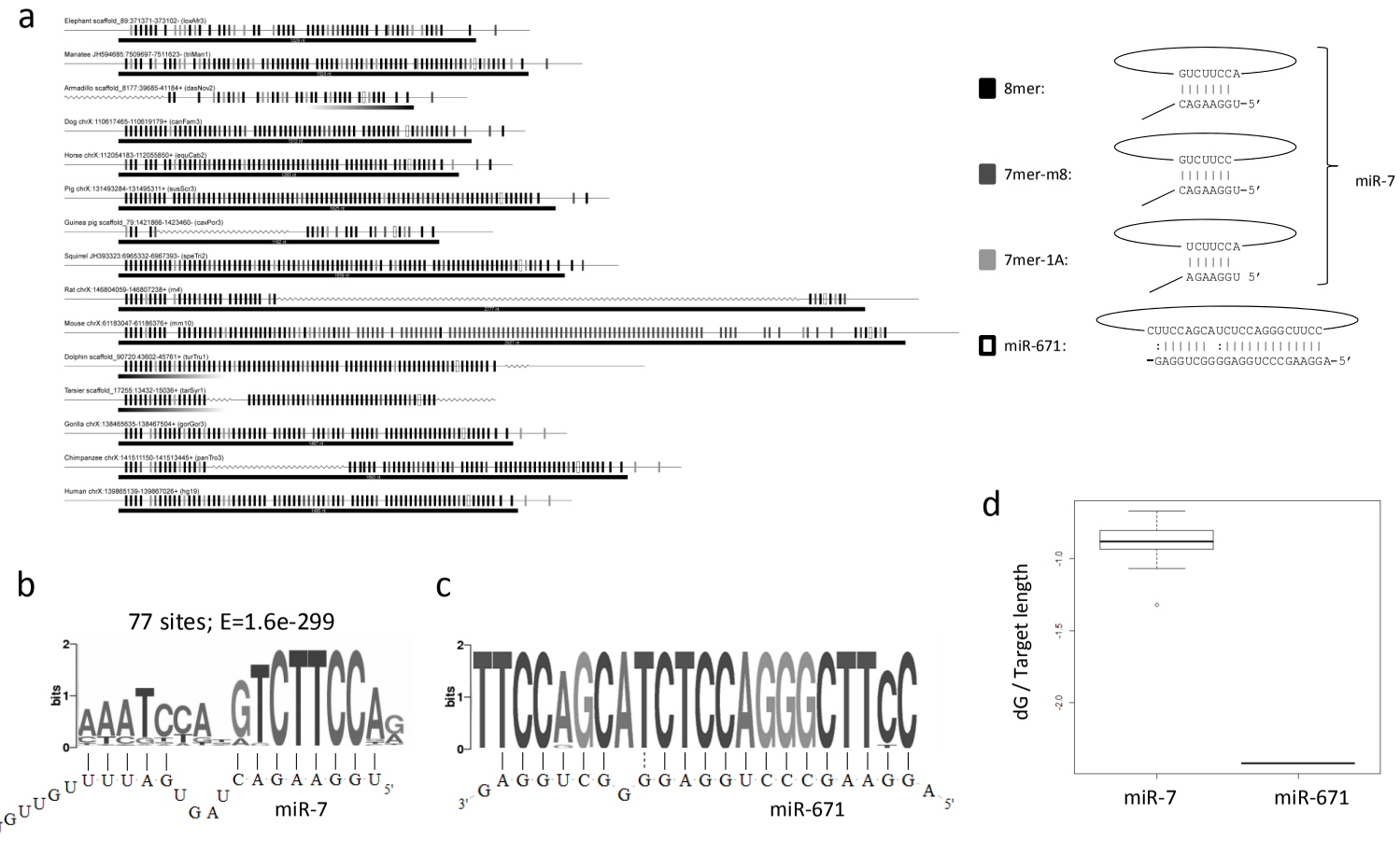
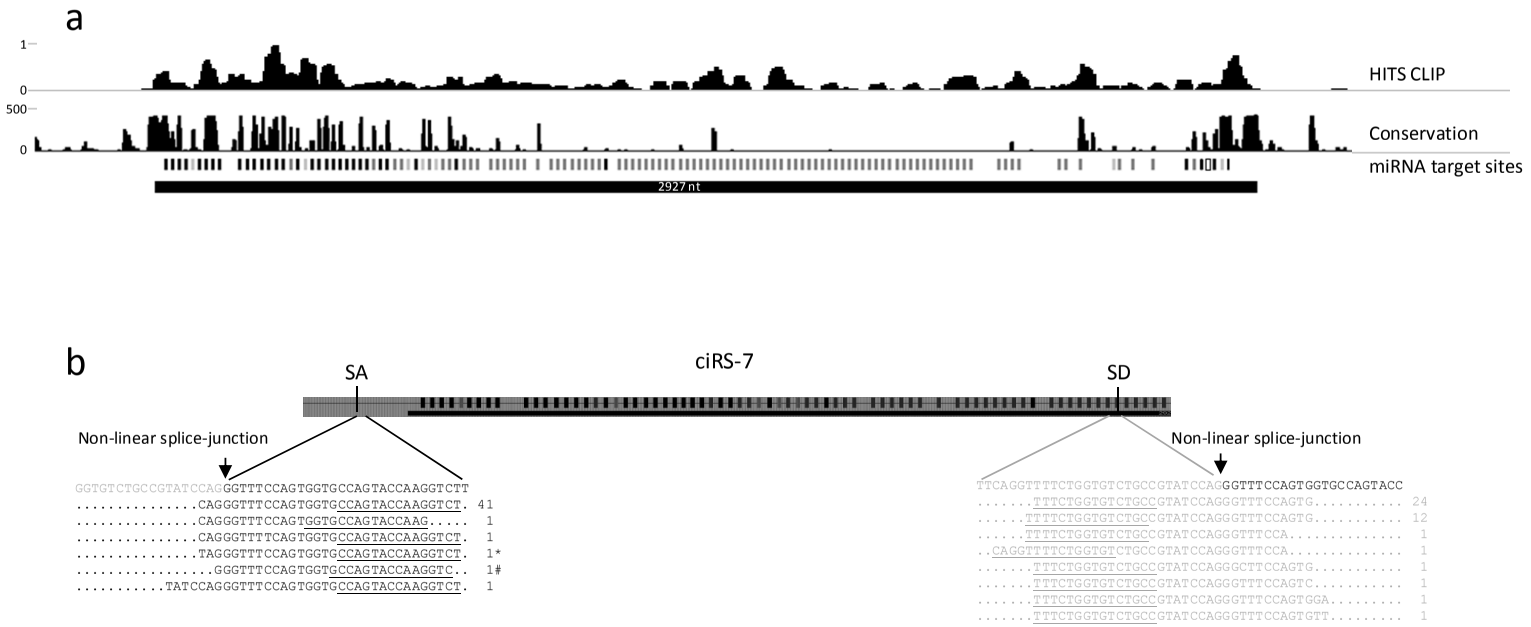
doi:10.1038/nature11993

