[도1a]

sgRNAs	Direction	Mismatch 0	Mismatch 1	Mismatch 2	Indel ratio(%)
sgRNA_CDS_Sp#1	-	1	0	0	76.80
sgRNA_CDS_Sp#2	-	1	0	0	21.20
sgRNA_CDS_Sp#3	+	1	0	0	66.30
sgRNA_CDS_Sp#4	-	1	0	0	65.90
sgRNA_CDS_Sp#5	-	1	0	0	66.50
sgRNA_CDS_Sp#6	+	1	0	0	43.10
sgRNA_CDS_Sp#7	-	1	0	0	75.00
sgRNA_CDS_Sp#8	+	1	0	0	19.90

[도1b]

sgRNAs	Direction	Mismatch 0	Mismatch 1	Mismatch 2	Indel ratio(%)
sgRNA_TATA_Sp#1	-	1	0	1	32.80
sgRNA_TATA_Sp#2	+	1	0	0	42.00
sgRNA_TATA_Sp#3	+	1	0	0	25.20
sgRNA_TATA_Sp#4	+	1	0	2	59.10
sgRNA_TATA_Sp#5	+	1	0	0	16.20
sgRNA_TATA_Sp#6	+	1	0	1	30.30
sgRNA_TATA_Sp#7	-	1	0	1	53.80
sgRNA_TATA_Sp#8	+	1	0	0	7.30
sgRNA_TATA_Sp#9	-	1	0	1	26.90
sgRNA_TATA_Sp#10	-	1	0	1	15.90
sgRNA_TATA_Sp#11	-	1	0	2	24.20
sgRNA_TATA_Sp#12	-	1	0	2	38.20
sgRNA_TATA_Sp#13	-	1	0	0	6.90
sgRNA_TATA_Sp#14	+	1	0	1	62.00
sgRNA_TATA_Sp#15	+	1	0	1	54.60
sgRNA_TATA_Sp#16	-	1	0	1	32.50

[도1c]

sgRNAs	Direction	Mismatch 0	Mismatch 1	Mismatch 2	Indel ratio(%)
sgRNA_Enh_Sp#1	-	1	0	1	66.30
sgRNA_Enh_Sp#2	-	1	0	0	18.80
sgRNA_Enh_Sp#3	-	1	0	1	24.40
sgRNA_Enh_Sp#4	-	1	0	0	44.00
sgRNA_Enh_Sp#5	-	1	0	2	41.30
sgRNA_Enh_Sp#6	+	1	0	1	8.60
sgRNA_Enh_Sp#10	-	1	0	2	19.30
sgRNA_Enh_Sp#11	-	1	0	2	21.90
sgRNA_Enh_Sp#12	-	1	0	2	2.50
sgRNA_Enh_Sp#13	-	1	0	1	12.80
sgRNA_Enh_Sp#14	-	1	0	1	11.40
sgRNA_Enh_Sp#15	+	1	0	1	21.80
sgRNA_Enh_Sp#16	+	1	0	1	47.40

[도2a]

sgRNAs	Direction	Mismatch 0	Mismatch 1	Mismatch 2	Indel ratio(%)
sgRNA_CDS_Cj#1	-	1	0	0	12.90
sgRNA_CDS_Cj#2	-	1	0	0	3.90
sgRNA_CDS_Cj#3	+	1	0	0	0.10
sgRNA_CDS_Cj#4	+	1	0	0	2.80
sgRNA_CDS_Cj#5	-	1	0	0	0.70

[도2b]

sgRNAs	Direction	Mismatch 0	Mismatch 1	Mismatch 2	Indel ratio(%)
sgRNA_TATA_Cj#1	+	1	0	0	0.35
sgRNA_TATA_Cj#2	+	1	0	0	0.07
sgRNA_TATA_Cj#3	+	1	0	0	0.04
sgRNA_TATA_Cj#4	+	1	0	1	3.80
sgRNA_TATA_Cj#5	+	1	0	0	36.10
sgRNA_TATA_Cj#6	-	1	0	0	0.02
sgRNA_TATA_Cj#7	+	1	0	0	0.02
sgRNA_TATA_Cj#8	-	1	0	0	0.06
sgRNA_TATA_Cj#9	-	1	0	0	0.15
sgRNA_TATA_Cj#10	-	1	0	0	13.10
sgRNA_TATA_Cj#11	-	1	0	0	0.12

[도2c]

sgRNAs	Direction	Mismatch 0	Mismatch 1	Mismatch 2	Indel ratio(%)
sgRNA_Enh_Cj#1	+	1	0	0	16.70
sgRNA_Enh_Cj#2	+	1	0	0	0.07
sgRNA_Enh_Cj#3	-	1	0	0	0
sgRNA_Enh_Cj#4	+	1	0	0	1.18
sgRNA_Enh_Cj#5	-	1	0	0	0.03
sgRNA_Enh_Cj#6	-	1	0	0	0.05
sgRNA_Enh_Cj#7	-	1	0	0	0.09
sgRNA_Enh_Cj#8	+	1	0	0	0.28
sgRNA_Enh_Cj#9	-	1	0	0	41.40
sgRNA_Enh_Cj#10	+	1	0	0	0.45
sgRNA_Enh_Cj#11	-	1	0	0	0.55
sgRNA_Enh_Cj#12	+	1	0	0	2.83
sgRNA_Enh_Cj#13	+	1	0	0	0.03

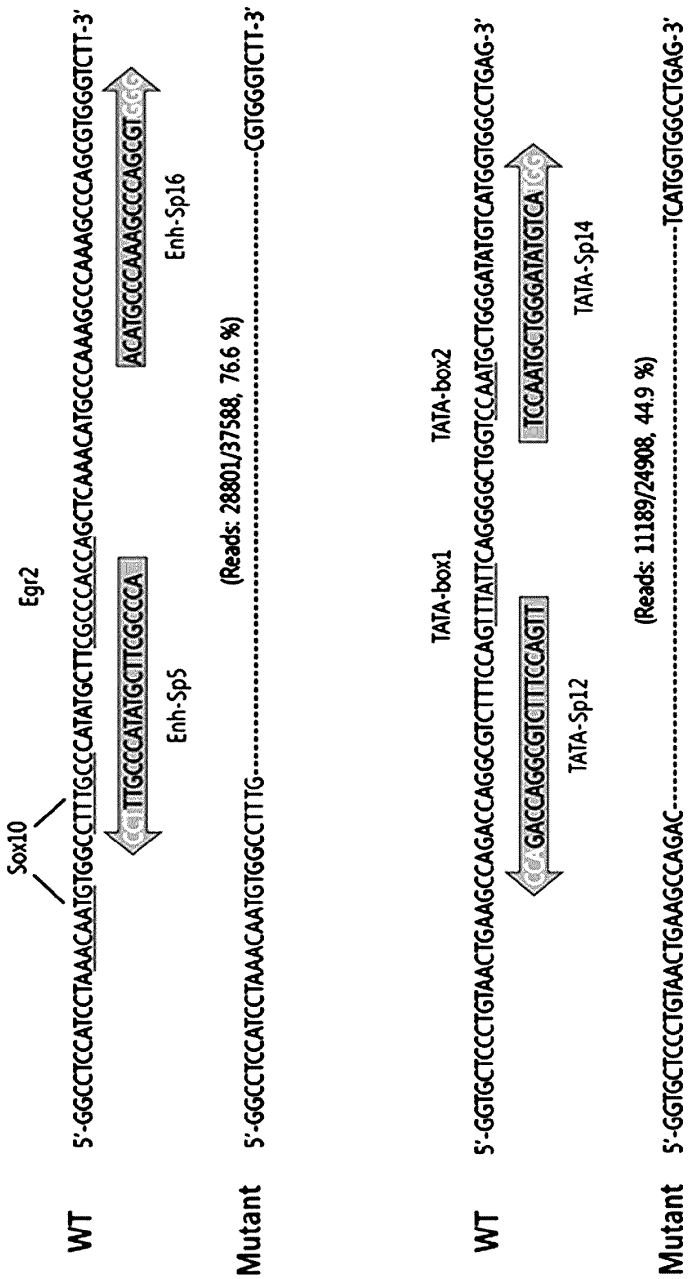
[도3]

