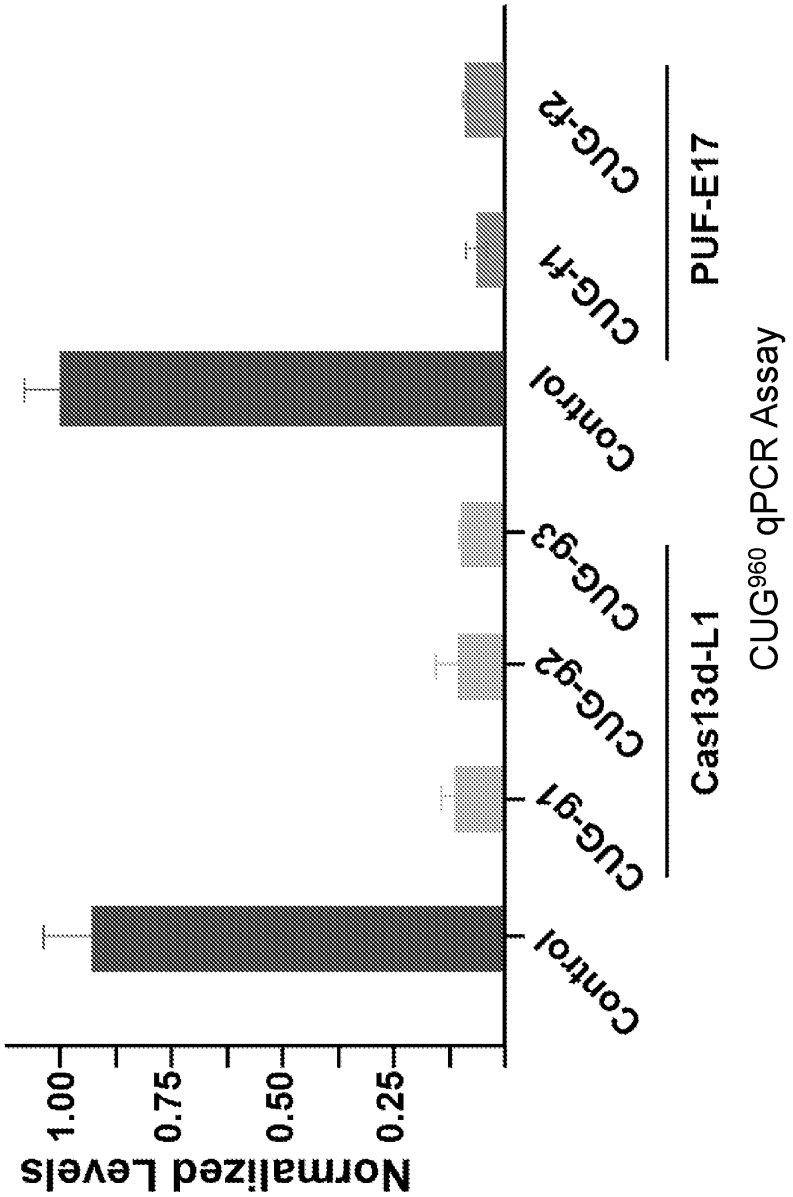

Normalized Levels
1.00
0.75
0.50
0.25
Control
CUG-g1
CUG-g2
CUG-g3
Control
CUG-f1
CUG-f2
Cas13d-L1
PUF-E17
CUG960 qPCR Assay

