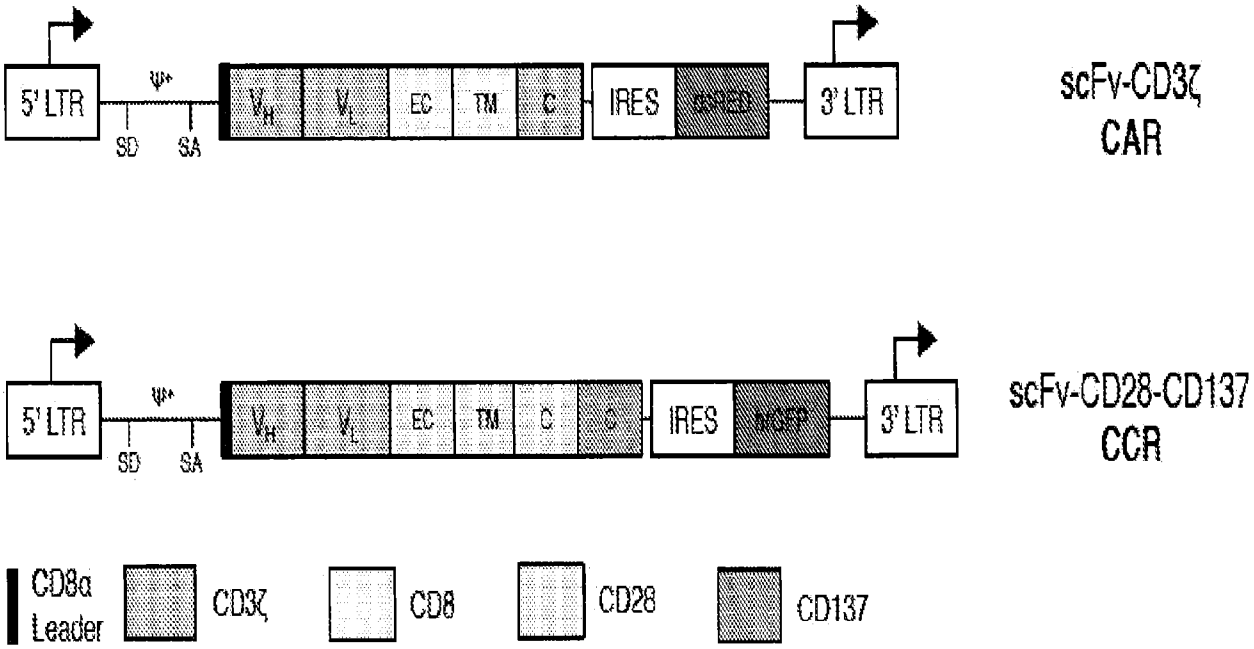

5' LTR
Ψ+
SD
SA
VH
VL
EC
TM
C
IRES
3' LTR
scFv-CD3ζ
CAR
5' LTR
Ψ+
SD
SA
VH
VL
EC
TM
C
C
IRES
3' LTR
scFv-CD28-CD137
CCR
CD8α
Leader
CD3ζ
CD8
CD28
CD137

