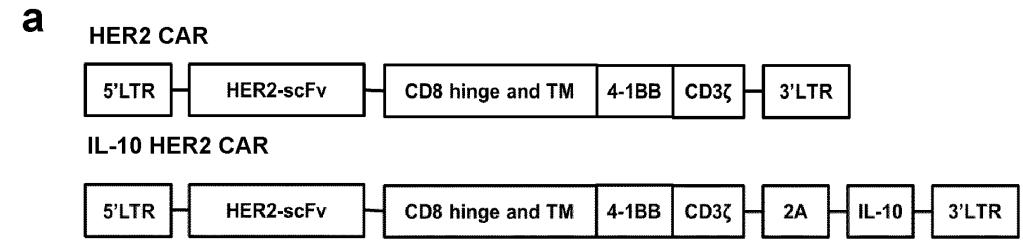
Figure 1 a, b


a
HER2 CAR
5'LTR
HER2-scFv
CD8 hinge and TM
4-1BB
CD3ζ
3'LTR
IL-10 HER2 CAR
5'LTR
HER2-scFv
CD8 hinge and TM
4-1BB
CD3ζ
2A
IL-10
3'LTR

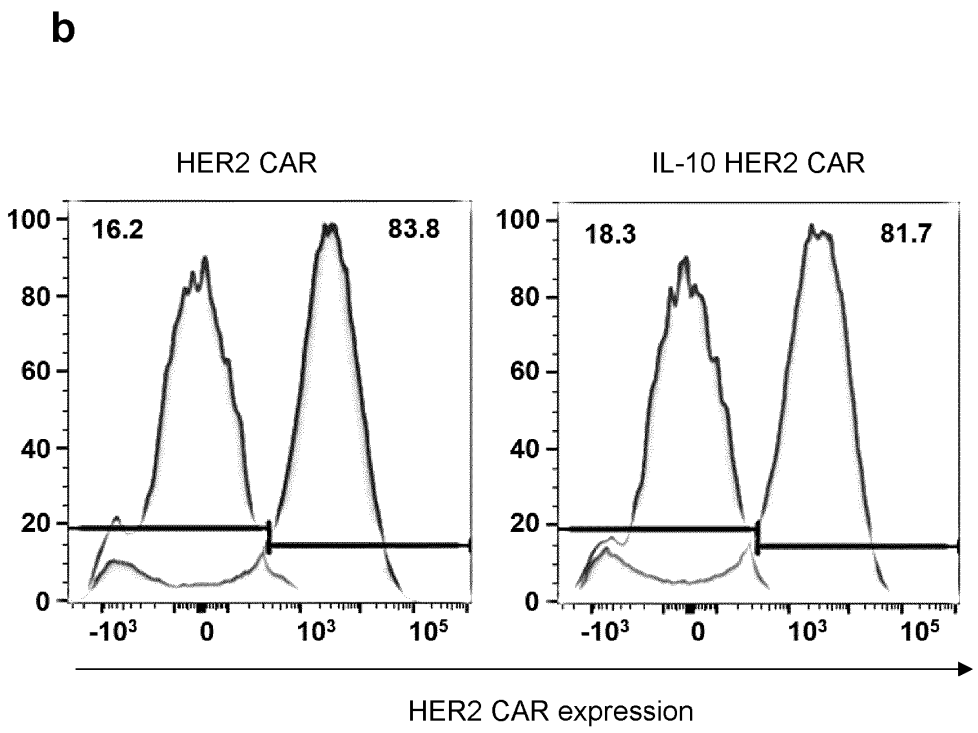

b
HER2 CAR
IL-10 HER2 CAR
16.2
83.8
18.3
81.7
100
80
60
40
20
0
-10³
0
10³
10⁵
HER2 CAR expression

Figure 1 c, d, e

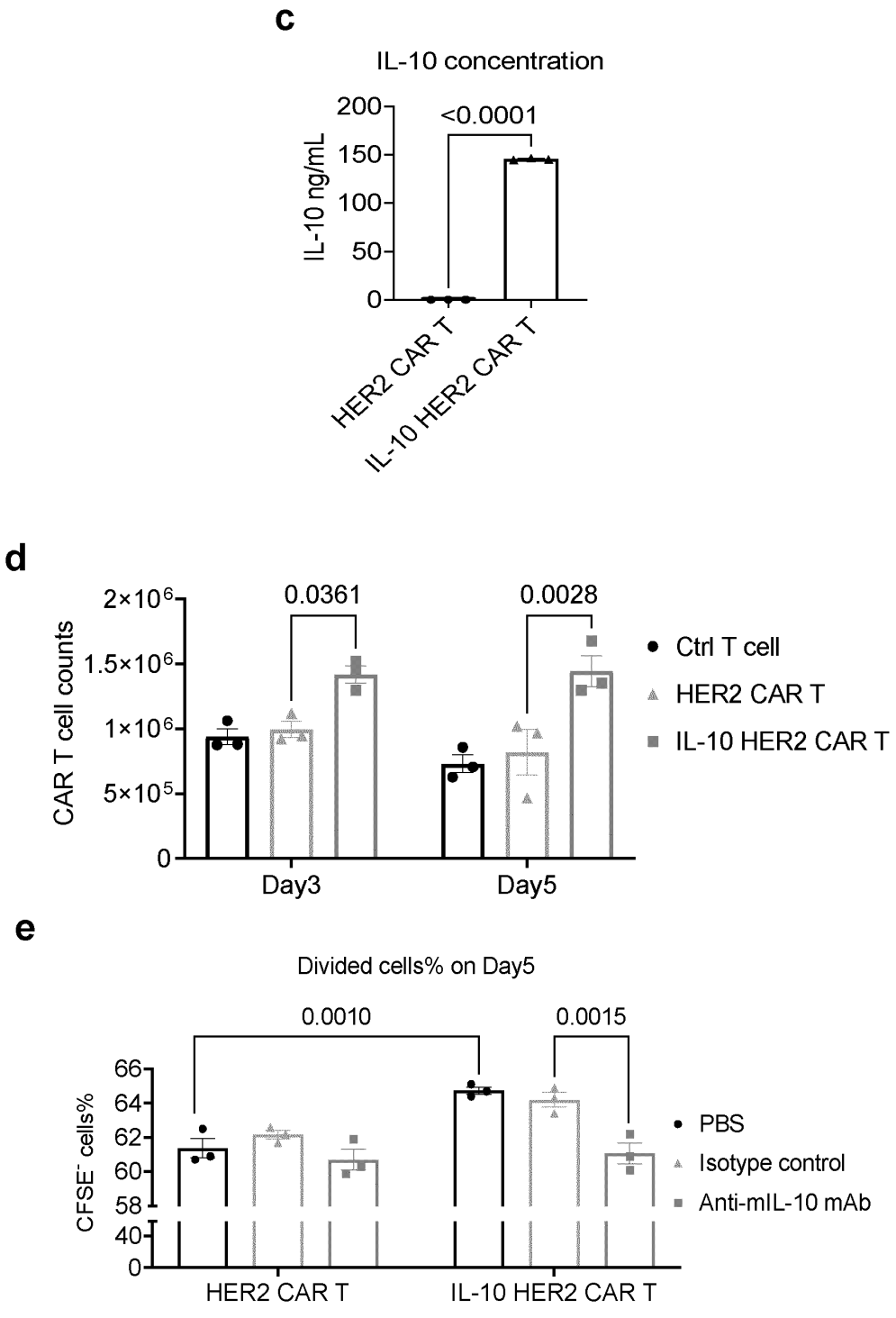
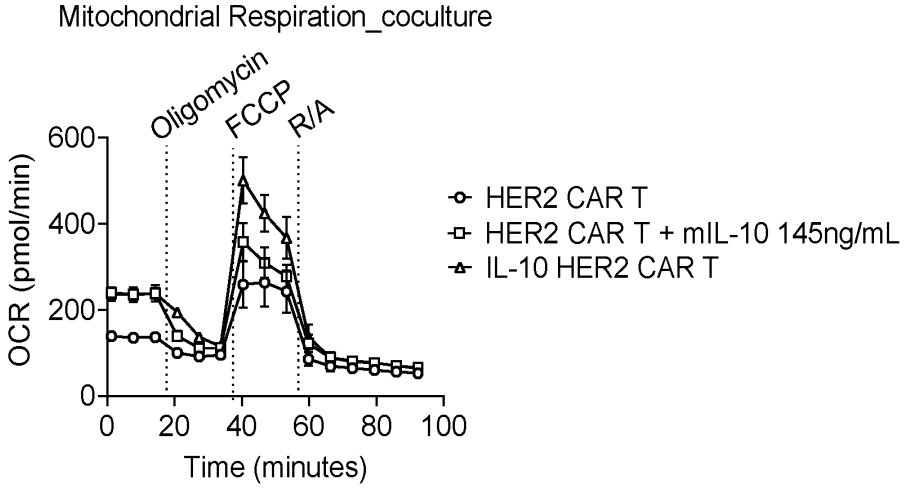

c
IL-10 concentration
IL-10 ng/mL
200
150
100
50
0
<0.0001
HER2 CAR T
IL-10 HER2 CAR T
d
CAR T cell counts
2×10^6
1.5×10^6
1×10^6
5×10^5
0
0.0361
0.0028
Day3
Day5
Ctrl T cell
HER2 CAR T
IL-10 HER2 CAR T
e
Divided cells% on Day5
CFSE- cells%
66
64
62
60
58
40
0
0.0010
0.0015
HER2 CAR T
IL-10 HER2 CAR T
PBS
Isotype control
Anti-mIL-10 mAb

