The functional circular RNA, ciRS-7 (CDR1as), is biosynthesized using back-splicing promoted by inverted msmmslisn-wide MIRs but not primate-specific *Alu*s

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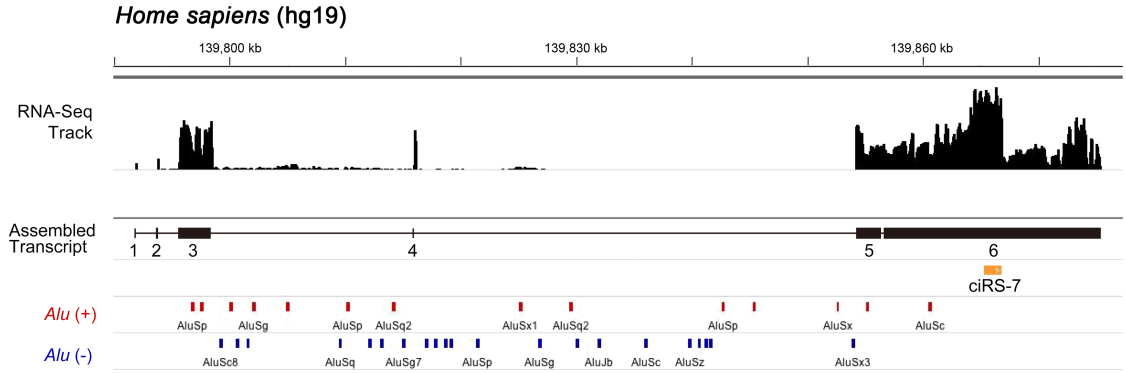

Figure S1. The ciRS-7 exon does not have flanking inverted *Alu* elements.
Modified screeenshot of human brain RNA-Seq data (GSE59612) mapped on the the human hg19 genome sequence is showing the assembled ciRS-7 precursor transcript. The transcript was assembled by StringTie. The locations of the human *Alu* elements, which were extracted from UCSC Reperat Masker database, are shown by bars (+ is the same strand as the ciRS-7 exon).

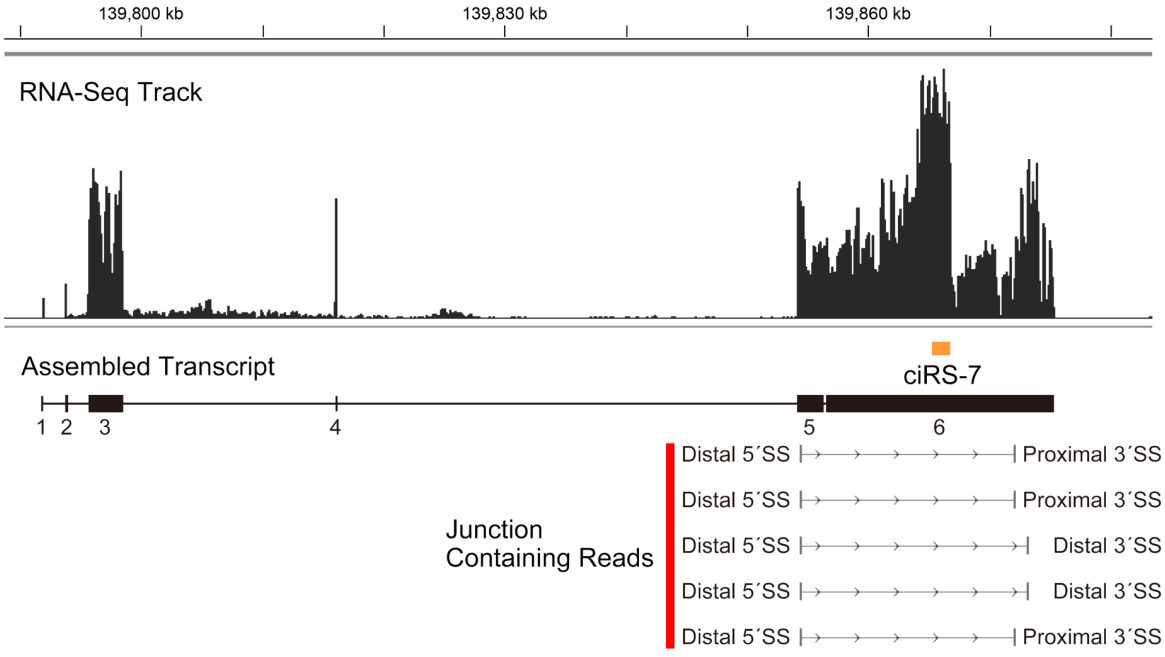

Figure S2. Alternative splicing events skip the ciRS-7 exon.
