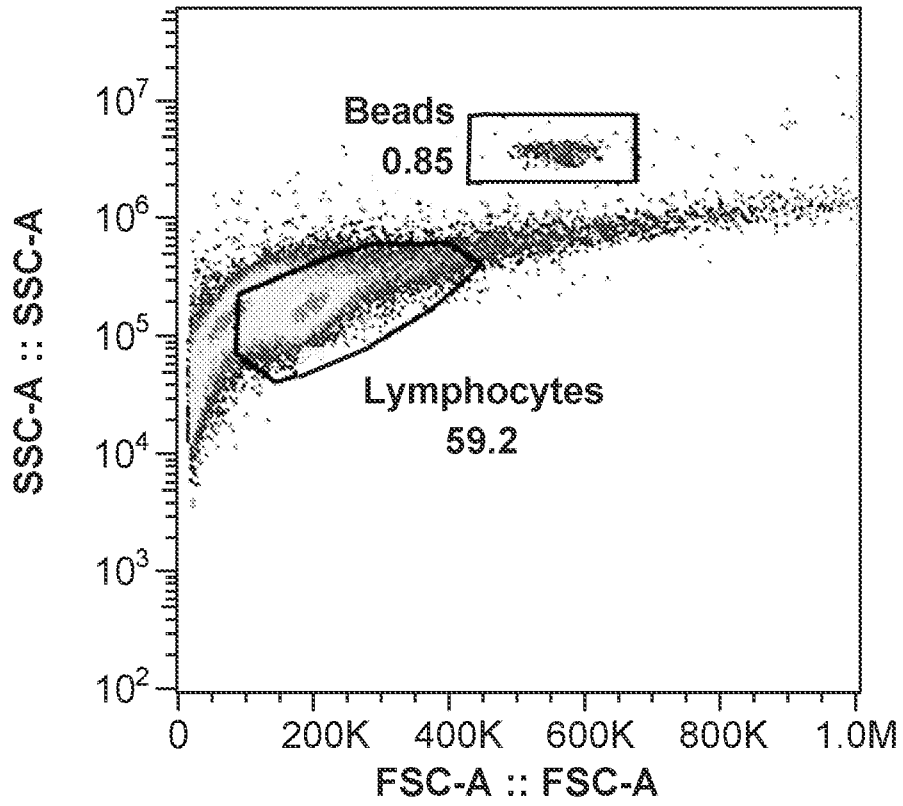

Beads
0.85
Lymphocytes
59.2
SSC-A :: SSC-A
FSC-A :: FSC-A
10^7
10^6
10^5
10^4
10^3
10^2
0
200K
400K
600K
800K
1.0M

