

**miRNAs regulate acute transcriptional changes in broiler embryos in response to  
modification of incubation temperature**

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**Supplementary Material**

**Table S1. Composition of feed stuff for broilers at respective phases**

Feeding phase	Starter until day 14	Grower until day 28	Finisher until day 35
Energy (MJ ME/kg)	12.4	12.6	12.7
Crude protein (%)	22	20	18
Methionine (%)	1.33	0.45	0.4
Lysine(%)	1.33	1.15	1.00
Calcium (%)	0.90	0.85	0.80

**Table S2. Primers used for quantitative real time PCR**

<b>miRNA</b>	<b>Primer sequence</b>
gga-miR-199	GTACAGTAGTCTGCACATTGG
gga-miR-30d	TGTAAACATCCCCGACTGGA
gga-miR-460a	CCTGCATTGTACACACTGTG
gga-miR-100	AACCCGTAGATCCGAACTTGT
gga-miR-133a	TTTGGTCCCCTTCAACCAGCT
gga-miR-222	GAGCTACATCTGGCTACTGG

1 **Table S3.** Summary of significant biological functions ( $p < 0.05$ ) of target mRNAs that are related to differentially expressed miRNAs for H10  
 2 treatment in embryonic breast and hind muscle tissues

Major category	Ingenuity biofunction	BH $p$ -value	Z-score	Top 10 genes	Differentially expressed miRNAs
<b>Breast H10</b>					
Cell maintenance, proliferation, differentiation, and replacement	Formation of actin filaments	1.58E-02	-0.728	<i>ARHGAP18, CDC42BPB, KIRREL, MYH11, NFATC1, PECAMI, PHLDB2, PIK3R1, PPF1A1, ROCK1</i>	miR-1825, miR-222, miR-739, miR-133, miR-133a-star, miR-17-3p, miR-2970, miR-971, miR-130c, miR-145, miR-363, miR-199a-3p, miR-460-5p, miR-1281, miR-212-star
	Formation of cytoskeleton	1.93E-02	-0.673		
	Interphase	1.09E-02	0.984	<i>CDKN1B, AKAP12, IL15, PIK3R1, SYK, TCF3, USP8, XRCC5, ADNP, ARFIP2</i>	miR-222, miR-289, miR-460-5p, miR-199a-3p, miR-212-star, miR-739, miR-578, miR-5109, miR-138, miR-1825, miR-133
	Proliferation of activated T lymphocytes	1.26E-02	1.446		
	Proliferation of thymocytes	3.06E-02	1.715		
	Organization of cytoskeleton	9.72E-03	1.874		
	Organization of cytoplasm	1.47E-02	1.874		
Organismal, organ, and tissue development	Organismal death	8.66E-03	-5.151	<i>ADNP, BPTF, CUL1, FBN2, HOXA3, JPH1, MYH11, PLXNB2, SLC25A25, TRIM33</i>	miR-222, miR-289, miR-460-5p, miR-1825, miR-971, let-7, miR-4530, miR-5109, miR-667-star
	Quantity of double-positive thymocyte	2.38E-02	0.128	<i>CDKN1B, KMT2E, NFATC1, TCF3, TPP2, F3, GUCY1A3, KLF5, SYK, CSPG4</i>	miR-222, miR-5109, miR-138, miR-3017a, miR-460-5p, miR-289, miR-1825, miR-4530, miR-130c, miR-145, miR-363, miR-133
	Formation of neointima	2.13E-03	0.152		
	Quantity of double-negative T lymphocyte	8.92E-03	0.447		
	Vasculogenesis	3.17E-02	0.882		
	Thermogenesis	2.89E-02	1		
Nutrient metabolism	Synthesis of polysaccharide	1.73E-02	0.447	<i>CHPF, CHST3, CX3CL1, GALNT5, GBE1, GPAM, PPP1R3B, PPP1R3C, ZFPM2, ADRB2</i>	miR-199a-3p, miR-212-star, miR-460-5p, miR-5109, miR-133, miR-133a-star, miR-147, miR-2970, miR-1281, miR-667-star, miR-199-star, miR-289
	Metabolism of polysaccharide	1.86E-02	0.816		
	Synthesis of carbohydrate	3.68E-02	1.807		
	Metabolism of phosphatidylinositol	1.89E-02	1.91		

