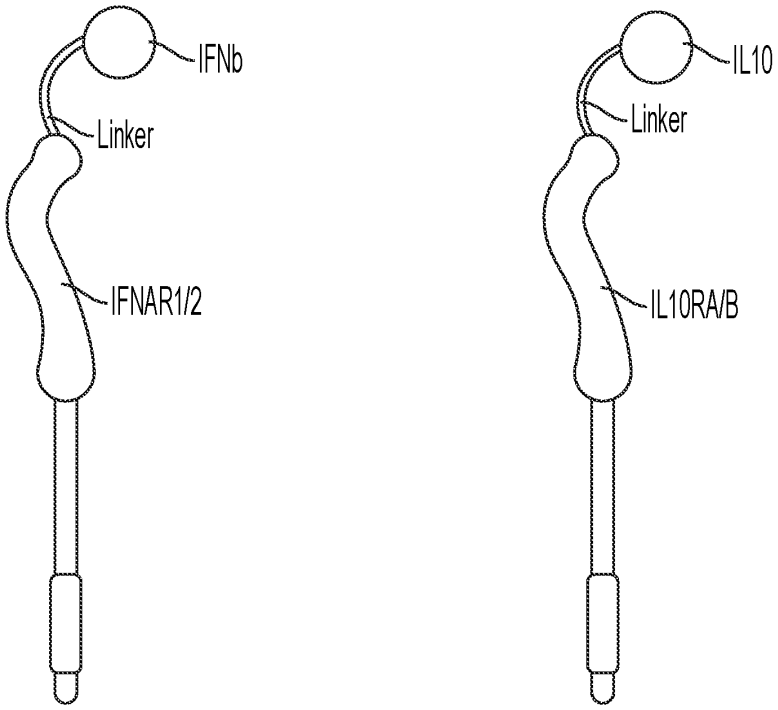


Figure 1

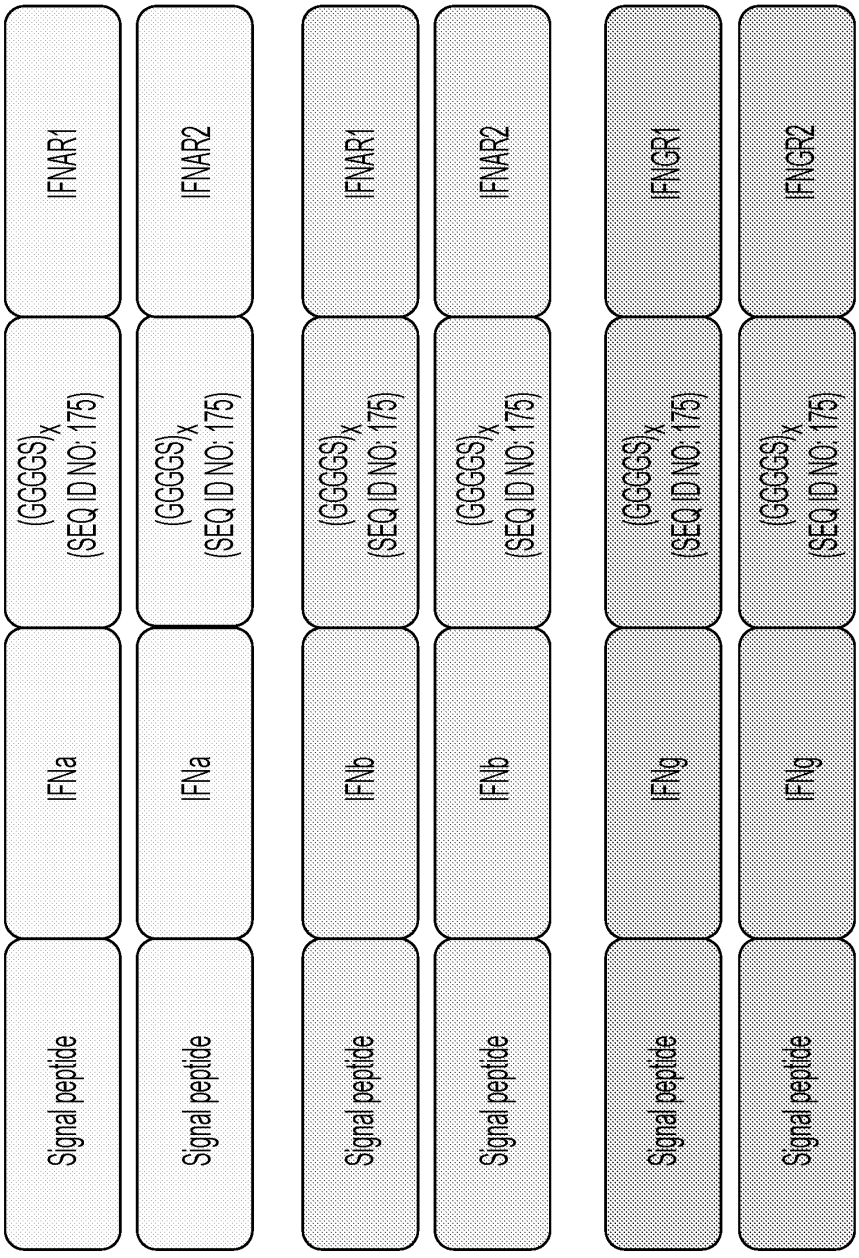

Signal peptide
IFNa
(GGGGS)x (SEQ ID NO: 175)
IFNAR1
Signal peptide
IFNa
(GGGGS)x (SEQ ID NO: 175)
IFNAR2
Signal peptide
IFNb
(GGGGS)x (SEQ ID NO: 175)
IFNAR1
Signal peptide
IFNb
(GGGGS)x (SEQ ID NO: 175)
IFNAR2
Signal peptide
IFNg
(GGGGS)x (SEQ ID NO: 175)
IFNGR1
Signal peptide
IFNg
(GGGGS)x (SEQ ID NO: 175)
IFNGR2

Figure 2

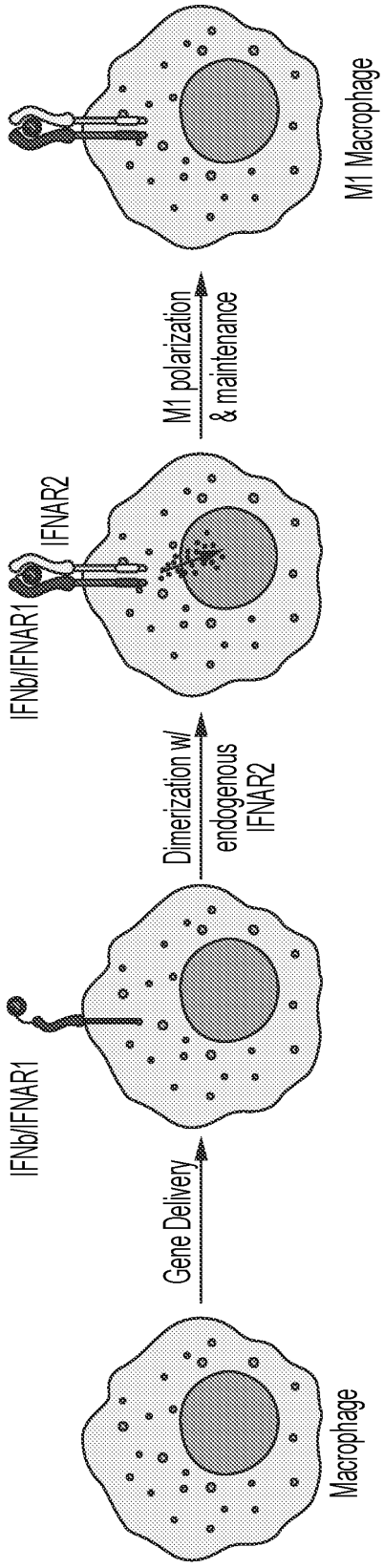

Macrophage
Gene Delivery
IFNb/IFNAR1
Dimerization w/
endogenous
IFNAR2
IFNb/IFNAR1
IFNAR2
M1 polarization
& maintenance
M1 Macrophage

