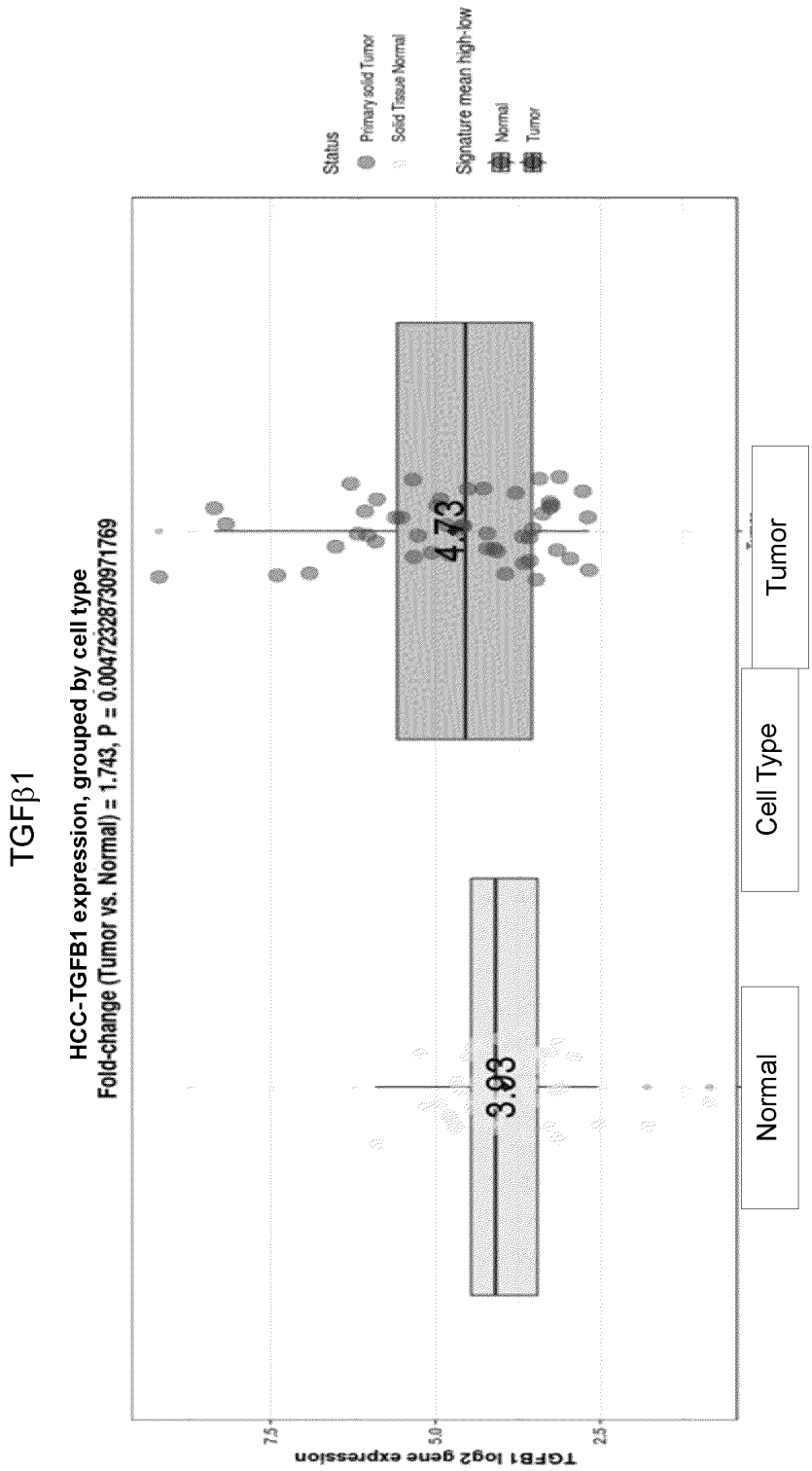

TGFβ1
HCC-TGFB1 expression, grouped by cell type
Fold-change (Tumor vs. Normal) = 1.743, P = 0.0047232873097176 9
Status
Primary solid Tumor
Solid Tissue Normal
Signature mean high-low
Normal
Tumor
4.73
3.03
TGFB1 log2 gene expression
7.5
5.0
2.5
Normal
Cell Type
Tumor

Fig. 1A

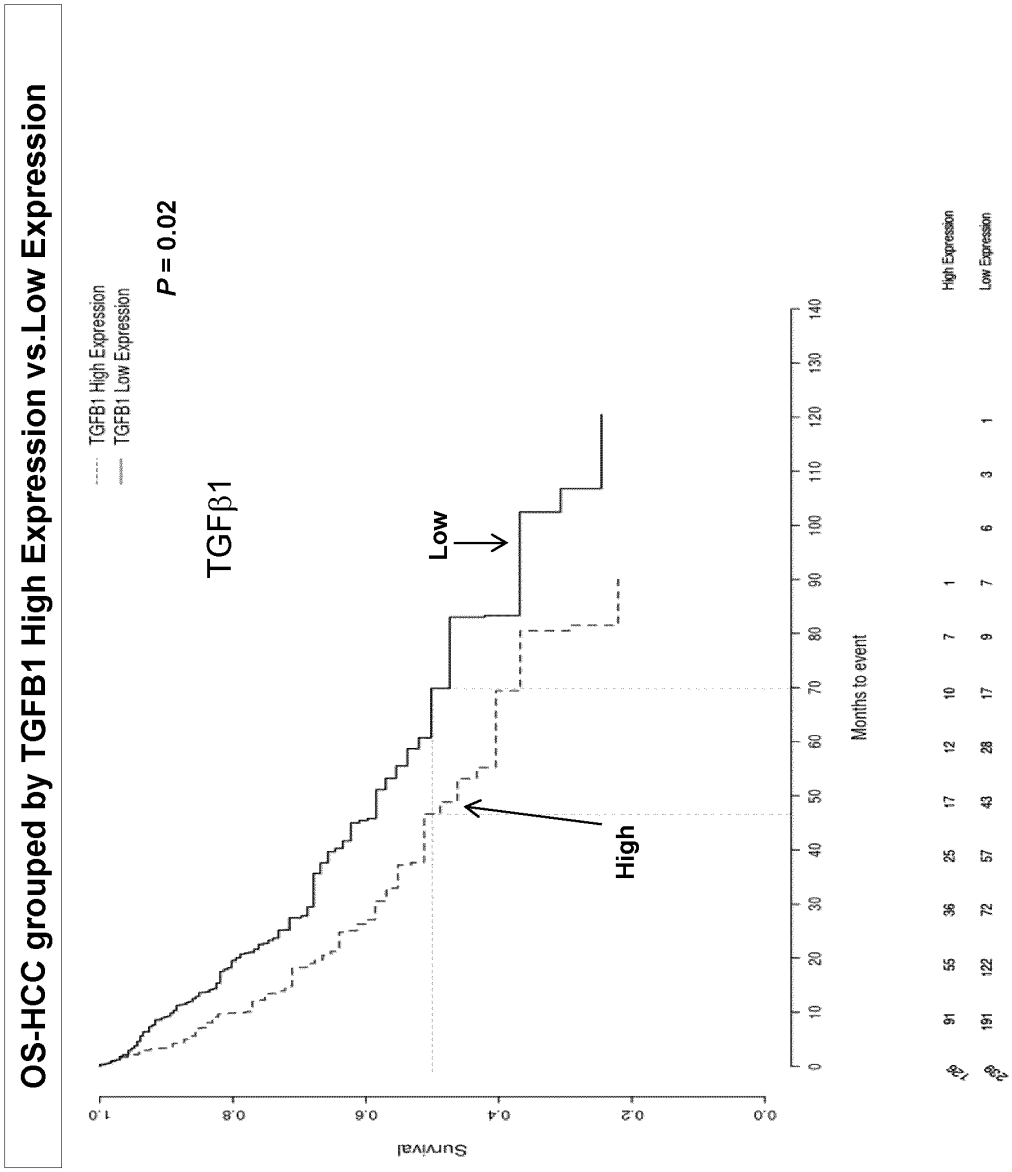

OS-HCC grouped by TGFB1 High Expression vs.Low Expression
TGFB1 High Expression
TGFB1 Low Expression
P = 0.02
TGFβ1
Low
High
Survival
Months to event
High Expression
Low Expression

