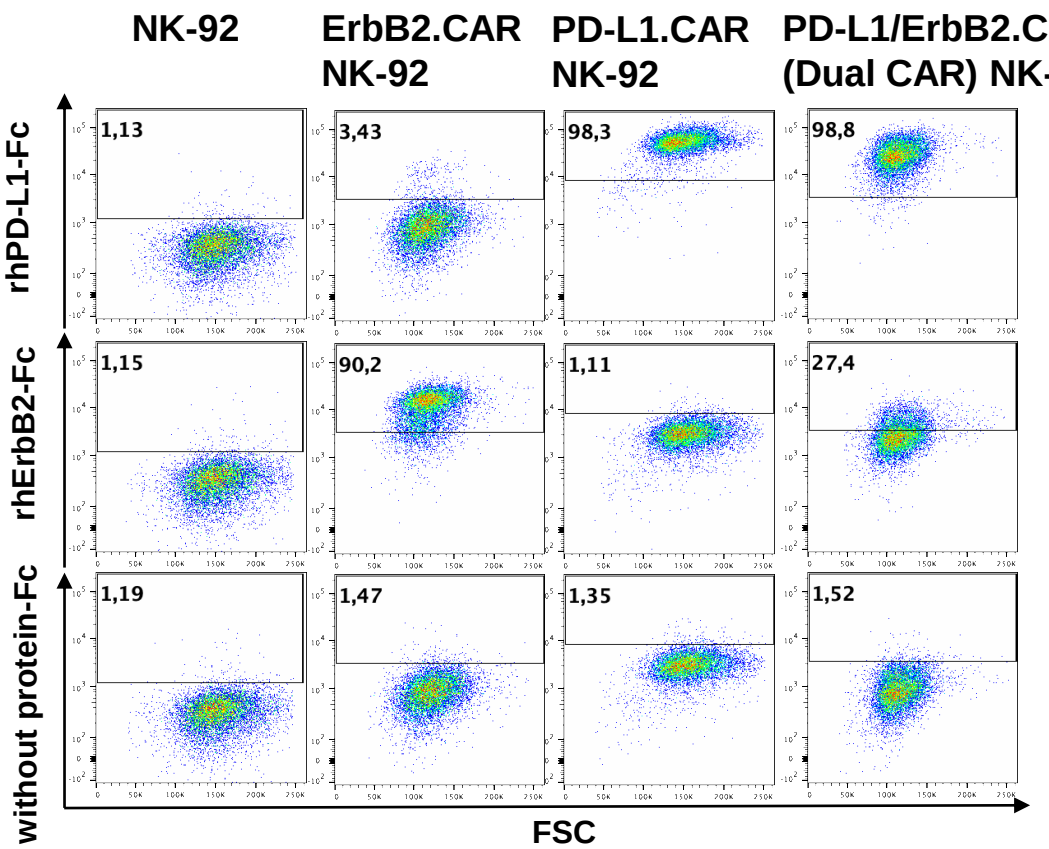
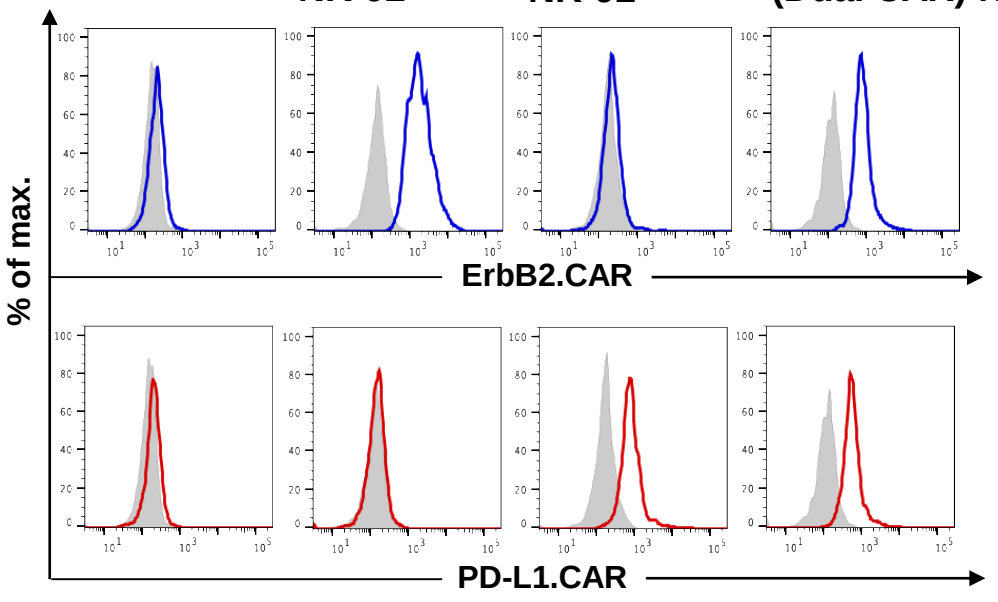
# Fig. S1

**A**

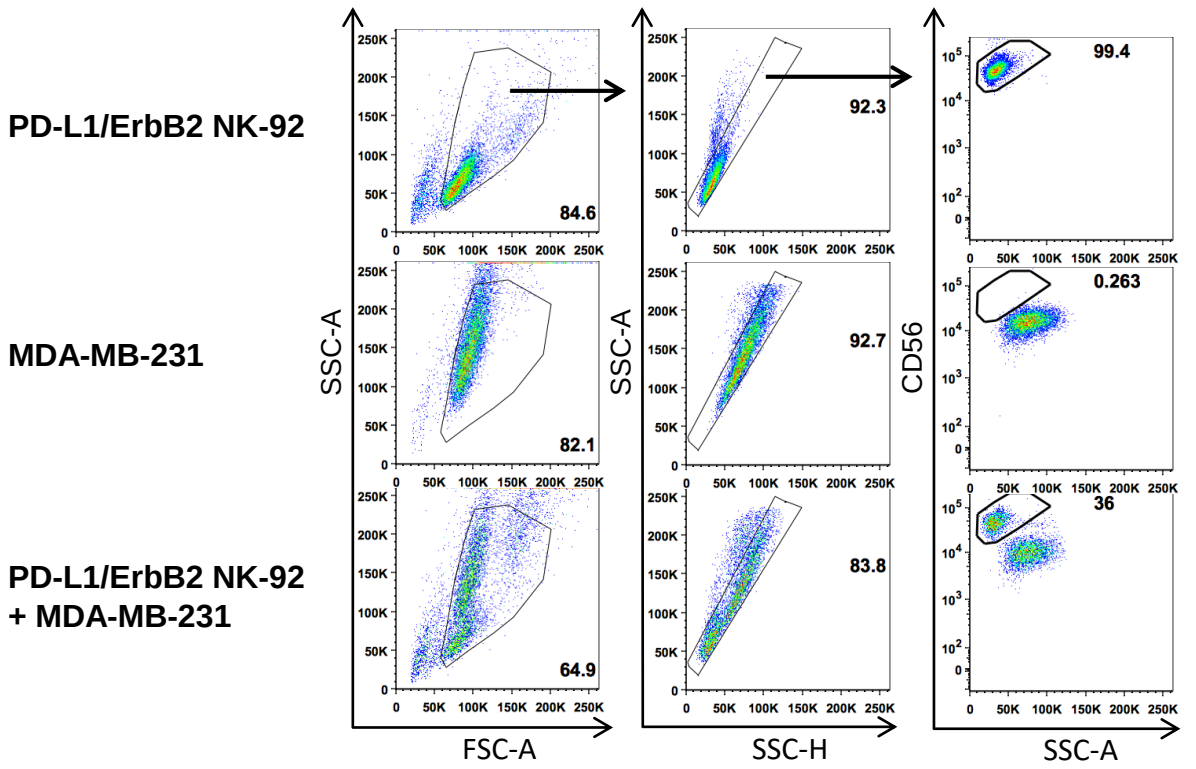

NK-92
ErbB2.CAR NK-92
PD-L1.CAR NK-92
PD-L1/ErbB2.CAR (Dual CAR) NK-92
rhPD-L1-Fc
rhErbB2-Fc
without protein-Fc
1,13
3,43
98,3
98,8
1,15
90,2
1,11
27,4
1,19
1,47
1,35
1,52
FSC

**B**

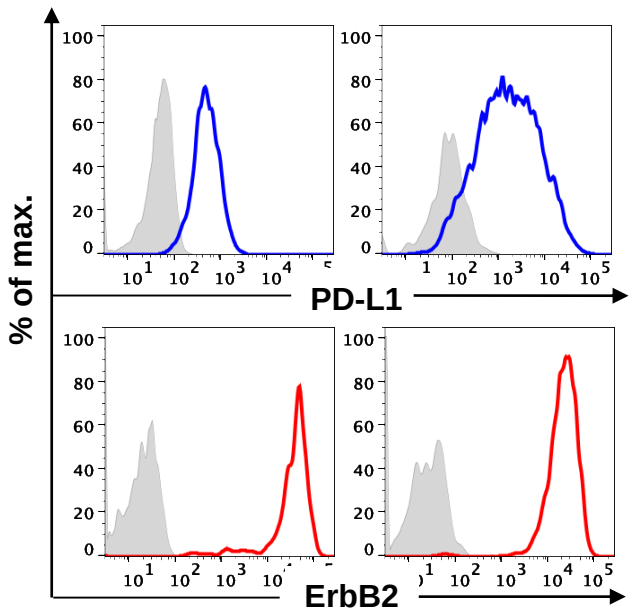

NK-92
ErbB2.CAR NK-92
PD-L1.CAR2 NK-92
PD-L1/ErbB2.CAR2 (Dual CAR) NK-92
% of max.
ErbB2.CAR
PD-L1.CAR

# Fig. S2

**A**


PD-L1/ErbB2 NK-92
MDA-MB-231
PD-L1/ErbB2 NK-92
+ MDA-MB-231
SSC-A
FSC-A
SSC-H
CD56
84.6
92.3
99.4
82.1
92.7
0.263
64.9
83.8
36

