

Rolling Circle cDNA Synthesis Uncovers Circular RNA Splice Variants

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Supplementary Materials: Figure S1. Validation of circRNA expression using divergent primers. **Figure S2.** Splice variants of hsa_circ_0001566. **Figure S3.** Splice variants of hsa_circ_0007822. **Figure S4.** Splice variants of hsa_circ_0084615. **Figure S5.** Splice variants of hsa_circ_0009156. **Figure S6.** Splice variants of hsa_circ_0071410. **Figure S7.** Potential miRNA targets of circRNA splice variants. **Figure S8.** Potential RBPs associated with circRNA splice variants. **Supplementary Table S1.** Sequences of the DNA primers used to in this study. **Supplementary Table S2.** Gene Ontology analysis for target genes of miRNAs associated with has_circ_007127 sequence reported in circBase and the splice variants.

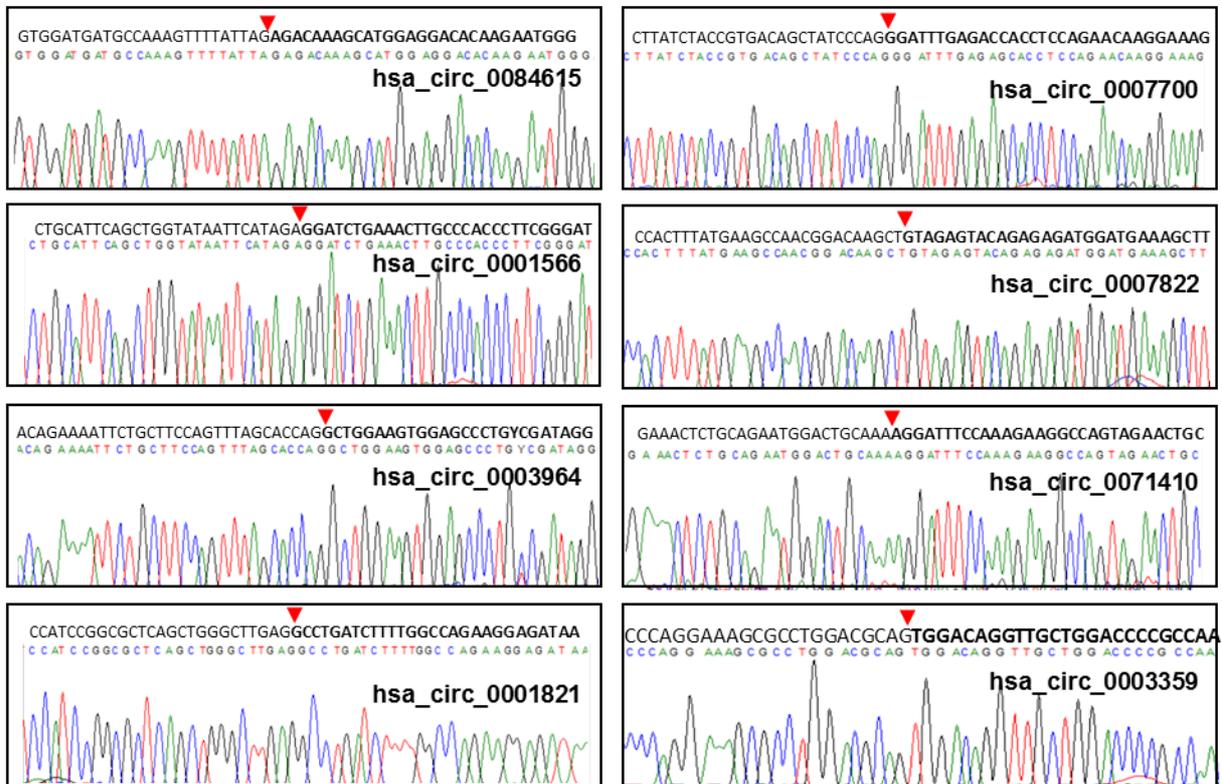


Figure S1. Validation of circRNA expression using divergent primers. RT-PCR products amplified with divergent primers were purified and verified by Sanger sequencing to confirm the identity of the backsplice junction sequences.

