FIG. 1

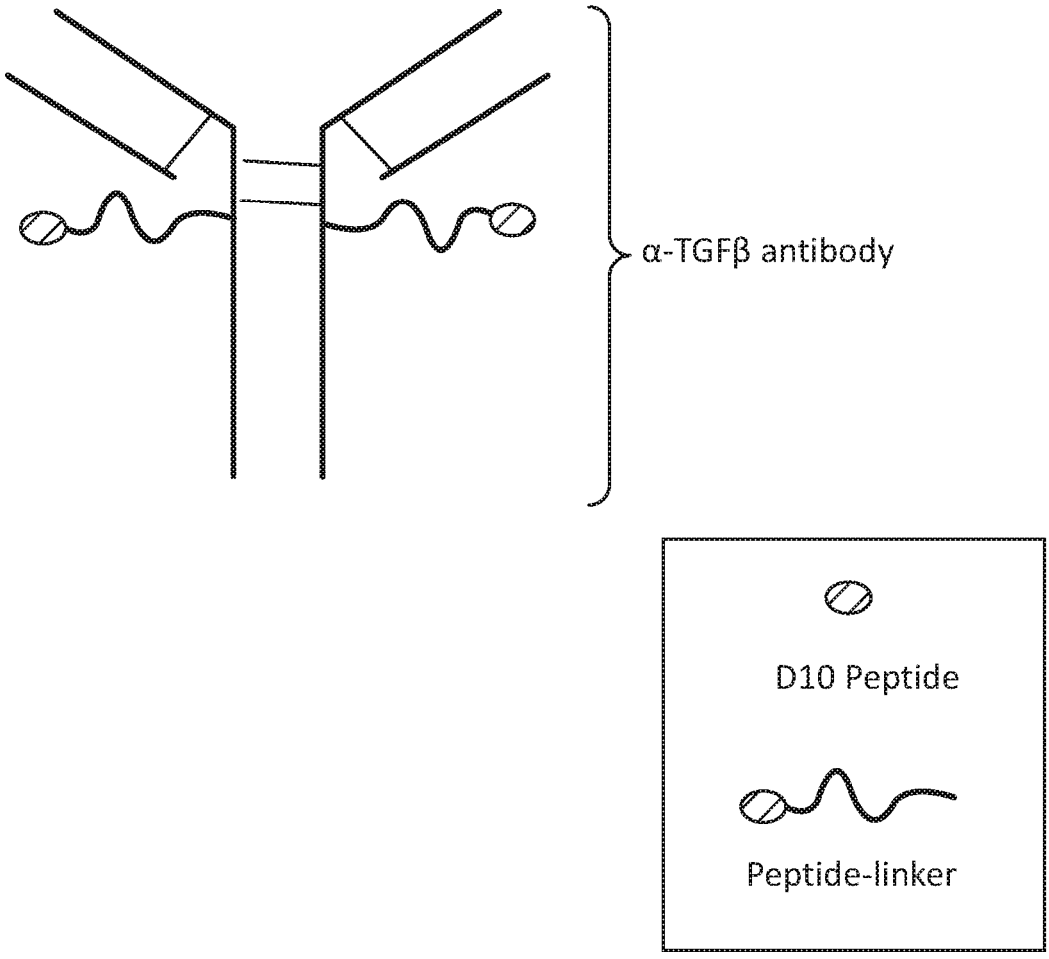
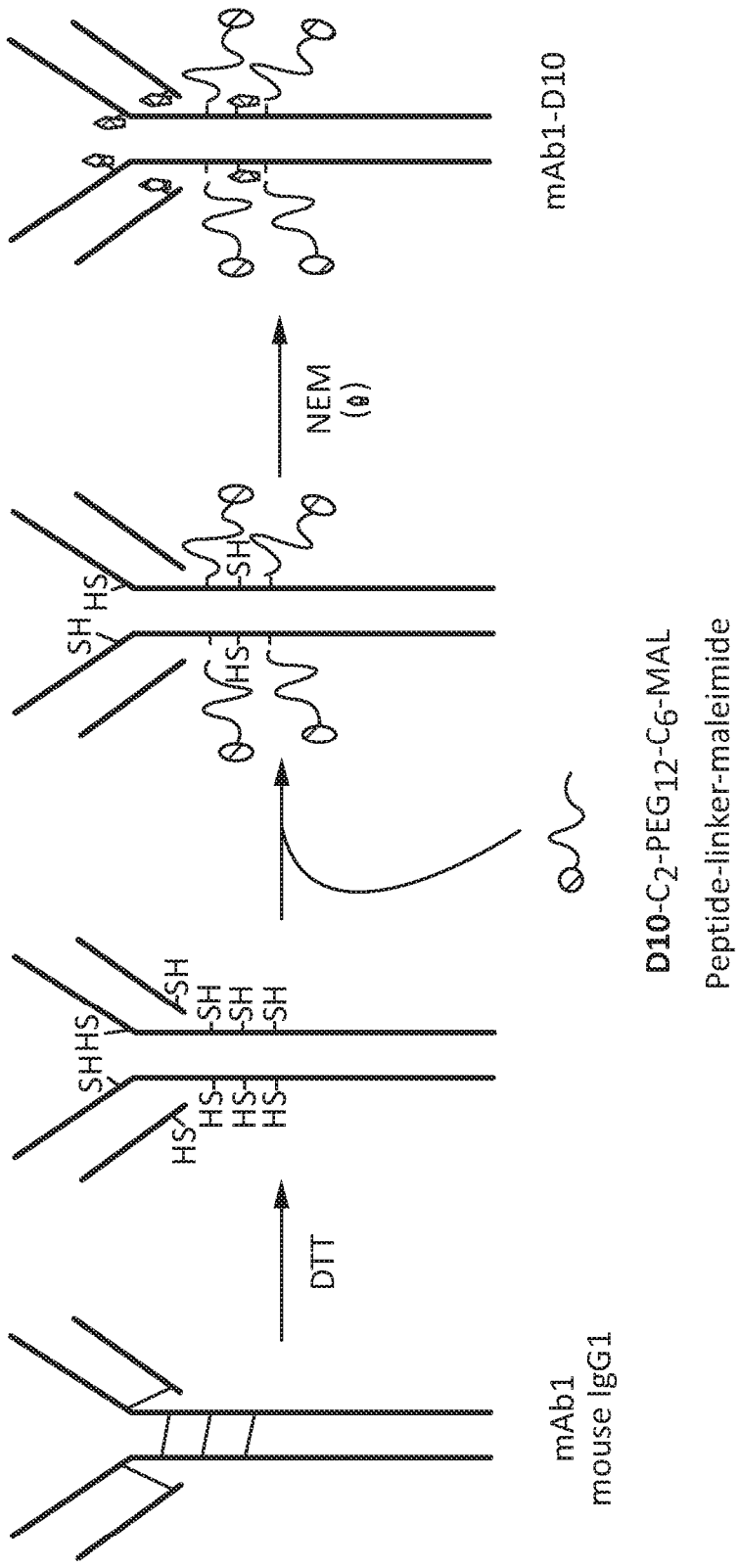


FIG. 2


mAb1
mouse IgG1
DTT
D10-C2-PEG12-C6-MAL
Peptide-linker-maleimide
NEM
(8)
mAb1-D10
SH
HS

