

SUPPORTING DATA

Distant Coupling between RNA Editing and Alternative Splicing of the Osmosensitive Cation Channel Tmem63b

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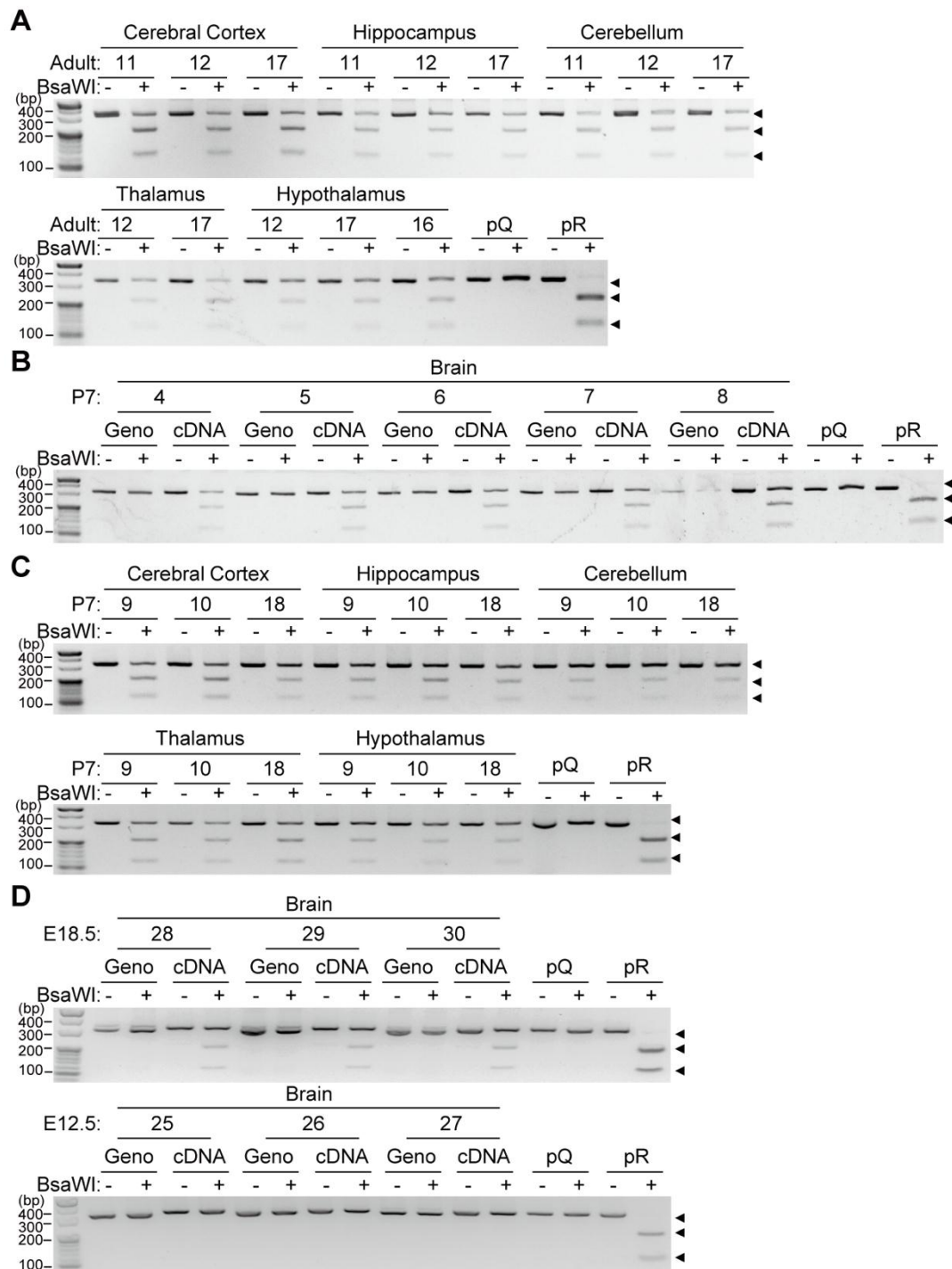


Figure S1. Q/R editing of *Tmem63b* in different brain regions and ages. A, Q/R editing of *Tmem63b* in different brain regions from three adult mice with codes labelled above. B, Q/R editing of *Tmem63b* in brains from P7 mice with codes labelled above. C, Q/R editing of *Tmem63b* in different brain regions from P7 mice with codes labelled above. D, Q/R editing of *Tmem63b* in brains of embryonic (E12.5, E18.5) mice with codes labelled above.

