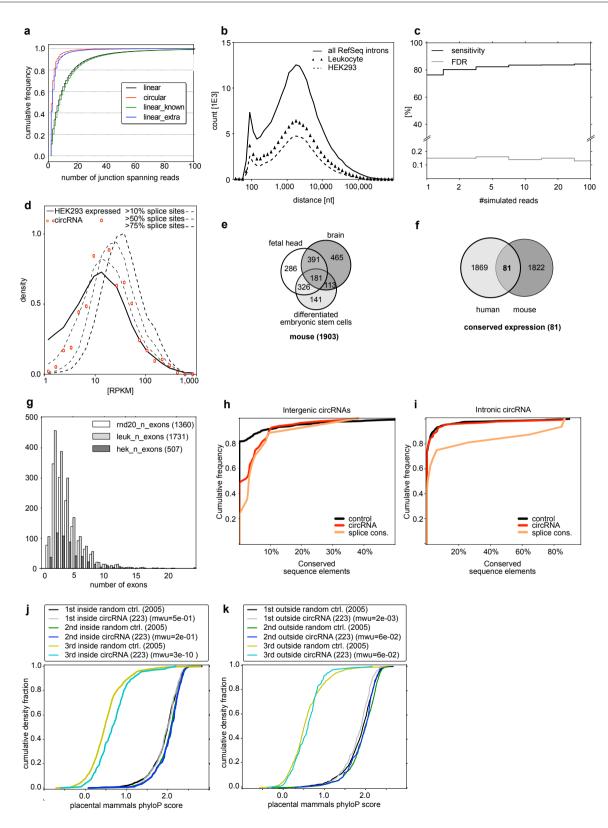
# SUPPLEMENTARY INFORMATION



Supplementary Figure 1 Computational screen and statistical analysis a, Cumulative frequency of the number of reads that span linear and circular splice sites

At the same cutoffs that were used to call the reported 1950 human circRNAs, our screen identifies 101,456 linear exon-exon junctions. Of these, 89% correspond to annotated RefSeq and non-coding RNA exon-exon junctions. By this comparison we find additional 11,521 exon-exon junctions that may be found in more sensitive but less stringently validated gene annotations.

The circular splice junction candidates that we identify in our screen are generally covered by much less reads than linear junctions (about ten times less on average).

#### b, The algorithm recovers annotated introns.

The logarithmic length histogram of detected linearly spliced introns in HEK293 (dashed lines) and leukocyte data (triangles) recapitulates the RefSeq intron length distribution (solid line).

### c, Sensitivity and false discovery rate estimates

Using chains of randomly chosen, consecutive internal exons as shown in **g**), we simulated reads spanning the circularizing splice junction. Performing the complete analysis on these synthetic data (~10.000 simulated circRNAs) yields a sensitivity (recovered simulated circRNAs over total simulated circRNAs) of > 75%, even for low coverage of the splice site. The false discovery rate (reported circRNAs that were not simulated divided by the number of all reported circRNAs) is below 0.2%.

**d**, **The sensitivity of splice site detection depends only weakly on gene expression.** Histograms of gene expression levels obtained from polyA+ RNA sequencing in HEK293 cells. The number of reads per kilobase of exon per million mapped reads (RPKM) reflects mRNA abundance. Genes that are predicted to give rise to circRNAs (red circles) are not specifically enriched for high expression, (solid line: all genes). circRNAs from lowly expressed genes are detected less frequently, comparable to the loss of sensitivity observed

for linear splicing (black dashed line: genes with > 75% of annotated splice sites recovered, gray dashed line: >50%, light gray: >10%).

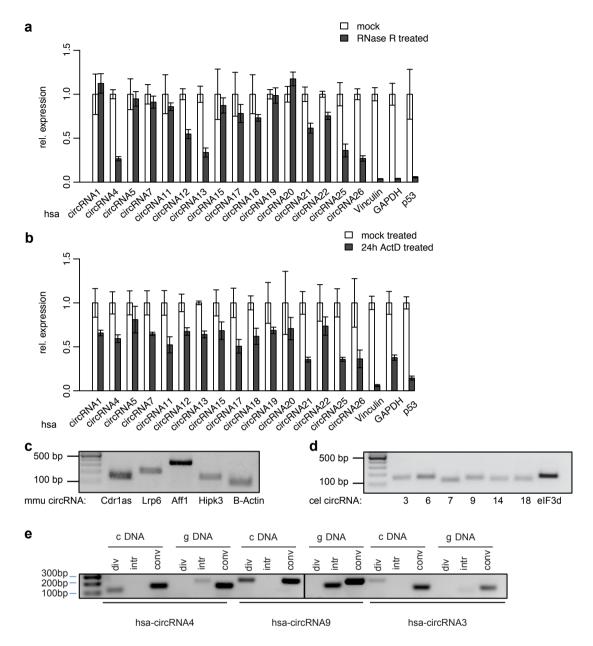
e, Mouse circRNAs. Venn diagram of circRNAs detected in mouse tissues.

**f**, **Conserved circularization**. We find 81 circRNAs with orthologous splice sites, detected independently in human and mouse data.

**g**, **Exon number distribution for human circRNAs** (gray:leukocytes, dark gray:HEK293 cells) and matched random exon controls, used for (c) (white).

**h**, Conserved sequence element (**Methods**) content of intergenic human circRNAs (red) significantly exceeds random intergenic controls (black), P < 5e-10, Mann-Whitney-U, n=81. Same for circRNAs with conserved splice sites (orange, P < 1e-6, Mann-Whitney-U, n=17). **i**, **Intronic circRNAs** show enrichment of conserved sequence elements if flanking splice sites are conserved (orange, P < 0.07, Mann-Whitney-U, n=16), but not significant for the complete set (red, P > 0.5, Mann-Whitney-U, n=215). Controls (black) randomly drawn from same introns.

