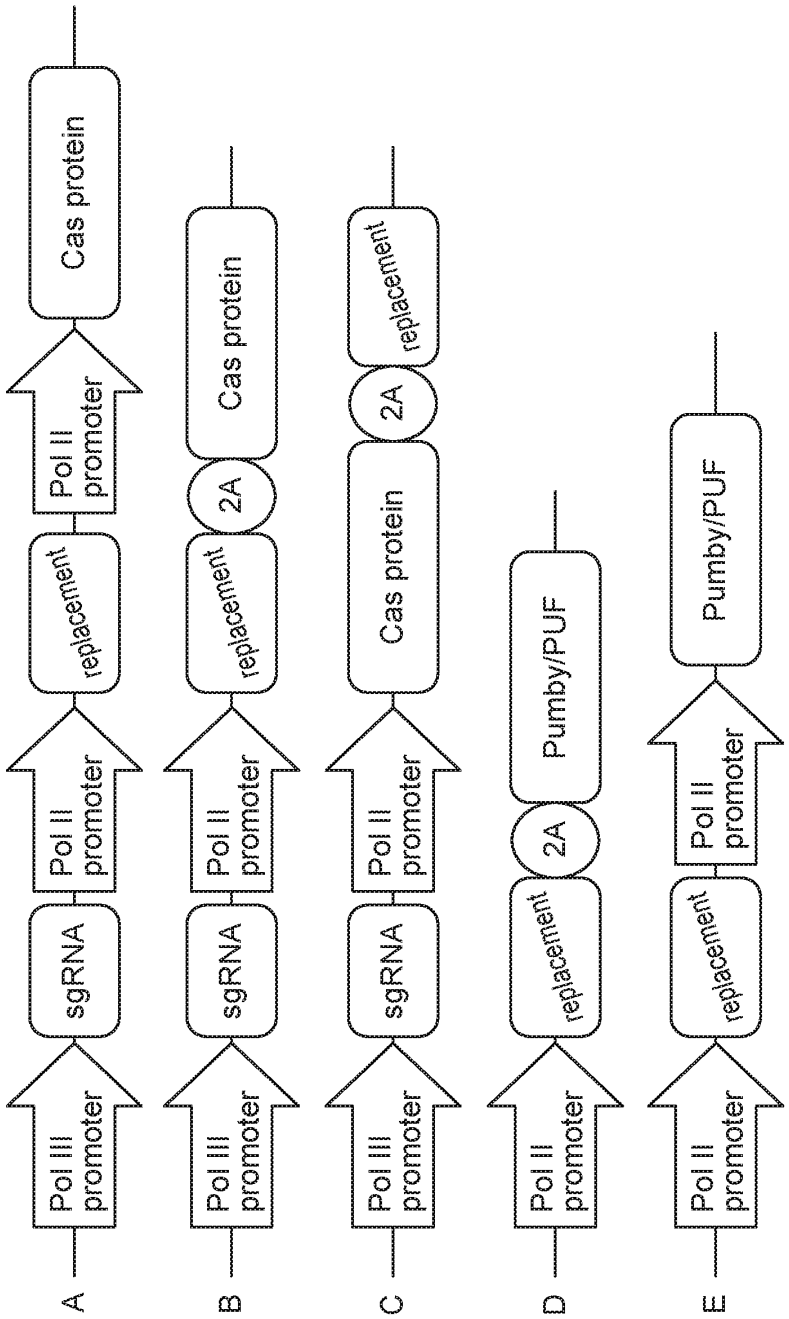

A
Pol III promoter
sgRNA
Pol II promoter
replacement
Pol II promoter
Cas protein
B
Pol III promoter
sgRNA
Pol II promoter
replacement
2A
Cas protein
C
Pol III promoter
sgRNA
Pol II promoter
Cas protein
2A
replacement
D
Pol II promoter
replacement
2A
Pumby/PUF
E
Pol II promoter
replacement
Pol II promoter
Pumby/PUF

