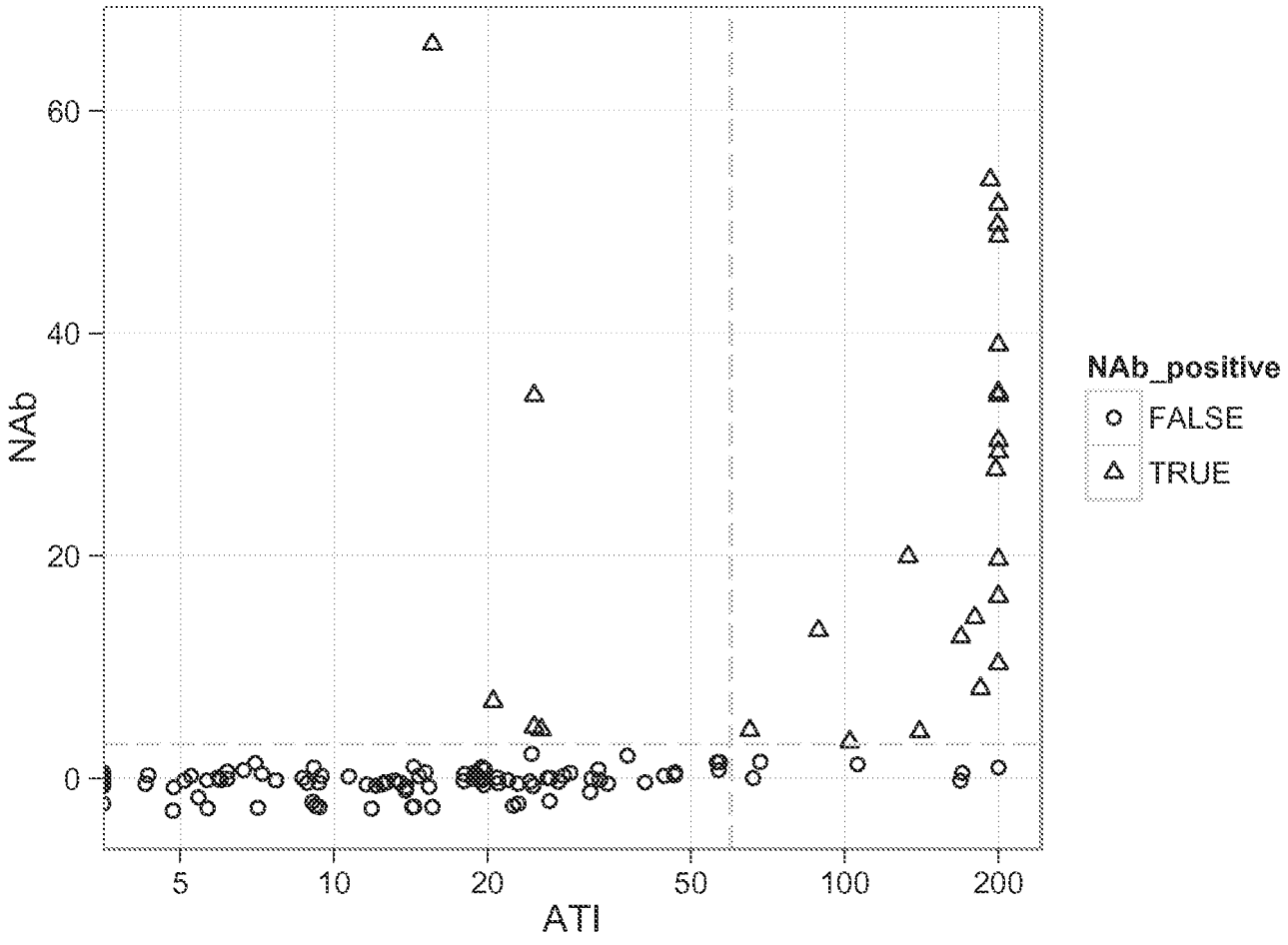


FIG. 1

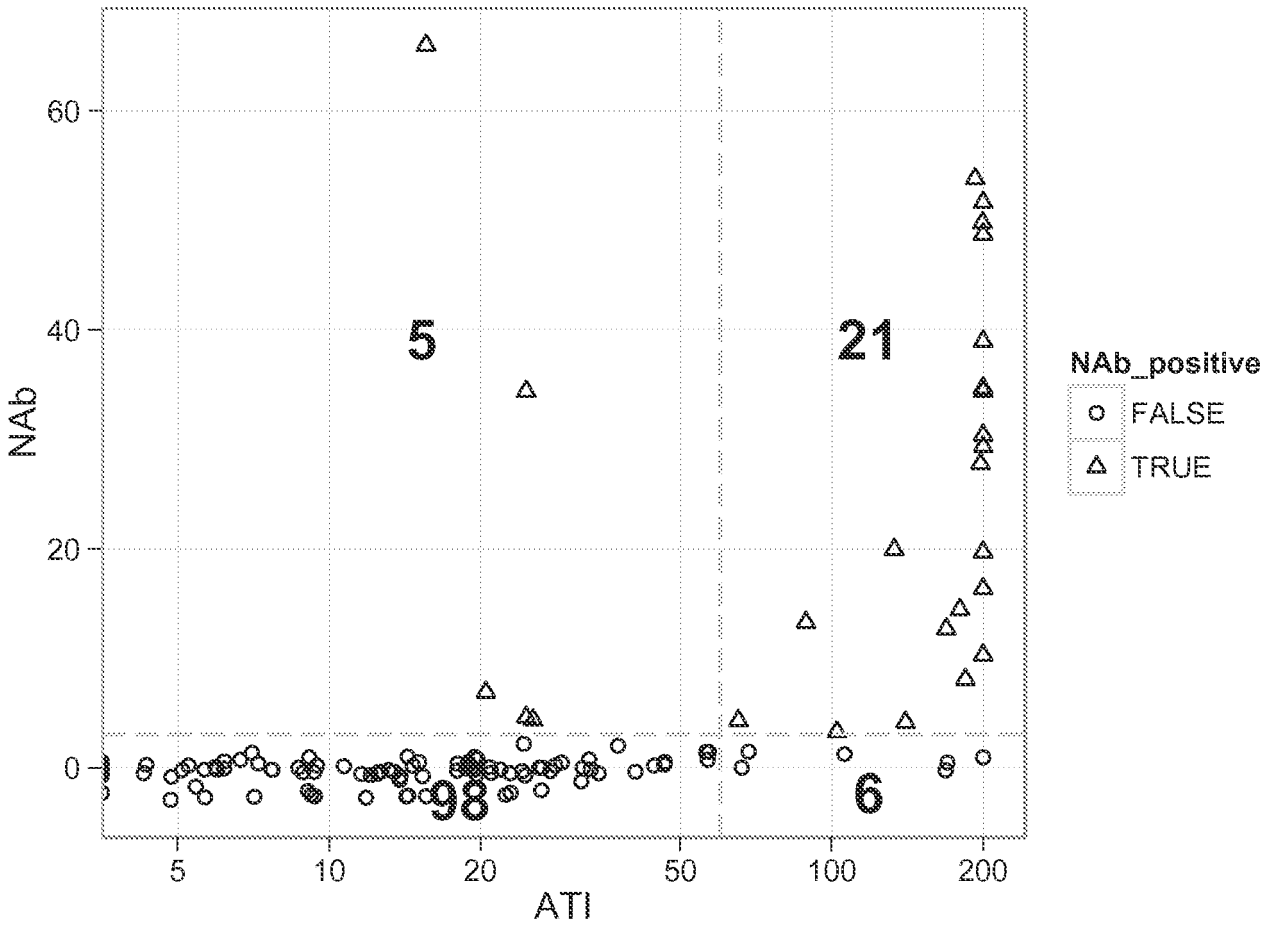

NAb
ATI
60
40
20
0
5
10
20
50
100
200
5
21
98
6
NAb_positive
FALSE
TRUE

FIG. 2

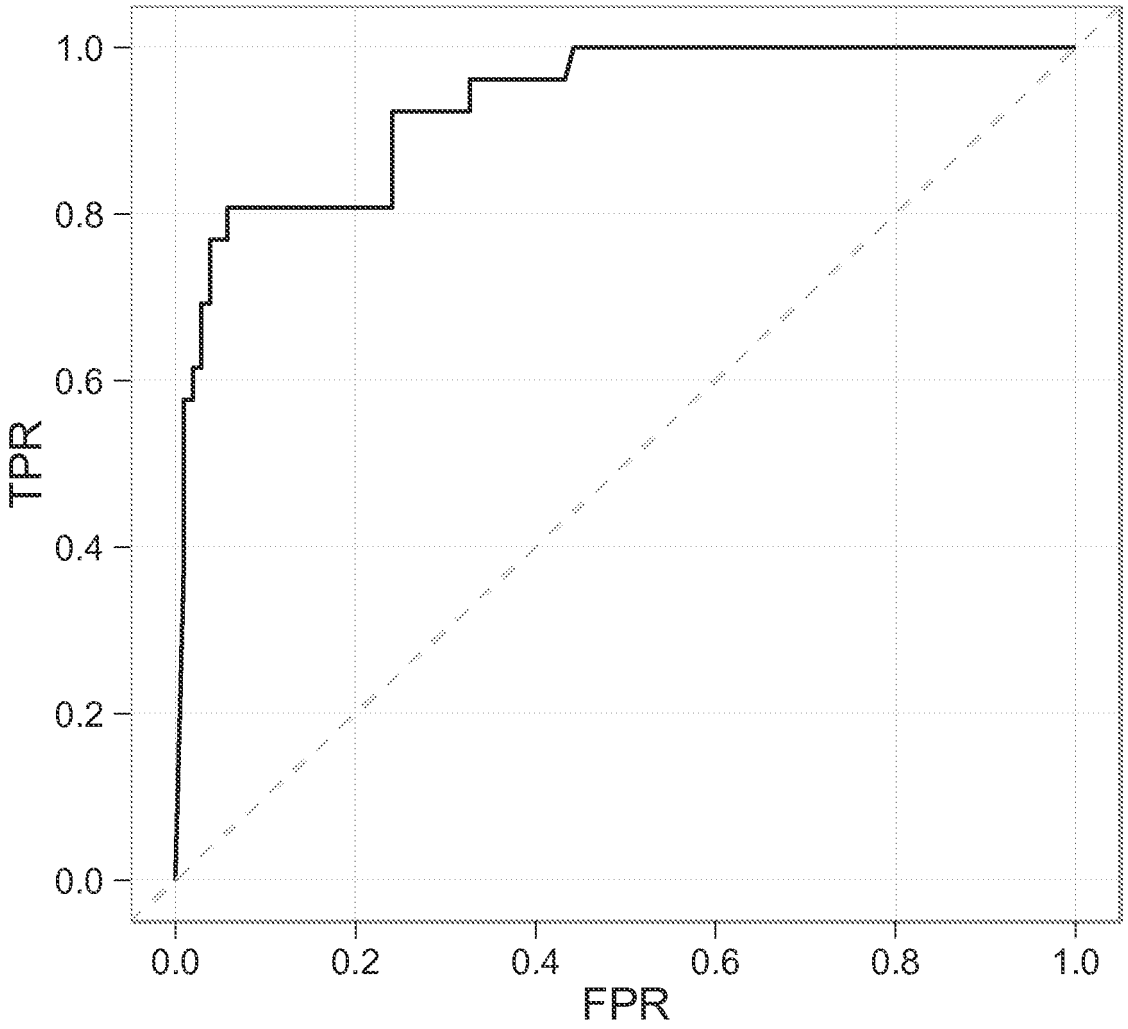


FIG. 3

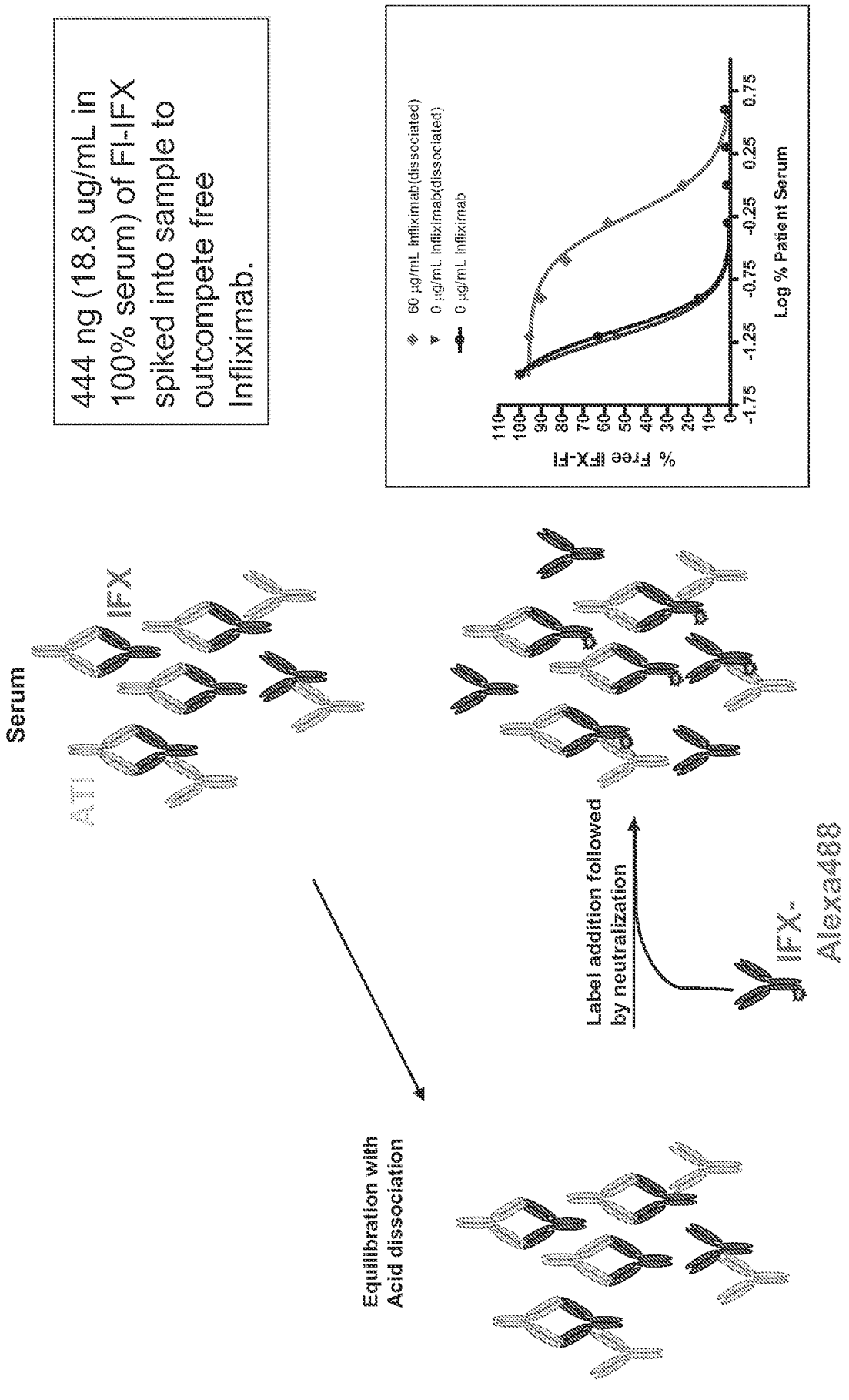
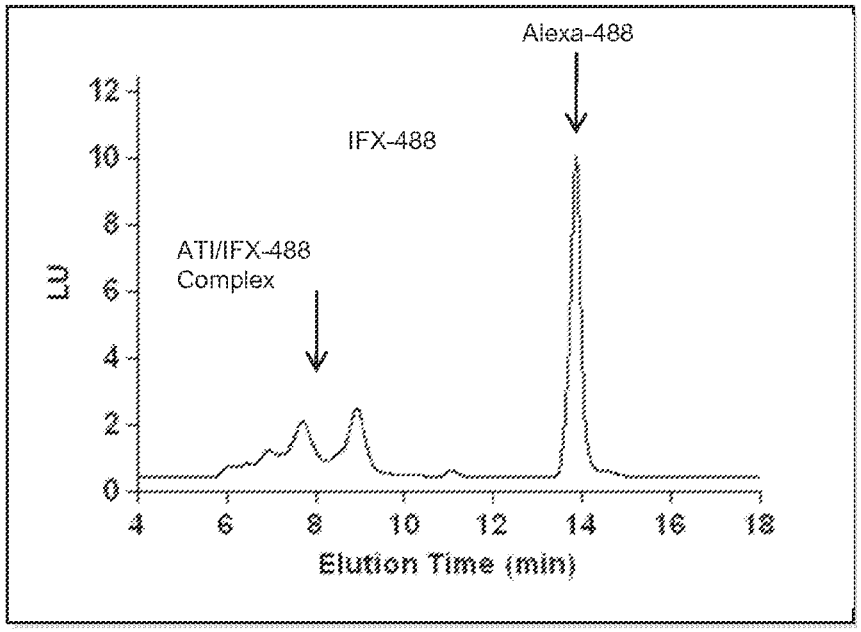
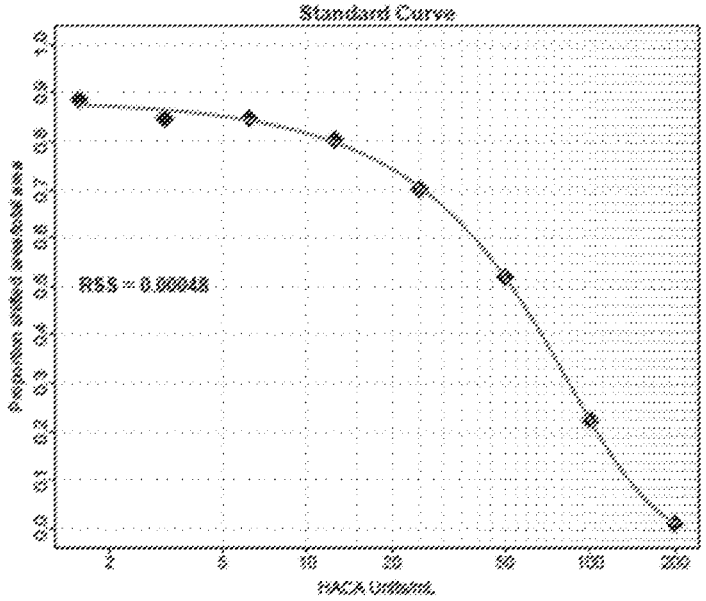

444 ng (18.8 ug/mL in 100% serum) of Fl-IFX spiked into sample to outcompete free Infliximab.
60 µg/mL Infliximab(dissociated)
0 µg/mL Infliximab(dissociated)
0 µg/mL Infliximab
% Free IFX-Fl
Log % Patient Serum
110
100
90
80
70
60
50
40
30
20
10
0
-1.75
-1.25
-0.75
-0.25
0.25
0.75
Serum
ATI
IFX
Equilibration with Acid dissociation
Label addition followed by neutralization
IFX-Alexa488

FIG. 4





