

## SUPPLEMENTARY FOR

### miRNA analysis reveals novel dysregulated pathways in amyotrophic lateral sclerosis

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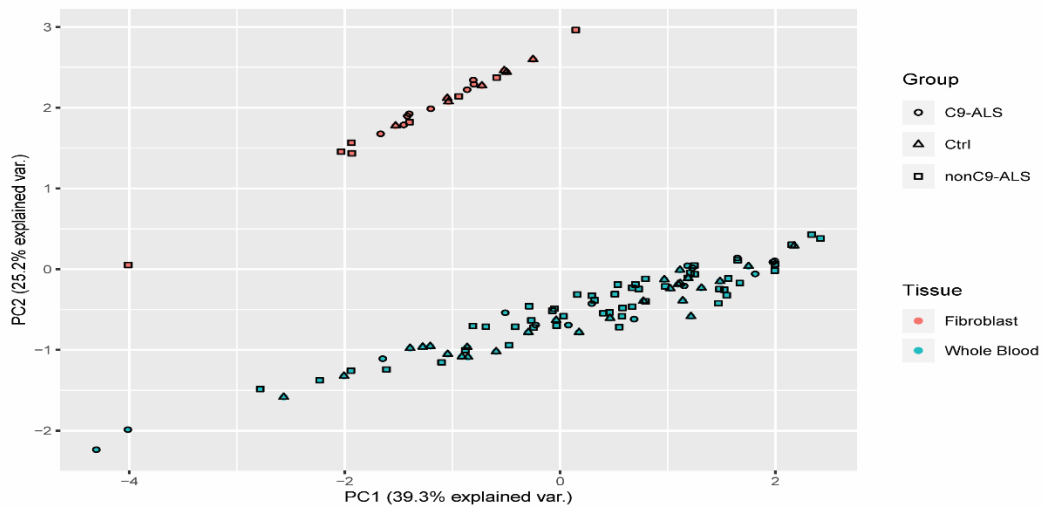
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## Supplementary Figures

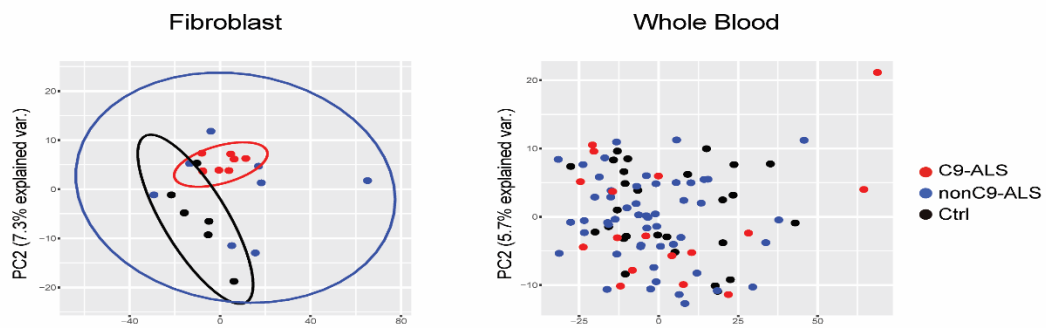
### Supplementary Figure S1. Trends of miRNA levels in fibroblasts and whole blood from C9-ALS and nonC9-ALS versus controls

(A) Principal component analysis of overall miRNA distribution in fibroblasts (red) and whole blood (cyan). Groups are represented by node shape, C9-ALS (circles, nonC9-ALS (squares), and controls (triangles). (B) Principal component analysis of miRNA distribution by tissue (fibroblasts, left; whole blood, right) in C9-ALS (red), nonC9-ALS (blue), and control (black) samples.

