Figure 1A

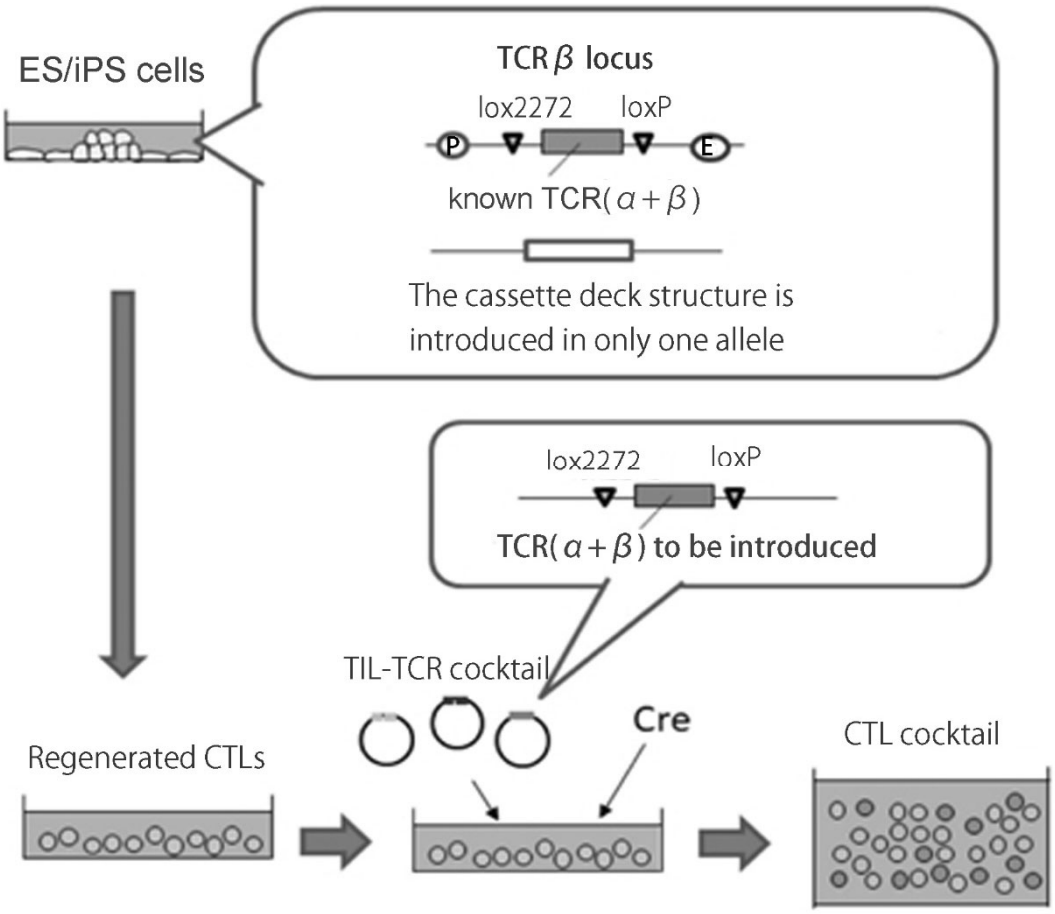

ES/iPS cells
TCRβ locus
lox2272
loxP
P
E
known TCR(α+β)
The cassette deck structure is introduced in only one allele
lox2272
loxP
TCR(α+β) to be introduced
TIL-TCR cocktail
Cre
Regenerated CTLs
CTL cocktail

Figure 1B

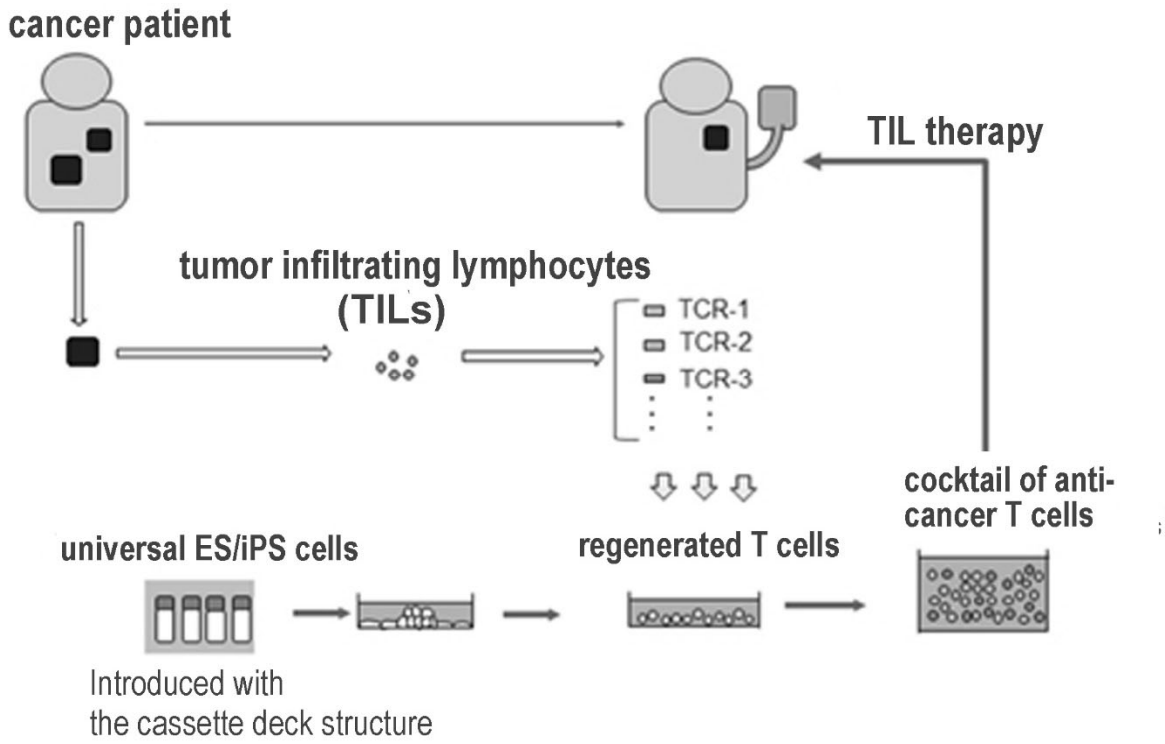

cancer patient
TIL therapy
tumor infiltrating lymphocytes
(TILs)
TCR-1
TCR-2
TCR-3
cocktail of anti-
cancer T cells
universal ES/iPS cells
regenerated T cells
Introduced with
the cassette deck structure