Supplementary Table 1: Primers and probes. Sequences are written in 5'-3' direction.

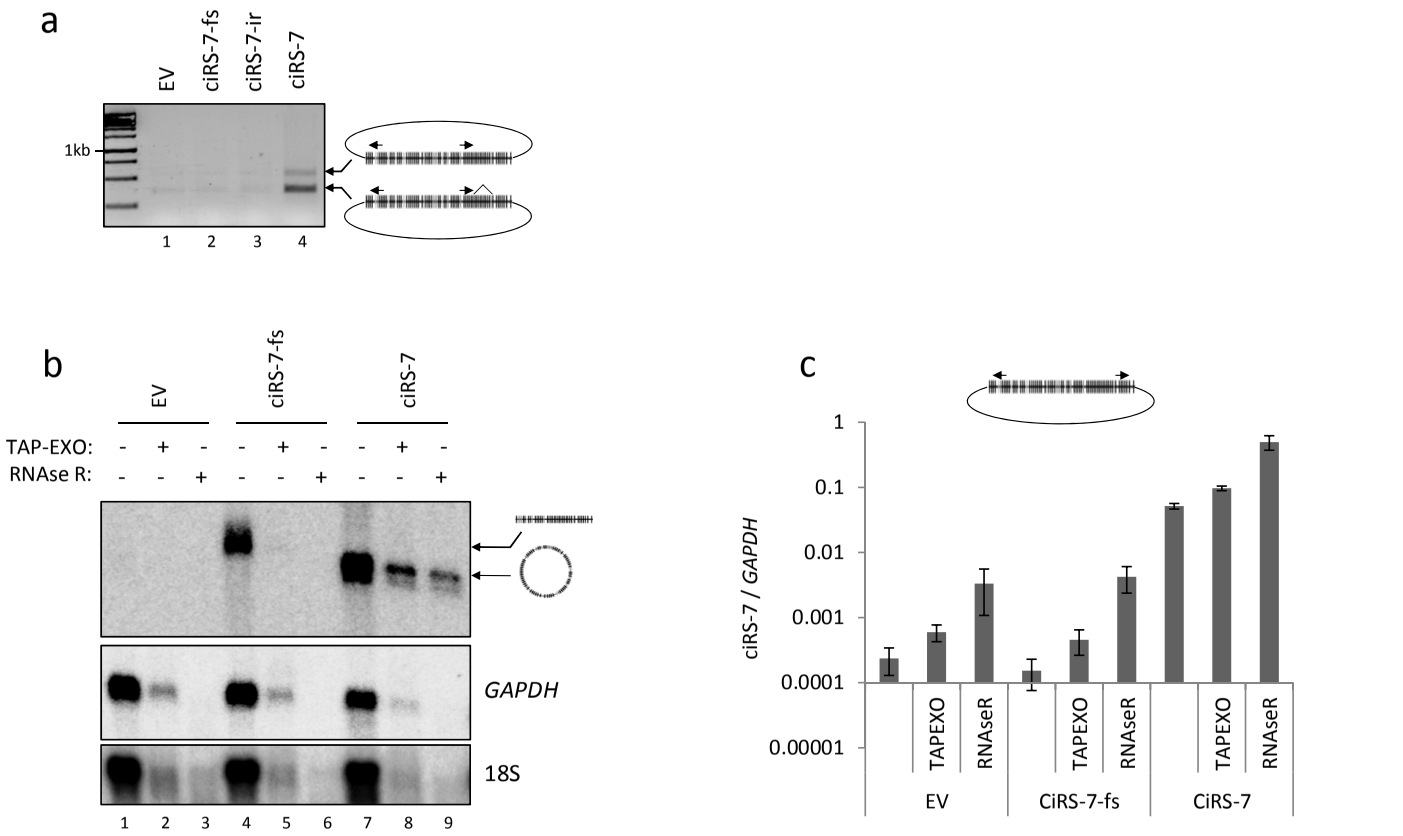
Vector construction	
ciRS-7 forward	GACCCAAGCTTCTGTAAGAGTAGTCTCATGATGT
ciRS-7 reverse	TTCAGGCGGCCGCCAAACTGCAGTACTGTTGGTTCAT
ciRS-7ir forward	TAGAACTCGAGCTGTAAGAGTAGTCTCATGATGT
ciRS-7ir reverse	TAGAACTCGAGTGGTTACTCTGGACATTGAT
ciRS-7fs forward	TTCAGAAGCTTAGGGTTTCCGATGGCACCT
ciRS-7fs reverse	TAGAACTCGAGCTGGATATTGCAGACACTGG
Sry forward	TTCAGGGATCCTGGACTAGGGAGGTCCTGAA
Sry reverse	TAGAAGCGGCCGCAACCCAAAAGGAAGGAGCAG
miR-7-1 forward	ATAGAAGCGGCCGCGCCATGGTGTCTCAACCTTT
miR-7-1 reverse	ATAGAAGTCGACGCAATGTTAAGTGTCAAGAAAAATG
miR-138 forward	ATAGAAGCGGCCGCGTCATCCTGTCTCCCCTCCT
miR-138 reverse	TAGAAGTCGACGGTTTCCTCACAGGCAGGT
miR-7-psiCheck forward	TAGAACTCGAGACAACAAAATCACTAGTCTTCCATCTAGAATGAC
miR-7-psiCheck reverse	GTCATTCTAGATGGAAGACTAGTGATTTTGTTGTCTCGAGTTCTA
ciRS-7-psiCheck forward	TTCAGCTCGAGGGTTTCCGATGGCACCTG
ciRS-7-psiCheck reverse	TTCAGGCGGCCGCCTGGATATTGCAGACACTG
Sry-psiCheck forward	TAGAACTCGAGCATTTCAGATCTTGATTTTTAGTGT
Sry-psiCheck reverse	GTCATGCTAGCCAGTGATGTCAGCTGTTAGTAAGT
EGFR-psiCheck forward	AATTGCGGCCGCCACGGAGGATAGTATGAGC
EGFR-psiCheck reverse	AATTTCTAGATTCATTGAGACAAAAATCAAATAC
qPCR / RT-PCR	
ciRS-7 forward	ACGTCTCCAGTGTGCTGA
ciRS-7 reverse	CTTGACACAGGTGCCATC
ciRS-7 (2) forward	ATAGCTTCCGAAAAATCCAG
GAPDH forward	GTCAGCCGCATCTTCTTTTG
