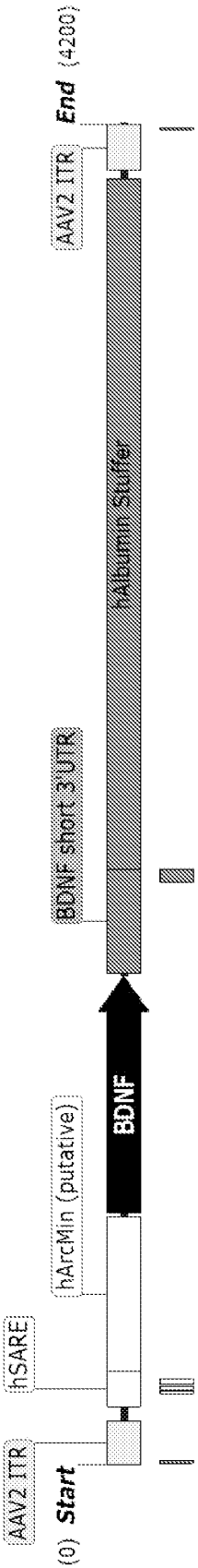


pTR141-hSARE-ArcMin-BDNF-short3'UTR (AAV2 ITR - AAV2 ITR)

4200 bp

FIG. 1

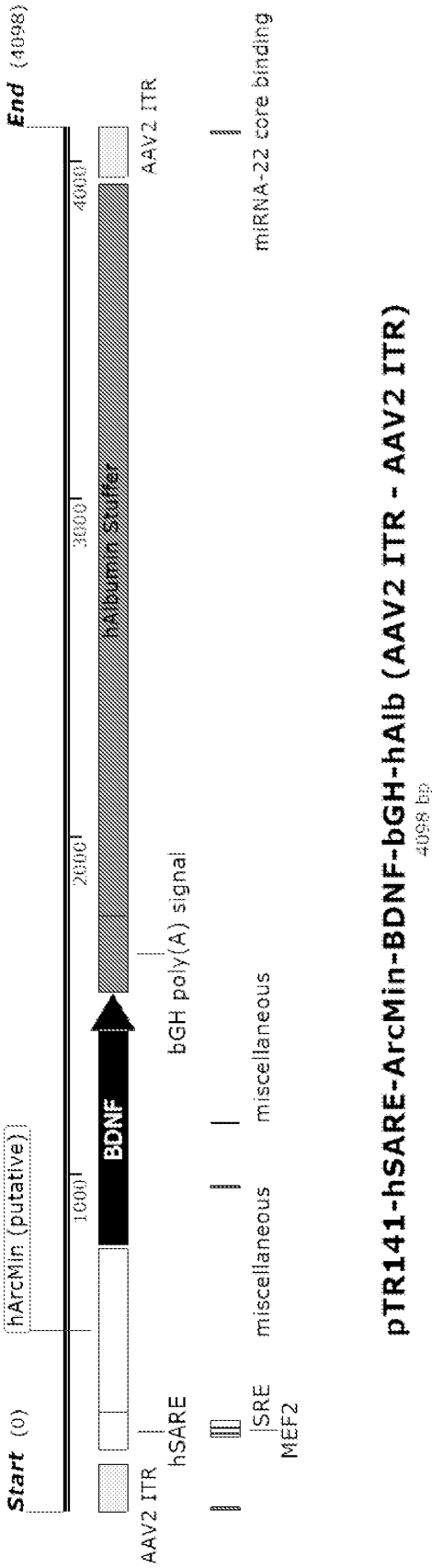

Start (0)
End (4098)
hArcMin (putative)
1000
2000
3000
4000
BDNF
hAlbumin Stuffer
AAV2 ITR
hSARE
SRE
MEF2
miscellaneous
miscellaneous
bGH poly(A) signal
miscellaneous
miRNA-22 core binding
AAV2 ITR
pTR141-hSARE-ArcMin-BDNF-bGH-hAlb (AAV2 ITR - AAV2 ITR)
4098 bp

FIG. 2

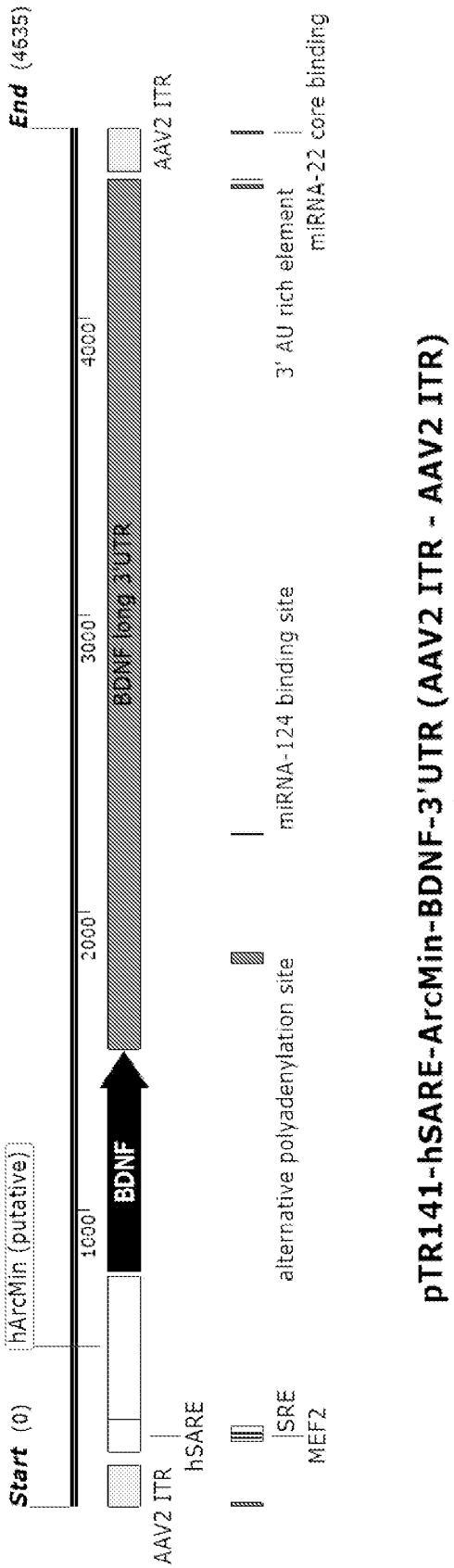

Start (0)
End (4635)
hArcMin (putative)
1000
2000
3000
4000
BDNF
BDNF long 3'UTR
AAV2 ITR
AAV2 ITR
hSARE
SRE
MEF2
alternative polyadenylation site
miRNA-124 binding site
3' AU rich element
miRNA-22 core binding
pTR141-hSARE-ArcMin-BDNF-3'UTR (AAV2 ITR - AAV2 ITR)
4635 bp

