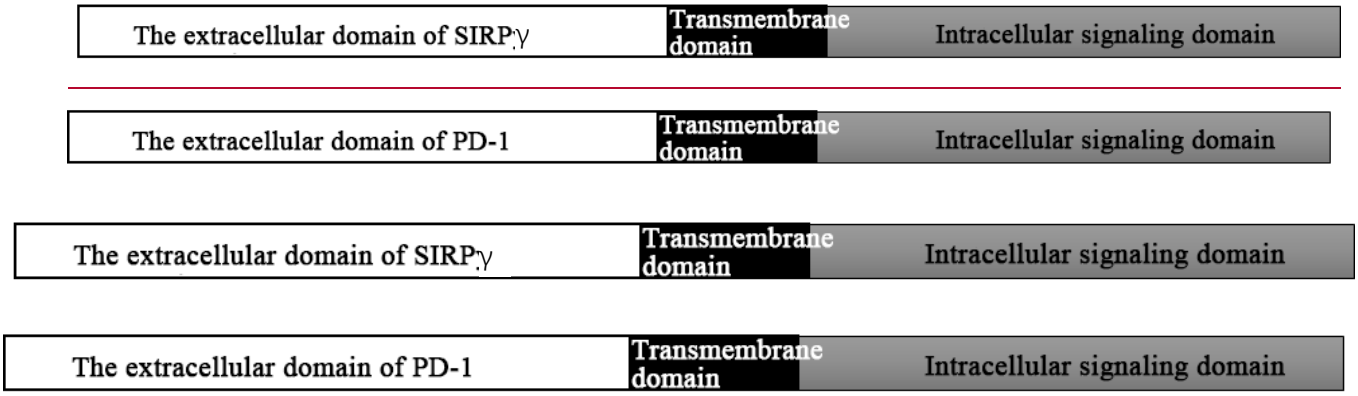


Figure 1

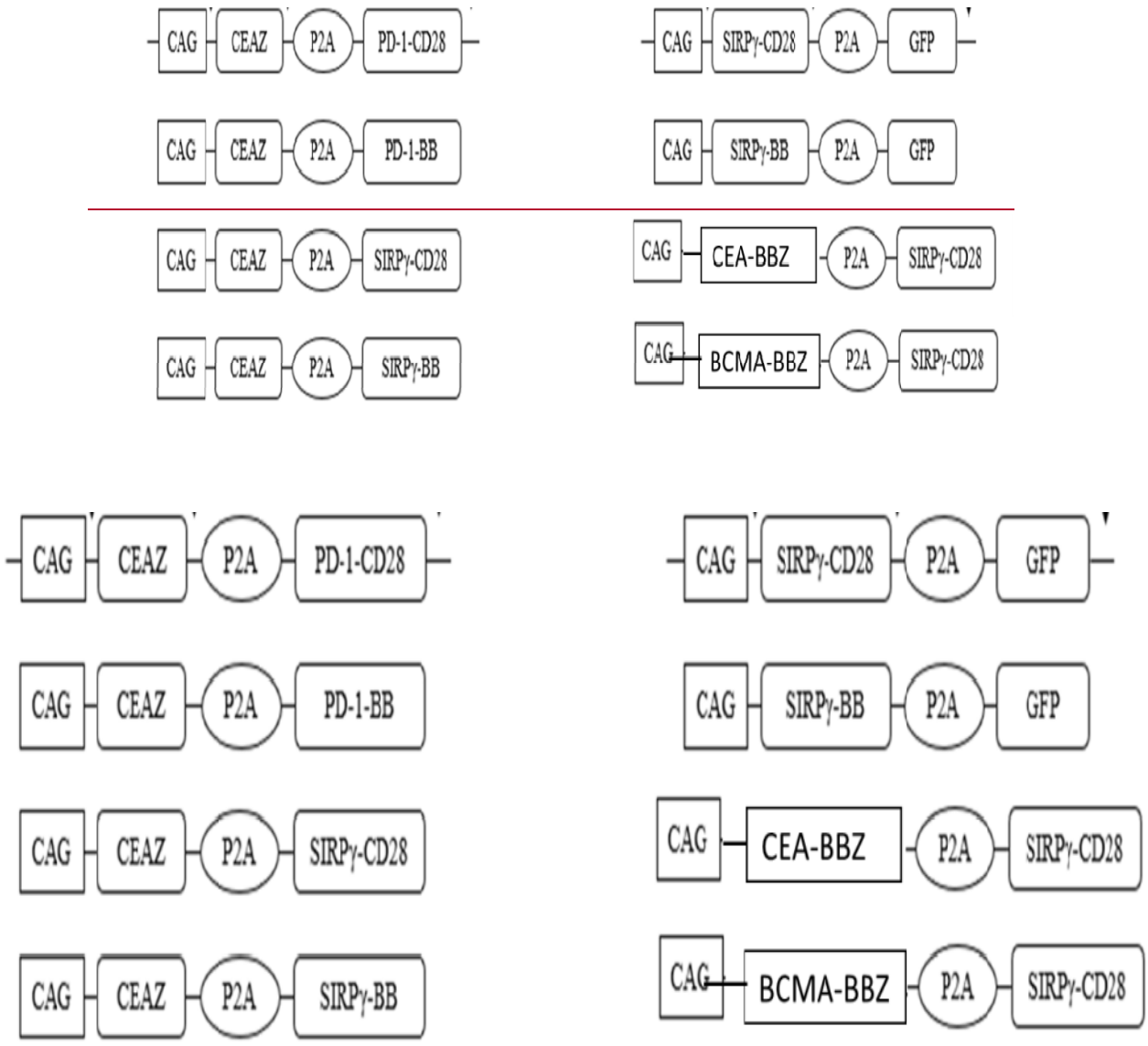


Figure 2

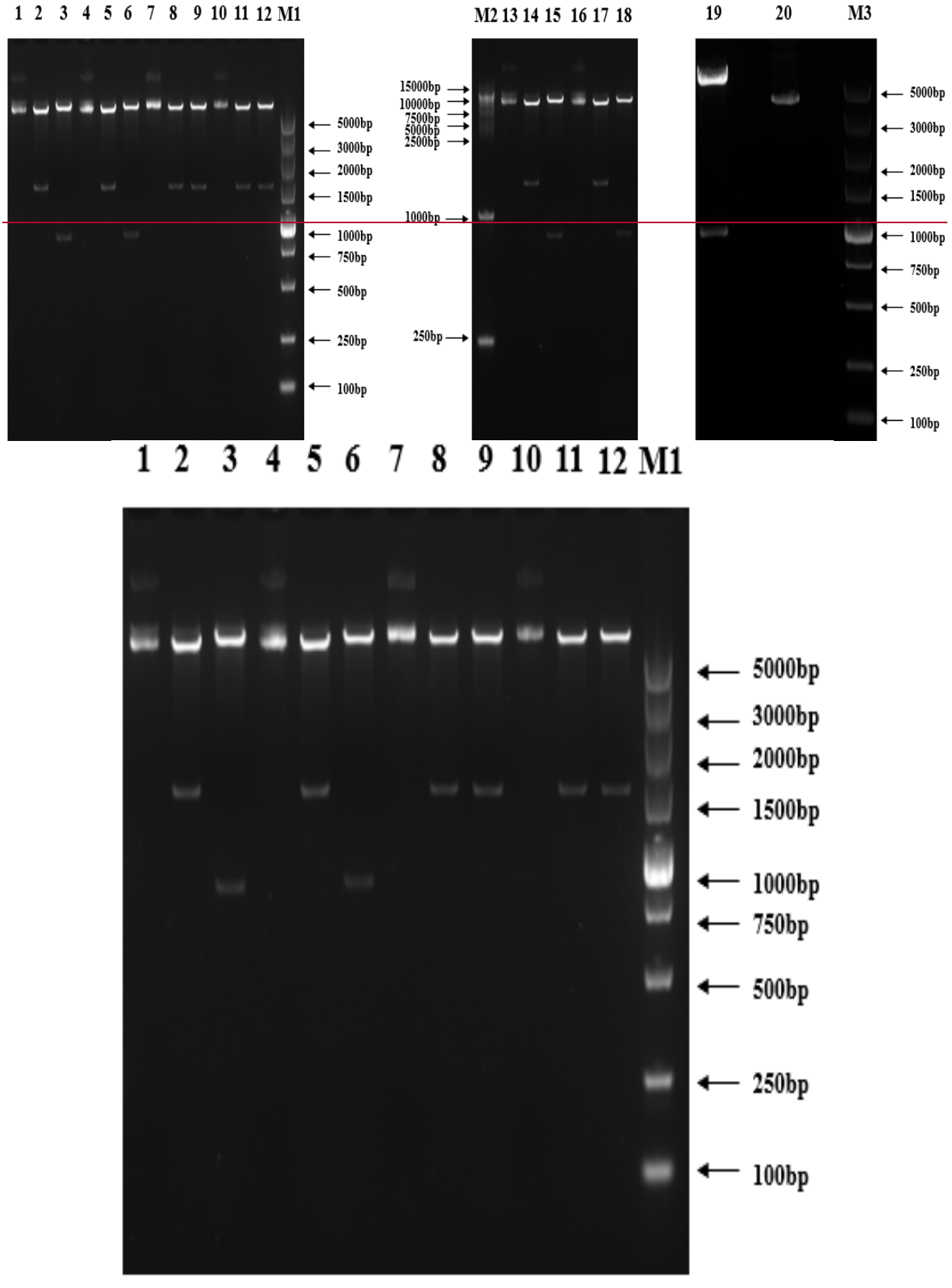

1 2 3 4 5 6 7 8 9 10 11 12 M1
M2 13 14 15 16 17 18
19 20 M3
15000bp
10000bp
7500bp
5000bp
2500bp
1000bp
250bp
5000bp
3000bp
2000bp
1500bp
1000bp
750bp
500bp
250bp
100bp
1 2 3 4 5 6 7 8 9 10 11 12 M1
5000bp
3000bp
2000bp
1500bp
1000bp
750bp
500bp
250bp
100bp

Figure 3

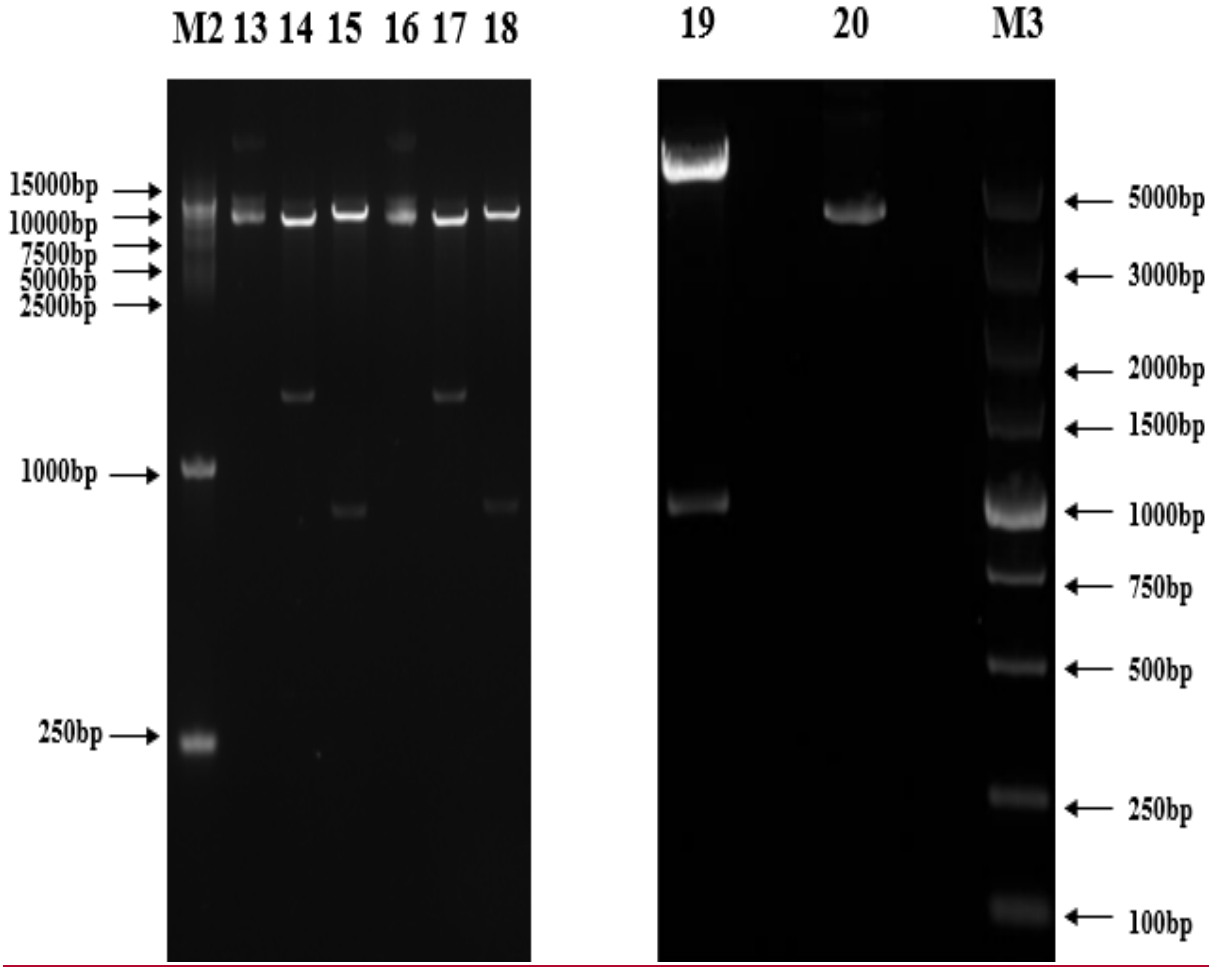
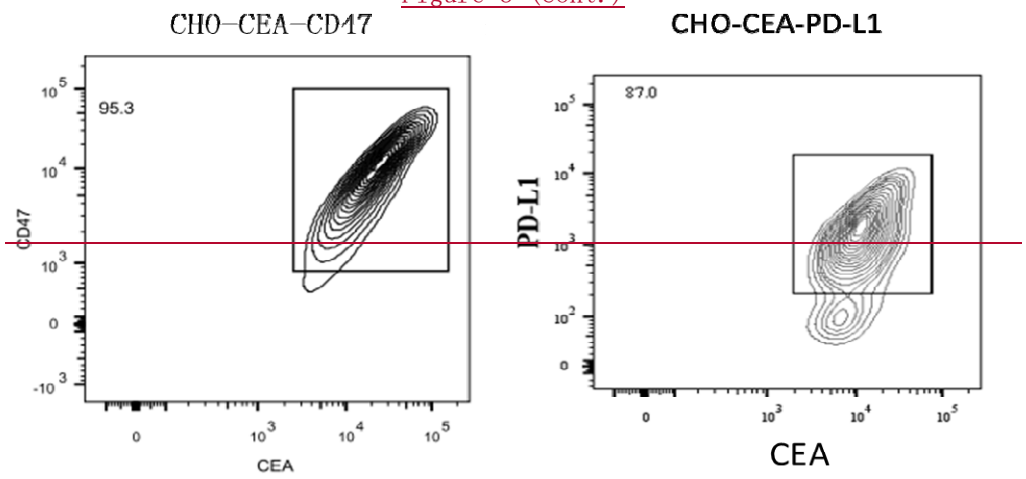
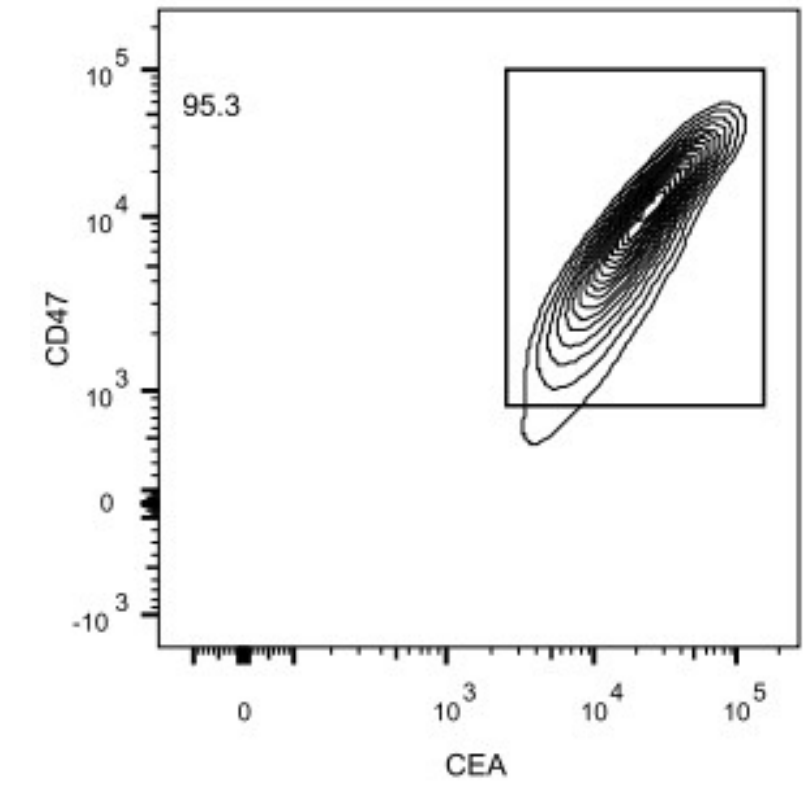


Figure 3 (Cont.)




