FIG. 1A

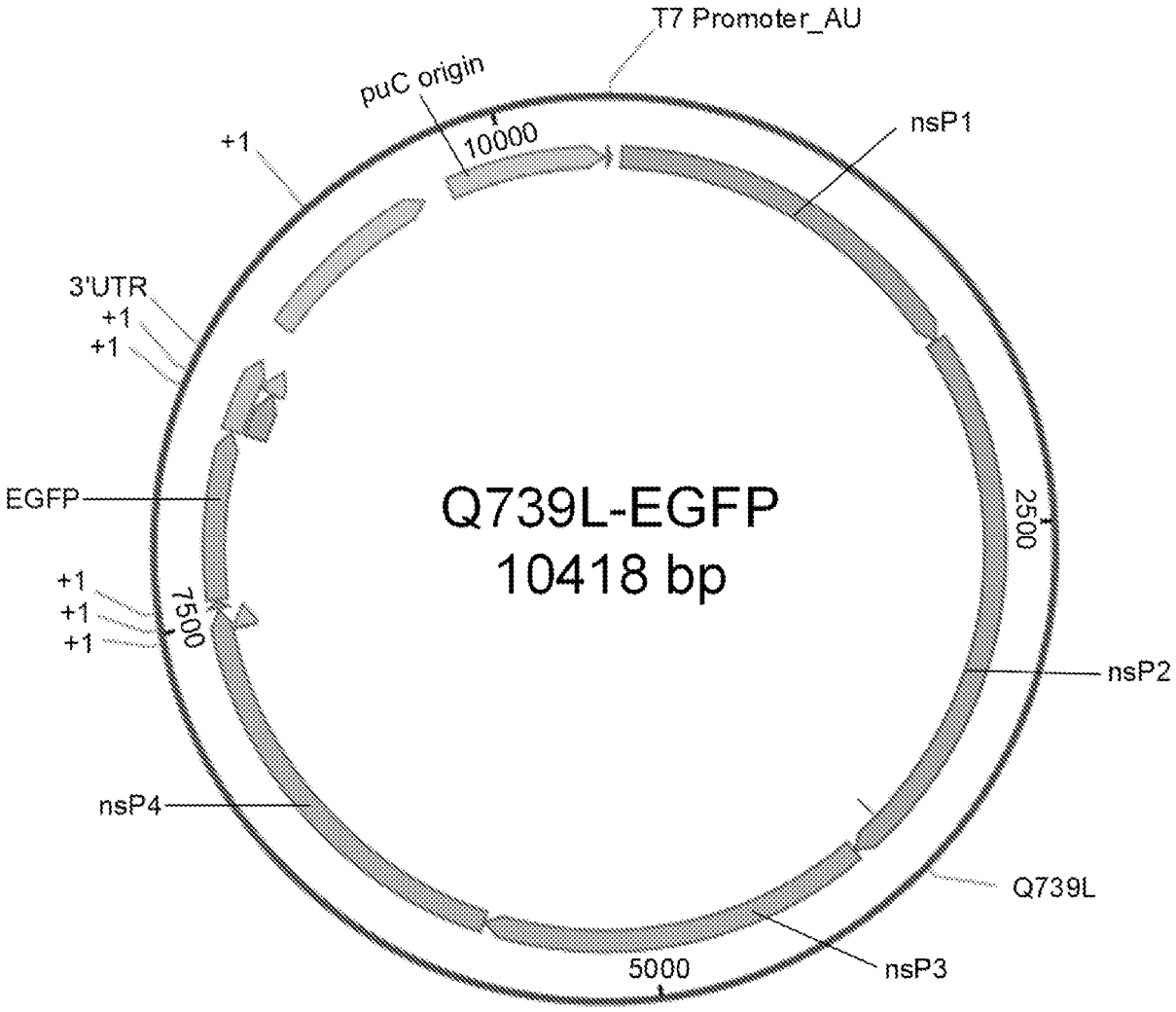

T7 Promoter_AU
puC origin
10000
+1
nsP1
3'UTR
+1
+1
EGFP
Q739L-EGFP
10418 bp
2500
+1
+1
+1
7500
nsP2
nsP4
Q739L
5000
nsP3

FIG. 1B

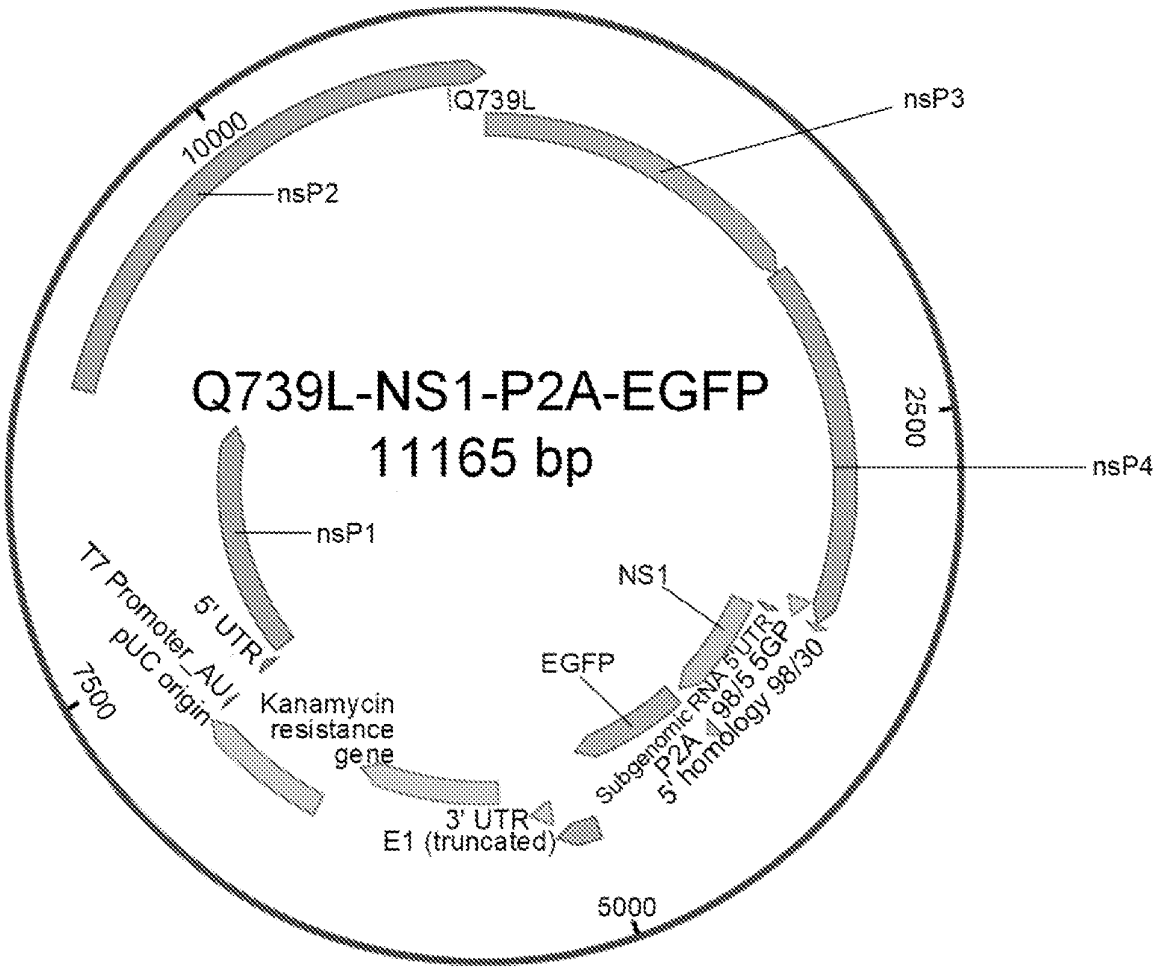

Q739L-NS1-P2A-EGFP
11165 bp
10000
2500
5000
7500
Q739L
nsP3
nsP2
nsP4
nsP1
NS1
5' UTR
T7 Promoter_AU
pUC origin
Kanamycin resistance gene
EGFP
Subgenomic RNA 5' UTR
P2A
98/5 5GP
5' homology 98/30
3' UTR
E1 (truncated)