- Samples containing various concentrations of ATI (standards or unknowns) equilibrated with fluorescently labeled Infliximab-(IFX-488) injected on size exclusion column in 2% serum
- Large IFX-488/ATI complexes elute first, followed by smaller complexes and then unbound IFX-488 and the Alexa488 loading control
- Unknown concentrations determined by interpolation from standard curve
- Detection of IFX follows similar methodology

Parameter	IFX [µg/mL]	ATI (U/mL)
LOD	0.10	6.22
LLOQ (= cutoff)	0.98	6.75
ULOQ	34.00	150

FIG. 5

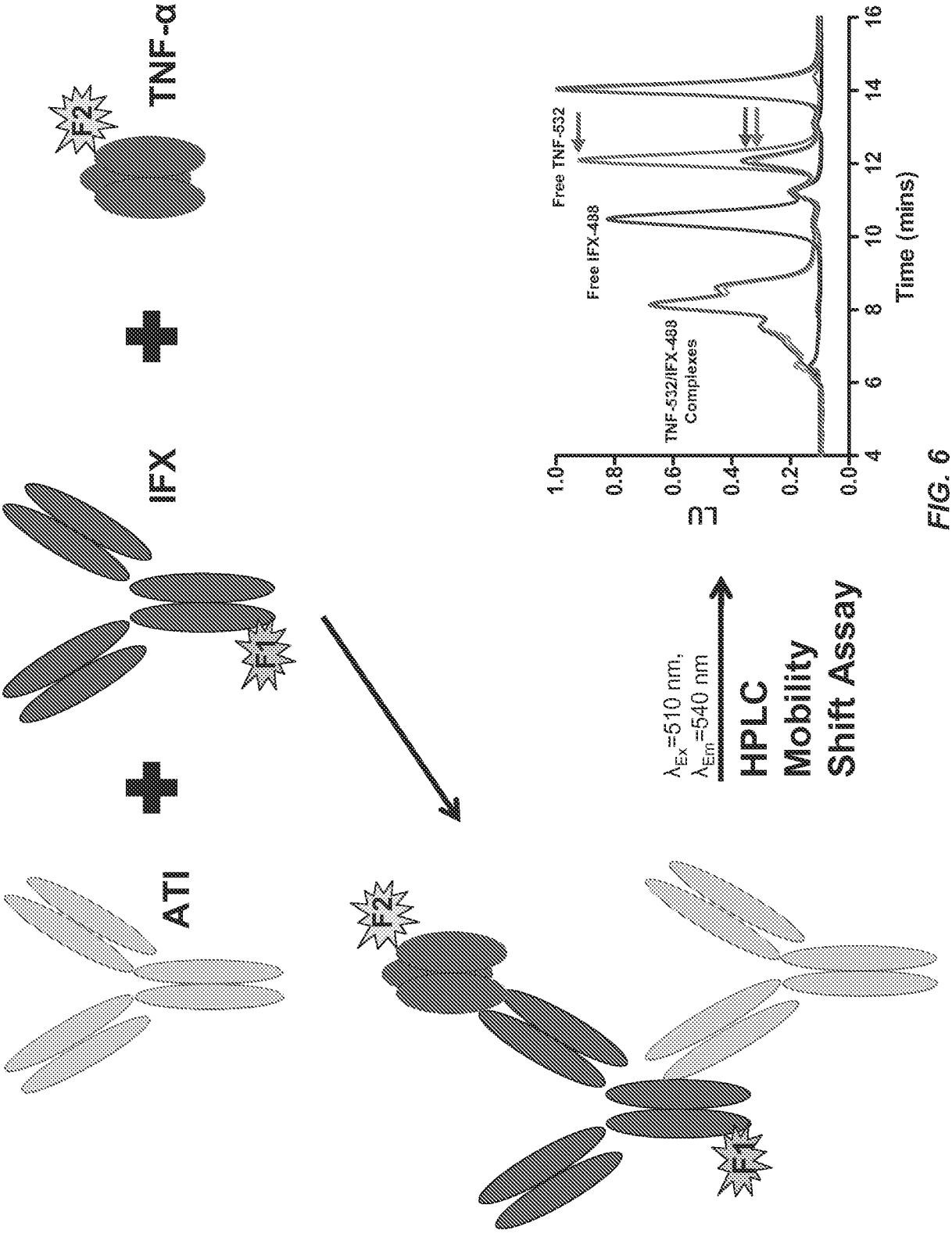

TNF-α
F2
IFX
F1
ATI
F2
F1
λ_{Ex}=510 nm,
λ_{Em}=540 nm
HPLC
Mobility
Shift Assay
NHS
TNF-532
IFX-488
TNF-532/IFX-488
Patient A Serum
Free TNF-532
Free IFX-488
TNF-532/IFX-488
Complexes
FU
Time (mins)
1.0
0.8
0.6
0.4
0.2
0.0
4
6
8
10
12
14
16

