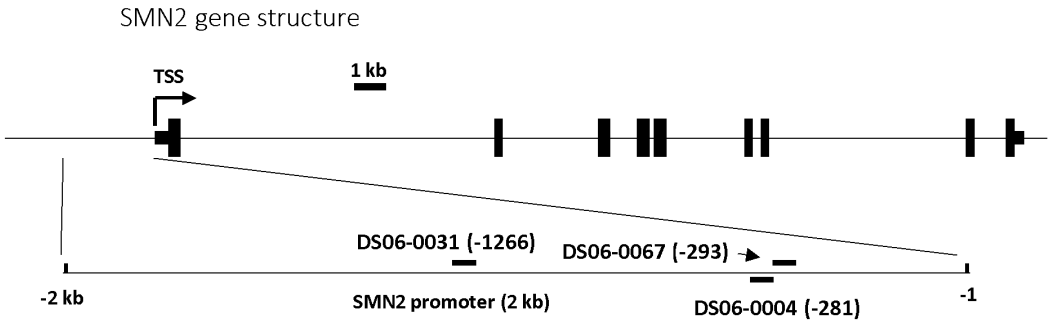
SMN2 gene structure
TSS
1 kb
DS06-0031 (-1266)
DS06-0067 (-293)
-2 kb
SMN2 promoter (2 kb)
DS06-0004 (-281)
-1

FIG. 1A

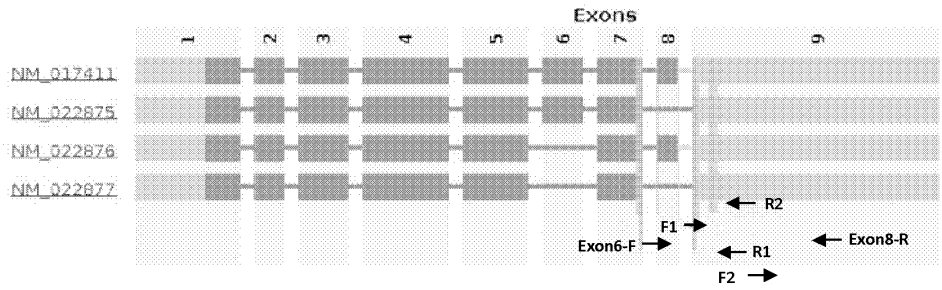
Exons
1
2
3
4
5
6
7
8
9
NM_017411
NM_022875
NM_022876
NM_022877
R2
F1
Exon6-F
R1
Exon8-R
F2

FIG. 1B

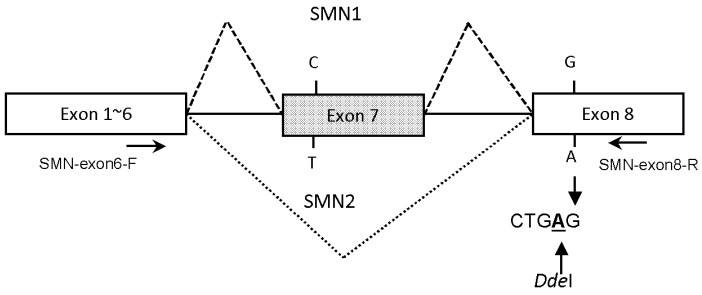

FIG. 2A

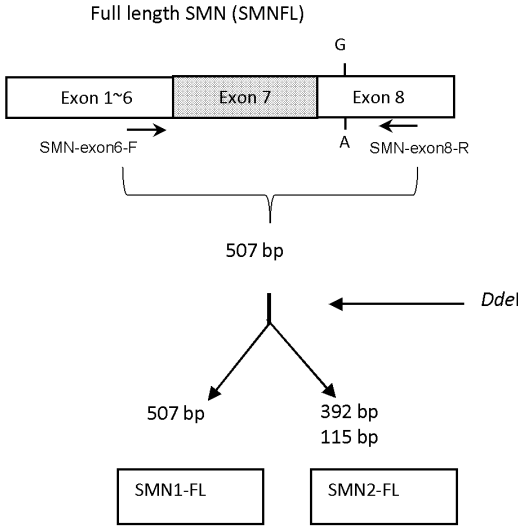

FIG. 2B

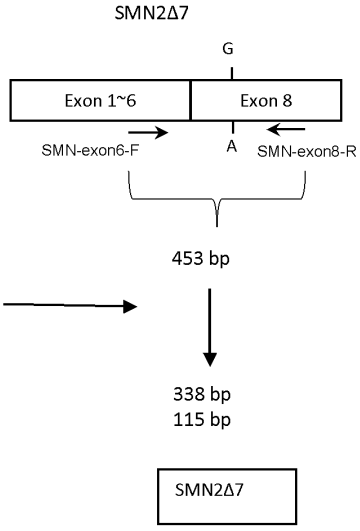

FIG. 2C

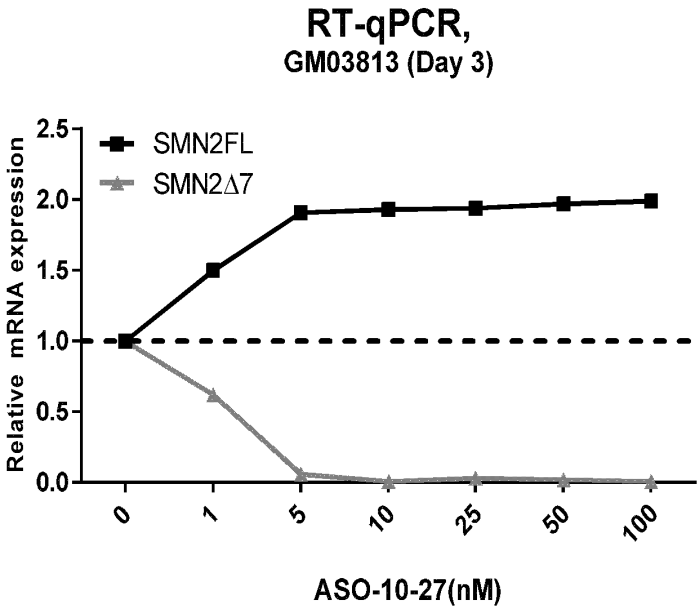
RT-qPCR,
GM03813 (Day 3)
Relative mRNA expression
SMN2FL
SMN2Δ7
2.5
2.0
1.5
1.0
0.5
0.0
0
1
5
10
25
50
100
ASO-10-27(nM)

FIG. 3A

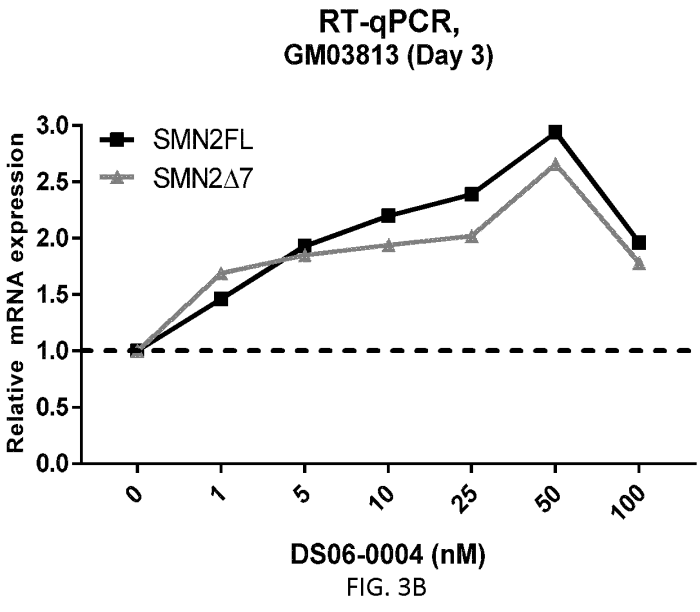
RT-qPCR,
GM03813 (Day 3)
Relative mRNA expression
SMN2FL
SMN2Δ7
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
5
10
25
50
100
DS06-0004 (nM)

