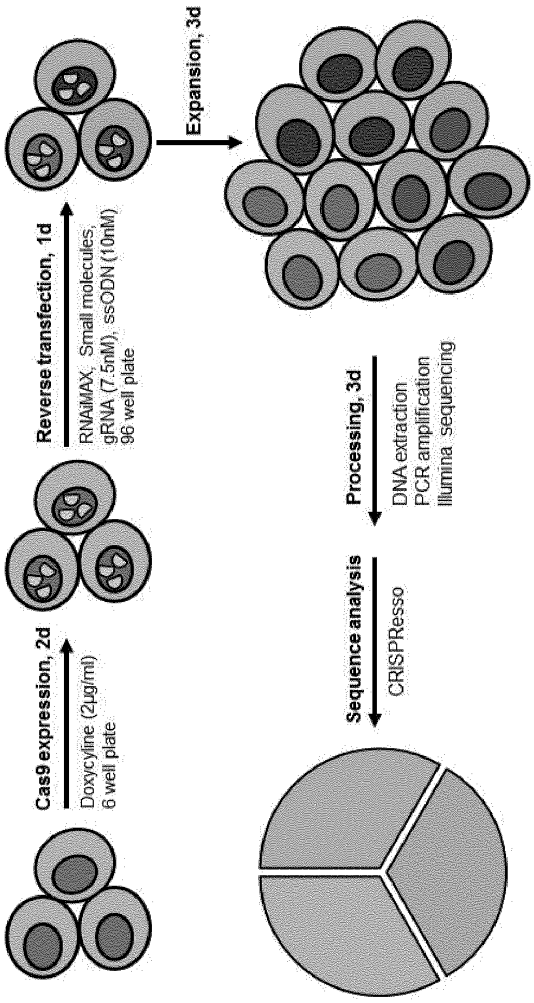

Cas9 expression, 2d
Doxycyline (2µg/ml)
6 well plate
Reverse transfection, 1d
RNAiMAX, Small molecules,
gRNA (7.5nM), ssODN (10nM)
96 well plate
Expansion, 3d
Processing, 3d
DNA extraction
PCR amplification
Illumina sequencing
Sequence analysis
CRISPResso

Fig. 1:

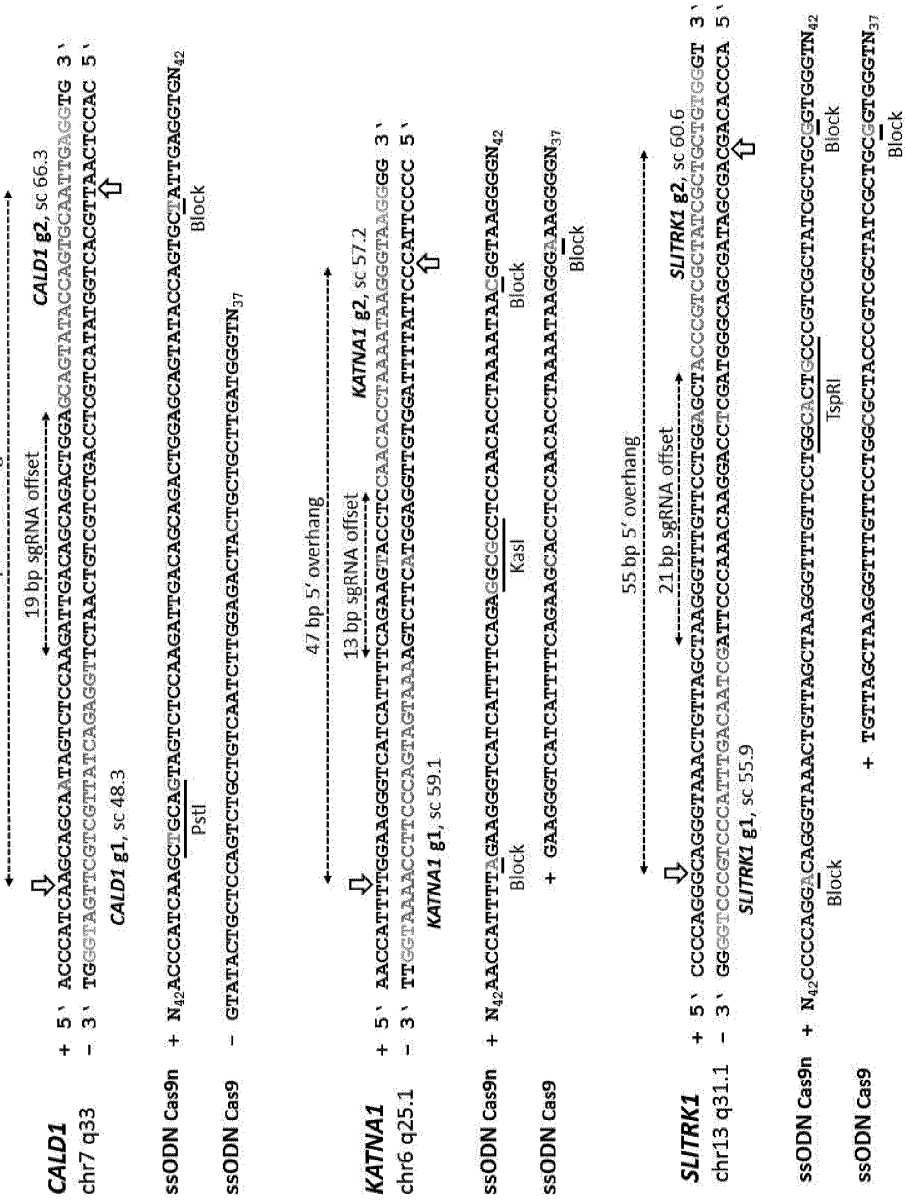

53 bp 5' overhang
19 bp sgRNA offset
CALD1 g2, sc 66.3
CALD1
chr7 q33
+ 5' ACCCATCAAGCAGCAATAGTCTCCAAGATTGACAGCAGACTGGAGCAGTATACCAGTGCAATTGAGGTG 3'
- 3' TGGGTAGTTCGTCGTTATCAGAGGTTCTAACTGTCGTCTGACCTCGTCATATGGTCACGTTAACTCCAC 5'
CALD1 g1, sc 48.3
ssODN Cas9n + N42ACCCATCAAGCTGCAGTAGTCTCCAAGATTGACAGCAGACTGGAGCAGTATACCAGTGCTATTGAGGTGN42
PstI
Block
ssODN Cas9 - GTATACTGCTCCAGTCTGCTGTCAATCTTGGAGACTACTGCTGCTTGATGGGTTN37
47 bp 5' overhang
13 bp sgRNA offset
KATNA1 g2, sc 57.2
KATNA1
chr6 q25.1
+ 5' AACCATTTTGGAAGGGTCATCATTTTCAGAAGTACCTCCAACACCTAAAATAAGGGTAAGGGG 3'
- 3' TTGGTAAAACCTTCCCAGTAGTAAAAGTCTTCATGGAGGTTGTGGATTTTATTCCCATTCCCC 5'
KATNA1 g1, sc 59.1
ssODN Cas9n + N42AACCATTTTAGAAGGGTCATCATTTTCAGAGGCGCCTCCAACACCTAAAATAACGGTAAGGGGN42
Block
KasI
Block
ssODN Cas9 + GAAGGGTCATCATTTTCAGAAGCACCTCCAACACCTAAAATAAGGGAAGGGGN37
Block
55 bp 5' overhang
21 bp sgRNA offset
SLITRK1 g2, sc 60.6
SLITRK1
chr13 q31.1
+ 5' CCCCAGGGGCAGGGTAAACTGTTAGCTAAGGGTTTGTTCCTGGAGCTACCCGTCGCTATCGCTGCTGTGGGT 3'
- 3' GGGGTCCCCGTCCCATTTGACAATCGATTCCCAAACAAGGACCTCGATGGGCAGCGATAGCGACGACACCCA 5'
SLITRK1 g1, sc 55.9
ssODN Cas9n + N42CCCCAGGGCAGGGTAAACTGTTAGCTAAGGGTTTGTTCCTGGCACTGCCCGTCGCTATCGCTGCGGTGGGTN42
Block
TspRI
Block
ssODN Cas9 + TGTTAGCTAAGGGTTTGTTCCTGGCGCTACCCGTCGCTATCGCTGCGGTGGGTN37
Block

