Figure 1

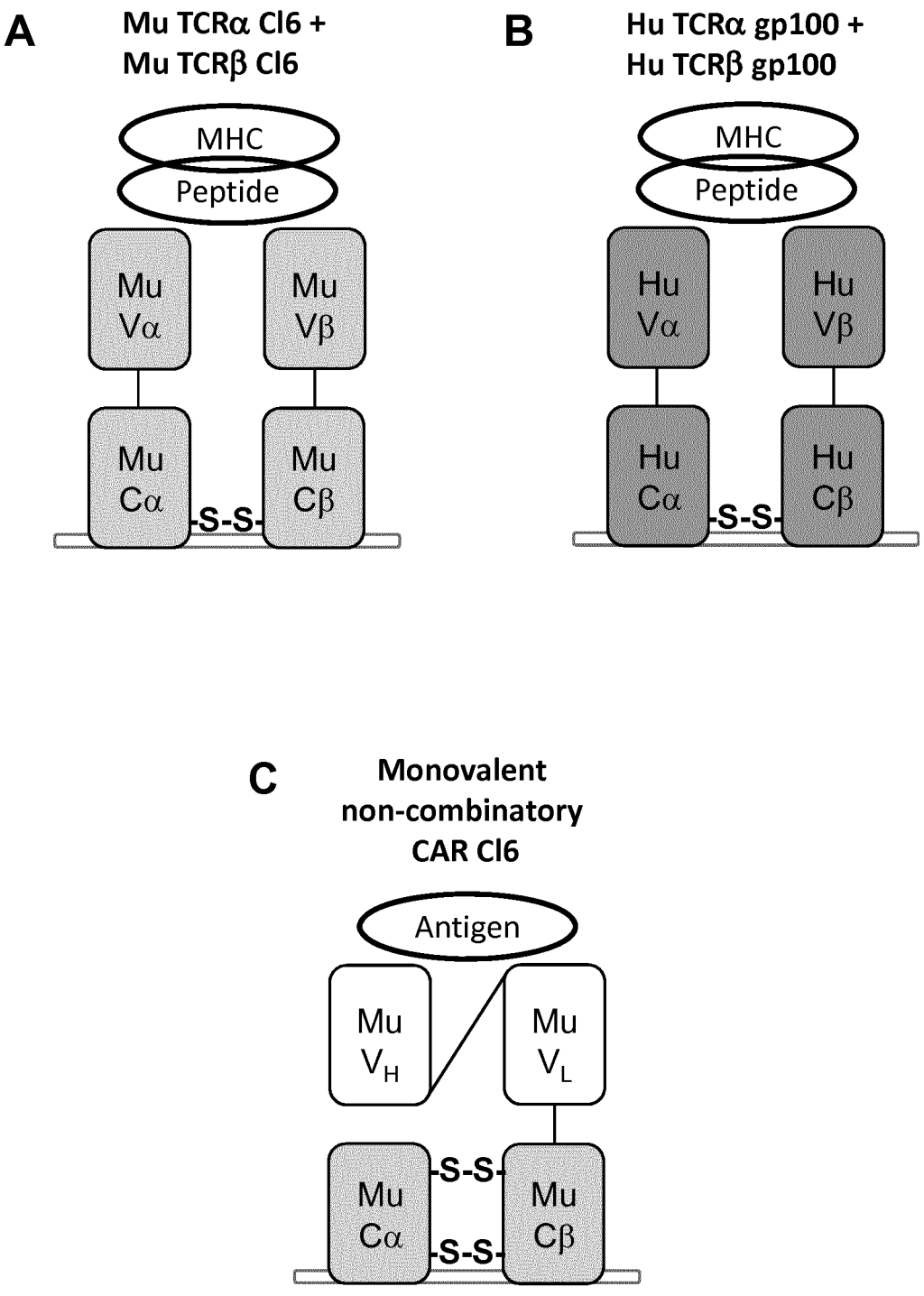

A
Mu TCRα Cl6 +
Mu TCRβ Cl6
MHC
Peptide
Mu Vα
Mu Vβ
Mu Cα
Mu Cβ
S-S
B
Hu TCRα gp100 +
Hu TCRβ gp100
MHC
Peptide
Hu Vα
Hu Vβ
Hu Cα
Hu Cβ
S-S
C
Monovalent
non-combinatory
CAR Cl6
Antigen
Mu V_H
Mu V_L
Mu Cα
Mu Cβ
S-S
S-S

Figure 1

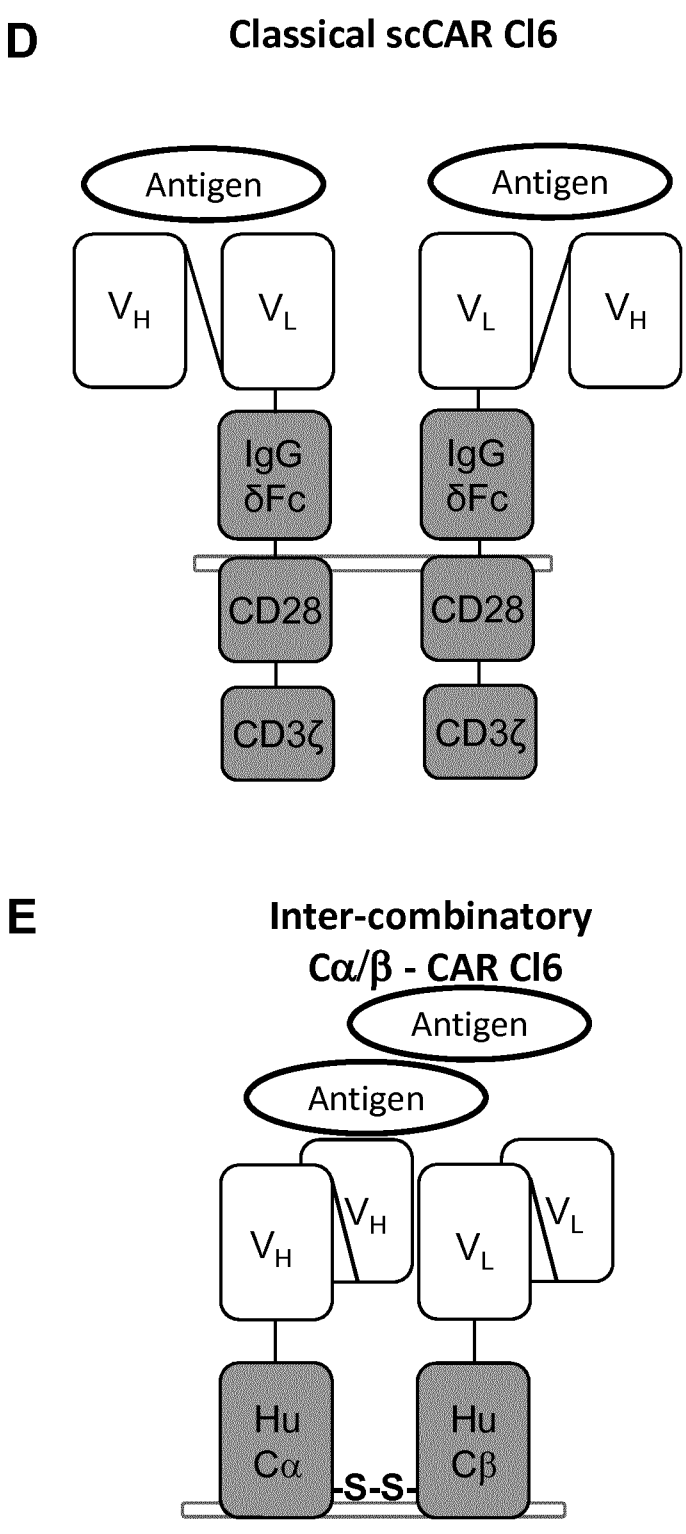
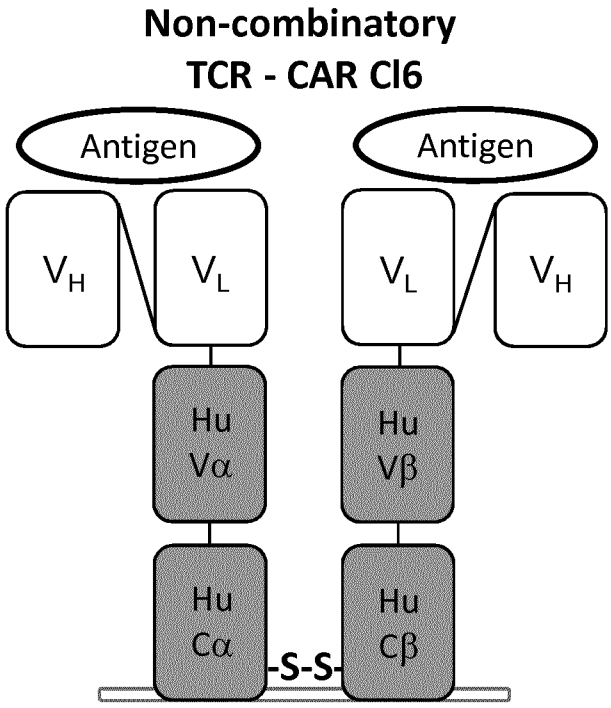