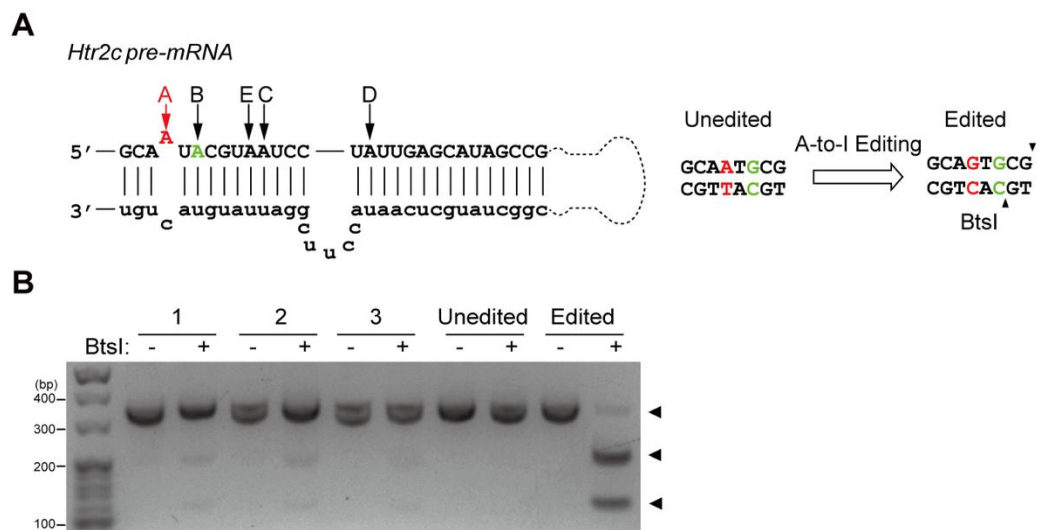


Figure S2. The A-to-I editing of 'A' site in *Htr2c* minigene co-transfected with *Adar1*. *A*, schematic representation of dsRNA hairpin structure formed by exon 5 and intron 5. The reported editing sites, 'A' to 'E', were marked in arrows. In order to evaluate the editing efficiency of 'A' site (red) individually, the adjacent 'B' site (green) was mutated to the edited G. In this mutant, BtsI endonuclease recognizing site (right panel) was introduced after A-to-I editing at 'A' site. *B*, the A-to-I editing of 'A' site in *Htr2c* minigene co-transfected with *Adar1* in HEK 293 cells. The fragment (340bp) amplified by specific primers were partially digested into two bands (122bp and 218bp) by BtsI. The experiments were repeated for three times.

