Figure 1

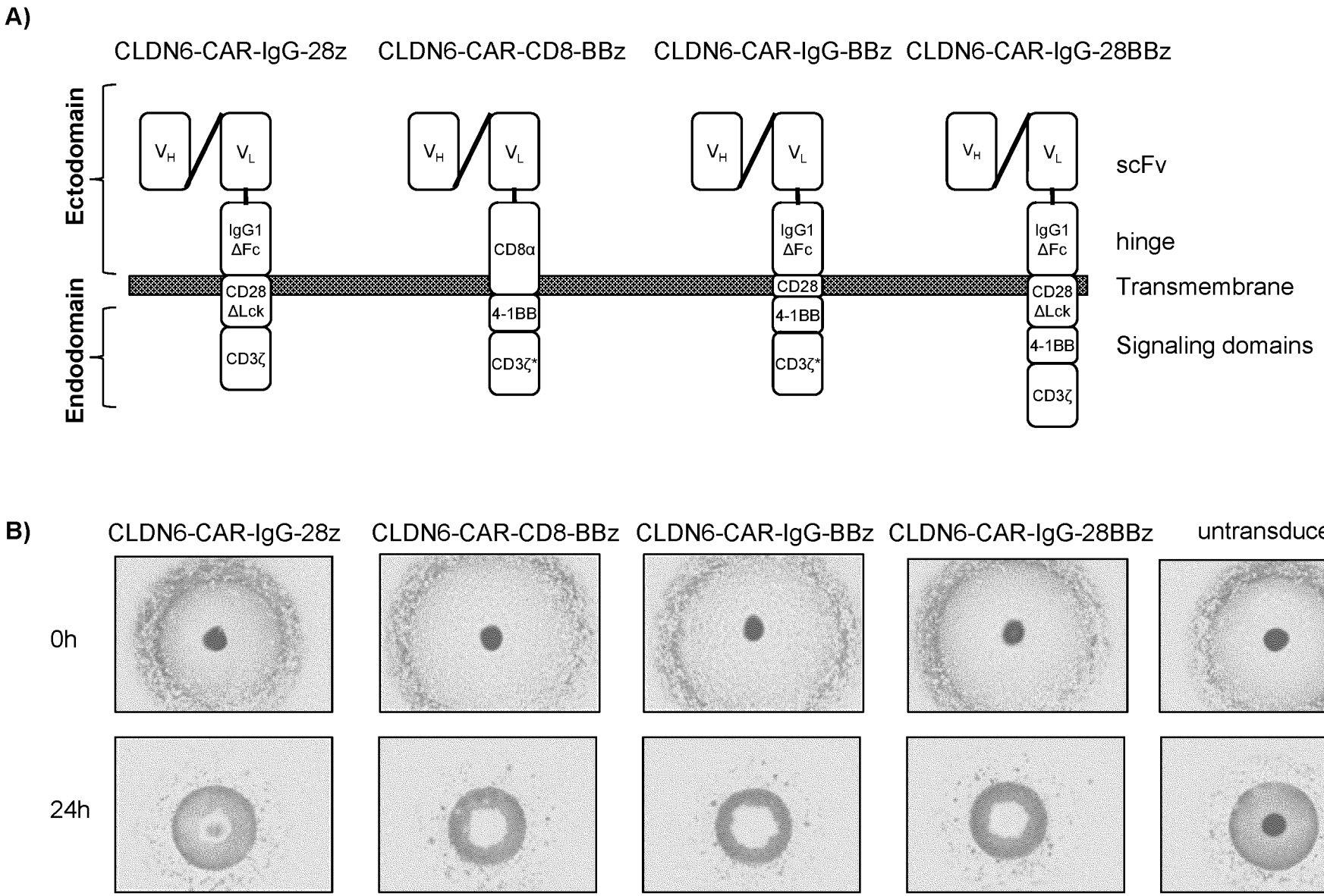

A)
CLDN6-CAR-IgG-28z
CLDN6-CAR-CD8-BBz
CLDN6-CAR-IgG-BBz
CLDN6-CAR-IgG-28BBz
Ectodomain
Endodomain
VH
VL
IgG1 ΔFc
CD8α
CD28 ΔLck
CD28
4-1BB
CD3ζ
CD3ζ*
scFv
hinge
Transmembrane
Signaling domains
B)
CLDN6-CAR-IgG-28z
CLDN6-CAR-CD8-BBz
CLDN6-CAR-IgG-BBz
CLDN6-CAR-IgG-28BBz
untransduced
0h
24h

Figure 2

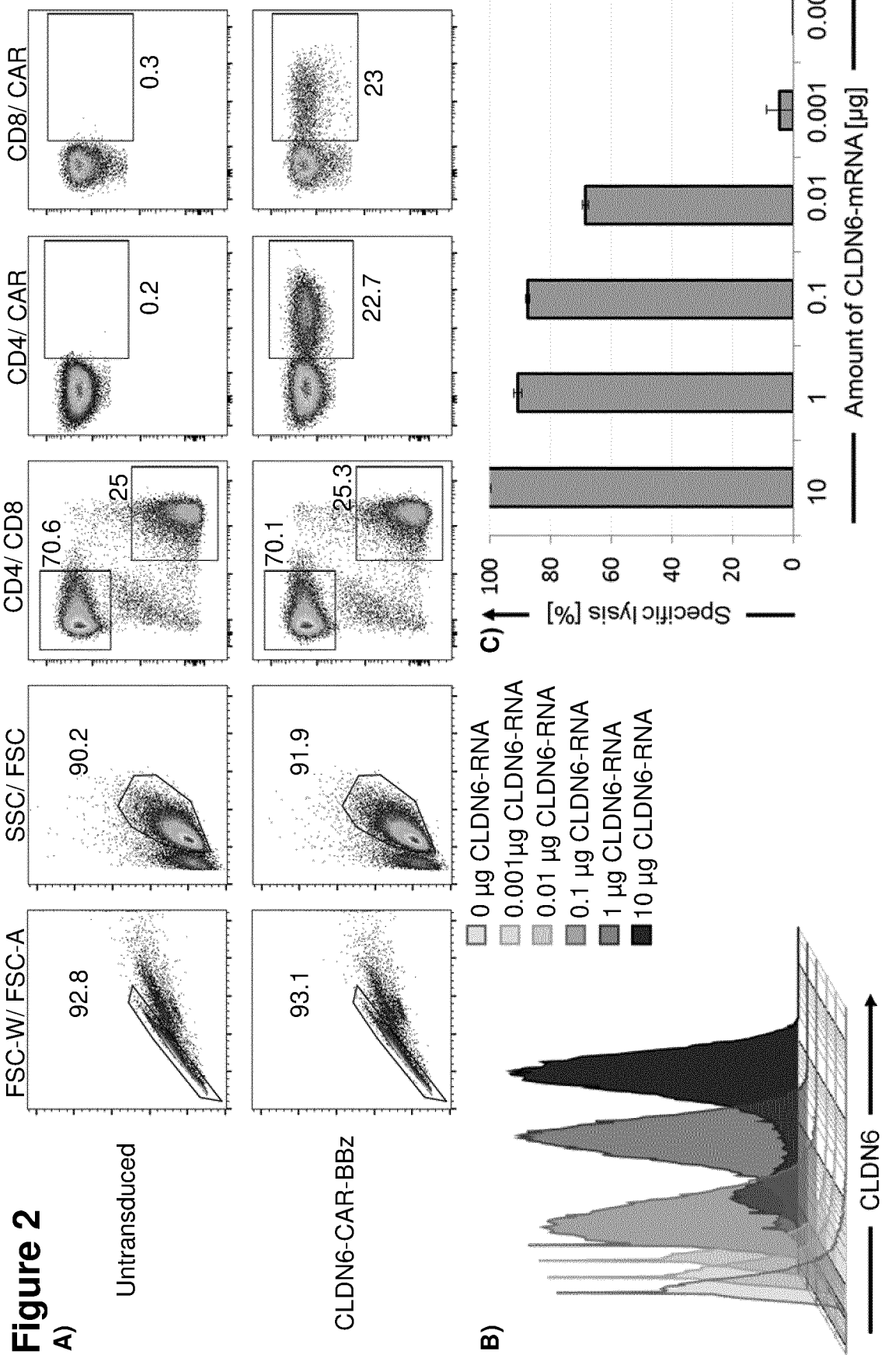
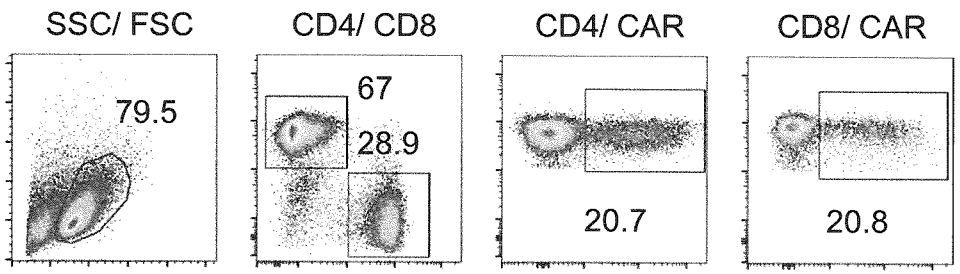

