SUPPLEMENTAL DATA

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cardiac ATP measurement – Cardiac pieces of 10 mg were used to determine ATP levels. Heart pieces were dissolved in ice-cold 0.1% Trichloro-acetic acid and centrifuged. Supernatant was resuspended in 50mM Tris-acetate containing 2mM EDTA, pH 7.8 and a portion was used to measure ATP. The measurement was performed with the ATP determination kit (Invitrogen) according to the instructions of the manufacturer.

MicroRNA Microarrays - RNA samples were sent to Ocean Ridge Biosciences (ORB, Palm Beach Gardens, FL) for analysis using custom multi-species microarrays containing 697 probes covering 707 mouse mature microRNAs present in Sanger 14.0 miRBase database. The array also contains 892 probes covering 902 human mature microRNAs and 388 probes covering 388 mouse mature microRNAs. The sensitivity of the microarray is such that it could detect as low as 20 amoles of synthetic microRNA being hybridized along with each sample. The microarrays were produced by Microarrays Inc. (Huntsville, Alabama), and consisted of epoxide glass substrates that had been spotted in triplicate with each probe.

Sample Processing - Quality of the total RNA samples was assessed using UV spectrophotometry and agarose gel electrophoresis. The samples were DNAse digested and low-molecular weight (LMW) RNA was isolated by ultrafiltration through YM-100 columns (Millipore) and subsequent purification using the RNeasy MinElute Clean-Up Kit (Qiagen). The LMW RNA samples were 3'-end labeled with Oyster-550 fluorescent dye using the Flash Tag RNA labeling Kit (Genisphere). Labeled LMW RNA samples were hybridized to the MicroRNA microarrays according to conditions recommended in the Flash Tag RNA labeling Kit manual. The microarrays were scanned on an Axon Genepix 4000B scanner, and data was extracted from images using GenePix V4.1 software.

Data Pre-processing - Spot intensities were obtained for the 4860 features on each microarray by subtracting the median local background from the median local foreground for each spot. Detection Thresholds for each array were determined by calculating the 10% trim mean intensity of the negative controls spots and adding 5X the standard deviation of the background (non-spot area). The spot intensities and the Threshold (T) were transformed by taking the log (base 2) of each value. The normalization factor (N) for each microarray was determined by obtaining the 20% trim mean of the mouse probes intensities above threshold in all samples. The log2-transformed spot intensities for all 4860 features were normalized, by subtracting N from each spot intensity, and scaled by adding the grand mean of N across all microarrays. The mean probe intensities for each of the 697 mouse probes on each of the 8 arrays were then determined by averaging the triplicate spot intensities. Spots flagged as poor quality during data extraction were omitted prior to averaging. The 697 mouse non-control log2-transformed, normalized, and averaged probe intensities were filtered to obtain a list of 318 mouse microRNA probes showing probe intensity above T in all samples from at least one treatment group. For statistical purposes, mouse probes saturated in 3 or more samples were removed and thus 295 non-saturated mouse probes intensities were obtained.

Microarray Quality Control - Each array contains probes targeting 11 different synthetic miRNAs, each of which is added at a mass of 200 amoles to each RNA sample prior to labeling and hybridization. Sensitivity of the microarray hybridization was confirmed by detection of hybridization signal for all 11 spikes well above the detection threshold. The array also contains a set of specificity control probes complementary to three different miRNAs. Each specificity control includes a perfect match, single mismatch, double mismatch, and shuffled version of the probe. Specificity of the hybridization was confirmed by detection of hybridization signal on the microarray for the perfect match probes and not the double mismatch and shuffled version of the probes. Reproducibility of the arrays was determined by

monitoring the hybridization intensity for the triplicate mouse spots on each array. The sensitivity, specificity, and reproducibility data for the arrays were compiled into a Quality Control report.