Genetic information and nucleic acid processing	Transcription of DNA	2.11E-02	0.313	<i>ADRB2, XPO1, ABRA, BACH1, CDKN1B, DCP1A, HOXC8, ILF2, JMJD1C, KDM7A</i>	miR-5109, miR-199a-3p, miR-212-star, miR-460-5p, miR-289, miR-222, miR-133, miR-3148, miR-93, miR-1825, miR-4530
	Hydrolysis of nucleotide	1.71E-02	1.941		
Cell signaling and interaction	Response of helper T lymphocytes	1.62E-02	-1	<i>CD276, NFATC1, NFATC2, PIK3R1</i>	miR-133, miR-130c, miR-145, miR-363, miR-1563, miR-17-3p, miR-2970, miR-425-5p, miR-739
	Hydrolysis of GTP	1.07E-02	1.4	<i>ADRB2, ARHGAP10, ARHGAP5, RANBP2, XPO1</i>	miR-199a-3p, miR-212-star, miR-460-5p, miR-222, miR-1825, miR-289, miR-4530
Response to stimuli	Adhesion of monocytes	1.84E-02	0.623	<i>CX3CL1, F3, IL15, ROCK1</i>	miR-460-5p, miR-5109, miR-138, miR-212-star
<b>Hind H10</b>					
Cell maintenance, proliferation, differentiation, and replacement	Formation of cytoskeleton	0.0275	-1.172	<i>ARHGAP18, DLC1, MYH11, PIK3R1, PPF1A1, ROCK1, AKAP11, TRAF6, MTM1, ACTA1</i>	miR-222a-star, miR-1915, miR-199a-5p, miR-4454, miR-4592, miR-638, miR-64b, miR-2861, miR-3960, miR-3885-5p
	Development of cytoplasm	0.0379	-1.172		
	Formation of actin stress fibers	0.0181	-0.849		
	Formation of filaments	0.0065	-0.342	<i>AKAP11, ARHGAP5, CELSR1, DLC1, IQGAP1, MTM1, MTUS1, PDZD8, PIK3R1, PLXNB2</i>	miR-1915, miR-199a-5p, miR-4454, miR-4592, miR-638, miR-64b, miR-1207-5p, miR-22-star, miR-4689, miR-3885-5p, miR-2861, miR-3960
	Organization of cytoplasm	0.0347	1.219		
	Organization of cytoskeleton	0.049	1.219		
Organismal, organ, and tissue development	Perinatal death	0.0407	-3.39	<i>AKAP6, ARHGAP5, ARID4B, HPGD, KMT2E, MAN2A1, MTM1, MYH11, PIK3R1, RAB3C</i>	miR-212, miR-199a-5p, miR-3885-5p, miR-4592, miR-2861, miR-3960, miR-638, miR-1915, miR-4454
	Size of body	0.00111	3.567	<i>ACTA1, AKAP6, ARHGAP5, ARID4B, CHD7, CUL4B, GAMT, GPAM, ITSN1, KCNJ16</i>	miR-222a-star, miR-212, miR-199a-5p, miR-3885-5p, miR-1915, miR-638, miR-2861
Nutrient metabolism	Concentration of phosphatidic acid	0.0069	0.532	<i>MTM1, FIG4, GPAM, MGLL, NT5E, PIK3R1, PLCB1, PNPLA8</i>	miR-425-5p, miR-638, miR-199a-5p, miR-212, miR-2861, miR-3960, miR-4592, miR-222
	Hydrolysis of carbohydrate	0.0411	1.972		
Genetic information and nucleic acid processing	Phosphorylation of protein	0.0124	0.896	<i>N4BP2, AKAP11, AKAP6, ARHGAP5, CCDC88C, CD7, CDKL5, CPNE3, EEF2K, FZD1</i>	miR-1915, miR-199a-5p, miR-4454, miR-4592, miR-638, miR-212, miR-4689, miR-222, miR-1207-5p, miR-22-star
	Hydrolysis of nucleotide	0.0377	1.067		

