Supporting Information

Arashiro et al. 10.1073/pnas.0901573106

SI Text

RNA Isolation. The muscle tissue was ground into fine powder in dry ice. Then, 6 ml of TRIReagent was added, homogenized for 1 min and the homogenate was split into 4 different tubes. After the incubation for 5 min, 300 μl of chloroform was added and the solution was shaken vigorously for 1 min. The tubes were incubated for 15 min at room temperature and then centrifuged at $12,000 \times g$ for 15 min at 4 °C. The supernatant was transferred to a fresh tube, 750 μl of isopropanol was added and mixed. After the incubation for 10 min at room temperature, the solution was centrifuged at $12,000 \times g$ for 15 min at 4 °C for RNA precipitation. The RNA pellets from the 4 tubes were mixed and washed with 1-ml 75% ethanol. The pellets were resuspended in nuclease-free water, mixed by pipettation, and incubated at 55 °C for 15 min. The RNA quality was observed through gel electrophoresis and Experion (Bio-Rad).

Microarray Assay. One microgram of total RNA was copied by reverse transcriptase using a T7-Oligo(dT) Promoter Primer and SuperScript II in the first-strand cDNA synthesis reaction. Following RNase H-mediated second-strand cDNA synthesis, the double-stranded cDNA was purified using a "Sample Cleanup Module" kit (Affymetrix). The cDNA served as a template in the subsequent in vitro transcription reaction. The in vitro transcription was carried out at 37 °C for 16 h in the presence of T7 RNA Polymerase and a biotinylated nucleotide analog/ribonucleotide mix for complementary RNA (cRNA) amplification and biotin labeling. The biotinylated cRNA was purified with a "Sample Cleanup Module" kit (Affymetrix) and quantified (NanoDrop company). Twenty micrograms of cRNA was fragmented randomly to 35 to 200 bases at 94 °C for 35 min. Fifteen micrograms of fragmented cRNA was hybridized to U133 Plus 2.0 chips for 16 h at 45 °C with rotation. The chips were washed and stained by the Affymetrix Fluidics Station 450, using instructions and reagents provided by Affymetrix. This step involves removal of nonhybridized probes and incubation with Streptavidin Phycoerythrin to detect bound probes.

Microarray Data Analysis. Each sample transcriptome profile is regarded as a 54,675-dimensional vector of measured probe-expression signals. There were a total of 15 individual sample profiles: 5 control, 5 asymptomatic, 5 affected. Asymptomatic sample number 14 was excluded from subsequent analysis because less than 5% of its total probes had a "present" Affymetrix detection call.

The entire dataset was normalized sample-wise by linear regression to the reference sample with the highest average linear correlation against all other samples. This reference sample was control sample number 3.

A probe-by-probe differential fold analysis was performed between any 2 sample populations (control vs. asymptomatic, control vs. affected, asymptomatic vs. affected) using a geometric logarithmic-fold method on the normalized dataset with the minimal probe-signal threshold set to 50 (1, 2). The difference in average logarithmic signals (AvgLF) of a microarray probe-gene between the 2 sample populations was calculated. If a given probe has normalized signals $50 \leq a1 \leq a2 \leq \ldots \leq aJ$ and $50 \leq b1 \leq b2 \leq \ldots \leq bK$ in populations A and B, respectively, then AvgLF of B relative to A is defined as $\text{mean}(\log(b\text{'s})) - \text{mean}(\log(a\text{'s}))$. Furthermore, we define NoisLF as the maximal difference in logarithmic signals from a common population. In this example, NoisLF for the probe in A is $\text{mean}(\log(aJ2)) - \text{mean}(\log(aJ1))$, where J1 ranges from 1 to [J/2], J2 range from [J/2]+1 to J (if J is even) or [J/2]+2 to J (if J is odd), where [J/2] is the largest integer less than or equal to J/2. An AvgLF value for a probe is considered significant if its magnitude exceeds log (2) and the NoisLF from all 3 sample populations. Twelve probes (11 unique genes) were significantly differentially expressed across control–asymptomatic, 56 probes (56 unique genes) across control–affected, and 147 probes (140 unique genes) across asymptomatic–affected by this criterion.

