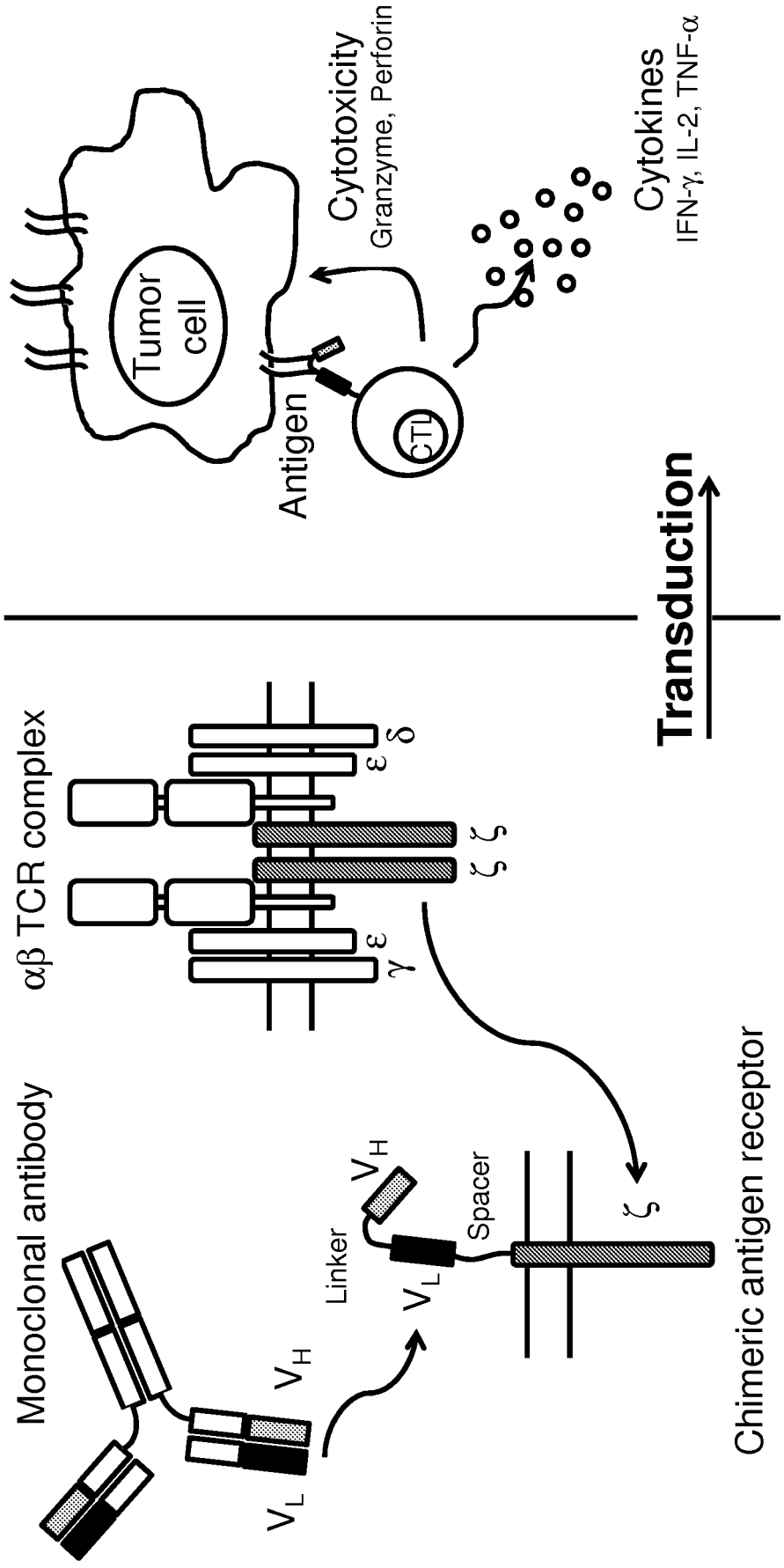

Monoclonal antibody
αβ TCR complex
Tumor cell
Antigen
CTL
Cytotoxicity
Granzyme, Perforin
Cytokines
IFN-γ, IL-2, TNF-α
Transduction
V_H
V_L
Linker
Spacer
ε
γ
ζ ζ
δ
ζ
Chimeric antigen receptor

FIGURE 1

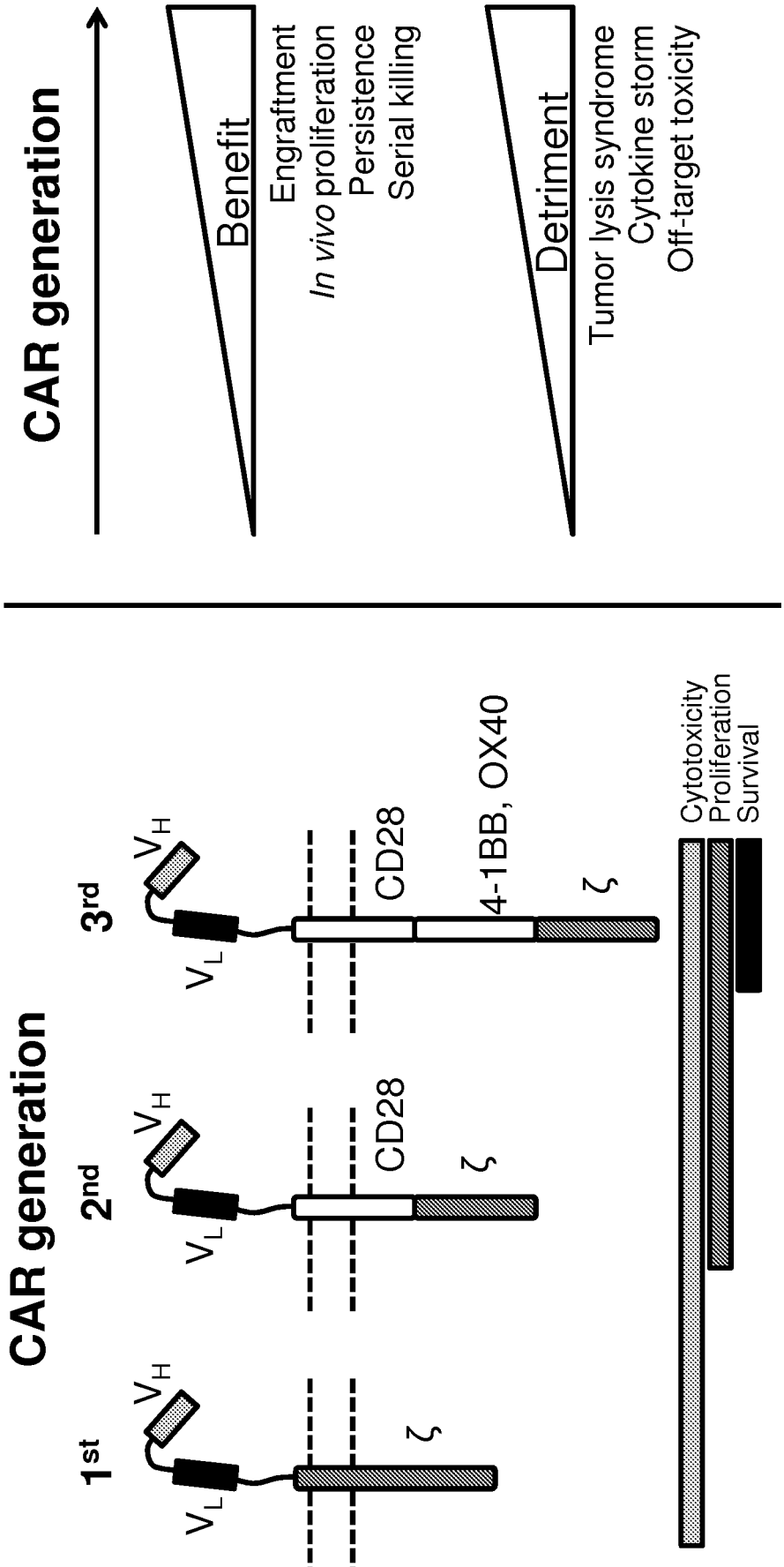

CAR generation
Benefit
Engraftment
In vivo proliferation
Persistence
Serial killing
Detriment
Tumor lysis syndrome
Cytokine storm
Off-target toxicity
CAR generation
1st
2nd
3rd
VH
VL
CD28
4-1BB, OX40
ζ
Cytotoxicity
Proliferation
Survival