FIG. 3B

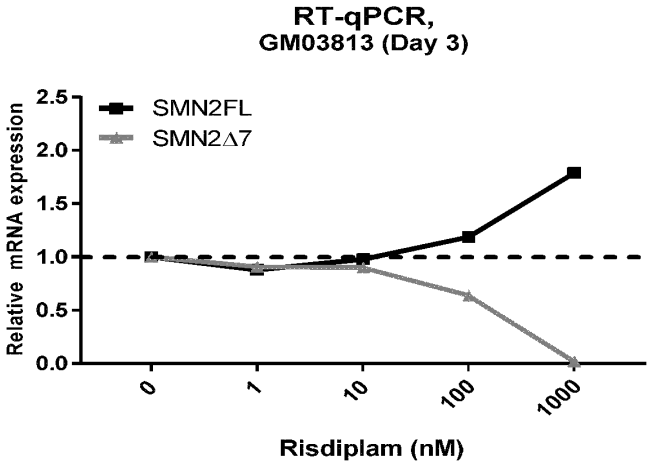

FIG. 3C

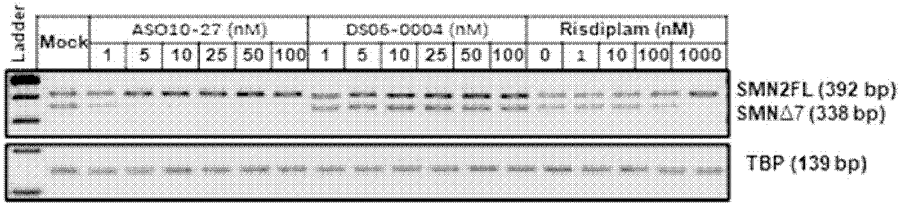

FIG. 3D

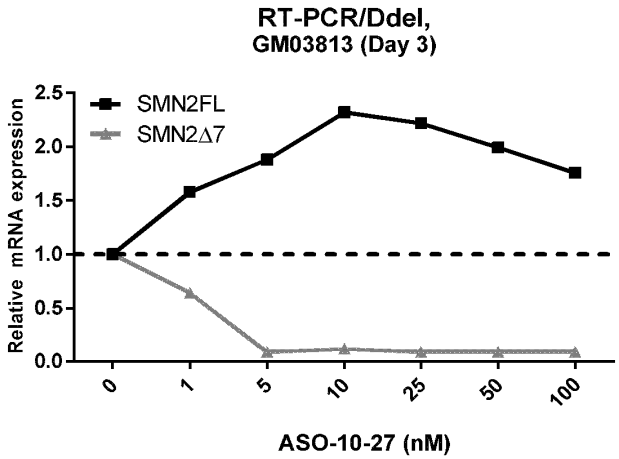

FIG. 3E

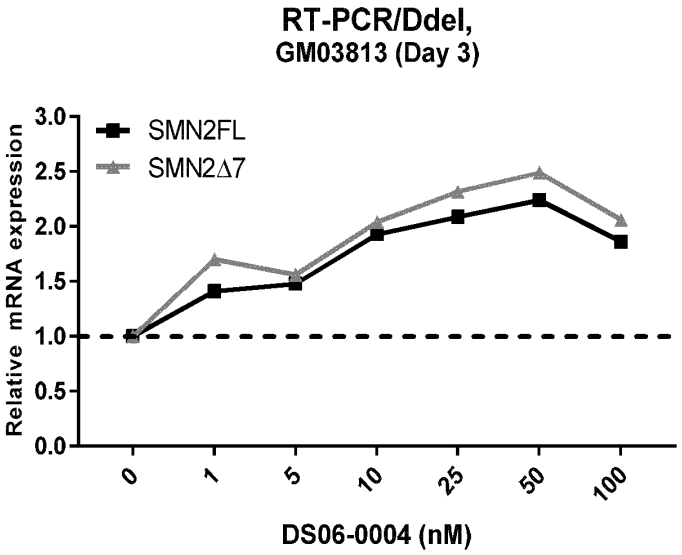
RT-PCR/DdeI,
GM03813 (Day 3)
Relative mRNA expression
3.0
2.5
2.0
1.5
1.0
0.5
0.0
SMN2FL
SMN2Δ7
0
1
5
10
25
50
100
DS06-0004 (nM)

FIG. 3F

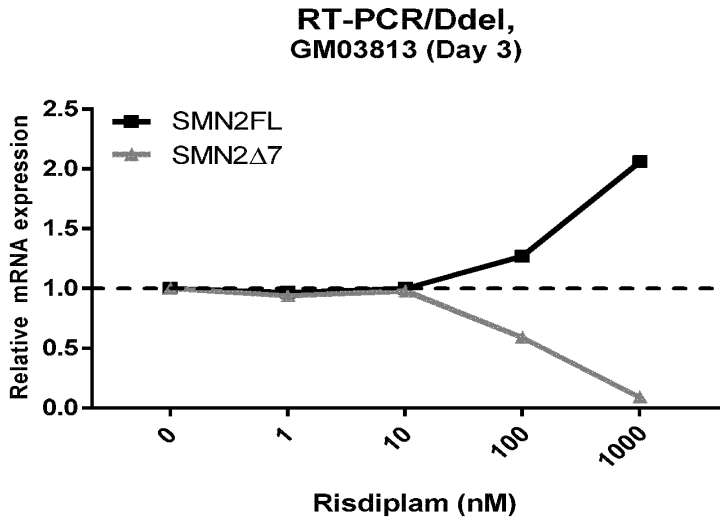
RT-PCR/DdeI,
GM03813 (Day 3)
Relative mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
SMN2FL
SMN2Δ7
0
1
10
100
1000
Risdiplam (nM)

FIG. 3G

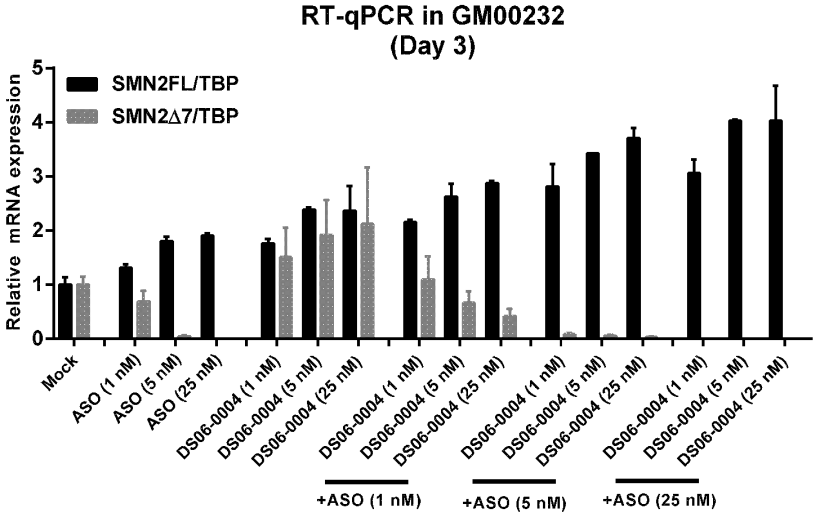
RT-qPCR in GM00232
(Day 3)
SMN2FL/TBP
SMN2Δ7/TBP
Relative mRNA expression
0
1
2
3
4
5
Mock
ASO (1 nM)
ASO (5 nM)
ASO (25 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
+ASO (1 nM)
+ASO (5 nM)
+ASO (25 nM)

FIG. 4A

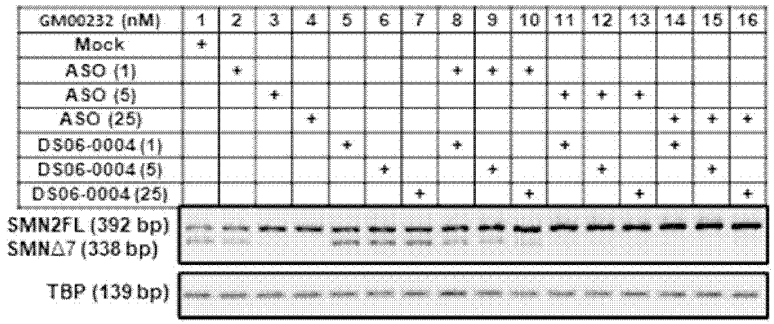
GM00232 (nM) 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Mock +
ASO (1) + + + +
ASO (5) + + + + +
ASO (25) + + + + + +
DS06-0004 (1) + + + +
DS06-0004 (5) + + + +
DS06-0004 (25) + + + +
SMN2FL (392 bp)
SMNΔ7 (338 bp)
TBP (139 bp)