RT-PCR Validation of Microarray Data. Starting with 500 ng of total RNA, cDNA syntheses were accomplished as above with Random Primers (Invitrogen) and SuperScript II (Invitrogen) in 20-μl reactions. TaqMan Gene Expression Assays (Applied Biosystems) for each sample were performed in duplicate in 96-well optical plates on cDNA equivalent to 100 ng of total RNA. The 20-μl reactions contained 5.0 μl nuclease-free water, 10.0 μl 2× TaqMan Universal PCR Master Mix (Applied Biosystems), 1.0 μl 20× TaqMan Gene Expression Assay Mix (consisting of unlabeled PCR primers and FAM dye-labeled MGB probe; Applied Biosystems), and 4.0 μl cDNA. For each gene, one no-target control, with no cDNA, was also performed. Thermal cycling conditions were 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles at 95 °C for 15 seconds and 60 °C for 1 min. Data were collected using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) and were analyzed through comparative C_T method ($\Delta\Delta Ct$). β-actin was used as an endogenous control as it was not observed as differentially expressed among the 3 groups (affected, asymptomatic, and control) according to microarray data.

1. Haslett JN, et al. (2002) Gene expression comparison of biopsies from Duchenne muscular dystrophy (DMD) and normal skeletal muscle. *Proc Natl Acad Sci USA* 99:15000–15005.

2. Zhao Q, et al. (2002) Identification of genes expressed with temporal-spatial restriction to developing cerebellar neuron precursors by a functional genomic approach. *Proc Natl Acad Sci USA* 99:5704–5709.
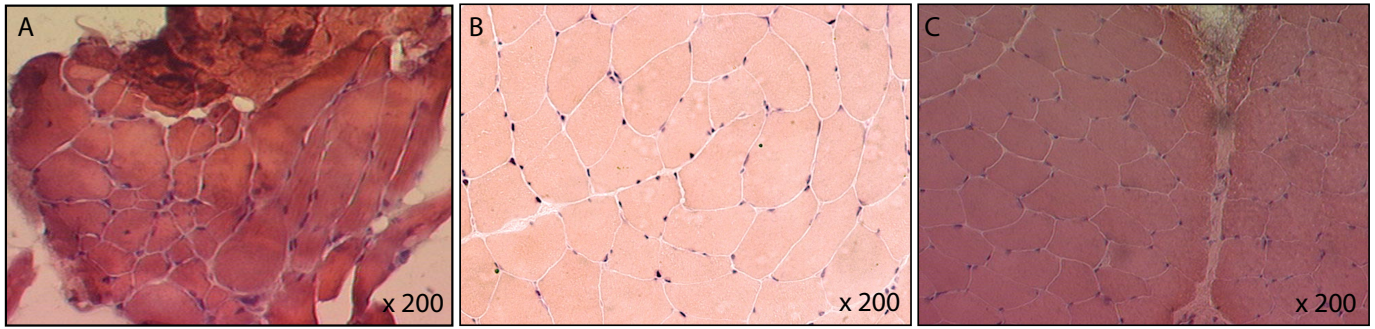
Fig. S1. Muscle tissues from biopsies stained with Hematoxylin & Eosin, in which we can observe nonspecific histological features of muscular dystrophy, such as variability of fiber sizes with atrophy, centralized nuclei, spliting, fibrosis in affected patient (*A*), and relatively normal histology in asymptomatic carrier (*B*) and normal control (*C*).

Table S1. Expression fold-changes of significantly upregulated genes in affected vs. asymptomatic

Probe set	Symbol	Loci	Fold-change (affected/asymptomatic)
204639_at	ADA	20q12–q13.11	4.17
208937_s_at	ID1	20q11	3.80
227337_at	ANKRD37	4q35.1	3.35
209118_s_at	TUBA3	12q12–12q14.3	2.97
223391_at	SGPP1	14q23.2	2.61
202458_at	PRSS23	11q14.1	2.60
44783_s_at	HEY1	8q21	2.58
219197_s_at	SCUBE2	11p15.3	2.40
226077_at	FLJ31951	5q33.3	2.40
200947_s_at	GLUD1	10q23.3	2.35
205794_s_at	NOVA1	14q	2.24
224827_at	UBTD2	5q35.1	2.20
227029_at	C14orf24	14q13.2	2.08

Table S2. Expression fold-changes of significantly downregulated genes in affected vs. asymptomatic

