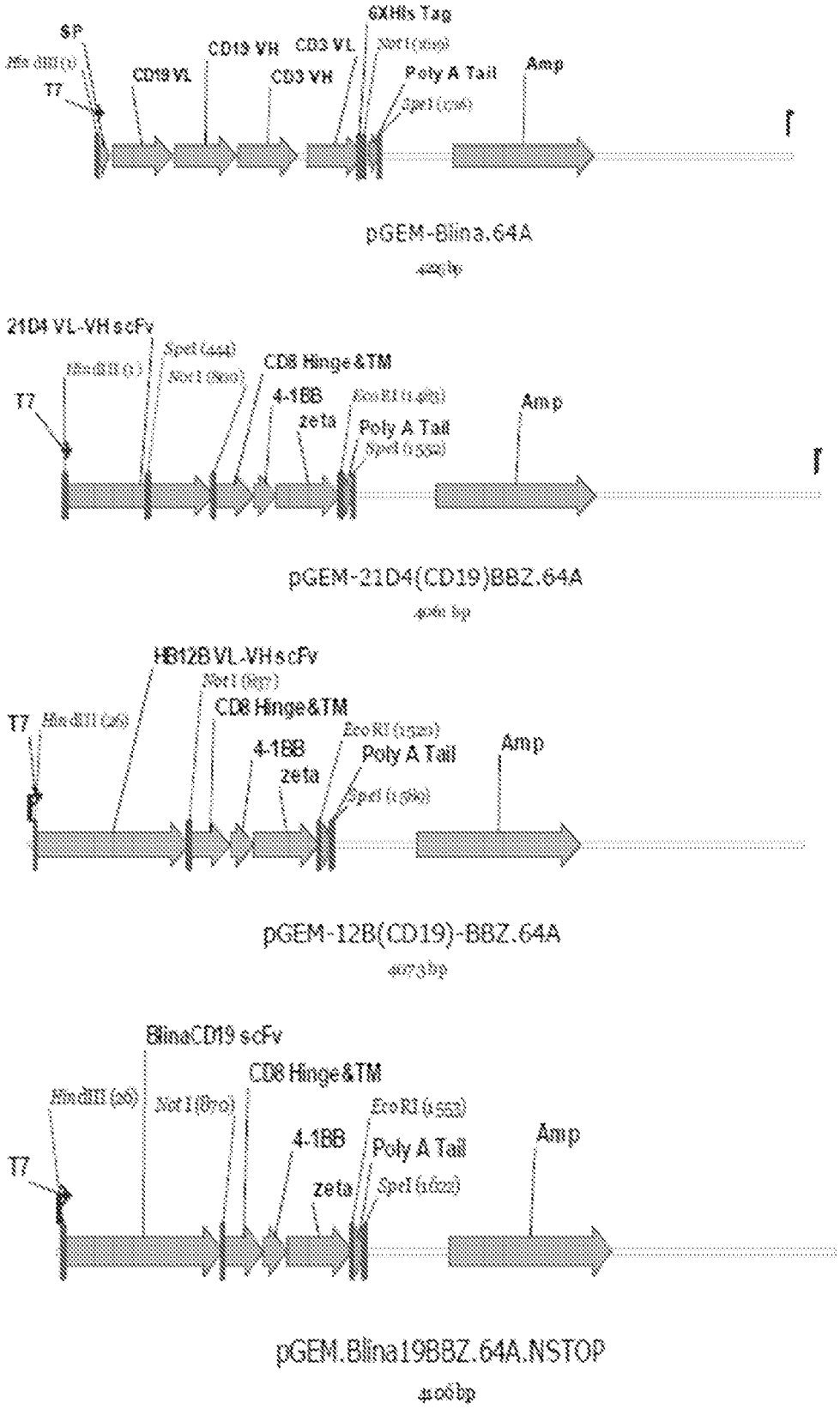

SP
CD19 VL
CD19 VH
CD3 VL
CD3 VH
6XHis Tag
Poly A Tail
Amp
T7
pGEM-Blina.64A
21D4 VL-VH scFv
CD8 Hinge&TM
4-1BB
zeta
Poly A Tail
Amp
T7
pGEM-21D4(CD19)BBZ.64A
HB12B VL-VH scFv
CD8 Hinge&TM
4-1BB
zeta
Poly A Tail
Amp
T7
pGEM-12B(CD19)-BBZ.64A
BlinaCD19 scFv
CD8 Hinge&TM
4-1BB
zeta
Poly A Tail
Amp
T7
pGEM.Blina19BBZ.64A.NSTOP

Figure 1

Testing Blina Expression and Function

EP #	RNA
1. CD19-CAR	10ug
2. CD19-CAR	5ug
3. CD19-CAR	5ug + Blina 10ug
4. Blina	10ug
5. GFP	5ug

After EP, mix immediately, culture overnight

6. CD19 5ug/GFP (mixture of 1ml EP 2 + 1ml EP 5)
7. CD19 5ug+ Blina 10ug/GFP(mixture of 1ml EP 3 + 1ml EP 5)
8. Blina 10ug/GFP (mixture of 1ml EP 4 + 1ml EP 5)

Mix before CAR staining and CD107a assay

9. CD19 5ug/GFP 2hr (mixture of 1ml EP 2 + 1ml EP 5)
10. CD19 5ug+ Blina 10ug/GFP 2hr(mixture of 1ml EP 3 + 1ml EP 5)
11. Blina 10ug/GFP 2hr(mixture of 1ml EP 4 + 1ml EP 5)
12. NO EP

