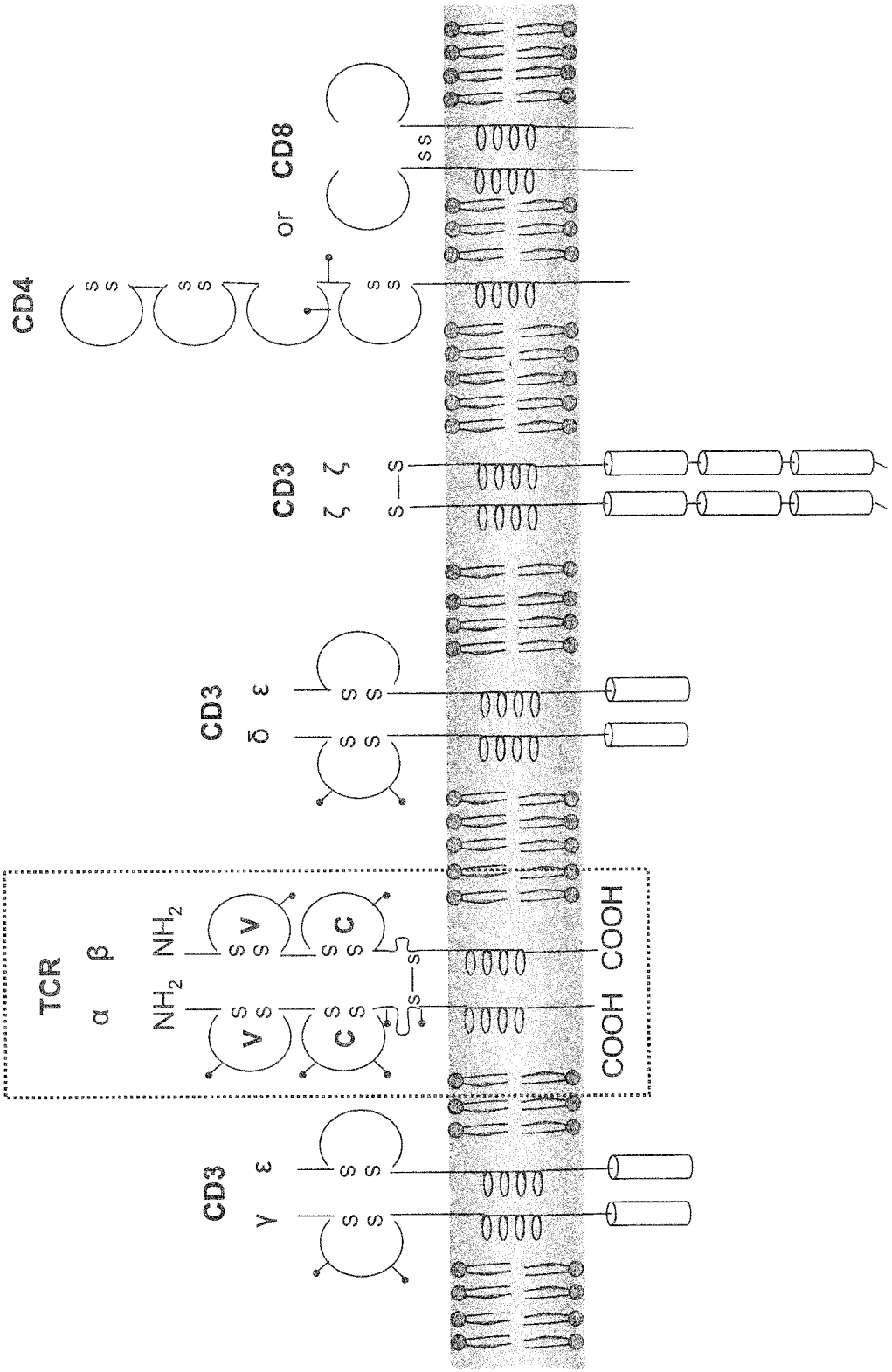


Figure 1

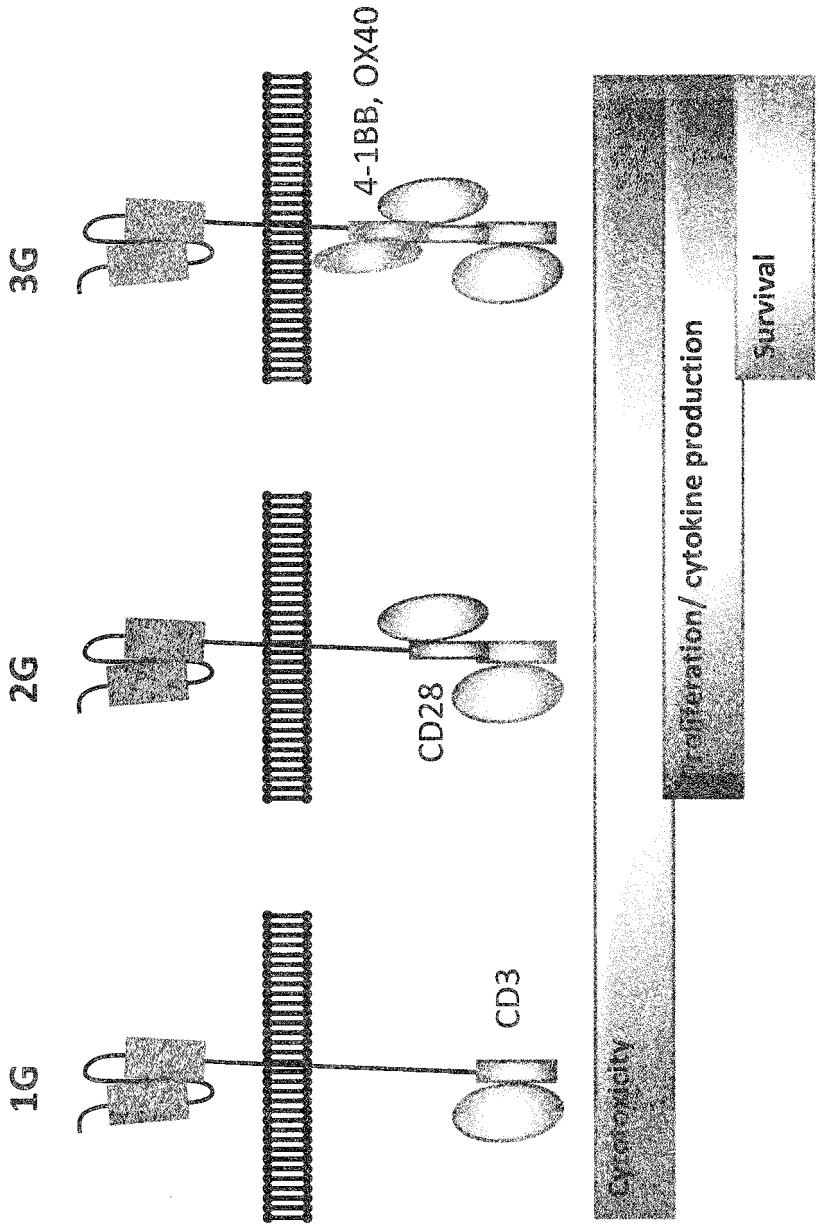

1G
2G
3G
CD3
CD28
4-1BB, OX40
Cytotoxicity
Proliferation/ cytokine production
Survival

