Supplementary Material: Development of novel chemically-modified nucleic acid molecules for efficient inhibition of human *MAPT* gene expression

Madhuri Chakravarthy, Suxiang Chen, Tao Wang and Rakesh N. Veedu

Table S1. List of first-generation DNAzymes and their sequences. Red nucleotides represent nucleotides in the catalytic loop. Blue nucleotides represent the cleavage site. Blue underlined nucleotides represent the cleavage site on the target sequence.

Name	Sequence	Target Sequence	Target
RNV547	ATCTTCCA GGCTAGCTACAACGA CACTTCG	CGAAGTGATGGAAGAT	Exon 1
RNV548	CAGCGTGA GGCTAGCTACAACGA CTTCCAT	ATGGAAGATCACGCTG	Exon 1
RNV549	CCCCCTGA GGCTAGCTACAACGA CTTTCCT	AGGAAAGATCAGGGGG	Exon 1
RNV550	TTGGTGCA GGCTAGCTACAACGA GGTGTAG	CTACACCATGCACCAA	Exon 1
RNV551	TCCTCAGA GGCTAGCTACAACGA CCGTCCT	AGGACGGATCTGAGGA	Exon 2
RNV552	TCTTAGCA GGCTAGCTACAACGA CAGAGGT	ACCTCTGATGCTAAGA	Exon 2
RNV553	CTCCCTCA GGCTAGCTACAACGA CCACTAA	TTAGTGGATGAGGGAG	Exon 3
RNV554	TTCTGGGA GGCTAGCTACAACGA CTCCGTG	CACGGAGATCCCAGAA	Exon 3
RNV555	GTCTCCAA GGCTAGCTACAACGA GCCTGCT	AGCAGGCATTGGAGAC	Exon 3
RNV556	TTTTGTCA GGCTAGCTACAACGA CGCTTCC	GGAAGCGATGACAAAA	Exon 5
RNV557	TGTGGCGA GGCTAGCTACAACGA CTTCGTT	AACGAAGATCGCCACA	Exon 7
RNV558	TGCTGGAA GGCTAGCTACAACGA CCTGGTG	CACCAGGATTCCAGCA	Exon 7
RNV559	TCCCCTGA GGCTAGCTACAACGA TTTGGAG	CTCCAAAATCAGGGGA	Exon 9

RNV560	CCGCTGCGA GGCTAGCTACAACGA CCCCTGA	TCAGGGGATCGCAGCGG	Exon 9
RNV561	ACTTGACA GGCTAGCTACAACGA TCTTCAG	CTGAAGAATGTCAAGT	Exon 9
RNV562	TTG CCTAA GGCTAGCTACAACGA GAGCCAC	GTGGCTCATTAGGCAA	Exon 11
RNV563	GTTTATGA GGCTAGCTACAACGA GGATGTT	AACATCCATCATAAAC	Exon 11
RNV564	CTGGTTTA GGCTAGCTACAACGA GATGGAT	ATCCATCATAAACCAG	Exon 11
RNV565	TTCTCAGA GGCTAGCTACAACGA TTTACTT	AAGTAAAATCTGAGAA	Exon 12
RNV566	GGACCCAA GGCTAGCTACAACGA CTTCGAC	GTCGAAGATTGGGTCC	Exon 12
RNV567	GGGTGATA GGCTAGCTACAACGA TGTCCAG	CTGGACAATATCACCC	Exon 12
RNV568	GTGGGTGA GGCTAGCTACAACGA ATTGTCC	GGACAATATCACCCAC	Exon 12
RNV569	GTACACGA GGCTAGCTACAACGA CTCCGCC	GGCGGAGATCGTGTAC	Exon 13
RNV570	TGCTGAGA GGCTAGCTACAACGA GCCGTGG	CCACGGCATCTCAGCA	Exon 13
RNV571	AGGAGACA GGCTAGCTACAACGA TGCTGAG	CTCAGCAATGTCTCCT	Exon 13
RNV572	CATGTCGA GGCTAGCTACAACGA GCTGCCG	CGGCAGCATCGACATG	Exon 13
RNV573	GTCTACCA GGCTAGCTACAACGA GTCGATG	CATCGACATGGTAGAC	Exon 13