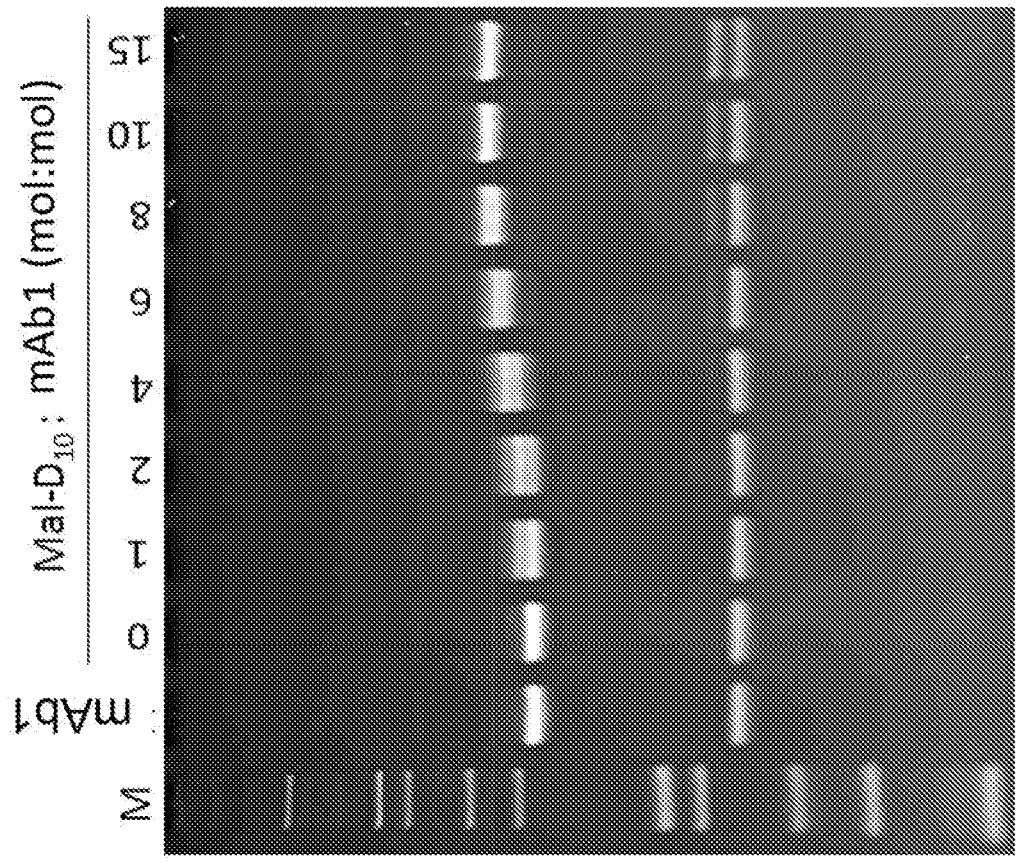


FIG. 3A

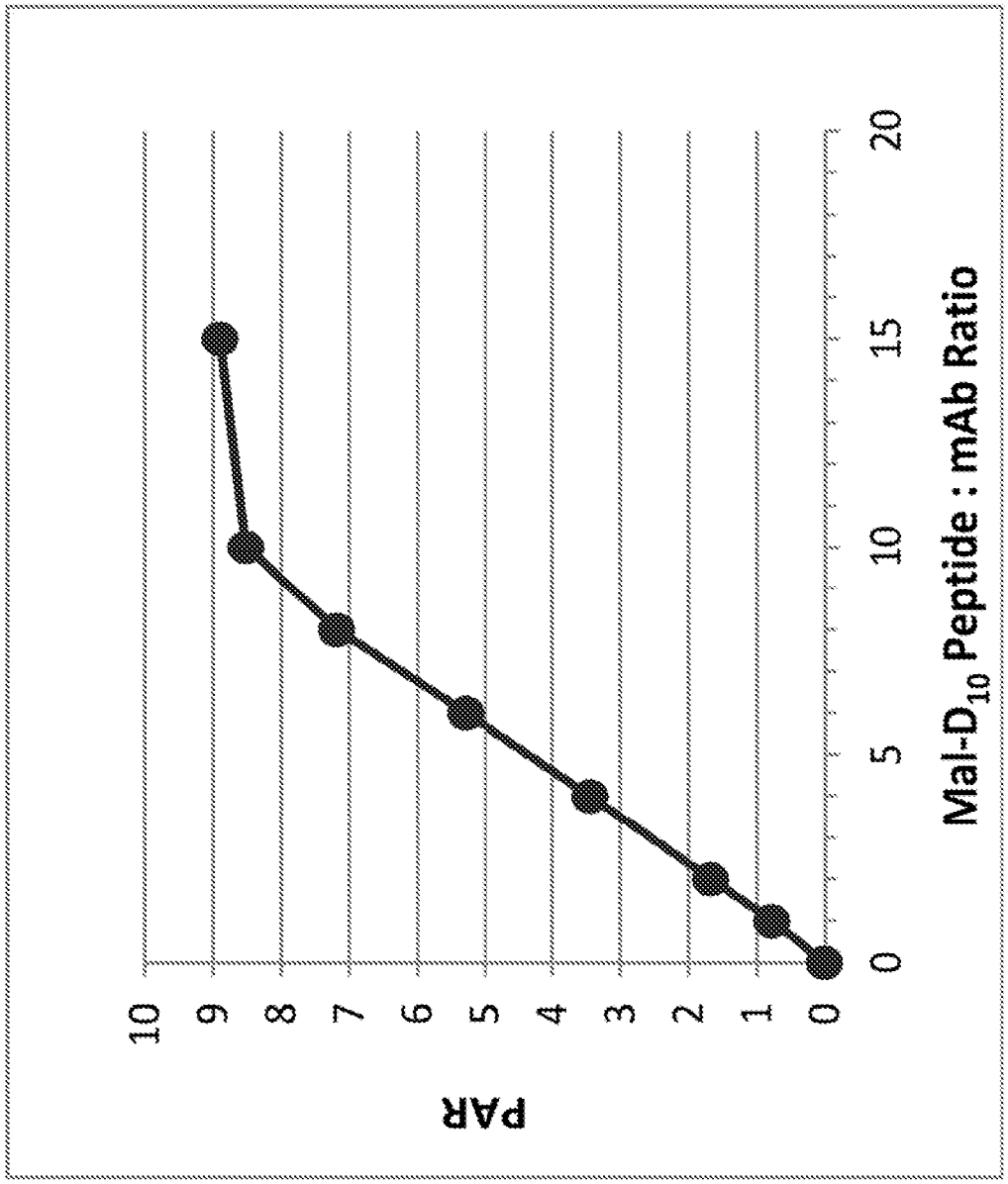


FIG. 3B

FIG. 4A

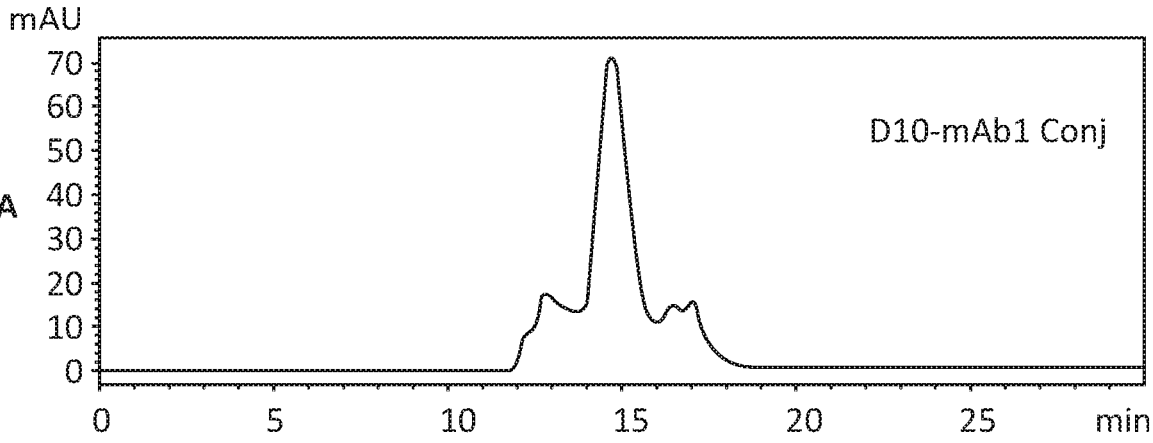


FIG. 4B

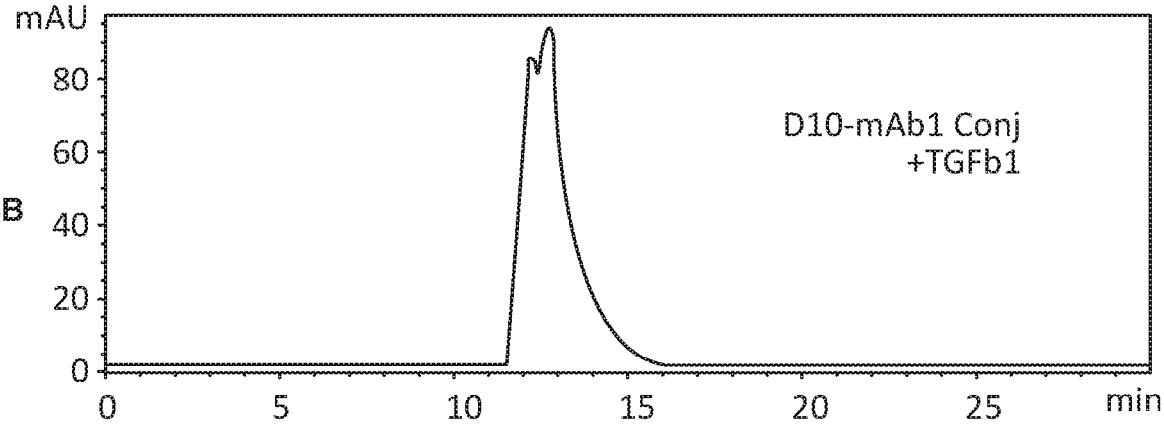


FIG. 4C

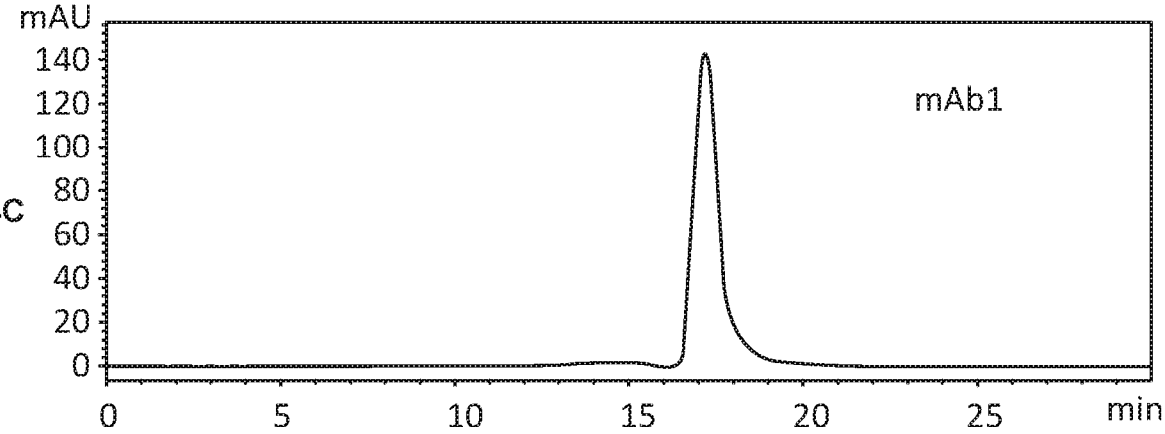


FIG. 4D

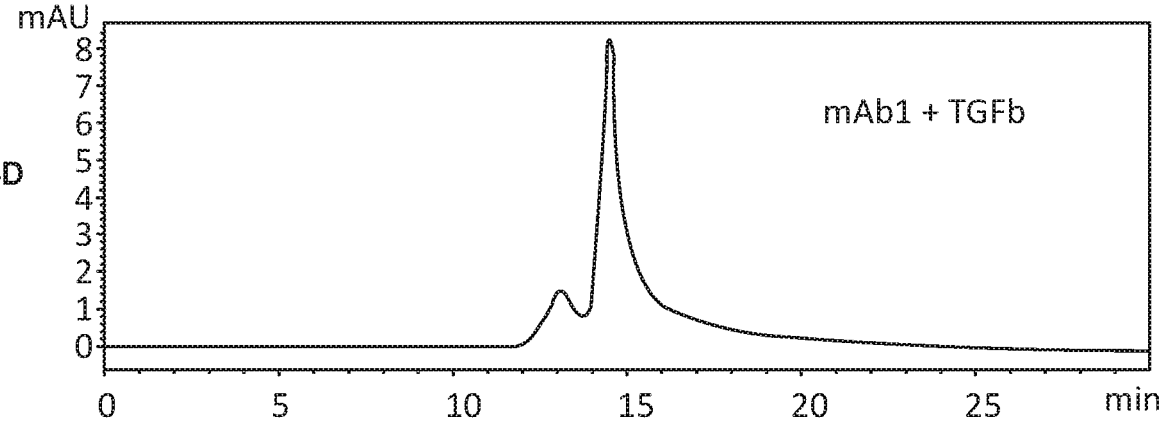


FIG. 4E

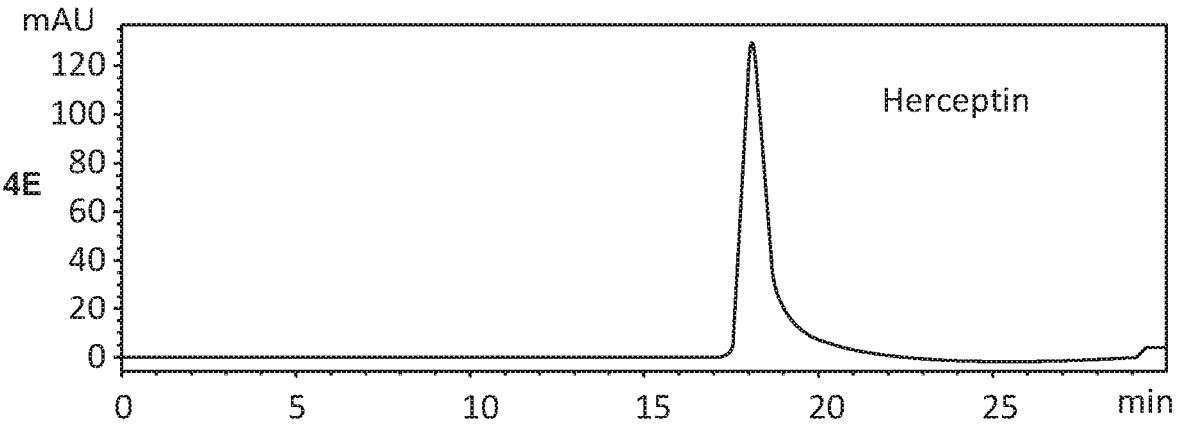


FIG. 4F

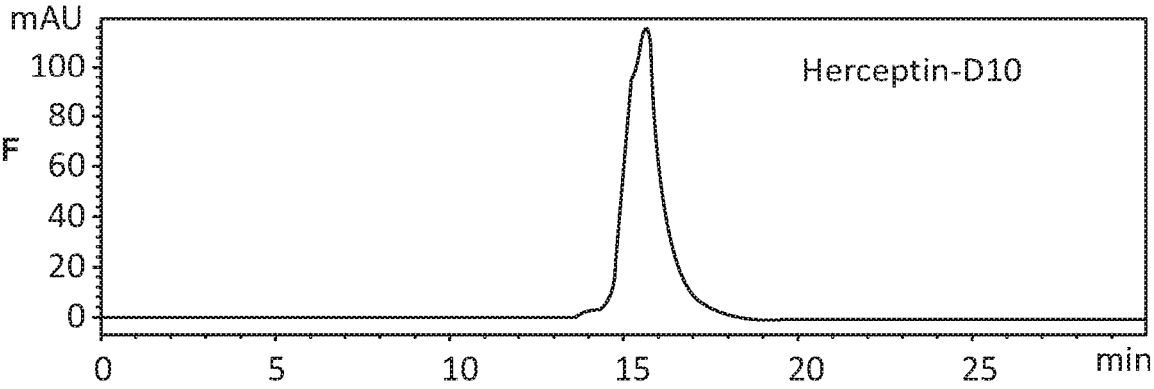
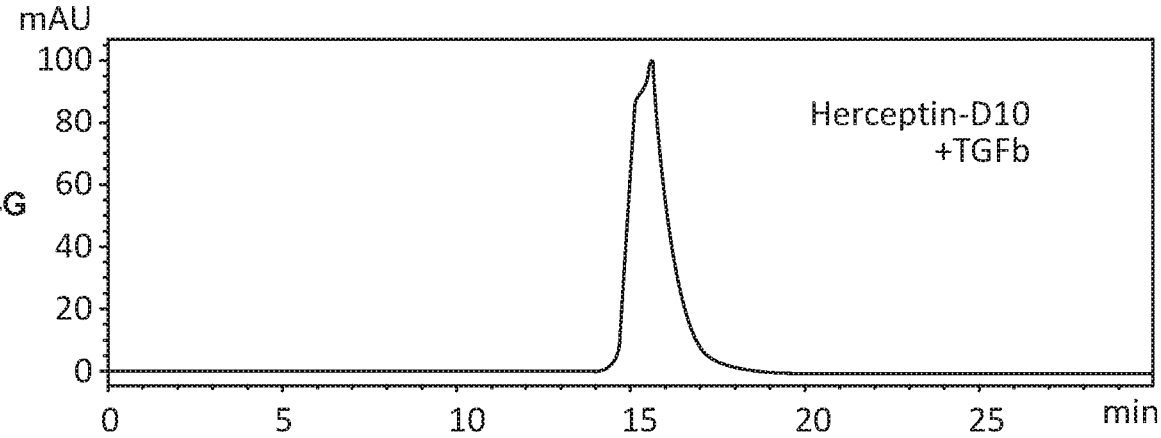


FIG. 4G



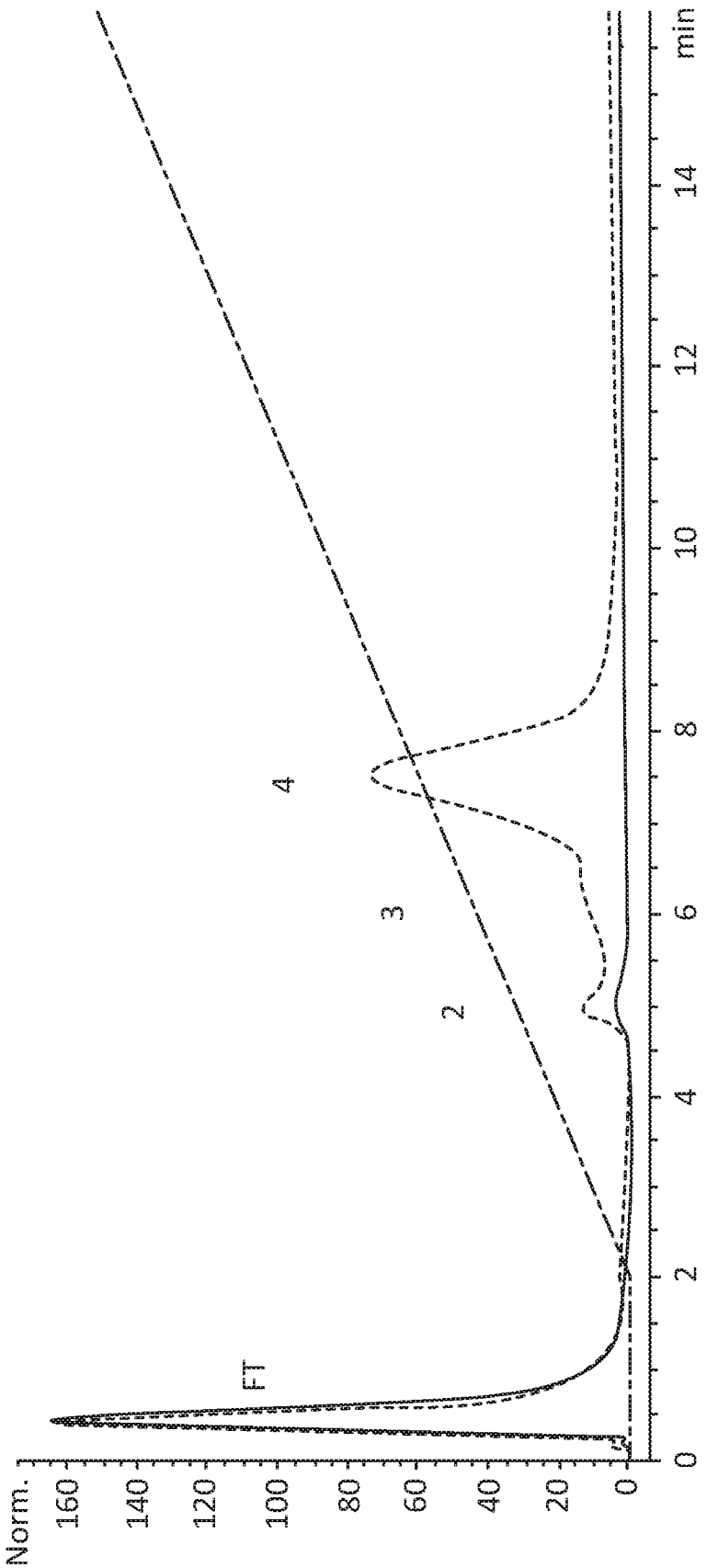


FIG. 5A

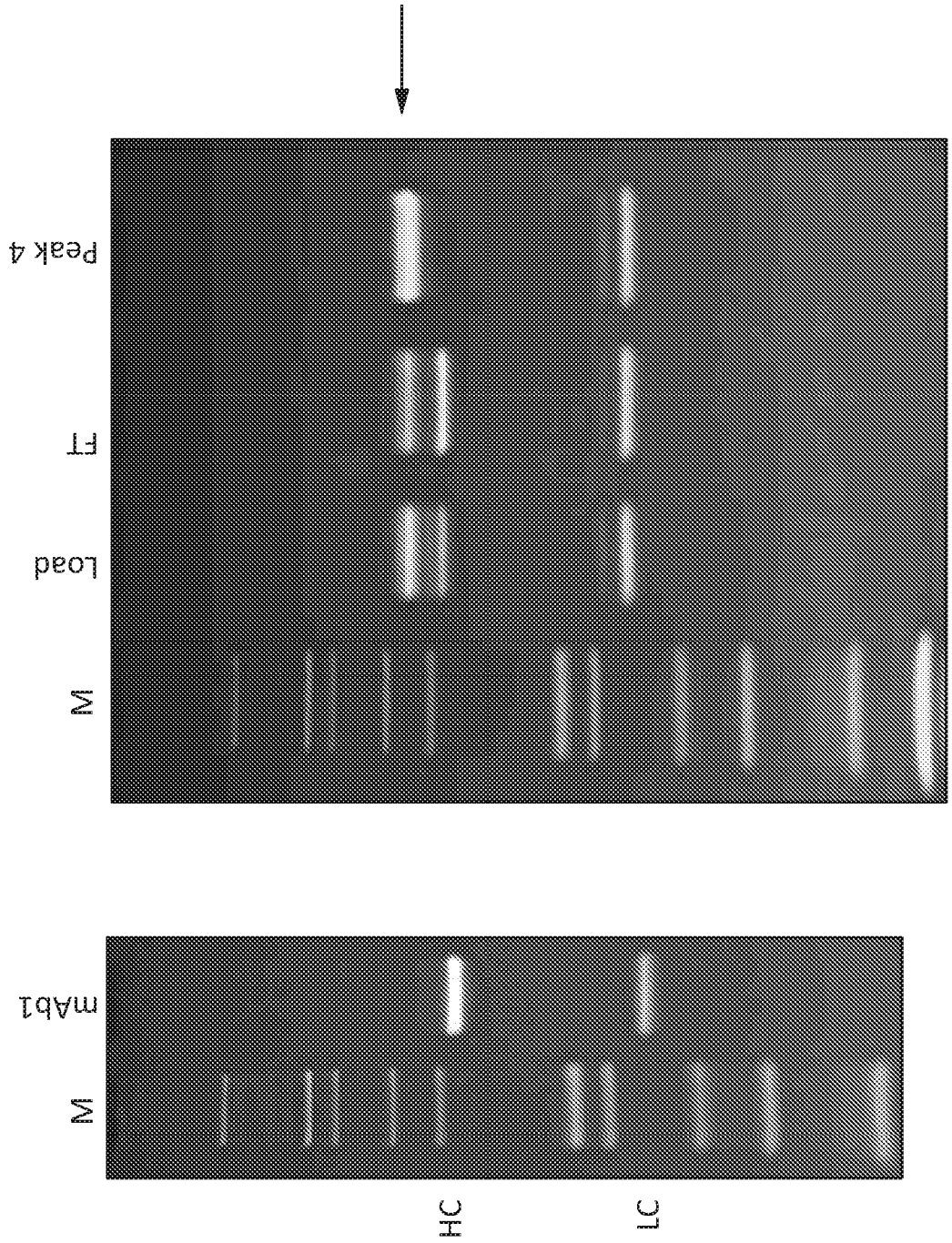
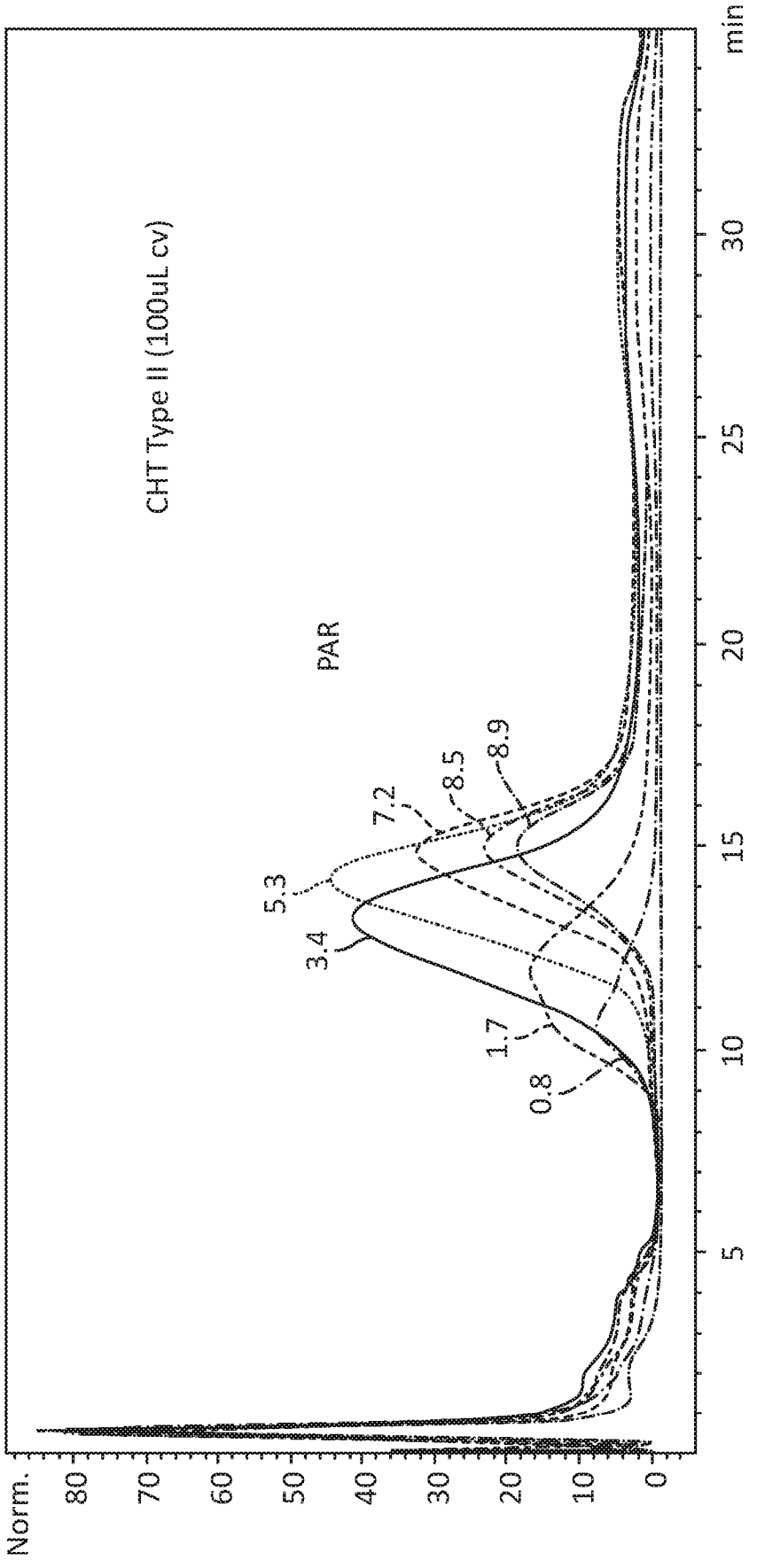


FIG. 5B

FIG. 6A


CHT Type II (100uL cv)
PAR
5.3
3.4
7.2
8.5
8.9
1.7
0.8
Norm.
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
min

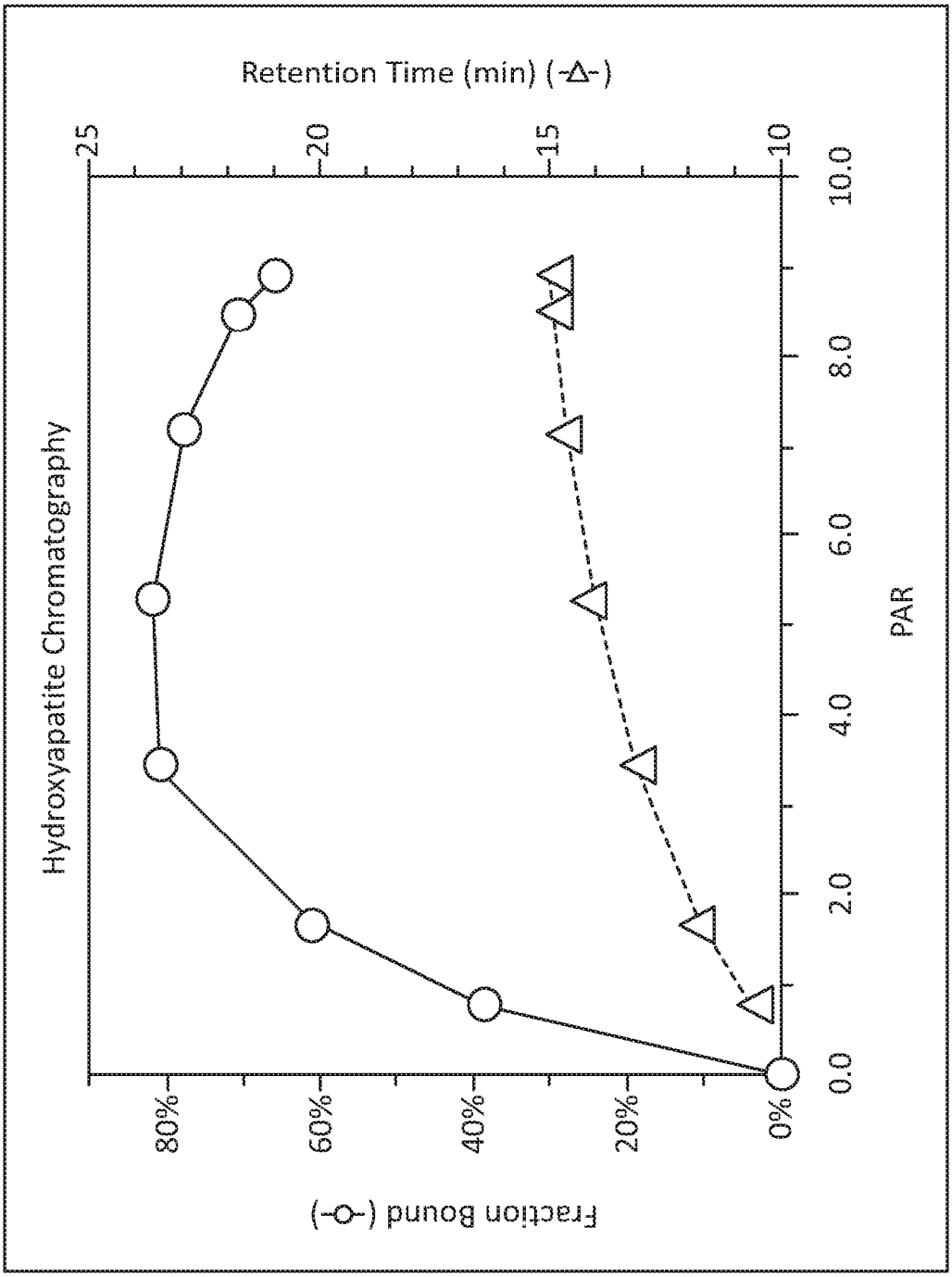

Hydroxyapatite Chromatography
Retention Time (min) (-Δ-)
25
20
15
10
Fraction Bound (-O-)
80%
60%
40%
20%
0%
0.0
2.0
4.0
6.0
8.0
10.0
PAR