FIG. 1

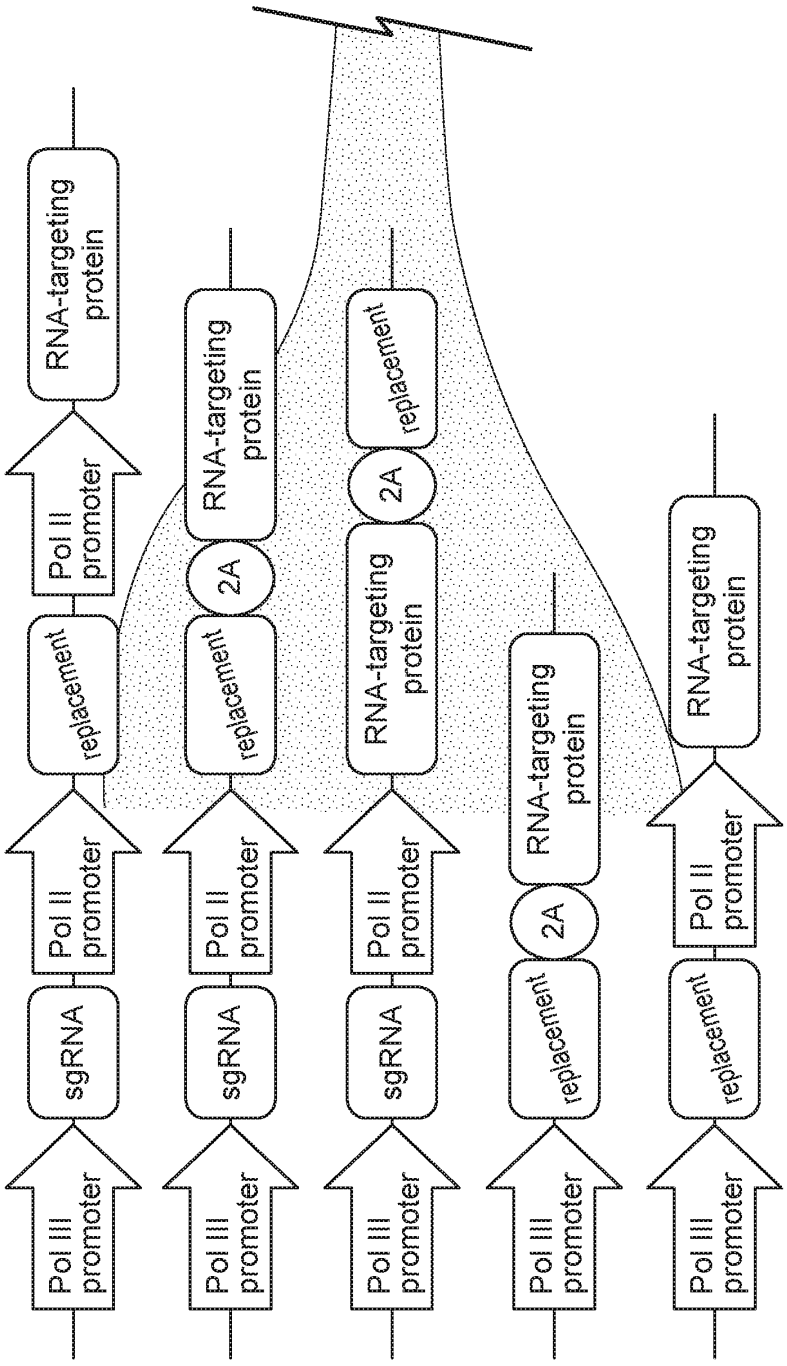

Pol III promoter
sgRNA
Pol II promoter
replacement
RNA-targeting protein
Pol III promoter
sgRNA
Pol II promoter
replacement
2A
RNA-targeting protein
Pol III promoter
sgRNA
Pol II promoter
RNA-targeting protein
2A
replacement
Pol III promoter
replacement
2A
RNA-targeting protein
Pol III promoter
replacement
Pol II promoter
RNA-targeting protein

