FIG. 1

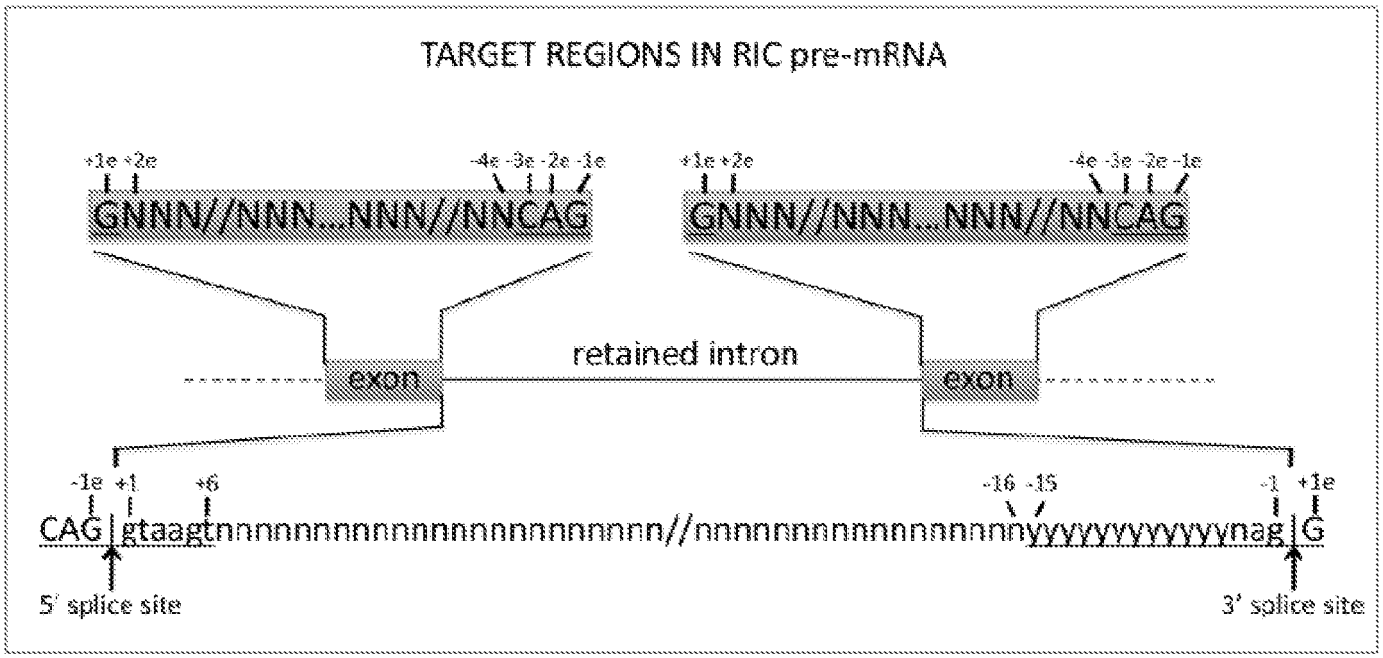

TARGET REGIONS IN RIC pre-mRNA
+1e +2e
-4e -3e -2e -1e
GNNN//NNN...NNN//NNCAG
+1e +2e
-4e -3e -2e -1e
GNNN//NNN...NNN//NNCAG
exon
retained intron
exon
-1e +1
+6
-16 -15
-1
+1e
CAG|gtaagtnnnnnnnnnnnnnnnnnnnnnnnn//nnnnnnnnnnnnnnnnnnnnyyyyyyyyyyyyynag|G
5' splice site
3' splice site

FIG. 2A

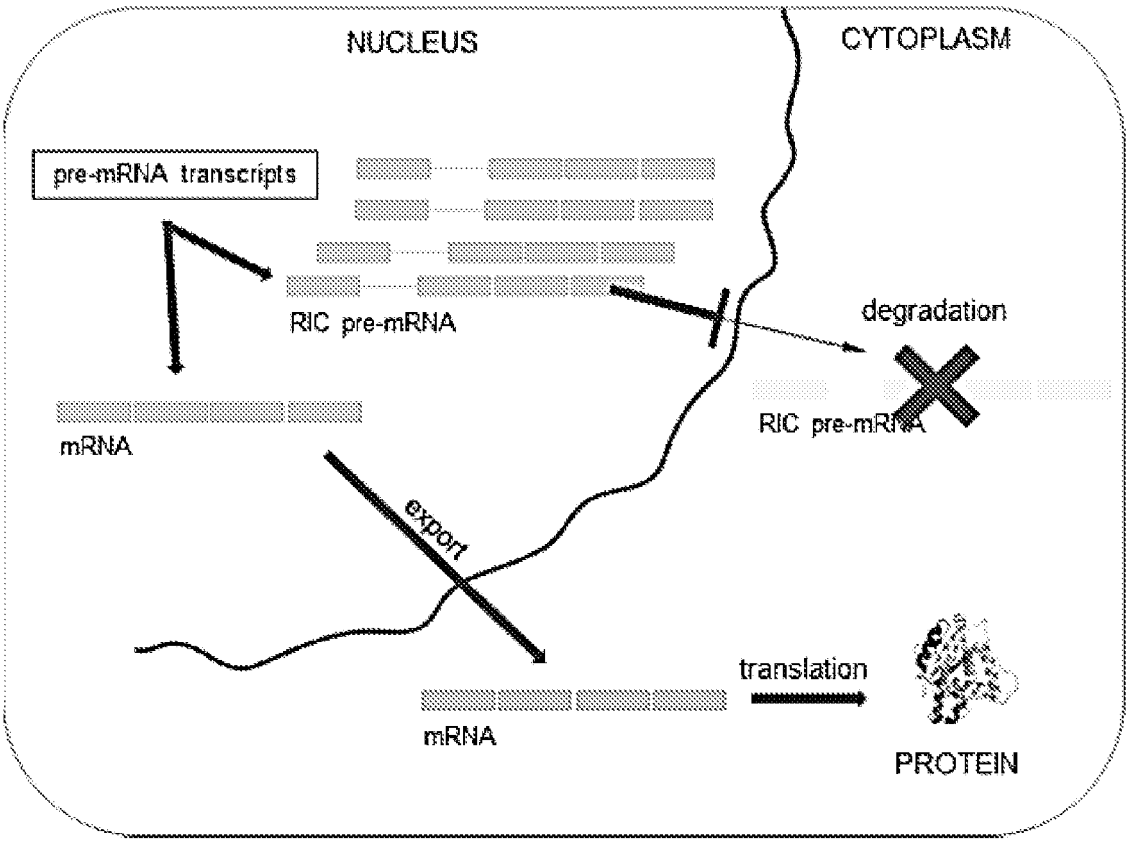

NUCLEUS
CYTOPLASM
pre-mRNA transcripts
RIC pre-mRNA
degradation
RIC pre-mRNA
mRNA
export
translation
mRNA
PROTEIN

FIG. 2B

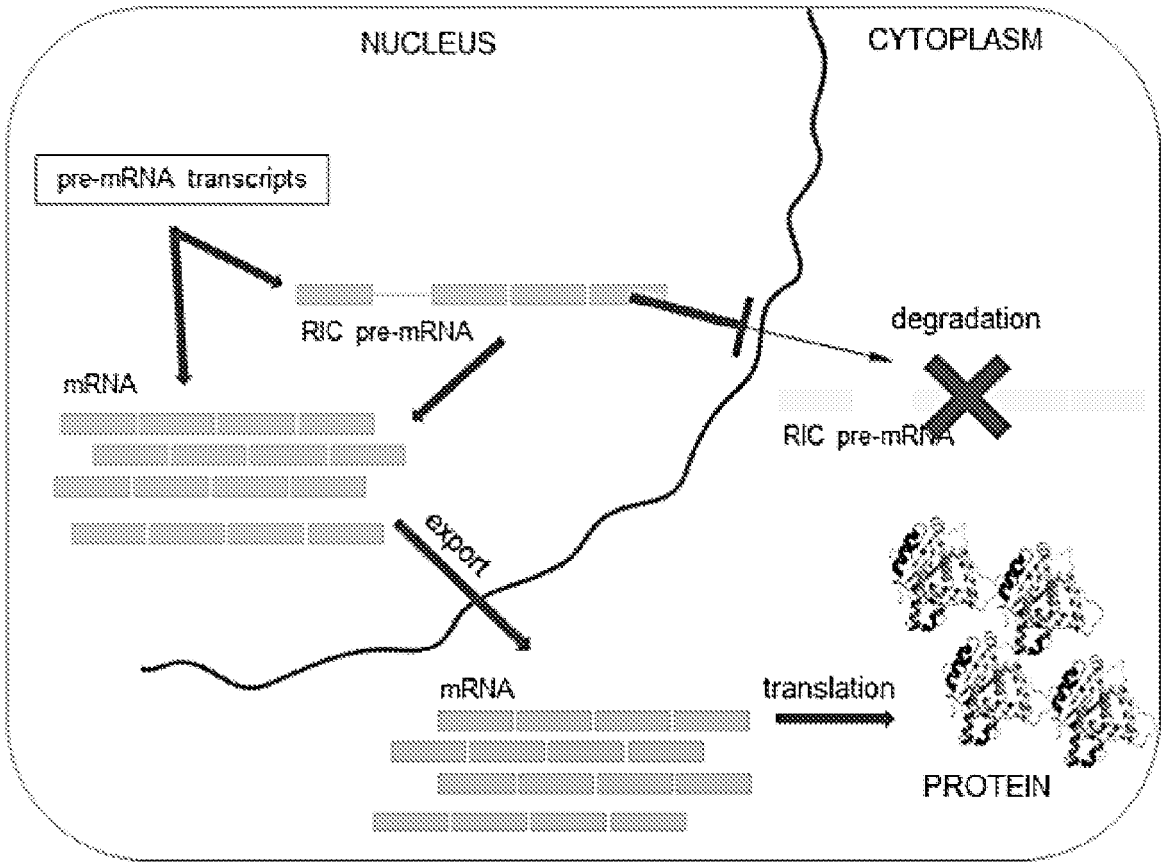

NUCLEUS
CYTOPLASM
pre-mRNA transcripts
RIC pre-mRNA
degradation
mRNA
RIC pre-mRNA
export
mRNA
translation
PROTEIN

FIG. 3

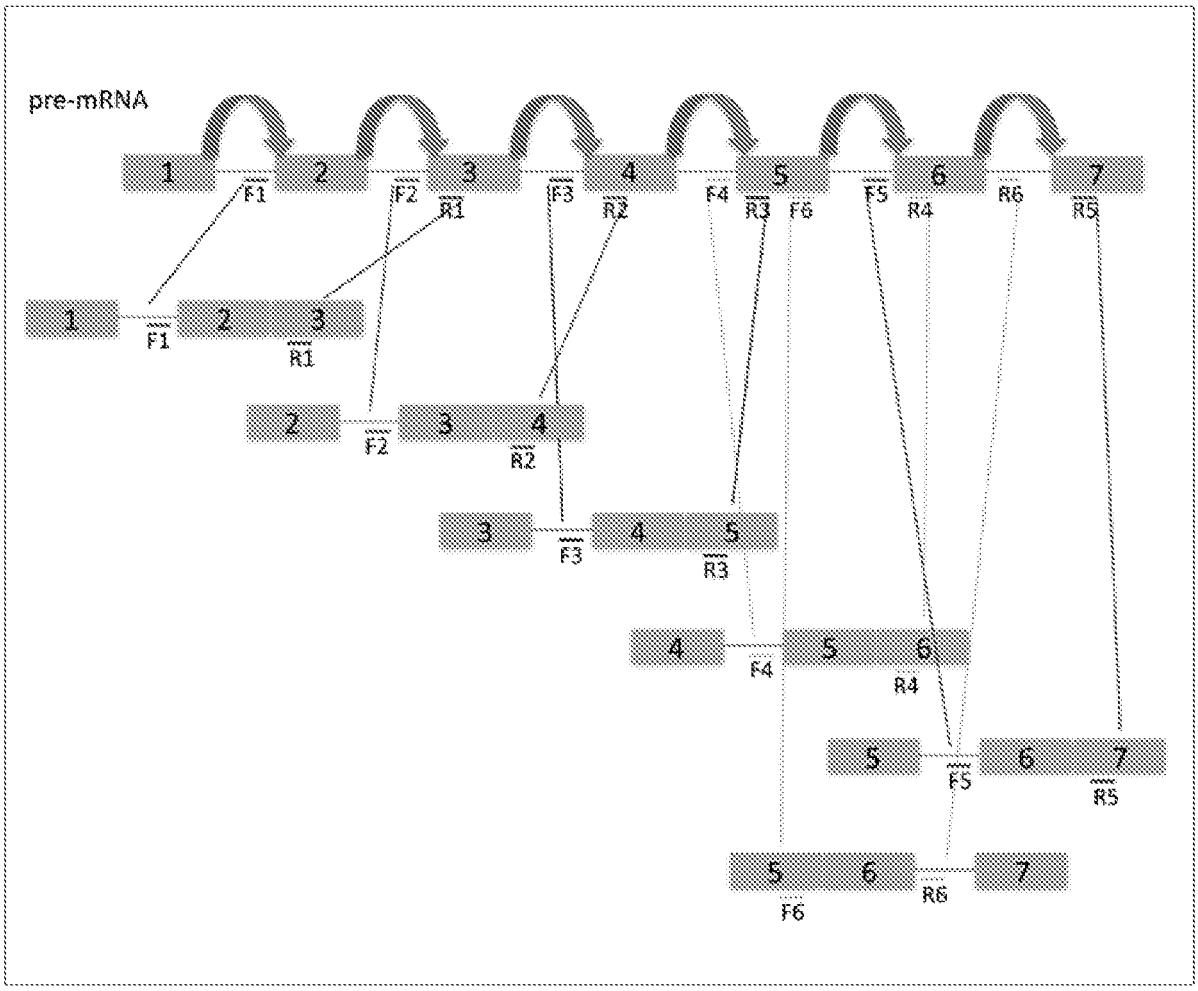

pre-mRNA
1
2
3
4
5
6
7
F1
F2
R1
F3
R2
F4
R3
F6
F5
R4
R6
R5
F1
R1
F2
R2
F3
R3
F4
R4
F5
R5
F6
R6

FIG. 4

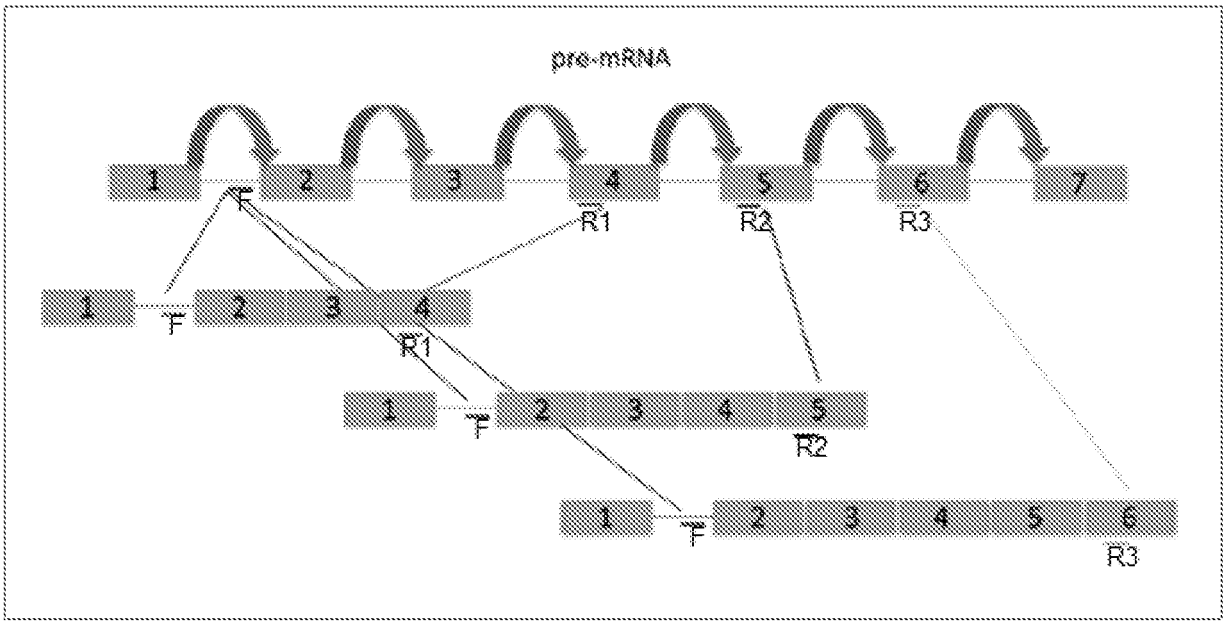

pre-mRNA
1
2
3
4
5
6
7
F
R1
R2
R3
F
R1
F
R2
F
R3

FIG. 5

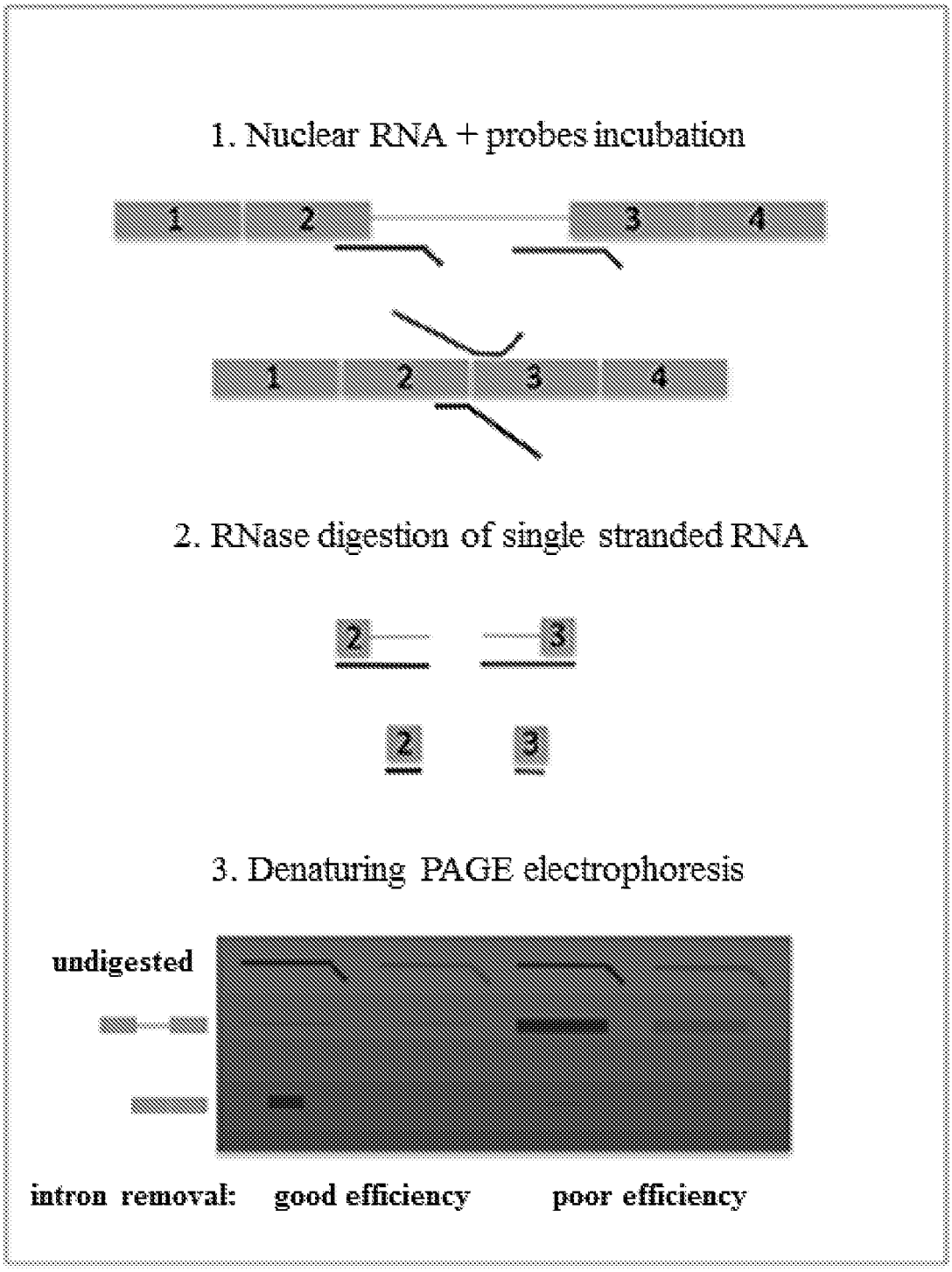

1. Nuclear RNA + probes incubation
1
2
3
4
1
2
3
4
2. RNase digestion of single stranded RNA
2
3
2
3
3. Denaturing PAGE electrophoresis
undigested
intron removal:
good efficiency
poor efficiency

FIG. 6A

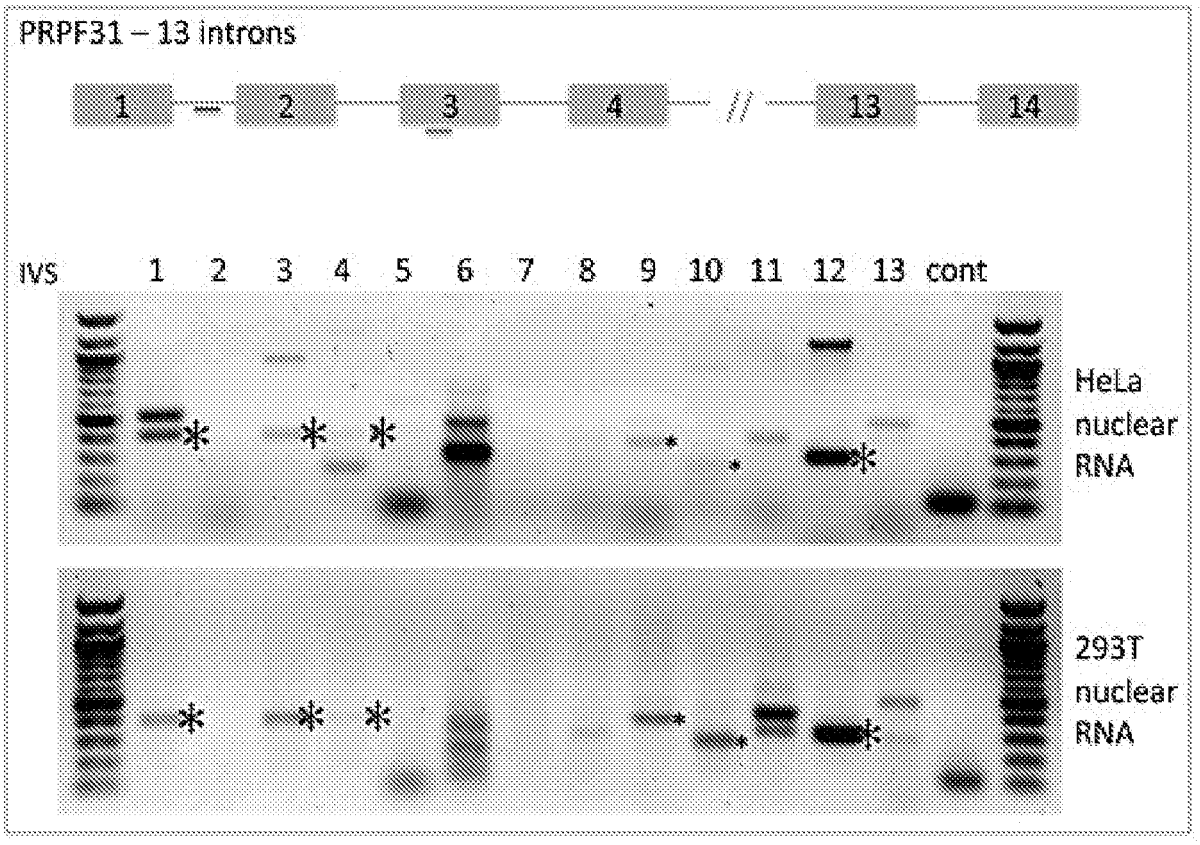

PRPF31 – 13 introns
1
2
3
4
13
14
IVS 1 2 3 4 5 6 7 8 9 10 11 12 13 cont
HeLa nuclear RNA
293T nuclear RNA