Figure 3A

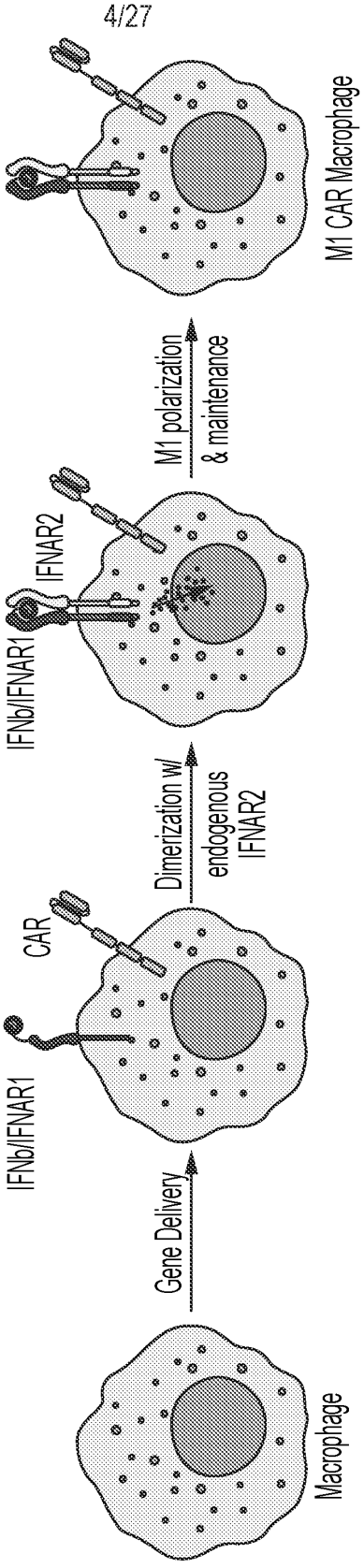

Macrophage
Gene Delivery
IFNb/IFNAR1
CAR
Dimerization w/
endogenous
IFNAR2
IFNb/IFNAR1
IFNAR2
M1 polarization
& maintenance
M1 CAR Macrophage

Figure 3B

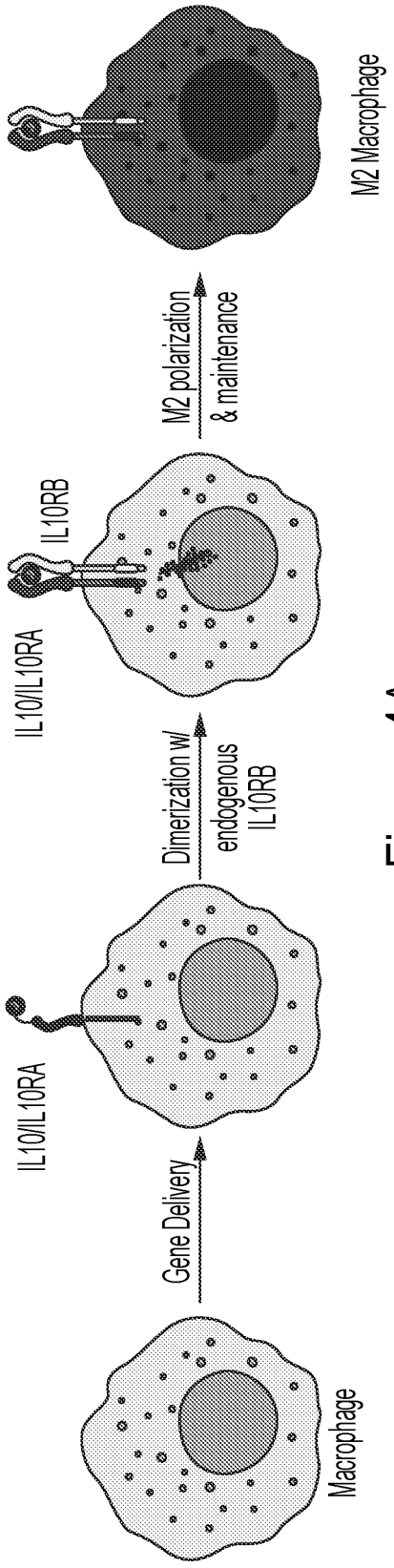

Macrophage
Gene Delivery
IL10/IL10RA
Dimerization w/
endogenous
IL10RB
IL10/IL10RA
IL10RB
M2 polarization
& maintenance
M2 Macrophage

Figure 4A

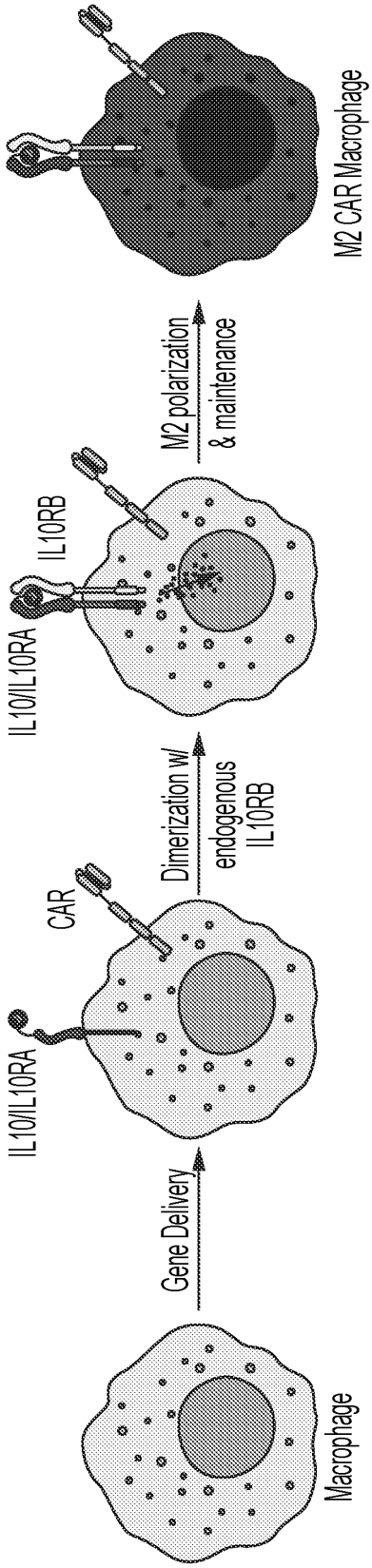

Macrophage
Gene Delivery
IL10/IL10RA
CAR
Dimerization w/
endogenous
IL10RB
IL10/IL10RA
IL10RB
M2 polarization
& maintenance
M2 CAR Macrophage

Figure 4B

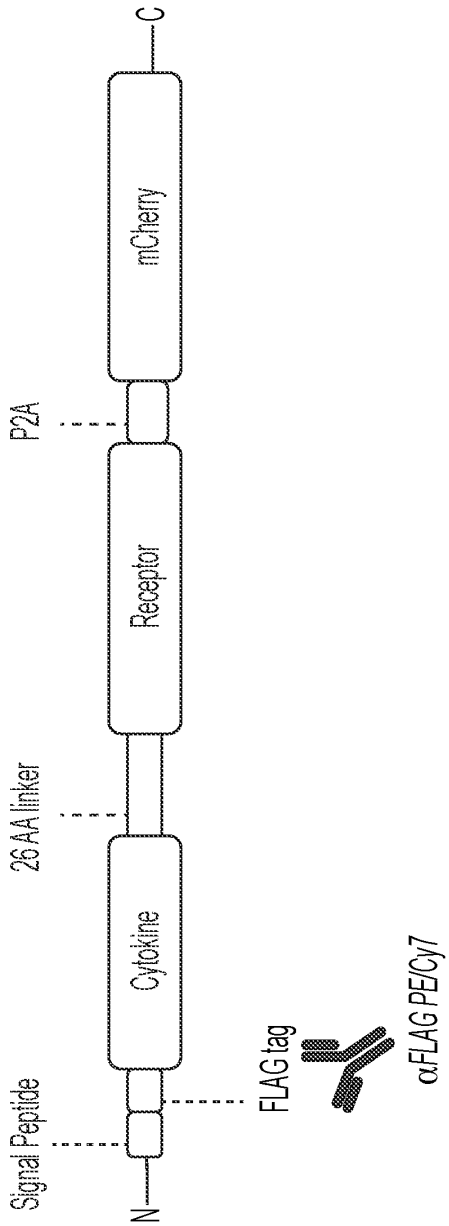

C
mCherry
P2A
Receptor
26 AA linker
Cytokine
FLAG tag
αFLAG PE/Cy7
Signal Peptide
N

