【FIGURES】

【Fig. 1a】

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

【Fig. 1b】

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

【Fig. 1c】

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

【Fig. 2a】

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

【Fig. 2b】

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

【Fig. 2c】

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

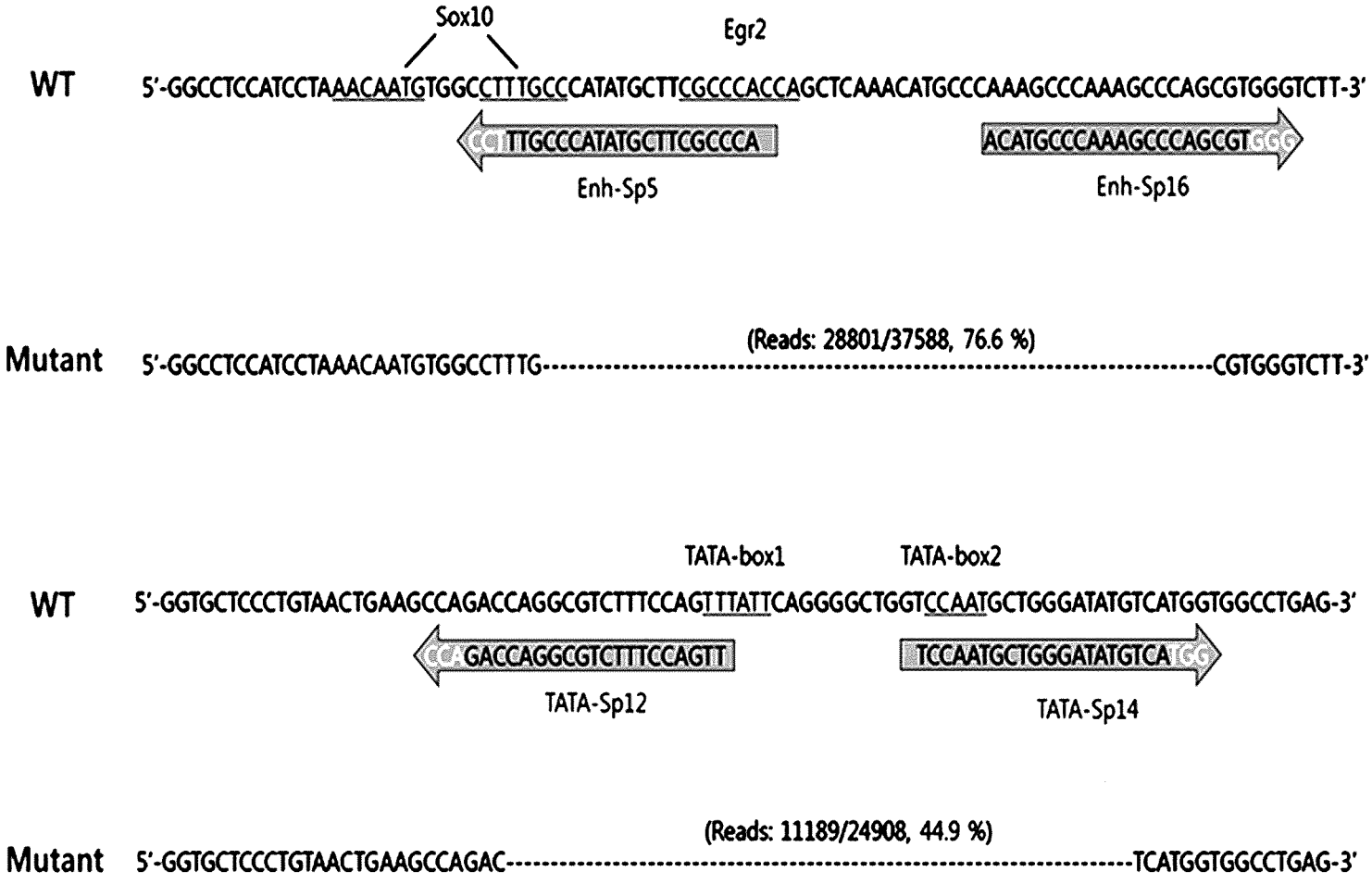
【Fig. 3】

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

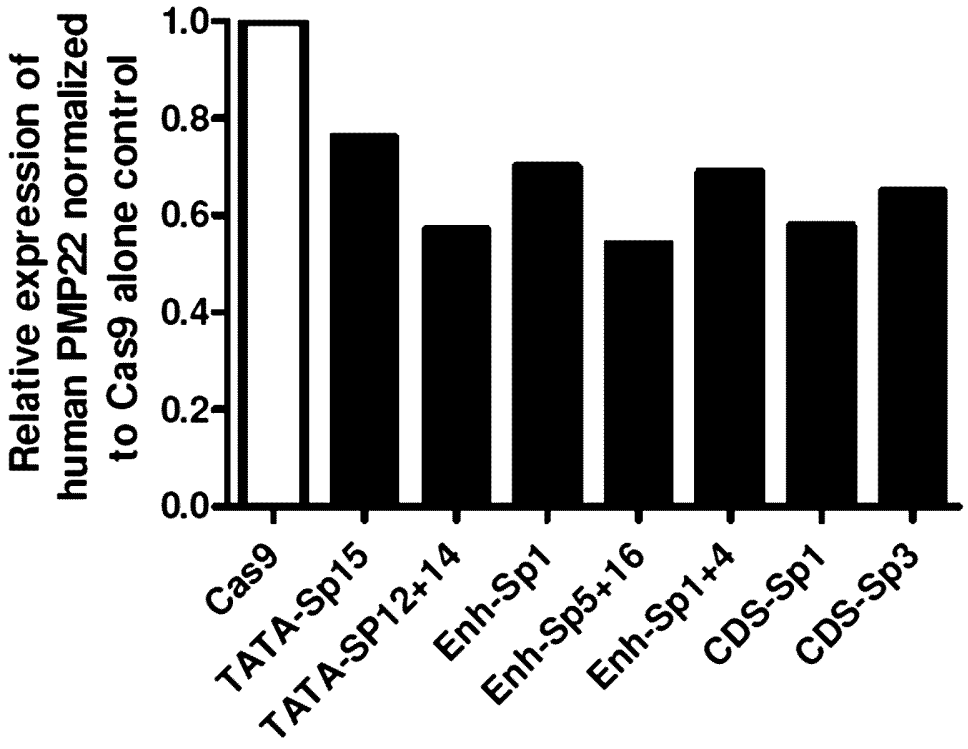
【Fig. 4】

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

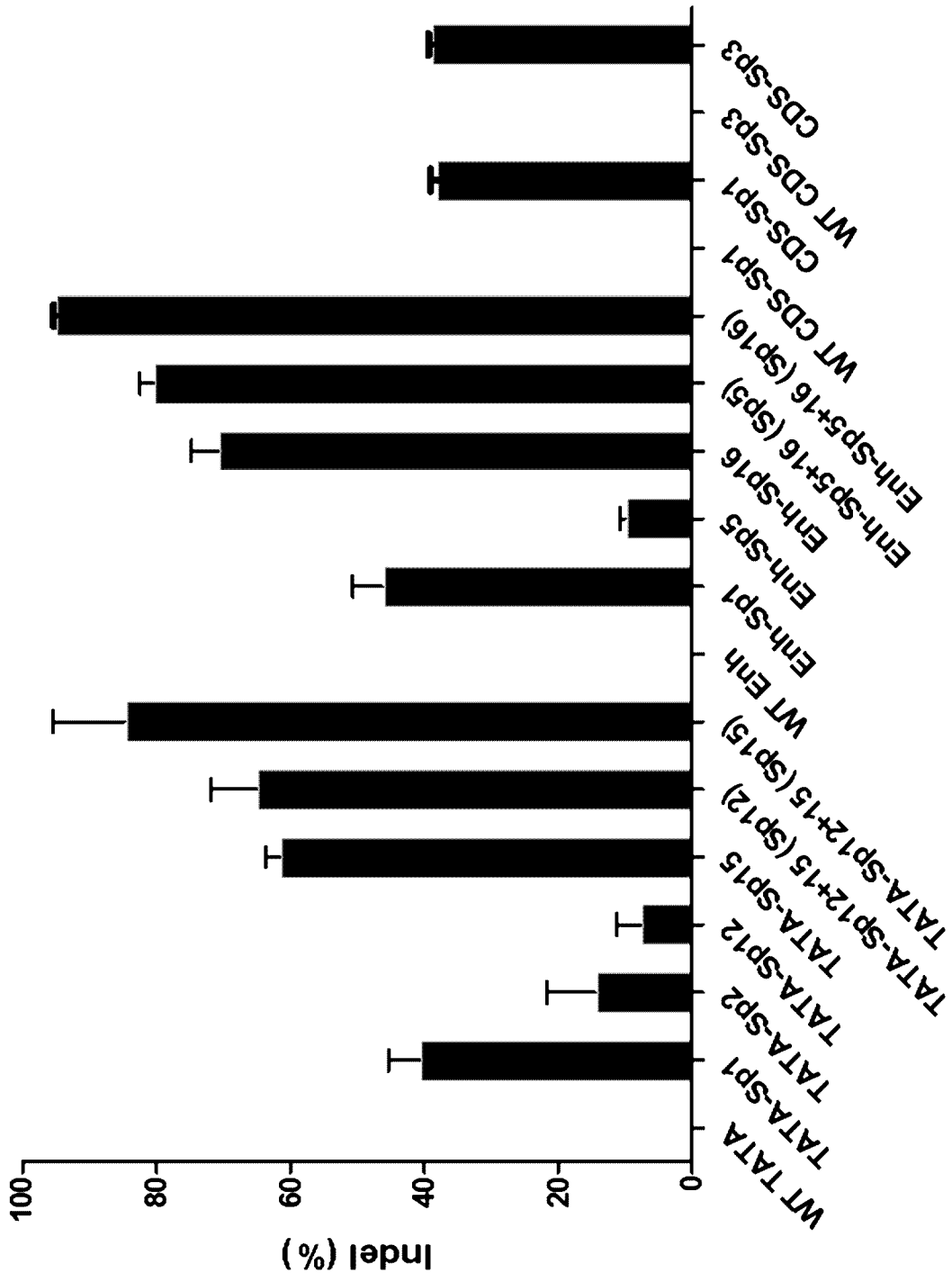
【Fig. 5】


Sox10
Egr2
WT
5'-GGCCTCCATCCTAAACAATGTGGCCTTTGCCCATATGCTTCGCCCACCAGCTCAAACATGCCCAAAGCCCAAAGCCCAGCGTGGGTCTT-3'
CCTTGCCCATATGCTTCGCCCA
ACATGCCCAAAGCCCAGCGTGGG
Enh-Sp5
Enh-Sp16
(Reads: 28801/37588, 76.6 %)
Mutant
5'-GGCCTCCATCCTAAACAATGTGGCCTTTG----------CGTGGGTCTT-3'
TATA-box1
TATA-box2
WT
5'-GGTGCTCCCTGTAACTGAAGCCAGACCAGGCGTCTTTCCAGTTTATTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGTGGCCTGAG-3'
CCGACCAGGCGTCTTTCCAGTT
TCCAATGCTGGGATATGTCAGG
TATA-Sp12
TATA-Sp14
(Reads: 11189/24908, 44.9 %)
Mutant
5'-GGTGCTCCCTGTAACTGAAGCCAGAC----------TCATGGTGGCCTGAG-3'