FIG. 1A

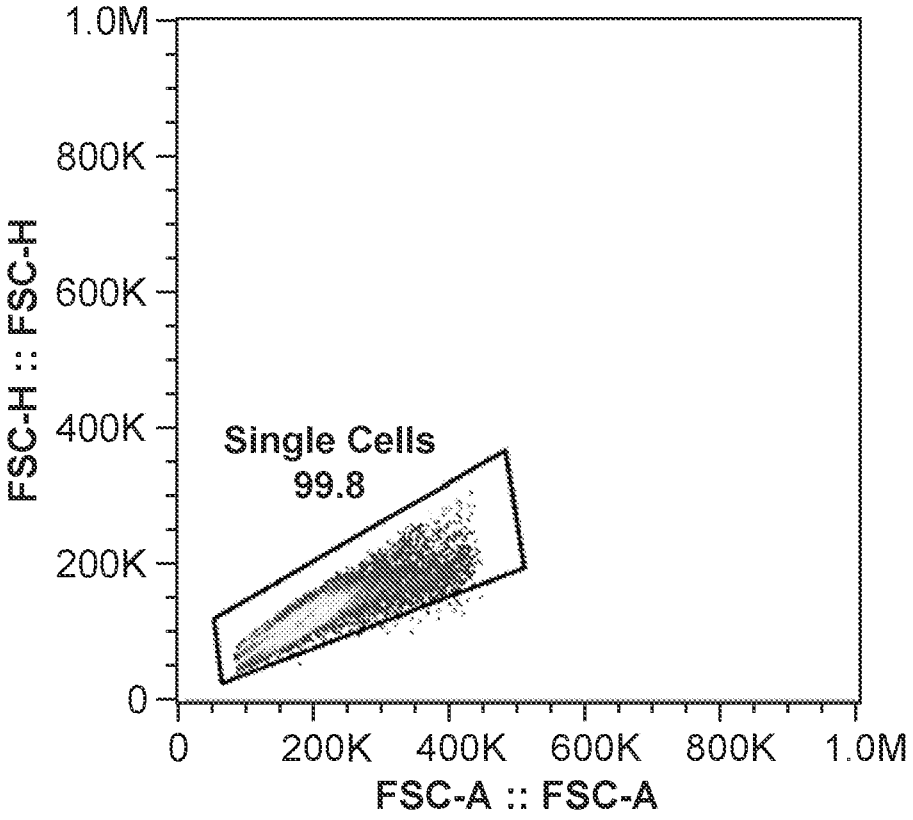

Single Cells
99.8
FSC-H :: FSC-H
FSC-A :: FSC-A
1.0M
800K
600K
400K
200K
0
0
200K
400K
600K
800K
1.0M

FIG. 1B

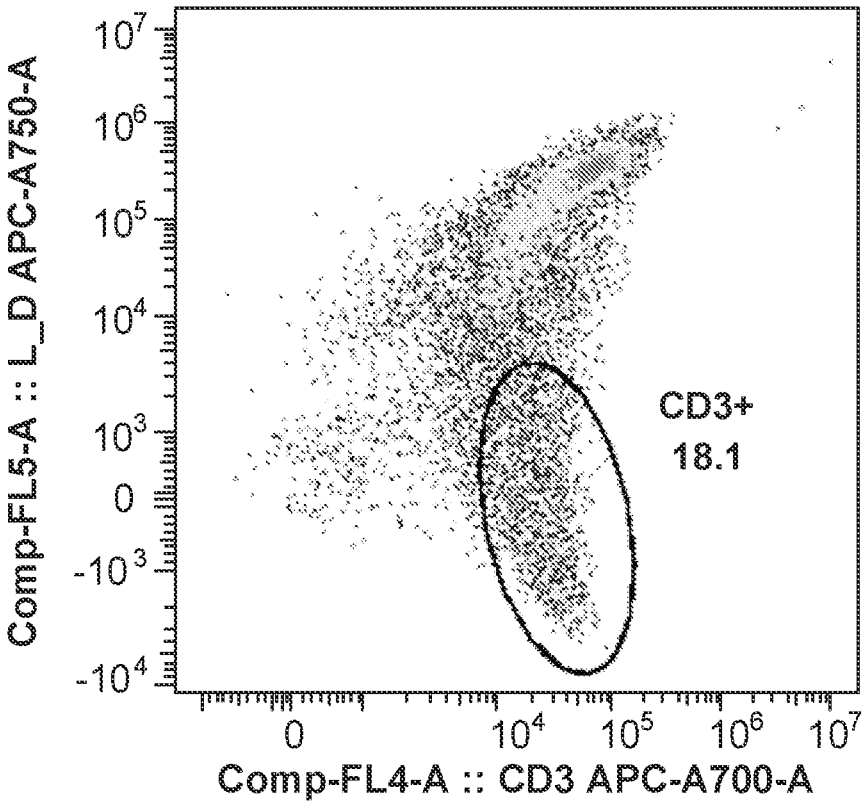

Comp-FL5-A :: L_D APC-A750-A
Comp-FL4-A :: CD3 APC-A700-A
CD3+
18.1
10^7
10^6
10^5
10^4
10^3
0
-10^3
-10^4
0
10^4
10^5
10^6
10^7