FIG. 6B

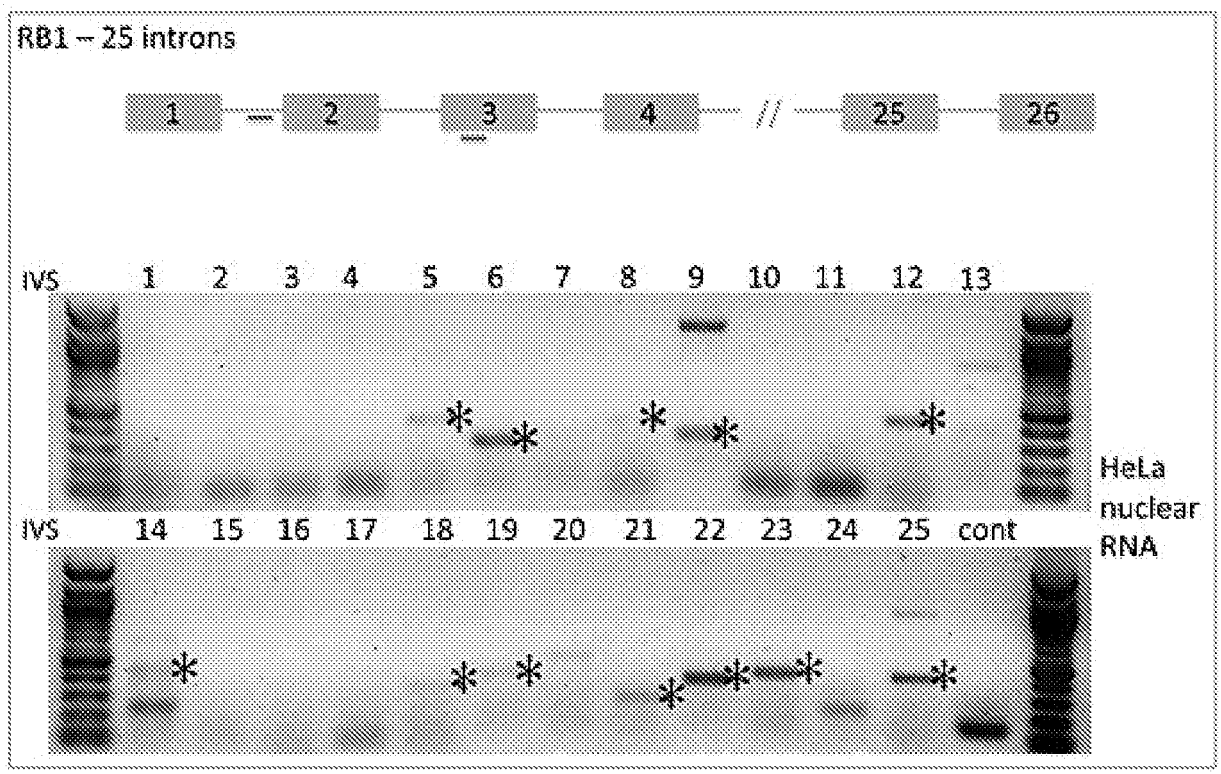

RB1 – 25 introns
1
2
3
4
25
26
IVS 1 2 3 4 5 6 7 8 9 10 11 12 13
IVS 14 15 16 17 18 19 20 21 22 23 24 25 cont
HeLa nuclear RNA

FIG. 6C

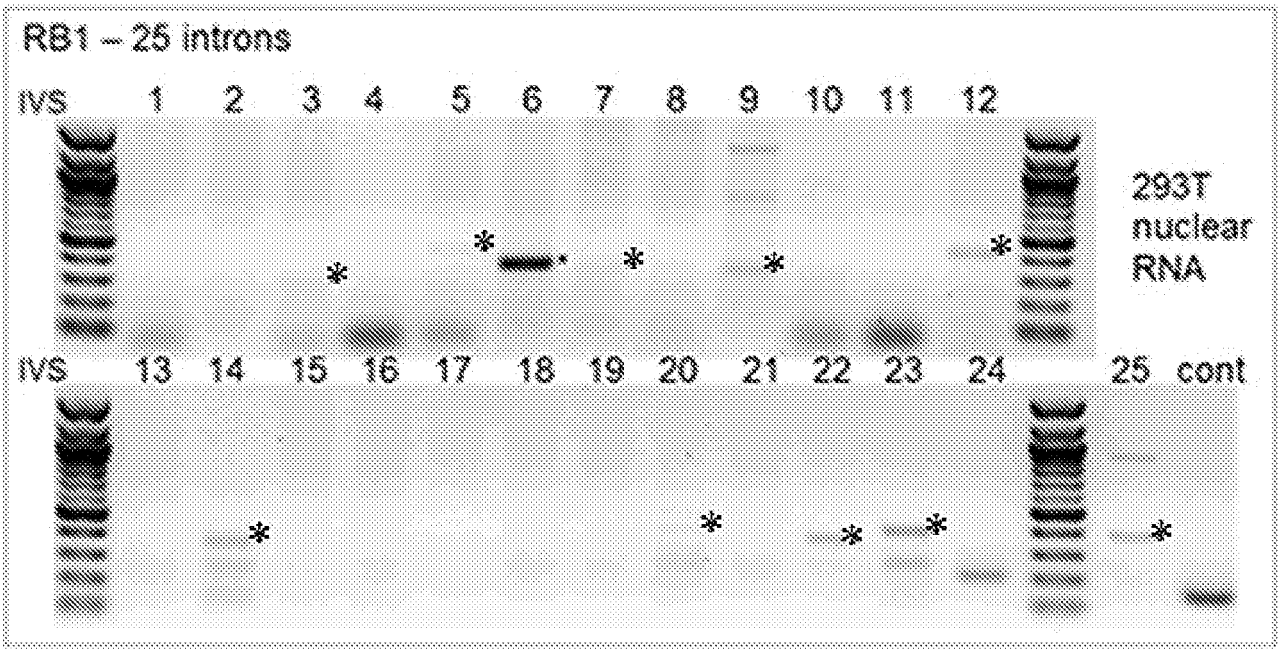

RB1 – 25 introns
IVS 1 2 3 4 5 6 7 8 9 10 11 12
293T nuclear RNA
IVS 13 14 15 16 17 18 19 20 21 22 23 24 25 cont

FIG. 6D

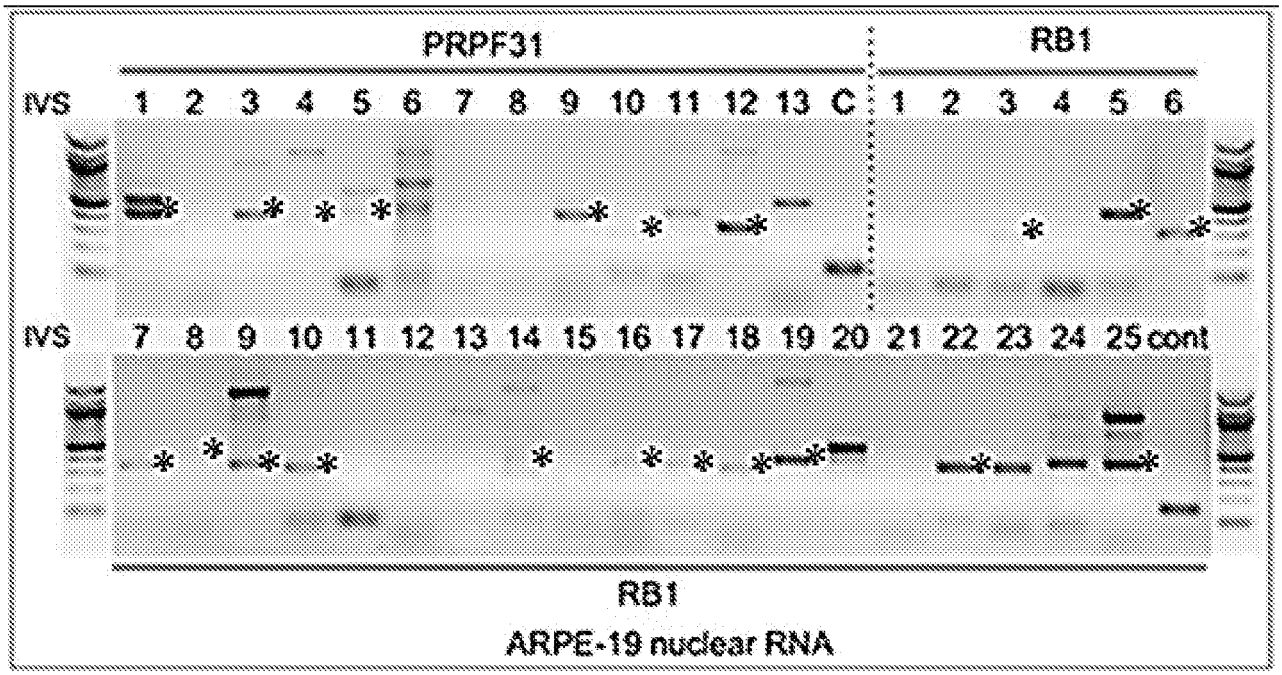

PRPF31
RB1
IVS 1 2 3 4 5 6 7 8 9 10 11 12 13 C 1 2 3 4 5 6
IVS 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 cont
RB1
ARPE-19 nuclear RNA

FIG. 6E

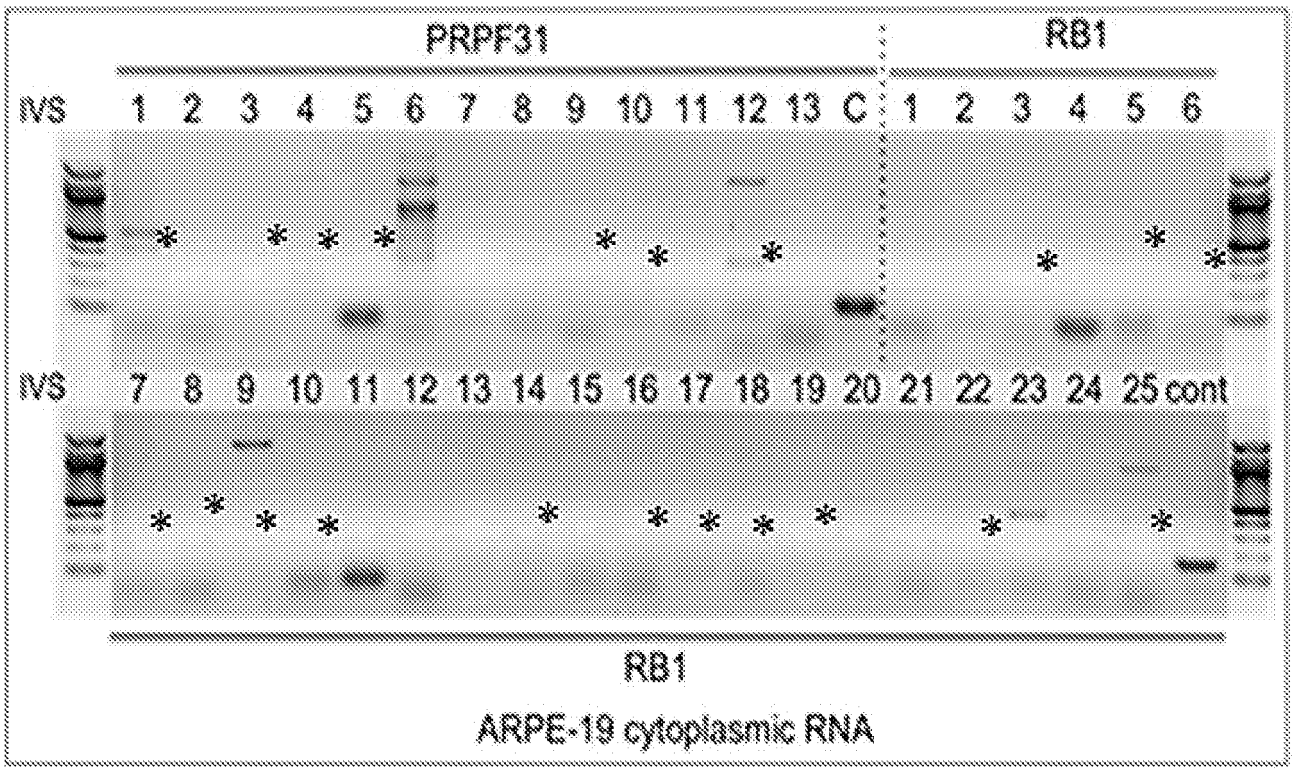

PRPF31
RB1
IVS 1 2 3 4 5 6 7 8 9 10 11 12 13 C 1 2 3 4 5 6
IVS 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 cont
RB1
ARPE-19 cytoplasmic RNA