D
Classical scCAR Cl6
Antigen
Antigen
VH
VL
VL
VH
IgG δFc
IgG δFc
CD28
CD28
CD3ζ
CD3ζ
E
Inter-combinatory Cα/β - CAR Cl6
Antigen
Antigen
VH
VH
VL
VL
Hu Cα
-S-S-
Hu Cβ

Figure 1

F


Non-combinatory
TCR - CAR Cl6
Antigen
Antigen
V_H
V_L
V_L
V_H
Hu
Vα
Hu
Vβ
Hu
Cα
Hu
Cβ
-S-S-

G

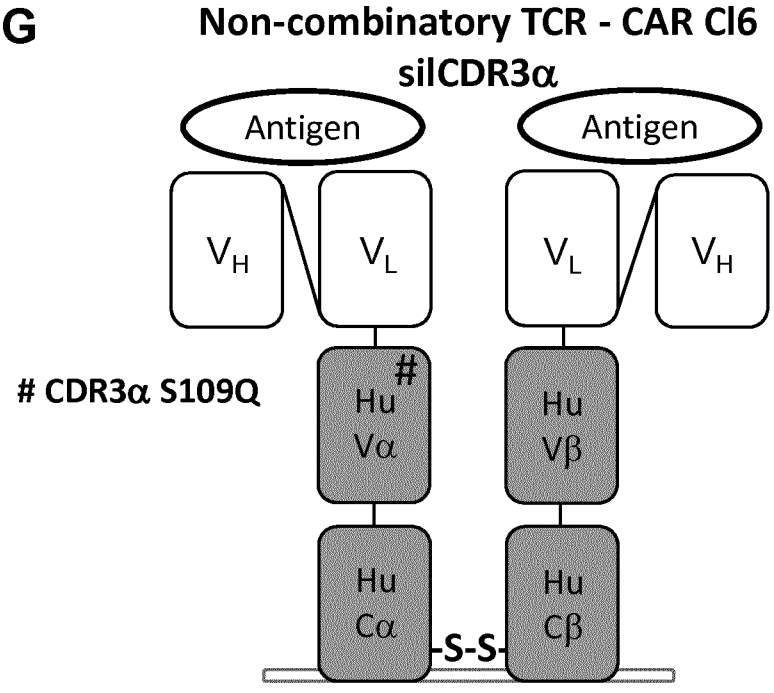
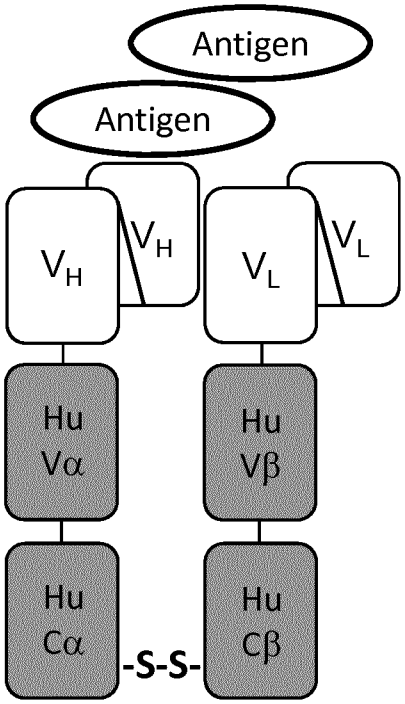

Non-combinatory TCR - CAR Cl6
silCDR3α
Antigen
Antigen
V_H
V_L
V_L
V_H
CDR3α S109Q
#
Hu
Vα
Hu
Vβ
Hu
Cα
Hu
Cβ
-S-S-

Figure 1

H **Inter-combinatory TCR - CAR Cl6**



I **Inter-combinatory TCR - CAR Cl6 silCDR3α**

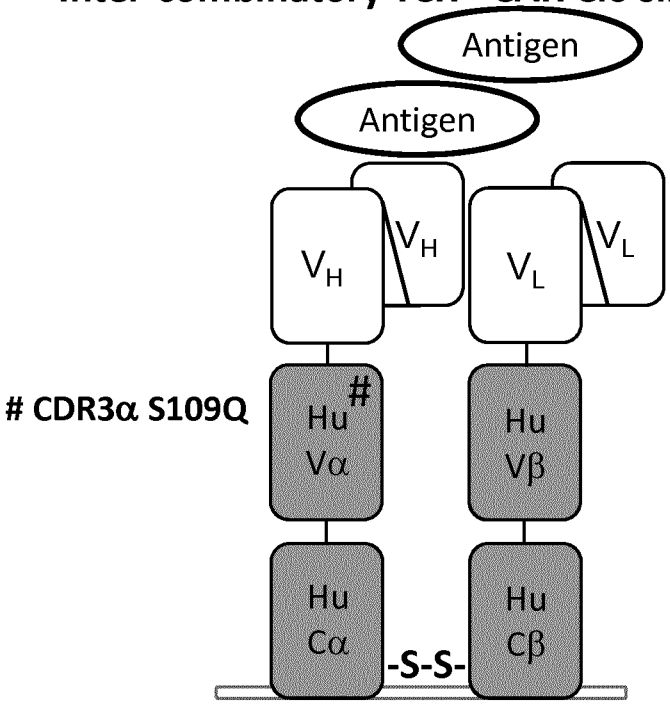


Figure 2A

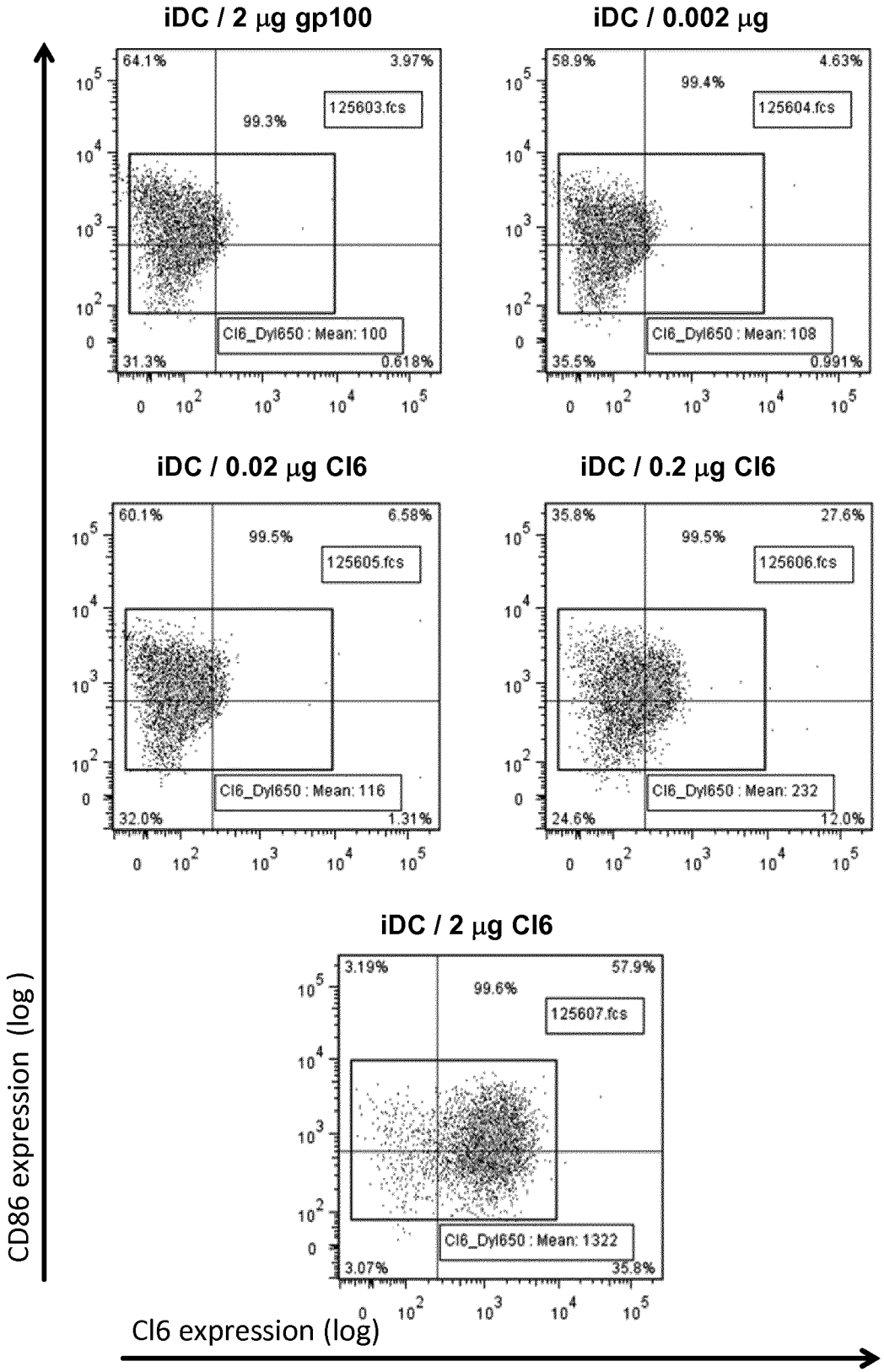

iDC / 2 µg gp100
64.1%
3.97%
99.3%
125603.fcs
Cl6_Dyl650 : Mean: 100
31.3%
0.618%
iDC / 0.002 µg
58.9%
4.63%
99.4%
125604.fcs
Cl6_Dyl650 : Mean: 108
35.5%
0.991%
iDC / 0.02 µg Cl6
60.1%
6.58%
99.5%
125605.fcs
Cl6_Dyl650 : Mean: 116
32.0%
1.31%
iDC / 0.2 µg Cl6
35.8%
27.6%
99.5%
125606.fcs
Cl6_Dyl650 : Mean: 232
24.6%
12.0%
iDC / 2 µg Cl6
3.19%
57.9%
99.6%
125607.fcs
Cl6_Dyl650 : Mean: 1322
3.07%
35.8%
CD86 expression (log)
Cl6 expression (log)

