FIGURE 1

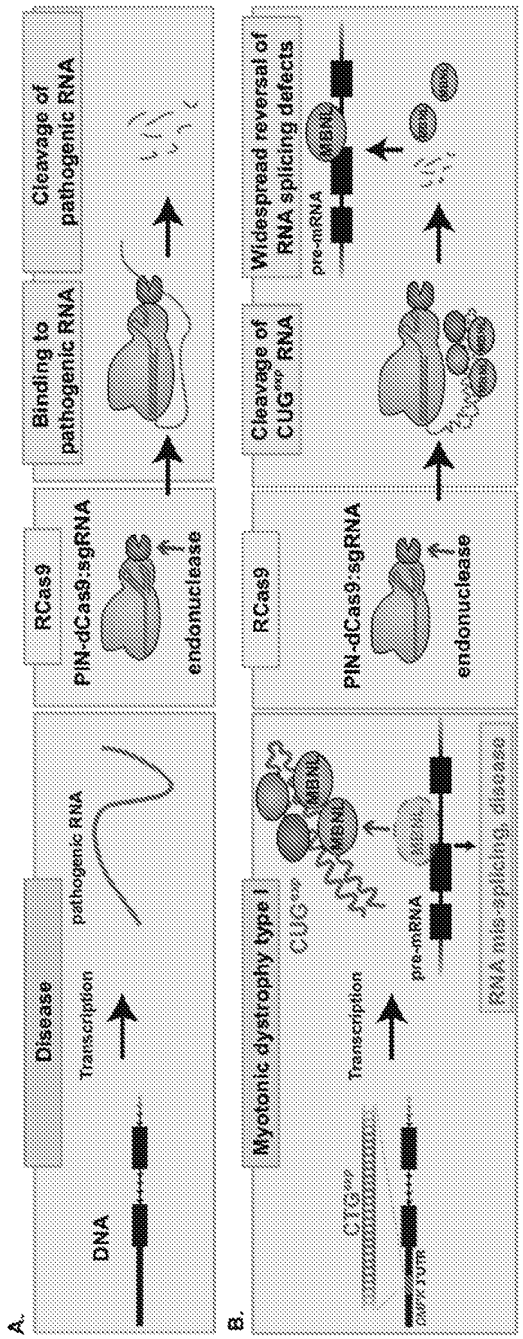

A.
Disease
DNA
Transcription
pathogenic RNA
RCas9
PIN-dCas9:sgRNA
endonuclease
Binding to pathogenic RNA
Cleavage of pathogenic RNA
B.
Myotonic dystrophy type I
CTG^exp
Transcription
pre-mRNA
MBNL
RNA mis-splicing, disease
RCas9
PIN-dCas9:sgRNA
endonuclease
Cleavage of CUG^exp RNA
Widespread reversal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