Fig. 1B

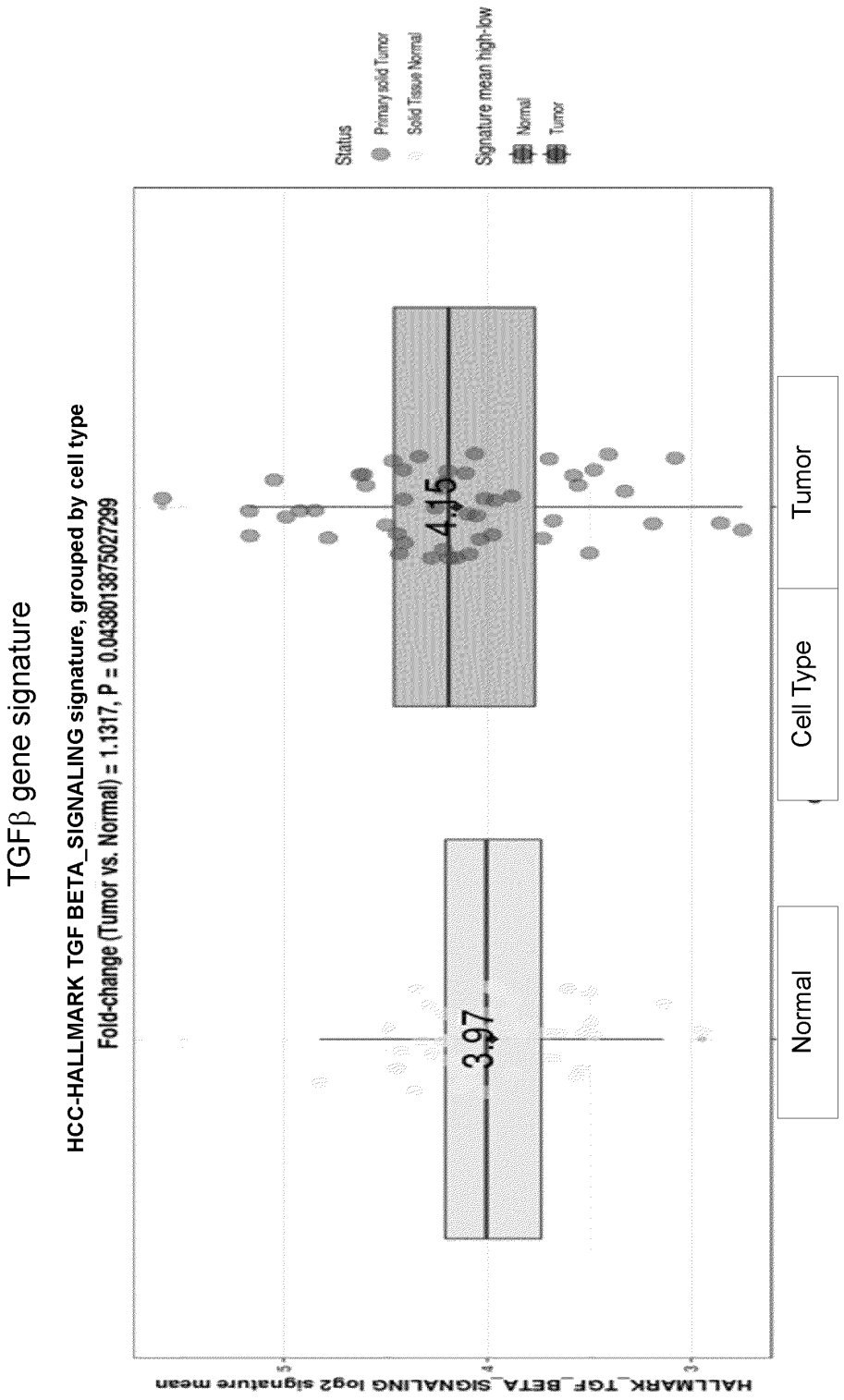

TGFβ gene signature
HCC-HALLMARK TGF BETA_SIGNALING signature, grouped by cell type
Fold-change (Tumor vs. Normal) = 1.1317, P = 0.043801387502729
Status
Primary solid Tumor
Solid Tissue Normal
Signature mean high-low
Normal
Tumor
4.15
3.97
Normal
Tumor
Cell Type
HALLMARK_TGF_BETA_SIGNALING log2 signature mean

Fig. 1C

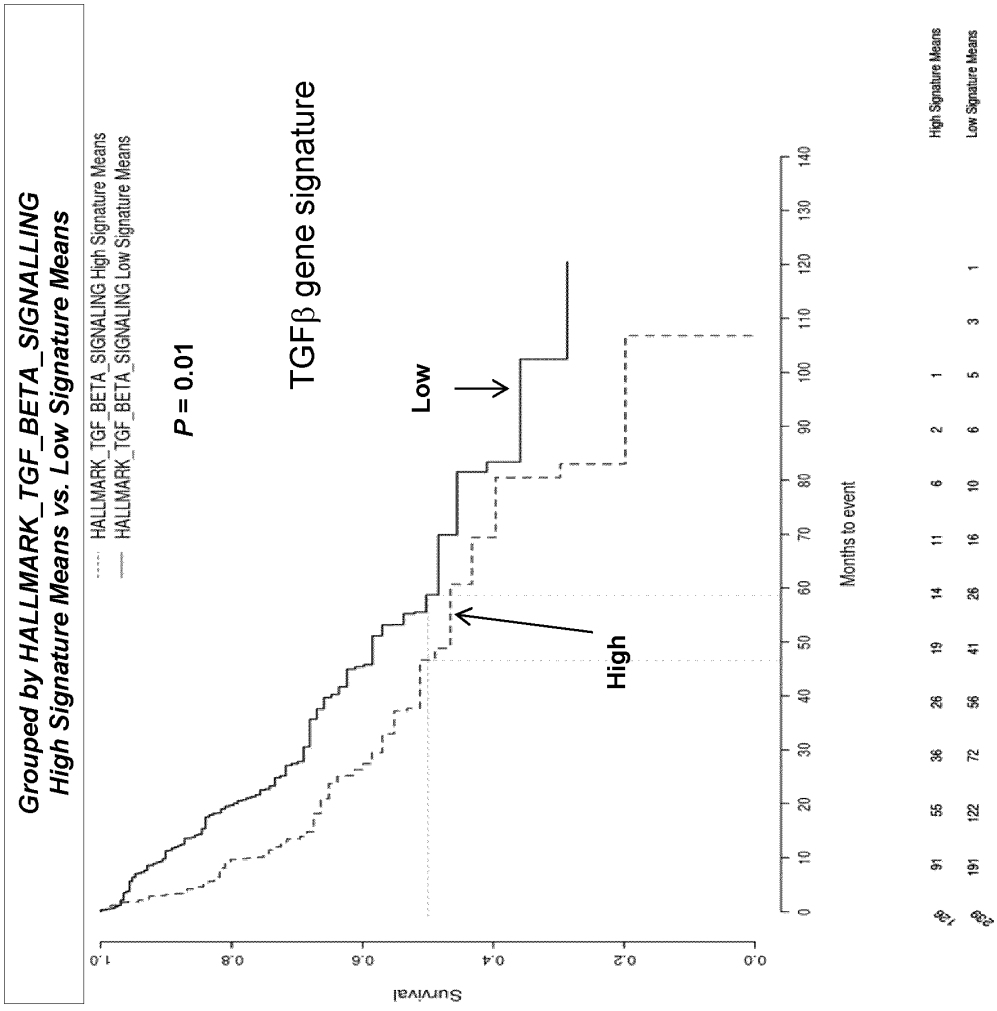

Grouped by HALLMARK_TGF_BETA_SIGNALLING
High Signature Means vs. Low Signature Means
HALLMARK_TGF_BETA_SIGNALING High Signature Means
HALLMARK_TGF_BETA_SIGNALING Low Signature Means
P = 0.01
TGFβ gene signature
Low
High
Survival
Months to event
High Signature Means
Low Signature Means

Fig. 1D

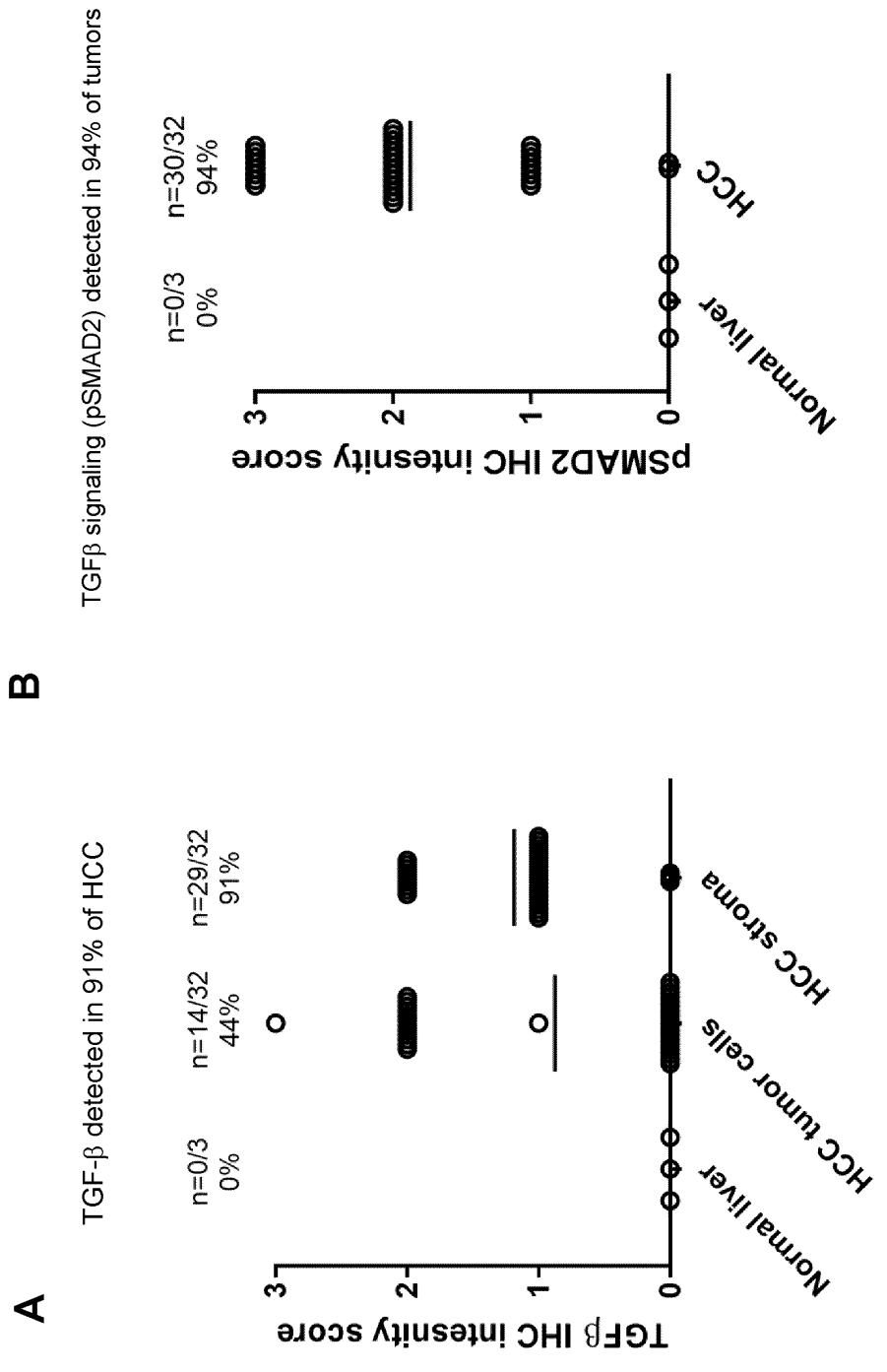

A
TGF-β detected in 91% of HCC
n=0/3
0%
n=14/32
44%
n=29/32
91%
TGFβ IHC intensity score
0
1
2
3
Normal liver
HCC tumor cells
HCC stroma
B
TGFβ signaling (pSMAD2) detected in 94% of tumors
n=0/3
0%
n=30/32
94%
pSMAD2 IHC intensity score
0
1
2
3
Normal liver
HCC