Figure 2B

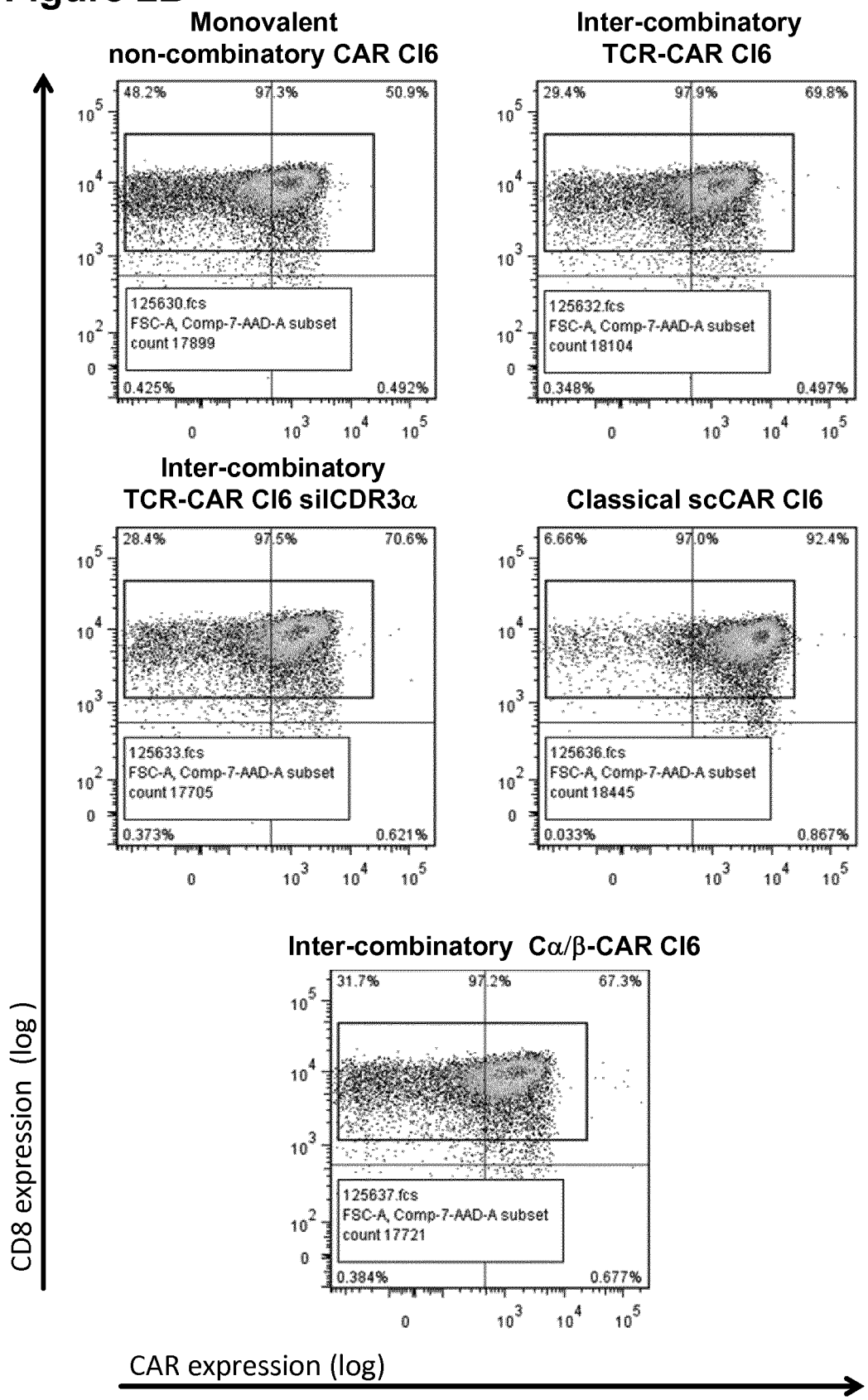

Monovalent
non-combinatory CAR Cl6
48.2%
97.3%
50.9%
125630.fcs
FSC-A, Comp-7-AAD-A subset
count 17899
0.425%
0.492%
Inter-combinatory
TCR-CAR Cl6
29.4%
97.9%
69.8%
125632.fcs
FSC-A, Comp-7-AAD-A subset
count 18104
0.348%
0.497%
Inter-combinatory
TCR-CAR Cl6 silCDR3α
28.4%
97.5%
70.6%
125633.fcs
FSC-A, Comp-7-AAD-A subset
count 17705
0.373%
0.621%
Classical scCAR Cl6
6.66%
97.0%
92.4%
125636.fcs
FSC-A, Comp-7-AAD-A subset
count 18445
0.033%
0.867%
Inter-combinatory Cα/β-CAR Cl6
31.7%
97.2%
67.3%
125637.fcs
FSC-A, Comp-7-AAD-A subset
count 17721
0.384%
0.677%
CD8 expression (log)
CAR expression (log)