Name	sgRNAs	More than minimum frequency	Insertions	Deletions	Indel ratio(%)
TATA WT		26730	28	70	0.40
TATA-1	sgRNA_TATA_Sp#15	30466	3110	6357	31.10
TATA-2	sgRNA_TATA_Sp#12	9286	0	6929	74.60
	sgRNA_TATA_Sp#14	5651	118	4972	90.10
Enh WT		27917	0	11	0.00
Enh-3	sgRNA_Enh_Sp#1	32148	8744	10126	58.70
Enh-4	sgRNA_Enh_Sp#5	37486	23	30928	82.60
	sgRNA_Enh_Sp#16	37277	69	35000	94.10
Enh-5	sgRNA_Enh_Sp#1	30576	2782	14544	56.70
	sgRNA_Enh_Sp#4	30399	217	11894	39.80
CDS-SP1 WT		35424	0	40	0.10
CDS-SP1	sgRNA_CDS_Sp#1	32206	4613	9077	42.50
CDS-SP3 WT		21997	0	0	0.00
CDS-SP3	sgRNA_CDS_Sp#3	27511	3441	11877	55.70

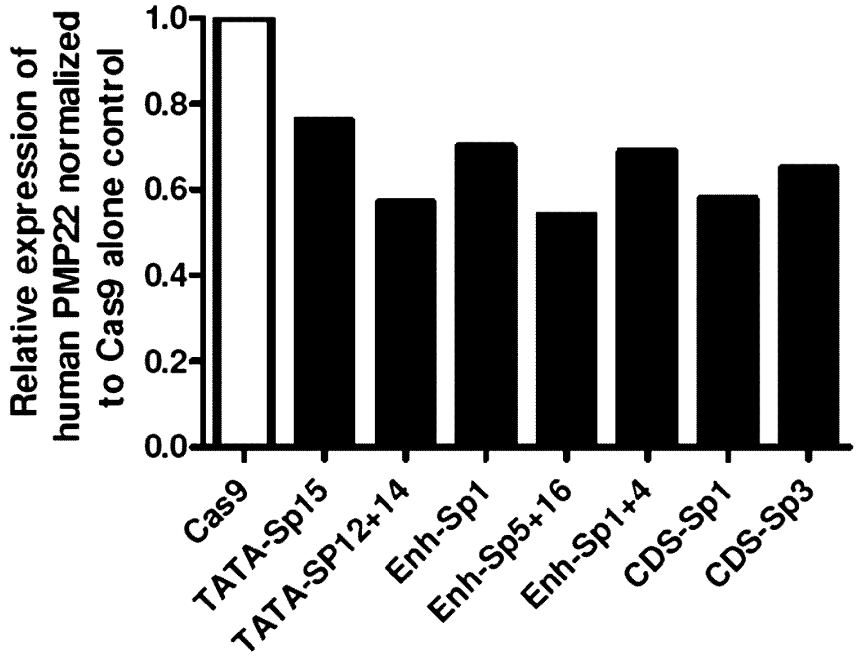
[도4]

sgRNAs	CDS-SP1		CDS-SP3	
	Reads	Ratio	Reads	Ratio
Total indels	13690	1	15312	1
3N±1, 3N±2	10063	0.74	12795	0.84
3N±0	3627	0.26	2517	0.16

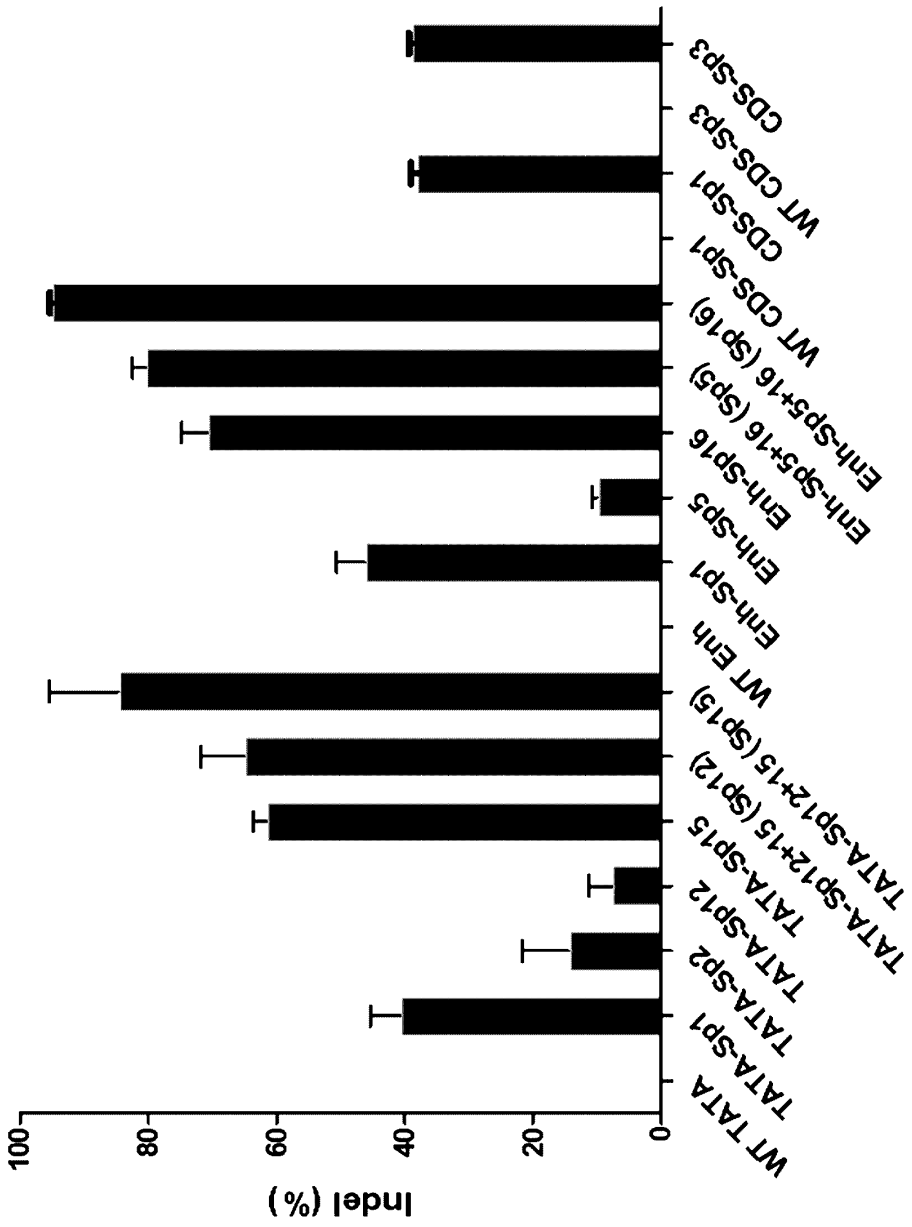
[도5]


Sox10
Egr2
WT
5'-GGCCTCCATCCTAAACAATGTGGCCTTTGCCCATATGCTTCGCCCACCAGCTCAAACATGCCCAAAGCCCAAGCCCAGCGTGGGTCTT-3'
TTGCCCATATGCTTCGCCCA
ACATGCCCAAAGCCCAGCGT
Enh-Sp5
Enh-Sp16
Mutant
5'-GGCCTCCATCCTAAACAATGTGGCCTTTG
(Reads: 28801/37588, 76.6 %)
CGTGGGTCTT-3'
TATA-box1
TATA-box2
WT
5'-GGTGCTCCCTGTAACTGAAGCCAGACCAGGCGTCTTTCCAGTTTATTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGTGGCCTGAG-3'
GACCAGGCGTCTTTCCAGTT
TCCAATGCTGGGATATGTCA
TATA-Sp12
TATA-Sp14
Mutant
5'-GGTGCTCCCTGTAACTGAAGCCAGAC
(Reads: 11189/24908, 44.9 %)
TCATGGTGGCCTGAG-3'