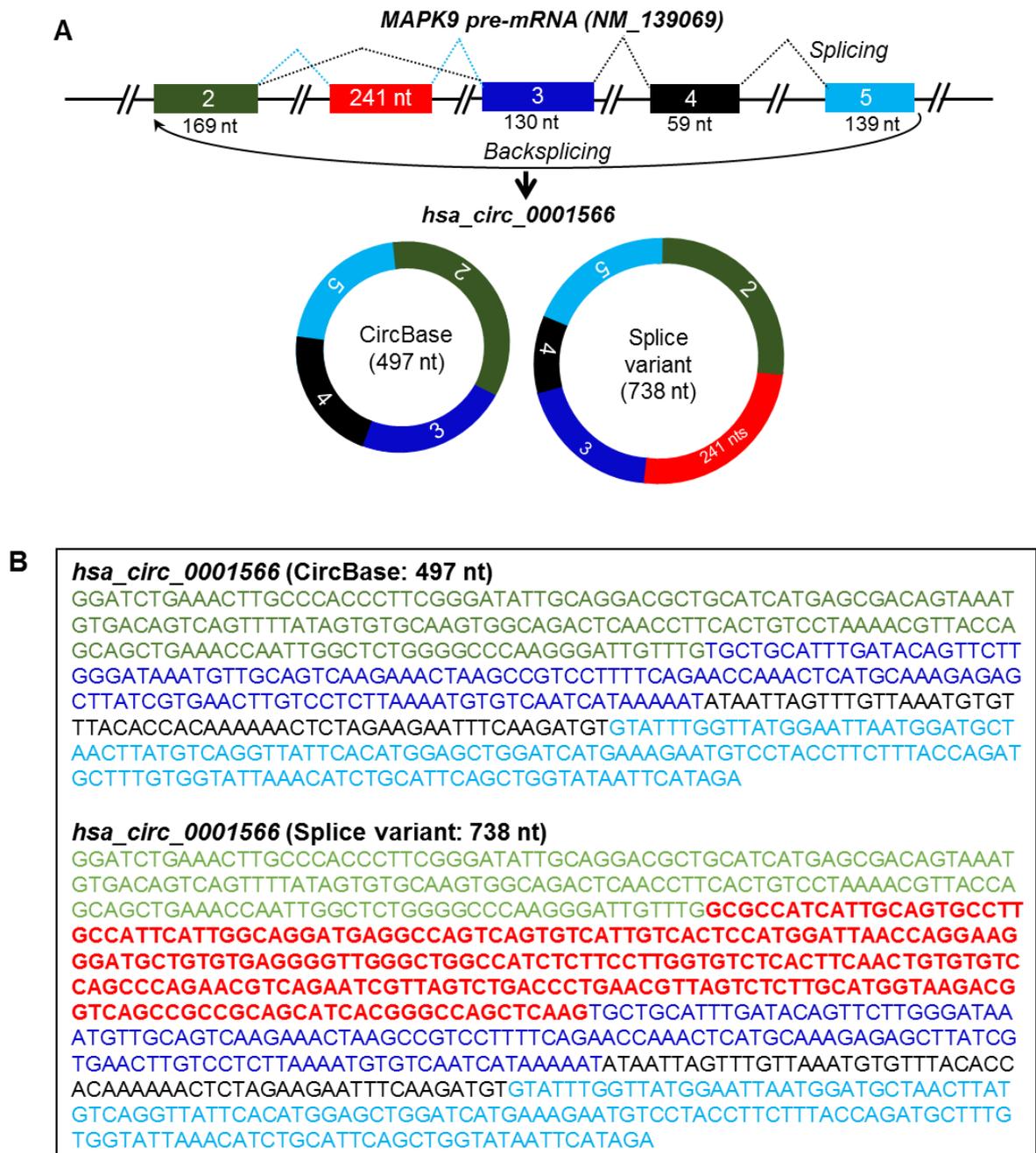
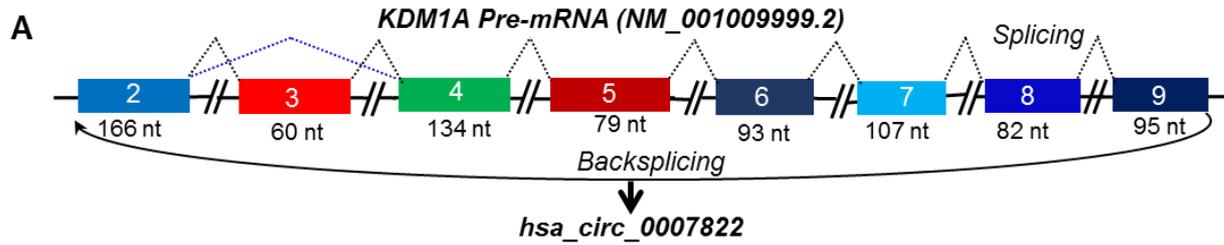