The assembled ciRS-7 precursor tarnscript (see Fig. S1) is shown together with the identified alternative splicing events. The junction containing reads revealed the active alternative 5′ and 3′ splice sites within the exons 5 and 6.

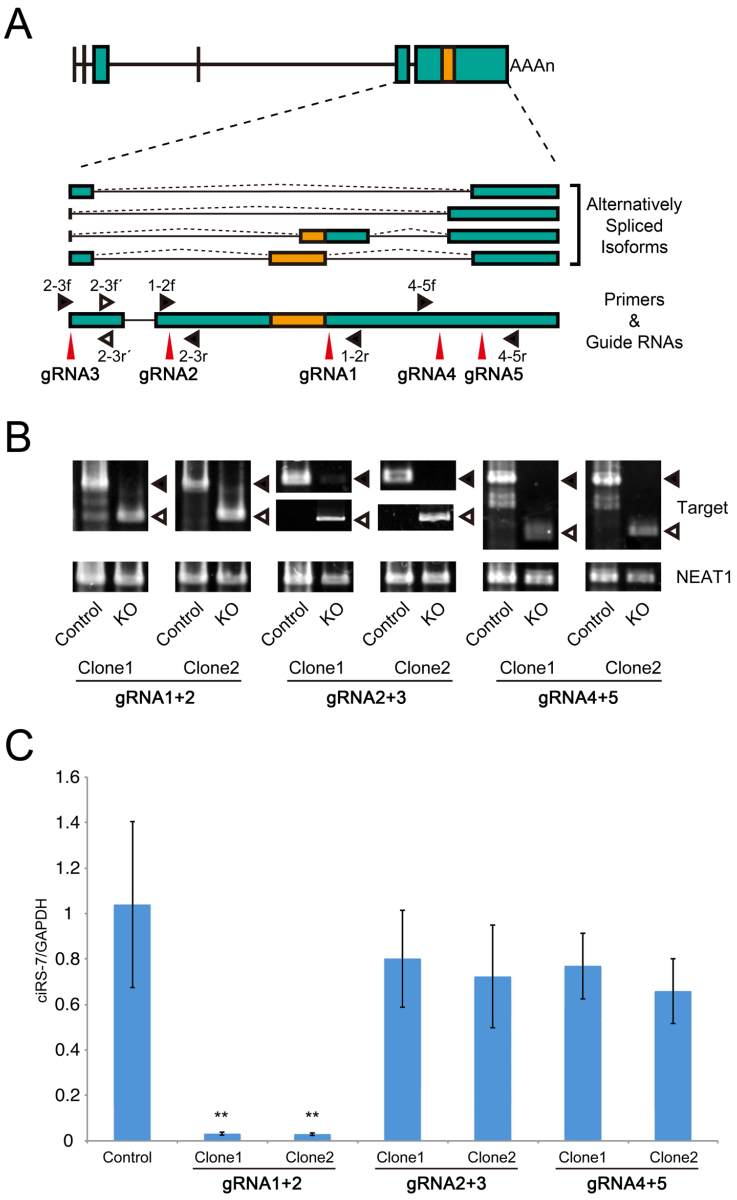

Figure S3. CRISPR/Cas9-mediated ciRS-7 genomic deletion of the flanking alternative splice sites confirms 'Back-splicing pathway' for ciRS-7 biosynthesis.
