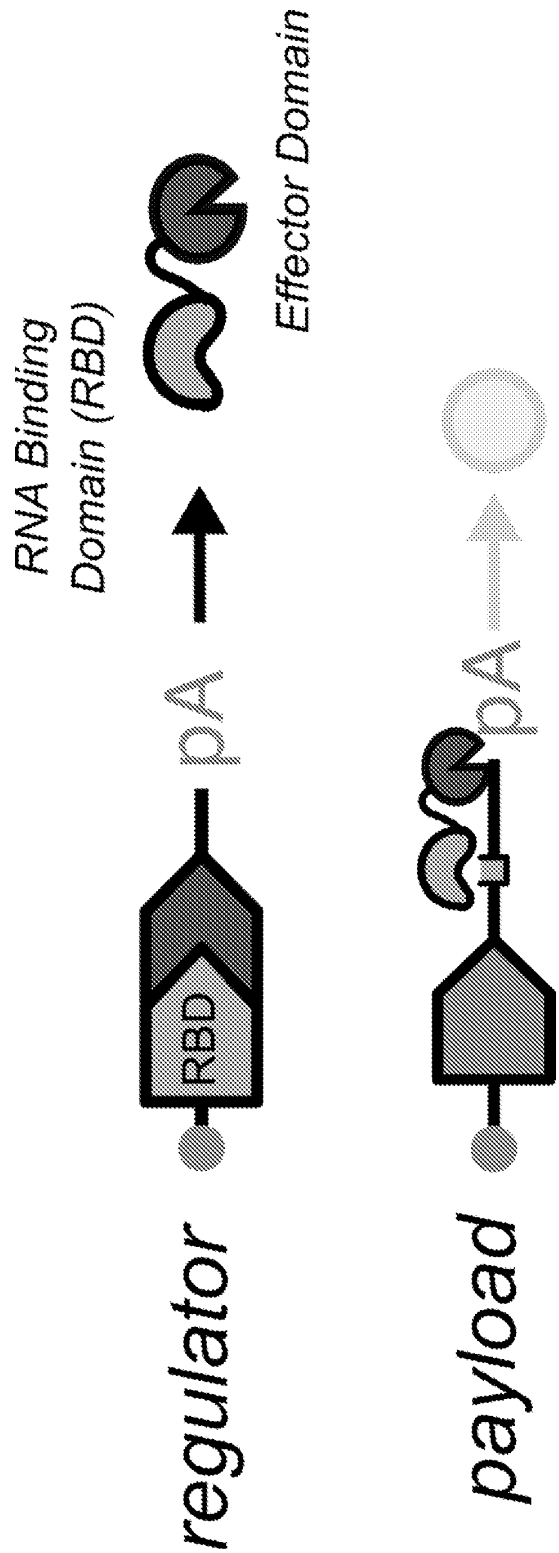

RNA Binding
Domain (RBD)
Effector Domain
RBD
pA
pA
regulator
payload

FIG. 1.

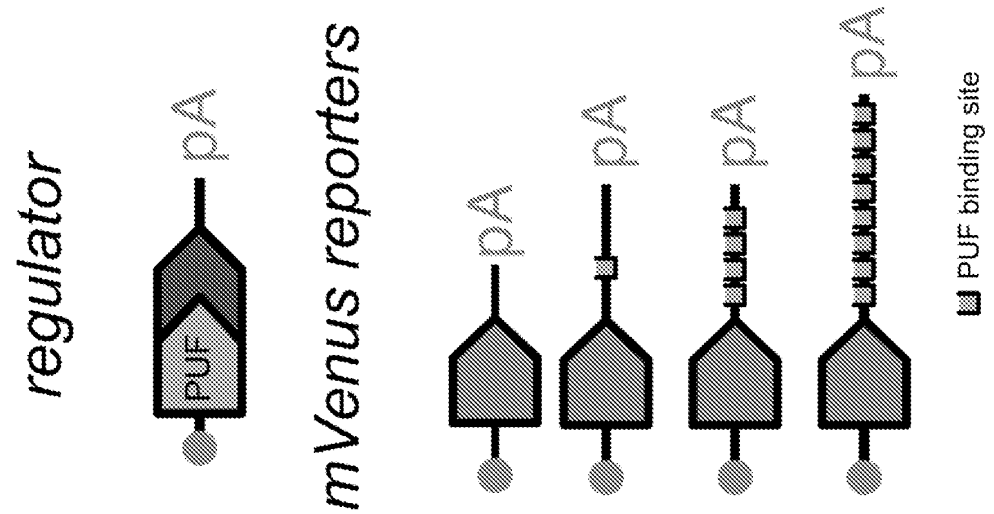
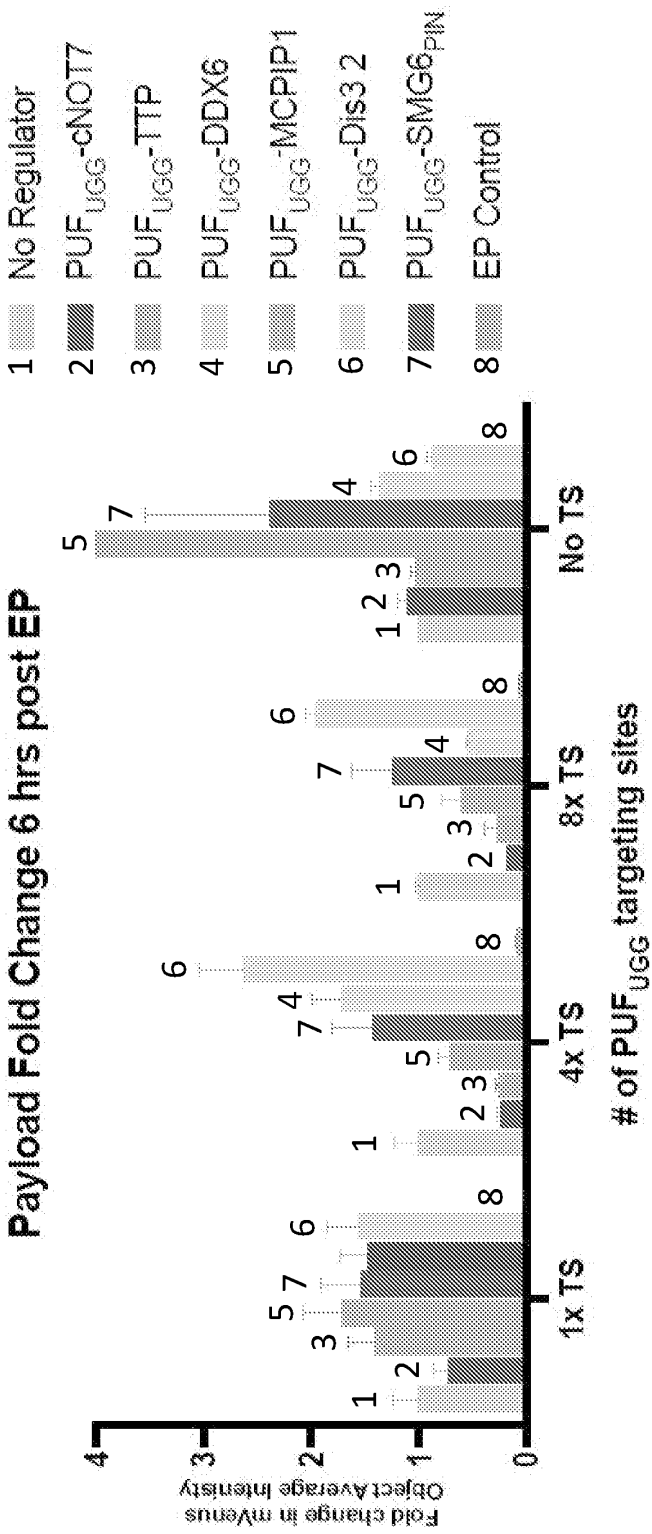

regulator
PUF
pA
mVenus reporters
pA
pA
pA
pA
PUF binding site

FIG. 2.

FIG. 3.


Payload Fold Change 6 hrs post EP
Fold change in mVenus ObjectAverage Intensity
0
1
2
3
4
1x TS
4x TS
8x TS
No TS
# of PUF$_{UGG}$ targeting sites
1 No Regulator
2 PUF$_{UGG}$-cNOT7
3 PUF$_{UGG}$-TTP
4 PUF$_{UGG}$-DDX6
5 PUF$_{UGG}$-MCPIP1
6 PUF$_{UGG}$-Dis3 2
7 PUF$_{UGG}$-SMG6$_{PIN}$
8 EP Control

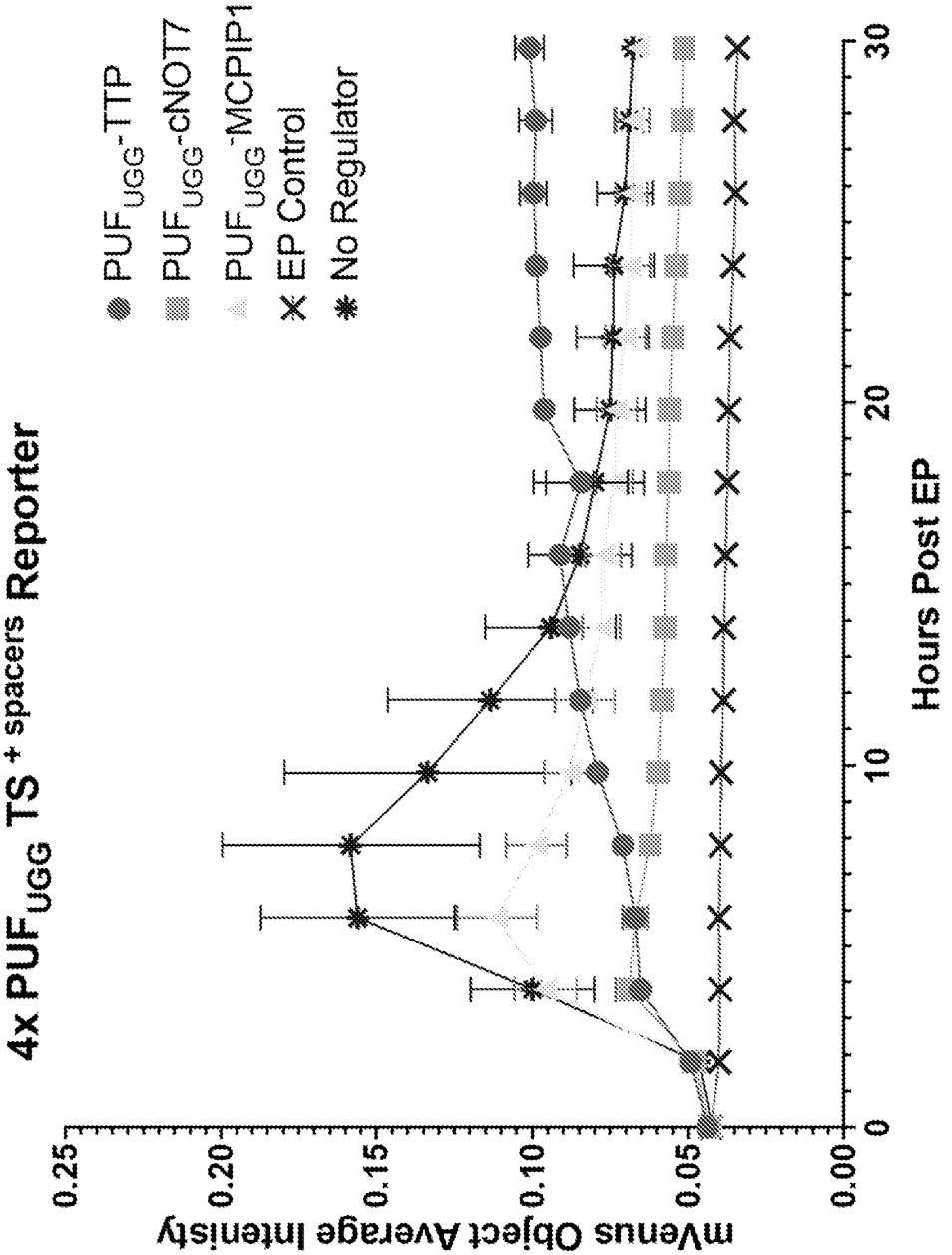

4x PUF$_{UGG}$ TS + spacers Reporter
PUF$_{UGG}$-TTP
PUF$_{UGG}$-cNOT7
PUF$_{UGG}$-MCPIP1
EP Control
No Regulator
mVenus Object Average Intenisty
0.25
0.20
0.15
0.10
0.05
0.00
0
10
20
30
Hours Post EP

FIG. 4.

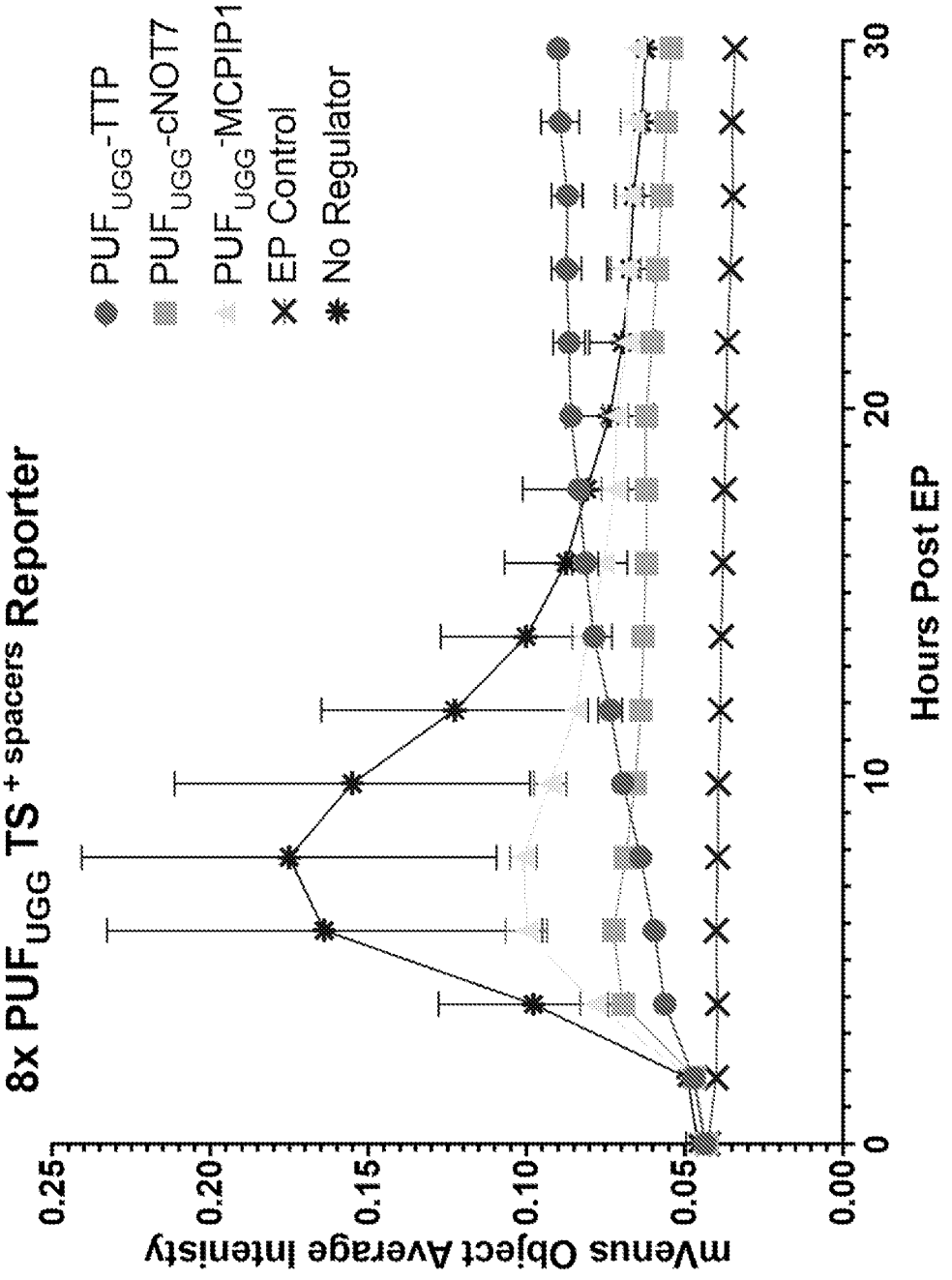

8x PUF$_{UGG}$ TS $^{+ spacers}$ Reporter
PUF$_{UGG}$-TTP
PUF$_{UGG}$-cNOT7
PUF$_{UGG}$-MCPIP1
EP Control
No Regulator
mVenus Object Average Intenisty
0.25
0.20
0.15
0.10
0.05
0.00
0
10
20
30
Hours Post EP

FIG. 5.