FIG. 6B

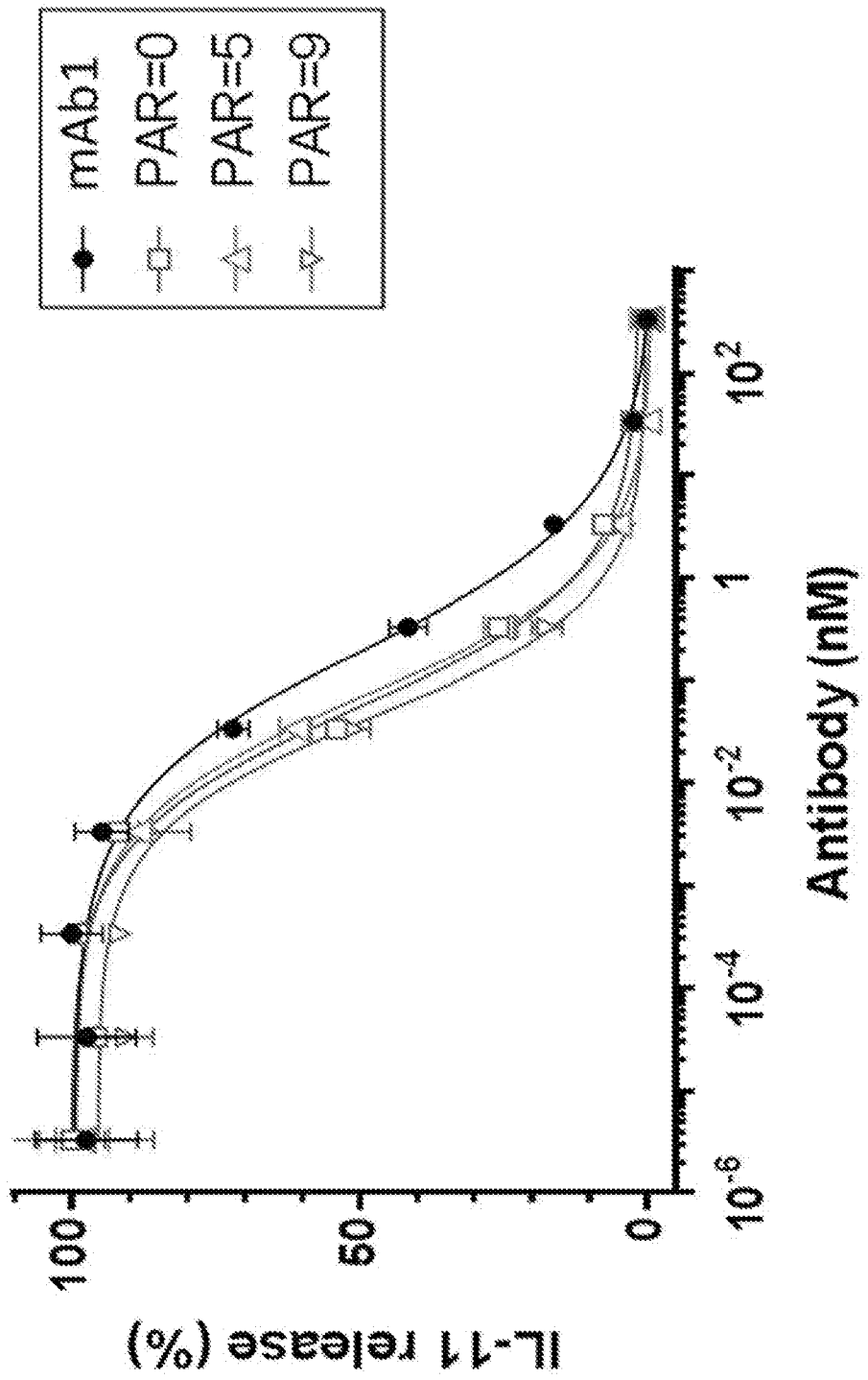
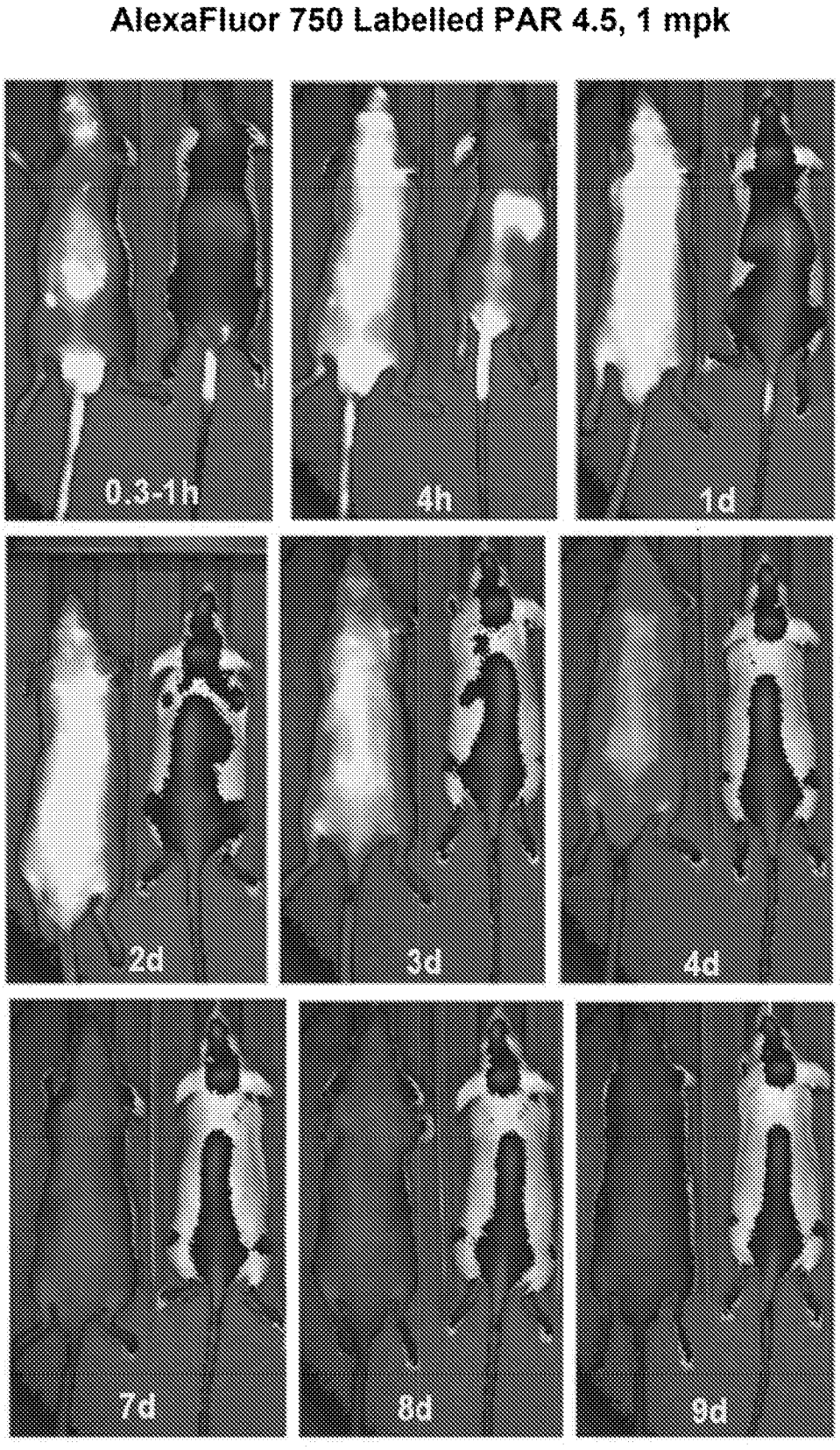

mAb1
PAR=0
PAR=5
PAR=9
IL-11 release (%)
100
50
0
10^{-6}
10^{-4}
10^{-2}
1
10^{2}
Antibody (nM)

FIG. 7

FIG. 8A


AlexaFluor 750 Labelled PAR 4.5, 1 mpk
0.3-1h
4h
1d
2d
3d
4d
7d
8d
9d

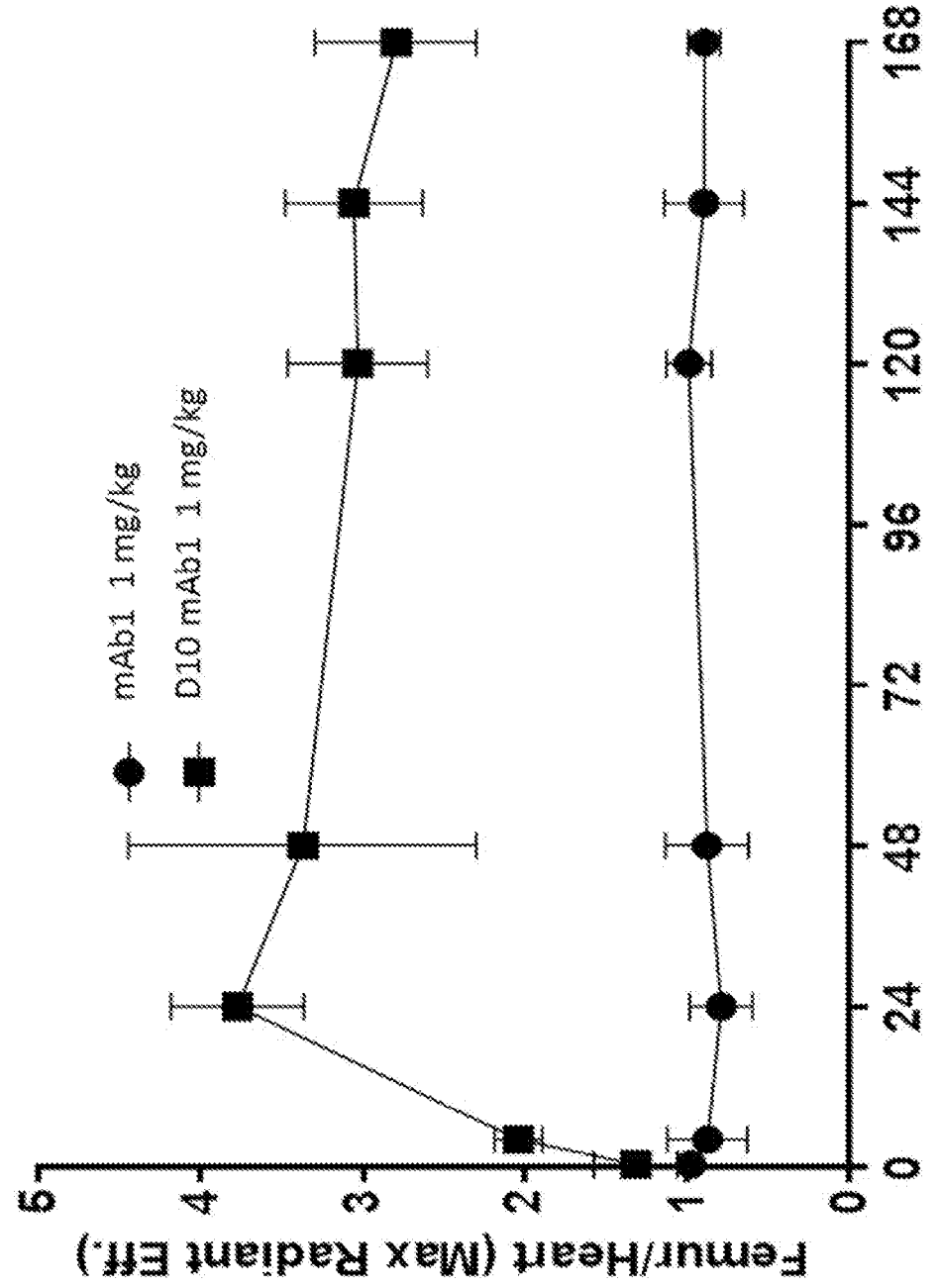
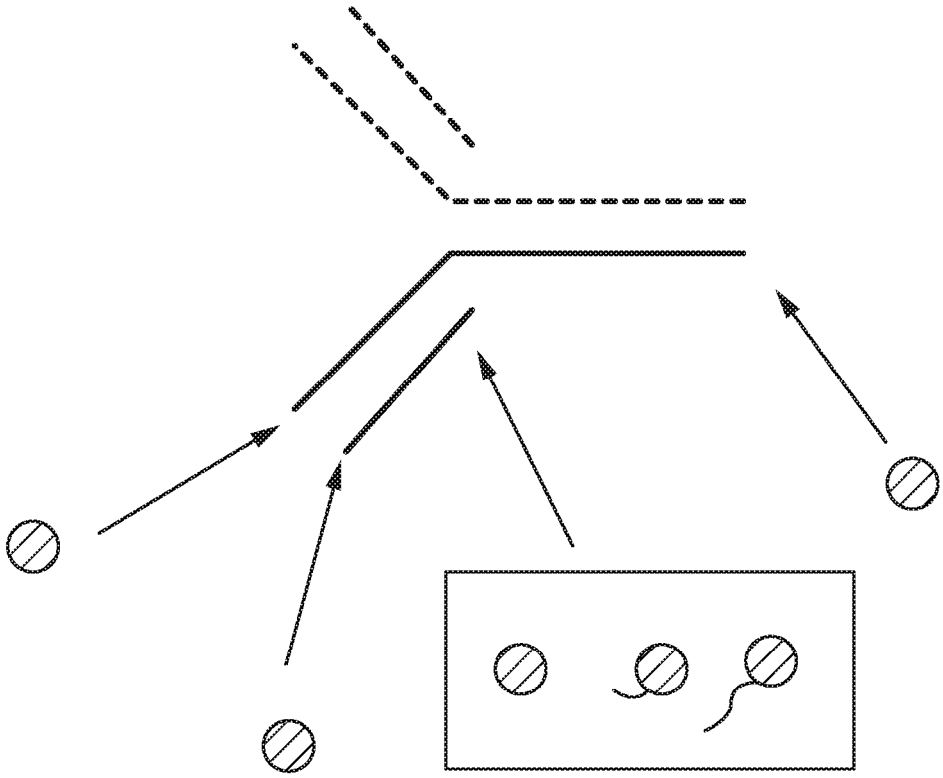

Bone:Blood Ratio
mAb1 1 mg/kg
D10 mAb1 1 mg/kg
Femur/Heart (Max Radiant Eff.)
0
1
2
3
4
5
0
24
48
72
96
120
144
168
Hours Post-Dose

FIG. 8B



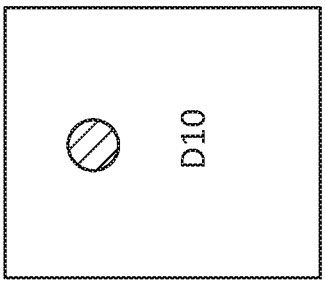


FIG. 9A

FIG. 9B

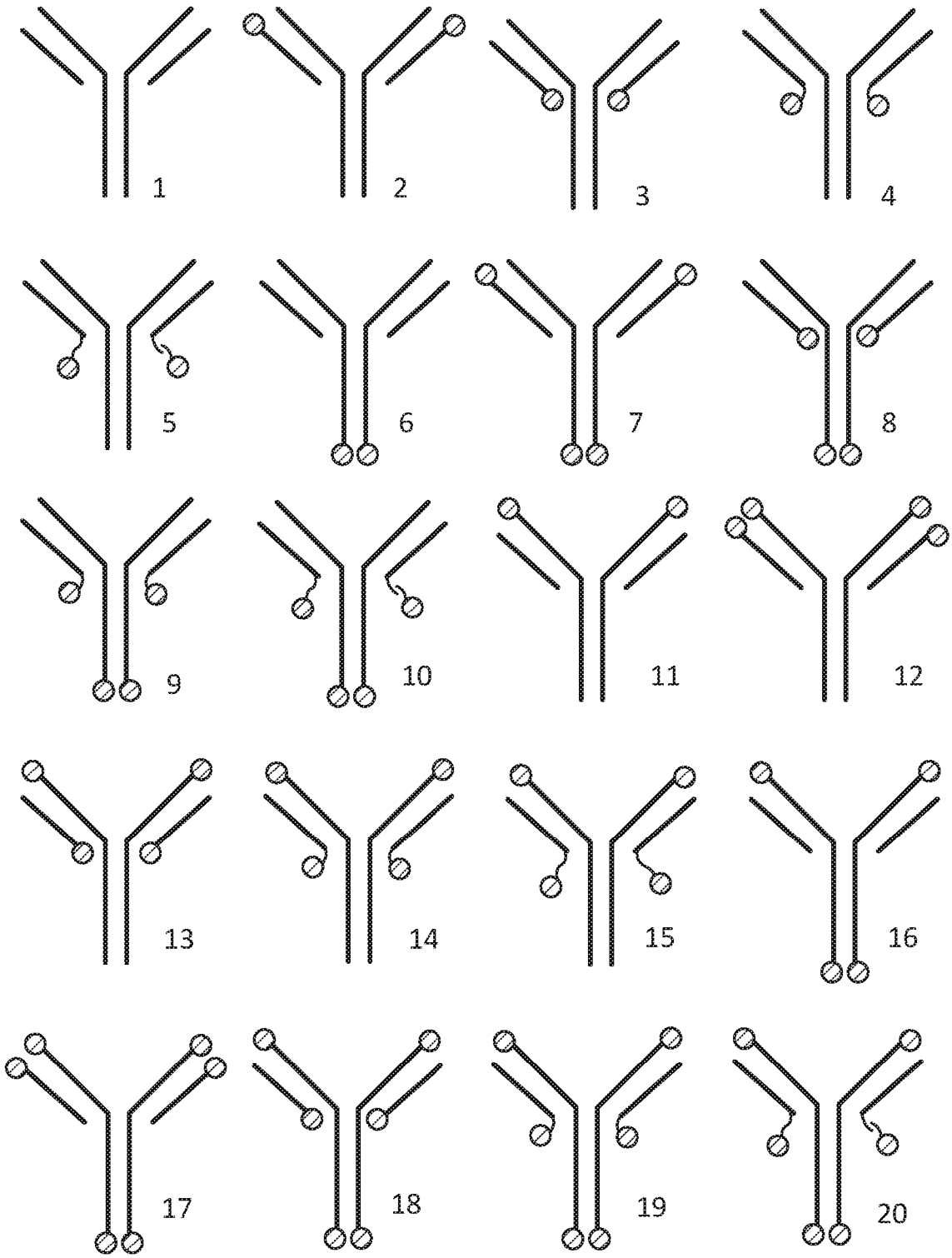
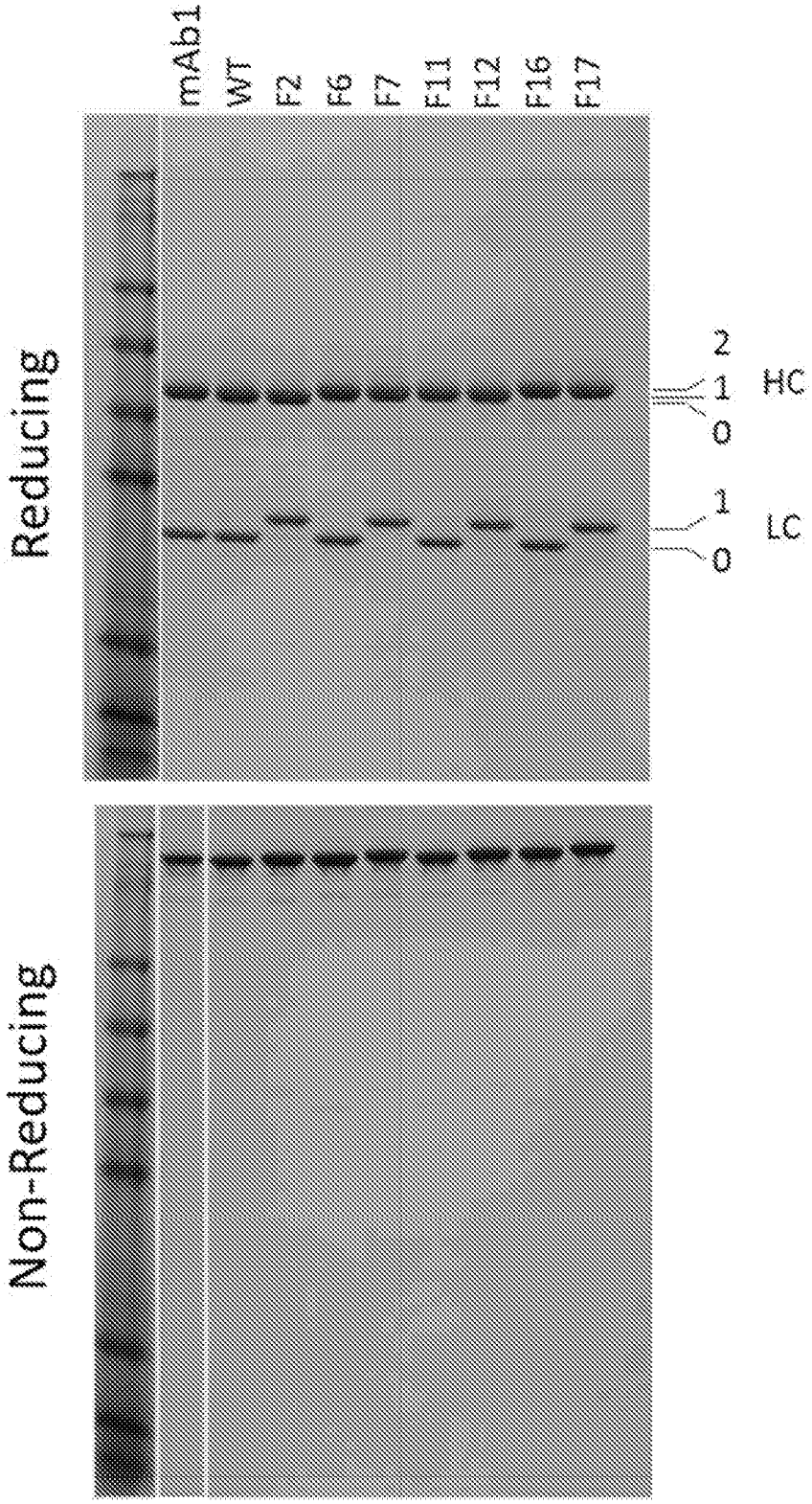