FIG. 7A

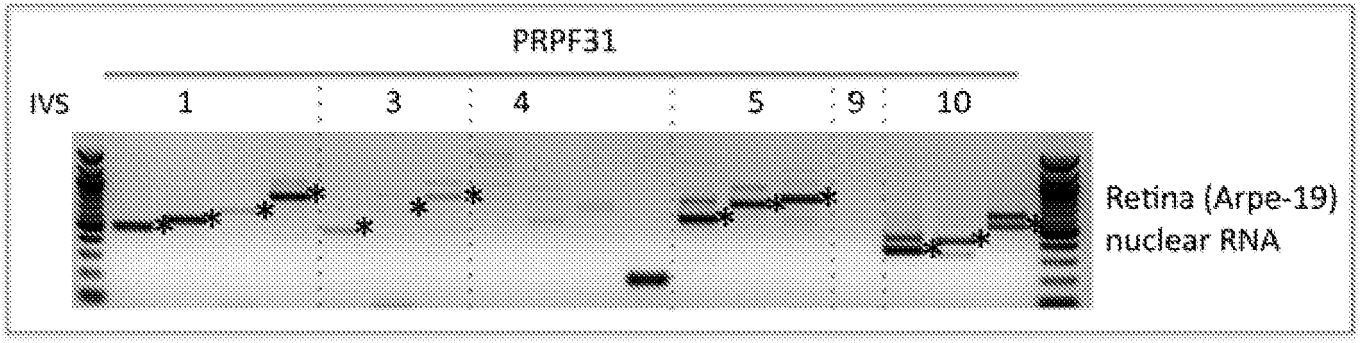

PRPF31
IVS 1 3 4 5 9 10
Retina (Arpe-19)
nuclear RNA

FIG. 7B

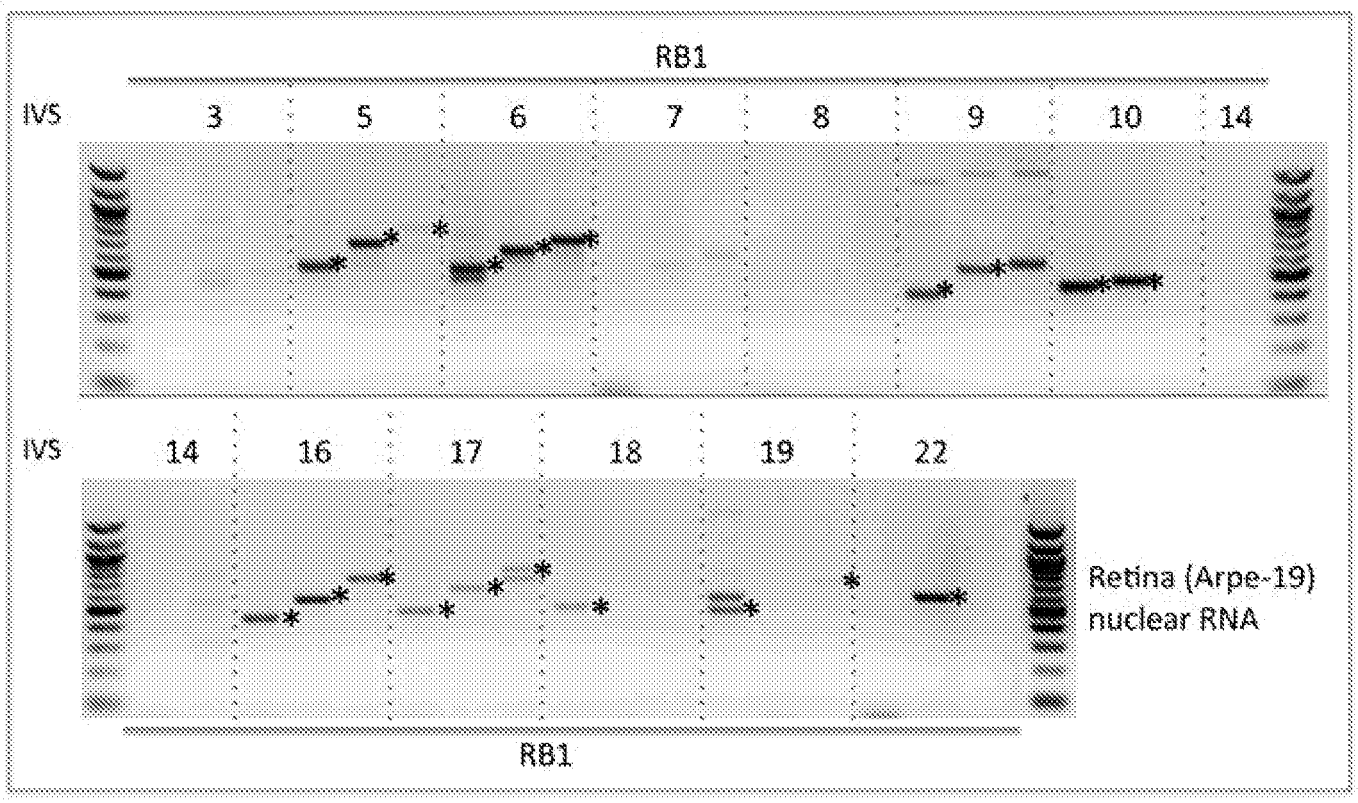

RB1
IVS
3
5
6
7
8
9
10
14
IVS
14
16
17
18
19
22
Retina (Arpe-19)
nuclear RNA
RB1

FIG. 8A

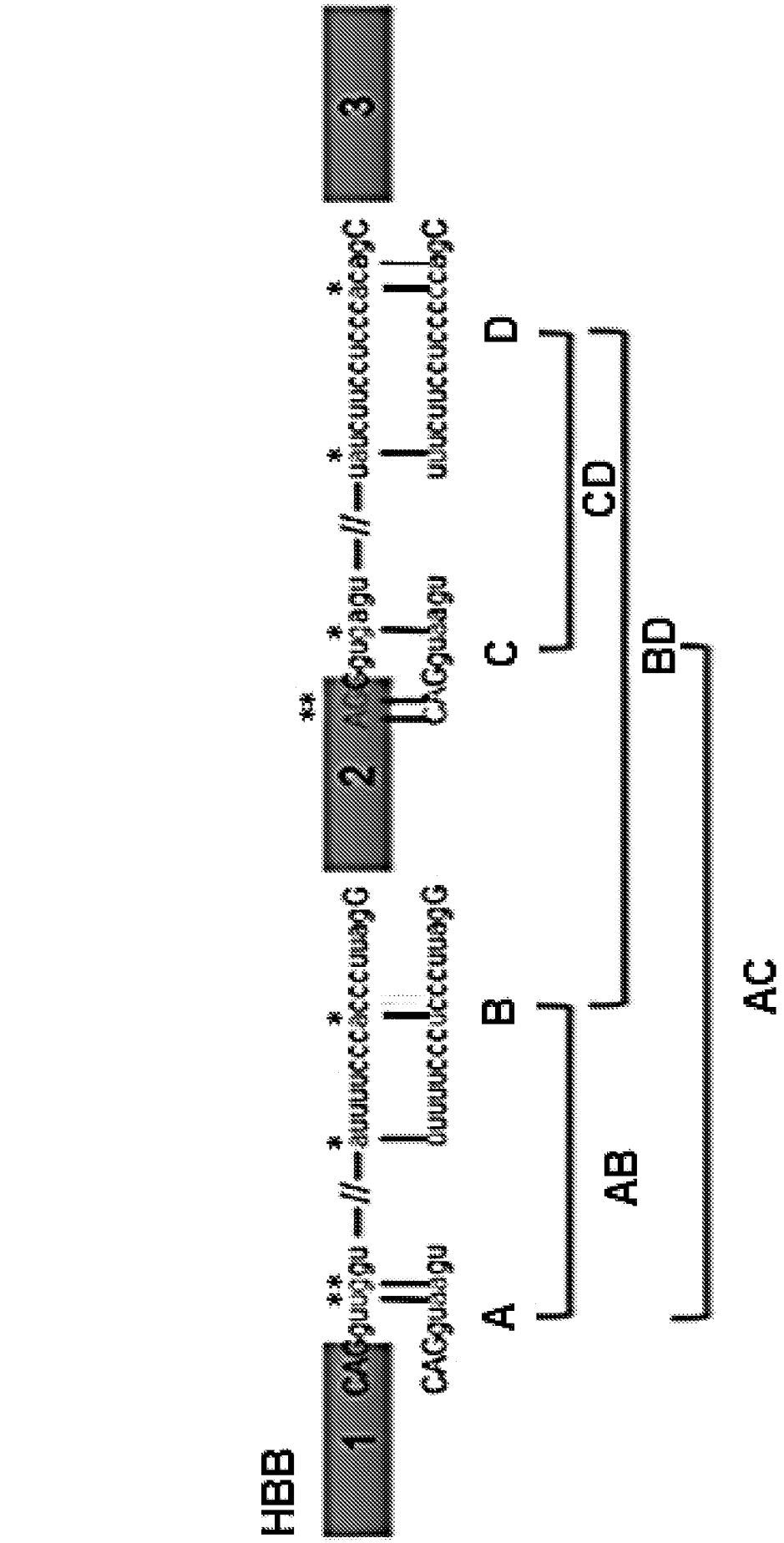


FIG. 8B

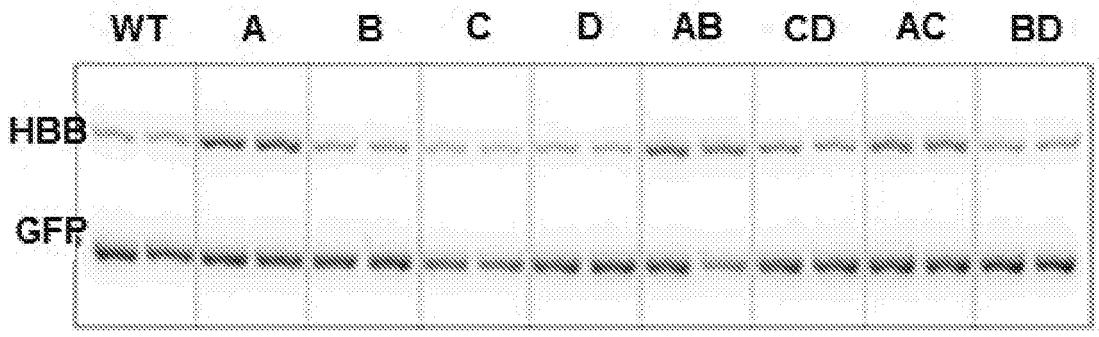


FIG. 8C

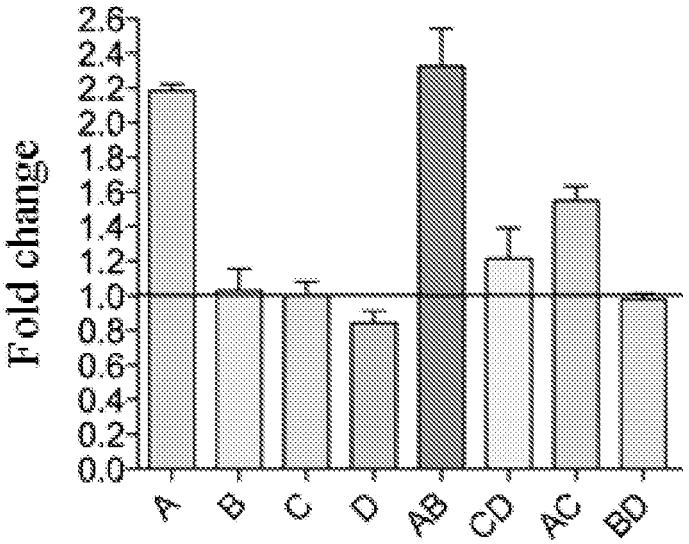


FIG. 9A

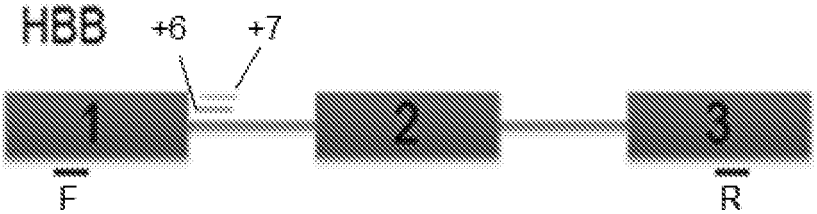


FIG. 9B

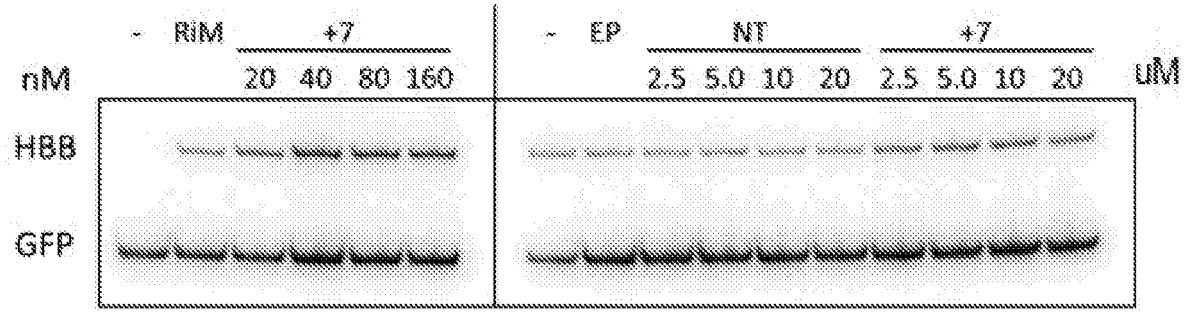

- RIM +7
nM
20 40 80 160
- EP NT +7
2.5 5.0 10 20 2.5 5.0 10 20
uM
HBB
GFP

FIG. 9C

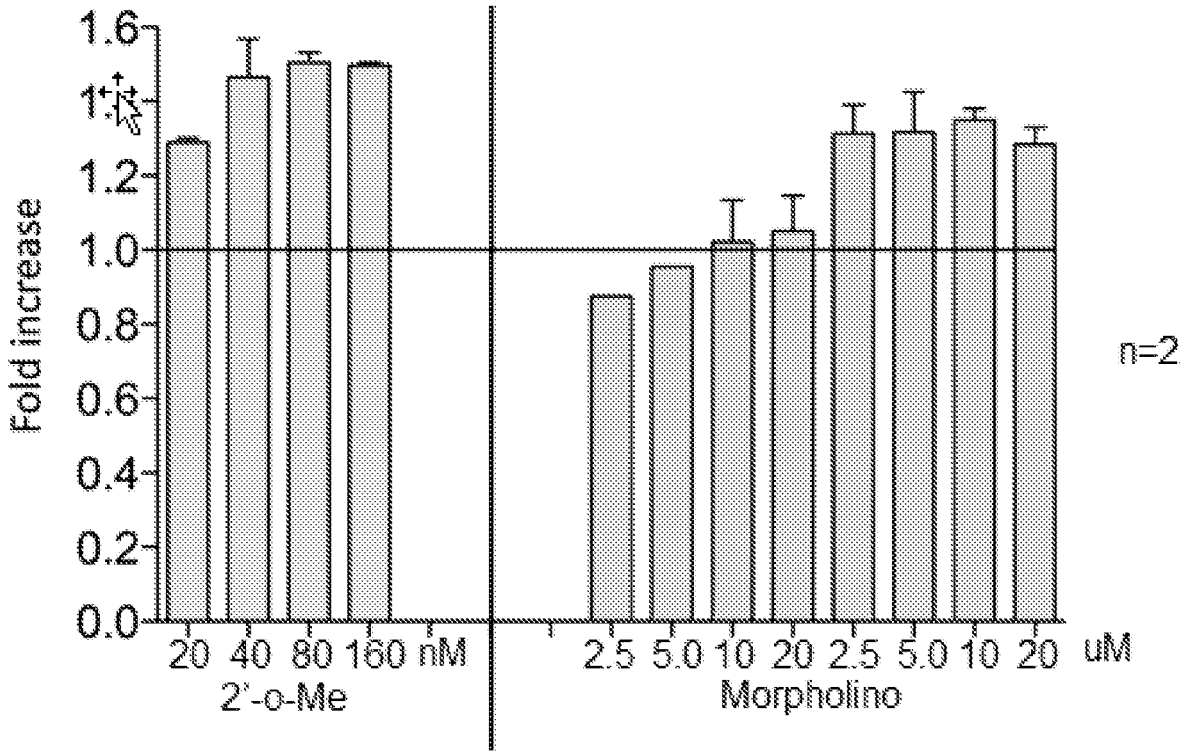

Fold increase
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
20 40 80 160 nM
2'-o-Me
2.5 5.0 10 20 2.5 5.0 10 20 uM
Morpholino
n=2

FIG. 10A

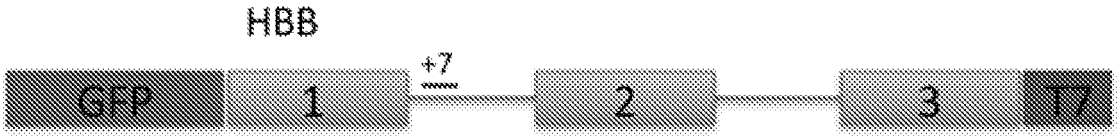


FIG. 10B

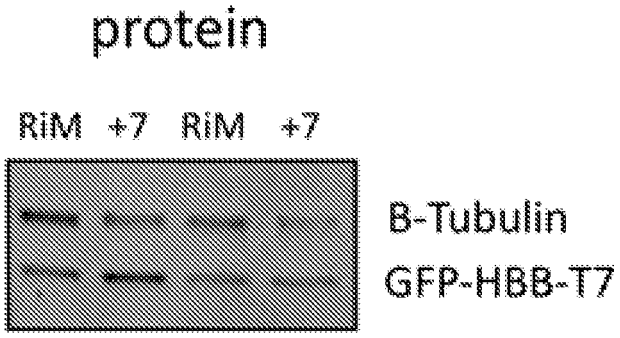


FIG. 10C

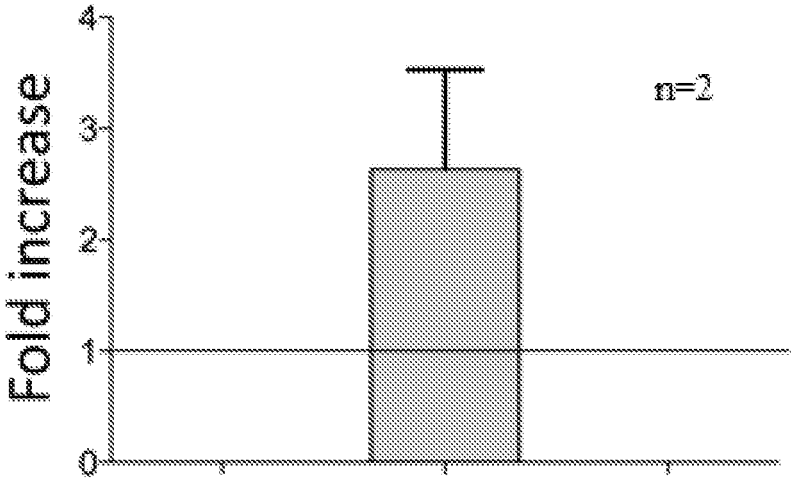


FIG. 11

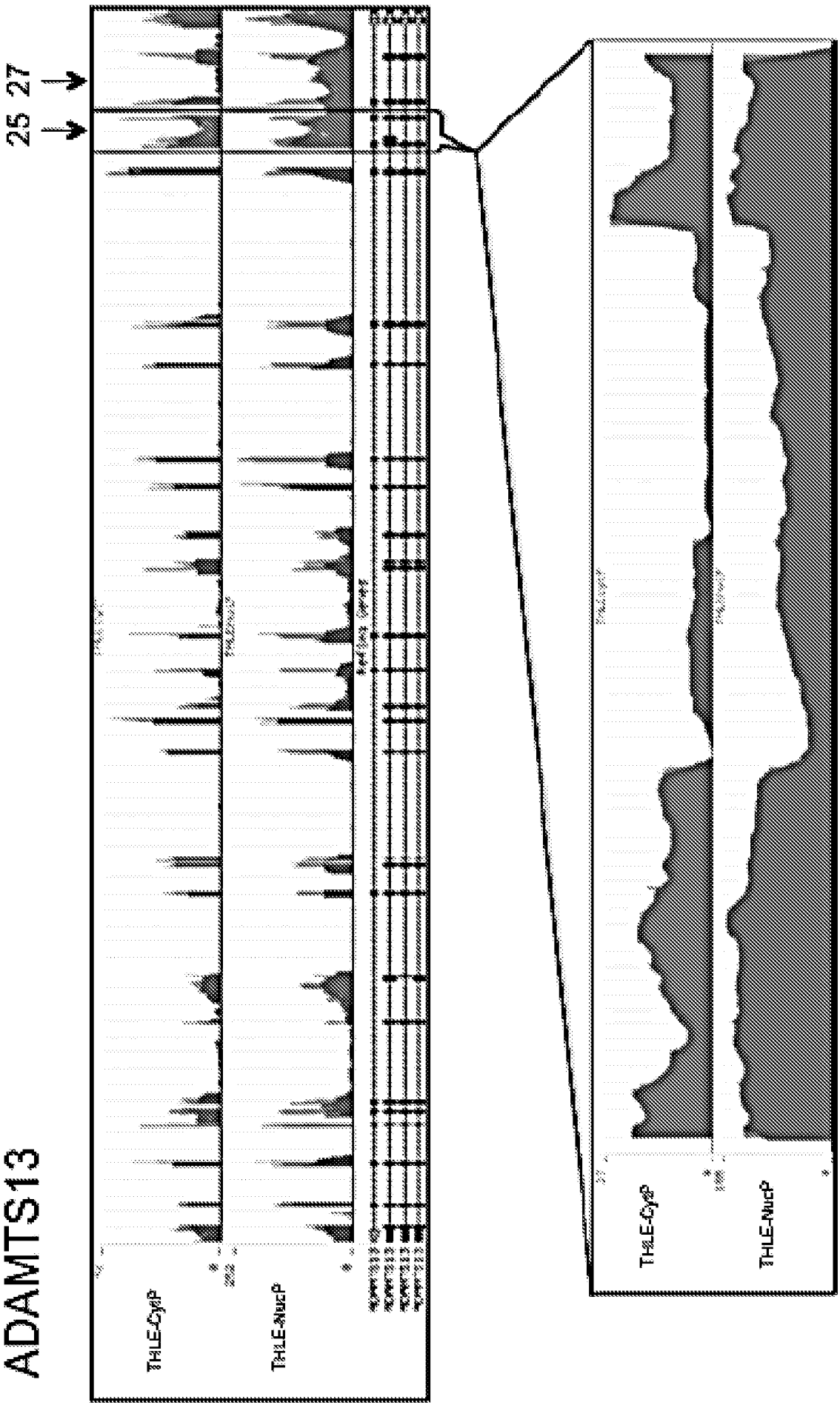
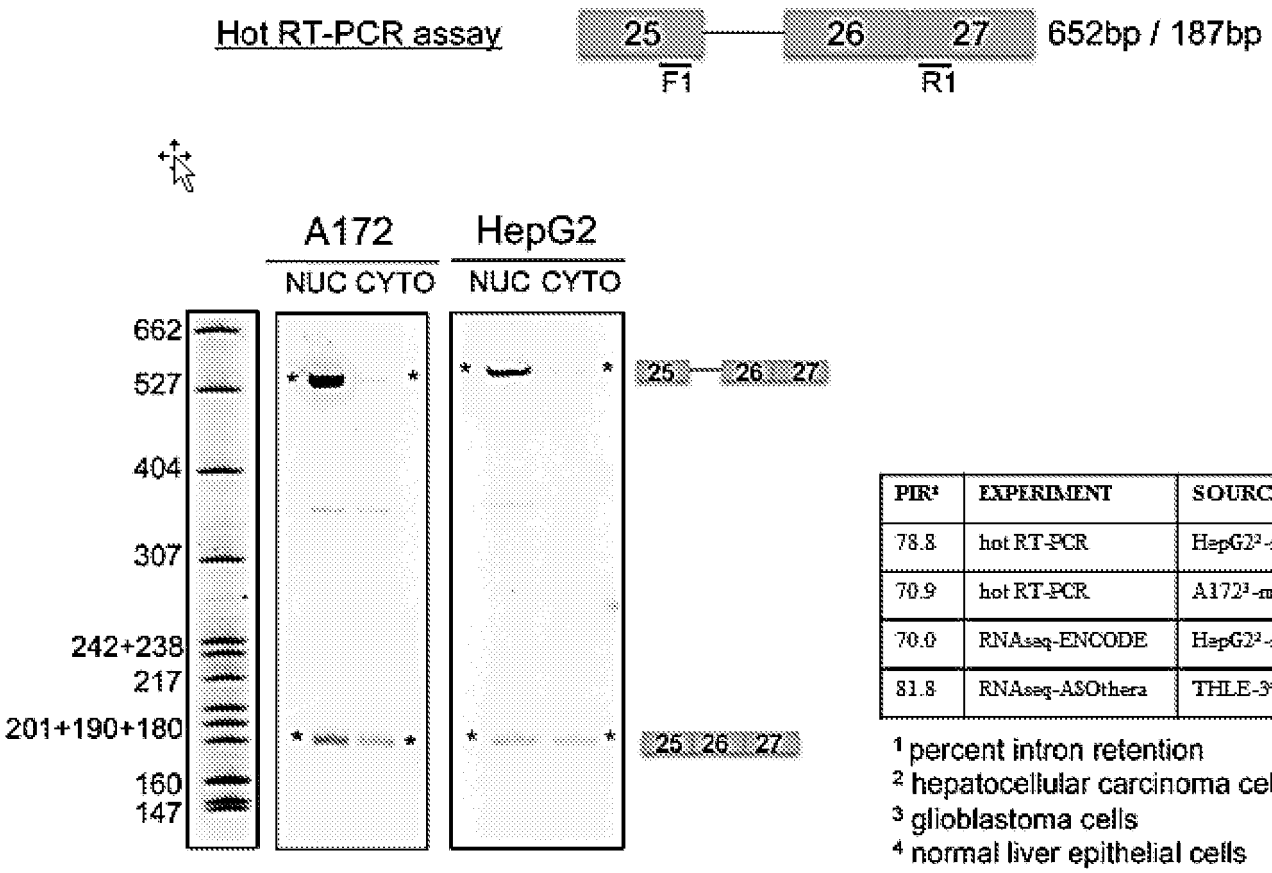


FIG. 12



PIR[1]	EXPERIMENT	SOURCE
78.8	hot RT-PCR	HepG2[2]-nuc
70.9	hot RT-PCR	A172[3]-nuc
70.0	RNAseq-ENCODE	HepG2[2]-nuc
81.8	RNAseq-ASOthera	THLE-3[4]-nuc

[1] percent intron retention
[2] hepatocellular carcinoma cells
[3] glioblastoma cells
[4] normal liver epithelial cells

FIG. 13

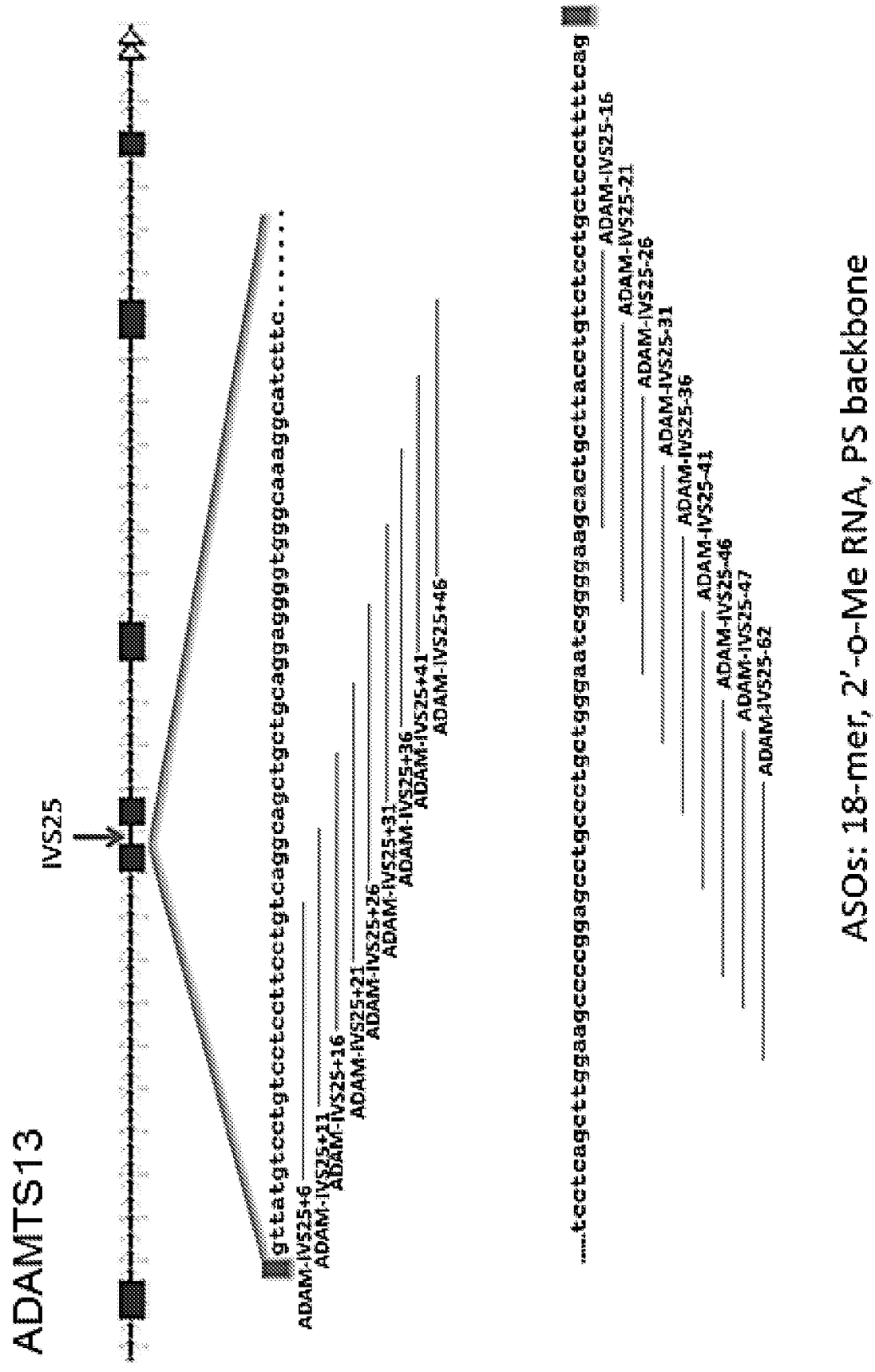

ADAMTS13
IVS25
gttatgtcctgtcctccttcctgtcaggcagctgctgcaggaggggtgggcaaaggcatcttc.....
ADAM-IVS25+6
ADAM-IVS25+11
ADAM-IVS25+16
ADAM-IVS25+21
ADAM-IVS25+26
ADAM-IVS25+31
ADAM-IVS25+36
ADAM-IVS25+41
ADAM-IVS25+46
....tcctcagcttggaagccccggagcctgccctgctgggaatcgggaagcactgcttacctgtctcctgctttcctttcag
ADAM-IVS25-16
ADAM-IVS25-21
ADAM-IVS25-26
ADAM-IVS25-31
ADAM-IVS25-36
ADAM-IVS25-41
ADAM-IVS25-46
ADAM-IVS25-47
ADAM-IVS25-62
ASOs: 18-mer, 2'-o-Me RNA, PS backbone

FIG. 14

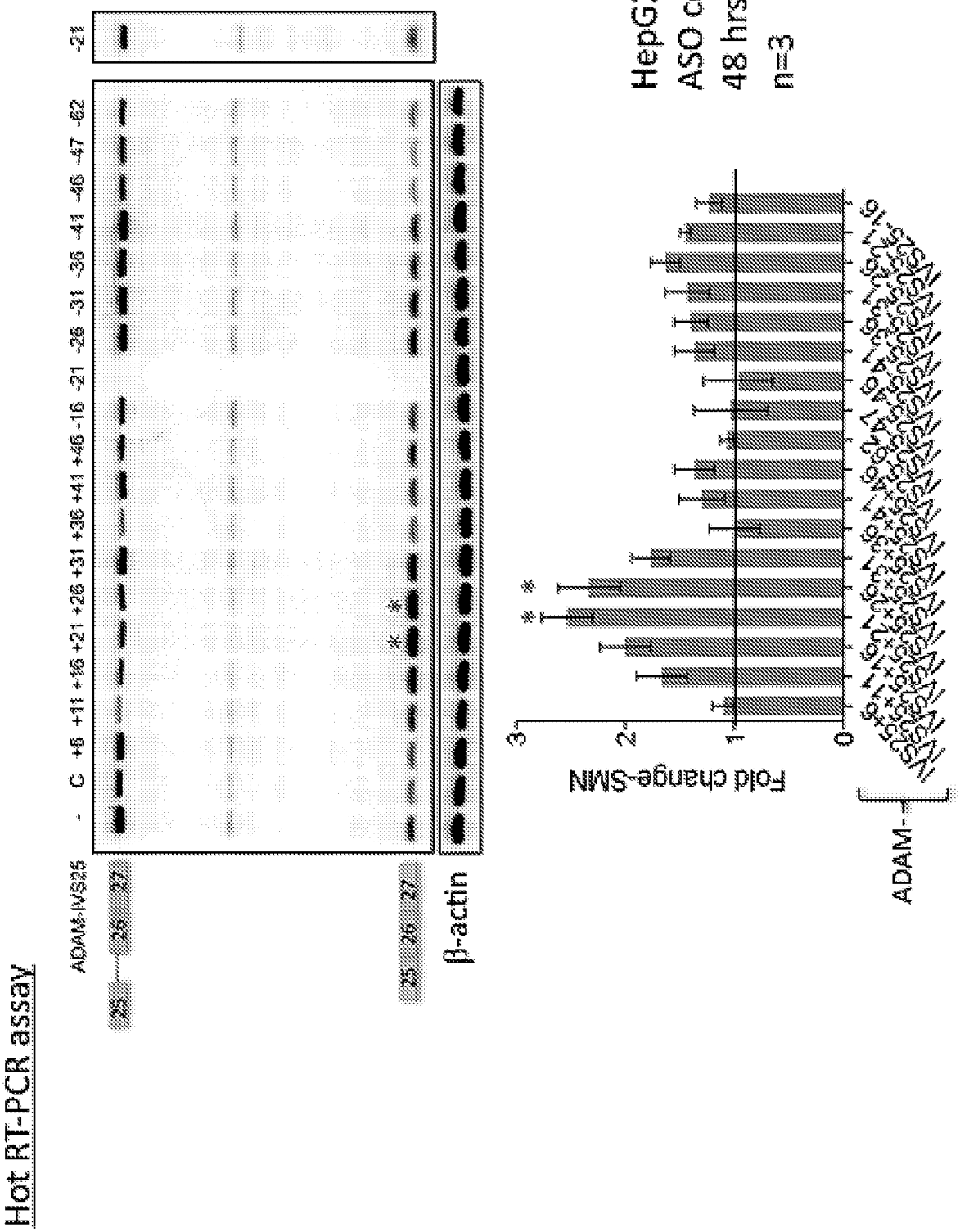

Hot RT-PCR assay
ADAM-IVS25
25 26 27
- C +6 +11 +16 +21 +26 +31 +36 +41 +46 -16 -21 -26 -31 -36 -41 -46 -47 -62 -21
β-actin
25 26 27
HepG2
ASO conc.: 60 nM
48 hrs
n=3
Fold change-SMN
ADAM-

