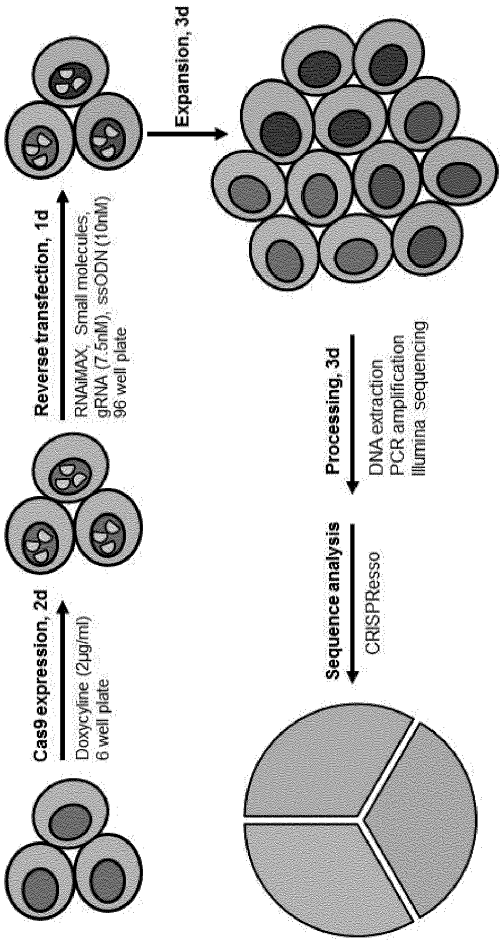
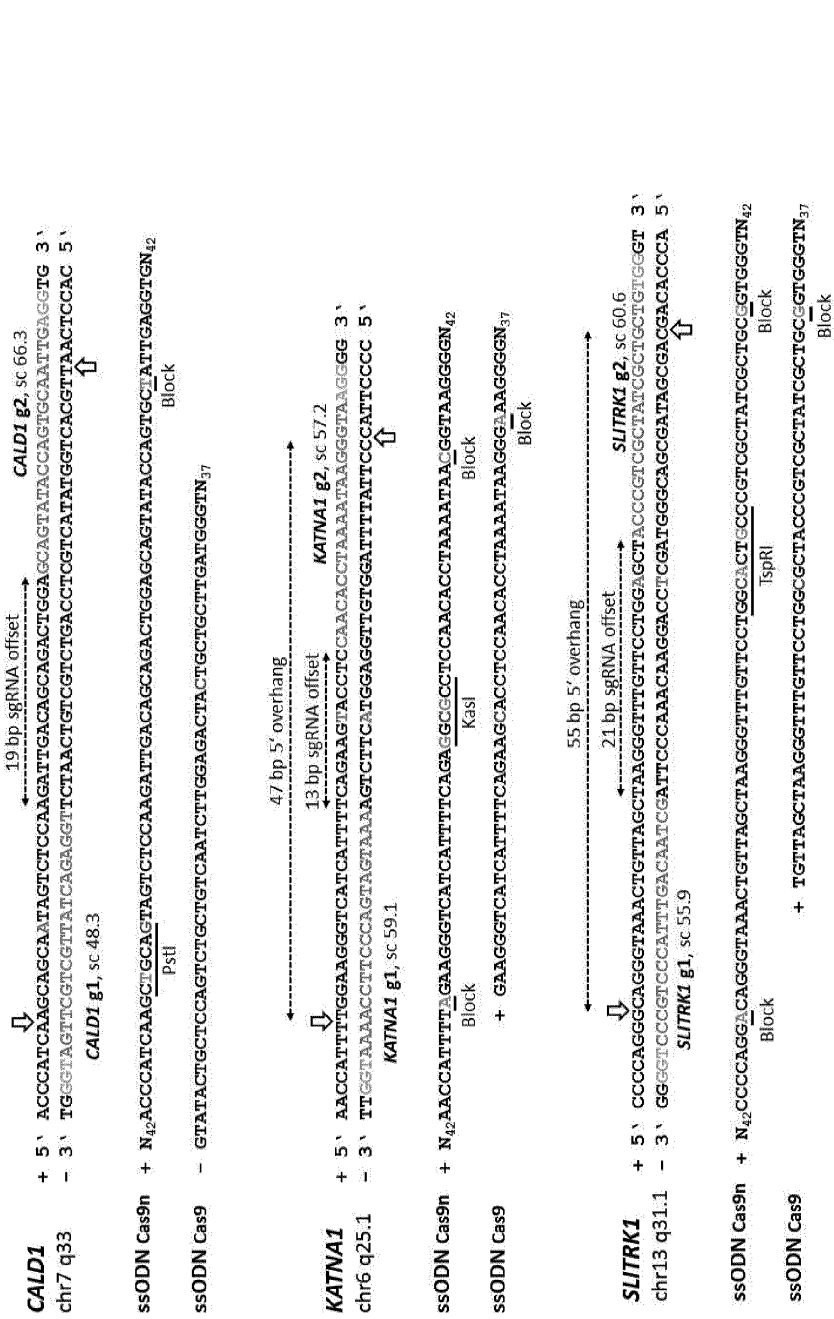

Cas9 expression, 2d
Doxycyline (2µg/ml)
6 well plate
Reverse transfection, 1d
RNAiMAX, Small molecules,
gRNA (7.5nM), ssODN (10nM)
96 well plate
Expansion, 3d
Processing, 3d
DNA extraction
PCR amplification
Illumina sequencing
Sequence analysis
CRISPResso

Fig. 1:

Fig. 2:


53 bp 5' overhang
19 bp sgRNA offset
CALD1 g2, sc 66.3
CALD1
chr7 q33
+ 5' ACCCATCAAGCAGCAATAGTCTCCAAGATTGACAGCAGACTGGAGCAGTATACCAGTGCAAATTGAGGTG 3'
- 3' TGGGTAGTTCGTCGTTATCAGAGGTTCTAACTGTCGTCTGACCTCGTCATATGGTCACGTTAACTCCAC 5'
CALD1 g1, sc 48.3
ssODN Cas9n + N42ACCCATCAAGCTGCAGTAGTCTCCAAGATTGACAGCAGACTGGAGCAGTATACCAGTGCTATTGAGGTGN42
PstI
Block
ssODN Cas9 - GTATACTGGTCCAGTCTGCTGTCAATCTTGGAGACTACTGCTGCTTGATGGGTTN37
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 + N42AACCATTTTAGAAGGGTCATCATTTTCAGAGGCGCCTCCAACACCTAAAATAAGGTAAGGGGN42
Block
KasI
Block
ssODN Cas9 + GAAGGGTCATCATTTTCAGAAGCACCTCCAACACCTAAAATAAGGGAAAGGGGN37
Block
55 bp 5' overhang
21 bp sgRNA offset
SLITRK1 g2, sc 60.6
SLITRK1
chr13 q31.1
+ 5' CCCCAGGGCAGGGTAAACTGTTAGCTAAGGGTTTGTTCCTGGAGCTACCCGTCGCTATCGCTGCGGTGGGTN42 3'
- 3' GGGGTCCCGTCCCATTTGACAATCGATTCCCAAACAAGGACCTCGATGGGCAGCGATAGCGACGACACCCA 5'
SLITRK1 g1, sc 55.9
ssODN Cas9n + N42CCCCAGGAAGGGTAAACTGTTAGCTAAGGGTTTGTTCCTGGCACTGCCCGTCGCTATCGCTGCGGTGGGTN42
Block
TspRI
Block
ssODN Cas9 + TGTTAGCTAAGGGTTTGTTCCTGGCGCTACCCGTCGCTATCGCTGCGGTGGGTN37
Block