Figure 2

Figure 3

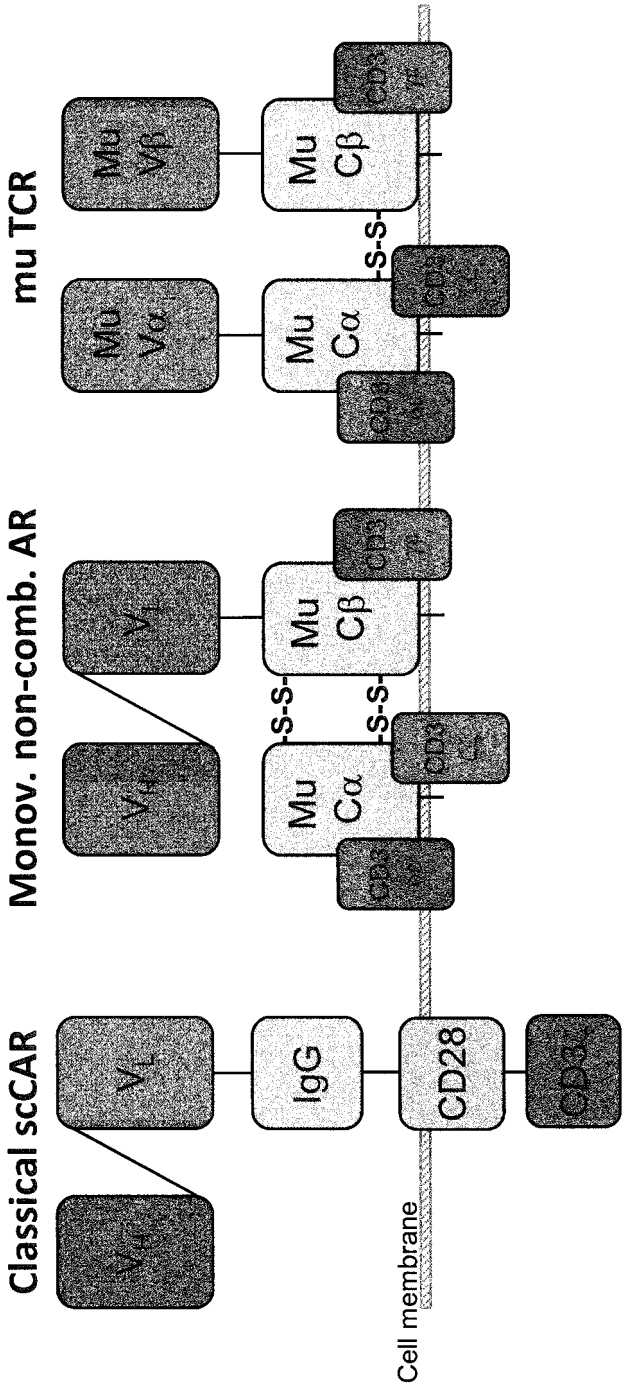

Classical scCAR
Monov. non-comb. AR
mu TCR
IgG
CD28
CD3ζ
Cell membrane
Mu Cβ
Mu Cα
Mu Vβ
Mu Vα
-S-S-

Figure 4A

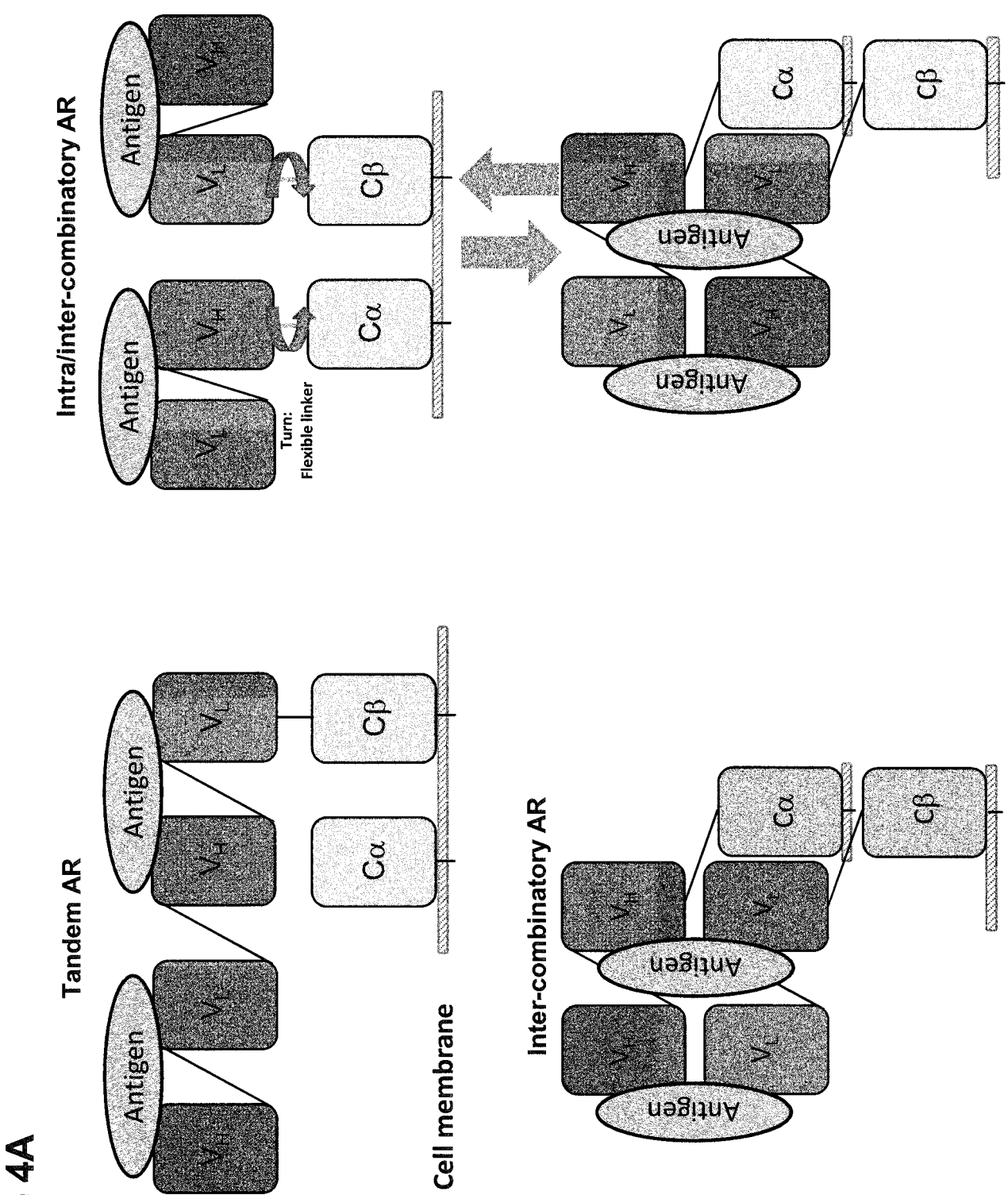
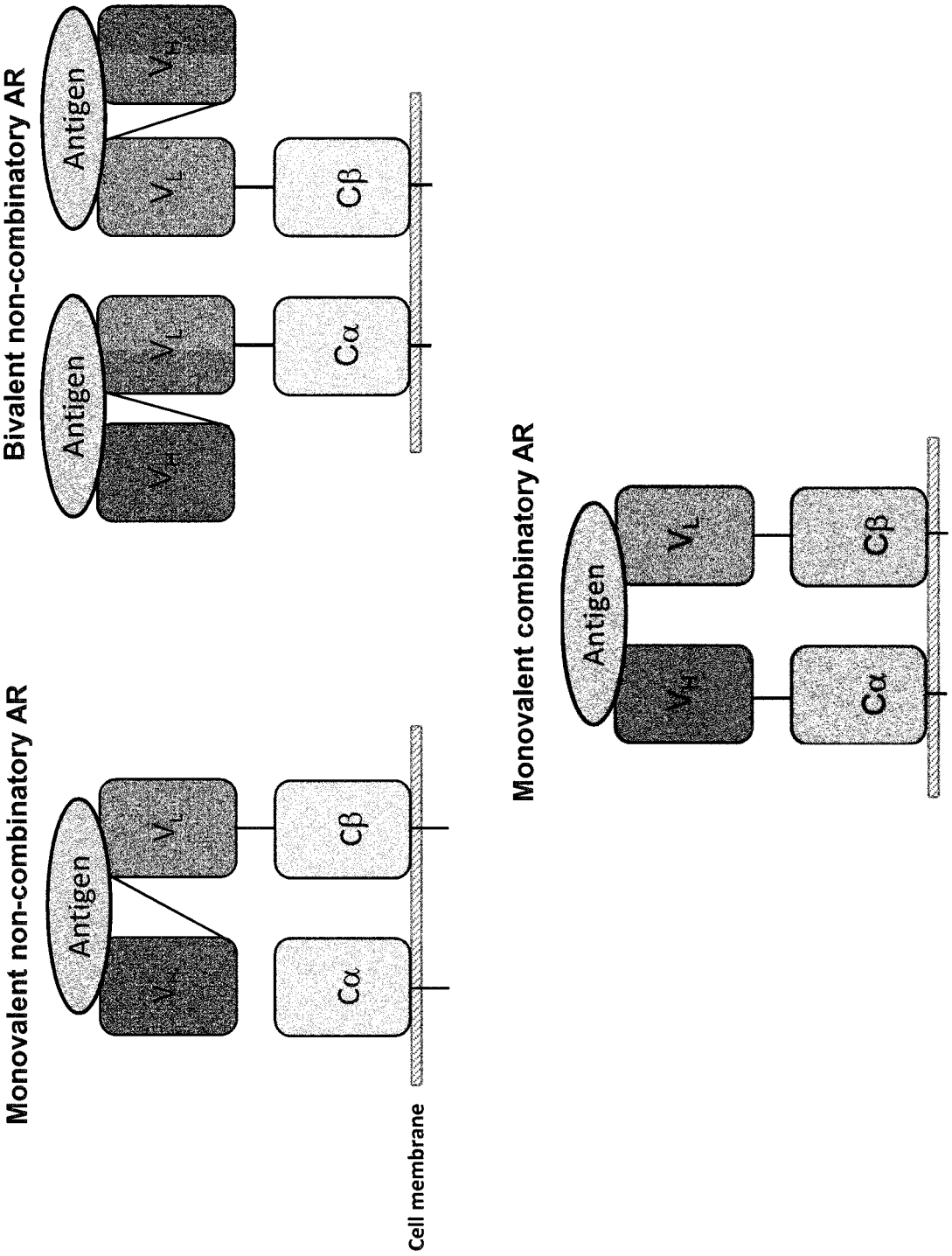

