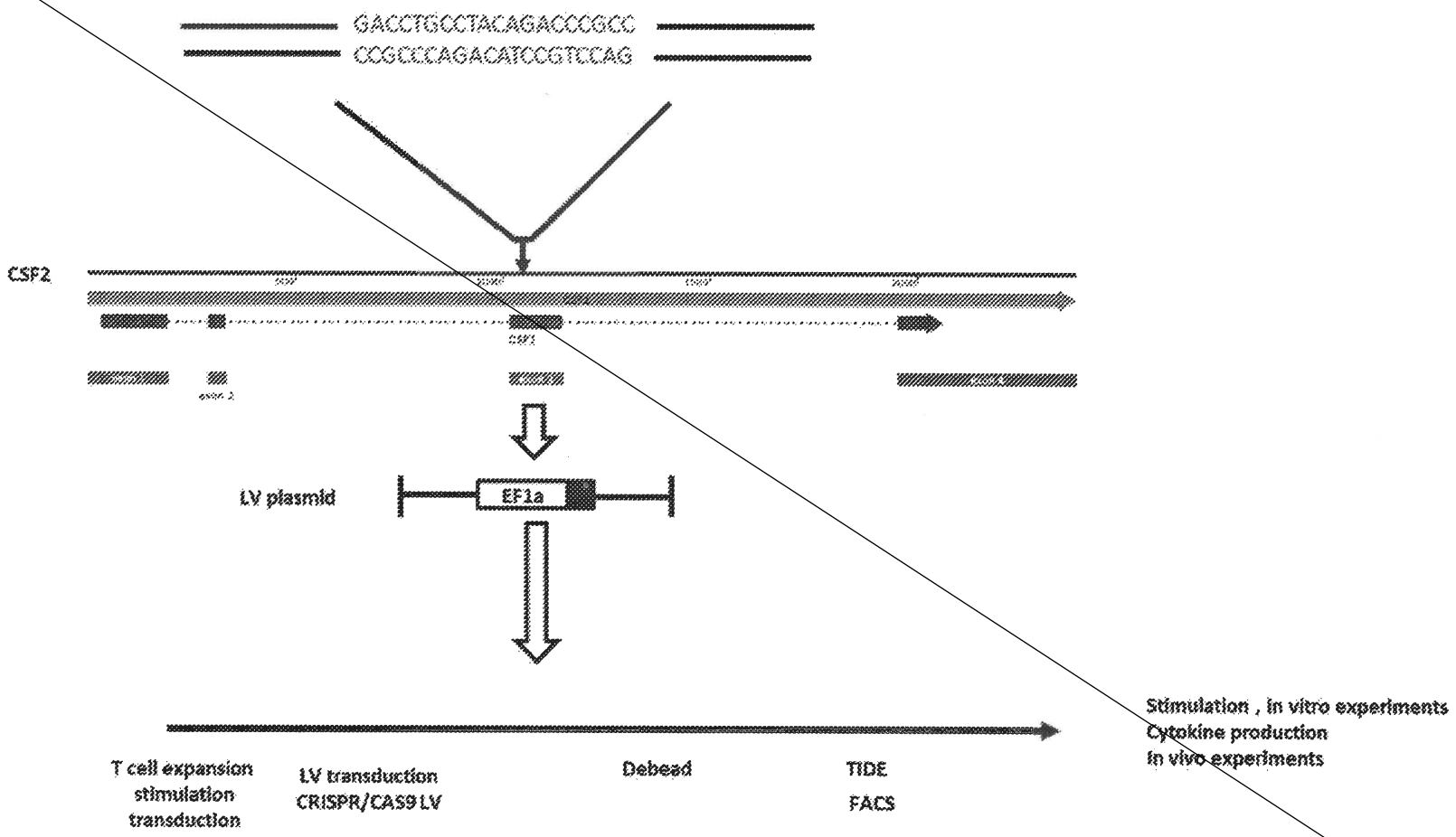
GACCTGCCTACAGACCCGCC
CCGCCCAGACATCCGTCCAG
CSF2
LV plasmid
EF1a
T cell expansion
stimulation
transduction
LV transduction
CRISPR/CAS9 LV
Debead
TIDE
FACS
Stimulation , in vitro experiments
Cytokine production
In vivo experiments

FIG. 1

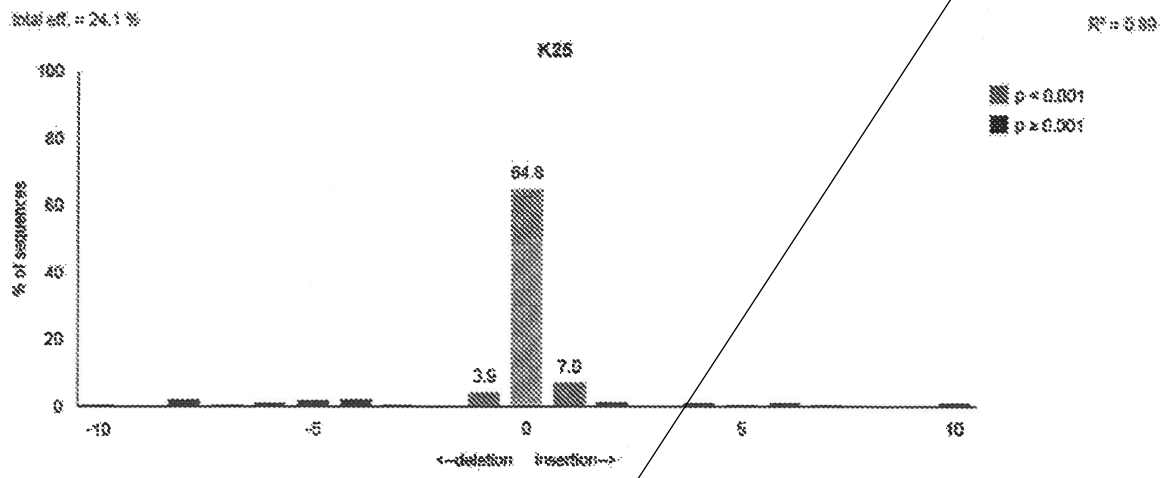
K25
% of sequences
64.8
3.9
7.8
-10
-5
0
5
10
<--deletion
insertion-->

FIG. 2A

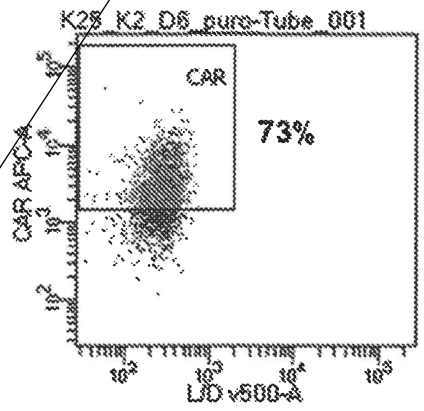
K25_K2_D6_puro-Tube_001
CAR
73%
CAR APC-A
LD v500-A

FIG. 2B

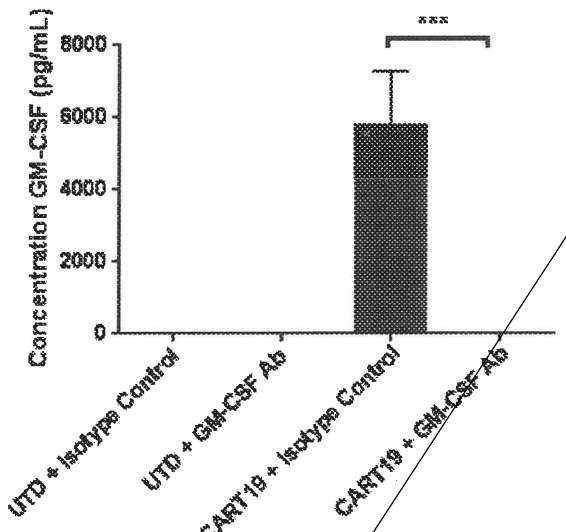
Concentration GM-CSF (pg/mL)
8000
6000
4000
2000
0
***
UTD + Isotype Control
UTD + GM-CSF Ab
CART19 + Isotype Control
CART19 + GM-CSF Ab