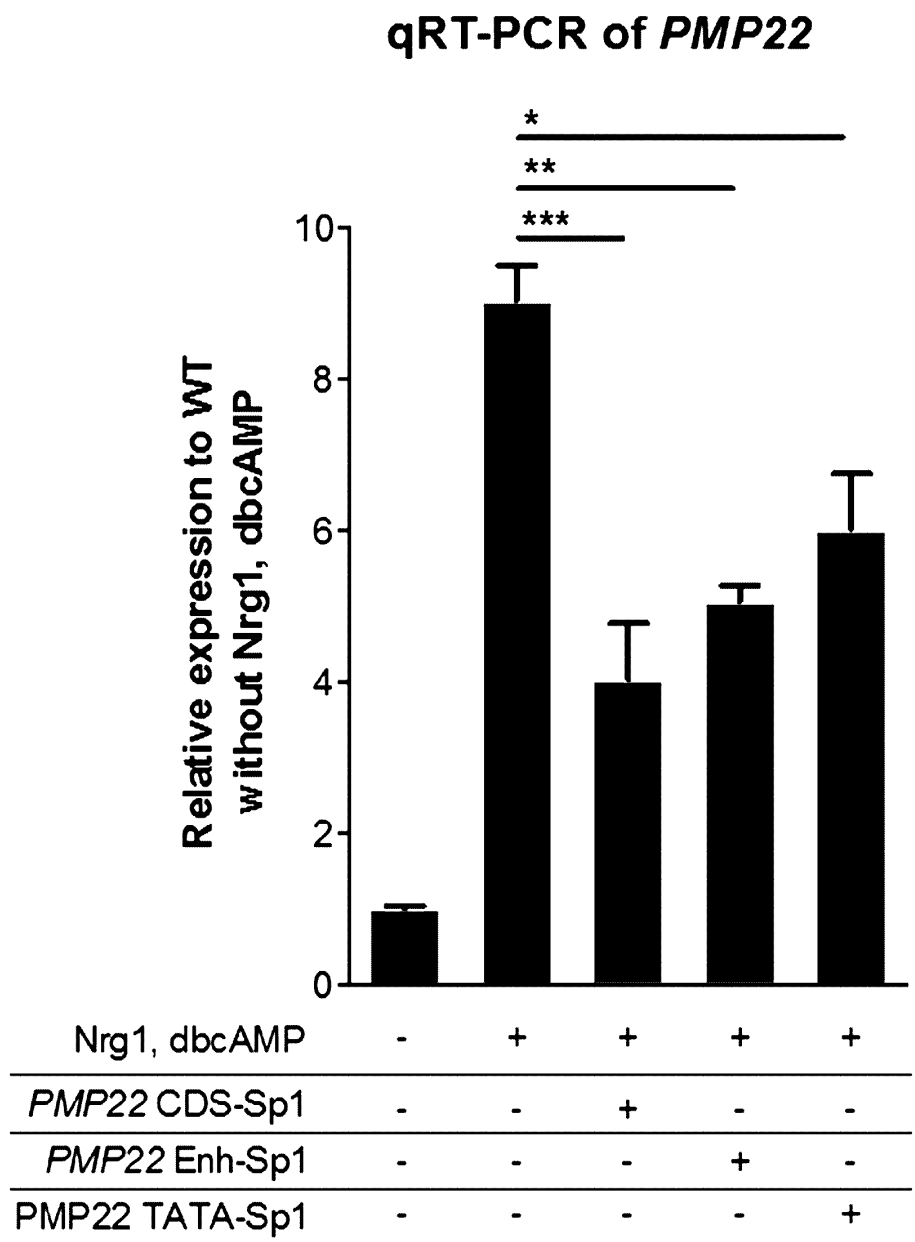
[도6]


Relative expression of human PMP22 normalized to Cas9 alone control
1.0
0.8
0.6
0.4
0.2
0.0
Cas9
TATA-Sp15
TATA-SP12+14
Enh-Sp1
Enh-Sp5+16
Enh-Sp1+4
CDS-Sp1
CDS-Sp3

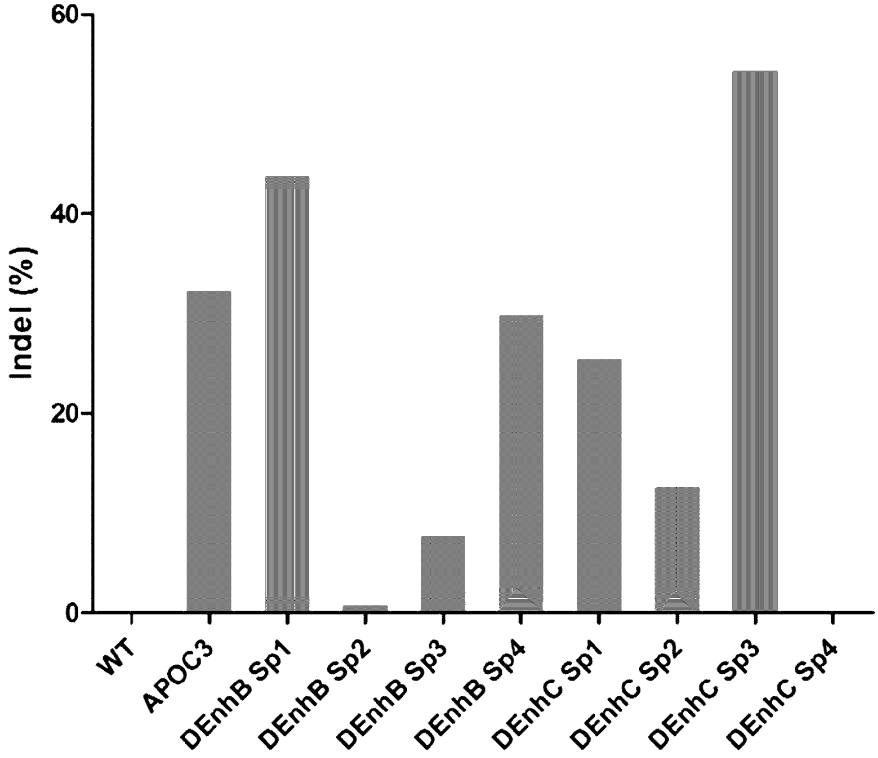
[도7a]


Indel (%)
0
20
40
60
80
100
WT TATA
TATA-Sp1
TATA-Sp2
TATA-Sp12
TATA-Sp15
TATA-Sp12+15 (Sp12)
TATA-Sp12+15 (Sp15)
WT Enh
Enh-Sp1
Enh-Sp5
Enh-Sp16
Enh-Sp5+16 (Sp5)
Enh-Sp5+16 (Sp16)
WT CDS-Sp1
CDS-Sp1
WT CDS-Sp3
CDS-Sp3

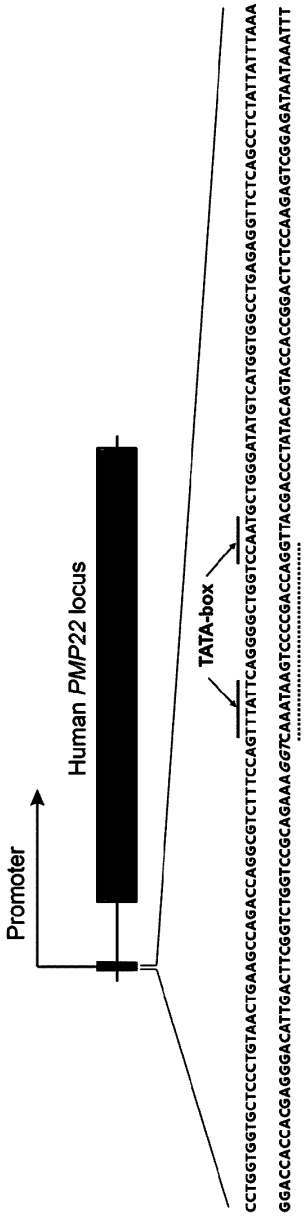
[도7b]


qRT-PCR of PMP22
*
**
***
Relative expression to WT without Nrg1, dbcAMP
10
8
6
4
2
0
Nrg1, dbcAMP - + + + +
PMP22 CDS-Sp1 - - + - -
PMP22 Enh-Sp1 - - - + -
PMP22 TATA-Sp1 - - - - +

