Figure 1

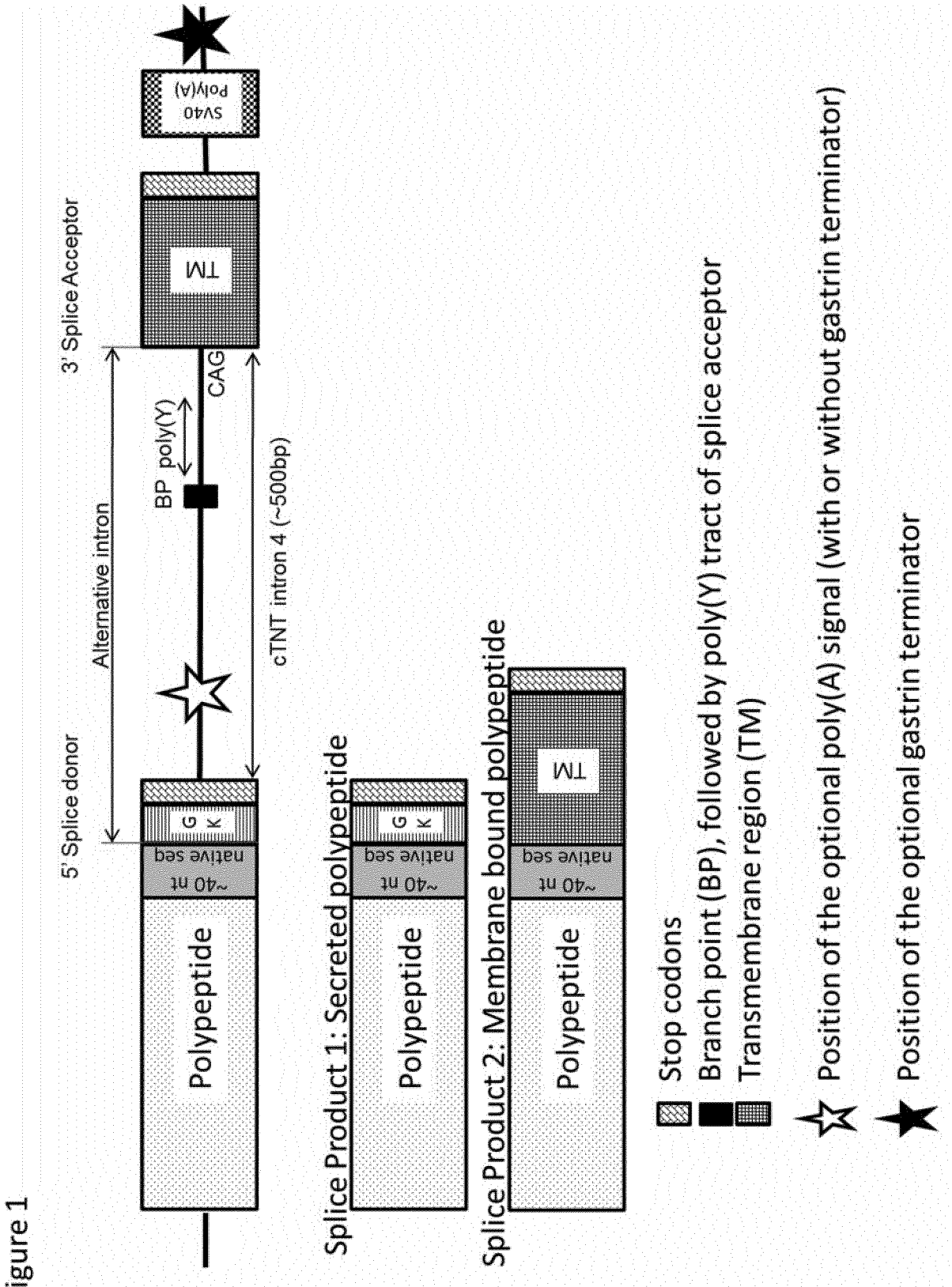

5' Splice donor
Alternative intron
3' Splice Acceptor
BP poly(Y)
CAG
cTNT intron 4 (~500bp)
~40 nt native seq
G K
Polypeptide
TM
SV40 Poly(A)
Splice Product 1: Secreted polypeptide
Splice Product 2: Membrane bound polypeptide
Stop codons
Branch point (BP), followed by poly(Y) tract of splice acceptor
Transmembrane region (TM)
Position of the optional poly(A) signal (with or without gastrin terminator)
Position of the optional gastrin terminator

Figure 2

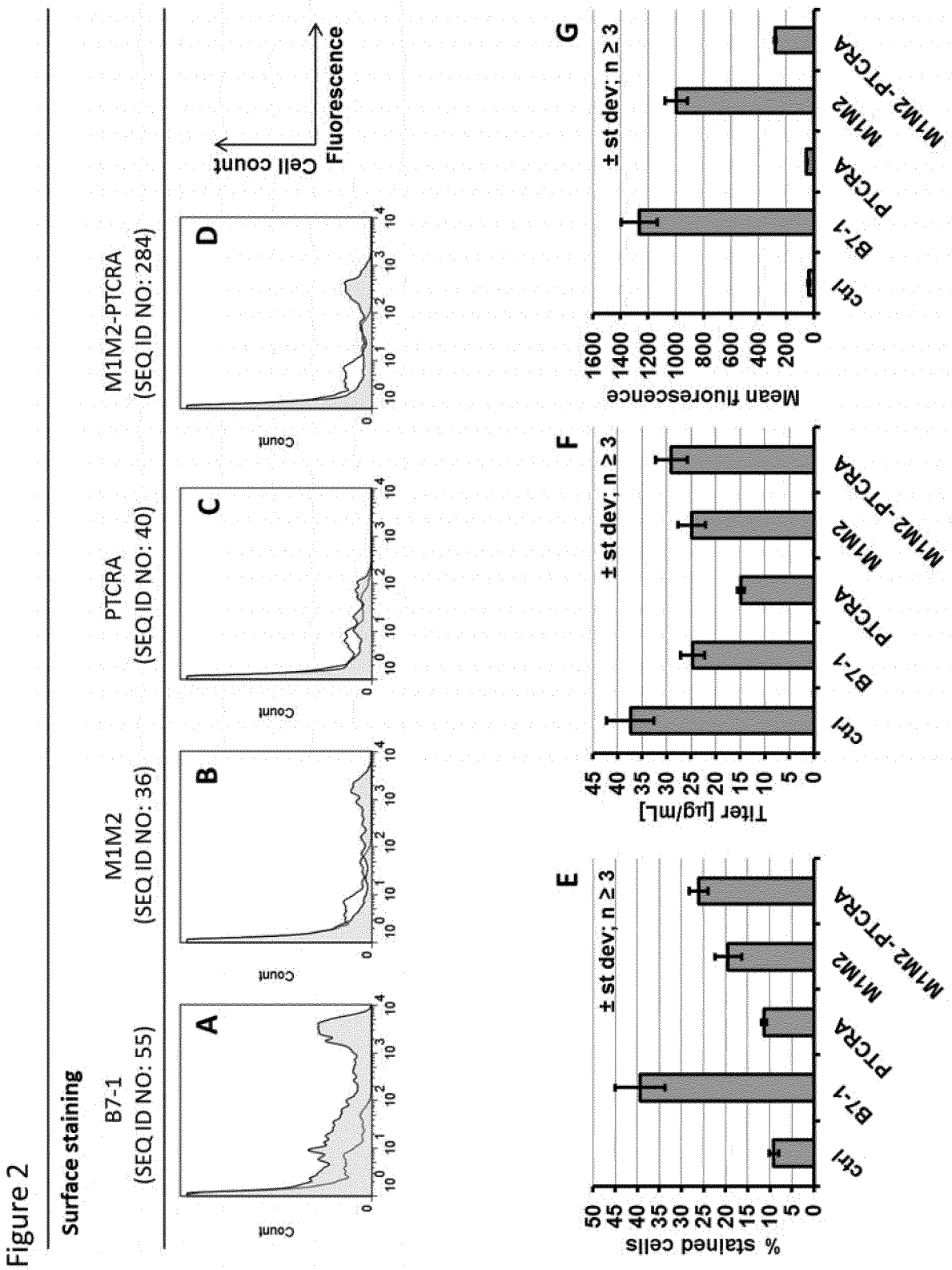

Surface staining
B7-1 (SEQ ID NO: 55)
M1M2 (SEQ ID NO: 36)
PTCRA (SEQ ID NO: 40)
M1M2-PTCRA (SEQ ID NO: 284)
A
B
C
D
Count
Cell count
Fluorescence
E
F
G
% stained cells
Titer [µg/mL]
Mean fluorescence
± st dev; n ≥ 3
ctrl
B7-1
PTCRA
M1M2
M1M2-PTCRA

Figure 3

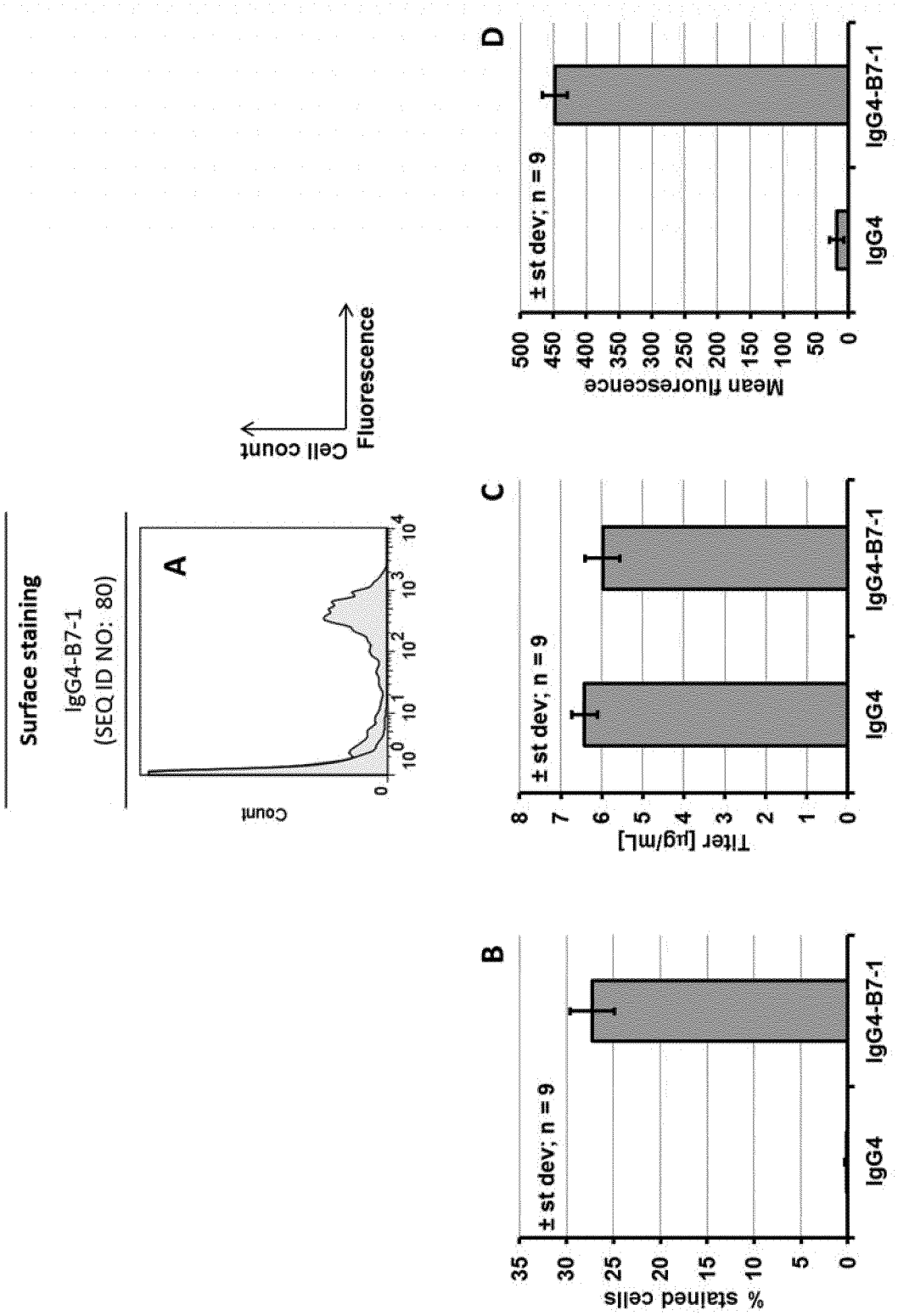

Surface staining
IgG4-B7-1
(SEQ ID NO: 80)
A
Count
Cell count
Fluorescence
B
% stained cells
± st dev; n = 9
IgG4
IgG4-B7-1
C
Titer [µg/mL]
± st dev; n = 9
IgG4
IgG4-B7-1
D
Mean fluorescence
± st dev; n = 9
IgG4
IgG4-B7-1

Figure 4

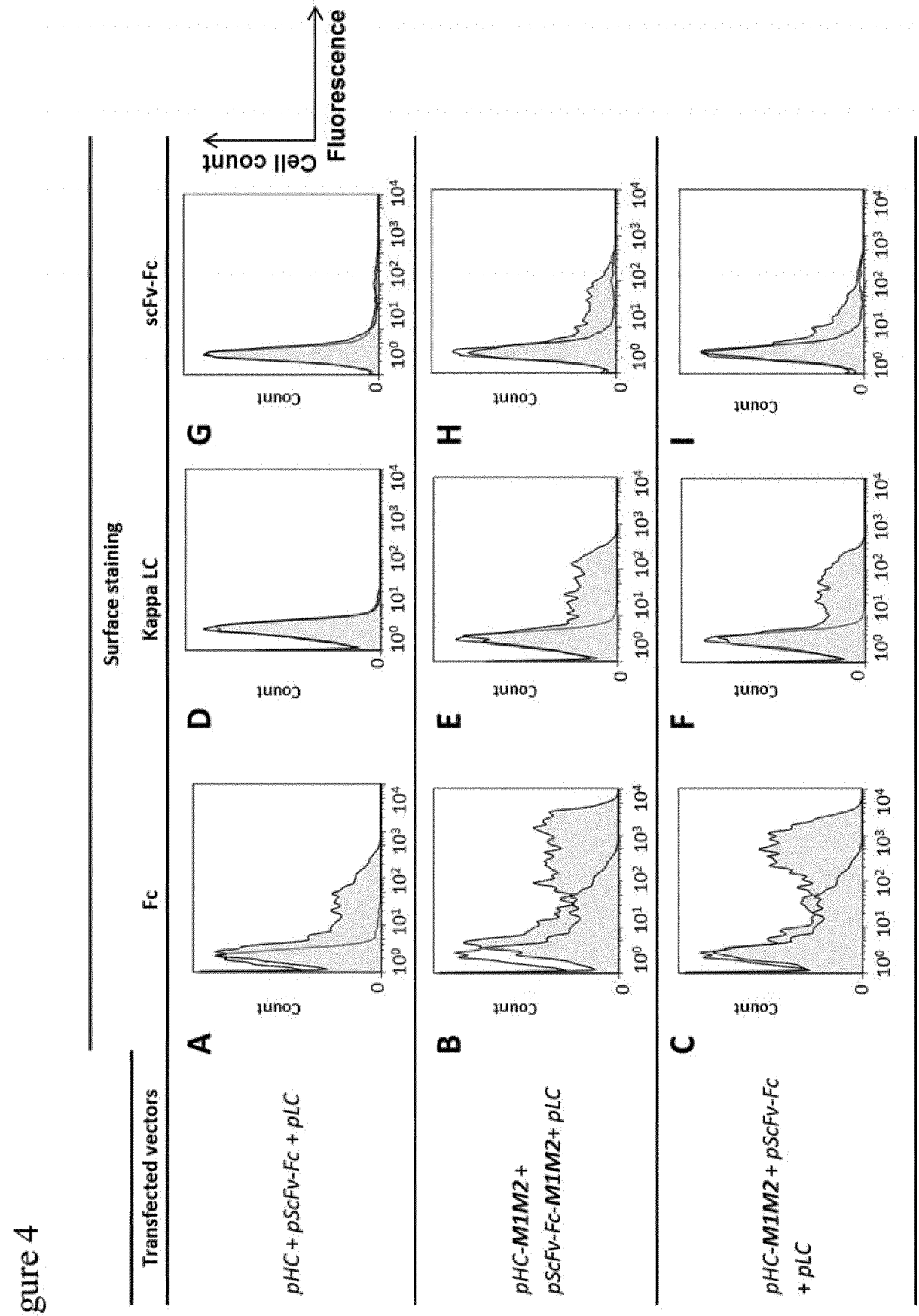

Surface staining
Transfected vectors
Fc
Kappa LC
scFv-Fc
Cell count
Fluorescence
A
B
C
D
E
F
G
H
I
pHC + pScFv-Fc + pLC
pHC-M1M2 + pScFv-Fc-M1M2+ pLC
pHC-M1M2 + pScFv-Fc + pLC
Count
0
10^0 10^1 10^2 10^3 10^4