Fig. 2

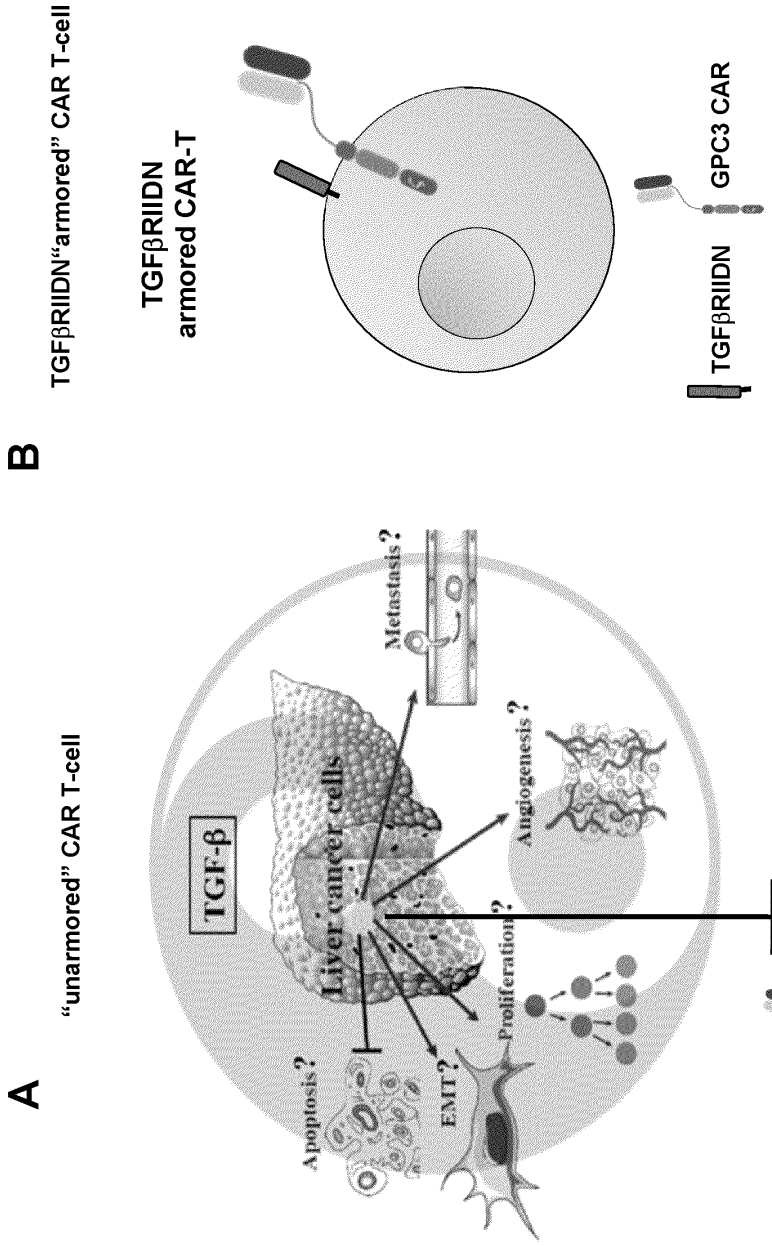

A
"unarmored" CAR T-cell
TGF-β
Liver cancer cells
Metastasis?
Angiogenesis ?
Proliferation?
EMT?
Apoptosis?
B
TGFβRIIDN"armored" CAR T-cell
TGFβRIIDN
armored CAR-T
GPC3 CAR
TGFβRIIDN

Fig. 3

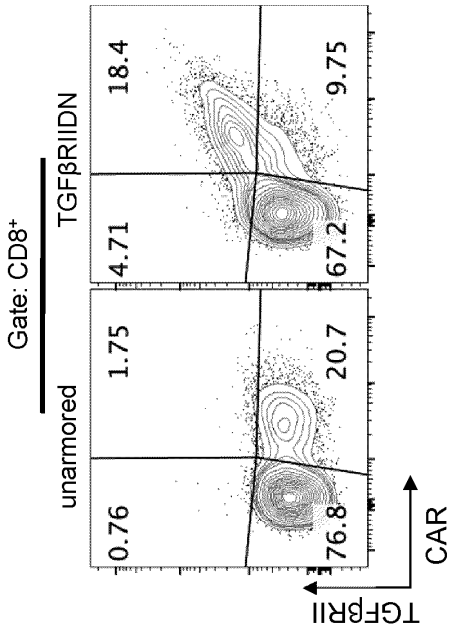

Gate: CD8+
unarmored
TGFβRIIDN
0.76
1.75
4.71
18.4
76.8
20.7
67.2
9.75
TGFβRII
CAR

Fig. 4

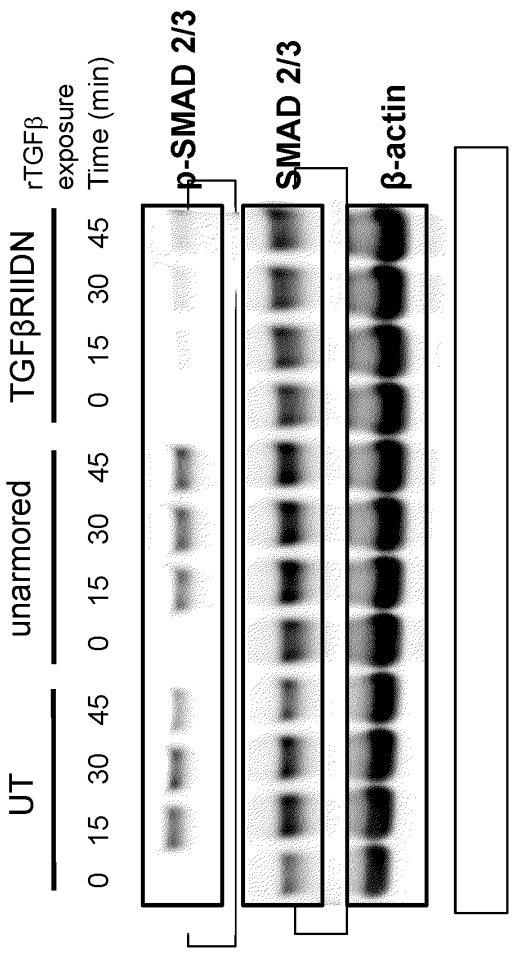

rTGFβ exposure Time (min)
TGFβRIIDN
unarmored
UT
0 15 30 45 0 15 30 45 0 15 30 45
p-SMAD 2/3
SMAD 2/3
β-actin