Figure 2C

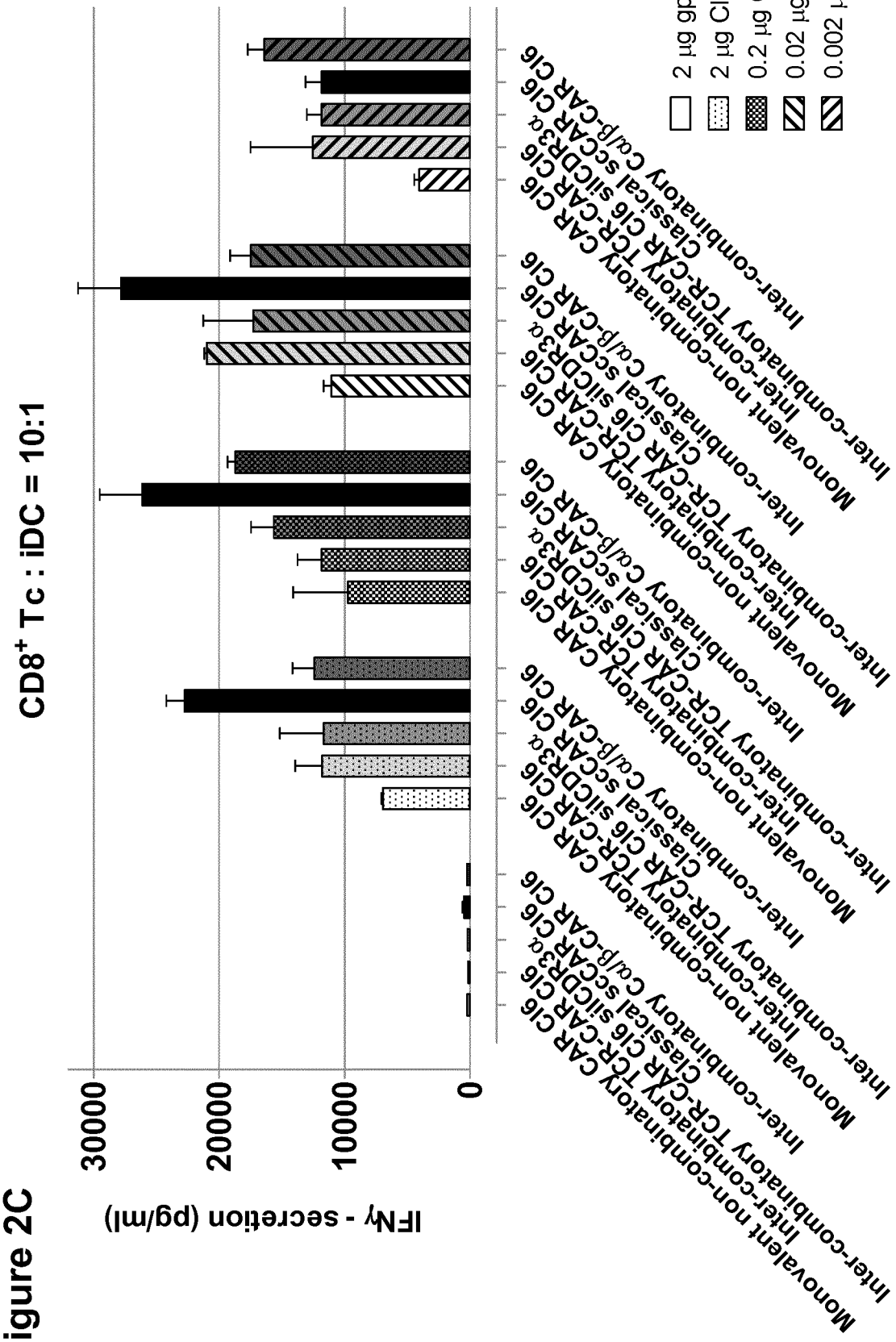
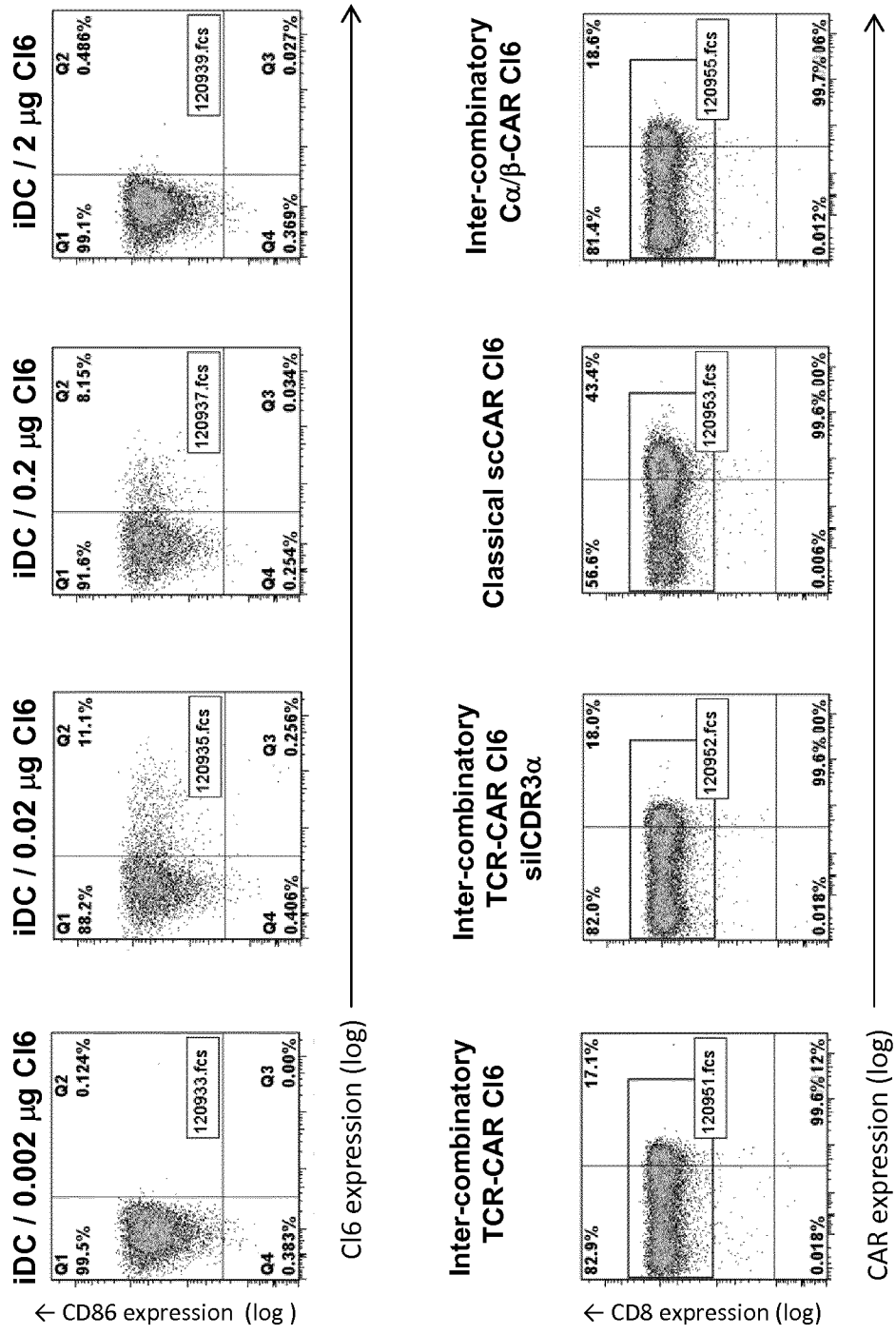

CD8+ Tc : iDC = 10:1
IFNγ - secretion (pg/ml)
30000
20000
10000
0
2 µg gp100
2 µg Cl6
0.2 µg Cl6
0.02 µg Cl6
0.002 µg Cl6
Monovalent non-combinatory CAR Cl6
Inter-combinatory TCR-CAR Cl6
Inter non-combinatory TCR-CAR Cl6
Classical siICDR3α scCAR Cl6
Inter-combinatory Cα/β-CAR Cl6

Figure 3


A
iDC / 0.002 µg CI6
iDC / 0.02 µg CI6
iDC / 0.2 µg CI6
iDC / 2 µg CI6
Q1 99.5%
Q2 0.124%
Q4 0.383%
Q3 0.00%
120933.fcs
Q1 88.2%
Q2 11.1%
Q4 0.406%
Q3 0.256%
120935.fcs
Q1 91.6%
Q2 8.15%
Q4 0.254%
Q3 0.034%
120937.fcs
Q1 99.1%
Q2 0.486%
Q4 0.369%
Q3 0.027%
120939.fcs
← CD86 expression (log)
CI6 expression (log)
B
Inter-combinatory TCR-CAR CI6
Inter-combinatory TCR-CAR CI6 silCDR3α
Classical scCAR CI6
Inter-combinatory Cα/β-CAR CI6
82.9%
17.1%
0.018%
99.6%12%
120951.fcs
82.0%
18.0%
0.018%
99.6%00%
120952.fcs
56.6%
43.4%
0.006%
99.6%00%
120953.fcs
81.4%
18.6%
0.012%
99.7%06%
120955.fcs
← CD8 expression (log)
CAR expression (log)