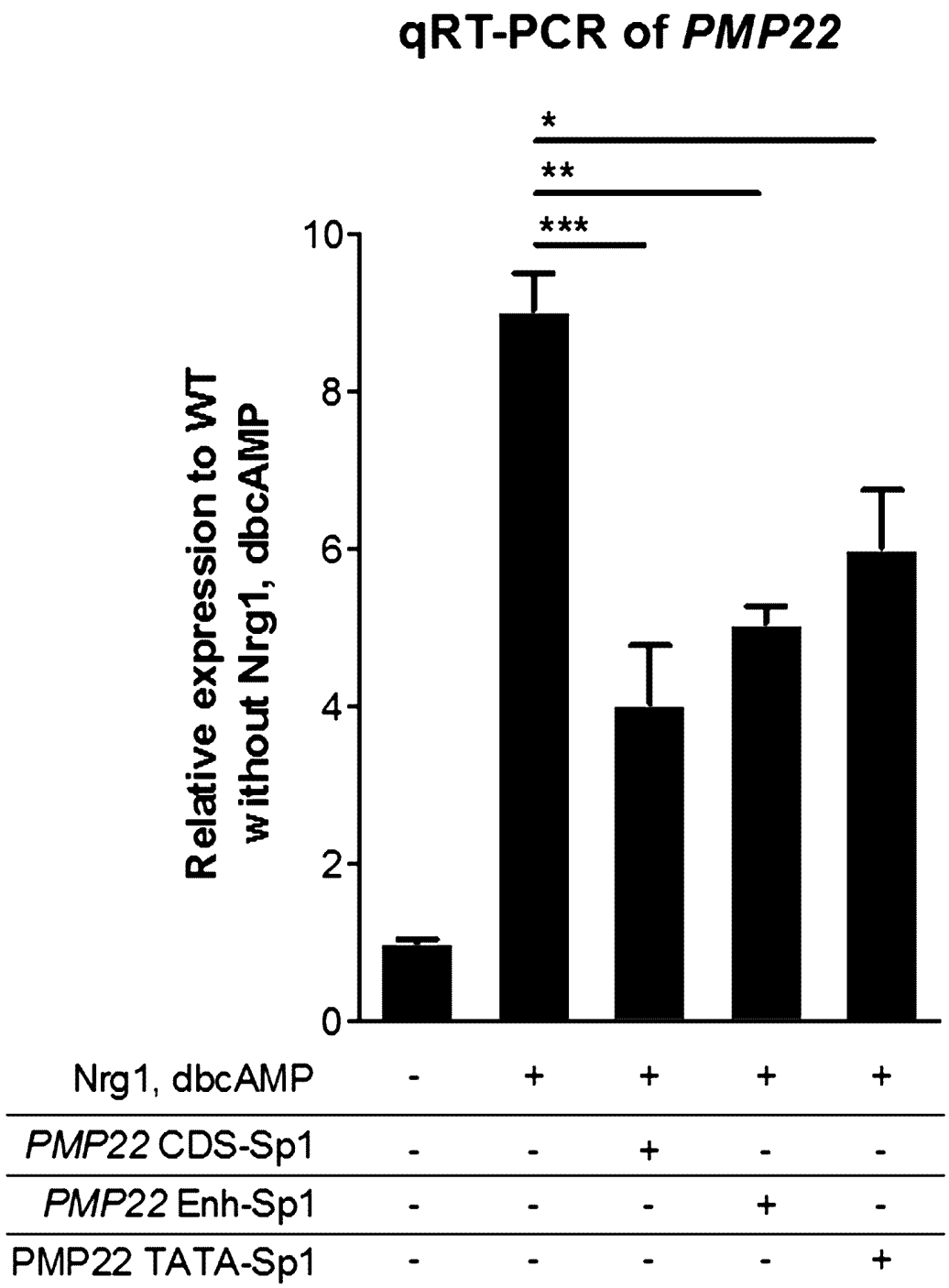
【Fig. 6】


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

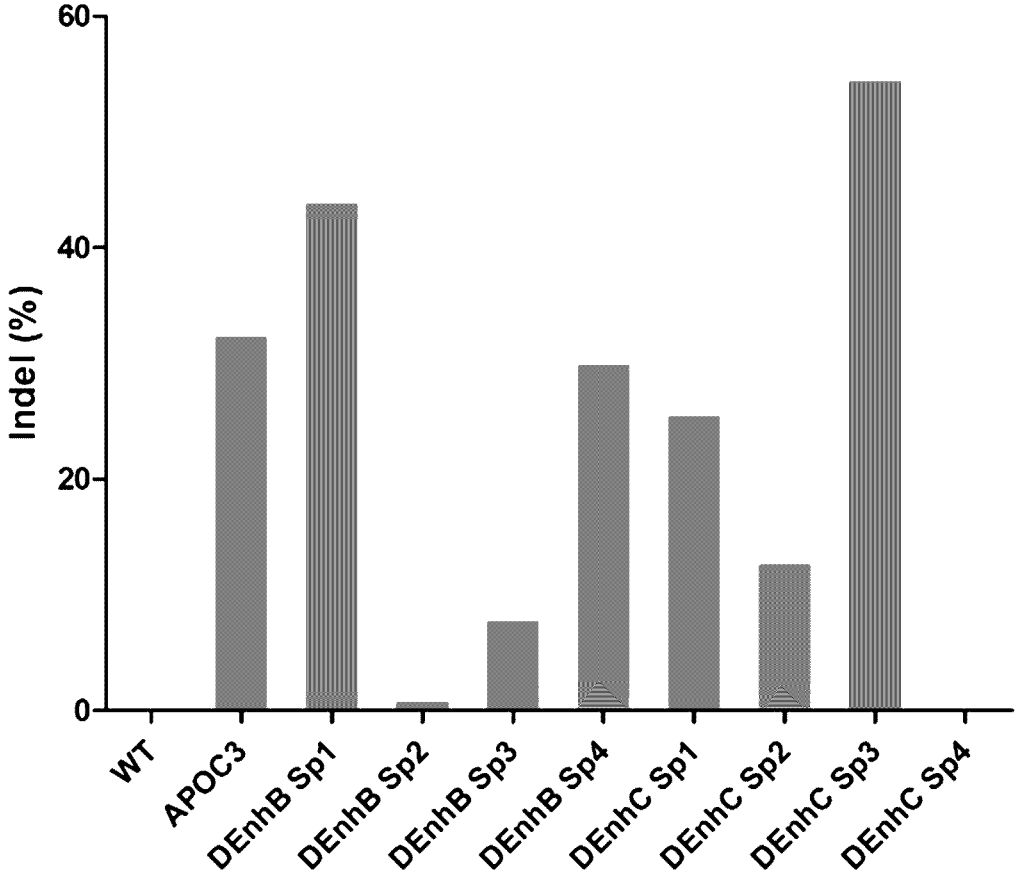
【Fig. 7a】


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

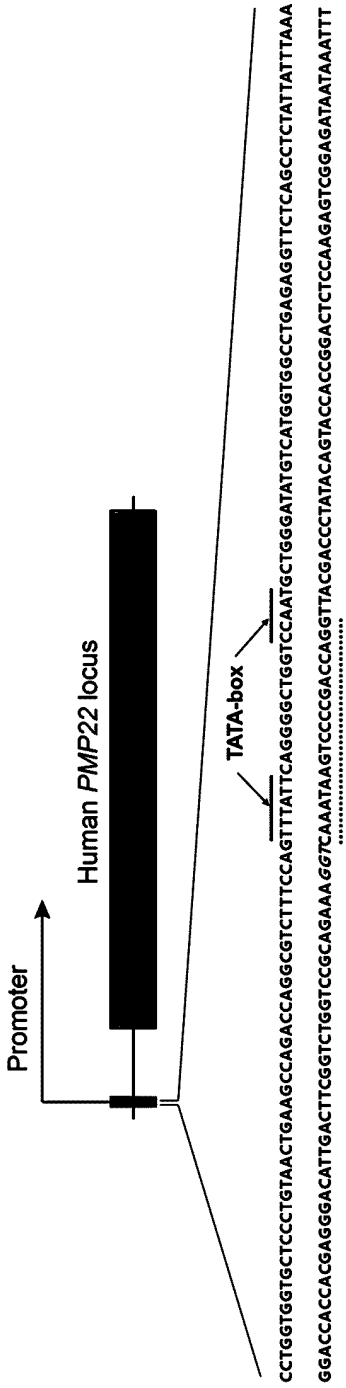
【Fig. 7b】


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 - - - - +

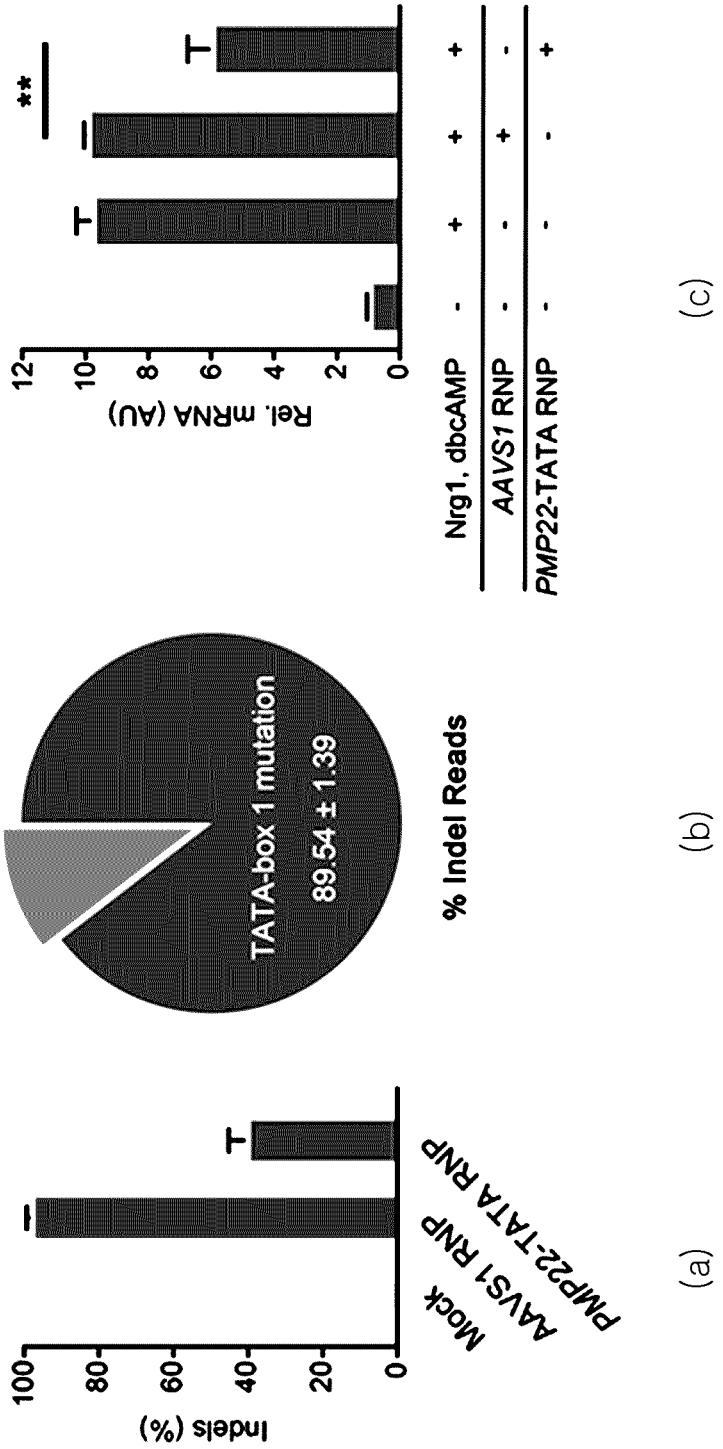
【Fig. 7c】


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