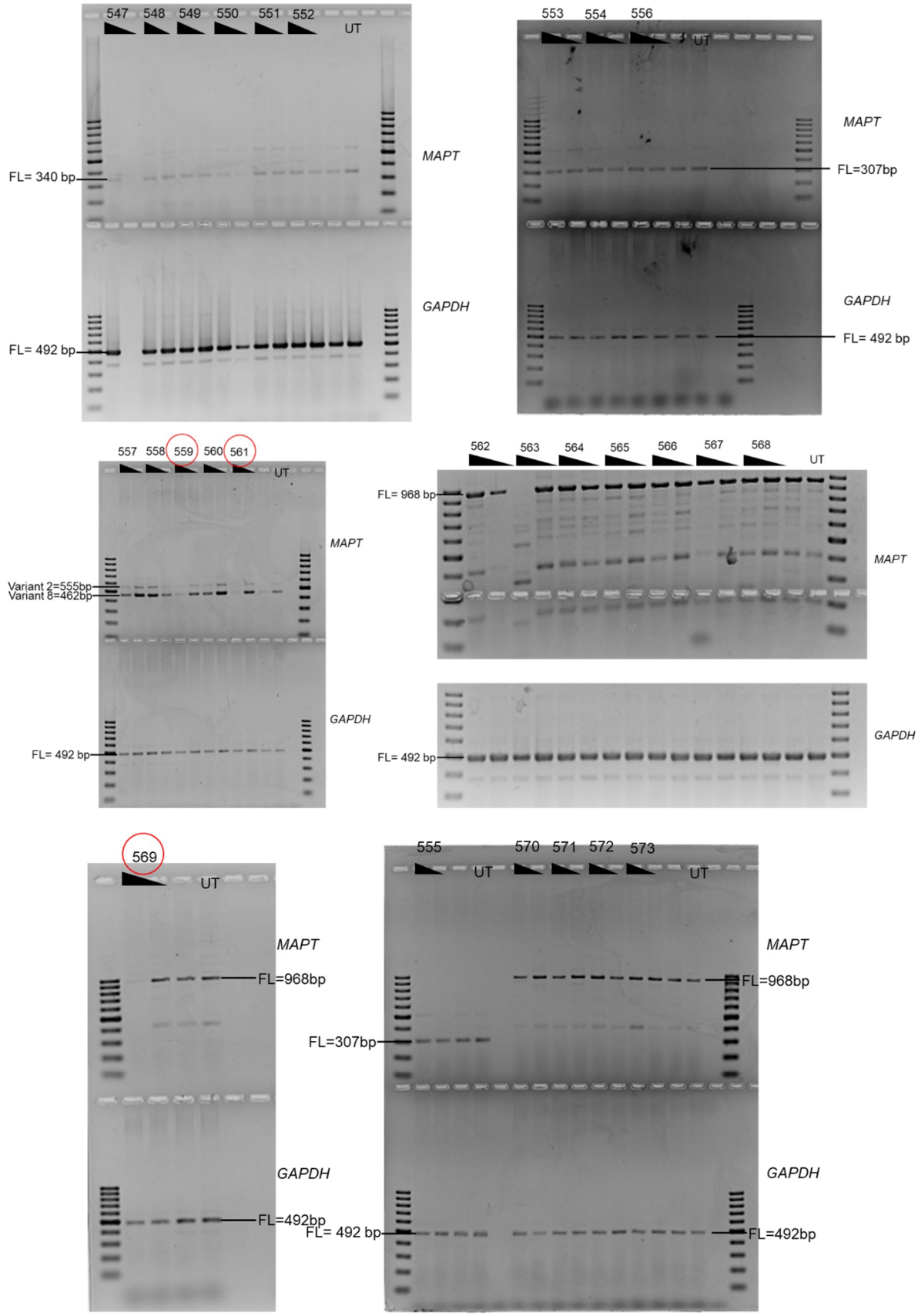

Figure S1: Representative RT-PCR products of the *MAPT* and *GAPDH* transcripts from SH-SY5Y cells after treatment with DNAzyme at 400 nM, and 50 nM concentrations. The RT-PCR products after treatment with RNV547, RNV548, RNV549, RNV550, RNV551, RNV552, RNV553, RNV554, RNV555, RNV556, RNV557, RNV558, RNV559, RNV560, RNV561, RNV562, RNV563, RNV564, RNV565, RNV566, RNV567, RNV568, RNV569, RNV570, RNV571, RNV572 and RNV573 are shown here. The

RNA from RNV547- RNV552 treated samples were amplified using primer set 1. The RNA from RNV553-RNV556 treated samples were amplified using primer set 2. The RNA from RNV557-561 treated samples were amplified using primer set 3. The RNA from RNV562-RNV573 treated samples were amplified using primer set 4. RNV FL, full-length; UT, untreated; *GAPDH* was used as a loading control.

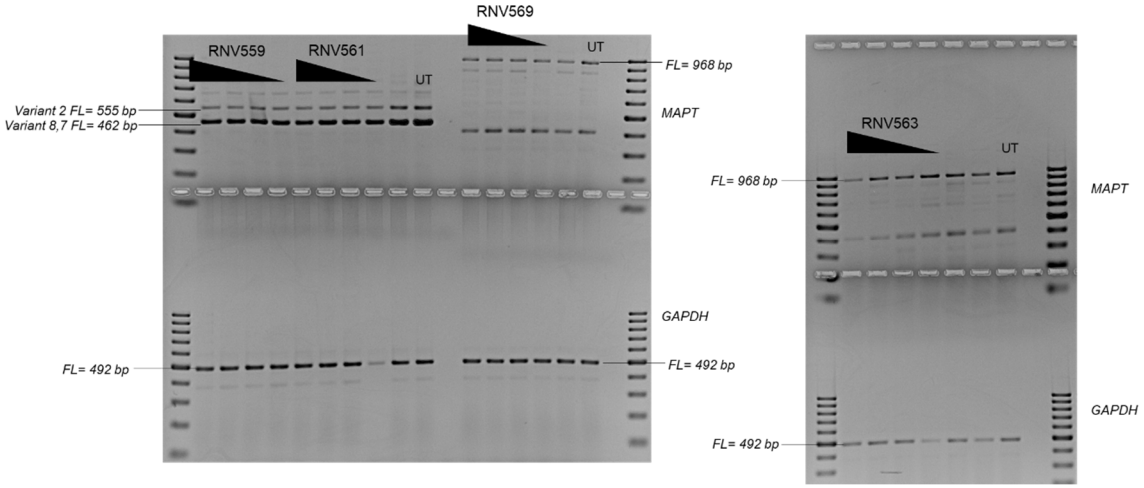

Figure S2: Representative RT-PCR products of the *MAPT* and *GAPDH* transcripts from SH-SY5Y cells after treatment with DNAzyme at 400 nM, 200 nM, 100 nM and 50 nM concentrations. The RT-PCR products after treatment with RNV559, RNV561, RNV563 and RNV563 are shown here. The RNA from RNV559 and RNV561 treated samples were amplified using primer set 2. The RNA from RNV563 and RNV569 treated samples were amplified using primer set 4. FL, full-length; UT, untreated; *GAPDH* was used as a loading control. [The gel in this figure is the original gel representing the gel in Figure 2 of the article. The cropped gel has been shown in Figure 2 of the article due to other unimportant samples that exist between the desired samples.].

Table S2: The average activity of 1st generation DNAzymes (at 400 nM concentration) in SH-SY5Y cells (knockdown of *MAPT* transcript).

Name	Average Activity of DNAzyme in SH-SY5Y cells
559	0%
561	26%
563	58%
569	0%

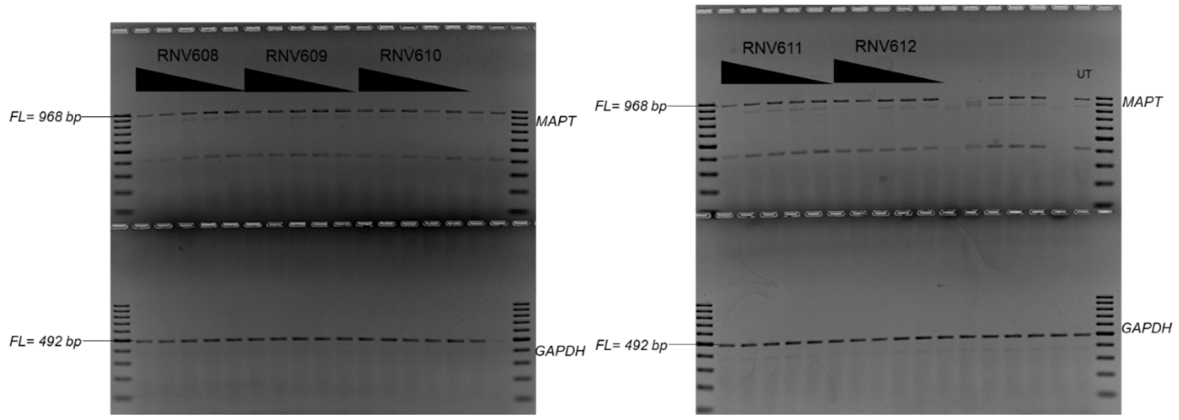

Figure S3: Representative RT-PCR products of the *MAPT* and *GAPDH* transcripts from SH-SY5Y cells after treatment with DNAzyme at 400 nM, 200 nM, 100 nM and 50 nM concentrations. The RT-PCR

products after treatment with RNV608, RNV609, RNV610, 611 and RNV612 are shown here and were amplified using primer set 4. FL, full-length; UT, untreated; *GAPDH* was used as a loading control.