**A**

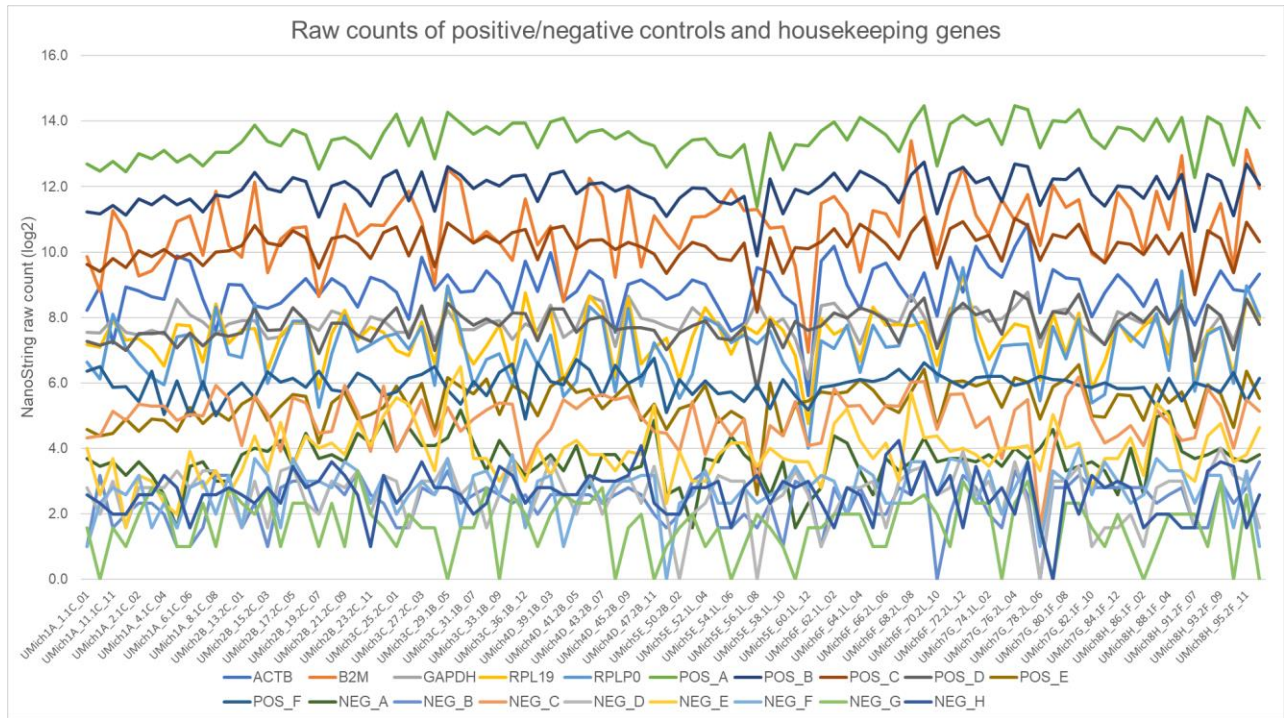


**B**



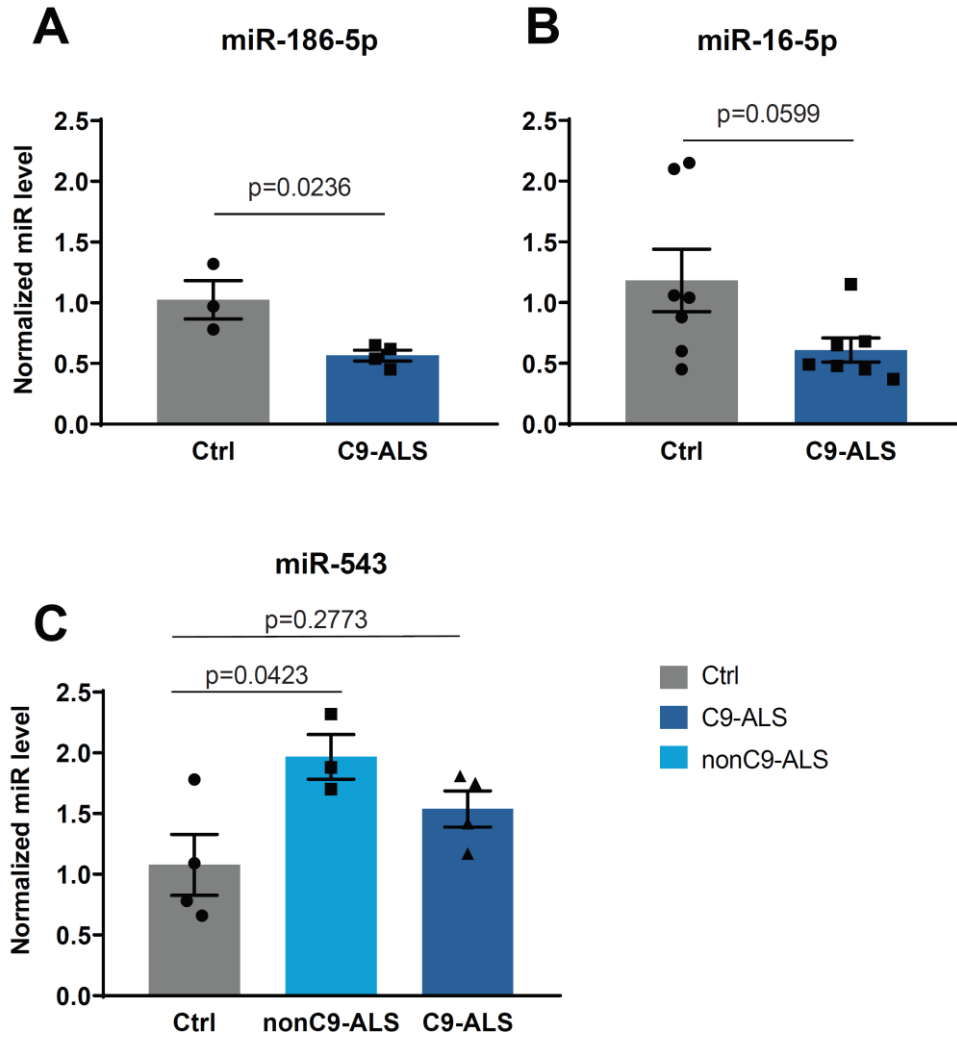
### Supplementary Figure S2. Trends of positive and negative control and reference (housekeeping gene) probes