FIG. 15

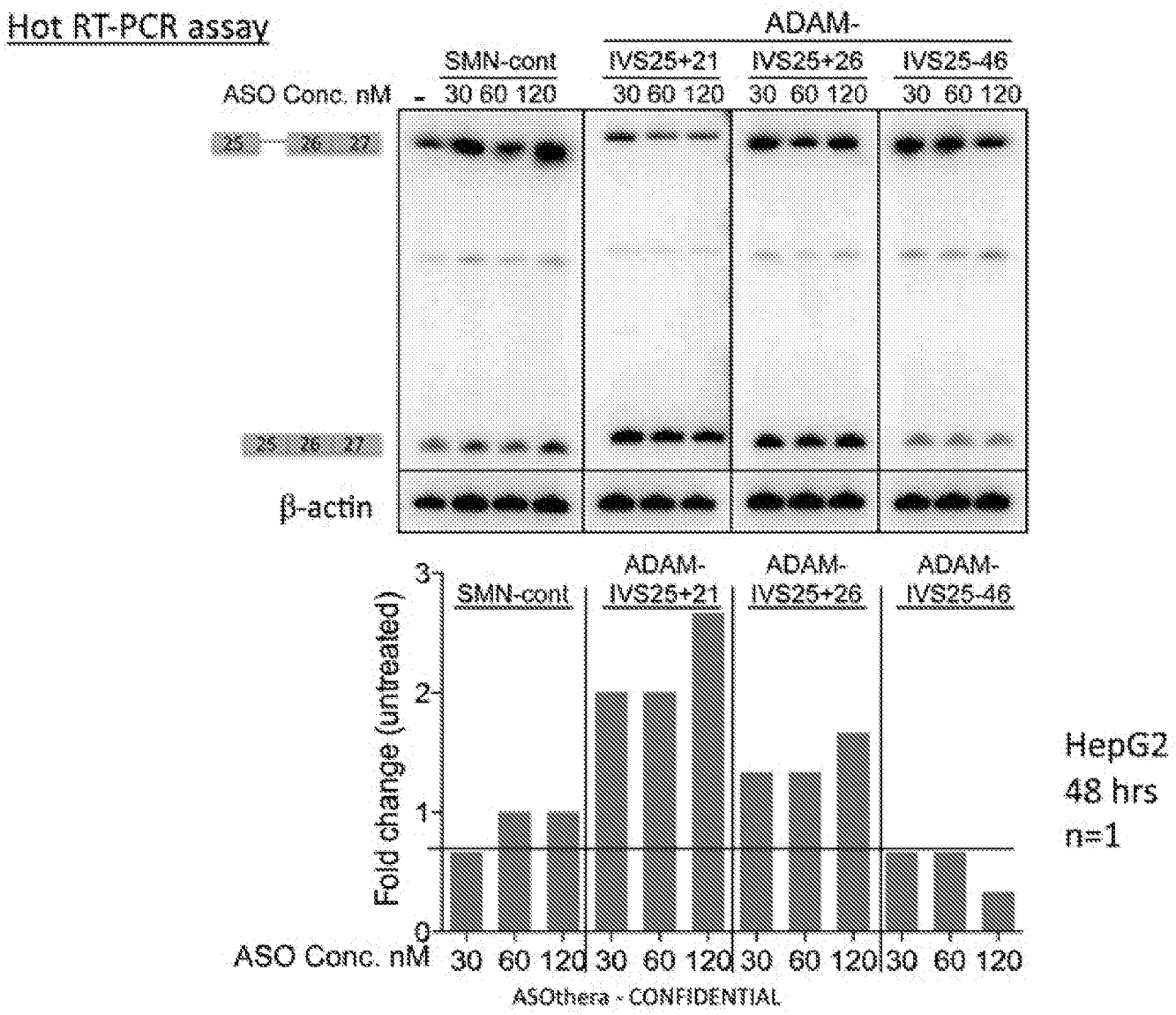

Hot RT-PCR assay
ADAM-
SMN-cont
IVS25+21
IVS25+26
IVS25-46
ASO Conc. nM - 30 60 120 30 60 120 30 60 120 30 60 120
25 26 27
25 26 27
β-actin
SMN-cont
ADAM-IVS25+21
ADAM-IVS25+26
ADAM-IVS25-46
Fold change (untreated)
0
1
2
3
ASO Conc. nM 30 60 120 30 60 120 30 60 120 30 60 120
HepG2
48 hrs
n=1
ASOthera - CONFIDENTIAL

FIG. 16

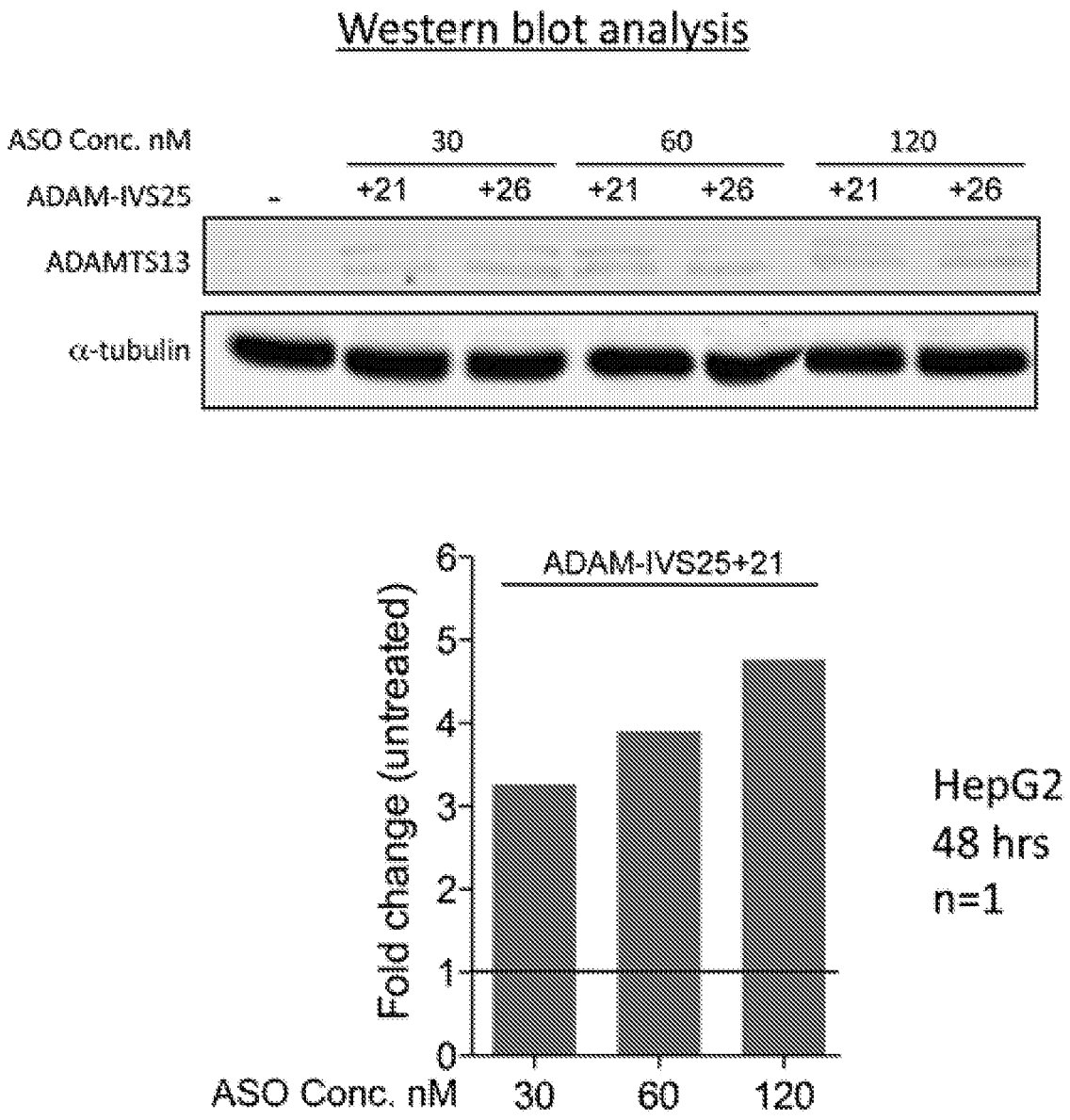

Western blot analysis
ASO Conc. nM
30
60
120
ADAM-IVS25
-
+21
+26
+21
+26
+21
+26
ADAMTS13
α-tubulin
ADAM-IVS25+21
Fold change (untreated)
0
1
2
3
4
5
6
ASO Conc. nM
30
60
120
HepG2
48 hrs
n=1

FIG. 17

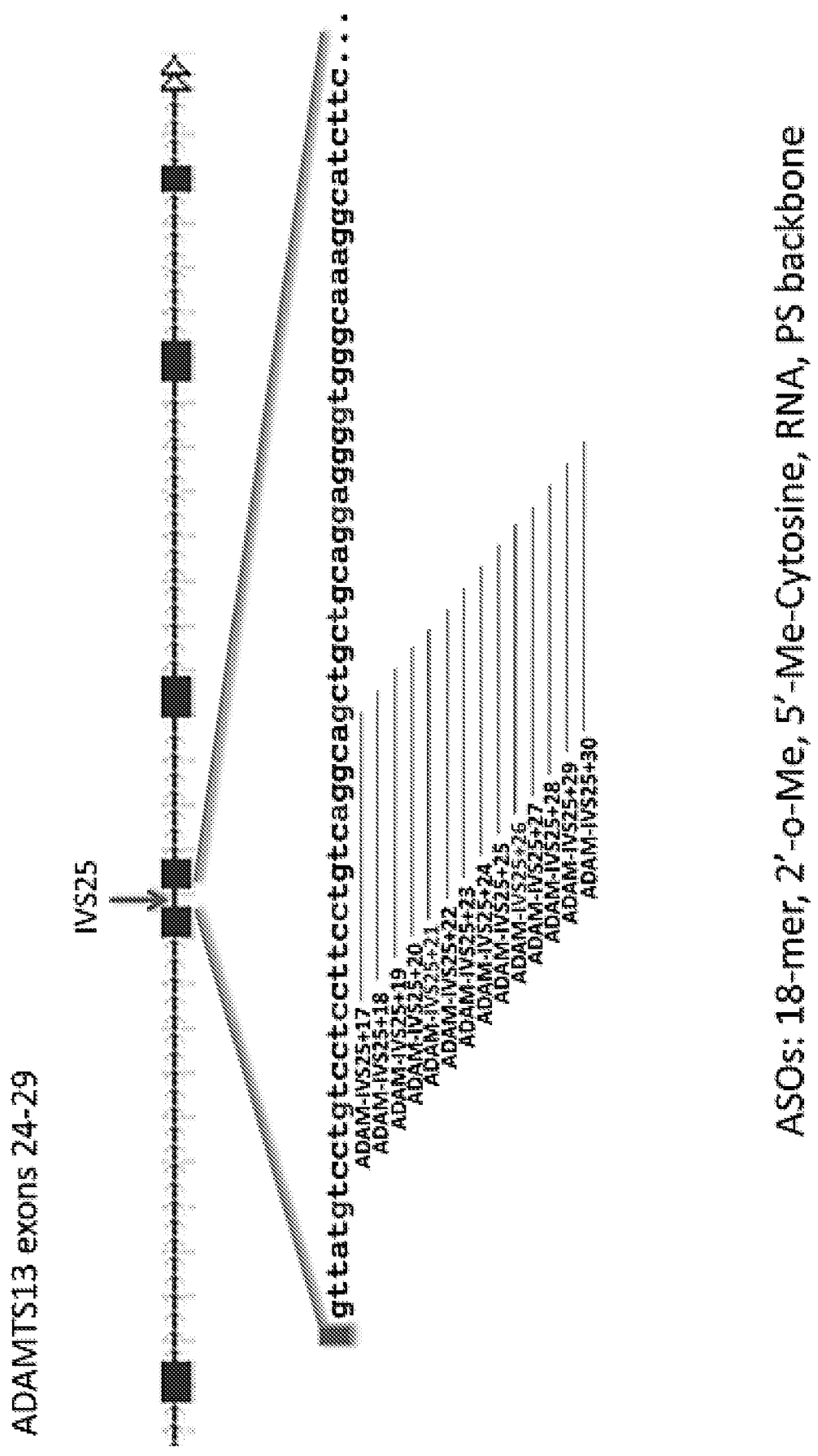

ADAMTS13 exons 24-29
IVS25
gttatgtcctgtcctccttcctgtcaggcagctgctgcaggagggtgggcaaaggcatcttc...
ADAM-IVS25+17
ADAM-IVS25+18
ADAM-IVS25+19
ADAM-IVS25+20
ADAM-IVS25+21
ADAM-IVS25+22
ADAM-IVS25+23
ADAM-IVS25+24
ADAM-IVS25+25
ADAM-IVS25+26
ADAM-IVS25+27
ADAM-IVS25+28
ADAM-IVS25+29
ADAM-IVS25+30
ASOs: 18-mer, 2'-o-Me, 5'-Me-Cytosine, RNA, PS backbone

FIG. 18

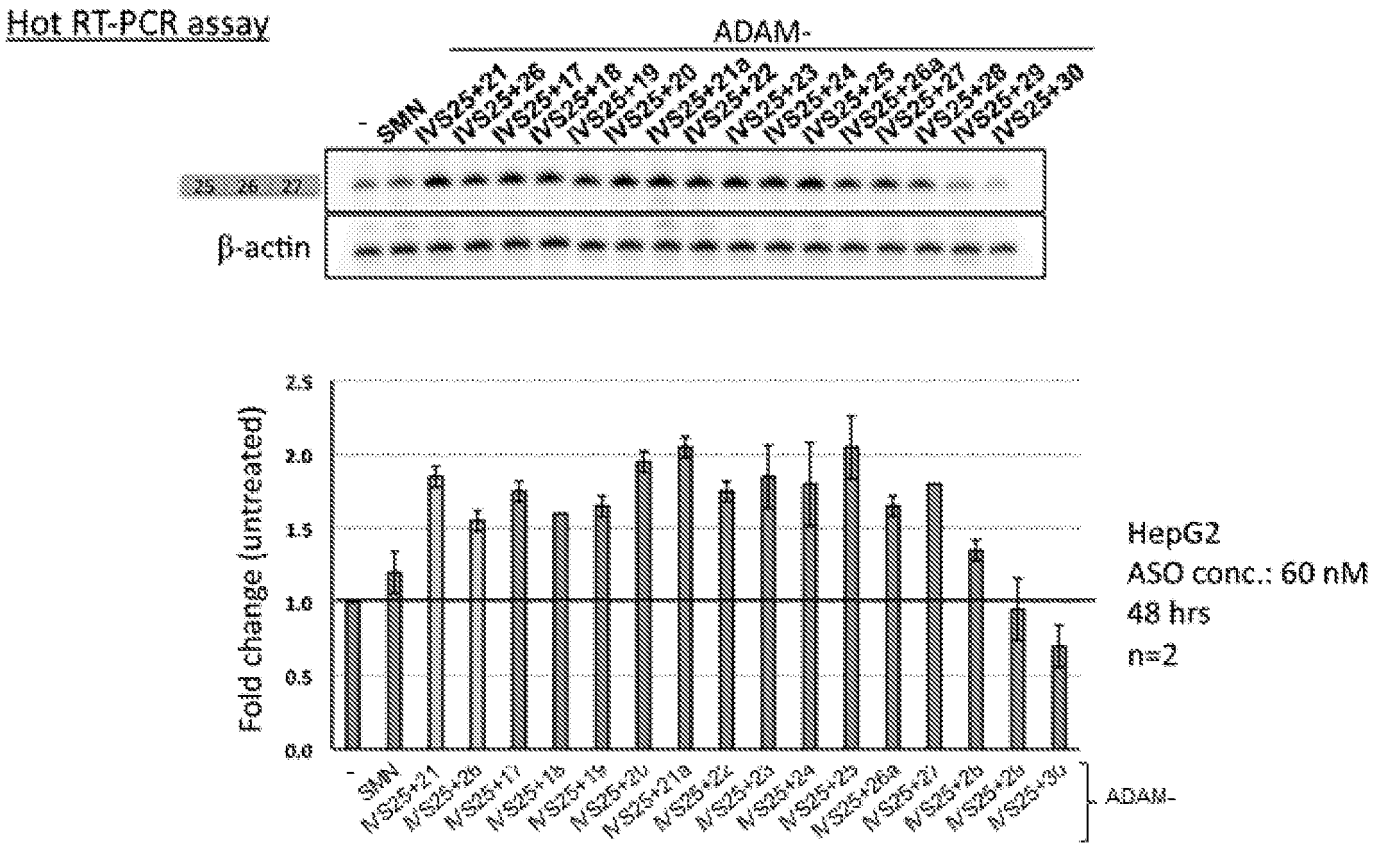

Hot RT-PCR assay
ADAM-
- SMN IVS25+21 IVS25+26 IVS25+17 IVS25+18 IVS25+19 IVS25+20 IVS25+21a IVS25+22 IVS25+23 IVS25+24 IVS25+25 IVS25+26a IVS25+27 IVS25+28 IVS25+29 IVS25+30
25 26 27
β-actin
Fold change (untreated)
2.5
2.0
1.5
1.0
0.5
0.0
HepG2
ASO conc.: 60 nM
48 hrs
n=2
ADAM-

FIG. 19

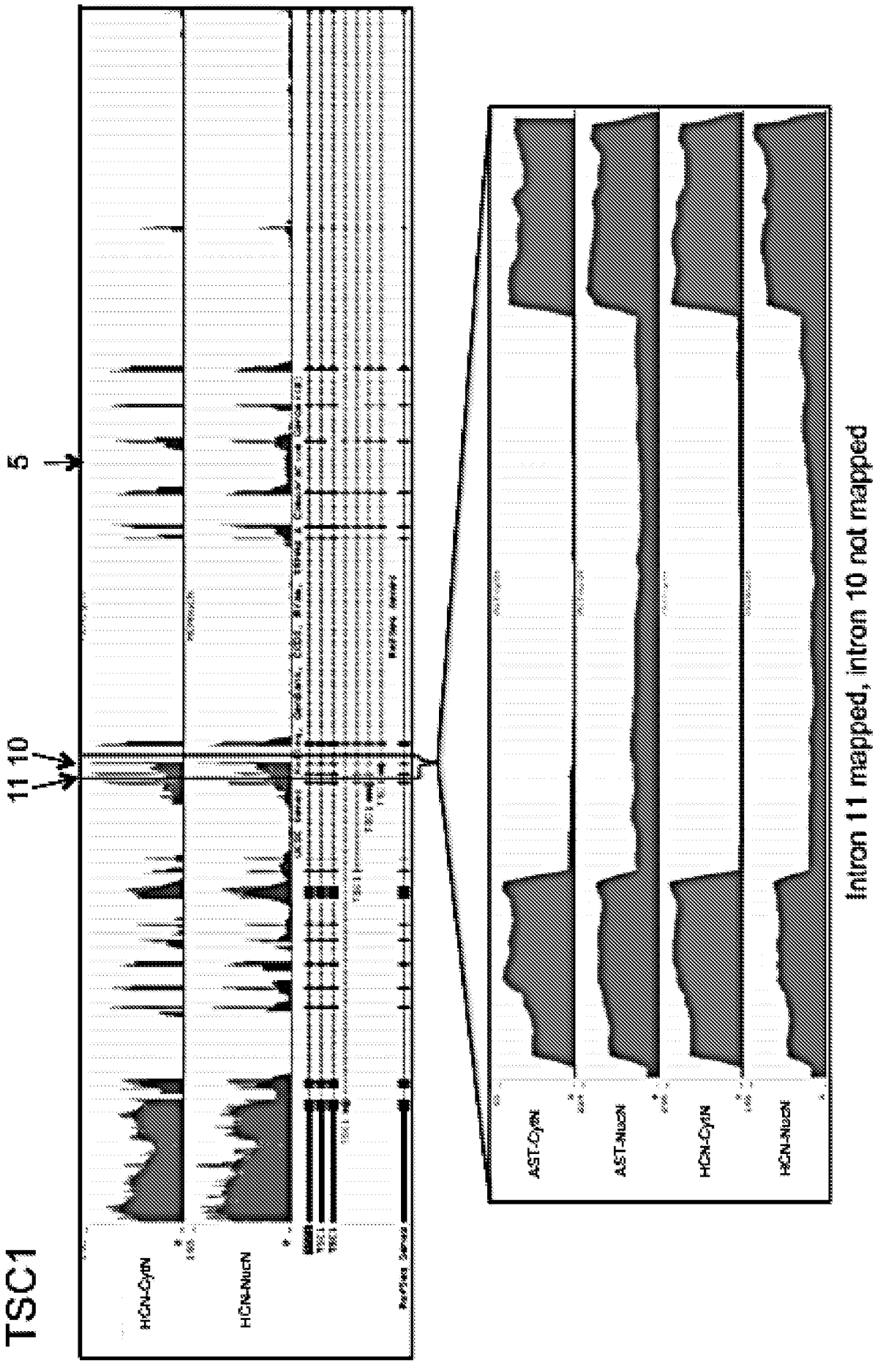
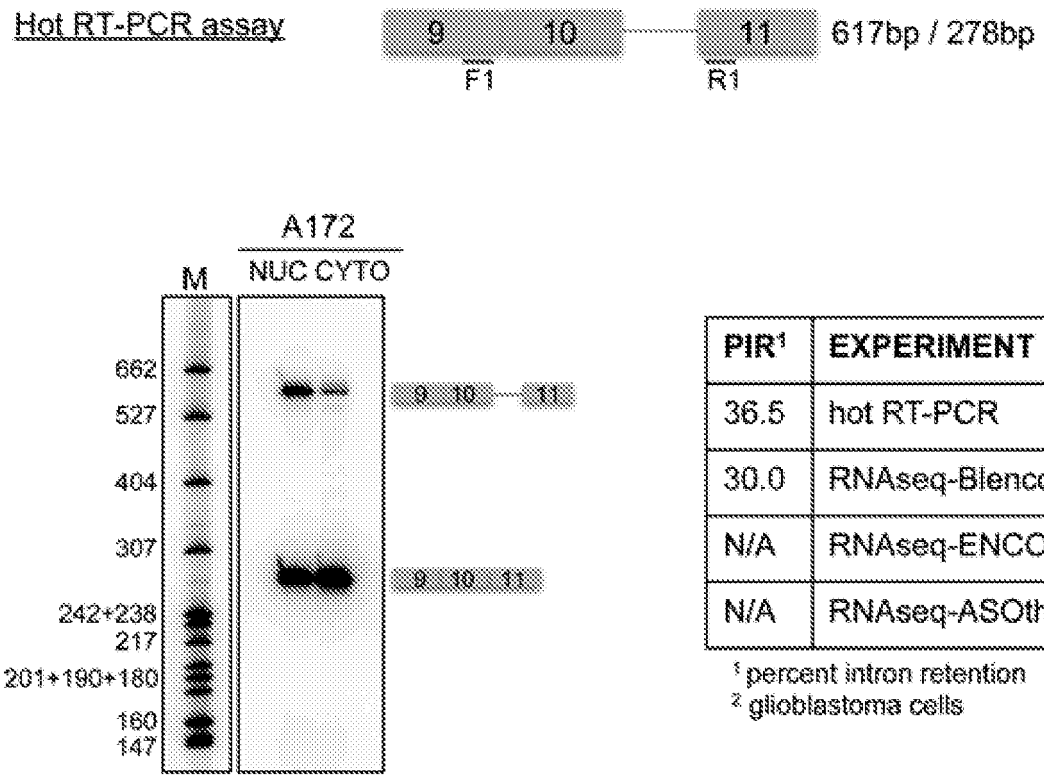

TSC1
11 10
5
HCN-Cyt/N
HCN-Nuc/N
AST-Cyt/N
AST-Nuc/N
HCN-Cyt/N
HCN-Nuc/N
Intron 11 mapped, intron 10 not mapped

FIG. 20



PIR[1]	EXPERIMENT	SOURCE
36.5	hot RT-PCR	A172[2]-nuc
30.0	RNAseq-Blencowe	Brain
N/A	RNAseq-ENCODE	N/A
N/A	RNAseq-ASOthera	N/A