FIG. 6

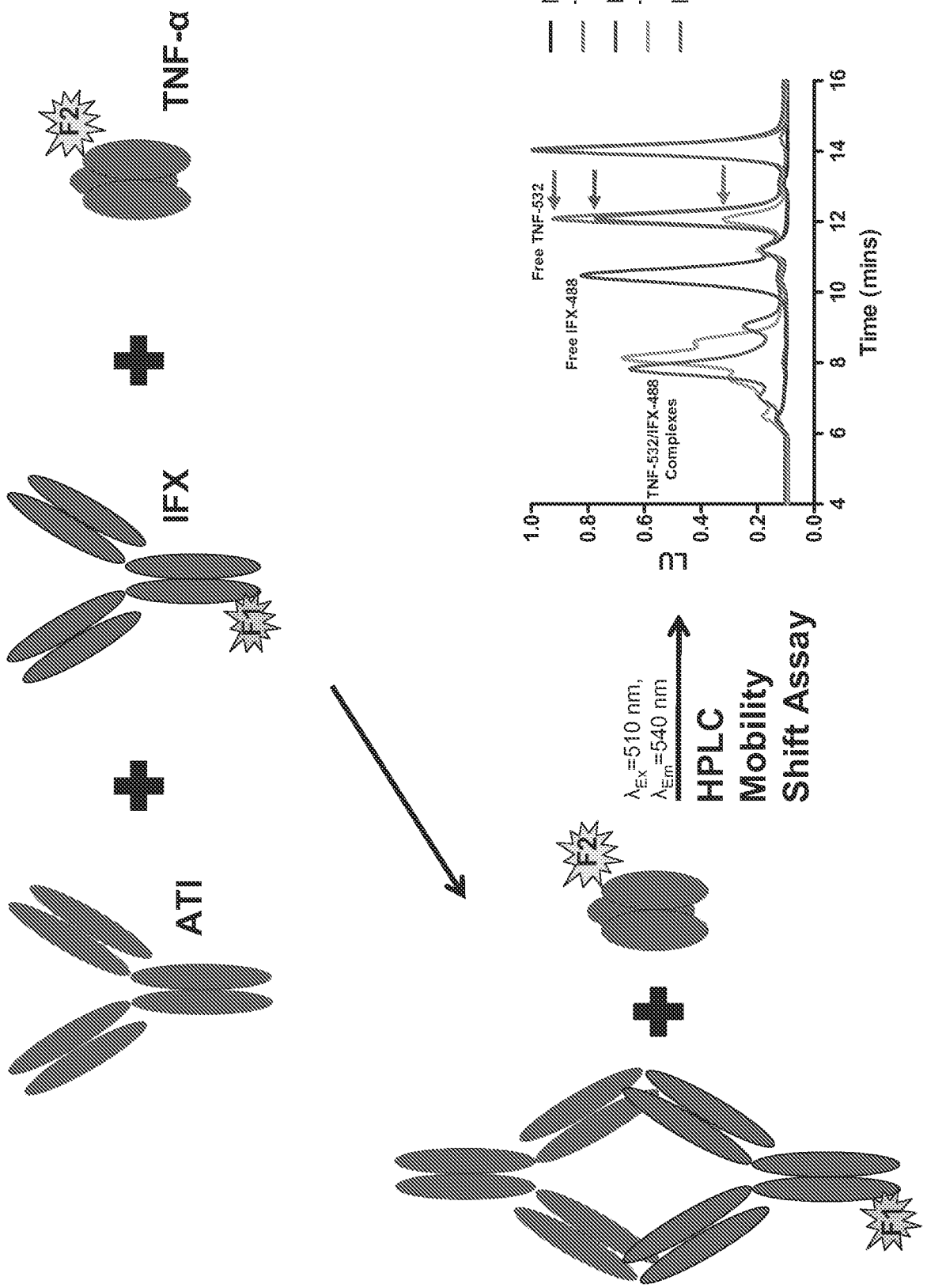
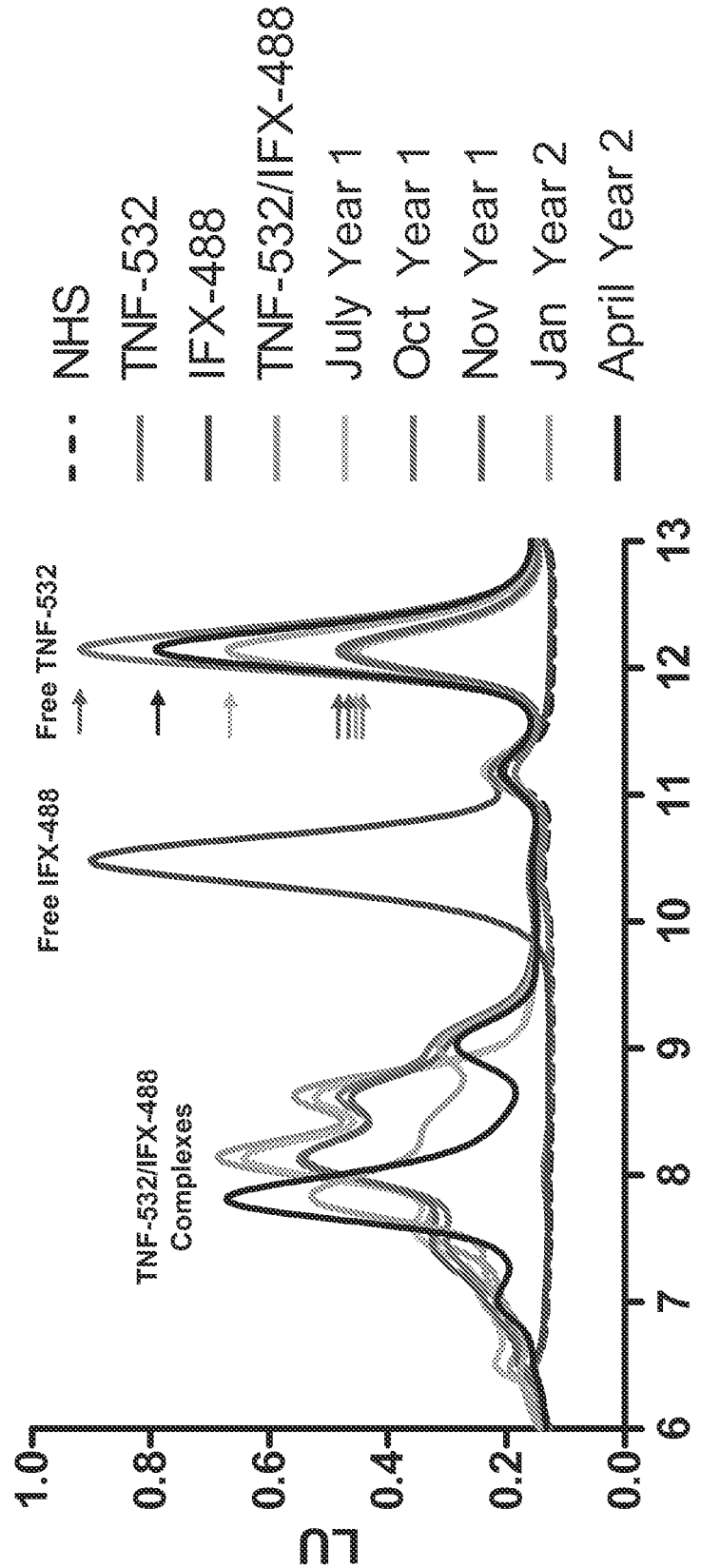

TNF-α
F2
IFX
F1
ATI
F2
F1
λ_{Ex}=510 nm,
λ_{Em}=540 nm
HPLC
Mobility
Shift Assay
NHS
TNF-532
IFX-488
TNF-532/IFX-488
Patient B Serum
Free TNF-532
Free IFX-488
TNF-532/IFX-488
Complexes
1.0
0.8
0.6
0.4
0.2
0.0
4
6
8
10
12
14
16
Time (mins)

FIG. 7



Collection Date	IFX [µg/mL]	ATI [U/mL]	Collection Date	IFX [µg/mL]	ATI [U/mL]
July Year 1	<0.98	12.07	Jan Year 2	<0.98	>150 U/mL
Oct Year 1	<0.98	>150 U/mL	April Year 2	<0.98	>150 U/mL
Nov Year 1	<0.98	>150 U/mL			

FIG. 8

Collection Date	IFX [µg/mL]	ATI [U/mL]
July Year 1	<0.98	12.07
Oct Year 1	<0.98	>150 U/mL
Nov Year 1	<0.98	>150 U/mL
Jan Year 2	<0.98	>150 U/mL
April Year 2	<0.98	>150 U/mL

Peak area of TNF532/IFX488 complex subtracted from Free TNF area of all samples and then % of free TNF calculated

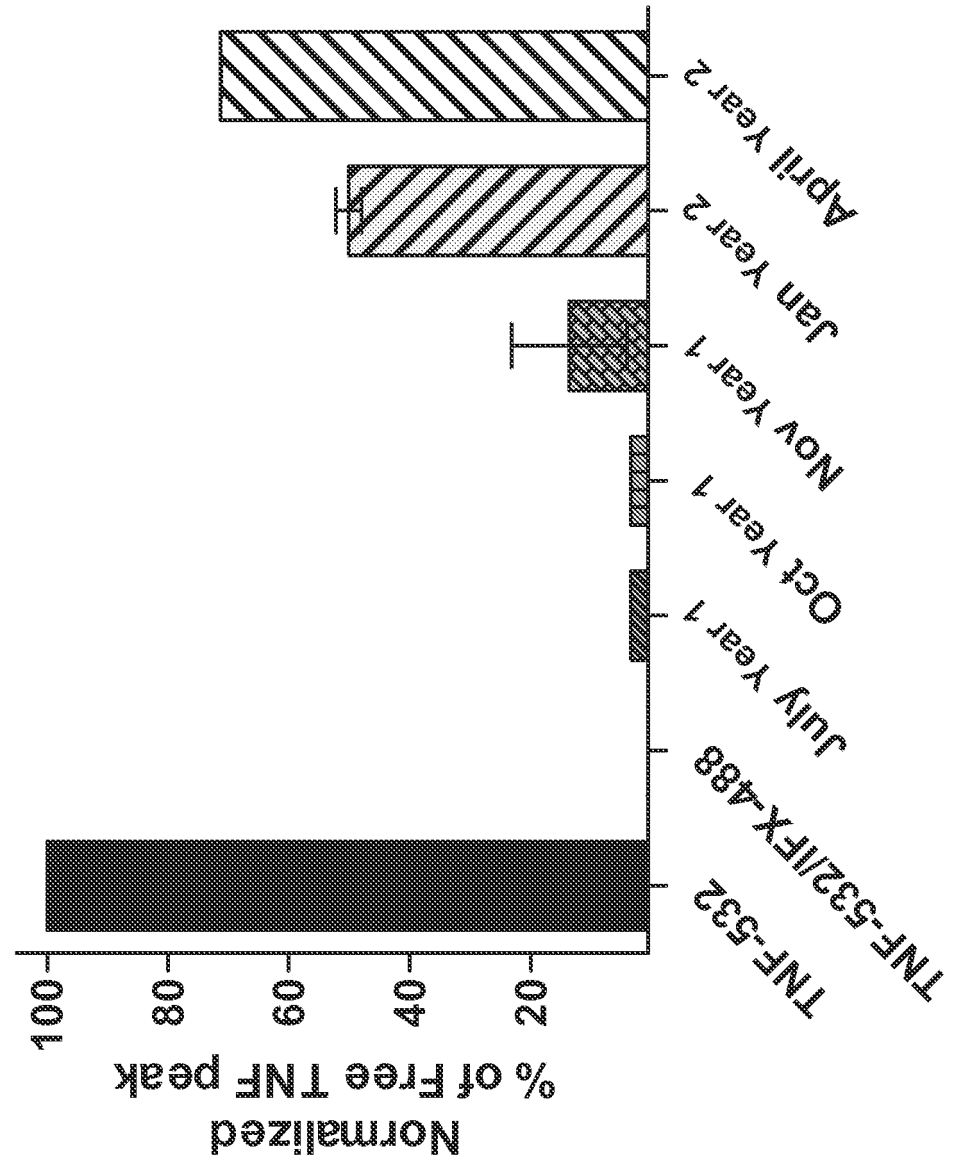


FIG. 9

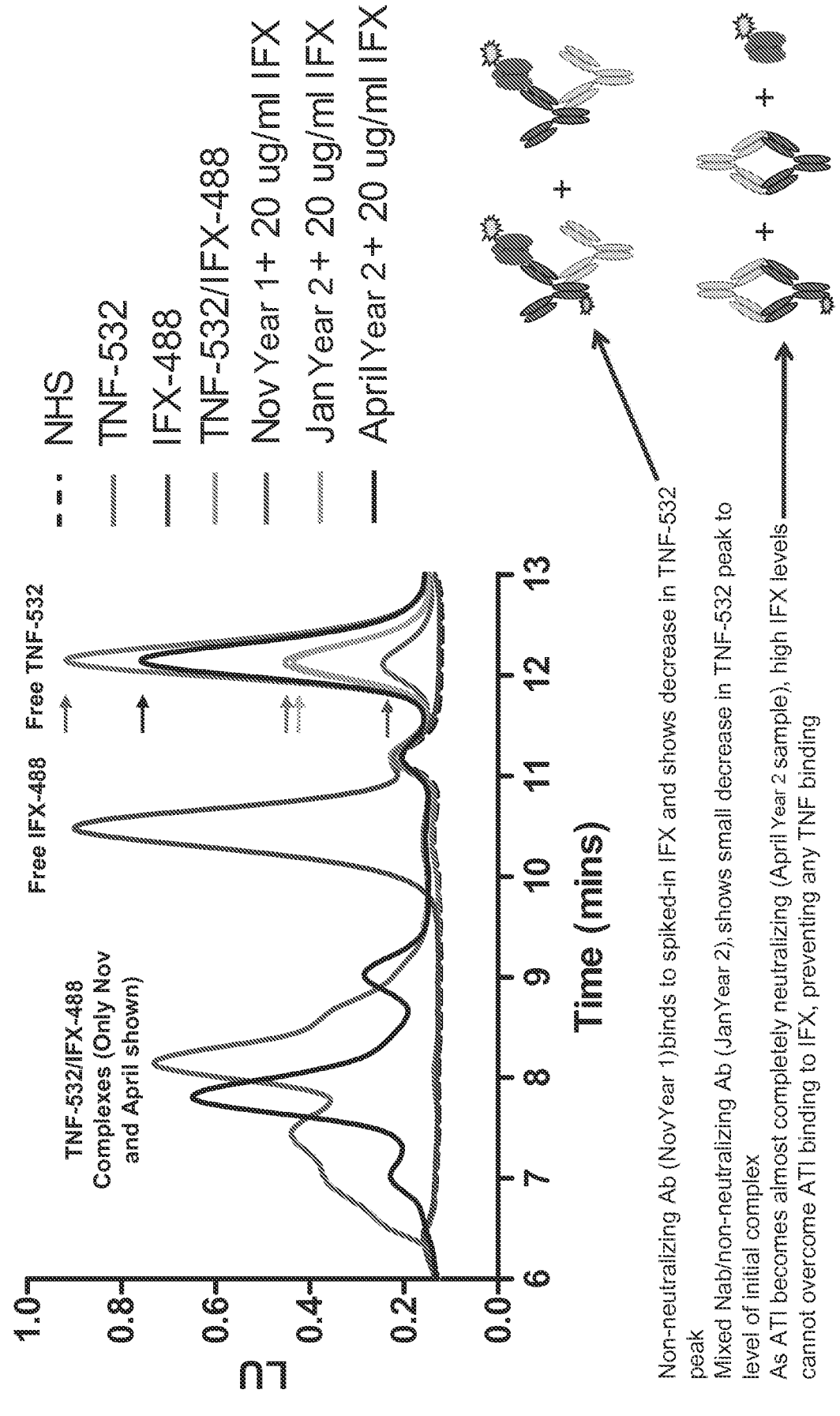

NHS
TNF-532
IFX-488
TNF-532/IFX-488
Nov Year 1+ 20 ug/ml IFX
Jan Year 2+ 20 ug/ml IFX
April Year 2+ 20 ug/ml IFX
Free IFX-488
Free TNF-532
TNF-532/IFX-488 Complexes (Only Nov and April shown)
1.0
0.8
0.6
0.4
0.2
0.0
6
7
8
9
10
11
12
13
Time (mins)
1. Non-neutralizing Ab (NovYear 1)binds to spiked-in IFX and shows decrease in TNF-532 peak
2. Mixed Nab/non-neutralizing Ab (JanYear 2), shows small decrease in TNF-532 peak to level of initial complex
3. As ATI becomes almost completely neutralizing (April Year 2 sample), high IFX levels cannot overcome ATI binding to IFX, preventing any TNF binding