FIGURE 2

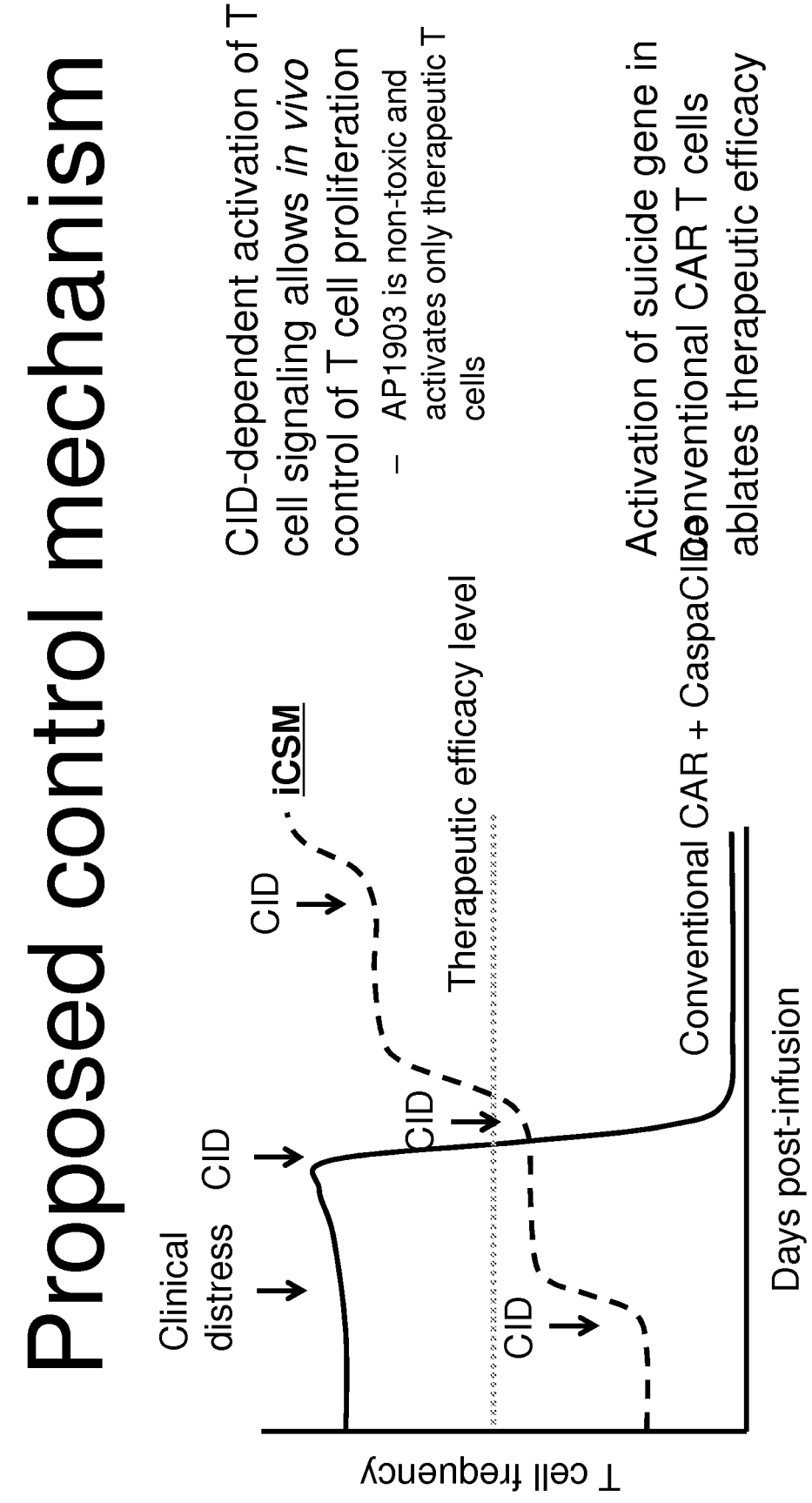

Proposed control mechanism
CID-dependent activation of T cell signaling allows *in vivo* control of T cell proliferation
– AP1903 is non-toxic and activates only therapeutic T cells
Activation of suicide gene in conventional CAR T cells ablates therapeutic efficacy
Clinical distress
CID
CID
iCSM
CID
CID
Therapeutic efficacy level
Conventional CAR + CaspaCIDe
T cell frequency
Days post-infusion

FIGURE 3

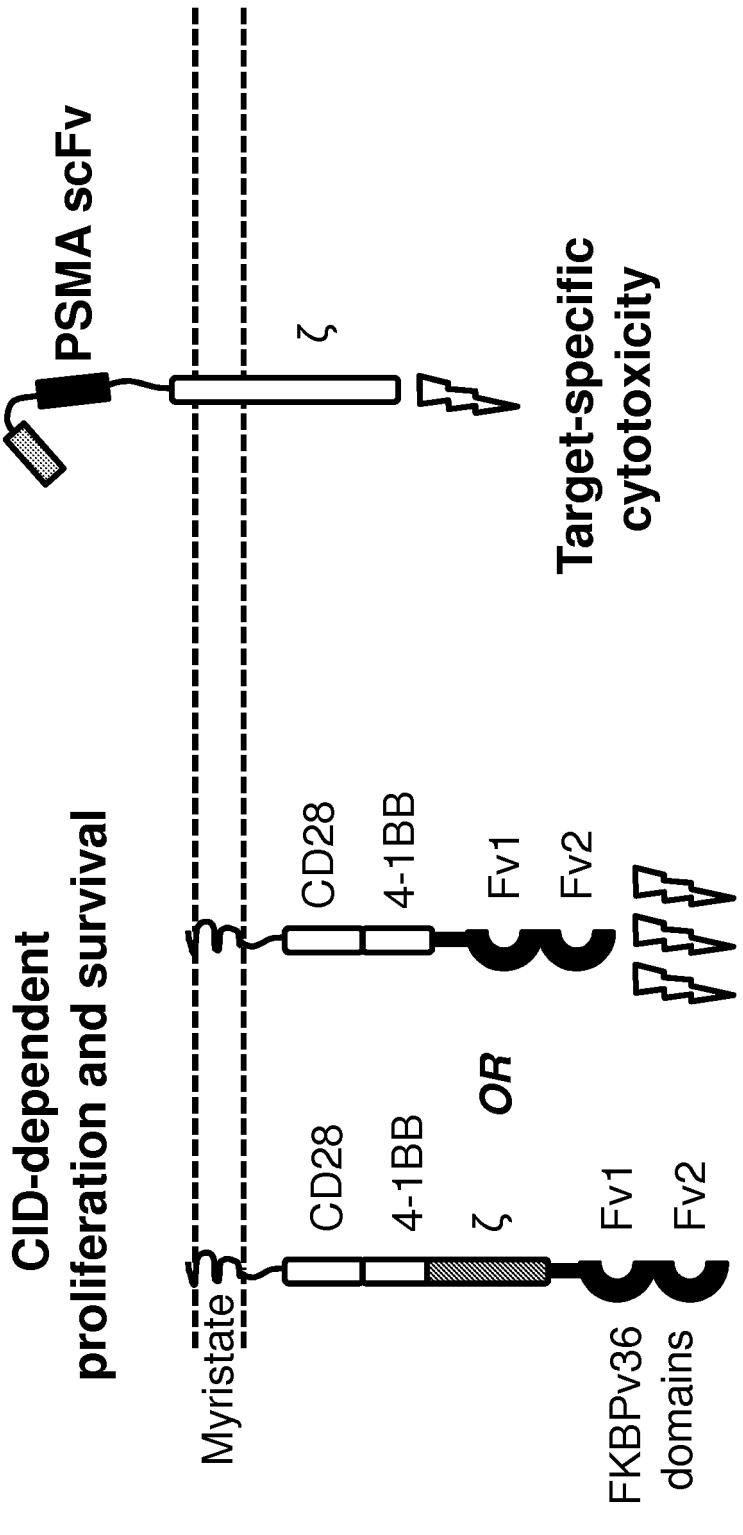

PSMA scFv
ζ
Target-specific cytotoxicity
CID-dependent proliferation and survival
CD28
4-1BB
Fv1
Fv2
Myristate
CD28
4-1BB
ζ
OR
Fv1
Fv2
FKBPv36 domains