Figure 1 f, g

f



g

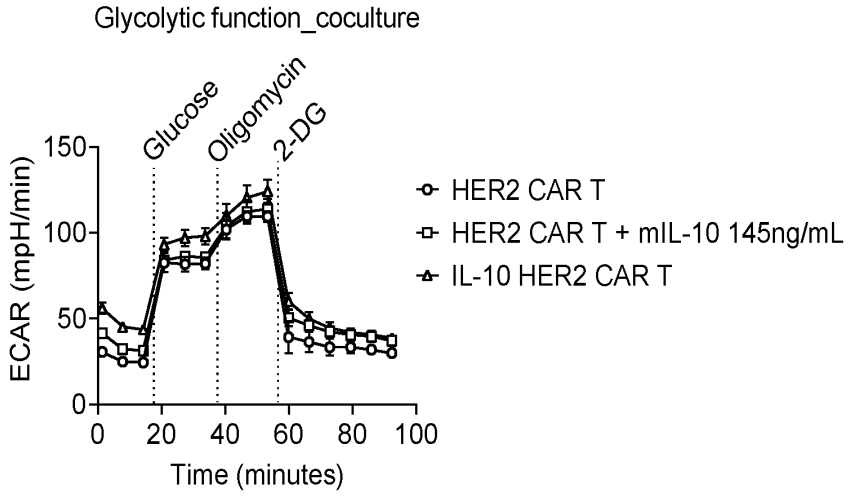
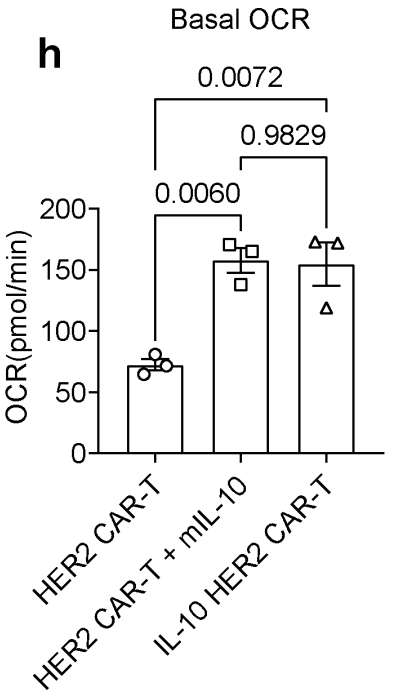


Figure 1 h, i


h
Basal OCR
0.0072
0.9829
0.0060
OCR(pmol/min)
200
150
100
50
0
HER2 CAR-T
HER2 CAR-T + mIL-10
IL-10 HER2 CAR-T

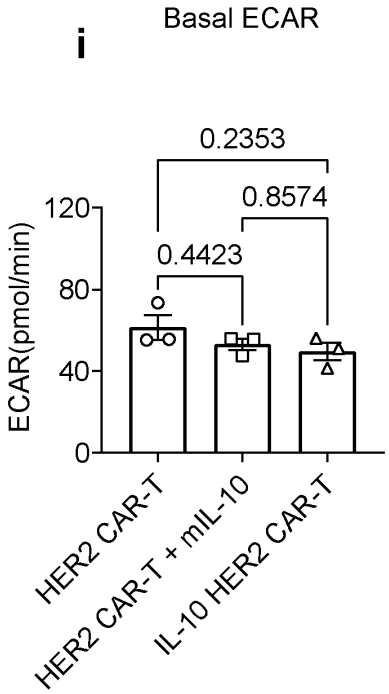

i
Basal ECAR
0.2353
0.8574
0.4423
ECAR(pmol/min)
120
80
40
0
HER2 CAR-T
HER2 CAR-T + mIL-10
IL-10 HER2 CAR-T

Figure 1 j

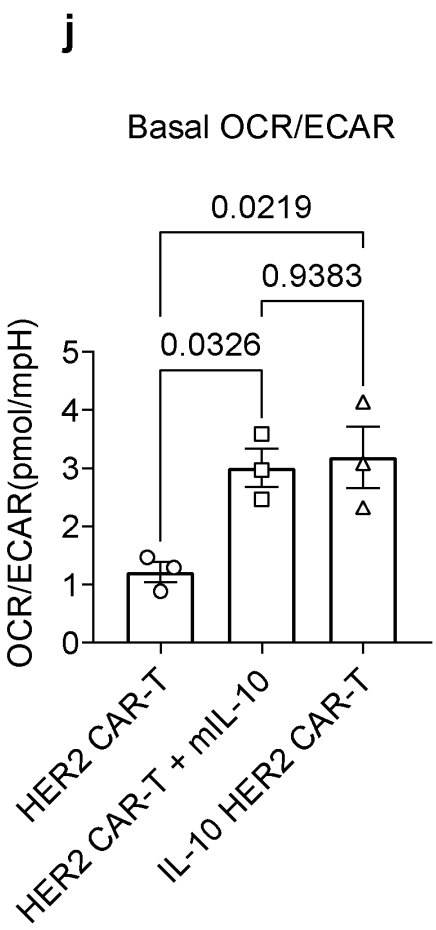

j
Basal OCR/ECAR
0.0219
0.9383
0.0326
OCR/ECAR(pmol/mpH)
5
4
3
2
1
0
HER2 CAR-T
HER2 CAR-T + mIL-10
IL-10 HER2 CAR-T