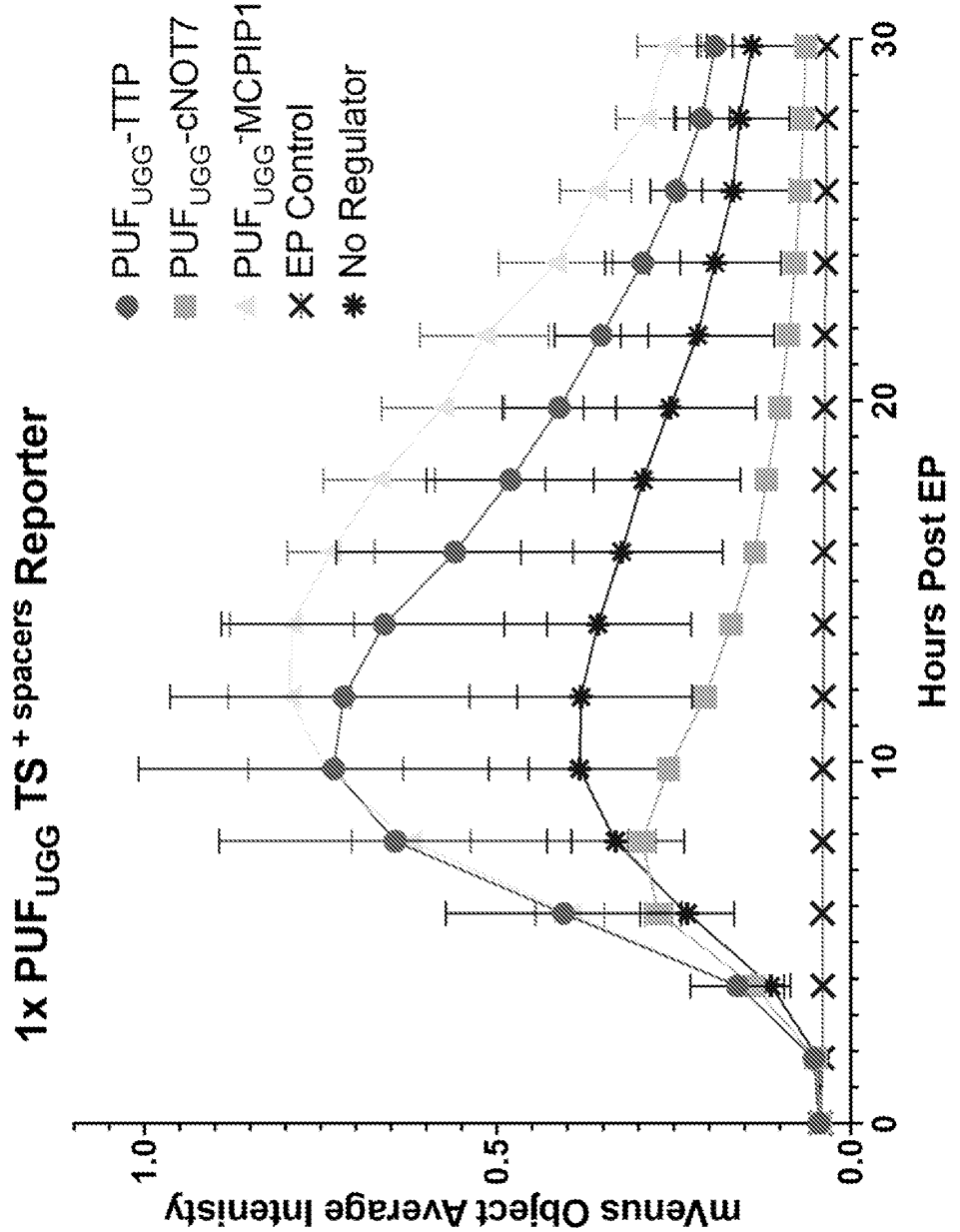

1x PUF$_{UGG}$ TS $^{+ spacers}$ Reporter
PUF$_{UGG}$-TTP
PUF$_{UGG}$-cNOT7
PUF$_{UGG}$-MCPIP1
EP Control
No Regulator
mVenus Object Average Intenisty
0.0
0.5
1.0
0
10
20
30
Hours Post EP

FIG. 6.

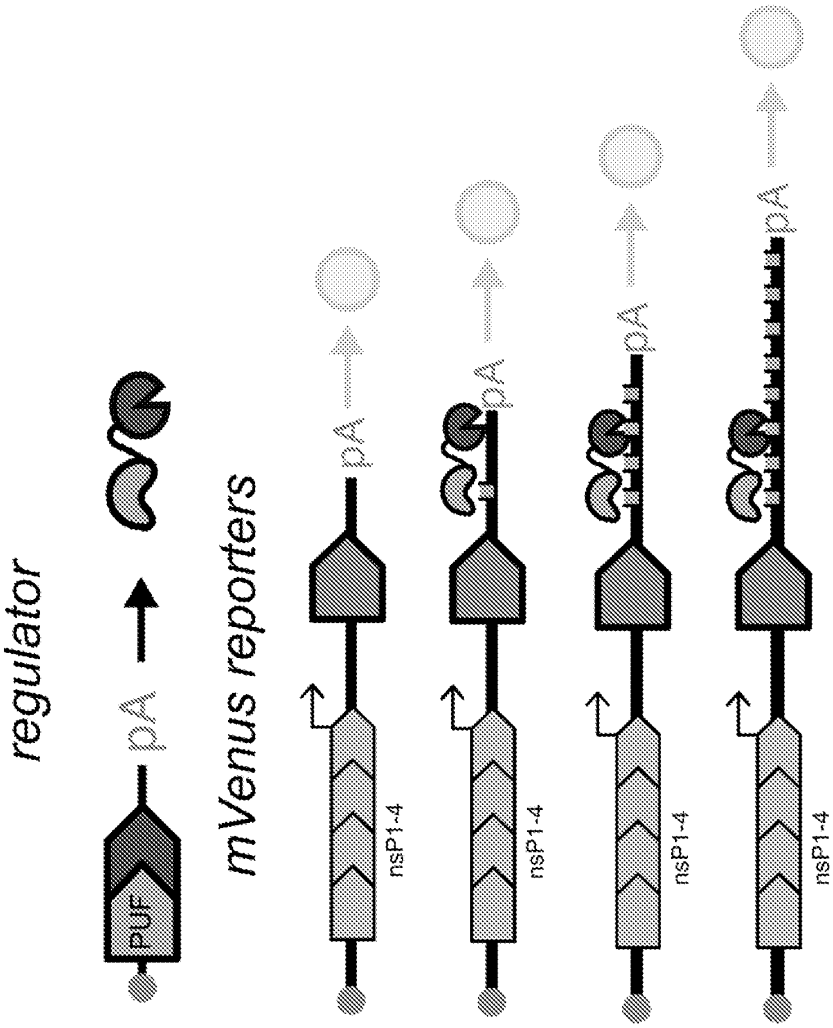
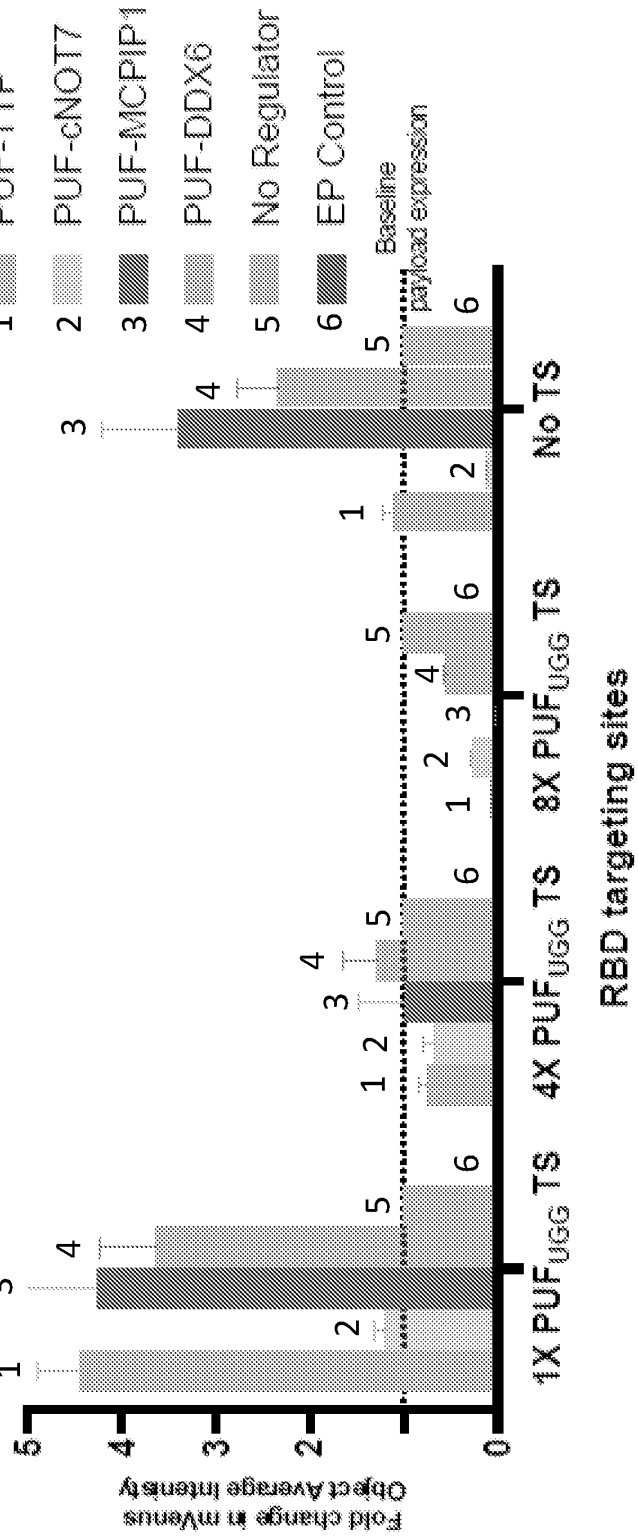

regulator
PUF
pA
mVenus reporters
nsP1-4
pA
nsP1-4
pA
nsP1-4
pA
nsP1-4
pA

FIG. 7.

FIG. 8.


Payload Fold Change 20 hrs post EP
Fold change in mVenus Object Average Intensity
RBD targeting sites
1X PUF$_{UGG}$ TS
4X PUF$_{UGG}$ TS
8X PUF$_{UGG}$ TS
No TS
PUF-TTP
PUF-cNOT7
PUF-MCPIP1
PUF-DDX6
No Regulator
EP Control
Baseline payload expression