FIG. 2A Strand-mCherry

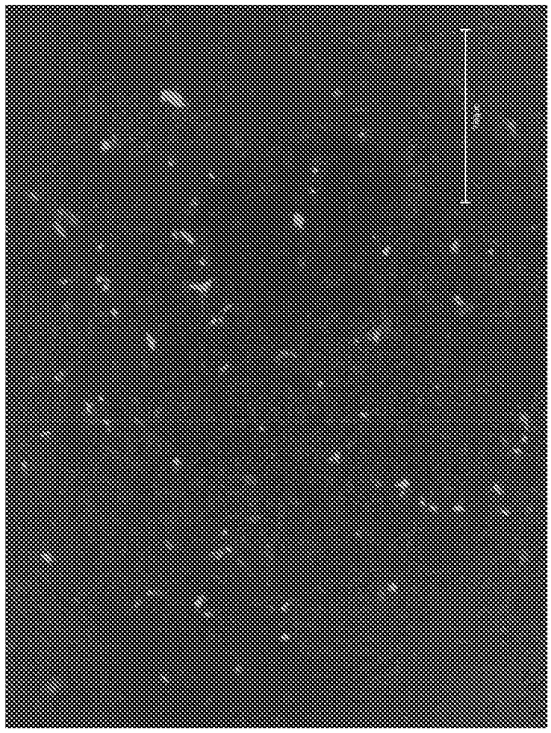


FIG. 2B mCherry modRNA

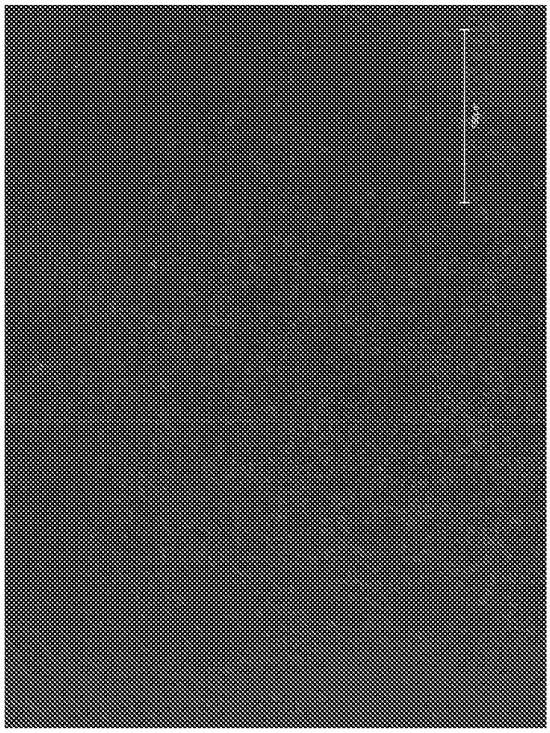


FIG. 2C Strand-mCherry + NS1 modRNA

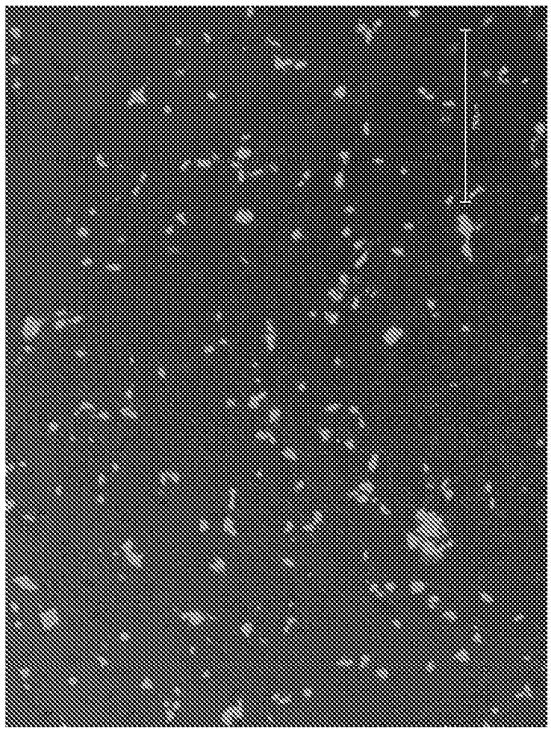


FIG. 2D

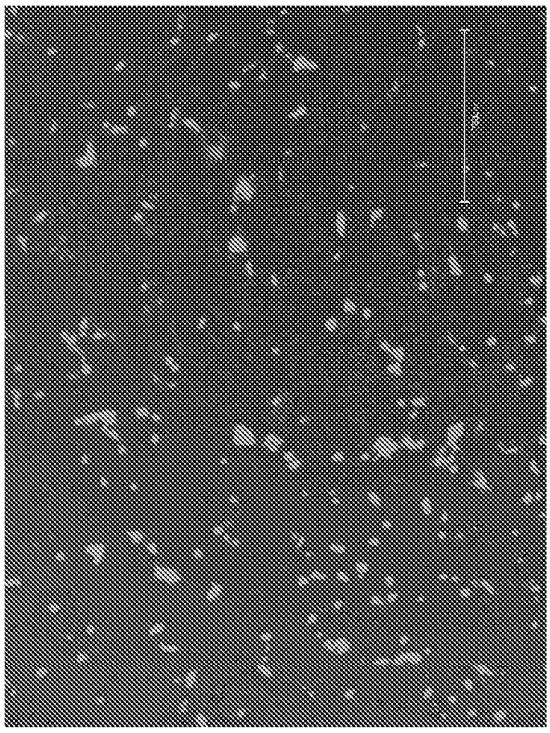


FIG. 2E

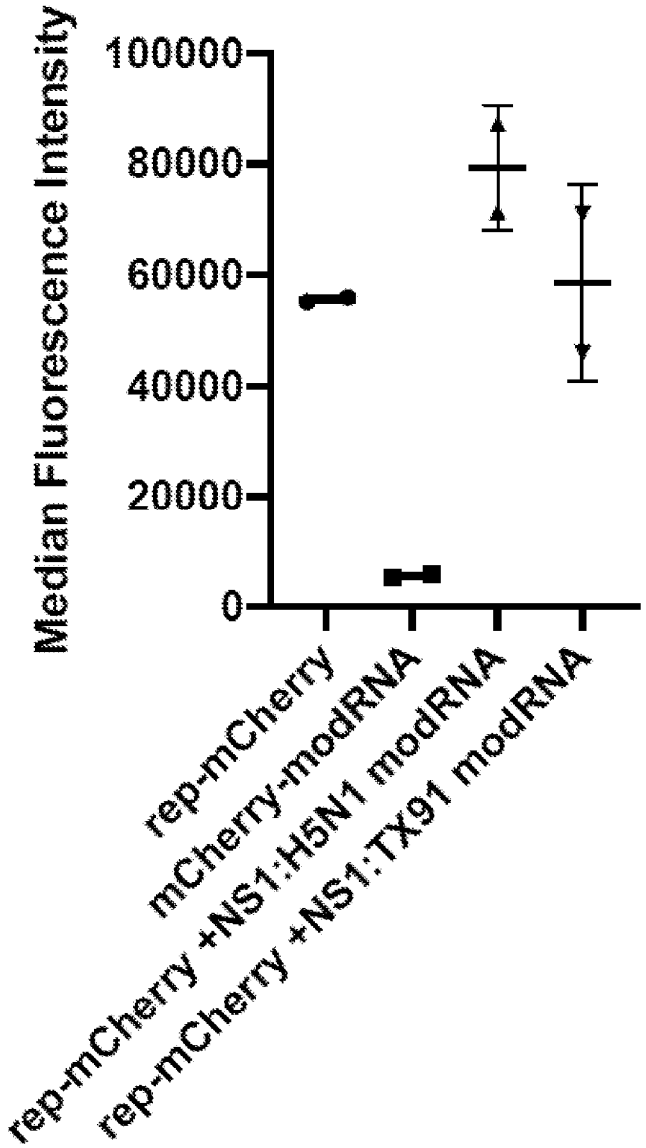

Median Fluorescence Intensity
100000
80000
60000
40000
20000
0
rep-mCherry
mCherry-modRNA
rep-mCherry +NS1:H5N1 modRNA
rep-mCherry +NS1:TX91 modRNA

FIG. 3A mCherry modRNA

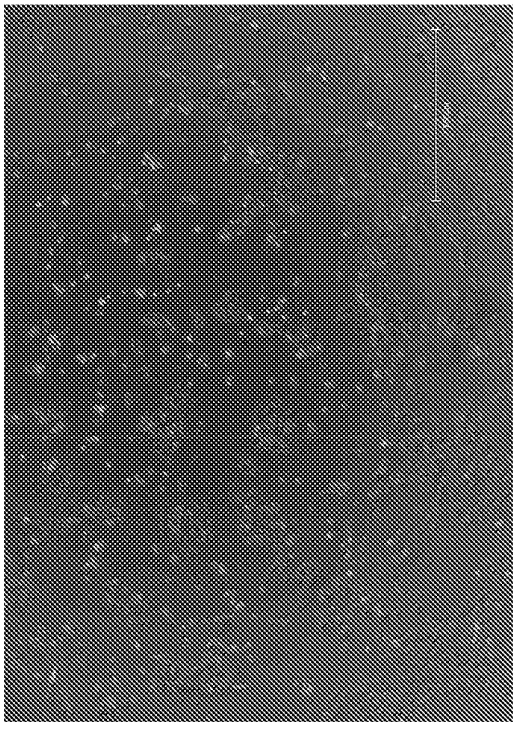


FIG. 3B Strand-mCherry

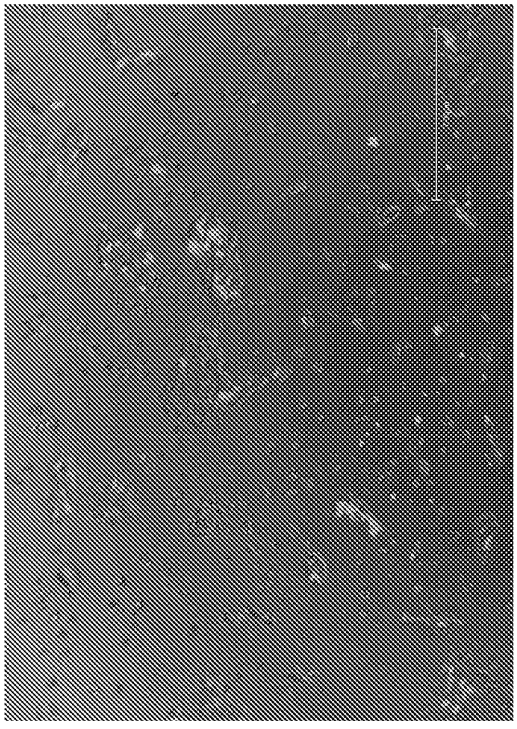


FIG. 3C Strand-mCherry + NS1 modRNA

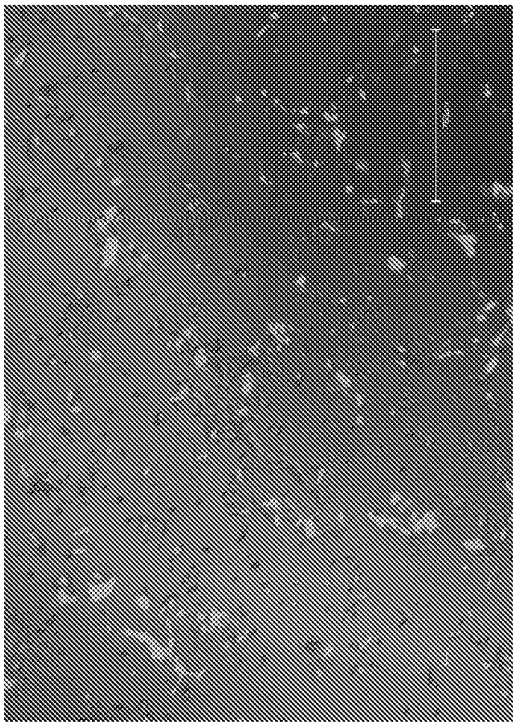


FIG. 3D

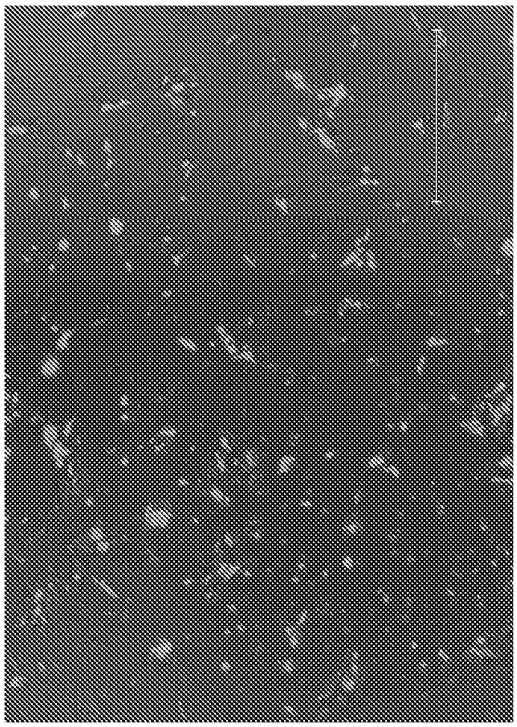


FIG. 3E

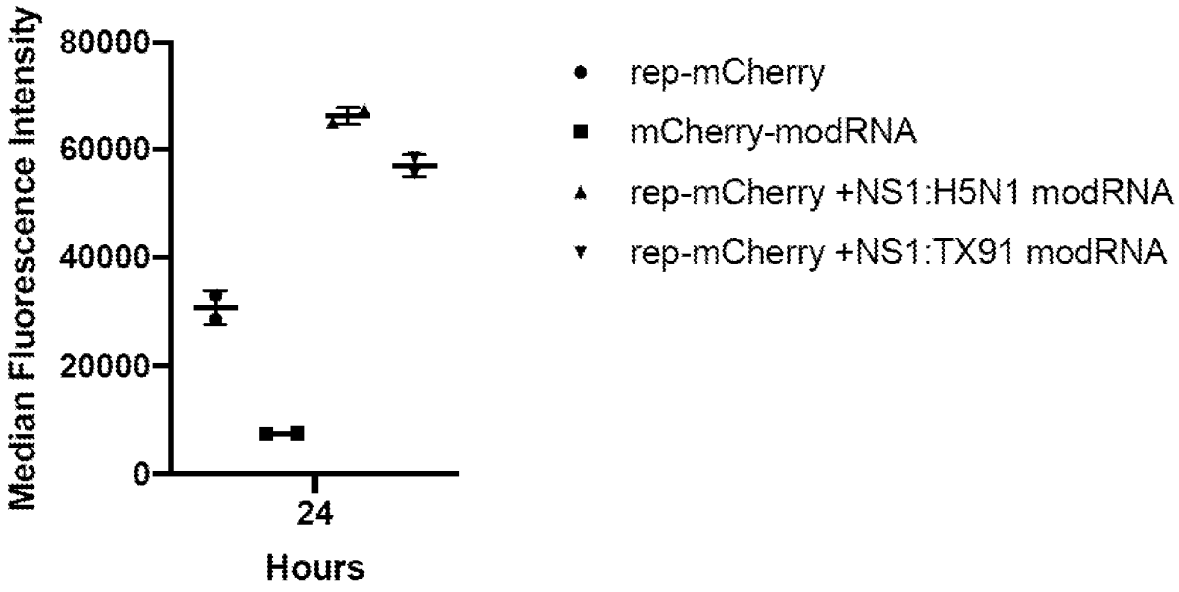

Median Fluorescence Intensity
80000
60000
40000
20000
0
24
Hours
rep-mCherry
mCherry-modRNA
rep-mCherry +NS1:H5N1 modRNA
rep-mCherry +NS1:TX91 modRNA