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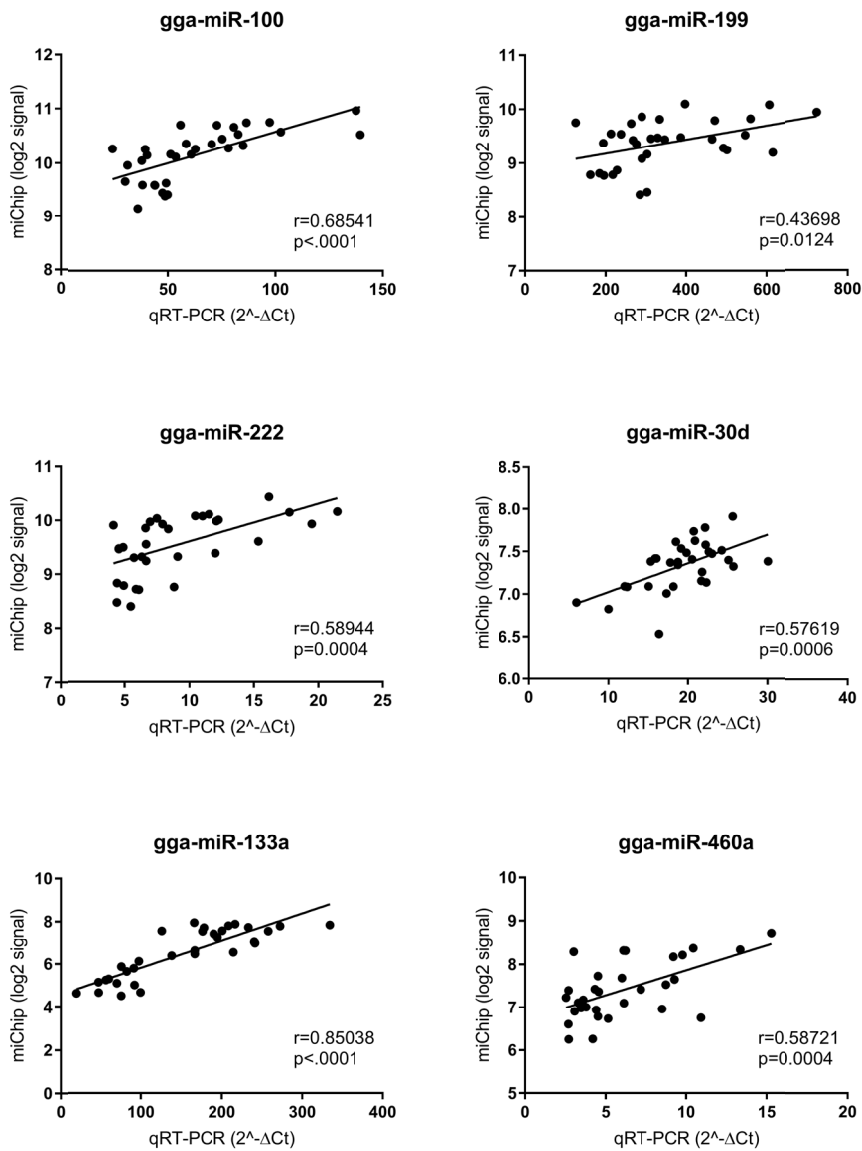
5 **Table S4.** Summary of significant biological functions ( $p < 0.05$ ) of target mRNAs that are related to differentially expressed miRNAs for L13  
6 treatment in embryonic breast and hind muscle tissues