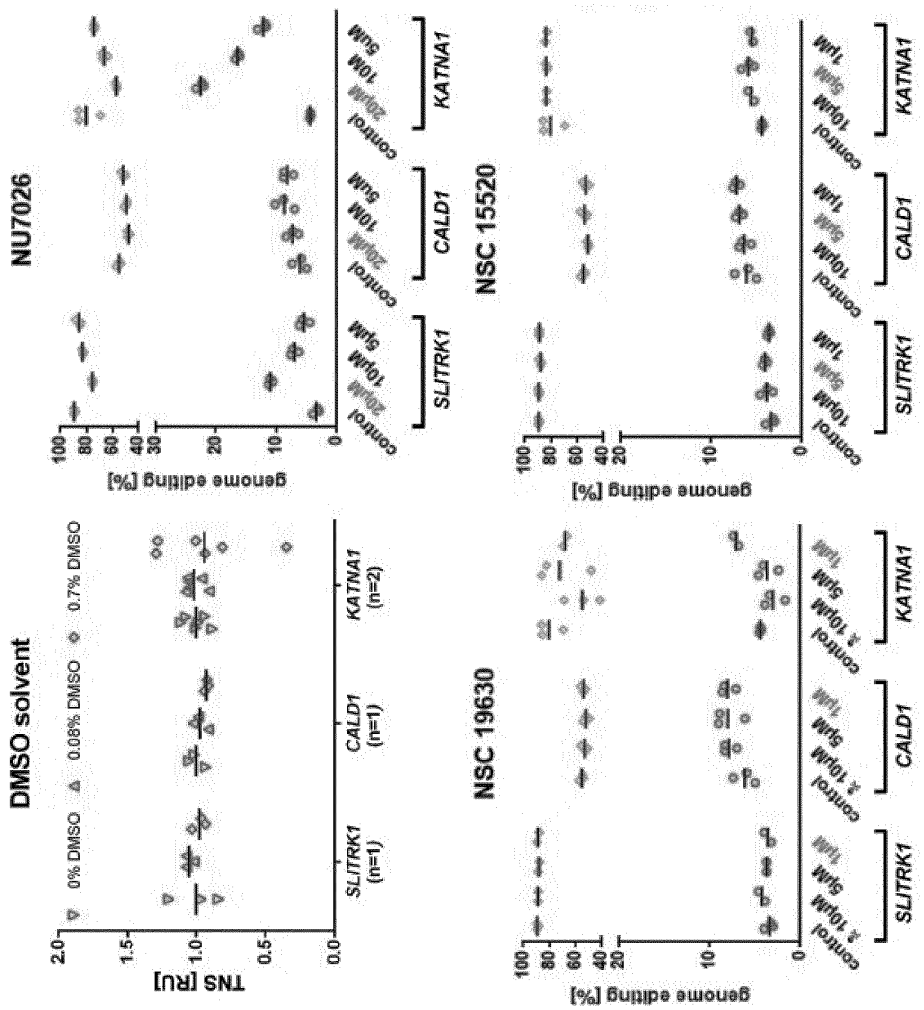

DMSO solvent
0% DMSO
0.08% DMSO
0.7% DMSO
TNS [RU]
SLITRK1 (n=1)
CALD1 (n=1)
KATNA1 (n=2)
NU7026
genome editing [%]
control
2.5µM
10µM
5µM
SLITRK1
CALD1
KATNA1
NSC 15520
NSC 19630
1µM
5µM
10µM
x 10µM

Fig. 3:

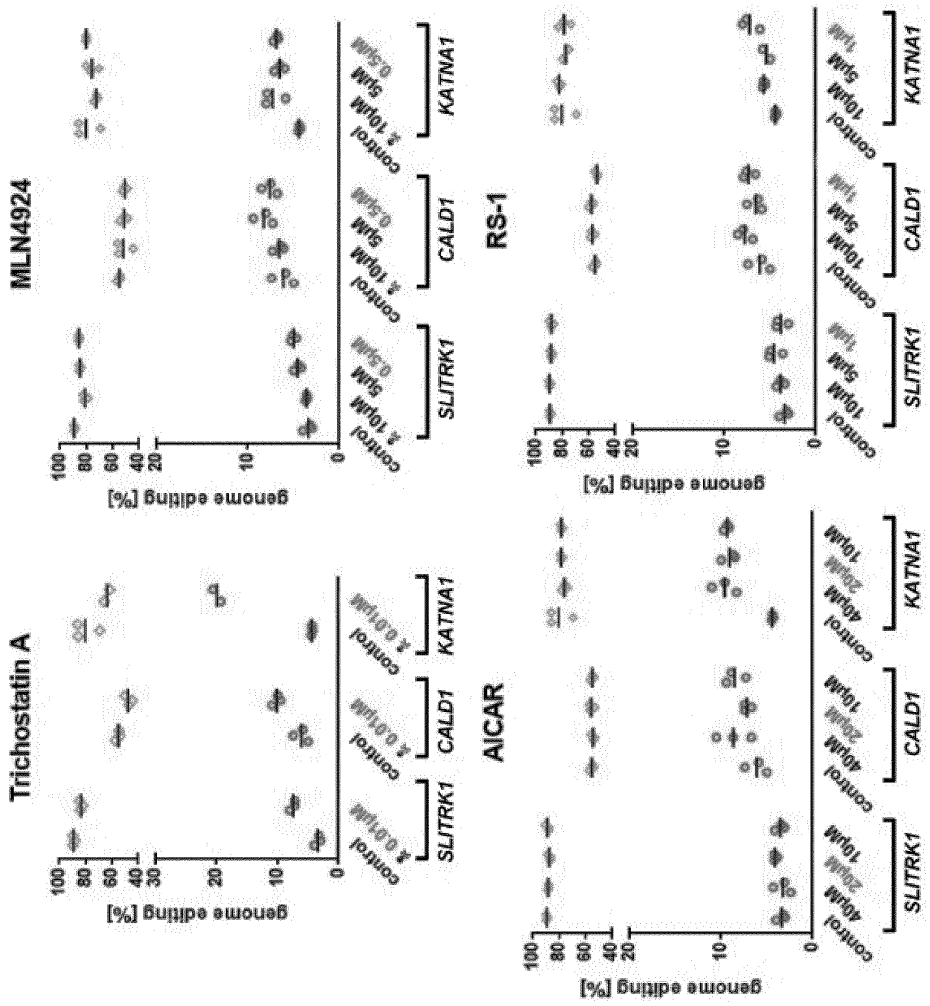


Fig. 3 (continued)

