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Supplemental Information

Artificial MicroRNAs Targeting *C9orf72* Can Reduce Accumulation of Intra-nuclear Transcripts in ALS and FTD Patients

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Supplementary material

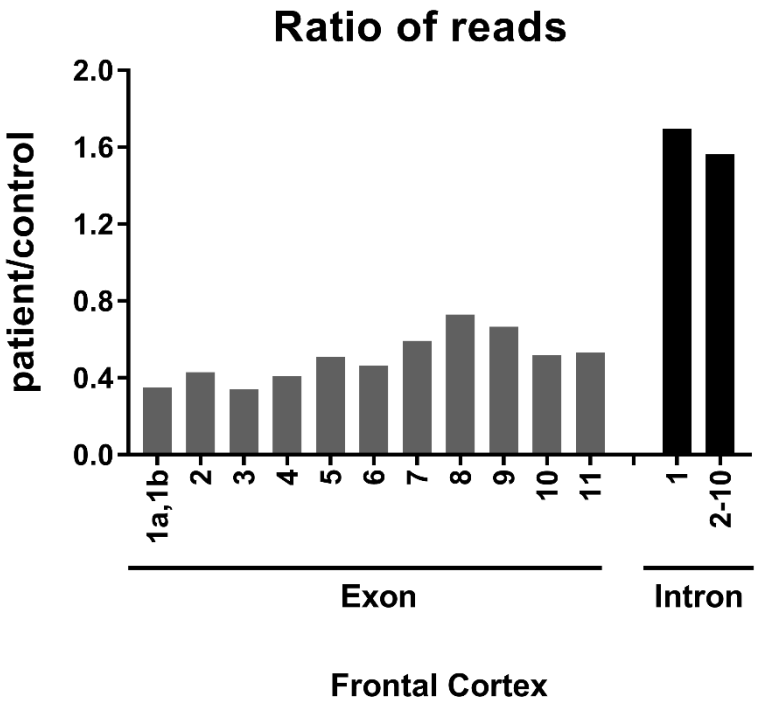

Figure S1. Ratio of reads between C9-ALS patients and controls in cortex. The total amount of reads counted in different intronic and exonic regions from C9-ALS patients were divided by the total amount of reads from the same region of control donors.