Figure 4 (contd)

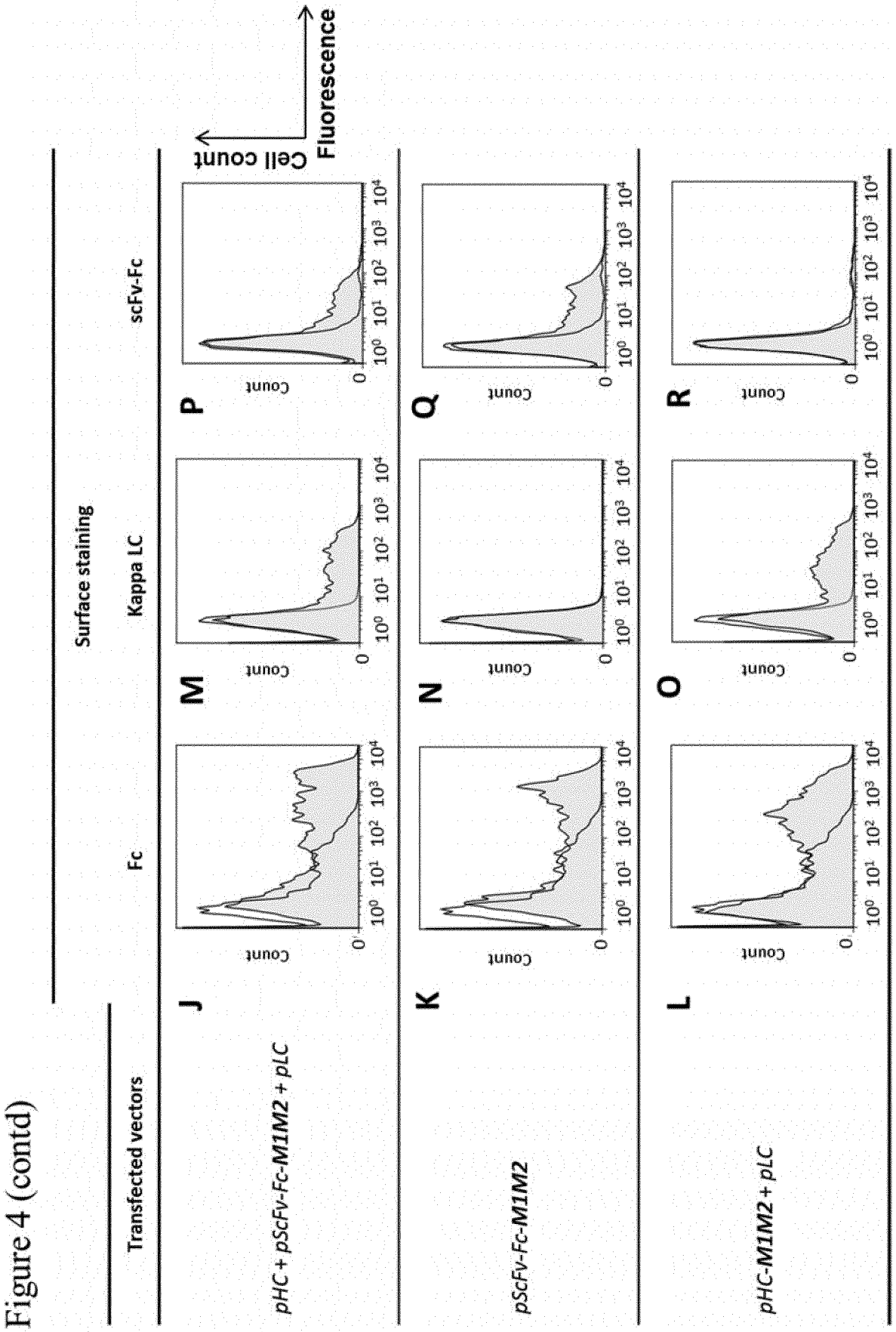


Figure 5

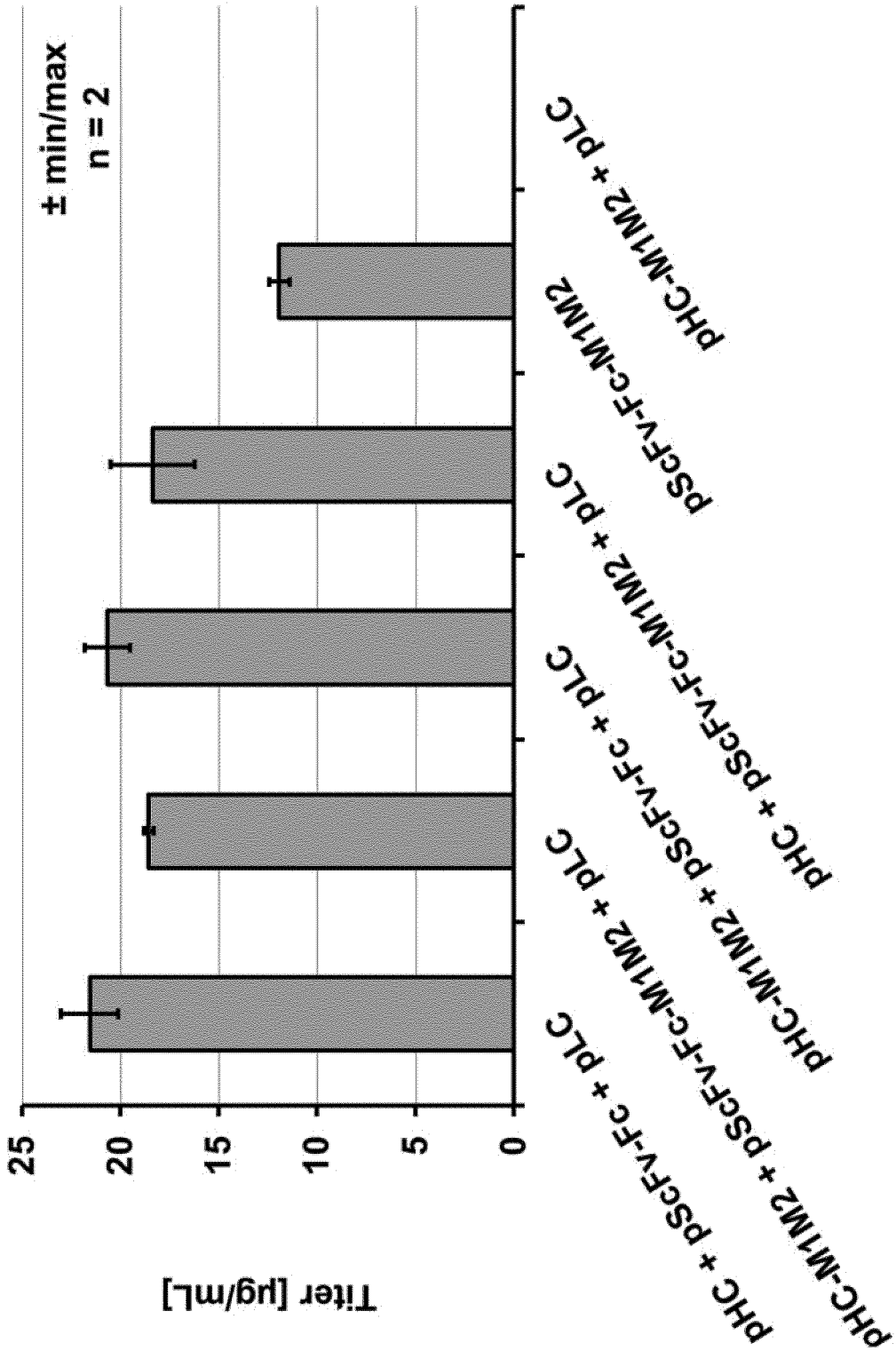

± min/max
n = 2
Titer [µg/mL]
0
5
10
15
20
25
pHC + pSCFv-FC + pLC
pHC-M1M2 + pSCFv-FC-M1M2 + pLC
pHC-M1M2 + pSCFv-FC + pLC
pHC + pSCFv-FC-M1M2 + pLC
pSCFv-FC-M1M2
pHC-M1M2 + pLC

Figure 6

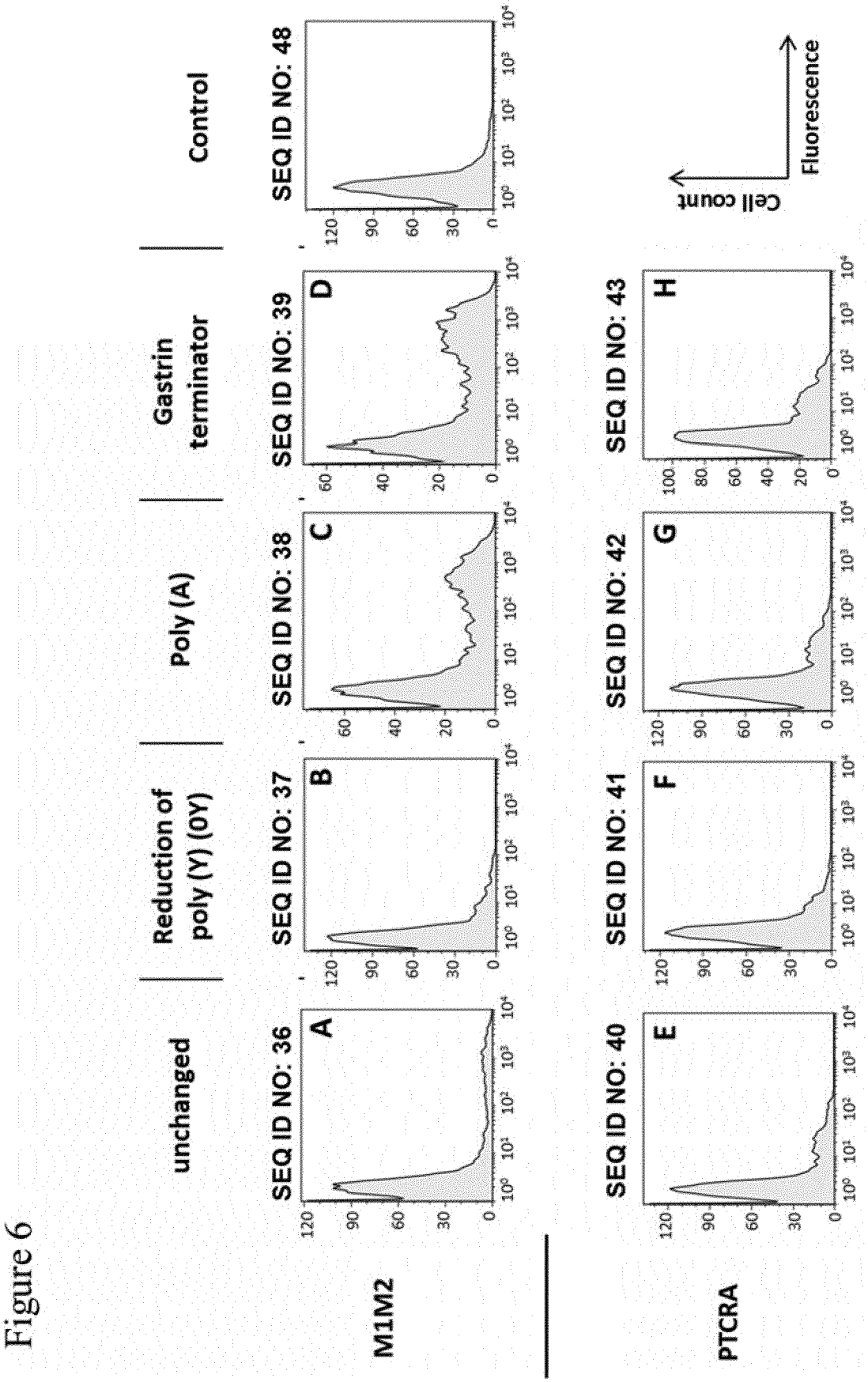

unchanged
Reduction of poly (Y) (0Y)
Poly (A)
Gastrin terminator
Control
M1M2
PTCRA
SEQ ID NO: 36
SEQ ID NO: 37
SEQ ID NO: 38
SEQ ID NO: 39
SEQ ID NO: 48
SEQ ID NO: 40
SEQ ID NO: 41
SEQ ID NO: 42
SEQ ID NO: 43
A
B
C
D
E
F
G
H
Cell count
Fluorescence

Figure 6 (contd)