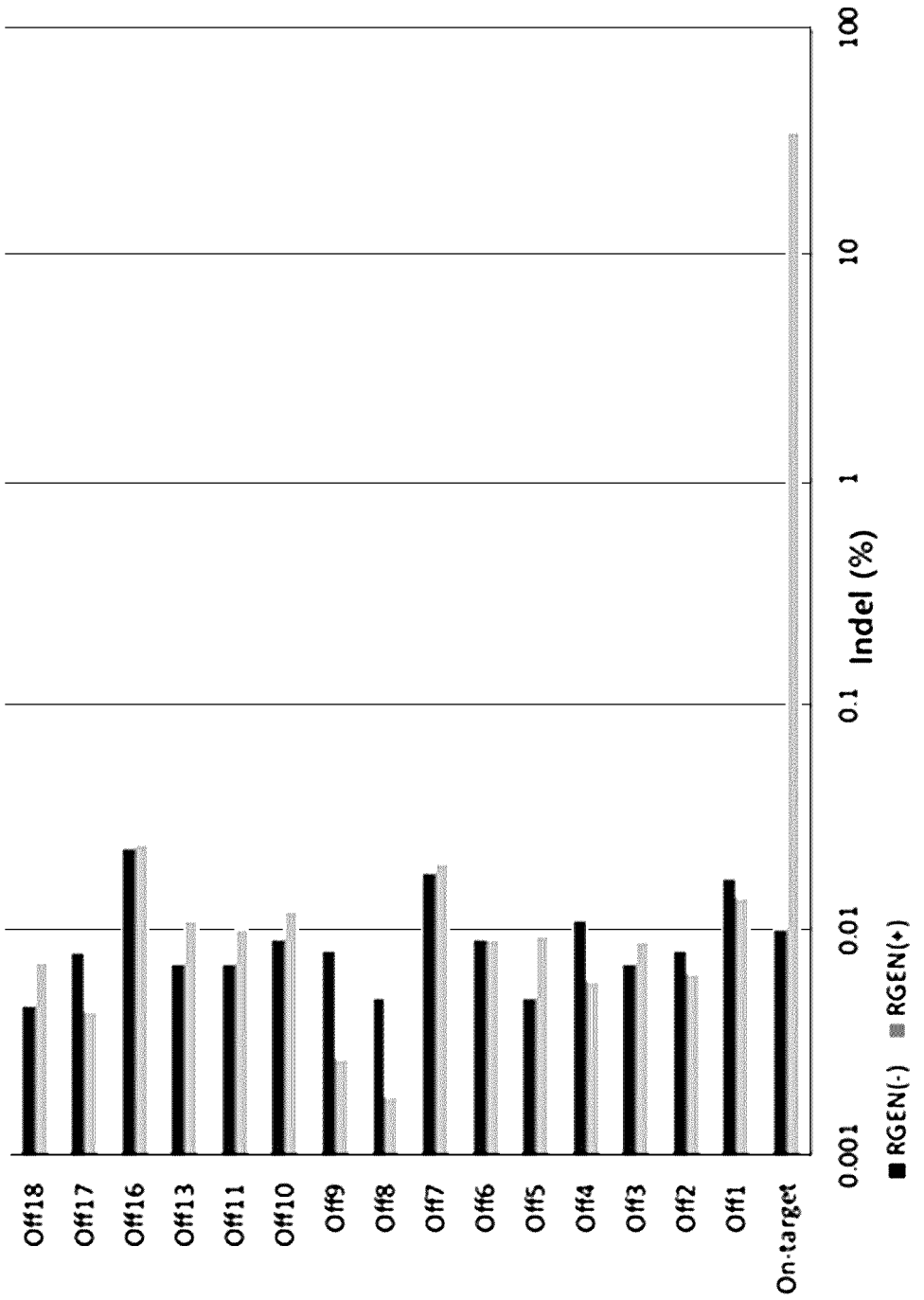
【Fig. 8a】


Promoter
Human PMP22 locus
TATA-box
sgRNA
CCTGGTGGTGCTCCCTGTAACTGAAGCCAGACCAGGCGTCTTTCCAGTTTATTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGTGGCCTGAGAGGTTCTCAGCCTCTATTATTTAAA
GGACCACCACGAGGGACATTGACTTCGGTCTGGTCCGCAGAAAGGTCAAATAAGTCCCGACCAGGTTACGACCCTATACAGTACCACCGGACTCTCCAAGAGTCGGAGATAATAAATTT

【Fig. 8b】


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)