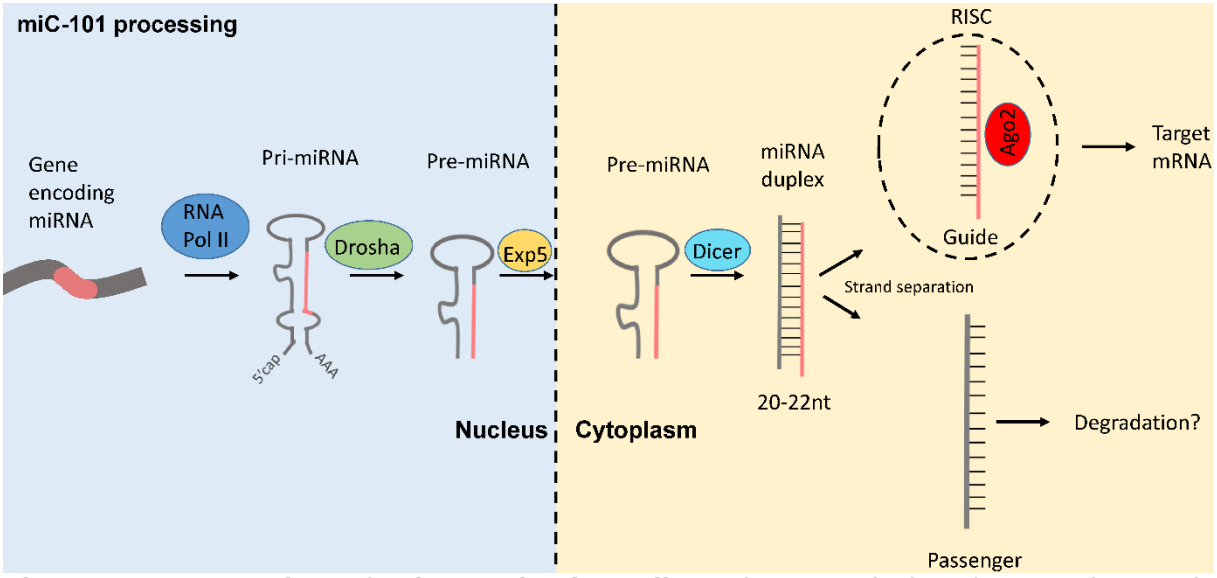

Figure S2. Processing of miC-101 in the cell. In the canonical pathway, miC-101 is transcribed by RNA pol II and produces a primary miRNA (pri-miC) hairpin, which is processed by Drosha to generate a precursor miRNA (pre-miC). The pre-miC is transported by exportin 5 (exp 5) into the cytoplasm for further processing by Dicer to produce a miC duplex of ~20-22 basepairs. An Argonaute (Ago2) protein binds the duplex and incorporates the mature miC-101 guide strand in the RNA-induced silencing complex (RISC) to suppress downstream target gene expression. The passenger strand of the miC-101 duplex is usually discarded but can also be functional.[1]

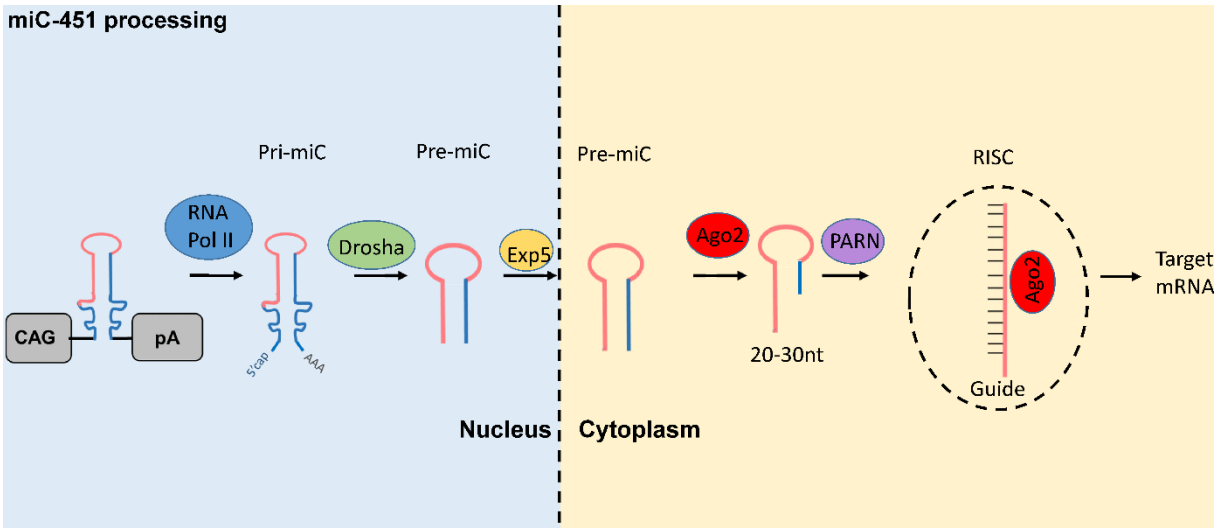

Figure S3. Processing of miC-451 in the cell. In the non-canonical pathway**,** miC-451 follows the same Drosha dependent processing in the nucleus into a pre-miC. The pre-miC-451 is too short to be cleaved by dicer and is directly loaded into Ago proteins. Ago2 cleaves the 3′ arm of pre-miR-451 by its slicer activity and yields a ~30-nt intermediate that is further trimmed by PARN to generate a mature miC of ~23nt. The trimming of the miC-451 is not essential for efficiency and miC-451 can be functional with mature guide strands longer than the mature length.[2]