Table S3. The average activity of 2nd generation DNAzymes (at 400 nM concentration) in SH-SY5Y cells (knockdown of *MAPT* transcript).

Name	Average Activity of DNAzymes in SH-SY5Y cells
608	0%
609	0%
610	15%
611	0%
612	2%

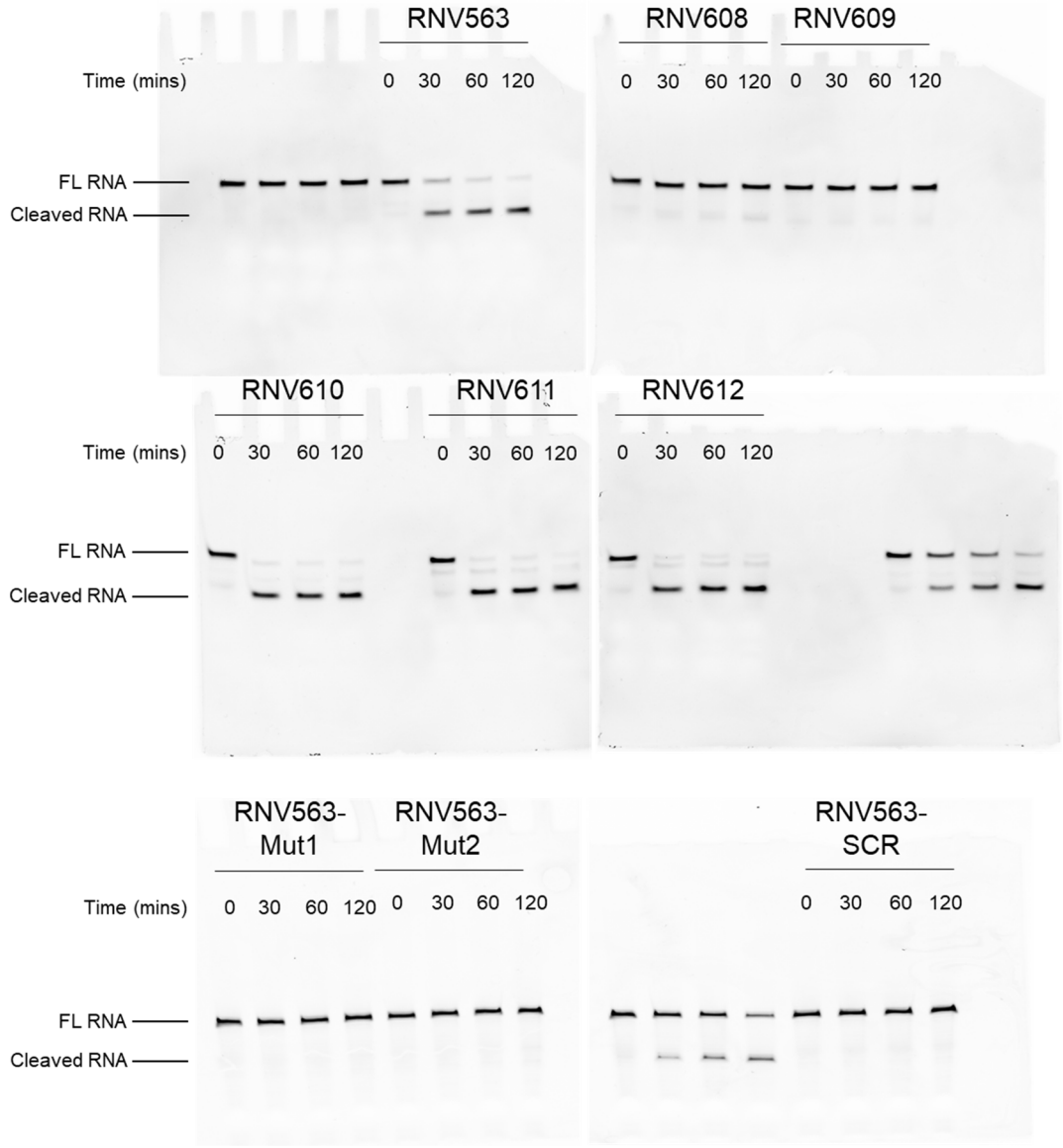

Figure S4. *In vitro* cleavage of the FAM-conjugated *MAPT* RNA template composed of exon 11 region (34 nucleotides) by RNV563 and its derivatives. FL RNA, full-length; FAM-conjugated RNA; cleaved RNA; the cleaved FAM-conjugated *MAPT* RNA (22 nucleotides long). The FAM- conjugated template RNA is a small region of the *MAPT* transcript complementary to the hybridisation arms of the DNAzymes of interest. [The gel in this figure is the original gel representing the gel in Figure 3 of the article. The cropped gel has been shown in Figure 3 of the article due to other unimportant samples or unwanted spaces that exist between the desired samples.].

Table S4. List of 2′-OMePS AOs and their sequences. In the sequences, capital letters denote bases from the exon regions, and small letters denote bases from the intronic region.