95.3
CD47
CEA
10^5
10^4
10^3
0
-10^3
0
10^3
10^4
10^5

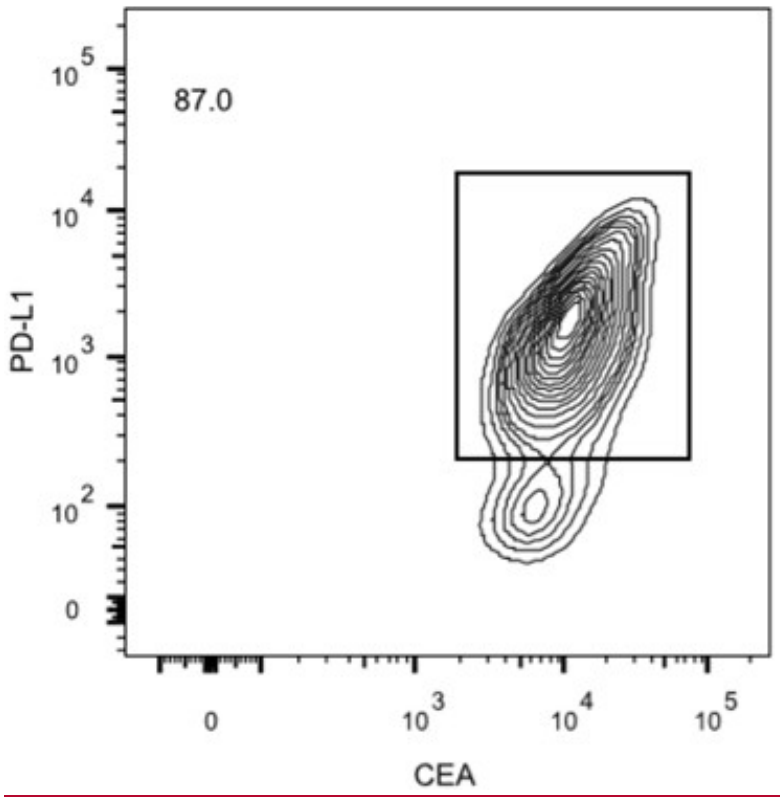
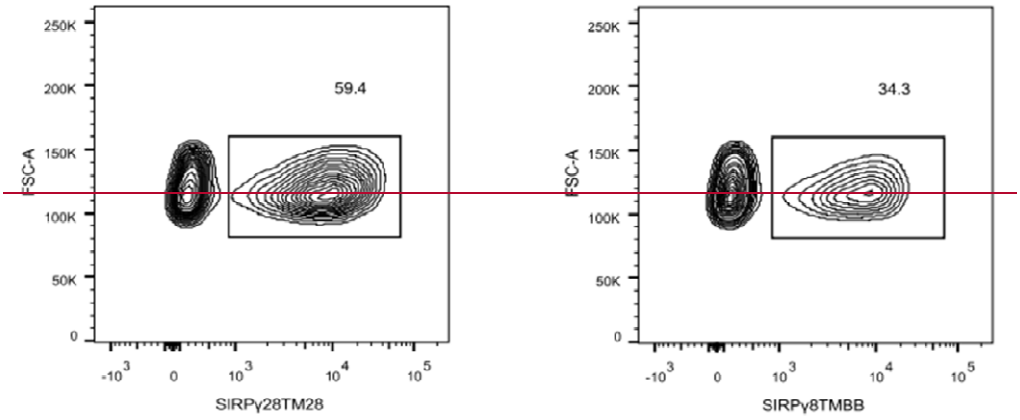
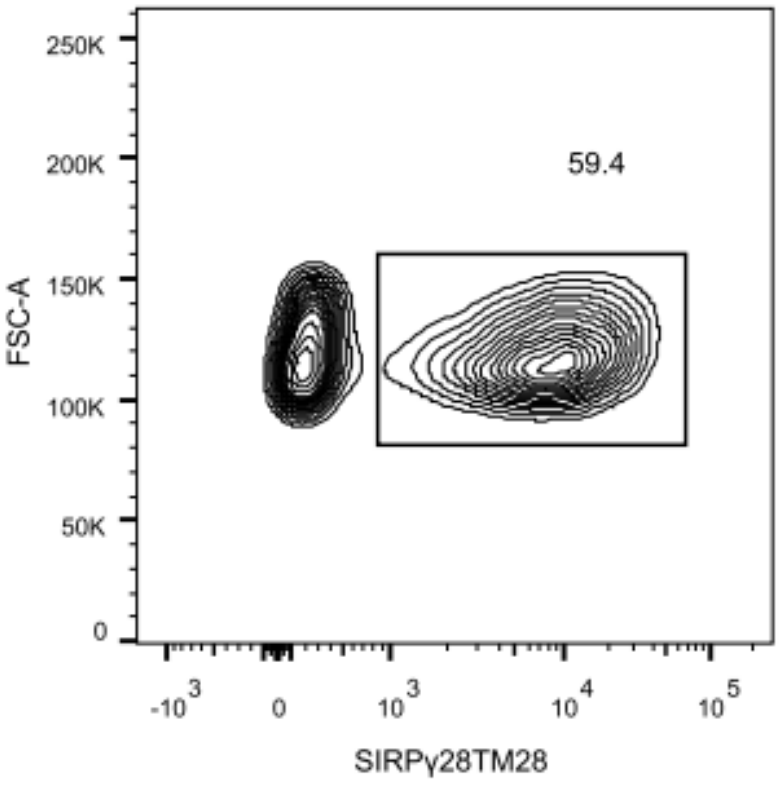

87.0
PD-L1
CEA
10^5
10^4
10^3
10^2
0
0
10^3
10^4
10^5

Figure 4


59.4
34.3
FSC-A
SIRPγ28TM28
SIRPγ8TMBB


59.4
FSC-A
SIRPγ28TM28

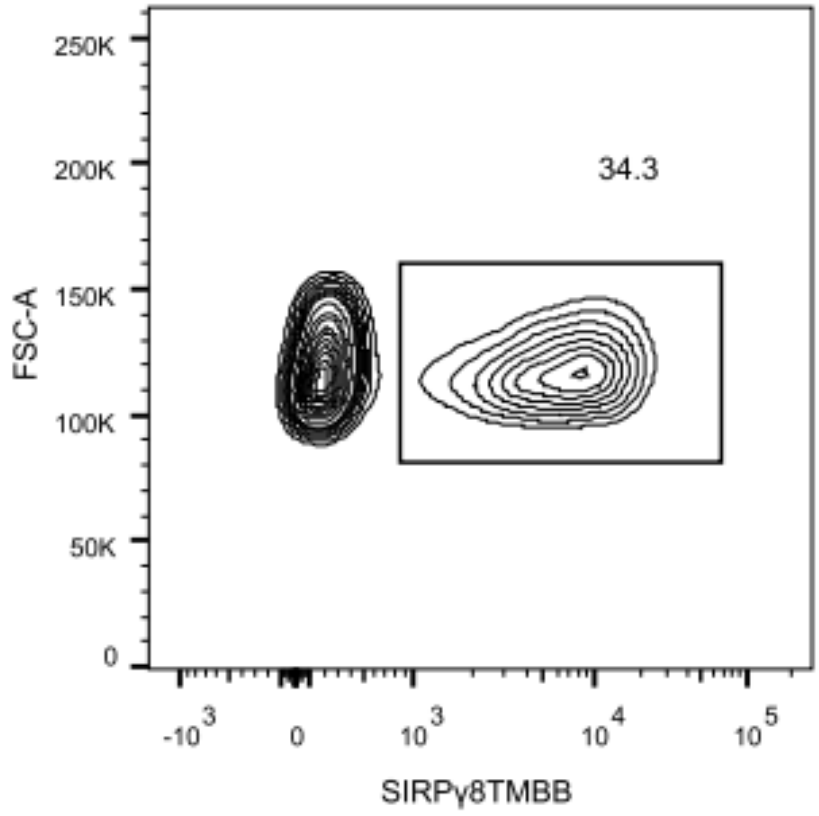
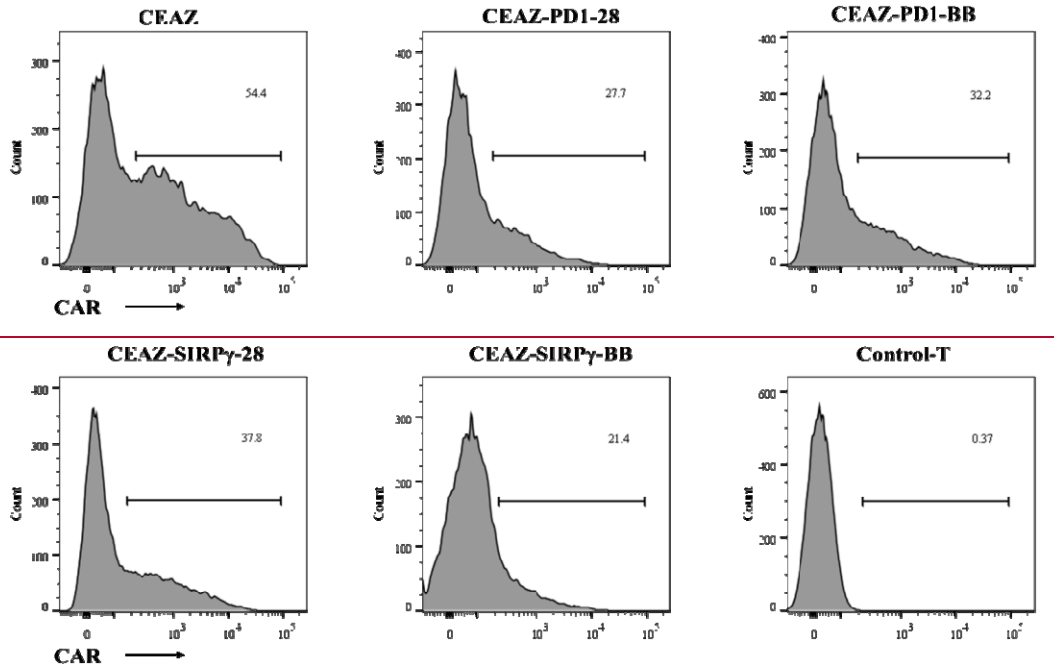

250K
200K
150K
100K
50K
0
FSC-A
34.3
-10^3
0
10^3
10^4
10^5
SIRPγ8TMBB

Figure 5a


CEAZ
54.4
CEAZ-PD1-28
27.7
CEAZ-PD1-BB
32.2
CEAZ-SIRPγ-28
37.8
CEAZ-SIRPγ-BB
21.4
Control-T
0.37
Count
CAR

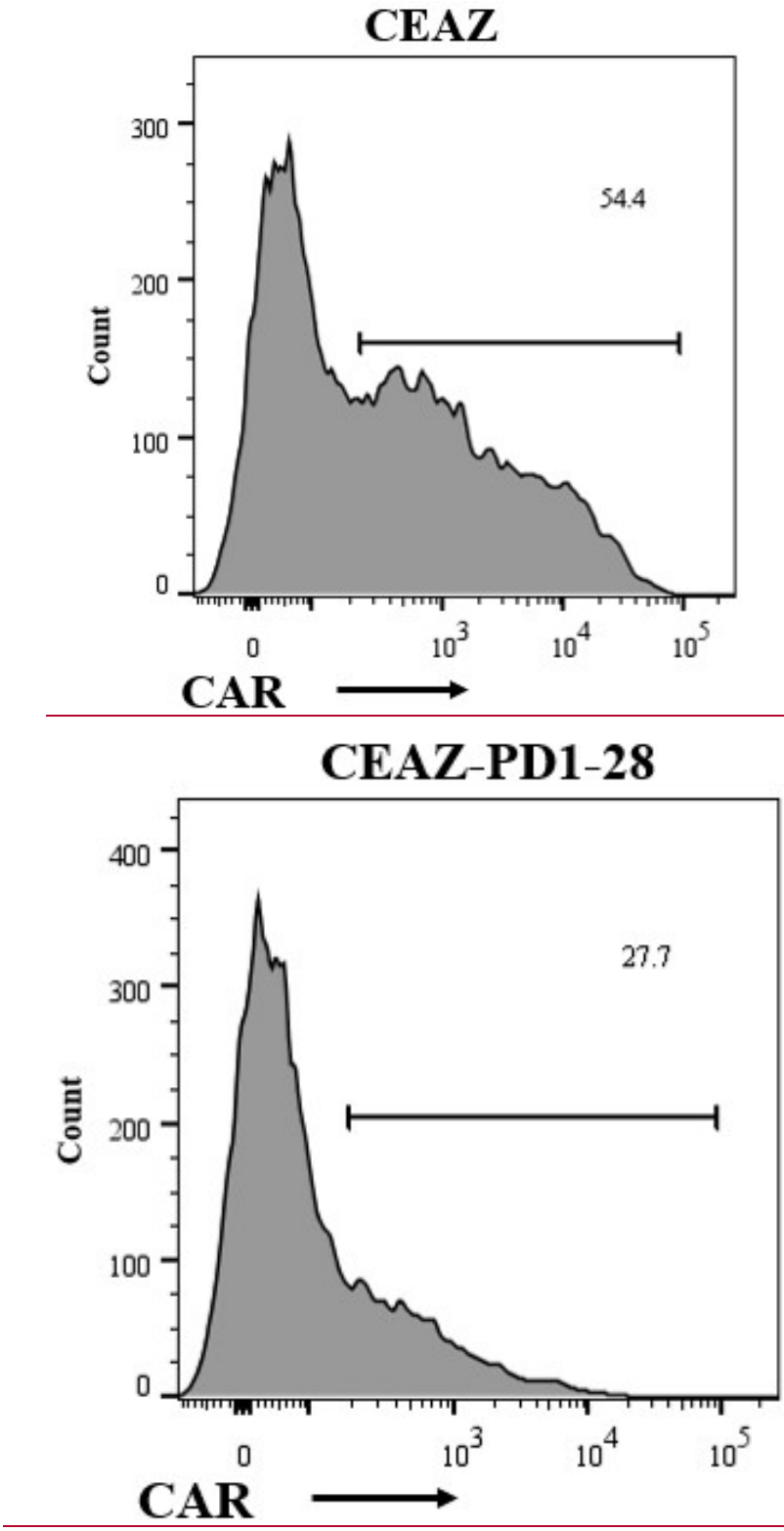

CEAZ
300
200
100
0
Count
54.4
0
10^3
10^4
10^5
CAR
CEAZ-PD1-28
400
300
200
100
0
Count
27.7
0
10^3
10^4
10^5
CAR

Figure 5b

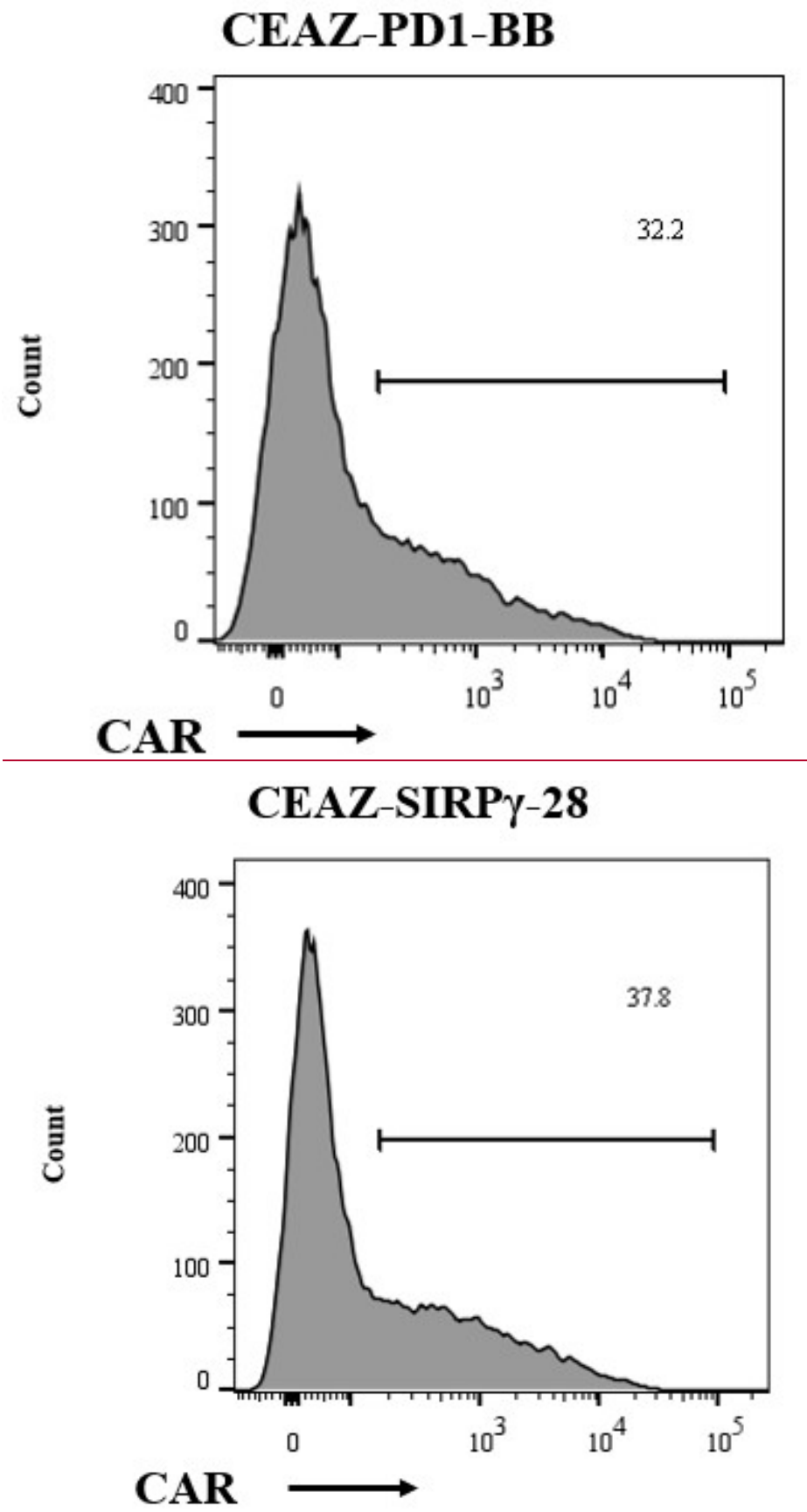
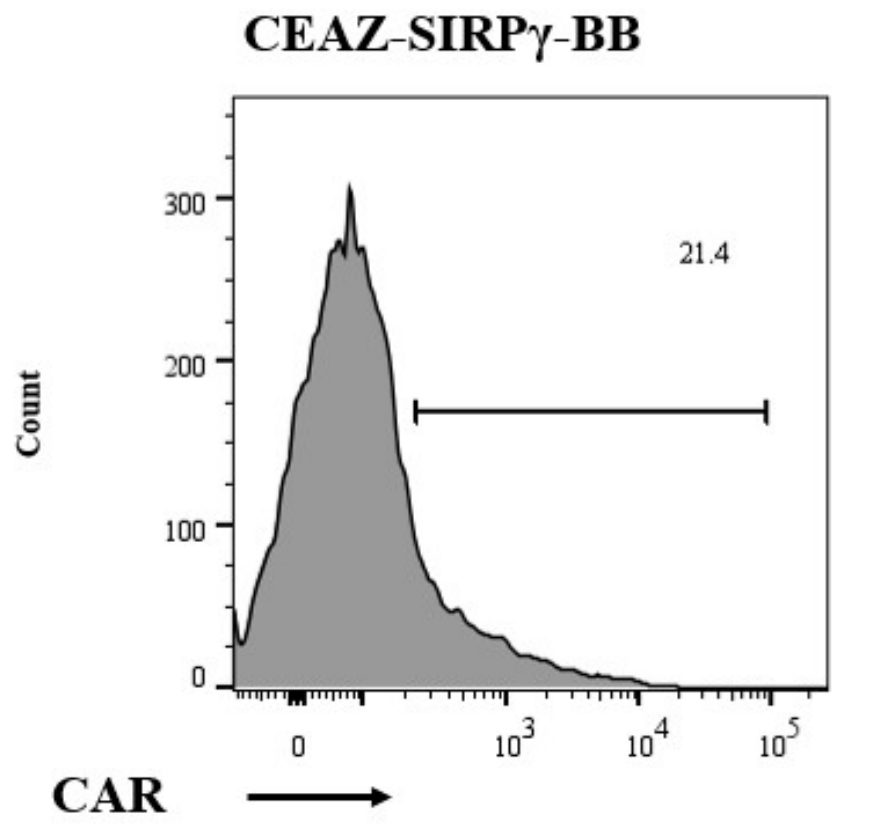


Figure 5b (Cont.)