The log<sub>2</sub>-transformed raw counts of the positive control, negative control, and reference gene probes, that are embedded in the NanoString nCount system, are depicted in line plots. The normalization of the raw count data was done by NanoStringDiff based on these quality control probes, which showed relatively consistent patterns across samples.



### Supplementary Figure S3. qPCR validation of differential miRNAs in fibroblasts

Dysregulated differential miRNAs in fibroblasts were validated by qPCR. Results were normalized to RNU48 and presented as fold-change calculated by the  $2^{-\Delta\Delta C_T}$  method for (A) miR-186-5p [C9-ALS, n=4; Control (Ctrl), n=3], (B) miR-165p (C9-ALS, n=7; Ctrl, n=7), (C) miR-543 (C9-ALS, n=4; nonC9-ALS, n=3; Ctrl, n=4). Analysis by Student's t-test in panels A and B and one-way ANOVA in panel C; data are represented as mean  $\pm$  standard error of the mean.



## Supplementary Tables

### Supplementary Table S1. Differential miRNAs in fibroblasts

All differential miRNAs (DmiRNAs) in fibroblasts were identified by NanoStringDiff between C9-ALS and nonC9-ALS versus controls and were deemed significant with p-value < 0.05.

DmiRNAs are ordered based on the sharing between C9-ALS and nonC9-ALS and then by log(fold-change) within each subgroup.

Fibroblast					
miRNA ID	Subgroup	C9-ALS versus Control		nonC9-ALS versus Control	
		logFC	p-value	logFC	p-value
hsa-miR-516a-3p	Shared	28.02	2.56E-03	27.52	2.55E-02
hsa-miR-153	Shared	27.73	1.05E-02	27.75	9.86E-03
hsa-miR-548ak	Shared	26.21	8.11E-09	26.01	7.55E-08
hsa-miR-663b	Shared	24.71	2.33E-03	25.92	5.15E-07
hsa-miR-141-3p	Shared	22.73	9.95E-05	22.07	3.12E-03
hsa-miR-217	Shared	21.90	2.18E-03	21.91	3.29E-03
hsa-miR-1260b	Shared	18.45	1.30E-02	19.60	2.70E-05
hsa-miR-302b-3p	Shared	3.35	1.07E-05	2.53	5.14E-03
hsa-miR-548ah-5p	Shared	2.97	4.69E-04	2.66	4.12E-03
hsa-miR-942	Shared	2.58	2.55E-03	3.14	7.25E-05
hsa-miR-1180	Shared	2.11	3.72E-06	1.80	1.79E-04
hsa-miR-542-5p	Shared	1.95	2.42E-02	1.82	4.28E-02
hsa-miR-4443	Shared	1.28	1.90E-03	1.38	8.84E-04
hsa-miR-7-5p	Shared	1.17	3.41E-02	1.79	4.88E-04
hsa-miR-216a	Shared	0.95	1.69E-02	1.15	3.98E-03
hsa-miR-1183	Shared	0.93	3.75E-02	1.05	2.02E-02
hsa-miR-128	Shared	-0.87	9.45E-03	-0.76	2.38E-02
hsa-miR-197-3p	Shared	-0.88	3.41E-03	-1.09	3.35E-04
hsa-miR-92a-3p	Shared	-1.00	2.71E-03	-0.91	6.16E-03
hsa-let-7f-5p	Shared	-1.15	4.75E-04	-0.67	4.11E-02
hsa-miR-30b-5p	Shared	-1.24	1.73E-04	-0.92	5.38E-03
hsa-miR-30c-5p	Shared	-1.33	9.29E-05	-0.88	9.71E-03
hsa-miR-484	Shared	-1.53	8.64E-05	-0.76	4.02E-02
hsa-miR-3195	Shared	-1.54	1.08E-04	-2.06	1.32E-06
hsa-miR-92b-3p	Shared	-1.55	2.75E-05	-0.75	3.46E-02
hsa-miR-138-5p	Shared	-1.57	5.73E-03	-1.19	3.52E-02
hsa-miR-593-3p	Shared	-1.67	1.74E-05	-1.00	7.64E-03
hsa-miR-4516	Shared	-3.41	4.56E-11	-3.02	3.40E-09
hsa-miR-1234	Shared	-3.50	9.49E-07	-1.57	2.87E-03

