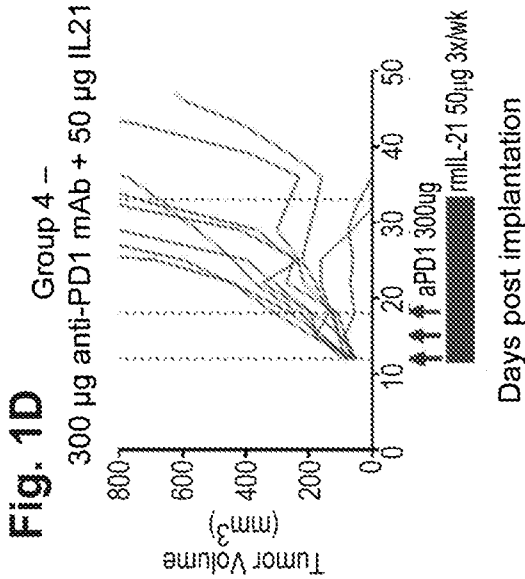
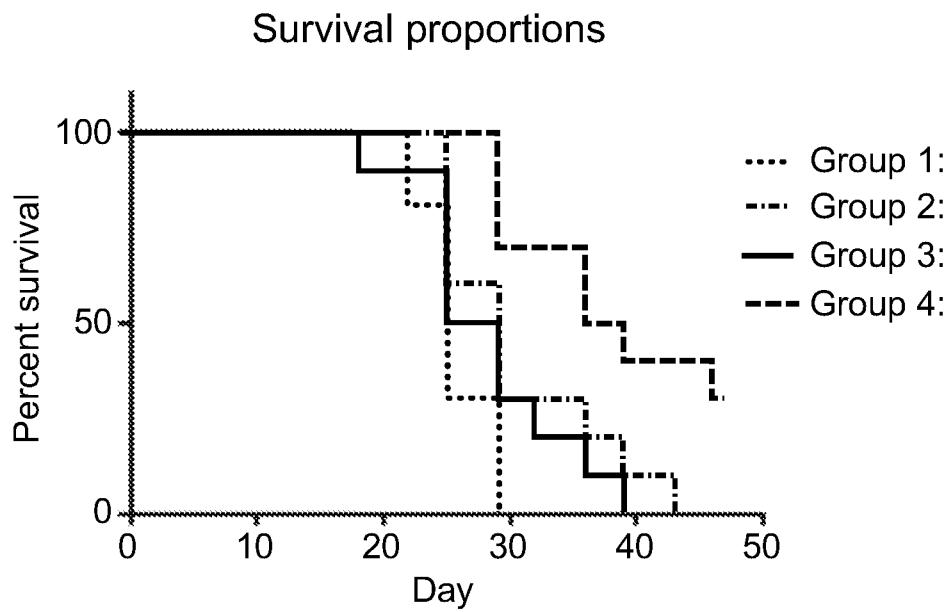
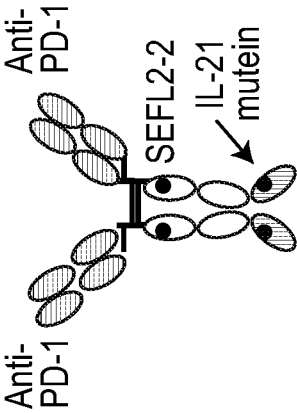


FIG. 2

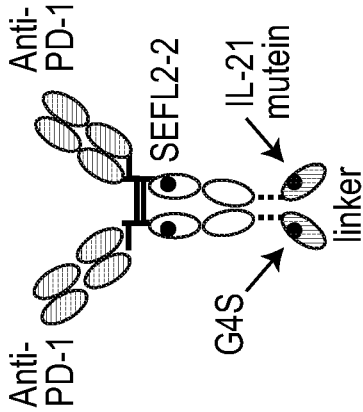
Iso vs IL-21, p=ns, 0.1269
Iso vs α PD-1 mAb, p=0.0408
Iso combo, p=0.0002
 α PD-1 mAb vs. combo, p=0.0143
IL-21 vs. combo, p=0.0047

FIGURE 4A



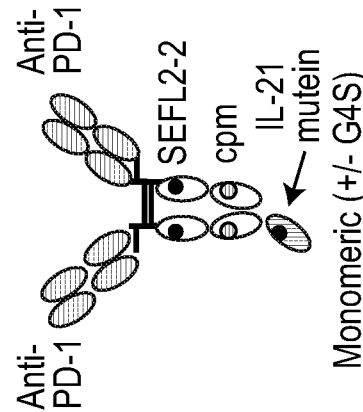
α PD1 x IL21 homodimer
(no linker)

FIGURE 4B



α PD1 x IL21 homodimer
with G4S linker

FIGURE 4C



α PD1 x monomeric IL-21

Fig. 5

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

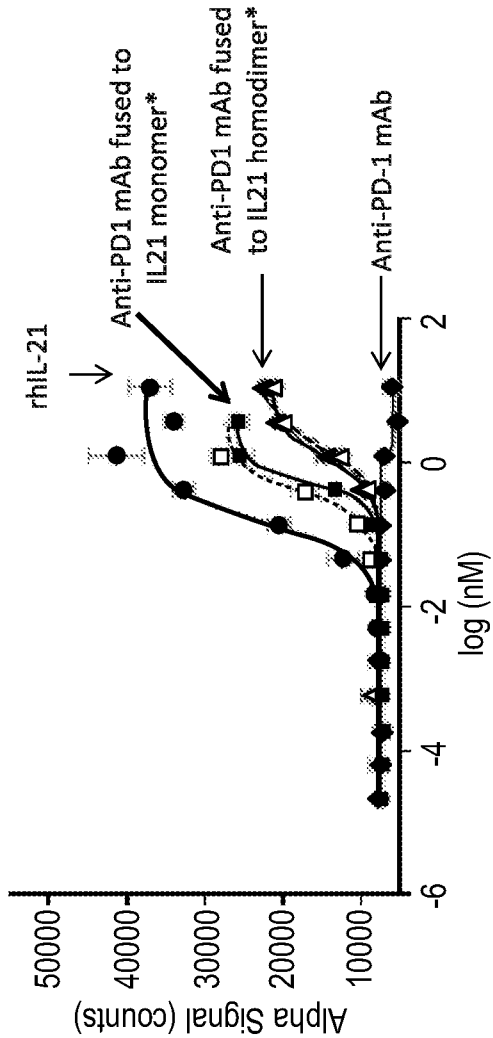
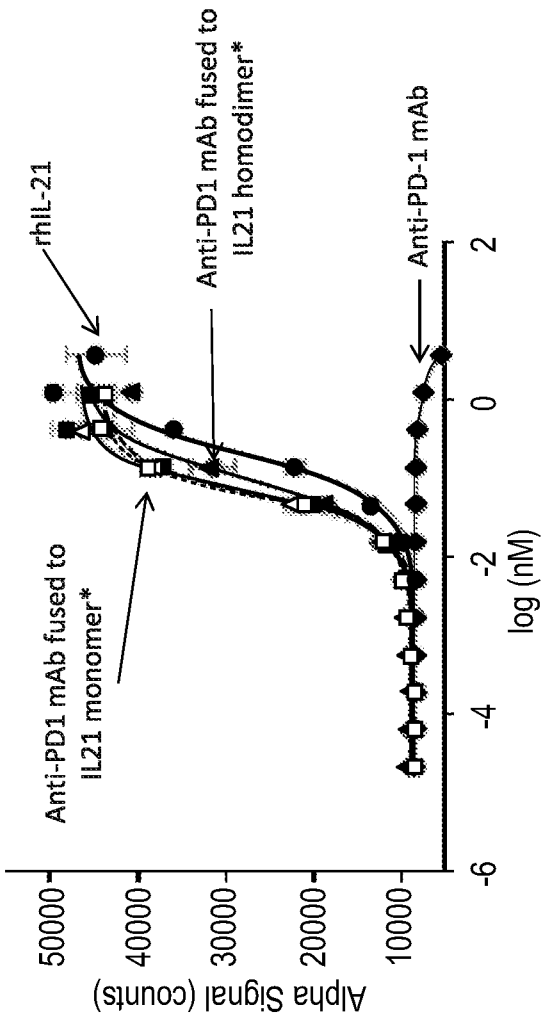
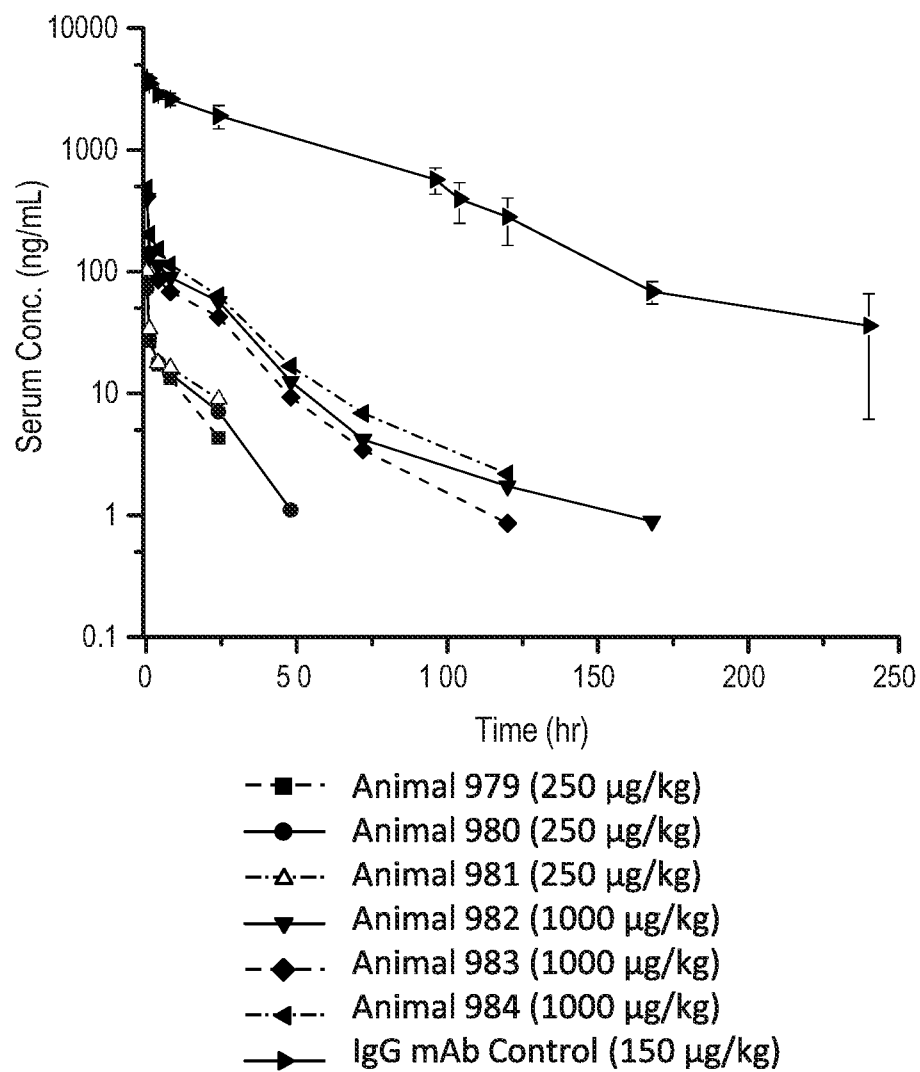
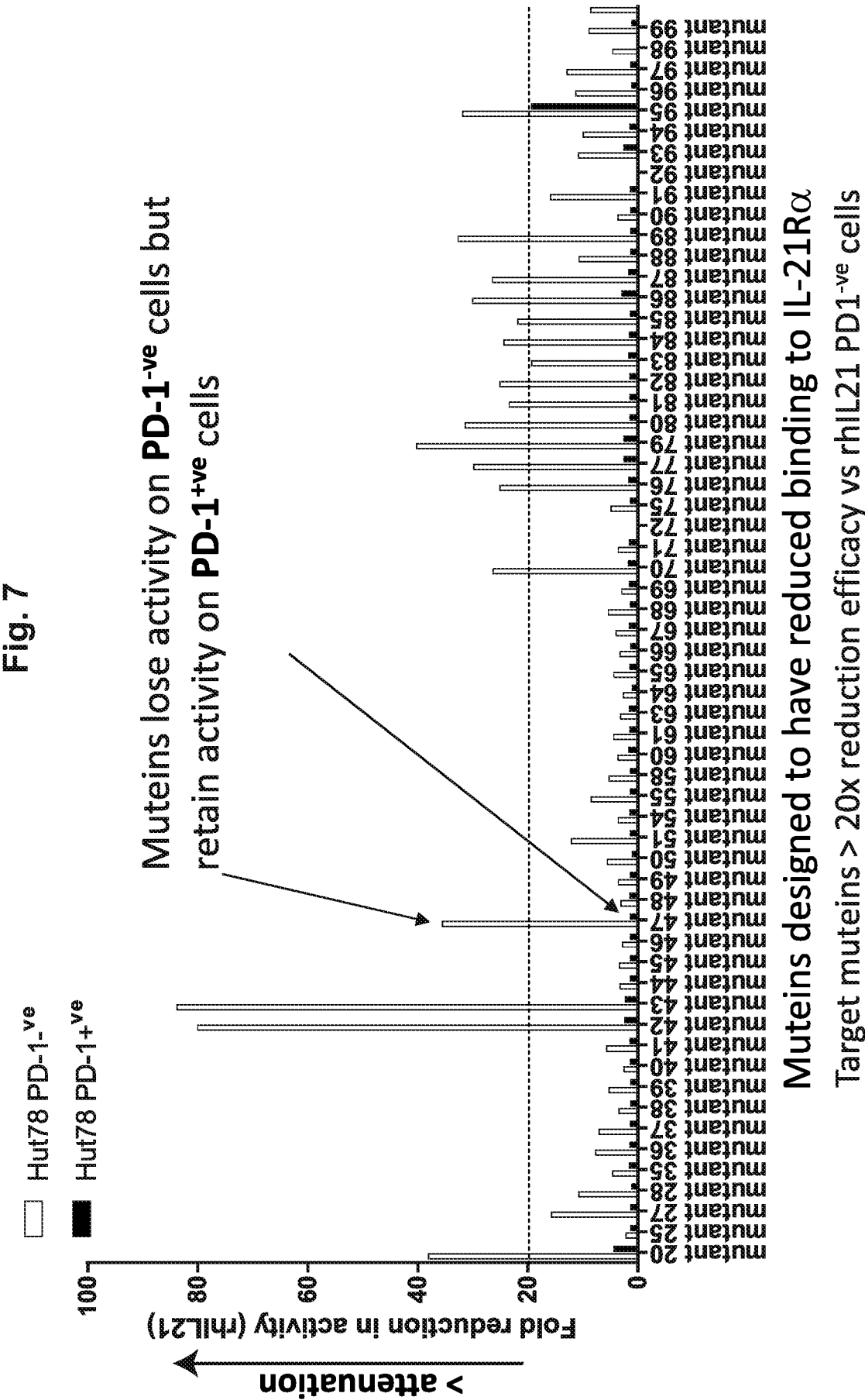