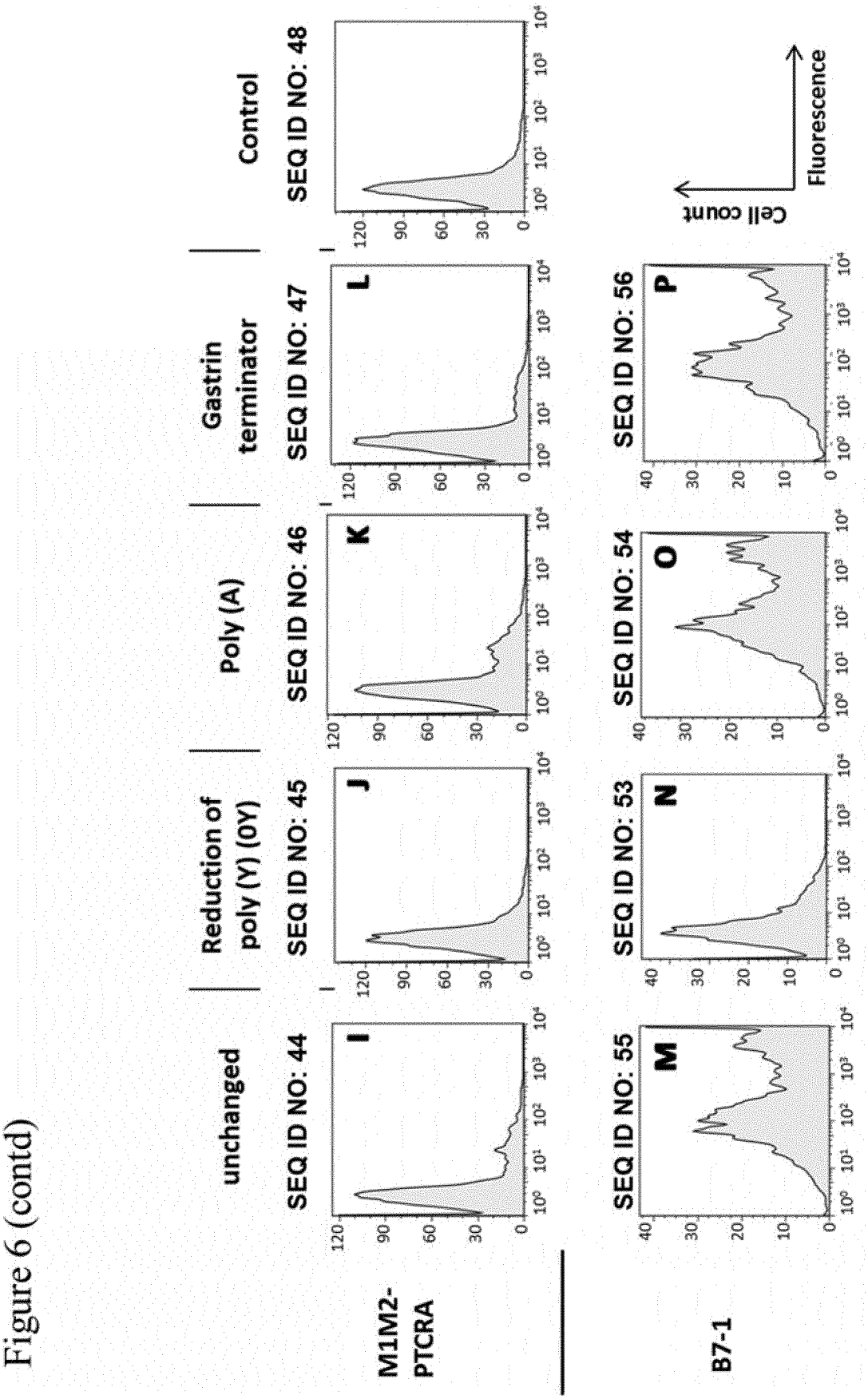


Figure 7

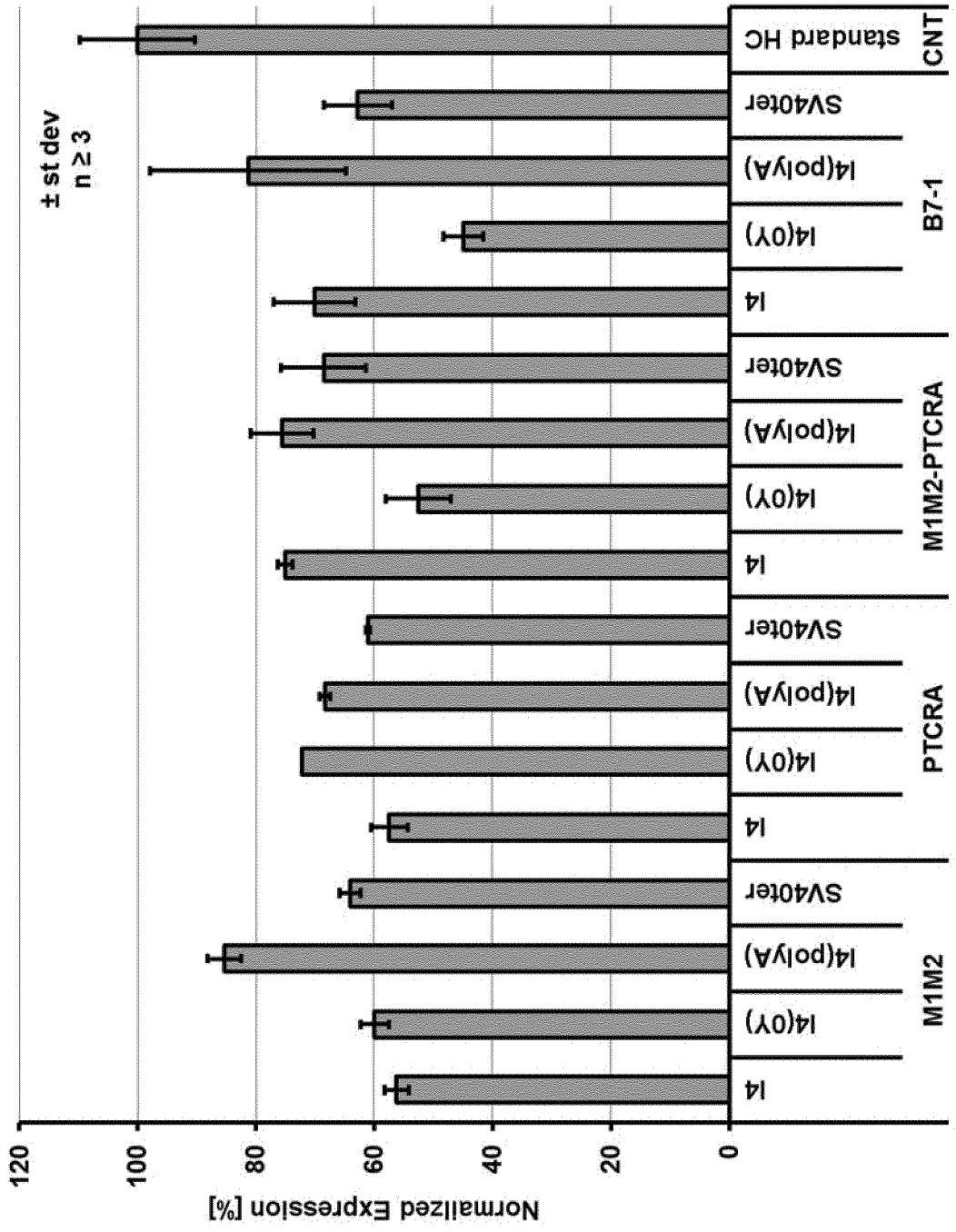

Normalized Expression [%]
120
100
80
60
40
20
0
± st dev
n ≥ 3
M1M2
PTCRA
M1M2-PTCRA
B7-1
CNT
I4
I4(0Y)
I4(polyA)
SV40ter
standard HC

Figure 8

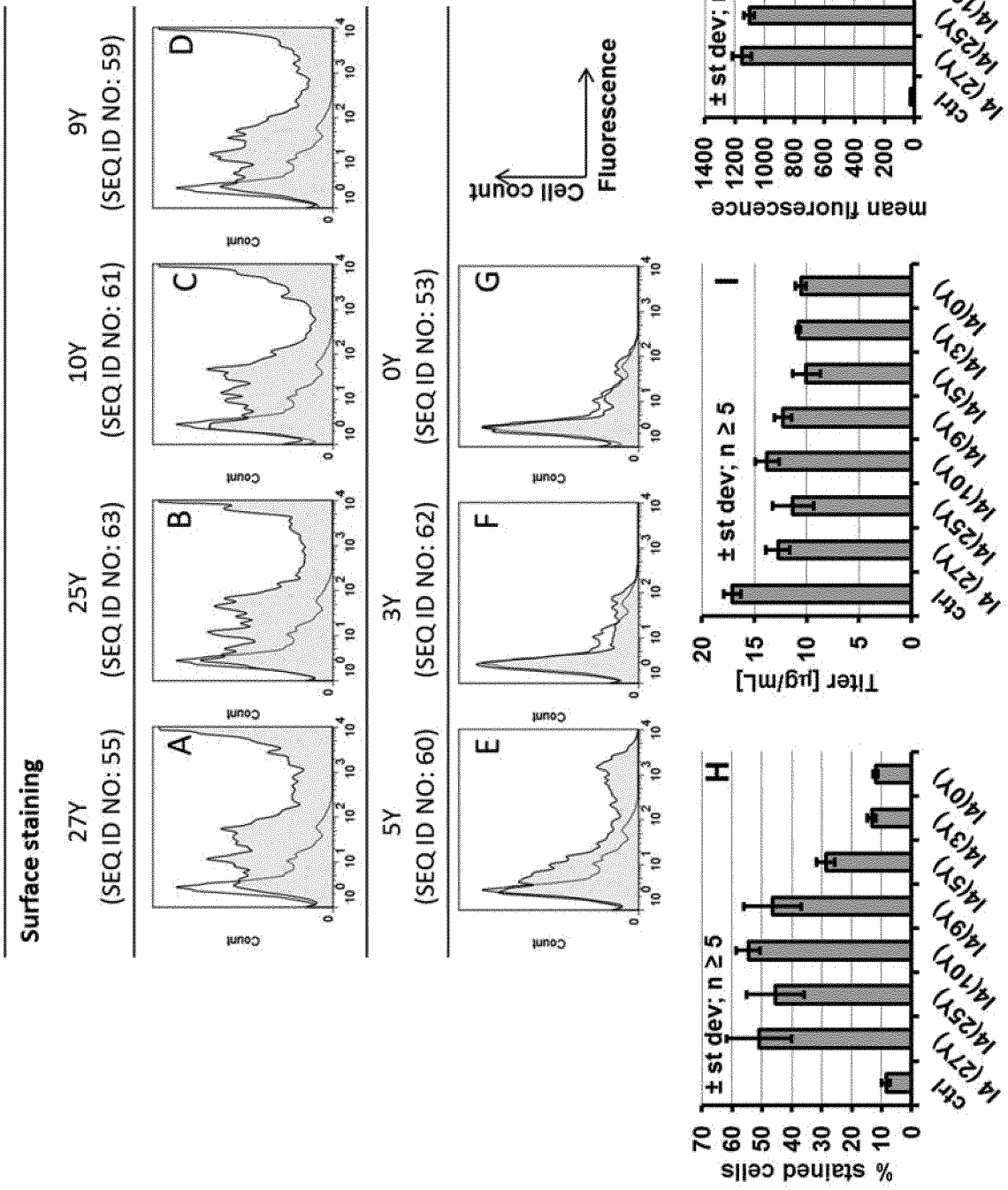

Surface staining
27Y (SEQ ID NO: 55)
25Y (SEQ ID NO: 63)
10Y (SEQ ID NO: 61)
9Y (SEQ ID NO: 59)
5Y (SEQ ID NO: 60)
3Y (SEQ ID NO: 62)
0Y (SEQ ID NO: 53)
A
B
C
D
E
F
G
Count
Cell count
Fluorescence
H
I
J
% stained cells
Titer [µg/mL]
mean fluorescence
± st dev; n ≥ 5
ctrl
14 (27Y)
14(25Y)
14(10Y)
14(9Y)
14(5Y)
14(3Y)
14(0Y)

Figure 9

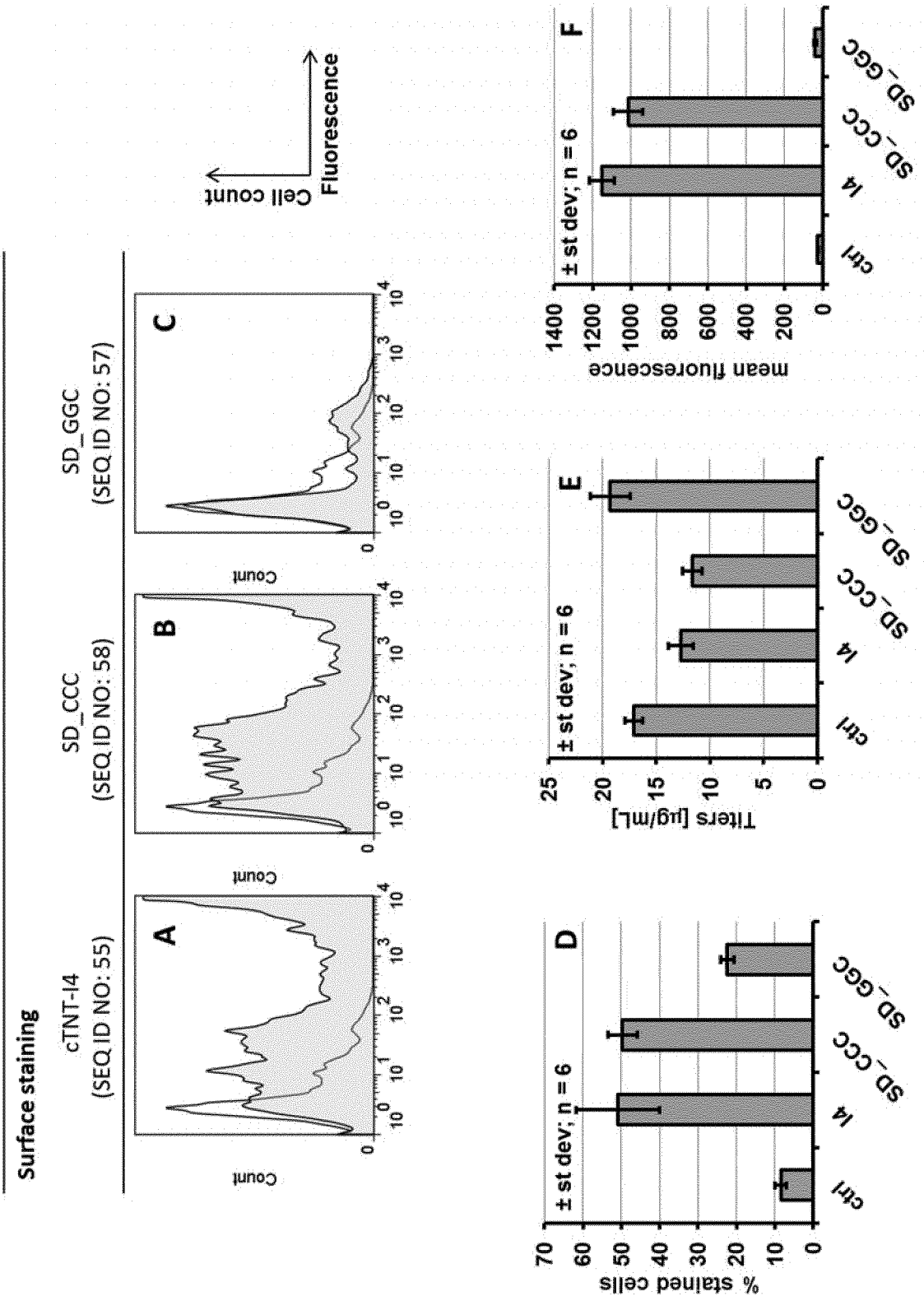

Surface staining
cTNT-I4
(SEQ ID NO: 55)
SD_CCC
(SEQ ID NO: 58)
SD_GGC
(SEQ ID NO: 57)
A
B
C
Count
Cell count
Fluorescence
D
E
F
% stained cells
Titers [µg/mL]
mean fluorescence
± st dev; n = 6
ctrl
I4
SD_CCC
SD_GGC

