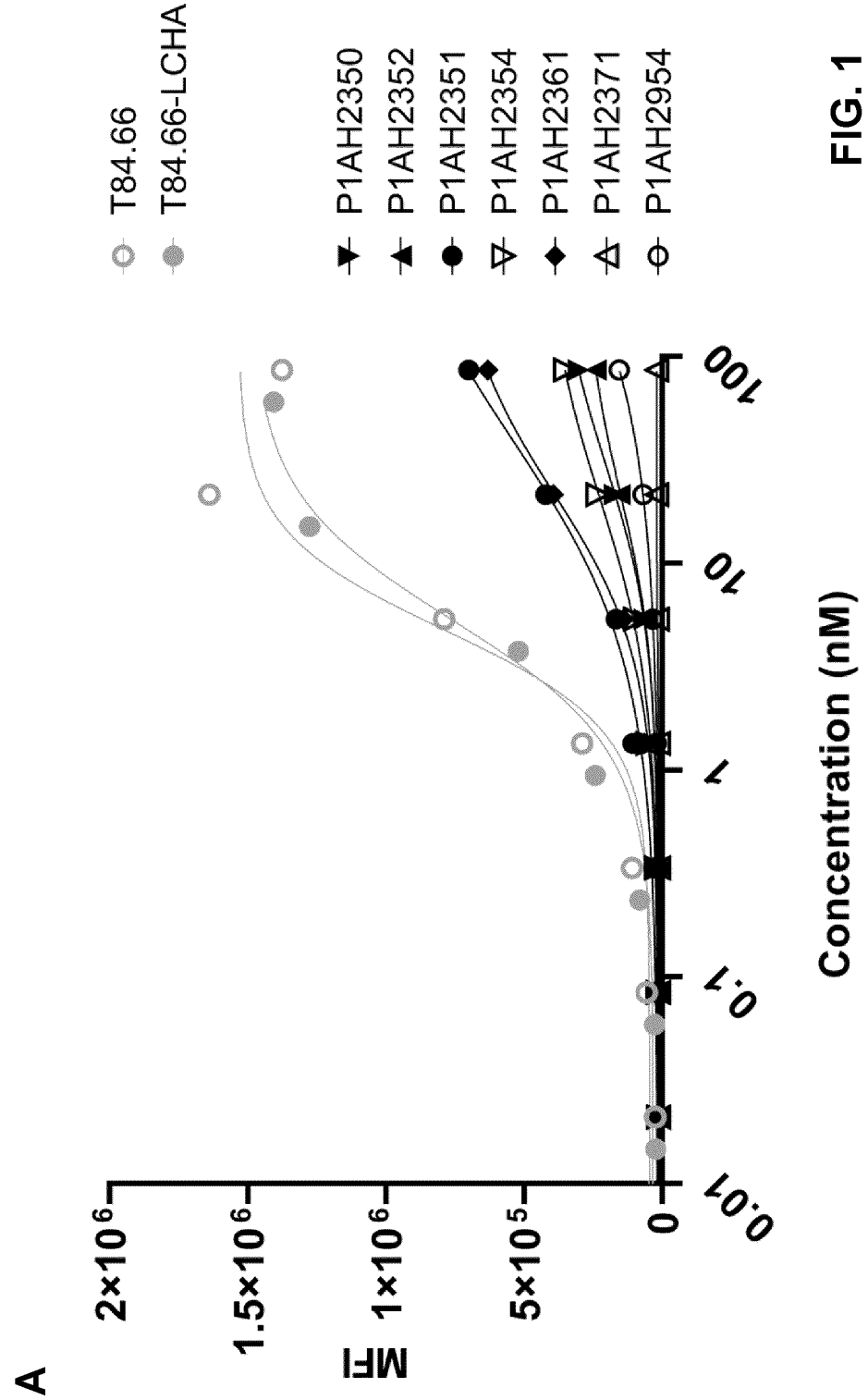

A
MFI
2×10^6
1.5×10^6
1×10^6
5×10^5
0
0.01
0.1
1
10
100
Concentration (nM)
T84.66
T84.66-LCHA
P1AH2350
P1AH2352
P1AH2351
P1AH2354
P1AH2361
P1AH2371
P1AH2954