GAPDH reverse	GCGCCCAATACGACCAAATC
Sry forward	AGTTCCACGACCAGCAGCT
Sry reverse	CAGCTGCTTGCTGATCTCTGTA
SNCA forward	CTGCTGCTGAGAAAACCA
SNCA reverse	CCTTGGTTTTGGAGCCTA
EGFR forward	CGAGGGCAAATACAGCTT
EGFR reverse	GCCGTCTTCCTCCATCT
IRS2 forward	TCTTGTCCCACCACTTGA
IRS2 reverse	CAGTGCTGAGCGTCTTCT
Oligo-dTN	GCGAGCACAGAATTAATACGACTCACTATAGGTTTTTTTTTTTTVN
3RACE reverse	GCGAGCACAGAATTAATACGACT
Probes	
miR-7 probe	ACAACAAAATCACTAGTCTTCCA
miR-769 probe	AGCTCAGAACCCAGAGGTCTCA
miR-138 probe	CGGCCTGATTCACAACACCAGCT
miR-15b probe	TGTAAACCATGATGTGCTGCTA
ciRS-7 probe	TTGGAAGACTTGAAGTCGCTGGAAGACCCGGAGTTGTTGGAAGACCTTGACACAGGTGCC
Sry probe	GGCTGCCAATAAAAGCTTTGCTGGTTTTTGGAGTACAGGTGTGCAGCTCTACTCCAGTCT
18S probe	TTACCGCGGCTGCTGGCACCAGACTTGCCCTCCAATGGATCCTCGTTAAAGGATTTAAAGTGGACTCATTCCAATTACAG
ciRS-7 (hsa) FISH probe (amino-allyl-T underlined)	TACATGGATTTGTTGGAAGACATGGATTTTCTGGAAGACATGGATTTTCT
ciRS-7 (mmu) FISH probe (amino-allyl-T underlined)	TTCTGGGAAGACTTGGATTTTCGGGAAGACTTGGATTTTCGGGAAGACTT
ciRS-7 (mmu) AP-probe	TGGGAAGACTCGGATTTCTGGGAAGACT
miR-7 FISH/AP-probe (LNA underlined)	ACAACAAAATCACTAGTCTTCCA
miR-449 FISH Probe (LNA underlined)	ACCAGCTAACAATACACTGCCA
Gapdh AP-probe	CCTGCTTCACCACCTTCTTGATGTCA
siRNA / miRNA	
miR-138 biotin guide	AGCTGGTGTTGTGAATCAGGCCG[Btn]
miR-138 passenger	GCCTGATTCACAACACCAGCGCC
miR-7 biotin guide	TGGAAGACTAGTGATTTTGTTGT[Btn]
miR-7 passenger	CAACAAAATCACTAGTCTACCTAA



