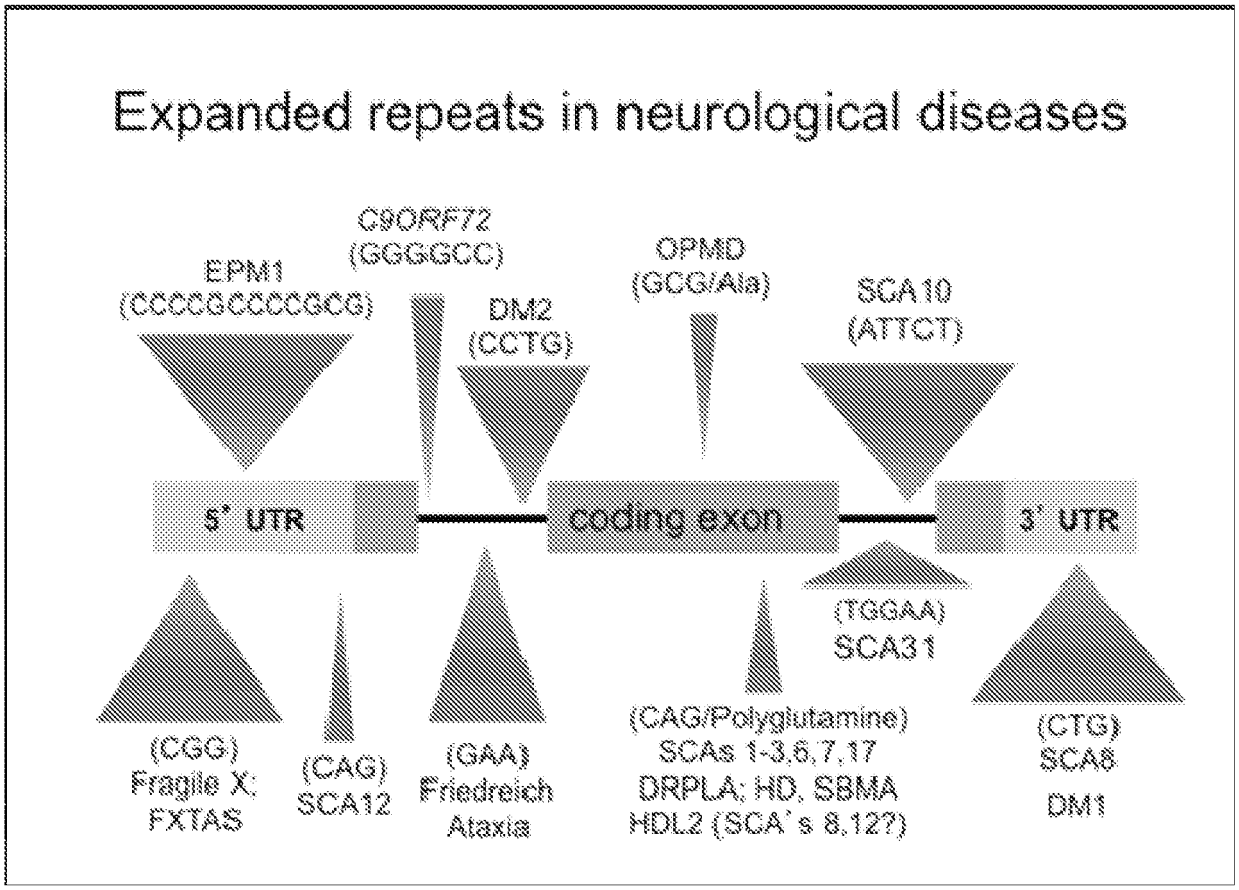

Expanded repeats in neurological diseases
C9ORF72
(GGGGCC)
EPM1
(CCCCGCCCCGCG)
DM2
(CCTG)
OPMD
(GCG/Ala)
SCA10
(ATTCT)
5' UTR
coding exon
3' UTR
(TGGAA)
SCA31
(CGG)
Fragile X;
FXTAS
(CAG)
SCA12
(GAA)
Friedreich
Ataxia
(CAG/Polyglutamine)
SCAs 1-3,6,7,17
DRPLA; HD, SBMA
HDL2 (SCA's 8,12?)
(CTG)
SCA8
DM1

FIG. 1

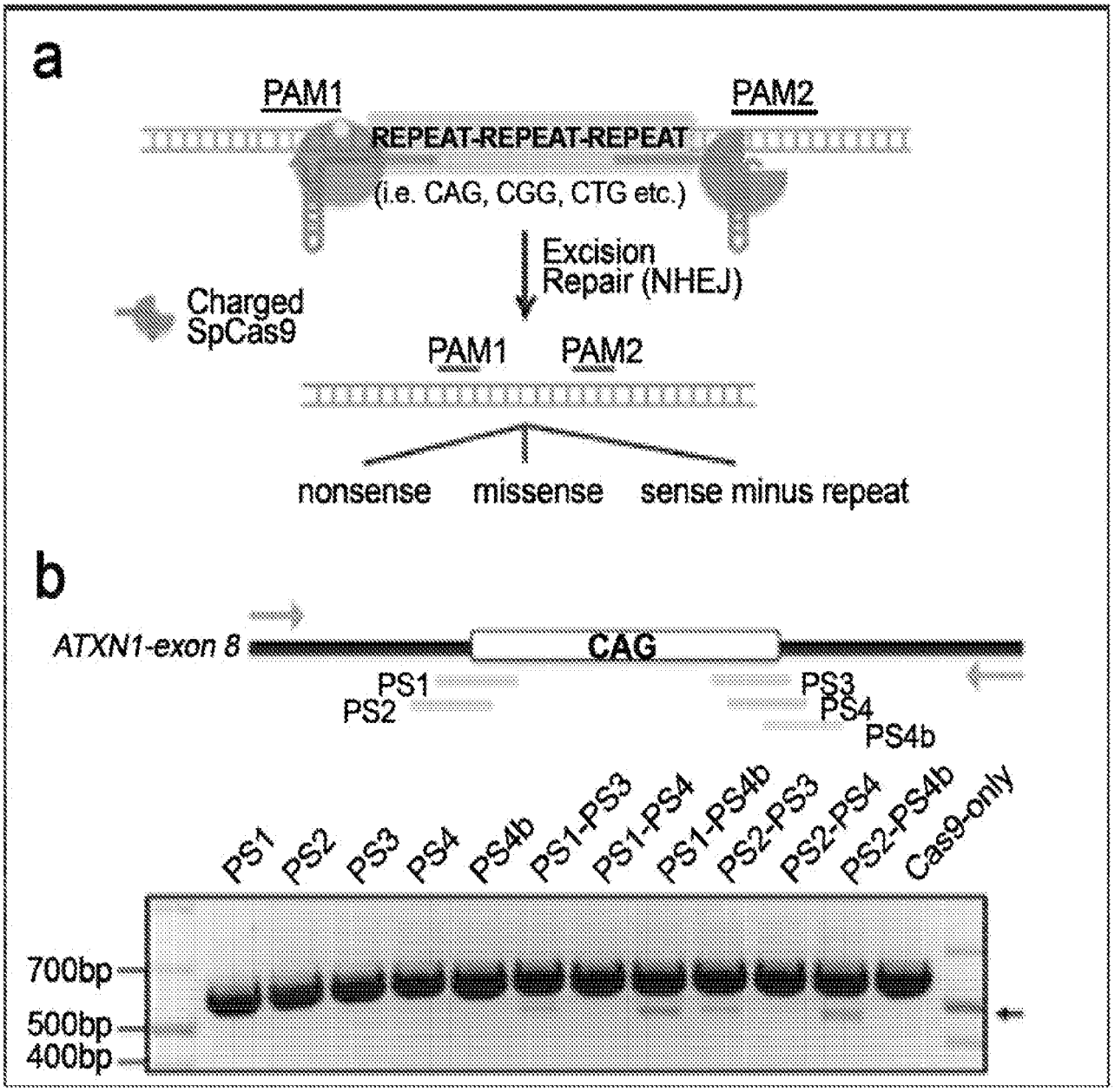

a
PAM1
PAM2
REPEAT-REPEAT-REPEAT
(i.e. CAG, CGG, CTG etc.)
Excision
Repair (NHEJ)
Charged
SpCas9
PAM1
PAM2
nonsense
missense
sense minus repeat
b
ATXN1-exon 8
CAG
PS1
PS2
PS3
PS4
PS4b
PS1
PS2
PS3
PS4
PS4b
PS1-PS3
PS1-PS4
PS1-PS4b
PS2-PS3
PS2-PS4
PS2-PS4b
Cas9-only
700bp
500bp
400bp