FIG. 2

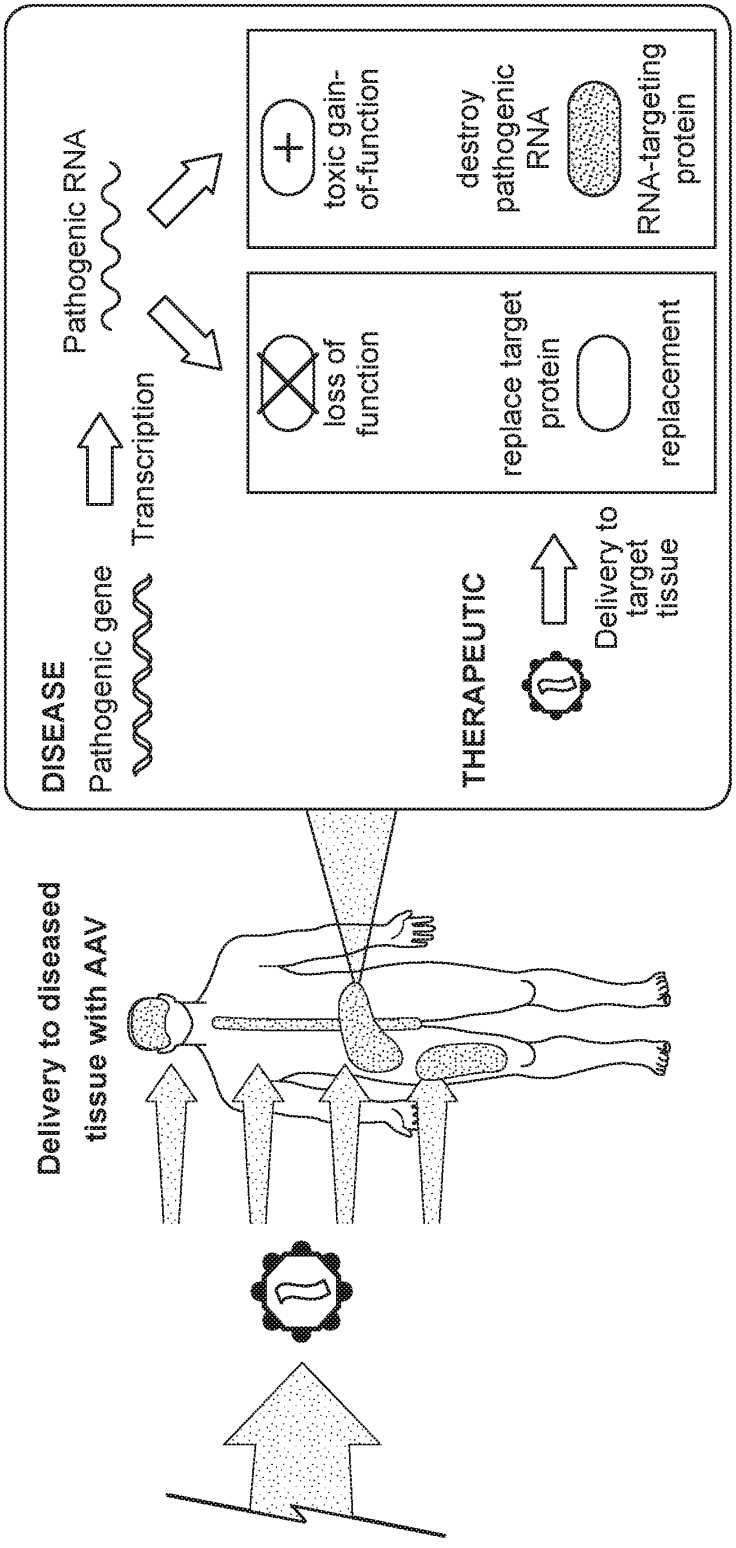

DISEASE
Pathogenic gene
Transcription
Pathogenic RNA
toxic gain-of-function
destroy pathogenic RNA
RNA-targeting protein
loss of function
replace target protein
replacement
THERAPEUTIC
Delivery to target tissue
Delivery to diseased tissue with AAV

FIG. 2 (Cont.)