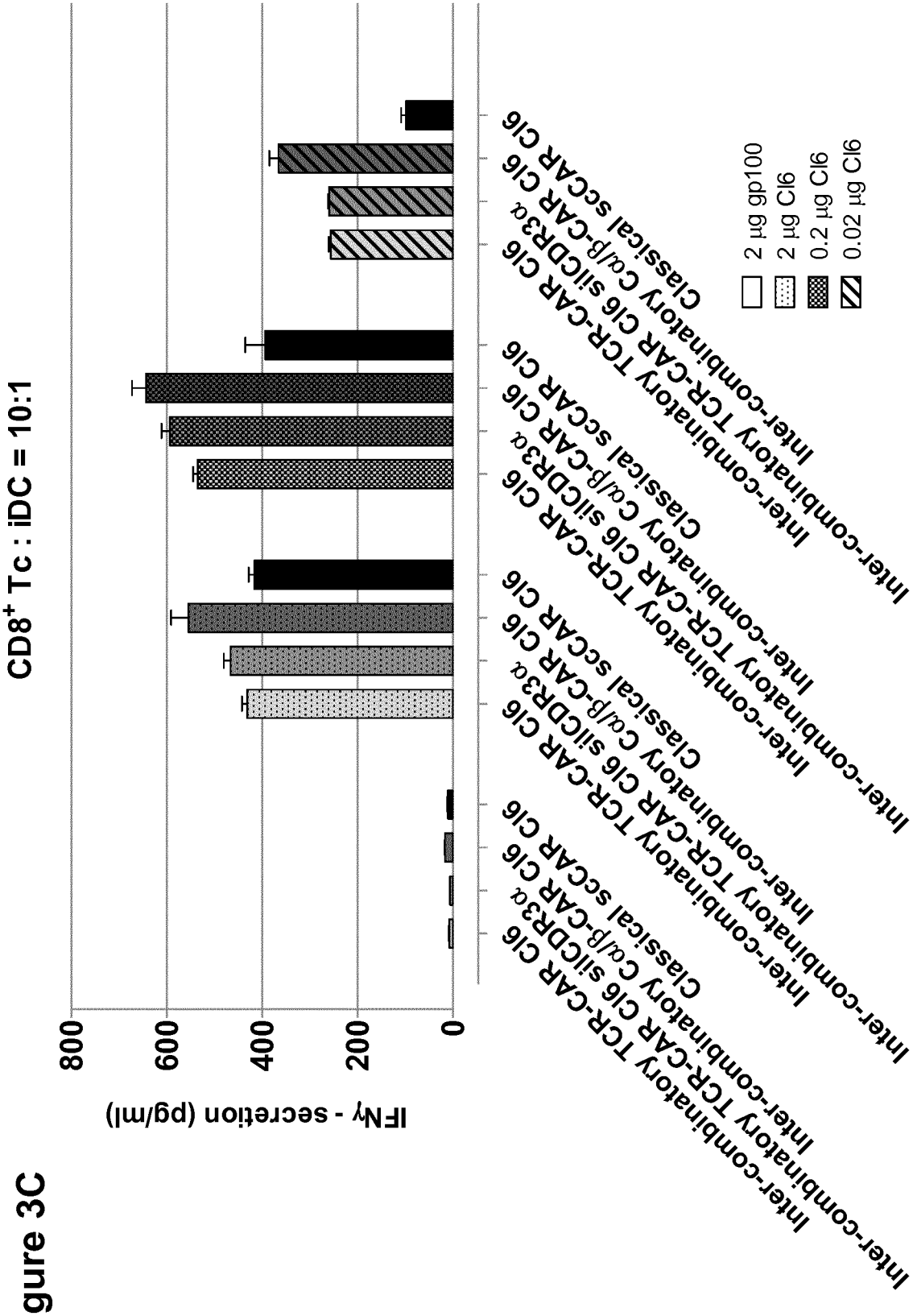

CD8+ Tc : iDC = 10:1
IFNγ - secretion (pg/ml)
0
200
400
600
800
2 µg gp100
2 µg Cl6
0.2 µg Cl6
0.02 µg Cl6
Inter-combinatory TCR-CAR Cl6
Inter-combinatory TCR-CAR Cl6 siICDR3α Cl6
Classical scCAR Cl6
Inter-combinatory Cαβ-CAR Cl6
Inter-combinatory TCR-CAR Cl6
Inter-combinatory TCR-CAR Cl6 siICDR3α Cl6
Classical scCAR Cl6
Inter-combinatory Cαβ-CAR Cl6
Inter-combinatory TCR-CAR Cl6
Inter-combinatory TCR-CAR Cl6 siICDR3α Cl6
Classical scCAR Cl6
Inter-combinatory Cαβ-CAR Cl6
Inter-combinatory TCR-CAR Cl6
Inter-combinatory TCR-CAR Cl6 siICDR3α Cl6
Classical scCAR Cl6
Inter-combinatory Cαβ-CAR Cl6

Figure 3C

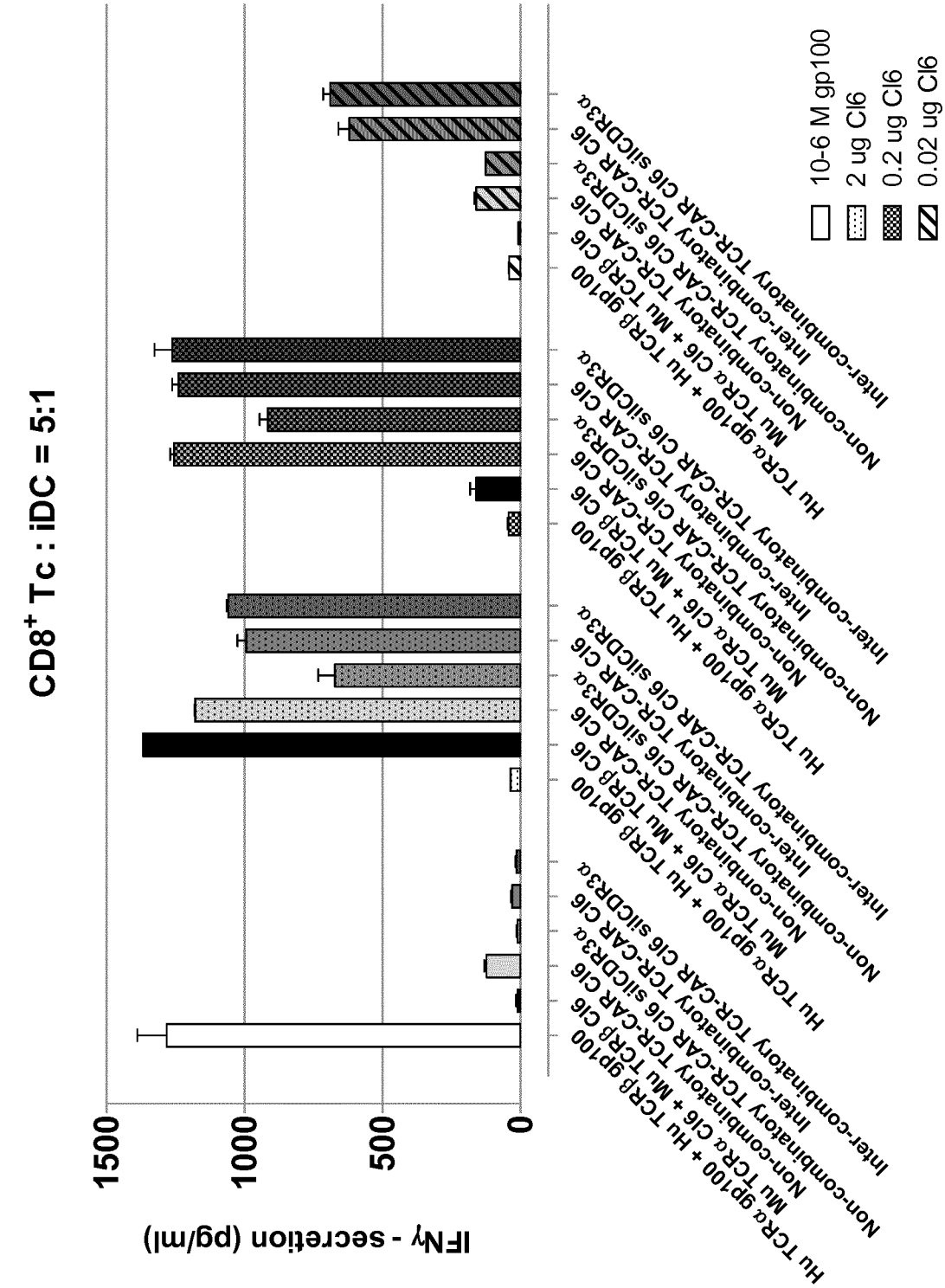

CD8+ Tc : iDC = 5:1
IFNγ - secretion (pg/ml)
1500
1000
500
0
10-6 M gp100
2 ug Cl6
0.2 ug Cl6
0.02 ug Cl6
Hu TCRα gp100 + Hu TCRβ gp100
Mu TCRα Cl6 + Mu TCRβ Cl6
Non-combinatory TCR-CAR Cl6 siICDR3α Cl6
Inter-combinatory TCR-CAR Cl6 siICDR3α Cl6
Hu TCRα gp100 + Hu TCRβ gp100
Mu TCRα Cl6 + Mu TCRβ Cl6
Non-combinatory TCR-CAR Cl6 siICDR3α Cl6
Inter-combinatory TCR-CAR Cl6 siICDR3α Cl6
Hu TCRα gp100 + Hu TCRβ gp100
Mu TCRα Cl6 + Mu TCRβ Cl6
Non-combinatory TCR-CAR Cl6 siICDR3α Cl6
Inter-combinatory TCR-CAR Cl6 siICDR3α Cl6
Hu TCRα gp100 + Hu TCRβ gp100
Mu TCRα Cl6 + Mu TCRβ Cl6
Non-combinatory TCR-CAR Cl6 siICDR3α Cl6
Inter-combinatory TCR-CAR Cl6 siICDR3α Cl6

