Fig. 1

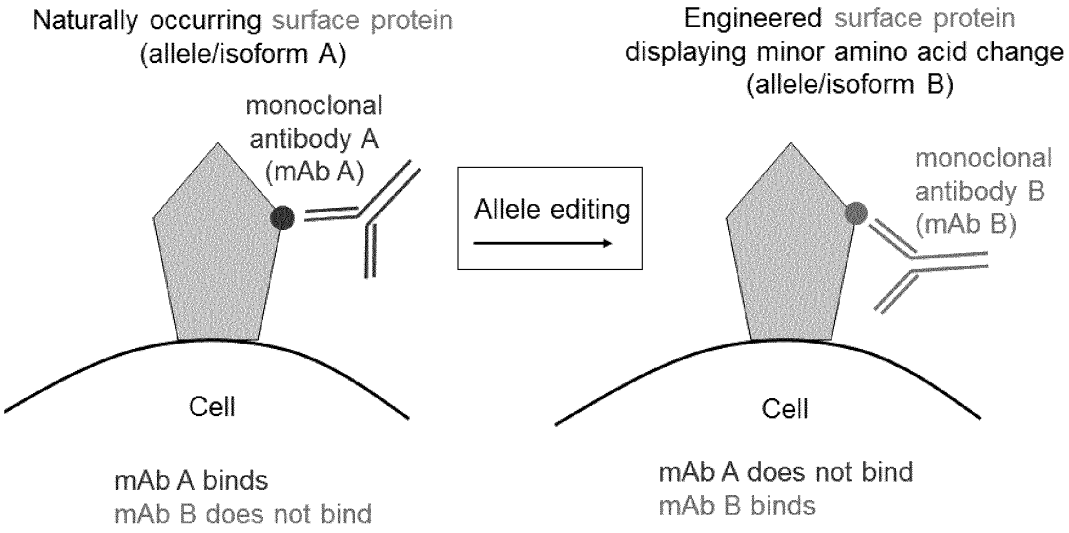

Naturally occurring surface protein
(allele/isoform A)
monoclonal
antibody A
(mAb A)
Cell
mAb A binds
mAb B does not bind
Allele editing
Engineered surface protein
displaying minor amino acid change
(allele/isoform B)
monoclonal
antibody B
(mAb B)
Cell
mAb A does not bind
mAb B binds

Fig. 2

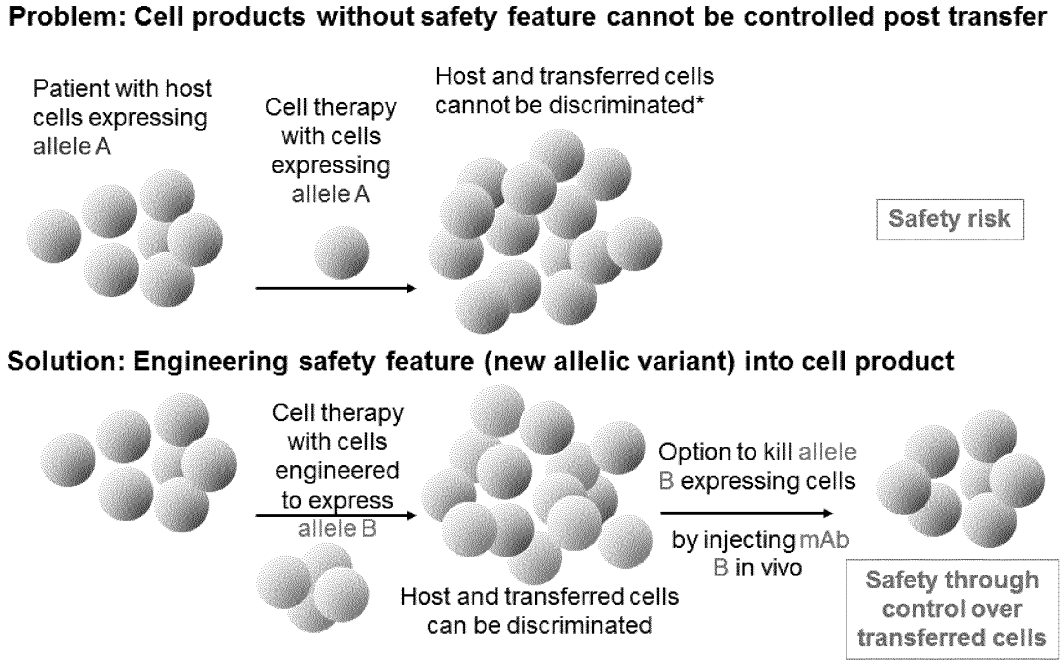

Problem: Cell products without safety feature cannot be controlled post transfer
Patient with host
cells expressing
allele A
Cell therapy
with cells
expressing
allele A
Host and transferred cells
cannot be discriminated*
Safety risk
Solution: Engineering safety feature (new allelic variant) into cell product
Cell therapy
with cells
engineered
to express
allele B
Host and transferred cells
can be discriminated
Option to kill allele
B expressing cells
by injecting mAb
B in vivo
Safety through
control over
transferred cells

Fig. 3

A) Two isoforms of CD90 differ by 1 nucleotide

Genomic difference: 1nt



B) CD90.1 and CD90.2 isoforms can be discriminated by two specific mAb

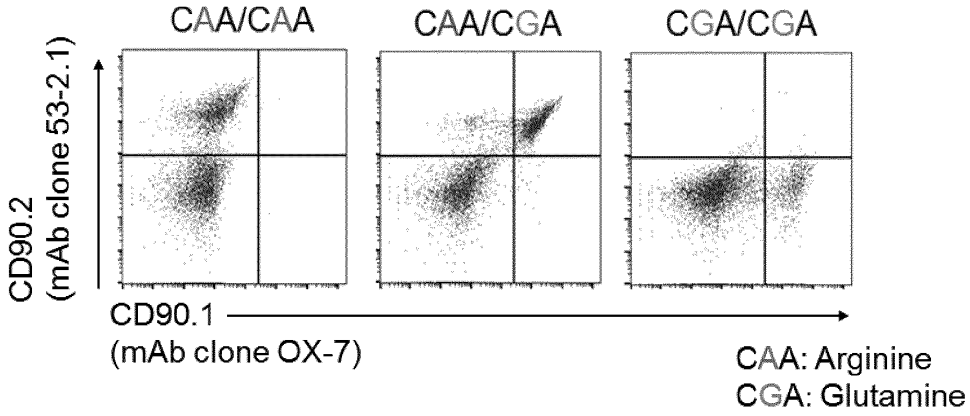


Fig. 4

Allele editing in primary T cells

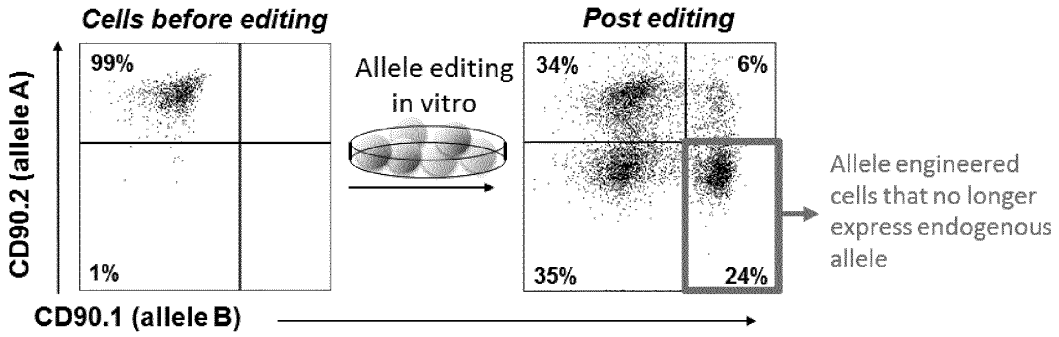


Fig. 5

A) Two isoforms of CD45 differ by 1 nucleotide

CD45.2 AAAAGGCTAATACTTCAATTTGTTTGGAGTGGAAAACAAAAAACCTTGATTTCAGAAAATGCA
CD45.1 AAAAGGCTAATACTTCAATTTGTTTGGAGTGGAAAACAGAAAACCTTGATTTCAGAAAATGCA

B) Conversion of CD45.2 to CD45.1 (allele editing in primary T cells)