FIGURE 4

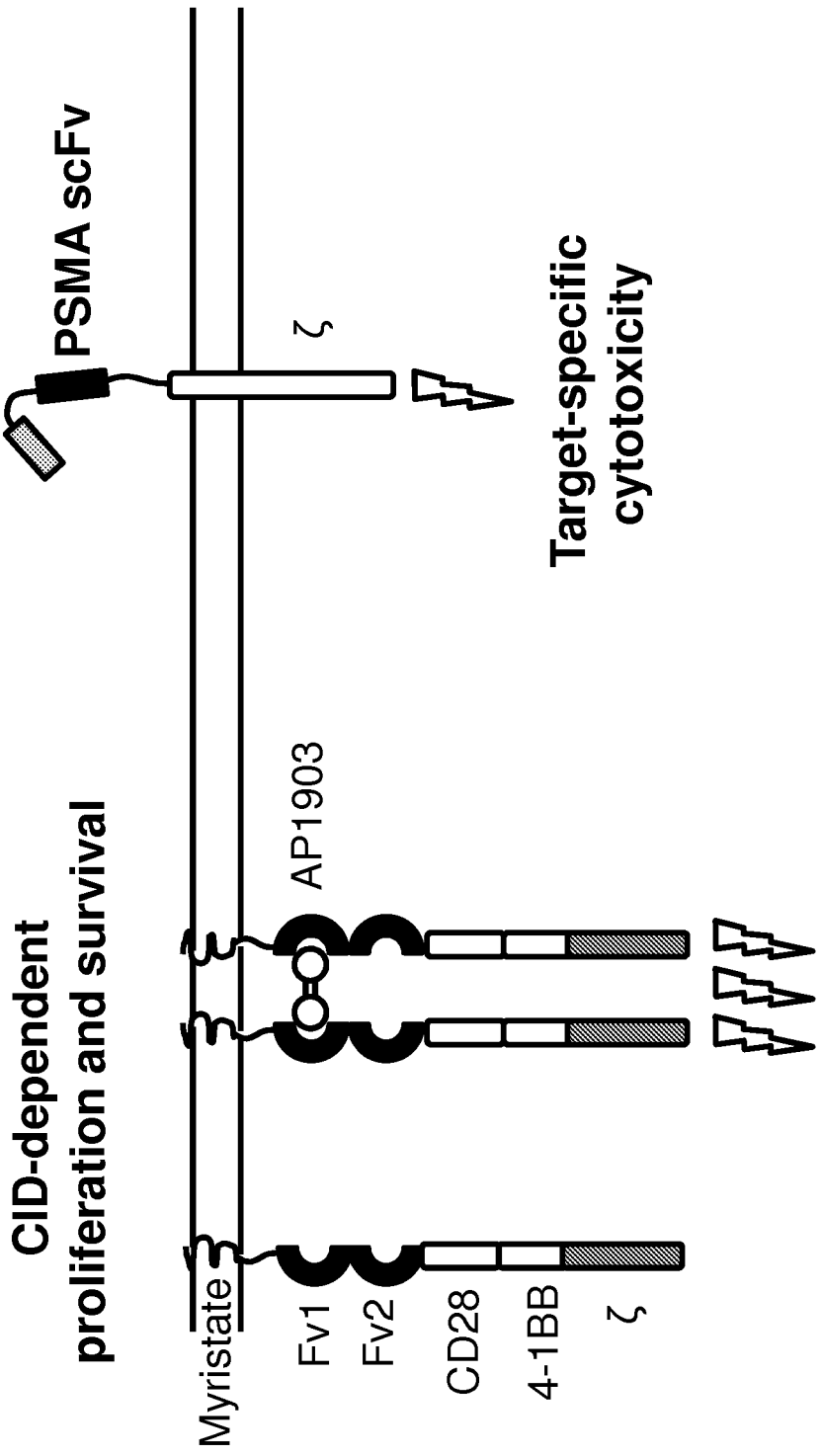

PSMA scFv
ζ
Target-specific cytotoxicity
CID-dependent proliferation and survival
AP1903
Myristate
Fv1
Fv2
CD28
4-1BB
ζ

FIGURE 5

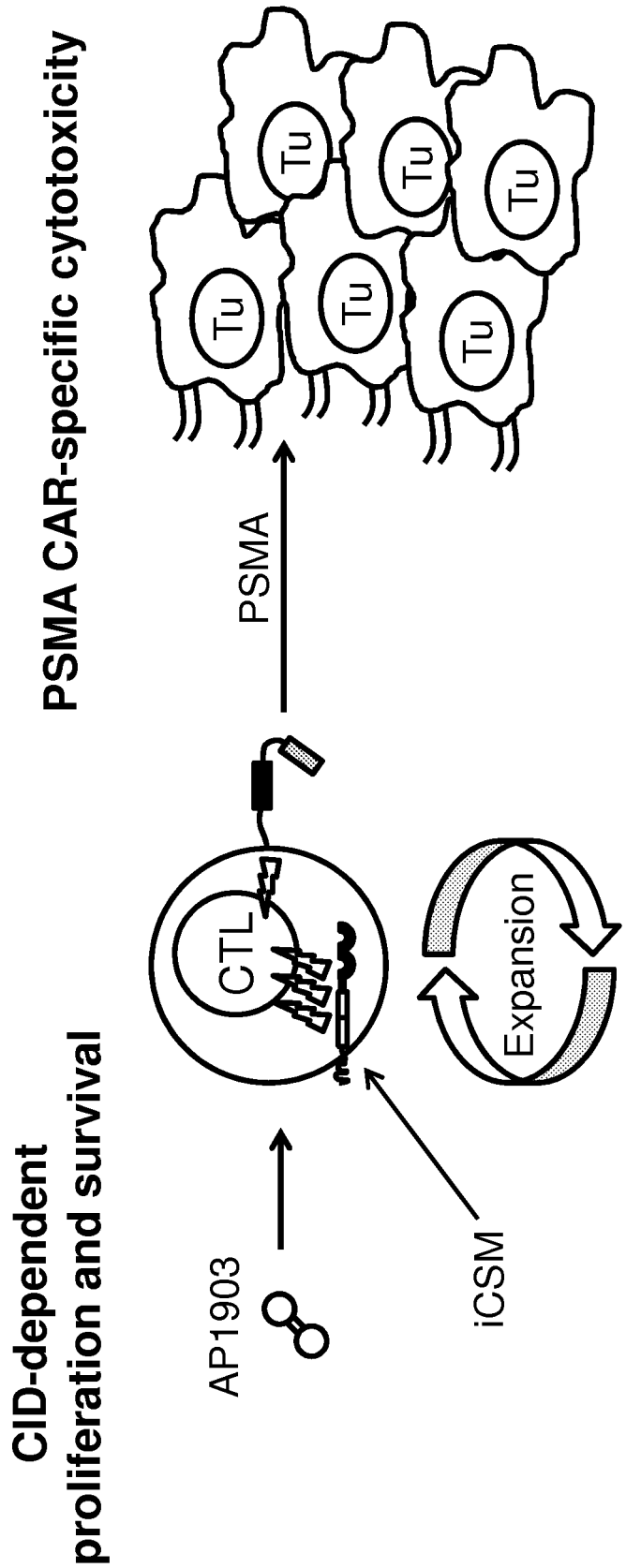

PSMA CAR-specific cytotoxicity
PSMA
Tu
Tu
Tu
Tu
Tu
Tu
CTL
Expansion
AP1903
iCSM
CID-dependent
proliferation and survival

FIGURE 6

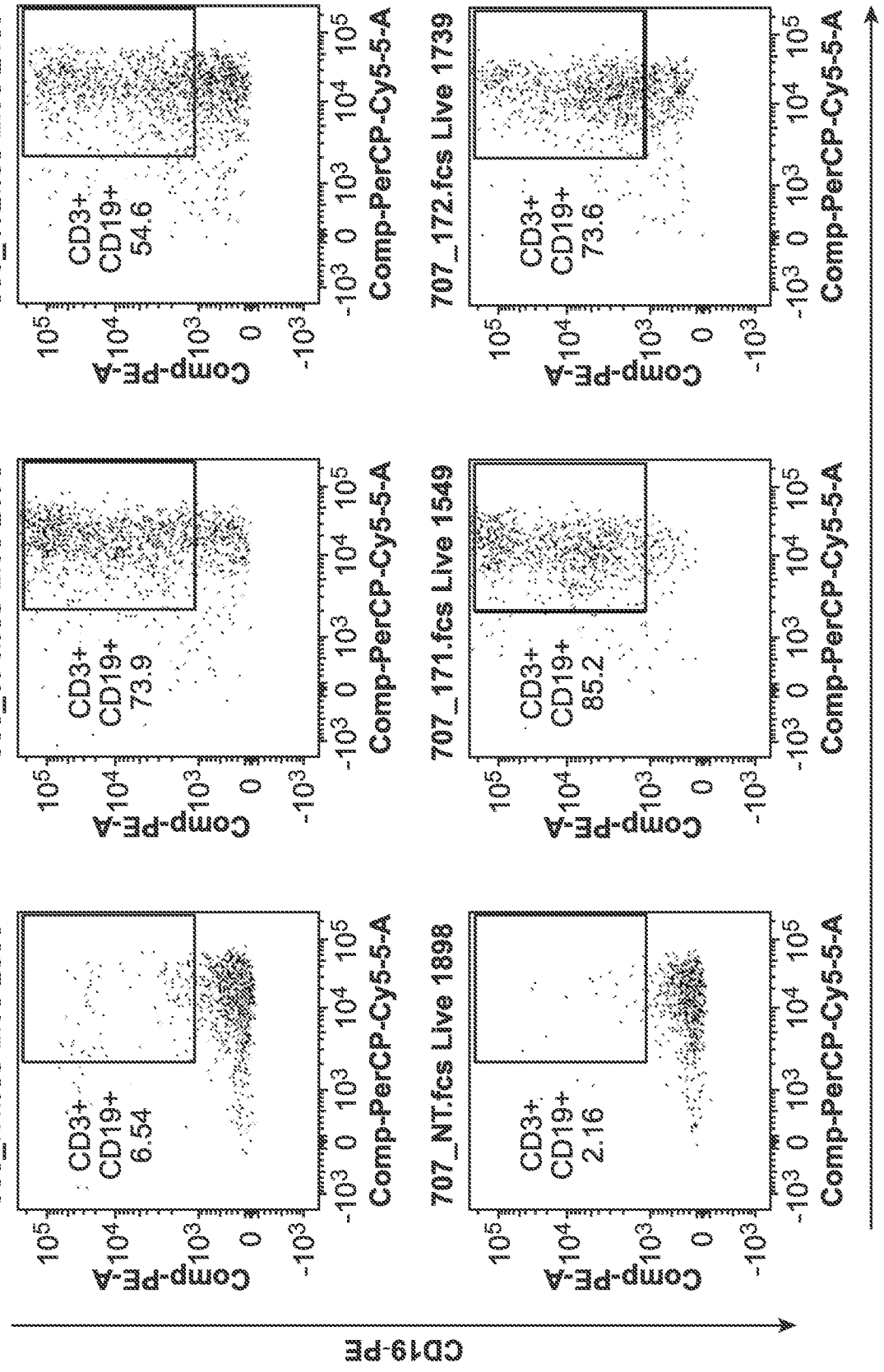

063_NT.fcs Live 2830
CD3+
CD19+
6.54
063_171.fcs Live 2178
CD3+
CD19+
73.9
063_172.fcs Live 2487
CD3+
CD19+
54.6
707_NT.fcs Live 1898
CD3+
CD19+
2.16
707_171.fcs Live 1549
CD3+
CD19+
85.2
707_172.fcs Live 1739
CD3+
CD19+
73.6
Comp-PE-A
Comp-PerCP-Cy5-5-A
CD19-PE
CD3-PerCP.Cy5.5