[1] percent intron retention
[2] glioblastoma cells

FIG. 21

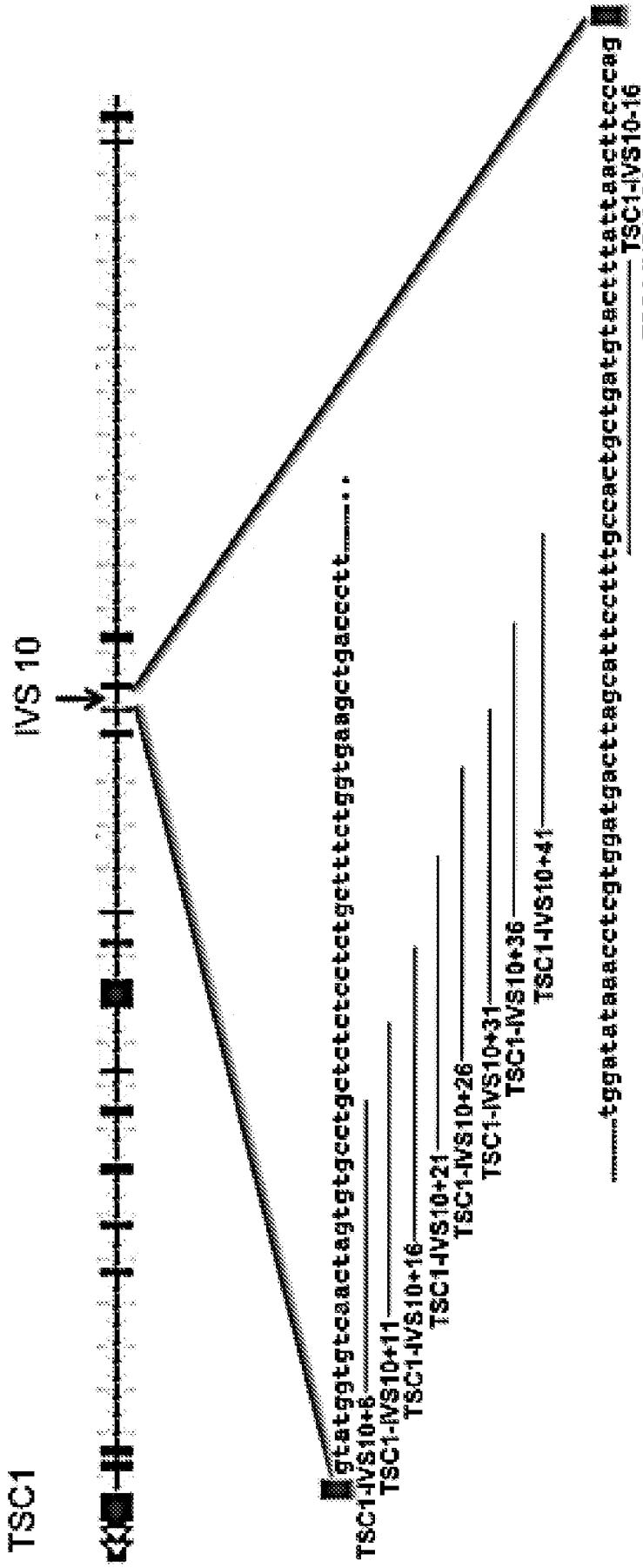

TSC1
IVS 10
gtatgggtgtcaactagtgtgcctgctctctcctctgctttctggtgaagctgaccctt
TSC1-IVS10+6
TSC1-IVS10+11
TSC1-IVS10+16
TSC1-IVS10+21
TSC1-IVS10+26
TSC1-IVS10+31
TSC1-IVS10+36
TSC1-IVS10+41
tggataaaacctcgtggatgacttagcatttccttgccactgctgatgtacttattaacttcccag
TSC1-IVS10-16
TSC1-IVS10-21
TSC1-IVS10-26
TSC1-IVS10-31
TSC1-IVS10-36
TSC1-IVS10-41
TSC1-IVS10-46
TSC1-IVS10-51

FIG. 22

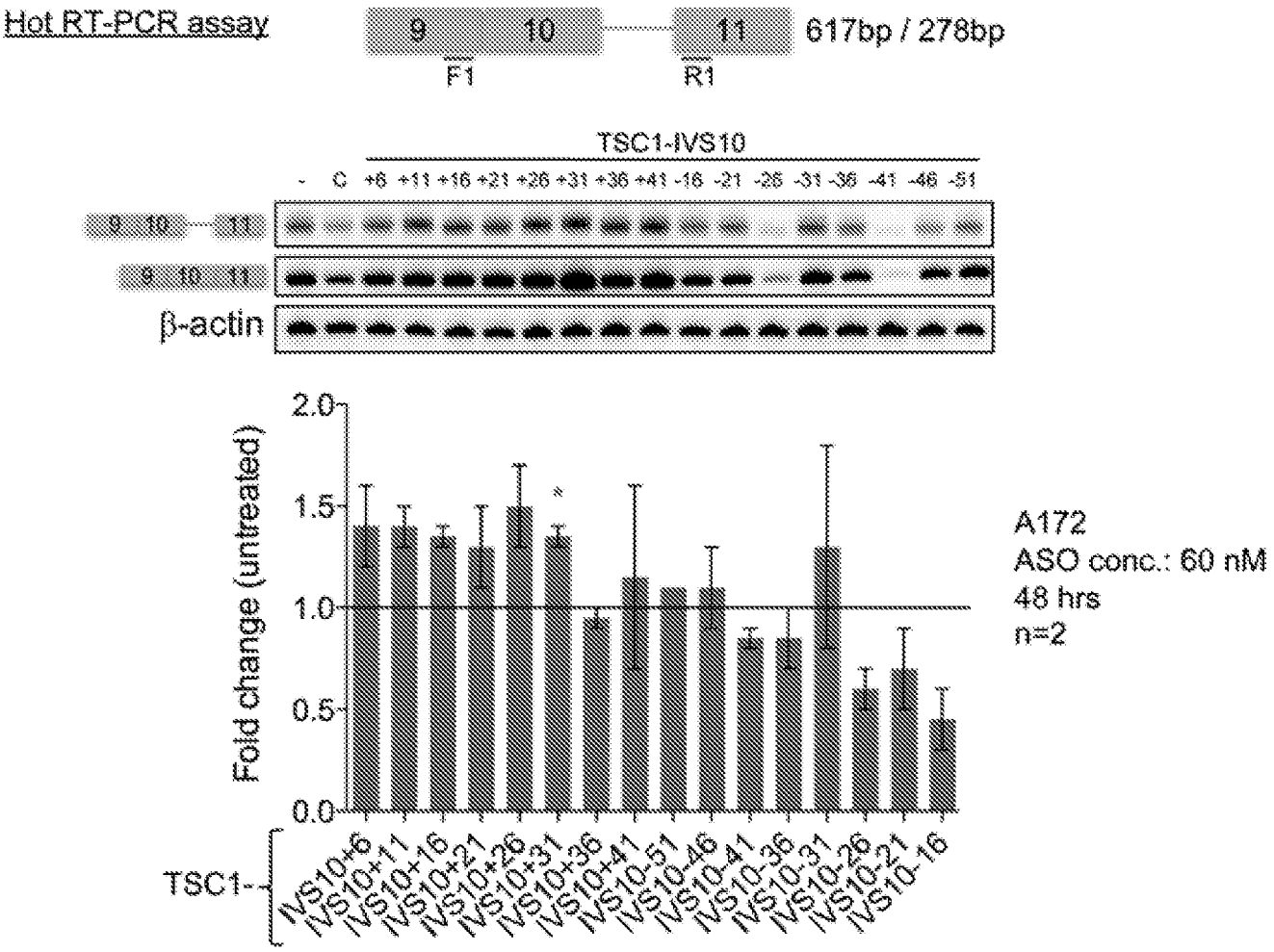

Hot RT-PCR assay
9
10
11
617bp / 278bp
F1
R1
TSC1-IVS10
- C +6 +11 +16 +21 +26 +31 +36 +41 -16 -21 -26 -31 -36 -41 -46 -51
9 10 11
9 10 11
β-actin
Fold change (untreated)
2.0
1.5
1.0
0.5
0.0
TSC1-
IVS10+6
IVS10+11
IVS10+16
IVS10+21
IVS10+26
IVS10+31
IVS10+36
IVS10+41
IVS10-51
IVS10-46
IVS10-41
IVS10-36
IVS10-31
IVS10-26
IVS10-21
IVS10-16
A172
ASO conc.: 60 nM
48 hrs
n=2

FIG. 23

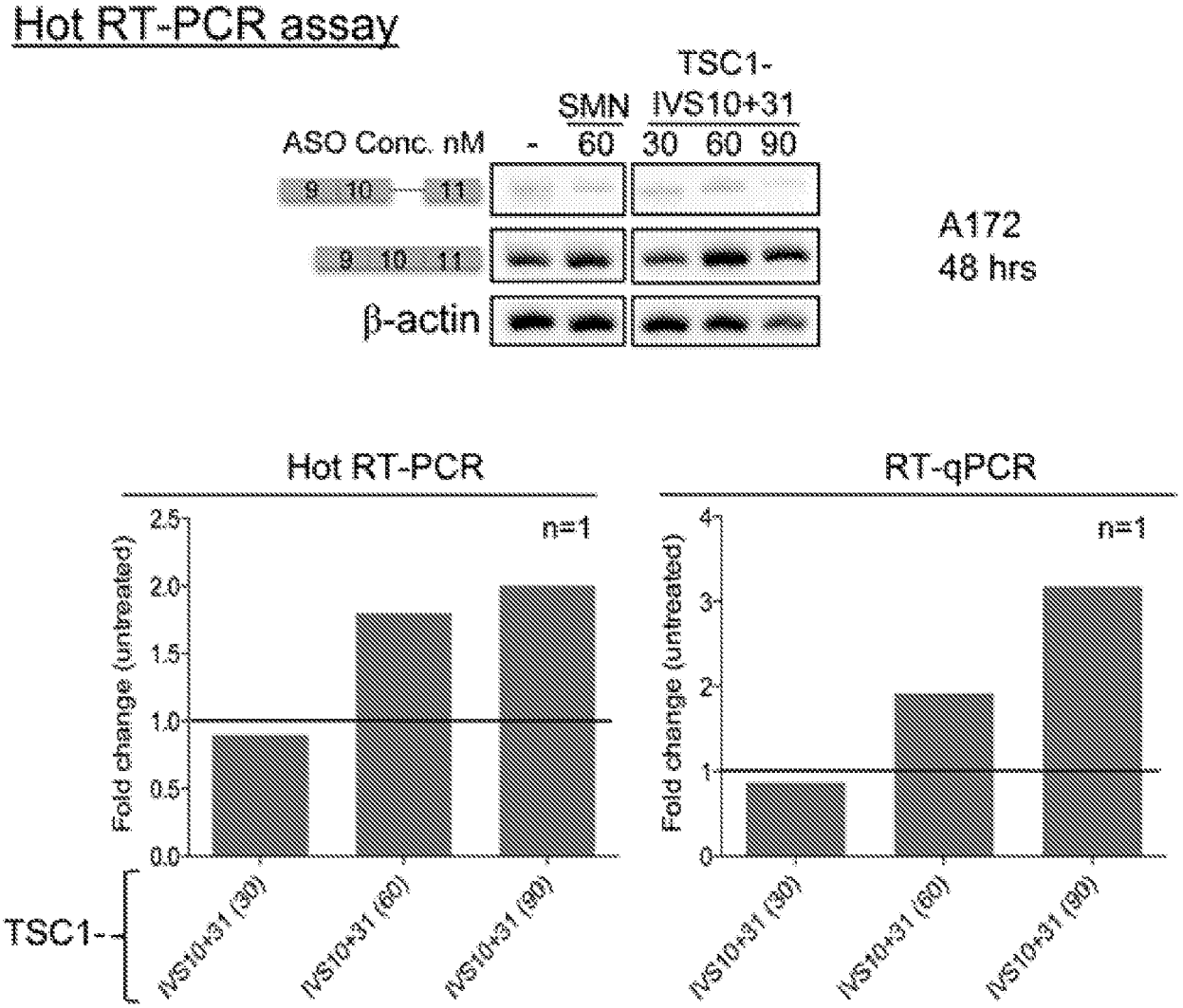

Hot RT-PCR assay
TSC1-
SMN
IVS10+31
ASO Conc. nM - 60 30 60 90
9 10 11
9 10 11
β-actin
A172
48 hrs
Hot RT-PCR
RT-qPCR
n=1
n=1
Fold change (untreated)
Fold change (untreated)
2.5
2.0
1.5
1.0
0.5
0.0
4
3
2
1
0
TSC1-
IVS10+31 (30)
IVS10+31 (60)
IVS10+31 (90)
IVS10+31 (30)
IVS10+31 (60)
IVS10+31 (90)

FIG. 24

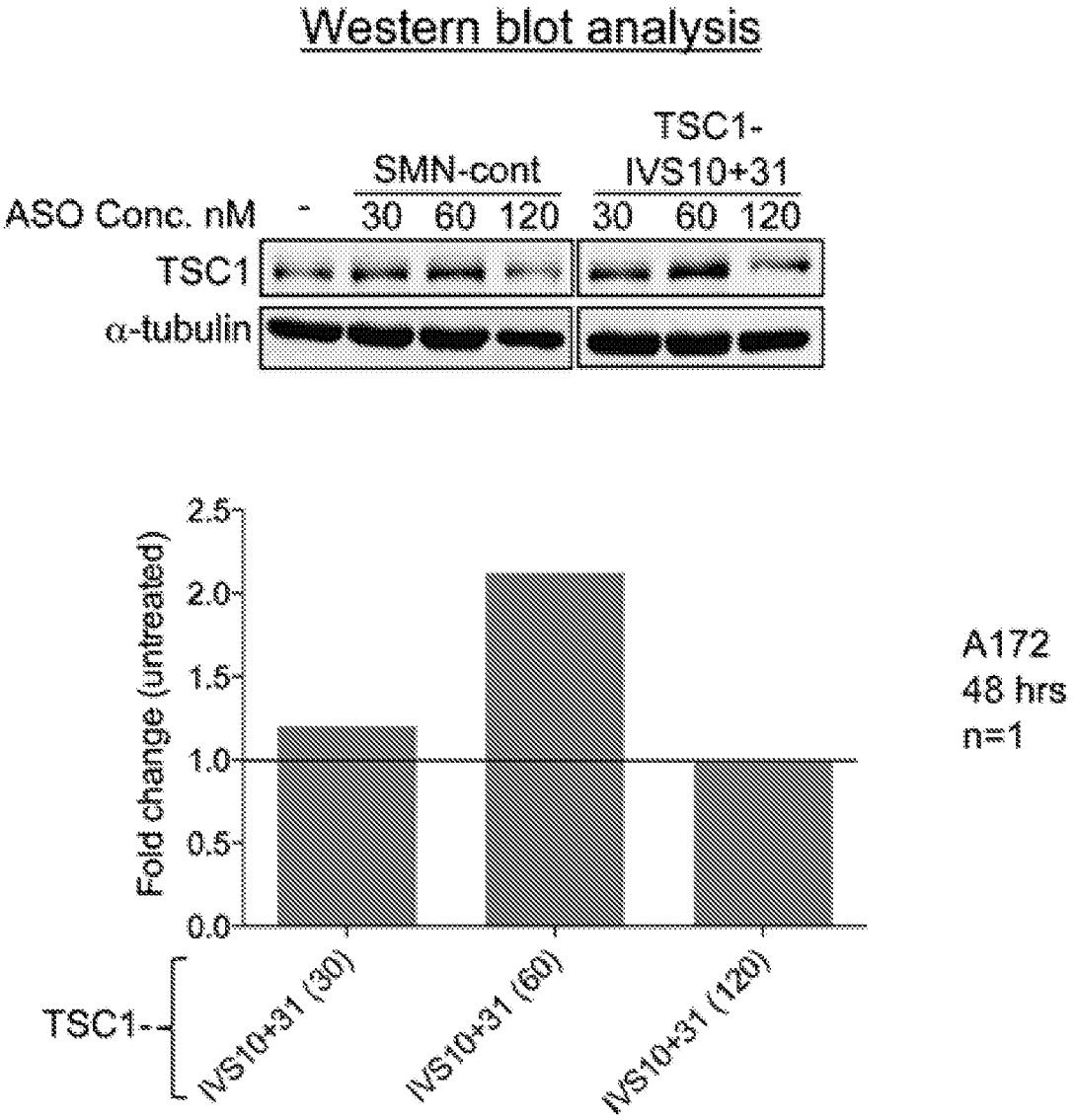

Western blot analysis
TSC1-
SMN-cont
IVS10+31
ASO Conc. nM - 30 60 120 30 60 120
TSC1
α-tubulin
Fold change (untreated)
2.5
2.0
1.5
1.0
0.5
0.0
TSC1-
IVS10+31 (30)
IVS10+31 (60)
IVS10+31 (120)
A172
48 hrs
n=1

FIG. 25

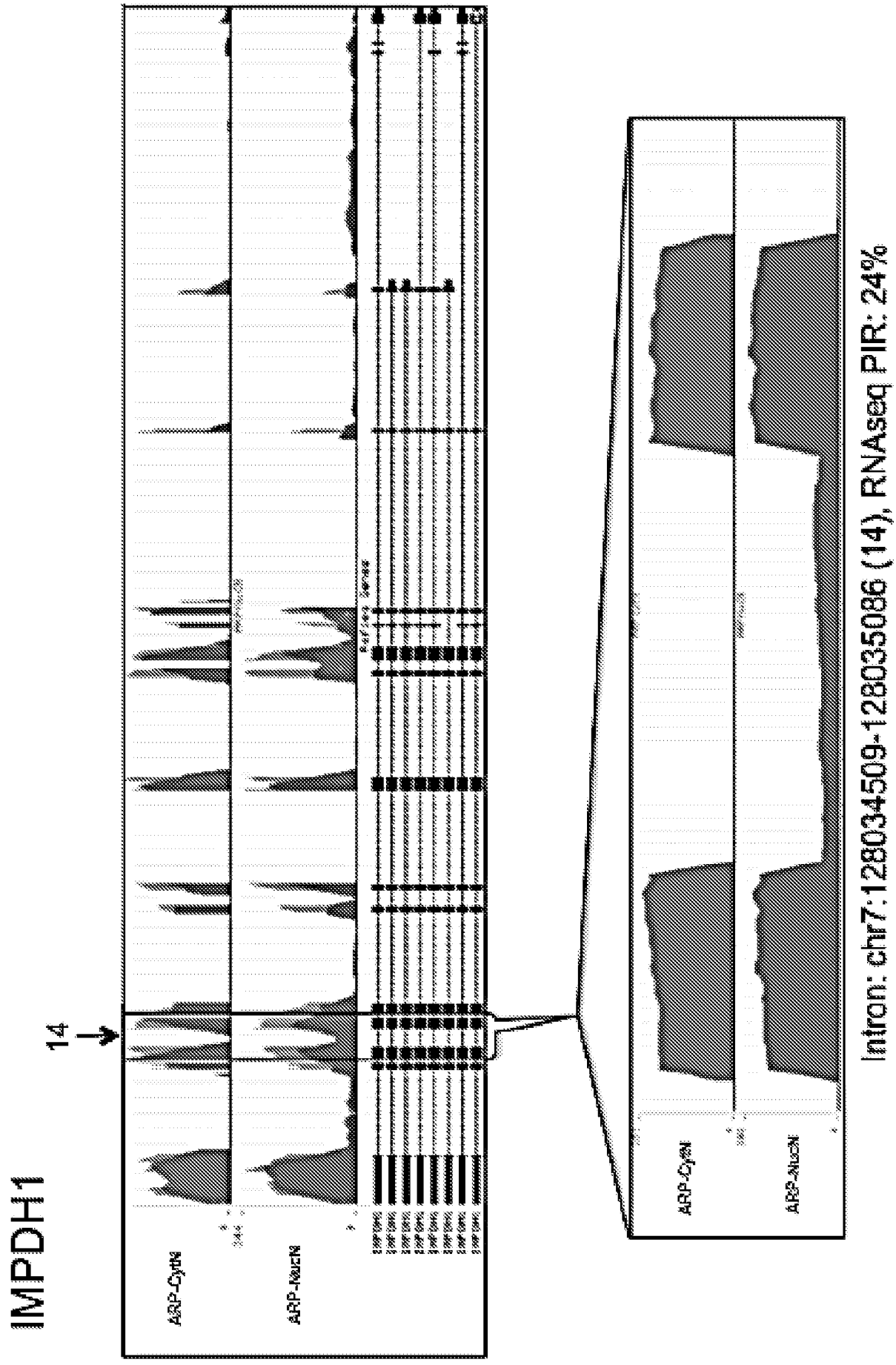

IMPDH1
14
ARP-Cyt%
ARP-Nuc%
ARP-Cyt%
ARP-Nuc%
Intron: chr7:128034509-128035086 (14), RNAseq PIR: 24%

FIG. 26

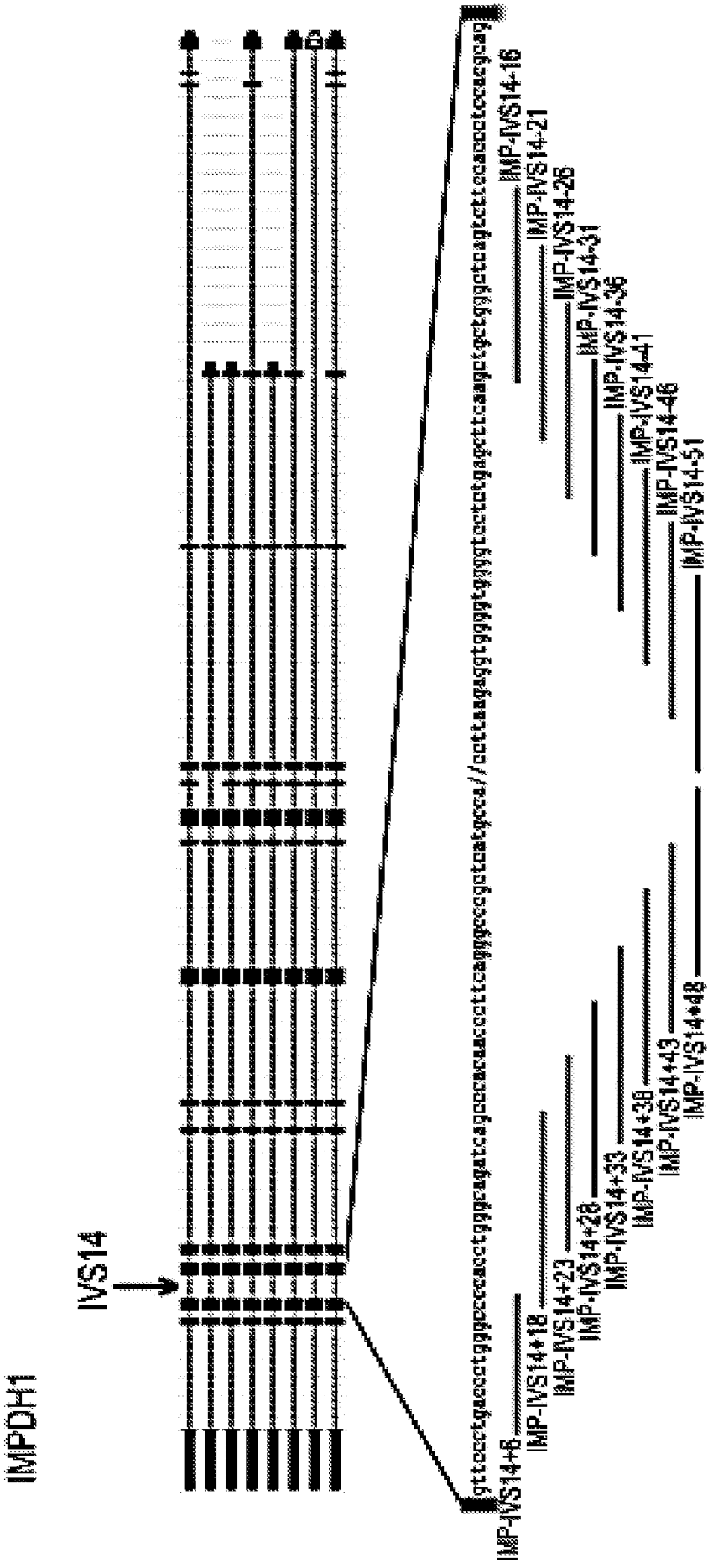

IMPDH1
IVS14
IMP-IVS14+6
IMP-IVS14+18
IMP-IVS14+23
IMP-IVS14+28
IMP-IVS14+33
IMP-IVS14+38
IMP-IVS14+43
IMP-IVS14+48
IMP-IVS14-16
IMP-IVS14-21
IMP-IVS14-26
IMP-IVS14-31
IMP-IVS14-36
IMP-IVS14-41
IMP-IVS14-46
IMP-IVS14-51