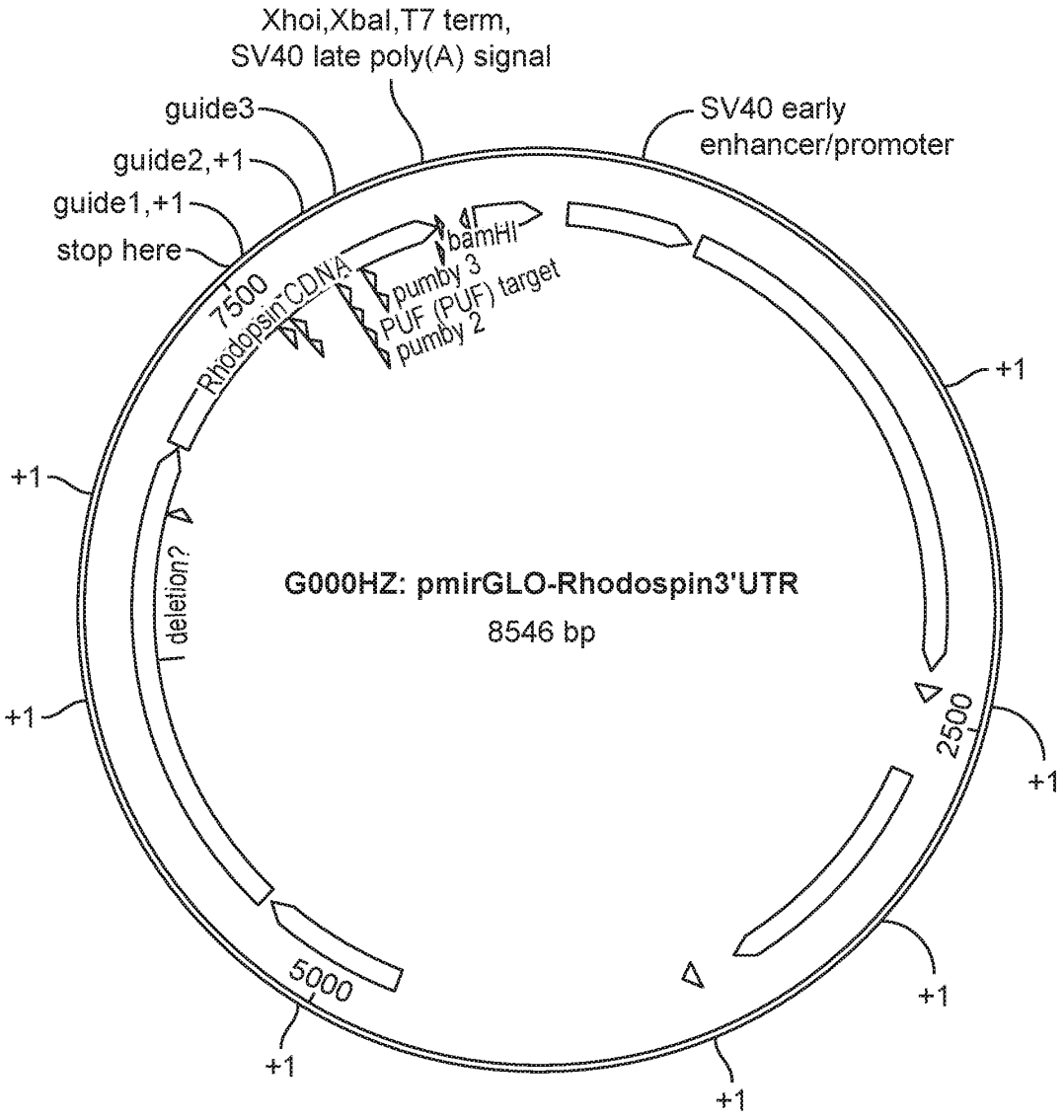

Xhoi,Xbal,T7 term,
SV40 late poly(A) signal
guide3
guide2,+1
guide1,+1
stop here
SV40 early
enhancer/promoter
7500
Rhodopsin CDNA
pumby 3
PUF (PUF) target
pumby 2
bamHI
+1
+1
deletion?
G000HZ: pmirGLO-Rhodospin3'UTR
8546 bp
+1
+1
2500
+1
5000
+1
+1
+1