Figure 2

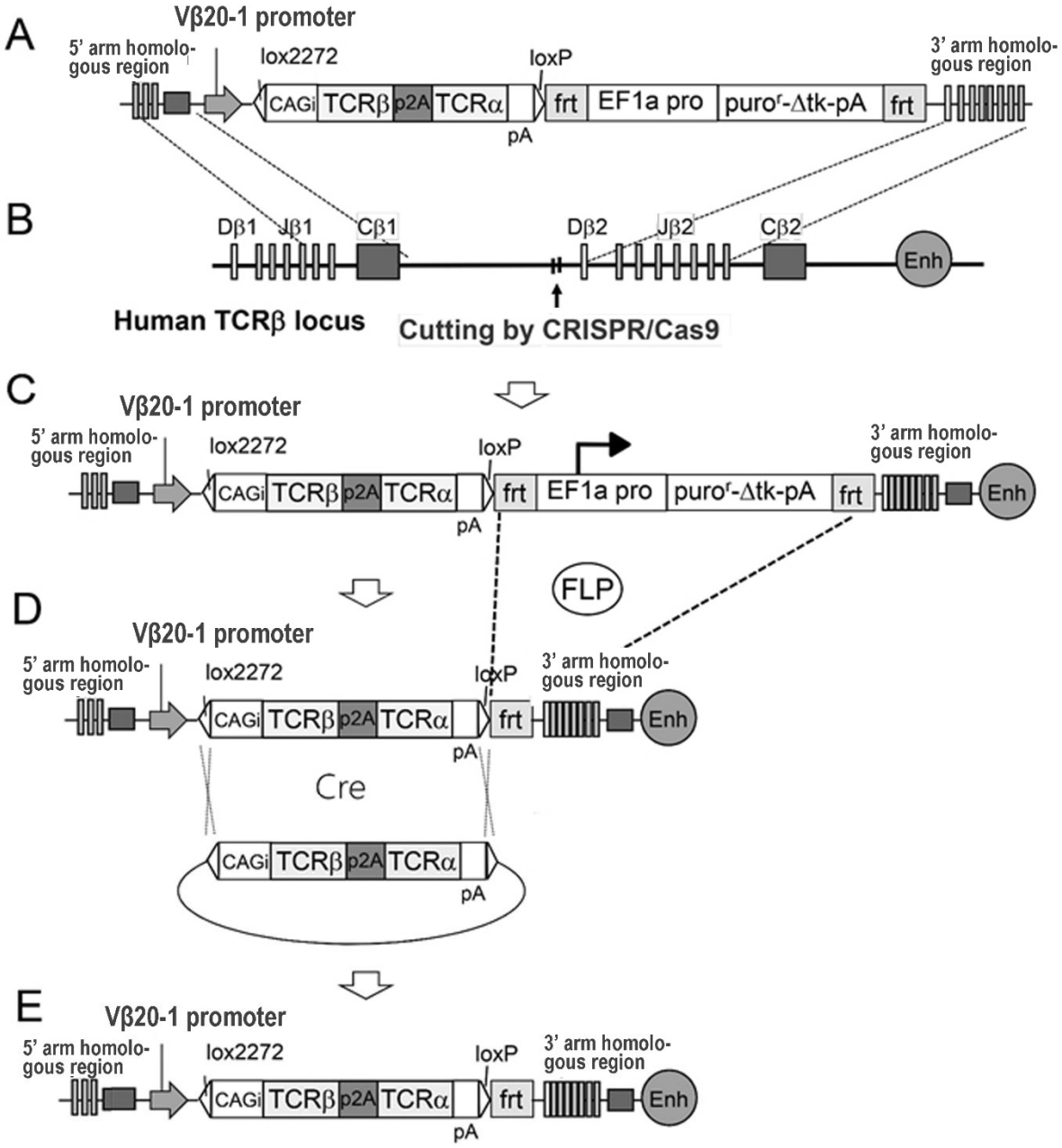

A
Vβ20-1 promoter
5' arm homologous region
lox2272
loxP
3' arm homologous region
CAGi
TCRβ
p2A
TCRα
pA
frt
EF1a pro
puroʳ-Δtk-pA
frt
B
Dβ1
Jβ1
Cβ1
Dβ2
Jβ2
Cβ2
Enh
Human TCRβ locus
Cutting by CRISPR/Cas9
C
Vβ20-1 promoter
5' arm homologous region
lox2272
loxP
3' arm homologous region
CAGi
TCRβ
p2A
TCRα
pA
frt
EF1a pro
puroʳ-Δtk-pA
frt
Enh
FLP
D
Vβ20-1 promoter
5' arm homologous region
lox2272
loxP
3' arm homologous region
CAGi
TCRβ
p2A
TCRα
pA
frt
Enh
Cre
CAGi
TCRβ
p2A
TCRα
pA
E
Vβ20-1 promoter
5' arm homologous region
lox2272
loxP
3' arm homologous region
CAGi
TCRβ
p2A
TCRα
pA
frt
Enh

Figure 3

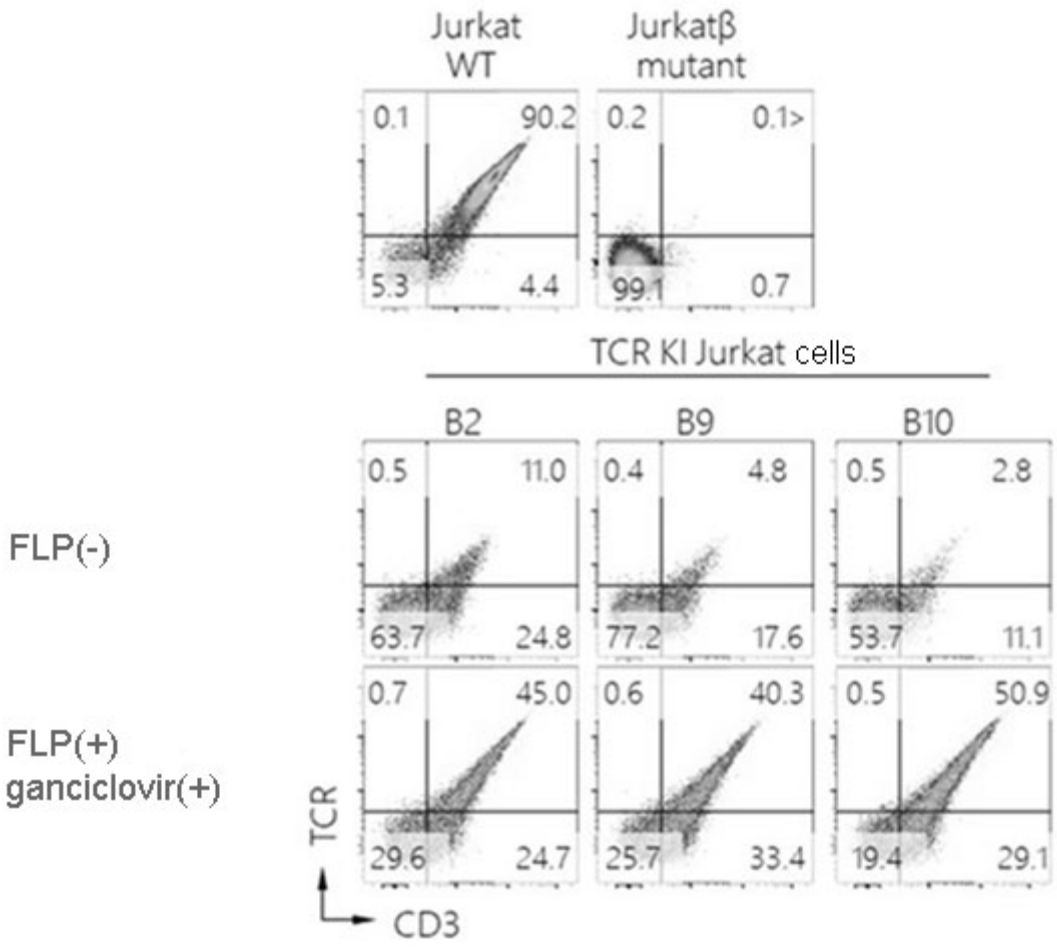

Jurkat WT
Jurkatβ mutant
0.1
90.2
5.3
4.4
0.2
0.1>
99.1
0.7
TCR KI Jurkat cells
B2
B9
B10
FLP(-)
FLP(+) ganciclovir(+)
0.5
11.0
63.7
24.8
0.4
4.8
77.2
17.6
0.5
2.8
53.7
11.1
0.7
45.0
29.6
24.7
0.6
40.3
25.7
33.4
0.5
50.9
19.4
29.1
TCR
CD3