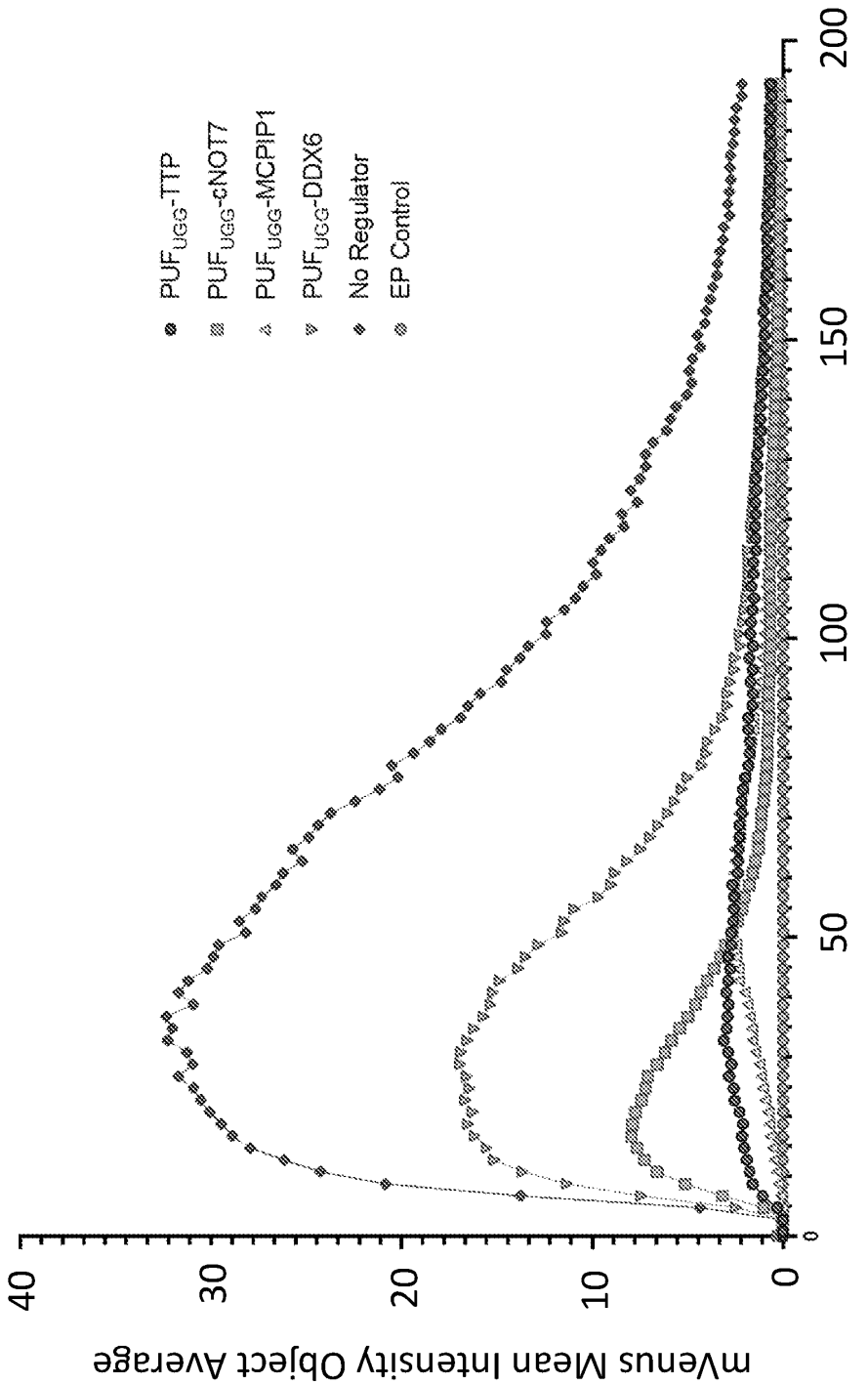
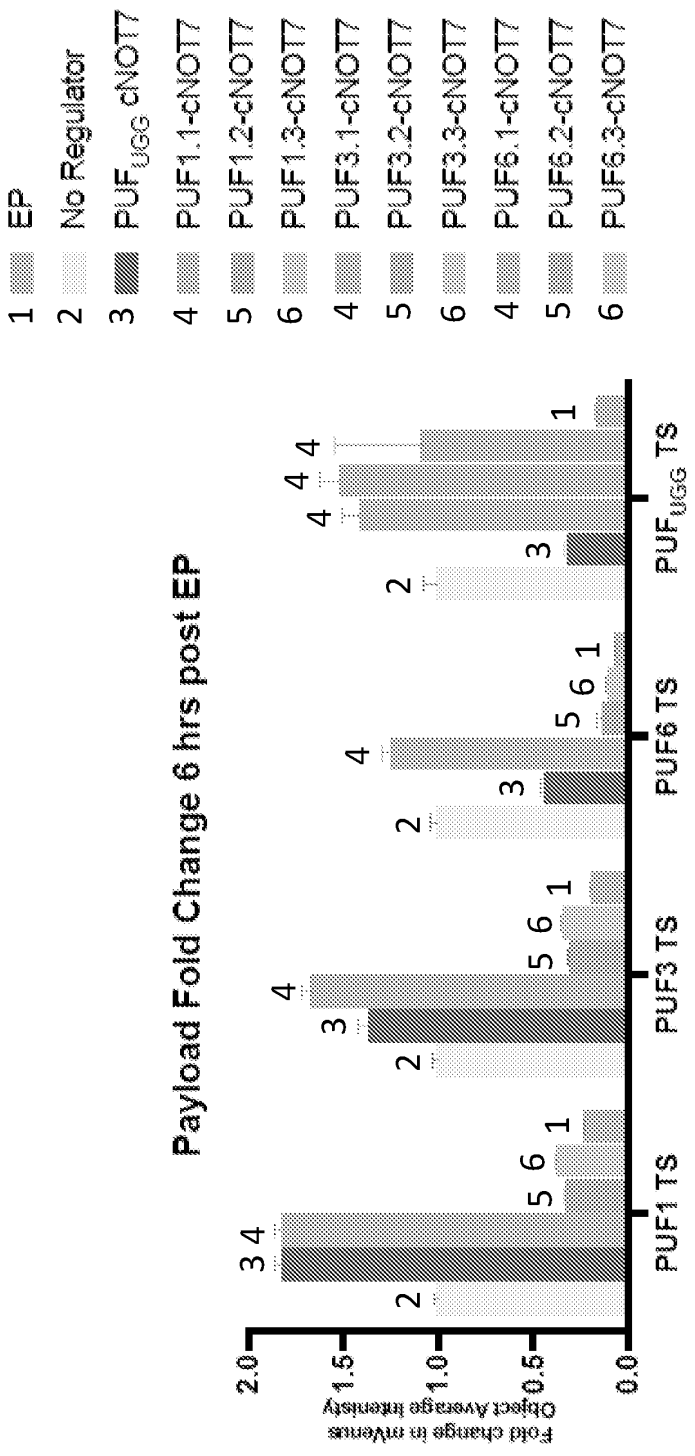

8x PUF$_{UGG}$ Payload expression timecourse
PUF$_{UGG}$-TTP
PUF$_{UGG}$-cNOT7
PUF$_{UGG}$-MCPIP1
PUF$_{UGG}$-DDX6
No Regulator
EP Control
mVenus Mean Intensity Object Average
0
10
20
30
40
0
50
100
150
200
Time (hrs)

FIG. 9.

FIG. 10.


1 EP
2 No Regulator
3 PUF$_{UGG}$ cNOT7
4 PUF1.1-cNOT7
5 PUF1.2-cNOT7
6 PUF1.3-cNOT7
4 PUF3.1-cNOT7
5 PUF3.2-cNOT7
6 PUF3.3-cNOT7
4 PUF6.1-cNOT7
5 PUF6.2-cNOT7
6 PUF6.3-cNOT7
Payload Fold Change 6 hrs post EP
Fold change in nVenus Object Average Intensity
2.0
1.5
1.0
0.5
0.0
PUF1 TS
PUF3 TS
PUF6 TS
PUF$_{UGG}$ TS

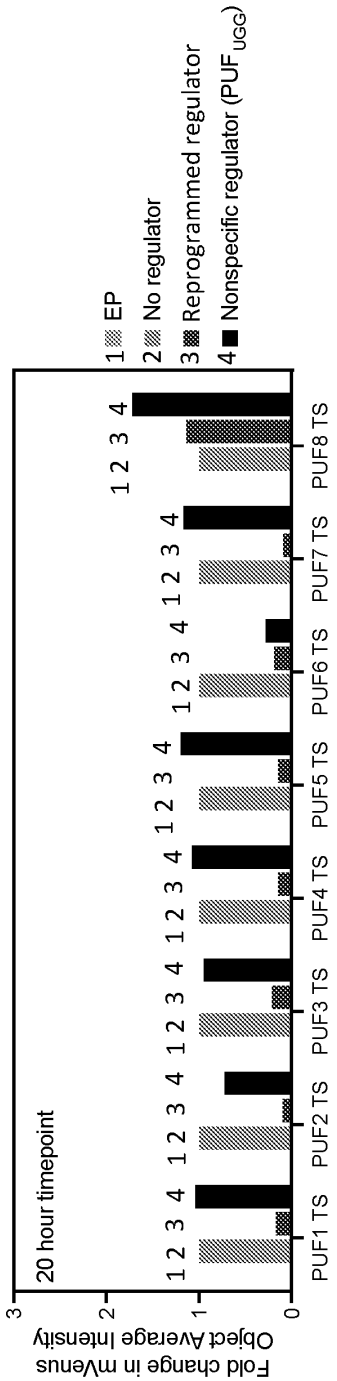


FIG. 11A

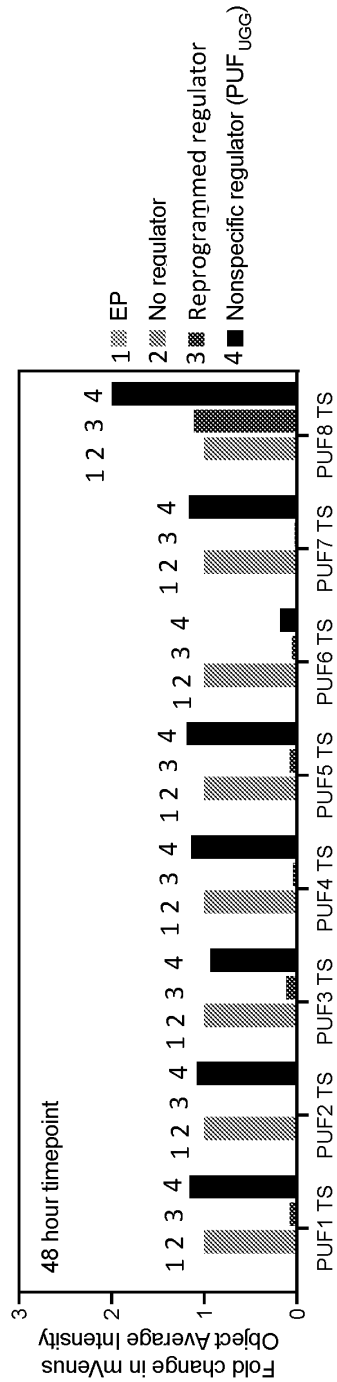


FIG. 11B

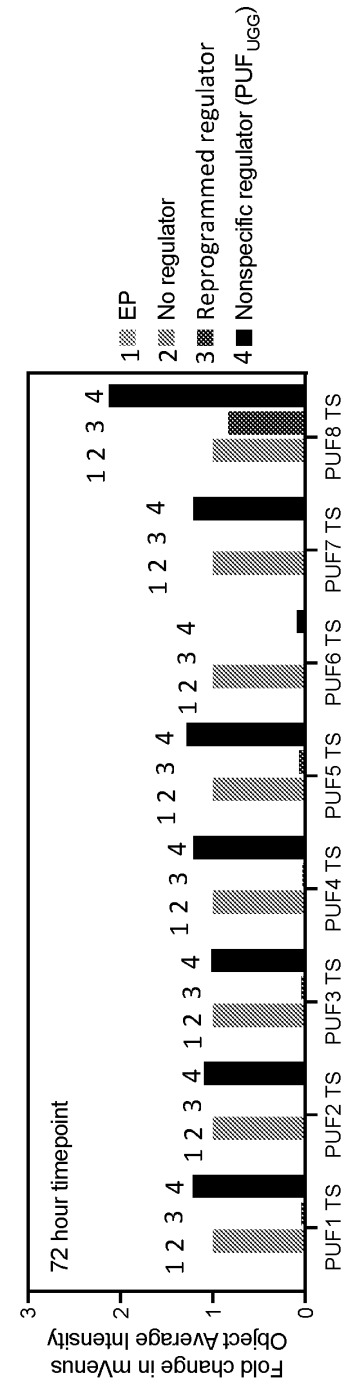


FIG. 11C

FIG. 11D

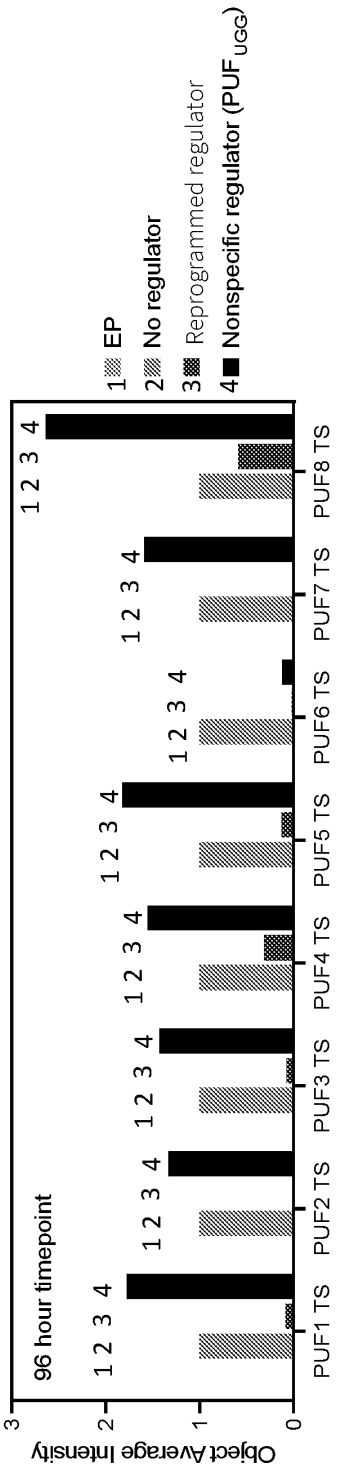
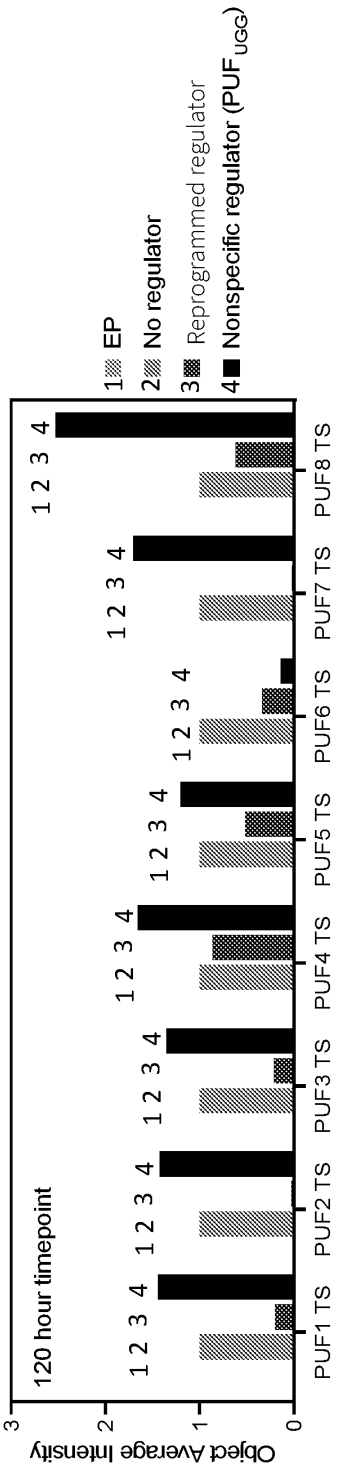


FIG. 11E



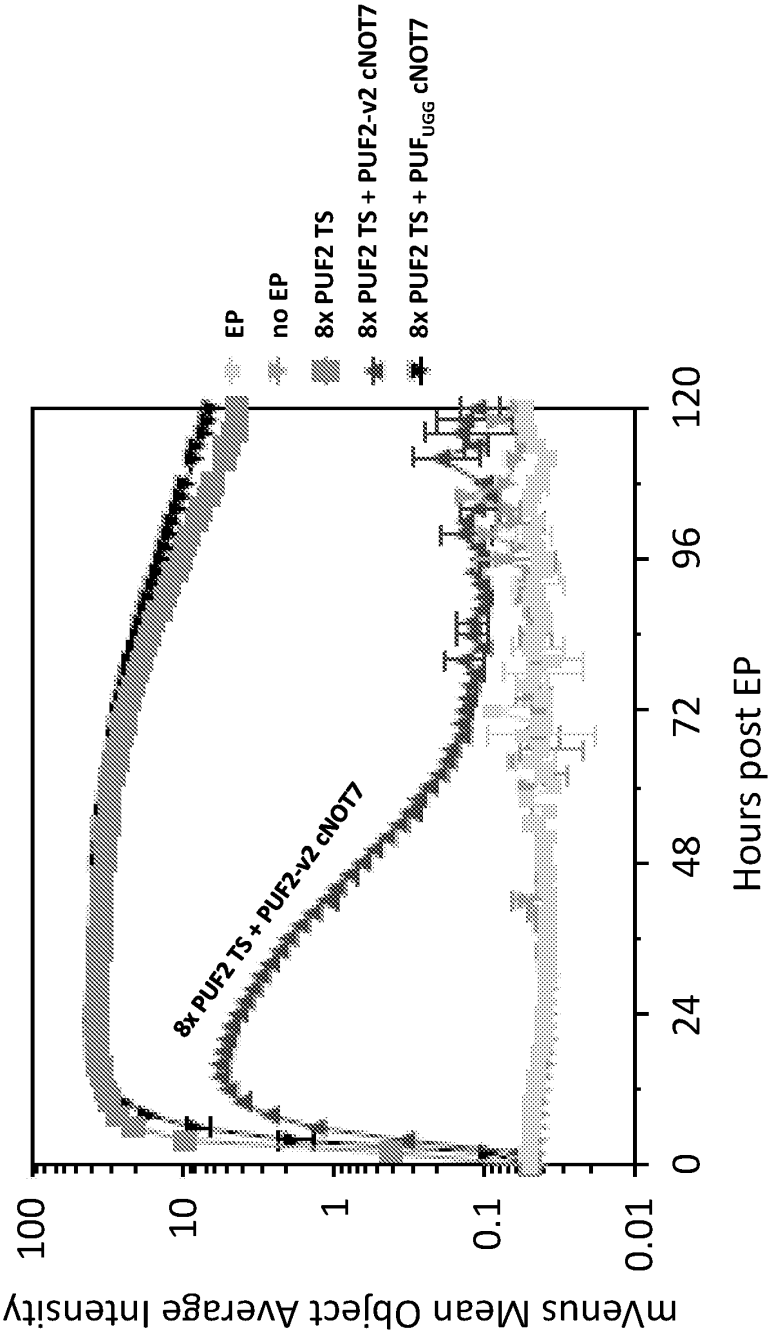

mVenus Mean Object Average Intensity
100
10
1
0.1
0.01
0
24
48
72
96
120
Hours post EP
EP
no EP
8x PUF2 TS
8x PUF2 TS + PUF2-v2 cNOT7
8x PUF2 TS + PUF2-v2 cNOT7
8x PUF2 TS + PUF$_{UGG}$ cNOT7

FIG. 12A.

