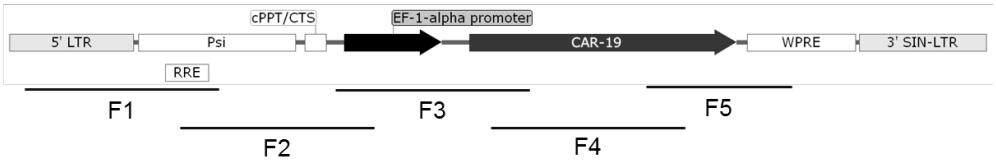
(A) Old vector including $P_{EF1\alpha}$-L; long promoter (531 bp)



(B) New vector including $P_{EF1\alpha}$-S; short promoter (212 bp)

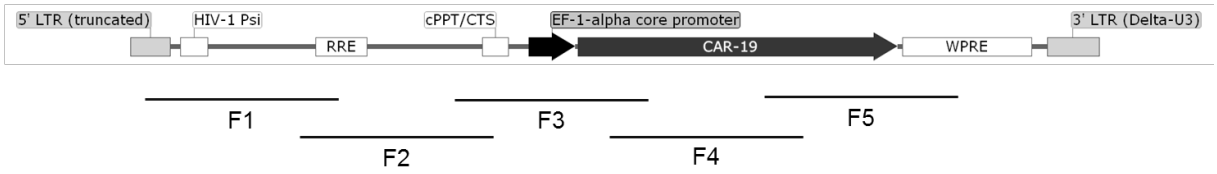


Figure 1

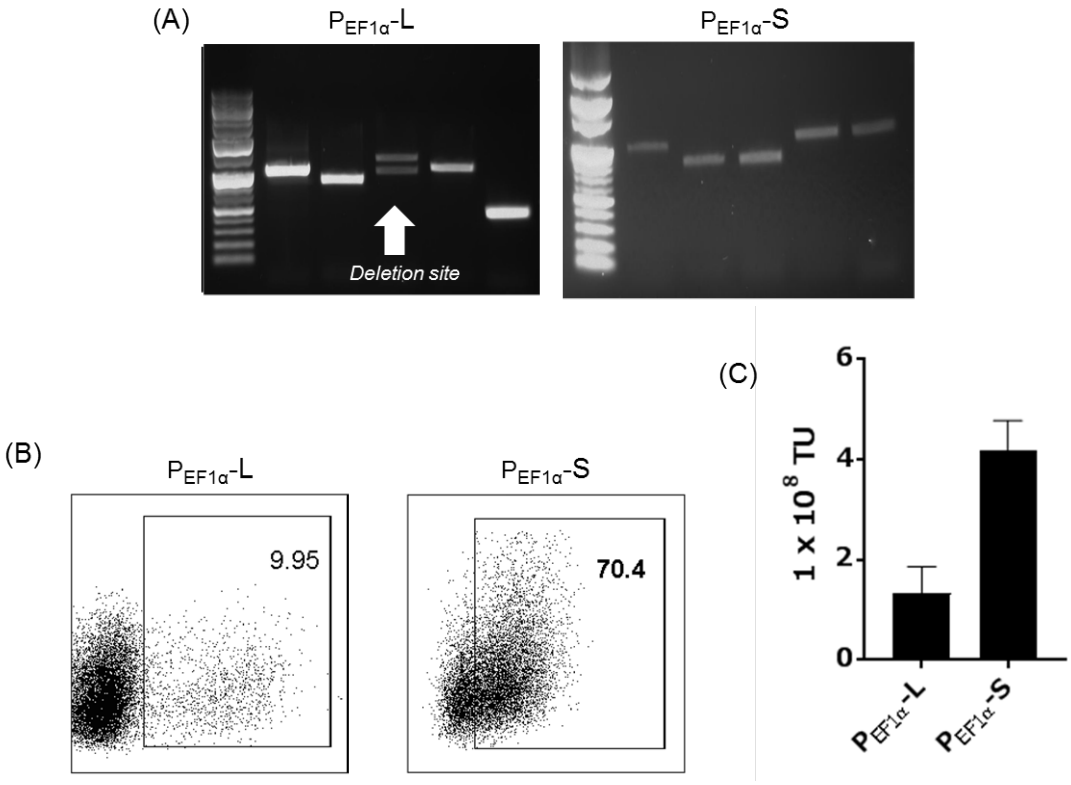

(A)
$P_{EF1\alpha}$-L
$P_{EF1\alpha}$-S
Deletion site
(B)
$P_{EF1\alpha}$-L
$P_{EF1\alpha}$-S
9.95
70.4
(C)
1 x 10^8 TU
6
4
2
0
$P_{EF1\alpha}$-L
$P_{EF1\alpha}$-S