Figure 4

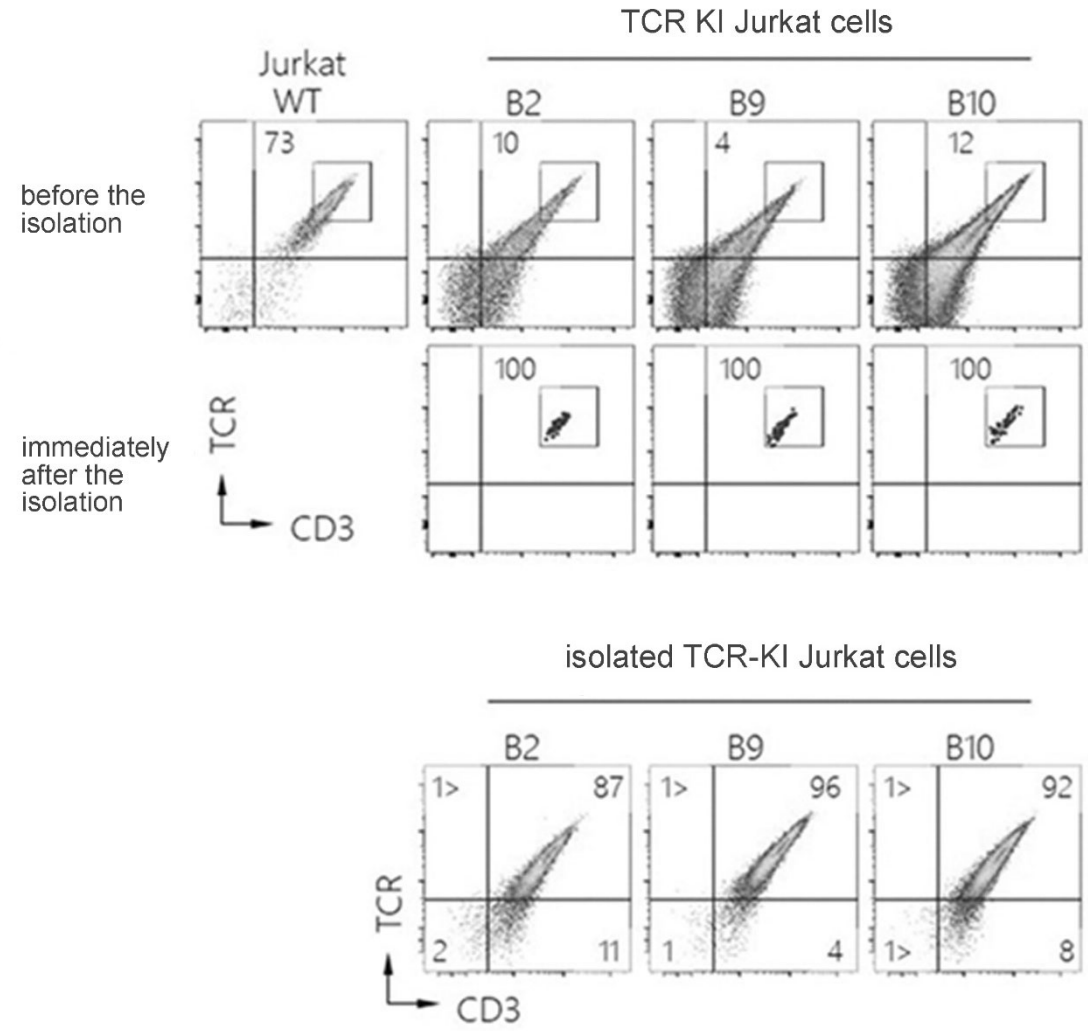

TCR KI Jurkat cells
Jurkat WT
B2
B9
B10
before the isolation
immediately after the isolation
73
10
4
12
100
100
100
TCR
CD3
isolated TCR-KI Jurkat cells
B2
B9
B10
1>
87
1>
96
1>
92
2
11
1
4
1>
8
TCR
CD3

Figure 5A

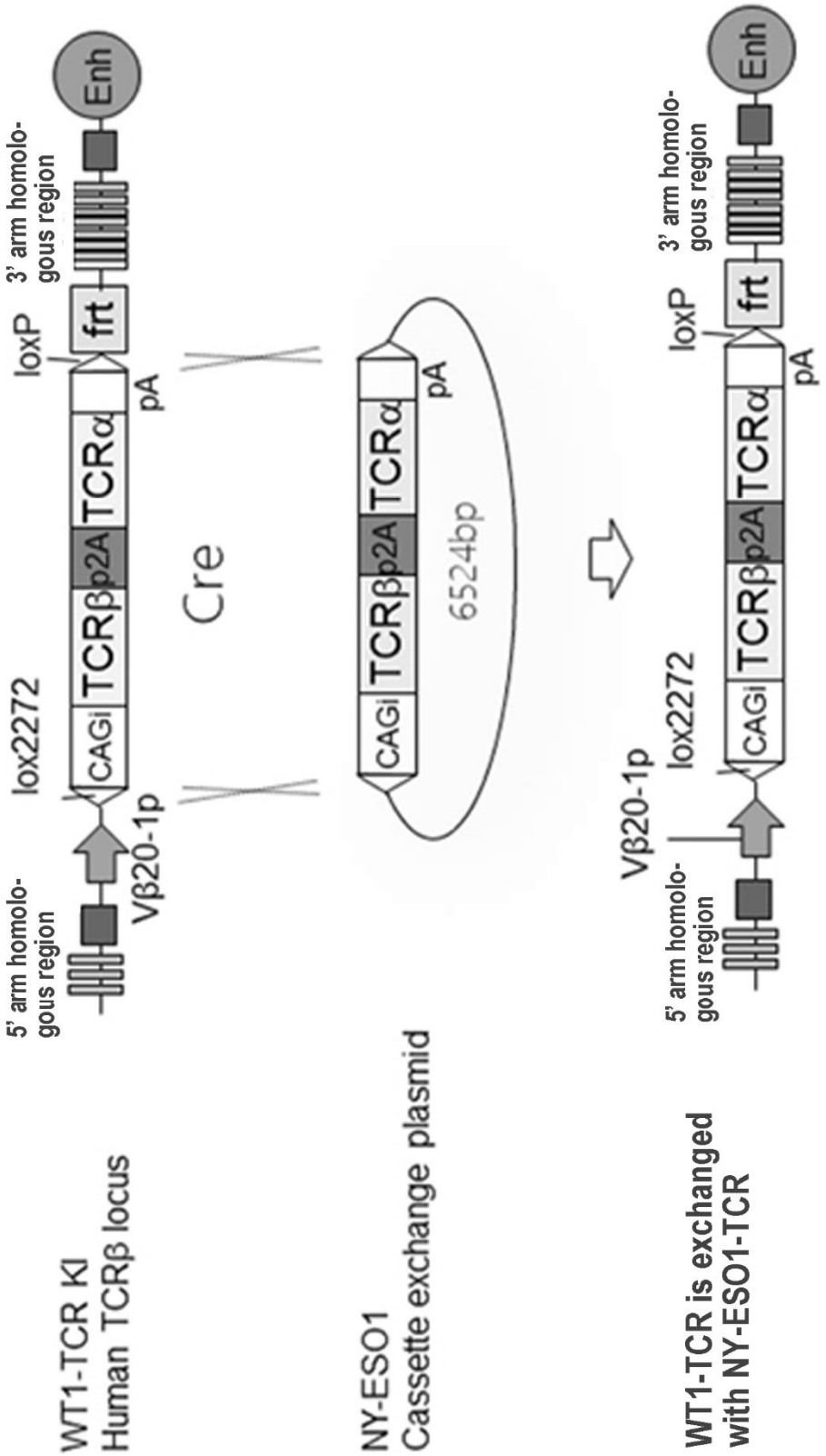

WT1-TCR KI
Human TCRβ locus
5' arm homolo-gous region
Vβ20-1p
lox2272
CAGi
TCRβ
p2A
TCRα
pA
loxP
frt
3' arm homolo-gous region
Enh
Cre
NY-ESO1
Cassette exchange plasmid
6524bp
WT1-TCR is exchanged with NY-ESO1-TCR