FIG. 7

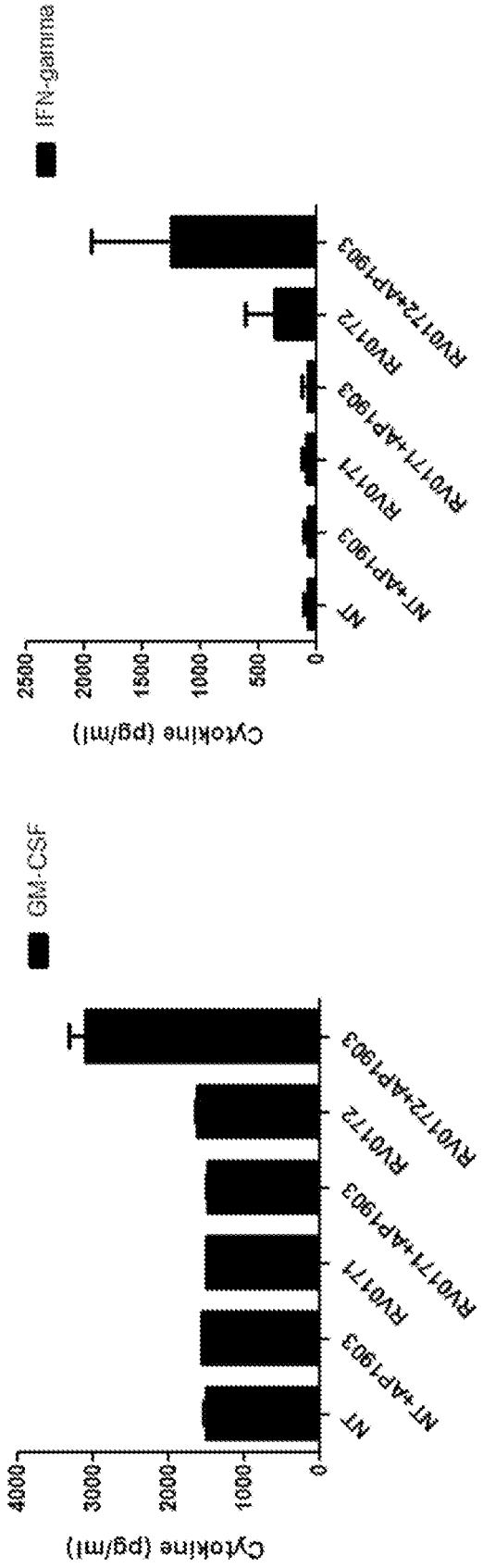

IFN-gamma
Cytokine (pg/ml)
2500
2000
1500
1000
500
0
NT
NT+AP1903
RV017.1
RV017.1+AP1903
RV017.2
RV017.2+AP1903
GM-CSF
Cytokine (pg/ml)
4000
3000
2000
1000
0
NT
NT+AP1903
RV017.1
RV017.1+AP1903
RV017.2
RV017.2+AP1903

Fig. 8

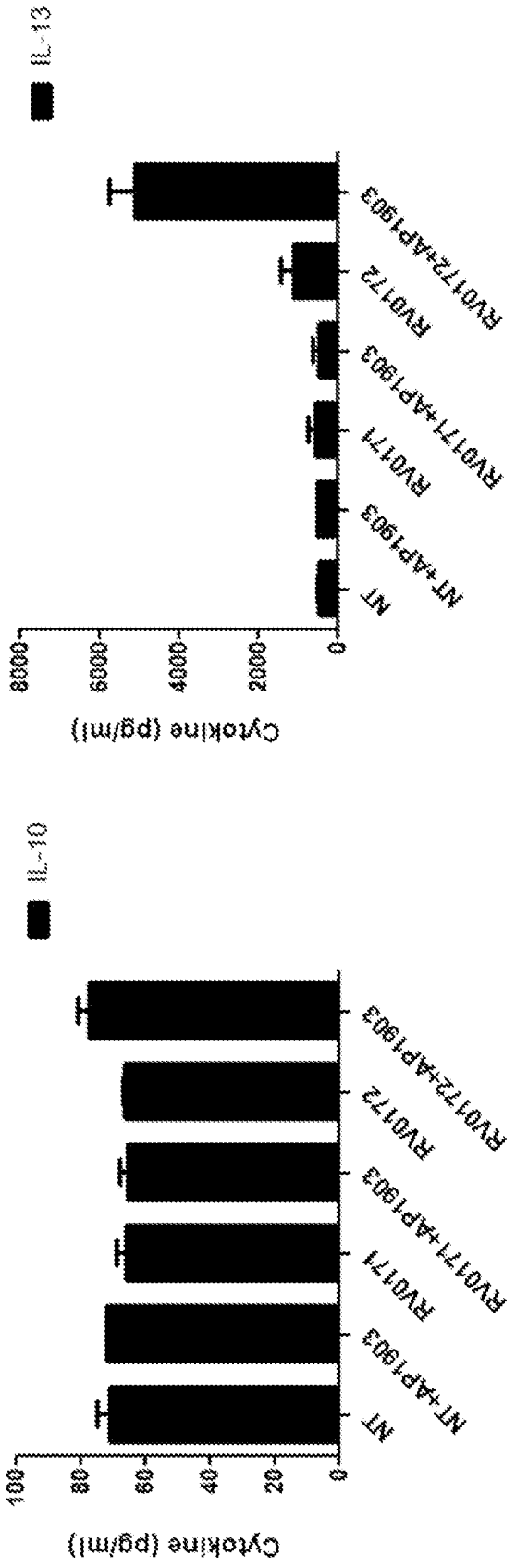

IL-10
Cytokine (pg/ml)
0
20
40
60
80
100
NT
NT+AP1903
RV017T
RV017T+AP1903
RV017Z
RV017Z+AP1903
IL-13
Cytokine (pg/ml)
0
2000
4000
6000
8000
NT
NT+AP1903
RV017T
RV017T+AP1903
RV017Z
RV017Z+AP1903

Fig. 9

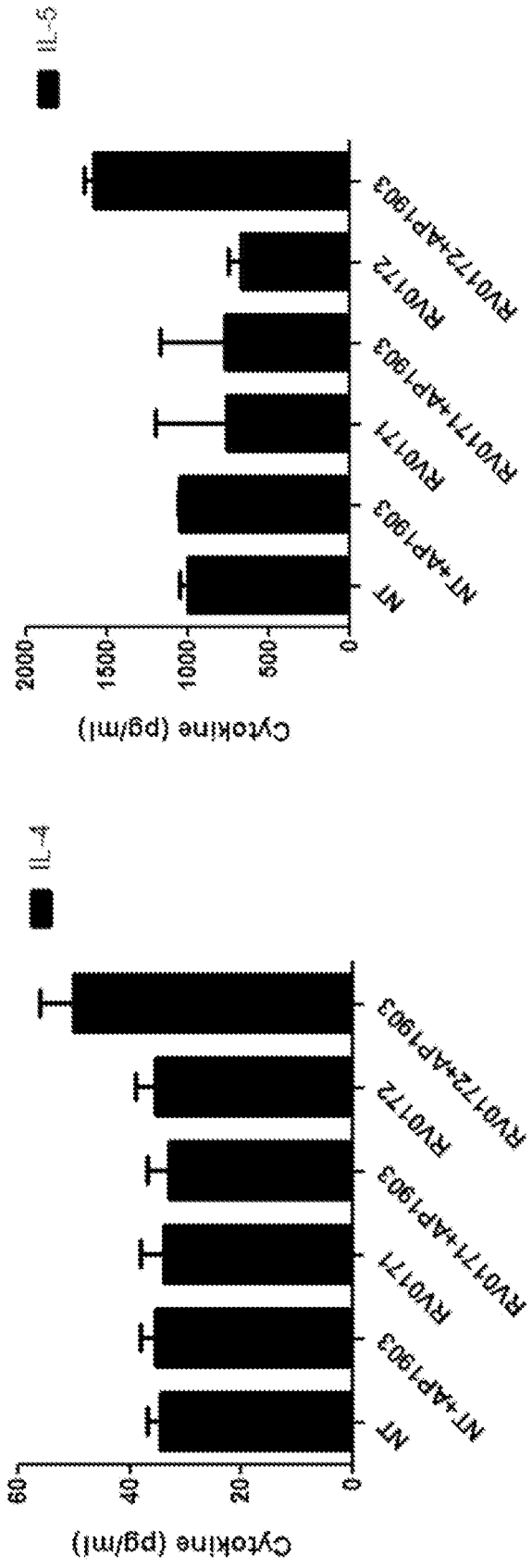
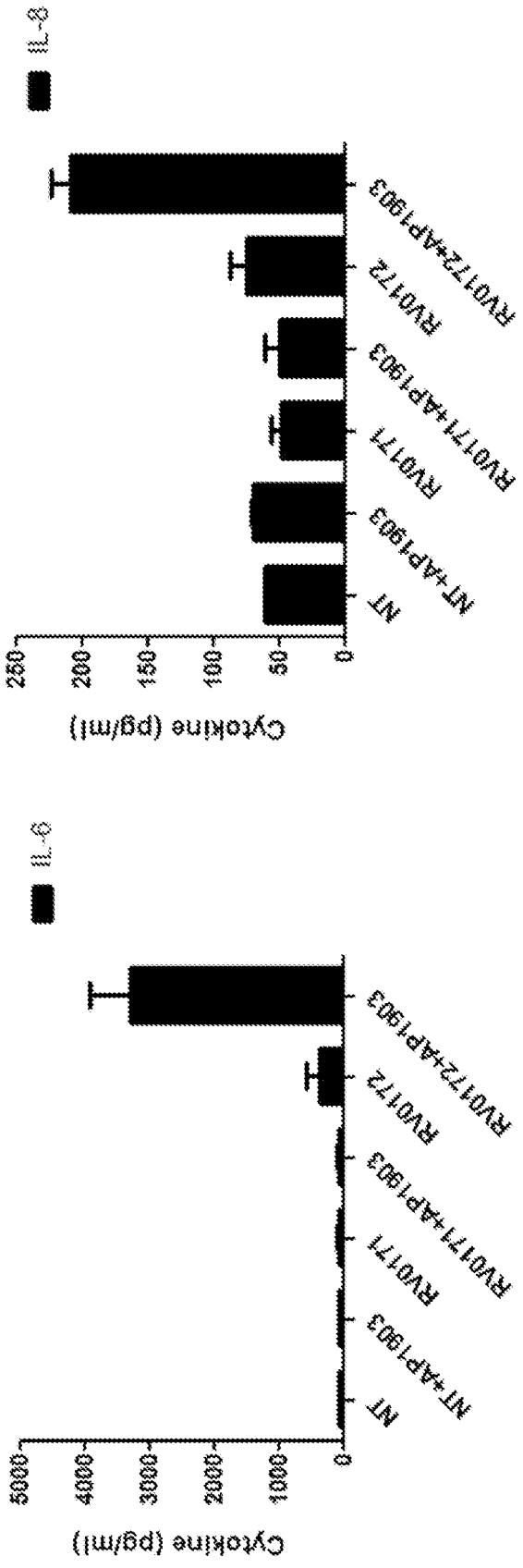
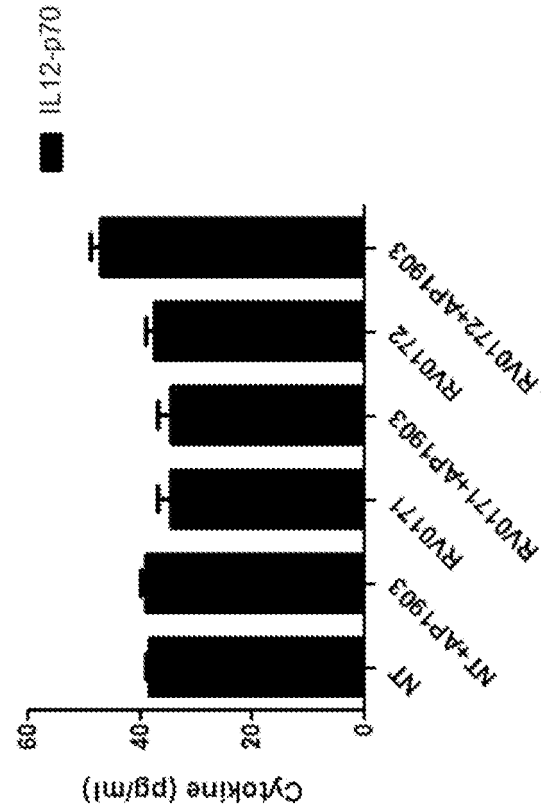