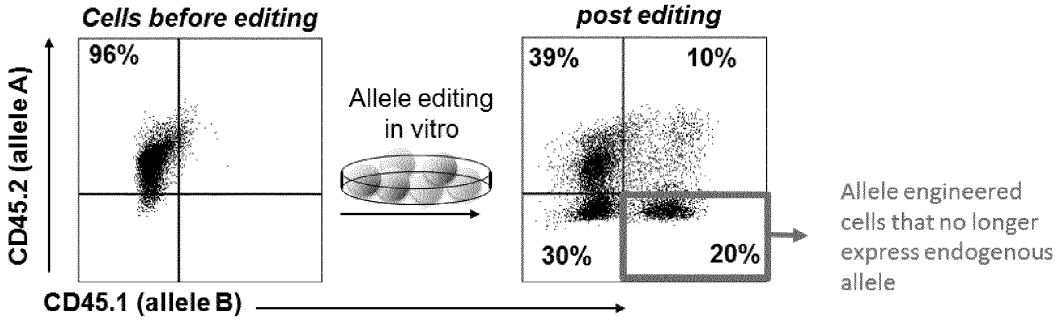


Fig. 6

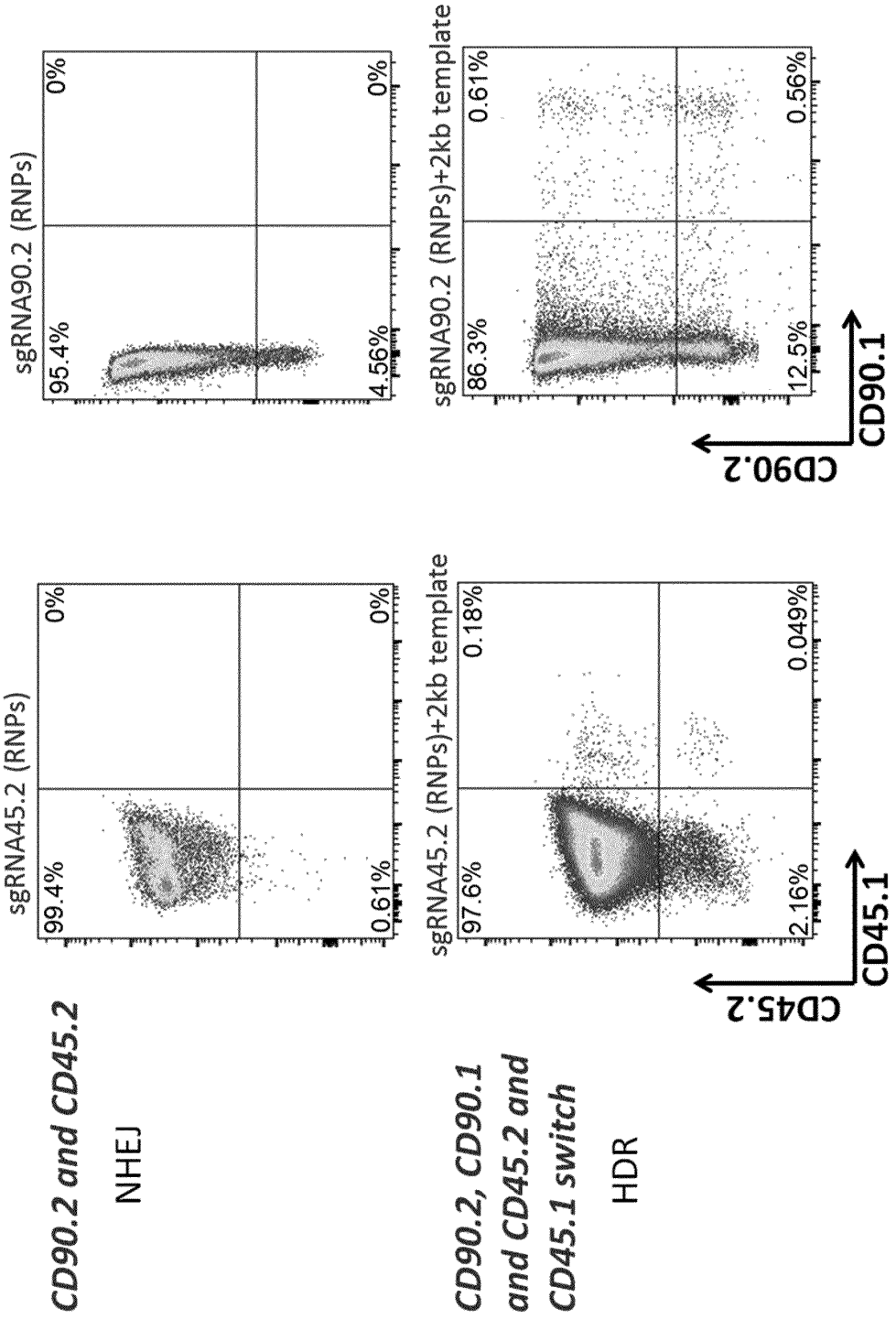
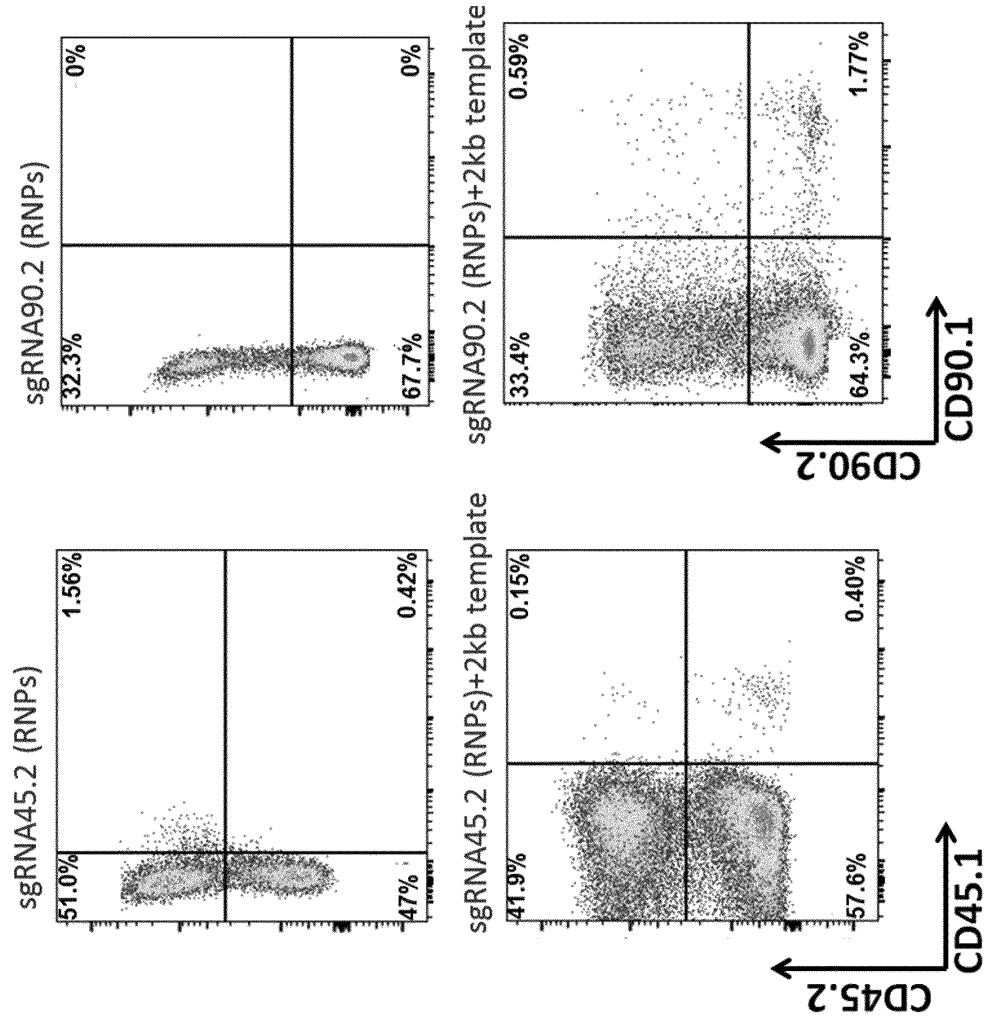

CD90.2 and CD45.2
NHEJ
sgRNA45.2 (RNPs)
99.4%
0%
0.61%
0%
sgRNA45.2 (RNPs)+2kb template
97.6%
0.18%
2.16%
0.049%
CD45.2
CD45.1
CD90.2, CD90.1 and CD45.2 and CD45.1 switch
HDR
sgRNA90.2 (RNPs)
95.4%
0%
4.56%
0%
sgRNA90.2 (RNPs)+2kb template
86.3%
0.61%
12.5%
0.56%
CD90.2
CD90.1

Fig. 7



CD90.2 and CD45.2

NHEJ

CD90.2, CD90.1 and CD45.2 and CD45.1 switch

HDR

Fig. 8

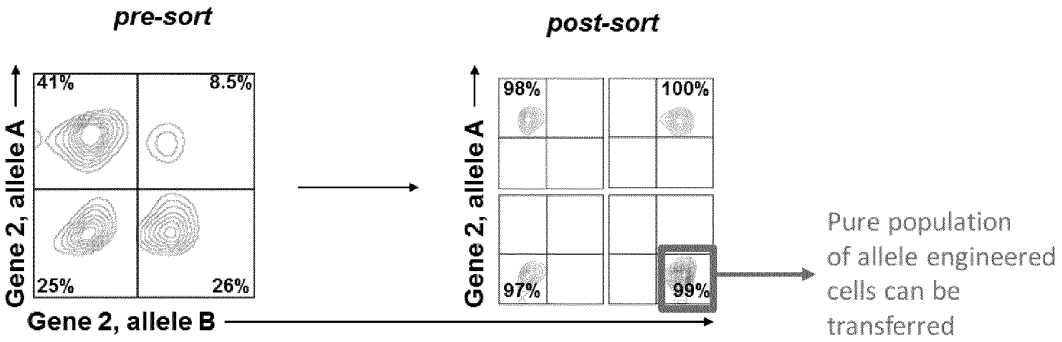

pre-sort
post-sort
41%
8.5%
25%
26%
Gene 2, allele A
Gene 2, allele B
98%
100%
97%
99%
Gene 2, allele A
Pure population of allele engineered cells can be transferred

Fig. 9

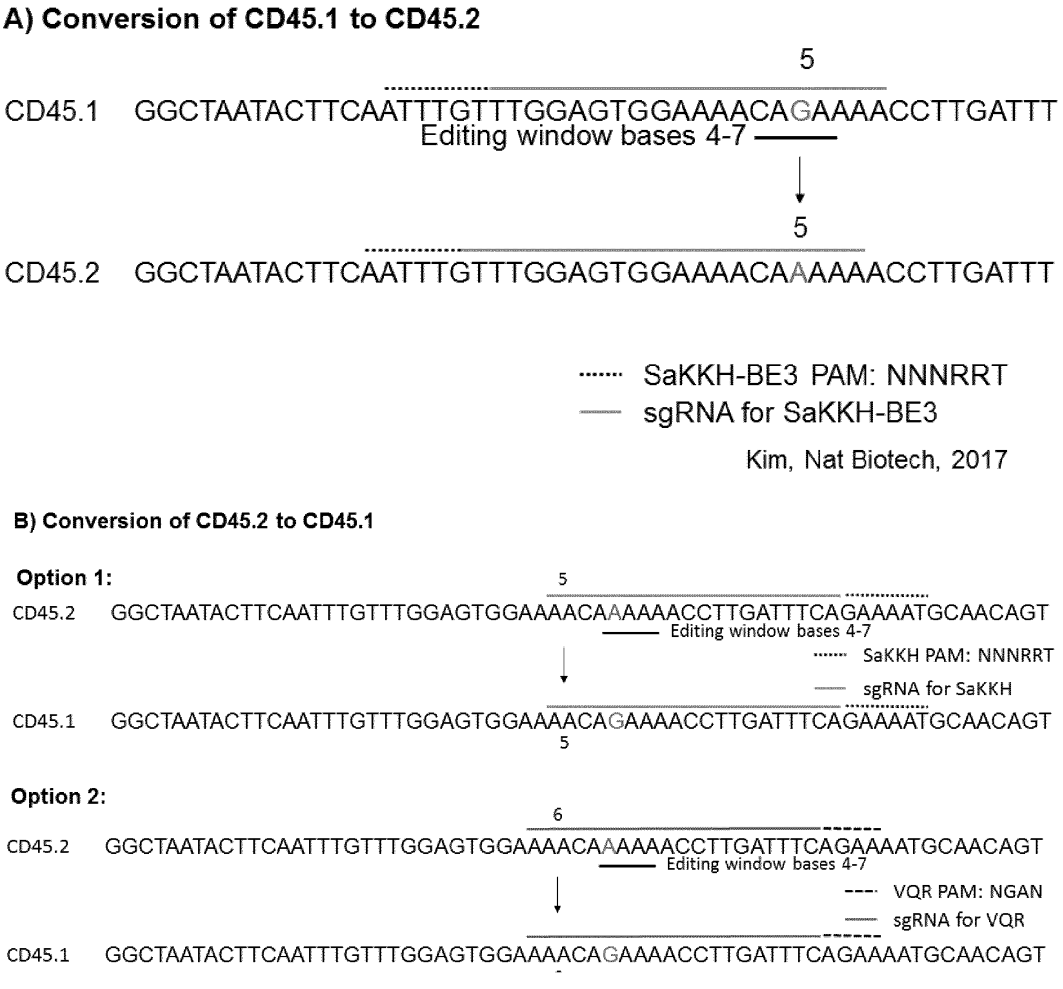

A) Conversion of CD45.1 to CD45.2
5
CD45.1 GGCTAATACTTCAATTTGTTTGGAGTGGAAAACAGAAAACCTTGATTT
Editing window bases 4-7
5
CD45.2 GGCTAATACTTCAATTTGTTTGGAGTGGAAAACAAAAAACCTTGATTT
SaKKH-BE3 PAM: NNNRRT
sgRNA for SaKKH-BE3
Kim, Nat Biotech, 2017
B) Conversion of CD45.2 to CD45.1
Option 1:
5
CD45.2 GGCTAATACTTCAATTTGTTTGGAGTGGAAAACAAAAAACCTTGATTTCAGAAAATGCAACAGT
Editing window bases 4-7
SaKKH PAM: NNNRRT
sgRNA for SaKKH
CD45.1 GGCTAATACTTCAATTTGTTTGGAGTGGAAAACAGAAAACCTTGATTTCAGAAAATGCAACAGT
5
Option 2:
6
CD45.2 GGCTAATACTTCAATTTGTTTGGAGTGGAAAACAAAAAACCTTGATTTCAGAAAATGCAACAGT
Editing window bases 4-7
VQR PAM: NGAN
sgRNA for VQR
CD45.1 GGCTAATACTTCAATTTGTTTGGAGTGGAAAACAGAAAACCTTGATTTCAGAAAATGCAACAGT