**j**, Coding sequence phyloP conservation score distributions of first and second codon positions match between circRNAs and controls, in contrast the 3rd codon position is significantly more conserved in circRNAs (P < 3e-10 n=223 Mann-Whitney-U (mwu)(also main Fig1. f). **k**, The conservation score distributions in the remaining parts of the CDS (outside the circRNA or control) do not differ significantly for codon positions two and three. For the first codon position, the controls are actually more conserved, P < 2e-3 n=223 Mann-Whitney-U (mwu)), therefore conservative.



# Supplementary Figure 2 Validation of circRNAs

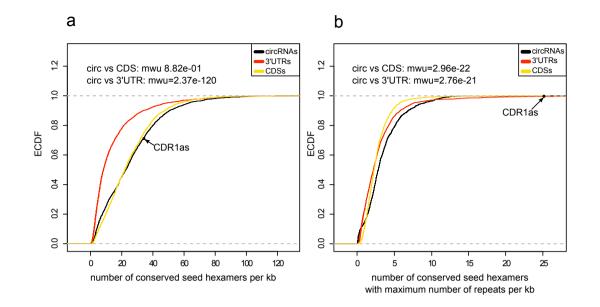
#### a, circRNAs are RNase R resistant.

16 human (hsa) circRNA candidates were tested in a RNase R assay for exonuclease resistance. VINCULIN, GAPDH and p53 specific primers were used as linear controls, error:stdev.

**b**, **circRNA expression after transcriptional block.** HEK293 cells were Actinomycin D treated and circRNA expression was assayed by qPCR (controls as in a)

**c,d. Expression of mouse and** *C. elegans* **circRNAs.** RT-PCRs using divergent primers detect circRNAs in mouse (mmu) and *C. elegans* mixed stage (cel).

e, RT-PCR of human circRNA candidates. RT-PCR was performed on cDNA (gDNA as control) using divergent or convergent primers for three circRNAs that span at least one intron. Additionally convergent intron specific primers were used (Methods). hsa-circRNA 9 corresponds to AFF1 (Fig. 1e).



# Supplementary Figure 3 Human circRNAs are significantly enriched for repetitions of conserved miRNA seed matches.

**a**, Empirical cumulative distribution function (ECDF) of the number of conserved miRNA seed matches (hexamers, miRNA position 2-7) per kilobase of inferred spliced sequence **(Methods)** of circRNAs (black). A seed match is considered to be conserved when it is present in 5/5 different mammals spanning the evolutionary distance from man to dog **(Methods)**. Analogous ECDFs were plotted for coding exons (n=3873) and 3'UTRs (n=3182) with similar length distributions as human circRNAs (n=1950). For the statistical comparisons Mann-Whitney-U test was used. **b**, Similar to (**a**) but counting only the number of those miRNA seed matches that are repeated most often. CDR1as is marked as a circle.

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Cow/1-893
Naked_mole/1-1145
Rabbit/1-1572
Elephant/1-1371
Marmoset/1-1311
Pig/1-1652
Dog/1-1356
Horse/1-1309
Lamprey/1-1177

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Human/1 Gorilla/1-1511 Macaque/1-1582 Guinea Pig/1-1236 Cow/1-893 Naked\_mole/1-1145 Rabbit/1-1572 Elephant/1-1371 Marmoset/1-1371 Marmoset/1-1371 Dog/1-1356 Horse/1-1309 Lamprey/1-1177

Human/1 Gorilla/1-1511 Macaque/1-1582 Guinea\_Pig/1-1236 Cow/1-893 Naked\_mole/1-1145 Rabbit/1-1572 Blephant/1-1311 Marmoset/1-1311 Marmoset/1-1316 Borse/1-1356 Borse/1-1309 Lamprey/1-1177

Human/1 Gorilla/1-1511 Macaque/1-1582 Guinea\_Pig/1-1236 Cov/1-893 Naked\_mole/1-1145 Rabbit/1-1572 Elephant/1-1311 Marmoset/1-1311 Marmoset/1-1310 Dog/1-1356 Horse/1-1309 Lamprey/1-1177

Human/1 Gorilla/1-1511 Macaque/1-1582 Guinea\_Pig/1-1236 Cov/1-893 Naked.mole/1-1145 Rabbit/1-1572 Elephant/1-1371 Marmoset/1-1311 Marmoset/1-1310 Dog/1-1356 Dog/1-1350 Lamprey/1-1177

Human/1 Gorilla/1-1511 Macaque/1-1582 Guinea Piq/1-1236 Cow/1-893 Naked\_mole/1-1145 Rabbit/1-1572 Elephant/1-1371 Marmoset/1-1371 Marmoset/1-1371 Horse/1-1309 Lamprey/1-1177

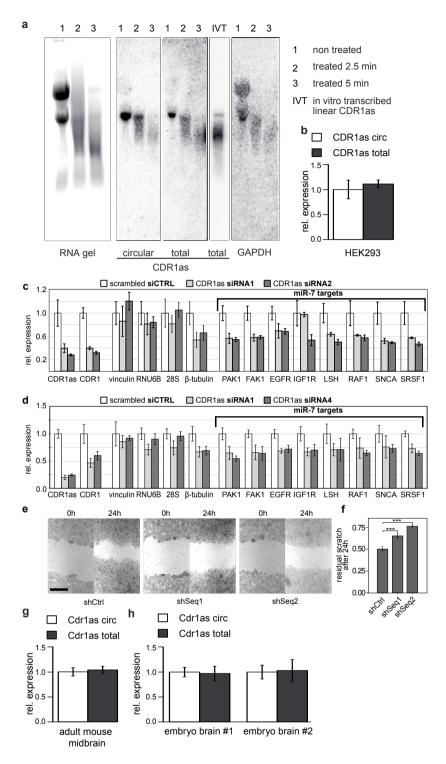
Human/1 Gorilla/1-1511 Macaque/1-1582 Guinea\_Pig/1-1282 Ow/1-893 Naked\_mole/1-1145 Rabbit/1-1572 Elephant/1-1371 Marmoset/1-1311 Marmoset/1-1310 Dog/1-1356 Horse/1-1309 Lamprey/1-1177