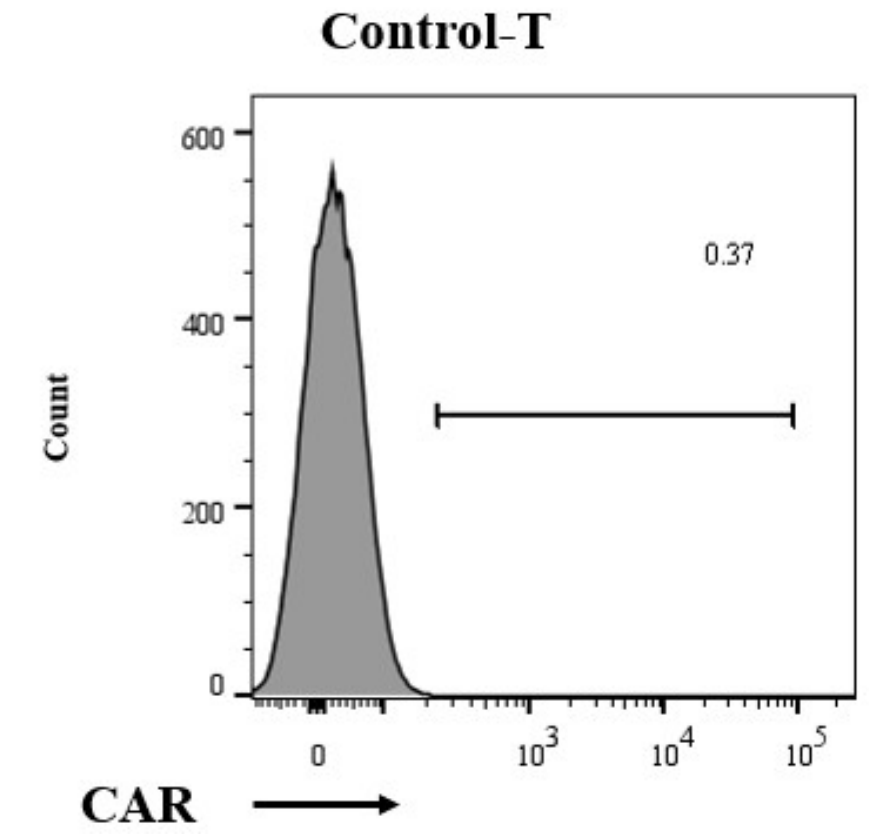
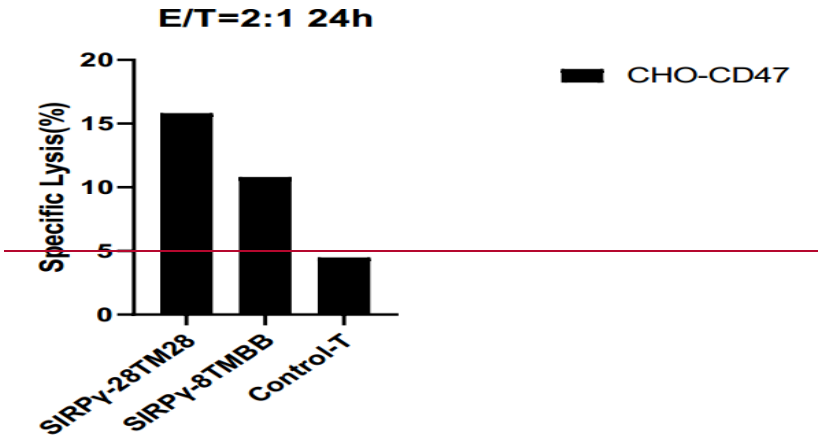


Figure 5b (Cont.)


E/T=2:1 24h
CHO-CD47
Specific Lysis(%)
20
15
10
5
0
SIRPγ-28TM28
SIRPγ-8TMBB
Control-T

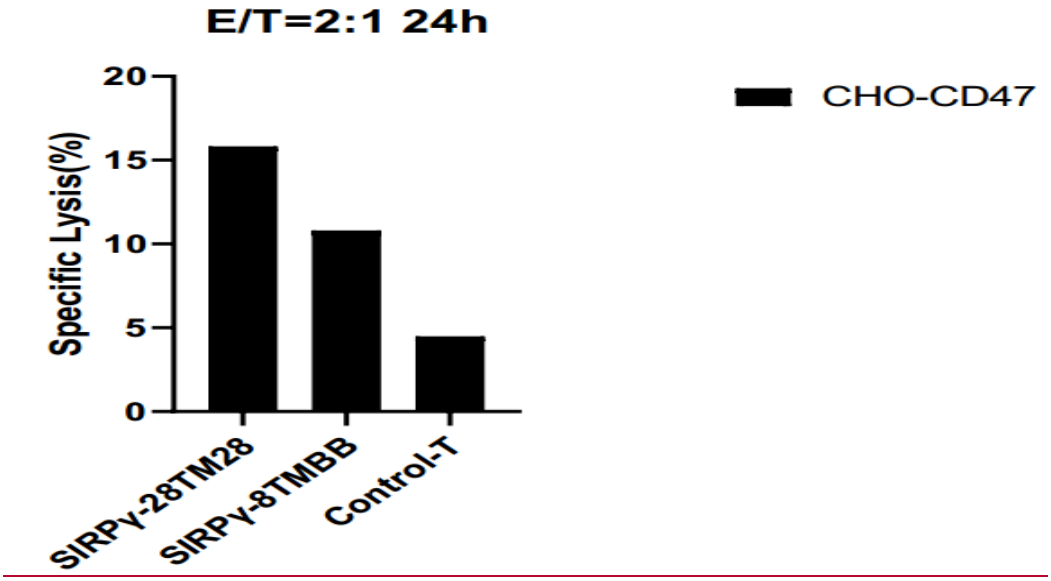

E/T=2:1 24h
CHO-CD47
Specific Lysis(%)
20
15
10
5
0
SIRPγ-28TM28
SIRPγ-8TMBB
Control-T

Figure 6a

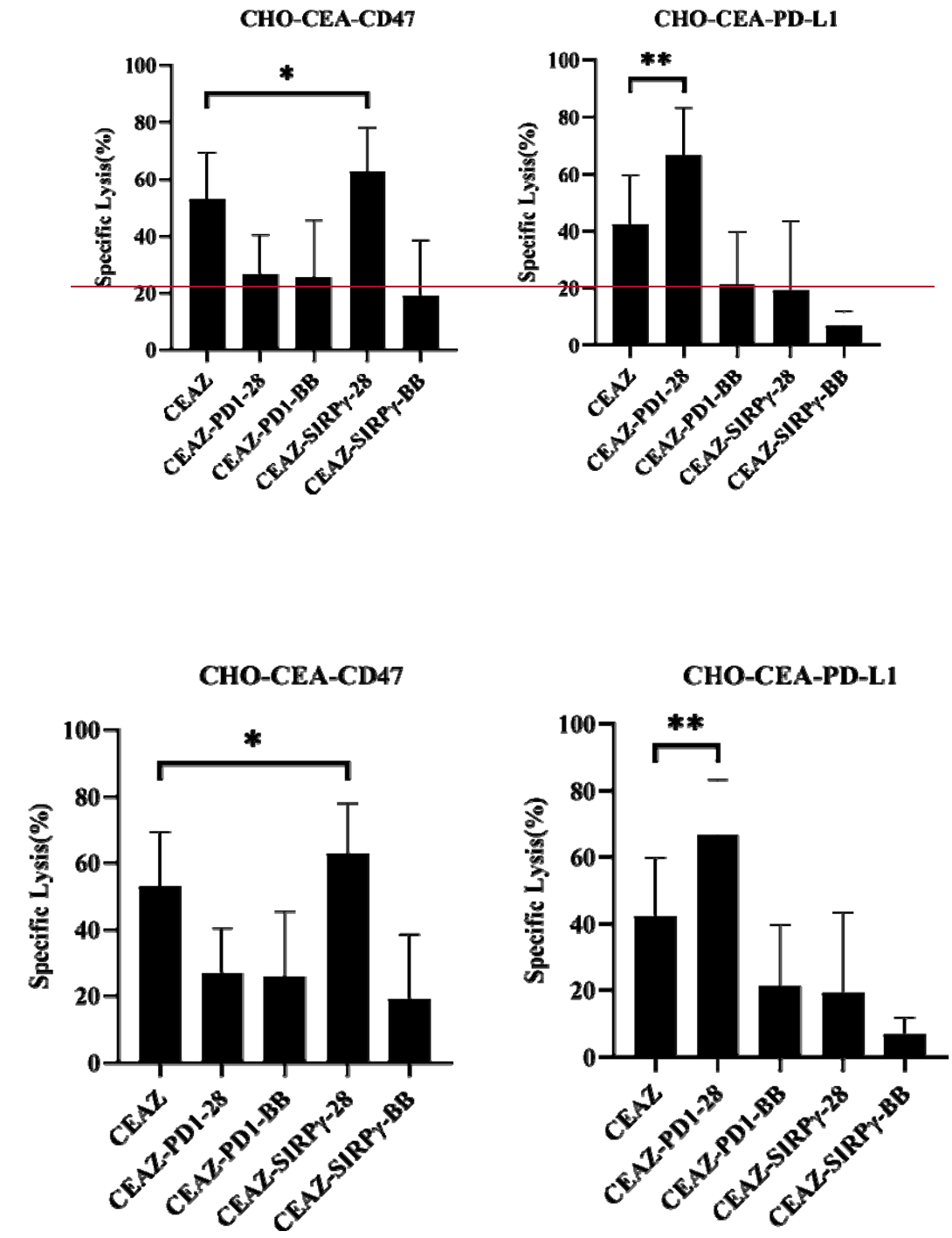

CHO-CEA-CD47
Specific Lysis(%)
100
80
60
40
20
0
*
CEAZ
CEAZ-PD1-28
CEAZ-PD1-BB
CEAZ-SIRPγ-28
CEAZ-SIRPγ-BB
CHO-CEA-PD-L1
Specific Lysis(%)
100
80
60
40
20
0
**
CEAZ
CEAZ-PD1-28
CEAZ-PD1-BB
CEAZ-SIRPγ-28
CEAZ-SIRPγ-BB
CHO-CEA-CD47
Specific Lysis(%)
100
80
60
40
20
0
*
CEAZ
CEAZ-PD1-28
CEAZ-PD1-BB
CEAZ-SIRPγ-28
CEAZ-SIRPγ-BB
CHO-CEA-PD-L1
Specific Lysis(%)
100
80
60
40
20
0
**
CEAZ
CEAZ-PD1-28
CEAZ-PD1-BB
CEAZ-SIRPγ-28
CEAZ-SIRPγ-BB

Figure 6b

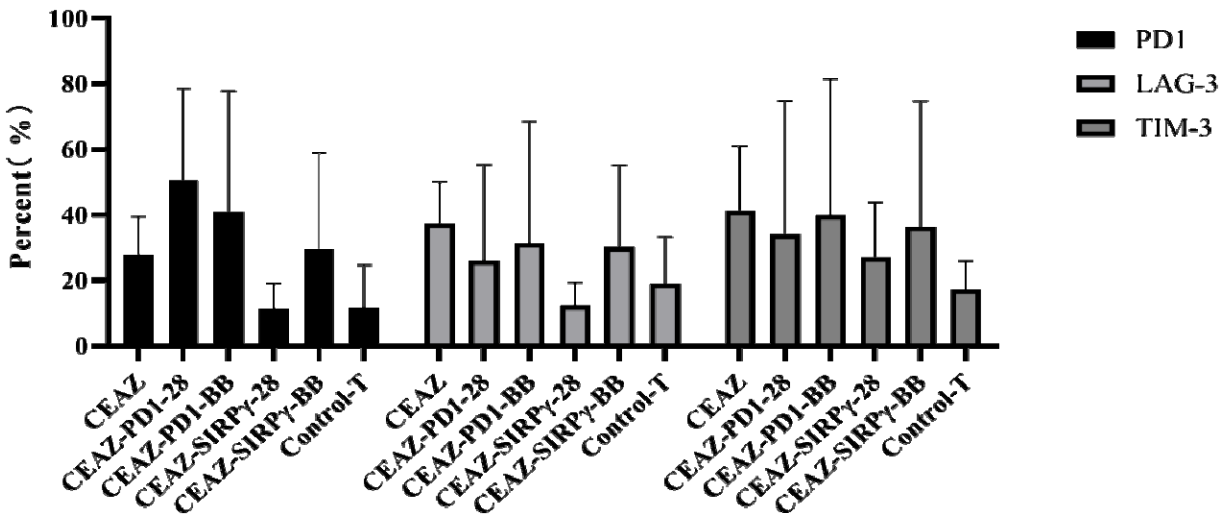
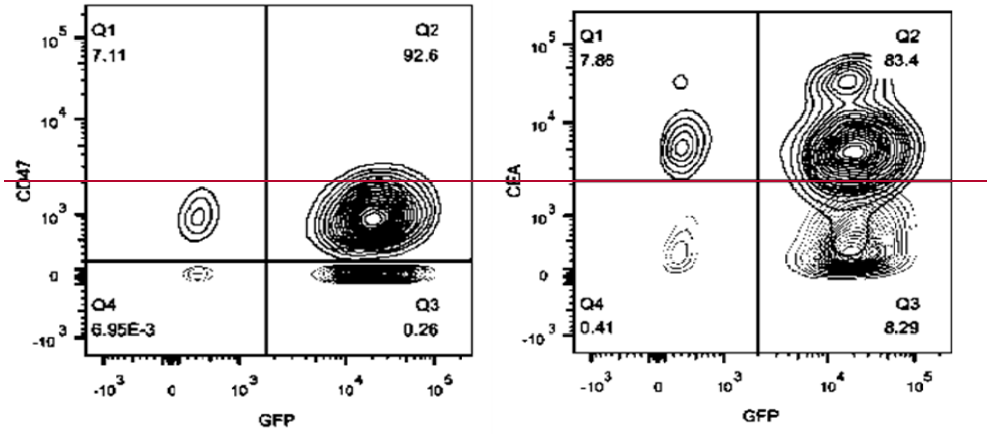
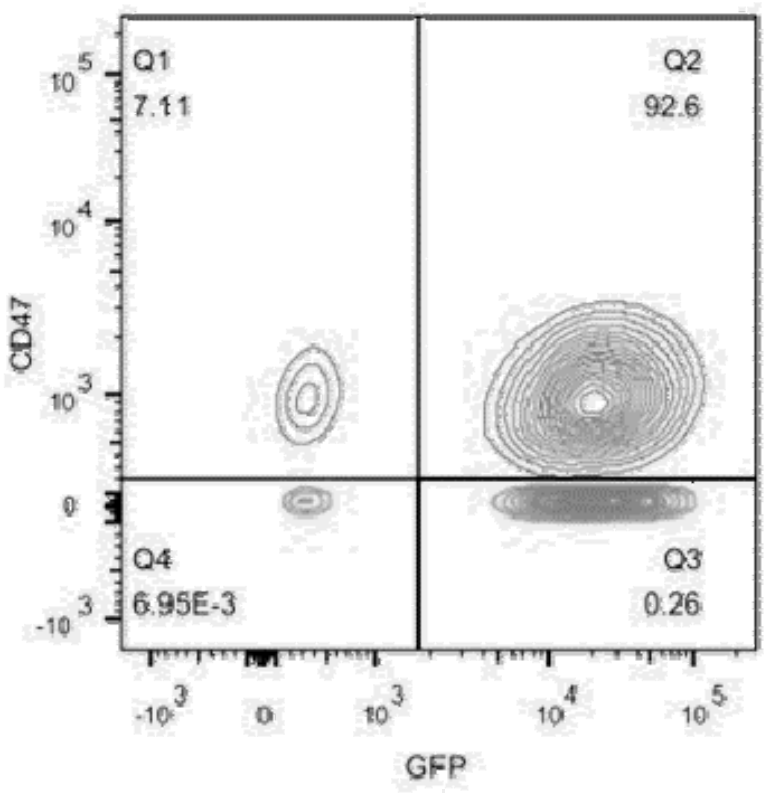

Percent(%)
100
80
60
40
20
0
PD1
LAG-3
TIM-3
CEAZ
CEAZ-PD1-28
CEAZ-PD1-BB
CEAZ-SIRPγ-28
CEAZ-SIRPγ-BB
Control-T

Figure 7

DLD-1-CEA-luc-GFP





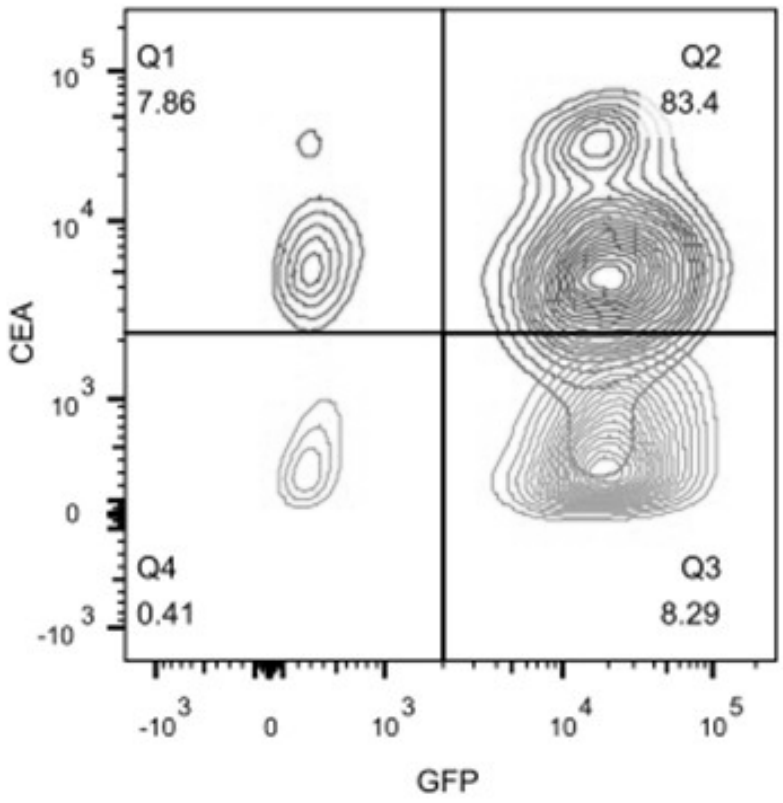
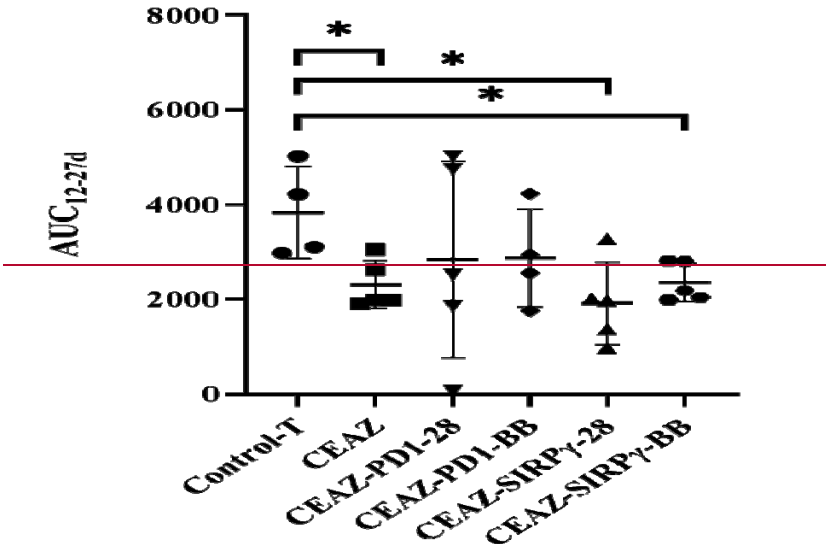