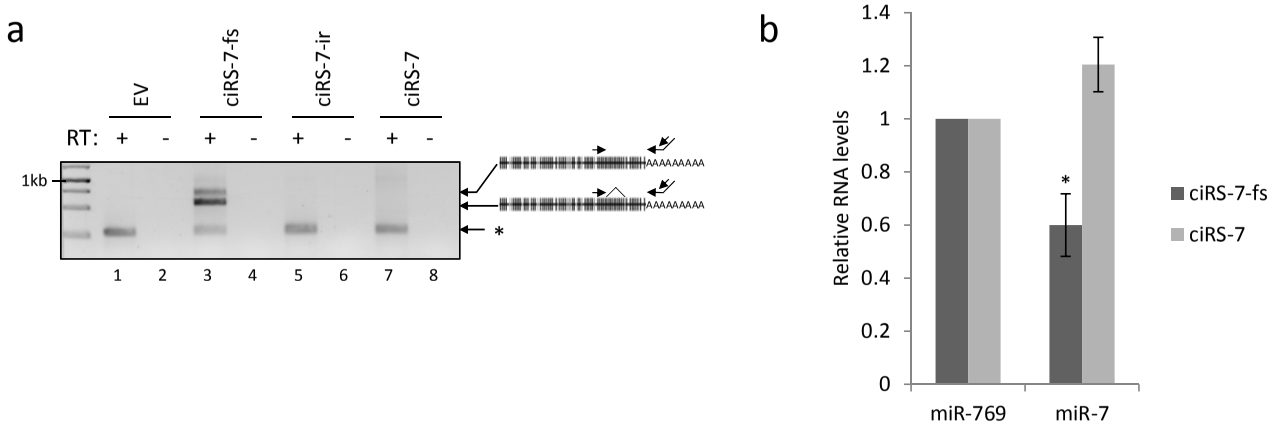
Supplementary Figure 1: Evolutionary conservation of ciRS-7. a, Organization of ciRS-7 locus in selected mammalian species. miR-7 and miR-671 target sites are denoted according to legend shown to the right. miR-671 target site is defined as the 22mer, TTCCAGCATCTCCAGGGCTTCC, allowing 1 mismatch. The boundaries of ciRS-7 (black horizontal bar) is based on the position of conserved splice donor (TCCAGGTATT) and splice acceptor (TCCAAT[AG]TC[CT]AGGG, optional nucleotides are shown in brackets) sites. The crinkled lines represent gaps in the genome assembly. **b**, Weblogo of the MEME[1] computed highest ranking 6-17mer occurring between 2 and 300 times within human ciRS-7. The motif is depicted with base pairing potential to miR-7. Not all sites predicted by MEME have perfect seed sequences, explaining the discrepancy between the 77 sites predicted by MEME and the 73 seed sites depicted in (a) **c**, Weblogo of the identified miR-671 target sites from all placental mammals investigated, as represented by non-filled boxes in (a) (except elephant, where no miR-671 sites was found) depicted with base pairing potential to miR-671. **d**, Boxplot showing thermodynamic predictions using MultiRNAFold (ver 1.1[2]) of 73 human miR-7 target sites (17mers) upon base pairing with miR-7 and the miR-671 target site (22mer) upon base pairing with miR-671 normalised to target site length.



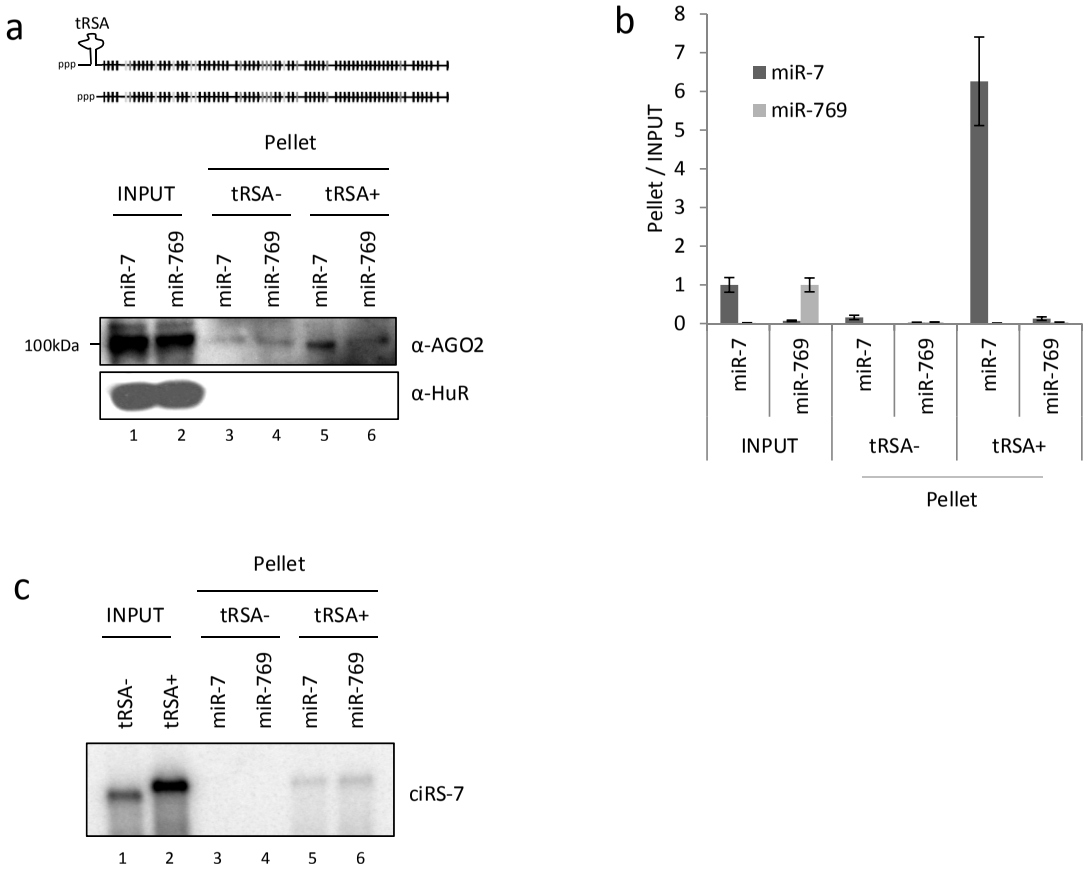
Supplementary Figure 2: HITS-CLIP reads derived from non-linear RNA. a, Schematic representation of the genomic locus encoding the murine ciRS-7 with predicted miR-7 and miR-671 target sites (as in Fig. 1a) and mammalian conservation scores. Distribution of Ago2 HITS-CLIP reads[3] from mouse brain is shown above. **b**, All reads from Ago2 HITS-CLIP[3] were trimmed to 15 nt from the 5'- or 3'-end, generating a set of 15-mers reads reflecting the 3' end or 5' end of each read, respectively. Subsequently, the 3' ends were mapped towards the 5' end of murine ciRS-7 (black sequence), whereas the 5' ends of all reads (15mers) were mapped towards the 3' end of murine ciRS-7 (grey sequence). For each perfectly mapped 15-mer (underlined), the untrimmed read was recovered and aligned to non-linear spliced ciRS-7 sequence (black-grey chimeric sequence). Read count is depicted to the right of each sequence. (*) denotes sequence not in support of non-linear splicing of ciRS-7. (#) denotes ambiguous sequence.