FIG. 27

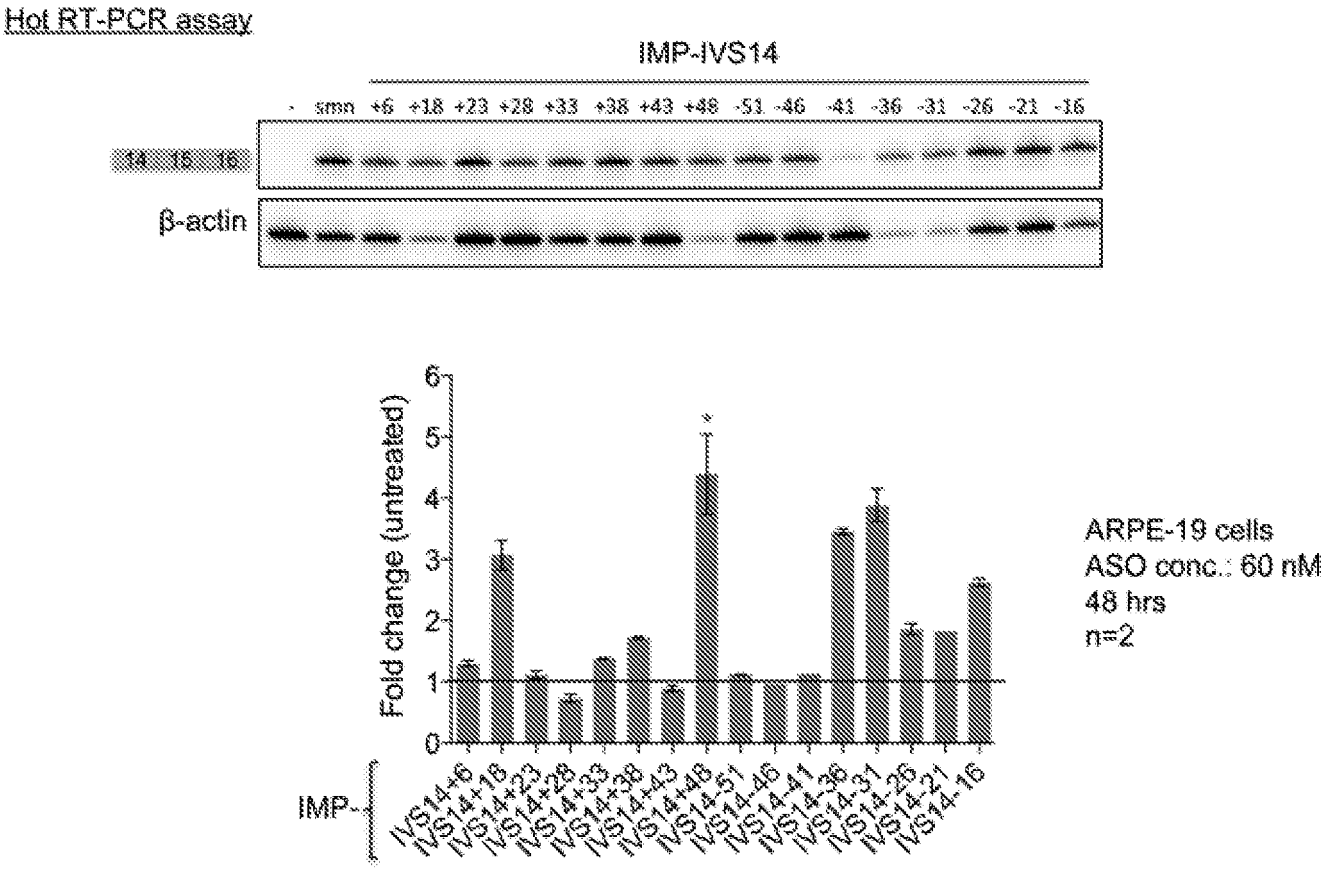

Hot RT-PCR assay
IMP-IVS14
- smn +6 +18 +23 +28 +33 +38 +43 +48 -51 -46 -41 -36 -31 -26 -21 -16
14 15 16
β-actin
Fold change (untreated)
0
1
2
3
4
5
6
IMP-
IVS14+6
IVS14+18
IVS14+23
IVS14+28
IVS14+33
IVS14+38
IVS14+43
IVS14+48
IVS14-51
IVS14-46
IVS14-41
IVS14-36
IVS14-31
IVS14-26
IVS14-21
IVS14-16
ARPE-19 cells
ASO conc.: 60 nM
48 hrs
n=2

FIG. 28

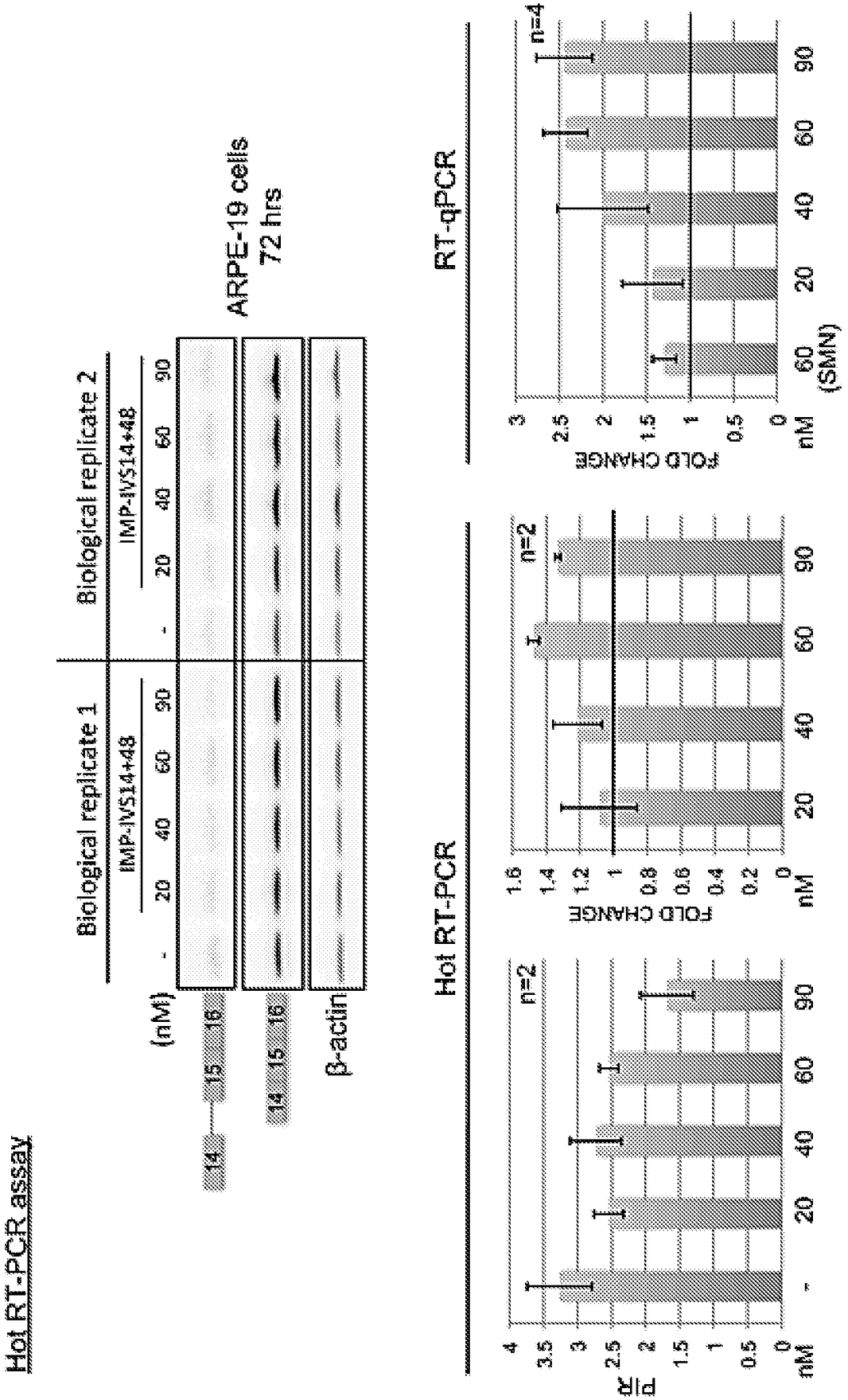

Hot RT-PCR assay
Biological replicate 1
Biological replicate 2
IMP-IVS14+48
(nM)
- 20 40 60 90
ARPE-19 cells
72 hrs
14 15 16
β-actin
Hot RT-PCR
RT-qPCR
PIR
FOLD CHANGE
n=2
n=4
nM
(SMN)

FIG. 29

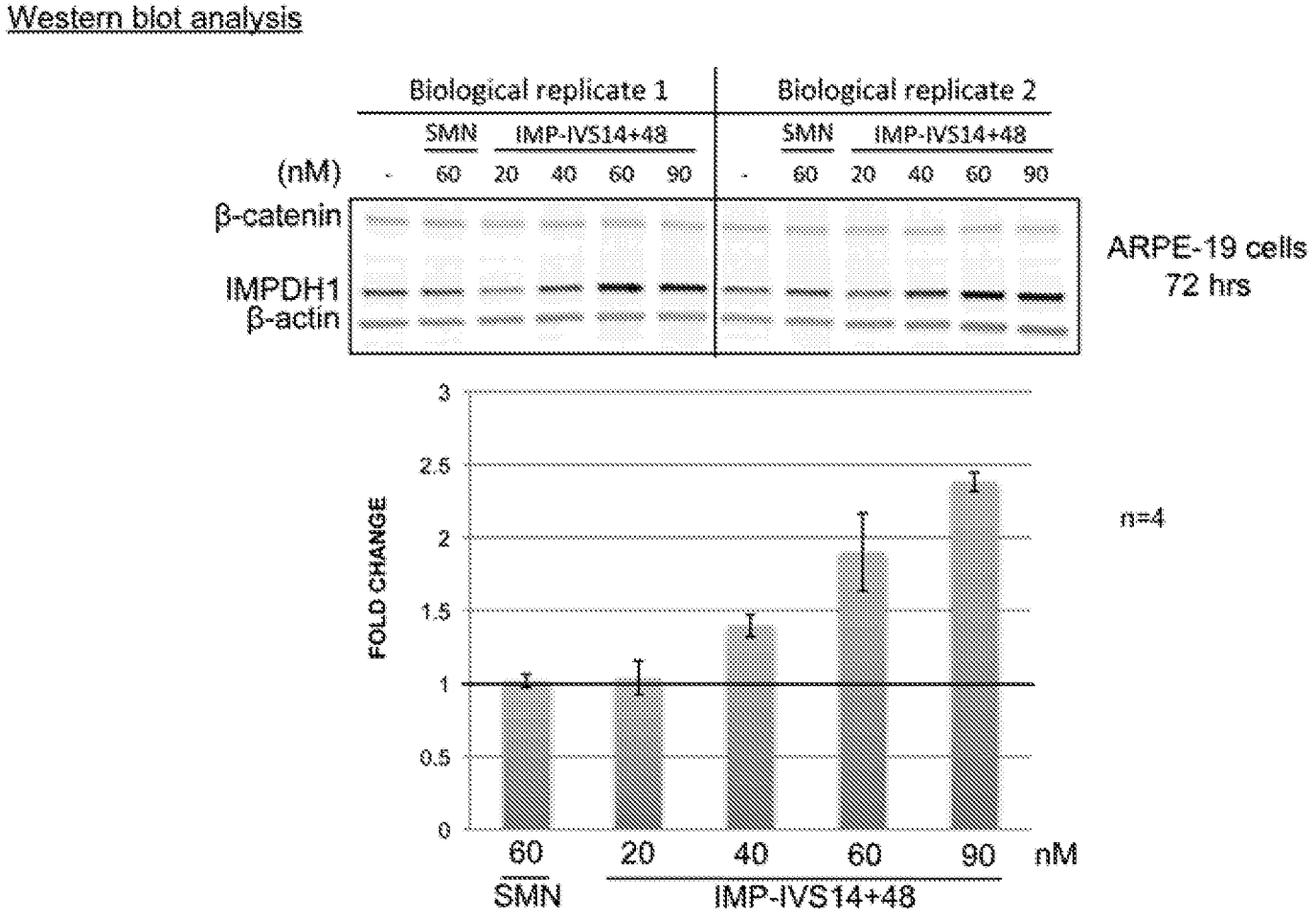

Western blot analysis
Biological replicate 1
Biological replicate 2
SMN
IMP-IVS14+48
(nM)
- 60 20 40 60 90
β-catenin
IMPDH1
β-actin
ARPE-19 cells
72 hrs
FOLD CHANGE
3
2.5
2
1.5
1
0.5
0
n=4
60 20 40 60 90 nM
SMN
IMP-IVS14+48

FIG. 30

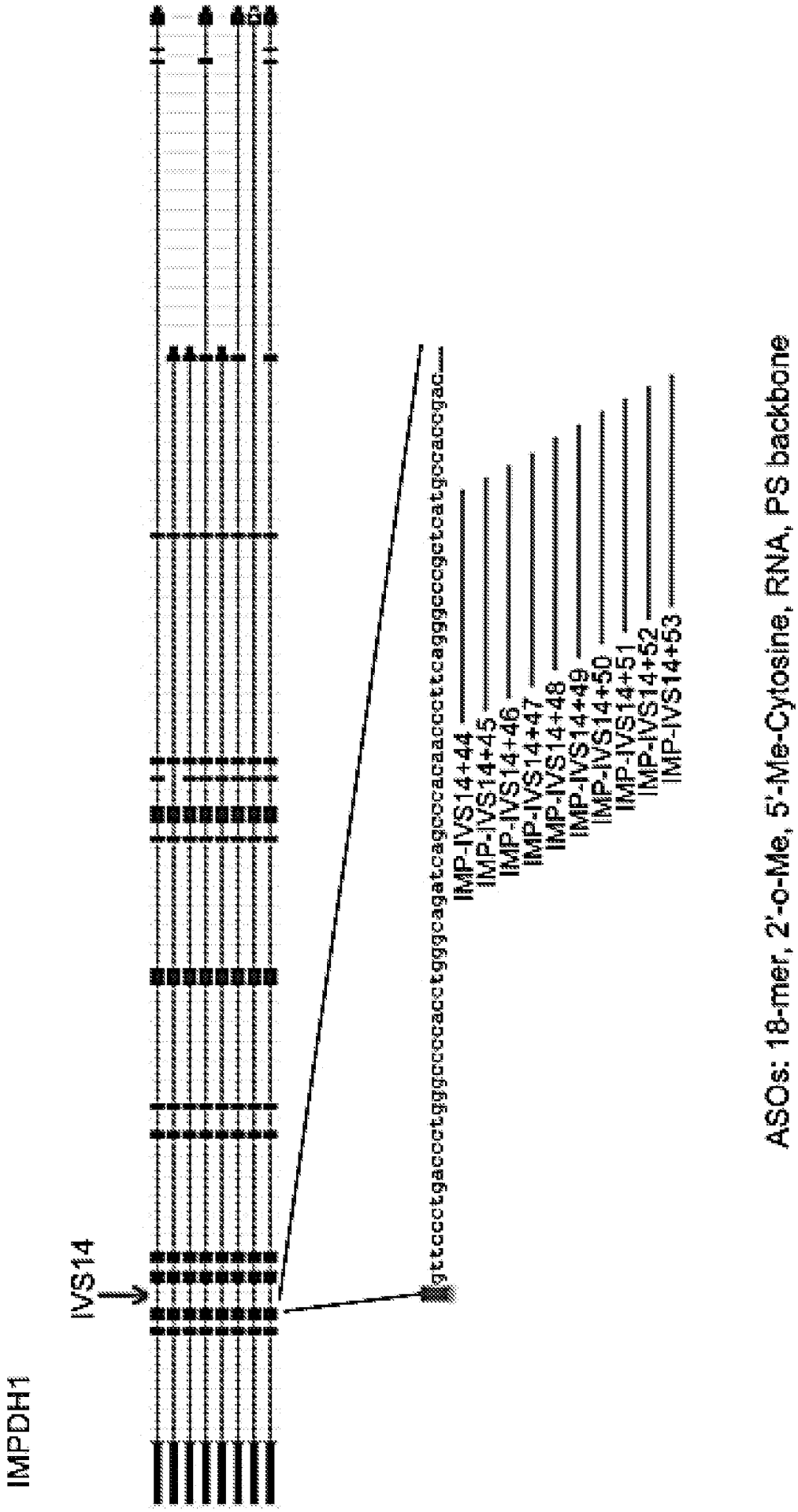

IMPDH1
IVS14
gtccctgaccctgggccccacctgggcagatcagcccacaacccttcagggcccgctcatgccaccgac
IMP-IVS14+44
IMP-IVS14+45
IMP-IVS14+46
IMP-IVS14+47
IMP-IVS14+48
IMP-IVS14+49
IMP-IVS14+50
IMP-IVS14+51
IMP-IVS14+52
IMP-IVS14+53
ASOs: 18-mer, 2'-o-Me, 5'-Me-Cytosine, RNA, PS backbone

FIG. 31

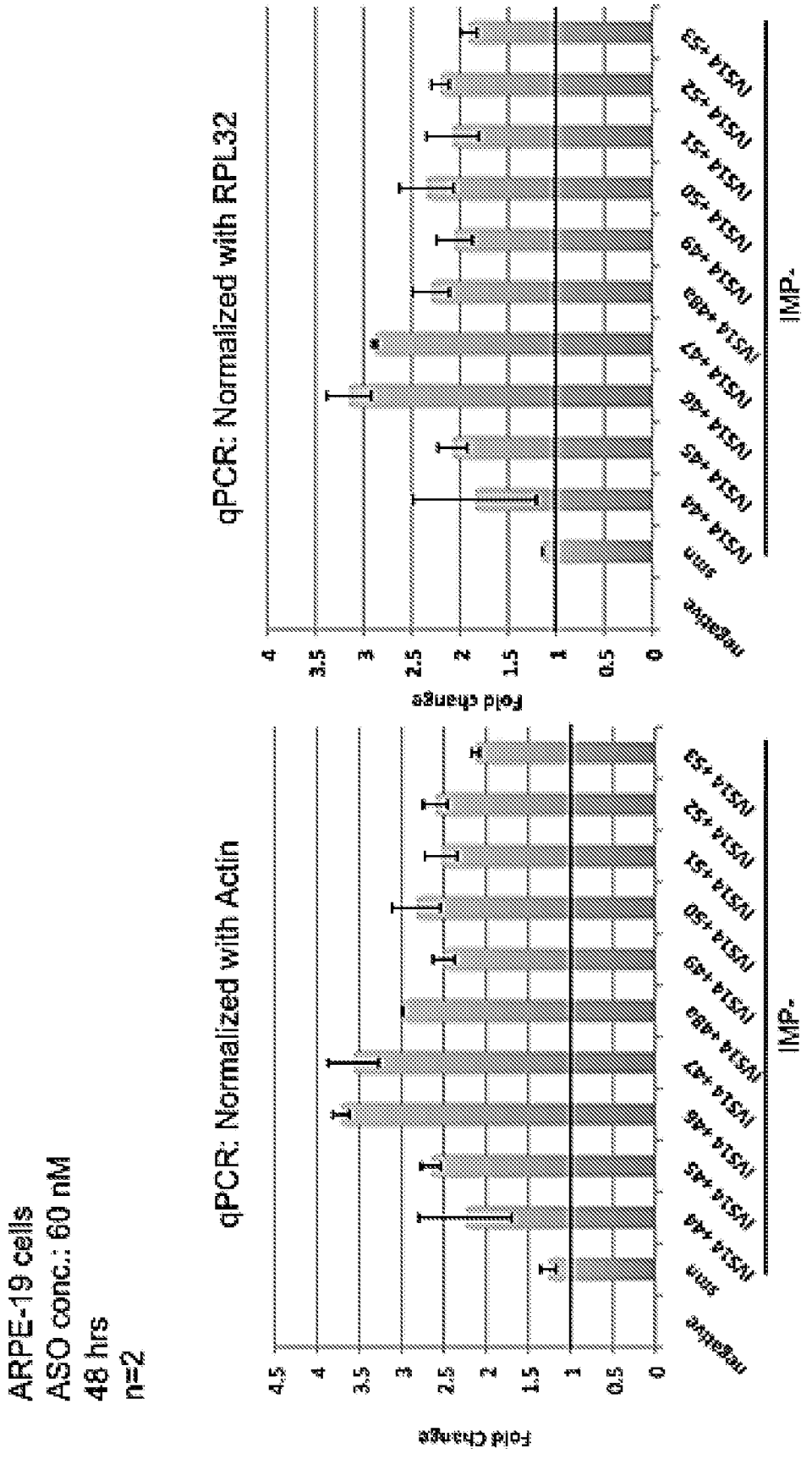

ARPE-19 cells
ASO conc.: 60 nM
48 hrs
n=2
qPCR: Normalized with Actin
Fold Change
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
negative
smn
IVSI+44
IVSI+45
IVSI+46
IVSI+47
IVSI+48a
IVSI+48
IVSI+49
IVSI+50
IVSI+51
IVSI+52
IVSI+53
IMP-
qPCR: Normalized with RPL32
Fold change
4
3.5
3
2.5
2
1.5
1
0.5
0
negative
smn
IVSI+44
IVSI+45
IVSI+46
IVSI+47
IVSI+48a
IVSI+48
IVSI+49
IVSI+50
IVSI+51
IVSI+52
IVSI+53
IMP-