Figure 1A

T Cell Groups

1. Mock
2. 19z1
3. P28BB
4. 19z1 + P28BB

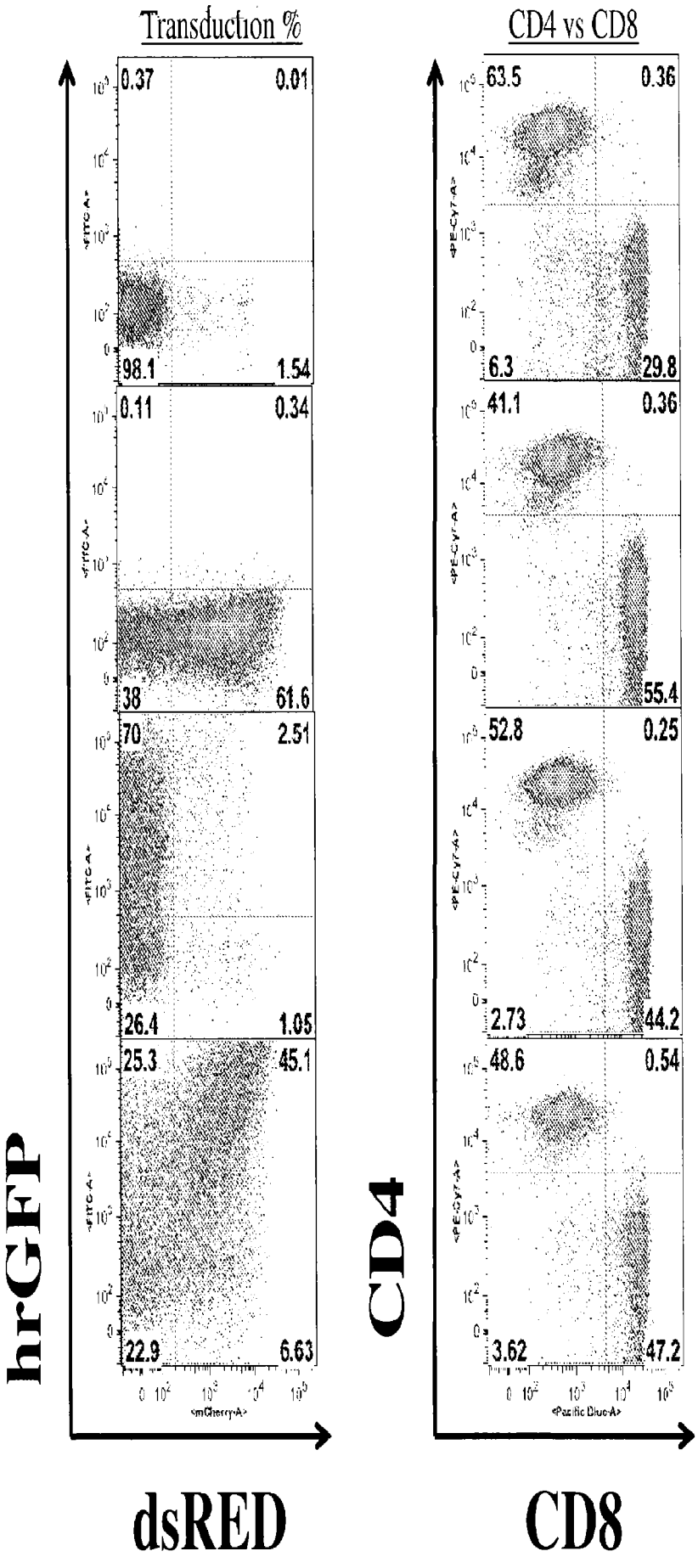


Figure 1B

Transduction of CTLs with Different and Multiple CARs

T Cell Groups	SFG Retroviral Transduction
1. Mock	N/A
2. 19z1	α-CD19 – CD8-CD3zeta | IRES | dsRED
3. P28BB	α-PSMA – CD28 – 4-1BB | IRES | hrGFP
4. 19z1 + P28BB	α-CD19 – CD8-CD3zeta | IRES | dsRED + α-PSMA – CD28 – 4-1BB | IRES | hrGFP

Figure 1C

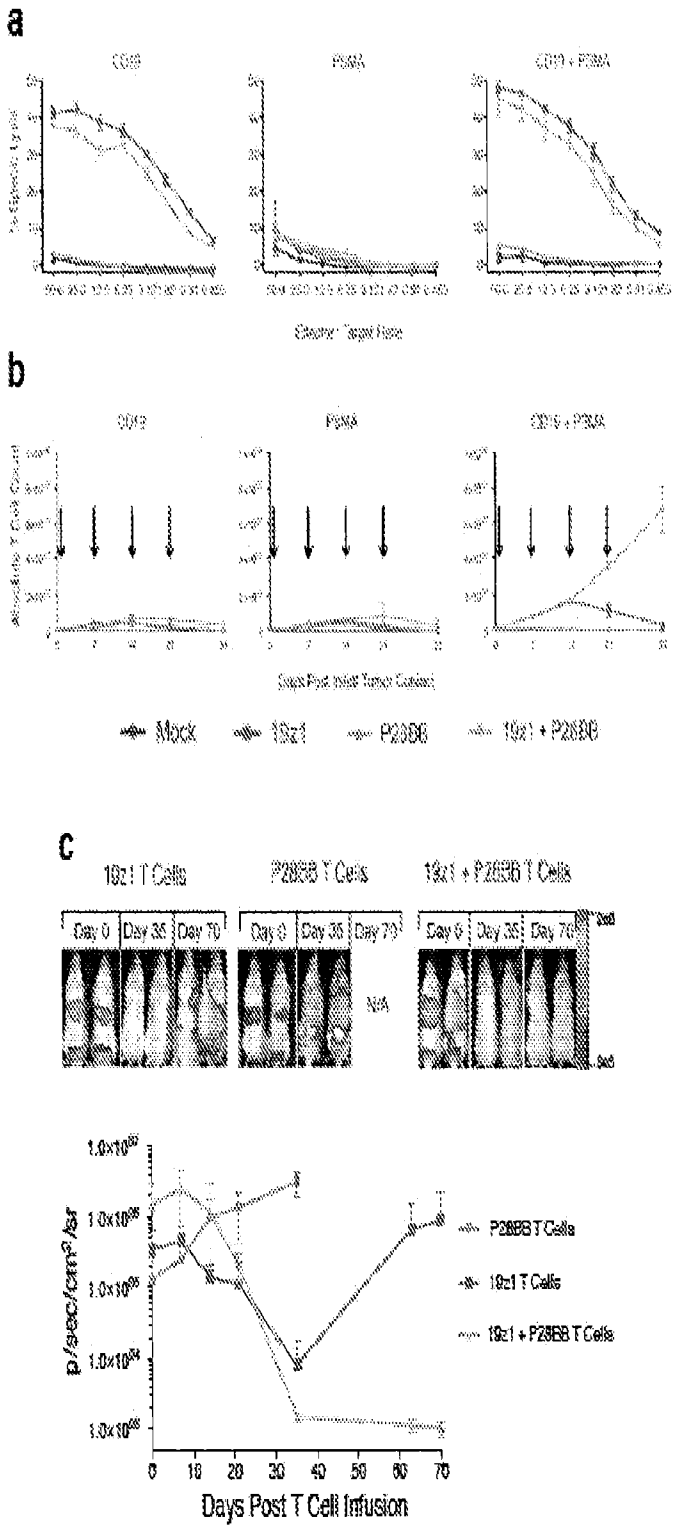

a
b
Mock
19z1
P28BB
19z1 + P28BB
c
19z1 T Cells
P28BB T Cells
19z1 + P28BB T Cells
Day 0
Day 35
Day 70
N/A
P28BB T Cells
19z1 T Cells
19z1 + P28BB T Cells
Days Post T Cell Infusion