Fig. 5

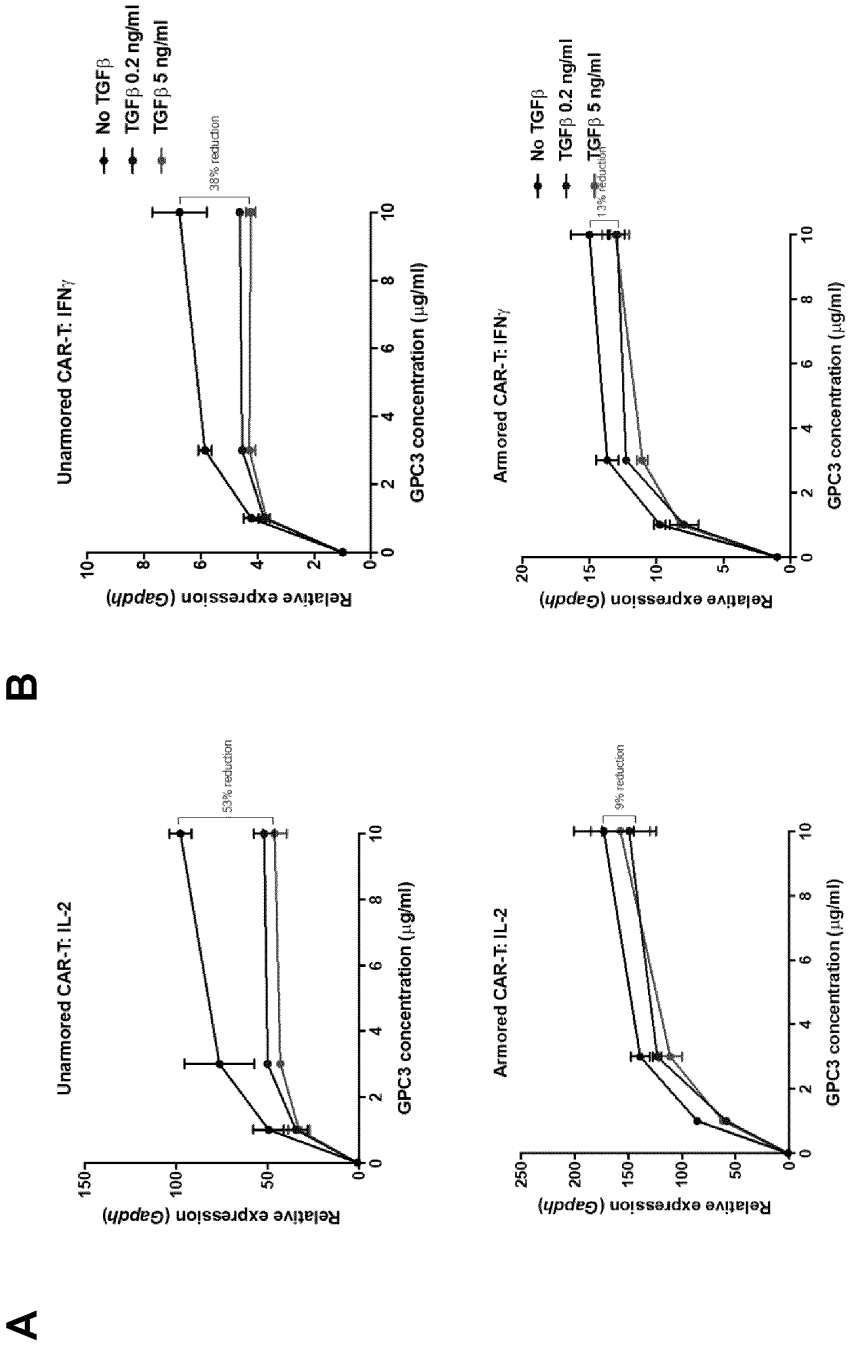

A
B
Unarmored CAR-T: IL-2
Armored CAR-T: IL-2
Unarmored CAR-T: IFNγ
Armored CAR-T: IFNγ
No TGFβ
TGFβ 0.2 ng/ml
TGFβ 5 ng/ml
53% reduction
9% reduction
38% reduction
13% reduction
Relative expression (Gapdh)
GPC3 concentration (µg/ml)

Fig. 6

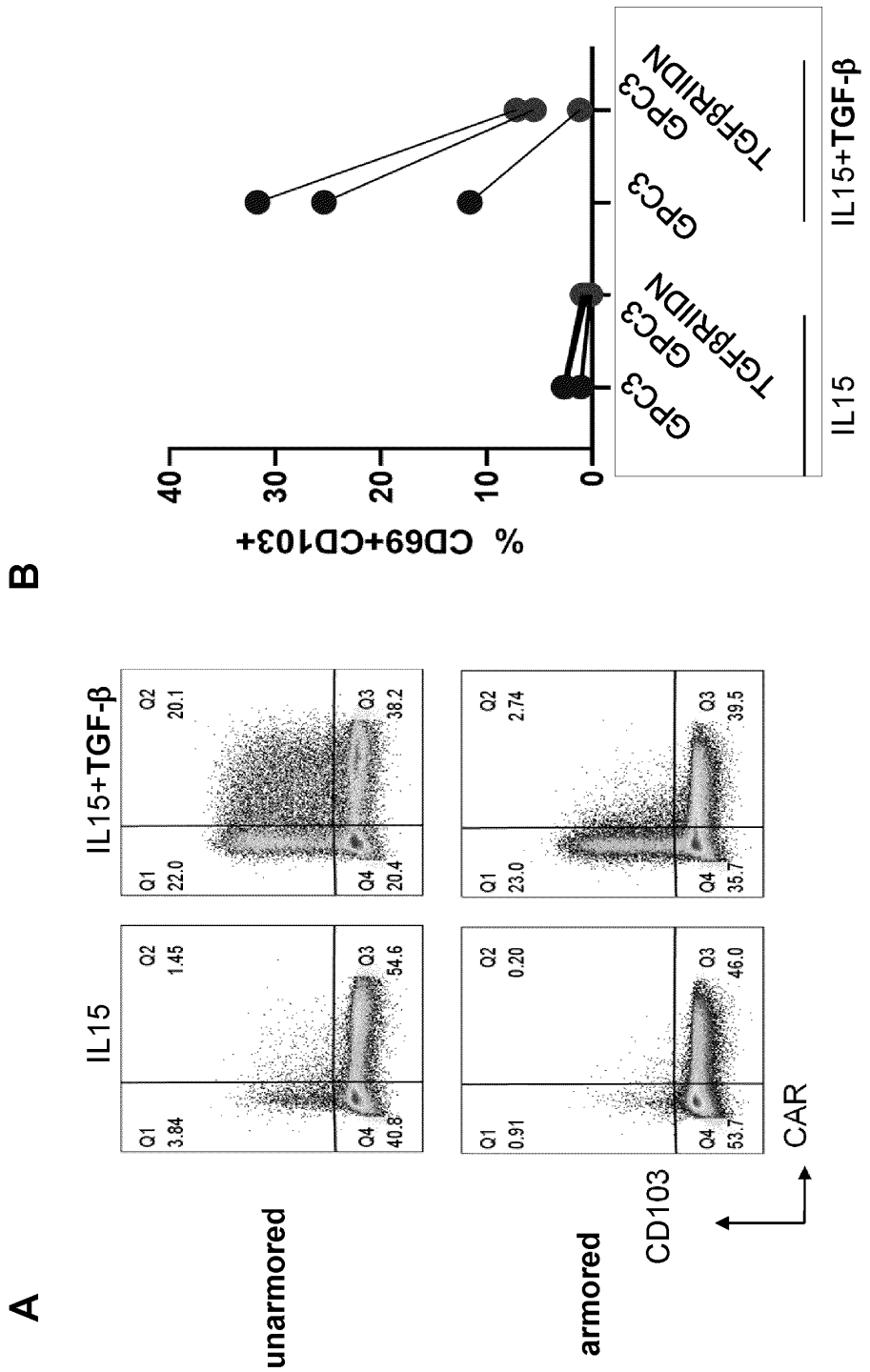

A
B
IL15
IL15+TGF-β
unarmored
armored
CD103
CAR
Q1 3.84
Q2 1.45
Q3 54.6
Q4 40.8
Q1 22.0
Q2 20.1
Q3 38.2
Q4 20.4
Q1 0.91
Q2 0.20
Q3 46.0
Q4 53.7
Q1 23.0
Q2 2.74
Q3 39.5
Q4 35.7
% CD69+CD103+
40
30
20
10
0
GPC3
GPC3
TGFBRIIDN
GPC3
GPC3
TGFBRIIDN
IL15
IL15+TGF-β

Fig. 7

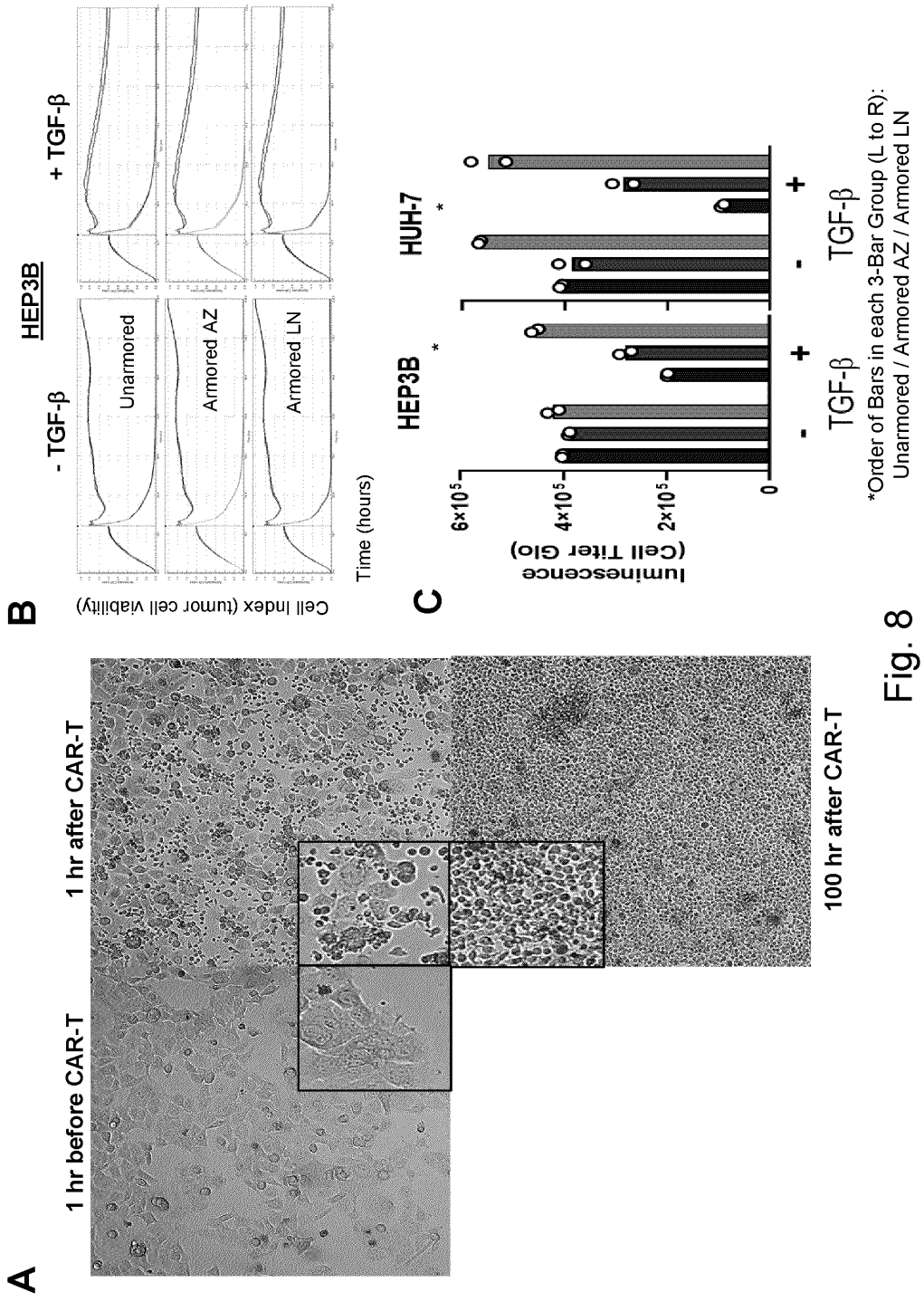

A
1 hr before CAR-T
1 hr after CAR-T
100 hr after CAR-T
B
HEP3B
- TGF-β
+ TGF-β
Unarmored
Armored AZ
Armored LN
Cell Index (tumor cell viability)
Time (hours)
C
HEP3B
HUH-7
Luminescence (Cell Titer Glo)
6×10^5
4×10^5
2×10^5
0
- + - +
TGF-β TGF-β
*Order of Bars in each 3-Bar Group (L to R):
Unarmored / Armored AZ / Armored LN