Human/1
Gorilla(1-1511
Macaque/1-1582
Guinea\_Pig/1-1236
Cov/1-893
Naked\_mole/1-1145
Rabbit/1-1572
Habbit/1-1572
Marmoset/1-1311
Marmoset/1-1310
Dog/1-1356
Dog/1-1350
Lamprey/1-1177

	-ATTTGGG	TCTTCC	TG-AAAATC-	TACGTCT	<mark>FCCA</mark> AAAAA-G	CCATGTCTT	CCA GAAAATCCACA T	CTTCCA ATGGC	-CTCCAGGTCTT	CCAGACTATCC.	ATGTCTTCCA	GAAAATCCTT	TCTTC
	ATTTGGG	TCTTCC	TG-AAAATC-	TACGTCT	TCCA	CCATGTCTT	CCAGAAAATCCACA	CTTCCA ATGGC	-CTCCAGGTCTT	CCAGACTATCC.	ATGTCTTCCA	GAAAATCCTT	TCTTC
ATA	-ATTCGGG	TCTTCC	TG-AAAATC-	TACATCT	CCAAAAAA-G	CCATGTCTT	CCA GAAAATCCAC GT	CTTCCA ATGGC	-CTCCAGGTCTT	CCAGACTATCC	ATGTCTTCCA	GAAAATCCTT	TCTTC
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							CCAGAAAATCCTCGT				ATGTAGTCCA		
							CCA GACAATTCAG GT						
							C TGAAAATCCAG GT						
ACA	-GTCCATG	TCTTCC	G-AGAAGC-	CGTGTCT	TCCAACAACTT	CCAGGTCTT	CCAGCAAATCCACAT	CTTCCAACAAC	AATCCAGGTCTT	COGGACAATTC	GGGTCTTCCT		
ATA	ATTCGGG		TG-AAAATC-	TACGTCT	TCCA	CCATGTCTT	CCA GAAAATCCAC GT	CTTCCA	-ATCCAG	CCAGACTGTCC	ATCTTCCA		
							CCA GGAAATCCAT GT						
							CCAGGAAATCCATGT						
ACA	ATTTGGG		TG-AAAATC-	CACCTCT	CCAGAAAA-C	CCATGTCTT	CCA GGAAATCCAT GT	CTTCCAACGAC	-CTCCAGCTCTT	CAGCCTATCC	AGTCTTCCA		
AAG	ATTCAGATTT	IGTTCTTCA	AG-AAGATCT	CAGATTTGTCT	TCCAGAAGATC	CCAGATTTGTCTT	CCA GAATATTCCAGA	TTT	GTGTT	CCAGAATATTC	CAGATTTGT		
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TCTAT	TAGCTTCCAA	AAAATCCAG	GTCTTCCAGG	AAATCTGTGTC	TCCAGCAAGT	CCACGTCTTCCAA		-CARAGCCATC	TCTTCCAGACTA	TCCACCTCTTC	GAAAATCC	TTGTCTTCCCT	CAAATO
TCCAT	PACCTTCCAN	ANATCONC	GTCTTCCA GTCTTCCCCCCCCCCCCCCCCCCCCCCCCCC	AAATCTCTCTCTCTC	TTCCACCAABT	CCACCTCTTCCAA		-CANACCONT	TOTTCONGACTA	TCCACCTCTTC	CAAAATCC	rr <u>crcrrcc</u> er	CARATC
			<u></u>			oene <mark>ororroon</mark> n			onorm				CAAATC
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G	GCTTCCGAAAA	AATCCAGG-	TCTTC	CAGGAAATC	CGTGTCTT	CCAGCAAATCCAC	GTCTTCCA ACAAAGC	CATGTCTTCCA	TCAAATTAATG-	TCTTCC	GCCTACTTG	FGTCTTCCA	;
							GTCTTCCA						
G	GCTTCCAGAA	CATCCACG-	TCTTC	CAGGAAATC	CTC	CCAACAAATCCAC		GTCTTCCA	TCACTTTCATG-	TTTTCT	GCCTTCCTA	CATTTTCCCAA	·
0	CTTCCAGGAZ	AATCCTTG-	лелте	CAACGAAGG	TGTGTCTT	CCAGAAAATCCTC	GTCTTCC GACACCTC	CAGGTCTTCCA	GCATCTCCAGG-	GCTTCC	AGCA	TCTGAT	
6	GCTTCAAAAA	AATCTATG-	TCTTC	GAAAATC	CANCITORIU	ACAAATTCA-			TCAGTTTTATC-	TCTTTC	AGCCTTCCTA	C.C.C.C.C.C.A.C	
0	CTTCCARGA	AATCCACC-	TCTTC	TGCTANTC	TATCTOT	CCAGCAAATCCAC	GACTTCCAAGAAAGC	TGTCTTCCT	TCABATTCATC-		GTCTACCTA	GTCTTCCDAC	AAAGCC
	CTTCCACA ~ ~	AATCCACC		CAGCTATC.	CGTCTCT	CAGAAAATTTA	GTCTTCCA	CATCTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCT	CAAAATTTATC		AGAAAATCCA	CTCTTCC2	
							STCTTCCA GACTATC						
	SCITCCGAAAA	AAICCAGG-	mommo	CHORANATC	mcch mcmm	GONGCARATCCAC	CITCCA RARAGE	CATGICITCCA	CARAMITANIG-	memmee	CONTRACTO	GICTICCARC	
		ARICCAIG-	TCTTC	CAAAAGG	IGCA	GRAAATCCAT	STCTTCCAACAAACC	IGIGICITICCA	GAAAATCCAIG=	<u>nemee</u>		GICTICCAGA	MANICC
G	SCTTCCAGAAA	AATCCATG-		GGAAATC	TATGTCTT	CCAGCAAACCCTT	GTCTTCCAAAAGC	TGTGTCTTCCA	TCAAATTCATG-		GCCTACCCA	GTCTTCCAAC	;