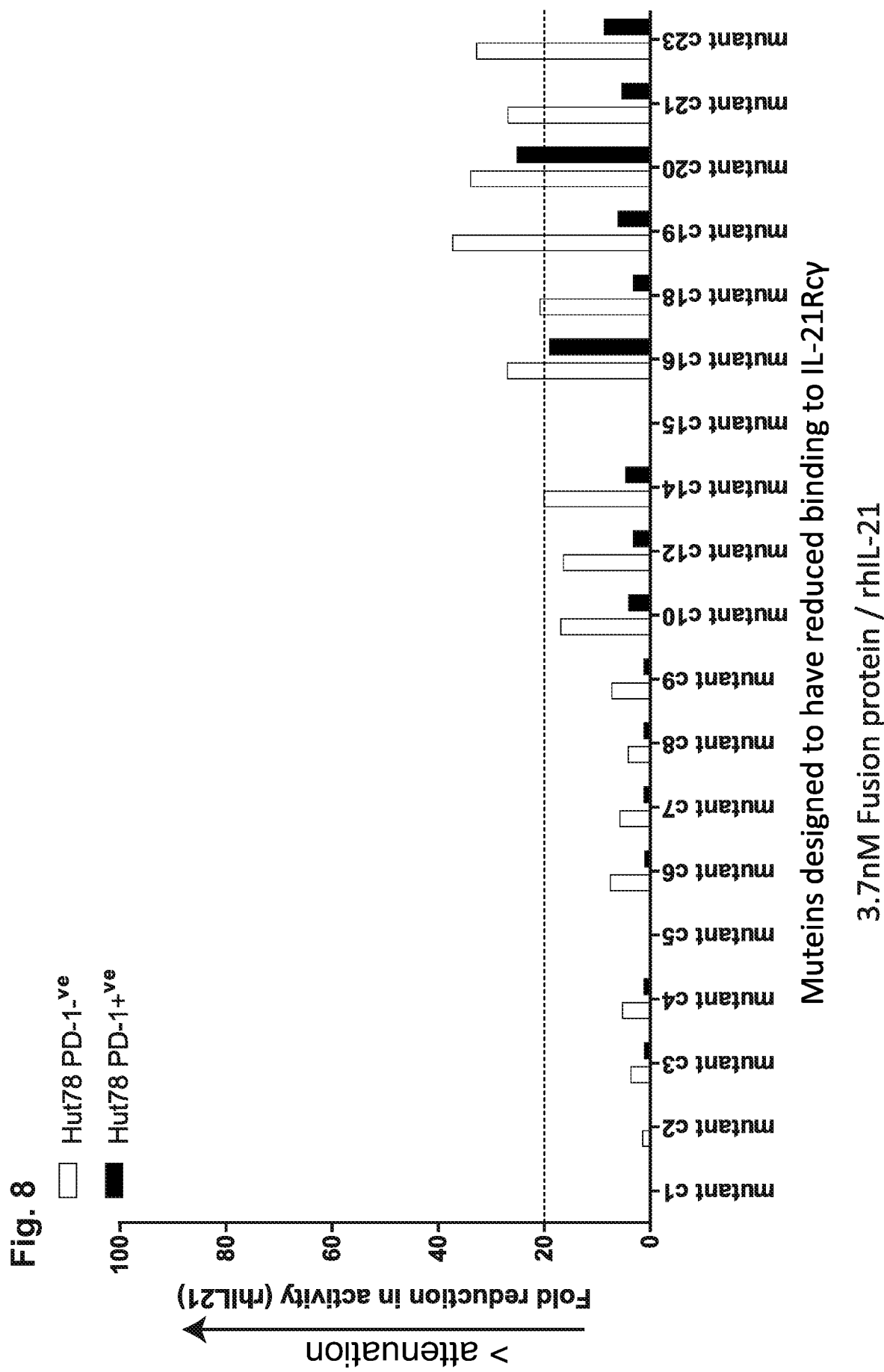
FIGURE 5B:
pSTAT3 AlphaLISA Hut78 T cells:
PD-1^{+ve}



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

Fig. 6





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

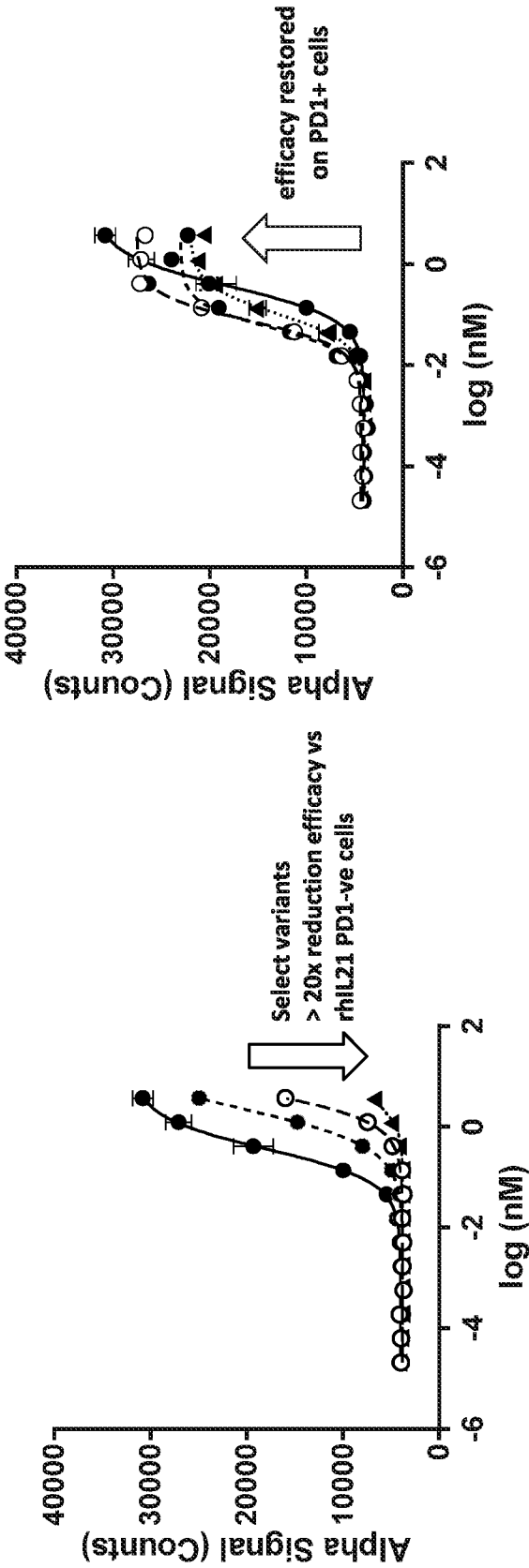
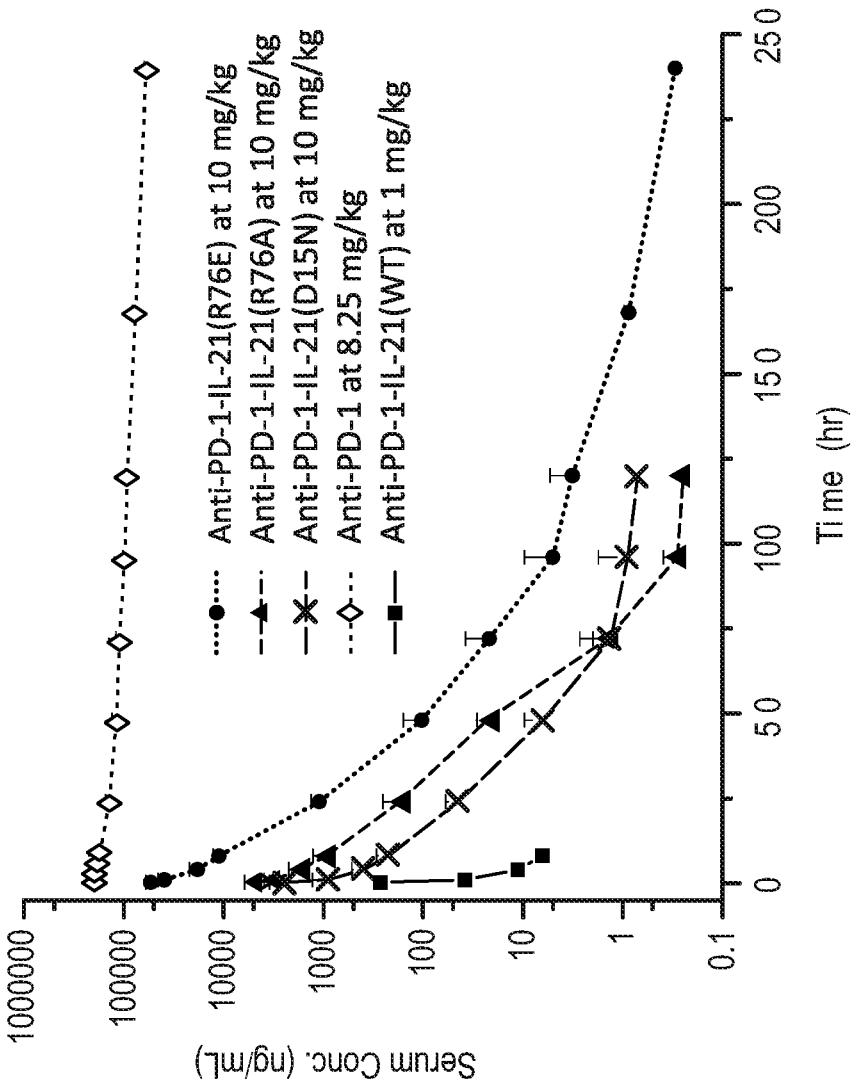