Fig. 10

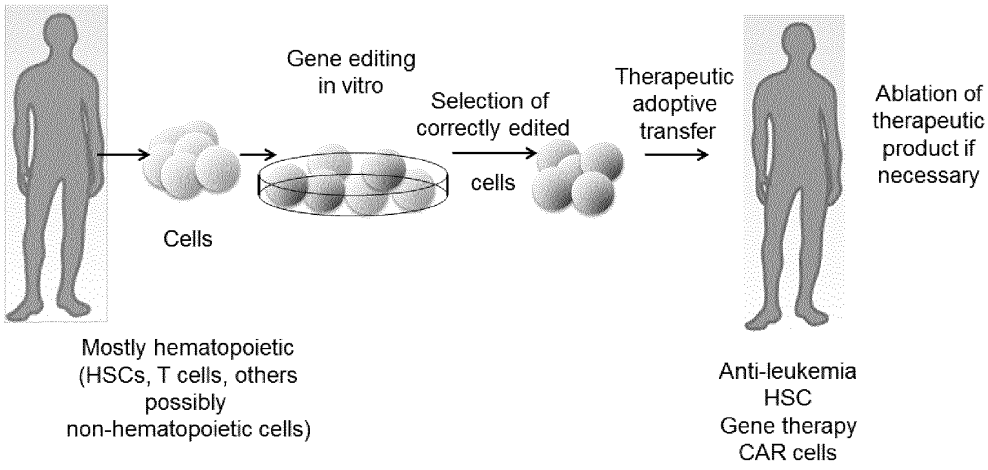

Gene editing
in vitro
Selection of
correctly edited
cells
Therapeutic
adoptive
transfer
Ablation of
therapeutic
product if
necessary
Cells
Mostly hematopoietic
(HSCs, T cells, others
possibly
non-hematopoietic cells)
Anti-leukemia
HSC
Gene therapy
CAR cells

Fig. 11

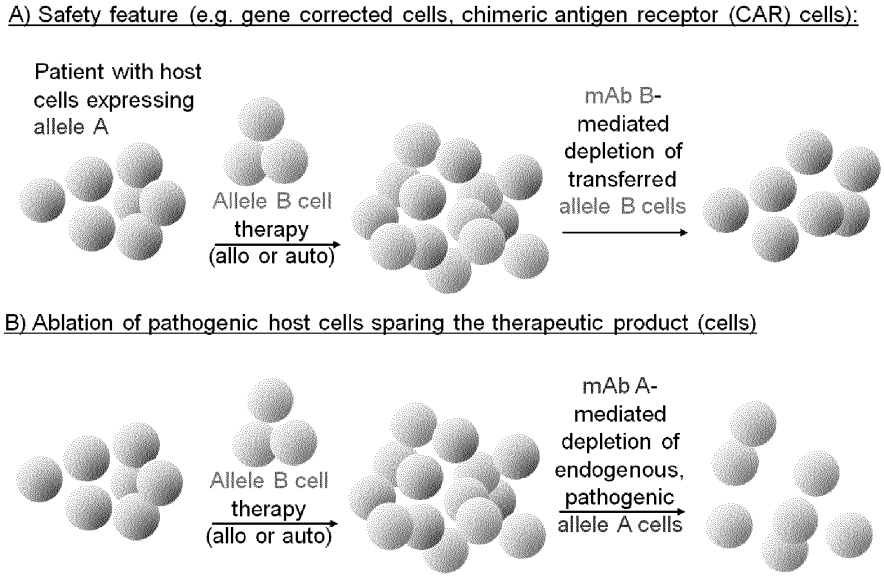

A) Safety feature (e.g. gene corrected cells, chimeric antigen receptor (CAR) cells):
Patient with host
cells expressing
allele A
Allele B cell
therapy
(allo or auto)
mAb B-
mediated
depletion of
transferred
allele B cells
B) Ablation of pathogenic host cells sparing the therapeutic product (cells)
Allele B cell
therapy
(allo or auto)
mAb A-
mediated
depletion of
endogenous,
pathogenic
allele A cells

Fig. 12

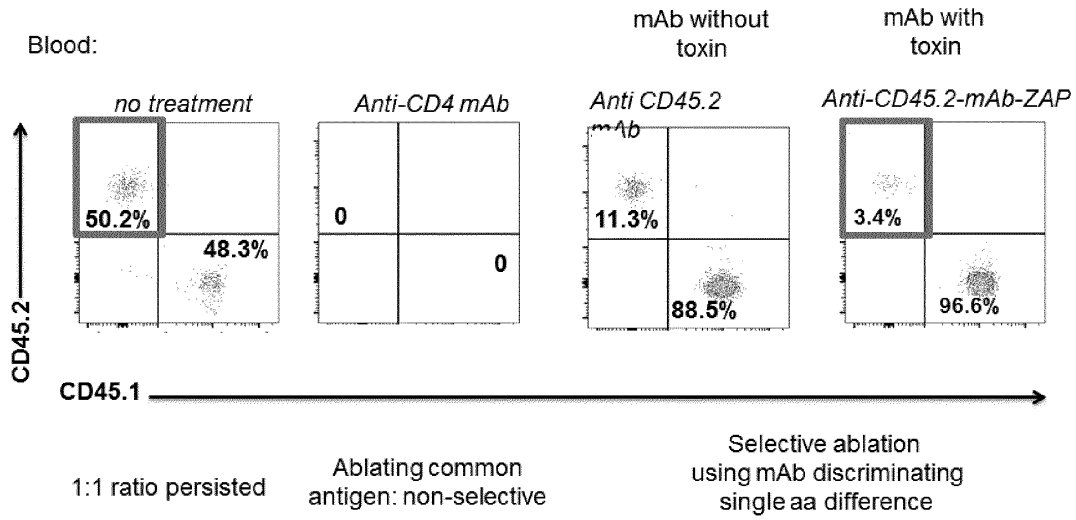

Blood:
mAb without toxin
mAb with toxin
no treatment
Anti-CD4 mAb
Anti CD45.2 mAb
Anti-CD45.2-mAb-ZAP
50.2%
48.3%
0
0
11.3%
88.5%
3.4%
96.6%
CD45.2
CD45.1
1:1 ratio persisted
Ablating common antigen: non-selective
Selective ablation using mAb discriminating single aa difference

Fig. 13

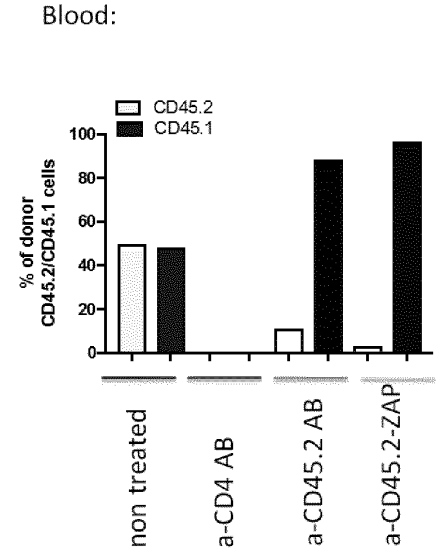

Blood:
CD45.2
CD45.1
% of donor CD45.2/CD45.1 cells
100
80
60
40
20
0
non treated
a-CD4 AB
a-CD45.2 AB
a-CD45.2-ZAP

Fig. 14

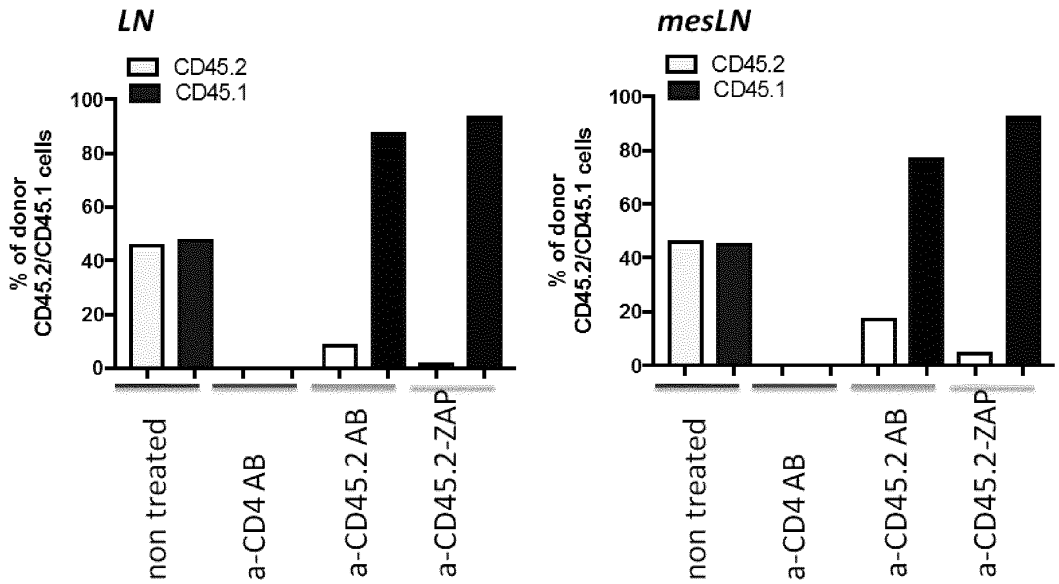

LN
CD45.2
CD45.1
% of donor CD45.2/CD45.1 cells
100
80
60
40
20
0
non treated
a-CD4 AB
a-CD45.2 AB
a-CD45.2-ZAP
mesLN
CD45.2
CD45.1
% of donor CD45.2/CD45.1 cells
non treated
a-CD4 AB
a-CD45.2 AB
a-CD45.2-ZAP