FIG. 1

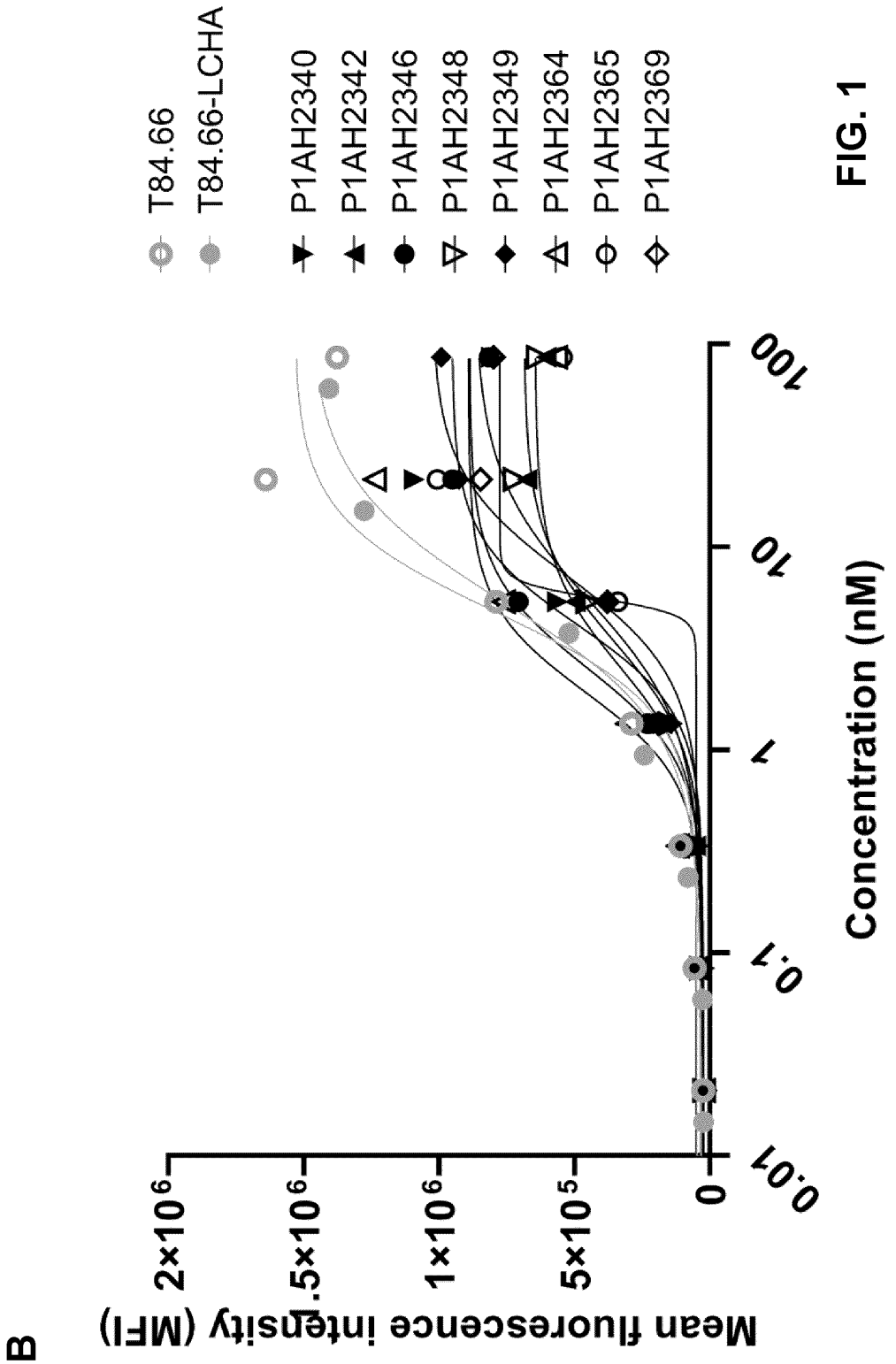

B
Mean fluorescence intensity (MFI)
2×10^6
1.5×10^6
1×10^6
5×10^5
0
0.01
0.1
1
10
100
Concentration (nM)
T84.66
T84.66-LCHA
P1AH2340
P1AH2342
P1AH2346
P1AH2348
P1AH2349
P1AH2364
P1AH2365
P1AH2369