Fig. 8

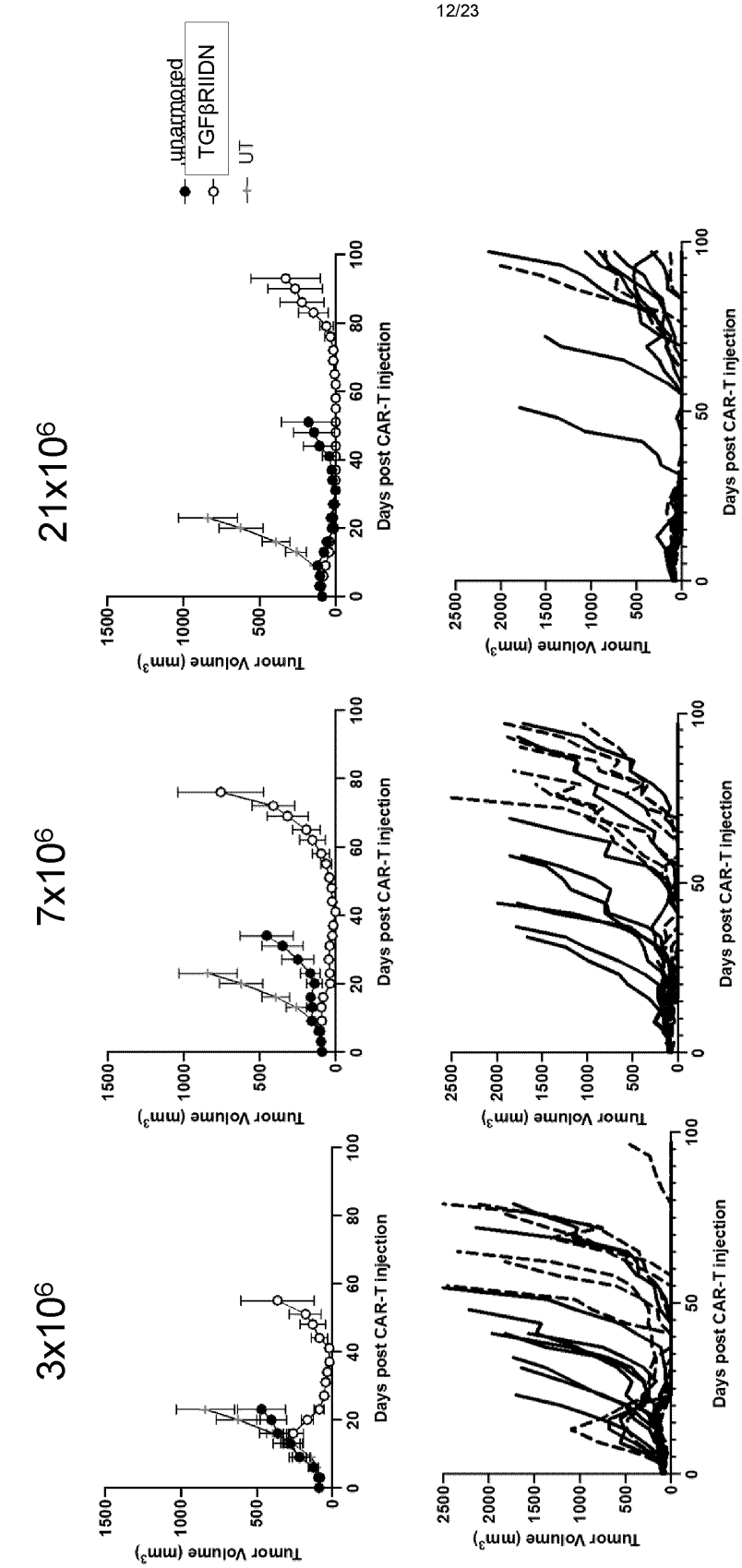

3x10^6
7x10^6
21x10^6
unarmored
TGFβRIIDN
UT
Tumor Volume (mm³)
Days post CAR-T injection
unarmored
TGFβRIIDN

Fig.9

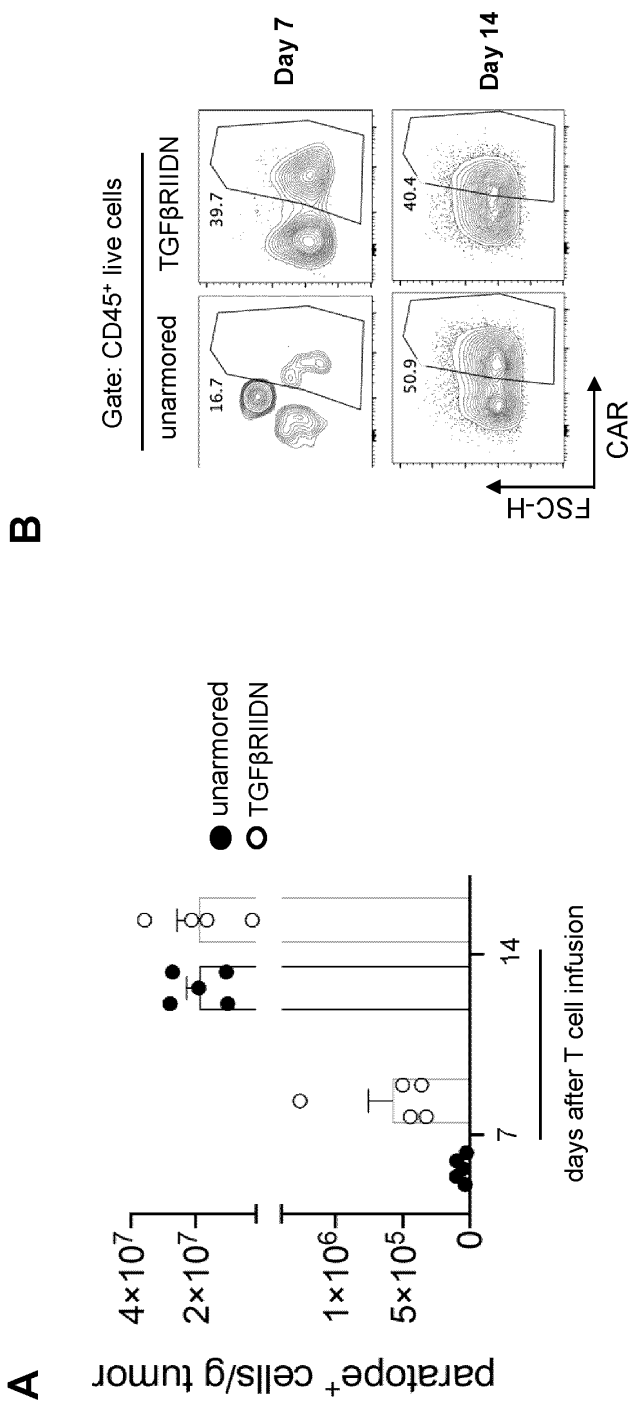

A
paratope+ cells/g tumor
4×10^7
2×10^7
1×10^6
5×10^5
0
unarmored
TGFβRIIDN
7
14
days after T cell infusion
B
Gate: CD45+ live cells
unarmored
TGFβRIIDN
Day 7
Day 14
16.7
39.7
50.9
40.4
FSC-H
CAR