FIG. 10

Collection Date	IFX [µg/mL]	ATI [U/mL]
Nov Year 1	<0.98	>150 U/mL
Jan Year 2	<0.98	>150 U/mL
April Year 2	<0.98	>150 U/mL

Peak area of TNF532/IFX488 complex subtracted from Free TNF area of all samples and then % of free TNF calculated

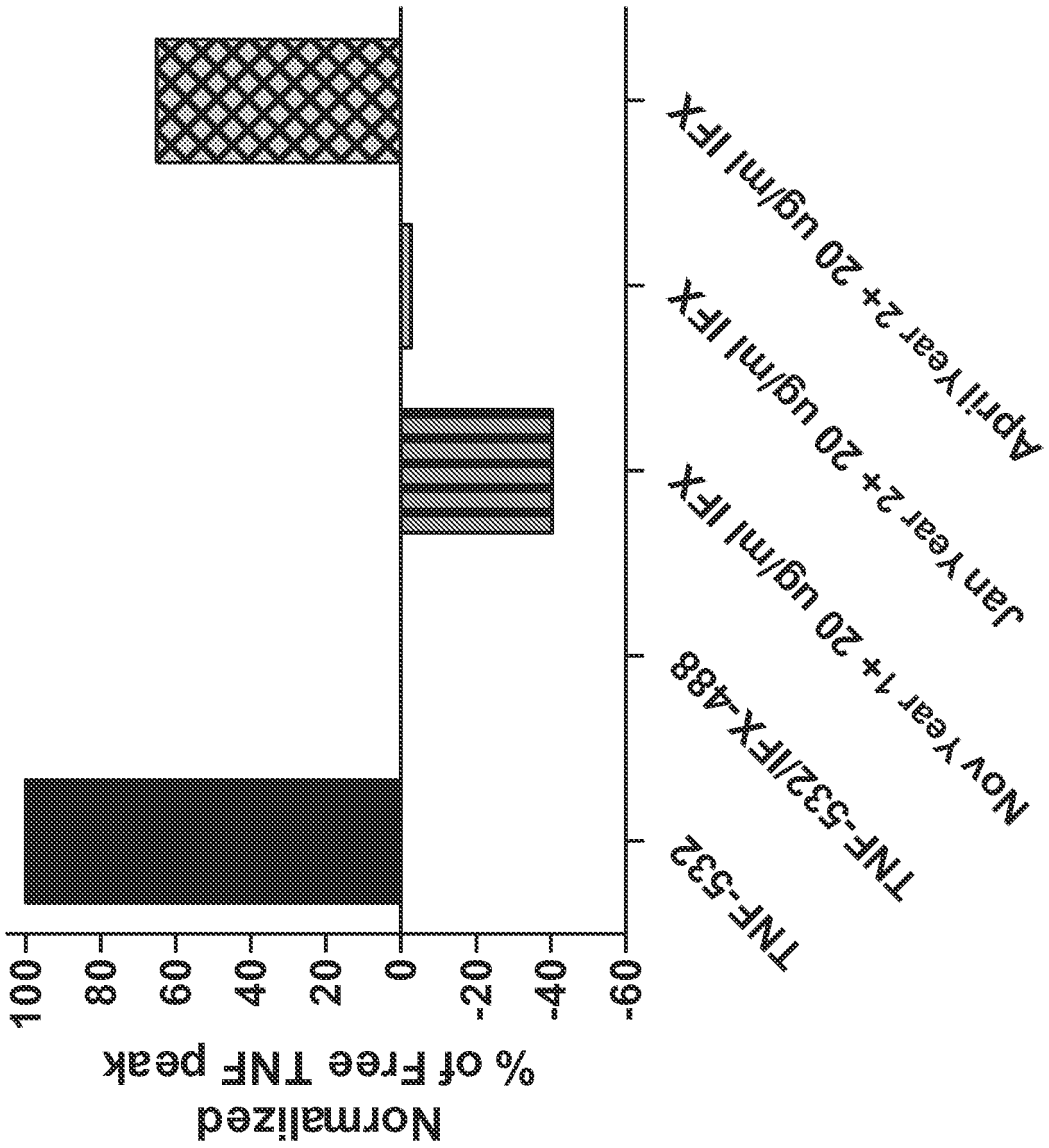
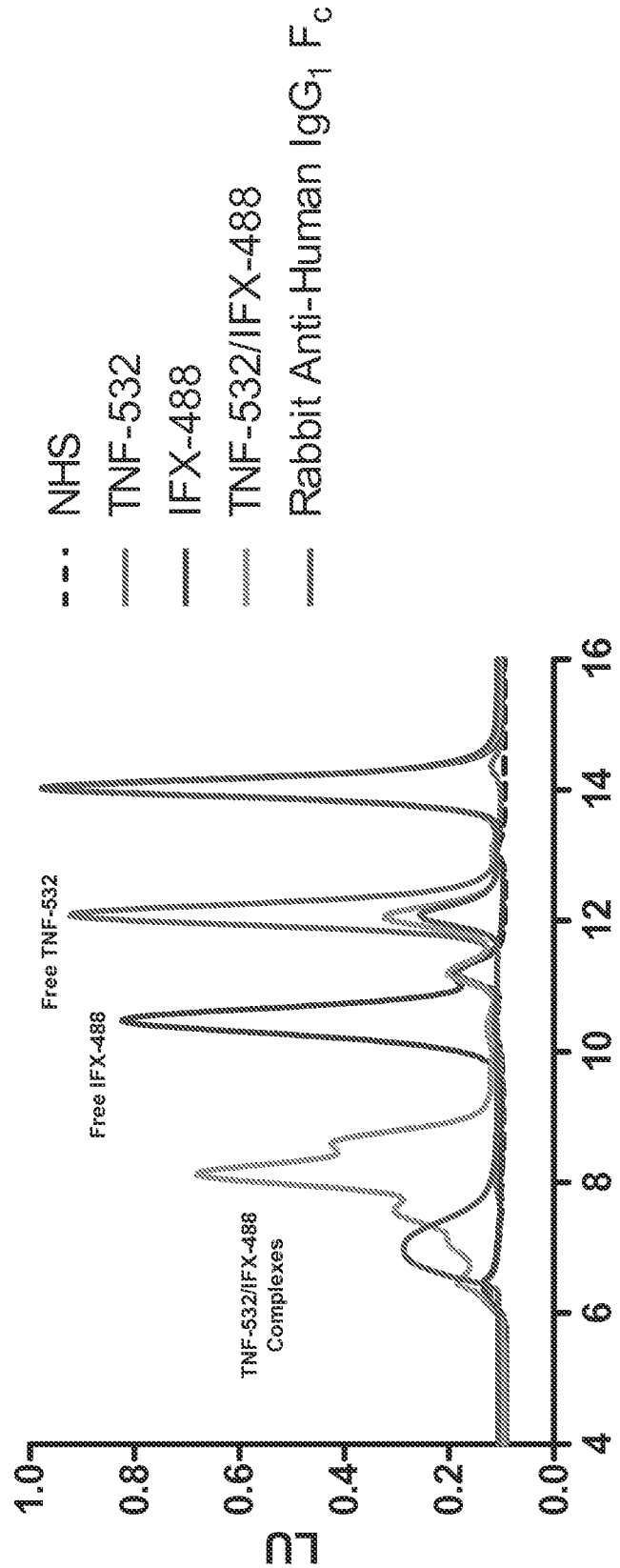
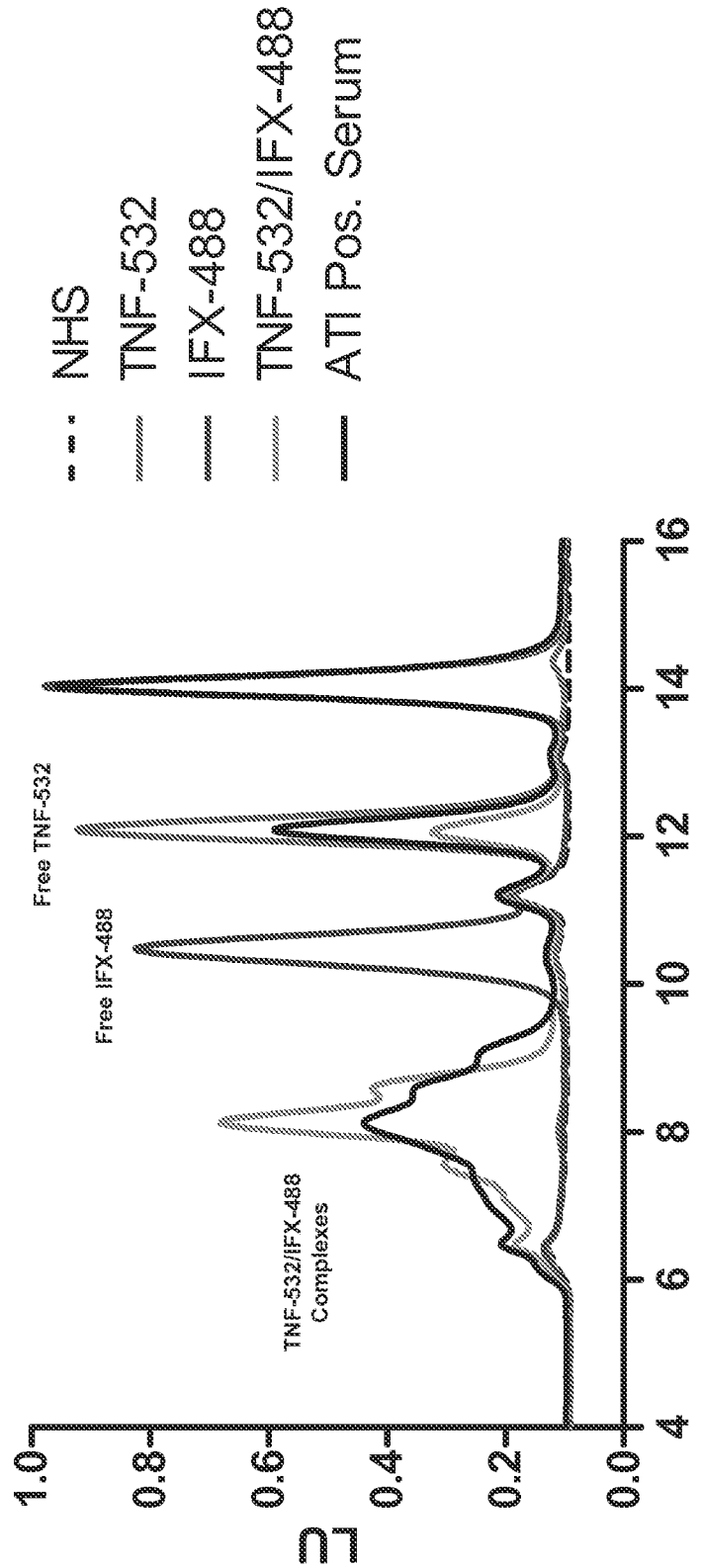