FIG. 10


mAb1
WT
F2
F6
F7
F11
F12
F16
F17
Reducing
2
1
0
HC
1
0
LC
Non-Reducing

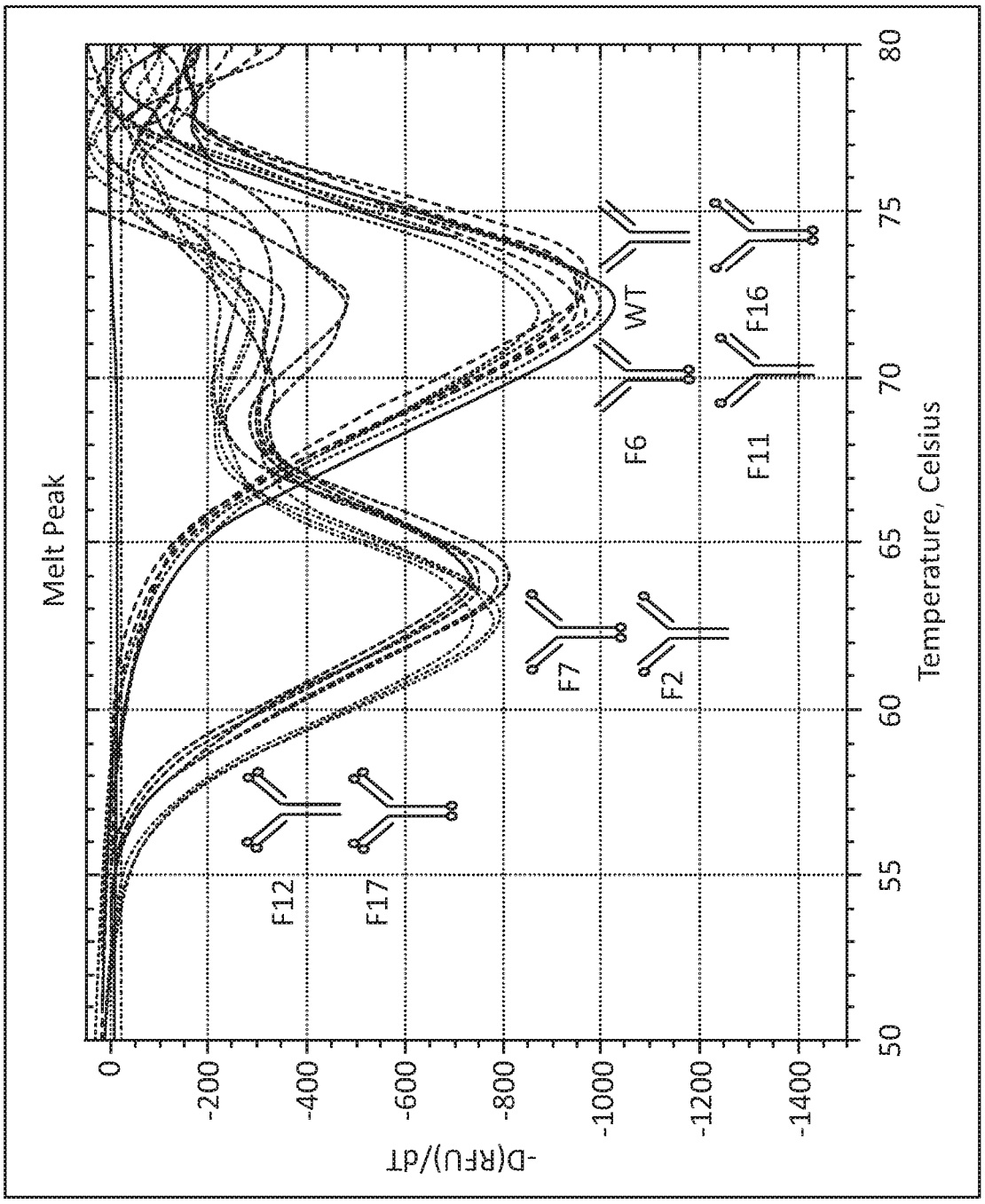


FIG. 11

SyPRO Orange; Conjugate (PAR 4.5) showed a broad Tm (65-72°)

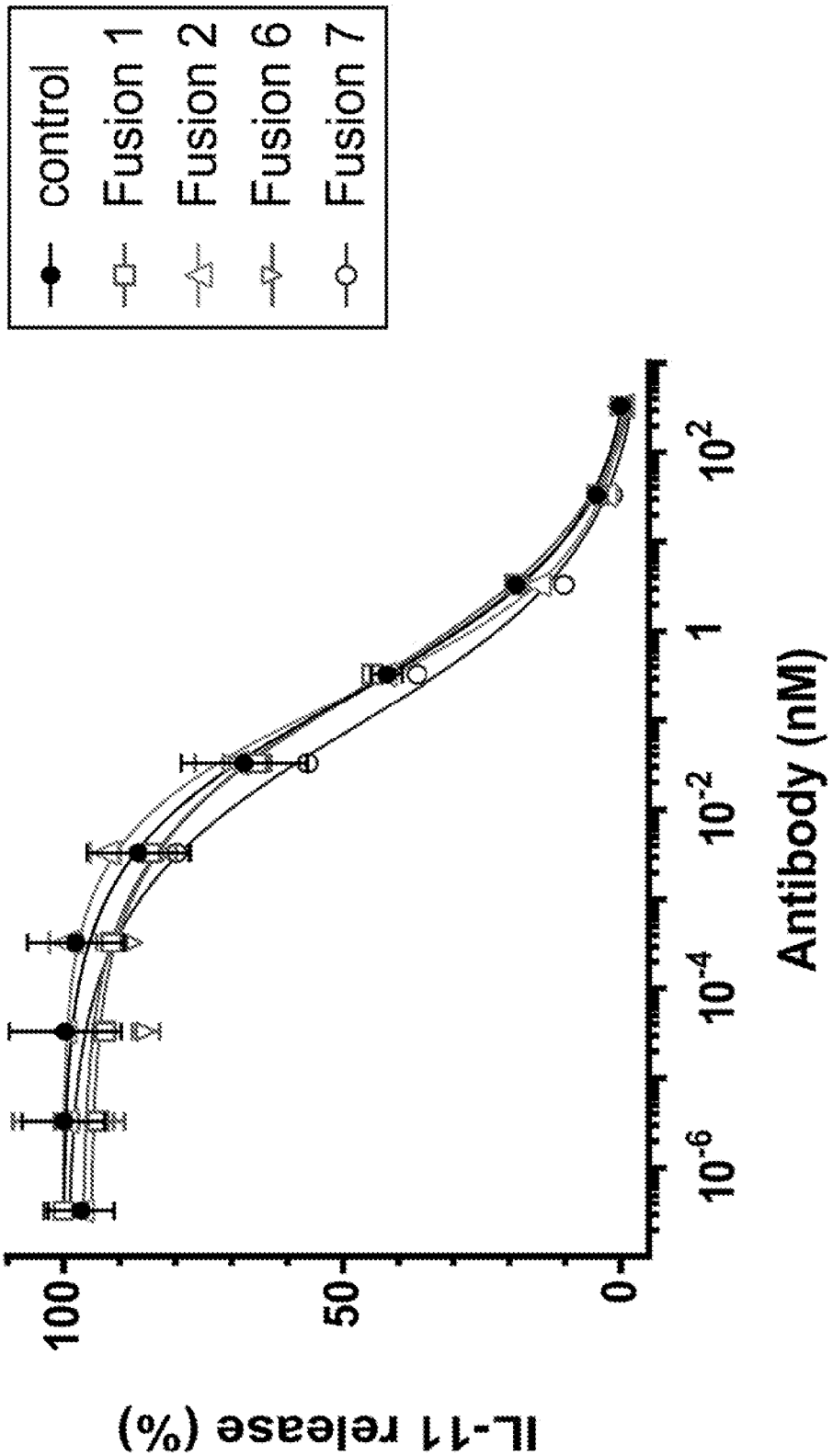

control
Fusion 1
Fusion 2
Fusion 6
Fusion 7
IL-11 release (%)
100
50
0
10^{-6}
10^{-4}
10^{-2}
1
10^{2}
Antibody (nM)

FIG. 12A

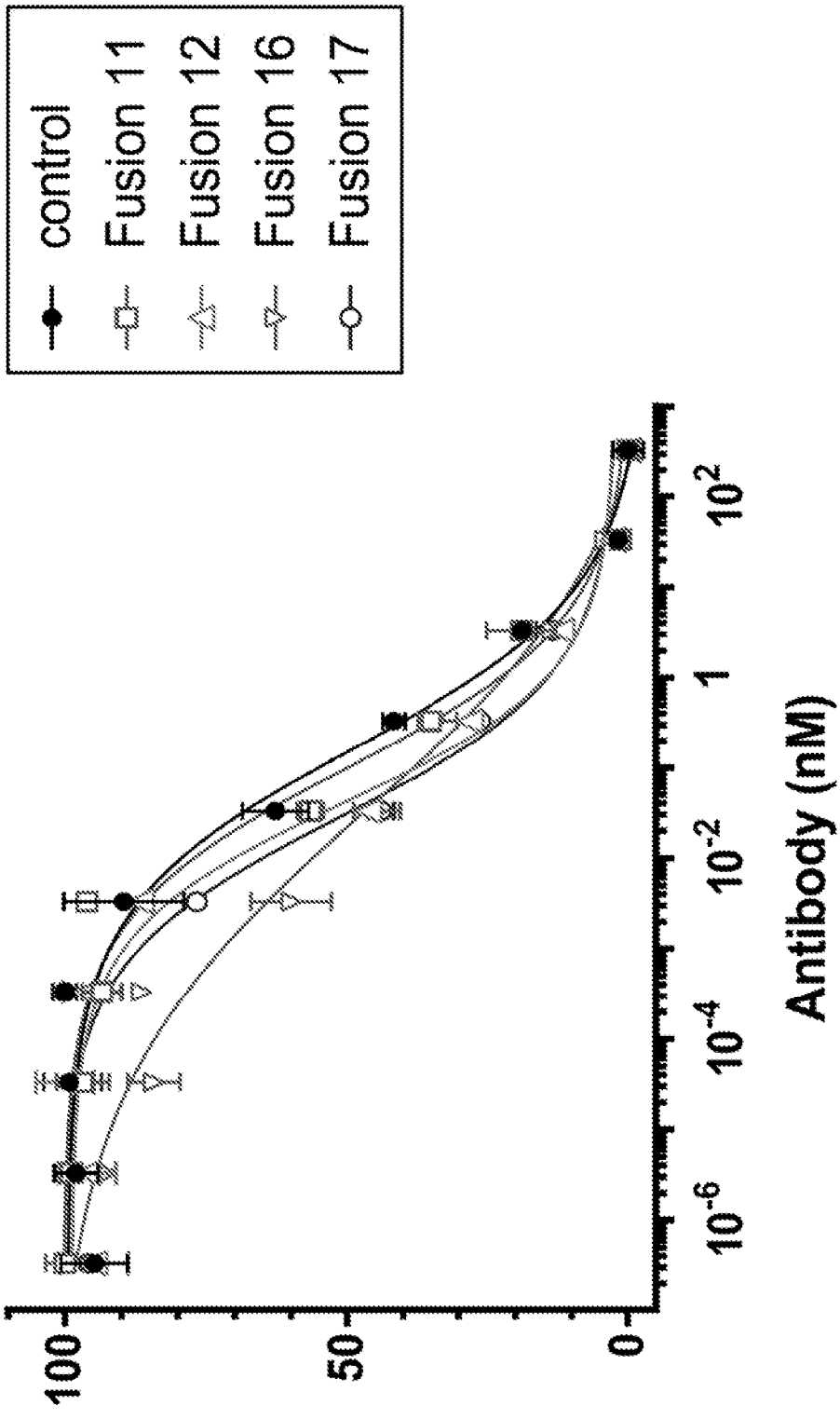

control
Fusion 11
Fusion 12
Fusion 16
Fusion 17
IL-11 release (%)
100
50
0
10^{-6}
10^{-4}
10^{-2}
1
10^{2}
Antibody (nM)

FIG. 12B

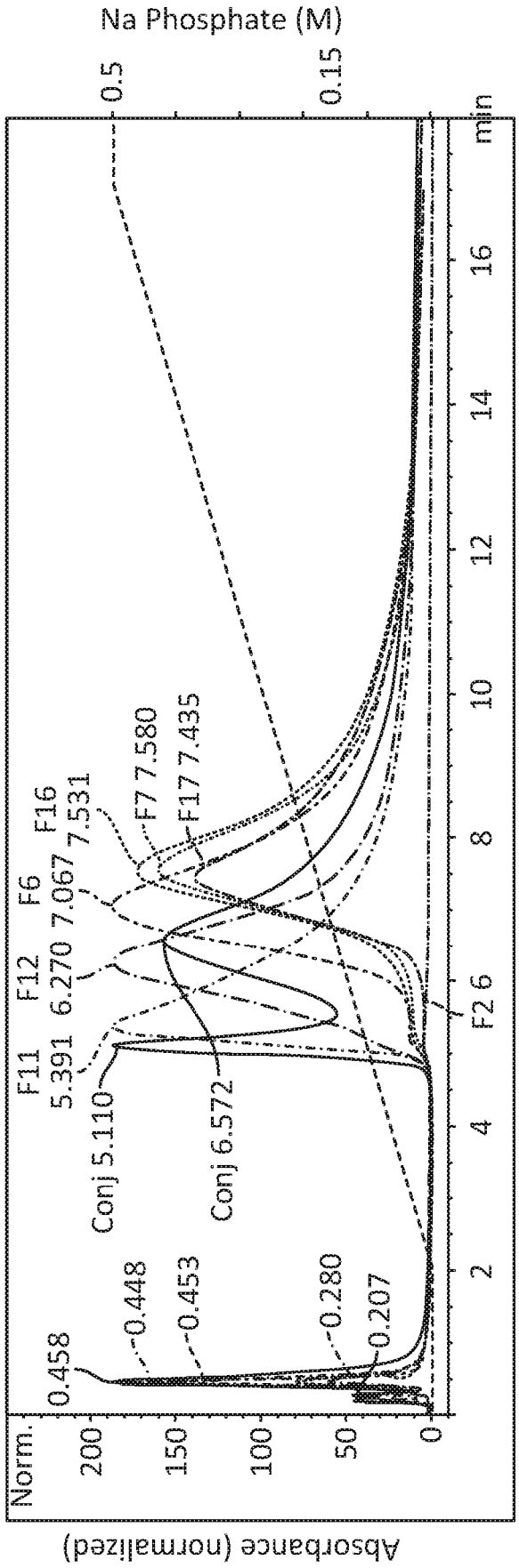
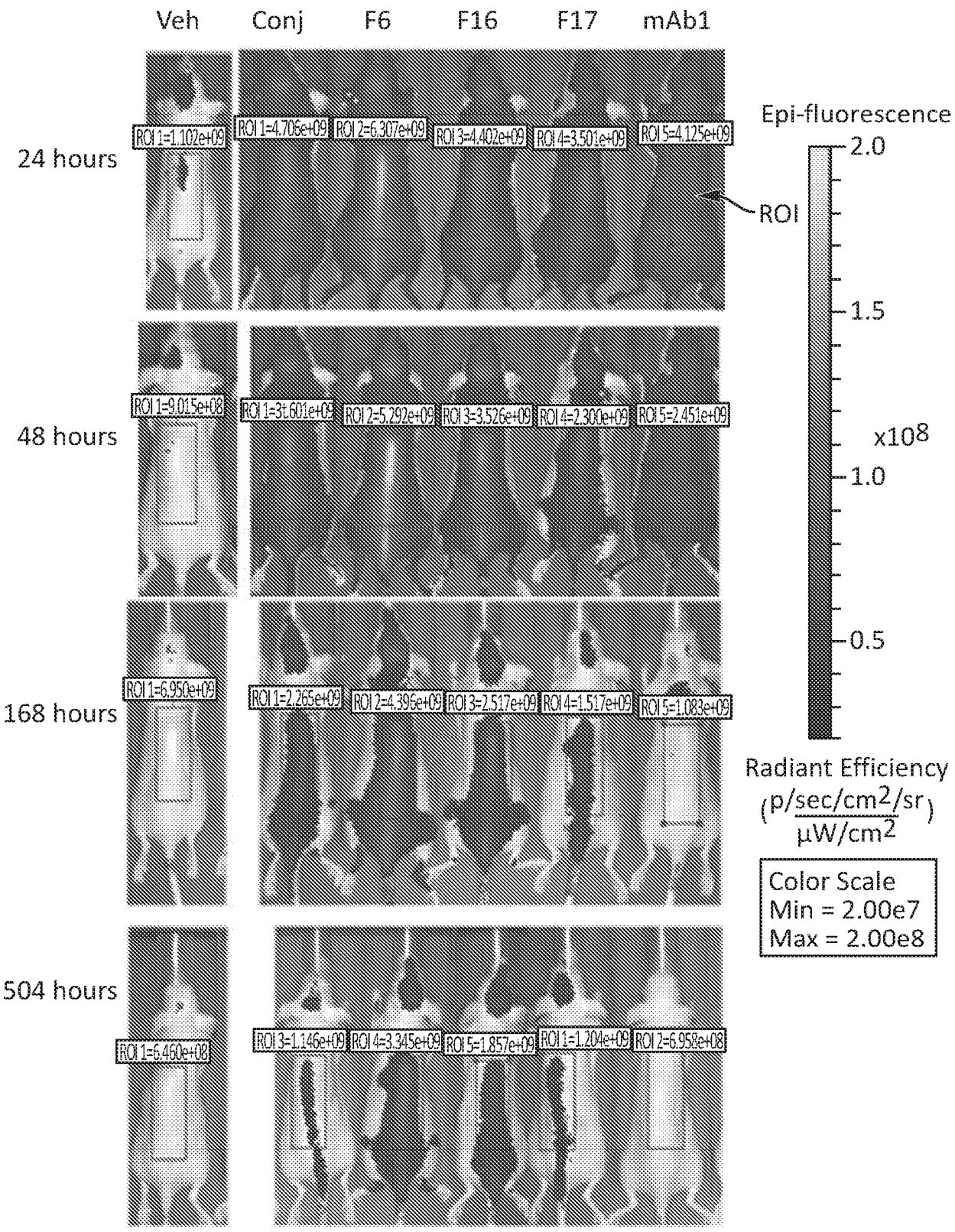

Na Phosphate (M)
0.5
0.15
min
16
14
12
10
8
6
4
2
Retention Time (min)
F2
F16 7.531
F7 7.580
F17 7.435
F6 7.067
F12 6.270
F11 5.391
Conj 5.110
Conj 6.572
0.458
0.448
0.453
0.280
0.207
Norm.
200
150
100
50
0
Absorbance (normalized)

FIG. 13

FIG. 14


Veh
Conj
F6
F16
F17
mAb1
24 hours
48 hours
168 hours
504 hours
ROI 1=1.102e+09
ROI 1=4.706e+09
ROI 2=6.307e+09
ROI 3=4.402e+09
ROI 4=3.501e+09
ROI 5=4.125e+09
ROI 1=9.015e+08
ROI 1=3t.601e+0
ROI 2=5.292e+09
ROI 3=3.526e+09
ROI 4=2.300e+09
ROI 5=2.451e+09
ROI 1=6.950e+09
ROI 1=2.265e+09
ROI 2=4.396e+09
ROI 3=2.517e+09
ROI 4=1.517e+09
ROI 5=1.083e+09
ROI 1=6.460e+08
ROI 3=1.146e+09
ROI 4=3.345e+09
ROI 5=1.857e+09
ROI 1=1.204e+09
ROI 2=6.958e+08
Epi-fluorescence
ROI
2.0
1.5
x10^8
1.0
0.5
Radiant Efficiency
(p/sec/cm2/sr / µW/cm2)
Color Scale
Min = 2.00e7
Max = 2.00e8