FIG. 1C

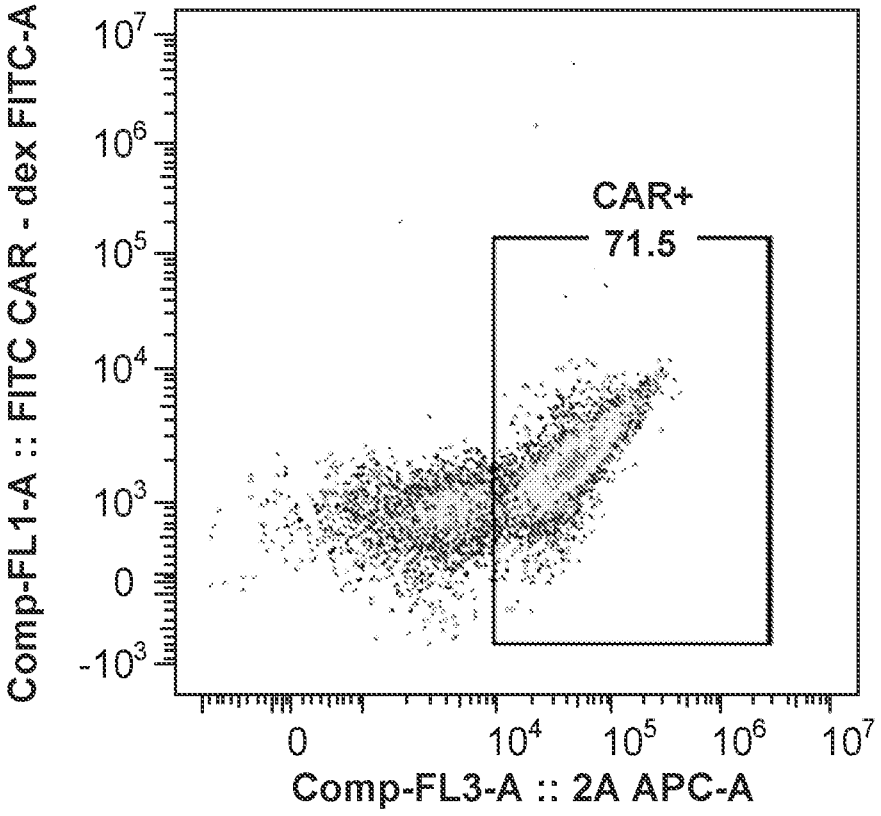

Comp-FL1-A :: FITC CAR - dex FITC-A
Comp-FL3-A :: 2A APC-A
CAR+
71.5
10^7
10^6
10^5
10^4
10^3
0
-10^3
0
10^4
10^5
10^6
10^7