Figure 5B

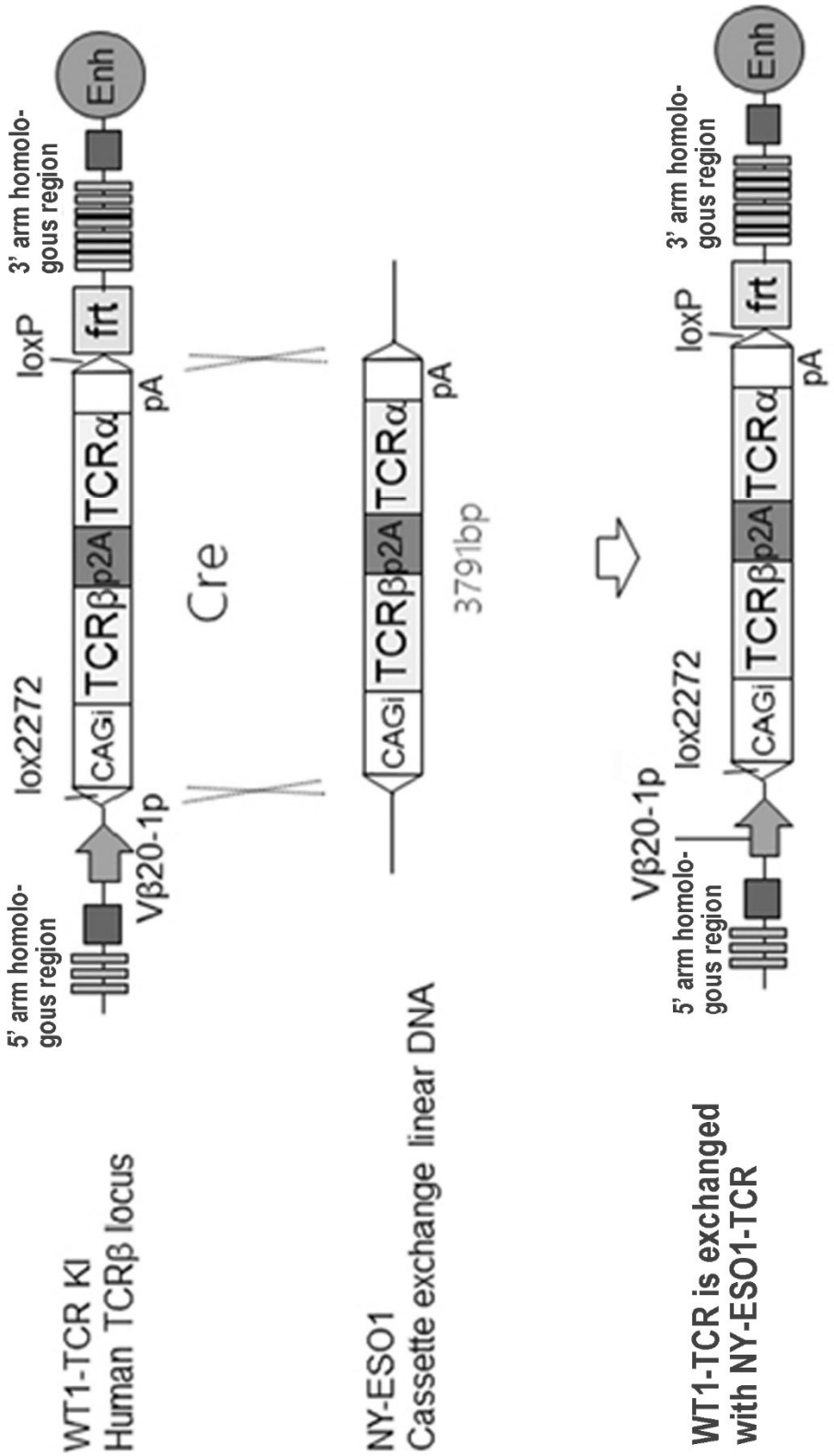

WT1-TCR KI
Human TCRβ locus
5' arm homolo-gous region
Vβ20-1p
lox2272
CAGi
TCRβ
p2A
TCRα
pA
loxP
frt
3' arm homolo-gous region
Enh
Cre
NY-ESO1
Cassette exchange linear DNA
3791bp
WT1-TCR is exchanged with NY-ESO1-TCR

Figure 6

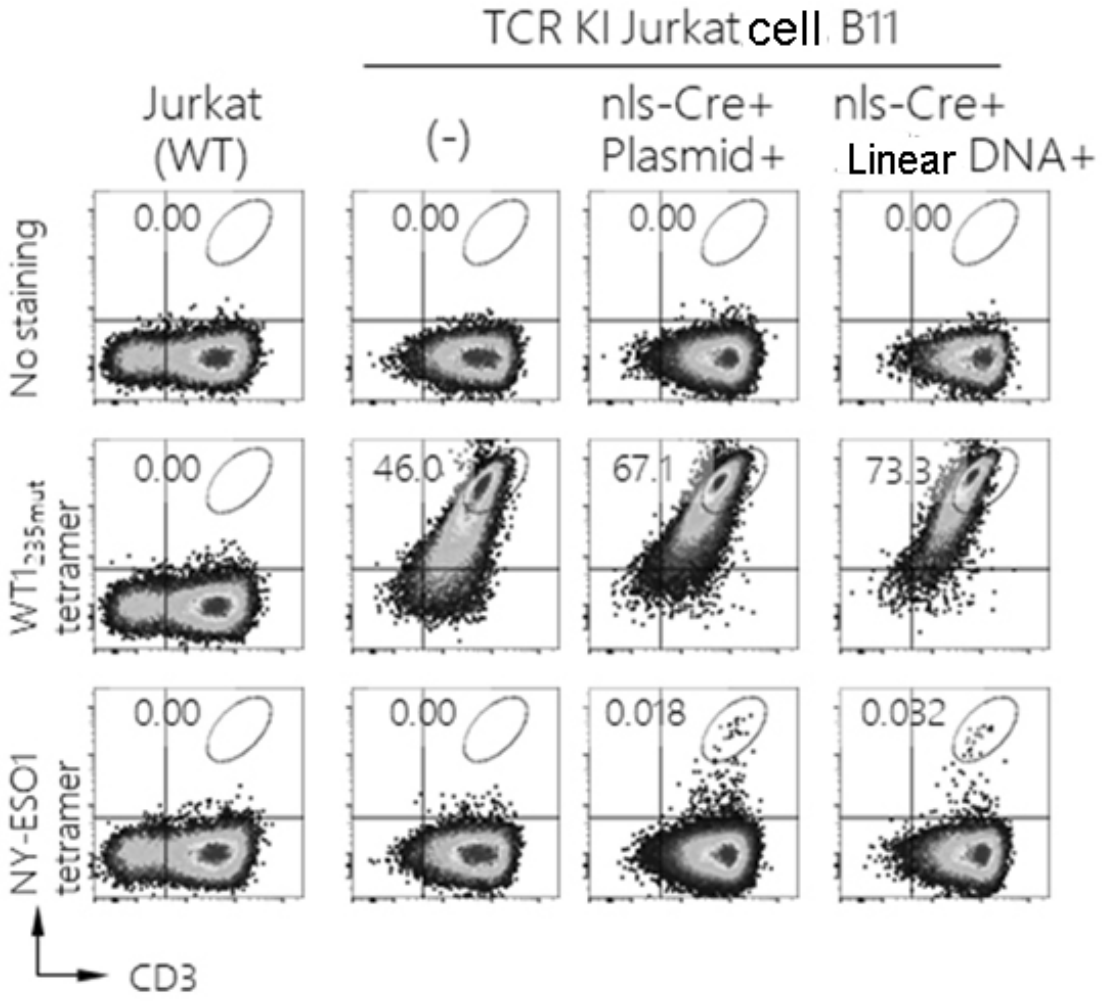

TCR KI Jurkat cell B11
Jurkat (WT)
(-)
nls-Cre+ Plasmid+
nls-Cre+ Linear DNA+
No staining
WT1235mut tetramer
NY-ESO1 tetramer
0.00
0.00
0.00
0.00
0.00
46.0
67.1
73.3
0.00
0.00
0.018
0.032
CD3