Figure 2

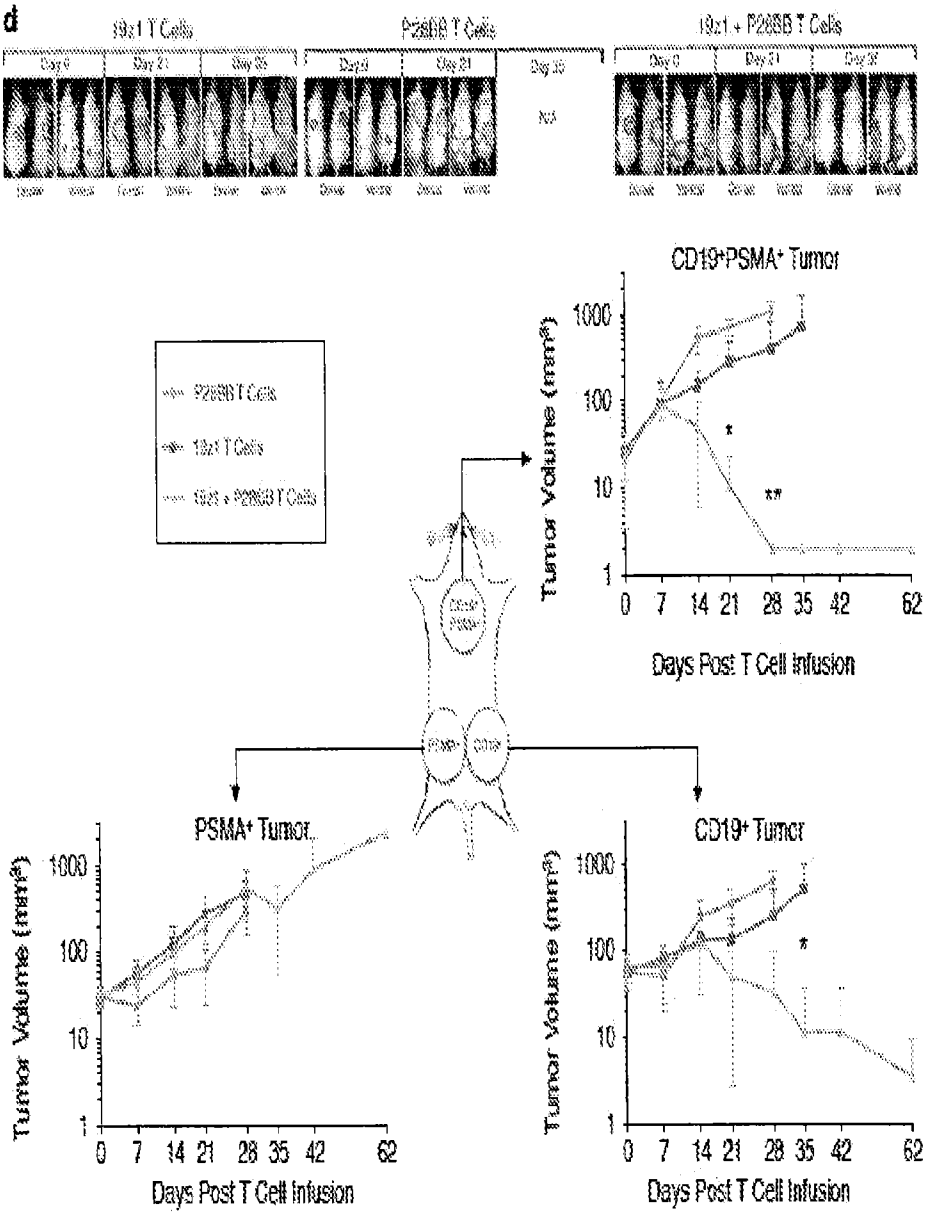


Figure 2 continued

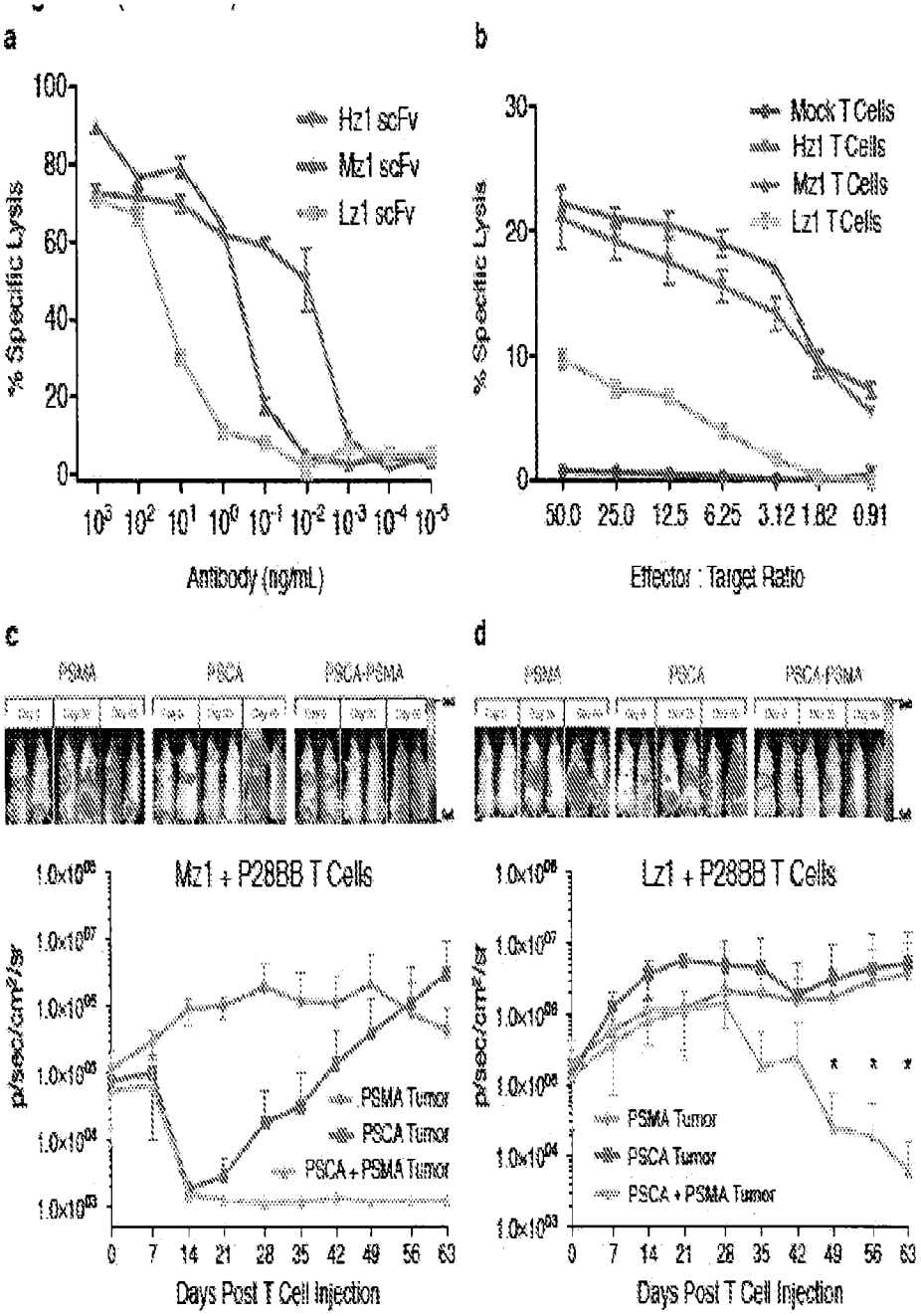

a
% Specific Lysis
Hz1 scFv
Mz1 scFv
Lz1 scFv
Antibody (ng/mL)
b
% Specific Lysis
Mock T Cells
Hz1 T Cells
Mz1 T Cells
Lz1 T Cells
Effector : Target Ratio
c
PSMA
PSCA
PSCA-PSMA
Mz1 + P28BB T Cells
p/sec/cm²/sr
PSMA Tumor
PSCA Tumor
PSCA + PSMA Tumor
Days Post T Cell Injection
d
PSMA
PSCA
PSCA-PSMA
Lz1 + P28BB T Cells
p/sec/cm²/sr
PSMA Tumor
PSCA Tumor
PSCA + PSMA Tumor
Days Post T Cell Injection