Figure 2

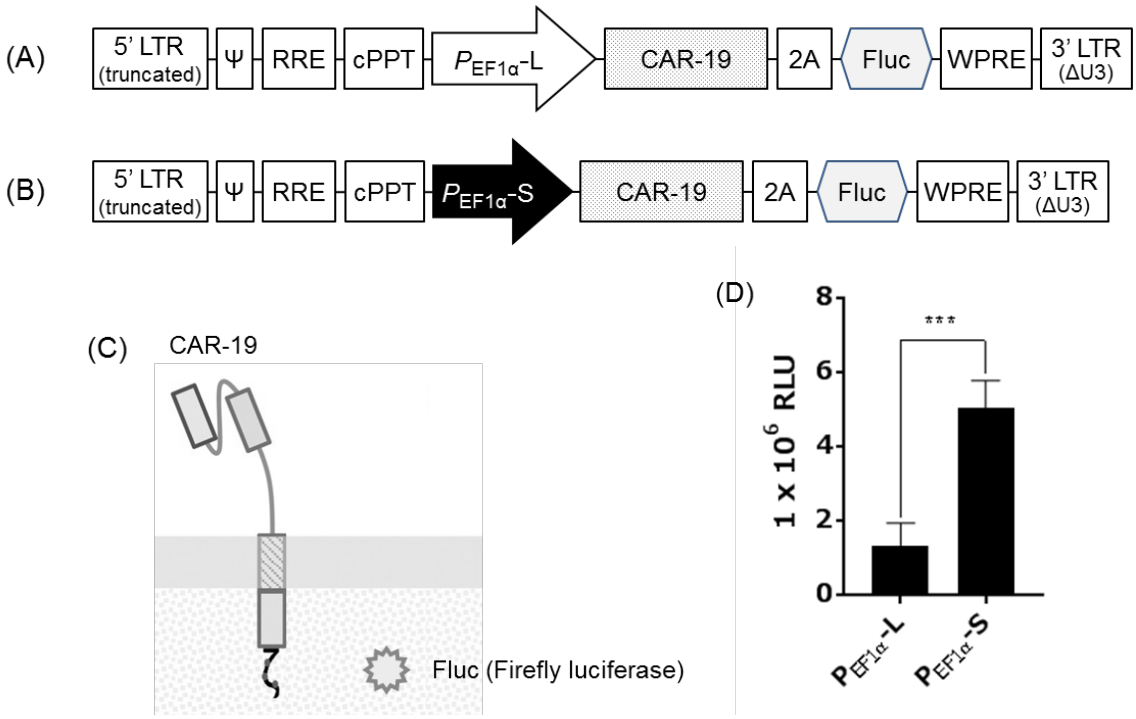

(A)
5' LTR (truncated)
Ψ
RRE
cPPT
$P_{EF1\alpha}$-L
CAR-19
2A
Fluc
WPRE
3' LTR (ΔU3)
(B)
5' LTR (truncated)
Ψ
RRE
cPPT
$P_{EF1\alpha}$-S
CAR-19
2A
Fluc
WPRE
3' LTR (ΔU3)
(C)
CAR-19
Fluc (Firefly luciferase)
(D)
1 x 10^6 RLU
8
6
4
2
0
***
$P_{EF1\alpha}$-L
$P_{EF1\alpha}$-S