Figure 7A

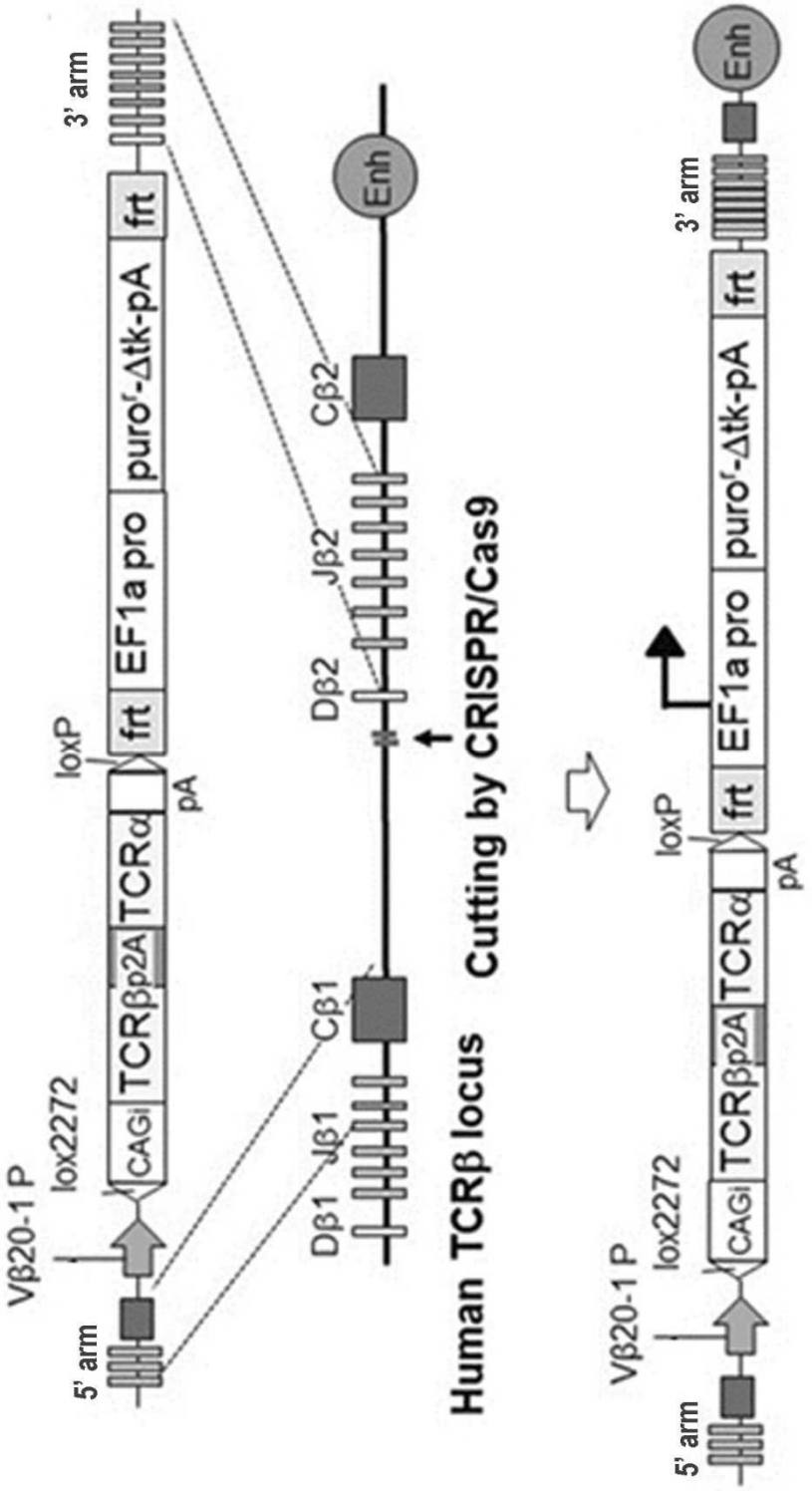

Vβ20-1 P
lox2272
CAGi
TCRβp2A
TCRα
pA
loxP
frt
EF1a pro
puroʳ-Δtk-pA
frt
3' arm
5' arm
Dβ1
Jβ1
Cβ1
Dβ2
Jβ2
Cβ2
Enh
Human TCRβ locus
Cutting by CRISPR/Cas9

Figure 7B

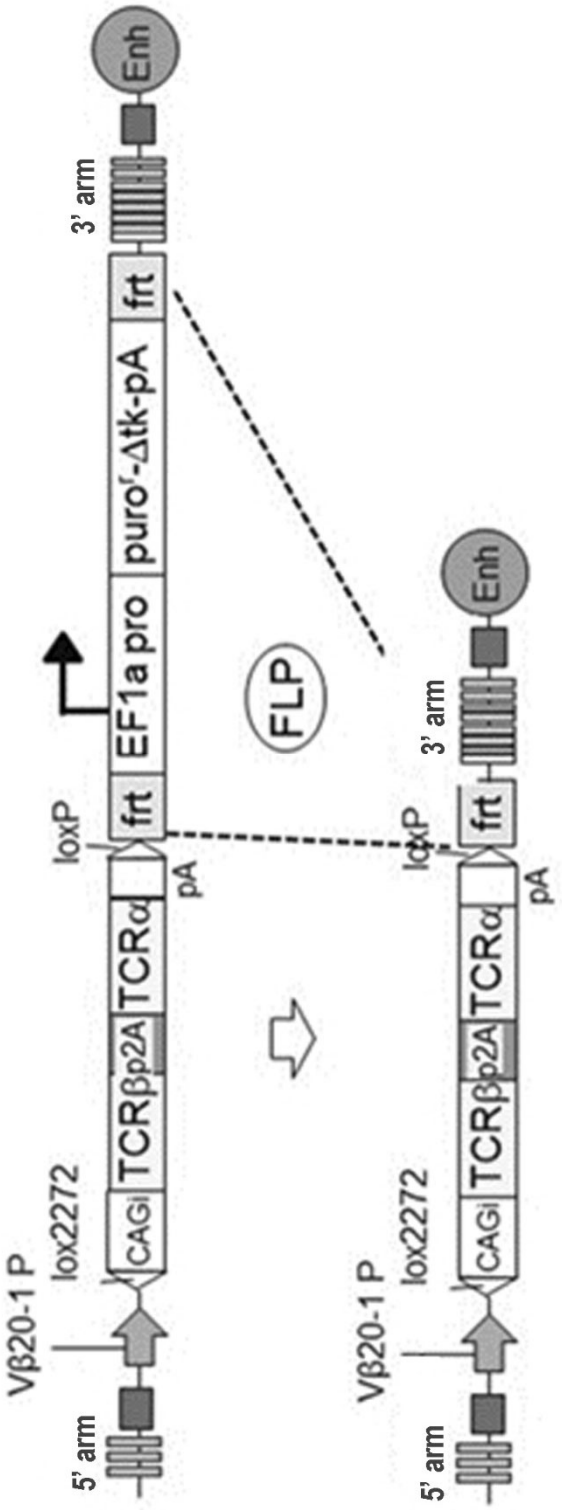

Enh
3' arm
frt
puro^r-Δtk-pA
EF1a pro
frt
loxP
pA
TCRα
TCRβp2A
CAGi
lox2272
Vβ20-1 P
5' arm
FLP
Enh
3' arm
frt
loxP
pA
TCRα
TCRβp2A
CAGi
lox2272
Vβ20-1 P
5' arm