FIG. 4B

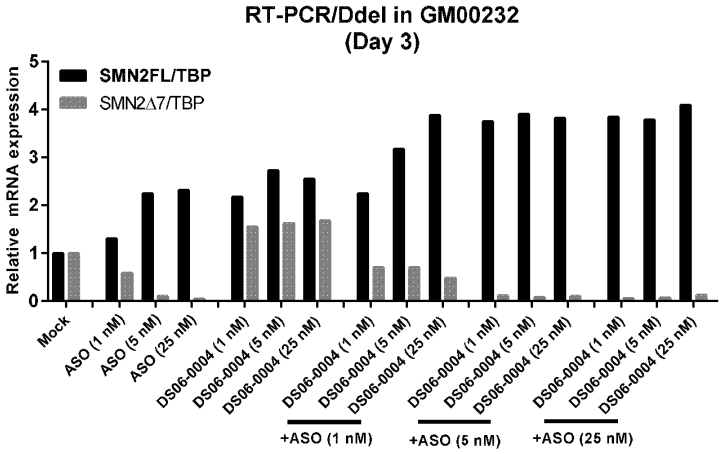

FIG. 4C

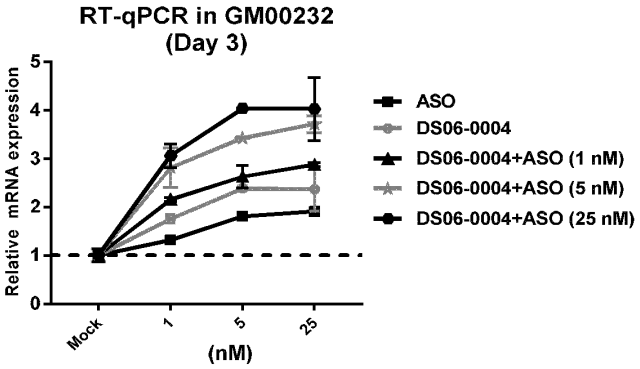

FIG. 4D

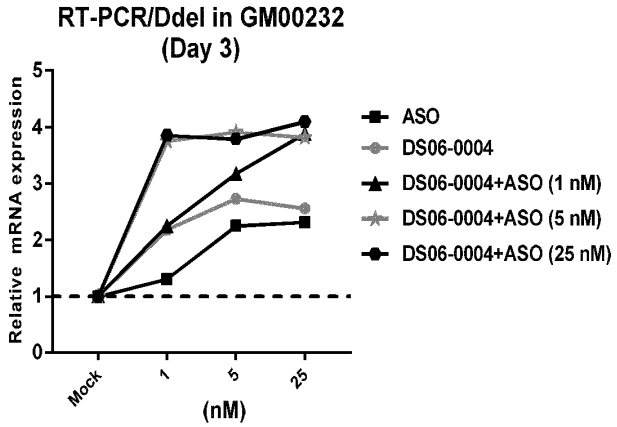

FIG. 4E

GM00232 (nM)	1	2	3	4	8	9	10	11	12	13	14	15	16
Mock	+												
ASO (1)		+			+	+	+						
ASO (5)			+					+	+	+			
ASO (25)				+							+	+	+
DS06-0004 (1)					+			+			+		
DS06-0004 (5)						+			+			+	
DS06-0004 (25)							+			+			+

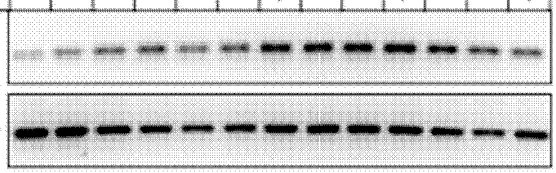

FIG. 5A

GM00232 (nM)	1	5	6	7	8	9	10	11	12	13	14	15	16
Mock	+												
ASO (1)					+	+	+						
ASO (5)								+	+	+			
ASO (25)											+	+	+
DS06-0004 (1)		+			+			+			+		
DS06-0004 (5)			+			+			+			+	
DS06-0004 (25)				+			+			+			+

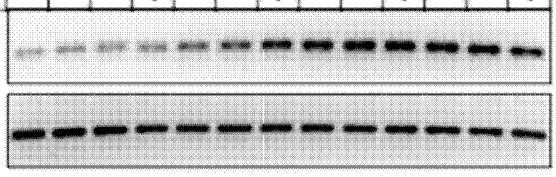

FIG. 5B

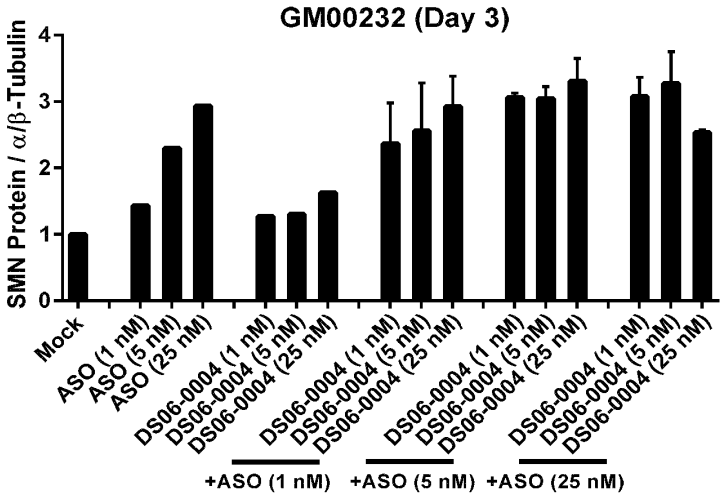
GM00232 (Day 3)
SMN Protein / α/β-Tubulin
0
1
2
3
4
Mock
ASO (1 nM)
ASO (5 nM)
ASO (25 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
+ASO (1 nM)
+ASO (5 nM)
+ASO (25 nM)

FIG. 5C

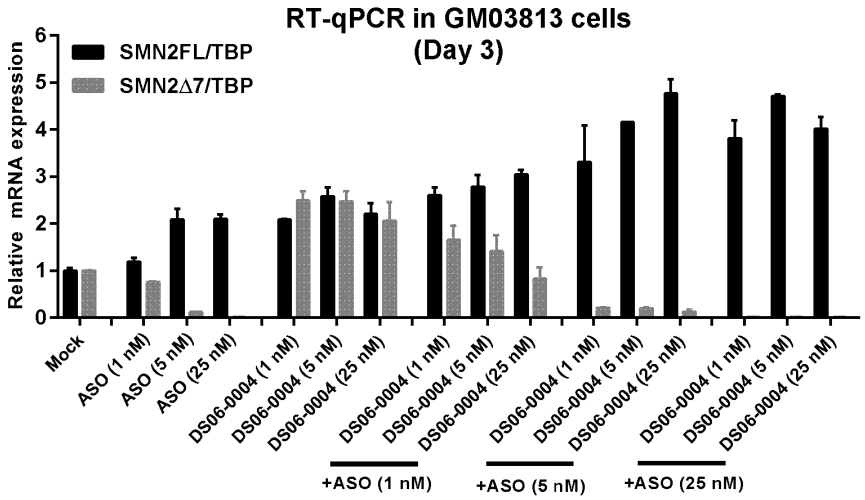
RT-qPCR in GM03813 cells
(Day 3)
SMN2FL/TBP
SMN2Δ7/TBP
Relative mRNA expression
0
1
2
3
4
5
6
Mock
ASO (1 nM)
ASO (5 nM)
ASO (25 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
+ASO (1 nM)
+ASO (5 nM)
+ASO (25 nM)