Figure 3

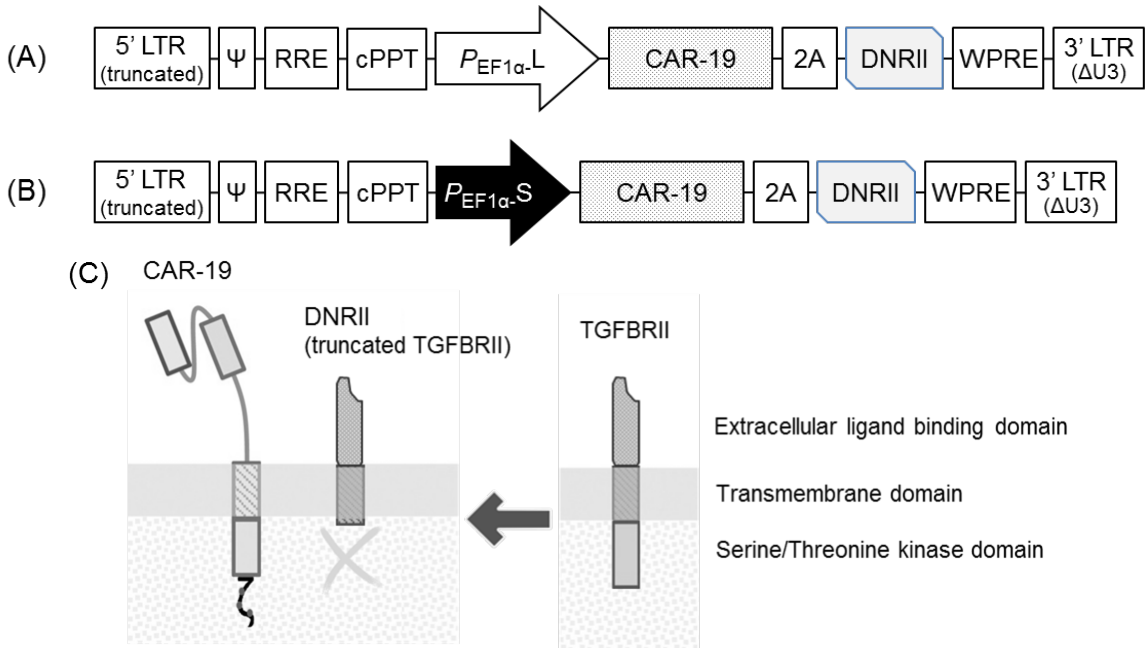

(A)
5' LTR (truncated)
Ψ
RRE
cPPT
$P_{EF1\alpha}$-L
CAR-19
2A
DNRII
WPRE
3' LTR (ΔU3)
(B)
5' LTR (truncated)
Ψ
RRE
cPPT
$P_{EF1\alpha}$-S
CAR-19
2A
DNRII
WPRE
3' LTR (ΔU3)
(C)
CAR-19
DNRII (truncated TGFBRII)
TGFBRII
Extracellular ligand binding domain
Transmembrane domain
Serine/Threonine kinase domain

Figure 4

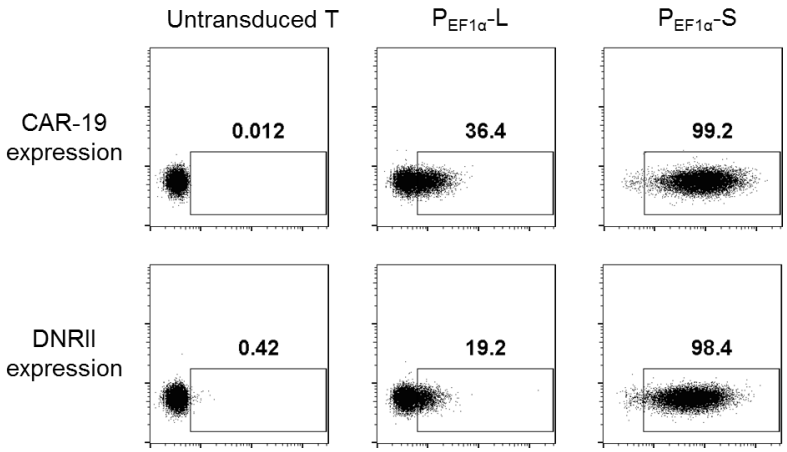

Untransduced T
$P_{EF1\alpha}$-L
$P_{EF1\alpha}$-S
CAR-19 expression
0.012
36.4
99.2
DNRII expression
0.42
19.2
98.4