Figure 2

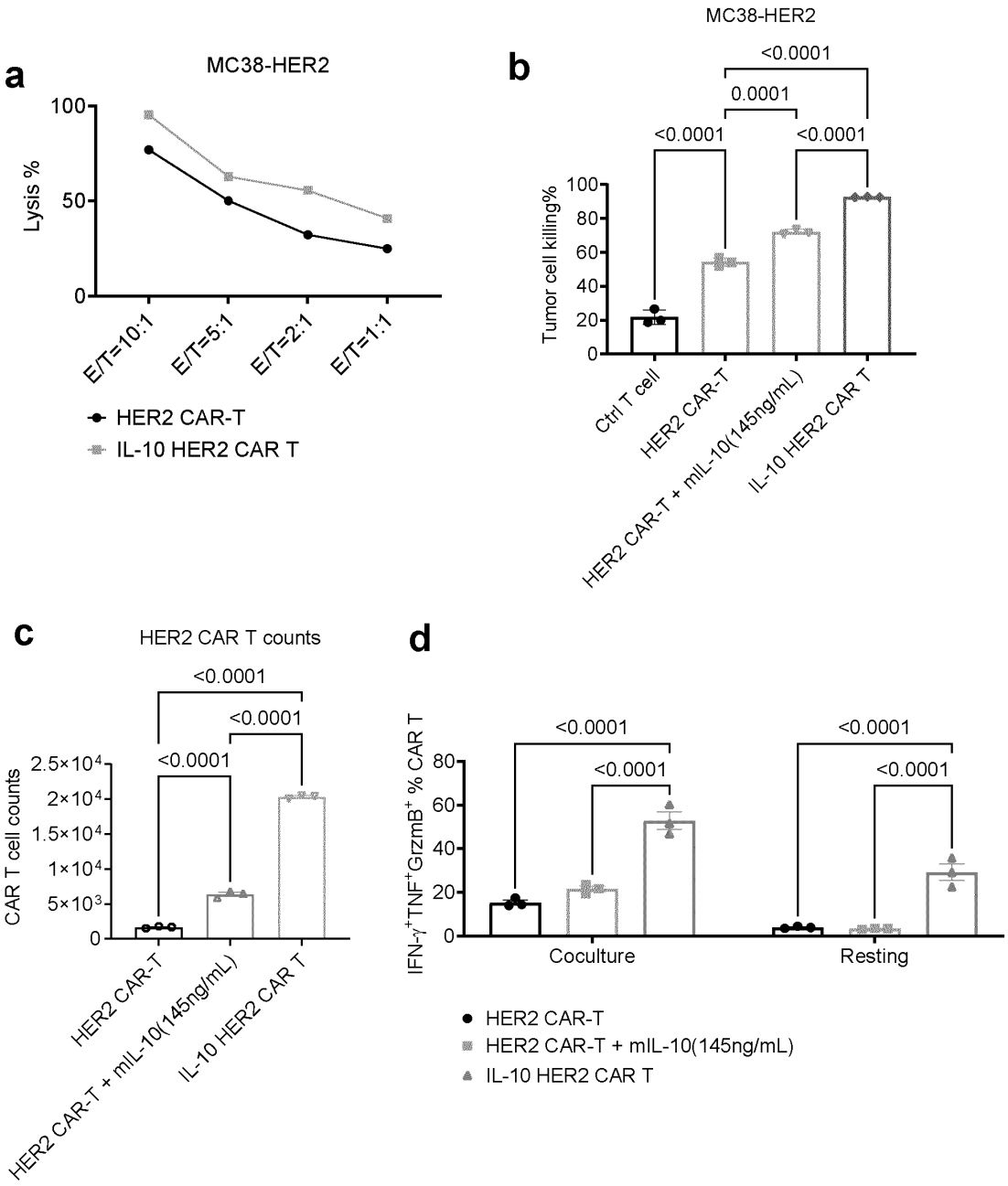

a
MC38-HER2
Lysis %
100
50
0
E/T=10:1
E/T=5:1
E/T=2:1
E/T=1:1
HER2 CAR-T
IL-10 HER2 CAR T
b
MC38-HER2
<0.0001
0.0001
<0.0001
<0.0001
Tumor cell killing%
100
80
60
40
20
0
Ctrl T cell
HER2 CAR-T
HER2 CAR-T + mIL-10(145ng/mL)
IL-10 HER2 CAR T
c
HER2 CAR T counts
<0.0001
<0.0001
<0.0001
CAR T cell counts
2.5×10⁴
2×10⁴
1.5×10⁴
1×10⁴
5×10³
0
HER2 CAR-T
HER2 CAR-T + mIL-10(145ng/mL)
IL-10 HER2 CAR T
d
<0.0001
<0.0001
<0.0001
<0.0001
IFN-γ⁺TNF⁺GrzmB⁺ % CAR T
80
60
40
20
0
Coculture
Resting
HER2 CAR-T
HER2 CAR-T + mIL-10(145ng/mL)
IL-10 HER2 CAR T

Figure 3

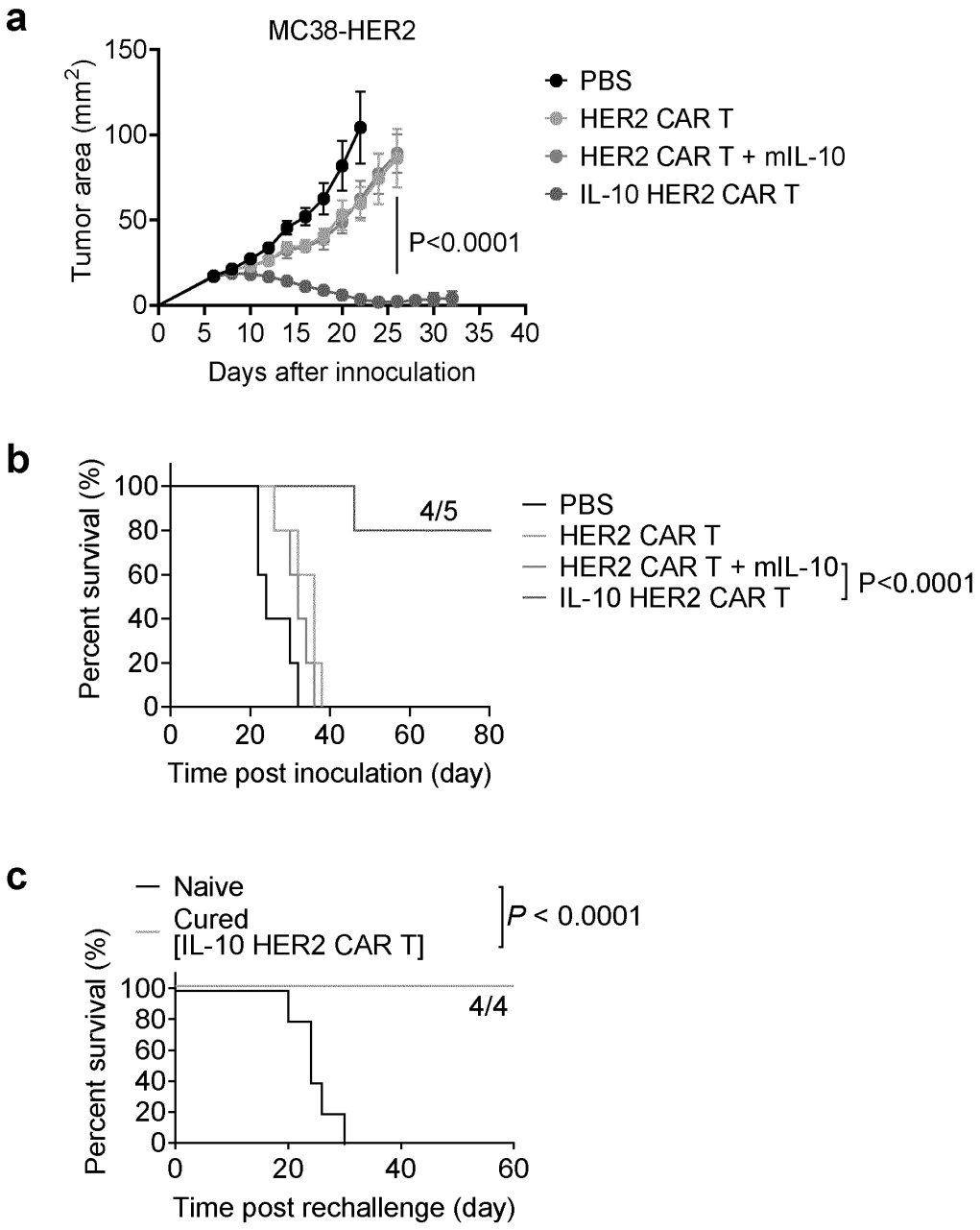
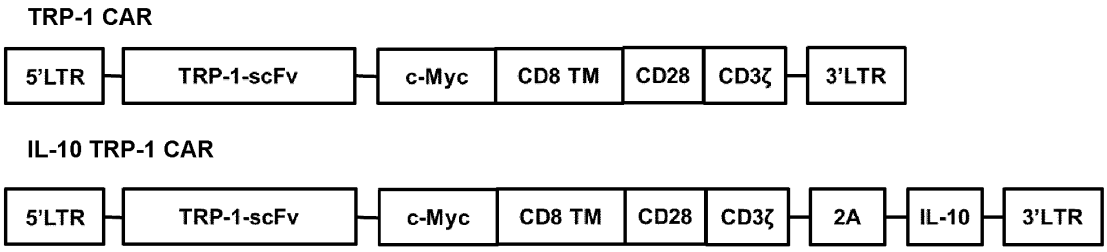

a
MC38-HER2
Tumor area (mm2)
150
100
50
0
0 5 10 15 20 25 30 35 40
Days after innoculation
P<0.0001
PBS
HER2 CAR T
HER2 CAR T + mIL-10
IL-10 HER2 CAR T
b
Percent survival (%)
100
80
60
40
20
0
0 20 40 60 80
Time post inoculation (day)
4/5
PBS
HER2 CAR T
HER2 CAR T + mIL-10
IL-10 HER2 CAR T
P<0.0001
c
Naive
Cured
[IL-10 HER2 CAR T]
P < 0.0001
Percent survival (%)
100
80
60
40
20
0
0 20 40 60
Time post rechallenge (day)
4/4