Fig. 2:

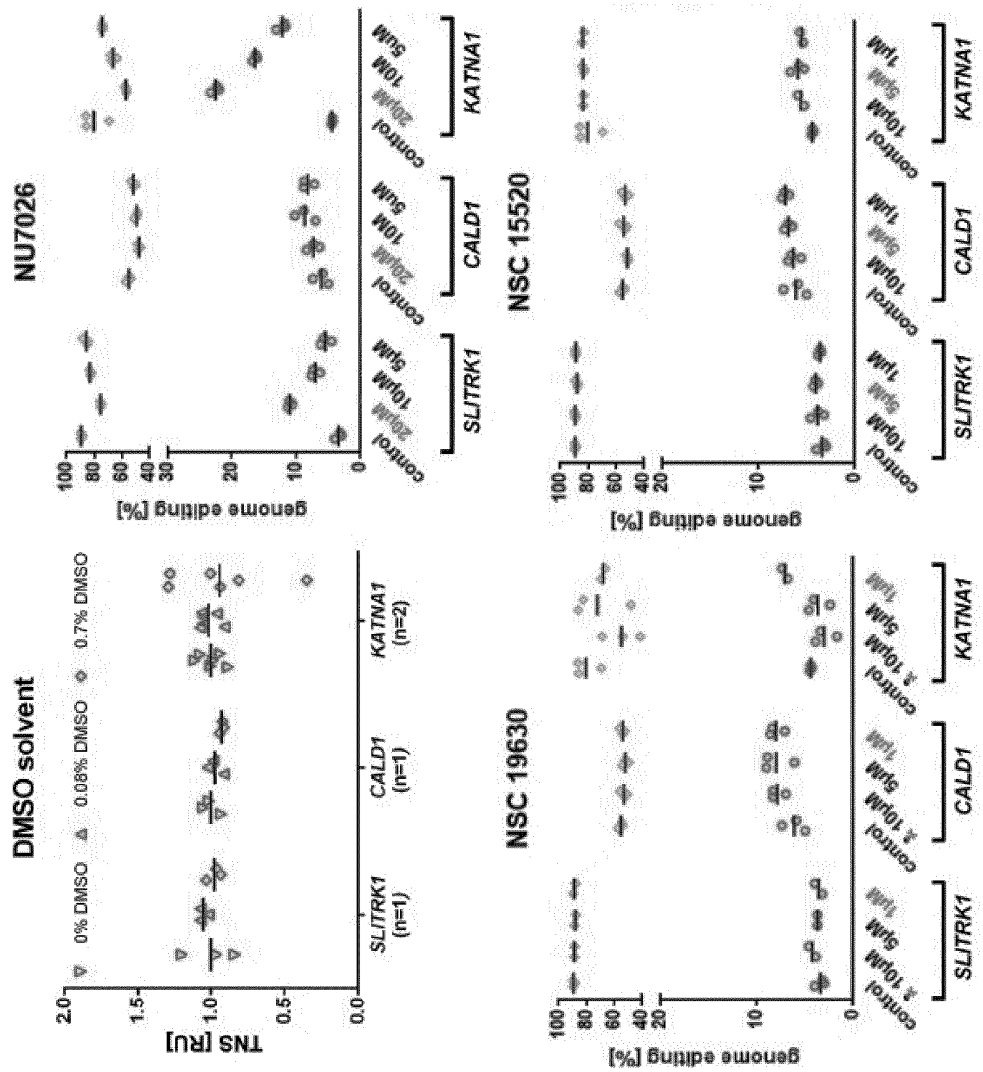

DMSO solvent
0% DMSO
0.08% DMSO
0.7% DMSO
TNS [RU]
SLITRK1 (n=1)
CALD1 (n=1)
KATNA1 (n=2)
NU7026
NSC 15520
NSC 19630
genome editing [%]
control
2.5µM
5µM
10µM
20µM
1µM
SLITRK1
CALD1
KATNA1

Fig. 3:

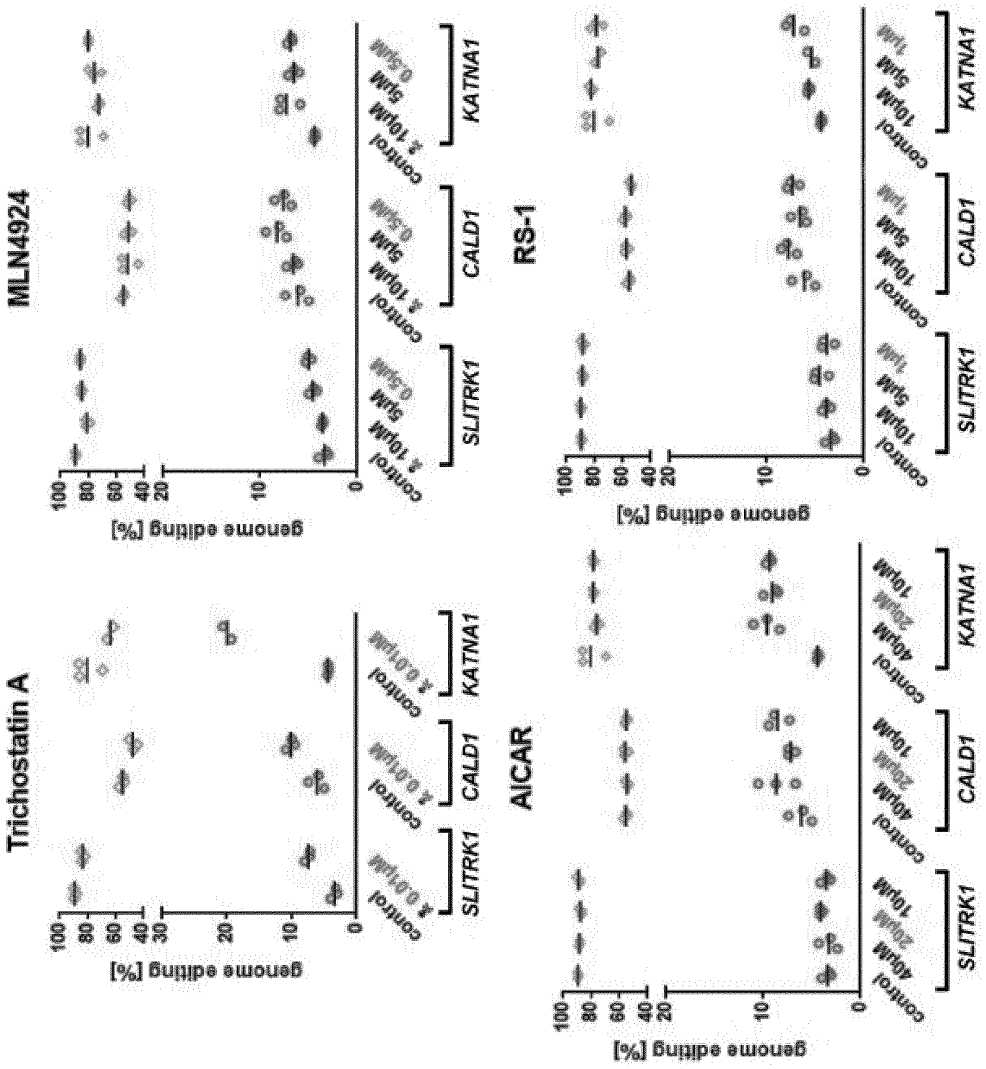


Fig. 3 (continued)