FIG. 1

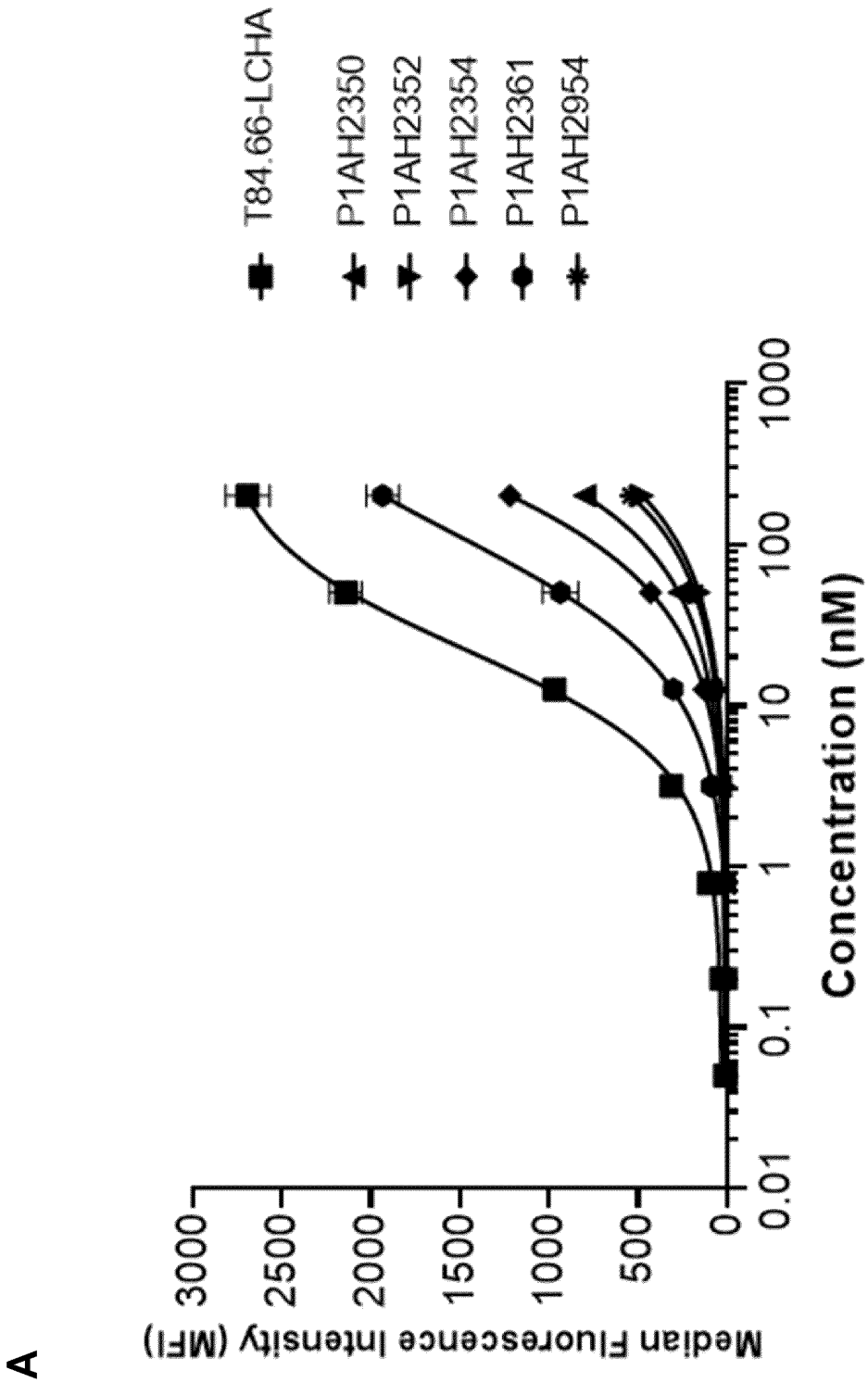

A
Median Fluorescence Intensity (MFI)
3000
2500
2000
1500
1000
500
0
0.01
0.1
1
10
100
1000
Concentration (nM)
T84.66-LCHA
P1AH2350
P1AH2352
P1AH2354
P1AH2361
P1AH2954

FIG. 2

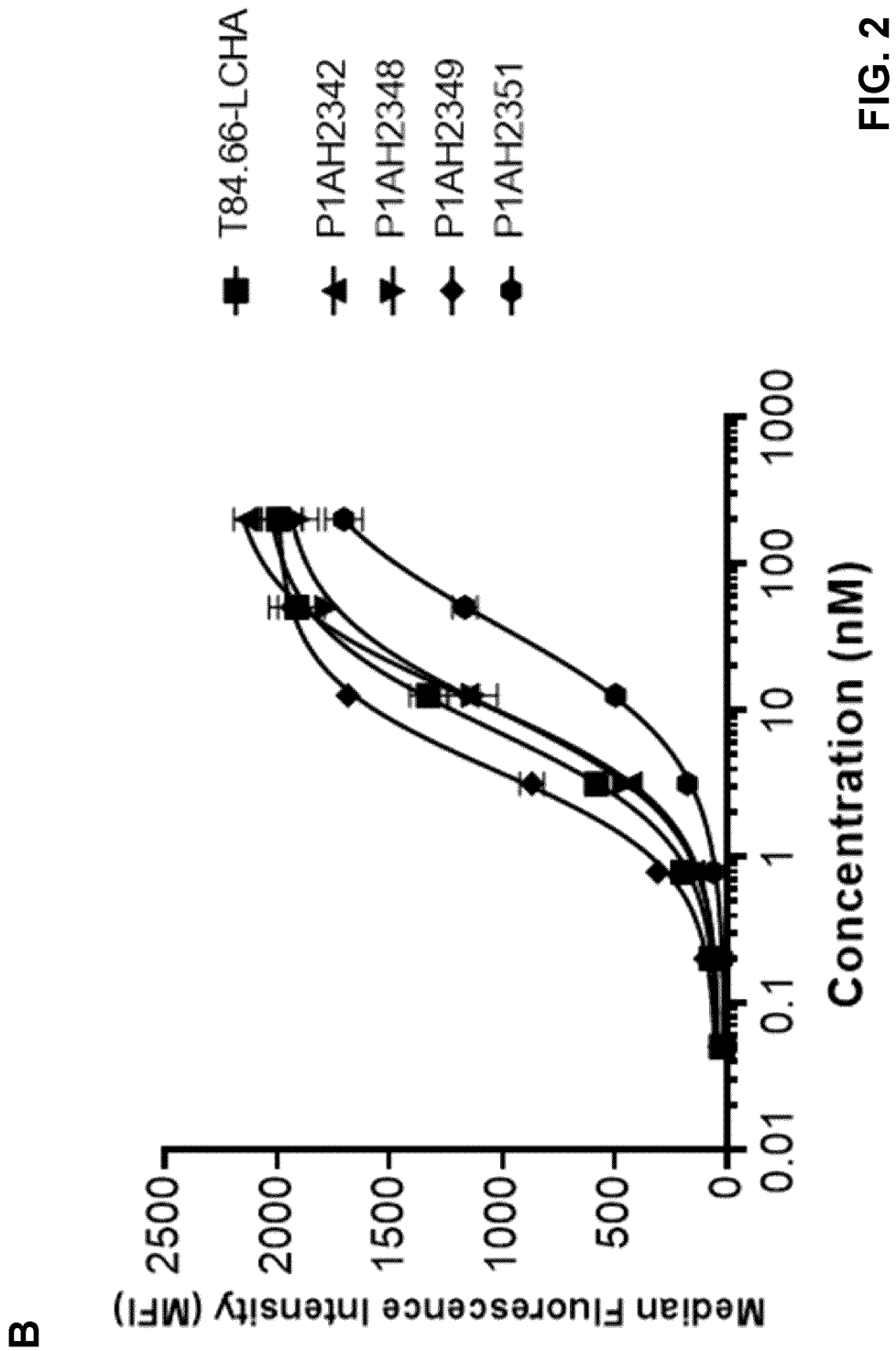

B
Median Fluorescence Intensity (MFI)
2500
2000
1500
1000
500
0
0.01
0.1
1
10
100
1000
Concentration (nM)
T84.66-LCHA
P1AH2342
P1AH2348
P1AH2349
P1AH2351