Figure S2. Splice variants of *hsa_circ_0001566*. (A) Schematic representation of the *MAPK9* pre-mRNA and the biogenesis of *hsa_circ_0001566* splice variants produced by alternative splicing. Boxes and straight lines represent exons and introns, respectively. The black dotted lines represent splicing while blue dotted lines represent alternative splicing. (B) Spliced full-length sequences of circBase and splice variants of *hsa_circ_0001566*.

**B*****hsa_circ_0007822* (circBase: 816 nt)**

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GTAGAGTACAGAGAGATGGATGAAAGCTTGGCCAACCTCTCAGAAGATGAGTATTATTCAGAAGAA
GAGAGAAATGCCAAAGCAGAGAAGGAAAAGAAGCTTCCCCCACCACCCCTCAAGCCCCACCTGA
GGAAGAAAATGAAAGTGAGCCTGAAGAACCATCGGGGCAAGCAGGAGGACTTCAAGACGCAGT
TCTGGAGGGTATGGAGACGGCCAAGCATCAGGTGTGGAGGGCGCAGCTTCCAGAGCCGACTTC
CTCATGACCGGATGACTTCTCAAGAAGCAGCCTGTTTTCCAGATATTATCAGTGGACCACAACAGA
CCCAGAAGGTTTTTCTTTTCATTAGAAACCGCACACTGCAGTTGTGGTTGGATAATCCAAAGATTCA
GCTGACATTTGAGGCTACTCTCCAACAATTAGAAGCACCTTATAACAGTGATACTGTGCTTGTCCAC
CGAGTTCACAGTTATTTAGAGCGTCATGGTCTTATCAACTTCGGCATCTATAAGAGGATAAAACCC
TACCAACTAAAAAGACAGGAAAGGTAATTATTATAGGCTCTGGGGTCTCAGGCTTGGCAGCAGCTC
GACAGTTACAAAGTTTTGGAATGGATGTCACACTTTTTGGAAGCCAGGGATCGTGTGGGTGGACGA
GTTGCCACATTTGCAAAGGAAACTATGTAGCTGATCTTGGAGCCATGGTGGTAACAGGTCTTGGA
GGGAATCCTATGGCTGTGGTCAGCAAACAAGTAAATATGGAAGTGGCCAAGATCAAGCAAAAATGC
CCACTTTATGAAGCCAACGGACAAGCT

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***hsa_circ_0007822* (Splice variant: 756 nt)**

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GTAGAGTACAGAGAGATGGATGAAAGCTTGGCCAACCTCTCAGAAGATGAGTATTATTCAGAAGAA
GAGAGAAATGCCAAAGCAGAGAAGGAAAAGAAGCTTCCCCCACCACCCCTCAAGCCCCACCTGA
GGAAGAAAATGAAAGTGAGCCTGAAGAACCATCGGGTGTGGAGGGCGCAGCTTCCAGAGCCGA
CTTCTCATGACCGGATGACTTCTCAAGAAGCAGCCTGTTTTCCAGATATTATCAGTGGACCACAA
CAGACCCAGAAGGTTTTTCTTTTCATTAGAAACCGCACACTGCAGTTGTGGTTGGATAATCCAAAGA
TTCAGCTGACATTTGAGGCTACTCTCCAACAATTAGAAGCACCTTATAACAGTGATACTGTGCTTGT
CCACCGAGTTCACAGTTATTTAGAGCGTCATGGTCTTATCAACTTCGGCATCTATAAGAGGATAAAA
CCCTACCAACTAAAAAGACAGGAAAGGTAATTATTATAGGCTCTGGGGTCTCAGGCTTGGCAGCA
GCTCGACAGTTACAAAGTTTTGGAATGGATGTCACACTTTTTGGAAGCCAGGGATCGTGTGGGTGGA
CGAGTTGCCACATTTGCAAAGGAAACTATGTAGCTGATCTTGGAGCCATGGTGGTAACAGGTCTT
GGAGGGAATCCTATGGCTGTGGTCAGCAAACAAGTAAATATGGAAGTGGCCAAGATCAAGCAAAA
ATGCCCACTTTATGAAGCCAACGGACAAGCT