Figure 3

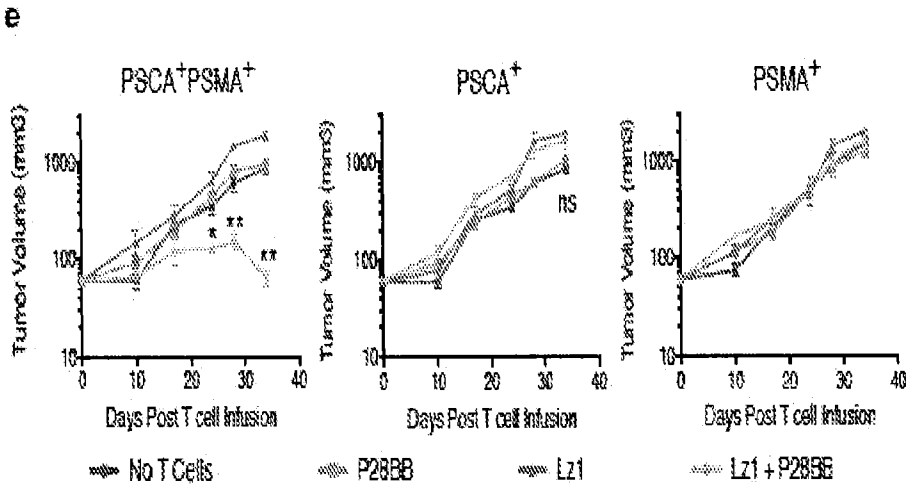


Figure 3 continued

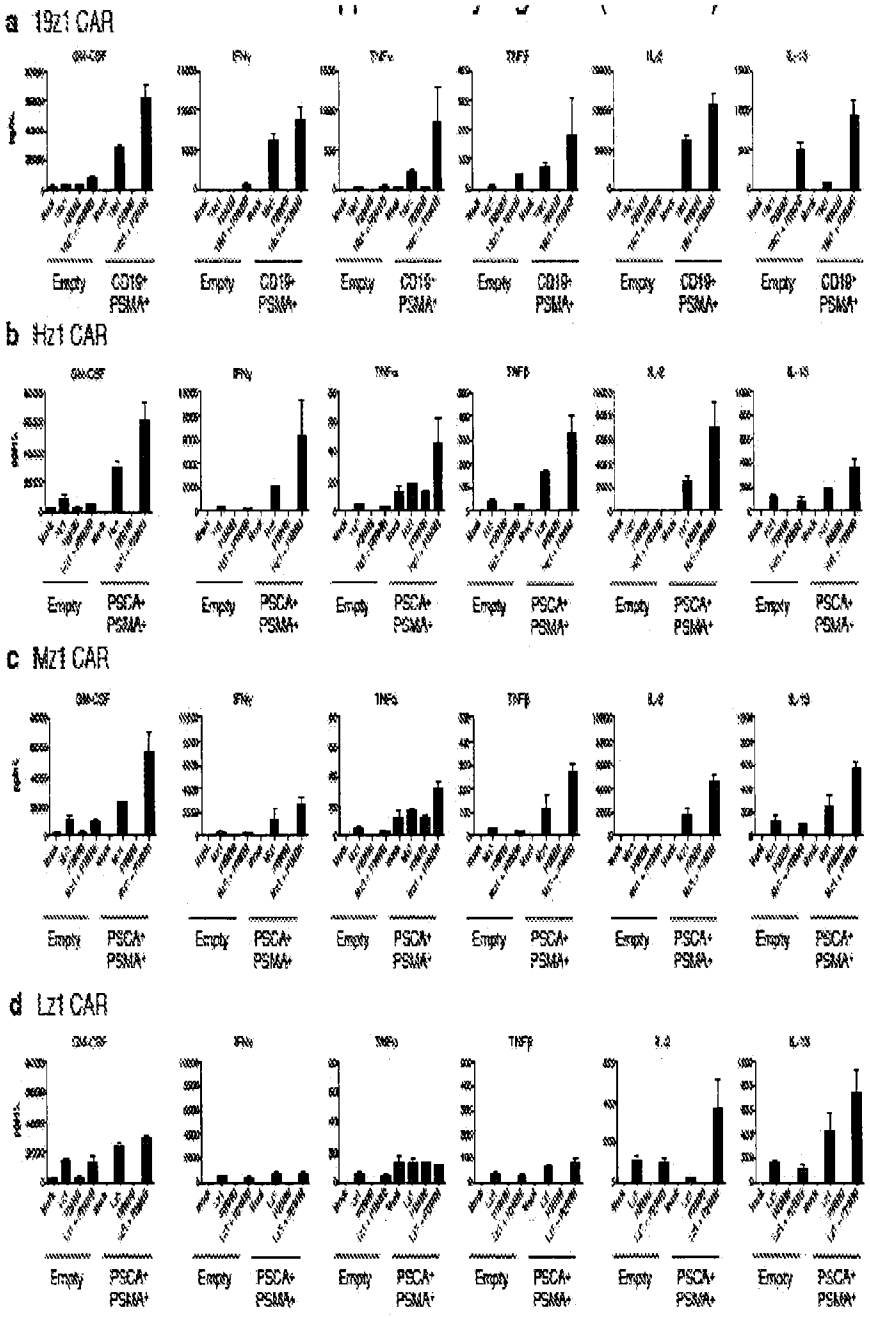

a 19z1 CAR
b Hz1 CAR
c Mz1 CAR
d Lz1 CAR
Empty
CD19+ PSMA+
PSCA+ PSMA+

Figure 4

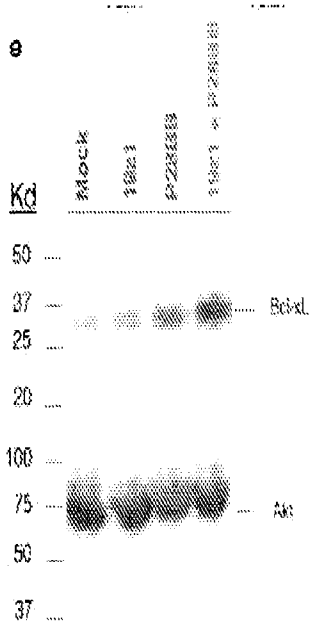