(A) Schematic structures of the ciRS-7 precursor and its alternatively spliced isoforms (orange box indicates ciRS-7 exon). The positions of the guide RNAs (gRNA1–gRNA5) targetting the alternative splice sites, and PCR primers for detecting deleted sites (filled triangles) and those for detecting un-deleted sites (open triangeles) are also indicated.
(B) The targeted ciRS-7 genomic deletions in HEK-293 cells were verified by genomic PCR. The indicated three pairs of gRNAs were used to delete the alternative splice sites. PCR primers indicated in (A) were used for detecting deleted sites (open triangles) and non-deleted sites (filled triangles). PCR primers for the *NEAT1* gene were used as control.
(C) The effects of the alternative splice site deletions, described in (A), on ciRS-7 production was analyzed by quantitative RT–PCR with ciRS-7 primers and the control GAPDH primers. The ciRS-7 expression levels were normalized to the control expression level of GAPDH (ciRS-7/GAPDH). The results were plotted as ratios to the value of control wild-type cells. Means ± standard deviation (SD) are given for three independent experiments (**P < 0.01).

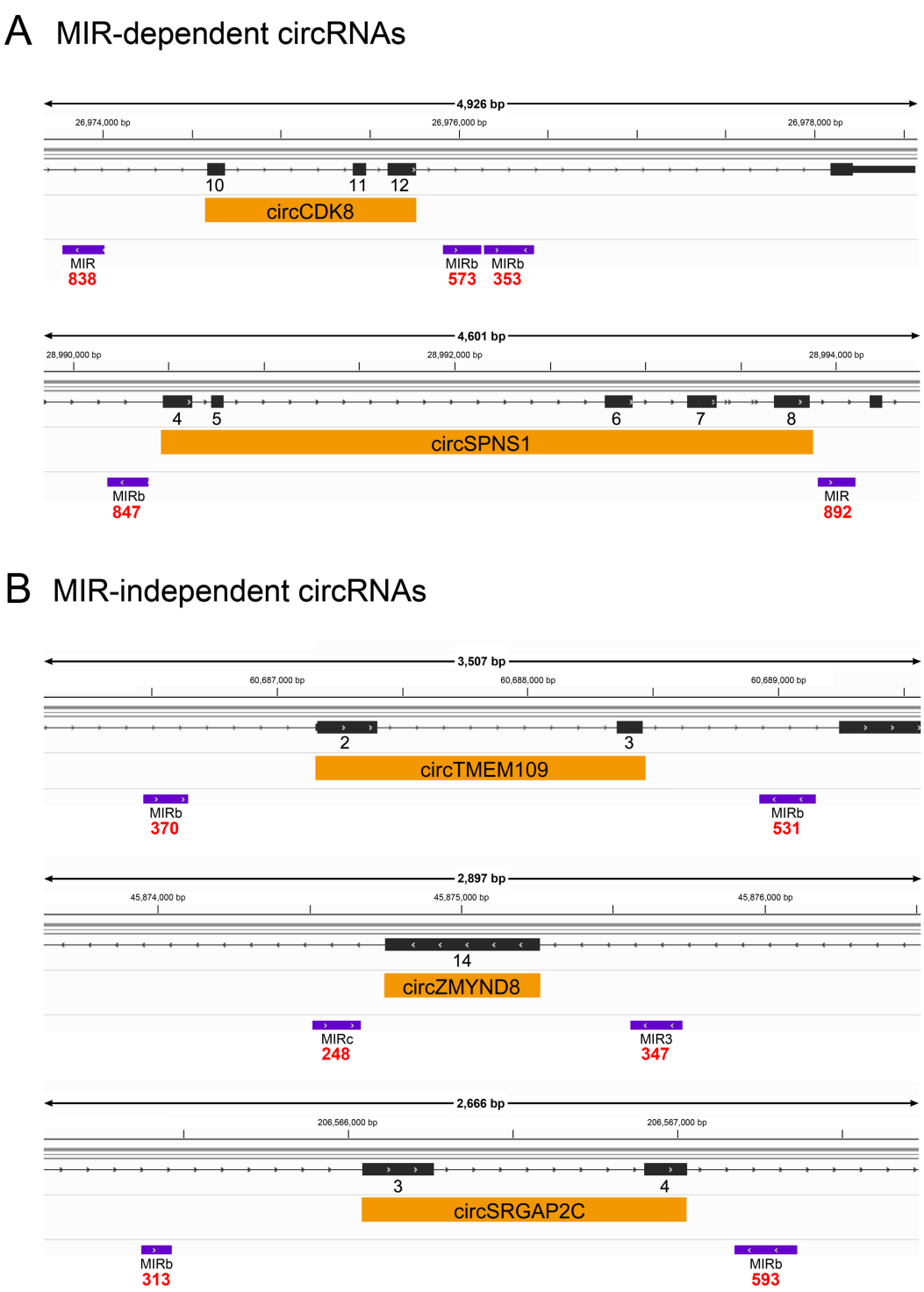

Figure S4. Identified another human circRNAs revealed different MIR-dependency.