miC	mature miRNA (5' to 3' Guide strand)	lenght	reads	% reads	mature miRNA (5' to 3' passenger strand)	nt leng	reads	% reads
miC2_101								
Total expression values: 1E+06								
5' passenger	1 TCACCCTCAGCGAGTACTGTGA	22	636099	57,6%	1 ACAGTACTCGCTGAGGGTTGACG	23	11703	1,1%
u cuggc u gucua	2 CACCCTCAGCGAGTACTGTGA	21	265198	24,0%	2 ACAGTACTCGCTGAGGGTTGAC	22	4773	0,4%
gcc cacaguacucgcugaggg ugac u	3 TCACCCTCAGCGAGTACTGTG	21	73556	6,7%	3 ACAGTACTCGCTGAGGGTTG	20	4136	0,4%
cgg gugucaugagcgacuccc acug u	4 CACCCTCAGCGAGTACTGTGAG	22	23444	2,1%	4 ACAGTACTCGCTGAGGGTTGACT	23	4102	0,4%
a uagga aaauc	5 TCACCCTCAGCGAGTACTGTGT	22	21217	1,9%	5 ACAGTACTCGCTGAGGGTTGA	21	3800	0,3%
3' guide	6 CACCCTCAGCGAGTACTGTGAGA	23	20599	1,9%	6 ACAGTACTCGCTGAGGGTT	19	1618	0,1%
	7 CACCCTCAGCGAGTACTGTGAA	22	18592	1,7%	7 ACAGTACTCGCTGAGGGTTGACA	23	1042	0,1%
	8 CACCCTCAGCGAGTACTGTG	20	14071	1,3%	8 CACAGTACTCGCTGAGGGTTG	21	774	0,1%
			total	97,1%			total	2,9%
miC4_101								
Total expression values: 3,40E+06								
5' passenger	1 CTAGCGCGCGACTCCTGAGTT	21	1955837	63,2%	1 CTCAGGAGTCGCGCGCTGA	19	47230	1,5%
u cuggc g cucua	2 CTAGCGCGCGACTCCTGAGTTA	22	378744	12,2%	2 ACTCAGGAGTCGCGCGCTGA	20	5763	0,2%
gcc acucaggaugucgcgcgcu aggg u	3 CTAGCGCGCGACTCCTGAGTTT	22	334594	10,8%	3 CTCAGGAGTCGCGCGCTG	18	5187	0,2%
cgg ugaguccucagcgcgcga uccu u	4 CTAGCGCGCGACTCCTGAGT	20	192640	6,2%	4 CTCAGGAGTCGCGCGCTGAGGG	22	4382	0,1%
a uaggu aaauc	5 CTAGCGCGCGACTCCTGAGTTAA	23	61917	2,0%	5 CTCAGGAGTCGCGCGCTGAGGGT	23	3151	0,1%
3' guide	6 CTAGCGCGCGACTCCTGAGTTG	22	37869	1,2%	6 CACTCAGGAGTCGCGCGCTGA	21	3128	0,1%
	7 CCTAGCGCGCGACTCCTGAGTT	22	34766	1,1%	7 CTCAGGAGTCGCGCGCTGT	19	2365	0,1%
	8 TAGCGCGCGACTCCTGAGTT	20	24587	0,8%	8 CTCAGGAGTCGCGCGCTGAGGGA	23	2311	0,1%
			Total	97,6%			Total	2,4%
miC32_101								
Total expression values: 3,46E+06								
5' passenger	1 TCCTTACTCTAGGACCAAGAAT	22	2606713	79,5%	1 TCTTGGTCCTAGAGTAACGGACG	22	24762	0,8%