FIG. 6A

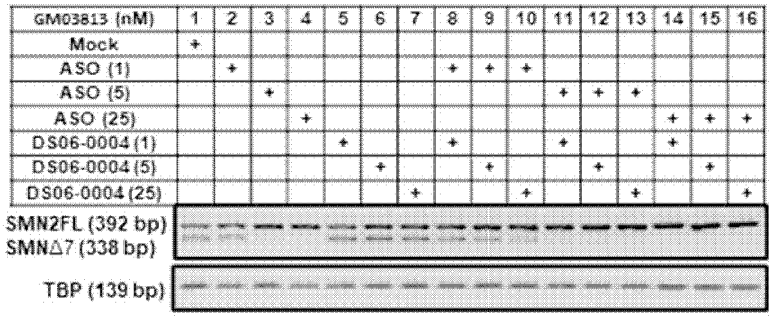
GM03813 (nM) 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Mock
ASO (1)
ASO (5)
ASO (25)
DS06-0004 (1)
DS06-0004 (5)
DS06-0004 (25)
SMN2FL (392 bp)
SMNΔ7 (338 bp)
TBP (139 bp)

FIG. 6B

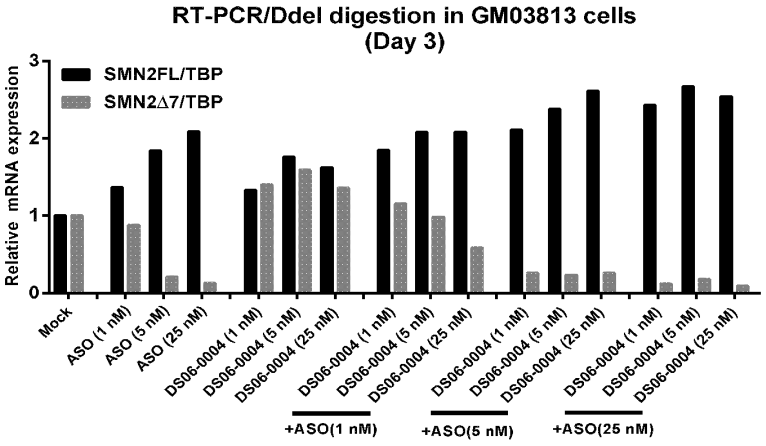

FIG. 6C

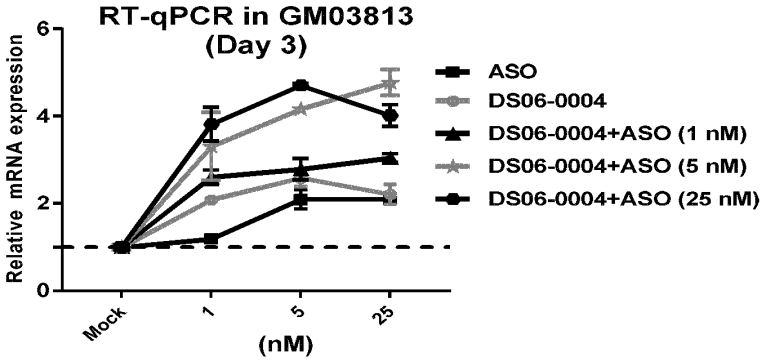

FIG. 6D

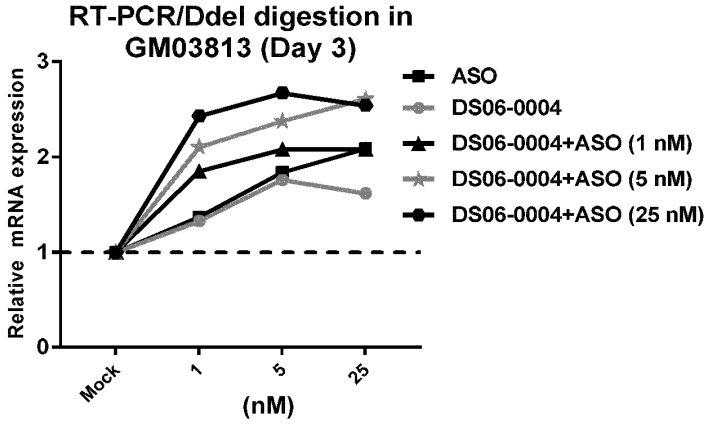

FIG. 6E

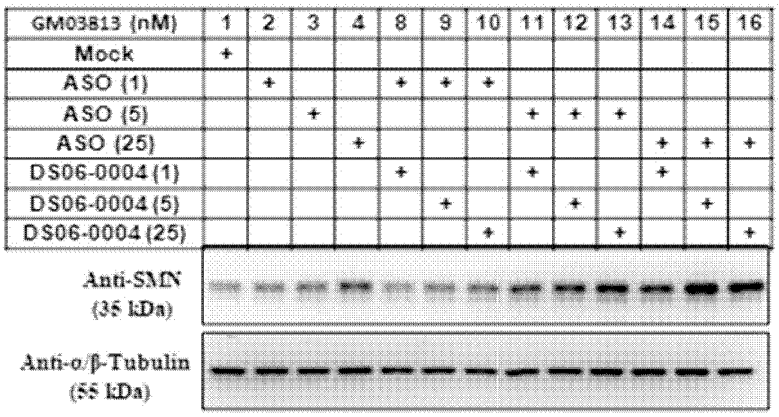
GM03813 (nM) 1 2 3 4 8 9 10 11 12 13 14 15 16
Mock
ASO (1)
ASO (5)
ASO (25)
DS06-0004 (1)
DS06-0004 (5)
DS06-0004 (25)
Anti-SMN (35 kDa)
Anti-α/β-Tubulin (55 kDa)

FIG. 7A

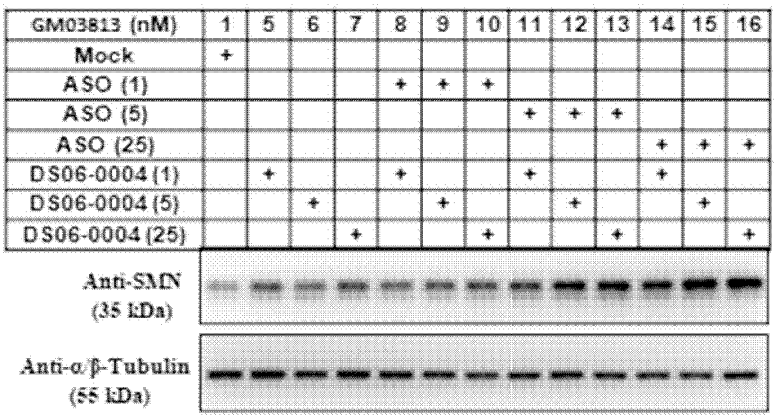
GM03813 (nM) 1 5 6 7 8 9 10 11 12 13 14 15 16
Mock
ASO (1)
ASO (5)
ASO (25)
DS06-0004 (1)
DS06-0004 (5)
DS06-0004 (25)
Anti-SMN (35 kDa)
Anti-α/β-Tubulin (55 kDa)