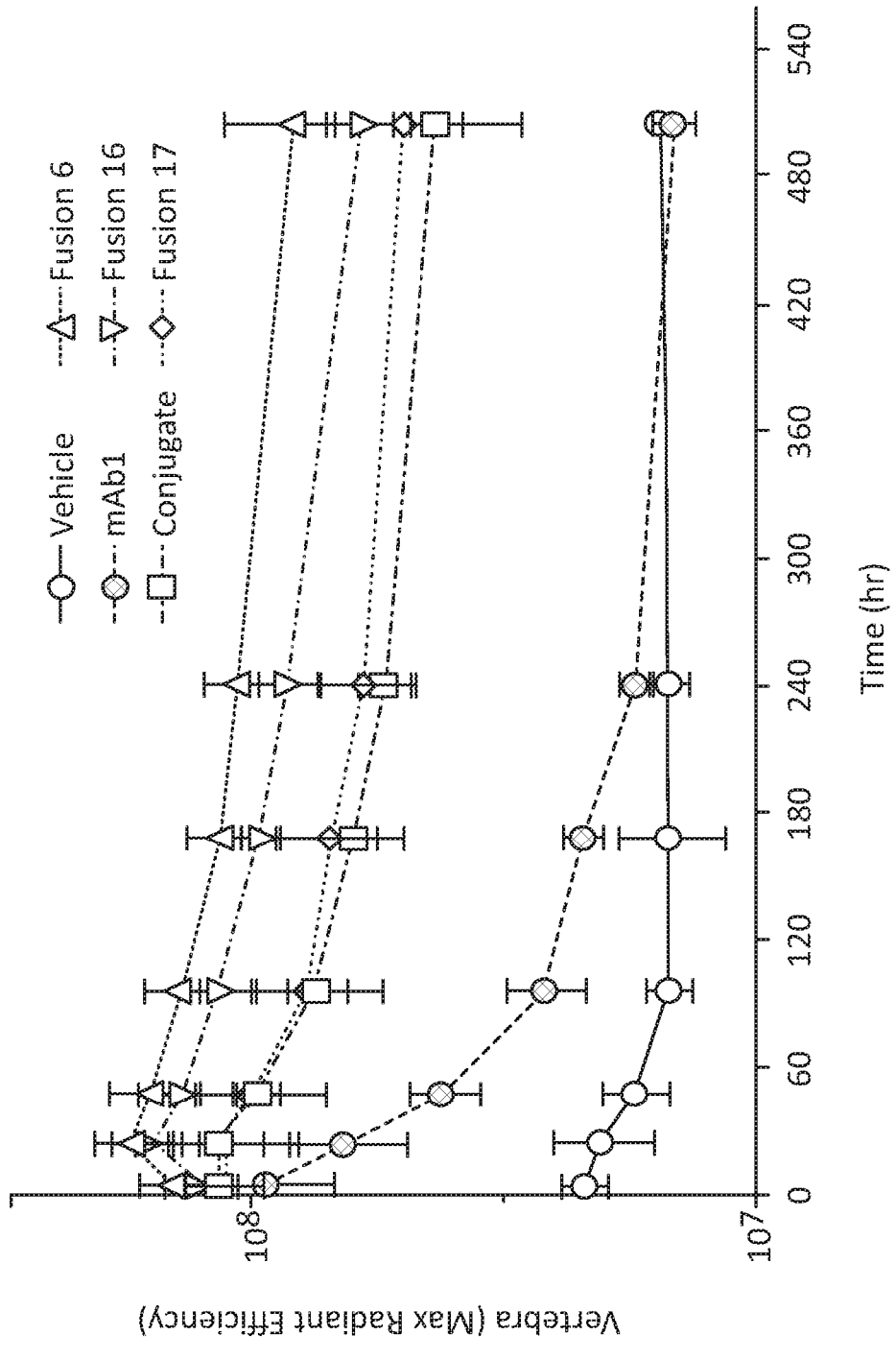

Vehicle
mAb1
Conjugate
Fusion 6
Fusion 16
Fusion 17
Vertebra (Max Radiant Efficiency)
10^8
10^7
0
60
120
180
240
300
360
420
480
540
Time (hr)

FIG. 15

FIG. 16

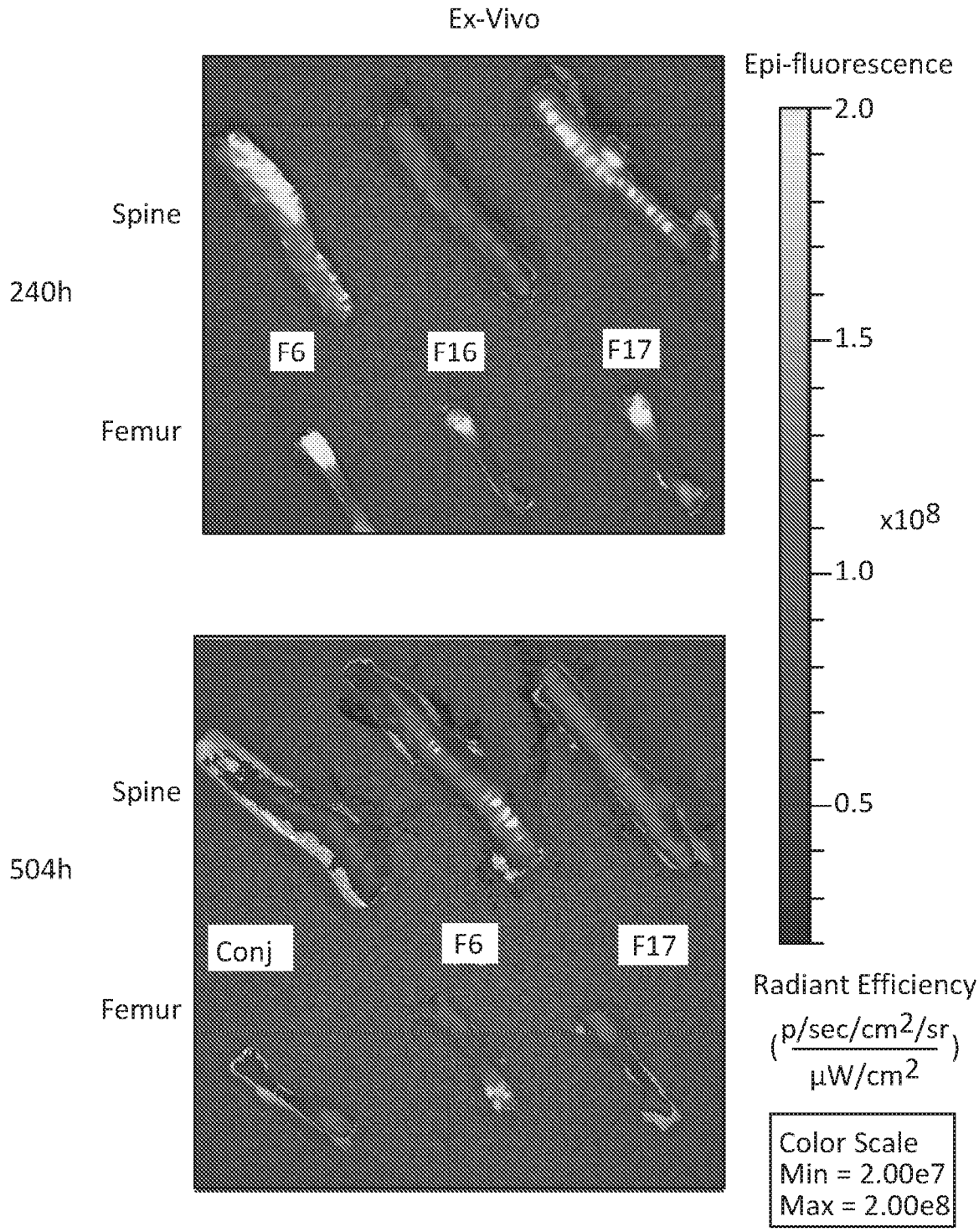

Ex-Vivo
Epi-fluorescence
2.0
1.5
1.0
0.5
x10^8
Spine
240h
Femur
F6
F16
F17
Spine
504h
Femur
Conj
F6
F17
Radiant Efficiency
(p/sec/cm2/sr / µW/cm2)
Color Scale
Min = 2.00e7
Max = 2.00e8

FIG. 17A

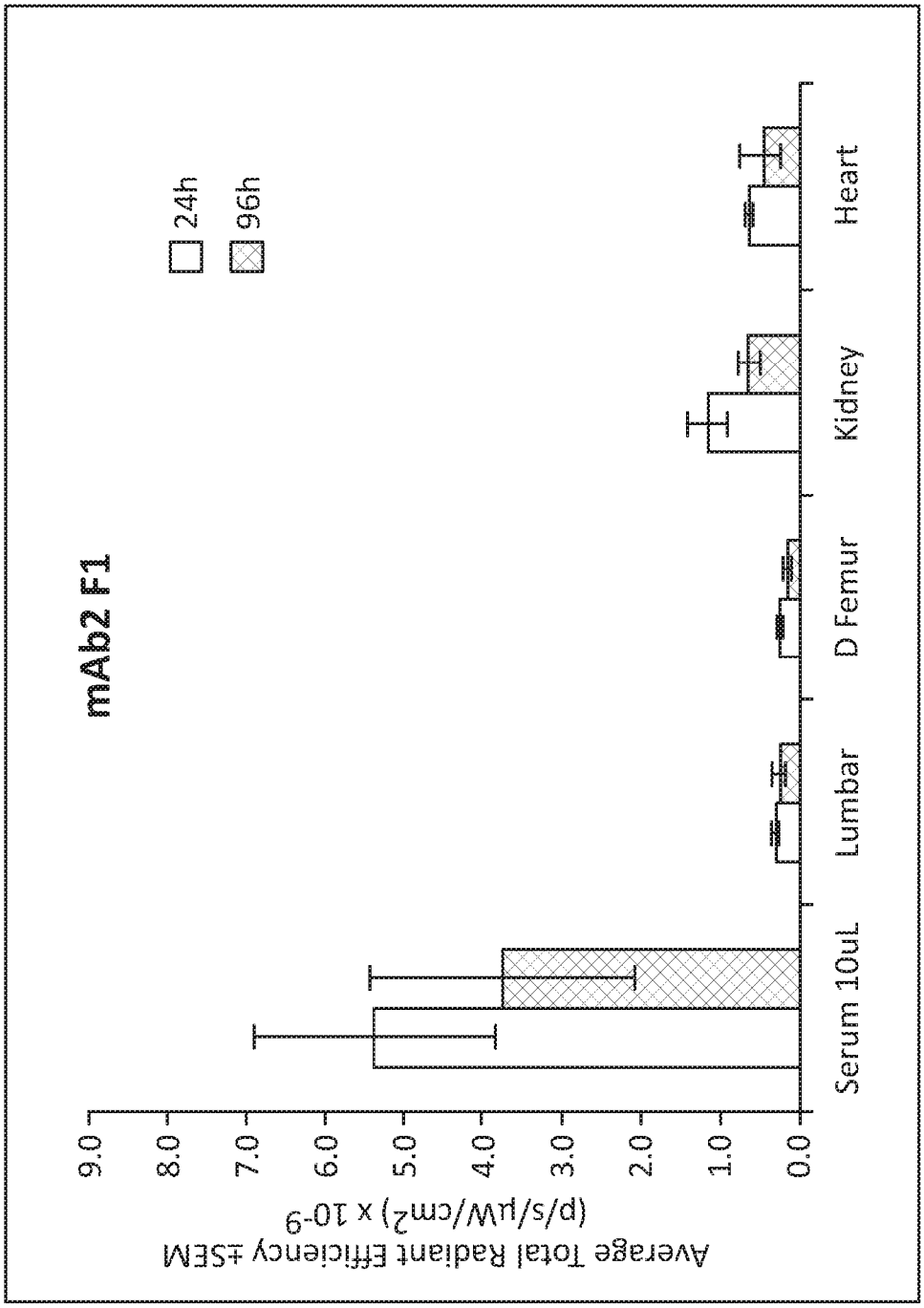
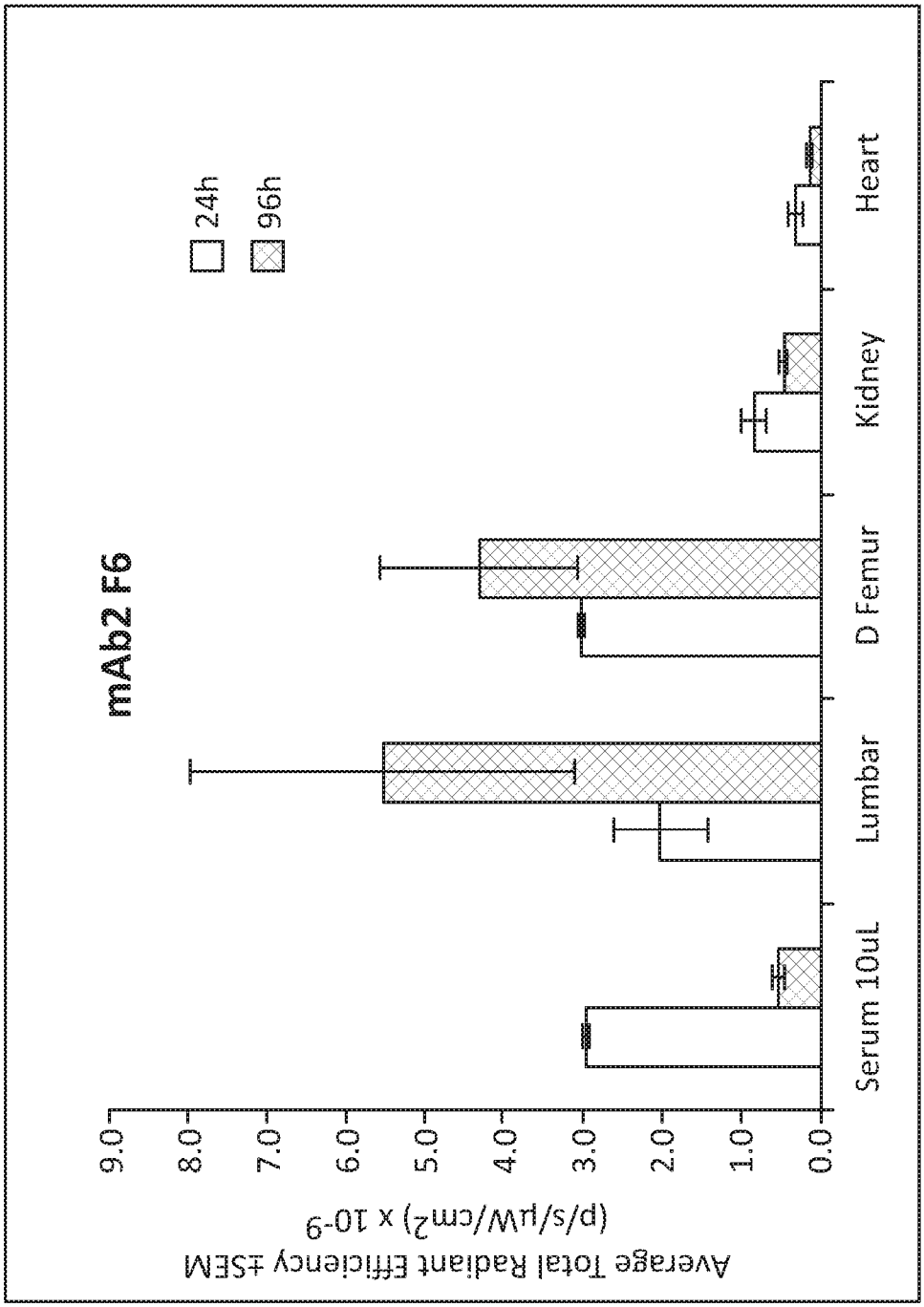

mAb2 F1
24h
96h
Average Total Radiant Efficiency ±SEM
(p/s/μW/cm2) x 10-9
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Serum 10uL
Lumbar
D Femur
Kidney
Heart

FIG. 17B


mAb2 F6
24h
96h
Average Total Radiant Efficiency ±SEM
(p/s/μW/cm2) x 10-9
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Serum 10uL
Lumbar
D Femur
Kidney
Heart

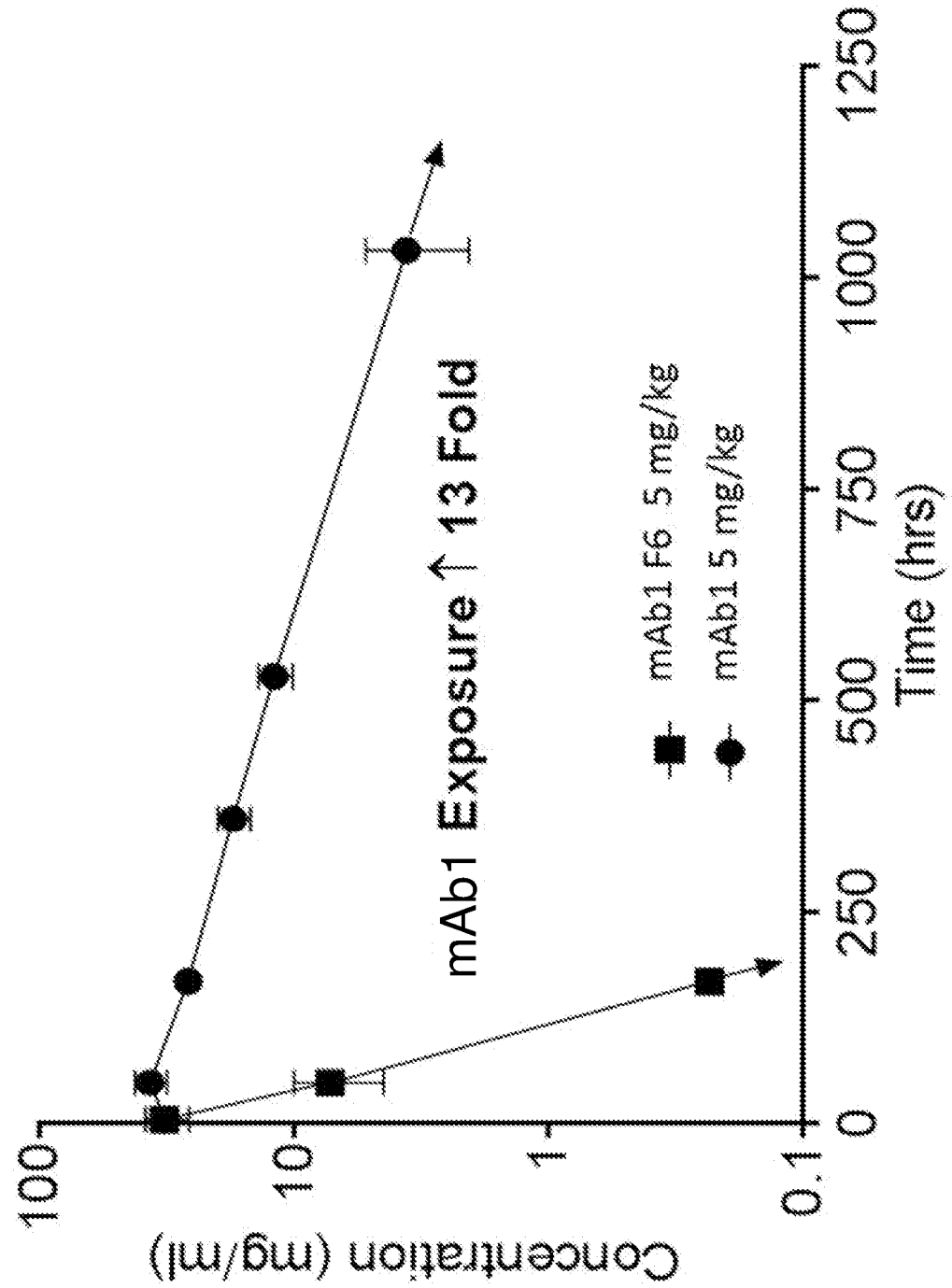

Concentration (mg/ml)
100
10
1
0.1
0
250
500
750
1000
1250
Time (hrs)
mAb1 Exposure ↑ 13 Fold
mAb1 F6 5 mg/kg
mAb1 5 mg/kg