FIG. 7B

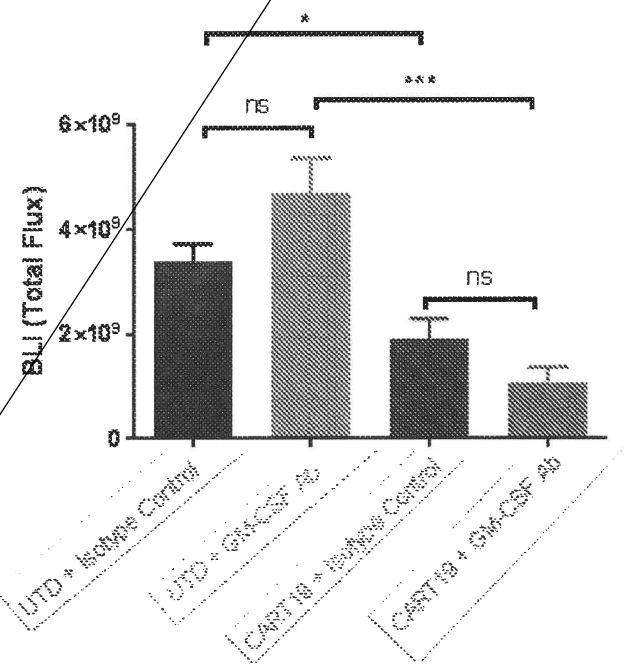
BLI (Total Flux)
6×10^9
4×10^9
2×10^9
0
*
ns
***
ns
UTD + Isotype Control
UTD + GM-CSF Ab
CART19 + Isotype Control
CART19 + GM-CSF Ab

FIG. 7C

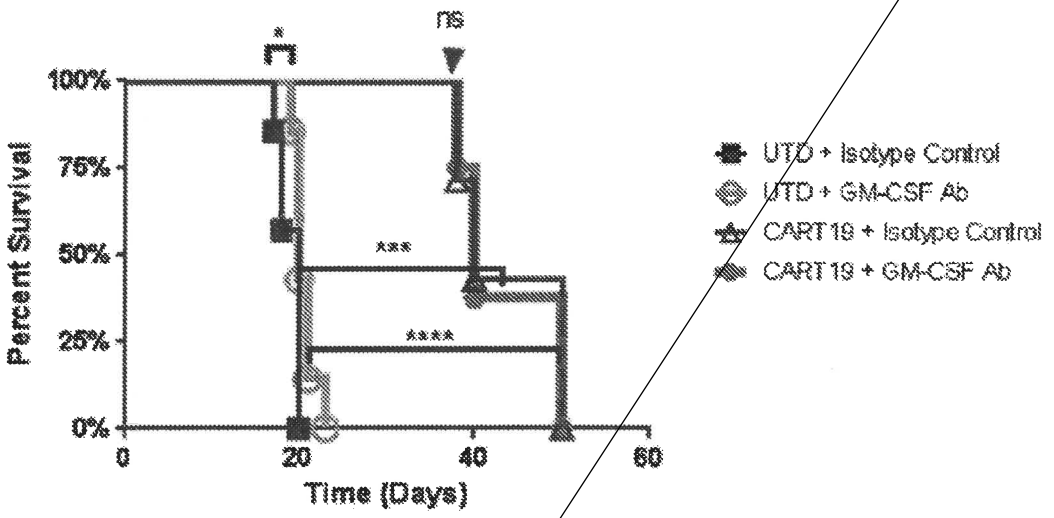
*
ns
100%
75%
50%
25%
0%
Percent Survival
***
****
0
20
40
60
Time (Days)
UTD + Isotype Control
UTD + GM-CSF Ab
CART19 + Isotype Control
CART19 + GM-CSF Ab

FIG. 8

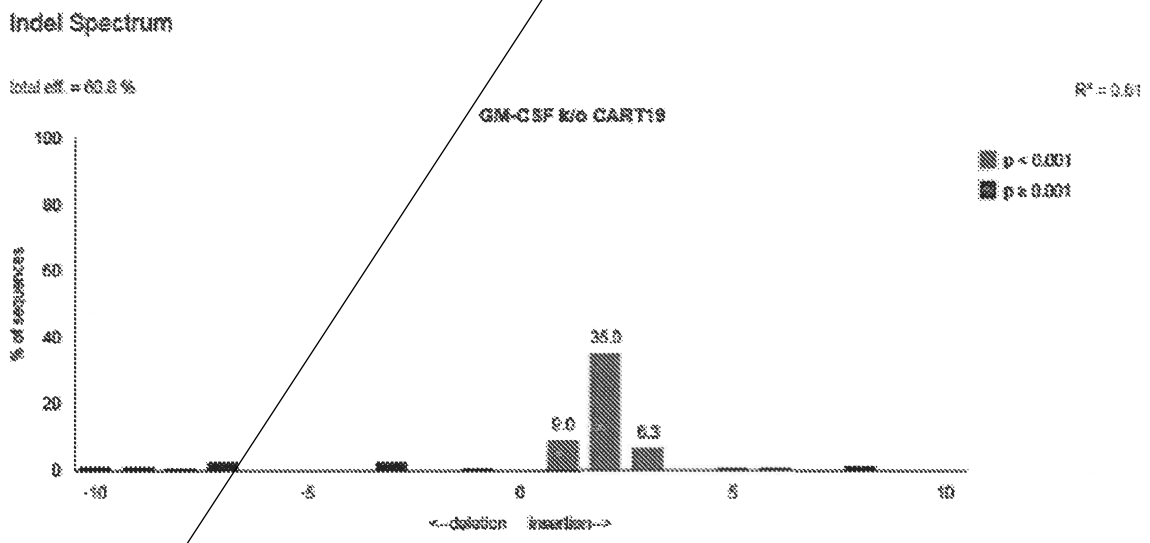
Indel Spectrum
GM-CSF k/o CART19
% of sequences
9.0
35.0
6.3
-10
-5
0
5
10
<--deletion
insertion-->
p < 0.001
p ≥ 0.001