Fig. 9A
PD1^{-ve}

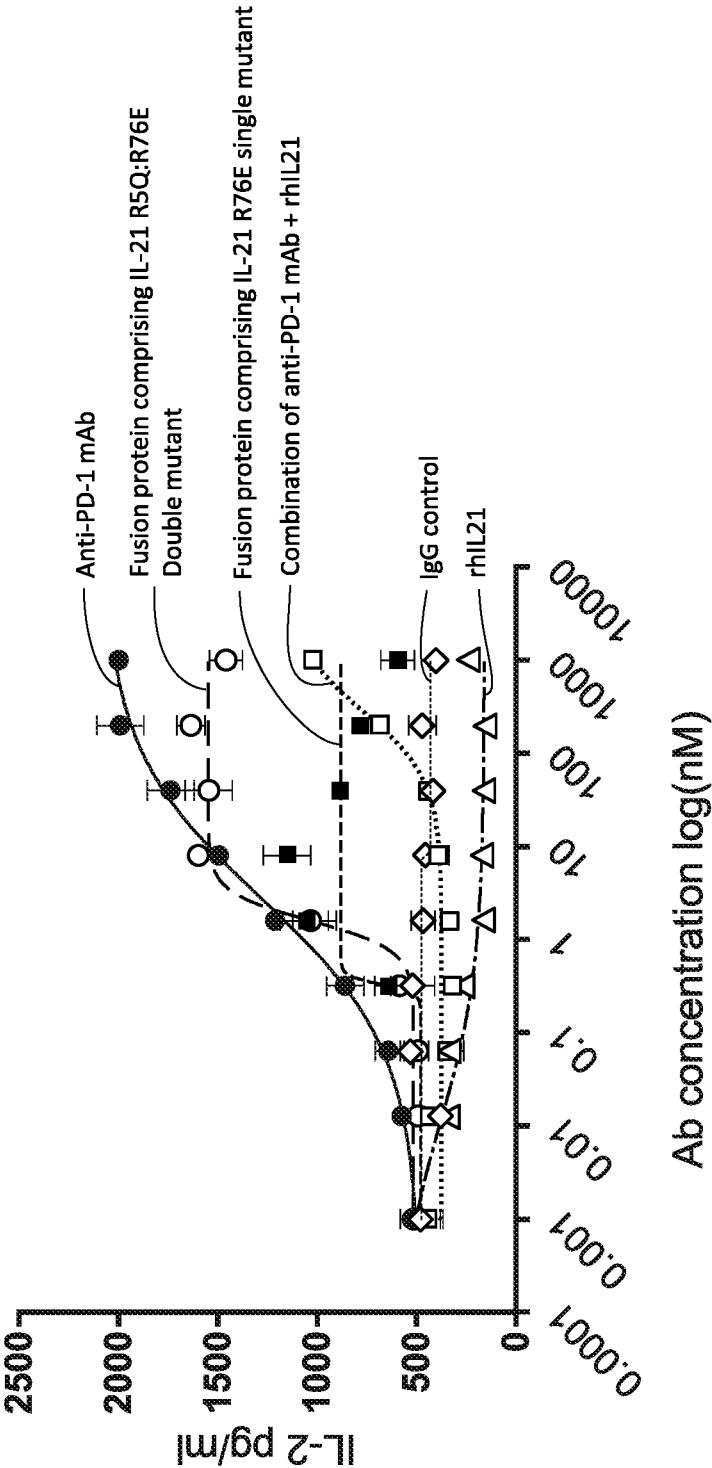
Fig. 9B
PD1^{+ve}

Fig. 10



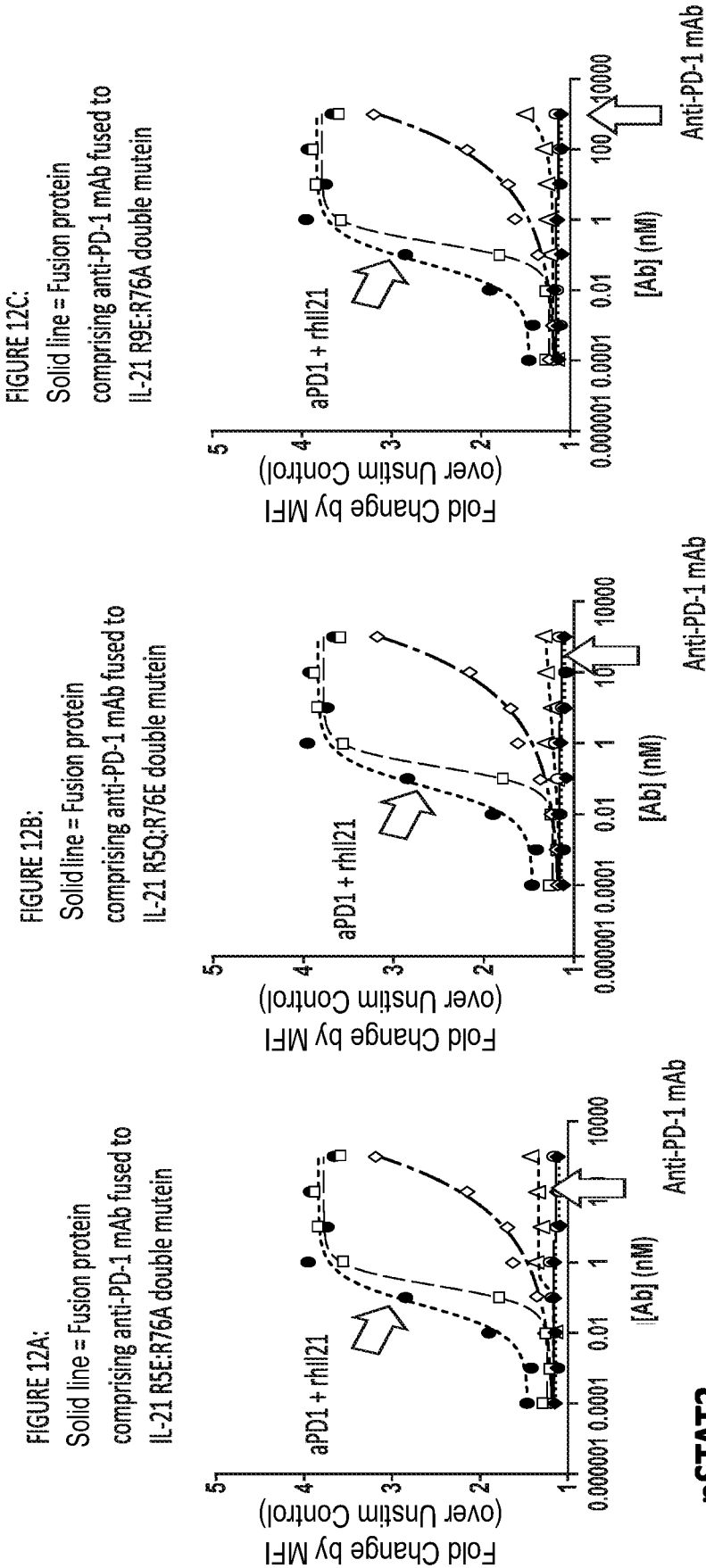
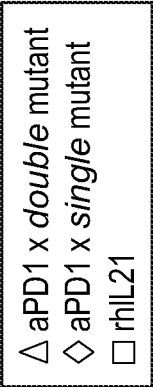
Anti-PD-1 mAb-IL-21 mutein fusion proteins exhibited altered distribution compared to parent anti-PD-1 mAb

Fig. 11



MLR – Day 3

Fig. 12



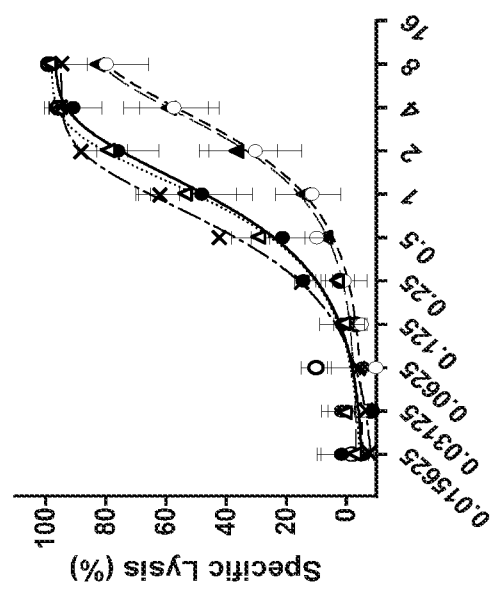
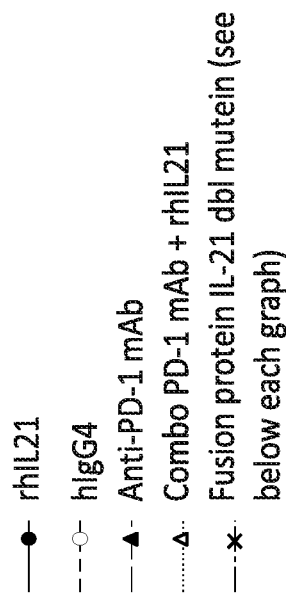


Fig. 13A
Double mutein = IL-21
R5E/R76A

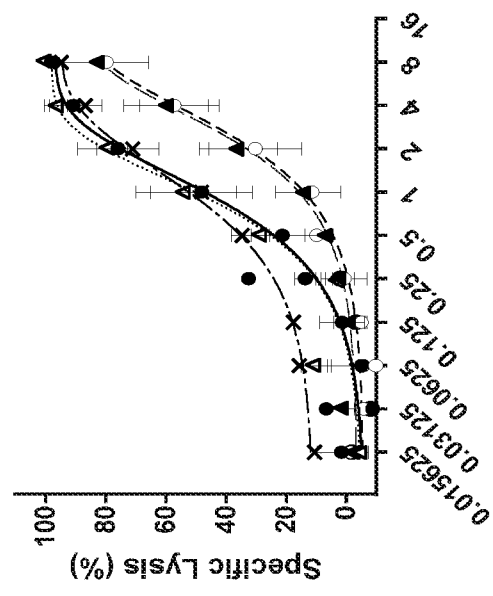


Fig. 13B
Double mutein = IL-21
R5Q/R76E

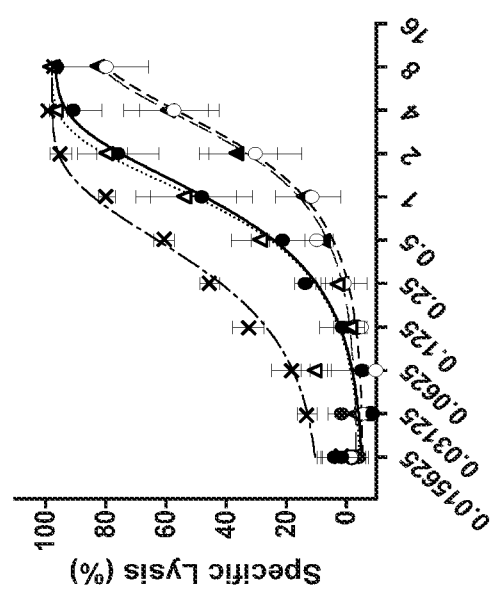
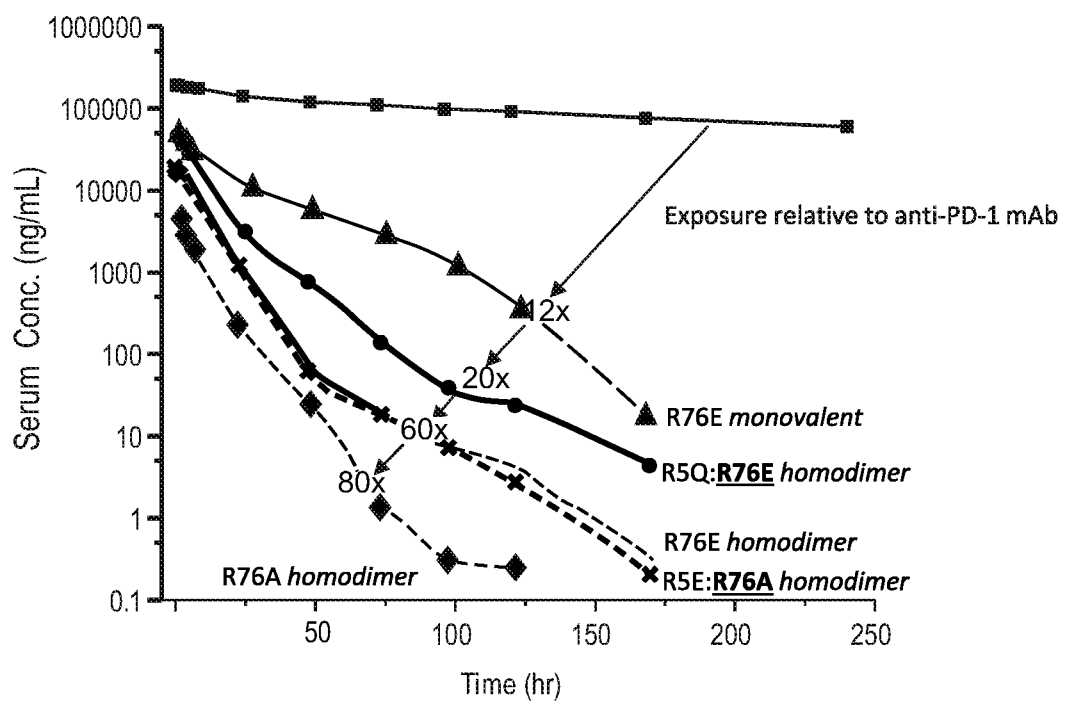


Fig. 13C
Double mutein = IL-21
R9E/R76A

31

Fig. 14



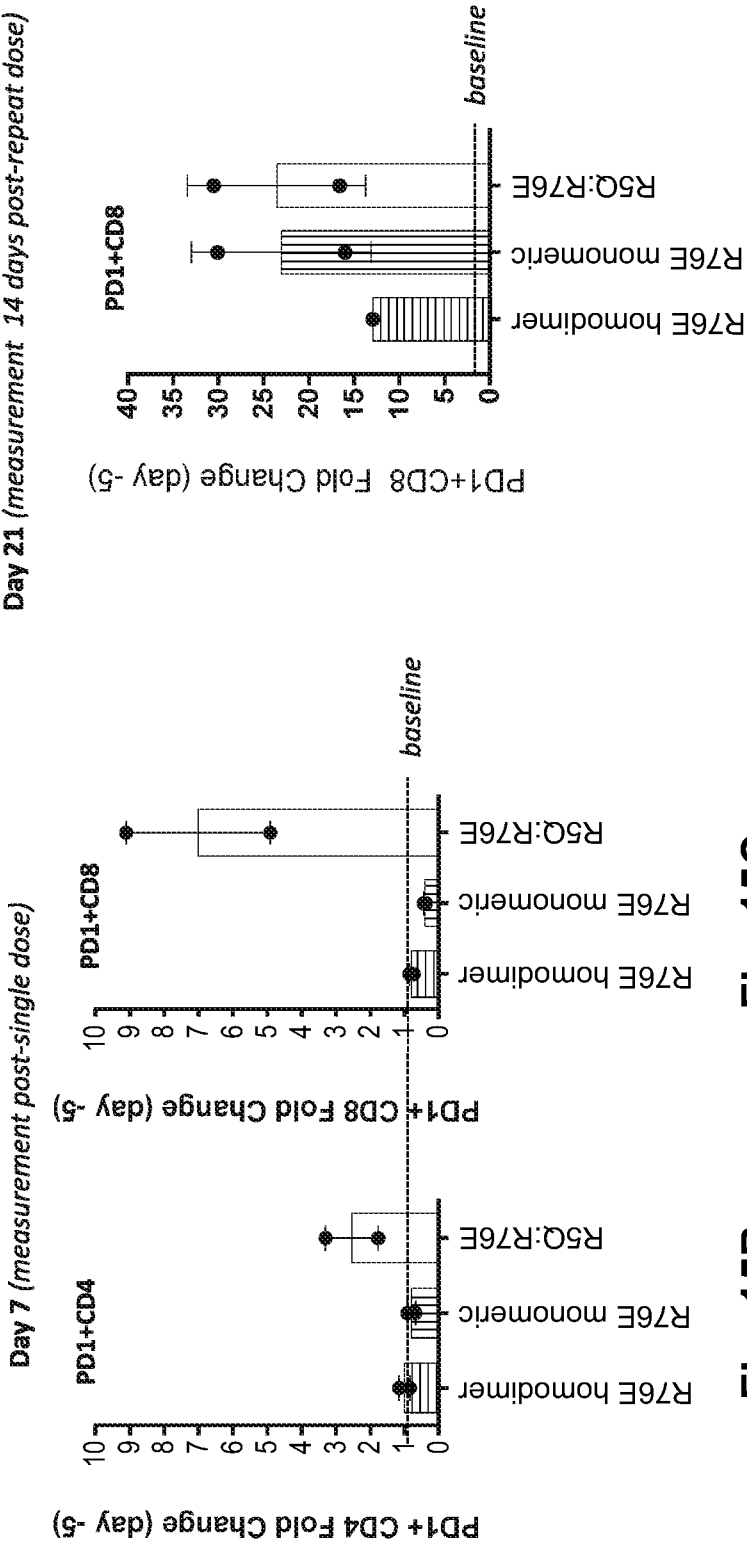
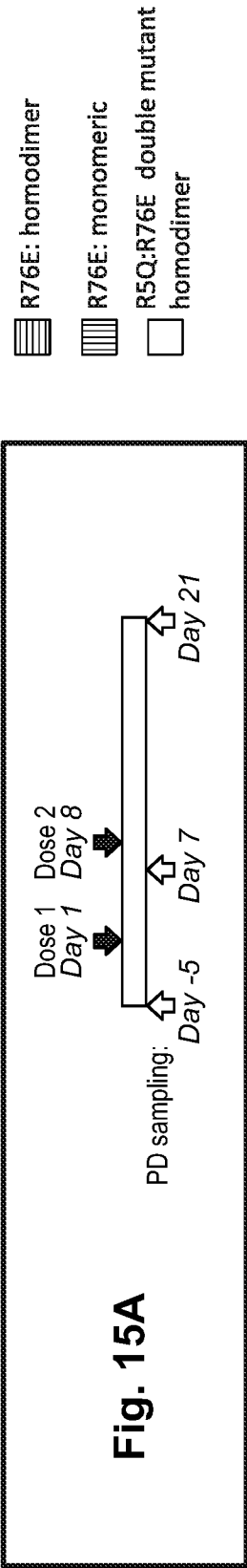