Figure 8

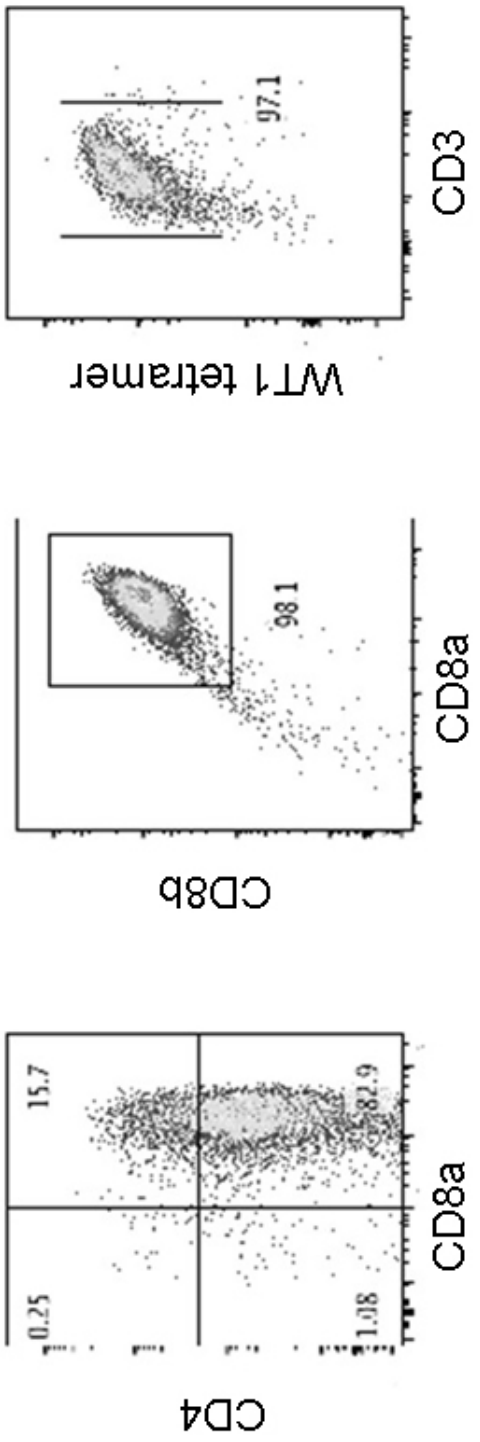


Figure 9

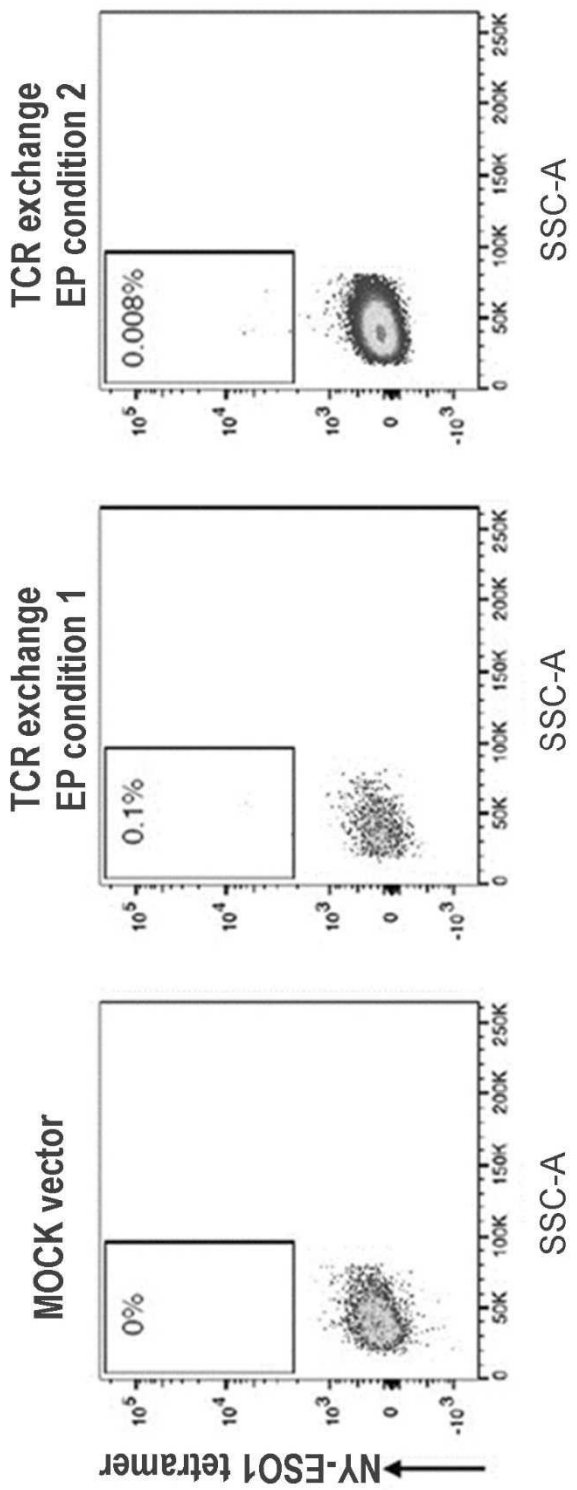

MOCK vector
0%
TCR exchange
EP condition 1
0.1%
TCR exchange
EP condition 2
0.008%
SSC-A
NY-ESO1 tetramer

Figure 10

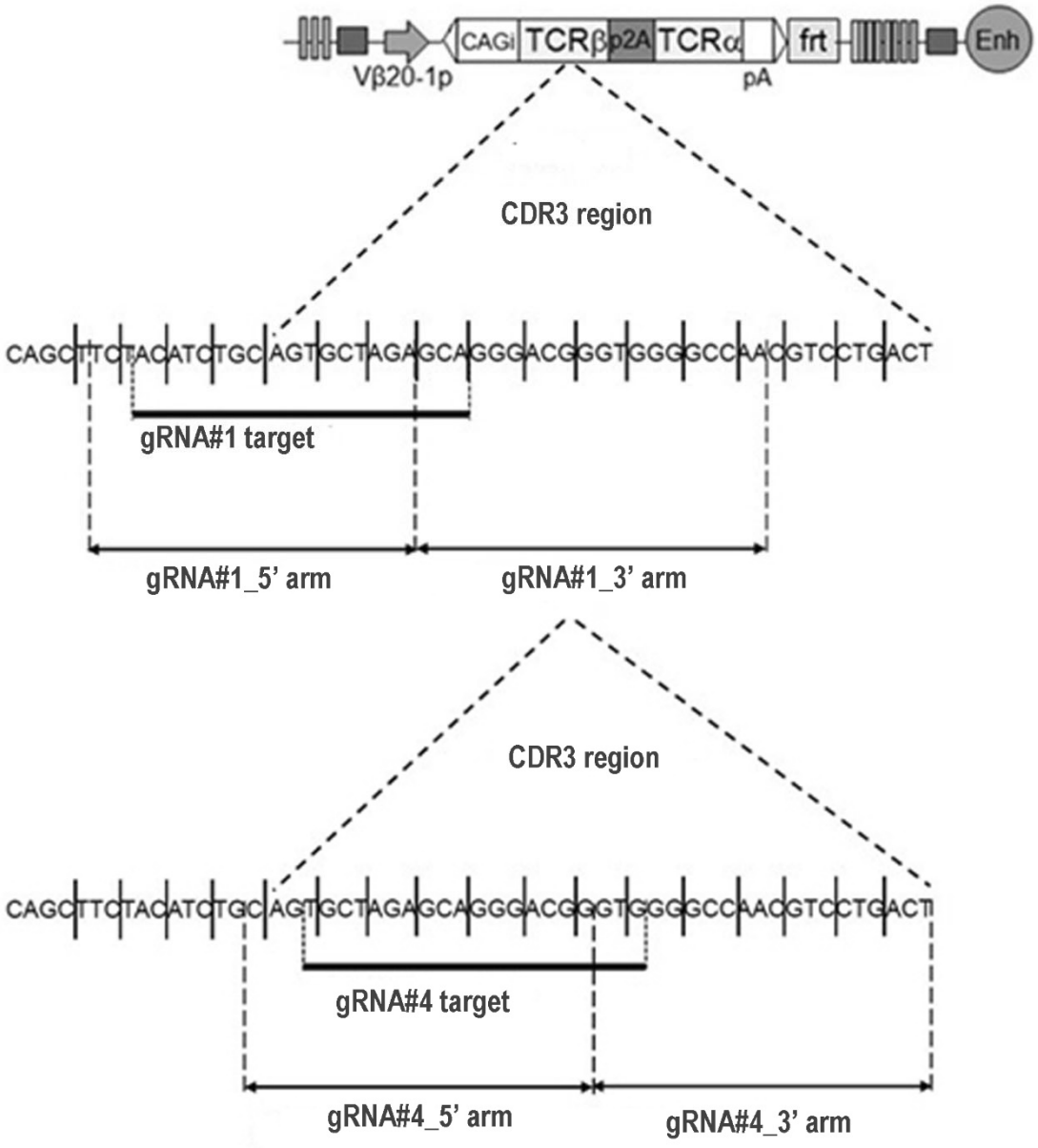

CAGi
TCRβ
p2A
TCRα
frt
Enh
Vβ20-1p
pA
CDR3 region
CAGCTTCTACATCTGCAGTGCTAGAGCAGGGACGGGTGGGGCCAACGTCCTGACT
gRNA#1 target
gRNA#1_5' arm
gRNA#1_3' arm
CDR3 region
CAGCTTCTACATCTGCAGTGCTAGAGCAGGGACGGGTGGGGCCAACGTCCTGACT
gRNA#4 target
gRNA#4_5' arm
gRNA#4_3' arm