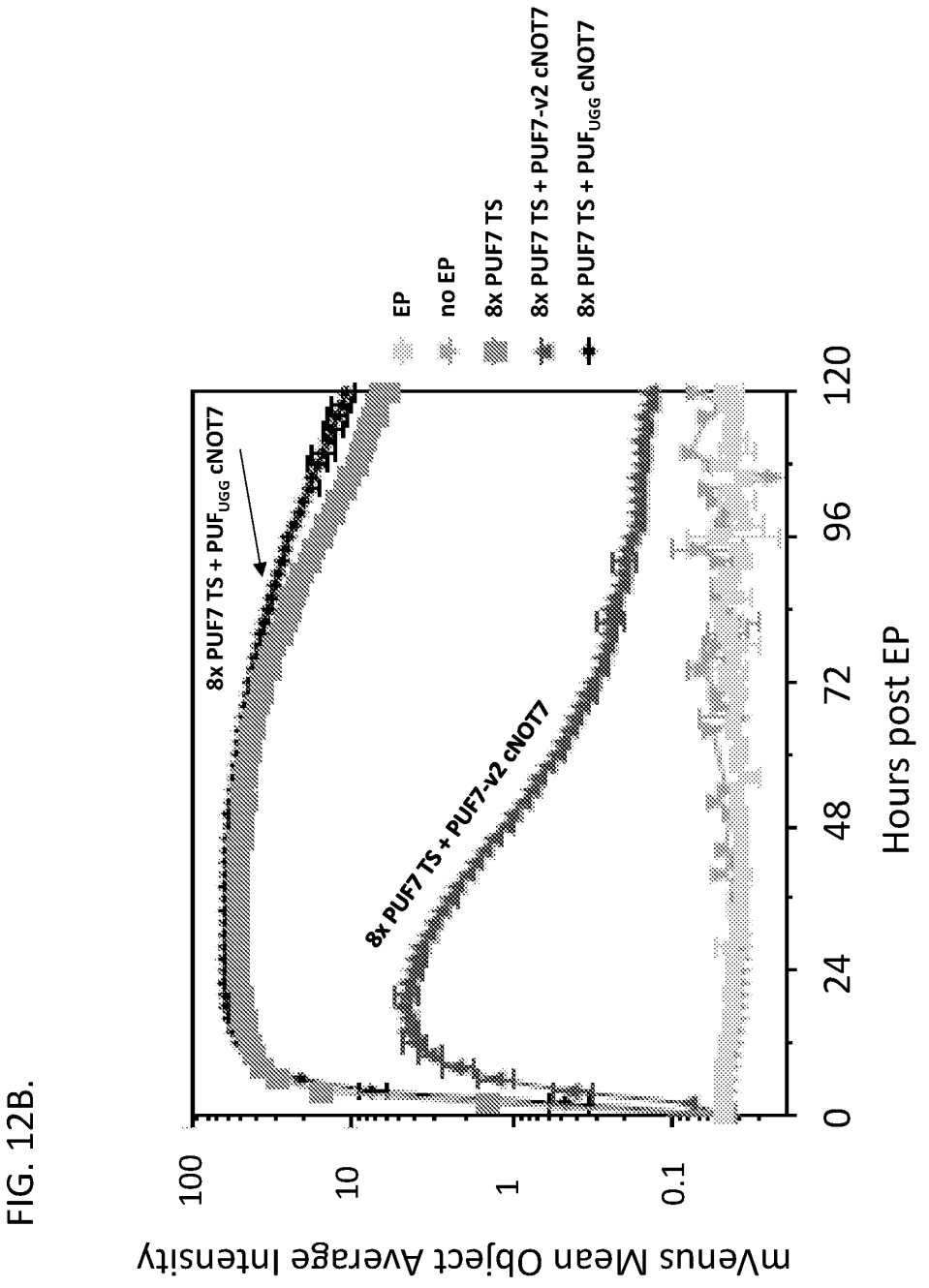

mVenus Mean Object Average Intensity
100
10
1
0.1
0
24
48
72
96
120
Hours post EP
EP
no EP
8x PUF7 TS
8x PUF7 TS + PUF7-v2 cNOT7
8x PUF7 TS + PUF$_{UGG}$ cNOT7
8x PUF7 TS + PUF$_{UGG}$ cNOT7
8x PUF7 TS + PUF7-v2 cNOT7

FIG. 12B.

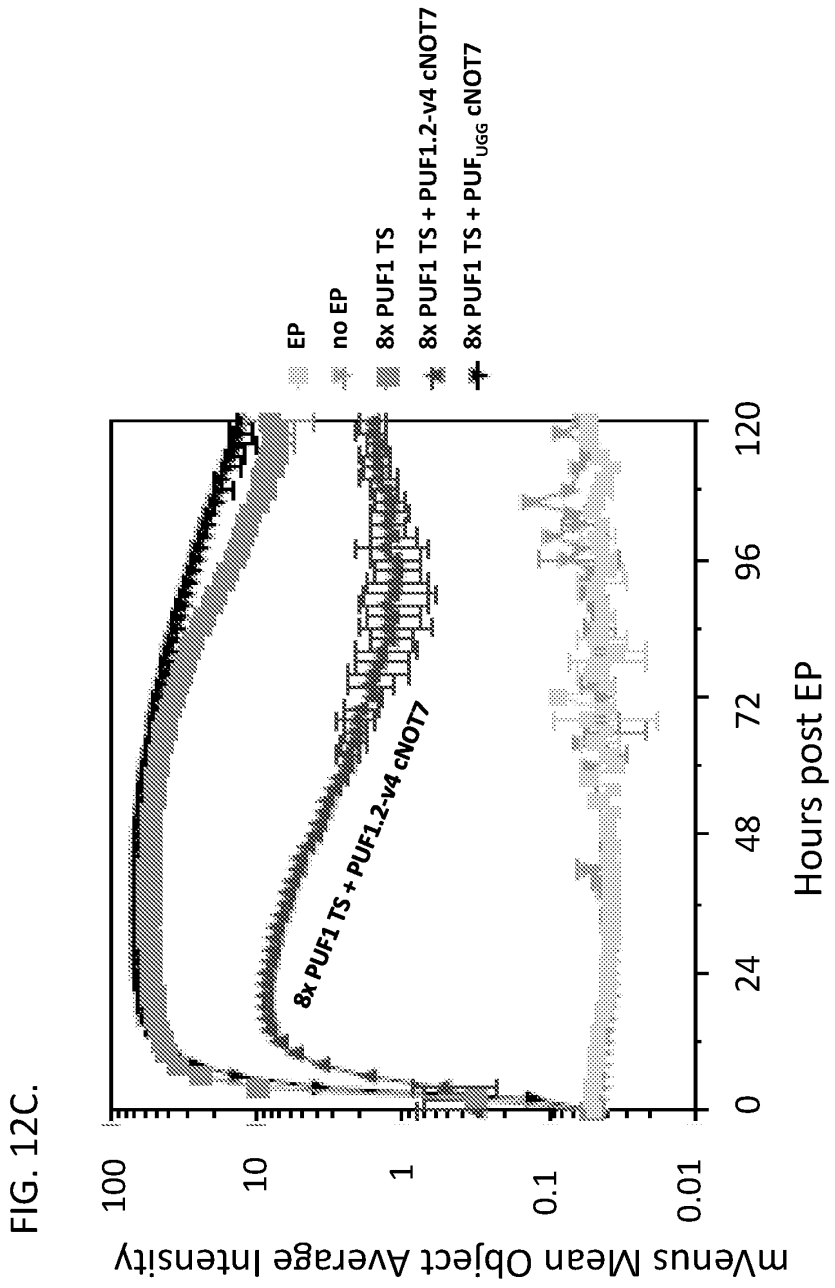

EP
no EP
8x PUF1 TS
8x PUF1 TS + PUF1.2-v4 cNOT7
8x PUF1 TS + PUF$_{UGG}$ cNOT7
8x PUF1 TS + PUF1.2-v4 cNOT7
mVenus Mean Object Average Intensity
100
10
1
0.1
0.01
0
24
48
72
96
120
Hours post EP

FIG. 12C.

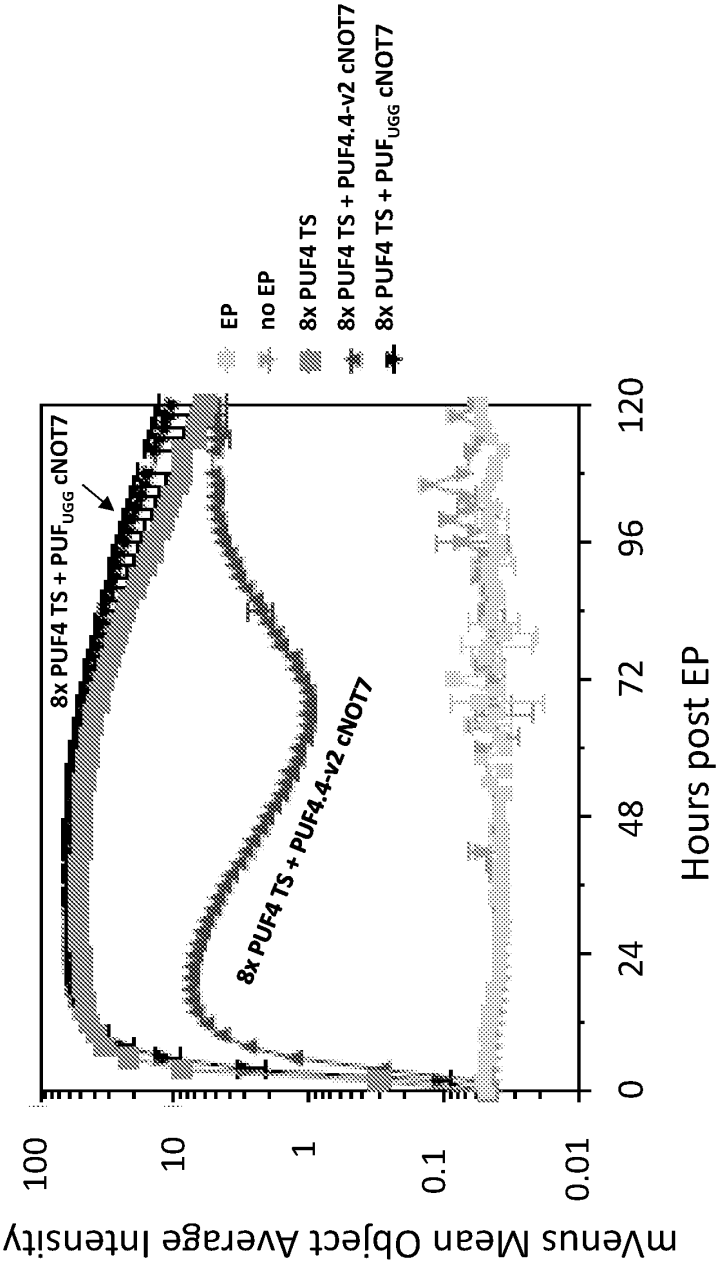

mVenus Mean Object Average Intensity
100
10
1
0.1
0.01
0
24
48
72
96
120
Hours post EP
EP
no EP
8x PUF4 TS
8x PUF4 TS + PUF4.4-v2 cNOT7
8x PUF4 TS + PUF$_{UGG}$ cNOT7
8x PUF4 TS + PUF$_{UGG}$ cNOT7
8x PUF4 TS + PUF4.4-v2 cNOT7

FIG. 12D.