FIG. 1D

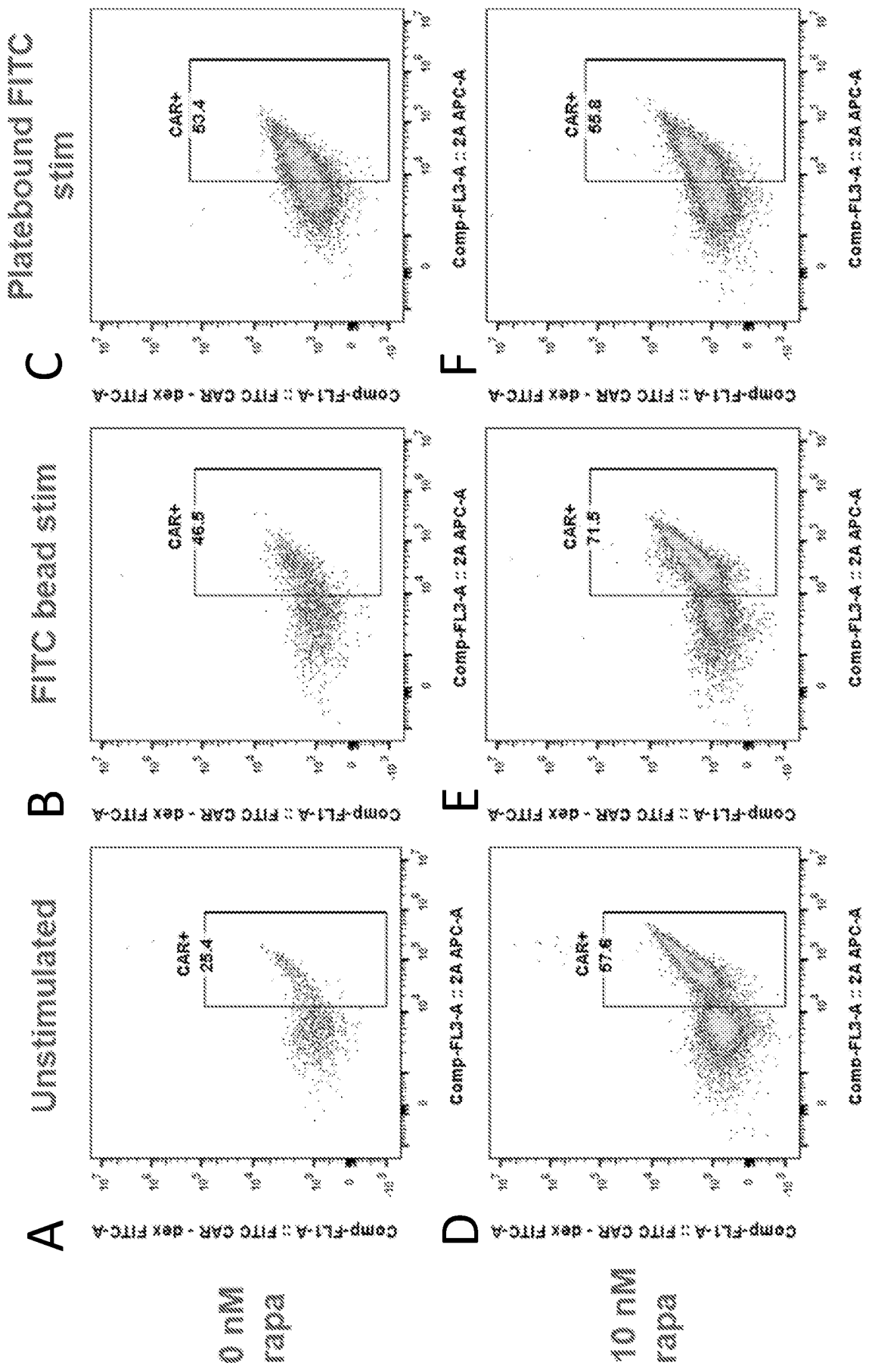

A
Unstimulated
B
FITC bead stim
C
Platebound FITC stim
0 nM rapa
10 nM rapa
D
E
F
CAR+
25.4
CAR+
46.5
CAR+
53.4
CAR+
67.6
CAR+
71.5
CAR+
65.8
Comp-FL1-A :: FITC CAR - dex FITC-A
Comp-FL3-A :: 2A APC-A