FIG. 1

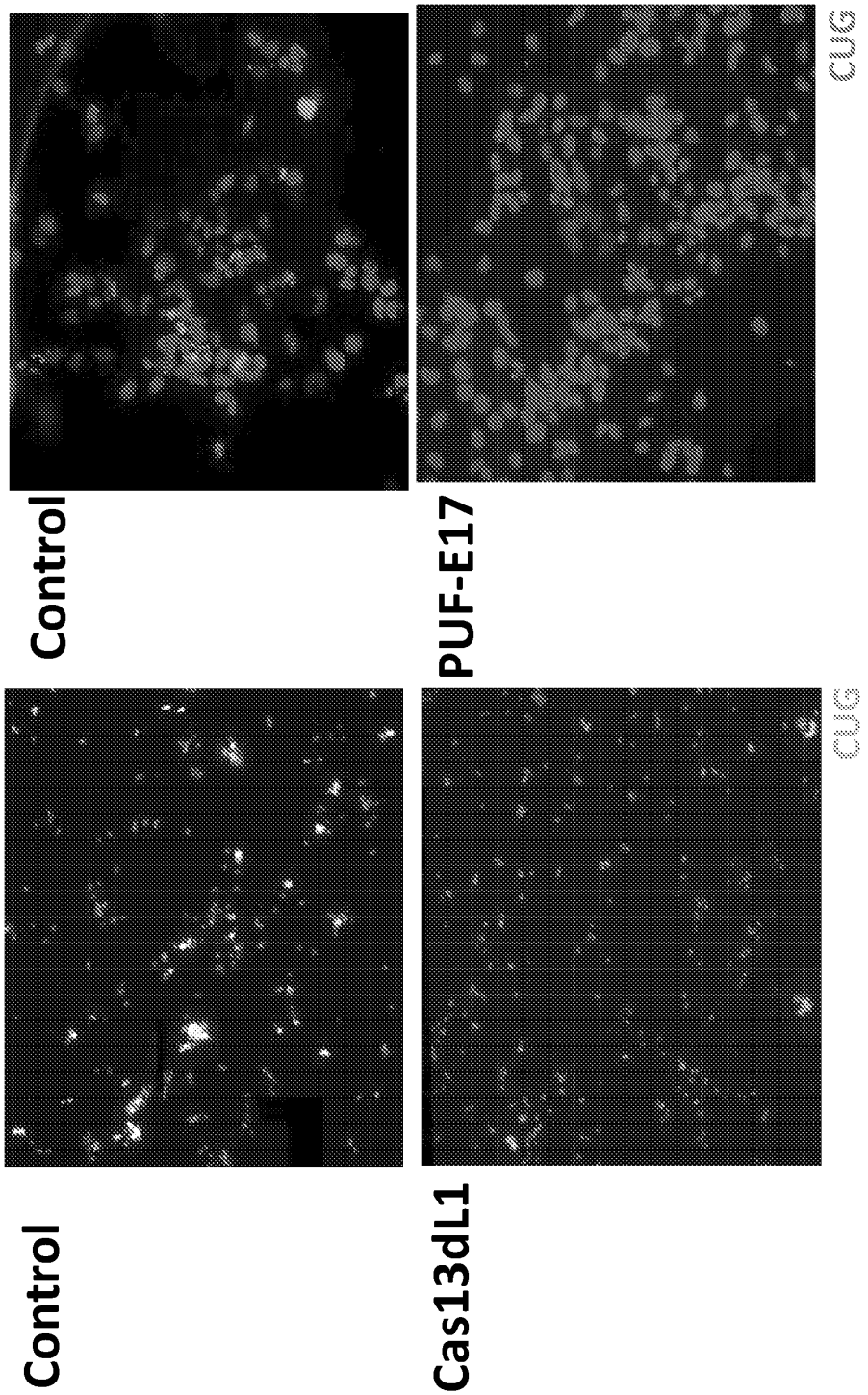


FIG. 2

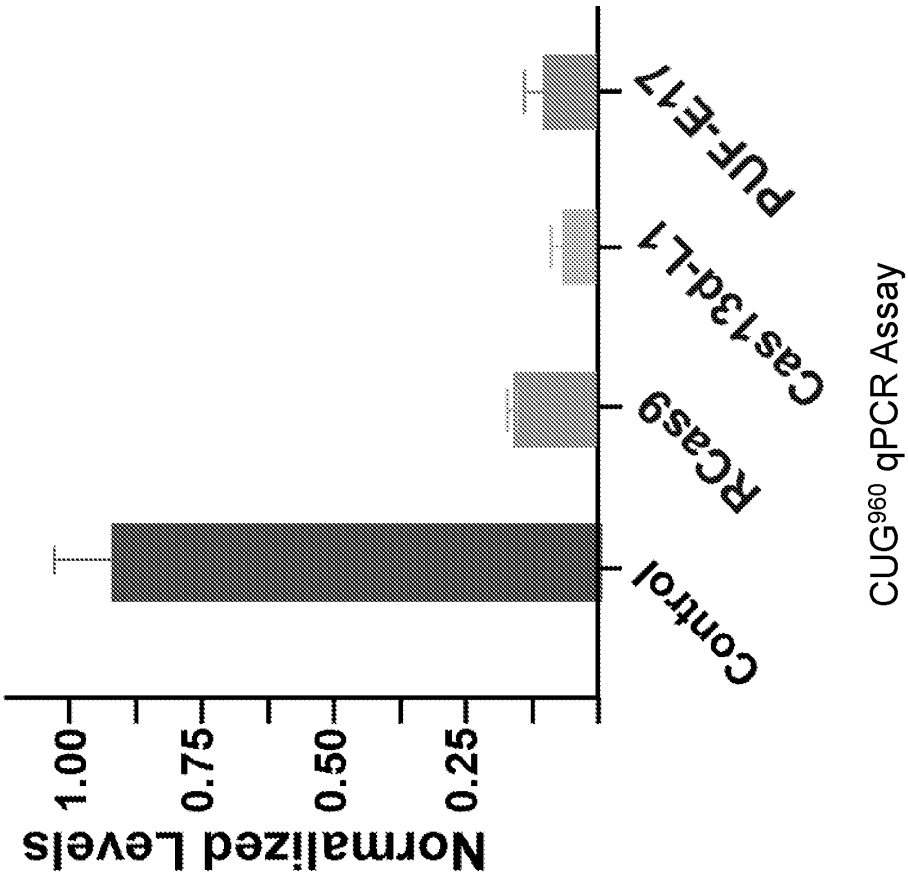

Normalized Levels
1.00
0.75
0.50
0.25
Control
RCas9
Cas13d-L1
PUF-E17
CUG960 qPCR Assay