【Fig. 9a】


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

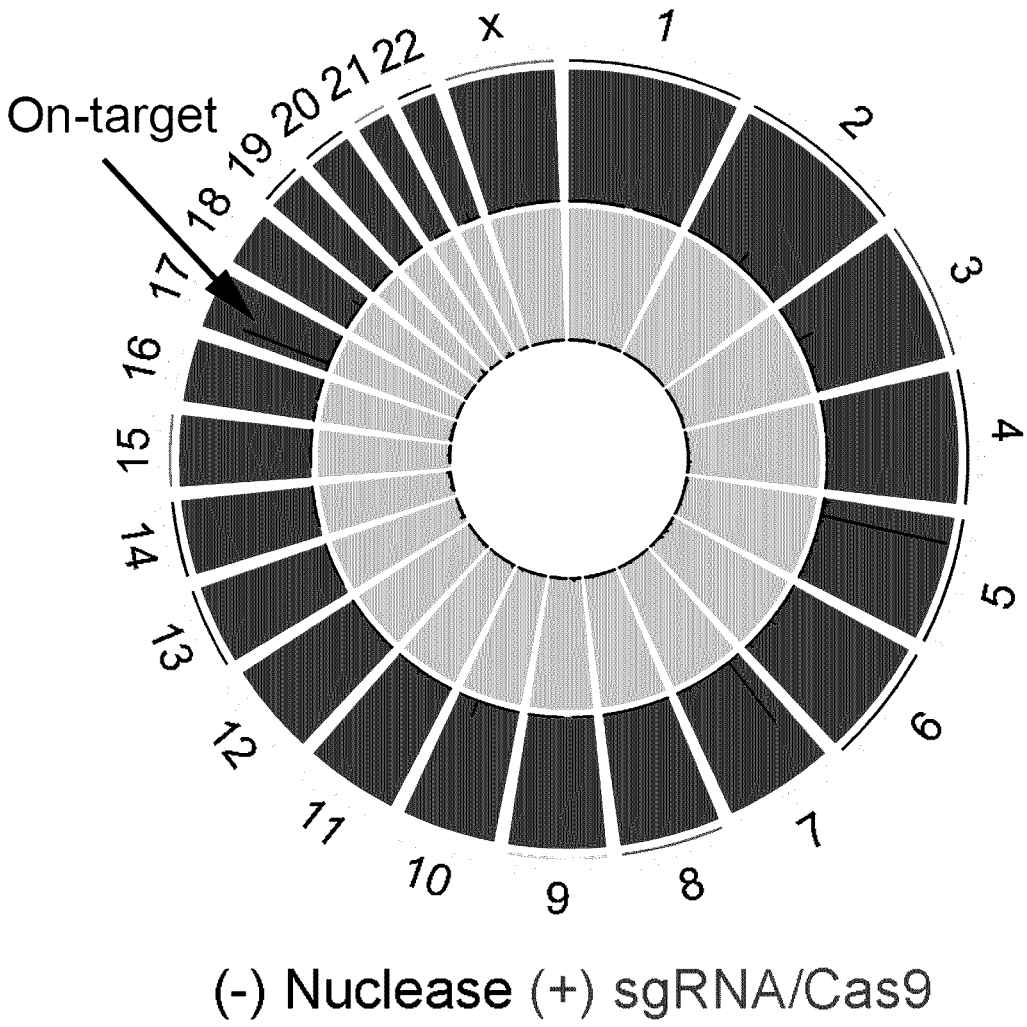
【Fig. 9b】

Indel	Local Sequence	Frequency (%)
WT	ACTGAAGCCAGACCAGGCGTCTTTCCAG**TTTATT**CAGGGGCTGG *TCC*AATGCTGGGATATGTCATGGTGGCCTGAGAGGTTCTCAGCCTC	
-1	ACTGAAGCCAGACCAGGCGTCTTTCCAG-TTATTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGCGGCCTGAGAGGTTCTCAGCCTC	14.00
-2	ACTGAAGCCAGACCAGGCGTCTTTCCAGTT--TTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGTGGCCTGAGAGGTTCTCAGCCTC	8.86
+1	ACTGAAGCCAGACCAGGCGTCTTTCCAGTTTTATTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGCGGCCTGAGAGGTTCTCAGCCTC	3.84
-3	ACTGAAGCCAGACCAGGCGTCTTTCCAGT---TTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGCGGCCTGAGAGGTTCTCAGCCTC	3.53
-4	ACTGAAGCCAGACCAGGCGTCTTTCCAG----TTCAGGGGCTGGTCCAATGCTGGGATATGTCATGGCGGCCTGAGAGGTTCTCAGCCTC	2.81

【Fig. 9c】

	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	GtACgAGCCCCCTGAATAAAt*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	GaACCAGCCCCCTGAtTAgAC*CAG*	Intergenic
Off14	chr18	77536261	GtACCAGCCaCTGAAaAAAC*AGG*	Intergenic
Off15	chr11	30065750	GtACCAGCCCCCTGcAaAAAC*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)

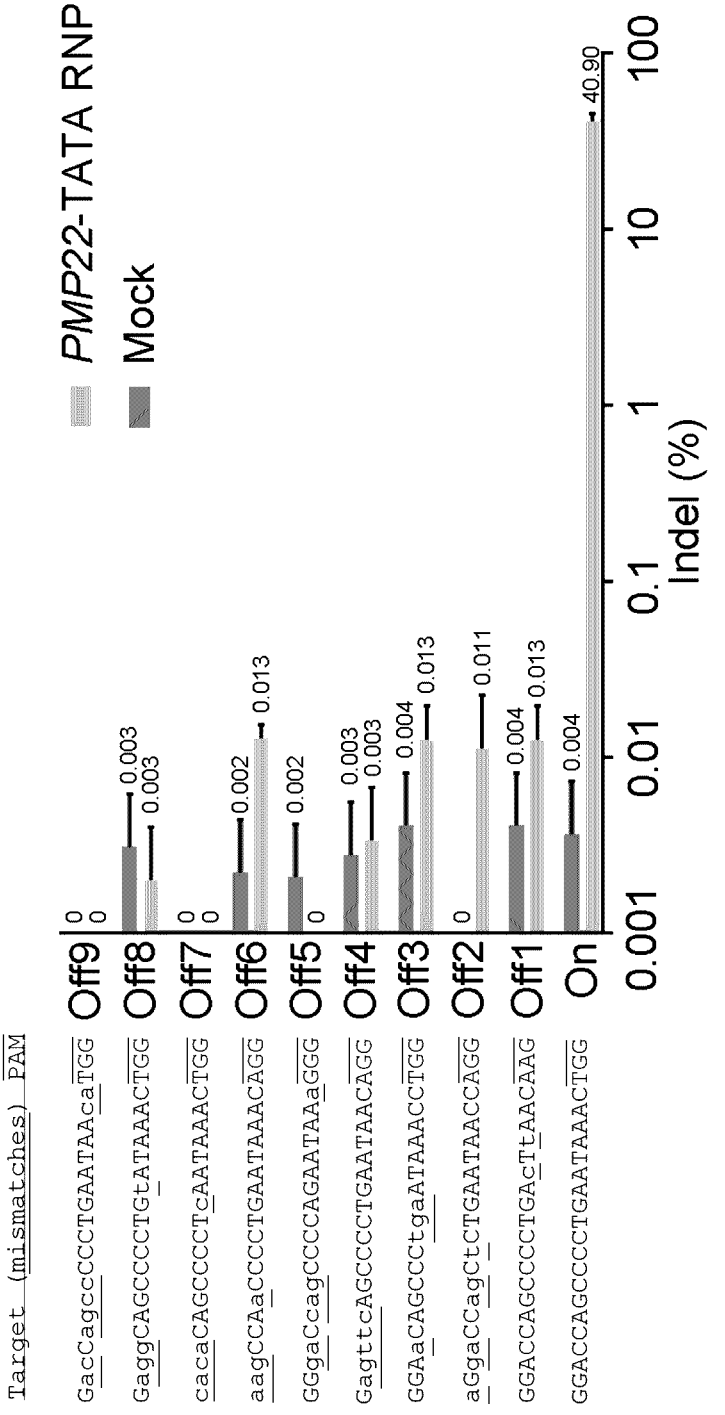
【Fig. 10a】


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

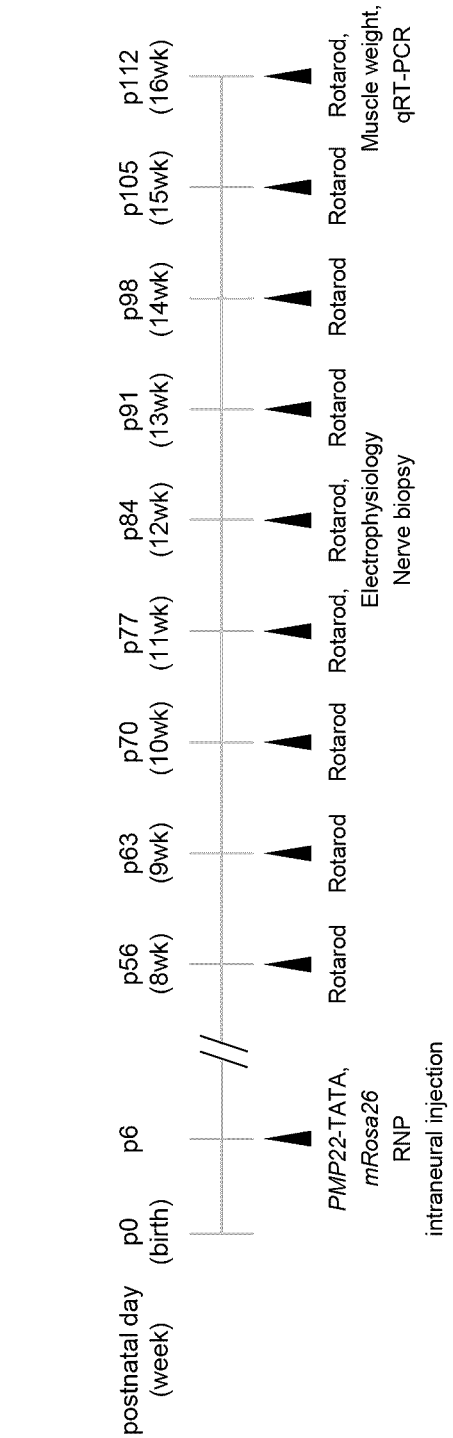
【Fig. 10b】

	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	cacaCAGCCCCTcAATAAAC*TGG*	ALPK2 (Intron)
Off8	chr22	27477459	GaggCAGCCCCTGtATAAAC*TGG*	Intergenic
Off9	chr6	91586787	GacCagccCCCTGAATAAca*TGG*	Intergenic