FIG. 2A-2F

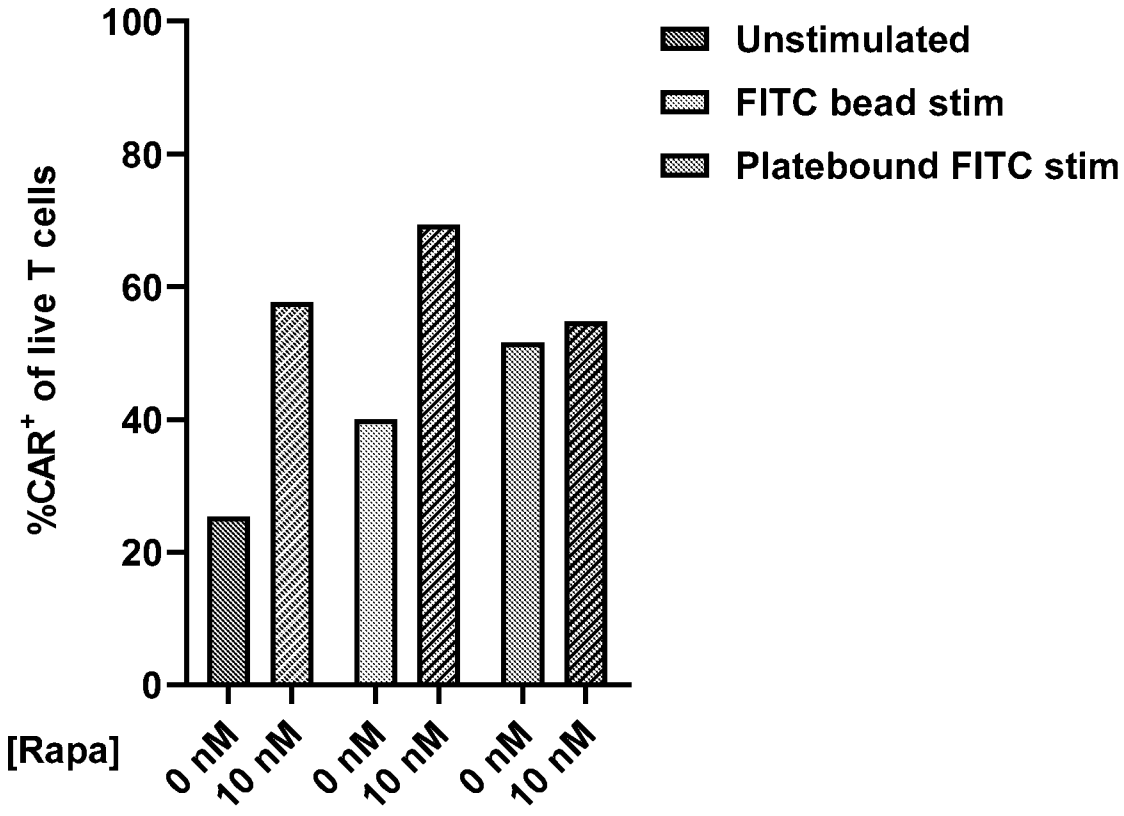

100
80
60
40
20
0
%CAR+ of live T cells
[Rapa]
0 nM
10 nM
0 nM
10 nM
0 nM
10 nM
Unstimulated
FITC bead stim
Platebound FITC stim

FIG. 3

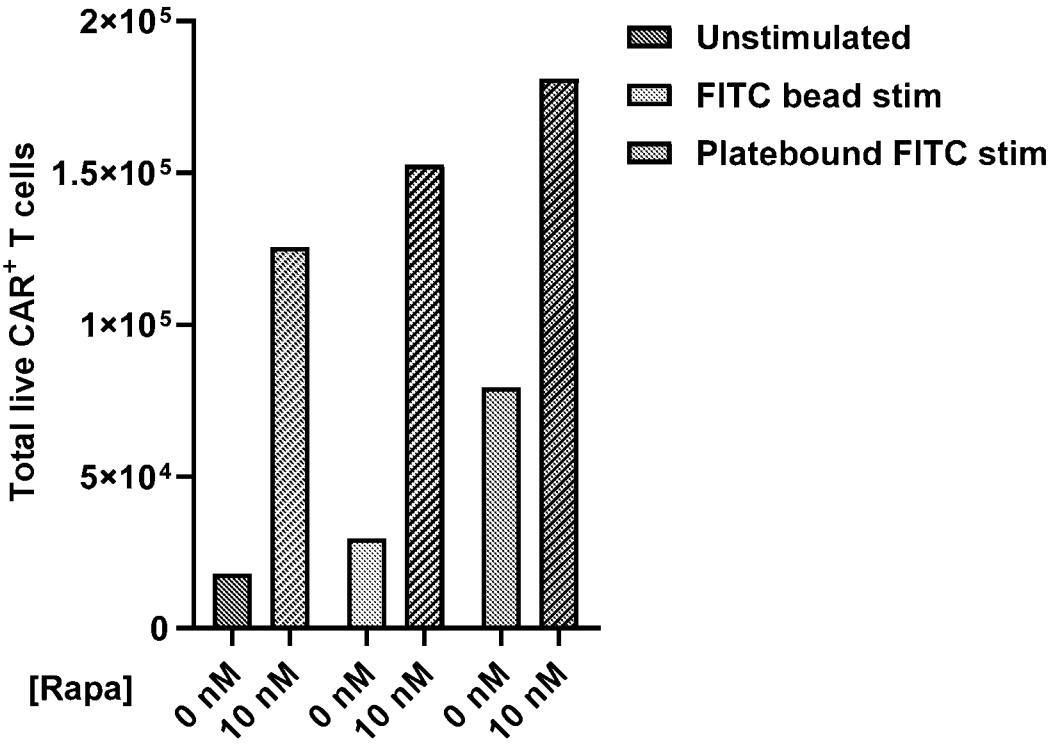

2×10^5
1.5×10^5
1×10^5
5×10^4
0
Total live CAR+ T cells
[Rapa]
0 nM
10 nM
0 nM
10 nM
0 nM
10 nM
Unstimulated
FITC bead stim
Platebound FITC stim