Figure 4A

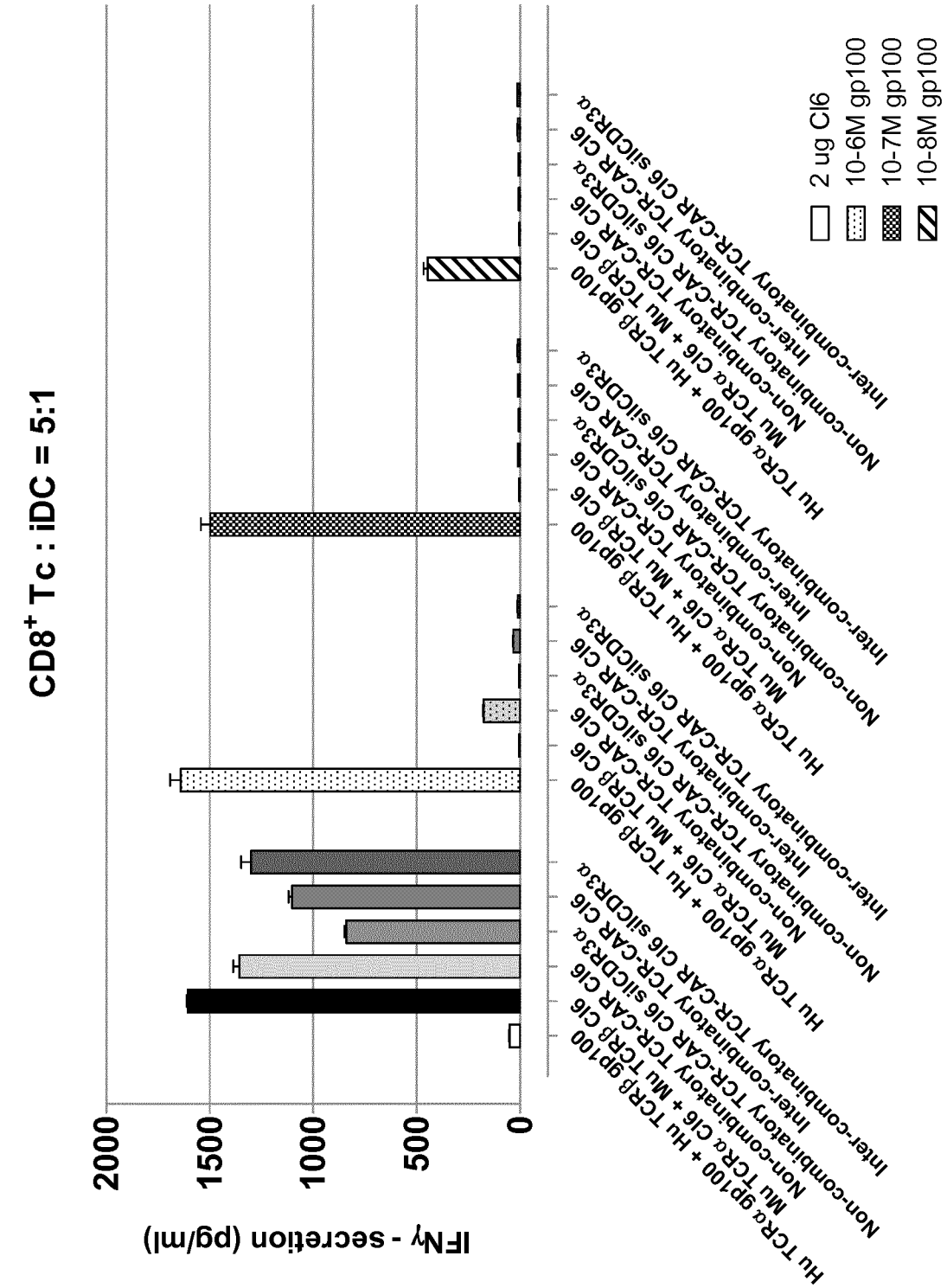

CD8+ Tc : iDC = 5:1
IFNγ - secretion (pg/ml)
2000
1500
1000
500
0
2 ug Cl6
10-6M gp100
10-7M gp100
10-8M gp100
Hu TCRα gp100 + Hu TCRβ gp100
Mu TCRα Cl6 + Mu TCRβ Cl6
Non-combinatory TCR-CAR Cl6 siICDR3α Cl6
Inter-combinatory TCR-CAR Cl6 siICDR3α Cl6
Hu TCRα gp100 + Hu TCRβ gp100
Mu TCRα Cl6 + Mu TCRβ Cl6
Non-combinatory TCR-CAR Cl6 siICDR3α Cl6
Inter-combinatory TCR-CAR Cl6 siICDR3α Cl6
Hu TCRα gp100 + Hu TCRβ gp100
Mu TCRα Cl6 + Mu TCRβ Cl6
Non-combinatory TCR-CAR Cl6 siICDR3α Cl6
Inter-combinatory TCR-CAR Cl6 siICDR3α Cl6
Hu TCRα gp100 + Hu TCRβ gp100
Mu TCRα Cl6 + Mu TCRβ Cl6
Non-combinatory TCR-CAR Cl6 siICDR3α Cl6
Inter-combinatory TCR-CAR Cl6 siICDR3α Cl6

Figure 4B

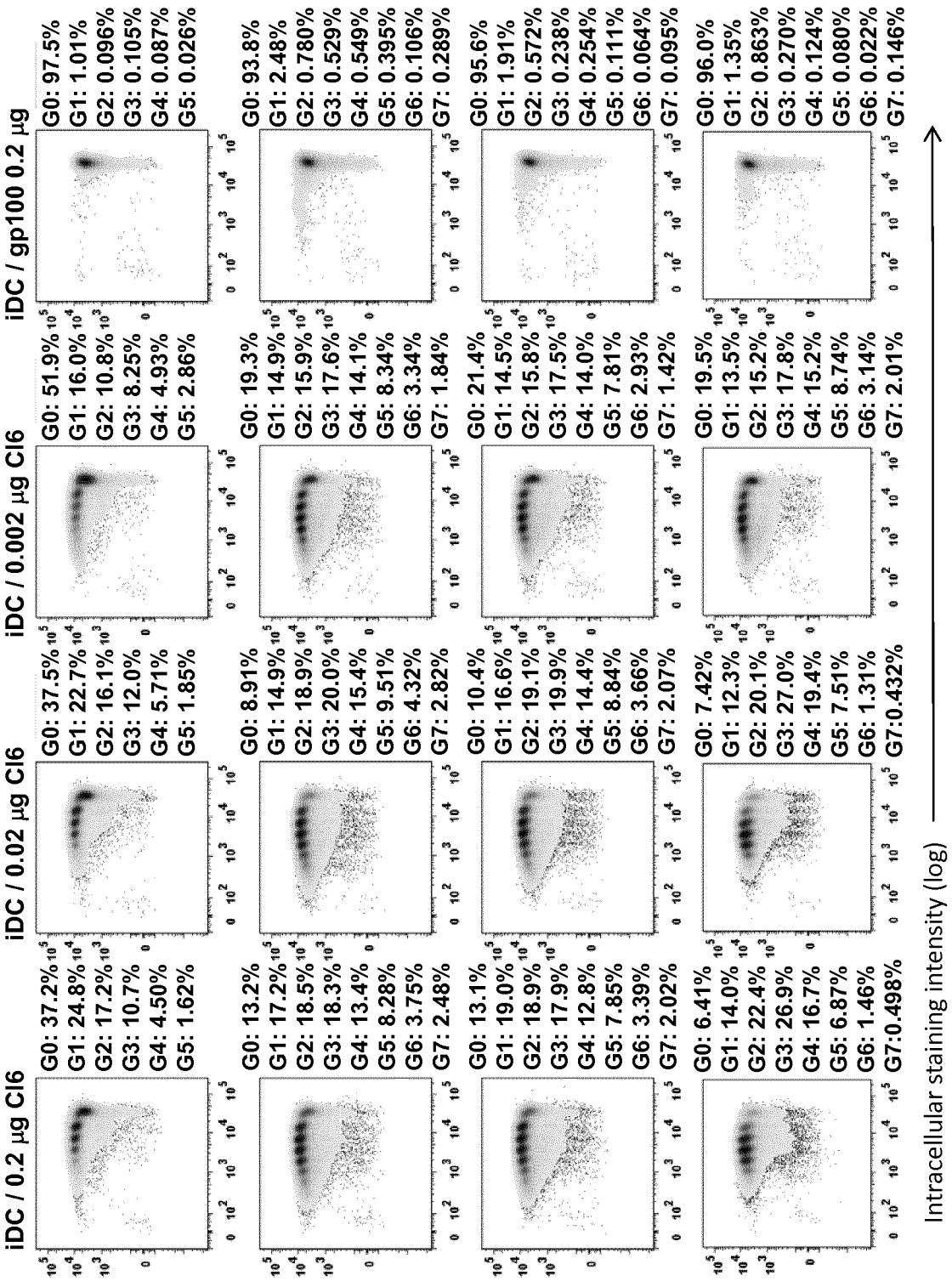


Figure 5A
iDC / 0.2 µg Cl6
iDC / 0.02 µg Cl6
iDC / 0.002 µg Cl6
iDC / gp100 0.2 µg
Monovalent non-combinatory CAR Cl6
Inter-combinatory TCR-CAR Cl6
Inter-combinatory TCR-CAR Cl6 silCDR3α
Classical scCAR Cl6
CD8 expression (log)
Intracellular staining intensity (log)
G0: 37.2%
G1: 24.8%
G2: 17.2%
G3: 10.7%
G4: 4.50%
G5: 1.62%
G0: 37.5%
G1: 22.7%
G2: 16.1%
G3: 12.0%
G4: 5.71%
G5: 1.85%
G0: 51.9%
G1: 16.0%
G2: 10.8%
G3: 8.25%
G4: 4.93%
G5: 2.86%
G0: 97.5%
G1: 1.01%
G2: 0.096%
G3: 0.105%
G4: 0.087%
G5: 0.026%
G0: 13.2%
G1: 17.2%
G2: 18.5%
G3: 18.3%
G4: 13.4%
G5: 8.28%
G6: 3.75%
G7: 2.48%
G0: 8.91%
G1: 14.9%
G2: 18.9%
G3: 20.0%
G4: 15.4%
G5: 9.51%
G6: 4.32%
G7: 2.82%
G0: 19.3%
G1: 14.9%
G2: 15.9%
G3: 17.6%
G4: 14.1%
G5: 8.34%
G6: 3.34%
G7: 1.84%
G0: 93.8%
G1: 2.48%
G2: 0.780%
G3: 0.529%
G4: 0.549%
G5: 0.395%
G6: 0.106%
G7: 0.289%
G0: 13.1%
G1: 19.0%
G2: 18.9%
G3: 17.9%
G4: 12.8%
G5: 7.85%
G6: 3.39%
G7: 2.02%
G0: 10.4%
G1: 16.6%
G2: 19.1%
G3: 19.9%
G4: 14.4%
G5: 8.84%
G6: 3.66%
G7: 2.07%
G0: 21.4%
G1: 14.5%
G2: 15.8%
G3: 17.5%
G4: 14.0%
G5: 7.81%
G6: 2.93%
G7: 1.42%
G0: 95.6%
G1: 1.91%
G2: 0.572%
G3: 0.238%
G4: 0.254%
G5: 0.111%
G6: 0.064%
G7: 0.095%
G0: 6.41%
G1: 14.0%
G2: 22.4%
G3: 26.9%
G4: 16.7%
G5: 6.87%
G6: 1.46%
G7:0.498%
G0: 7.42%
G1: 12.3%
G2: 20.1%
G3: 27.0%
G4: 19.4%
G5: 7.51%
G6: 1.31%
G7:0.432%
G0: 19.5%
G1: 13.5%
G2: 15.2%
G3: 17.8%
G4: 15.2%
G5: 8.74%
G6: 3.14%
G7: 2.01%
G0: 96.0%
G1: 1.35%
G2: 0.863%
G3: 0.270%
G4: 0.124%
G5: 0.080%
G6: 0.022%
G7: 0.146%