Figure 10

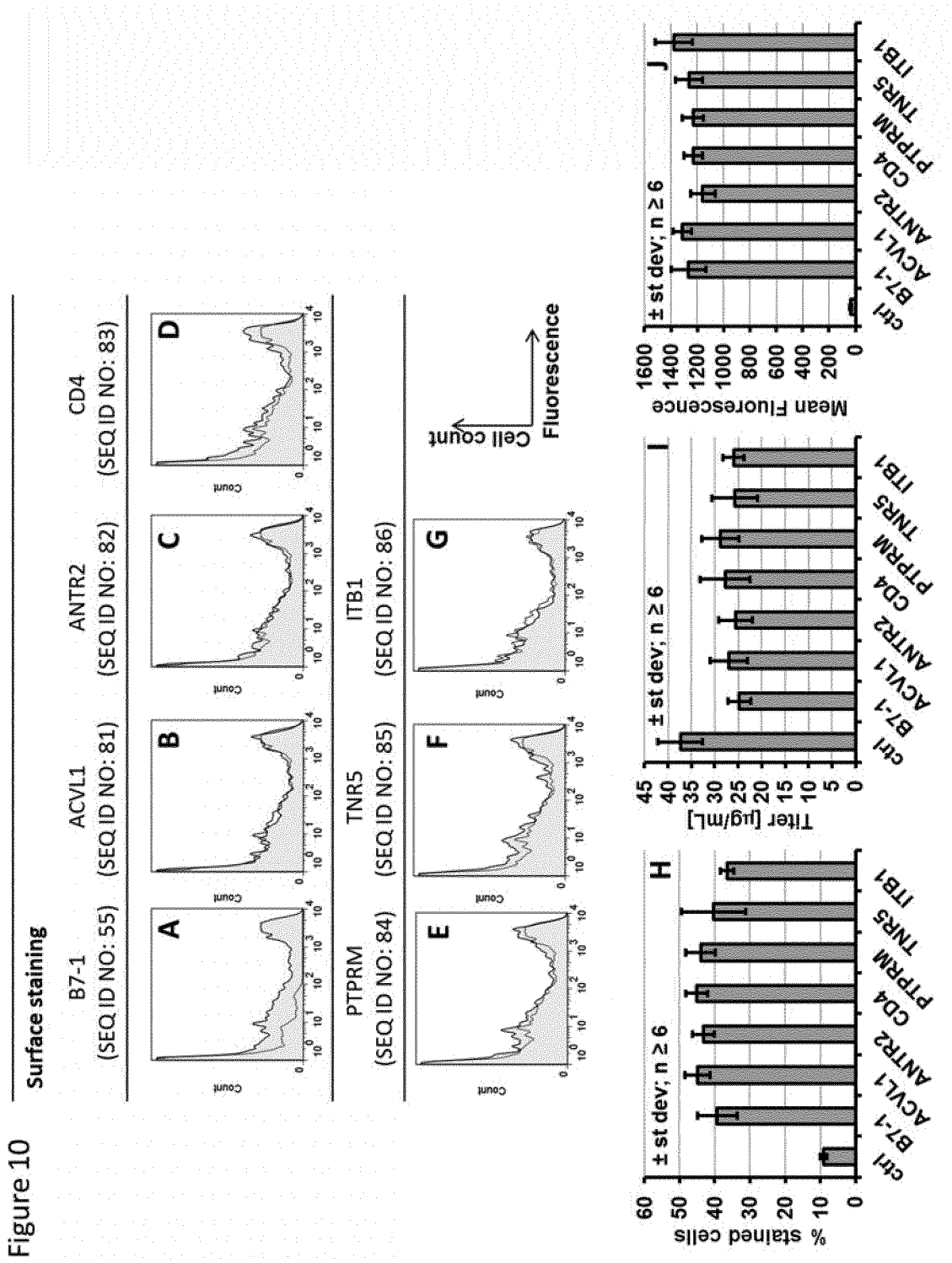
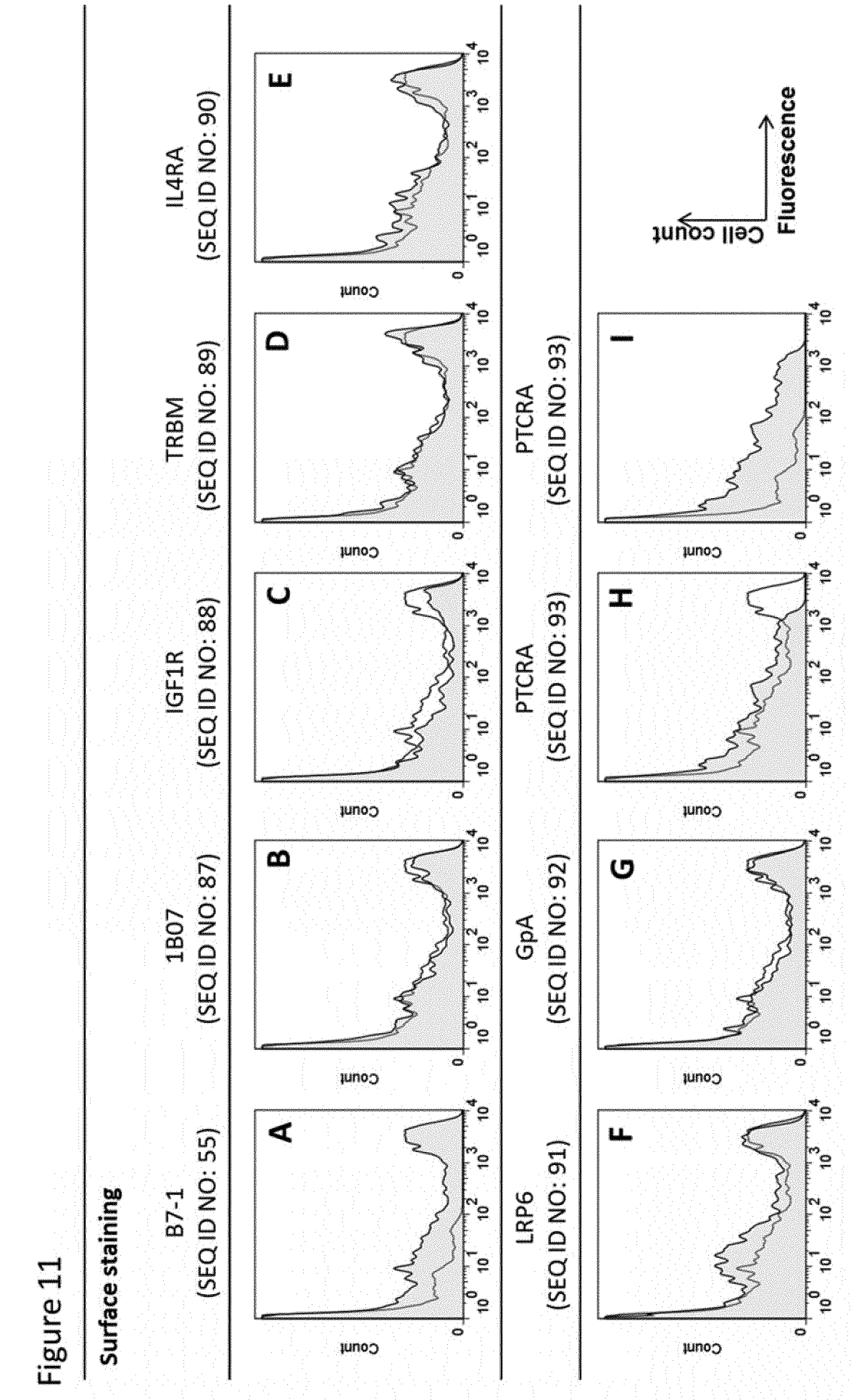
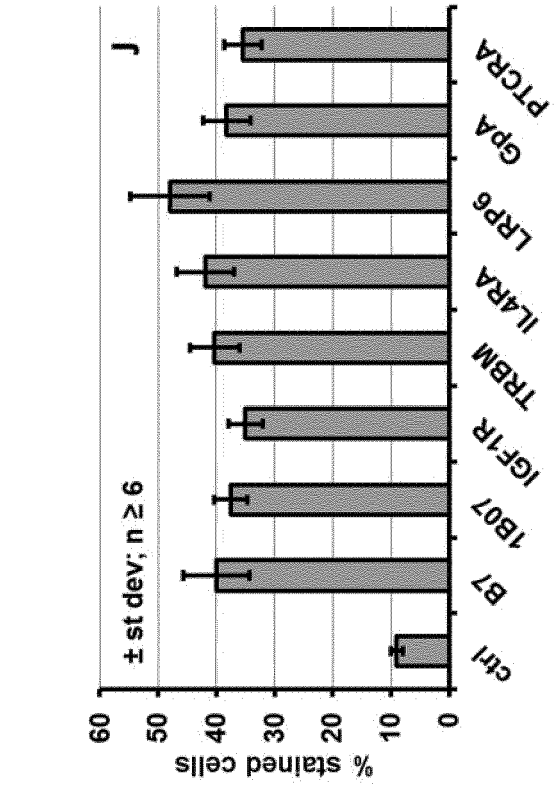
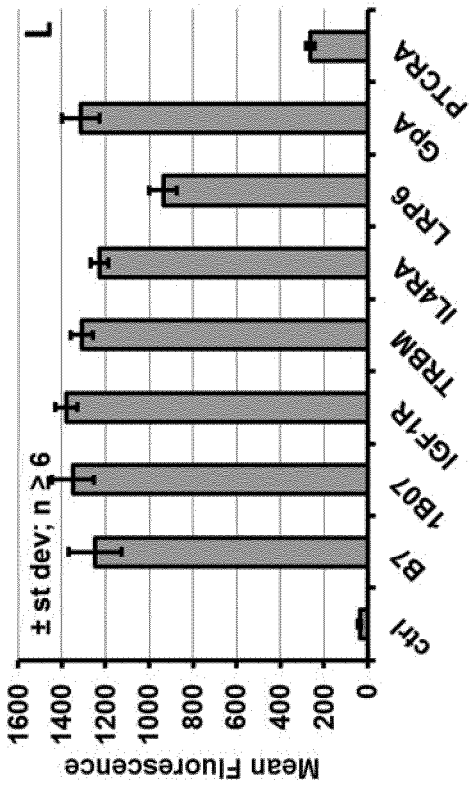

Surface staining
B7-1 (SEQ ID NO: 55)
ACVL1 (SEQ ID NO: 81)
ANTR2 (SEQ ID NO: 82)
CD4 (SEQ ID NO: 83)
PTPRM (SEQ ID NO: 84)
TNR5 (SEQ ID NO: 85)
ITB1 (SEQ ID NO: 86)
A
B
C
D
E
F
G
Count
Cell count
Fluorescence
H
% stained cells
± st dev; n ≥ 6
I
Titer [µg/mL]
J
Mean Fluorescence
ctrl
B7-1
ACVL1
ANTR2
CD4
PTPRM
TNR5
ITB1

Figure 11


Surface staining
B7-1 (SEQ ID NO: 55)
1B07 (SEQ ID NO: 87)
IGF1R (SEQ ID NO: 88)
TRBM (SEQ ID NO: 89)
IL4RA (SEQ ID NO: 90)
LRP6 (SEQ ID NO: 91)
GpA (SEQ ID NO: 92)
PTCRA (SEQ ID NO: 93)
PTCRA (SEQ ID NO: 93)
A
B
C
D
E
F
G
H
I
Count
Cell count
Fluorescence





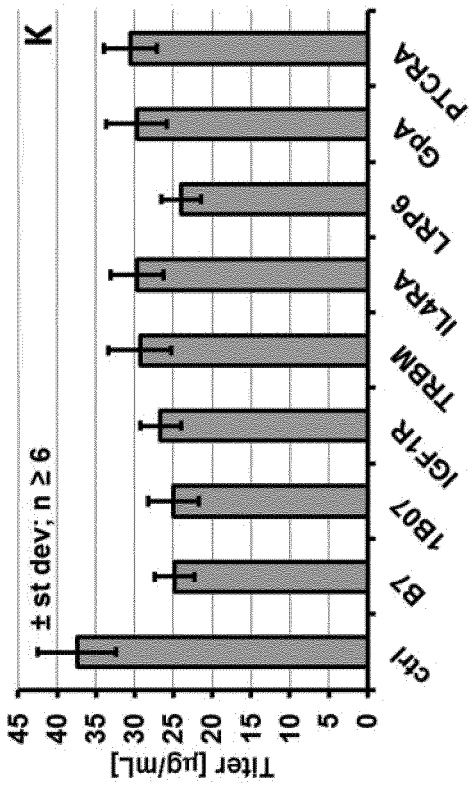
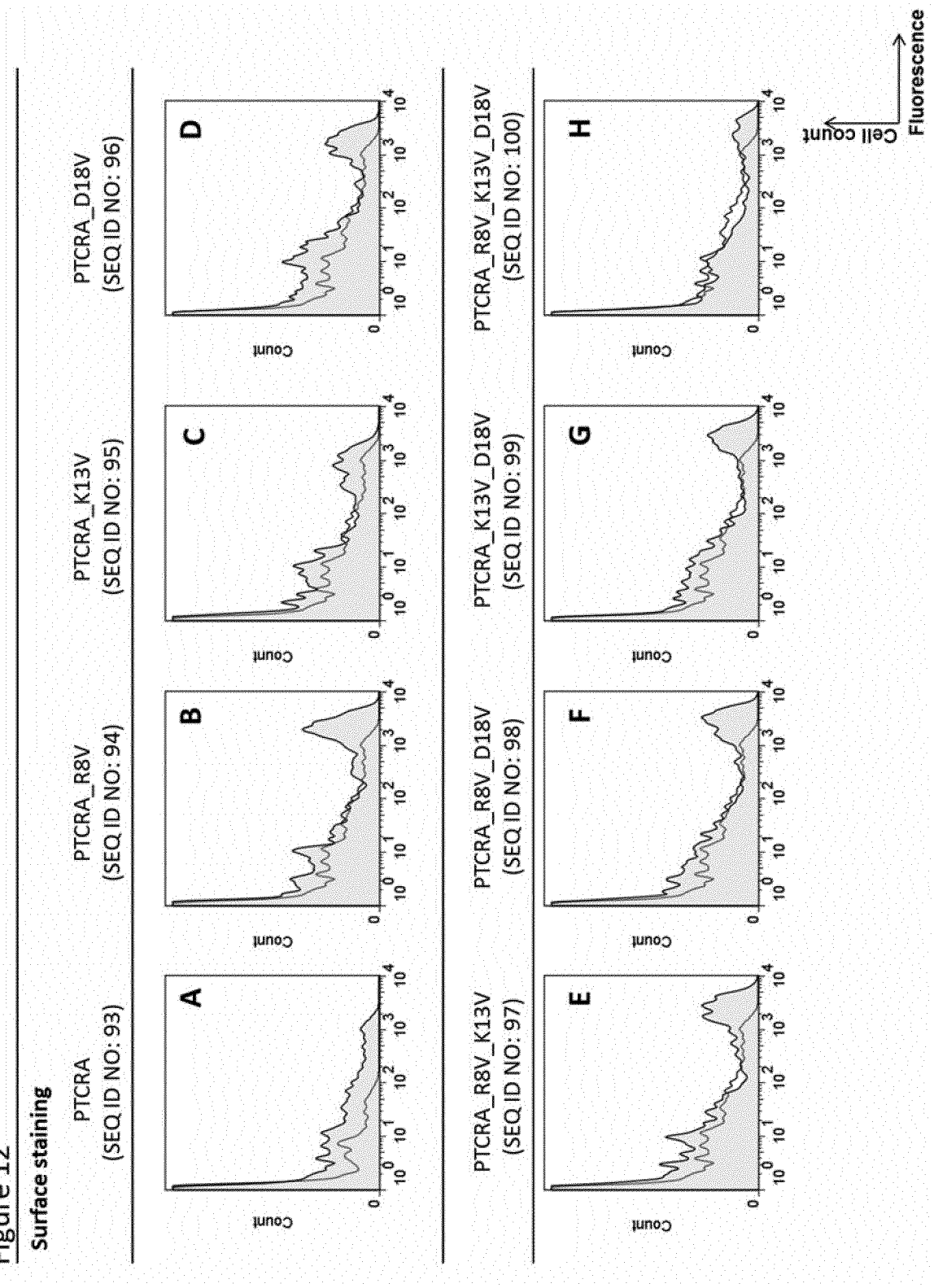


Figure 11 (contd)

Figure 12


Surface staining
PTCRA (SEQ ID NO: 93)
PTCRA_R8V (SEQ ID NO: 94)
PTCRA_K13V (SEQ ID NO: 95)
PTCRA_D18V (SEQ ID NO: 96)
PTCRA_R8V_K13V (SEQ ID NO: 97)
PTCRA_R8V_D18V (SEQ ID NO: 98)
PTCRA_K13V_D18V (SEQ ID NO: 99)
PTCRA_R8V_K13V_D18V (SEQ ID NO: 100)
A
B
C
D
E
F
G
H
Count
Cell count
Fluorescence