IL-5
Cytokine (pg/ml)
2000
1500
1000
500
0
NT
NT+AAP1903
RV0171
RV0171+AAP1903
RV0172
RV0172+AAP1903
IL-4
Cytokine (pg/ml)
60
40
20
0
NT
NT+AAP1903
RV0171
RV0171+AAP1903
RV0172
RV0172+AAP1903

Fig. 10

Fig. 11


IL-8
Cytokine (pg/ml)
250
200
150
100
50
0
NT
NT+AP1903
RV017T
RV017T+AP1903
RV017Z
RV017Z+AP1903
IL-6
Cytokine (pg/ml)
5000
4000
3000
2000
1000
0
NT
NT+AP1903
RV017T
RV017T+AP1903
RV017Z
RV017Z+AP1903


IL12-p70
Cytokine (pg/ml)
0
20
40
60
NT
NT+AP1903
RV017T
RV017T+AP1903
RV017T2
RV017T2+AP1903

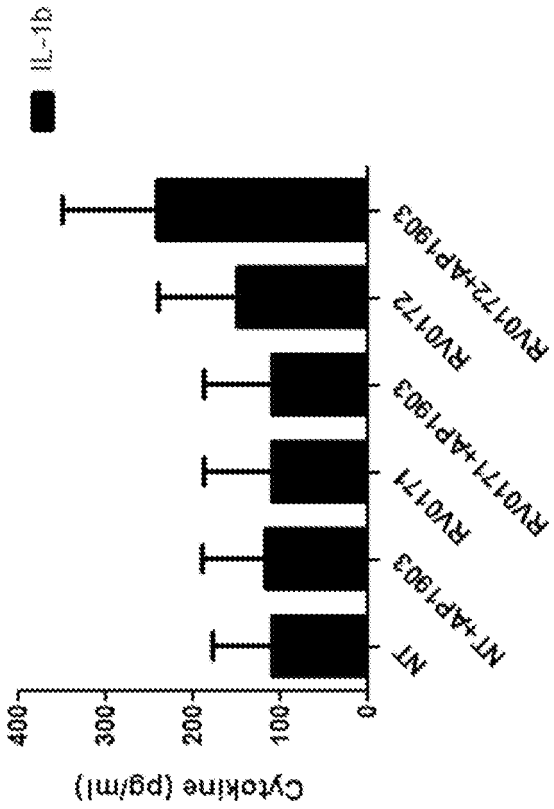

IL-1b
Cytokine (pg/ml)
0
100
200
300
400
NT
NT+AP1903
RV017T
RV017T+AP1903
RV017T2
RV017T2+AP1903

Fig. 12

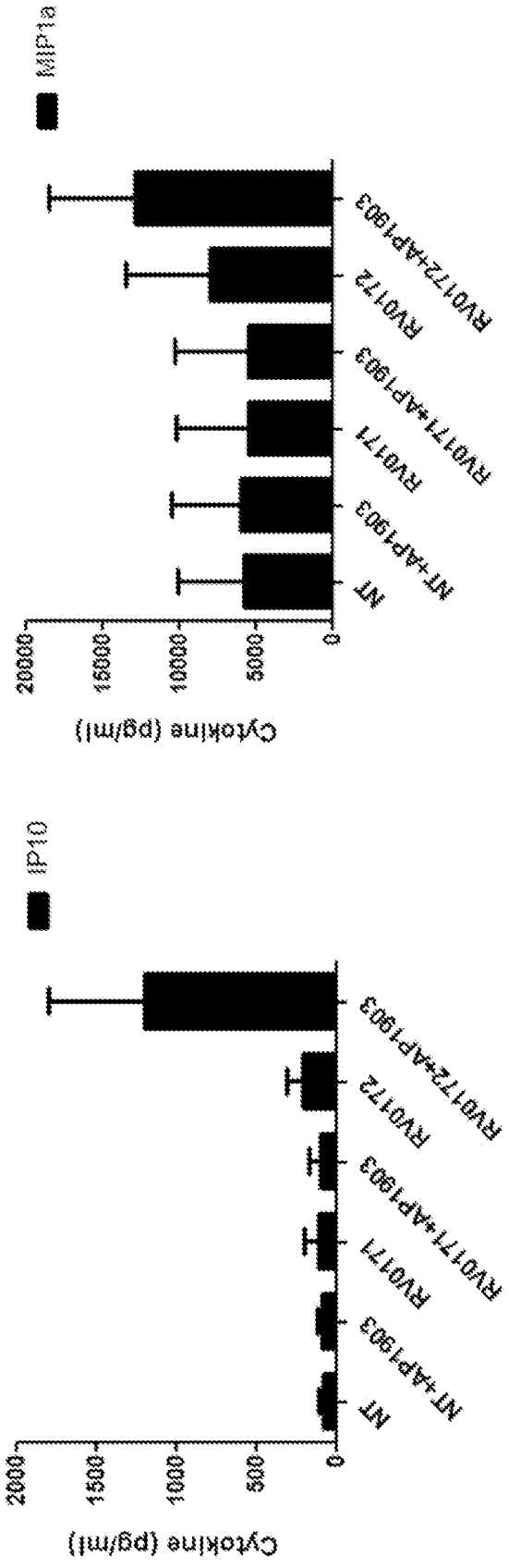

MIP1a
Cytokine (pg/ml)
20000
15000
10000
5000
0
NT
NT+AP1903
RV0171
RV0171+AP1903
RV0172
RV0172+AP1903
IP10
Cytokine (pg/ml)
2000
1500
1000
500
0
NT
NT+AP1903
RV0171
RV0171+AP1903
RV0172
RV0172+AP1903