AO Number	AO Name	AO Sequence	Target
AO1	MAPT E1A(+11+35)	TCCATCACTTCGAACTCCTGGCGGG	Exon 1
AO2	MAPT E1A(+41+65)	TCCCCCAACCCGTACGTCCCAGCGT	Exon 1
AO3	MAPT E1A(+91+115)	TGTCACCCTCTTGGTCTTGGTGCAT	Exon 1
AO4	MAPT E4A(+1+25)	GTGTCTCCAATGCCTGCTTCTTCAG	Exon 4
AO5	MAPT E4A(+26+50)	AGCAGCTTCGTCTTCCAGGCTGGGG	Exon 4
AO6	MAPT E5A(+16+40)	TCGCTTCCAGTCCCGTCTTTGCTTT	Exon 5
AO7	MAPT E5D(+3-22)	ctccgtggcatcgtcagcttacCTT	Exon 5
AO8	MAPT E7A(+26+50)	GGAGGGGCTGCTCCCCGCGGTGTGG	Exon 7
AO9	MAPT E7A(+46+70)	TGGCCTGGCCCTTCTGGCCTGGAGG	Exon 7
AO10	MAPT E7A(+71+95)	GTTTTTGCTGGAATCCTGGTGGCGT	Exon 7
AO11	MAPT E7A(+97+121)	TGGGTGGTGTCTTTGGAGCGGGCGG	Exon 7
AO12	MAPT E7D(+5-20)	caagagaacgttcttcttacCAGAG	Exon 7
AO13	MAPT E9A(+1+25)	CGATCCCCTGATTTTGGAGGTTCAC	Exon 9
AO14	MAPT E9A(+111+135)	TACGGACCACTGCCACCTTCTTGGG	Exon 9
AO15	MAPT E9A(+159+183)	GGGCTGTCTGCAGGCGGCTCTTGGC	Exon 9
AO16	MAPT E9A(+196+220)	TTGGACTTGACATTCTTCAGGTCTG	Exon 9
AO17	MAPT E9A(+226+250)	TGGTGCTTCAGGTTCTCAGTGGAGC	Exon 9
AO18	MAPT E9D(+21-4)	tcacCTTCCCGCCTCCCGGCTGGTG	Exon 9
AO19	MAPT E12A(-5+20)	TACTTCCACCTGGCCACCTCctaga	Exon 12
AO20	MAPT E12A(+21+45)	CCTTGAAGTCAAGCTTCTCAGATTT	Exon 12
AO21	MAPT E12A(+46+70)	GACCCAATCTTCGACTGGACTCTGT	Exon 12
AO22	MAPT E12A(+81+95)	AGGGACGTGGGTGATATTGTCCAGG	Exon 12

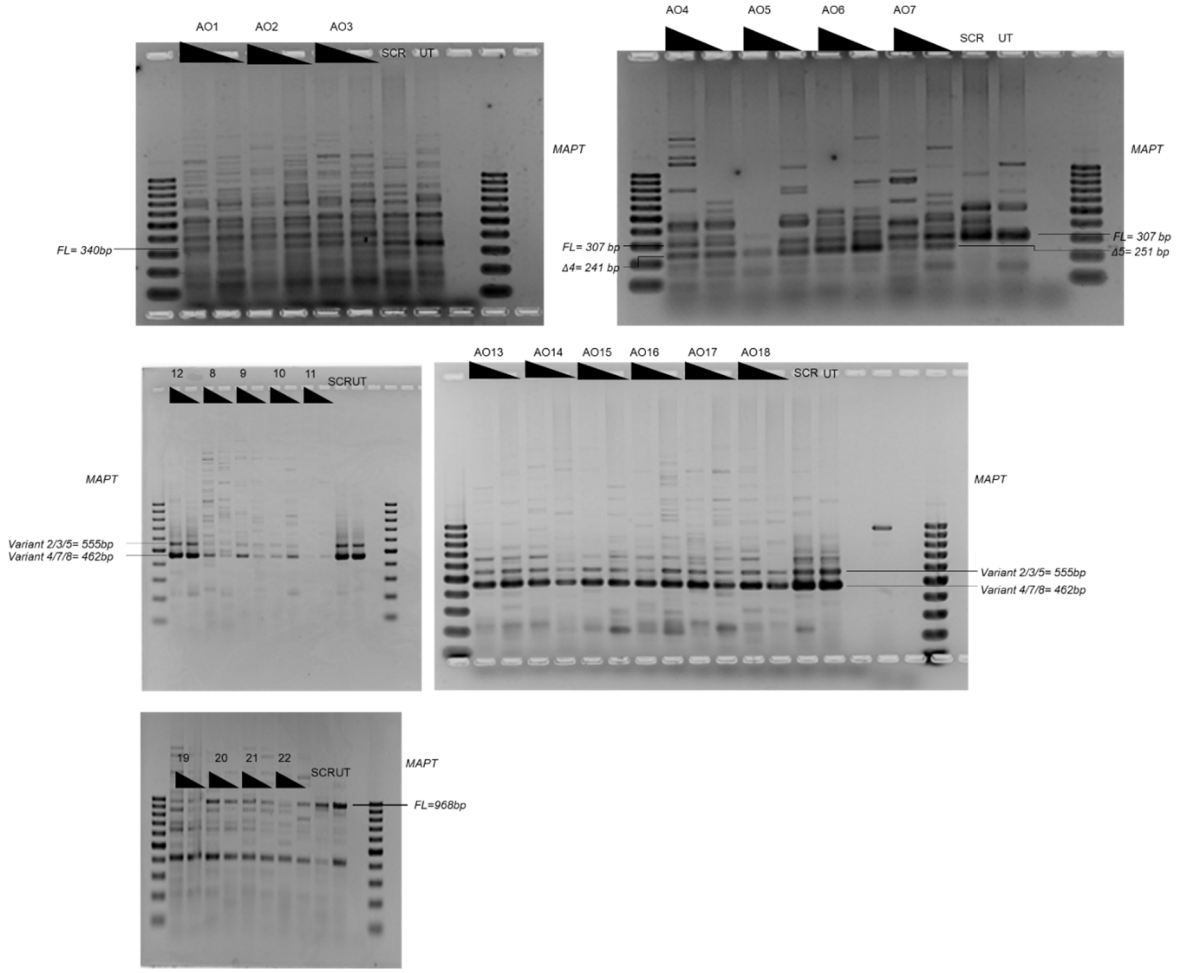

Figure S5: Representative RT-PCR products of the *MAPT* transcripts from SH-SY5Y cells after treatment with splice-modulating AOs at 400 nM, and 50 nM concentrations. The RT-PCR products after treatment with AOs 1-22 are shown here. The RNA from AOs 1-3 were amplified using primer set 2. The RNA from AOs 4-18 were amplified using primer set 3. The RNA from AOs 19-22 were amplified using primer set 4. FL, full-length; UT, untreated; SCR, scrambled sequence.