Figure 4 a, b

a



b

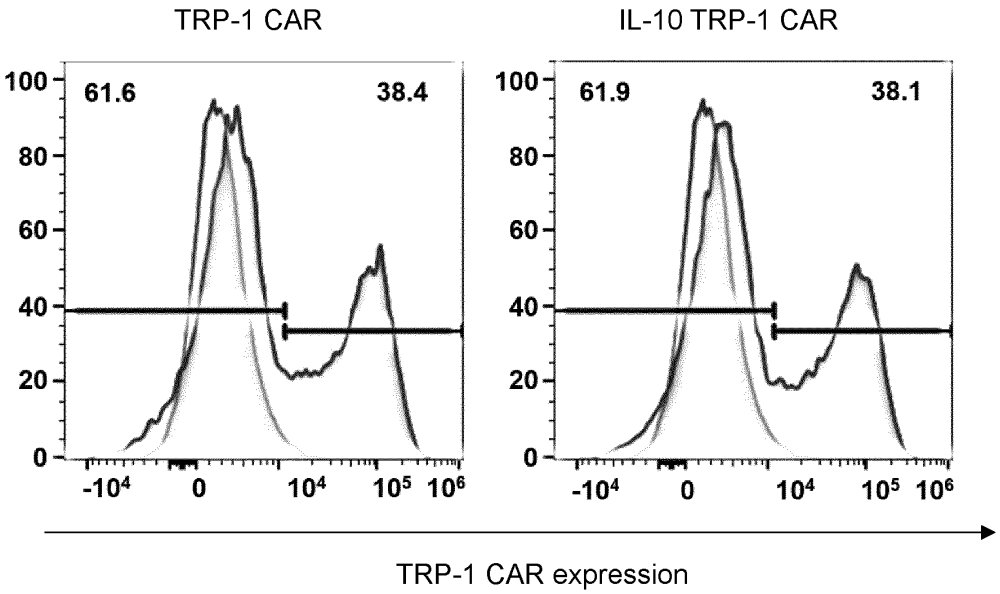


Figure 4 c, d

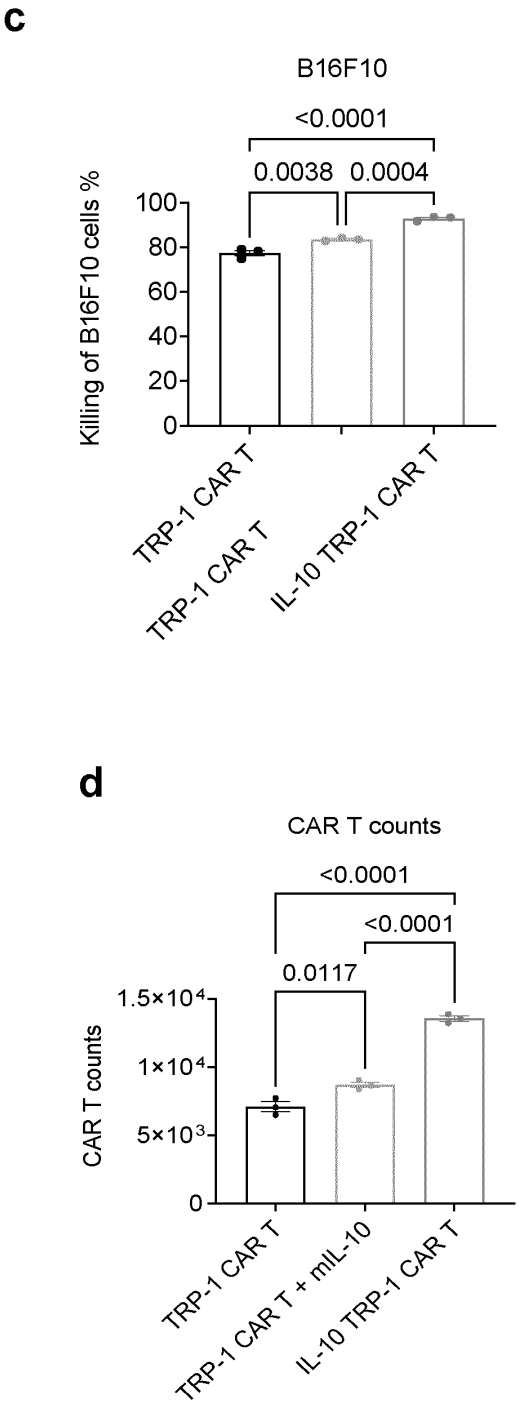
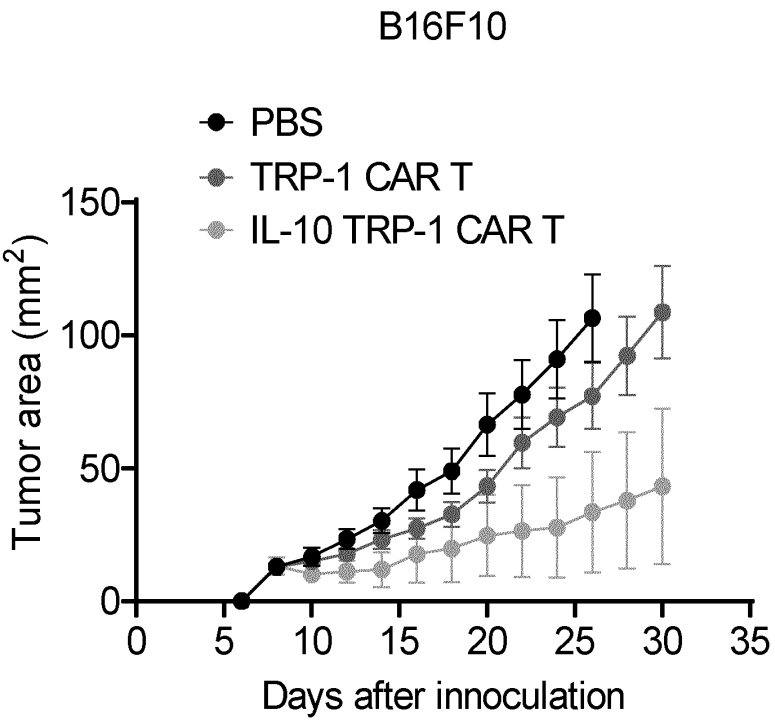

c
B16F10
<0.0001
0.0038
0.0004
Killing of B16F10 cells %
100
80
60
40
20
0
TRP-1 CAR T
TRP-1 CAR T
IL-10 TRP-1 CAR T
d
CAR T counts
<0.0001
<0.0001
0.0117
CAR T counts
1.5×10⁴
1×10⁴
5×10³
0
TRP-1 CAR T
TRP-1 CAR T + mIL-10
IL-10 TRP-1 CAR T

Figure 4 e, f

e



f

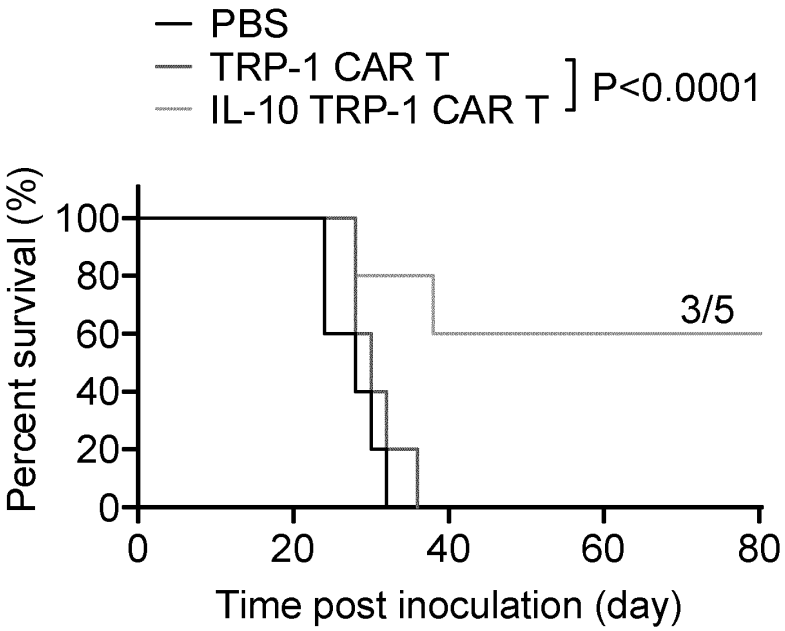
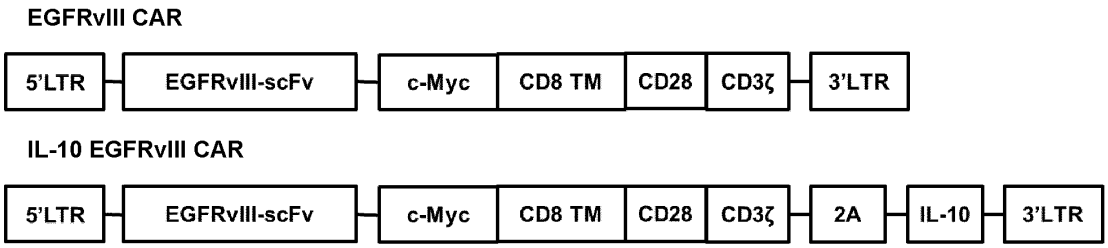