FIG. 11



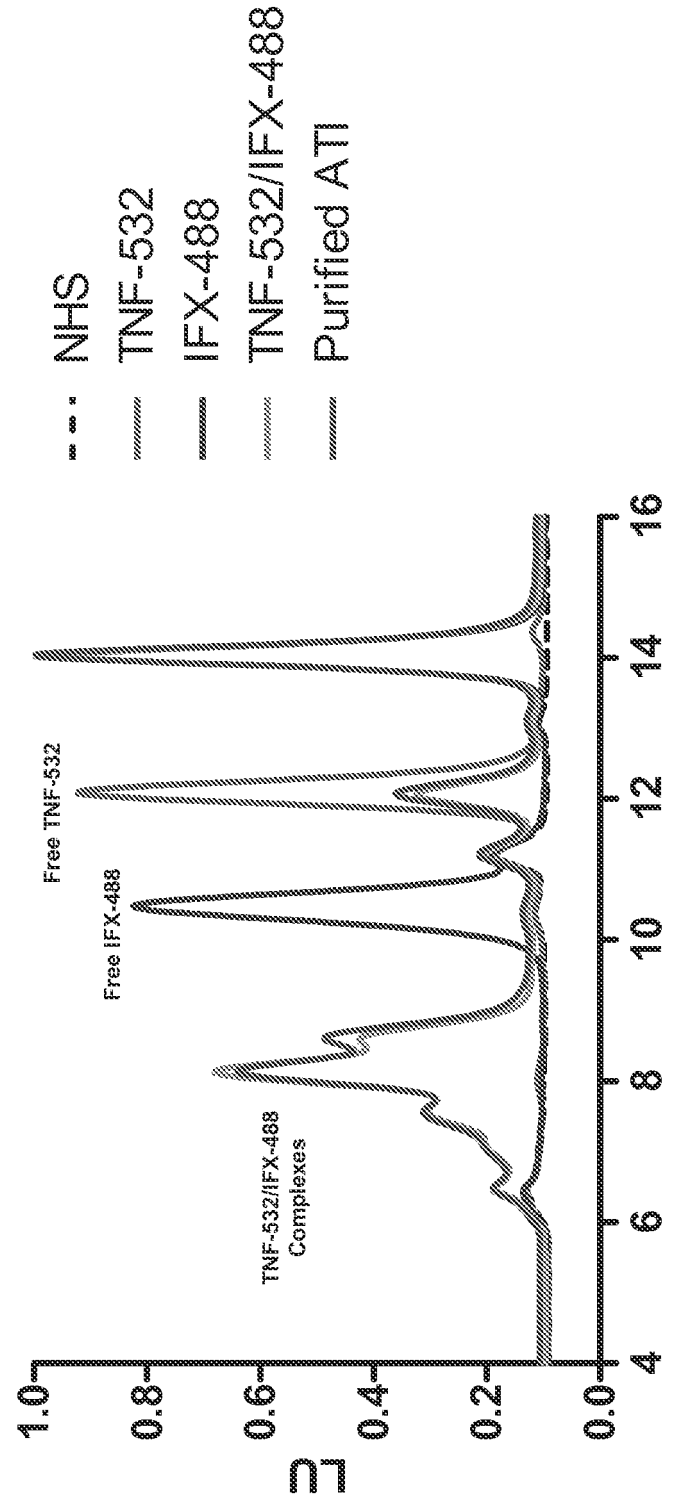
Rabbit Anti-Human IgG_1 F_c: Non-neutralizing Ab Control

FIG. 12



ATI Pos. Serum: Mixed Nab/Non-neutralizing Ab Control

FIG. 13



Purification of ATI from ATI Positive Serum results in loss of weaker affinity Nab

FIG. 14

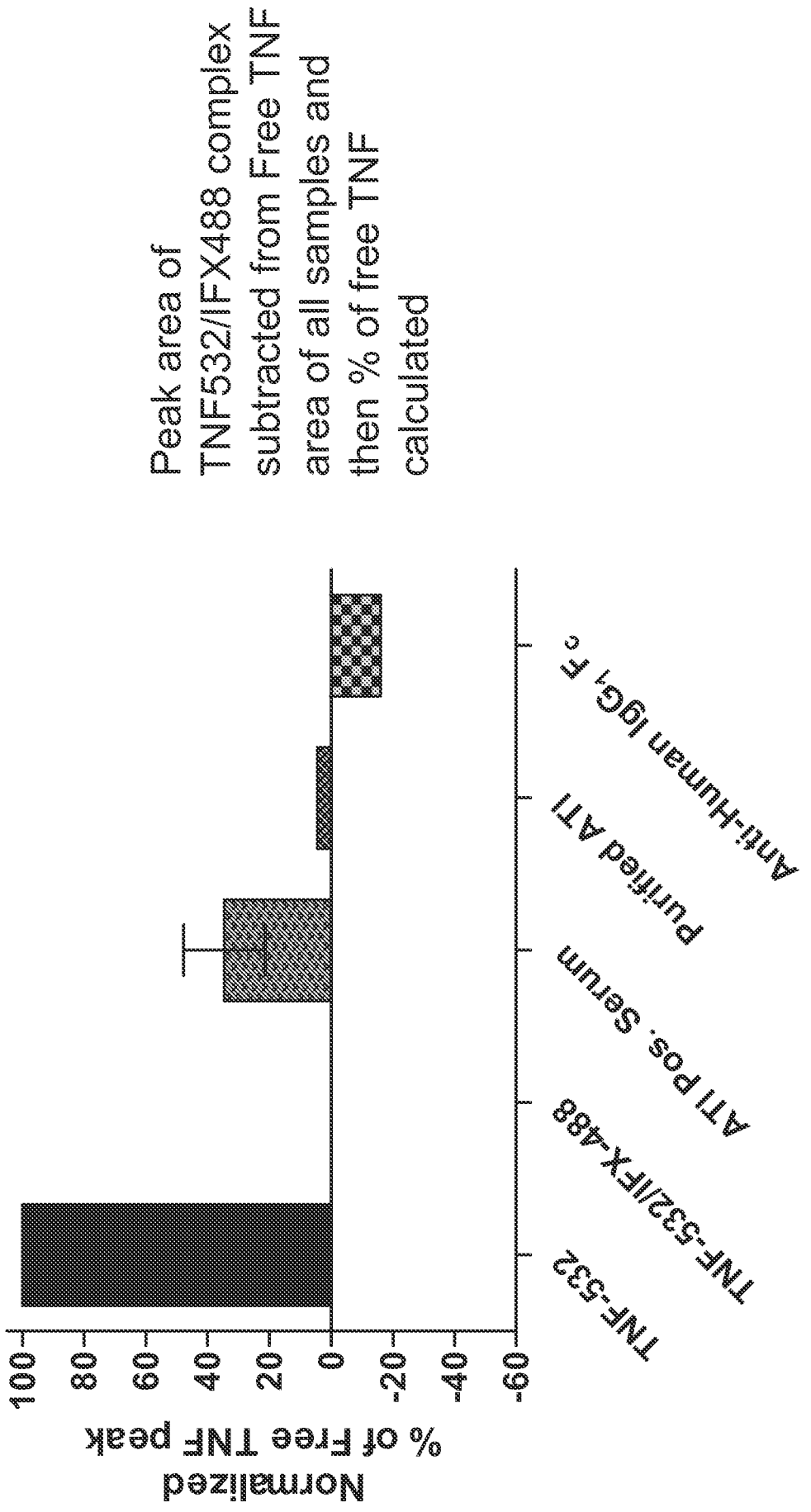

Peak area of TNF532/IFX488 complex subtracted from Free TNF area of all samples and then % of free TNF calculated
Normalized % of Free TNF peak
100
80
60
40
20
0
-20
-40
-60
TNF-532
TNF-532/IFX-488
ATI Pos. Serum
Purified ATI
Anti-Human IgG1 Fc

FIG. 15

Sample ID	Week #	IFX [µg/mL]	ATI [U/mL]
10113A	7	26.14	17.09
10113B	14	19.26	14.37
10113C	22	17.09	15.12
10113D	30	7.22	10.16

Induction: Weeks 1-14
Maintenance: Weeks 22-50

Peak area of TNF532/IFX488 complex subtracted from all samples and then % of free TNF calculated

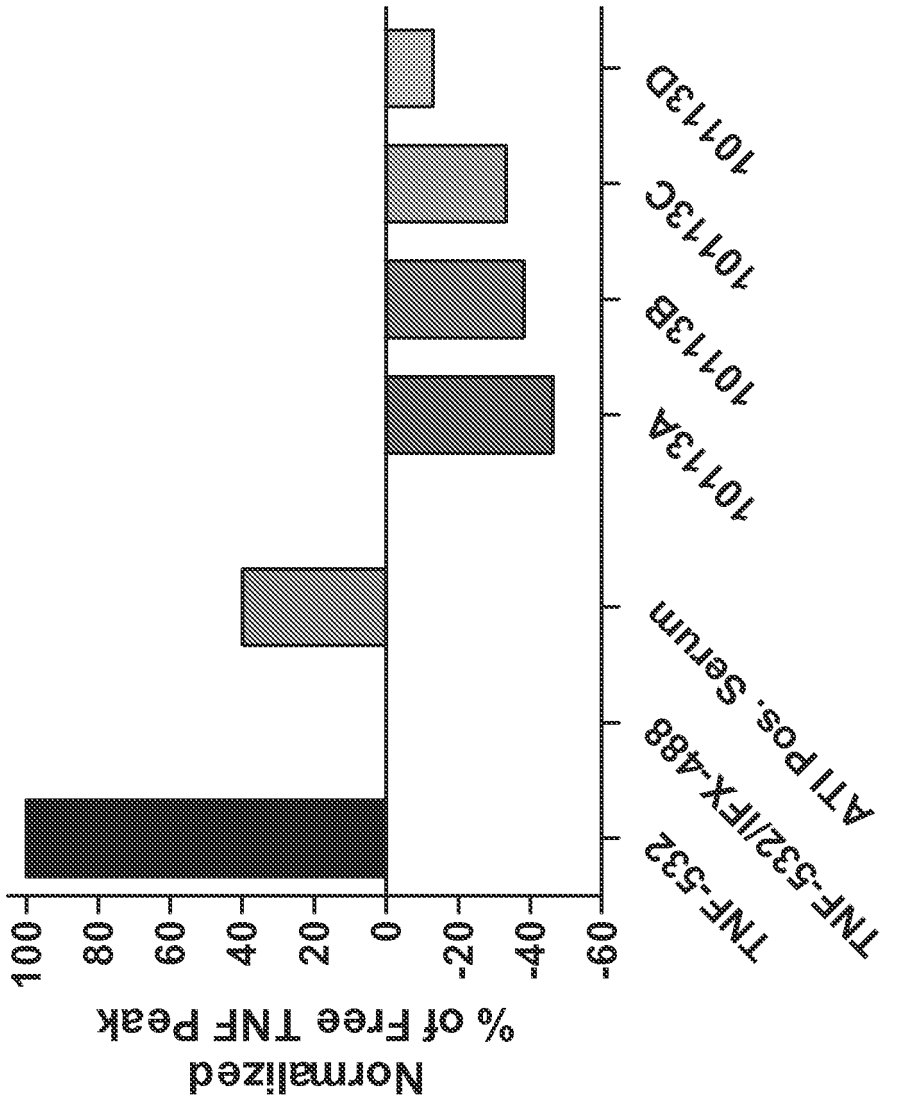


FIG. 16

Sample ID	Week #	IFX [µg/mL]	ATI [U/mL]
10509A	22	12.77	11.42
10509B	46	5.63	10.15
10509C	50	27.24	17.57

Induction: Weeks 1-14
Maintenance: Weeks 22-50

Peak area of TNF532/IFX488 complex subtracted from Free TNF area of all samples and then % of free TNF calculated

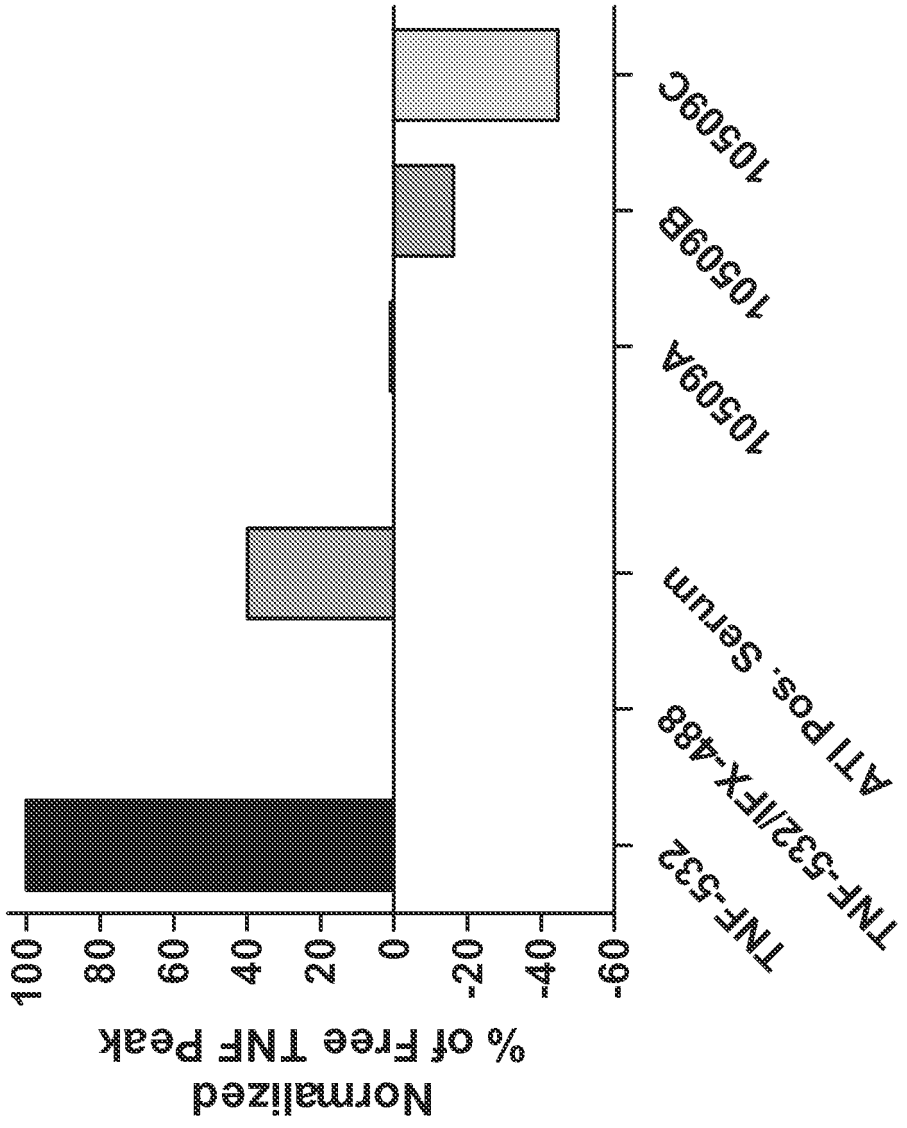


FIG. 17

Sample ID	Week #	IFX [µg/mL]	ATI [U/mL]
10111	46	8.76	13.59
11505	14	11.42	12.66
11903	60	20.95	11.79
10308	14	1.09	54.64