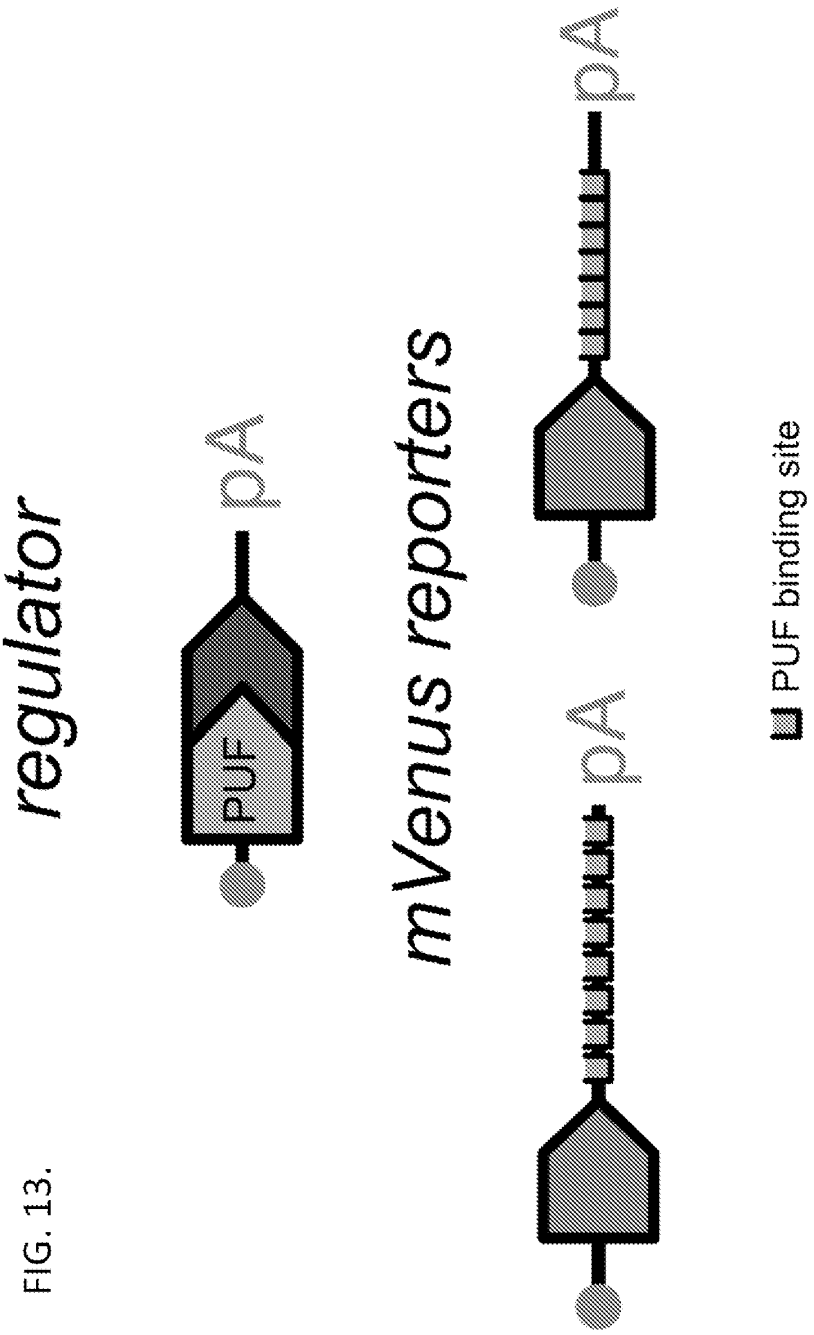


FIG. 13.

FIG. 14.

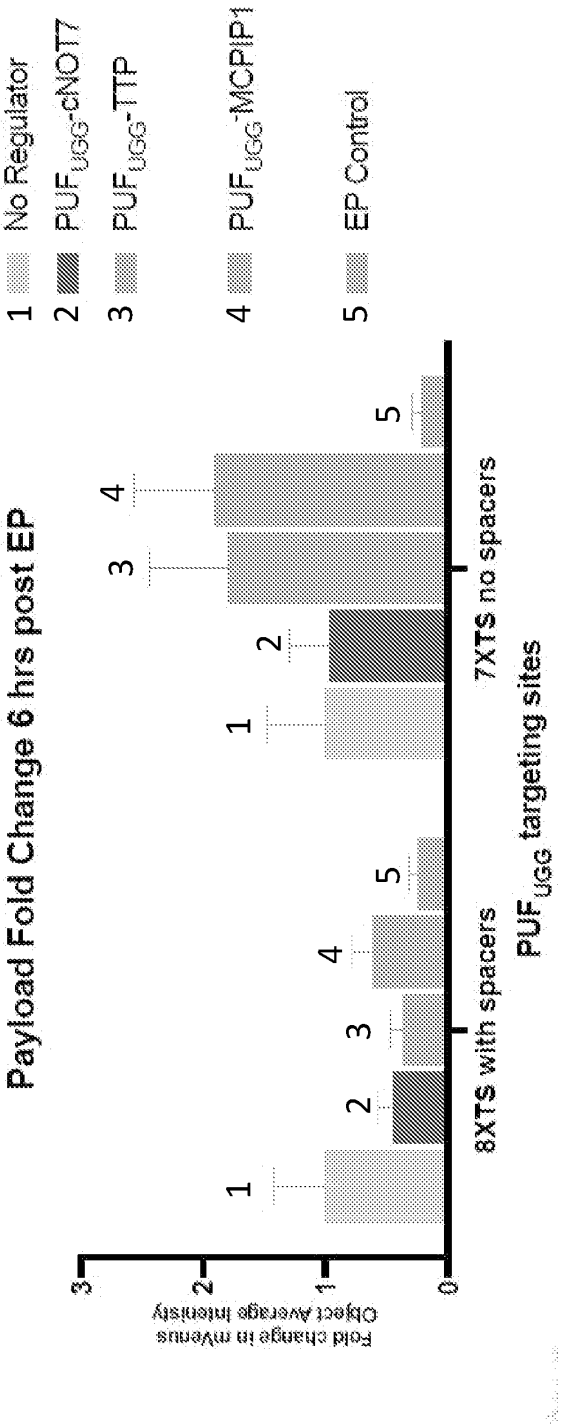

Payload Fold Change 6 hrs post EP
1 No Regulator
2 PUF$_{UGG}$-cNOT7
3 PUF$_{UGG}$-TTP
4 PUF$_{UGG}$-MCPIP1
5 EP Control
Fold change in mVenus Object Average Intensity
0
1
2
3
8XTS with spacers
7XTS no spacers
PUF$_{UGG}$ targeting sites

FIG. 15.

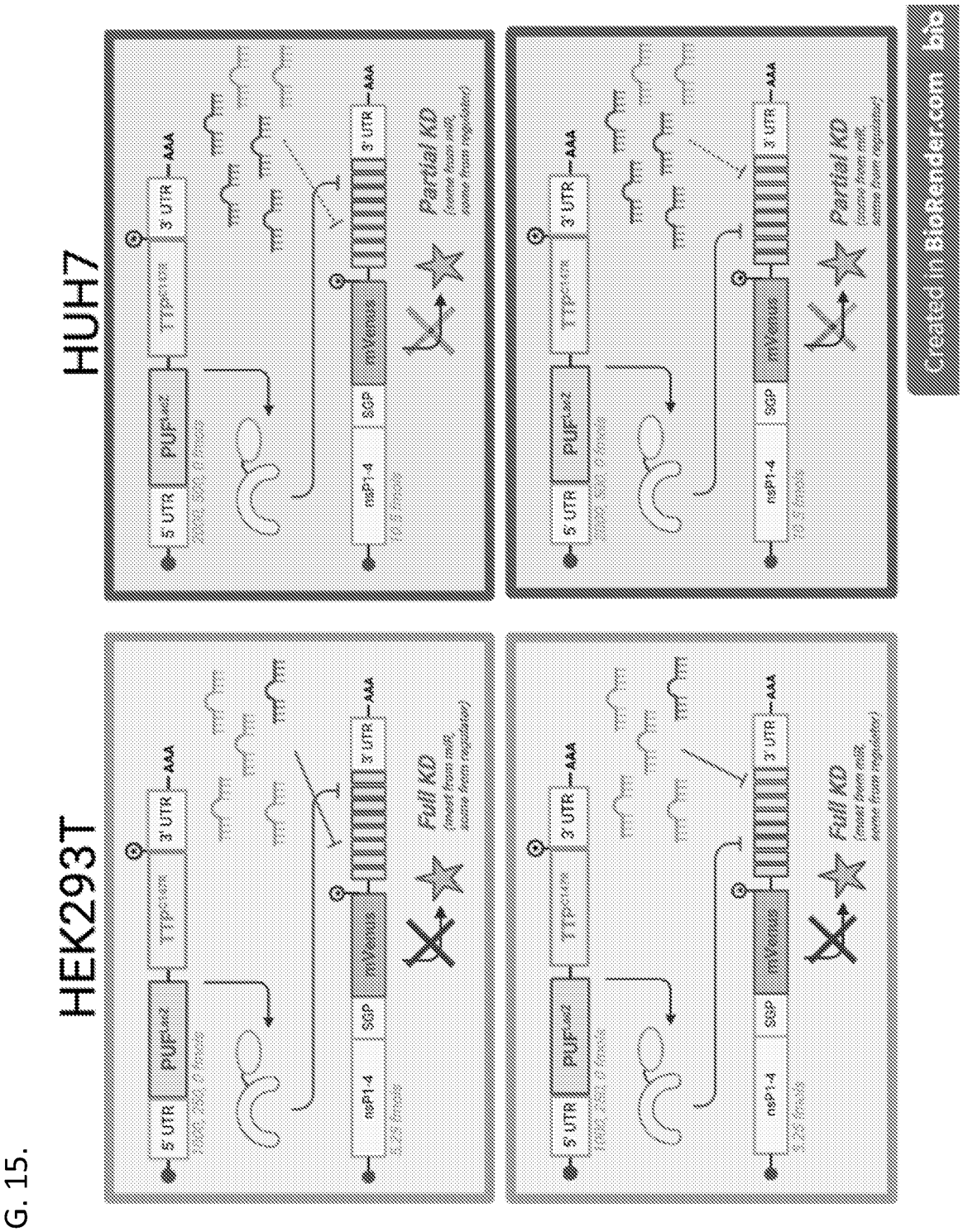
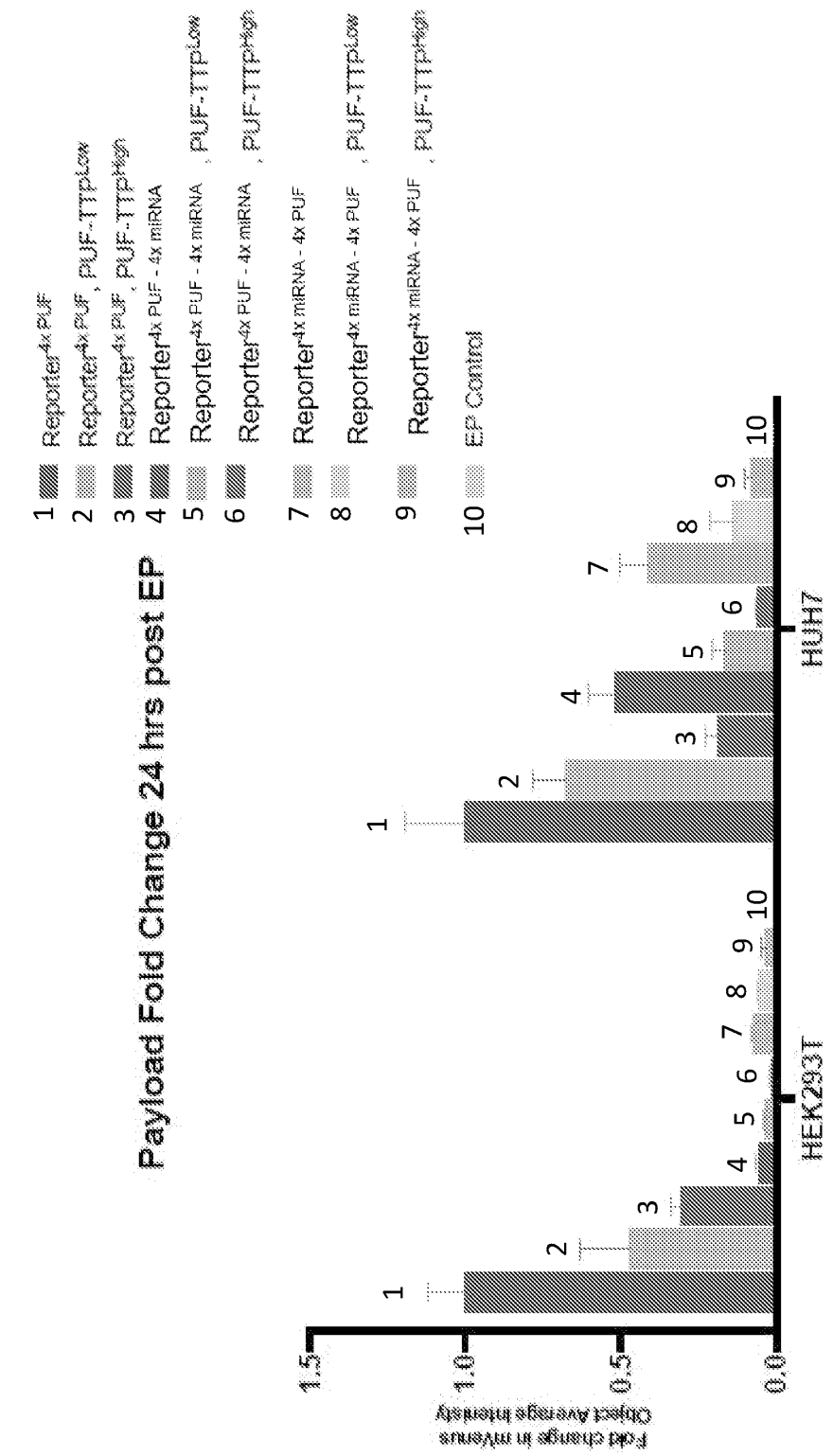

HEK293T
HUH7
5' UTR
PUF-like RBD 8 Pumilio
TTP-like
3' UTR
AAA
nsP1-4
SGP
mNeonGreen
Full KO
(most from miR, some from regulator)
Partial KO
(some from miR, some from regulator)
Created in BioRender.com

FIG. 16.


Payload Fold Change 24 hrs post EP
1 Reporter4x PUF
2 Reporter4x PUF, PUF-TTPLow
3 Reporter4x PUF, PUF-TTPHigh
4 Reporter4x PUF - 4x miRNA
5 Reporter4x PUF - 4x miRNA, PUF-TTPLow
6 Reporter4x PUF - 4x miRNA, PUF-TTPHigh
7 Reporter4x miRNA - 4x PUF
8 Reporter4x miRNA - 4x PUF, PUF-TTPLow
9 Reporter4x miRNA - 4x PUF, PUF-TTPHigh
10 EP Control
Fold change in mVenus Object Average Intensity
1.5
1.0
0.5
0.0
HEK293T
HUH7

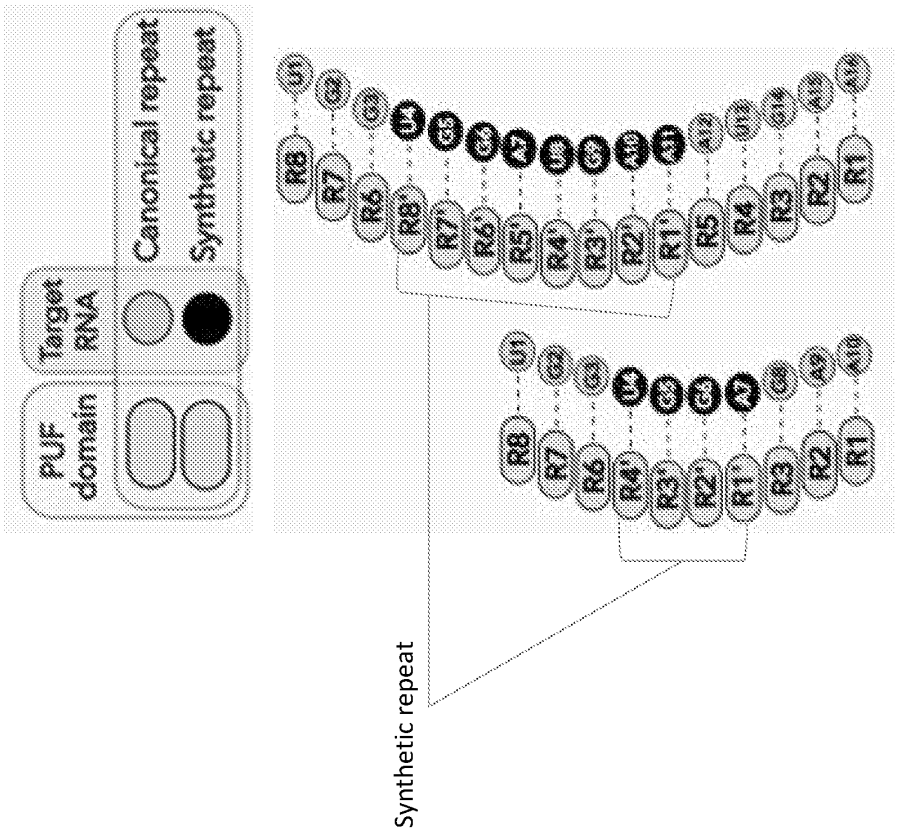

PUF domain
Target RNA
Canonical repeat
Synthetic repeat
Synthetic repeat

FIG. 17A.