Figure 5B

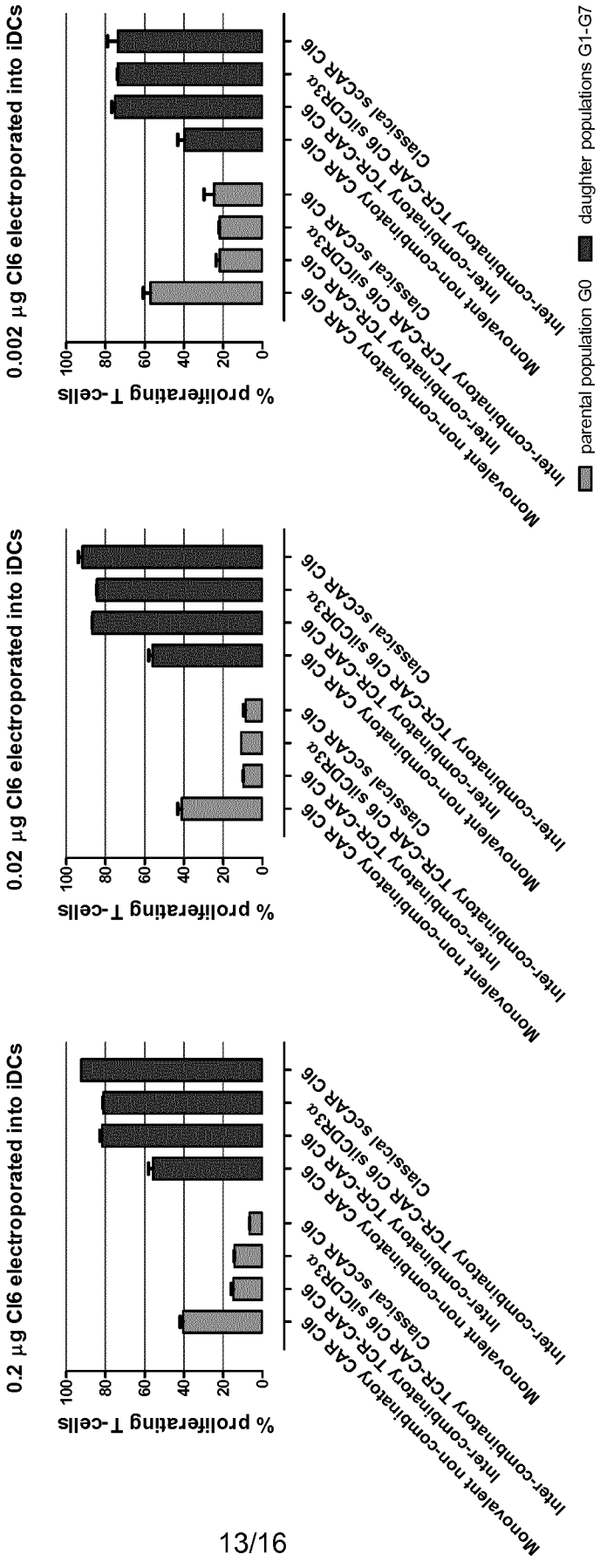

0.2 µg CI6 electroporated into iDCs
0.02 µg CI6 electroporated into iDCs
0.002 µg CI6 electroporated into iDCs
% proliferating T-cells
0
20
40
60
80
100
parental population G0
daughter populations G1-G7
Monovalent non-combinatory CAR CI6
Inter-combinatory TCR-CAR CI6
Monovalent non-combinatory CAR CI6
Classical scCAR CI6
Inter-combinatory TCR-CAR CI6 siICDR3α
Inter-combinatory TCR-CAR CI6

Figure 6

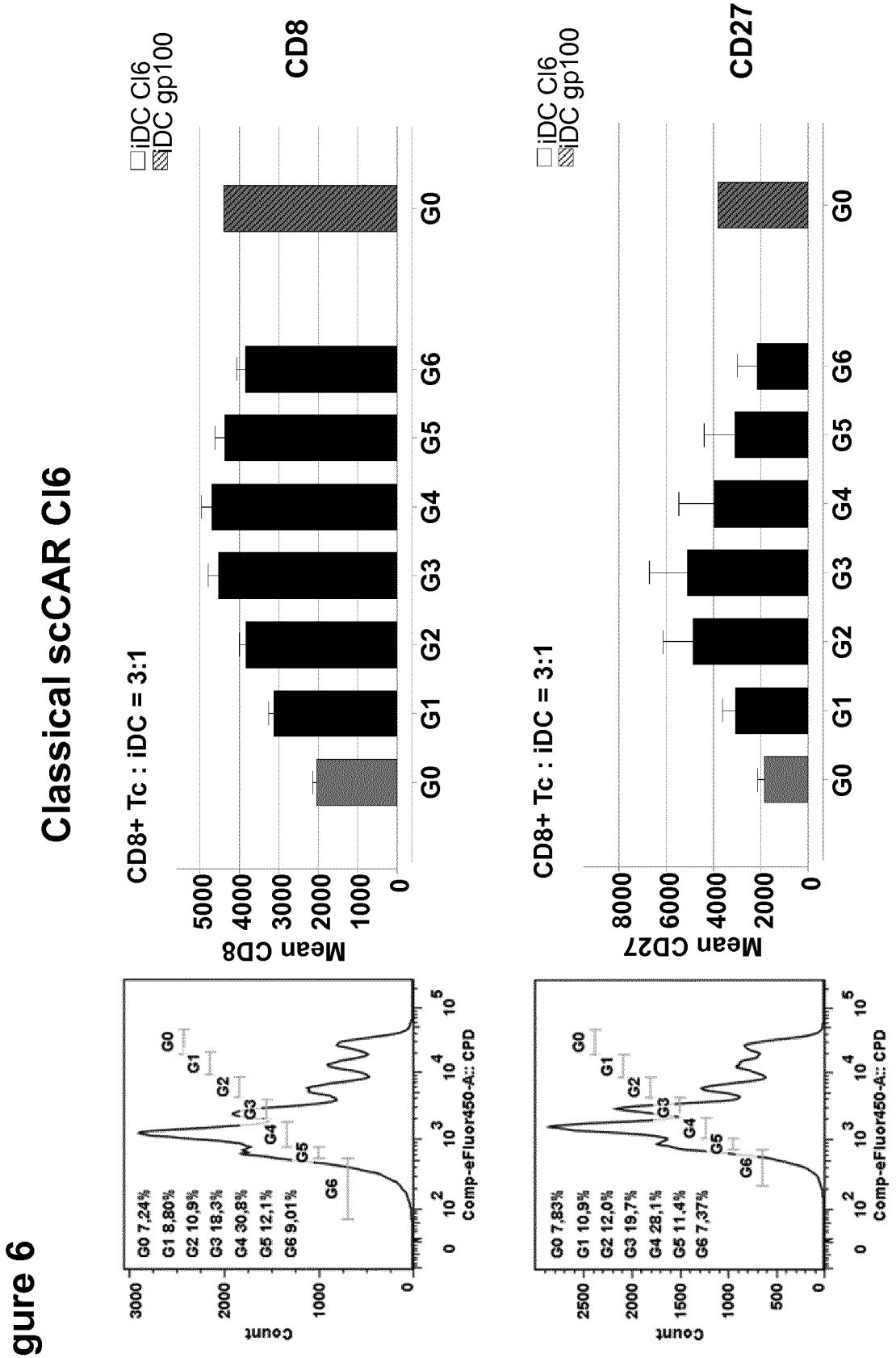

Classical scCAR Cl6
CD8
CD27
iDC Cl6
iDC gp100
CD8+ Tc : iDC = 3:1
Mean CD8
Mean CD27
G0
G1
G2
G3
G4
G5
G6
Count
Comp-eFluor450-A:: CPD
G0 7,24%
G1 8,80%
G2 10,9%
G3 18,3%
G4 30,8%
G5 12,1%
G6 9,01%
G0 7,83%
G1 10,9%
G2 12,0%
G3 19,7%
G4 28,1%
G5 11,4%
G6 7,37%