**B**


BXPC-3 ErbB2 OE
Calu-3 PDL1 OE
% of max.
PD-L1
ErbB2

**C**

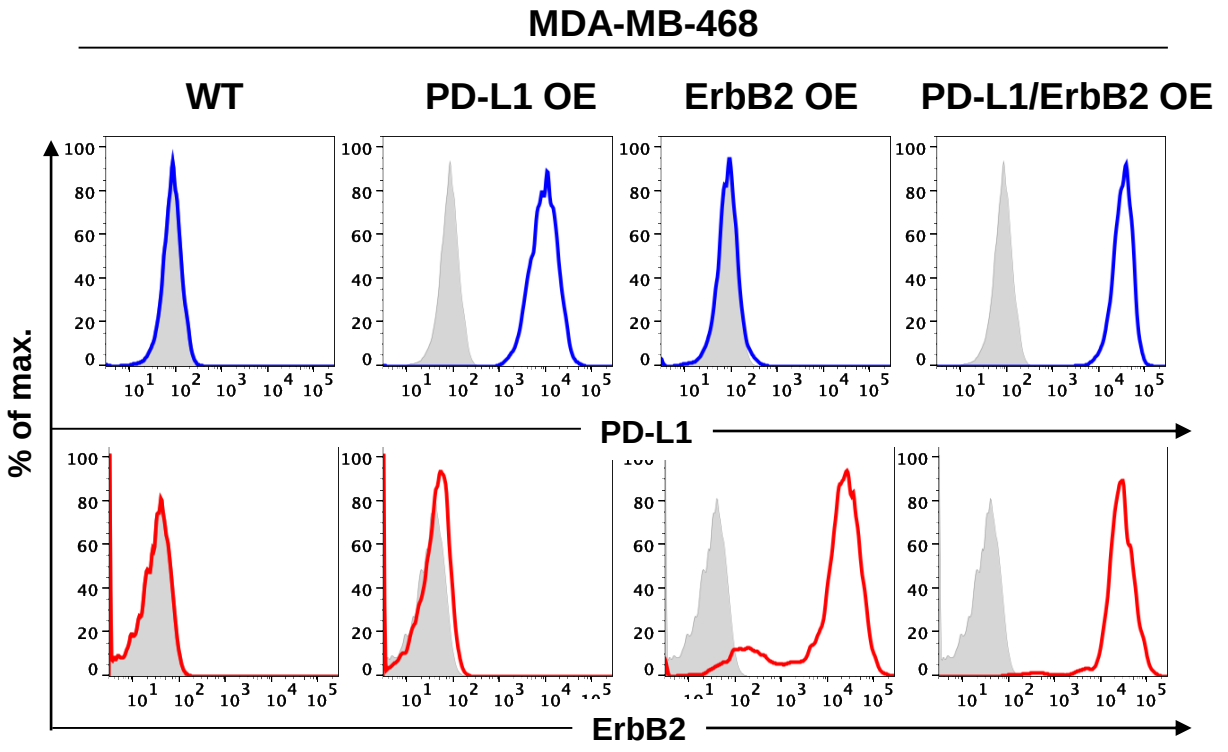
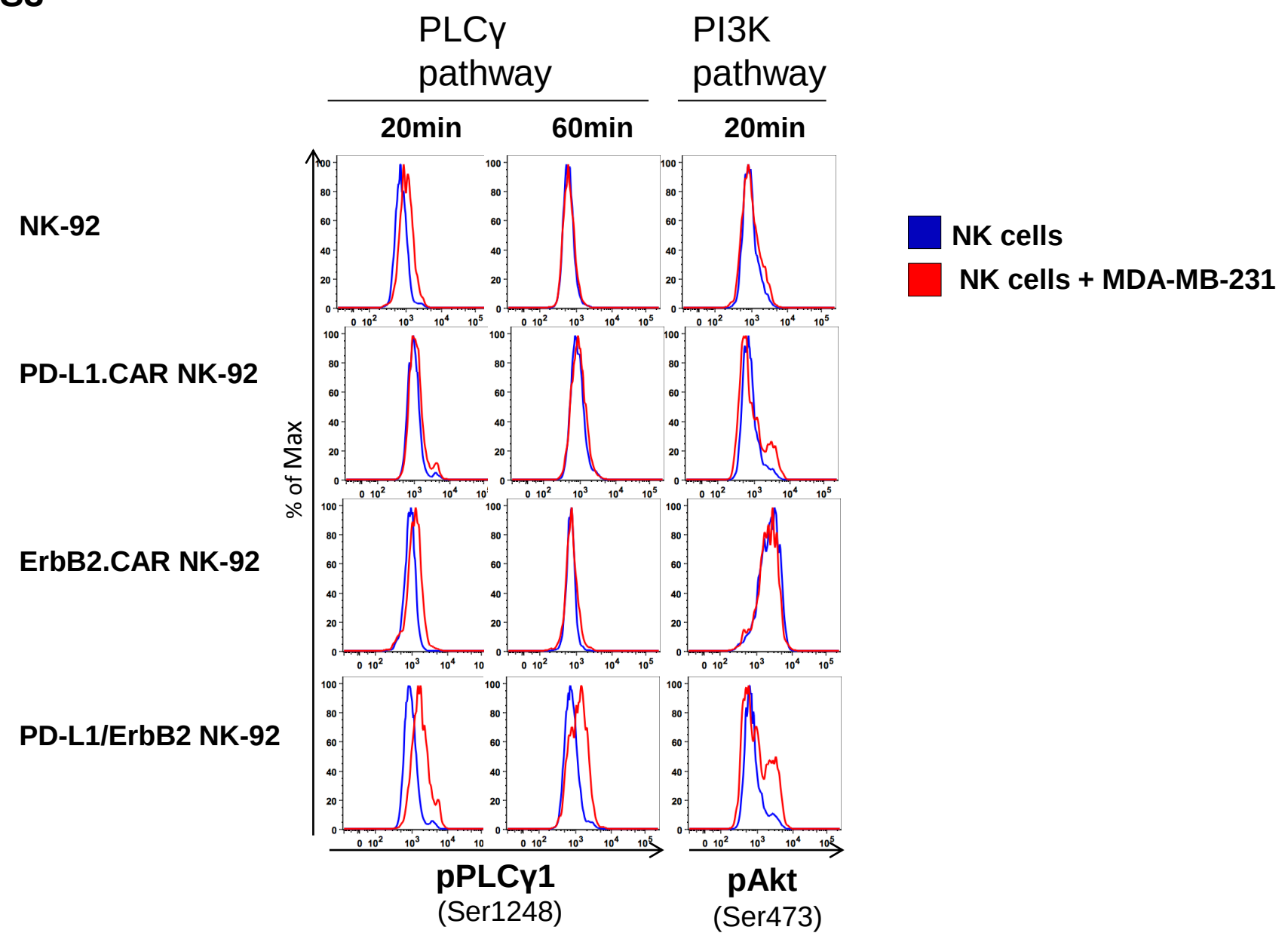
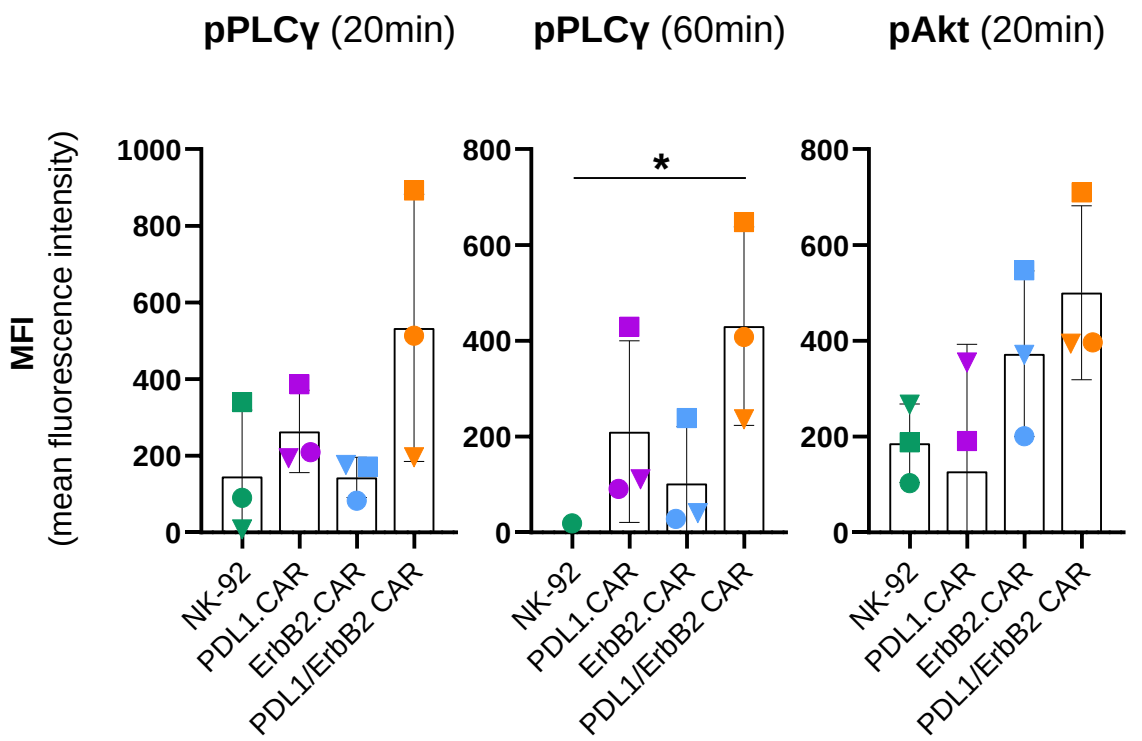

MDA-MB-468
WT
PD-L1 OE
ErbB2 OE
PD-L1/ErbB2 OE
% of max.
PD-L1
ErbB2

Fig. S3

A

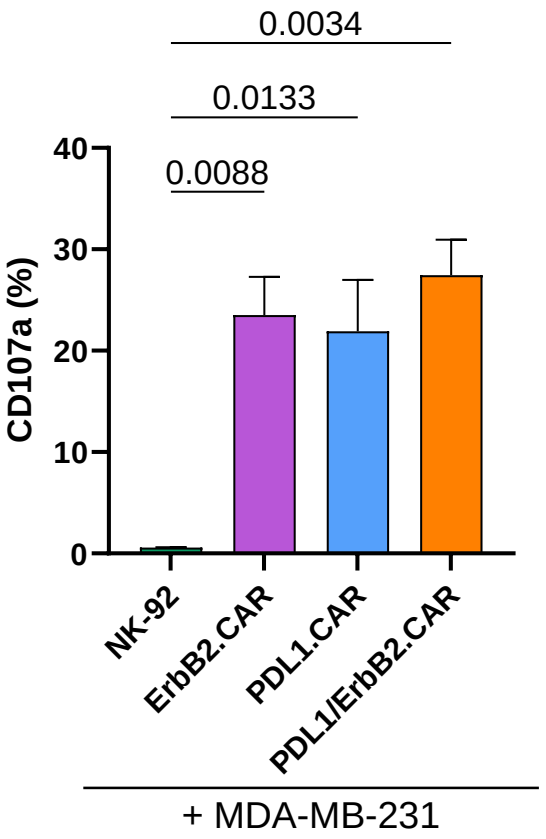

PLCγ pathway
PI3K pathway
20min
60min
20min
NK-92
PD-L1.CAR NK-92
ErbB2.CAR NK-92
PD-L1/ErbB2 NK-92
% of Max
NK cells
NK cells + MDA-MB-231
pPLCγ1 (Ser1248)
pAkt (Ser473)

B


pPLCγ (20min)
pPLCγ (60min)
pAkt (20min)
MFI (mean fluorescence intensity)
*
NK-92
PDL1.CAR
ErbB2.CAR
PDL1/ErbB2 CAR