Q1
7.86
Q2
83.4
Q4
0.41
Q3
8.29
CEA
GFP
10^5
10^4
10^3
0
-10^3
-10^3
0
10^3
10^4
10^5

Figure 8a


8000
6000
4000
2000
0
AUC_{12-27d}
*
*
*
Control-T
CEAZ
CEAZ-PD1-28
CEAZ-PD1-BB
CEAZ-SIRPγ-28
CEAZ-SIRPγ-BB

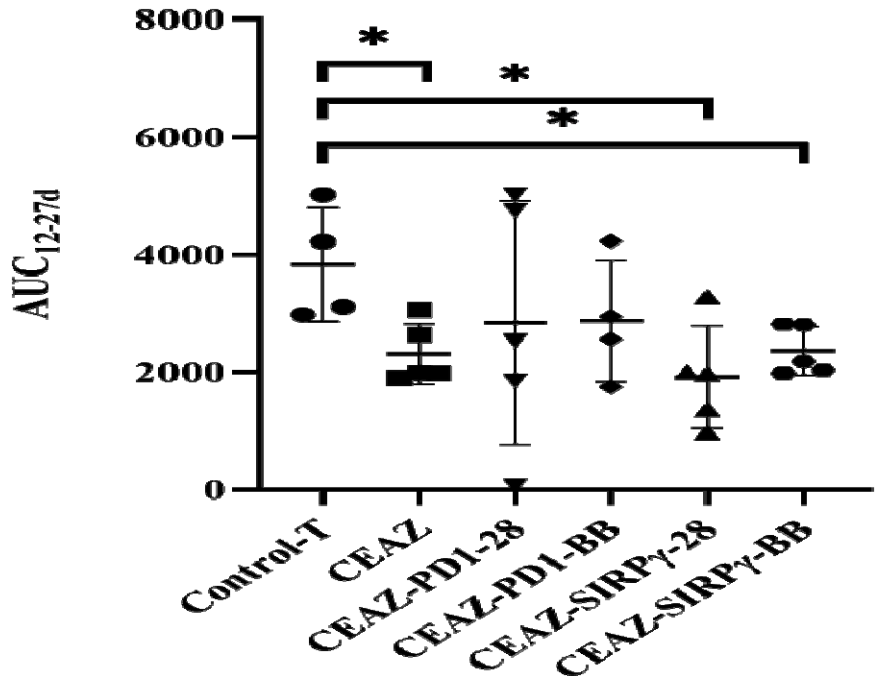


Figure 8b

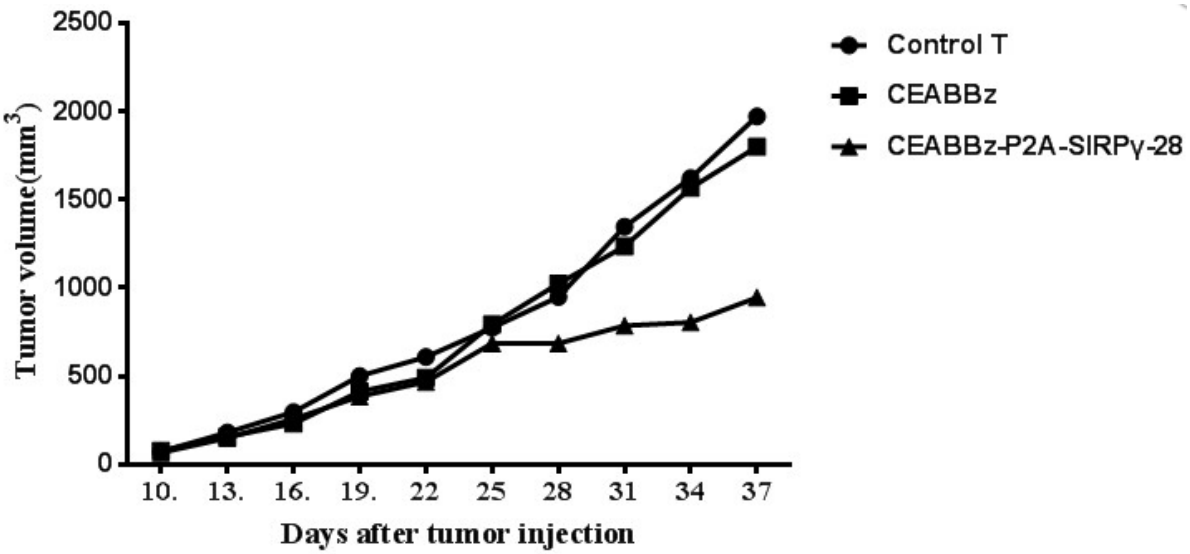


Figure 9

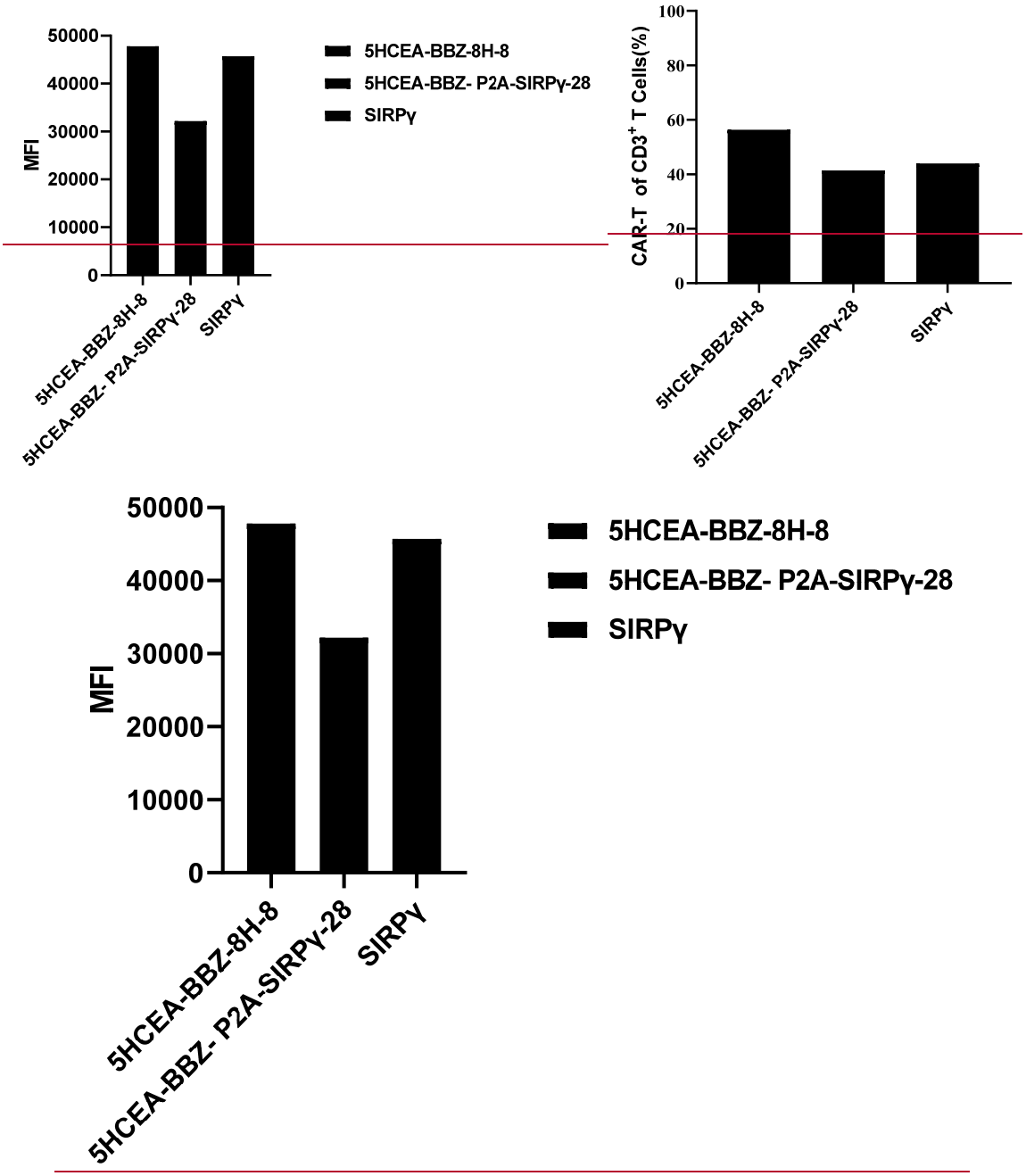

MFI
50000
40000
30000
20000
10000
0
5HCEA-BBZ-8H-8
5HCEA-BBZ- P2A-SIRPγ-28
SIRPγ
CAR-T of CD3+ T Cells(%)
100
80
60
40
20
0

Figure 10

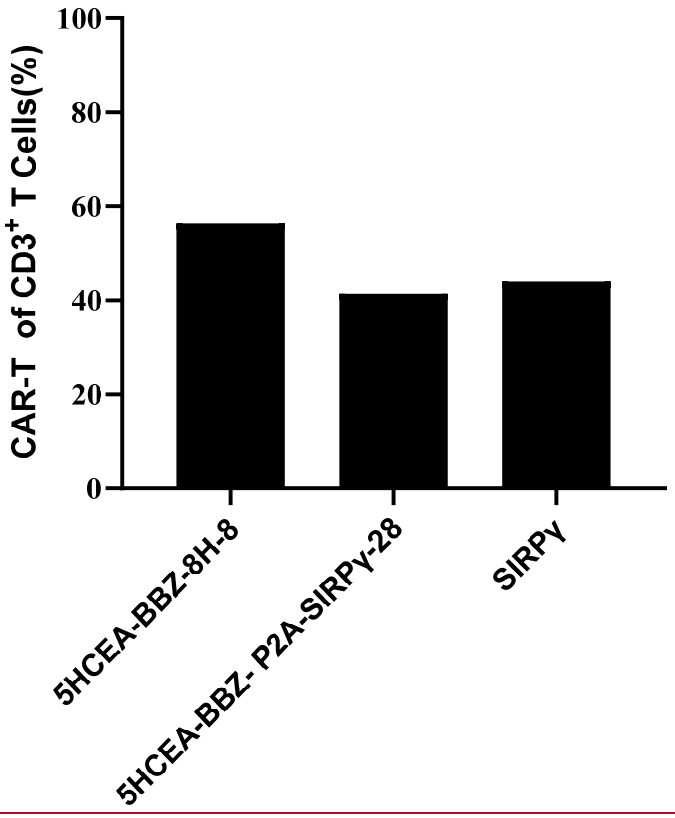


Figure 11

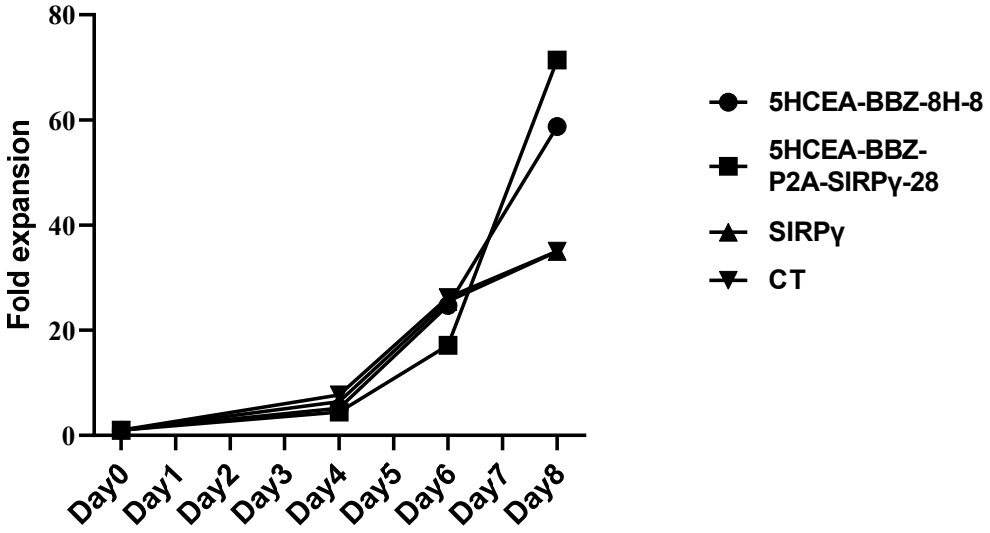
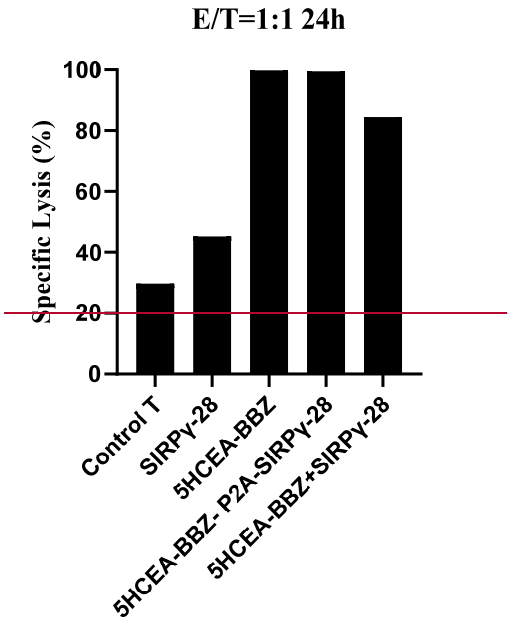


Figure 12


E/T=1:1 24h
Specific Lysis (%)
100
80
60
40
20
0
Control T
SIRPγ-28
5HCEA-BBZ
5HCEA-BBZ- P2A-SIRPγ-28
5HCEA-BBZ+SIRPγ-28

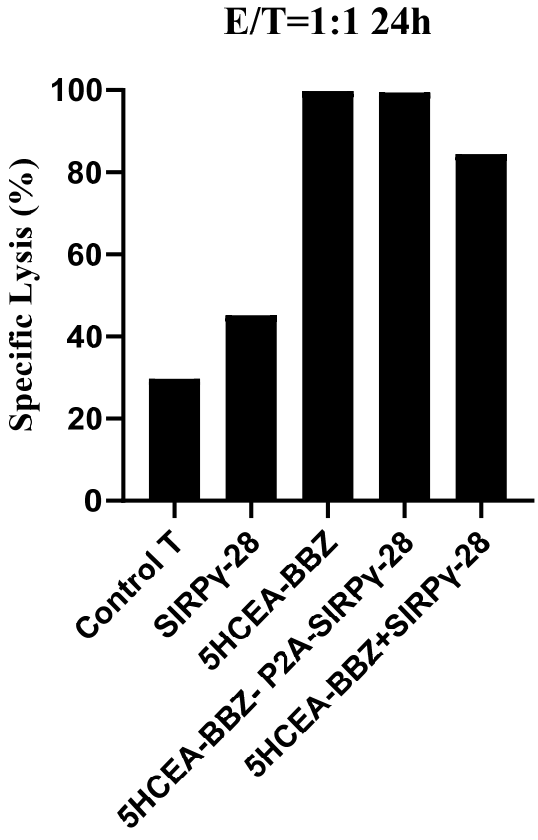
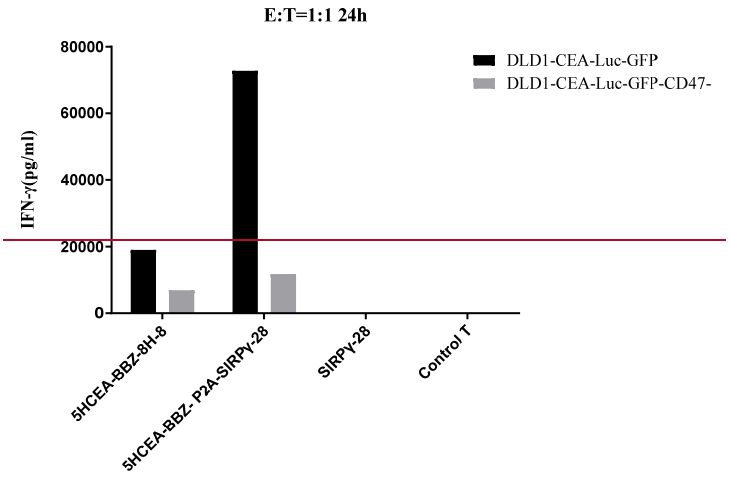

E/T=1:1 24h
Specific Lysis (%)
100
80
60
40
20
0
Control T
SIRPγ-28
5HCEA-BBZ
5HCEA-BBZ- P2A-SIRPγ-28
5HCEA-BBZ+SIRPγ-28

Figure 13


E:T=1:1 24h
80000
60000
40000
20000
0
IFN-γ(pg/ml)
DLD1-CEA-Luc-GFP
DLD1-CEA-Luc-GFP-CD47-
5HCEA-BBZ-8H-8
5HCEA-BBZ- P2A-SIRPγ-28
SIRPγ-28
Control T