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		<del>STETTCO</del> GG	AAAATCCAC						AAAGC AAATCCAC AAATCTGT AAAAGTAC	GTCTTCCA GGCTTCCAGAA GTCTTCCAGAA	AATC		CATG
		<u>STCTTCC</u> GG	AAAATCCAC						AAAGC AAATCCAC AAATCTGT AAAAGTAC GGGAAAATCCAC	GTCTTCCA GGCTTCCAGAA GTCTTCCAGAA	AATC		CATG
		<del>STETTCO</del> GG	AAAATCCAC						AAAGC AAATCCAC AAATCTGT AAAAGTAC GGGAAAATCCAC	GTCTTCCA GGCTTCCAGAA GTCTTCCAGAA	AATC		CATG
TCCA	AGAAGGGTGC	GTCTTCO GG	AAAATCCAC	TCTTCCA	AGGTGC	TCCGGAAAATCCA	C <b>TCTTCCA</b> ACAAAG	GGTGC <mark>GTCTTC</mark>	AAAGC AAATCCAC AAATCTGT AAAATCGAC GGAAAATCCAC AAATC AAAT	GTCTTCCAGCA GGCTTCCAGAA GTCTTCCAACA GTCTTCCAACA	AATC AAGG AAGGGTGC <mark>GT</mark>	CTTCC	CATG
TCCA	AGAAGGGTGC	GTCTTCO GG	AAAATCCAC	TCTTCCA	AGGTGC	TCCGGAAAATCCA	C <b>TCTTCCA</b> ACAAAG	GGTGC <mark>GTCTTC</mark>	AAAGC AAATCCAC AAATCTGT AAAATCGAC GGAAAATCCAC AAATC AAAT	GTCTTCCAGCA GGCTTCCAGAA GTCTTCCAACA GTCTTCCAACA	AATC AAGG AAGGGTGC <mark>GT</mark>	CTTCC	CATG
TCCA	AGAAGGGTGC	GTCTTCO GG	AAAATCCAC	TCTTCCA	AGGTGC	TCCGGAAAATCCA	C <b>TCTTCCA</b> ACAAAG	GGTGC <mark>GTCTTC</mark>	AAAGC AAATCCAC AAATCTGT AAAATCGAC GGAAAATCCAC AAATC AAAT	GTCTTCCAGCA GGCTTCCAGAA GTCTTCCAACA GTCTTCCAACA	AATC AAGG AAGGGTGC <mark>GT</mark>	CTTCC	CATG
TCCA	AGAAGGGTGC	GTCTTCO GG	AAAATCCAC	TCTTCCA	AGGTGC	TCCGGAAAATCCA	C <b>TCTTCCA</b> ACAAAG	GGTGC <mark>GTCTTC</mark>	AAAGC AAATCCAC AAATCTGT AAAATCGAC GGAAAATCCAC AAATC AAAT	GTCTTCCAGCA GGCTTCCAGAA GTCTTCCAACA GTCTTCCAACA	AATC AAGG AAGGGTGC <mark>GT</mark>	CTTCC	CATG
+1650 AACA AACAA.	AGAAGGGTGC D -AAGGTAT <mark>GTG</mark> AA	CTTCCAACA	AAAATCCAC <mark>G</mark> AA AAGGTAC <mark>GTC</mark>	TCTTCCPACAA	AGGGTGC <mark>STCT</mark> GGTAC <mark>STCTTC</mark> GGTAC <mark>STCTTC</mark> GGTAC <mark>STCTTC</mark>	CCGGAAAATCCA CAGAAAATCCACG CAGAAAATCCATG CAGAAAATCCATG CAGAAATCCATG	CTTTCCAACAAAG TCTTCCAACCAAGC- TCTTCCAACCAAGC- TCTTCCAACCAAGC- TCTTCCAACCAAGC-	GGTGC	AAAGC 	GICTICCAGA GCCTICCAGA GICTICCAACA GICTICCAACA GICTICCAACA TICCACAAAAT TICCAGAAAAT TICCAGAAAAT	AATC	TTTCCGGAAAA TCTTCCAG-A TCTTCCAG-A TCTTCCGG-A	CATG TACC ATCCACC 
+1650 AACA AACAA.	AGAAGGGTGC D -AAGGTAT <mark>GTG</mark> AA	CTTCCAACA	AAAATCCAC <mark>G</mark> AA AAGGTAC <mark>GTC</mark>	TCTTCCPACAA	AGGGTGC <mark>STCT</mark> GGTAC <mark>STCTTC</mark> GGTAC <mark>STCTTC</mark> GGTAC <mark>STCTTC</mark>	CCGGAAAATCCA CAGAAAATCCACG CAGAAAATCCATG CAGAAAATCCATG CAGAAATCCATG	CTTTCCAACAAAG TCTTCCAACCAAGC- TCTTCCAACCAAGC- TCTTCCAACCAAGC- TCTTCCAACCAAGC-	GGTGC	AAAGC 	GICTICCAGA GCCTICCAGA GICTICCAACA GICTICCAACA GICTICCAACA TICCACAAAAT TICCAGAAAAT TICCAGAAAAT	AATC	TTTCCGGAAAA TCTTCCAG-A TCTTCCAG-A TCTTCCGG-A	CATG TACC ATCCACC 