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Figure S3. Splice variants of *hsa_circ_0007822*. (A) Schematic representation of the *hsa_circ_0007822* splice variants biogenesis from *KDM1A* pre-mRNA. Boxes and straight lines represent exons and introns, respectively. The black dotted lines represent splicing while blue dotted lines represent alternative splicing. (B) Spliced full-length sequences of splice variants of *hsa_circ_0007822*. The text colors correspond to the colors of the exon boxes in panel A.

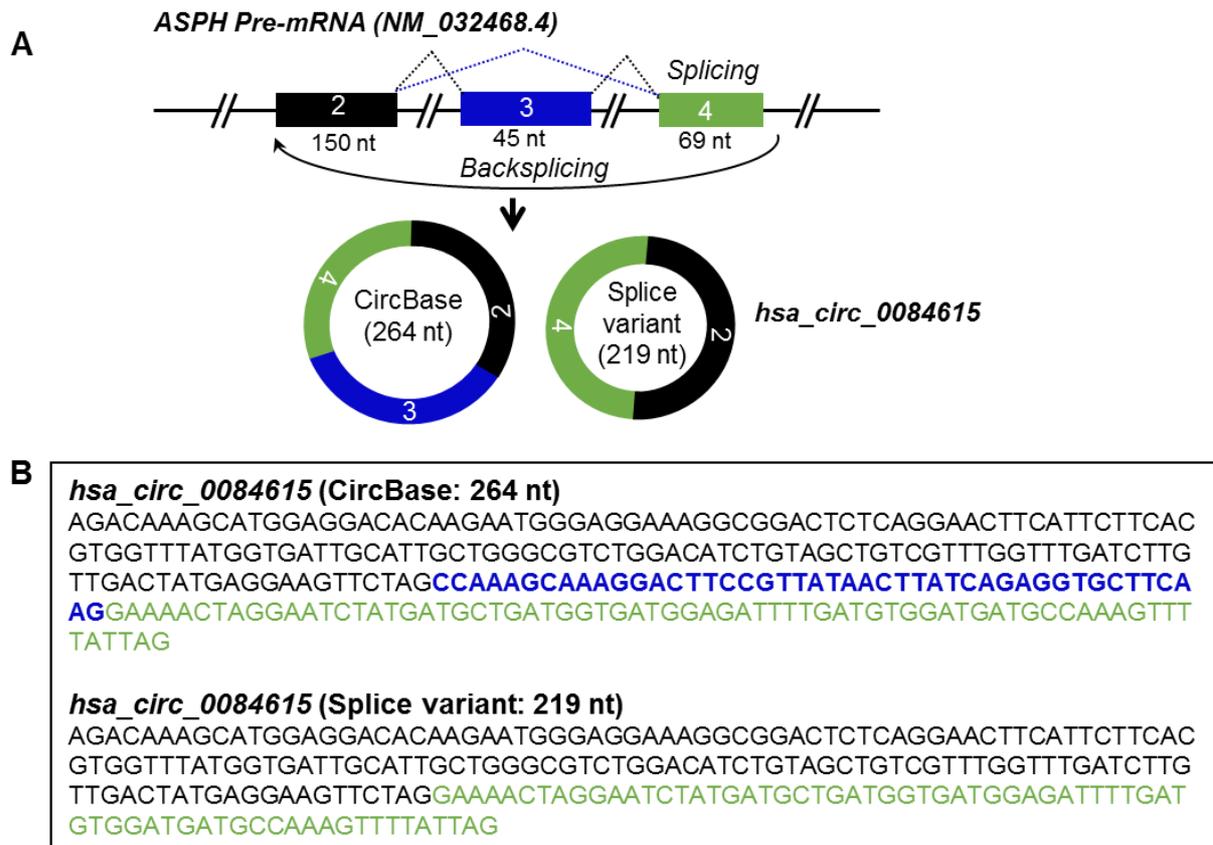


Figure S4. Splice variants of *hsa_circ_0084615*. (A) Schematic representation of the *hsa_circ_0084615* splice variants biogenesis from *ASPH* pre-mRNA. Boxes and straight lines represent exons and introns, respectively. The black dotted lines represent splicing while blue dotted lines represent alternative splicing. (B) Spliced full-length sequences of splice variants of *hsa_circ_0084615*. The text colors correspond to the colors of the exon boxes in panel A.