FIG. 7B

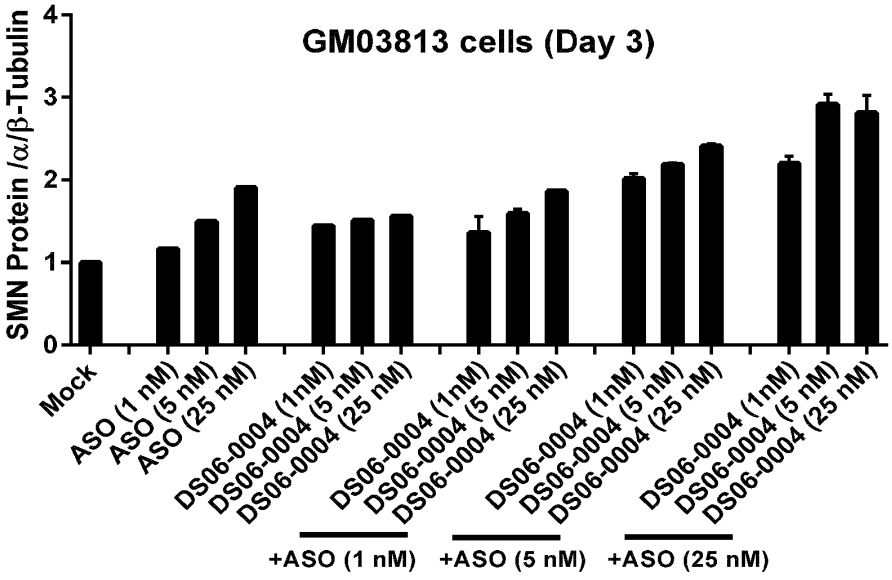
GM03813 cells (Day 3)
SMN Protein /α/β-Tubulin
0
1
2
3
4
Mock
ASO (1 nM)
ASO (5 nM)
ASO (25 nM)
DS06-0004 (1nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
DS06-0004 (1nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
DS06-0004 (1nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
DS06-0004 (1nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
+ASO (1 nM)
+ASO (5 nM)
+ASO (25 nM)

FIG. 7C

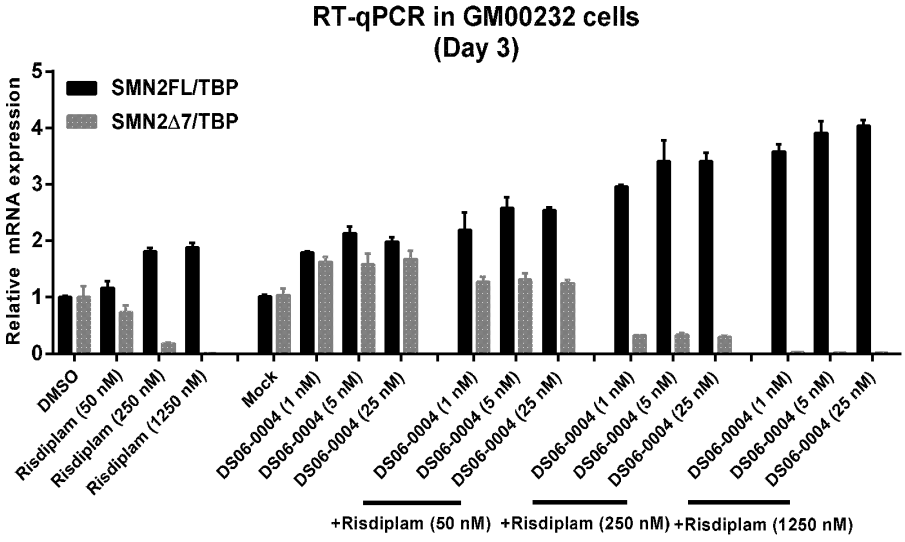

FIG. 8A

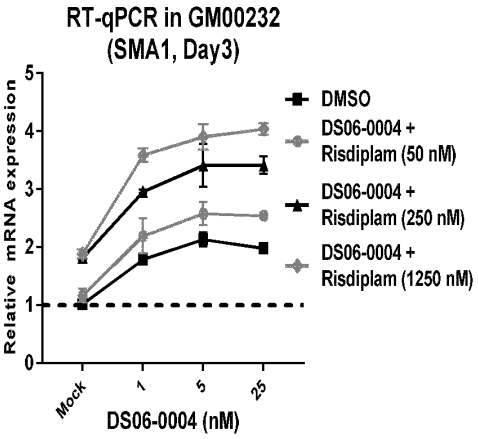

FIG. 8B

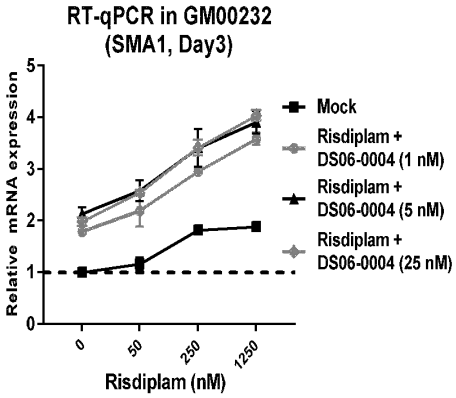

FIG. 8C

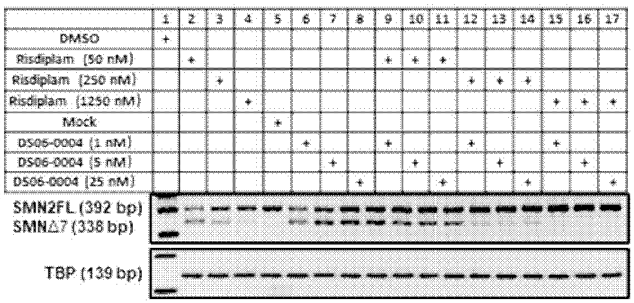

FIG. 8D

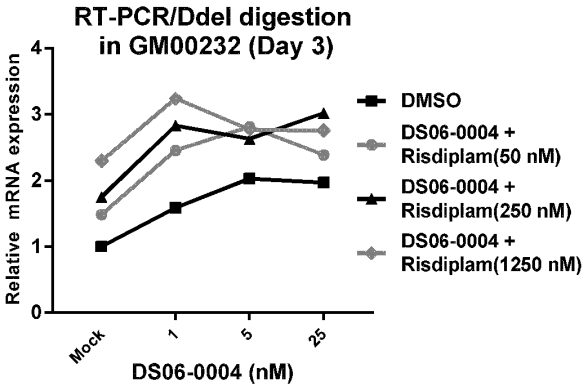

FIG. 8E

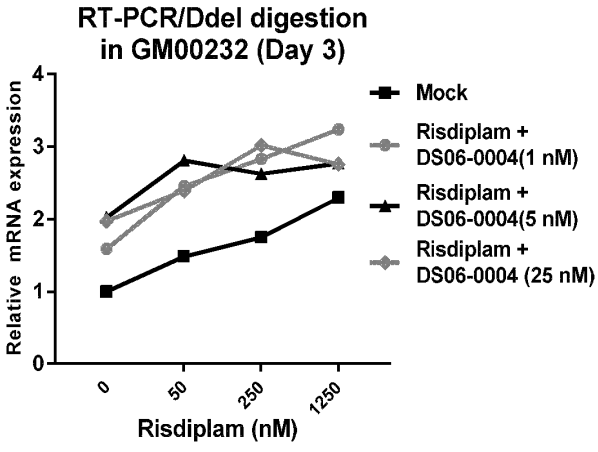

FIG. 8F

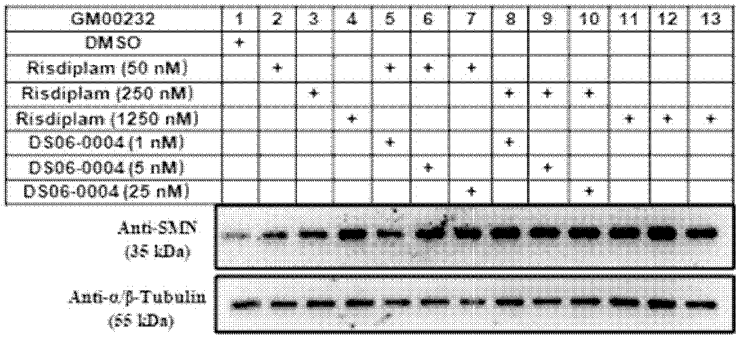
GM00232 1 2 3 4 5 6 7 8 9 10 11 12 13
DMSO + + + + + + + + + + + + +
Risdiplam (50 nM) + + + + + + + + + + + + +
Risdiplam (250 nM) + + + + + + + + + + + + +
Risdiplam (1250 nM) + + + + + + + + + + + + +
DS06-0004 (1 nM) + + + + + + + + + + + + +
DS06-0004 (5 nM) + + + + + + + + + + + + +
DS06-0004 (25 nM) + + + + + + + + + + + + +
Anti-SMN (35 kDa)
Anti-α/β-Tubulin (55 kDa)