+1650 AACA AACAA.	AGAAGGGTGC D -AAGGTAT <mark>GTG</mark> AA	CTTCCAACA	AAAATCCAC <mark>G</mark> AA AAGGTAC <mark>GTC</mark>	TCTTCCPACAA	AGGGTGC <mark>STCT</mark> GGTAC <mark>STCTTC</mark> GGTAC <mark>STCTTC</mark> GGTAC <mark>STCTTC</mark>	CCGGAAAATCCA CAGAAAATCCACG CAGAAAATCCATG CAGAAAATCCATG CAGAAATCCATG	CTTTCCAACAAAG TCTTCCAACCAAGC- TCTTCCAACCAAGC- TCTTCCAACCAAGC- TCTTCCAACCAAGC-	GGTGC	AAAGC 	GICTICCAGA GCCTICCAGA GICTICCAACA GICTICCAACA GICTICCAACA TICCACAAAAT TICCAGAAAAT TICCAGAAAAT	AATC	TTTCCGGAAAA TCTTCCAG-A TCTTCCAG-A TCTTCCGG-A	CATG TACC ATCCACC 
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+1650 AACA AACAA. AACAA.	AGAAGGGTGC ) -AAGGTATGTC -AAGGTACGTC -AAGGTACGTC	CTTCCA CTTCCA ACA	AAAATCCACG	TTTCCAACAA	AGGGTGCGTCT GGTACGTCTTC GGTACGTCTTC 	C GGAAAATCCA GGAAAATCCACG GAGAAAATCCACG GGAAAATCCACG GGAAAATCCACG GGAAAATCCACG GGAAAATCCACG GGAAAATCCATG GGAAAATCCATG	CTCTTCOTACAAAG	GGTGC	AAAGC	GIGTICOLCAAA GGCTTCCAGAA GGCTTCCAGAA GGCTTCCACA GGCTTCCACAAAA TTCCAGAAAAAT TTCCAGAAAAAT TTCCAAAAAA TTCCAAAAAAA	AATCAAGG	TCTCCGGAAAA TCTTCCACA TCTTCCACA TCTTCCGGAA TCT- TCT- TCTC- TCTCCGGAA	CATG TACC TTCCACC 
+1650 AACA AACAA. AACAA.	AGAAGGGTGC ) -AAGGTATGTC -AAGGTACGTC -AAGGTACGTC	CTTCCA CTTCCA ACA	AAAATCCACG	TTTCCAACAA	AGGGTGCGTCT GGTACGTCTTC GGTACGTCTTC 	C GGAAAATCCA GGAAAATCCACG GAGAAAATCCACG GGAAAATCCACG GGAAAATCCACG GGAAAATCCACG GGAAAATCCACG GGAAAATCCATG GGAAAATCCATG	CTCTTCOTACAAAG	GGTGC	AAAGC	GIGTICOLCAAA GGCTTCCAGAA GGCTTCCAGAA GGCTTCCACA GGCTTCCACAAAA TTCCAGAAAAAT TTCCAGAAAAAT TTCCAAAAAA TTCCAAAAAAA	AATCAAGG	TCTCCGGAAAA TCTTCCACA TCTTCCACA TCTTCCGGAA TCT- TCT- TCTC- TCTCCGGAA	CATG TACC TTCCACC 
тосла +1650 Даса-, Дасаа, Даса, Gaat.	AGAAGGGTAC D-AAGGTATGTO -AAGGTACGTO -AAGGTACGTO -AAGGTACGTO	CTTCCAACA CTTCCAACA CTTCCAACA CTTCCAACA	AAAATCCACS	TCTTCCAACAA	AGGGTGCGTCT GGTACGTCTTC GGTACGTCTTC GGTACGTCTTC GGTACGTCTTC GTGTACGTCTTC GCTACGTCTTC GGTACGTCTTC	CLGAAAATCCAC CLGAAAATCCAC CLGAAAATCCAC CLGAAAATCCAC CLGAAAATCCAC CLGAAAATCCAC CLGAAAATCCAC CLGAAAATCCAC CLGAAAATCCAC CLGAAAATCCAC CLGAAAATCCAC	CTTTCCIACCAAGC- CTTTCCIACCAAGC- CTTTCCIACCAAGC- CTTTCCIACCAAGC- CTTTCCIACCAAGC- CTTTCCIACCAAGC- CTTTCCIACCAAGC- CTTTCCIACCAAGC- CTTTCCIACAAGC- CTTTCCIACAAGC- CTTTCCIACAAGC-	GGTGC		Grotticolaca Grotticolaca Grotticolaca Grotticolaca Grotticolaca Tricolacaaat Tricolacaaag Tricolacaaag Tricolacaaag	AATC AAGGGTGC T CCACG CCACG CCACA CCACG CCACG CCACG	TTCC GGAAAA TCTTCCAG-A TCTTCCAG-A TCTTCCAGAA TCTTCCAGAA TCTTCCAGAAC TCTTCCAGAAC	CATG TAC TTCCAC 
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<u>тсса</u> а +1650 Ласа. Ласа. Даса. , Даса. , Даса. , Даса.	AGAAGGTACGT AAGGTACGTC AAGGTACGTC AAGGTACGTC AAGGTACGTC	CTTCCAACA CTTCCAACA CTTCCAACA CTTCCAACA CTTCCAACA	AAAATCCACG AAGGTACGTC AAGGTACGTC AA	TTCCAACAA	AGGGTGC CICIT GGTAC CICITC GGTAC CICITC GGTAC CICITC GGTAC CICITC GGTAC CICITC CICITC CICITC GGTAC CICITC GGTAC CICITC GGTAC CICITC GGTAC CICITC CICITC TAC CICITC	GGAAAATCCAC GGAAAATCCAC GGAAAATCCAC GGAAAATCCAC GGAAAATCCAC GGAAAATCCAC GGAAAATCCAC GGAAAATCCAC GGAAAATCCAC GGAAAATCCAC GGAAAATCCAC	CTTCTTCCAACCAAGC CTTCCCAACCAAGC CTTCCCAACCAAGC CTTTCCCAACCAAGC CTTTCCCAACCAAGC CTTTCCCAACCAAGC CTTTCCCAACCAAGC CTTTCCCAACCAAGC CTTTCCCAACCAAGC CTTTCCCAACCAAGC CTTTCCCAACCAAGC CTTTCCCAACCAAGC CTTTCCCAACCAAGC CTTTCCCAACCAAGC CTTTCCCAACCAAGC	GGTGC DY CTTC CTCTTCC AAC		STOTFOL CAGA GGCTTCCAGAA GGCTTCCAGAA GGCTTCCAGAA TTGCAGAAAAA TTGCAGAAAAA TTGCAGAAAAAT TTGCAGAAAAAT TTGCAGAAAAAT TTGCAGAAAAAT	AATC	ICTICCAGA ICTICCAGA ICTICCAGA ICTICCAGA ICTICCAGA ICTICCAGAA ICTICCAGAA ICTICCAGAA ICTICCAAC ICTICCAAC	CATG TACC ATCCACC 