Tandem AR
Antigen
V_H
V_L
Cα
Cβ
Cell membrane
Intra/inter-combinatory AR
Antigen
V_H
V_L
Turn:
Flexible linker
Cα
Cβ
Antigen
V_H
V_L
Antigen
V_H
V_L
Cα
Cβ
Inter-combinatory AR
Antigen
V_H
V_L
Antigen
V_H
V_L
Cα
Cβ

Figure 4B


Monovalent non-combinatory AR
Antigen
V_H
V_L
Cα
Cβ
Cell membrane
Bivalent non-combinatory AR
Antigen
V_H
V_L
Cα
Cβ
Antigen
V_H
V_L
Cβ
Monovalent combinatory AR
Antigen
V_L
V_H
Cβ
Cα

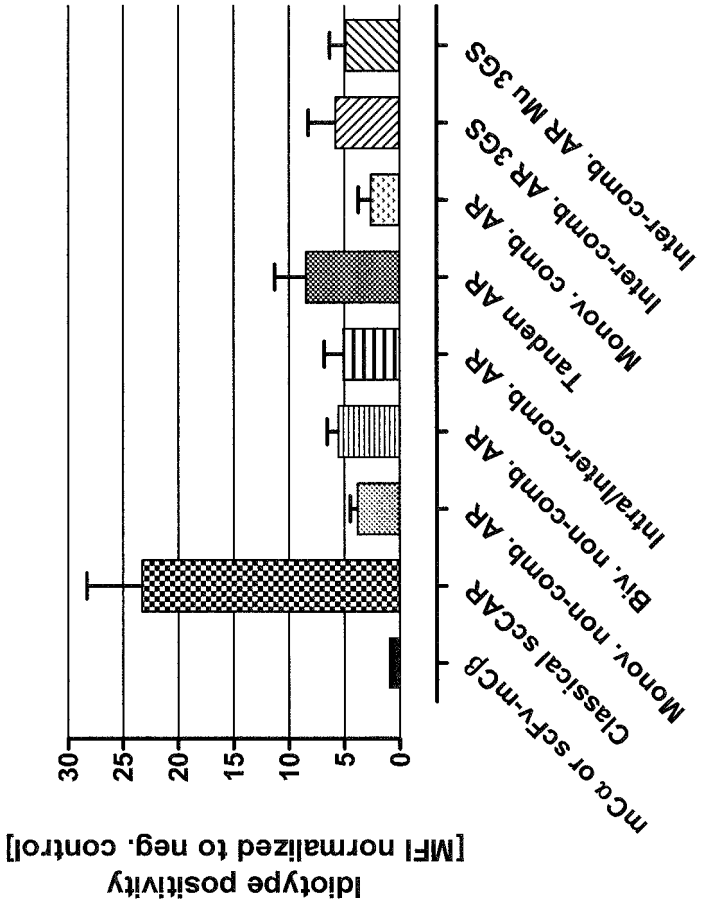

Idiotype positivity
[MFI normalized to neg. control]
0
5
10
15
20
25
30
mCα or scFv-mCp
Classical scCAR
Monov. non-comb. AR
Biv. non-comb. AR
Intra/Inter-comb. AR
Tandem AR
Monov. comb. AR
Inter-comb. AR 3GS
Inter-comb. AR Mu 3GS

Figure 5

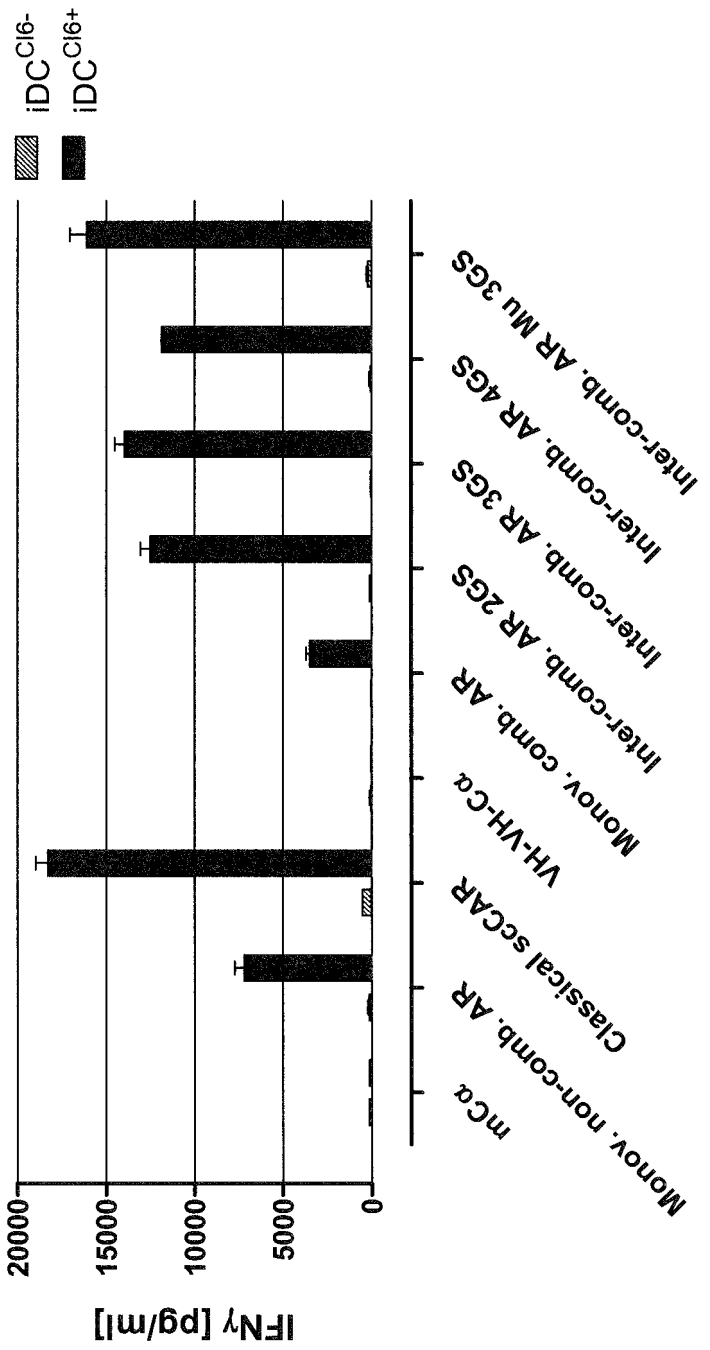

iDC^CI6-
iDC^CI6+
IFNγ [pg/ml]
20000
15000
10000
5000
0
mCα
Monov. non-comb. AR
Classical scCAR
VH-VH-Cα
Monov. comb. AR
Inter-comb. AR 2GS
Inter-comb. AR 3GS
Inter-comb. AR 4GS
Inter-comb. AR Mu 3GS