Induction: Weeks 1-14
Maintenance: Weeks 22-50

Peak area of TNF532/IFX488 complex subtracted from Free TNF area of all samples and then % of free TNF calculated

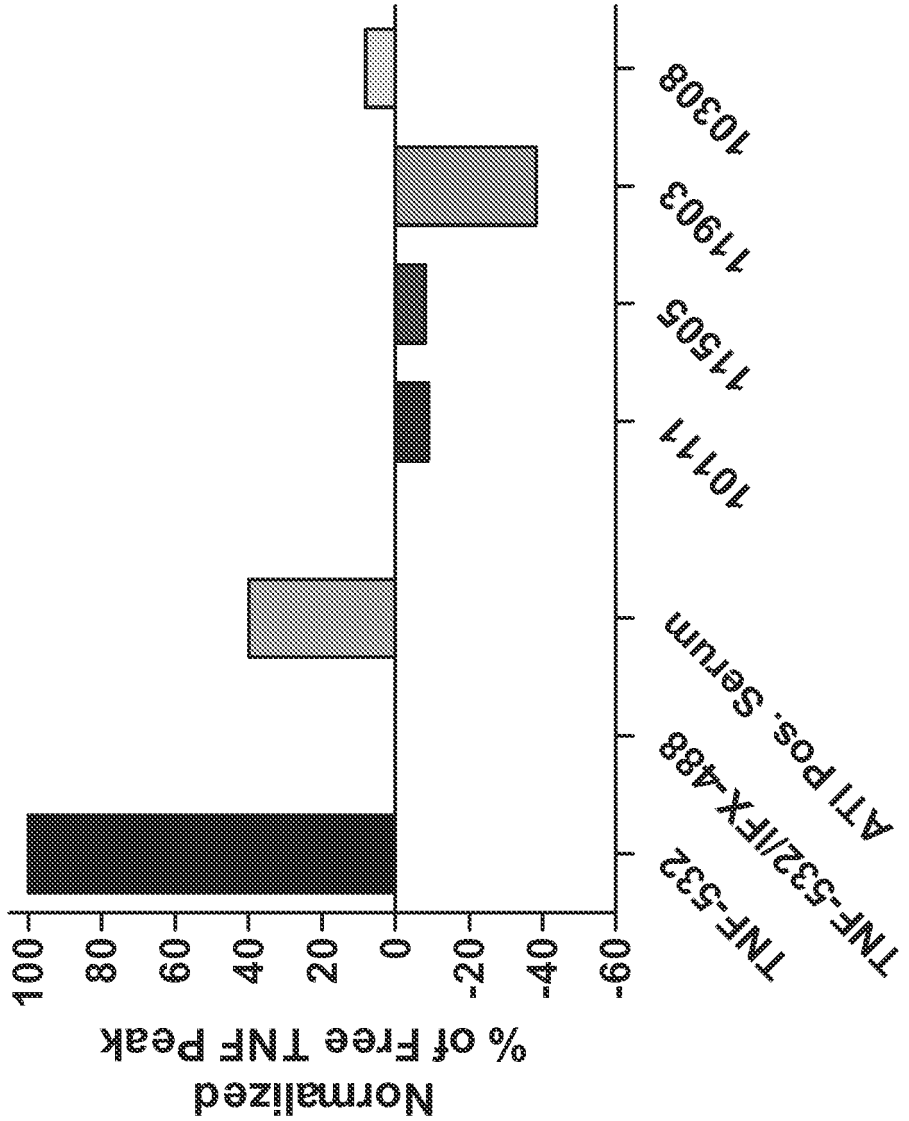


FIG. 18

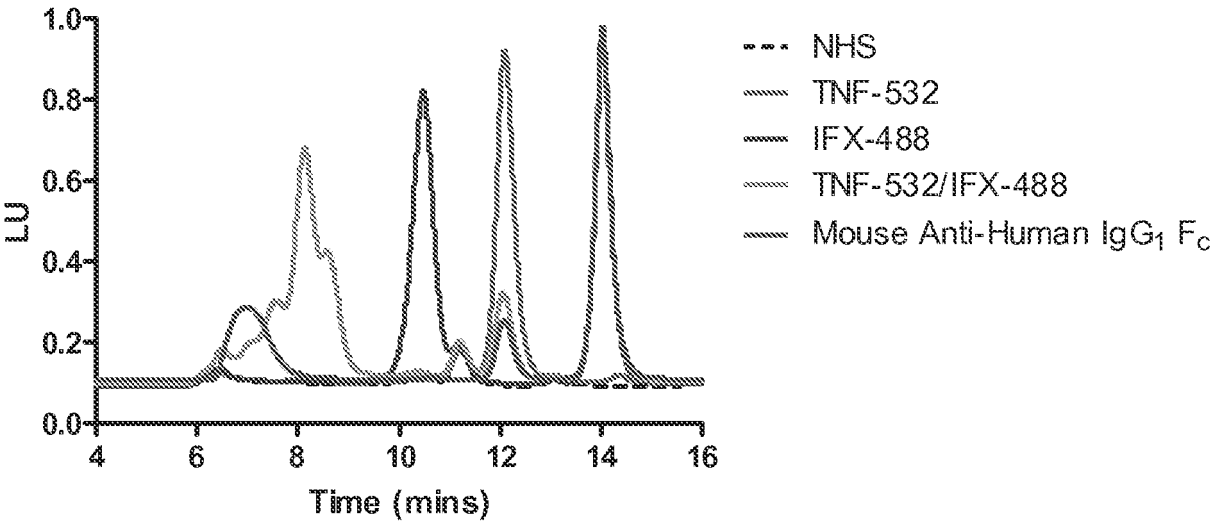
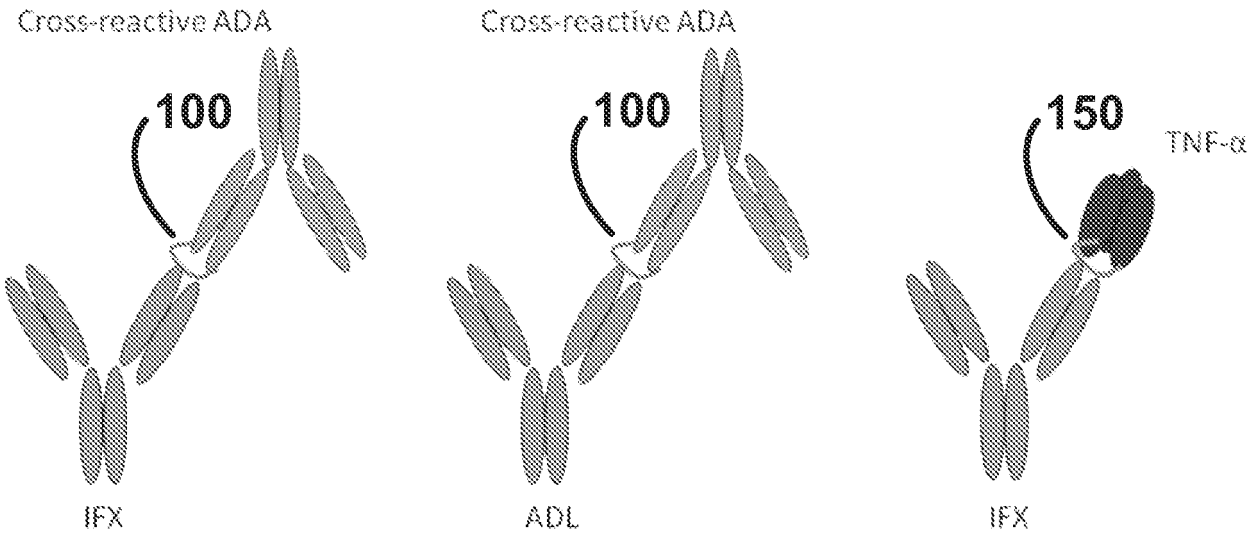

1.0
0.8
0.6
0.4
0.2
0.0
LU
4
6
8
10
12
14
16
Time (mins)
NHS
TNF-532
IFX-488
TNF-532/IFX-488
Mouse Anti-Human IgG1 Fc

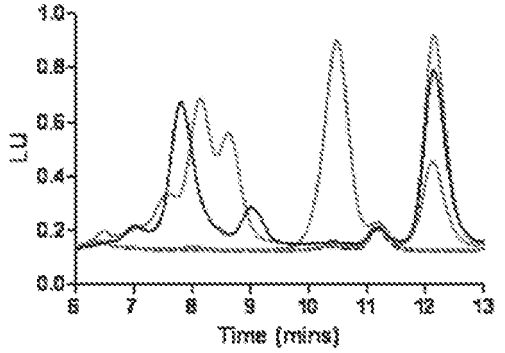
FIG. 19



Binding site of ADA **(100)** mimics binding site of TNF **(150)** , can therefore bind to multiple anti-TNF drugs

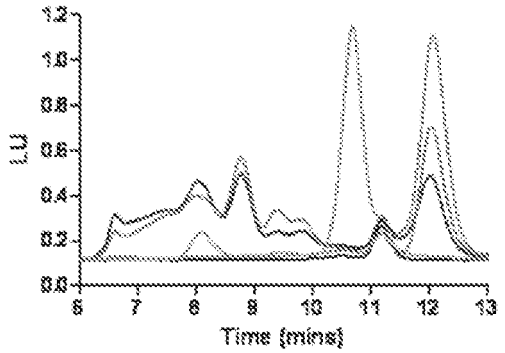
FIG. 20

Patient Study #1 – UC Patient



71% of free TNF

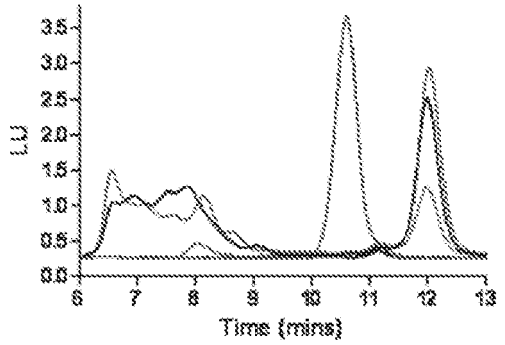
Collection Date	IFX [µg/mL]	ATI [U/mL]	Confirmed positive (>20 µg/mL neutralizing activity)
GRD0125	<0.08	>150 U/mL	Yes



21% of free TNF

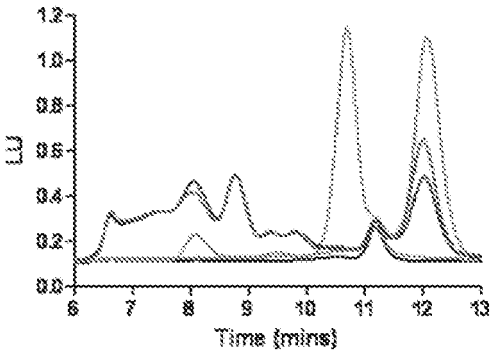
Patient will likely not respond to adalimumab

Patient Study #2 – CD Patient



69% of free TNF

Collection Date	IFX [µg/mL]	ATI [U/mL]	Confirmed positive (>20 µg/mL neutralizing activity)
PL_CL01956.7	<0.08	356.82 U/mL	Yes

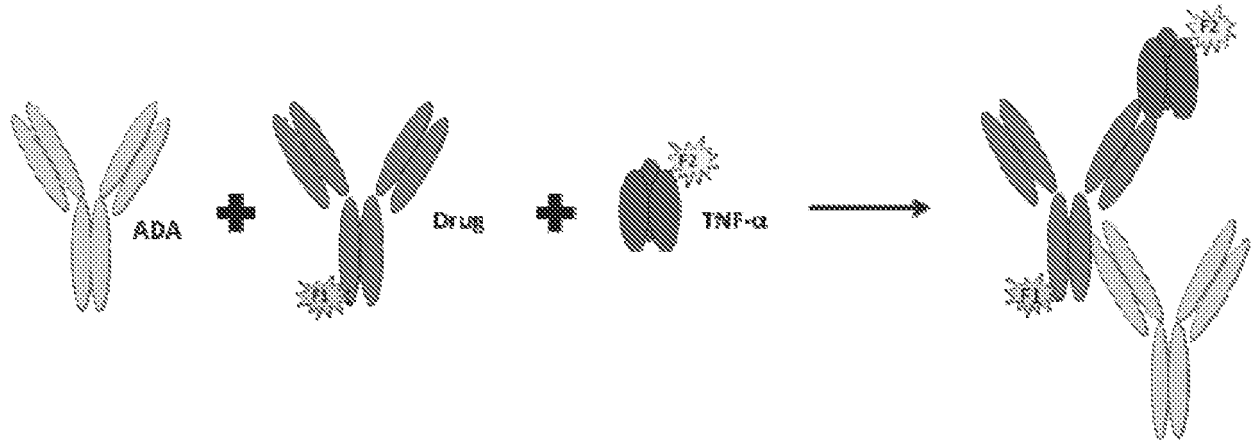


21% of free TNF

Patient will likely not respond to adalimumab

FIG. 21

Binding (non-NAB) Antibody



Neutralizing Antibody (NAB)