u cuggc c gucua	2 TCCTTACTCTAGGACCAAGAATT	23	269810	8,2%	2 TCTTGGTCCTAGAGTAACGGA	21	16762	0,5%
gcc ucuugguccuagaguaa ggac u	3 CCTTACTCTAGGACCAAGAAT	21	142674	4,4%	3 TCTTGGTCCTAGAGTAACGG	20	7759	0,2%
cgg aagaaccaggaucucauu ccug u	4 CCTTACTCTAGGACCAAGAATT	22	75024	2,3%	4 TTCTTGGTCCTAGAGTAACGGA	22	6829	0,2%
a uaggu aaauc	5 TCCTTACTCTAGGACCAAGAA	21	37463	1,1%	5 TCTTGGTCCTAGAGTAACGGAC	22	6535	0,2%
3' guide	6 TCCTTACTCTAGGACCAAGAATTT	24	36928	1,1%	6 TCTTGGTCCTAGAGTAACG	19	6513	0,2%
	7 TCCTTACTCTAGGACCAAGAAC	22	16022	0,5%	7 TTCTTGGTCCTAGAGTAACGG	21	6217	0,2%
	8 TCCTTACTCTAGGACCAAGA	20	13809	0,4%	8 TCTTGGTCCTAGAGTAACGGACGT	24	4317	0,1%
			total	97,6%			total	2,4%
miC33_101								
Total expression values: 1E+06								
5' passenger	1 GCCTTACTCTAGGACCAAGAAT	22	304832	25,5%	1 CTTGGTCCTAGAGTAAGGGCC	21	10472	0,9%
u cuggc g gucua	2 GCCTTACTCTAGGACCAAGA	20	260199	21,7%	2 TCTTGGTCCTAGAGTAAGGG	20	3471	0,3%
gcc ucuugguccuagaguaag gcca u	3 GCCTTACTCTAGGACCAAGAA	21	193939	16,2%	3 TCTTGGTCCTAGAGTAAGG	19	2371	0,2%
cgg agaaccaggaucucauuc cggu u	4 GCCTTACTCTAGGACCAAGAATT	23	143923	12,0%	4 CTTGGTCCTAGAGTAAGGGC	20	2109	0,2%
a uagga aaauc	5 GCCTTACTCTAGGACCAAGACC	22	82239	6,9%	5 TCTTGGTCCTAGAGTAAGGGC	21	1873	0,2%
3' guide	6 GCCTTACTCTAGGACCAAGAAA	22	73716	6,2%	6 TCTTGGTCCTAGAGTAAGGGCC	22	1442	0,1%
	7 GCCTTACTCTAGGACCAAGAAG	22	69331	5,8%	7 CTTGGTCCTAGAGTAAGGGCCA	22	1299	0,1%
	8 GCCTTACTCTAGGACCAAGACT	22	44100	3,7%	8 CTTGGTCCTAGAGTAAGGG	19	1184	0,1%
			total	98,0%			total	2,0%
miC46_101								
Total expression values: 2E+05								
5' passenger	1 TTATCTTCAGGTTCCGAAGAGA	22	10318	5,6%	1 TCTTCGGAACCTGAAGATTGACG	23	126624	68,3%
u cuggc u gucua	2 TTATCTTCAGGTTCCGAAGAG	21	340	0,2%	2 TCTTCGGAACCTGAAGATTGAC	22	16393	8,8%
gcc cucuucggaaccugaaga ugac u	3 TTATCTTCAGGTTCCGAAGAGT	22	240	0,1%	3 TCTTCGGAACCTGAAGATTGACGT	24	15479	8,3%
cgg gagaagccuuggacuucu auug u	4 TTATCTTCAGGTTCCGAAGAGAA	23	199	0,1%	4 TCTTCGGAACCTGAAGATTGACT	23	5010	2,7%
a uagga aaauc	5 TATCTTCAGGTTCCGAAGAGA	21	198	0,1%	5 TCTTCGGAACCTGAAGATTGA	21	4479	2,4%