FIG. 3F

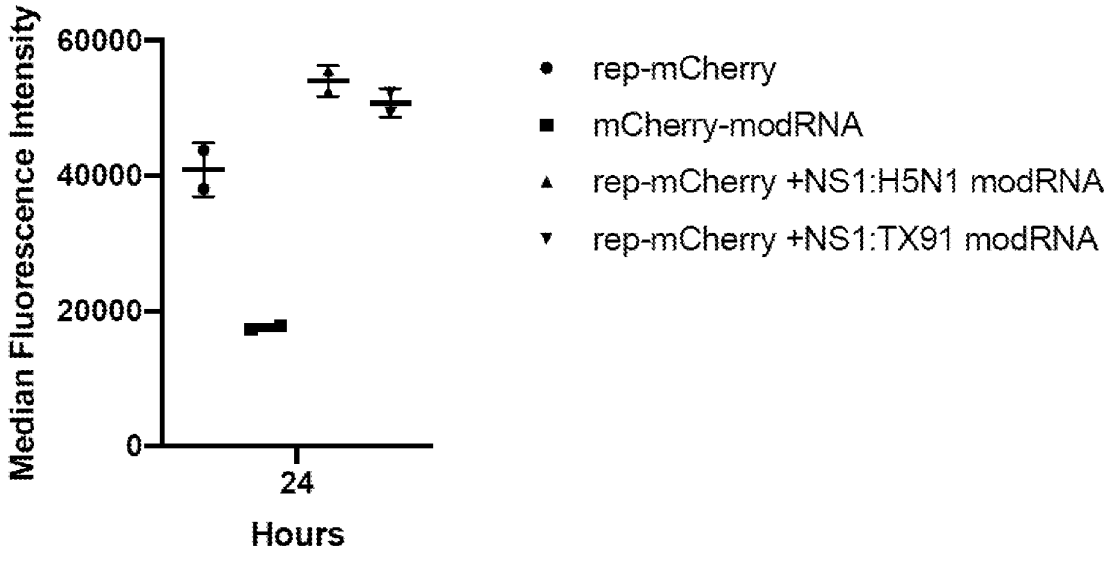

Median Fluorescence Intensity
60000
40000
20000
0
24
Hours
rep-mCherry
mCherry-modRNA
rep-mCherry +NS1:H5N1 modRNA
rep-mCherry +NS1:TX91 modRNA

FIG. 4A

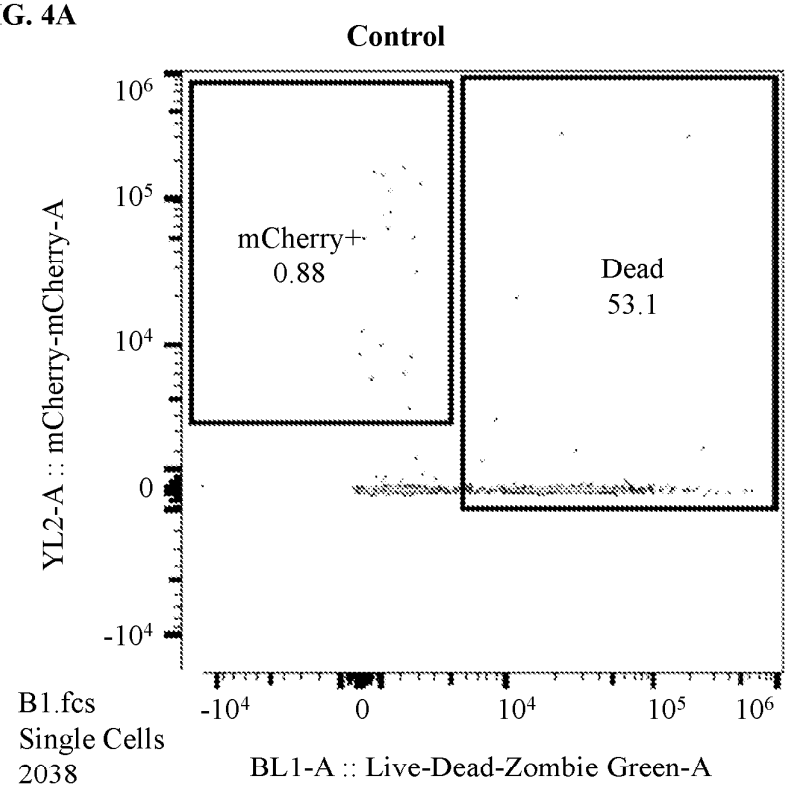

Control
mCherry+
0.88
Dead
53.1
YL2-A :: mCherry-mCherry-A
BL1-A :: Live-Dead-Zombie Green-A
B1.fcs
Single Cells
2038

FIG. 4B

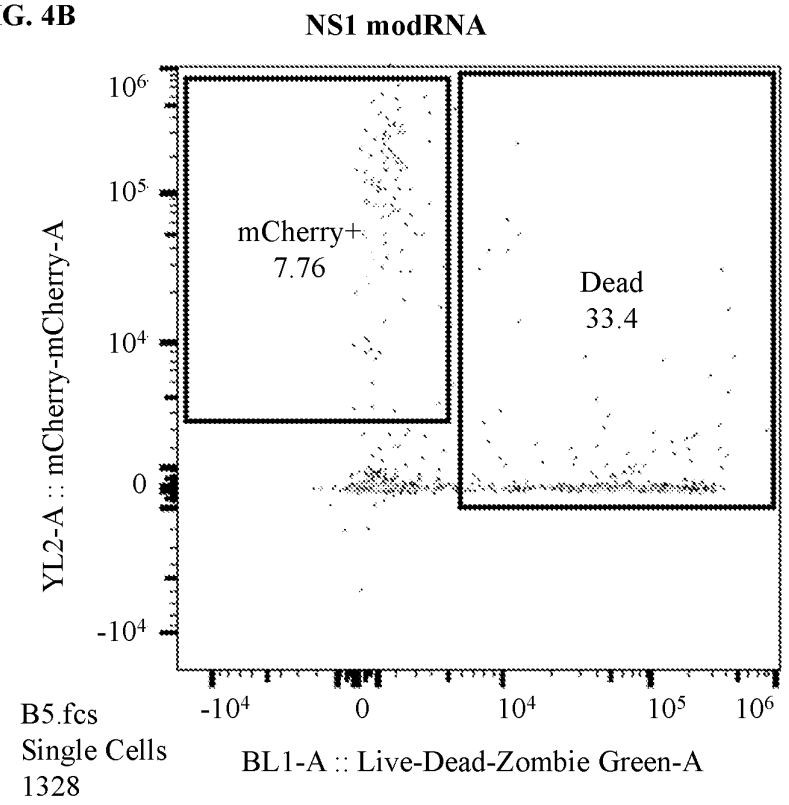

NS1 modRNA
mCherry+
7.76
Dead
33.4
YL2-A :: mCherry-mCherry-A
BL1-A :: Live-Dead-Zombie Green-A
B5.fcs
Single Cells
1328

FIG. 4C

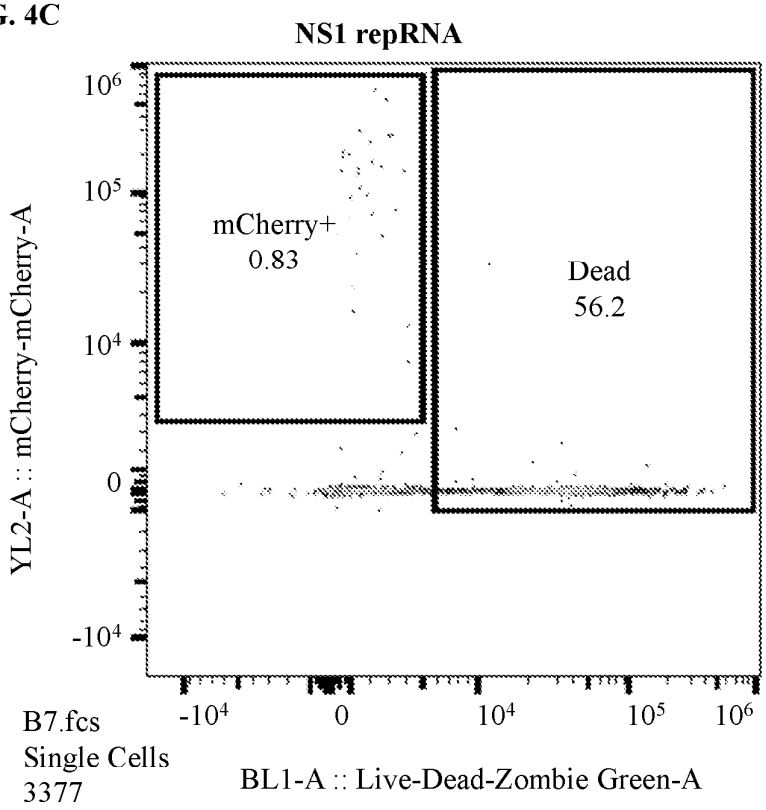

NS1 repRNA
mCherry+
0.83
Dead
56.2
YL2-A :: mCherry-mCherry-A
10^6
10^5
10^4
0
-10^4
-10^4
0
10^4
10^5
10^6
B7.fcs
Single Cells
3377
BL1-A :: Live-Dead-Zombie Green-A

FIG. 4D

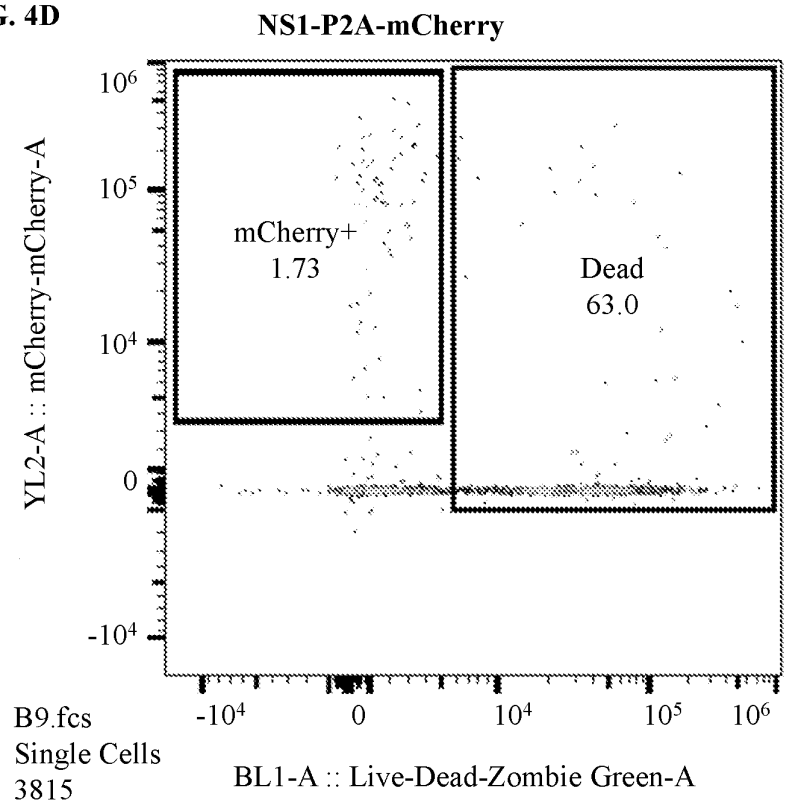

NS1-P2A-mCherry
mCherry+
1.73
Dead
63.0
YL2-A :: mCherry-mCherry-A
10^6
10^5
10^4
0
-10^4
-10^4
0
10^4
10^5
10^6
B9.fcs
Single Cells
3815
BL1-A :: Live-Dead-Zombie Green-A