Fibroblast					
miRNA ID	Subgroup	C9-ALS versus Control		nonC9-ALS versus Control	
		logFC	p-value	logFC	p-value
hsa-miR-4488	Shared	-23.75	6.22E-15	-5.85	4.02E-10
hsa-miR-1246	C9-unique	40.41	0.00E+00		
hsa-miR-369-5p	C9-unique	30.93	6.52E-04		
hsa-miR-515-5p	C9-unique	29.00	2.20E-03		
hsa-miR-524-3p	C9-unique	28.00	3.70E-02		
hsa-miR-4458	C9-unique	27.69	1.14E-02		
hsa-miR-922	C9-unique	25.79	9.40E-03		
hsa-miR-3180	C9-unique	25.53	2.33E-02		
hsa-miR-105-5p	C9-unique	25.35	2.42E-02		
hsa-miR-215	C9-unique	22.38	2.72E-03		
hsa-miR-550b-3p	C9-unique	2.14	4.49E-05		
hsa-miR-33a-5p	C9-unique	1.75	2.57E-02		
hsa-miR-761	C9-unique	1.59	2.36E-02		
hsa-miR-369-3p	C9-unique	1.43	4.27E-02		
hsa-miR-548a-5p	C9-unique	1.36	6.38E-03		
hsa-miR-34c-3p	C9-unique	1.07	4.95E-02		
hsa-miR-193a-5p	C9-unique	-0.62	4.84E-02		
hsa-miR-409-3p	C9-unique	-0.66	4.54E-02		
hsa-miR-139-5p	C9-unique	-0.70	3.51E-02		
hsa-miR-579	C9-unique	-0.70	3.12E-02		
hsa-miR-16-5p	C9-unique	-0.72	1.65E-02		
hsa-miR-29a-3p	C9-unique	-0.73	3.08E-02		
hsa-miR-145-5p	C9-unique	-0.78	2.17E-02		
hsa-miR-489	C9-unique	-0.80	3.23E-02		
hsa-miR-365a-3p	C9-unique	-0.83	7.87E-03		
hsa-miR-137	C9-unique	-0.84	1.98E-02		
hsa-miR-107	C9-unique	-0.89	1.57E-03		
hsa-miR-455-3p	C9-unique	-0.92	1.21E-02		
hsa-miR-26a-5p	C9-unique	-0.94	6.15E-03		
hsa-miR-376b	C9-unique	-0.95	1.76E-02		
hsa-miR-186-5p	C9-unique	-1.06	6.14E-03		
hsa-miR-214-3p	C9-unique	-1.20	8.55E-05		
hsa-miR-204-5p	C9-unique	-1.41	1.01E-02		
hsa-miR-597	nonC9-unique			26.49	5.31E-03
hsa-miR-378f	nonC9-unique			25.55	1.42E-03
hsa-miR-133b	nonC9-unique			25.37	3.23E-03
hsa-miR-548n	nonC9-unique			25.27	9.71E-03
hsa-miR-1323	nonC9-unique			24.76	7.53E-03