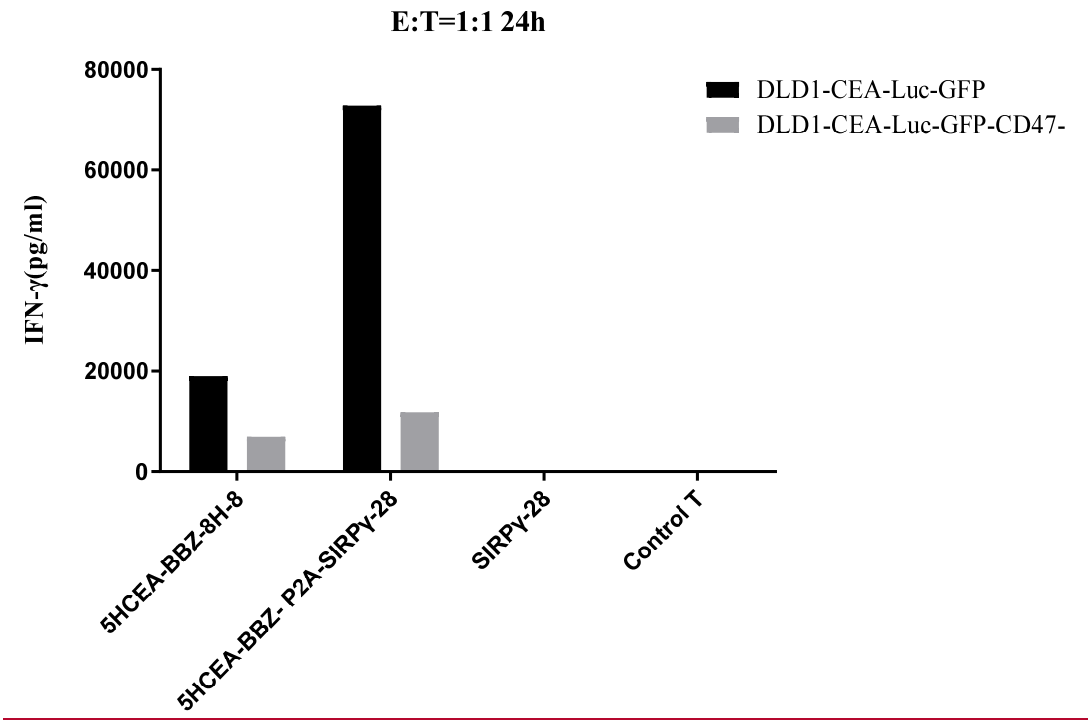

E:T=1:1 24h
80000
60000
40000
20000
0
IFN-γ(pg/ml)
DLD1-CEA-Luc-GFP
DLD1-CEA-Luc-GFP-CD47-
5HCEA-BBZ-8H-8
5HCEA-BBZ- P2A-SIRPγ-28
SIRPγ-28
Control T

Figure 14

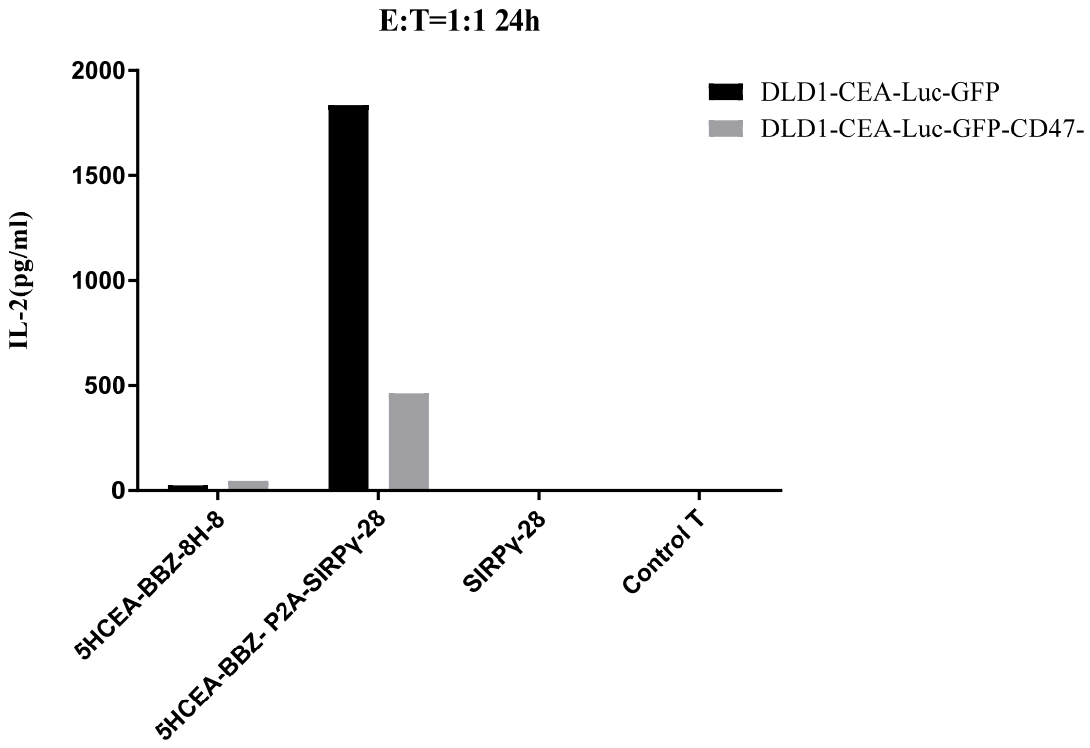


Figure 15

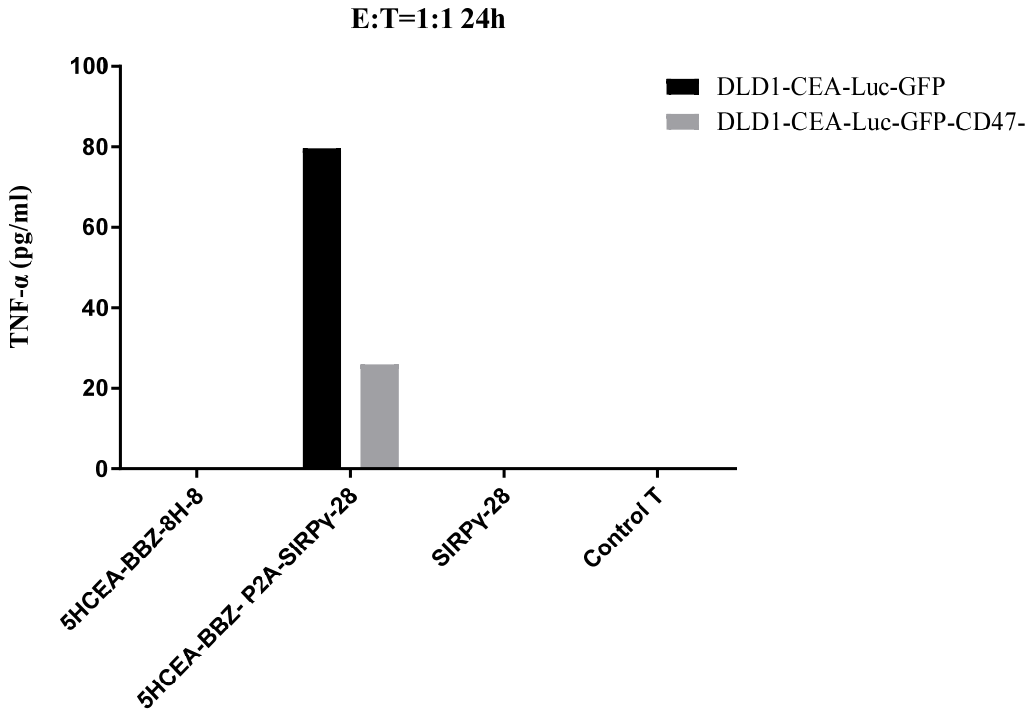
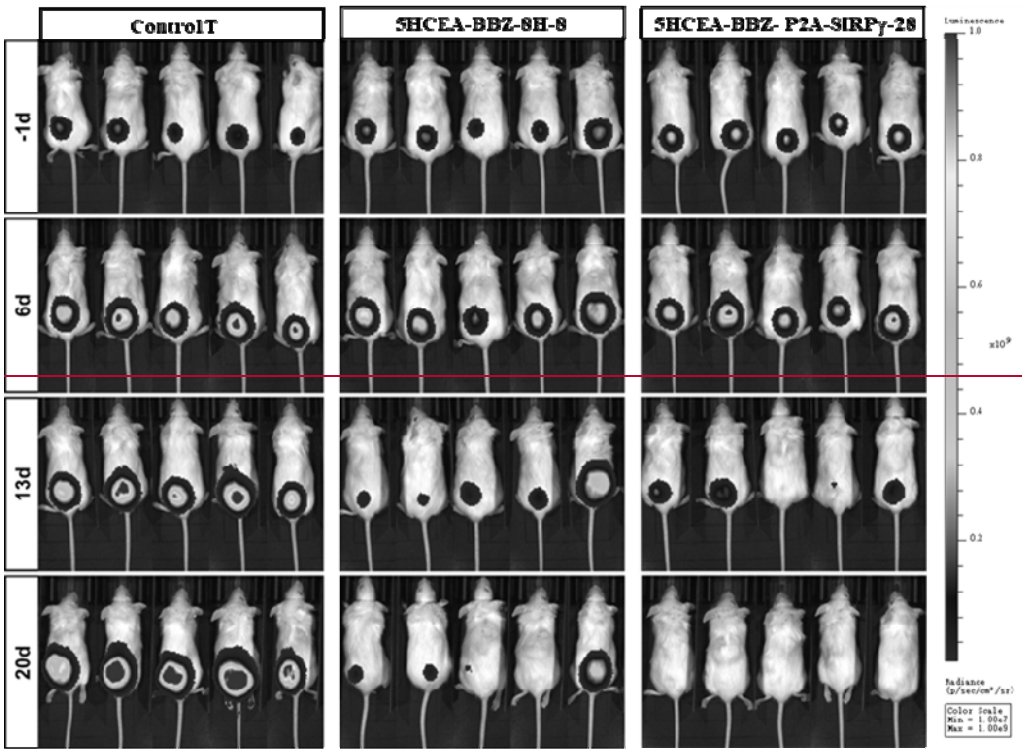


Figure 16


ControlT
5HCEA-BBZ-8H-8
5HCEA-BBZ- P2A-SIRPγ-28
-1d
6d
13d
20d
Luminescence
1.0
0.8
0.6
x10⁹
0.4
0.2
Radiance
(p/sec/cm²/sr)
Color Scale
Min = 1.00e7
Max = 1.00e9

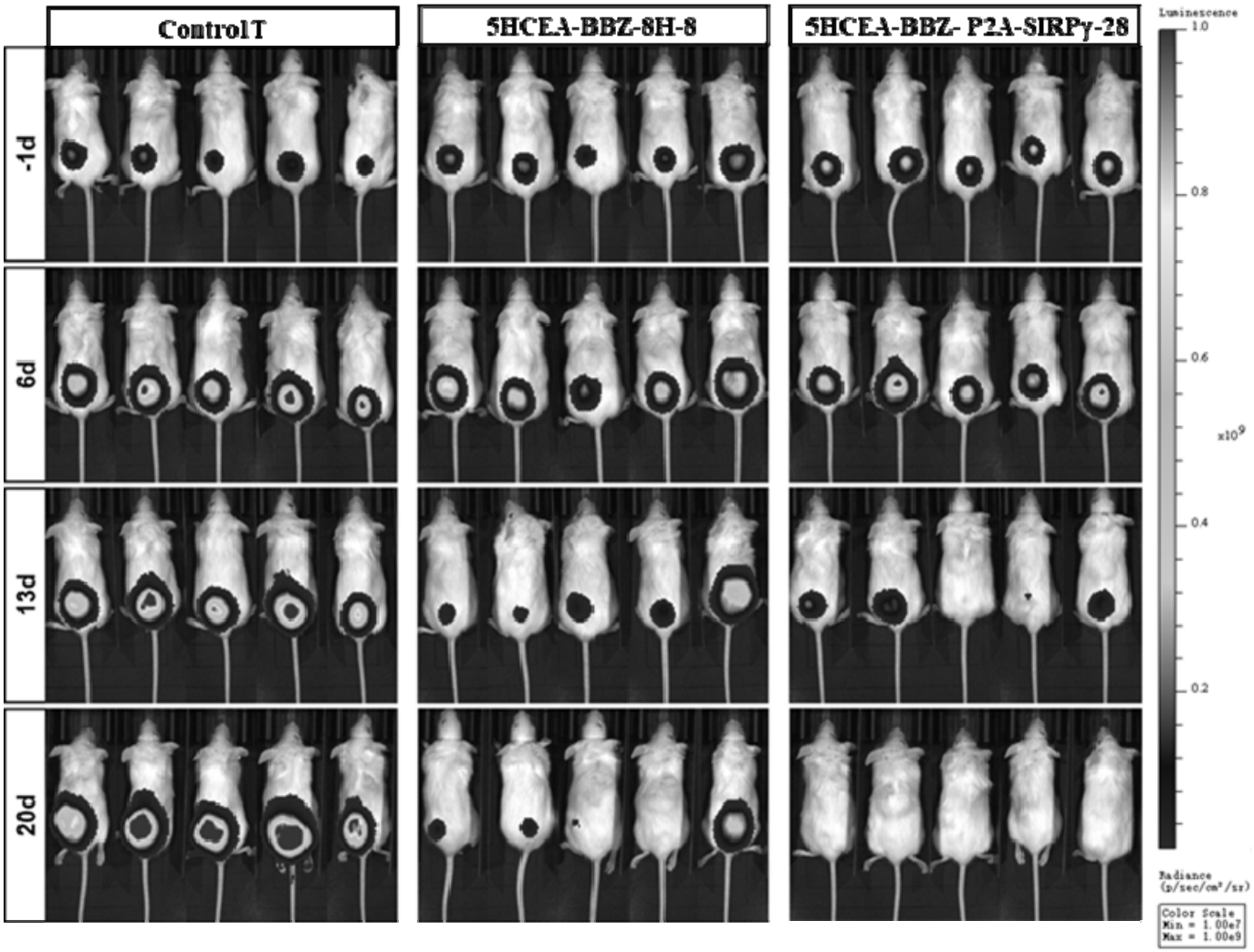

Control T
5HCEA-BBZ-8H-8
5HCEA-BBZ- P2A-SIRPγ-28
-1d
6d
13d
20d
Luminescence
1.0
0.8
0.6
x10^9
0.4
0.2
Radiance
(p/sec/cm²/sr)
Color Scale
Min = 1.00e7
Max = 1.00e9

Figure 17

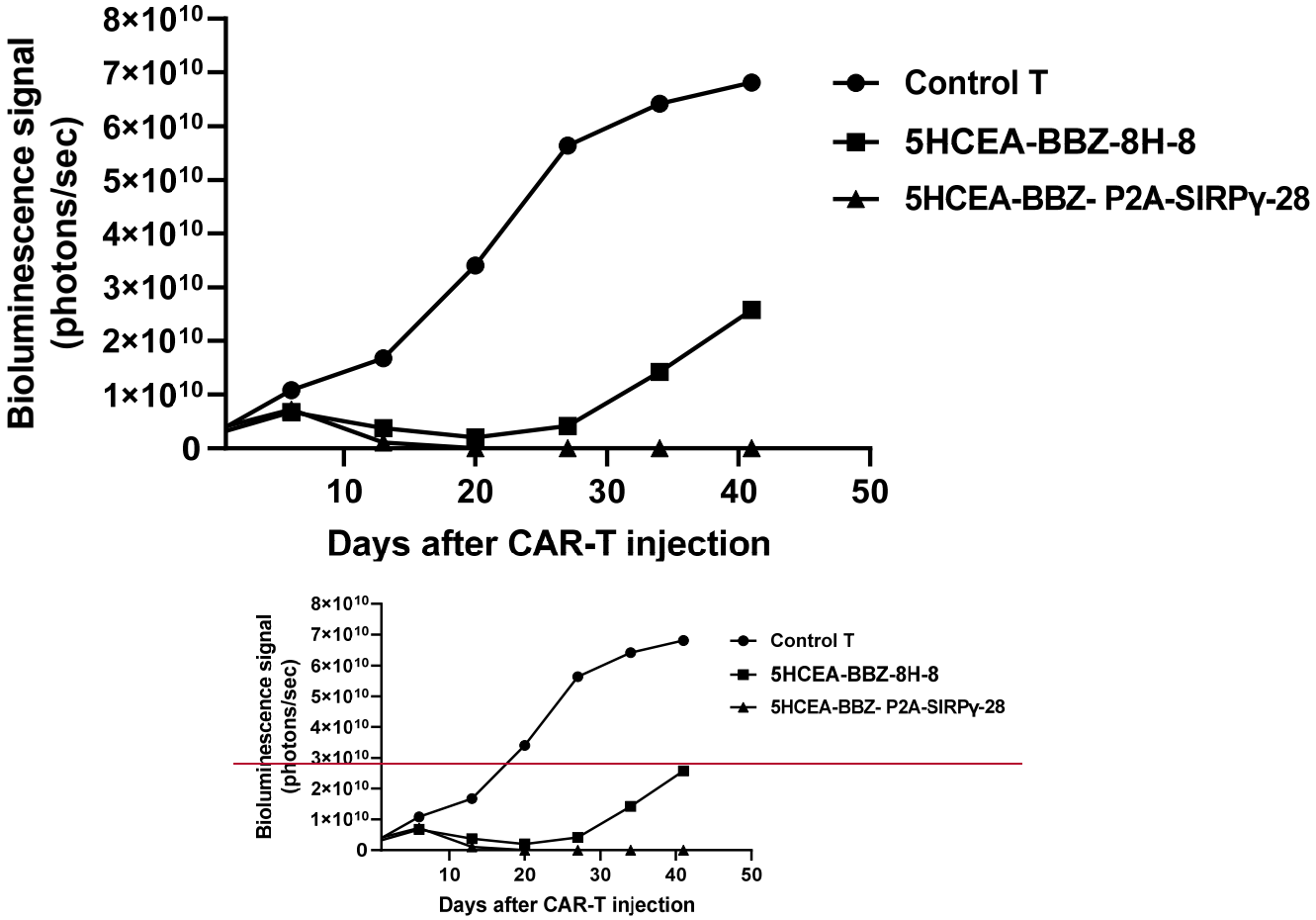
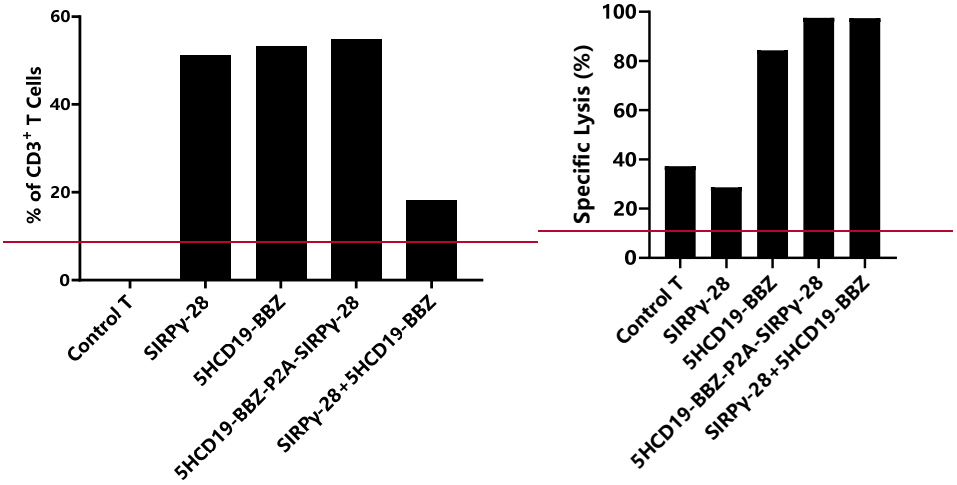