FIG. 4E

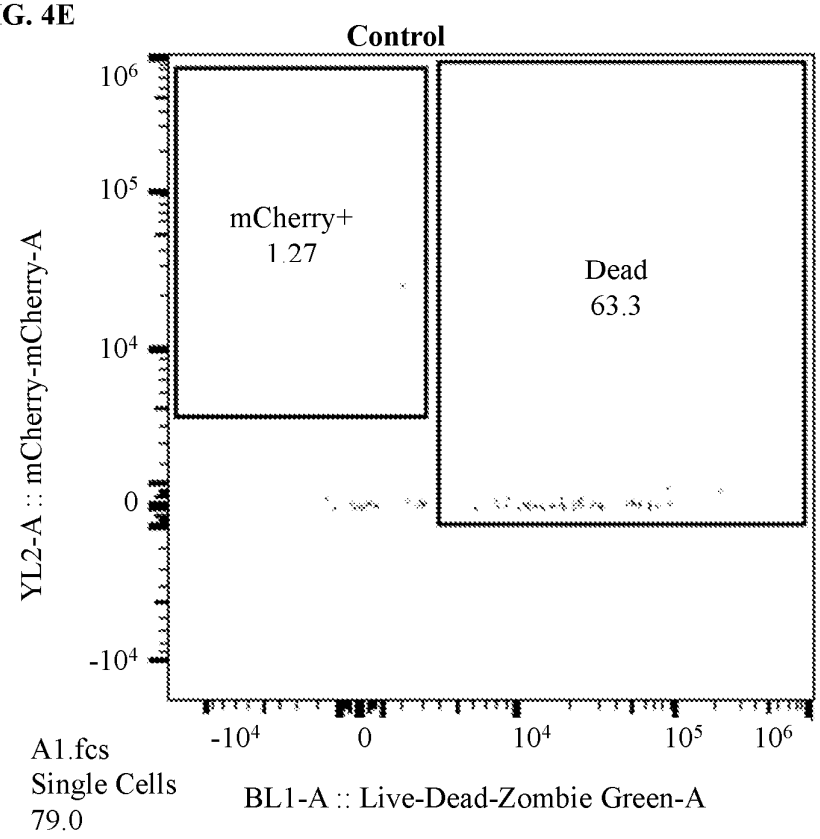

Control
mCherry+
1.27
Dead
63.3
YL2-A :: mCherry-mCherry-A
10^6
10^5
10^4
0
-10^4
-10^4
0
10^4
10^5
10^6
A1.fcs
Single Cells
79.0
BL1-A :: Live-Dead-Zombie Green-A

FIG. 4F

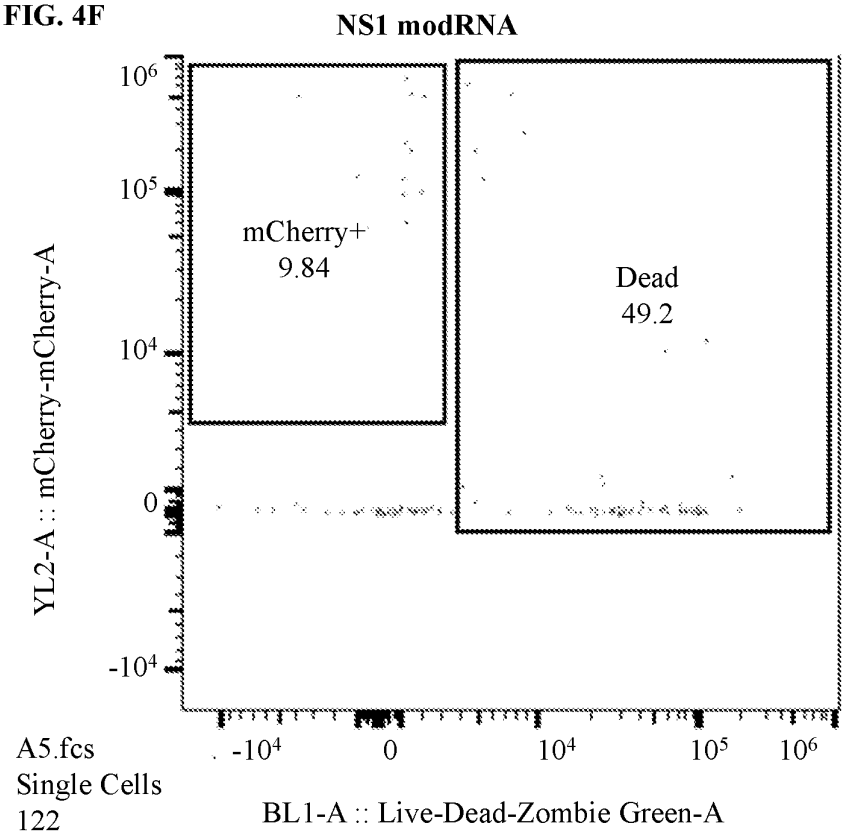

NS1 modRNA
mCherry+
9.84
Dead
49.2
YL2-A :: mCherry-mCherry-A
10^6
10^5
10^4
0
-10^4
-10^4
0
10^4
10^5
10^6
A5.fcs
Single Cells
122
BL1-A :: Live-Dead-Zombie Green-A

FIG. 4G

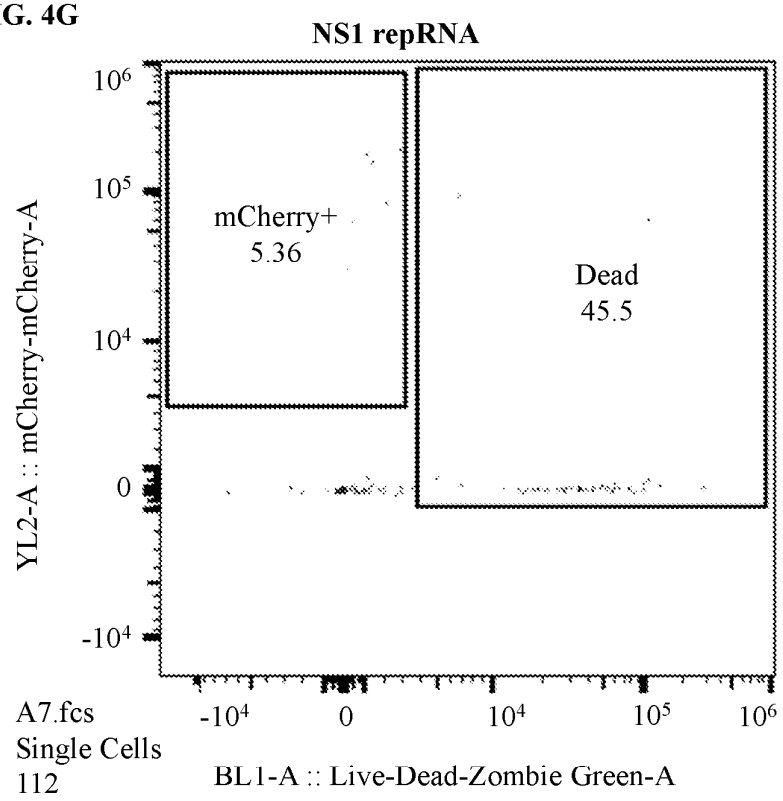
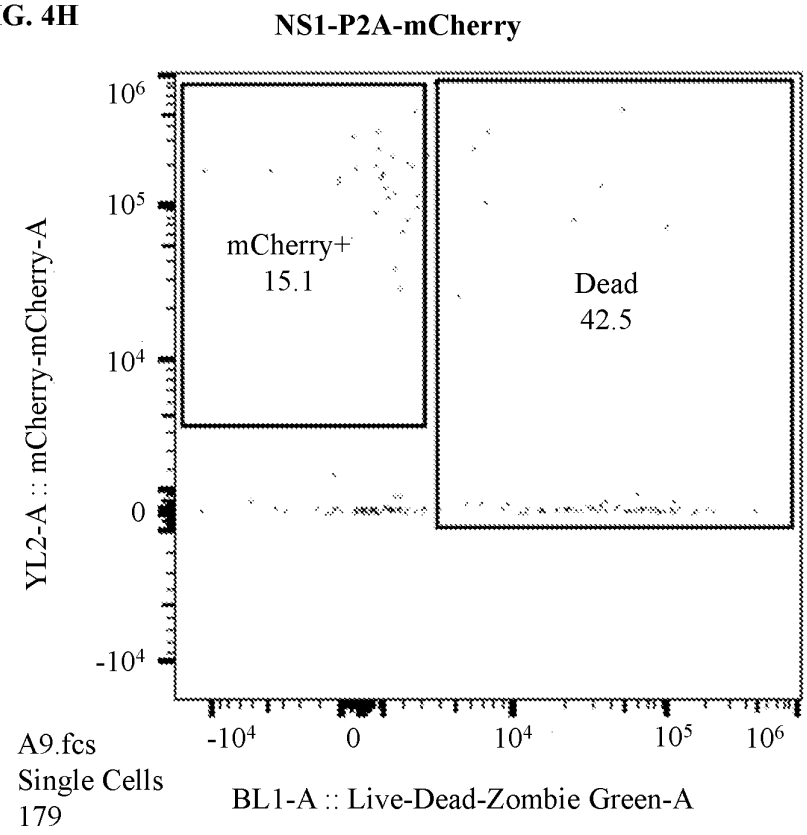

NS1 repRNA
mCherry+
5.36
Dead
45.5
YL2-A :: mCherry-mCherry-A
10^6
10^5
10^4
0
-10^4
-10^4
0
10^4
10^5
10^6
A7.fcs
Single Cells
112
BL1-A :: Live-Dead-Zombie Green-A

FIG. 4H


NS1-P2A-mCherry
mCherry+
15.1
Dead
42.5
YL2-A :: mCherry-mCherry-A
10^6
10^5
10^4
0
-10^4
-10^4
0
10^4
10^5
10^6
A9.fcs
Single Cells
179
BL1-A :: Live-Dead-Zombie Green-A