Figure 6A

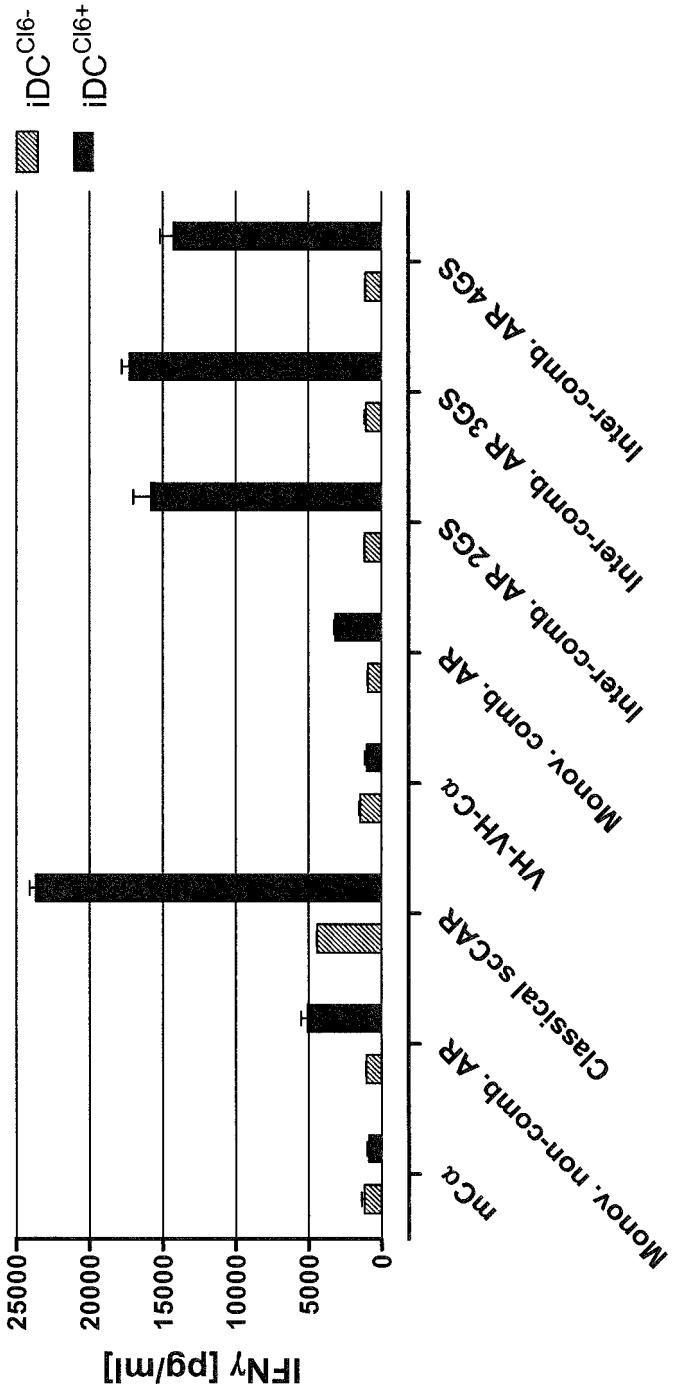

iDC^Cl6-
iDC^Cl6+
IFNγ [pg/ml]
25000
20000
15000
10000
5000
0
mCα
Monov. non-comb. AR
Classical scCAR
VH-VH-Cα
Monov. comb. AR
Inter-comb. AR 2GS
Inter-comb. AR 3GS
Inter-comb. AR 4GS

Figure 6B

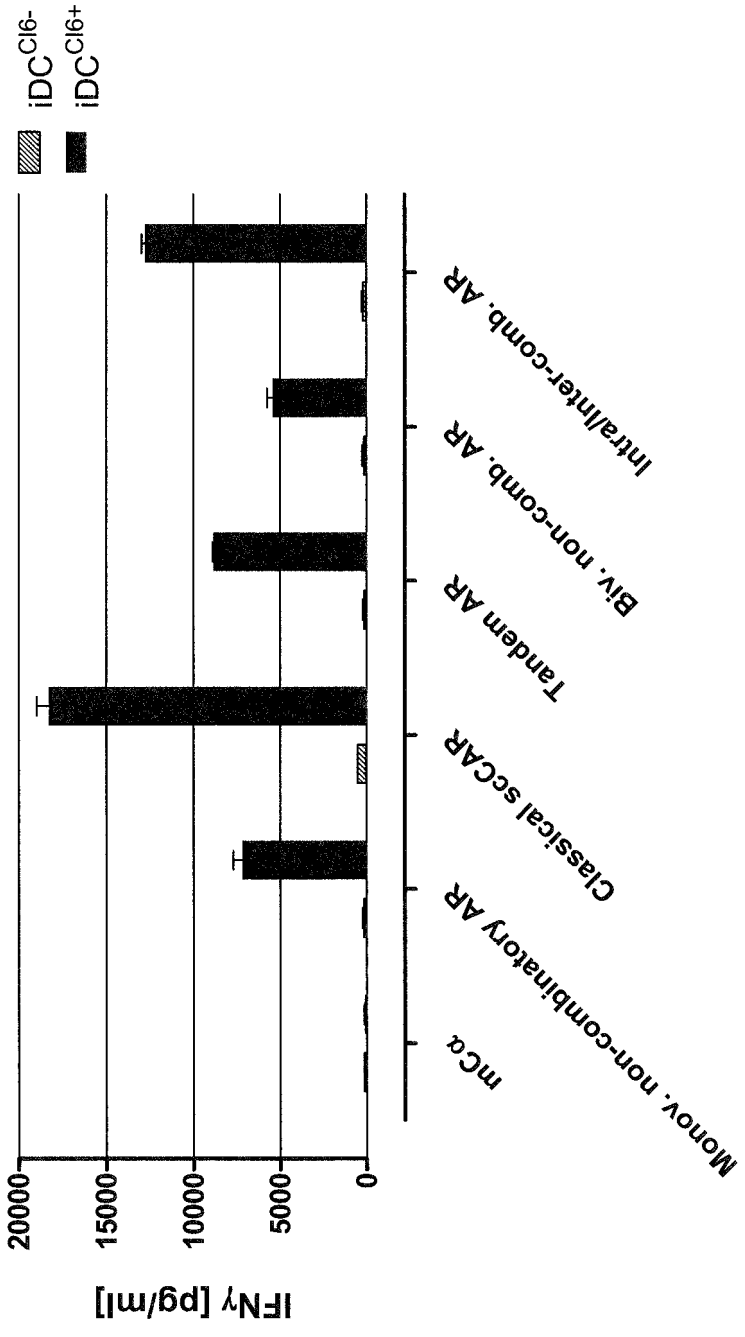

iDC^CI6-
iDC^CI6+
IFNγ [pg/ml]
20000
15000
10000
5000
0
mCα
Monov. non-combinatory AR
Classical scCAR
Tandem AR
Biv. non-comb. AR
Intra/Inter-comb. AR

Figure 6C

Figure 7

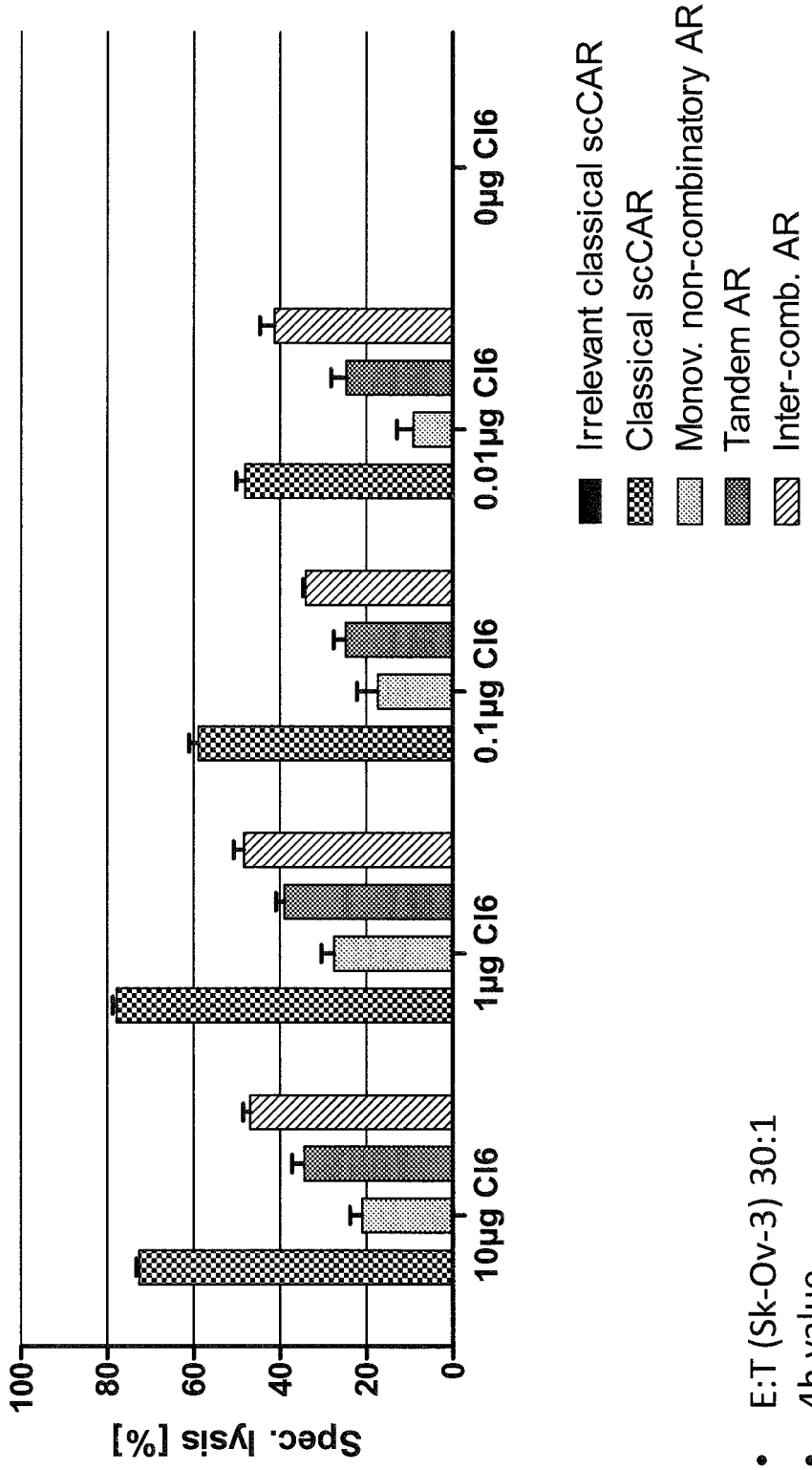

Spec. lysis [%]
100
80
60
40
20
0
10µg CI6
1µg CI6
0.1µg CI6
0.01µg CI6
0µg CI6
Irrelevant classical scCAR
Classical scCAR
Monov. non-combinatory AR
Tandem AR
Inter-comb. AR
• E:T (Sk-Ov-3) 30:1
• 4h value