The loci of two MIR-dependent circRNAs **(A)** and three MIR-independent circRNAs **(B)** are shown. The positions of the host genes (with red numbered exons), circRNA exons (in orange), and the identified inverted MIR elements (in purple) are indicated. Red numbers indicate Smith-Waterman (SW) alignment scores of these MIR elements. SW alignment scores of MIRs in MIR-dependent human ciRS-7 (see Fig. 2A) are 945 (upstream) and 1077 (downstream).

Table S1. List of all the synthetic oligonucleotides unsed in the experiments.

Primer DNAs for plasmids construction		
ciRS-7-5´/BamHI	5'-AAAGGATCCACCCCTGGGTTATTGGGTTGTGGAGAATCA-3'	For pcDNA3-ciRS-7
ciRS-7-3´/XhoI	5'-AAACTCGAGACCTTTGGATCACATCATTCTATCATGATT-3'	
ciRS-7-Δ5´MIR/BamHI	5'-AAAGGATCCATTATTATAACATGAATAAGCATTATGCAA-3'	For pcDNA3-ciRS-7ΔMIR
ciRS-7-Δ3´MIR/XhoI	5'-AAACTCGAGTCTTTGGTCATGTAAGACCAATGATAAAAA-3'	
circCDK8-5´/BamHI	5'-AAAGAATTCAATGCAGTCTCAGTCCAGCAG-3'	For pcDNA3-circCDK8
circCDK8-3´/XhoI	5'-TTTCTCGAGCACTCGGGACCTTATCAGTTT-3'	
circCDK8-Δ5´MIR-R	5'-ATCACACAGCTCTGTACTTTGTAAGTTGTG-3'	For pcDNA3-circCDK8Δ5´MIR
circCDK8-Δ5´MIR -F	5'-TCTGGGACTTGATGAAATGGCTTTTCTTCT-3'	
circCDK8-Δ3´MIR-R	5'-TGTCAGTACTTTTCCATGCCAGTAACATTT-3'	For pcDNA3-circCDK8Δ3´MIR
circCDK8-Δ3´MIR-F	5'-GCTATTGTTTTGGAACAATGTTTCACAGTG-3'	
circSPNS1-infusion-F	5'-TGACGATAAAGGATCCAGGGGTTGTCTTATTTAGA-3'	For pcDNA3-circSPNS1
circSPNS1-infusion-R	5'-TAGATGCATGCTCGACATCTATTAACTAATTTAATCCT-3'	
circSPNS1-Δ5´MIR-F	5'-TGACGATAAAGGATCTGGGAGCGGGCTGGTGAG-3'	For pcDNA3-circSPNS1ΔMIR
circSPNS1-Δ3´MIR-R	5'-TAGATGCATGCTCGAGACCCACGCCATCCCCCT-3'	
circTMEM109/BamHI	5'-AAAGGATCCGGAGACAATACAGCAGAGTTG-3'	For pcDNA3-circTMEM109
circTMEM109/XhoI	5'-TTTCTCGAGACATTGCAAAGGAATAAGCCG-3'	
circTMEM109-Δ5´MIR/BamHI	5'-AAAGGATCCTGAATGTTAGCTTTTTATTAA-3'	For pcDNA3-circTMEM109Δ5,3MIR
circTMEM109-Δ3´MIR/XhoI	5'-TTTCTCGAGTGGTGGCGGAAGTTCTTTTTT-3'	
circSRGAP2C/BamHI	5'-CCCAGATCTATCCAGAGGAGAGTCGGTGTT-3'	For pcDNA3-circSRGAP2C