FIG. 3

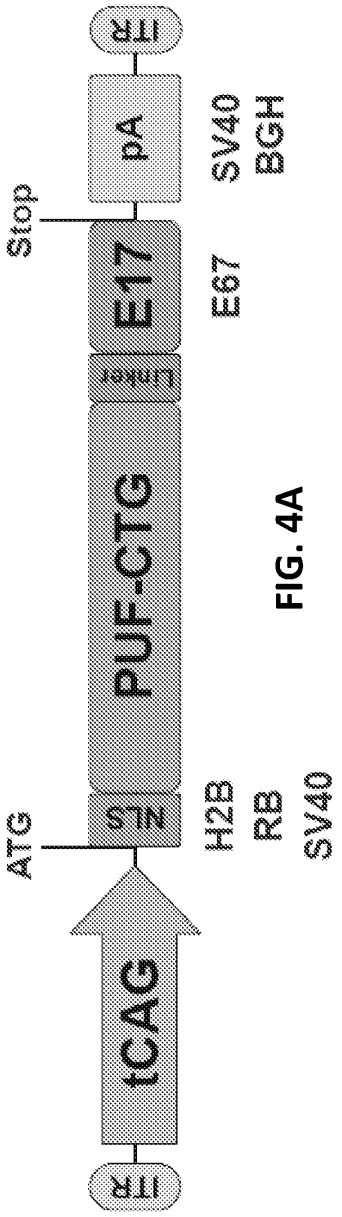


FIG. 4A

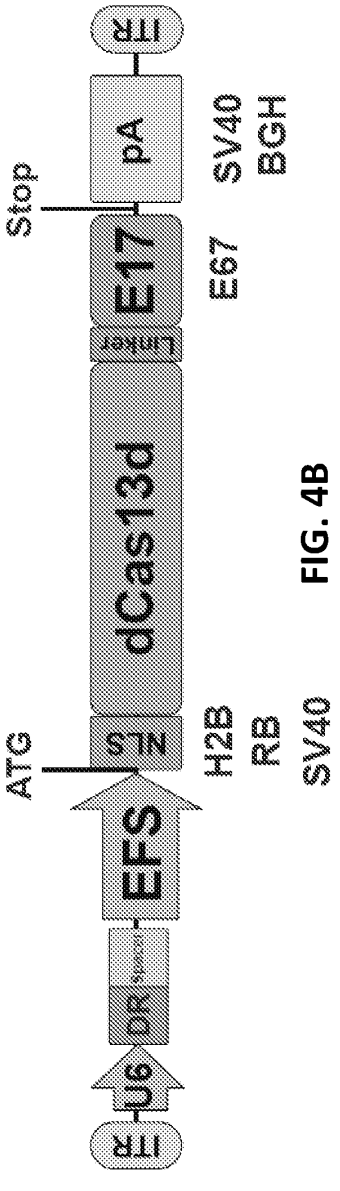


FIG. 4B

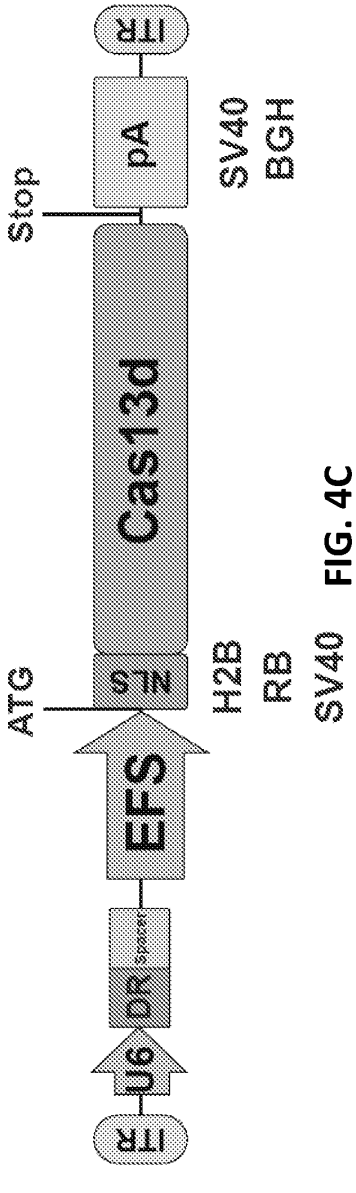


FIG. 4C

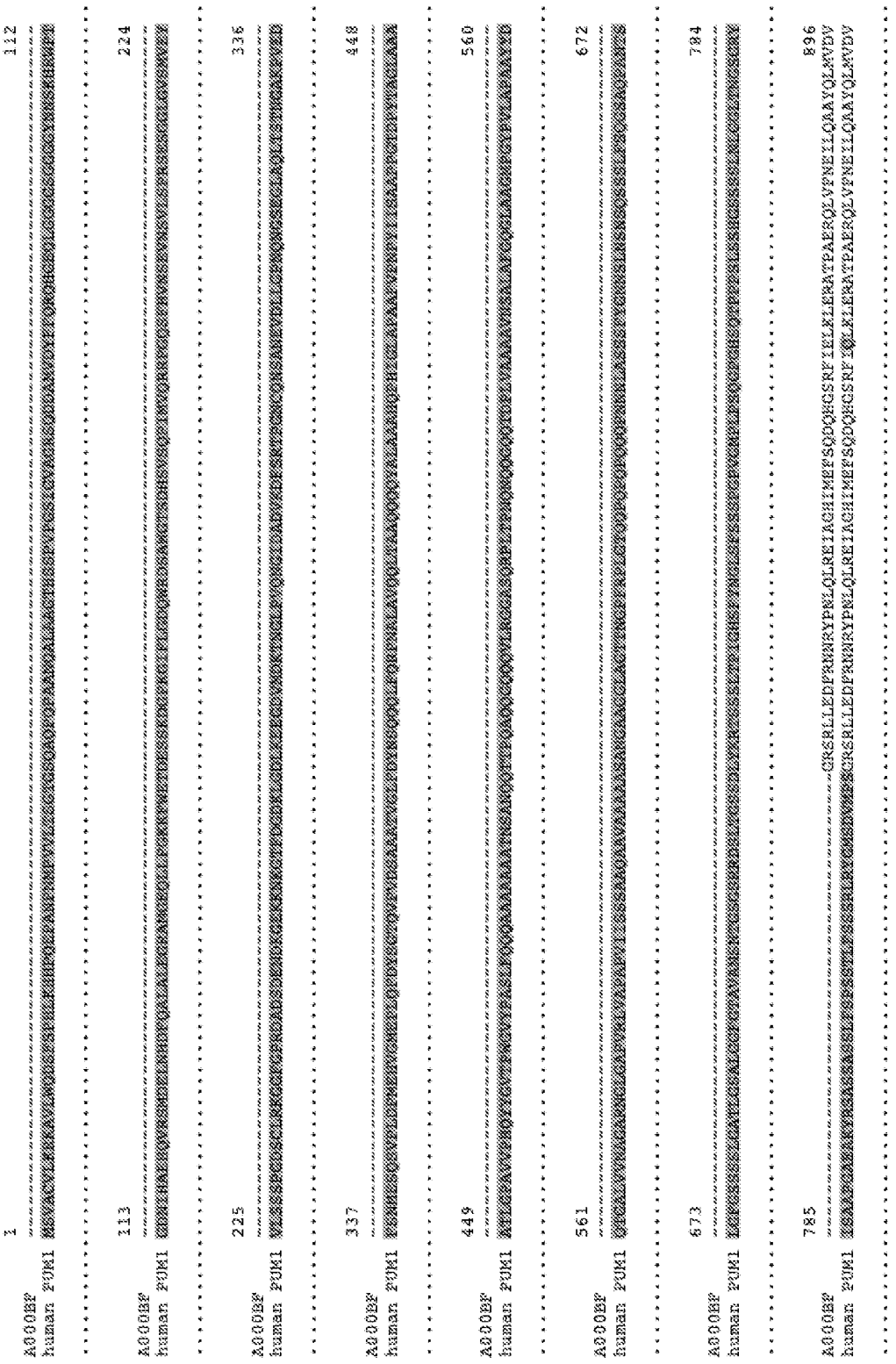


FIG. 5

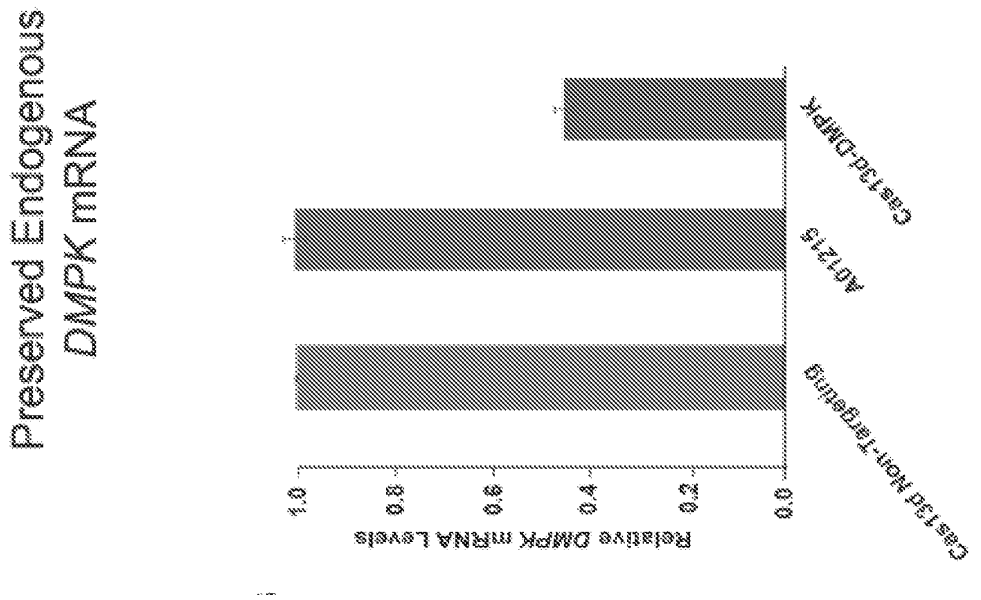

Preserved Endogenous
DMPK mRNA
Relative DMPK mRNA Levels
1.0
0.8
0.6
0.4
0.2
0.0
Cas13d Non-Targeting
A01215
Cas13d-DMPK