Figure 11

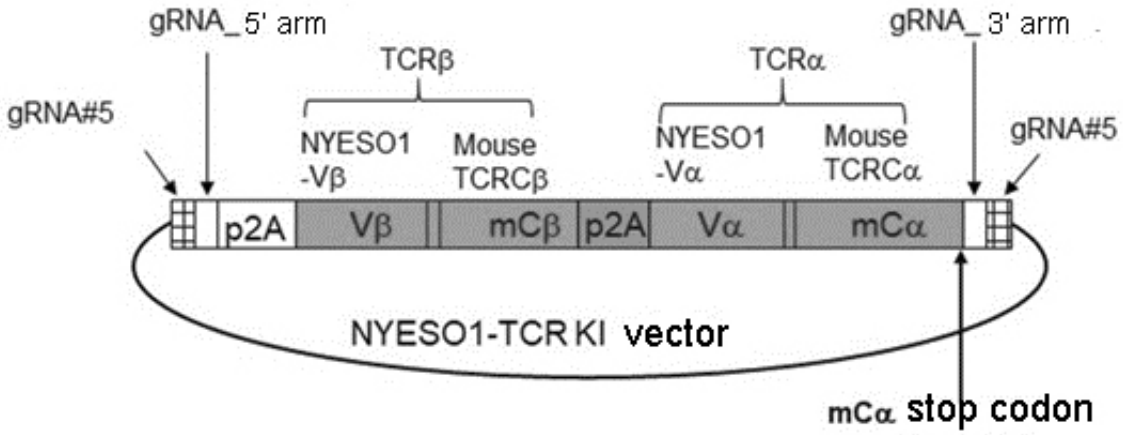


Figure 12

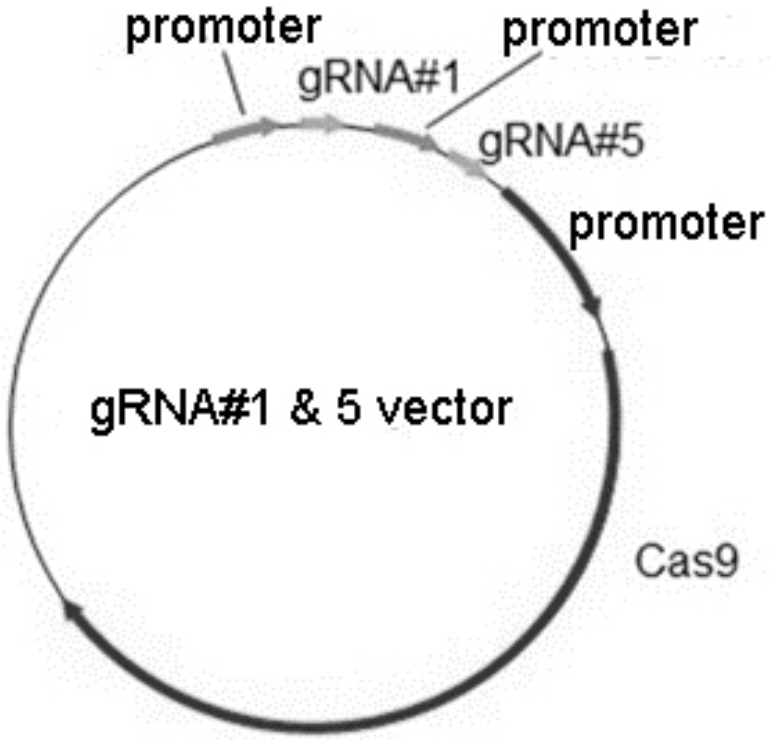


Figure 13

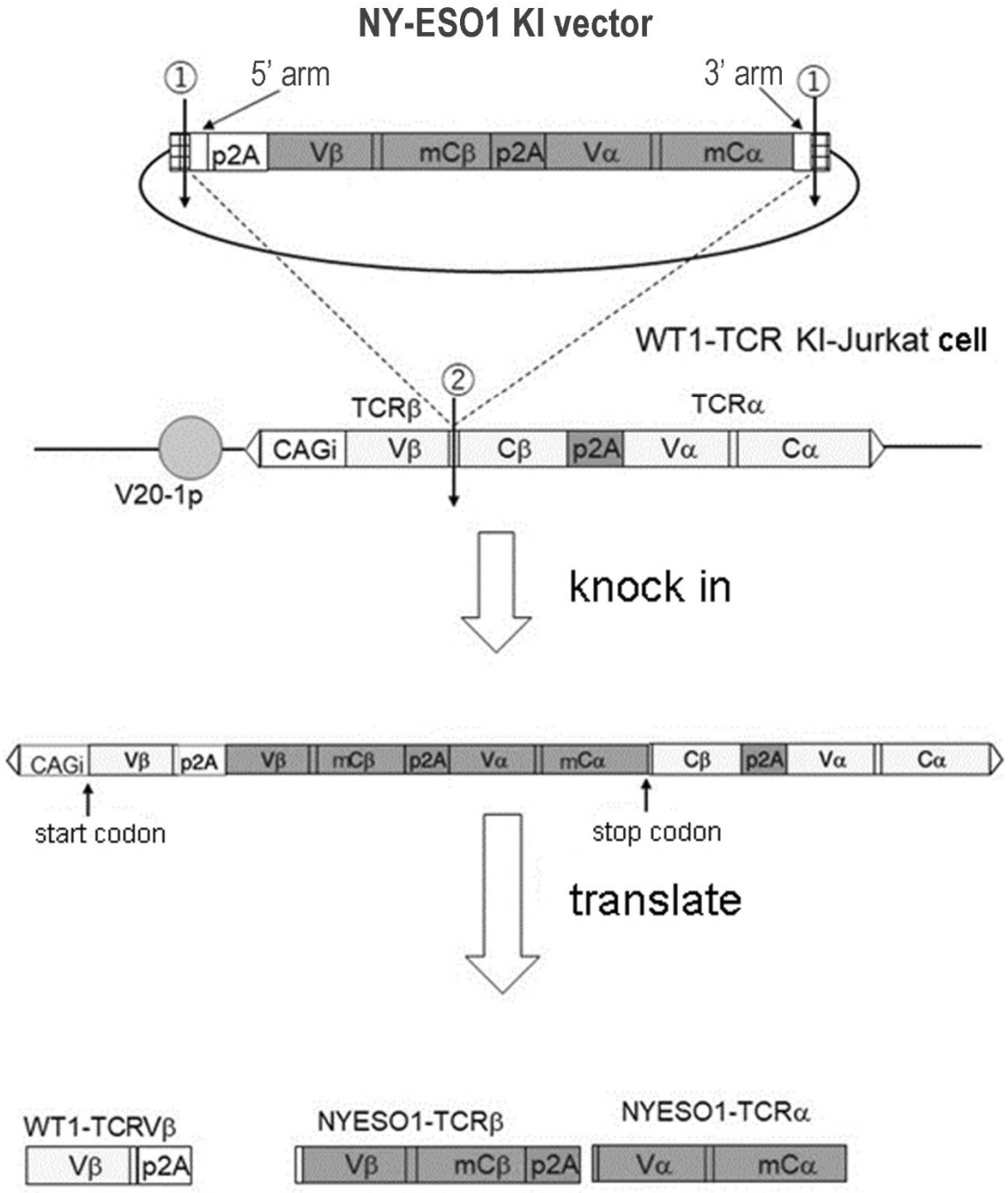

NY-ESO1 KI vector
5' arm
3' arm
p2A
Vβ
mCβ
p2A
Vα
mCα
WT1-TCR KI-Jurkat cell
TCRβ
TCRα
CAGi
Vβ
Cβ
p2A
Vα
Cα
V20-1p
knock in
CAGi
Vβ
p2A
Vβ
mCβ
p2A
Vα
mCα
Cβ
p2A
Vα
Cα
start codon
stop codon
translate
WT1-TCRVβ
Vβ
p2A
NYESO1-TCRβ
Vβ
mCβ
p2A
NYESO1-TCRα
Vα
mCα