A)
FSC-W/ FSC-A
SSC/ FSC
CD4/ CD8
CD4/ CAR
CD8/ CAR
Untransduced
CLDN6-CAR-BBz
92.8
90.2
70.6
25
0.2
0.3
93.1
91.9
70.1
25.3
22.7
23
B)
CLDN6
0 µg CLDN6-RNA
0.001µg CLDN6-RNA
0.01 µg CLDN6-RNA
0.1 µg CLDN6-RNA
1 µg CLDN6-RNA
10 µg CLDN6-RNA
C)
Specific lysis [%]
100
80
60
40
20
0
10
1
0.1
0.01
0.001
0.0001
Amount of CLDN6-mRNA [µg]

Figure 3

A)



B)

CLDN6+ and CLDN6- tumor cell lines

	Name	CLDN6 pos/neg	Origin
1	LCLC-103H	negative	Lung
2	Mel-526	negative	Skin
3	SK-MEL-37	negative	Skin
4	SK-OV-3	negative	Ovary
5	23132-87	negative	Gastic
6	Colo-699-N	negative	Lung
7	HEK-293	negative	Embryonic kidney
8	MDA-MB-231	negative	Breast
9	PA-1-SC12	positive	Ovary
10	NIH-OvCar-3	positive	Ovary
11	JAR	positive	Embryo
12	NEC-8	positive	Embryo