FIG. 3

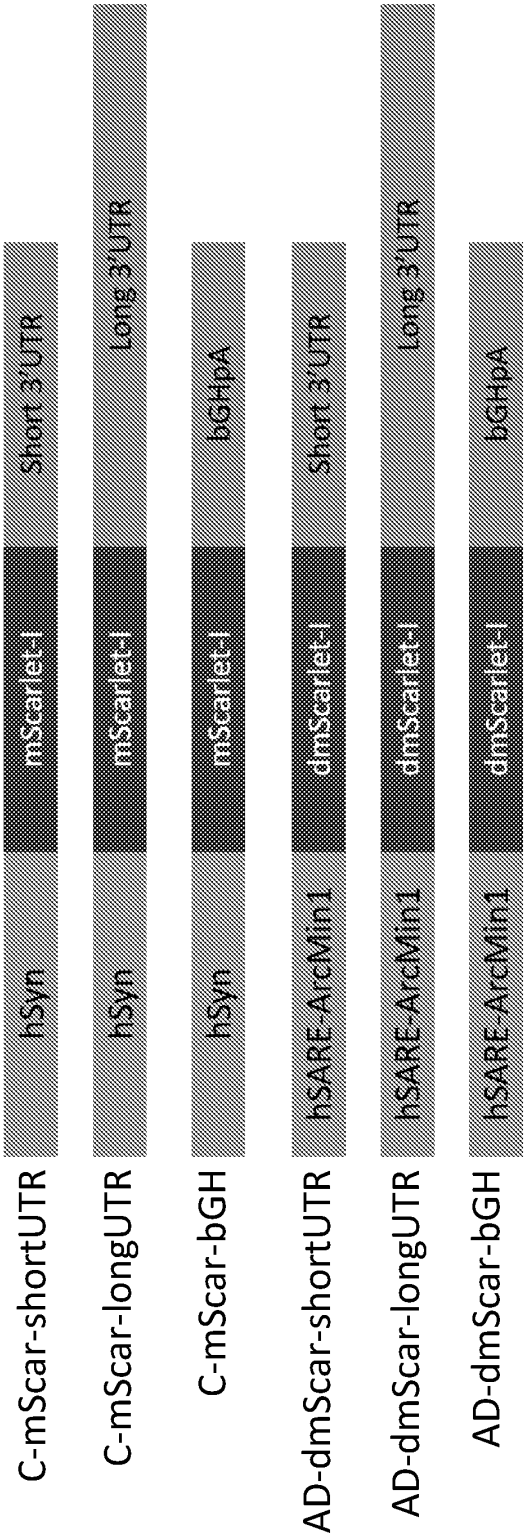

C-mScar-shortUTR
hSyn
mScarlet-I
Short 3'UTR
C-mScar-longUTR
hSyn
mScarlet-I
Long 3'UTR
C-mScar-bGH
hSyn
mScarlet-I
bGHpA
AD-dmScar-shortUTR
hSARE-ArcMin1
dmScarlet-I
Short 3'UTR
AD-dmScar-longUTR
hSARE-ArcMin1
dmScarlet-I
Long 3'UTR
AD-dmScar-bGH
hSARE-ArcMin1
dmScarlet-I
bGHpA

FIG. 4

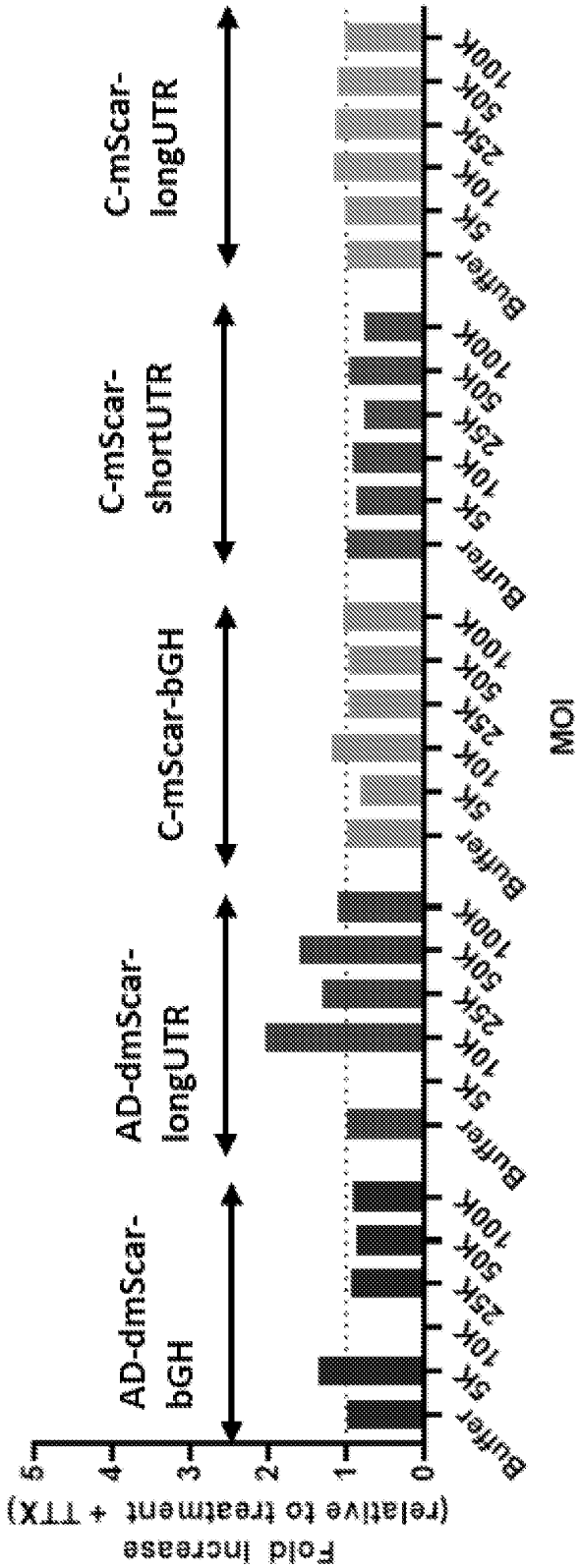

Fold Increase
(relative to treatment + TTX)
0
1
2
3
4
5
AD-dmScar-bGH
AD-dmScar-longUTR
C-mScar-bGH
C-mScar-shortUTR
C-mScar-longUTR
Buffer
5K
10K
25K
50K
100K
MOI

FIG. 5A

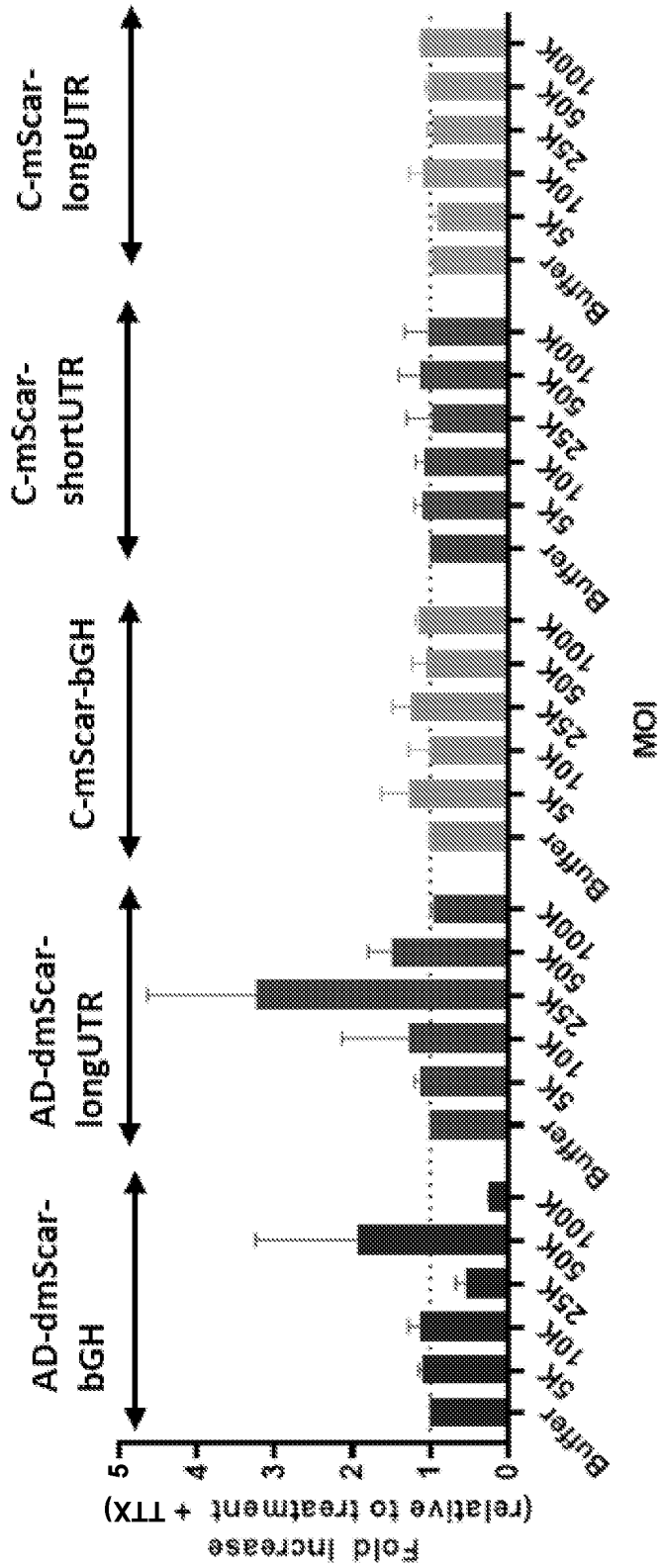

Fold Increase
(relative to treatment + TTX)
0
1
2
3
4
5
Buffer
5K
10K
25K
50K
100K
MOI
AD-dmScar-bGH
AD-dmScar-longUTR
C-mScar-bGH
C-mScar-shortUTR
C-mScar-longUTR

