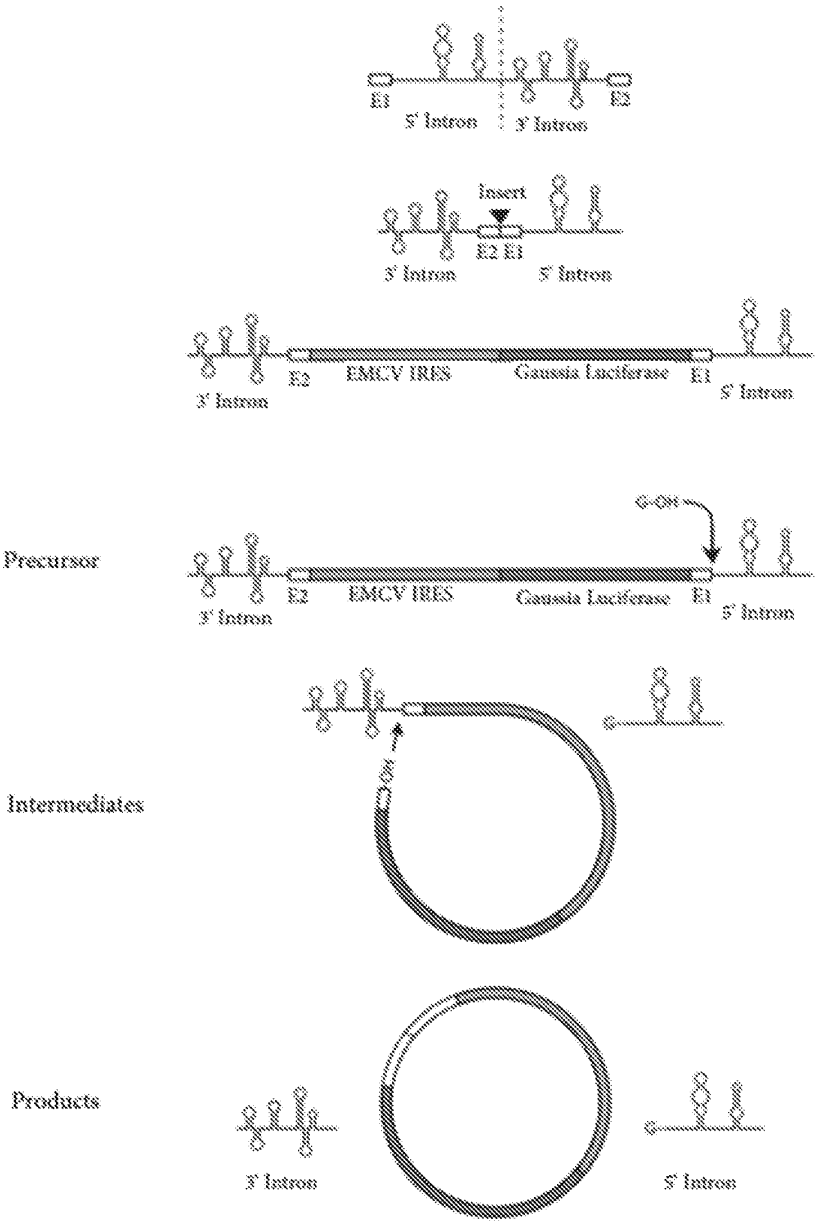

E1
E2
5' Intron
3' Intron
Insert
E2 E1
5' Intron
3' Intron
E2
EMCV IRES
Gaussia Luciferase
E1
3' Intron
5' Intron
G-OH
Precursor
E2
EMCV IRES
Gaussia Luciferase
E1
3' Intron
5' Intron
Intermediates
Products
3' Intron
5' Intron

FIG. 1A

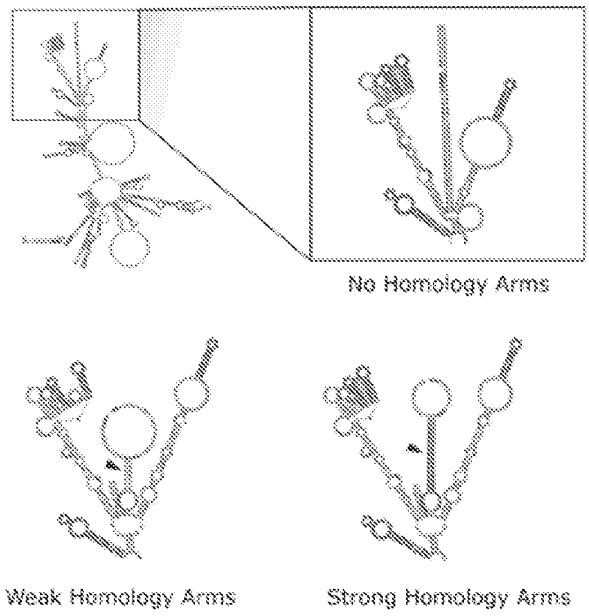

No Homology Arms
Weak Homology Arms
Strong Homology Arms

FIG. 1B

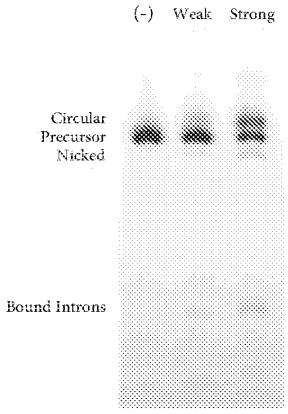

(-) Weak Strong
Circular
Precursor
Nicked
Bound Introns

FIG. 1C

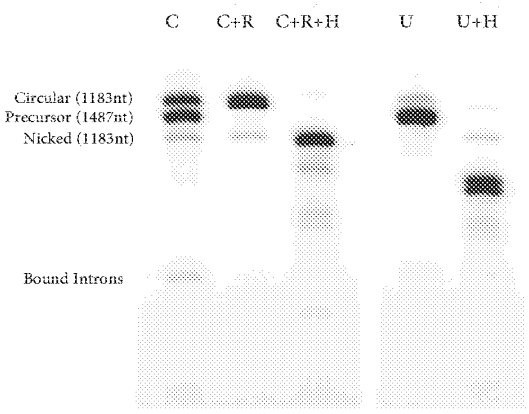

C
C+R
C+R+H
U
U+H
Circular (1183nt)
Precursor (1487nt)
Nicked (1183nt)
Bound Introns

FIG. 1D

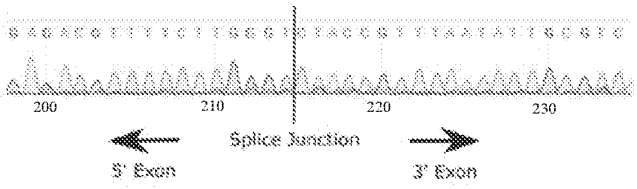

200
210
220
230
Splice Junction
5' Exon
3' Exon

FIG. 1E

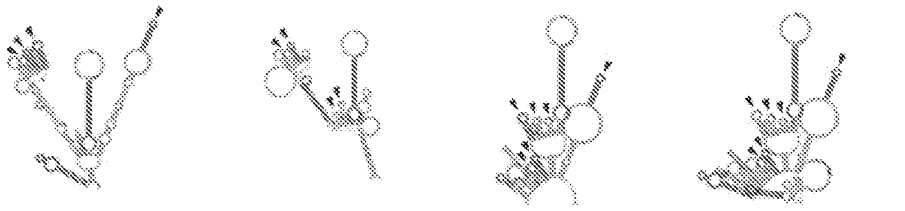

(-) No Spacer
(D) Disruptive Spacer
(P1) Permissive Spacer 1
(P2) Permissive Spacer 2

FIG. 1F

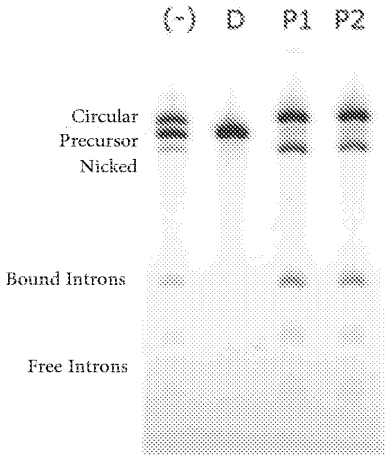

(-)
D
P1
P2
Circular
Precursor
Nicked
Bound Introns
Free Introns

FIG. 1G

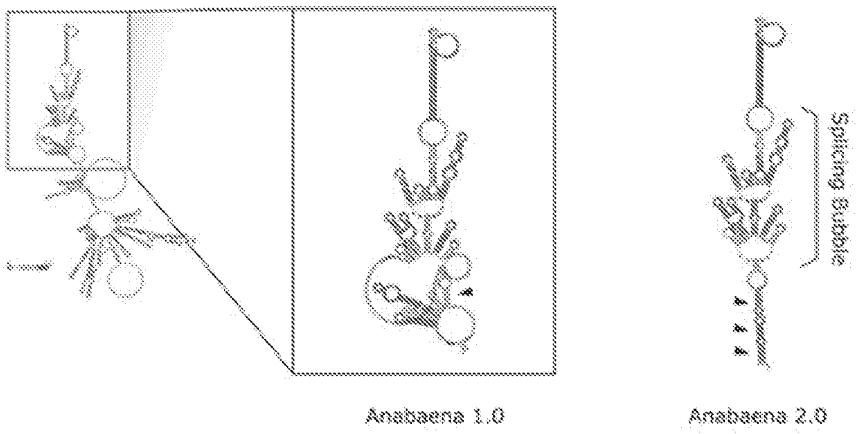

Splicing Guides
Anabaena 1.0
Anabaena 2.0

FIG. 1H

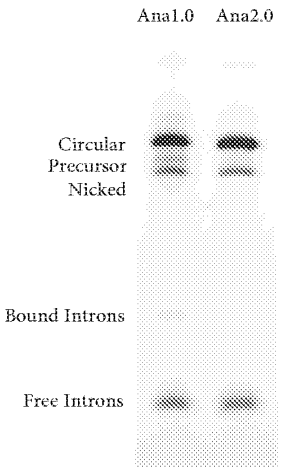

Ana1.0
Ana2.0
Circular
Precursor
Nicked
Bound Introns
Free Introns

FIG. 1I

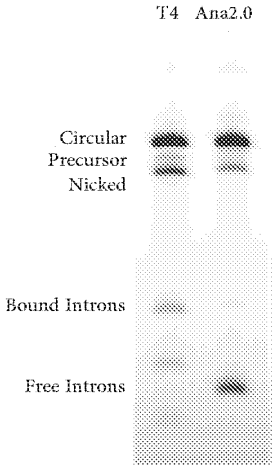

T4 Ana2.0
Circular
Precursor
Nicked
Bound Introns
Free Introns

FIG. 1J

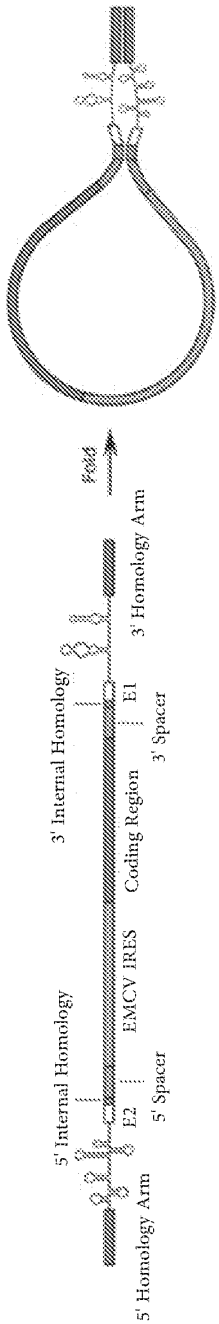

Read
3' Homology Arm
3' Internal Homology
E1
3' Spacer
Coding Region
EMCV IRES
5' Internal Homology
E2
5' Spacer
5' Homology Arm

FIG. 2A

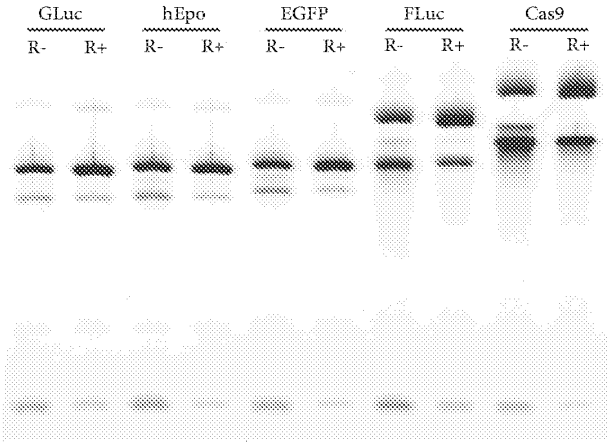

GLuc
hEpo
EGFP
FLuc
Cas9
R-
R+
R-
R+
R-
R+
R-
R+
R-
R+

FIG. 2B

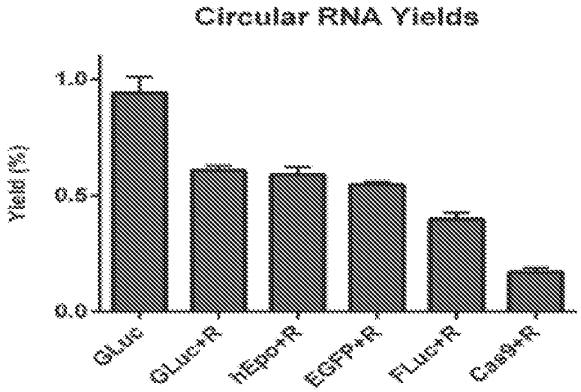

Circular RNA Yields
Yield (%)
1.0
0.5
0.0
GLuc
GLuc+R
hEpo+R
EGFP+R
FLuc+R
Cas9+R

FIG. 2C

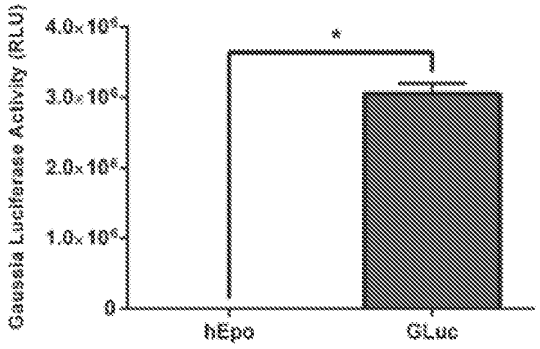

Gaussia Luciferase Activity (RLU)
4.0×10⁶
3.0×10⁶
2.0×10⁶
1.0×10⁶
0
*
hEpo
GLuc

FIG. 2D

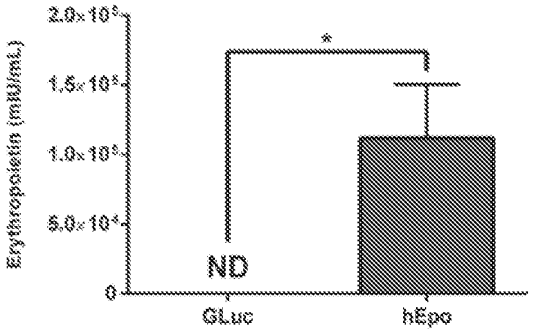

Erythropoietin (mIU/mL)
2.0×10⁵
1.5×10⁵
1.0×10⁵
5.0×10⁴
0
*
ND
GLuc
hEpo

FIG. 2E

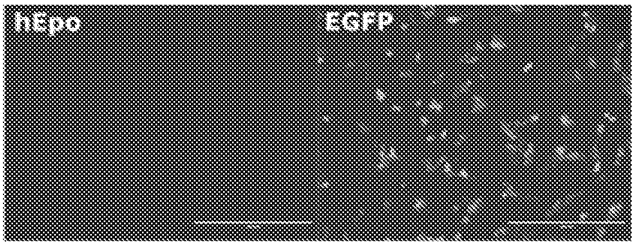

hEpo
EGFP

FIG. 2F

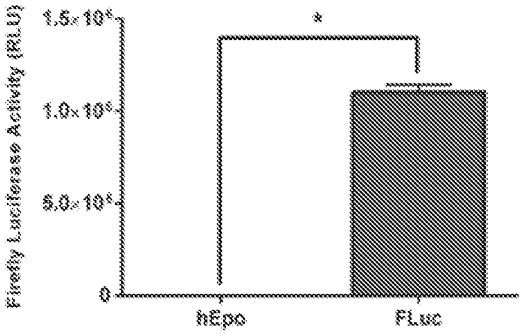

Firefly Luciferase Activity (RLU)
1.5×10⁶
1.0×10⁶
5.0×10⁵
0
*
hEpo
FLuc

FIG. 2G

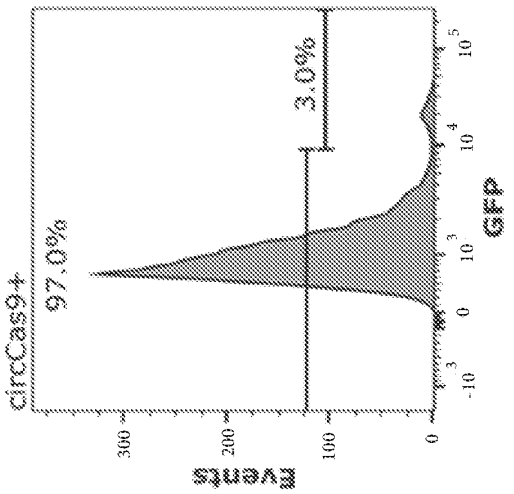


FIG. 2I

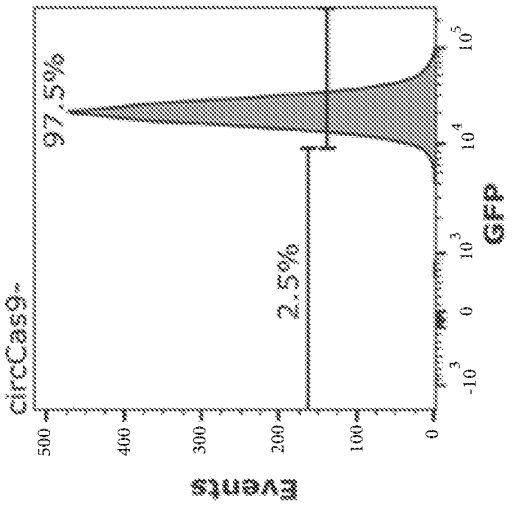


FIG. 2H

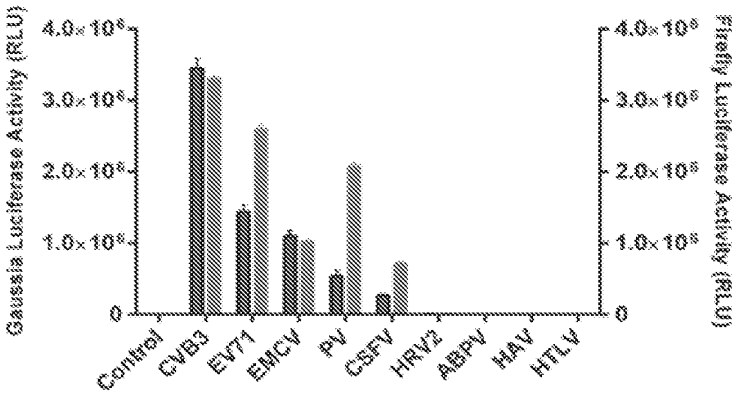

Gaussia Luciferase Activity (RLU)
Firefly Luciferase Activity (RLU)
4.0×10⁶
3.0×10⁶
2.0×10⁶
1.0×10⁶
0
Control
CVB3
EV71
EMCV
PV
CSFV
HRV2
ABPV
HAV
HTLV

FIG. 3A

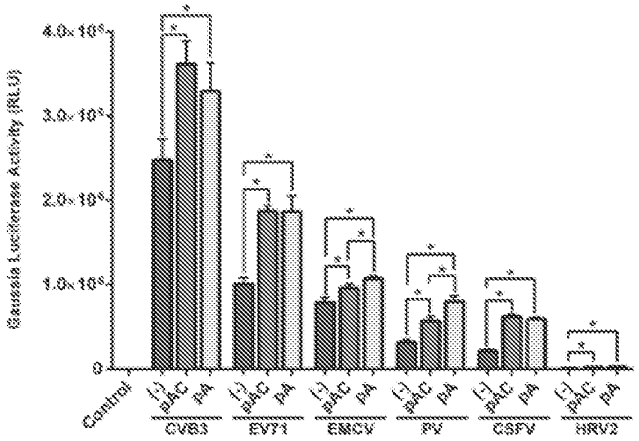

Gaussia Luciferase Activity (RLU)
4.0×10⁶
3.0×10⁶
2.0×10⁶
1.0×10⁶
0
Control
(-)
pAC
pA
CVB3
EV71
EMCV
PV
CSFV
HRV2