Figure 5

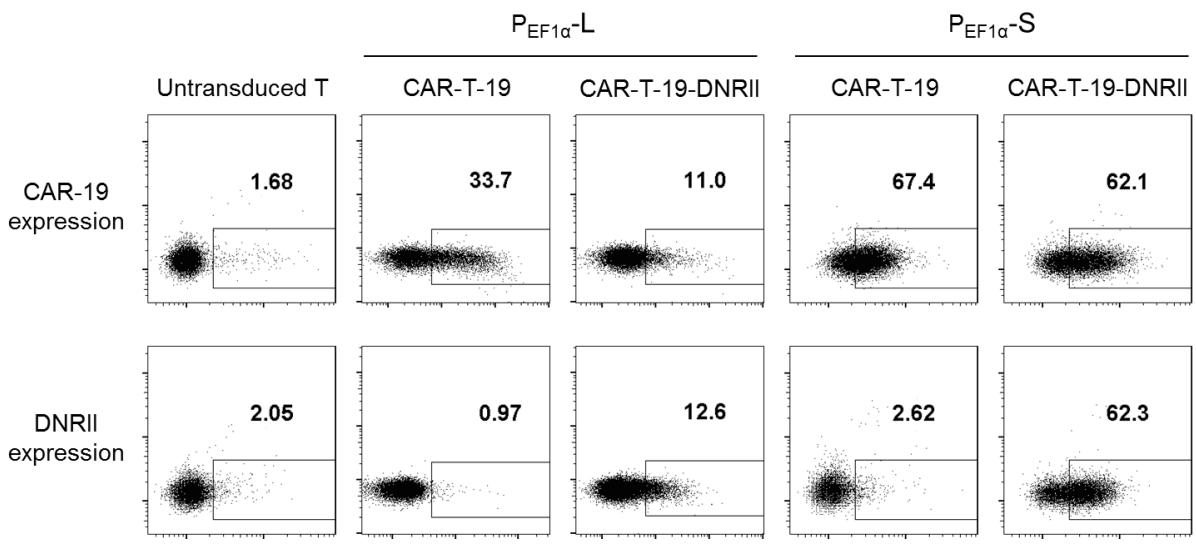

$P_{EF1\alpha}$-L
$P_{EF1\alpha}$-S
Untransduced T
CAR-T-19
CAR-T-19-DNRII
CAR-T-19
CAR-T-19-DNRII
CAR-19 expression
1.68
33.7
11.0
67.4
62.1
DNRII expression
2.05
0.97
12.6
2.62
62.3

Figure 6

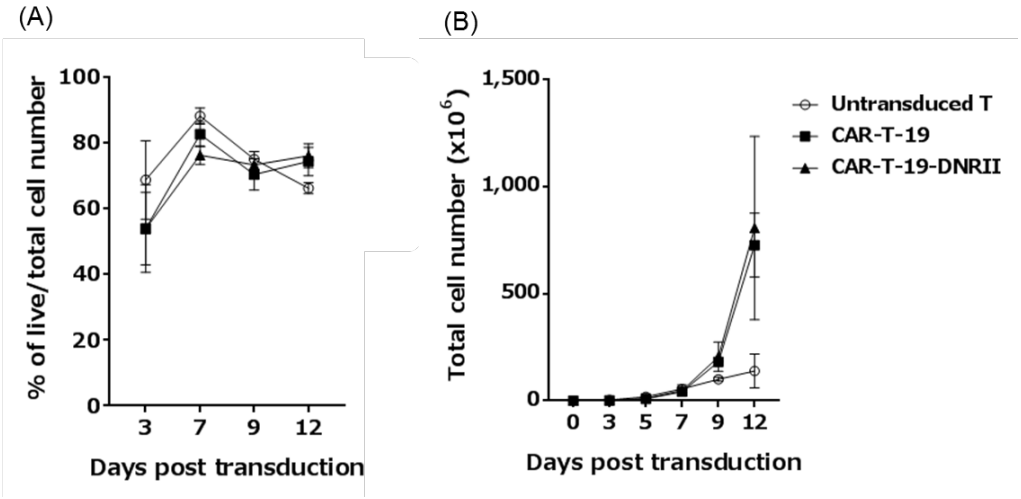

(A)
% of live/total cell number
100
80
60
40
20
0
3
7
9
12
Days post transduction
(B)
Total cell number (x10^6)
1,500
1,000
500
0
0
3
5
7
9
12
Days post transduction
Untransduced T
CAR-T-19
CAR-T-19-DNRII