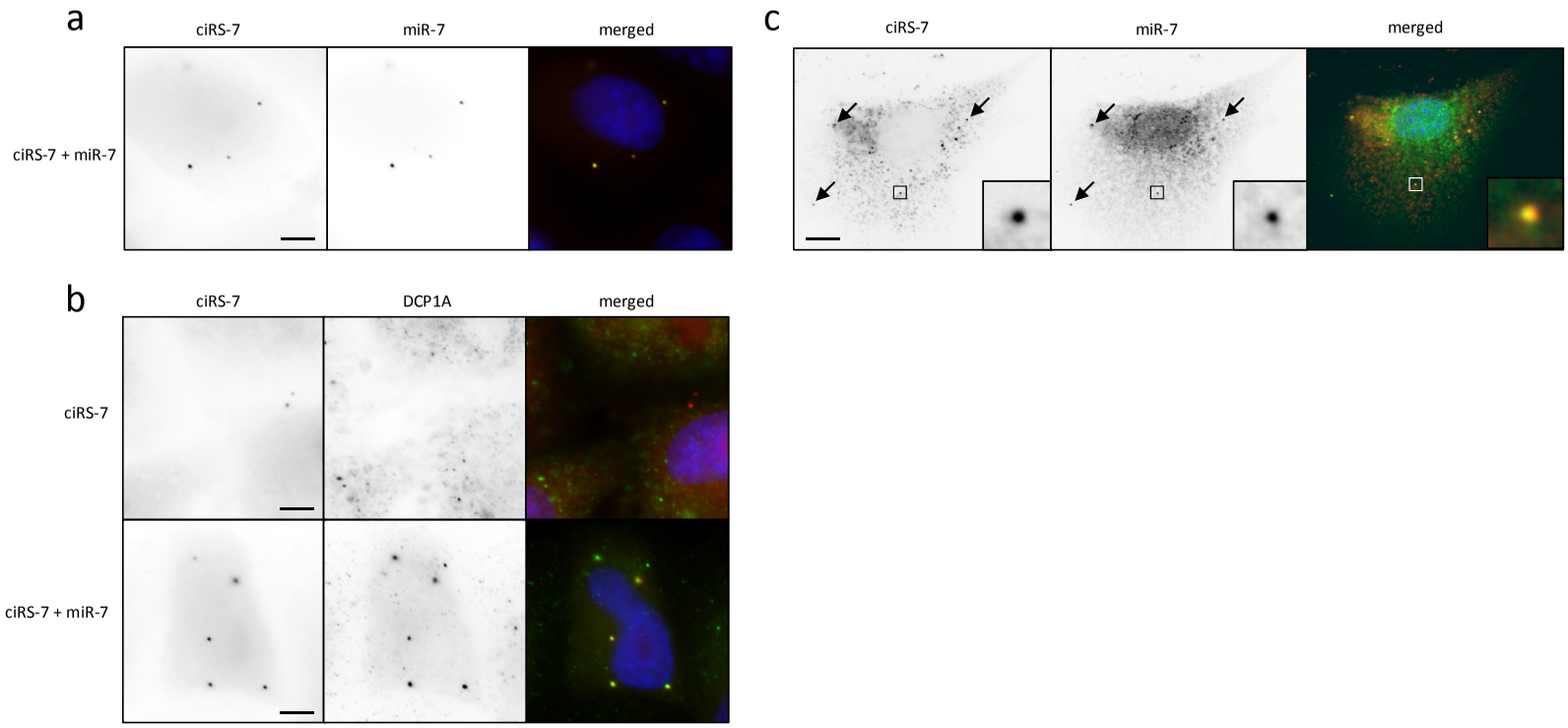
Supplementary Figure 3: Circularization of ciRS-7. a, RT-PCR on RNA from HEK293 cells transfected with pcDNA3 (EV), pcDNA3-ciRS-7-fs, pcDNA3-ciRS-7-ir, and pcDNA3-ciRS-7, as denoted above, using outwards facing primers (ciRS-7 (2) forward / ciRS-7 reverse) as depicted. The upper and lower band corresponds to inclusion or exclusion of previously identified intron[4] as indicated to the right. **b, c**, Northern blot (**b**) or qRT-PCR (**c**) on RNA from HEK293 cells transfected with empty vector (pcDNA3; EV), pcDNA3-ciRS-7-fs, and pcDNA3-ciRS-7. Prior to analysis, the RNA was either digested with Tobacco Acid Pyrophosphate that removes the 5' cap followed by Terminator 5' Exonuclease treatment (TAP-EXO), or with RNase R, as indicated. Error bars represent s.d. (n=4).