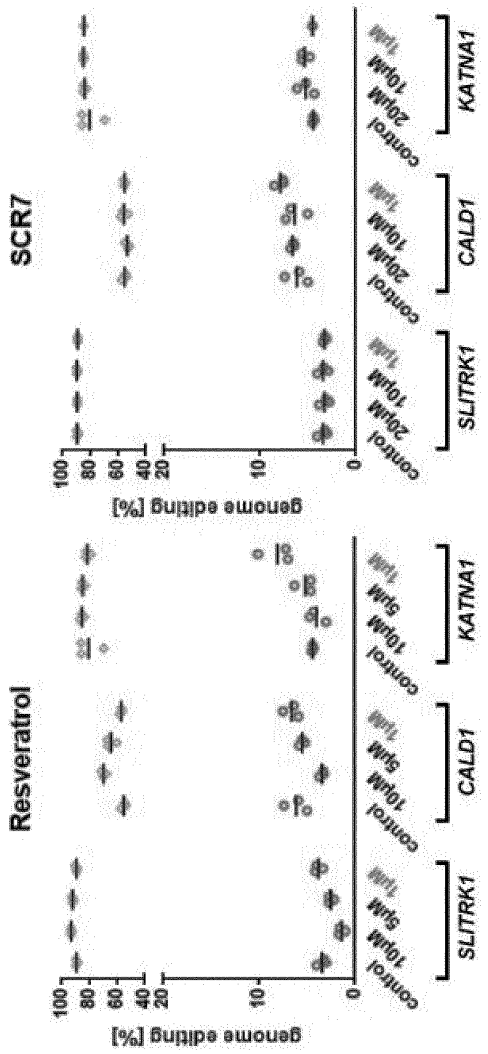


Fig. 3 (continued)

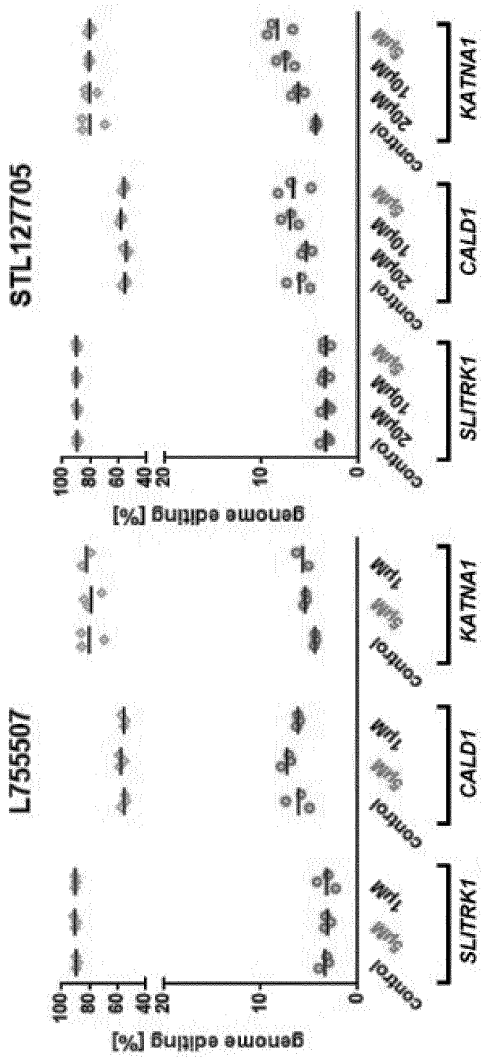


Fig. 3 (continued)

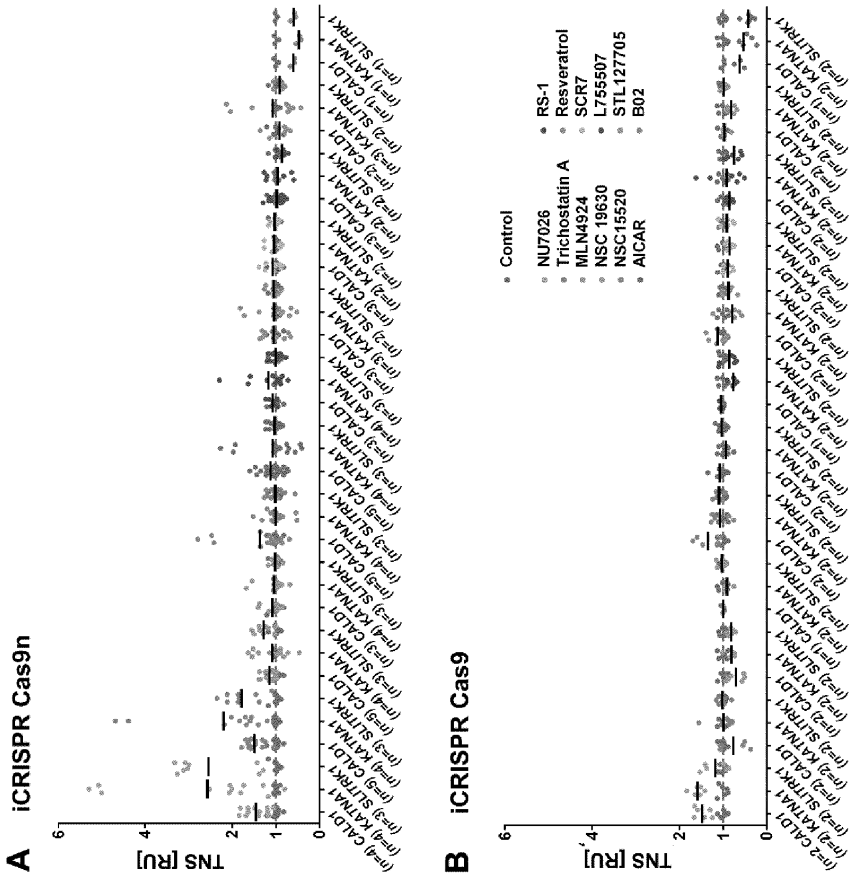

A
iCRISPR Cas9n
TNS [RU]
B
iCRISPR Cas9
TNS [RU]
Control
NU7026
Trichostatin A
MLN4924
NSC 19630
NSC15520
AICAR
RS-1
Resveratrol
SCR7
L755507
STL127705
B02

Fig. 4:

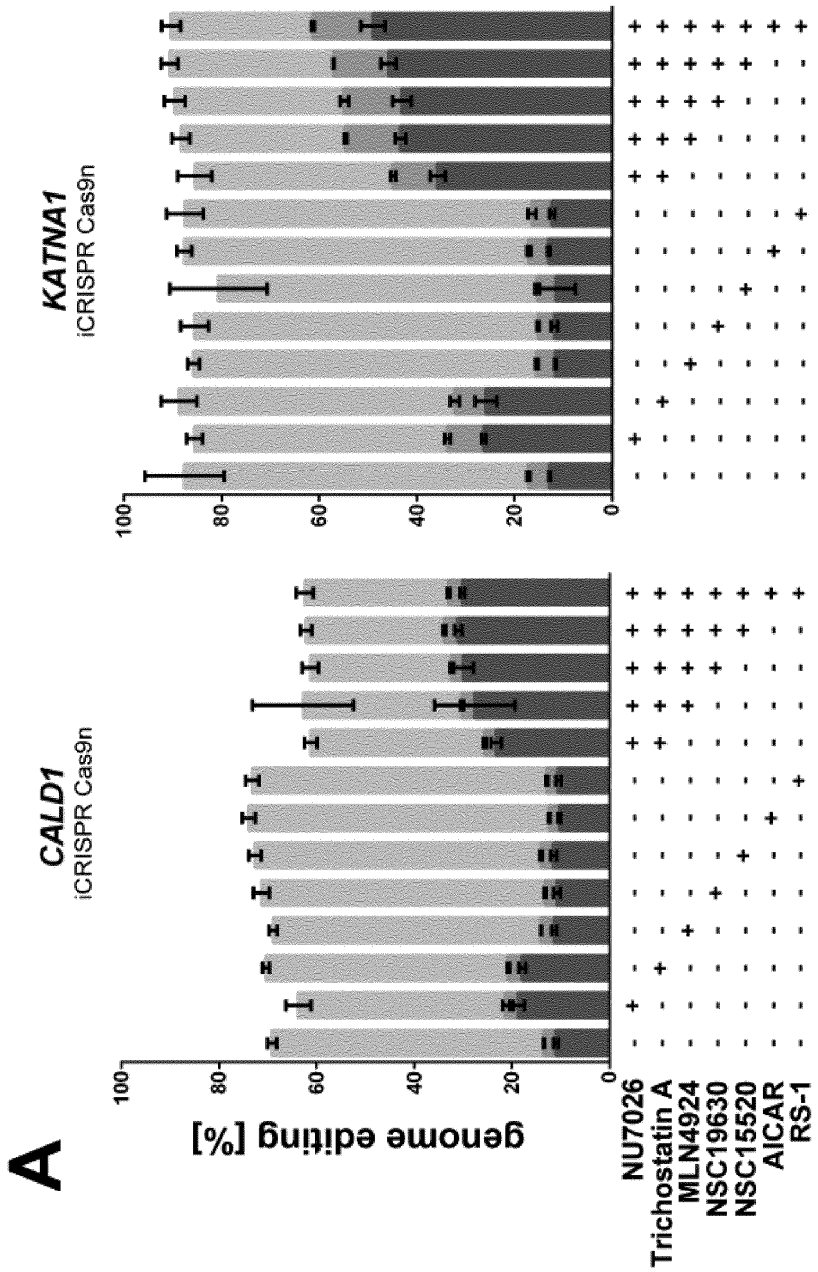

A
CALD1
iCRISPR Cas9n
KATNA1
iCRISPR Cas9n
genome editing [%]
100
80
60
40
20
0
NU7026
Trichostatin A
MLN4924
NSC19630
NSC15520
AICAR
RS-1

Fig. 5:

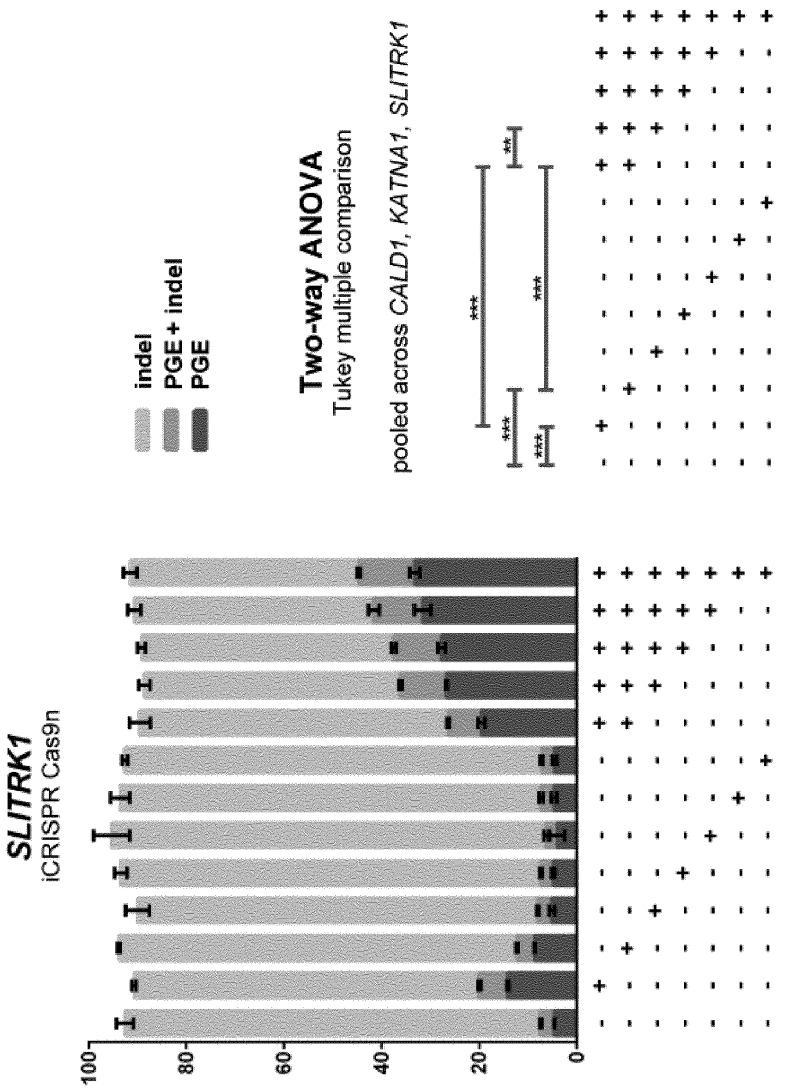


Fig. 5A (continued)

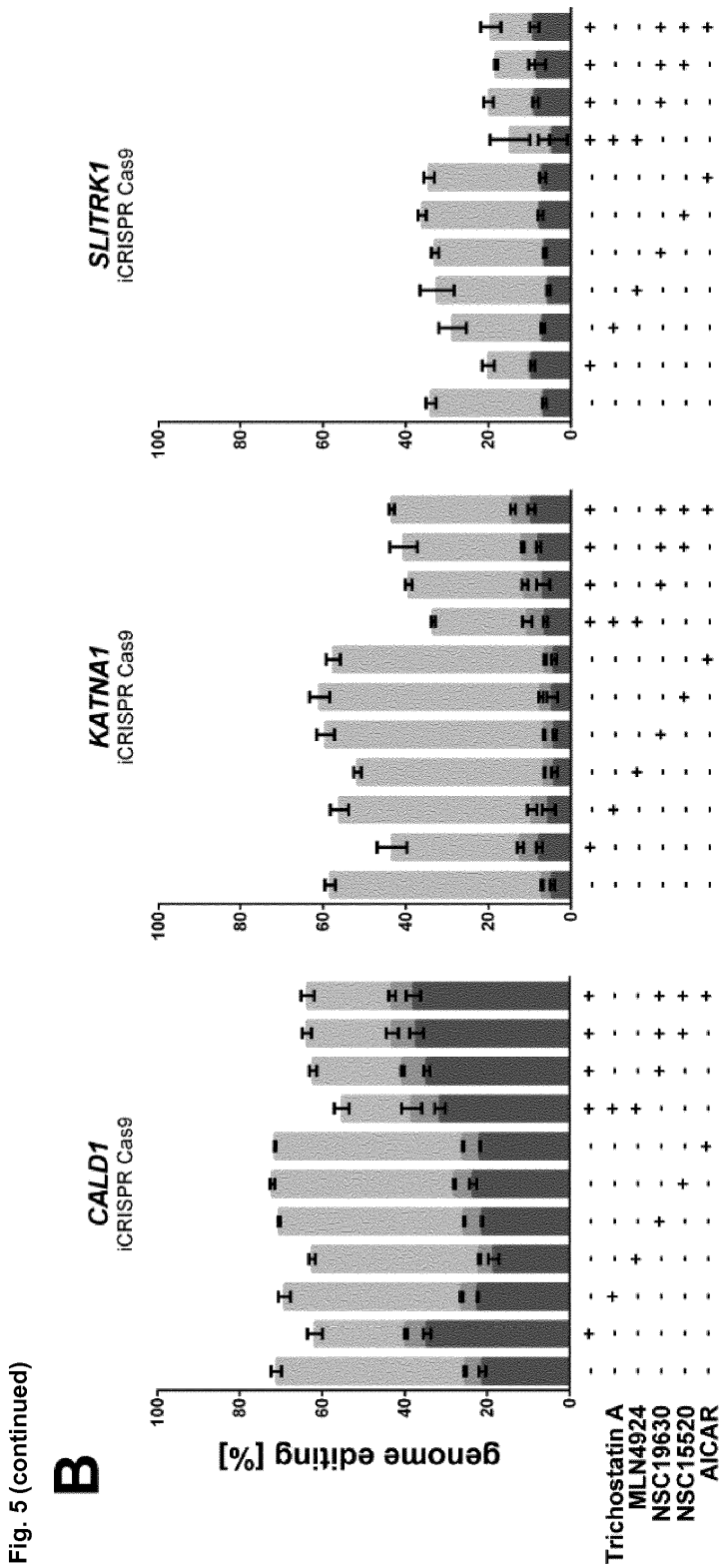


Fig. 5 (continued)