Figure 5 a, b

a



b

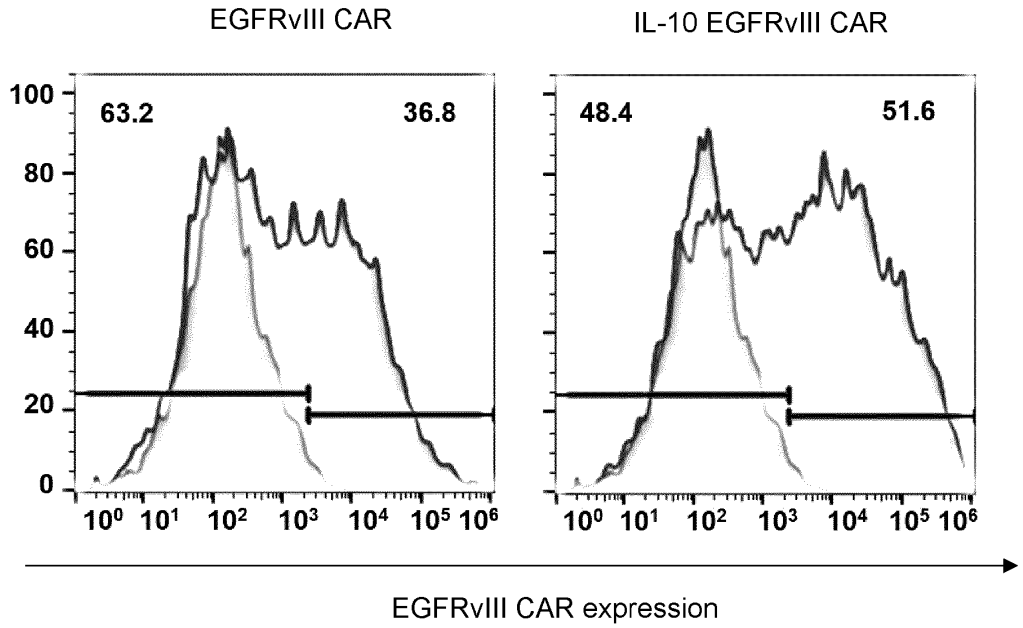


Figure 5 c, d, e

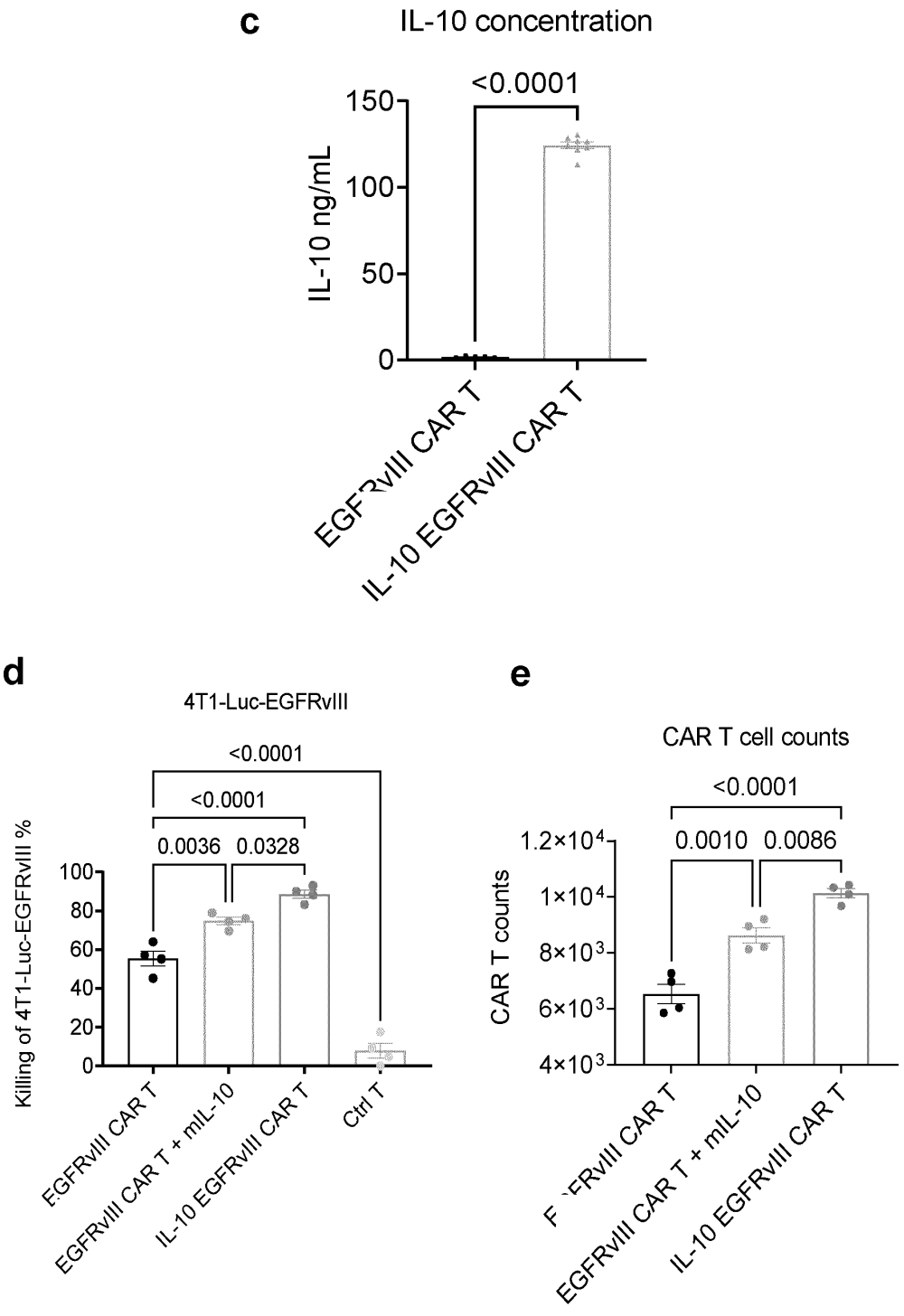

c
IL-10 concentration
<0.0001
150
100
50
0
IL-10 ng/mL
EGFRvIII CAR T
IL-10 EGFRvIII CAR T
d
4T1-Luc-EGFRvIII
<0.0001
<0.0001
0.0036
0.0328
100
80
60
40
20
0
Killing of 4T1-Luc-EGFRvIII %
EGFRvIII CAR T
EGFRvIII CAR T + mIL-10
IL-10 EGFRvIII CAR T
Ctrl T
e
CAR T cell counts
<0.0001
0.0010
0.0086
1.2×10^4
1×10^4
8×10^3
6×10^3
4×10^3
CAR T counts
EGFRvIII CAR T
EGFRvIII CAR T + mIL-10
IL-10 EGFRvIII CAR T

Figure 5 f, g, h

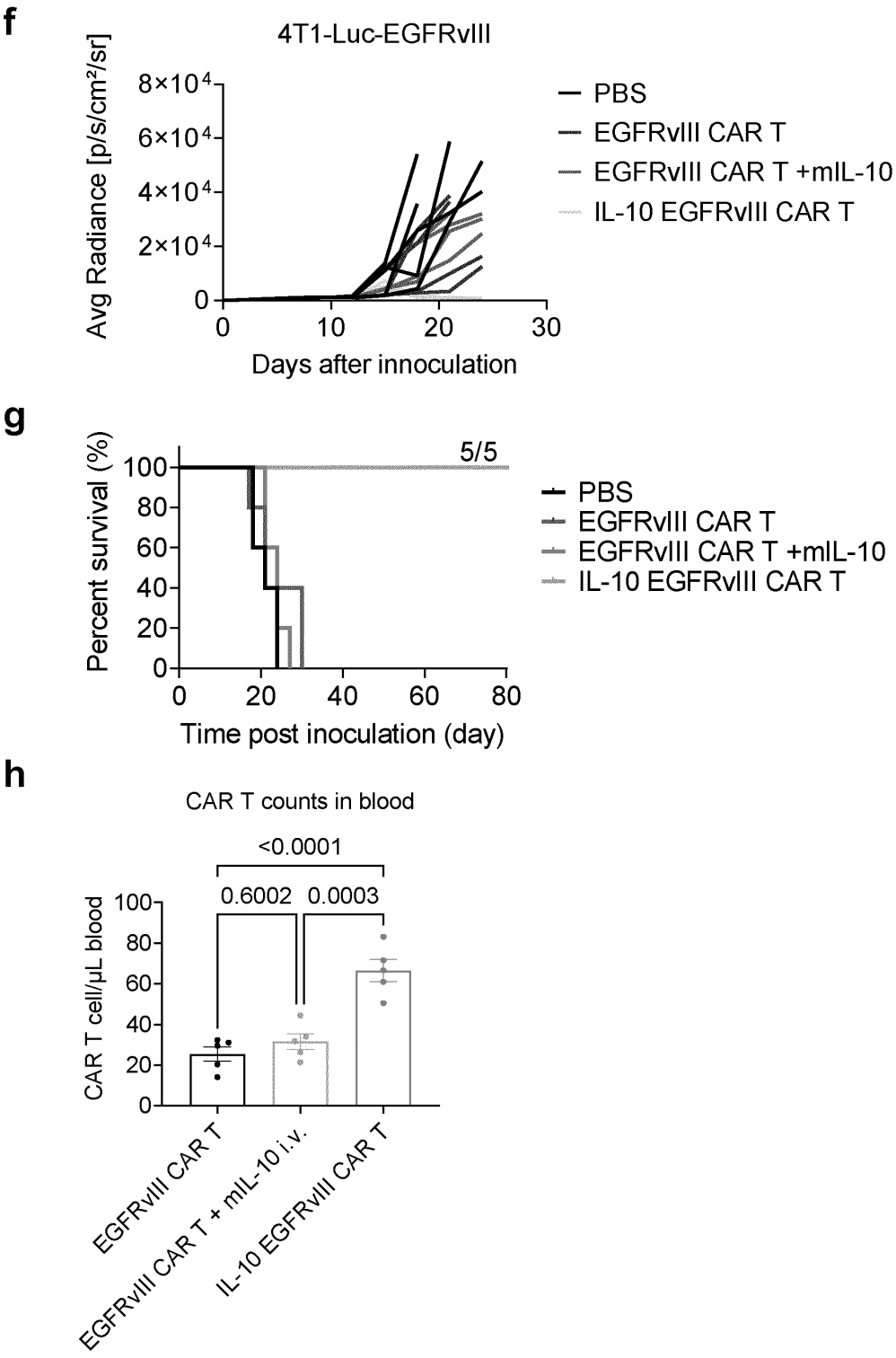

f
4T1-Luc-EGFRvIII
Avg Radiance [p/s/cm²/sr]
8×10⁴
6×10⁴
4×10⁴
2×10⁴
0
0
10
20
30
Days after innoculation
PBS
EGFRvIII CAR T
EGFRvIII CAR T +mIL-10
IL-10 EGFRvIII CAR T
g
Percent survival (%)
100
80
60
40
20
0
0
20
40
60
80
Time post inoculation (day)
5/5
PBS
EGFRvIII CAR T
EGFRvIII CAR T +mIL-10
IL-10 EGFRvIII CAR T
h
CAR T counts in blood
<0.0001
0.6002
0.0003
CAR T cell/µL blood
100
80
60
40
20
0
EGFRvIII CAR T
EGFRvIII CAR T + mIL-10 i.v.
IL-10 EGFRvIII CAR T