FIG. 2

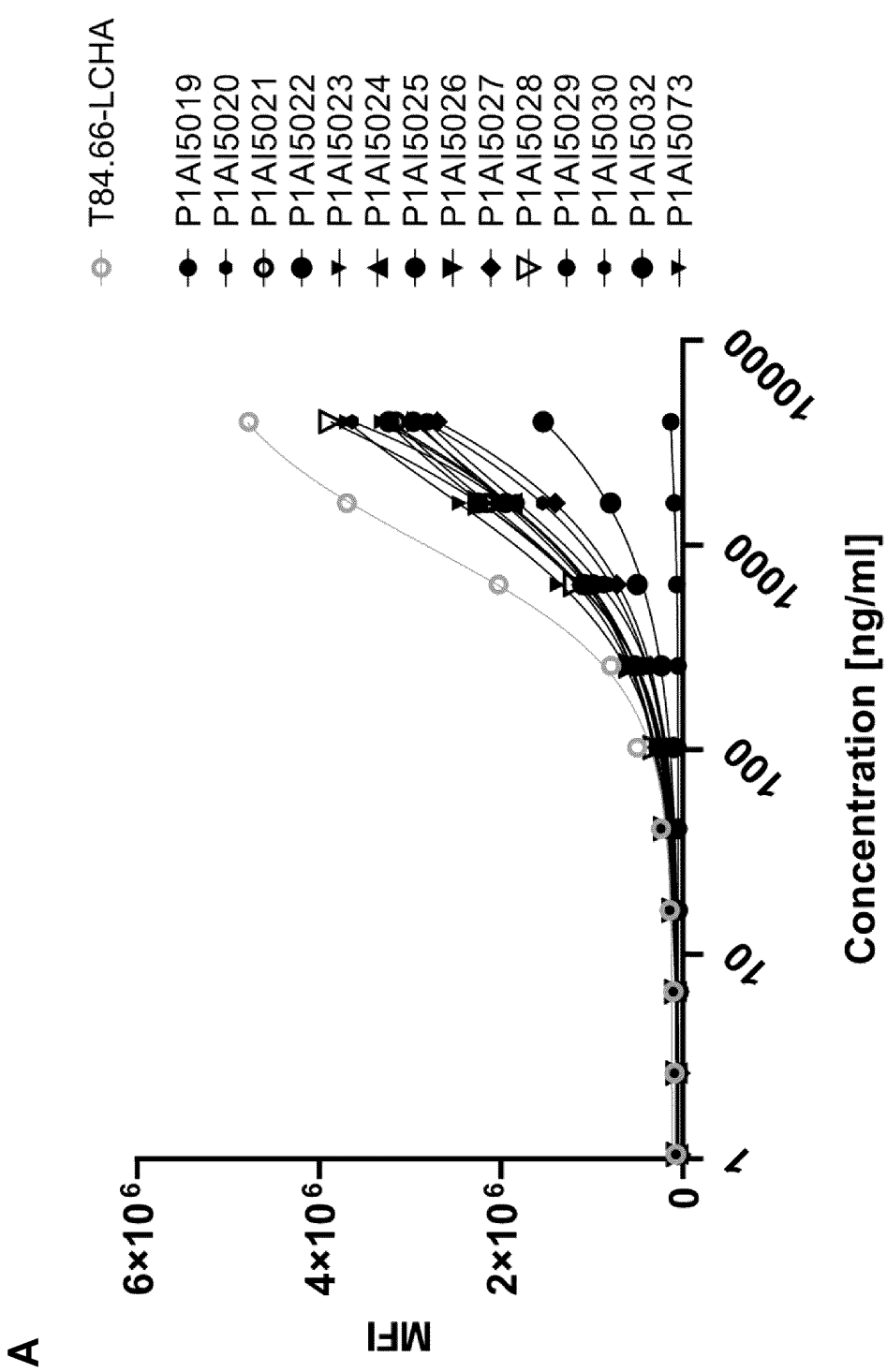

A
MFI
6×10^6
4×10^6
2×10^6
0
1
10
100
1000
10000
Concentration [ng/ml]
T84.66-LCHA
P1AI5019
P1AI5020
P1AI5021
P1AI5022
P1AI5023
P1AI5024
P1AI5025
P1AI5026
P1AI5027
P1AI5028
P1AI5029
P1AI5030
P1AI5032
P1AI5073