Probe set	Symbol	Loci	Fold-change (affected/asymptomatic)
210163_at	CXCL11	4q21.2	0.08
240453_at	C20orf26	20p11.23	0.09
210797_s_at	OASL	12q24.2	0.09
1560750_at	LOC151121	2q21.1	0.09
230331_at	LOC196541	13q33.1	0.09
1569112_at	SLC44A5	1p31.1	0.09
1552779_a_at	SLC44A5	1p31.1	0.11
227262_at	HAPLN3	15q26.1	0.12
220340_at	KIAA1772	18q11.1-q11.2	0.12
211122_s_at	CXCL11	4q21.2	0.12
205890_s_at	UBD	6p21.3	0.12
1558705_at	ATOH8	2p11.2	0.12
244106_at	KCNQ5	6q14	0.13
235504_at	GREM2	1q43	0.13
239465_at	UQCRC2	16p12	0.14
205660_at	OASL	12q24.2	0.14
233613_x_at	REXO2	11q23.1-q23.2	0.14
236108_at	KIAA1632	18q12.3-q21.1	0.15
211807_x_at	PCDHGB5	5q31	0.15
232469_x_at	SSBP3	1p32.3	0.15
217141_at	BTBD7	14q32.12-q32.13	0.15
1553015_a_at	RECQL4	8q24.3	0.15
233059_at	KCNJ3	2q24.1	0.15
232961_at	DNAPTP6	2q33.1	0.15
241824_at	FOSL2	2p23.3	0.15
244670_at	CDKL1	14q22.1	0.16
208159_x_at	DDX11	12p11	0.16
1555385_at	B4GALNT1	12q13.3	0.16
235157_at	PARP14	3q21.1	0.16
222833_at	AYTL1	16q12.2	0.16
210536_s_at	SPAM1	7q31.3	0.16
1570227_at	COQ9	16q13	0.17
211194_s_at	TP73L	3q28	0.17
214671_s_at	ABR	17p13.3	0.17
215159_s_at	NADK	1p36.33-p36.21	0.17
221010_s_at	SIRT5	6p23	0.17
210443_x_at	OGFR	20q13.3	0.17
1553992_s_at	NBR2	17q21	0.17
205067_at	IL1B	2q14	0.17
236897_at	IL17RB	3p21.1	0.17
238627_at	TRAPPC2L	16q24.3	0.17
229377_at	GRTP1	13q34	0.17
207348_s_at	LIG3	17q11.2-q12	0.17
233251_at	STRBP	9q33.3	0.17
233959_at	LOC221442	6p21.1	0.18
239373_at	ARFGEF2	20q13.13	0.18
1557595_at	GINS2	16q24.1	0.18
207306_at	TCF15	20p13	0.18
233981_at	RCL1	9p24.1-p23	0.18
238628_s_at	TRAPPC2L	16q24.3	0.18
39248_at	AQP3	9p13	0.18
1569930_at	DCUN1D4	4q12	0.18
232311_at	B2M	15q21-q22.2	0.19
207735_at	RNF125	18q12.1	0.19
233992_x_at	ZNF445	3p21.32	0.19
229617_x_at	AP2A1	19q13.33	0.19
1552326_a_at	CCDC11	18q21.1	0.19
243682_at	NCOR1	17p11.2	0.19
217282_at	MAN1A2	1p13	0.19
236913_at	C17orf45	17p11.2	0.19
220476_s_at	C1orf183	1p13.2	0.19
232767_at	IGSF4	11q23.2	0.19
231985_at	MICAL3	22q11.21	0.19
232612_s_at	ATG16L1	2q37.1	0.19