Figure 5A

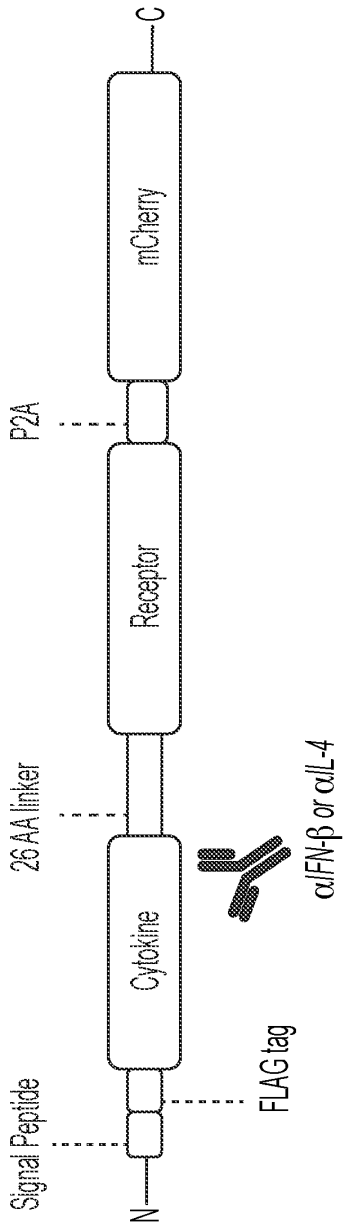

C
mCherry
P2A
Receptor
26 AA linker
Cytokine
αIFN-β or αIL-4
FLAG tag
Signal Peptide
N

Figure 5B

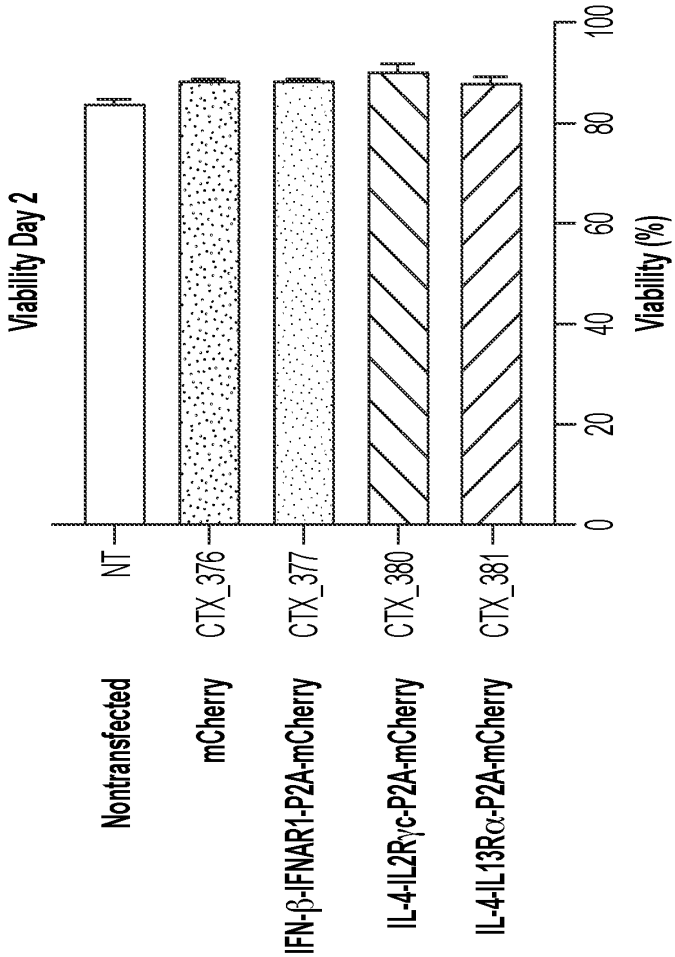

Viability Day 2
Viability (%)
0
20
40
60
80
100
NT
CTX_376
CTX_377
CTX_380
CTX_381
Nontransfected
mCherry
IFN-β-IFNAR1-P2A-mCherry
IL-4-IL2Rγc-P2A-mCherry
IL-4-IL13Rα-P2A-mCherry

Figure 6A

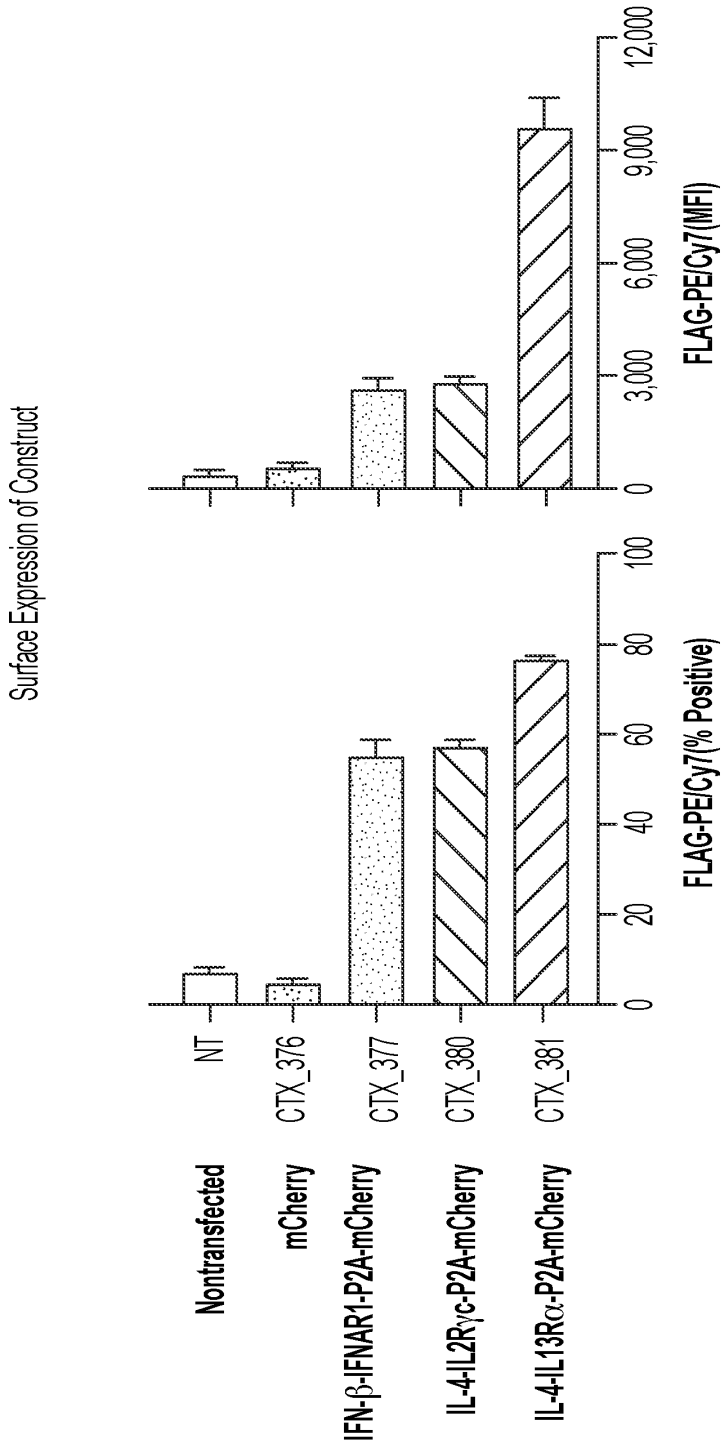

Surface Expression of Construct
FLAG-PE/Cy7(% Positive)
0
20
40
60
80
100
FLAG-PE/Cy7(MFI)
0
3,000
6,000
9,000
12,000
NT
CTX_376
CTX_377
CTX_380
CTX_381
Nontransfected
mCherry
IFN-β-IFNAR1-P2A-mCherry
IL-4-IL2Rγc-P2A-mCherry
IL-4-IL13Rα-P2A-mCherry