Major category	Ingenuity biofunction	BH $p$ -value	Z-score	Top 10 genes	Differentially expressed miRNAs
<b>Breast EmL13</b>					
Cell maintenance, proliferation, differentiation, and replacement	Outgrowth of neurites	0.0131	-1.506	<i>CD226, CD80, LRRC8A, NOTCH2, PATZ1, PRKCQ, RUNX2, TCF3, PTGS1, SRF</i>	miR-92a, miR-4900a, miR-344d-2-star, let-7, miR-93, miR-1677, miR-222, miR-4651, miR-762, miR-92b, miR-1908, miR-2436-5p, miR-460-5p, miR-130c, miR-199b, miR-312-5p
	Differentiation of T lymphocytes	0.0215	-0.294		
	Homeostasis of leukocytes	0.0176	-0.156		
	T cell development	0.0214	-0.125		
	Engulfment of leukocytes	0.0029	1.664	<i>MIF, DNMI, SOATI, TICAMI, CLIC4, CTNND1, JAM3, PICK1, RNF2, SRF</i>	miR-5109, miR-2016, let-7, miR-1, miR-17-3p, miR-302a, miR-93, miR-124, miR-1908, miR-222, miR-460-5p, miR-92a, miR-30e, miR-263a-star, miR-1270, miR-199b, miR-4651
	Formation of cells	0.0143	1.709		
	Homing	0.0251	1.947		
	Proliferation of splenocytes	0.0042	2		
Engulfment of cells	0.0060	2.008			
Organismal, organ, and tissue development	Organismal death	0.0004	-4.096	<i>AMER1, RUNX2, ARHGAP1, ARID1A, DHCR7, DNMI, INPP5E, PIP5K1C, PTGS1, TCF3</i>	miR-93, let-7, miR-199b, miR-1, miR-17-3p, miR-302a, miR-1908, miR-2436-5p, miR-460-5p, miR-762, miR-92b, miR-130c, miR-312-5p
	Perinatal death	0.0116	-2.926		
	Neonatal death	0.0077	-2.578		
	Size of bone	0.0117	-1.673		
	Accumulation of leukocytes	0.0235	1.744	<i>CD80, NOTCH2, PRKCQ, TCF3, MIF, ARHGAP1, HIVEP3, IP6K1, RUNX2, SRF</i>	miR-93, miR-4900a, let-7, miR-1677, miR-222, miR-4651, miR-762, miR-92a, miR-92b, miR-130c, miR-199b, miR-312-5p
	Quantity of lymphatic system component	0.0196	1.815		
	Development of body trunk	0.0309	1.964		
	Generation of lymphocytes	0.0188	1.987		
Quantity of double-positive thymocyte	0.0117	2			
Nutrient metabolism	Uptake of D-glucose	0.0064	1.432	<i>JUP, MIF, NTRK3, PRKAB2,</i>	miR-5109, let-7, miR-1575, miR-1908, miR-