circSRGAP2C/XhoI	5'-TTTCTCGAGATGTCAGGGGGAGCTGGATAC-3'	
circSRGAP2C-Δ5´MIR/BamHI	5'-CCCAGATCTCAATGTTTATTAAGTGGTAGA-3'	For pcDNA3-circSRGAP2CΔ5,3MIR
circSRGAP2C-Δ3´MIR/XhoI	5'-TTTCTCGAGGCATTGTAACTCTTTAGTTAA-3'	
circZMYNDR8/BamHI	5'-CGGAATTCGGCCTAGACACAAAGGATCAAG-3'	For pcDNA3-circZMYNDR8
circZMYNDR8/XhoI	5'-TTTCTCGAGTGGAAAAGAAGCTGCTCAGAC-3'	
circZMYNDR8-Δ5´MIR/BamHI	5'-CCCAGATCTCAATGTTTATTAAGTGGTAGA-3'	For pcDNA3-circZMYNDR8Δ5,3MIR
circZMYNDR8-Δ3´MIR/XhoI	5'-TTTCTCGAGGGTCACATAACTTTTGATAGT-3'	
Primer DNAs for splicing products analysis		
ciRS-7-F(nested)	5'-TAAGGATGGCCCAGAAGGAG-3'	For PCR detection of alternatively spliced products from ciRS-7 precursor
ciRS-7-R(nested)	5'-GGAACAGTTAGGGATCCCTT-3'	
ciRS-7-F	5'-TCCGCGCCTTTGAGAGCTTTGGAACGATAT-3'	
ciRS-7-R	5'-CAGAAACAATGGCAATTATAATAGTTAAAC-3'	
circSPNS1-F(nested)	5'-TGCTCTTCCTCTGACAGTCTC-3'	For PCR detection of circSPNS1 cDNA
circSPNS1-R(nested)	5'-AAGAGGTCGGCAATGAGAGTG-3'	
circSPNS1-F	5'-GCGCGATGGTGGAATAACTG -3'	
circSPNS1-R	5'-TTCCTCTTCCTGTCCCTTGC -3'	
circCDK8-F	5'-TGAGAGTTGTTCCTCCTACCAC-3'	For PCR detection of circCDK8 cDNA
circCDK8-R	5'-TCCTGCATAGCCTGTTCTGAG-3'	
circTMEM109-F	5'-TTCTGTGGCCTTCTTTGCTC-3'	For PCR detection of circTMEM109 cDNA
circTMEM109-R	5'-AACACATGCTTTCCCCATGG-3'	
circZMYNDR8-F	5'-AGGAACCCAAAGAACCATCTCC-3'	For PCR detection of circZMYNDR8 cDNA
circZMYNDR8-R	5'-TTCTCAGAATCCTCCGAATCGC-3'	
circSRGAP2C-F	5'-CAACCAATGCATCTGTCTTCAAG-3'	For PCR detection of circSRGAP2C cDNA
circSRGAP2C-R	5'-GCTGTCGGCATTGTACATGTG-3'	
ciRS-7-F	5'-ACGTCTCCAGTGTGCTGA-3'	For PCR detection of ciRS-7 cDNA
ciRS-7-R	5'-GCTGTCGGCATTGTACATGTG-3'	
GAPDH-F	5'-AGCCACATCGCTCAGACAC-3'	For PCR detection of GAPDH cDNA
GAPDH-R	5'-GCCCAATACGACCAAATCC-3'	
pcDNA3 3´UTR-F	5'-ATGCATCTAGAGGGCCCTATTC-3'	For PCR detection of circRNA precursor cDNAs from reporter pDNA3-plasmids
pcDNA3 3´UTR-R	5'-AACAACAGATGGGCTGGCAAC-3'	