Fig. 15B

Fig. 15C

Fig. 15D

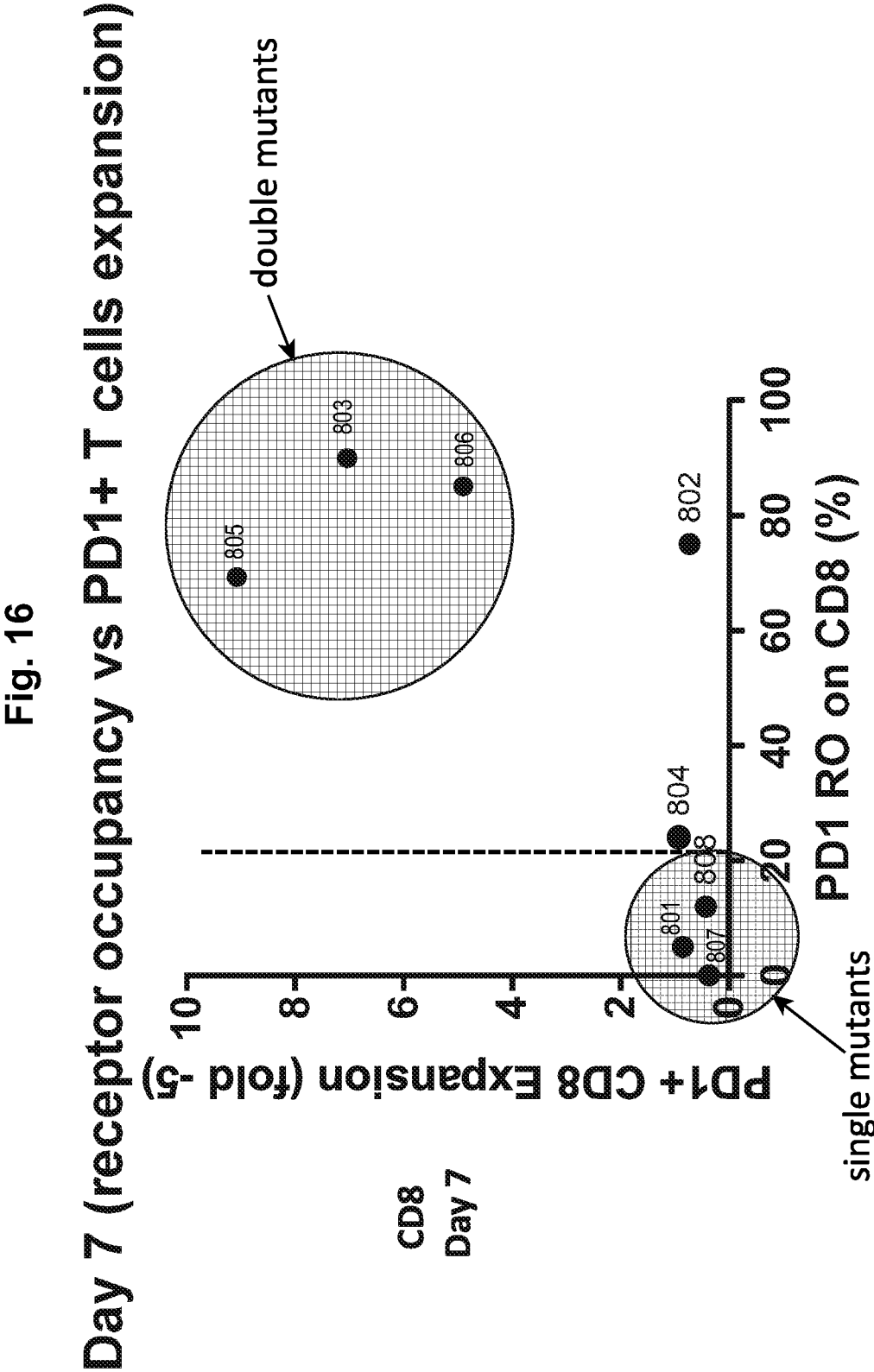
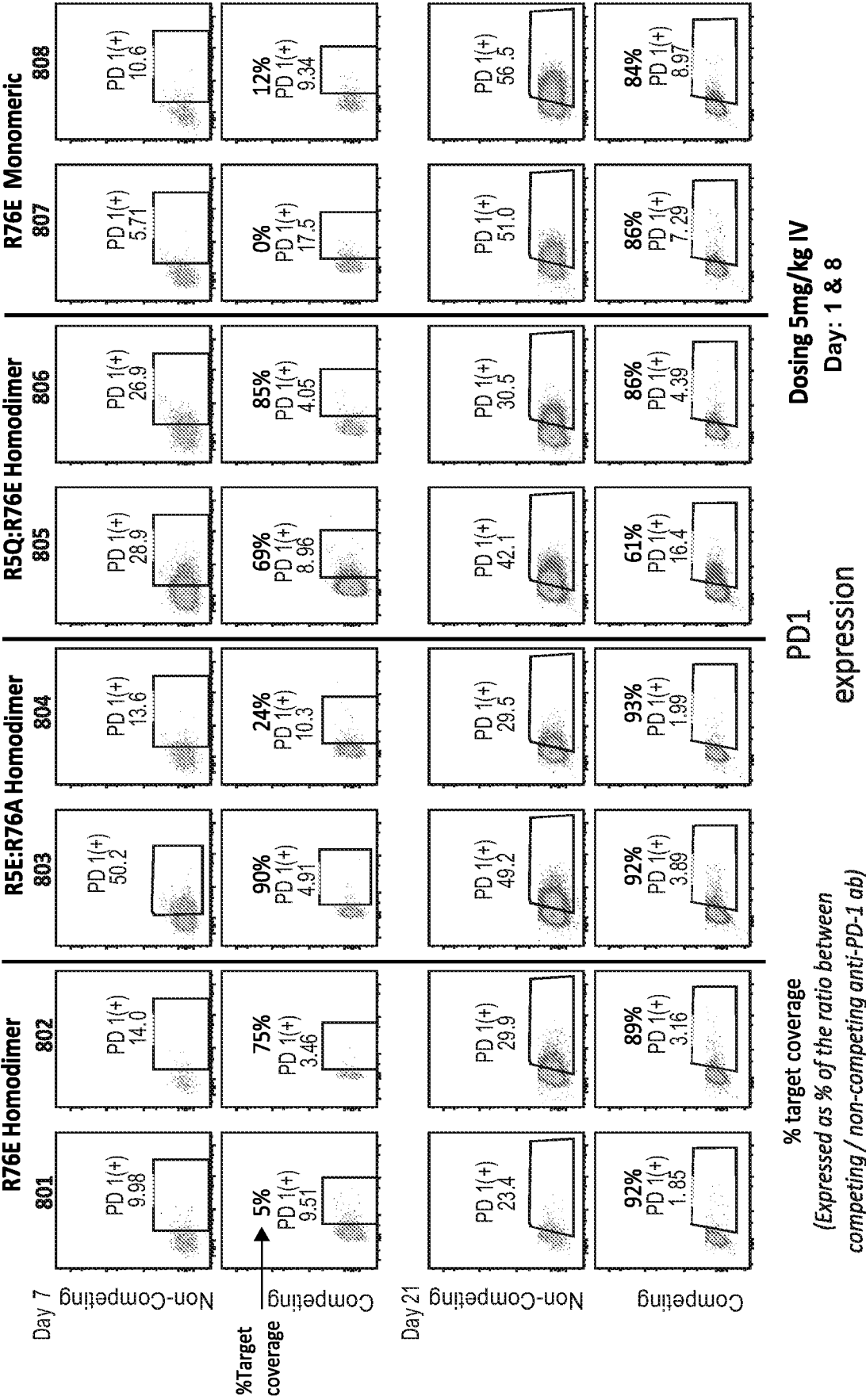