Figure 6

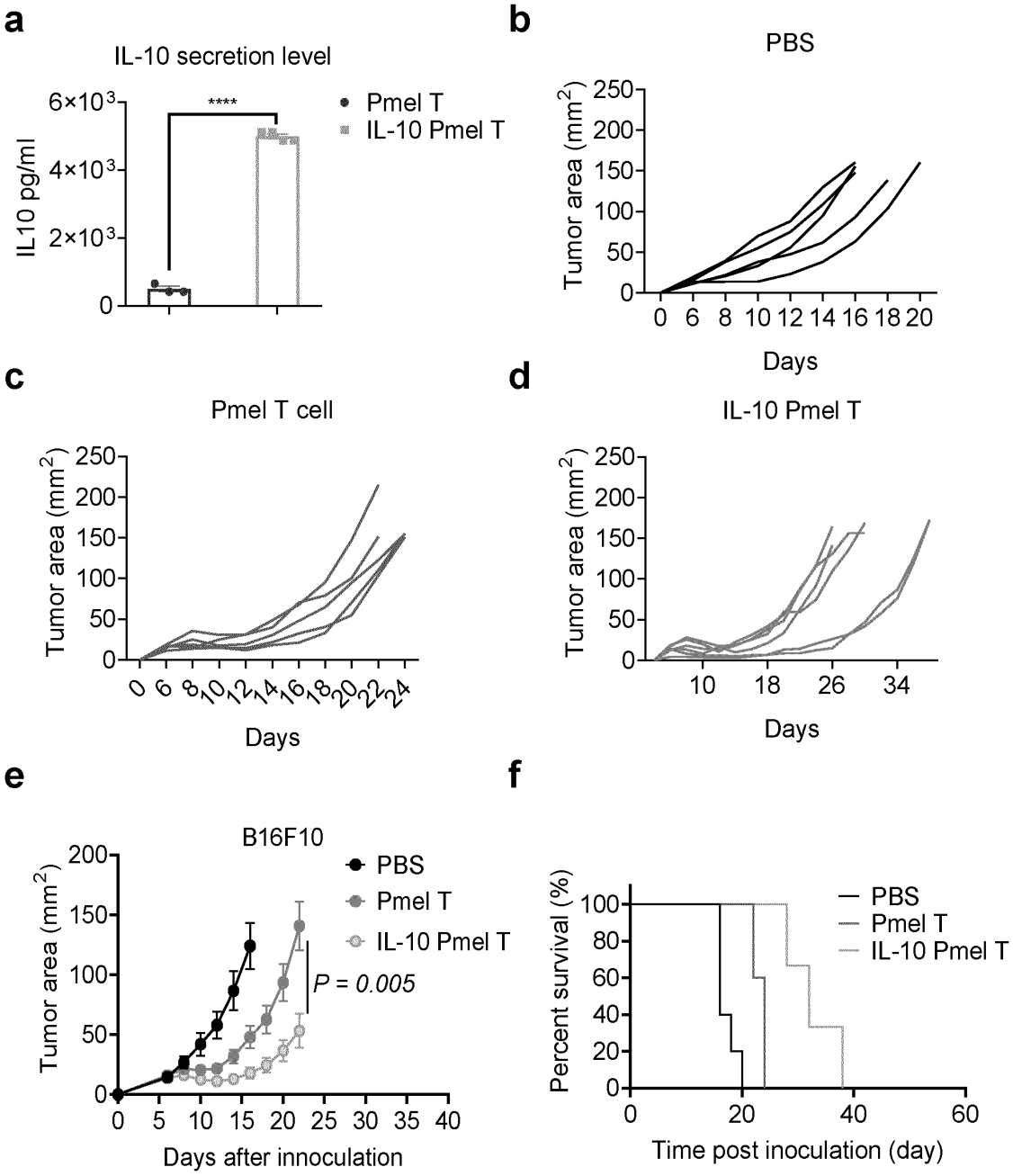
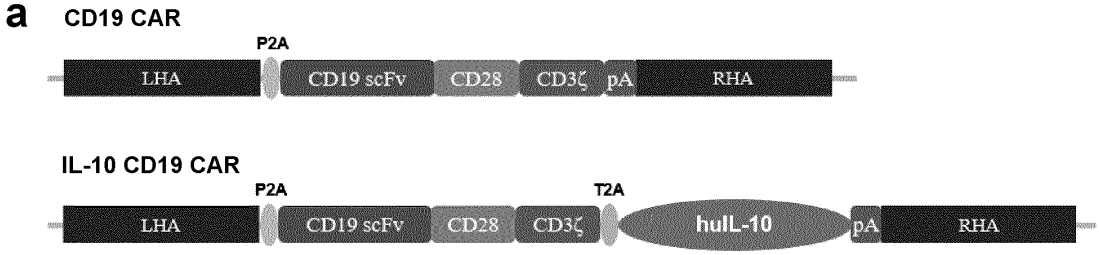

a
IL-10 secretion level
IL10 pg/ml
6×10³
4×10³
2×10³
0
****
Pmel T
IL-10 Pmel T
b
PBS
Tumor area (mm²)
250
200
150
100
50
0
0 6 8 10 12 14 16 18 20
Days
c
Pmel T cell
Tumor area (mm²)
0 6 8 10 12 14 16 18 20 22 24
Days
d
IL-10 Pmel T
Tumor area (mm²)
10 18 26 34
Days
e
B16F10
Tumor area (mm²)
200
150
100
50
0
PBS
Pmel T
IL-10 Pmel T
P = 0.005
0 5 10 15 20 25 30 35 40
Days after innoculation
f
Percent survival (%)
100
80
60
40
20
0
PBS
Pmel T
IL-10 Pmel T
0 20 40 60
Time post inoculation (day)

Figure 7 a,b


a
CD19 CAR
P2A
LHA
CD19 scFv
CD28
CD3ζ
pA
RHA
IL-10 CD19 CAR
P2A
T2A
LHA
CD19 scFv
CD28
CD3ζ
hulL-10
pA
RHA