Fibroblast					
miRNA ID	Subgroup	C9-ALS versus Control		nonC9-ALS versus Control	
		logFC	p-value	logFC	p-value
hsa-miR-548ae	nonC9-unique			23.62	1.19E-02
hsa-miR-367-3p	nonC9-unique			21.40	3.86E-02
hsa-miR-627	nonC9-unique			20.75	3.19E-02
hsa-miR-142-3p	nonC9-unique			15.94	6.98E-04
hsa-miR-518b	nonC9-unique			4.40	4.00E-03
hsa-miR-769-5p	nonC9-unique			3.16	1.05E-02
hsa-miR-412	nonC9-unique			3.08	1.38E-02
hsa-miR-514b-5p	nonC9-unique			2.01	3.80E-04
hsa-miR-95	nonC9-unique			1.62	6.28E-03
hsa-miR-1290	nonC9-unique			1.25	2.36E-03
hsa-miR-1233	nonC9-unique			1.23	3.33E-03
hsa-miR-4455	nonC9-unique			0.96	6.37E-03
hsa-miR-338-3p	nonC9-unique			0.96	1.97E-02
hsa-miR-612	nonC9-unique			0.92	2.68E-02
hsa-miR-548ai	nonC9-unique			0.91	3.16E-02
hsa-miR-135b-5p	nonC9-unique			0.89	3.78E-02
hsa-miR-543	nonC9-unique			0.59	4.44E-02
hsa-miR-27a-3p	nonC9-unique			-0.91	1.04E-02
hsa-miR-3161	nonC9-unique			-1.15	4.00E-02
hsa-miR-362-3p	nonC9-unique			-1.81	6.80E-03



**Supplementary Table S2. Differential miRNAs in whole blood**

All differential miRNAs (DmiRNAs) in whole blood were identified by NanoStringDiff between C9-ALS and nonC9-ALS versus controls and were deemed significant with p-value < 0.05. DmiRNAs are ordered based on the sharing between C9-ALS and nonC9-ALS and then by log(fold-change) within each subgroup.

Whole Blood					
miRNA ID	Subgroup	C9-ALS versus Control		nonC9-ALS versus Control	
		logFC	p-value	logFC	p-value
hsa-miR-96-5p	Shared	30.35	1.53E-03	28.71	1.75E-02
hsa-miR-483-3p	Shared	27.49	2.47E-04	26.85	6.67E-04
hsa-miR-515-3p	Shared	25.76	5.01E-08	23.48	9.49E-05
hsa-miR-215-5p	Shared	25.43	7.25E-04	25.70	1.43E-05
hsa-miR-665	Shared	25.41	2.64E-05	25.33	2.52E-06
hsa-miR-575	Shared	24.39	3.35E-04	23.78	7.39E-04
hsa-miR-877-5p	Shared	23.96	1.04E-04	23.54	1.29E-05
hsa-miR-154-5p	Shared	22.89	4.12E-05	22.18	2.15E-04
hsa-miR-574-5p	Shared	20.16	4.02E-03	21.99	2.98E-06
hsa-miR-548j-3p	Shared	20.03	2.77E-02	19.62	3.57E-02
hsa-miR-339-5p	Shared	2.74	2.56E-03	3.24	2.14E-05
hsa-miR-1290	Shared	2.18	2.64E-02	2.16	1.02E-02
hsa-miR-128-3p	Shared	2.14	1.86E-02	1.85	1.47E-02
hsa-miR-30b-5p	Shared	1.28	2.70E-03	0.82	1.53E-02
hsa-miR-28-3p	Shared	1.26	7.06E-03	0.95	1.09E-02
hsa-miR-26a-5p	Shared	1.19	9.21E-03	0.96	7.97E-03
hsa-miR-324-3p	Shared	1.17	4.46E-02	1.56	5.72E-04
hsa-miR-30c-5p	Shared	1.10	4.58E-03	0.78	1.02E-02
hsa-miR-361-5p	Shared	0.93	8.30E-03	0.58	3.32E-02
hsa-miR-484	Shared	0.90	1.34E-02	0.79	5.59E-03
hsa-miR-92a-3p	Shared	0.69	3.21E-02	0.55	2.60E-02
hsa-let-7b-5p	Shared	-0.82	4.13E-02	-0.75	7.55E-03
hsa-miR-2110	Shared	-1.52	3.77E-04	-0.63	2.73E-02
hsa-miR-671-3p	Shared	-6.48	1.60E-02	-3.65	1.10E-02
hsa-miR-885-5p	C9-unique	31.60	4.19E-03		
hsa-miR-146b-5p	C9-unique	27.69	1.65E-02		
hsa-miR-3614-3p	C9-unique	26.29	4.00E-11		
hsa-miR-501-3p	C9-unique	25.59	1.59E-08		
hsa-miR-4536-5p	C9-unique	25.35	4.88E-03		
hsa-miR-1197	C9-unique	24.38	1.79E-03		
hsa-miR-345-3p	C9-unique	23.84	4.56E-11		