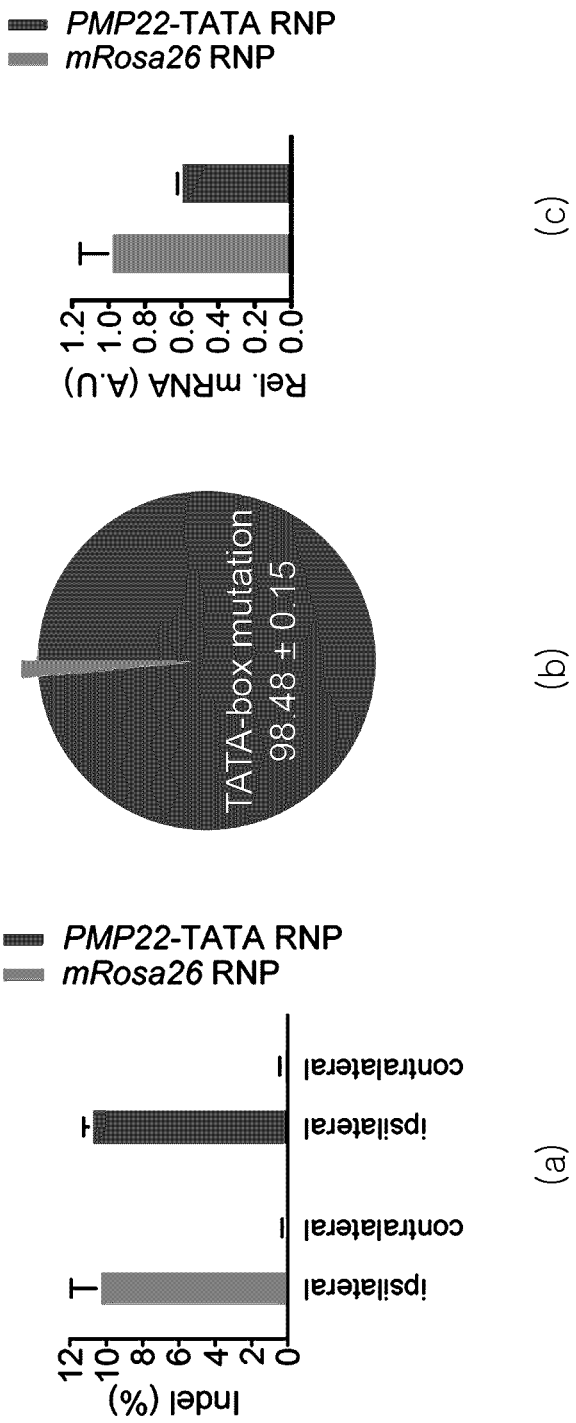
【Fig. 10c】


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

【Fig. 11】


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, Rotarod, Rotarod
Electrophysiology
Nerve biopsy
Rotarod
Rotarod
Rotarod, Muscle weight,
qRT-PCR