	Uptake of carbohydrate	0.0109	1.964	<i>PRKCQ, RAPGEF1, SRF, SLC35B4</i>	2309, miR-4651, miR-762, miR-1677, miR-222, miR-92a, miR-92b, miR-2413, miR-4900a, miR-1, miR-93, miR-199b
Genetic information and nucleic acid processing	Transcription of DNA	0.0366	0.749	<i>ARID1A, NOTCH2, RUNX2, SRF, TCF3, CRTCL1, PRKCQ, AGO1, ATNI, BCL9</i>	let-7, miR-93, miR-3128, miR-312-5p, miR-1677, miR-222, miR-4651, miR-762, miR-92a, miR-92b, miR-130c, miR-199b
	Transcription of RNA	0.0327	1.137		
	Expression of RNA	0.0263	1.445		
	Transactivation of RNA	0.0065	1.666		
	Transactivation	0.0004	1.671		
Molecular transport	Transport of protein	0.0049	1.067	<i>FRAS1, IPO9, JUP, LRP1, NDFIP2, NUP214, XPO5, XPO6, CLIC4, CLNS1A</i>	miR-5109, miR-312-5p, miR-199-star, miR-199b, miR-2413, miR-31, miR-762, miR-93, let-7, miR-17-3p, miR2275b-5p, miR-1908, miR-4651, miR-30e, miR-263a-star
	Transport of molecule	0.0226	1.21		
Cell signaling and interaction	Adhesion of mononuclear leukocytes	0.0259	0.152	<i>MIF, PIP5K1C, RALGDS, RAPGEF1, CD80, CLIC4, DNMI, IPO9, JAM3, JUP</i>	miR-4900a, miR-5109, let-7, miR-1, miR-17-3p, miR-302a, miR-93, miR-124, miR-1908, miR-222, miR-460-5p, miR-92a, miR-2413, miR-4651, miR-762
	Adhesion of connective tissue cells	0.0128	1		
	Phagocytosis by macrophages	0.0131	1		
	Adhesion of immune cells	0.0317	1.299		
Response to stimuli	Migration of phagocytes	0.0102	0.408	<i>ARHGAP1, JAM3, PRKCQ, SRF, CD80, LRP1, MIF</i>	miR-93, miR-4900a, miR-124, miR-1908, miR-222, miR-460-5p, miR-5109, miR-92a, let-7, miR-17-3p, miR2275b-5p, miR-1677, miR-4651, miR-762, miR-92b, miR-199b
	Migration of neutrophils	0.0117	1.695		
<b>Hind EmL13</b>					
Cell maintenance, proliferation, differentiation, and replacement	Cell death	0.0234	0.915	<i>ADAMTS20, BLVRA, CTSG, IRS1, MKI67, OSBP2, PCMT1, PRPS1, RCAN2, SRF</i>	miR-138, miR-222, miR-132, miR-383, miR-271, miR-1245b-3p, miR-2137, miR-3042
	Apoptosis	0.0085	1.269		
Organismal, organ, and tissue development	Growth of connective tissue	0.0090	-1	<i>IRS1, RAPGEF1, RUNX2, SRF</i>	miR-271, miR-2137, miR-130c
Response to stimuli	Cell movement of neutrophils	0.0106	1.701	<i>CTSG, PRKCQ, SRF, TSC1</i>	miR-138, miR-222, miR-271, miR-383