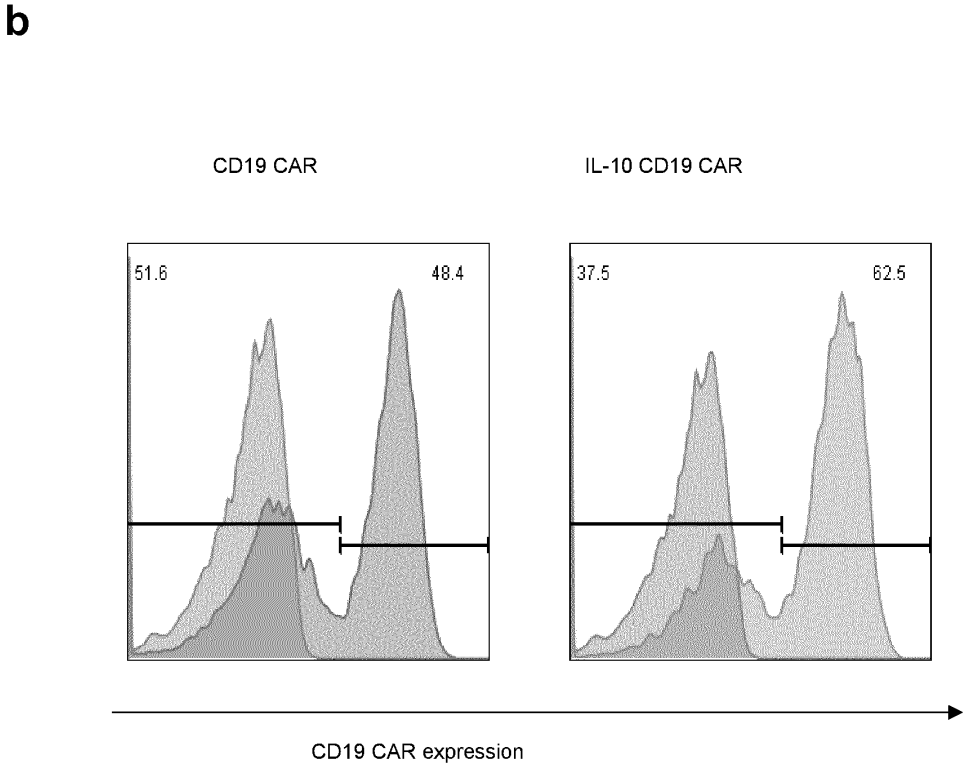

b
CD19 CAR
IL-10 CD19 CAR
51.6
48.4
37.5
62.5
CD19 CAR expression

Figure 7 c, d, e

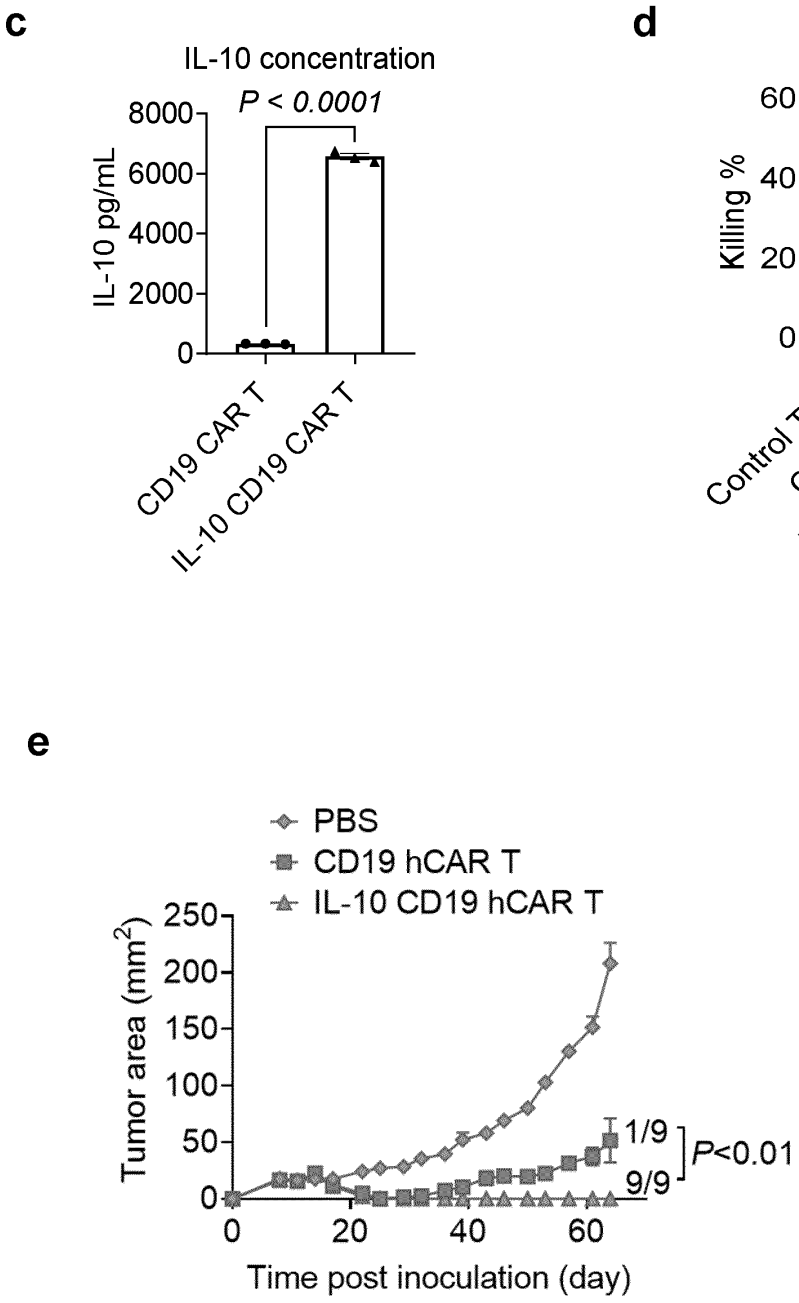

c
IL-10 concentration
P < 0.0001
IL-10 pg/mL
8000
6000
4000
2000
0
CD19 CAR T
IL-10 CD19 CAR T
d
P= 0.0026
Killing %
60
40
20
0
Control T cells
CD19 CAR T
IL-10 CD19 CAR T
e
PBS
CD19 hCAR T
IL-10 CD19 hCAR T
Tumor area (mm2)
250
200
150
100
50
0
0
20
40
60
Time post inoculation (day)
1/9
9/9
P<0.01

Figure 8

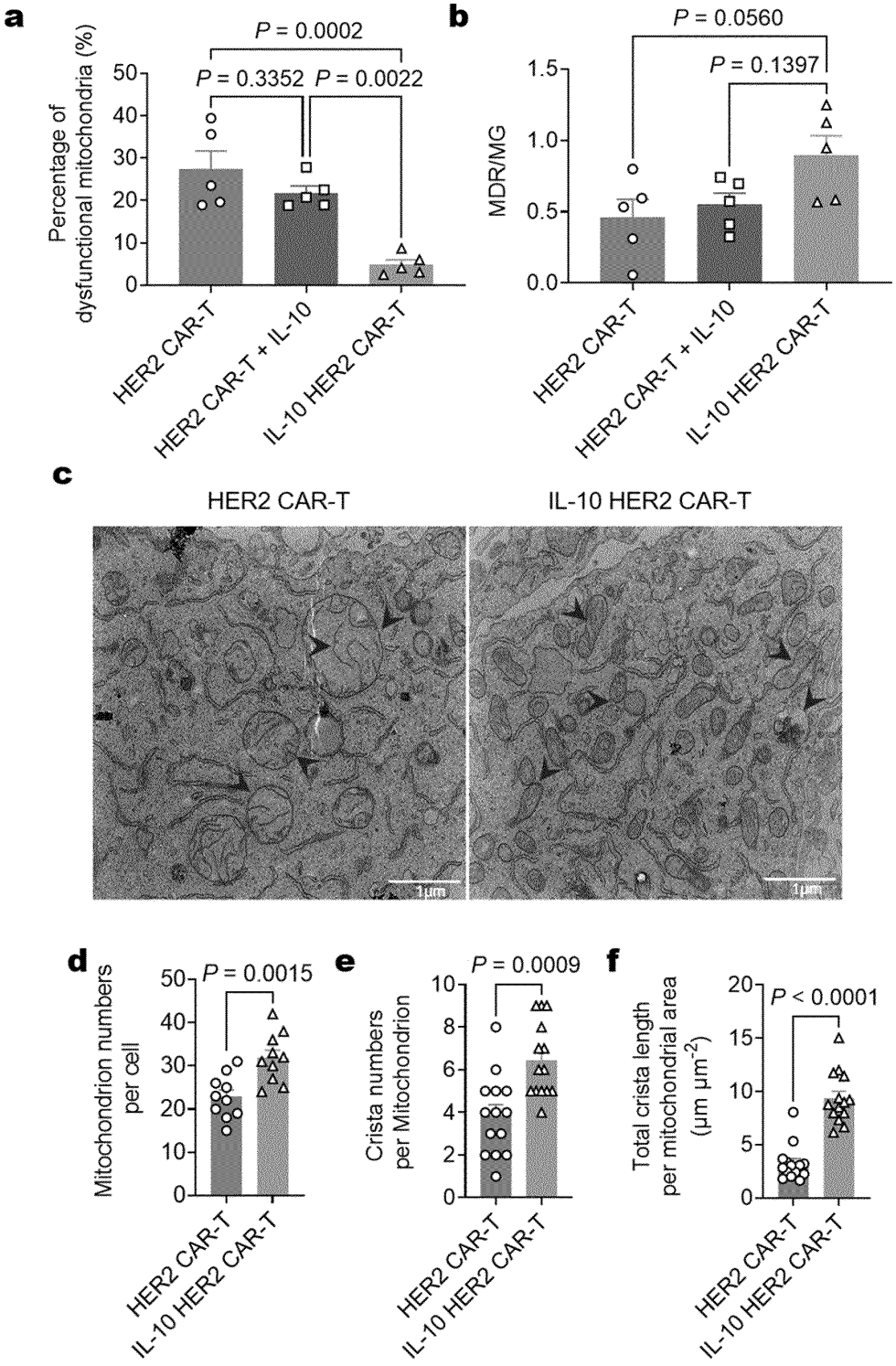

a
Percentage of dysfunctional mitochondria (%)
P = 0.0002
P = 0.3352
P = 0.0022
HER2 CAR-T
HER2 CAR-T + IL-10
IL-10 HER2 CAR-T
b
MDR/MG
P = 0.0560
P = 0.1397
c
HER2 CAR-T
IL-10 HER2 CAR-T
1μm
d
Mitochondrion numbers per cell
P = 0.0015
e
Crista numbers per Mitochondrion
P = 0.0009
f
Total crista length per mitochondrial area (μm μm-2)
P < 0.0001