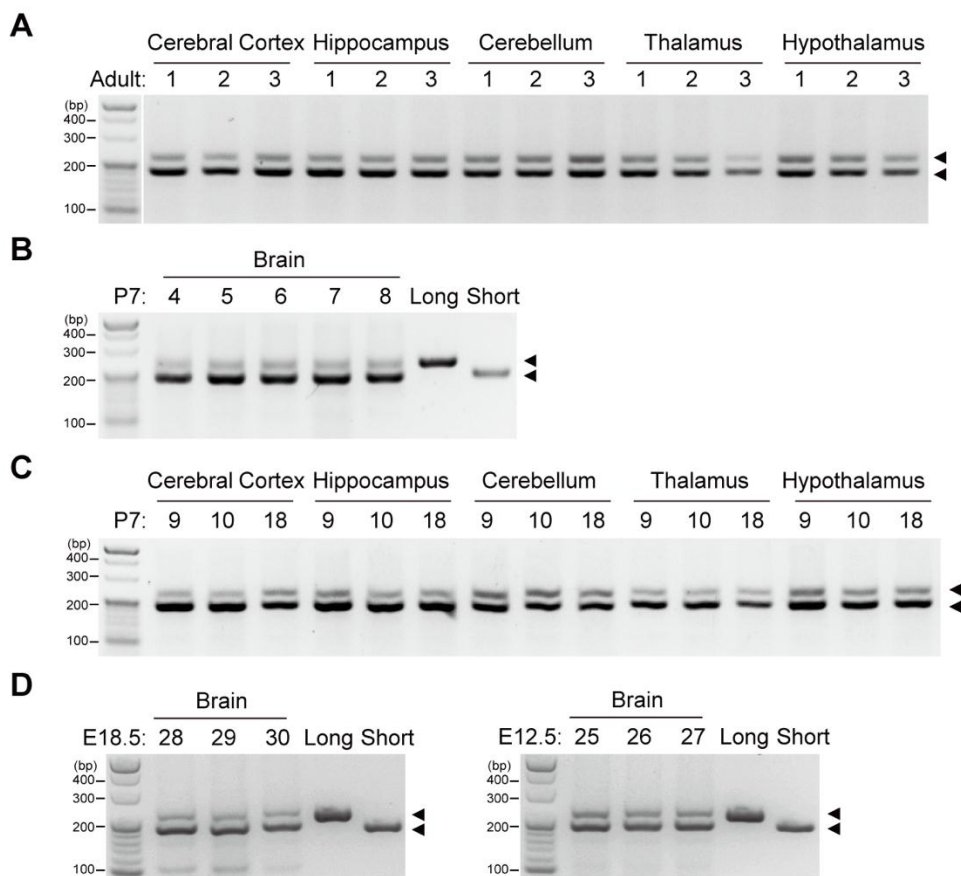


Figure S3. Alternative splicing of *Tmem63b* exon 4 in different brain regions and ages. *A*, alternative splicing of *Tmem63b* exon 4 in different brain regions from three adult mice with codes labelled above. *B*, alternative splicing of *Tmem63b* exon 4 in brains of P7 mice with codes labelled above. *C*, alternative splicing of *Tmem63b* exon 4 in different brain regions from three P7 mice with codes labelled above. *D*, alternative splicing of *Tmem63b* exon 4 in brains of embryonic (E12.5, E18.5) mice with codes labelled above.