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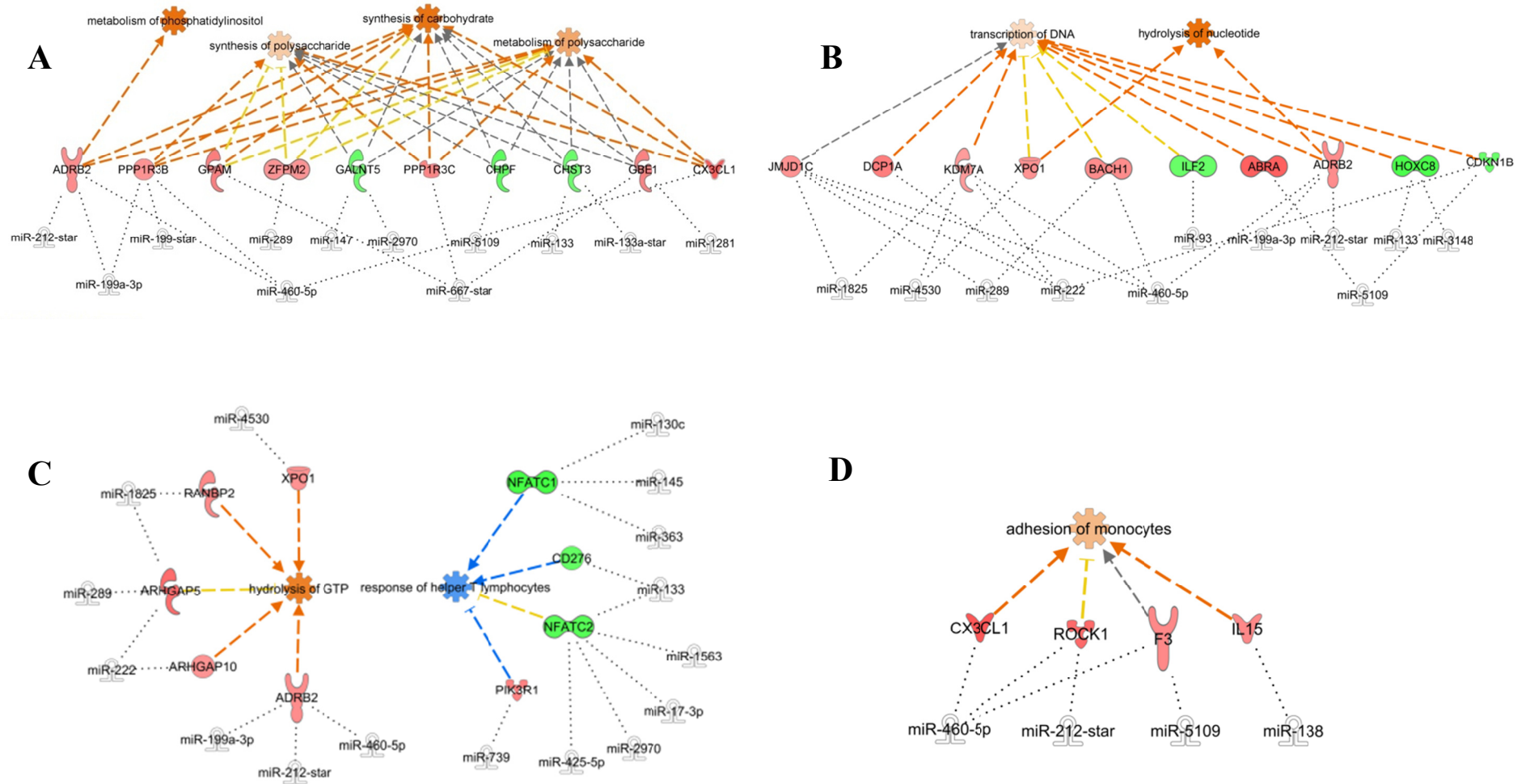


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11 **Figure S1.** Expression levels of miRNAs obtained from the same samples of embryonic  
 12 breast muscle (n=32) by microarray and quantitative real time PCR respectively, with  
 13 Pearson correlation coefficient r and p-values.

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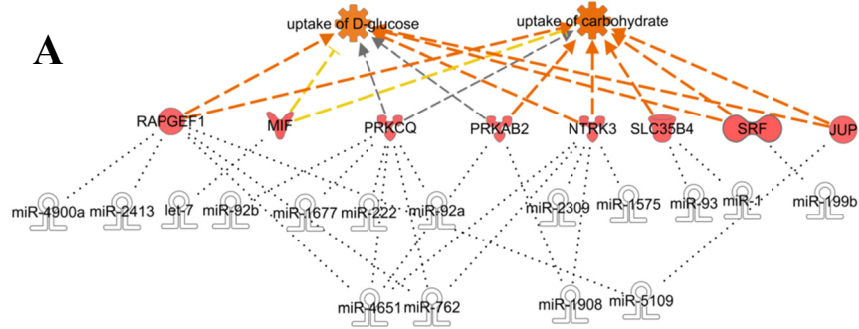


34 **Figure S2.** miRNA–mRNA regulatory networks. Representative gene regulatory networks derived from H10 treatment in breast muscle that are  
35 related to major functional categories 3 (A), 4 (B), 6 (C), and 8 (D). Activated pathways are orange, deactivated pathways are blue. The networks  
36 were generated through the use of IPA (QIAGEN Inc., <https://www.qiagenbio-informatics.com/products/ingenuity-pathway-analysis>).