FIG. 6B

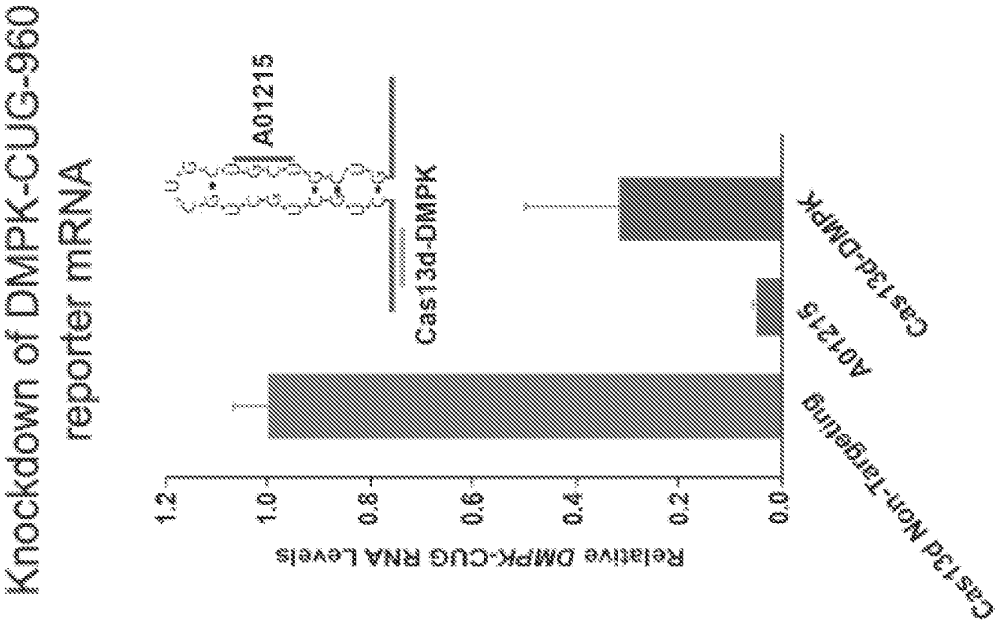
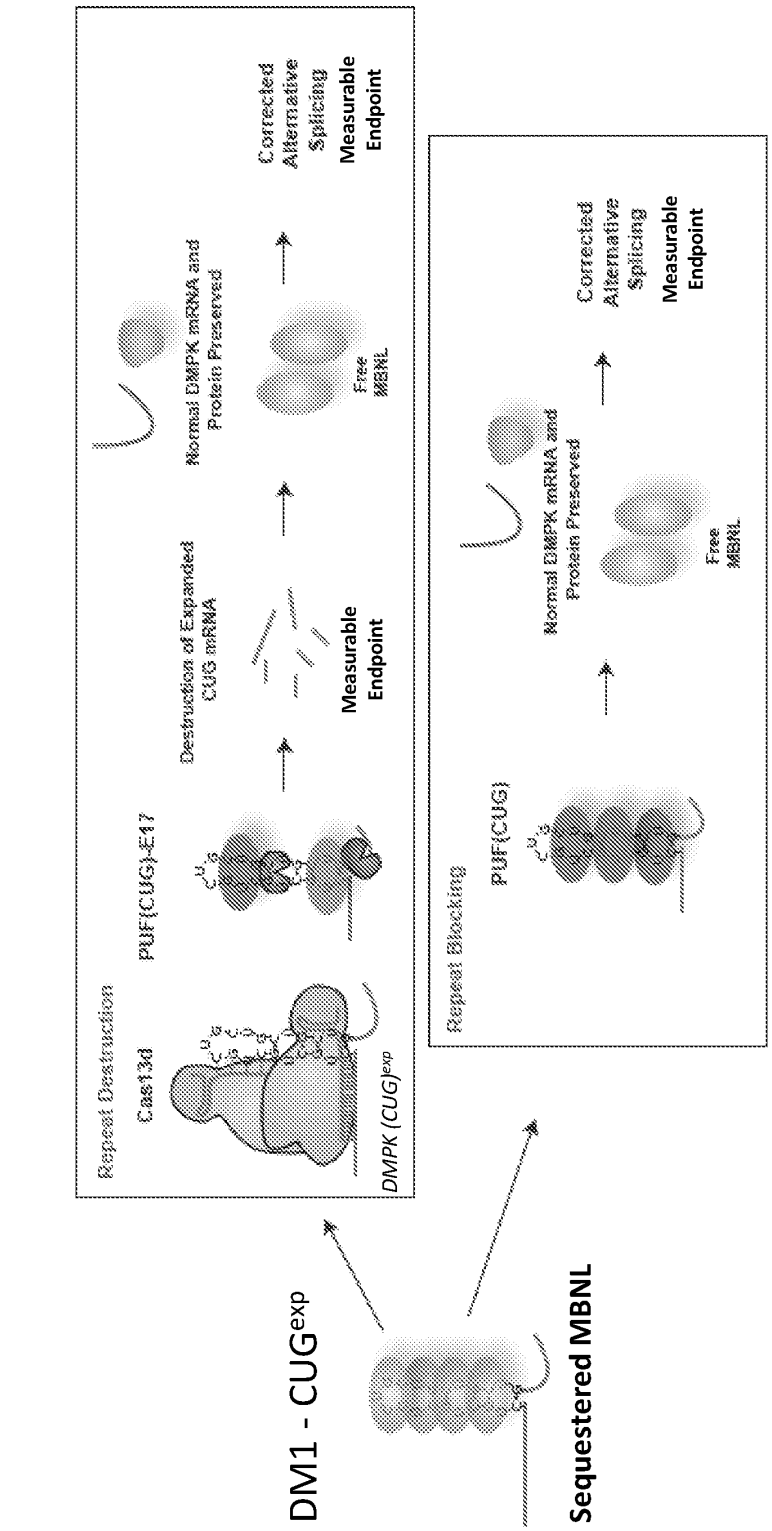