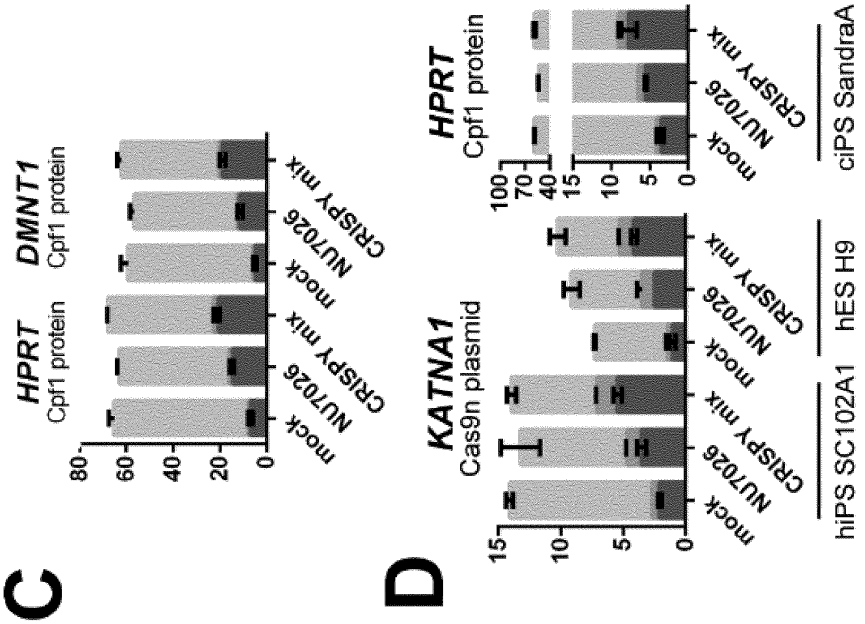


Fig. 5 (continued)

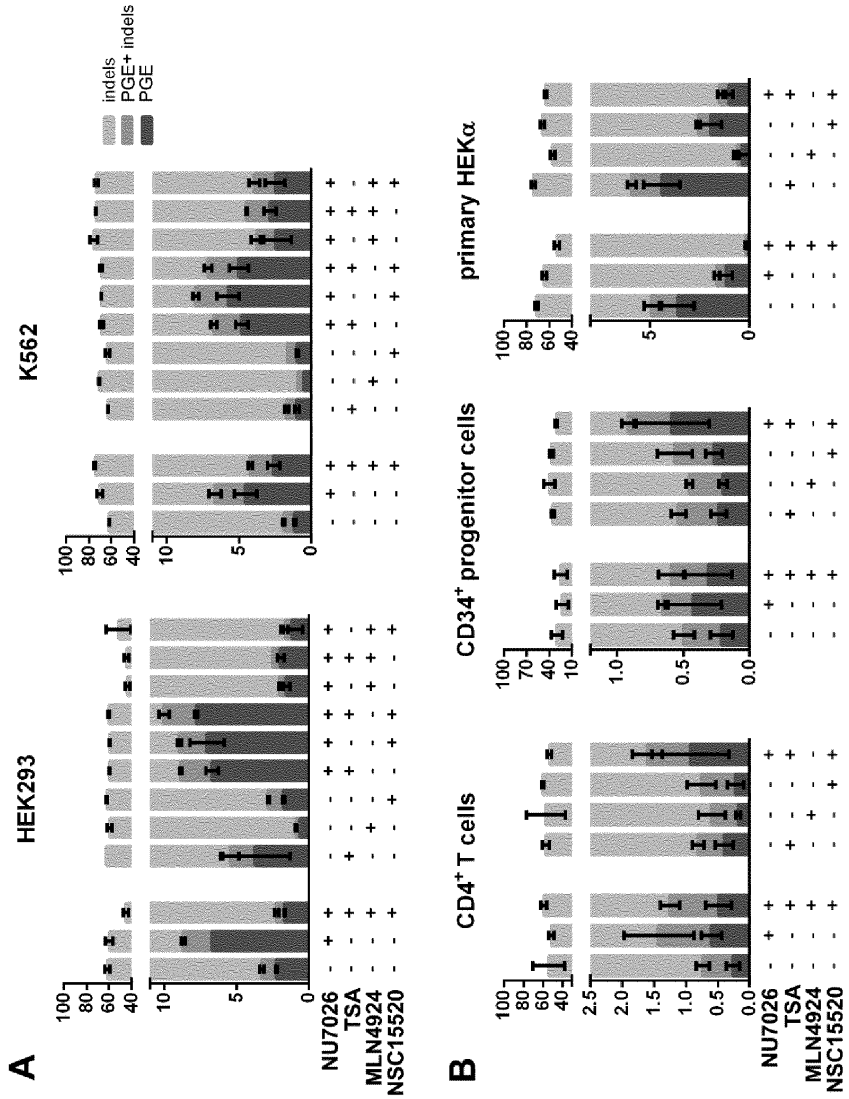
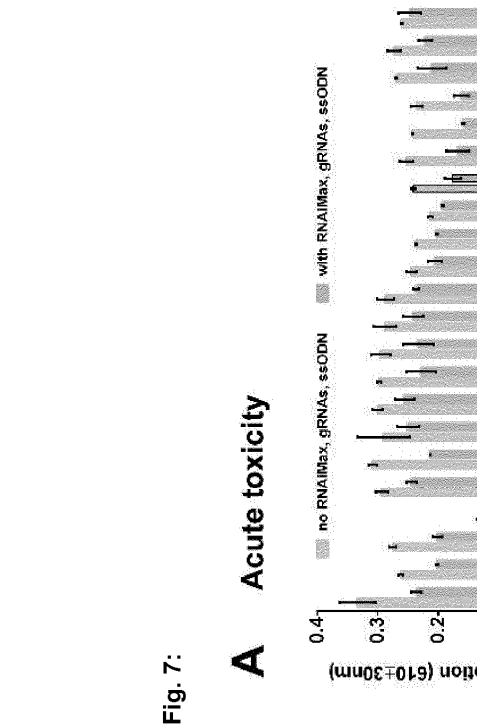

A
HEK293
K562
indels
PGE+ indels
PGE
NU7026
TSA
MLN4924
NSC15520
B
CD4+ T cells
CD34+ progenitor cells
primary HEKα