FIG. 3

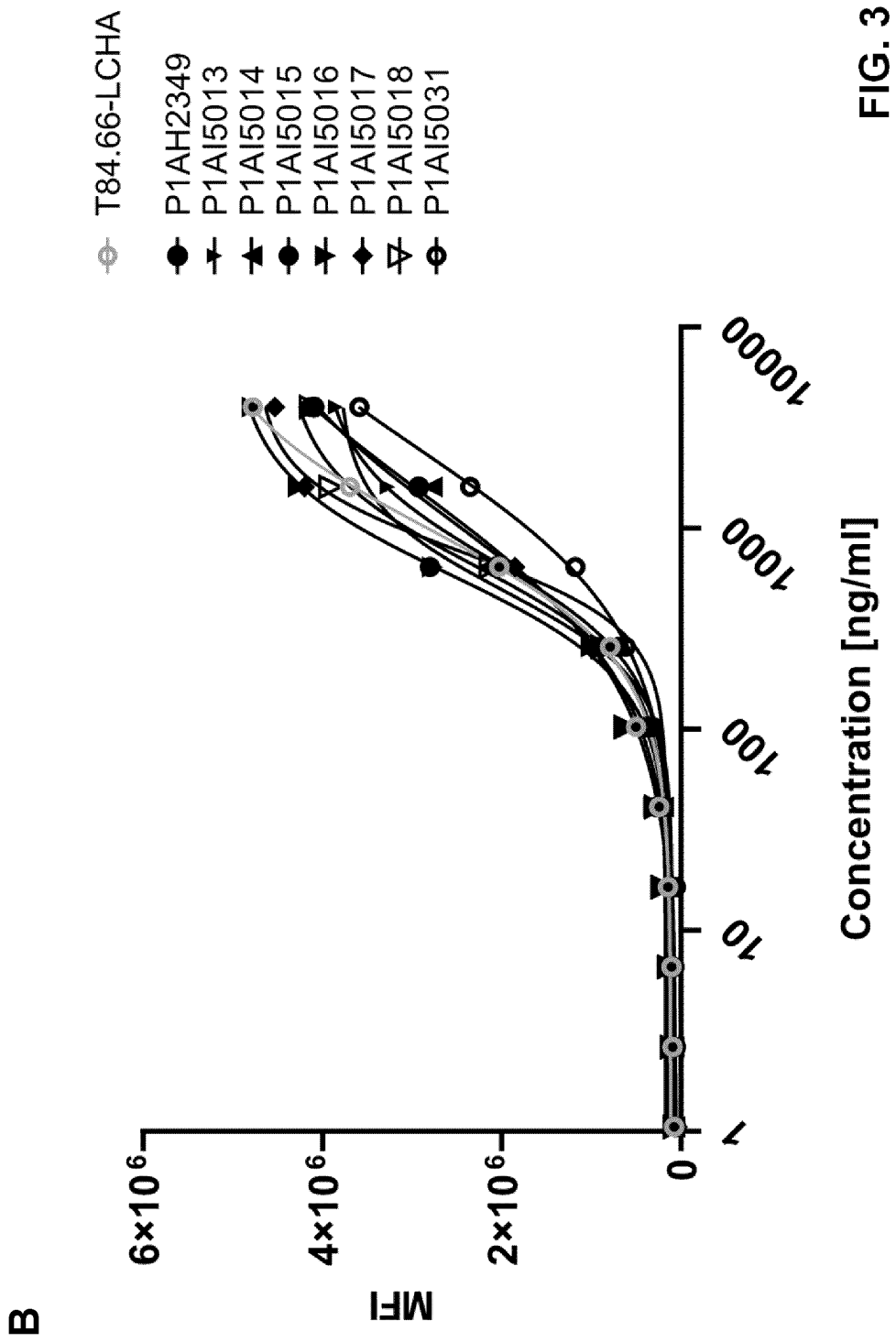

B
MFI
6×10^6
4×10^6
2×10^6
0
1
10
100
1000
10000
Concentration [ng/ml]
T84.66-LCHA
P1AH2349
P1AI5013
P1AI5014
P1AI5015
P1AI5016
P1AI5017
P1AI5018
P1AI5031

