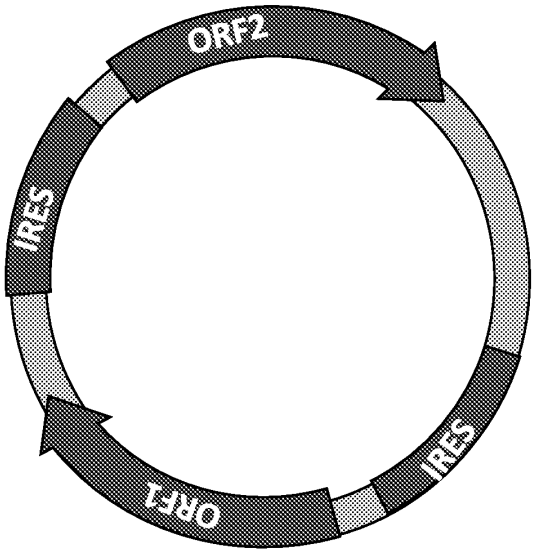
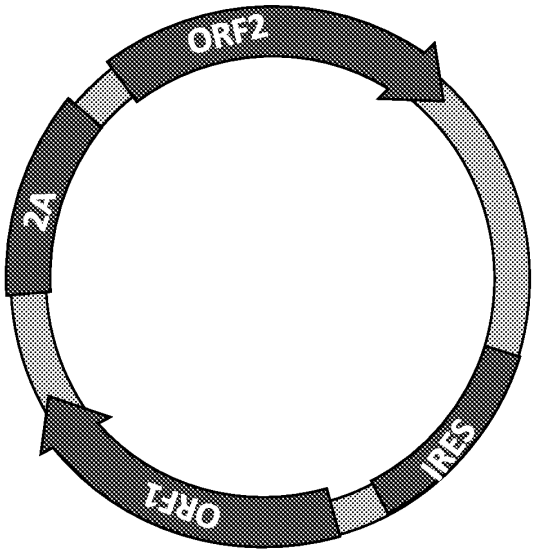
FIG. 1A



Example	ORF1	ORF2	Wherein
A	Immunogen 1	Immunogen 2	Immunogen 1 and Immunogen 2 are mutational variants of one another.
B	Immunogen 1	Immunogen 2	Immunogen 1 and Immunogen 2 are derived from different polypeptides but identify the same target.
C	Immunogen 1	Immunogen 2	Immunogen 1 and Immunogen 2 are derived from different polypeptides but identify different targets.
D	Immunogen	Adjuvant	ORF2 encodes for the expression of a polypeptide adjuvant.
E	Adjuvant	Immunogen	ORF1 encodes for the expression of a polypeptide adjuvant.

FIG. 1B



Example	ORF1	ORF2	Wherein
A	Immunogen 1	Immunogen 2	Immunogen 1 and Immunogen 2 are mutational variants of one another.
B	Immunogen 1	Immunogen 2	Immunogen 1 and Immunogen 2 are derived from different polypeptides but identify the same target.
C	Immunogen 1	Immunogen 2	Immunogen 1 and Immunogen 2 are derived from different polypeptides but identify different targets.
D	Immunogen	Adjuvant	ORF2 encodes for the expression of a polypeptide adjuvant.
E	Adjuvant	Immunogen	ORF1 encodes for the expression of a polypeptide adjuvant.

FIG. 2

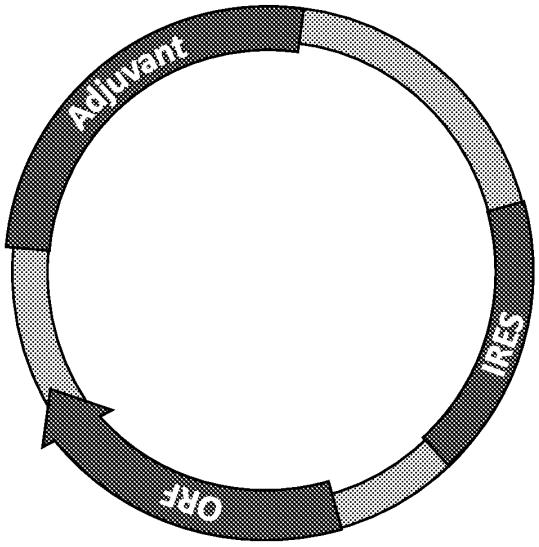
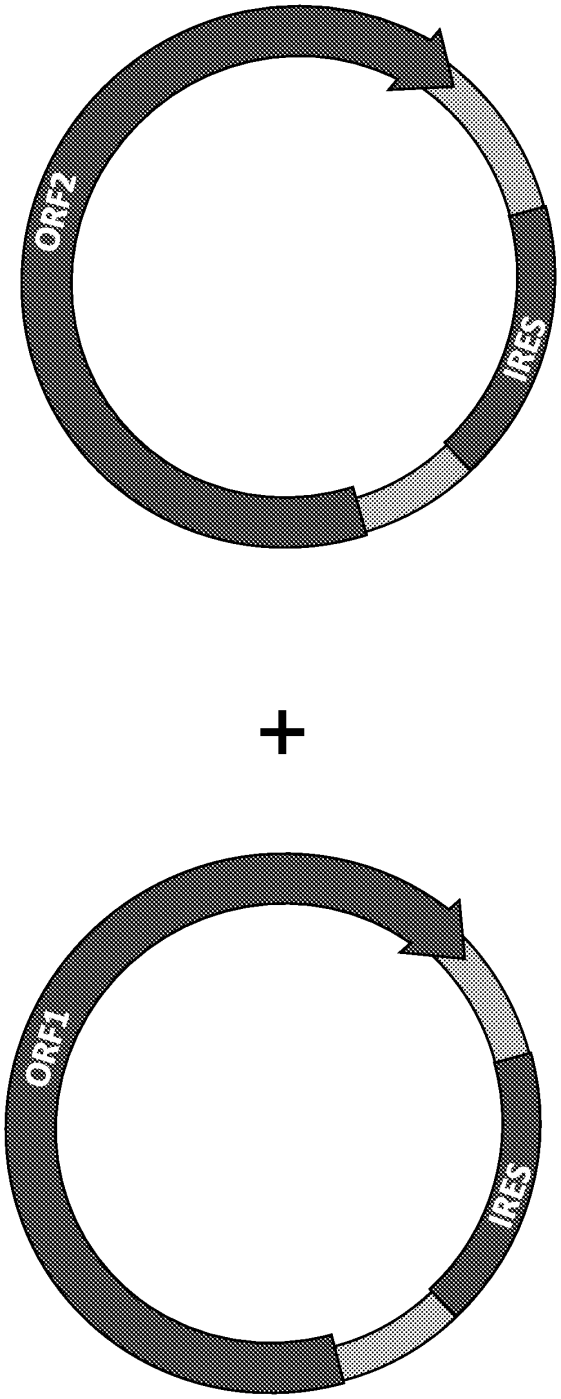


FIG. 3



Example	ORF1	ORF2	Wherein
A	Immunogen 1	Immunogen 2	Immunogen 1 and Immunogen 2 are mutational variants of one another.
B	Immunogen 1	Immunogen 2	Immunogen 1 and Immunogen 2 are derived from different polypeptides but identify the same target.
C	Immunogen 1	Immunogen 2	Immunogen 1 and Immunogen 2 are derived from different polypeptides but identify different targets.
D	Immunogen	Adjuvant	ORF2 encodes for the expression of a polypeptide adjuvant.
E	Adjuvant	Immunogen	ORF1 encodes for the expression of a polypeptide adjuvant.

FIG. 4

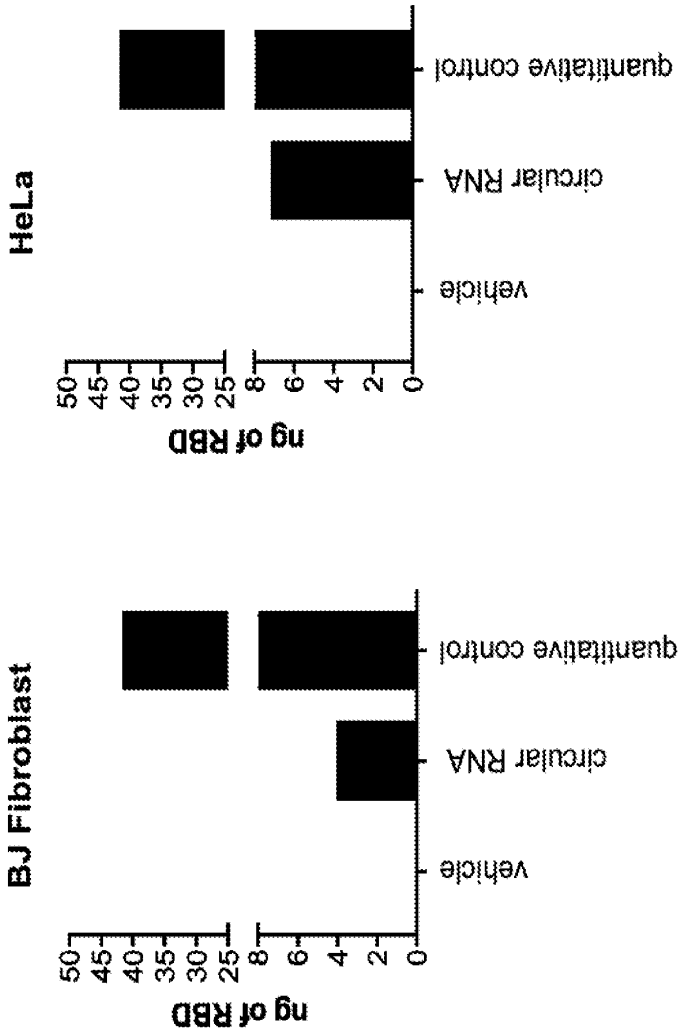

HeLa
ng of RBD
50
45
40
35
30
25
8
6
4
2
0
vehicle
circular RNA
quantitative control
BJ Fibroblast
ng of RBD
50
45
40
35
30
25
8
6
4
2
0
vehicle
circular RNA
quantitative control

FIG. 5

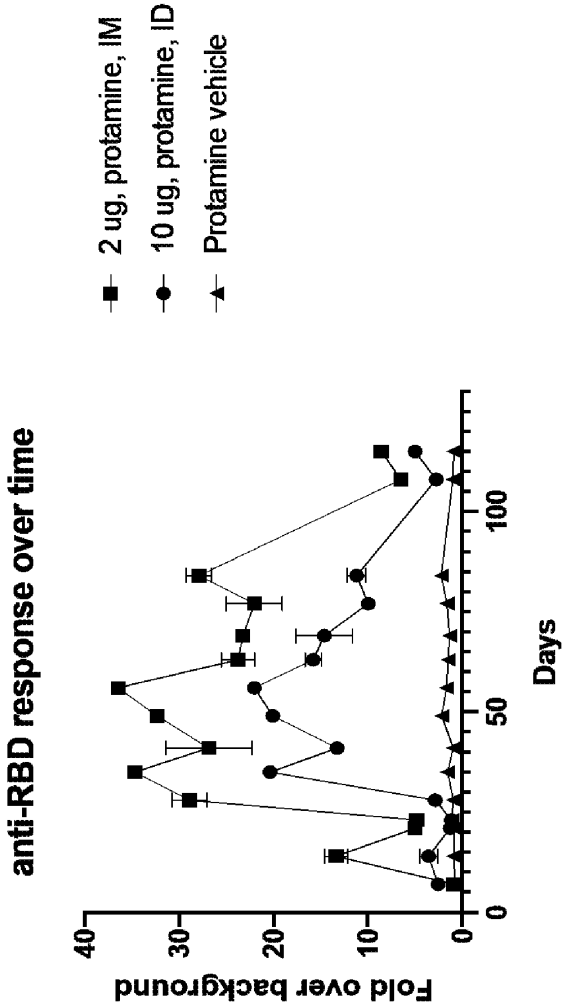

anti-RBD response over time
2 ug, protamine, IM
10 ug, protamine, ID
Protamine vehicle
Fold over background
0
10
20
30
40
0
50
100
Days

FIG. 6

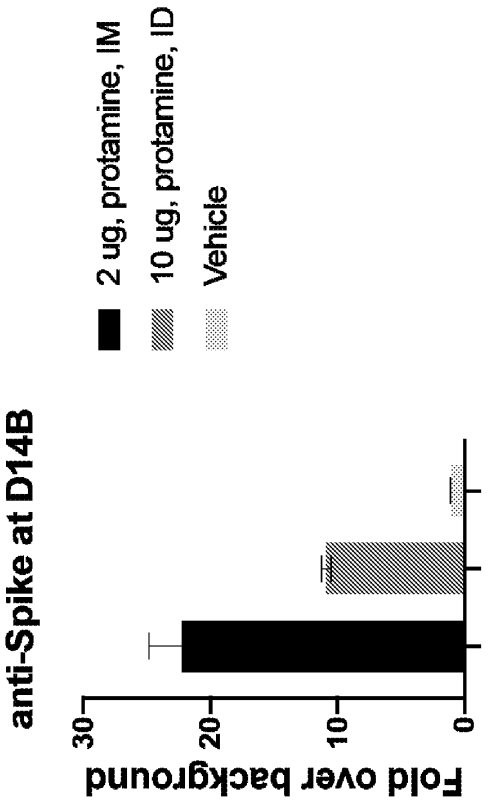
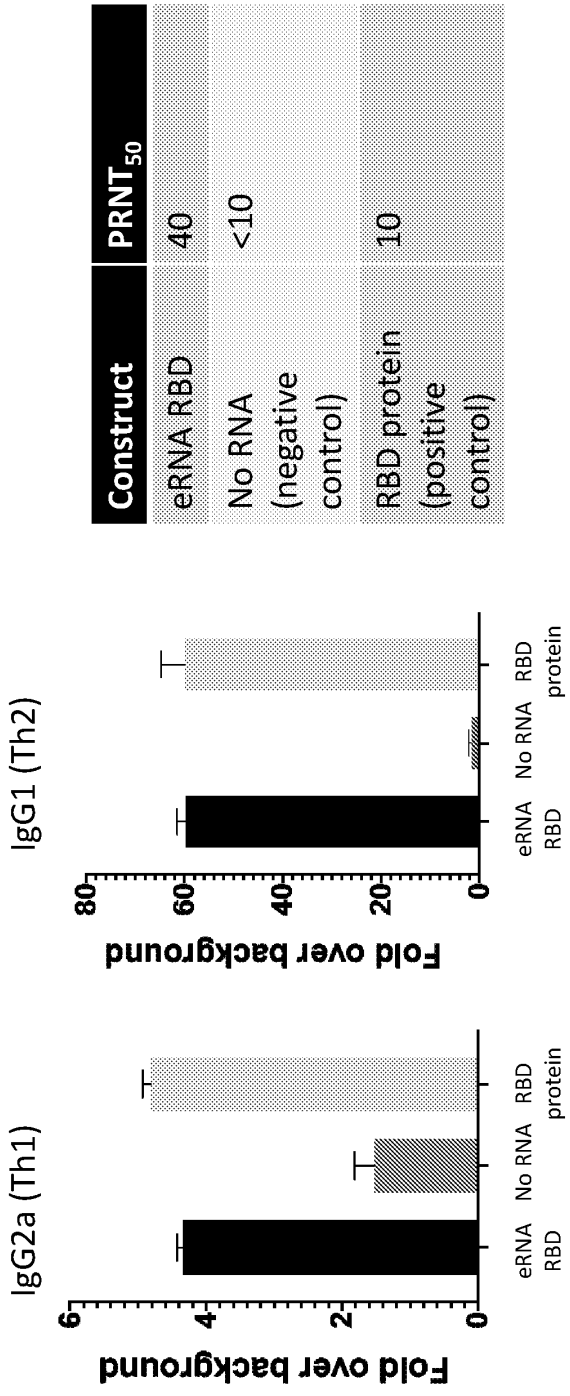

anti-Spike at D14B
Fold over background
30
20
10
0
2 ug, protamine, IM
10 ug, protamine, ID
Vehicle

FIG. 7



Construct	$PRNT_{50}$
eRNA RBD	40
No RNA (negative control)	<10
RBD protein (positive control)	10

FIG. 8

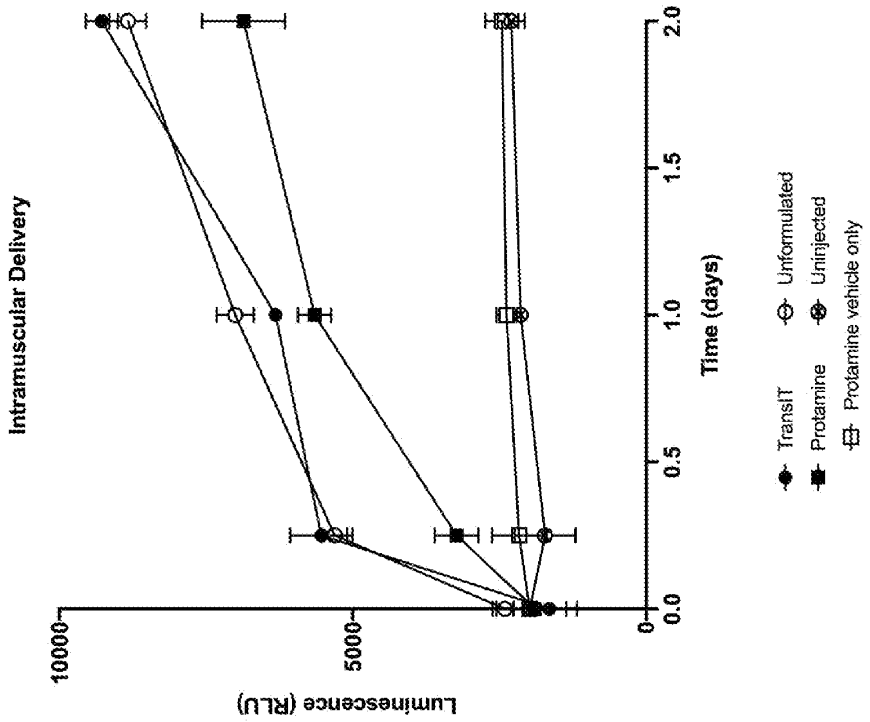

Intramuscular Delivery
Luminescence (RLU)
10000
5000
0
0.0
0.5
1.0
1.5
2.0
Time (days)
TransIT
Protamine
Protamine vehicle only
Unformulated
Uninjected

FIG. 9

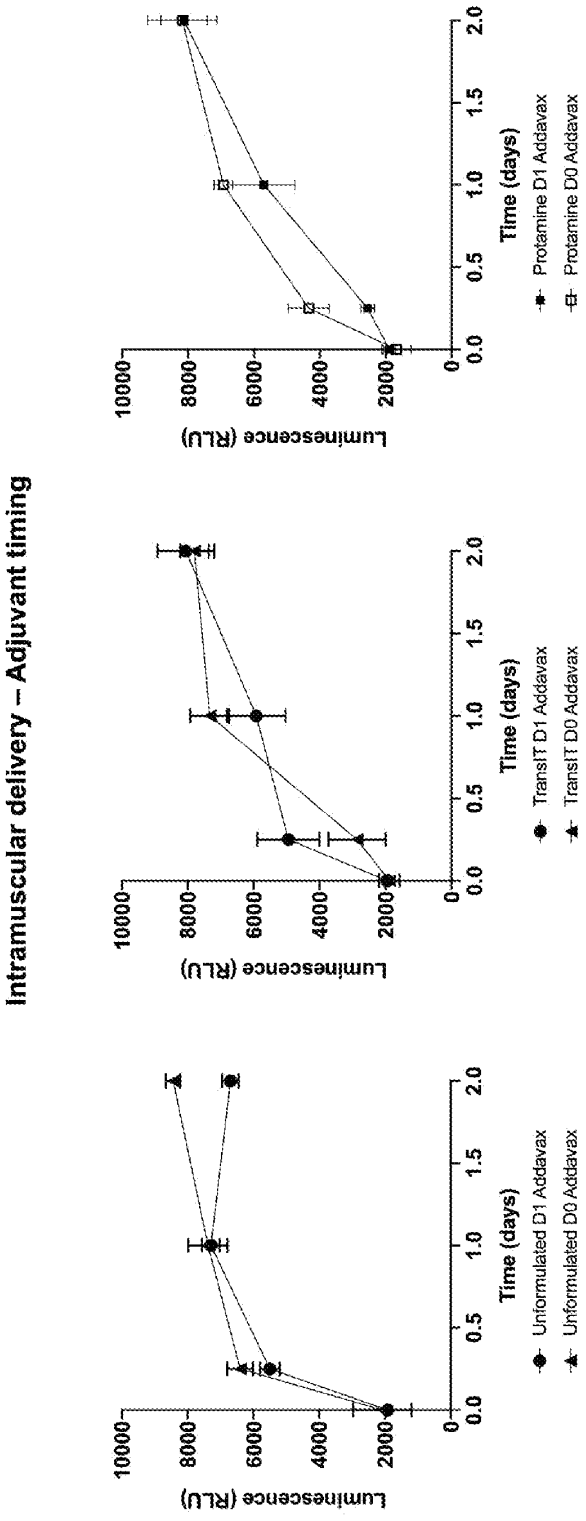

Intramuscular delivery – Adjuvant timing
Luminescence (RLU)
10000
8000
6000
4000
2000
0
0.0
0.5
1.0
1.5
2.0
Time (days)
Unformulated D1 Addavax
Unformulated D0 Addavax
TransIT D1 Addavax
TransIT D0 Addavax
Protamine D1 Addavax
Protamine D0 Addavax

FIG. 10

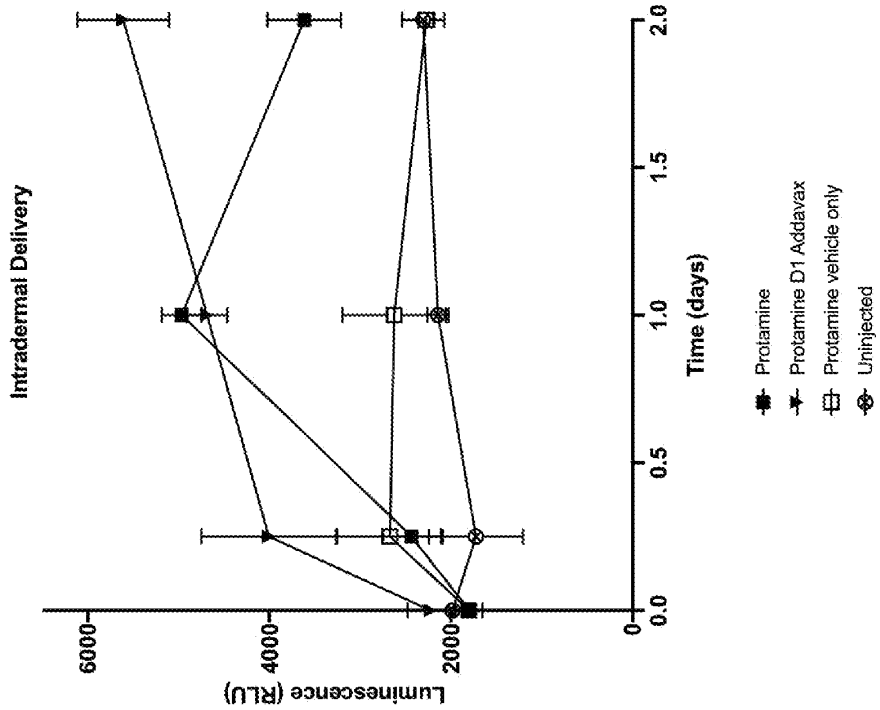

Intradermal Delivery
Luminescence (RLU)
6000
4000
2000
0
0.0
0.5
1.0
1.5
2.0
Time (days)
Protamine
Protamine D1 Addavax
Protamine vehicle only
Uninjected

FIG. 11

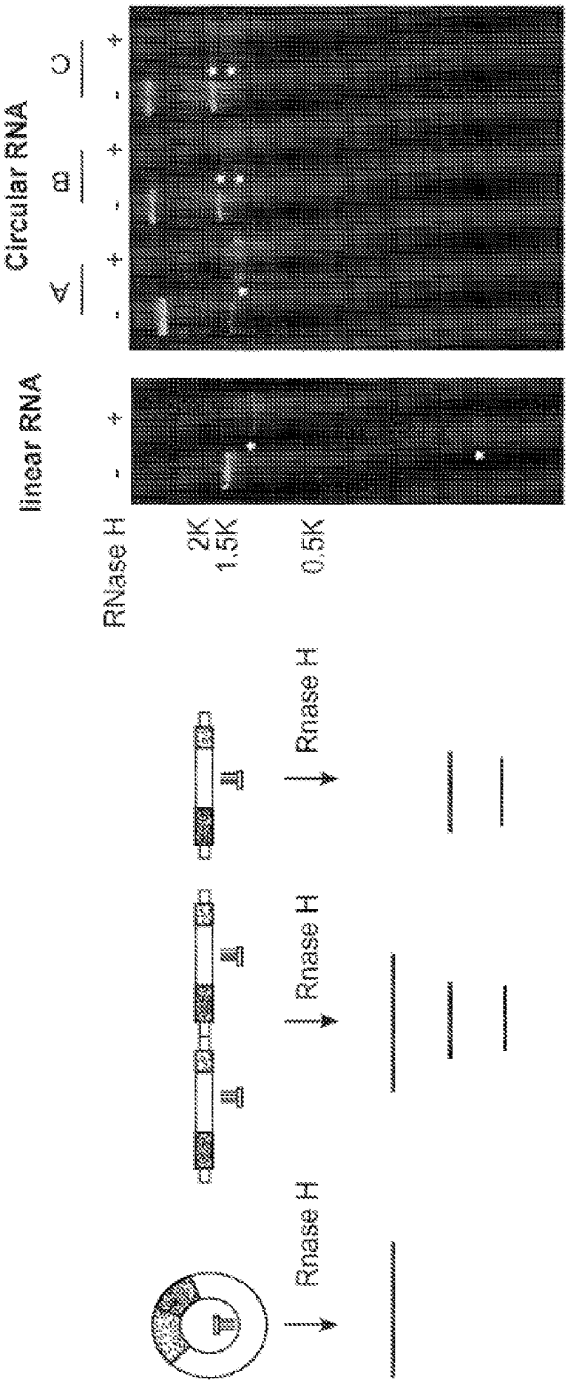

Circular RNA
A
B
C
linear RNA
RNase H
2K
1.5K
0.5K
Rnase H
Rnase H
Rnase H

FIG. 12

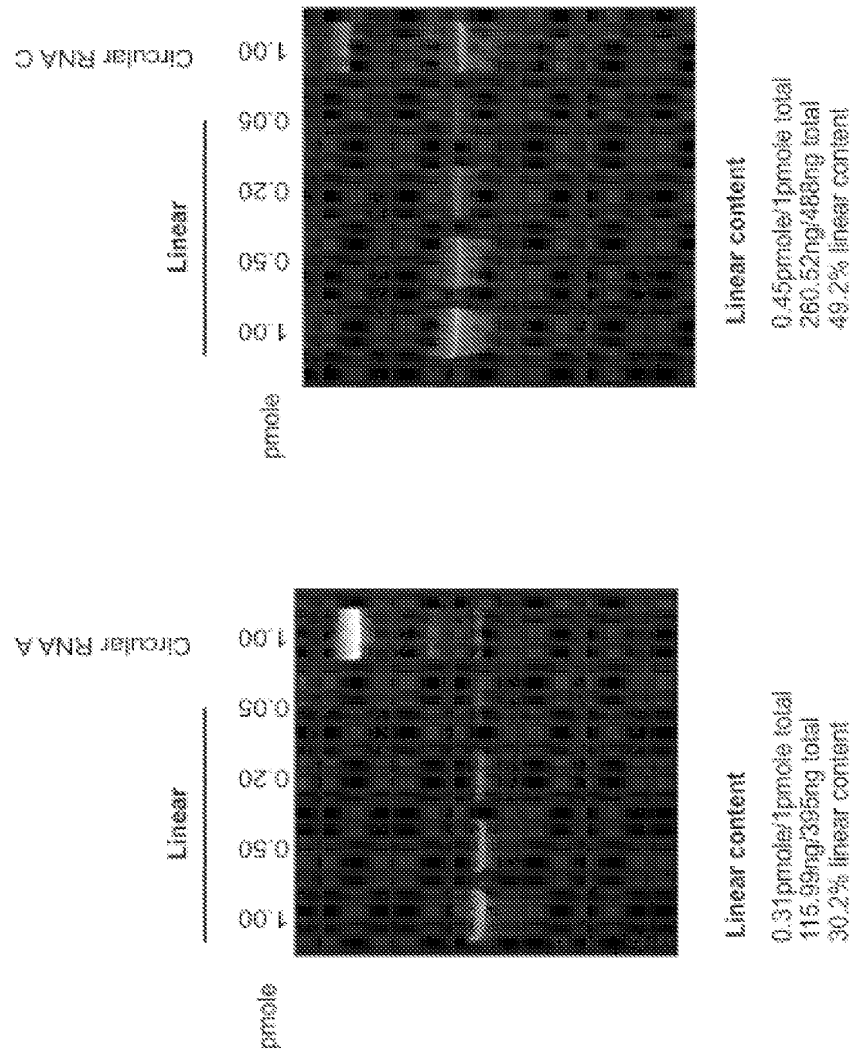

Circular RNA C
Linear
1.00
0.50
0.20
0.05
1.00
pmole
Linear content
0.45pmole/1pmole total
260.52ng/498ng total
49.2% linear content
Circular RNA A
Linear
1.00
0.50
0.20
0.05
1.00
pmole
Linear content
0.31pmole/1pmole total
115.69ng/385ng total
30.2% linear content

FIG. 13

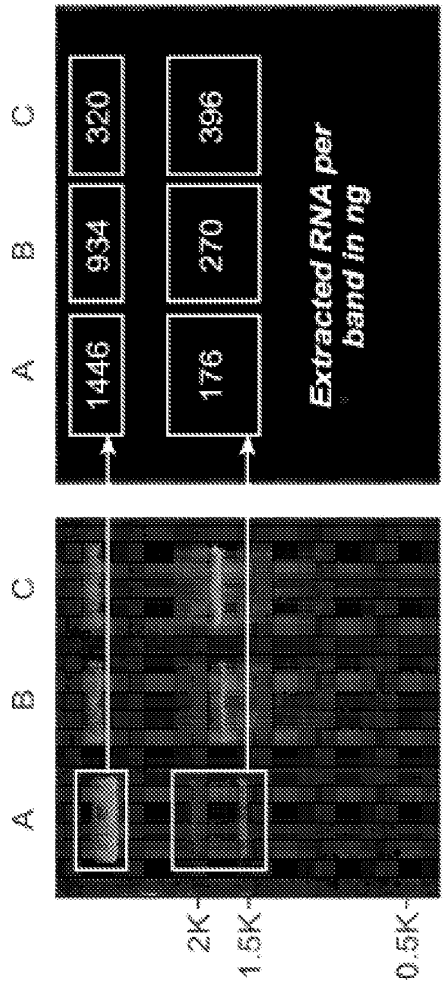

A
B
C
2K
1.5K
0.5K
A
B
C
1446
934
320
176
270
396
Extracted RNA per band in ng

FIG. 14

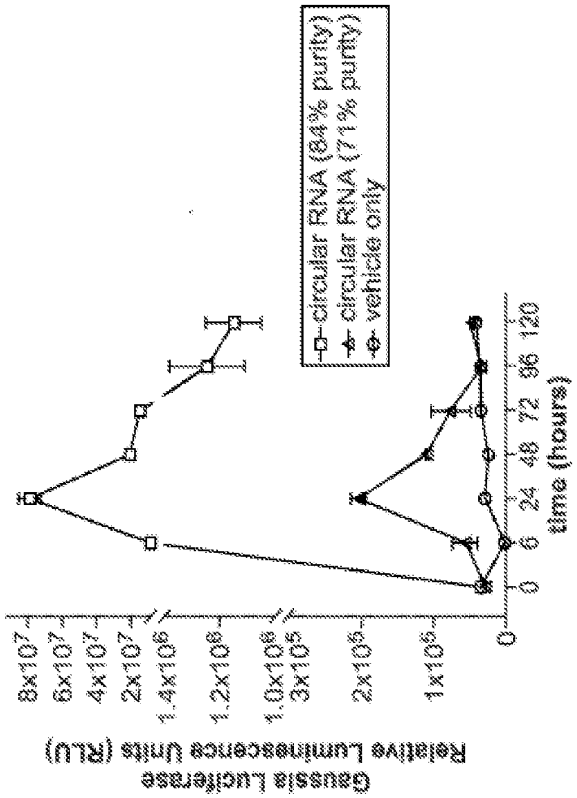

circular RNA (84% purity)
circular RNA (71% purity)
vehicle only
Gaussia Luciferase
Relative Luminescence Units (RLU)
8x10^7
6x10^7
4x10^7
2x10^7
1.4x10^6
1.2x10^6
1.0x10^6
3x10^5
2x10^5
1x10^5
0
0
6
24
48
72
96
120
time (hours)

FIG. 15

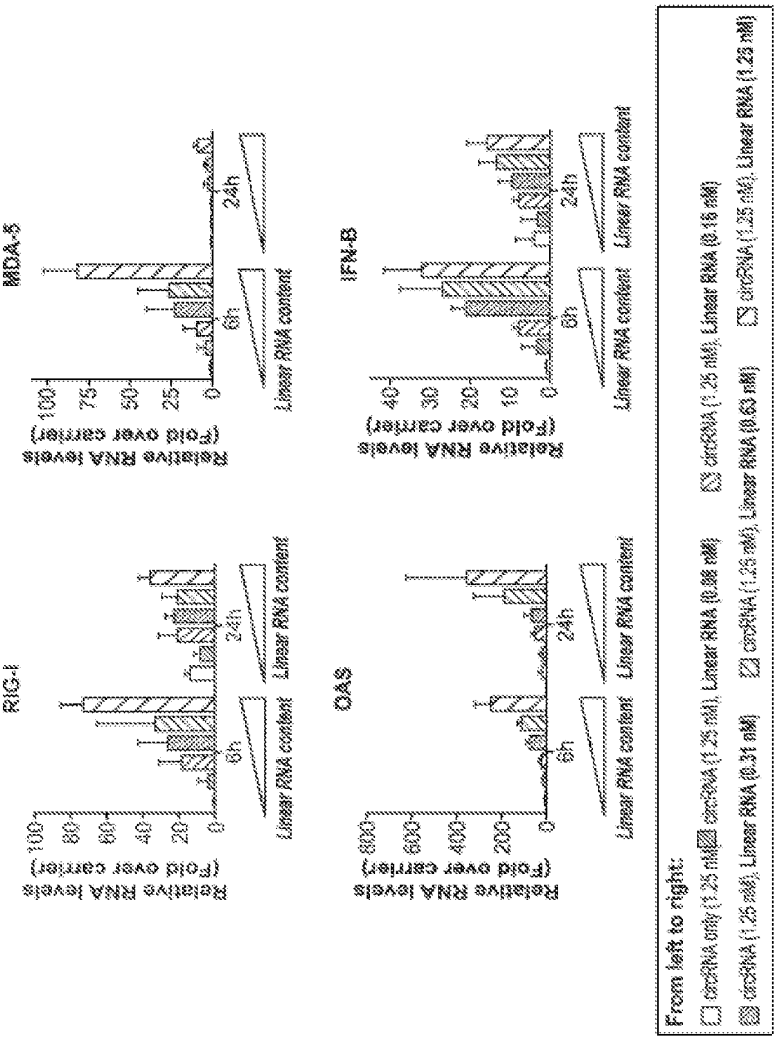

RIG-I
MDA-5
OAS
IFN-B
Relative RNA levels (Fold over carrier)
6h
24h
Linear RNA content
From left to right:
circRNA only (1.25 nM)
circRNA (1.25 nM), Linear RNA (0.86 nM)
circRNA (1.25 nM), Linear RNA (0.31 nM)
circRNA (1.25 nM), Linear RNA (0.63 nM)
circRNA (1.25 nM), Linear RNA (0.16 nM)
circRNA (1.25 nM), Linear RNA (1.23 nM)

FIG. 16

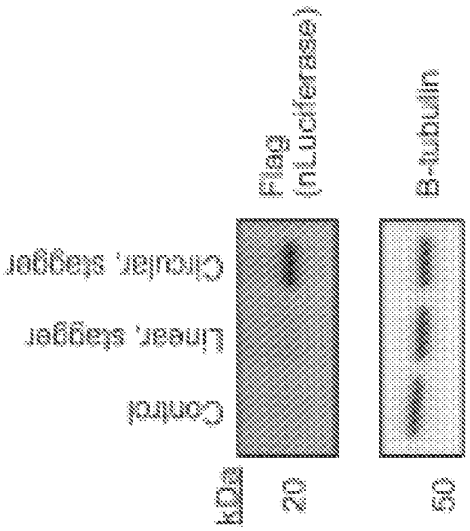

Flag
(nLuciferase)
β-tubulin
Circular, stagger
Linear, stagger
Control
kDa
20
50

FIG. 17

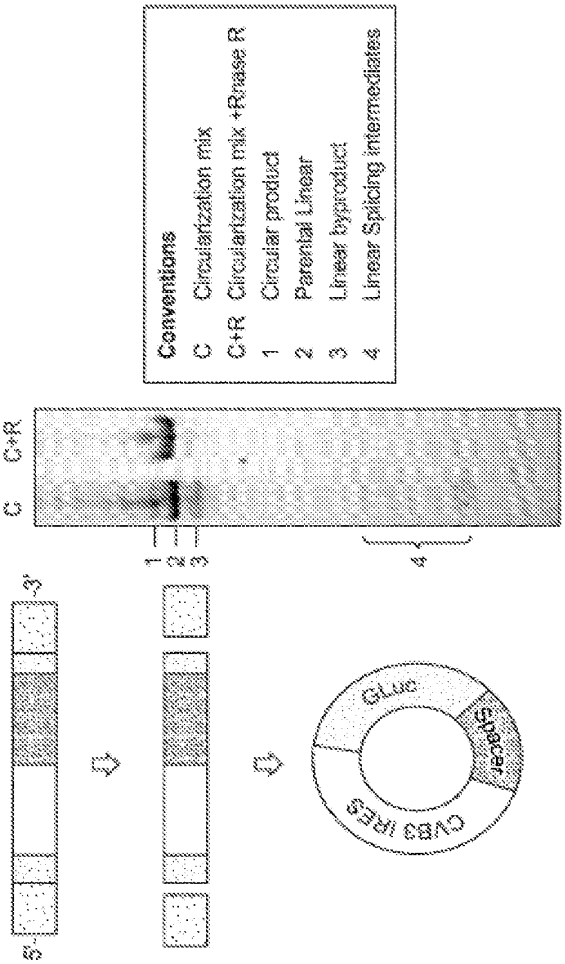

Conventions
C Circularization mix
C+R Circularization mix +Rnase R
1 Circular product
2 Parental Linear
3 Linear byproduct
4 Linear Splicing intermediates
C
C+R
1
2
3
4
5'
3'
GLuc
Secret.
CVB3 IRES

FIG. 18

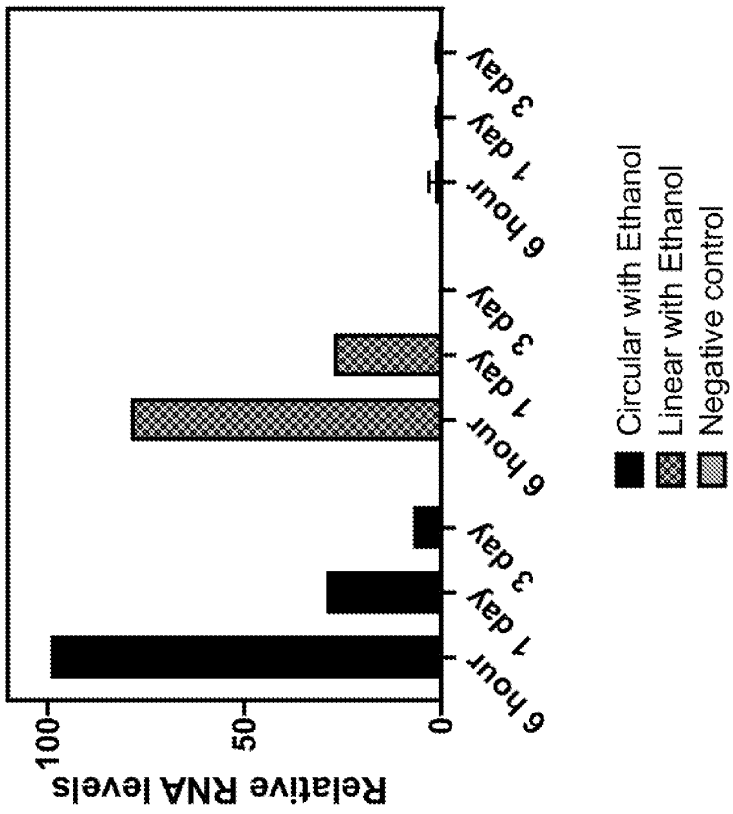

Relative RNA levels
100
50
0
6 hour
1 day
3 day
6 hour
1 day
3 day
6 hour
1 day
3 day
Circular with Ethanol
Linear with Ethanol
Negative control

FIG. 19A

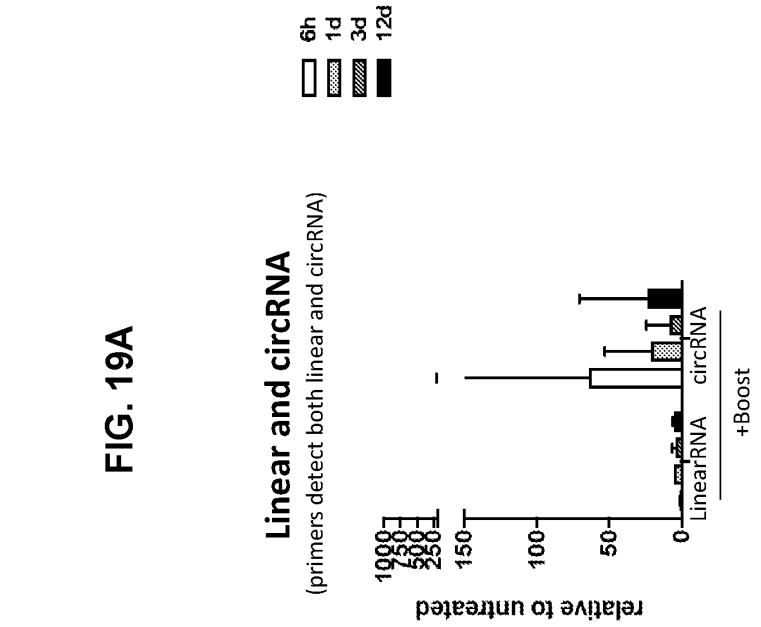


FIG. 19B

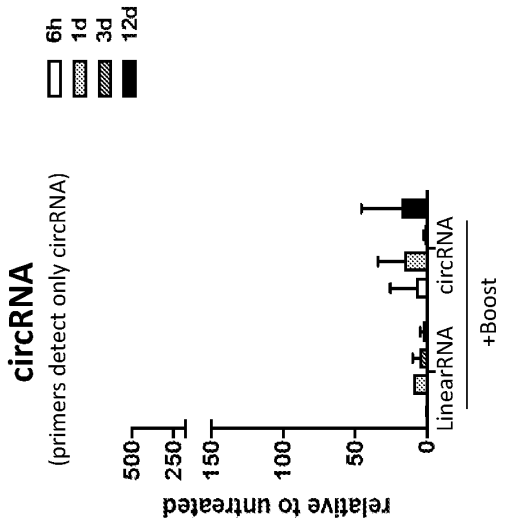


FIG. 20

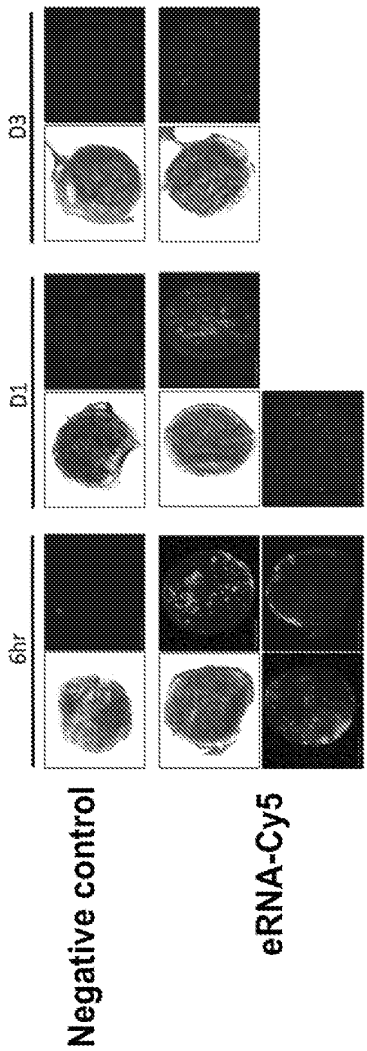


FIG. 21

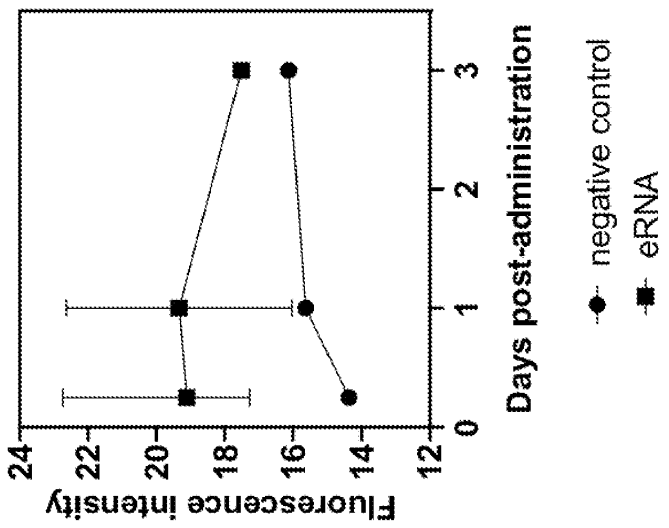
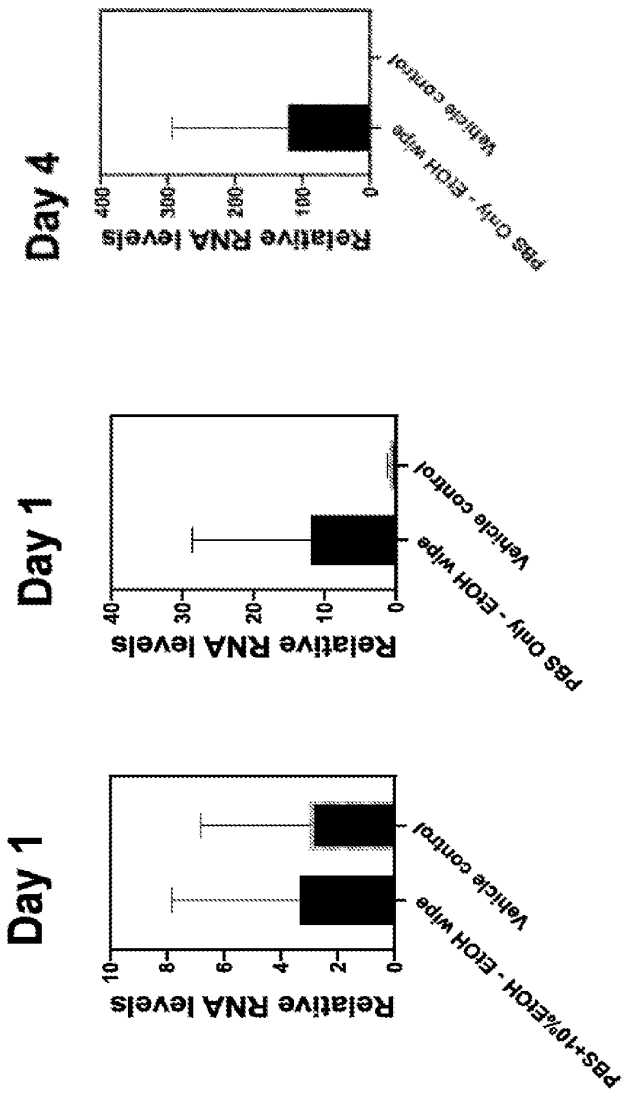

Fluorescence intensity
24
22
20
18
16
14
12
0
1
2
3
Days post-administration
negative control
eRNA

FIG. 22


Day 4
Relative RNA levels
400
300
200
100
0
PBS Only - EtOH wipe
Vehicle control
Day 1
Relative RNA levels
40
30
20
10
0
PBS Only - EtOH wipe
Vehicle control
Day 1
Relative RNA levels
10
8
6
4
2
0
PBS+10%EtOH - EtOH wipe
Vehicle control

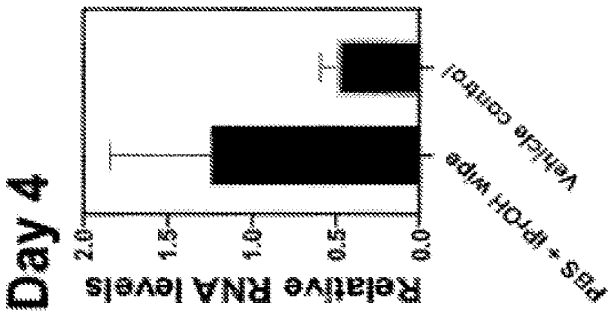

Day 4
Relative RNA levels
2.0
1.5
1.0
0.5
0.0
PBS + IPrOH wipe
Vehicle Control

FIG. 23

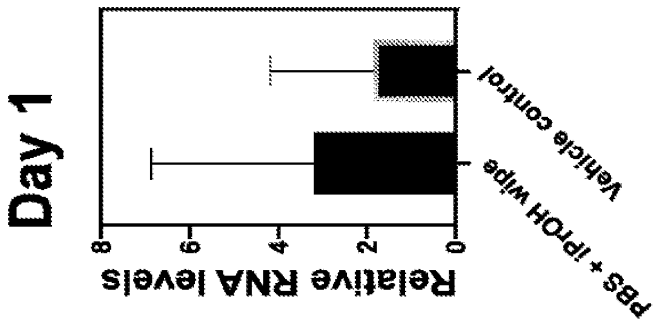

Day 1
Relative RNA levels
8
6
4
2
0
PBS + IPrOH wipe
Vehicle control

FIG. 24

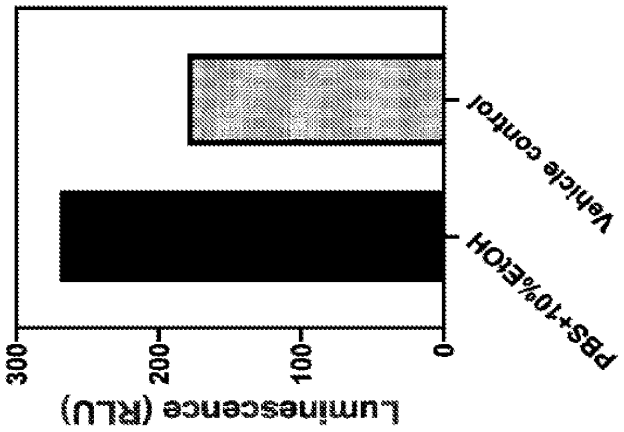


FIG. 25

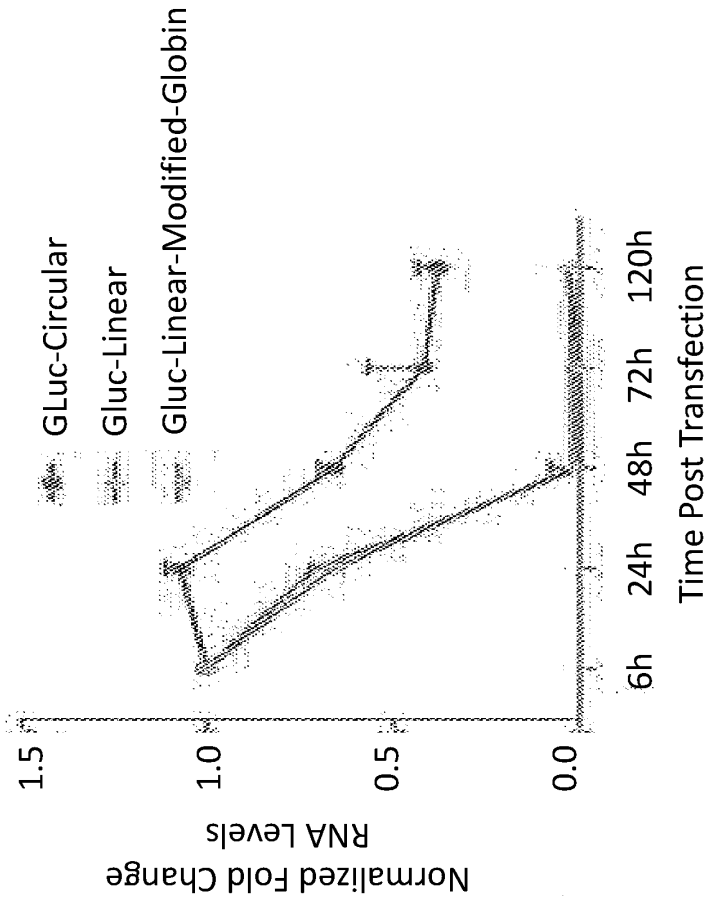

GLuc-Circular
Gluc-Linear
Gluc-Linear-Modified-Globin
1.5
1.0
0.5
0.0
Normalized Fold Change
RNA Levels
6h
24h
48h
72h
120h
Time Post Transfection

FIG. 26

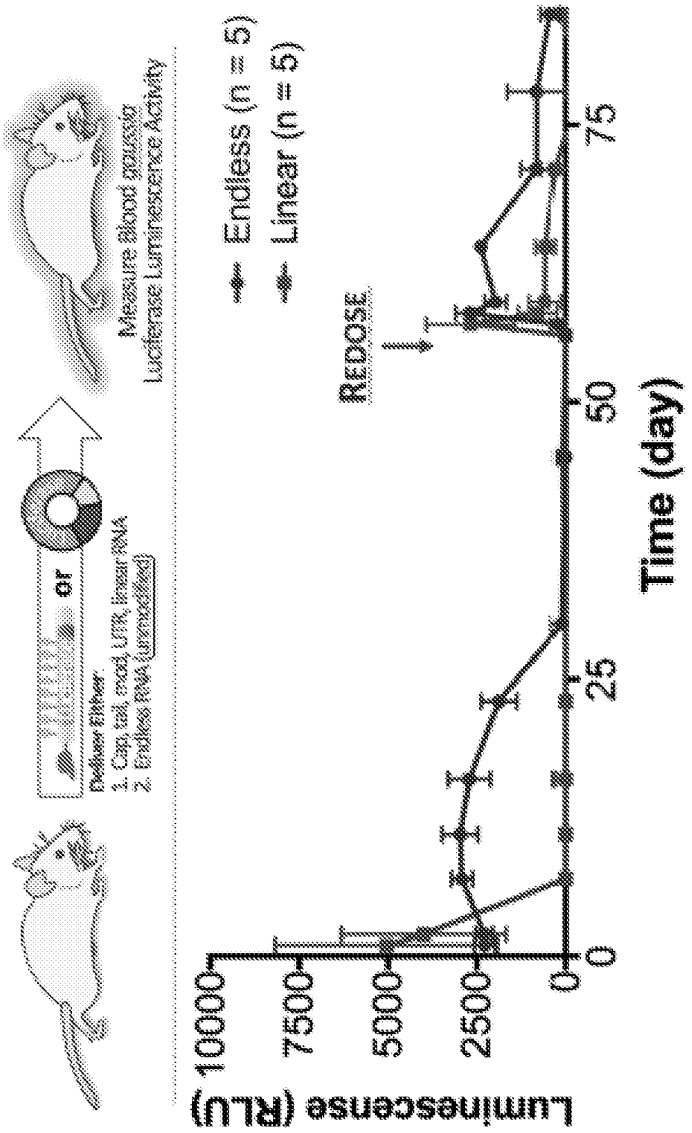

Deliver either
1. Cap, tail, mod, UTR, linear RNA
2. Endless RNA (unmodified)
or
Measure Blood gaussia
Luciferase Luminescence Activity
Endless (n = 5)
Linear (n = 5)
REDOSE
Luminescense (RLU)
10000
7500
5000
2500
0
0
25
50
75
Time (day)

FIG. 27

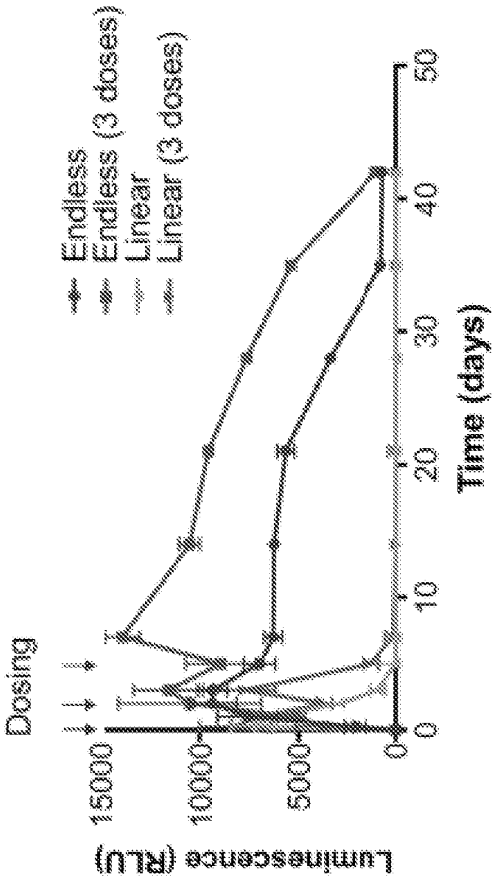

Endless
Endless (3 doses)
Linear
Linear (3 doses)
Dosing
15000
10000
5000
0
Luminescence (RLU)
0
10
20
30
40
50
Time (days)

FIG. 28

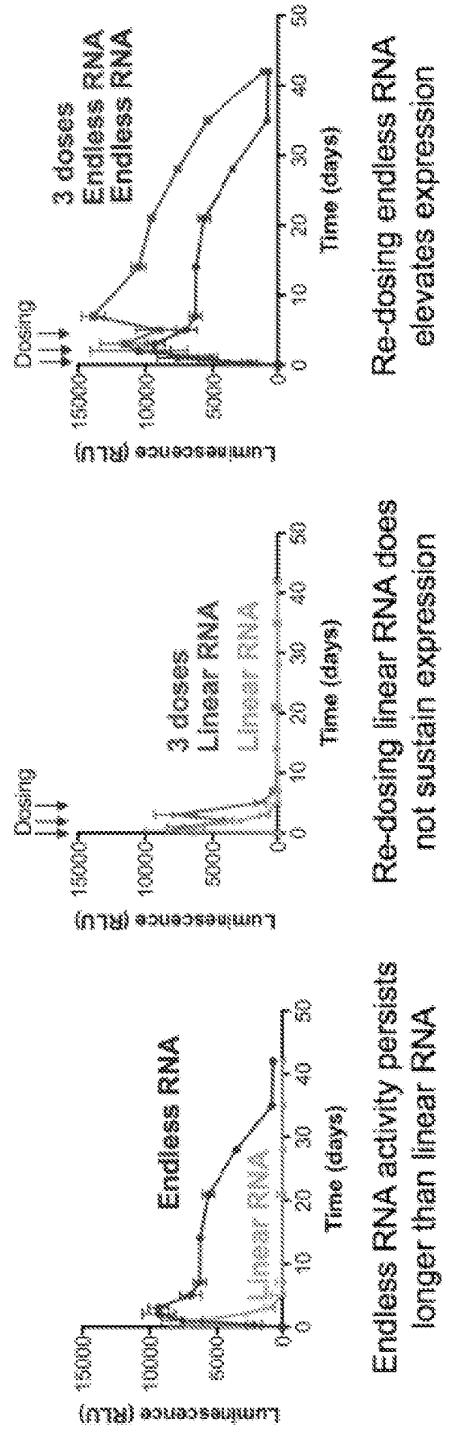

Endless RNA activity persists longer than linear RNA
Endless RNA
Linear RNA
Luminescence (RLU)
Time (days)
Re-dosing linear RNA does not sustain expression
3 doses
Linear RNA
Linear RNA
Dosing
Re-dosing endless RNA elevates expression
3 doses
Endless RNA
Endless RNA

FIG. 29

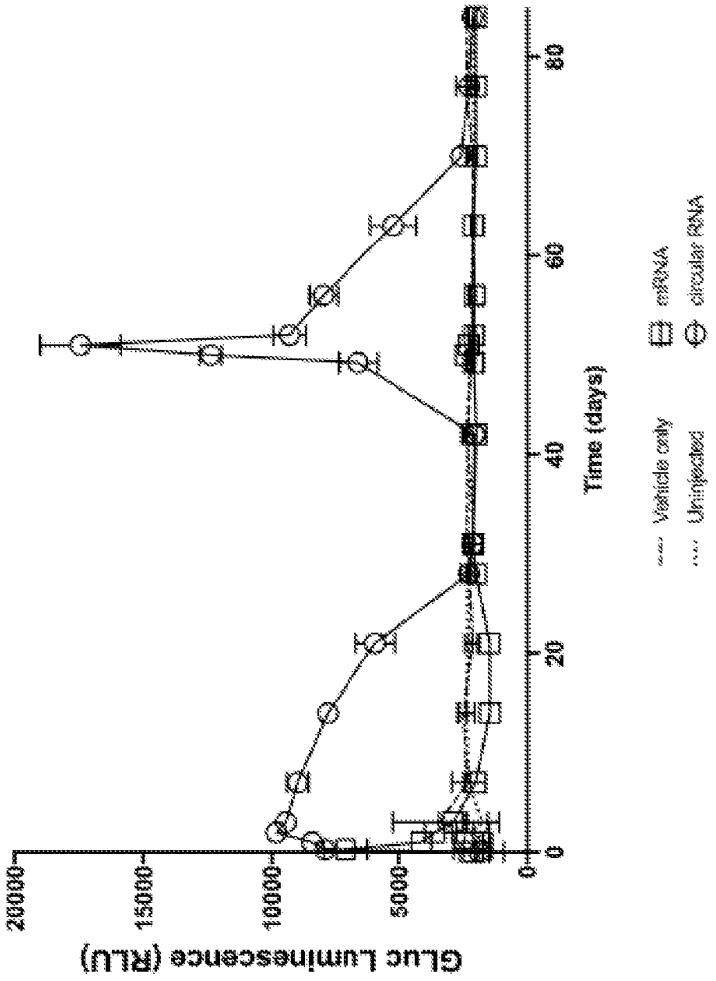

GLuc Luminescence (RLU)
20000
15000
10000
5000
0
0
20
40
60
80
Time (days)
Vehicle only
Uninjected
eRNA
circular RNA

FIG. 30

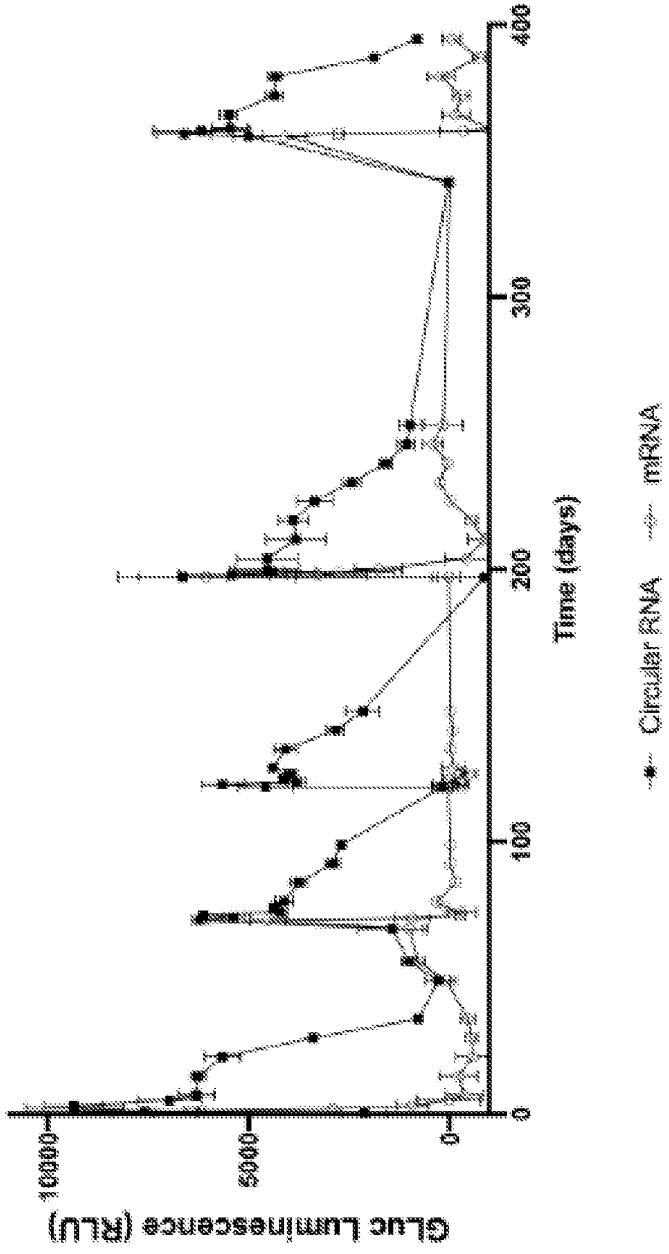

Gluc Luminescence (RLU)
10000
5000
0
0
100
200
300
400
Time (days)
Circular RNA
mRNA

FIG. 31B

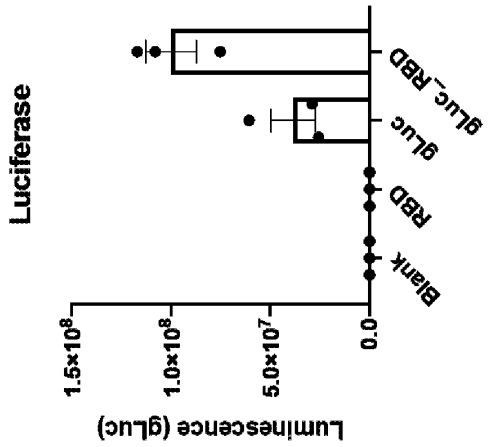


FIG. 31A

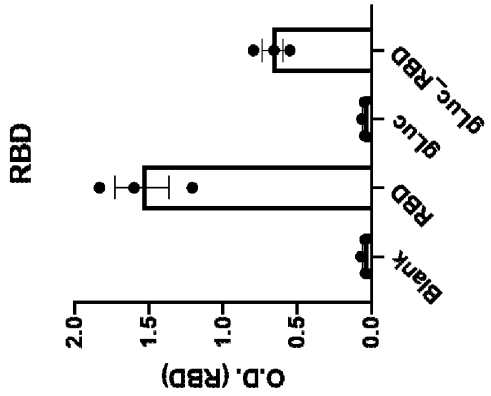


FIG. 32A

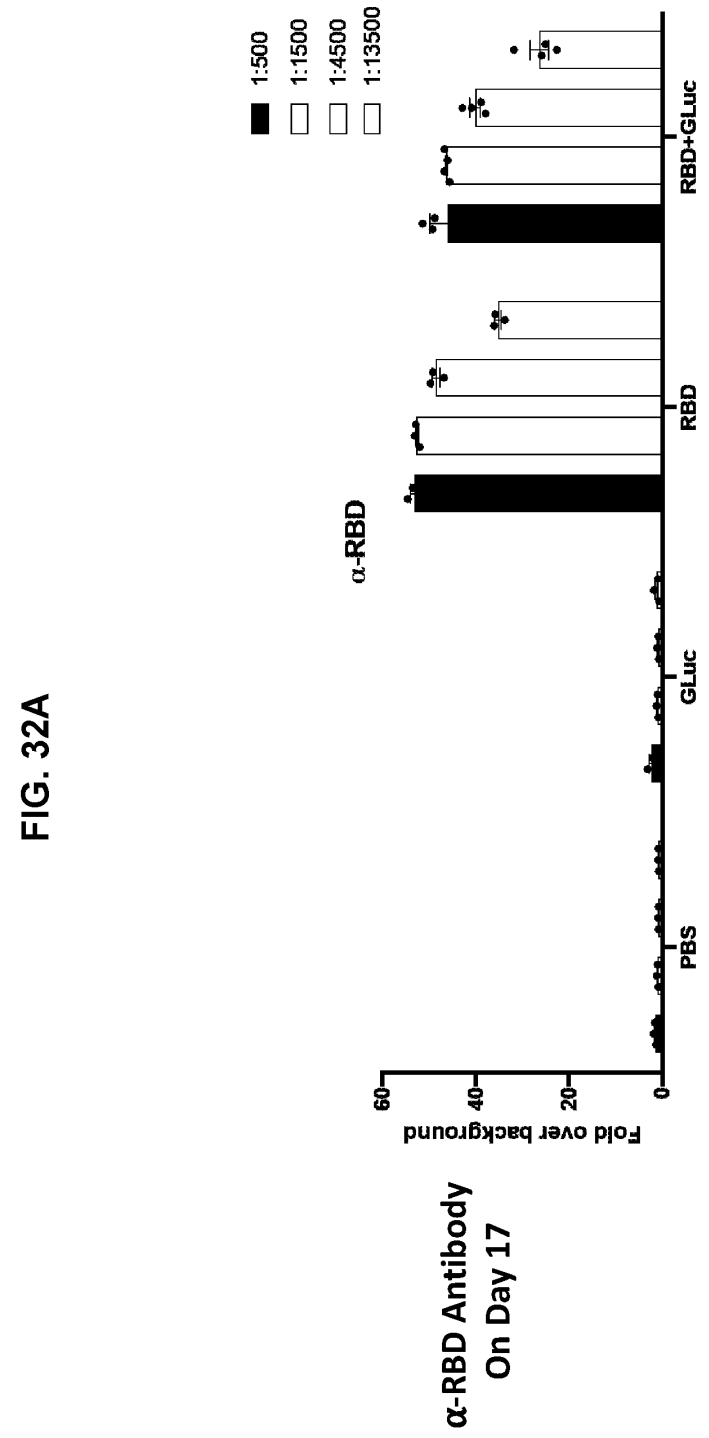

α-RBD Antibody
On Day 17
Fold over background
0
20
40
60
α-RBD
1:500
1:1500
1:4500
1:13500
PBS
GLuc
RBD
RBD+GLuc

FIG. 32B

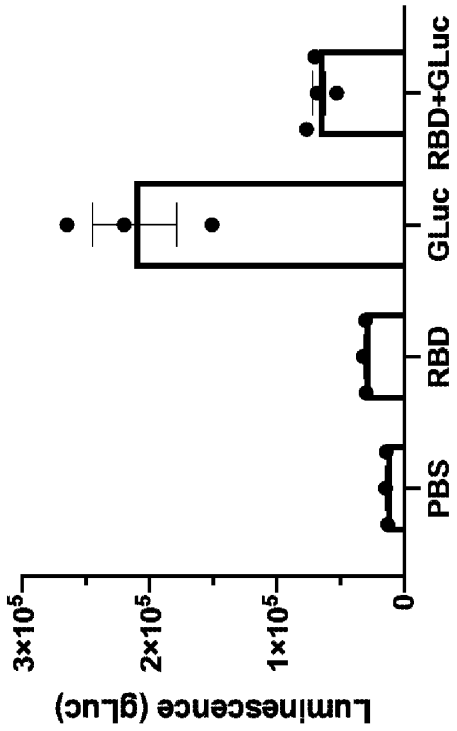

Luciferase
On Day 2
Luminescence (gLuc)
3×10^5
2×10^5
1×10^5
0
PBS
RBD
GLuc
RBD+GLuc

FIG. 33A

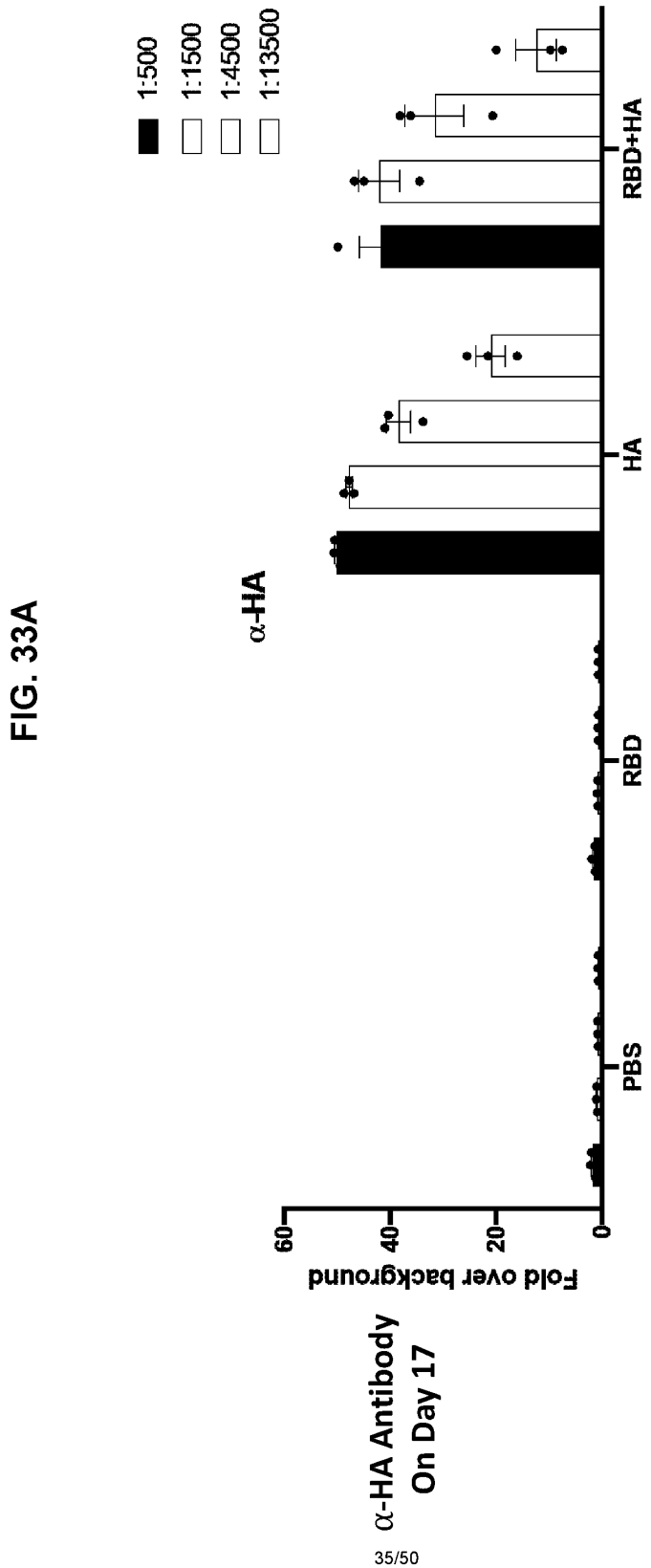

1:500
1:1500
1:4500
1:13500
α-HA
Fold over background
0
20
40
60
PBS
RBD
HA
RBD+HA
α-HA Antibody
On Day 17

FIG. 33B

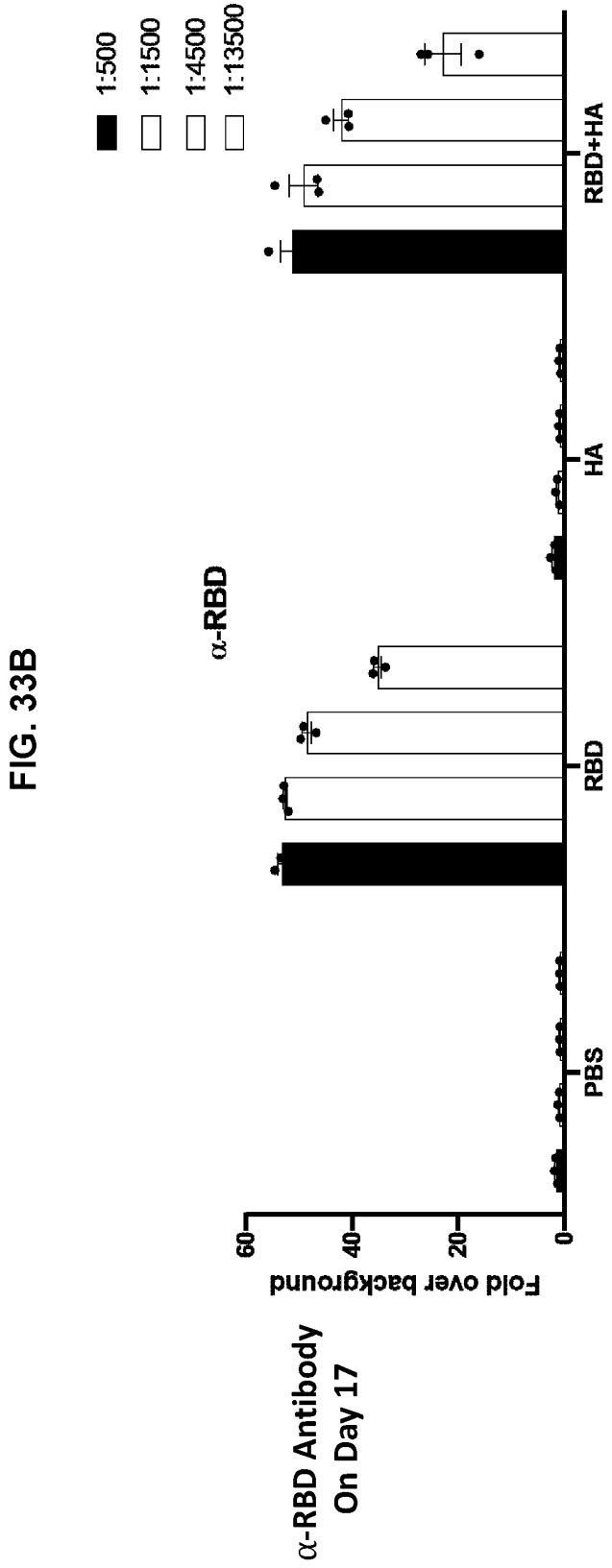

α-RBD Antibody
On Day 17
α-RBD
Fold over background
60
40
20
0
1:500
1:1500
1:4500
1:13500
PBS
RBD
HA
RBD+HA

FIG. 34A

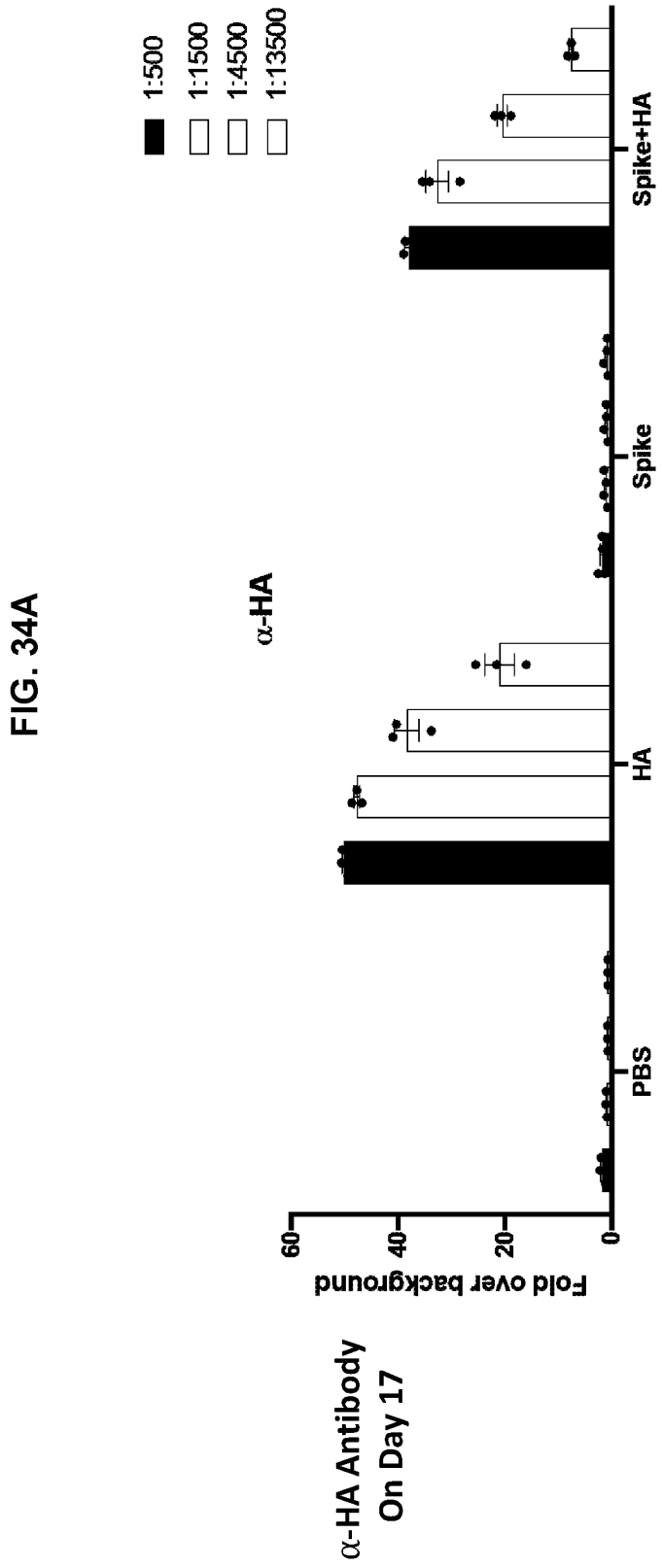

α-HA Antibody
On Day 17
α-HA
Fold over background
60
40
20
0
1:500
1:1500
1:4500
1:13500
PBS
HA
Spike
Spike+HA

FIG. 34B

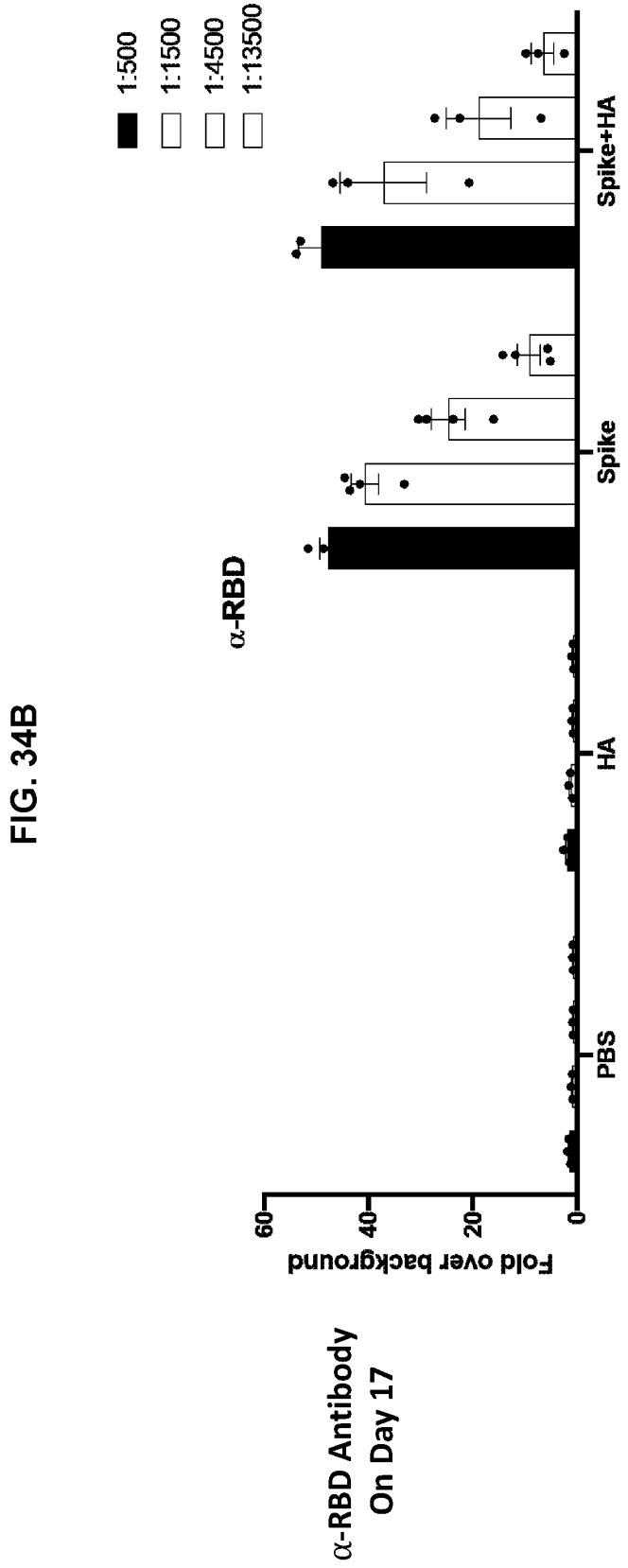

α-RBD Antibody
On Day 17
α-RBD
Fold over background
0
20
40
60
PBS
HA
Spike
Spike+HA
1:500
1:1500
1:4500
1:13500

FIG. 35

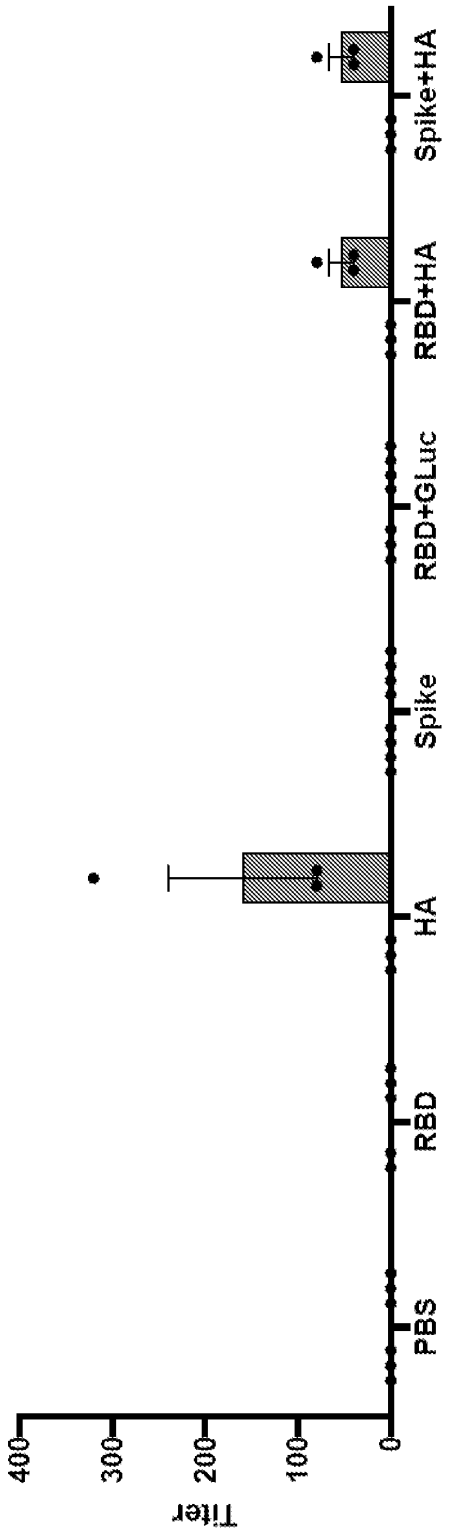

Day 2
Day 17
Titer
400
300
200
100
0
PBS
RBD
HA
Spike
RBD+GLuc
RBD+HA
Spike+HA

FIG. 36

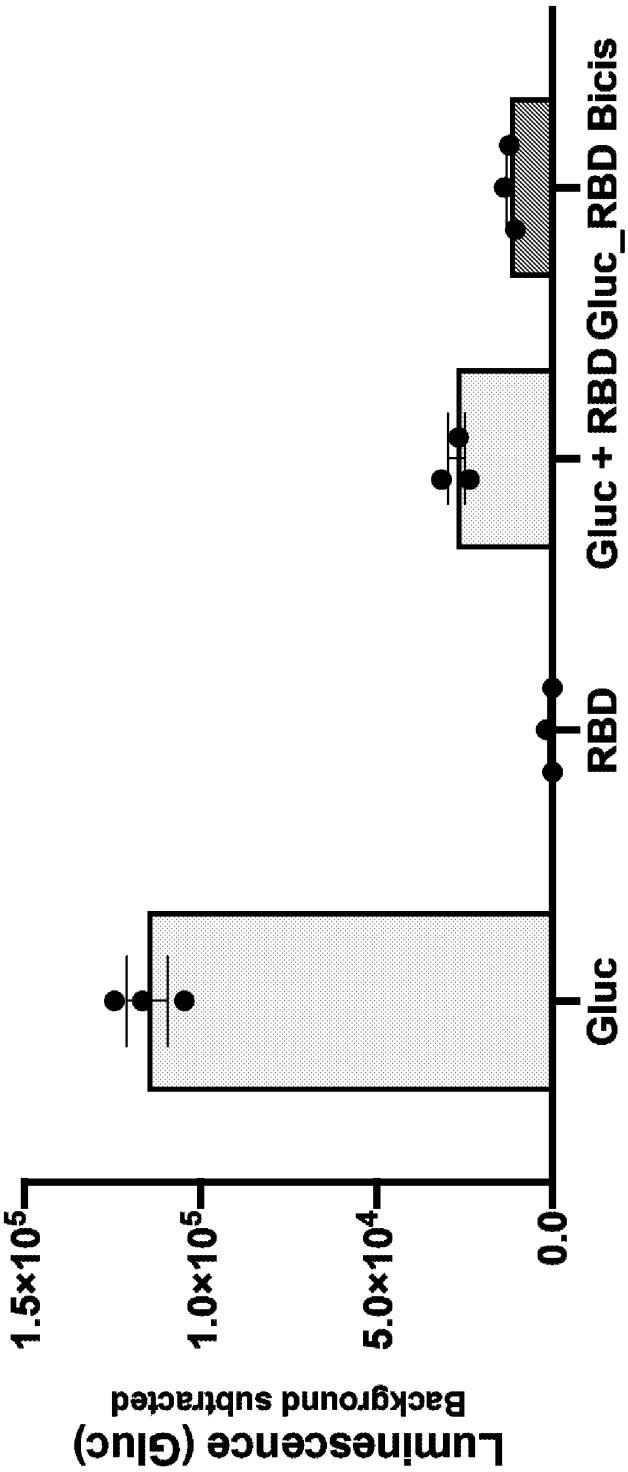
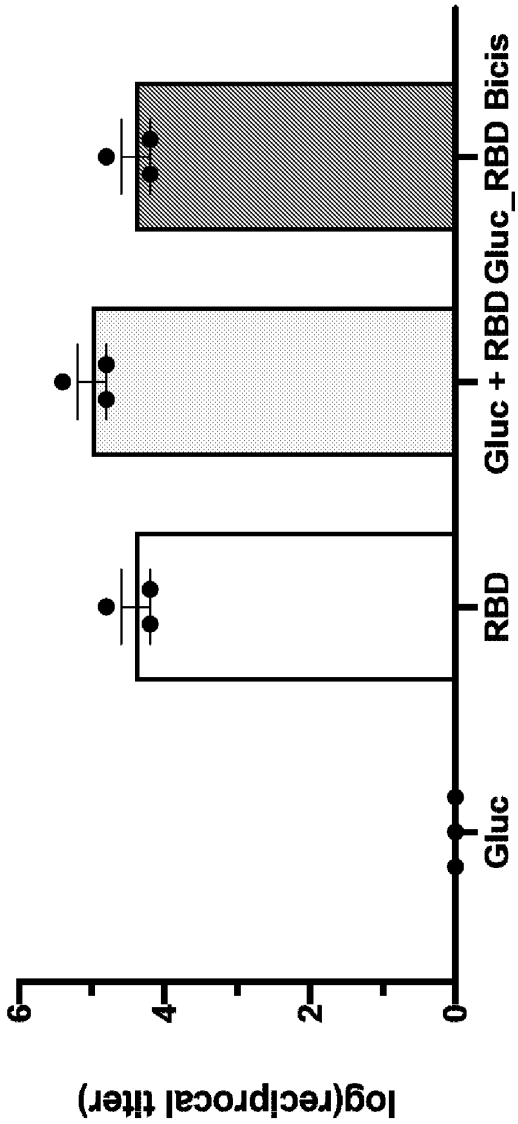

Luminescence (Gluc)
Background subtracted
1.5×10^5
1.0×10^5
5.0×10^4
0.0
Gluc
RBD
Gluc + RBD
Gluc_RBD Bicis

FIG. 37A



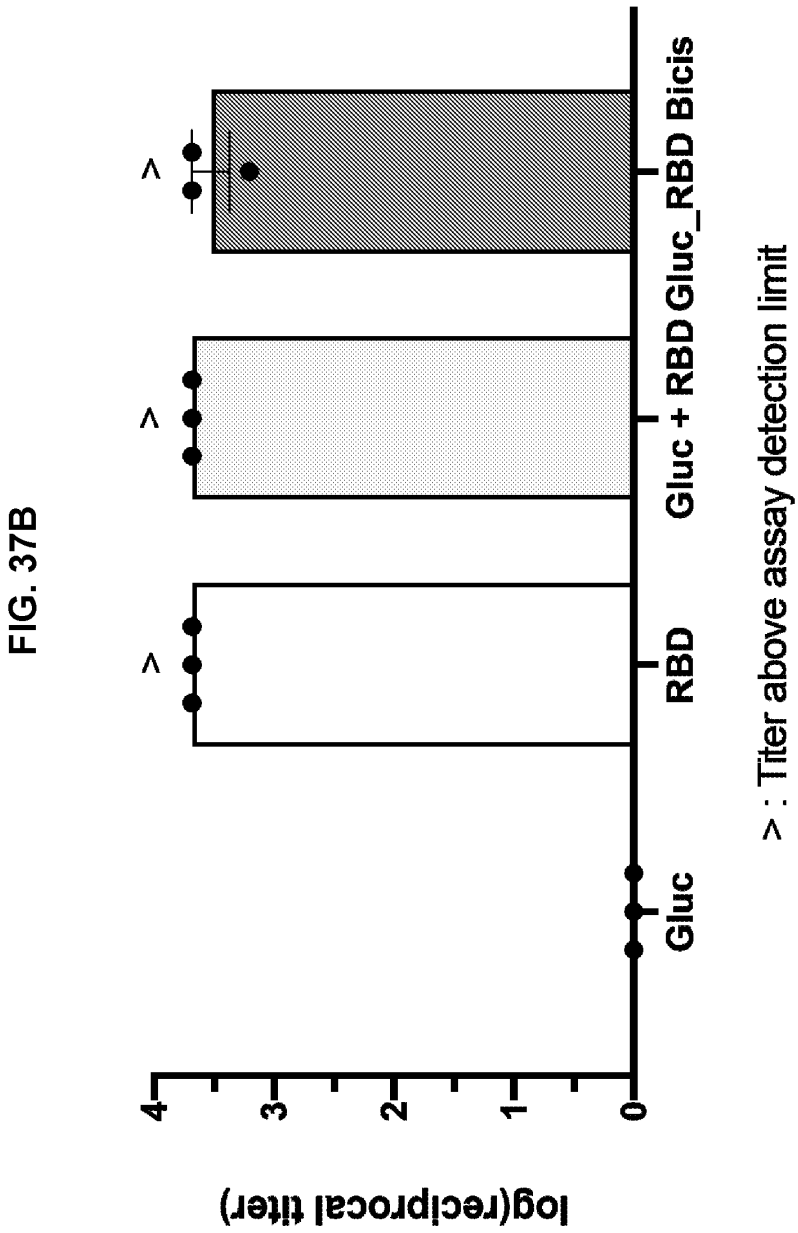

log(reciprocal titer)
4
3
2
1
0
Gluc
RBD
Gluc + RBD
Gluc_RBD Bicis
>
>
>
> : Titer above assay detection limit

FIG. 37B

FIG. 38

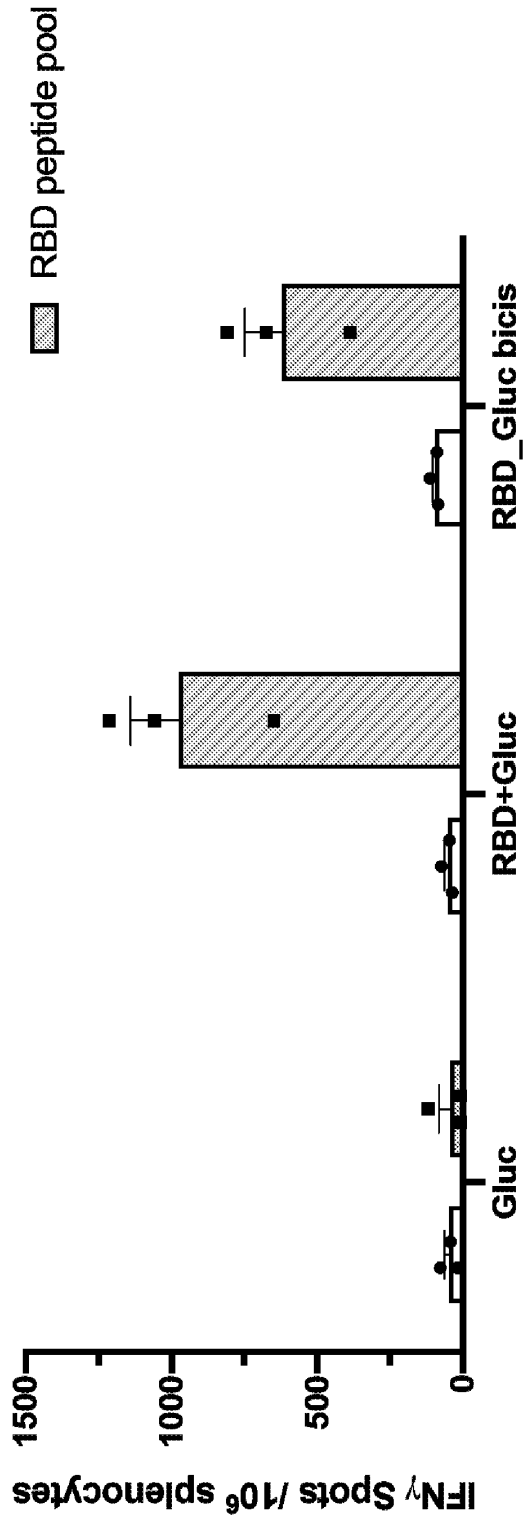

Unstimulated
RBD peptide pool
IFNγ Spots /10^6 splenocytes
1500
1000
500
0
Gluc
RBD+Gluc
RBD_Gluc bicis

FIG. 39

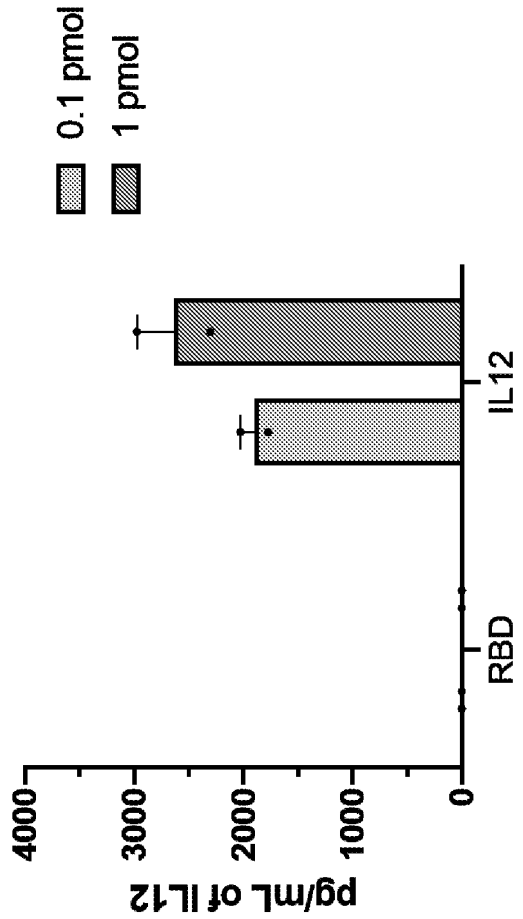


FIG. 40A

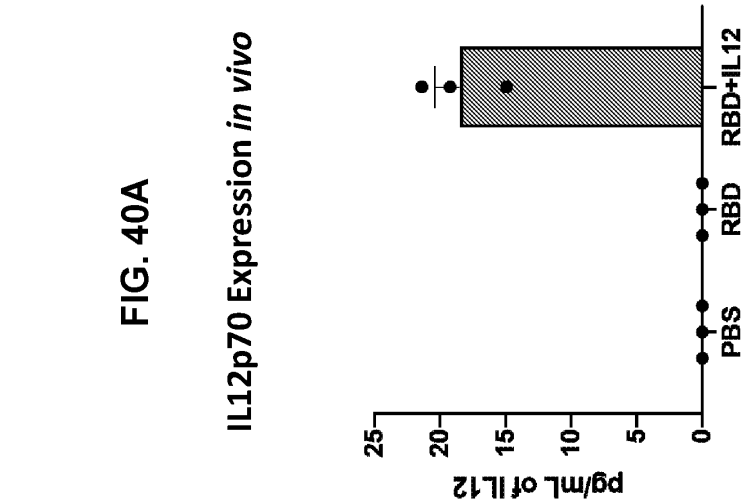


FIG. 40B

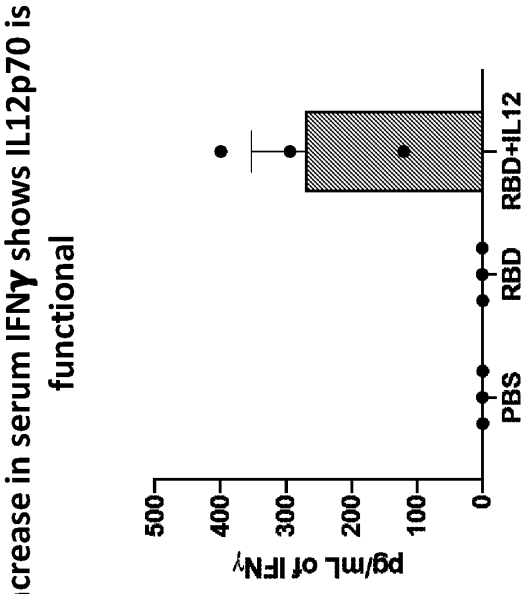


FIG. 41A

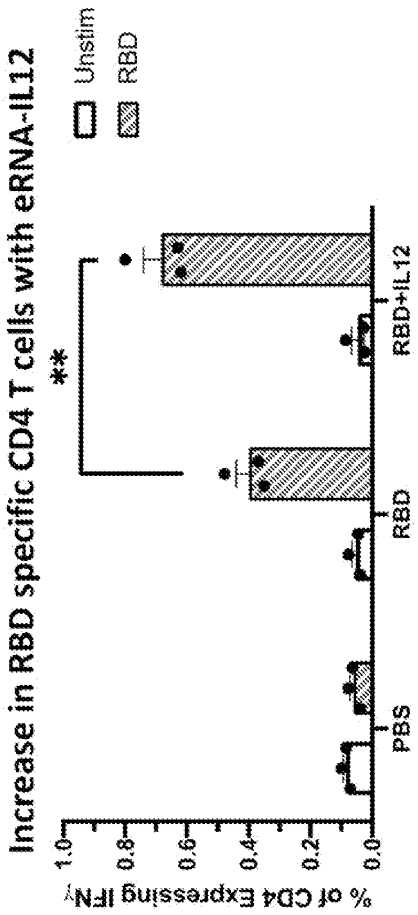

Increase in RBD specific CD4 T cells with eRNA-IL12
Unstim
RBD
**
% of CD4 Expressing IFNγ
1.0
0.8
0.6
0.4
0.2
0.0
PBS
RBD
RBD+IL12

FIG. 41B

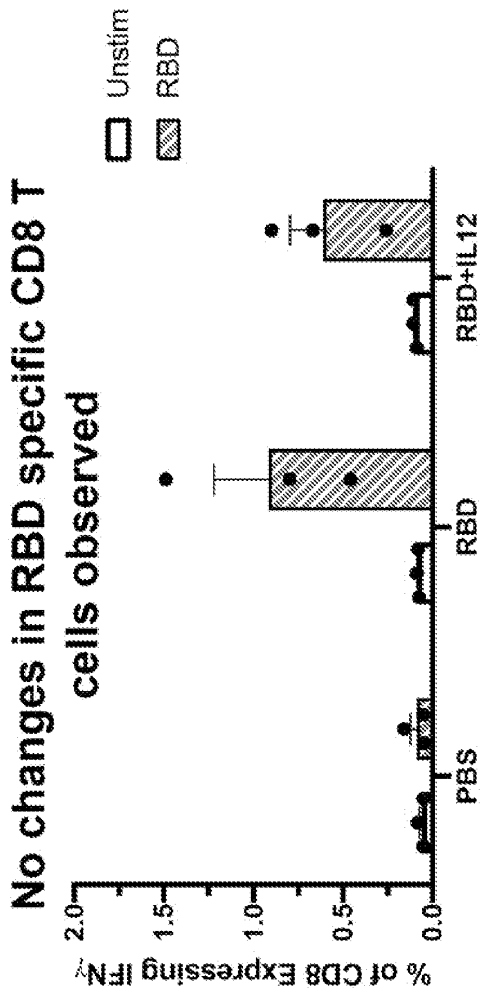

No changes in RBD specific CD8 T cells observed
Unstim
RBD
% of CD8 Expressing IFNγ
2.0
1.5
1.0
0.5
0.0
PBS
RBD
RBD+IL12

FIG. 41C

RBD specific CD4s express higher IFNγ with eRNA-IL12

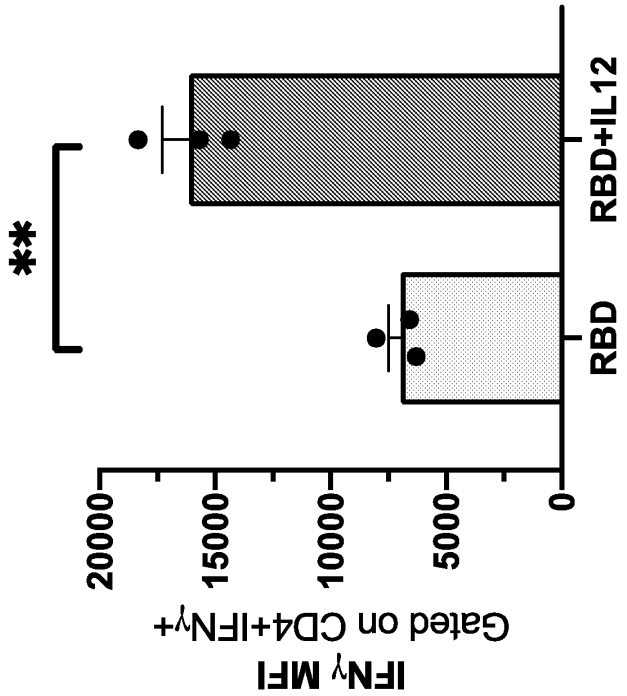


FIG. 41D

RBD specific CD8s express higher IFNγ with eRNA-IL12

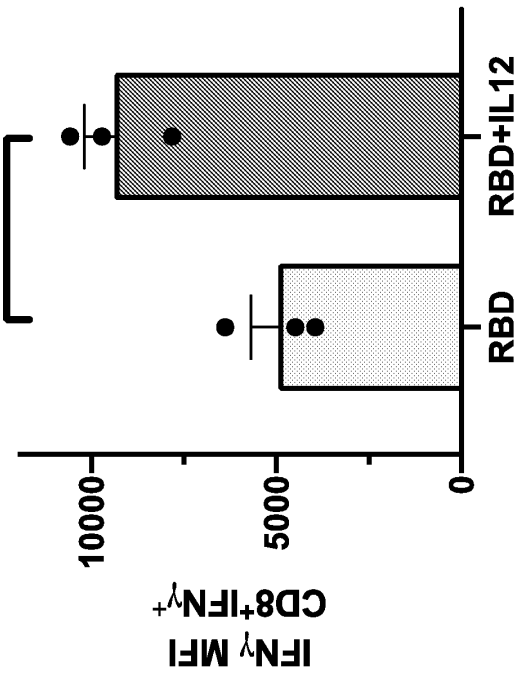
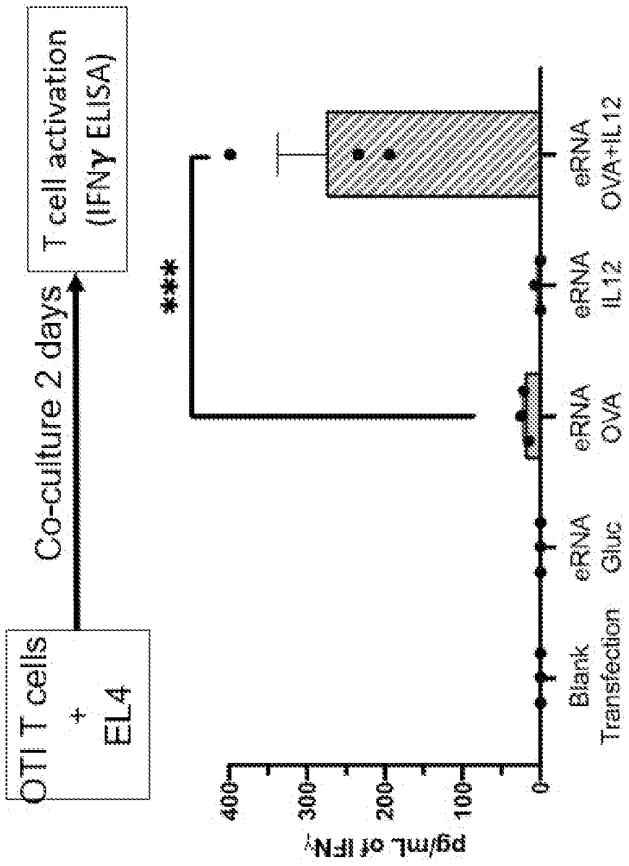


FIG. 42


OTI T cells
+
EL4
Co-culture 2 days
T cell activation
(IFNγ ELISA)
***
pg/mL of IFNγ
400
300
200
100
0
Blank
Transfection
eRNA
Gluc
eRNA
OVA
eRNA
IL12
eRNA
OVA+IL12