FIG. 3B

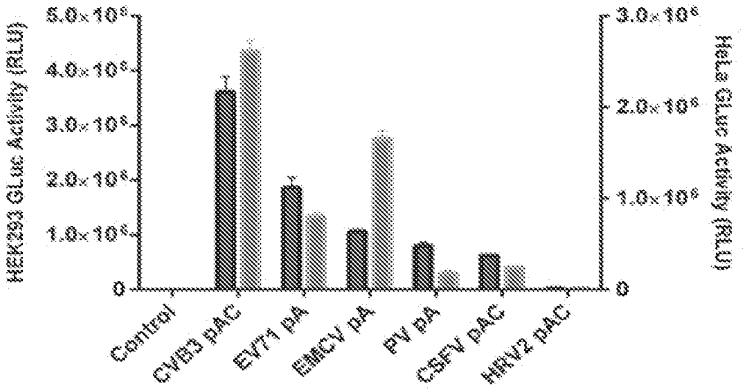

HEK293 GLuc Activity (RLU)
HeLa GLuc Activity (RLU)
5.0×10^6
4.0×10^6
3.0×10^6
2.0×10^6
1.0×10^6
0
3.0×10^6
2.0×10^6
1.0×10^6
0
Control
CVB3 pAC
EV71 pA
EMCV pA
PV pA
CSFV pAC
HRV2 pAC

FIG. 3C

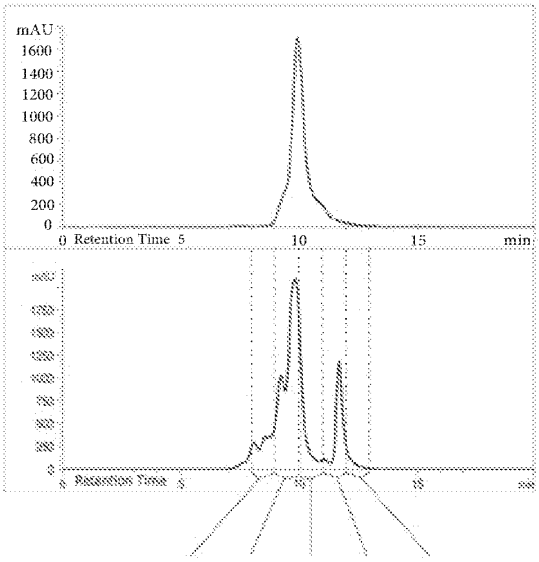

mAU
1600
1400
1200
1000
800
600
400
200
0
0 Retention Time 5 10 15 min

FIG. 3D

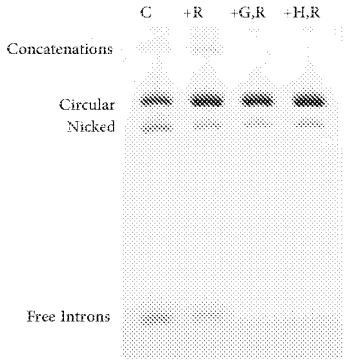


FIG. 3E

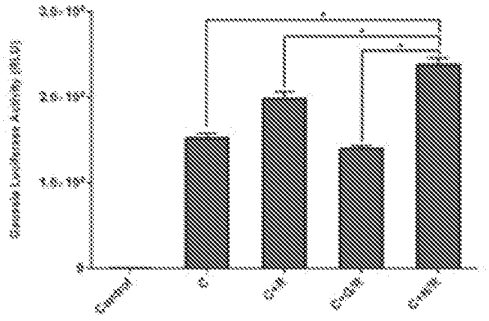


FIG. 3F

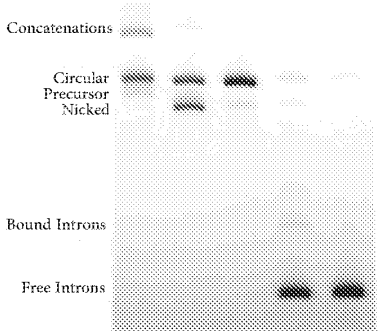


FIG. 3G

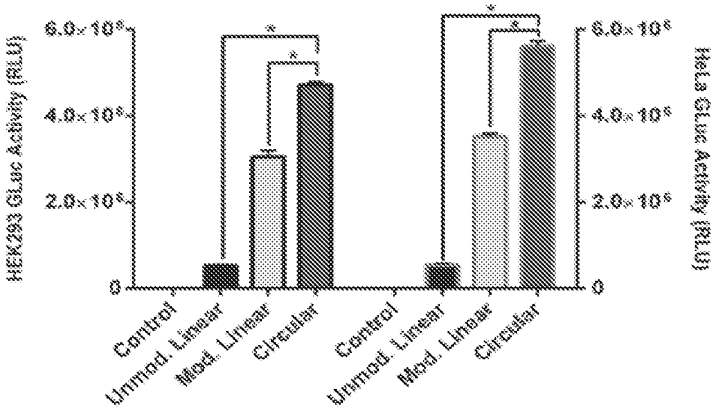

HEK293 GLuc Activity (RLU)
HeLa GLuc Activity (RLU)
6.0×10⁶
4.0×10⁶
2.0×10⁶
0
Control
Unmod. Linear
Mod. Linear
Circular
*

FIG. 3H

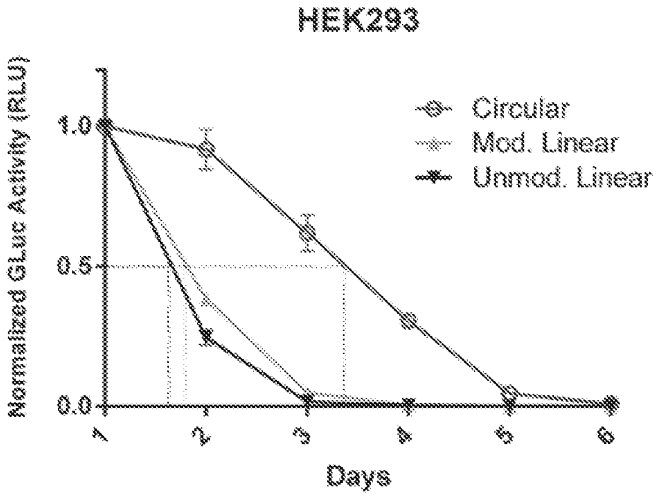

HEK293
Normalized GLuc Activity (RLU)
1.0
0.5
0.0
1
2
3
4
5
6
Days
Circular
Mod. Linear
Unmod. Linear

FIG. 3I

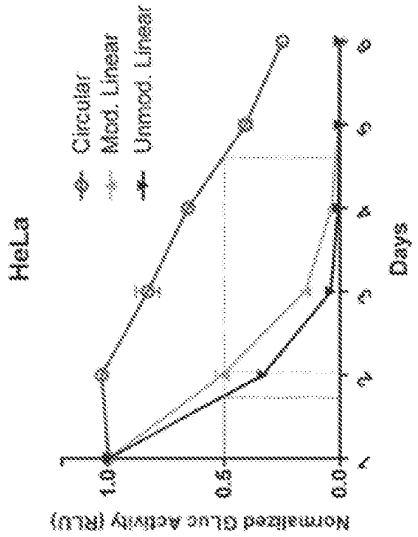

HeLa
Circular
Mod. Linear
Unmod. Linear
Normalized GLuc Activity (RLU)
1.0
0.5
0.0
1
2
3
4
5
6
Days

FIG. 3K

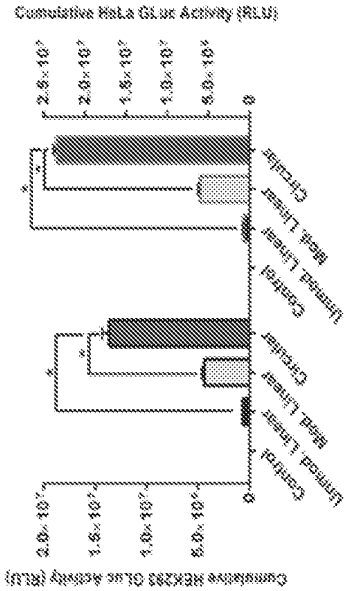

Cumulative HeLa GLuc Activity (RLU)
2.5×10⁷
2.0×10⁷
1.5×10⁷
1.0×10⁷
5.0×10⁶
0
Circular
Mod. Linear
Unmod. Linear
Control
Circular
Mod. Linear
Unmod. Linear
Control
Cumulative HEK293 GLuc Activity (RLU)
2.0×10⁷
1.5×10⁷
1.0×10⁷
5.0×10⁶
0

FIG. 3J

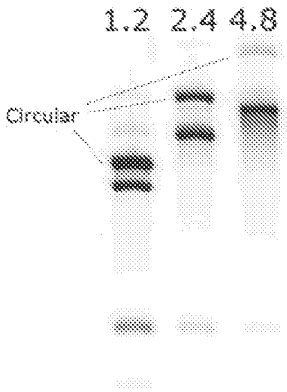

1.2
2.4
4.8
Circular

FIG. 4A

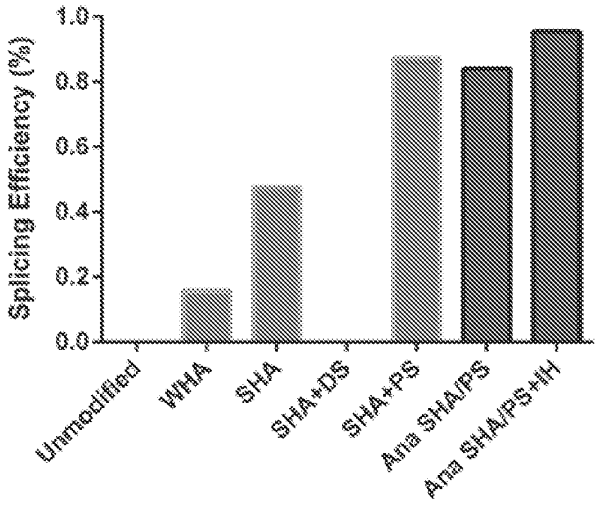

Splicing Efficiency (%)
1.0
0.8
0.6
0.4
0.2
0.0
Unmodified
WHA
SHA
SHA+DS
SHA+PS
Ana SHA/PS
Ana SHA/PS+H

FIG. 4B

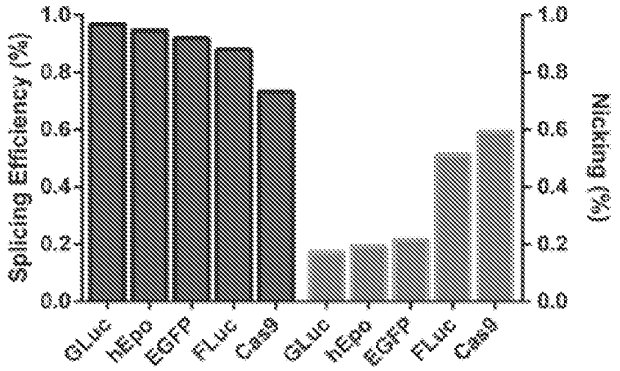

Splicing Efficiency (%)
Nicking (%)
1.0
0.8
0.6
0.4
0.2
0.0
GLuc
hEpo
EGFP
FLuc
Cas9

FIG. 5A

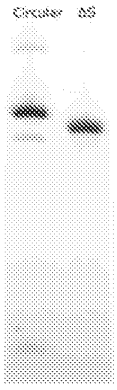


FIG. 5B

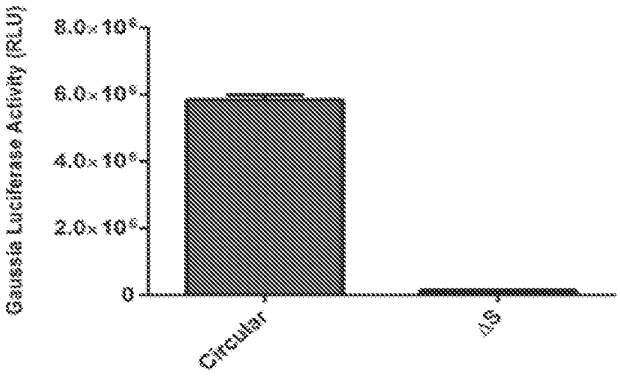

Gaussia Luciferase Activity (RLU)
8.0×10⁶
6.0×10⁶
4.0×10⁶
2.0×10⁶
0
Circular
ΔS

FIG. 5C

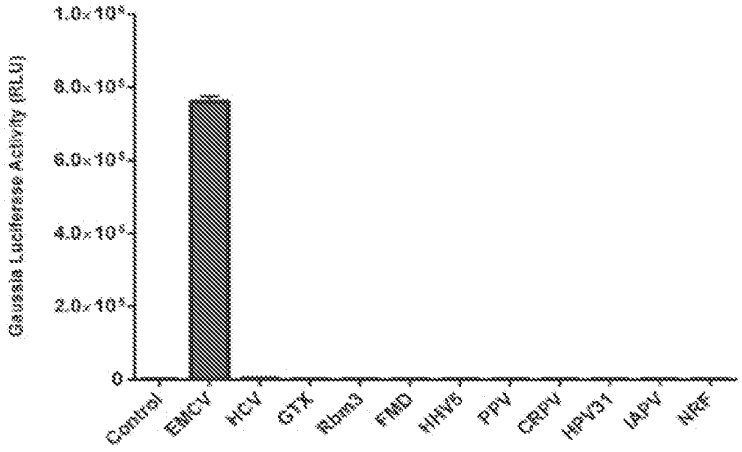

Gaussia Luciferase Activity (RLU)
1.0×10⁶
8.0×10⁵
6.0×10⁵
4.0×10⁵
2.0×10⁵
0
Control
EMCV
HCV
GTX
Rhm3
FMD
HHV6
PPV
CRPV
HPV31
IAPV
NRF

FIG. 6A

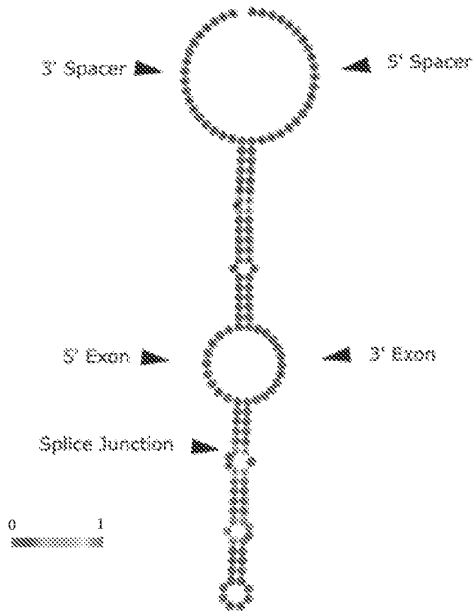


FIG. 6B

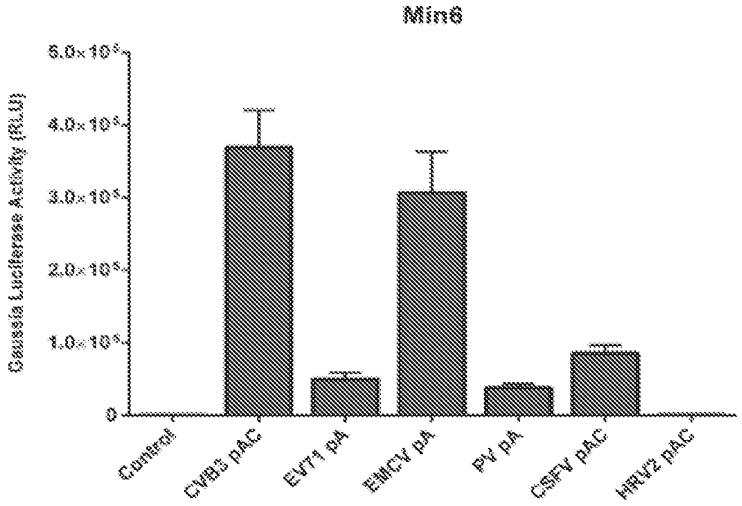

Min6
Gaussia Luciferase Activity (RLU)
5.0×10⁵
4.0×10⁵
3.0×10⁵
2.0×10⁵
1.0×10⁵
0
Control
CVB3 pAC
EV71 pA
EMCV pA
PV pA
CSFV pAC
HRV2 pAC

FIG. 6C

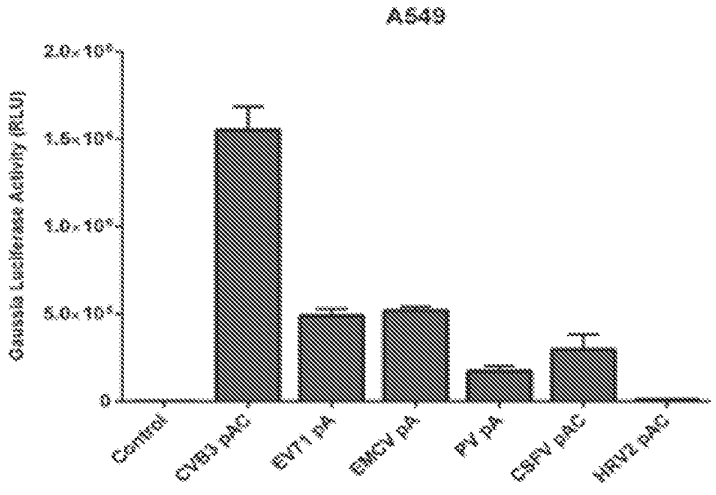

A549
Gaussia Luciferase Activity (RLU)
2.0×10⁶
1.5×10⁶
1.0×10⁶
5.0×10⁵
0
Control
CVB3 pAC
EV71 pA
EMCV pA
PV pA
CSFV pAC
HRV2 pAC

FIG. 6D

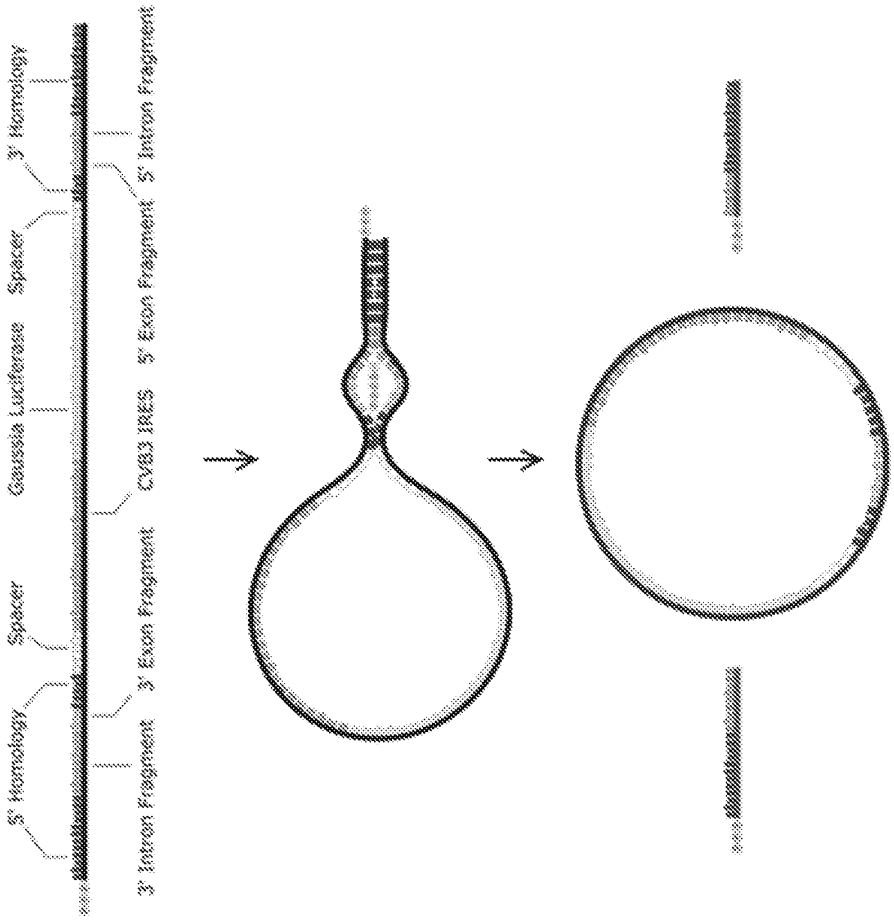

5' Homology
Spacer
3' Exon Fragment
CVB3 IRES
Gaussia Luciferase
Spacer
5' Exon Fragment
3' Homology
3' Intron Fragment
5' Intron Fragment