Figure 3

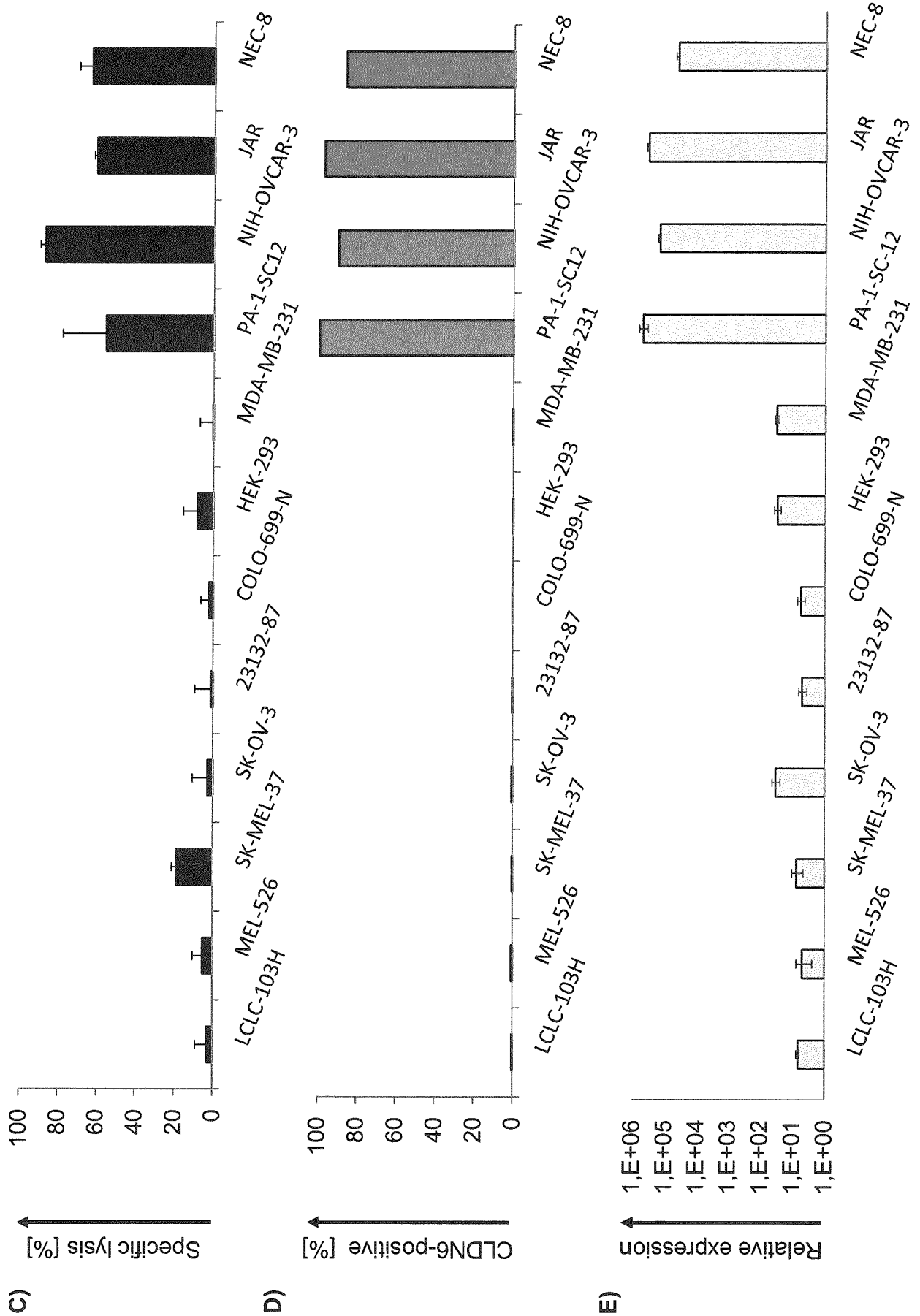

C)
Specific lysis [%]
100
80
60
40
20
0
LCLC-103H
MEL-526
SK-MEL-37
SK-OV-3
23132-87
COLO-699-N
HEK-293
MDA-MB-231
PA-1-SC12
NIH-OVCAR-3
JAR
NEC-8
D)
CLDN6-positive [%]
100
80
60
40
20
0
LCLC-103H
MEL-526
SK-MEL-37
SK-OV-3
23132-87
COLO-699-N
HEK-293
MDA-MB-231
PA-1-SC12
NIH-OVCAR-3
JAR
NEC-8
E)
Relative expression
1,E+06
1,E+05
1,E+04
1,E+03
1,E+02
1,E+01
1,E+00
LCLC-103H
MEL-526
SK-MEL-37
SK-OV-3
23132-87
COLO-699-N
HEK-293
MDA-MB-231
PA-1-SC-12
NIH-OVCAR-3
JAR
NEC-8

Figure 4

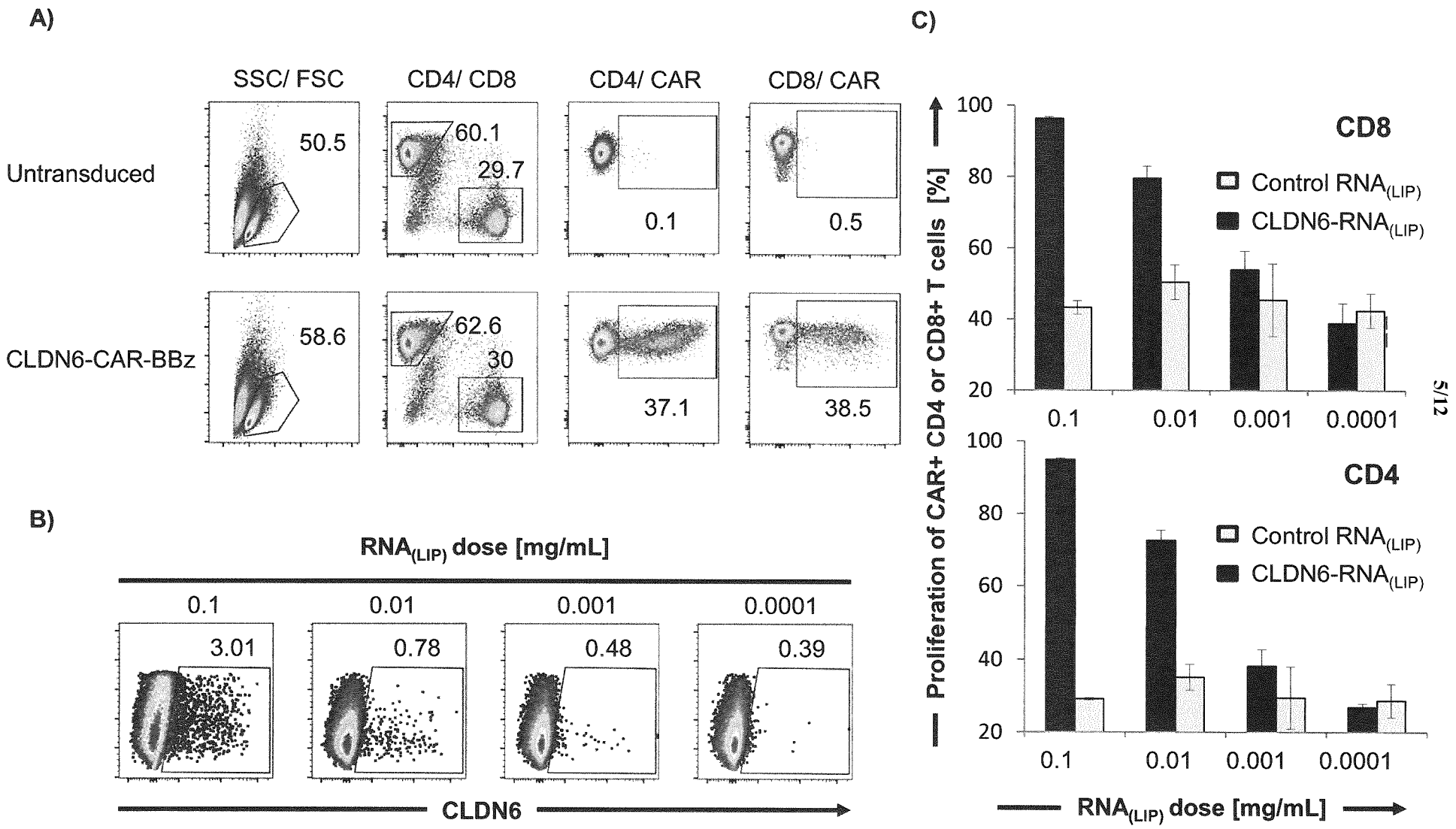
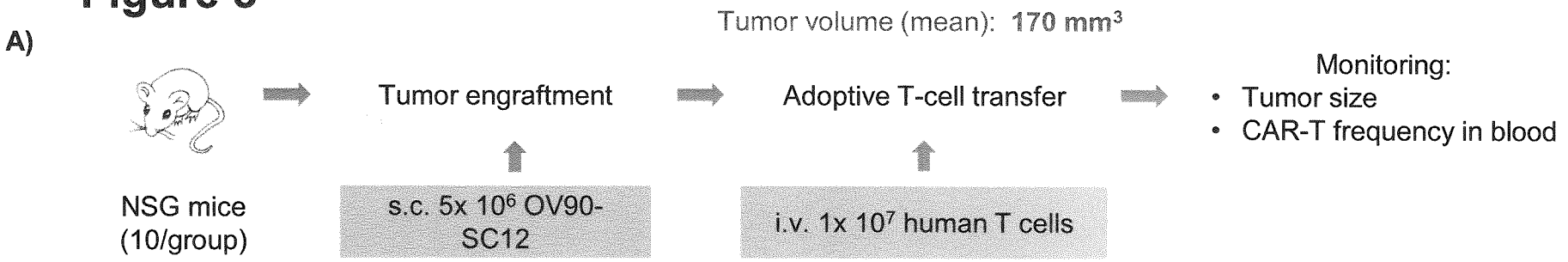
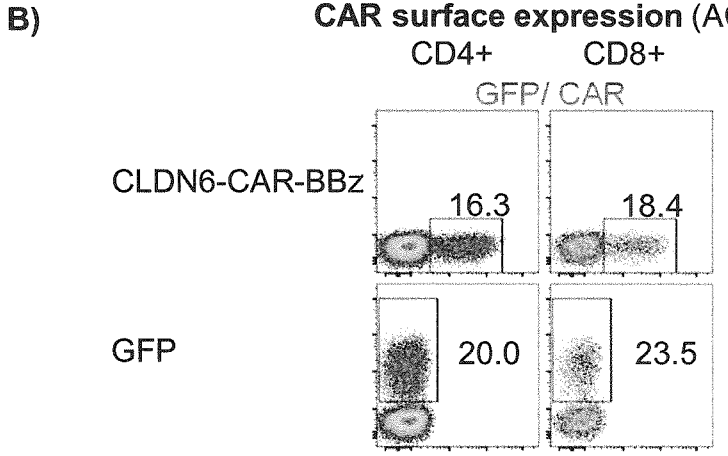
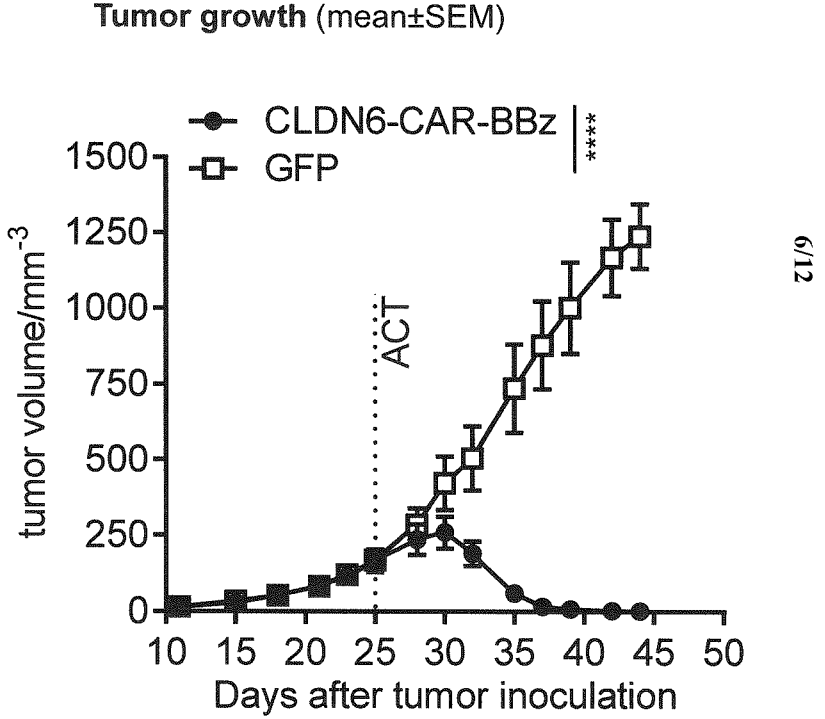