Figure 4 continued

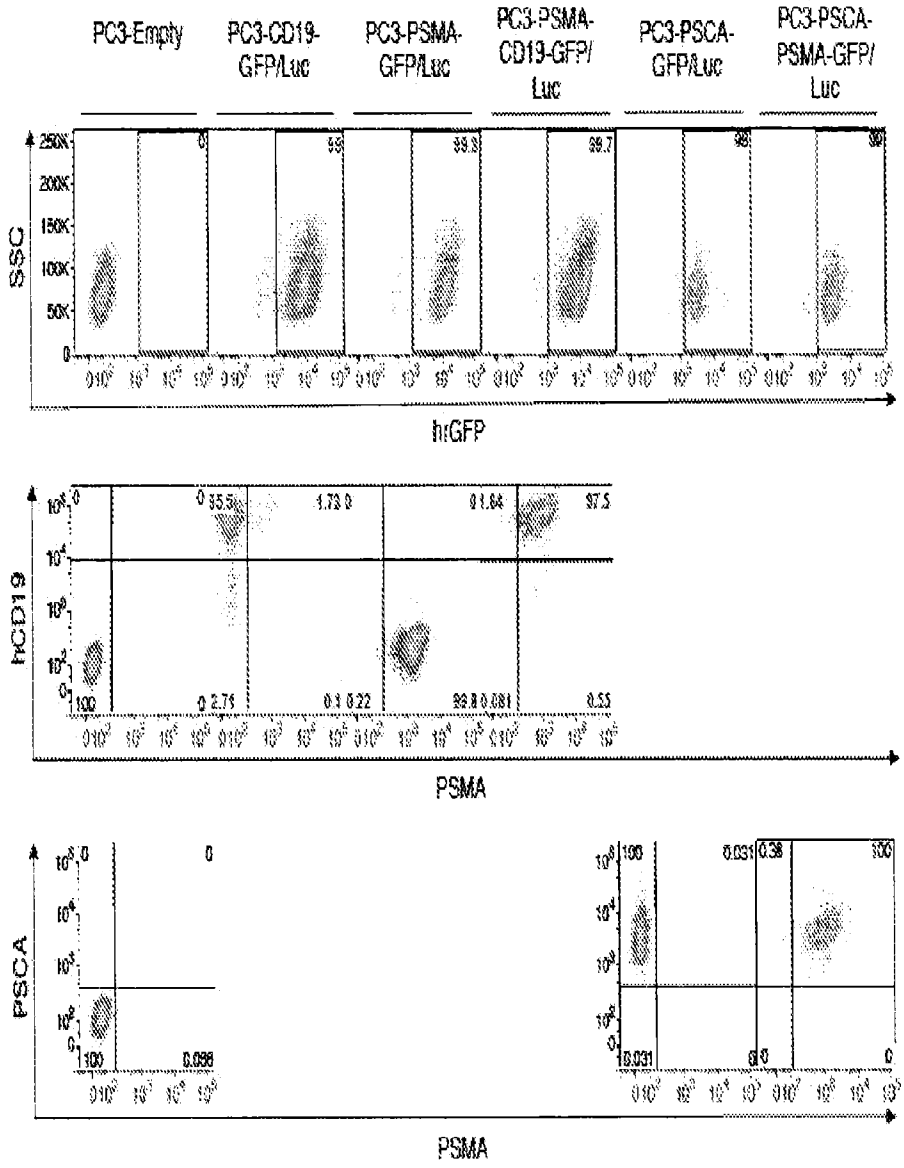

PC3-Empty
PC3-CD19-GFP/Luc
PC3-PSMA-GFP/Luc
PC3-PSMA-CD19-GFP/Luc
PC3-PSCA-GFP/Luc
PC3-PSCA-PSMA-GFP/Luc
SSC
hrGFP
hCD19
PSMA
PSCA
PSMA