FIG. 18A

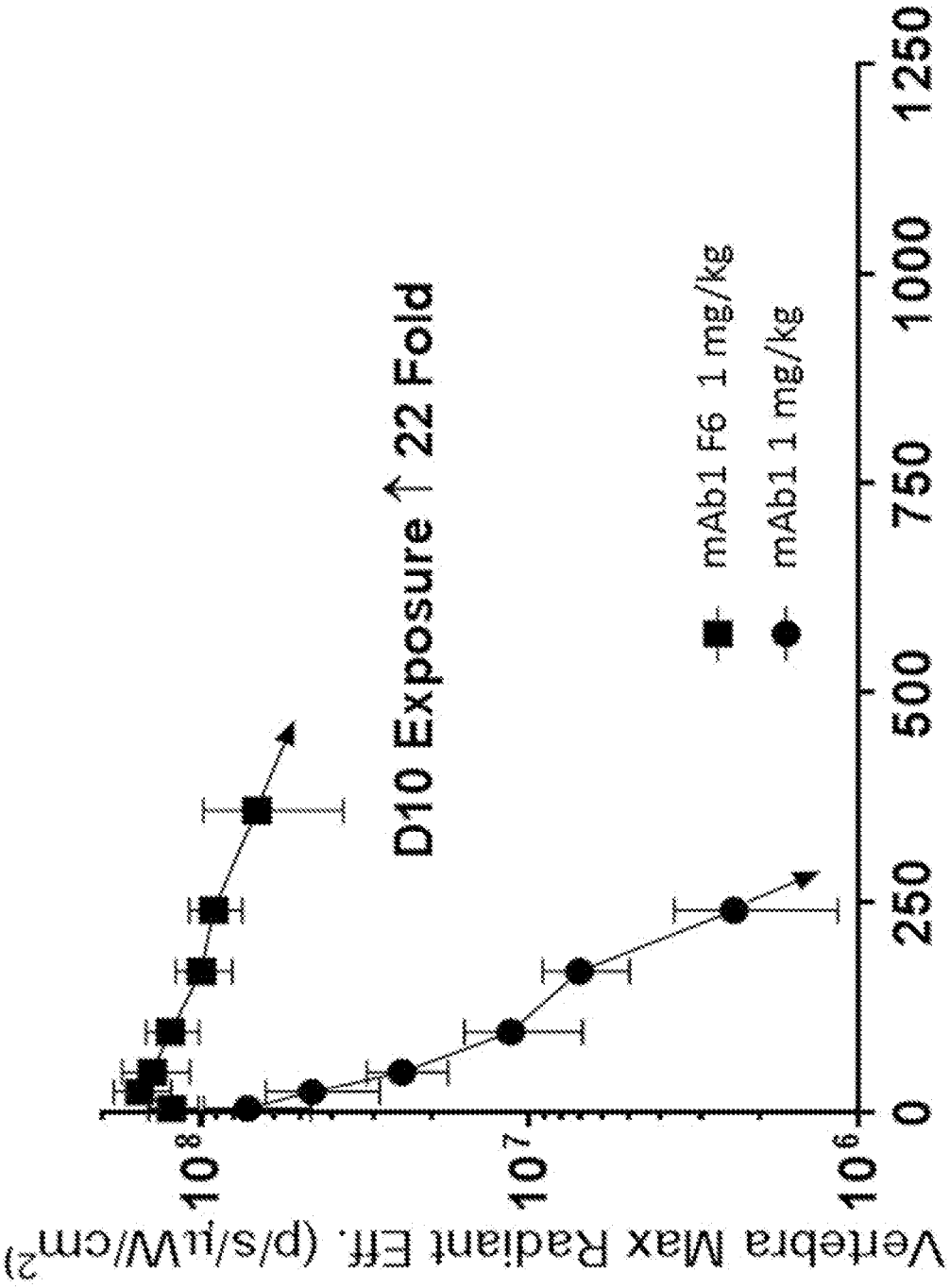

Vertebra Max Radiant Eff. (p/s/μW/cm²)
10⁸
10⁷
10⁶
0
250
500
750
1000
1250
Time (hrs)
D10 Exposure ↑ 22 Fold
mAb1 F6 1 mg/kg
mAb1 1 mg/kg

FIG. 18B

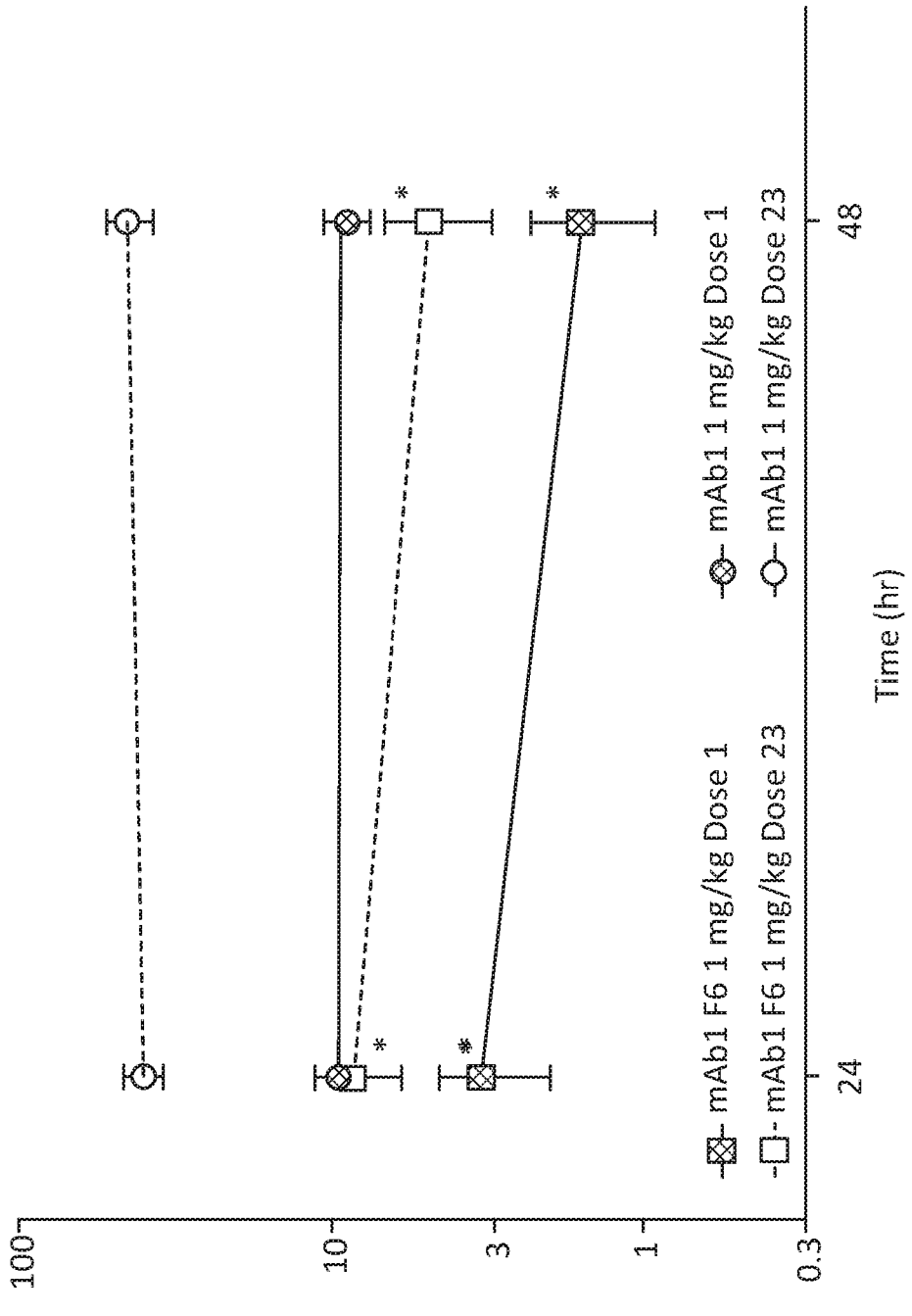

100
10
3
1
0.3
Concentration (µg/ml)
24
48
Time (hr)
mAb1 F6 1 mg/kg Dose 1
mAb1 F6 1 mg/kg Dose 23
mAb1 1 mg/kg Dose 1
mAb1 1 mg/kg Dose 23
*
*
*
*

FIG. 19

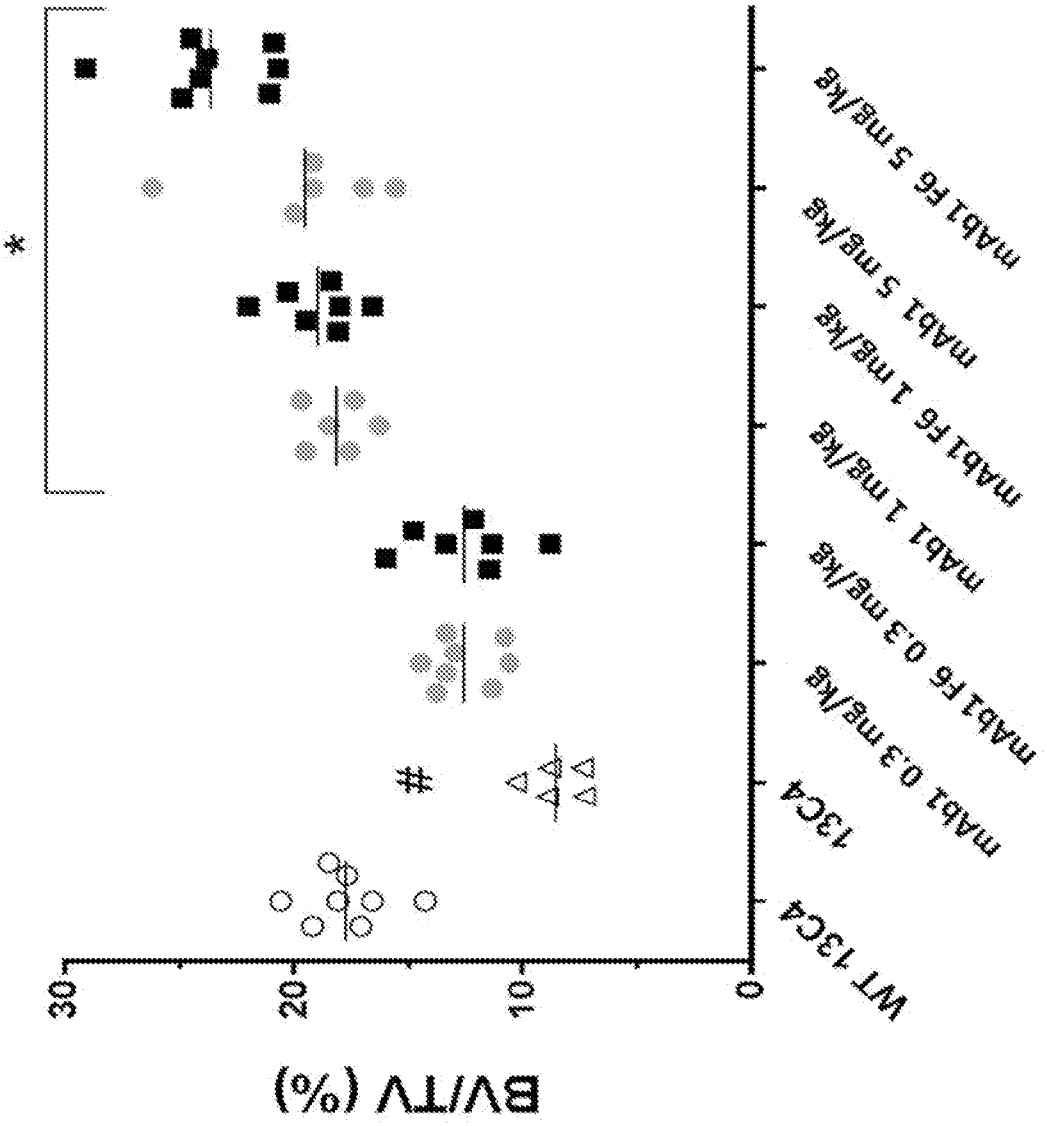

BV/TV (%)
30
20
10
0
WT 13C4
13C4
mAb1 0.3 mg/kg
mAb1 F6 0.3 mg/kg
mAb1 1 mg/kg
mAb1 F6 1 mg/kg
mAb1 5 mg/kg
mAb1 F6 5 mg/kg
*
#

FIG. 20

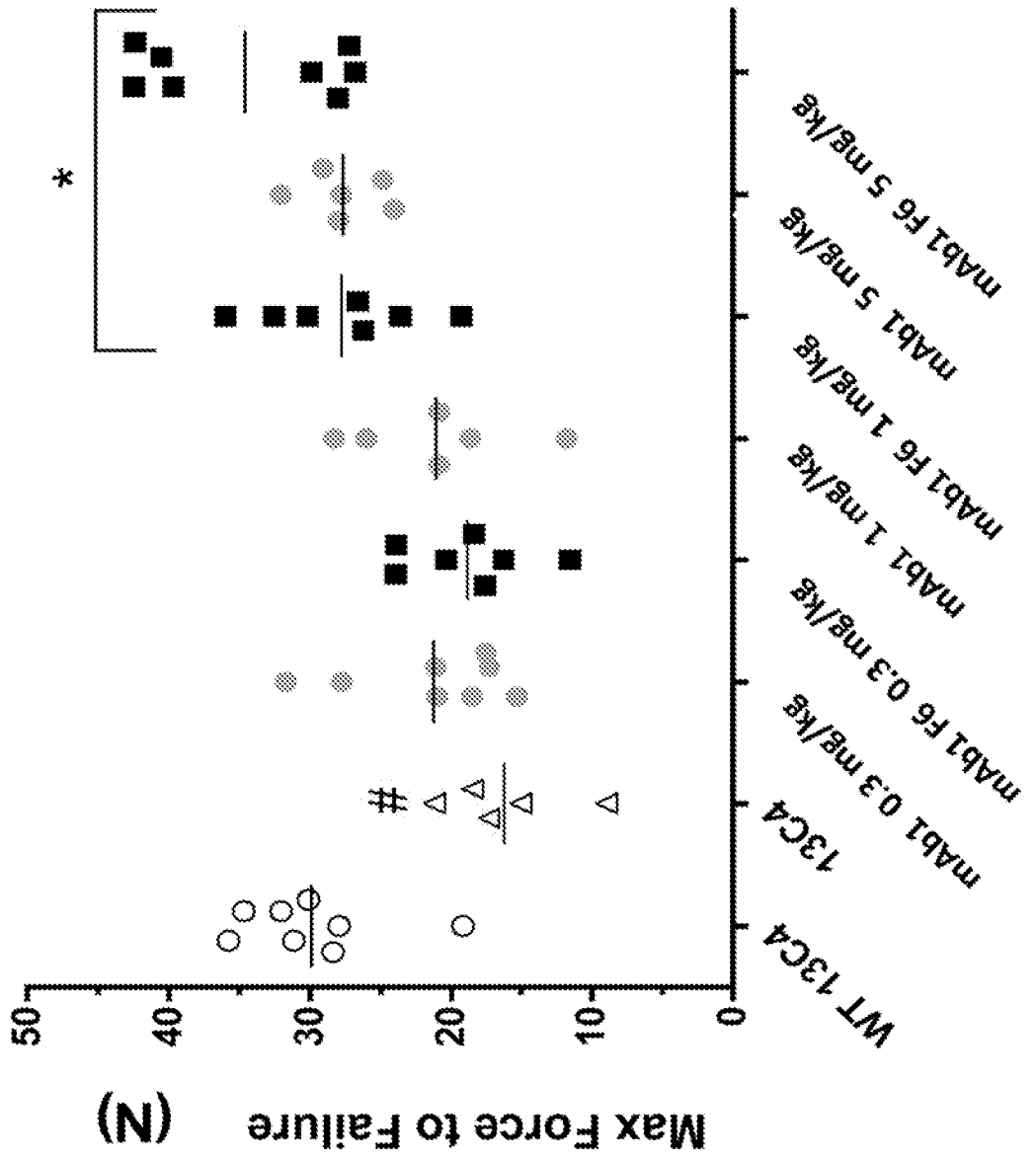

Max Force to Failure (N)
50
40
30
20
10
0
*
#
WT 13C4
13C4
mAb1 0.3 mg/kg
mAb1 F6 0.3 mg/kg
mAb1 1 mg/kg
mAb1 F6 1 mg/kg
mAb1 5 mg/kg
mAb1 F6 5 mg/kg

FIG. 21

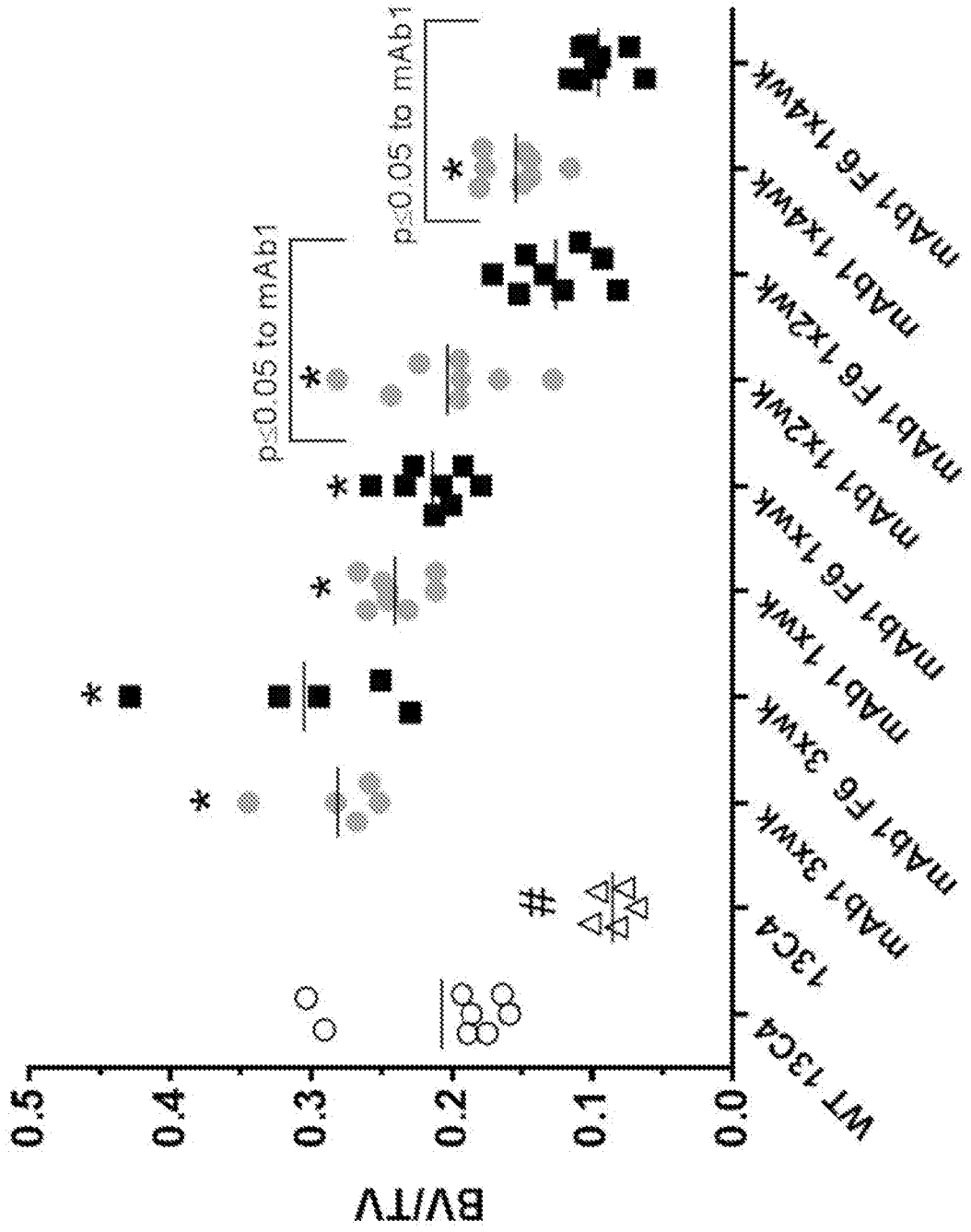

BV/TV
0.5
0.4
0.3
0.2
0.1
0.0
p≤0.05 to mAb1
p≤0.05 to mAb1
*
*
*
*
*
#
WT 13C4
13C4
mAb1 3xwk
mAb1 F6 3xwk
mAb1 1xwk
mAb1 F6 1xwk
mAb1 1x2wk
mAb1 F6 1x2wk
mAb1 1x4wk
mAb1 F6 1x4wk

FIG. 22

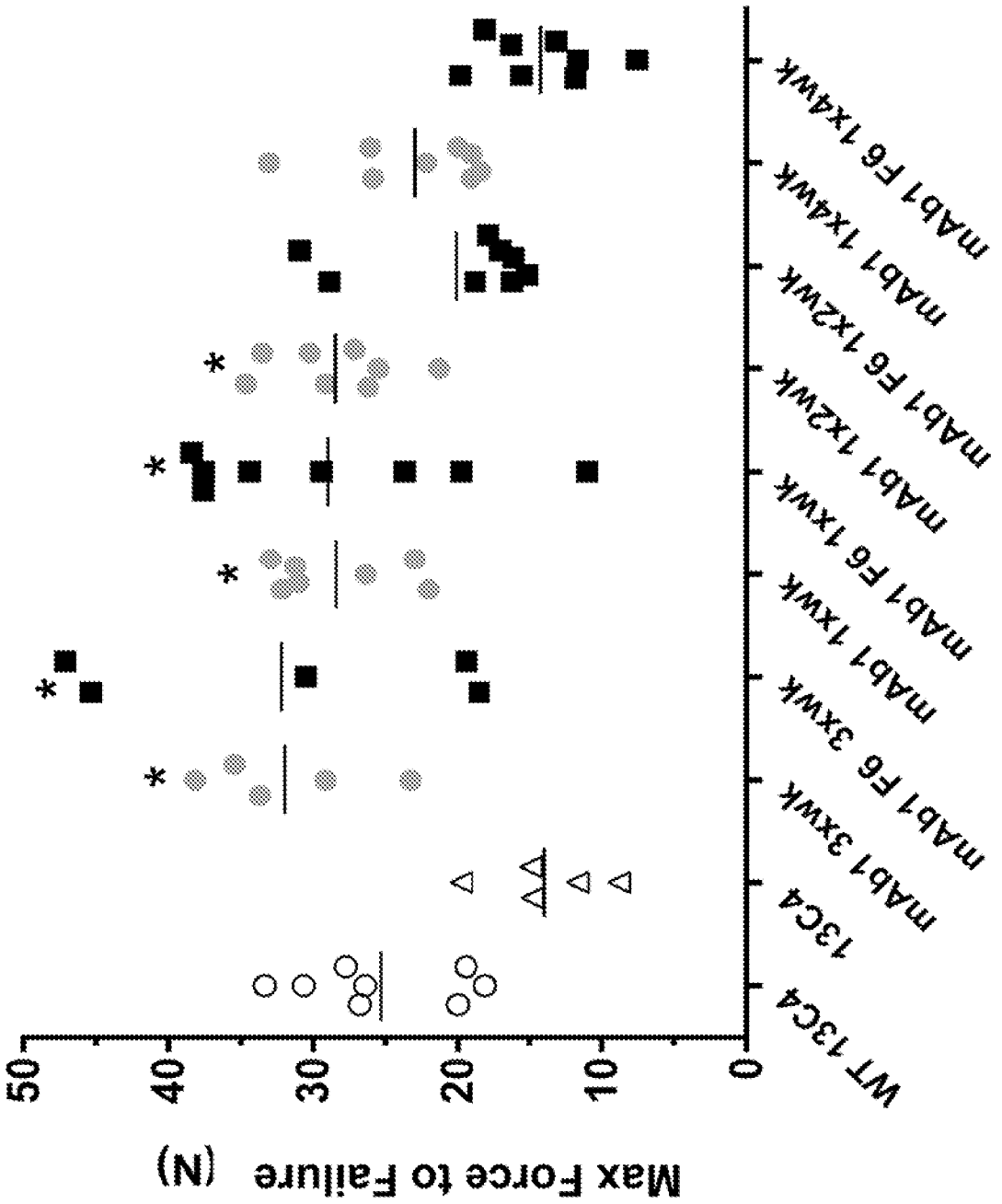

Max Force to Failure (N)
50
40
30
20
10
0
WT 13C4
13C4
mAb1 3xwk
mAb1 F6 3xwk
mAb1 1xwk
mAb1 F6 1xwk
mAb1 1x2wk
mAb1 F6 1x2wk
mAb1 1x4wk
mAb1 F6 1x4wk