Figure 8

A

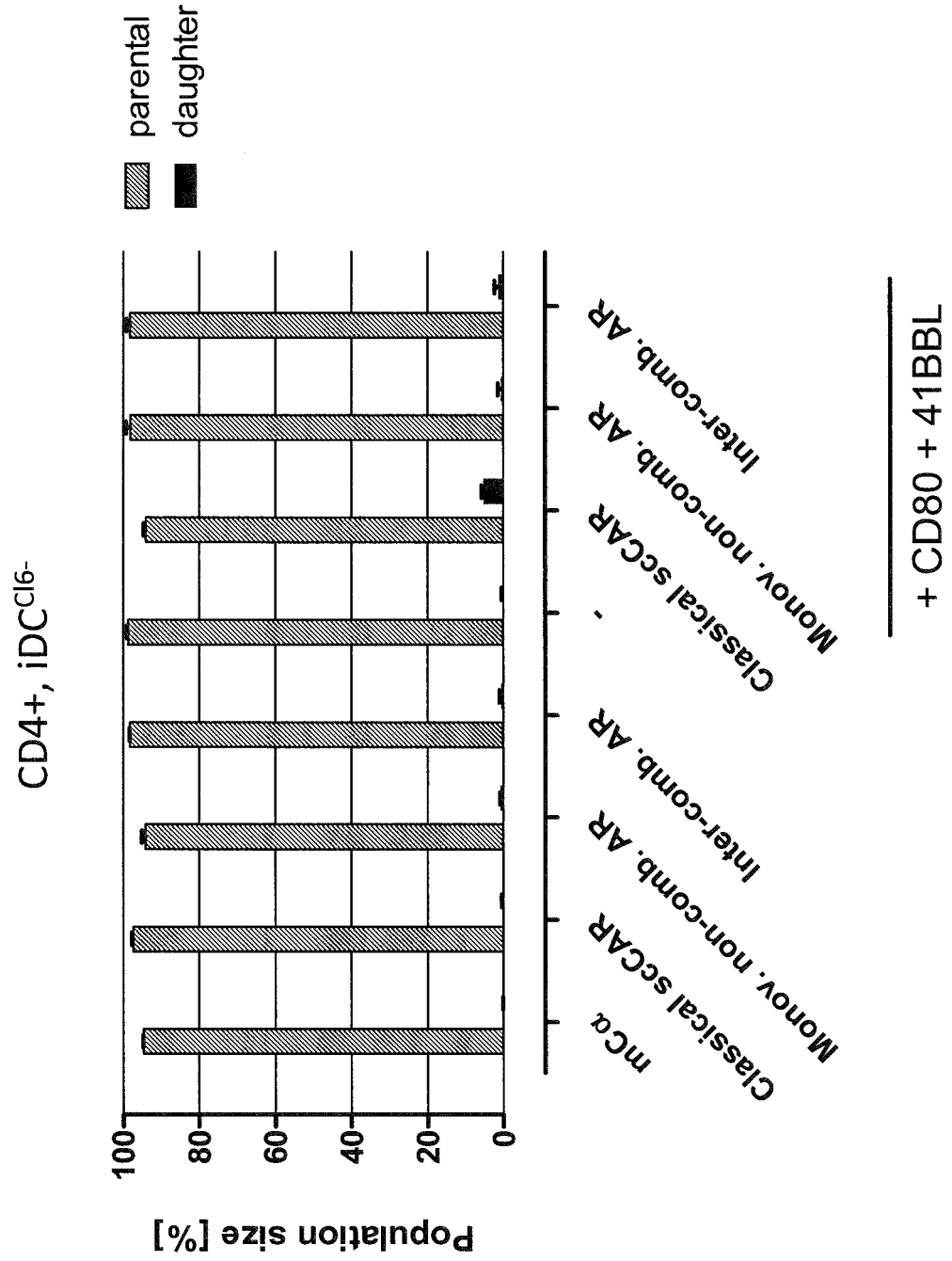

parental
daughter
CD4+, iDC^Cl6-
Population size [%]
100
80
60
40
20
0
mCα
Classical scCAR
Monov. non-comb. AR
Inter-comb. AR
-
Classical scCAR
Monov. non-comb. AR
Inter-comb. AR
+ CD80 + 41BBL