Differential Expression Analysis - For statistical analysis, samples were binned in two groups (Control and Treated). The log2-transformed and normalized spot intensities for the 295 non-saturated and detectable mouse probes were examined for differences between the groups by 1-way ANOVA using National Institute of Ageing (NIA) Array Analysis software (1). This ANOVA was conducted using the Bayesian Error Model and 20 degrees of freedom. A total of 71 probes showed significant differences with $P < 0.03$ and $FDR < 0.1$. The statistical significance was determined using the False Discovery Rate (FDR) method which was proposed by Benjamini and Hochberg (2). It is the proportion of false positives among all probes with P values lower or equal to the P value of the probes that we consider significant. It can also be viewed as an equivalent of a P-value in experiments with multiple hypotheses testing. FDR is an intermediate method between the P-value and Bonferroni correction (multiplying P-value by the total number of probes). The equation is:

$$FDR_r = \min_{i \geq r} \left[\frac{p_i N}{i} \right]$$

where r is the rank of a probe ordered by increasing P values, p_i is the P value for probe with rank i, and N is the total number of probes tested. FDR value increases monotonously with increasing P value.

Hierarchical Clustering Of MicroRNA Array Data - Data for the 295 detectable mouse probes were clustered using Cluster 3.0 software (3). Genes were median centered prior to hierarchical clustering. Hierarchical clustering was conducted using Centered Correlation as the similarity metric and Average Linkage as the clustering method. Intensity scale shown is arbitrary.

References

(1) Sharov, A.A., Dudekula, D.B., Ko, M.S.H. (2005) Principal component and significance analysis of microarrays with NIA Array Analysis tool. Bioinformatics. 21(10): 2548-9.

(2) Benjamini, Y. & Hochberg, Y., (1995). J Roy Stat Soc B 57: 289-300

(3) De Hoon, M. J. L., Imoto, S., Nolan, J. Nolan, and Miyano, S. (2004) Open Source Clustering Software. Bioinformatics, 20 (9): 1453-1454.

SUPPLEMENTAL FIGURE AND TABLE LEGENDS

Supplementary Table 1: Human and mouse primers used for qRT-PCR gene expression analyses of this study.

Supplementary Table 2: Cardiac miR expression changes in LPS-treated C57BL/6 mice.as determined by miR array. Indicated values represent fold-changes vs. saline-treated C57BL/6 mice; NS indicates non-significant changes, ND indicates non-detected miRs, *p<0.05.

Supplementary Figure 1: Cardiac ATP levels are reduced in sepsis – ATP levels in hearts obtained from C57BL/6 mice that were treated with 5mg/kg LPS; n=4, *p<0.05.

Supplementary Figure 2: **Heat Map for heart failure-associated miRs in cardiac RNA obtained from C57BL/6 mice that were treated with 5mg/kg LPS -** Each column represents a single sample, and each row represents a single miR. Green color represents lower than median level of miR expression; black color represents median level of miR expression; red color represents higher than median level of miR expression. Legend units: 0.33 = differs from median probe intensity by 0.33 unit.

Supplementary Figure 3: JNK1 is activated in LPS-treated *Jnk2*$^{-/-}$ mice and inhibits PPARα gene expression – (A) Western blot of JNK1 and β-actin protein levels obtained from hearts of 10-12 weeks old C57BL/6 and *Jnk2*$^{-/-}$ mice. (B) Fractional shortening as measured by 2D-echocardiography of 10-12 weeks old *Jnk2*$^{-/-}$ and C57BL/6 mice that were treated with 5mg/kg LPS; n=5, **p<0.01. (C) Western blot of pJNK, total JNK1, pc-Jun-Ser63, pc-Jun-Ser73 and β-actin protein levels obtained from hearts of 10-12 weeks old *Jnk2*$^{-/-}$ mice that were treated with 5mg/kg LPS. (D), PPARα, PGC-1α, PGC-1β, PPARγ, PPARδ, FATP, LpL, CD36 and Cpt1 mRNA levels in hearts of 10-12 weeks old JNK2-/- and C57BL/6 mice that were treated with 5mg/kg LPS; n=5, *p<0.05, **p<0.01, $^{+}$p<0.001. (E) TNFα, IL-1α and IL-6 mRNA levels in hearts of 10-12 weeks old *Jnk2*$^{-/-}$ and C57BL/6 mice that were treated with 5mg/kg LPS; n=5, **p<0.01, $^{+}$p<0.001.