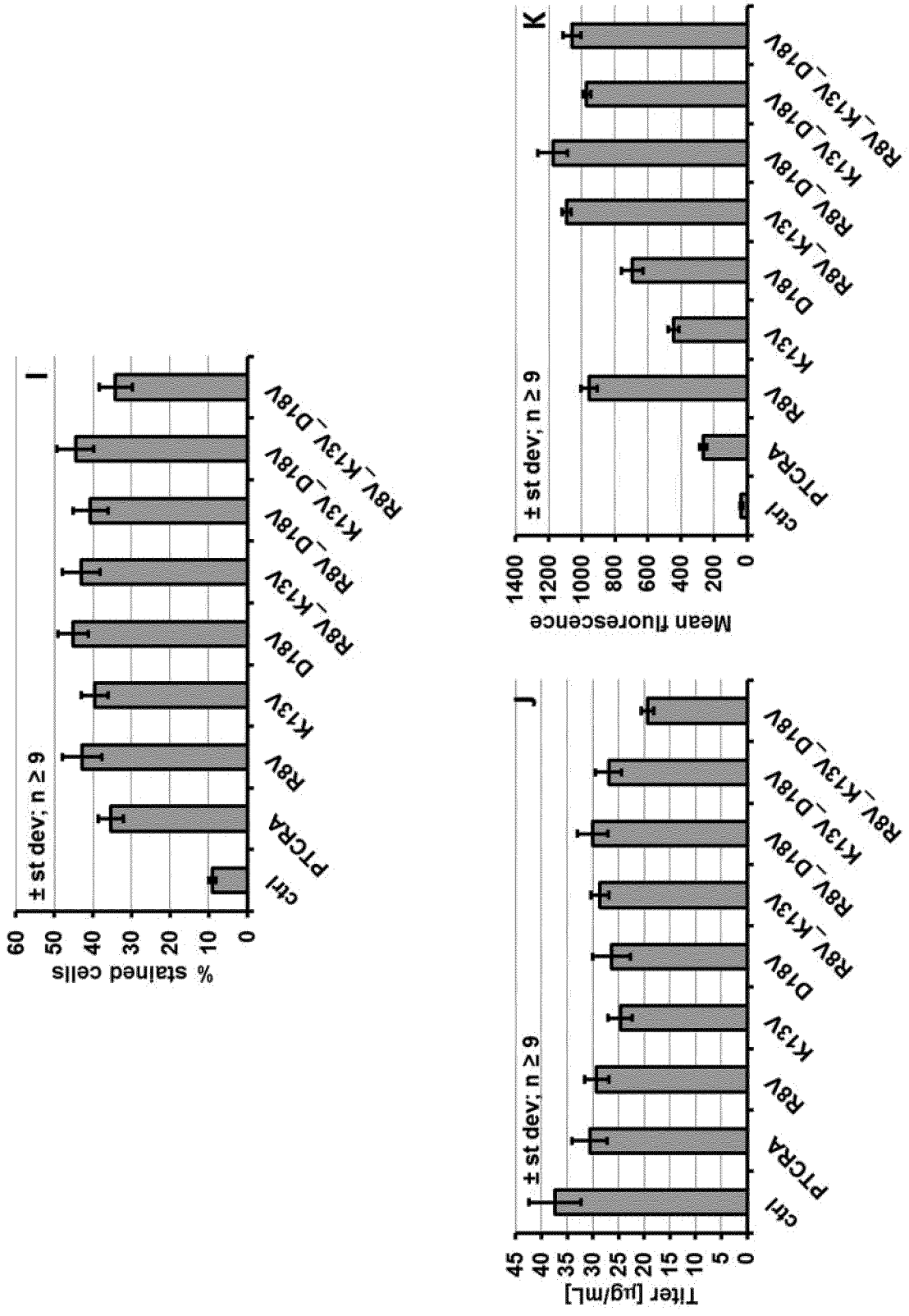


Figure 12 (contd)

Figure 13

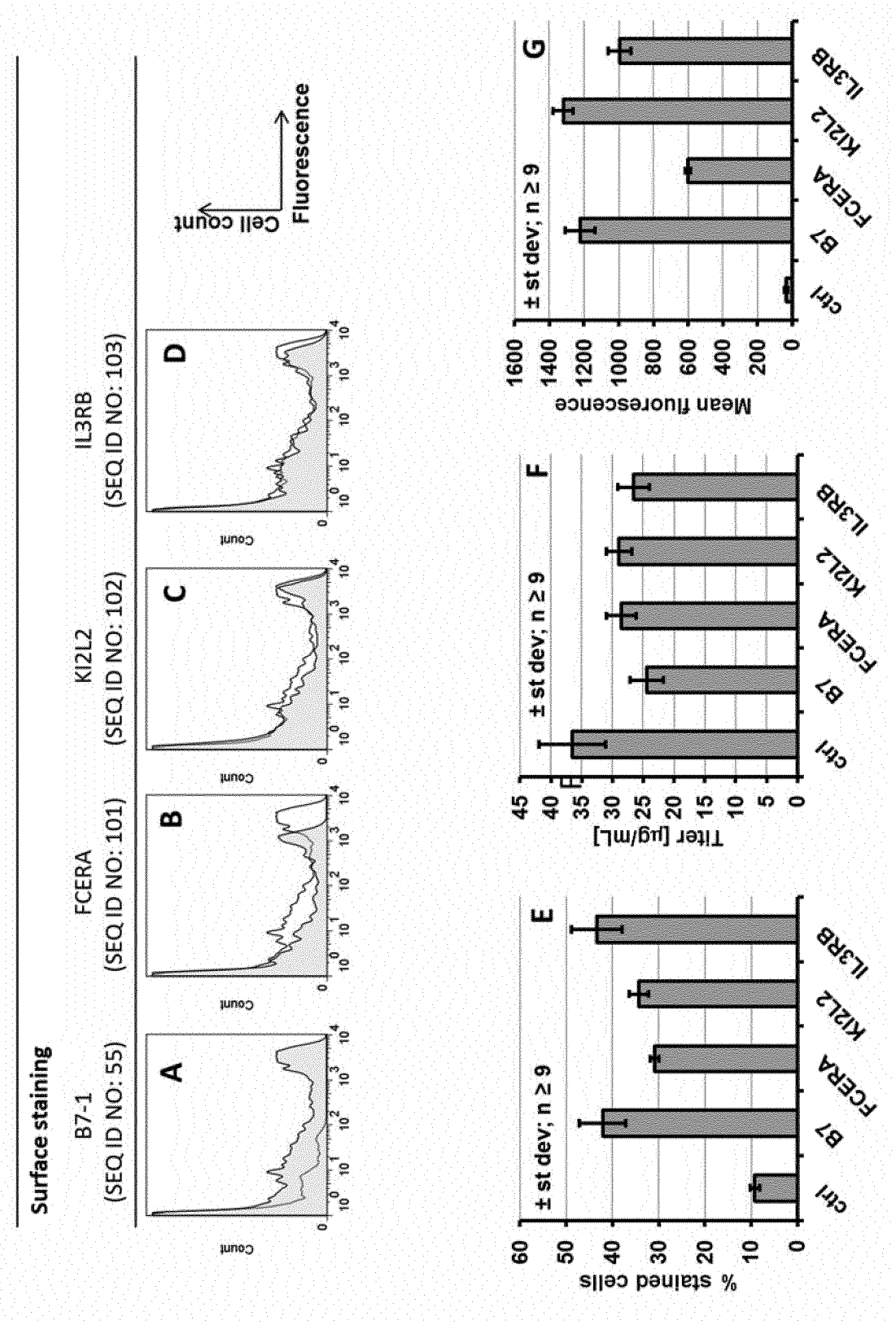

Surface staining
B7-1 (SEQ ID NO: 55)
FCERA (SEQ ID NO: 101)
KI2L2 (SEQ ID NO: 102)
IL3RB (SEQ ID NO: 103)
A
B
C
D
Count
Cell count
Fluorescence
E
% stained cells
± st dev; n ≥ 9
ctrl
B7
FCERA
KI2L2
IL3RB
F
Titer [µg/mL]
G
Mean fluorescence

Figure 14

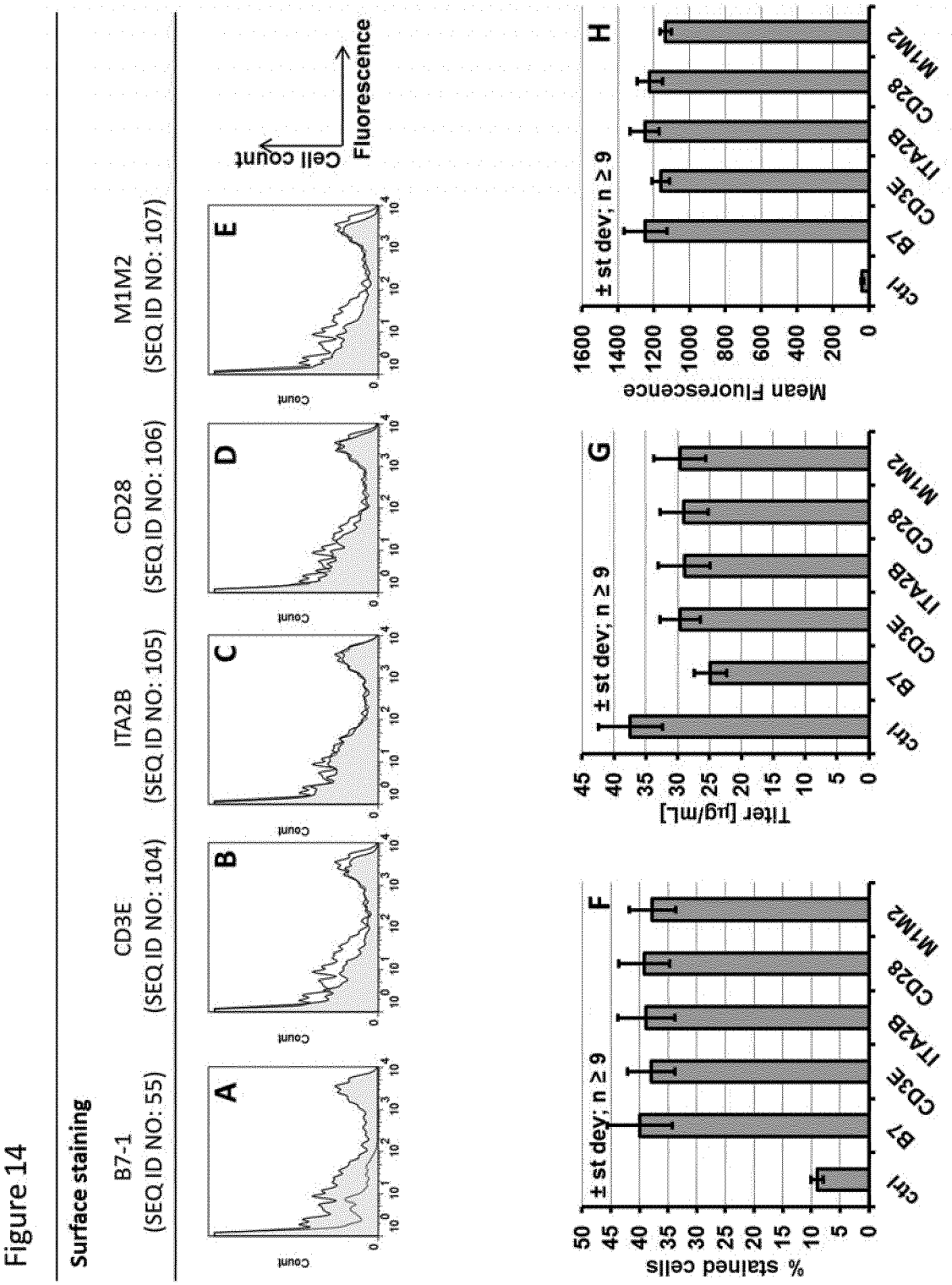

Surface staining
B7-1 (SEQ ID NO: 55)
CD3E (SEQ ID NO: 104)
ITA2B (SEQ ID NO: 105)
CD28 (SEQ ID NO: 106)
M1M2 (SEQ ID NO: 107)
A
B
C
D
E
Count
Cell count
Fluorescence
F
% stained cells
± st dev; n ≥ 9
G
Titer [µg/mL]
H
Mean Fluorescence
ctrl
B7
CD3E
ITA2B
CD28
M1M2

Figure 15

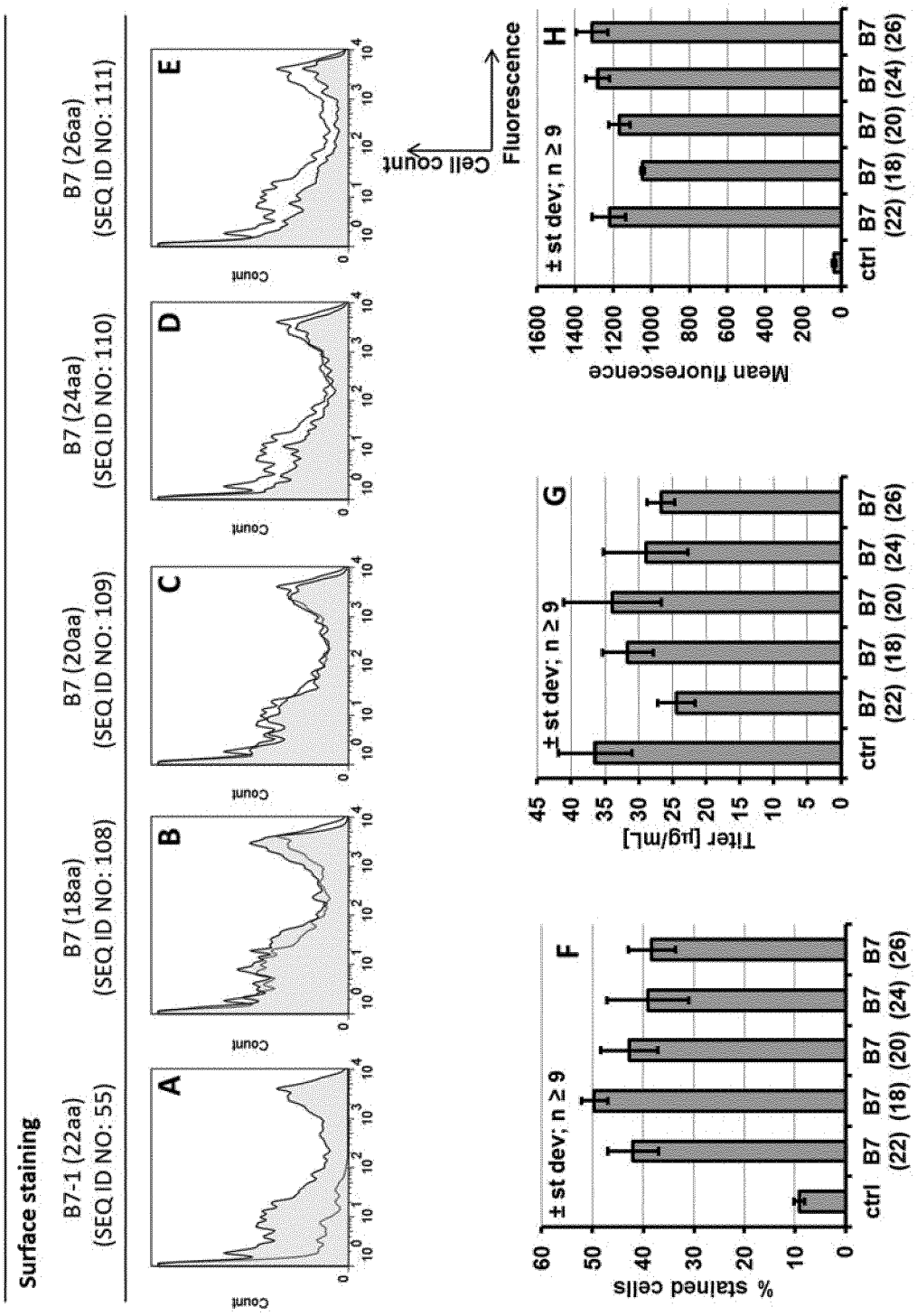

Surface staining
B7-1 (22aa) (SEQ ID NO: 55)
B7 (18aa) (SEQ ID NO: 108)
B7 (20aa) (SEQ ID NO: 109)
B7 (24aa) (SEQ ID NO: 110)
B7 (26aa) (SEQ ID NO: 111)
A
B
C
D
E
Count
Cell count
Fluorescence
% stained cells
Titer [µg/mL]
Mean fluorescence
± st dev; n ≥ 9
F
G
H
ctrl
B7 (22)
B7 (18)
B7 (20)
B7 (24)
B7 (26)