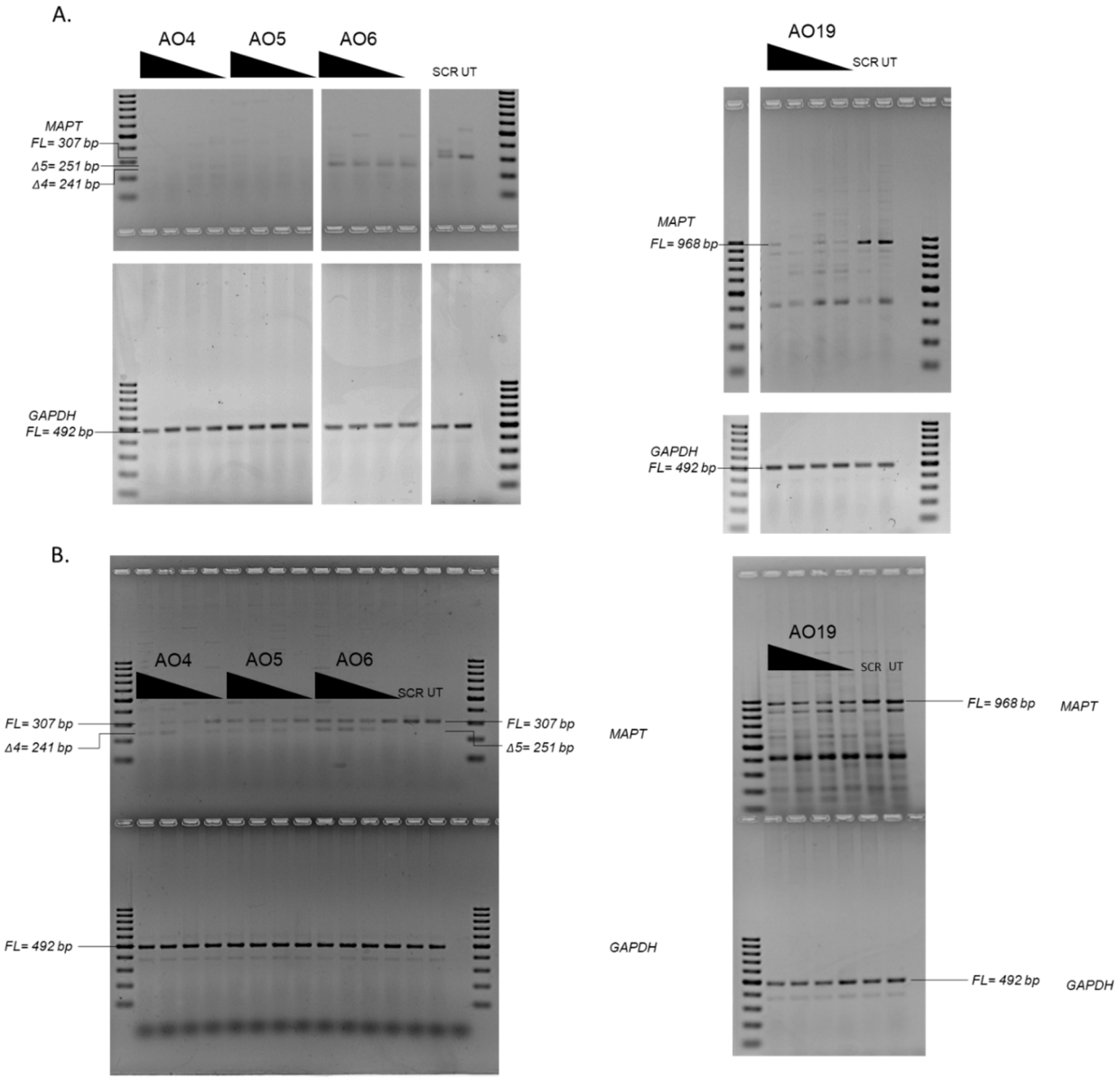

Figure S6. A. Representative RT-PCR products of the *MAPT* transcripts from SH-SY5Y cells after treatment with AO4, AO5, AO6, and AO19 at 400 nM, 200 nM, 100 nM and 50 nM concentrations. B. Representative RT-PCR products of the *MAPT* and *GAPDH* transcripts from SH-SY5Y cells after treatment with AO4, AO5, AO6, and AO19 at 50 nM, 25 nM, 12.5 nM and 6.25 nM concentrations. AO4 targets exon 4, AO5 and AO6 target exon 5, and AO19 targets exon 12 of the *MAPT* transcript. The RNA form AO4, AO5 and AO6 treated samples were amplified using primer set 2. The RNA from AO19 treated samples were amplified using primer set 4. FL, full-length; UT, untreated; SCR, scrambled sequence; *GAPDH* was used as a loading control. [The gel in this figure is the original gel representing the gel in Figure 4 of the article. The cropped gel has been shown in Figure 4 of the article due to unwanted spaces that exist on the gel.].