3' guide	6 TTATCTTCAGGTTCCGAAG	19	151	0,1%	6 TCTTCGGAACCTGAAGATTGACGTCTATTCTAAAG	35	2069	1,1%
	7 TTATCTTCAGGTTCCGA	17	144	0,1%	7 TCTTCGGAACCTGAAGATTGACGTT	25	1939	1,0%
	8 TTATCTTCAGGTTCCGAA	18	105	0,1%	8 CTTCGGAACCTGAAGATTGACG	22	1779	1,0%
			total	6,3%			total	93,7%
miC49_101								
Total expression values: 3,70E+05								
5' passenger	1 GCTCATCCTATGTTCAAGCTCG	22	142575	42,9%	1 AGCTTGAACATAGGATGACGTC	22	73189	22,0%
u cuagc c gucua	2 CTCATCCTATGTTCAAGCTCG	21	34782	10,5%	2 AGCTTGAACATAGGATGACGT	21	14962	4,5%
gcc agcuugaacauaggauga gucu u	3 GCTCATCCTATGTTCAAGCTCT	22	21281	6,4%	3 AGCTTGAACATAGGATGACGTCT	23	8609	2,6%
cgg ucgaacuuguauccuacu cggg u	4 CTCATCCTATGTTCAAGCTCGG	22	5304	1,6%	4 GCTTGAACATAGGATGACGTC	21	6126	1,8%
a uaggc aaauc	5 GCTCATCCTATGTTCAAGCTC	21	4672	1,4%	5 AGCTTGAACATAGGATGACG	20	4119	1,2%
3' guide	6 GCTCATCCTATGTTCAAGCTCGT	23	4018	1,2%	6 AGCTTGAACATAGGATGACGTCA	23	2509	0,8%
	7 CTCATCCTATGTTCAAGCTCGA	22	3894	1,2%	7 CAGCTTGAACATAGGATGACGT	22	2150	0,6%
	8 GCTCATCCTATGTTCAAGCTCGA	23	3608	1,1%	8 AGCTTGAACATAGGATGACGTT	22	905	0,3%
			total	66,2%			total	33,8%
miC50_101								
Total expression values: 1E+06								
5' passenger	1 AGGGACACTACAAGGTAGTATT	22	455665	35,7%	1 TACTACCTTGTAGTGTCCCCTCG	23	349594	27,4%
u cuggc c gucua	2 AGGGACACTACAAGGTAGTAT	21	73542	5,8%	2 TACTACCTTGTAGTGTCCCCTCGT	24	85200	6,7%
gcc auacuaccuuguaguguc ccuc u	3 AGGGACACTACAAGGTAGTA	20	15195	1,2%	3 TACTACCTTGTAGTGTCCCCTC	22	65164	5,1%
cgg uaugauggaacaucacag ggag u	4 GGGACACTACAAGGTAGTATT	21	12306	1,0%	4 TACTACCTTGTAGTGTCCCCTCT	23	62606	4,9%
a uaggu aaauc	5 AGGGACACTACAAGGTAGTATTT	23	9717	0,8%	5 TACTACCTTGTAGTGTCCCCTCGA	24	61009	4,8%
3' guide	6 AGGGACACTACAAGGTAGTATTA	23	5461	0,4%	6 TACTACCTTGTAGTGTCCCCT	21	38667	3,0%
	7 GGGACACTACAAGGTAGTATTG	22	3372	0,3%	7 ATACTACCTTGTAGTGTCCCCTCT	24	20936	1,6%
	8 GGGACACTACAAGGTAGTAT	20	2861	0,2%	8 TACTACCTTGTAGTGTCCCCTCGTT	25	15182	1,2%
			total	45,3%				54,7%