Probe set	Symbol	Loci	Fold-change (affected/asymptomatic)
213889_at	PIGL	17p12-p11.2	0.19
216629_at	SRRM2	16p13.3	0.19
203276_at	LMNB1	5q23.3-q31.1	0.19
1558340_at	DIXDC1	11q23.1	0.19
1555464_at	IFIH1	2p24.3-q24.3	0.20
229286_at	MAGEE1	Xq13.3	0.20
200879_s_at	EPAS1	2p21-p16	0.20
207978_s_at	NR4A3	9q22	0.20
223859_at	EPB41L4B	9q31-q32	0.20
238594_x_at	DUSP8	11p15.5	0.20
211818_s_at	PDE4C	19p13.11	0.20
205714_s_at	ZMYND10	3p21.3	0.20
1557690_x_at	NPAS2	2q11.2	0.20
213748_at	TRIM66	11p15.4	0.20
1565759_at	RPL13	16q24.3 17p11.2	0.20
225777_at	C9orf140	9q34.3	0.20
227513_s_at	LRRFIP1	2q37.3	0.21
243874_at	LPP	3q28	0.21
1568702_a_at	WDR71	11q13.4	0.21
214095_at	SHMT2	12q12-q14	0.21
211135_x_at	LILRB2	19q13.4	0.21
232629_at	PROK2	3p21.1	0.21
236274_at	EIF3S9	7p22.2	0.21
222963_s_at	IL1RAPL1	Xp22.1-p21.3	0.21
207319_s_at	CDC2L5	7p13	0.21
203439_s_at	STC2	5q35.2	0.21
1565935_at	LOC91431	4q25	0.22
1570414_x_at	FLJ13197	4p14	0.22
224556_s_at	LHX6	9q33.2	0.22
216968_at	MASP2	1p36.3-p36.2	0.22
213403_at	MGC11332	2q12.1	0.22
215607_x_at	SMEK1	14q32.12	0.22
235776_x_at	LOC389772	-	0.22
238127_at	GAS6	13q34	0.22
1564242_at	TRPM2	21q22.3	0.22
237583_at	LOC399978	11q24.3	0.22
232319_at	GGPS1	1q43	0.22
238581_at	GBP5	1p22.2	0.23
206356_s_at	GNAL	18p11.22-p11.21	0.23
232588_at	STAG1	3q22.3	0.23
220873_at	REPS2	Xp22.2	0.23
234525_at	DKFZP761C1711	-	0.23
216026_s_at	POLE	12q24.3	0.23
228230_at	PRIC285	20q13.33	0.23
1565436_s_at	MLL	11q23	0.23
213006_at	CEBPD	8p11.2-p11.1	0.23
1570541_s_at	LOC400759	1p22.2	0.23
214511_x_at	FCGR1A	1q21.2-q21.3	0.23
219324_at	NOL12	22q13.1	0.24
240509_s_at	GREM2	1q43	0.24
212523_s_at	KIAA0146	8q11.21	0.24
232711_at	PKNOX2	11q24.2	0.24
228439_at	BATF2	11q13.1	0.24
213983_s_at	SCC-112	4p14	0.24
222595_s_at	DIDO1	20q13.33	0.24
39549_at	NPAS2	2q11.2	0.24
207999_s_at	ADARB1	21q22.3	0.25
223820_at	RBP5	12p13.31	0.25
204769_s_at	TAP2	6p21.3	0.25
227675_at	LRSAM1	9q33.3-q34.11	0.26
219776_s_at	FLJ11125	8p21.2	0.26
206610_s_at	F11	4q35	0.27
220097_s_at	TMEM104	17q25.1	0.28
232753_at	ZNF346	5q35.2	0.28
222937_s_at	MMP28	17q11-q21.1	0.28

Probe set	Symbol	Loci	Fold-change (affected/asymptomatic)
226130_at	LOC441876	1p36.21	0.29
204918_s_at	MLLT3	9p22	0.30
238919_at	PCDH9	13q14.3-q21.1	0.34
51146_at	PIGV	1p36.11	0.35
206561_s_at	AKR1B10	7q33	0.49

Table S3. Expression fold-changes of significantly up-regulated genes in affected vs. control

Probe set	Symbol	Loci	Fold-change (affected/control)
217022_s_at	IGHA1	14q32.33	19.98
206717_at	MYH8	17p13.1	6.41
213247_at	SVEP1	9q32	5.73
213502_x_at	LOC91316	22q11.23	5.20
226818_at	MPEG1	11q12.1	4.06
229800_at	DCAMKL1	13q13	3.96
202149_at	NEDD9	6p25-p24	3.11
232304_at	PELI1	2p13.3	3.02
1557905_s_at	CD44	11p13	2.98
205794_s_at	NOVA1	14q	2.94
226621_at	OSMR	5p13.1	2.91
217478_s_at	HLA-DMA	6p21.3	2.85
203088_at	FBLN5	14q32.1	2.67
221031_s_at	APOLD1	12p13.1	2.44
212412_at	PDLIM5	4q22	2.36
201005_at	CD9	12p13.3	2.23
222816_s_at	ZCCHC2	18q21.33	2.21
225464_at	FRMD6	14q22.1	2.12
208319_s_at	RBM3	Xp11.2	2.02
214946_x_at	FAM21C	10q11.1	2.00

Table S4. Expression fold-changes of significantly down-regulated genes in affected vs. control