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1001A	AGAAGGGTGC - AAGGTAT - AAGGTAT - AAGGTAT - AAGGTAT - AAGGTAT - AAGGTAT - AAGGTAT - AAGGTAC  - AAGGTAC        -		AAAATCCACG AAATC		AGGGTGC 0111 SGTAC 01111 GTAC 011111 GTAC 011111 GTAC 011111 GTAC 0111111 GTAC 01111	CLGAAAATCCAC GGAAAATCCAC GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GACAATCCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GGAAATCCCAT GGAAATCCCAT GGAAATCCCAT AGGAGATCCCAG ACAGGCCAATA AACTGGCTCAATA AACTGGCTCAATA					AATC		
1001A	AGAAGGGTGC AAGGTATGTC AAGGTATGTC AAGGTATGTC TRATTCATGTC TRATTCATGTC TRATTCATGTC TRATCATGTC CTAGGGTGC CTAAG CTACC CTAAG CTACC CTAAG CTACC CTAAG CTACC CTACG CTACC CTACG CTACC CTACG CTACC CTACG CTACC CTACG CTACC C	STICCAACA STICCAACAACA STICCAACAACAACAACAACAACAACAACAACAACAACAACA	AAAATCCAC		AGGGTGC 2101 SGTAC 210126 SGTAC 210126 SGTAC 201012 SGTAC 201012 SG	GAAAATCCAC GGAAAATCCAC GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GACAATCCAT GACAATCCAT GACAATCCAT GGAAGTCCTAT AACTGGCTCATT AACTGGCTCATT AGCAGGCTCATT					AATC		
1001A	AGAAGGGTGC - AAGGTAT - AAGGTAT - AAGGTAT - AAGGTAT - AAGGTAT - AAGGTAT - AAGGTAT - AAGGTAC - AAGGTAC		AAAATCCACG AAAATCCACG AAAT		AGGGTGC 0111 SGTAC 01111 SGTAC 011111 SGTAC 01111 SGTAC 011111 SGTAC 011111 SGTAC 011111 SGTAC 011111 SGTAC 011111 SGTAC 011111 SGTAC 011111 SGTAC 011111 SGTAC 011111 SGTAC 0111111 SGTAC 0111111 SGTAC 0111111 SGTAC 011111111 SGTAC 01111111111 SGTAC 011111111111111111111111	CLGAAAATCCAC GGAAAATCCAC GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GGAATCCCAG GACATCCCAG GCATCTCCAG GCATCTCCAG GGAATCCCAT AACTGGCTCATA AACTGGCTCATA AACTGGCTCATA AACTGGCTCATA AACGGGCTCATA AACGGGCTCATA					AATC- AAGGGTGC 271 AAGGGTGC 271 CCACG- CCACG		
	AGAAGGGTGC AAGGTATGT AAGGTATGT AAGGTATGT TRATTCATGT TRATTCATGT TRATTCATGT TRATCATGT AGGTACGT CTAAG CTA		AAAATCCAC		AGGGTGC 21217 SGTAC 212174 SGTAC 212174 SGTAC 212174 SGTAC 212174 CGT47444 CGT4744 CGT						AATC		
1001A +1650 ACA-	AGAAGGGTGC - AGGTAT - AGGTAT - AGGTAT - AGGTAT - AGGTAT - AGGTAT - AGGTAT - AGGTAT - AGGTAC  - AGGTAC        -	CTTCONACA TT	AAAATCCACG		AGGGTGC 2111 SGTAC 21011C SGTAC 21011C SG	CLGAAAATCCAC GGAAAATCCAC GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GGAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GACAATCCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GAAAATCCAT GGAATCCCAG GACATCCCAG GCATCTCCAG GCATCTCCAG GCATCTCCAG GGAAATCCCAT ACGGCCCATA AACTGGCCAATA AACGGGCCAATA AGCGGCCCAATA AGCGGGCTCATA AGCGGCCCAATA					AATC- AAGGGTGC 271 AAGGGTGC 271 CCACG- CCACG		