FIG. 3

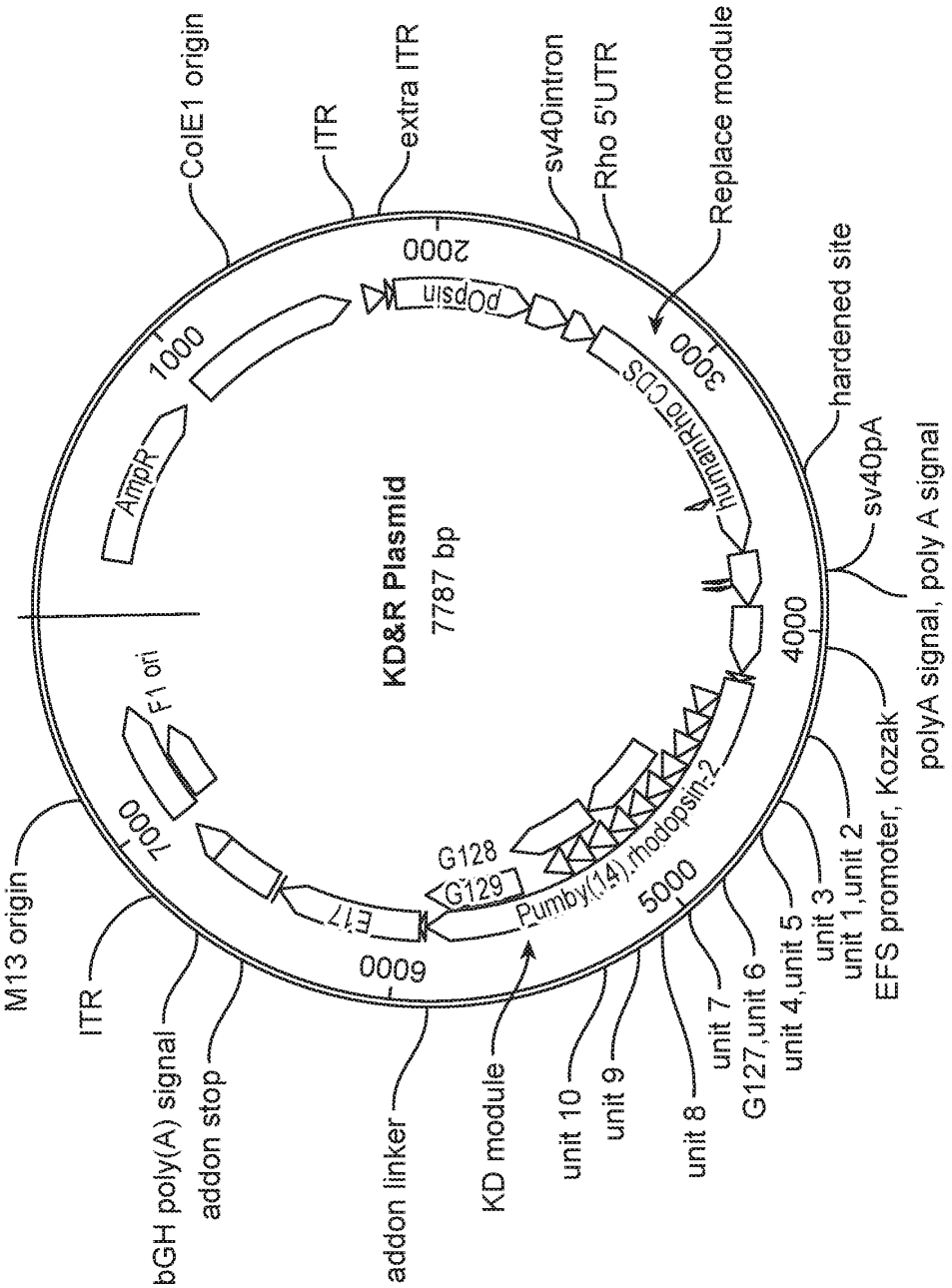

KD&R Plasmid
7787 bp
1000
2000
3000
4000
5000
6000
7000
ColE1 origin
ITR
extra ITR
sv40intron
Rho 5'UTR
Replace module
hardened site
sv40pA
polyA signal, poly A signal
EFS promoter, Kozak
unit 1,unit 2
unit 3
unit 4,unit 5
G127,unit 6
unit 7
unit 8
unit 9
unit 10
KD module
addon linker
bGH poly(A) signal
addon stop
ITR
M13 origin
AmpR
F1 ori
E17
G129
G128
Pumby(14).rhodopsin:2
hunanRho CDS
POpsin

FIG. 4

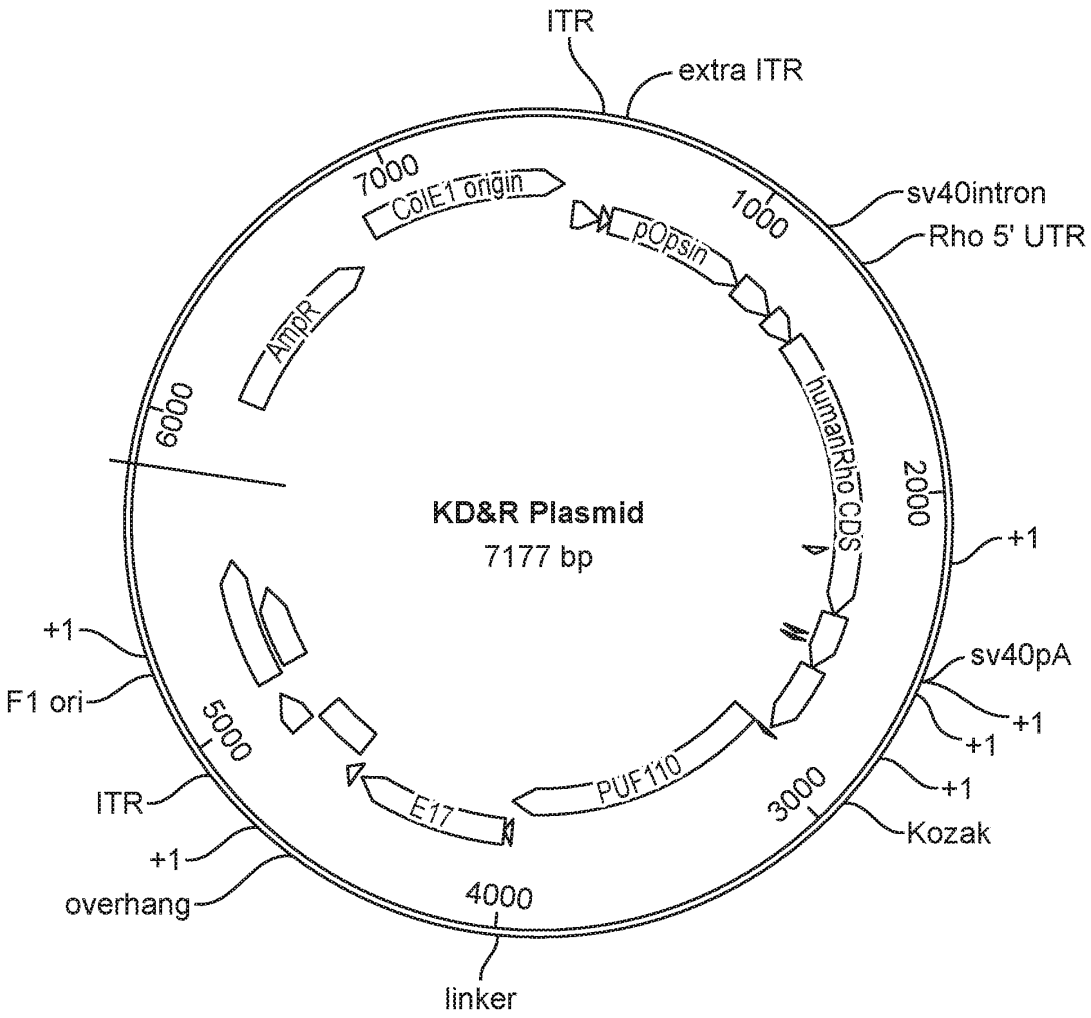

ITR
extra ITR
7000
ColE1 origin
1000
sv40intron
Rho 5' UTR
pOpsin
AmpR
6000
humanRho CDS
2000
KD&R Plasmid
7177 bp
+1
+1
sv40pA
F1 ori
5000
+1
+1
ITR
PUF110
E17
3000
+1
Kozak
+1
overhang
4000
linker

FIG. 5

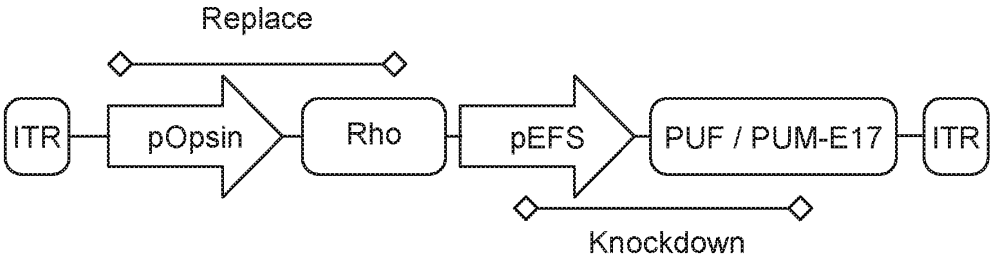

Replace
ITR
pOpsin
Rho
pEFS
PUF / PUM-E17
ITR
Knockdown

FIG. 6A

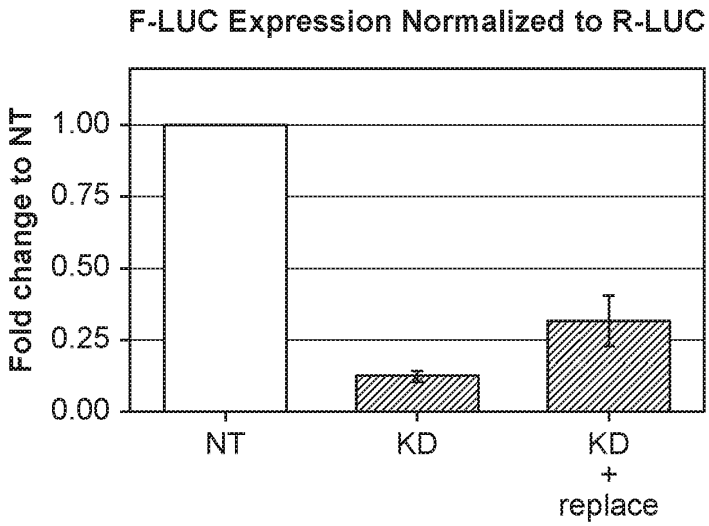

F-LUC Expression Normalized to R-LUC
Fold change to NT
1.00
0.75
0.50
0.25
0.00
NT
KD
KD
+
replace

FIG. 6B

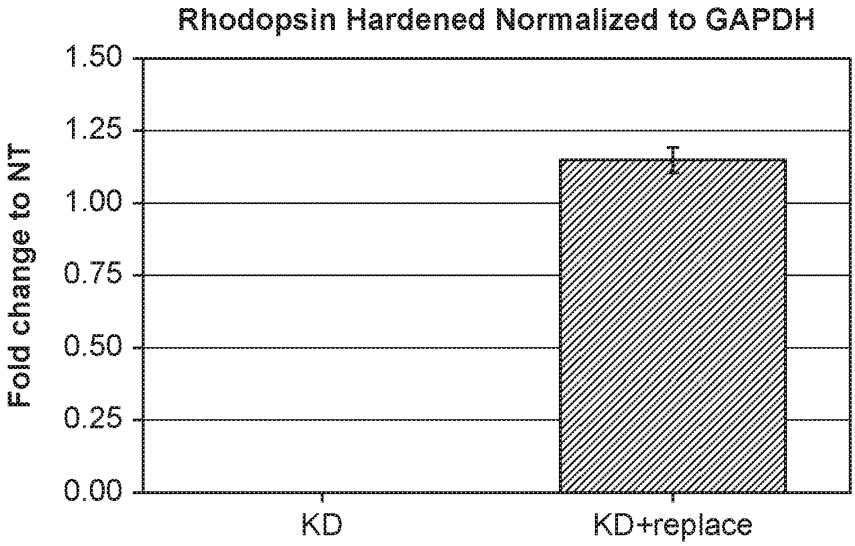

Rhodopsin Hardened Normalized to GAPDH
Fold change to NT
1.50
1.25
1.00
0.75
0.50
0.25
0.00
KD
KD+replace

FIG. 6C

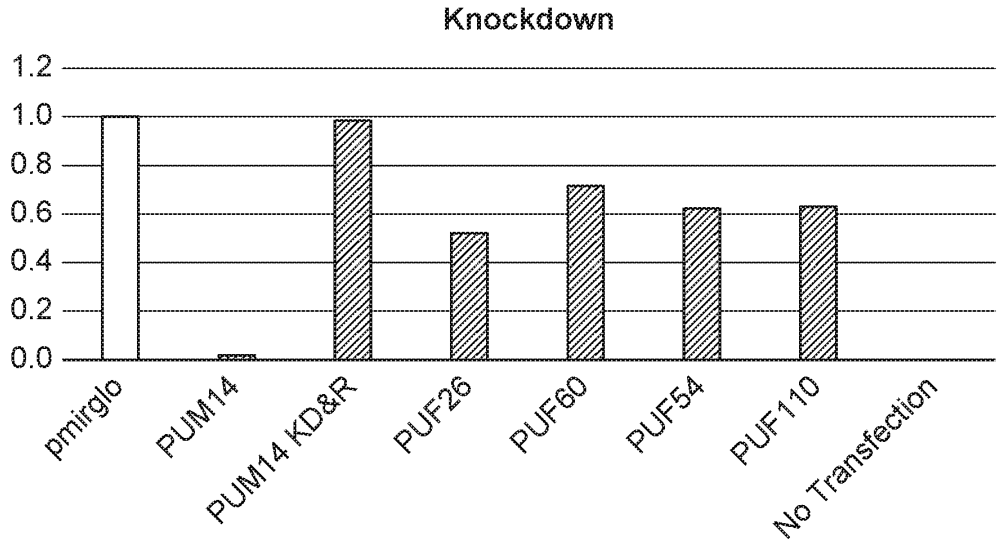

Knockdown
1.2
1.0
0.8
0.6
0.4
0.2
0.0
pmirglo
PUM14
PUM14 KD&R
PUF26
PUF60
PUF54
PUF110
No Transfection

FIG. 7A

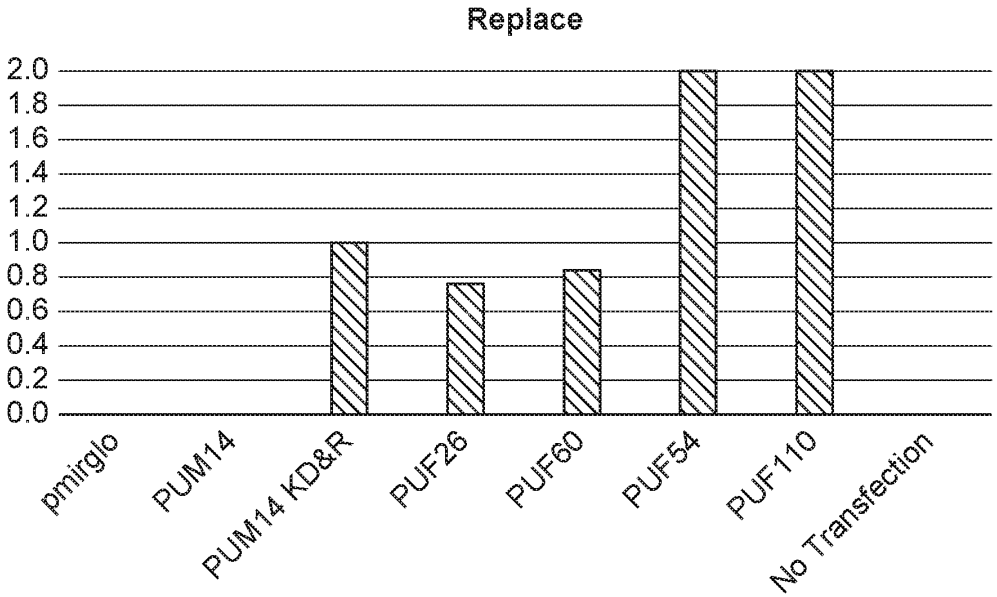

Replace
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
pmirglo
PUM14
PUM14 KD&R
PUF26
PUF60
PUF54
PUF110
No Transfection

