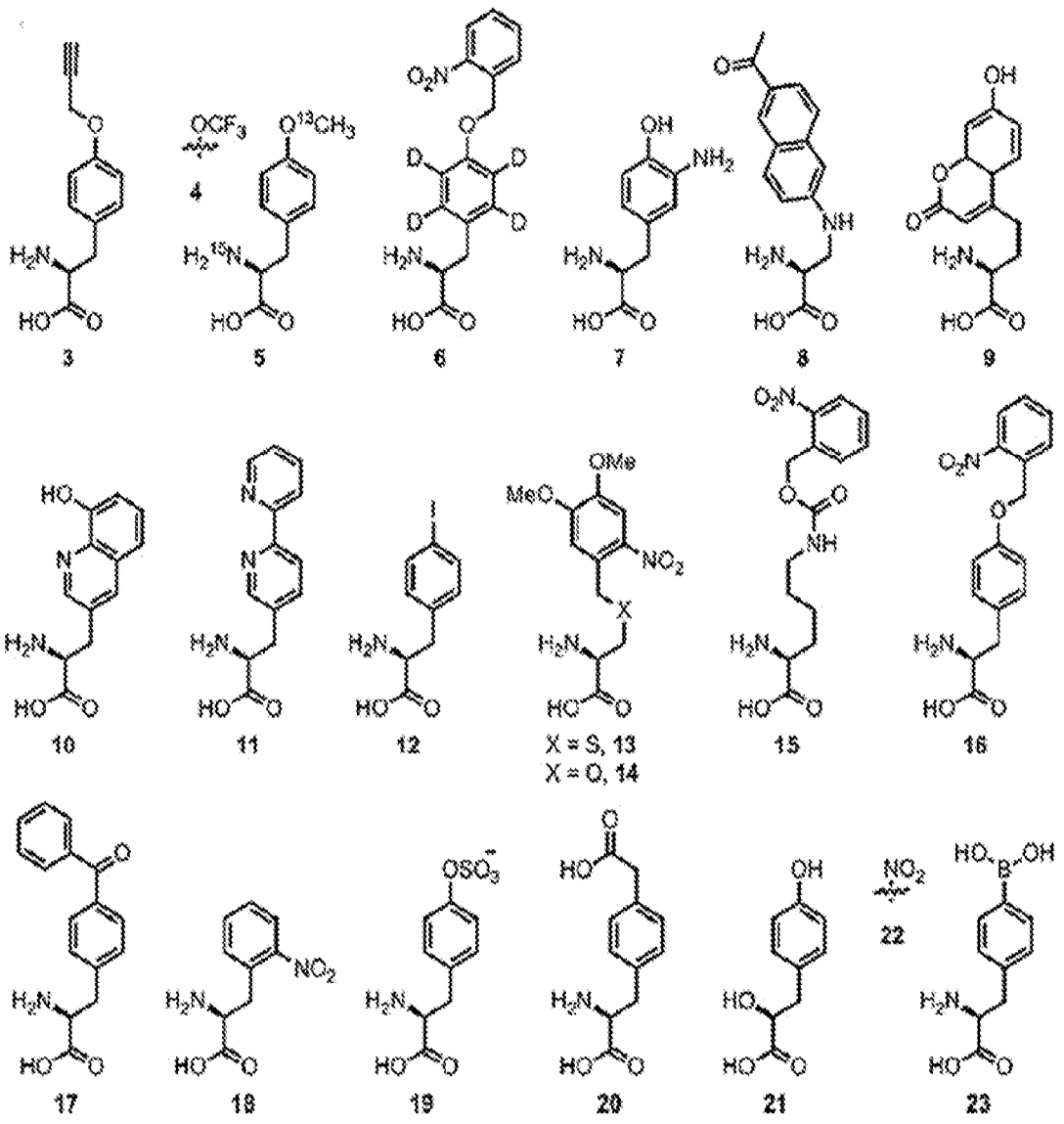


FIG. 1

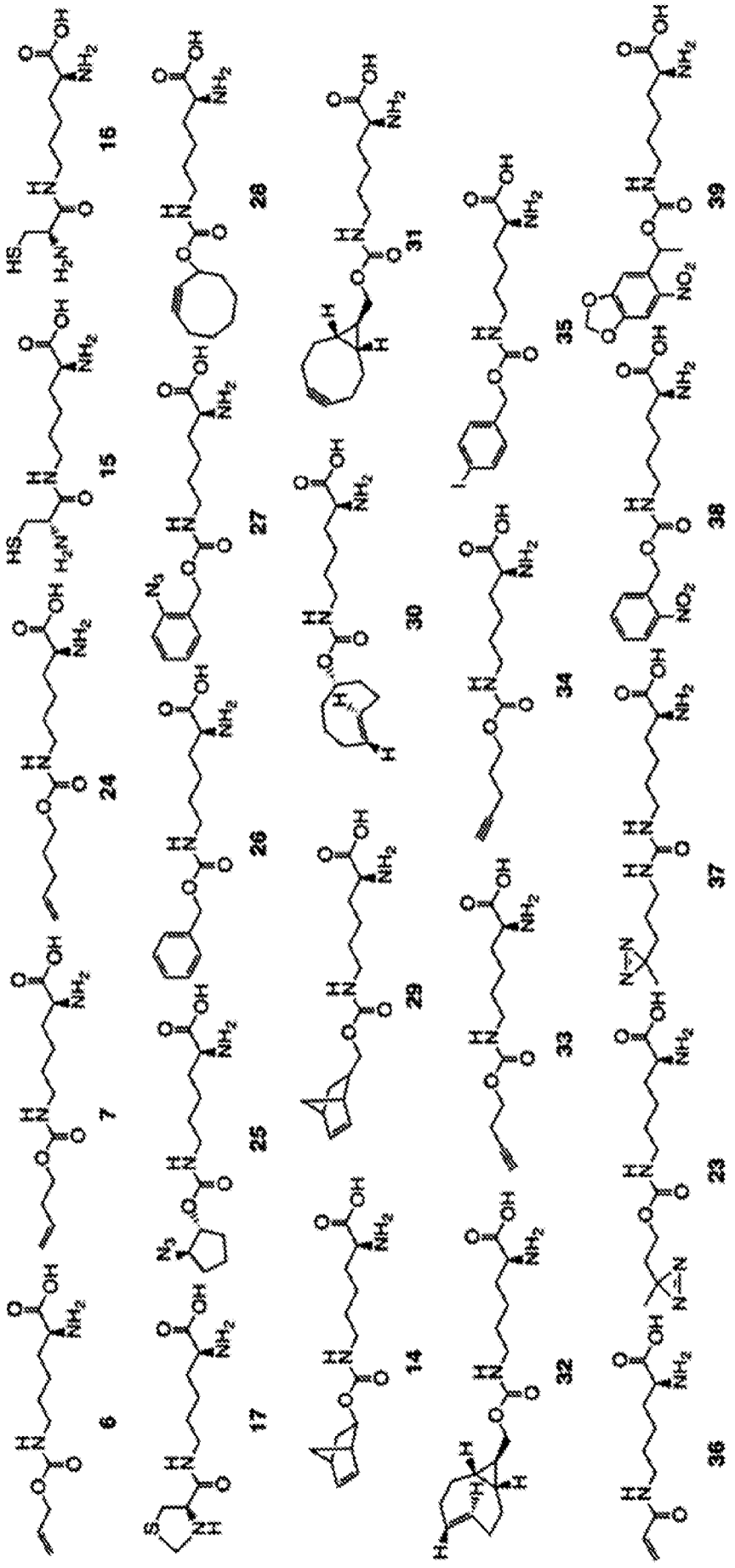


FIG. 2A

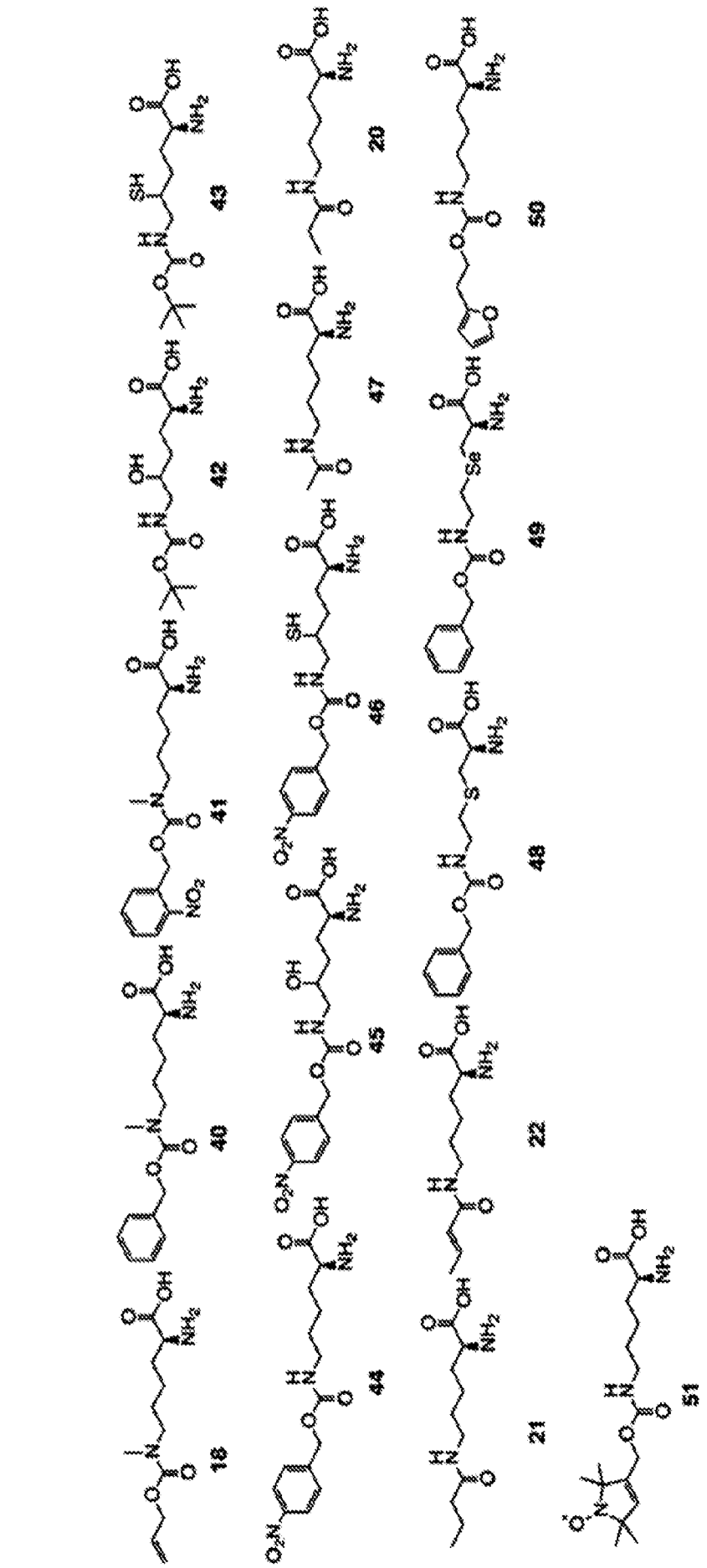


FIG. 2A (cont.)

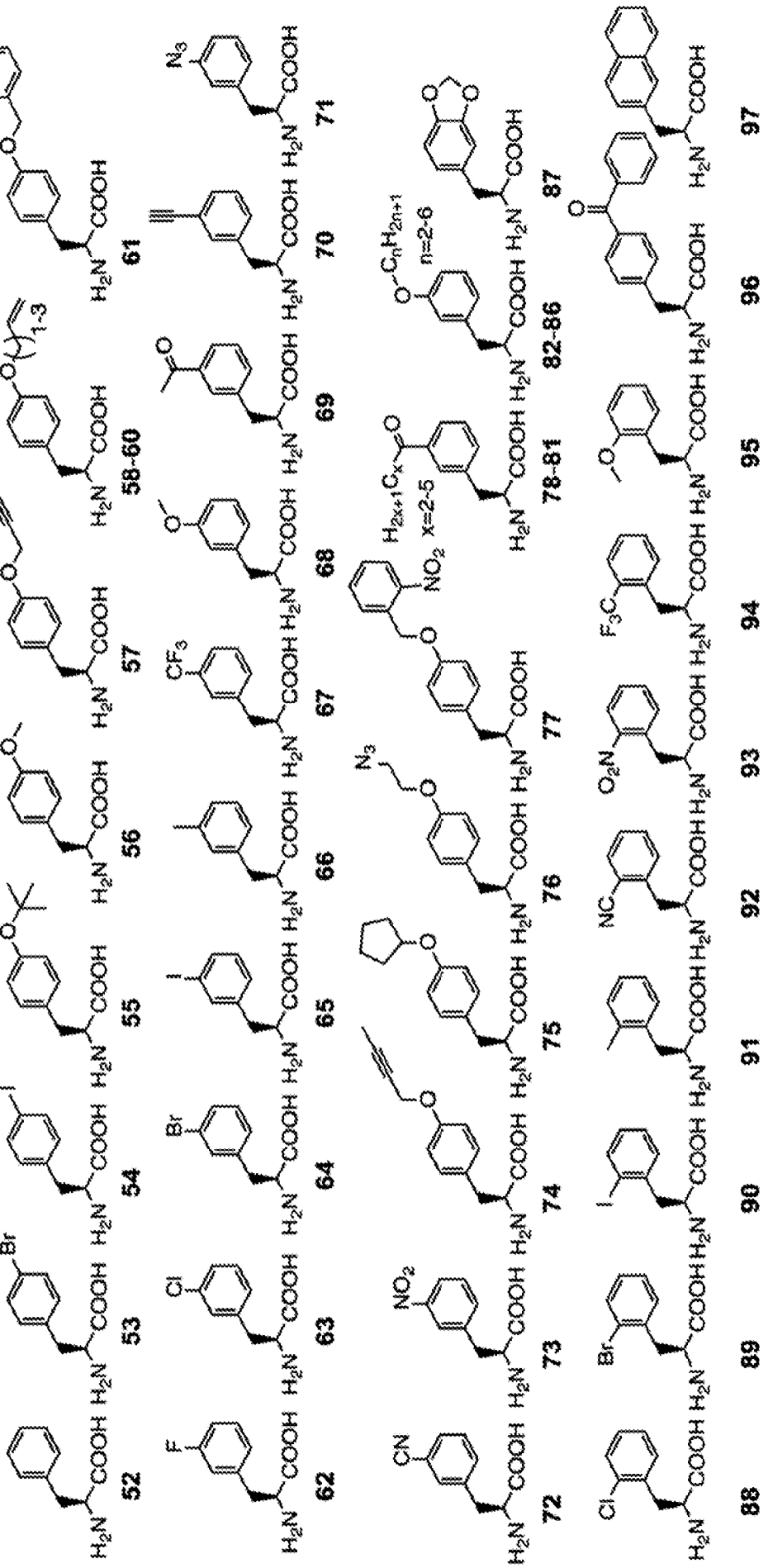

52
53
54
55
56
57
58-60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78-81
82-86
87
88
89
90
91
92
93
94
95
96
97
n=2-6
x=2-5