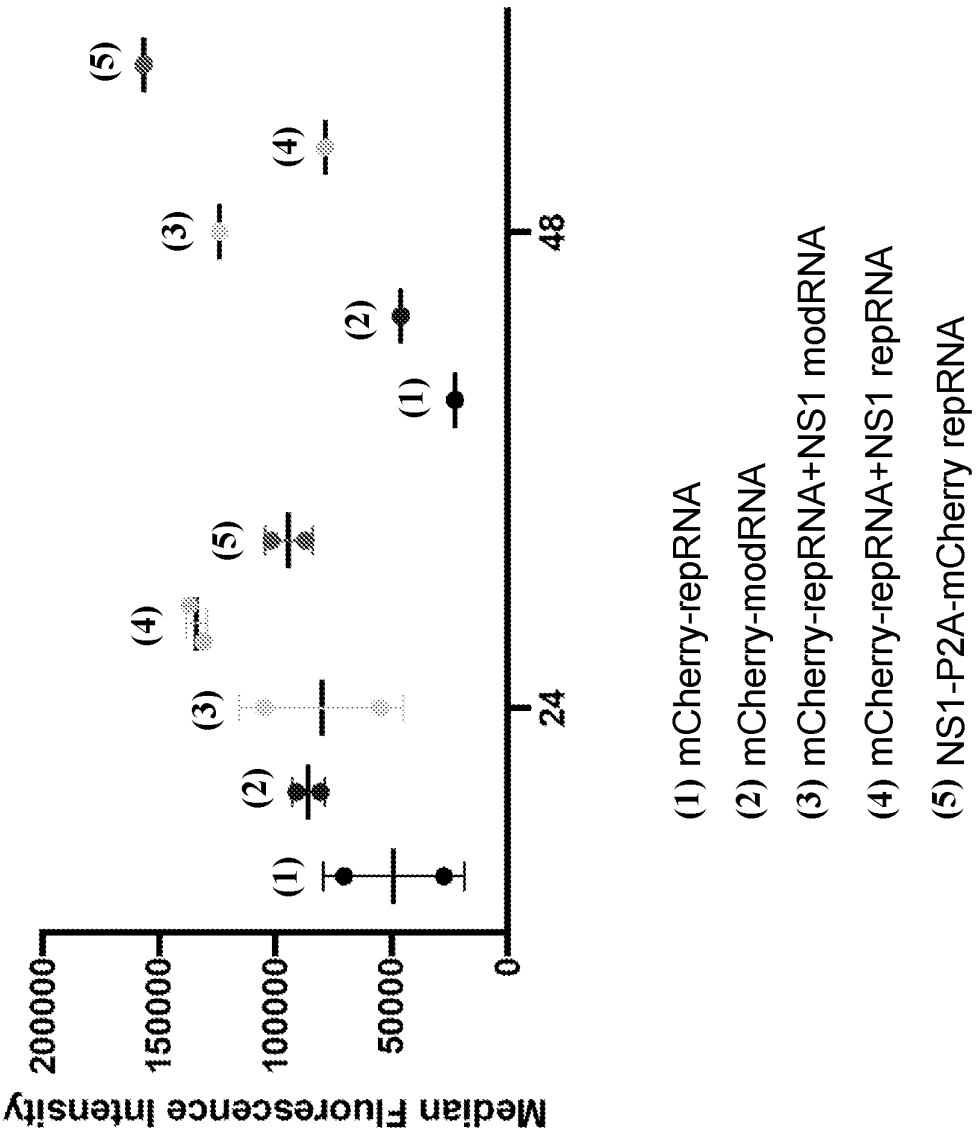

Median Fluorescence Intensity
200000
150000
100000
50000
0
24
48
(1)
(2)
(3)
(4)
(5)
(1) mCherry-repRNA
(2) mCherry-modRNA
(3) mCherry-repRNA+NS1 modRNA
(4) mCherry-repRNA+NS1 repRNA
(5) NS1-P2A-mCherry repRNA

FIG. 4I

FIG. 4J

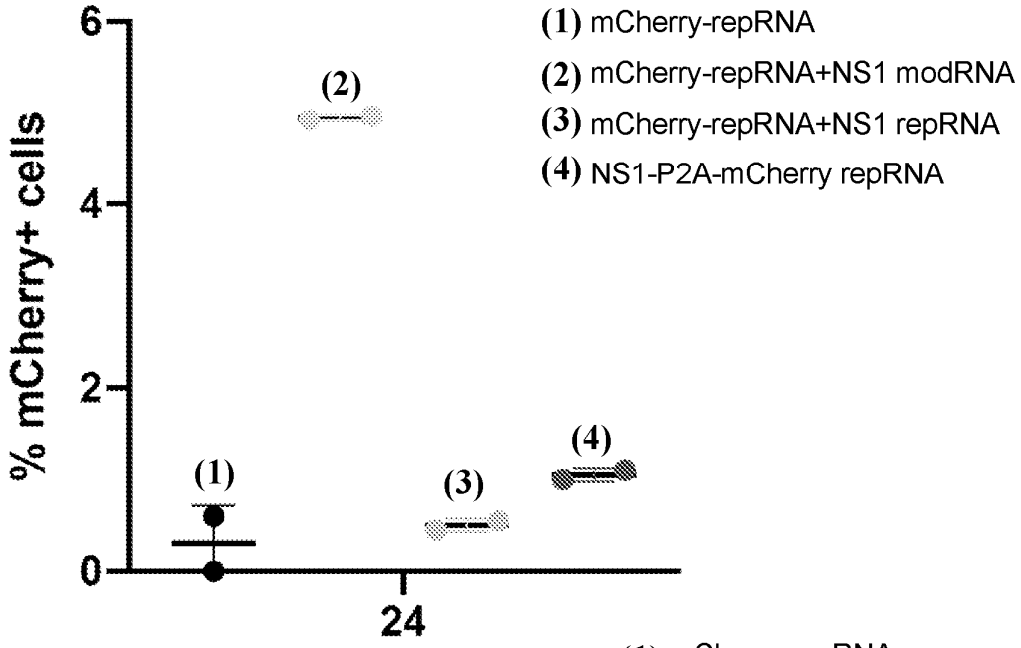

(1) mCherry-repRNA
(2) mCherry-repRNA+NS1 modRNA
(3) mCherry-repRNA+NS1 repRNA
(4) NS1-P2A-mCherry repRNA
% mCherry+ cells
6
4
2
0
(1)
(2)
(3)
(4)
24

FIG. 4K

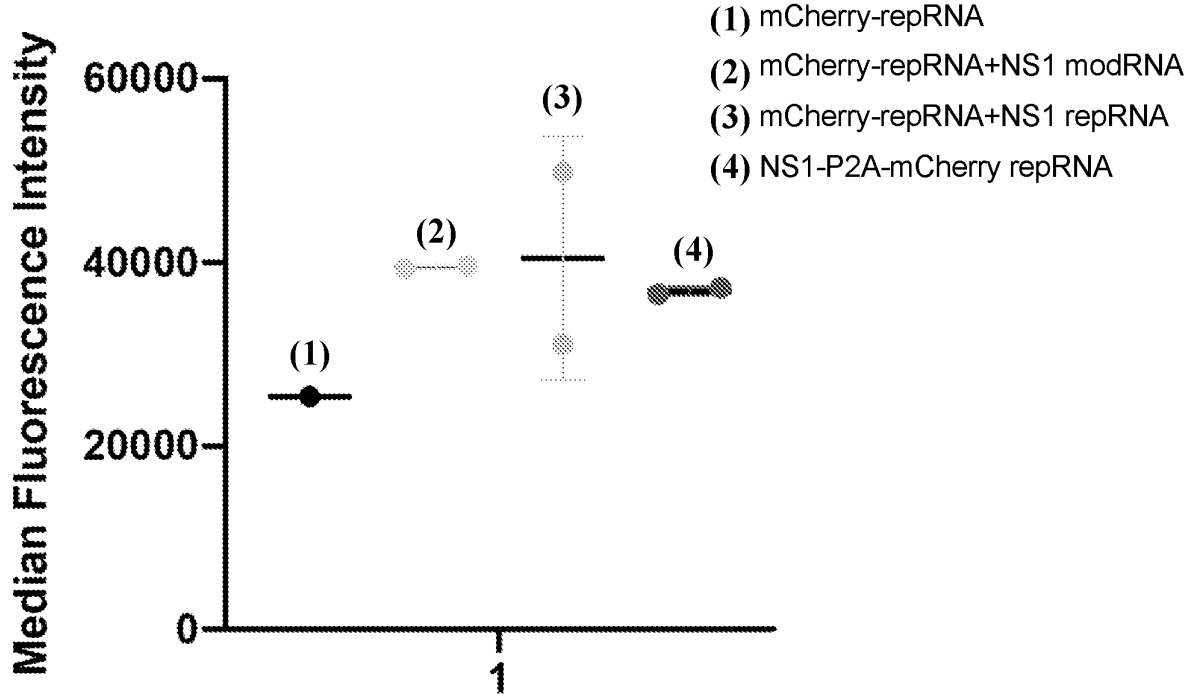

(1) mCherry-repRNA
(2) mCherry-repRNA+NS1 modRNA
(3) mCherry-repRNA+NS1 repRNA
(4) NS1-P2A-mCherry repRNA
Median Fluorescence Intensity
60000
40000
20000
0
(1)
(2)
(3)
(4)
1