Figure 6B

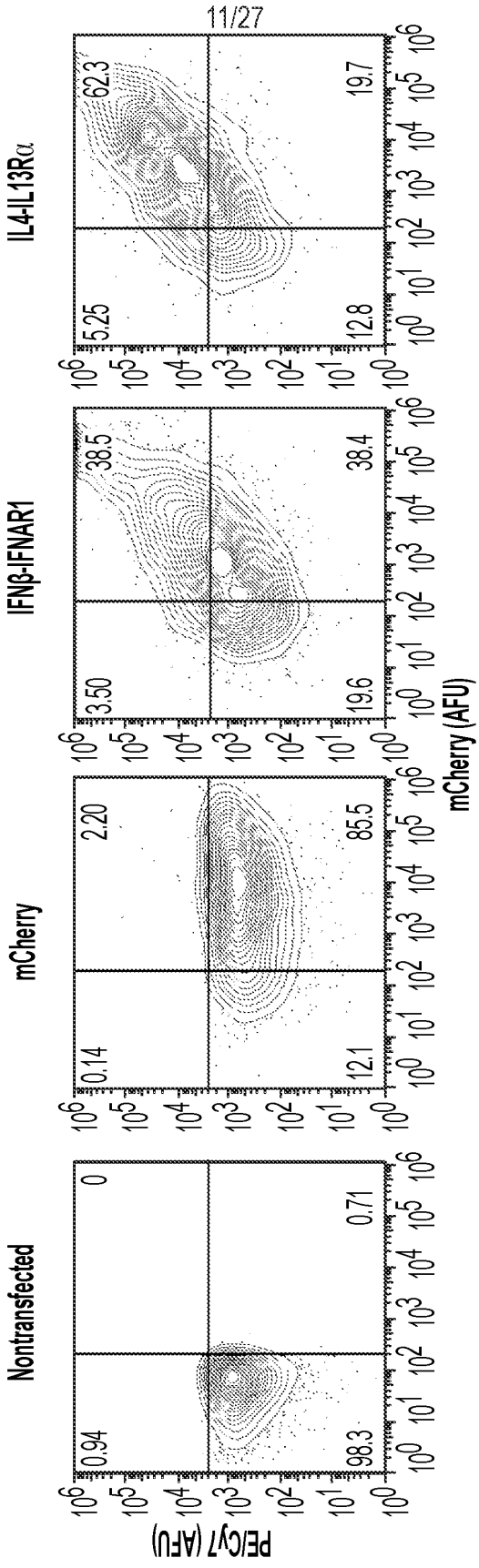

Nontransfected
mCherry
IFNβ-IFNAR1
IL4-IL13Rα
PE/Cy7 (AFU)
mCherry (AFU)
0.94
98.3
0
0.71
0.14
12.1
2.20
85.5
3.50
19.6
38.5
38.4
5.25
12.8
62.3
19.7

Figure 7

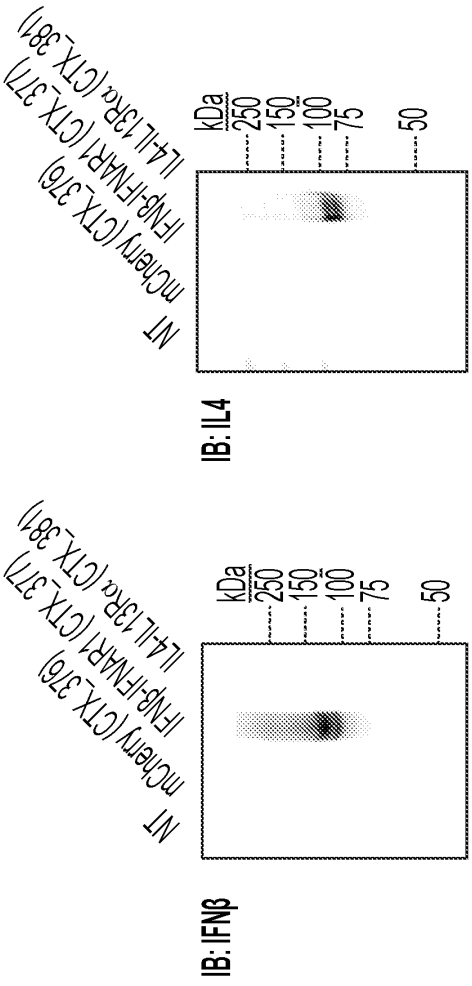

kDa
250
150
100
75
50
NT
mCherry (CTX_376)
IFNβ-IFNAR1 (CTX_377)
IL4-IL13Rα (CTX_381)
IB: IL4
IB: IFNβ

Figure 8

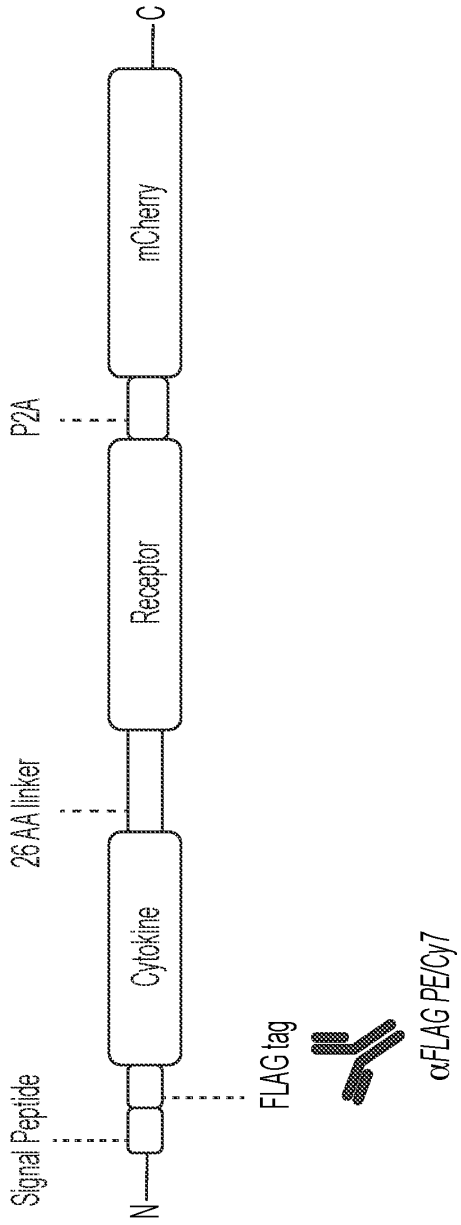

C
mCherry
P2A
Receptor
26 AA linker
Cytokine
FLAG tag
αFLAG PE/Cy7
Signal Peptide
N

Figure 9

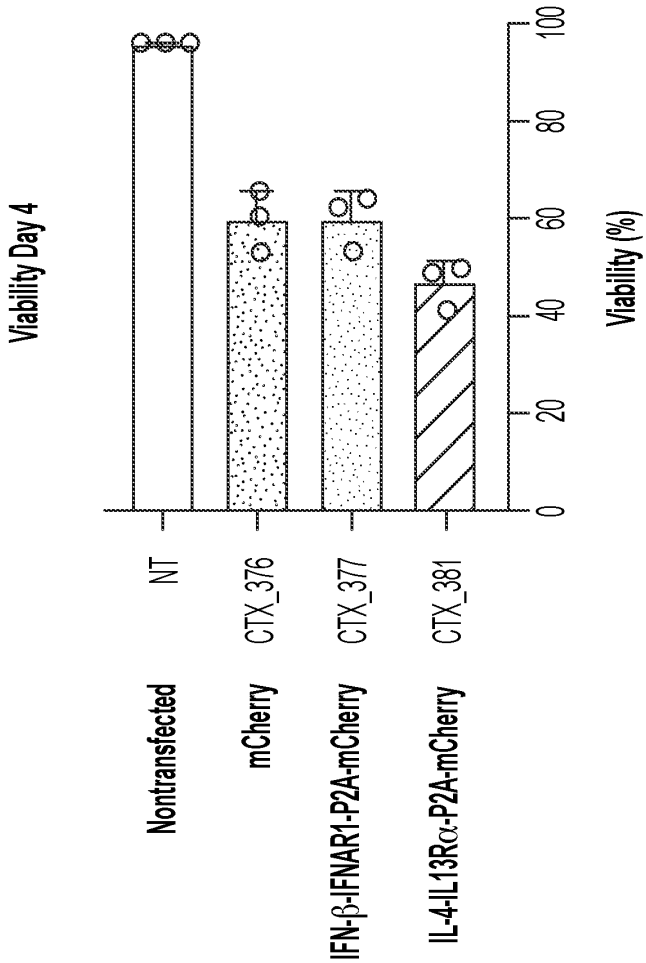

Viability Day 4
Viability (%)
0
20
40
60
80
100
NT
CTX_376
CTX_377
CTX_381
Nontransfected
mCherry
IFN-β-IFNAR1-P2A-mCherry
IL-4-IL13Rα-P2A-mCherry