Knockdown of DMPK-CUG-960
reporter mRNA
Relative DMPK-CUG RNA Levels
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A01215
Cas13d-DMPK
Cas13d Non-Targeting
A01215
Cas13d-DMPK

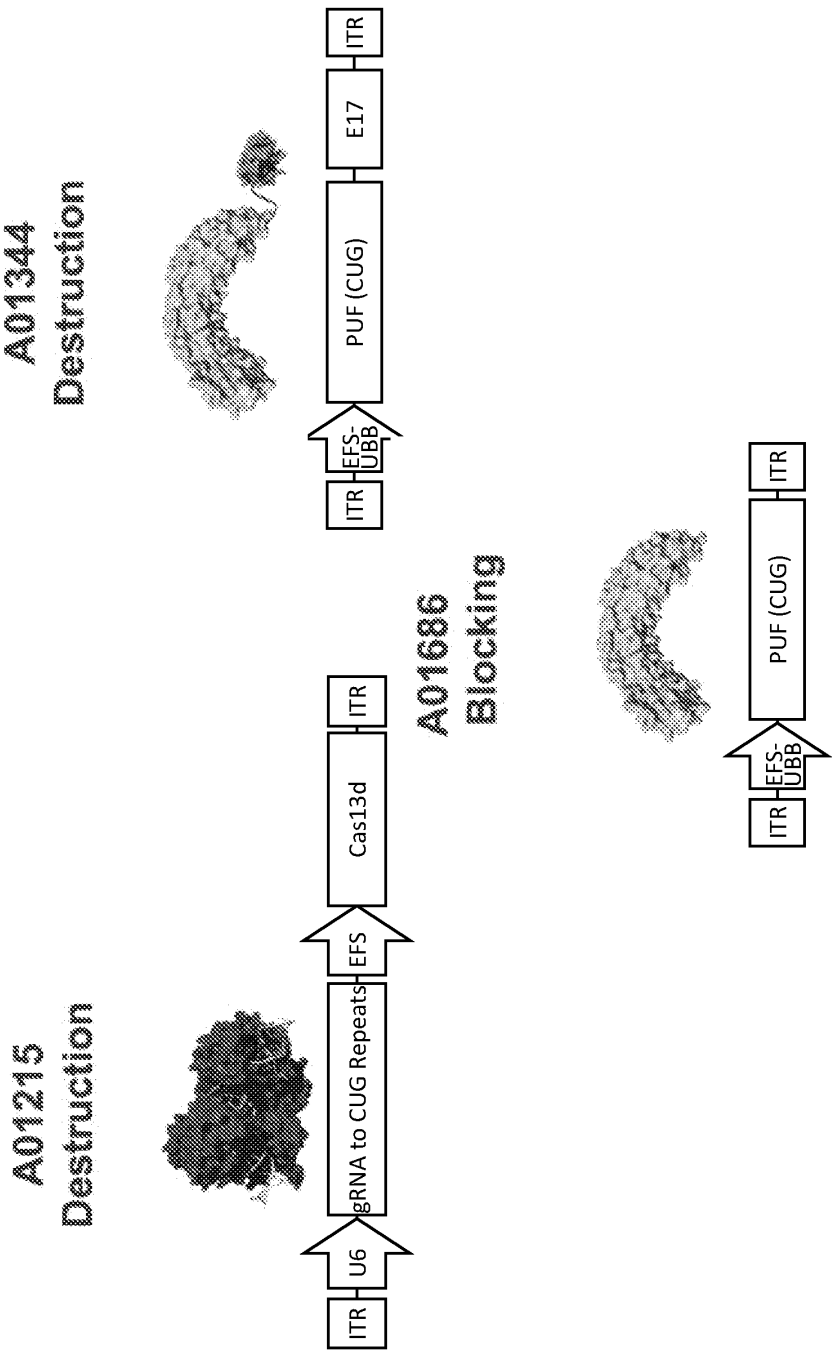
FIG. 6A



Repeat Destruction: Cas13d-CUG and PUF(CUG)-E17 bind to the expanded CUG repeats and destroy the toxic RNA. This leads to clearance of nuclear RNA foci and release of MBNL. Free MBNL can function normally to regulate alternative splicing reverse disease-associated missplicing and missplicing associated functional deficits of DM1.

Repeat Blocking: PUF(CUG) binds to expanded CUG and blocks the repeats releasing MBNL. Free MBNL can function normally to regulate alternative splicing reverse disease-associated missplicing and missplicing associated functional deficits of DM1.

FIG. 7



A01215 - Cas13d with cognate CUG Targeting gRNA (not a previously published Cas13d ortholog)
A01344 - PUF (Human PUM1 derived) protein engineered to target and cleave repetitive CUG RNA and fused to a human endonuclease domain (E17)
A01686 - PUF (Human PUM1 derived) protein engineered to target and bind repetitive CUG RNA

FIG. 8

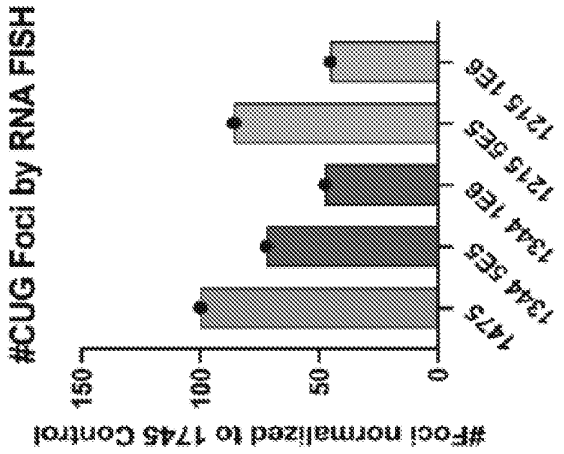

#CUG Foci by RNA FISH
#Foci normalized to 1745 Control
150
100
50
0
1475
1344 ΔE5
1344 1E6
1215 ΔE5
1215 1E6