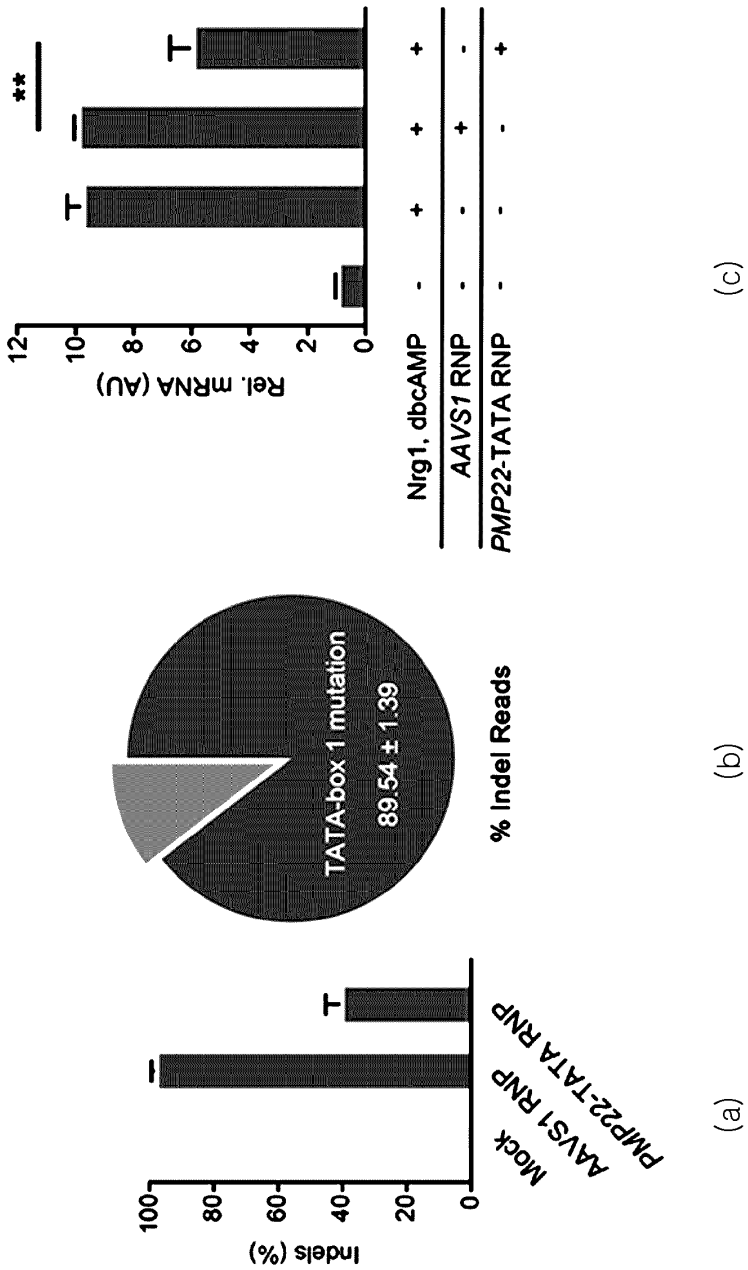
[도7c]


60
40
20
0
Indel (%)
WT
APOC3
DEnhB Sp1
DEnhB Sp2
DEnhB Sp3
DEnhB Sp4
DEnhC Sp1
DEnhC Sp2
DEnhC Sp3
DEnhC Sp4

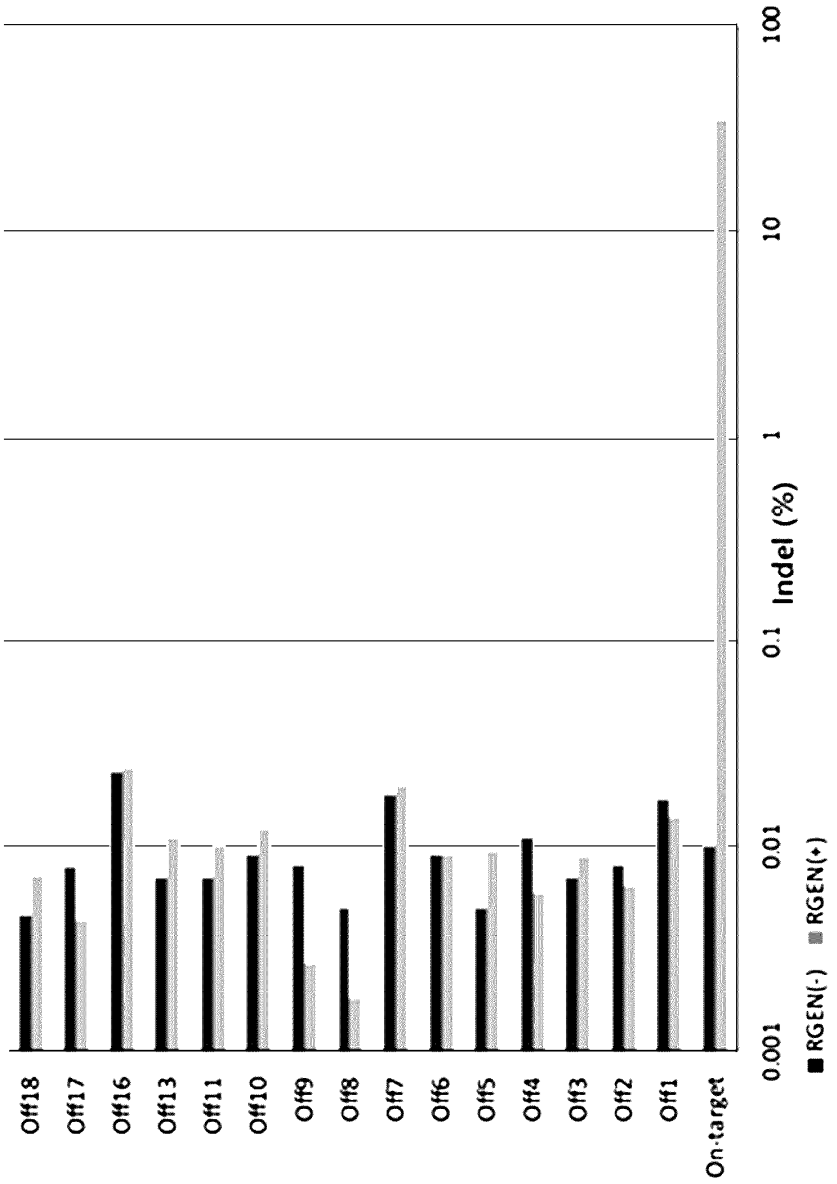
[도8a]


Promoter
Human PMP22 locus
TATA-box
sgRNA
CCTGGTGGTGCTCCCTGTAACTGAAGCCAGACCAGGCGTCTTTCCAGTTTATTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGTGGCCTGAGAGGTTCTCAGCCTCTATTATTTAAA
GGACCACCACGAGGGACATTGACTTCGGTCTGGTCCGCAGAAA GGTCAAATAAGTCCCCGACCAGGTTACGACCCTATACAGTACCACCGGACTCTCCAAGAGTCGGAGATAATAAATTT

[도8b]


Indels (%)
100
80
60
40
20
0
Mock
AAVS1 RNP
PMP22-TATA RNP
(a)
TATA-box 1 mutation
89.54 ± 1.39
% Indel Reads
(b)
Rel. mRNA (AU)
12
10
8
6
4
2
0
**
Nrg1, dbcAMP - + + +
AAVS1 RNP - - + -
PMP22-TATA RNP - - - +
(c)

[도9a]


100
10
1
0.1
0.01
0.001
Indel (%)
RGEN(-)
RGEN(+)
Off18
Off17
Off16
Off13
Off11
Off10
Off9
Off8
Off7
Off6
Off5
Off4
Off3
Off2
Off1
On-target