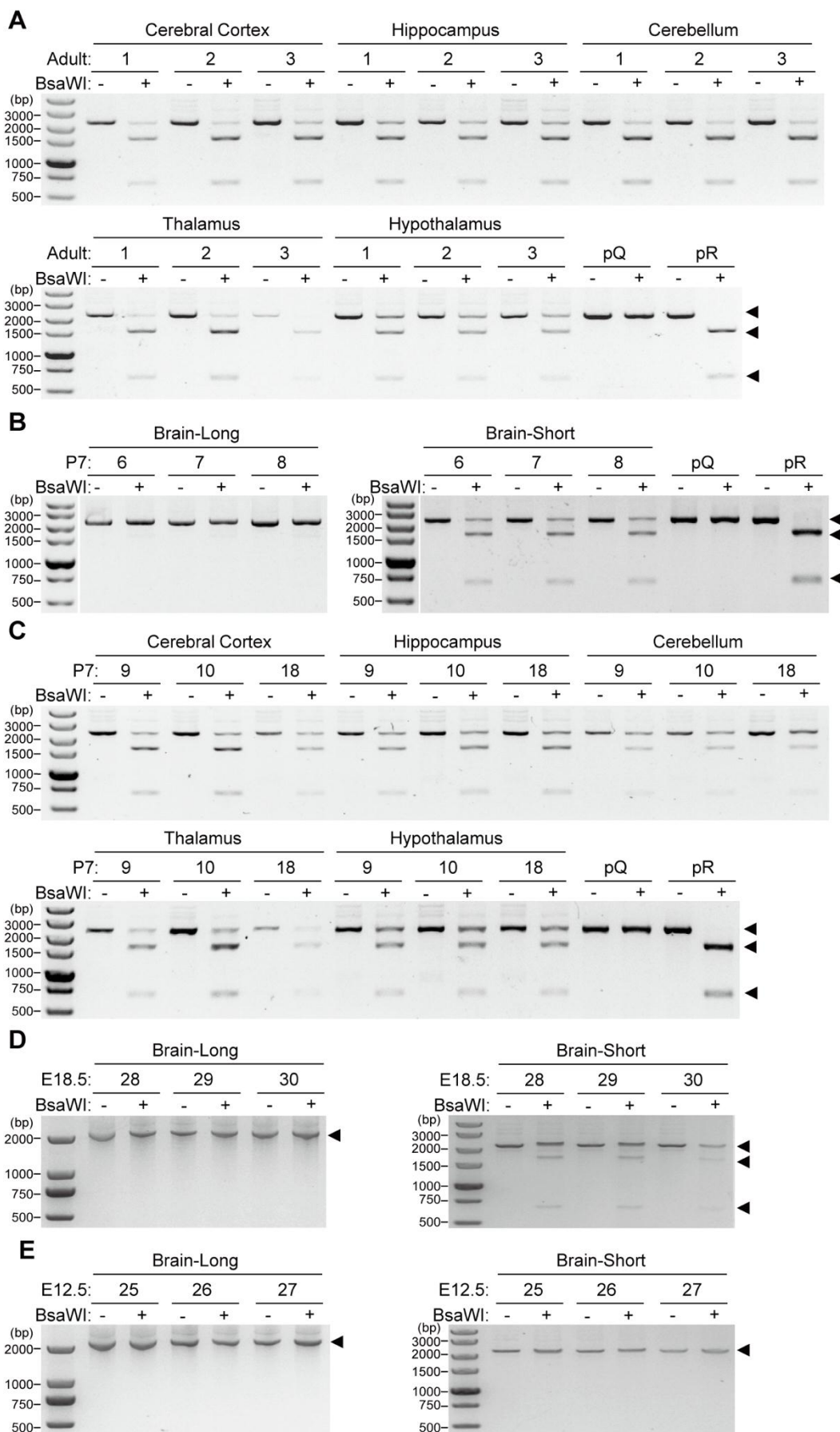


Figure S4. The coupling between exon 4 alternative splicing and Q/R editing of *Tmem63b* in different brain regions and ages. *A*, Q/R editing of short form *Tmem63b* in different brain regions from three adult mice with codes labelled above. *B*, Q/R editing of long form (left) and short form (right) *Tmem63b* in brains of p7 mice with codes labelled above. *C*, Q/R editing of short form *Tmem63b* in brain regions from three P7 mice with codes labelled above. *D and E*, Q/R editing of long form (left) and short form (right) *Tmem63b* in brains of embryonic (E12.5, E18.5) mice with codes labelled above.

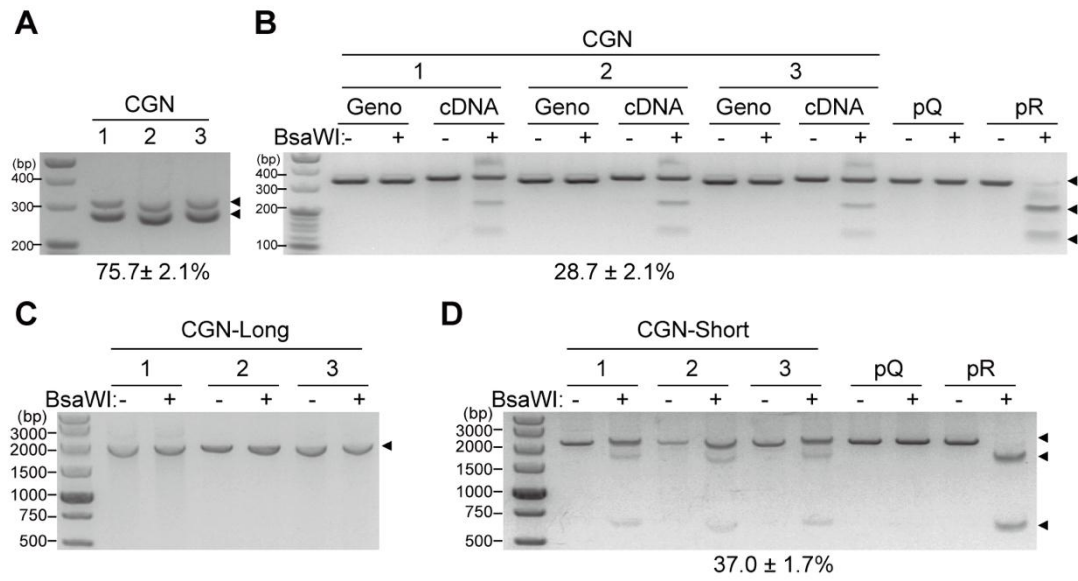


Figure S5. Alternative splicing (A) and Q/R editing (B) of endogenous *Tmem63b* in primary cultured CGNs. C and D, Q/R editing of long form and short form *Tmem63b* in CGNs. The experiments were repeated in three independent culturing of CGNs.