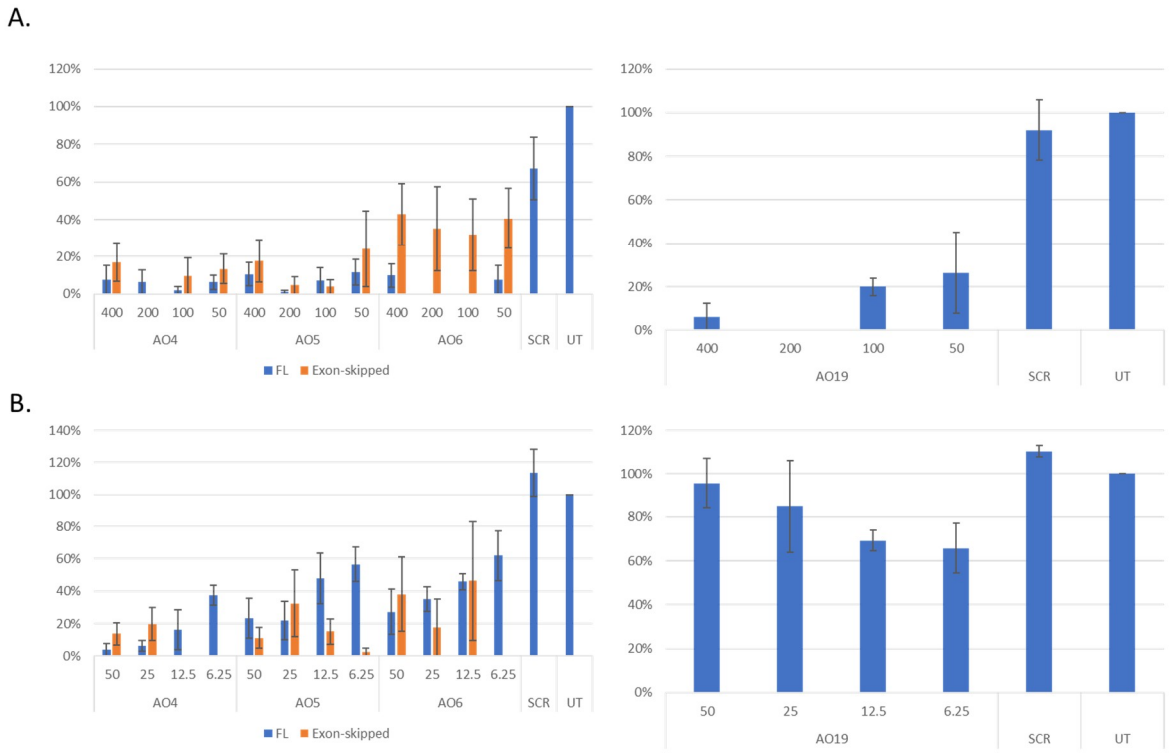

Figure S7. Densitometry analysis of RT-PCR products (three replicates) using AO4, AO6, AO7 and AO19 showed downregulation and exon-skipping of *MAPT* transcript in SH-SY5Y cells *in vitro*. Concentrations of AOs used include 400 nM, 200 nM, 100 nM and 50 nM. The error bars represent the standard error of mean. B. Densitometry analysis of RT-PCR products (more than two replicates) using AO4, AO6, AO7 and AO19 showed downregulation and exon-skipping of *MAPT* transcript in SH-SY5Y cells *in vitro*. Concentrations of AOs used include 50 nM, 25 nM, 12.5 nM and 6.25 nM. The error bars represent the standard error of mean.

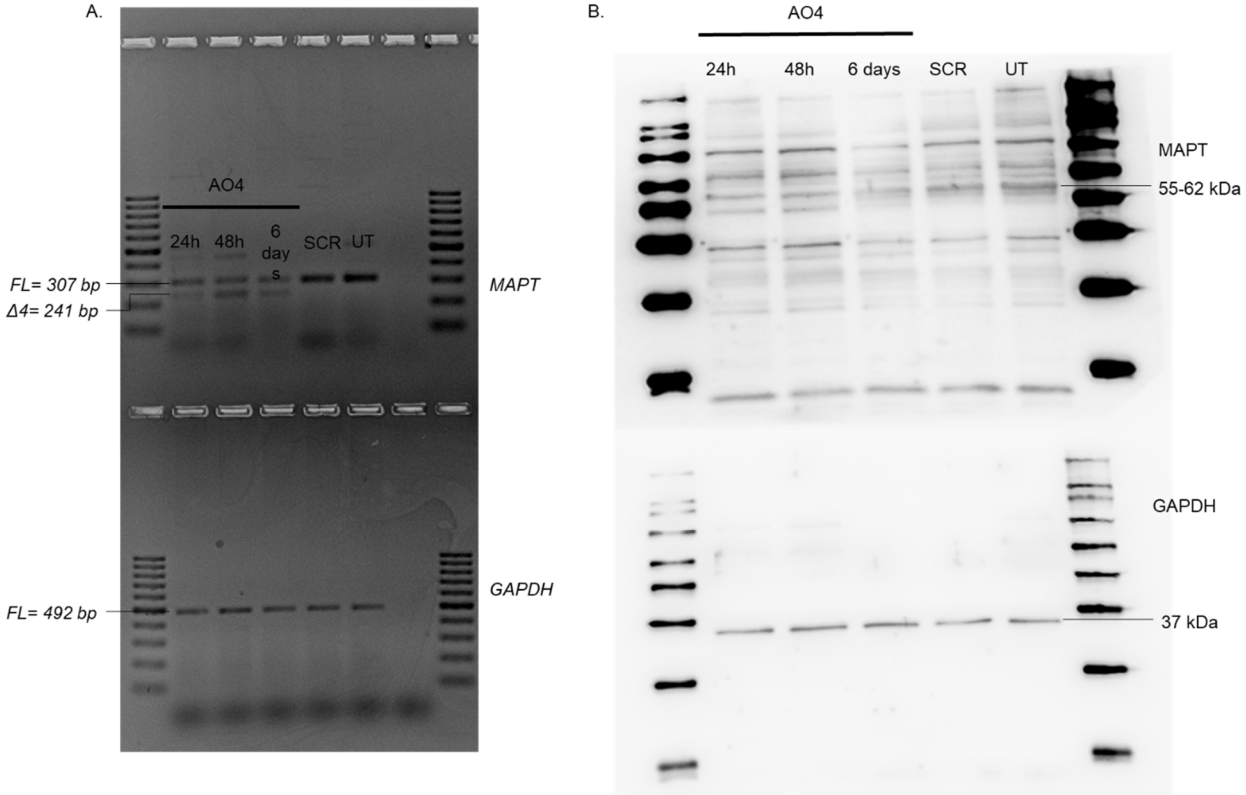

Figure S8. A. Representative RT-PCR products of the MAPT transcripts from SH-SY5Y cells after treatment with AO4 50 nM concentration and incubation of AO for 24 h, 48 h and six days. AO4 targets exon 4 of the *MAPT* transcript. **B**. Representative protein products of the *MAPT* and *GAPDH* transcripts from SH-SY5Y cells after treatment with AO4 at 50 nM concentrations and incubation of AO for 24 h, 48 h and six days. AO4 targets exon 4 of the *MAPT* transcript. The RNA from AO4 treated samples for the RT-PCR were amplified using primer set 2. FL, full-length; UT, untreated; SCR, scrambled sequence; *GAPDH* was used as a loading control. [The gel in this figure is the original gel representing the gel in Figure 5 of the article. The cropped gel has been shown in Figure 5 of the article due to unwanted spaces, other unimportant samples, and non-specific bands that exist on the gel and membrane.].