Table S1. miC-101 processing by NGS. Sequence distribution (%) of guide- and passenger strands of reads mapping to miC2_101, miC4_101, miC32_101, miC33_101, miC46_101, miC49_101 and miC50_101. Human embryonic kidney (HEK)293T cells were transfected with 250ng of the constructs. RNA was isolated 48 hours post-transfection and small RNA NGS was performed. The scaffold for each candidate is shown in the first column. Based on miRBase, the predicted guide and passenger strand sequences of the cellular pri-

miRNA scaffolds are indicated in red and blue, respectively. The 5’ and 3’ flanking nucleotides are indicated in black.

miC	mature miRNA (5' to 3' Guide strand)	lenght	reads	% reads
miC38_451				
Total expression values: 6,21E+04				
5' guide c a g a a u u g g g a u g g c a a g g c a a u c u c u g u c u u g g c a g a c c c u a u c g u u c c g u u a g a g a c a g a a c c g c a a u c a 3'	1 AGCAATCTCTGTCTTGGCAACAGCC	25	12263	22,2%
	2 AGCAATCTCTGTCTTGGCAACAGCCAT	27	11500	20,8%
	3 AGCAATCTCTGTCTTGGCAACAGCCAAT	28	7931	14,3%
	4 AGCAATCTCTGTCTTGGCAACAGCCAAGA	29	4655	8,4%
	5 AGCAATCTCTGTCTTGGCAACAGCCAAG	28	3896	7,0%
	6 AGCAATCTCTGTCTTGGCAACAGCCAA	27	2978	5,4%
	7 AGCAATCTCTGTCTTGGCAACAGCCT	26	2973	5,4%
	8 AGCAATCTCTGTCTTGGCAACAGC	23	2773	5,0%
	9 AGCAATCTCTGTCTTGGCAACAGCCATT	28	2732	4,9%
	10 AGCAATCTCTGTCTTGGCAACAGCCAAGT	29	1779	3,2%
	11 AGCAATCTCTGTCTTGGCAACAGCCAAA	28	1048	1,9%
	12 AGCAATCTCTGTCTTGGCAACA	22	805	1,5%
			total	100,0%
miC39_451				
Total expression values: 2,05E+05				
5' guide c a g u g u u g g g a u g g c a a g a a a g c a a u c u c u g u c u u g g a c c c u a u c g u u c u u u c g u u a g a g a c a g a a c a a u c a 3'	1 TAAAGCAATCTCTGTCTTGGCA	22	76916	39,1%
	2 TAAAGCAATCTCTGTCTTGGCAA	23	75442	38,4%
	3 TAAAGCAATCTCTGTCTTGGCAAA	24	13838	7,0%
	4 TAAAGCAATCTCTGTCTTGGCAAAG	25	11180	5,7%
	5 TAAAGCAATCTCTGTCTTGGC	21	6379	3,2%
	6 TAAAGCAATCTCTGTCTTGGCAAT	24	2638	1,3%
	7 AAAGCAATCTCTGTCTTGGCAA	22	2314	1,2%
	8 TAAAGCAATCTCTGTCTTGGCAAGA	26	2238	1,1%
	9 TAAAGCAATCTCTGTCTTGGCAT	23	1613	0,8%
	10 AAAGCAATCTCTGTCTTGGCA	21	1502	0,8%
	11 AAAGCAATCTCTGTCTTGGCAAG	24	1248	0,6%
	12 TAAAGCAATCTCTGTCTTGGCAAGT	26	1243	0,6%
			total	99,9%
miC43_451				
Total expression values: 1,36E+04				
5' guide c a g u u u u g g g a u g g c a a g u g g c a a g a a a a g u u a u u c g a c c c u a u c g u u c a c c g u u c u u u u c a a u a a u a a u c c 3'	1 TTGGCAAGAAAAGTTATTTCTCAAT	25	5312	42,7%
	2 TTGGCAAGAAAAGTTATTTCTCAATAACT	29	1853	14,9%
	3 TTGGCAAGAAAAGTTATTTCTCA	23	1387	11,1%