FIG. 32

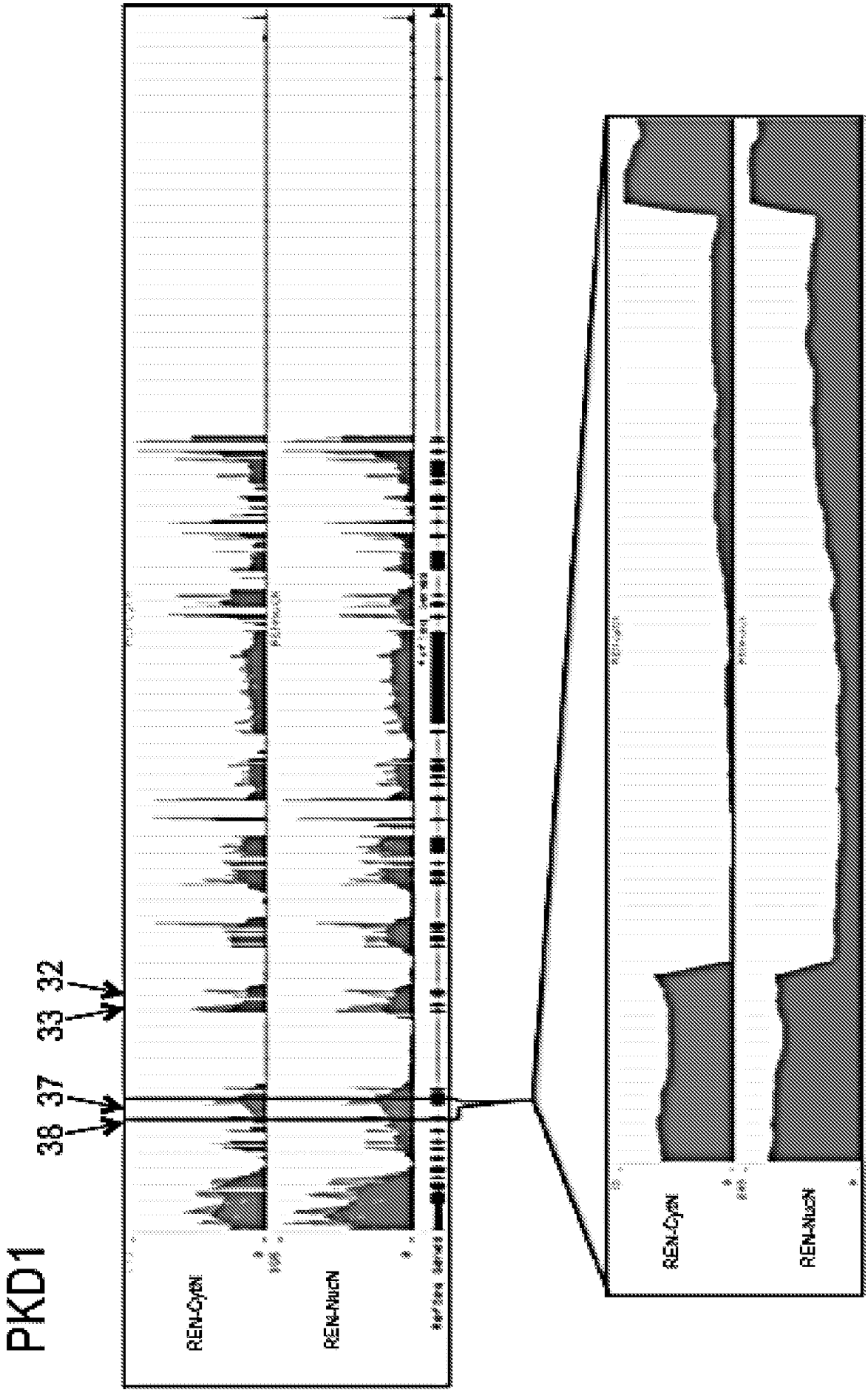


FIG. 33

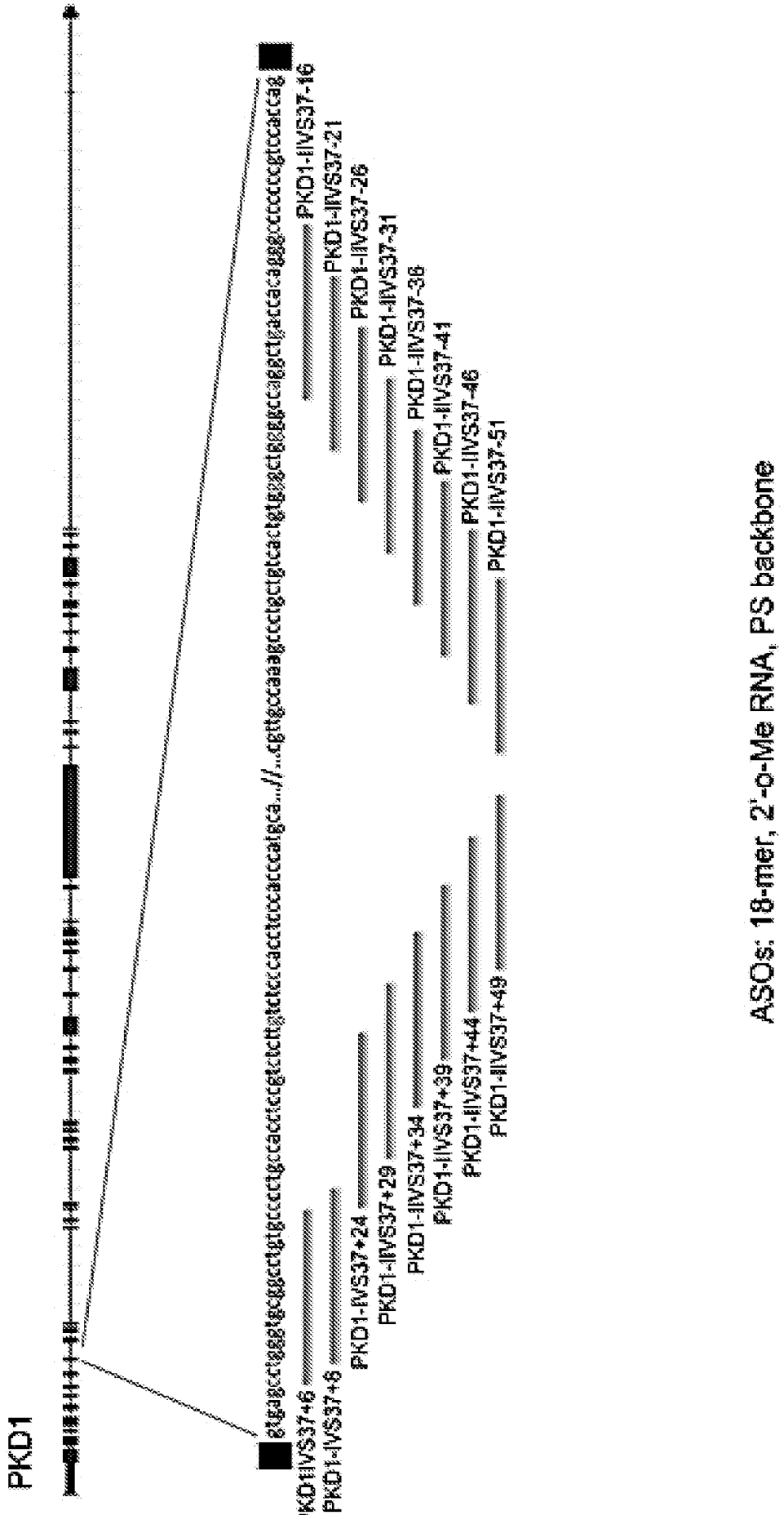

PKD1
PKD1IVS37+6
PKD1-IVS37+6
PKD1-IVS37+24
PKD1-IVS37+29
PKD1-IVS37+34
PKD1-IVS37+39
PKD1-IVS37+44
PKD1-IVS37+49
PKD1-IVS37-16
PKD1-IVS37-21
PKD1-IVS37-26
PKD1-IVS37-31
PKD1-IVS37-36
PKD1-IVS37-41
PKD1-IVS37-46
PKD1-IVS37-51
ASOs: 18-mer, 2'-o-Me RNA, PS backbone

FIG. 34

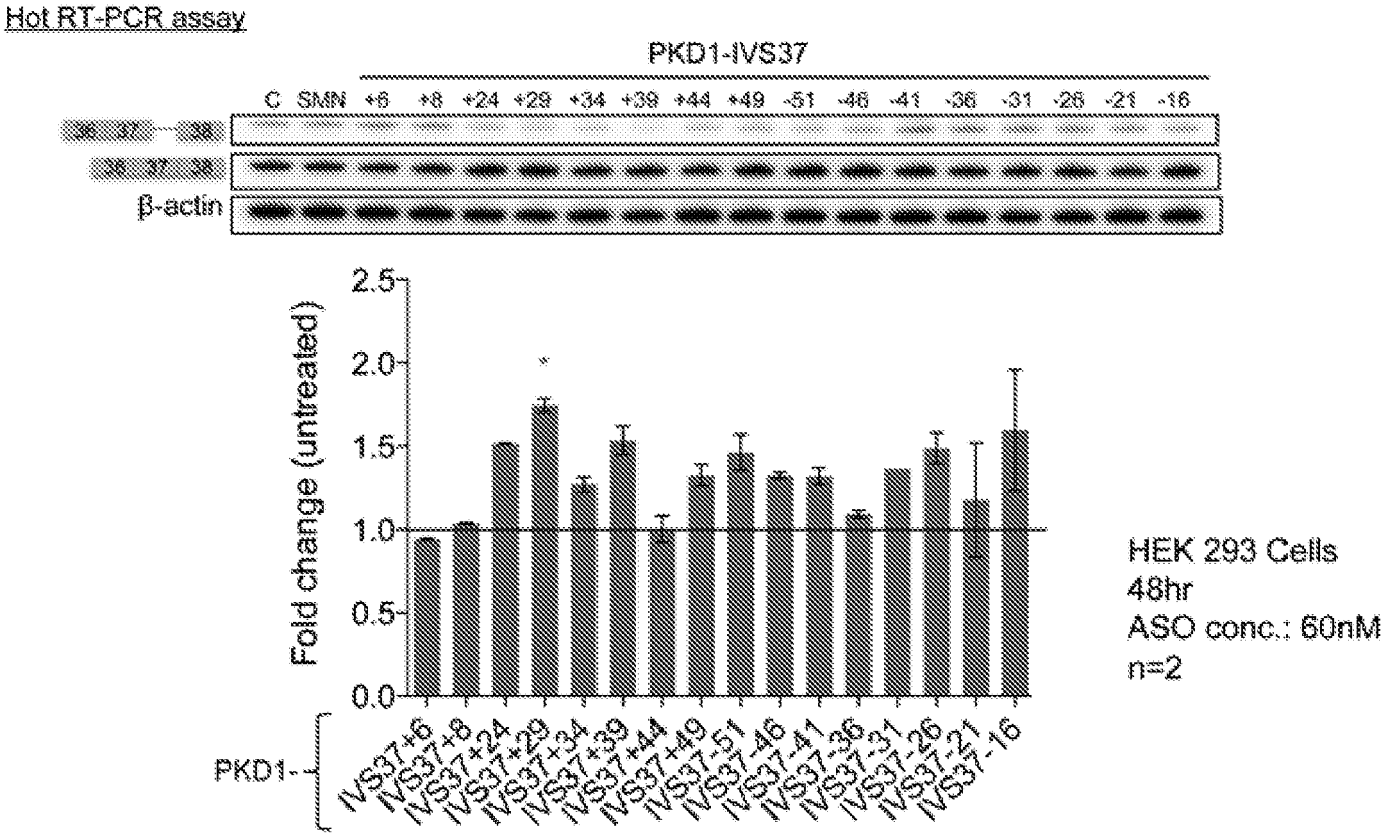

Hot RT-PCR assay
PKD1-IVS37
C SMN +6 +8 +24 +29 +34 +39 +44 +49 -51 -46 -41 -36 -31 -26 -21 -16
β-actin
Fold change (untreated)
2.5
2.0
1.5
1.0
0.5
0.0
PKD1
IVS37+6
IVS37+8
IVS37+24
IVS37+29
IVS37+34
IVS37+39
IVS37+44
IVS37+49
IVS37-51
IVS37-46
IVS37-41
IVS37-36
IVS37-31
IVS37-26
IVS37-21
IVS37-16
HEK 293 Cells
48hr
ASO conc.: 60nM
n=2

FIG. 35

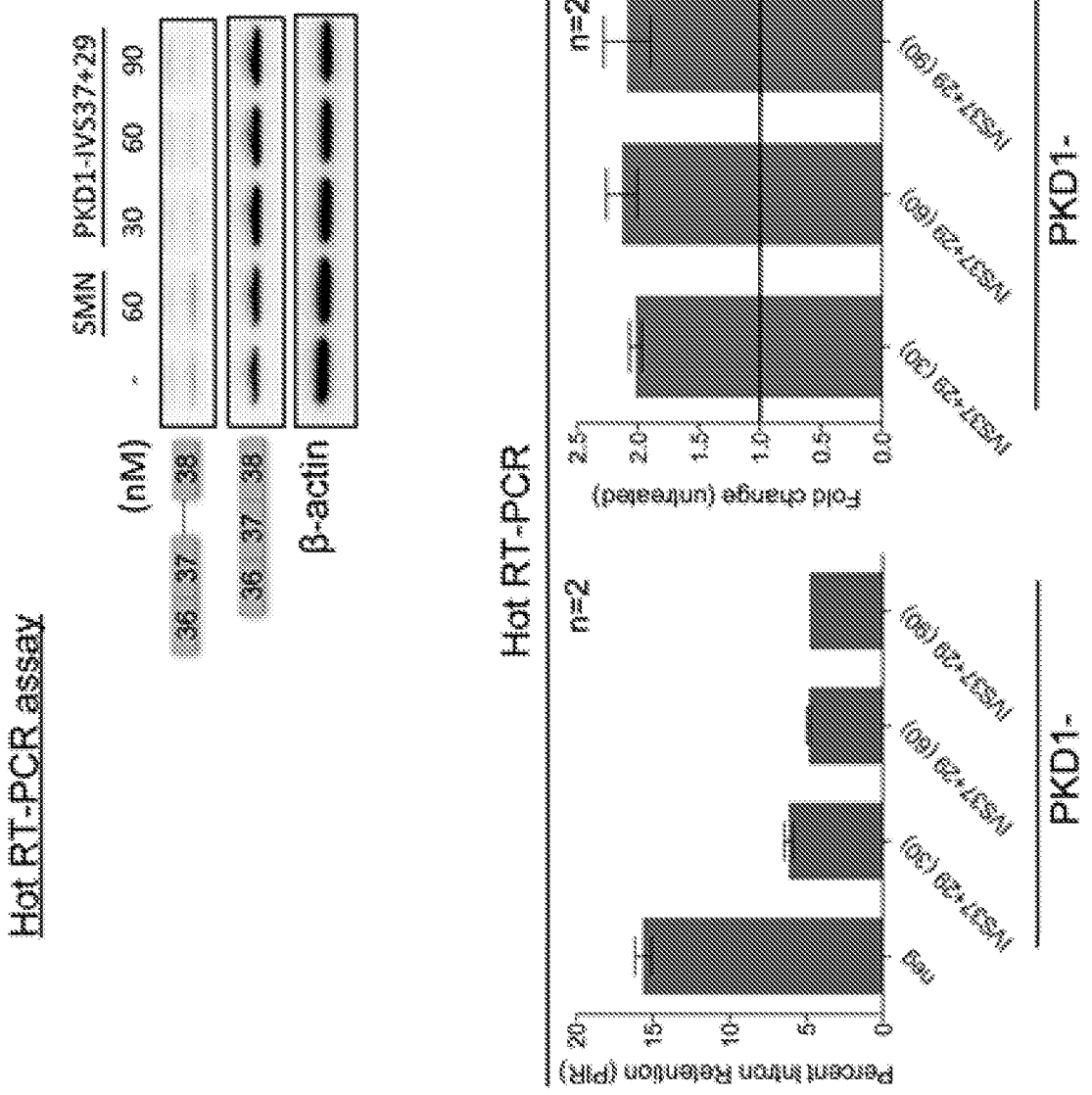

Hot RT-PCR assay
SMN
PKD1-IVS37+29
(nM)
-
60
30
60
90
38
36
37
β-actin
HEK 293 Cells
48hr
n=2
Hot RT-PCR
n=2
Percent Intron Retention (PIR)
Fold change (untreated)
PKD1-
RT-qPCR
n=4
Fold change (untreated)
PKD1-

FIG. 36

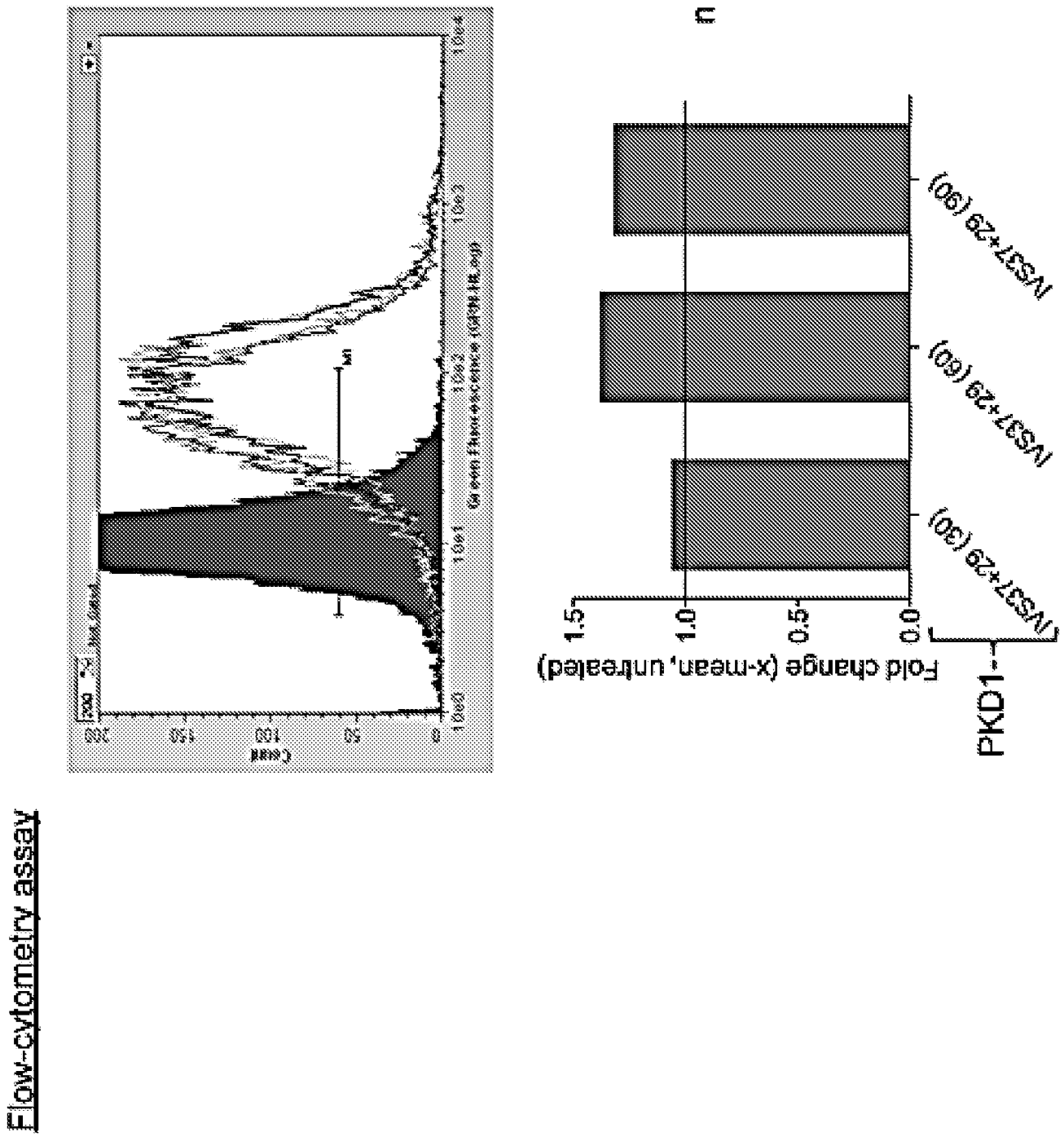

Flow-cytometry assay
HEK 293 Cells
72hrs
Mouse IgG (Filled)
Untransfected
PKD1-IVS37+29 (30nM)
PKD1-IVS37+29 (60nM)
PKD1-IVS37+29 (90nM)
Count
Green Fluorescence (GRN-HLog)
n = 1
Fold change (x-mean, untreated)
1.5
1.0
0.5
0.0
PKD1-IVS37+29 (30)
PKD1-IVS37+29 (60)
PKD1-IVS37+29 (90)

FIG. 37

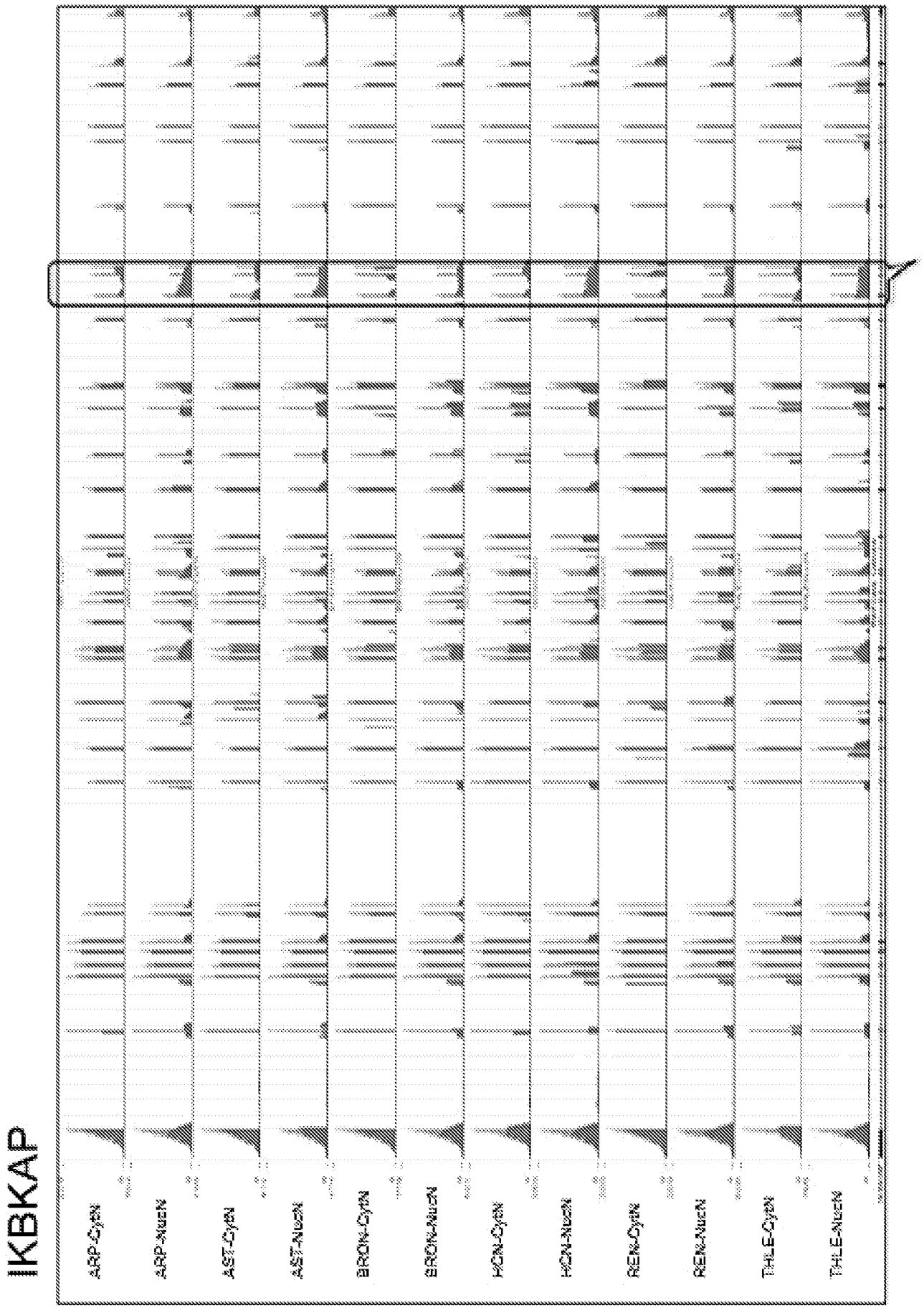

IKBKAP
ARP-Cyt
ARP-Nuc
AST-Cyt
AST-Nuc
BRON-Cyt
BRON-Nuc
HON-Cyt
HON-Nuc
REN-Cyt
REN-Nuc
THLE-Cyt
THLE-Nuc