Figure 10A

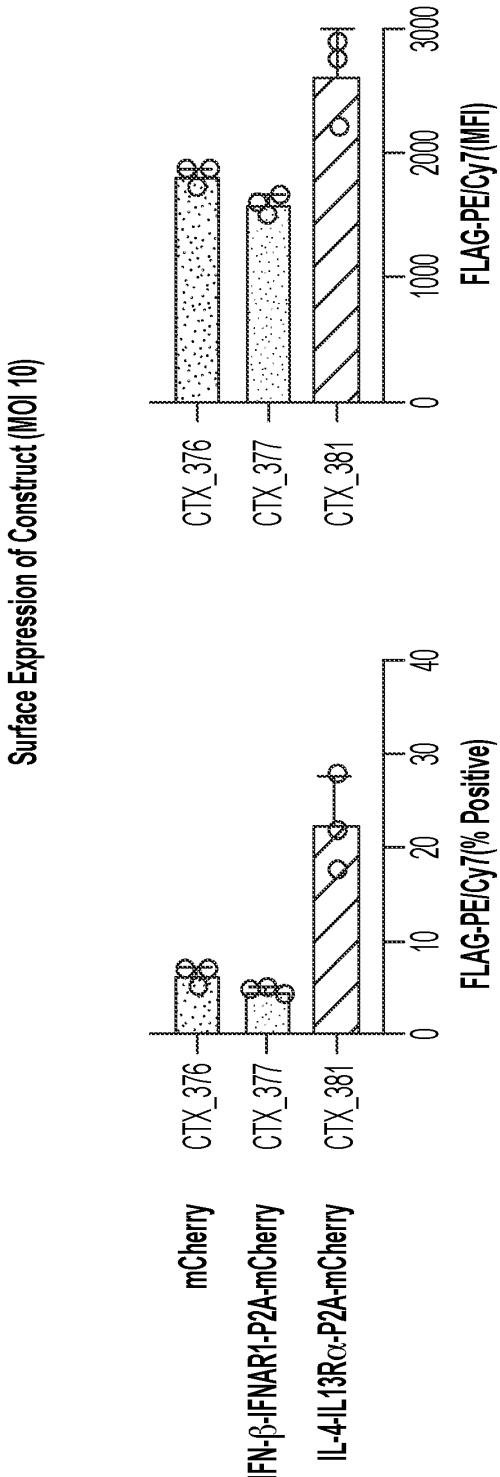

Surface Expression of Construct (MOI 10)
mCherry
IFN-β-IFNAR1-P2A-mCherry
IL-4-IL13Rα-P2A-mCherry
CTX_376
CTX_377
CTX_381
0
10
20
30
40
FLAG-PE/Cy7(% Positive)
0
1000
2000
3000
FLAG-PE/Cy7(MFI)

Figure 10B

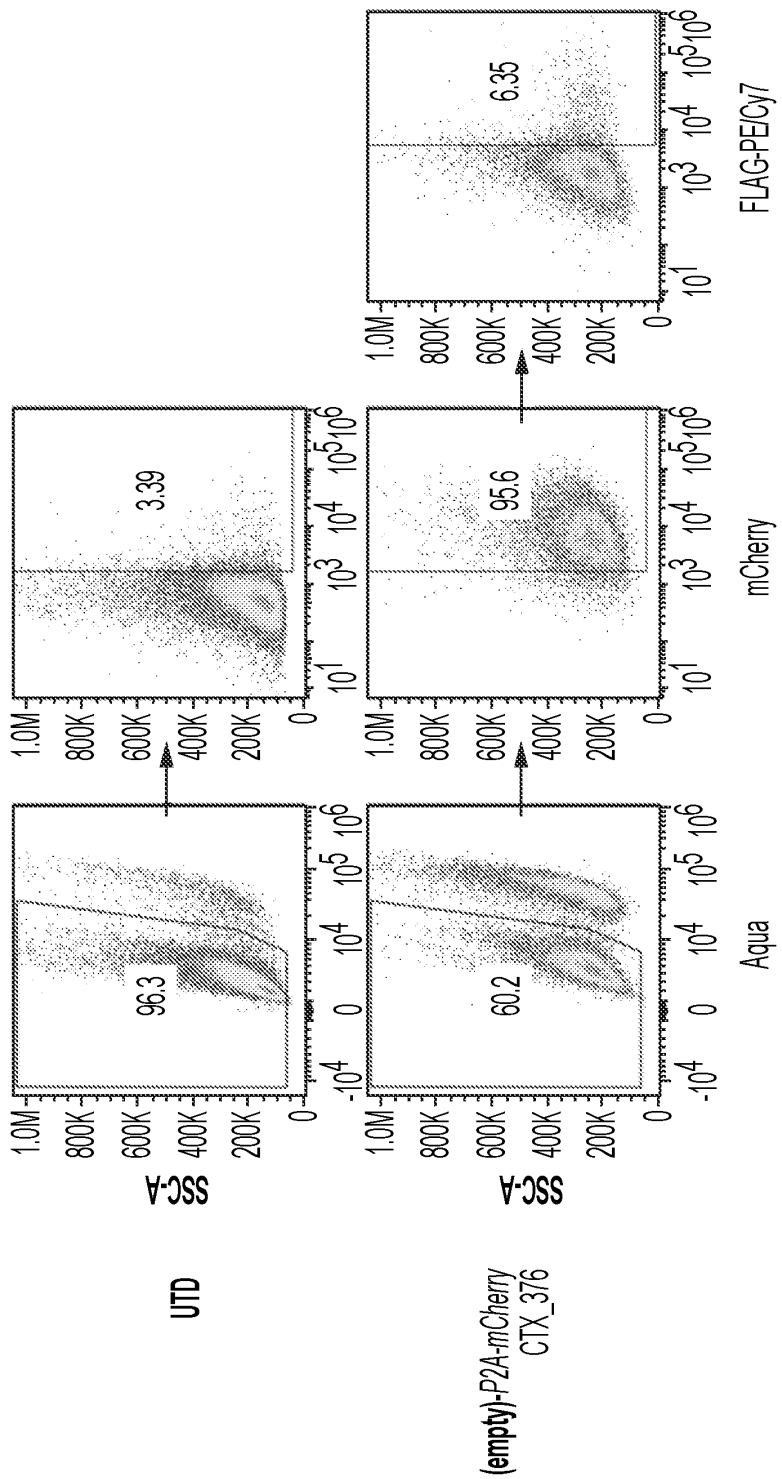

UTD
(empty)-P2A-mCherry
CTX_376
SSC-A
Aqua
mCherry
FLAG-PE/Cy7
96.3
3.39
60.2
95.6
6.35
1.0M
800K
600K
400K
200K
0
-10^4
0
10^4
10^5
10^6
10^1
10^3