Table S1. Q/R Editing in different brain regions of P7 and adult mice (Fig 2D)

Editing (%)	Cx	Hi	Cb	Th	Hy
P7	53.0±1.4, n=3	37.3±2.3, n=3	19.7±0.6, n=3	52.0±1.0, n=3	30.0±1.7, n=3
Adult	64.8±2.2, n=5	53.6±3.0, n=5	58.0±1.2, n=5	65.0±2.9, n=4	36.5±4.4, n=6

Table S2. Alternative splicing in different brain regions of P7 and adult mice (Fig 5D)

Editing (%)	Cx	Hi	Cb	Th	Hy
P7	80.5±0.7, n=3	76.0±1.7, n=3	65.0±1.7, n=3	76.0±0.0, n=3	69.0±1.7, n=3
Adult	75.3±3.1, n=6	73.7±3.4, n=6	68.3±3.9, n=6	76.0±6.5, n=4	67.4±3.1, n=5

Table S3. Q/R Editing of short form *Tmem63b* in different brain regions of P7 and adult mice (Fig 6F)

Editing (%)	Cx	Hi	Cb	Th	Hy
P7	74.5±0.7, n=3	60.7±1.2, n=3	42.5±2.1, n=3	78.0±0.0, n=3	52.3±1.5, n=3
Adult	84.6±2.8, n=5	74.0±2.0, n=5	85.7±1.5, n=6	85.3±2.5, n=3	61.3±3.3, n=4

TableS4. Primers for cloning and amplifications

Gene	Primer	Sequence (5'-3')
<i>Tmem63b</i>	Tmem63b-Fw	5'-ATTCGCGGCCGCTAGCATGCTGCCGTTCTTGCTG-3'
	Tmem63b-Re	5'-GAAGCTTGAGCTCGAGTTACTGGTGAATCTCATTC-3'
<i>Adar1</i>	Adar1-Fw	5'-ATTCGCGGCCGCTAGCATGTCTCAAGGGTTCAGG-3'
	Adar1-Re	5'-GAAGCTTGAGCTCGAGTCAGTCATTGGGTACTGGAC-3'
<i>Adar2</i>	Adar2-Fw	5'-ATTCGCGGCCGCTAGCATGGATATAGAAGATGAAGAG-3'
	Adar2-Re	5'-GAAGCTTGAGCTCGAGTCAGGGAGTGAAGGAGAAC-3'
Editing segment	Ex19-Fw	5'-ATTCGCGGCCGCTAGCGTGTGTGTTCCCTGCCCGAC-3'
	Ex21-Re	5'-GAAGCTTGAGCTCGAGCCGTGCGCATGGTGGAGAAG-3'
<i>Htr2c</i>	Ht(BG)-Ex5-Fw	5'-ATTCGCGGCCGCTAGCGGACCGGTATGTAGCAATGCGTAATC-3'
	Ht-In5-Re	5'-GAAGCTTGAGCTCGAGGACAACCGATCAAACGCAATG-3'
	Ex3-Fw	5'-ATTCGCGGCCGCTAGCGCCCTGCTGTTCTTATTC-3'
	Ex5-Re(fs)	5'-ATGCGTGACTATTGTCCCTTTGGTCAAAG-3'
	Ex19-Fw(fs)	5'-ACAATAGTCACGCATGTGTGTGTTCCCTGCCCGAC-3'
	Ex21-Re	5'-GAAGCTTGAGCTCGAGCCGTGCGCATGGTGGAGAAG-3'
	Ex4(CA)-Fw	5'-GAGAGGGAGCGAGTGGAACAGGAATACAATGTGGGGCATCAG
Splicing-editing fusing minigene	(TC)Ex4-Re	CCCCCTC-3' 5'-CACTCGCTCCCTCTCCTGCCGGCGAAGCGAGGGGACAGAGAAC AGAAAG-3'
	NovaM-Fw1	5'-CAAACAAGTGACTGGTGGTTGC-3'
	NovaM-Re1	5'-GCAACCACCAGTCACTTGTTTG-3'
	NovaM-Fw2	5'-CTCTGTCCGGTGACTGGTC-3'
	NovaM-Re2	5'-GACCAGTCACCGACAGAG-3'
	NovaM-Fw3	5'-CAGGGCCTCAGTACTGCTGCAC-3'
	NovaM-Re3	5'-GTGCAGCAGTACTGAGGCCCTG-3'
<i>I3</i>	TM63B-In3-Fw	5'-CAAAGAATTCGCGGCCGCGTAAGAAAGAGCCCCGAGCCTG-3'
	TM63B-In3-Re	5'-ATCTAGAGTCGCGGCCGCTGGGGACAGAGAACAGAAAG-3'
<i>I4</i>	TM63B-In4-Fw	5'-CAAAGAATTCGCGGCCGCGTATGTGGGGCATCAGCCCCCTC-3'
	TM63B-In4-Re	5'-ATCTAGAGTCGCGGCCGCTGGGGGGTGGGGAGACAG-3'
<i>I3E4I4</i>	TM63B-In3-Fw	5'-CAAAGAATTCGCGGCCGCGTAAGAAAGAGCCCCGAGCCTG-3'
	TM63B-In4-Re	5'-ATCTAGAGTCGCGGCCGCTGGGGGGTGGGGAGACAG-3'
Mut1	Mut1-Fw	5'-CTGGGGCCCTGCTCGGGGAAC-3'
	Mut1-Re	5'-GTTCCCCCGAGCAGGGCCAGGTGCCTACCGAAGGGCAC-3'
Mut2	Mut2-Fw	5'-CACCGCCGCGCCGGTCTG-3'
	Mut2-Re	5'-CAGGACCCGCGCGGCGGTG-3'
Mut3	Mut3-Fw	5'-CGCCGCGACCTGGGCCCTG-3'
	Mut3-Re	5'-CAGGGCCAGGTCGCGGCG-3'
Mut4	Mut4-Fw	5'-CACCGCCGCGCTGGGACCTG-3'
	Mut4-Re	5'-CAGGTCCCAGCGCGGCGGTG-3'
Mut5	Mut5-Fw	5'-CACCGCCGCGGCGGACCTG-3'
	Mut5-Re	5'-CAGGTCCCAGCGCGGCGGTG-3'