Fig. 10

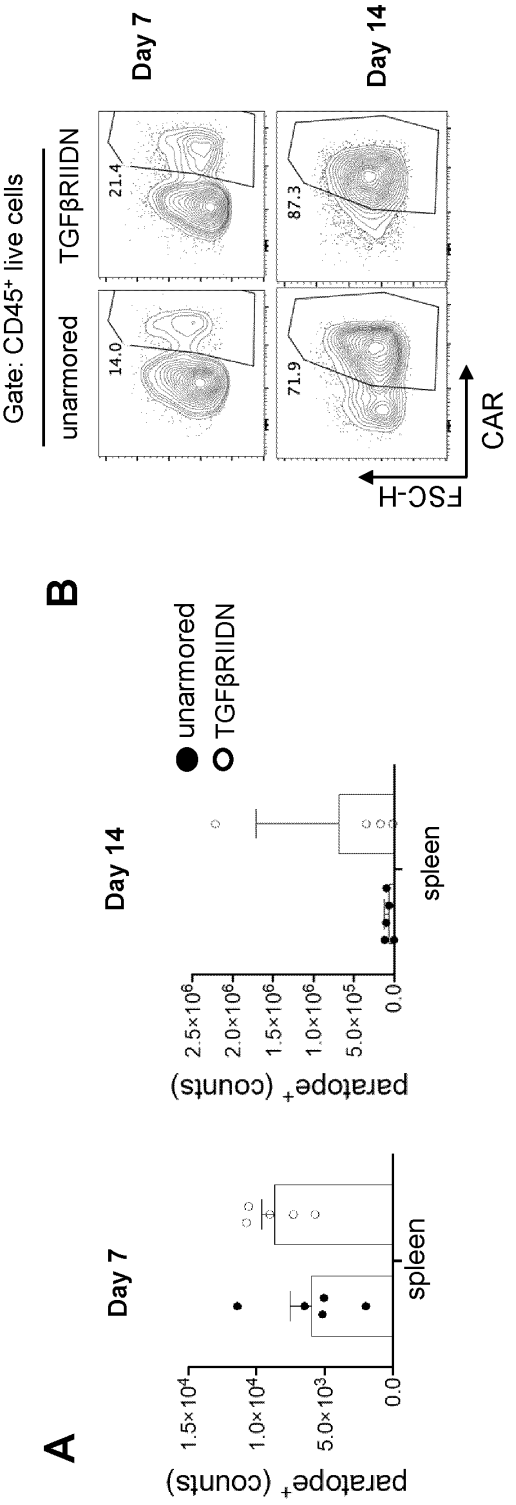

A
Day 7
paratope+ (counts)
1.5×10^4
1.0×10^4
5.0×10^3
0.0
spleen
B
Day 14
unarmored
TGFβRIIDN
paratope+ (counts)
2.5×10^6
2.0×10^6
1.5×10^6
1.0×10^6
5.0×10^5
0.0
spleen
Gate: CD45+ live cells
unarmored
TGFβRIIDN
Day 7
Day 14
14.0
21.4
71.9
87.3
FSC-H
CAR

Fig. 11

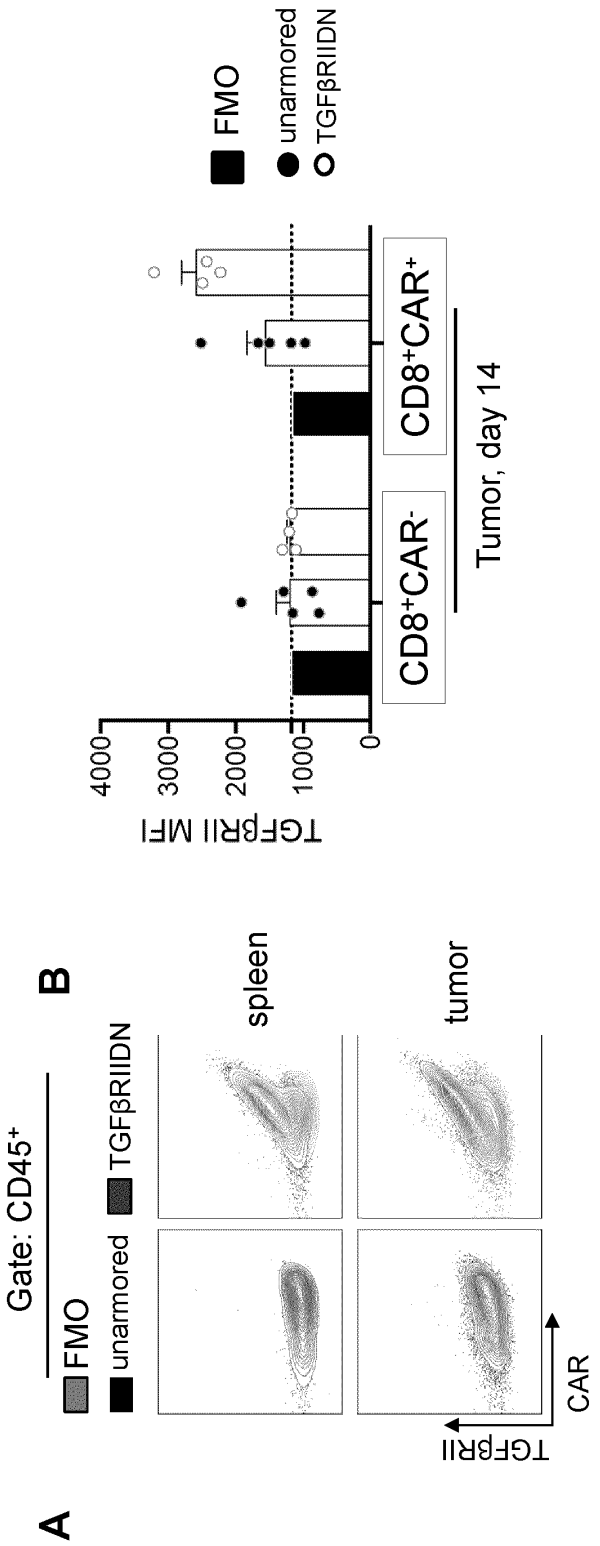

A
Gate: CD45+
FMO
unarmored
TGFβRIIDN
spleen
tumor
TGFβRII
CAR
B
FMO
unarmored
TGFβRIIDN
TGFβRII MFI
4000
3000
2000
1000
0
CD8+CAR-
CD8+CAR+
Tumor, day 14

Fig. 12

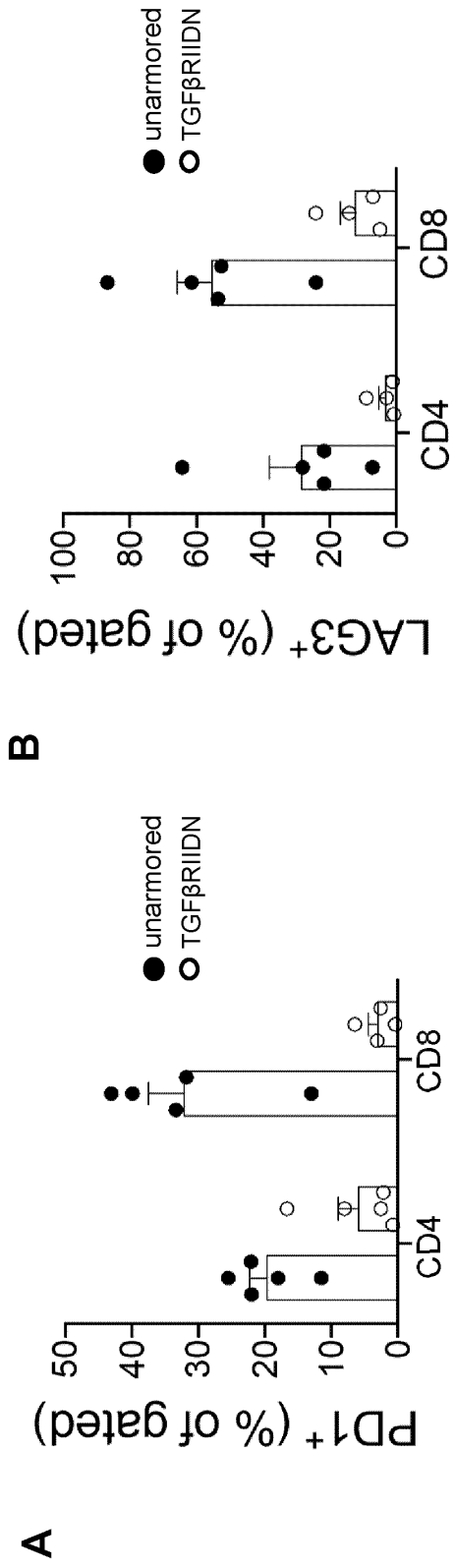

A
PD1+ (% of gated)
50
40
30
20
10
0
CD4
CD8
unarmored
TGFβRIIDN
B
LAG3+ (% of gated)
100
80
60
40
20
0
CD4
CD8
unarmored
TGFβRIIDN

Fig. 13

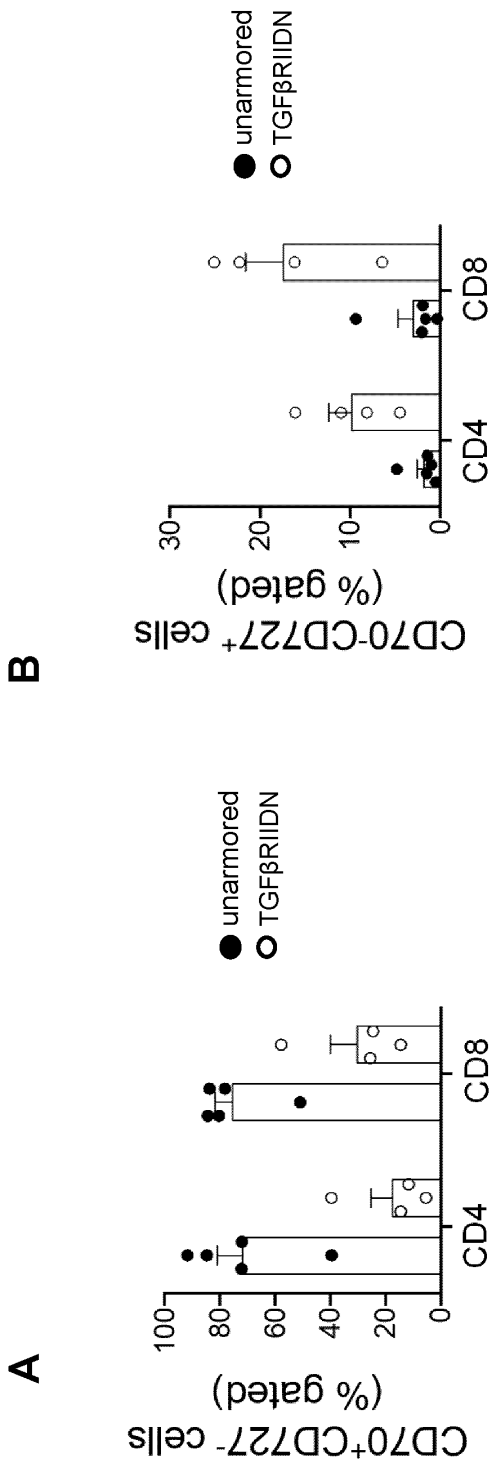

A
CD70+CD727- cells
(% gated)
100
80
60
40
20
0
CD4
CD8
unarmored
TGFβRIIDN
B
CD70-CD727+ cells
(% gated)
30
20
10
0
CD4
CD8
unarmored
TGFβRIIDN