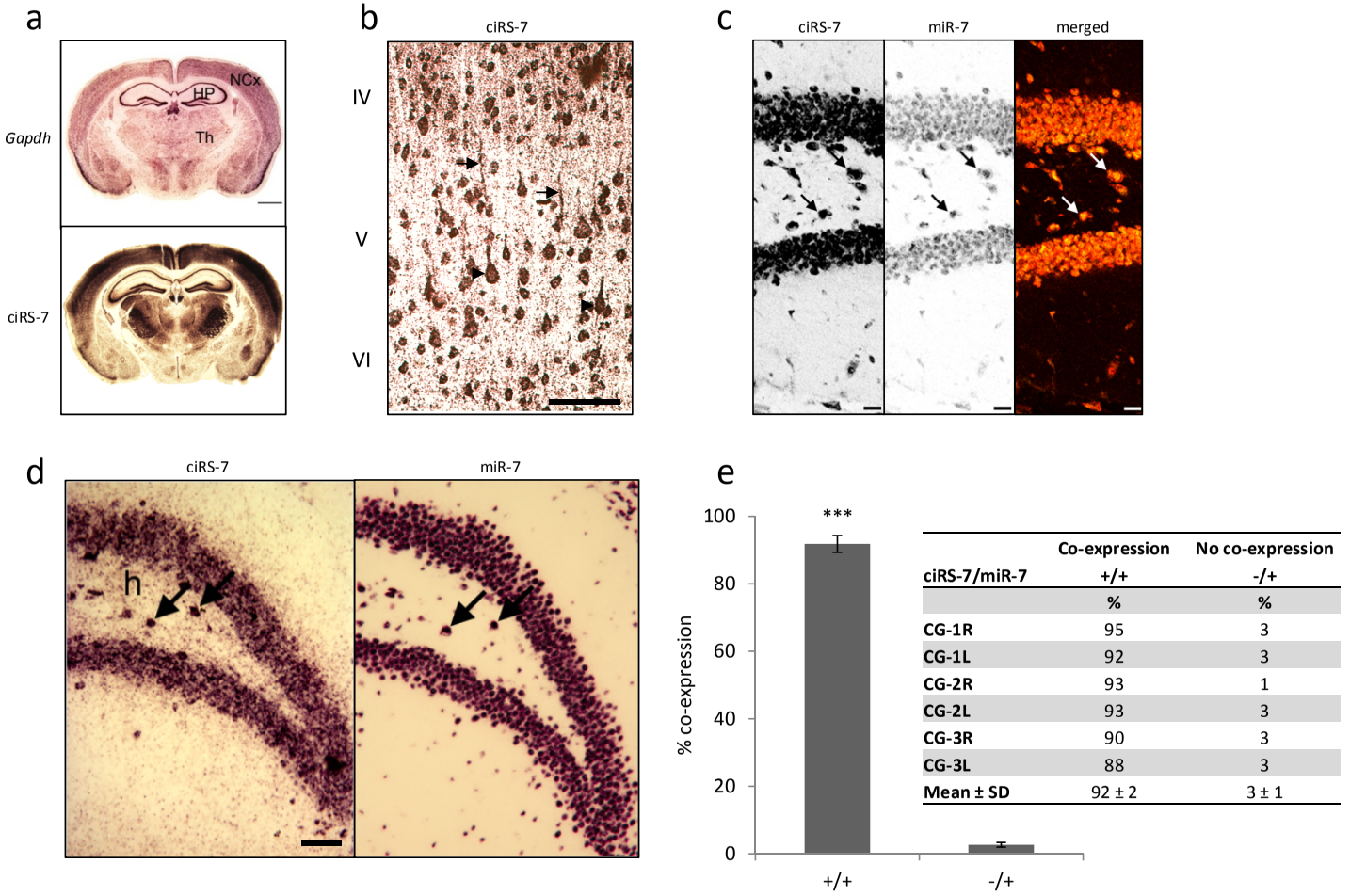
Supplementary Figure 4: Linear version of ciRS-7 is sensitive towards miR-7. a, RT-PCR on RNA from HEK293 cells transfected with pcDNA3 (EV), pcDNA3-ciRS-7-fs, pcDNA3-ciRS-7-ir, and pcDNA3-ciRS-7 using the 3'RACE approach. The upper and lower band corresponds to inclusion or exclusion of previously identified intron[4] as indicated to the right. **b**, Quantification of ciRS-7 levels derived from transfections with pcDNA3-ciRS-7-fs and pcDNA3-ciRS-7 along with plasmids expressing either miR-7 (pJEBB-7) or miR-769 (pJEBB-769) based on northern blot signal intensities. Error bars represent s.e.m (n=3). *, $p<0.05$.



Supplementary Figure 5: Streptavidin-aptamer capture of AGO2 and miR-7. a, b, T7-transcripts covering the entire ciRS-7 sequence with or without a streptavidin aptamer (tRSA) sequence in the 5' end (as schematically depicted above), were incubated with cell lysate pre-transfected with either miR-7 (pJEBB-7) or miR-769 (pJEBB-769) expression vectors. After streptavidin capture, the input and bound fraction were analysed by western blot using anti-AGO2 antibody (α-AGO2) or anti-HuR (control; α -HuR) (**a**) or by miRNA taqman® qPCR detecting miR-7 (dark bars) or miR-769 as a control (light grey bars) normalised to RNU48 and INPUT (**b**). Error bars represent s.d. (n=3). **c,** Northern blot of T7-transcribed ciRS-7 with (tRSA+) or without tRSA sequence (tRSA-) from prior (INPUT; T7 transcripts alone) and after cell lysate incubation and streptavidin capture (Pellet).