Fig. 17



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

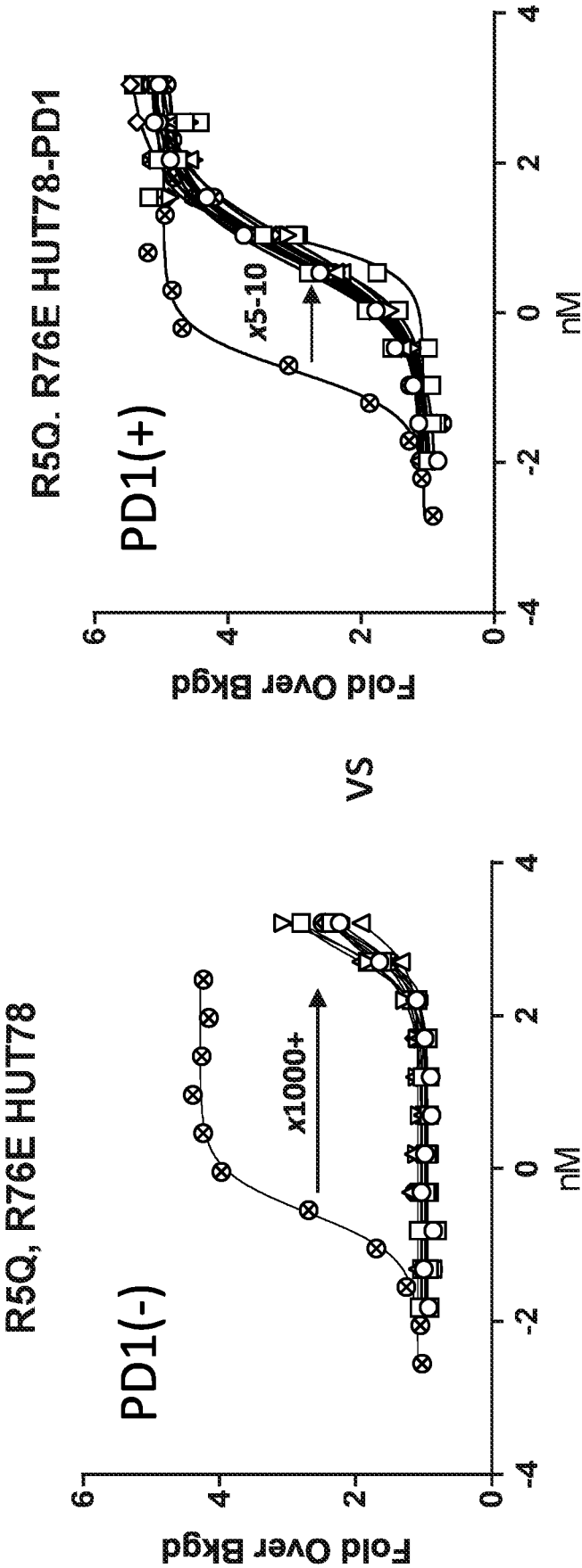


Fig. 18A

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

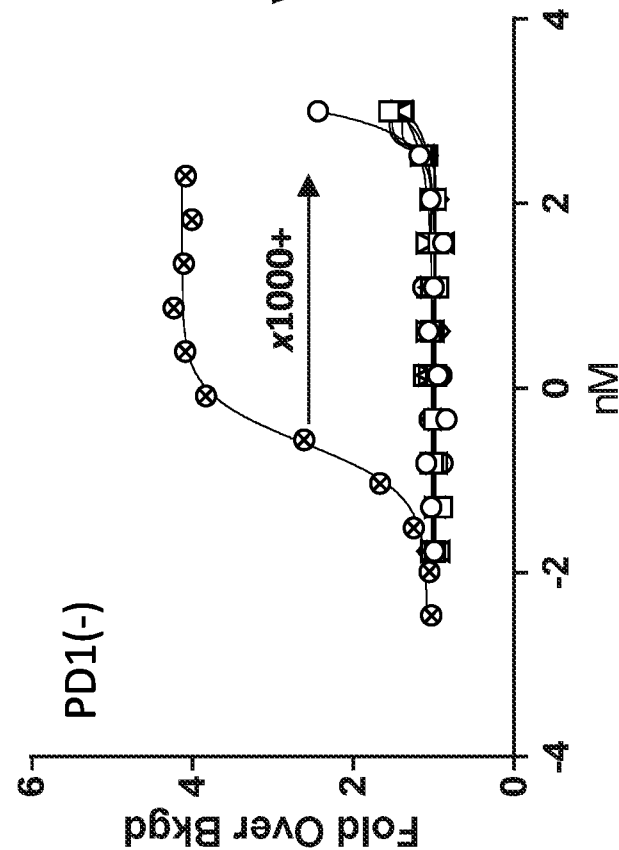


Fig. 19A

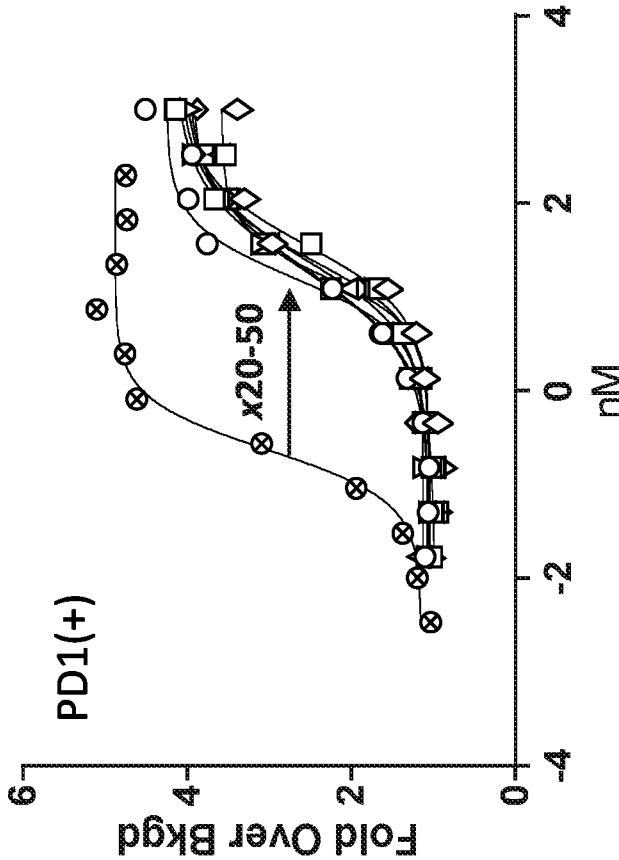


Fig. 19B

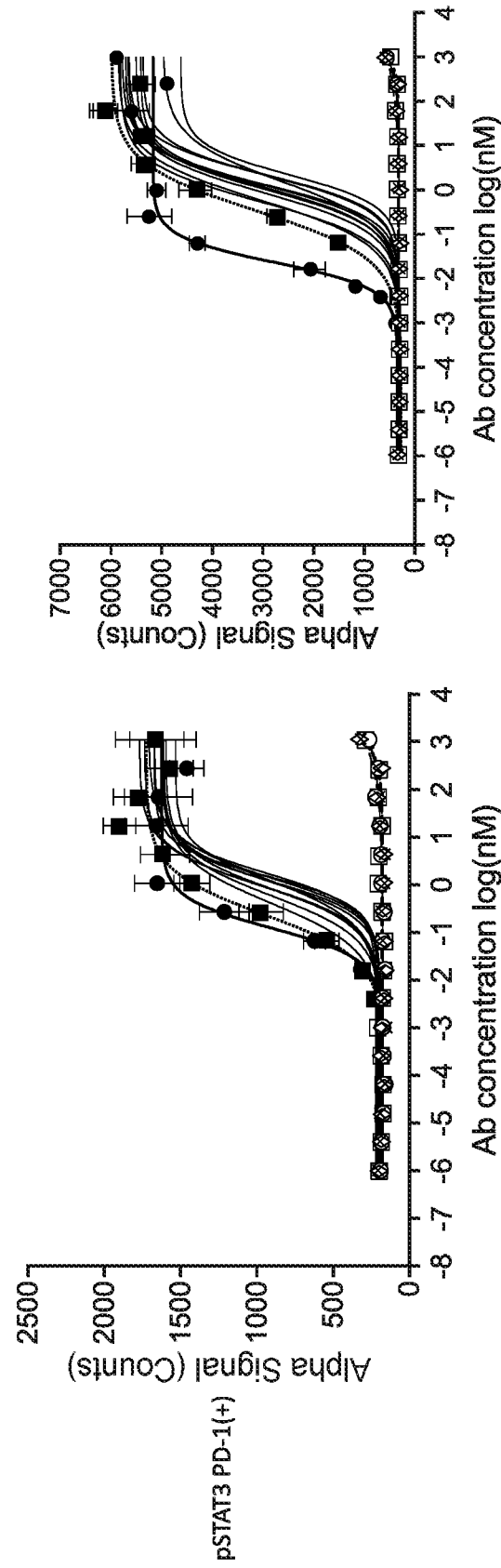
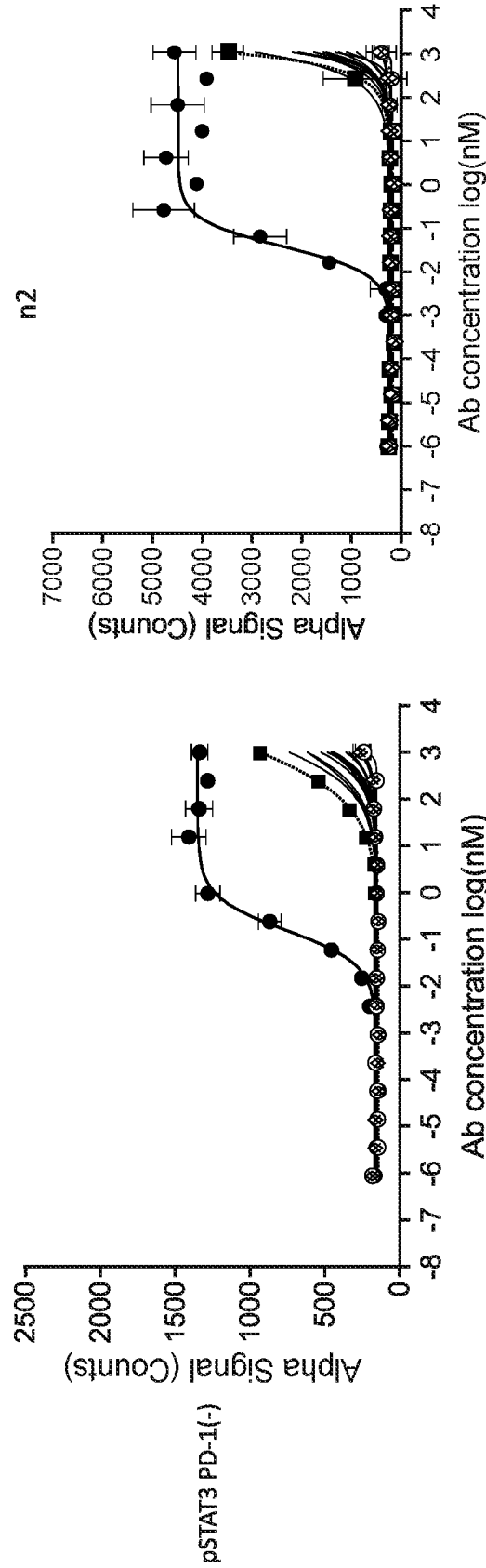


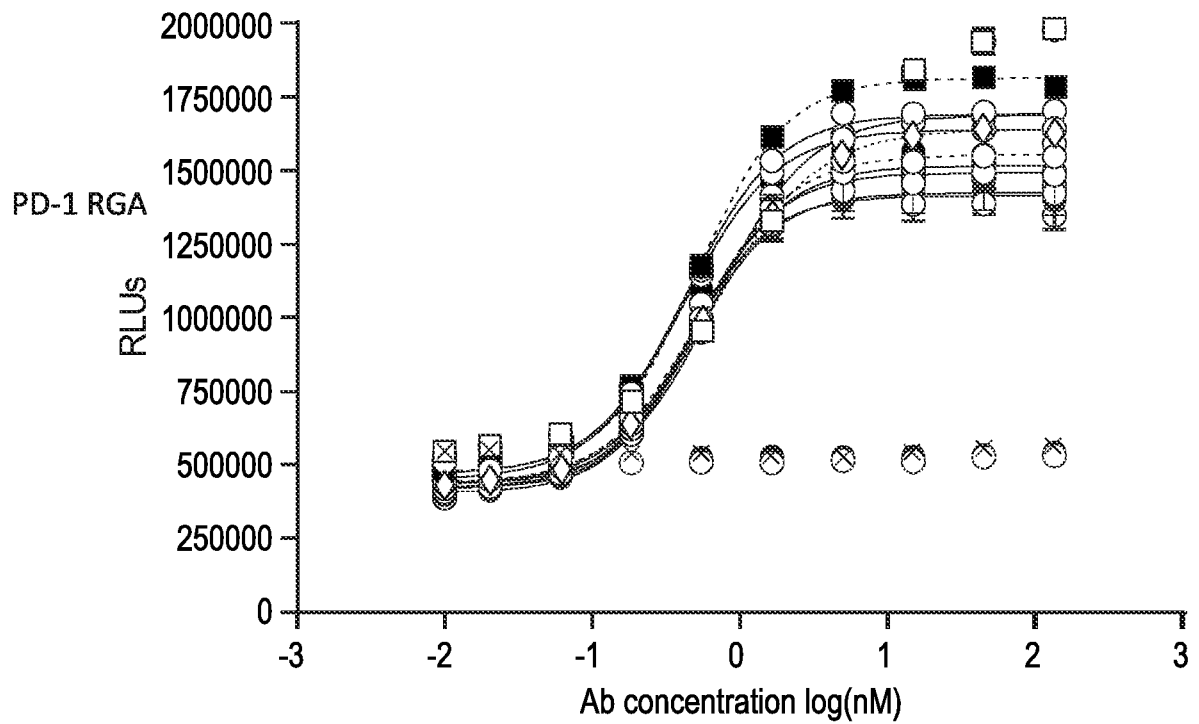
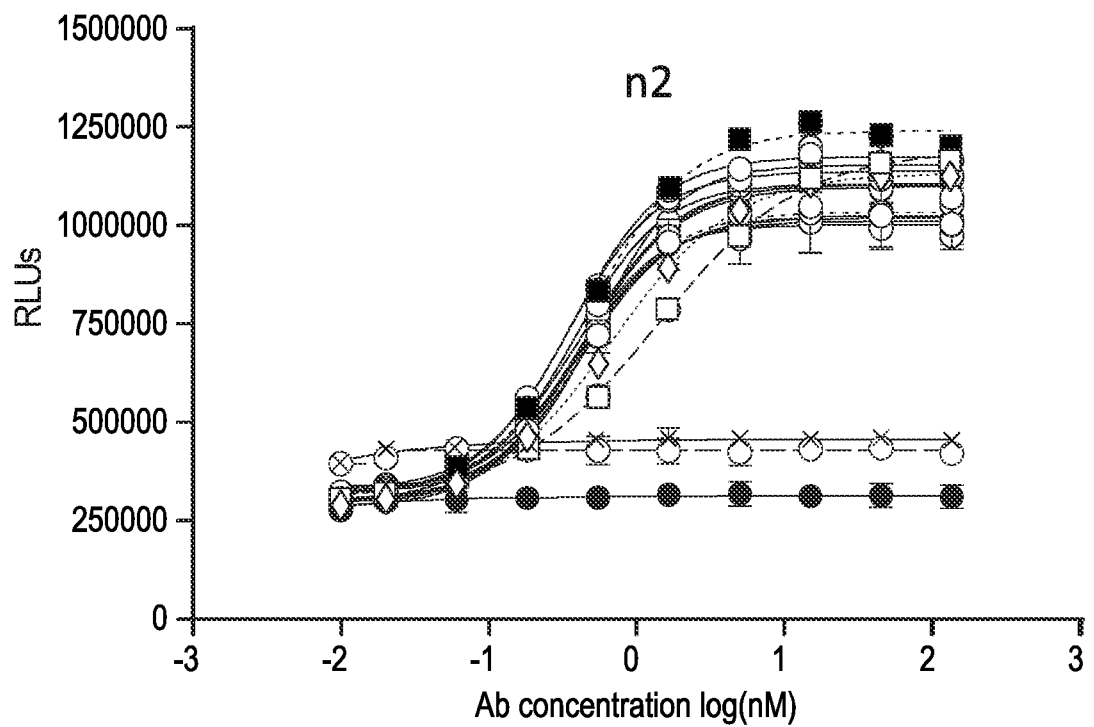
Fig. 21A**Fig. 21B**