Fig. 15

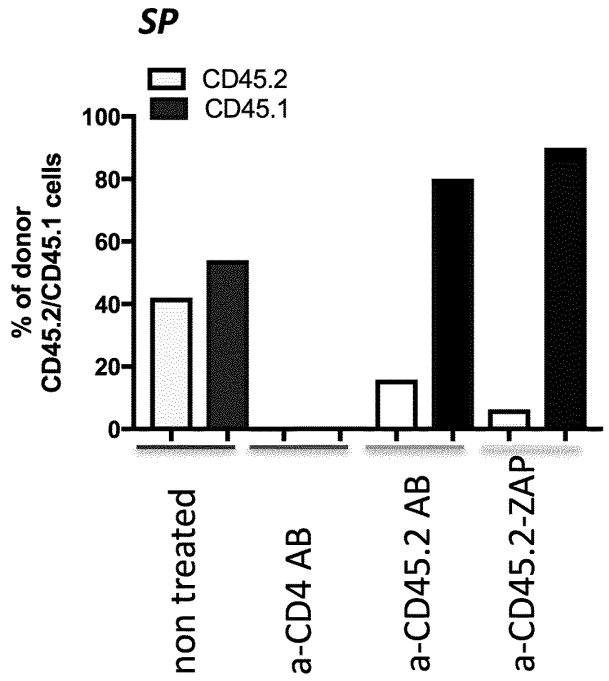

SP
CD45.2
CD45.1
% of donor CD45.2/CD45.1 cells
100
80
60
40
20
0
non treated
a-CD4 AB
a-CD45.2 AB
a-CD45.2-ZAP

Fig. 16

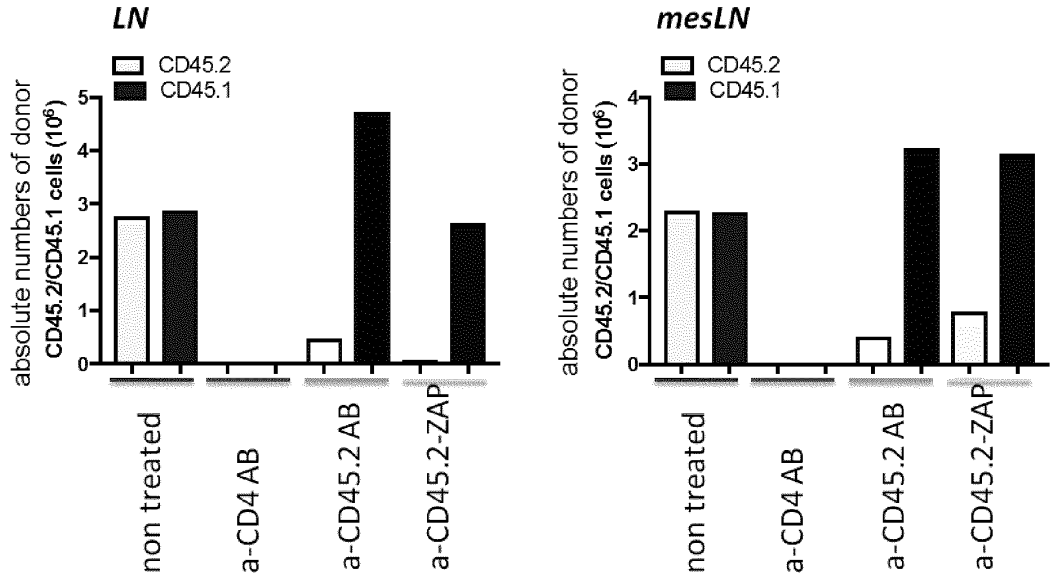

LN
CD45.2
CD45.1
absolute numbers of donor CD45.2/CD45.1 cells (10^6)
0
1
2
3
4
5
non treated
a-CD4 AB
a-CD45.2 AB
a-CD45.2-ZAP
mesLN
CD45.2
CD45.1
absolute numbers of donor CD45.2/CD45.1 cells (10^6)
0
1
2
3
4
non treated
a-CD4 AB
a-CD45.2 AB
a-CD45.2-ZAP

Fig. 17

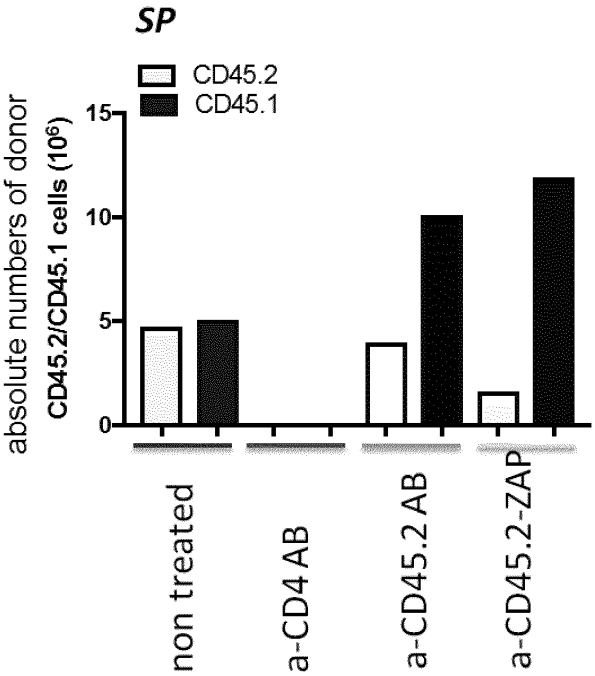

SP
CD45.2
CD45.1
absolute numbers of donor CD45.2/CD45.1 cells (10^6)
0
5
10
15
non treated
a-CD4 AB
a-CD45.2 AB
a-CD45.2-ZAP

Fig. 18

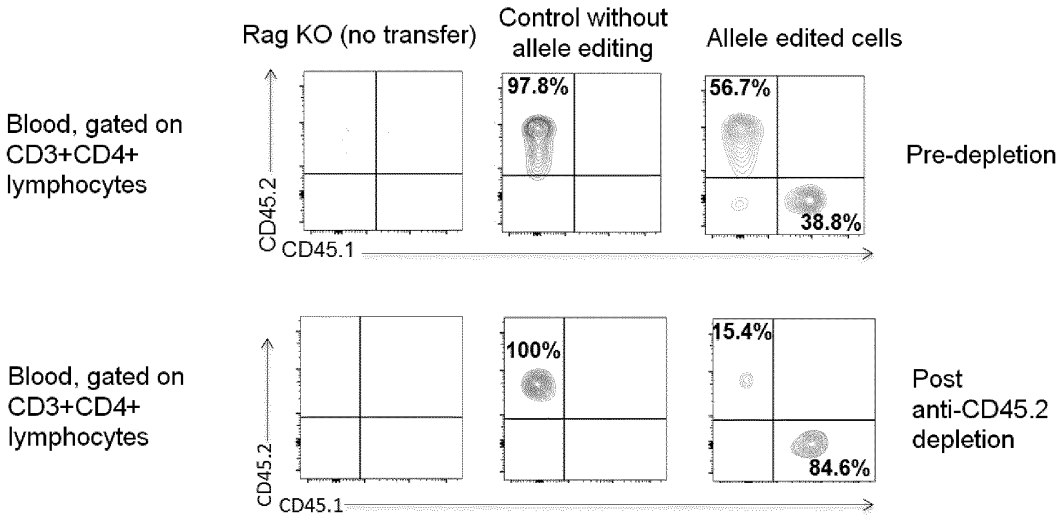

Rag KO (no transfer)
Control without allele editing
Allele edited cells
Blood, gated on CD3+CD4+ lymphocytes
CD45.2
CD45.1
97.8%
56.7%
38.8%
Pre-depletion
Blood, gated on CD3+CD4+ lymphocytes
100%
15.4%
84.6%
Post anti-CD45.2 depletion

Fig. 19

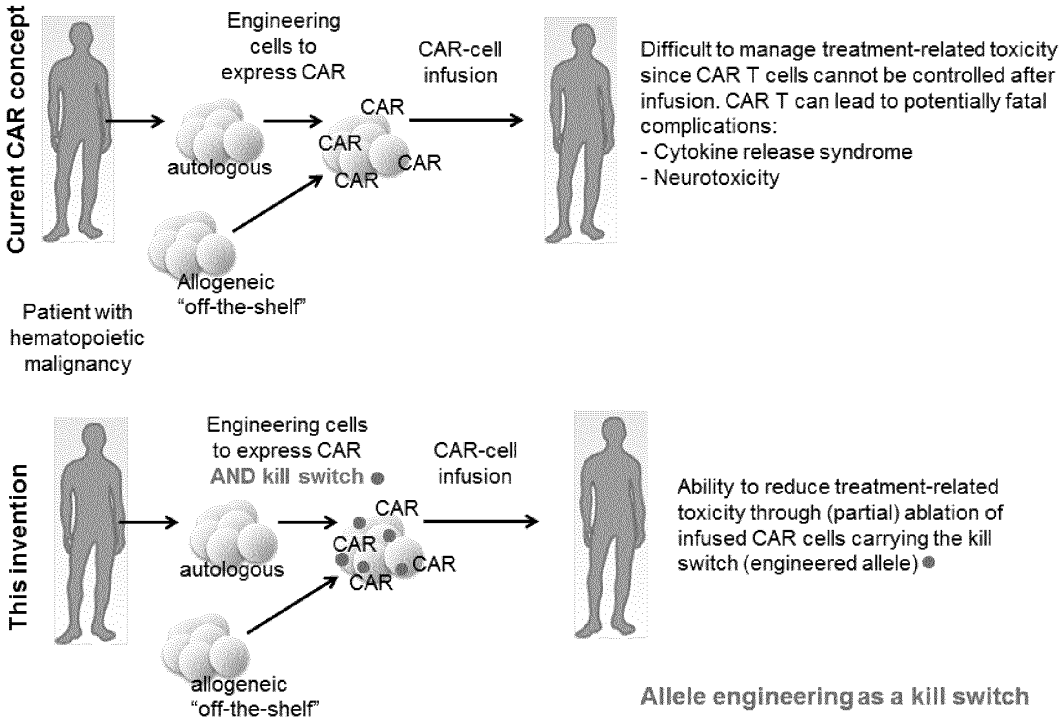

Current CAR concept
Engineering cells to express CAR
CAR-cell infusion
autologous
CAR
Allogeneic "off-the-shelf"
Patient with hematopoietic malignancy
Difficult to manage treatment-related toxicity since CAR T cells cannot be controlled after infusion. CAR T can lead to potentially fatal complications:
- Cytokine release syndrome
- Neurotoxicity
This invention
Engineering cells to express CAR
AND kill switch
CAR-cell infusion
autologous
allogeneic "off-the-shelf"
Ability to reduce treatment-related toxicity through (partial) ablation of infused CAR cells carrying the kill switch (engineered allele)
Allele engineering as a kill switch