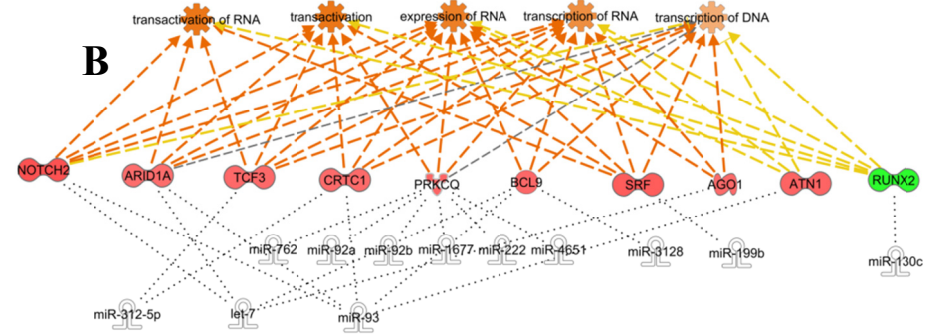


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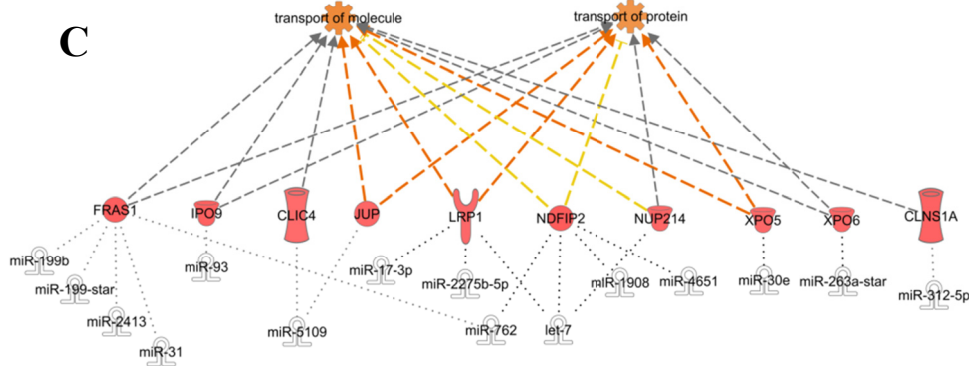
**A**



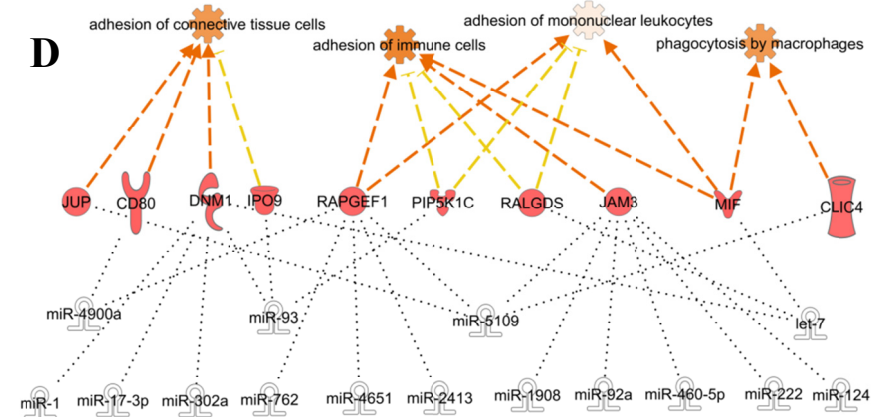
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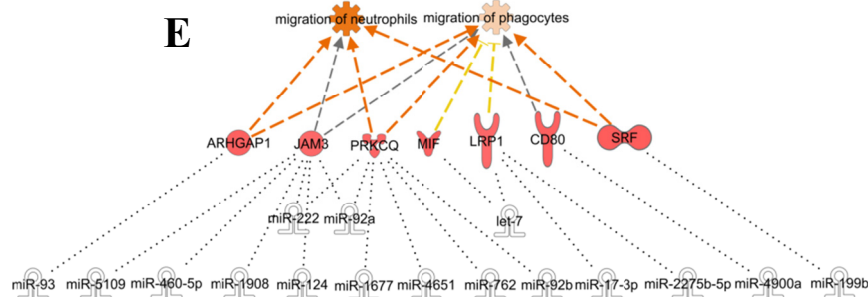
**C**