FIG. 9

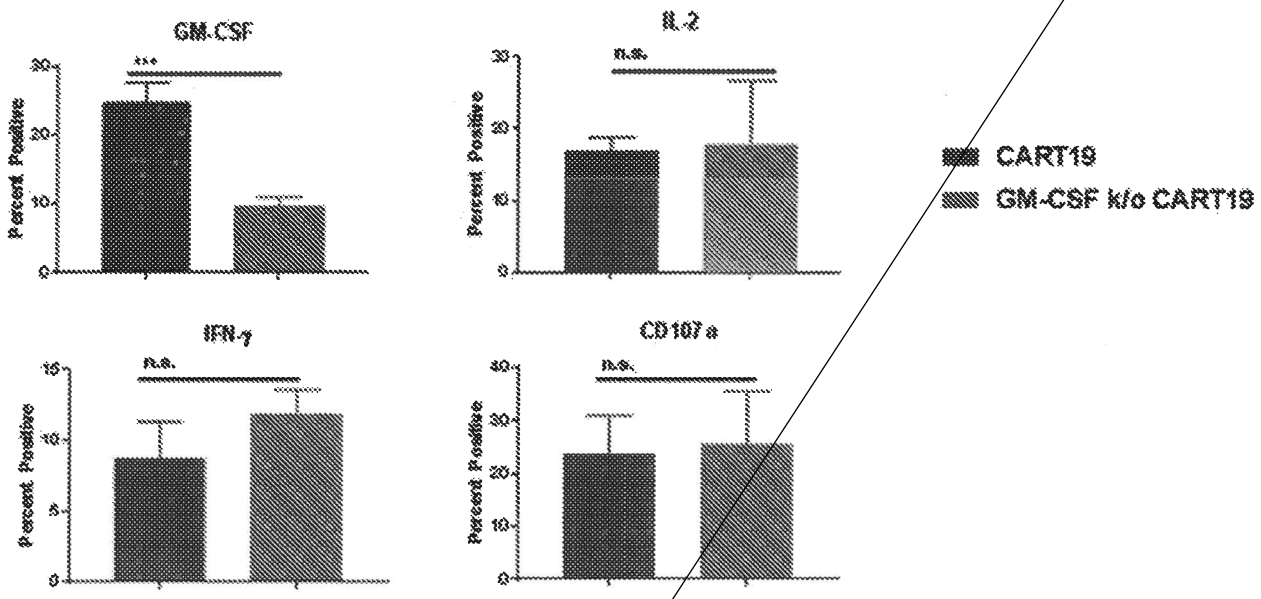

FIG. 10A

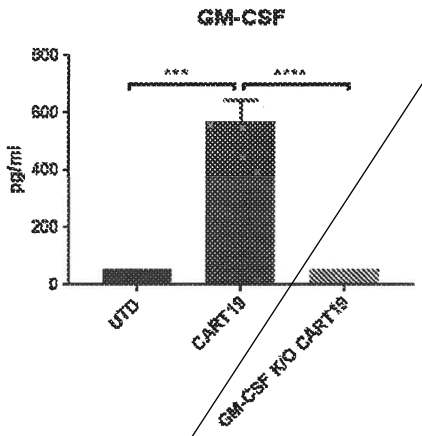

FIG. 10B

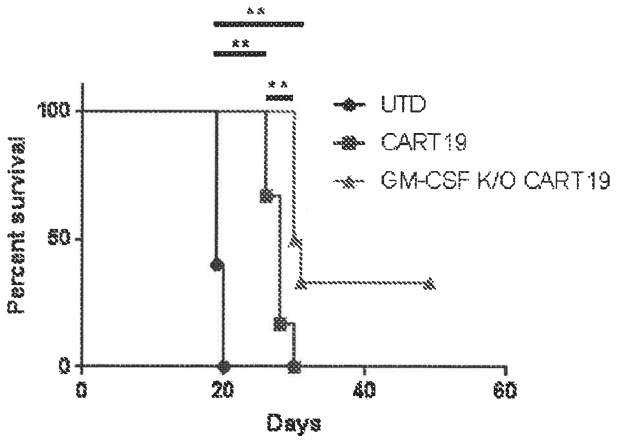

FIG. 10C

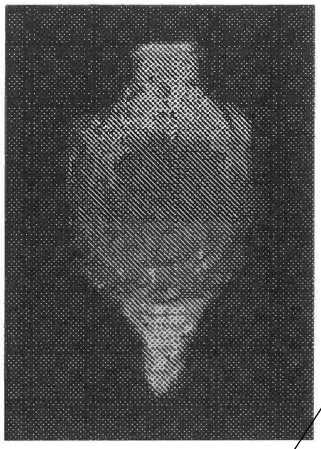

FIG. 12C

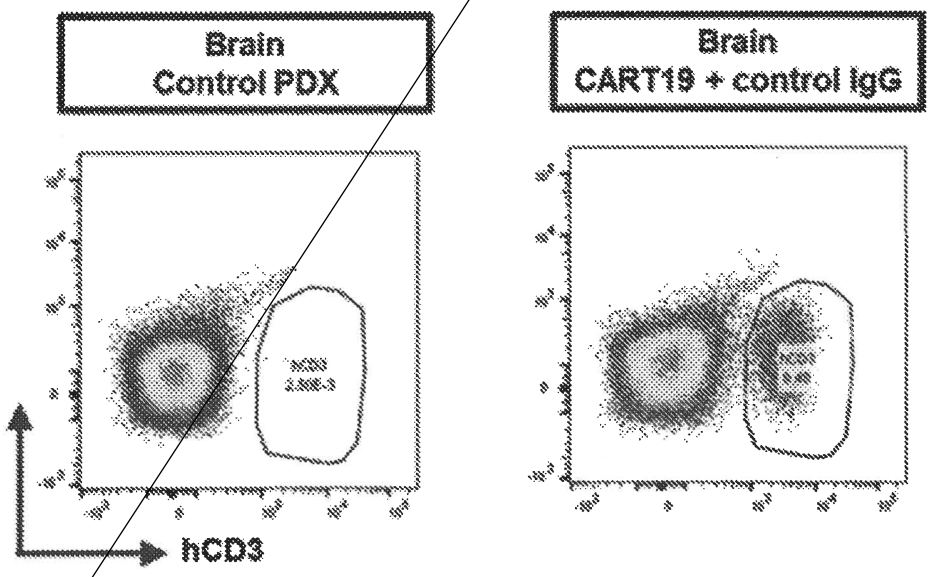

FIG. 12D

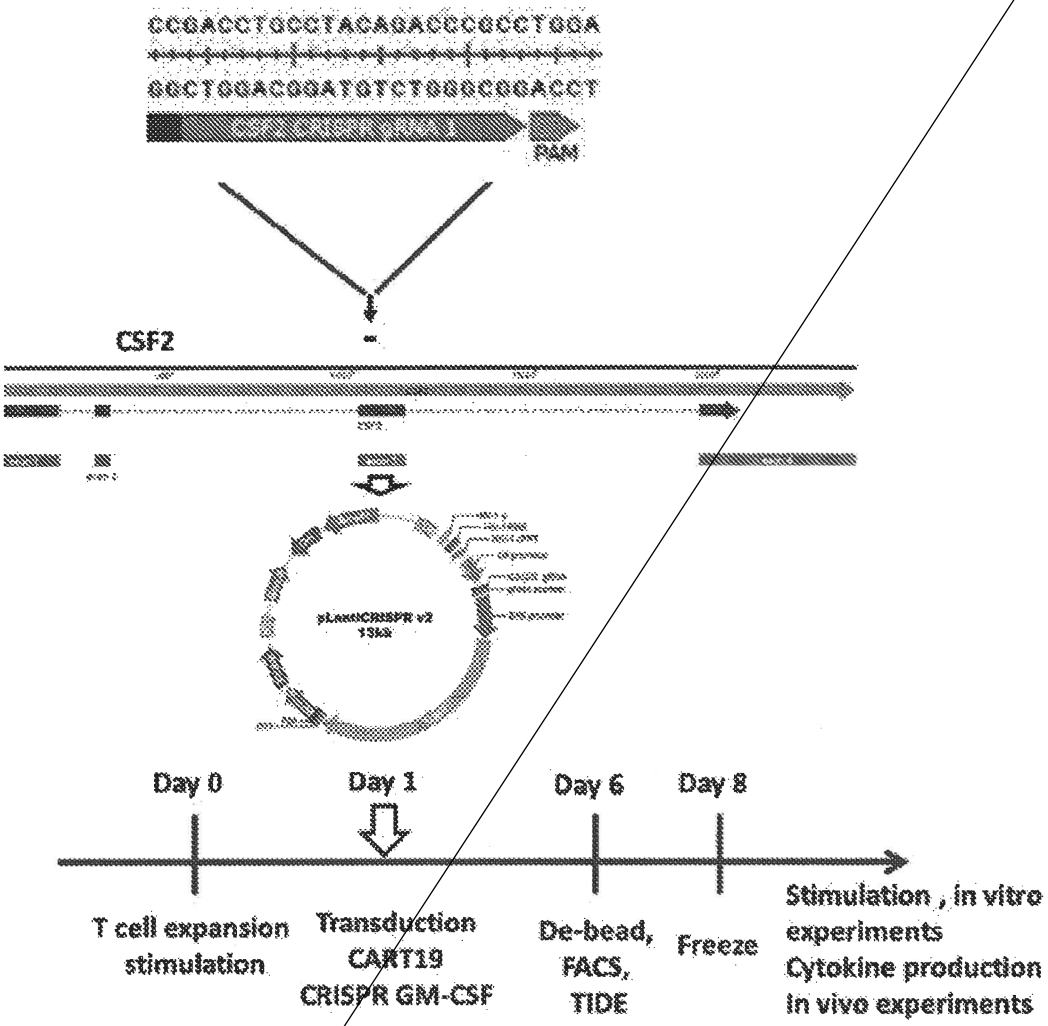

FIG. 15A

gRNA/Primer	Sequence
CSF2 Primer For	TGACTACAGAGAGGCACAGA
CSF2 Primer Rev	TCACCTCTGACCTCATTAACC
CSF2 CRISPR gRNA 1	GACCTGCCTACAGACCCGCC

FIG. 15B