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

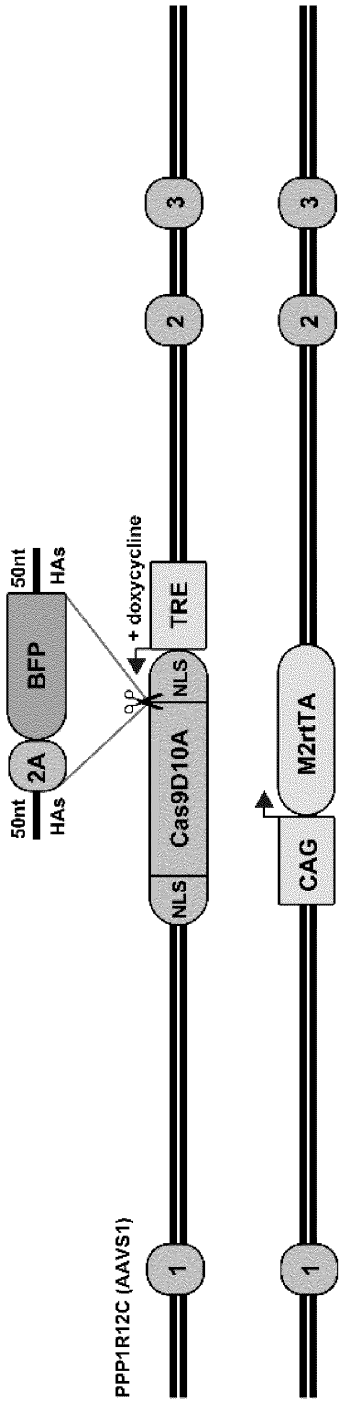

A
PPP1R12C (AAVS1)
1
2
3
CAG
M2rtTA
NLS
Cas9D10A
NLS
TRE
+ doxycycline
50nt
HAs
2A
BFP
50nt
HAs

Fig. 8:

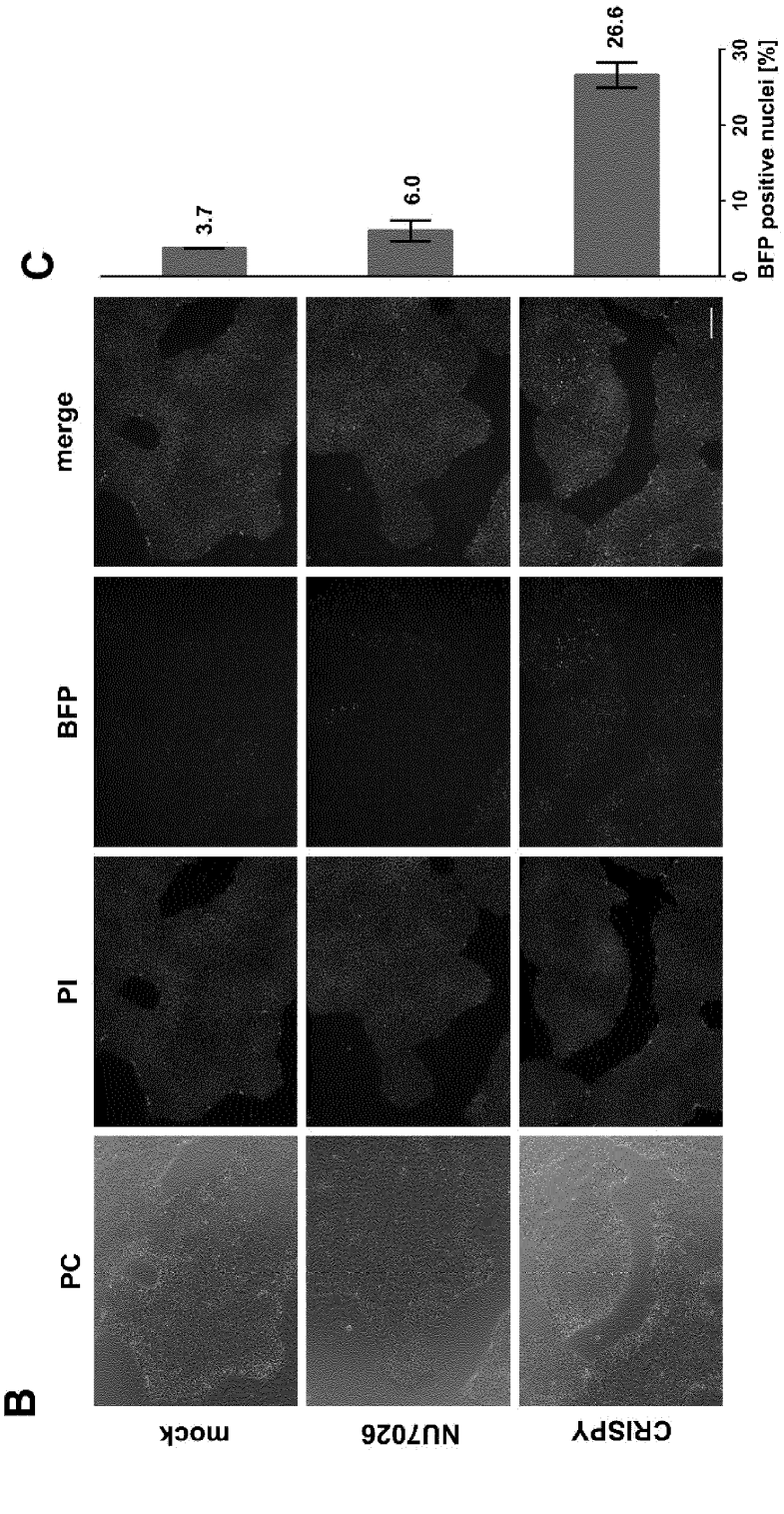


Fig. 8 (continued)