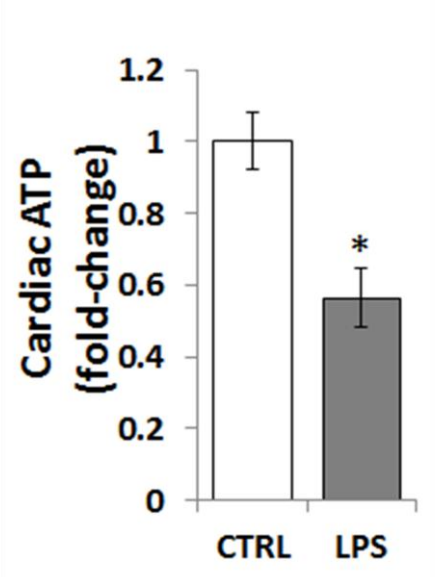
Supplementary Table 1

Species	Gene	Forward primer	Reverse primer
Homo *sapiens*	*b-actin*	5´-GACAGGATGCAGAAGGAGATTCT-3´	5’-TGATCCACATCTGCTGGAAGGT-3’
	pparα	5'-CTGTCGGGATGTCACACAAC-3'	5'-CCGCAAACACCTACTGGATT-3'
	cd36	5’-ATTGGTCAAGCCAGCT-3’	5’-TGTAGGCTCATCCACTAC-3’
	cpt1	5'-CTCCTTTCCTTGCTGAGGTG-3'	5'-TCTCGCCTGCAATCATGTAG-3'
Mus *musculus*	*18s*	5'-CCATCCAATCGGTAGTAGCG-3'	5'-GTAACCCGTTGAACCCCATT-3'
	pparα	5'-TGCAAACTTGGACTTGAACG-3'	5'-GATCAGCATCCCGTCTTTGT-3'
	cd36	5'-TGTGTTTGGAGGCATTCTCA-3'	5'-TGGGTTTTGCACATCAAAGA-3'
	cpt1	5'-CCCATGTGCTCCTACCAGAT-3'	5'-CCTTGAAGAAGCGACCTTTG-3'
	pgc-1α	5'-CACGCAGCCCTATTCA-3'	5'-GTCGTACCTGGGCCTA-3'
	pgc-1β	5´-AACCCAACCAGTCTCACAGG-3'	5'-CTCCTAGGGGCCTTTGTTTC-3'
	fatp	5'-GCGTTTCGATGGTTATGT-3'	5'-TTGAGTTAGGGTCCAACTG-3'
	lpl	5'-GCTGGTGGGAAATGATGTG-3'	5'-TGGACGTTGTCTAGGGGGTA-3'
	pparg	5'-GAGTGTGACGACAAGATTTG-3'	5'-GGTGGGCCAGAATGGCATCT-3'
	ppard	5'-TGGAGCTCGATGACAGTGAC-3'	5'-GTACTGGCTGTCAGGGTGGT-3'
	il-1α	5'-GCAACGGGAAGATTCTGAAG-3'	5'-TGACAAACTTCTGCCTGACG-3'
	il-6	5'-CCGGAGAGGAGACTTCACAG-3'	5'-TCCACGATTTCCCAGAGAAC-3'
	tnfα	5'-ACGGCATGGATCTCAAAGAC-3'	5'-GTGGGTGAGGAGCACGTAGT-3'
	glut4	5'-ACTCTTGCCACACAGGCTCT-3'	5'-CCTTGCCCTGTCAGGTATGT-3'
	pdk4	5'-CCGCTGTCCATGAAGCA-3'	5'-GCAGAAAAGCAAAGGACGTT-3'
	αmhc	5'-GGCACAGAAGATGCTGACAA-3'	5'-CTGCCCCTTGGTGACATACT-3'
	bmhc	5'-CTTCAACCACCACATGTTCG-3'	5'-TCTCGATGAGGTCAATGCAG-3'