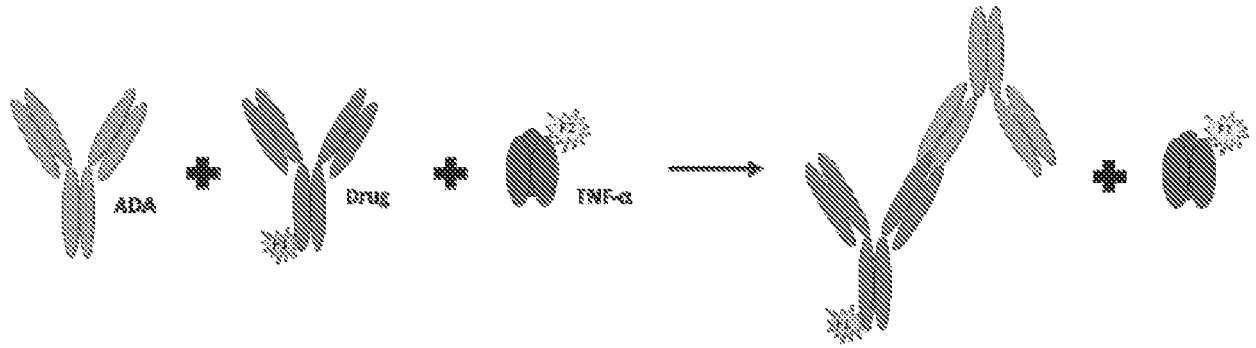


FIG. 22

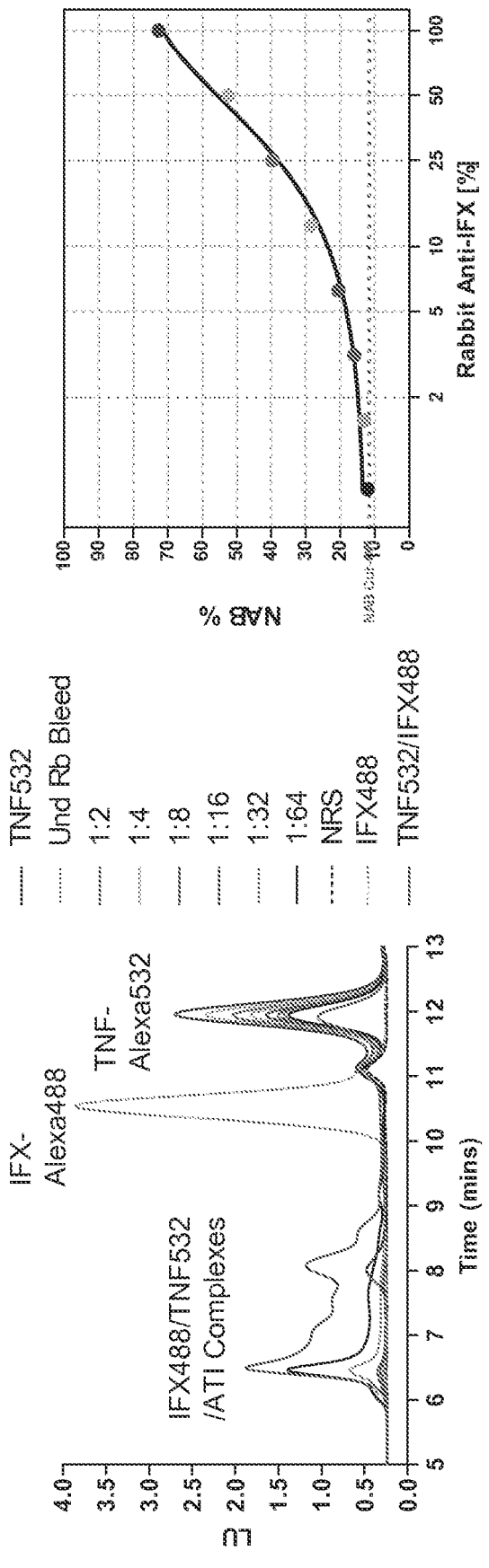

IFX-
Alexa488
TNF-
Alexa532
IFX488/TNF532
/ATI Complexes
Time (mins)
TNF532
Und Rb Bleed
1:2
1:4
1:8
1:16
1:32
1:64
NRS
IFX488
TNF532/IFX488
% MAB
Rabbit Anti-IFX [%]

FIG. 23

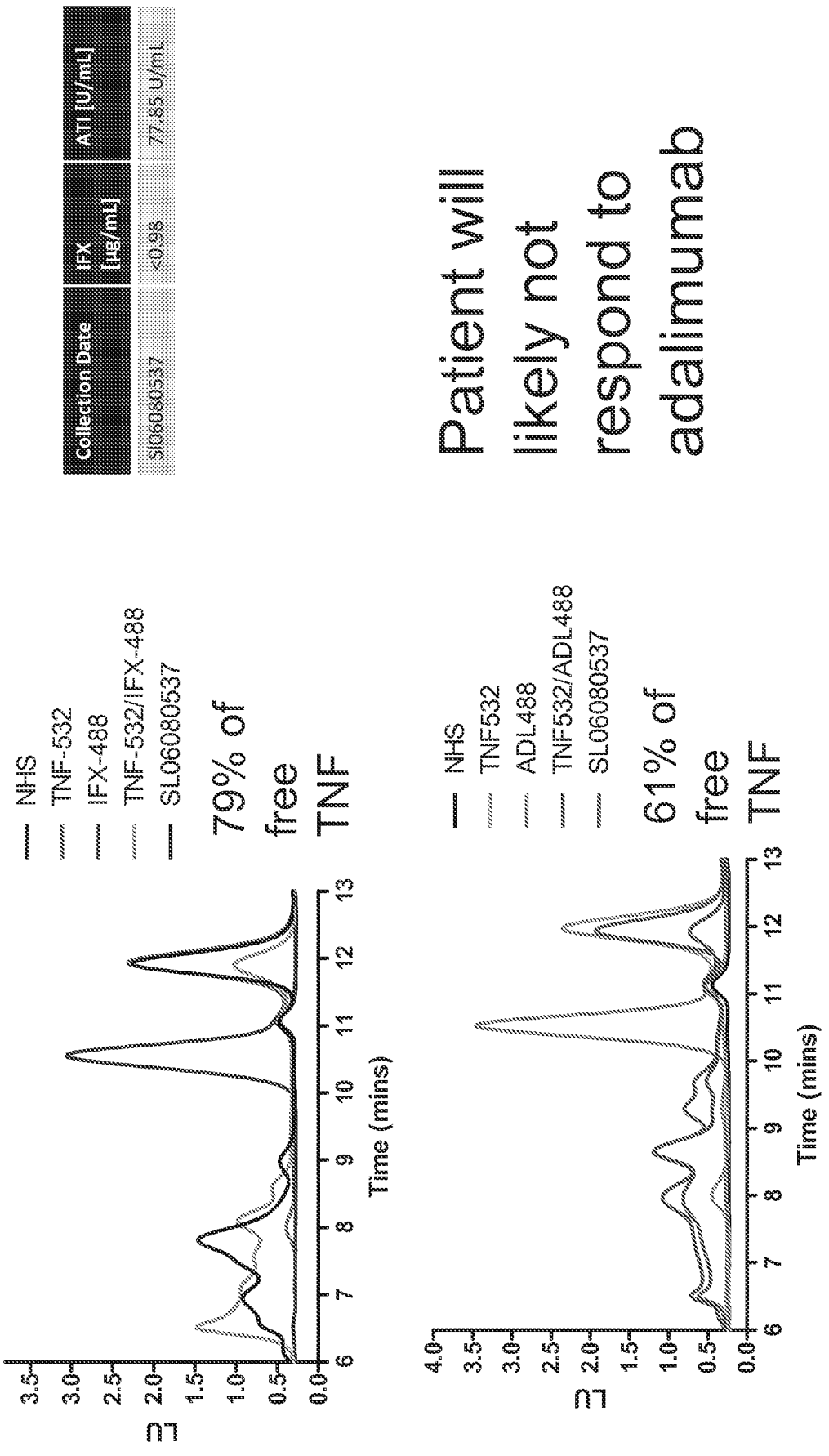

Collection Date
IFX [µg/mL]
ATI [U/mL]
SL06080537
<0.98
77.85 U/mL
Patient will likely not respond to adalimumab
NHS
TNF-532
IFX-488
TNF-532/IFX-488
SL06080537
79% of free TNF
Time (mins)
NHS
TNF532
ADL488
TNF532/ADL488
SL06080537
61% of free TNF
Time (mins)

FIG. 24

Collection Date	IFX [µg/mL]	ATI [U/mL]
SL07141009	<0.98	147.35 U/mL

Patient will likely not respond to adalimumab

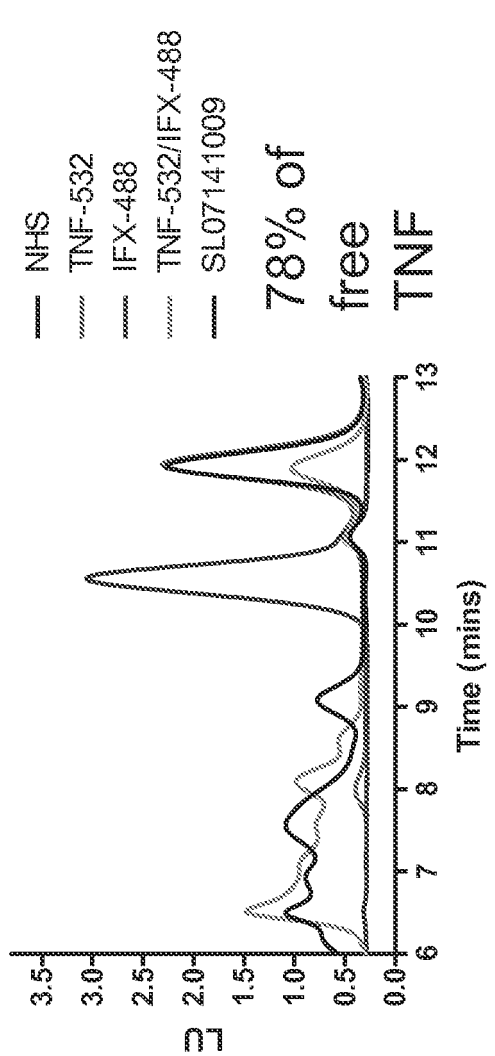


FIG. 25

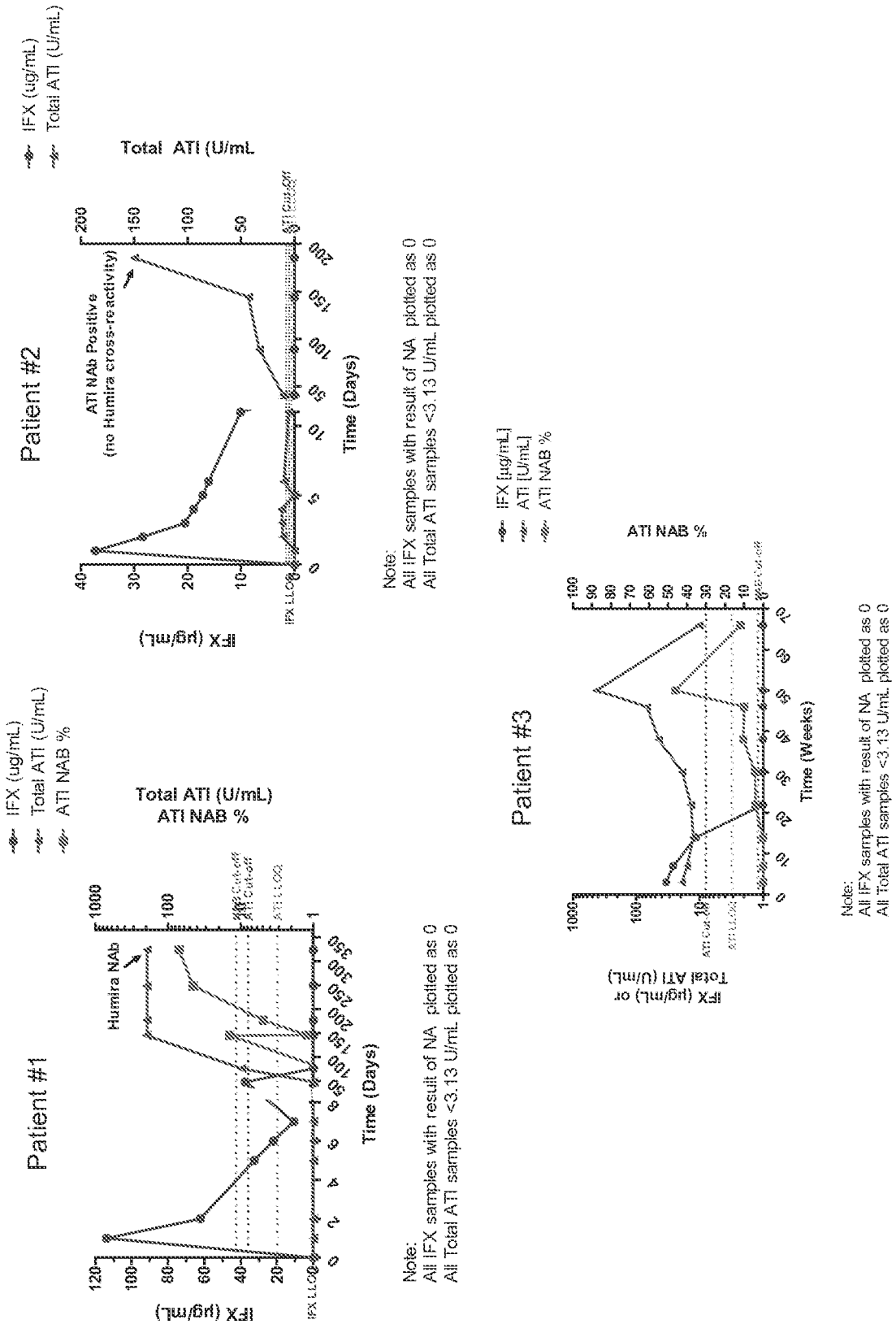

Patient #1
IFX (ug/mL)
Total ATI (U/mL)
ATI NAB %
Total ATI (U/mL)
ATI NAB %
Humira NAb
IFX (µg/mL)
Time (Days)
Note:
All IFX samples with result of NA plotted as 0
All Total ATI samples <3.13 U/mL plotted as 0
Patient #2
IFX (ug/mL)
Total ATI (U/mL)
Total ATI (U/mL)
ATI NAb Positive
(no Humira cross-reactivity)
IFX (µg/mL)
Time (Days)
Note:
All IFX samples with result of NA plotted as 0
All Total ATI samples <3.13 U/mL plotted as 0
Patient #3
IFX [µg/mL]
ATI [U/mL]
ATI NAB %
ATI NAB %
IFX (µg/mL) or
Total ATI (U/mL)
Time (Weeks)
Note:
All IFX samples with result of NA plotted as 0
All Total ATI samples <3.13 U/mL plotted as 0