FIG. 2A-B

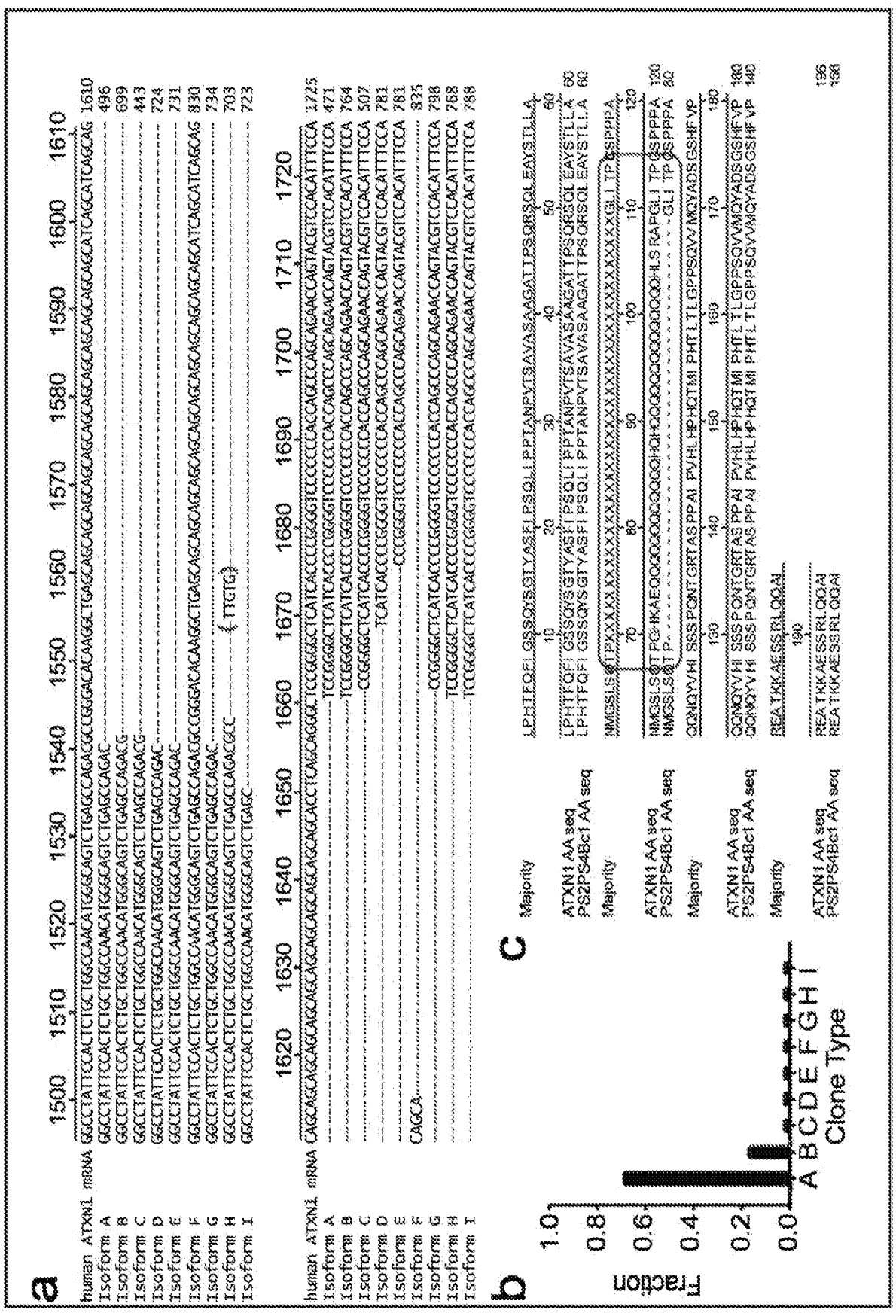

a
1500 1510 1520 1530 1540 1550 1560 1570 1580 1590 1600 1610
human ATXN1 mRNA
Isoform A
Isoform B
Isoform C
Isoform D
Isoform E
Isoform F
Isoform G
Isoform H
Isoform I
1620 1630 1640 1650 1660 1670 1680 1690 1700 1710 1720
b
Fraction
1.0
0.8
0.6
0.4
0.2
0.0
A B C D E F G H I
Clone Type
c
Majority
ATXN1 AA seq
PS2PS4Bc1 AA seq