Probe set	Symbol	Loci	Fold-change (affected/control)
230331_at	LOC196541	13q33.1	0.04
232185_at	C20orf132	20q11.22	0.15
1558331_at	SIRT2	19q13	0.16
243816_at	ZNF70	22q11.2 22q11.23	0.17
208159_x_at	DDX11	12p11	0.17
205232_s_at	PAFAH2	1p34.3	0.17
229377_at	GRTP1	13q34	0.17
240016_at	SENP6	6q13-q14.3	0.17
214095_at	SHMT2	12q12-q14	0.17
219241_x_at	SSH3	11q13.1	0.17
216026_s_at	POLE	12q24.3	0.18
213889_at	PIGL	17p12-p11.2	0.18
215781_s_at	TOP3B	22q11.22	0.19
1555385_at	B4GALNT1	12q13.3	0.19
206395_at	DGKG	3q27-q28	0.20
232612_s_at	ATG16L1	2q37.1	0.20
215554_at	GPLD1	6p22.3-p22.2	0.20
200879_s_at	EPAS1	2p21-p16	0.21
219324_at	NOL12	22q13.1	0.23
215099_s_at	RXRB	6p21.3	0.23
1570414_x_at	FLJ13197	4p14	0.24
222595_s_at	DIDO1	20q13.33	0.24
211807_x_at	PCDHGB5	5q31	0.24
212996_s_at	URB1	21q22.11	0.24
1558705_at	ATOH8	2p11.2	0.24
215607_x_at	SMEK1	14q32.12	0.24
235983_at	TRAK2	2q33	0.24
213403_at	MGC11332	2q12.1	0.25
232469_x_at	SSBP3	1p32.3	0.26
1568702_a_at	WDR71	11q13.4	0.26
1570227_at	COQ9	16q13	0.29
233613_x_at	REXO2	11q23.1-q23.2	0.29
232753_at	ZNF346	5q35.2	0.30
1565759_at	RPL13	16q24.3 17p11.2	0.33
51146_at	PIGV	1p36.11	0.36
238919_at	PCDH9	13q14.3-q21.1	0.40

Table S5. Expression of genes from 4q35

Probe set	Gene	Name	Fold-change (asy/cont)	Fold-change (asy/aff)	Fold-change (aff/cont)
216473_x_at 208201_at	*DUX4*	Double homeobox, 4	No call	No call	No call
234830_at	*FRG2*	FSHD region gene 2 protein	No call	No call	No call
204145_at	*FRG1*	FSHD region gene 1	−1.07	−1.08	1.01
235535_x_at	*FRG1*	FSHD region gene 1	1.65	1.67	−1.01
206610_s_at	*F11*	Coagulation factor XI	1.82	<u>3.71</u>	−2.04
210170_at	*PDLIM3*	PDZ and LIM domain 3	−1.23	−1.47	1.19
238592_at	*PDLIM3*	PDZ and LIM domain 3	−1.08	−1.23	1.14
209621_s_at	*PDLIM3*	PDZ and LIM domain 3	−1.24	−1.19	−1.05
227337_at	*ANKRD37*	Ankyrin repeat domain 37	−1.22	<u>−3.35</u>	2.76
202825_at	*ANT1*	Solute carrier family 25	−1.09	−1.34	1.23

Underlined fold-changes represent those deemed significant.

Table S6. Expression fold-change in affected and asymptomatic carriers relative to healthy controls

Gene	Fold-change: affected ($n = 4$)	Fold-change: asymptomatic ($n = 6$)
CXCL9	8.2 (2.7–24.8)	3.7 (0.8–18.1)
CXCL10	2.3 (1.3–4.2)	4.3 (0.6–31.7)
CXCL11	2.9 (1.0–7.9)	2.2 (0.3–14.2)
STATH	386.0 (22.6–6,579.3)	501.8 (142.0–1,773.0)
LOC91431	1.5 (0.5–4.6)	2.0 (0.9–4.5)
PRIC285	0.5 (0.1–2.4)	0.7 (0.2–2.6)
LPP	2.1 (0.6–6.9)	1.7 (0.6–4.3)

Additional samples from affected and unrelated asymptomatic carriers were included in the analysis.