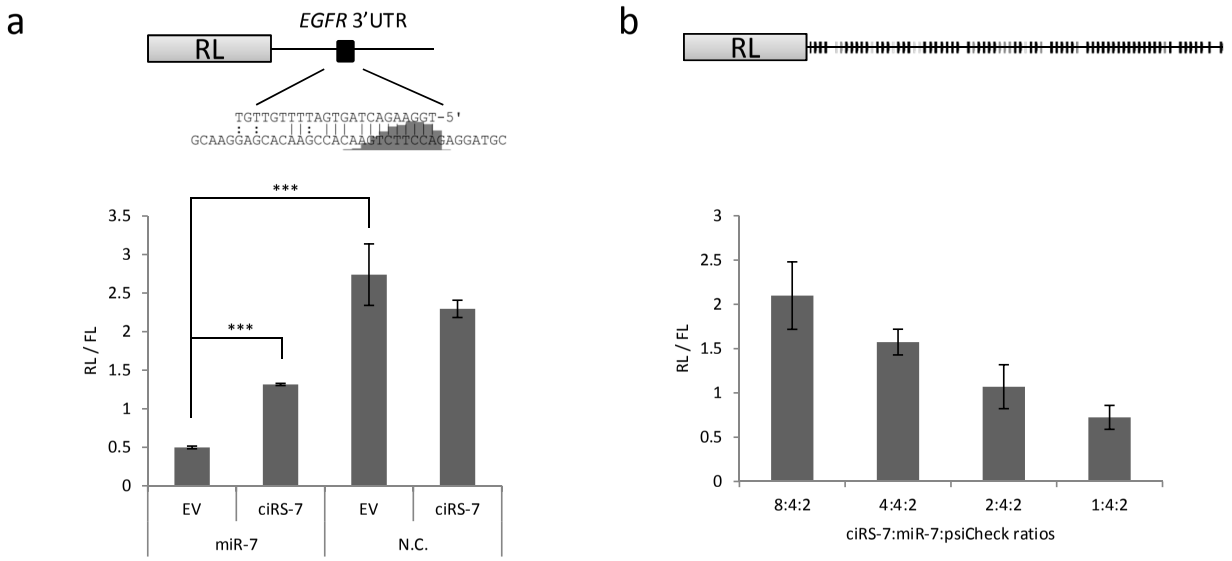


Supplementary Figure 6: Intracellular localization of ciRS-7 and miR-7. a, b, RNA-FISH and IF-FISH on HeLa cells transfected with constructs as indicated (as in Fig 2c-d). **c**, RNA-FISH on primary cells extracted from mouse brain using Cy3-labelled ciRS-7 probe (left panel), Cy5-labelled murine miR-7 probe (middle panel) and merged (right panel). Scale bars, 5 μm.

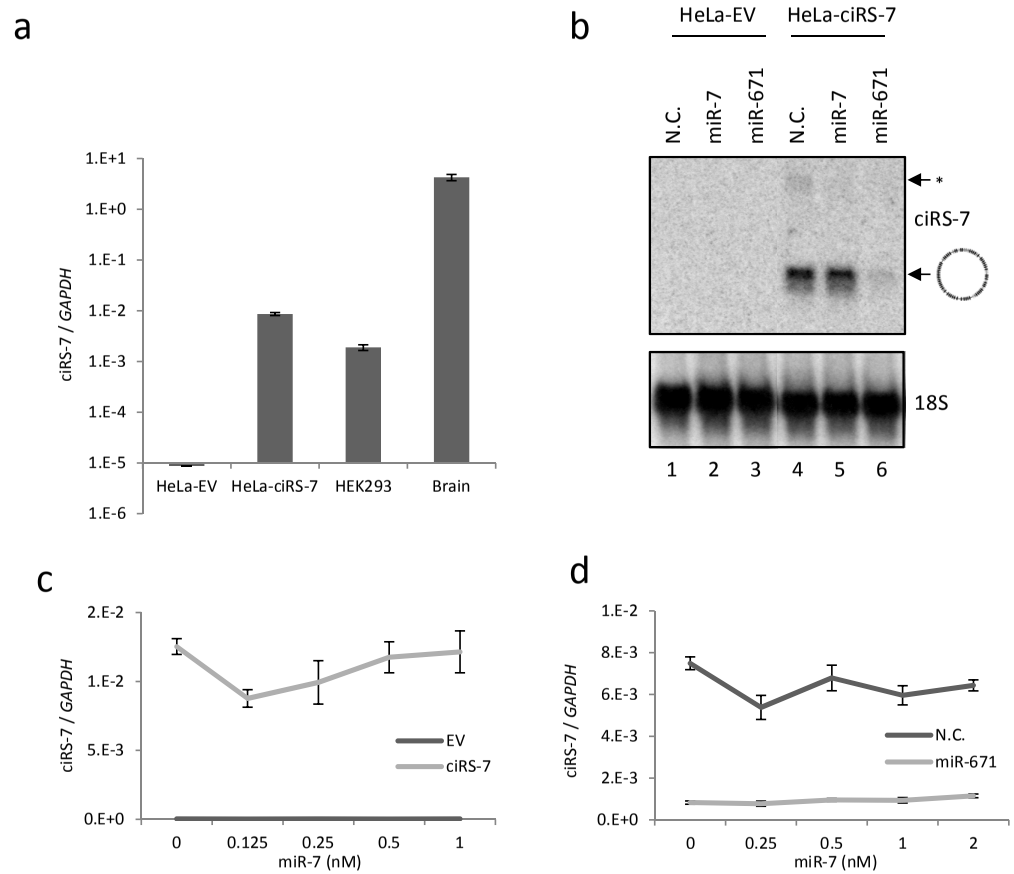


ciRS-7/miR-7	Co-expression +/+	No co-expression -/+
	%	%
CG-1R	95	3
CG-1L	92	3
CG-2R	93	1
CG-2L	93	3
CG-3R	90	3
CG-3L	88	3
Mean ± SD	92 ± 2	3 ± 1