FIG. 5B

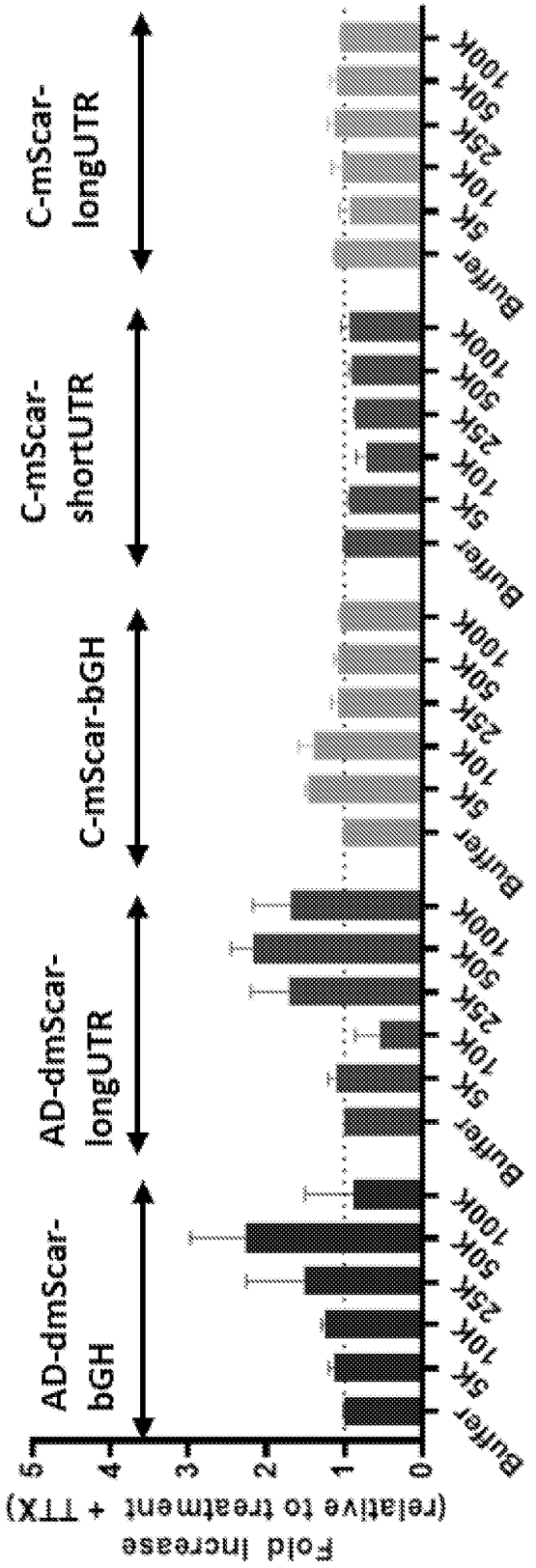

Fold Increase
(relative to treatment + TTX)
0
1
2
3
4
5
AD-dmScar-bGH
AD-dmScar-longUTR
C-mScar-bGH
C-mScar-shortUTR
C-mScar-longUTR
Buffer
5K
10K
25K
50K
100K
MOI

FIG. 5C

FIG. 6A

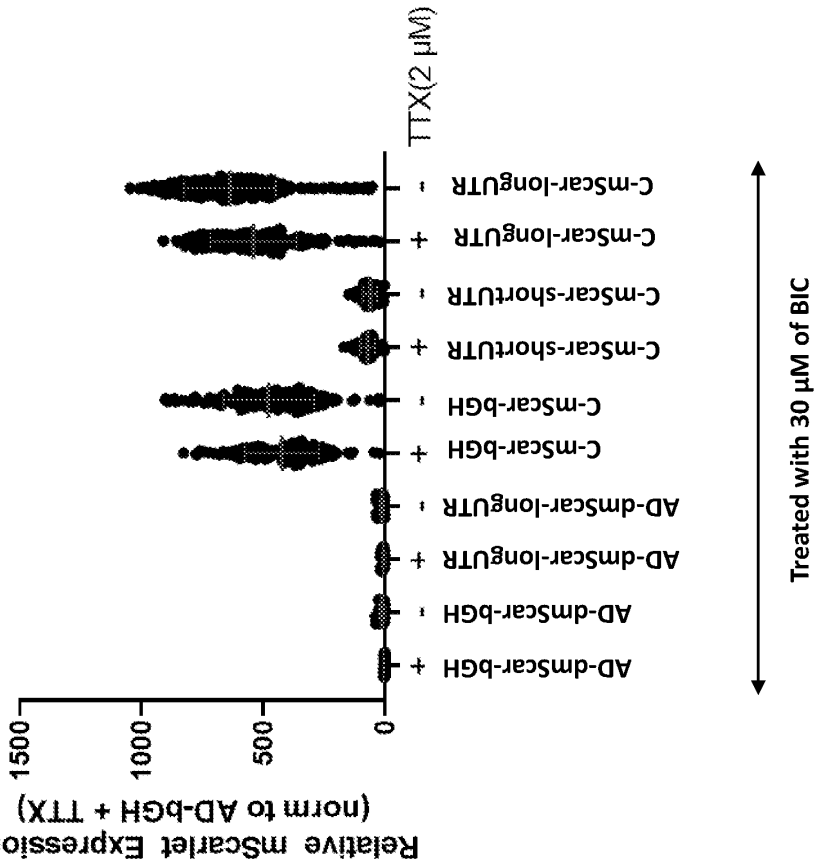

Relative mScarlet Expression
(norm to AD-bGH + TTX)
1500
1000
500
0
TTX(2 μM)
AD-dmScar-bGH + - + - + - + - + -
C-mScar-bGH
C-mScar-shortUTR
C-mScar-longUTR
Treated with 30 μM of BIC