Bioluminescence signal (photons/sec)
8×10^10
7×10^10
6×10^10
5×10^10
4×10^10
3×10^10
2×10^10
1×10^10
0
10
20
30
40
50
Days after CAR-T injection
Control T
5HCEA-BBZ-8H-8
5HCEA-BBZ- P2A-SIRPγ-28

Figure 18


% of CD3+ T Cells
60
40
20
0
Control T
SIRPγ-28
5HCD19-BBZ
5HCD19-BBZ-P2A-SIRPγ-28
SIRPγ-28+5HCD19-BBZ
Specific Lysis (%)
100
80
60
40
20
0
Control T
SIRPγ-28
5HCD19-BBZ
5HCD19-BBZ-P2A-SIRPγ-28
SIRPγ-28+5HCD19-BBZ

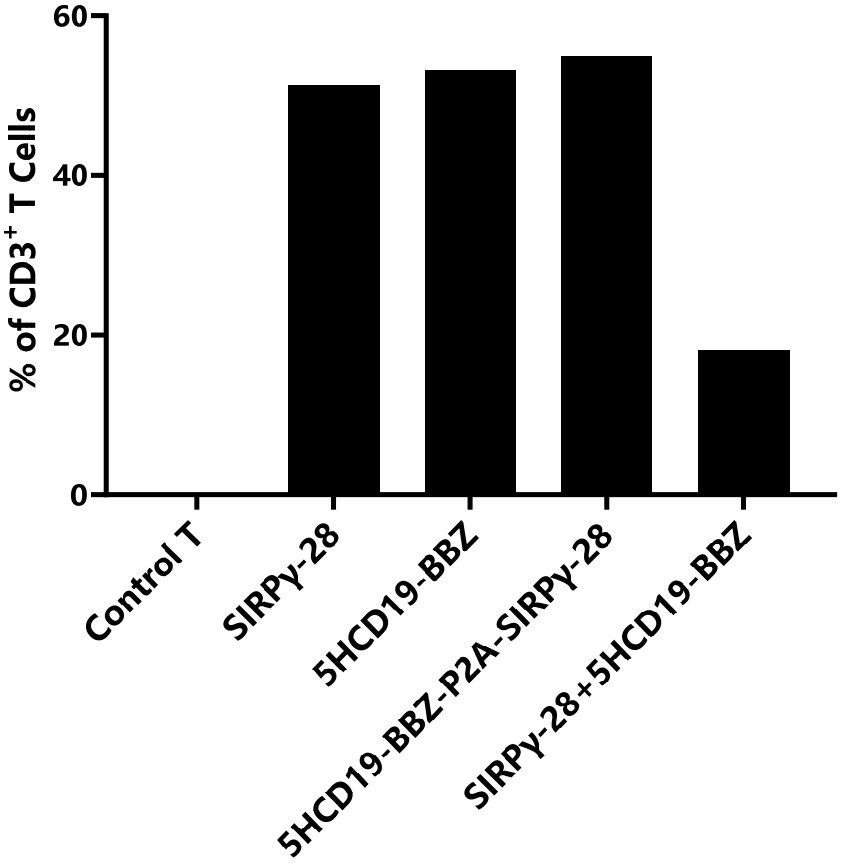


Figure 19

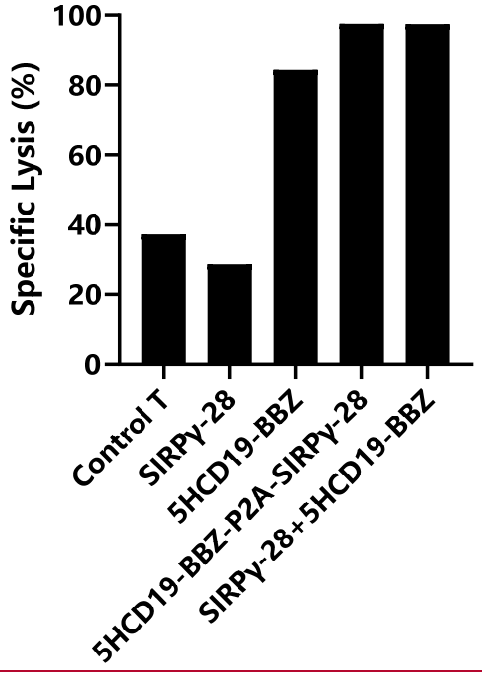
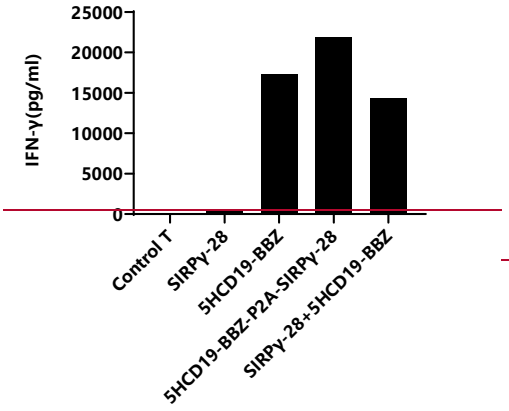
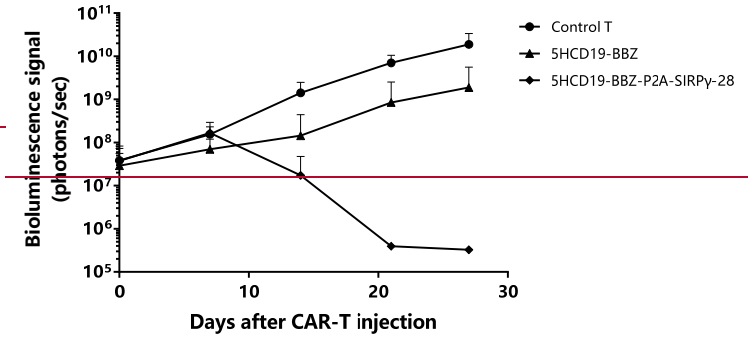


Figure 20


IFN-γ(pg/ml)
25000
20000
15000
10000
5000
0
Control T
SIRPγ-28
5HCD19-BBZ
5HCD19-BBZ-P2A-SIRPγ-28
SIRPγ-28+5HCD19-BBZ


Bioluminescence signal (photons/sec)
Days after CAR-T injection
Control T
5HCD19-BBZ
5HCD19-BBZ-P2A-SIRPγ-28

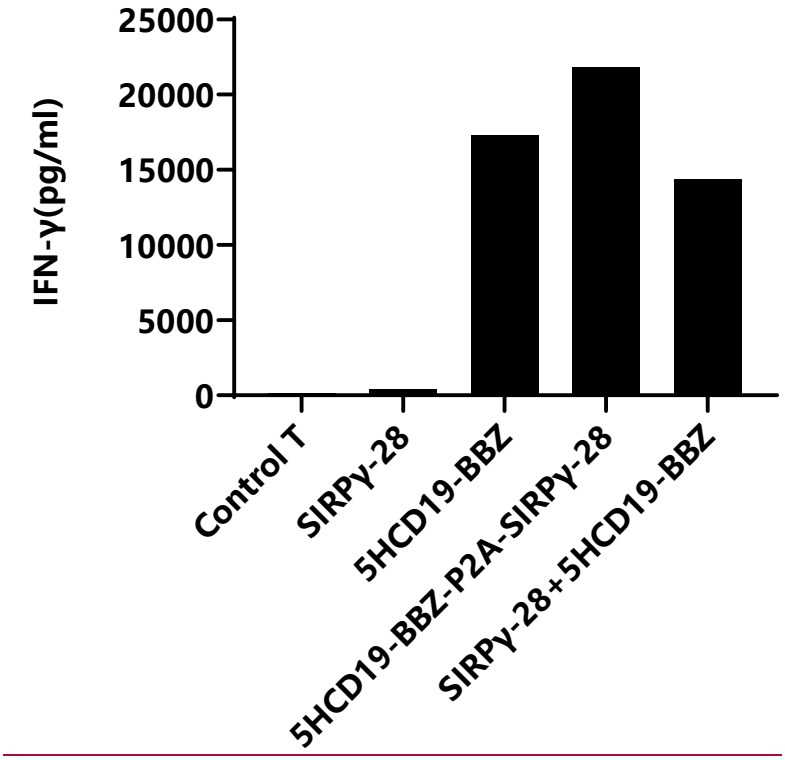

IFN-γ(pg/ml)
25000
20000
15000
10000
5000
0
Control T
SIRPγ-28
5HCD19-BBZ
5HCD19-BBZ-P2A-SIRPγ-28
SIRPγ-28+5HCD19-BBZ

Figure 21

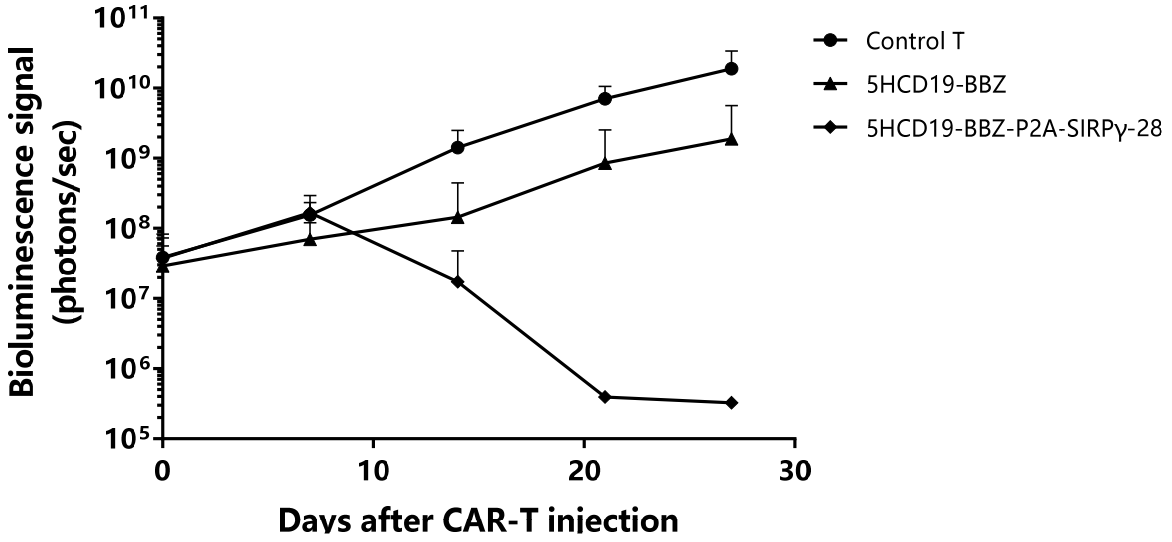


Figure 22

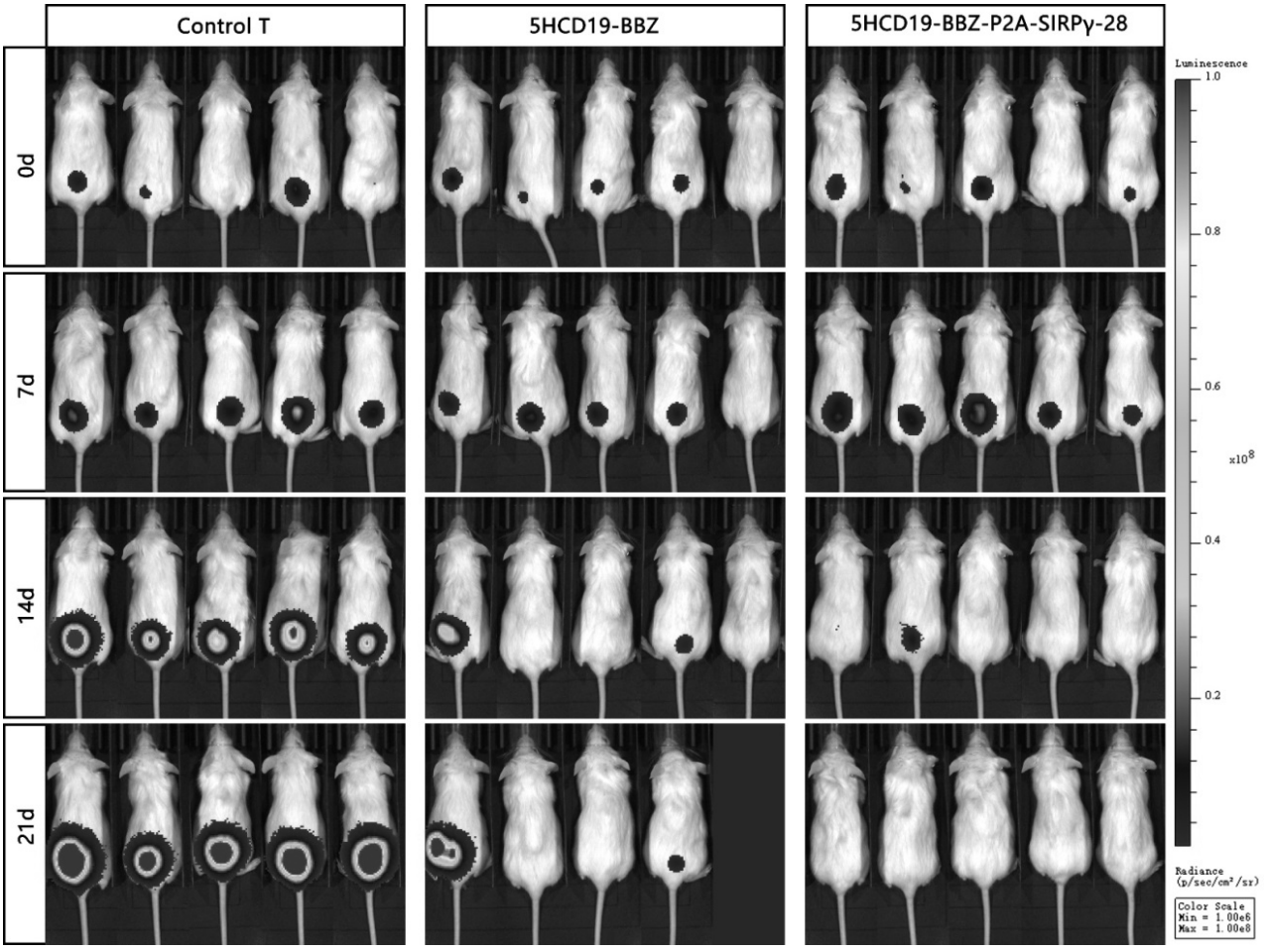
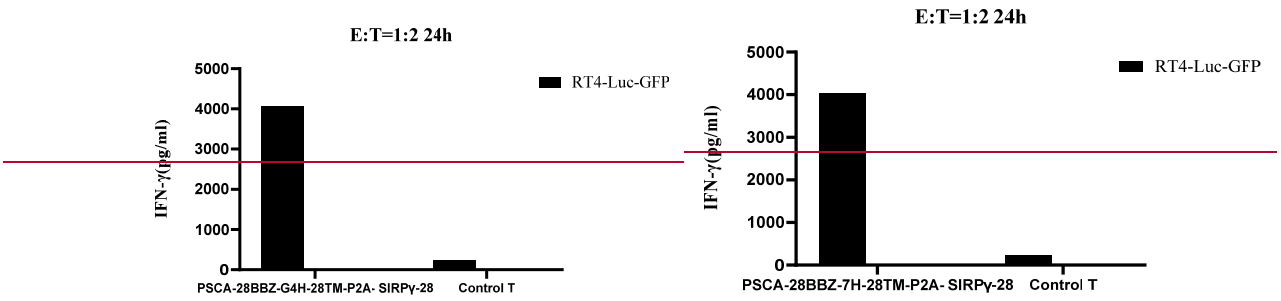


Figure 23



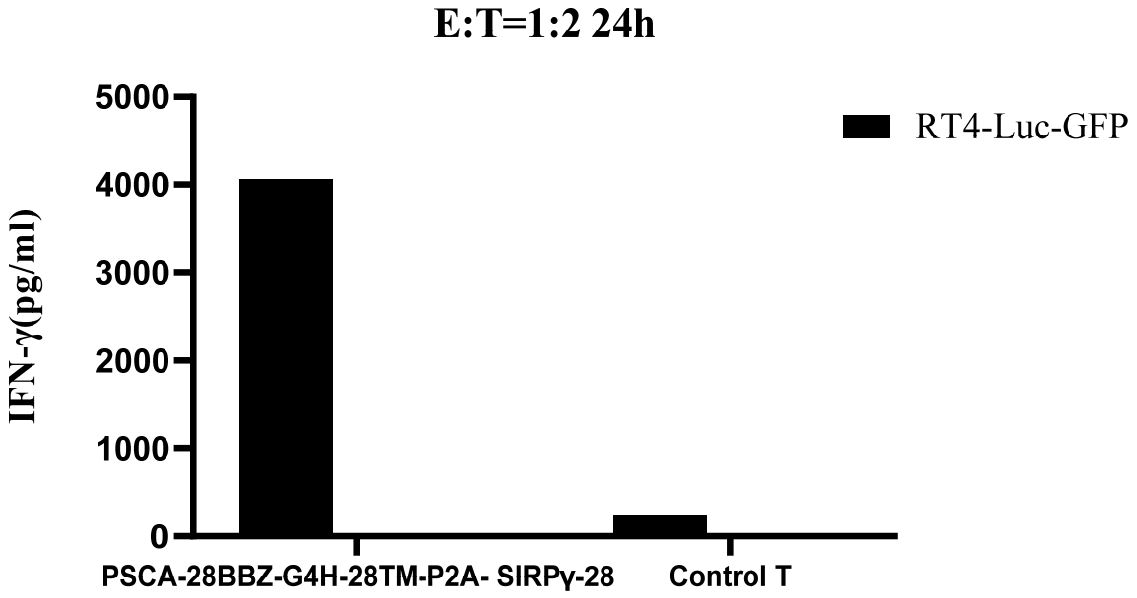


Figure 24

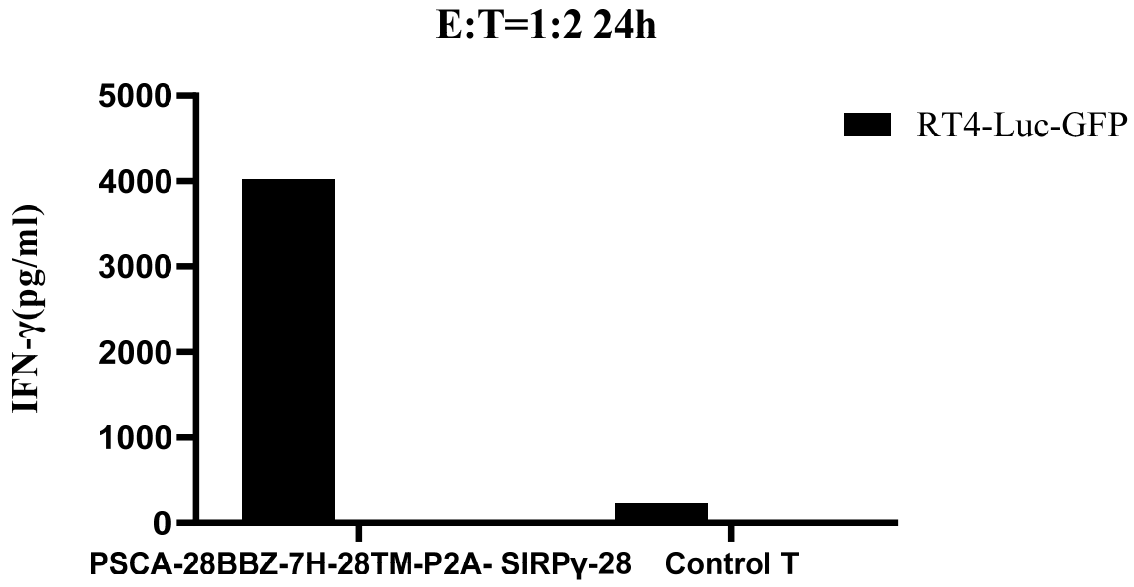
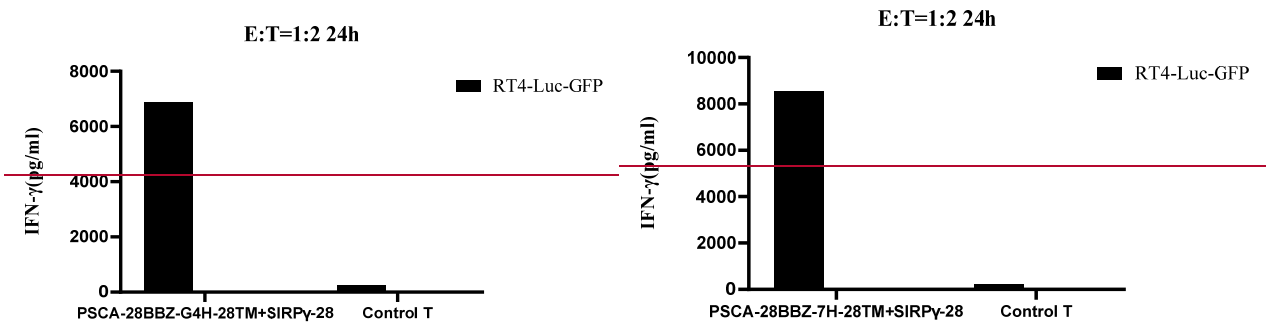