FIG. 5A

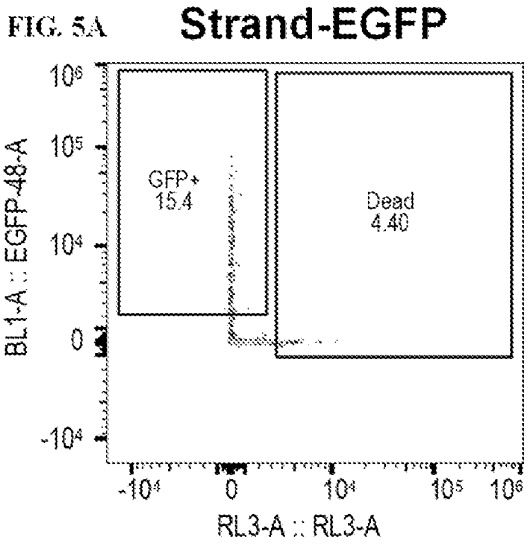


FIG. 5B

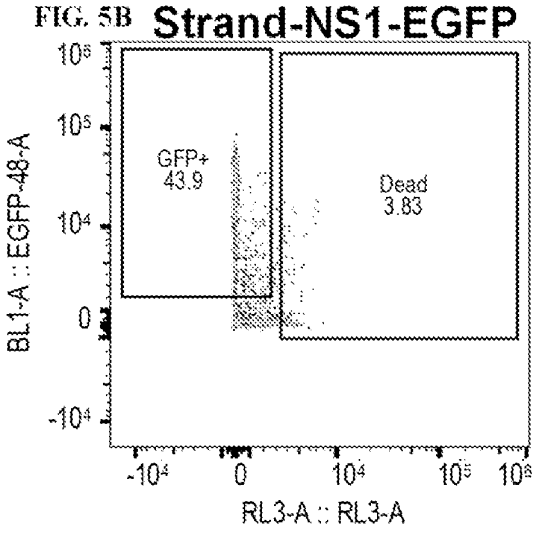


FIG. 5C

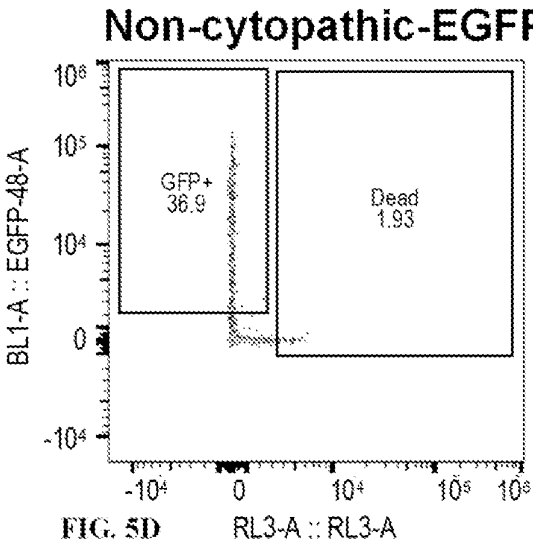


FIG. 5D

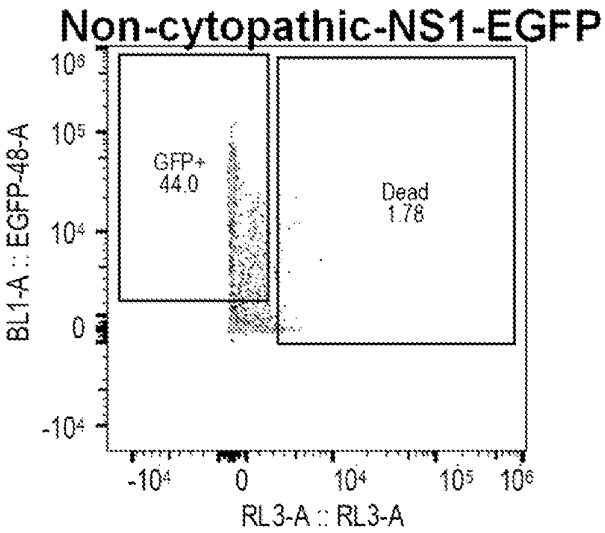


FIG. 5E

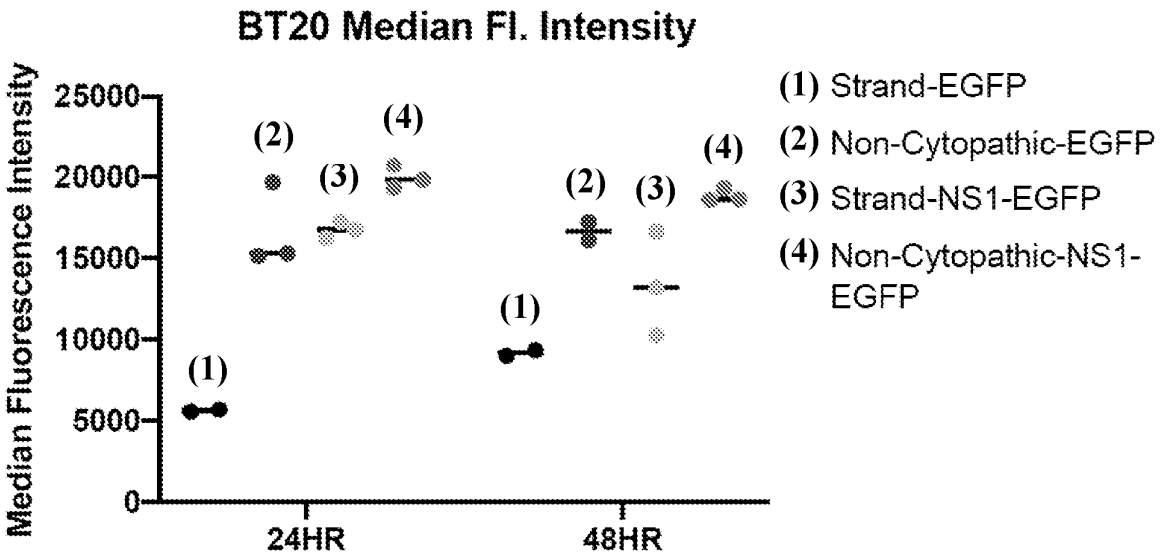
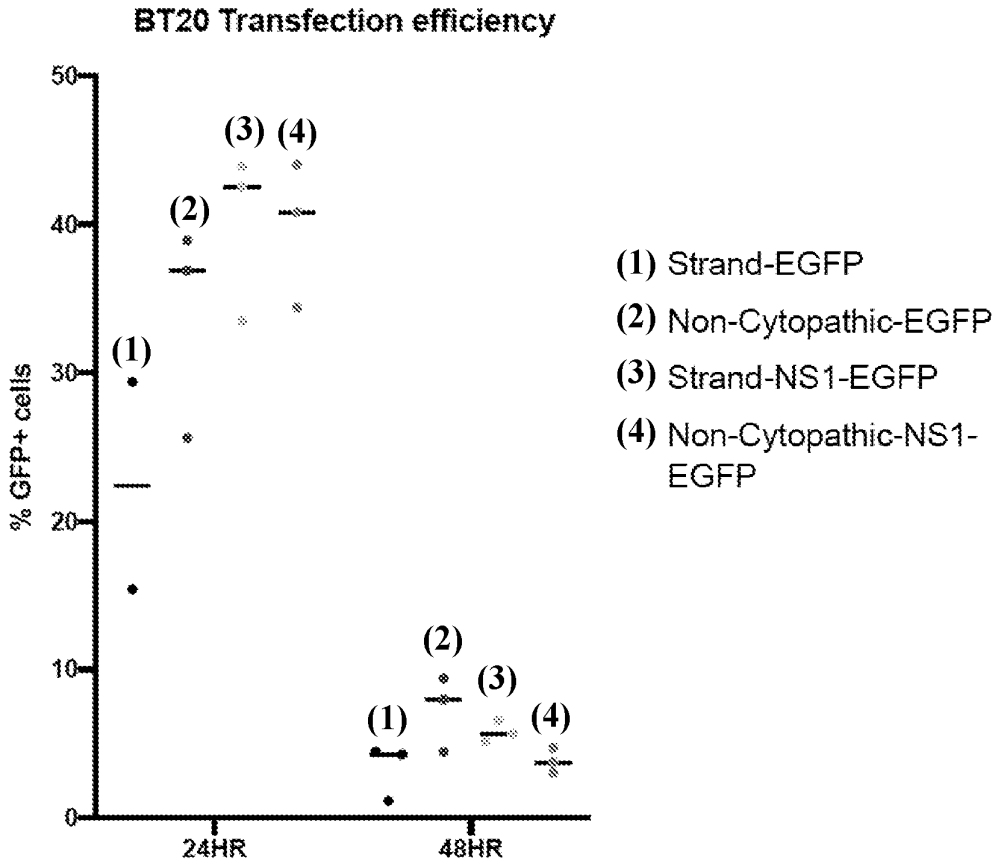

BT20 Median Fl. Intensity
Median Fluorescence Intensity
25000
20000
15000
10000
5000
0
24HR
48HR
(1)
(2)
(3)
(4)
(1) Strand-EGFP
(2) Non-Cytopathic-EGFP
(3) Strand-NS1-EGFP
(4) Non-Cytopathic-NS1-EGFP

FIG. 5F


BT20 Transfection efficiency
% GFP+ cells
50
40
30
20
10
0
24HR
48HR
(1)
(2)
(3)
(4)
(1) Strand-EGFP
(2) Non-Cytopathic-EGFP
(3) Strand-NS1-EGFP
(4) Non-Cytopathic-NS1-EGFP

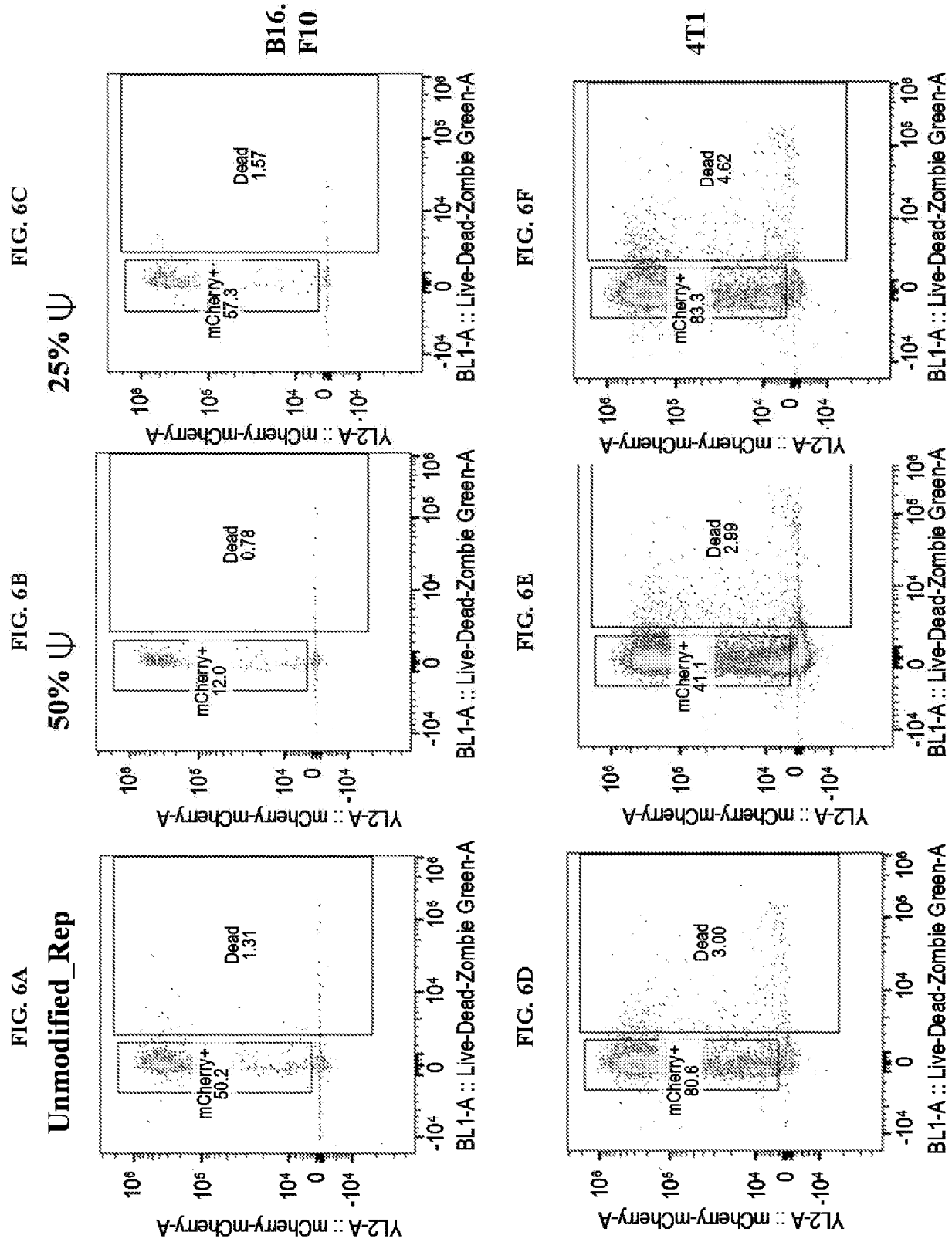

FIG. 6A
Unmodified_Rep
FIG. 6B
50% Ψ
FIG. 6C
25% Ψ
B16.
F10
FIG. 6D
FIG. 6E
FIG. 6F
4T1
mCherry+
80.6
Dead
3.00
mCherry+
41.1
Dead
2.99
mCherry+
83.3
Dead
4.62
mCherry+
50.2
Dead
1.31
mCherry+
12.0
Dead
0.78
mCherry+
57.3
Dead
1.57
YL2-A :: mCherry-mCherry-A
BL1-A :: Live-Dead-Zombie Green-A