FIG. 3

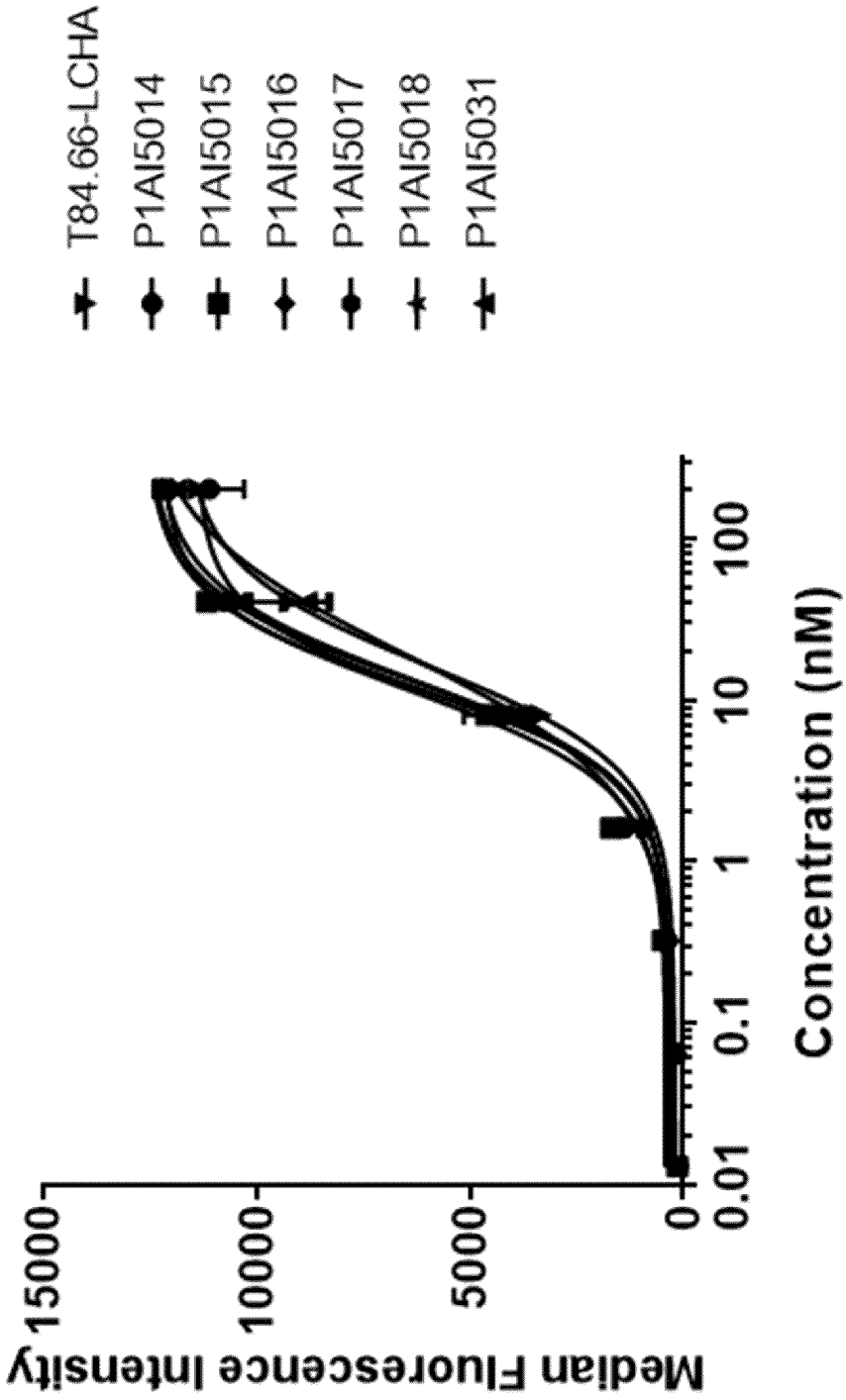

T84.66-LCHA
P1AI5014
P1AI5015
P1AI5016
P1AI5017
P1AI5018
P1AI5031
Median Fluorescence Intensity
15000
10000
5000
0
0.01
0.1
1
10
100
Concentration (nM)