FIG. 6B

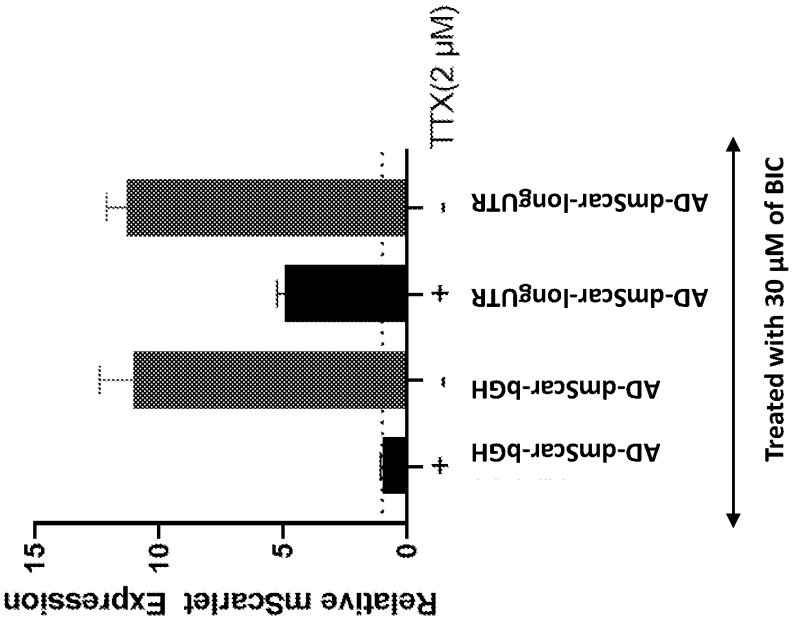
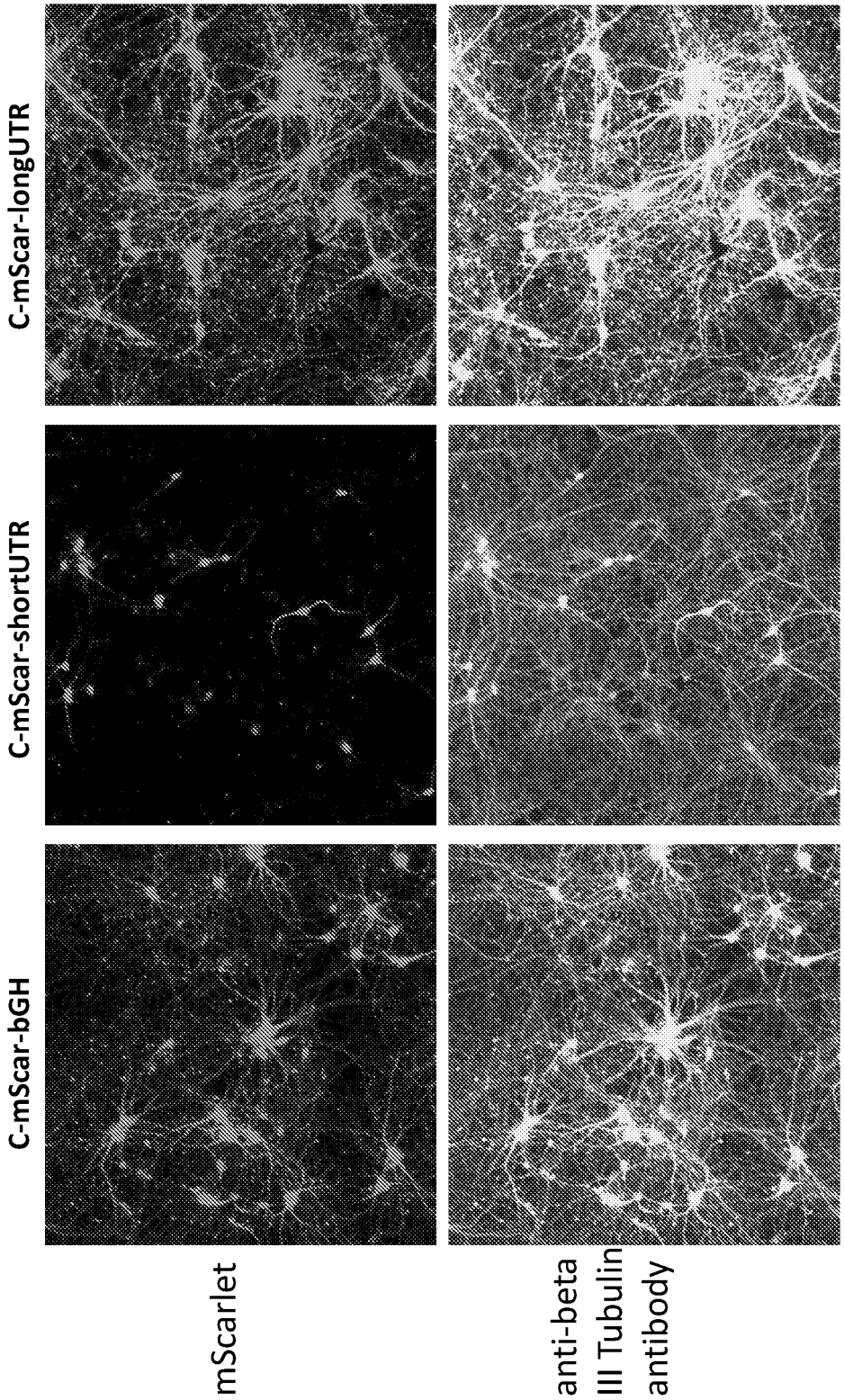

Relative mScarlet Expression
15
10
5
0
TTX(2 μM)
AD-dmScar-bGH + - + - + -
AD-dmScar-longUTR
Treated with 30 μM of BIC

FIG. 7


C-mScar-longUTR
C-mScar-shortUTR
C-mScar-bGH
mScarlet
anti-beta III Tubulin antibody

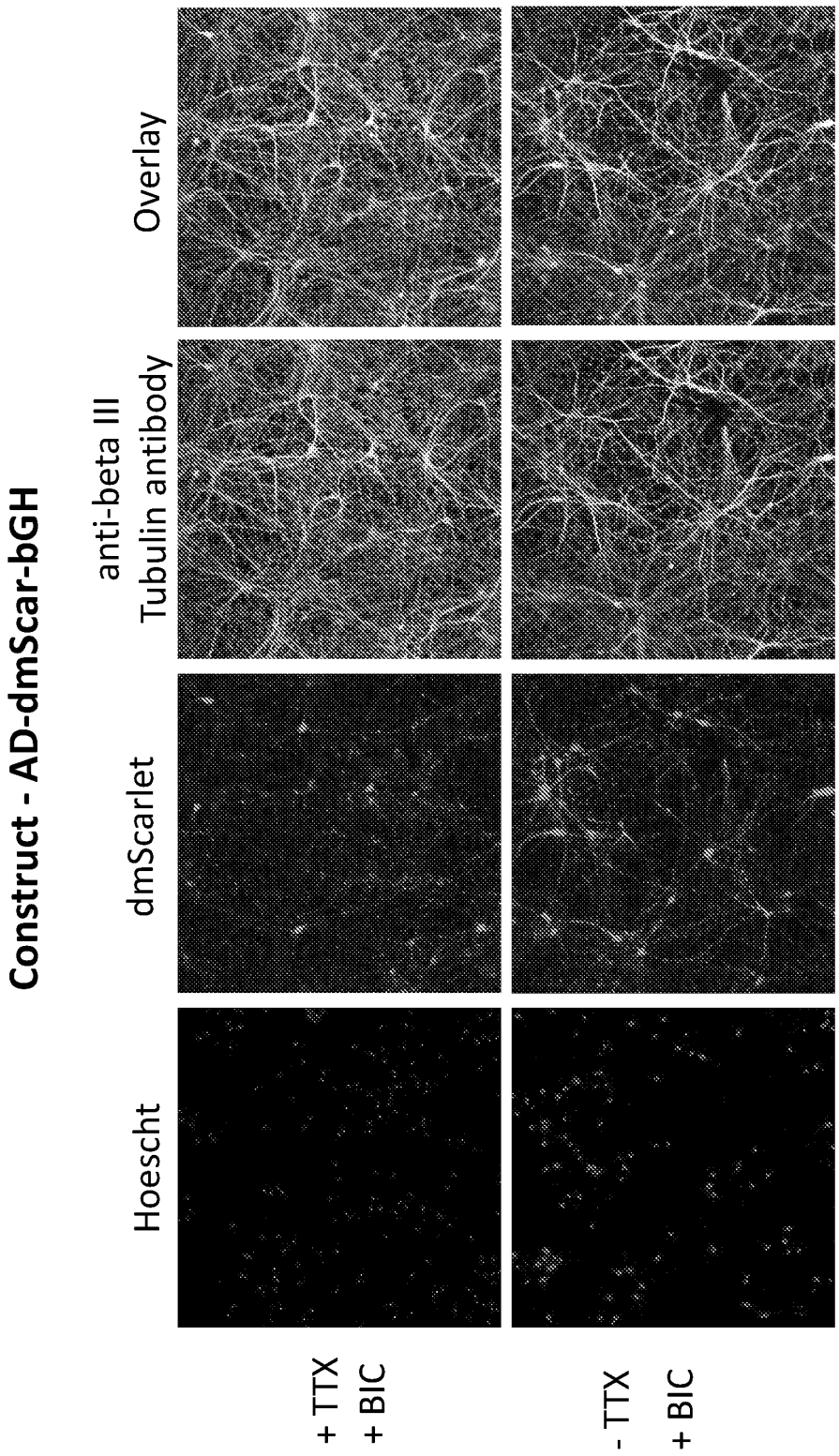

Construct - AD-dmScar-bGH
Hoescht
dmScarlet
anti-beta III Tubulin antibody
Overlay
+ TTX + BIC
- TTX + BIC