A)
SSC/ FSC
CD4/ CD8
CD4/ CAR
CD8/ CAR
Untransduced
CLDN6-CAR-BBz
50.5
60.1
29.7
0.1
0.5
58.6
62.6
30
37.1
38.5
B)
RNA(LIP) dose [mg/mL]
0.1
0.01
0.001
0.0001
3.01
0.78
0.48
0.39
CLDN6
C)
CD8
CD4
Control RNA(LIP)
CLDN6-RNA(LIP)
Proliferation of CAR+ CD4 or CD8+ T cells [%]
100
80
60
40
20
0.1
0.01
0.001
0.0001
RNA(LIP) dose [mg/mL]

Figure 5


A)
Tumor volume (mean): 170 mm3
Tumor engraftment
Adoptive T-cell transfer
Monitoring:
• Tumor size
• CAR-T frequency in blood
NSG mice
(10/group)
s.c. 5x 10^6 OV90-SC12
i.v. 1x 10^7 human T cells


B)
CAR surface expression (ACT)
CD4+
CD8+
GFP/ CAR
CLDN6-CAR-BBz
GFP
16.3
18.4
20.0
23.5


C)
Tumor growth (mean±SEM)
CLDN6-CAR-BBz
GFP
****
tumor volume/mm-3
1500
1250
1000
750
500
250
0
ACT
10 15 20 25 30 35 40 45 50
Days after tumor inoculation

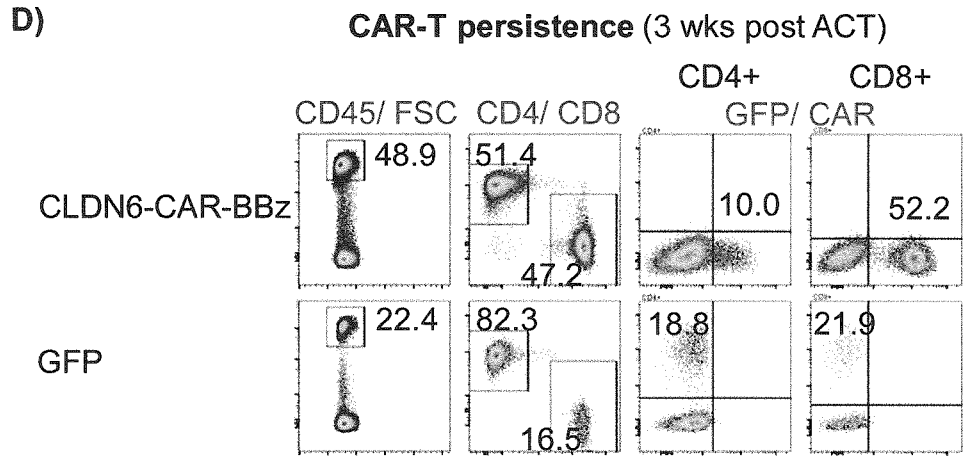

D)
CAR-T persistence (3 wks post ACT)
CD4+
CD8+
CD45/ FSC
CD4/ CD8
GFP/ CAR
CLDN6-CAR-BBz
GFP
48.9
51.4
47.2
10.0
52.2
22.4
82.3
16.5
18.8
21.9

Figure 6

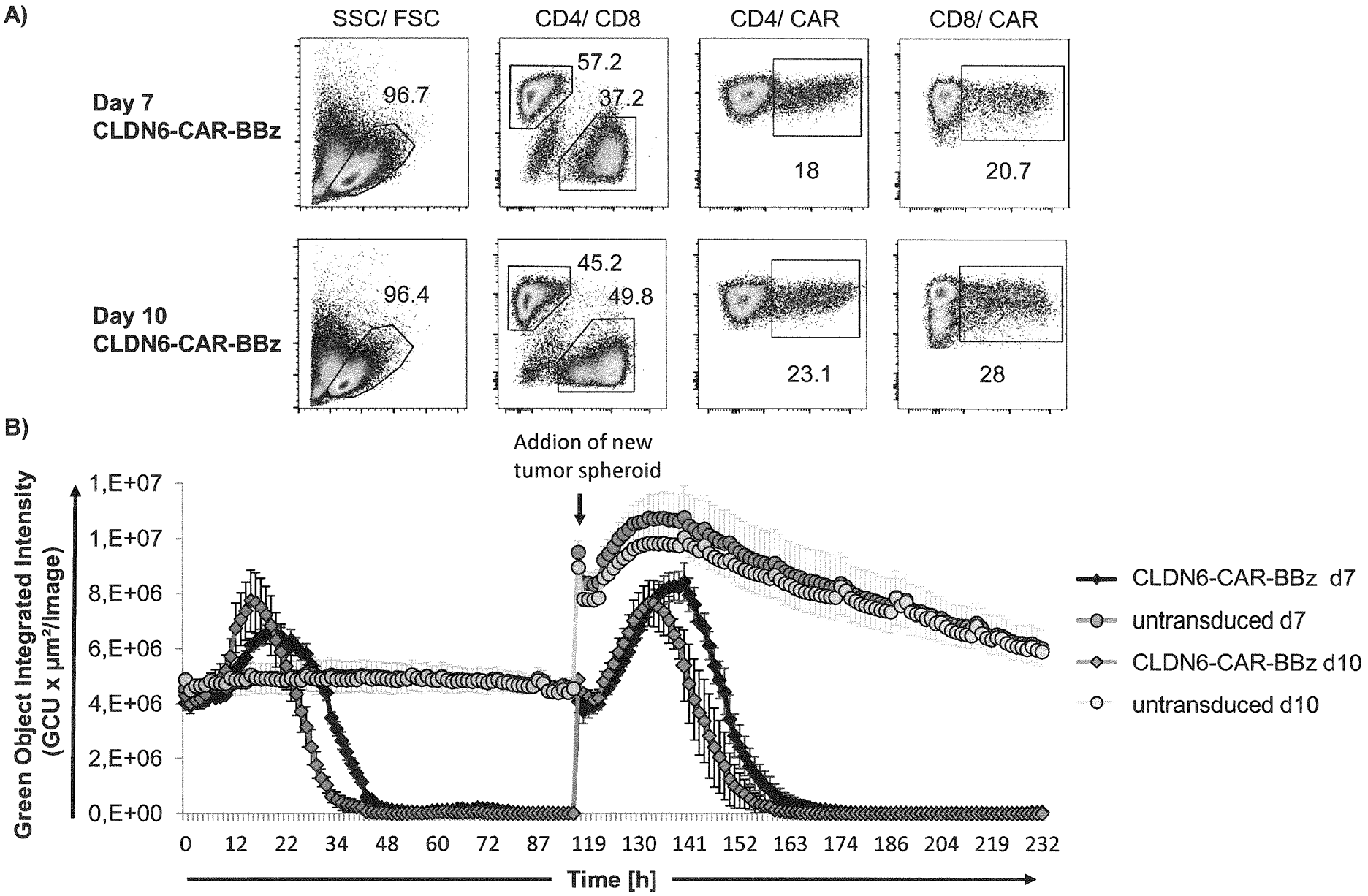

A)
SSC/ FSC
CD4/ CD8
CD4/ CAR
CD8/ CAR
Day 7
CLDN6-CAR-BBz
96.7
57.2
37.2
18
20.7
Day 10
CLDN6-CAR-BBz
96.4
45.2
49.8
23.1
28
B)
Addion of new
tumor spheroid
Green Object Integrated Intensity
(GCU x µm²/Image)
1,E+07
1,E+07
8,E+06
6,E+06
4,E+06
2,E+06
0,E+00
0
12
22
34
48
60
72
87
119
130
141
152
163
174
186
204
219
232
Time [h]
CLDN6-CAR-BBz d7
untransduced d7
CLDN6-CAR-BBz d10
untransduced d10

Figure 7

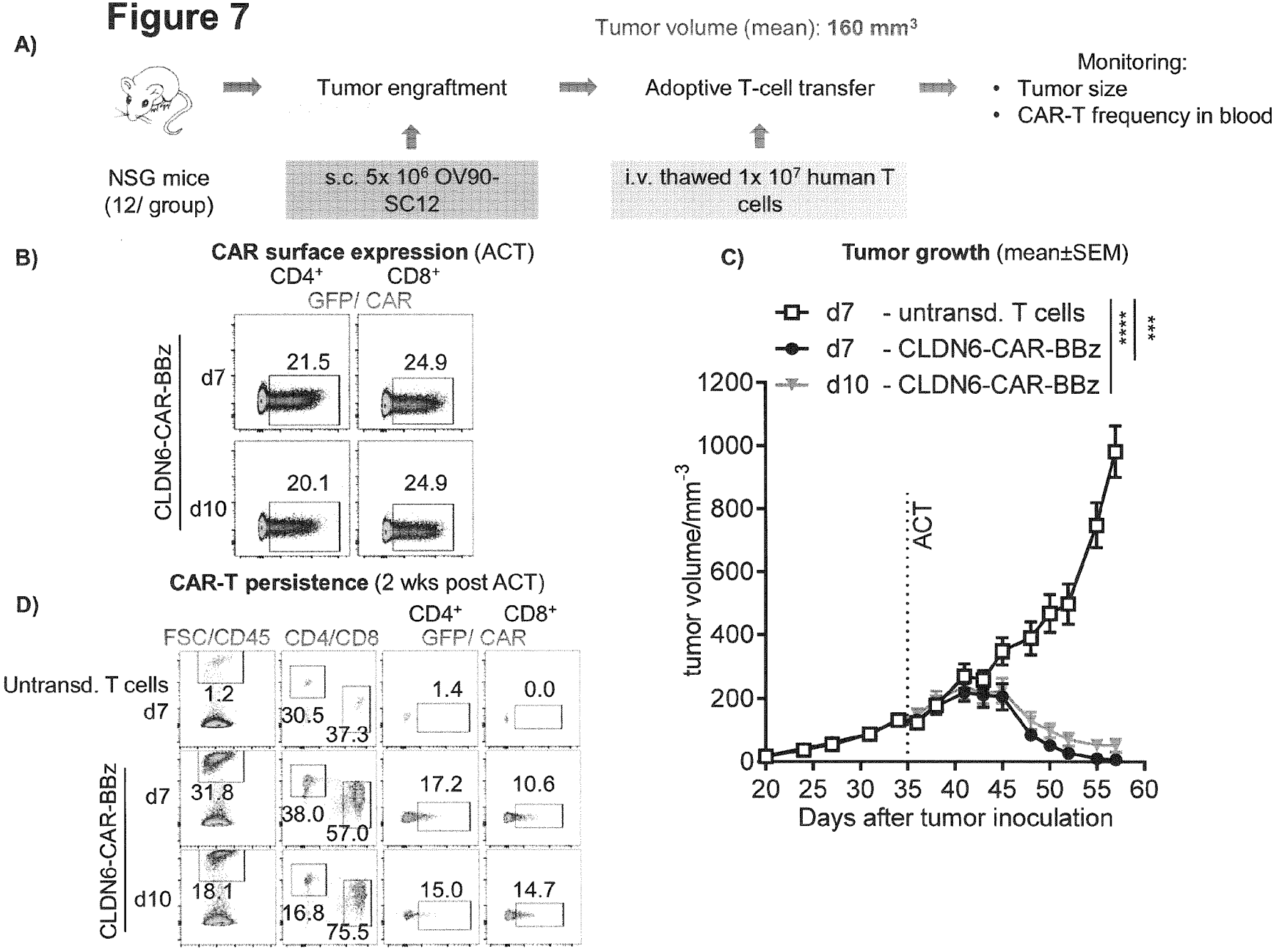

A)
Tumor volume (mean): 160 mm3
Tumor engraftment
Adoptive T-cell transfer
Monitoring:
• Tumor size
• CAR-T frequency in blood
NSG mice (12/ group)
s.c. 5x 10^6 OV90-SC12
i.v. thawed 1x 10^7 human T cells
B)
CAR surface expression (ACT)
CD4+
CD8+
GFP/ CAR
CLDN6-CAR-BBz
d7
d10
21.5
24.9
20.1
24.9
C)
Tumor growth (mean±SEM)
d7 - untransd. T cells
d7 - CLDN6-CAR-BBz
d10 - CLDN6-CAR-BBz
****
***
tumor volume/mm-3
ACT
Days after tumor inoculation
D)
CAR-T persistence (2 wks post ACT)
FSC/CD45
CD4/CD8
GFP/ CAR
Untransd. T cells
d7
1.2
30.5
37.3
1.4
0.0
CLDN6-CAR-BBz
d7
31.8
38.0
57.0
17.2
10.6
d10
18.1
16.8
75.5
15.0
14.7

Figure 8

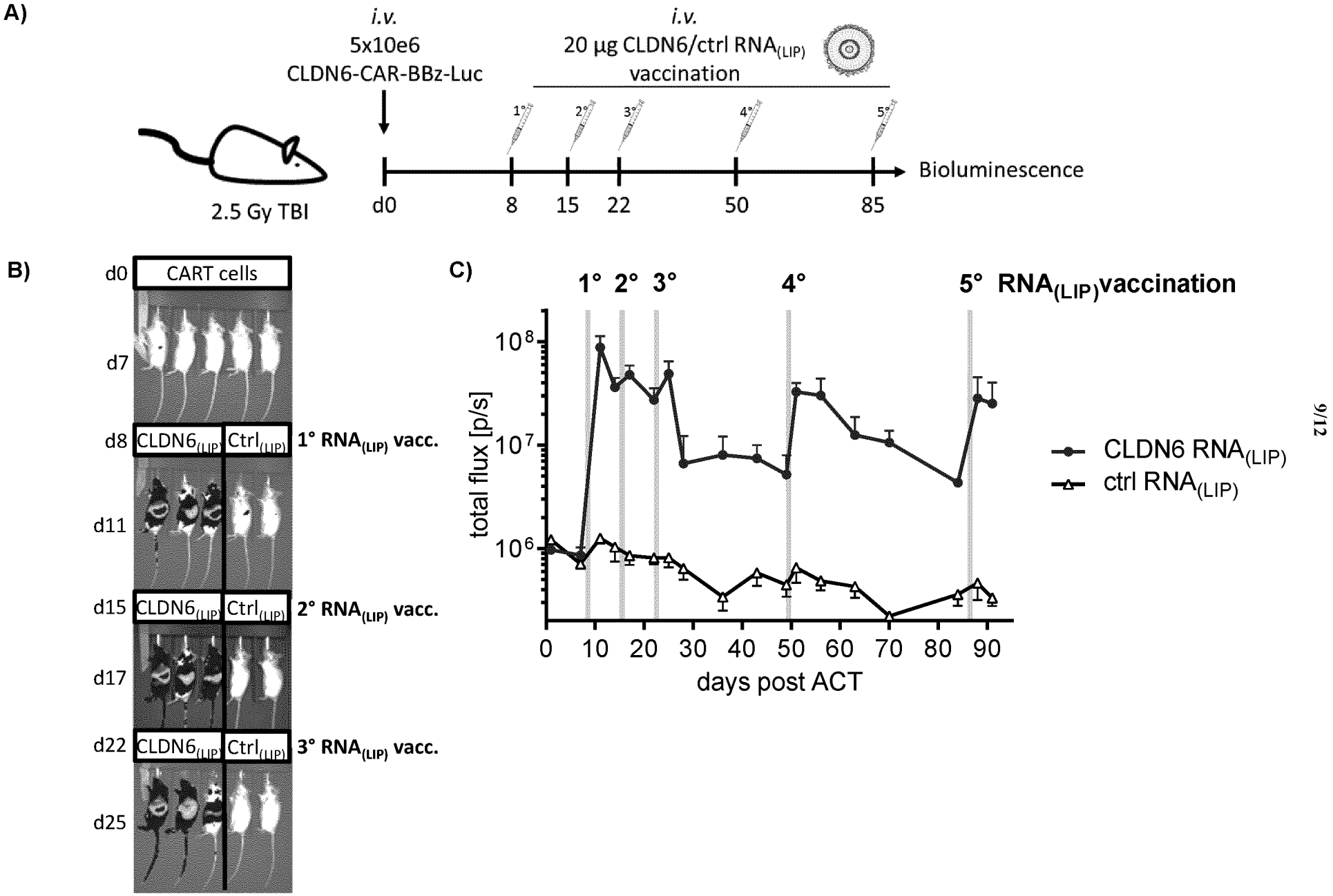
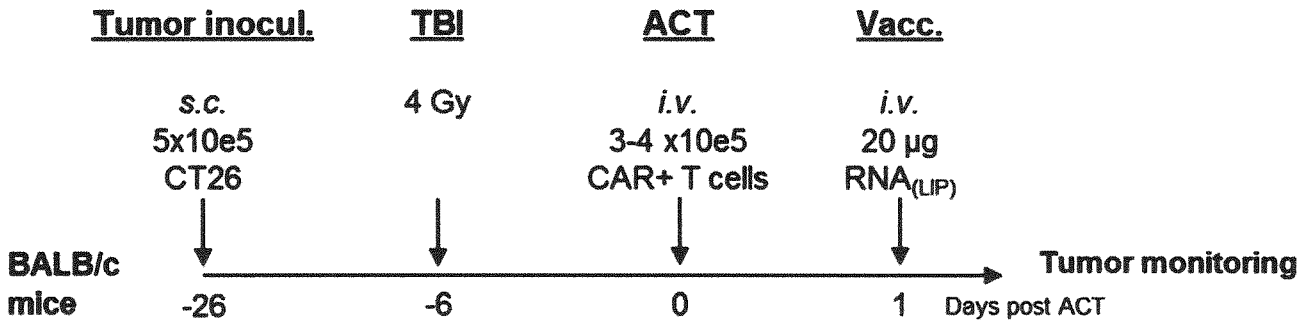

A)
i.v.
5x10e6
CLDN6-CAR-BBz-Luc
i.v.
20 µg CLDN6/ctrl RNA(LIP)
vaccination
1°
2°
3°
4°
5°
2.5 Gy TBI
d0
8
15
22
50
85
Bioluminescence
B)
d0
CART cells
d7
d8
CLDN6(LIP)
Ctrl(LIP)
1° RNA(LIP) vacc.
d11
d15
CLDN6(LIP)
Ctrl(LIP)
2° RNA(LIP) vacc.
d17
d22
CLDN6(LIP)
Ctrl(LIP)
3° RNA(LIP) vacc.
d25
C)
1° 2° 3°
4°
5° RNA(LIP)vaccination
total flux [p/s]
10^8
10^7
10^6
0 10 20 30 40 50 60 70 80 90
days post ACT
CLDN6 RNA(LIP)
ctrl RNA(LIP)

Figure 9

A)


Tumor inocul.
TBI
ACT
Vacc.
s.c.
5x10e5
CT26
4 Gy
i.v.
3-4 x10e5
CAR+ T cells
i.v.
20 µg
RNA(LIP)
BALB/c
mice
-26
-6
0
1
Days post ACT
Tumor monitoring

B)

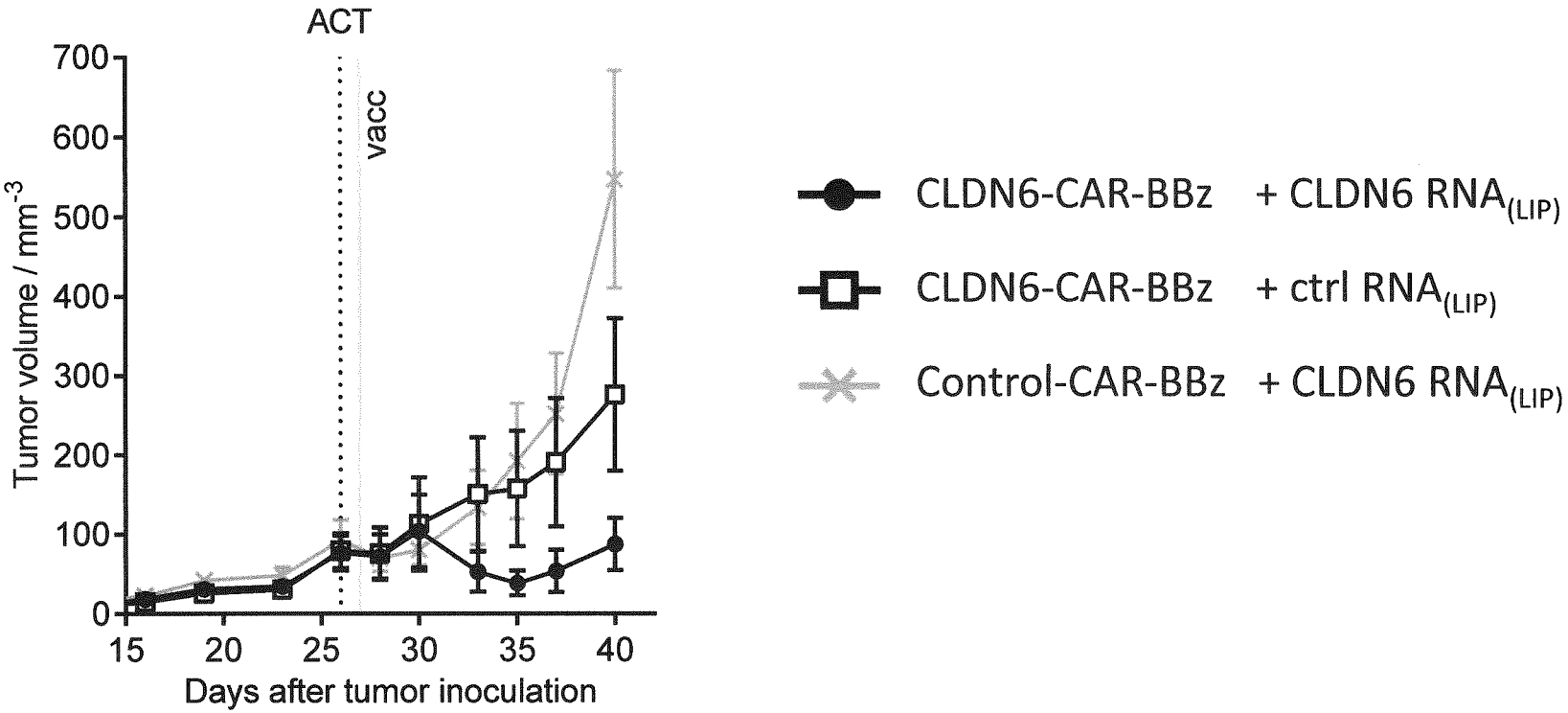
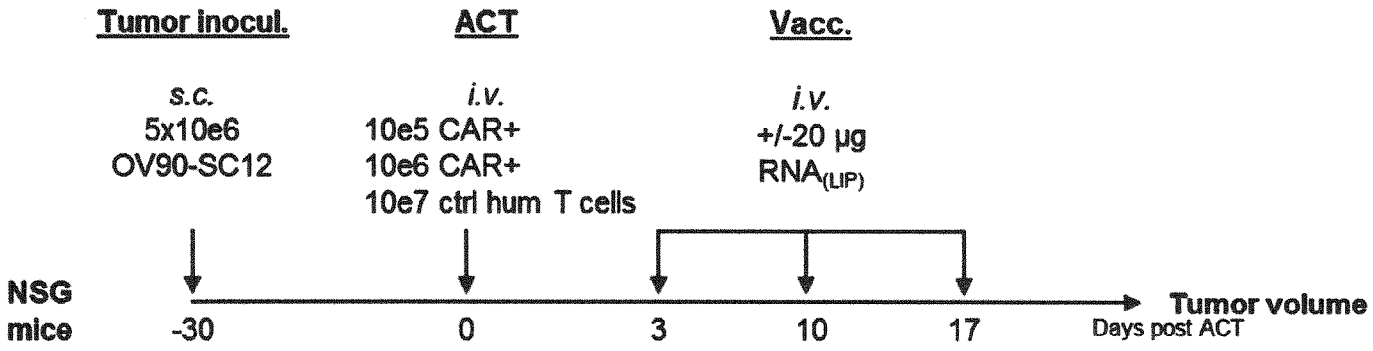
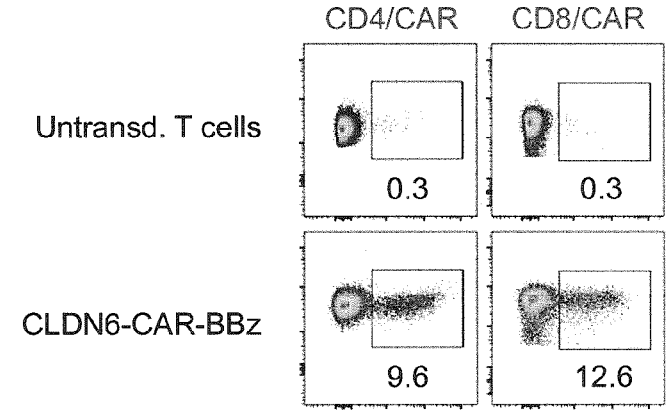

ACT
vacc
Tumor volume / mm-3
700
600
500
400
300
200
100
0
15
20
25
30
35
40
Days after tumor inoculation
CLDN6-CAR-BBz + CLDN6 RNA(LIP)
CLDN6-CAR-BBz + ctrl RNA(LIP)
Control-CAR-BBz + CLDN6 RNA(LIP)

Figure 10

A)



B)



C)

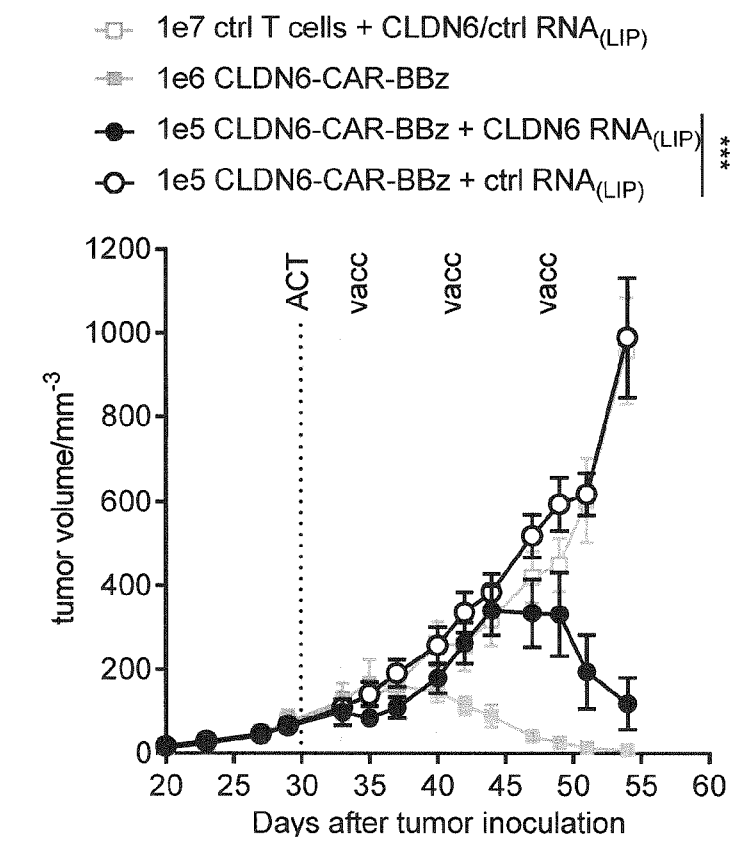
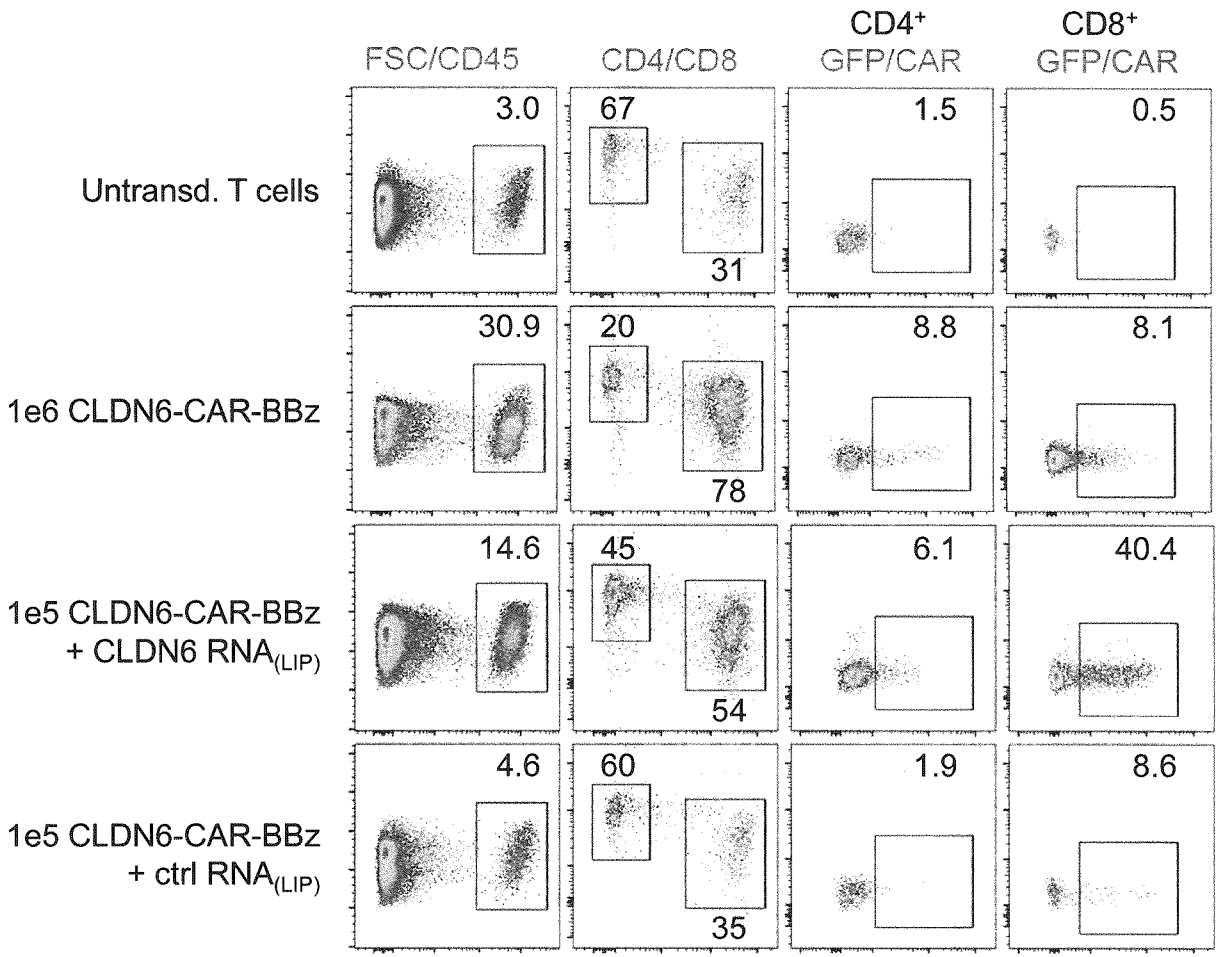


Figure 10

D)


CAR-T persistence (2.5 wks post ACT)
FSC/CD45
CD4/CD8
CD4+ GFP/CAR
CD8+ GFP/CAR
Untransd. T cells
3.0
67
31
1.5
0.5
1e6 CLDN6-CAR-BBz
30.9
20
78
8.8
8.1
1e5 CLDN6-CAR-BBz + CLDN6 RNA(LIP)
14.6
45
54
6.1
40.4
1e5 CLDN6-CAR-BBz + ctrl RNA(LIP)
4.6
60
35
1.9
8.6