	4 TTGGCAAGAAAAGTTATTTCTCAATAAC	28	1253	10,1%
	5 TTGGCAAGAAAAGTTATTTCTCAATA	26	1015	8,2%
	6 TTGGCAAGAAAAGTTATTTCTCAATAACTT	30	556	4,5%
	7 TTGGCAAGAAAAGTTATTTCTCAA	24	525	4,2%
	8 TTGGCAAGAAAAGTTATTTCTCAATAA	27	272	2,2%
	9 TTGGCAAGAAAAGTTATTTCTC	22	228	1,8%
	10 TTGGCAAGAAAAGTTATTTCT	23	54	0,4%
	11			0,0%
	12			0,0%
			total	100,0%
miC49_451				
Total expression values : 1,37E+05				
5' guide c a g a g u u g g g a u g g c a a g g c u c a u c c u a u g u u c a a c g a c c c u a u c g u u c c g a g u a g g a u a c a a g u u u a a u c c 3'	1 AGCTCATCCTATGTTCAAGCTCTT	24	76725	58,6%
	2 AGCTCATCCTATGTTCAAGCTCTTG	25	14652	11,2%
	3 AGCTCATCCTATGTTCAAGCTCTTGA	26	13511	10,3%
	4 AGCTCATCCTATGTTCAAGCTCT	23	11970	9,1%
	5 AGCTCATCCTATGTTCAAGCTCTTGAAC	28	6402	4,9%
	6 AGCTCATCCTATGTTCAAGCTCTTGT	26	2060	1,6%
	7 AGCTCATCCTATGTTCAAGCTCTTGAA	27	1982	1,5%
	8 AGCTCATCCTATGTTCAAGCTCTTT	25	1321	1,0%
	9 AGCTCATCCTATGTTCAAGCTCTTGAACA	29	693	0,5%
	10 GCTCATCCTATGTTCAAGCTCTT	23	634	0,5%
	11 AGCTCATCCTATGTTCAAGCTCTTA	25	534	0,4%
	12 AGCTCATCCTATGTTCAAGCTCTTGAT	27	406	0,3%
			total	100,0%
miC50_451				
Total expression values : 1,06E+04				
5' guide c a g u u u u g g g a u g g c a a g g g a c a c u a c a a g g u a g a g a c c c u a u c g u u c c c c u g u g a u g u u c c a u c u a a a u u 3'	1 TGGGACACTACAAGGTAGTATTCTACCTT	29	5354	55,9%
	2 TGGGACACTACAAGGTAGTATTCTACCT	28	961	10,0%
	3 TGGGACACTACAAGGTAGTATT	22	611	6,4%
	4 TGGGACACTACAAGGTAGTATTC	23	560	5,8%
	5 TGGGACACTACAAGGTAGTATTCTACCTTT	30	485	5,1%
	6 TGGGACACTACAAGGTAGTATTCTACCTTG	30	461	4,8%
	7 TGGGACACTACAAGGTAGTATTCT	24	284	3,0%
	8 TGGGACACTACAAGGTAGTATTCTACC	27	274	2,9%
	9 TGGGACACTACAAGGTAGTATTCTACCTTGT	31	252	2,6%
	10 TGGGACACTACAAGGTAGTATTCTACCTTA	30	149	1,6%
	11 TGGGACACTACAAGGTAGTATTCTACCTA	29	126	1,3%
	12 GGGACACTACAAGGTAGTATTCTACCTT	28	73	0,8%
			total	100,0%

Table S2. miC-451 processing by NGS. Sequence distribution (%) of guide strands of reads mapping to miC38_451, miC39_451, miC43_451, miC49_451 and miC 50_451. Performed as described in table S1.

References

1. Miniarikova, J, Zanella, I, Huseinovic, A, van der Zon, T, Hanemaaijer, E, Martier, R, *et al.* (2016). Design, Characterization, and Lead Selection of Therapeutic miRNAs Targeting Huntingtin for Development of Gene Therapy for Huntington's Disease. *Mol. Ther. Nucleic Acids* **5**: e297.
2. Mayuko Yoda, Daniel Cifuentes, Natsuko Izumi, Yuriko Sakaguchi, Tsutomu Suzuki, AJG and YT (2013). PARN mediates 3′-end trimming of Argonaute2-cleaved precursor microRNAs **5**.