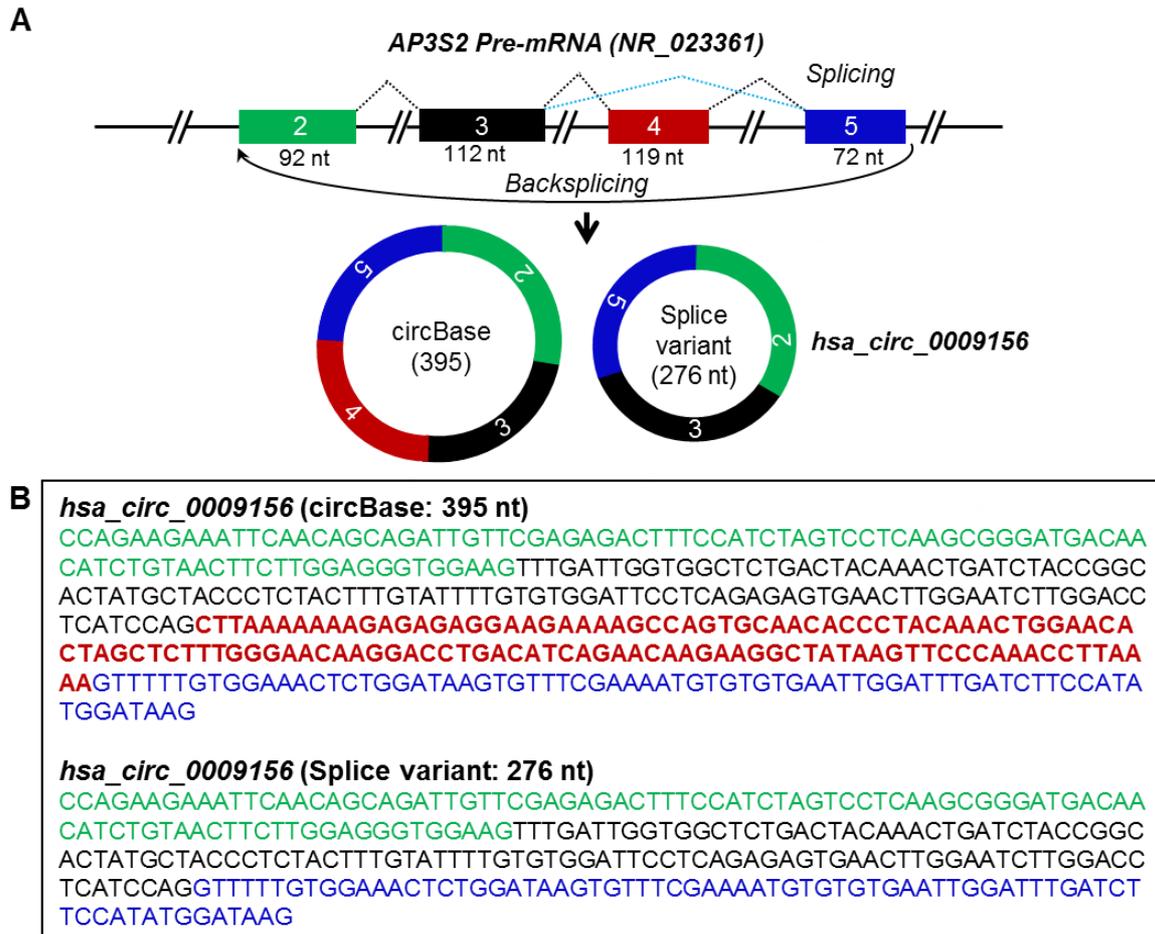
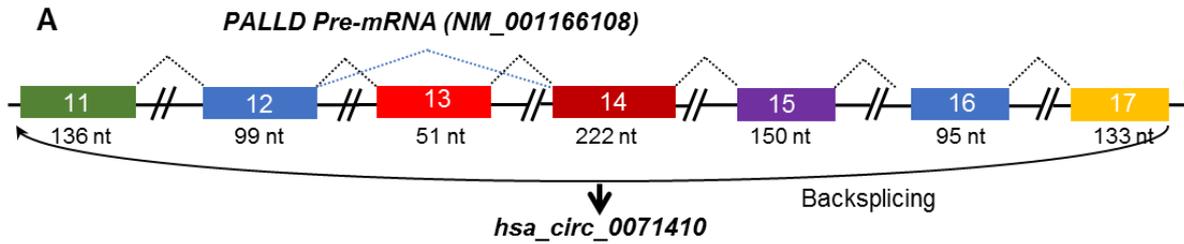