Figure 16

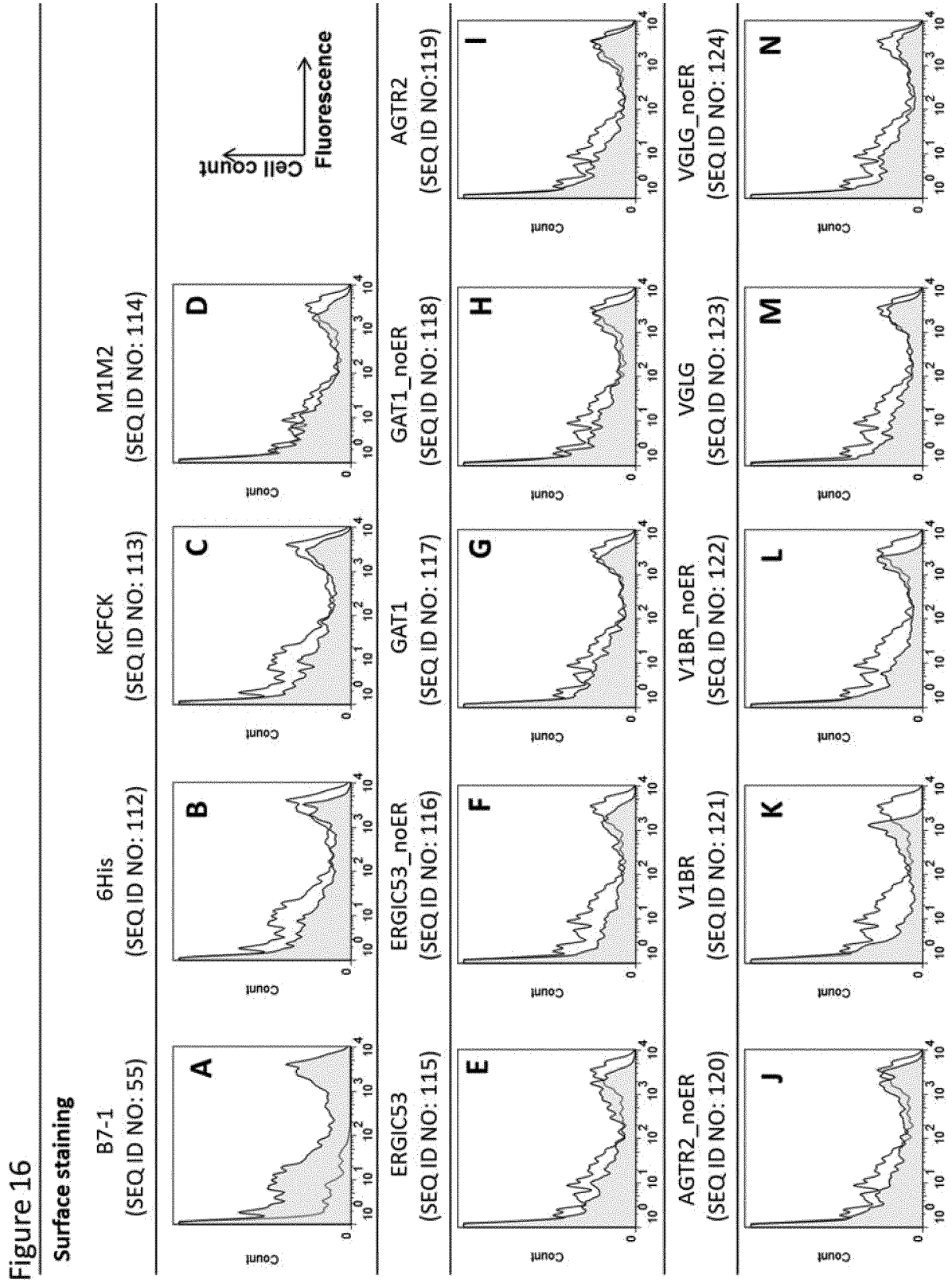

Surface staining
B7-1 (SEQ ID NO: 55)
6His (SEQ ID NO: 112)
KCFCK (SEQ ID NO: 113)
M1M2 (SEQ ID NO: 114)
A
B
C
D
Cell count
Fluorescence
Count
ERGIC53 (SEQ ID NO: 115)
ERGIC53_noER (SEQ ID NO: 116)
GAT1 (SEQ ID NO: 117)
GAT1_noER (SEQ ID NO: 118)
AGTR2 (SEQ ID NO:119)
E
F
G
H
I
AGTR2_noER (SEQ ID NO: 120)
V1BR (SEQ ID NO: 121)
V1BR_noER (SEQ ID NO: 122)
VGLG (SEQ ID NO: 123)
VGLG_noER (SEQ ID NO: 124)
J
K
L
M
N

Figure 16 (contd)

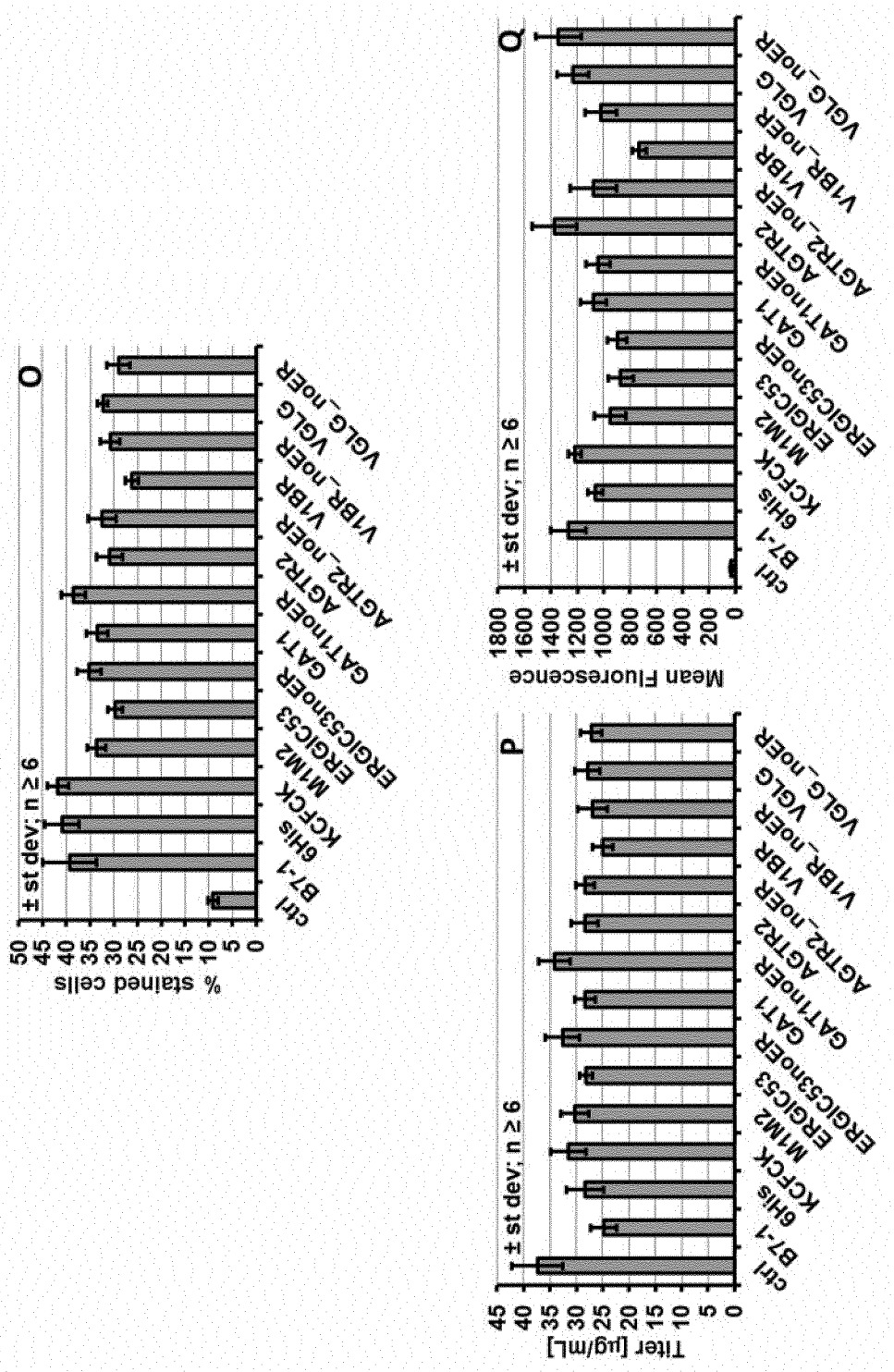


Figure 17

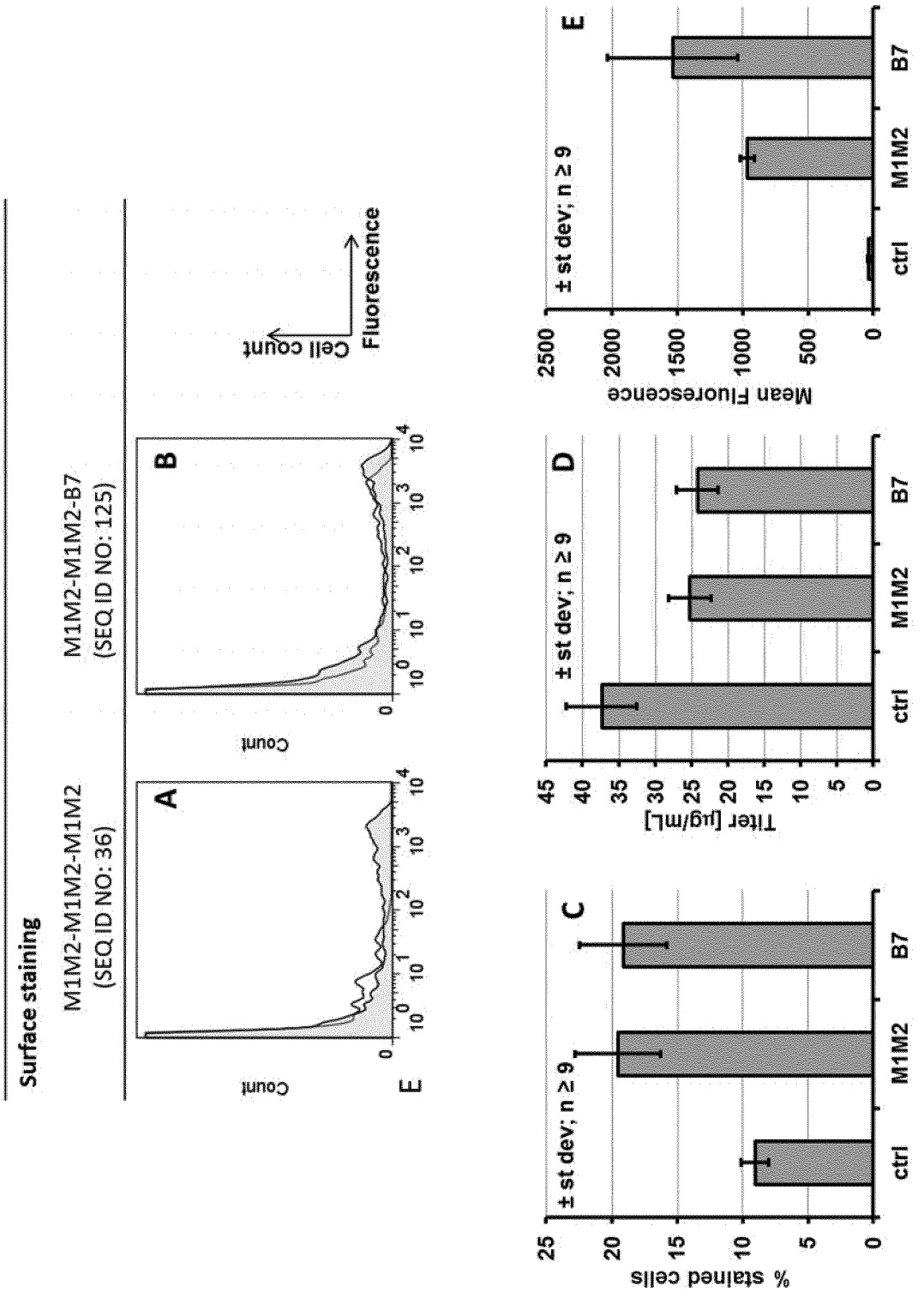
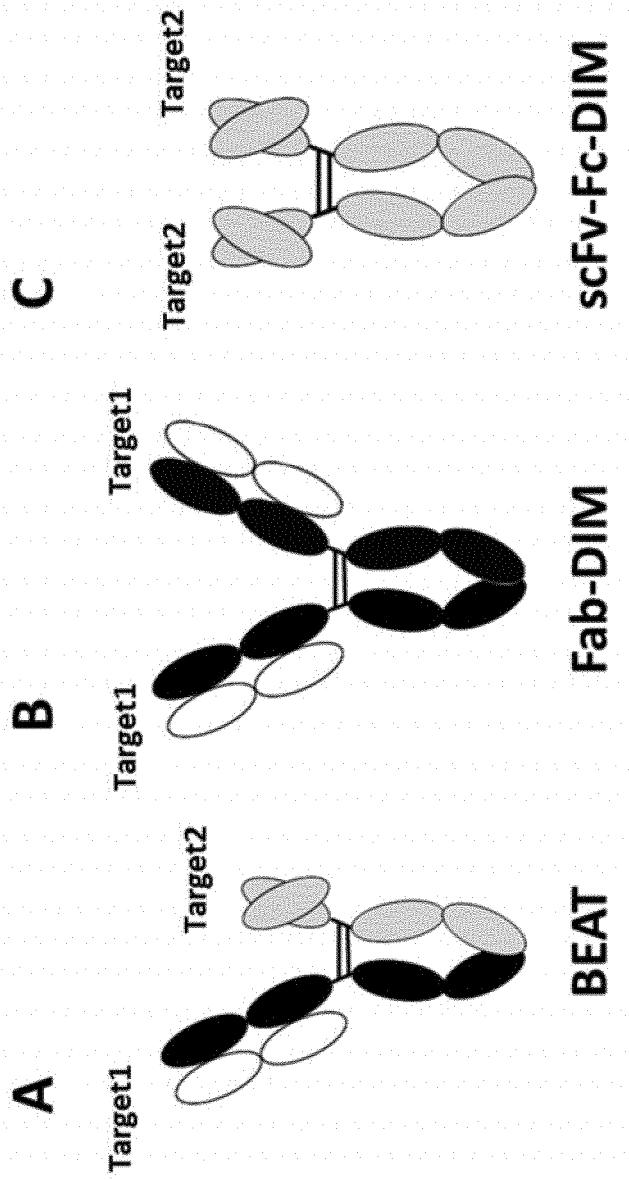

Surface staining
M1M2-M1M2-M1M2
(SEQ ID NO: 36)
M1M2-M1M2-B7
(SEQ ID NO: 125)
A
B
Count
Cell count
Fluorescence
E
% stained cells
± st dev; n ≥ 9
C
ctrl
M1M2
B7
Titer [µg/mL]
D
Mean Fluorescence
E