FIG. 4

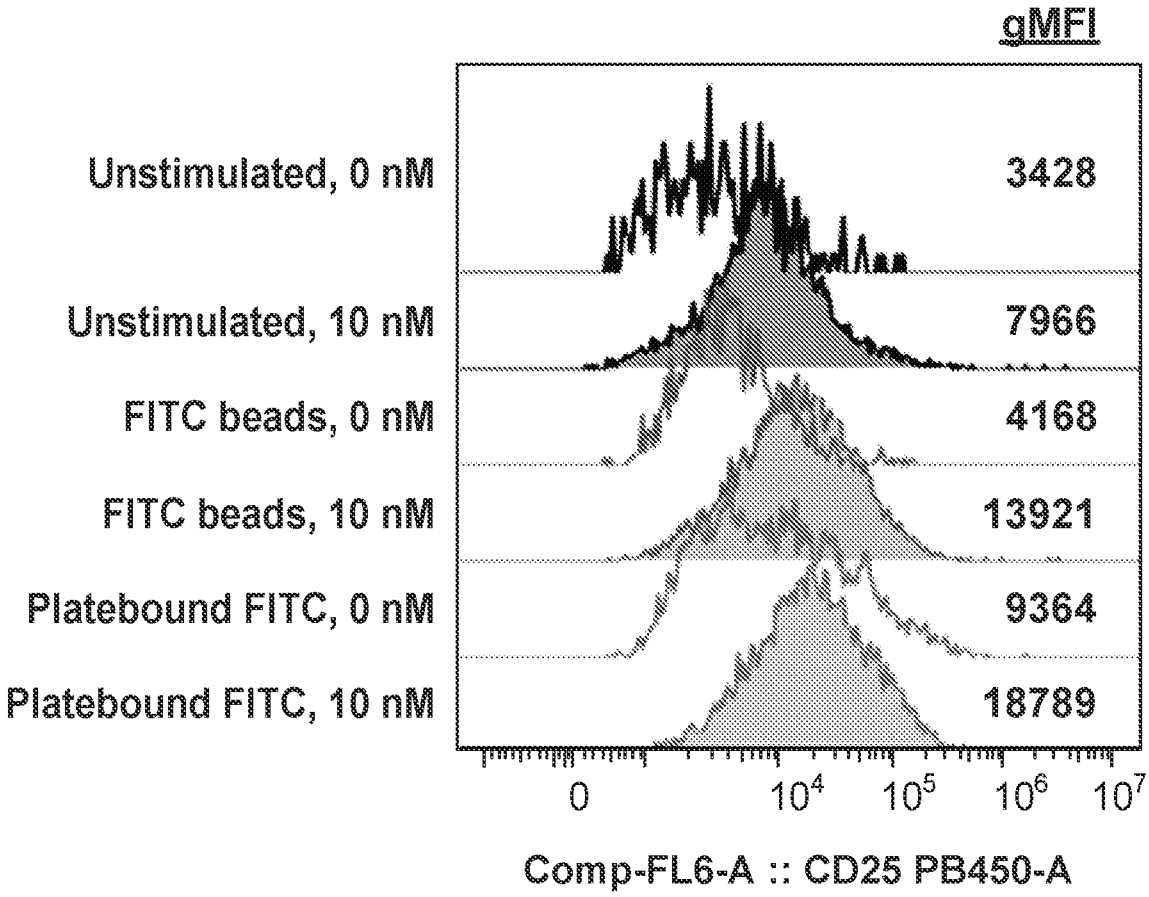

gMFI
Unstimulated, 0 nM
3428
Unstimulated, 10 nM
7966
FITC beads, 0 nM
4168
FITC beads, 10 nM
13921
Platebound FITC, 0 nM
9364
Platebound FITC, 10 nM
18789
0
10^4
10^5
10^6
10^7
Comp-FL6-A :: CD25 PB450-A