Figure 6

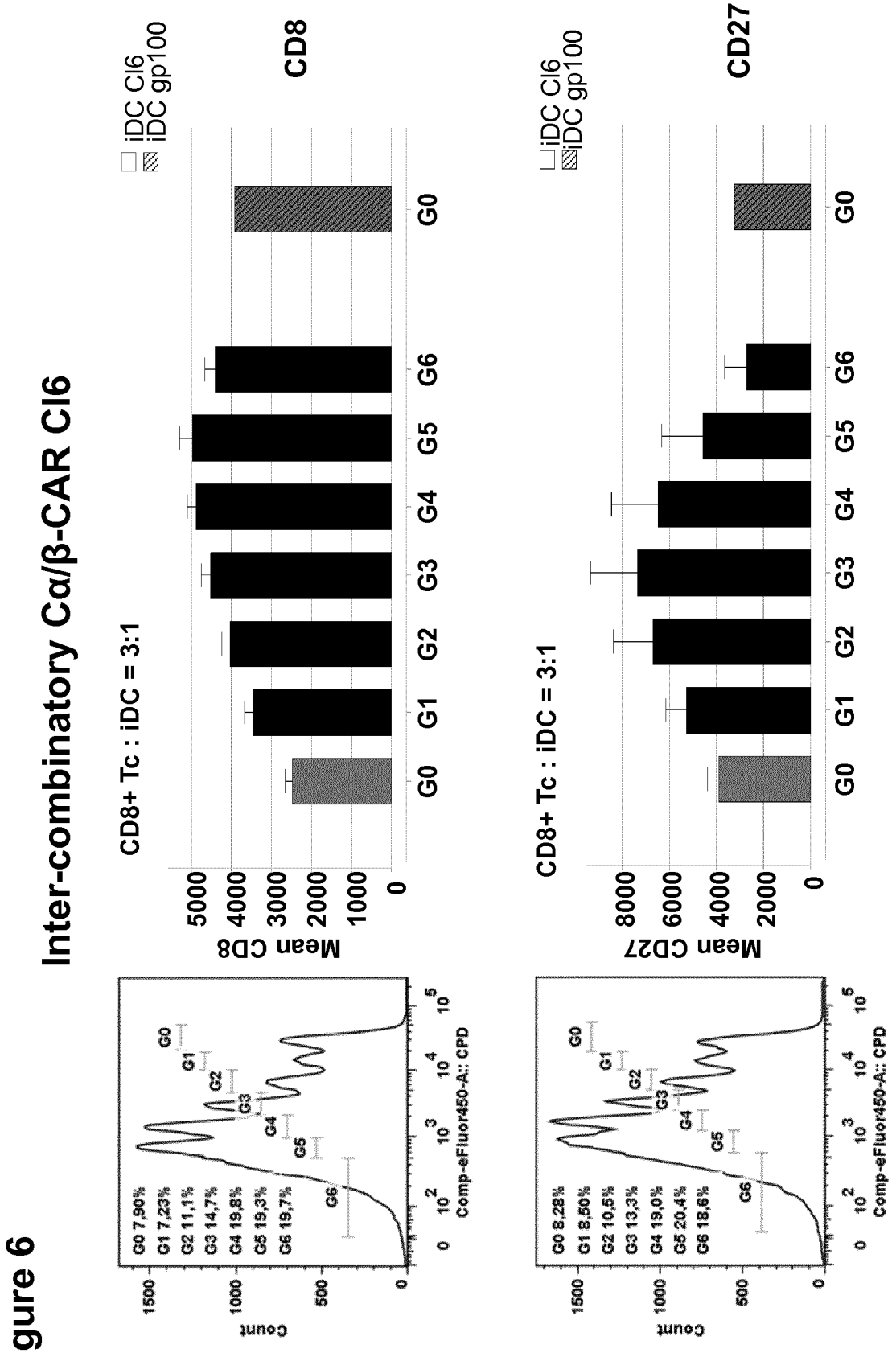
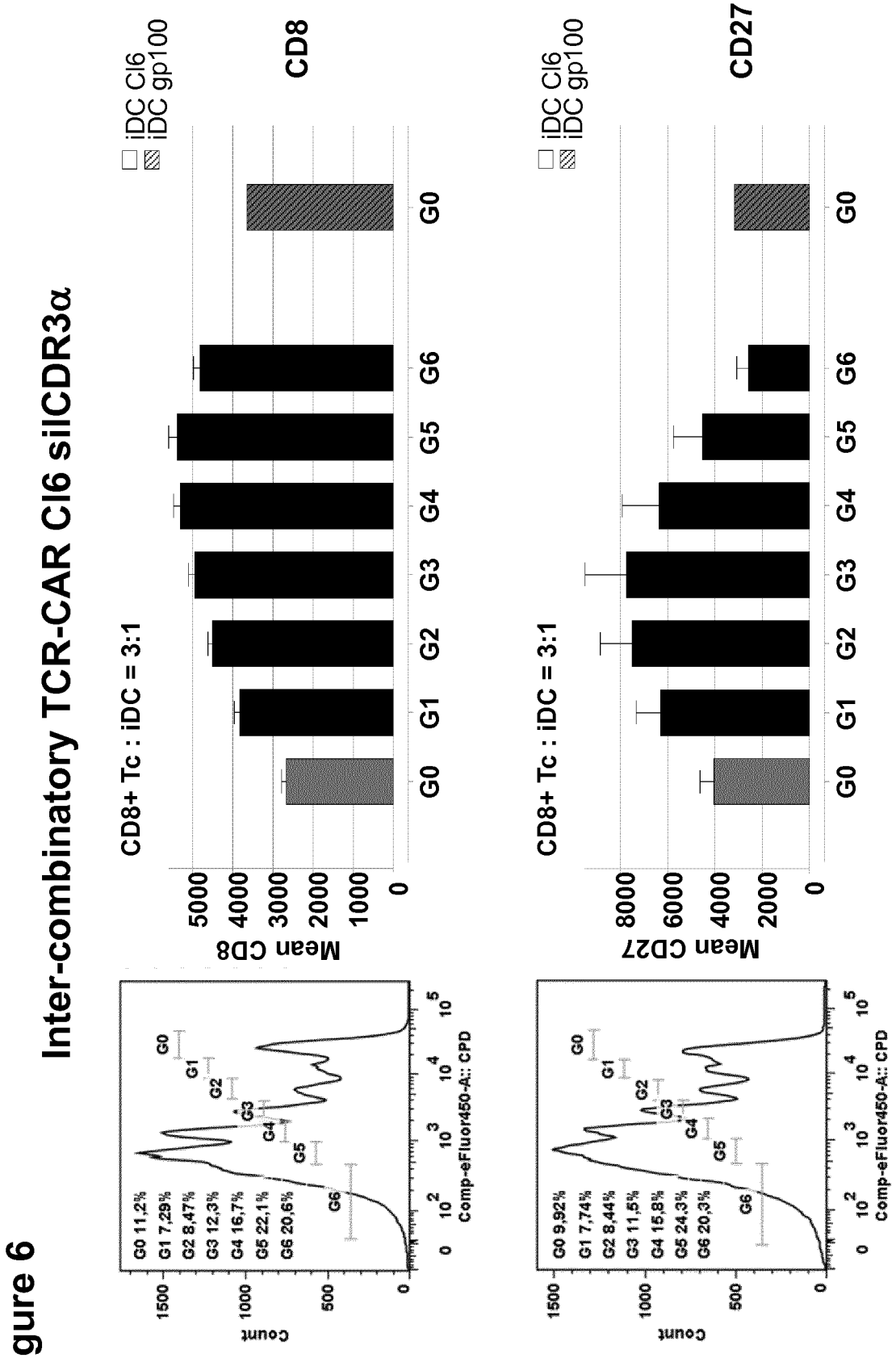

Inter-combinatory Cα/β-CAR CI6
CD8
CD27
iDC CI6
iDC gp100
CD8+ Tc : iDC = 3:1
Mean CD8
Mean CD27
G0
G1
G2
G3
G4
G5
G6
G0 7,90%
G1 7,23%
G2 11,1%
G3 14,7%
G4 19,8%
G5 19,3%
G6 19,7%
G0 8,28%
G1 8,50%
G2 10,5%
G3 13,3%
G4 19,0%
G5 20,4%
G6 18,6%
Count
Comp-eFluor450-A:: CPD

Figure 6


Inter-combinatory TCR-CAR CI6 siICDR3α
CD8
CD27
iDC CI6
iDC gp100
CD8+ Tc : iDC = 3:1
Mean CD8
Mean CD27
G0
G1
G2
G3
G4
G5
G6
G0 11,2%
G1 7,29%
G2 8,47%
G3 12,3%
G4 16,7%
G5 22,1%
G6 20,6%
G0 9,92%
G1 7,74%
G2 8,44%
G3 11,5%
G4 15,8%
G5 24,3%
G6 20,3%
count
Comp-eFluor450-A:: CPD