Figure 14A

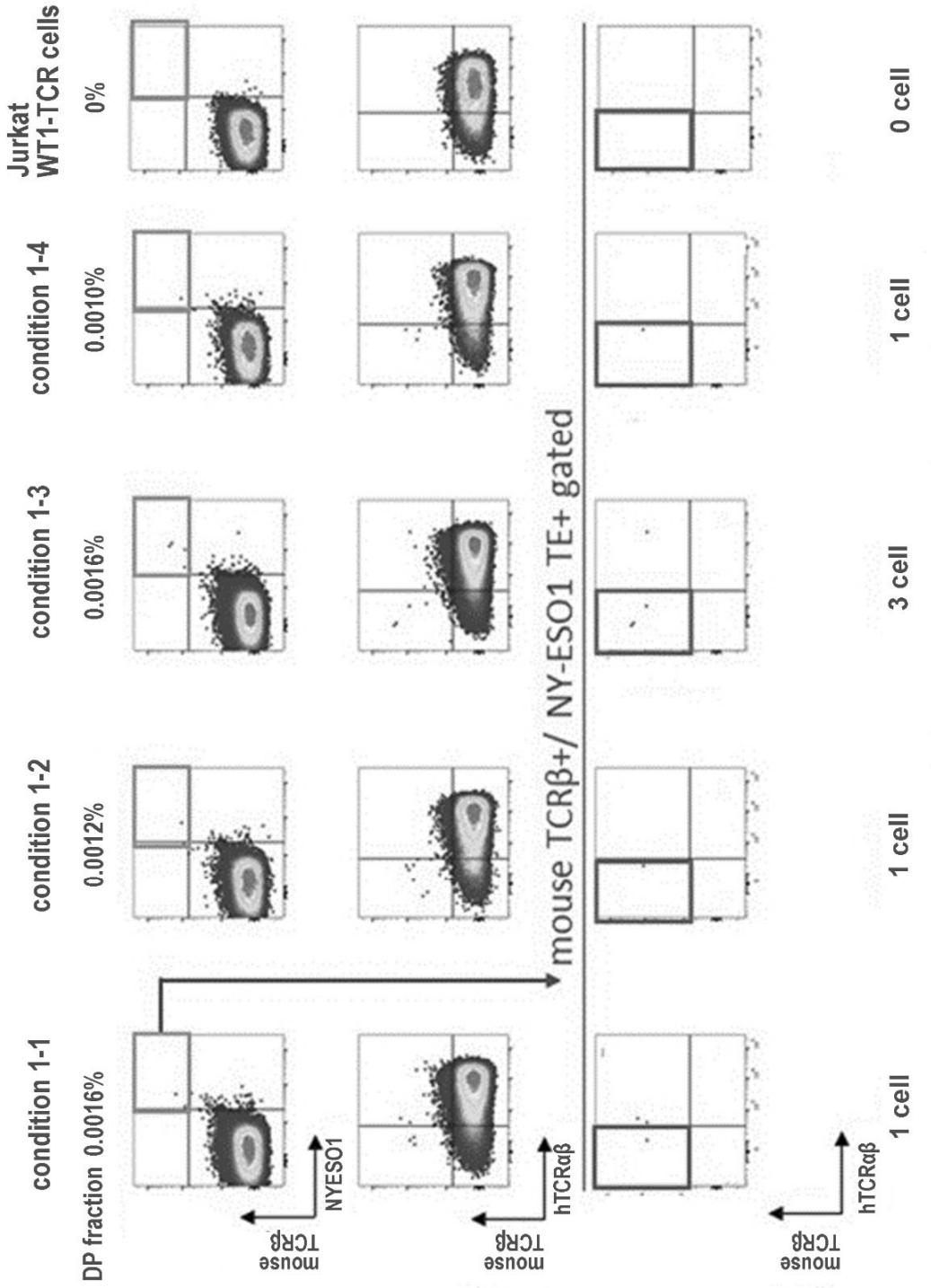
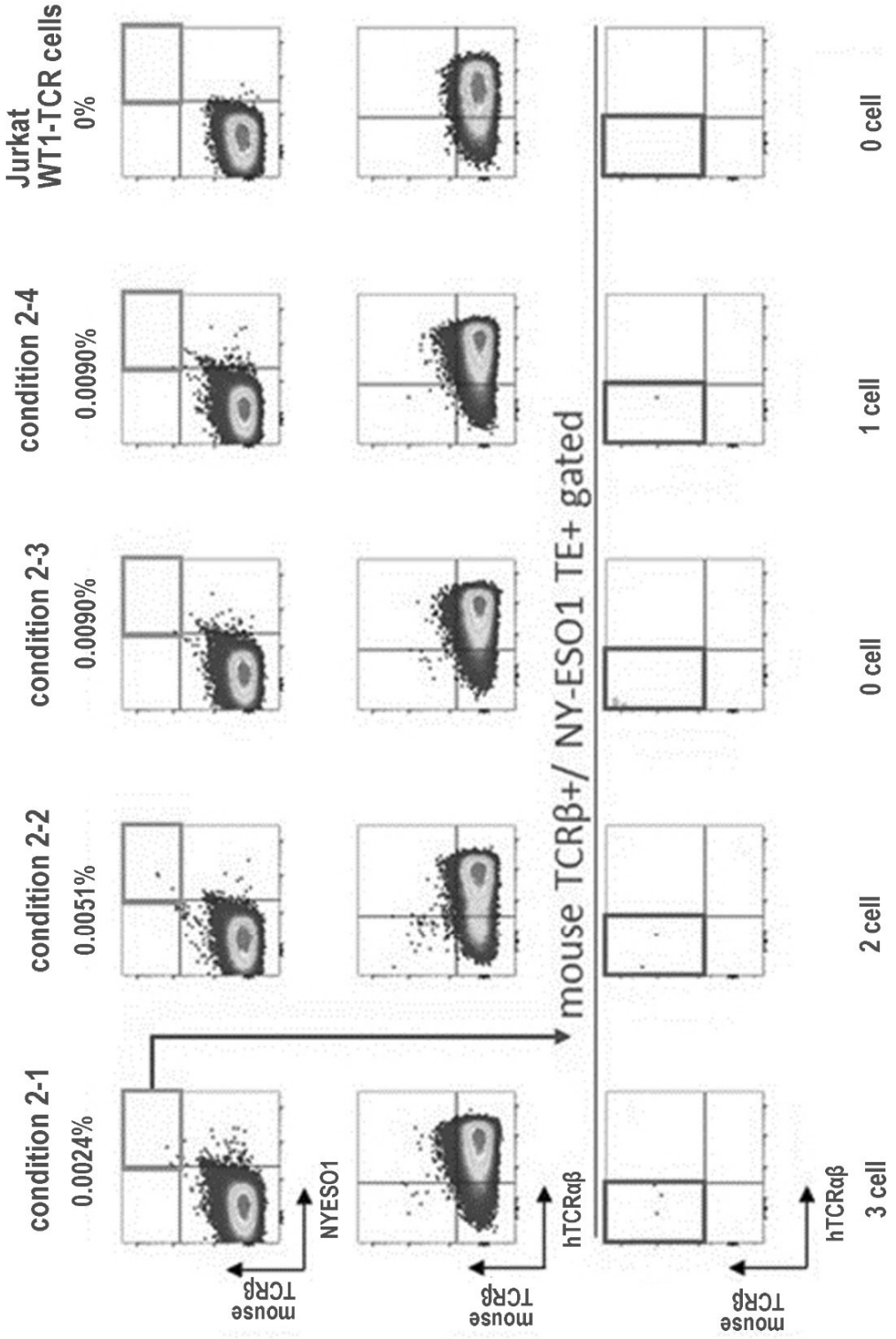

condition 1-1
condition 1-2
condition 1-3
condition 1-4
Jurkat WT1-TCR cells
DP fraction 0.0016%
0.0012%
0.0016%
0.0010%
0%
1 cell
1 cell
3 cell
1 cell
0 cell
mouse TCRβ+/ NY-ESO1 TE+ gated
mouse TCRβ
NYESO1
mouse TCRβ
hTCRαβ
mouse TCRβ
hTCRαβ

Figure 14B


Jurkat WT1-TCR cells
0%
condition 2-4
0.0090%
condition 2-3
0.0090%
condition 2-2
0.0051%
condition 2-1
0.0024%
mouse TCRβ+/ NY-ESO1 TE+ gated
0 cell
1 cell
0 cell
2 cell
3 cell
NYESO1
hTCRαβ
mouse TCRβ
mouse TCRβ
mouse TCRβ
hTCRαβ