Figure S5. Splice variants of *hsa_circ_0009156*. (A) Schematic representation of the biogenesis of the splice variants of *hsa_circ_0009156* from precursor *AP3S2* pre-mRNA. Boxes and straight lines represent exons and introns, respectively. The black dotted lines represent splicing while blue dotted lines represent alternative splicing. (B) Spliced full-length sequences of splice variants of *hsa_circ_0009156*. The text color corresponds to the color of the exon box in panel A.



B *hsa_circ_0071410* (circBase: 886 nt)

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AGGATTTCCAAAGAAGGCCAGTAGAACTGCTAGAATAGCCTCCGATGAGGAAATTCAAGGCACA
AAGGATGCTGTTATTCAAGACCTGGAACGAAAACCTCGCTTCAAGGAGGACCTCCTGAACAATG
GCCAGCCGAGGTTAACATACGAAGAAAGAATGGCTCGTCGACTGCTAGGTGCTGACAGTGCAA
CTGTCTTTAATATTCAGGAGCCAGAAGAGGAAACAGCTAATCAGGACATTGGTTCTCCTCATGC
TTCTGTAGGGAGTCTCTGGATGGTCAAAAAGGAATACAAAGTCTCCAGCTGTGAACAGAGACT
CATCAGTGAAATAGAGTACAGGCTAGAAAGGTCTCCTGTGGATGAATCAGGTGATGAAGTTCAG
TATGGAGATGTGCCTGTGGAATAATGGAATGGCACCATTCTTTGAGATGAAGCTGAAACATTACA
AGATCTTTGAGGGAATGCCAGTAACTTTACATGTAGAGTGGCTGGAAATCCAAAGCCAAAGAT
CTATTGGTTTAAAGATGGGAAGCAGATCTCTCAAAGAGTGATCACTACACCATTCAAAGAGATC
TCGATGGGACCTGCTCCCTCCATACCACAGCCTCCACCCTAGATGATGATGGGAATTATACAAT
TATGGCTGCAAACCCTCAGGGCCGCATCAGTTGTAAGTGGACGGCTAATGGTACAGGCTGTCAA
CCAAAGAGGTCTGAAGTCCCGGTCTCCCTCAGGCCATCCTCATGTCAGAAGGCCCTCGTTCTAG
ATCAAGGGACAGTGGAGACGAAAATGAACCAATTCAGGAGCGATTCTTCAGACCTCACTTCTTG
CAGGCTCCTGGAGATCTGACTGTTCAAGAAGGAAAACCTGCAGAATGGACTGCAA

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hsa_circ_0071410 (Splice variant: 835 nt)