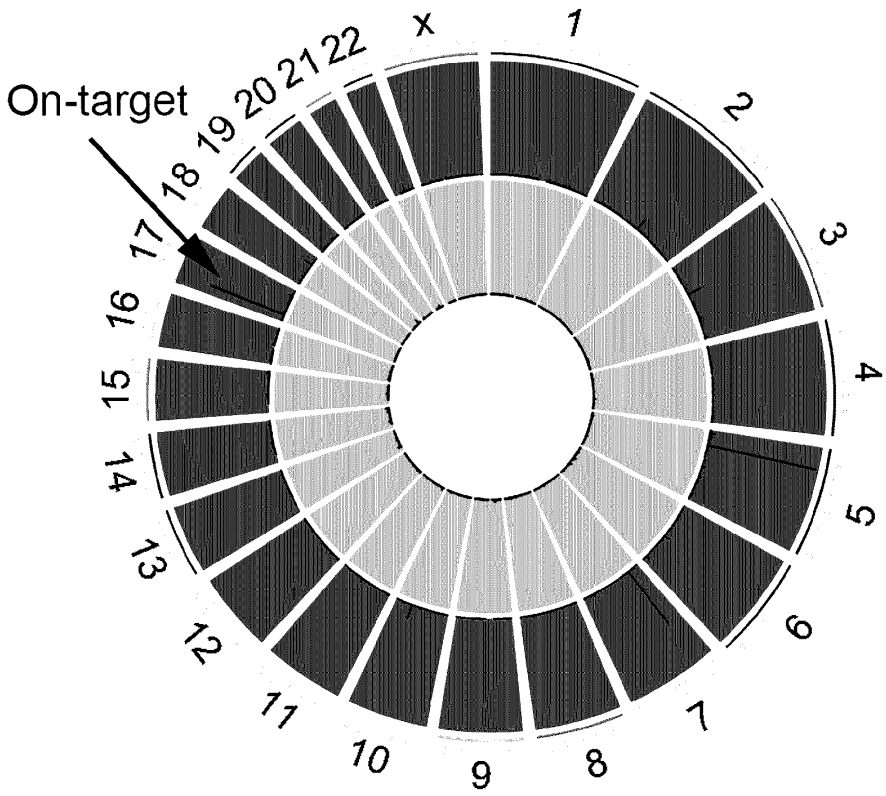
[도9b]

Indel	Local Sequence	Frequency (%)
WT	ACTGAAGCCAGACCAGGCGTCTTTCCAGTTTATTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGTGGCCTGAGAGGTTCTCAGCCTC	
-1	ACTGAAGCCAGACCAGGCGTCTTTCCAG-TTATTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGCGGCCTGAGAGGTTCTCAGCCTC	14.00
-2	ACTGAAGCCAGACCAGGCGTCTTTCCAGTT--TTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGTGGCCTGAGAGGTTCTCAGCCTC	8.86
+1	ACTGAAGCCAGACCAGGCGTCTTTCCAGTTTTTATTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGCGGCCTGAGAGGTTCTCAGCCTC	3.84
-3	ACTGAAGCCAGACCAGGCGTCTTTCCAGT---TTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGCGGCCTGAGAGGTTCTCAGCCTC	3.53
-4	ACTGAAGCCAGACCAGGCGTCTTTCCAG----TTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGCGGCCTGAGAGGTTCTCAGCCTC	2.81

[도9c]

	Location	Position	Target sequence (5' to 3')	Gene
On-target	chr17	15265347	GGACCAGCCCCTGAATAAAC*TGG*	hPMP22-TATA
Off1	chr5	135488419	GGACCAGCCaCaGAATAAAC*AAG*	Intergenic
Off2	chr8	140735957	tGACCAGtCCaTGAATAAAC*CAG*	PTK2 (Intron)
Off3	chr12	14124312	GGACCAGaCaCTGAATAtAC*CAG*	Intergenic
Off4	chr4	97775621	GGACCAGCCaCaGAATAAAt*TGG*	STPG2 (Intron)
Off5	chr5	26531145	GGAtCAGCCCCaGAATAAAt*TAG*	Intergenic
Off6	chr1	41780482	GGAgCAtCCCCaGAATAAAC*CAG*	HIVEP3 (Intron)
Off7	chr1	157564675	GGAtCAGCgtCTGAATAAAC*AAG*	Intergenic
Off8	chr13	20254256	aGACCAGCCCCaGAAcAAAC*AAG*	Intergenic
Off9	chr15	100401183	GtACgAGCCCCTGAATAAAt*AGG*	CERS3 (Exon)
Off10	chr6	26006396	GGACCAaaCaCTGAATAAAC*CAG*	Intergenic
Off11	chr20	10136908	GcACCAGCCaCTGAATtAAC*AAG*	SNAP25 (Intron)
Off12	chrX	7525146	GtACCAGCCaCTGAAaAAAC*AGG*	Intergenic
Off13	chr18	1972251	GaACCAGCCCCTGAtTAgAC*CAG*	Intergenic
Off14	chr18	77536261	GtACCAGCCaCTGAAaAAAC*AGG*	Intergenic
Off15	chr11	30065750	GtACCAGCCCCTGcAaAAAC*AGG*	Intergenic
Off16	chr11	30579429	GcACCAGgCCtTGAATAAAC*AAG*	MPP2D2 (Intron)
Off17	chr11	35726323	GGcCCAGCCaCTGAgTAAAC*TAG*	TRIM44 (Intron)
Off18	chr11	112468286	GGAattGCCCCTGAATAAAC*AAG*	RP11-65M17.3 (Intron)

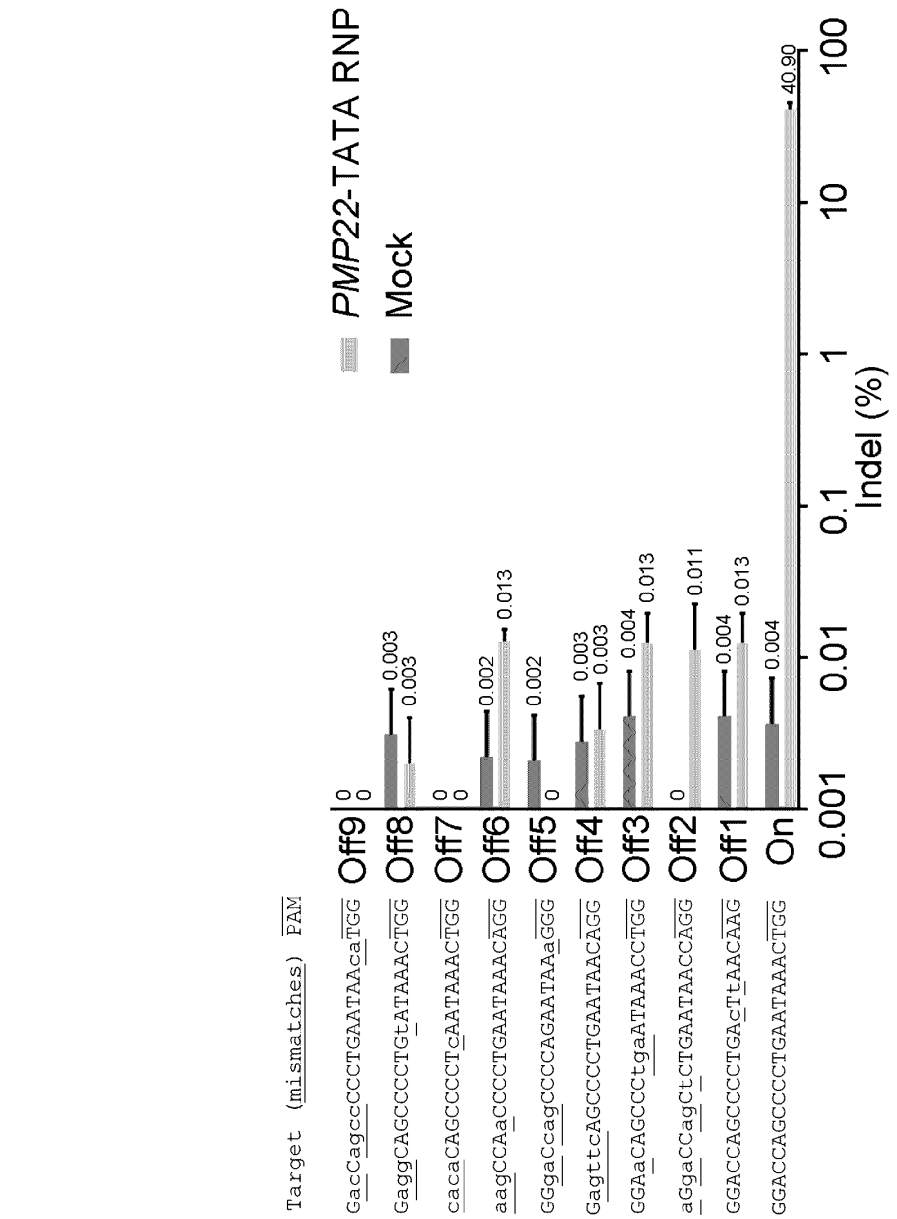
[도10a]