FIG. 7A

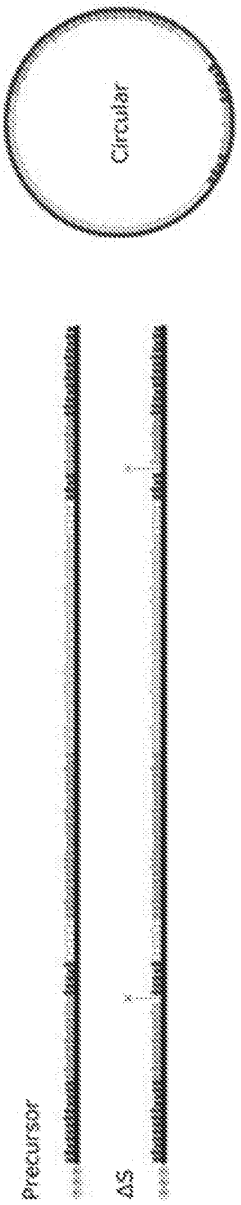


FIG. 7B


Purification Methods
Concentration
Circular Precursor Nicked
Linear

FIG. 7D

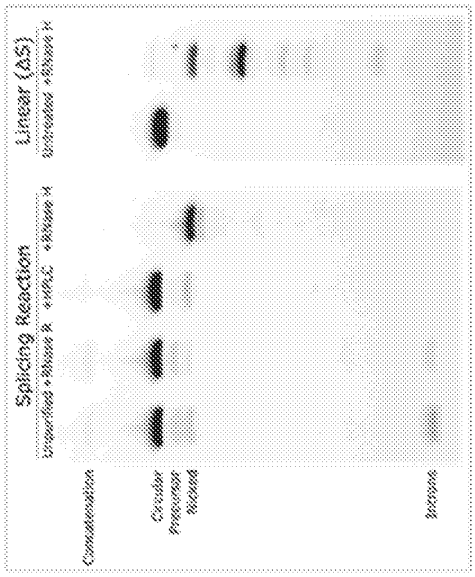

Splicing Reaction
Linear (AS)
Concentration
Circular Precursor Nicked
Linear

FIG. 7C

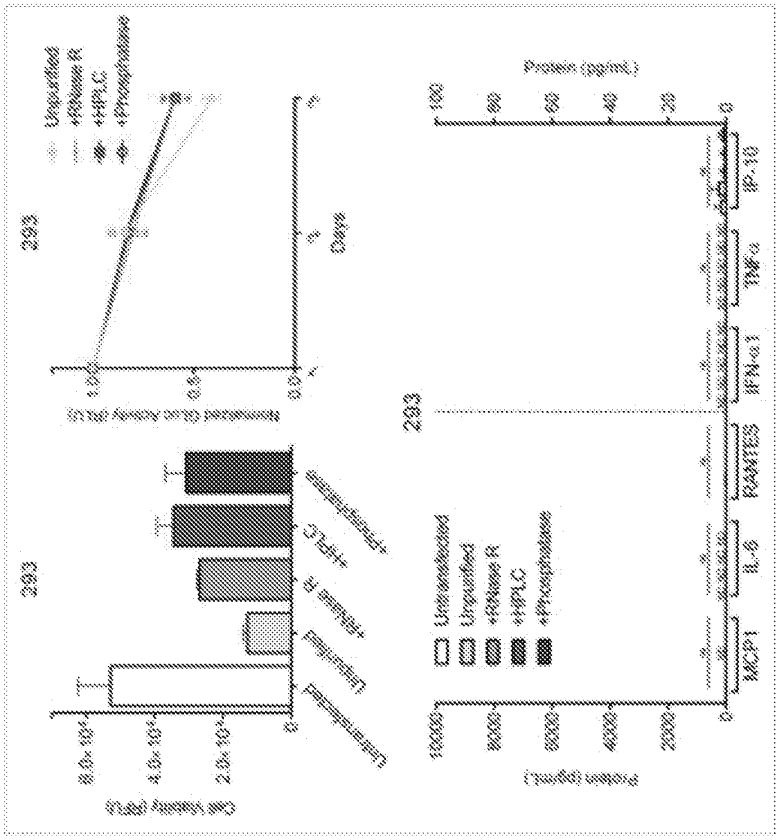

293
Unpurified
+RNase R
+HPLC
+Phosphatase
Days
293
Protein (pg/mL)
MCP1
IL-6
RANTES
IFN-α1
TNF-α
IP-10
293
Unpurified
+RNase R
+HPLC
+Phosphatase

FIG. 7E

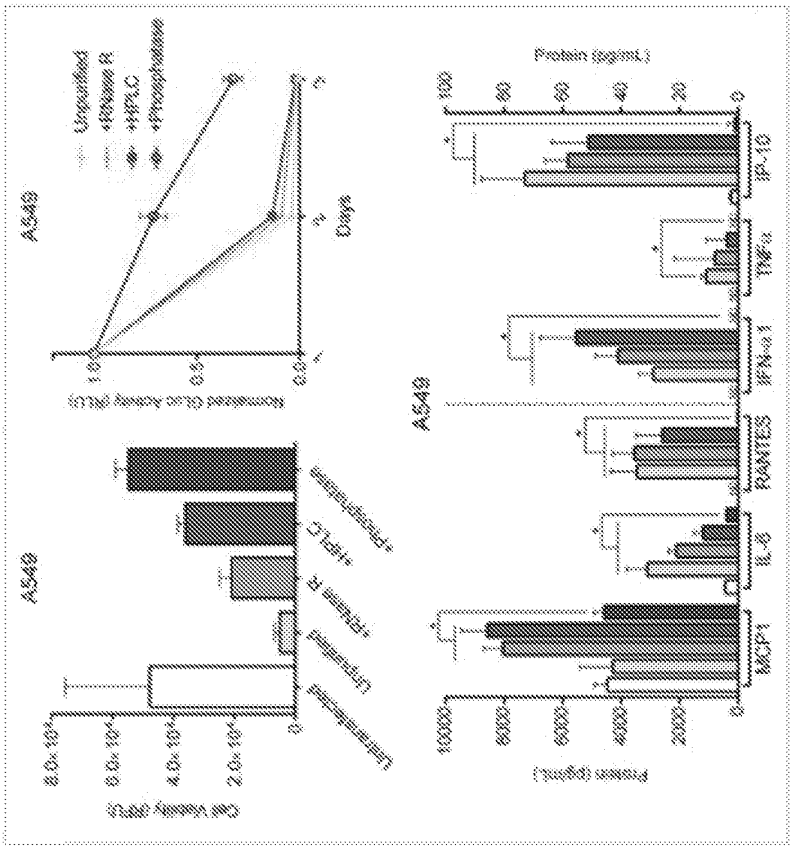

A549
Unpurified
+RIG-I R
+HMLC
+Phosphatase
Days
A549
Protein (pg/mL)
IP-10
TNF-α
IFN-α1
RANTES
IL-8
MCP1

FIG. 7F

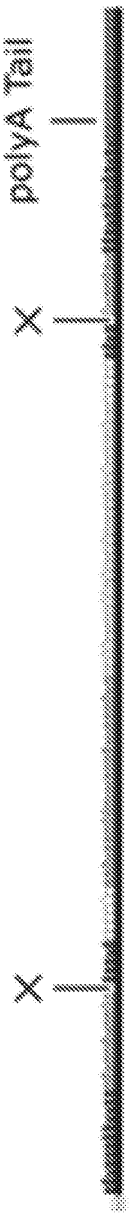


FIG. 7G

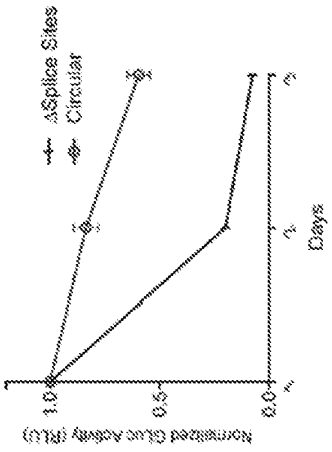


FIG. 7J

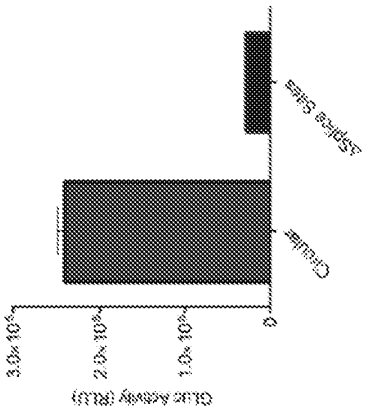


FIG. 7I

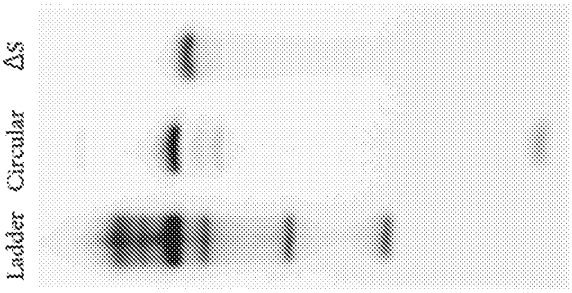


FIG. 7H

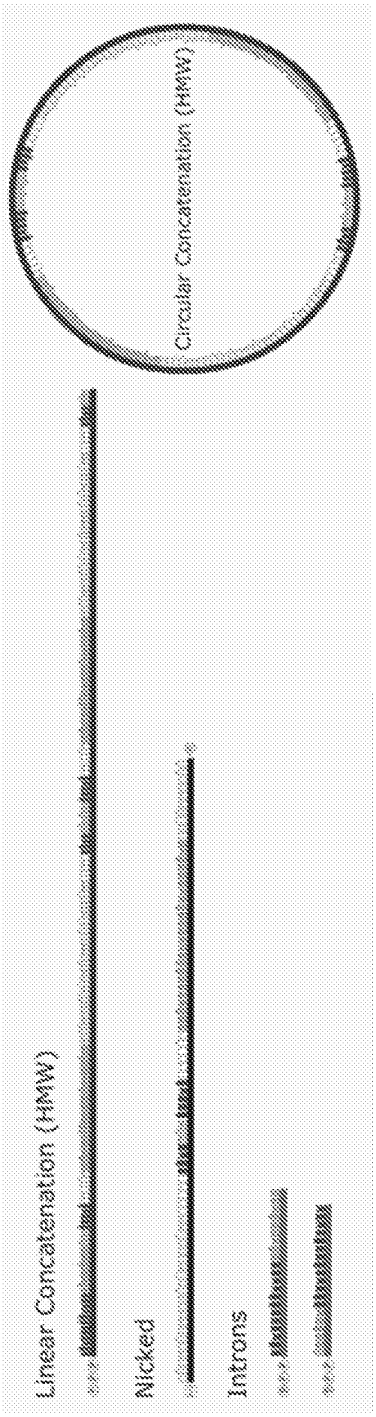

Circular Concatenation (HMW)
Linear Concatenation (HMW)
Nicked
Introns

FIG. 8A

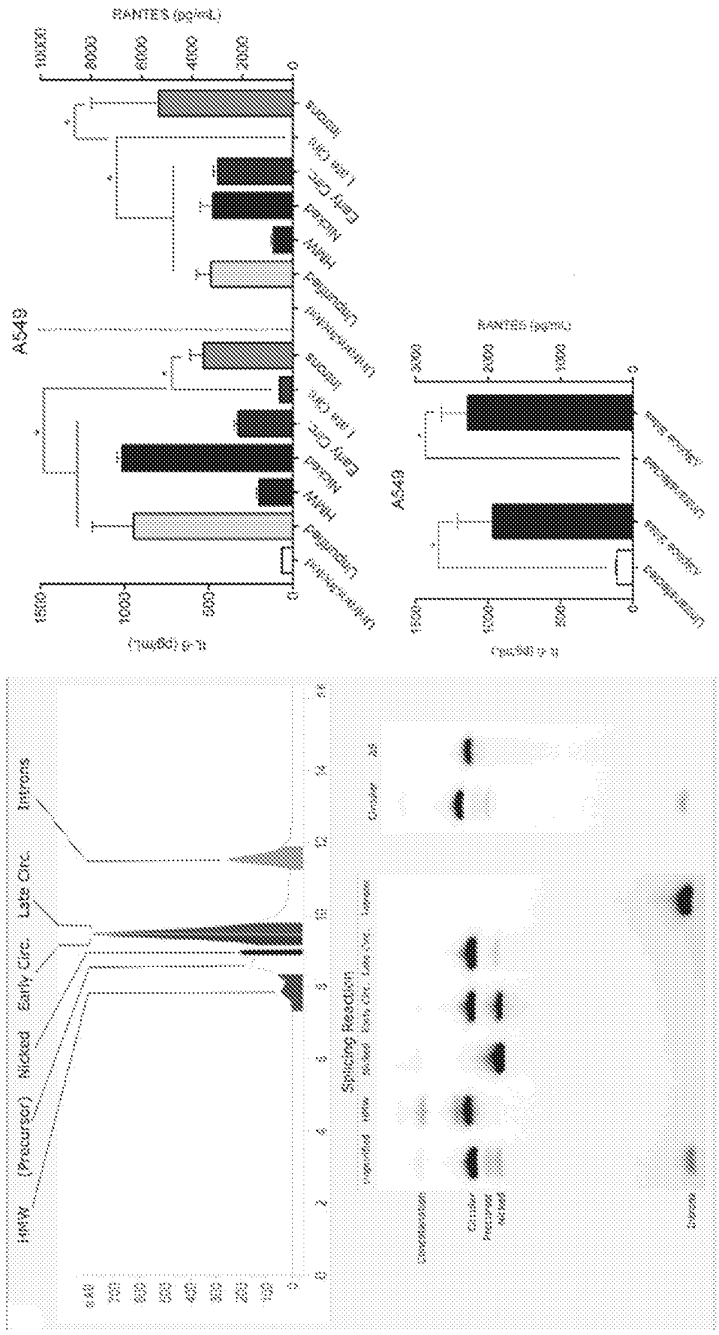

RANTES (pg/mL)
A549
Introns
Late Circ.
Early Circ.
Nicked
HWR (Precursor)
Splicing Reaction
Linear
Circular
Precursor
Nicked
Early Circ.
Late Circ.
Introns

FIG. 8C

FIG. 8B

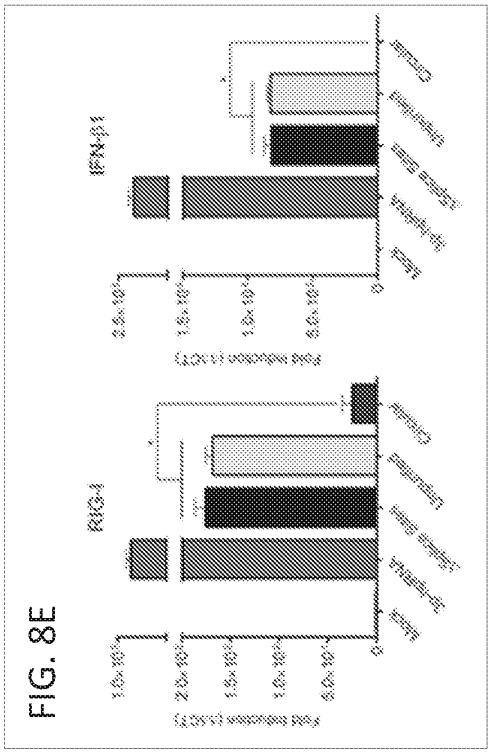
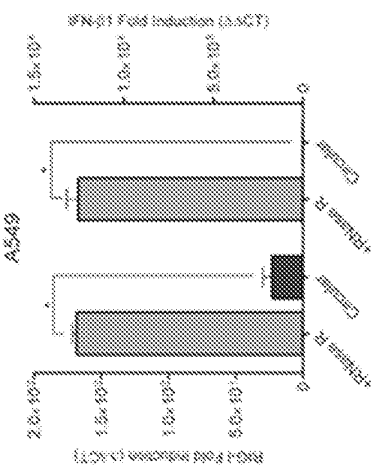

FIG. 8E
IFNβ1
RIG-I
Control
Poly(I:C)
Lipofectamine
Poly(I:C) + Lipofectamine
IFNα
IFNγ

FIG. 8F


A549
Control
Poly(I:C)
Lipofectamine
Poly(I:C) + Lipofectamine

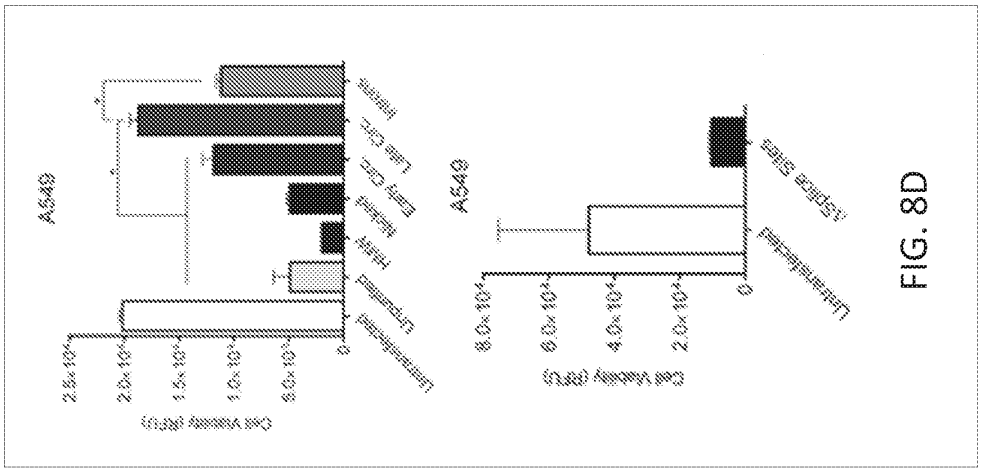

A549
A549
Control
Poly(I:C)
Lipofectamine
Poly(I:C) + Lipofectamine
IFNα
IFNγ

FIG. 8D

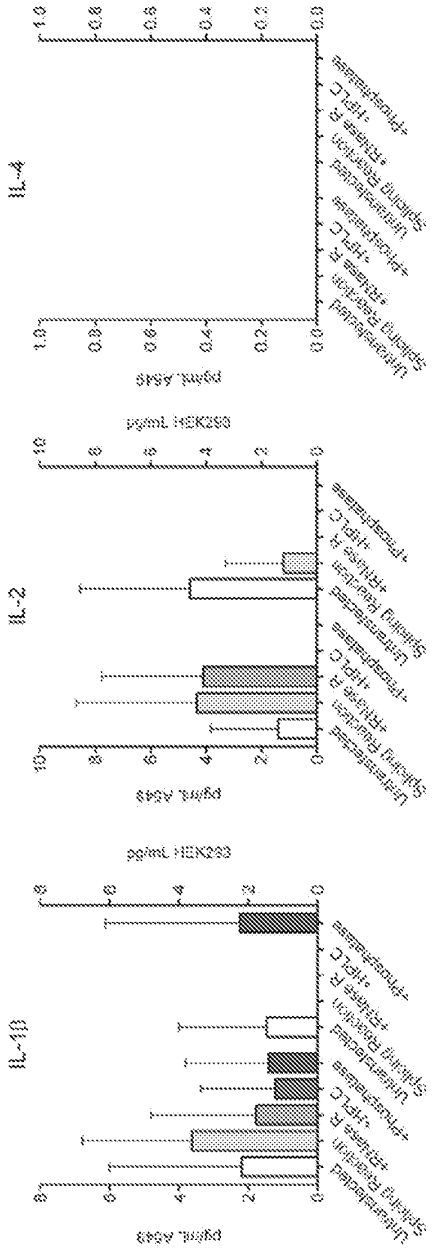

IL-4
IL-2
IL-1β
pg/mL HEK293
Unstimulated
3 days, Unstimulated
Restimulated
3 days, Restimulated