Figure 5

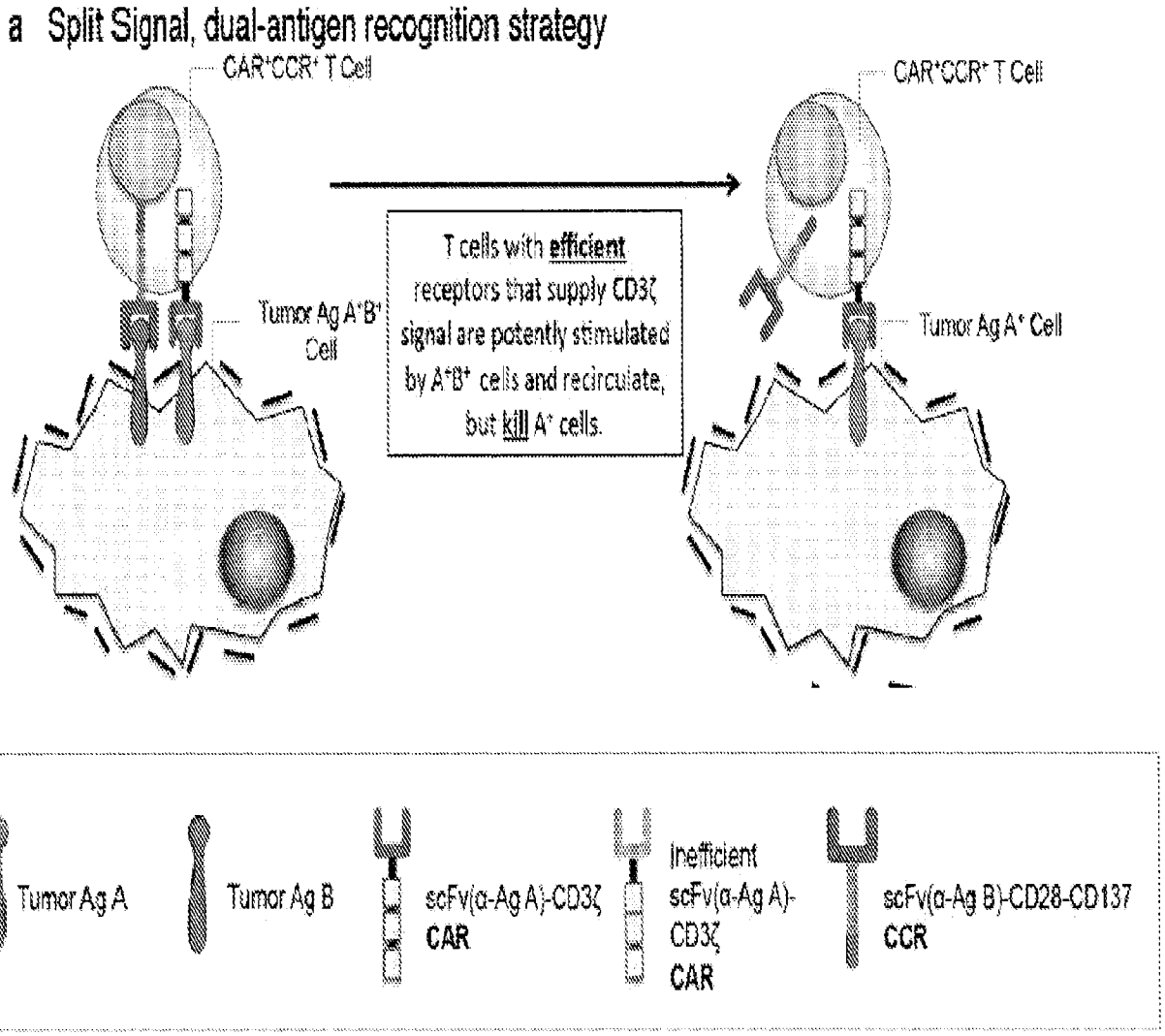

a Split Signal, dual-antigen recognition strategy
CAR+CCR+ T Cell
Tumor Ag A+B+ Cell
T cells with efficient receptors that supply CD3ζ signal are potently stimulated by A+B+ cells and recirculate, but kill A+ cells.