Fig. 14

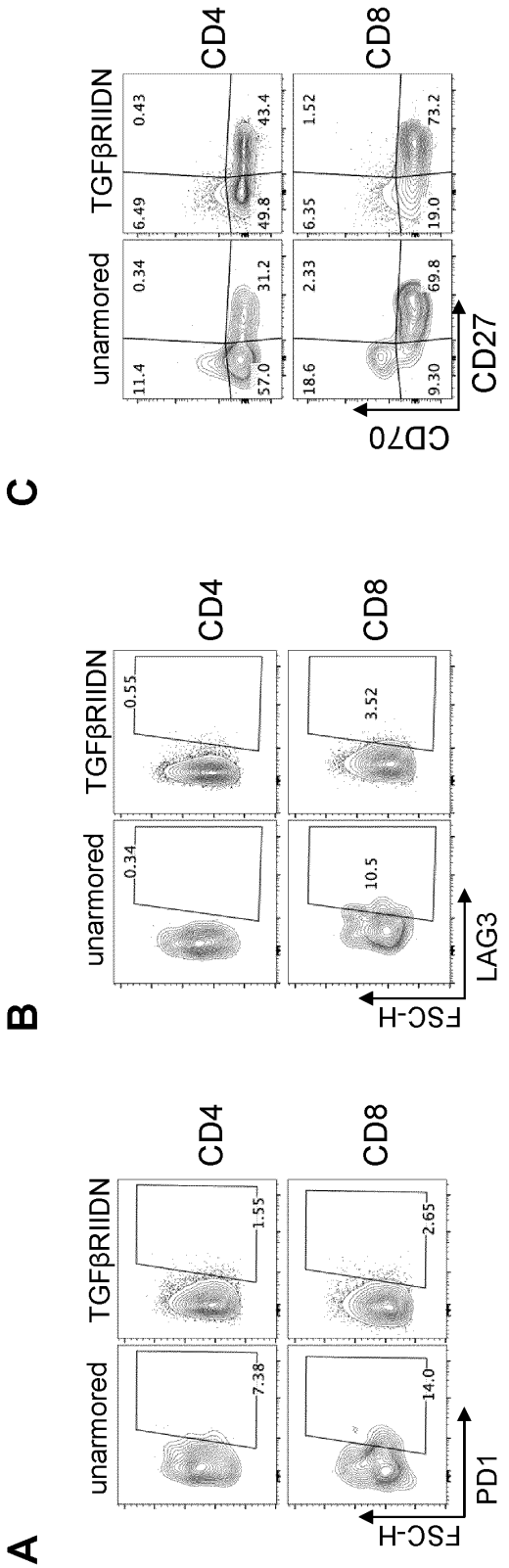

A
unarmored
TGFβRIIDN
CD4
CD8
7.38
14.0
1.55
2.65
FSC-H
PD1
B
unarmored
TGFβRIIDN
CD4
CD8
0.34
10.5
0.55
3.52
FSC-H
LAG3
C
unarmored
TGFβRIIDN
CD4
CD8
11.4
0.34
57.0
31.2
18.6
2.33
9.30
69.8
6.49
0.43
49.8
43.4
6.35
1.52
19.0
73.2
CD70
CD27

Fig. 15

Ex vivo analysis: higher IFNg in the serum of mice treated with armored CART

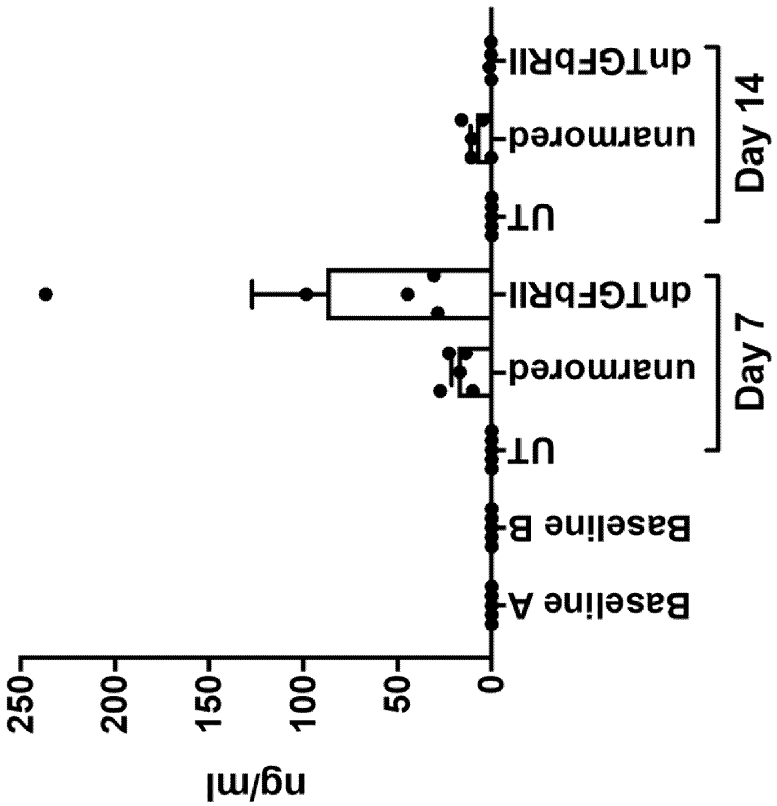


Fig. 16

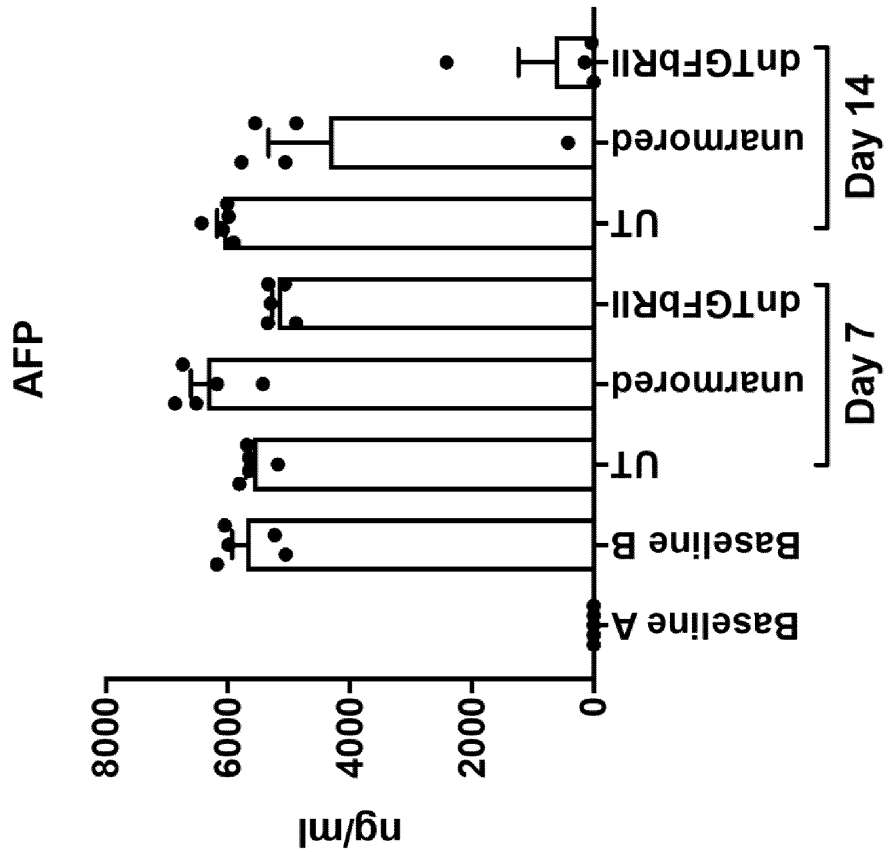

AFP
ng/ml
8000
6000
4000
2000
0
Baseline A
Baseline B
UT
unarmored
dnTGFbRII
UT
unarmored
dnTGFbRII
Day 7
Day 14