FIG. 3A-C

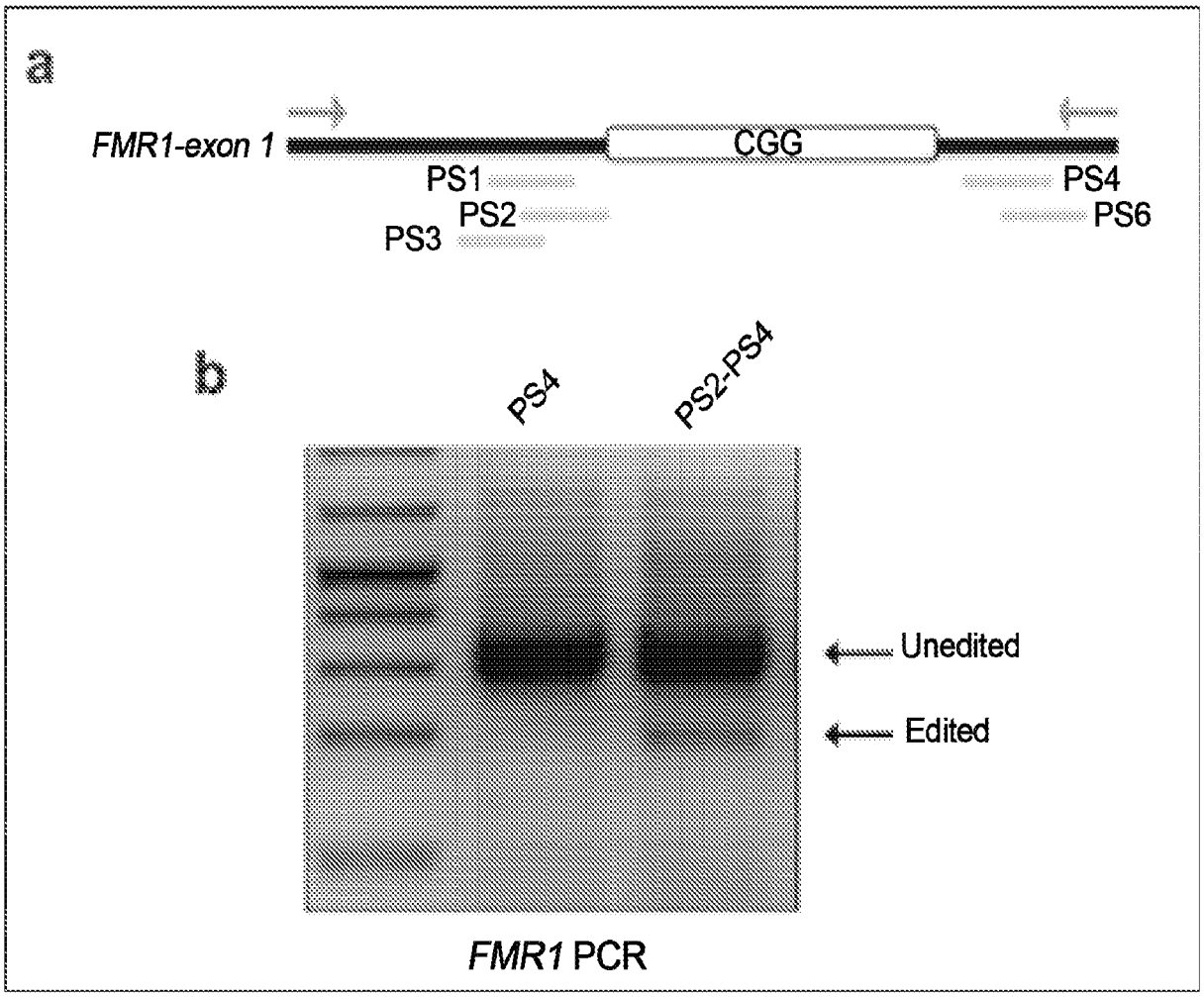

a
FMR1-exon 1
CGG
PS1
PS2
PS3
PS4
PS6
b
PS4
PS2-PS4
Unedited
Edited
FMR1 PCR

FIG. 4A-B

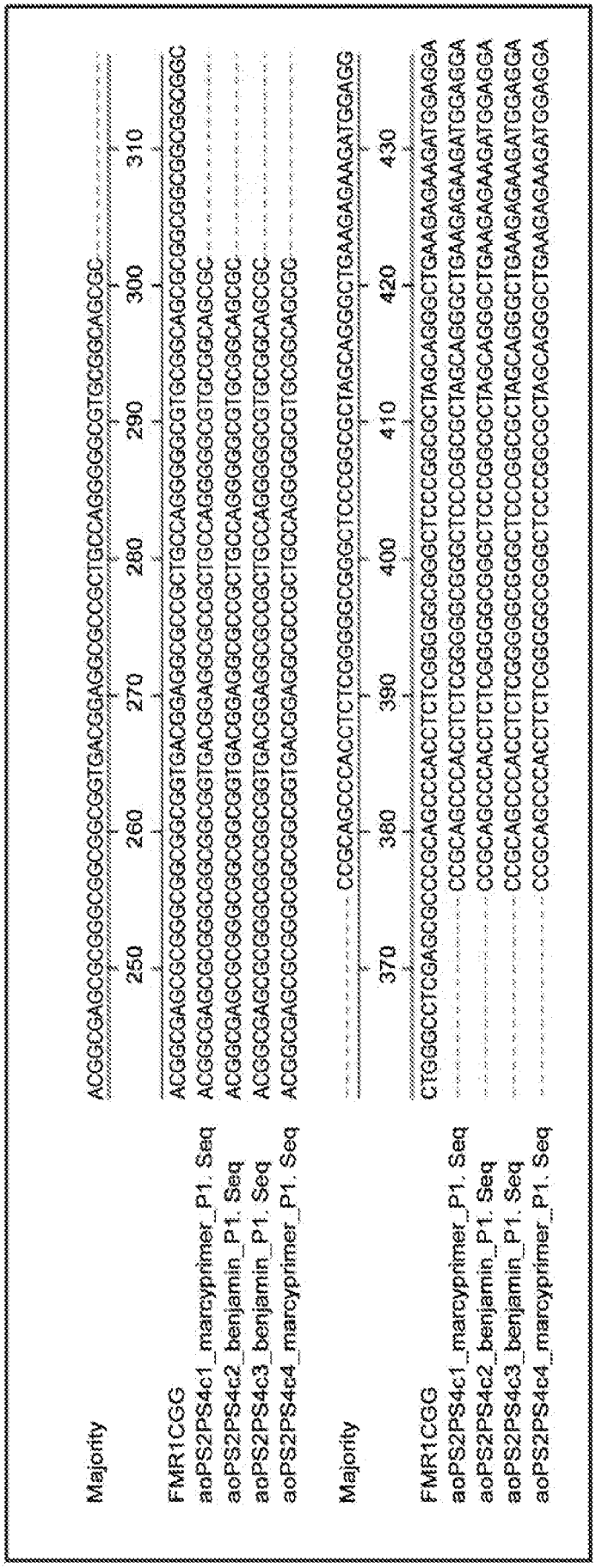

Majority
250 260 270 280 290 300 310
FMR1CGG
aoPS2PS4c1_marcyprimer_P1. Seq
aoPS2PS4c2_benjamin_P1. Seq
aoPS2PS4c3_benjamin_P1. Seq
aoPS2PS4c4_marcyprimer_P1. Seq
Majority
370 380 390 400 410 420 430
FMR1CGG
aoPS2PS4c1_marcyprimer_P1. Seq
aoPS2PS4c2_benjamin_P1. Seq
aoPS2PS4c3_benjamin_P1. Seq
aoPS2PS4c4_marcyprimer_P1. Seq

FIG. 5

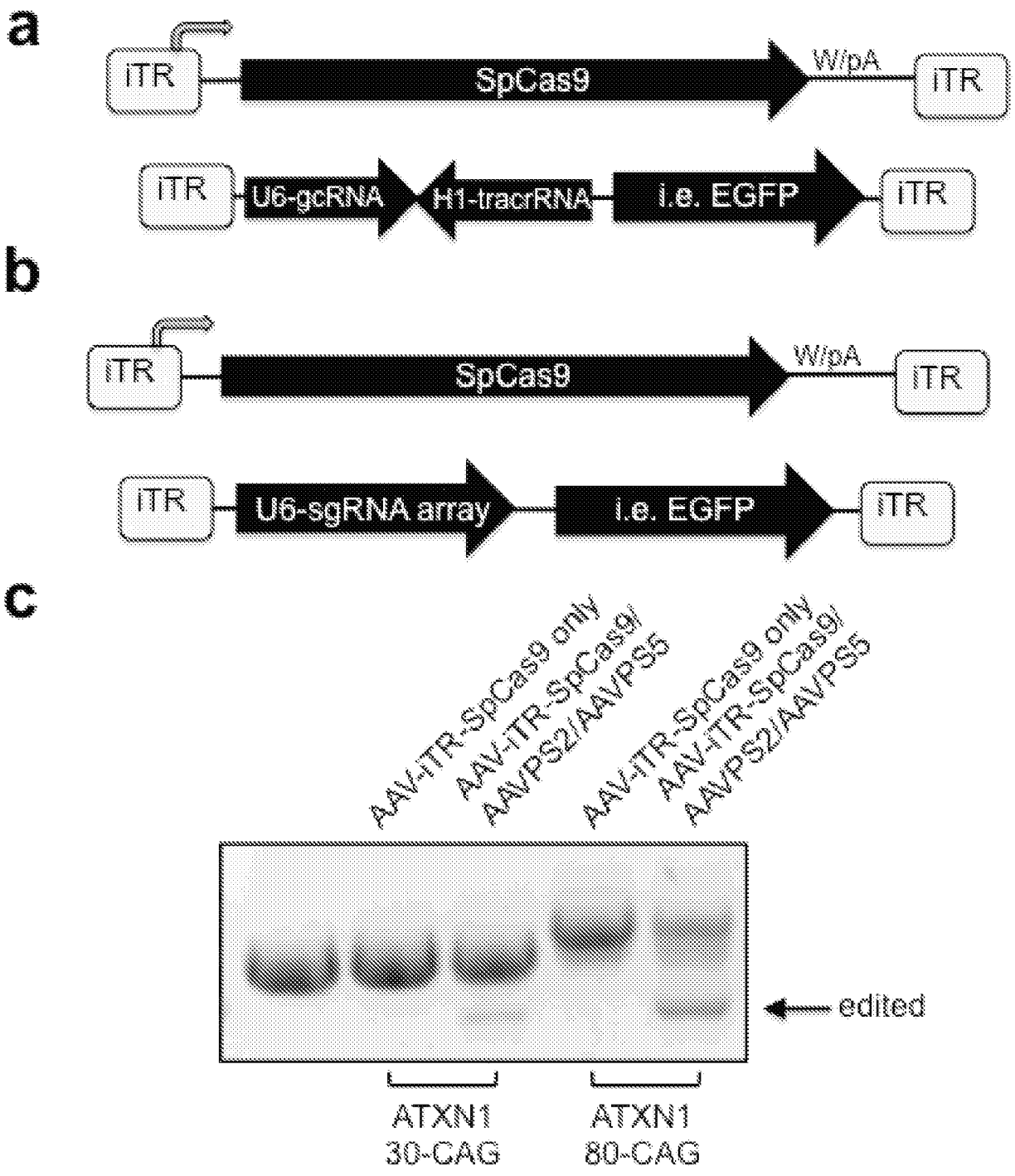
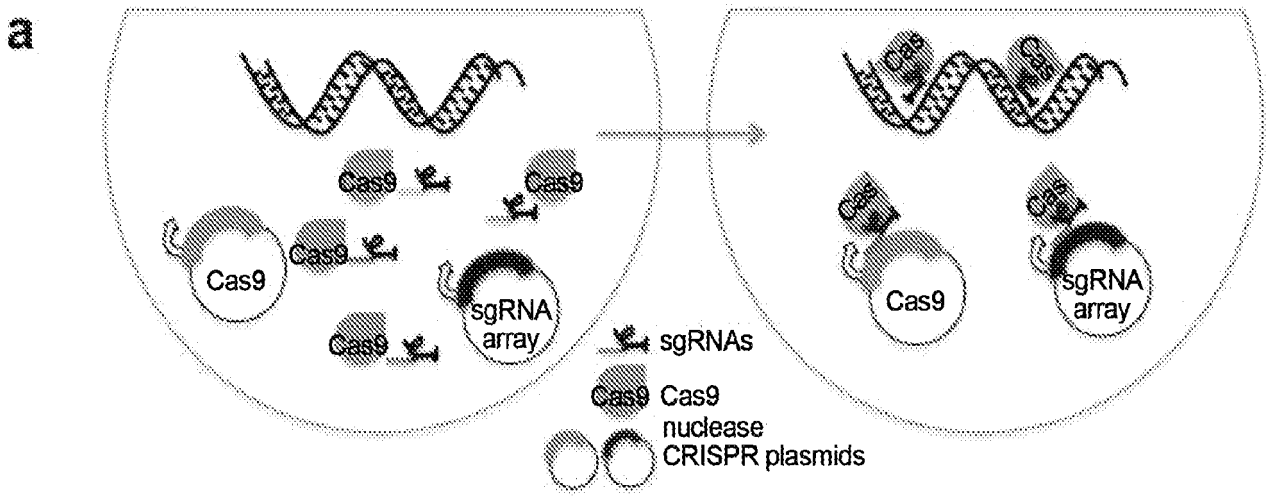

a
ITR
SpCas9
W/pA
ITR
ITR
U6-gcRNA
H1-tracrRNA
i.e. EGFP
ITR
b
ITR
SpCas9
W/pA
ITR
ITR
U6-sgRNA array
i.e. EGFP
ITR
c
AAV-ITR-SpCas9 only
AAV-ITR-SpCas9/AAVPS2/AAVPS5
AAV-ITR-SpCas9 only
AAV-ITR-SpCas9/AAVPS2/AAVPS5
edited
ATXN1 30-CAG
ATXN1 80-CAG

FIG. 6A-C


a
Cas9
Cas9
Cas9
Cas9
Cas9
sgRNA array
sgRNAs
Cas9 Cas9 nuclease
CRISPR plasmids
Cas9
Cas9
Cas9
sgRNA array

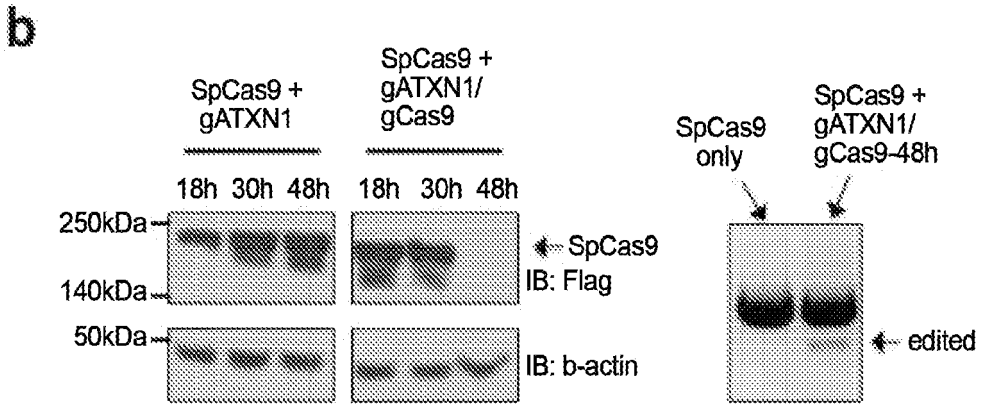

b
SpCas9 + gATXN1
SpCas9 + gATXN1/ gCas9
18h 30h 48h 18h 30h 48h
250kDa
140kDa
50kDa
SpCas9
IB: Flag
IB: b-actin
SpCas9 only
SpCas9 + gATXN1/ gCas9-48h
edited

FIG. 7A-B

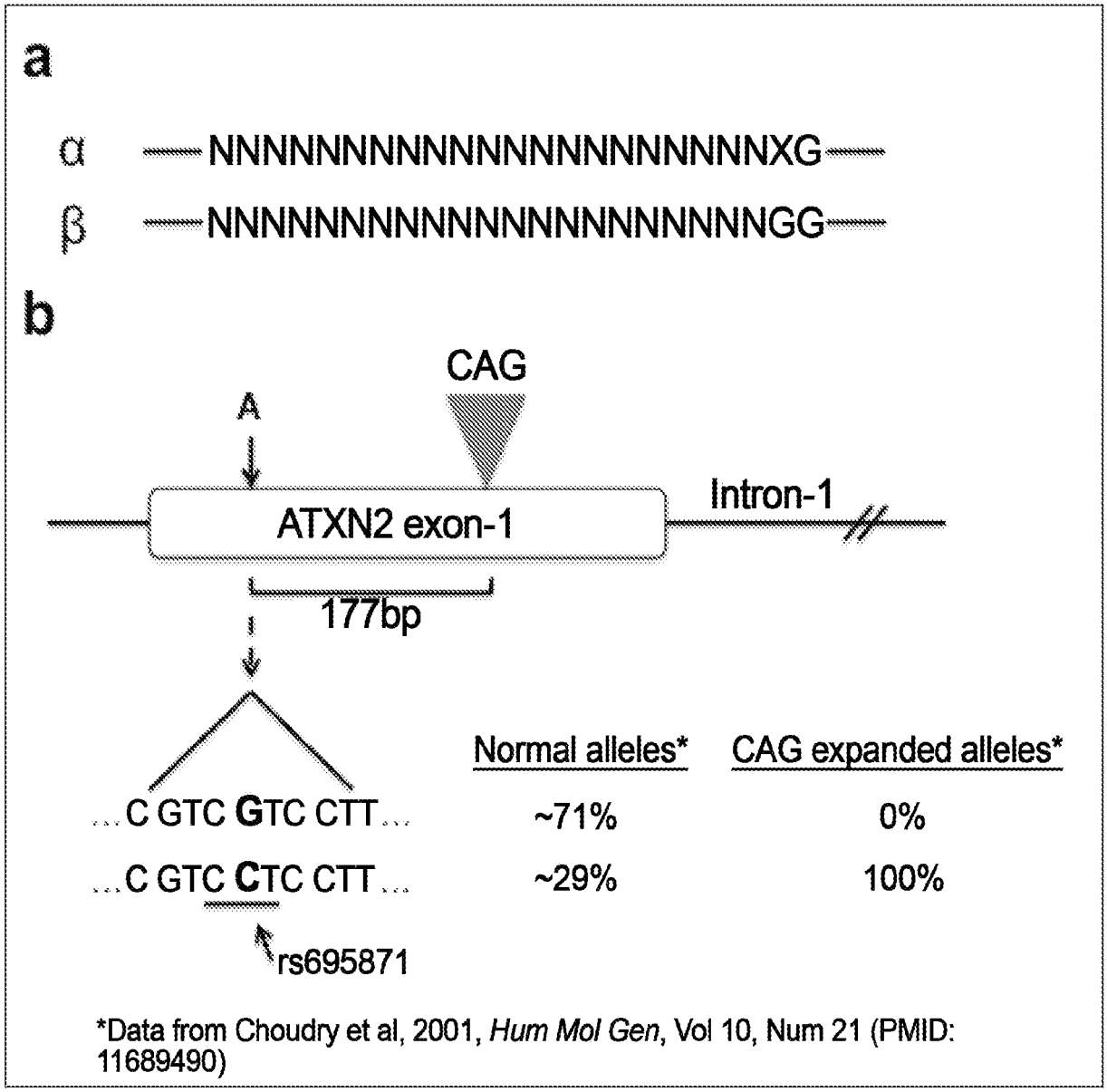
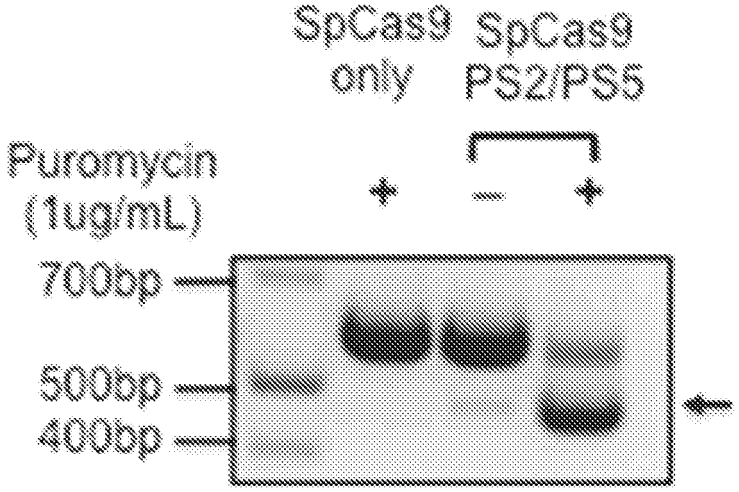

a
α NNNNNNNNNNNNNNNNNNNNNXG
β NNNNNNNNNNNNNNNNNNNNNGG
b
CAG
A
ATXN2 exon-1
Intron-1
177bp
Normal alleles*
CAG expanded alleles*
...C GTC GTC CTT...
~71%
0%
...C GTC CTC CTT...
~29%
100%
rs695871
*Data from Choudry et al, 2001, Hum Mol Gen, Vol 10, Num 21 (PMID: 11689490)

FIG. 8A-B

A


SpCas9 only
SpCas9 PS2/PS5
Puromycin (1ug/mL)
+
–
+
700bp
500bp
400bp

B

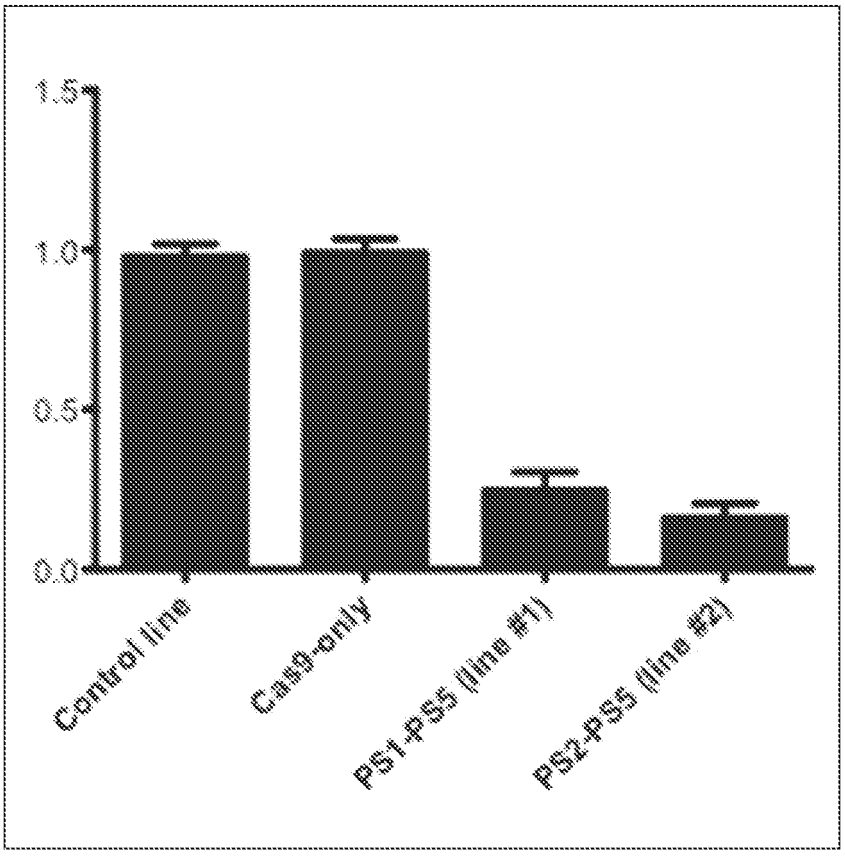

1.5
1.0
0.5
0.0
Control line
Cas9-only
PS1-PS5 (line #1)
PS2-PS5 (line #2)

FIG. 9A-B

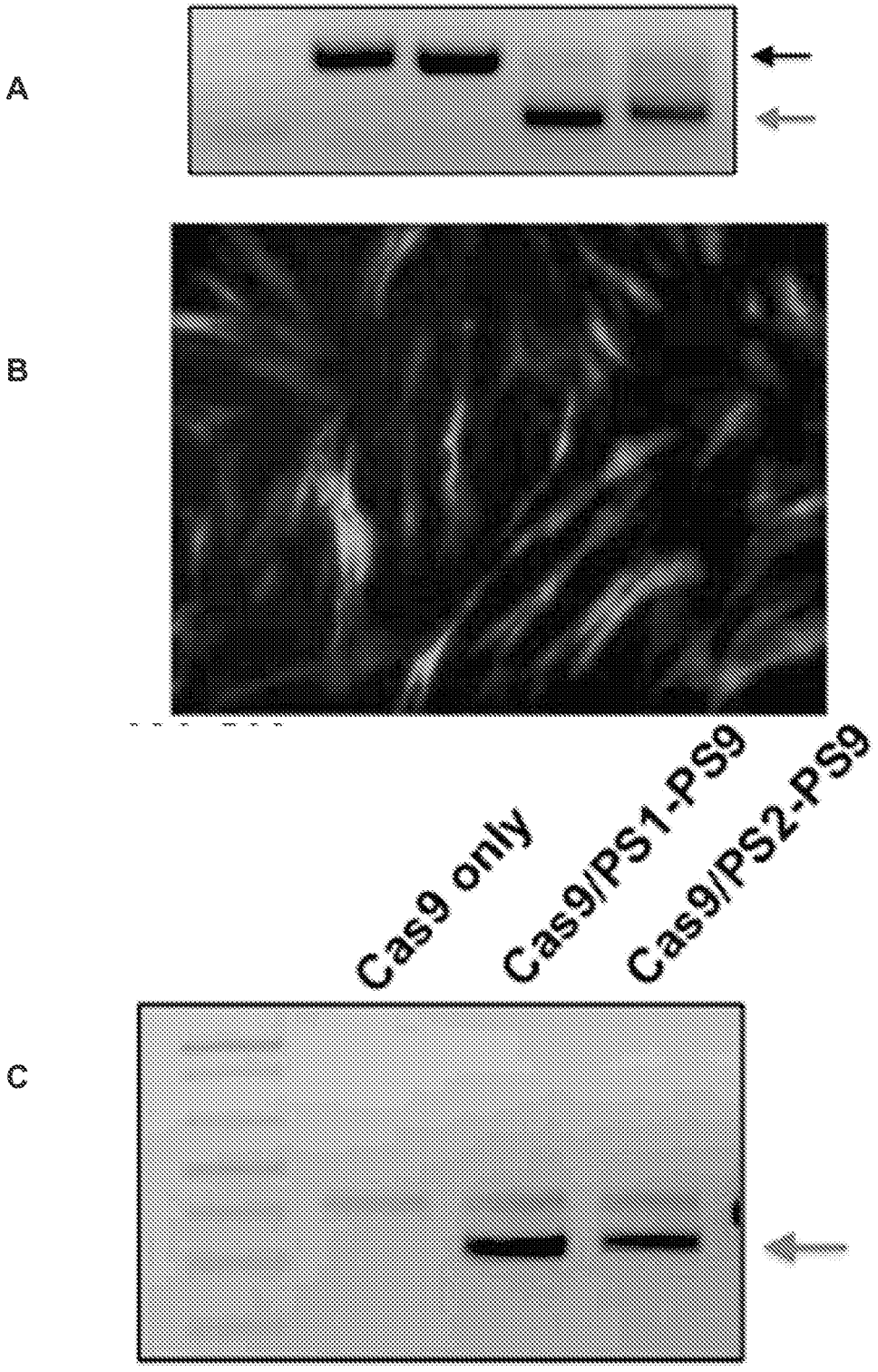


FIG. 10A-C

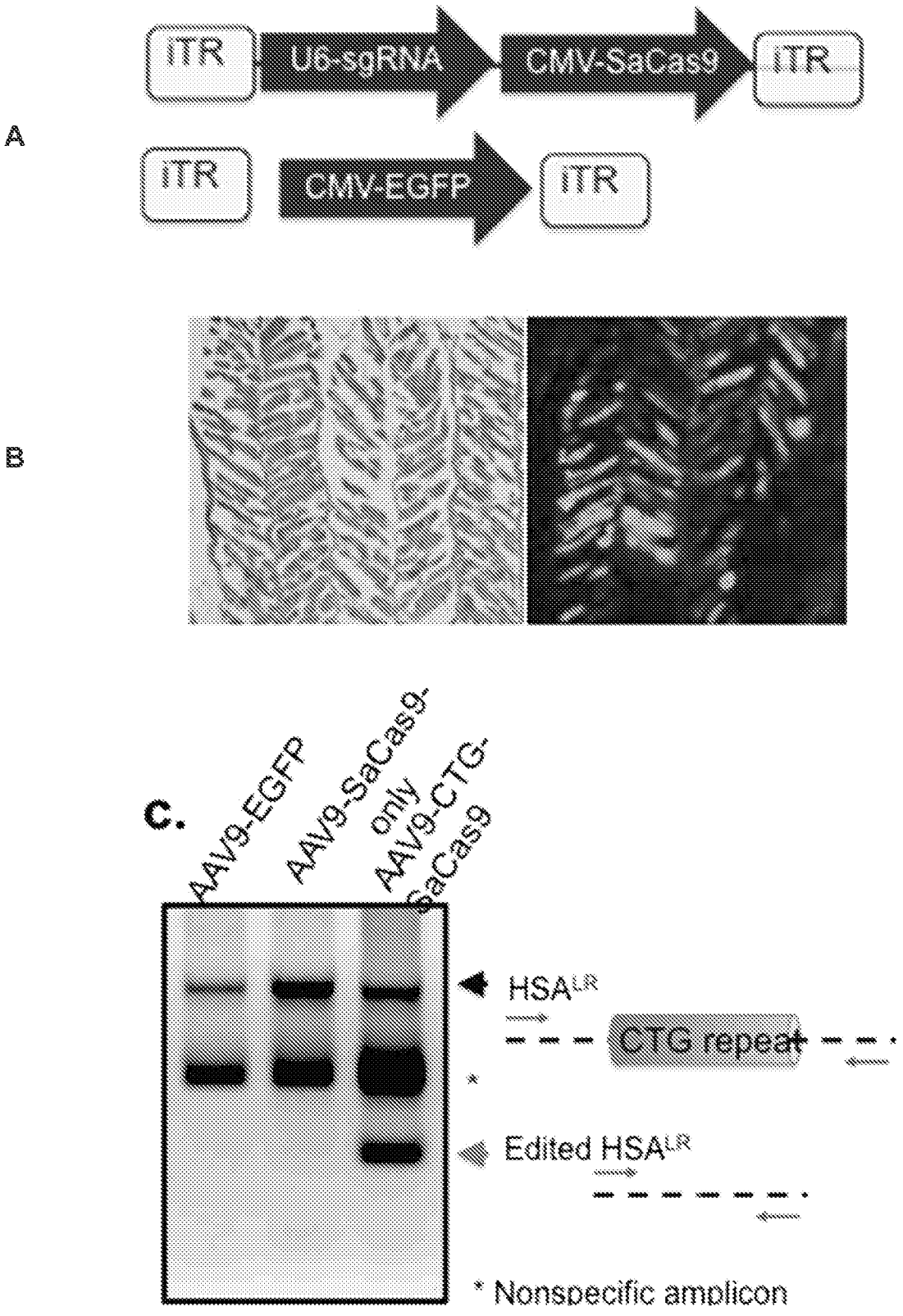

A
ITR
U6-sgRNA
CMV-SaCas9
ITR
ITR
CMV-EGFP
ITR
B
c.
AAV9-EGFP
AAV9-SaCas9-only
AAV9-CTG-SaCas9
HSALR
CTG repeat
*
Edited HSALR
* Nonspecific amplicon

FIG. 11-A-C

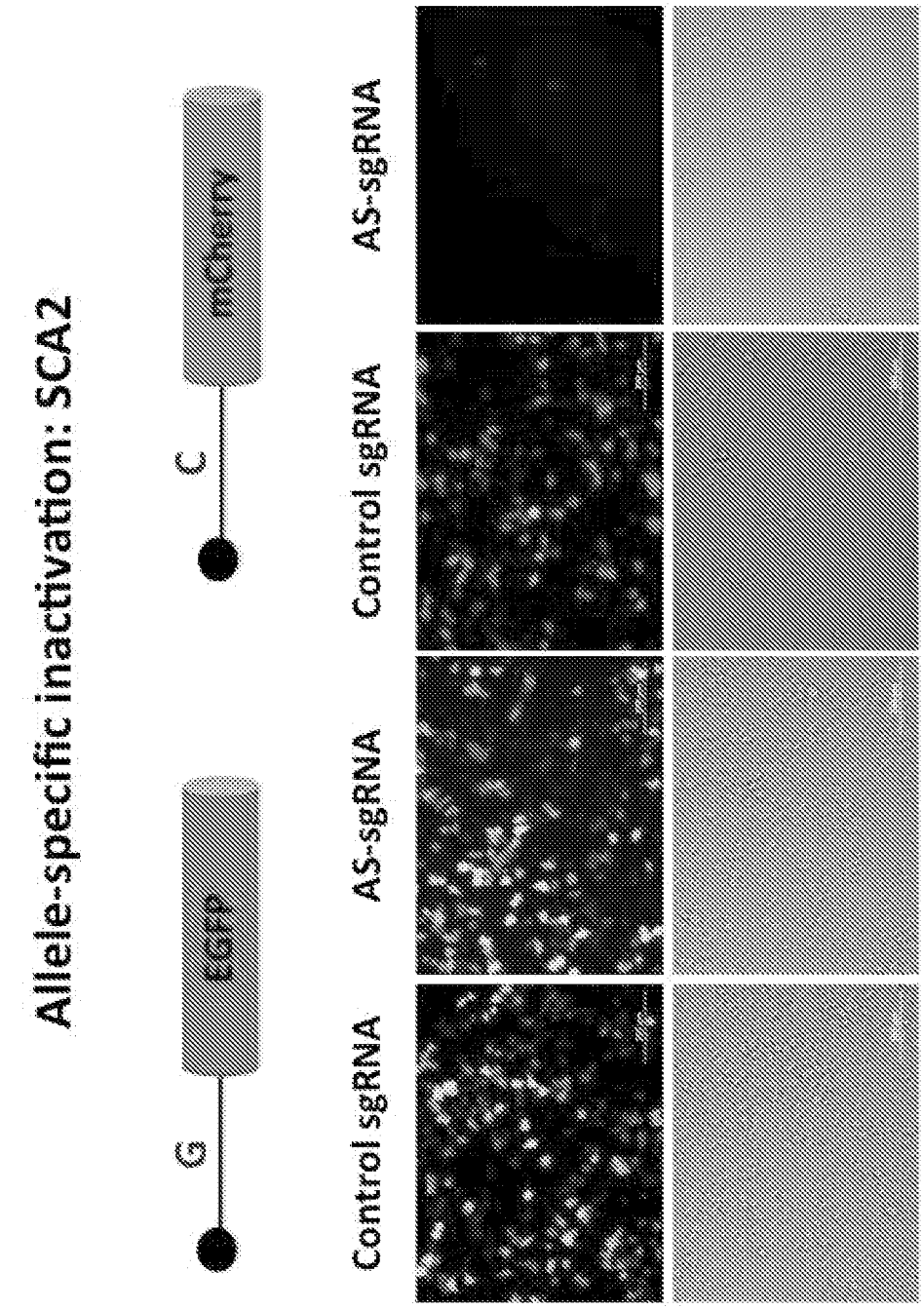
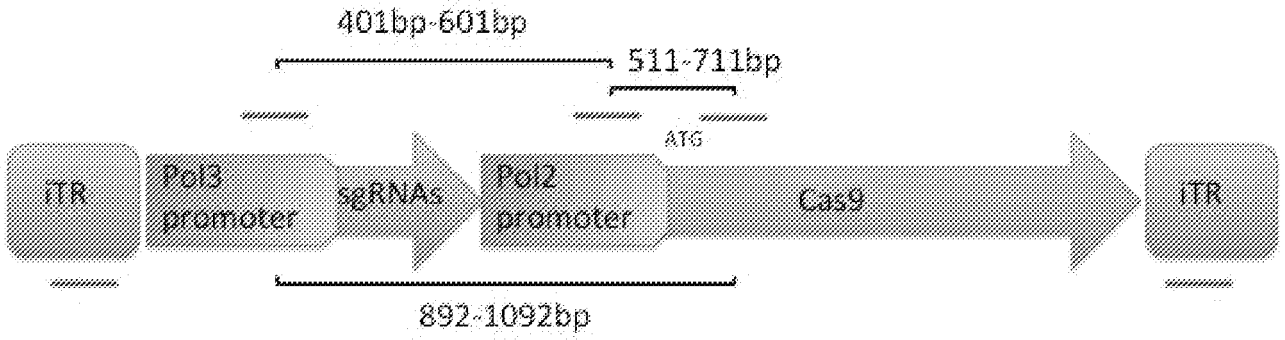

Allele-specific inactivation: SCA2
G
EGFP
C
mCherry
Control sgRNA
AS-sgRNA
Control sgRNA
AS-sgRNA

FIG. 12



Pol3 promoters such as the U6 or H1 promoters.

Pol2 promoters such as those mentioned throughout the application.

"Self-inactivating" sgRNAs (shown in red) can be expressed singly or in tandem array format from 1 up to 4 different guide sequences processed from 1 chimeric pol3 transcript.

Black brackets depict the expected DNA excisions resulting from double-stranded breaks when at least 2 different "Self-inactivating" sgRNAs are expressed in tandem arrays.

FIG. 13

Example/sequence of chimeric, tandem array transcript is shown below. The ATXN1aPS9 guide edits the ATXN1 locus while the U6aPS1 and CMVaPS1 guides inactivate the CRISPR/Cas9 system.

*Orange boxes denote 3 different guide sequences.

Examples of Self-inactivating target sequences using the SaCas9 system:

SI_Cas9aPS1
5'agcgtaatctggaacatcgt3'

SI_CMVaPS1
5'GGGACTTTCCATTGACGTCA3'

SI_modU6aPS1
5'ACAATTACTTTACAGTTAGG3'

SI_ITRaPS1
5'CTACAAGGAACCCCTAGTGA3'

SI_ITRaPS2
5'AGCGAGCGAGCGCGCAGAGA3'

FIG. 14

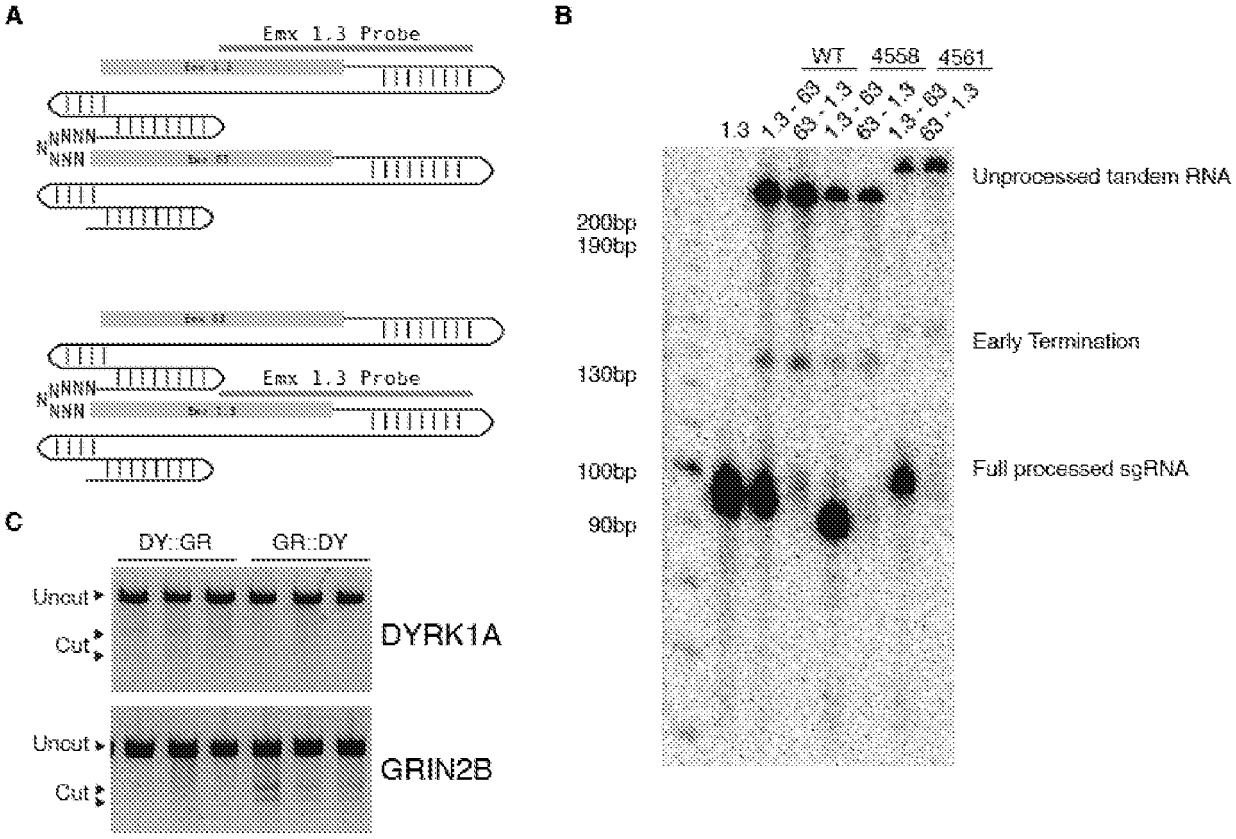

A
Emx 1.3 Probe
NNNNNNNNNNNNNNNNNNNN
Emx 1.3 Probe
B
WT
4558
4561
1.3
1.3 - 63
63 - 1.3
200bp
190bp
130bp
100bp
90bp
Unprocessed tandem RNA
Early Termination
Full processed sgRNA
C
DY::GR
GR::DY
Uncut
Cut
DYRK1A
GRIN2B

FIG. 15A-C

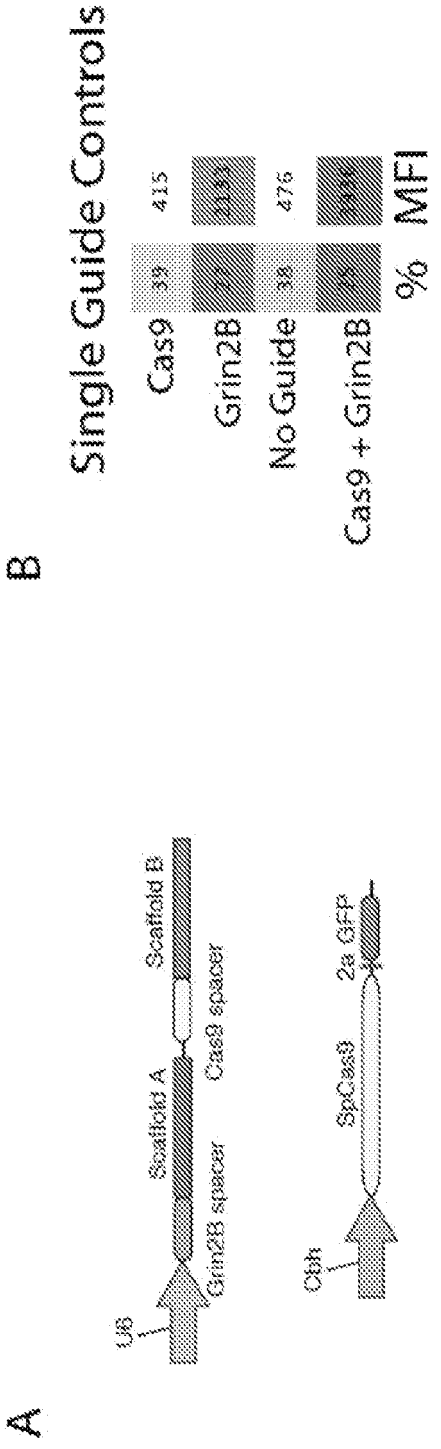

A
U6
Grin2B spacer
Scaffold A
Scaffold B
Cas9 spacer
Cbh
SpCas9
2a GFP
B
Single Guide Controls
% MFI
Cas9
39
415
Grin2B
No Guide
38
476
Cas9 + Grin2B

FIG. 16A-B

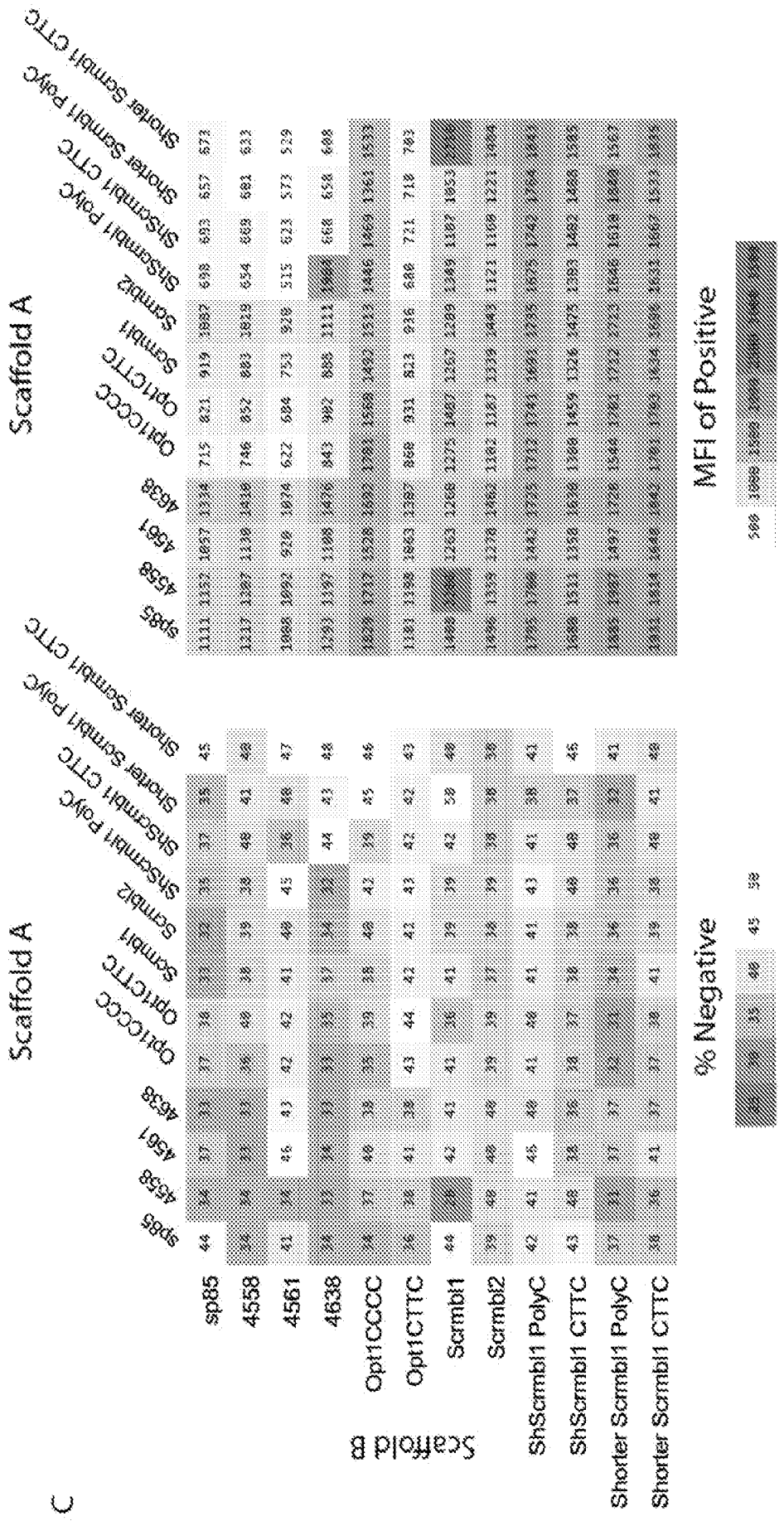

C
Scaffold B
sp85
4558
4561
4638
Opt1CCCC
Opt1CTTC
Scrmbl1
Scrmbl2
ShScrmbl1 PolyC
ShScrmbl1 CTTC
Shorter Scrmbl1 PolyC
Shorter Scrmbl1 CTTC
Scaffold A
sp85
4558
4561
4638
Opt1CCCC
Opt1CTTC
Scrmbl1
Scrmbl2
ShScrmbl1 PolyC
ShScrmbl1 CTTC
Shorter Scrmbl1 PolyC
Shorter Scrmbl1 CTTC
% Negative
Scaffold A
sp85
4558
4561
4638
Opt1CCCC
Opt1CTTC
Scrmbl1
Scrmbl2
ShScrmbl1 PolyC
ShScrmbl1 CTTC
Shorter Scrmbl1 PolyC
Shorter Scrmbl1 CTTC
MFI of Positive

FIG. 16C

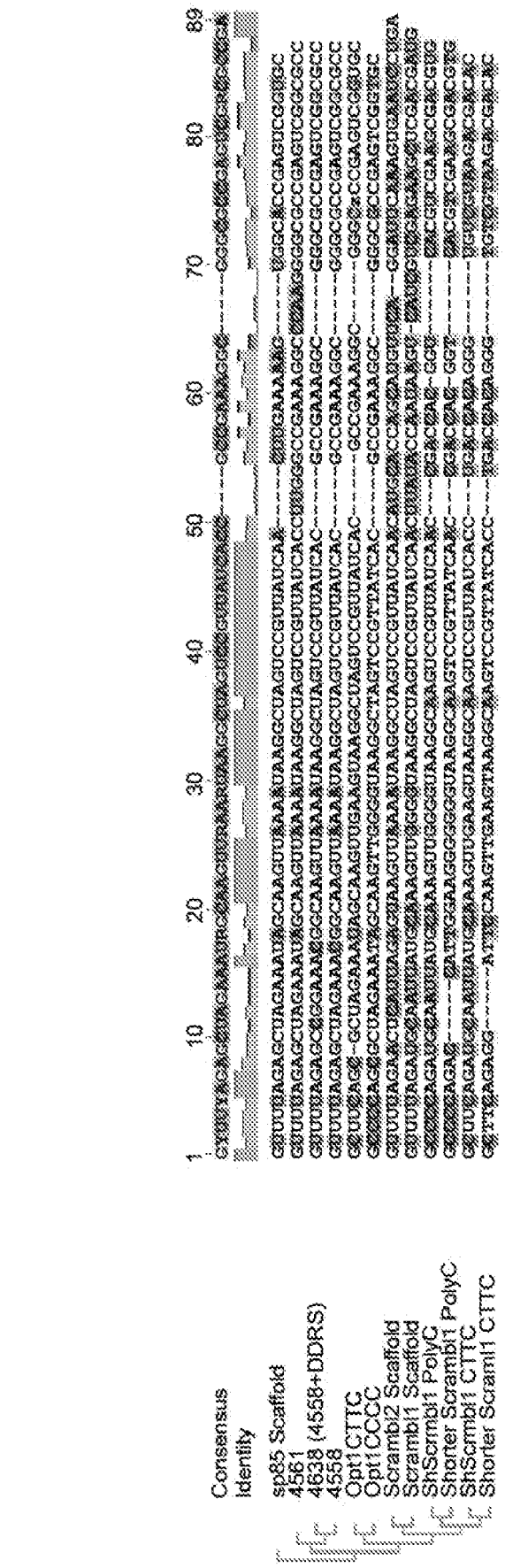

Consensus
Identity
sp85 Scaffold
4561
4638 (4558+DDRS)
4558
Opt1 CTTC
Opt1 CCCC
Scrambl2 Scaffold
Scrambl1 Scaffold
ShScrmbl1 PolyC
Shorter Scrambl1 PolyC
ShScrmbl1 CTTC
Shorter Scraml1 CTTC

FIG. 17

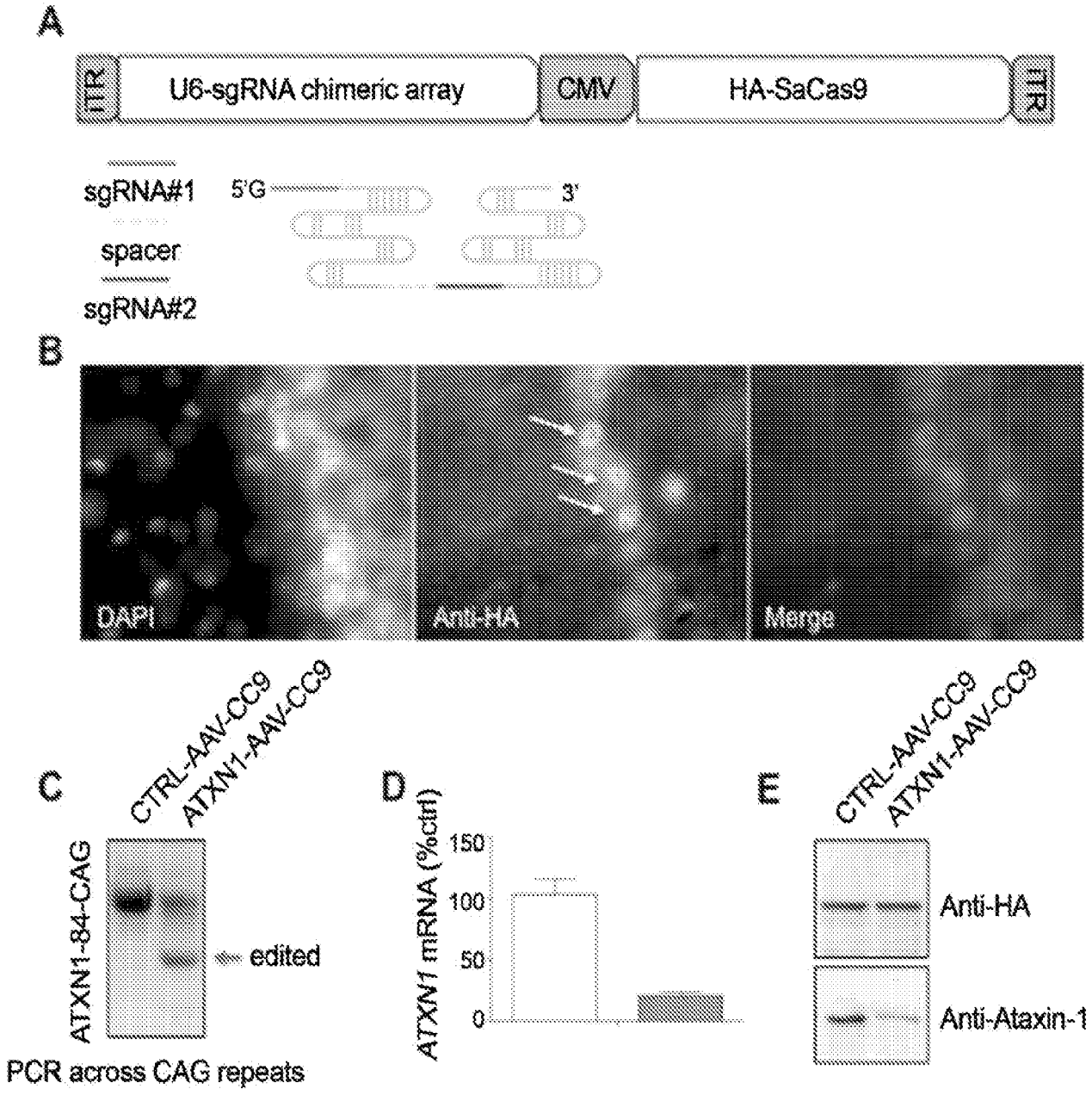

A
ITR
U6-sgRNA chimeric array
CMV
HA-SaCas9
ITR
sgRNA#1
spacer
sgRNA#2
5'G
3'
B
DAPI
Anti-HA
Merge
C
CTRL-AAV-CC9
ATXN1-AAV-CC9
ATXN1-84-CAG
edited
PCR across CAG repeats
D
ATXN1 mRNA (%ctrl)
150
100
50
0
E
CTRL-AAV-CC9
ATXN1-AAV-CC9
Anti-HA
Anti-Ataxin-1

FIG. 18A-E