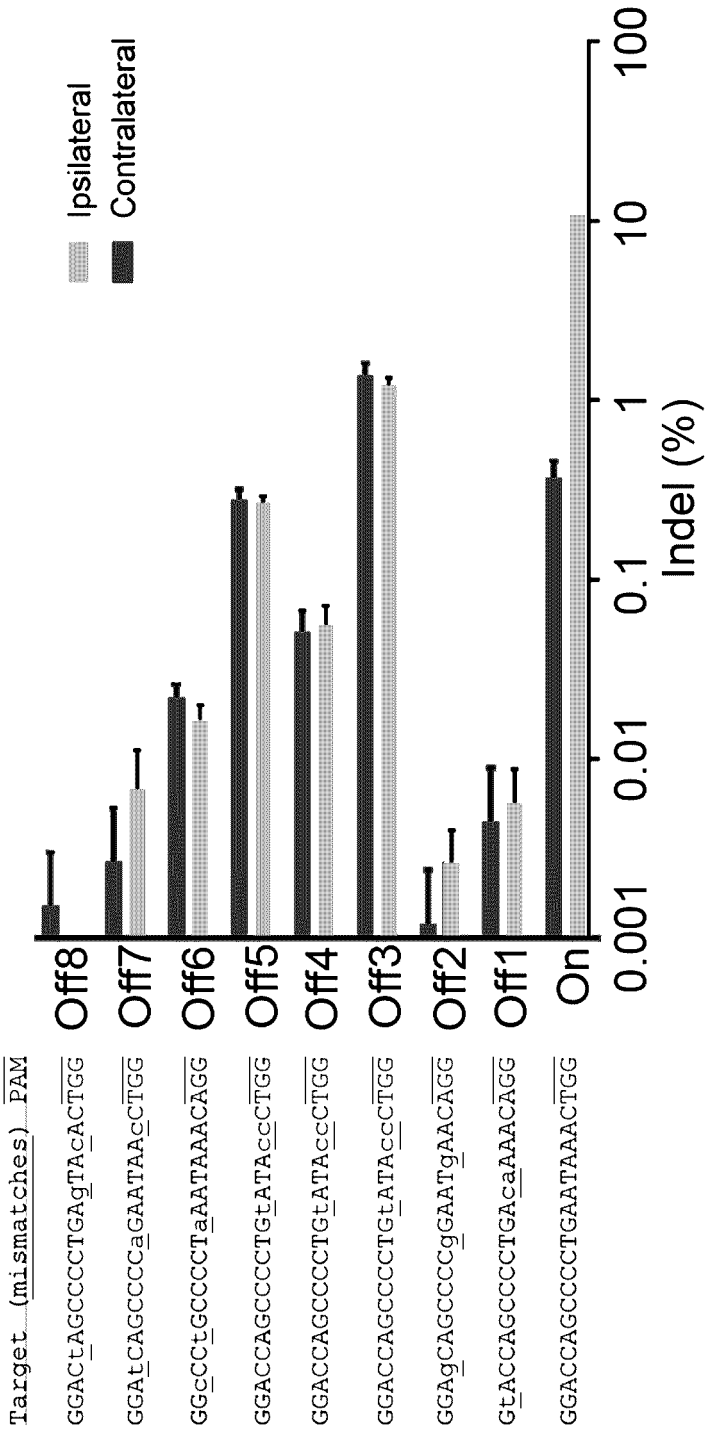
【Fig. 12】


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

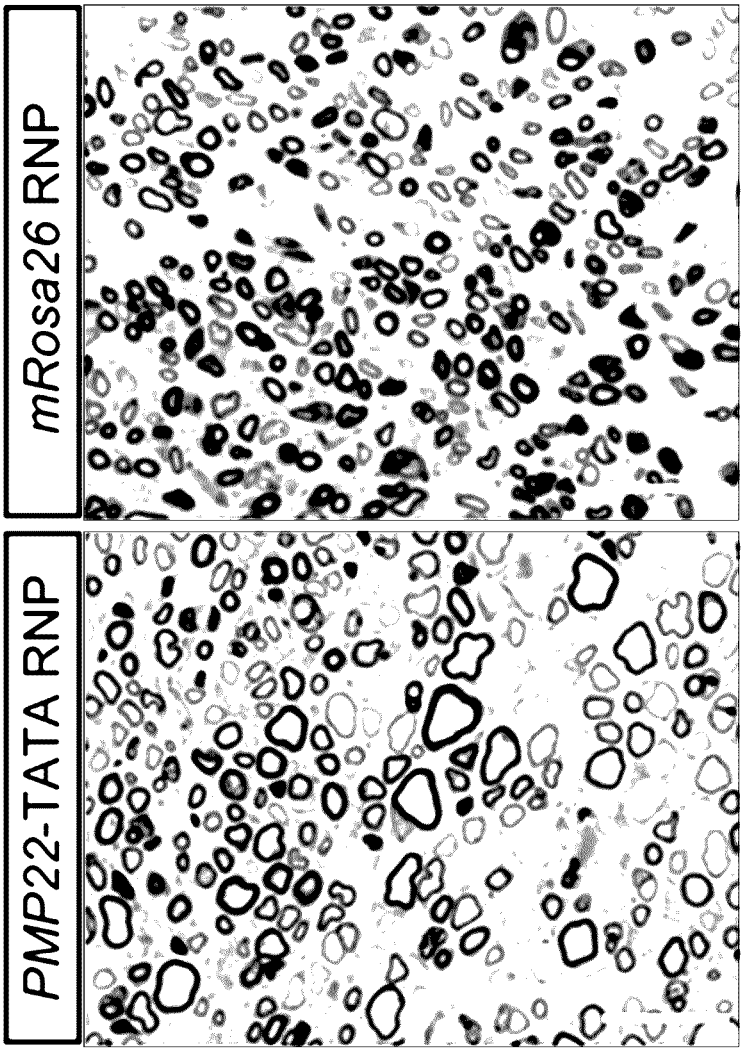
【Fig. 13a】

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

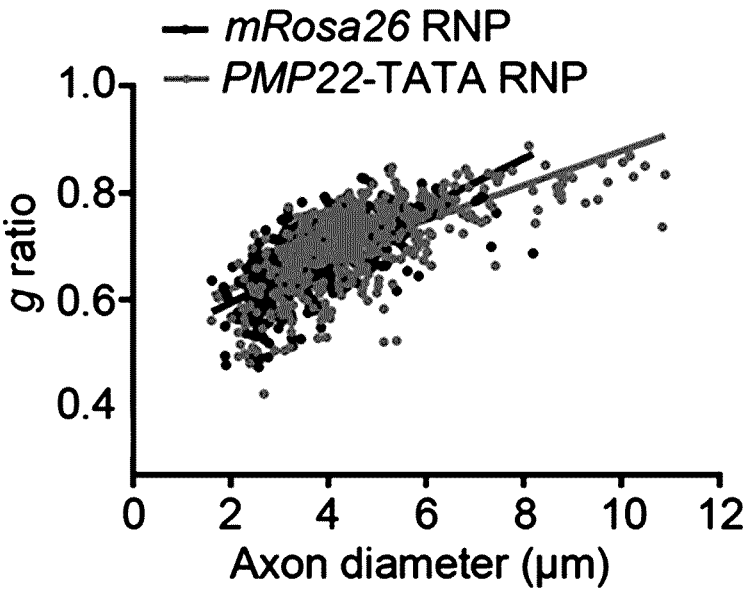