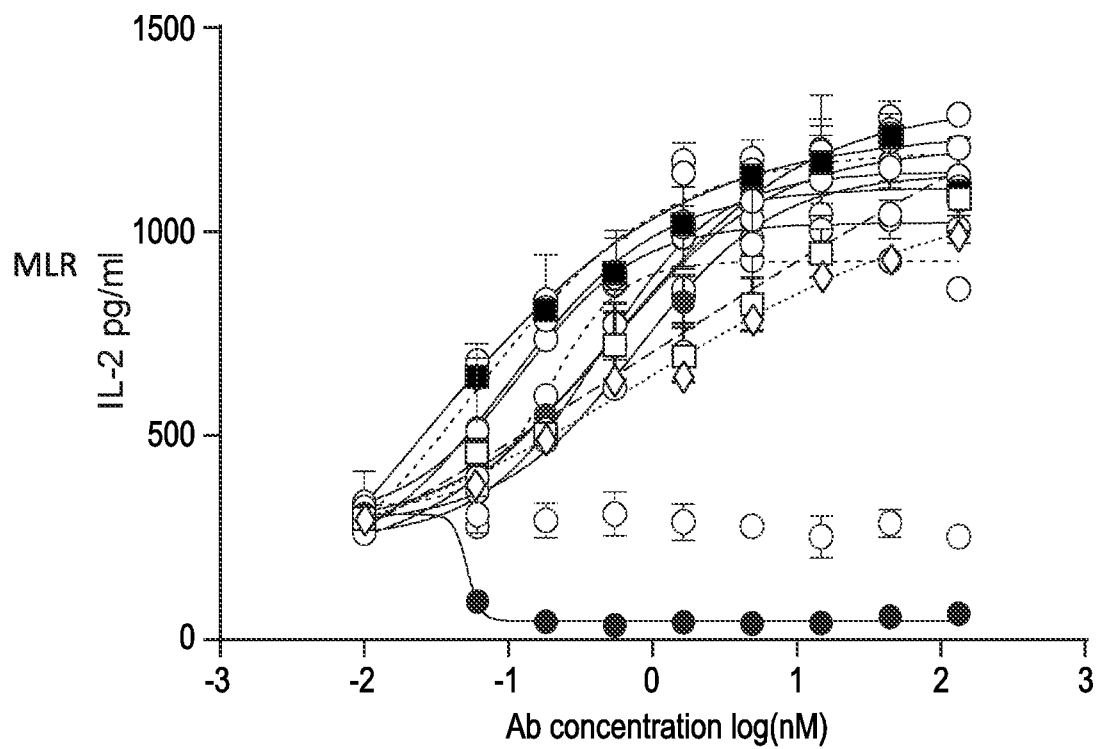
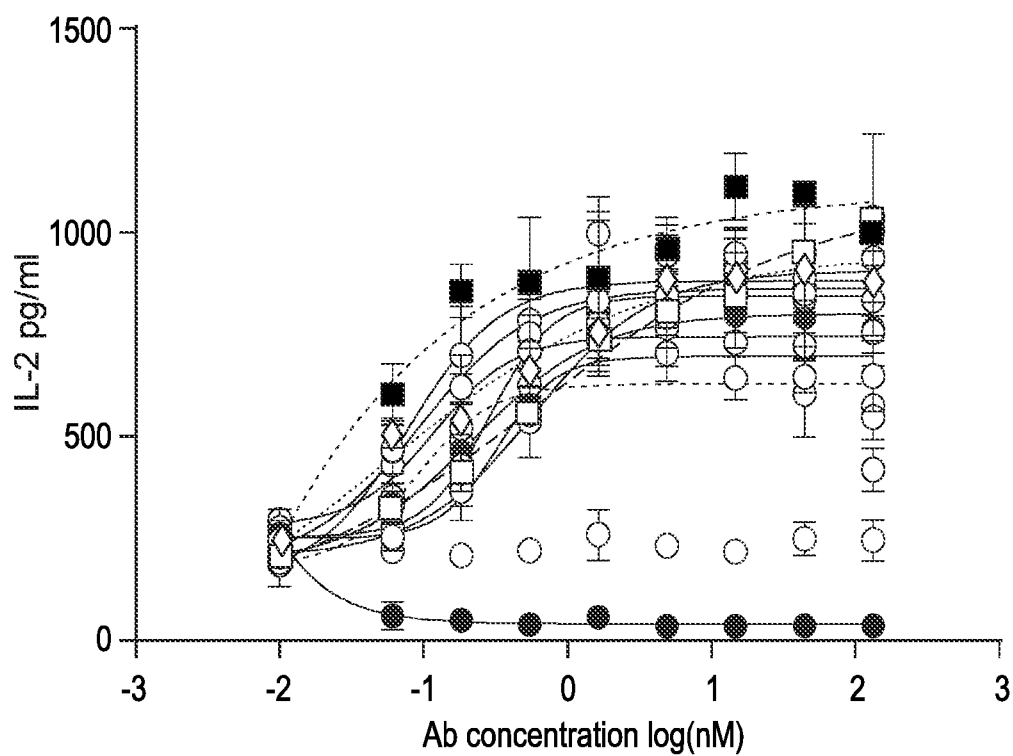
Fig. 21C**Fig. 21D**

Fig. 22A

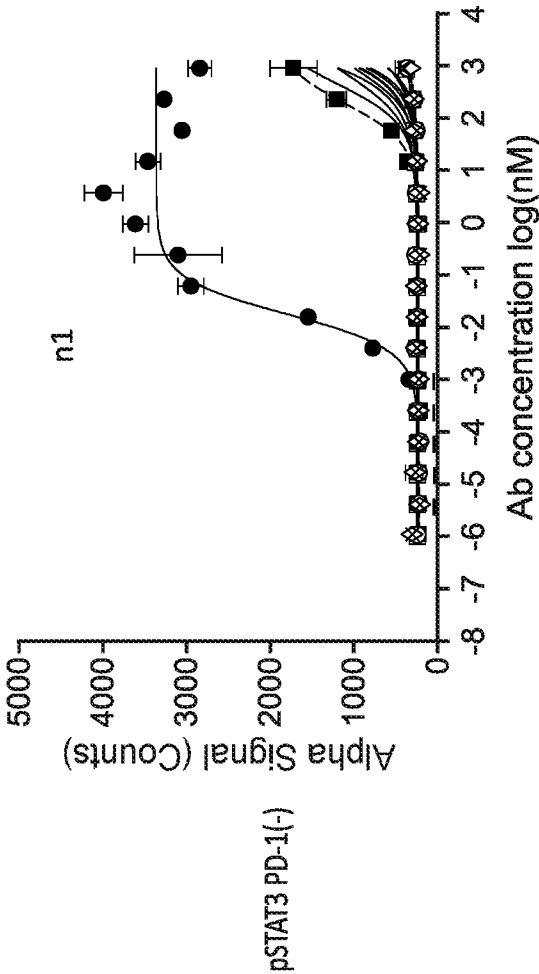


Fig. 22B

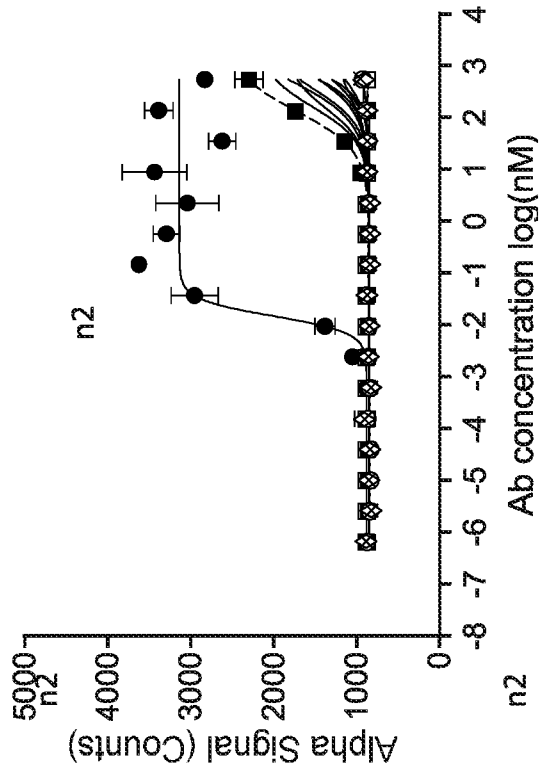


Fig. 22C

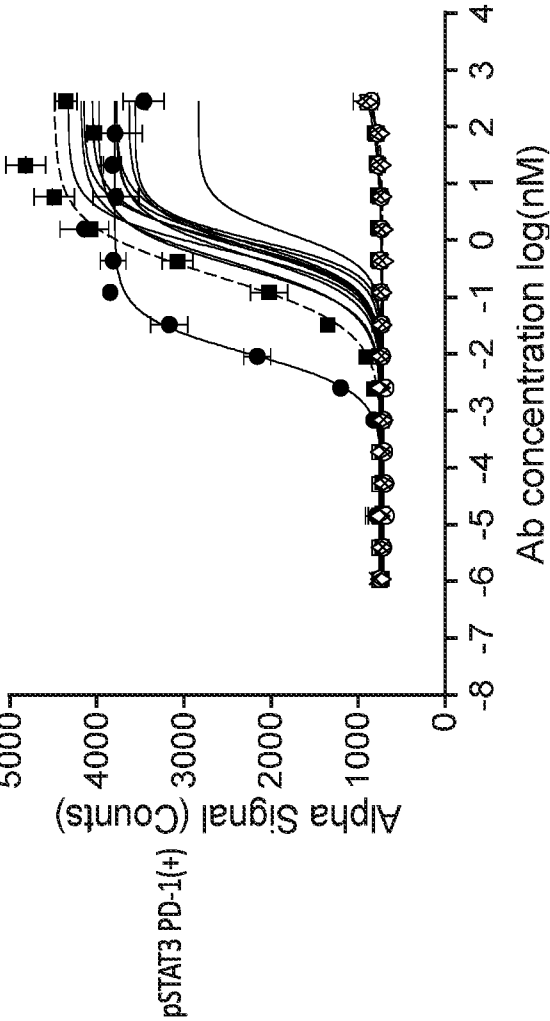
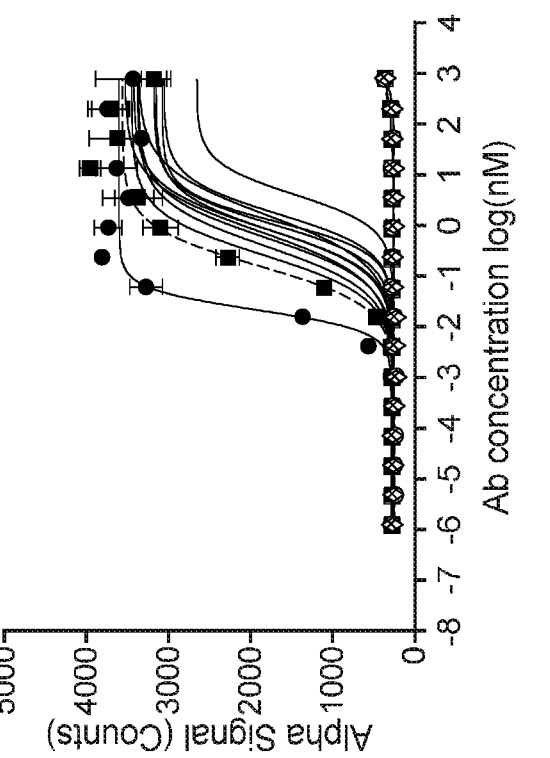


Fig. 22D



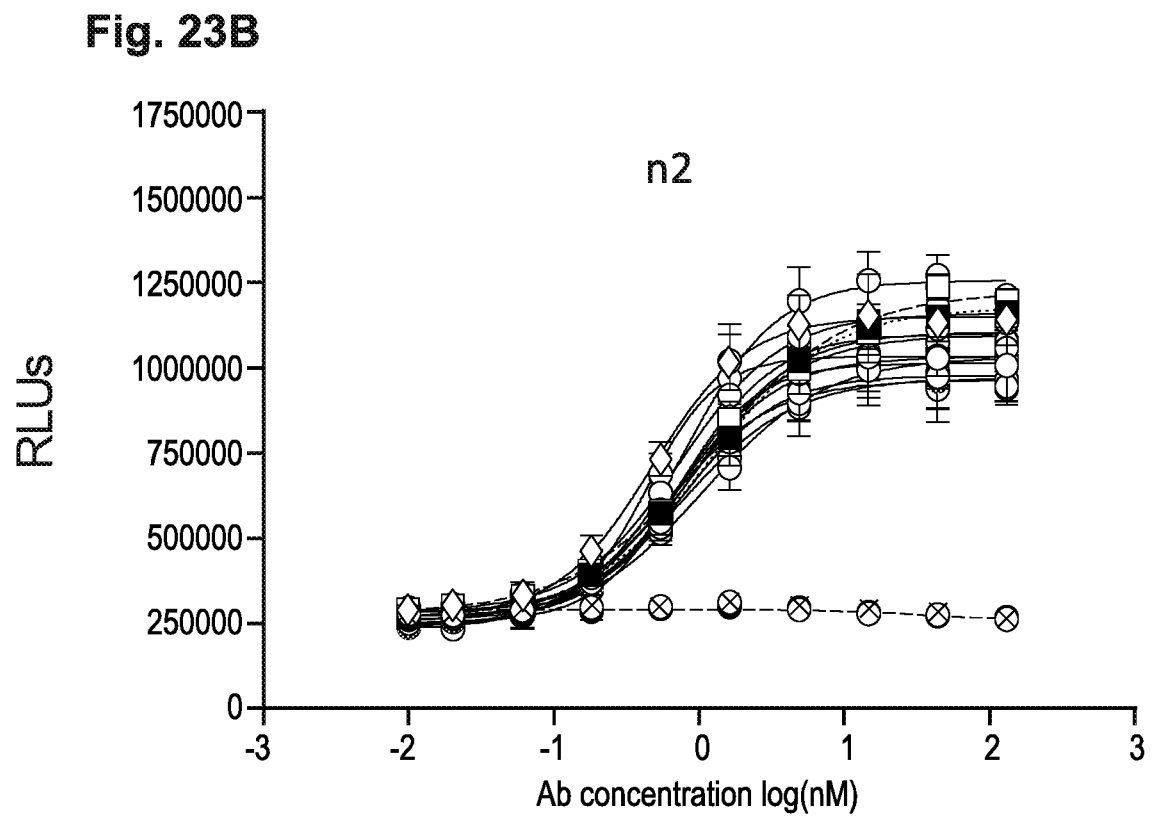
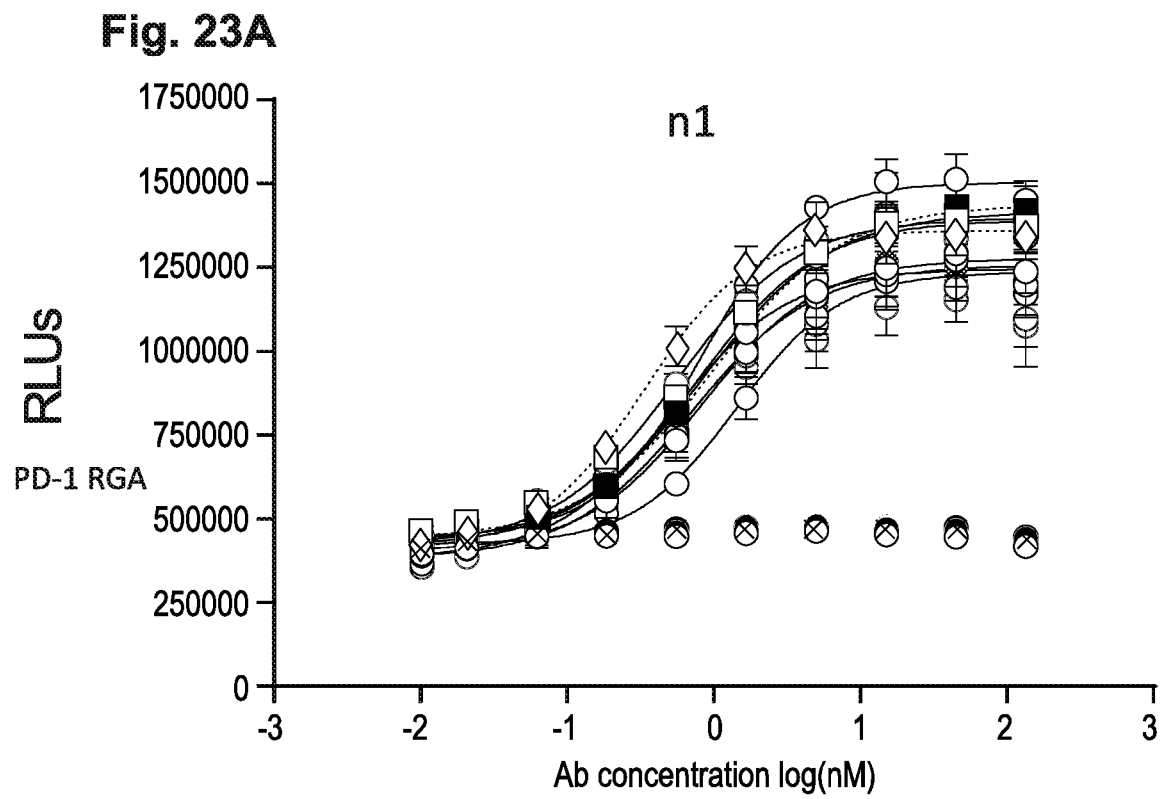