FIG. 9A

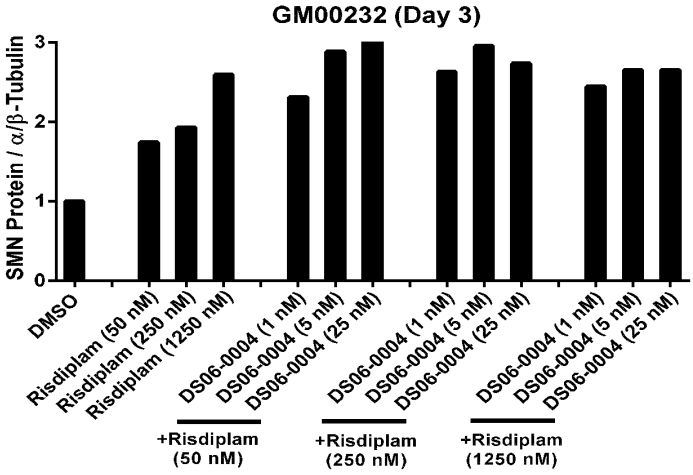
GM00232 (Day 3)
SMN Protein / α/β-Tubulin
0
1
2
3
DMSO
Risdiplam (50 nM)
Risdiplam (250 nM)
Risdiplam (1250 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
DS06-0004 (1 nM)
DS06-0004 (5 nM)
DS06-0004 (25 nM)
+Risdiplam (50 nM)
+Risdiplam (250 nM)
+Risdiplam (1250 nM)

FIG. 9B

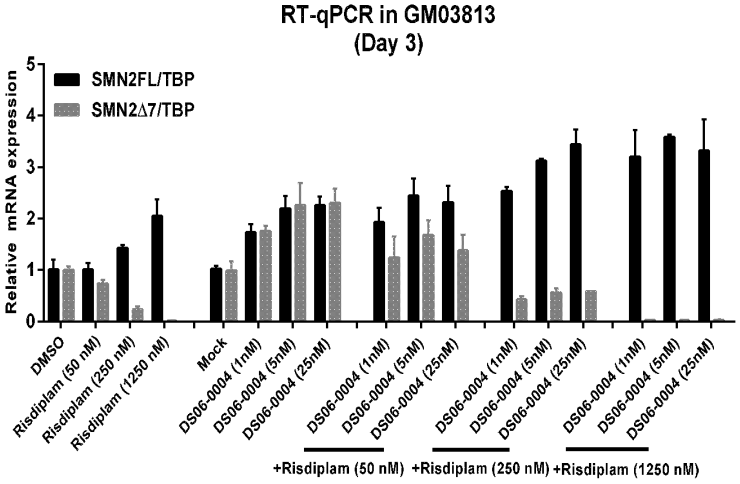

FIG. 10A

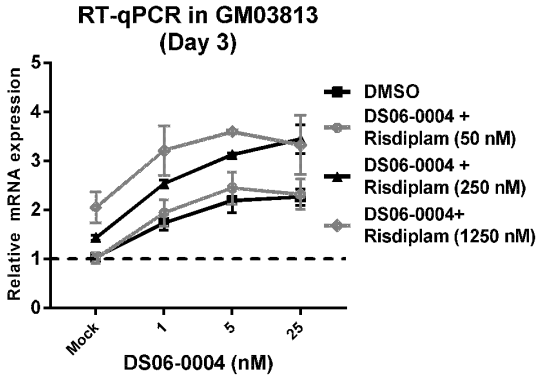

FIG. 10B

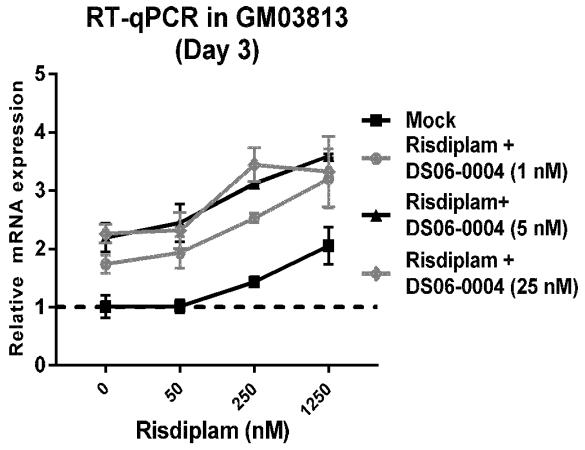

FIG. 10C

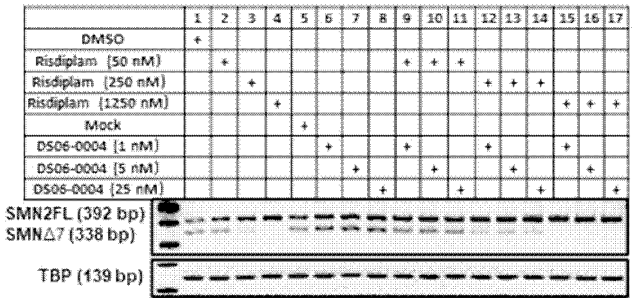

FIG. 10D

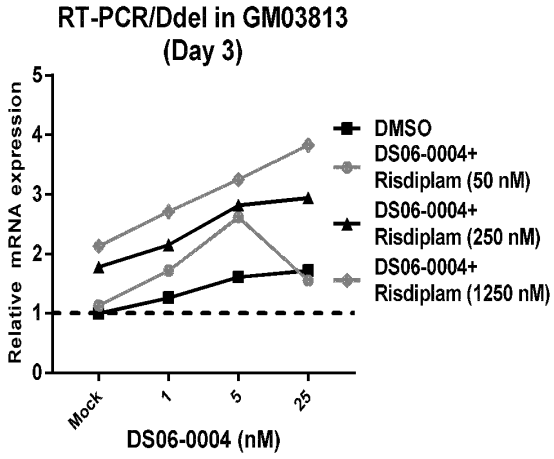

FIG. 10E

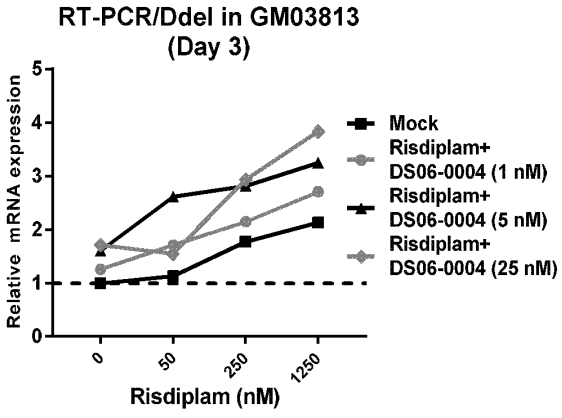

FIG. 10F

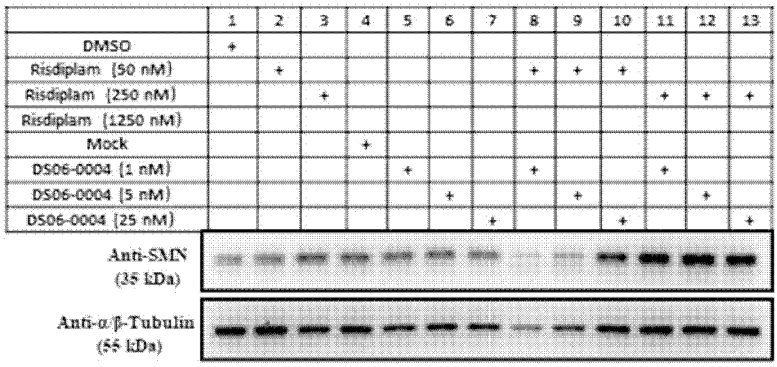

FIG. 11A

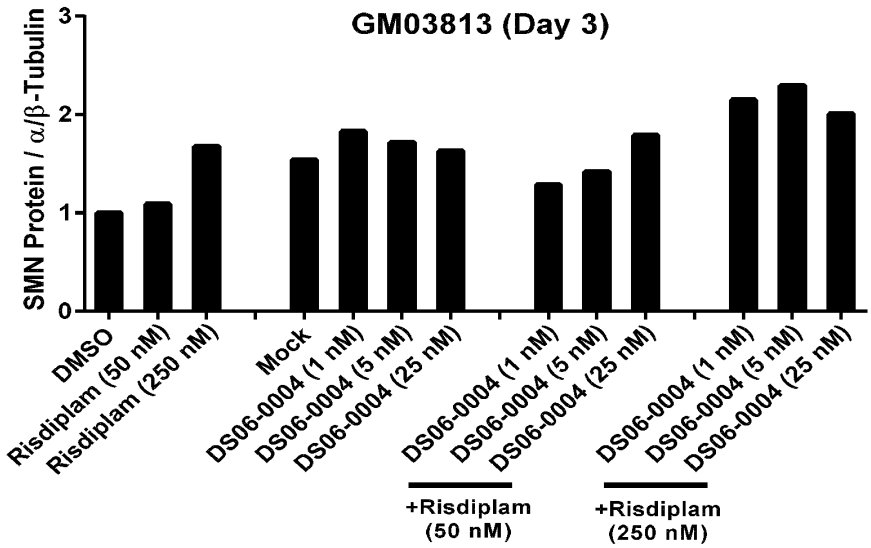

FIG. 11B

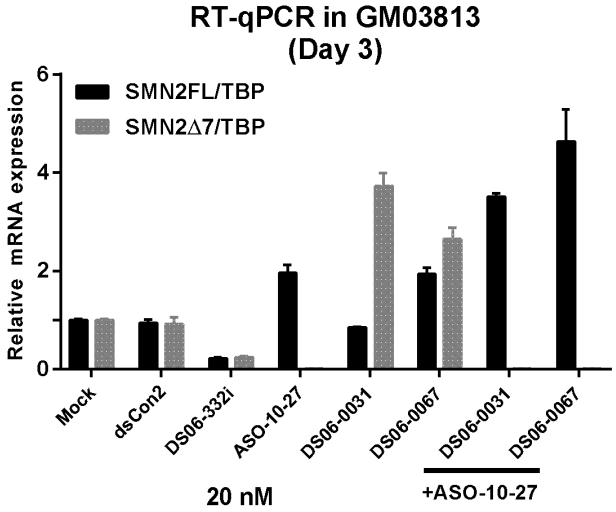

FIG. 12A

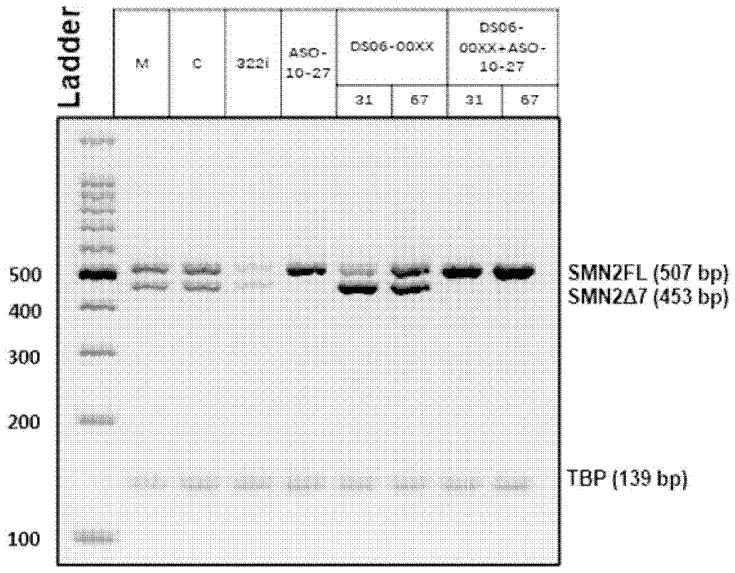

FIG. 12B

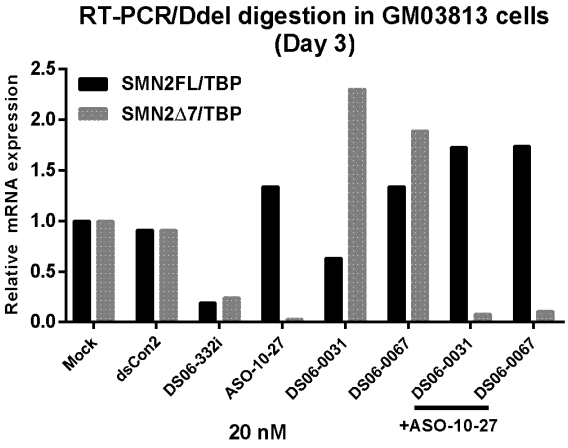

FIG. 12C

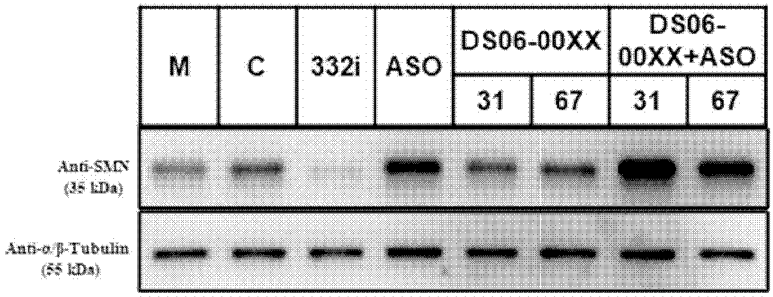

FIG. 12D

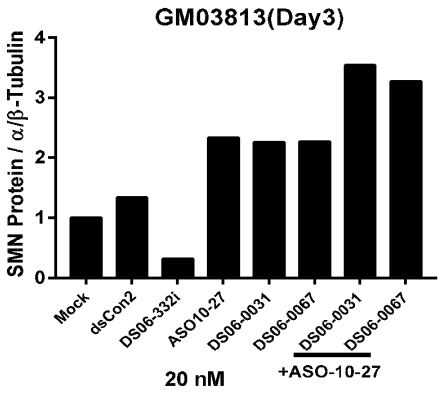

FIG. 12E

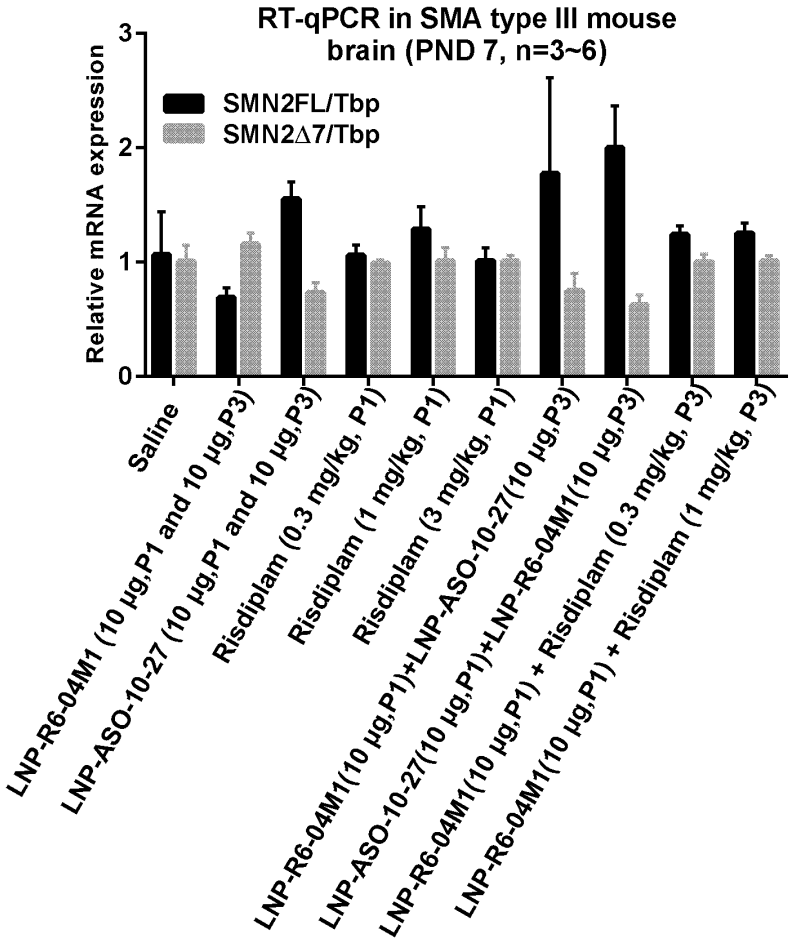
RT-qPCR in SMA type III mouse brain (PND 7, n=3~6)
Relative mRNA expression
SMN2FL/Tbp
SMN2Δ7/Tbp
0
1
2
3
Saline
LNP-R6-04M1 (10 μg,P1 and 10 μg,P3)
LNP-ASO-10-27 (10 μg,P1 and 10 μg,P3)
Risdiplam (0.3 mg/kg, P1)
Risdiplam (1 mg/kg, P1)
Risdiplam (3 mg/kg, P1)
LNP-R6-04M1(10 μg,P1)+LNP-ASO-10-27(10 μg,P3)
LNP-ASO-10-27(10 μg,P1)+LNP-R6-04M1(10 μg,P3)
LNP-R6-04M1(10 μg,P1) + Risdiplam (0.3 mg/kg, P3)
LNP-R6-04M1(10 μg,P1) + Risdiplam (1 mg/kg, P3)

FIG. 13A

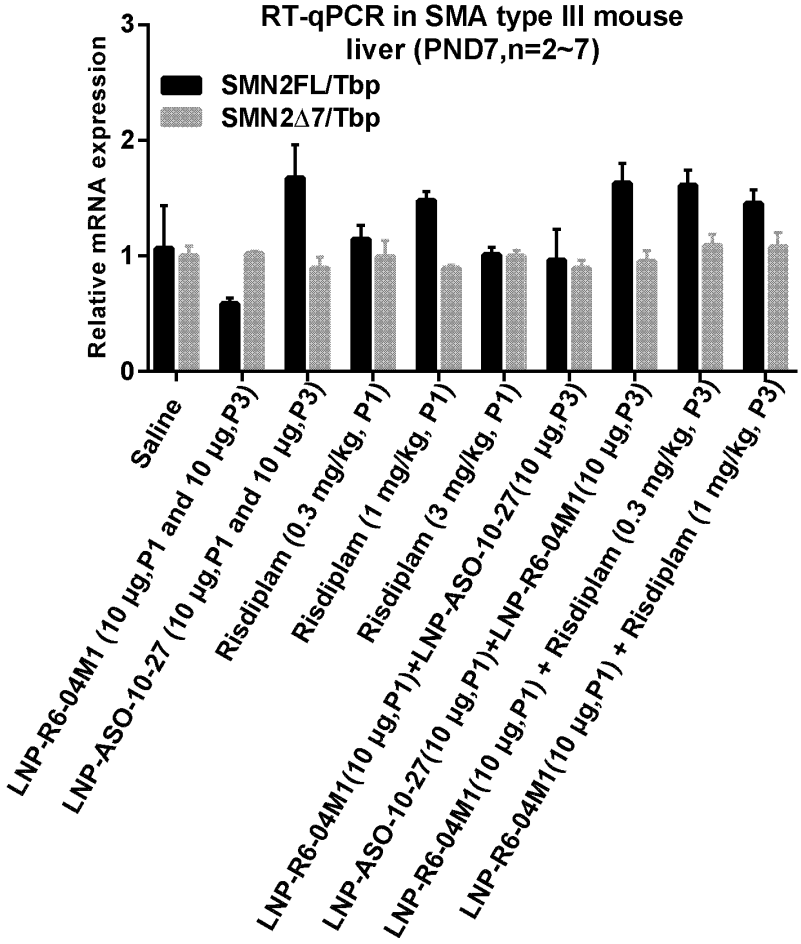
RT-qPCR in SMA type III mouse liver (PND7,n=2~7)
SMN2FL/Tbp
SMN2Δ7/Tbp
Relative mRNA expression
3
2
1
0
Saline
LNP-R6-04M1 (10 μg,P1 and 10 μg,P3)
LNP-ASO-10-27 (10 μg,P1 and 10 μg,P3)
Risdiplam (0.3 mg/kg, P1)
Risdiplam (1 mg/kg, P1)
Risdiplam (3 mg/kg, P1)
LNP-R6-04M1(10 μg,P1)+LNP-ASO-10-27(10 μg,P3)
LNP-ASO-10-27(10 μg,P1)+LNP-R6-04M1(10 μg,P3)
LNP-R6-04M1(10 μg,P1) + Risdiplam (0.3 mg/kg, P3)
LNP-R6-04M1(10 μg,P1) + Risdiplam (1 mg/kg, P3)

FIG. 13B

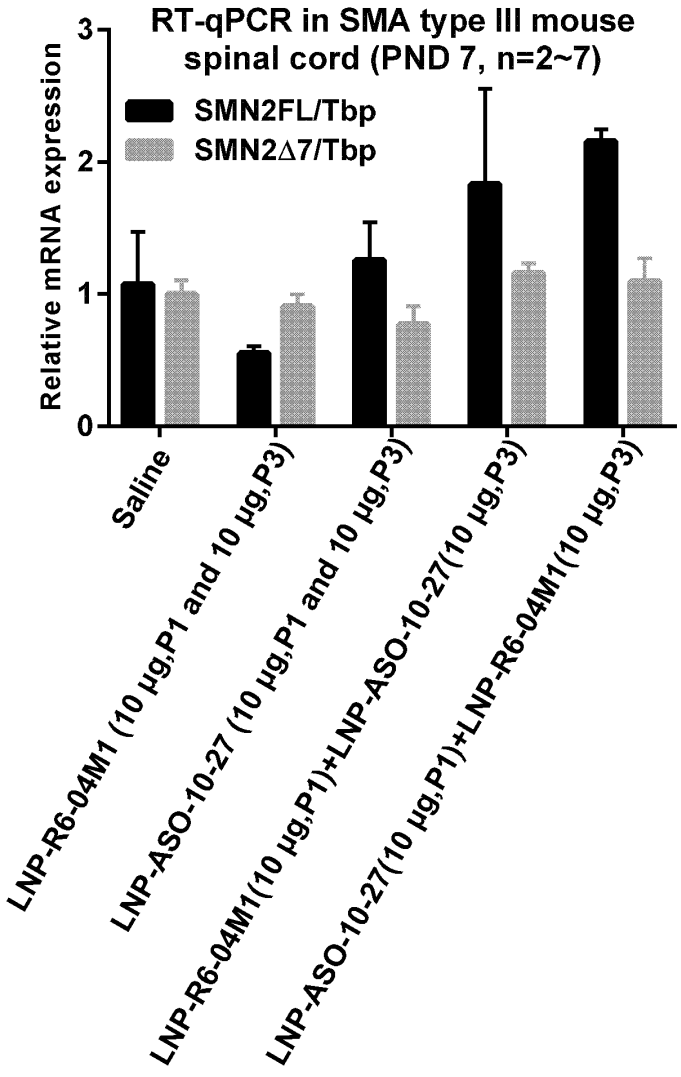
RT-qPCR in SMA type III mouse spinal cord (PND 7, n=2~7)
SMN2FL/Tbp
SMN2Δ7/Tbp
Relative mRNA expression
3
2
1
0
Saline
LNP-R6-04M1 (10 μg,P1 and 10 μg,P3)
LNP-ASO-10-27 (10 μg,P1 and 10 μg,P3)
LNP-R6-04M1(10 μg,P1)+LNP-ASO-10-27(10 μg,P3)
LNP-ASO-10-27(10 μg,P1)+LNP-R6-04M1(10 μg,P3)

FIG. 13C