Mut6	Mut6-Fw	5'-CACCGGCGCGCCGGGACCTG-3'
	Mut6-Re	5'-CAGGTCCC GGCGCGCCGGTG-3'
Mut7	Mut7-Fw	5'-CACCGGCGCGGTGGGTCCTG-3'
	Mut7-Re	5'-CAGGACCCACCGCGCCGGTG-3'
Mut8	Mut8-Fw	5'-GGACCTTCCGCCTGCTCG-3'
	Mut8-Re	5'-CGAGCAGGCGGAAGGTCC-3'
Mut9	Mut9-Fw	5'-GTGCCCTTCGGTTTCCACC-3'
	Mut9-Re	5'-GGTGGAAACCGAAGGGCAC-3'
Mut10	Mut10-Fw	5'-CTACGAGATCCAGTTTGGC-3'
	Mut10-Re	5'-GCCAAACTGGATCTCGTAG-3'
Mut11	Mut11-Fw	5'-CTACGAGTTGCAGTTTGGC-3'
	Mut11-Re	5'-GCCAAACTGCAACTCGTAG-3'
Mut12	Mut12-Fw	5'-CTACGAGTTCCACTTTGGC-3'
	Mut12-Re	5'-GCCAAAGTGGA ACTCGTAG-3'

Primer	Sequence (5'-3')
EDIFw1	5'-ACATTAGGTCCCTCACTC-3'
EDIRel	5'-TGCCTTACTAGAGTGGAG-3'
EDIFw2	5'-CTGGCTGAAGCAGCTATTCG-3'
EDIRe2	5'-TACCAGGTGCTTCAGCAGC-3'
SPFw	5'-TCTGGACTTCATGTGCTTTC-3'
SPRe	5'-TTGGTCAAAGTCGACAGAGC-3'
LFw	5'-AGAGGGAGCGAGTGGAACAG-3'
SFw	5'-TGACAGATGCAGACAGTGTG-3'
CR	5'-CCGCTCGAGTTACTGGTGAATCTCATTCTC-3'
E5Fw	5'-GACTTTGACCAAAGGGAC-3'
E5Re	5'-GTCCCTTTGGTCAAAGTC-3'
pCAGFw	5'-GCTAACCATGTTTCATGCCTTC-3'
pCAGRe	5'-CTGCAGAATTCGAAGCTTG-3'