Figure 8

B

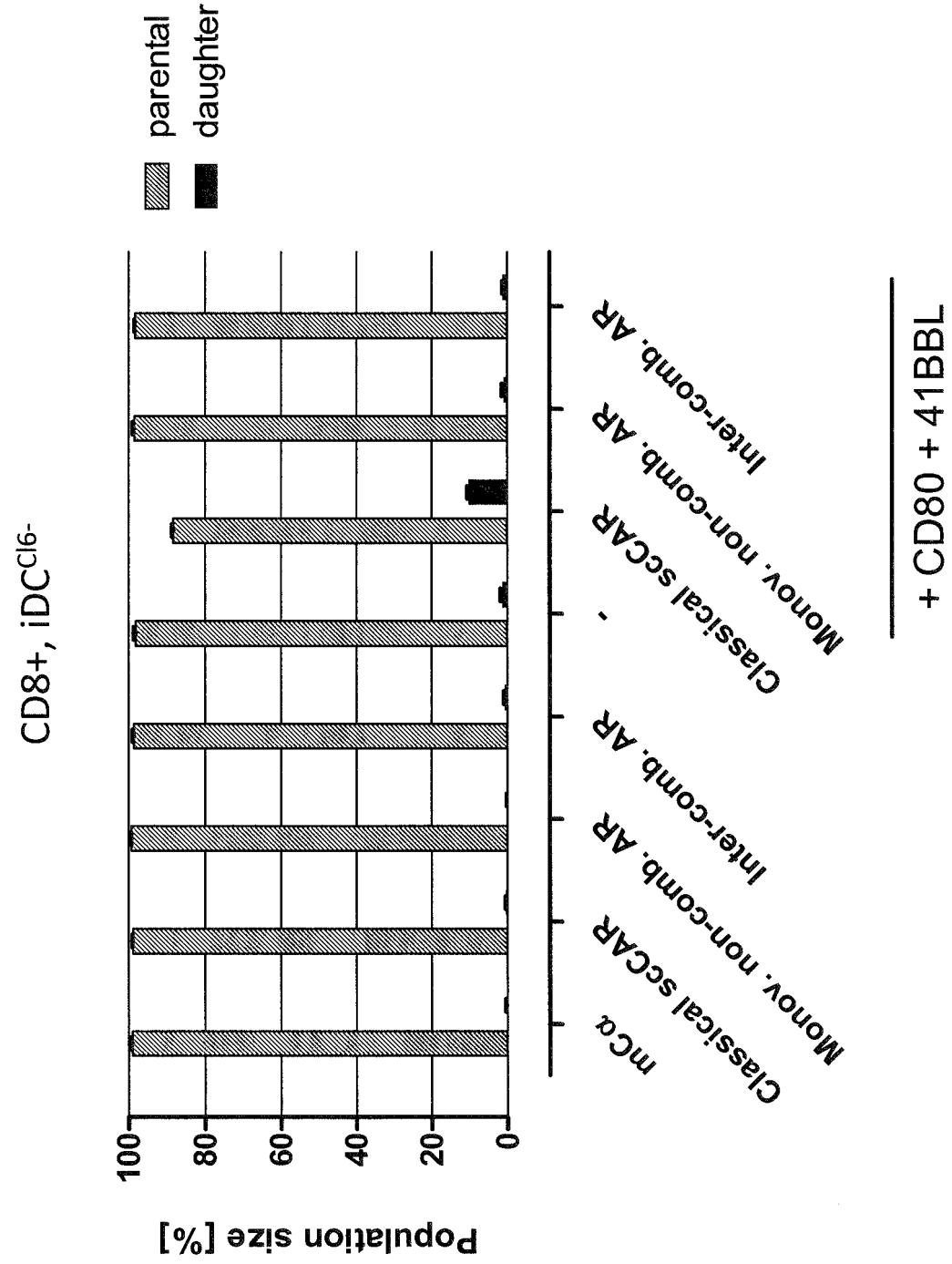
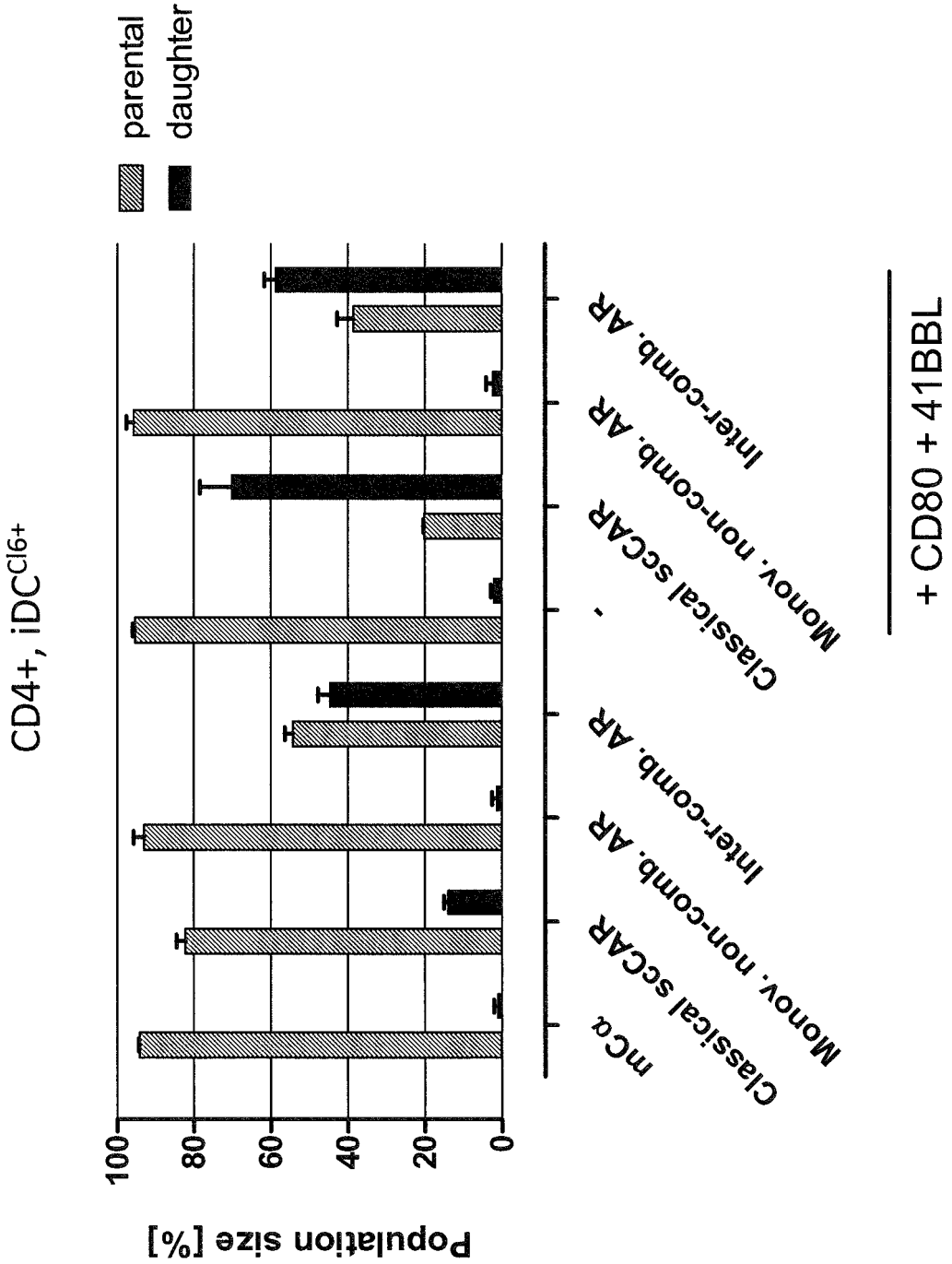

parental
daughter
CD8+, iDC^Cl6-
Population size [%]
0
20
40
60
80
100
mCα
Classical scCAR
Monov. non-comb. AR
Inter-comb. AR
Classical scCAR
-
Monov. non-comb. AR
Inter-comb. AR
+ CD80 + 41BBL

Figure 8

C


parental
daughter
CD4+, iDC^Cl6+
Population size [%]
100
80
60
40
20
0
mCα
Classical scCAR
Monov. non-comb. AR
Inter-comb. AR
Classical scCAR
-
Monov. non-comb. AR
Inter-comb. AR
+ CD80 + 41BBL

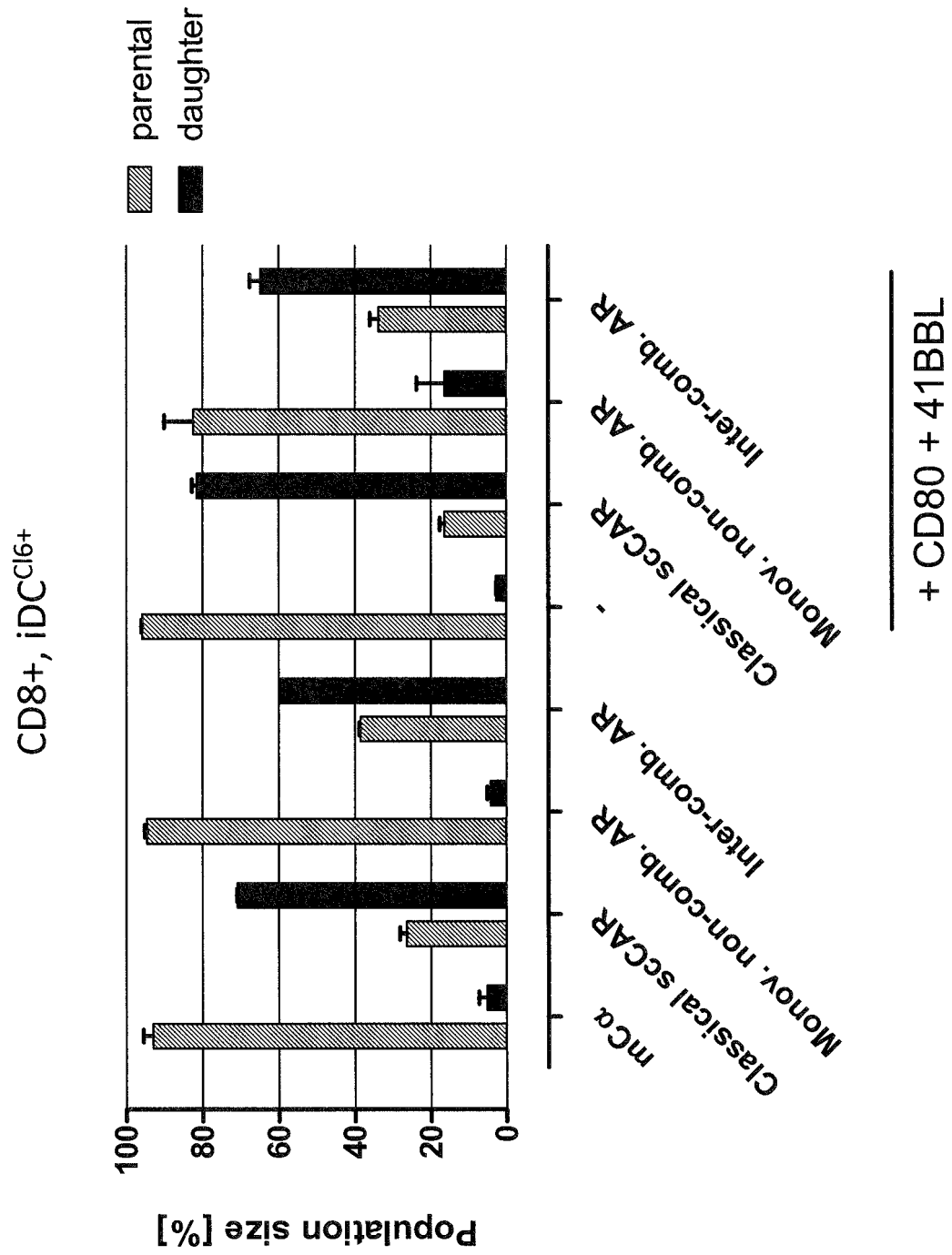

D
CD8+, iDC^Cl6+
parental
daughter
Population size [%]
100
80
60
40
20
0
mCα
Classical scCAR
Monov. non-comb. AR
Inter-comb. AR
-
Classical scCAR
Monov. non-comb. AR
Inter-comb. AR
+ CD80 + 41BBL