Figure 25



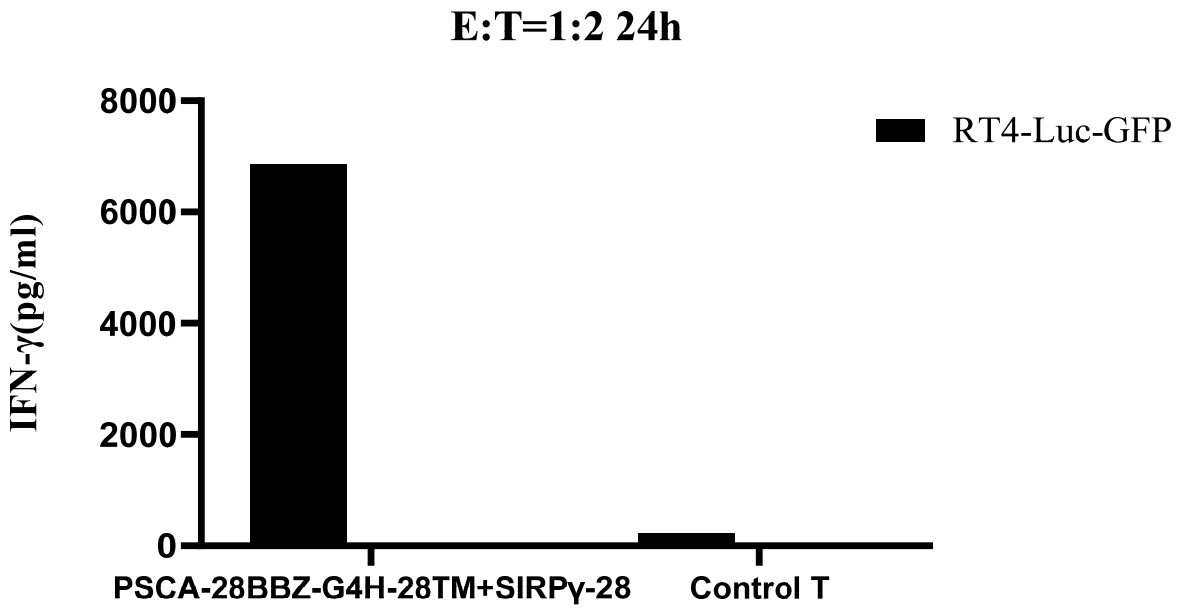


Figure 26

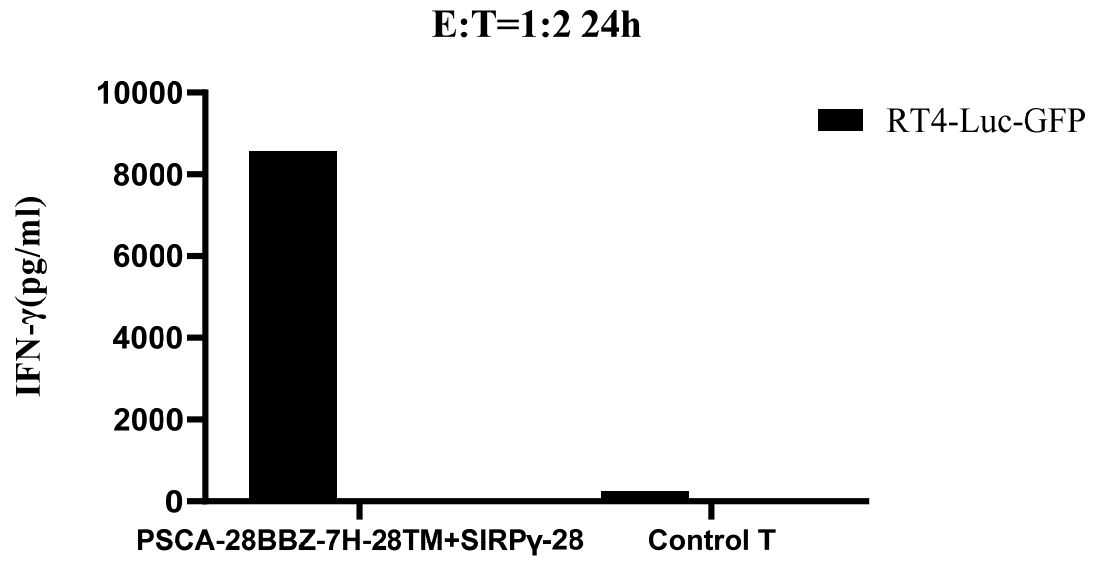
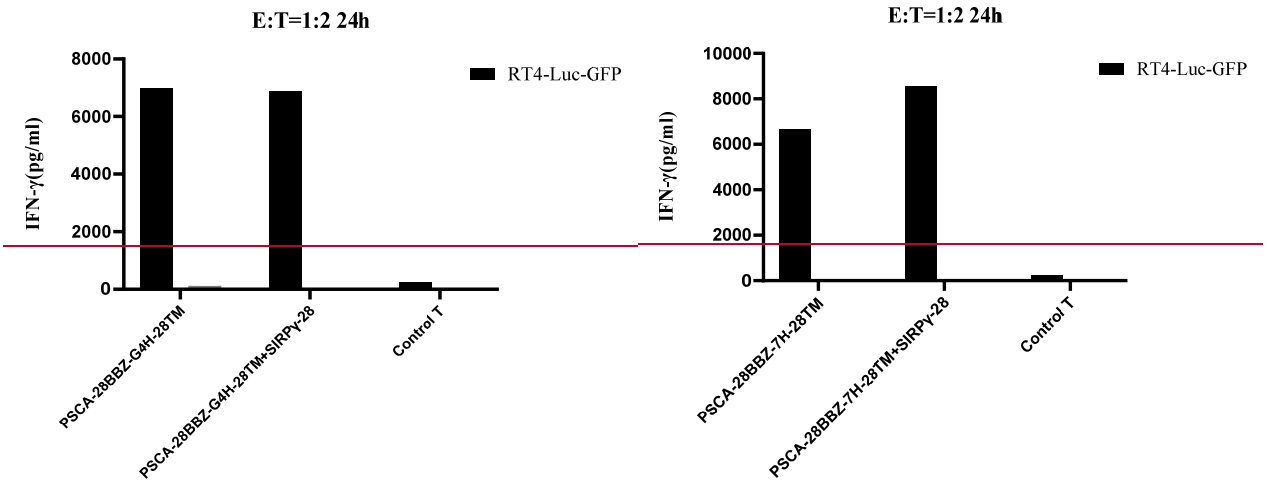


Figure 27


E:T=1:2 24h
IFN-γ(pg/ml)
8000
6000
4000
2000
0
RT4-Luc-GFP
PSCA-28BBZ-G4H-28TM
PSCA-28BBZ-G4H-28TM+SIRPγ-28
Control T
E:T=1:2 24h
IFN-γ(pg/ml)
10000
8000
6000
4000
2000
0
RT4-Luc-GFP
PSCA-28BBZ-7H-28TM
PSCA-28BBZ-7H-28TM+SIRPγ-28
Control T

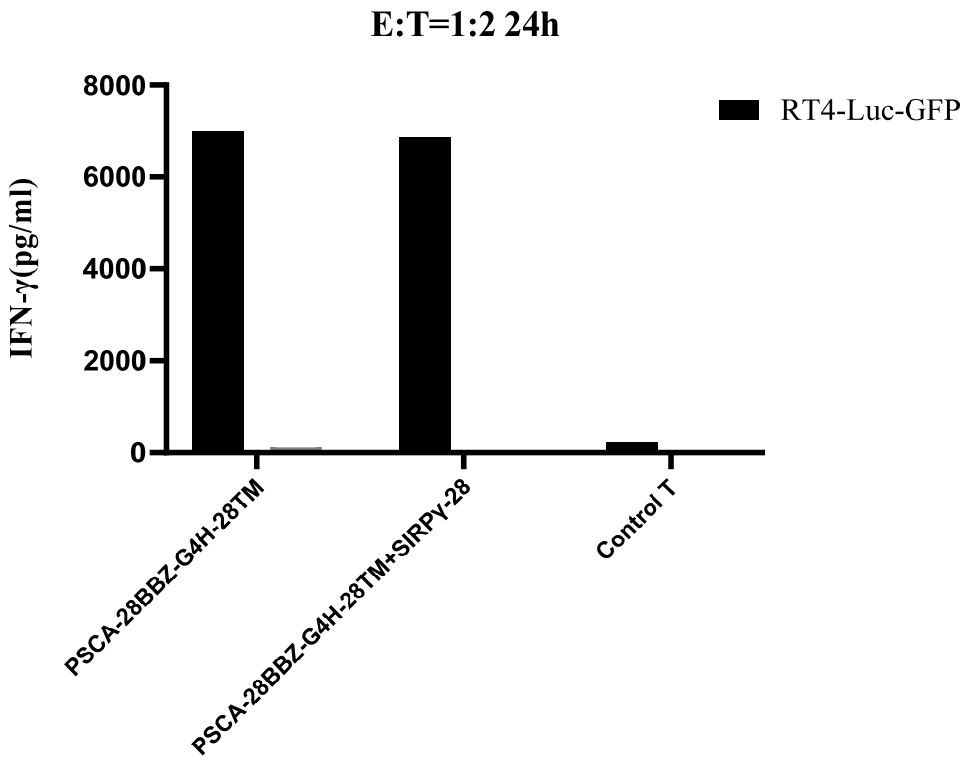

E:T=1:2 24h
IFN-γ(pg/ml)
8000
6000
4000
2000
0
RT4-Luc-GFP
PSCA-28BBZ-G4H-28TM
PSCA-28BBZ-G4H-28TM+SIRPγ-28
Control T

Figure 28

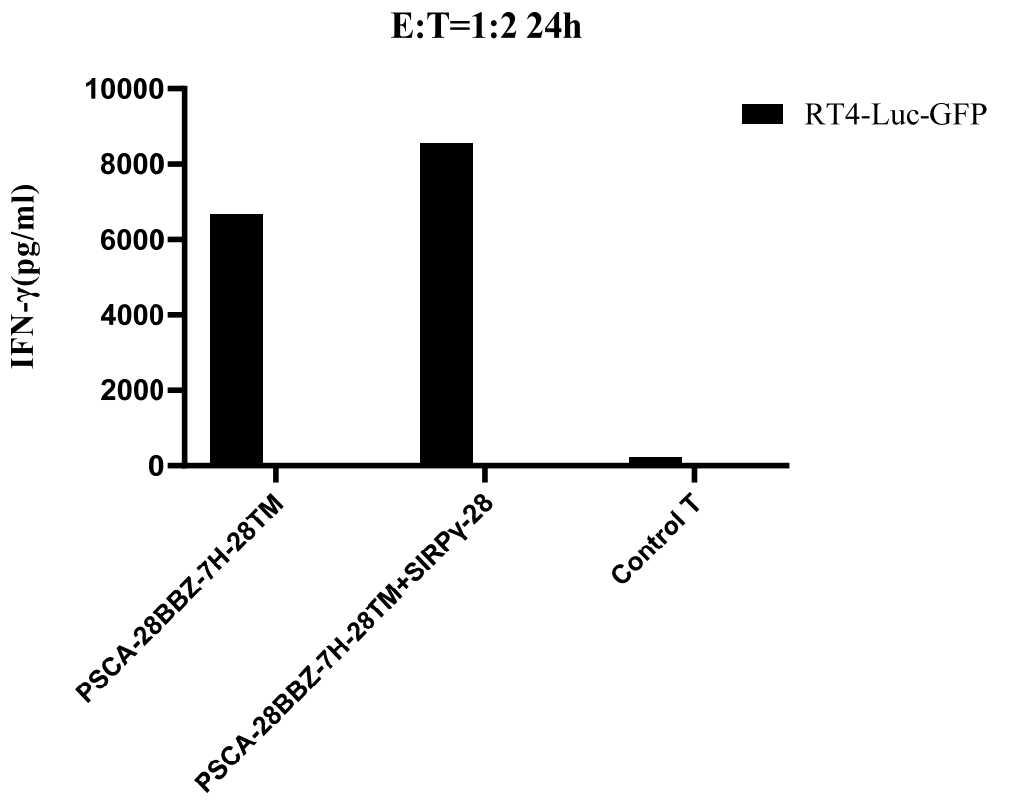
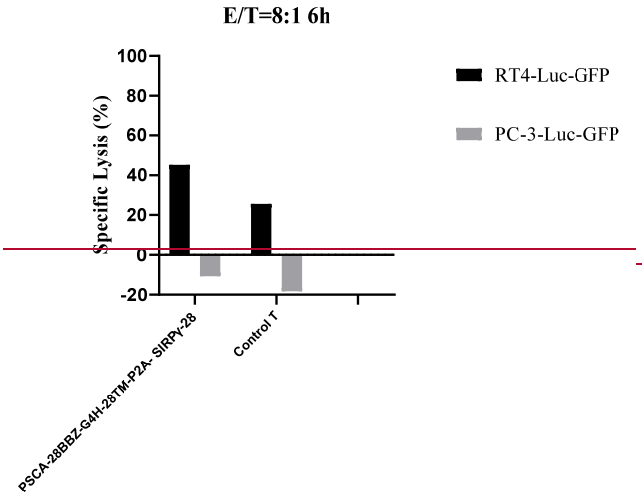
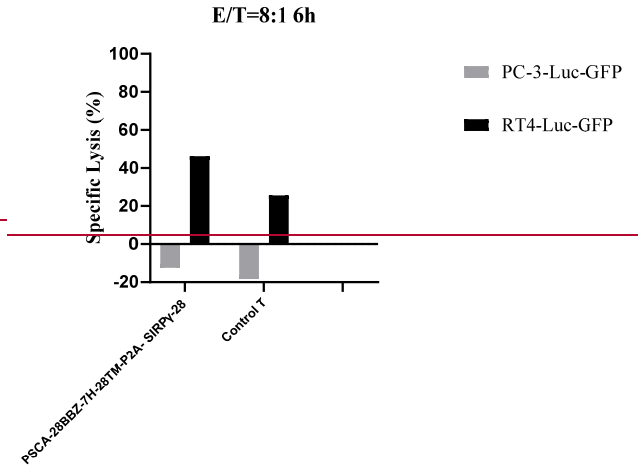

E:T=1:2 24h
RT4-Luc-GFP
IFN-γ(pg/ml)
10000
8000
6000
4000
2000
0
PSCA-28BBZ-7H-28TM
PSCA-28BBZ-7H-28TM+SIRPγ-28
Control T

Figure 29


E/T=8:1 6h
RT4-Luc-GFP
PC-3-Luc-GFP
Specific Lysis (%)
100
80
60
40
20
0
-20
PSCA-28BBZ-G4H-28TM-P2A- SIRPγ-28
Control T


E/T=8:1 6h
PC-3-Luc-GFP
RT4-Luc-GFP
Specific Lysis (%)
100
80
60
40
20
0
-20
PSCA-28BBZ-7H-28TM-P2A- SIRPγ-28
Control T