Figure 11A

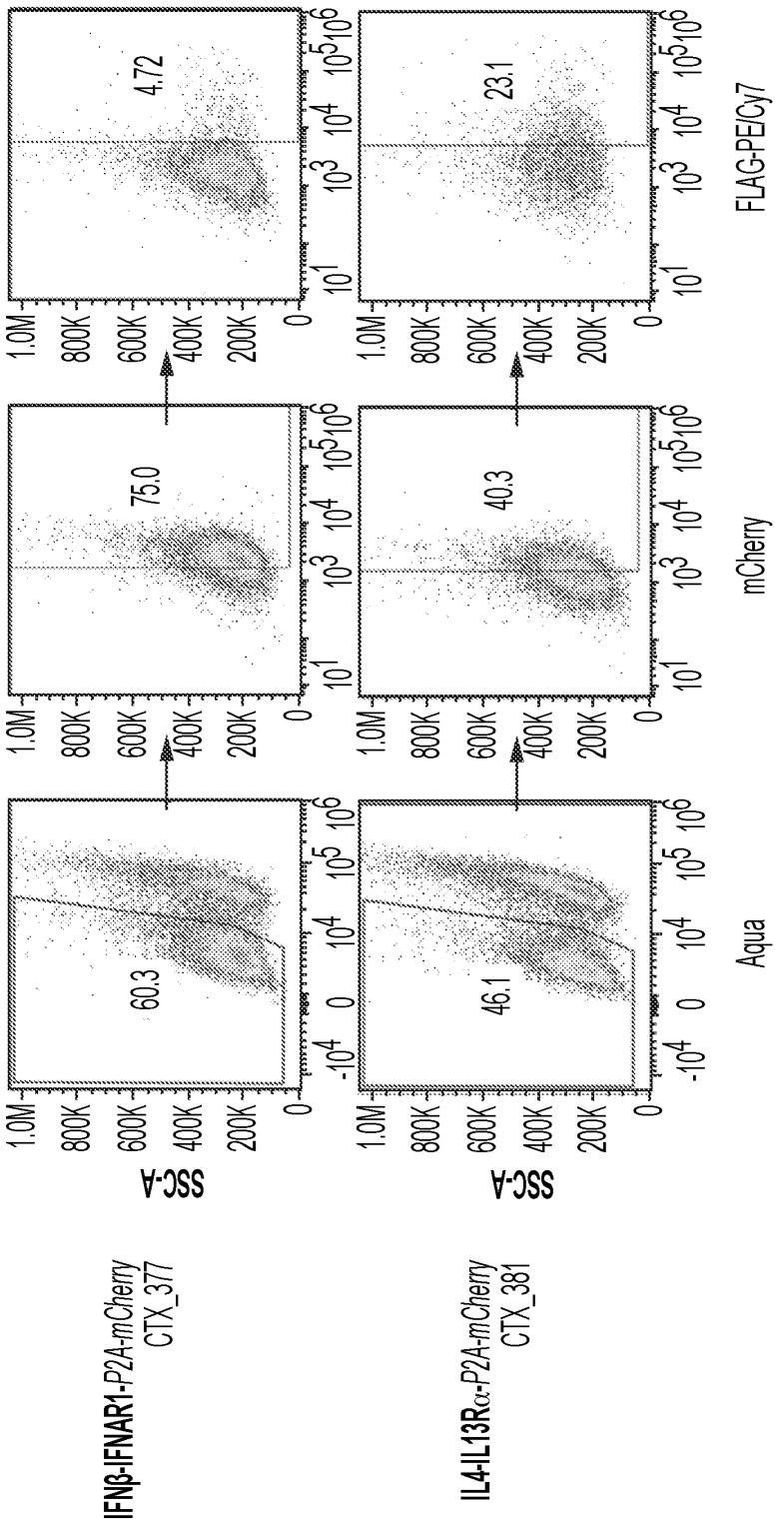

IFNβ-IFNAR1-P2A-mCherry
CTX_377
IL4-IL13Rα-P2A-mCherry
CTX_381
SSC-A
Aqua
mCherry
FLAG-PE/Cy7
60.3
75.0
4.72
46.1
40.3
23.1

Figure 11B

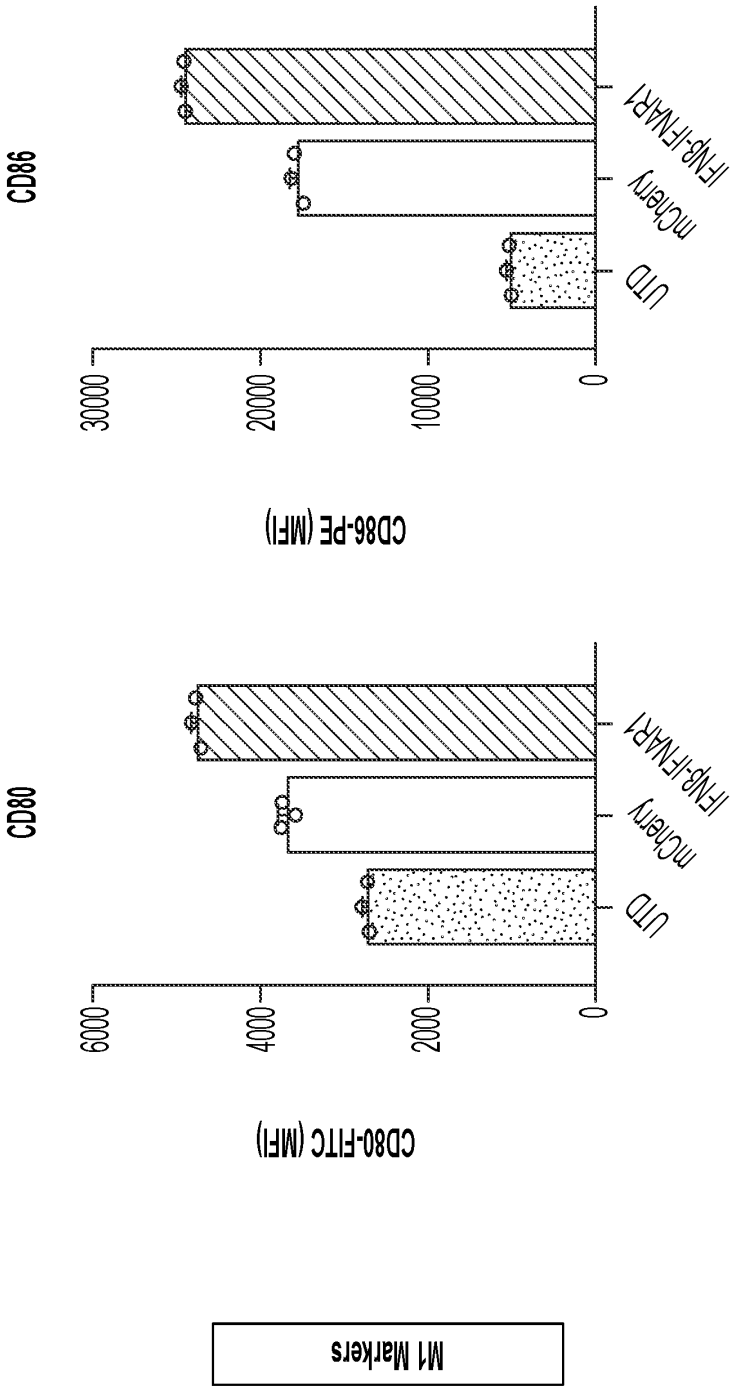

CD86
CD86-PE (MFI)
30000
20000
10000
0
UTD
mCherry
IFNβ-IFNAR1
CD80
CD80-FITC (MFI)
6000
4000
2000
0
UTD
mCherry
IFNβ-IFNAR1
M1 Markers

Figure 12A

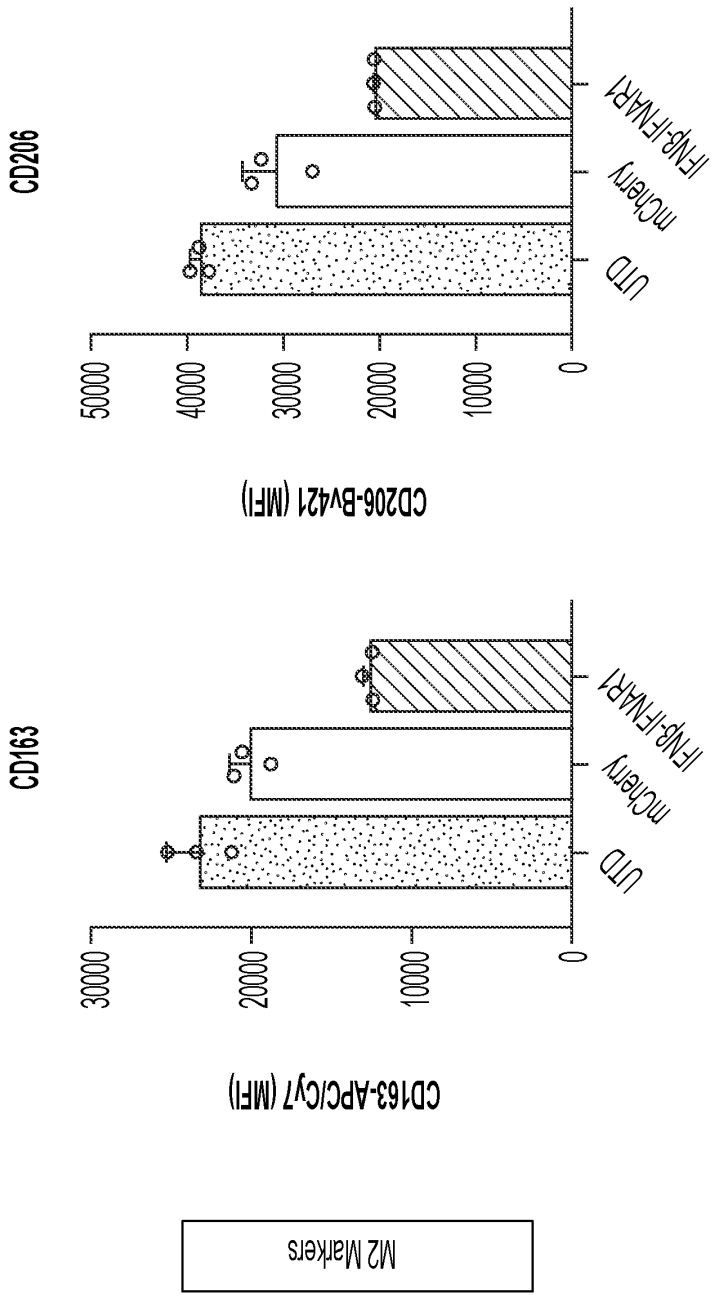

M2 Markers
CD163-APC/Cy7 (MFI)
30000
20000
10000
0
UTD
mCherry
IFNβ-IFNAR1
CD163
CD206-BV421 (MFI)
50000
40000
30000
20000
10000
0
UTD
mCherry
IFNβ-IFNAR1
CD206