## Supplementary Figure 4 CDR1as multiple species alignment.

We obtained sequences homologous to the human CDR1as sequence using BLAT and the UCSC Genome browser. If the reverse BLAT hit human CDR1as, we kept the sequence for further analysis. A multiple species alignment was built with MUSCLE. miR-7 seed matches are color coded by their strength (gray: 6mer, light blue: 7mer, dark blue: 8mer, black: 9mer).



# Supplementary Figure 5 circular CDR1as nicking, expression and knock down in HEK293 cells and circular *Cdr1as* expression in mouse brain

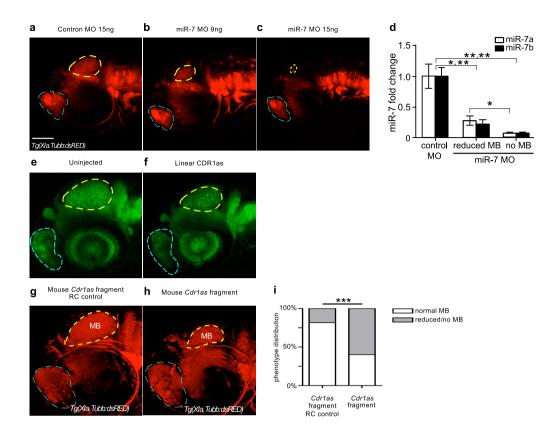
**a**, RNA nicking assay. Total RNA from HEK293 was subjected to RNA nicking (**Methods**) for the indicated time points. The "circular" probe spans CDR1as head-to-tail junction, "total" can detect linear as well as circular CDR1as. GAPDH: control. A linear IVT product of CDR1as serves as positive control.

b, qPCR analysis of CDR1as circularization in HEK293 (error:stdev).

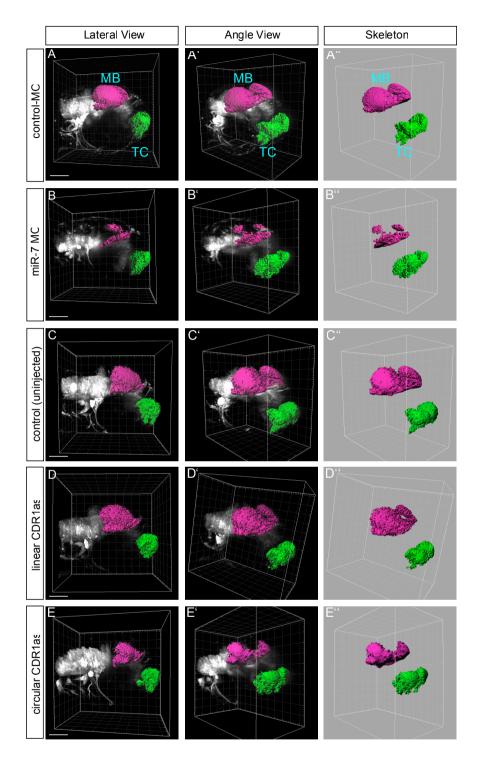
**c**, siRNA depletion of CDR1as induces repression of miR-7 target genes. siRNA knock down reduces levels of CDR1as and CDR1 mRNA by ~60%. Some house keeping genes are slightly affected, while 8/8 tested miR-7 targets are repressed, consistent with a release of miR-7 loaded RISC from CDR1as to the cytoplasm. **d**, A replicate of the experiment in (c) is shown with siRNA1 and the additional siRNA4.

e, Stable CDR1 knock down causes a cellular migration defect *in vitro*. 24 hours after disruption of a confluent cell monolayer, two cell lines derived from HEK293 cells with stable CDR1as knock downs (shSeq1, shSeq2) were significantly impaired in migrating into the scratch compared to control cell lines. Scalebar is 1mm.

**f**, **Quantification of the migration defect.** Three experiments were performed in quadruplicates and one in duplicate. Errorbars are standard deviation (P=0.002 shSeq1 vs shCtrl, P=1.2E-5 shSeq2 vs shCtrl, two-tailed T-test, unequal variance, n=14). **g,h**, *Cdr1as* is circular in adult midbrain and two mouse embryonic whole brains (error:stdev).

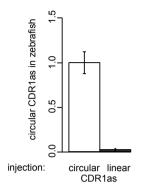


**Supplementary Figure 6 miR-7 MO/CDR1as phenotype in zebra fish a-c**, Representative confocal images of Tg(*Xia.Tubb:dsRED*) embryos at 48hpf injected with 15ng control MO **a**, 9ng miR-7 MO **b**, or 15ng miR-7 MO **c**, scale bar represents 100  $\mu$ m. Yellow dashed line indicates midbrain, blue dashed line indicates the telencephalon in **a-h**). **d**, qPCR analysis of *miR-7a* and *miR-7b* expression in control and *miR-7* morphants showing reduced midbrain (MB) or no MB. error:stdev \**P* < 0.05; \*\**P* < 0.01 in Student's *t*-test **e**,**f**, Representative confocal images of *Tg(huC:egfp)* animals after injection of a plasmid coding for linear CDR1as or unjected controls. **g**, Confocal z-stack projection from Tg(*Xia.Tubb:dsRED*) embryos at 48hpf in lateral view, after injection of mouse *Cdr1as* reverse complement (RC) control RNA or mouse *Cdr1as* RNA **h**. Yellow dashed line indicates midbrain, blue dashed line telencephalon. **i**, phenotype distributions of fish shown in (**g,h**), \*\*\**P* < 0.001; in Student's *t*-test.



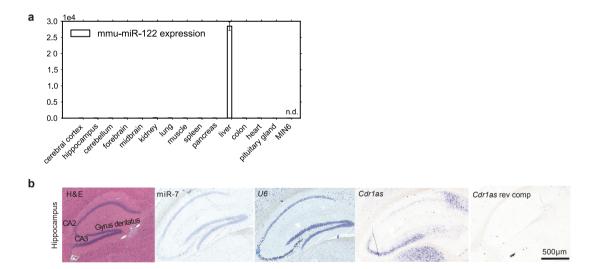
**Supplementary Figure 7 Volume measurement of zebra fish embryo midbrain and telencephalon based on confocal 3D stacks.** A-E: Midbrain and telencephalon volumes were calculated using Imaris 64x7.6.1 software based on high resolution 3D stacks obtained from Tg(*Xia.Tubb:dsRED*) embryos. Confocal images were taken at steps of 2µm, line scan 4 times, and 200 images were acquired per embryo. Midbrain (MB) was pseudo-colored in pink, telencephalon (TC) in green; other neuronal structures are in white. For clarity 3D projections are presented in lateral view (A-E), after rotation about the z-axis (A'-E'), and isolated from other brain structures (A''-E''). Embryos were injected with 15ng control MO (A), 9ng miR-7 MO (B). In a separate experiment, uninjected control embryos (C), were compared with embryos injected with plasmid expressing linear CDR1as (D), or circular

CDR1as (E). Note: the telencephalon structures (green) appear intact in miR-7 MO and CDR1as treated embryos. Dorsal is up, anterior to the right, posterior to the left. Scale bar:100µm. MB, midbrain; TC, telencephalon.