1. T cell: ND200 OKT stim
2. 100ul Eped T cell in 3ml pre-warmed R10 medium/well
3. EP Condition: 500V 70ms

Figure 2

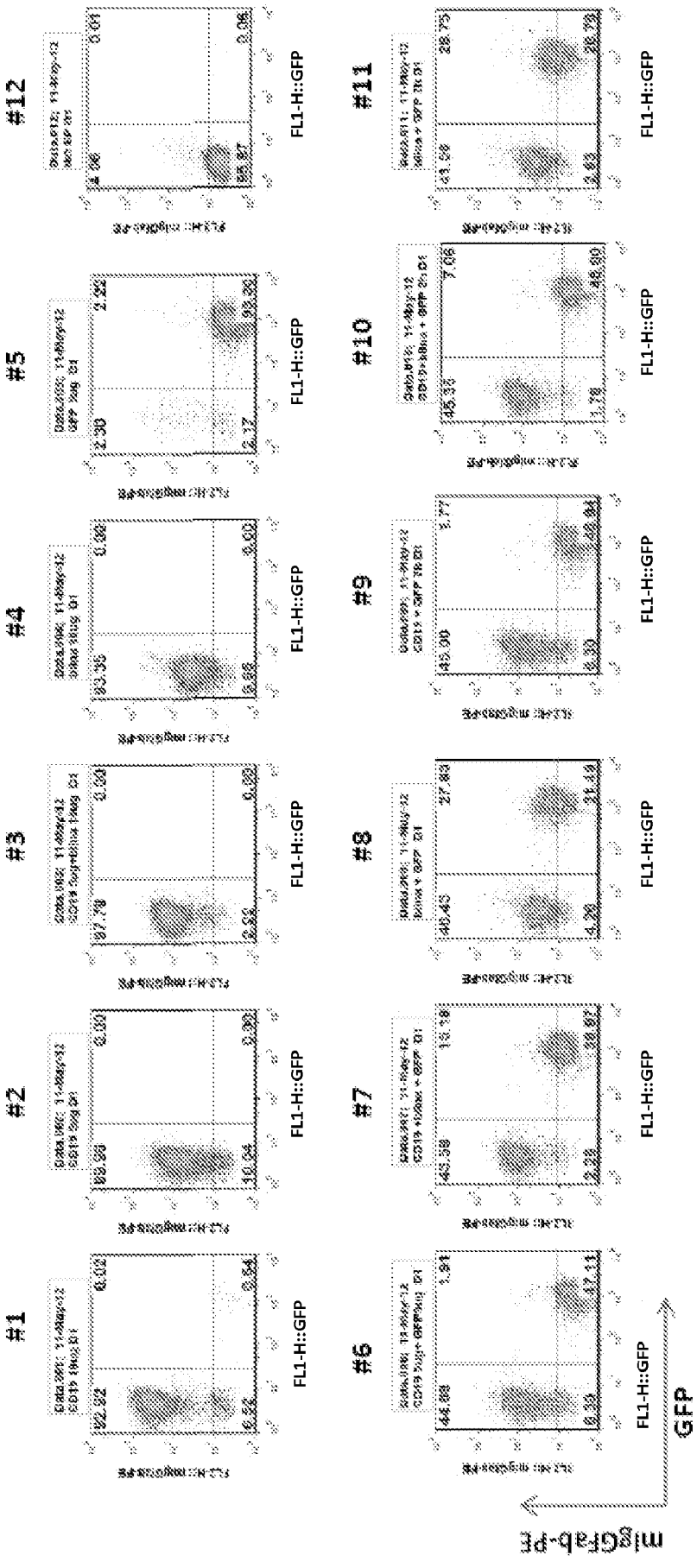


Figure 3

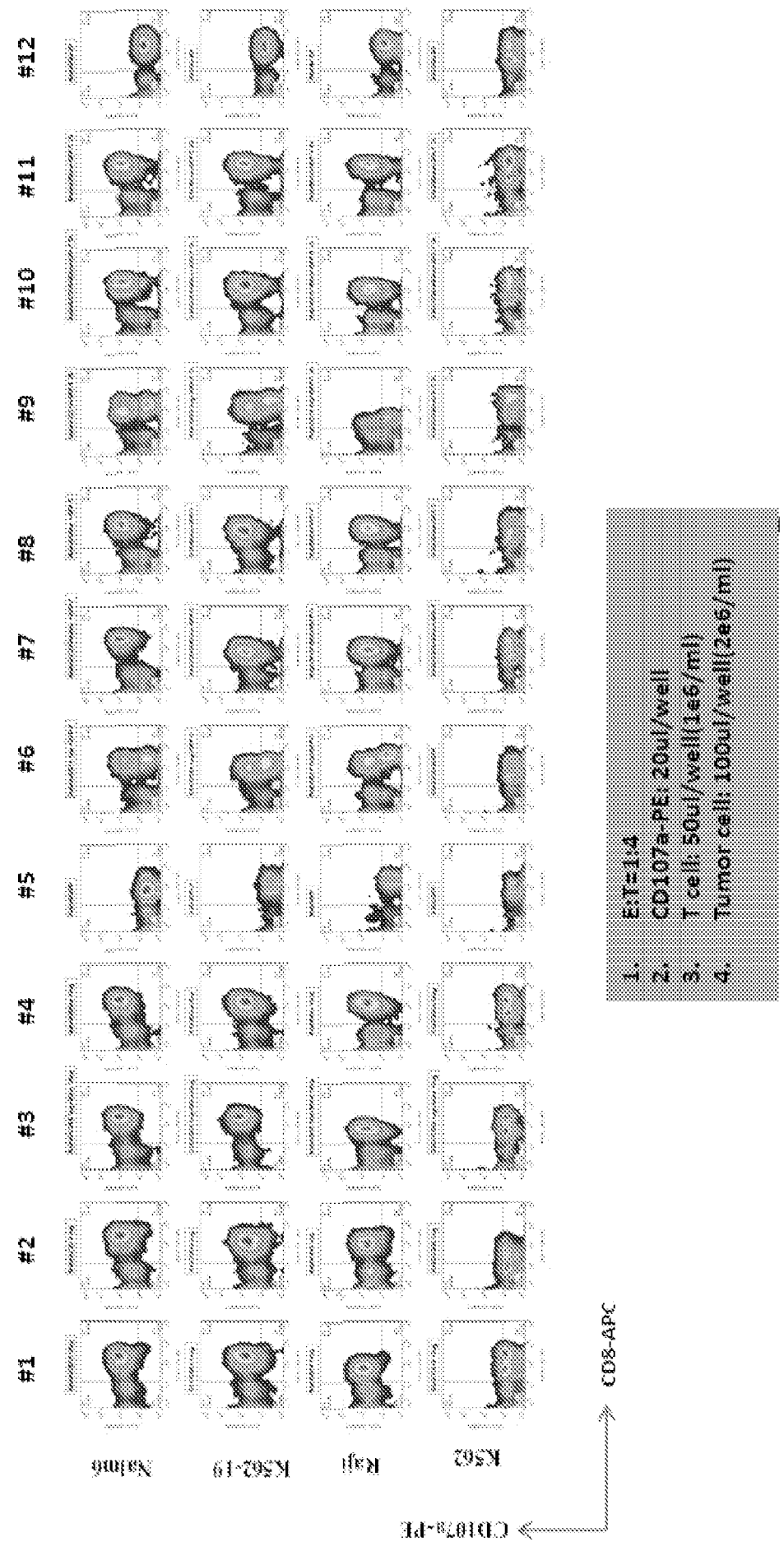

#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
#11
#12
K562
Raji
K562-19
Nalm6
CD107a-PE
CD8-APC
1. E:T=1:4