CAR+CCR+ T Cell
Tumor Ag A+ Cell
Tumor Ag A
Tumor Ag B
scFv(α-Ag A)-CD3ζ CAR
inefficient scFv(α-Ag A)-CD3ζ CAR
scFv(α-Ag B)-CD28-CD137 CCR

Figure 6A

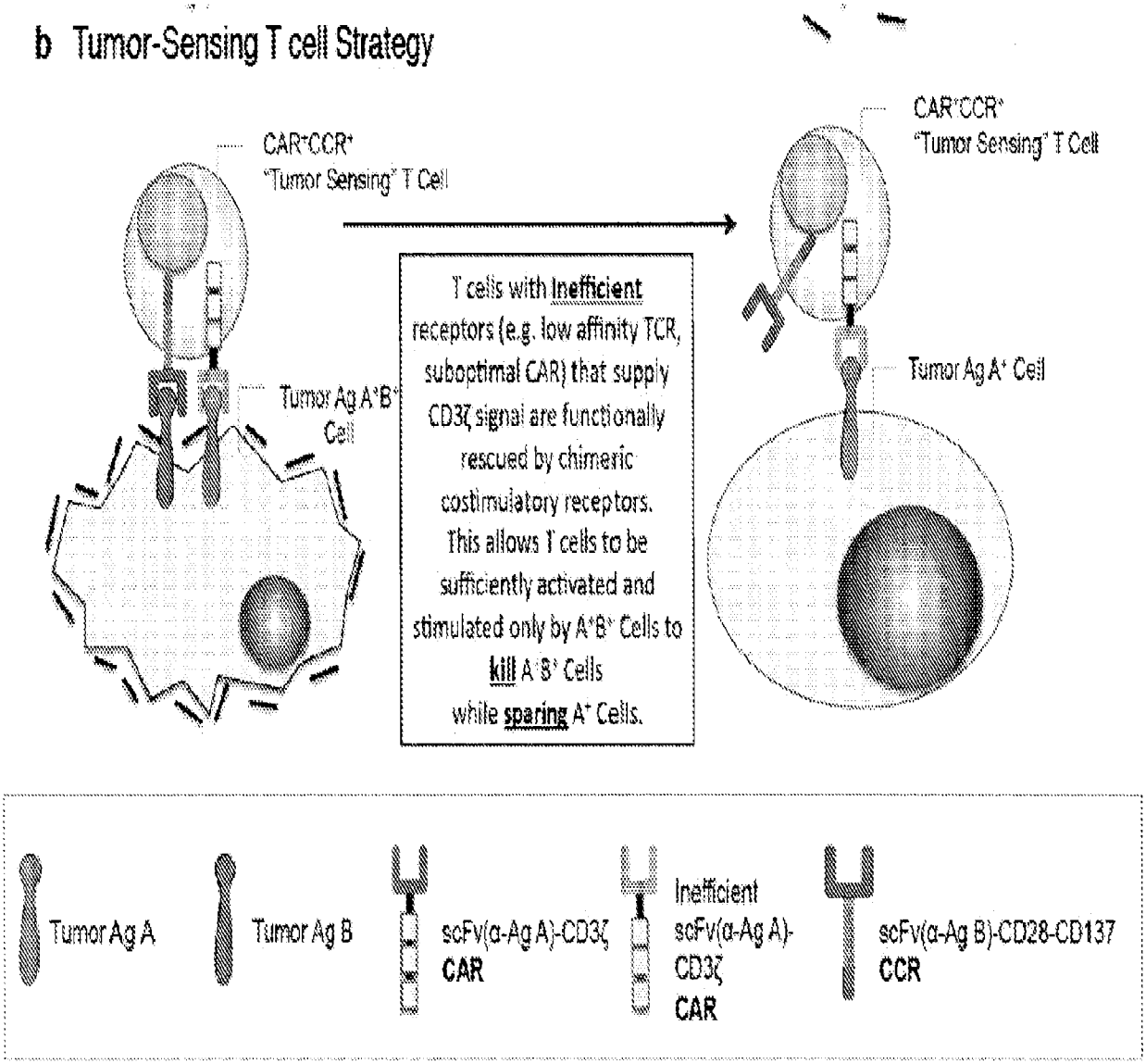
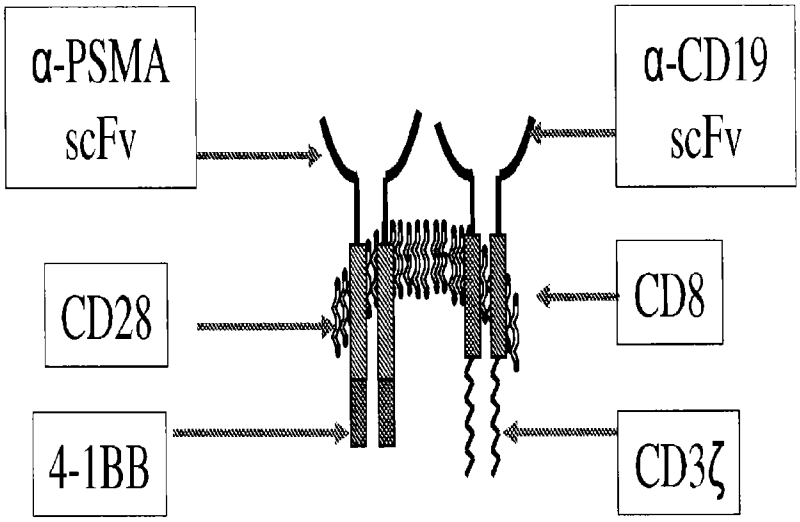
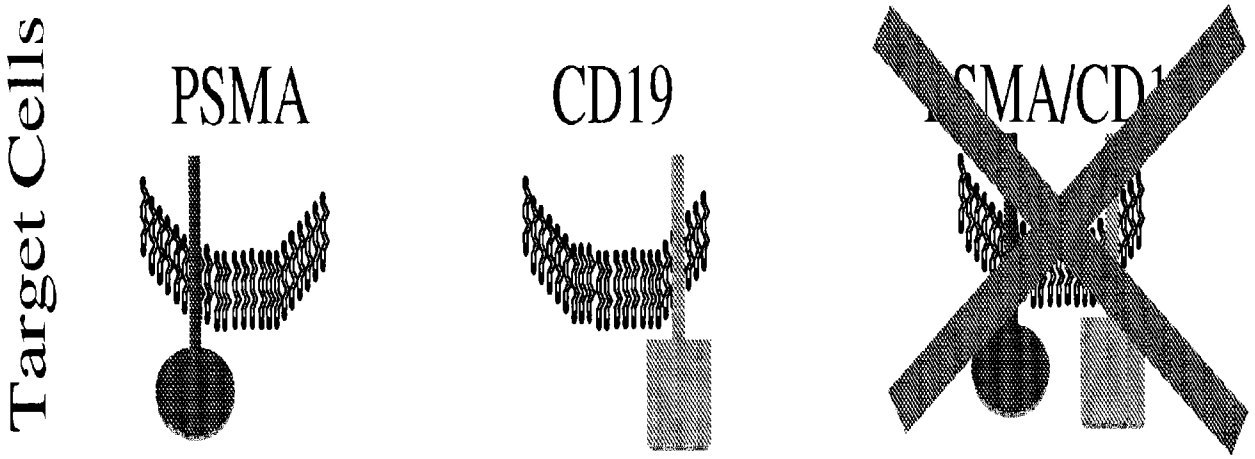