FIG. 23

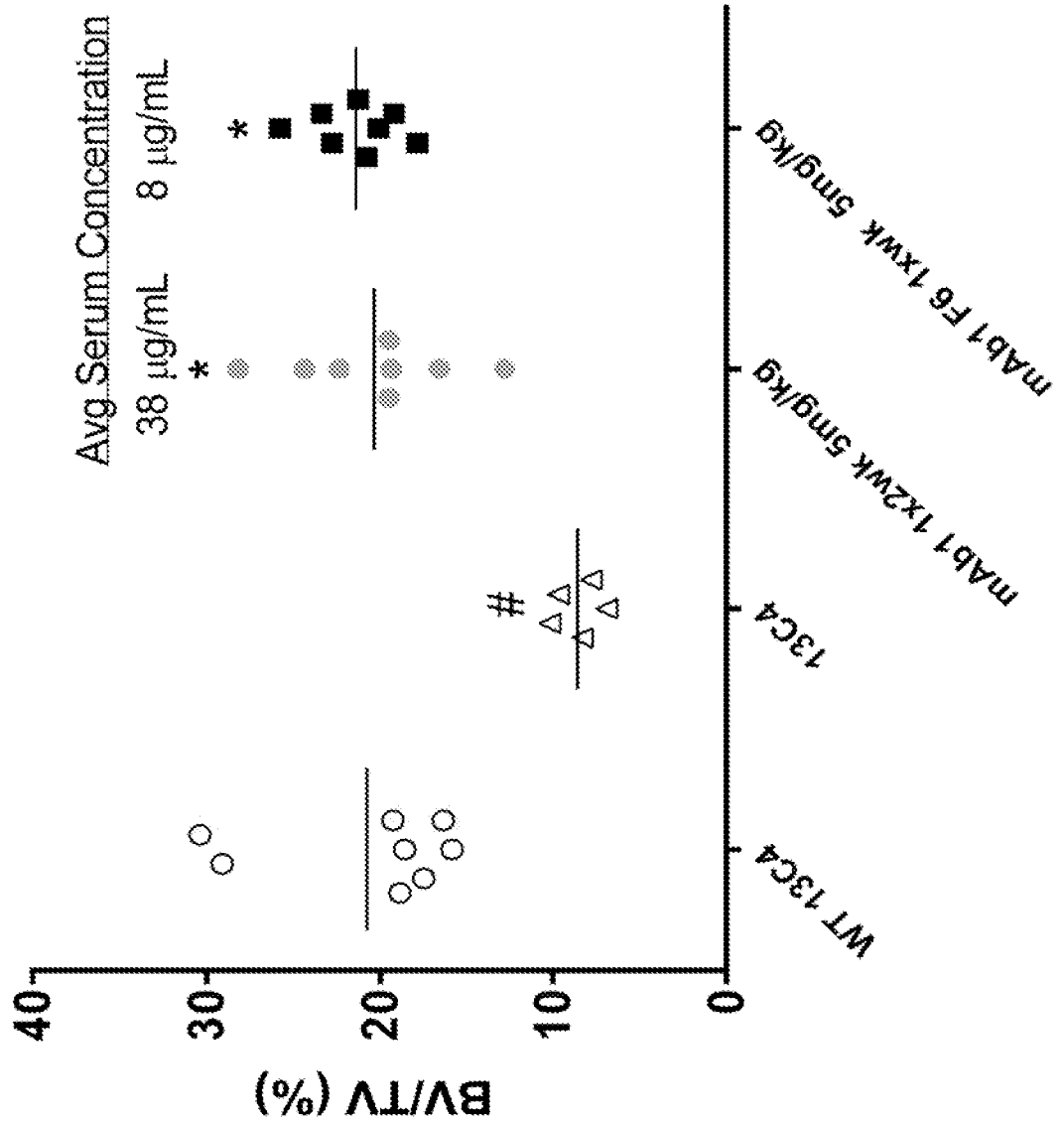

Avg Serum Concentration
38 µg/mL
8 µg/mL
*
*
#
BV/TV (%)
40
30
20
10
0
WT 13C4
13C4
mAb1 1x2wk 5mg/kg
mAb1 F6 1xwk 5mg/kg

FIG. 24

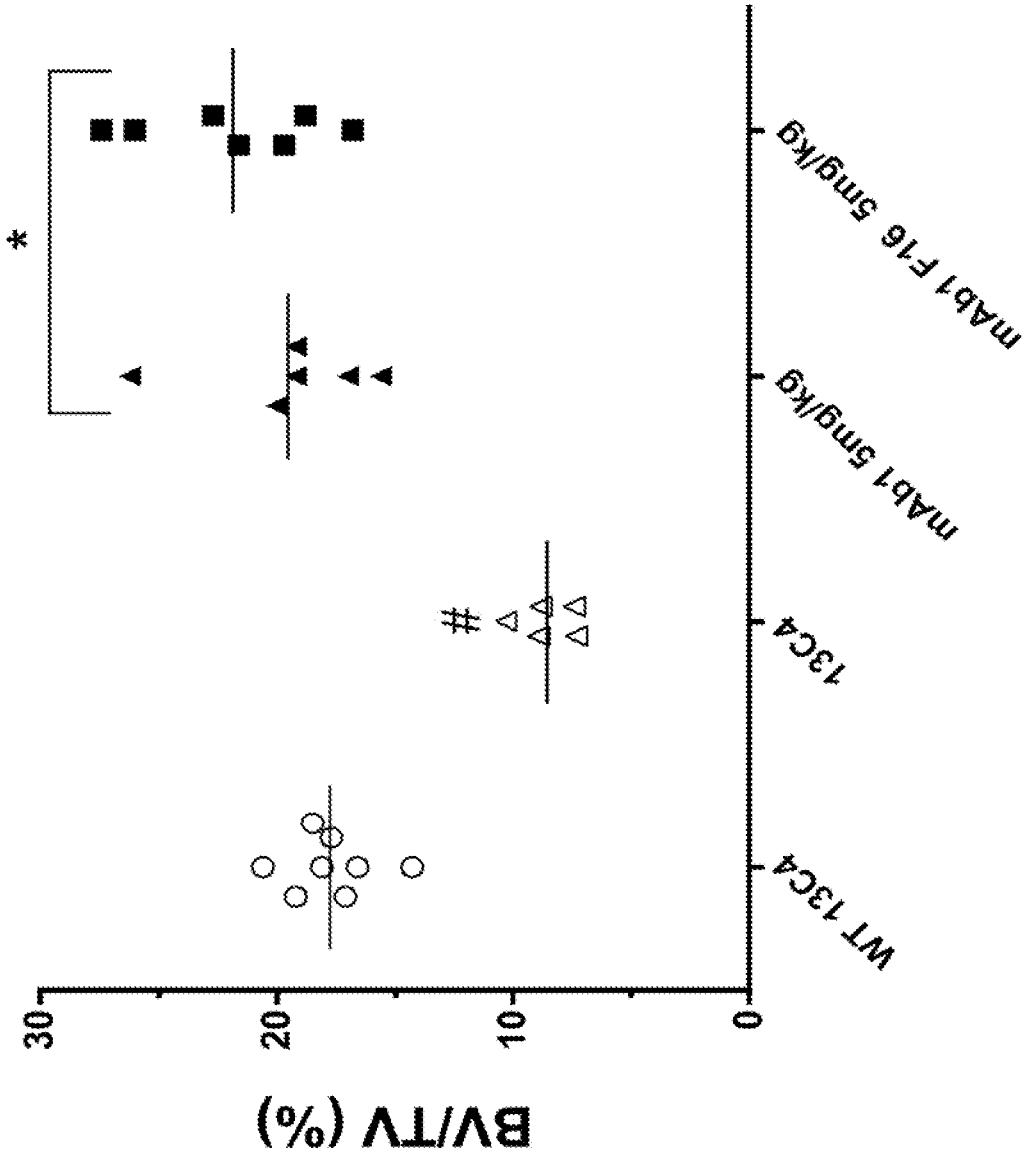


FIG. 25

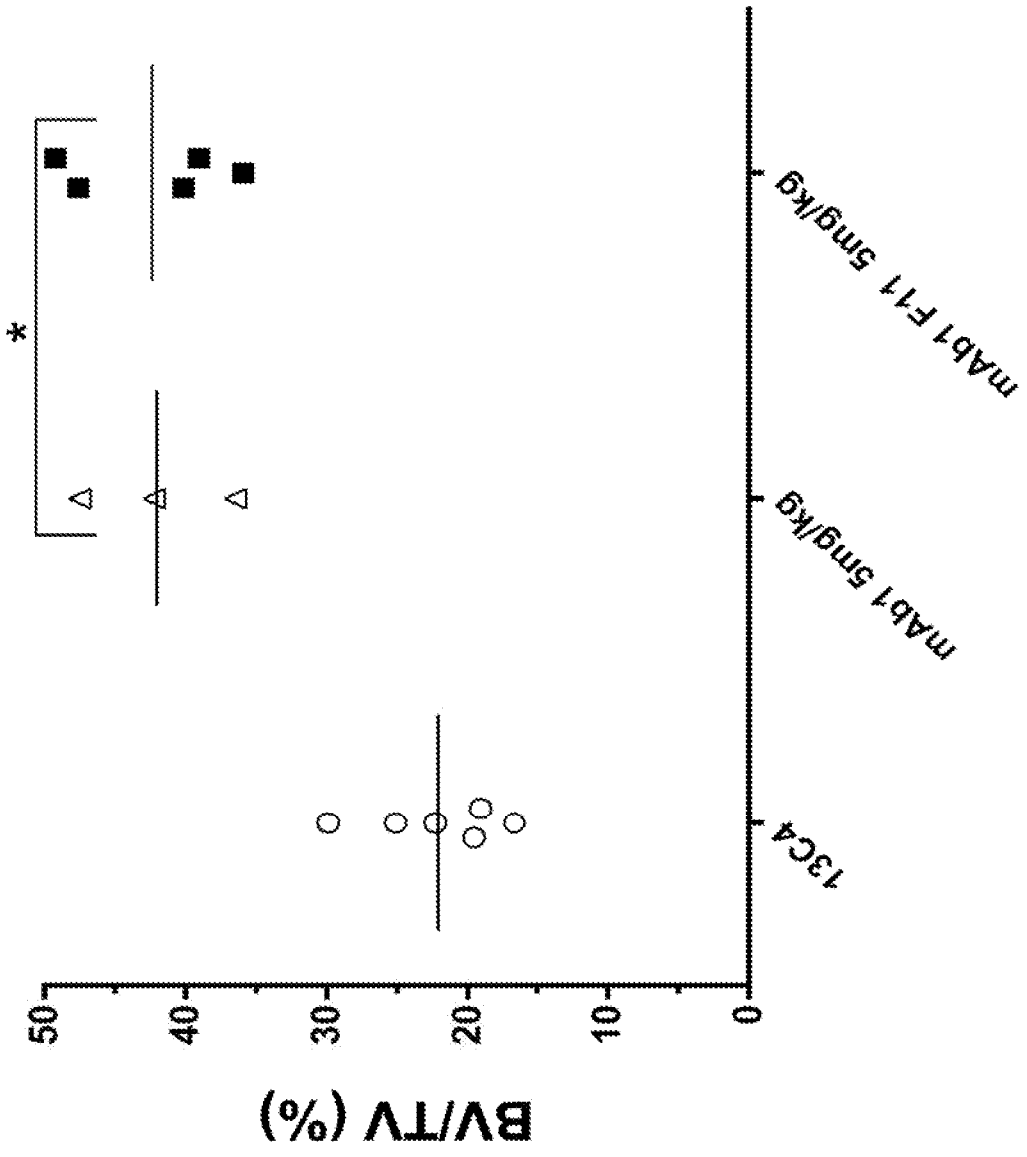


FIG. 26

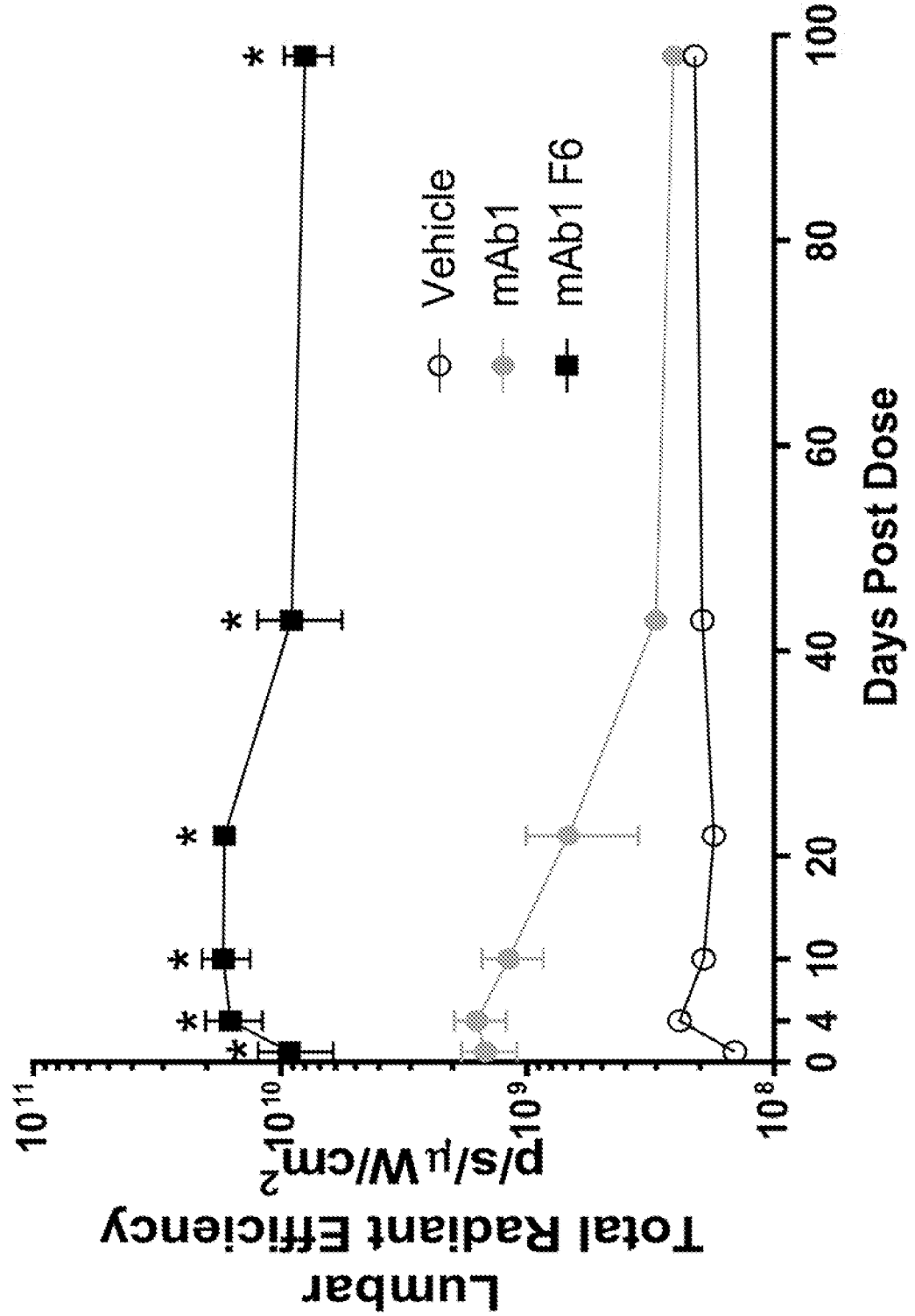

Lumbar
Total Radiant Efficiency
p/s/μW/cm²
10¹¹
10¹⁰
10⁹
10⁸
0 4 10 20 40 60 80 100
Days Post Dose
Vehicle
mAb1
mAb1 F6
*

FIG. 27

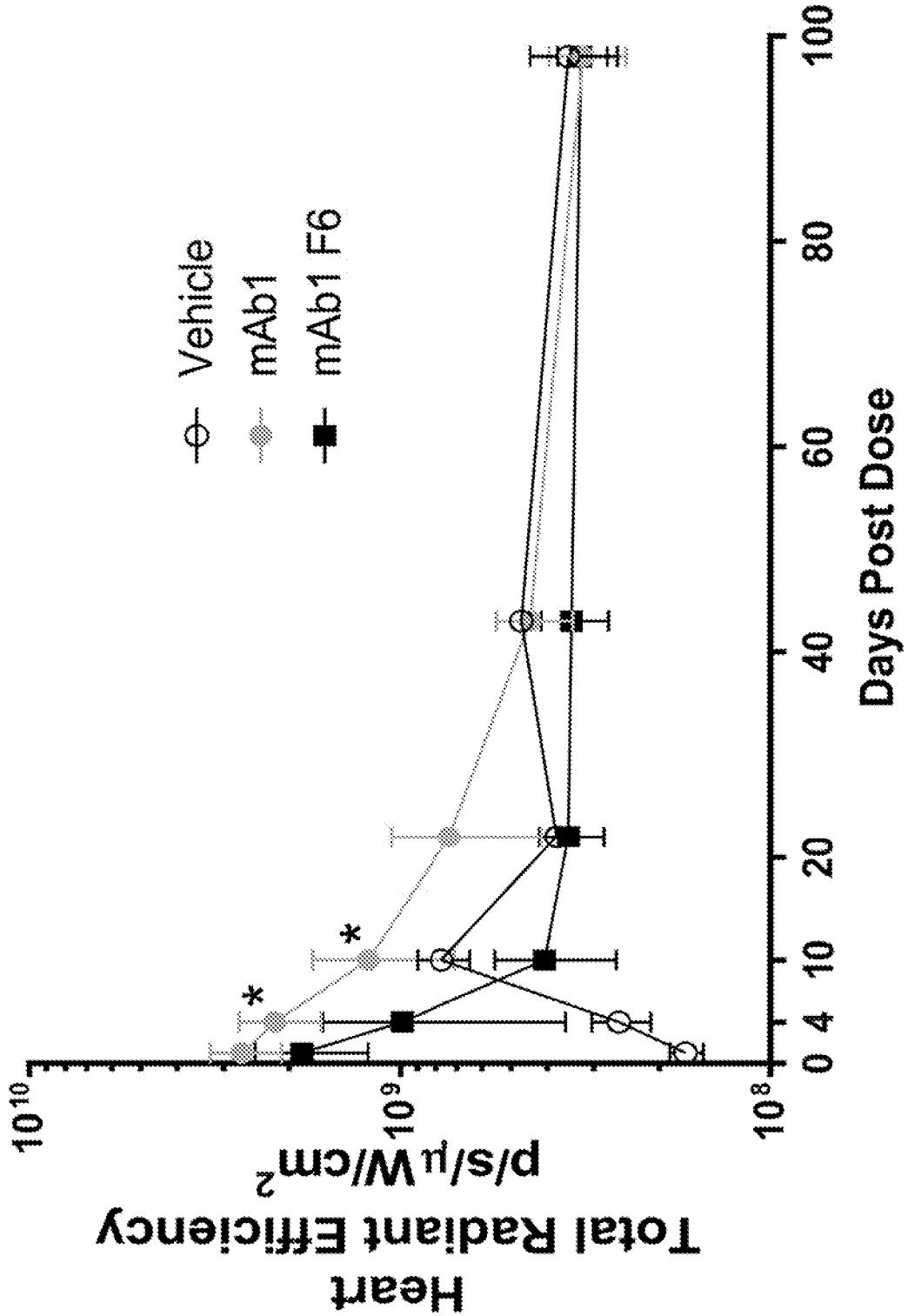

Heart
Total Radiant Efficiency
p/s/μW/cm²
10¹⁰
10⁹
10⁸
0 4 10 20 40 60 80 100
Days Post Dose
Vehicle
mAb1
mAb1 F6
*
*