2. CD107a-PE: 20ul/well
3. T cell: 50ul/well(1e6/ml)
4. Tumor cell: 100ul/well(2e6/ml)

Figure 4

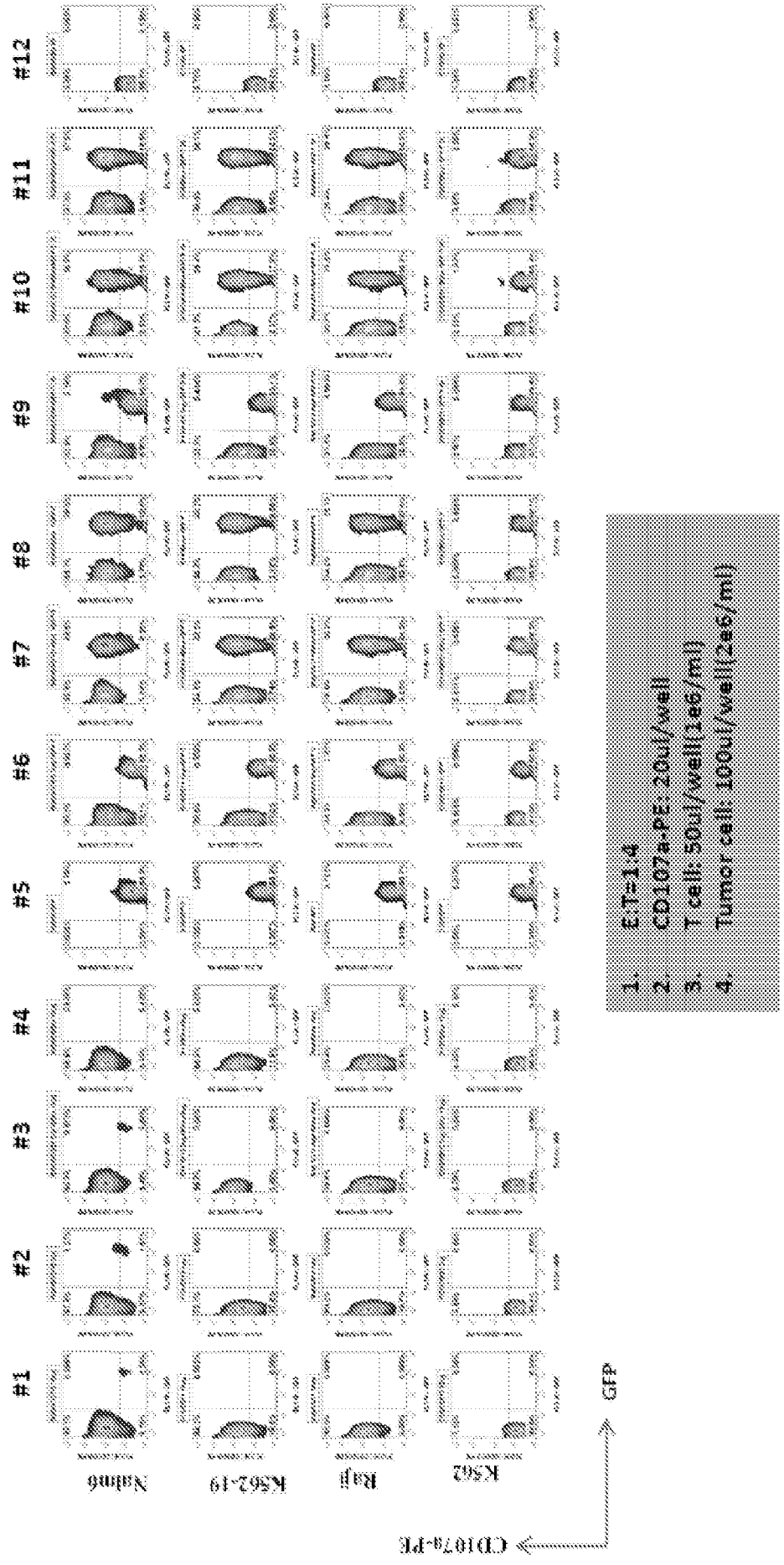

#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
#11
#12
Nalm6
K562-19
Raji
K562
CD107a-PE
GFP
1. E:T=1:4
2. CD107a-PE: 20ul/well
3. T cell: 50ul/well(1e6/ml)
4. Tumor cell: 100ul/well(2e6/ml)