FIG. 5

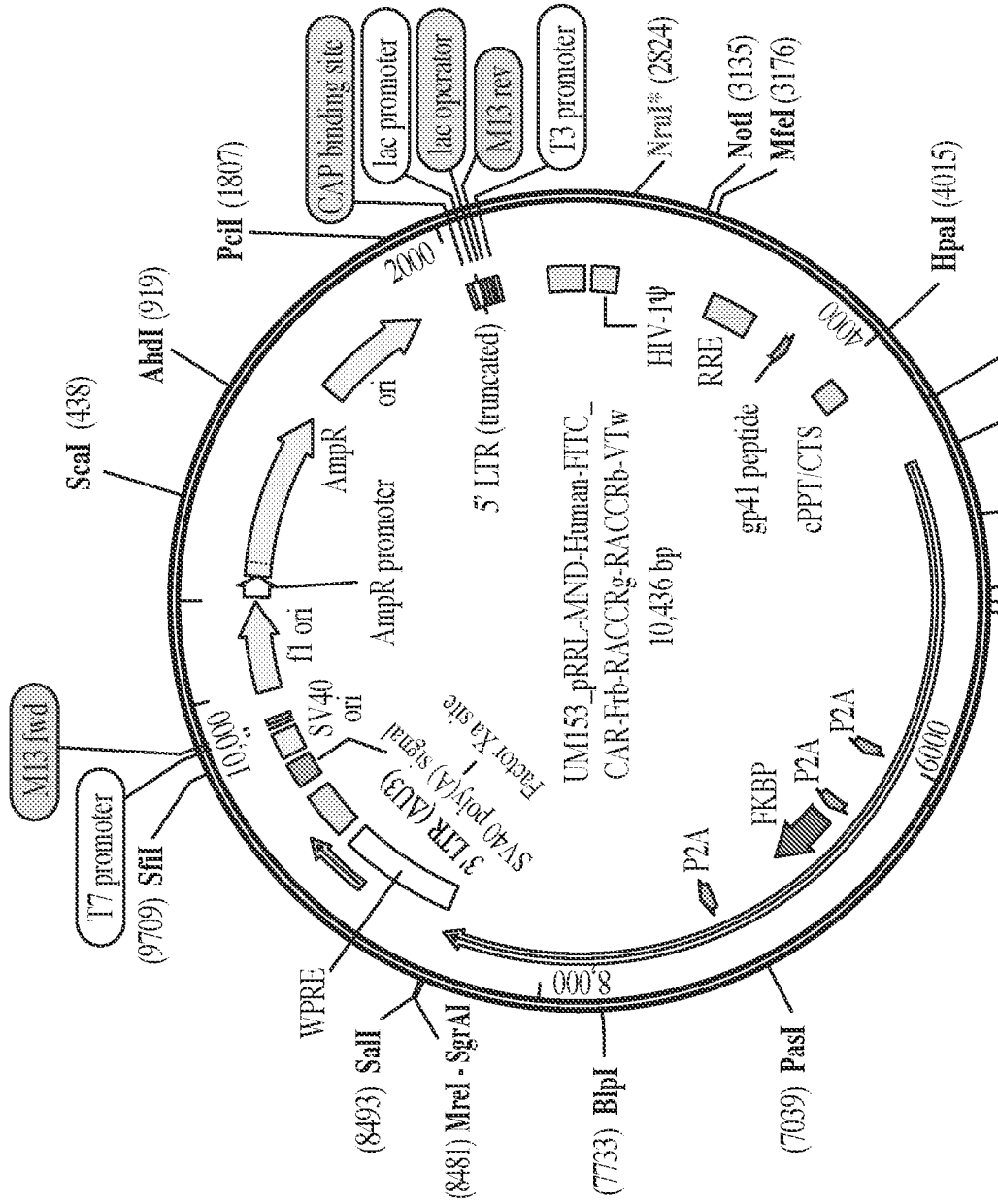

M13 fwd
T7 promoter
(9709) SfiI
ScaI (438)
AhdI (919)
PciI (1807)
CAP binding site
lac promoter
lac operator
M13 rev
T3 promoter
NruI* (2824)
NotI (3135)
MfeI (3176)
HpaI (4015)
XbaI (4346)
SbfI (4550)
PshAI (4861)
FseI (5157)
SmaI (5222)
TspMI - XmaI (5220)
(5264) Bsu36I
(7039) PasI
(7733) BlpI
(8481) MreI - SgrAI
(8493) SalI
WPRE
3' LTR (ΔU3)
SV40 poly(A) signal
Factor Xa site
SV40 ori
f1 ori
AmpR promoter
AmpR
ori
5' LTR (truncated)
HIV-1ψ
RRE
gp41 peptide
cPPT/CTS
P2A
FKBP
P2A
P2A
UM153_pRRL-MND-Human-FITC_
CAR-Frb-RACCRg-RACCRb-VTw
10,436 bp
2000
4000
6000
8000
10000