FIG. 28

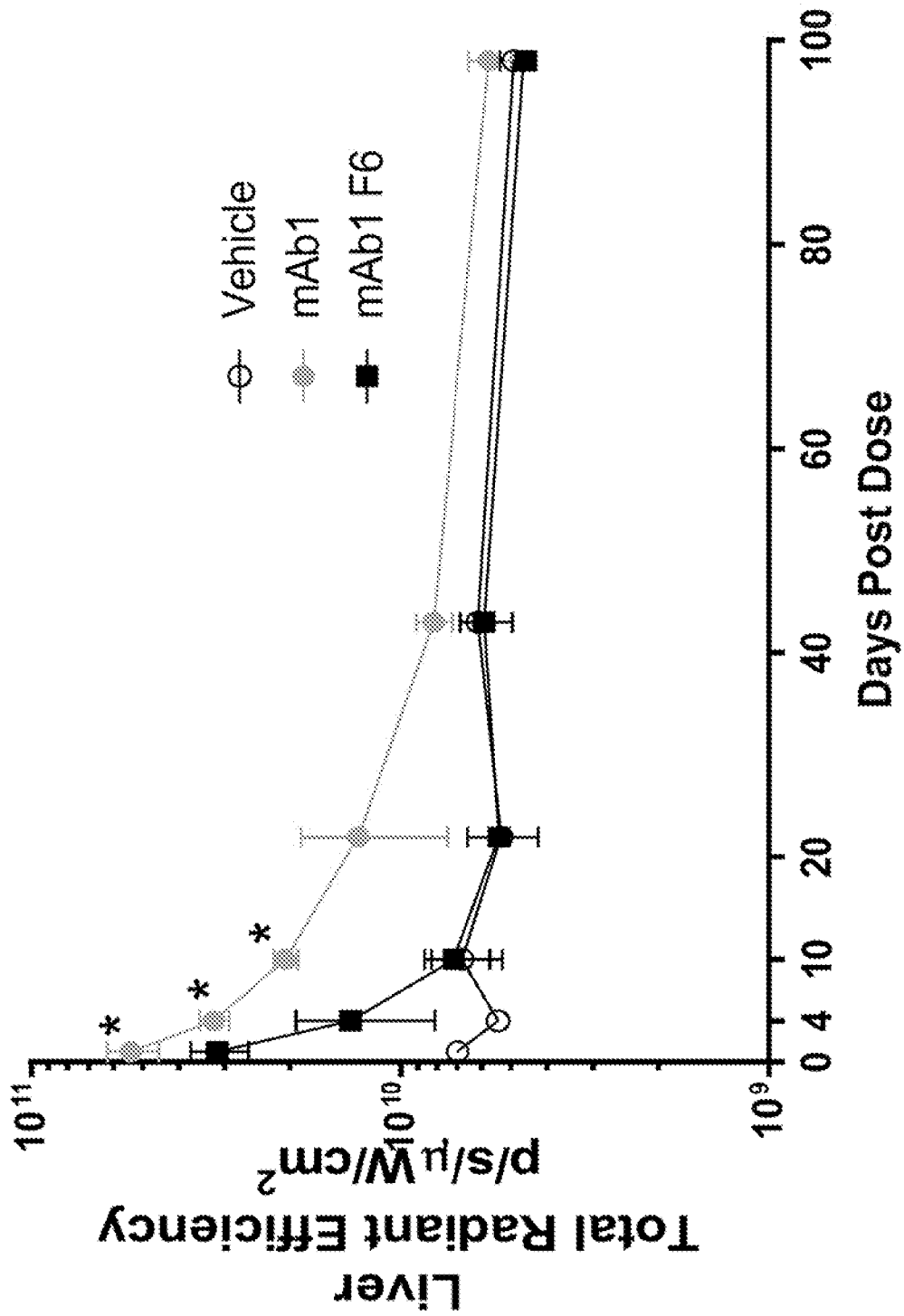

Liver
Total Radiant Efficiency
p/s/μW/cm²
10¹¹
10¹⁰
10⁹
0 4 10 20 40 60 80 100
Days Post Dose
Vehicle
mAb1
mAb1 F6
*
*
*

FIG. 29

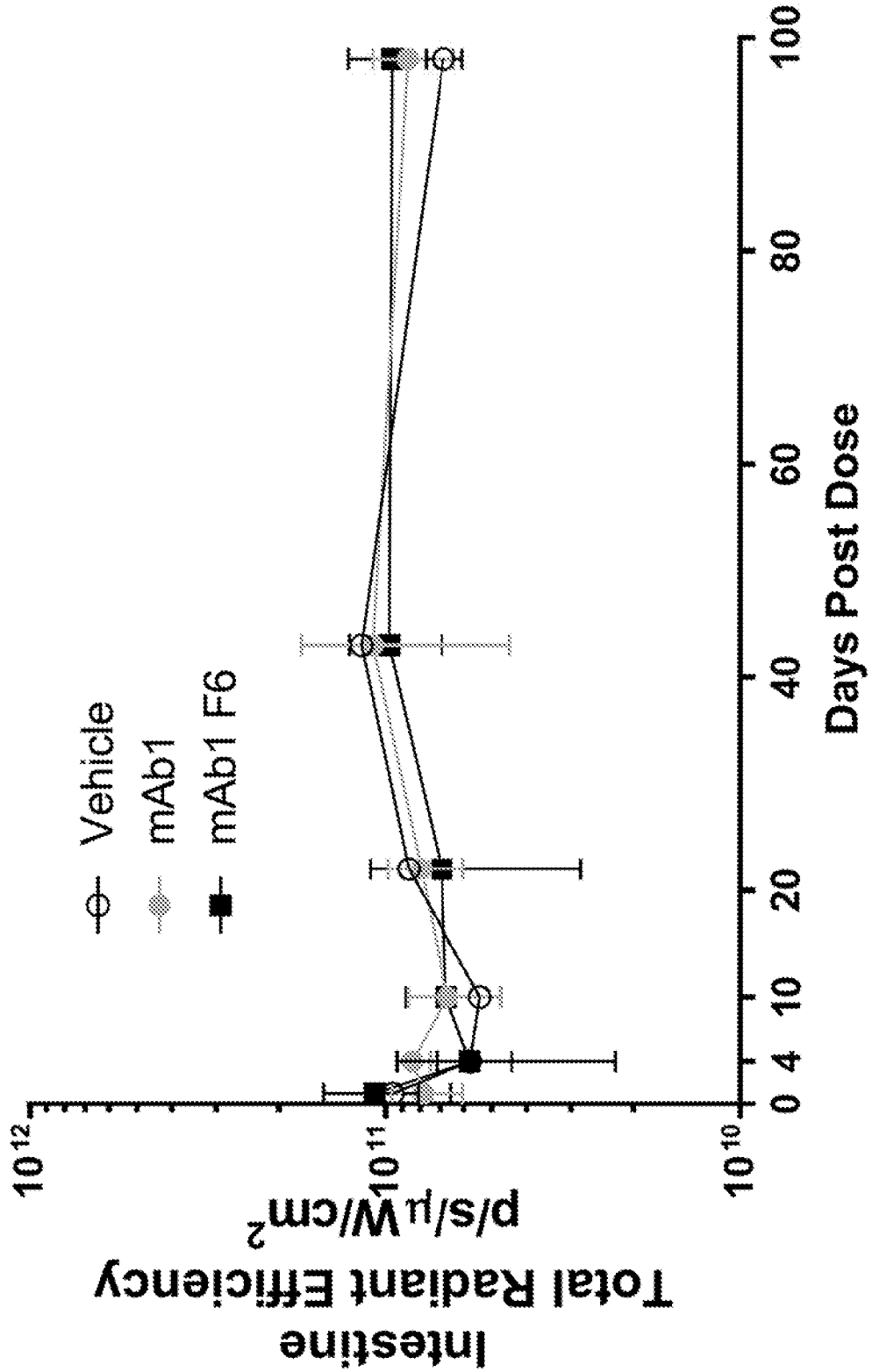
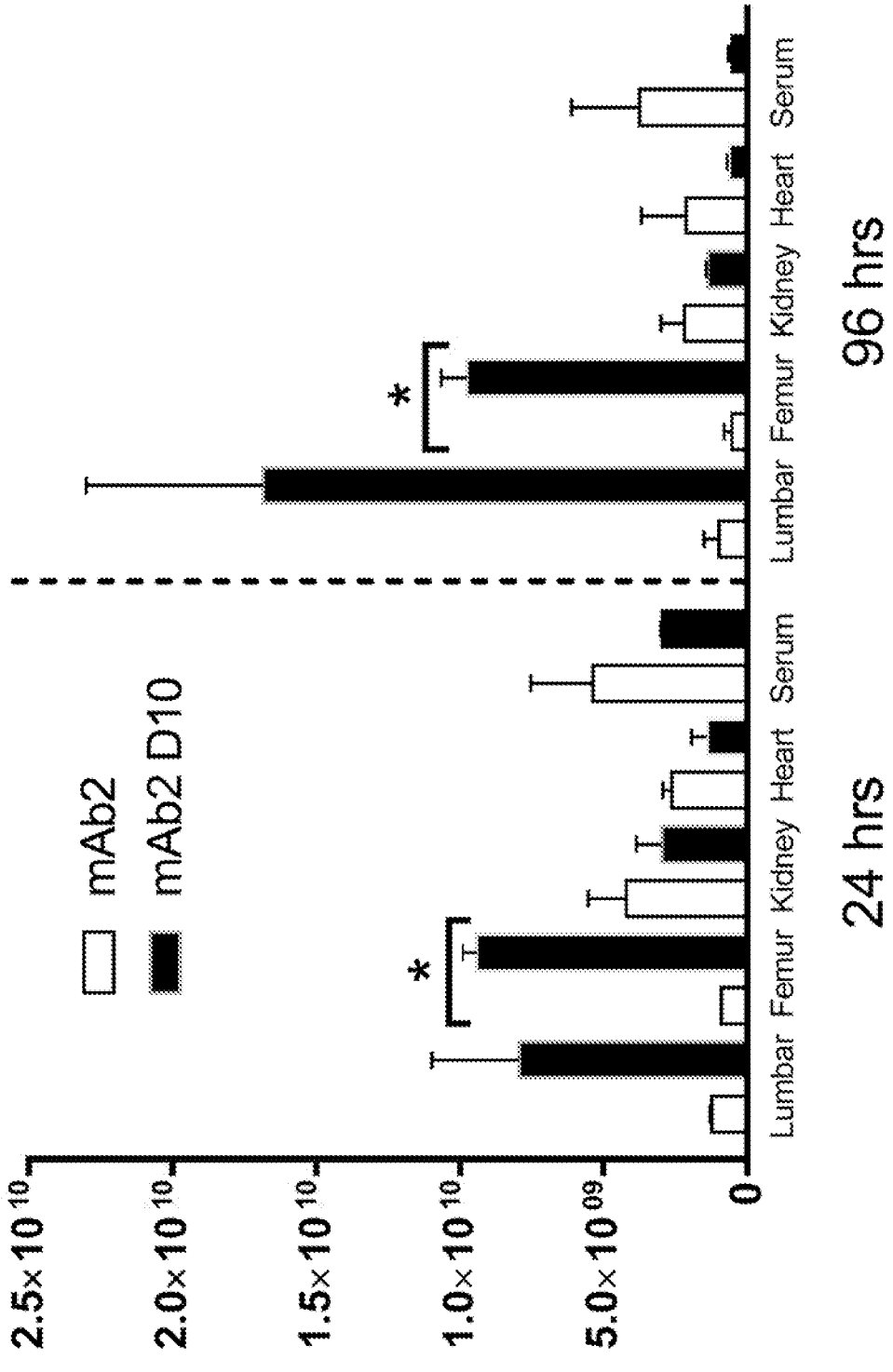

Intestine
Total Radiant Efficiency
p/s/μW/cm²
10¹²
10¹¹
10¹⁰
0 4 10 20 40 60 80 100
Days Post Dose
Vehicle
mAb1
mAb1 F6

FIG. 30

FIG. 31


Total Radiant Efficiency
p/s/μW/cm²
2.5×10¹⁰
2.0×10¹⁰
1.5×10¹⁰
1.0×10¹⁰
5.0×10⁰⁹
0
mAb2
mAb2 D10
*
*
Lumbar Femur Kidney Heart Serum
Lumbar Femur Kidney Heart Serum
24 hrs
96 hrs

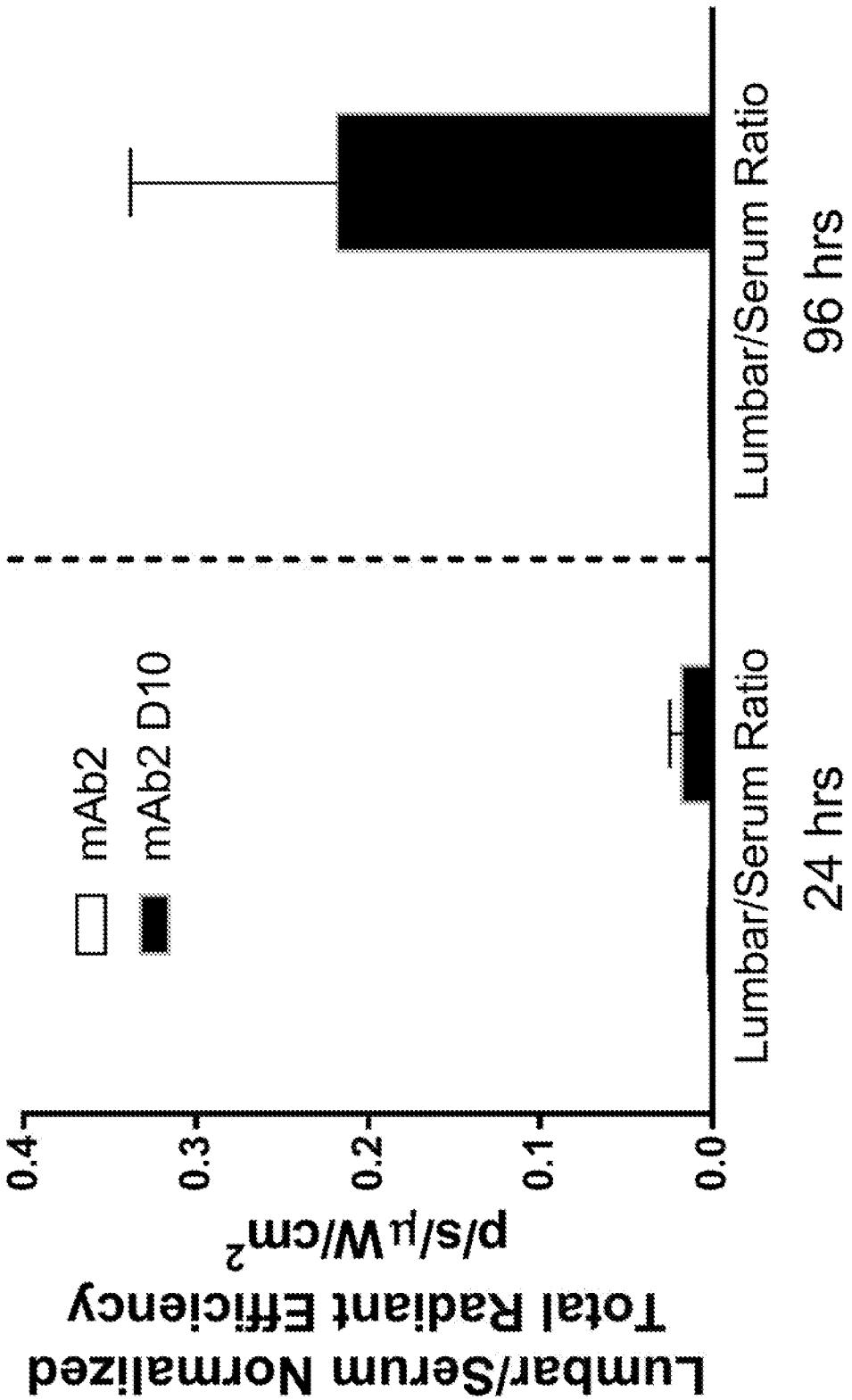

Lumbar/Serum Normalized
Total Radiant Efficiency
p/s/μW/cm²
0.4
0.3
0.2
0.1
0.0
mAb2
mAb2 D10
Lumbar/Serum Ratio
24 hrs
Lumbar/Serum Ratio
96 hrs

FIG. 32

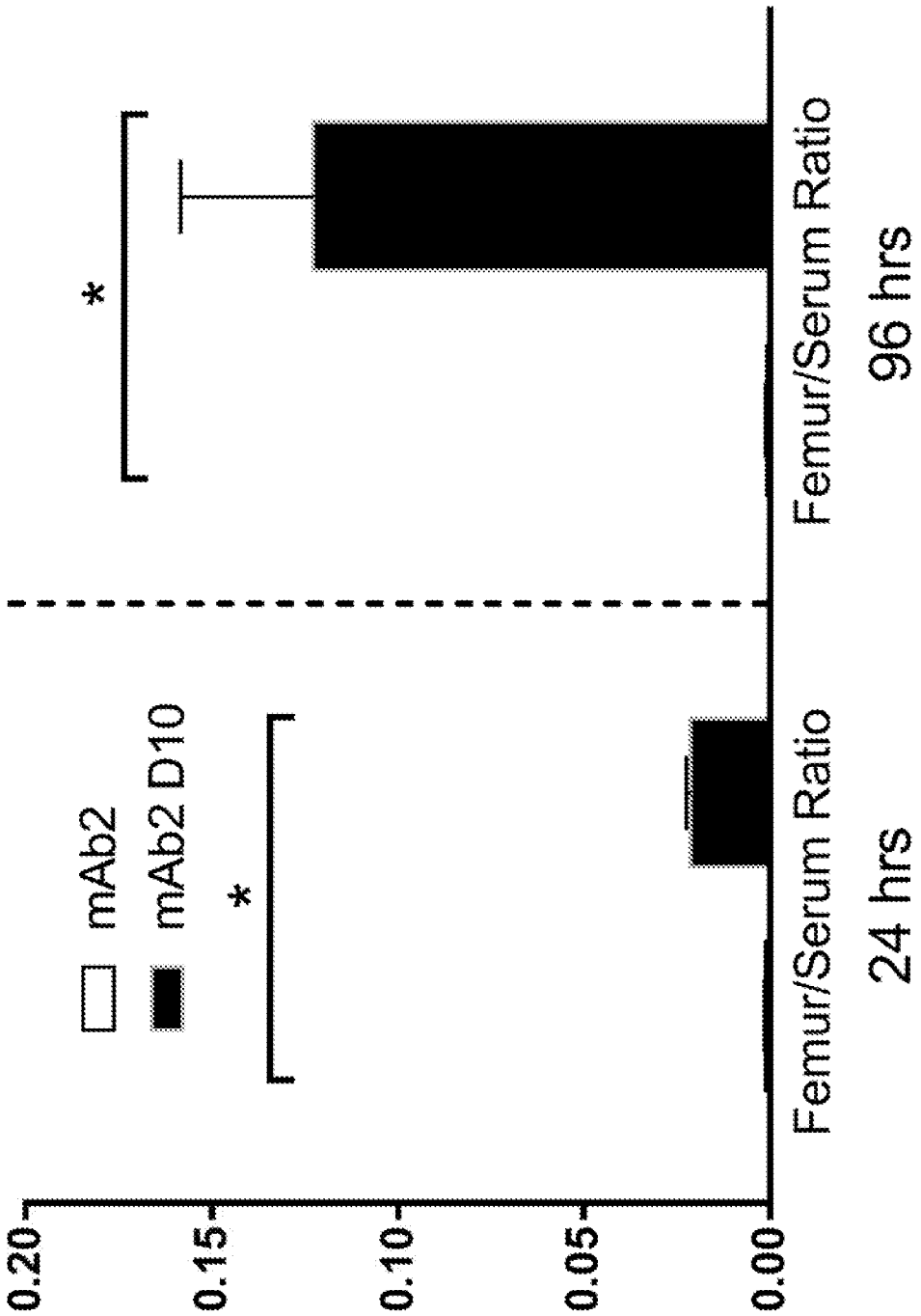

Femur/Serum Normalized
Total Radiant Efficiency
p/s/μW/cm²
0.20
0.15
0.10
0.05
0.00
mAb2
mAb2 D10
*
*
Femur/Serum Ratio
24 hrs
Femur/Serum Ratio
96 hrs

FIG. 33