Figure 5

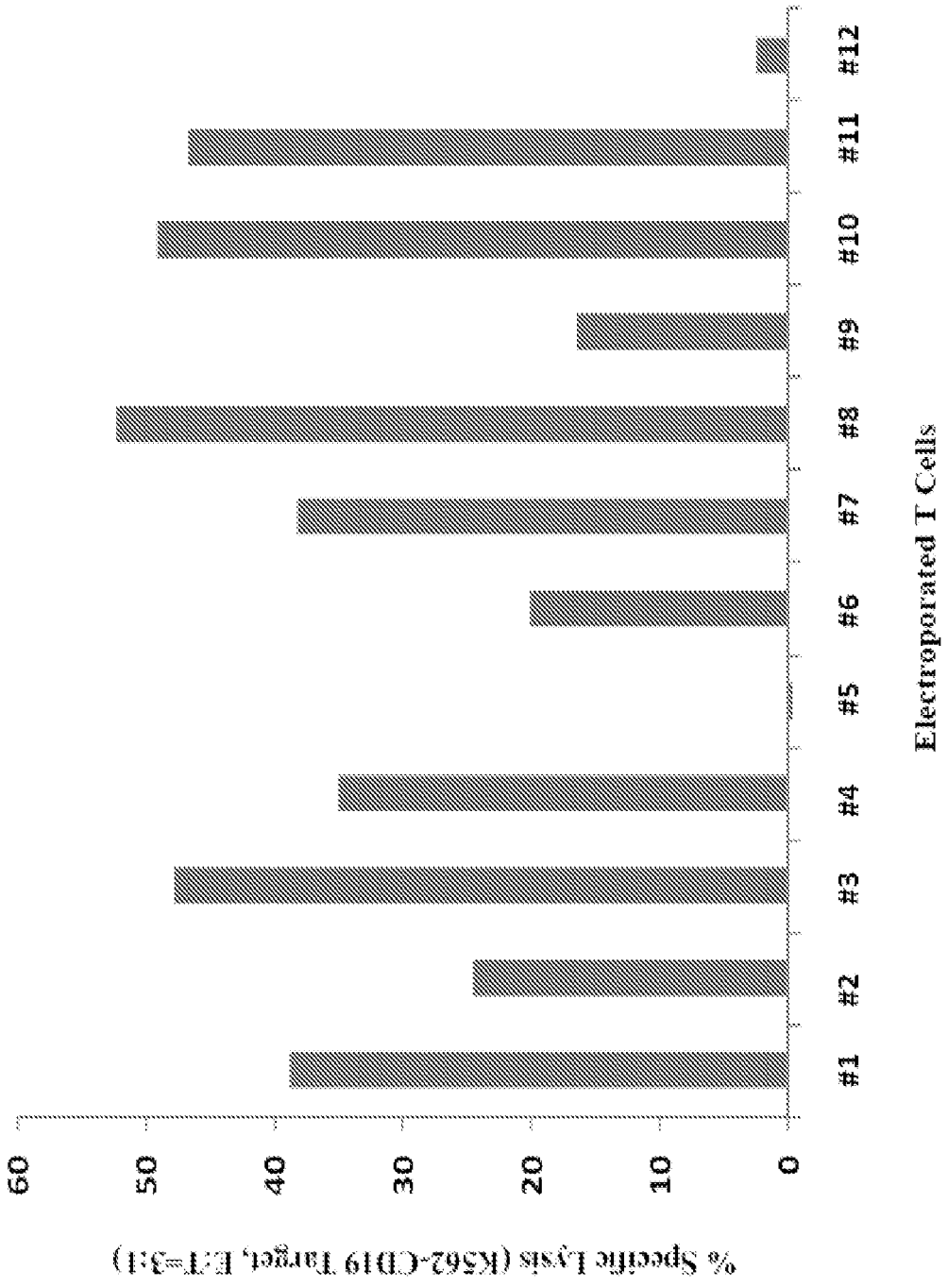

% Specific Lysis (K562-CD19 Target, E:T=3:1)
60
50
40
30
20
10
0
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
#11
#12
Electroporated T Cells