FIG. 2B

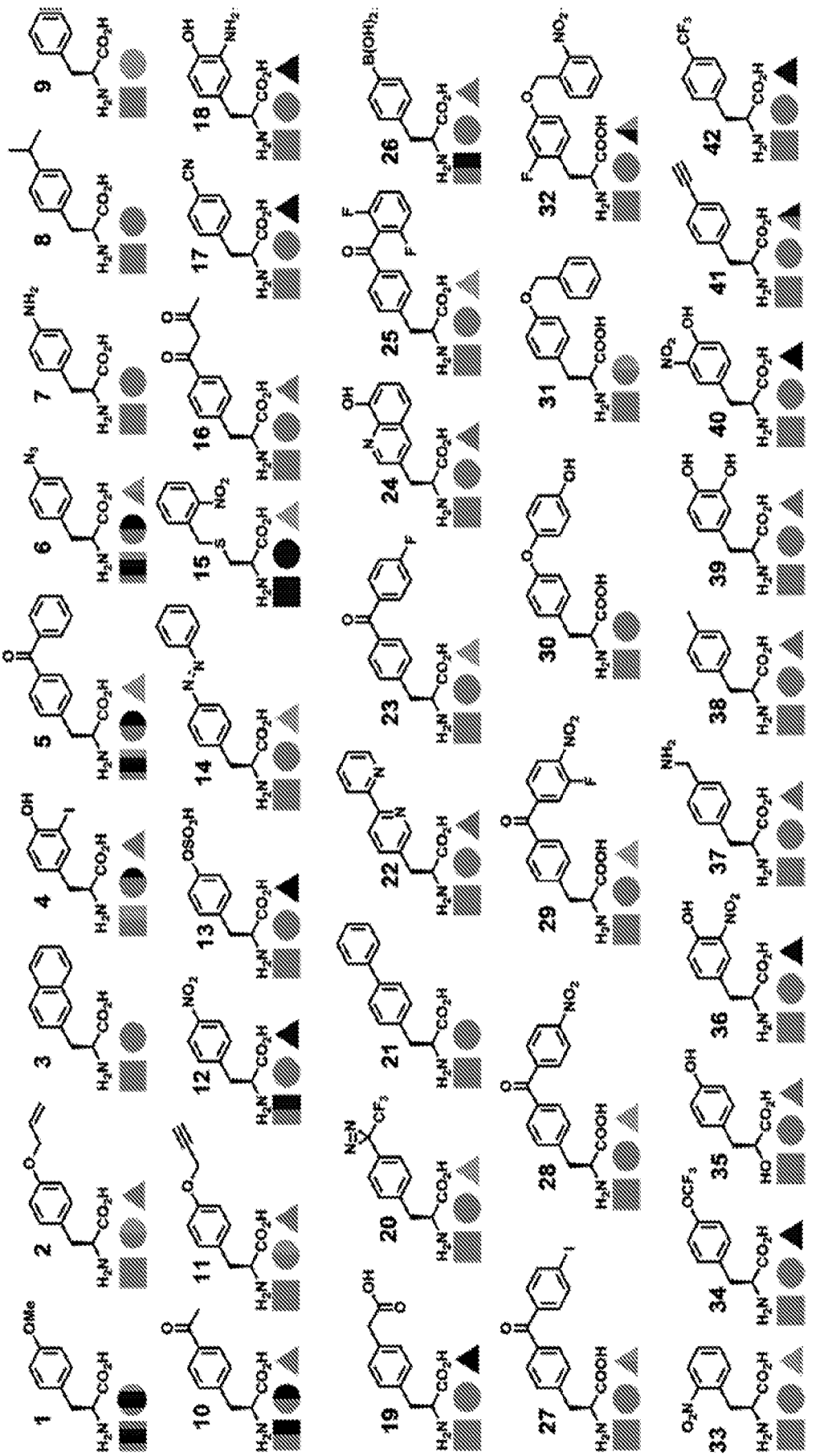


FIG. 3A

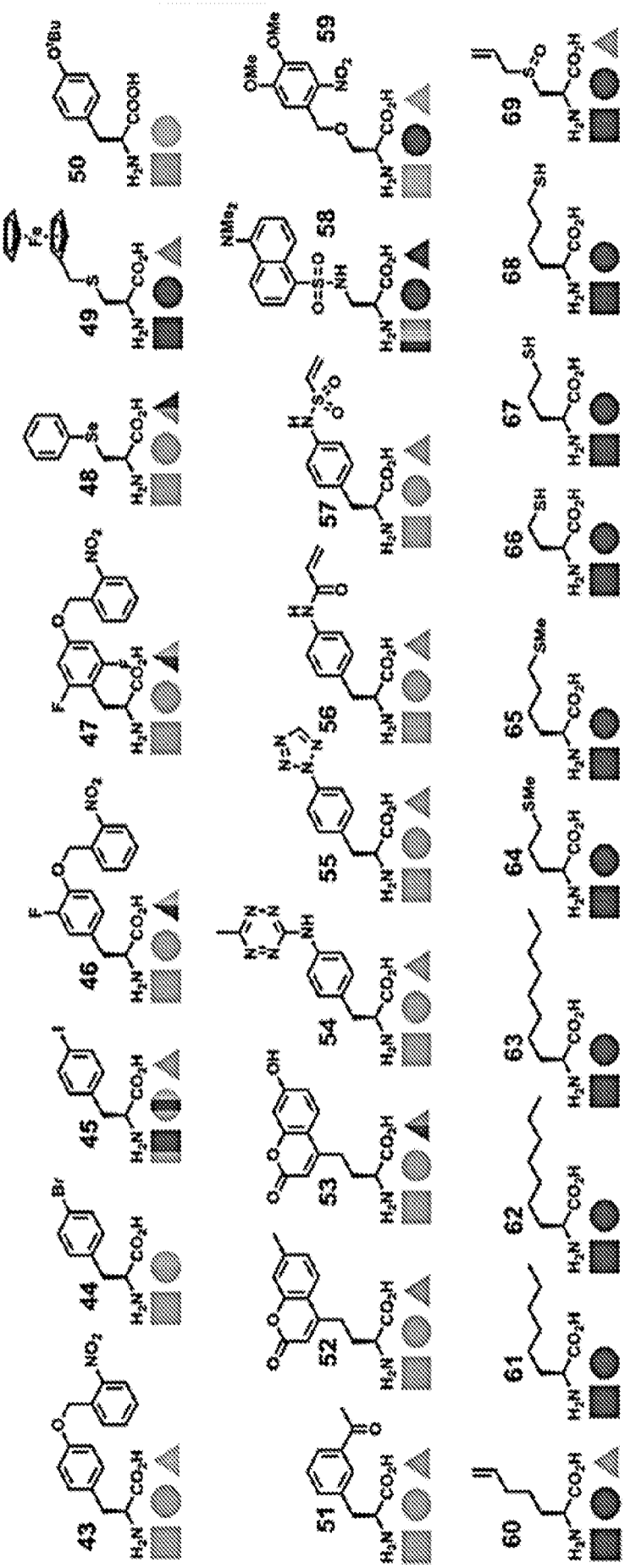


FIG. 3B

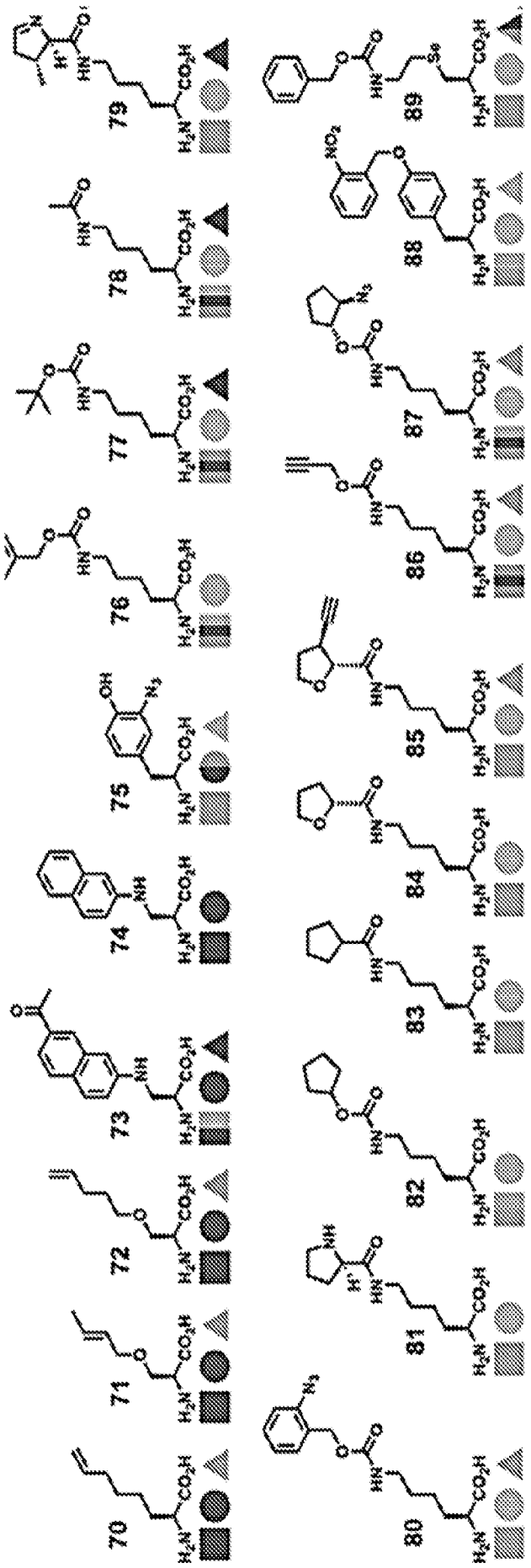


FIG. 3B (cont.)

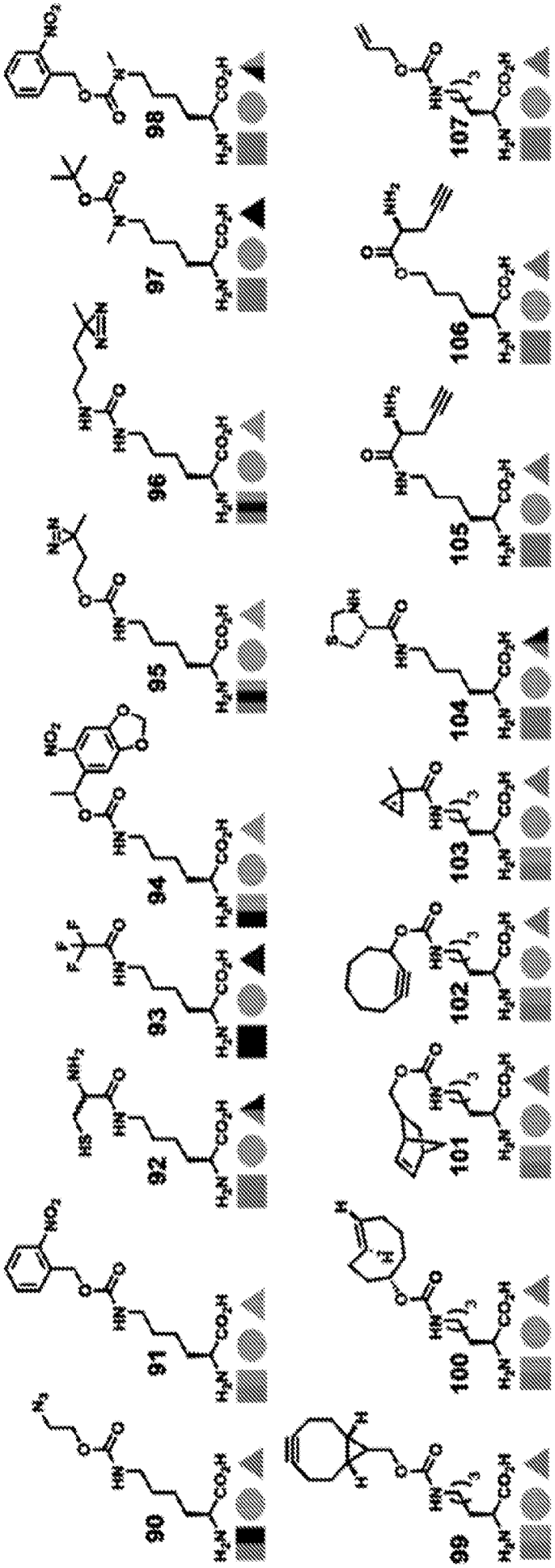


FIG. 3C

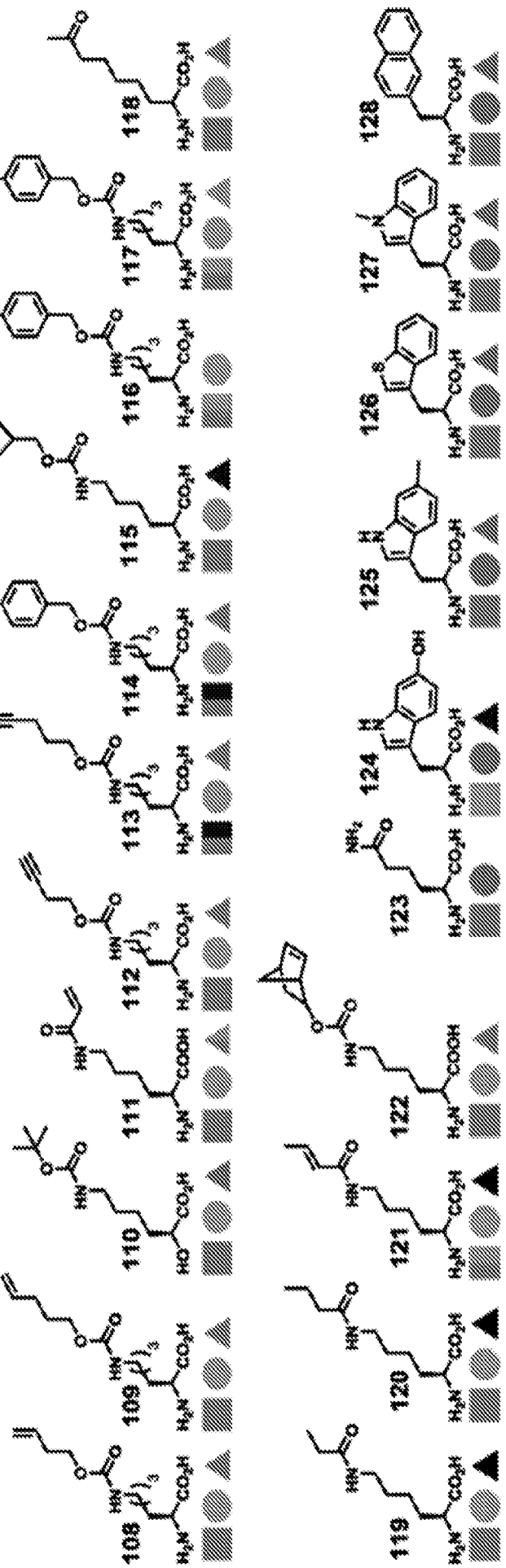

108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128

FIG. 3C (cont.)

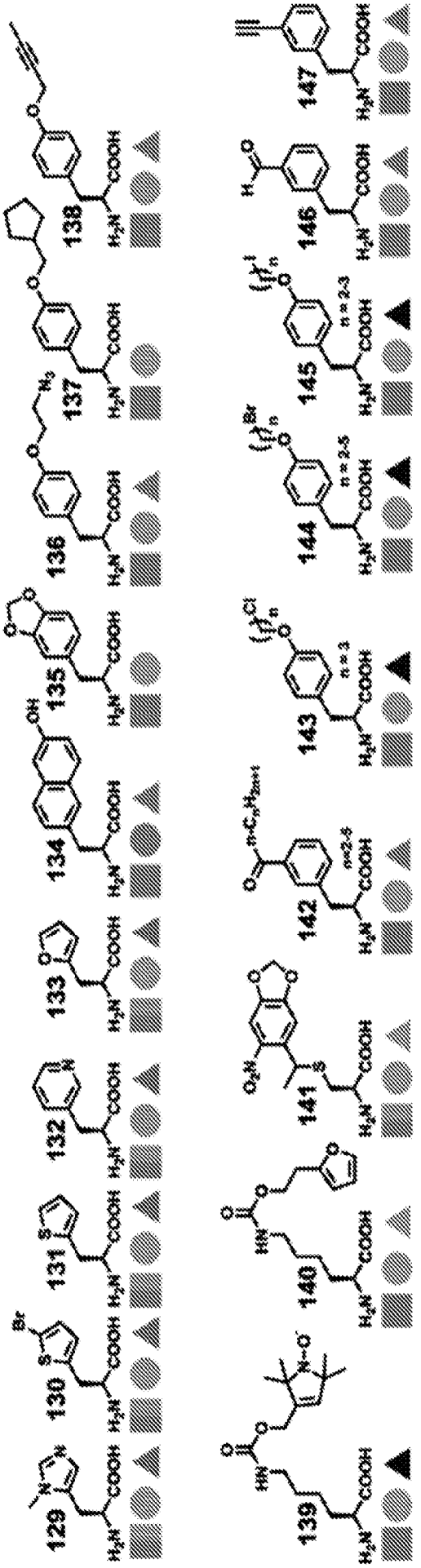


FIG. 3D

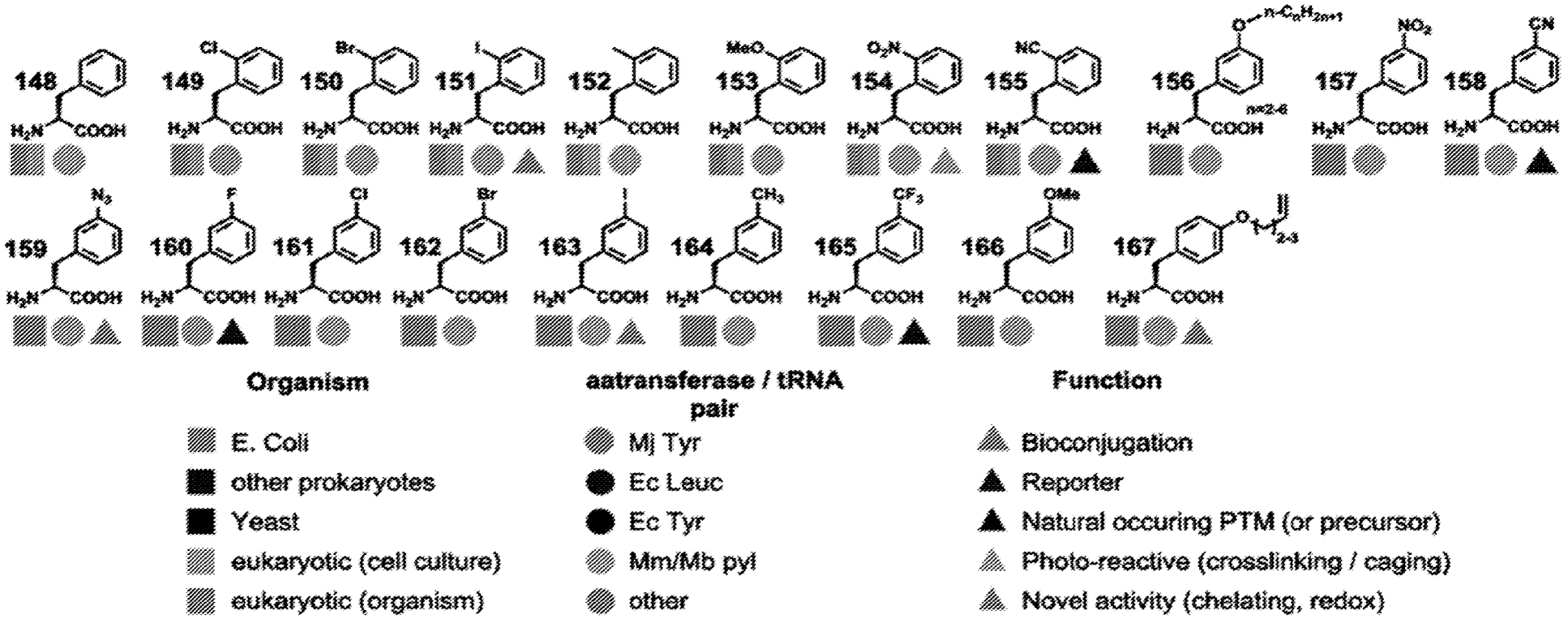

148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
Organism
E. Coli
other prokaryotes
Yeast
eukaryotic (cell culture)
eukaryotic (organism)
aatransferase / tRNA pair
Mj Tyr
Ec Leuc
Ec Tyr
Mm/Mb pyl
other
Function
Bioconjugation
Reporter
Natural occuring PTM (or precursor)
Photo-reactive (crosslinking / caging)
Novel activity (chelating, redox)

FIG. 3D (cont.)