FIG. 7B

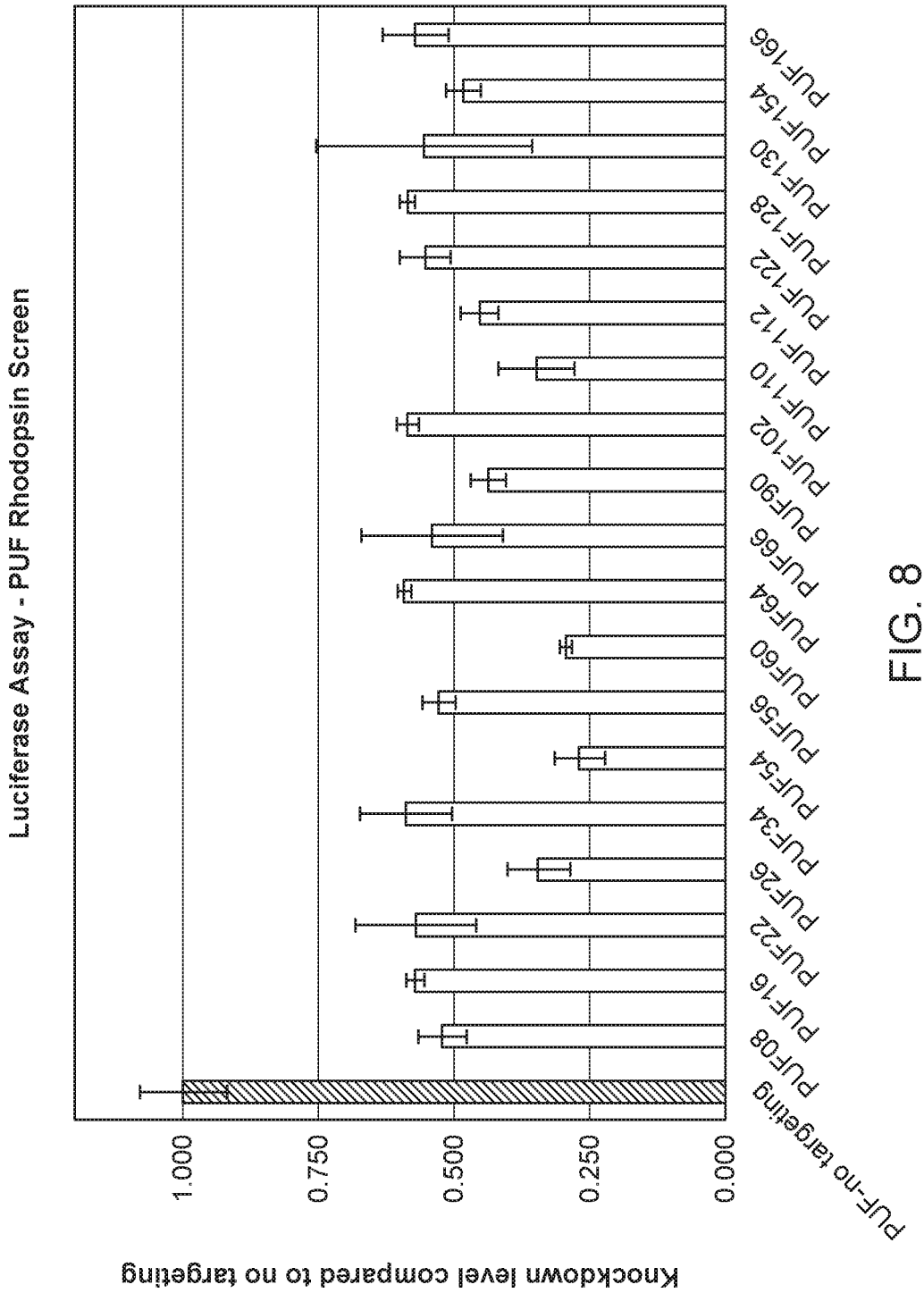

Luciferase Assay - PUF Rhodopsin Screen
Knockdown level compared to no targeting
1.000
0.750
0.500
0.250
0.000
PUF-no targeting
PUF08
PUF16
PUF22
PUF26
PUF34
PUF54
PUF56
PUF60
PUF64
PUF66
PUF90
PUF102
PUF110
PUF112
PUF122
PUF128
PUF130
PUF154
PUF166

FIG. 8