FIGURE 2

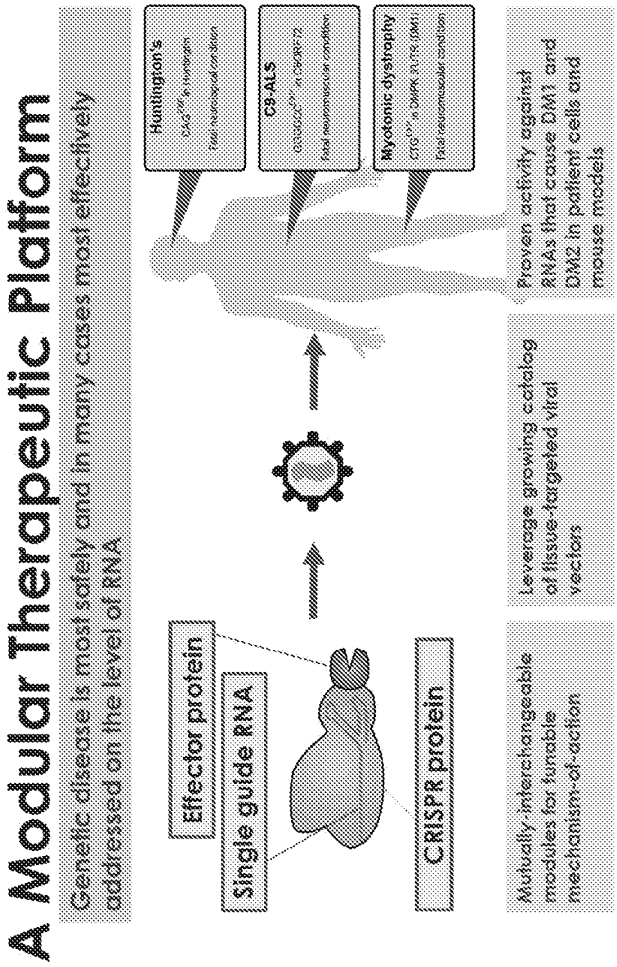

A Modular Therapeutic Platform
Genetic disease is most safely and in many cases most effectively addressed on the level of RNA
Huntington's
C9-ALS
Myotonic dystrophy
Effector protein
Single guide RNA
CRISPR protein
Mutually-interchangeable modules for tunable mechanism-of-action
Leverage growing catalog of tissue-targeted viral vectors
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)

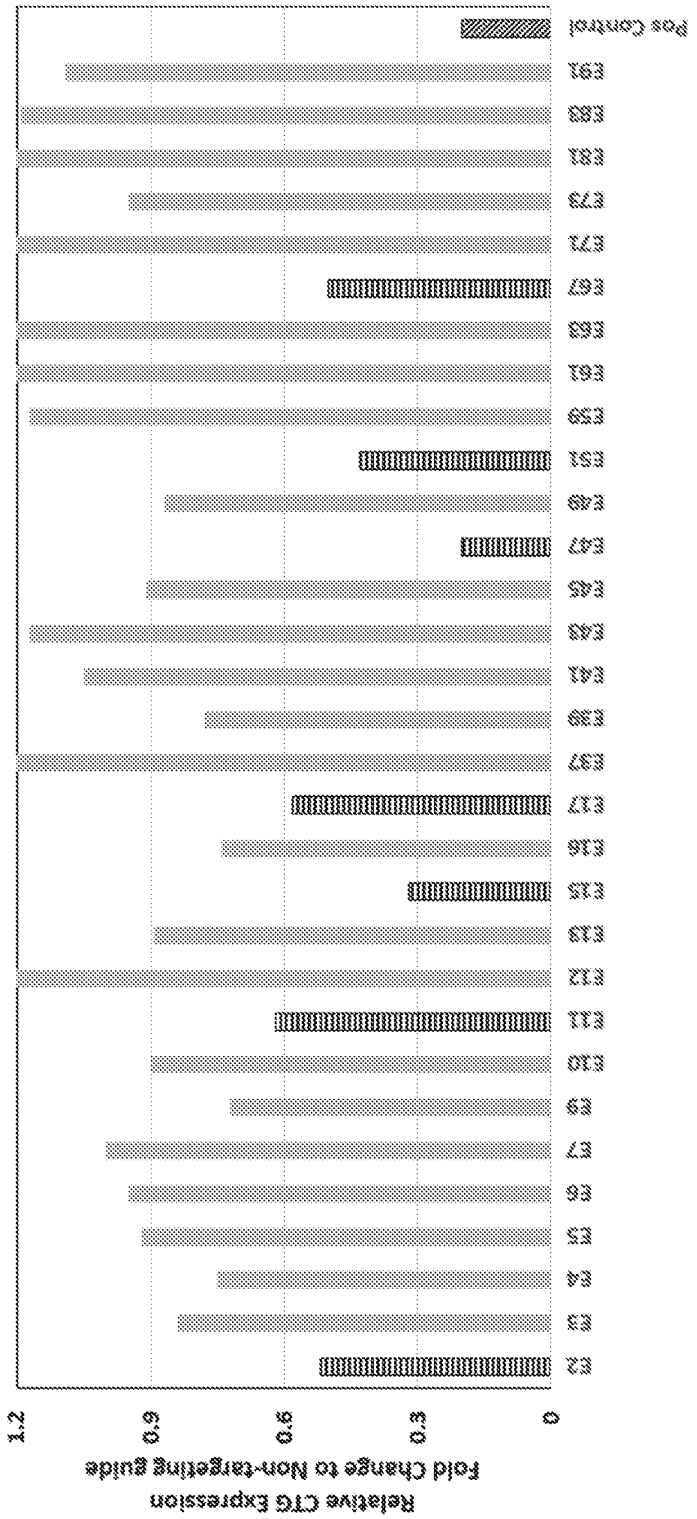

Relative CTG Expression
fold Change to Non-targeting guide
1.2
0.9
0.6
0.3
0
E2
E3
E4
E5
E6
E7
E9
E10
E11
E12
E13
E15
E16
E17
E37
E39
E41
E43
E45
E47
E49
E51
E59
E61
E63
E67
E71
E73
E81
E83
E91
Pos Control

FIGURE 4B

FIGURE 5A

SpyCas9 with N-terminal endonuclease: activity against luciferase mRNA
Endonuclease-C. jejuni Cas9 (nuclease inactive)
pU6
sgRNA
pEF1
endo
CjCas9
2A
T2

FIGURE 5B

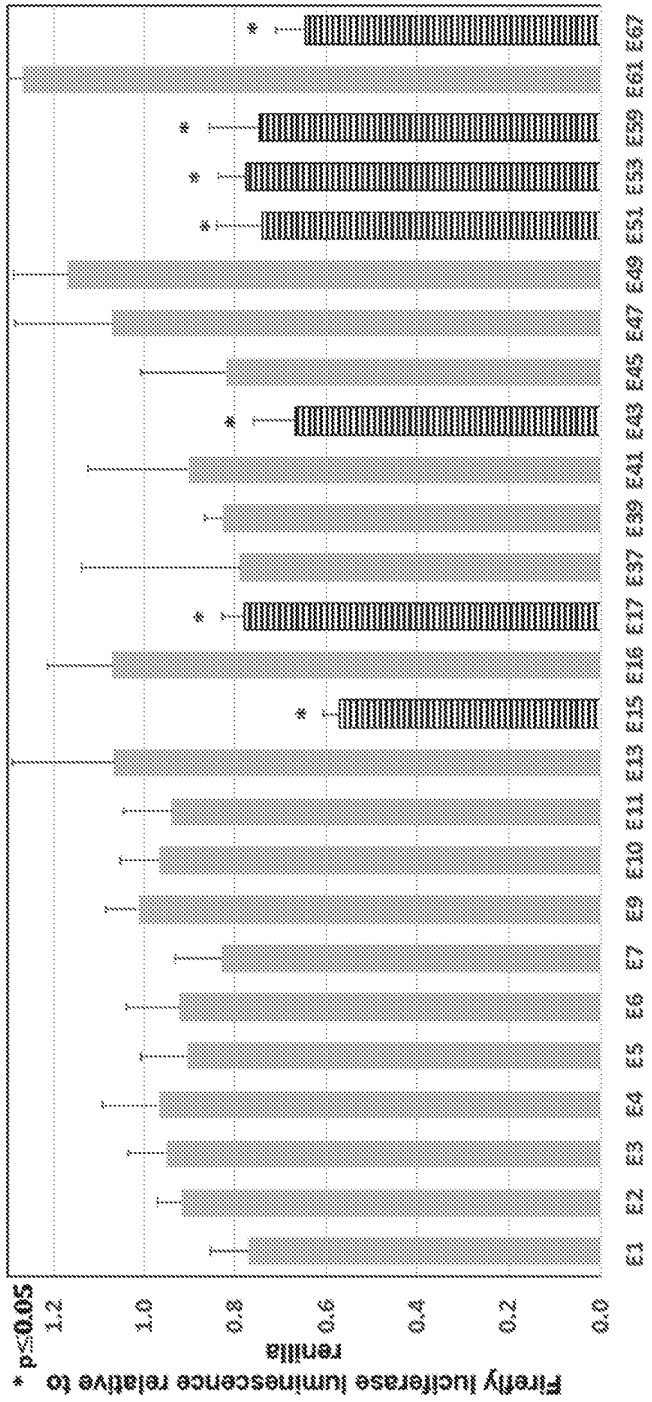

Firefly luciferase luminescence relative to renilla
* p≤0.05
1.2
1.0
0.8
0.6
0.4
0.2
0.0
E1
E2
E3
E4
E5
E6
E7
E9
E10
E11
E13
E15
E16
E17
E37
E39
E41
E43
E45
E47
E49
E51
E53
E59
E61
E67

FIGURE 6

Endonuclease Name Key

E1	RNAseI	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

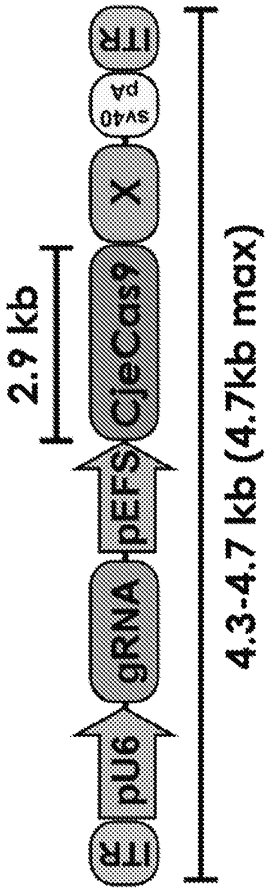

ITR
pU6
gRNA
pEFS
CjeCas9
X
SV40 pA
ITR
2.9 kb
4.3-4.7 kb (4.7kb max)
Endonuclease-
C. jejuni Cas9

FIGURE 7B

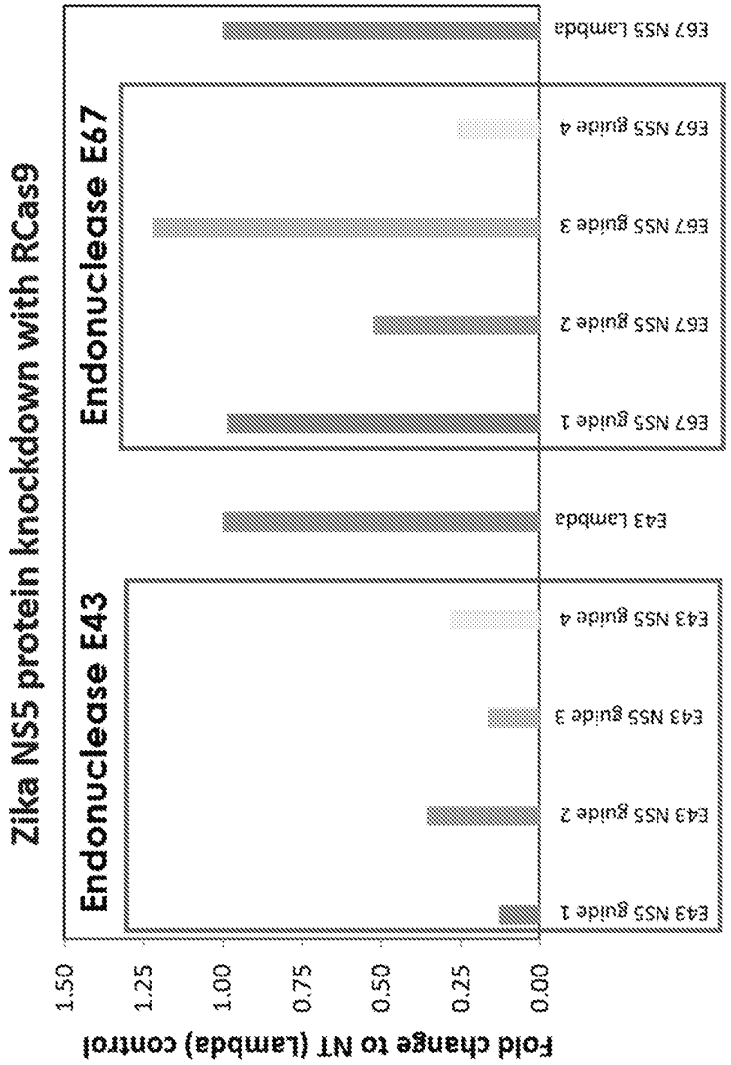

Zika NS5 protein knockdown with RCas9
Endonuclease E43
Endonuclease E67
Fold change to NT (lambda) control
1.50
1.25
1.00
0.75
0.50
0.25
0.00
E43 NS5 guide 1
E43 NS5 guide 2
E43 NS5 guide 3
E43 NS5 guide 4
E43 lambda
E67 NS5 guide 1
E67 NS5 guide 2
E67 NS5 guide 3
E67 NS5 guide 4
E67 NS5 lambda

FIGURE 8A

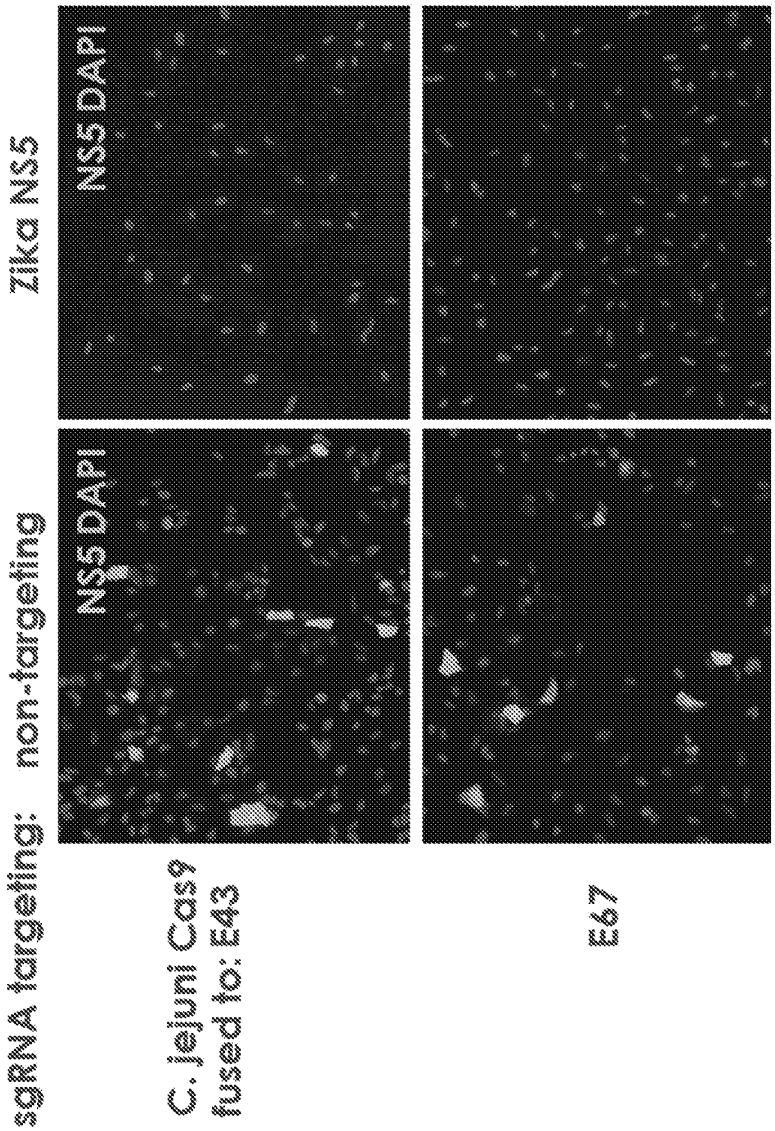

sgRNA targeting:
non-targeting
Zika NS5
C. jejuni Cas9 fused to: E43
NS5 DAPI
NS5 DAPI
E67