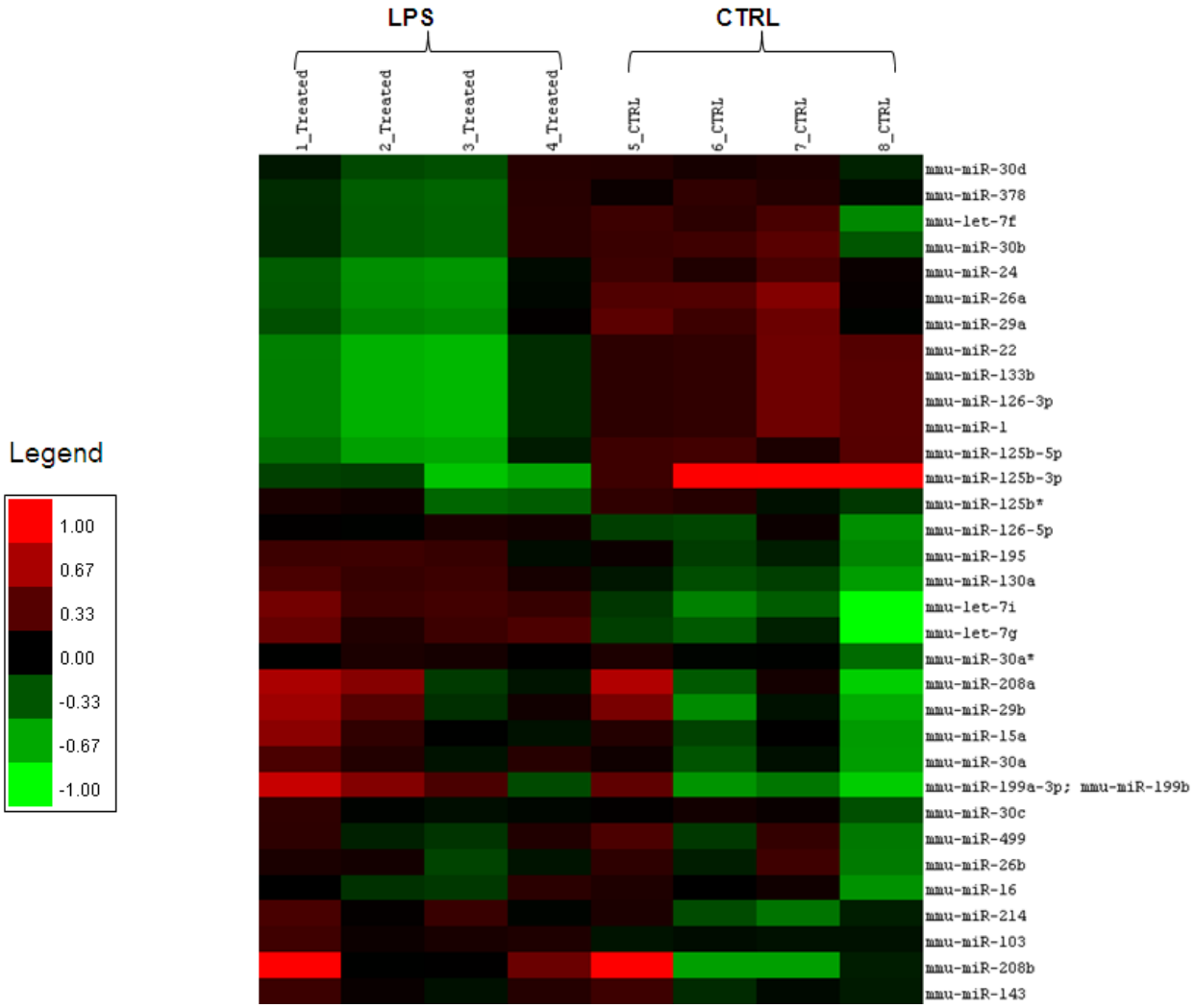
Supplementary Table 2

miR	Fold Change	miR	Fold Change	miR	Fold Change
miR-1	NS	miR-30b	NS	miR-199a-3p	1.62*
miR-15a	NS	miR-30c	NS	miR-208	NS
miR-16	NS	miR-30d	NS	miR-214	NS
miR-22	NS	miR-103	NS	miR-378	NS
miR-24	NS	miR-125b	NS	miR-499	NS
miR-26a	NS	miR-126	NS	let-7f	NS
miR-26b	NS	miR-130a	1.44*	let-7g	1.63*
miR-29a	NS	miR-133b	NS	let-7i	1.79*
miR-29b	NS	miR-143	NS		
miR-30a-5p	ND	miR-195	NS		

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3