Fig. 17

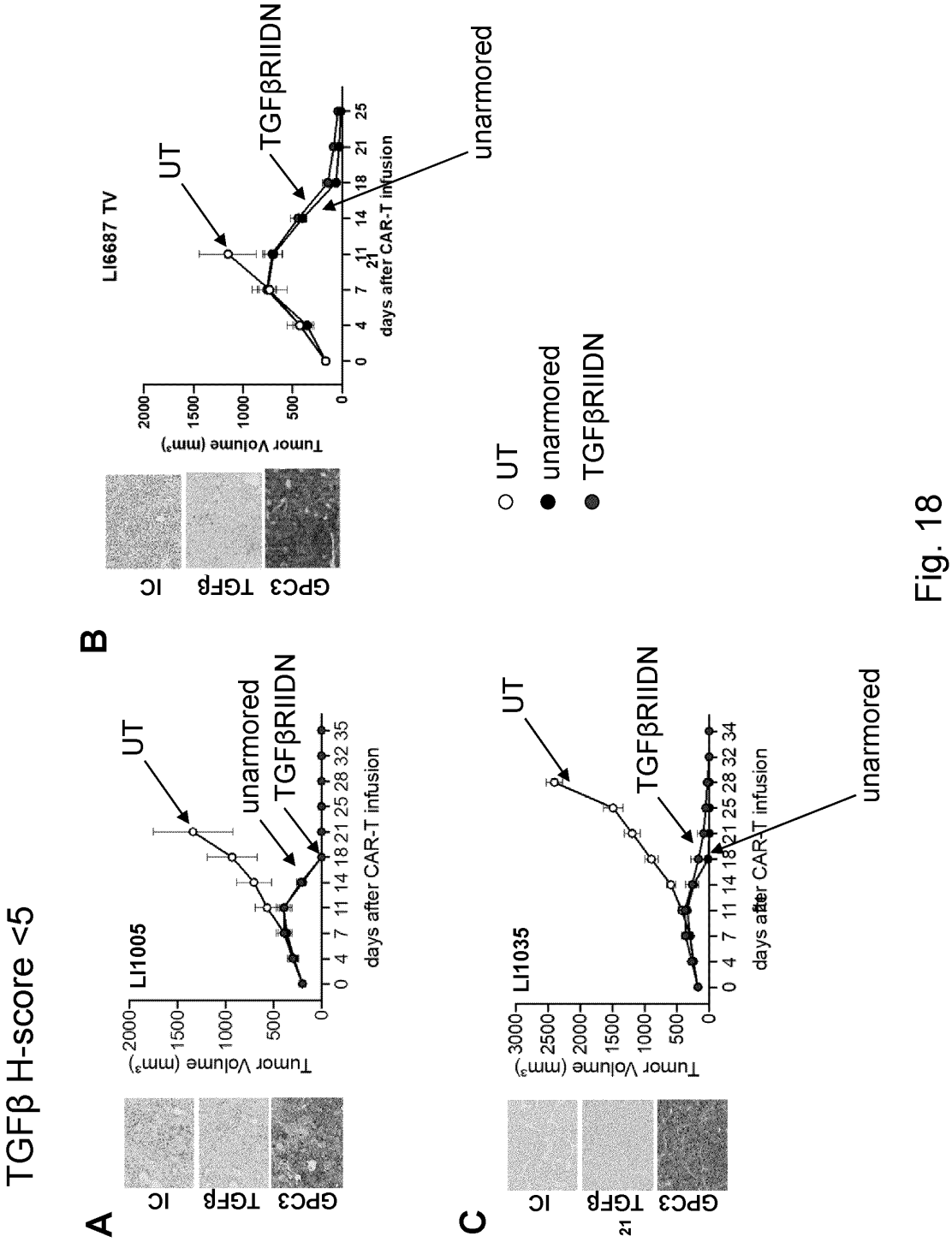

TGFβ H-score <5
A
GPC3
TGFβ
IC
LI1005
Tumor Volume (mm³)
days after CAR-T infusion
UT
unarmored
TGFβRIIDN
B
LI6687 TV
C
LI1035
○ UT
● unarmored
● TGFβRIIDN

Fig. 18

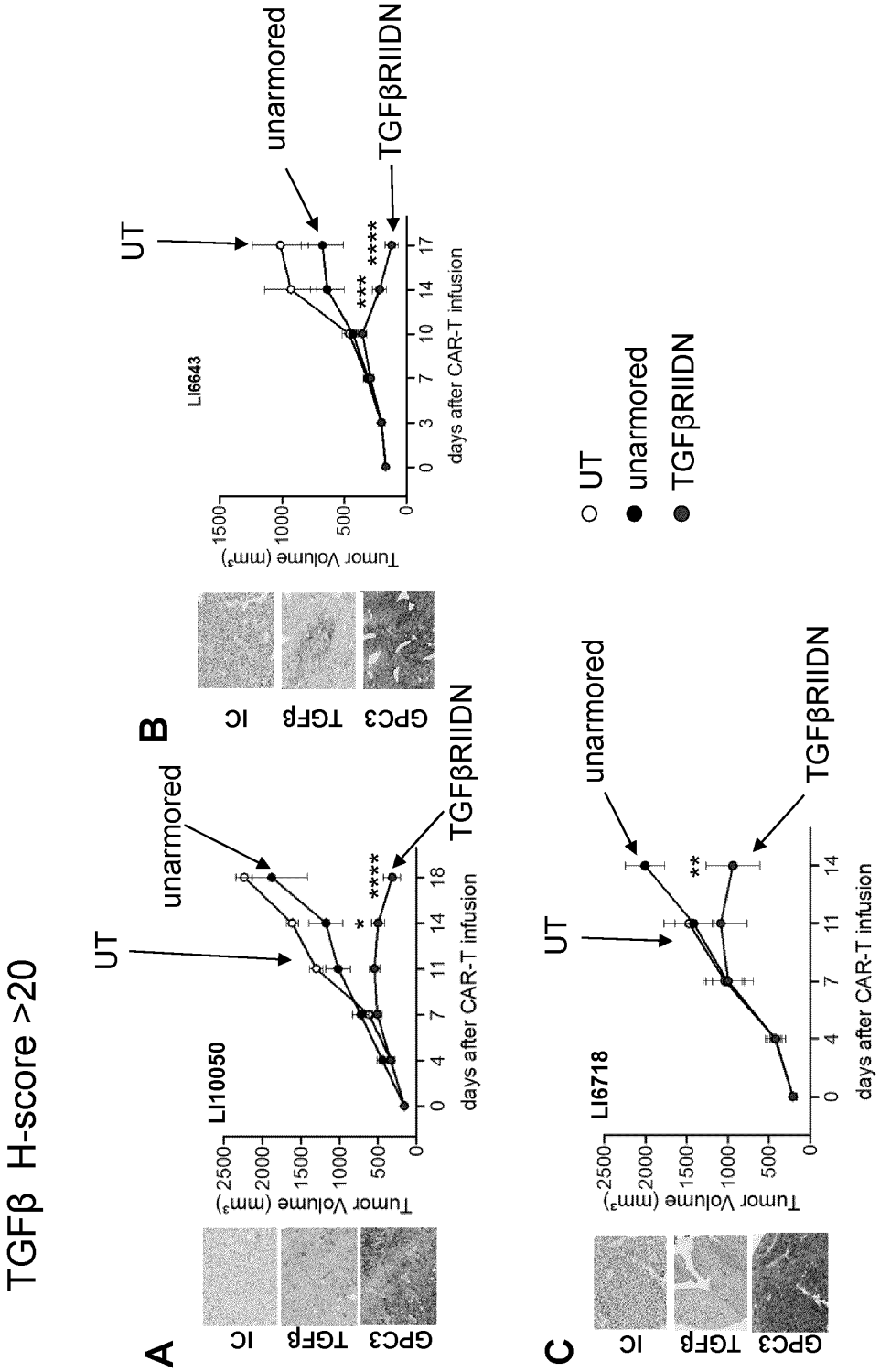
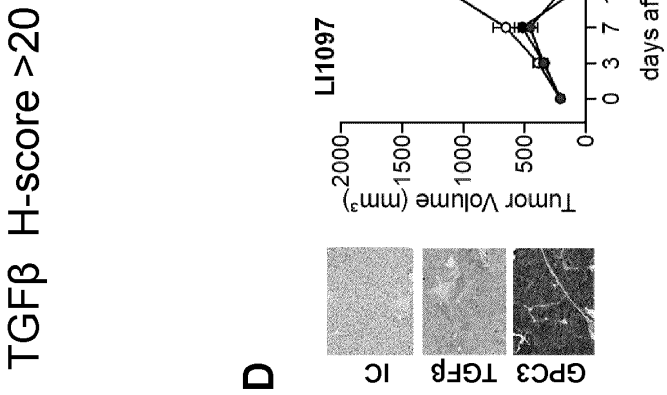

TGFβ H-score >20
A
B
C
UT
unarmored
TGFβRIIDN
LI10050
LI6643
LI6718
Tumor Volume (mm³)
days after CAR-T infusion
IC
TGFβ
GPC3
○ UT
● unarmored
● TGFβRIIDN

Fig. 19


TGFβ H-score >20
D
LI1097
Tumor Volume (mm³)
2000
1500
1000
500
0
0 3 7 10 14 17 21 24 28
days after CAR-T infusion
GPC3
TGFβ
IC

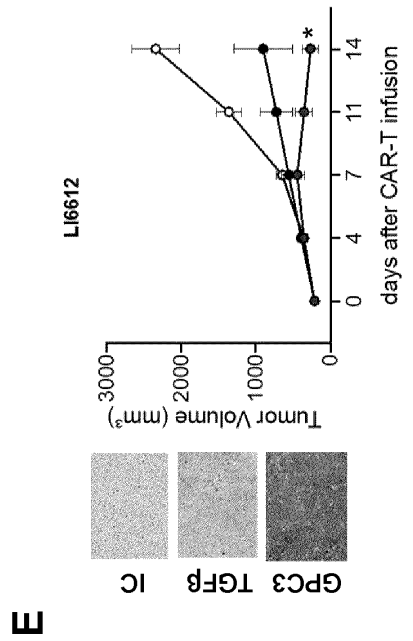

E
LI6612
Tumor Volume (mm³)
3000
2000
1000
0
0 4 7 11 14
days after CAR-T infusion
*
GPC3
TGFβ
IC

Fig. 19 (cont.)