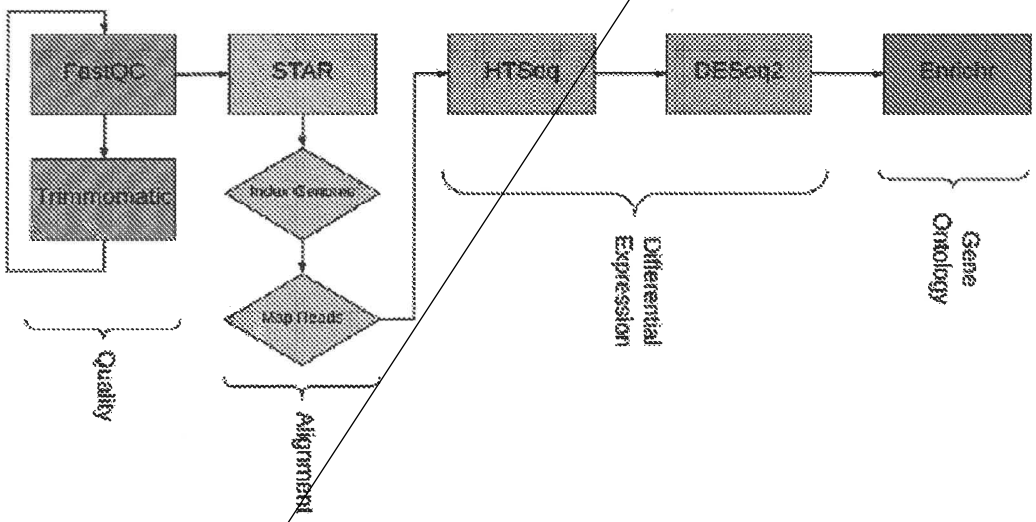
FastQC
STAR
HTSeq
DESeq2
Enrichr
Trimmomatic
Index Genome
Map Reads
Quality
Alignment
Differential Expression
Gene Ontology

FIG. 16

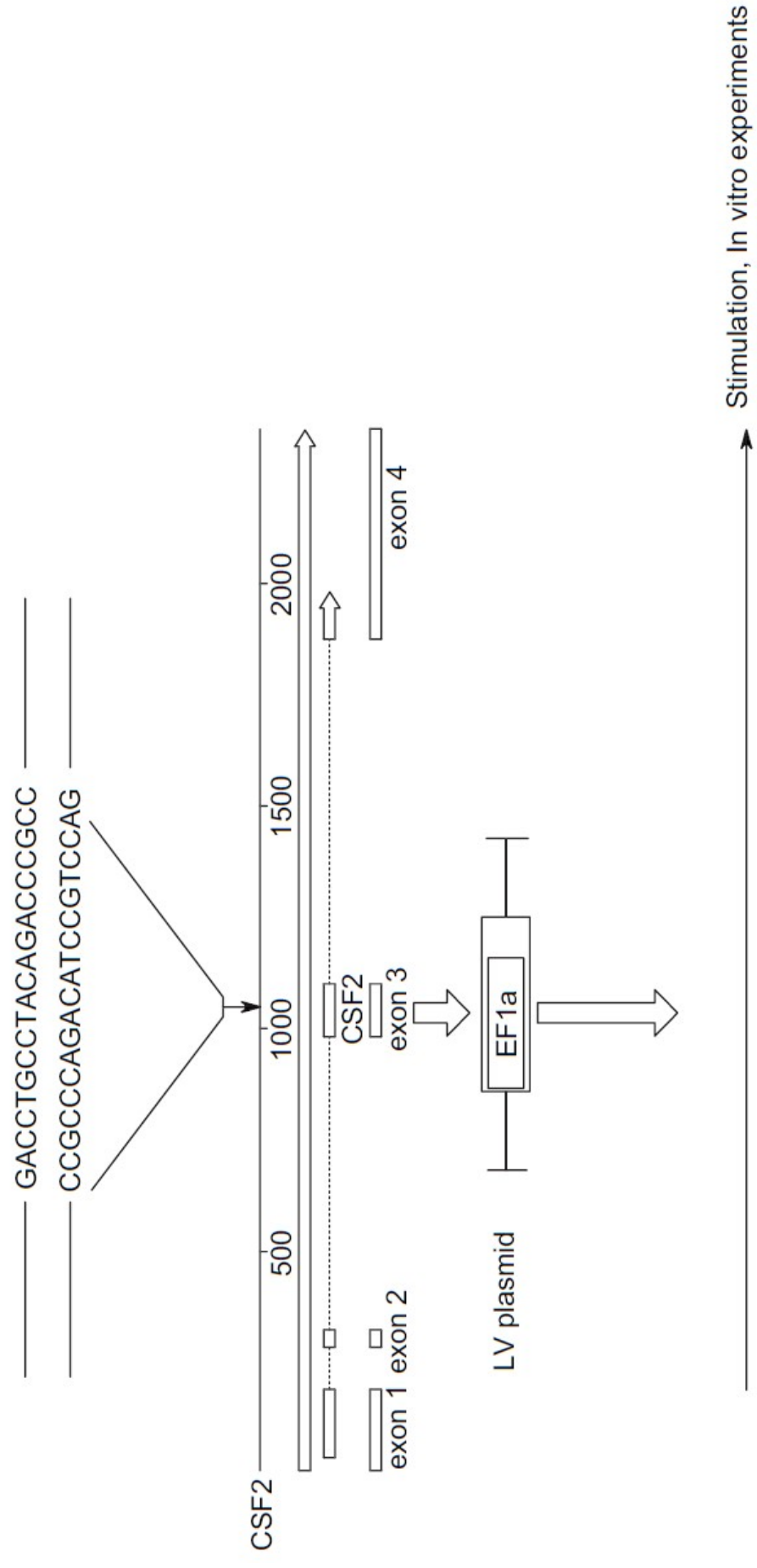
GACCTGCCTACAGACCCGCC
CCGCCCAGACATCCGTCCAG
CSF2
500
1000
1500
2000
exon 1
exon 2
CSF2
exon 3
exon 4
LV plasmid
EF1a
T cell expansion
stimulation
transduction
LV transduction
CRISPR/CAS9LV
Debead
TIDE
FACS
Stimulation, In vitro experiments
Cytokine production
in vivo experiments

FIG. 1

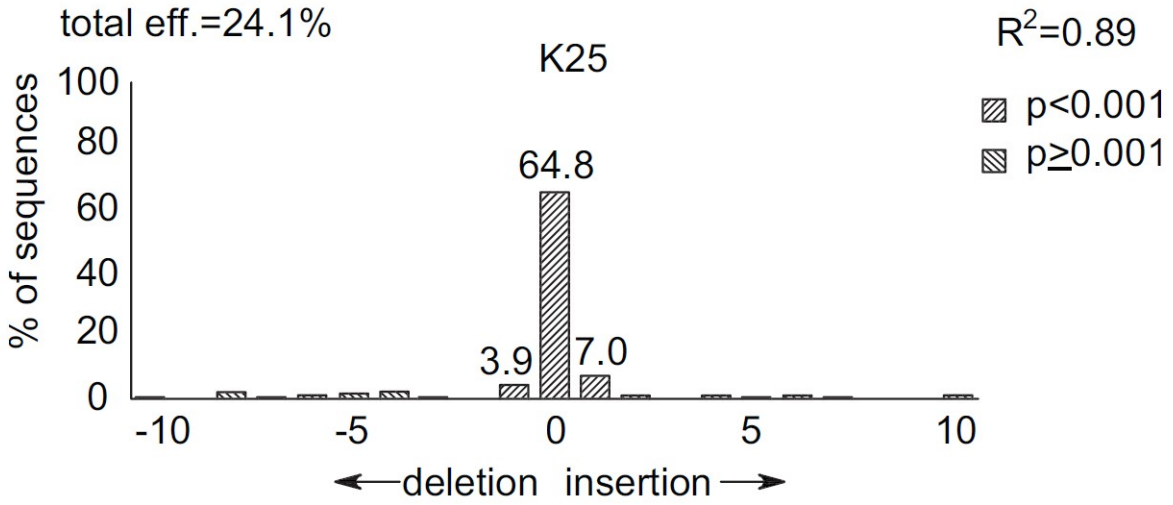
total eff.=24.1%
K25
R^2=0.89
p<0.001
p≥0.001
% of sequences
100
80
60
40
20
0
64.8
3.9
7.0
-10
-5
0
5
10
deletion
insertion

FIG. 2A

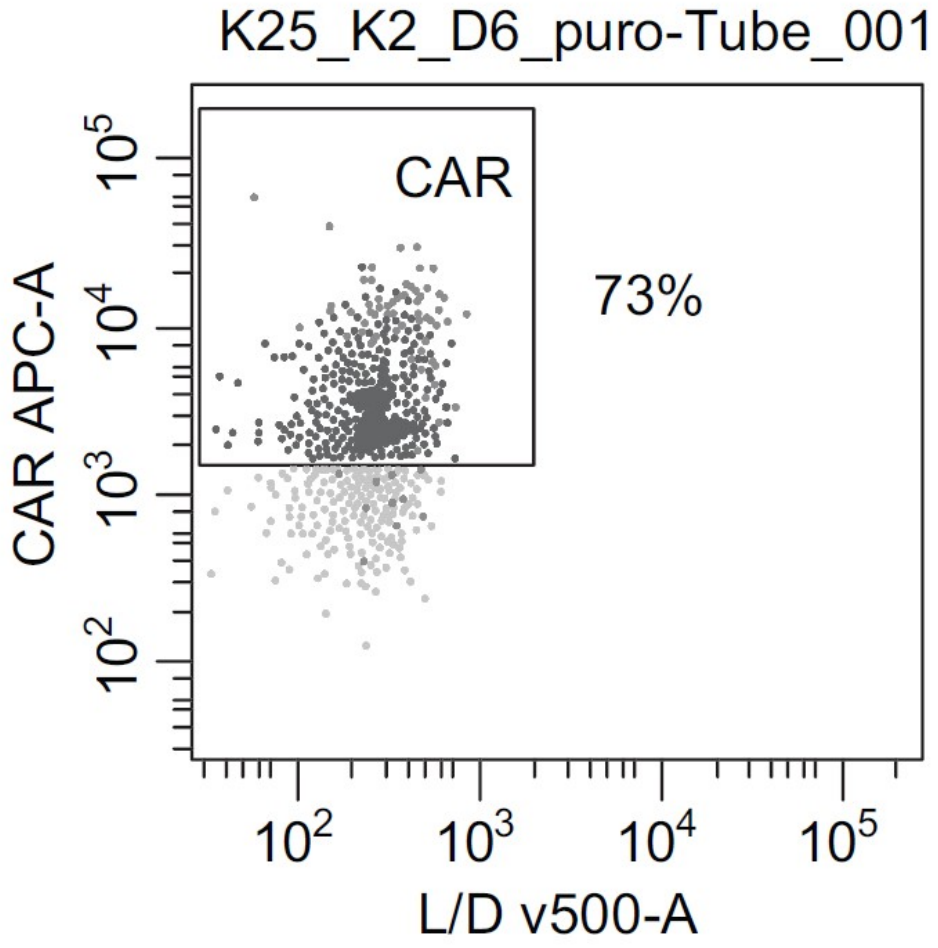
K25_K2_D6_puro-Tube_001
CAR
73%
CAR APC-A
10^2
10^3
10^4
10^5
L/D v500-A

FIG. 2B

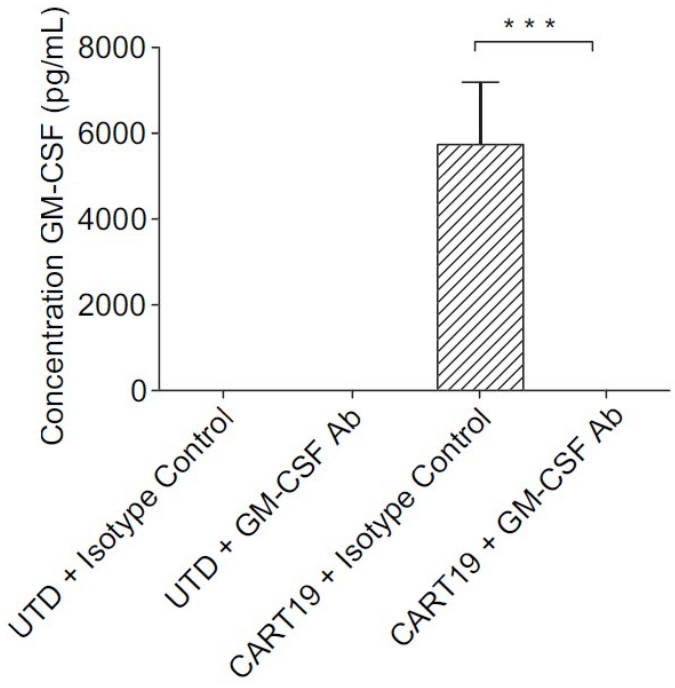
Concentration GM-CSF (pg/mL)
8000
6000
4000
2000
0
* * *
UTD + Isotype Control
UTD + GM-CSF Ab
CART19 + Isotype Control
CART19 + GM-CSF Ab

FIG. 7B

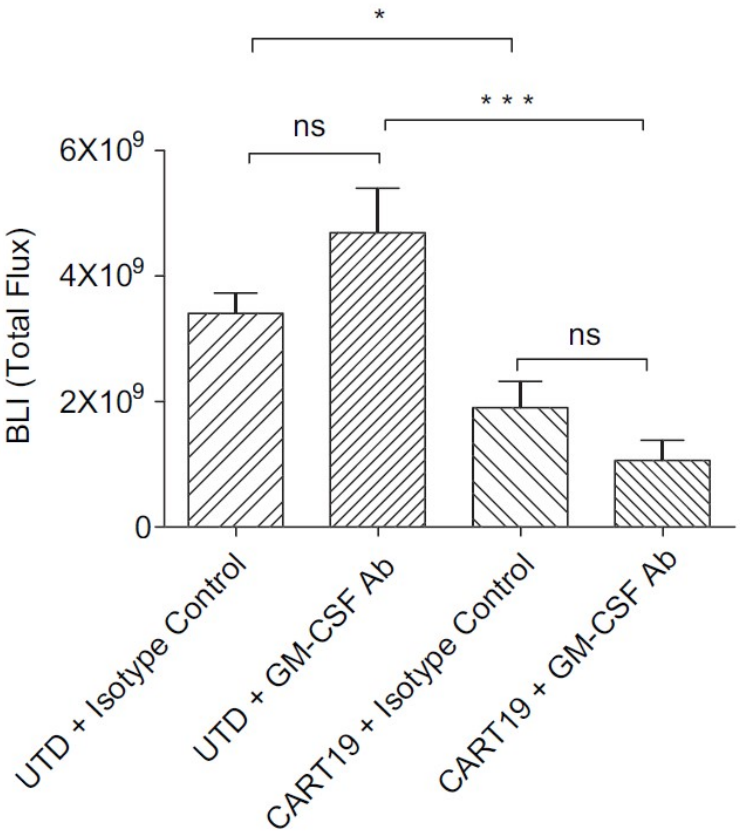
BLI (Total Flux)
6X10^9
4X10^9
2X10^9
0
*
* * *
ns
ns
UTD + Isotype Control
UTD + GM-CSF Ab
CART19 + Isotype Control
CART19 + GM-CSF Ab

FIG. 7C

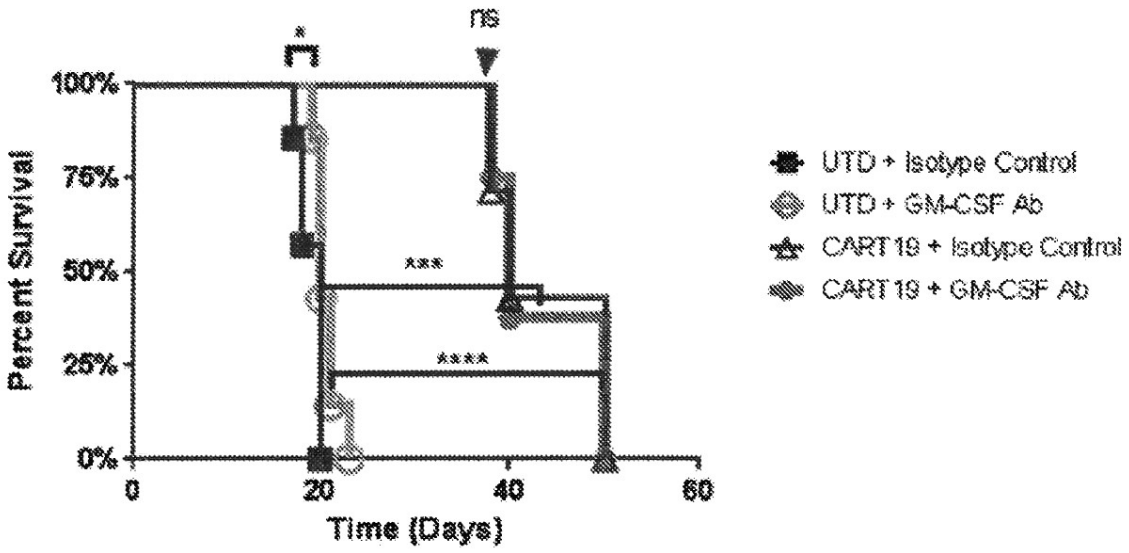
Percent Survival
100%
75%
50%
25%
0%
0
20
40
60
Time (Days)
*
ns
***
****
UTD + Isotype Control
UTD + GM-CSF Ab
CART19 + Isotype Control
CART19 + GM-CSF Ab

FIG. 8

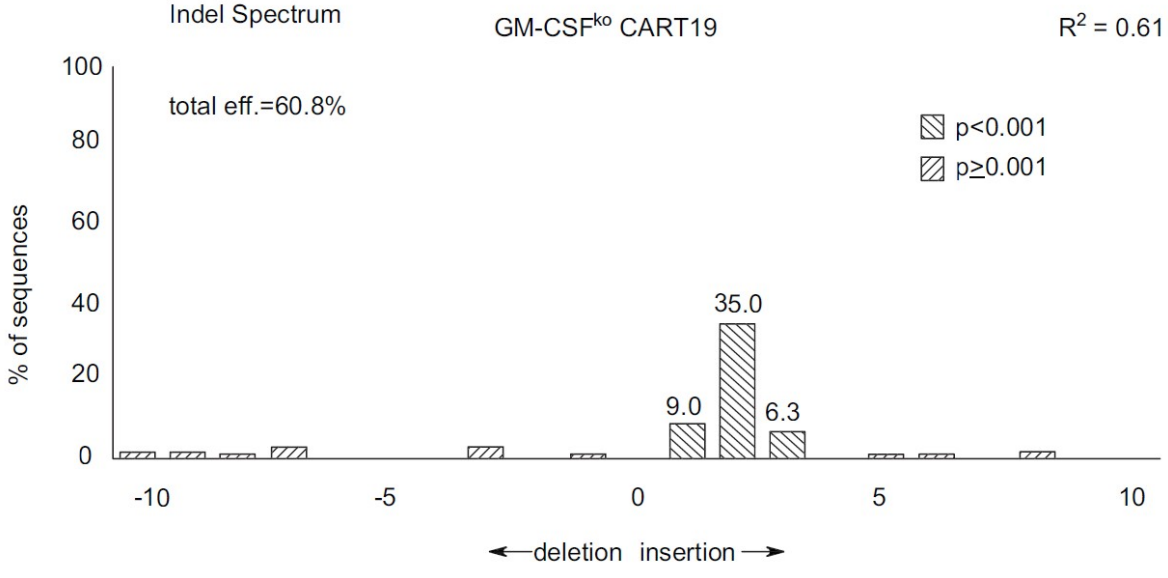
Indel Spectrum
GM-CSF^ko CART19
R^2 = 0.61
total eff.=60.8%
p<0.001
p≥0.001
% of sequences
100
80
60
40
20
0
35.0
9.0
6.3
-10
-5
0
5
10
deletion
insertion

FIG. 9

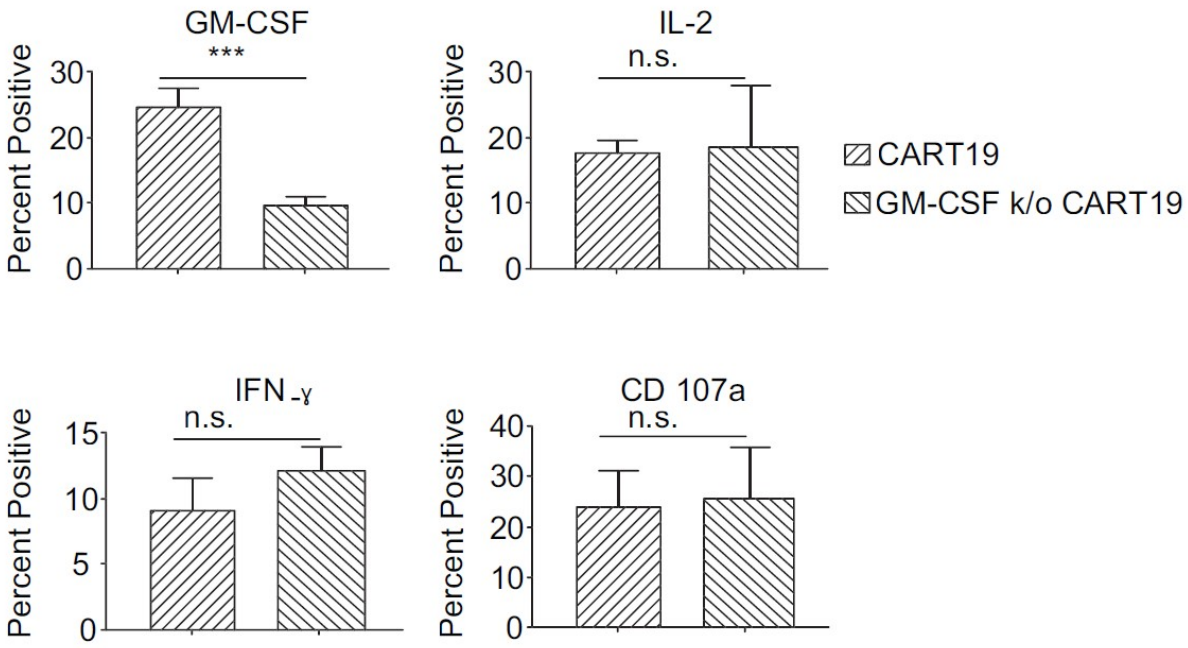

FIG. 10A

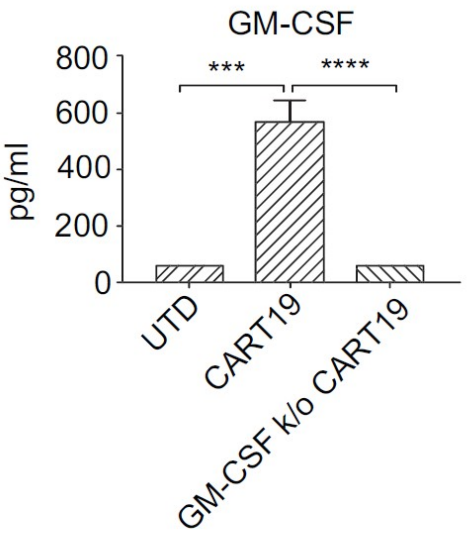

FIG. 10B

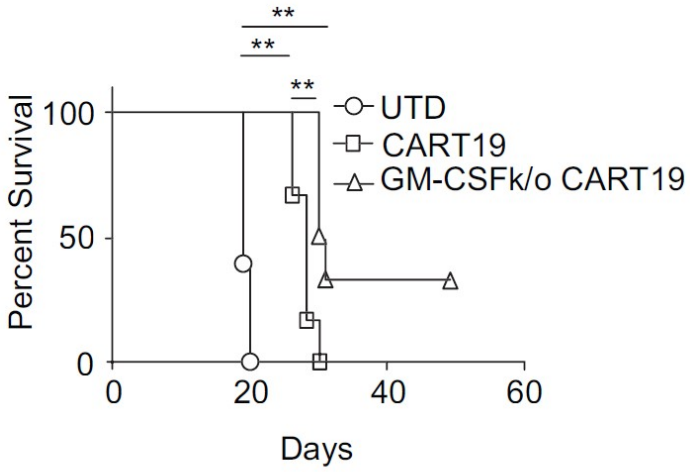

FIG. 10C

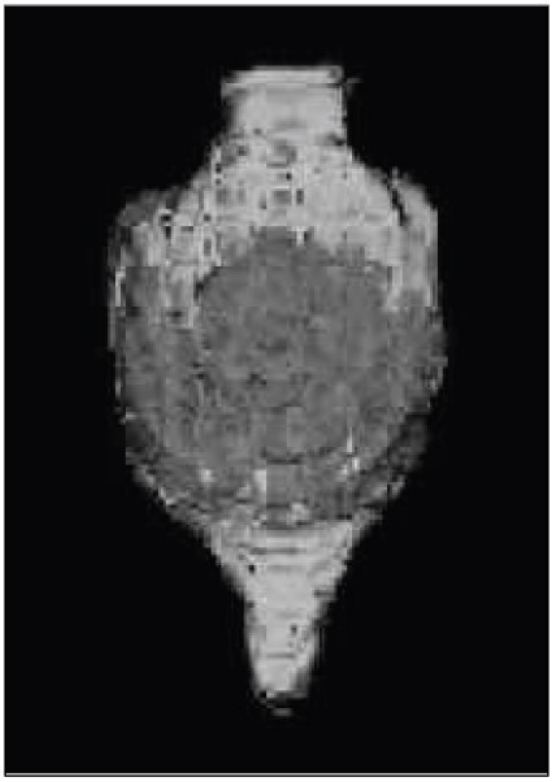

FIG. 12C

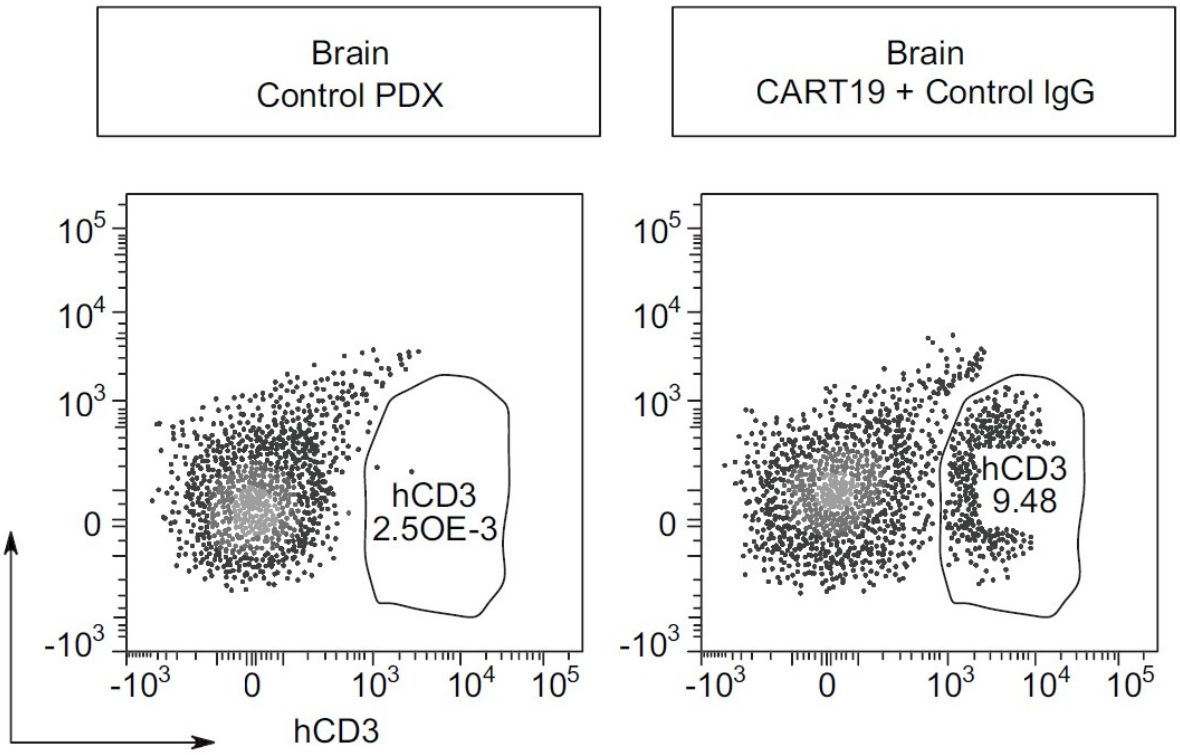

FIG. 12D

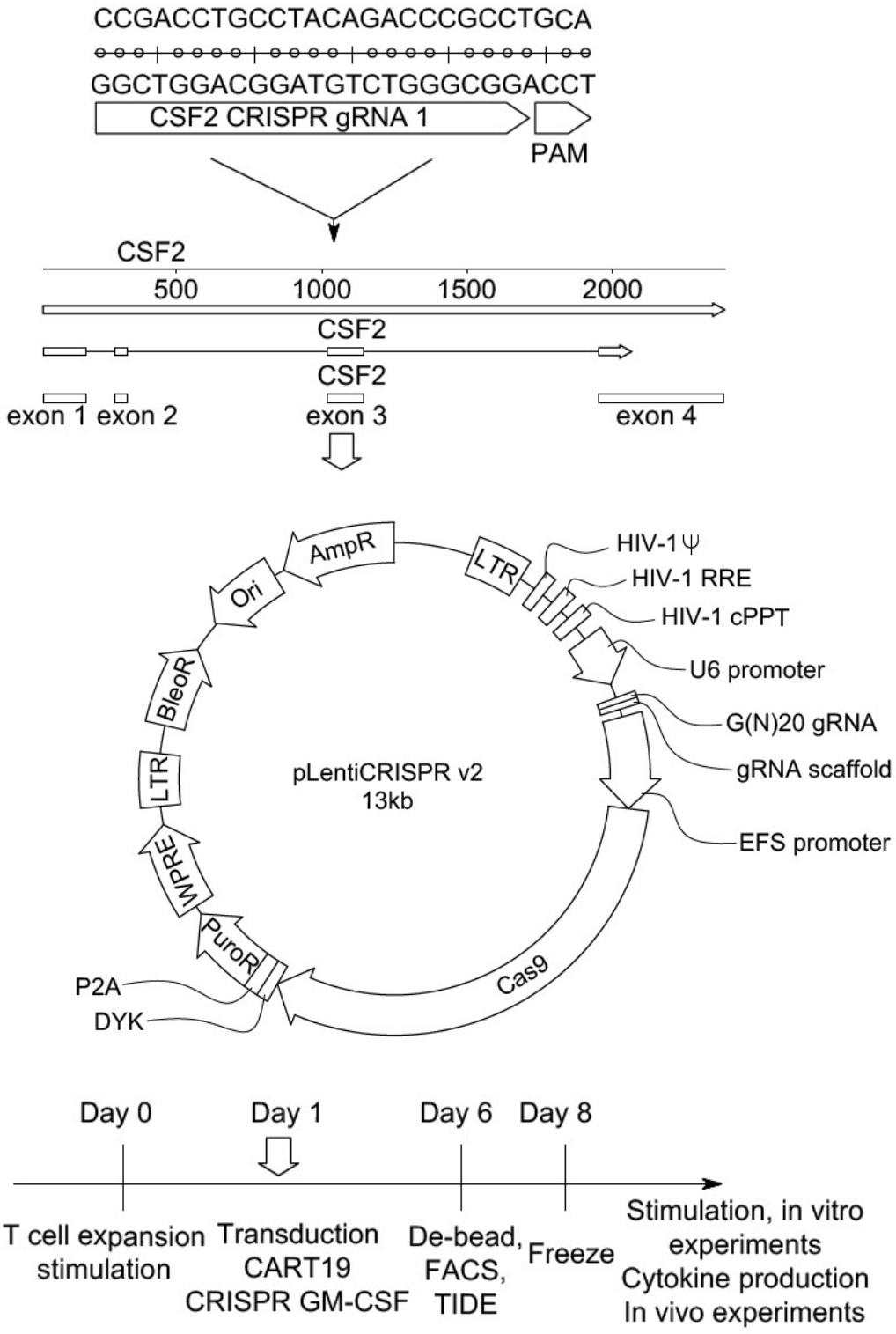
CCGACCTGCCTACAGACCCGCCTGCA
GGCTGGACGGATGTCTGGGCGGACCT
CSF2 CRISPR gRNA 1
PAM
CSF2
500
1000
1500
2000
CSF2
CSF2
exon 1
exon 2
exon 3
exon 4
AmpR
Ori
BleoR
LTR
WPRE
PuroR
P2A
DYK
Cas9
LTR
HIV-1 Ψ
HIV-1 RRE
HIV-1 cPPT
U6 promoter
G(N)20 gRNA
gRNA scaffold
EFS promoter
pLentiCRISPR v2
13kb
Day 0
Day 1
Day 6
Day 8
T cell expansion
stimulation
Transduction
CART19
CRISPR GM-CSF
De-bead,
FACS,
TIDE
Freeze
Stimulation, in vitro
experiments
Cytokine production
In vivo experiments

FIG. 15A

gRNA/Primer	Sequence
CSF2 Primer For	TGACTACAGAGAGGCACAGA
CSF2 Primer Rev	TCACCTCTGACCTCATTAACC
CSF2 CRISPR gRNA 1	GACCTGCCTACAGACCCGCC

FIG. 15B

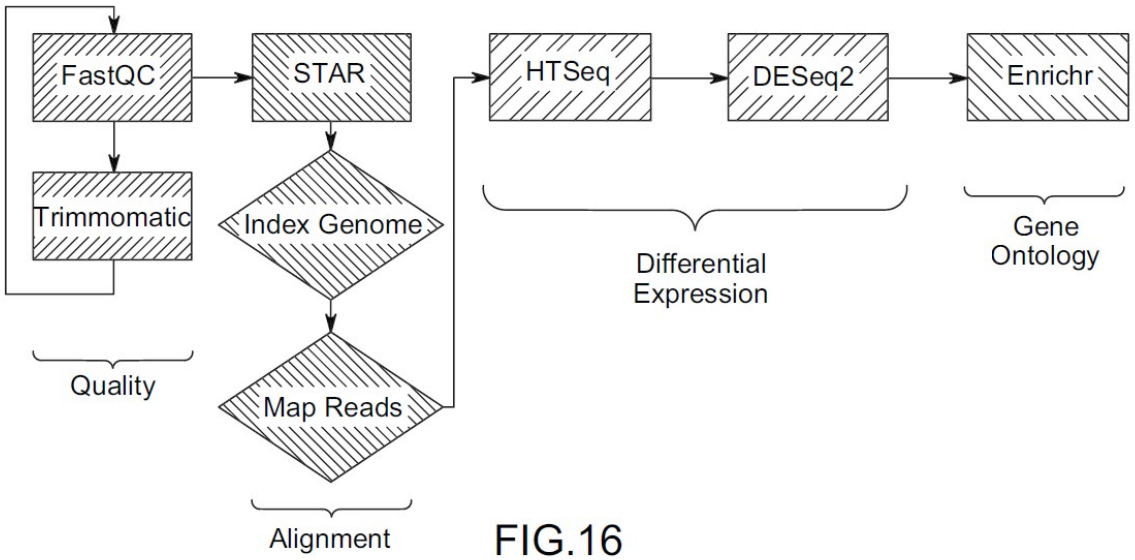

FIG.16