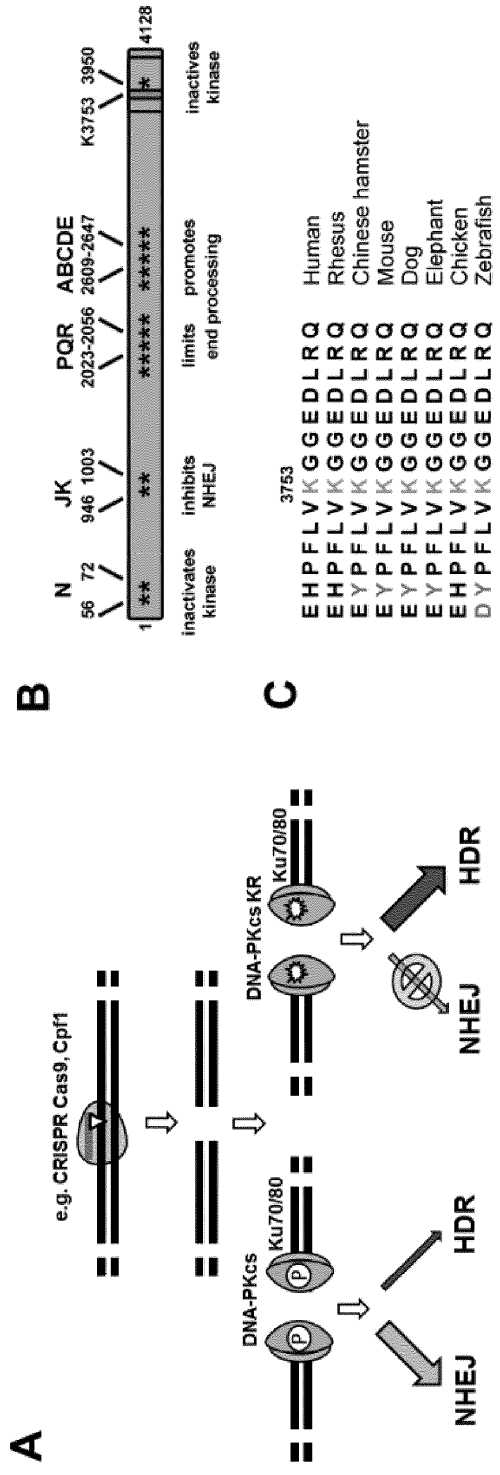

A
e.g. CRISPR Cas9, Cpf1
DNA-PKcs
Ku70/80
DNA-PKcs KR
Ku70/80
NHEJ
HDR
NHEJ
HDR
B
N
JK
PQR
ABCDE
56 72
946 1003
2023-2056 2609-2647
K3753 3950
4128
1
inactivates kinase
inhibits NHEJ
limits end processing
promotes
inactives kinase
C
3753
EHPFLVKGGEDLRQ Human
EHPFLVKGGEDLRQ Rhesus
EYPFLVKGGEDLRQ Chinese hamster
EYPFLVKGGEDLRQ Mouse
EYPFLVKGGEDLRQ Dog
EYPFLVKGGEDLRQ Elephant
EHPFLVKGGEDLRQ Chicken
DYPFLVKGGEDLRQ Zebrafish

Fig. 9:

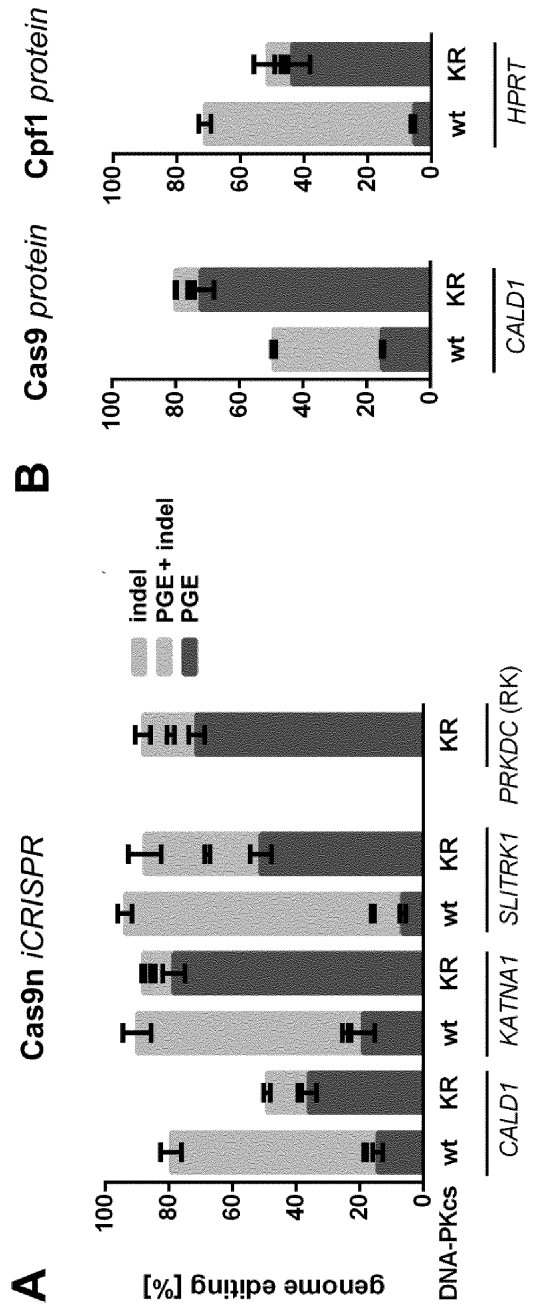

A
Cas9n iCRISPR
genome editing [%]
indel
PGE + indel
PGE
DNA-PKcs
wt
KR
wt
KR
wt
KR
wt
KR
CALD1
KATNA1
SLITRK1
PRKDC (RK)
B
Cas9 protein
Cpf1 protein
CALD1
HPRT

Fig. 10:

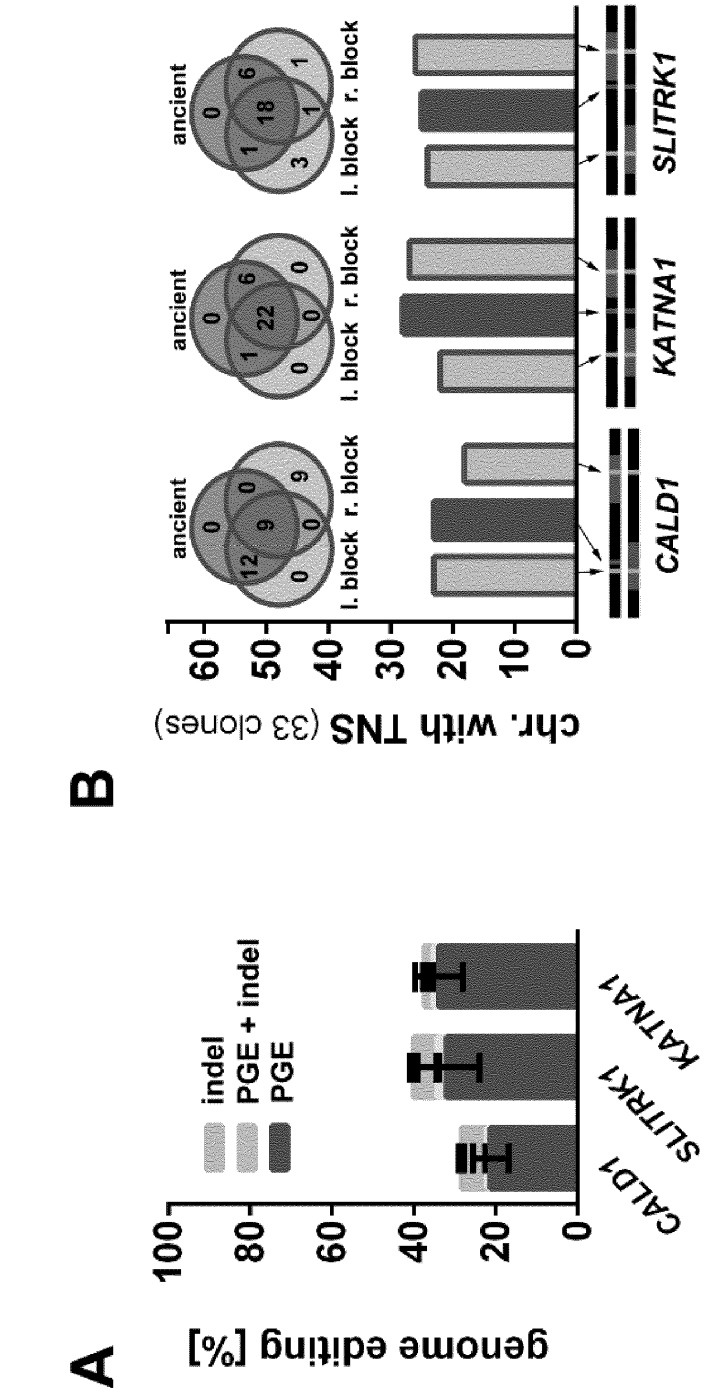

A
genome editing [%]
100
80
60
40
20
0
indel
PGE + indel
PGE
CALD1
SLITRK1
KATNA1
B
chr. with TNS (33 clones)
60
50
40
30
20
10
0
ancient
l. block
r. block
0
12
9
0
0
9
0
ancient
l. block
r. block
0
1
22
6
0
0
0
ancient
l. block
r. block
0
1
18
6
3
1
1
CALD1
KATNA1
SLITRK1

Fig. 11:

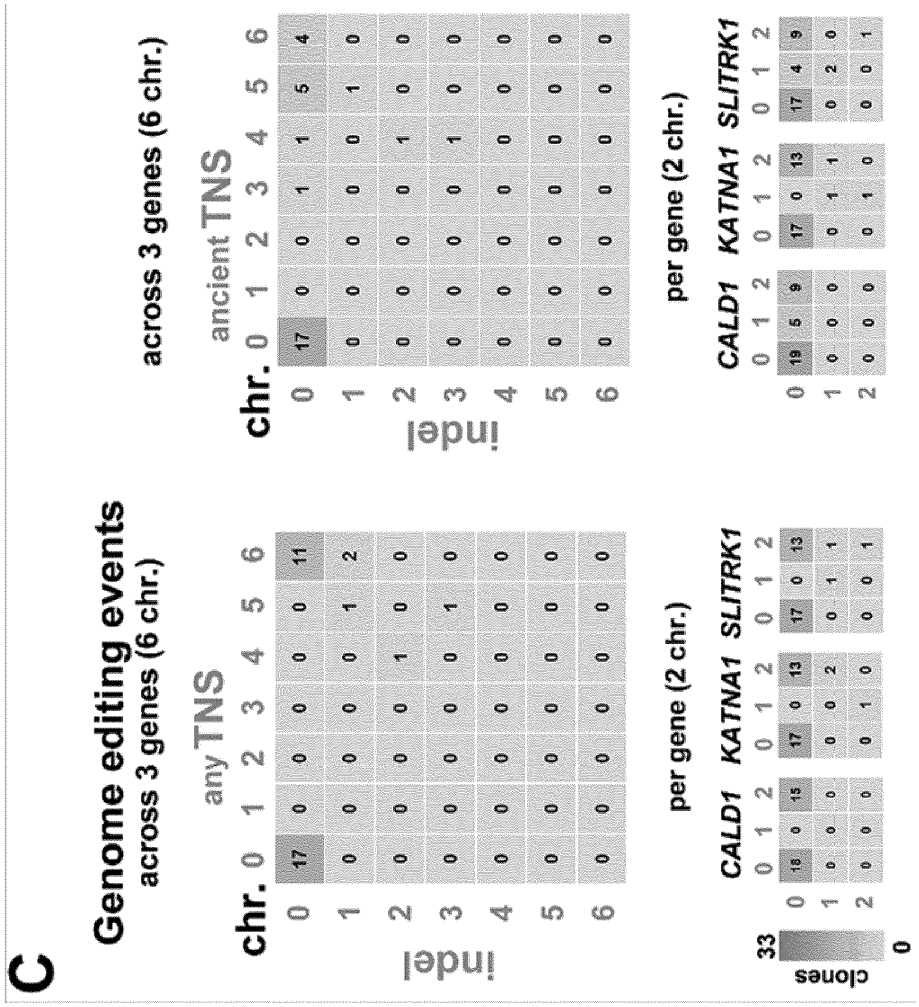


Fig. 11 (continued)

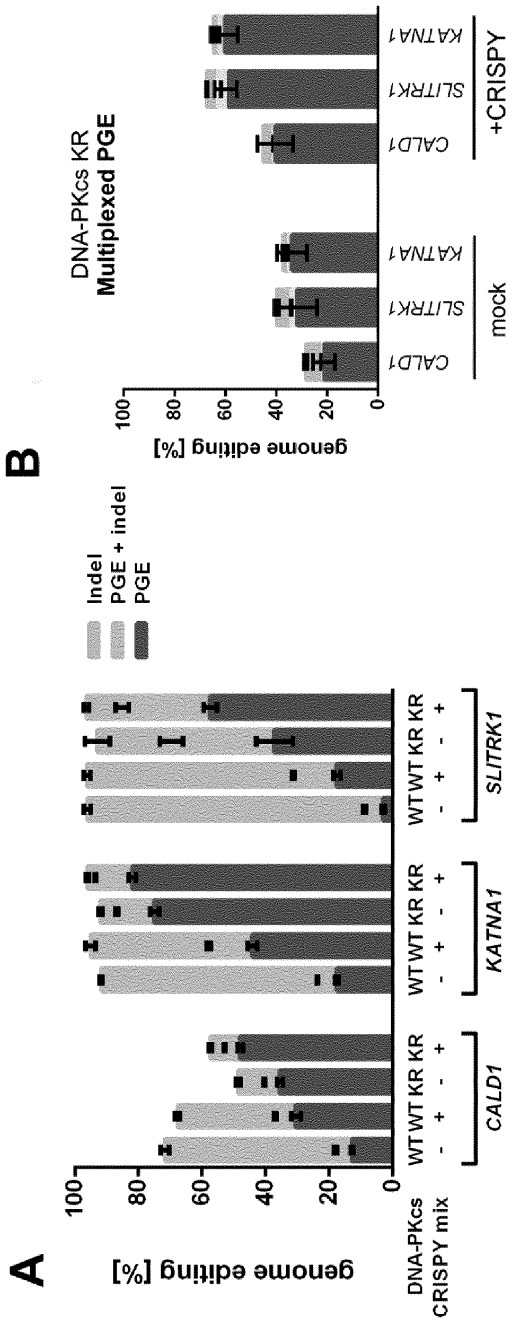

A
genome editing [%]
100
80
60
40
20
0
DNA-PKcs
CRISPY mix
WT WT KR KR
- + - +
CALD1
KATNA1
SLITRK1
Indel
PGE + indel
PGE
B
DNA-PKcs KR
Multiplexed PGE
genome editing [%]
CALD1
SLITRK1
KATNA1
mock
+CRISPY

Fig. 12:

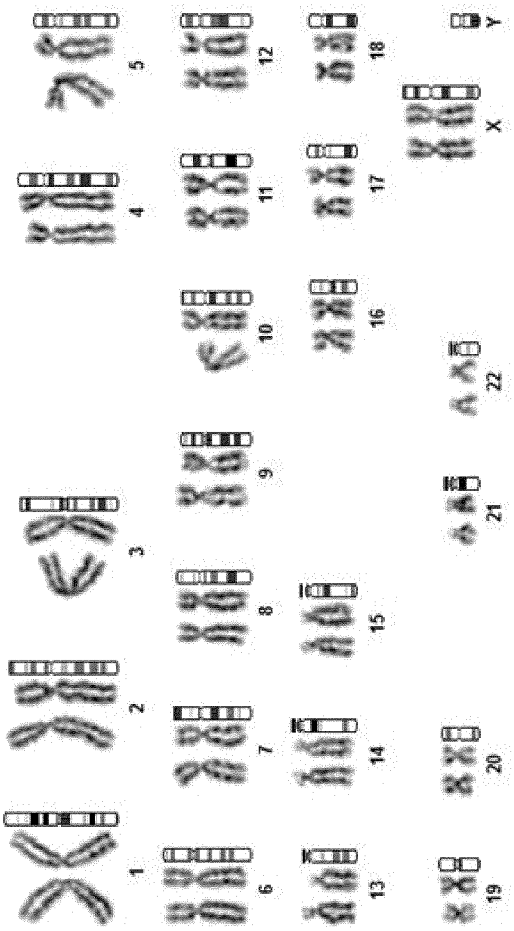


Fig. 13: