FIGURE 1

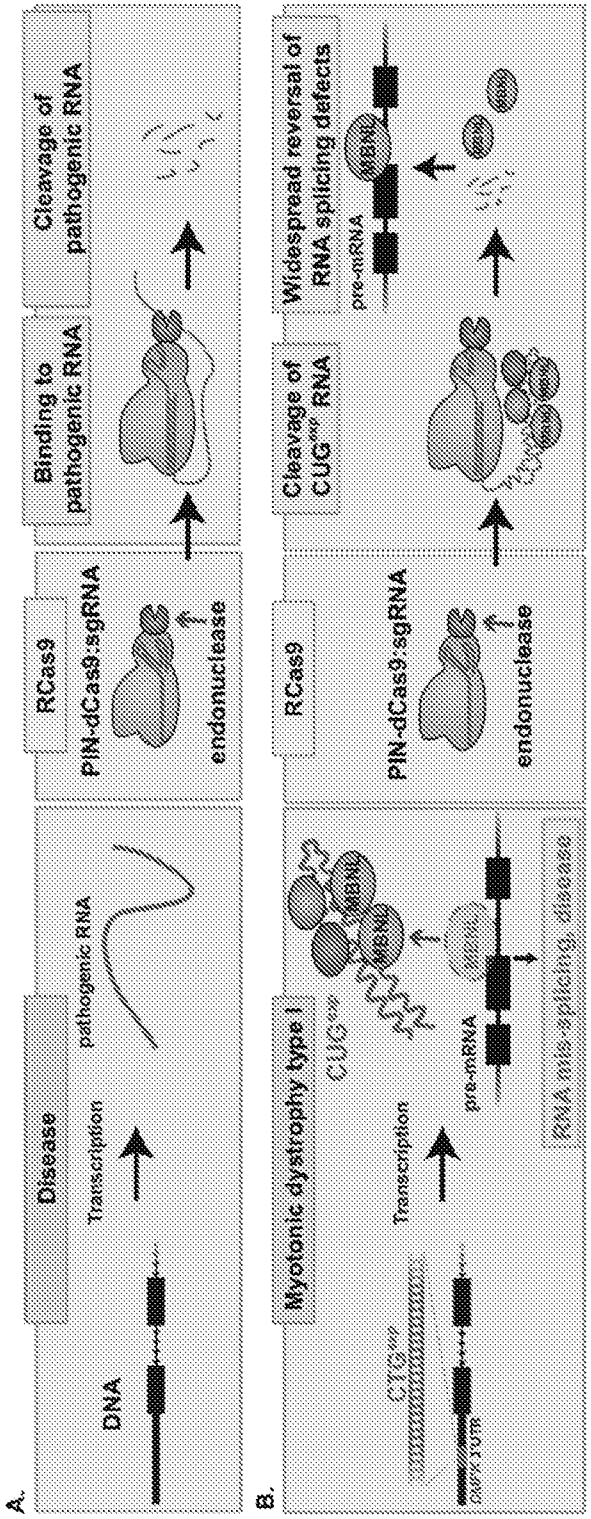

A.
Disease
Transcription
pathogenic RNA
DNA
RCas9
PIN-dCas9:sgRNA
endonuclease
Binding to pathogenic RNA
Cleavage of pathogenic RNA
B.
Myotonic dystrophy type I
Transcription
pre-mRNA
RNA mis-splicing, Disease
RCas9
PIN-dCas9:sgRNA
endonuclease
Cleavage of CUG^exp RNA
Widespread reversal of RNA splicing defects
pre-mRNA
MBNL

FIGURE 2

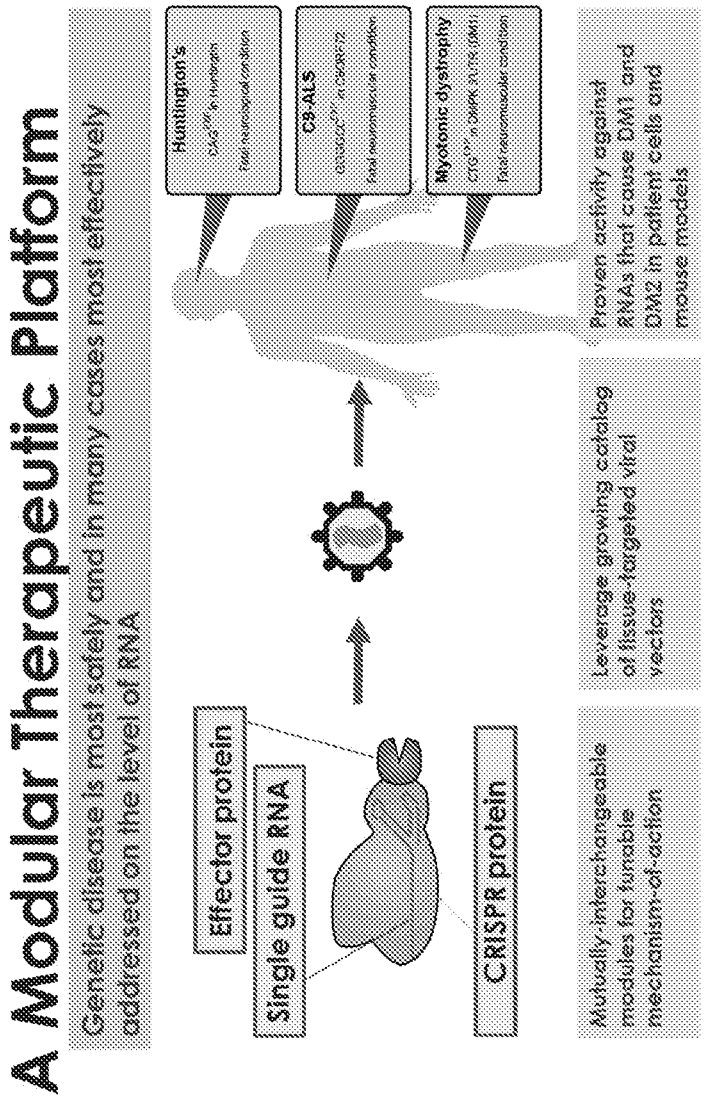
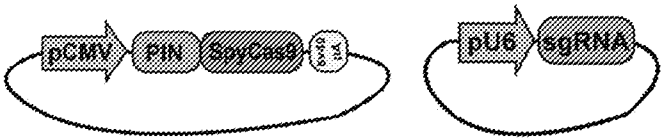

A Modular Therapeutic Platform
Genetic disease is most safely and in many cases most effectively addressed on the level of RNA
Effector protein
Single guide RNA
CRISPR protein
Mutually-interchangeable modules for tunable mechanism-of-action
Leverage growing catalog of tissue-targeted viral vectors
Huntington's
Fatal neurodegeneration
C9-ALS
Fatal neuromuscular condition
Myotonic dystrophy
Fatal neuromuscular condition
Proven activity against RNAs that cause DM1 and DM2 in patient cells and mouse models

FIGURE 3A

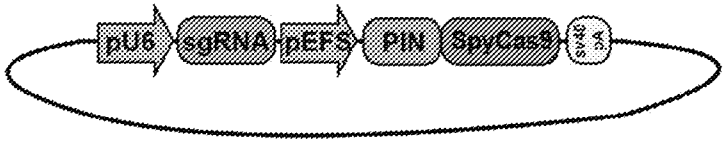
High expression positive control: "pos control"



Two plasmid system with PIN-SpyCas9 driven by a highly-active CMV promoter. This promoter is 5-20x as active as EFS.

FIGURE 3B

Low expression positive control: "P13"



Single plasmid system with lower expression of the fusion. Same architecture as the fusions involving new endonucleases

FIGURE 4A

CjeCas9 with N-terminal endonuclease: activity against CTG repeats
pU6
sgRNA
pEFS
endo
SpyCas9
SV40
pA
Endonuclease-C. jejuni Cas9 (nuclease inactive)

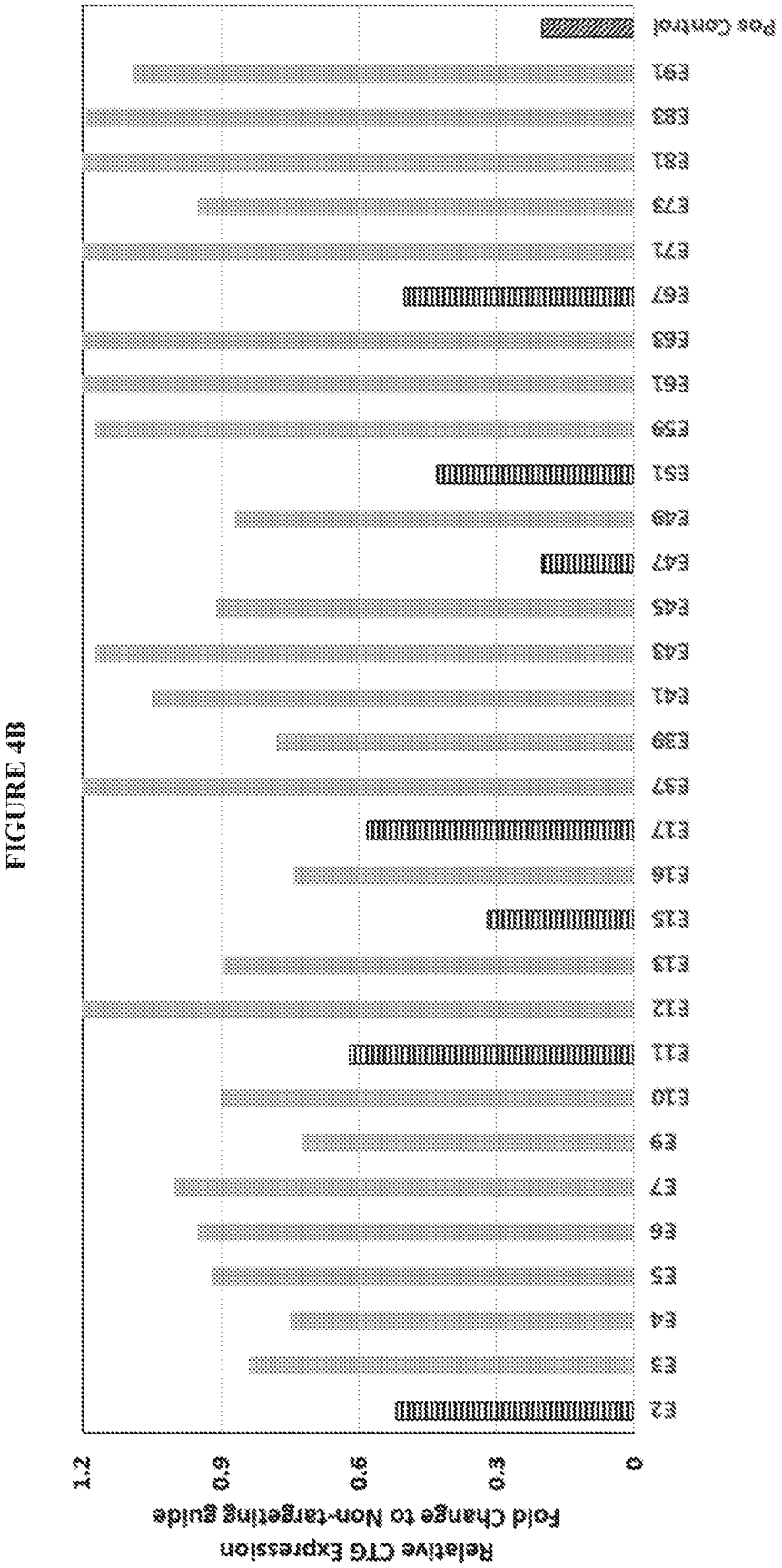

Relative CTG expression
fold Change to Non-targeting guide
1.2
0.9
0.6
0.3
0
E2
E3
E4
E5
E6
E7
E9
E10
E11
E12
E13
E15
E16
E17
E37
E39
E41
E43
E45
E47
E49
E51
E59
E61
E63
E67
E71
E73
E81
E83
E91
Pos Control

FIGURE 4B

FIGURE 5A

SpyCas9 with N-terminal endonuclease: activity against luciferase mRNA
pU6
sgRNA
pEF3
endo
CjeCas9
SV40 pA
Endonuclease-C. jejuni Cas9 (nuclease inactive)

FIGURE 5B

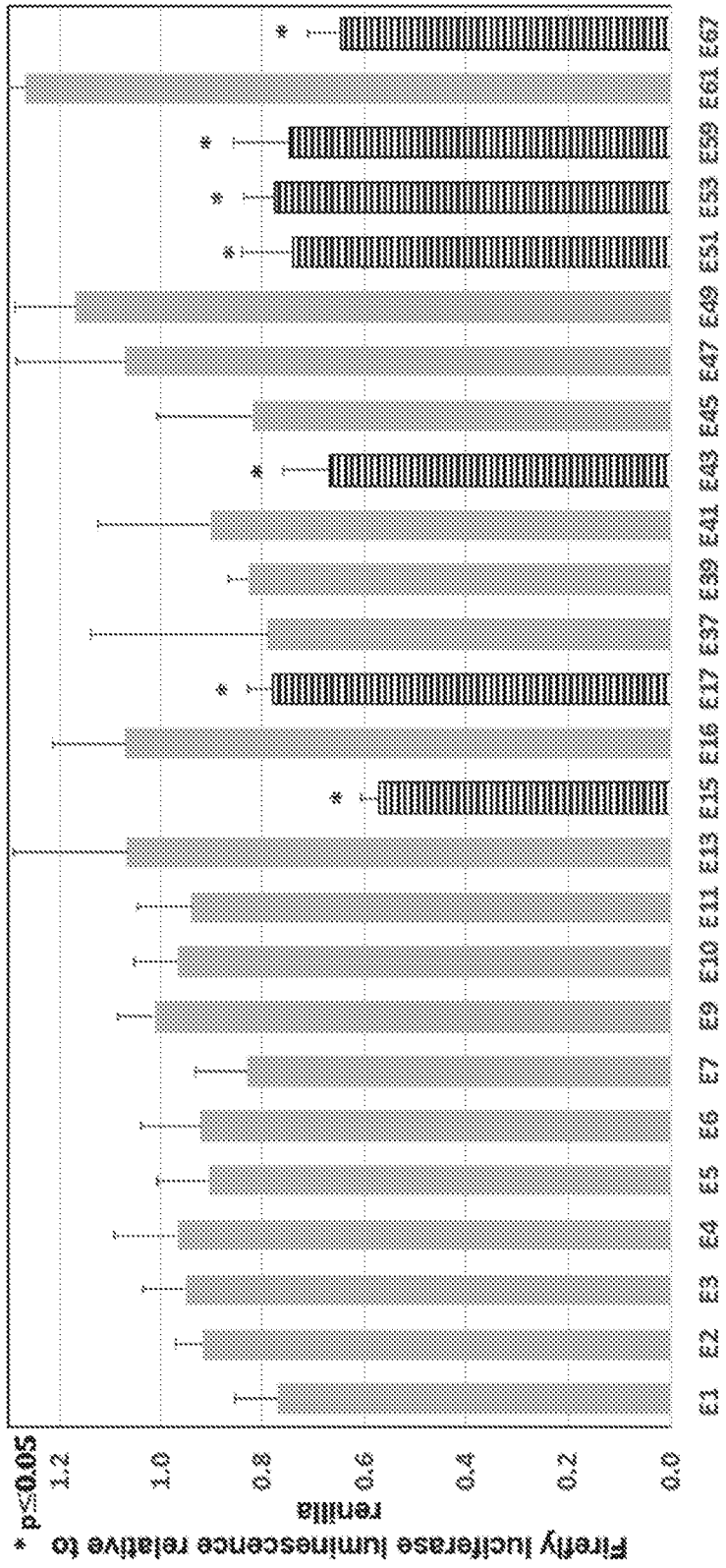

Firefly luciferase luminescence relative to renilla
0.0
0.2
0.4
0.6
0.8
1.0
1.2
* p≤0.05
E1
E2
E3
E4
E5
E6
E7
E9
E10
E11
E13
E15
E16
E17
E37
E39
E41
E43
E45
E47
E49
E51
E53
E59
E61
E67

FIGURE 6

Endonuclease Name Key

E1	RNAse1	E53	EXOG
E2	RNAse4	E55	ZC3H12D
E3	RNAse6	E57	ERN2
E4	NOB1	E59	ENDOG
E5	EndoV	E61	PELO
E6	FEN1	E63	YBEY
E7	SLFN14	E65	ENDOD1
E8	LACTB2	E67	CPSF4L
E9	RNAse7	E69	hCG_2002731
E10	RNAse8	E71	hCG_2002731
E11	RNAse2	E73	ERCC1
E12	ANG	E75	RAC1
E13	HRSP12	E77	APEX2
E14	RNAse6PL	E79	APEX2_1-350
E15	RNAseL	E81	RAA1_25-156
E16	RNAseT2	E83	RAB1
E17	ZC3H12A	E85	RNAseK
E37	RIDA	E87	DNA2_FL
E39	RNAse11	E89	RNASE1(K41R)
E41	PDL6	E91	RNASE1(K41R, D121E)
E43	NTHL1	E93	RNASE1(K41R, D121E, H119N)
E45	KIAA0391	E95	RNASE1(H119N)
E47	APEX1	E97	RNASE1(R39D, N67D, N88A, G89D, R91D, H119N)
E49	Ago2	E99	RNASE1(R39D, N67D, N88A, G89D, R91D, H119N, K41R, D121E)
E51	ZC3H12A	E100	RNAse1 (R39D, N67D, N88A, G89D, R91D, H119N, K41R, D121E)
		E101	RNASE1(R39D, N67D, N88A, G89D, R91D)

FIGURE 7A

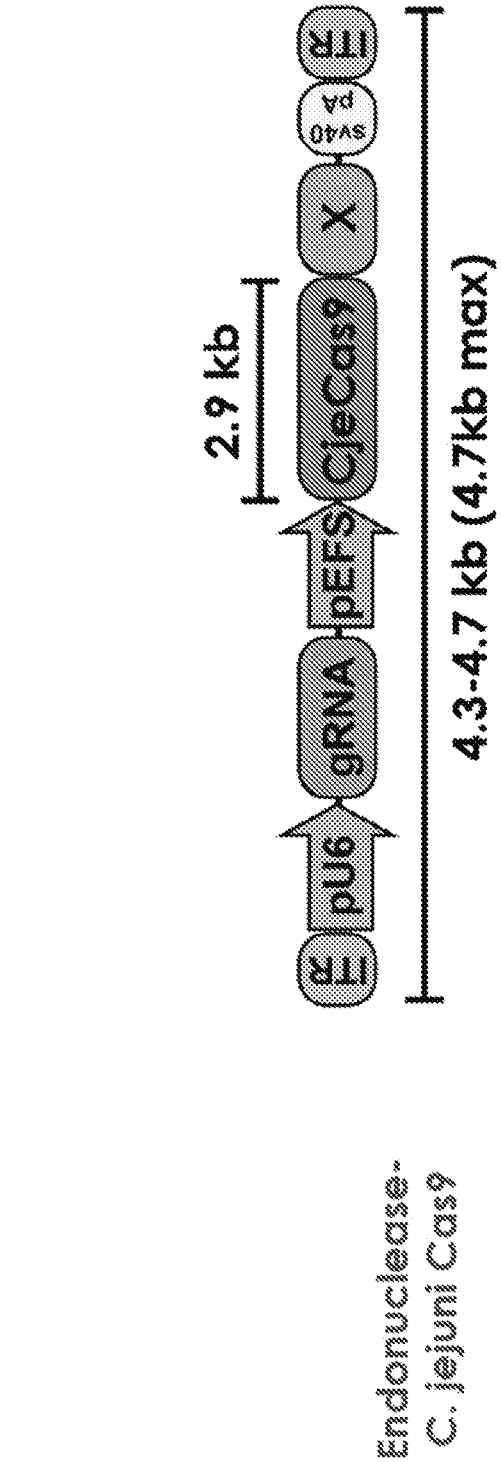

ITR
pU6
gRNA
pEFS
CjeCas9
X
SV40 pA
ITR
2.9 kb
4.3-4.7 kb (4.7kb max)
Endonuclease-
C. jejuni Cas9

FIGURE 7B

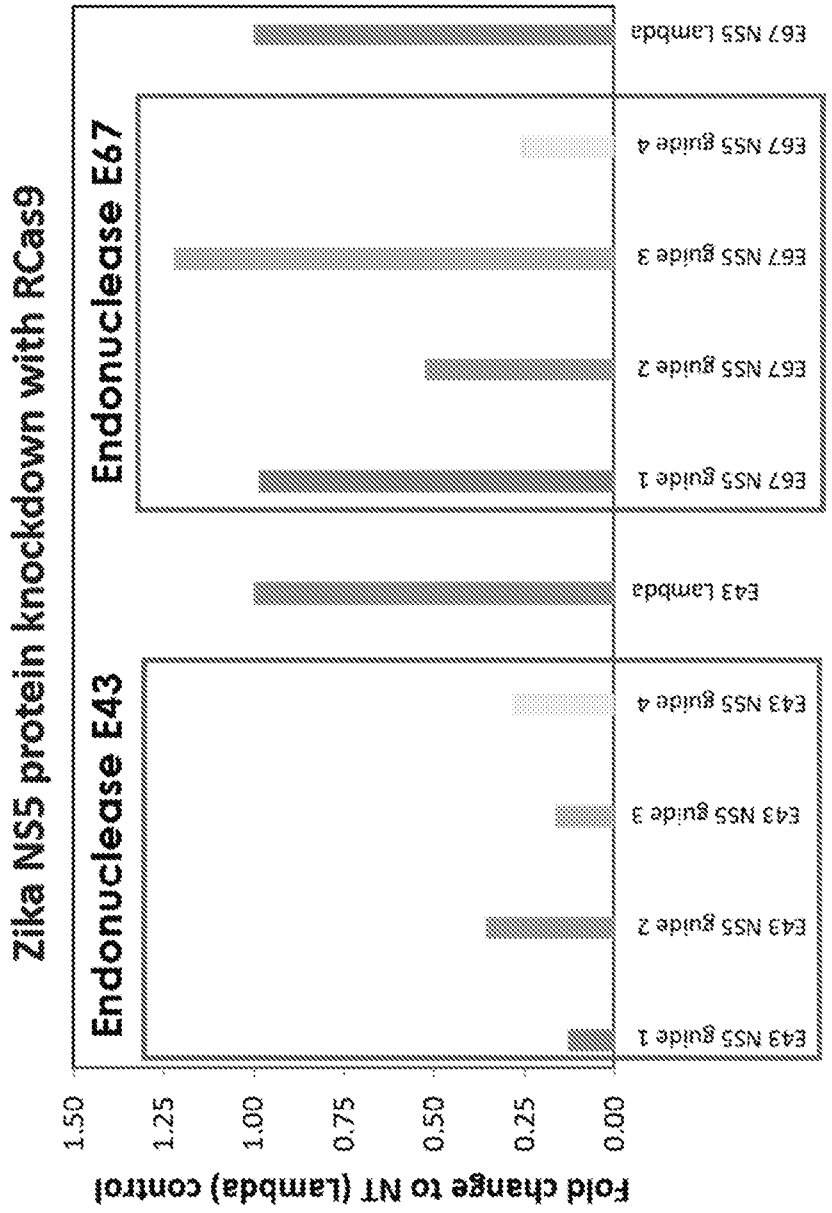
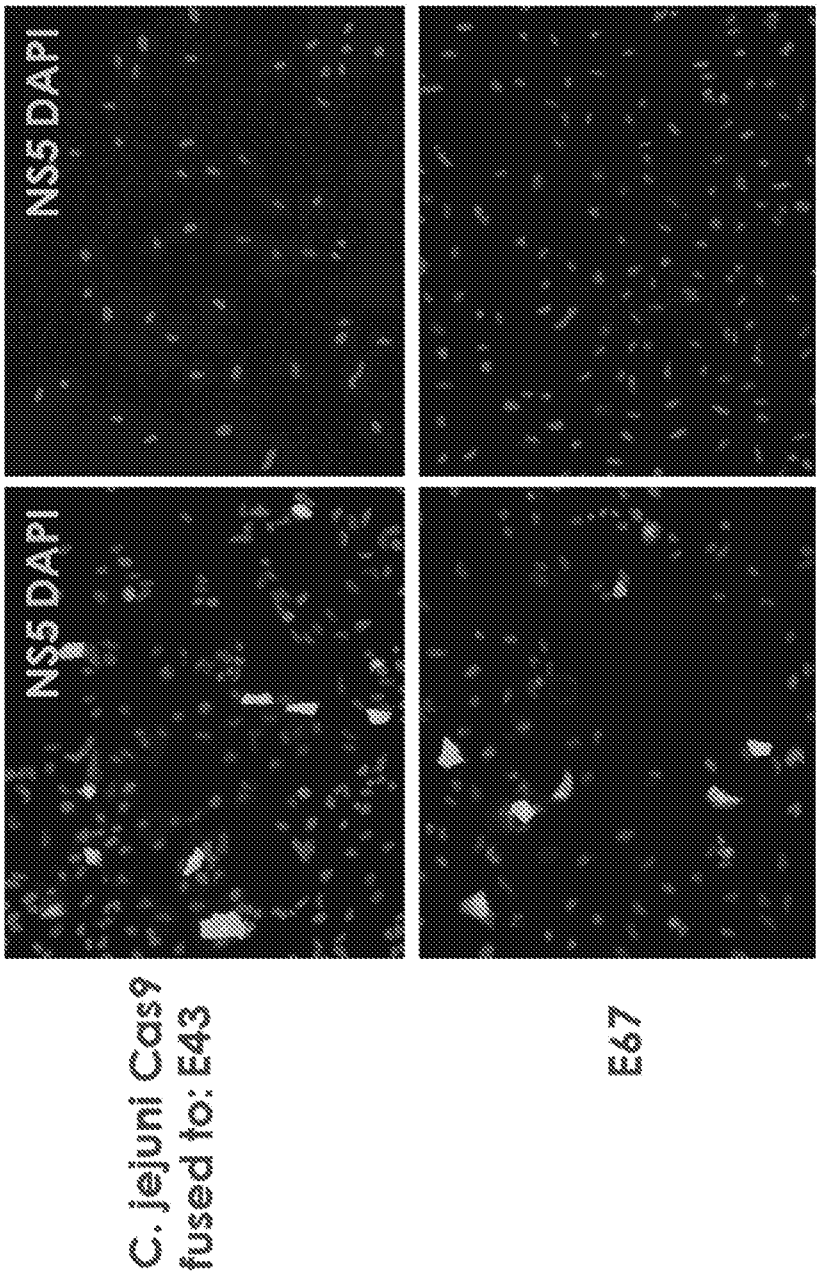

Zika NS5 protein knockdown with RCas9
Fold change to NT (lambda) control
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Endonuclease E43
E43 NS5 guide 1
E43 NS5 guide 2
E43 NS5 guide 3
E43 NS5 guide 4
E43 lambda
Endonuclease E67
E67 NS5 guide 1
E67 NS5 guide 2
E67 NS5 guide 3
E67 NS5 guide 4
E67 NS5 lambda

FIGURE 8A


sgRNA targeting: non-targeting
Zika NS5
NS5 DAPI
NS5 DAPI
C. jejuni Cas9 fused to: E43
E67

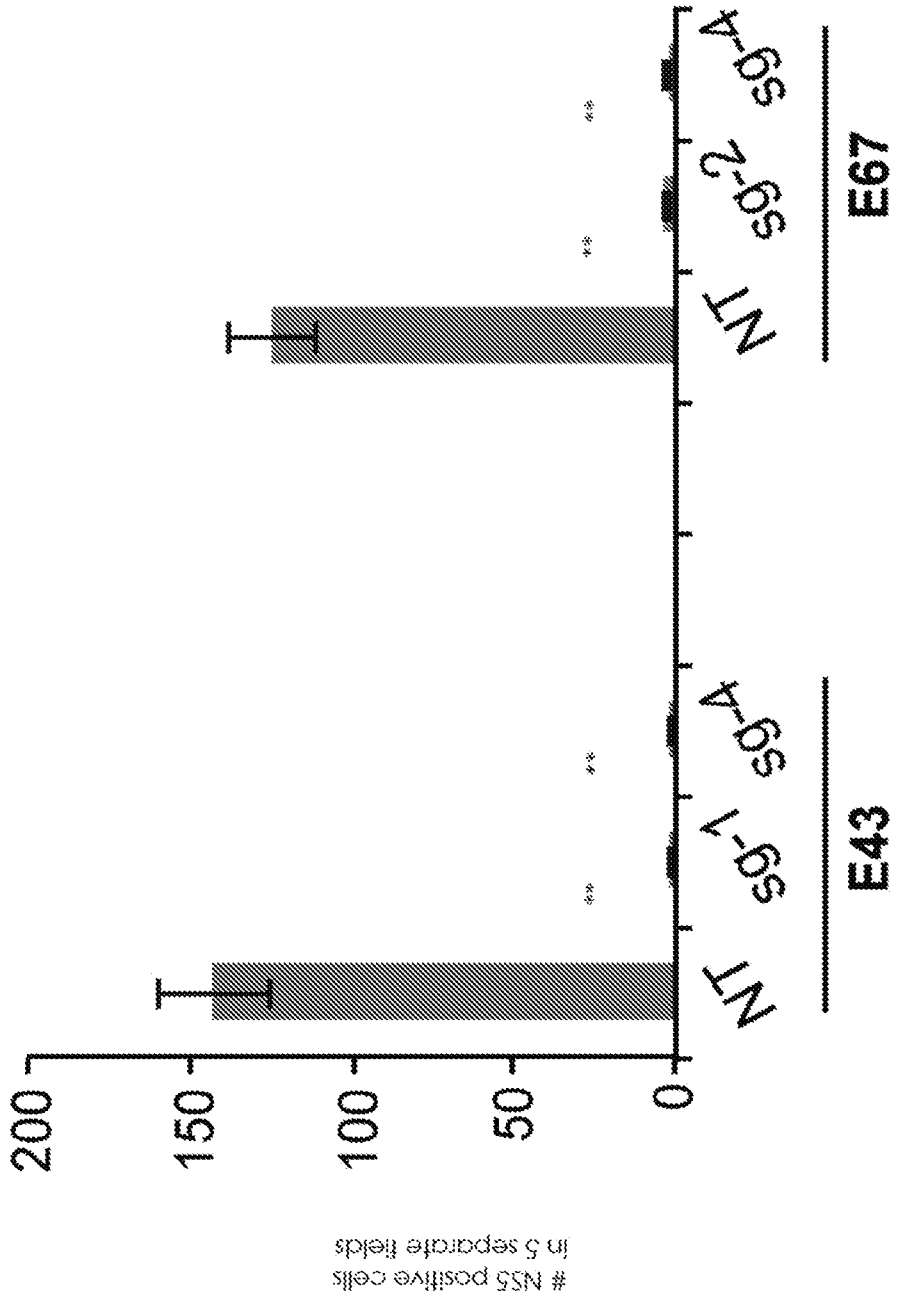
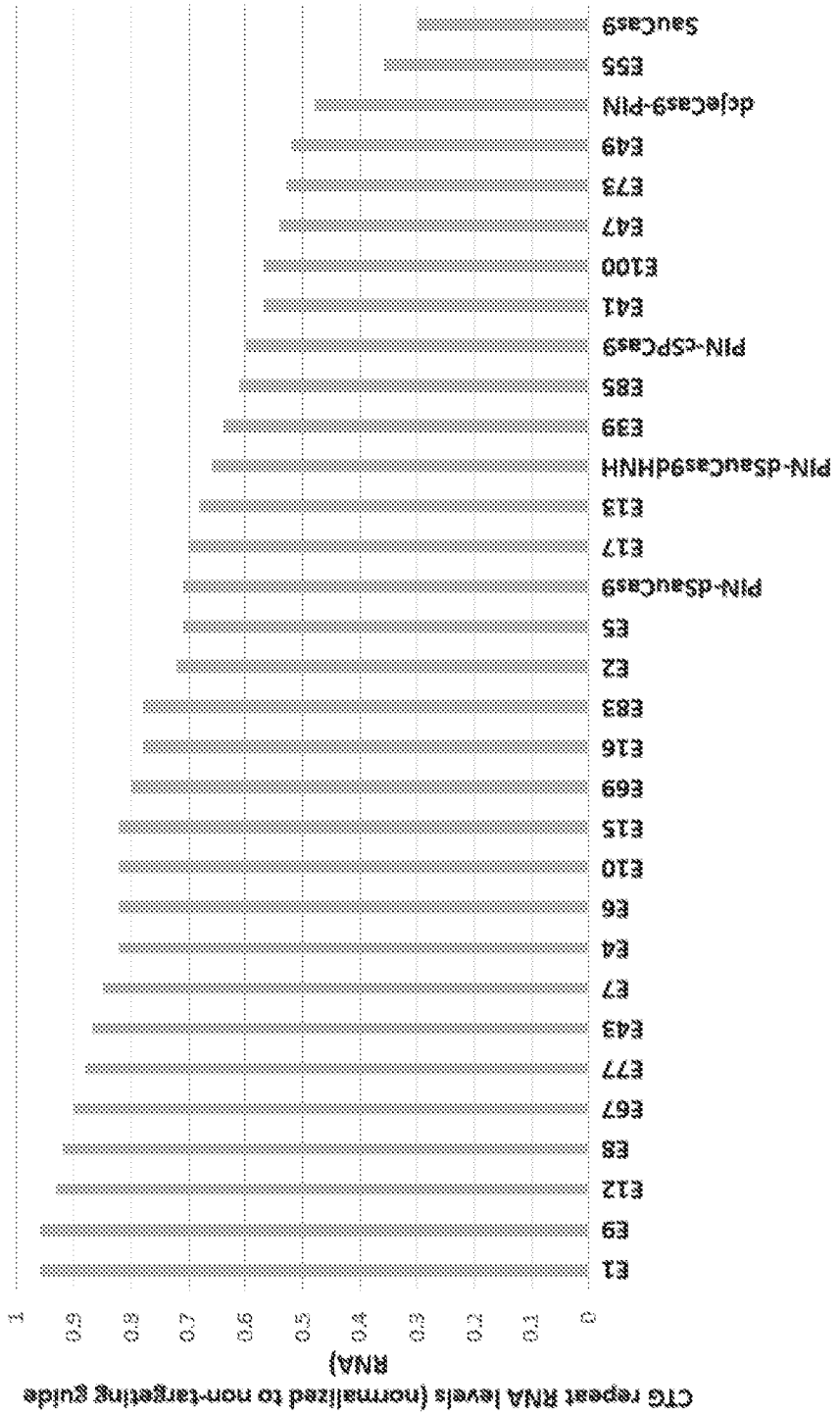

200
150
100
50
0
N55 positive cells
in 5 separate fields
NT
sg-1
sg-4
NT
sg-2
sg-4
E43
E67
**
**
**
**

FIGURE 8B

FIGURE 9


CTG repeat RNA levels (normalized to non-targeting guide RNA)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
E1
E9
E12
E8
E67
E77
E43
E7
E4
E6
E10
E15
E69
E16
E83
E2
E5
PIN-dSauCas9
E17
E13
PIN-dSauCas9dHNH
E39
E85
PIN-cSPCas9
E41
E100
E47
E73
E49
dCjeCas9-PIN
E55
SauCas9