FIG. 8

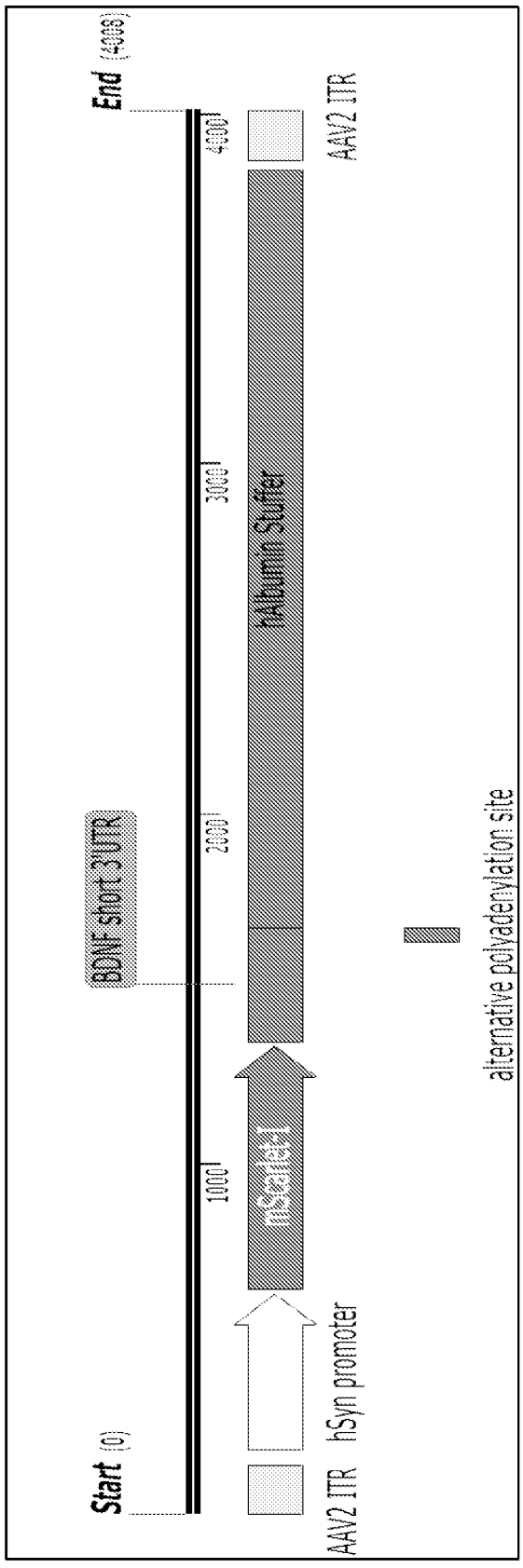

C-mScar-shortUTR
Start (0)
End (4098)
1000
2000
3000
4000
AAV2 ITR
hSyn promoter
mScarlet-I
BDNF short 3'UTR
rAlbumin Stuffer
AAV2 ITR
alternative polyadenylation site

FIG. 9A

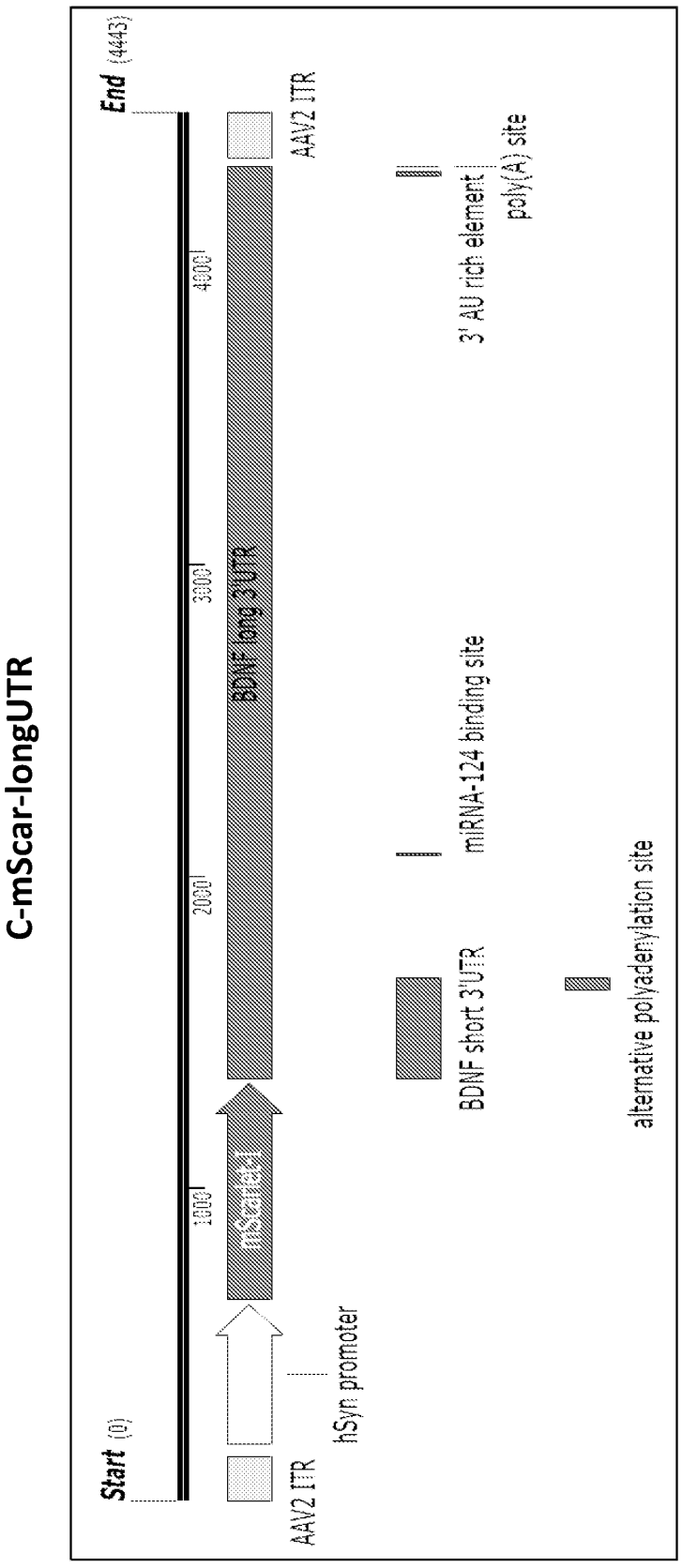

C-mScar-longUTR
Start (0)
End (4443)
1000
2000
3000
4000
AAV2 ITR
hSyn promoter
mScarlet
BDNF long 3'UTR
AAV2 ITR
BDNF short 3'UTR
miRNA-124 binding site
3' AU rich element
poly(A) site
alternative polyadenylation site