Figure 7

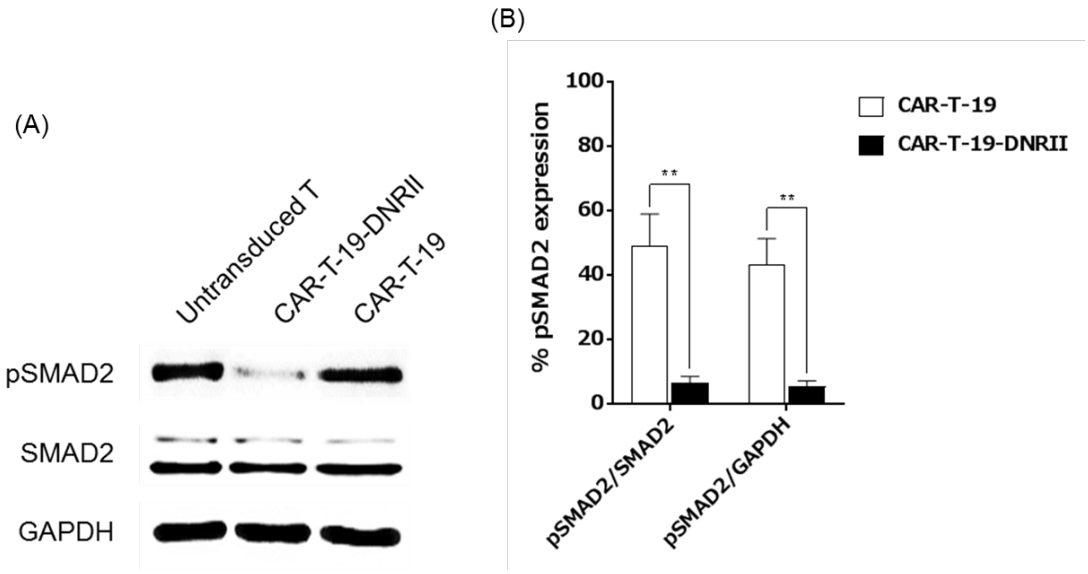

(A)
Untransduced T
CAR-T-19-DNRII
CAR-T-19
pSMAD2
SMAD2
GAPDH
(B)
% pSMAD2 expression
100
80
60
40
20
0
**
**
CAR-T-19
CAR-T-19-DNRII
pSMAD2/SMAD2
pSMAD2/GAPDH

Figure 8

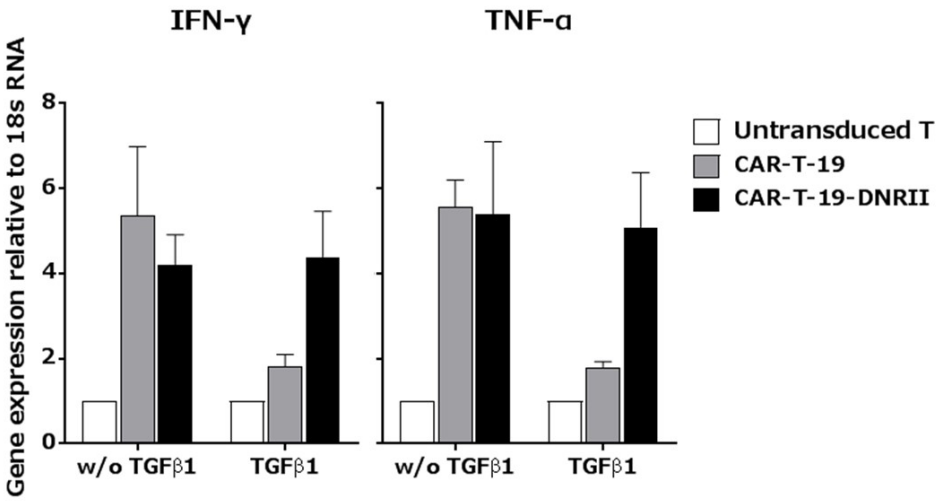

IFN-γ
TNF-α
Gene expression relative to 18s RNA
8
6
4
2
0
w/o TGFβ1
TGFβ1
w/o TGFβ1
TGFβ1
Untransduced T
CAR-T-19
CAR-T-19-DNRII

Figure 9

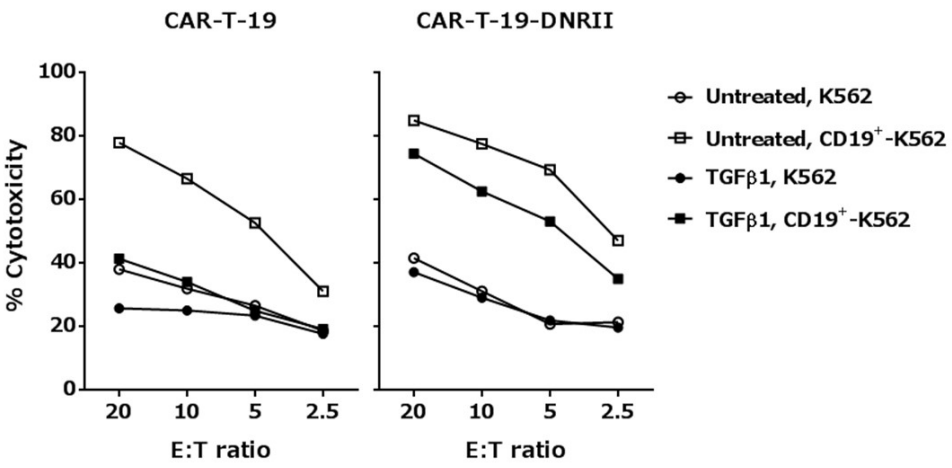

CAR-T-19
CAR-T-19-DNRII
% Cytotoxicity
100
80
60
40
20
0
20
10
5
2.5
E:T ratio
Untreated, K562
Untreated, CD19+-K562
TGFβ1, K562
TGFβ1, CD19+-K562

Figure 10