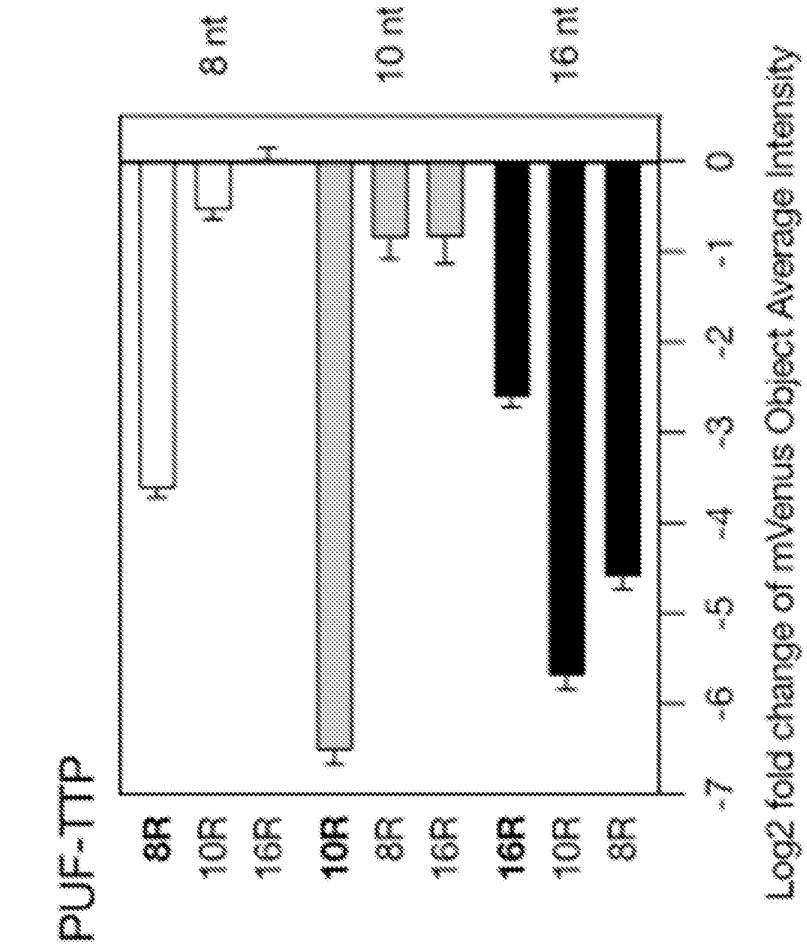

PUF-TTP
8 nt
10 nt
16 nt
8R
10R
16R
10R
8R
16R
16R
10R
8R
0
-1
-2
-3
-4
-5
-6
-7
Log2 fold change of mVenus Object Average Intensity

FIG. 17B.

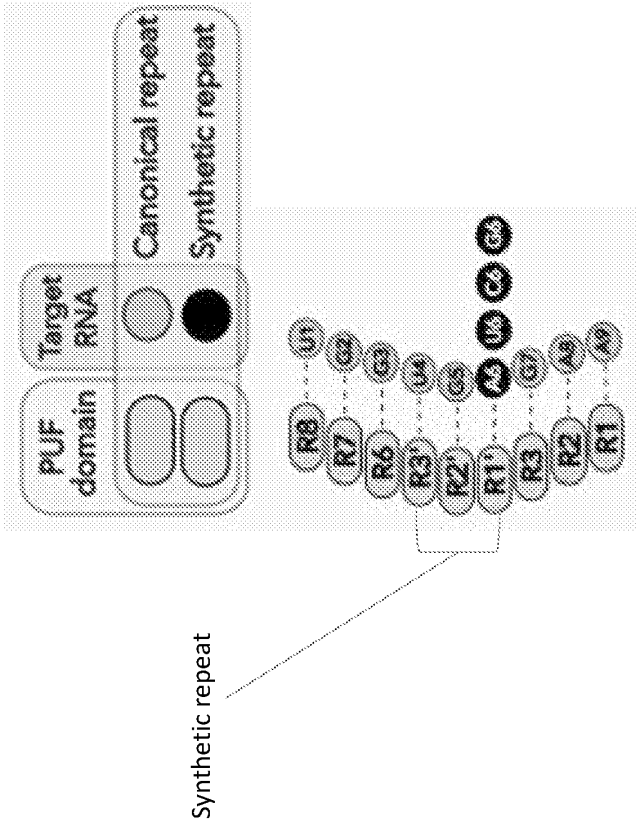

Canonical repeat
Synthetic repeat
Target RNA
PUF domain
G1
G2
G3
G4
G5
G6
G7
G8
R8
R7
R6
R5
R4
R3
R2
R1
Synthetic repeat

FIG. 18A.

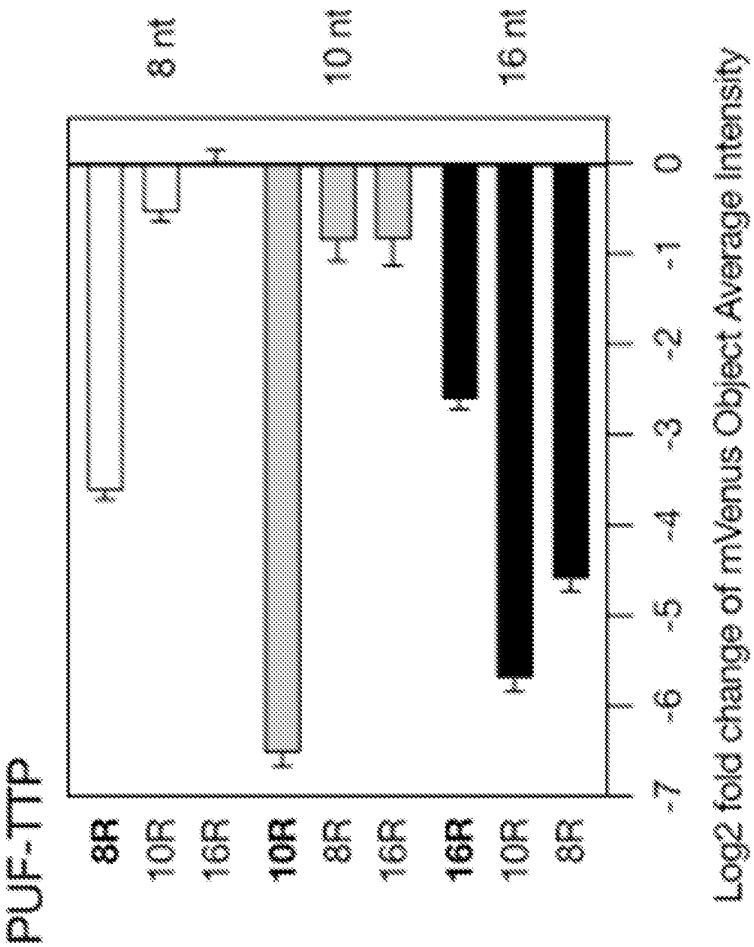

PUF-TTP
8R
10R
16R
10R
8R
16R
16R
10R
8R
8 nt
10 nt
16 nt
0
-1
-2
-3
-4
-5
-6
-7
Log2 fold change of mVenus Object Average Intensity

FIG. 18B.

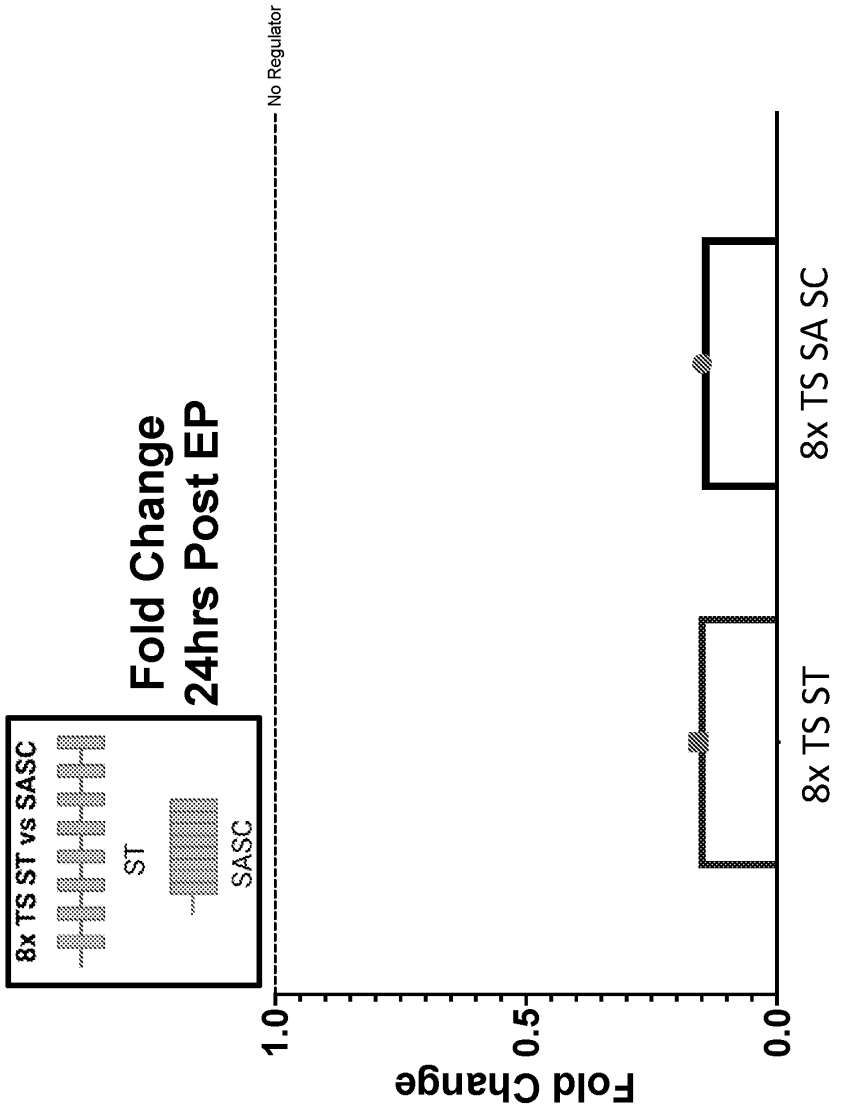

Fold Change
24hrs Post EP
8x TS ST vs SASC
ST
SASC
No Regulator
1.0
0.5
0.0
Fold Change
8x TS ST
8x TS SA SC

FIG. 19A.

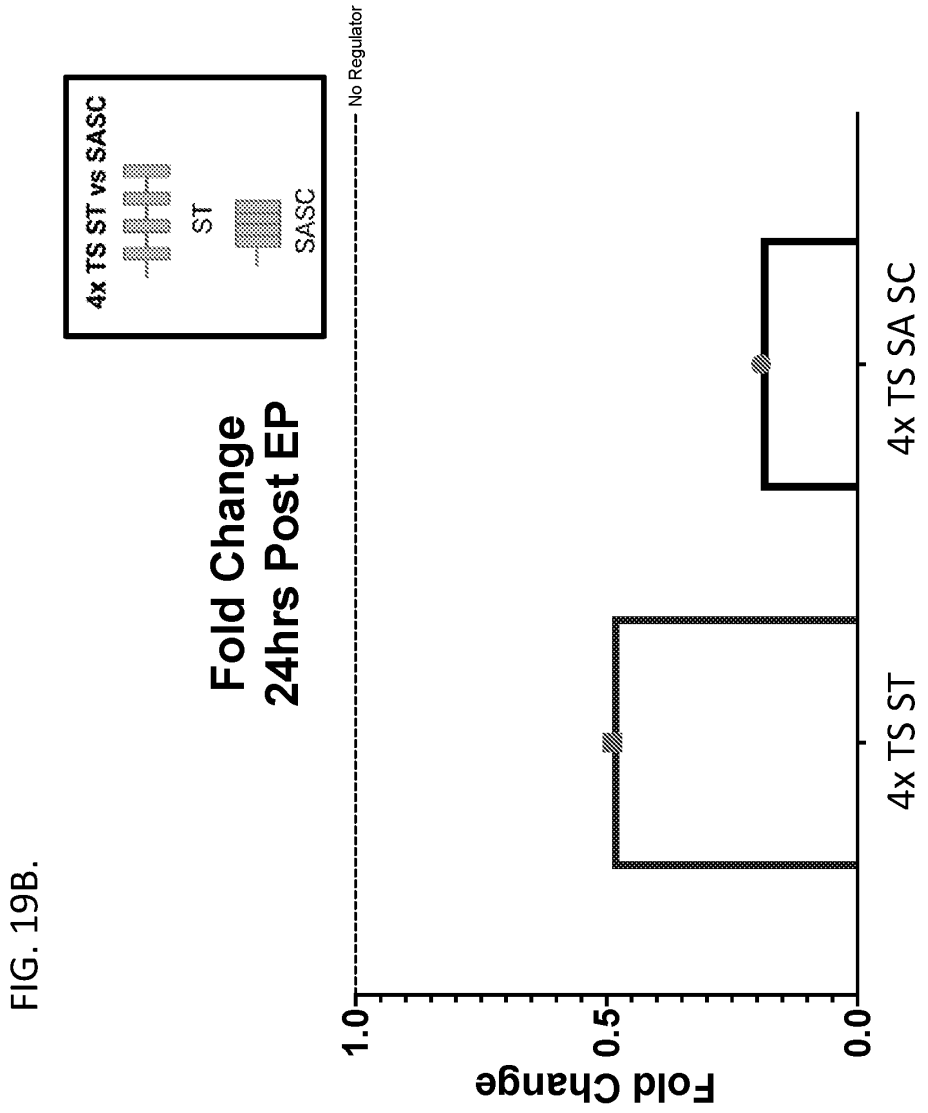
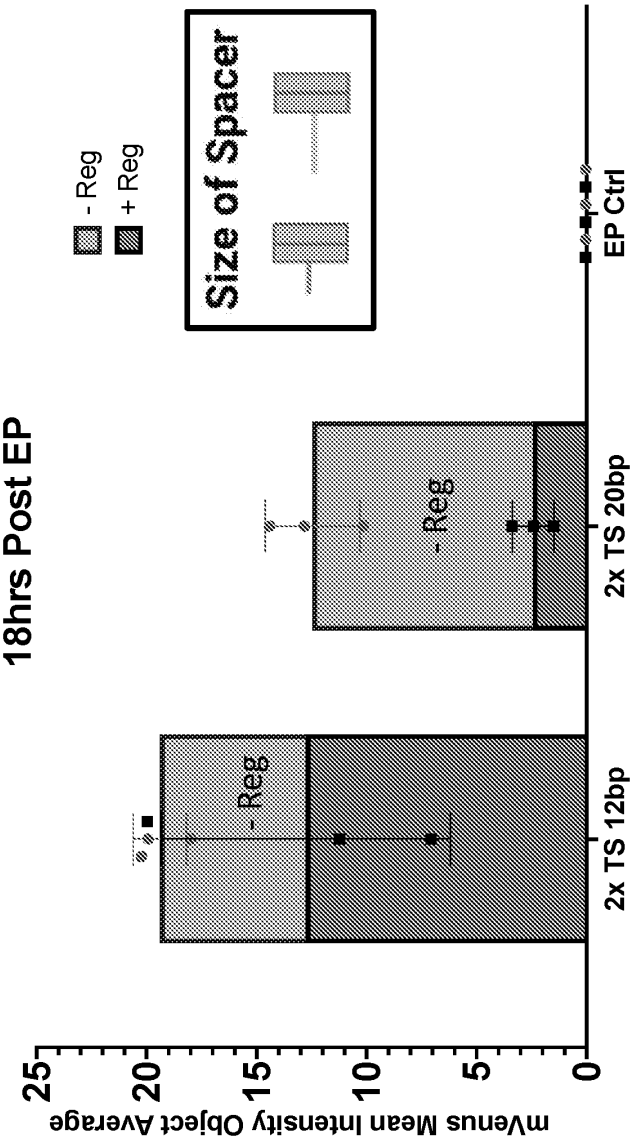