pcDNA5 FRT/TO 3´UTR-F	5'-AGTCTAGAGGGCCCGTTTAAAC-3'	For PCR detection of circRNA precursor cDNAs from reporter pDNA5-plasmids
pcDNA5 FRT/TO 3´UTR-R	5'-TTAGGAAAGGACAGTGGGAGTG-3'	
gRNA1-2F	5'-ACTGACTTCTTGTCTGCTCTG G-3'	For PCR detection of editing & un-editing by gRNA1+2
gRNA1-2R	5'-TGCCTGGATGTAGCAAATGC-3'	
gRNA2-3F	5'-TCCTAGATGGTGTGCTTCTCA C-3'	For PCR detection of editing by gRNA2+3
gRNA2-3R	5'-CAGTCACACAGCTGGTAATTG G-3'	
gRNA2-3F'	5'-ATCGGGGGCACACAAGAATC-3'	For PCR detection of un-editing by gRNA2+3
gRNA2-3R'	5'-TGCCATTGTCCTCAATCGTC-3'	
gRNA4-5F	5'-TTTCCTGCTCTGTAGAAAGTCA-3'	For PCR detection of editing & un-editing by gRNA4+5
gRNA4-5R	5'-TCAGCTTGTCAGATGCCAGA-3'	
Antisense 2'-*O*-Me RNAs for blocking alternative splice sites (*:phosphothiorate backbone, mN: 2'-O-methyl oxyribonucleotides)		
GFP-ASO	5'-mG*mC*mA*mC*mC*mA*mU*mC*mU*mU*mC*mU*mU*mC*mA*mA*mG*mG*mA-3'	For off-target negative control
ciRS-7-ASO1	5'-mA*mU*mC*mG*mG*mA*mA*mA*mC*mC*mC*mU*mG*mG*mA*mC*mA*mU*mU*mG-3'	For 3´ splice site of ciRS-7 exon
ciRS-7-ASO2	5'-mG*mU*mU*mA*mG*mA*mA*mU*mA*mC*mC*mU*mG*mG*mA*mU*mA*mU*mU*mG-3'	For 5' splice site of ciRS-7 exon
ciRS-7-ASO3	5'-mA*mA*mA*mG*mG*mC*mU*mA*mA*mC*mC*mA*mG*mU*mU*mU*mU*mU*mG*mU-3'	For prox. alternative 5' splice site
ciRS-7-ASO4	5'-mU*mA*mC*mC*mA*mA*mU*mG*mU*mG*mC*mU*mA*mA*mA*mA*mG*mA*mA*mA-3'	For dist. alternative 3' splice site
ciRS-7-ASO5	5'-mG*mU*mC*mU*mU*mC*mU*mC*mA*mC*mA*mG*mG*mA*mG*mG*mU*mA*mG*mC-3'	For dist. alternative 5' splice site
ciRS-7-ASO6	5'-mA*mU*mC*mC*mA*mG*mA*mG*mA*mU*mC*mU*mA*mC*mA*mA*mA*mU*mU*mA-3'	For prox. alternative 3' splice site
Guide RNAs for editing alternative splice sites		
gRNA1	5'TACTGTTGGTTCATAAGAT-3'	For positive control (gRNA1+2) & for deletion of alternative 5' splice site (gRNA2+3)
gRNA2	5'-CTCCACAACCCAATAACCCA-3'	
gRNA3	5'-AAGGTCAGGCTATCACGCTG-3'	
gRNA4	5'-TTTGAGCTAAATTGCCC-3'	For deletion of alternative 3' splice site (gRNA4+5)
gRNA5	5'-AACATCAATCTGCATTG-3'	