Figure 8

Figure 9

A

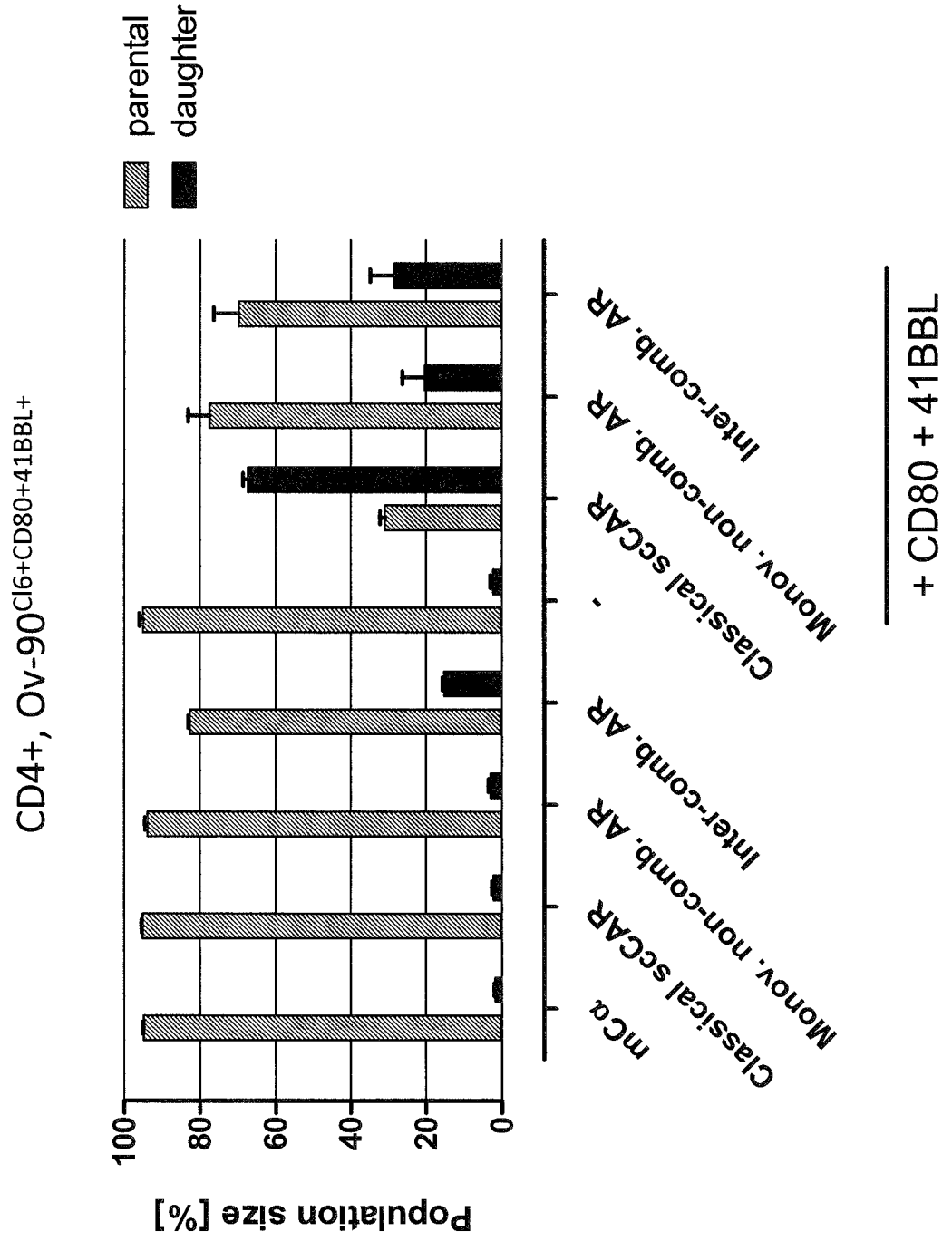
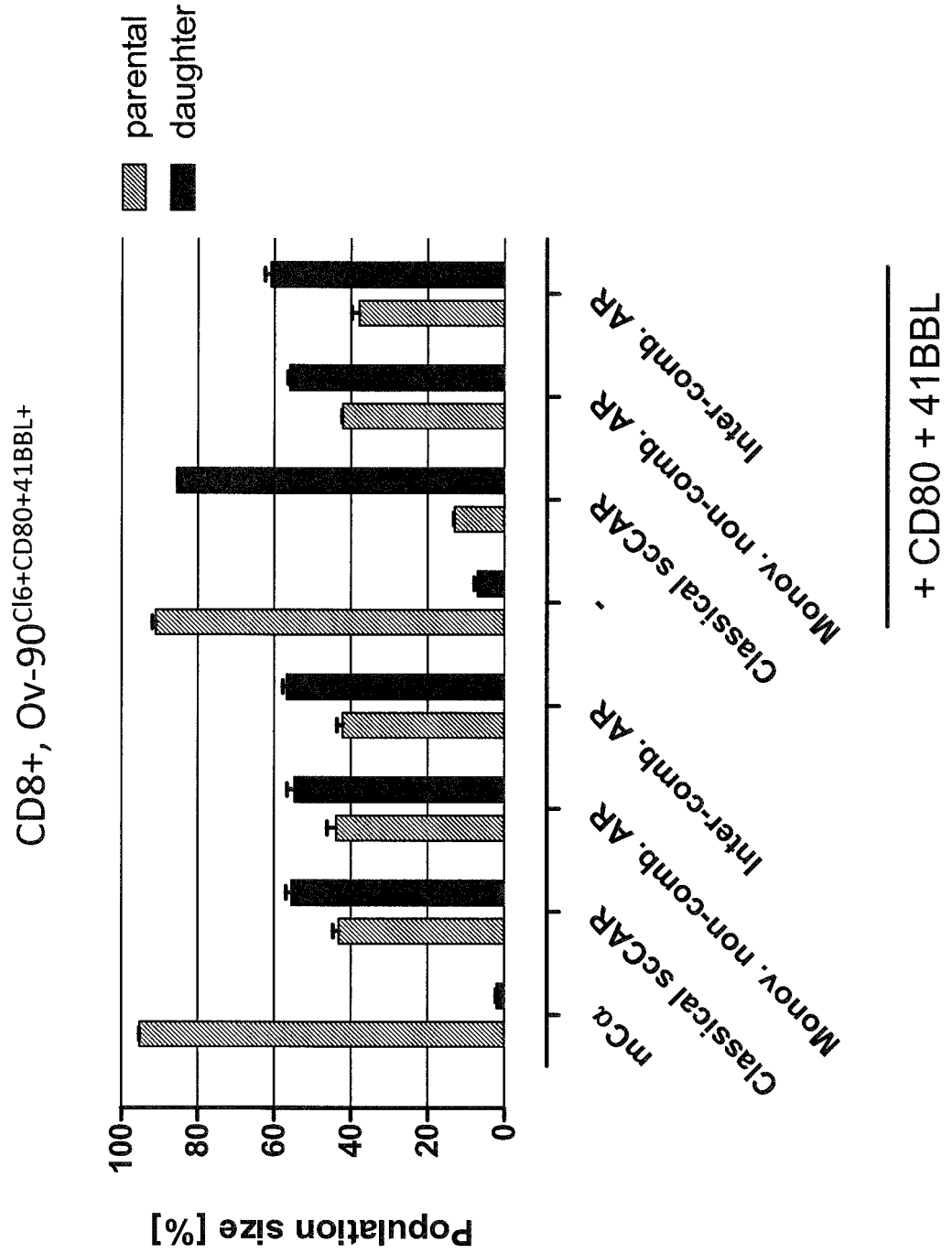

parental
daughter
CD4+, Ov-90Cl6+CD80+41BBL+
Population size [%]
100
80
60
40
20
0
mCα
Classical scCAR
Monov. non-comb. AR
Inter-comb. AR
Classical scCAR
-
Monov. non-comb. AR
Inter-comb. AR
+ CD80 + 41BBL

Figure 9

B


parental
daughter
CD8+, Ov-90Cl6+CD80+41BBL+
Population size [%]
100
80
60
40
20
0
mCα
Classical scCAR
Monov. non-comb. AR
Inter-comb. AR
Classical scCAR
-
Monov. non-comb. AR
Inter-comb. AR
+ CD80 + 41BBL