On-target
X
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
(-) Nuclease (+) sgRNA/Cas9

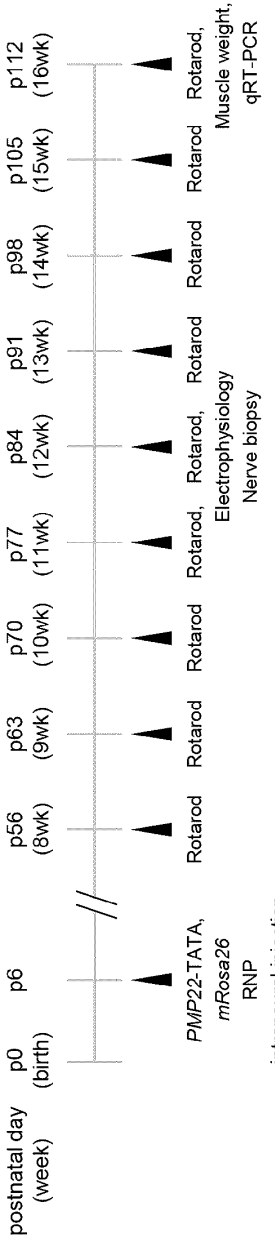
[도10b]

	Location	Position	Target sequence (5' to 3')	Gene
On-target	chr17	15265347	GGACCAGCCCCTGAATAAAC*TGG*	hPMP22-TATA
Off1	chr5	38420810	GGgaacagCCCTGAATAAAC*CTG*	EGFLAM (Intron)
Off2	chr7	28618099	aGgaCCagCtCTGAATAACC*AGG*	CREB5 (Intron)
Off3	chr5	38420811	GGAaCAGCCCtgaATAAACC*TGG*	EGFLAM (Intron)
Off4	chr10	93462291	GagttcAGCCCCTGAATAAC*AGG*	Intergenic
Off5	chr3	78627344	GGgaCcagCCCCAGAATAAa*GGG*	Intergenic
Off6	chr2	131586033	aagCCAaCCCCTGAATAAAC*AGG*	Intergenic
Off7	chr18	56254369	cacaCAGCCCCCTcAATAAAC*TGG*	ALPK2 (Intron)
Off8	chr22	27477459	GaggCAGCCCCCTGtATAAAC*TGG*	Intergenic
Off9	chr6	91586787	GacCagccCCCTGAATAAca*TGG*	Intergenic

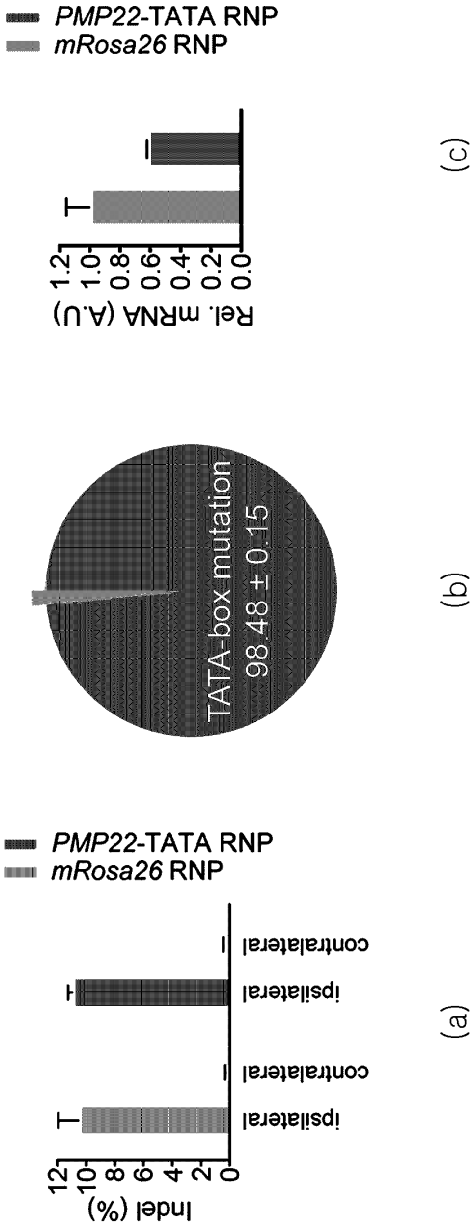
[도10c]


PMP22-TATA RNP
Mock
Indel (%)
0.001
0.01
0.1
1
10
100
Target (mismatches) PAM
GacCagccCCCTGAATAAcaTGG
GaggCAGCCCCTGtATAAACTGG
cacaCAGCCCCTcAATAAACTGG
aagCCAaCCCCTGAATAAACAGG
GGgaCcagCCCCAGAATAAaGGG
GagttcAGCCCCTGAATAACAGG
GGAaCAGCCCtgaATAAACCTGG
aGgaCCagCtCTGAATAACCAGG
GGACCAGCCCCTGAcTtAACAAG
GGACCAGCCCCTGAATAAACTGG
Off9
Off8
Off7
Off6
Off5
Off4
Off3
Off2
Off1
On
0
0
0.003
0.003
0
0
0.002
0.013
0.002
0
0.003
0.003
0.004
0.013
0
0.011
0.004
0.013
0.004
40.90

[도11]


postnatal day (week)
p0 (birth)
p6
p56 (8wk)
p63 (9wk)
p70 (10wk)
p77 (11wk)
p84 (12wk)
p91 (13wk)
p98 (14wk)
p105 (15wk)
p112 (16wk)
PMP22-TATA, mRosa26 RNP intraneural injection
Rotarod
Rotarod
Rotarod
Rotarod, Electrophysiology Nerve biopsy
Rotarod, Electrophysiology Nerve biopsy
Rotarod
Rotarod
Rotarod
Rotarod, Muscle weight, qRT-PCR

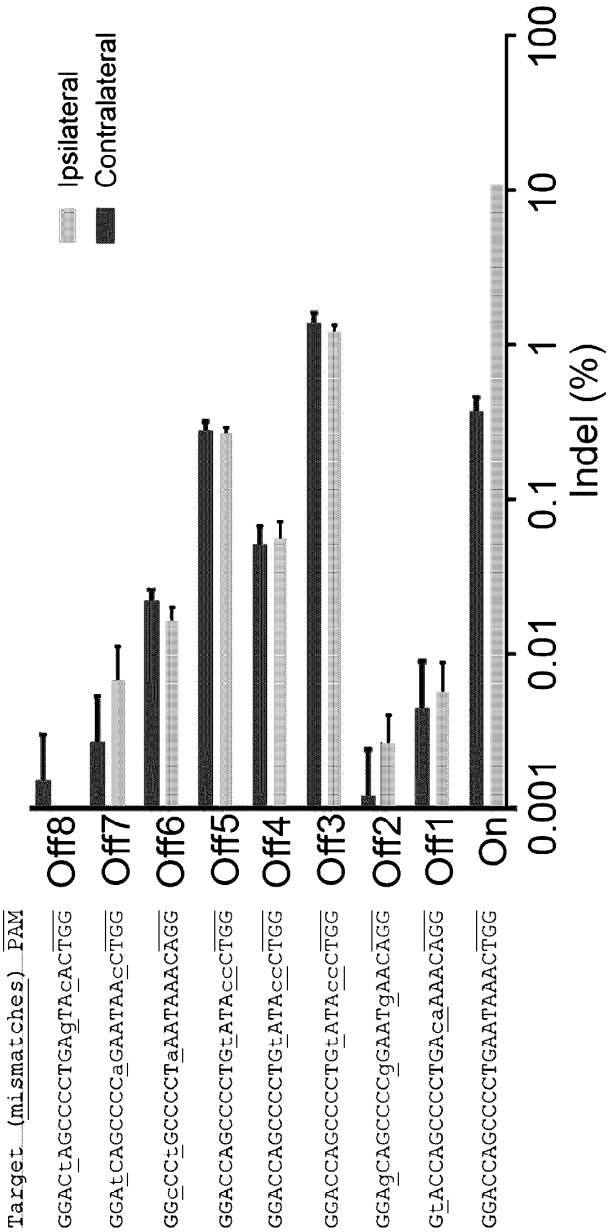
[도12]