Figure 12B

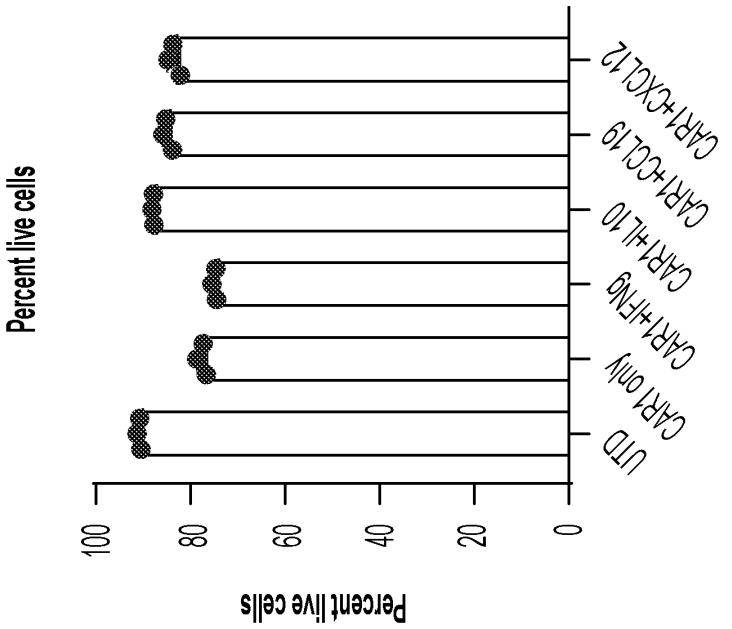

Percent live cells
100
80
60
40
20
0
Percent live cells
UTD
CAR1 only
CAR1+IFNg
CAR1+IL10
CAR1+CCL19
CAR1+CXCL12

Figure 13

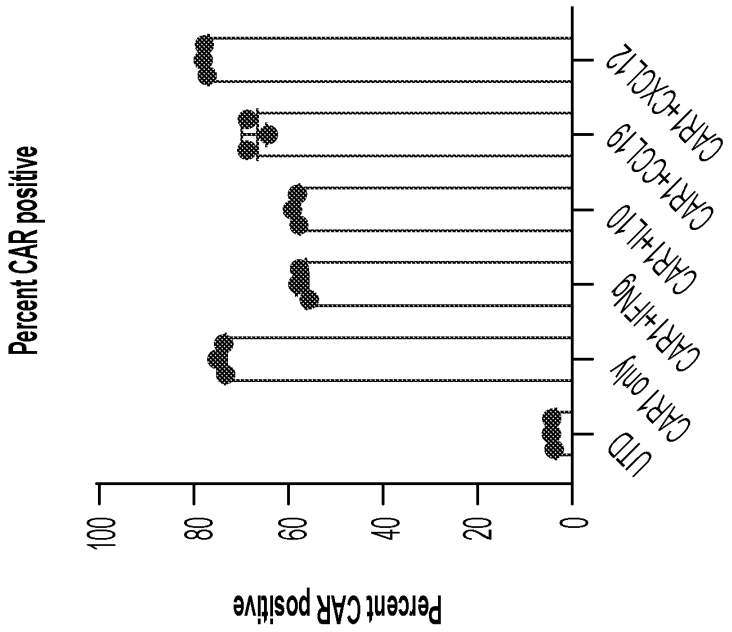

Percent CAR positive
Percent CAR positive
100
80
60
40
20
0
UTD
CAR1 only
CAR1+IFNg
CAR1+IL10
CAR1+CCL19
CAR1+CXCL12

Figure 14

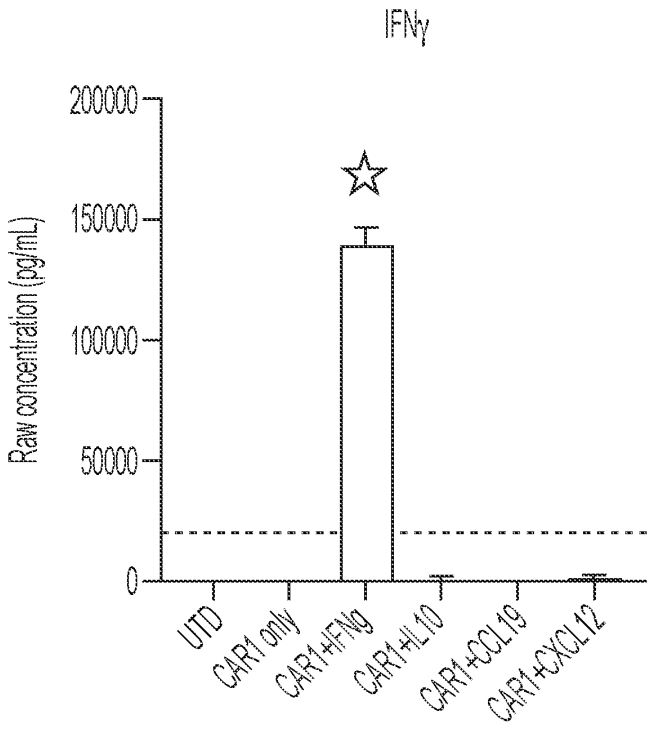

IFNγ
Raw concentration (pg/mL)
200000
150000
100000
50000
0
UTD
CAR1 only
CAR1+IFNg
CAR1+IL10
CAR1+CCL19
CAR1+CXCL12

Figure 15

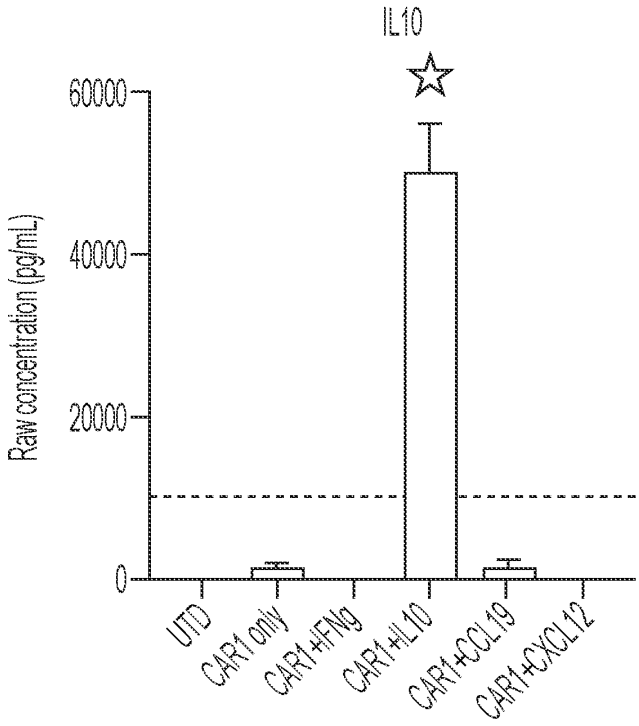

IL10
60000
40000
20000
0
Raw concentration (pg/mL)
UTD
CAR1 only
CAR1+IFNg
CAR1+IL10
CAR1+CCL19
CAR1+CXCL12

Figure 16

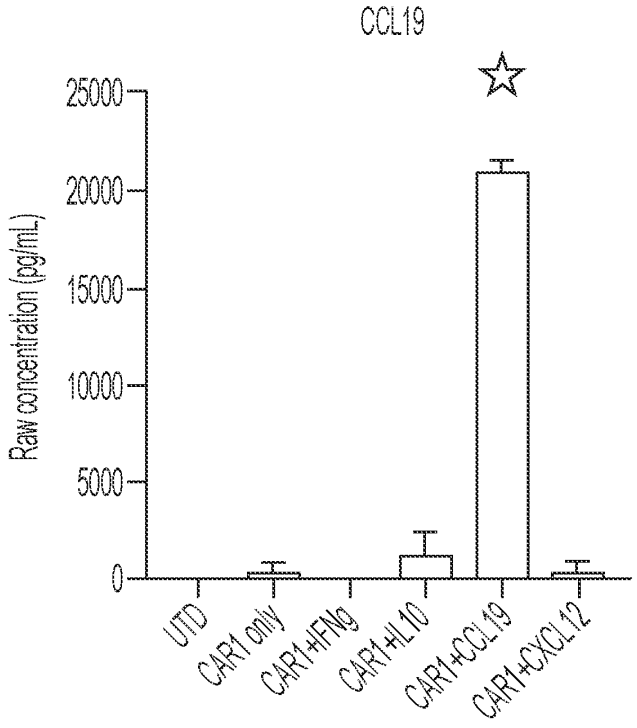

CCL19
Raw concentration (pg/mL)
25000
20000
15000
10000
5000
0
UTD
CAR1 only
CAR1+IFNg
CAR1+IL10
CAR1+CCL19
CAR1+CXCL12