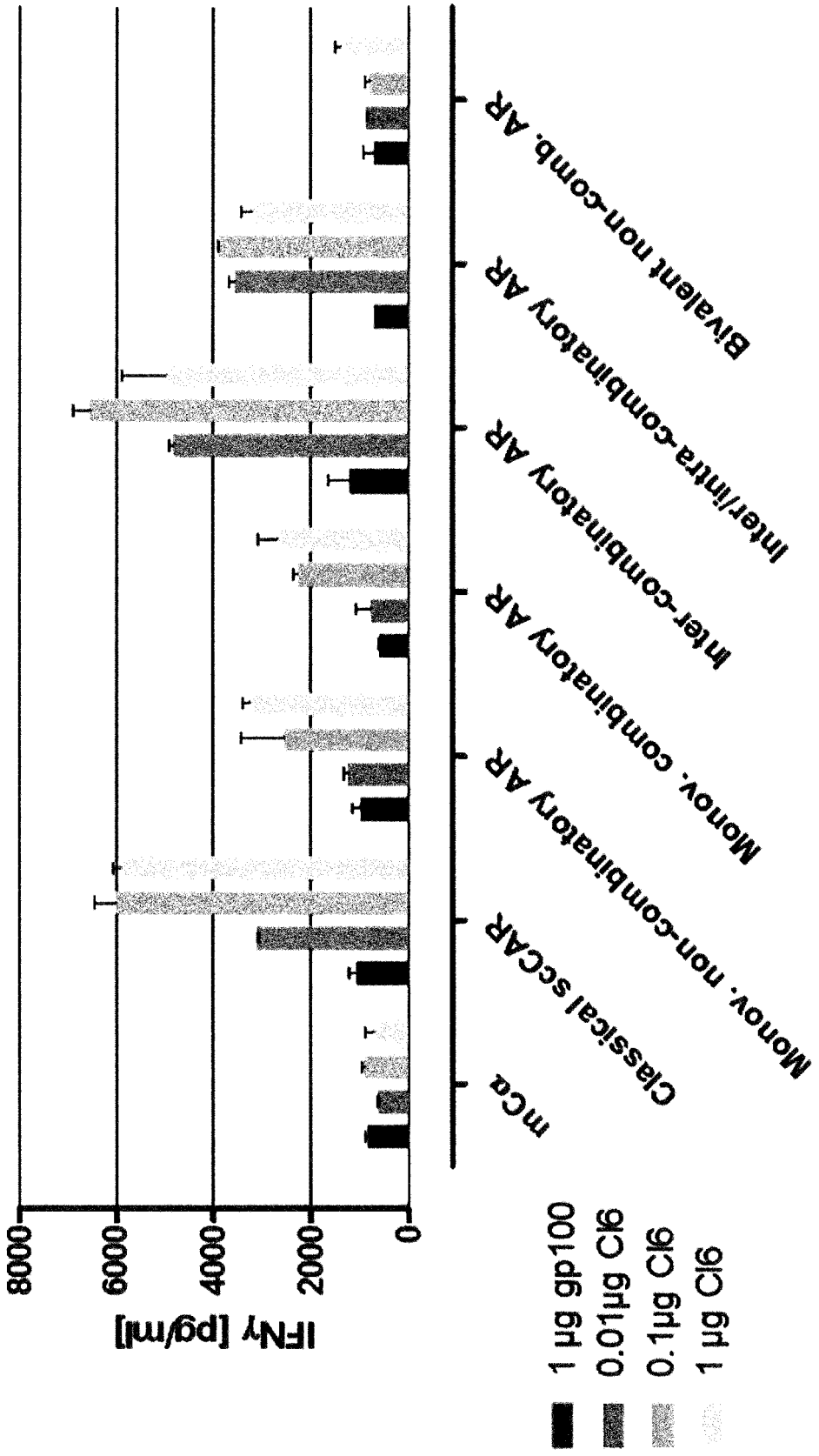
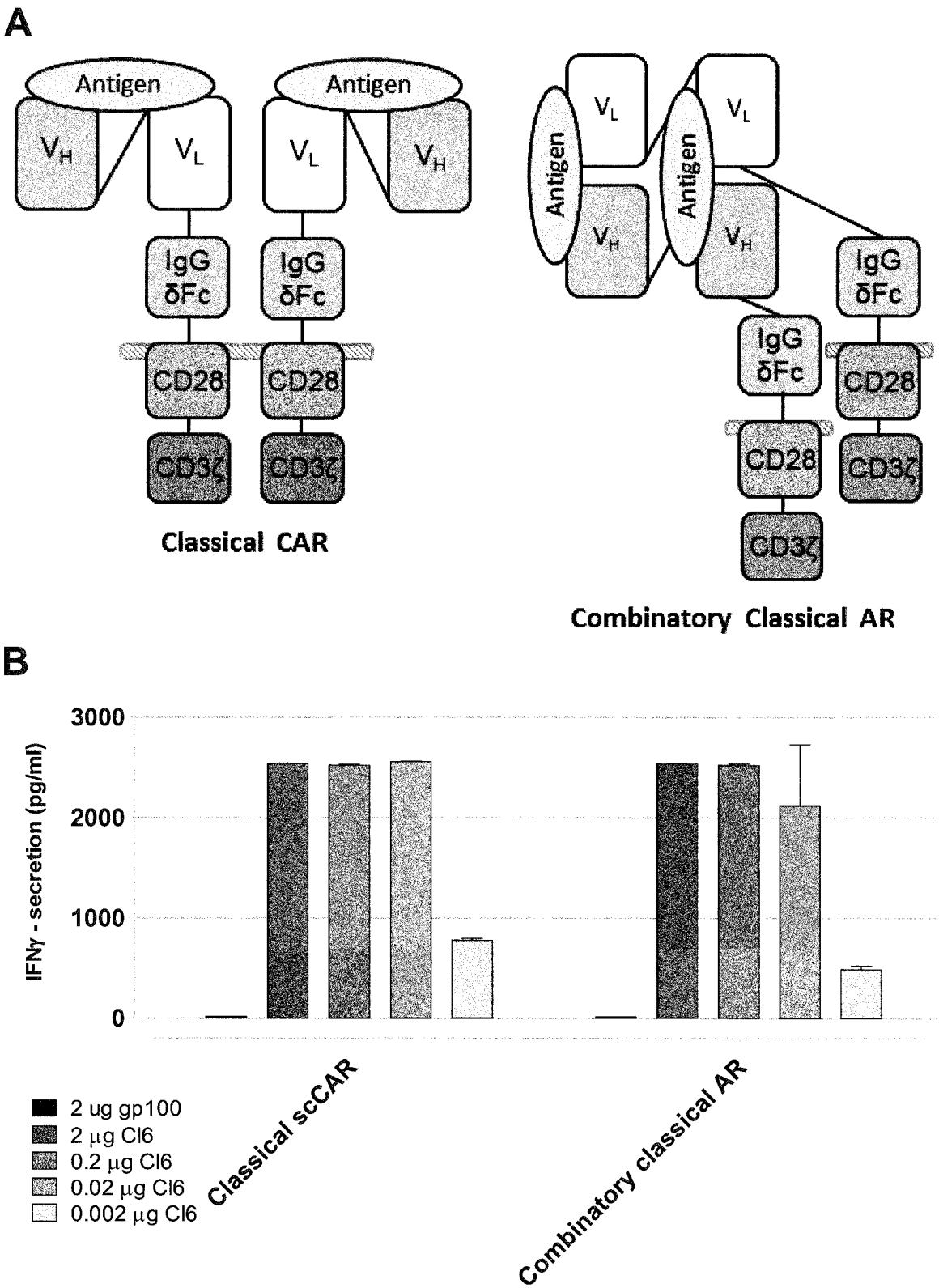

IFNγ [pg/ml]
8000
6000
4000
2000
0
mCα
Classical scCAR
Monov. non-combinatory AR
Monov. combinatory AR
Inter-combinatory AR
Inter/intra-combinatory AR
Bivalent non-comb. AR
1 µg gp100
0.01µg Cl6
0.1µg Cl6
1 µg Cl6

Figure 10

Figure 11


A
Antigen
Antigen
V_H
V_L
V_L
V_H
IgG δFc
IgG δFc
CD28
CD28
CD3ζ
CD3ζ
Classical CAR
Antigen
Antigen
V_L
V_L
V_H
V_H
IgG δFc
IgG δFc
CD28
CD28
CD3ζ
CD3ζ
Combinatory Classical AR
B
IFNγ - secretion (pg/ml)
3000
2000
1000
0
2 ug gp100
2 µg Cl6
0.2 µg Cl6
0.02 µg Cl6
0.002 µg Cl6
Classical scCAR
Combinatory classical AR