Figure 6

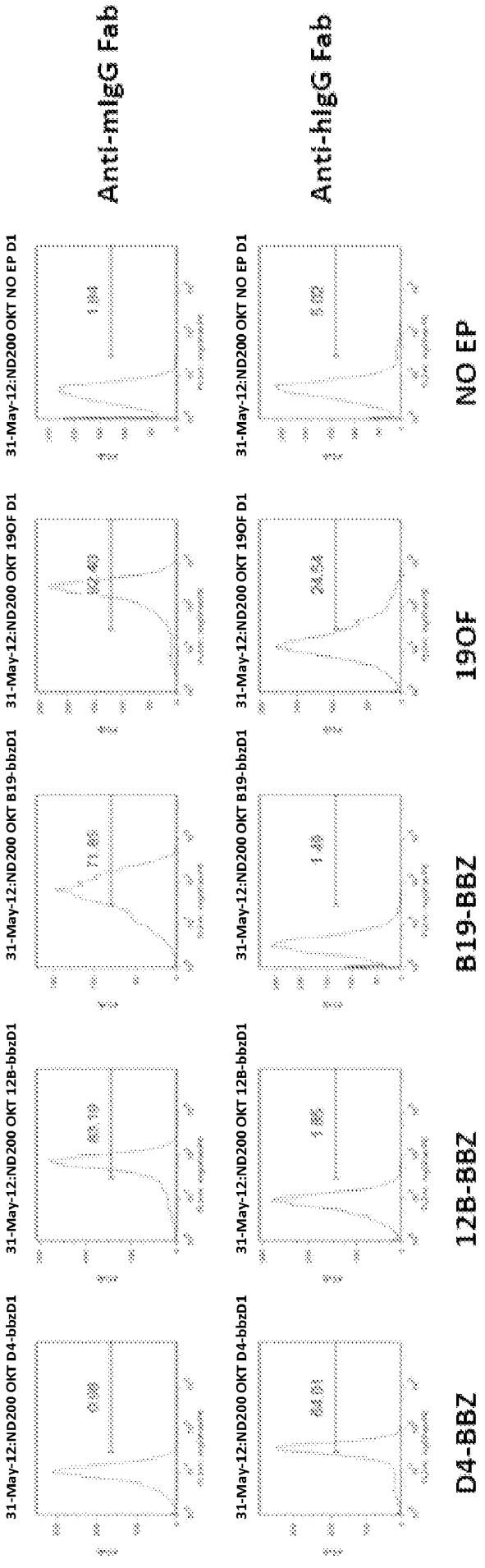


Figure 7

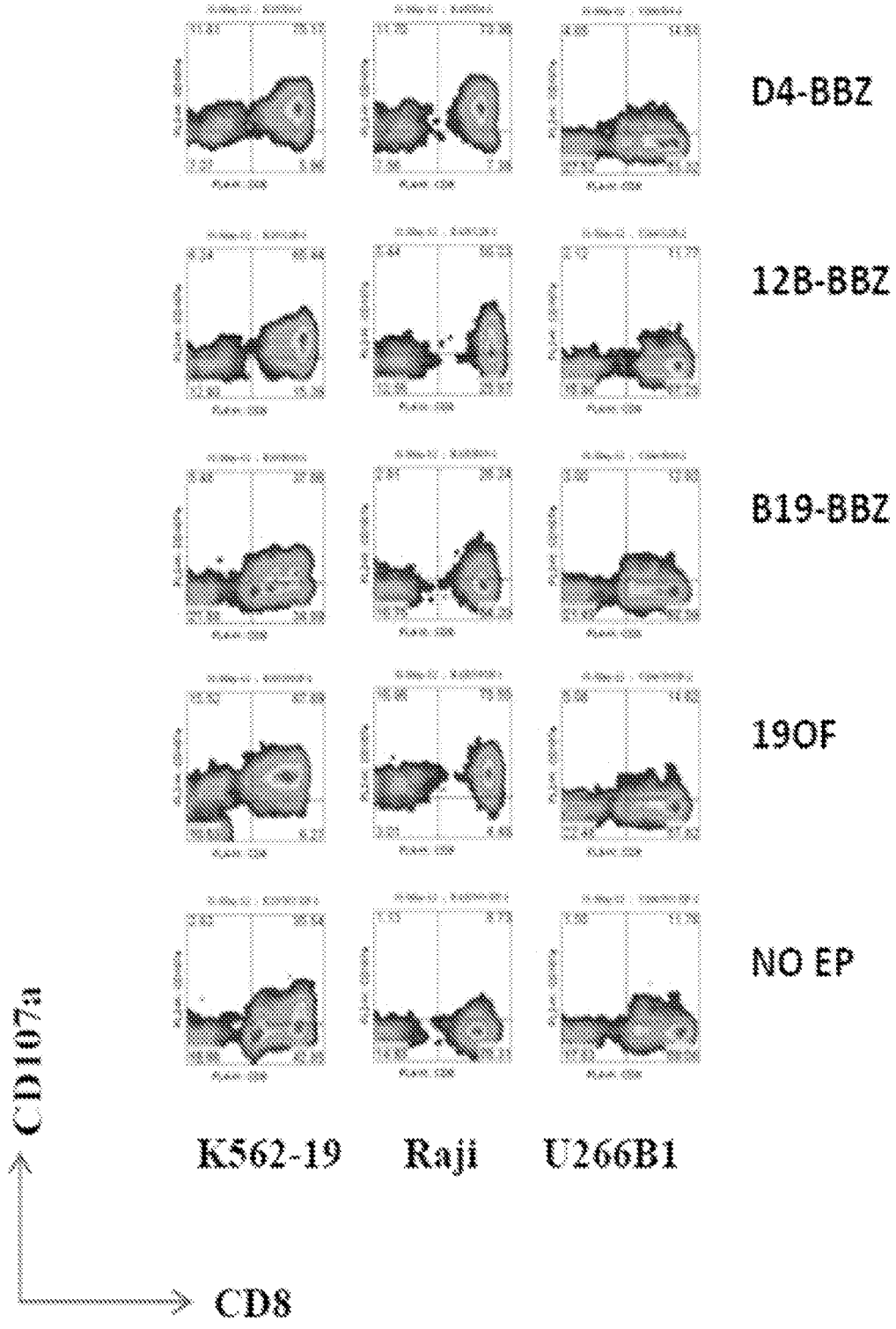

D4-BBZ
12B-BBZ
B19-BBZ
19OF
NO EP
K562-19
Raji
U266B1
CD107a
CD8

Figure 8

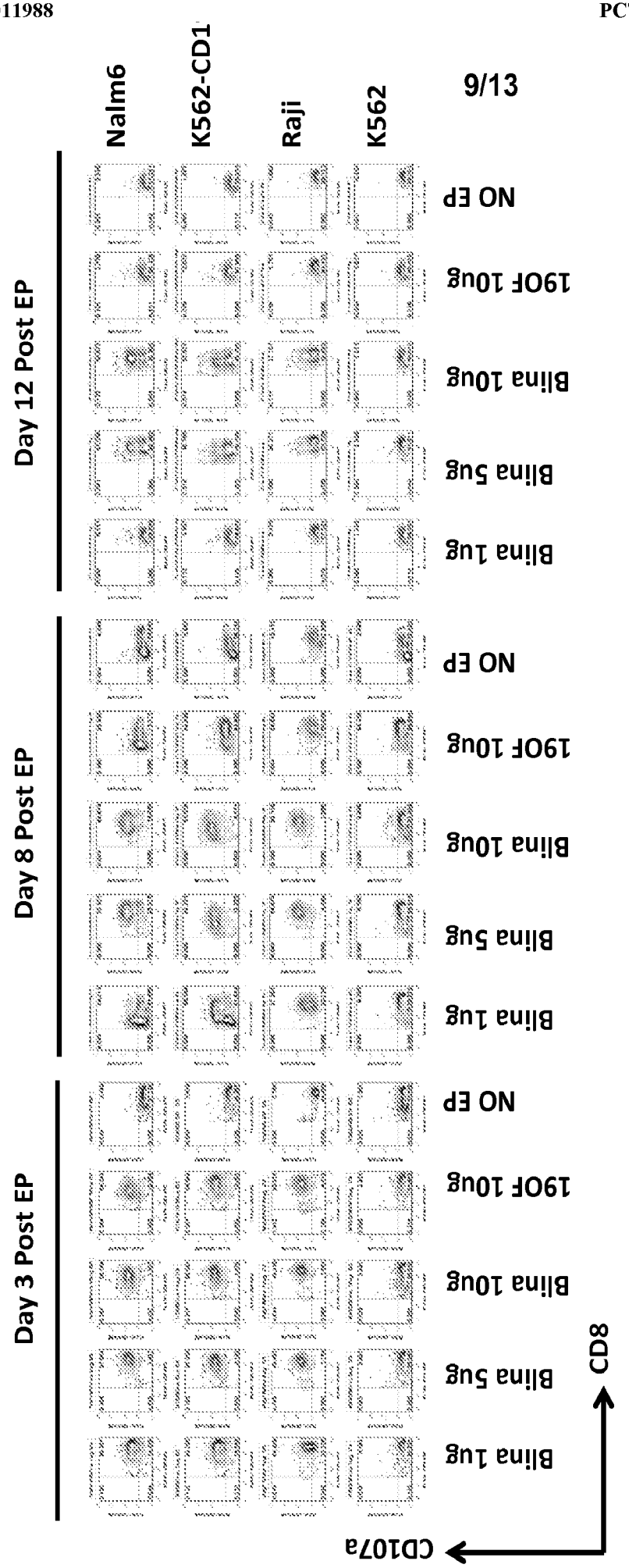

9/13
Nalm6
K562-CD1
Raji
K562
Day 12 Post EP