Fig. 13

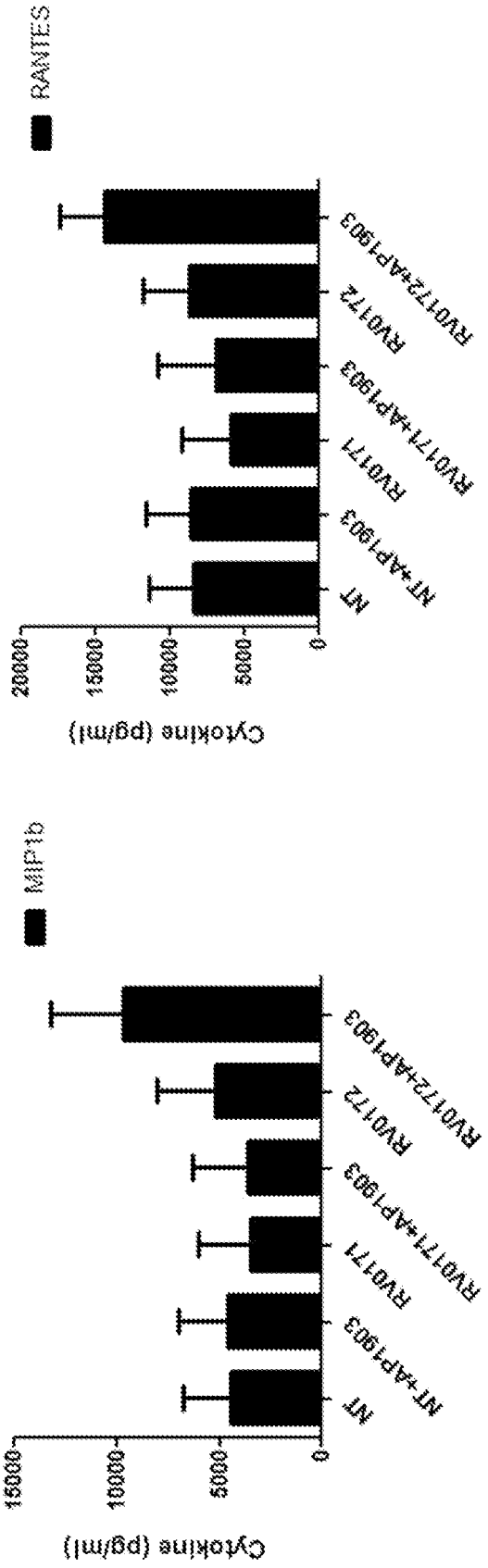
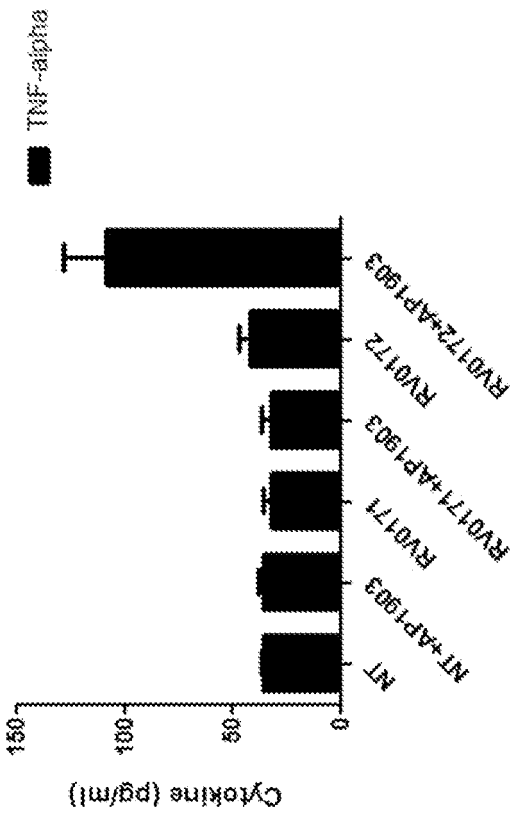

RANTES
Cytokine (pg/ml)
20000
15000
10000
5000
0
NT
NT+AP1903
RV0177
RV0177+AP1903
RV0172
RV0172+AP1903
MIP1b
Cytokine (pg/ml)
15000
10000
5000
0
NT
NT+AP1903
RV0177
RV0177+AP1903
RV0172
RV0172+AP1903

Fig. 14

Fig. 15


TNF-alpha
Cytokine (pg/ml)
150
100
50
0
NT
NT+AP1903
RV017
RV017+AP1903
RV017
RV017+AP1903

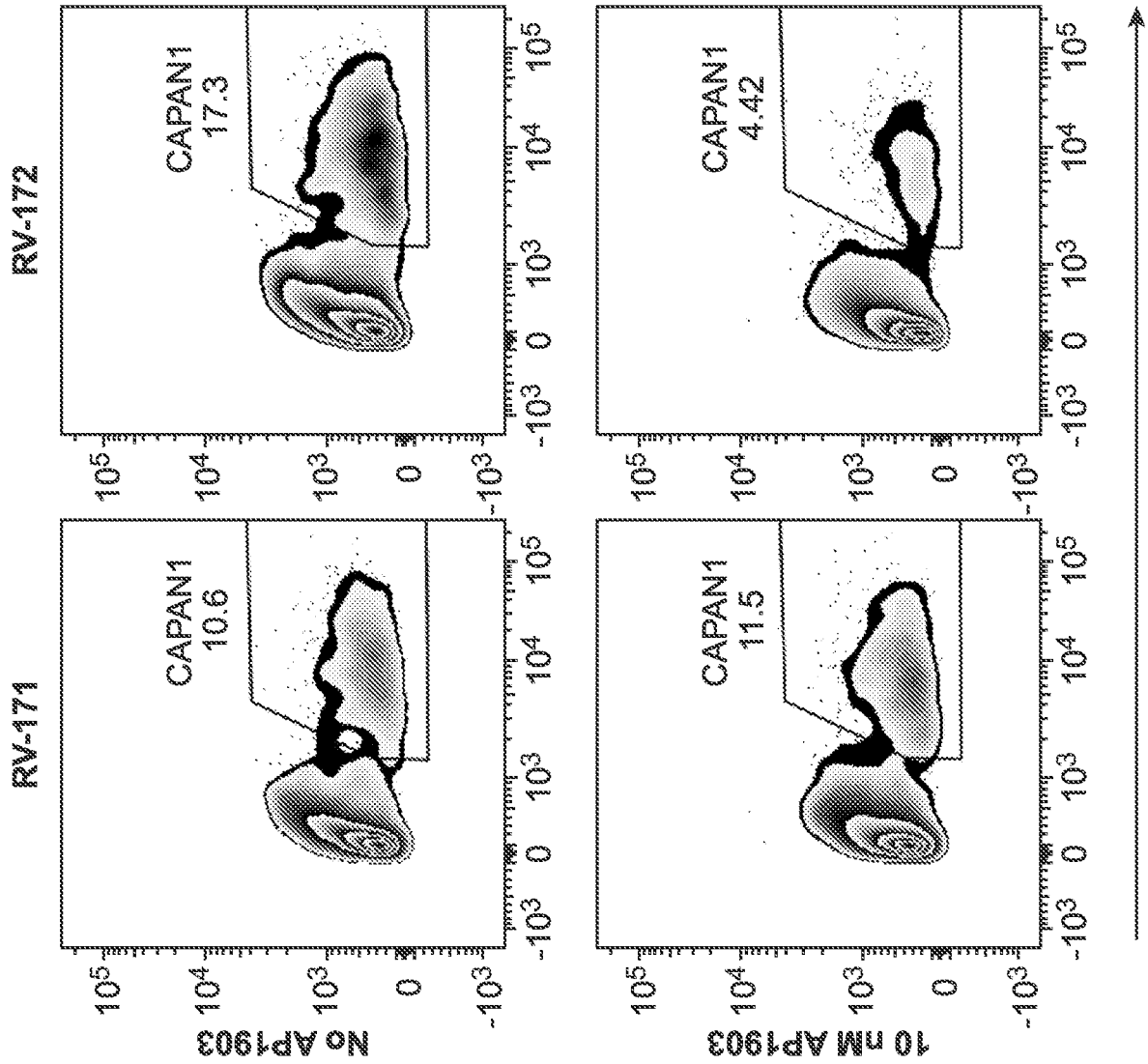

RV-171
RV-172
CAPAN1
10.6
CAPAN1
17.3
CAPAN1
11.5
CAPAN1
4.42
No AP1903
10 nM AP1903
CAPAN-1 (GFP)