FIG. 37
Continued

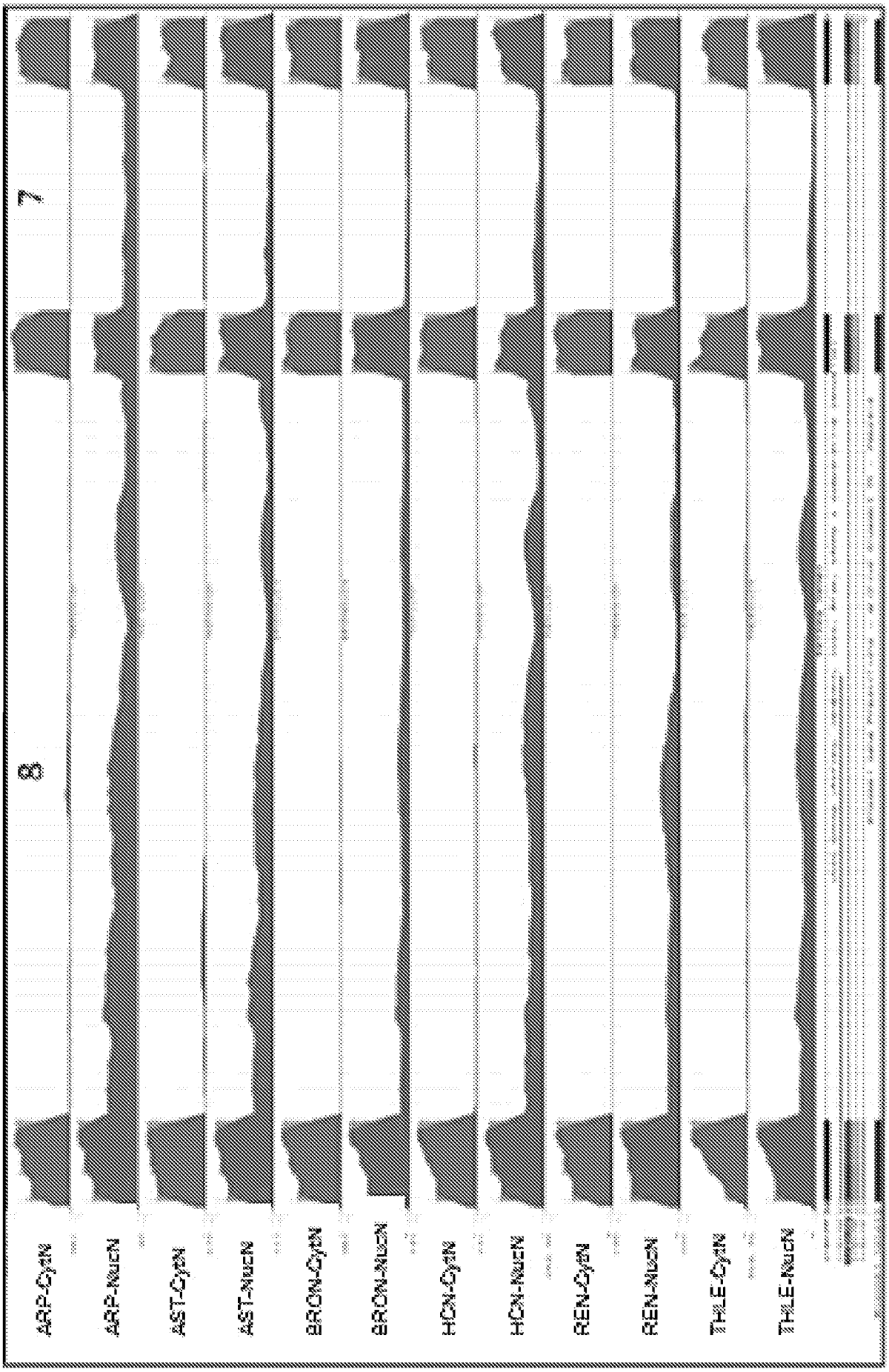
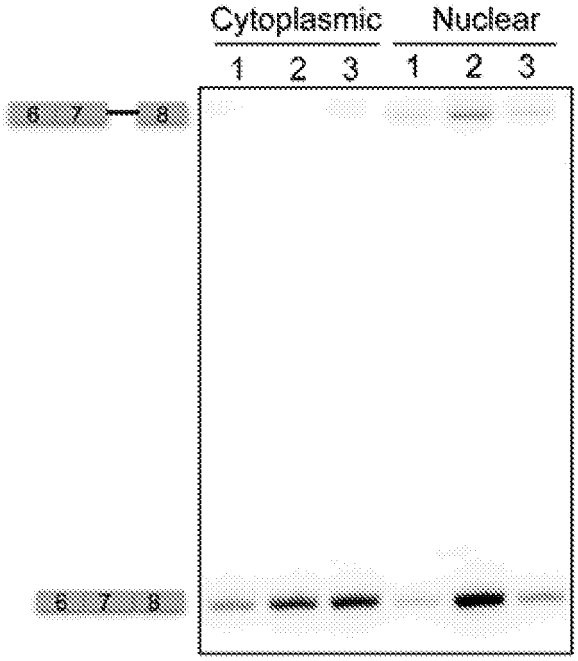


FIG. 38

Hot RT-PCR analysis



1. ARPE-19
2. HeLa
3. U2OS

PIR[1]	EXPERIMENT	SOURCE
35.5	hot RT-PCR	ARPE19[2]
18	hot RT-PCR	HeLa[3]
26	hot RT-PCR	U2OS[4]
33	RNAseq-ASOthera	ARPE-19

[1]Percent intron retention
[2]Retina epithelial
[3]Cervical carcinoma
[4]Osteosarcoma

FIG. 39

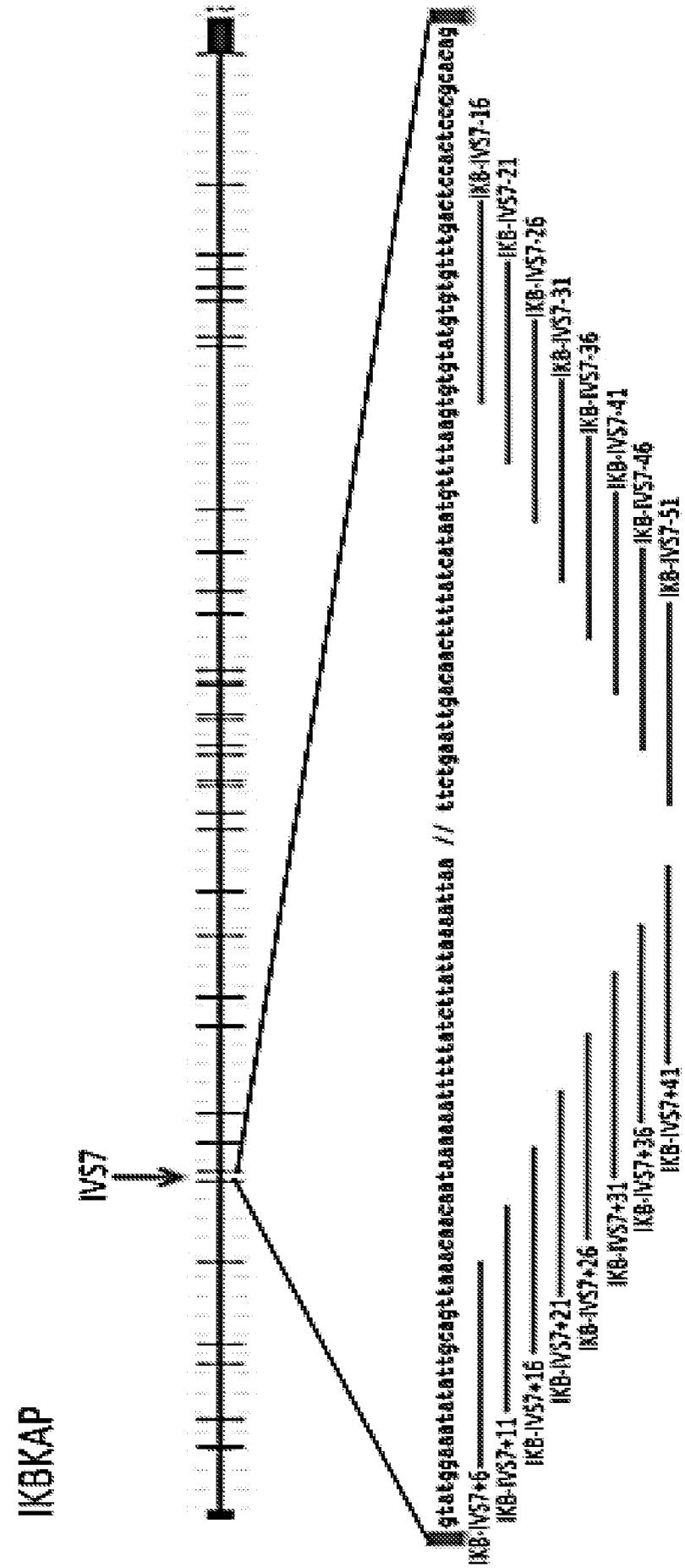

IKBKAP
IVS7
gtatggaaatatattgcagttaaacaacaatgaaaaatttttatctattaaaattaa // ttctgaattgacaacttttatcataatgttttaagtgtgtatgtgtttgactccactcccgcacag
IKB-IVS7+6
IKB-IVS7+11
IKB-IVS7+16
IKB-IVS7+21
IKB-IVS7+26
IKB-IVS7+31
IKB-IVS7+36
IKB-IVS7+41
IKB-IVS7-16
IKB-IVS7-21
IKB-IVS7-26
IKB-IVS7-31
IKB-IVS7-36
IKB-IVS7-41
IKB-IVS7-46
IKB-IVS7-51

FIG. 39
Continued

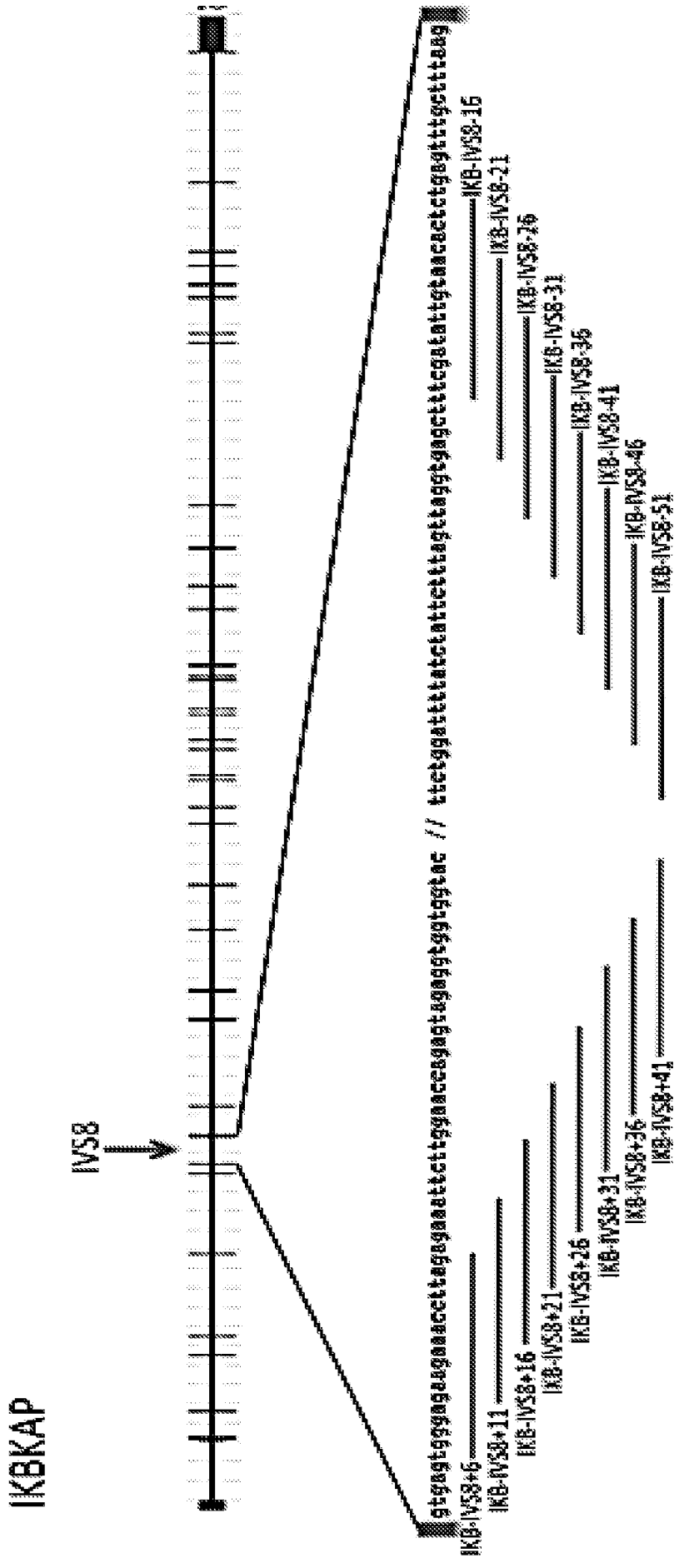


FIG. 40

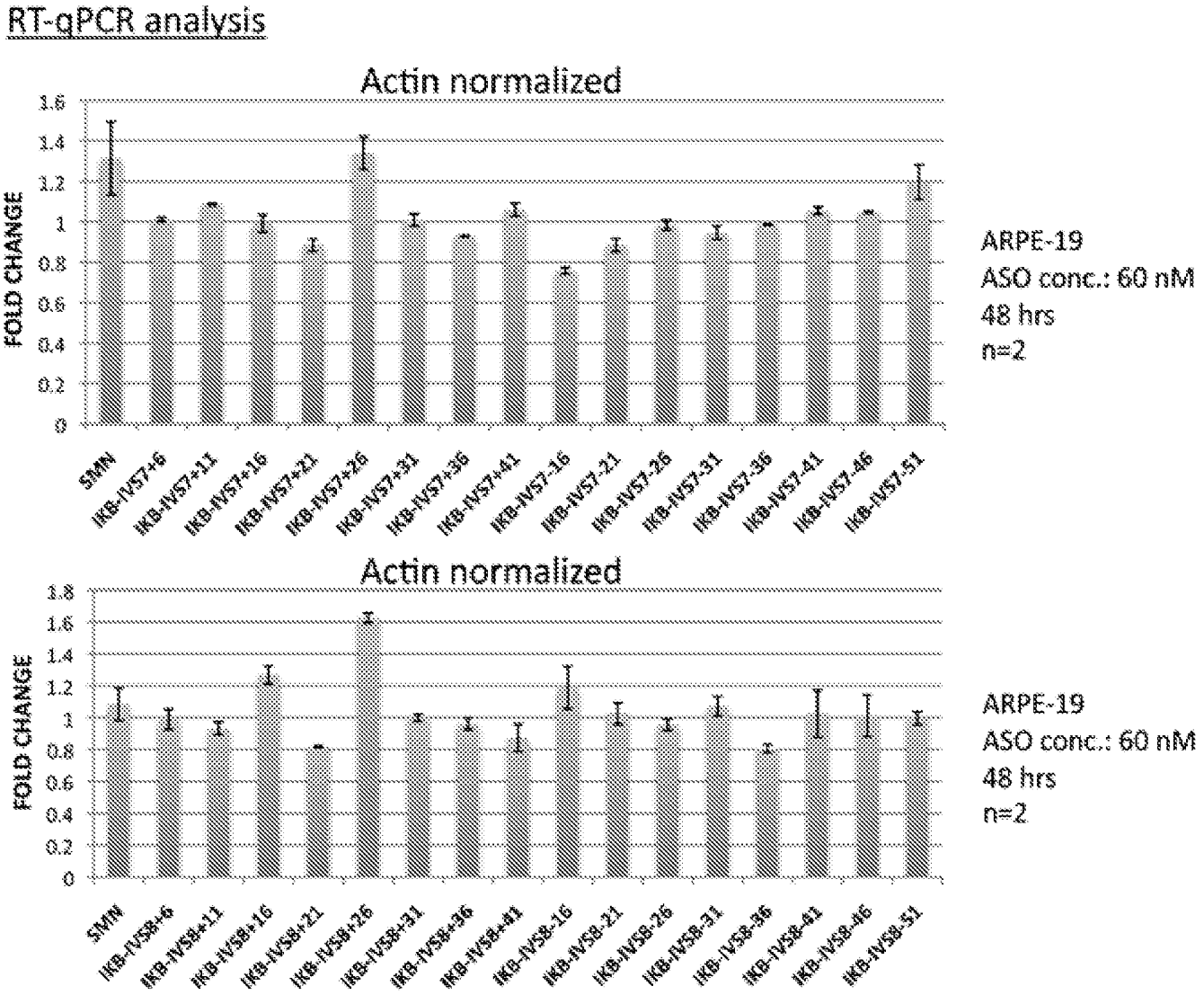

RT-qPCR analysis
Actin normalized
FOLD CHANGE
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
SMN
IKB-IVS7+6
IKB-IVS7+11
IKB-IVS7+16
IKB-IVS7+21
IKB-IVS7+26
IKB-IVS7+31
IKB-IVS7+36
IKB-IVS7+41
IKB-IVS7-16
IKB-IVS7-21
IKB-IVS7-26
IKB-IVS7-31
IKB-IVS7-36
IKB-IVS7-41
IKB-IVS7-46
IKB-IVS7-51
ARPE-19
ASO conc.: 60 nM
48 hrs
n=2
Actin normalized
FOLD CHANGE
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
SMN
IKB-IVS8+6
IKB-IVS8+11
IKB-IVS8+16
IKB-IVS8+21
IKB-IVS8+26
IKB-IVS8+31
IKB-IVS8+36
IKB-IVS8+41
IKB-IVS8-16
IKB-IVS8-21
IKB-IVS8-26
IKB-IVS8-31
IKB-IVS8-36
IKB-IVS8-41
IKB-IVS8-46
IKB-IVS8-51
ARPE-19
ASO conc.: 60 nM
48 hrs
n=2

FIG. 41

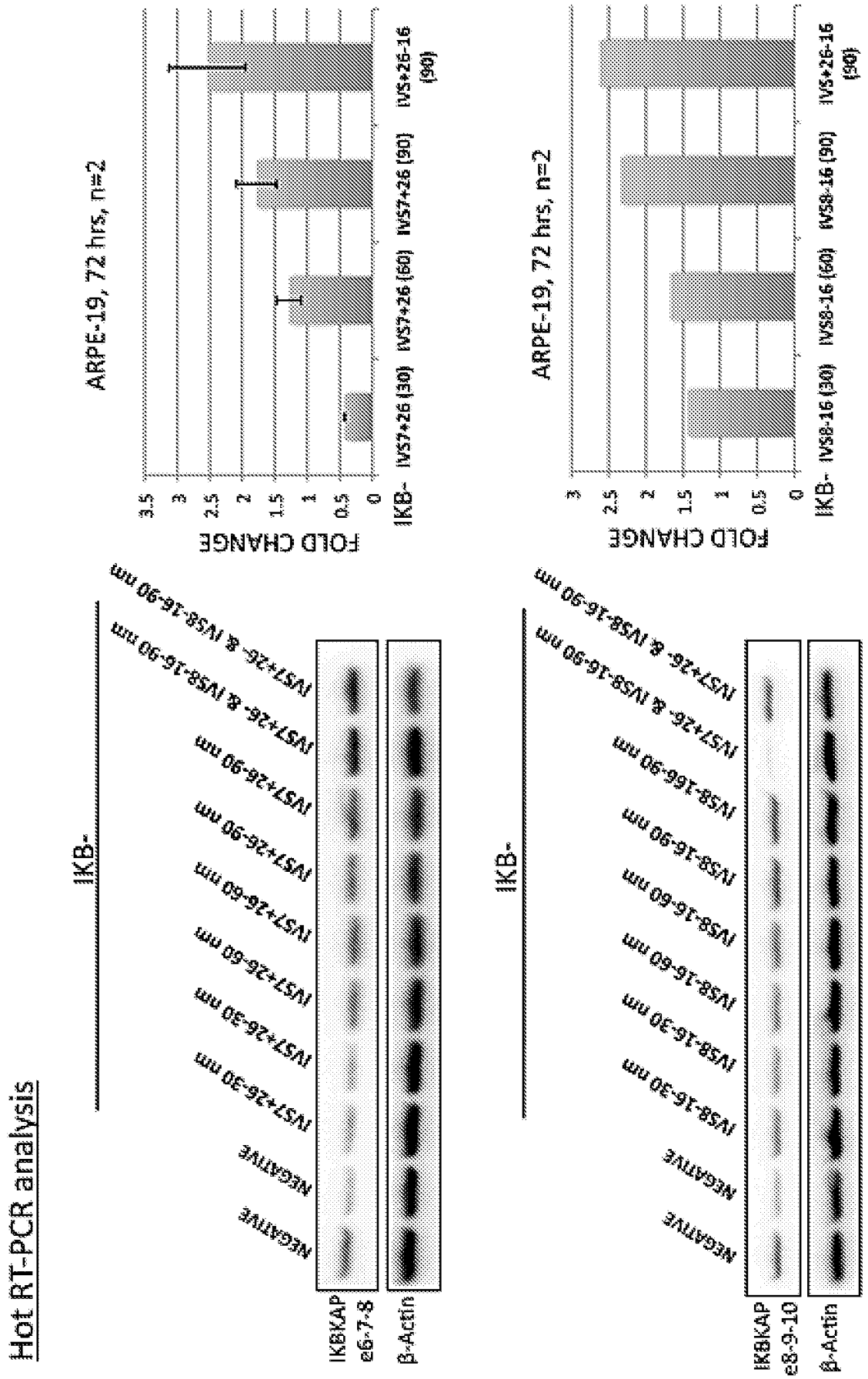
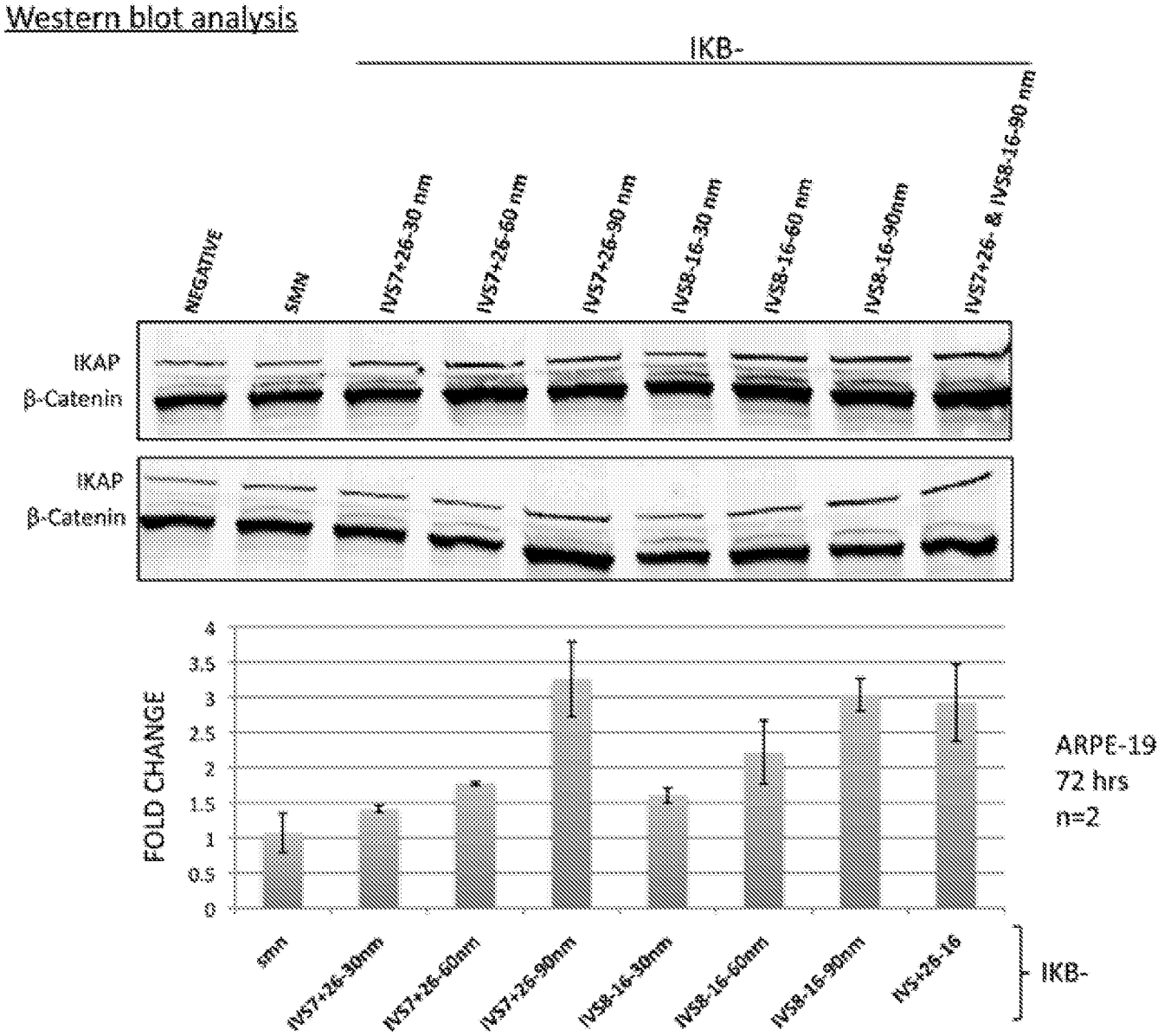

Hot RT-PCR analysis
IKB-
IKBKAP e6-7-8
β-Actin
NEGATIVE
IVS7+26-30 nm
IVS7+26-60 nm
IVS7+26-90 nm
IVS7+26- & IVS8-16-90 nm
ARPE-19, 72 hrs, n=2
FOLD CHANGE
IKB-
IVS7+26 (30)
IVS7+26 (60)
IVS7+26 (90)
IVS+26-16 (90)
IKBKAP e8-9-10
IVS8-16-30 nm
IVS8-16-60 nm
IVS8-16-90 nm
IVS8-16&-90 nm
IVS8-16 (30)
IVS8-16 (60)
IVS8-16 (90)

FIG. 42


Western blot analysis
IKB-
NEGATIVE
SMN
IVS7+26-30 nm
IVS7+26-60 nm
IVS7+26-90 nm
IVS8-16-30 nm
IVS8-16-60 nm
IVS8-16-90nm
IVS7+26- & IVS8-16-90 nm
IKAP
β-Catenin
IKAP
β-Catenin
FOLD CHANGE
4
3.5
3
2.5
2
1.5
1
0.5
0
smn
IVS7+26-30nm
IVS7+26-60nm
IVS7+26-90nm
IVS8-16-30nm
IVS8-16-60nm
IVS8-16-90nm
IVS+26-16
IKB-
ARPE-19
72 hrs
n=2