PMP22-TATA RNP
mRosa26 RNP
Indel (%)
12
10
8
6
4
2
0
ipsilateral
contralateral
ipsilateral
contralateral
(a)
TATA-box mutation
98.48 ± 0.15
(b)
PMP22-TATA RNP
mRosa26 RNP
Rel. mRNA (A.U)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
(c)

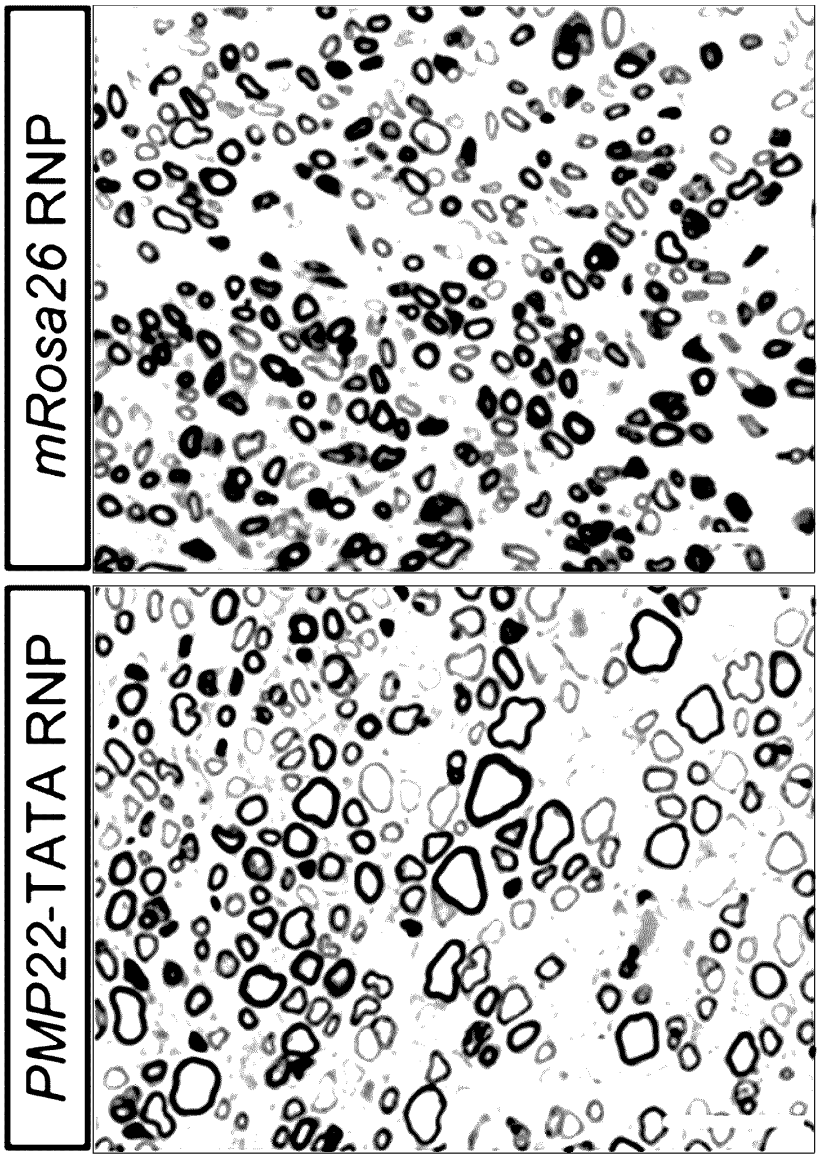
[도13a]

	Location	Position	Target sequence (5' to 3')	Gene
On-target	chr17	15265347	GGACCAGCCCCTGAATAAAC*TGG*	hPMP22-TATA
Off1	chr12	118558427	GtACCAGCCCCTGAcaAAAC*AGG*	Intergenic
Off2	chr1	74579514	GGAgCAGCCCCgGAATgAAC*AGG*	Zfp142 (Exon)
Off3	chr13	50187695	GGACCAGCCCCTGtATAccC*TGG*	Intergenic
Off4	chr13	50319559	GGACCAGCCCCTGtATAccC*TGG*	Intergenic
Off5	chr13	50623450	GGACCAGCCCCTGtATAccC*TGG*	Intergenic
Off6	chr2	29191358	GGcCCtGCCCCTaAATAAAC*AGG*	Intergenic
Off7	chr9	102823783	GGAtCAGCCCCaGAATAAcC*TGG*	Intergenic
Off8	chrX	101405421	GGACtAGCCCCTGAgTAcAC*TGG*	Zmym3 (Exon)

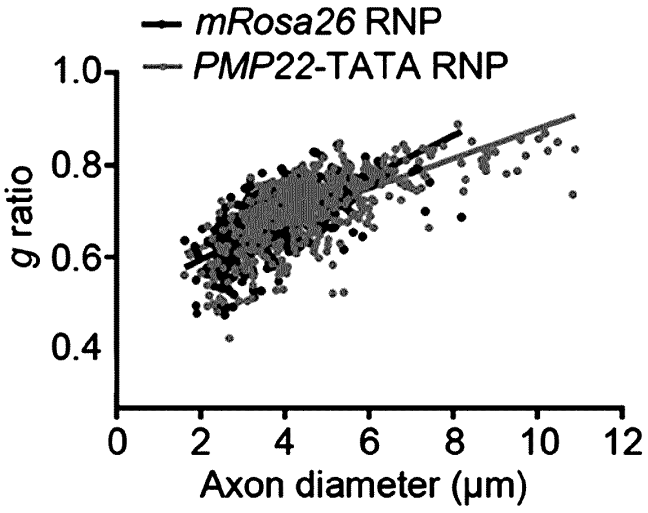
[도13b]


Ipsilateral
Contralateral
Indel (%)
0.001
0.01
0.1
1
10
100
Target (mismatches) PAM
Off8
Off7
Off6
Off5
Off4
Off3
Off2
Off1
On
GGACtAGCCCCTGAgTAcACTGG
GGAtCAGCCCCaGAATAAcCTGG
GGcCCtGCCCCTaAATAAACAGG
GGACCAGCCCCTGtATAccCTGG
GGACCAGCCCCTGtATAccCTGG
GGACCAGCCCCTGtATAccCTGG
GGAgCAGCCCCgGAATgAACAGG
GtACCAGCCCCTGAcaAAACAGG
GGACCAGCCCCTGAATAAACTGG

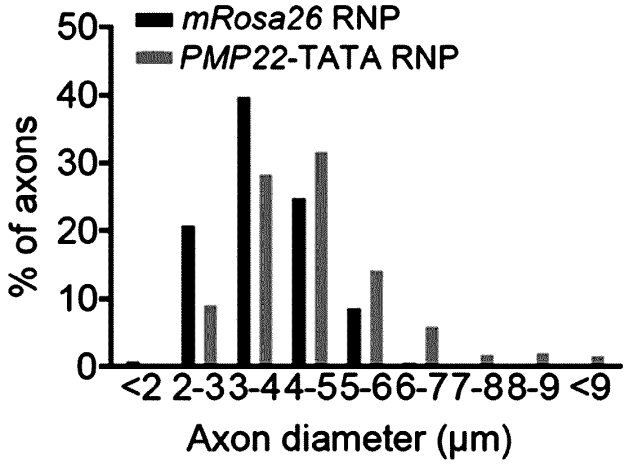
[도14a]



[도14b]