Day 8 Post EP
Day 3 Post EP
NO EP
19OF 10ug
Blina 10ug
Blina 5ug
Blina 1ug
CD107a
CD8

Figure 9A

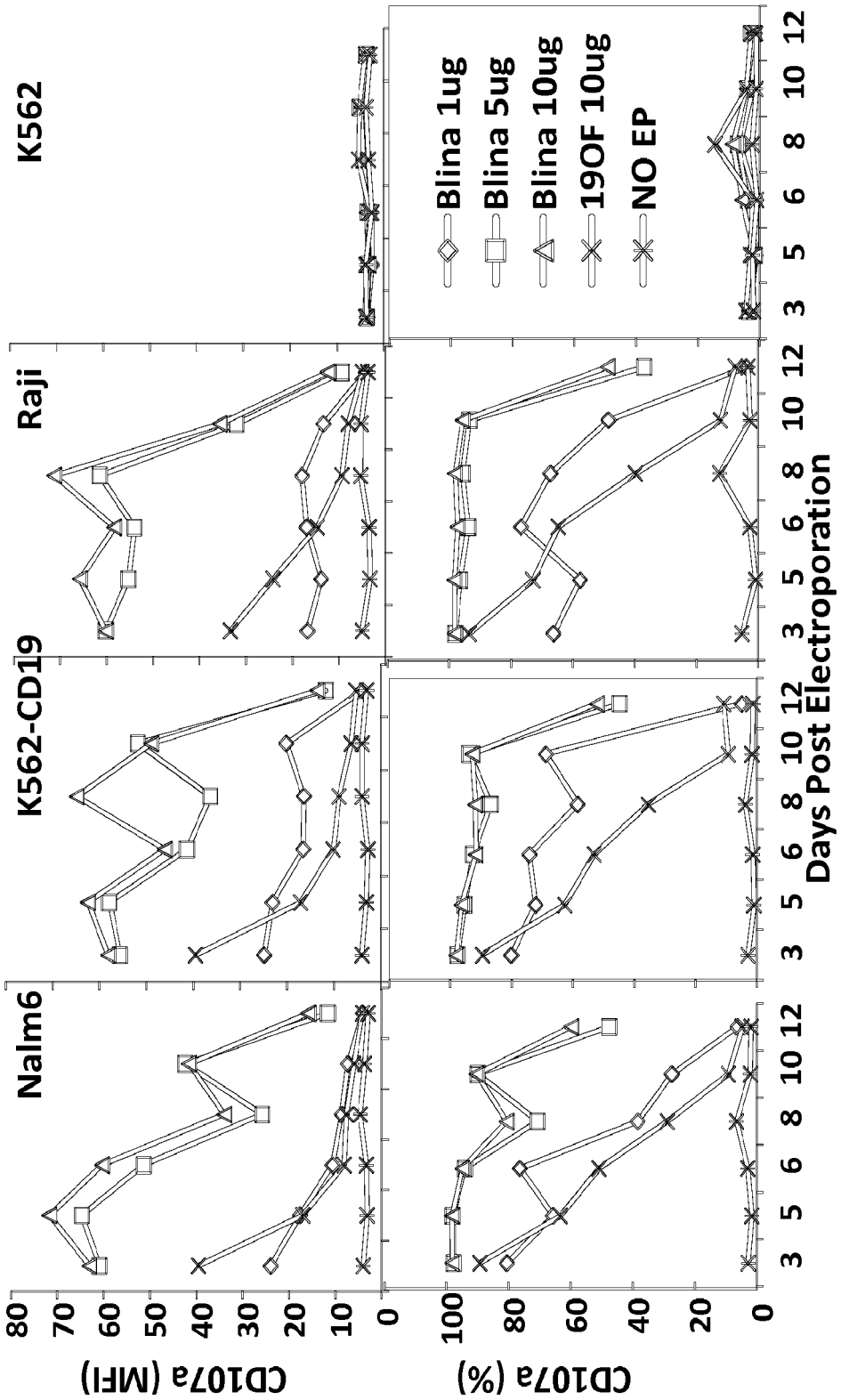

Nalm6
K562-CD19
Raji
K562
CD107a (MFI)
CD107a (%)
Days Post Electroporation
Blina 1ug
Blina 5ug
Blina 10ug
19OF 10ug
NO EP