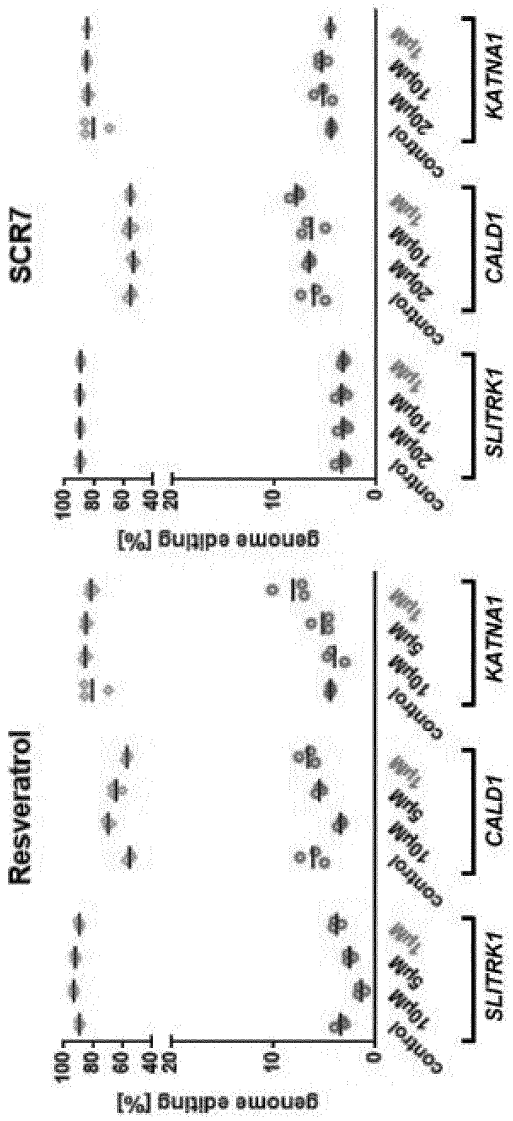


Fig. 3 (continued)

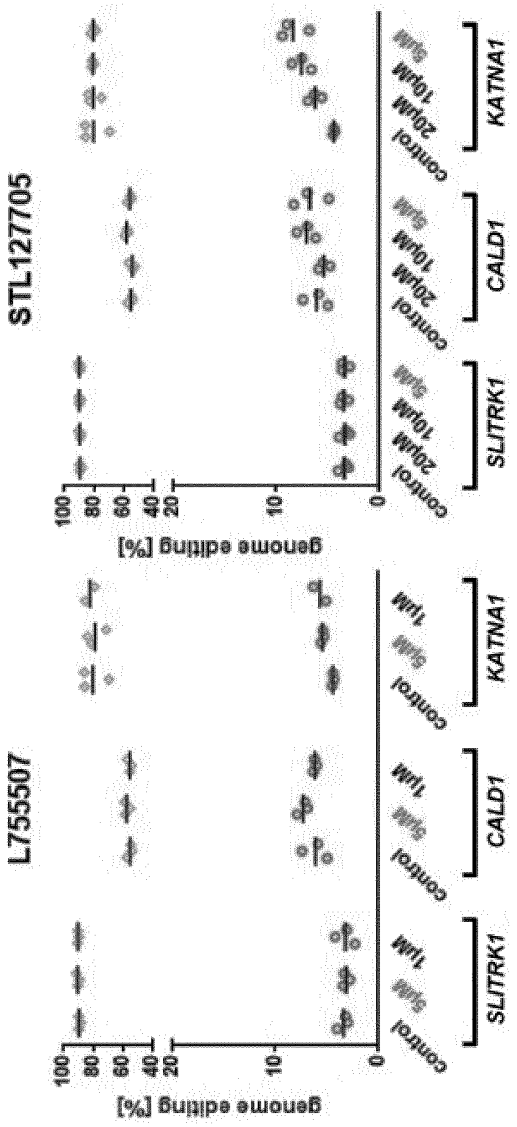


Fig. 3 (continued)

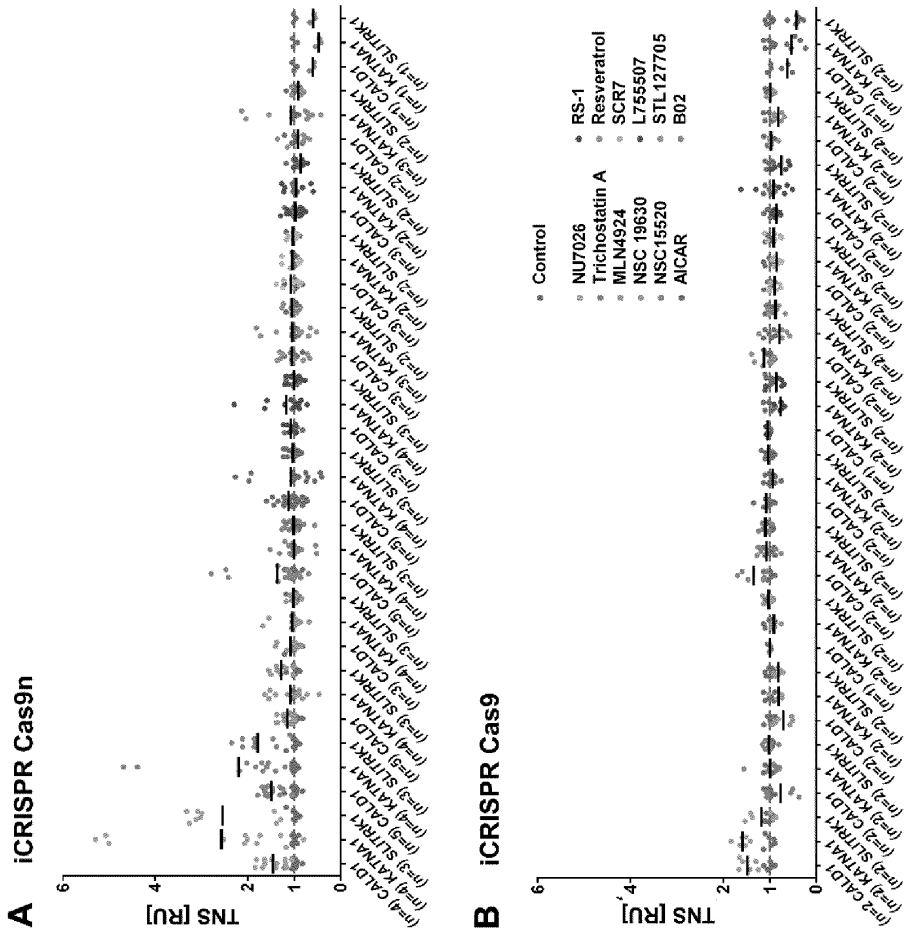

A
iCRISPR Cas9n
TNS [RU]
B
iCRISPR Cas9
TNS [RU]
Control
NU7026
Trichostatin A
MLN4924
NSC 19630
NSC15520
AICAR
RS-1
Resveratrol
SCR7
L755507
STL127705
B02

Fig. 4:

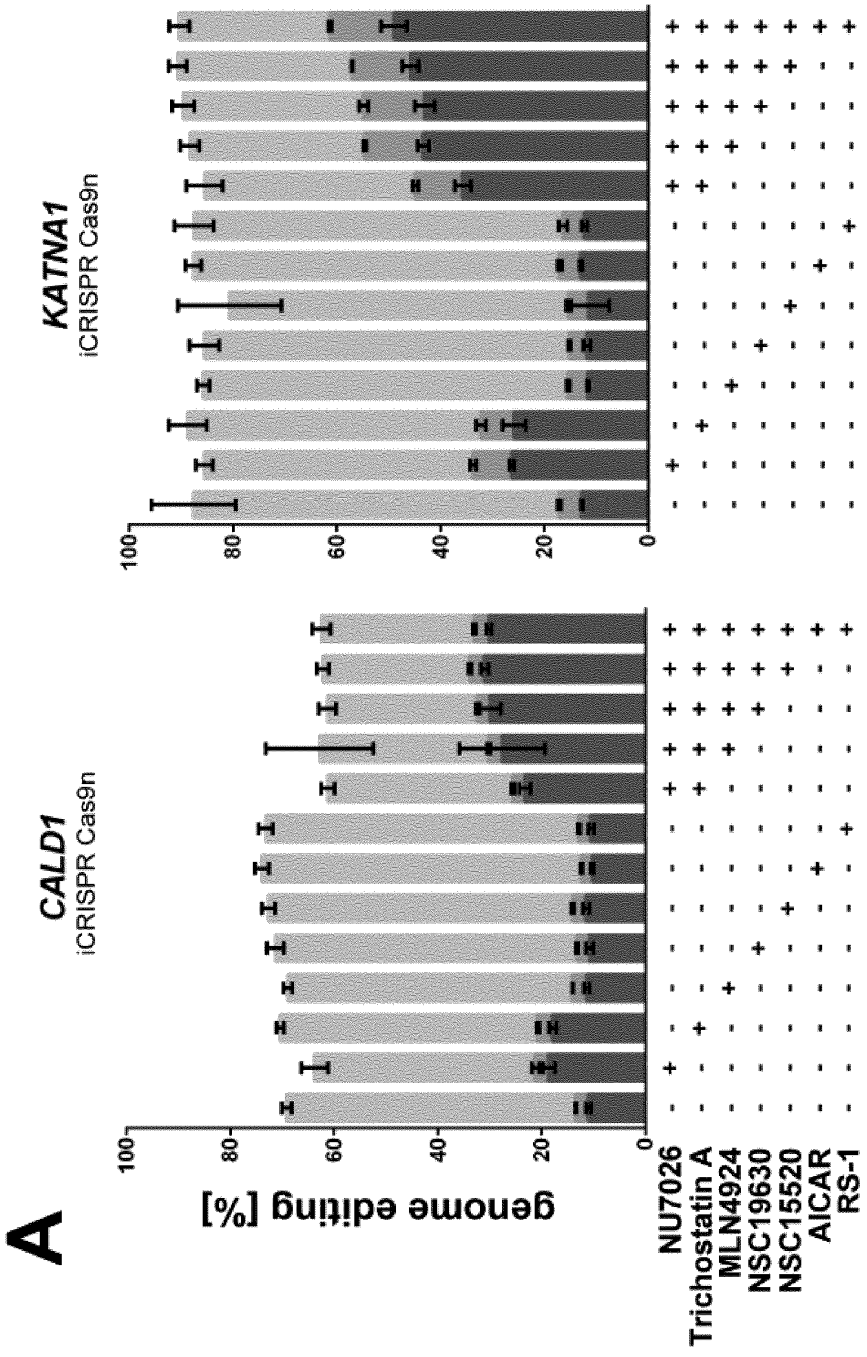

A
CALD1
iCRISPR Cas9n
KATNA1
iCRISPR Cas9n
genome editing [%]
0
20
40
60
80
100
NU7026
Trichostatin A
MLN4924
NSC19630
NSC15520
AICAR
RS-1

Fig. 5:

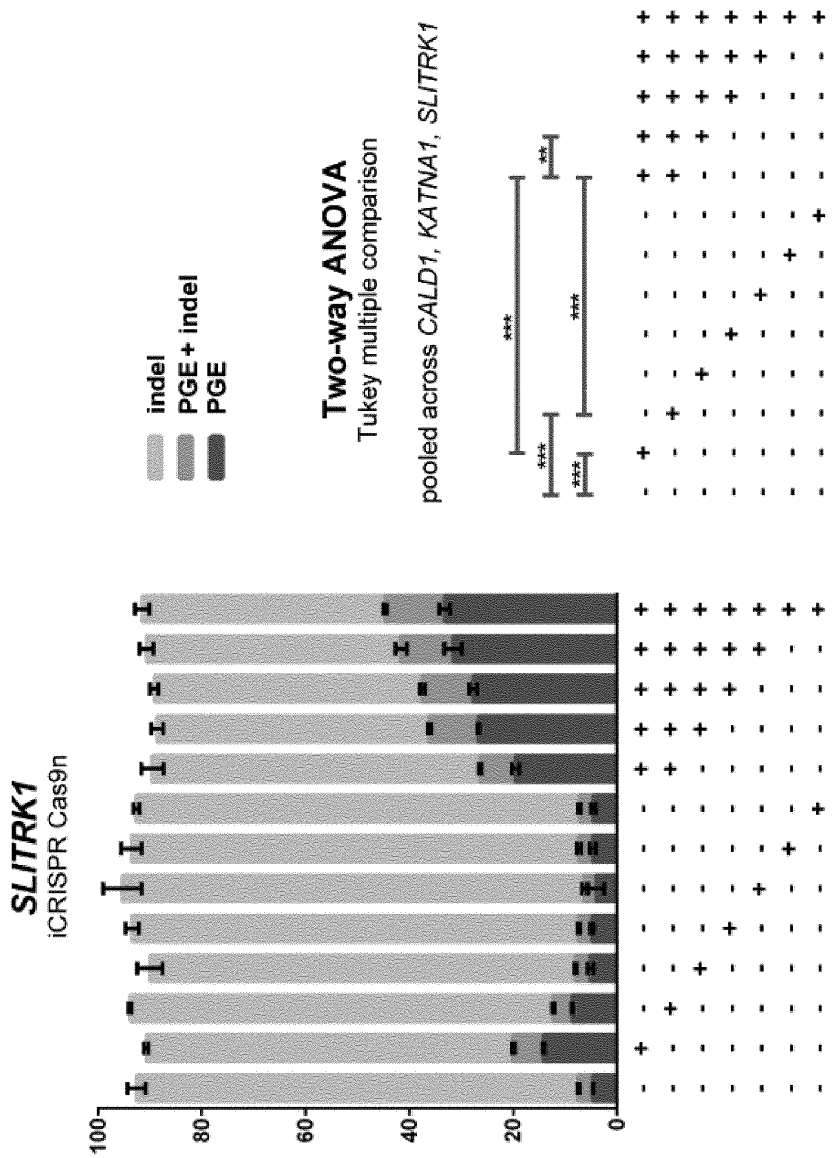


Fig. 5A (continued)

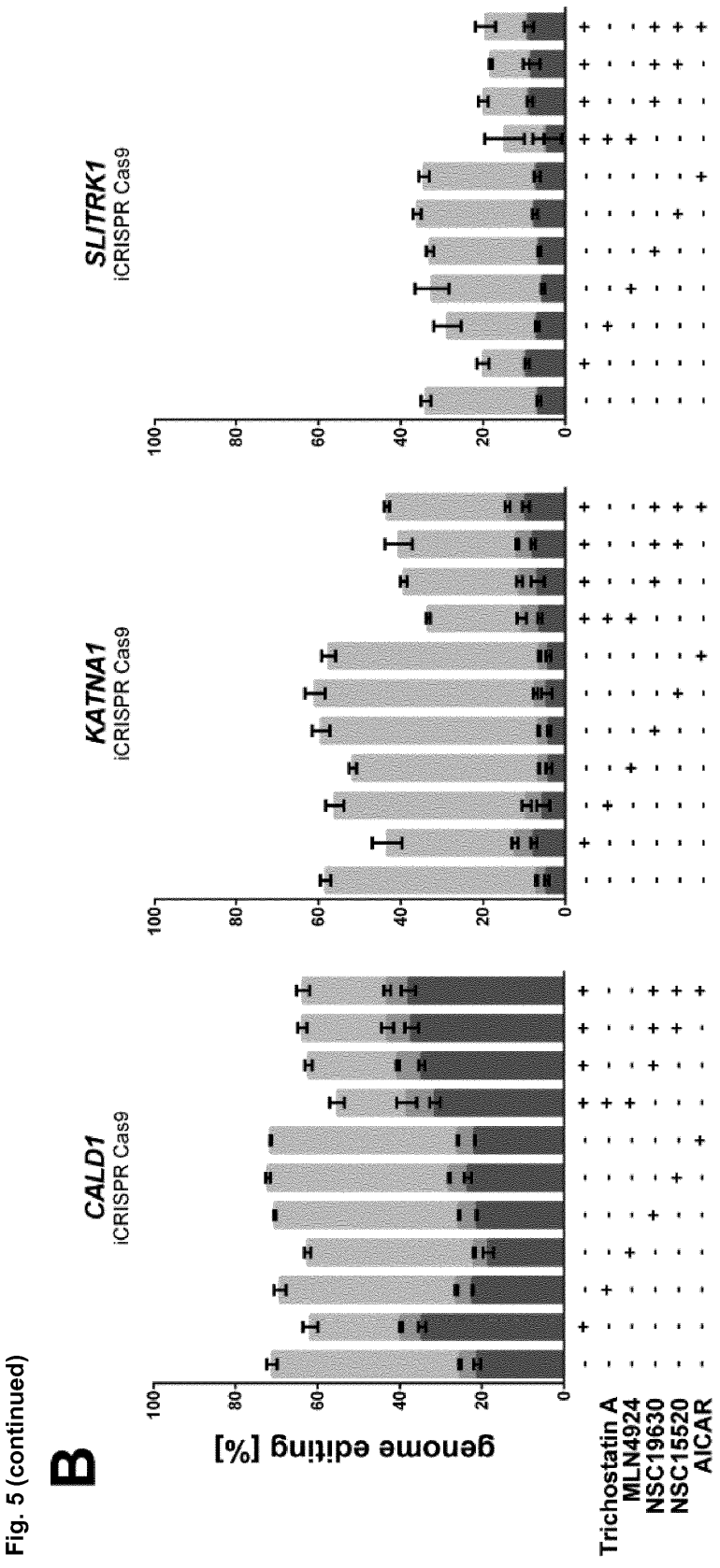


Fig. 5 (continued)

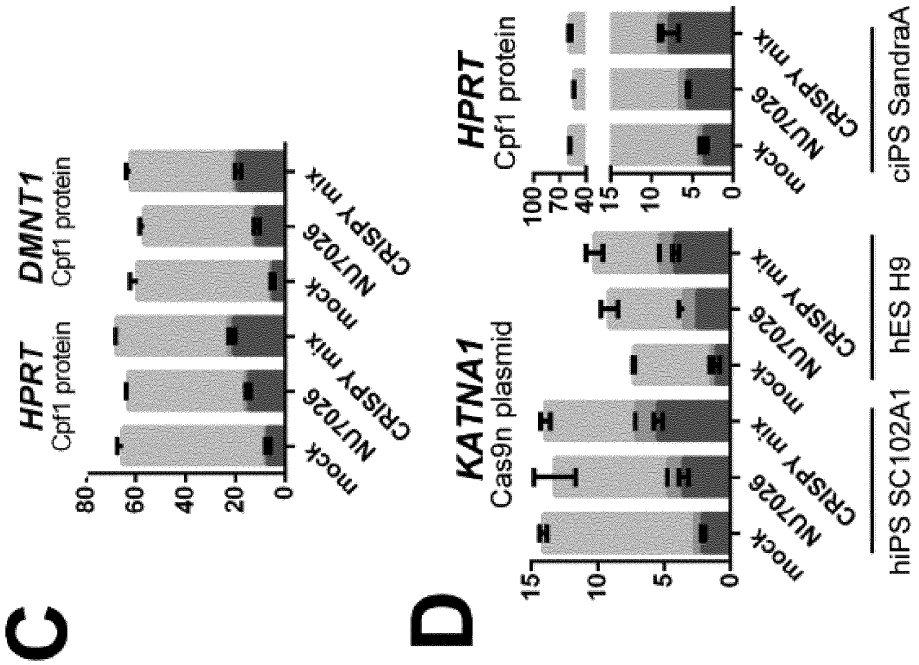


Fig. 5 (continued)

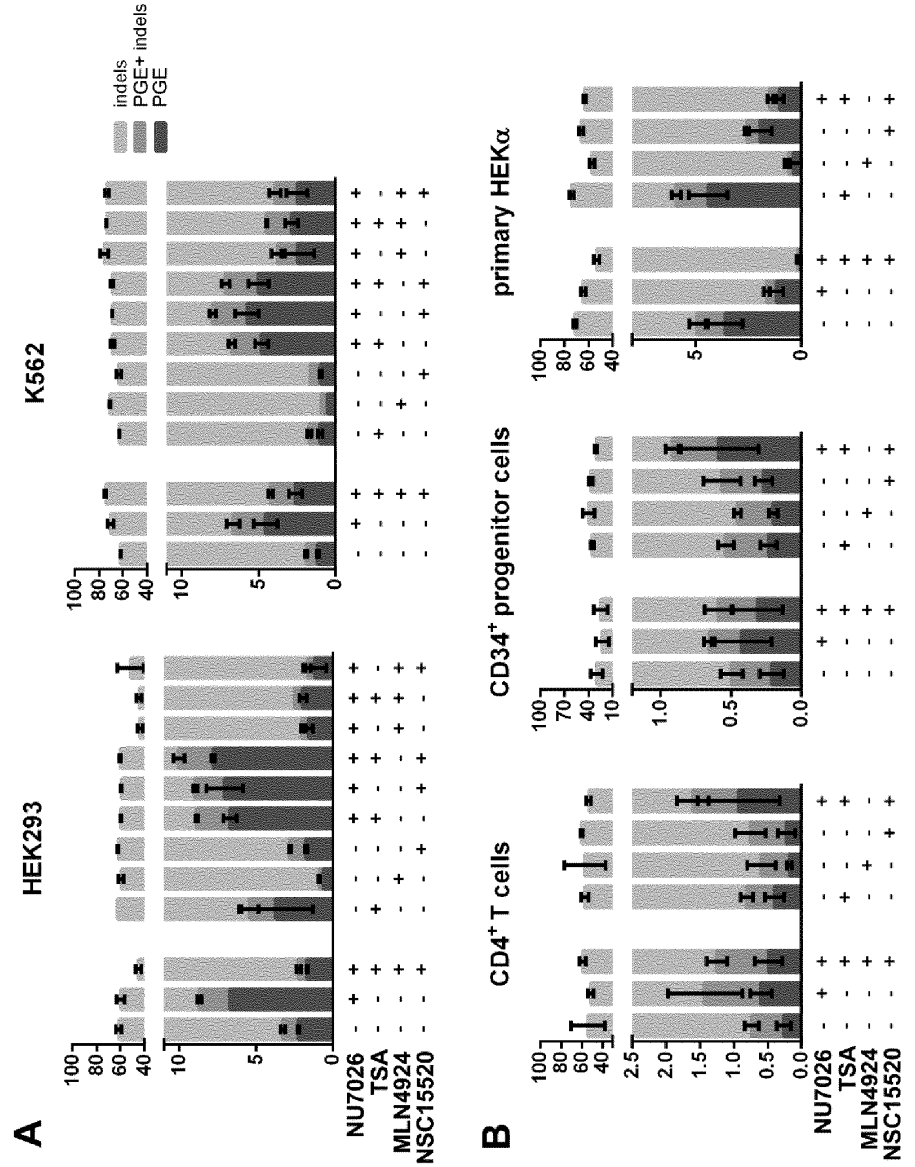

A
HEK293
K562
indels
PGE+ indels
PGE
NU7026
TSA
MLN4924
NSC15520
B
CD4+ T cells
CD34+ progenitor cells
primary HEKα

Fig. 6:

Fig. 7:

A Acute toxicity



B Long-term genotoxicity

5x (1 day passaging with mock or CRISPY mix, and RNAiMax; 3 days recovery)

sample	metaphases	band number	gray shades	healthy karyotype (46,XX)
mock (bulk)	20	400	3	20/20
CRISPY (bulk)	25	450	3	25/25
mock clone 1	25	400	3	21/25 (4 polyploid)
mock clone 2	25	450	3	25/25
mock clone 3	25	450	3	25/25
mock clone 4	25	450	3	25/25
mock clone 5	25	400	3	25/25
CRISPY clone 1	25	400	3	25/25
CRISPY clone 2	25	400	3	25/25
CRISPY clone 3	25	400	3	25/25
CRISPY clone 4	25	400	3	22/25 (3 polyploid)
CRISPY clone 5	25	400	3	25/25

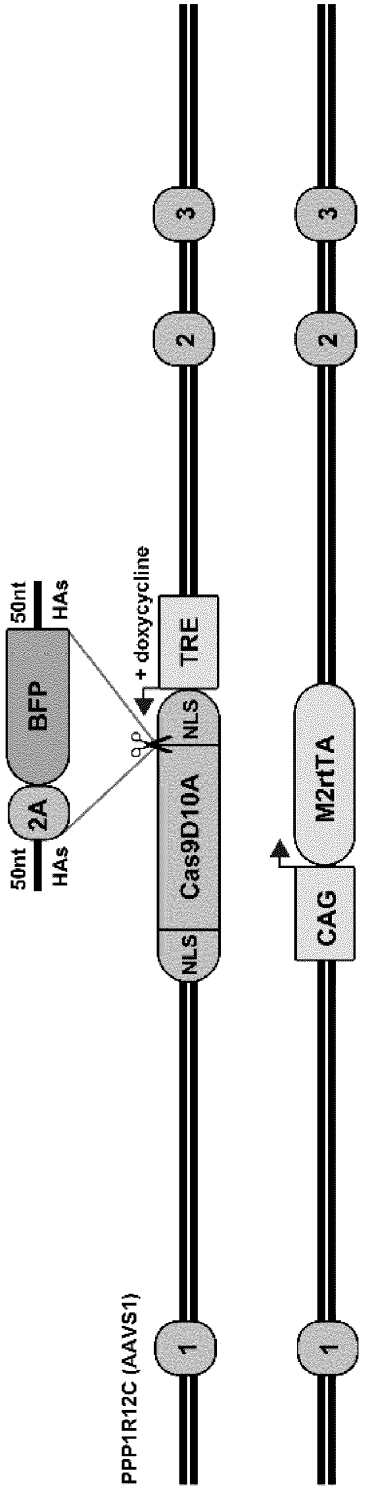

A
PPP1R12C (AAVS1)
1
2
3
50nt
HAs
BFP
2A
50nt
HAs
+ doxycycline
TRE
NLS
Cas9D10A
NLS
CAG
M2rtTA
1
2
3

Fig. 8:

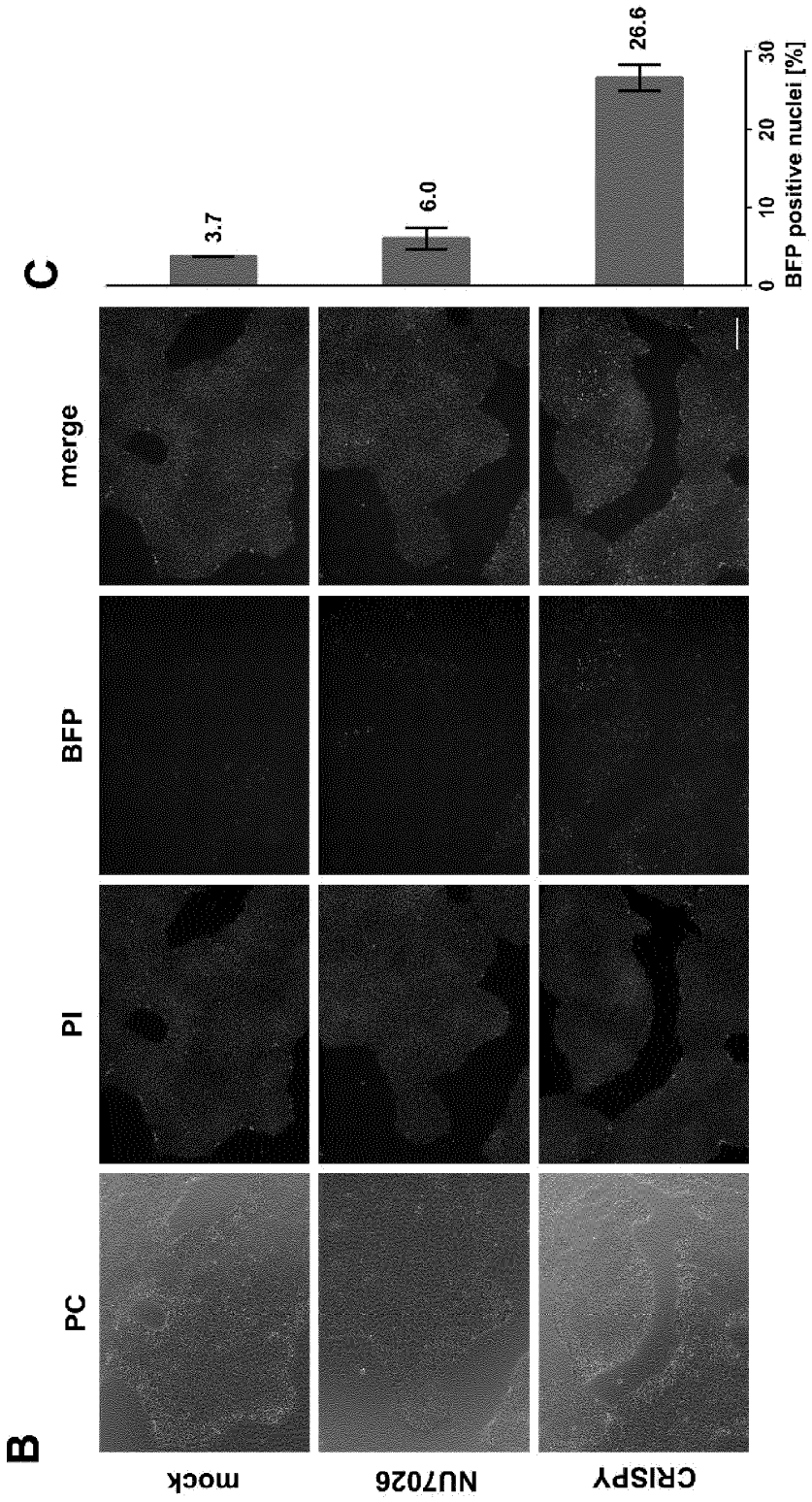


Fig. 8 (continued)

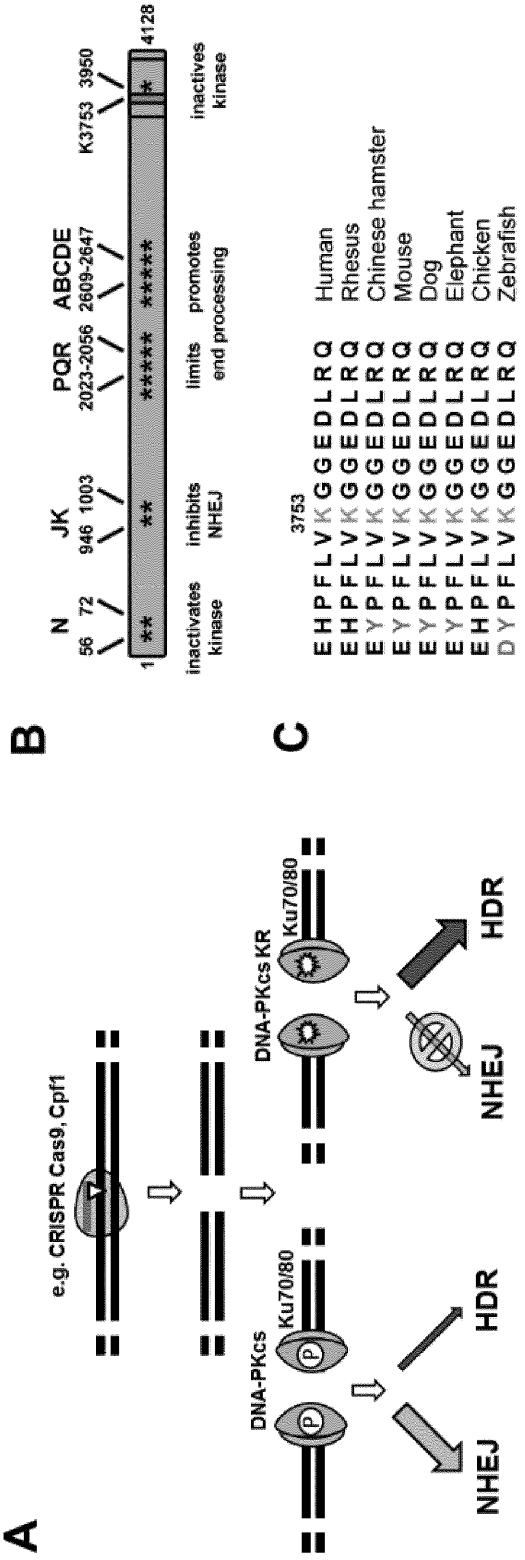

A
e.g. CRISPR Cas9, Cpf1
DNA-PKcs
Ku70/80
DNA-PKcs KR
Ku70/80
NHEJ
HDR
NHEJ
HDR
B
N
JK
PQR
ABCDE
1
56
72
946
1003
2023-2056
2609-2647
K3753
3950
4128
inactivates kinase
inhibits NHEJ
limits end processing
promotes
inactives kinase
C
3753
E H P F L V K G G E D L R Q Human
E H P F L V K G G E D L R Q Rhesus
E Y P F L V K G G E D L R Q Chinese hamster
E Y P F L V K G G E D L R Q Mouse
E Y P F L V K G G E D L R Q Dog
E Y P F L V K G G E D L R Q Elephant
E H P F L V K G G E D L R Q Chicken
D Y P F L V K G G E D L R Q Zebrafish

Fig. 9:

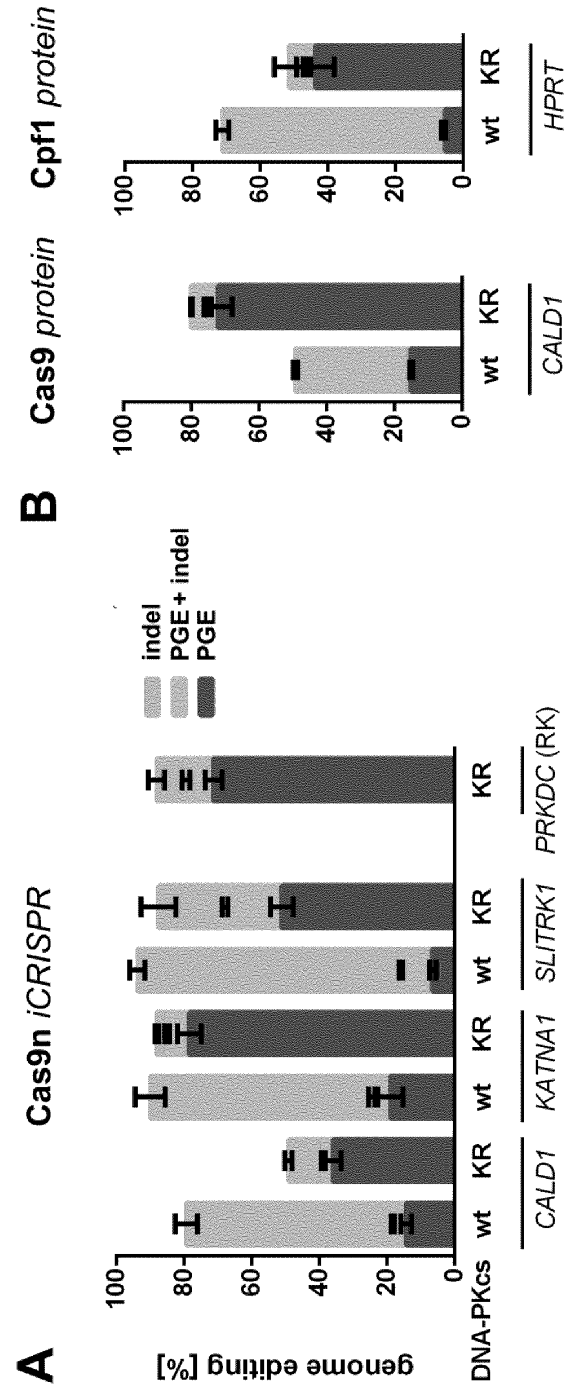
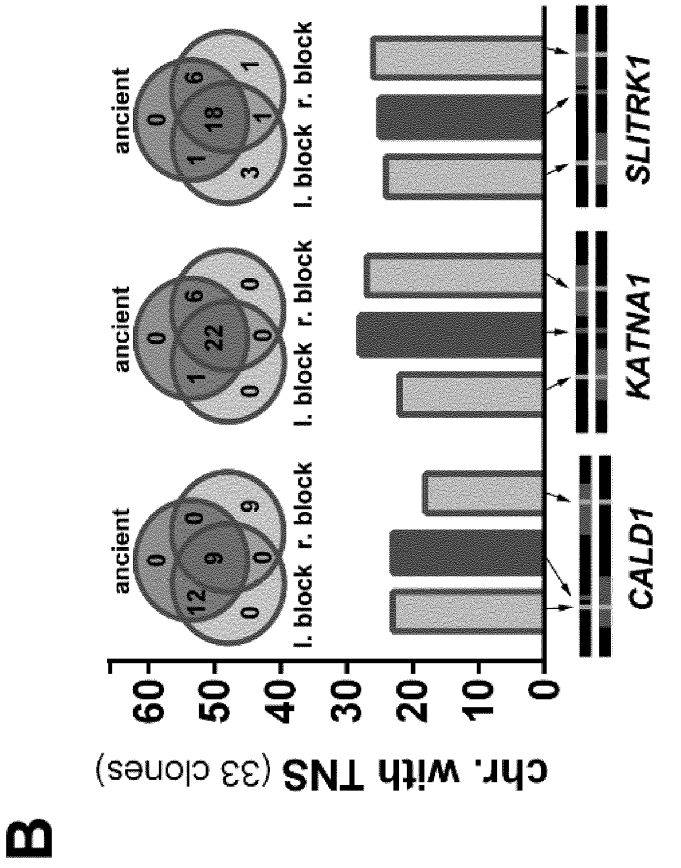

A
Cas9n iCRISPR
genome editing [%]
indel
PGE + indel
PGE
DNA-PKcs
wt
KR
wt
KR
wt
KR
wt
KR
CALD1
KATNA1
SLITRK1
PRKDC (RK)
B
Cas9 protein
Cpf1 protein
wt
KR
wt
KR
CALD1
HPRT

Fig. 10:


B
chr. with TNS (33 clones)
60
50
40
30
20
10
0
ancient
l. block
r. block
0
12
9
0
0
0
9
ancient
l. block
r. block
0
1
22
6
0
0
0
ancient
l. block
r. block
0
1
18
6
3
1
CALD1
KATNA1
SLITRK1

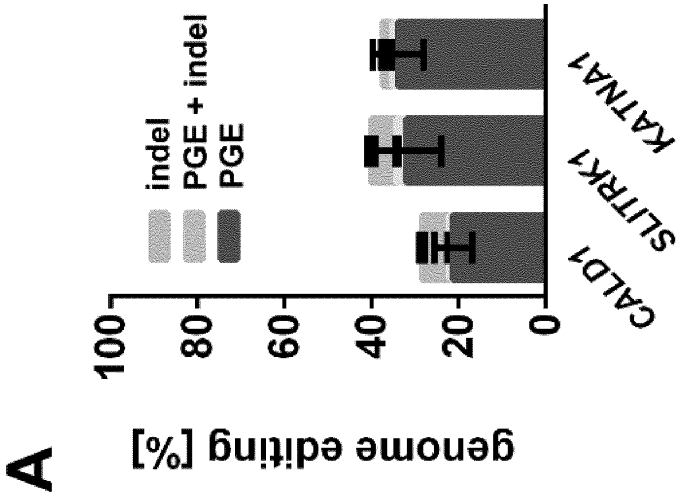

A
genome editing [%]
100
80
60
40
20
0
indel
PGE + indel
PGE
CALD1
SLITRK1
KATNA1

Fig. 11:

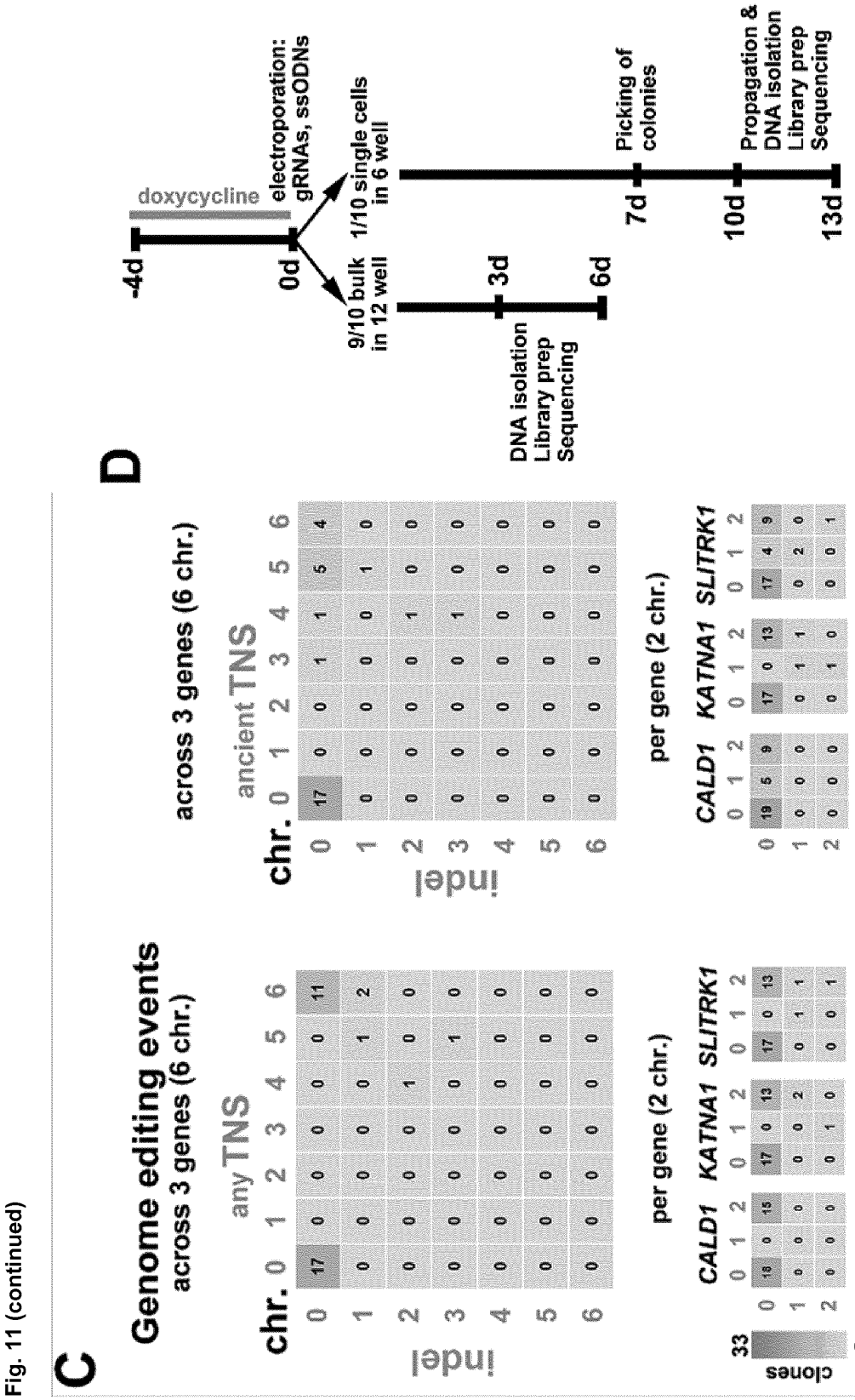


Fig. 11 (continued)

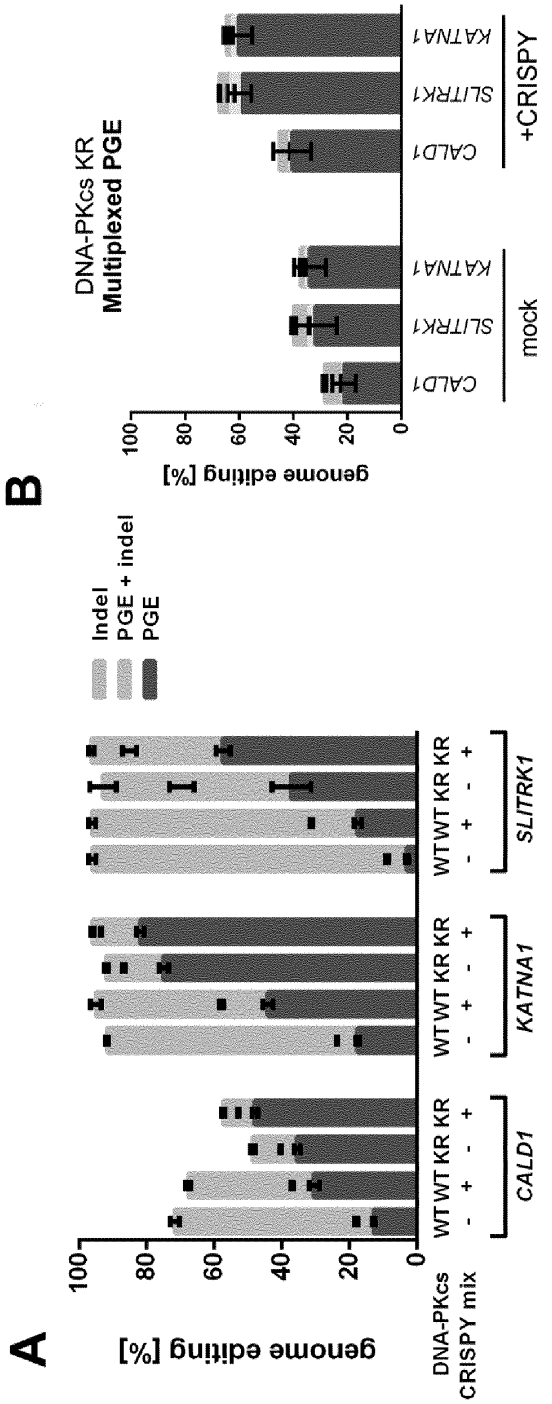

A
genome editing [%]
100
80
60
40
20
0
DNA-PKcs
CRISPY mix
WT WT KR KR
- + - +
CALD1
WT WT KR KR
- + - +
KATNA1
WT WT KR KR
- + - +
SLITRK1
Indel
PGE + indel
PGE
B
DNA-PKcs KR
Multiplexed PGE
genome editing [%]
100
80
60
40
20
0
CALD1
SLITRK1
KATNA1
mock
CALD1
SLITRK1
KATNA1
+CRISPY

Fig. 12:

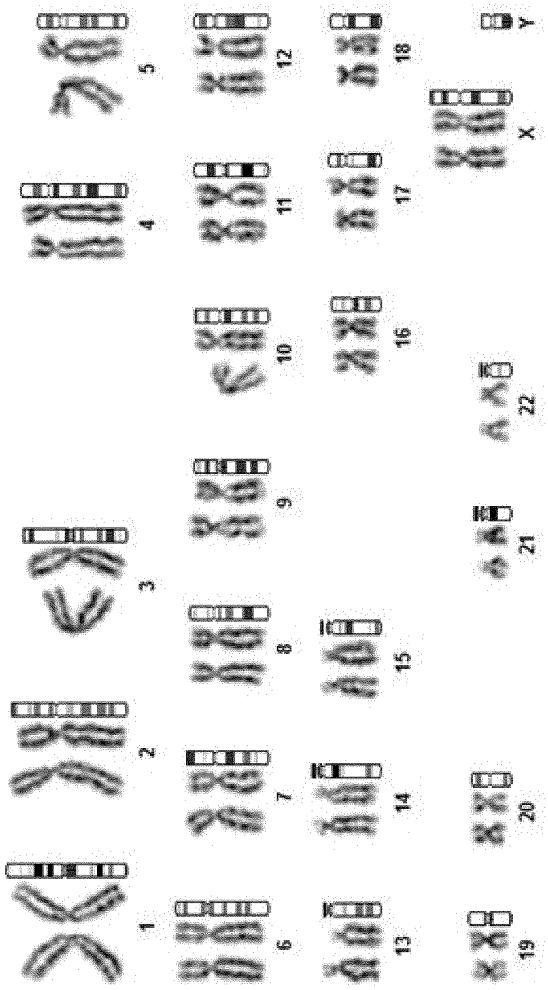


Fig. 13: