FIGURE 1

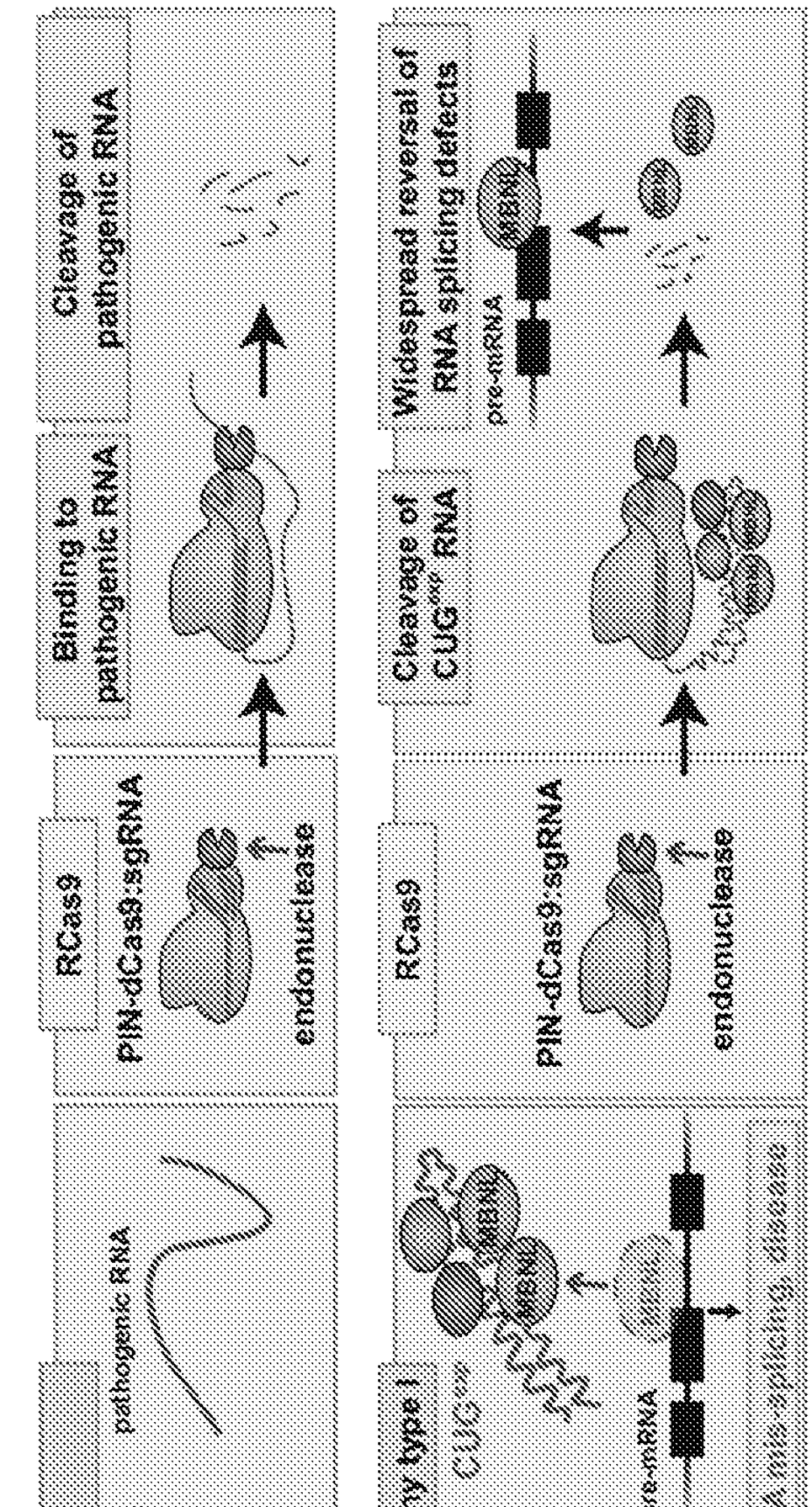

A.
DNA
Transcription
pathogenic RNA
Disease
B.
Myotonic dystrophy type 1
CUG exp
Transcription
pre-mRNA
RNA mis-splicing, disease
CTG exp
Gene X STR
PIN-dCas9:sgRNA
RCas9
endonuclease
Binding to pathogenic RNA
Cleavage of pathogenic RNA
Cleavage of CUG exp RNA
Widespread reversal of RNA splicing defects
pre-mRNA

FIGURE 2

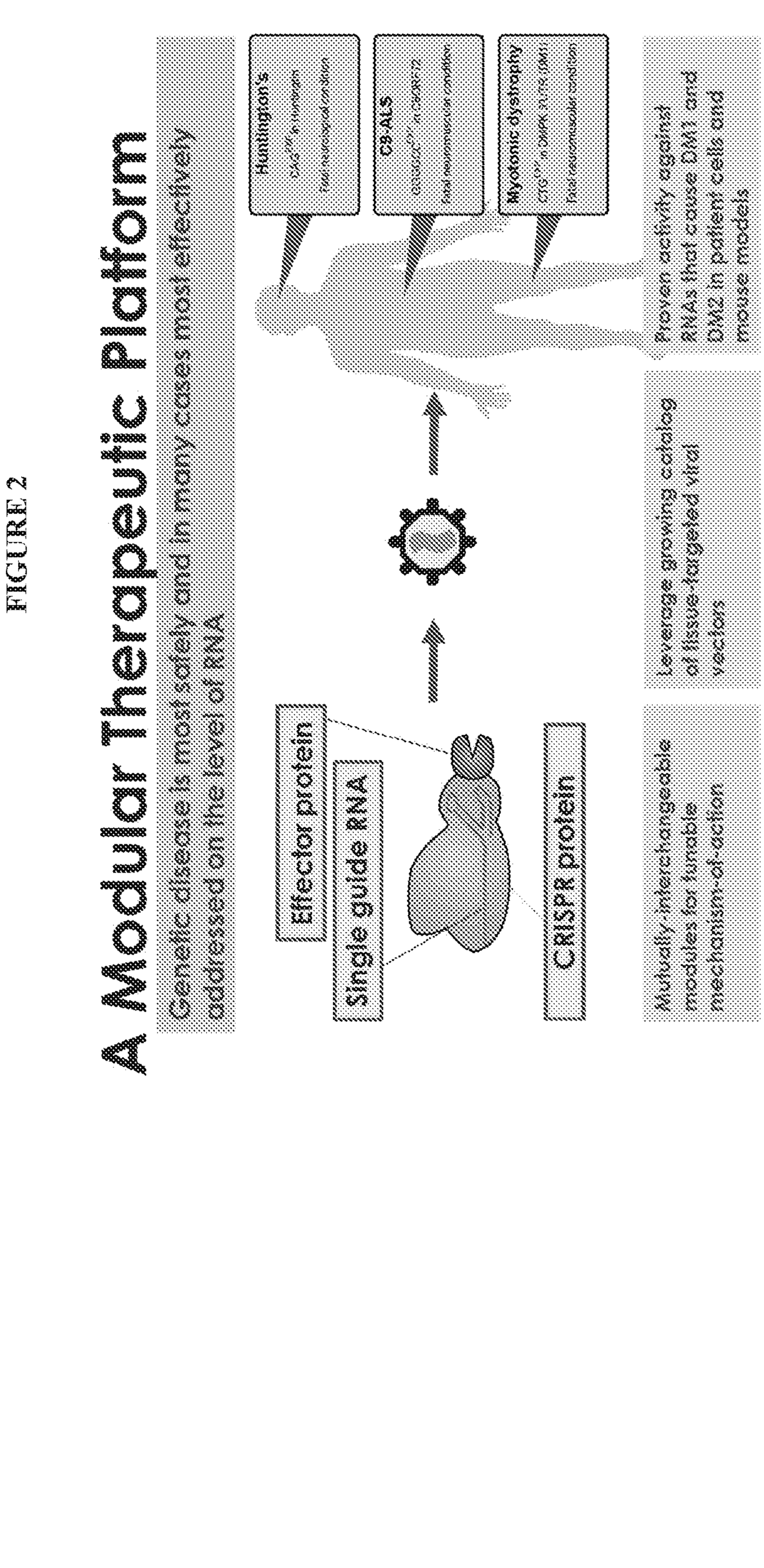
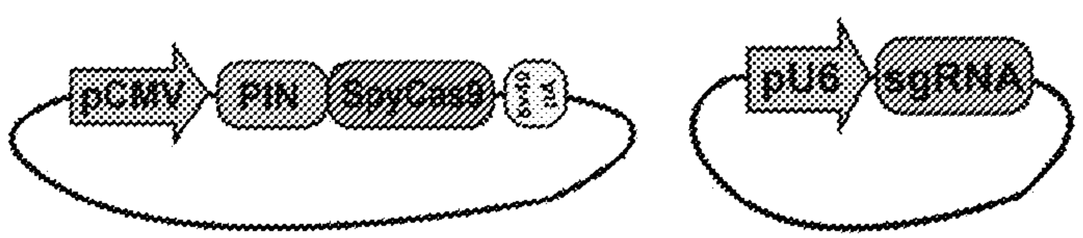

A Modular Therapeutic Platform
Genetic disease is most safely and in many cases most effectively addressed on the level of RNA
Huntington's
CAG-expansion in Huntingtin
Fatal neurodegeneration
C9-ALS
Hexanucleotide repeat in C9orf72
Fatal neurodegenerative condition
Myotonic dystrophy
CTG-expansion in DMPK 3'UTR (DM1)
Fatal neuromuscular condition
Proven activity against RNAs that cause DM1 and DM2 in patient cells and mouse models
Effector protein
Single guide RNA
CRISPR protein
Mutually interchangeable modules for tunable mechanism-of-action
Leverage growing catalog of tissue-targeted viral vectors

FIGURE 3A

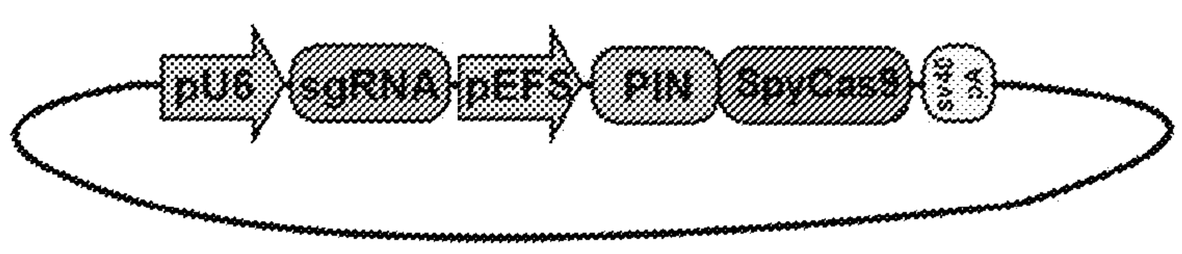
High expression positive control: "pos control"



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"



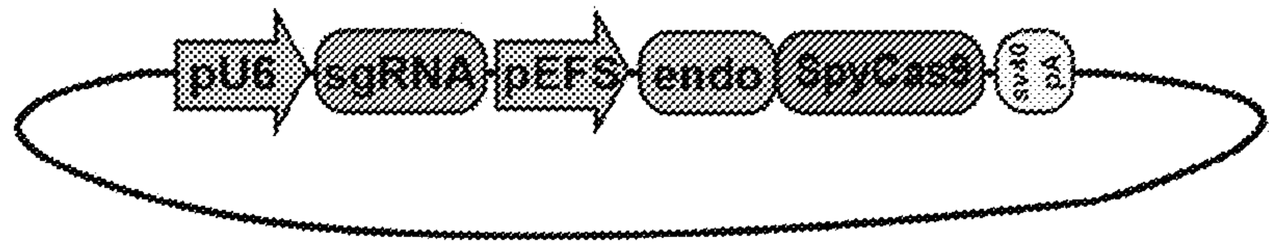
Single plasmid system with lower expression of the fusion. Same architecture as the fusions involving new endonucleases

FIGURE 4A

CjeCas9 with N-terminal endonuclease: activity against CTG repeats



Endonuclease-C. jejuni Cas9 (nuclease inactive)



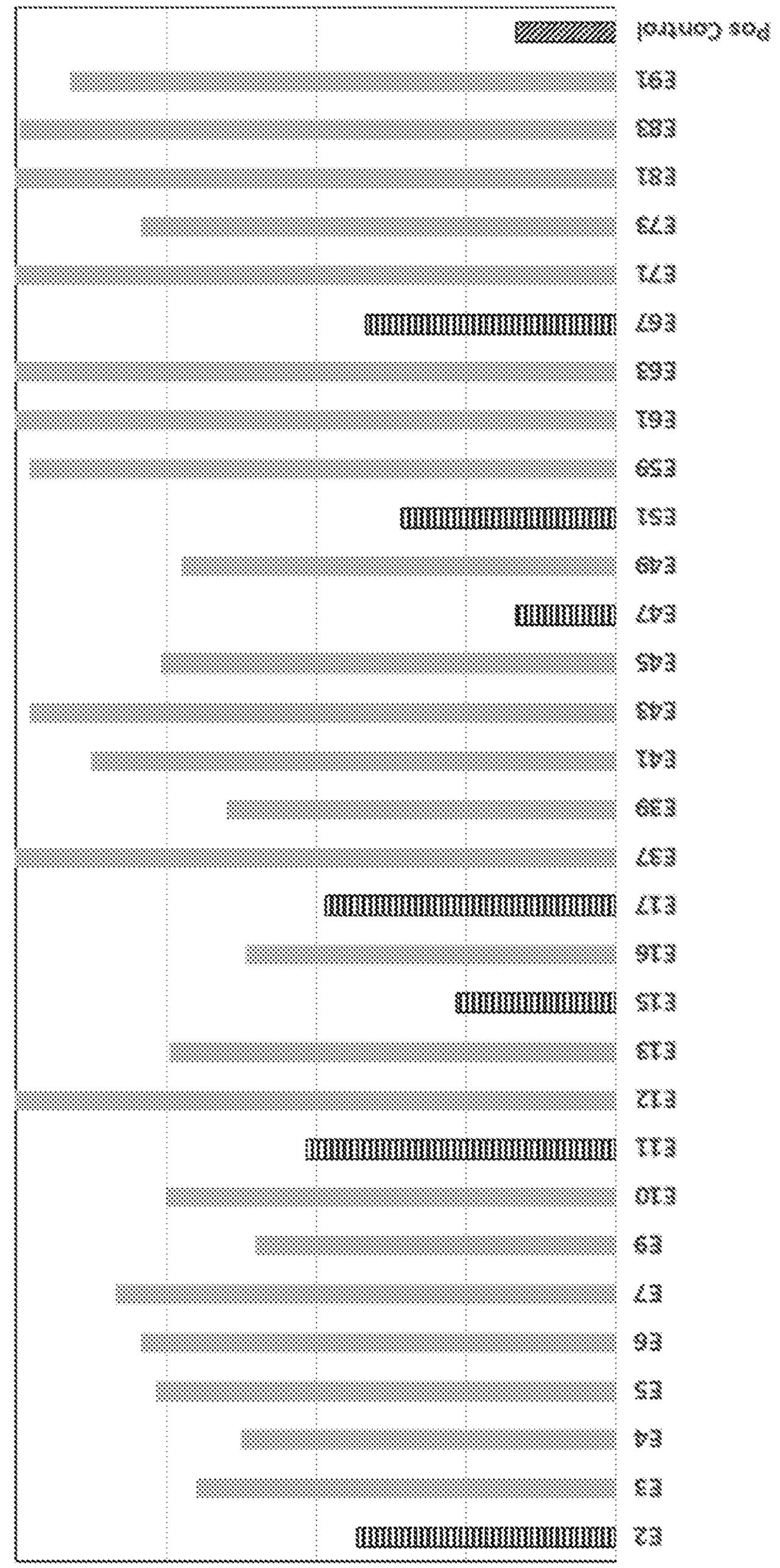
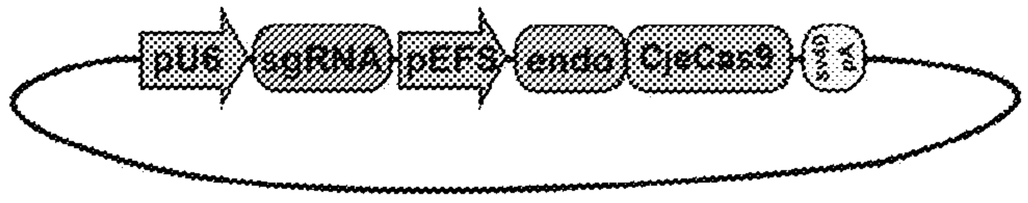

Non-targeting siRNA
E91
E89
E81
E73
E71
E67
E63
E61
E59
E51
E49
E47
E45
E43
E41
E39
E37
E17
E15
E13
E12
E10
E3
E7
E6
E5
E4
E2
1.2
0.9
0.6
0.3
0
Relative CTG expression
fold change to Non-targeting siRNA

FIGURE 4B

FIGURE 5A

SpyCas9 with N-terminal endonuclease: activity against luciferase mRNA





Endonuclease-C. jejuni Cas9 (nuclease inactive)

FIGURE 5B

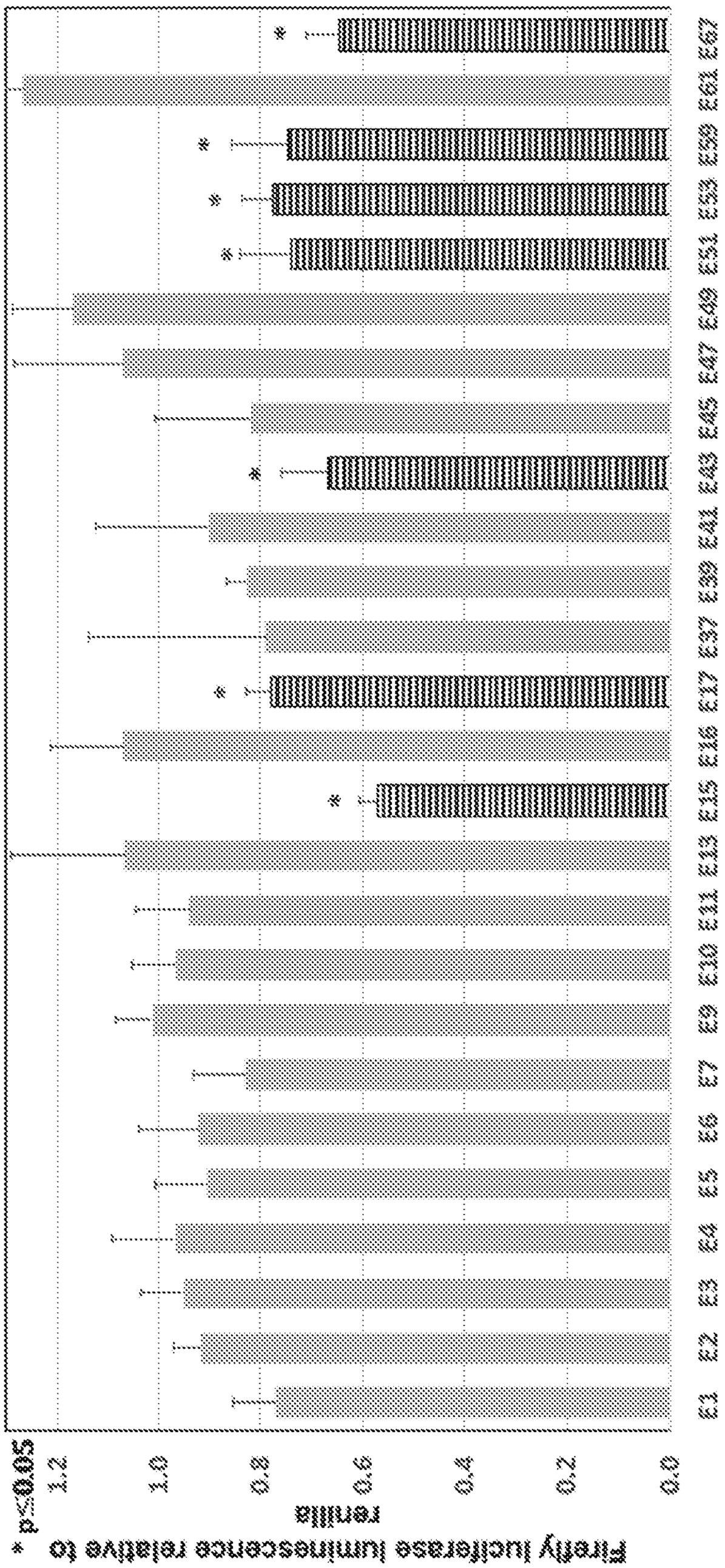

firefly luciferase luminescence relative to renilla
* p≤0.05
0.0
0.2
0.4
0.6
0.8
1.0
1.2
E1
E2
E3
E4
E5
E6
E7
E9
E10
E11
E13
E15
E16
E17
E37
E39
E41
E43
E45
E47
E49
E51
E61
E67

FIGURE 6

Endonuclease Name Key

E1	RNAse1	E53	EXOG
E2	RNAse4	E55	ZC3H13D
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)

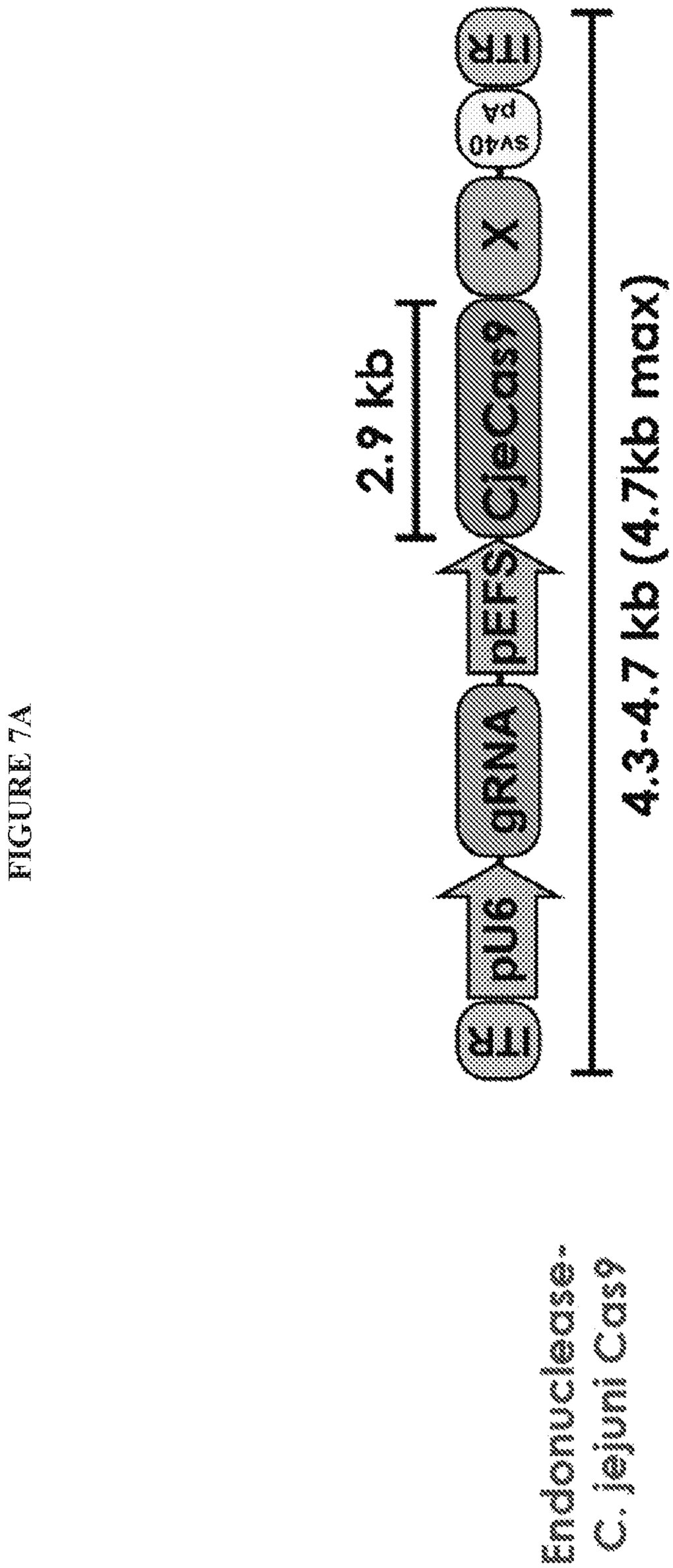


FIGURE 7A

Endonuclease-
C. jejuni Cas9

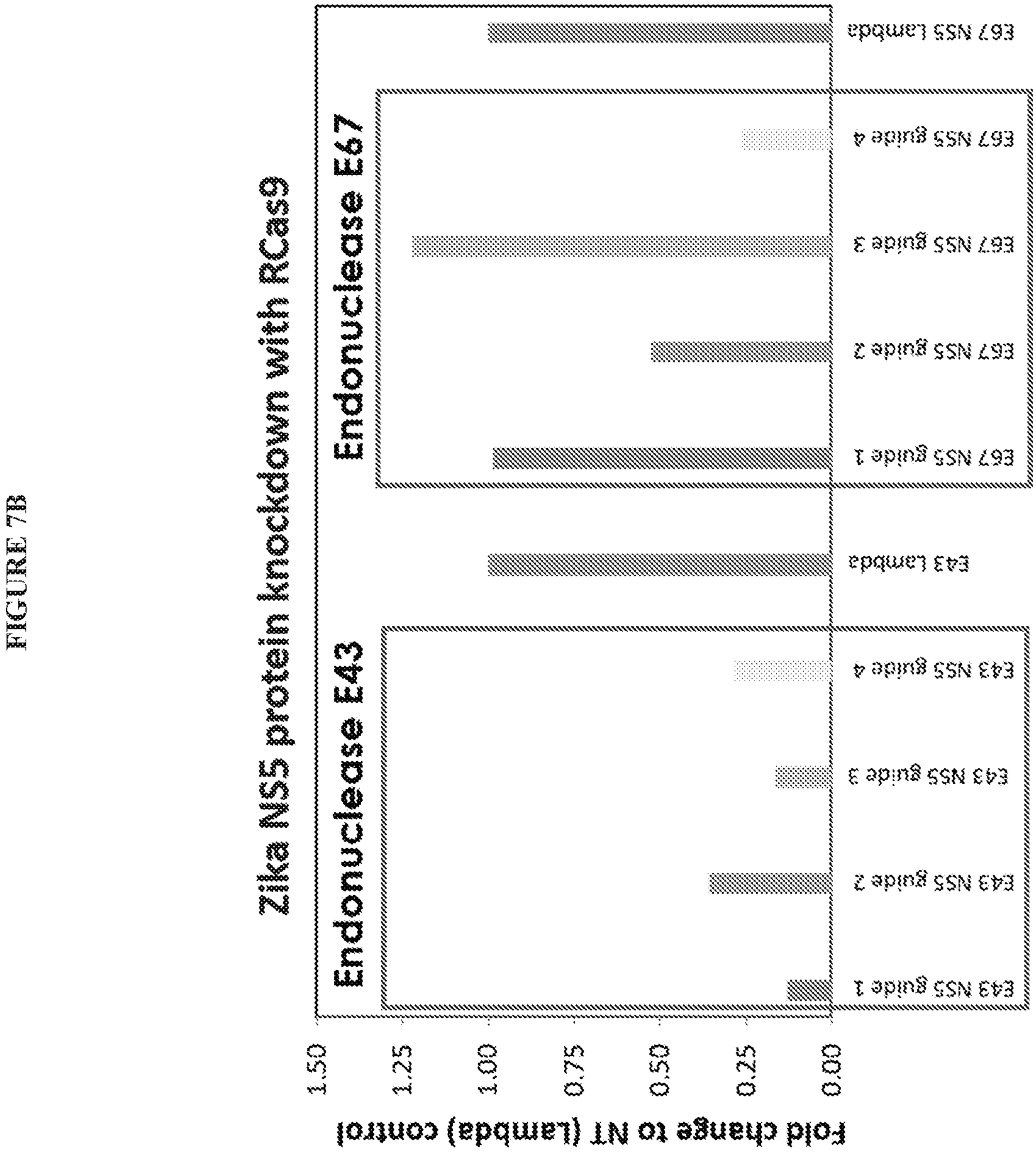

Zika NS5 protein knockdown with RCas9
Endonuclease E43
E43 NS5 guide 1
E43 NS5 guide 2
E43 NS5 guide 3
E43 NS5 guide 4
E43 lambda
Endonuclease E67
E67 NS5 guide 1
E67 NS5 guide 2
E67 NS5 guide 3
E67 NS5 guide 4
E67 NS5 lambda
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Fold change to NT (lambda) control

FIGURE 7B

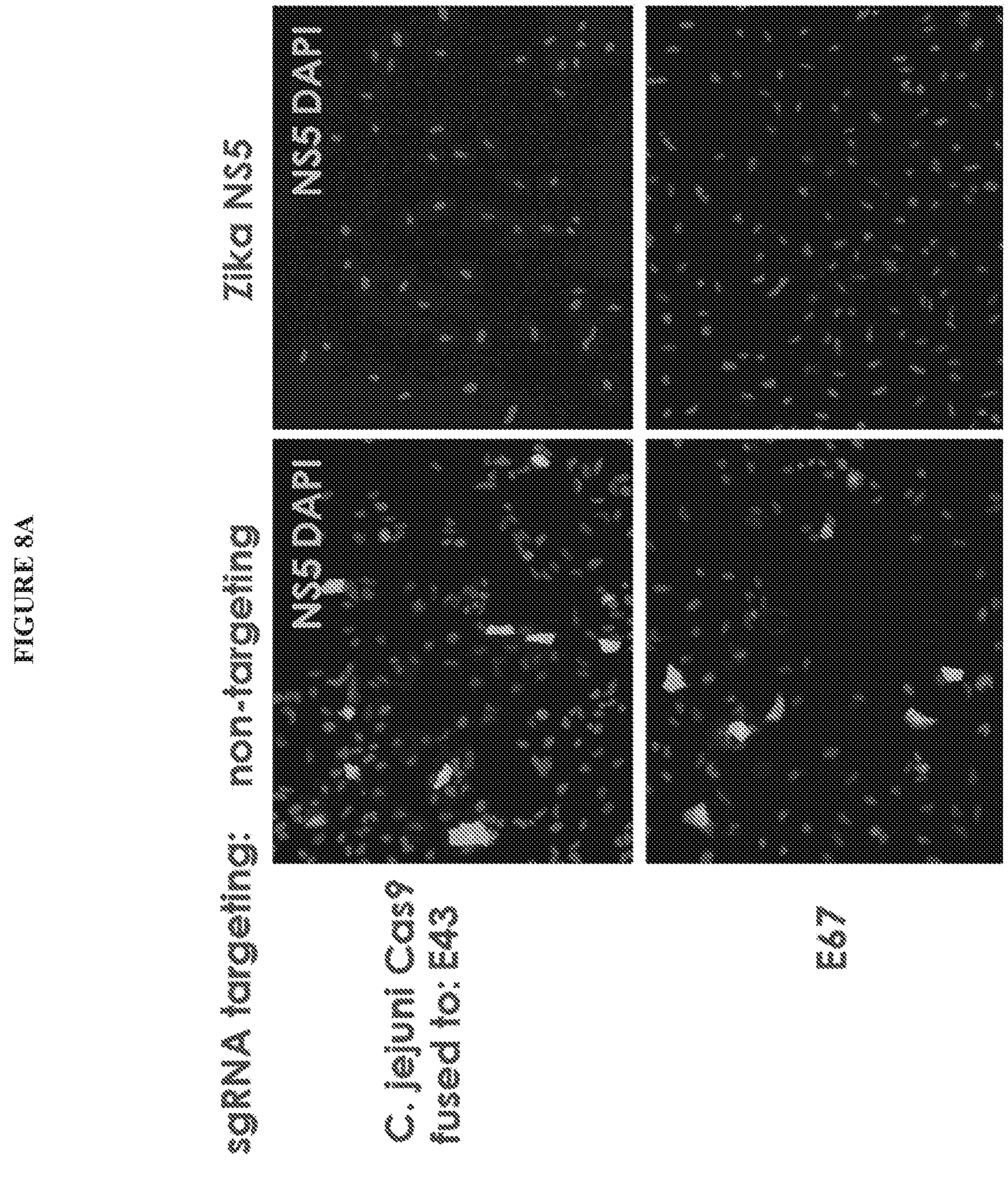

Zika NS5
NS5 DAPI
sgRNA targeting:
non-targeting
C. jejuni Cas9
fused to: E43
E67

FIGURE 8A

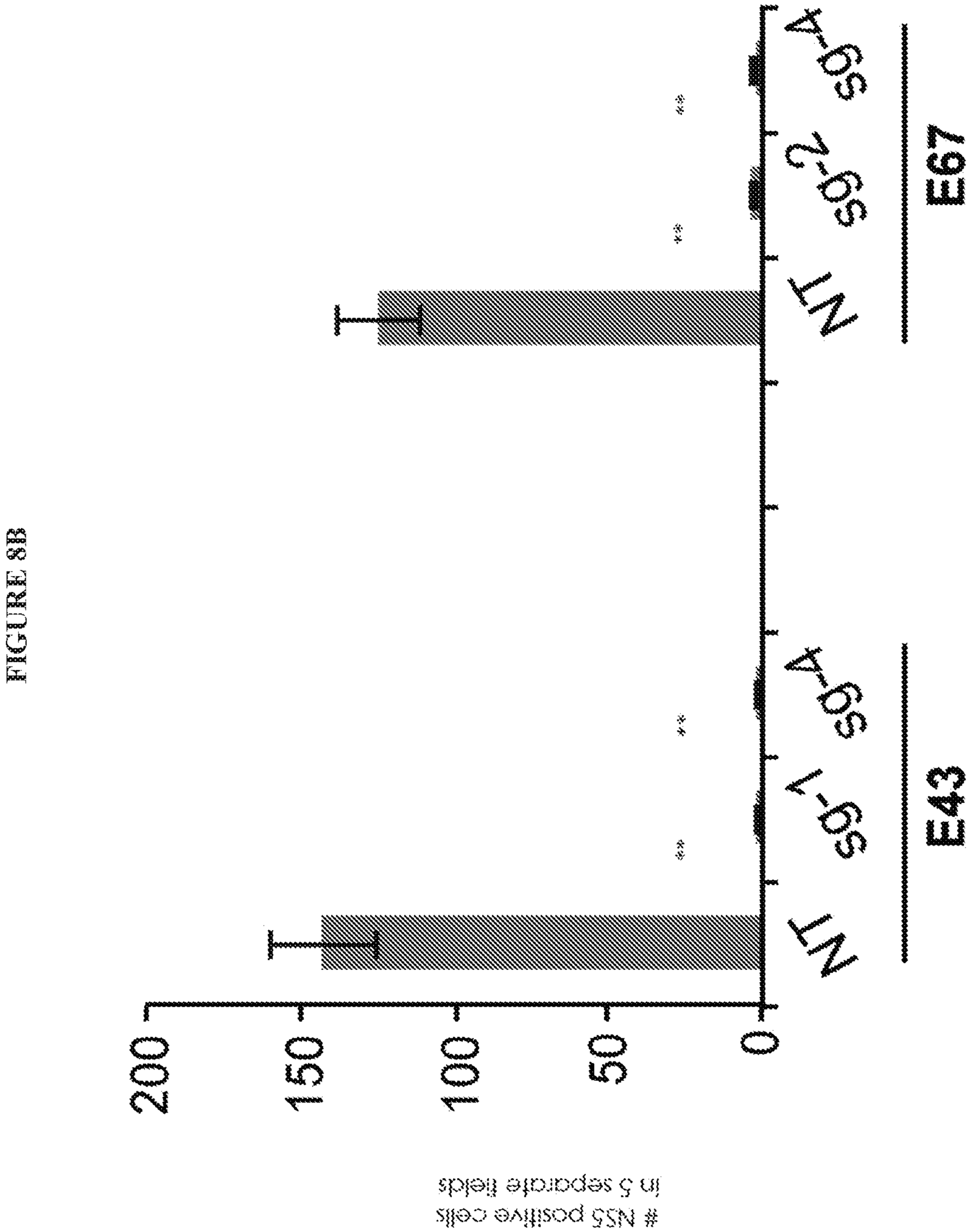

E43
E67
NT
sg-1
sg-2
sg-4
200
150
100
50
0
**
**
**
**
N55 positive cells
in 5 separate fields

FIGURE 8B

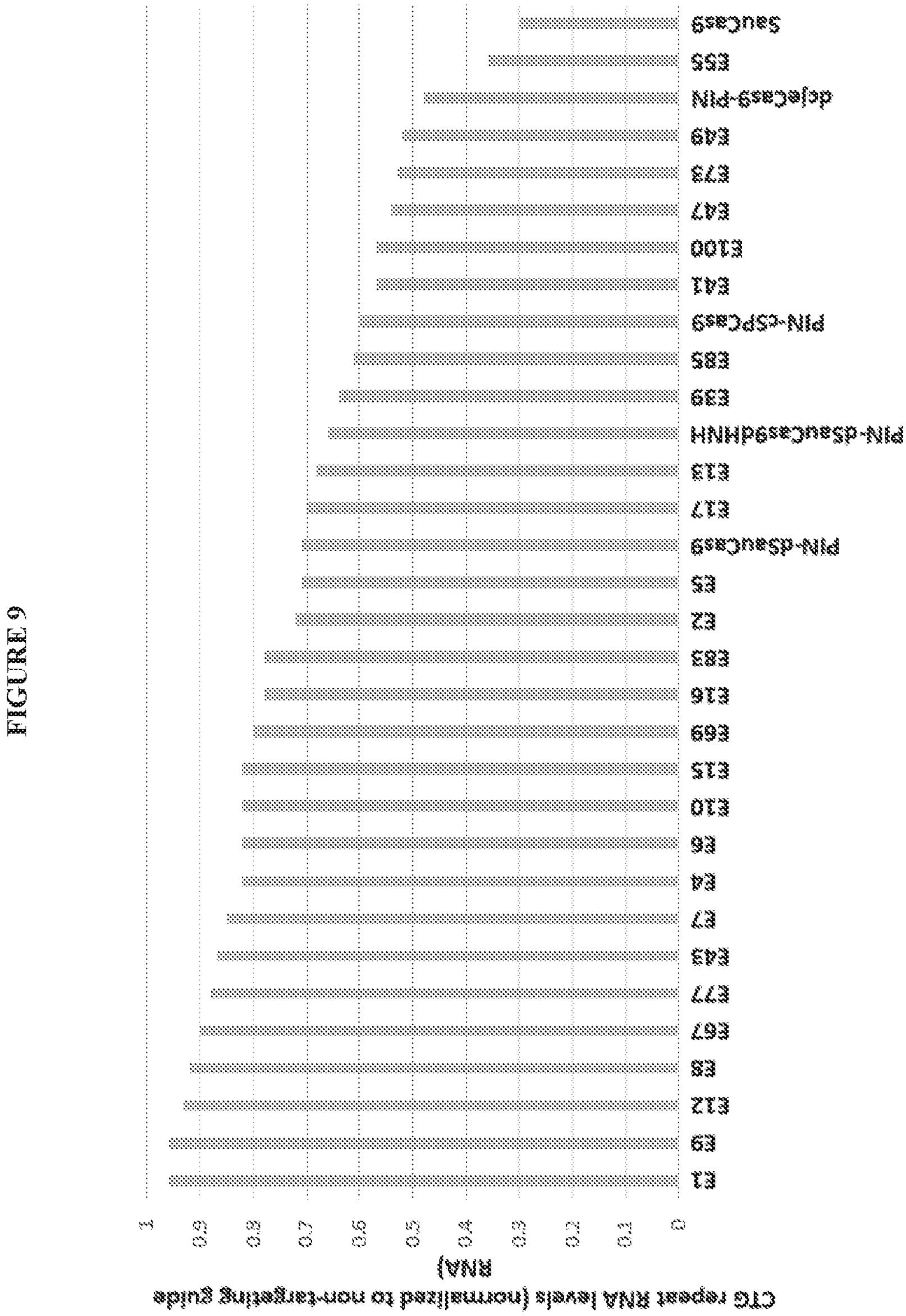

SauCas9
ES3
dCpf1-PIN
E49
E73
E47
E100
E41
PIN-dSpCas9
E85
E39
PIN-dSauCas9dHNH
E13
E17
PIN-dSauCas9
ES
E2
E83
E16
E69
E15
E10
E6
E4
E7
E43
E77
E67
E8
E12
E9
E1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
CTG repeat RNA levels (normalized to non-targeting guide RNA)

FIGURE 9

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

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"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