Figure 9B

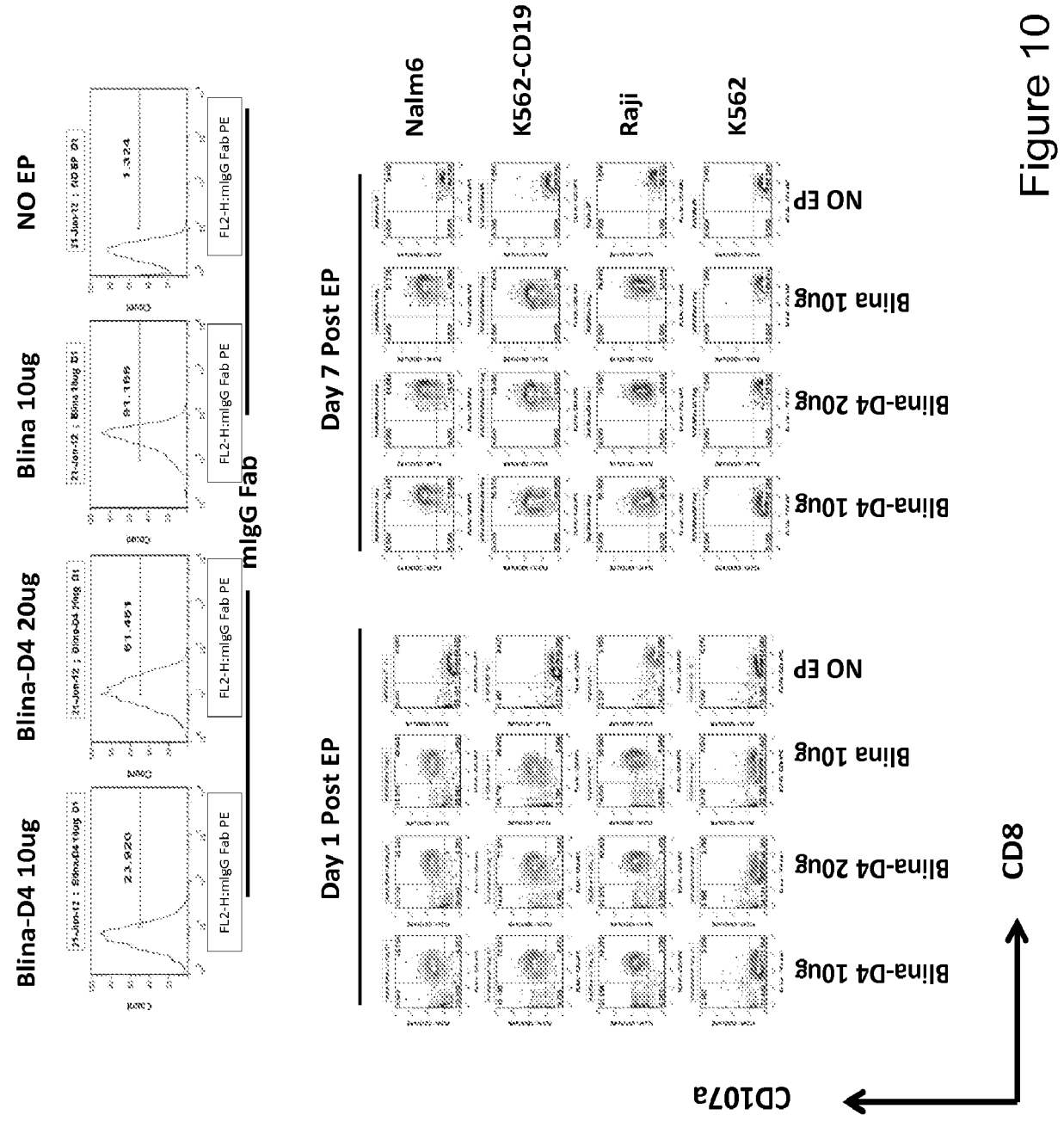

Blina-D4 10ug
Blina-D4 20ug
Blina 10ug
NO EP
FL2-H:mIgG Fab PE
mIgG Fab
Day 1 Post EP
Day 7 Post EP
Nalm6
K562-CD19
Raji
K562
Blina-D4 10ug
Blina-D4 20ug
Blina 10ug
NO EP
CD107a
CD8