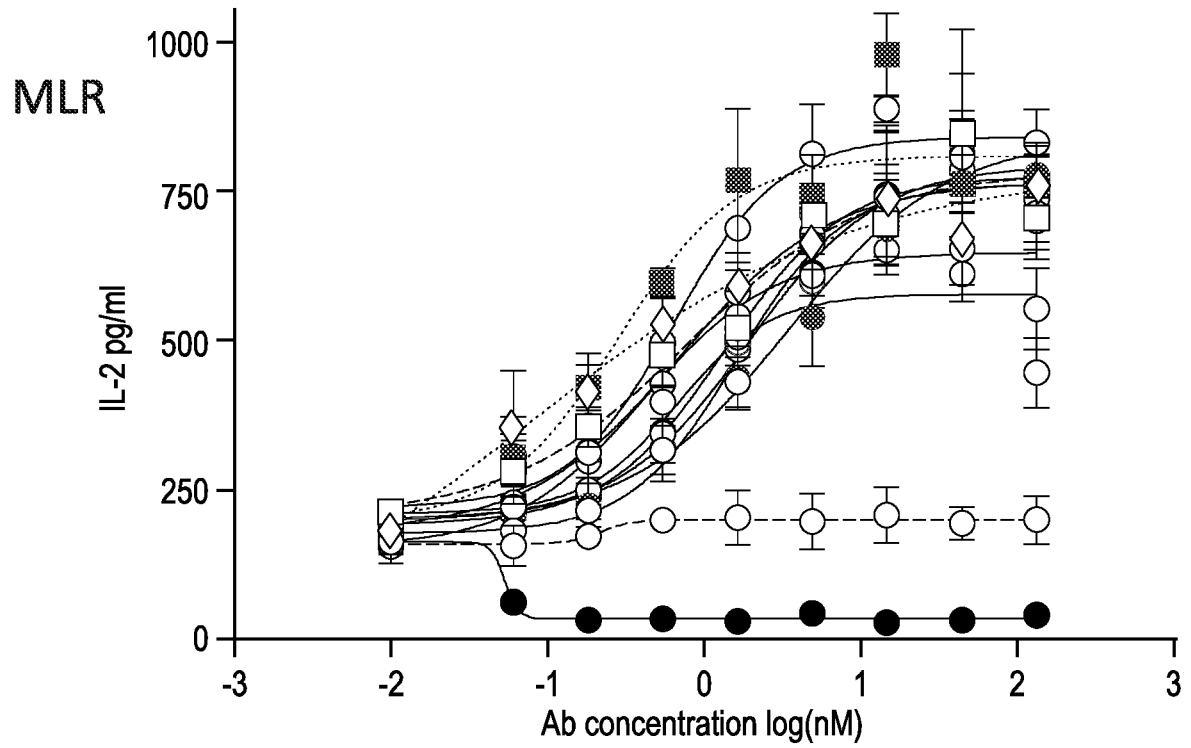
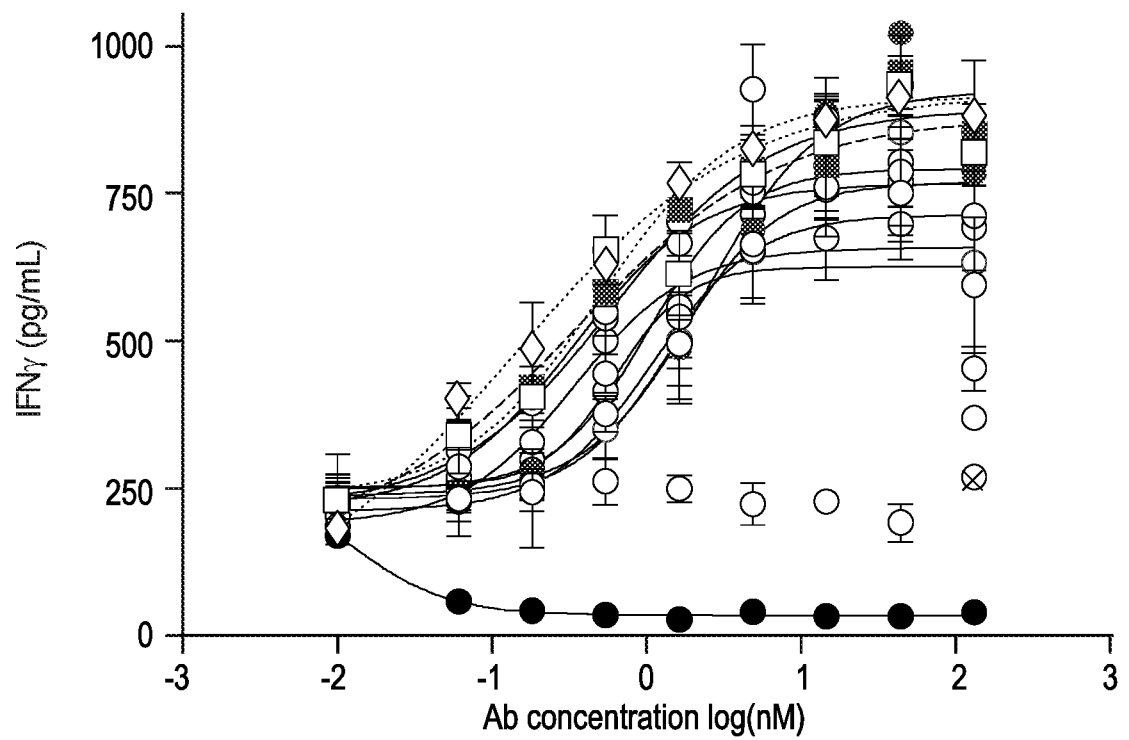
Fig. 23C**Fig. 23D**