Figure 18


A
Target1
Target2
BEAT
B
Target1
Target1
Fab-DIM
C
Target2
Target2
scFv-Fc-DIM

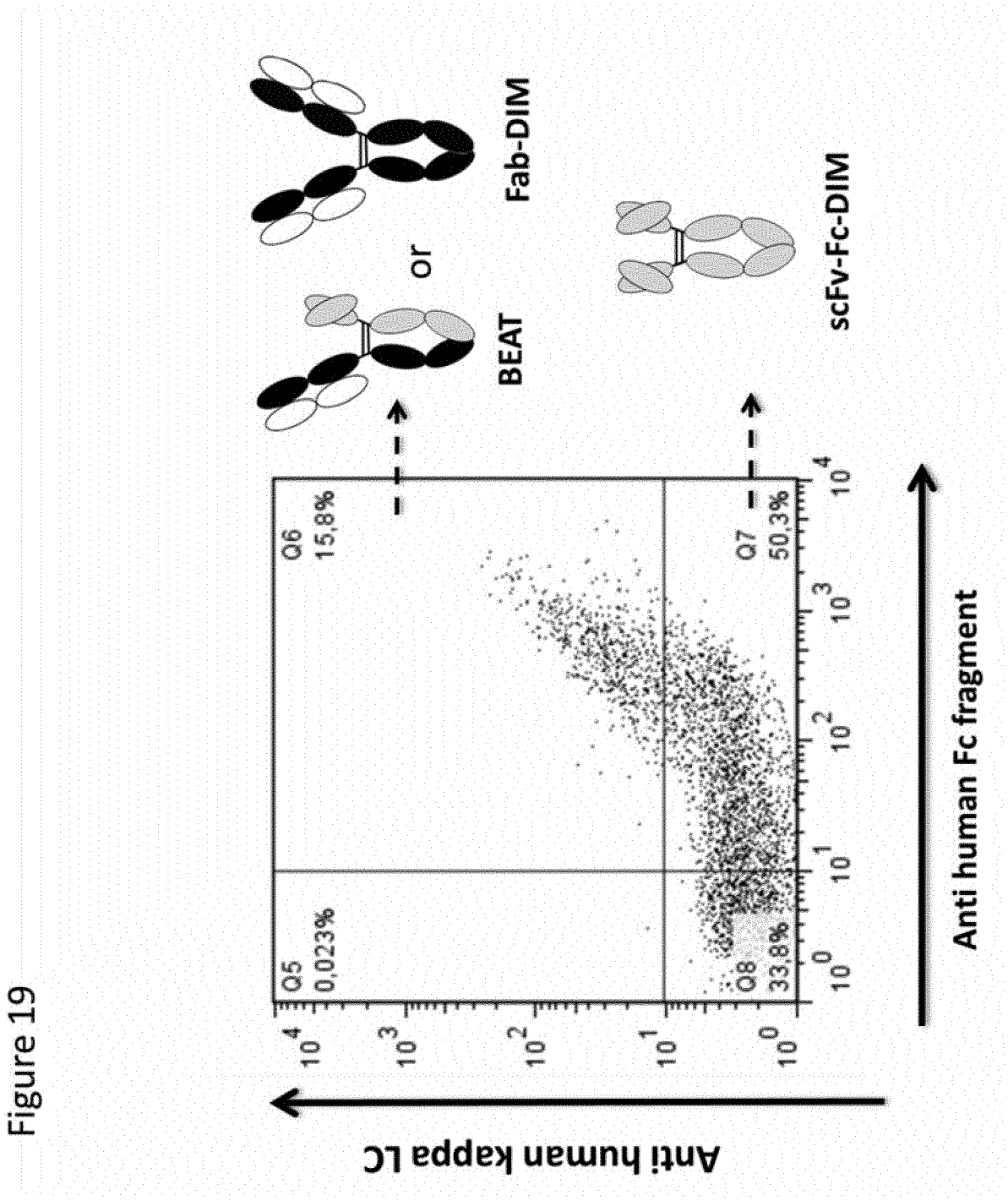

Fab-DIM
or
BEAT
scFv-Fc-DIM
Q6
15,8%
Q7
50,3%
Q5
0,023%
Q8
33,8%
Anti human kappa LC
Anti human Fc fragment

Figure 19

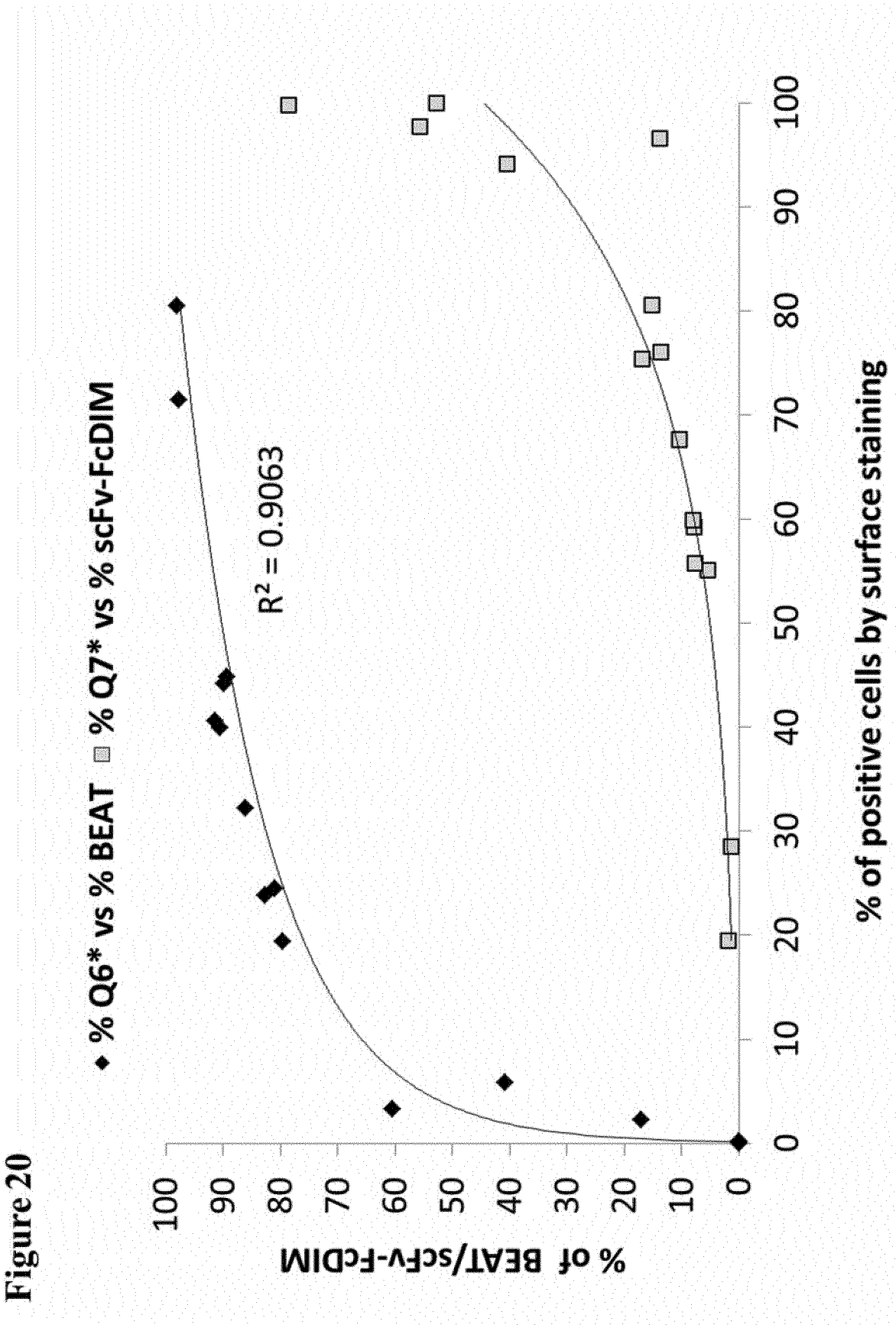
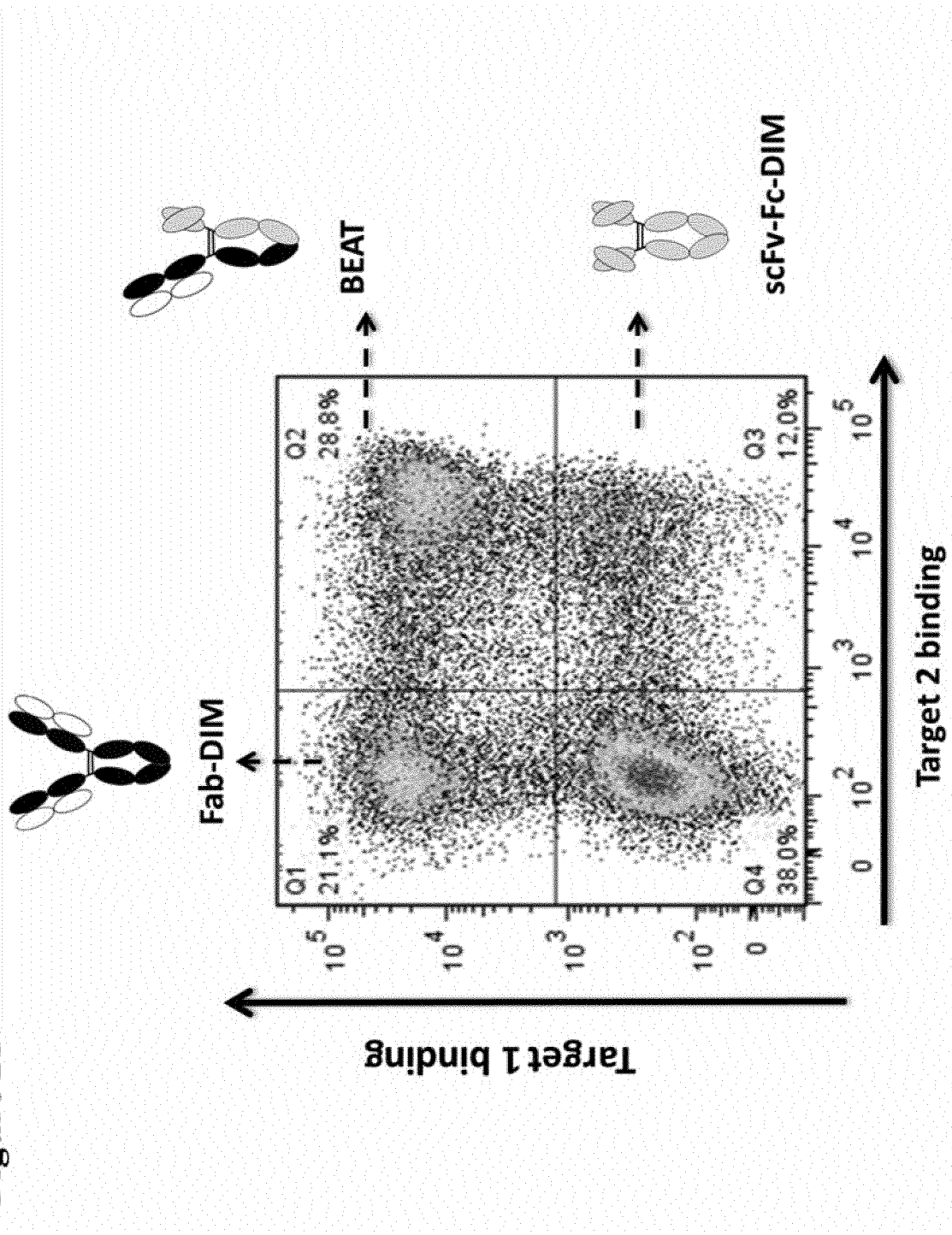

% Q6* vs % BEAT
% Q7* vs % scFv-FcDIM
$R^2 = 0.9063$
% of BEAT/scFv-FcDIM
% of positive cells by surface staining
0
10
20
30
40
50
60
70
80
90
100

Figure 20

Figure 21


Fab-DIM
BEAT
scFv-Fc-DIM
Q1
21,1%
Q2
28,8%
Q3
12,0%
Q4
38,0%
Target 1 binding
Target 2 binding
0
10^2
10^3
10^4
10^5

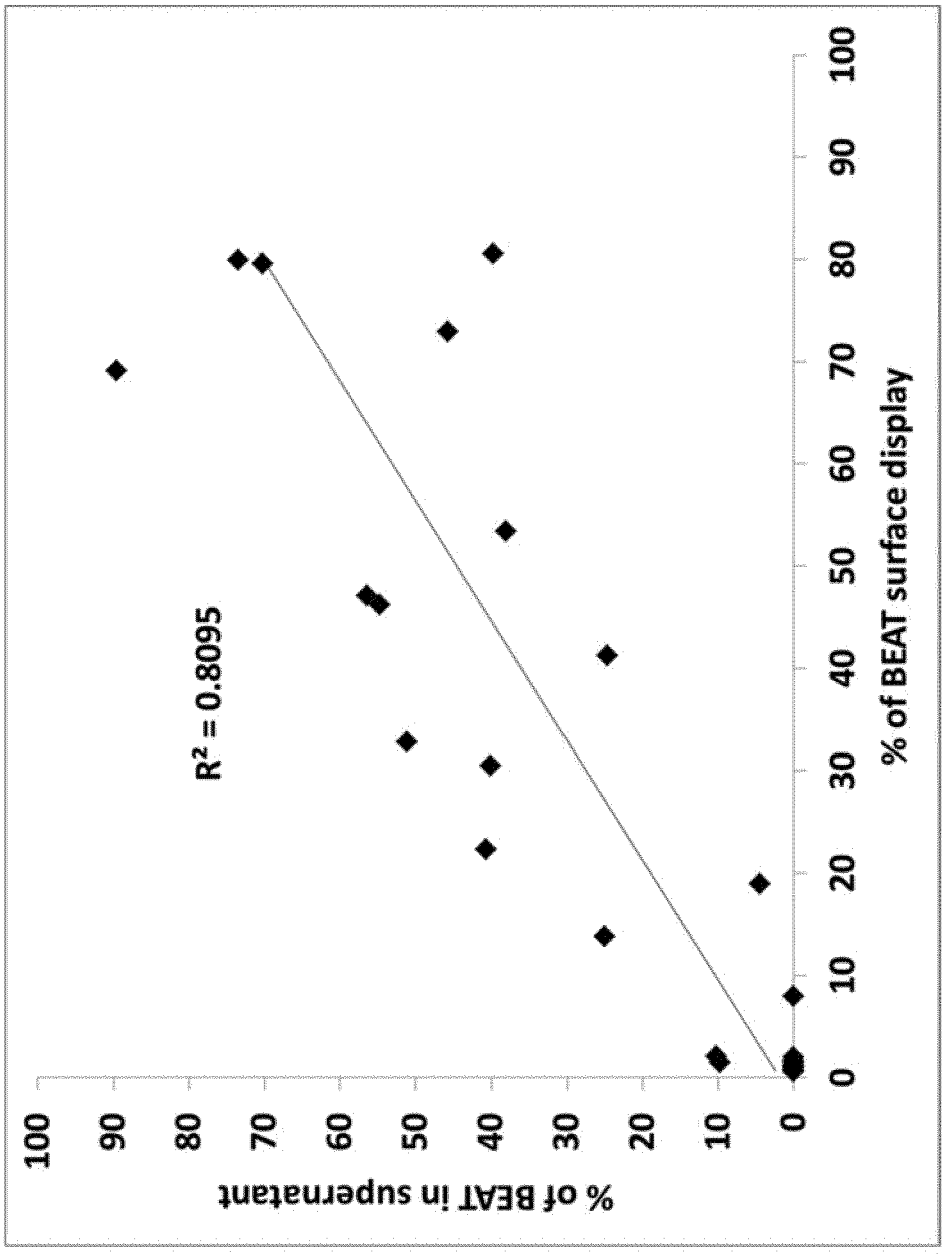

% of BEAT in supernatant
% of BEAT surface display
R² = 0.8095
0
10
20
30
40
50
60
70
80
90
100