Table S7. Muscle specimens available for the present study

Family	Sample	Diagnosis	Age/Gender (year)	Onset (year)	N° D4Z4 repeats	Muscle weakness	Other features	CK (IU/l)	Muscle
1	1	Affected	49/M	16	5	F,S,P,A	-	617.5	Biceps
	2	Asymptomatic carrier	69/F	-	5	-	-	ND	Biceps
	3	Normal control	41/F	-	-	-	-	72.5	Biceps
2	4	Affected	30/M	15	2	F,S,P,A	H	11	Deltoid
	5	Asymptomatic carrier	66/M	-	2	-	-	4	Deltoid
	6	Normal control	38/F	-	-	-	-	2.9	Deltoid
3	7	Affected	14/F	7	3	F,S	D	94.5	Deltoid
	8	Asymptomatic carrier	32/F	-	3	-	-	6.2	Deltoid
	9	Normal control	35/F	-	-	-	-	81	Deltoid
4	10	Affected	11/M	6	5	F,S,P	L	230.9	Biceps
	11	Asymptomatic carrier	54/M	-	5	-	-	8.6	Biceps
	12	Normal control	16/M	-	-	-	-	9.1	Biceps
5	13	Affected	38/M	21	8	S,P	-	16	Biceps
	14	Asymptomatic carrier	57/F	-	8	-	-	8.8	Biceps
	15	Normal control	39/M	-	-	-	-	10	Biceps
6	16	Asymptomatic carrier	40/F	-	8	-	-	ND	Biceps
7	17	Asymptomatic carrier	45/F	-	9	-	-	9.3	Biceps
8	18	Affected	57/F	ND	6	S	D	12.3	Biceps
9	19	Asymptomatic carrier	51/F	-	8	-	-	5.2	Biceps
10	20	Asymptomatic carrier	46/F	-	5	-	-	13.0	Biceps

A, ankle; D, depression; F, face; H, hearing loss; L, swallowing problem; ND, not described; P, pelvic girdle; S, shoulder girdle.

Table S8. miRNA:mRNA predicted targeting in FSHD

miRNA:mRNA	miRNA fold-change	Target fold-change	MAMI score
miR-19b: TRAK2	2.99	−4.09	0.67303
miR-19b: B4GALNT1	2.99	−5.17	0.50875
miR-17–3p: SMEK1	1.81	−4.14	0.41443
miR-18a: REXO2	2.24	−3.49	0.20708
let-7b: ATG16L1	1.65	−5.02	0.11277
let-7c: ATG16L1	1.69	−5.02	0.11277
let-7e: ATG16L1	1.77	−5.02	0.11277
let-7i: ATG16L1	1.86	−5.02	0.11277
miR-106a: ATG16L1	2.11	−5.02	0.11277
miR-106a: EPAS1	2.11	−4.75	0.11277
miR-106b: ATG16L1	3.04	−5.02	0.11277
miR-106b: EPAS1	3.04	−4.75	0.11277
miR-125a: ATOH8	1.83	−4.15	0.11277
miR-130a: ATG16L1	3.02	−5.02	0.11277
miR-130b: ATG16L1	2.03	−5.02	0.11277
miR-152: EPAS1	1.54	−4.75	0.11277
miR-155: PCDH9	4.4	−2.5	0.11277
miR-15a: PCDH9	2.53	−2.5	0.11277
miR-16: PCDH9	1.77	−2.5	0.11277
miR-17–5p: ATG16L1	2.12	−5.02	0.11277
miR-17–5p: EPAS1	2.12	−4.75	0.11277
miR-195: PCDH9	2.62	−2.5	0.11277
miR-199a*: RXRB	2.83	−4.31	0.11277
miR-19b: ATG16L1	2.99	−5.02	0.11277
miR-20a: ATG16L1	2.13	−5.02	0.11277
miR-20a: EPAS1	2.13	−4.75	0.11277
miR-20b: ATG16L1	2.47	−5.02	0.11277
miR-20b: EPAS1	2.47	−4.75	0.11277
miR-93: EPAS1	2.03	−4.75	0.11277
let-7b: PIGV	1.65	−2.76	0.09432
let-7i: COQ9	1.86	−3.5	0.09432
miR-140: COQ9	2.31	−3.5	0.09432
miR-140: RPL13	2.31	−3.07	0.09432
miR-154: COQ9	2.2	−3.5	0.09432
miR-199b: PIGL	3.41	−5.48	0.09432
miR-29a: RPL13	2.57	−3.07	0.09432
miR-29a: ZNF346	2.57	−3.33	0.09432
miR-29b: RPL13	2.47	−3.07	0.09432
miR-29b: ZNF346	2.47	−3.33	0.09432
miR-34a: DIDO1	5.56	−4.19	0.09432