FIG. 6G

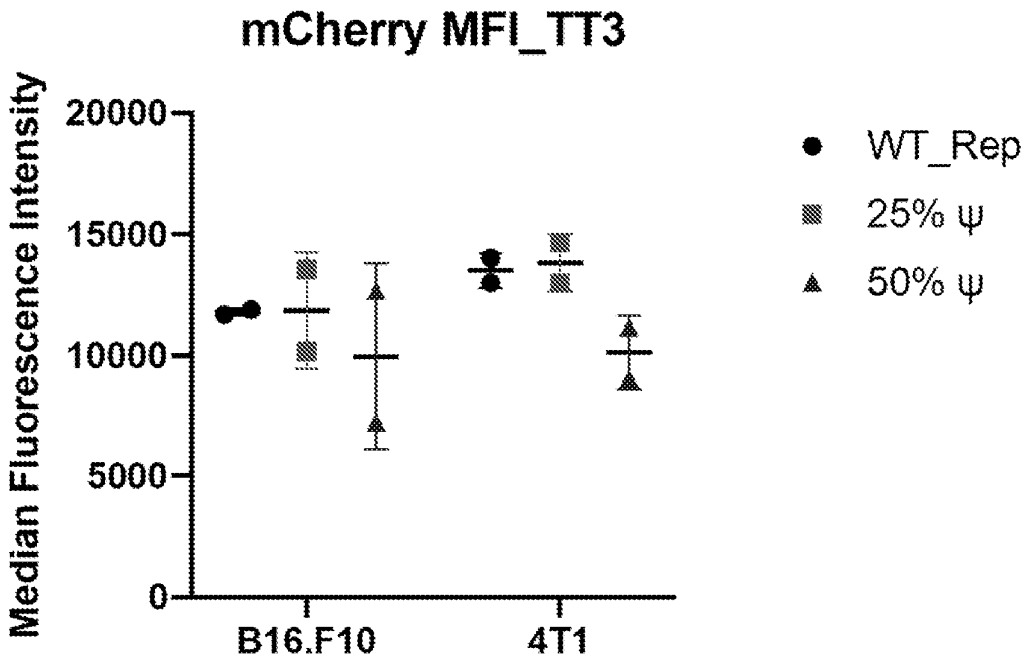
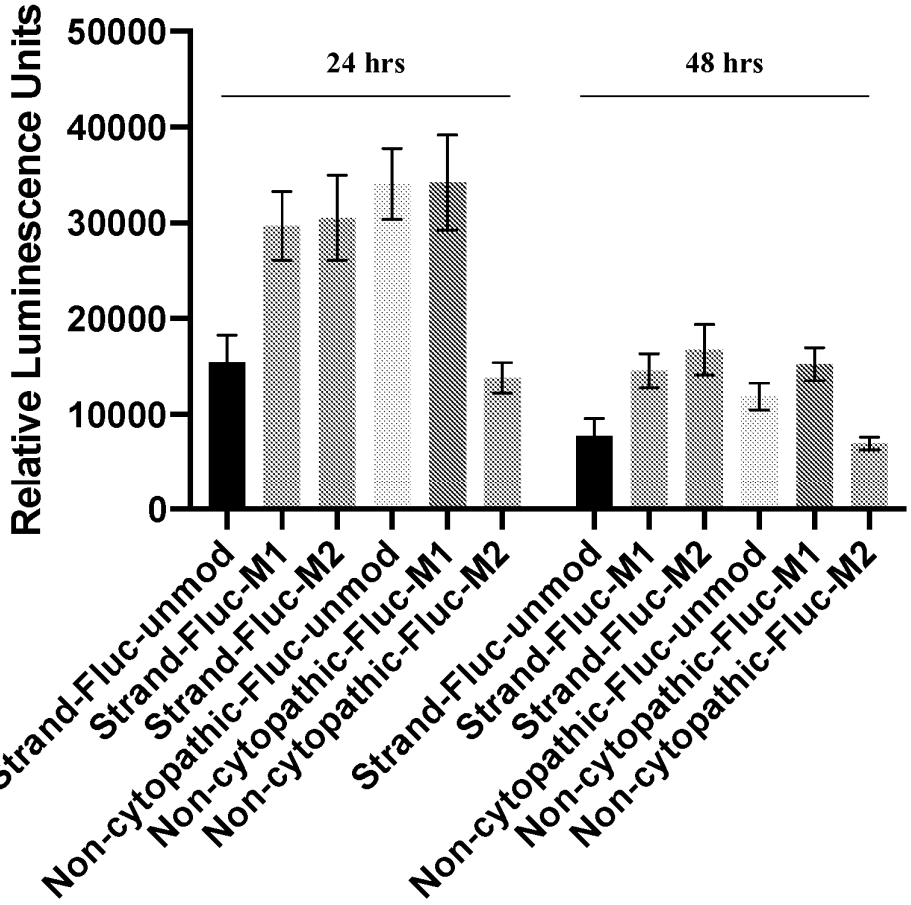

mCherry MFI_TT3
Median Fluorescence Intensity
20000
15000
10000
5000
0
B16.F10
4T1
WT_Rep
25% ψ
50% ψ

FIG. 7



M1- 1:3 U: Ψ
M2- 1:3 U: Ψ; 1:3 C:5me-C

FIG. 8A

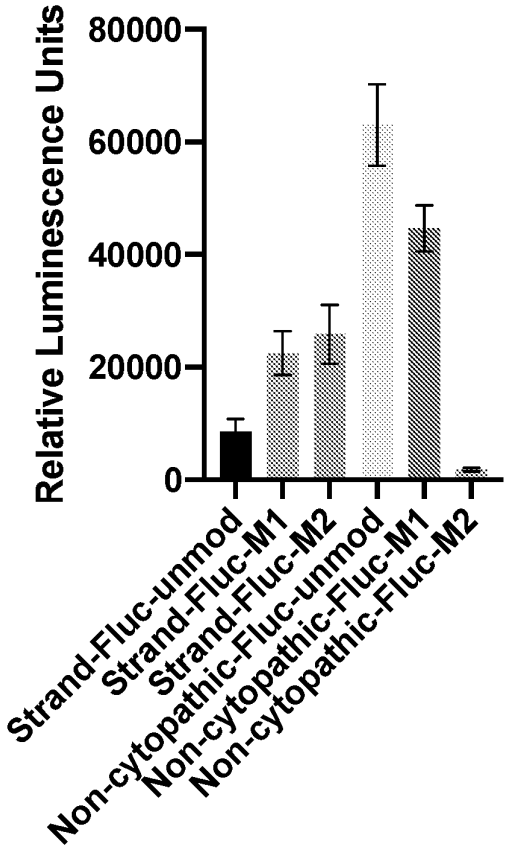
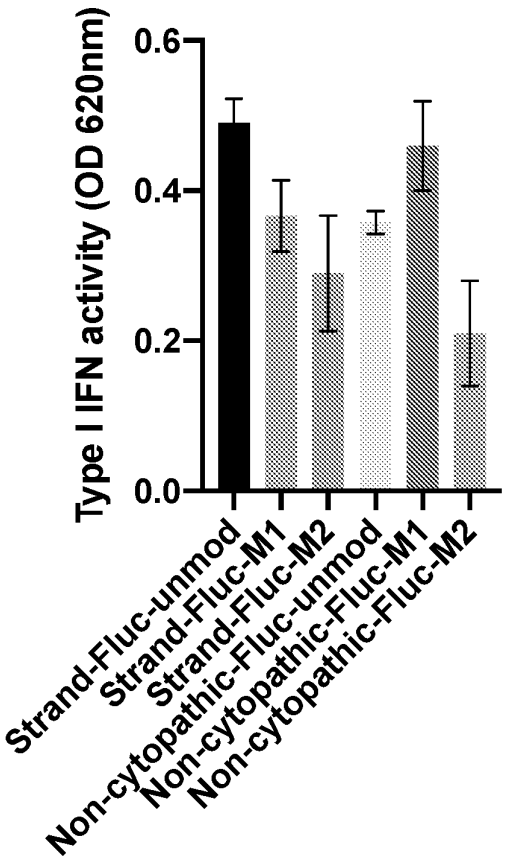


FIG. 8B



M1- 1:3 U: Ψ
M2- 1:3 U: Ψ; 1:3 C:5me-C

FIG. 9A

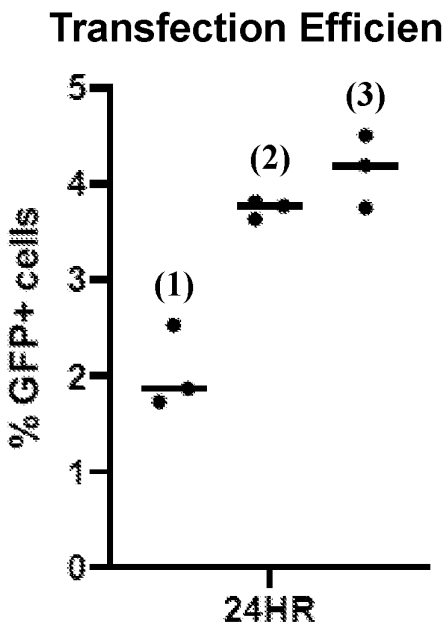
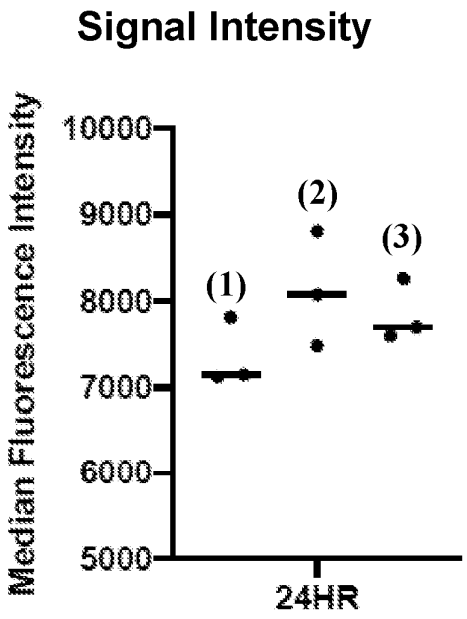


FIG. 9B

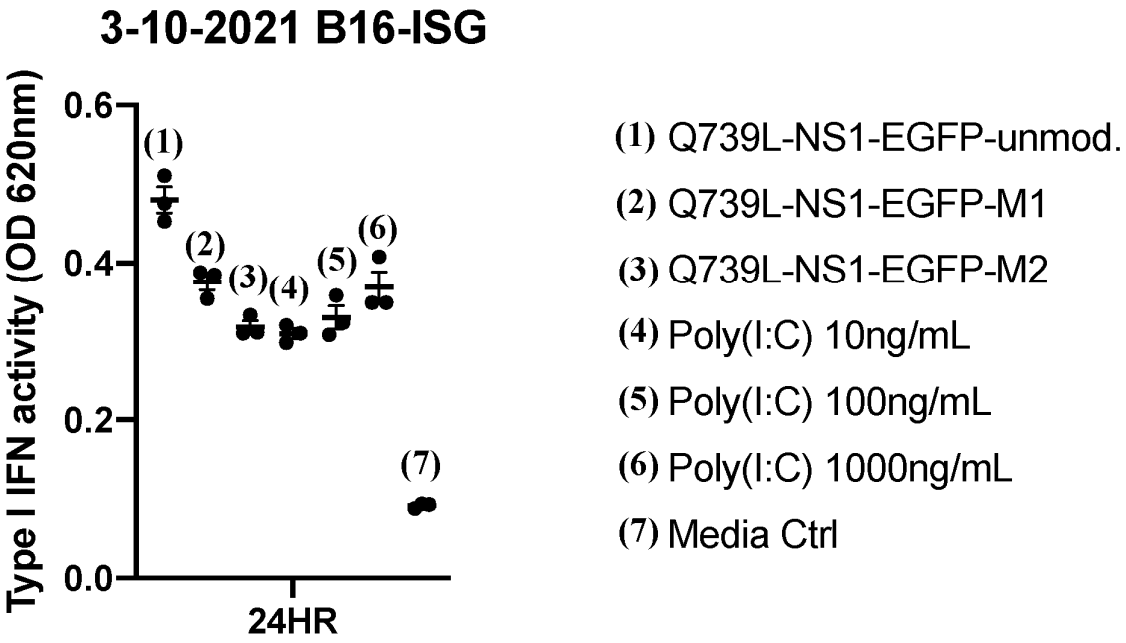


(1) Q739L-NS1-EGFP-unmod.