FIG. 4

FIG. 5

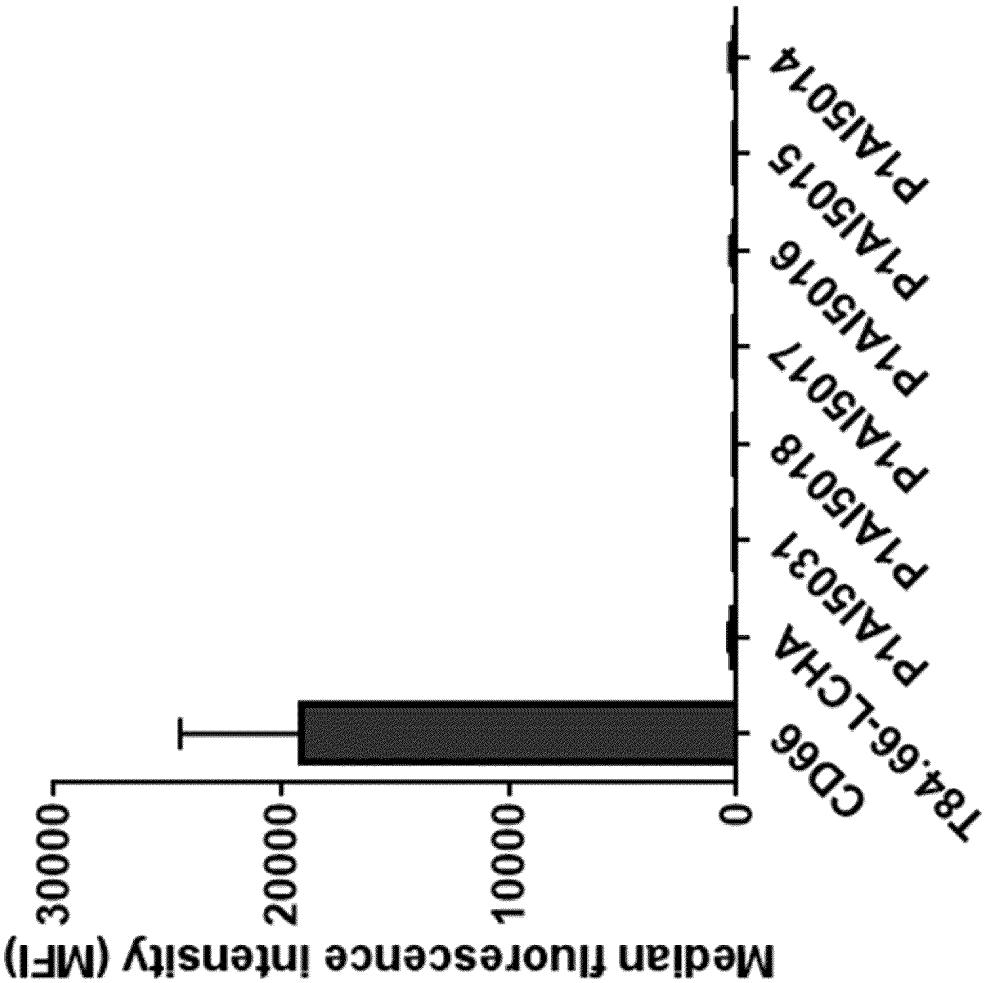
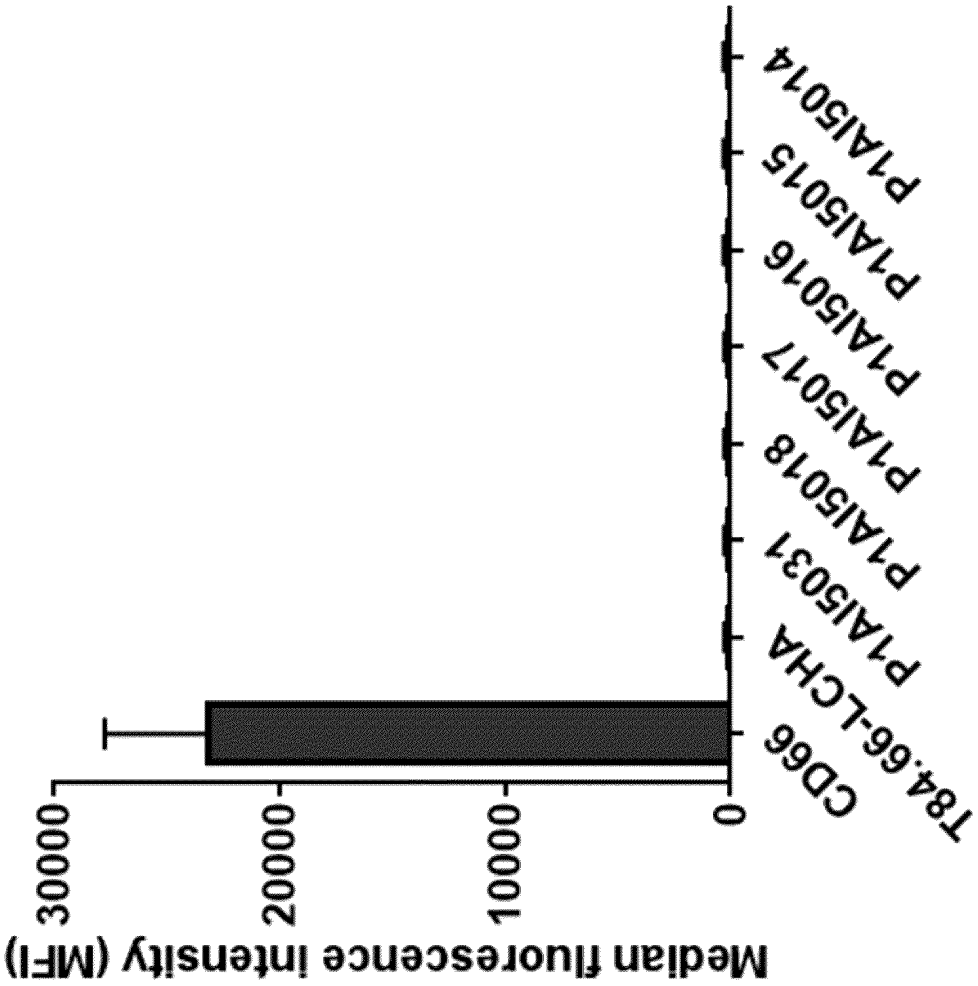
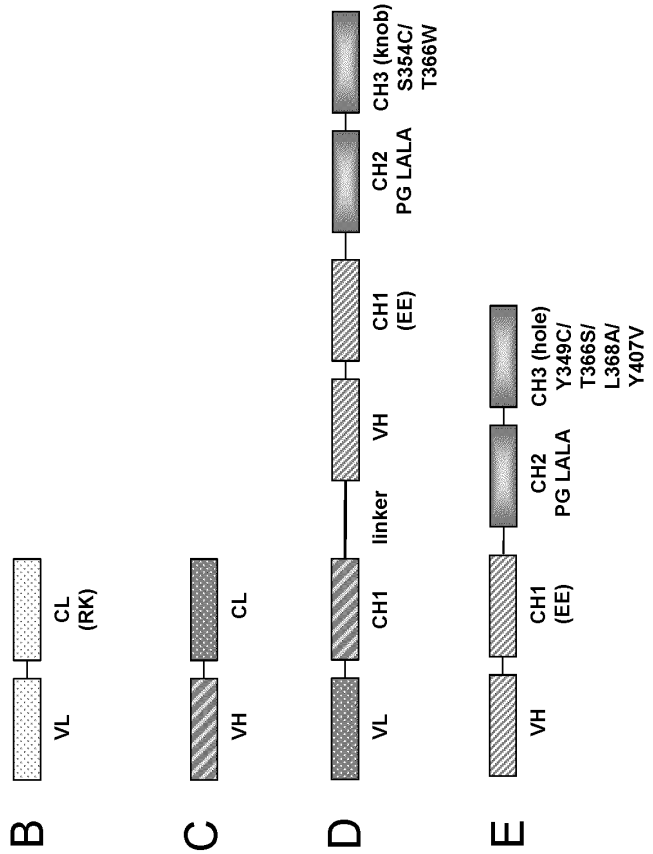