Figure 10

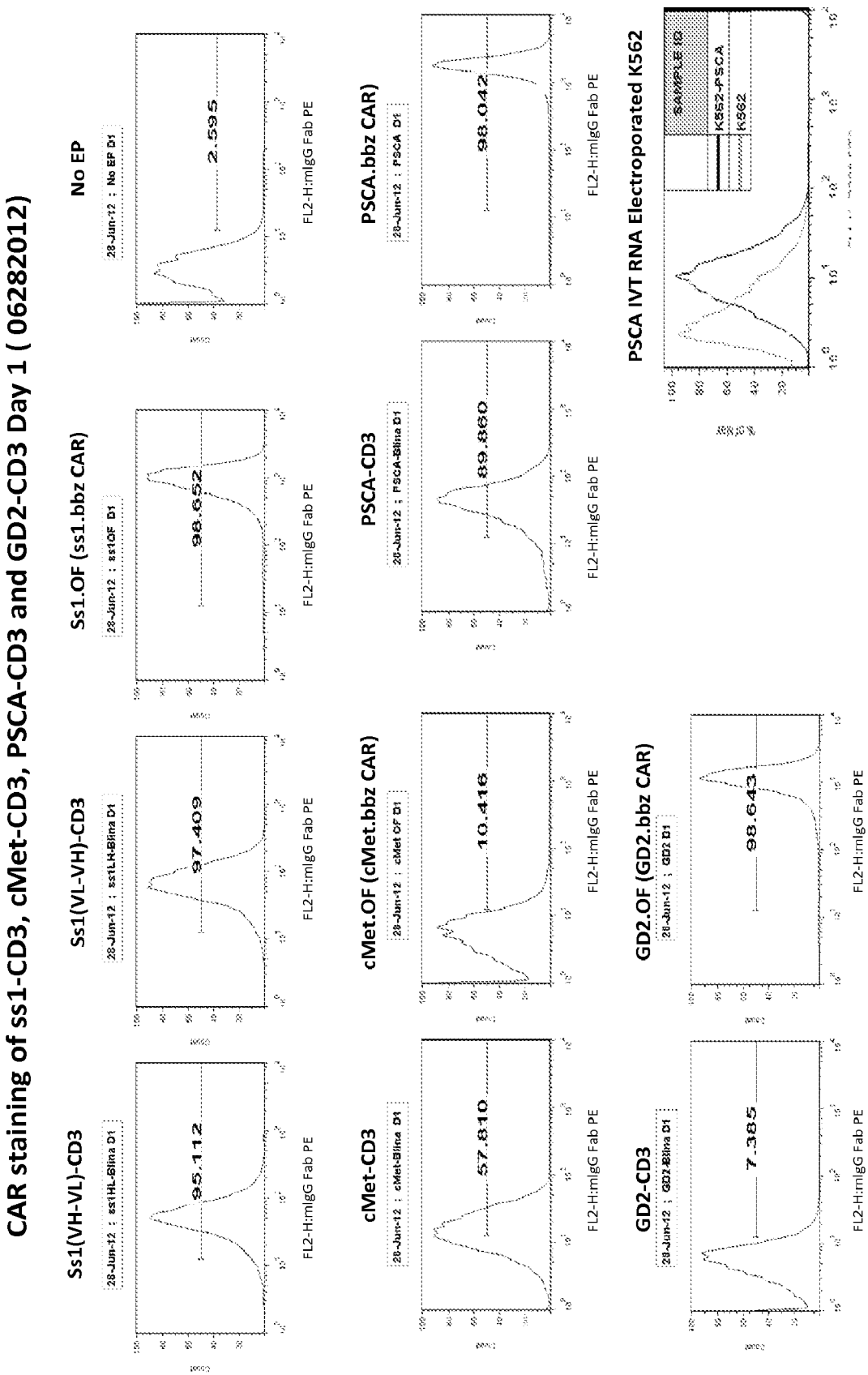

CAR staining of ss1-CD3, cMet-CD3, PSCA-CD3 and GD2-CD3 Day 1 (06282012)
Ss1(VH-VL)-CD3
Ss1(VL-VH)-CD3
Ss1.OF (ss1.bbz CAR)
No EP
cMet-CD3
cMet.OF (cMet.bbz CAR)
PSCA-CD3
PSCA.bbz CAR)
GD2-CD3
GD2.OF (GD2.bbz CAR)
PSCA IVT RNA Electroporated K562
95.112
97.409
98.652
2.595
57.810
10.416
89.860
98.042
7.385
98.643
FL2-H:mIgG Fab PE
SAMPLE ID
K562-PSCA
K562

Figure 11A

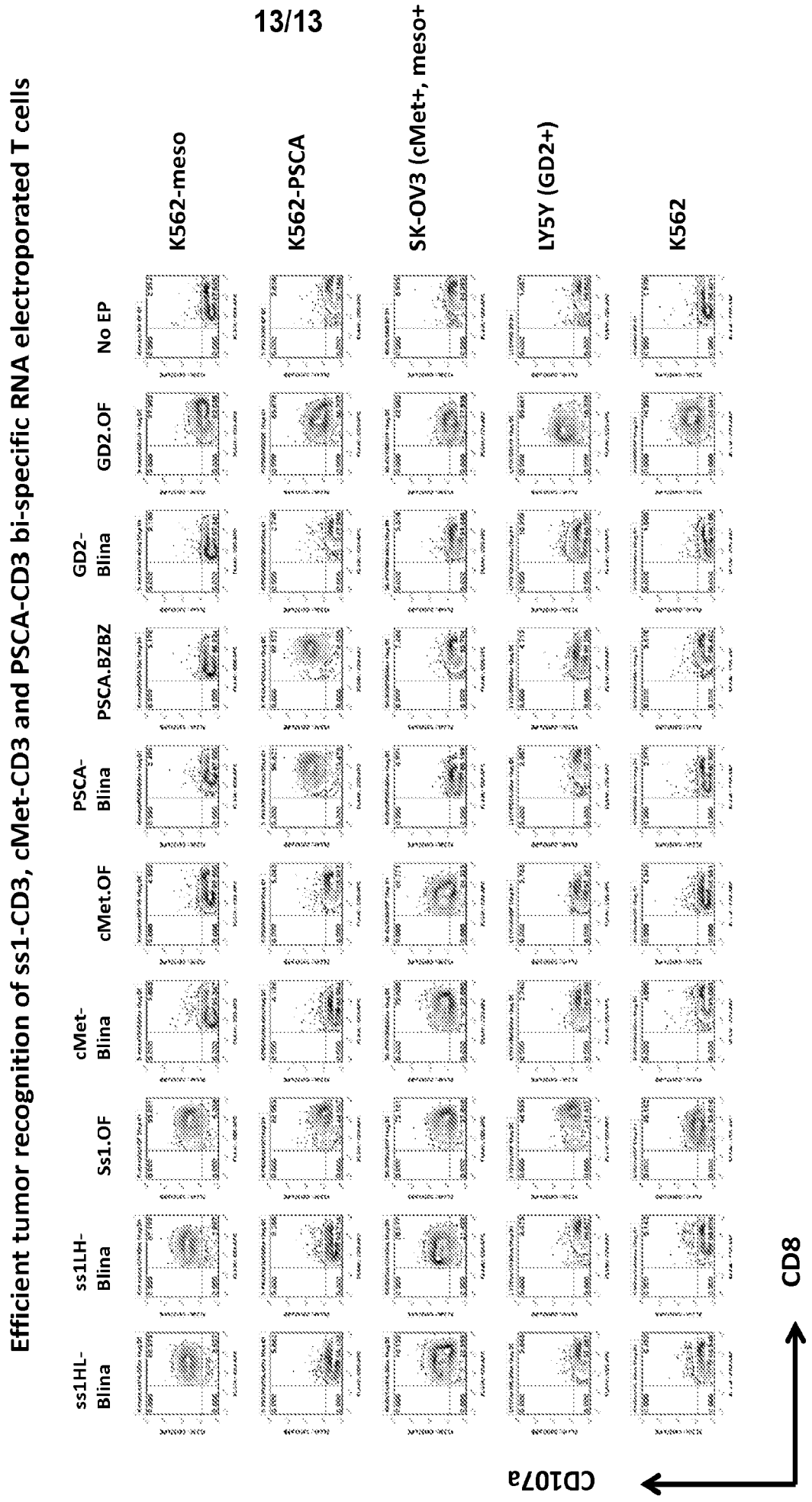

Efficient tumor recognition of ss1-CD3, cMet-CD3 and PSCA-CD3 bi-specific RNA electroporated T cells
ss1HL-Blina
ss1LH-Blina
Ss1.OF
cMet-Blina
cMet.OF
PSCA-Blina
PSCA.BZBZ
GD2-Blina
GD2.OF
No EP
K562-meso
K562-PSCA
SK-OV3 (cMet+, meso+
LY5Y (GD2+)
K562
CD107a
CD8

Figure 11B