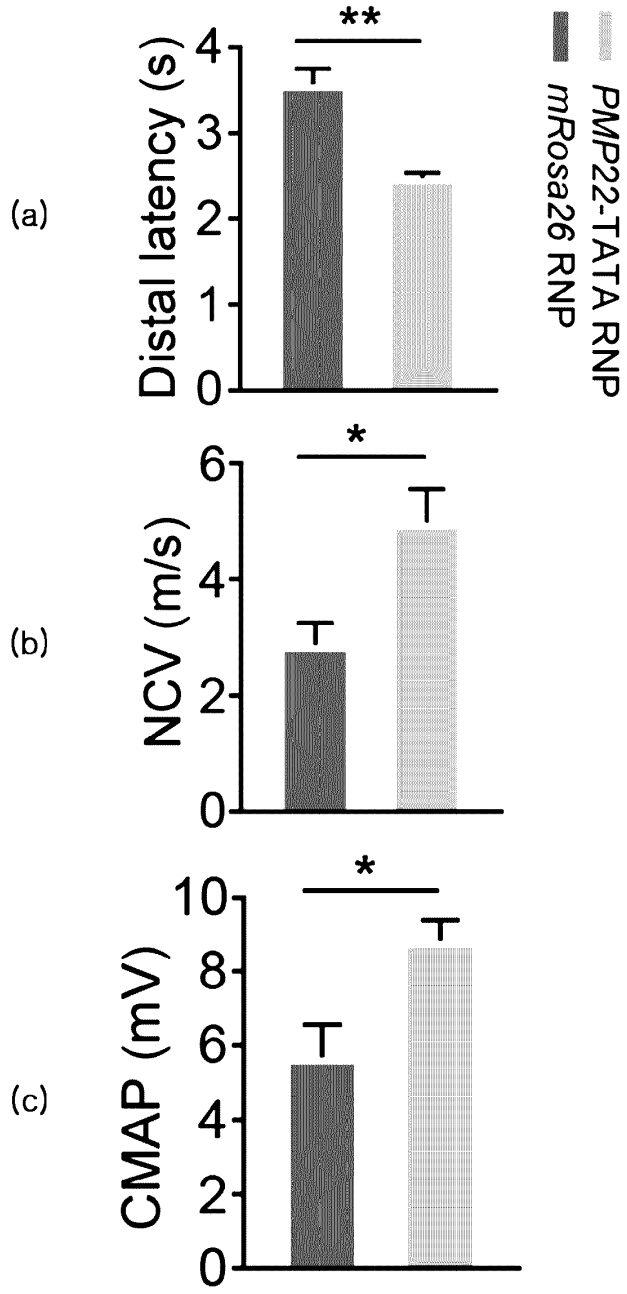

mRosa26 RNP
PMP22-TATA RNP
g ratio
1.0
0.8
0.6
0.4
0
2
4
6
8
10
12
Axon diameter (μm)

(a)

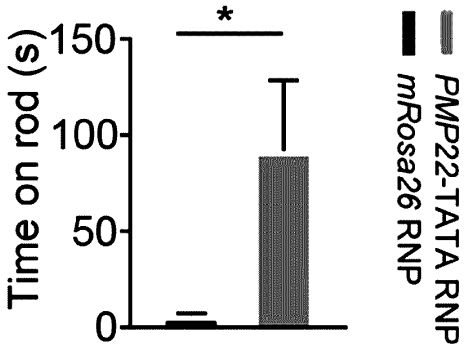

mRosa26 RNP
PMP22-TATA RNP
% of axons
50
40
30
20
10
0
<2 2-3 3-4 4-5 5-6 6-7 7-8 8-9 <9
Axon diameter (μm)

(b)

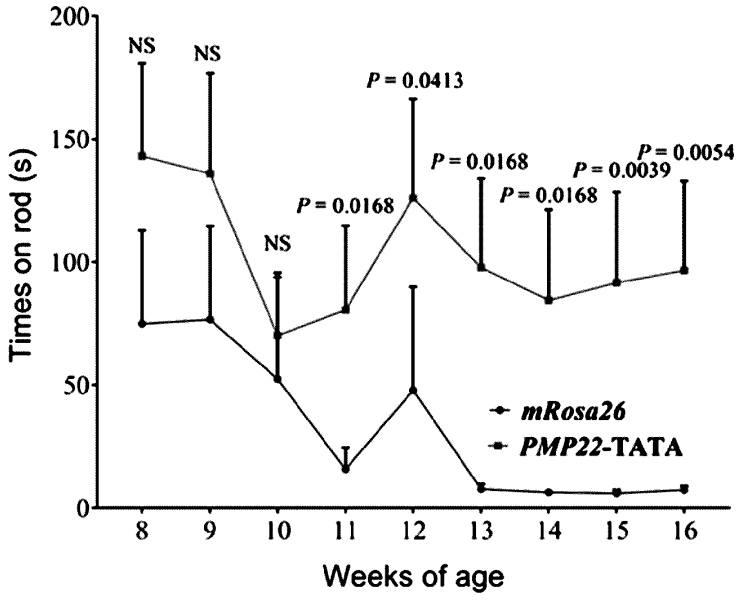
[도15]


(a)
Distal latency (s)
**
0
1
2
3
4
(b)
NCV (m/s)
*
0
2
4
6
(c)
CMAP (mV)
*
0
2
4
6
8
10
PMP22-TATA RNP
mRosa26 RNP

[도16a]

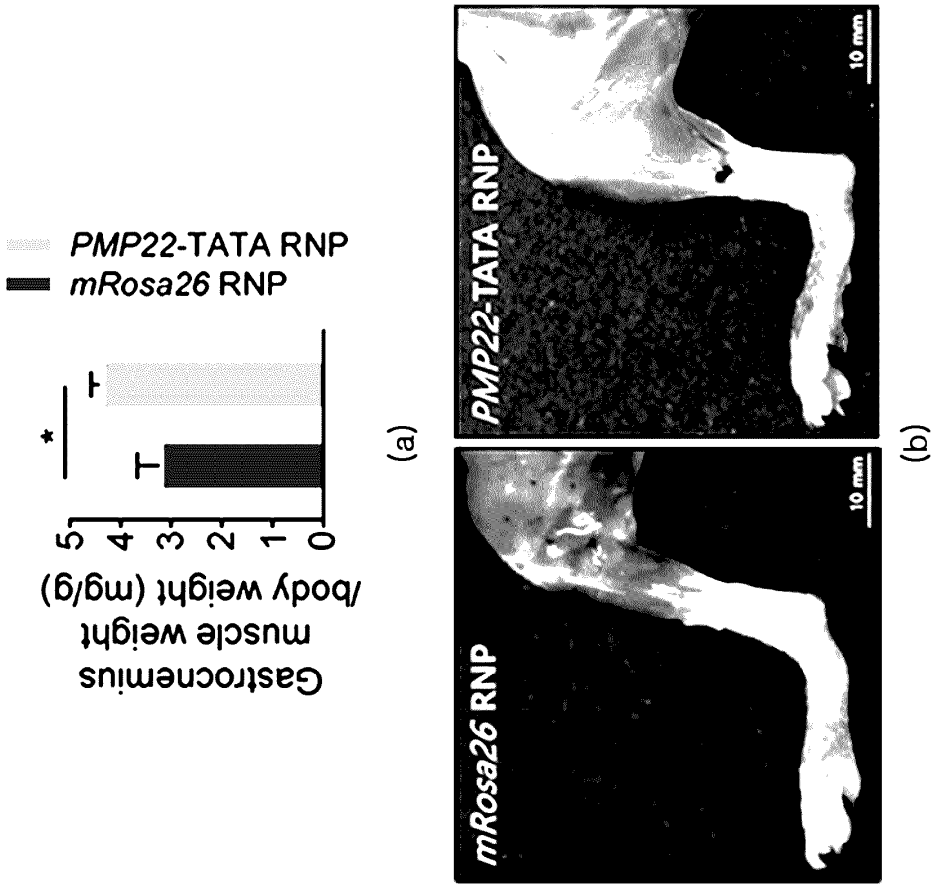

*
Time on rod (s)
150
100
50
0
mRosa26 RNP
PMP22-TATA RNP

(a)


Times on rod (s)
200
150
100
50
0
NS
NS
NS
P = 0.0413
P = 0.0168
P = 0.0168
P = 0.0168
P = 0.0039
P = 0.0054
mRosa26
PMP22-TATA
8
9
10
11
12
13
14
15
16
Weeks of age

(b)

[도16b]


PMP22-TATA RNP
mRosa26 RNP
Gastrocnemius muscle weight /body weight (mg/g)
0
1
2
3
4
5
*
(a)
PMP22-TATA RNP
mRosa26 RNP
10 mm
10 mm
(b)