FIG. 16

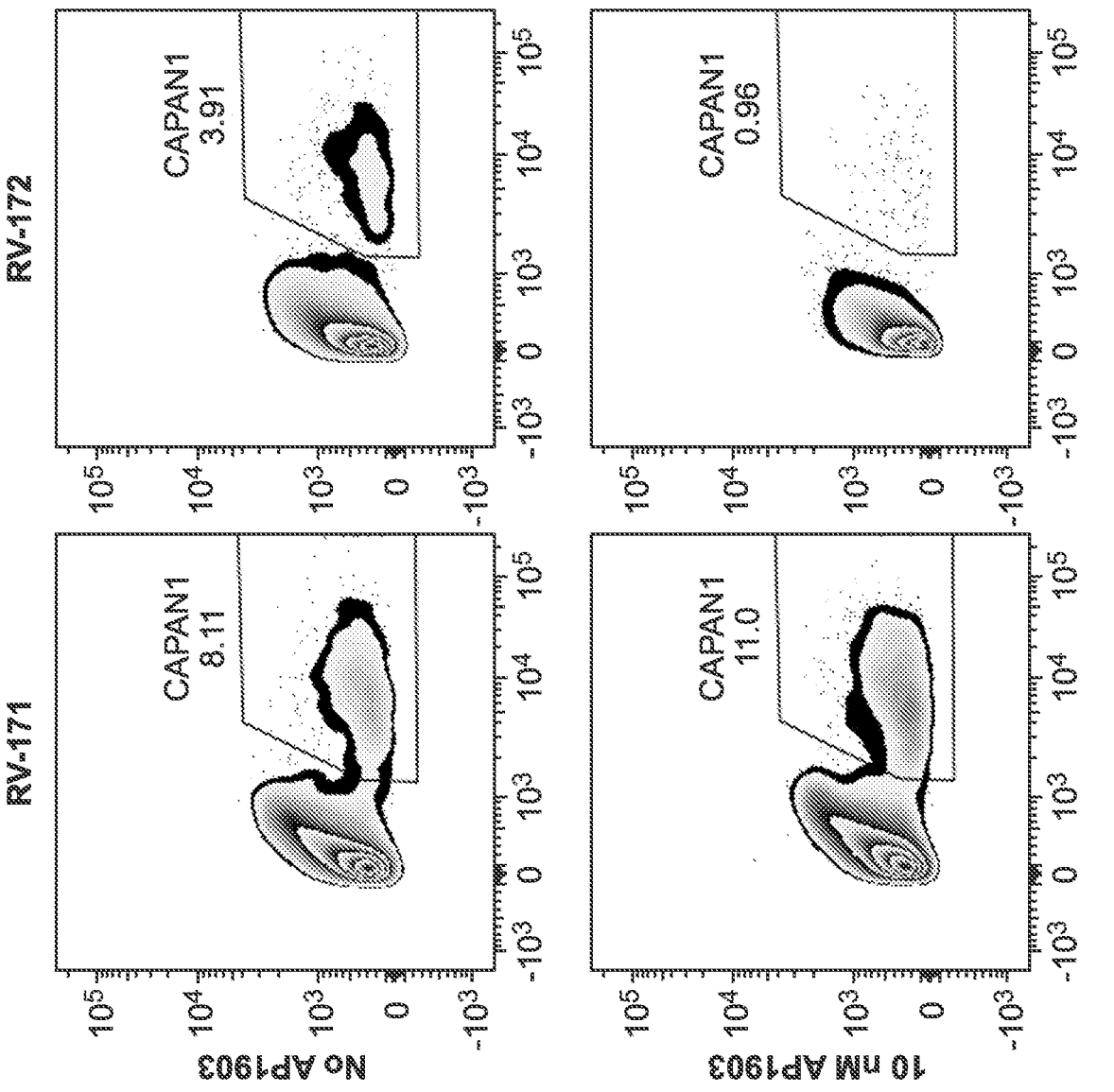

RV-171
RV-172
CAPAN1
8.11
CAPAN1
3.91
CAPAN1
11.0
CAPAN1
0.96
No AP1903
10 nM AP1903
CAPAN-1 (GFP)
10^5
10^4
10^3
0
-10^3

FIG. 17

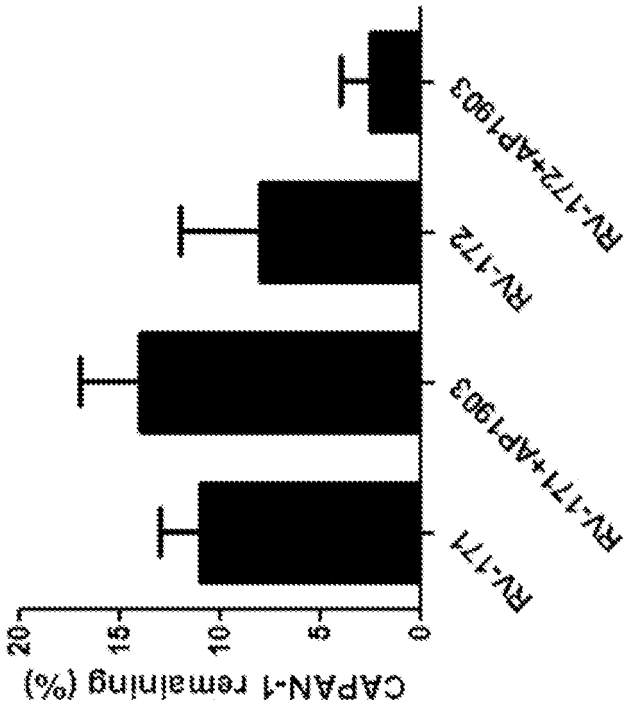

CAPAN-1 remaining (%)
0
5
10
15
20
RV-171
RV-171+AP1903
RV-172
RV-172+AP1903

Figure 18

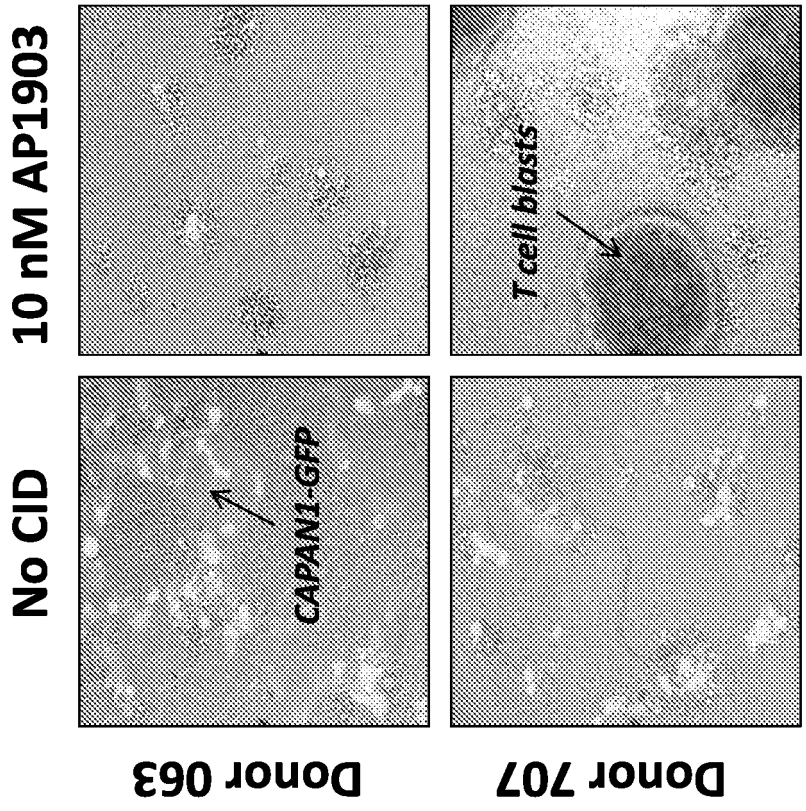

No CID
10 nM AP1903
CAPAN1-GFP
T cell blasts
Donor 063
Donor 707

Figure 19

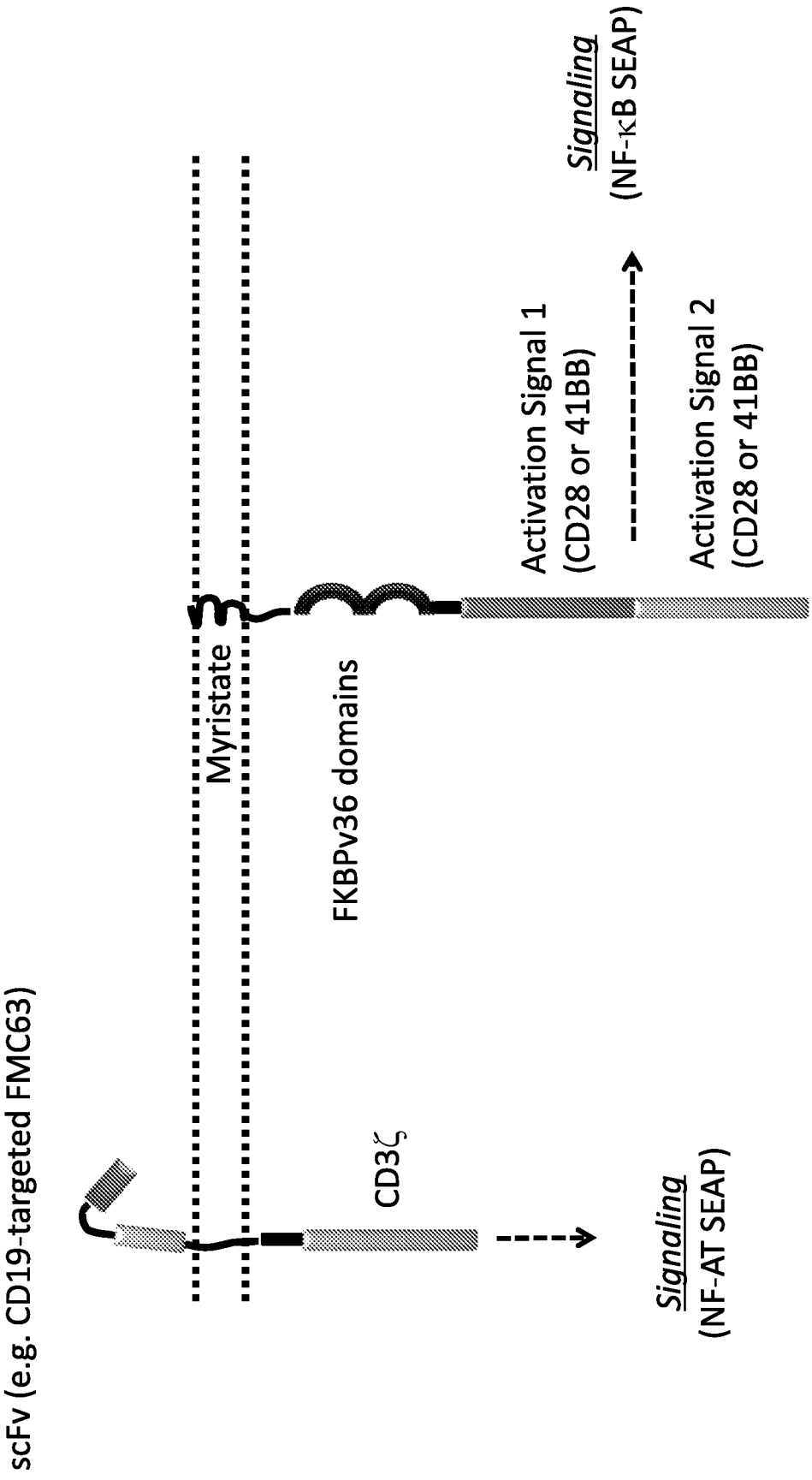

scFv (e.g. CD19-targeted FMC63)
Myristate
FKBPv36 domains
CD3ζ
Activation Signal 1
(CD28 or 41BB)
Activation Signal 2
(CD28 or 41BB)
Signaling
(NF-κB SEAP)
Signaling
(NF-AT SEAP)