Fig. 20

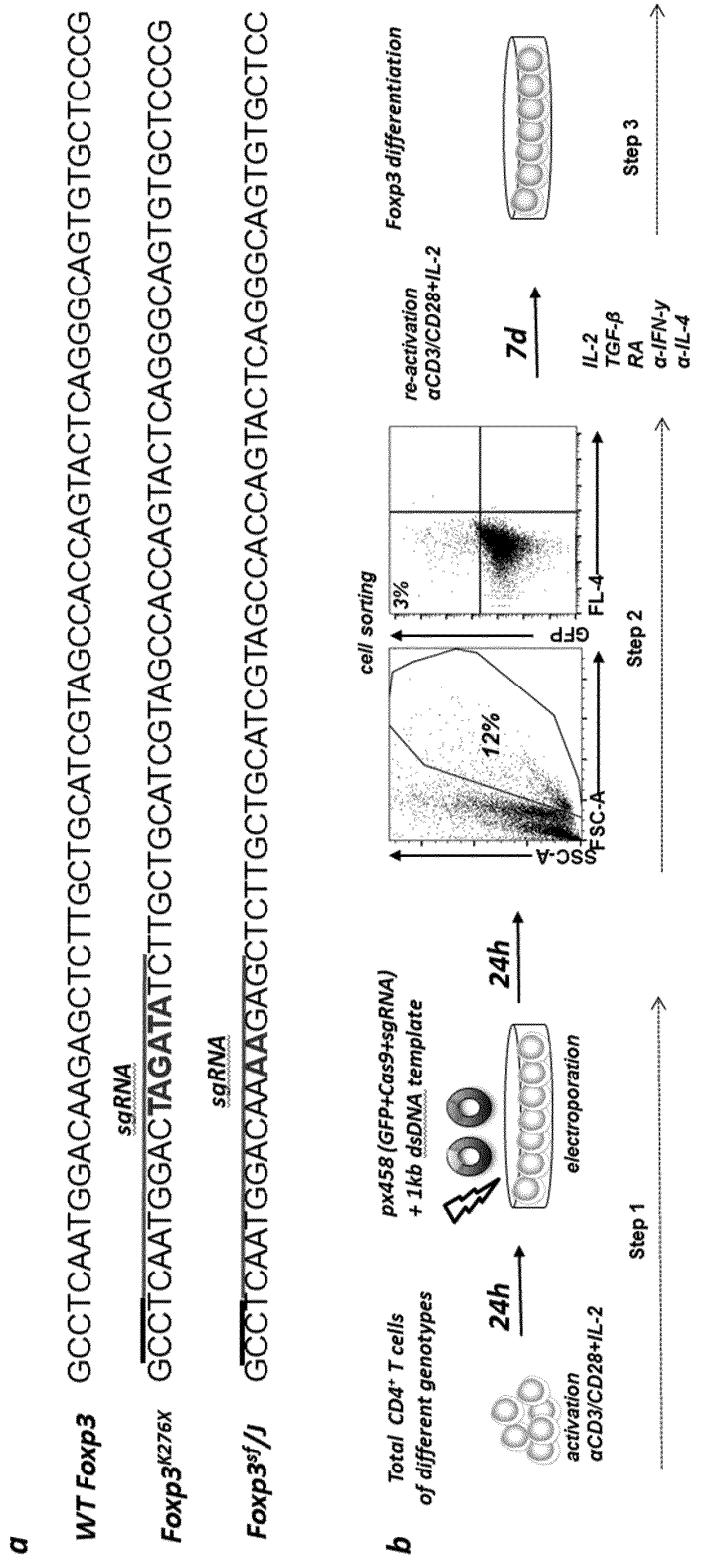

a
WT Foxp3
GCCTCAATGGACAAGAGCTCTTGCTGCATCGTAGCCACCAGTACTCAGGGCAGTGTGCTCCCG
sgRNA
Foxp3K276X
GCCTCAATGGACTAGATATCTTGCTGCATCGTAGCCACCAGTACTCAGGGCAGTGTGCTCCCG
sgRNA
Foxp3sf/J
GCCTCAATGGACAAAAGAGCTCTTGCTGCATCGTAGCCACCAGTACTCAGGGCAGTGTGCTCC
b
Total CD4+ T cells
of different genotypes
activation
αCD3/CD28+IL-2
24h
px458 (GFP+Cas9+sgRNA)
+ 1kb dsDNA template
electroporation
24h
Step 1
cell sorting
SSC-A
FSC-A
12%
GFP
FL-4
3%
Step 2
re-activation
αCD3/CD28+IL-2
7d
IL-2
TGF-β
RA
α-IFN-γ
α-IL-4
Foxp3 differentiation
Step 3

Fig. 20 (continued)