FIG. 8G

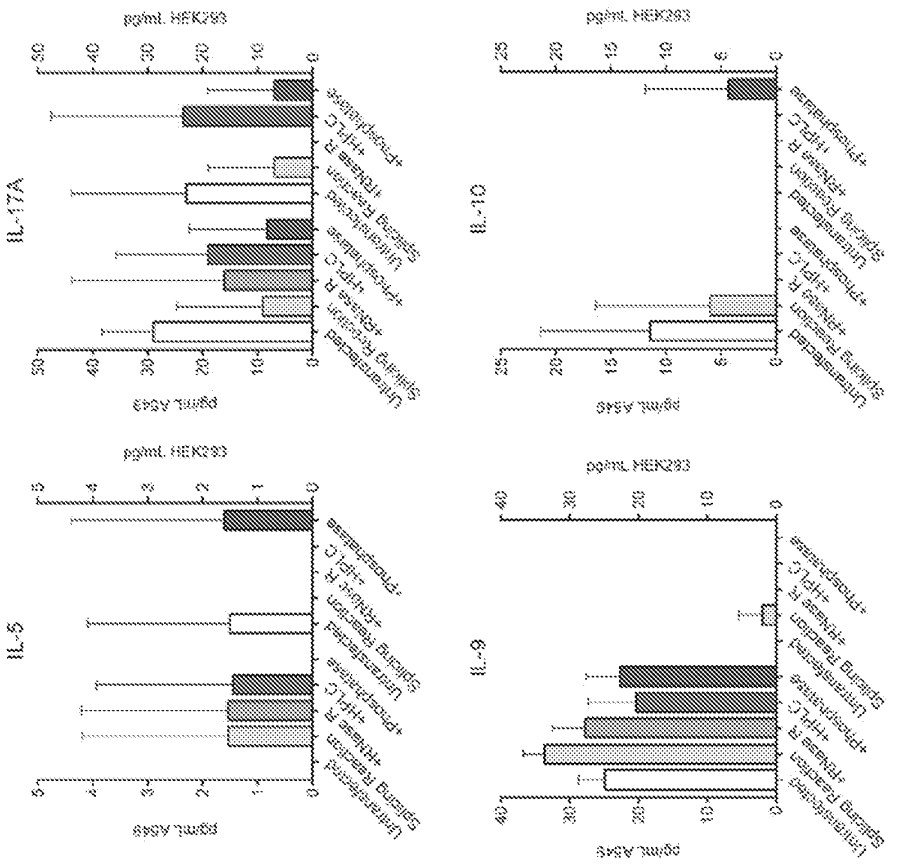

IL-17A
pg/mL A549
pg/mL HEK293
IL-5
pg/mL A549
pg/mL HEK293
IL-10
pg/mL A549
pg/mL HEK293
IL-9
pg/mL A549
pg/mL HEK293
Untransfected
Sparing Fraction
+Kinase R
+HPLC
+Phosphatase

FIG. 8G (cont'd)

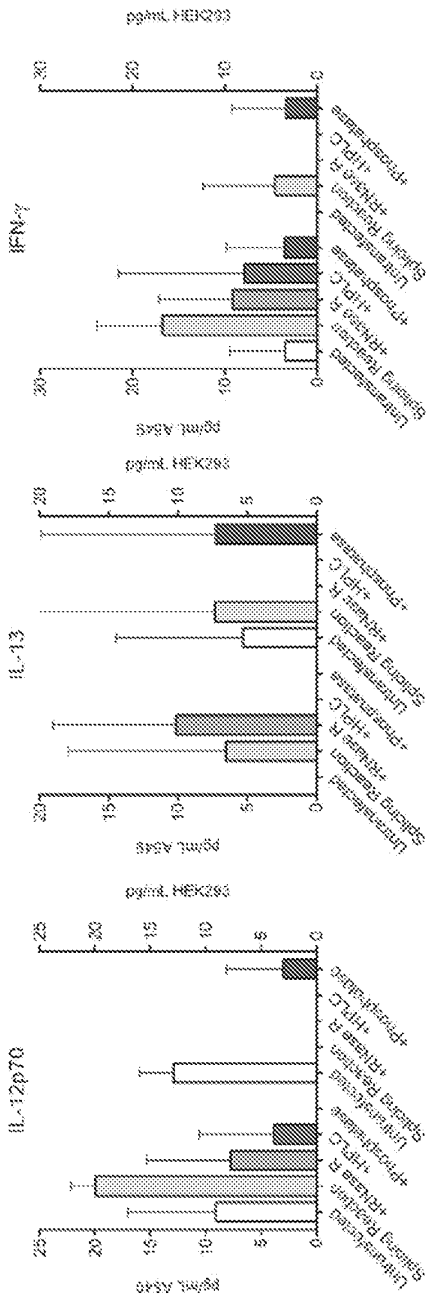

IFN-γ
IL-13
IL-12p70
pg/mL A549
pg/mL HEK293
Unstimulated
Stimulated Reaction
+Kinase A
+TBK1
+Phosphatase

FIG. 8G (cont'd)

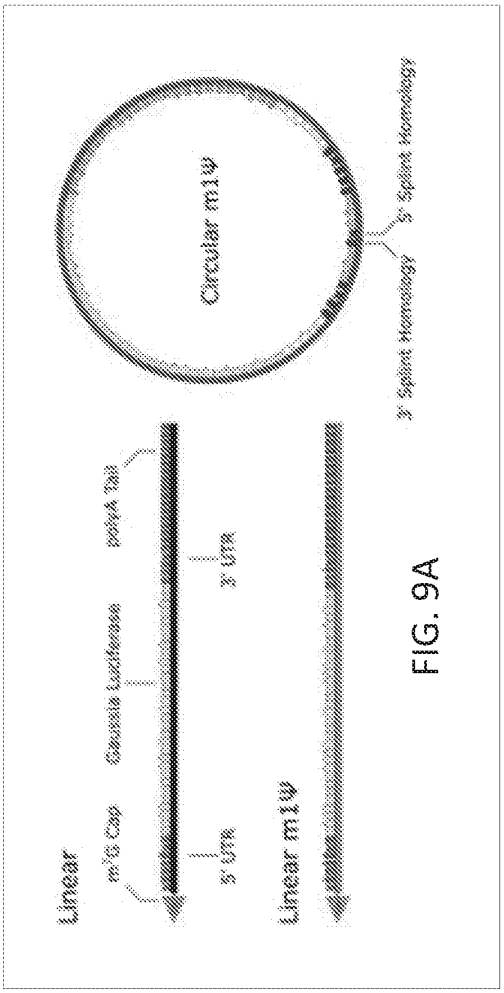

Linear
m7G Cap
Gaussia Luciferase
polyA Tail
5' UTR
3' UTR
Linear m1Ψ
Circular m1Ψ
3' Splint Homology
5' Splint Homology

FIG. 9A

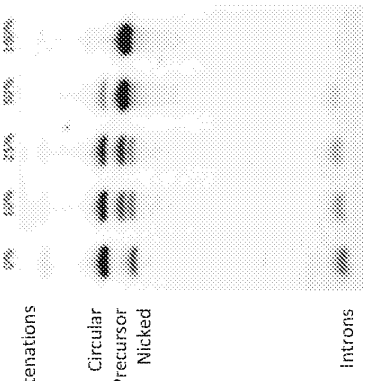

N1-Methylpseudouridine
0%
25%
50%
75%
100%
Concatenations
Circular Precursor
Nicked
Introns

FIG. 9B

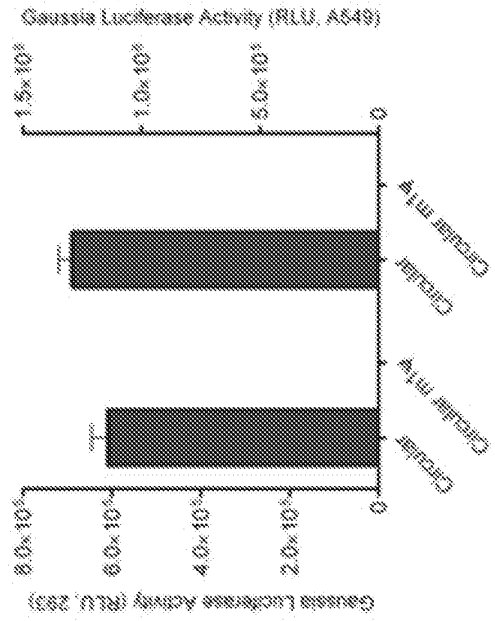

Gaussia Luciferase Activity (RLU, A549)
1.5x 10^6
1.0x 10^6
5.0x 10^5
0
Circular RNA
Linear RNA
Circular RNA
Linear RNA
8.0x 10^4
6.0x 10^4
4.0x 10^4
2.0x 10^4
0
Gaussia Luciferase Activity (RLU, 293)

FIG. 9D

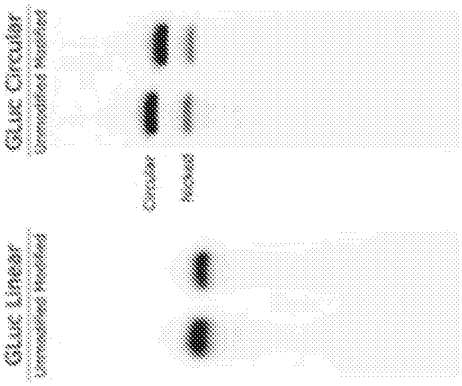

GLuc Circular
Unmodified Nucleotides
Circular
Linear
GLuc Linear
Unmodified Nucleotides

FIG. 9C

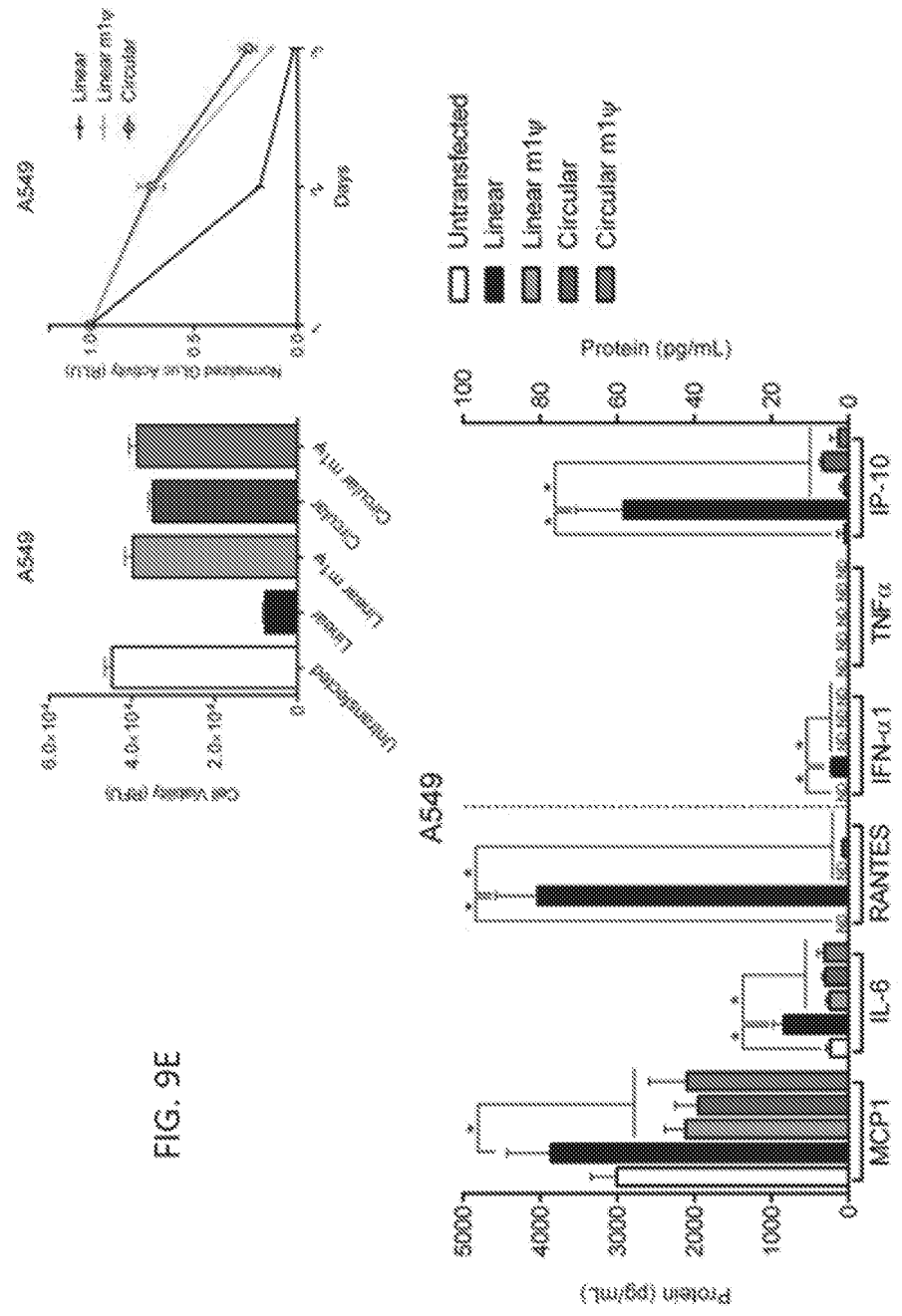

A549
Linear
Linear m1ψ
Circular
Days
A549
Untransfected
Linear
Linear m1ψ
Circular
Circular m1ψ
Protein (pg/mL)
IP-10
TNFα
IFN-α1
RANTES
IL-6
MCP1

FIG. 9E

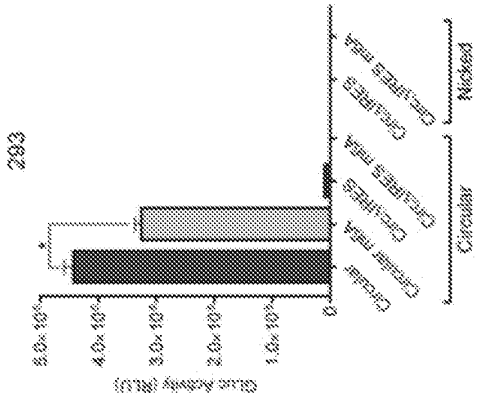

293
Circular
Nicked

FIG. 9G

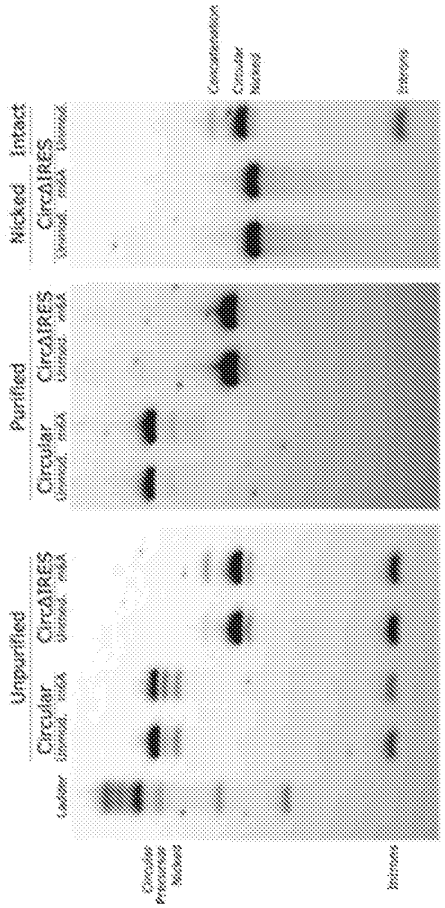

Unpurified
Purified
Nicked
Intact
Circular
CircAIRES
Concatemer
Circular
Nicked
Intact

FIG. 9F

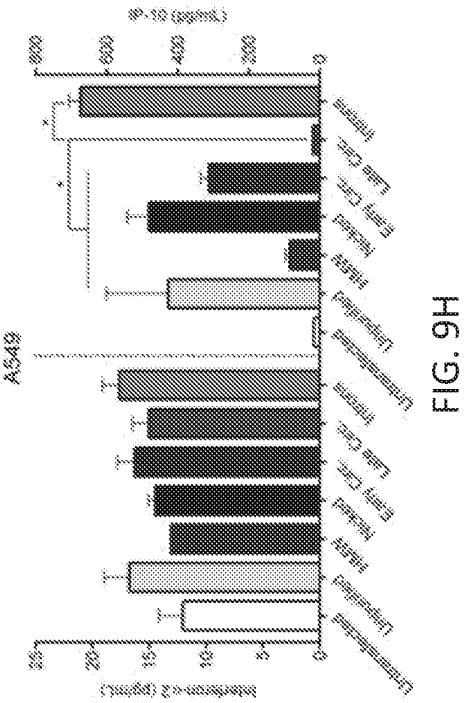


FIG. 9H

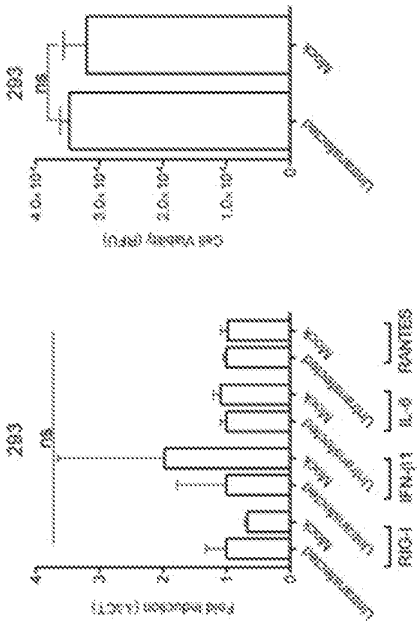


FIG. 9I

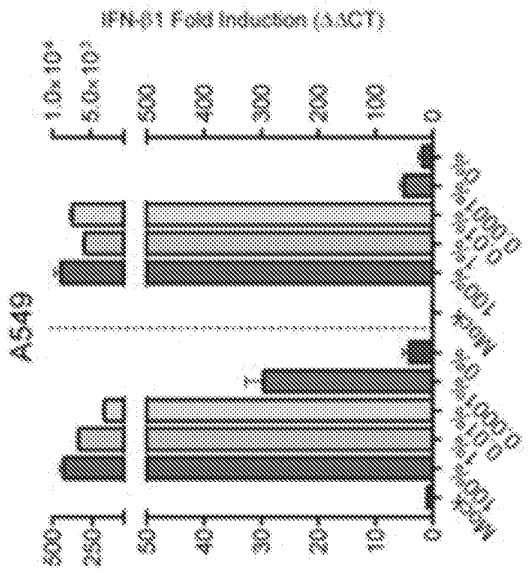

IFN-β1 Fold Induction (ΔΔCT)
1.0×10⁴
5.0×10³
600
400
300
200
100
0
A549
500
250
50
40
30
20
10
0

FIG. 9K

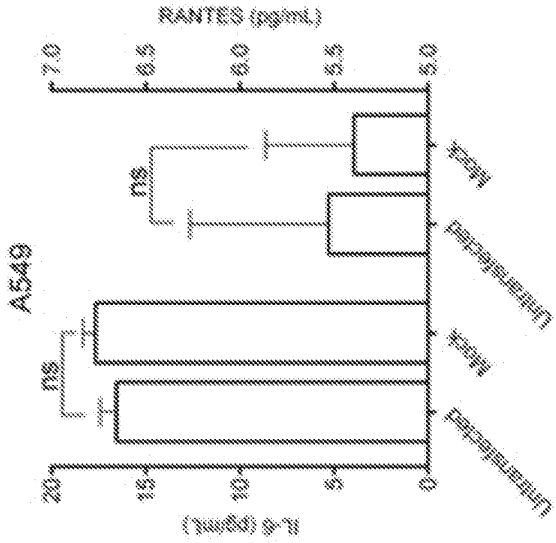

RANTES (pg/mL)
7.0
6.5
6.0
5.5
5.0
A549
ns
ns
20
15
10
5
0

FIG. 9J

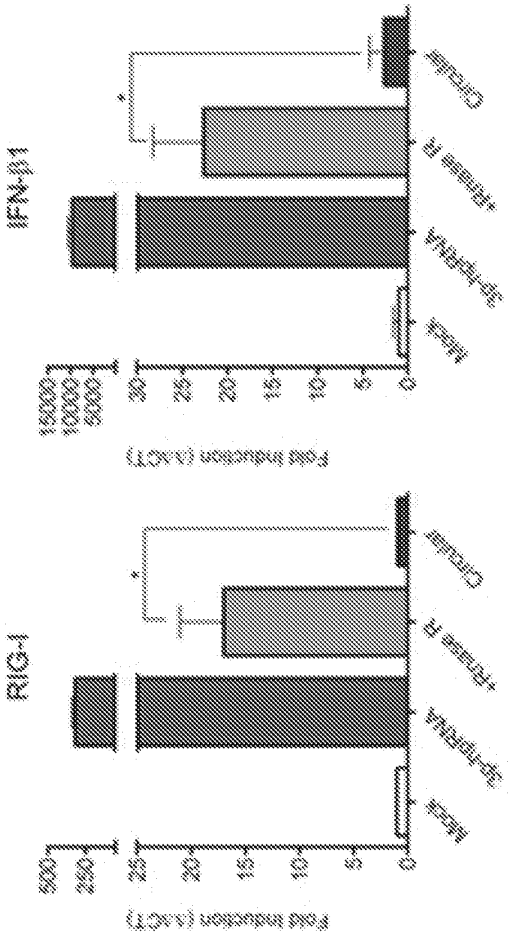

IFN-β1
RIG-I
Fold induction (ΔΔCT)
Media
3p-hpRNA
+Poly I:C
Circular
*

FIG. 9L

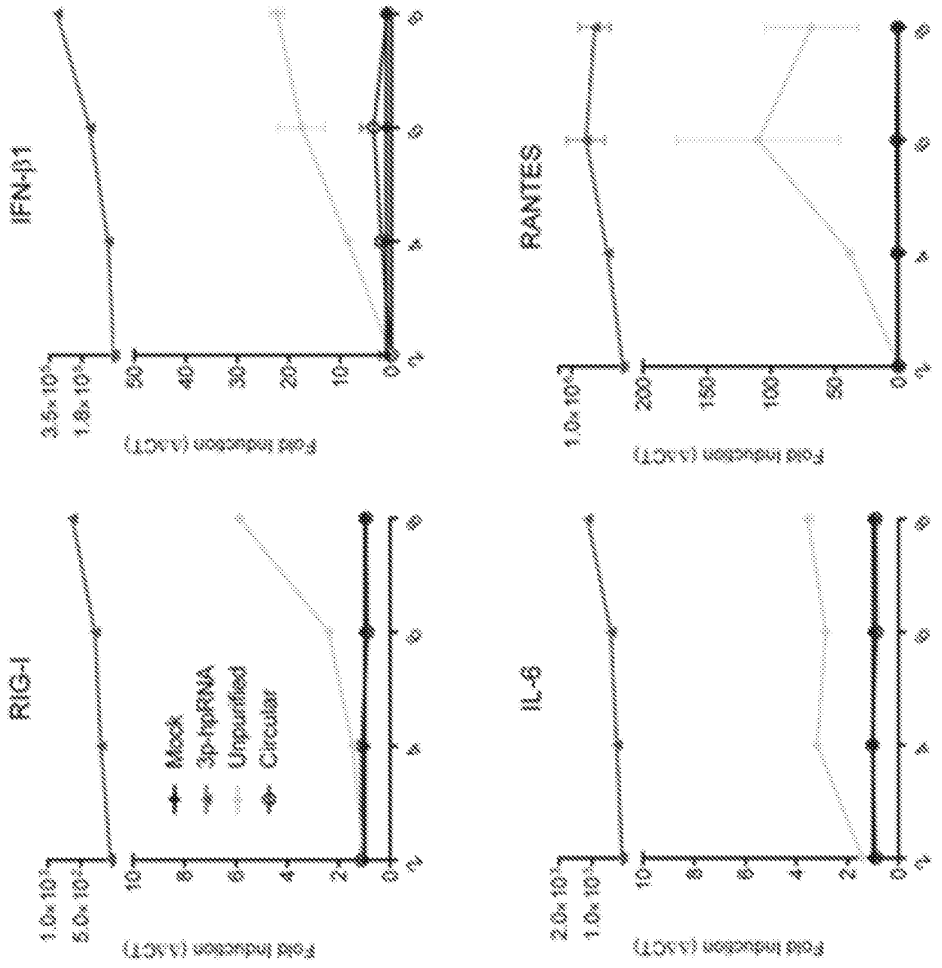

IFN-β1
RANTES
RIG-I
IL-8
Mock
3p-hpRNA
Unpurified
Circular
Fold Induction (-ΔΔCT)
1 h
4 h
8 h
24 h

FIG. 9M

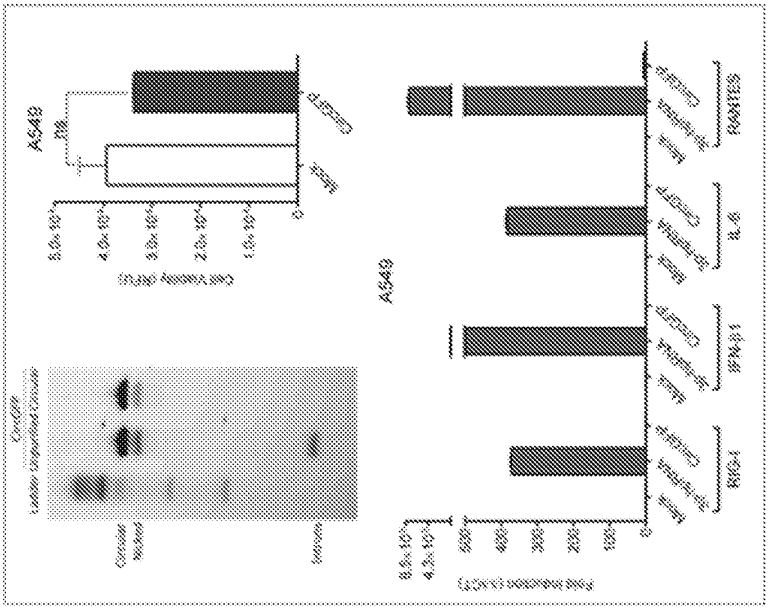


FIG. 9O

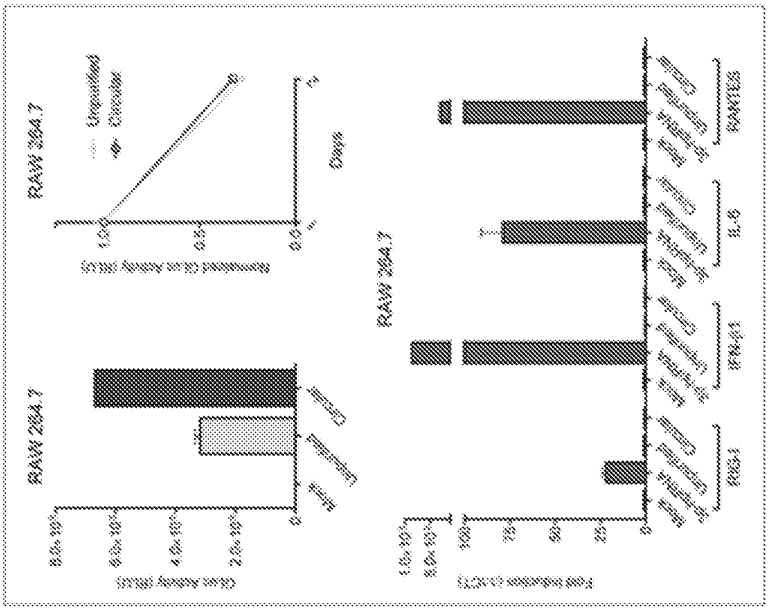


FIG. 9N

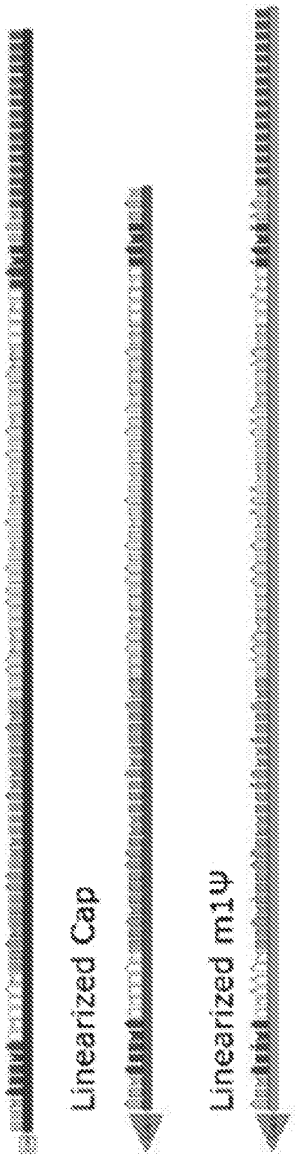


FIG. 10A

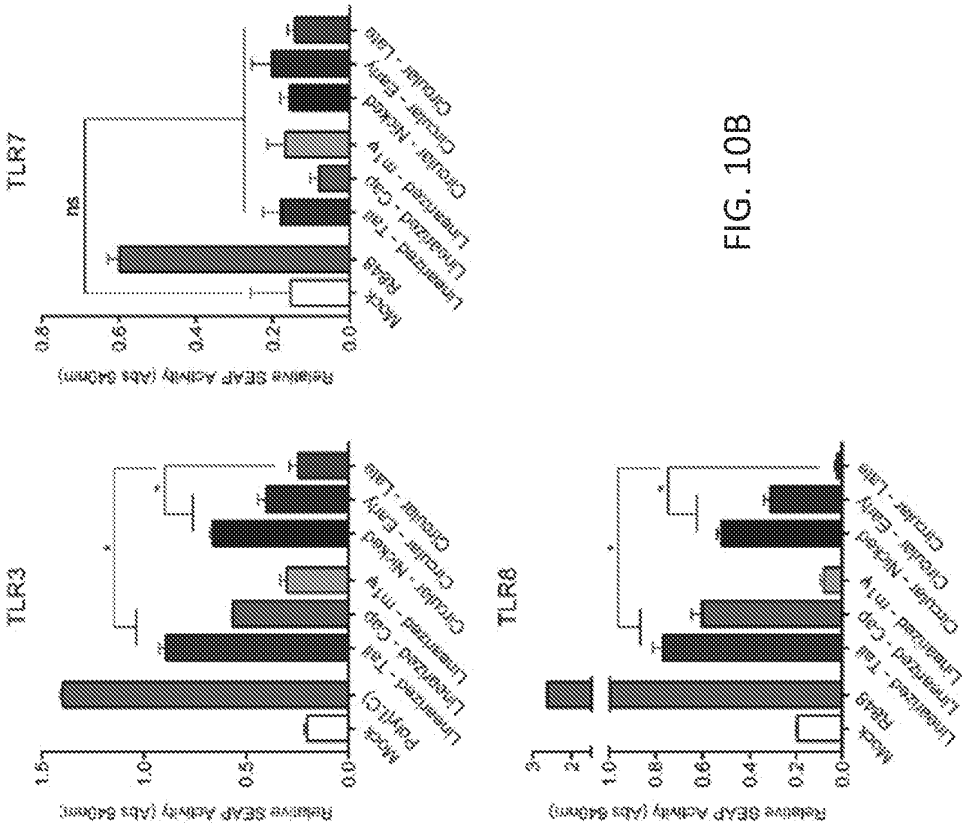

TLR7
TLR3
TLR8
Relative SEAP Activity (Abs 640nm)
Media
Rituximab
Unmethylated - Tail
Unmethylated - Cap
Unmethylated - m1ψ
Circular - Nicked
Circular - Early
Circular - Late
Linearized - Tail
Linearized - Cap
Linearized - m1ψ
Circular - Nicked
Circular - Early
Circular - Late
ns

FIG. 10B

FIG. 10C

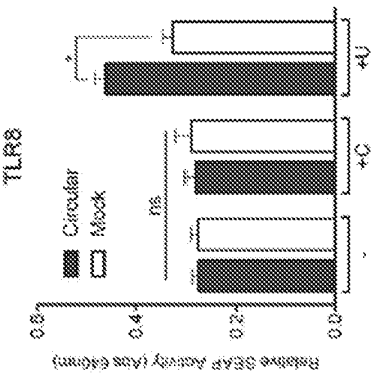


FIG. 10D

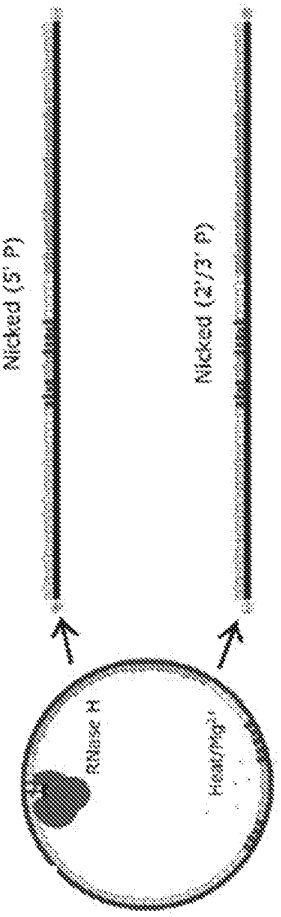
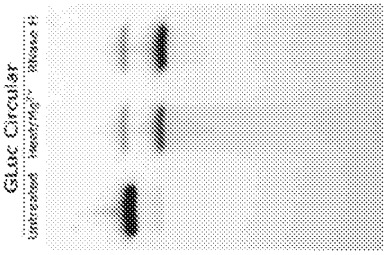


FIG. 10E



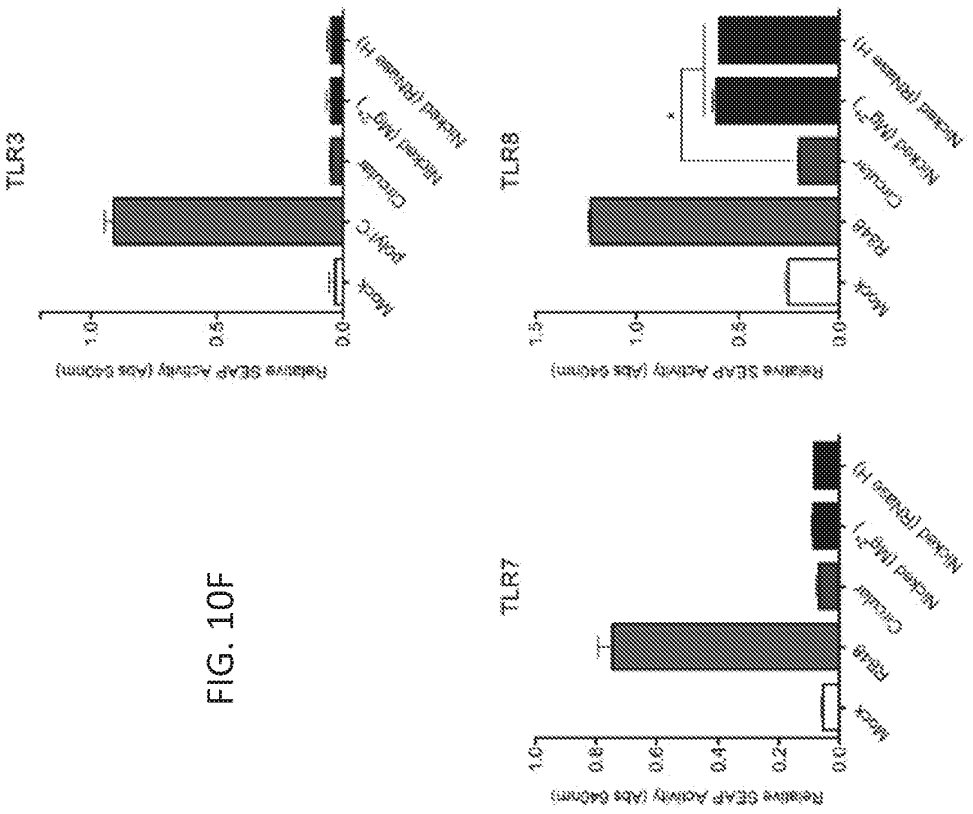

TLR3
TLR8
TLR7
Relative SEAP Activity (Abs 650nm)
1.5
1.0
0.5
0.0
0.8
0.6
0.4
0.2
Media
R848
CpG ODN
Cationic Lipid
Nucleic Acid Complex (1h)
Nucleic Acid Complex (4hr)
Nucleic Acid Complex (24hr)
*

FIG. 10F

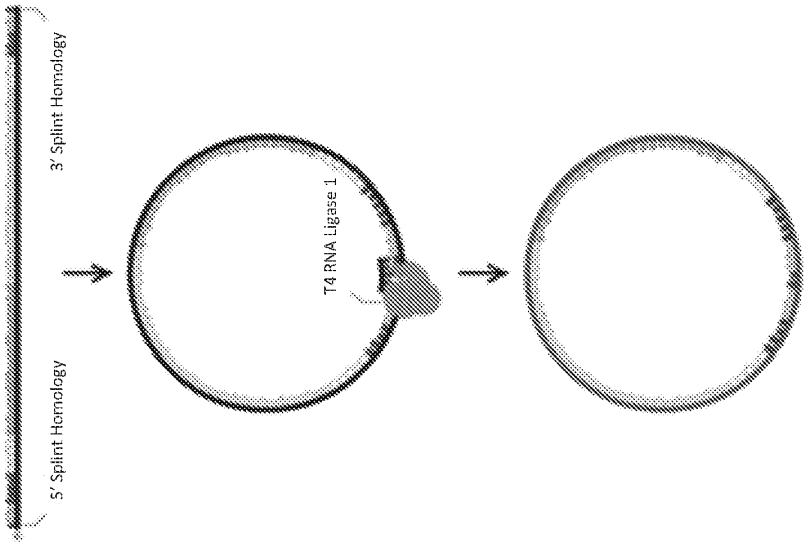


FIG. 10G

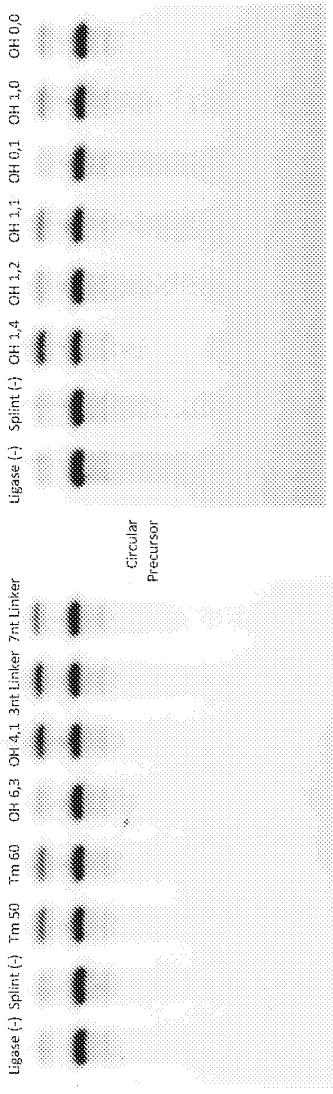


FIG. 10H

FIG. 10I

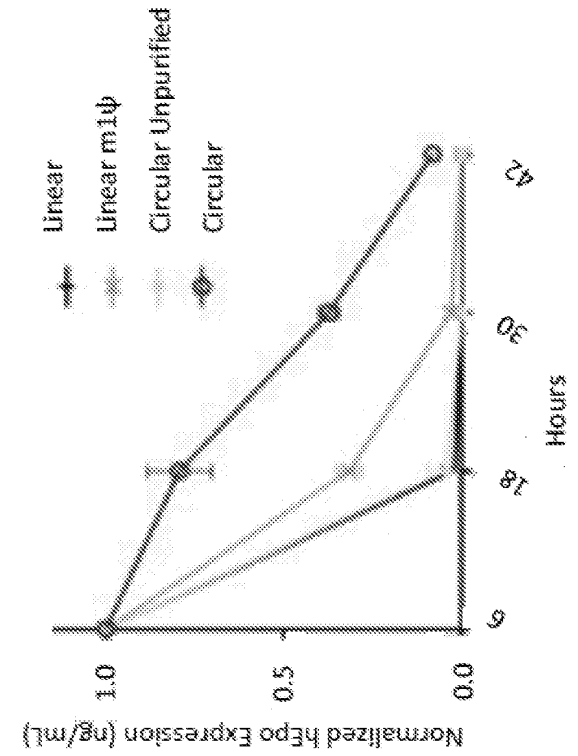


FIG. 11B

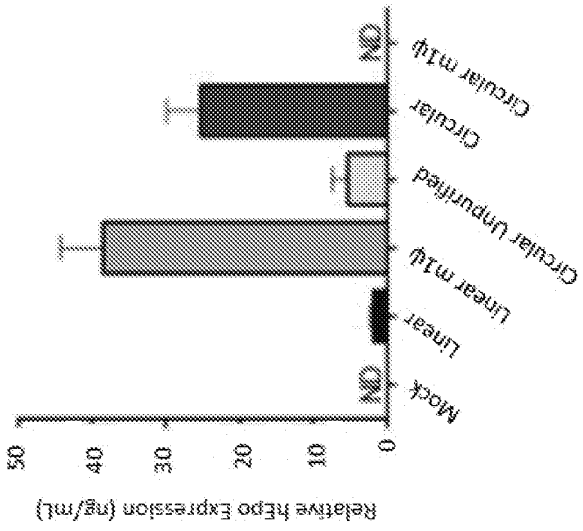


FIG. 11A

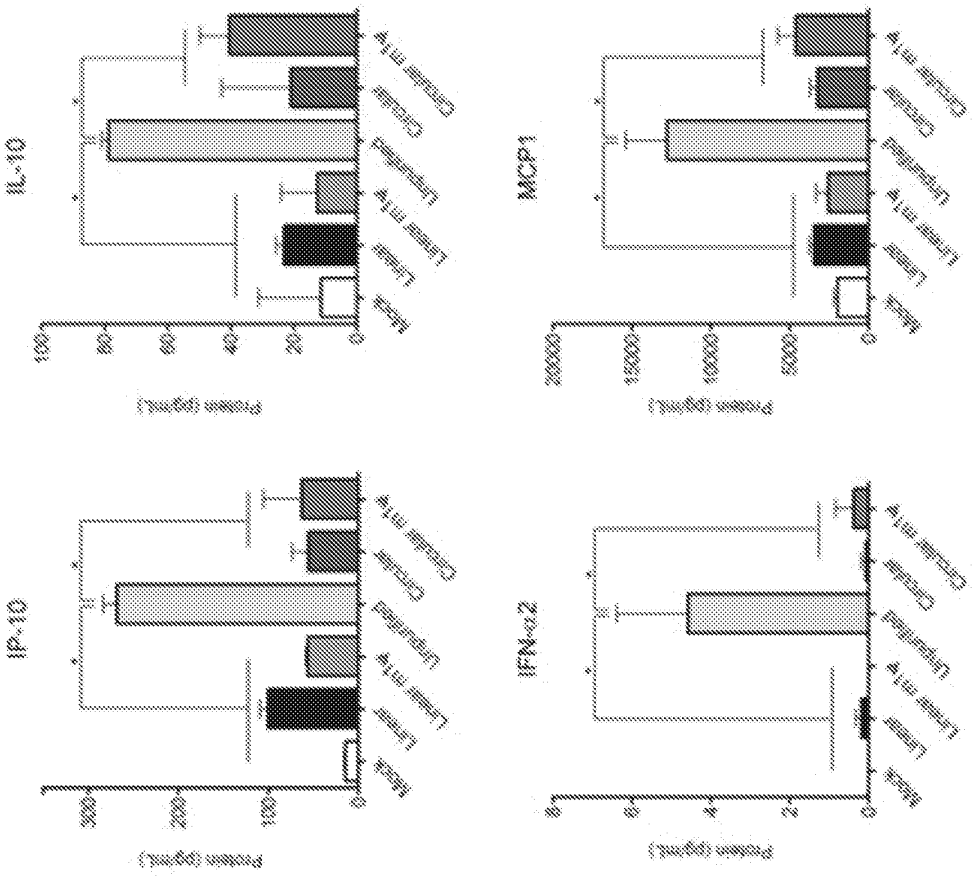

IL-10
IP-10
MCP1
IFN-α2
Protein (pg/mL)
Naïve
Influenza
Influenza + Naïve serum
Influenza + Influenza serum

FIG. 11C

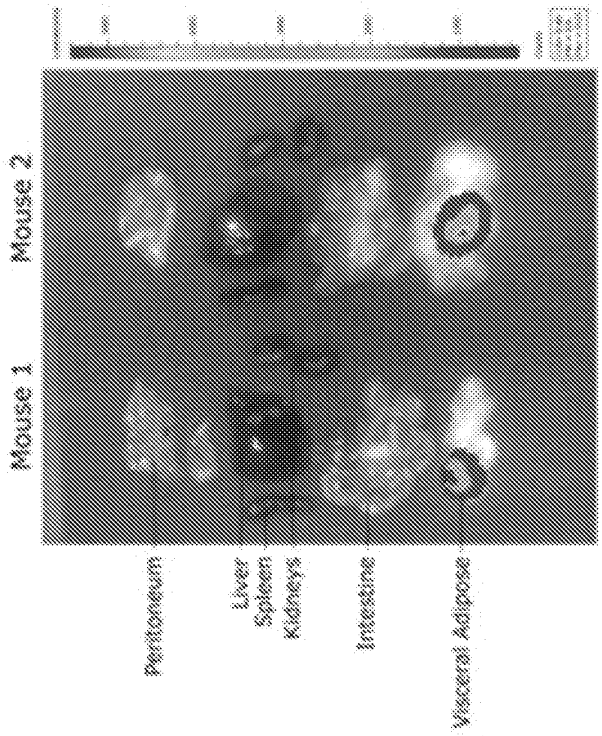

Mouse 1
Mouse 2
Peritoneum
Liver
Spleen
Kidneys
Intestine
Visceral Adipose

FIG. 11D

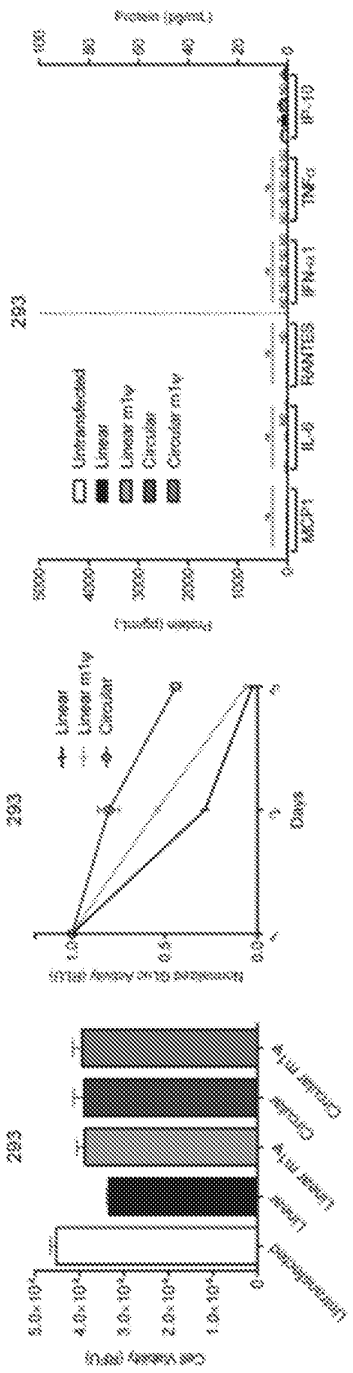

293
Untransfected
Linear
Linear m1ψ
Circular
Circular m1ψ
293
Linear
Linear m1ψ
Circular
Days
293
Circular m1ψ
Circular
Linear m1ψ
Linear
Untransfected

FIG. 11E

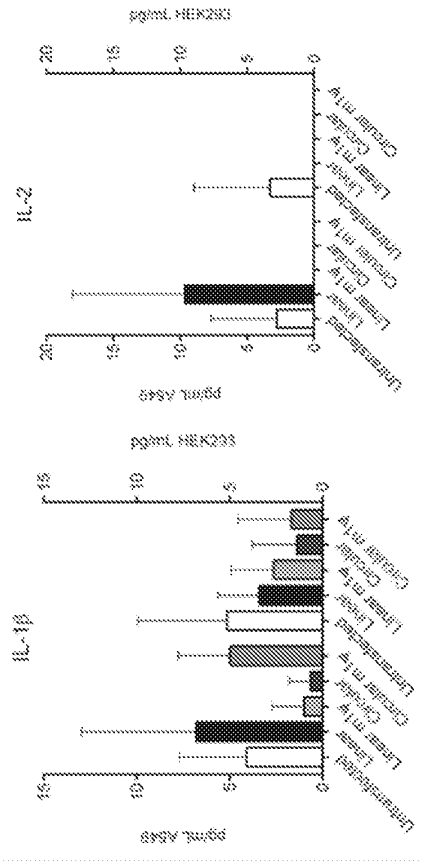

IL-2
IL-1β
IL-5
IL-4
pg/mL HEK293
pg/mL Ab49
Unstimulated
Liver Ab49
Liver R1V
Cecum Ab49
Cecum R1V

FIG. 11F

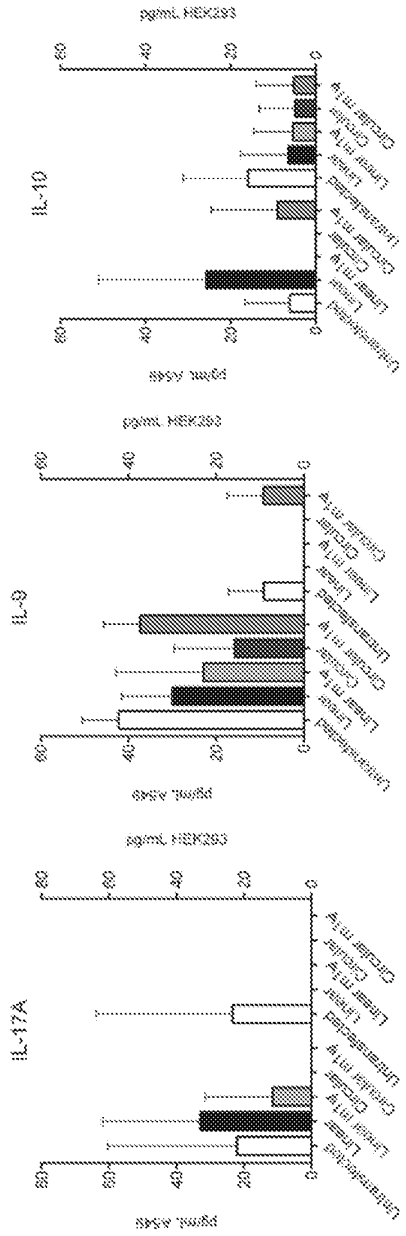


FIG. 11F (Cont'd)

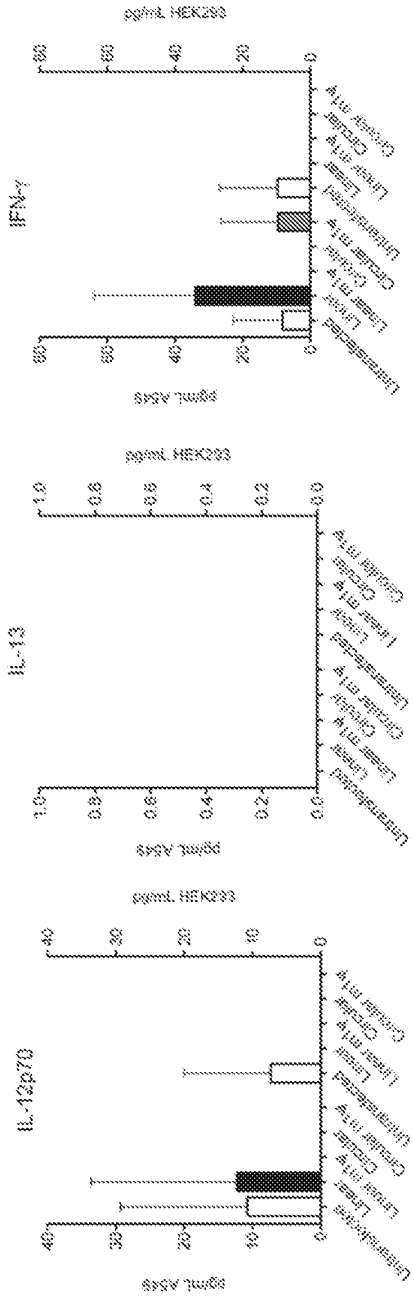


FIG. 11F (Cont'd)

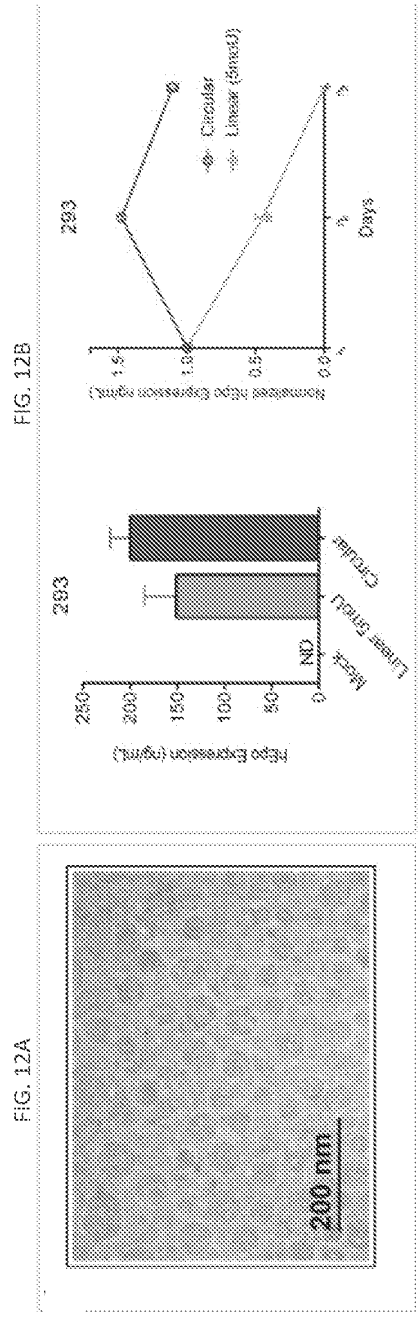
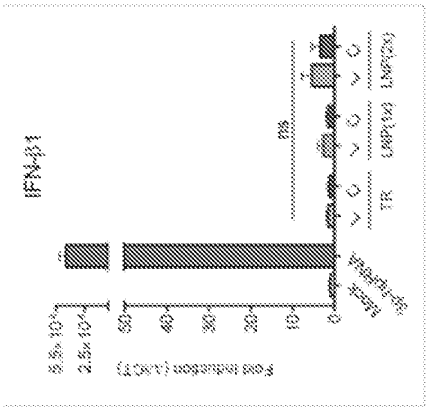


FIG. 12A

FIG. 12B



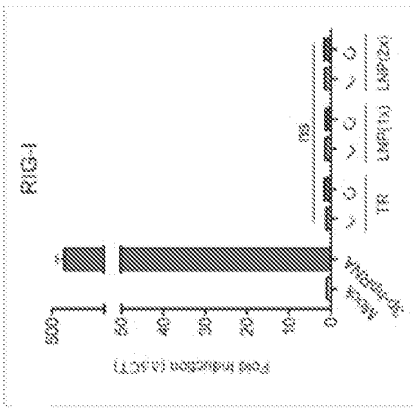


FIG. 12C

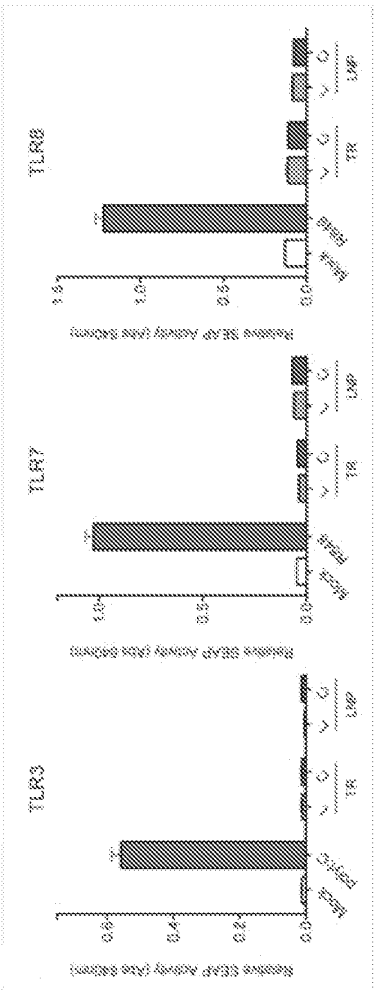


FIG. 12D

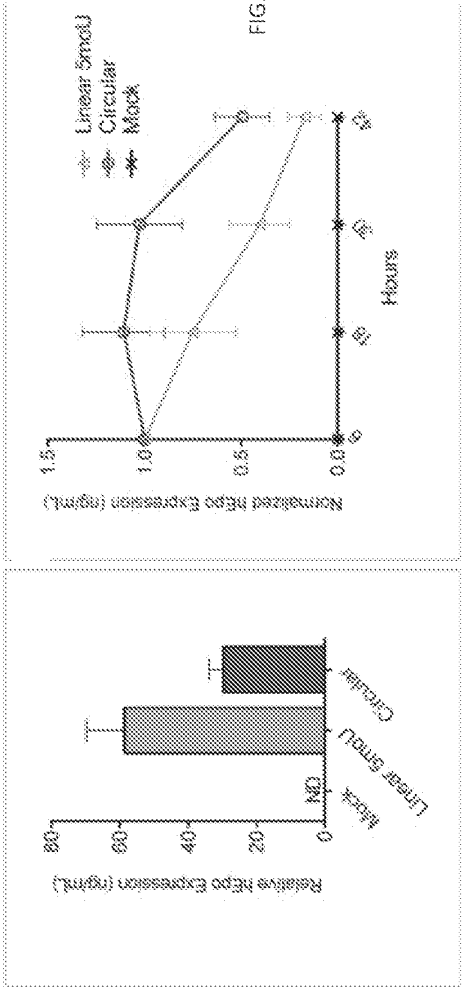


FIG. 12E

FIG. 12F

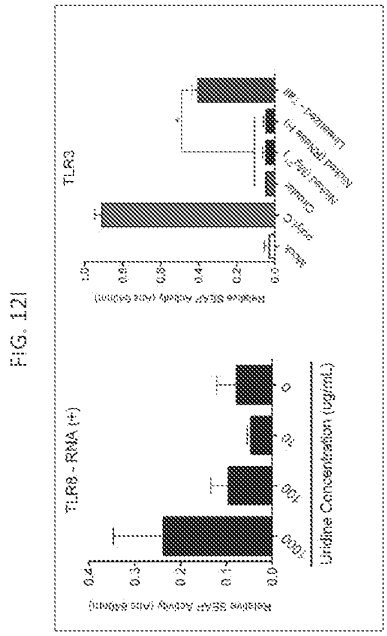


FIG. 12I

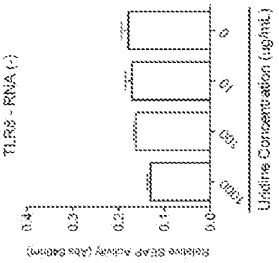


FIG. 12H

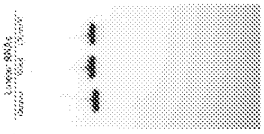


FIG. 12G

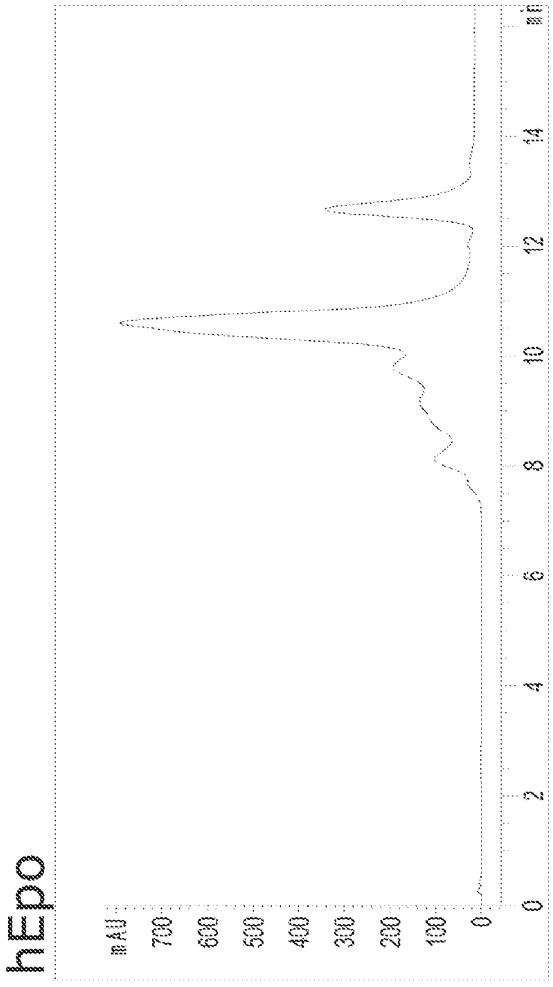


FIG. 13

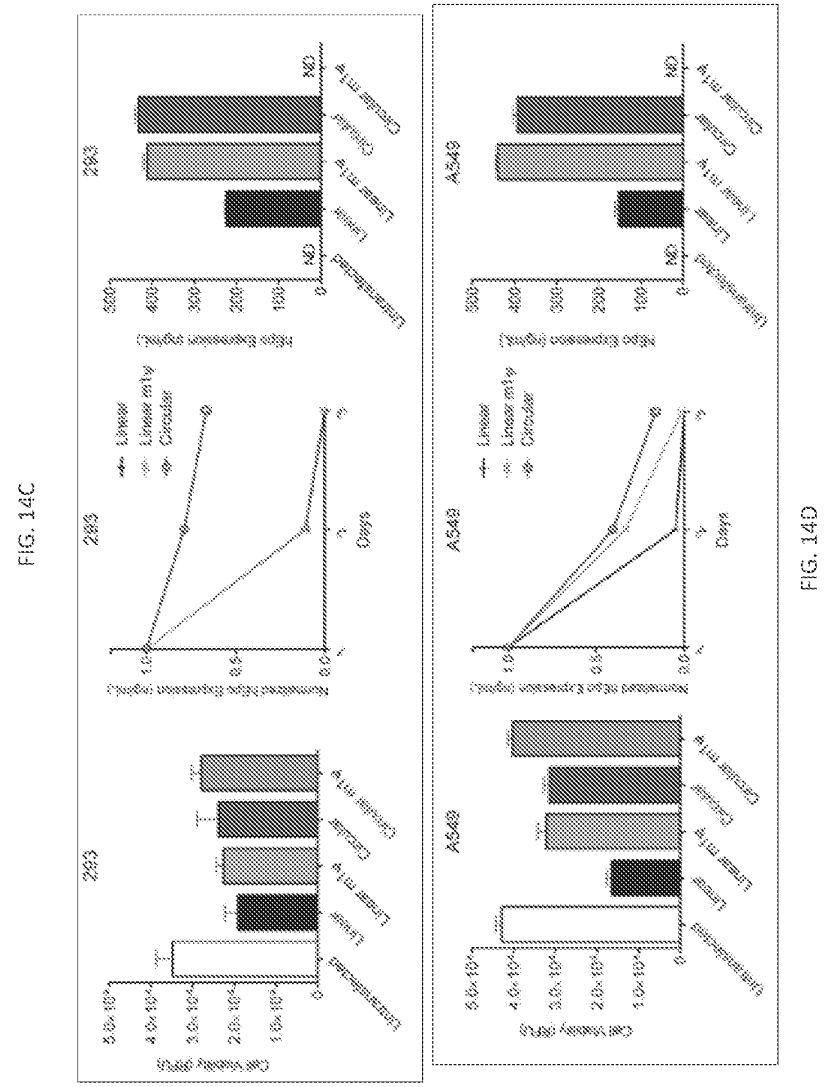


FIG. 14C

FIG. 14D

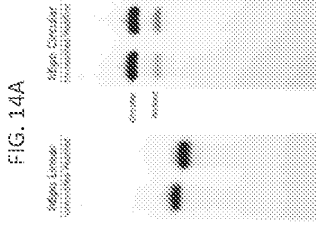


FIG. 14A

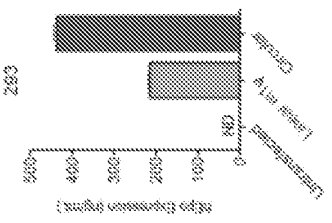


FIG. 14B

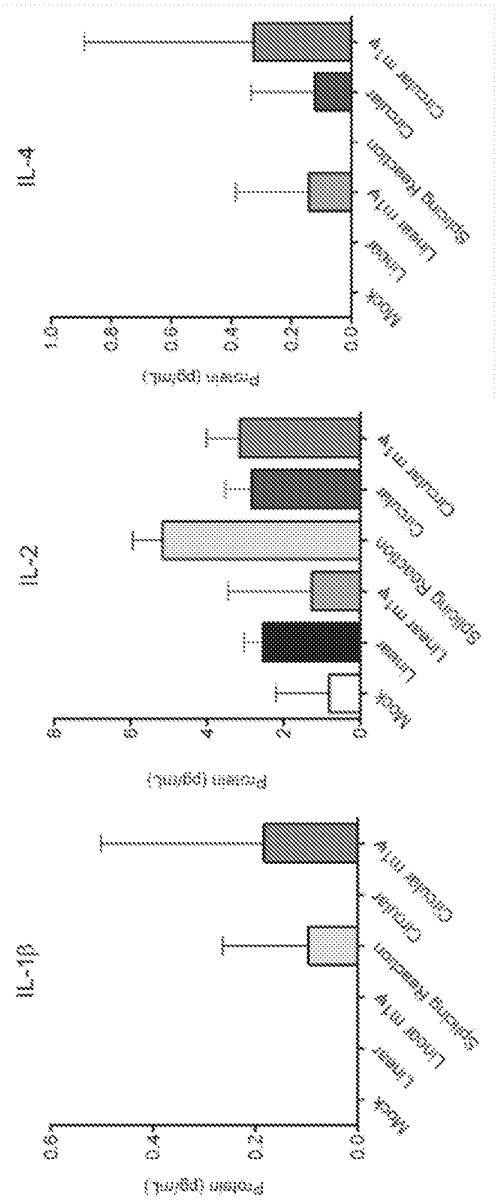

IL-4
IL-2
IL-1β
Protein (pg/mL)
Block
Linear
Linear m1γ
Spacing Reaction
Circular
Circular m1γ

FIG. 15

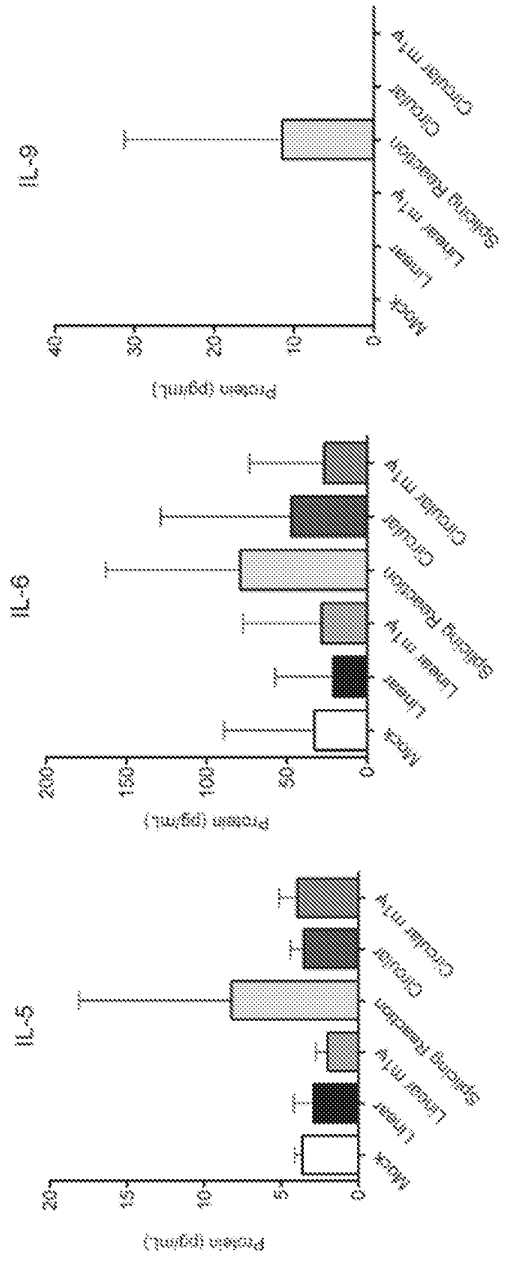

IL-5
IL-6
IL-9
Protein (pg/mL)
Mock
Linear
Linear m1y
Splicing Reaction
Circular
Circular m1y

FIG. 15 (Cont'd)

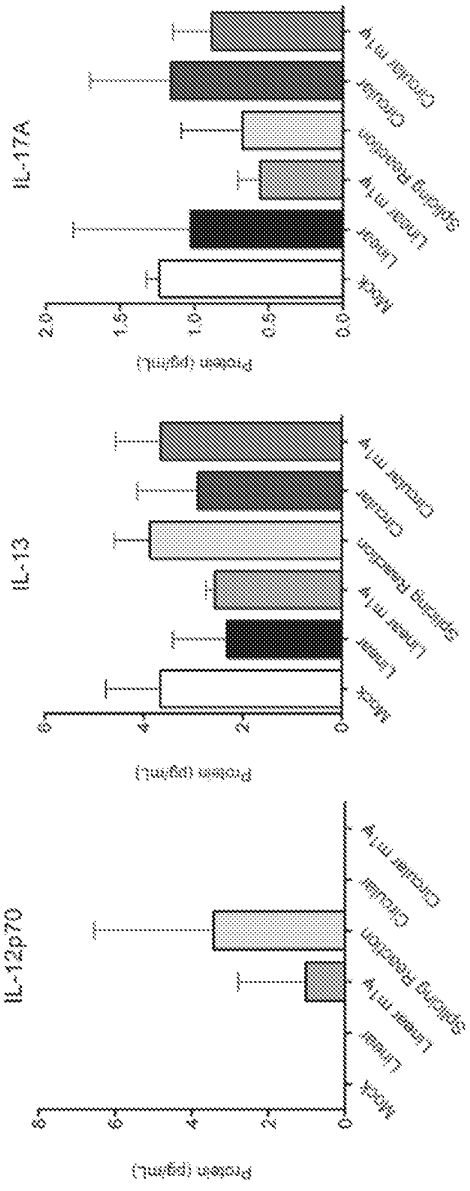

IL-17A
IL-13
IL-12p70
Protein (pg/mL)
Block
Linear
Linear m1/4
Splicing Reaction
Circular
Circular m1/4

FIG. 15 (Cont'd)

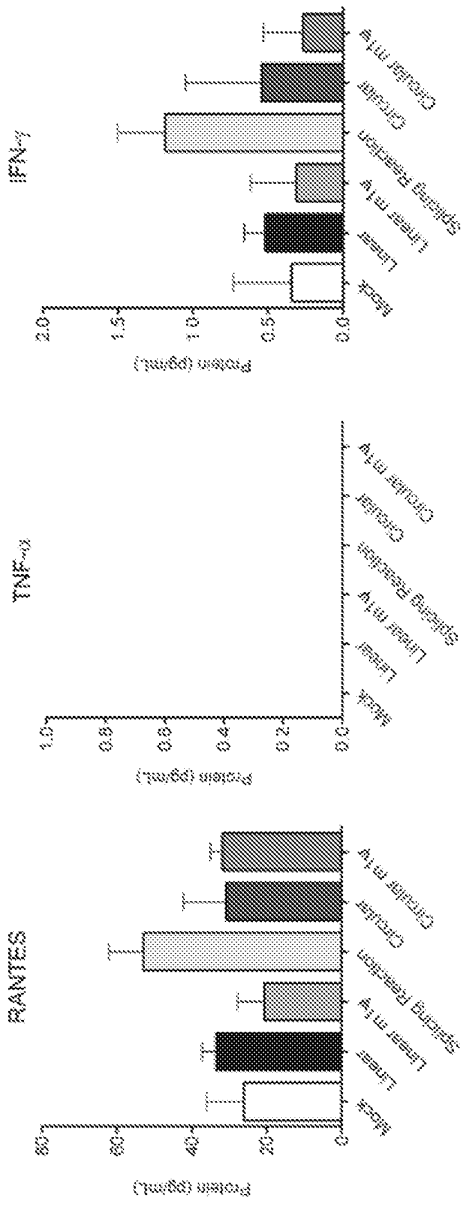

IFN-γ
TNF-α
RANTES
Protein (pg/mL)
Naïve
Lysed
Lysed mTeV
Splicing Reaction
Circular
Circular mTeV

FIG. 15 (Cont'd)

FIG. 16A

LNP-EPT Formulation	Polydispersity	Size (Intensity Mean) (nm)	Encapsulation Efficiency (%)
Smad1-mRNA	0.14 ± 0.02	92 ± 6	75 ± 6
Unpurified	0.13 ± 0.04	87 ± 7	75 ± 13
Circular	0.12 ± 0.03	95 ± 7	77 ± 14

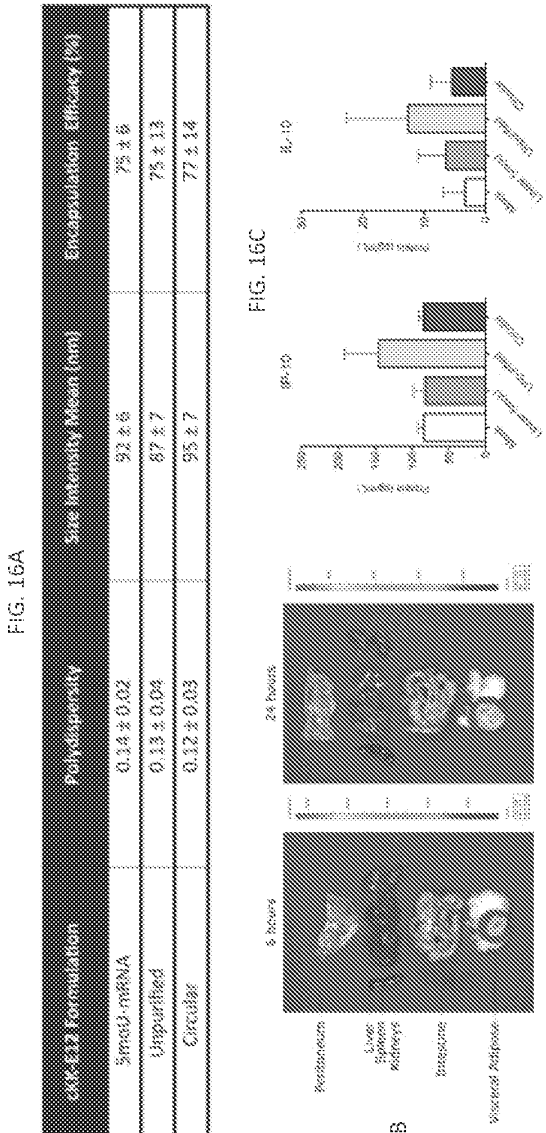


FIG. 16B

FIG. 16C

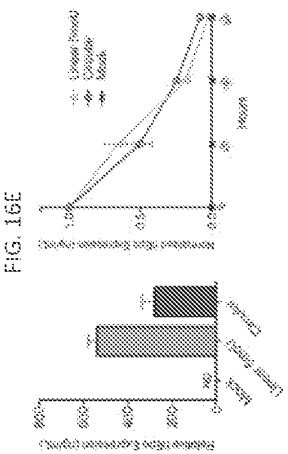


FIG. 16E

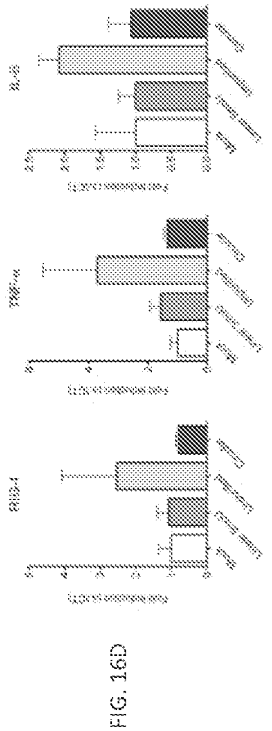


FIG. 16D

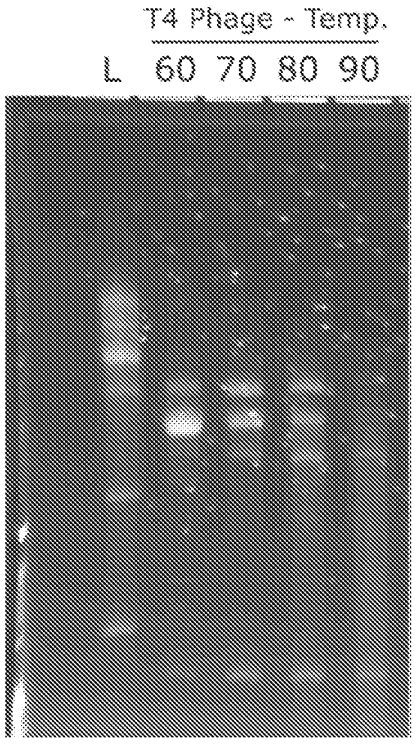


FIG. 17

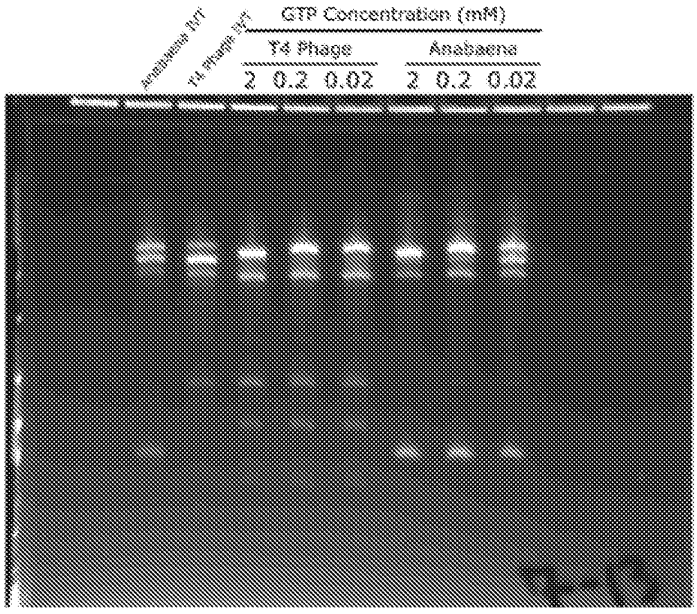


FIG. 18A

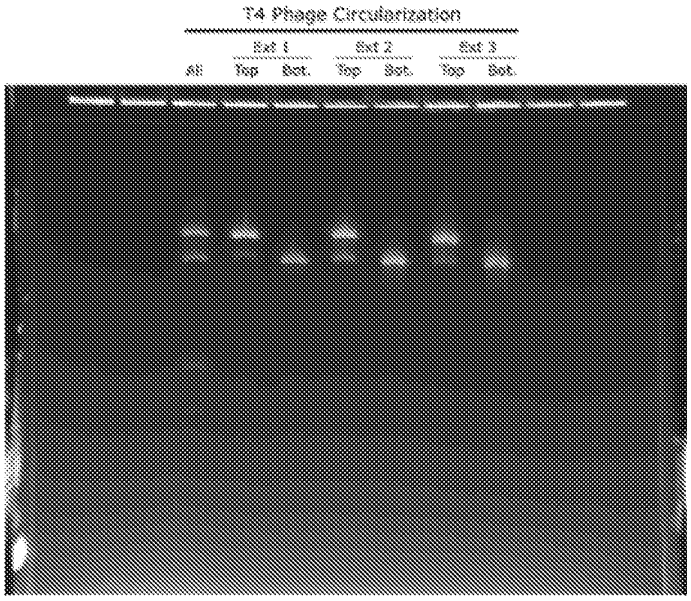


FIG. 18B

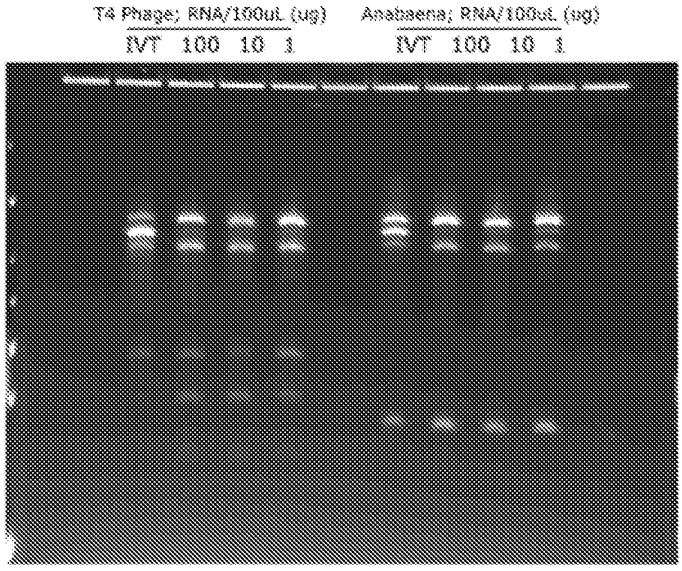


FIG. 18C

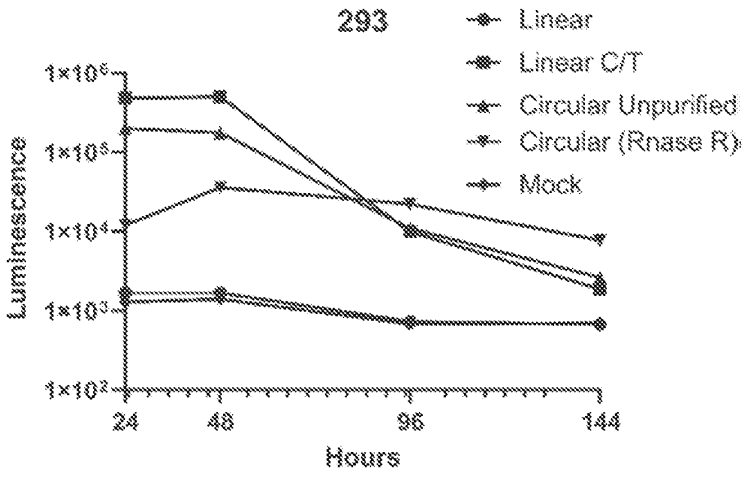


FIG. 19

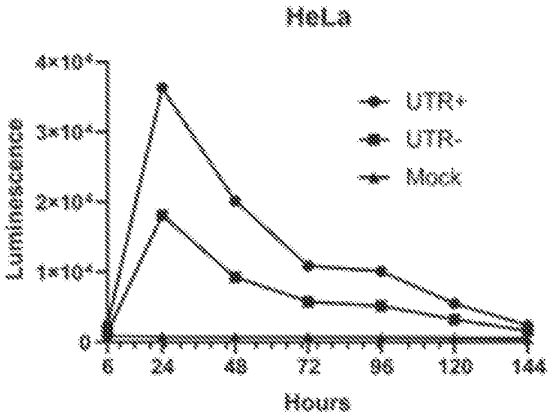


FIG. 20A

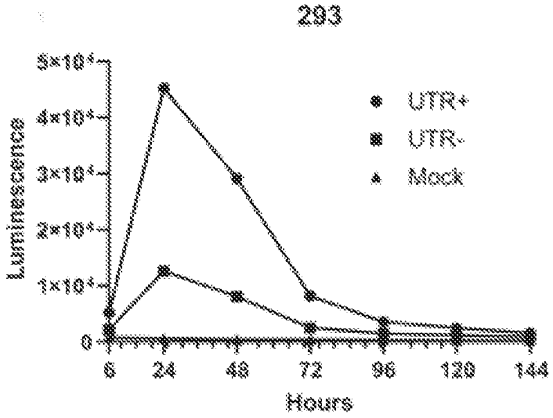


FIG. 20B

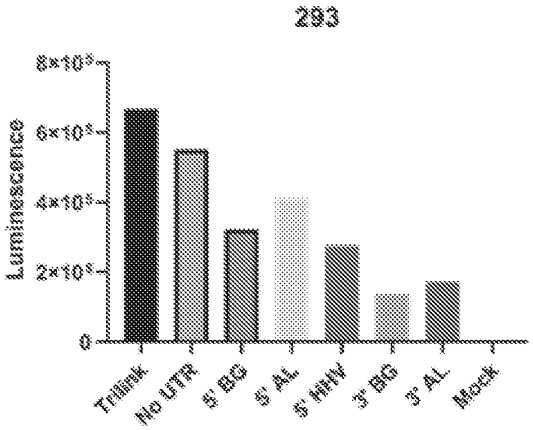


FIG. 20C

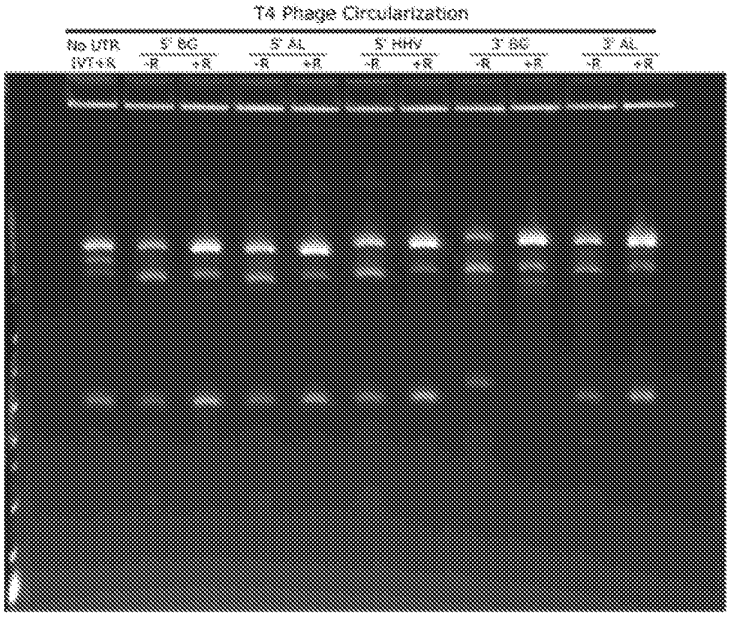


FIG. 20D

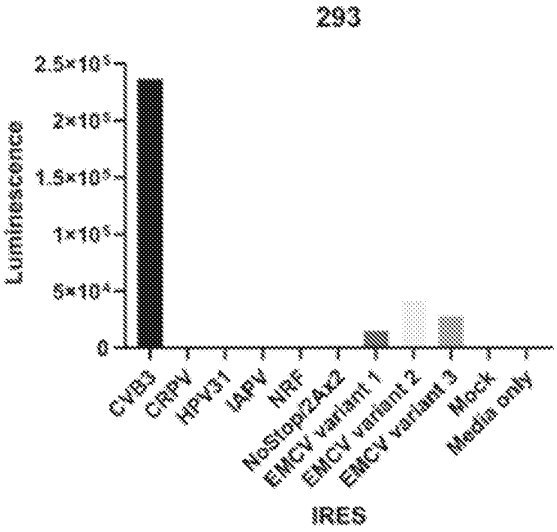


FIG. 21A

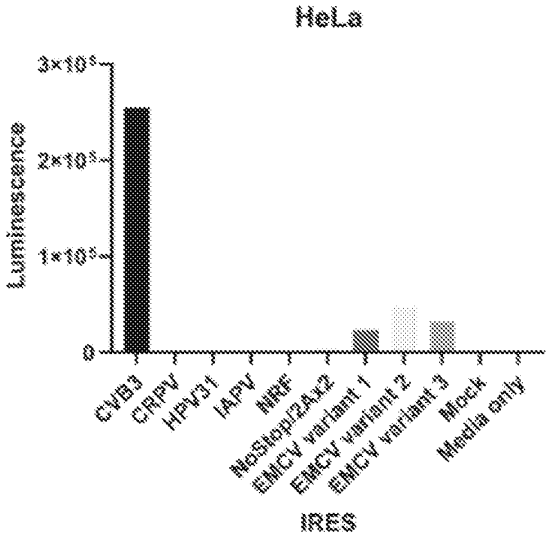


FIG. 21B

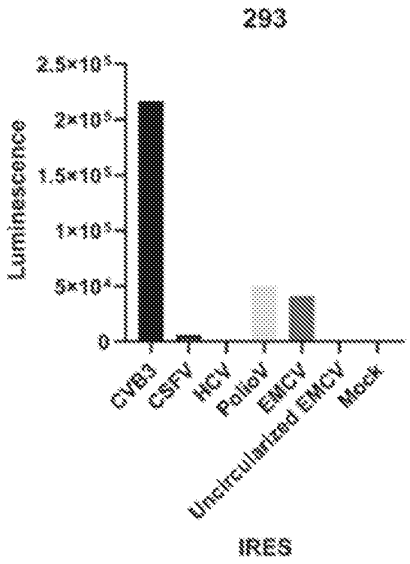


FIG. 21C

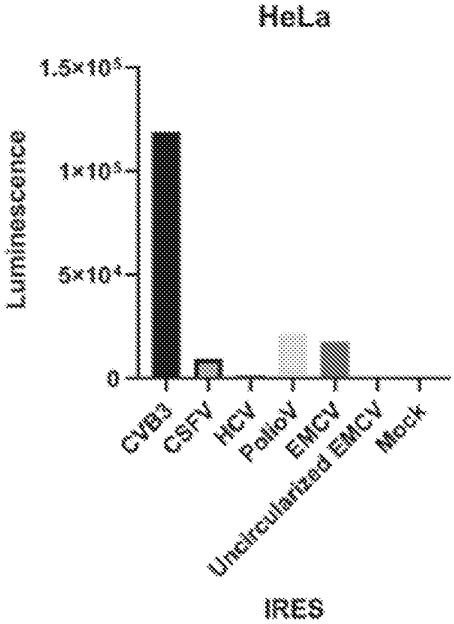


FIG. 21D

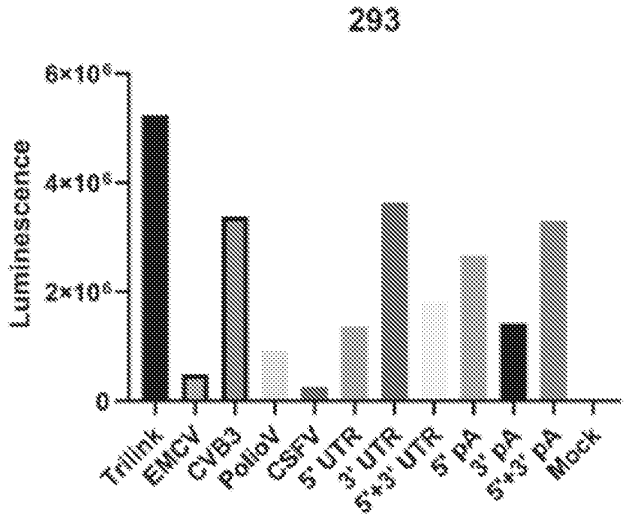


FIG. 21E

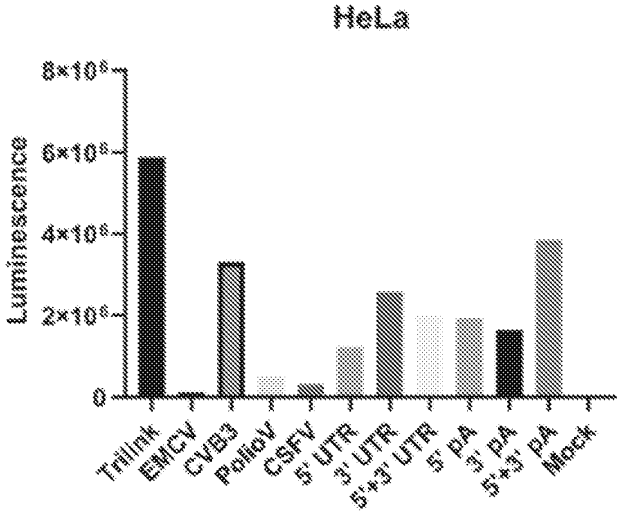


FIG. 21F

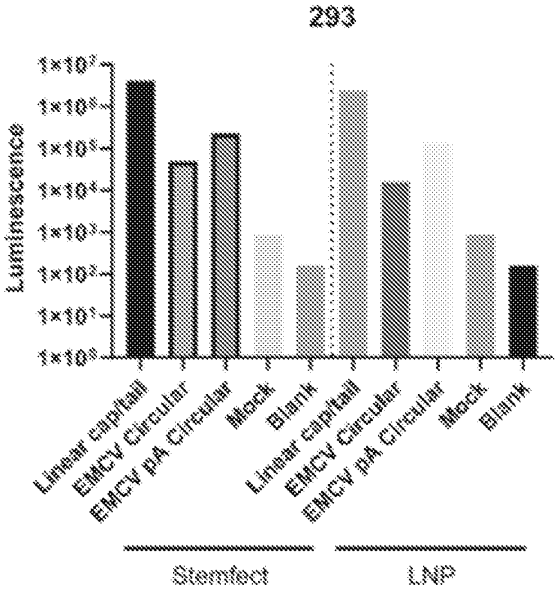

293
Luminescence
1×10⁷
1×10⁶
1×10⁵
1×10⁴
1×10³
1×10²
1×10¹
1×10⁰
Linear capHall
EMCV Circular
EMCV pA Circular
Mock
Blank
Linear capHall
EMCV Circular
EMCV pA Circular
Mock
Blank
Stemfect
LNP

FIG. 21G

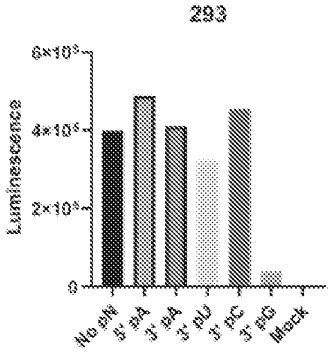


FIG. 22A

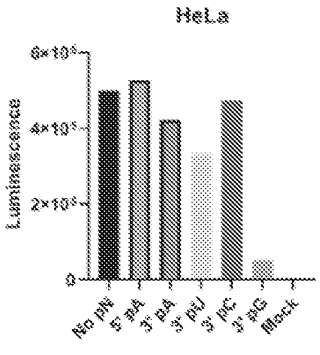


FIG. 22B

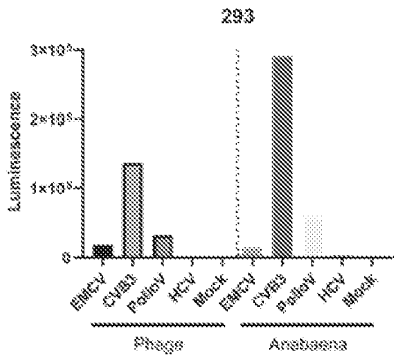


FIG. 23A

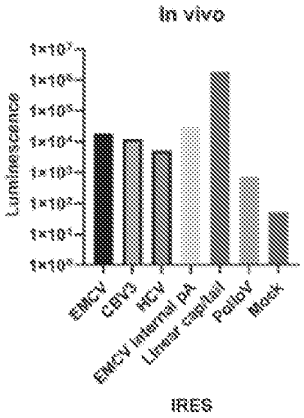


FIG. 23B

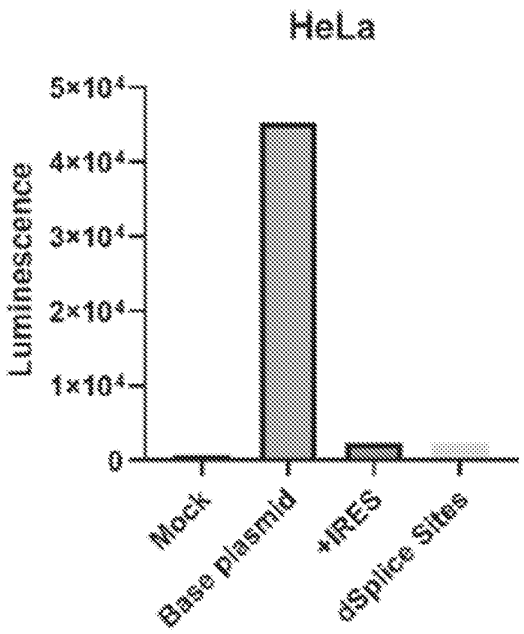

HeLa
Luminescence
5×10⁴
4×10⁴
3×10⁴
2×10⁴
1×10⁴
0
Mock
Base plasmid
+IRES
dSplice Sites

FIG. 24

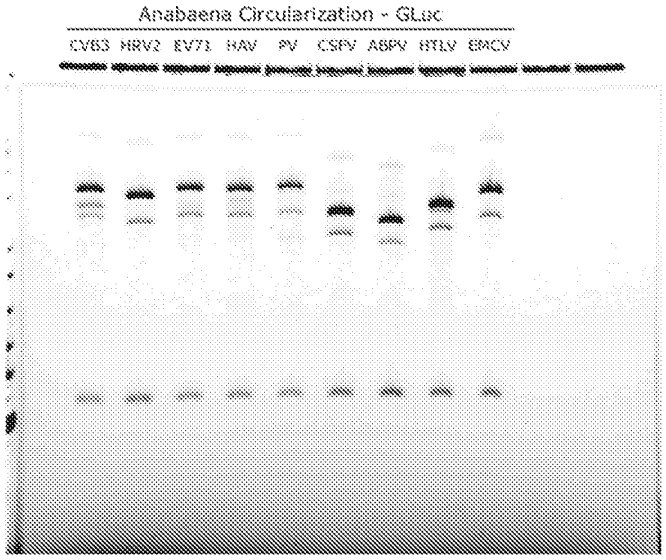


FIG. 25A

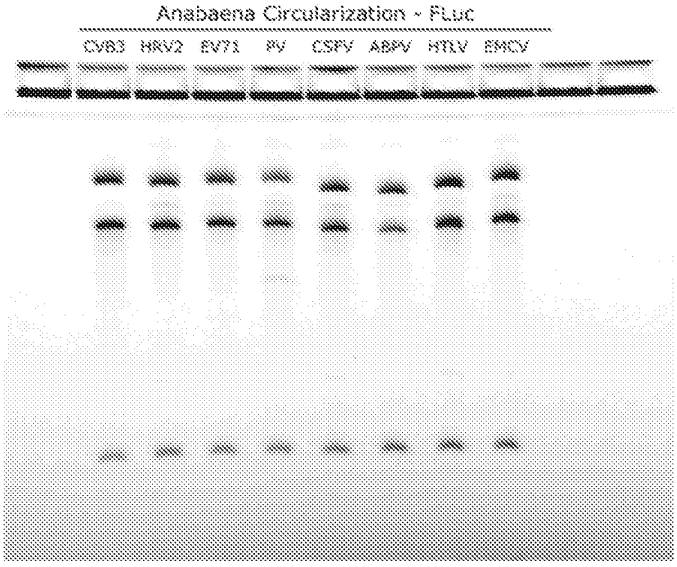


FIG. 25B

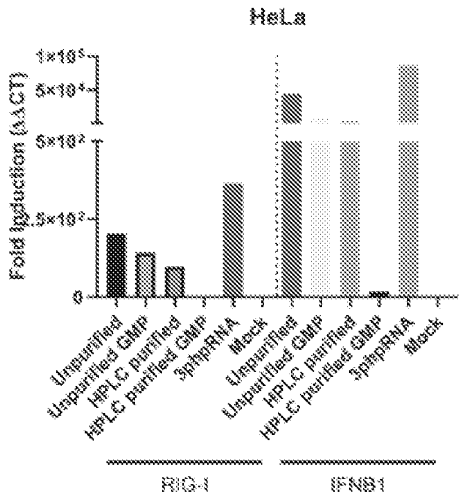


FIG. 26A

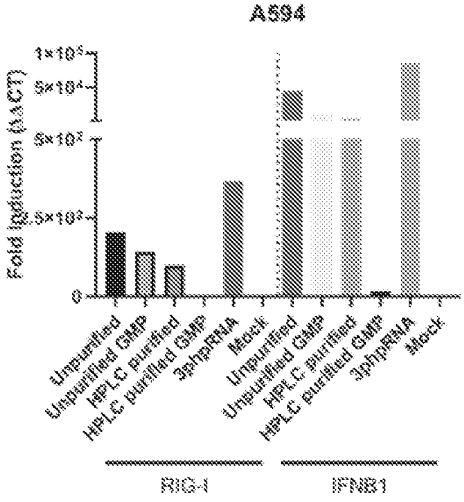


FIG. 26B