(2) Q739L-NS1-EGFP-M1

(3) Q739L-NS1-EGFP-M2

FIG. 9C



Type I Interferon Activity (SEAP colorimetric assay)

No Enhancer

FIG. 10A Lipid 1

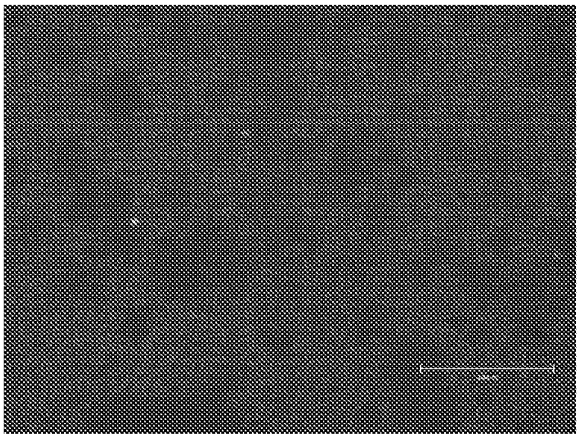


FIG. 10B Lipid 2-Chol.

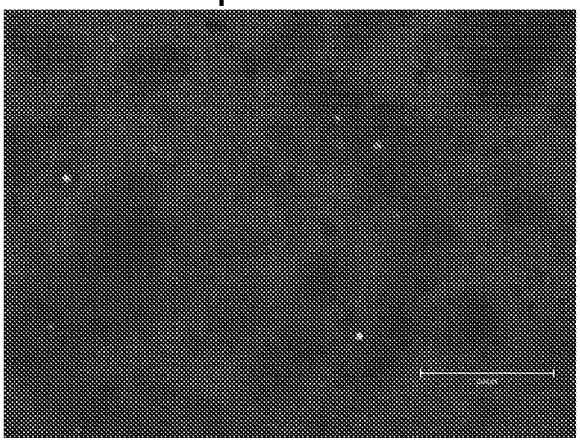
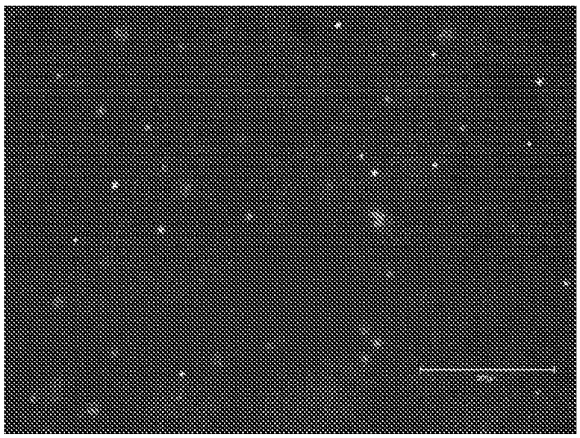


FIG. 10C Lipid 2-β-Sito.



Enhancer

FIG. 10D Lipid 1

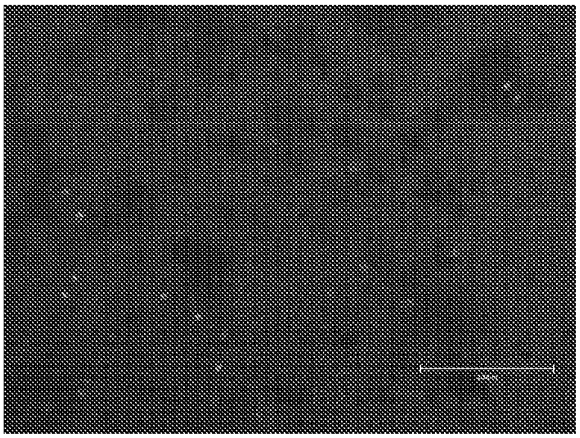


FIG. 10E Lipid 2-Chol.

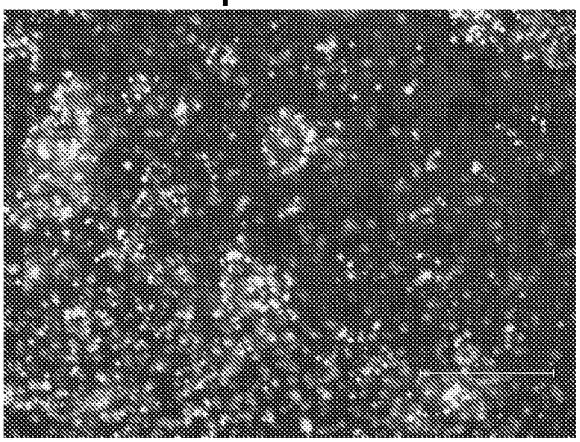


FIG. 10F Lipid 2-β-Sito.

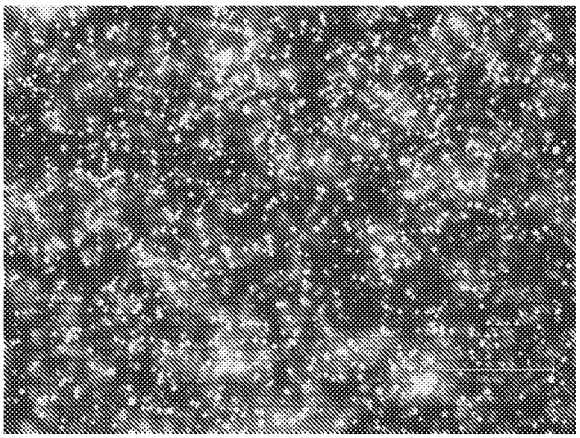


FIG. 11A

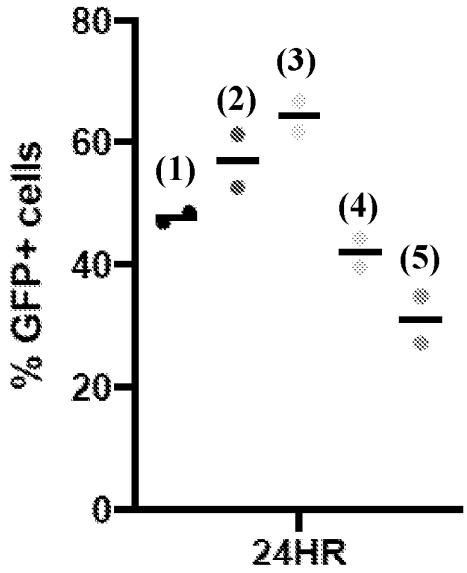


FIG. 11B

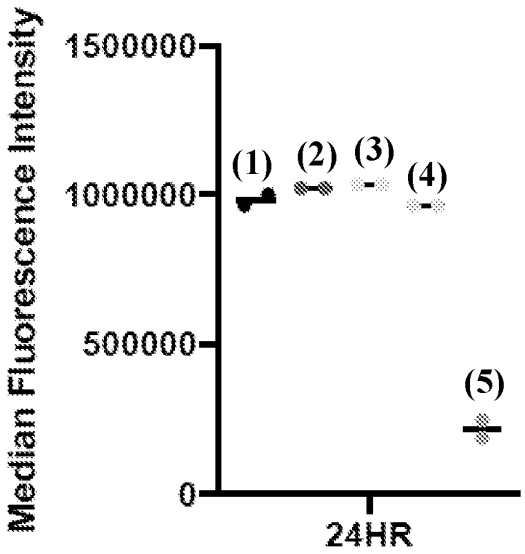
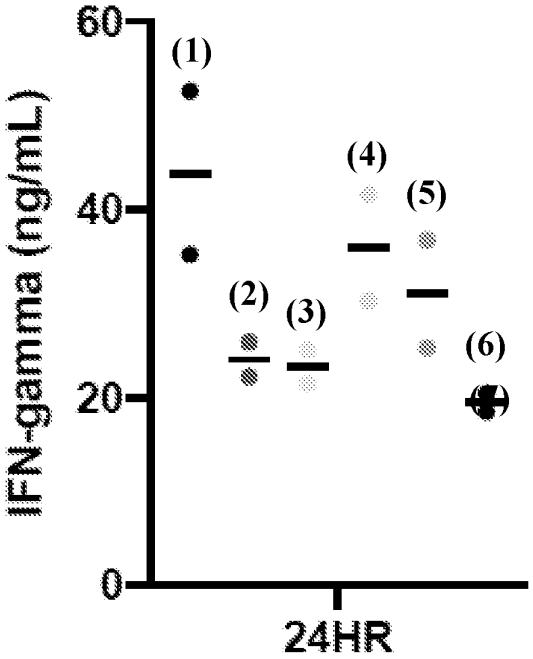


FIG. 11C



(1) Q739L-NS1-EGFP

(2) Q739L-NS1-EGFP-M1

(3) Q739L-NS1-EGFP-M2

(4) Q739L-NS1-EGFP +1% Poly(I:C)

(5) Q739L-NS1-EGFP +10% Poly(I:C)

(6) Untreated

FIG. 12A

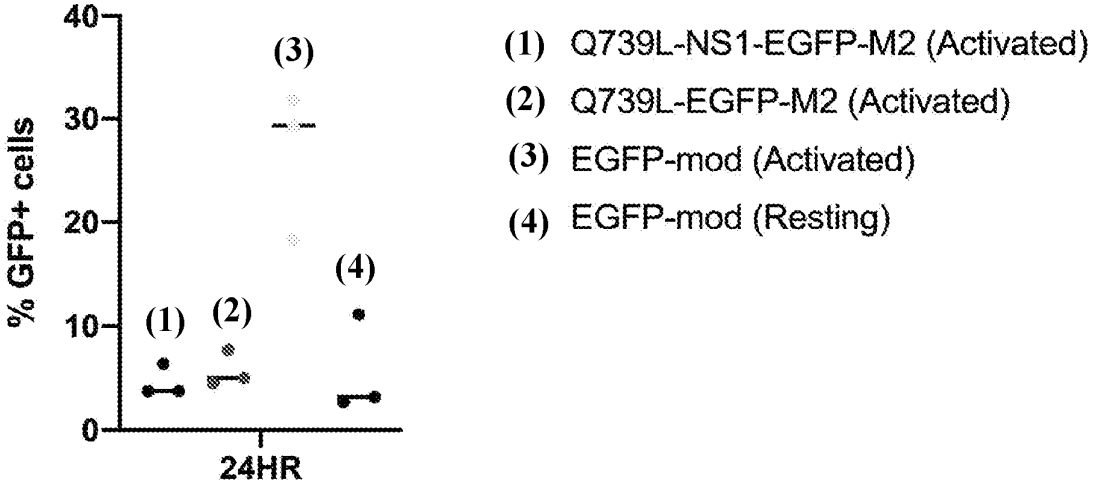


FIG. 12B