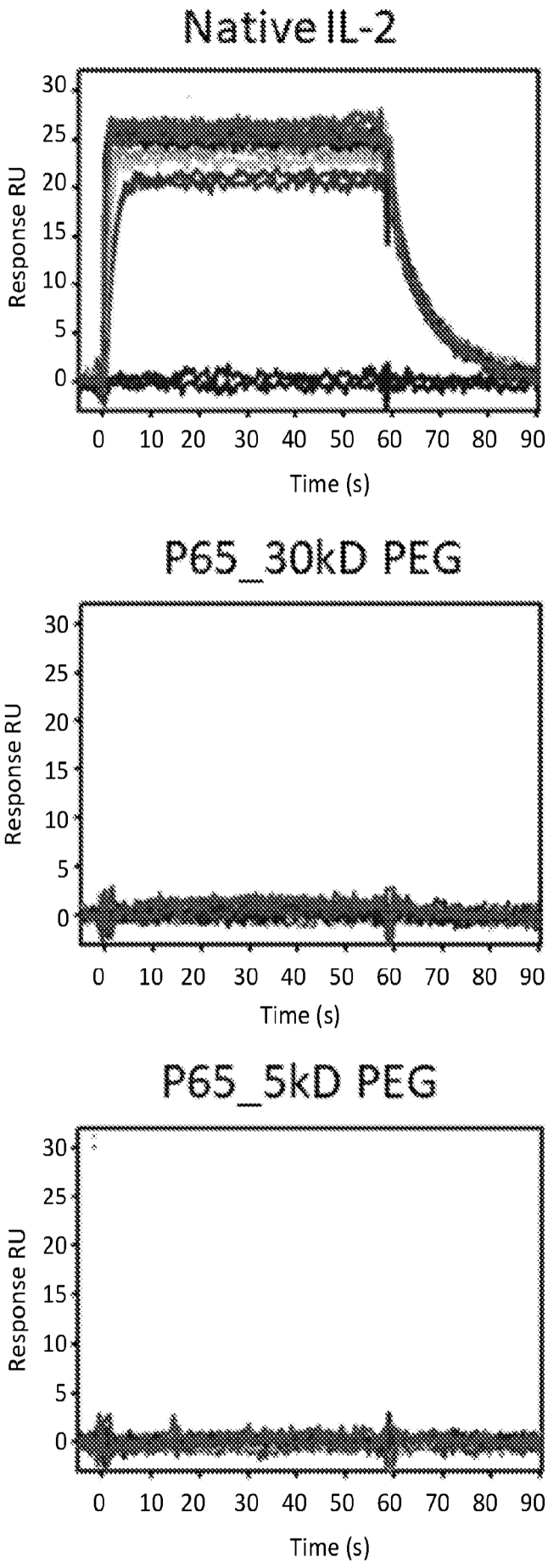
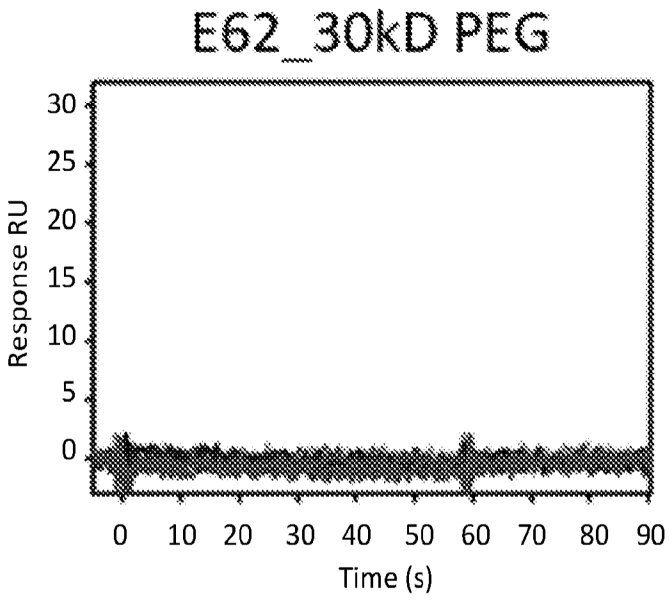

Native IL-2
Response RU
30
25
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90
Time (s)
P65_30kD PEG
Response RU
30
25
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90
Time (s)
P65_5kD PEG
Response RU
30
25
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90
Time (s)

FIG. 4A


E62_30kD PEG
Response RU
30
25
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90
Time (s)

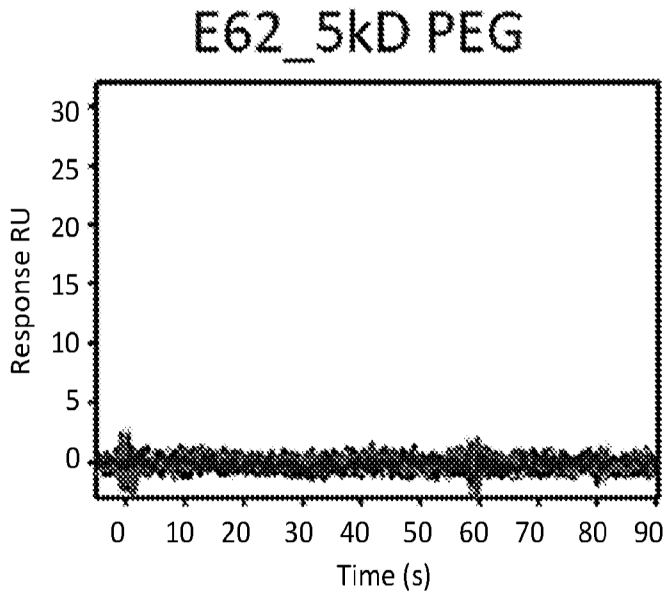

E62_5kD PEG
Response RU
30
25
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90
Time (s)

FIG. 4A (cont.)

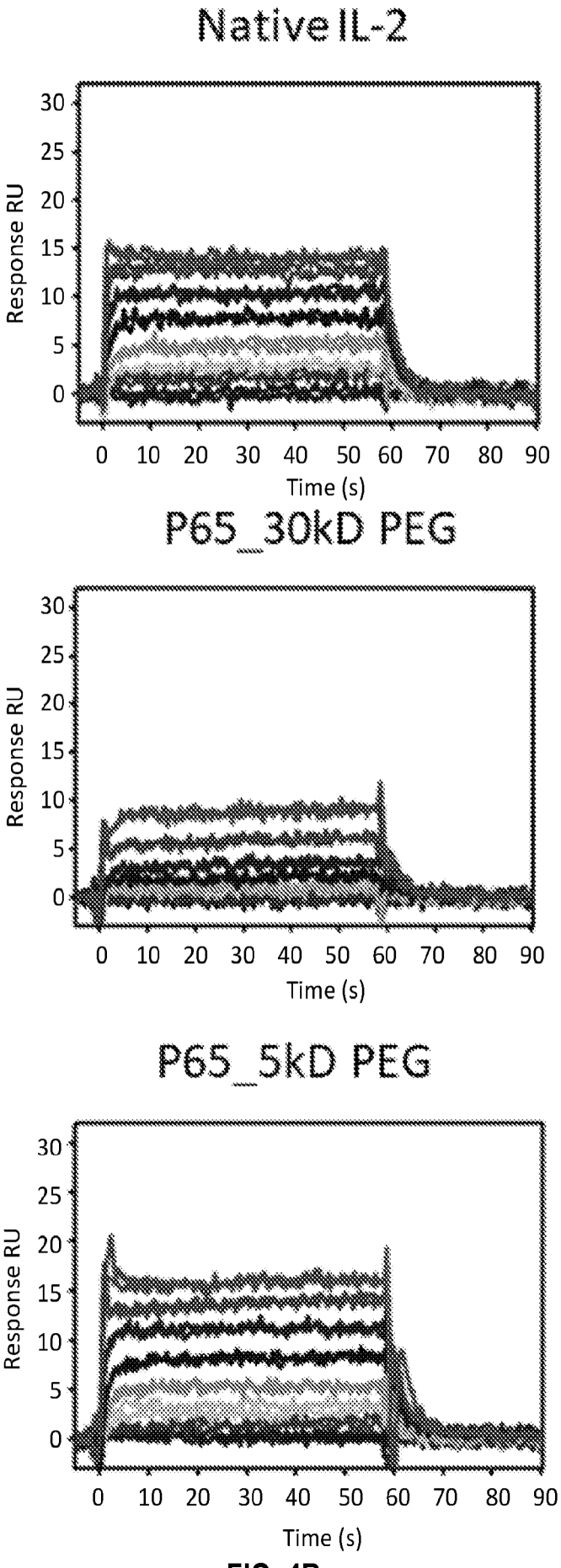
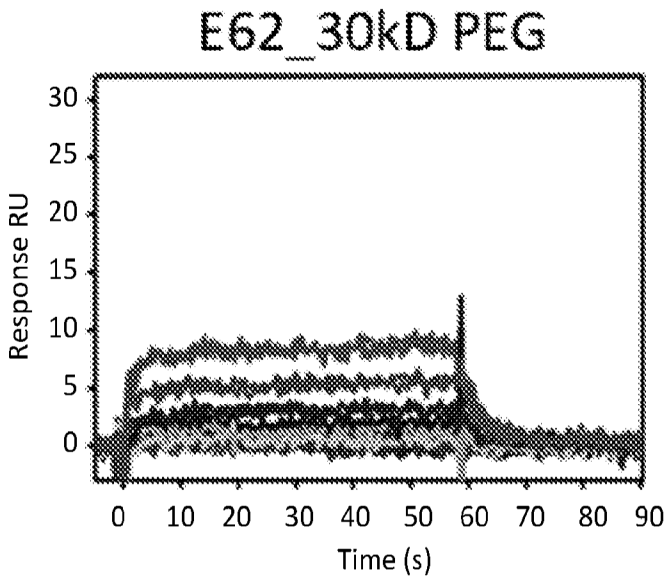

Native IL-2
Response RU
30
25
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90
Time (s)
P65_30kD PEG
Response RU
30
25
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90
Time (s)
P65_5kD PEG
Response RU
30
25
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90
Time (s)

FIG. 4B


E62_30kD PEG
Response RU
30
25
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90
Time (s)

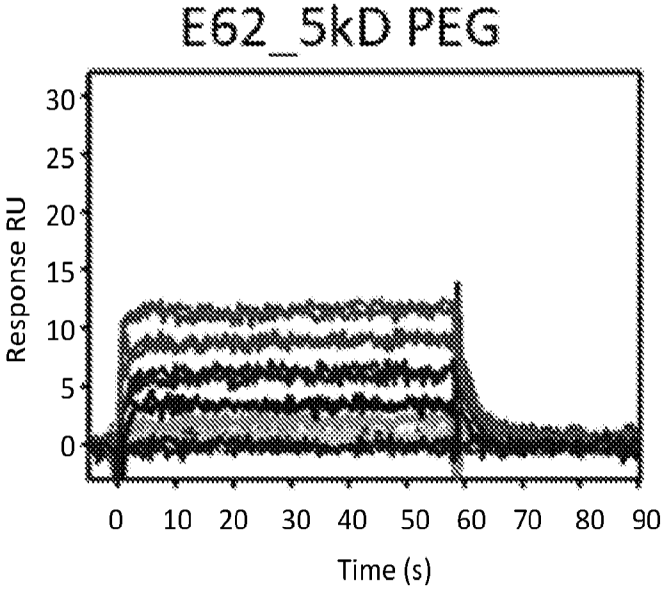

E62_5kD PEG
Response RU
30
25
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90
Time (s)

FIG. 4B (cont.)

rhIL-2

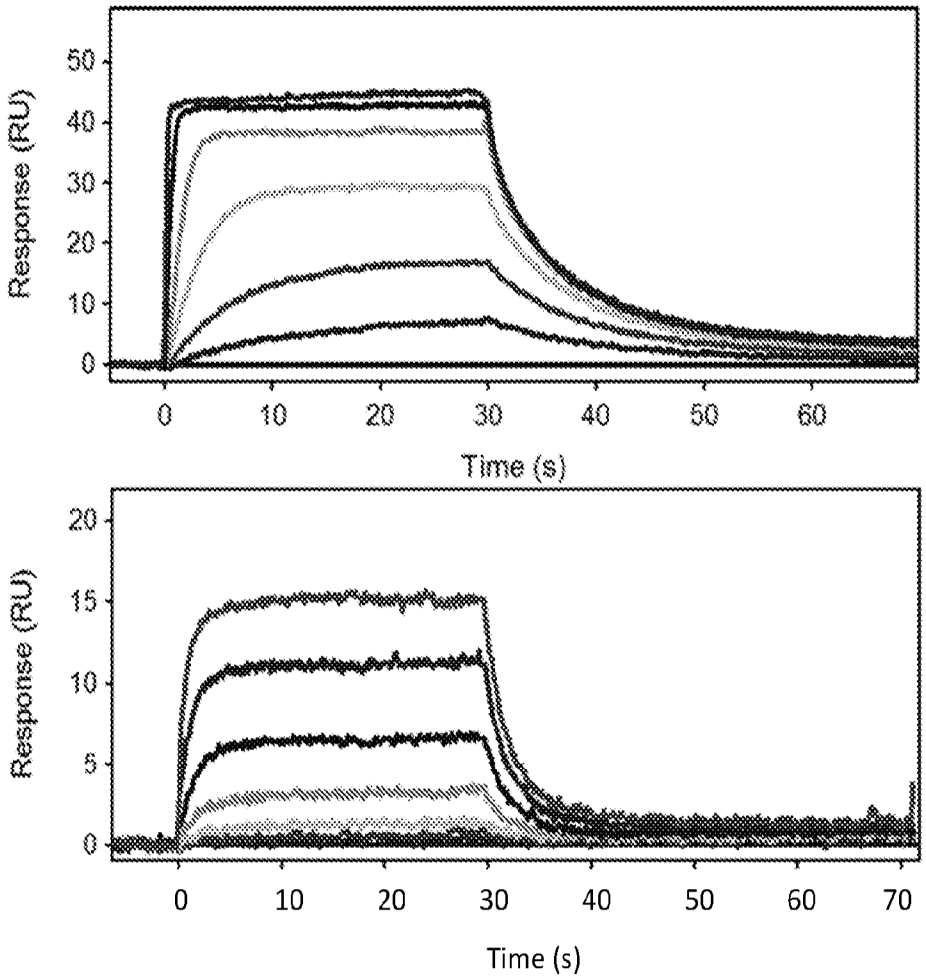

Response (RU)
50
40
30
20
10
0
0
10
20
30
40
50
60
Time (s)
IL-2 Rα
Response (RU)
20
15
10
5
0
0
10
20
30
40
50
60
70
Time (s)
IL-2 Rβ

FIG. 4C

F42_30kD

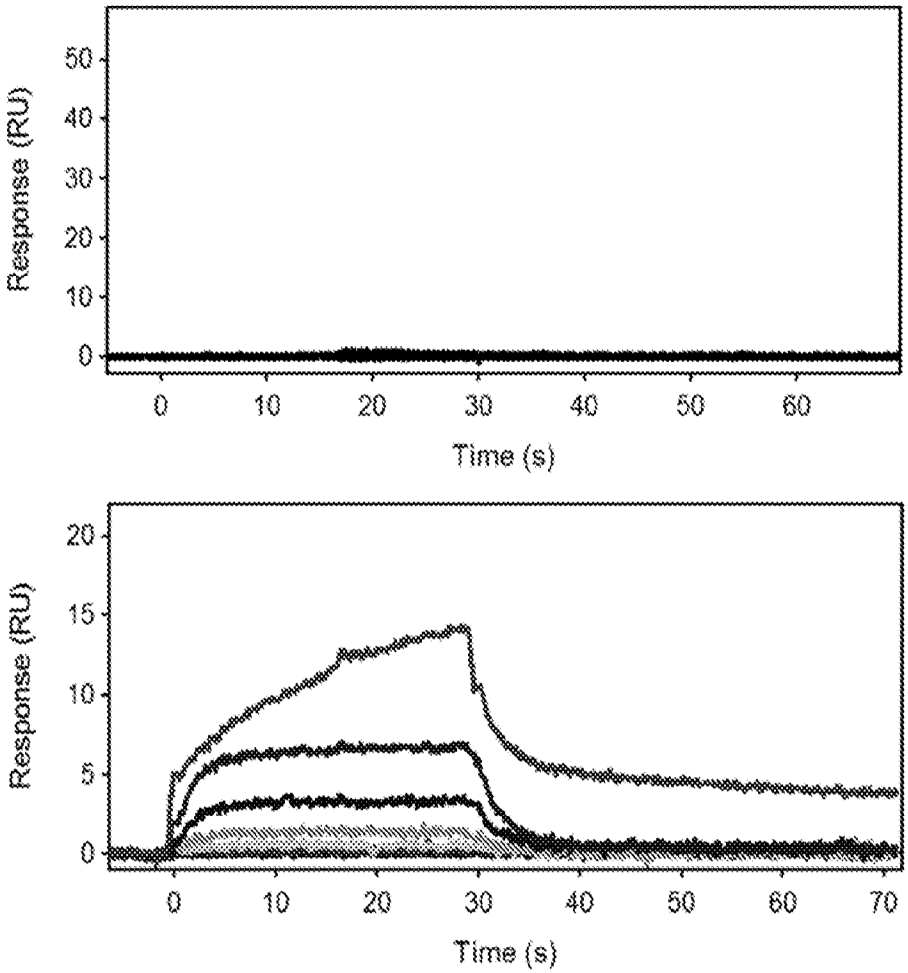


FIG. 4C (cont.)

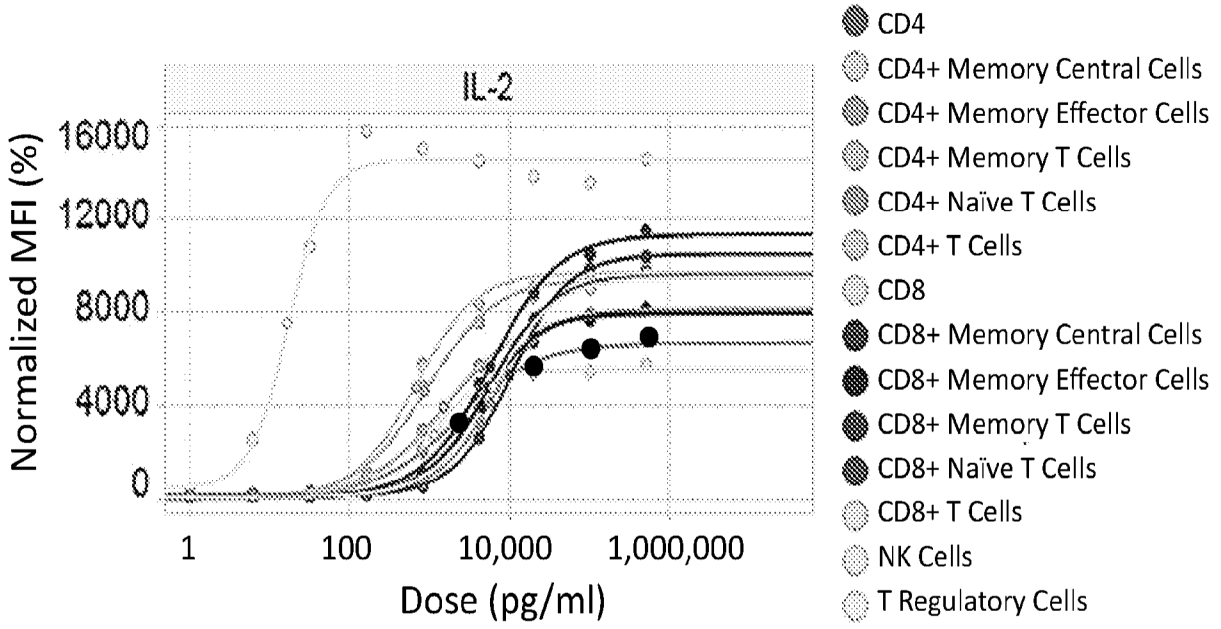

IL-2
Normalized MFI (%)
16000
12000
8000
4000
0
1
100
10,000
1,000,000
Dose (pg/ml)
CD4
CD4+ Memory Central Cells
CD4+ Memory Effector Cells
CD4+ Memory T Cells
CD4+ Naïve T Cells
CD4+ T Cells
CD8
CD8+ Memory Central Cells
CD8+ Memory Effector Cells
CD8+ Memory T Cells
CD8+ Naïve T Cells
CD8+ T Cells
NK Cells
T Regulatory Cells

FIG. 5A

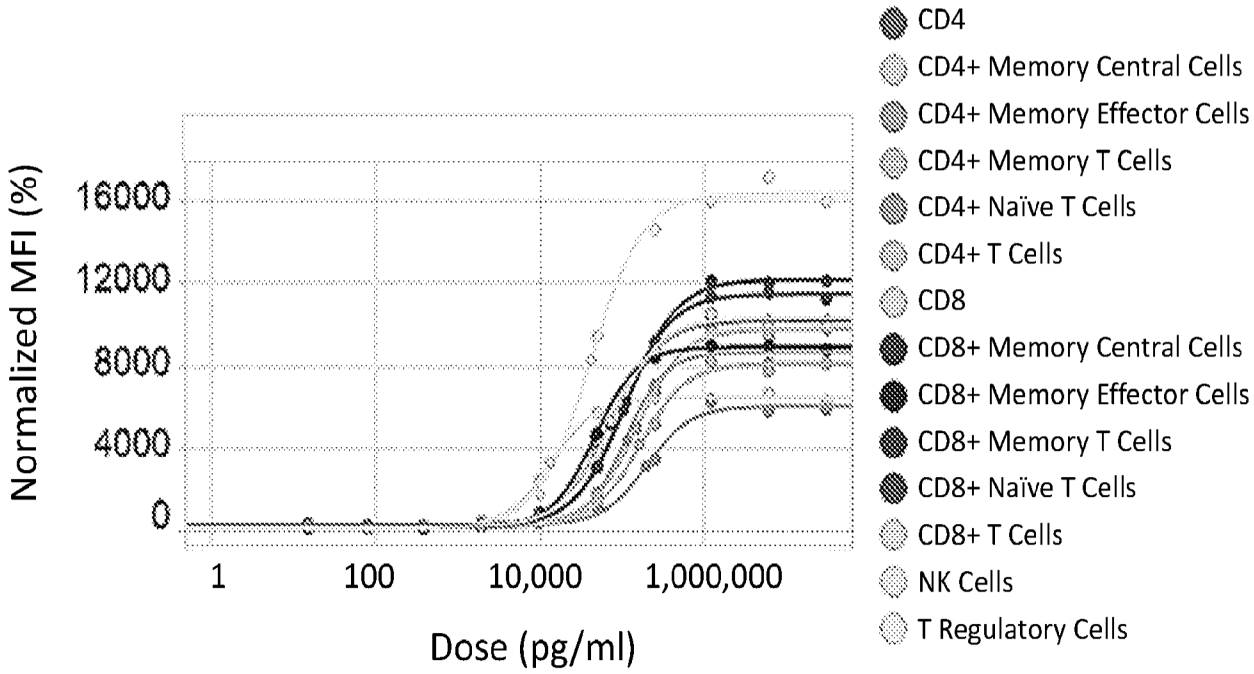

Normalized MFI (%)
16000
12000
8000
4000
0
1
100
10,000
1,000,000
Dose (pg/ml)
CD4
CD4+ Memory Central Cells
CD4+ Memory Effector Cells
CD4+ Memory T Cells
CD4+ Naïve T Cells
CD4+ T Cells
CD8
CD8+ Memory Central Cells
CD8+ Memory Effector Cells
CD8+ Memory T Cells
CD8+ Naïve T Cells
CD8+ T Cells
NK Cells
T Regulatory Cells

FIG. 5B

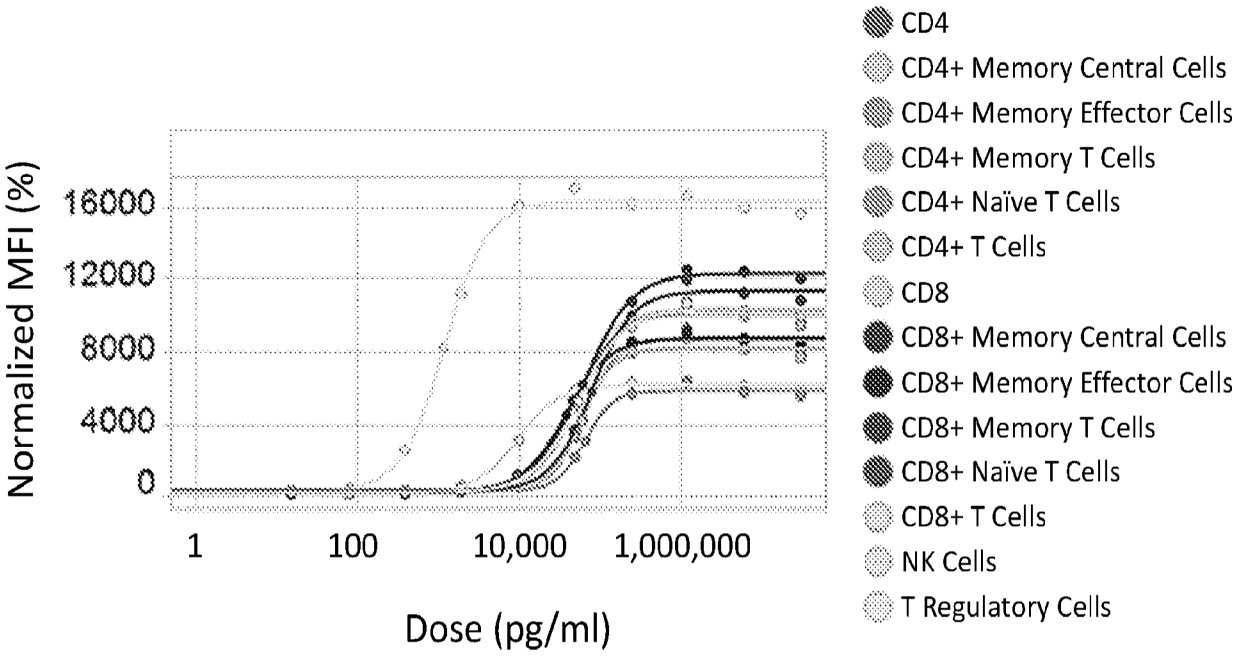

Normalized MFI (%)
16000
12000
8000
4000
0
1
100
10,000
1,000,000
Dose (pg/ml)
CD4
CD4+ Memory Central Cells
CD4+ Memory Effector Cells
CD4+ Memory T Cells
CD4+ Naïve T Cells
CD4+ T Cells
CD8
CD8+ Memory Central Cells
CD8+ Memory Effector Cells
CD8+ Memory T Cells
CD8+ Naïve T Cells
CD8+ T Cells
NK Cells
T Regulatory Cells

FIG. 5C

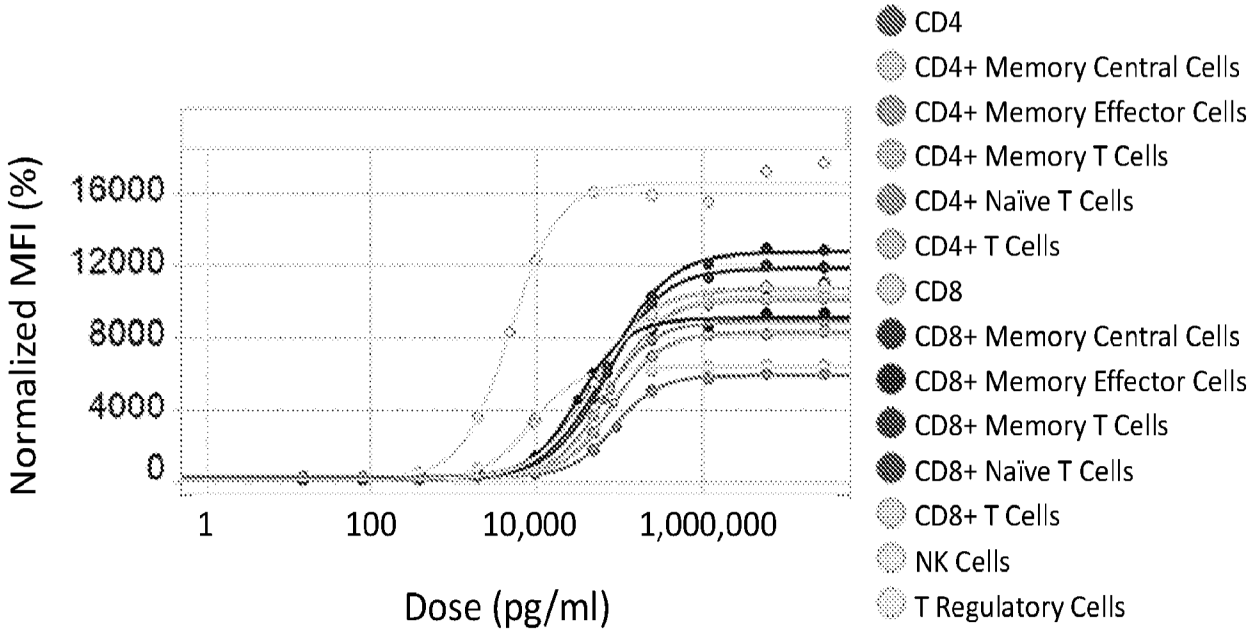

Normalized MFI (%)
16000
12000
8000
4000
0
1
100
10,000
1,000,000
Dose (pg/ml)
CD4
CD4+ Memory Central Cells
CD4+ Memory Effector Cells
CD4+ Memory T Cells
CD4+ Naïve T Cells
CD4+ T Cells
CD8
CD8+ Memory Central Cells
CD8+ Memory Effector Cells
CD8+ Memory T Cells
CD8+ Naïve T Cells
CD8+ T Cells
NK Cells
T Regulatory Cells

FIG. 5D

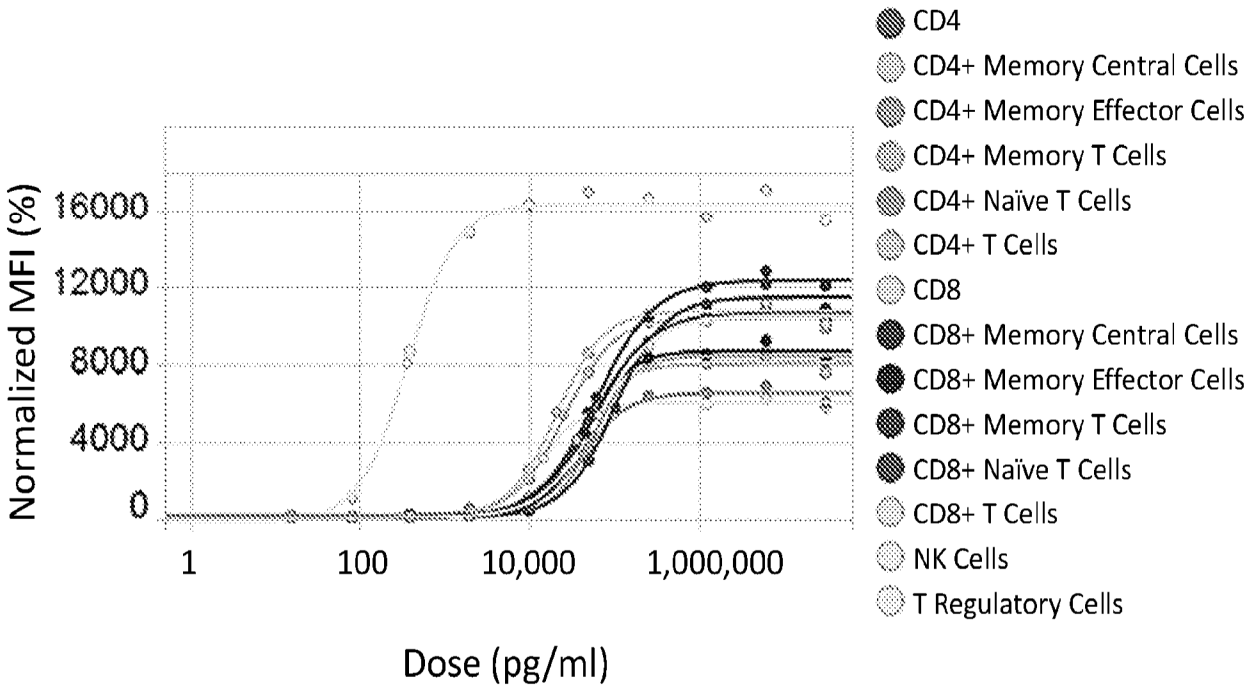

Normalized MFI (%)
16000
12000
8000
4000
0
1
100
10,000
1,000,000
Dose (pg/ml)
CD4
CD4+ Memory Central Cells
CD4+ Memory Effector Cells
CD4+ Memory T Cells
CD4+ Naïve T Cells
CD4+ T Cells
CD8
CD8+ Memory Central Cells
CD8+ Memory Effector Cells
CD8+ Memory T Cells
CD8+ Naïve T Cells
CD8+ T Cells
NK Cells
T Regulatory Cells

FIG. 5E

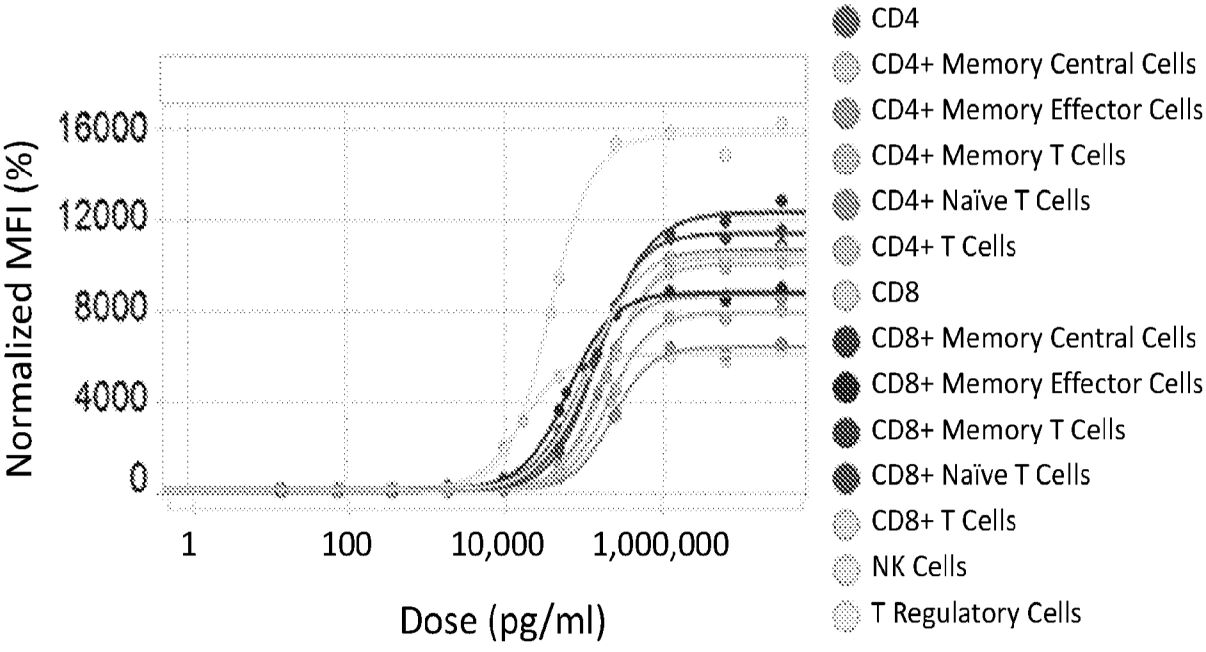

Normalized MFI (%)
16000
12000
8000
4000
0
1
100
10,000
1,000,000
Dose (pg/ml)
CD4
CD4+ Memory Central Cells
CD4+ Memory Effector Cells
CD4+ Memory T Cells
CD4+ Naïve T Cells
CD4+ T Cells
CD8
CD8+ Memory Central Cells
CD8+ Memory Effector Cells
CD8+ Memory T Cells
CD8+ Naïve T Cells
CD8+ T Cells
NK Cells
T Regulatory Cells

FIG. 5F

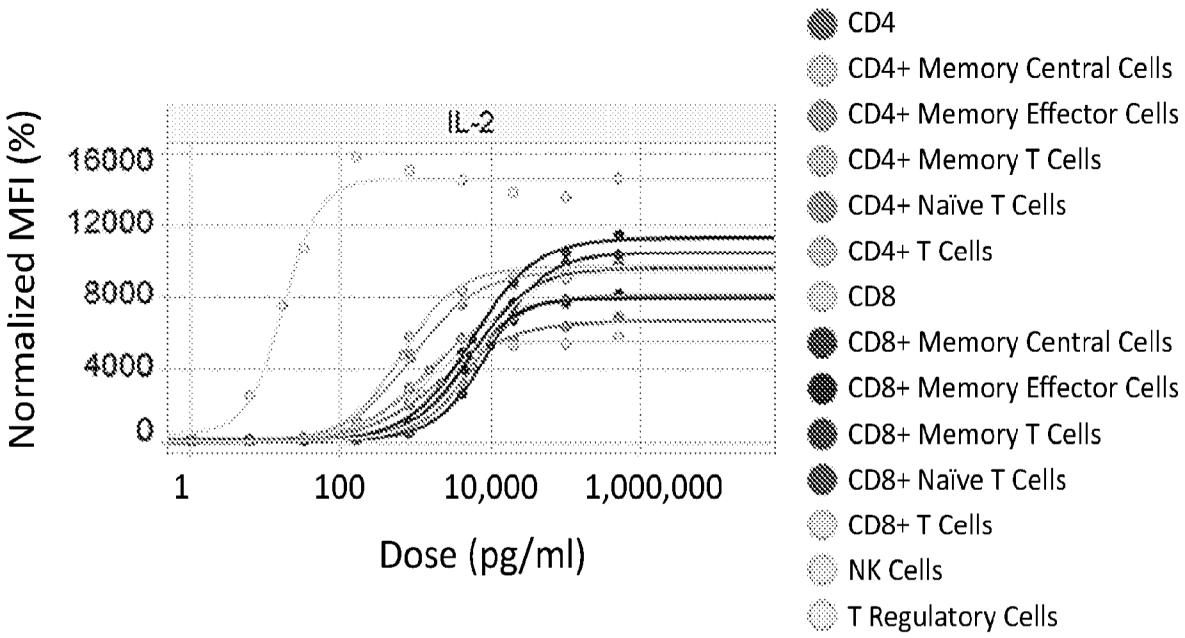

IL-2
Normalized MFI (%)
16000
12000
8000
4000
0
1
100
10,000
1,000,000
Dose (pg/ml)
CD4
CD4+ Memory Central Cells
CD4+ Memory Effector Cells
CD4+ Memory T Cells
CD4+ Naïve T Cells
CD4+ T Cells
CD8
CD8+ Memory Central Cells
CD8+ Memory Effector Cells
CD8+ Memory T Cells
CD8+ Naïve T Cells
CD8+ T Cells
NK Cells
T Regulatory Cells

FIG. 6A

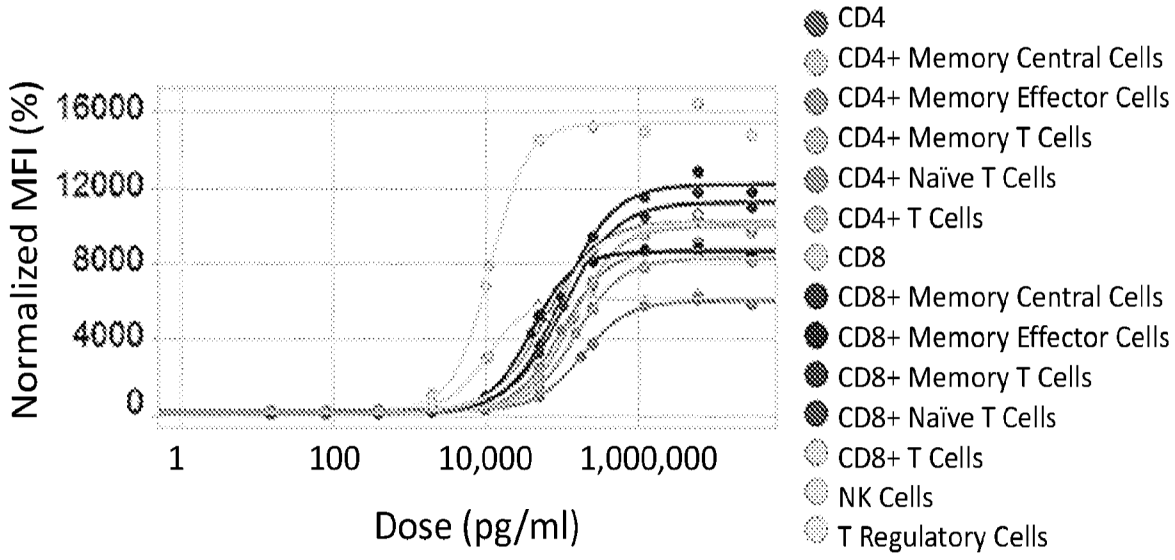

Normalized MFI (%)
16000
12000
8000
4000
0
1
100
10,000
1,000,000
Dose (pg/ml)
CD4
CD4+ Memory Central Cells
CD4+ Memory Effector Cells
CD4+ Memory T Cells
CD4+ Naïve T Cells
CD4+ T Cells
CD8
CD8+ Memory Central Cells
CD8+ Memory Effector Cells
CD8+ Memory T Cells
CD8+ Naïve T Cells
CD8+ T Cells
NK Cells
T Regulatory Cells

FIG. 6B

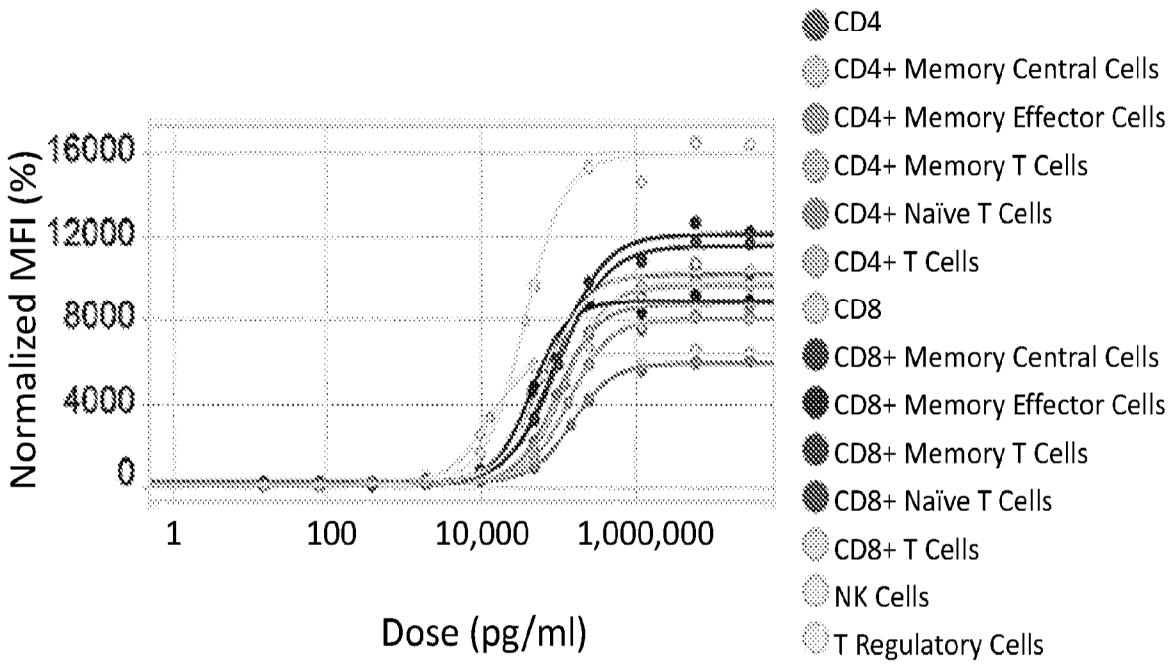

Normalized MFI (%)
16000
12000
8000
4000
0
1
100
10,000
1,000,000
Dose (pg/ml)
CD4
CD4+ Memory Central Cells
CD4+ Memory Effector Cells
CD4+ Memory T Cells
CD4+ Naïve T Cells
CD4+ T Cells
CD8
CD8+ Memory Central Cells
CD8+ Memory Effector Cells
CD8+ Memory T Cells
CD8+ Naïve T Cells
CD8+ T Cells
NK Cells
T Regulatory Cells

FIG. 6C

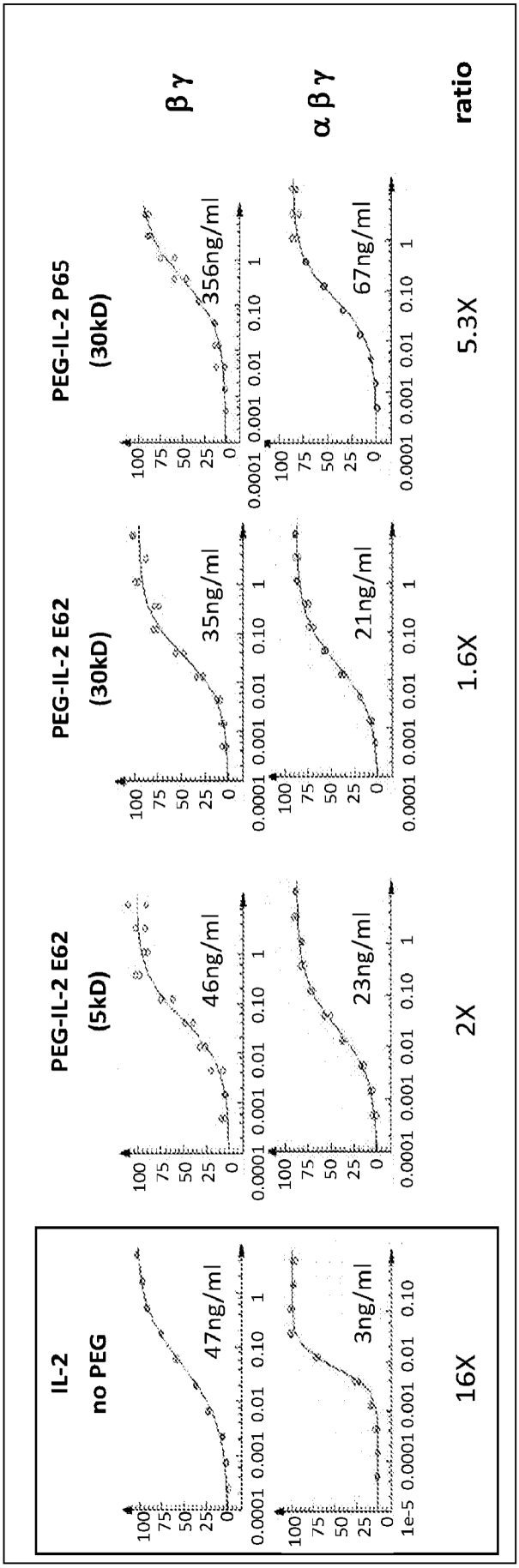

IL-2
no PEG
PEG-IL-2 E62
(5kD)
PEG-IL-2 E62
(30kD)
PEG-IL-2 P65
(30kD)
β γ
α β γ
ratio
47ng/ml
3ng/ml
46ng/ml
23ng/ml
35ng/ml
21ng/ml
356ng/ml
67ng/ml
16X
2X
1.6X
5.3X

FIG. 7

Mean (±SD) Plasma Concentration versus Time Profiles Following a Single IV Bolus Dose of aldesleukin or P65_30KD and E62_30KD, E62_5KD to Female C57BL/6 mice

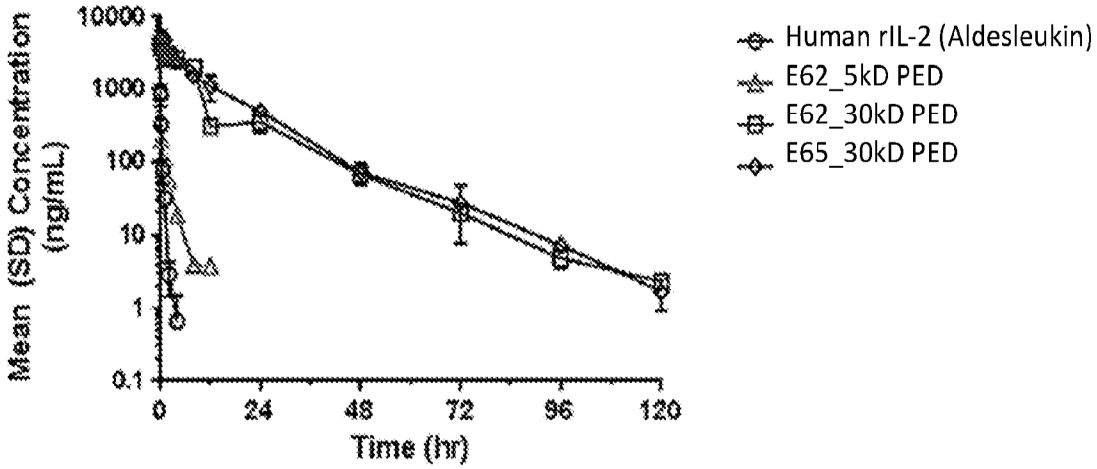


FIG. 8

Sustained increase in peripheral blood pSTAT5+ CD8+ T in response to P65_30KD

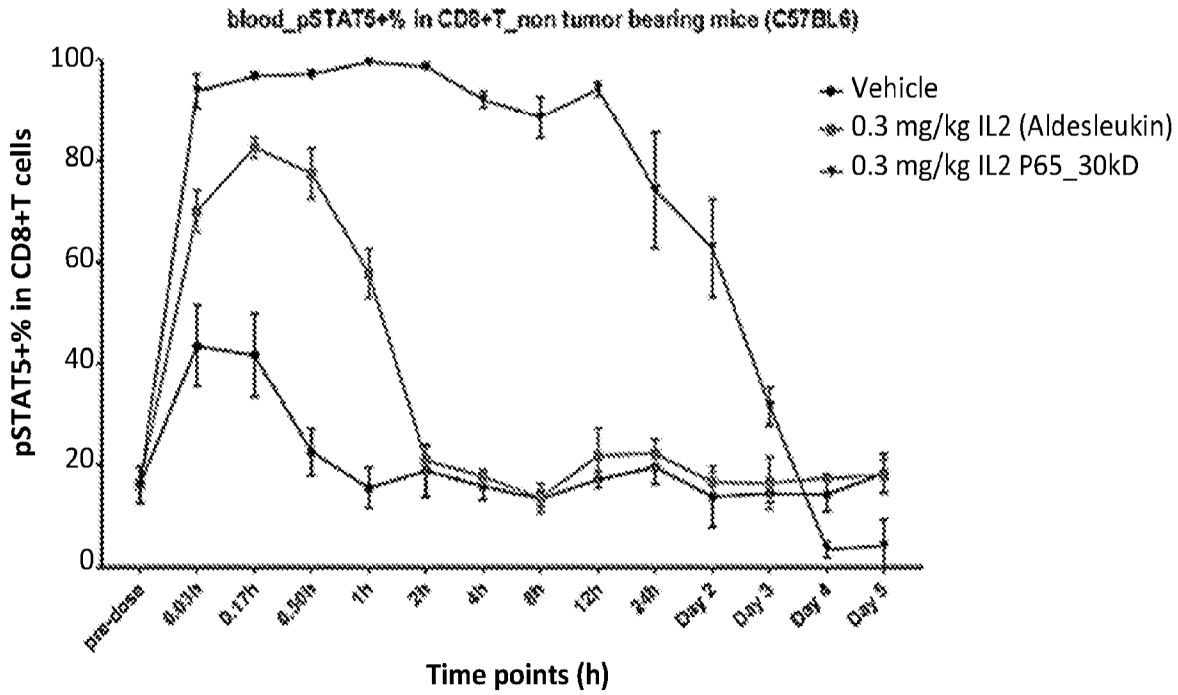


FIG. 9

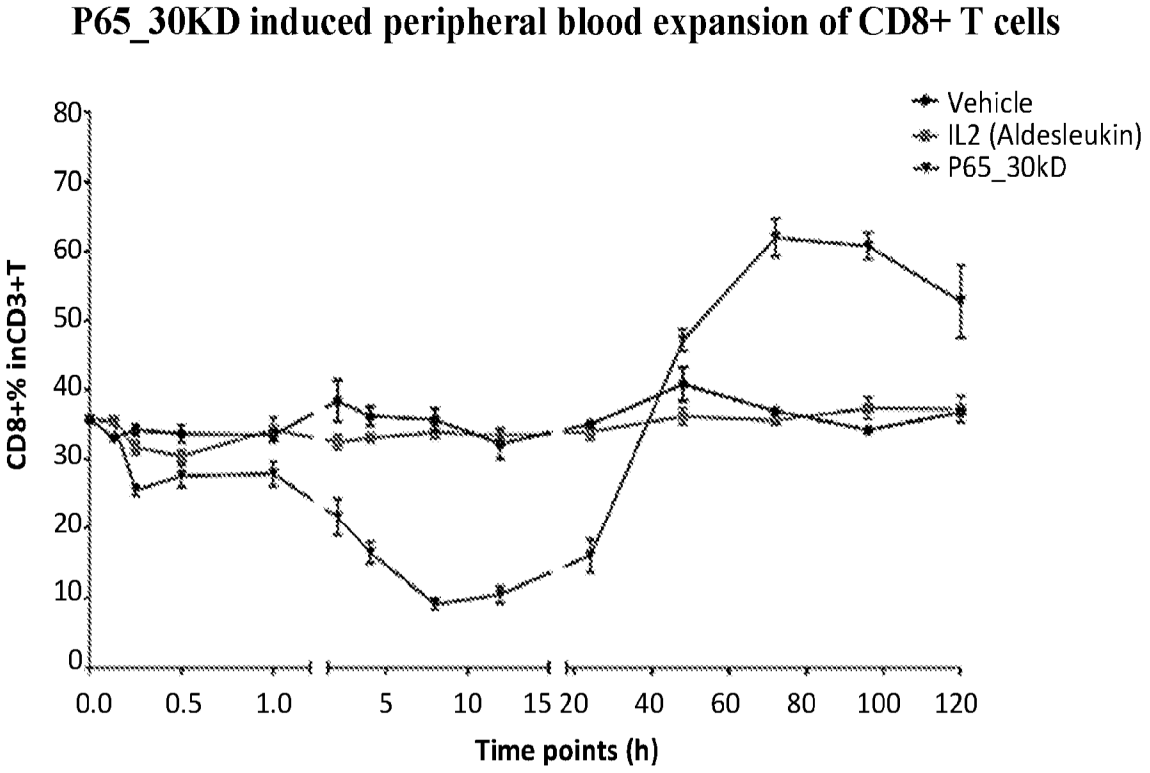


FIG. 10A

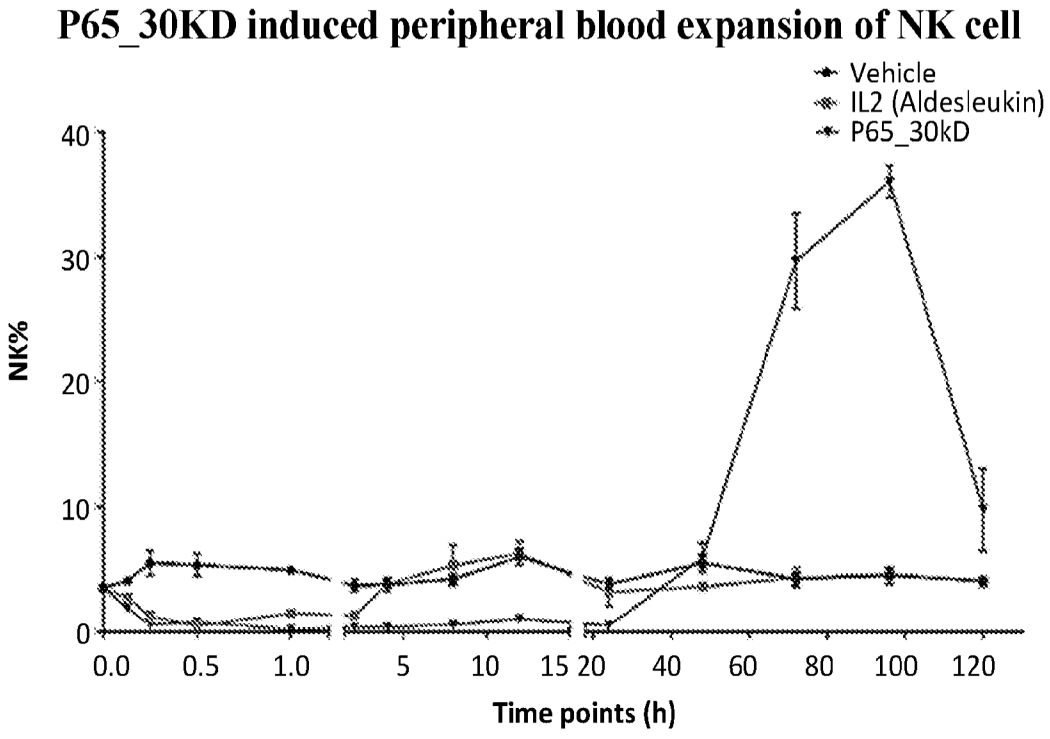


FIG. 10B

P65_30KD did not induce the proliferation of CD4+ Tregs

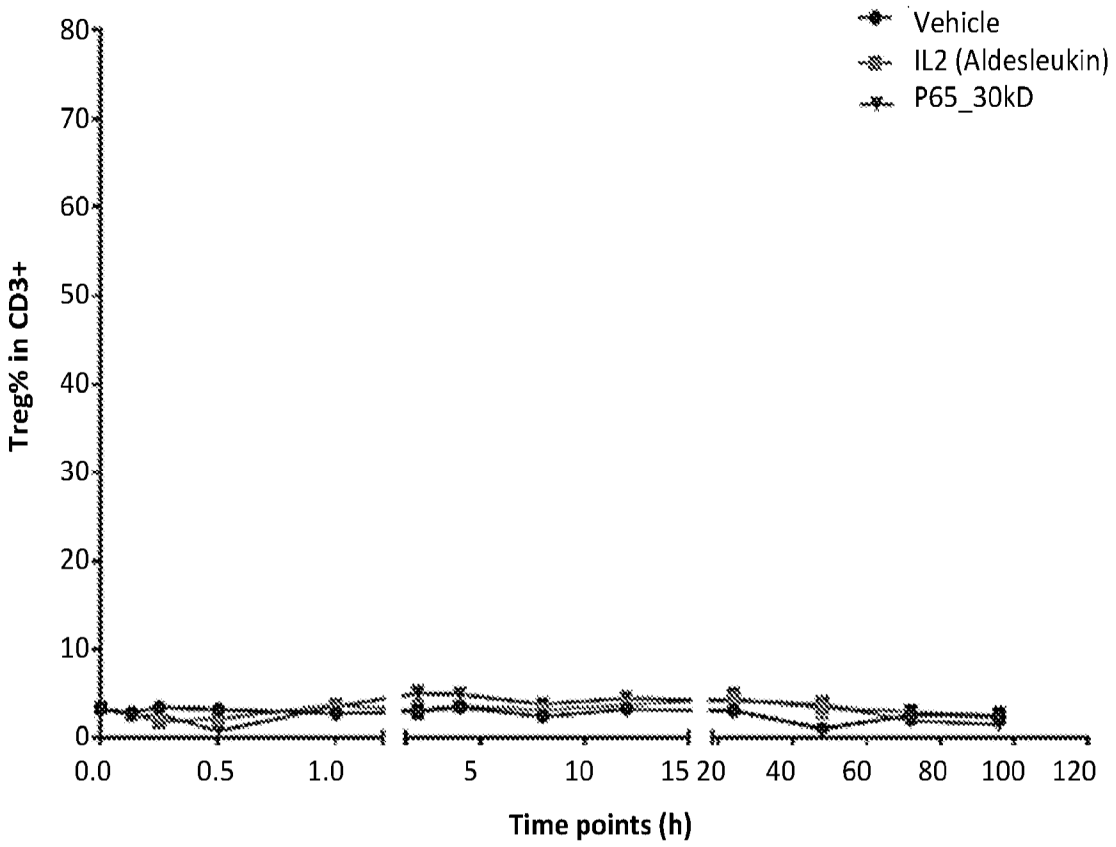


FIG. 10C

P65_30KD induces CD8+ T effector memory cell expansion

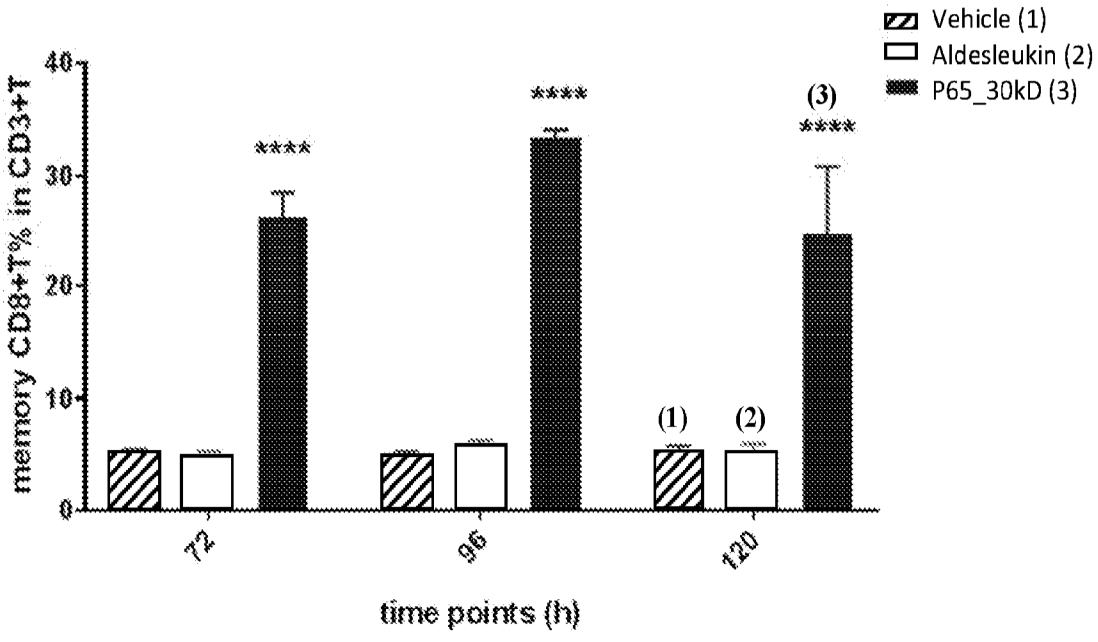


FIG. 11A

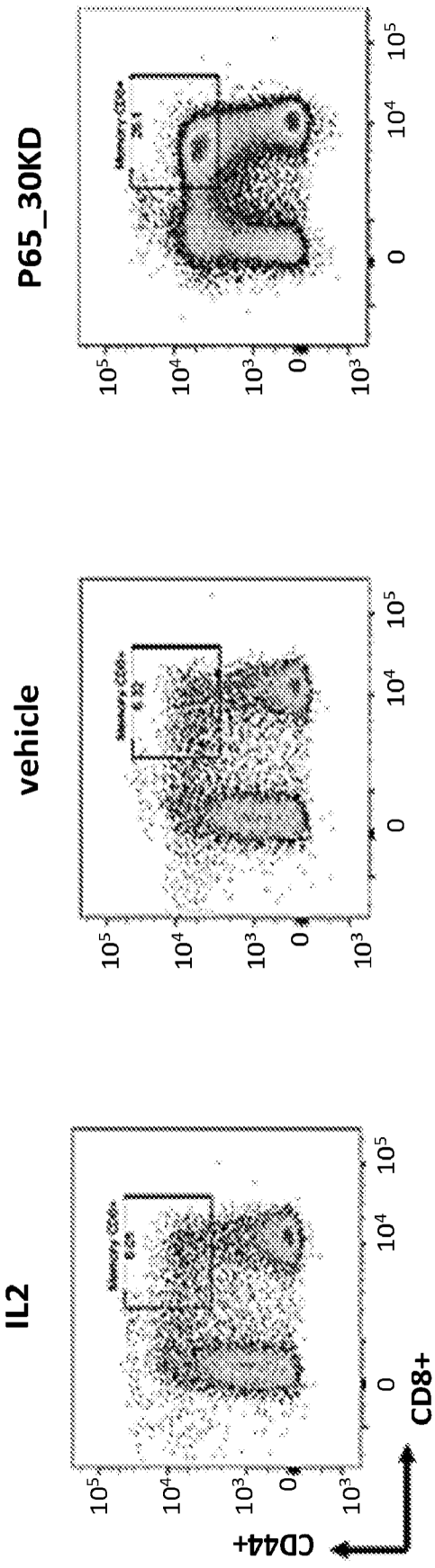


FIG. 11B

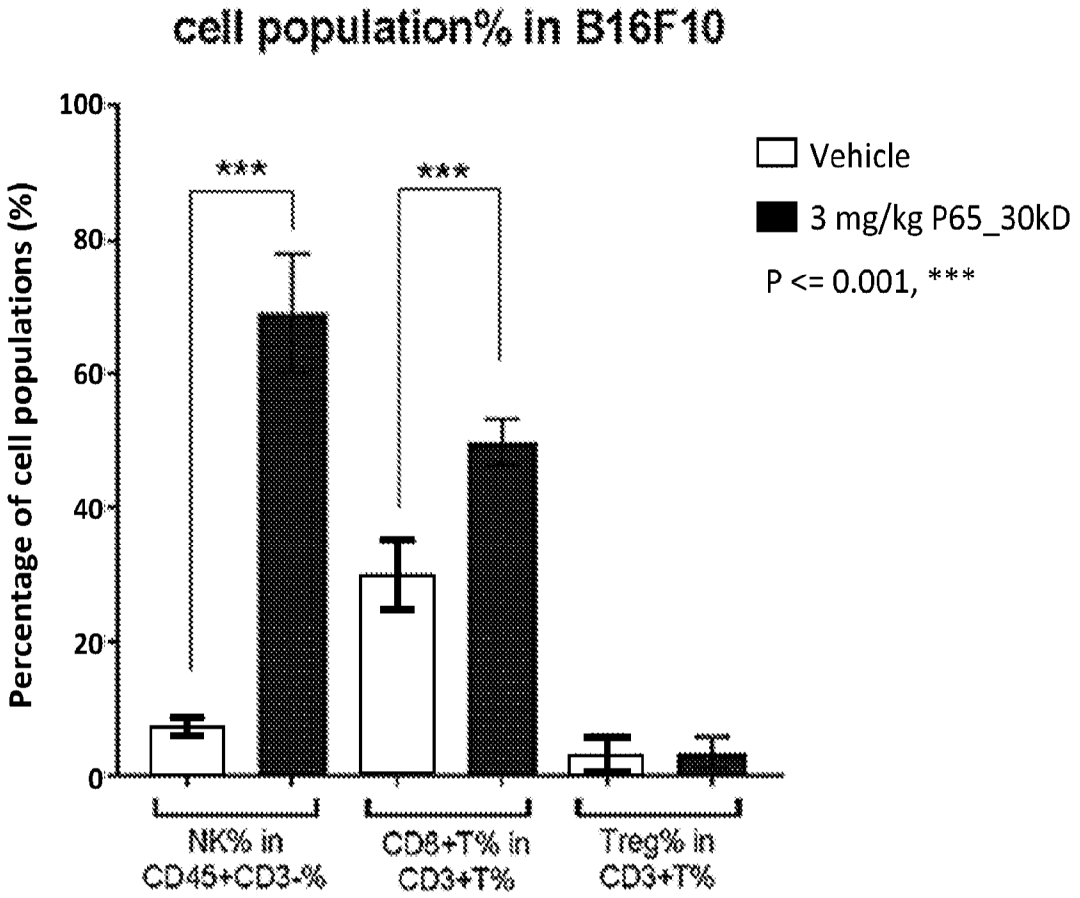


FIG. 12A

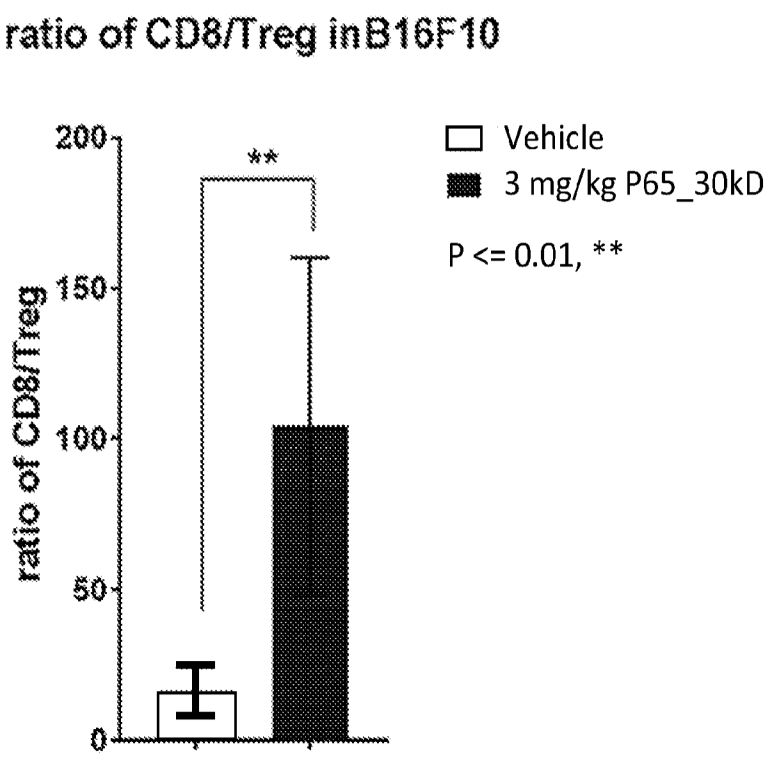


FIG. 12B

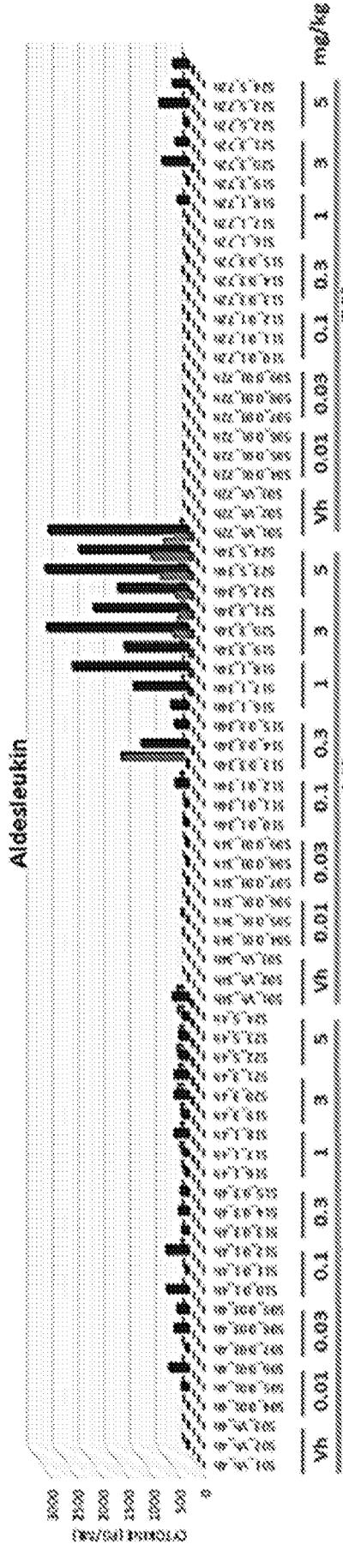


FIG. 13A

FIG. 13B

White Blood Cell, Lymphocyte and Eosinophil Counts (Mean ±SD) following a Single IV Dose of P65_30KD to Male Cynomolgus monkeys