Whole Blood					
miRNA ID	Subgroup	C9-ALS versus Control		nonC9-ALS versus Control	
		logFC	p-value	logFC	p-value
hsa-miR-587	C9-unique	23.69	7.75E-03		
hsa-miR-548g-3p	C9-unique	23.25	1.44E-03		
hsa-miR-10b-5p	C9-unique	22.60	4.72E-02		
hsa-miR-200a-3p	C9-unique	21.88	1.41E-02		
hsa-miR-2682-5p	C9-unique	16.83	1.80E-02		
hsa-miR-556-5p	C9-unique	3.32	3.16E-04		
hsa-miR-330-5p	C9-unique	2.61	1.71E-02		
hsa-miR-208b-5p	C9-unique	2.28	2.34E-03		
hsa-miR-128-1-5p	C9-unique	1.73	7.48E-04		
hsa-miR-1285-5p	C9-unique	1.73	5.21E-03		
hsa-miR-1183	C9-unique	1.53	2.28E-03		
hsa-miR-30e-3p	C9-unique	1.52	9.49E-03		
hsa-miR-337-3p	C9-unique	1.35	1.96E-02		
hsa-miR-186-5p	C9-unique	1.04	1.00E-02		
hsa-miR-150-5p	C9-unique	-0.54	4.06E-02		
hsa-miR-125a-5p	C9-unique	-0.62	4.65E-02		
hsa-miR-378i	C9-unique	-1.00	1.64E-02		
hsa-miR-1226-3p	C9-unique	-1.21	1.25E-02		
hsa-miR-320a	C9-unique	-1.23	2.88E-02		
hsa-miR-7-5p	C9-unique	-1.40	3.69E-02		
hsa-miR-378g	C9-unique	-2.93	7.41E-03		
hsa-miR-3065-5p	C9-unique	-3.16	4.00E-04		
hsa-miR-516b-5p	C9-unique	-19.70	9.48E-03		
hsa-miR-498	C9-unique	-19.80	2.94E-03		
hsa-miR-6511a-3p	C9-unique	-20.22	1.27E-03		
hsa-miR-1248	C9-unique	-20.74	8.44E-03		
hsa-miR-548ak	C9-unique	-21.32	1.29E-03		
hsa-miR-122-5p	C9-unique	-22.76	6.69E-05		
hsa-miR-3127-5p	C9-unique	-23.40	2.43E-05		
hsa-miR-3195	C9-unique	-23.61	6.18E-04		
hsa-miR-494-3p	C9-unique	-30.09	2.67E-05		
hsa-miR-642a-5p	nonC9-unique			29.98	7.05E-03
hsa-miR-1306-3p	nonC9-unique			29.49	2.17E-02
hsa-miR-875-3p	nonC9-unique			29.44	6.66E-04
hsa-miR-3918	nonC9-unique			26.78	3.49E-02
hsa-miR-543	nonC9-unique			25.36	3.63E-02
hsa-miR-944	nonC9-unique			24.59	1.08E-02
hsa-miR-371a-5p	nonC9-unique			20.09	6.89E-03

Whole Blood					
miRNA ID	Subgroup	C9-ALS versus Control		nonC9-ALS versus Control	
		logFC	p-value	logFC	p-value
hsa-miR-151b	nonC9-unique			19.94	9.03E-03
hsa-miR-320b	nonC9-unique			18.93	3.89E-02
hsa-miR-597-5p	nonC9-unique			5.69	2.41E-03
hsa-miR-548al	nonC9-unique			4.26	4.31E-02
hsa-miR-2117	nonC9-unique			1.20	4.59E-02
hsa-miR-4516	nonC9-unique			1.02	7.65E-03
hsa-miR-99a-5p	nonC9-unique			1.01	3.14E-02
hsa-miR-182-5p	nonC9-unique			0.75	1.77E-02
hsa-miR-197-3p	nonC9-unique			0.60	2.07E-02
hsa-miR-145-5p	nonC9-unique			0.60	2.63E-02
hsa-miR-331-3p	nonC9-unique			0.59	2.97E-02
hsa-miR-423-3p	nonC9-unique			0.54	4.57E-02
hsa-miR-1180-3p	nonC9-unique			-0.46	2.65E-02