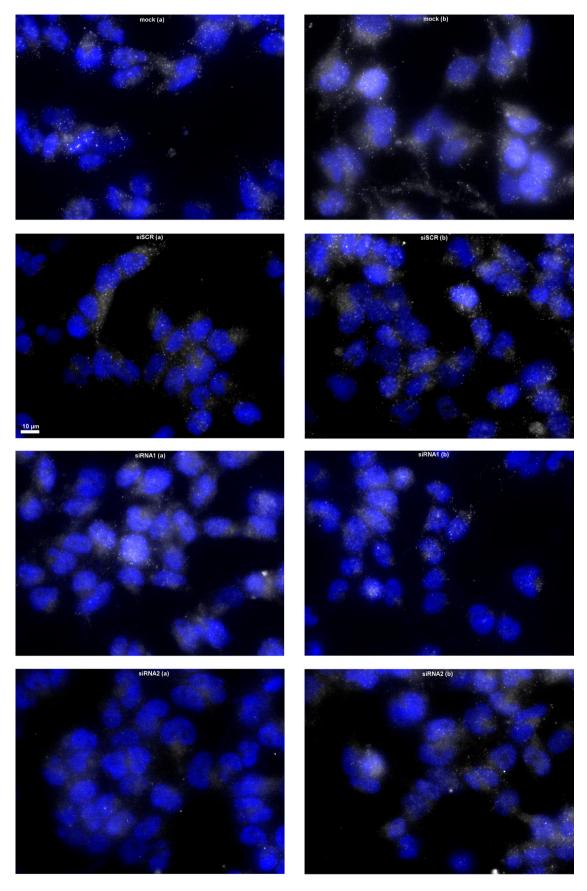
#### Supplementary Figure 8 circularized CDR1as in injected zebra fish

qPCR analysis of CDR1as transcripts in zebra fish 48hpf after injection of a plasmid coding for linear CDR1as or a plasmid coding for a transcript that can be circularized. PCR was performed using divergent primers, the signal from CDR1as linear injected fish is at background level. Data are normalized to  $\beta$ -actin (error:stdev).



# Supplementary Figure 9 miR-122 expression across mouse tissues and MIN6 cells, *Cdr1as* in mouse brain

**a**, miR-122 expression in 14 mouse tissues and MIN6 cells. As expected, miR-122 is highly and specifically expressed in liver. **b**, In situ hybridizations (ISH) were performed on paraffin sections showing *Cdr1as* expression in brains of adult C57Bl6/129Sv mice. A probe specific for the circular *Cdr1as* reverse complement serves as negative control. A histological staining (H&E) depicts the highly nucleated structures of the hippocampus (the areas of within the hippocampus CA2, CA3 neuronal layer and the Gyrus dentatus are labeled). ISH using double Digoxigenin labeled LNA probes detects miR-7 expression specifically in the hippocampus but not in the surrounding tissues. A U6 specific probe serves as positive control. The *Cdr1as* expression is most pronounced in the CA2 and CA3 area of the hippocampus whereas a scrambled LNA serves as negative control and yields no signal.



Supplementary Figure10 smRNA FISH of CDR1as in HEK293

species se	#miR-7 ed matches	length[nt]	6mer	7mer_1	7mer_2	8mer_1	8mer_2	9mer_1	other 11mer_1 matches
humar	74	1529	1	6	7	52	1	7	
gorilla	i 72	1511	1	6	7	51		7	
rhesus macaque	e 76	1582	1	6	11	51		7	
marmoset	62	1311		2	7	47		6	
mouse	139	2954	9	7	16	26	73	8	
rat	: 36	814	1	3	4	24		4	
naked mole-rat	52	1145		6	6	33		7	
guinea pig	22	744		3	6	11		2	
rabbit	: 74	1572	2	6	11	44	1	10	
pig	83	1652		1	15	58		9	
COW	34	893		1	3	27		1	2
horse	e 61	1309		3	8	43		7	
dog	65	1356	1	1	13	42		7	1
elephant	: 54	1371	1	12	6	32		3	
lamprey	38	1177		19	1	18			

## Supplementary Table 4 miR-7 seed matches in vertebrates.

We obtained sequences homologous to the human CDR1as sequence using BLAT and the UCSC Genome browser. If the reverse BLAT hit human CDR1as, we kept the sequence for further analysis. We counted miR-7 seed matches independently in the species used for our multiple species alignment.

smFISH CDR1as knock down control

	CDR1as relative expression	stdev
siSCR(control)	1.00	0.18
siRNA1	0.34	0.14
siRNA2	0.34	0.06

## Supplementary Table5 qPCR quantification of CDR1as in smFISH samples.

CDR1as expression was quantified by qPCR from the samples used for single molecule fluorescent *in situ* hybridization.

### CDR1as expression in stable knock down cell lines

	CDR1as relative expression	stdev
shCtrl	1.00	0.21
shRNA1	0.47	0.06
shRNA2	0.34	0.07

## Supplementary Table7 qPCR quantification of CDR1as in shCDR1as knock down cells.

CDR1as expression was quantified by qPCR from cell lines stably expressing shCDR1as shown in Supplementary Fig 5e.