FIG. 9B

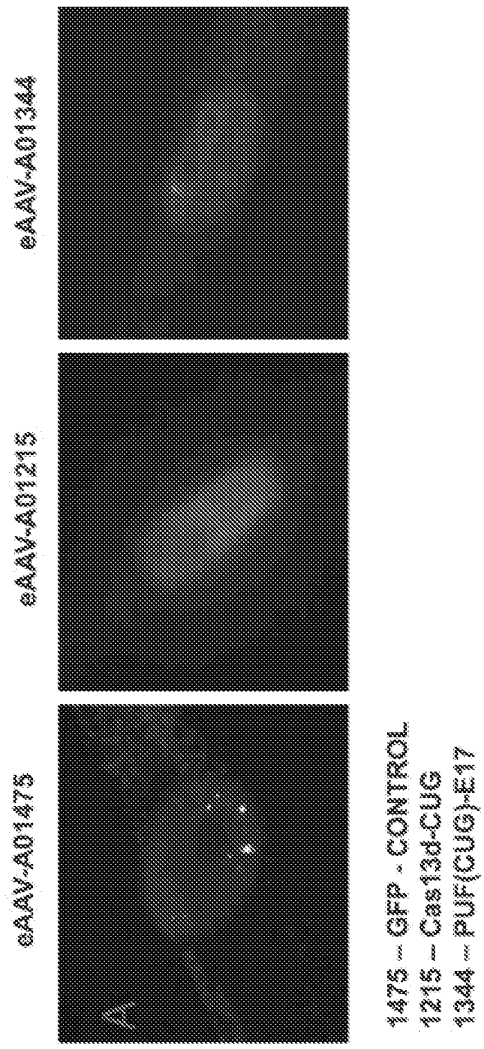

eAAV-A01475
eAAV-A01215
eAAV-A01344
1475 – GFP - CONTROL
1215 – Cas13d-CUG
1344 – PUF(CUG)-E17

FIG. 9A

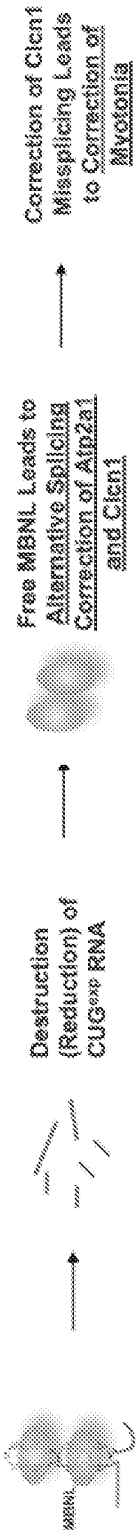


FIG. 10A

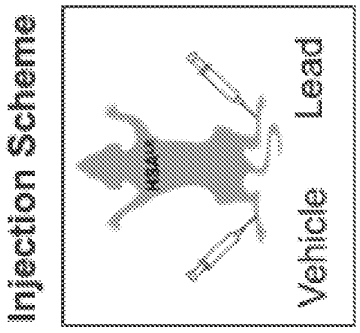


FIG. 10B

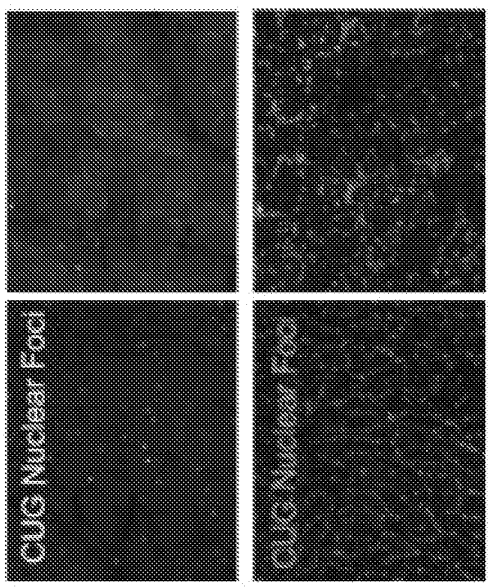