Fig. 20

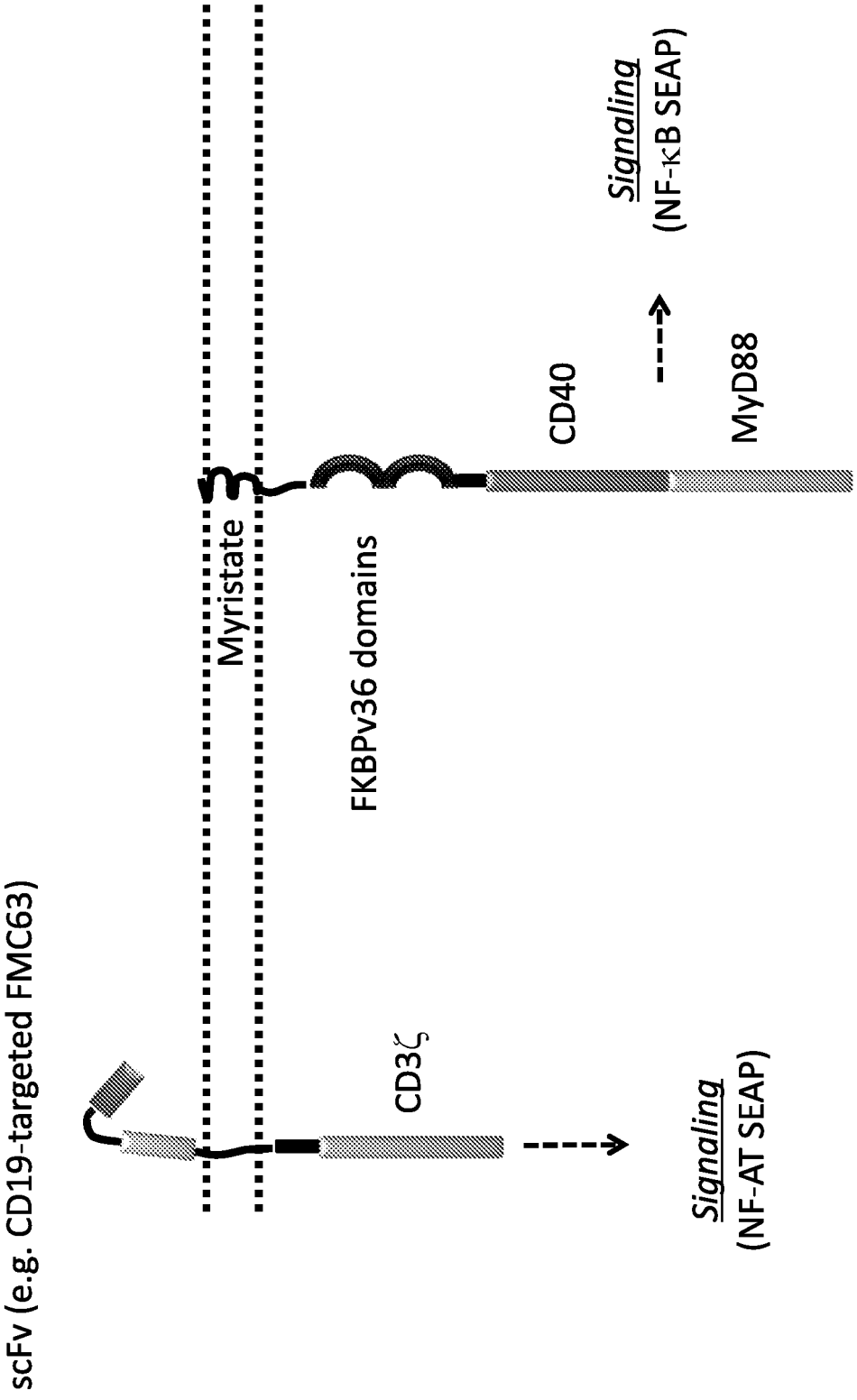

scFv (e.g. CD19-targeted FMC63)
Myristate
FKBPv36 domains
CD3ζ
CD40
MyD88
Signaling
(NF-κB SEAP)
Signaling
(NF-AT SEAP)

Fig. 21

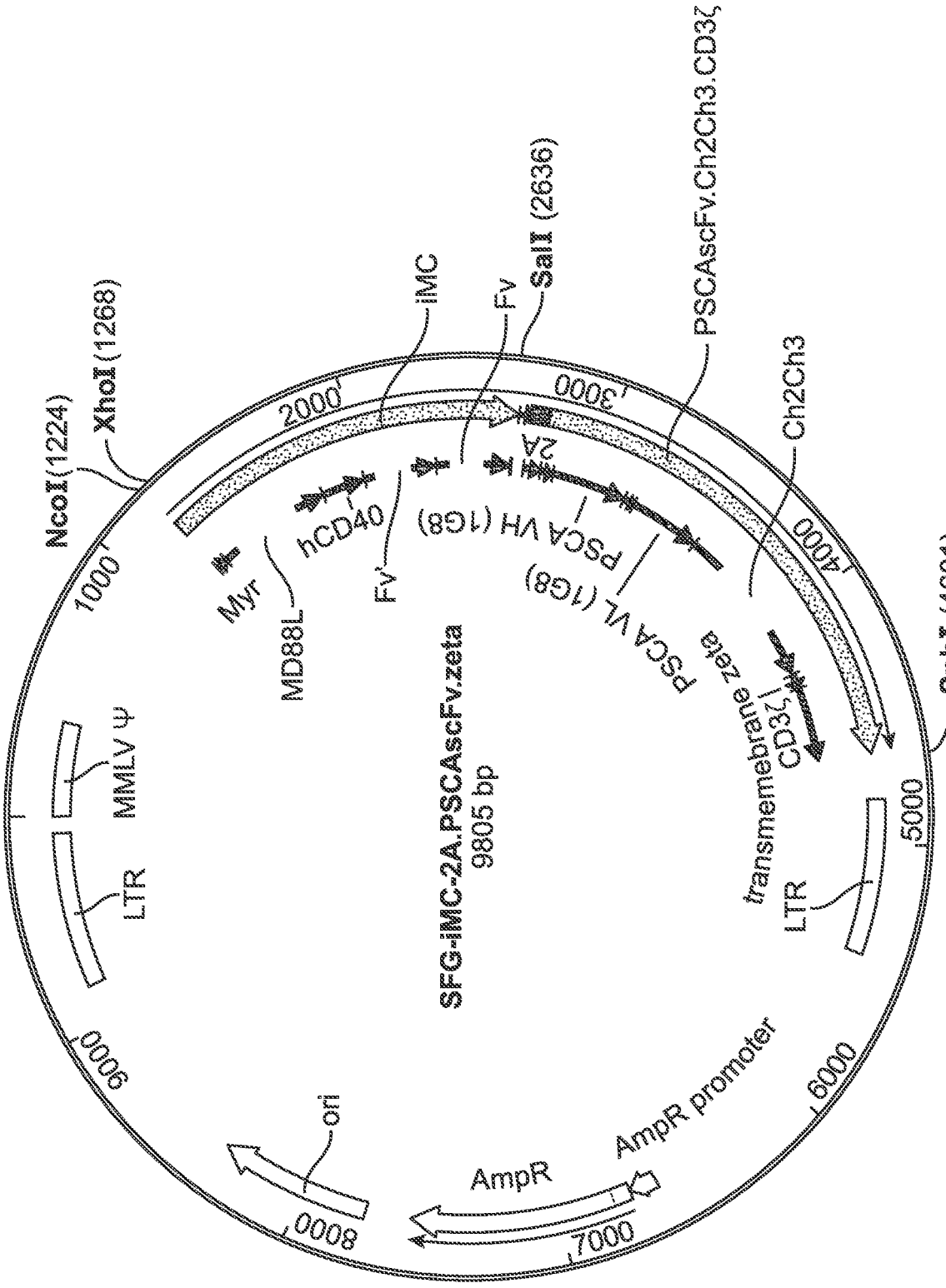

NcoI (1224)
XhoI (1268)
1000
2000
iMC
Fv
SalI (2636)
3000
PSCAscFv.Ch2Ch3.CD3ζ
Ch2Ch3
4000
SphI (4681)
5000
6000
7000
8000
9000
MMLV ψ
LTR
Myr
MD88L
hCD40
Fv'
2A
PSCA VH (1G8)
PSCA VL (1G8)
zeta
transmemebrane
CD3ζ
LTR
AmpR promoter
AmpR
ori
SFG-iMC-2A.PSCAscFv.zeta
9805 bp

FIG. 22