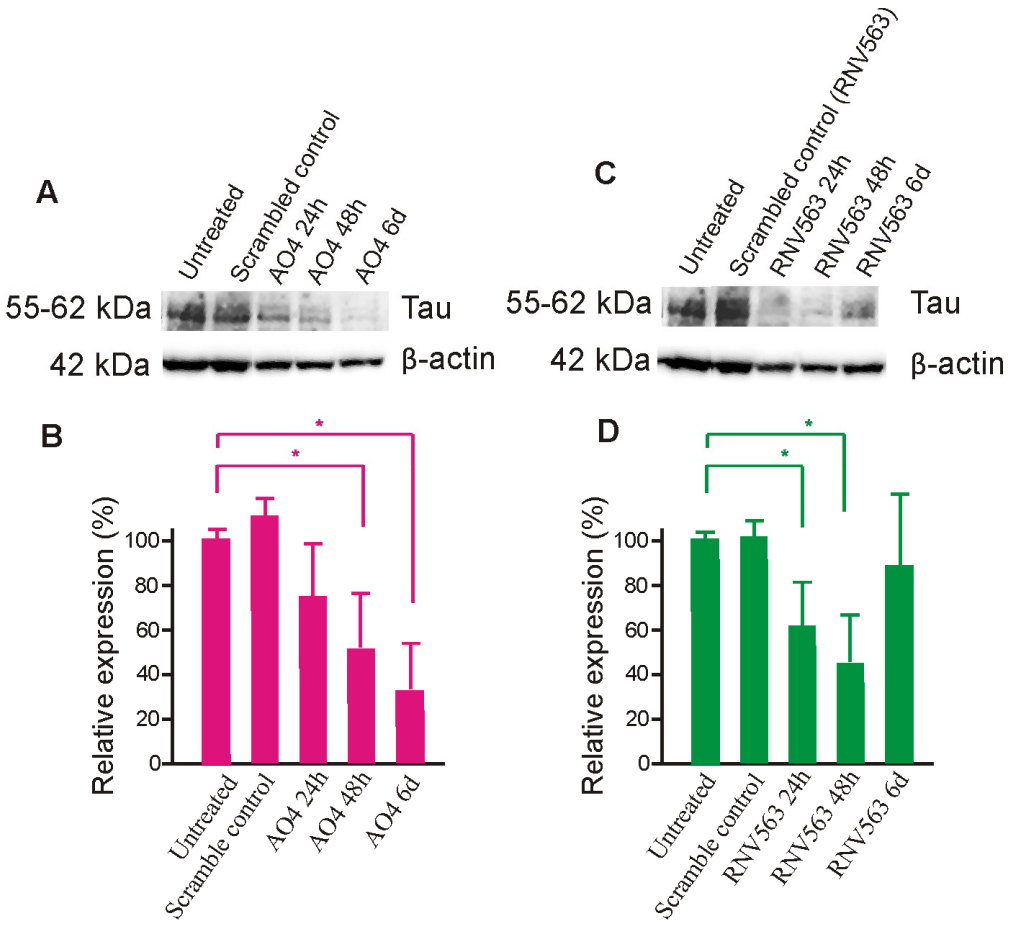

Figure S9. A. Representative protein products of the *MAPT* and *β-actin* transcripts from SH-SY5Y cells after treatment with AO4 at 50 nM concentrations and incubation of AO for 24 h, 48 h and six days. AO4 targets exon 4 of the *MAPT* transcript. **B**. Densitometry analysis of the western blot (three replicates) shown in A of this figure. The error bars represent the standard error of mean. **C**. Representative protein products of the *MAPT* and *β-actin* transcripts from SH-SY5Y cells after treatment with RNV563 at 400 nM concentrations and incubation of DNAzyme for 24 h, 48 h and six days. RNV563 targets exon 9 of the *MAPT* transcript. **D**. Densitometry analysis of the western blot (three replicates) shown in C of this figure. The error bars represent the standard error of mean.

Table S5. List of primers used, their sequences and the expected product lengths.

Primer Set	Primer pairs	Primer Sequences	Expected product length
MAPT Primer Set 1	MAPT6_Ex0Fa MAPT6_Ex3R	5′ TCCTCGCCTCTGTCGACTAT 3′ 5′ TCCTTCTGGGATCTCCGTGT 3′	Variant 2/8= 340 bp

MAPT Primer Set 2	MAPT6_Ex3F MAPT6_Ex7R	5′ GTGACAGCACCCTTAGTGGA 3′ 5′ GCGGGGTTTTTGCTGGAATC 3′	Variant 2/8= 307 bp
MAPT Primer Set 3	MAPT6_Ex5F MAPT6_Ex11R	5′ AAGACGGGACTGGAAGCGAT 3′ 5′ TGCTCAGGTCAACTGGTTTGT 3′	Variant 2/3/5= 555 bp Variant 4/7/8= 462 bp
MAPT Primer Set 4	MAPT6_Ex10F MAPT6_Ex13Ra	5′ TTAGCAACGTCCAGTCCAAGT 3′ 5′ AGGTTGACATCGTCTGCCTG 3′	Variant 2/3/5= 968 bp
GAPDH Primer set	GAPDH For GAPDH Rev	5′ GGACTCATGACCACAGTCCATGC 3′ 5′ TTACTCCTTGGAGGCCATGTGGG 3′	492 bp

Table S6. The PCR conditions for each primer set.

MAPT Primer Set 1 (50 ng each)	**Temperature**	**Time**	
	55°C	30 min	
	94°C	2 min	
	94°C	30 s	28 cycles
	55°C	1 min	
	68°C	2 min	
MAPT Primer Set 3 & 4 (50 ng each)	Temperature	Time	
	55°C	30 min	
	94°C	2 min	
	94°C	30 s	34 cycles
	60°C	1 min	
	68°C	2 min	
MAPT Primer Set 2 & 3 (50 ng each)	**Temperature**	**Time**	
	55°C	30 min	
	94°C	2 min	
	94°C	30 s	30 cycles
	60°C	1 min	
	68°C	2 min	
GAPDH Primer set (12.5 ng each)	**Temperature**	**Time**	
	55°C	30 min	
	94°C	2 min	
	94°C	30 s	16 cycles

Table S7. List of primers used, their binding co-ordinates, the expected full-length product size and the exon-skipped product size.

MAPT Primer Set	Primer pairs	Primer binding Co-ordinates	Expected full-length product size	Exon skipped product size
1	MAPT6_Ex0Fa MAPT6_Ex3R	Exon 0 (+283 +302) Exon 3 (+79+60)	Variant 2/8= 340 bp	ΔExon1=190bp
2	MAPT6_Ex3F MAPT6_Ex7R	Exon 3 (+2+21) Exon 7 (+99+80)	Variant 2/8= 307 bp	ΔExon4=241bp ΔExon5=251bp
3	MAPT6_Ex5F MAPT6_Ex11R	Exon 5 (+21+40) Exon 11 (+33+13)	Variant 2/3/5= 555 bp Variant 4/7/8= 462 bp	ΔExon7 Variant 2/3/5=428bp Variant 4/7/8=335bp ΔExon9 Variant 2/3/5=289bp

				Variant 4/7/8=196bp
4	MAPT6_Ex10F MAPT6_Ex13Ra	Exon 10 (+28+48) Exon 13 (+707+688)	Variant 2/3/5= 968 bp	ΔExon12=855bp