Median fluorescence intensity (MFI)
30000
20000
10000
0
CD66
T84.66-LCHA
P1AI50031
P1AI50018
P1AI50017
P1AI50016
P1AI50015
P1AI50014

FIG. 6


Median fluorescence intensity (MFI)
30000
20000
10000
0
CD66
T84.66-LCHA
P1A150314
P1A150184
P1A150174
P1A150164
P1A150154
P1A150144


B
VL
CL
(RK)
C
VH
CL
D
VL
CH1
linker
VH
CH1
(EE)
CH2
PG LALA
CH3 (knob)
S354C/
T366W
E
VH
CH1
(EE)
CH2
PG LALA
CH3 (hole)
Y349C/
T366S/
L368A/
Y407V

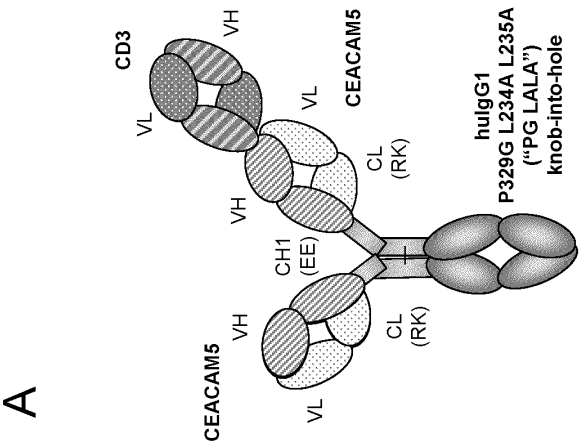

A
CEACAM5
VH
VL
CH1
(EE)
CL
(RK)
CD3
VH
VL
CEACAM5
VH
VL
CL
(RK)
huIgG1
P329G L234A L235A
("PG LALA")
knob-into-hole

FIG. 7

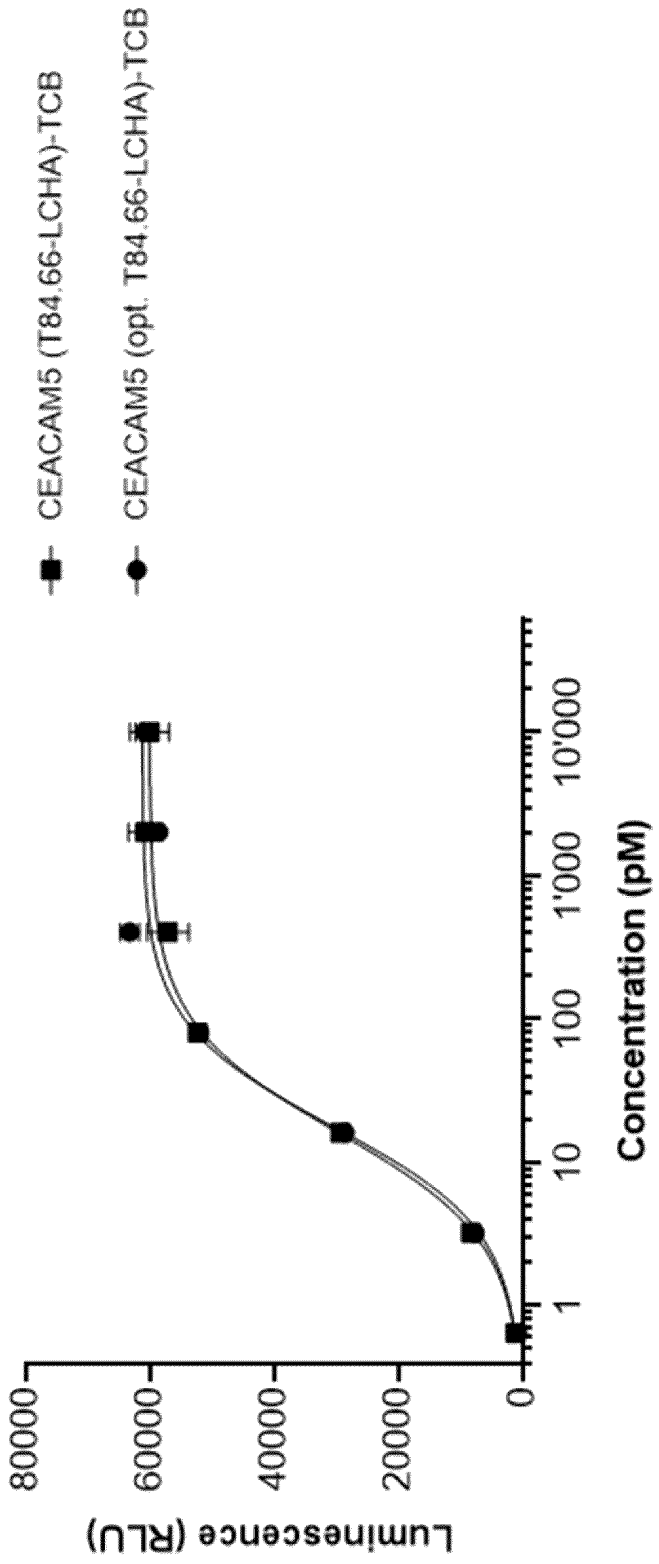

CEACAM5 (T84.66-LCHA)-TCB
CEACAM5 (opt. T84.66-LCHA)-TCB
Luminescence (RLU)
80000
60000
40000
20000
0
1
10
100
1'000
10'000
Concentration (pM)

FIG. 8