FIG. 10C

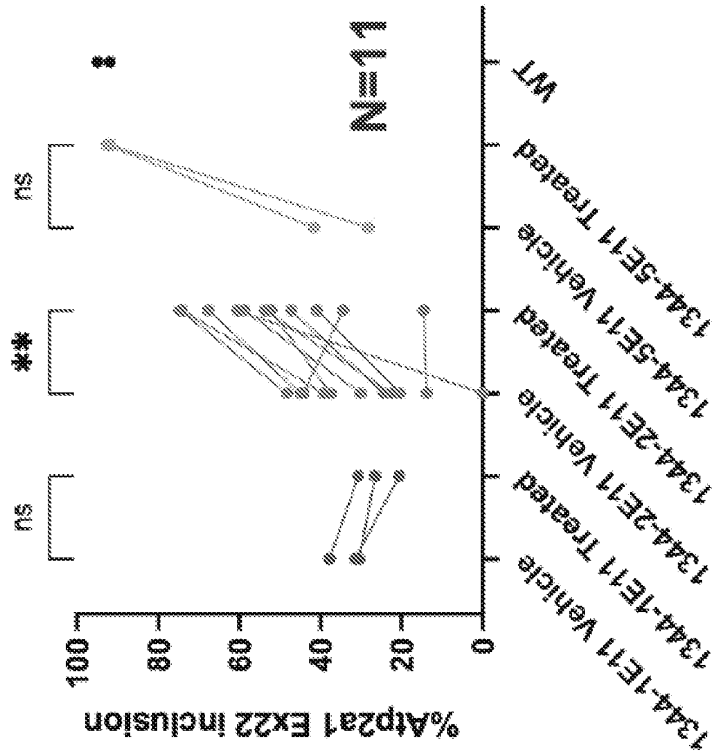


FIG. 10E

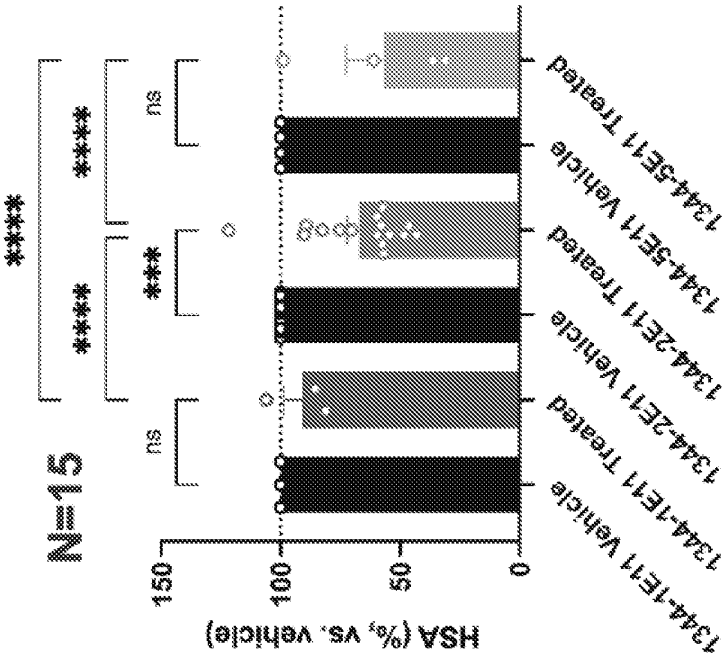


FIG. 10D

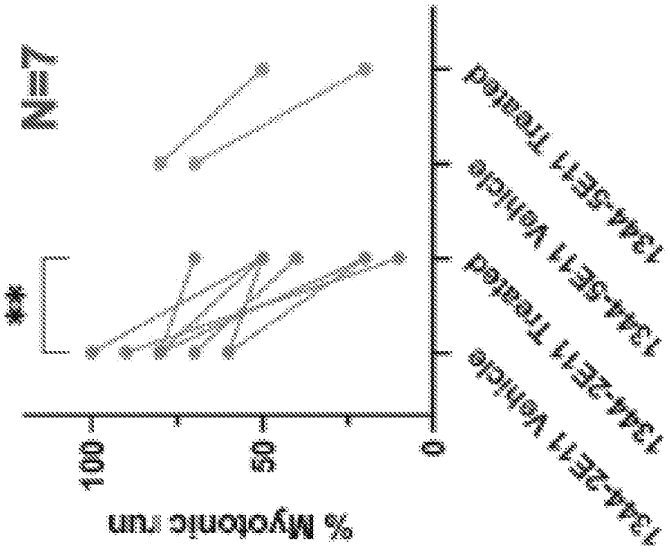


FIG. 10G

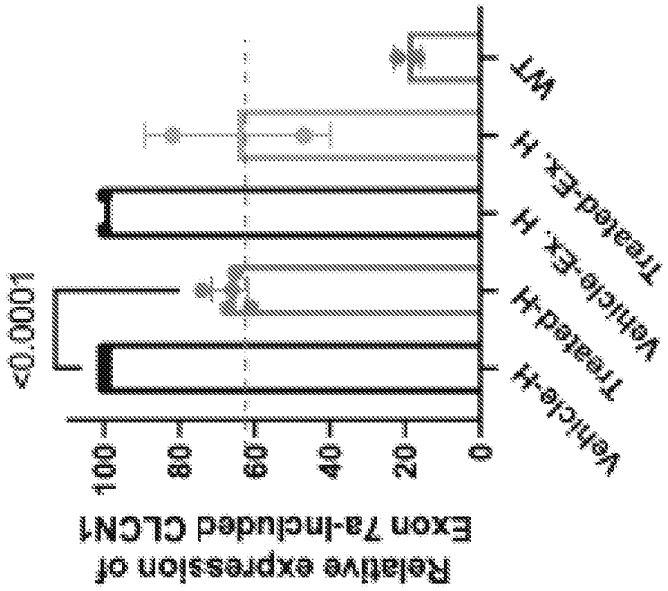


FIG. 10F

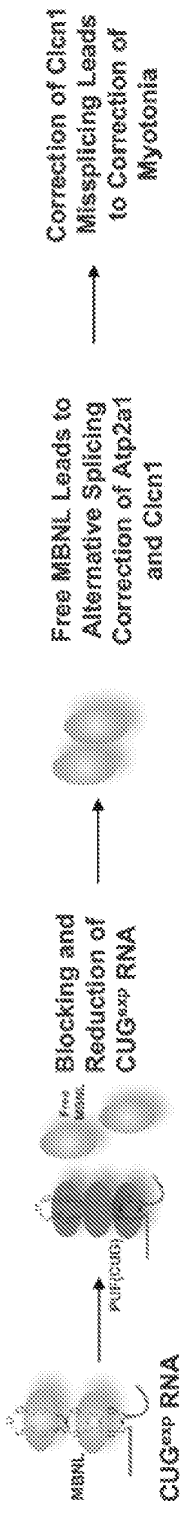
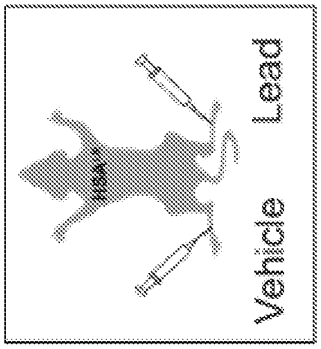