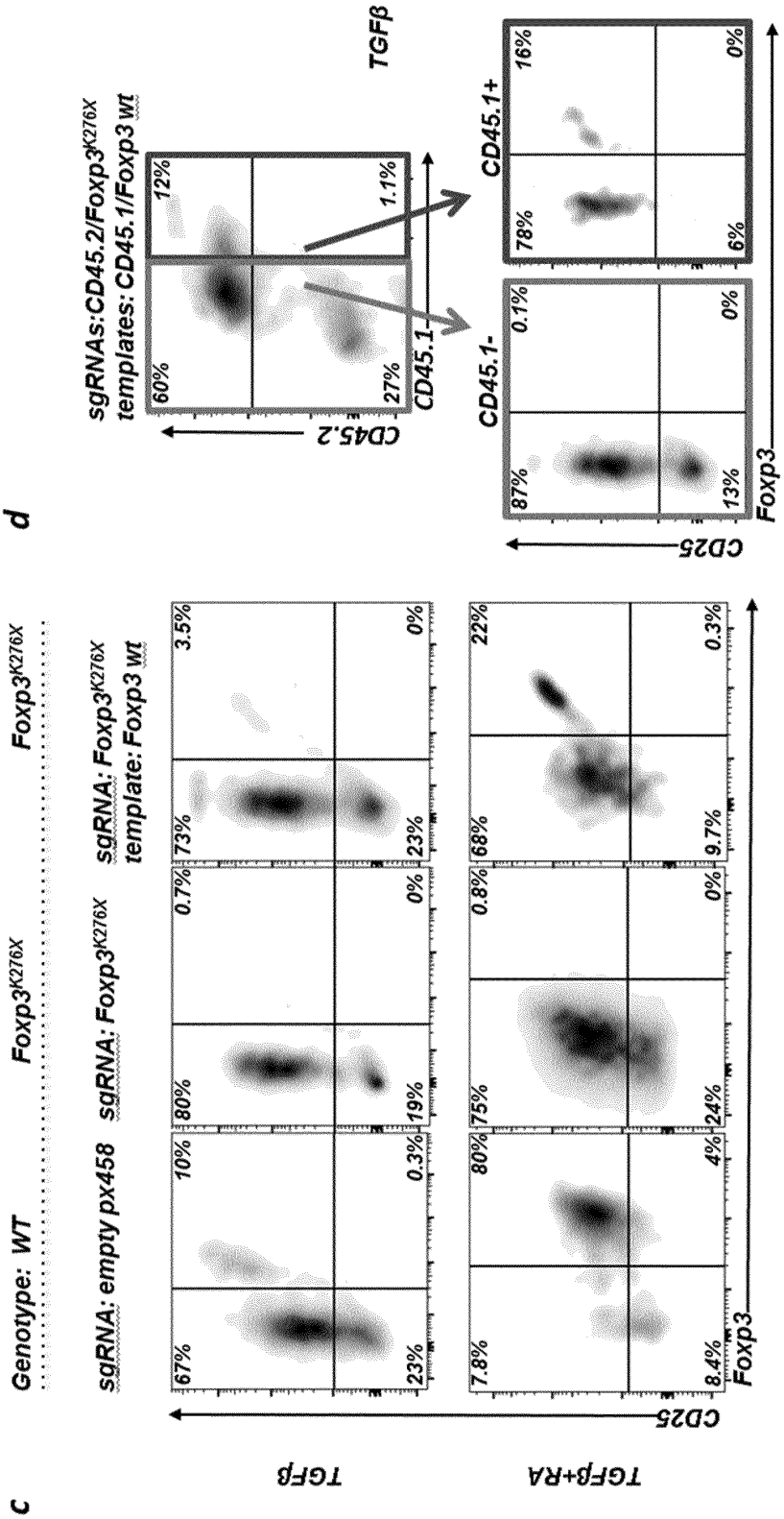


Fig. 21

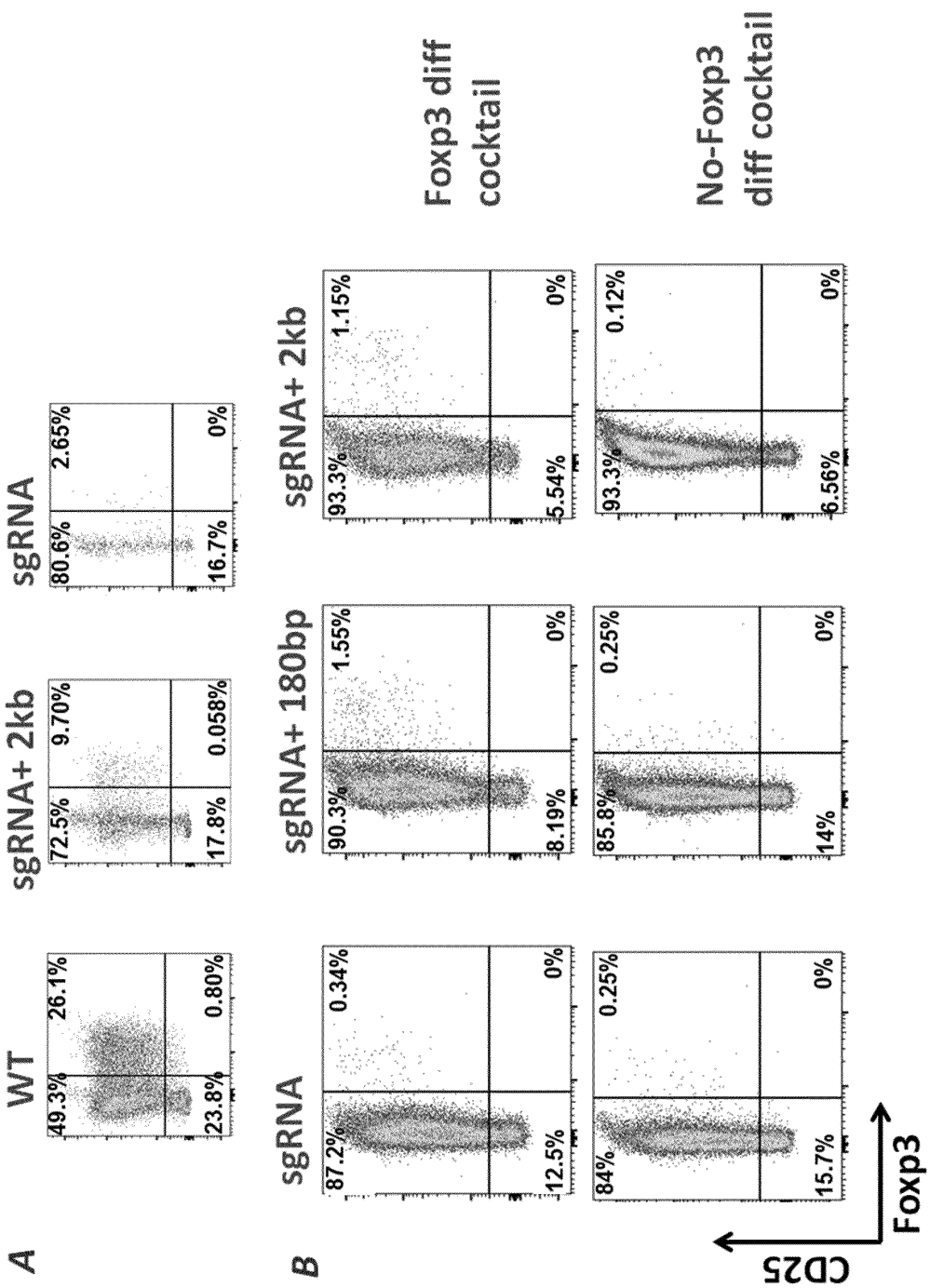

A
WT
sgRNA
sgRNA+ 2kb
sgRNA+ 180bp
Foxp3 diff cocktail
No-Foxp3 diff cocktail
B
49.3%
26.1%
23.8%
0.80%
80.6%
2.65%
16.7%
0%
72.5%
9.70%
17.8%
0.058%
87.2%
0.34%
12.5%
0%
90.3%
1.55%
8.19%
0%
85.8%
0.25%
14%
0%
84%
0.25%
15.7%
0%
93.3%
1.15%
5.54%
0%
93.3%
0.12%
6.56%
0%
CD25
Foxp3

Fig. 22

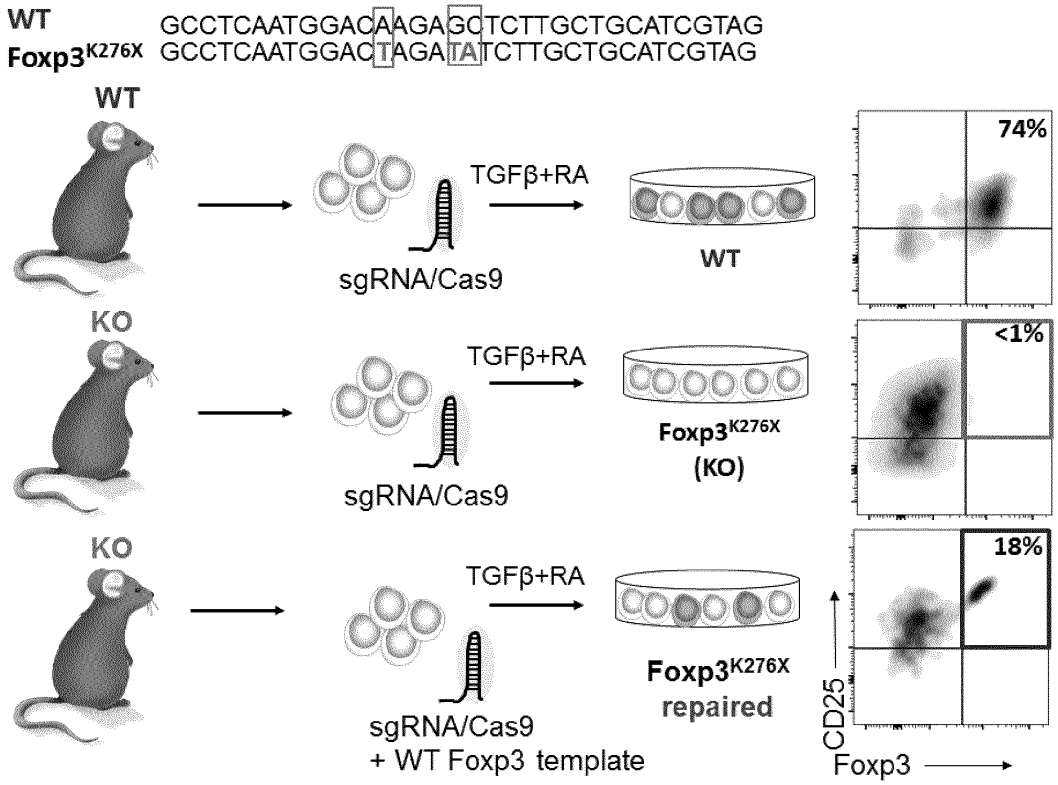

WT GCCTCAATGGACAAGAGCTCTTGCTGCATCGTAG
Foxp3K276X GCCTCAATGGACTAGATATCTTGCTGCATCGTAG
WT
KO
KO
TGFβ+RA
sgRNA/Cas9
WT
74%
Foxp3K276X
(KO)
<1%
Foxp3K276X
repaired
18%
sgRNA/Cas9
+ WT Foxp3 template
CD25
Foxp3

Fig. 23

CD4+ T cells
(KO or WT)
activation
αCD3/αCD28+IL-2
24h
WT: px458
KO: px458
Repair:px458 + 2kb dsDNA template
electroporation
24h
cell sorting
12%
SSC-A
FSC-A
3%
GFP
FL-4
Immunodeficient host
✓ IL-2 therapy
1ST PHASE :
3 SUBSEQUENT INJECTIONS
MAINTENANCE PHASE :
injections 2 X per week
S: day 7 post AT
✓ Weight measurements every 2 week

Fig. 24

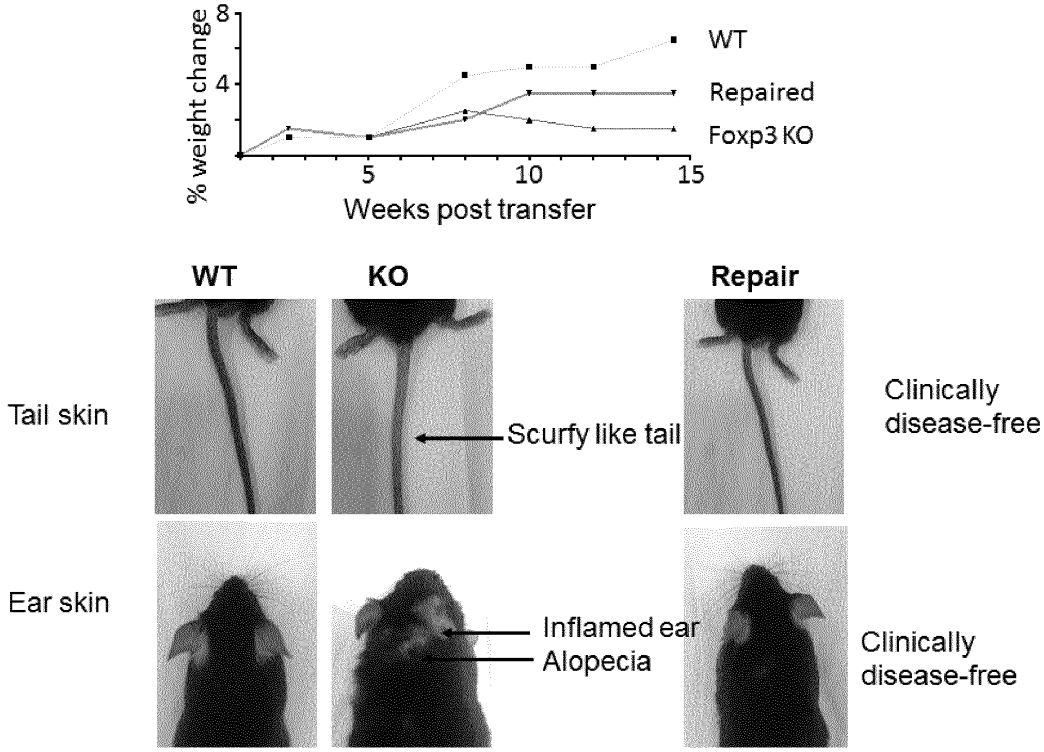

% weight change
8
4
5
10
15
Weeks post transfer
WT
Repaired
Foxp3 KO
WT
KO
Repair
Tail skin
Scurfy like tail
Clinically disease-free
Ear skin
Inflamed ear
Alopecia
Clinically disease-free