4x TS ST vs SASC
ST
SASC
Fold Change
24hrs Post EP
No Regulator
Fold Change
1.0
0.5
0.0
4x TS ST
4x TS SA SC

FIG. 19B.

FIG. 20.


Mean mVenus Expression
18hrs Post EP
mVenus Mean Intensity Object Average
25
20
15
10
5
0
- Reg
+ Reg
Size of Spacer
- Reg
- Reg
2x TS 12bp
2x TS 20bp
EP Ctrl

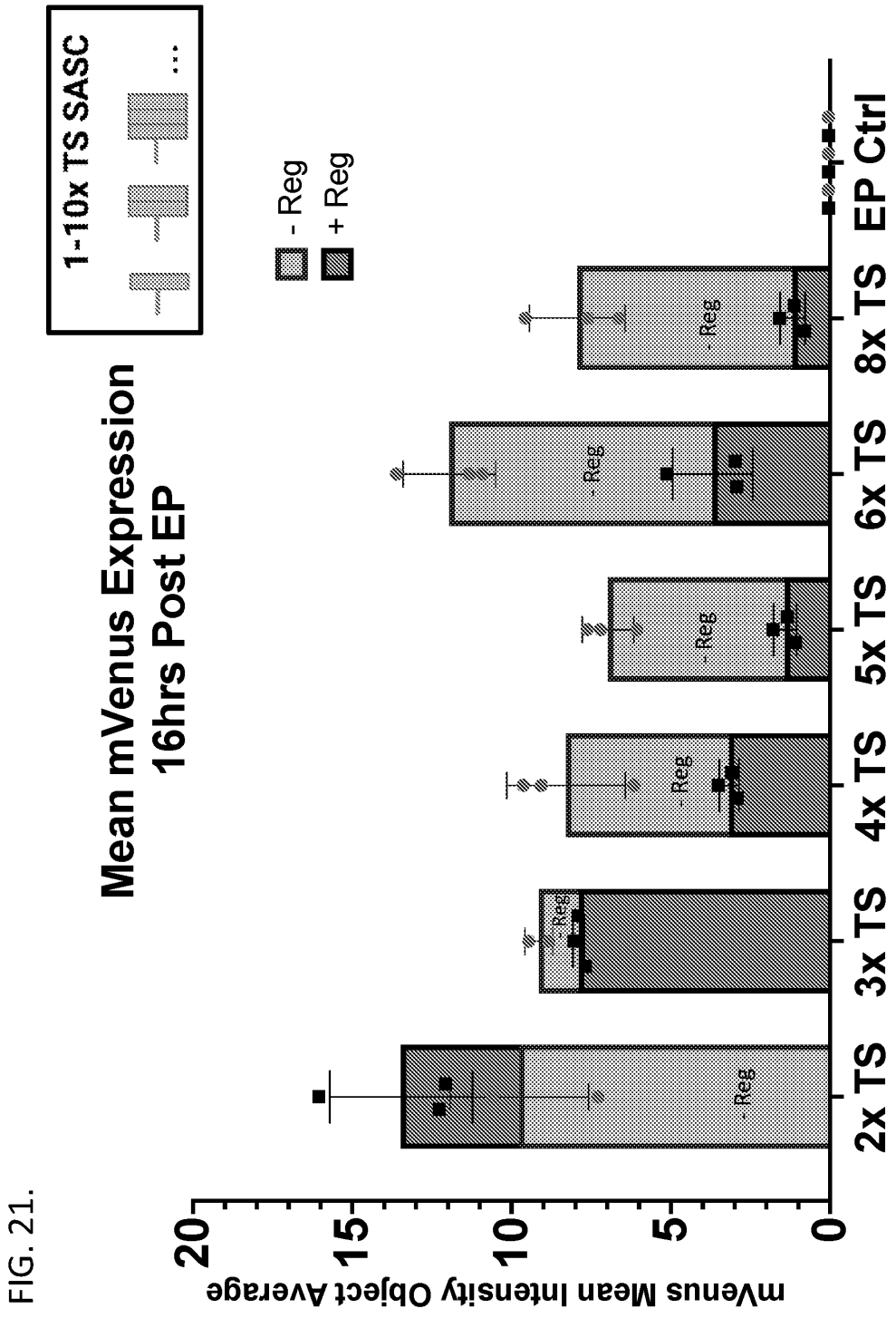

Mean mVenus Expression
16hrs Post EP
1-10x TS SASC
- Reg
+ Reg
mVenus Mean Intensity Object Average
0
5
10
15
20
2x TS
3x TS
4x TS
5x TS
6x TS
8x TS
EP Ctrl

FIG. 21.

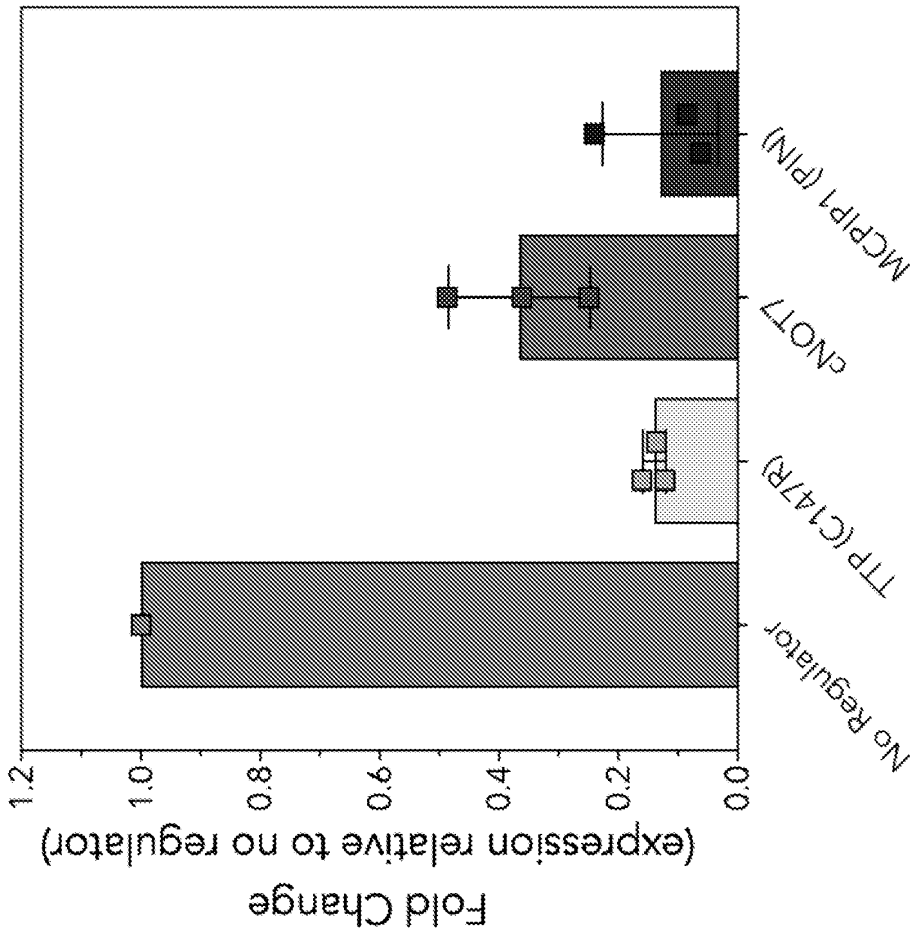

Fold Change
(expression relative to no regulator)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
No Regulator
TTP (C147R)
cNOT7
MCPIP1 (PIN)

FIG. 22.

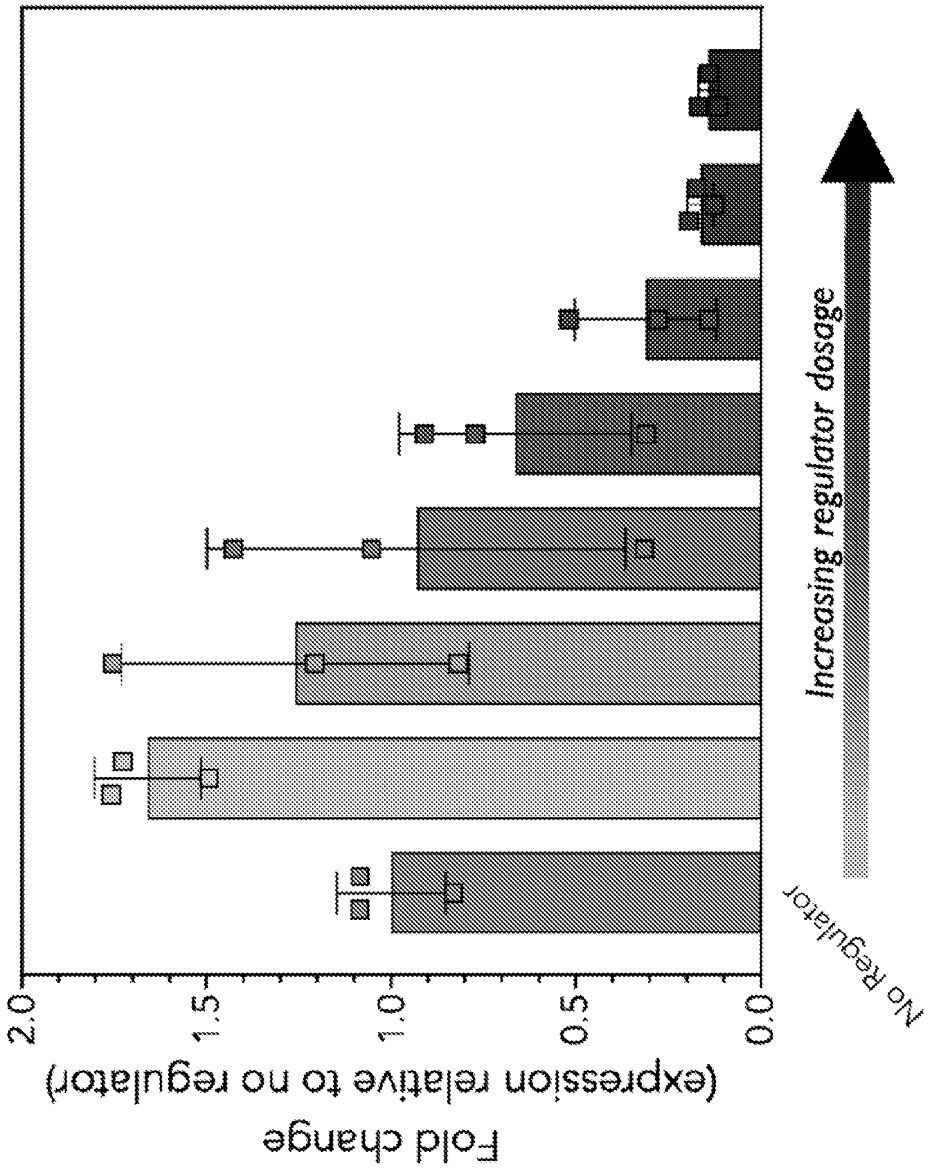

Fold change
(expression relative to no regulator)
2.0
1.5
1.0
0.5
0.0
No Regulator
Increasing regulator dosage

FIG. 23.

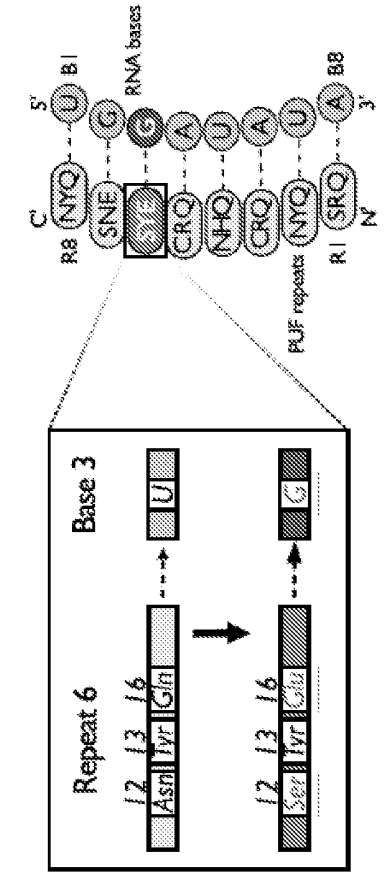


FIG. 24A.

FIG. 24B.