【Fig. 13b】


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

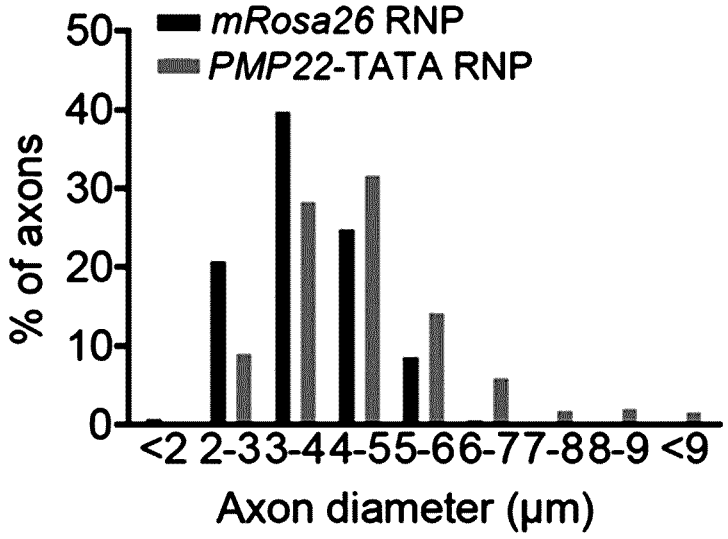
【Fig. 14a】



【Fig. 14b】

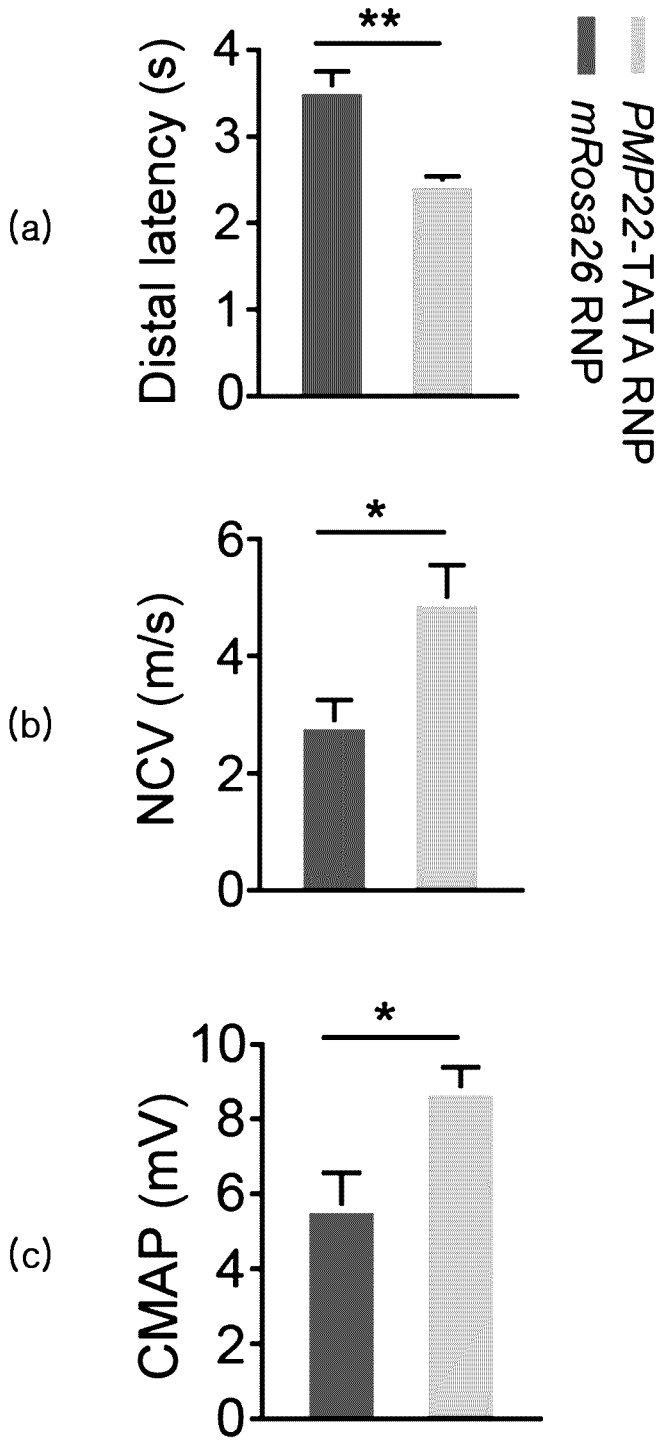

mRosa26 RNP
PMP22-TATA RNP
1.0
0.8
0.6
0.4
g ratio
0
2
4
6
8
10
12
Axon diameter (μm)

(a)