Figure 17

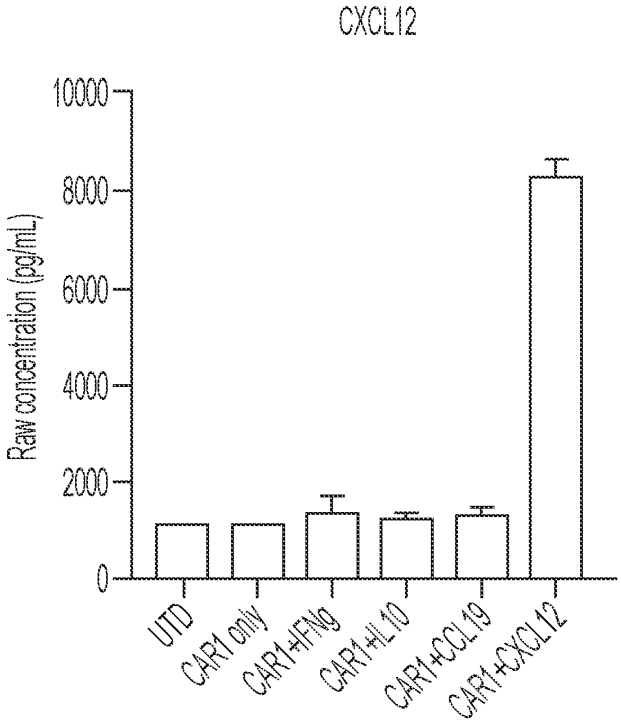

CXCL12
Raw concentration (pg/mL)
10000
8000
6000
4000
2000
0
UTD
CAR1 only
CAR1+IFNg
CAR1+IL10
CAR1+CCL19
CAR1+CXCL12

Figure 18

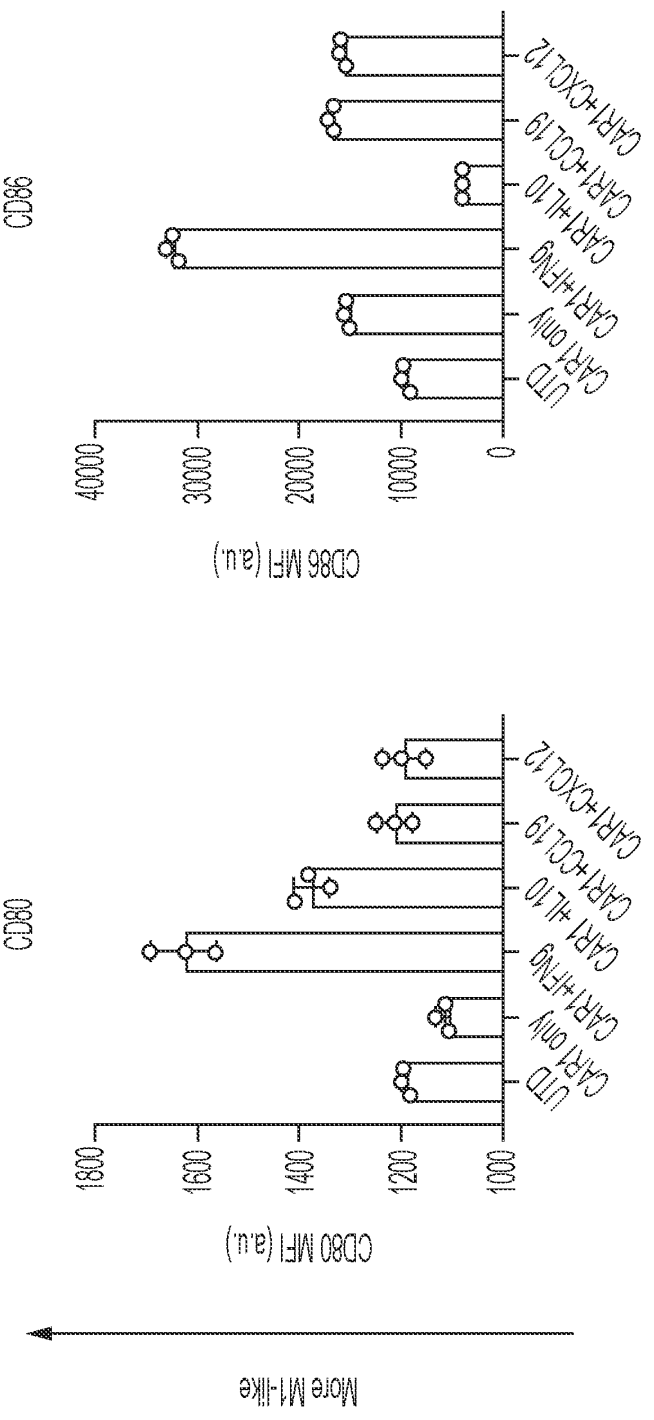

CD86
CD86 MFI (a.u.)
40000
30000
20000
10000
0
UTD
CAR1 only
CAR1+IFNg
CAR1+IL10
CAR1+CCL19
CAR1+CXCL12
CD80
CD80 MFI (a.u.)
1800
1600
1400
1200
1000
More M1-like

Figure 19

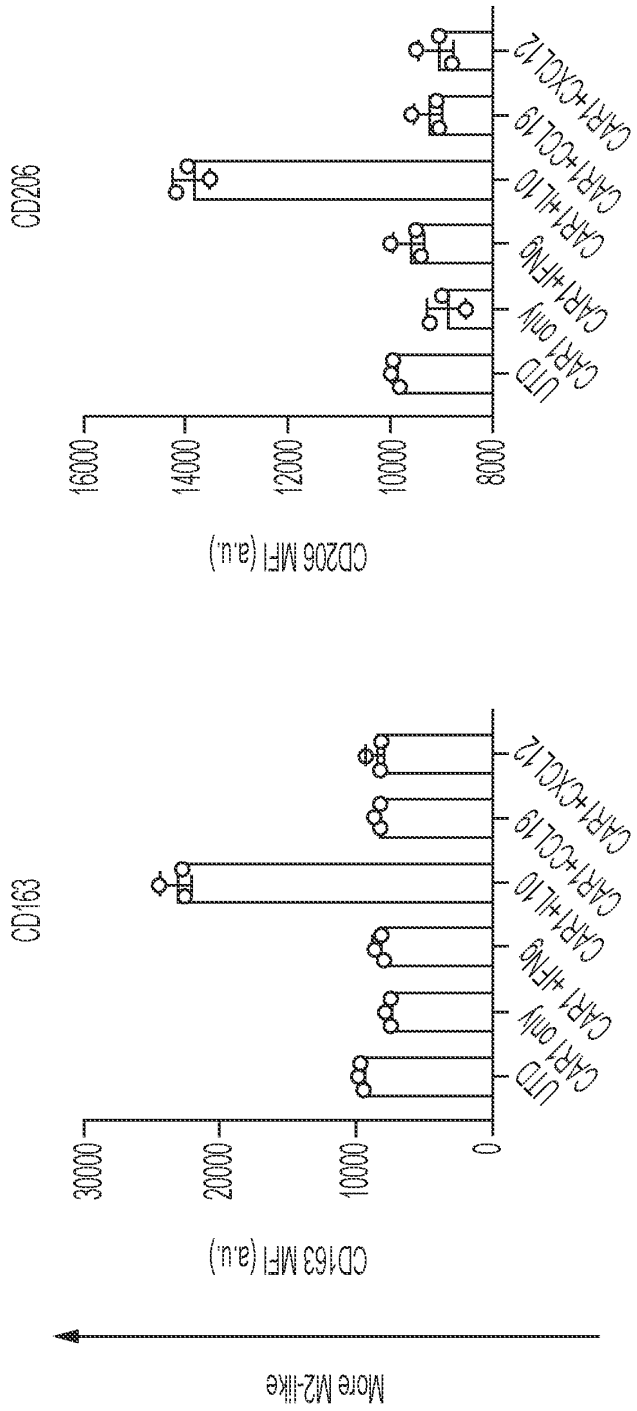

CD206
16000
14000
12000
10000
8000
CD206 MFI (a.u.)
UTD
CAR1 only
CAR1+IFNg
CAR1+IL10
CAR1+CCL19
CAR1+CXCL12
CD163
30000
20000
10000
0
CD163 MFI (a.u.)
More M2-like

Figure 20