Fig. 25

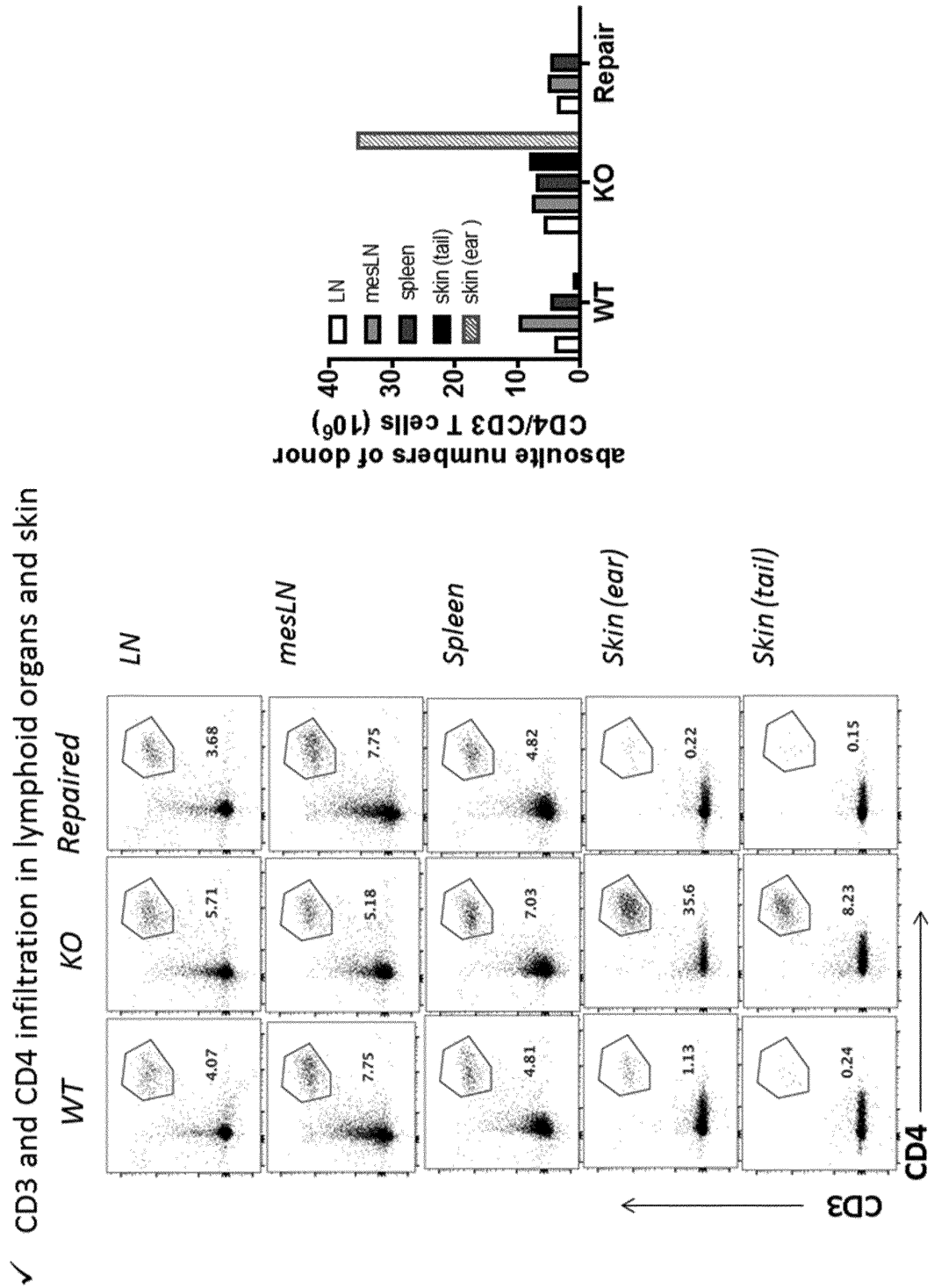

✓ CD3 and CD4 infiltration in lymphoid organs and skin
WT
KO
Repaired
LN
mesLN
Spleen
Skin (ear)
Skin (tail)
4.07
5.71
3.68
7.75
5.18
7.75
4.81
7.03
4.82
1.13
35.6
0.22
0.24
8.23
0.15
CD3
CD4
absolute numbers of donor CD4/CD3 T cells (10⁶)
LN
mesLN
spleen
skin (tail)
skin (ear)
WT
KO
Repair

Fig. 26

✓ Presence of CD25/Foxp3^{+} T_{reg} cells

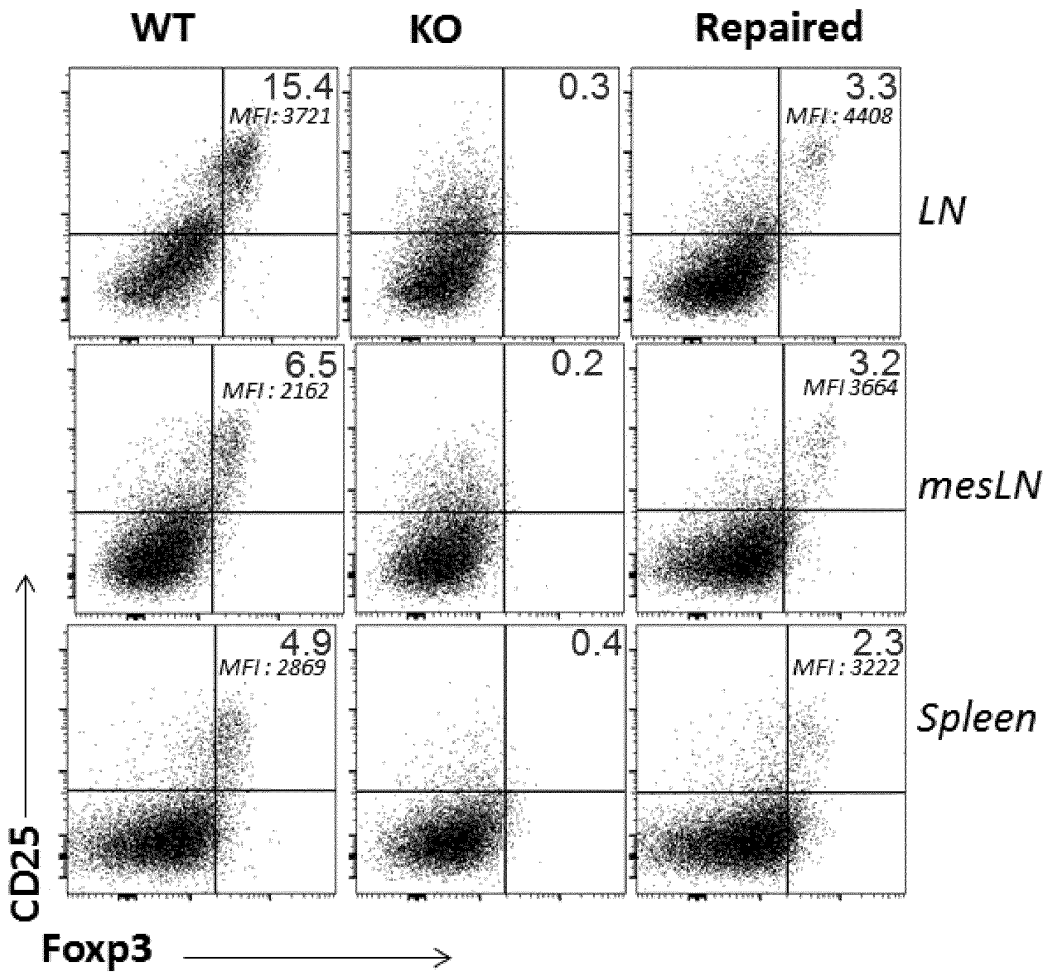


Fig. 27

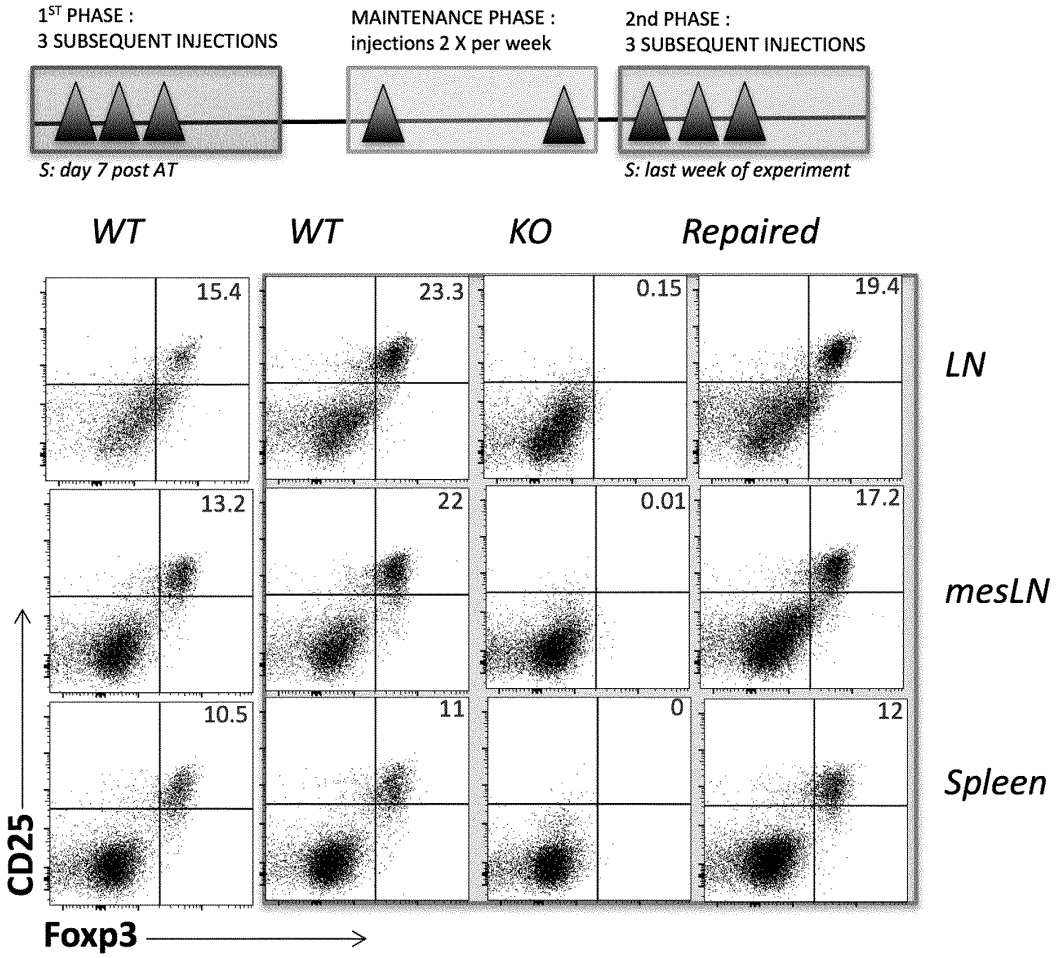


Fig. 28

✓ Repaired T_{reg} cells express similar levels of Treg cells associated surface molecules