FIG. 26

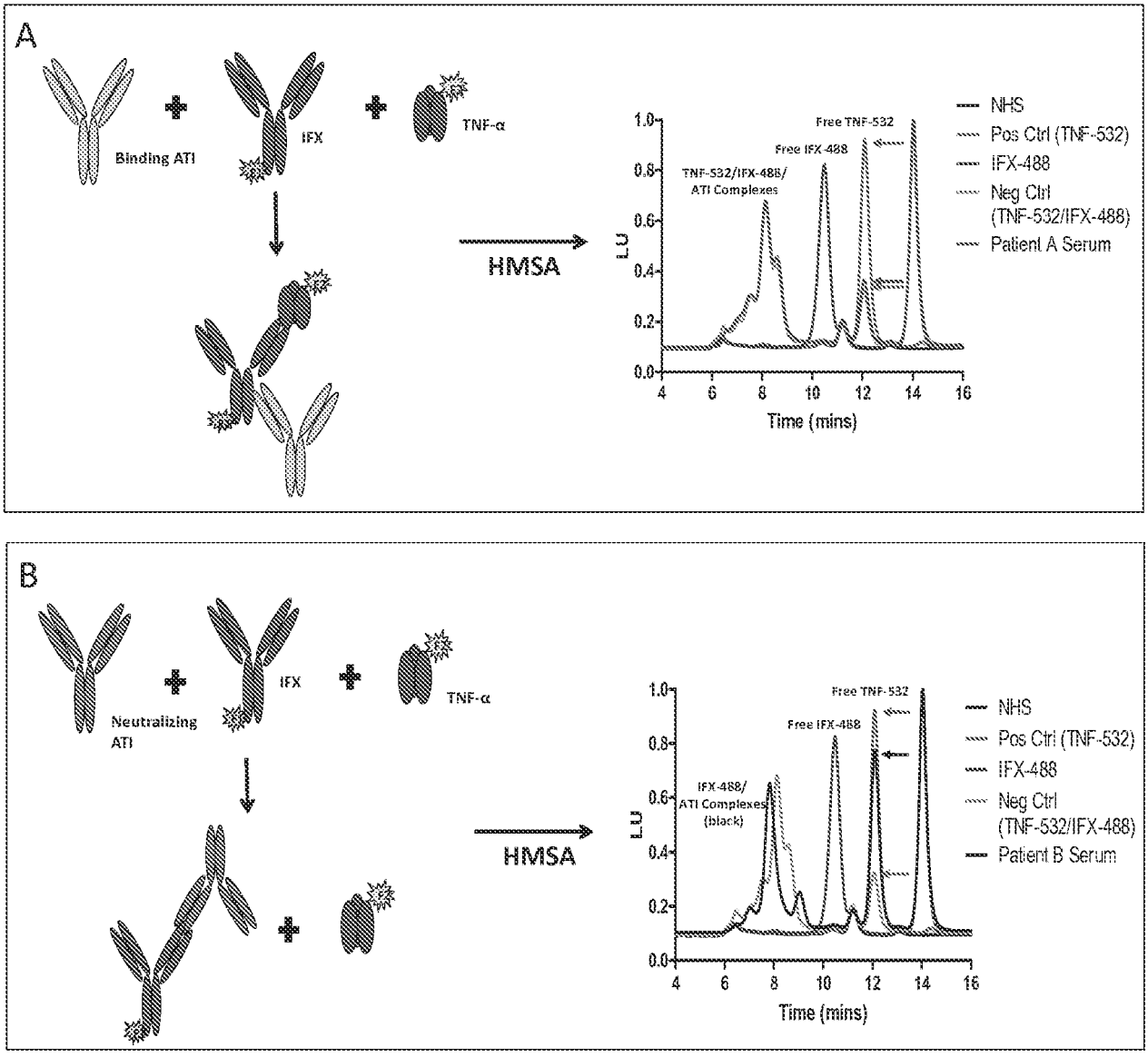

A
Binding ATI
IFX
TNF-α
HMSA
TNF-532/IFX-488/
ATI Complexes
Free IFX-488
Free TNF-532
LU
Time (mins)
NHS
Pos Ctrl (TNF-532)
IFX-488
Neg Ctrl
(TNF-532/IFX-488)
Patient A Serum
B
Neutralizing
ATI
IFX
TNF-α
HMSA
IFX-488/
ATI Complexes
(black)
Free IFX-488
Free TNF-532
LU
Time (mins)
NHS
Pos Ctrl (TNF-532)
IFX-488
Neg Ctrl
(TNF-532/IFX-488)
Patient B Serum

FIG. 27

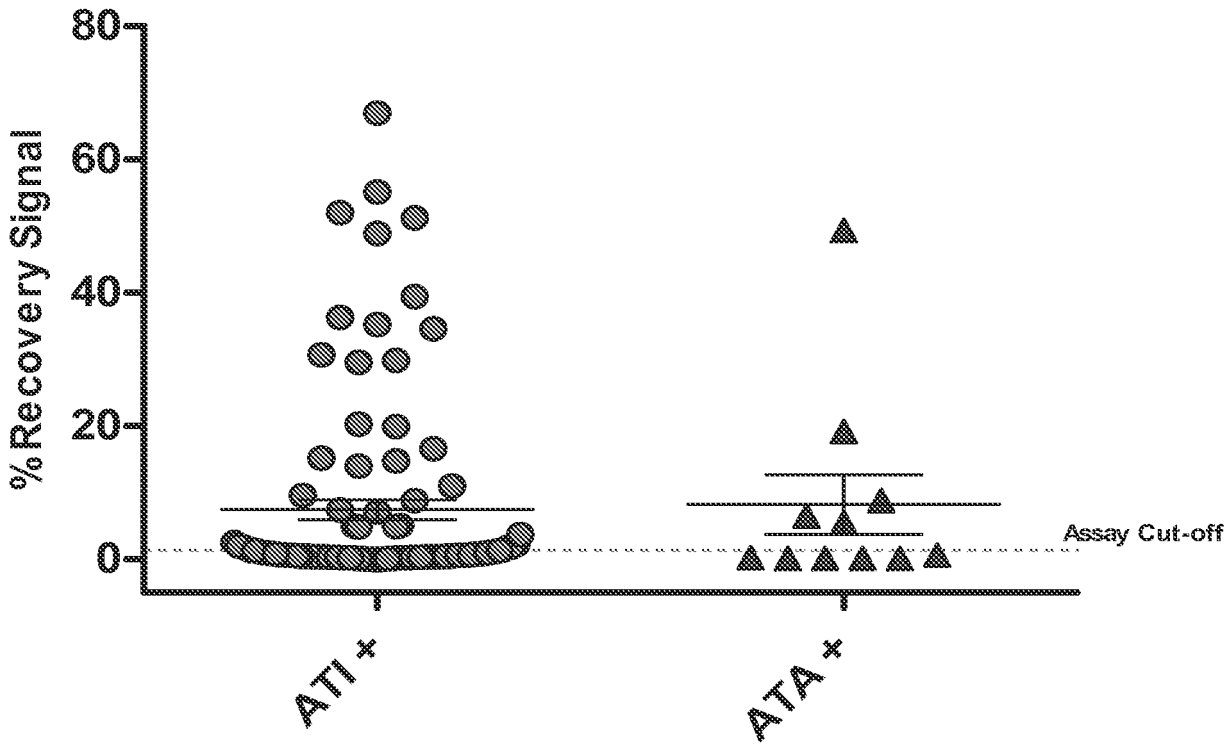


FIG. 28

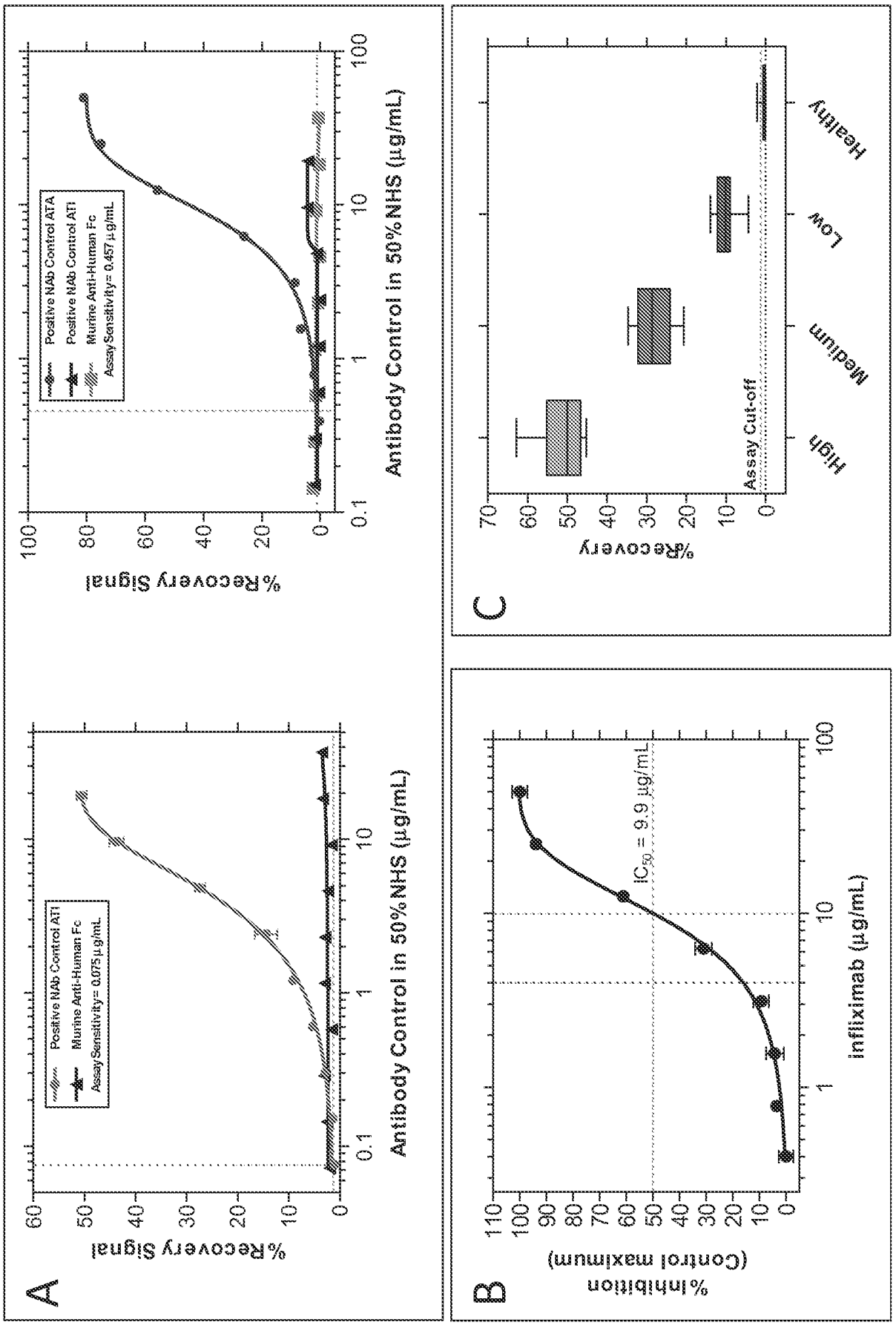

A
%Recovery Signal
Antibody Control in 50% NHS (µg/mL)
Positive NAb Control ATI
Murine Anti-Human Fc
Assay Sensitivity= 0.075 µg/mL
B
%Inhibition (Control maximum)
infliximab (µg/mL)
IC50 = 9.9 µg/mL
C
%Recovery
Assay Cut-off
High
Medium
Low
Healthy

FIG. 29