FIG. 11A



Injection Scheme

FIG. 11B

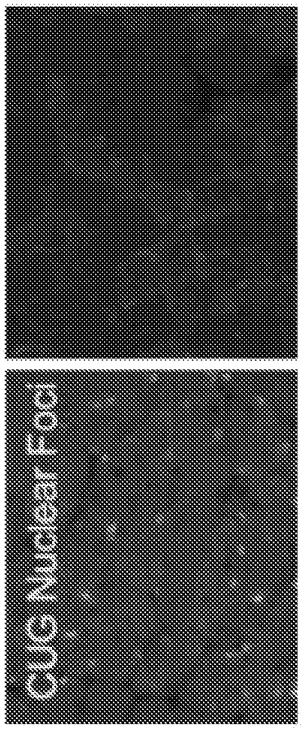


FIG. 11C

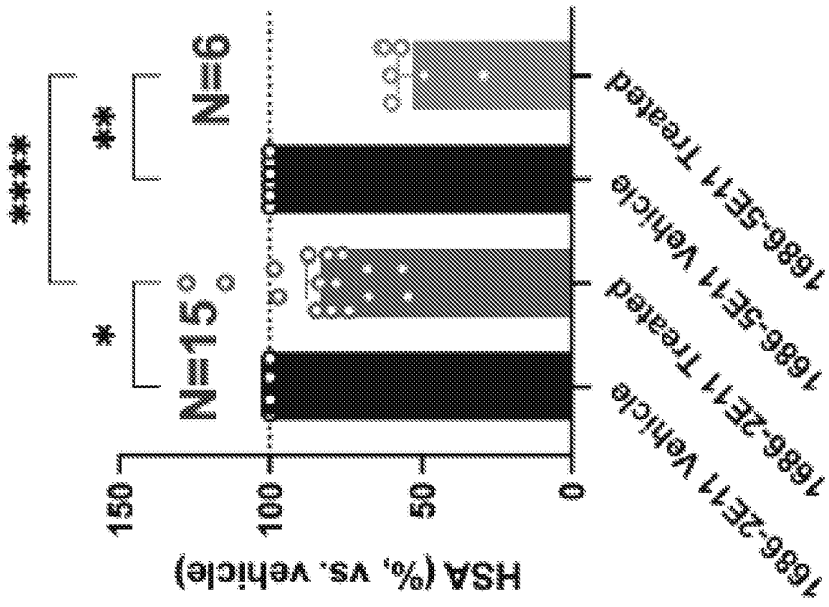


FIG. 11D

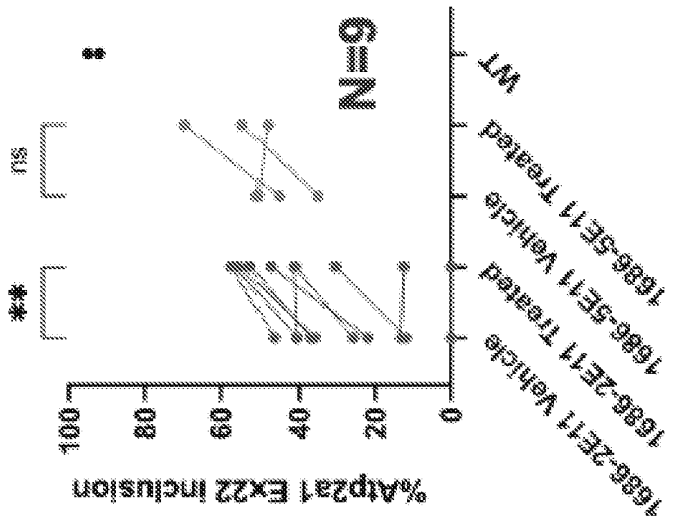


FIG. 11E

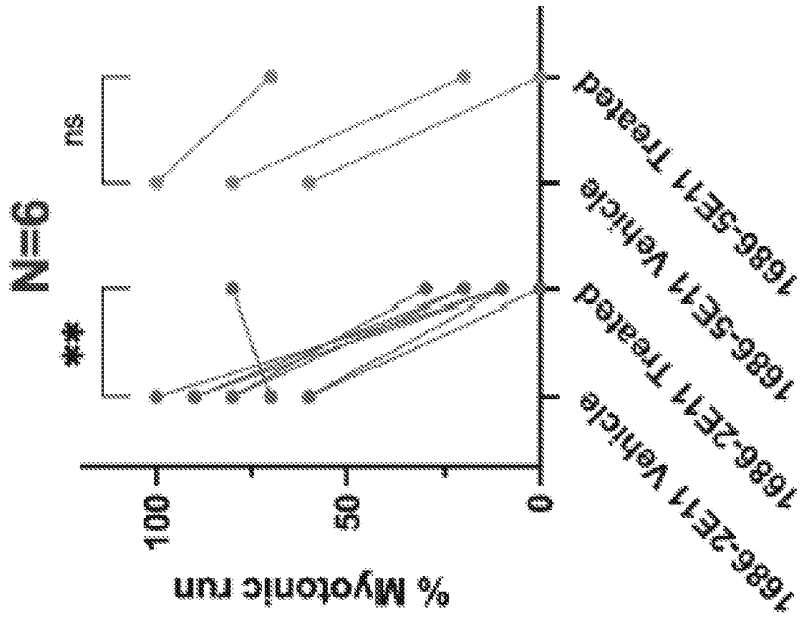


FIG. 11G

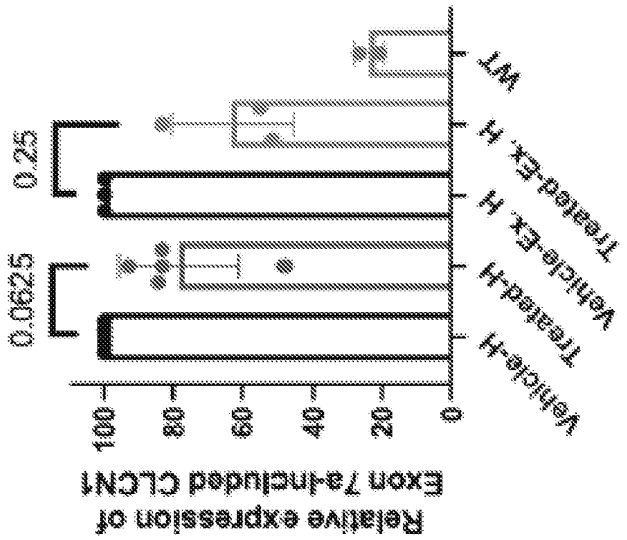


FIG. 11F

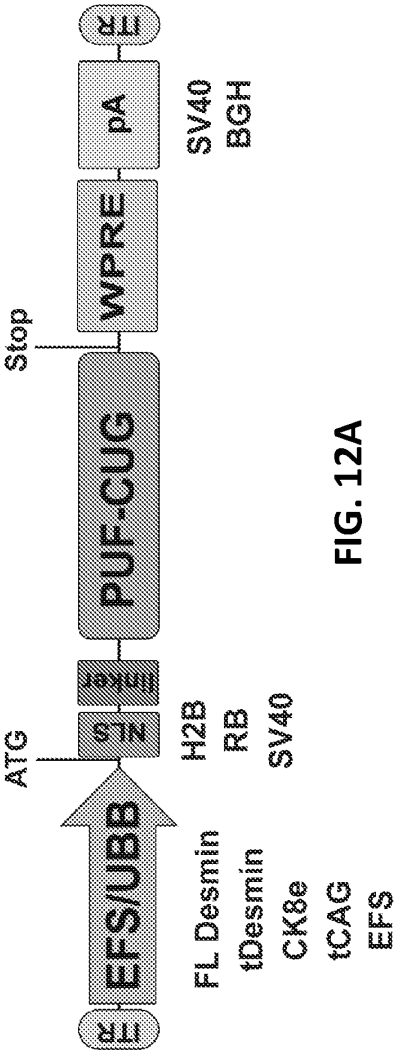


FIG. 12A

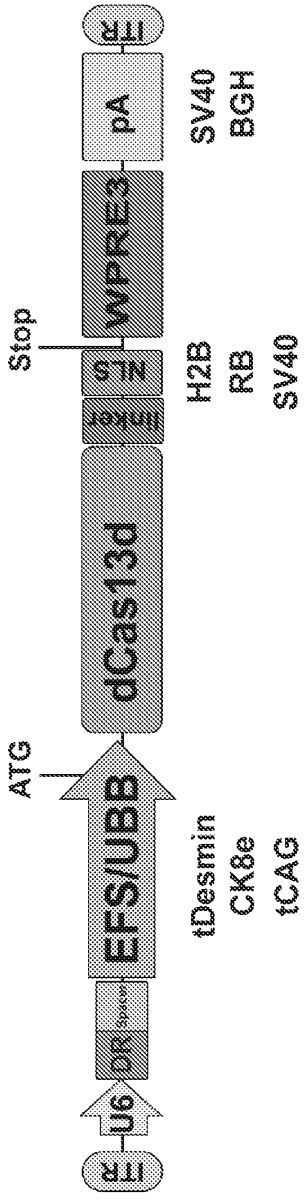


FIG. 12B