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AGGATTTCCAAAGAAGGCCAGTAGAACTGCTAGAATAGCCTCCGATGAGGAAATTCAAGGCACA
AAGGATGCTGTTATTCAAGACCTGGAACGAAAACCTCGCTTCAAGGAGGACCTCCTGAACAATG
GCCAGCCGAGGTTAACATACGAAGAAAGAATGGCTCGTCGACTGCTAGGTGCTGACAGTGCAA
CTGTCTTTAATATTCAGGAGCCAGAAGAGGAAACAGCTAATCAGGAATACAAAGTCTCCAGCTG
TGAACAGAGACTCATCAGTGAAATAGAGTACAGGCTAGAAAGGTCTCCTGTGGATGAATCAGGT
GATGAAGTTCAGTATGGAGATGTGCCTGTGGAATAATGGAATGGCACCATTCTTTGAGATGAAGC
TGAAACATTACAAGATCTTTGAGGGAATGCCAGTAACTTTACATGTAGAGTGGCTGGAAATCCA
AAGCCAAAGATCTATTGGTTTAAAGATGGGAAGCAGATCTCTCAAAGAGTGATCACTACACCAT
TCAAAGAGATCTCGATGGGACCTGCTCCCTCCATACCACAGCCTCCACCCTAGATGATGATGG
GAATTATACAATTATGGCTGCAAACCCTCAGGGCCGCATCAGTTGTAAGTGGACGGCTAATGGTA
CAGGCTGTCAACCAAGAGGTCTGAAGTCCCGGTCTCCCTCAGGCCATCCTCATGTCAGAAGG
CCTCGTTCTAGATCAAGGGACAGTGGAGACGAAAATGAACCAATTCAGGAGCGATTCTTCAGAC
CTCACTTCTTGACAGGCTCCTGGAGATCTGACTGTTCAAGAAGGAAAACCTGCAGAATGGACTG
CAA

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Figure S6. Splice variants of *hsa_circ_0071410*. (A) Schematic representation of the biogenesis of *hsa_circ_0071410* splice variants from *PALLD* pre-mRNA. Boxes and straight lines represent exons and introns, respectively. The black dotted lines represent splicing while blue dotted lines represent alternative splicing. (B) Spliced full-length sequences of splice variants of *hsa_circ_0071410*. The text colors correspond to the colors of the exon boxes in panel A.

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hsa_circ_0009156 (miRNA sites)	
Splice variant (276 nt)	circBase (395 nt)
hsa-miR-6503-3p (1)	hsa-miR-6503-3p (1)
hsa-miR-3692-3p (1)	hsa-miR-3692-3p (1)
hsa-miR-490-3p (1)	hsa-miR-320e (1)
	hsa-miR-5195-3p (1)
	hsa-miR-145-5p (1)
	hsa-miR-186-3p (1)

Figure S7. Potential miRNA targets of circRNA splice variants. MicroRNAs predicted by miRDB web tool to target splice variants of *hsa_circ_0009156*.

A		B	
hsa_circ_0009156 (RBP sites)		hsa_circ_0084615 (RBP sites)	
Splice variant (276 nt)	circBase (395 nt)	Splice variant (219 nt)	circBase (264 nt)
PTBP1 (13)	PTBP1 (16)	CUG-BP (16)	CUG-BP (17)
SNRNP70 (2)	SNRNP70 (4)	G3BP2 (1)	G3BP2 (2)
SRSF1 (7)	SRSF1 (5)	MBNL1 (14)	MBNL1 (13)
SRSF10 (4)	SRSF10 (9)	SRSF1 (13)	SRSF1 (12)
SRSF3 (29)	SRSF3 (40)	SRSF2 (17)	SRSF2 (16)
SRSF5 (6)	SRSF5 (7)	SRSF3 (20)	SRSF3 (14)
SRSF7 (5)	SRSF7 (7)	SRSF5 (7)	SRSF5 (5)
TRA2B (9)	TRA2B (19)	TRA2B (4)	TRA2B (3)
YBX2 (3)	YBX2 (7)		DAZAP1 (3)
	CNOT4 (1)		MSI1 (2)
	ENOX1 (2)		PABPC5 (2)
	FMR1 (3)		SRSF7 (1)
	FXR2 (1)		
	HNRNPA1 (3)		
	HNRNPH1 (4)		
	HNRNPK (1)		
	HNRNPL (3)		
	IGF2BP2 (2)		
	IGF2BP3 (2)		
	KHDRBS1 (5)		
	KHDRBS2 (4)		
	KHDRBS3 (6)		
	PABPC1 (7)		
	PABPC4 (7)		
	PABPN1 (9)		
	SART3 (7)		
	SRSF9 (1)		
	TRA2A (4)		

Figure S8. Potential RBPs associated with circRNA splice variants. Selected list of RBPs predicted by RBPmap web tool to target splice variants of *hsa_circ_0009156* (A), and *hsa_circ_0084615* (B).