FIG. 9B

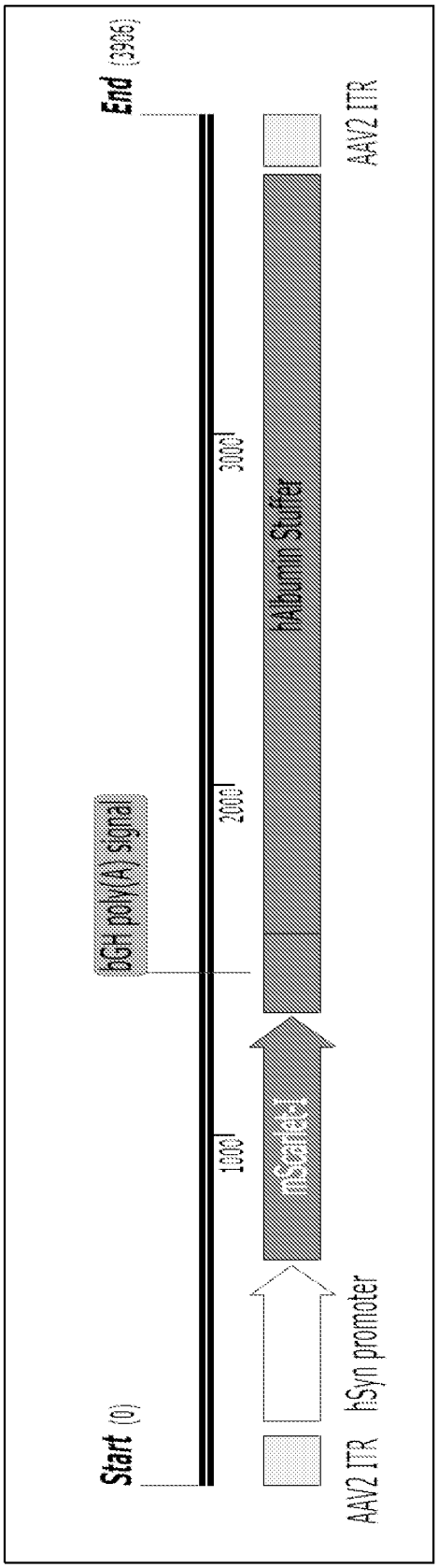

C-mScar-bGH
Start (0)
End (3906)
AAV2 ITR
hSyn promoter
mScarlet-I
1000
2000
3000
bGH poly(A) signal
hAlbumin Stuffer
AAV2 ITR

FIG. 9C

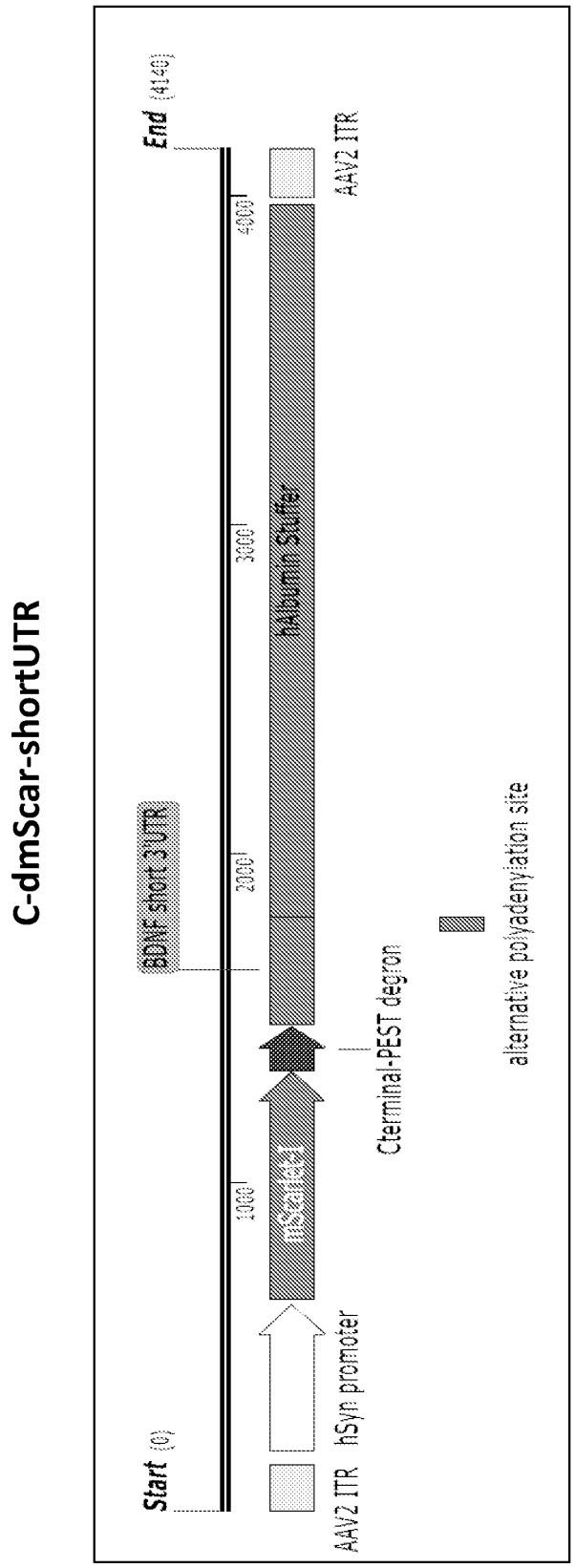

C-dmScar-shortUTR
Start (0)
End (4140)
AAV2 ITR
hSyn promoter
dmScarlet
BDNF short 3'UTR
hAlbumin Stuffer
AAV2 ITR
Cterminal-PEST degron
alternative polyadenylation site
1000
2000
3000
4000

FIG. 10A

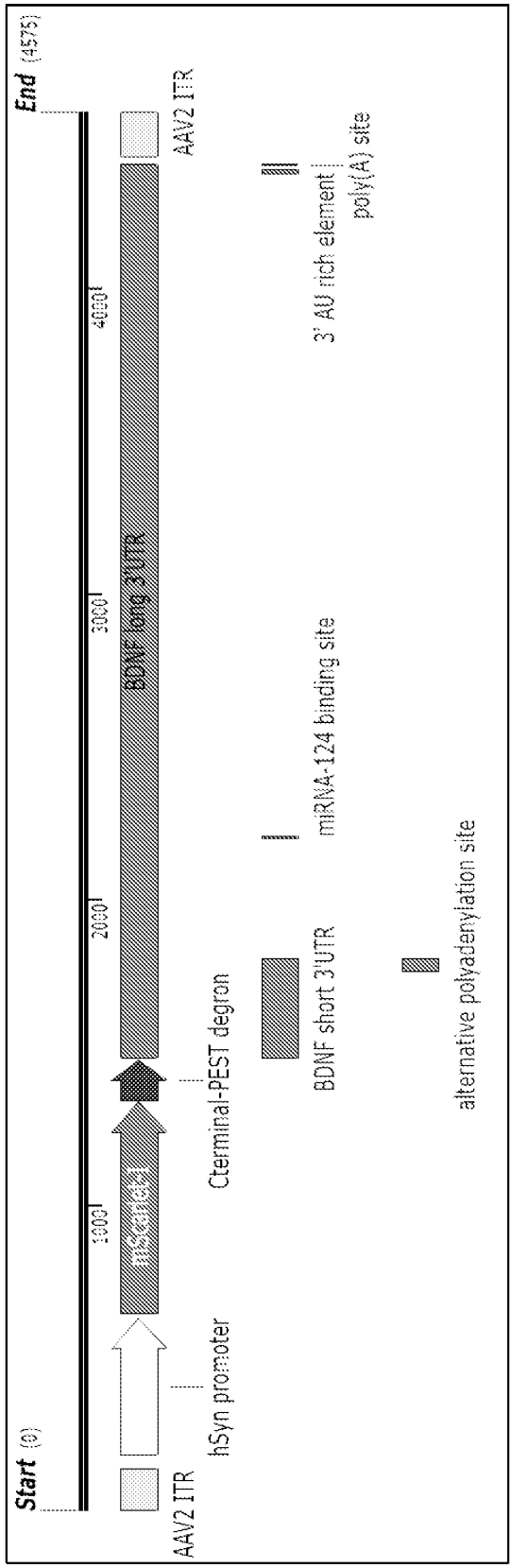

C-dmScar-longUTR
Start (0)
End (4575)
1000
2000
3000
4000
AAV2 ITR
hSyn promoter
mScarlet-I
Cterminal-PEST degron
BDNF long 3'UTR
BDNF short 3'UTR
miRNA-124 binding site
3' AU rich element
poly(A) site
alternative polyadenylation site
AAV2 ITR