b Tumor-Sensing T cell Strategy
CAR+CCR+
"Tumor Sensing" T Cell
Tumor Ag A+B+
Cell
T cells with Inefficient receptors (e.g. low affinity TCR, suboptimal CAR) that supply CD3ζ signal are functionally rescued by chimeric costimulatory receptors. This allows T cells to be sufficiently activated and stimulated only by A+B+ Cells to kill A-B+ Cells while sparing A+ Cells.
CAR+CCR+
"Tumor Sensing" T Cell
Tumor Ag A+ Cell
Tumor Ag A
Tumor Ag B
scFv(α-Ag A)-CD3ζ
CAR
Inefficient
scFv(α-Ag A)-
CD3ζ
CAR
scFv(α-Ag B)-CD28-CD137
CCR

Figure 6B

Dual-CAR Stimulation and Activation





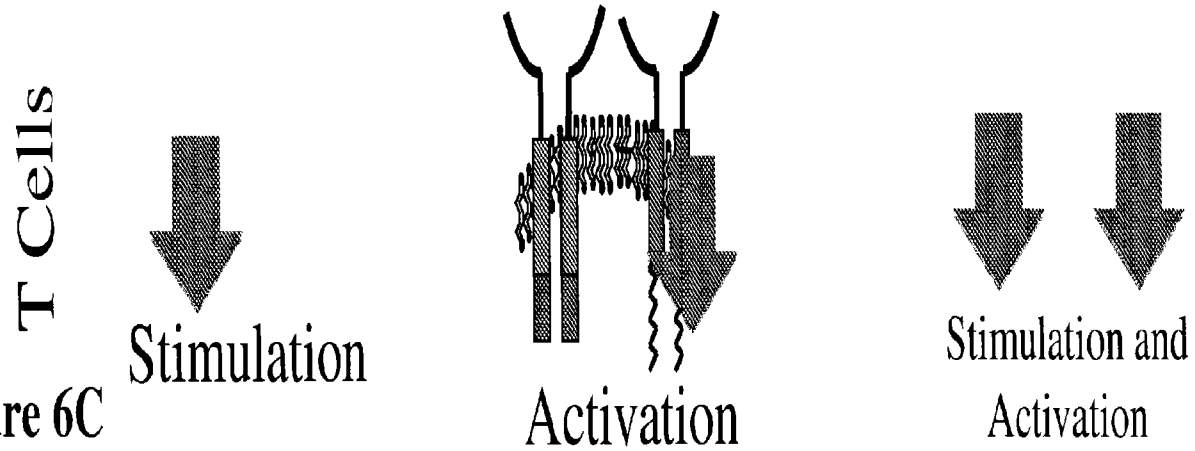


Figure 6C