Figure 22

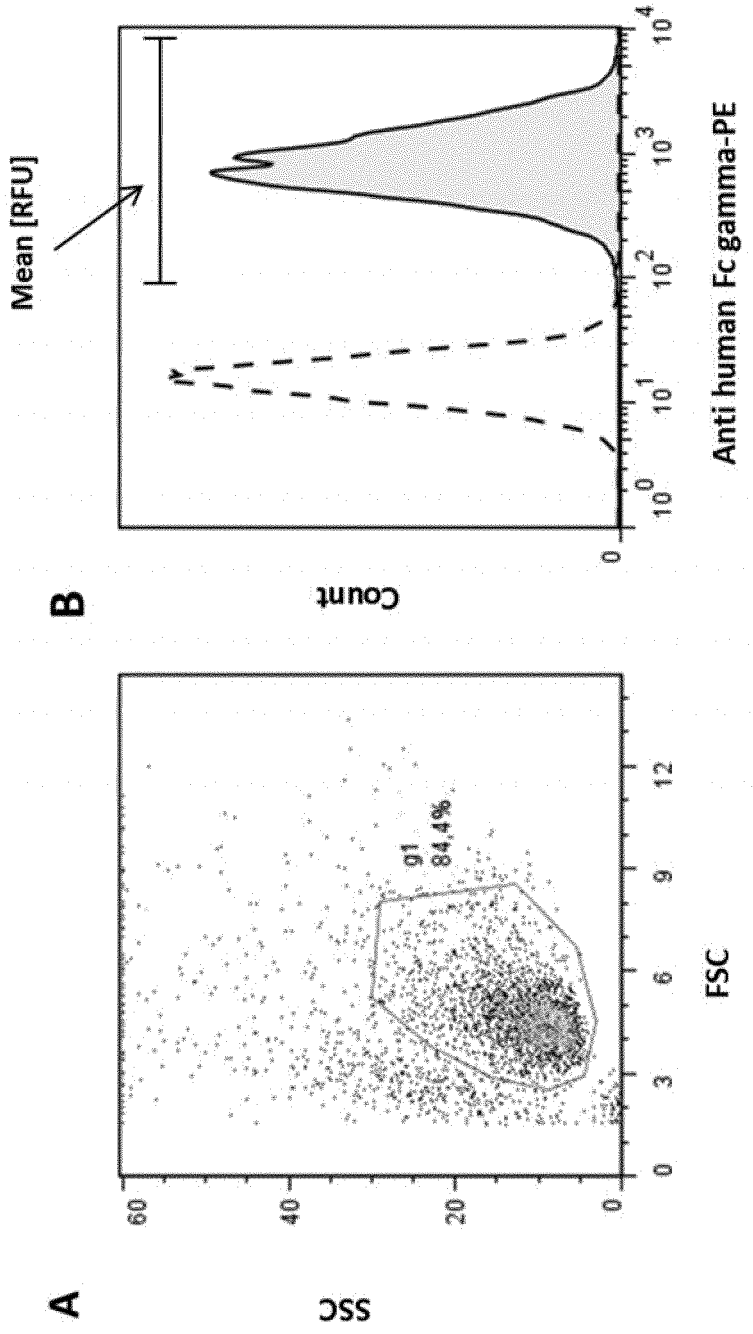


Figure 23

Figure 24

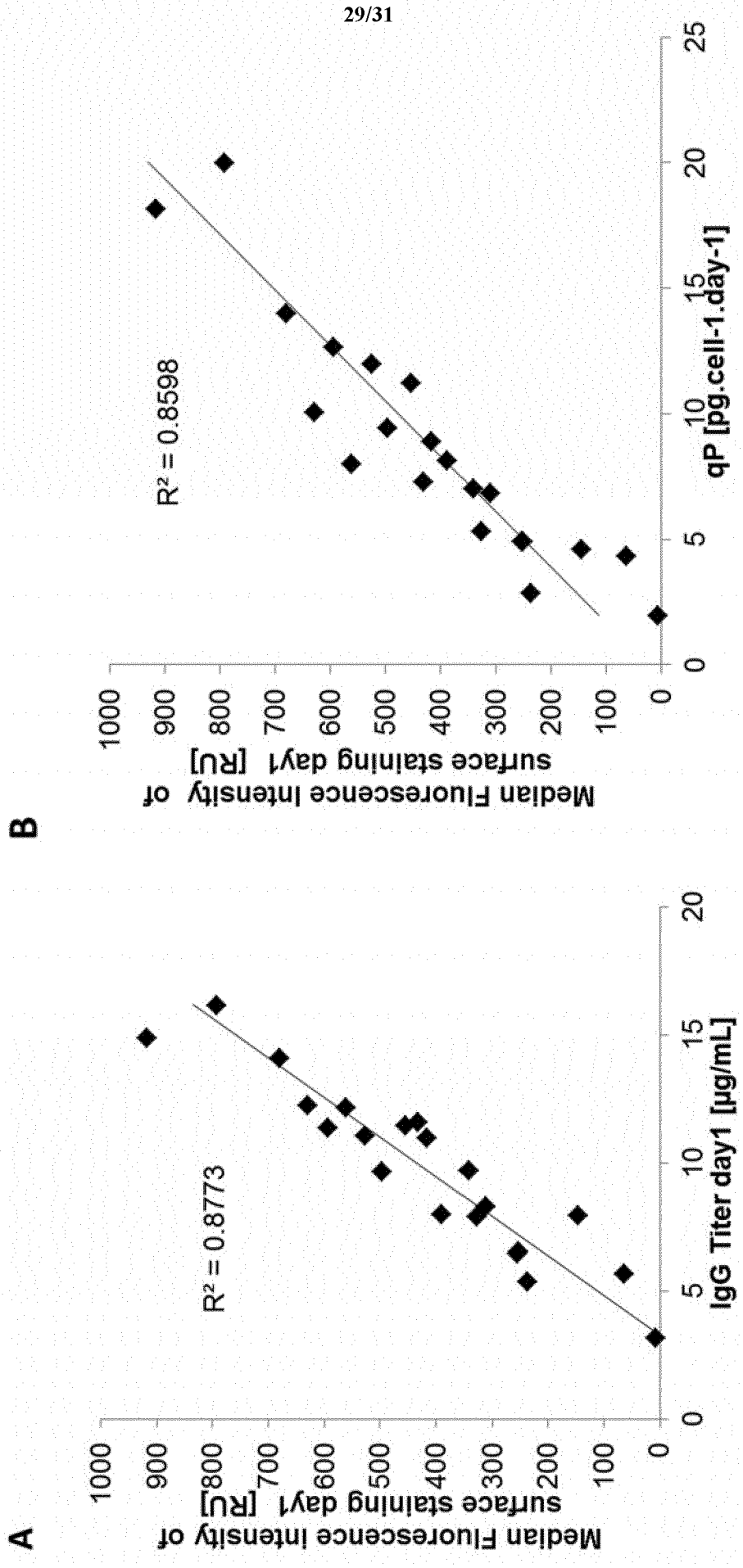

A
Median Fluorescence Intensity of surface staining day1 [RU]
IgG Titer day1 [µg/mL]
R² = 0.8773
B
Median Fluorescence Intensity of surface staining day1 [RU]
qP [pg.cell-1.day-1]
R² = 0.8598

Figure 25

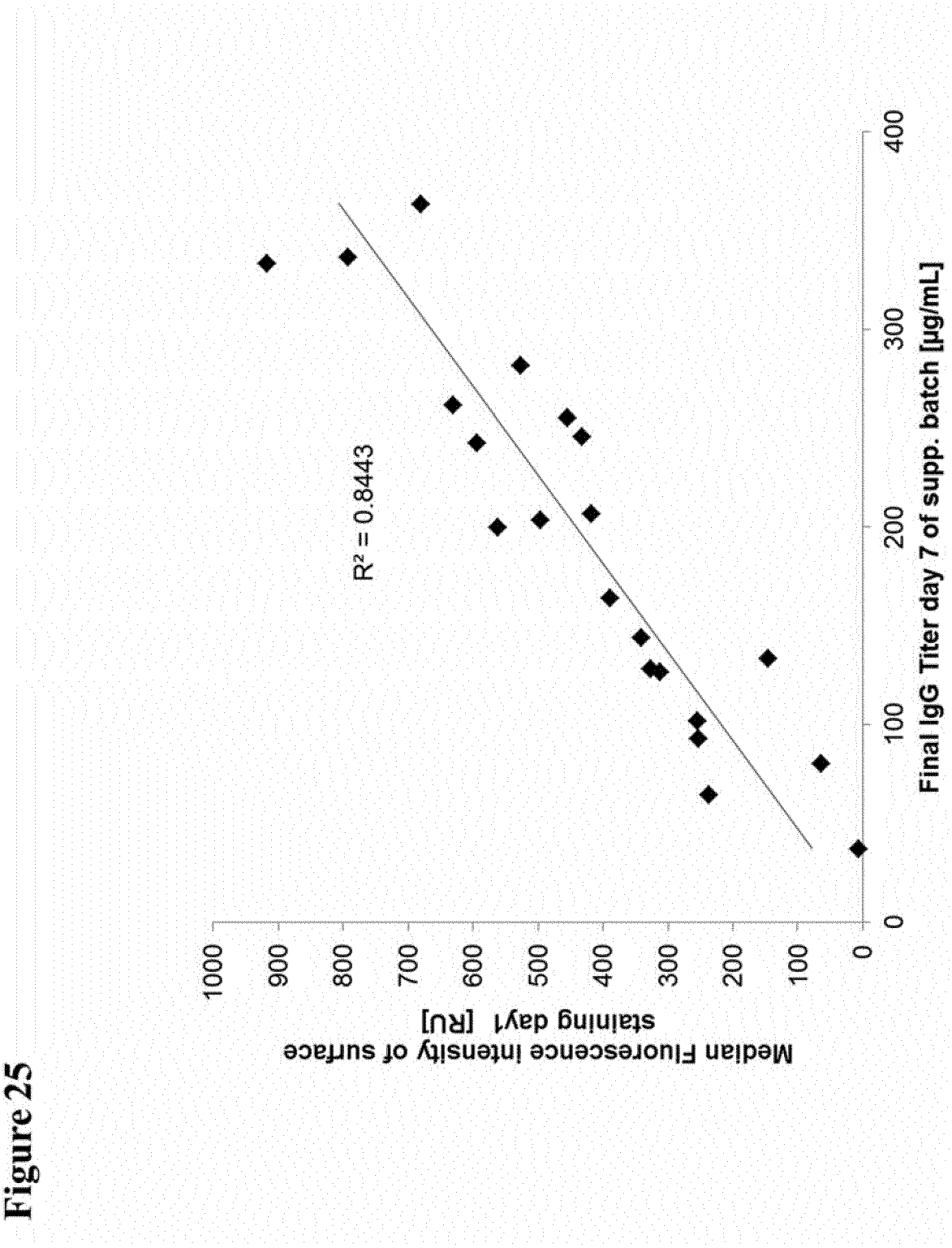
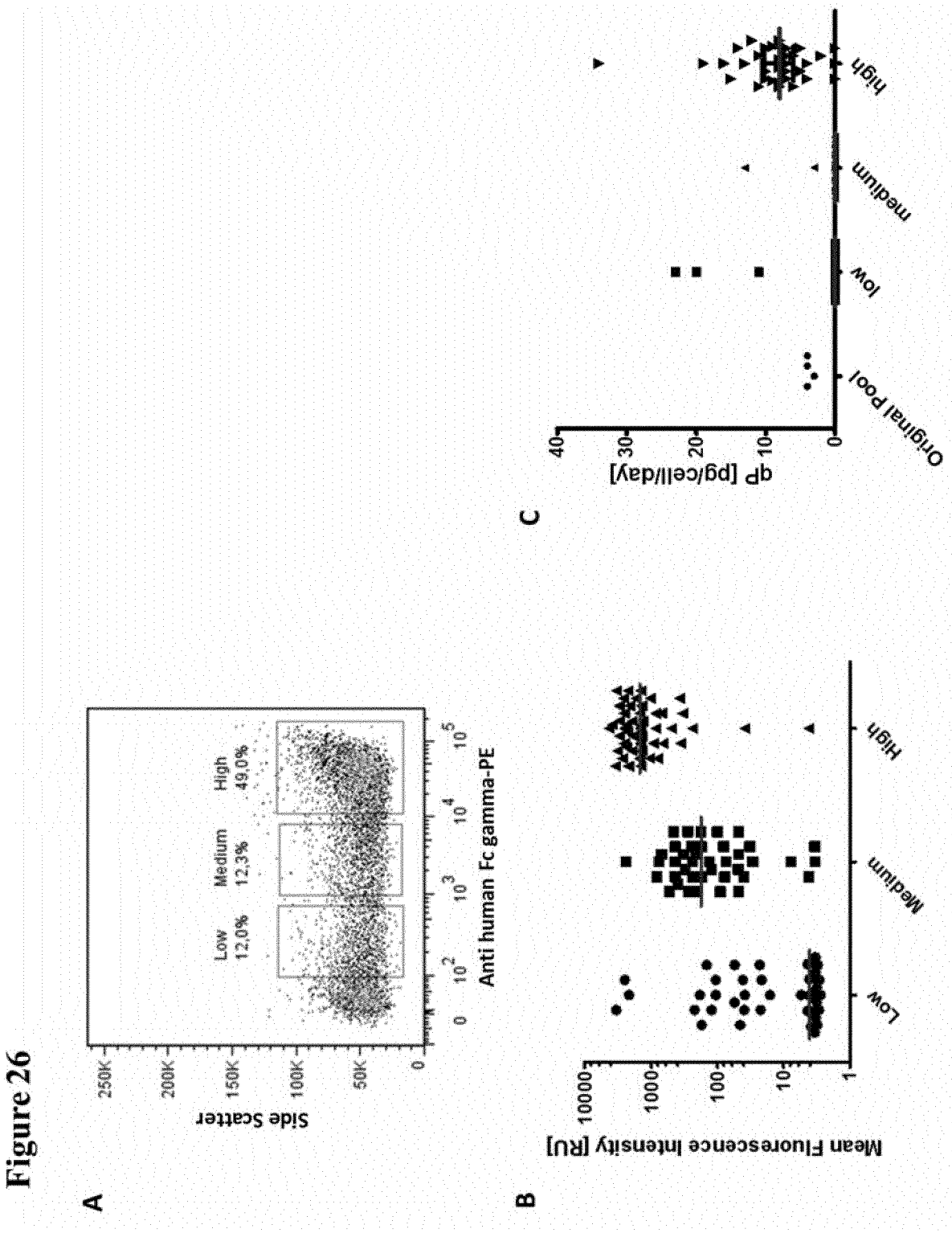

Median Fluorescence Intensity of surface staining day1 [RU]
1000
900
800
700
600
500
400
300
200
100
0
R² = 0.8443
0
100
200
300
400
Final IgG Titer day 7 of supp. batch [µg/mL]

Figure 26


A
Side Scatter
250K
200K
150K
100K
50K
0
Low
12,0%
Medium
12,3%
High
49,0%
0
10^2
10^3
10^4
10^5
Anti human Fc gamma-PE
B
Mean Fluorescence Intensity [RU]
10000
1000
100
10
1
Low
Medium
High
C
qP [pg/cell/day]
40
30
20
10
0
Original Pool
low
medium
high