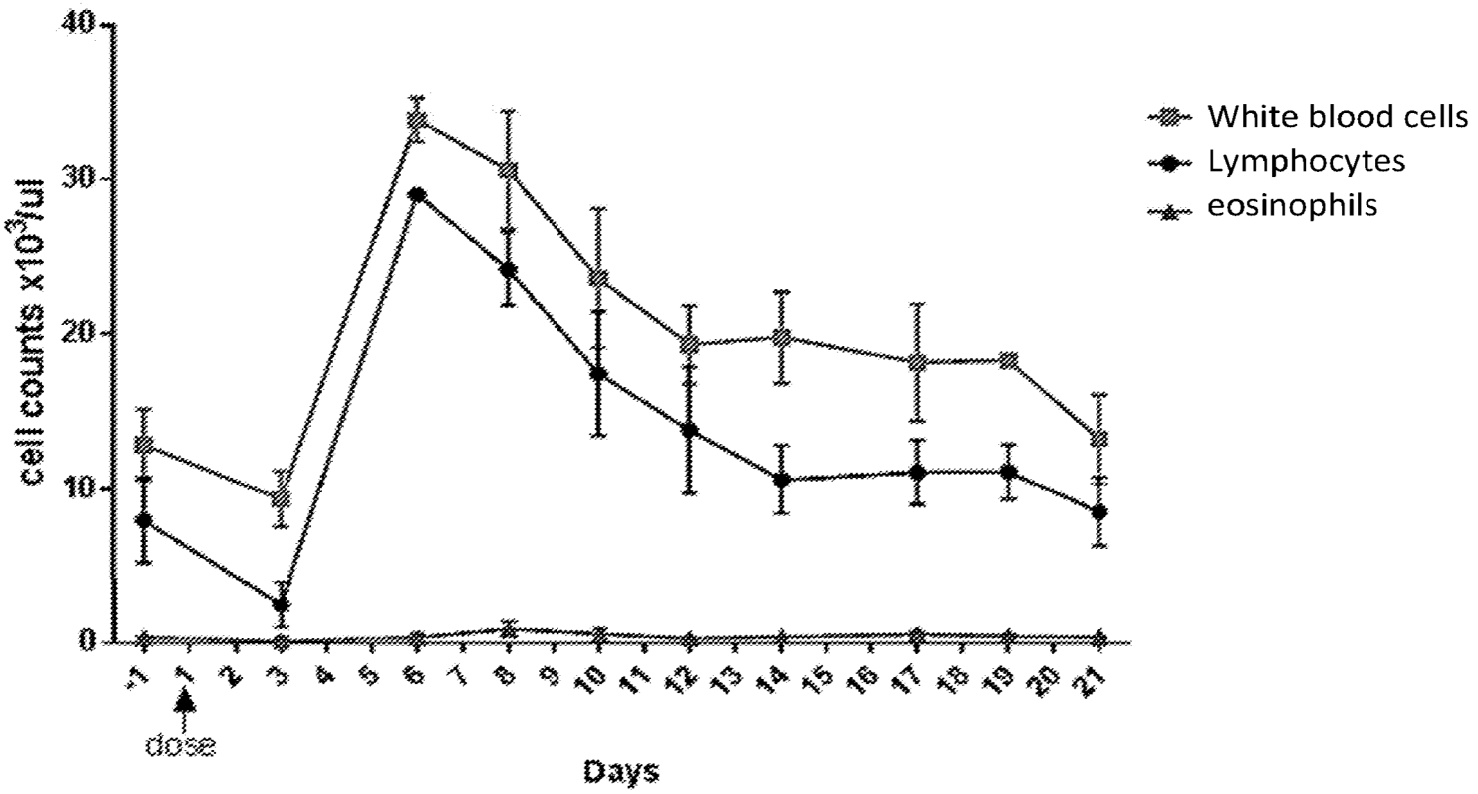


FIG. 14

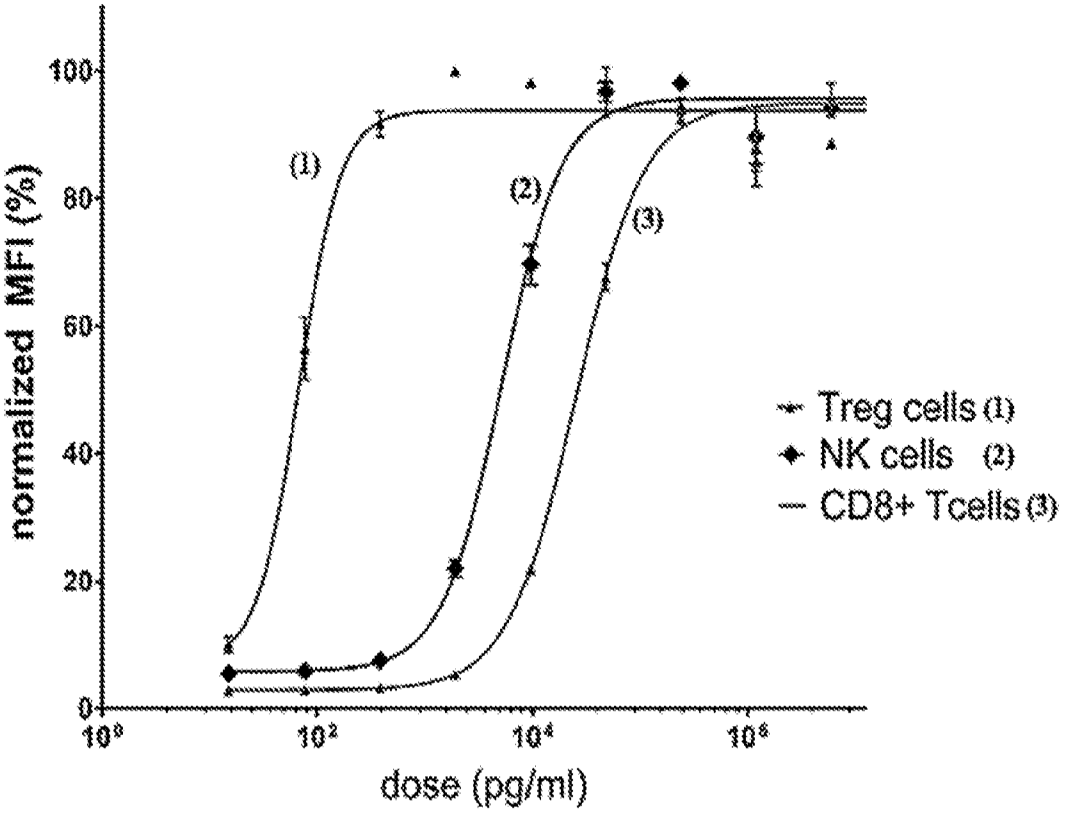


FIG. 15A

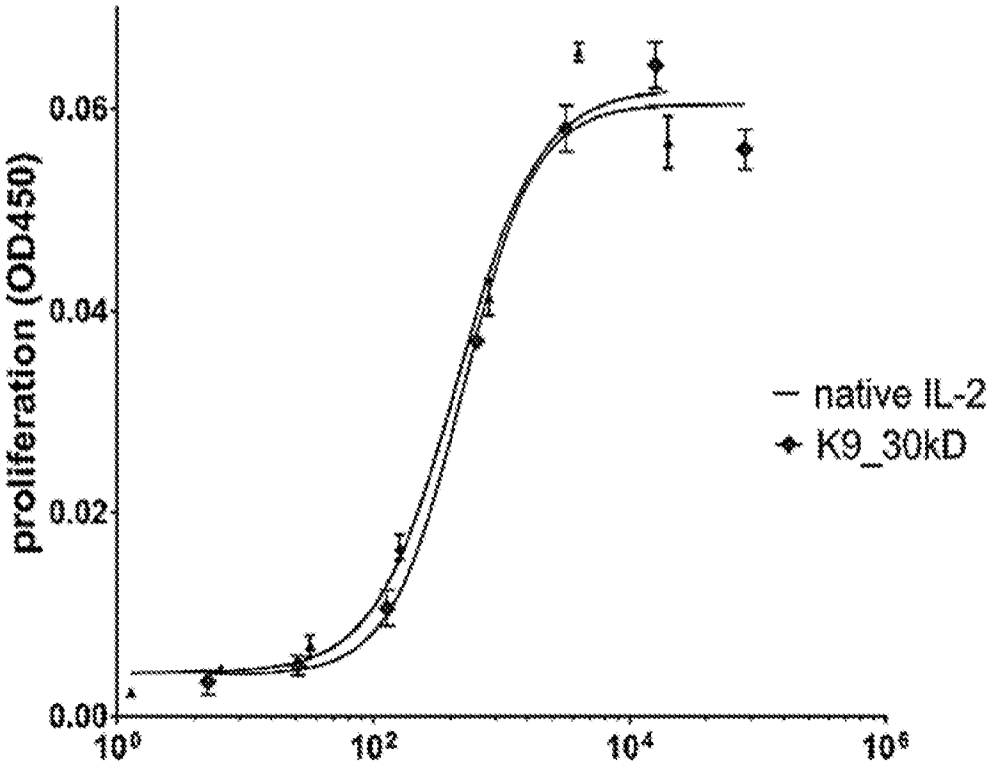


FIG. 15B

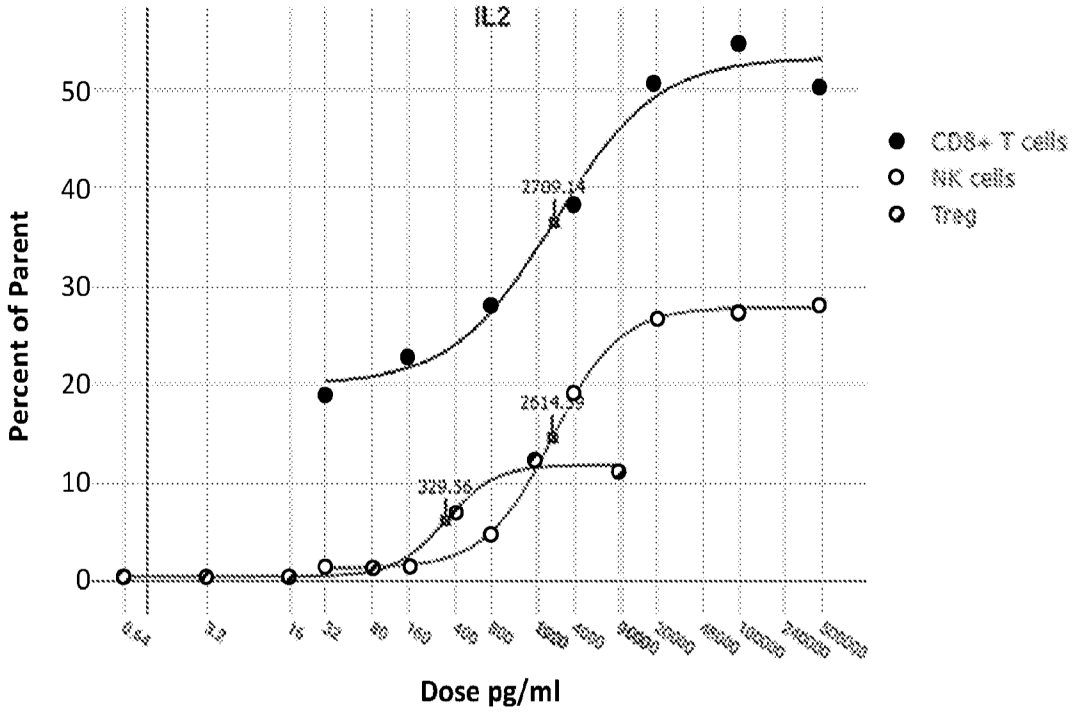


FIG. 16A

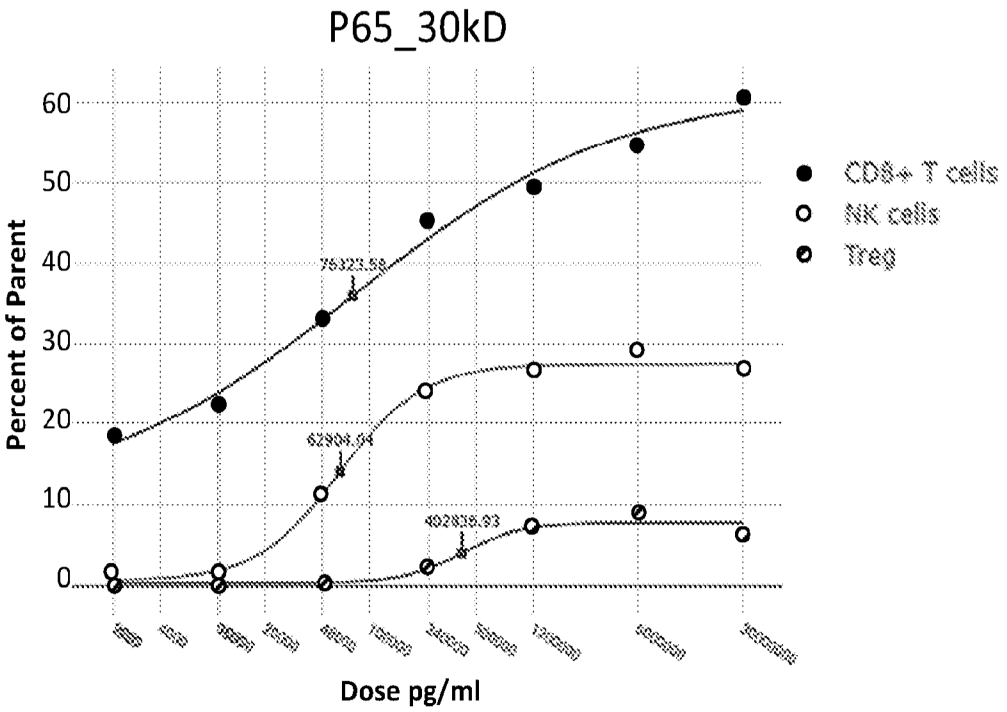


FIG. 16B

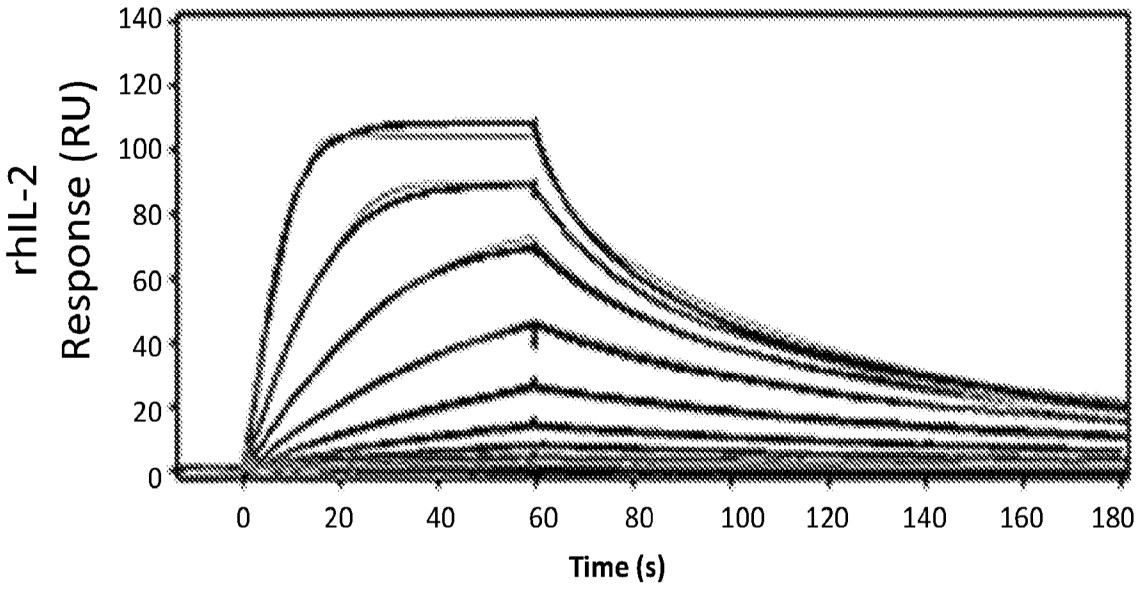


FIG. 17A

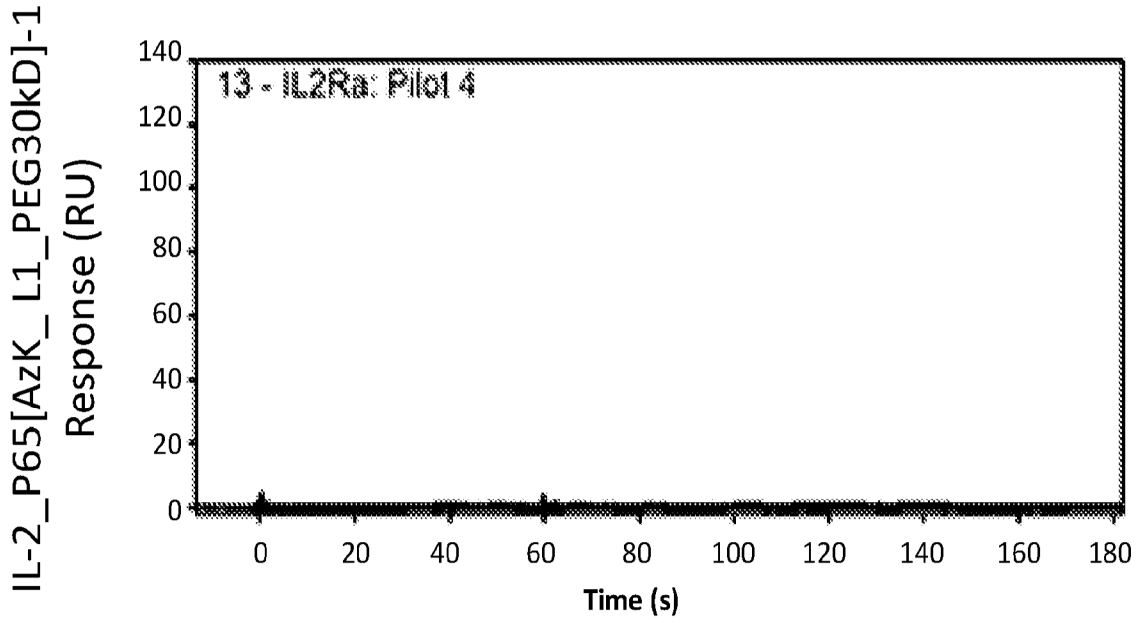


FIG. 17B

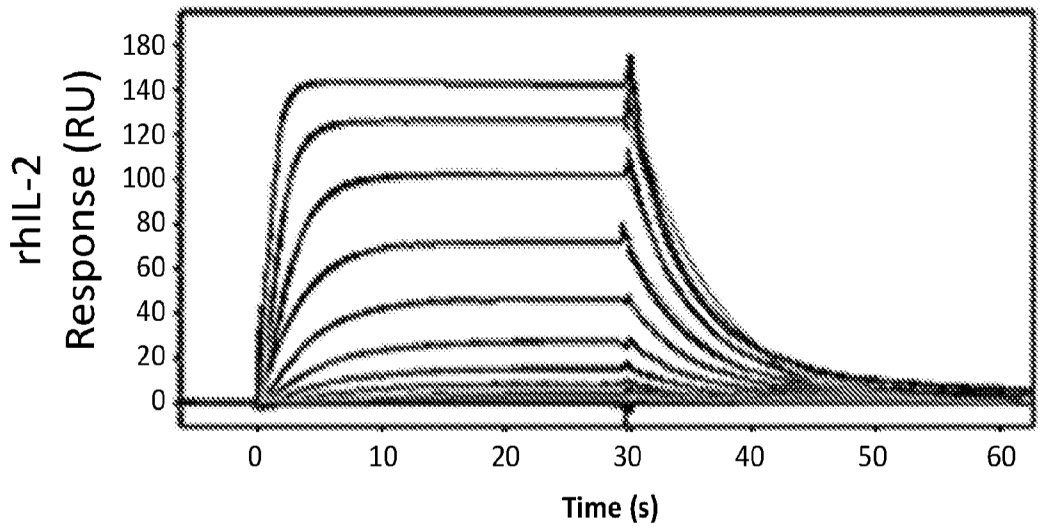


FIG. 17C

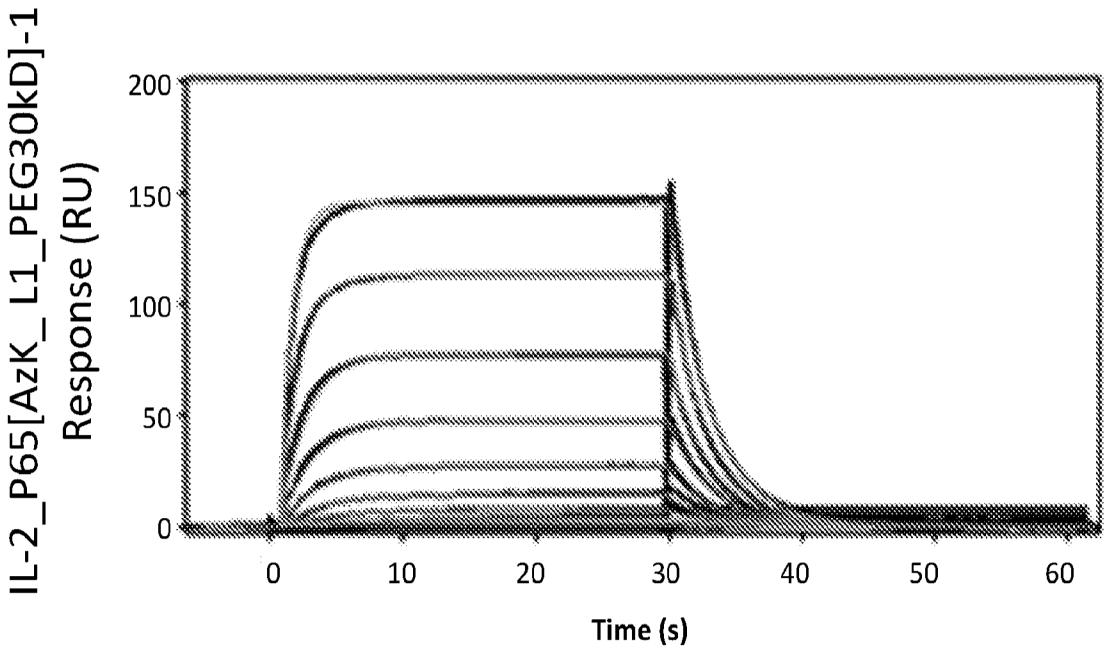


FIG. 17D

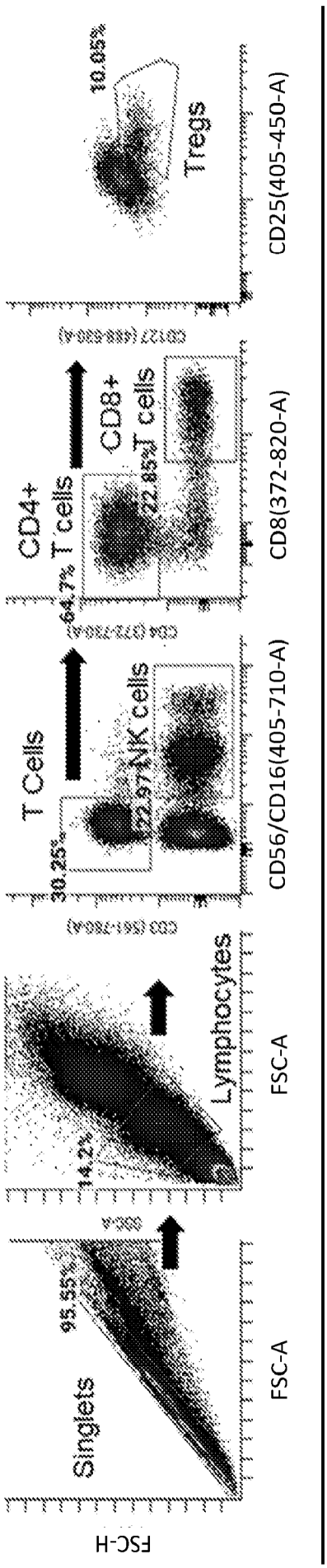

FSC-H