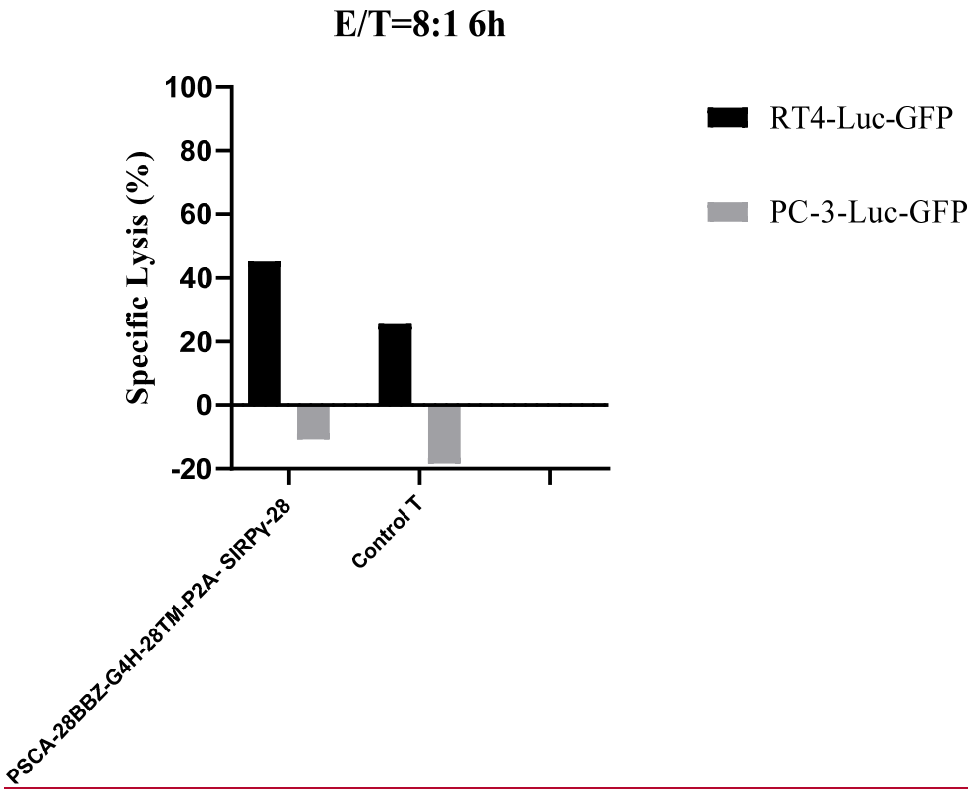


Figure 30

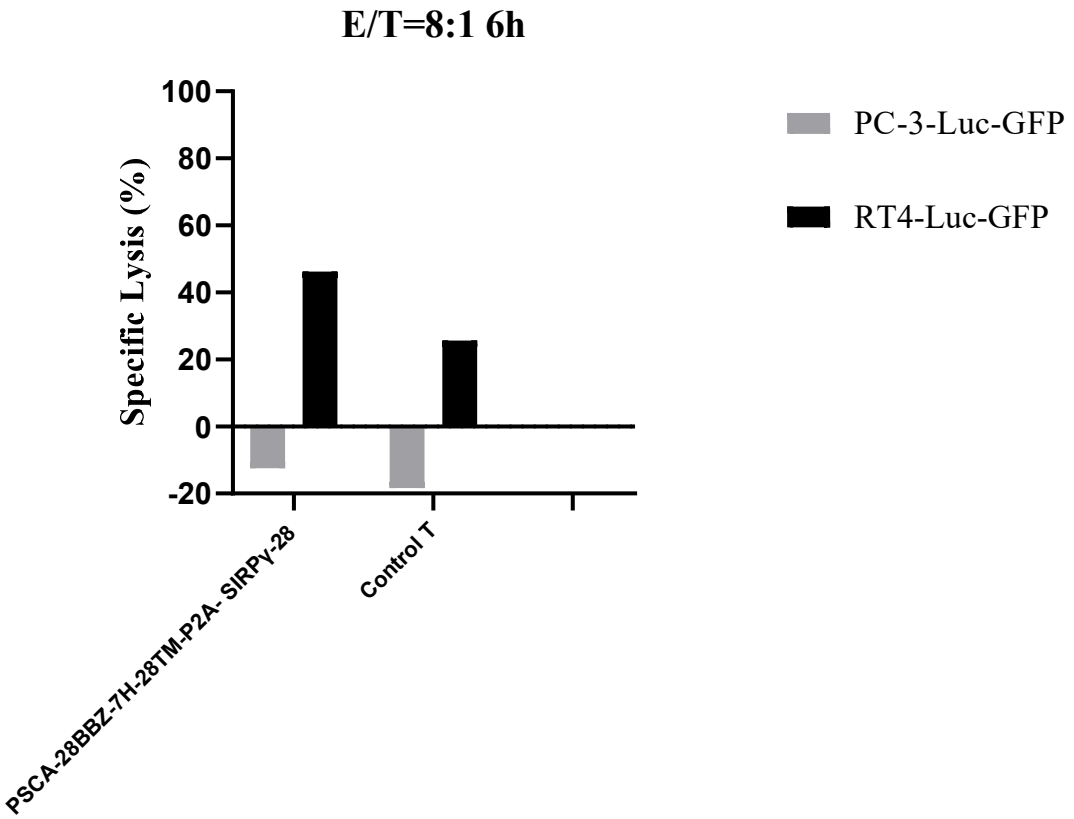
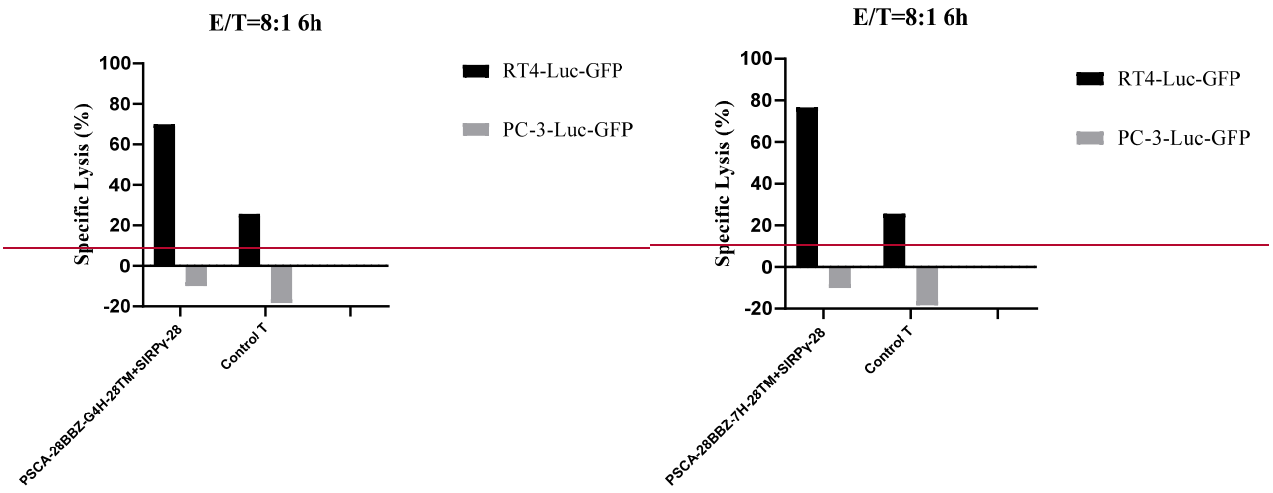


Figure 31


E/T=8:1 6h
Specific Lysis (%)
100
80
60
40
20
0
-20
RT4-Luc-GFP
PC-3-Luc-GFP
PSCA-28BBZ-G4H-28TM+SIRPγ-28
Control T
E/T=8:1 6h
Specific Lysis (%)
RT4-Luc-GFP
PC-3-Luc-GFP
PSCA-28BBZ-7H-28TM+SIRPγ-28
Control T

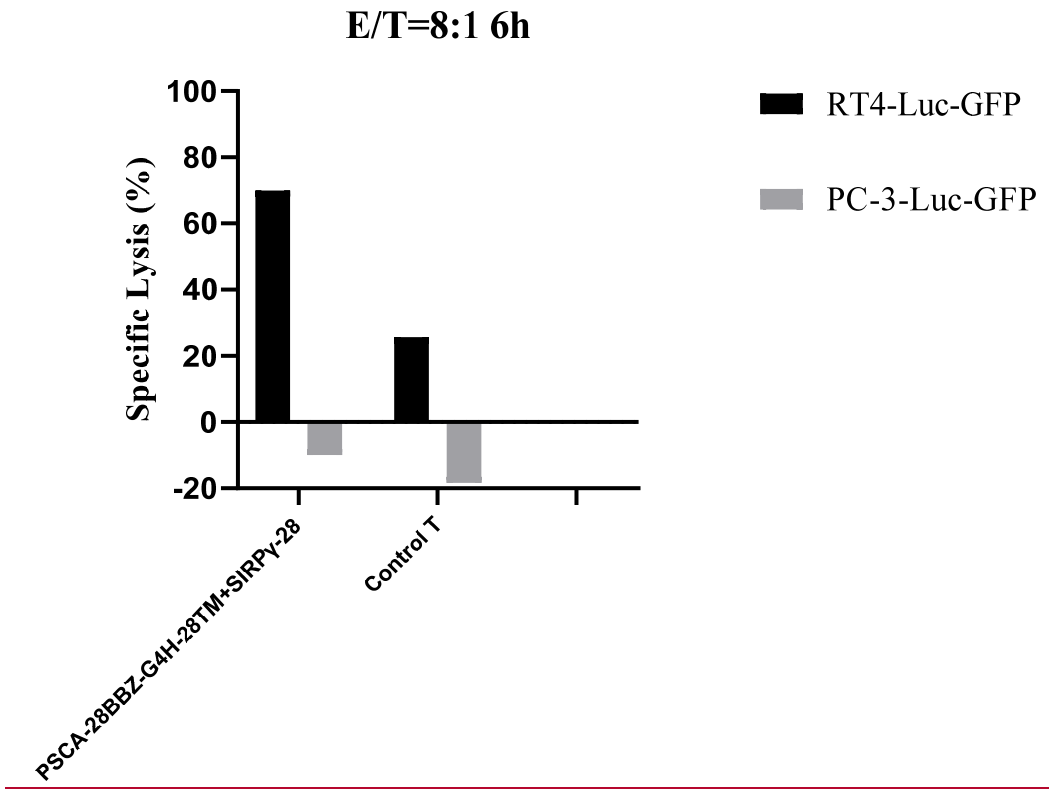

E/T=8:1 6h
Specific Lysis (%)
100
80
60
40
20
0
-20
RT4-Luc-GFP
PC-3-Luc-GFP
PSCA-28BBZ-G4H-28TM+SIRPγ-28
Control T

Figure 32

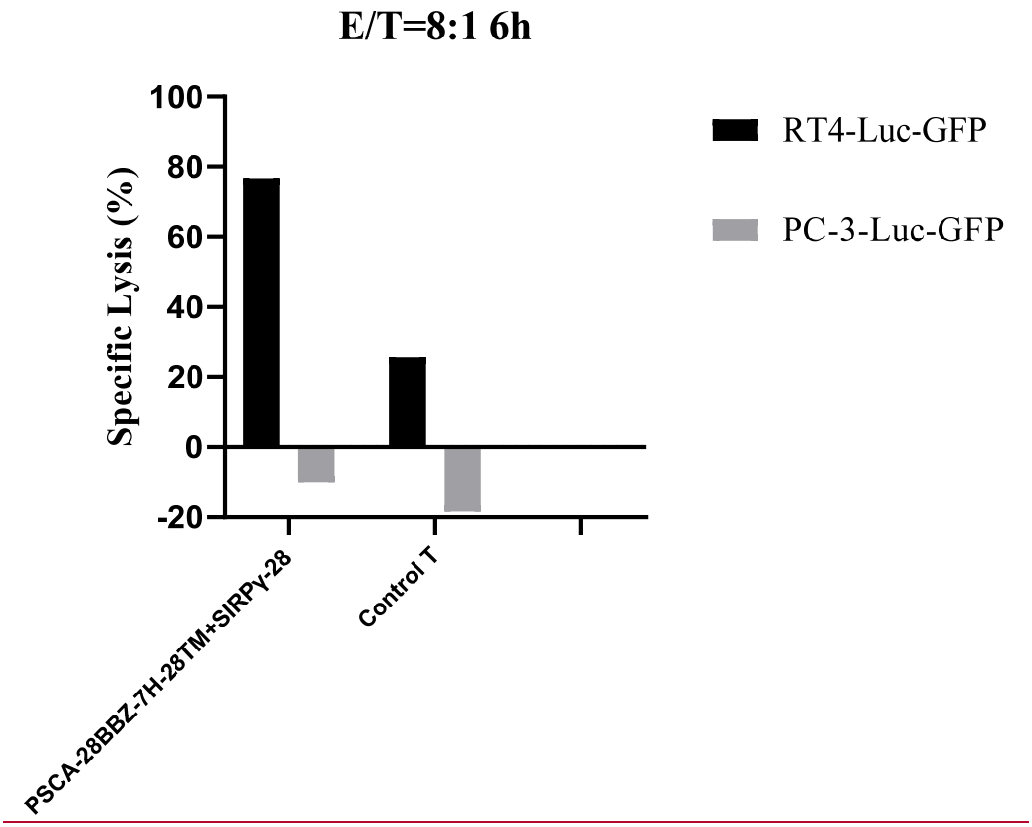
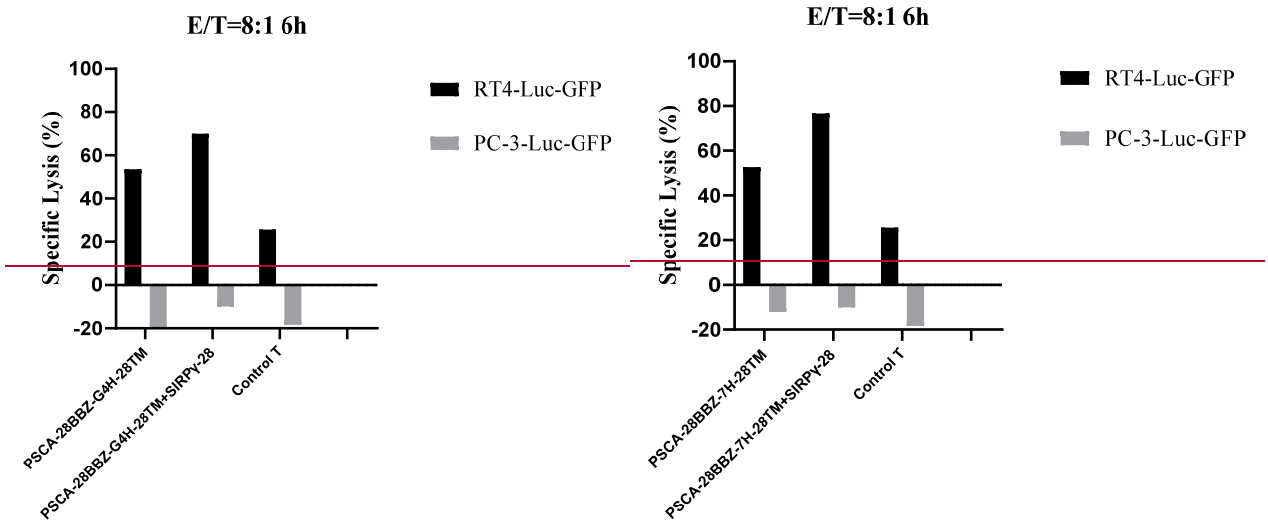

E/T=8:1 6h
Specific Lysis (%)
100
80
60
40
20
0
-20
RT4-Luc-GFP
PC-3-Luc-GFP
PSCA-28BBZ-7H-28TM+SIRPγ-28
Control T

Figure 33


E/T=8:1 6h
Specific Lysis (%)
100
80
60
40
20
0
-20
RT4-Luc-GFP
PC-3-Luc-GFP
PSCA-28BBZ-G4H-28TM
PSCA-28BBZ-G4H-28TM+SIRPγ-28
Control T
E/T=8:1 6h
Specific Lysis (%)
100
80
60
40
20
0
-20
RT4-Luc-GFP
PC-3-Luc-GFP
PSCA-28BBZ-7H-28TM
PSCA-28BBZ-7H-28TM+SIRPγ-28
Control T

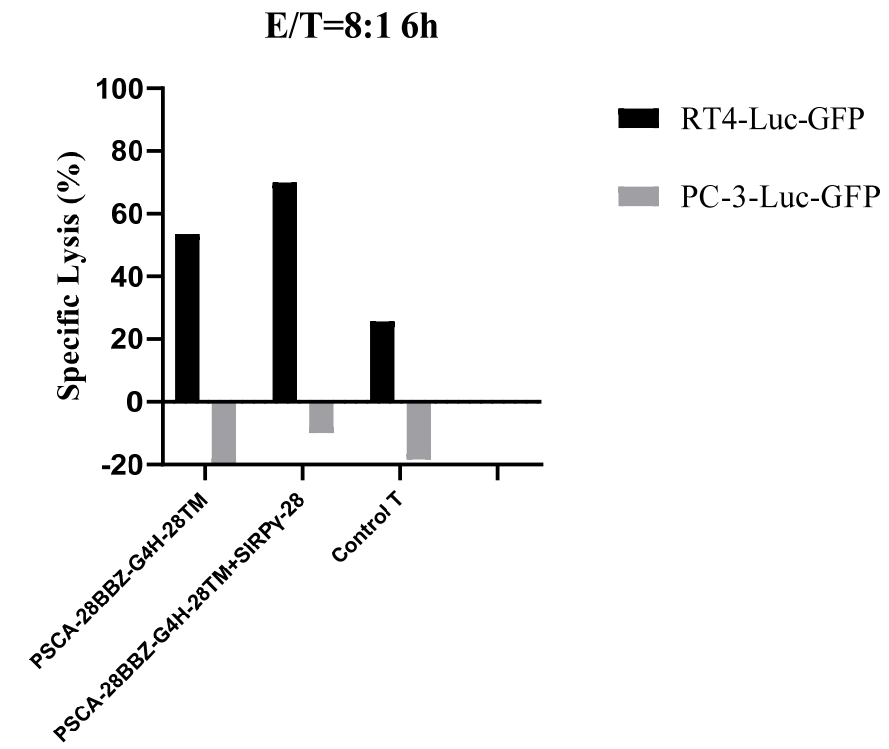


Figure 34

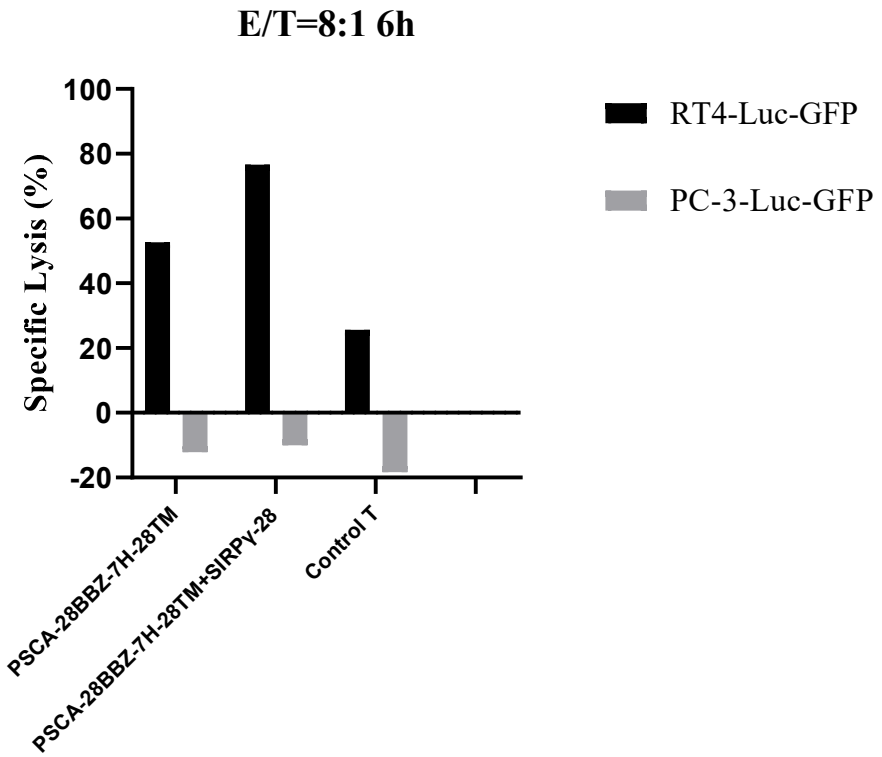


Figure 35