FIG. 6

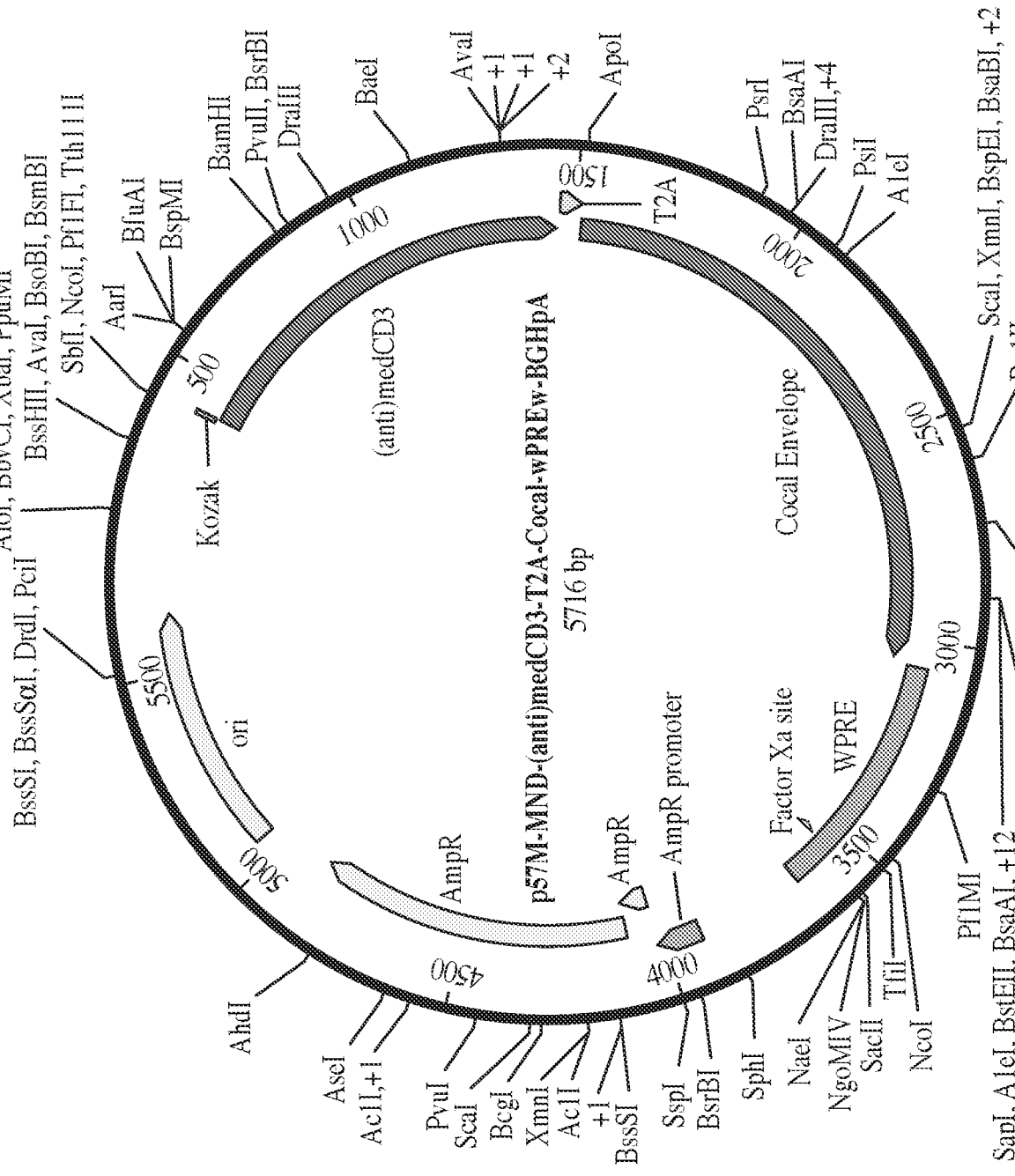

p57M-MND-(anti)medCD3-T2A-Cocal-wPREw-BGHpA
5716 bp
(anti)medCD3
T2A
Cocal Envelope
WPRE
Factor Xa site
AmpR promoter
AmpR
AmpR
ori
Kozak
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
AloI, BbvCI, XbaI, PpuMI
BssSI, BssSαI, DrdI, PciI
BssHII, AvaI, BsoBI, BsmBI
SbfI, NcoI, Pf1FI, Tth111I
AarI
BfuAI
BspMI
BamHI
PvuII, BsrBI
DraIII
BaeI
AvaI
+1
+1
+2
ApoI
PsrI
BsaAI
DraIII,+4
PsiI
A1eI
ScaI, XmnI, BspEI, BsaBI, +2
Bg1II
SphI, PvuII, A1oI
BspQI
SapI, A1eI, BstEII, BsaAI, +12
Pf1MI
NcoI
TfiI
SacII
NgoMIV
NaeI
SphI
SspI
BsrBI
+1
BssSI
Ac11
XmnI
BcgI
ScaI
PvuI
Ac11,+1
AseI
AhdI

FIG. 7