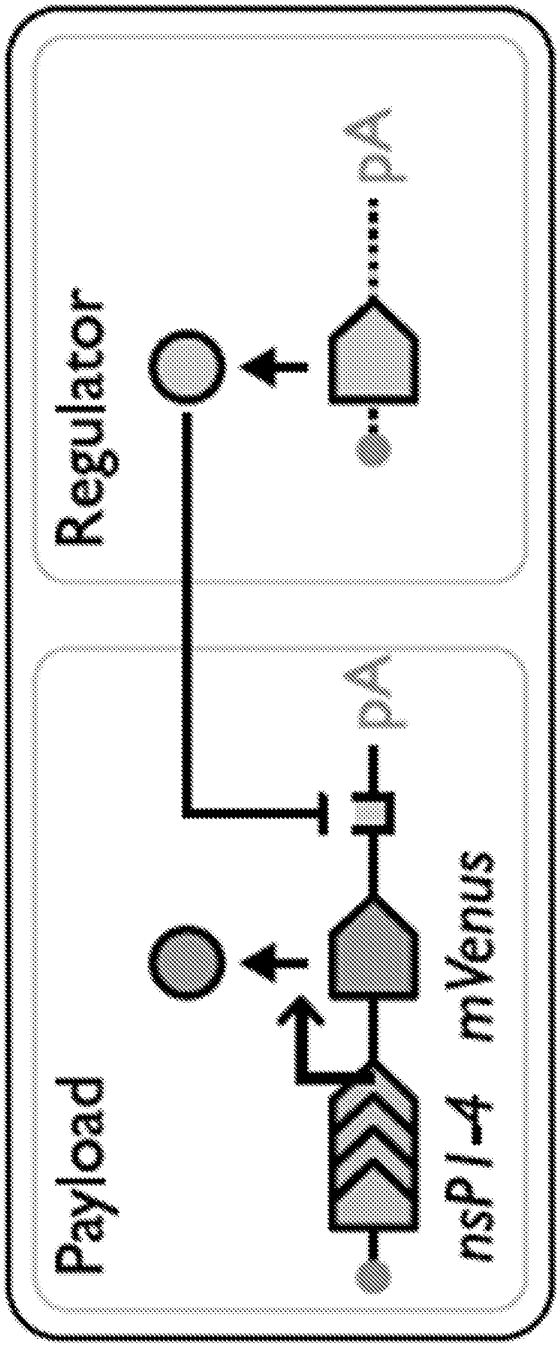


FIG. 24C.

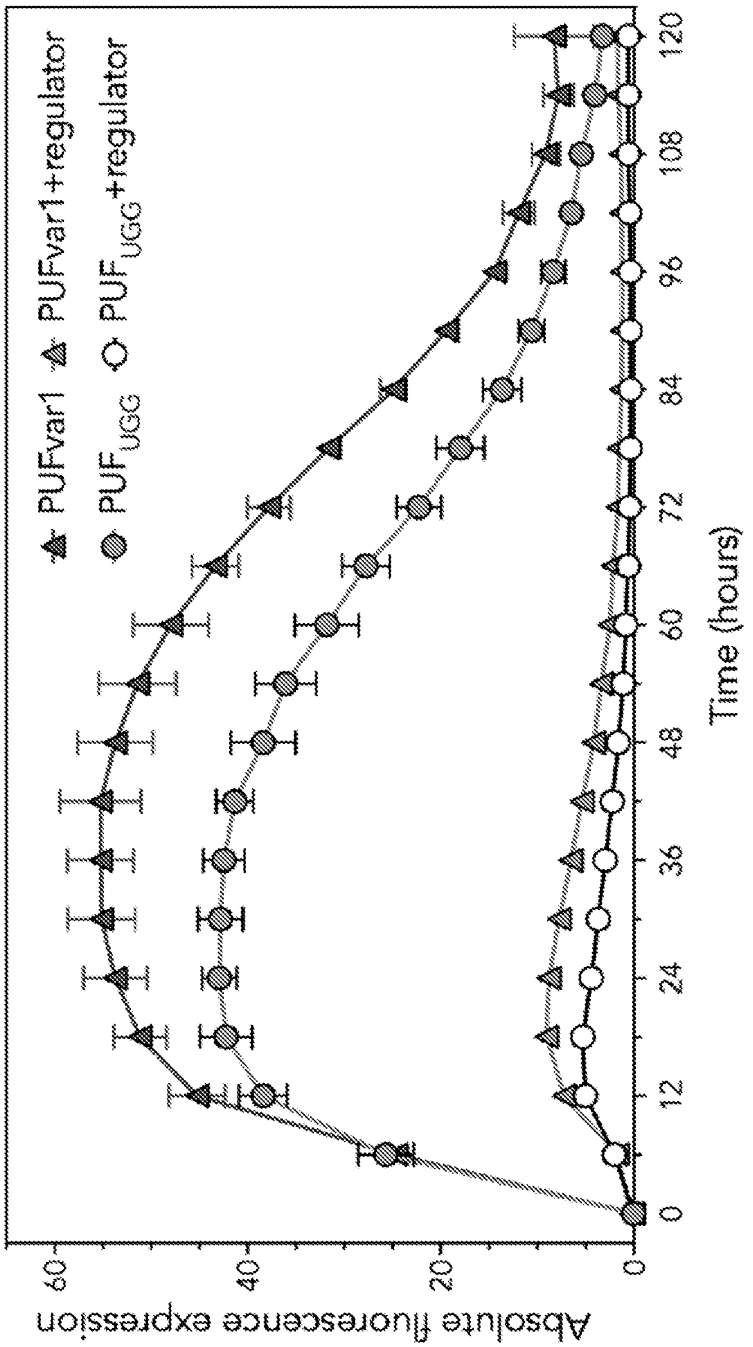

PUFvar1
PUFvar1+regulator
PUF$_{UGG}$
PUF$_{UGG}$+regulator
Absolute fluorescence expression
Time (hours)
0
20
40
60
0
12
24
36
48
60
72
84
96
108
120

FIG. 25.

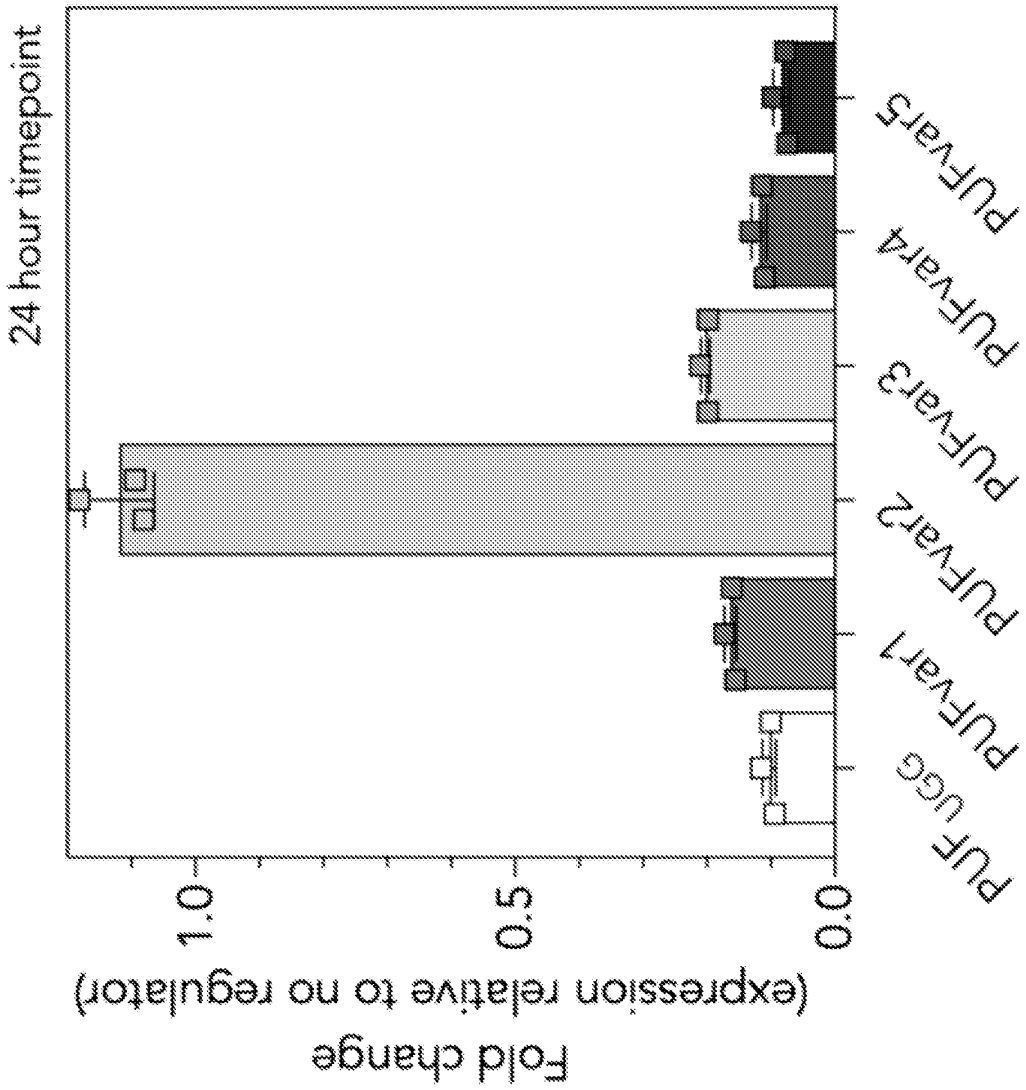
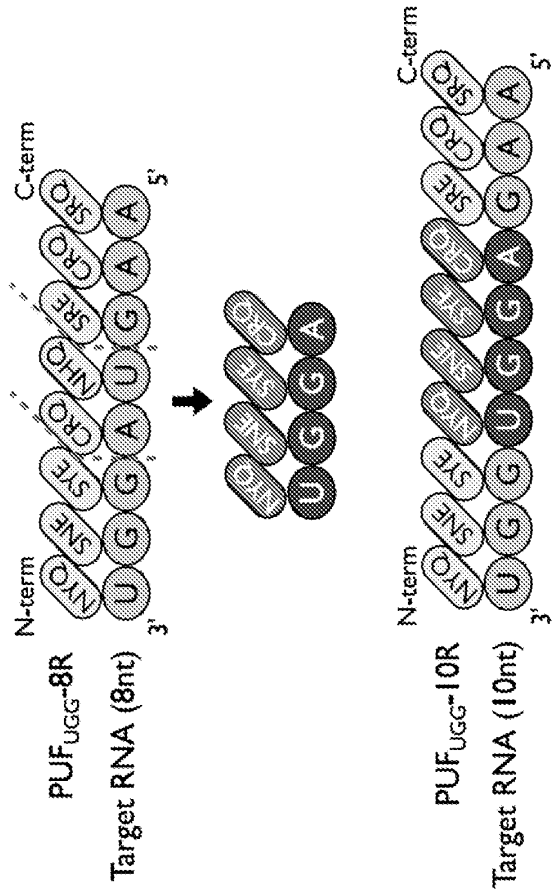

24 hour timepoint
Fold change
(expression relative to no regulator)
0.0
0.5
1.0
PUF UGG
PUFVar1
PUFVar2
PUFVar3
PUFVar4
PUFVar5

FIG. 26.

FIG. 27A.


N-term
C-term
PUF$_{UGG}$-8R
Target RNA (8nt)
3'
5'
PUF$_{UGG}$-10R
Target RNA (10nt)

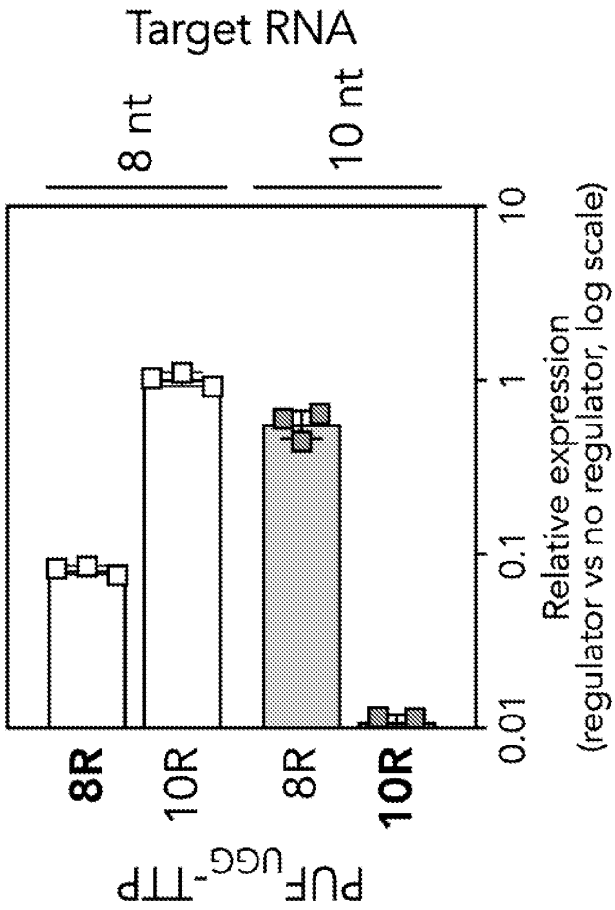

Target RNA
8 nt
10 nt
PUF$_{UGG}$-TTP
8R
10R
8R
10R
0.01
0.1
1
10
Relative expression
(regulator vs no regulator, log scale)

FIG. 27B.

FIG. 28A.

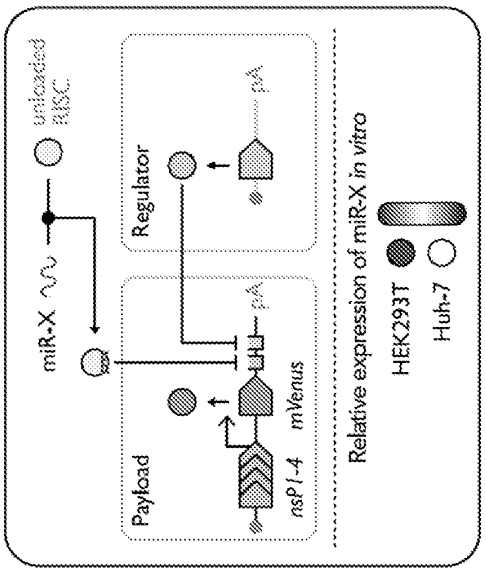


FIG. 28B.

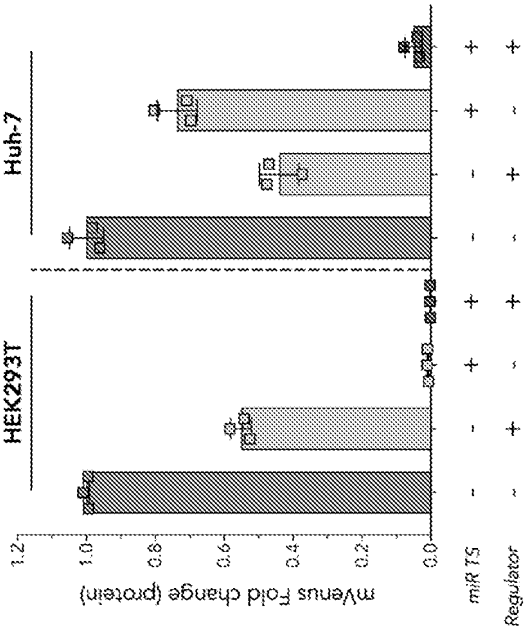


FIG. 28C.

FIG. 28D.