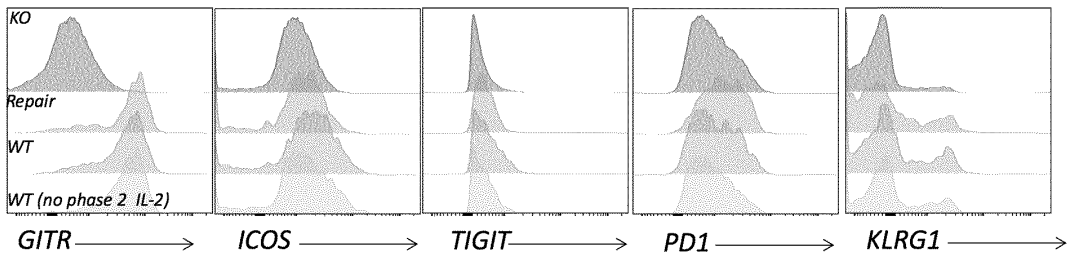


Fig. 29

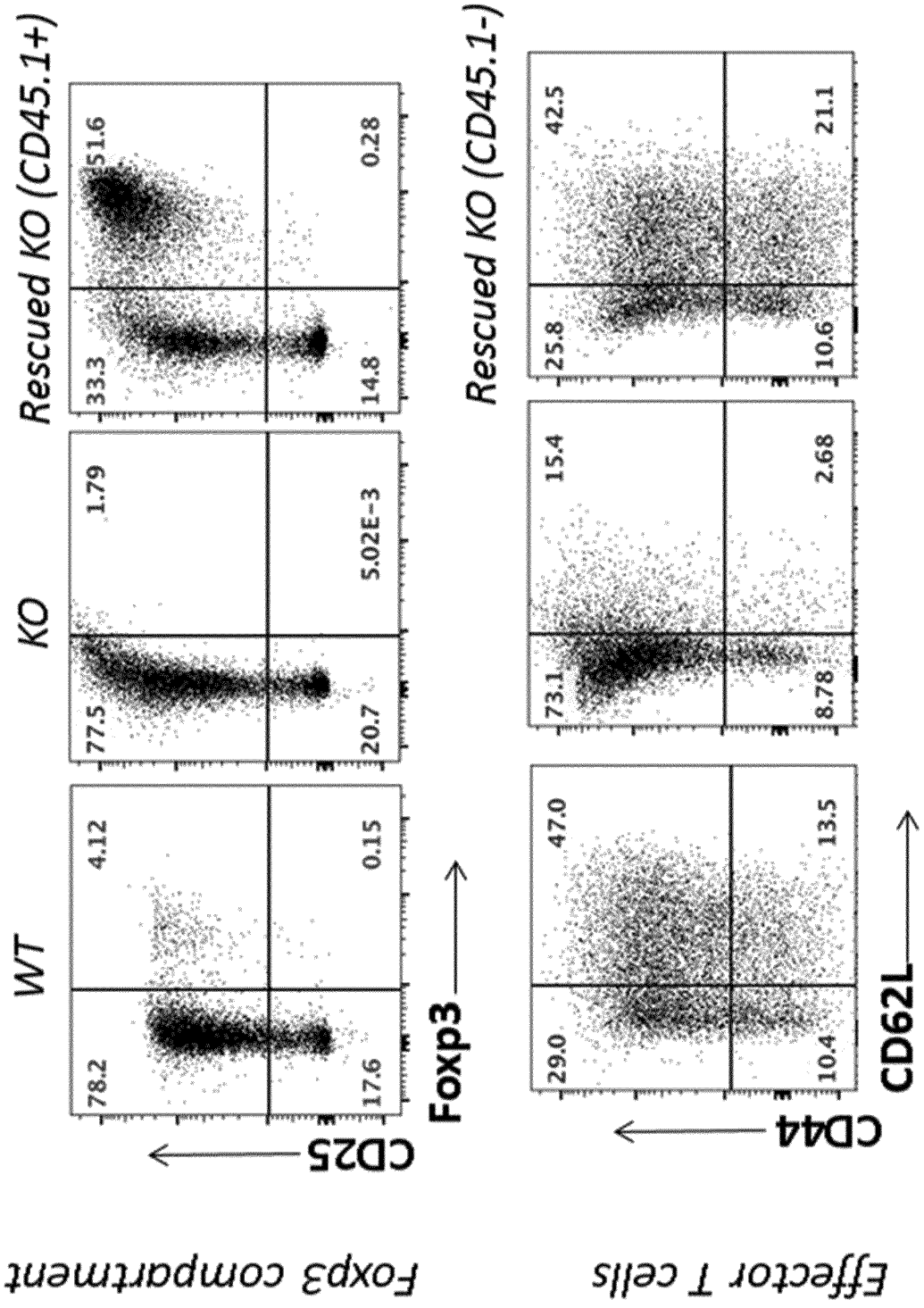

WT
KO
Rescued KO (CD45.1+)
Rescued KO (CD45.1-)
78.2
4.12
0.15
17.6
77.5
1.79
5.02E-3
20.7
33.3
51.6
14.8
0.28
29.0
47.0
13.5
10.4
73.1
15.4
2.68
8.78
25.8
42.5
10.6
21.1
CD25
Foxp3
CD44
CD62L
Foxp3 compartment
Effector T cells

Fig. 30

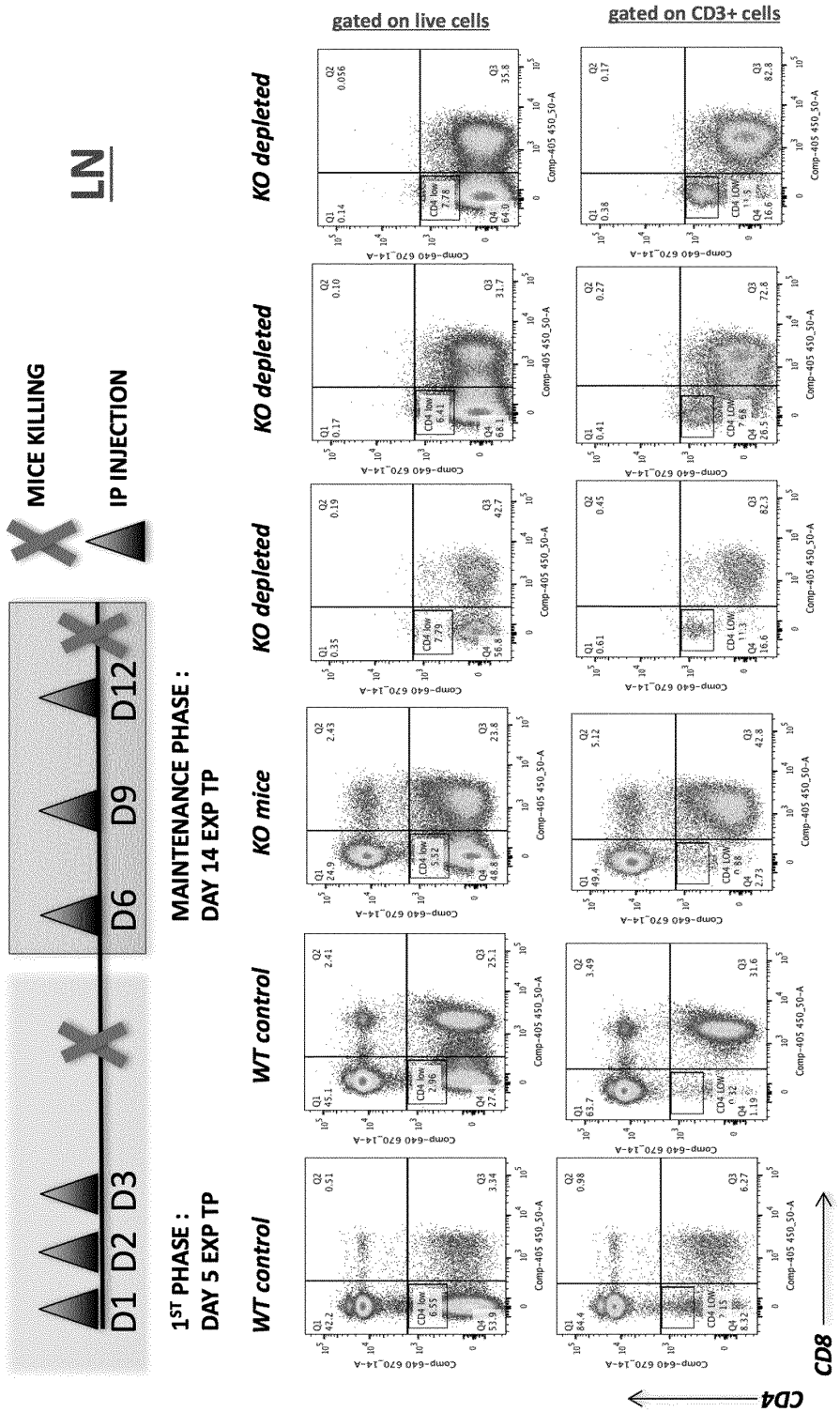

LN
MICE KILLING
IP INJECTION
D1 D2 D3
D6 D9 D12
1ST PHASE :
DAY 5 EXP TP
MAINTENANCE PHASE :
DAY 14 EXP TP
WT control
KO mice
KO depleted
KO depleted
gated on live cells
gated on CD3+ cells
CD4
CD8

Fig. 31

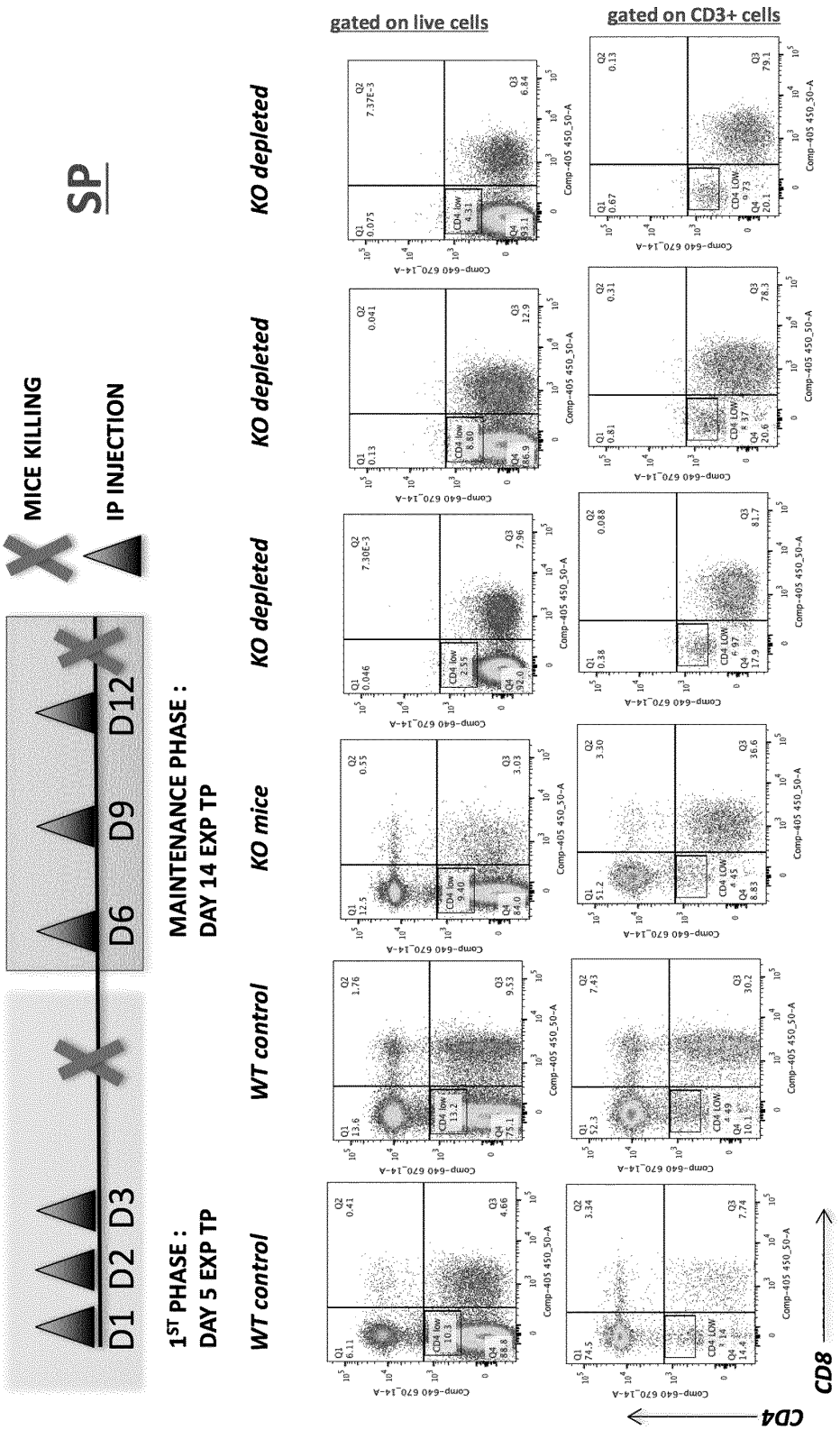

SP
MICE KILLING
IP INJECTION
D1 D2 D3
D6 D9 D12
1ST PHASE :
DAY 5 EXP TP
MAINTENANCE PHASE :
DAY 14 EXP TP
WT control
KO mice
KO depleted
KO depleted
gated on live cells
gated on CD3+ cells
CD4
CD8

Fig. 32