Singlets
95.55%
FSC-A
SSC-A
14.2%
Lymphocytes
FSC-A
CD3 (561-780-A)
30.25%
T Cells
22.97%NK cells
CD56/CD16(405-710-A)
CD4 (372-730-A)
64.7% T cells
CD4+
22.85%T cells
CD8+
CD8(372-820-A)
CD127 (488-530-A)
10.05%
Tregs
CD25(405-450-A)

FIG. 18

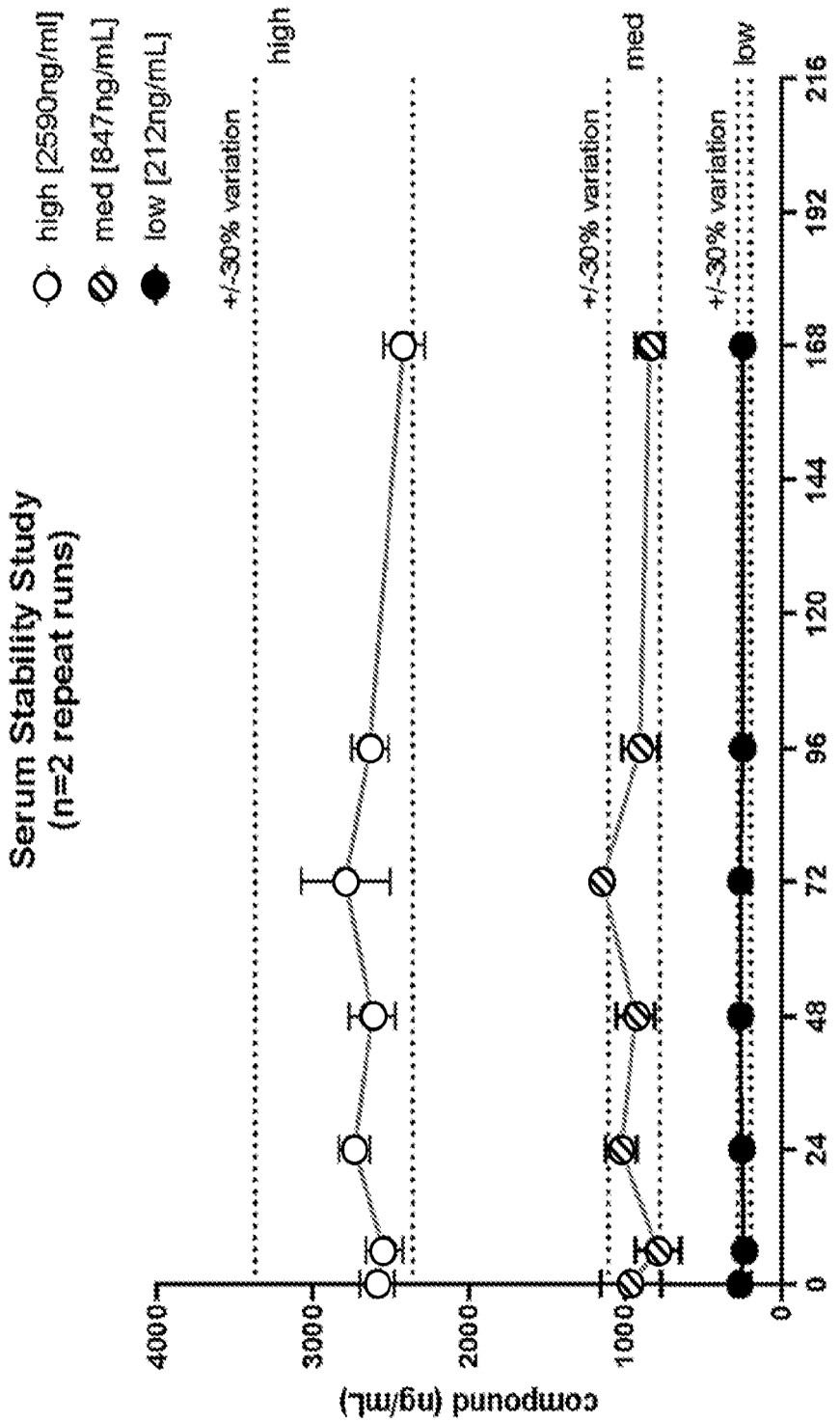

Serum Stability Study
(n=2 repeat runs)
high [2590ng/ml]
med [847ng/mL]
low [212ng/mL]
+/-30% variation
high
+/-30% variation
med
+/-30% variation
low
compound (ng/mL)
4000
3000
2000
1000
0
0
24
48
72
96
120
144
168
192
216
Time (h)

FIG. 19