FIGURE 8B

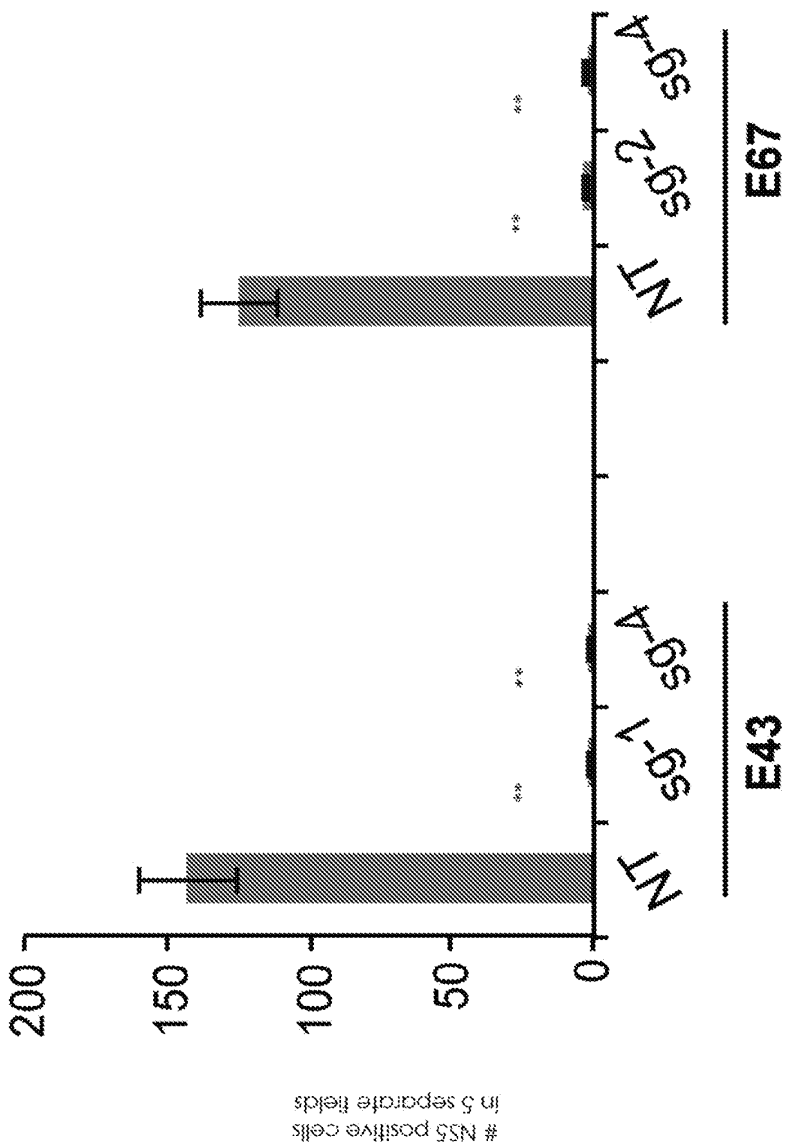
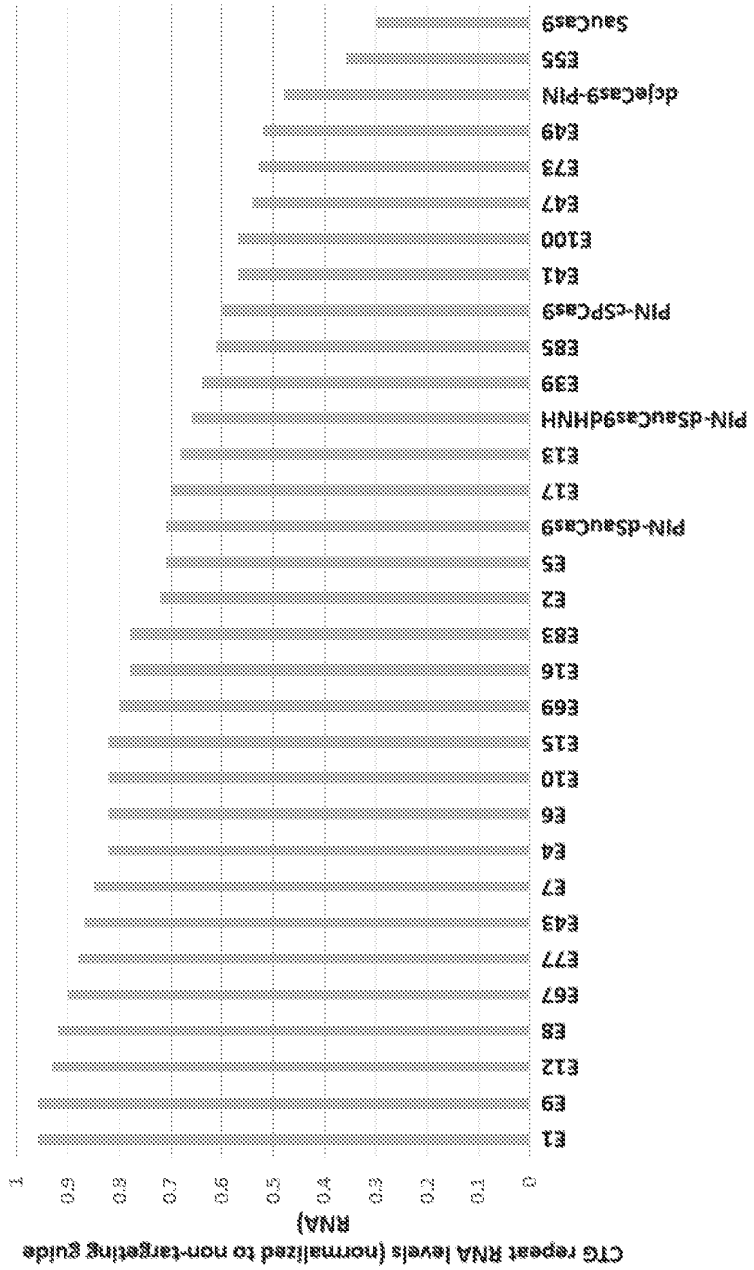

N55 positive cells
in 5 separate fields
200
150
100
50
0
NT
sg-1
sg-4
NT
sg-2
sg-4
E43
E67
**
**
**
**

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/036021

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 9/22; C12N 15/113 (2019.01)
CPC - C07K 2319/85; C12N 9/22; C12N 15/113; C12N 2310/20 (2019.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/199; 435/69.7 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2017/0145394 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 25 May 2017 (25.05.2017) entire document	1-12, 14-17, 19, 20 ---------- 13, 18
Y	US 2017/0088845 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA et al) 30 March 2017 (30.03.2017) entire document	13
Y	US 2013/0178513 A1 (ISIS PHARMACEUTICALS, INC. et al) 11 July 2013 (11.07.2013) entire document	18
A	US 2017/0314002 A1 (BIO-RAD LABORATORIES, INC.) 02 November 2017 (02.11.2017) entire document	1-20
A	WO 2015/089486 A2 (THE BROAD INSTITUTE INC. et al) 18 June 2015 (18.06.2015) entire document	1-20

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 September 2019	16 OCT 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774