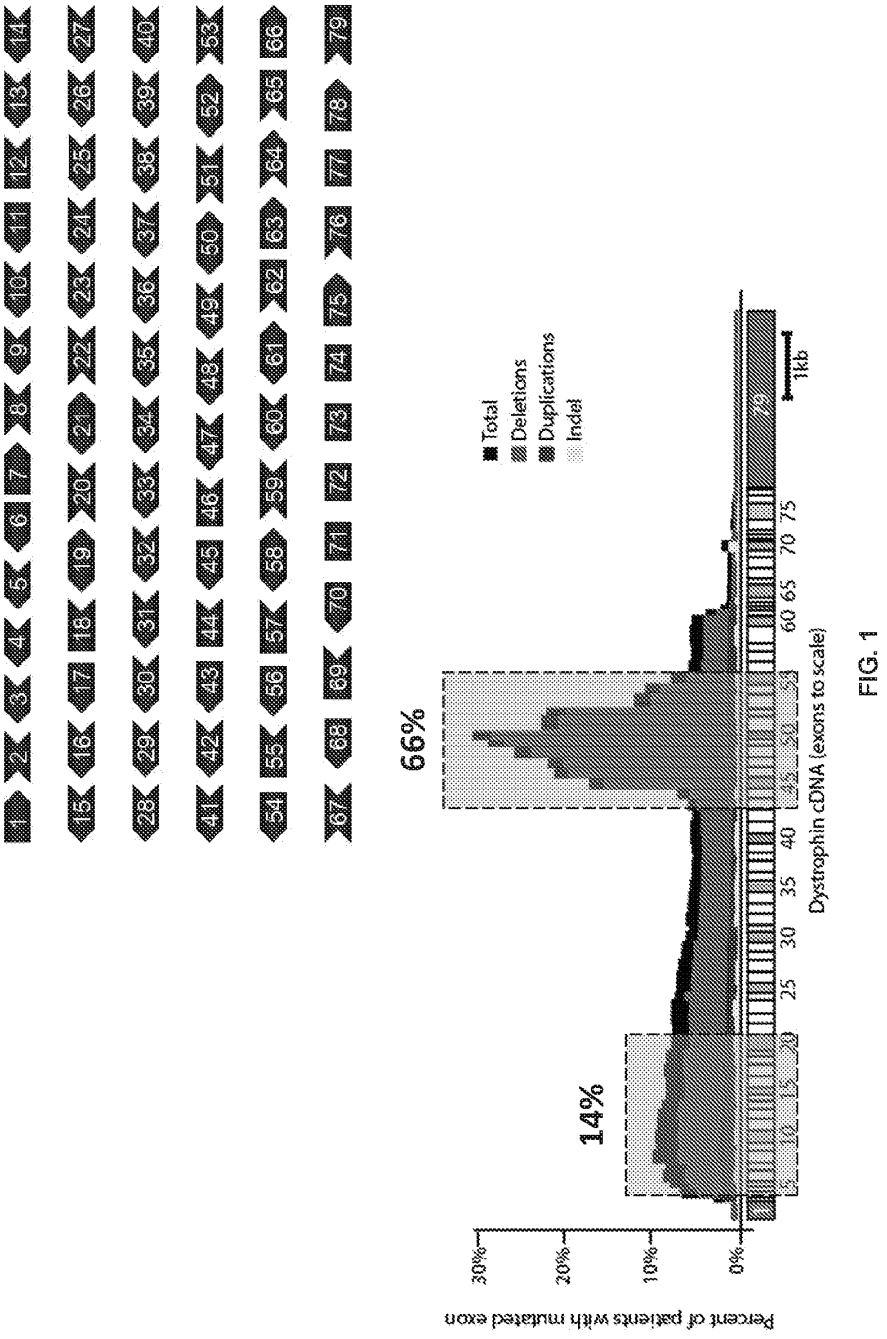

1 2 3 4 5 6 7 8 9 10 11 12 13 14
15 16 17 18 19 20 21 22 23 24 25 26 27
28 29 30 31 32 33 34 35 36 37 38 39 40
41 42 43 44 45 46 47 48 49 50 51 52 53
54 55 56 57 58 59 60 61 62 63 64 65 66
67 68 69 70 71 72 73 74 75 76 77 78 79
66%
14%
Total
Deletions
Duplications
Indel
1kb
Dystrophin cDNA (exons to scale)
Percent of patients with mutated exon
30%
20%
10%
0%

FIG. 1

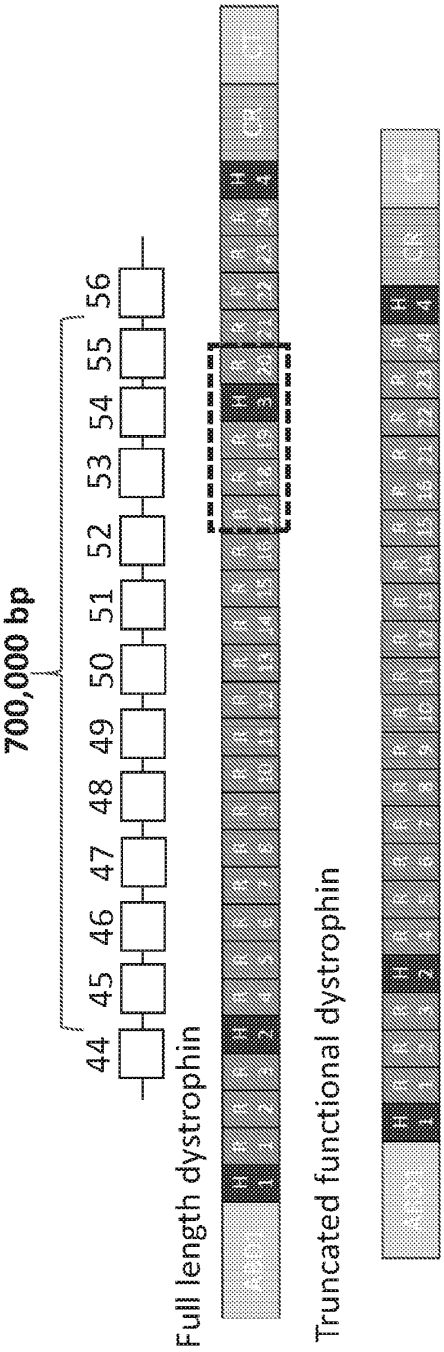

700,000 bp
44
45
46
47
48
49
50
51
52
53
54
55
56
Full length dystrophin
Truncated functional dystrophin

FIG. 2

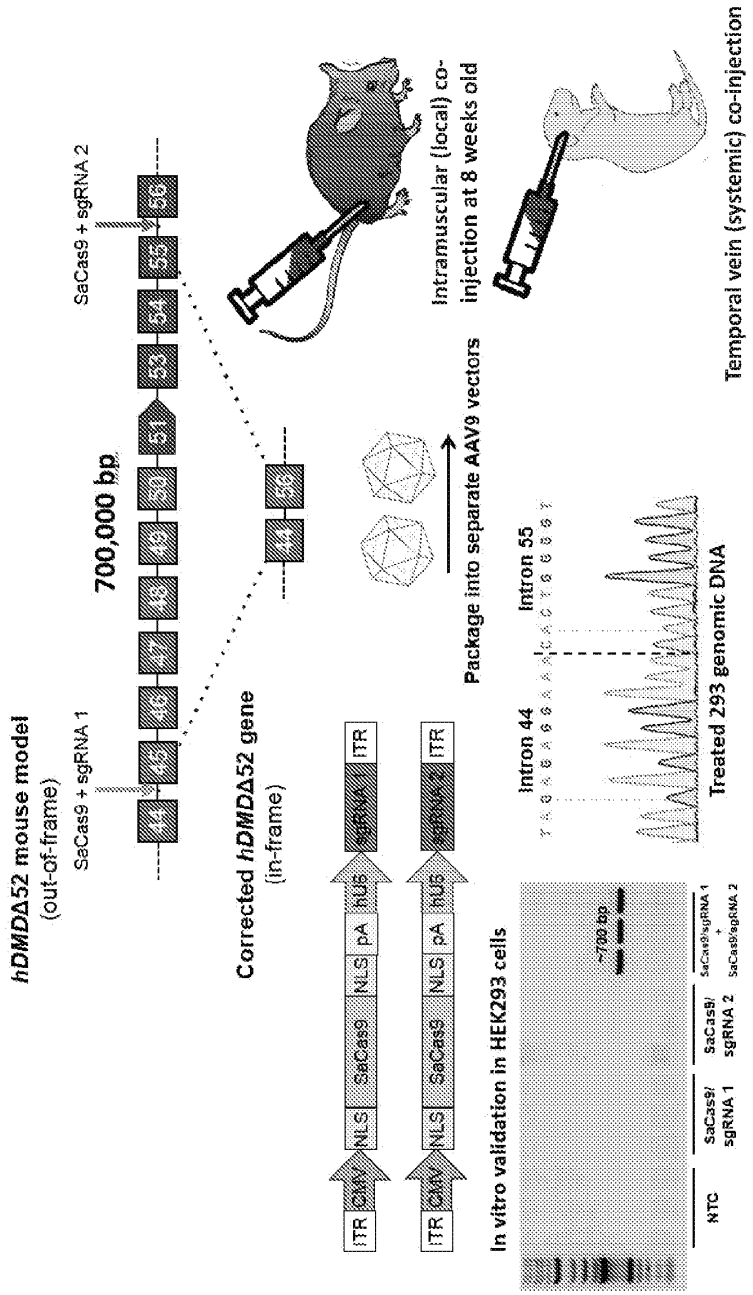

hDMDΔ52 mouse model
(out-of-frame)
SaCas9 + sgRNA 1
SaCas9 + sgRNA 2
700,000 bp
44
45
46
47
48
49
50
51
53
54
55
56
Corrected hDMDΔ52 gene
(in-frame)
44
56
Package into separate AAV9 vectors
Intramuscular (local) co-injection at 8 weeks old
Temporal vein (systemic) co-injection at 2 days postnatal
ITR
CMV
NLS
SaCas9
NLS
pA
hU6
sgRNA
ITR
In vitro validation in HEK293 cells
Intron 44
Intron 55
Treated 293 genomic DNA
~739 bp
NTC
SaCas9/ sgRNA 1
SaCas9/ sgRNA 2
SaCas9/sgRNA 1 + SaCas9/sgRNA 2

FIG. 3

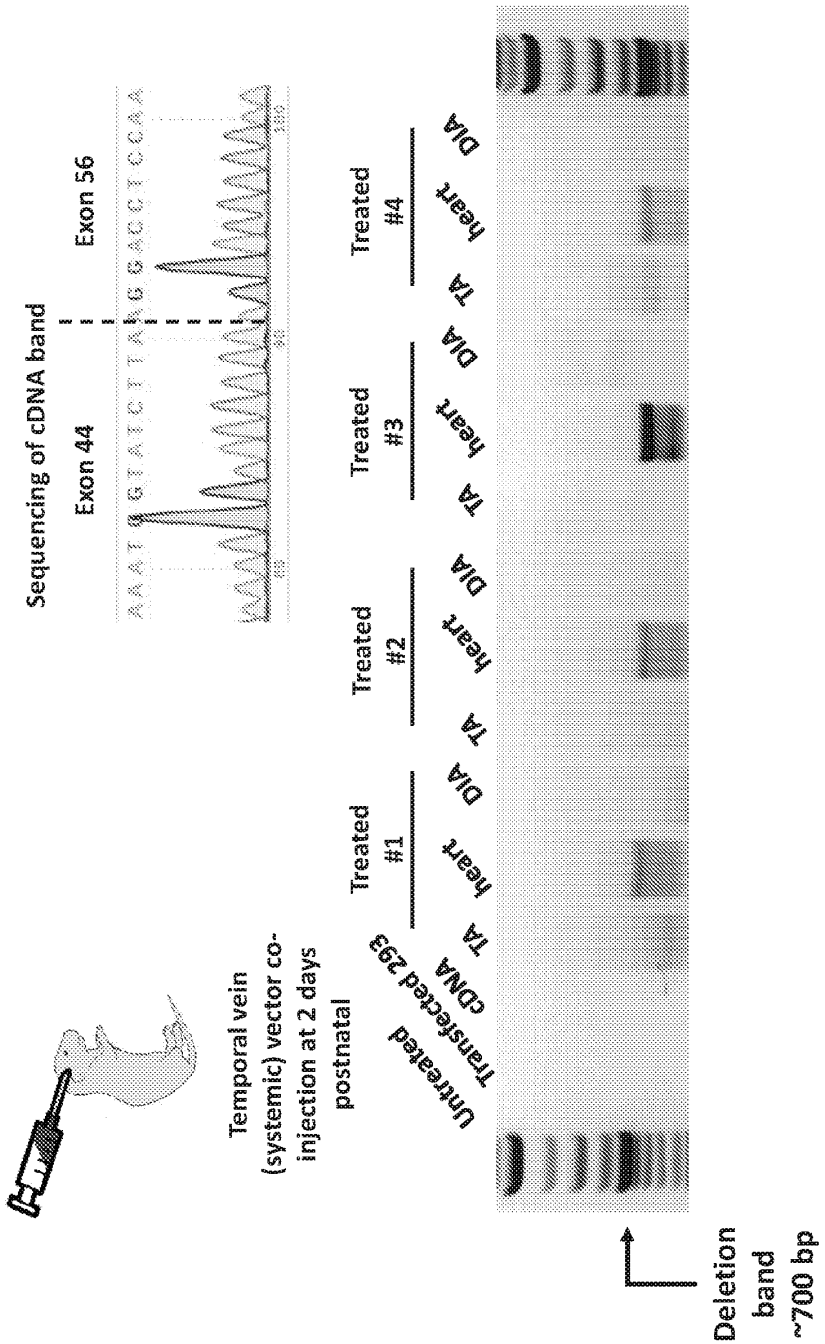

Sequencing of cDNA band
Exon 44
Exon 56
AAATGTATCTTATAGGACCTCCAA
Temporal vein (systemic) vector co-injection at 2 days postnatal
Untreated
Transfected 293 cDNA
Treated #1
Treated #2
Treated #3
Treated #4
TA
heart
DIA
Deletion band ~700 bp

FIG. 4

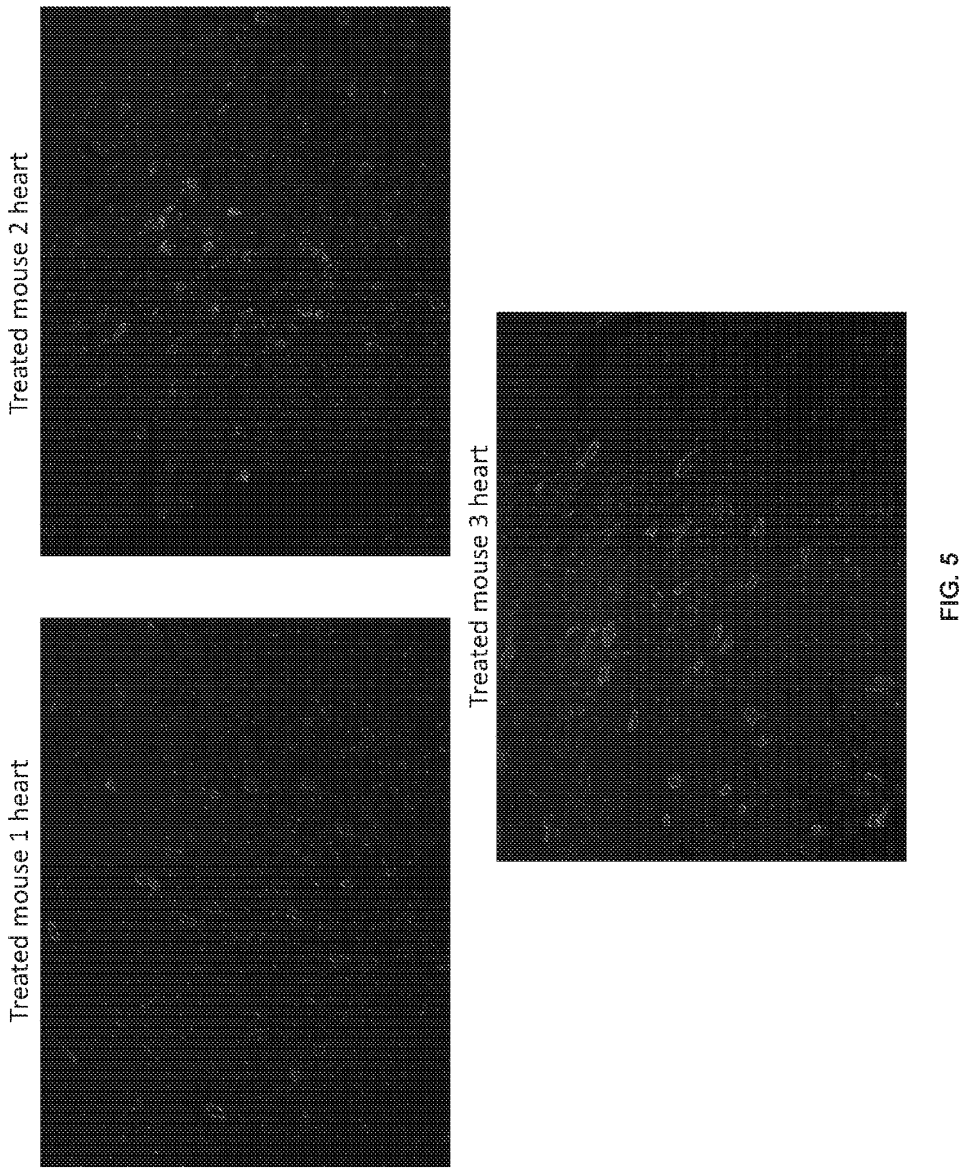

Treated mouse 2 heart
Treated mouse 1 heart
Treated mouse 3 heart

FIG. 5

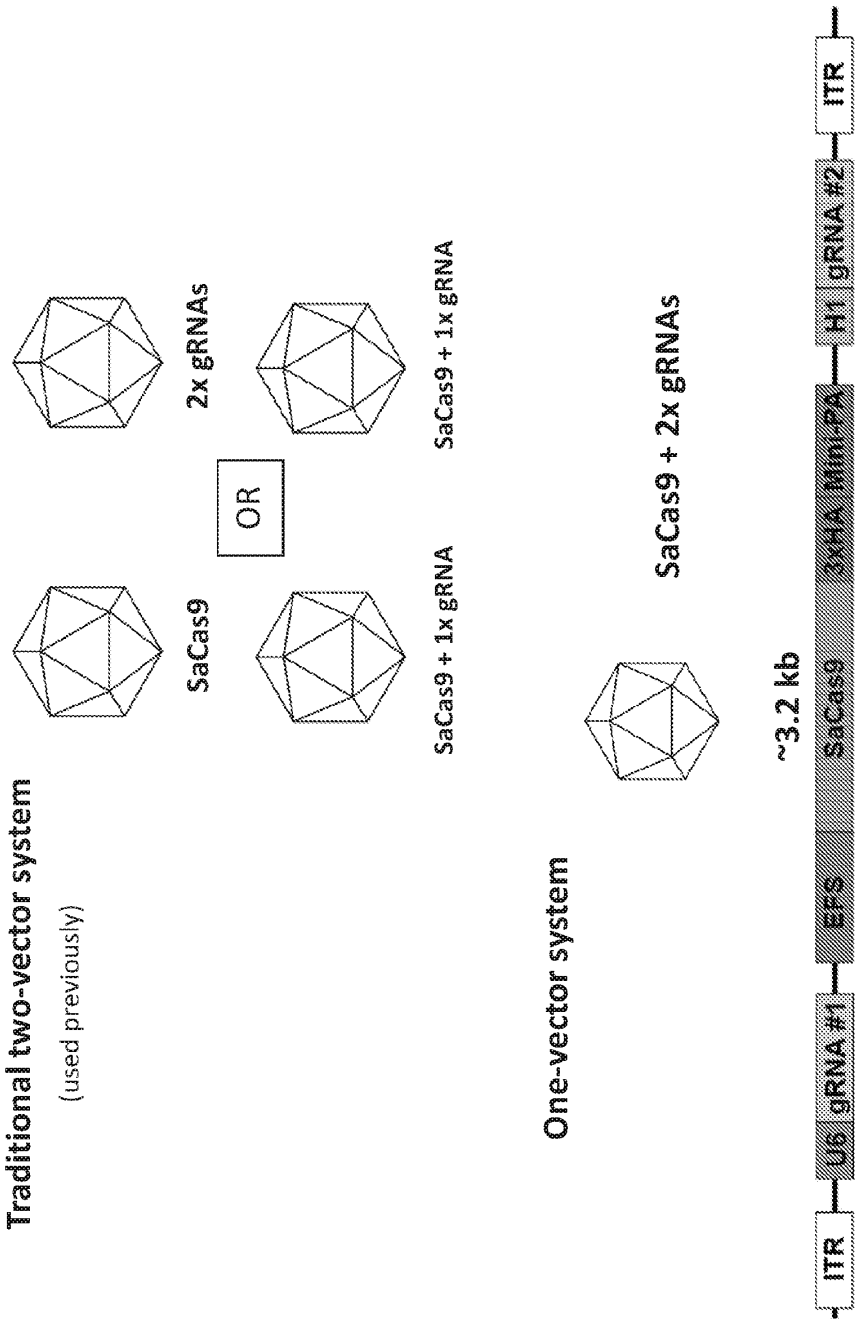

Traditional two-vector system
(used previously)
SaCas9
2x gRNAs
OR
SaCas9 + 1x gRNA
SaCas9 + 1x gRNA
SaCas9 + 1x gRNA
One-vector system
SaCas9 + 2x gRNAs
~3.2 kb
ITR
U6
gRNA #1
EFS
SaCas9
3xHA
Mini-PA
H1
gRNA #2
ITR

FIG. 6

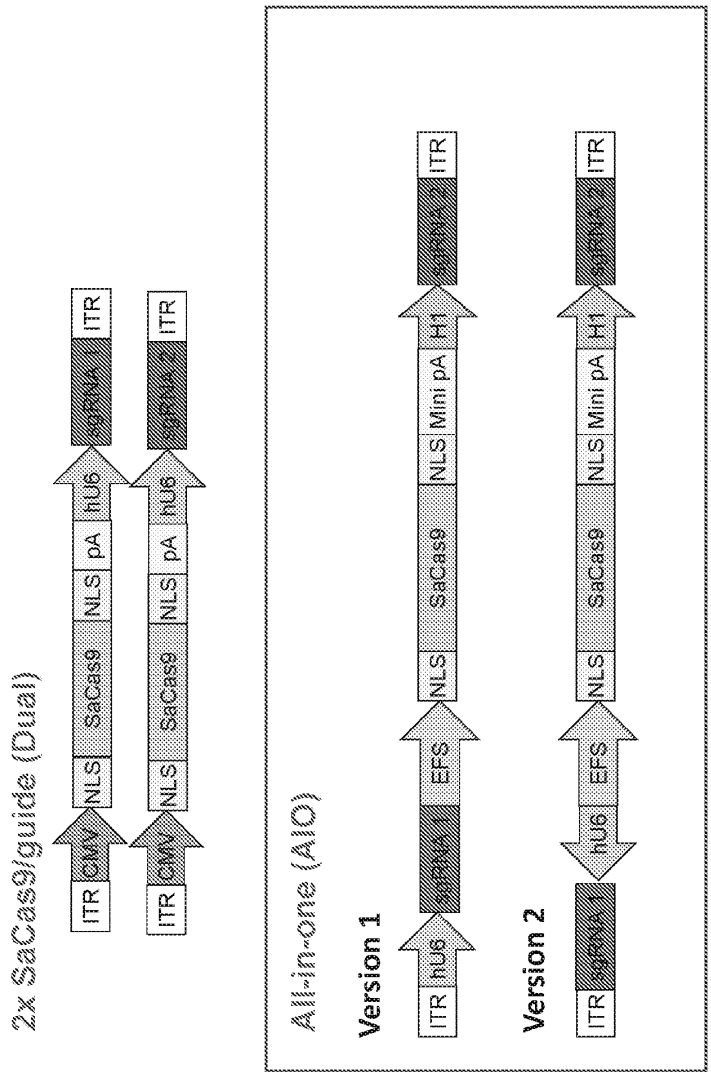

2x SaCas9/guide (Dual)
ITR
CMV
NLS
SaCas9
NLS
pA
hU6
gRNA 1
ITR
ITR
CMV
NLS
SaCas9
NLS
pA
hU6
gRNA 2
ITR
All-in-one (AIO)
Version 1
ITR
hU6
gRNA 1
EFS
NLS
SaCas9
NLS
Mini pA
H1
gRNA 2
ITR
Version 2
ITR
gRNA 1
hU6
EFS
NLS
SaCas9
NLS
Mini pA
H1
gRNA 2
ITR

FIG. 7

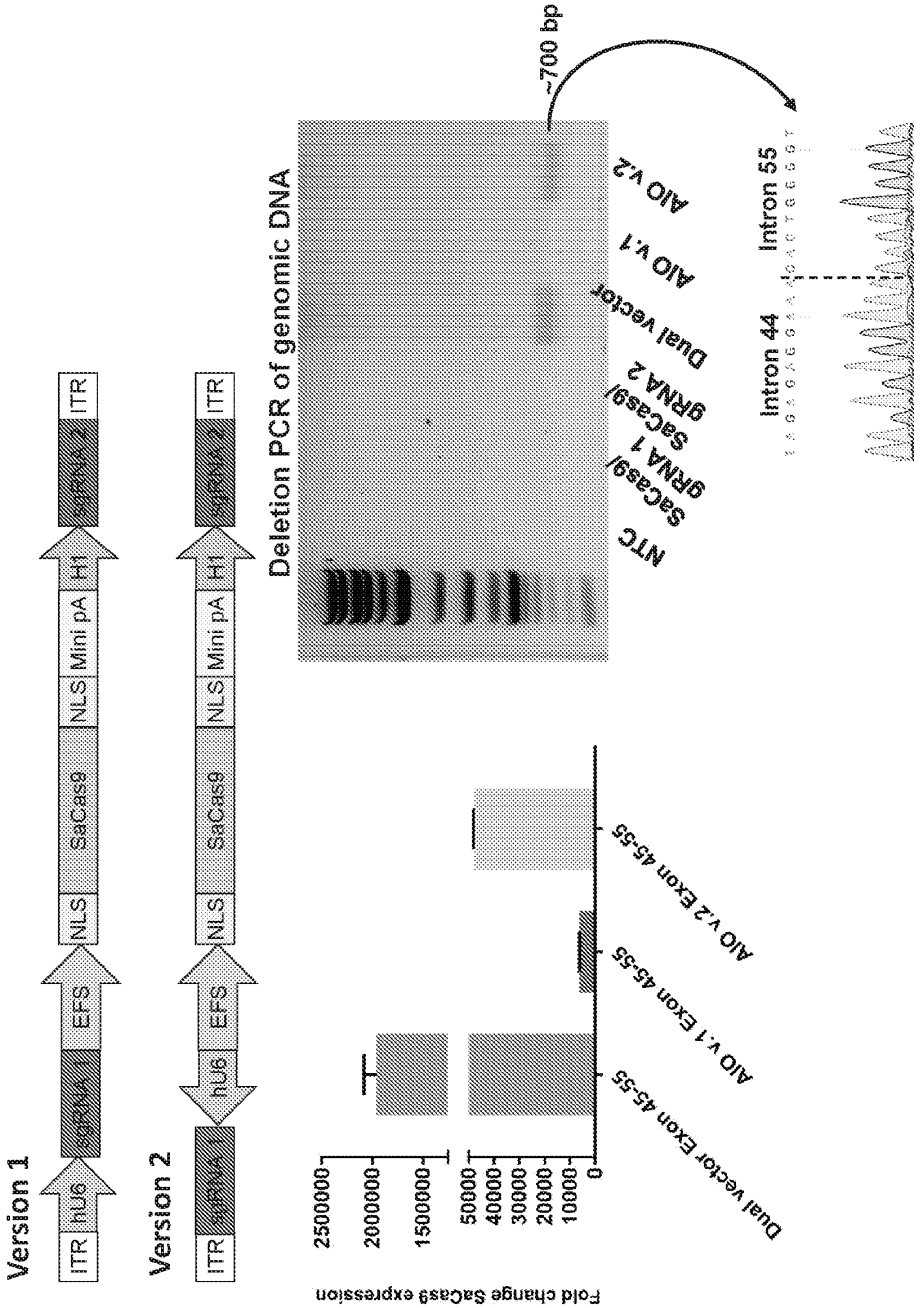

Version 1
ITR
hU6
sgRNA 1
EFS
NLS
SaCas9
NLS
Mini pA
H1
sgRNA 2
ITR
Version 2
ITR
sgRNA 1
hU6
EFS
NLS
SaCas9
NLS
Mini pA
H1
sgRNA 2
ITR
Deletion PCR of genomic DNA
~700 bp
NTC
SaCas9/ gRNA 1
SaCas9/ gRNA 2
Dual vector
AIO V.1
AIO V.2
Intron 44
Intron 55
Fold change SaCas9 expression
2500000
2000000
1500000
50000
40000
30000
20000
10000
0
Dual vector Exon 45-55
AIO V.1 Exon 45-55
AIO V.2 Exon 45-55

FIG. 8

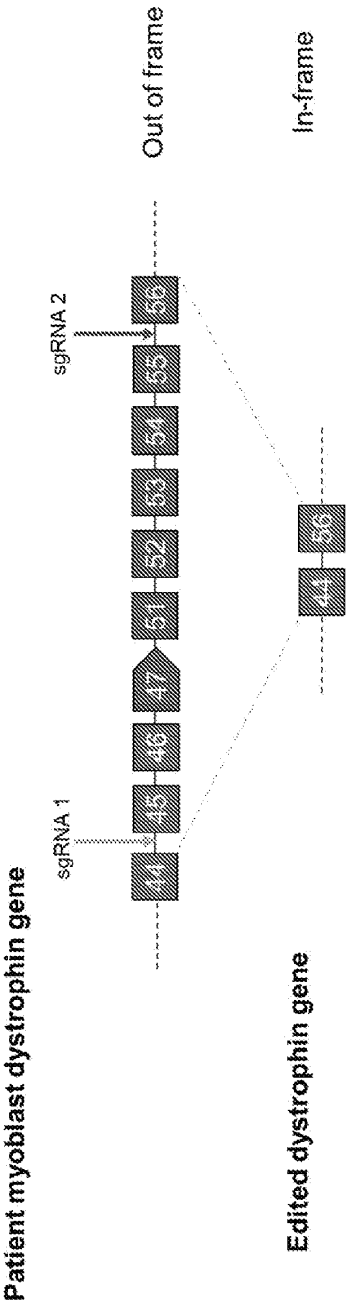

Patient myoblast dystrophin gene
sgRNA 1
sgRNA 2
44
45
46
47
51
52
53
54
55
59
Out of frame
In-frame
Edited dystrophin gene
44
59

FIG. 9A

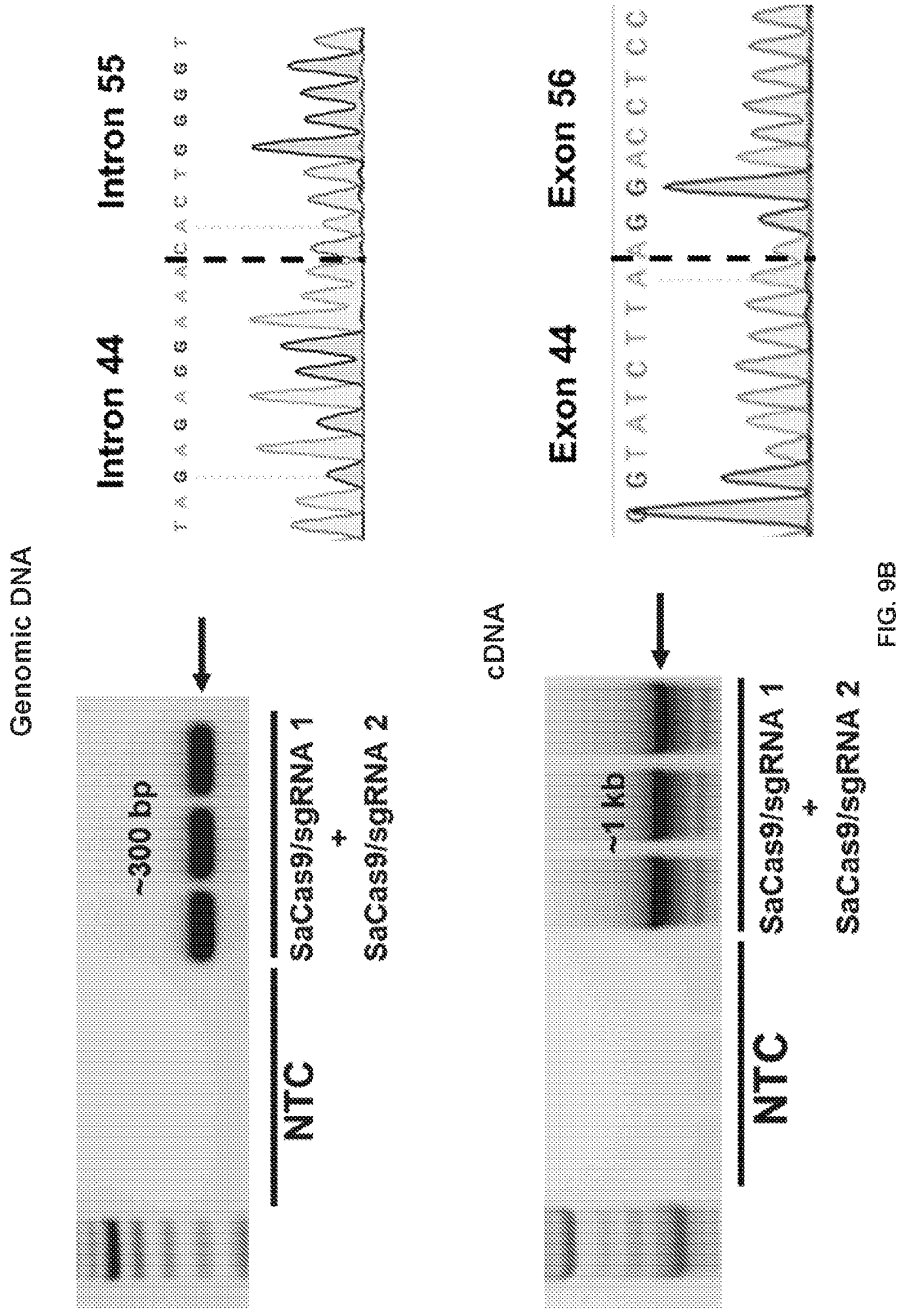

Genomic DNA
~300 bp
NTC
SaCas9/sgRNA 1
+
SaCas9/sgRNA 2
Intron 44
Intron 55
T A G A G G A A A C A C T G G G T
cDNA
~1 kb
NTC
SaCas9/sgRNA 1
+
SaCas9/sgRNA 2
Exon 44
Exon 56
G T A T C T T A G G A C C T C C

FIG. 9B

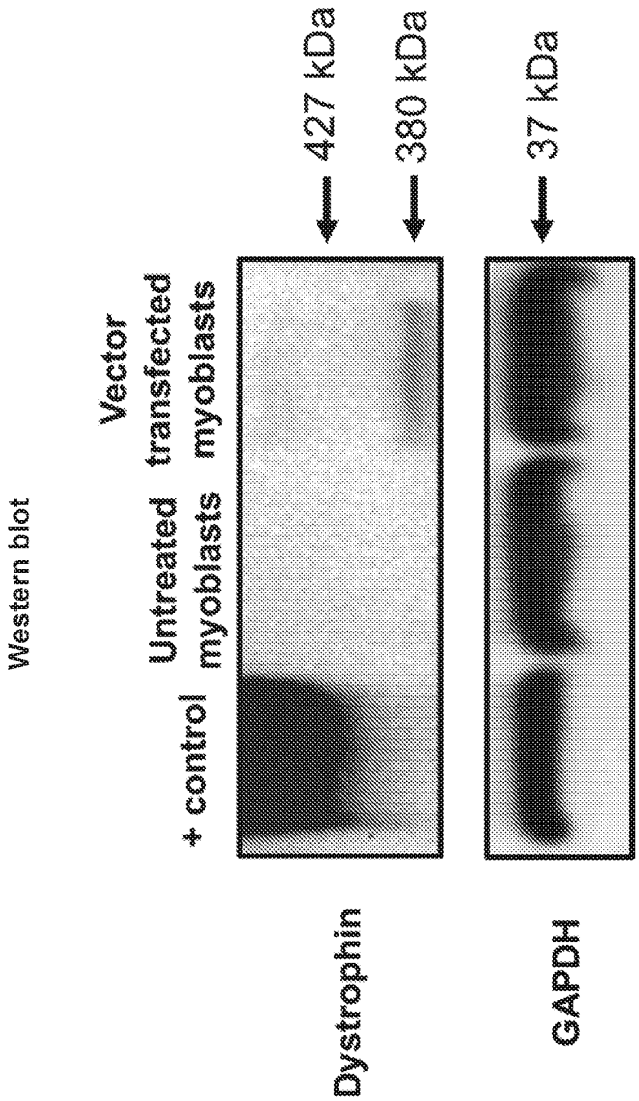

Western blot
+ control
Untreated myoblasts
Vector transfected myoblasts
427 kDa
380 kDa
37 kDa
Dystrophin
GAPDH

FIG. 9C

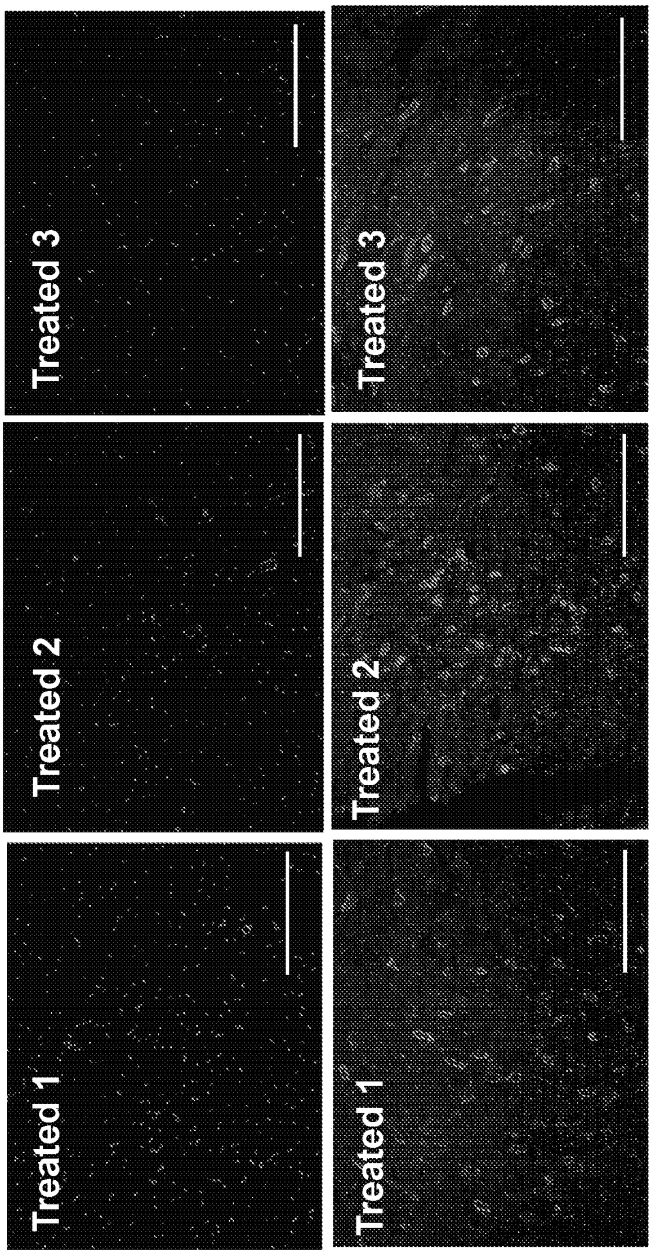

Treated 1
Treated 2
Treated 3
Treated 1
Treated 2
Treated 3
AAV9-CRISPR ROSA26
AAV9-CRISPR Exon 45-55
Dystrophin DAPI

FIG. 10

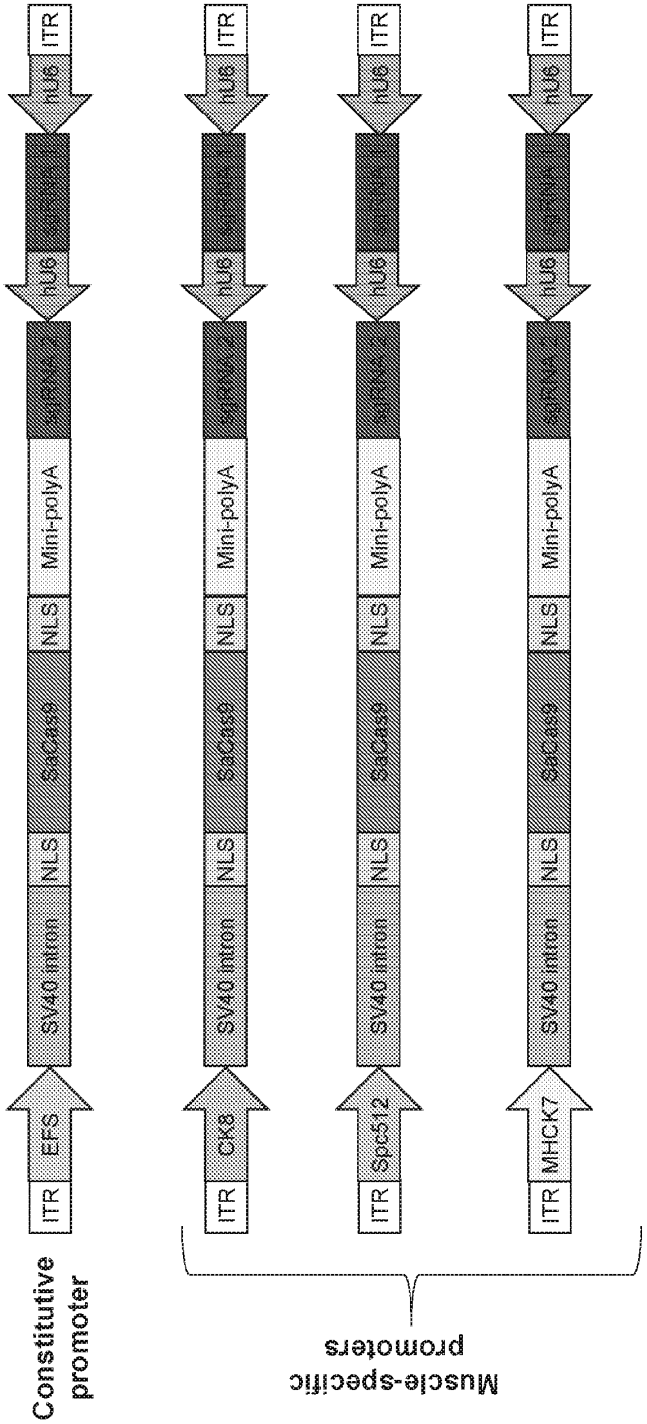

ITR
EFS
SV40 intron
NLS
SaCas9
NLS
Mini-polyA
hU6
ITR
Constitutive promoter
ITR
CK8
ITR
Spc512
ITR
MHCK7
Muscle-specific promoters

FIG. 11

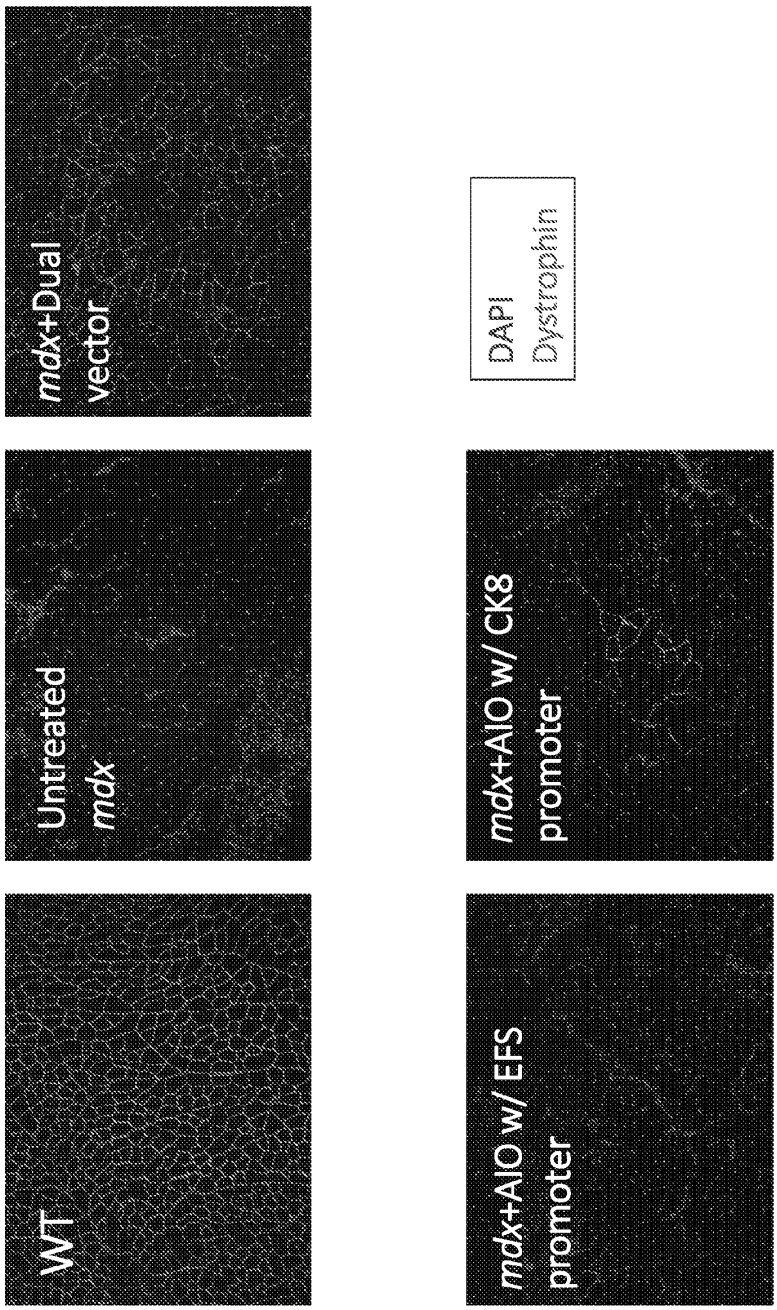

WT
Untreated mdx
mdx+Dual vector
mdx+AIO w/ EFS promoter
mdx+AIO w/ CK8 promoter
DAPI
Dystrophin

FIG. 12

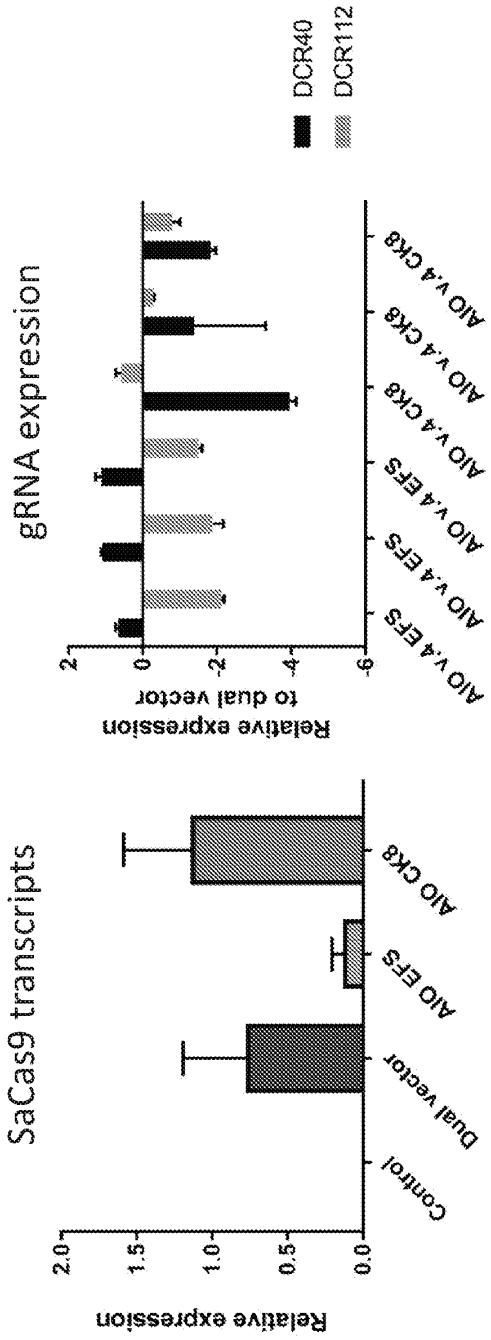

SaCas9 transcripts
Relative expression
2.0
1.5
1.0
0.5
0.0
Control
Dual vector
AIO EFS
AIO CK8
gRNA expression
Relative expression
to dual vector
2
0
-2
-4
-6
AIO v.4 EFS
AIO v.4 EFS
AIO v.4 EFS
AIO v.4 CK8
AIO v.4 CK8
AIO v.4 CK8
DCR40
DCR112

FIG. 13

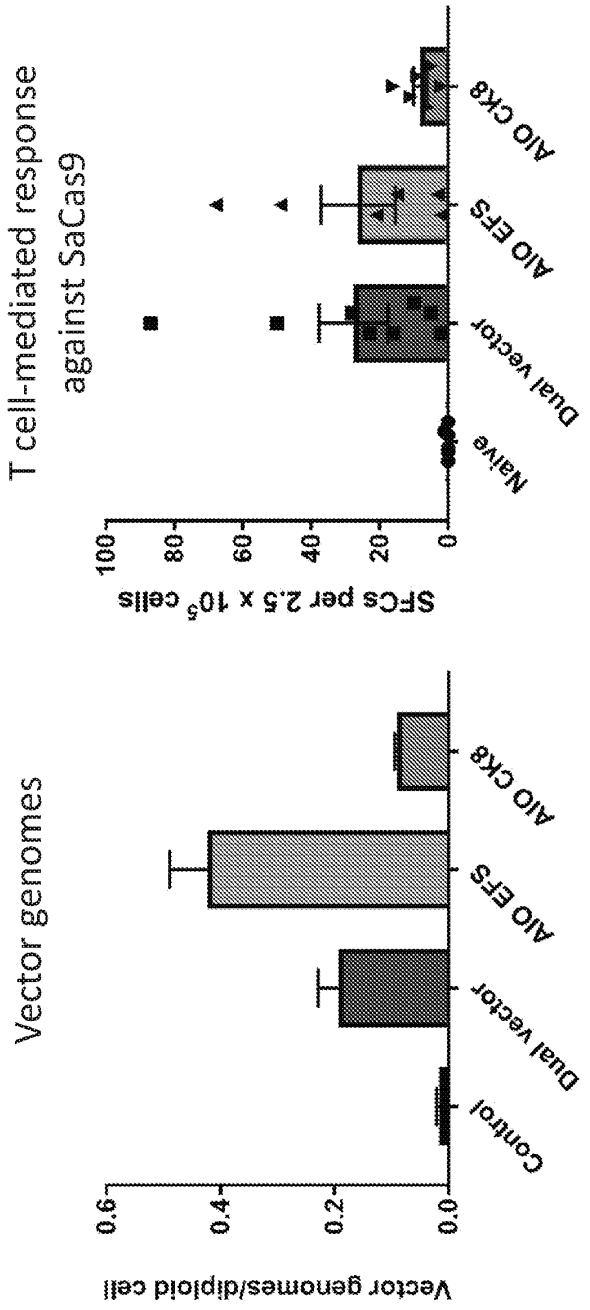

T cell-mediated response against SaCas9
SFCs per 2.5 x 10^5 cells
0
20
40
60
80
100
Naive
Dual vector
AIO EFS
AIO CK8
Vector genomes
Vector genomes/diploid cell
0.0
0.2
0.4
0.6
Control
Dual vector
AIO EFS
AIO CK8

FIG. 14