# Fig. S4

**A**


0.0034
0.0133
0.0088
CD107a (%)
40
30
20
10
0
NK-92
ErbB2.CAR
PDL1.CAR
PDL1/ErbB2.CAR
+ MDA-MB-231

**B**

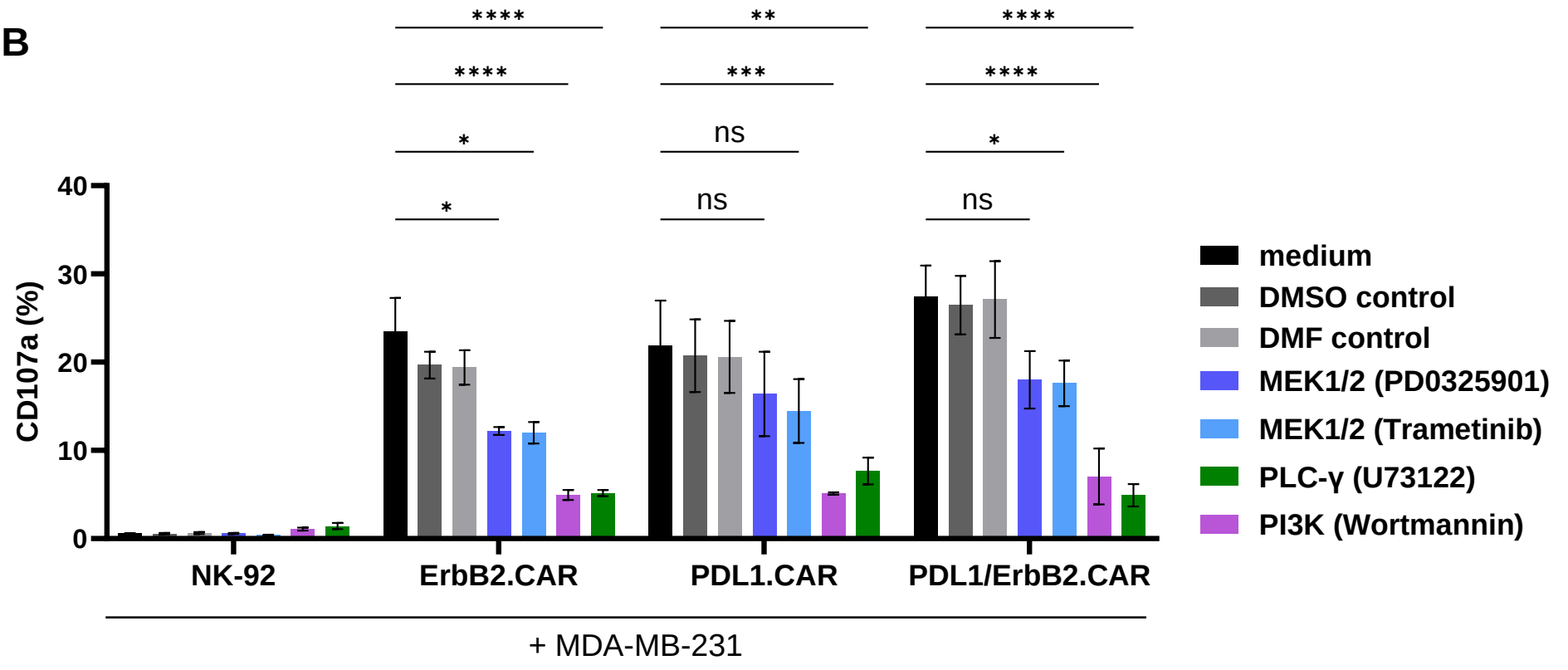
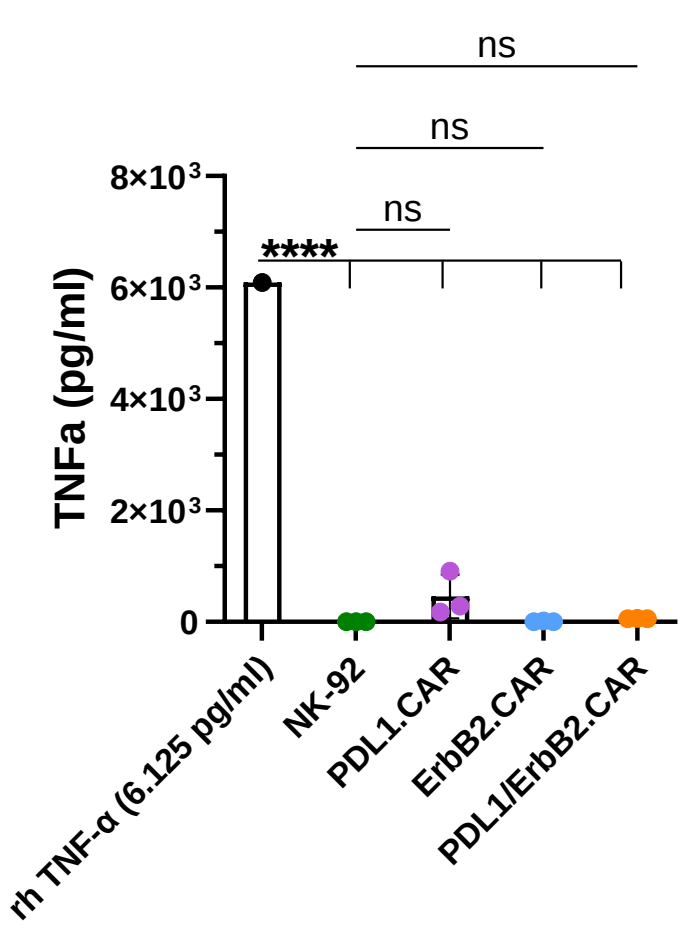
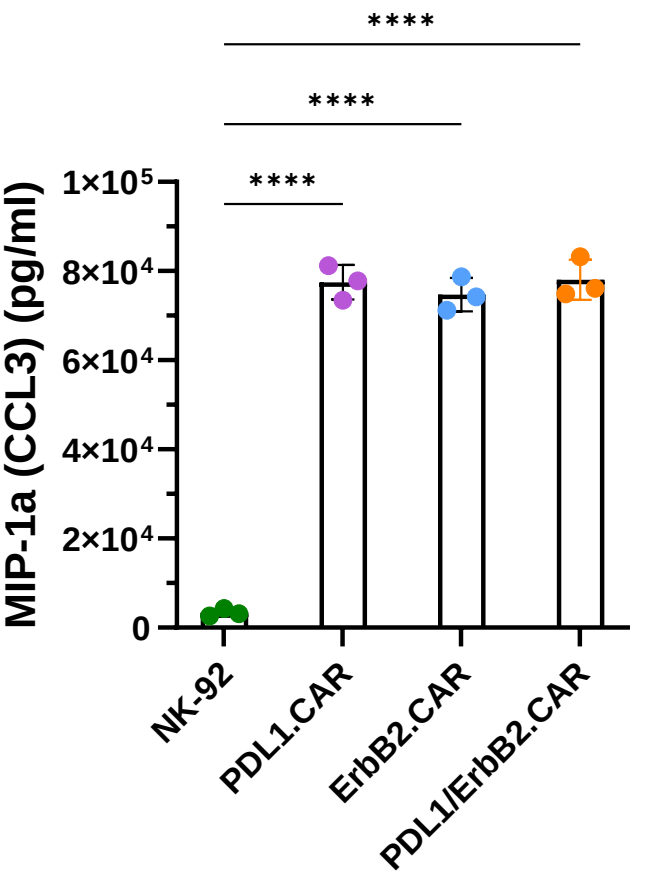
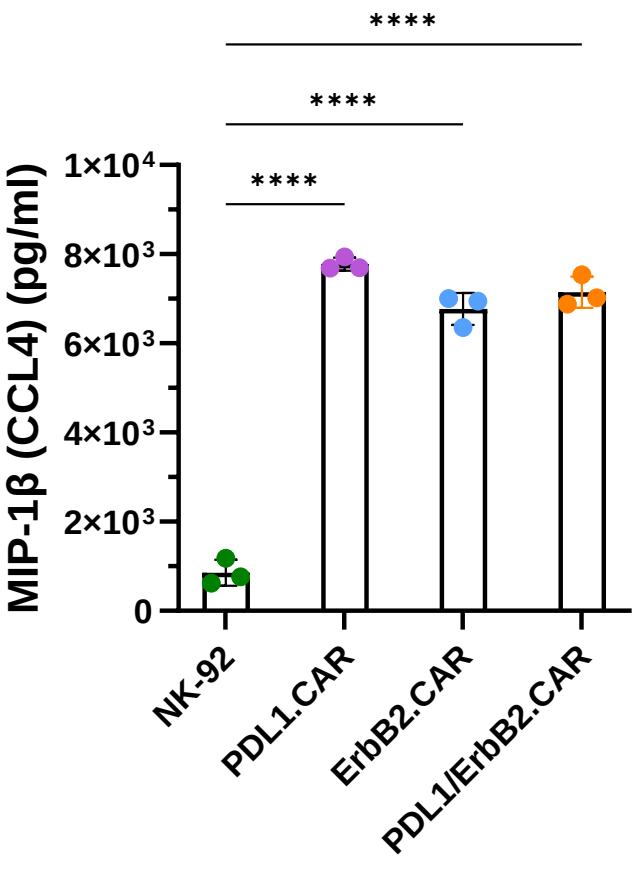

****
**
****
****
***
****
*
ns
*
*
ns
ns
CD107a (%)
40
30
20
10
0
NK-92
ErbB2.CAR
PDL1.CAR
PDL1/ErbB2.CAR
+ MDA-MB-231
medium
DMSO control
DMF control
MEK1/2 (PD0325901)
MEK1/2 (Trametinib)
PLC-γ (U73122)
PI3K (Wortmannin)

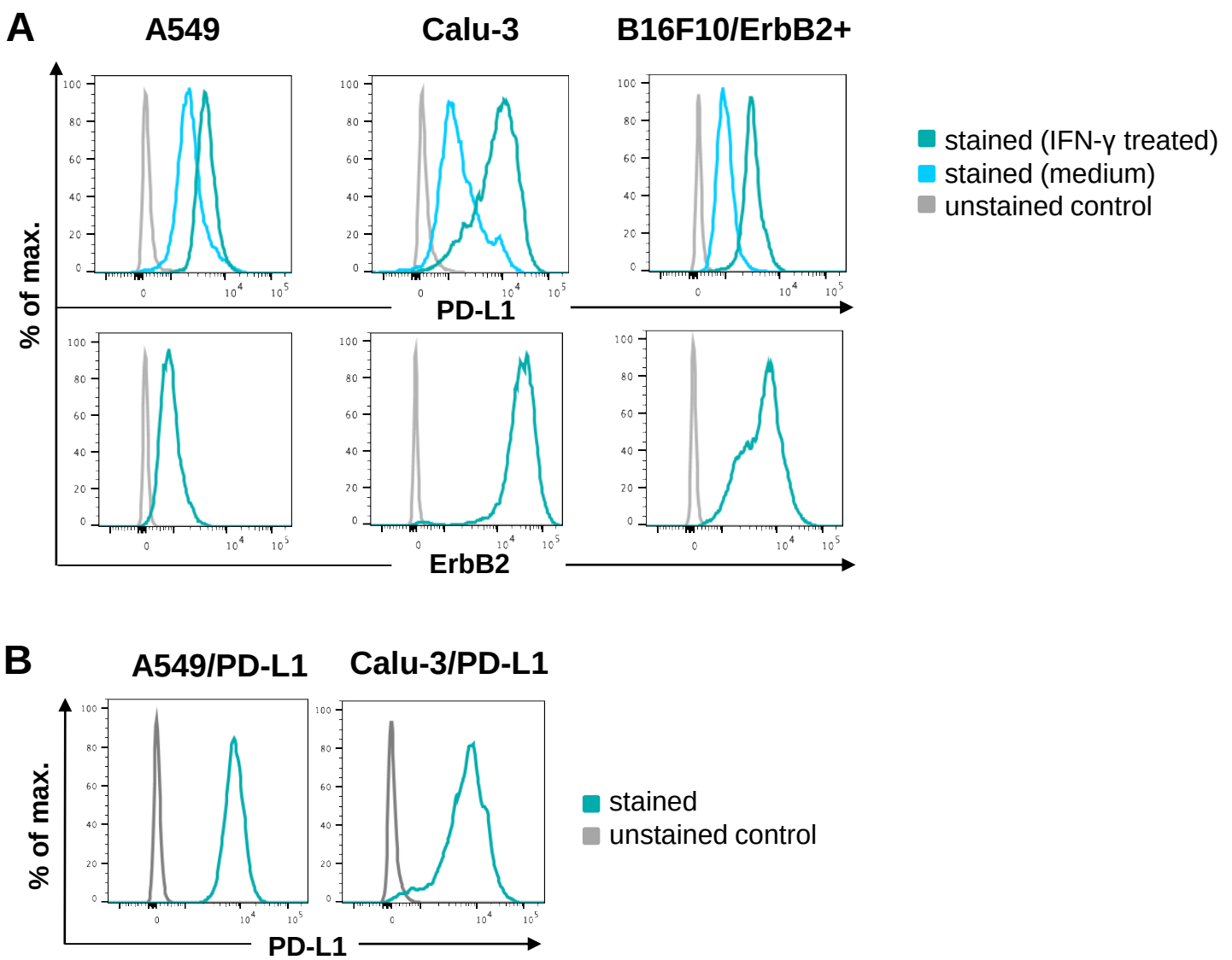
**Fig. S5**


ns
ns
ns
****
8×10³
6×10³
4×10³
2×10³
0
TNFa (pg/ml)
r'h TNF-α (6.125 pg/ml)
NK-92
PDL1.CAR
ErbB2.CAR
PDL1/ErbB2.CAR

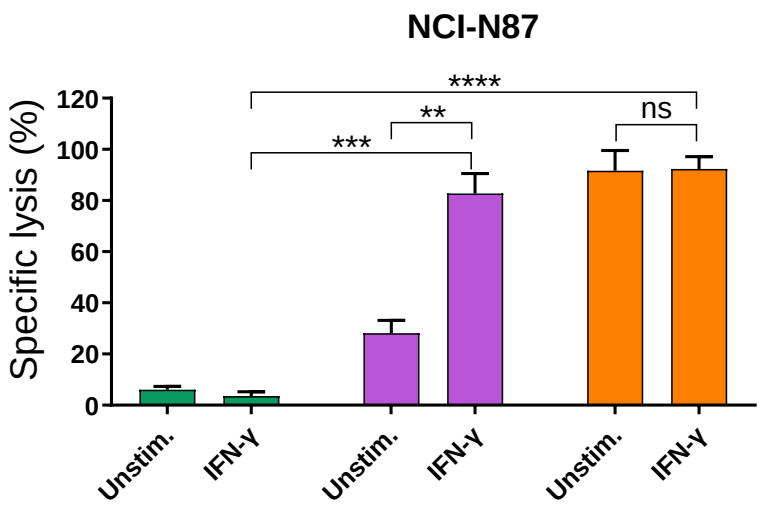

****
****
****
1×10⁵
8×10⁴
6×10⁴
4×10⁴
2×10⁴
0
MIP-1a (CCL3) (pg/ml)
NK-92
PDL1.CAR
ErbB2.CAR
PDL1/ErbB2.CAR

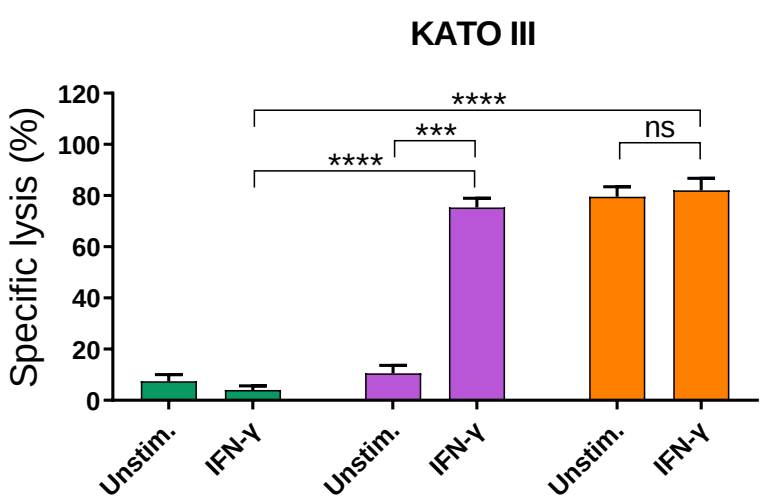

****
****
1×10⁴
8×10³
6×10³
4×10³
2×10³
0
MIP-1β (CCL4) (pg/ml)
NK-92
PDL1.CAR
ErbB2.CAR
PDL1/ErbB2.CAR

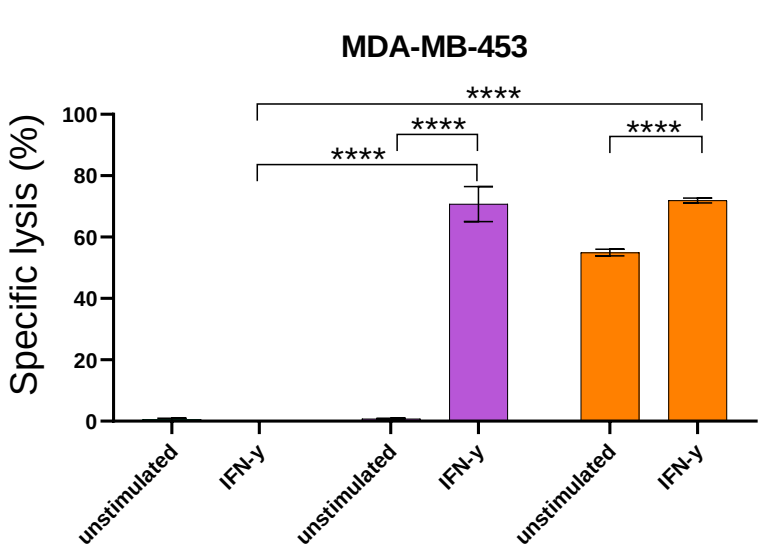
# Fig. S6


A
A549
Calu-3
B16F10/ErbB2+
% of max.
PD-L1
ErbB2
stained (IFN-γ treated)
stained (medium)
unstained control
B
A549/PD-L1
Calu-3/PD-L1
% of max.
PD-L1
stained
unstained control

# Fig. S7




NK-92
PDL1.CAR
PDL1/ErbB2.CAR

Fig. S8

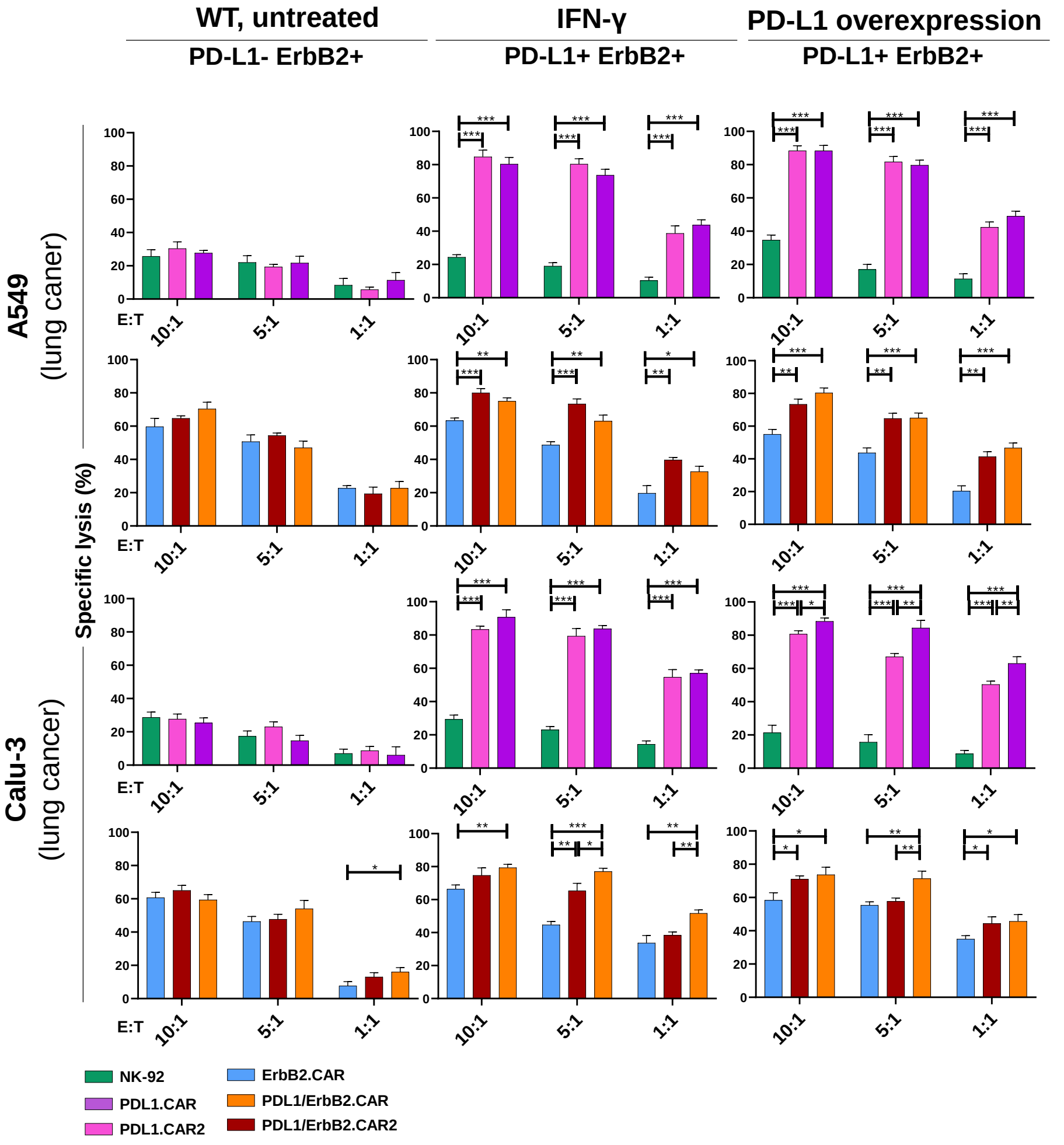

WT, untreated
IFN-γ
PD-L1 overexpression
PD-L1- ErbB2+
PD-L1+ ErbB2+
PD-L1+ ErbB2+
A549
(lung caner)
Calu-3
(lung cancer)
Specific lysis (%)
E:T
10:1
5:1
1:1
NK-92
PDL1.CAR
PDL1.CAR2
ErbB2.CAR
PDL1/ErbB2.CAR
PDL1/ErbB2.CAR2

Fig. S9

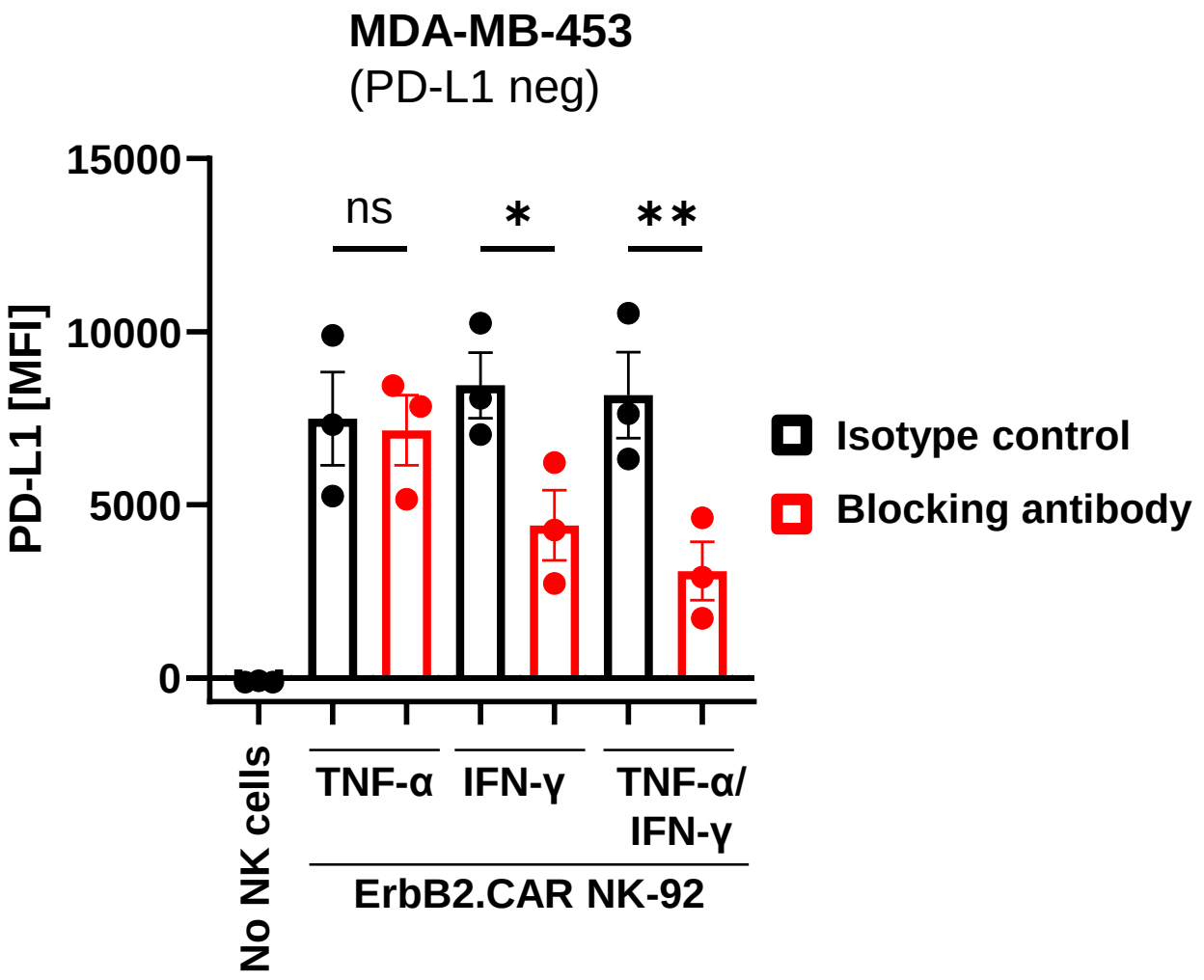
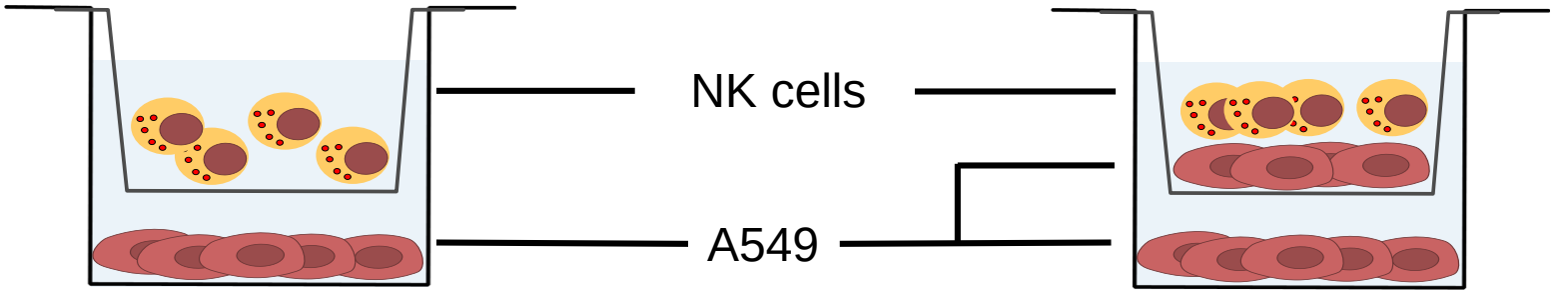
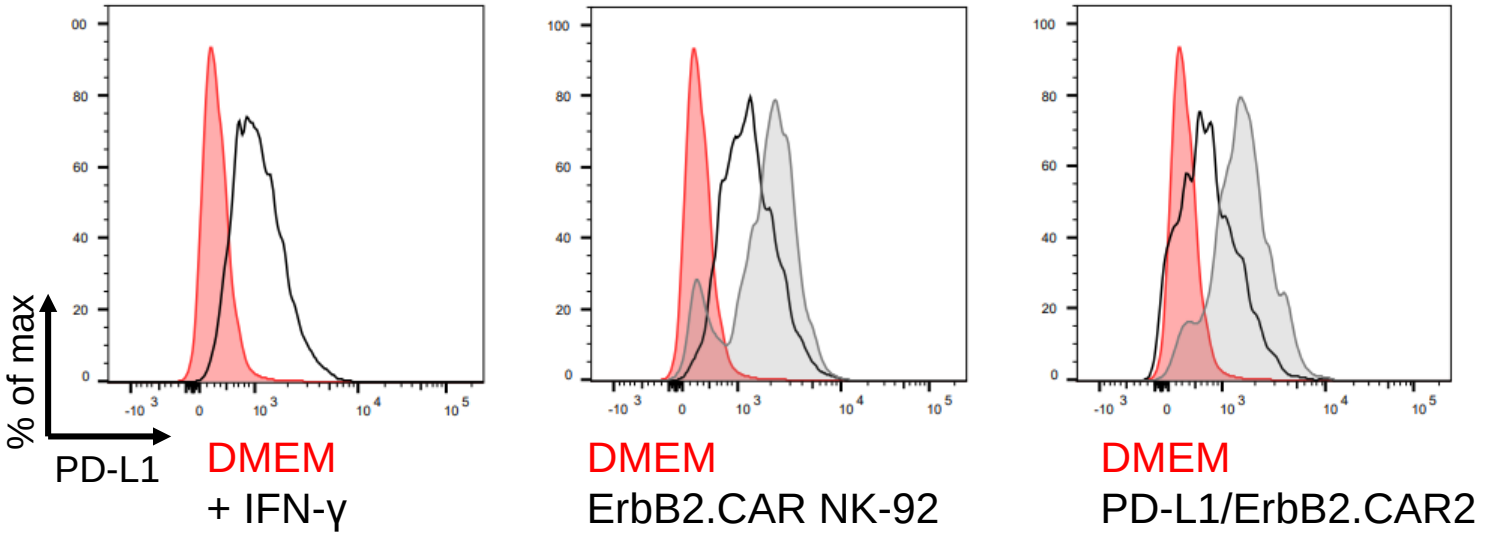

MDA-MB-453
(PD-L1 neg)
PD-L1 [MFI]
15000
10000
5000
0
ns
*
**
Isotype control
Blocking antibody
No NK cells
TNF-α
IFN-γ
TNF-α/
IFN-γ
ErbB2.CAR NK-92

**Fig. S10**

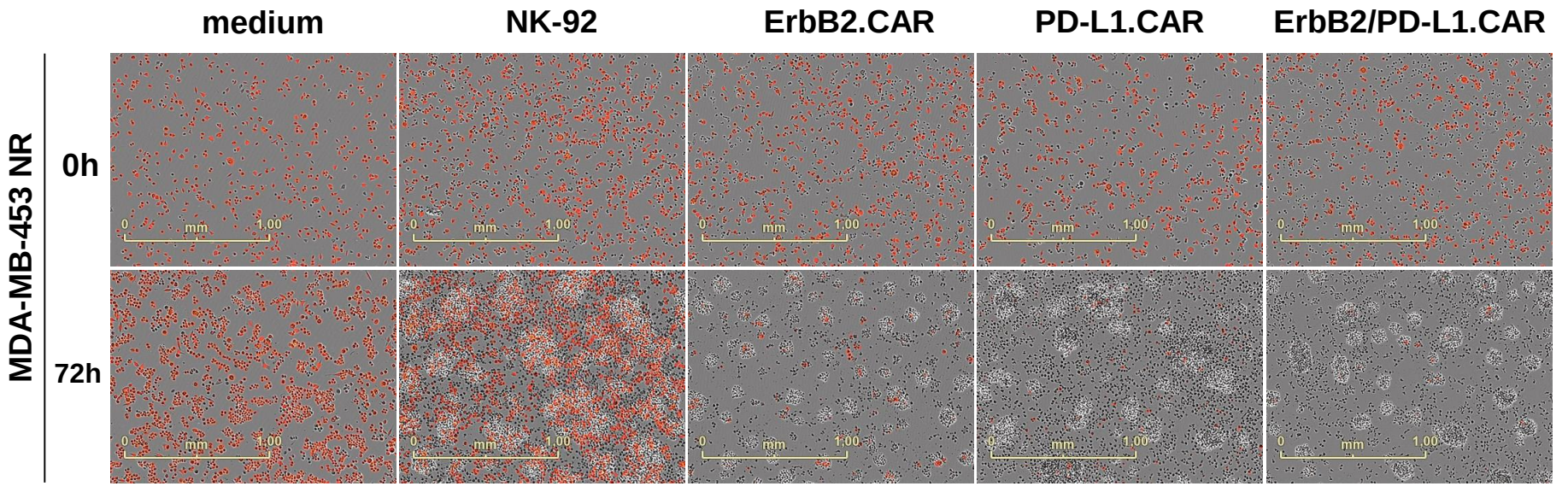
**A**


NK cells
A549

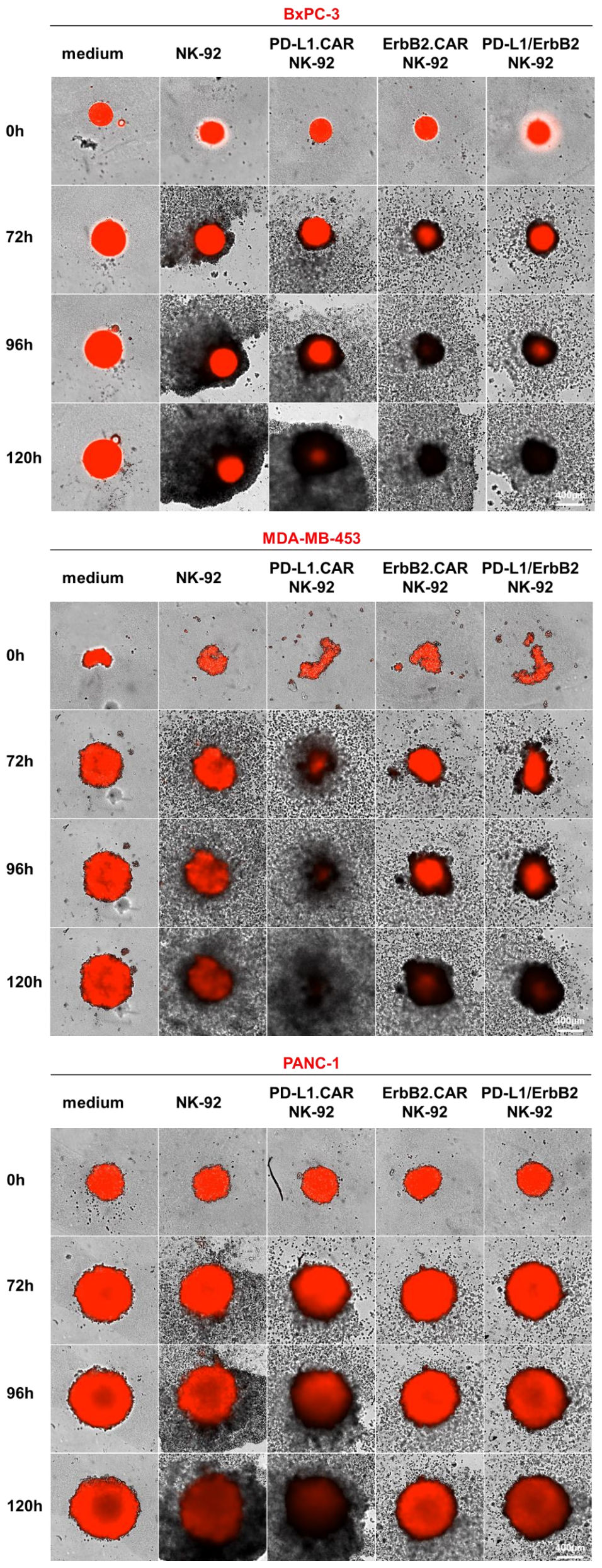
**B**


% of max
PD-L1
DMEM
+ IFN-γ
DMEM
ErbB2.CAR NK-92
ErbB2.CAR NK-92
+A549
DMEM
PD-L1/ErbB2.CAR2
PD-L1/ErbB2.CAR2
+A549

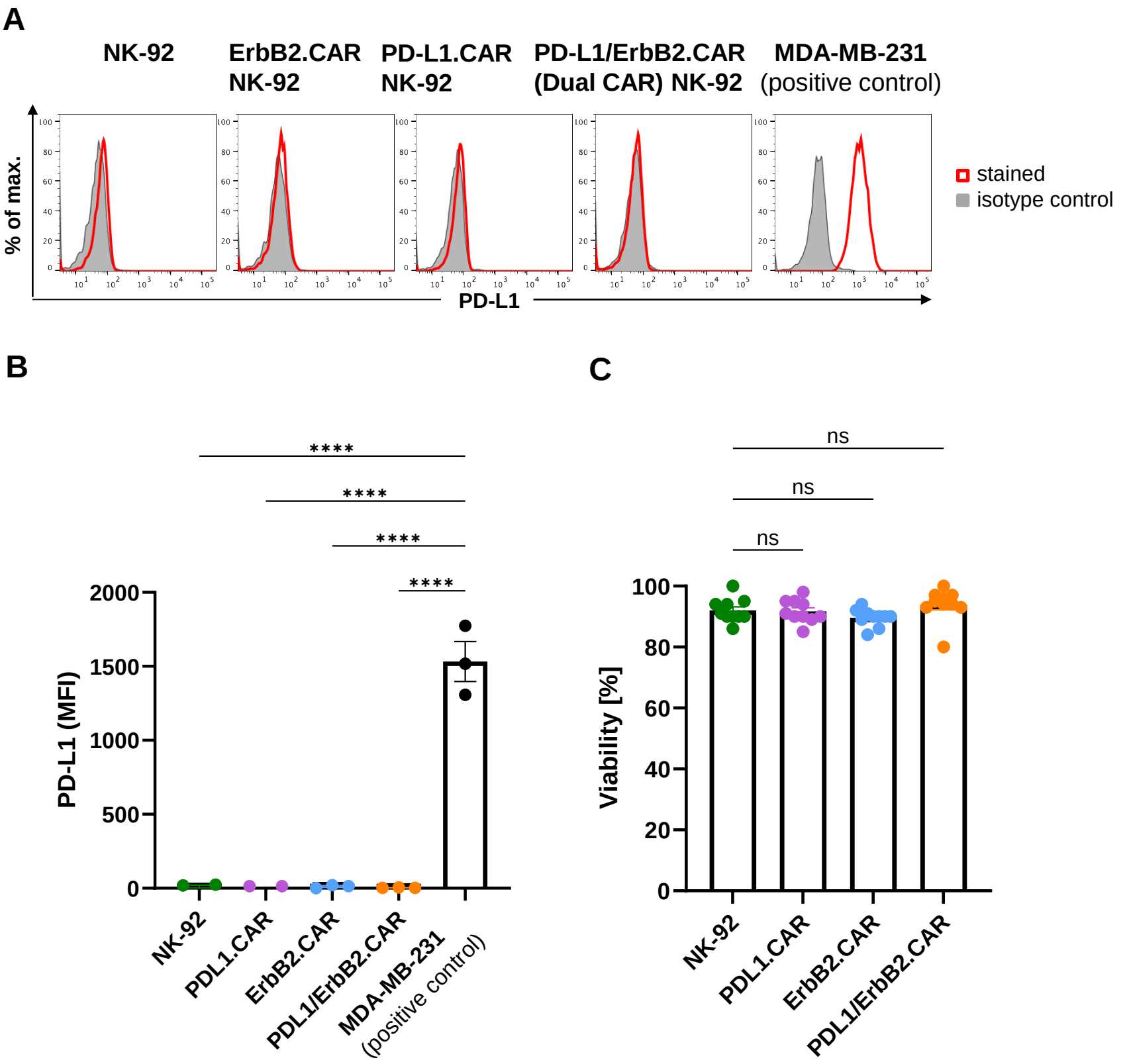
# Fig. S11


medium
NK-92
ErbB2.CAR
PD-L1.CAR
ErbB2/PD-L1.CAR
MDA-MB-453 NR
0h
72h
0
mm
1,00

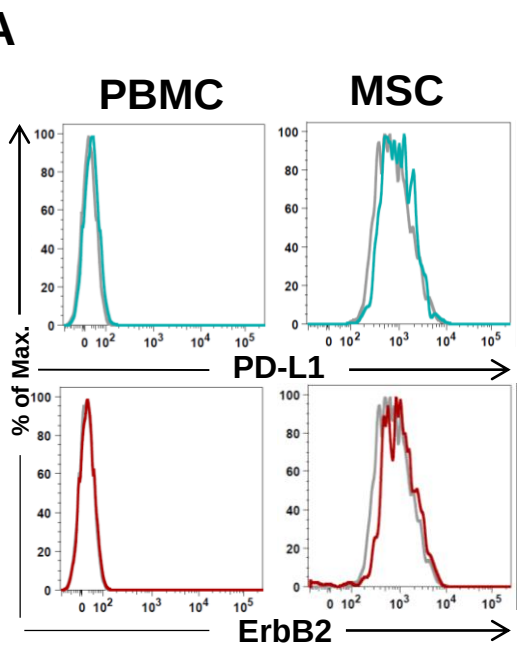
# Fig. S12

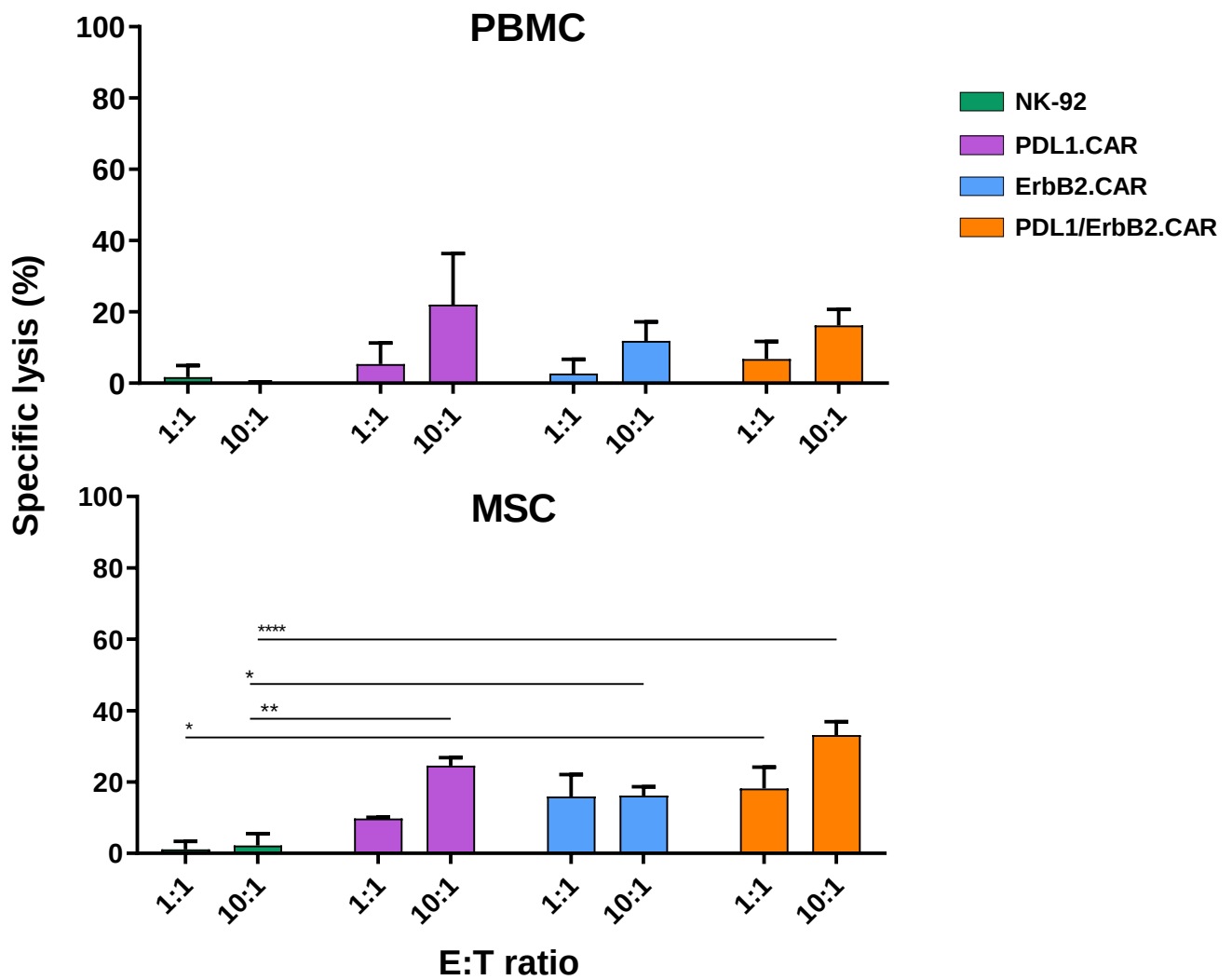

BxPC-3
medium
NK-92
PD-L1.CAR NK-92
ErbB2.CAR NK-92
PD-L1/ErbB2 NK-92
0h
72h
96h
120h
MDA-MB-453
medium
NK-92
PD-L1.CAR NK-92
ErbB2.CAR NK-92
PD-L1/ErbB2 NK-92
0h
72h
96h
120h
400μm
PANC-1
medium
NK-92
PD-L1.CAR NK-92
ErbB2.CAR NK-92
PD-L1/ErbB2 NK-92
0h
72h
96h
120h
400μm

# Fig. S13


A
NK-92
ErbB2.CAR NK-92
PD-L1.CAR NK-92
PD-L1/ErbB2.CAR (Dual CAR) NK-92
MDA-MB-231 (positive control)
% of max.
PD-L1
stained
isotype control
B
****
****
****
****
PD-L1 (MFI)
2000
1500
1000
500
0
NK-92
PDL1.CAR
ErbB2.CAR
PDL1/ErbB2.CAR
MDA-MB-231 (positive control)
C
ns
ns
ns
Viability [%]
100
80
60
40
20
0
NK-92
PDL1.CAR
ErbB2.CAR
PDL1/ErbB2.CAR

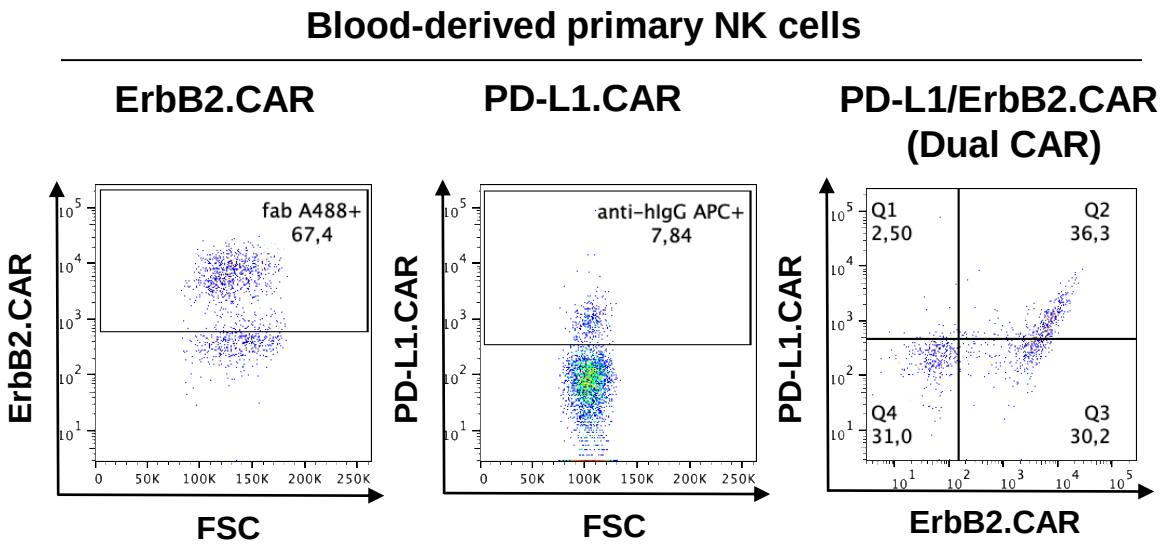
# Fig. S14


A
PBMC
MSC
% of Max.
PD-L1
ErbB2

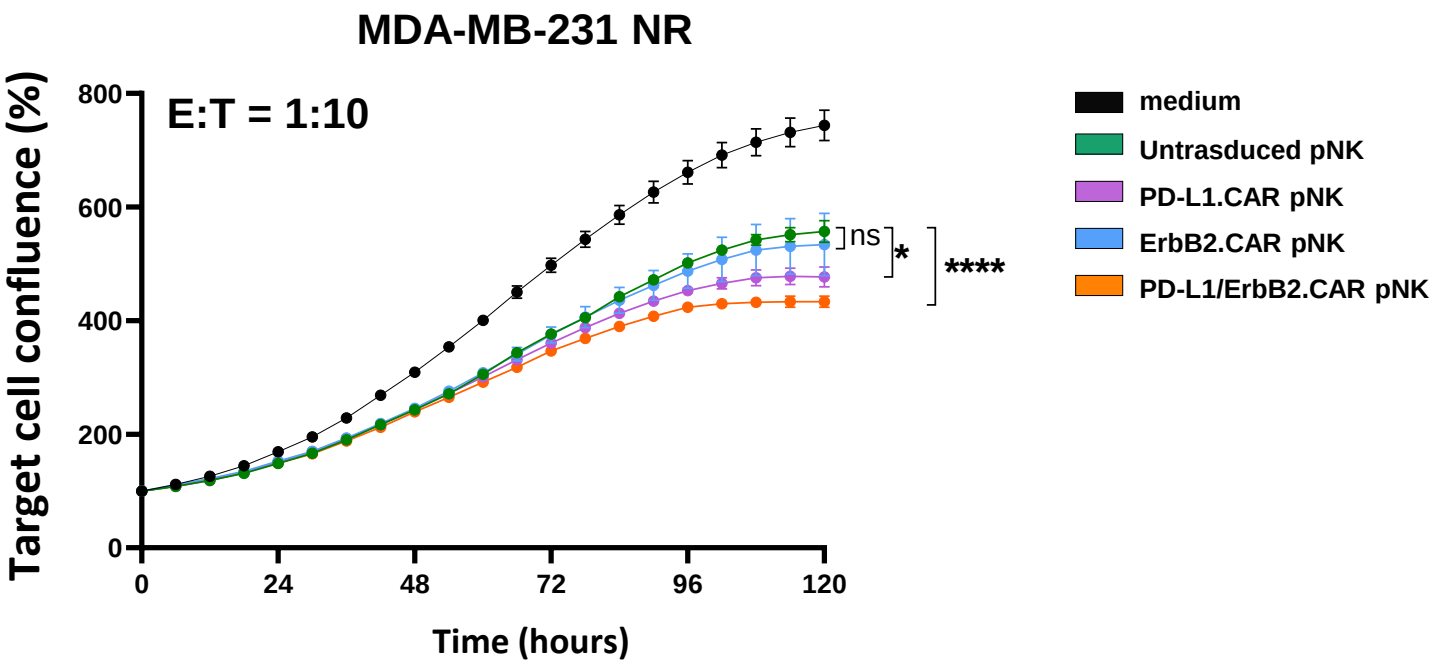

B
PBMC
MSC
Specific lysis (%)
E:T ratio
NK-92
PDL1.CAR
ErbB2.CAR
PDL1/ErbB2.CAR
1:1
10:1
****
*
**

# Fig. S15

**A**


Blood-derived primary NK cells
ErbB2.CAR
PD-L1.CAR
PD-L1/ErbB2.CAR
(Dual CAR)
fab A488+
67,4
anti-hIgG APC+
7,84
Q1
2,50
Q2
36,3
Q4
31,0
Q3
30,2
ErbB2.CAR
PD-L1.CAR
FSC
FSC
PD-L1.CAR
ErbB2.CAR

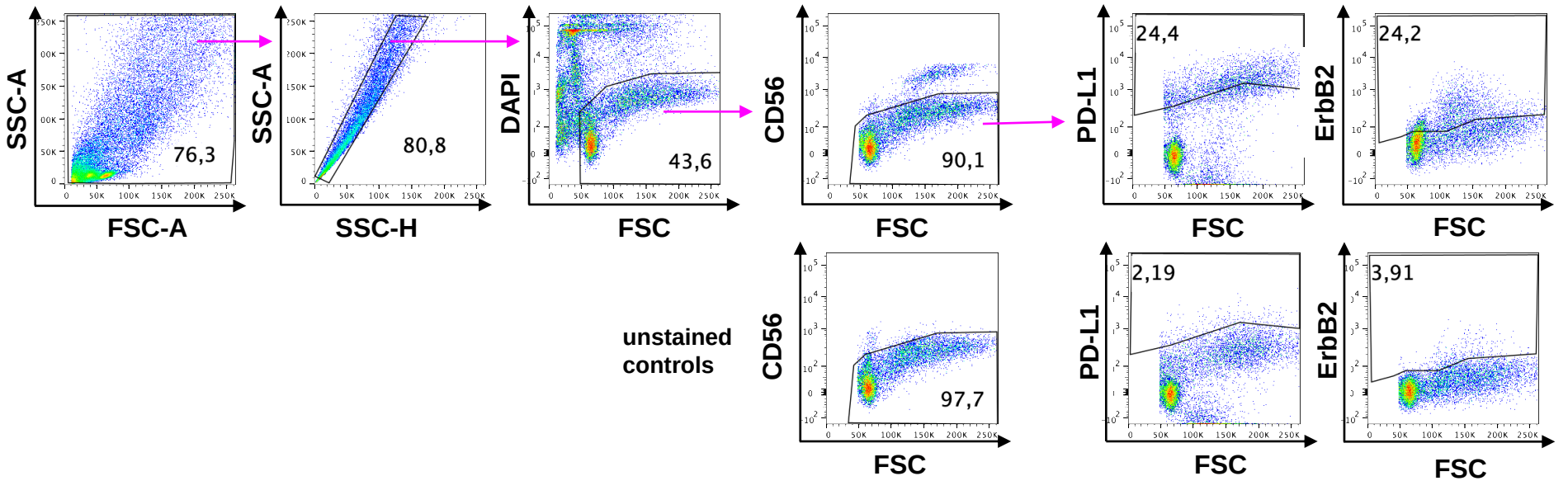
**B**


MDA-MB-231 NR
E:T = 1:10
Target cell confluence (%)
Time (hours)
ns
*
****
medium
Untrasduced pNK
PD-L1.CAR pNK
ErbB2.CAR pNK
PD-L1/ErbB2.CAR pNK

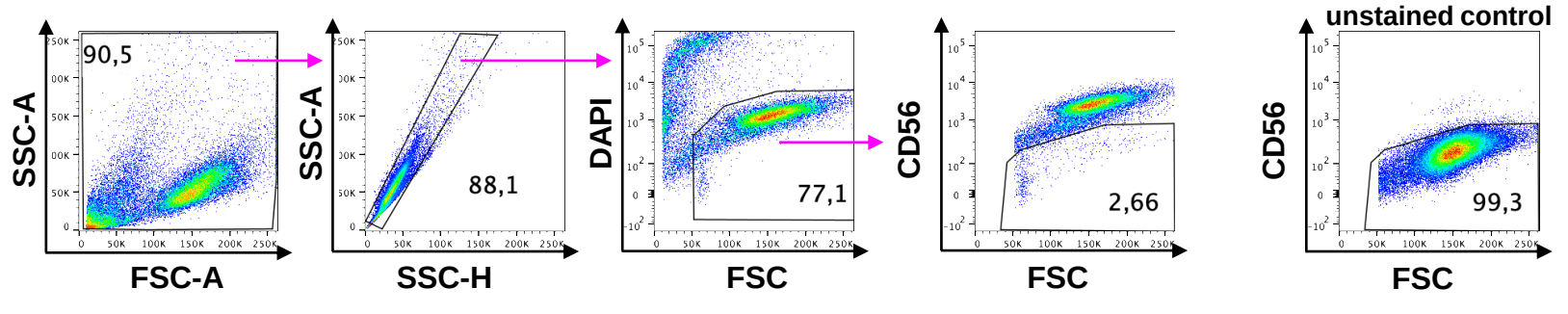
# Fig. S16

## A

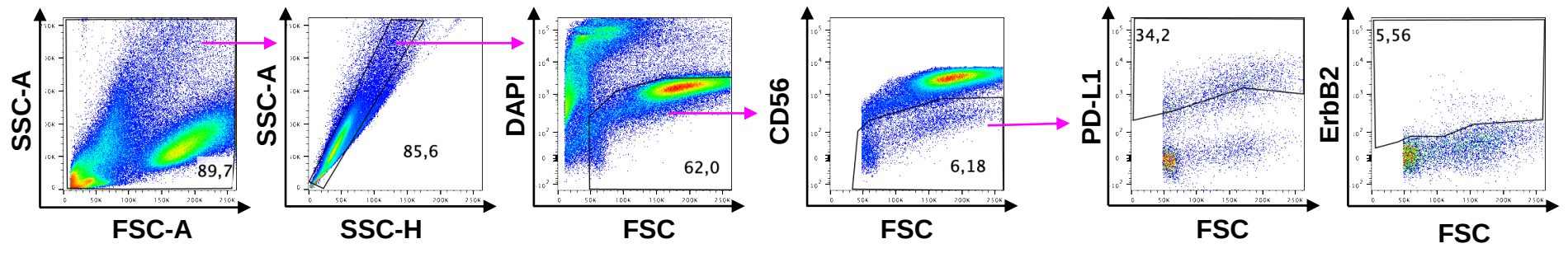
### Ascites cells



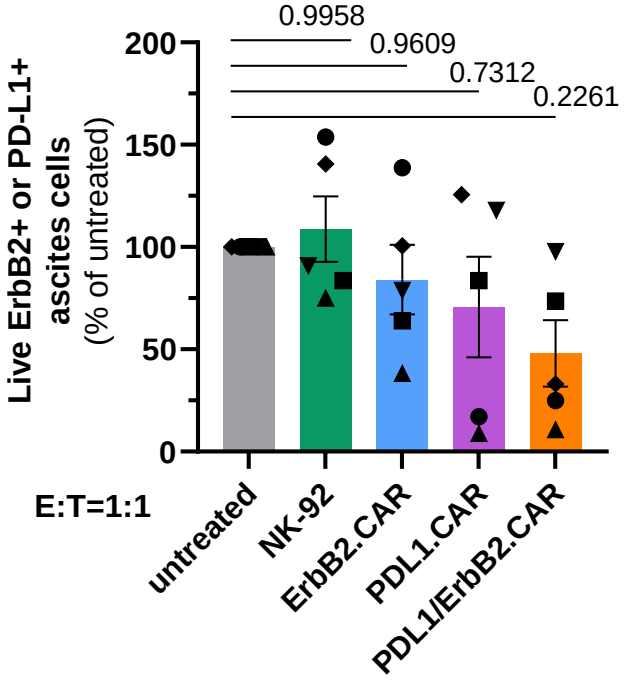

### PD-L1.CAR NK-92



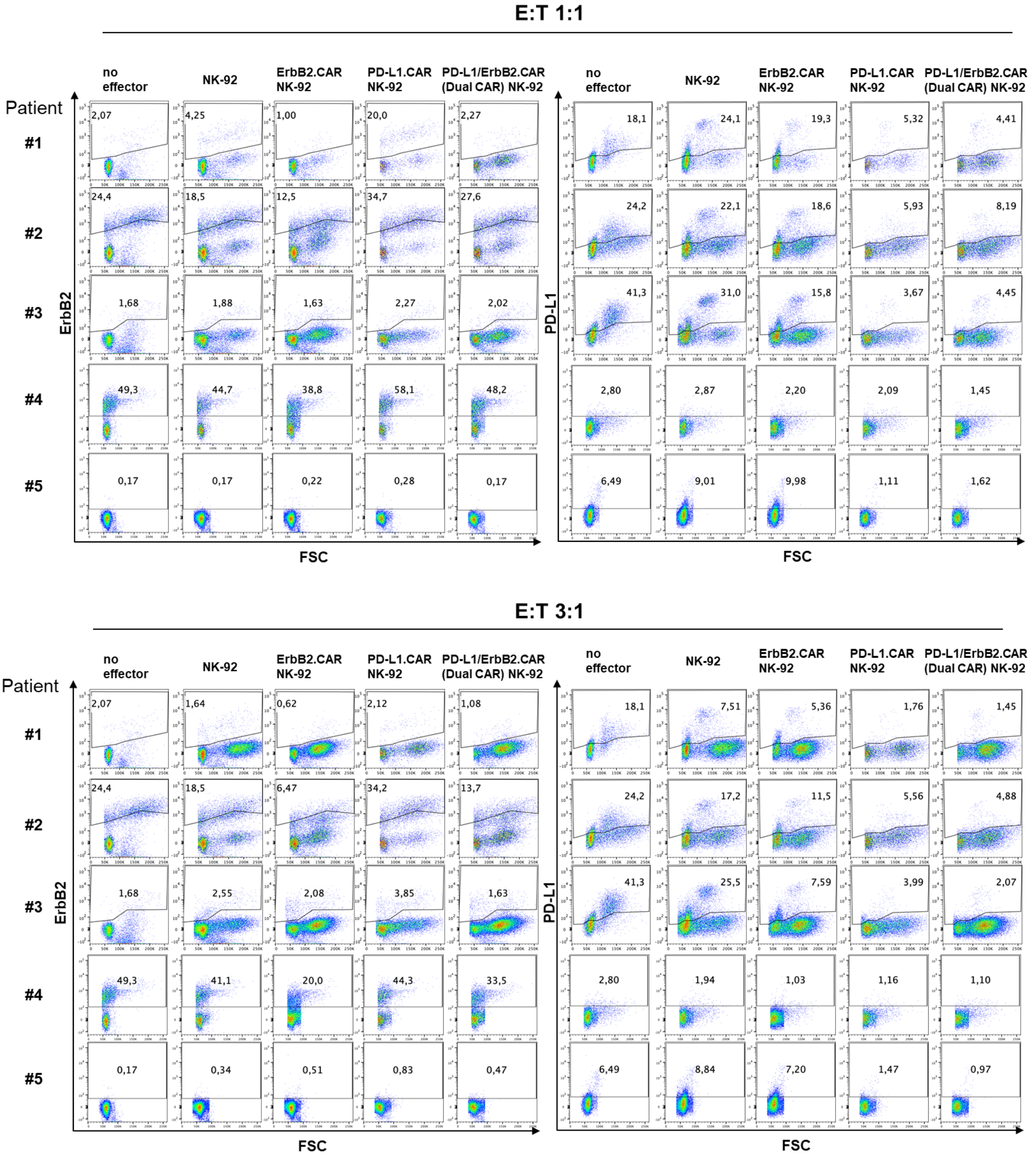

### PD-L1.CAR NK-92 + Ascites cells



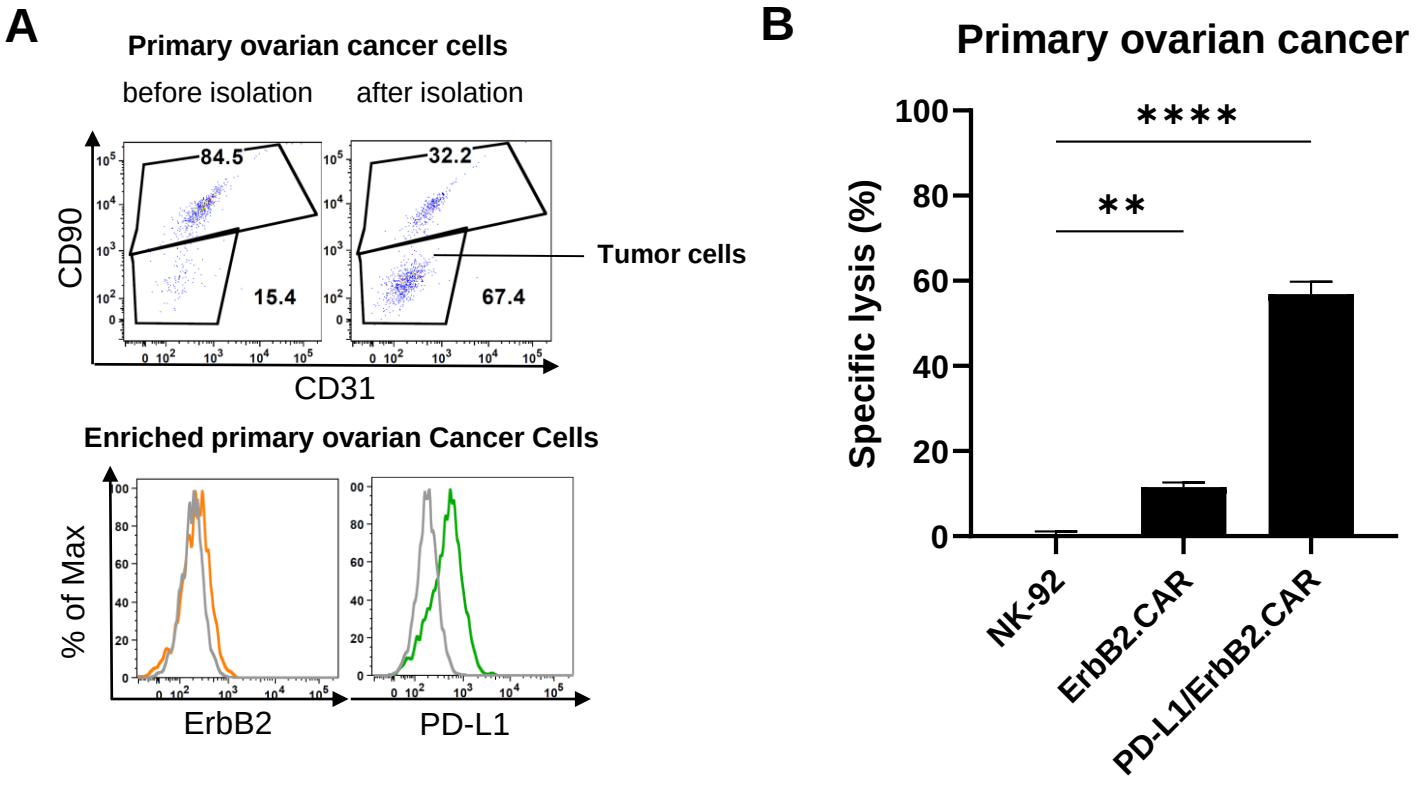

## B



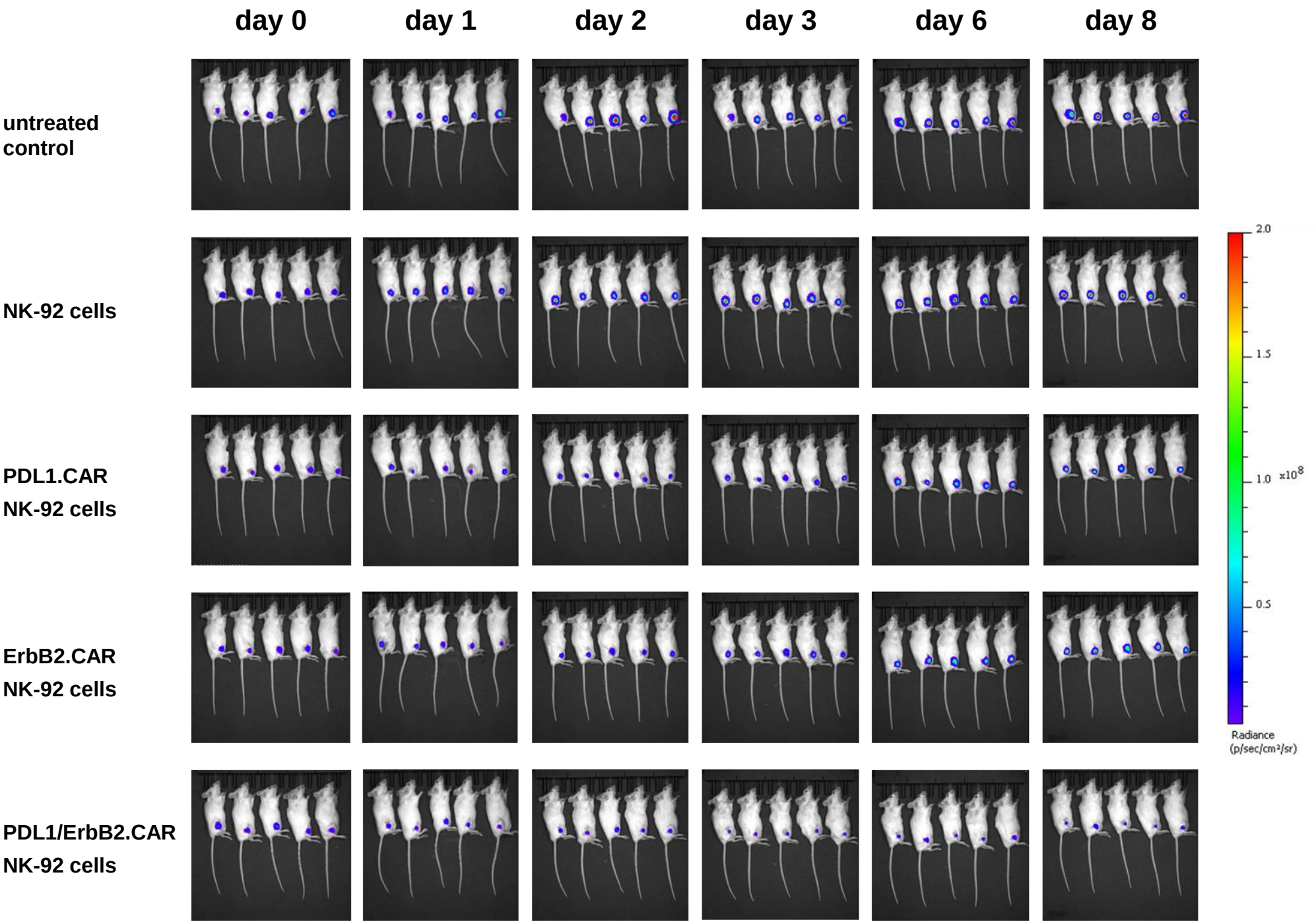
# Fig. S17


E:T 1:1
no effector
NK-92
ErbB2.CAR NK-92
PD-L1.CAR NK-92
PD-L1/ErbB2.CAR (Dual CAR) NK-92
Patient
#1
#2
#3
#4
#5
ErbB2
PD-L1
FSC
2,07
4,25
1,00
20,0
2,27
24,4
18,5
12,5
34,7
27,6
1,68
1,88
1,63
2,27
2,02
49,3
44,7
38,8
58,1
48,2
0,17
0,17
0,22
0,28
0,17
18,1
24,1
19,3
5,32
4,41
24,2
22,1
18,6
5,93
8,19
41,3
31,0
15,8
3,67
4,45
2,80
2,87
2,20
2,09
1,45
6,49
9,01
9,98
1,11
1,62
E:T 3:1
2,07
1,64
0,62
2,12
1,08
24,4
18,5
6,47
34,2
13,7
1,68
2,55
2,08
3,85
1,63
49,3
41,1
20,0
44,3
33,5
0,17
0,34
0,51
0,83
0,47
18,1
7,51
5,36
1,76
1,45
24,2
17,2
11,5
5,56
4,88
41,3
25,5
7,59
3,99
2,07
2,80
1,94
1,03
1,16
1,10
6,49
8,84
7,20
1,47
0,97

# Fig. S18


A
Primary ovarian cancer cells
before isolation
after isolation
84.5
15.4
32.2
67.4
CD90
CD31
Tumor cells
Enriched primary ovarian Cancer Cells
% of Max
ErbB2
PD-L1
B
Primary ovarian cancer
Specific lysis (%)
100
80
60
40
20
0
****
**
NK-92
ErbB2.CAR
PD-L1/ErbB2.CAR

# Fig. S19


day 0
day 1
day 2
day 3
day 6
day 8
untreated control
NK-92 cells
PDL1.CAR NK-92 cells
ErbB2.CAR NK-92 cells
PDL1/ErbB2.CAR NK-92 cells
2.0
1.5
1.0
0.5
x10^8
Radiance (p/sec/cm²/sr)