Fig. 24

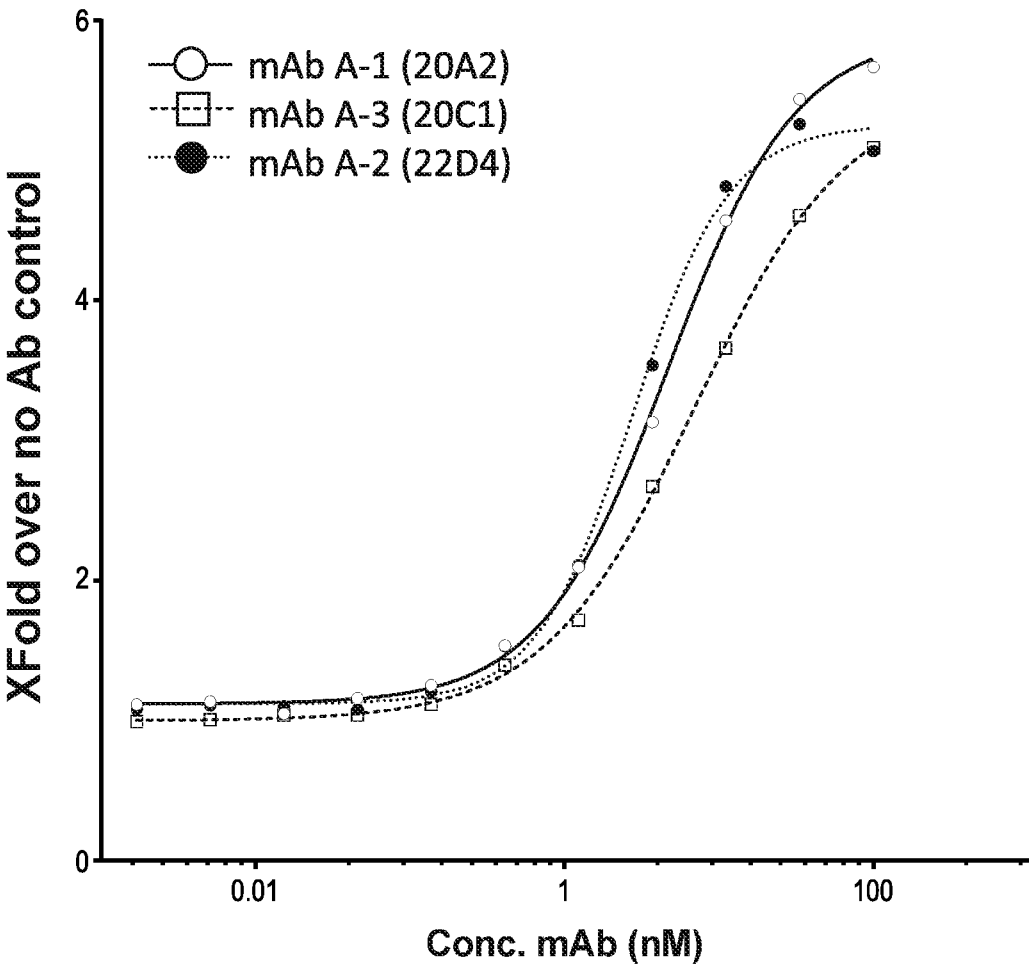


Fig. 25A

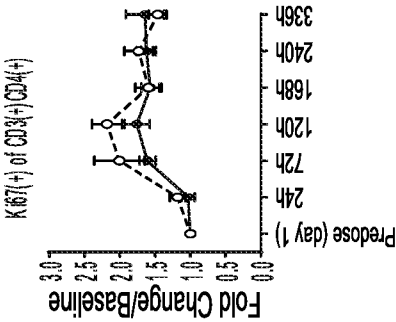


Fig. 25C

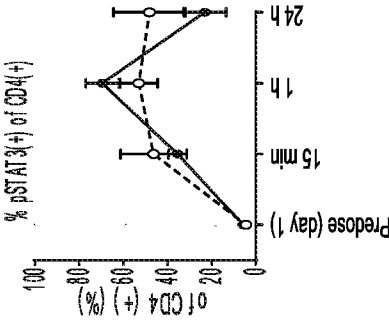


Fig. 25E

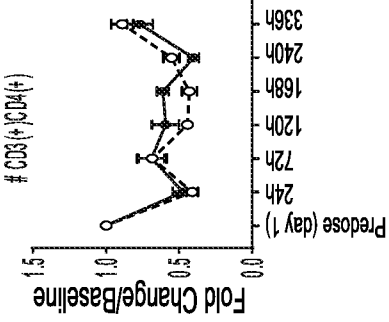


Fig. 25G

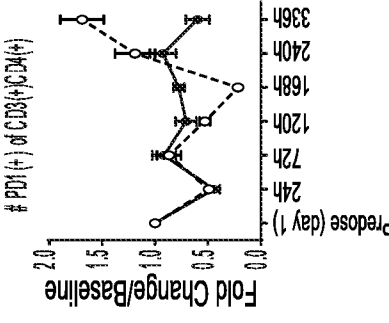


Fig. 25I

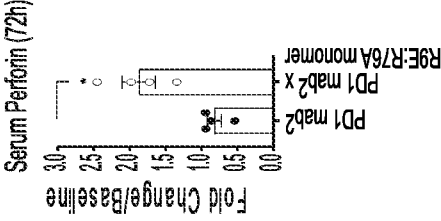


Fig. 25B

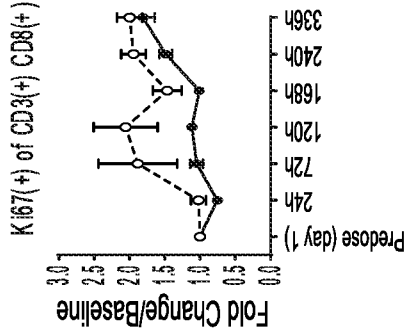


Fig. 25D

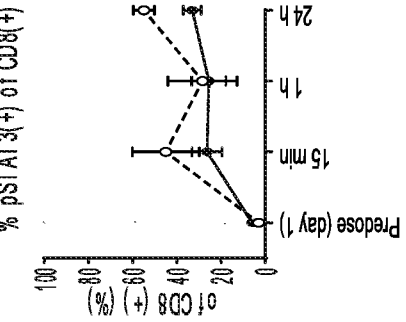


Fig. 25F

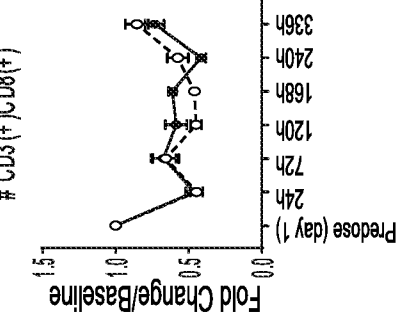


Fig. 25H

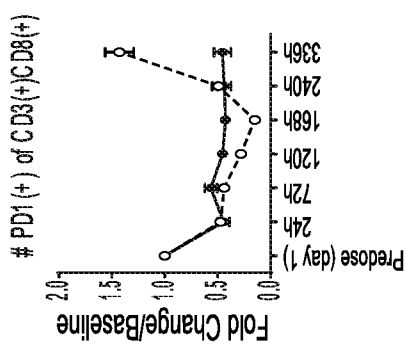
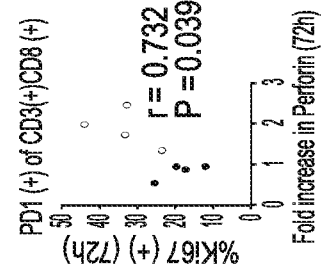


Fig. 25J

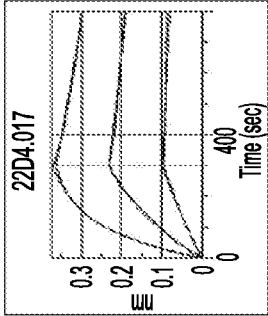


--○-- [22D4.017]-[R9E:R76A] (monomer)

—●— [22D4.017]

FIG. 26A

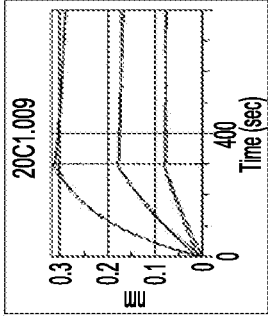
huPD-1 Ab 22D4.017



$K_D = 1.3$ nM

FIG. 26B

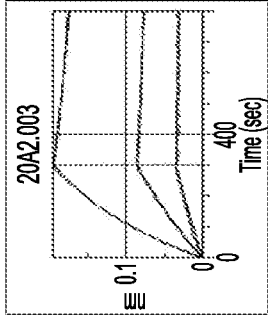
huPD-1 Ab 20C1.009



$K_D = 0.59$ nM

FIG. 26C

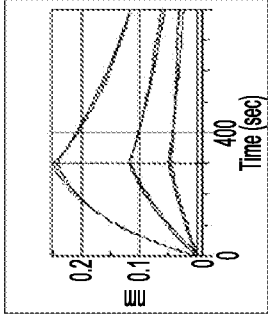
huPD1 Ab 20A2.003



$K_D = 1.7$ nM

FIG. 26D

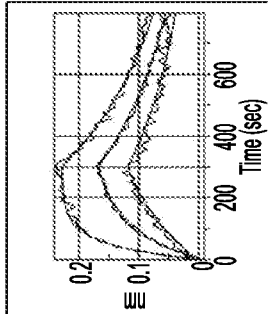
IgG1 anti-PD-1 mAb



$K_D = 8.4$ nM

FIG. 26E

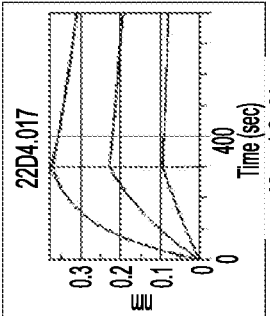
IgG4 anti-PD-1 mAb



$K_D = 3.9$ nM

FIG. 26F

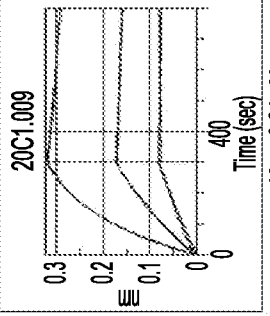
huPD-1 Ab 22D4.017



$K_D = 1.2$ nM

FIG. 26G

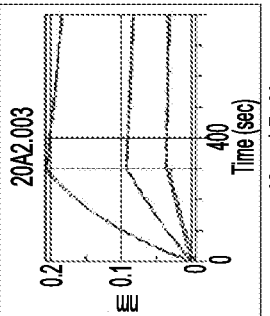
huPD-1 Ab 20C1.009



$K_D = 0.84$ nM

FIG. 26H

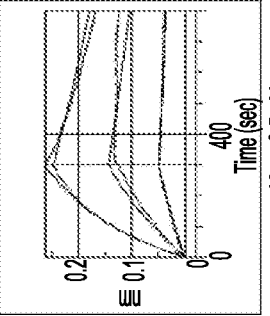
huPD1 Ab 20A2.003



$K_D = 1.7$ nM

FIG. 26I

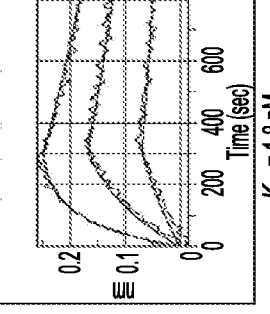
IgG1 anti-PD-1 mAb



$K_D = 3.5$ nM

FIG. 26J

IgG4 anti-PD-1 mAb



$K_D = 1.8$ nM

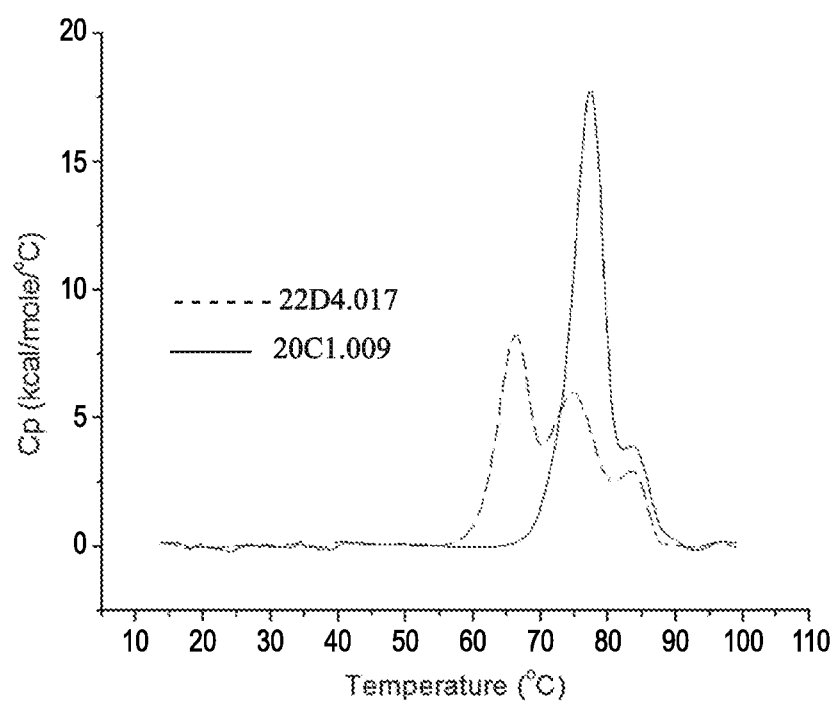
Fig. 27

Fig. 28

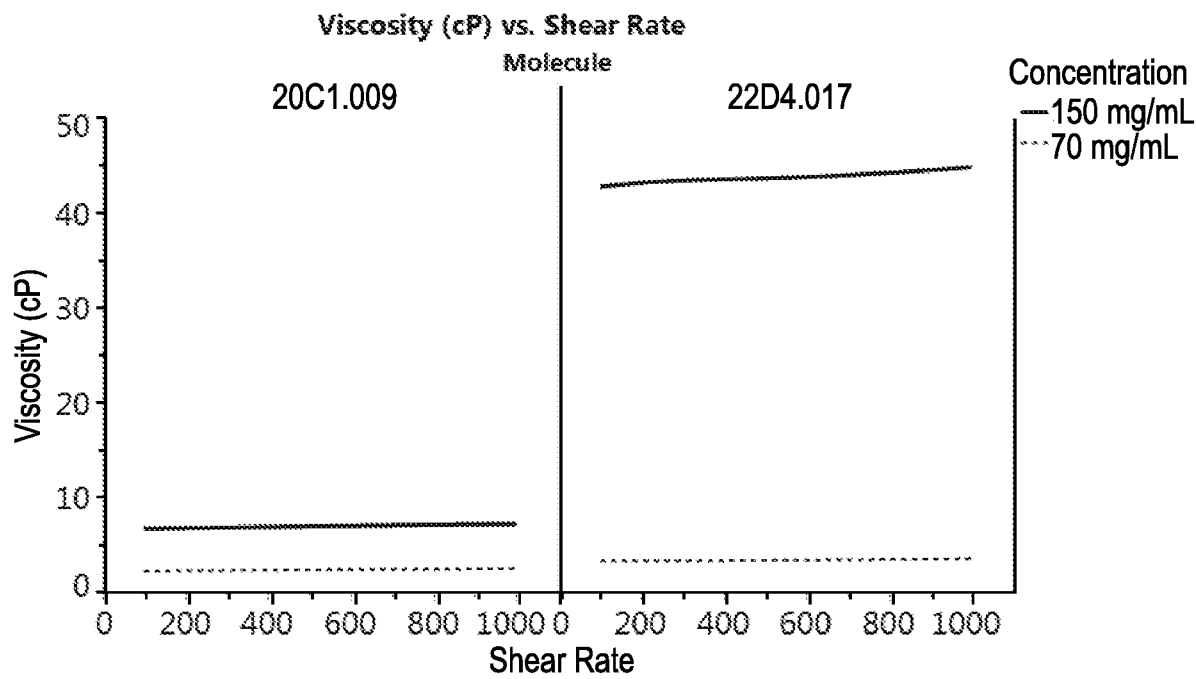


Fig. 29A

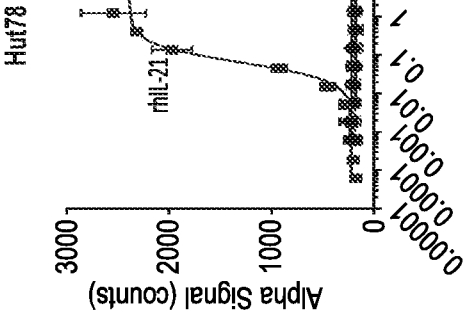


Fig. 29B

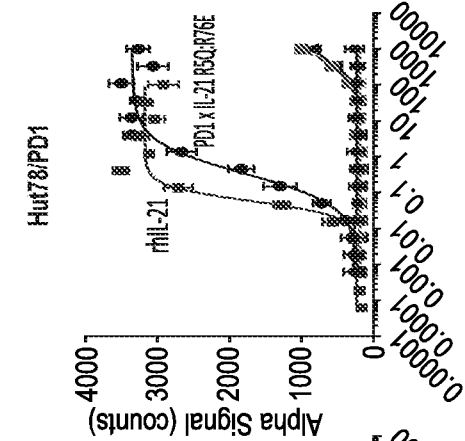


Fig. 29C

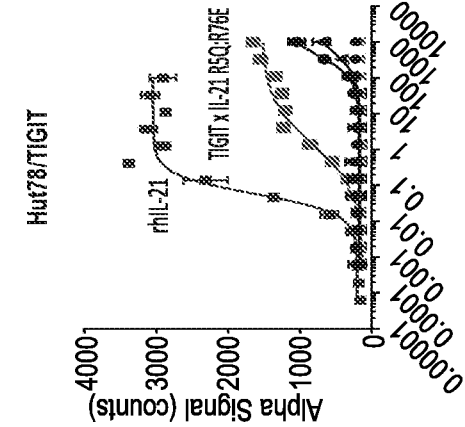
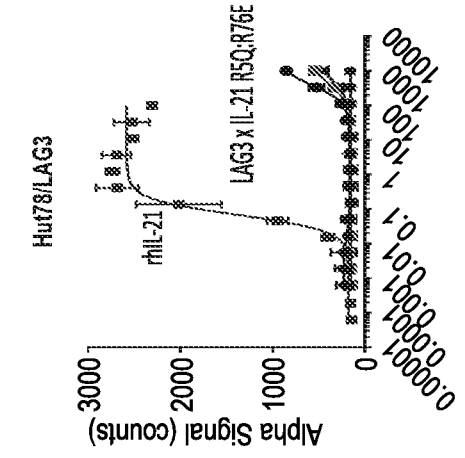


Fig. 29D



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

- hulG1
- rhIL-21
- 22D4 017
- TIGIT mAb
- LAG3 mAb
- PD1 mab x R5Q:R76E
- TIGIT mab x R5Q:R78E
- LAG3 mab x R5Q:R78E