FIG. 10B

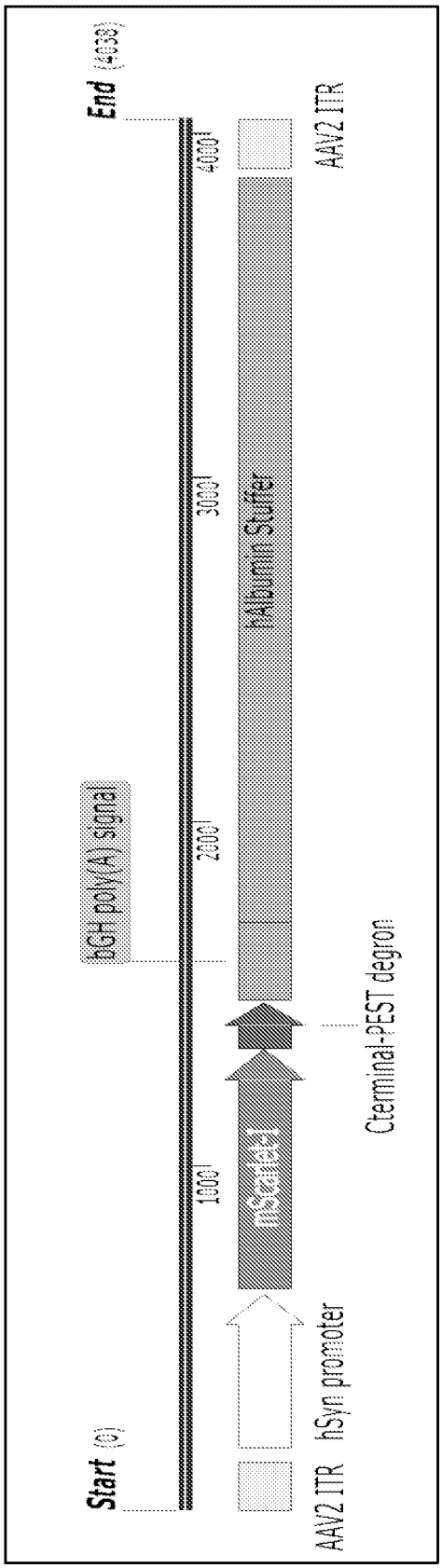

C-dmScar-bGH
Start (0)
End (4038)
AAV2 ITR
hSyn promoter
dmScarlet
Cterminal-PEST degron
hAlbumin Stuffer
bGH poly(A) signal
AAV2 ITR
1000
2000
3000
4000

FIG. 10C

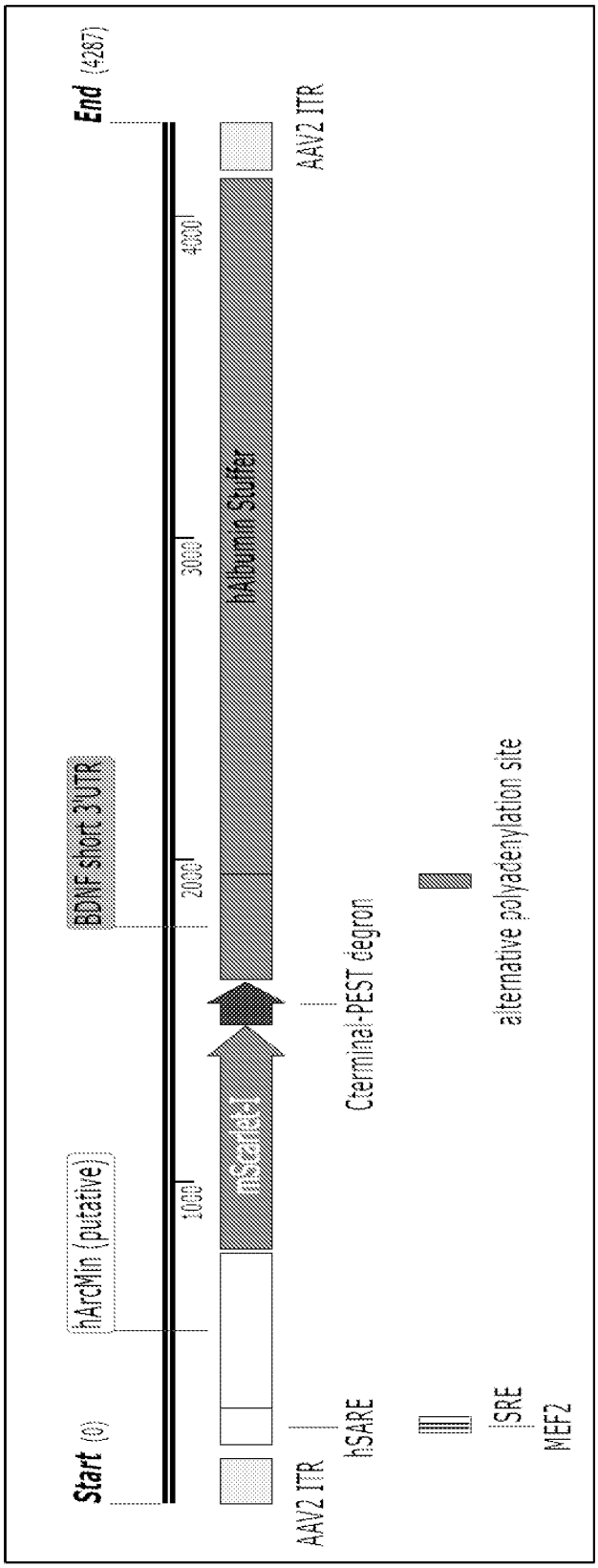

AD-dmScar-shortUTR
Start (0)
End (4287)
hArcMin (putative)
BDNF short 3'UTR
1000
2000
3000
4000
mScarlet3
hAlbumin Stuffer
AAV2 ITR
hSARE
SRE
MEF2
Cterminal PEST degron
alternative polyadenylation site
AAV2 ITR

FIG. 11A

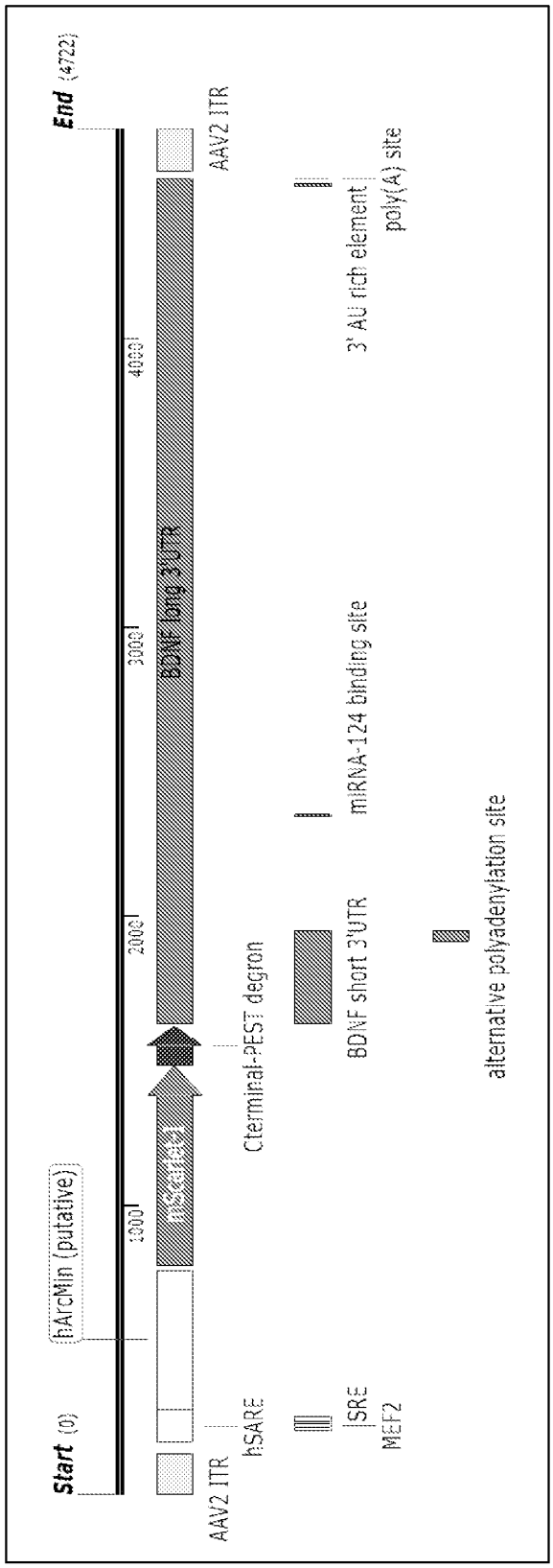

AD-dmScar-longUTR
Start (0)
hArcMin (putative)
1000
2000
3000
4000
End (4722)
AAV2 ITR
hSARE
SRE
MEF2
dmScarlet
Cterminal-PEST degron
BDNF short 3'UTR
BDNF long 3'UTR
miRNA-124 binding site
alternative polyadenylation site
3' AU rich element
poly(A) site
AAV2 ITR