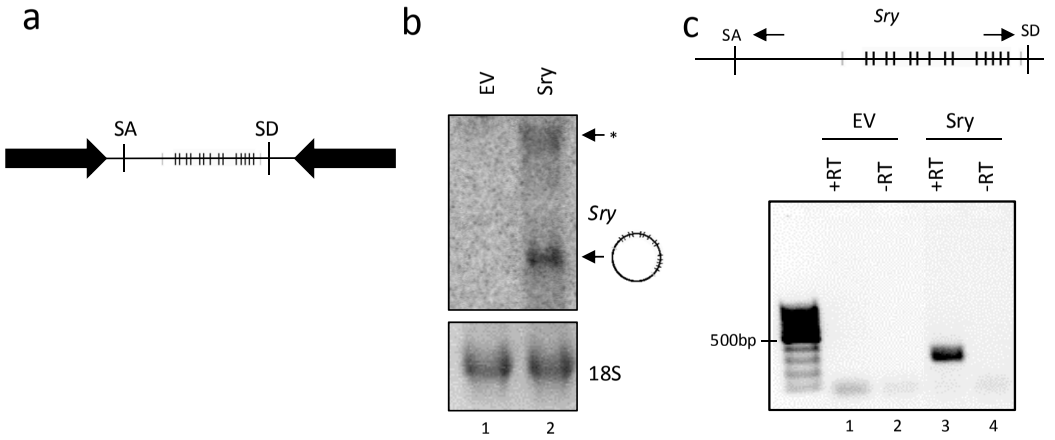
Supplementary Figure 7: Sub-brain expression of ciRS-7 and miR-7 in mouse. a, Mouse brains sections hybridized with alkaline phosphatase-coupled probes specific for *GAPDH* mRNA (top panel) or ciRS-7 (bottom panel). HP, hippocampus; NCx, neocortex; Th, thalamus. **b**, ciRS-7 is expressed in the neuronal cell body (arrowheads) and dendrites (arrows), here shown for layer IV-VI of somatosensory cortex in a 5µm thick section. **c**, Double fluorescence *in situ* hybridization showing co-expression between ciRS-7 (left panel) and miR-7 (middle panel), merged image (right panel) visualized by confocal microscopy. **d**, *In situ* hybridization for ciRS-7 (left panel) and miR-7 (right panel) performed on adjacent, 5 µm thick sections, which allows identification of co-expressing neurons (arrows), here shown for the dentate hilus (h). For presentation one of the digitalized images was laterally reversed. **e**, Quantitative data on % co-expression between ciRS-7 and miR-7 in cingulate gyrus (CG) layer IV-V. Table shows data obtained from right (R) and left (L) CG. Data are presented as mean ± SD. ***, $p < 0.001$. Bar: 1 mm (a), 100 µm (b), 10 µm (c) and 50 µm (d).



Supplementary Figure 8: Luciferase reporter assay. **a**, The 3'UTR of miR-7 target gene, *EGFR*, was inserted into psiCheck vector (schematic representation of target site depicted above). HEK293 cells in 12-well plates were co-transfected with 0.5 μg empty vector (EV) or pcDNA3-ciRS-7 together with 10 nM miR-7 mimic siRNA or 10 nM N.C. (negative control siRNA) and 0.1 μg psiCheck reporter plasmid containing the entire *EGFR* 3' UTR. Forty eight hours after transfection, relative luminescence was measured and plotted. **b**, Vectors expressing ciRS-7 (pcDNA3-ciRS-7), miR-7 (pJEBB-7) and psiCheck-ciRS-7 were transfected in various plasmid weight ratios as indicated. Forty eight hours after transfection, relative luminescence was measured and plotted. Error bars represent s.d. (n=3). ***, $p<0.001$.



Supplementary Figure 9: Stable expression of ciRS-7 in HeLa cells. a, qRT-PCR on RNA purified from HeLa-EV, HeLa-ciRS-7, HEK293 cells, and total brain quantifying ciRS-7 levels relative to *GAPDH* mRNA. **b,** Northern blot using 10 µg RNA from HeLa-EV (with empty vector expression cassette) or Hela-ciRS-7 (with ciRS-7 expression cassette). Cells were transfected with 10 nM non-related control (N.C.), miR-7 or miR-671 mimic siRNAs as indicated. The blot was probed with ciRS-7 and 18S (loading control). The asterix (*) denotes a high-molecular, non-circular RNA species produced from the ciRS-7 construct. **c, d,** RT-qPCR quantification of ciRS-7 in HeLa-ciRS-7 (light grey line) or HeLa-EV cells (dark grey line) transfected with gradient concentration of miR-7 (**c**) or in HeLa-ciRS-7 cells transfected with miR-671 mimic siRNA (light grey line) or N.C. (negative control; dark grey line) siRNA 24 hrs prior to transfections with miR-7 gradient (**d**). Error bars represent s.d. (n=4).



Supplementary Figure 10: Sponging of miR-138 by *Sry* circRNA expression. a, Schematic representation of *Sry* expression vector (pcDNA3-Sry) in which the tet-responsive CMV promoter from the pcDNA5/FRT/TO vector has been inserted in an inverted orientation downstream the *Sry* locus. **b,** Northern blot using 10 µg RNA from HEK293 cells transfected with either empty vector (EV, pcDNA3) or the *Sry* expression construct (pcDNA3-Sry). Blot was probed with *Sry* and 18S (upper and lower panel, respectively). Position of the circular *Sry* RNA is indicated. The asterix (*) denotes a high-molecular, non-circular RNA species produced from the *Sry* construct. **c,** RT-PCR on RNA from HEK293 cells transfected with either empty vector (EV, pcDNA3) or *Sry* expression vector (pcDNA3-Sry) using primers depicted with arrows above. RT reactions were performed with (+RT) or without reverse transcriptase (-RT) as control.

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