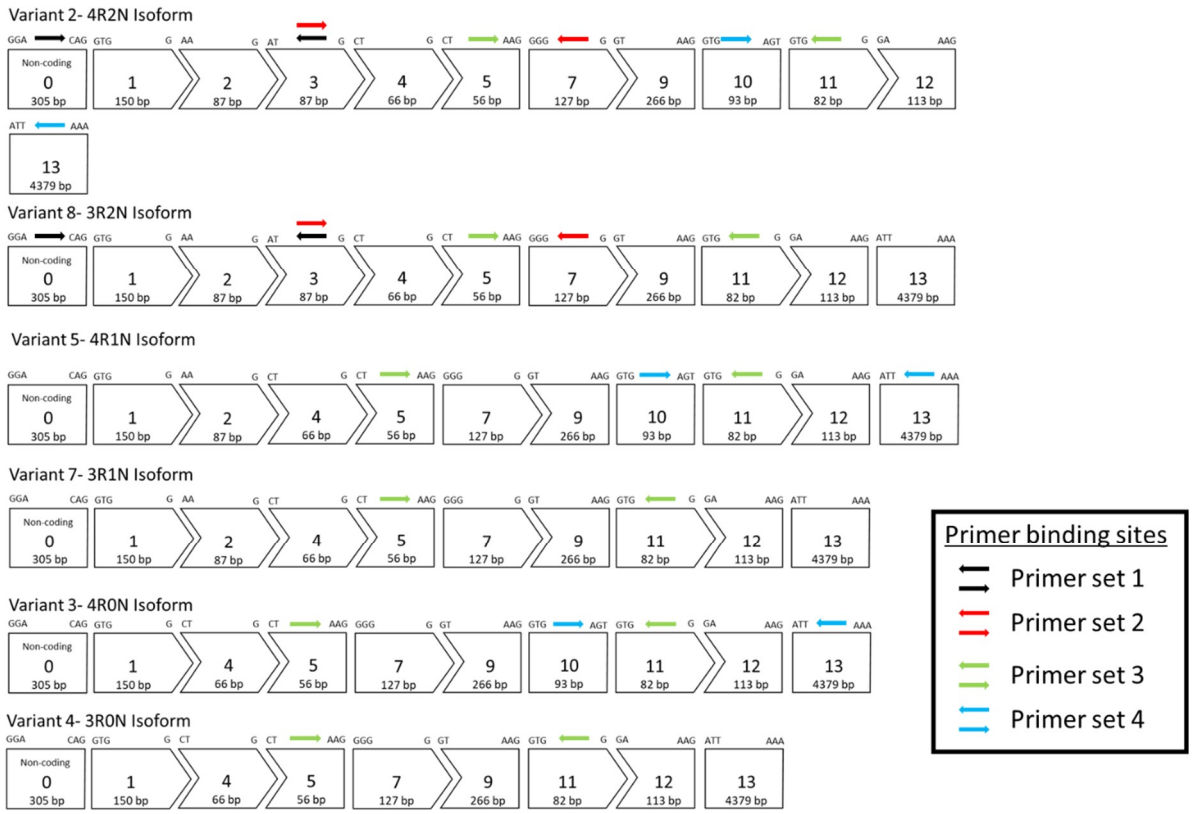

Figure S10. Exon map of the *MAPT* variants found in the human brain and the primer binding sites of the primer sets used in this study.

Figure S11. Sequence alignment of the full length band (968bp product) from RNV563 treated samples and the longest *MAPT* isoform, variant 2. .

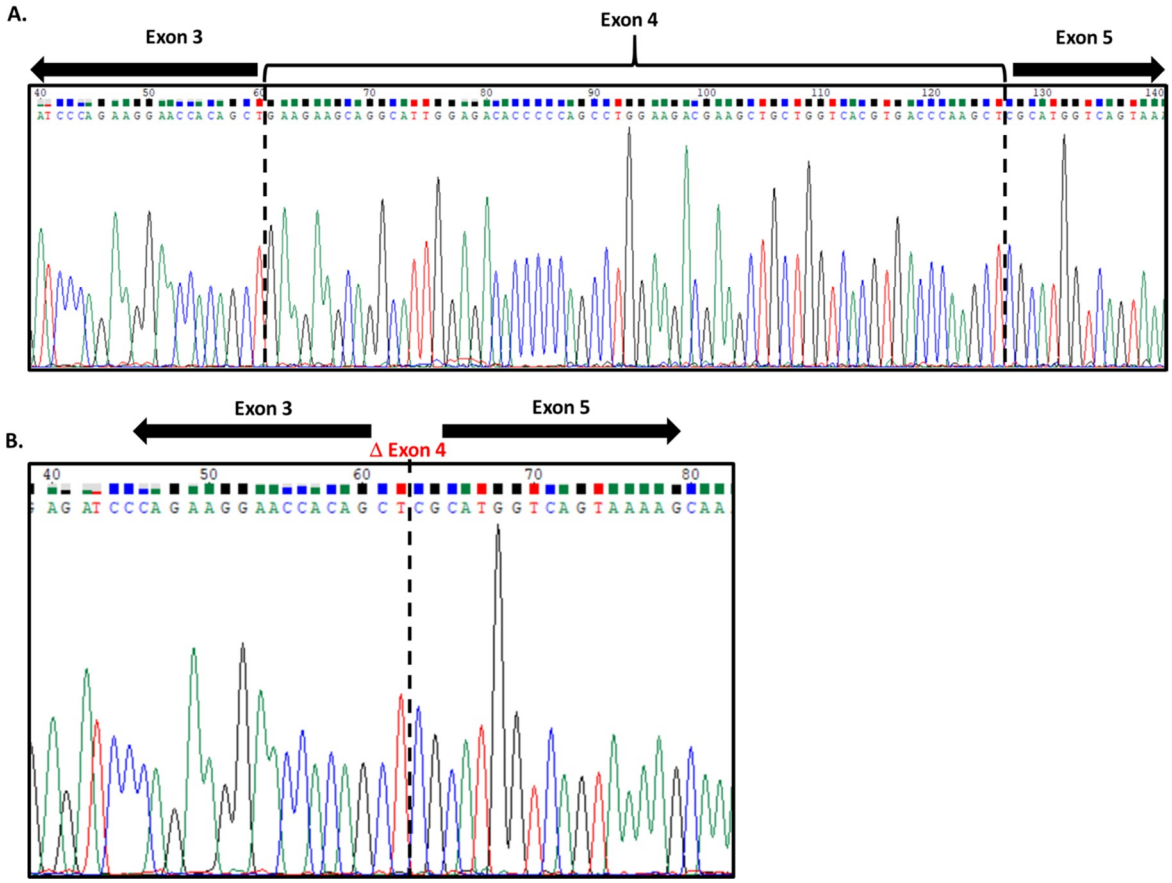

Figure S12. A. Exon 3- Exon 5 region of the sequencing of the FL (307 bp) product from AO4 treated sample. **B**. Exon 3- Exon 5 region of the sequencing of the exon-skipped (241 bp) product from AO treated sample.