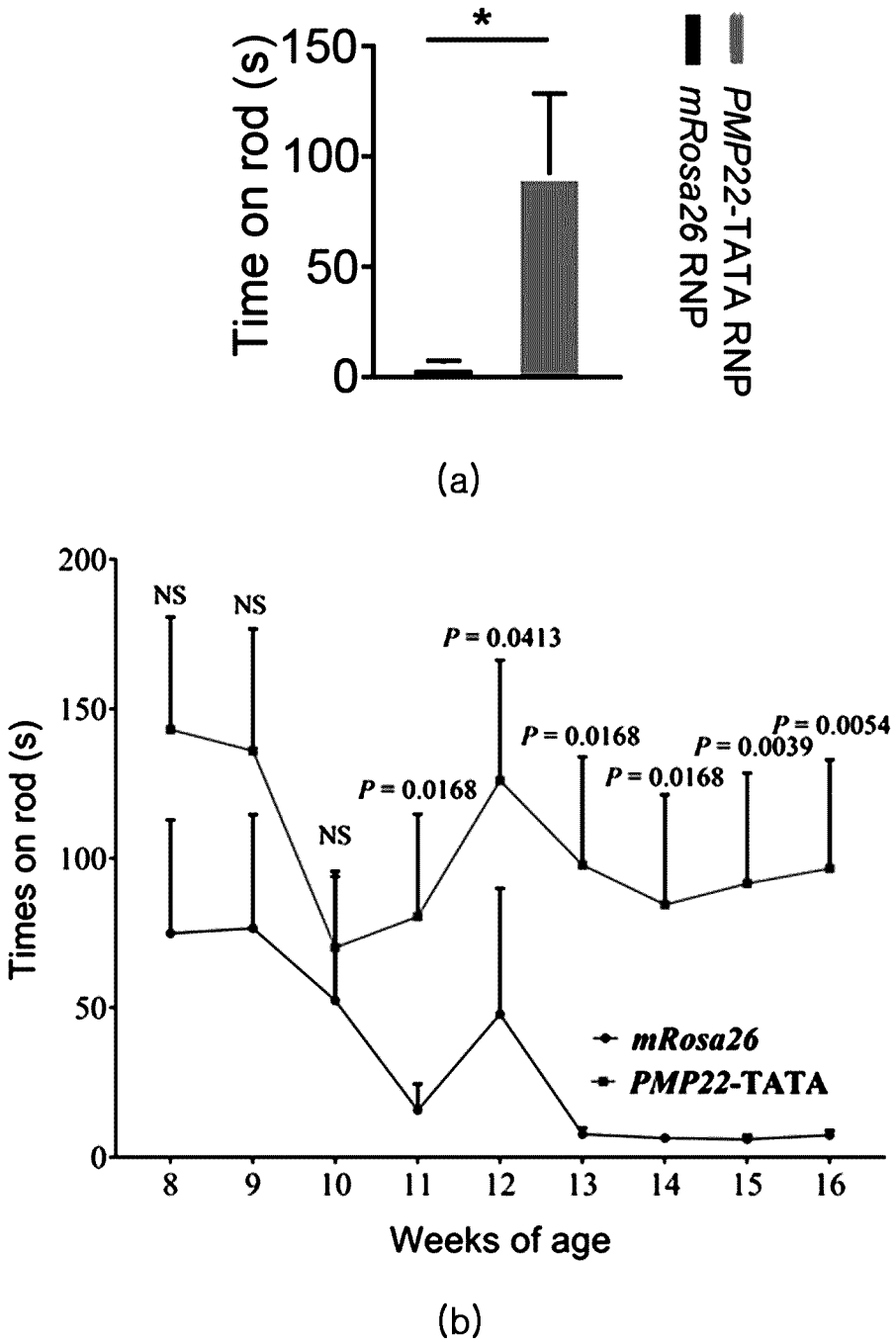

mRosa26 RNP
PMP22-TATA RNP
50
40
30
20
10
0
% of axons
<2 2-3 3-4 4-5 5-6 6-7 7-8 8-9 <9
Axon diameter (μm)

(b)

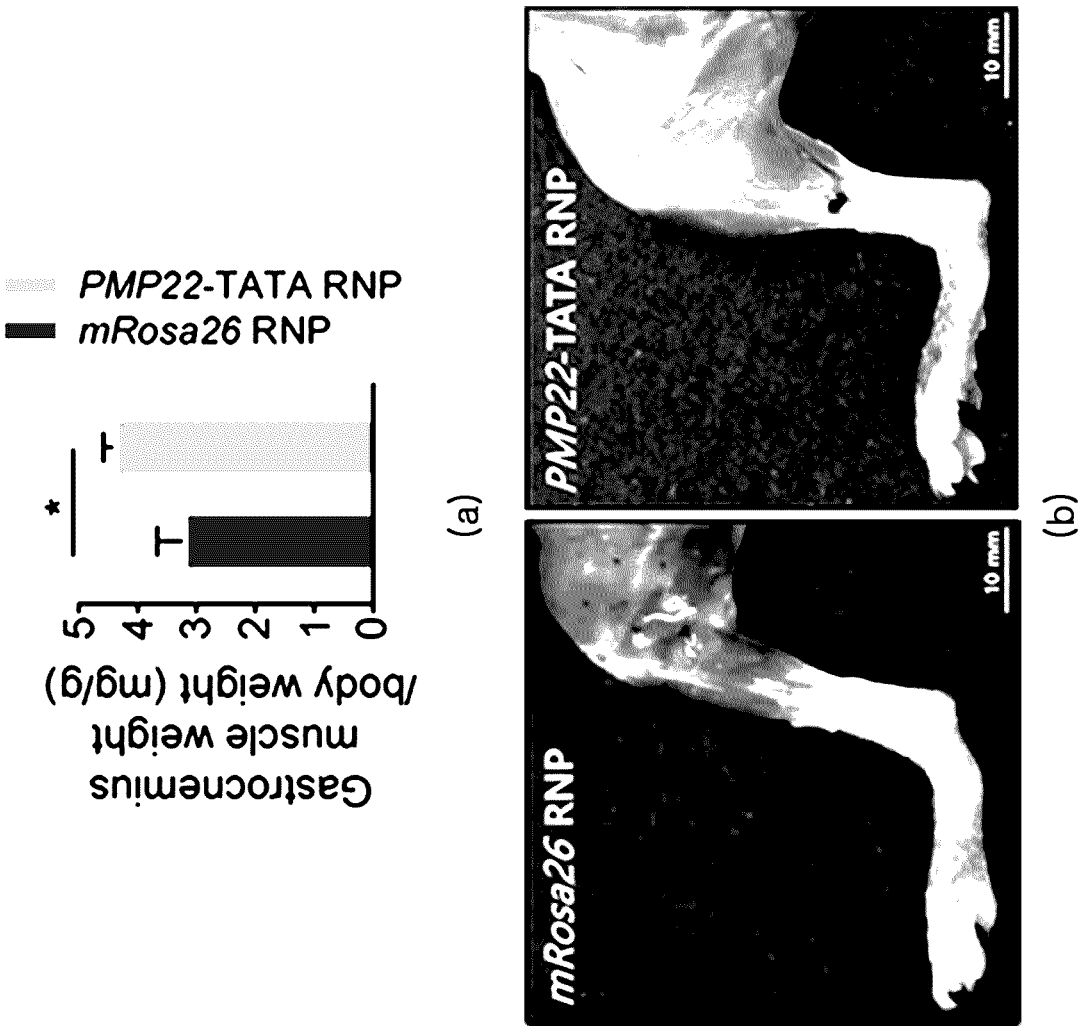
【Fig. 15】


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

【Fig. 16a】


*
Time on rod (s)
150
100
50
0
mRosa26 RNP
PMP22-TATA RNP
(a)
200
150
100
50
0
Times on rod (s)
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)

【Fig. 16b】


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