**D**



**E**



57 **Figure S3.** miRNA–mRNA regulatory networks. Representative gene regulatory networks derived from L13 treatment in breast muscle that are  
58 related to major functional categories 3 (A), 4 (B), 5 (C), 6 (D), and 8 (E). Activated pathways are orange. The networks were generated through  
59 the use of IPA (QIAGEN Inc., <https://www.qiagenbio-informatics.com/products/ingenuity-pathway-analysis>).  
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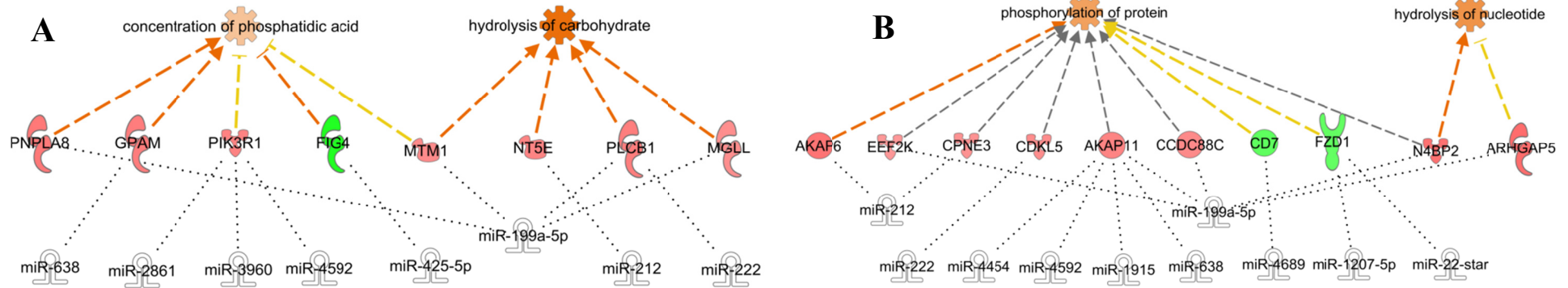
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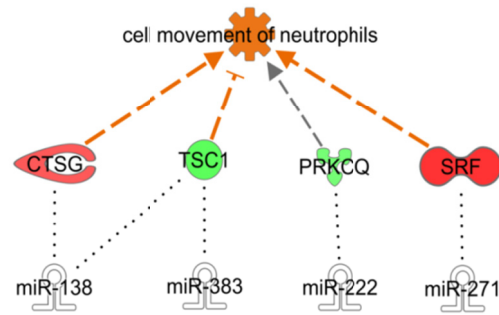
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**Figure S4.** miRNA-mRNA regulatory networks. Representative gene regulatory networks derived from H10 treatment in hind muscle that are related to major functional categories 3 (A) and 4 (B). Activated pathways are orange, while deactivated pathways are blue. The networks were generated through the use of IPA (QIAGEN Inc., <https://www.qiagenbio-informatics.com/products/ingenuity-pathway-analysis>).



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73 **Figure S5.** miRNA-mRNA regulatory networks. Representative gene regulatory networks derived from H10 treatment in hind muscle that are  
 74 related to major functional category 8. Activated pathways are orange. The networks were generated through the use of IPA (QIAGEN Inc.,  
 75 <https://www.qiagenbio-informatics.com/products/ingenuity-pathway-analysis>).