FIG. 11B

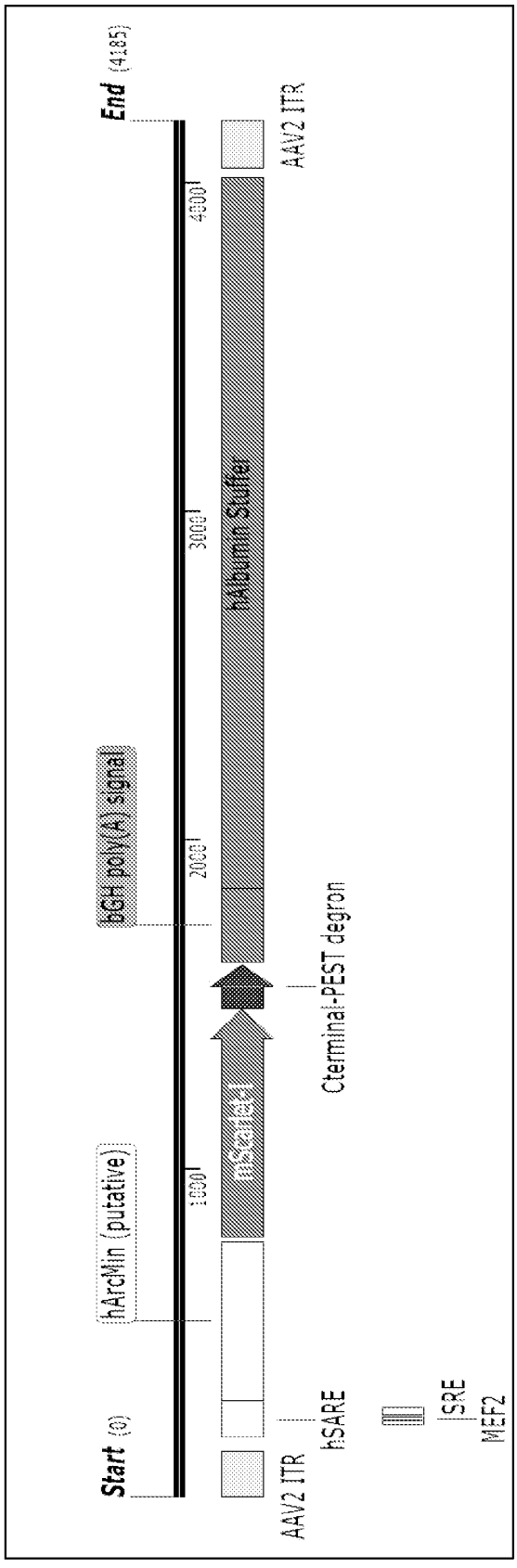

AD-dmScar-bGH
Start (0)
End (4185)
hArcMin (putative)
bGH poly(A) signal
1000
2000
3000
4000
AAV2 ITR
hSARE
ISRE
MEF2
mScarlet
Cterminal-PEST degron
hAlbumin Stuffer
AAV2 ITR

FIG. 11C

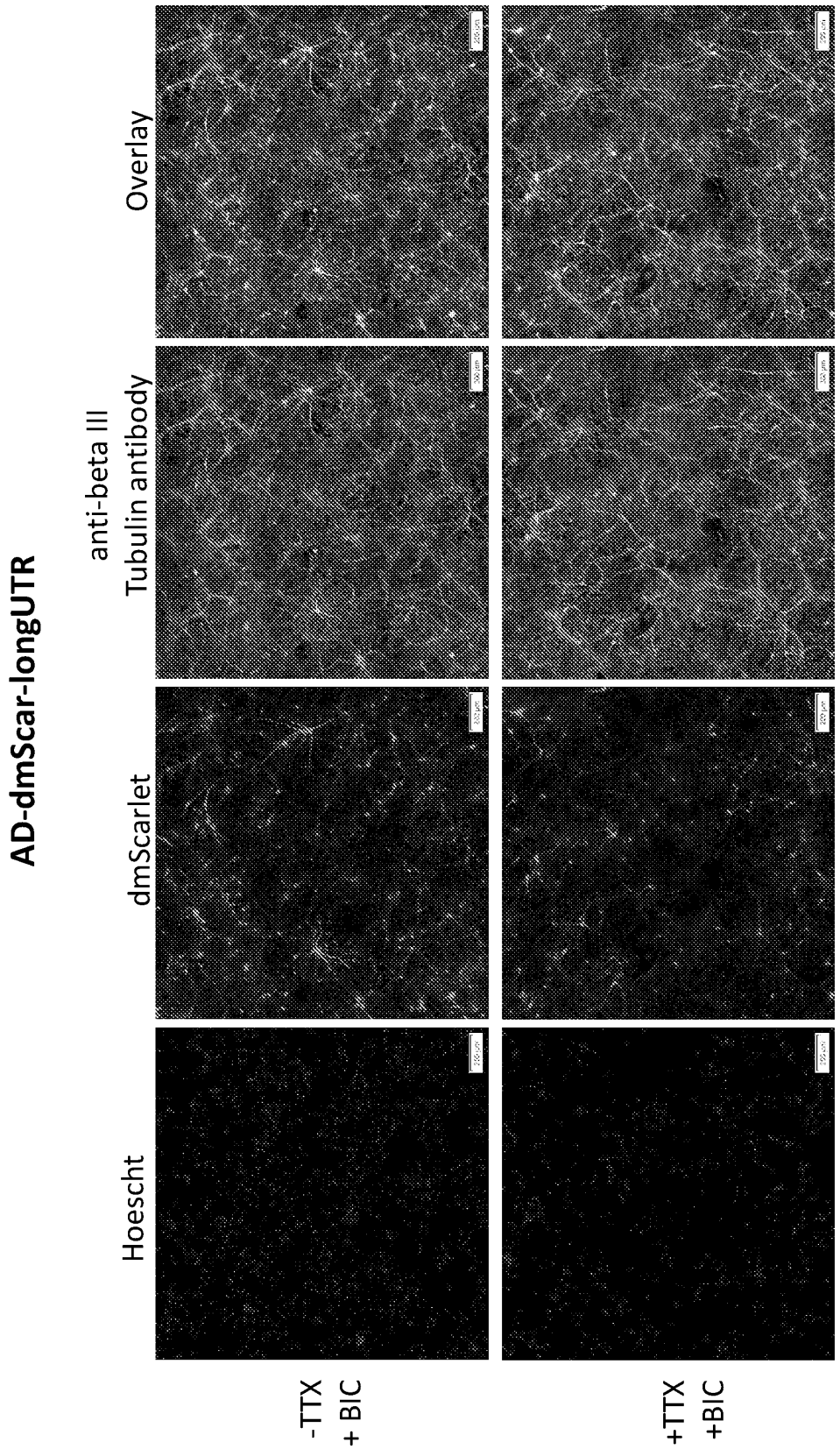

AD-dmScar-longUTR
Hoescht
dmScarlet
anti-beta III Tubulin antibody
Overlay
-TTX + BIC
+TTX +BIC

FIG. 12