Figure 9

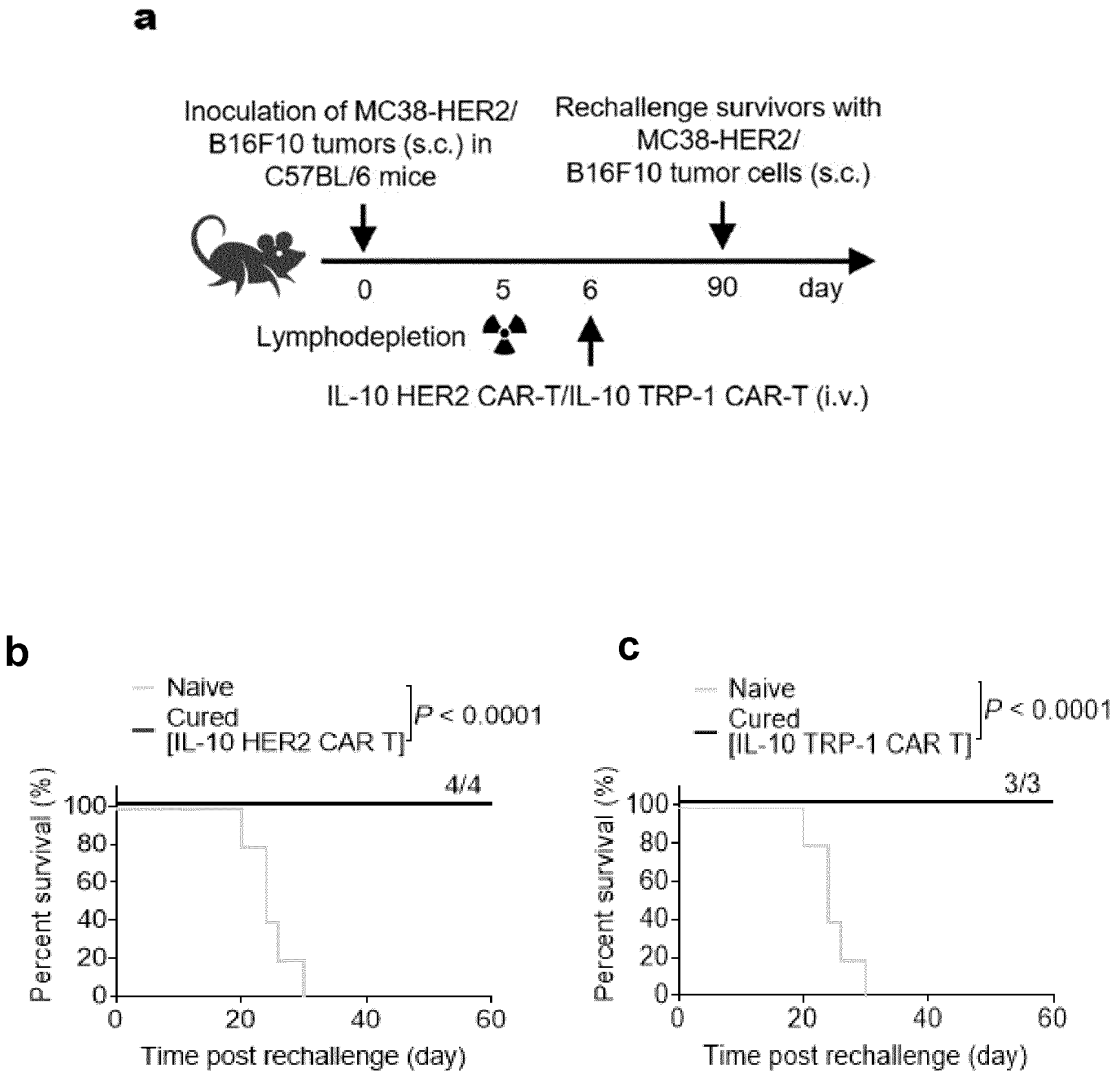
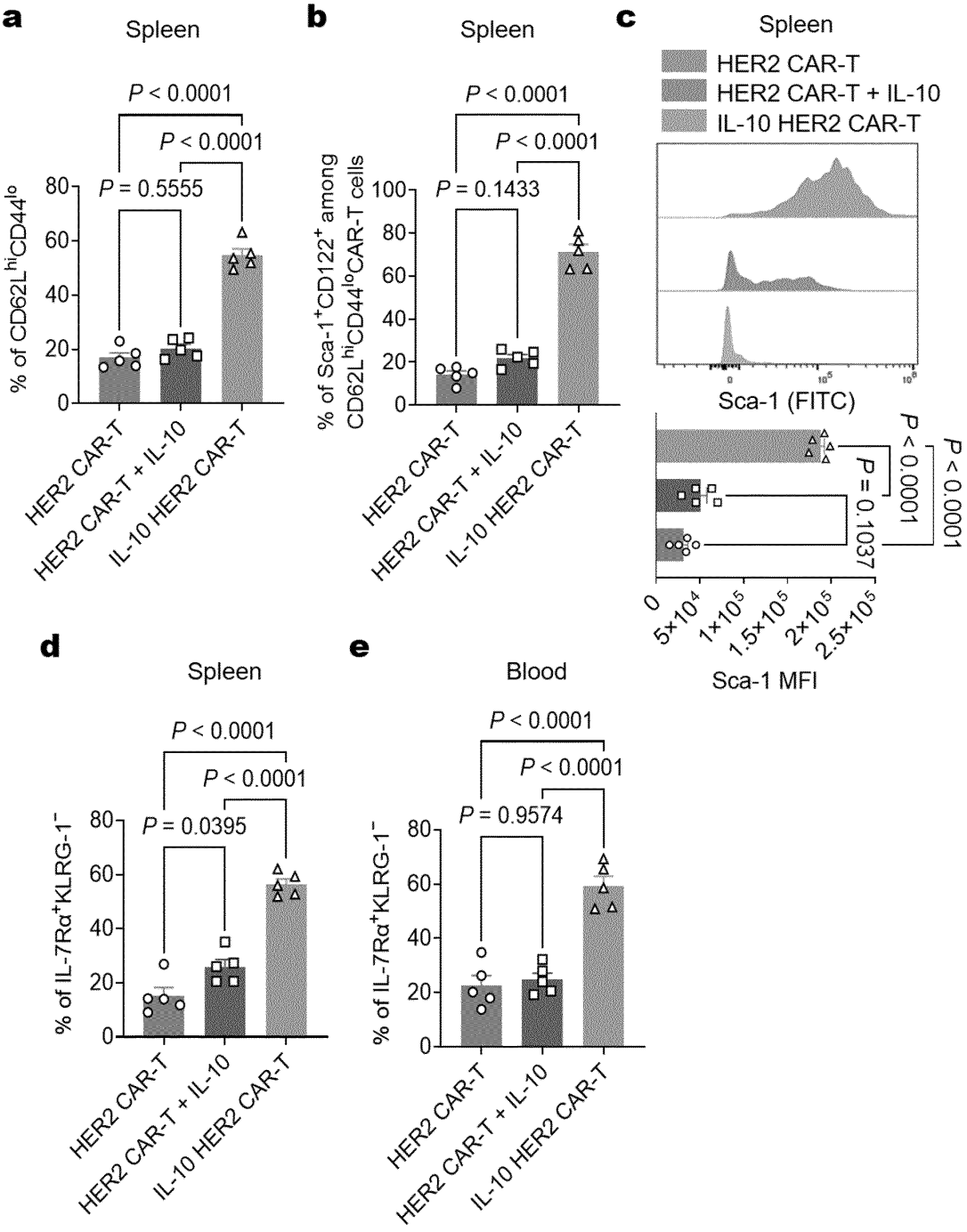

a
Inoculation of MC38-HER2/
B16F10 tumors (s.c.) in
C57BL/6 mice
Rechallenge survivors with
MC38-HER2/
B16F10 tumor cells (s.c.)
0
5
6
90
day
Lymphodepletion
IL-10 HER2 CAR-T/IL-10 TRP-1 CAR-T (i.v.)
b
Naive
Cured
[IL-10 HER2 CAR T]
P < 0.0001
4/4
Percent survival (%)
Time post rechallenge (day)
c
Naive
Cured
[IL-10 TRP-1 CAR T]
P < 0.0001
3/3
Percent survival (%)
Time post rechallenge (day)

Figure 10


a
Spleen
P < 0.0001
P < 0.0001
P = 0.5555
% of CD62L hiCD44lo
HER2 CAR-T
HER2 CAR-T + IL-10
IL-10 HER2 CAR-T
b
Spleen
P < 0.0001
P < 0.0001
P = 0.1433
% of Sca-1+CD122+ among CD62L hiCD44loCAR-T cells
c
Spleen
HER2 CAR-T
HER2 CAR-T + IL-10
IL-10 HER2 CAR-T
Sca-1 (FITC)
P < 0.0001
P = 0.1037
P < 0.0001
Sca-1 MFI
d
Spleen
P < 0.0001
P < 0.0001
P = 0.0395
% of IL-7Rα+KLRG-1−
e
Blood
P < 0.0001
P < 0.0001
P = 0.9574
% of IL-7Rα+KLRG-1−