SUPPLEMENTAL MATERIAL

Methods

Computational Analyses

To scan the mitochondrial genome for potential miRNA target sites, we used three independent algorithms, RNAhybrid, miRWalk and MicroCosm. The algorithmic core of RNAhybrid does not utilize any RNA folding or pairwise sequence alignment code, but rather, implements an algorithm that was specifically designed for RNA hybridization (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/submission.html). The miRWalk algorithm is based on a computational approach starting with a heptamer seed miRNA seed, and identifies complementation on the complete mitochondrial genome (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/). The MicroCosm target algorithm uses a weighted scoring system and rewards complementation at the 5' end of the miRNA (http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/info.html). A probability distribution of random matches for a subsequence in the given sequence is calculated. All prediction algorithms were run under default parameters.

Network Analyses

Network analyses were performed on altered miRNAs from the microarray analyses and on the predicated targets identified from the Computational Analyses (see above). Mitochondrially-encoded genes were mapped to corresponding gene objects in the Ingenuity Knowledge Base (http://ingenuity.com/). These mitochondrial genes, called focus genes, were overlaid into a global miRNA network developed from the information contained in the Ingenuity Pathways Knowledge Base. Networks of focus genes were

then algorithmically generated based on their connectivity. A network pathway is a graphical representation of the molecular relationships between individual miRNAs as well as miRNAs and mitochondrially-encoded mRNAs.

Supplemental Figure Legends

Supplementary Figure 1. Mitochondrial genome-encoded proteins and predicted mitomiR target networks in mouse cardiac mitochondrial subpopulations. (a) Prediction analyses for mitomiR targeting to mitochondrial genome-encoded mRNAs using bioinformatic databases RNAhybrid, miRWalk and Microcosm. Mitochondrial genes (Focus genes) were overlaid into a global mitomiR network developed from the information contained in the Ingenuity Pathways Knowledge Base. Focus gene networks were algorithmically generated based on their connectivity. The network pathway is a graphical representation of the molecular relationships between mitomiR and mitomiR or mitomiR and mitochondrial encoded gene. Those mitomiRs predicted to bind a single mitochondrial genome target are indicated in red with red lines, while those mitomiRs predicted to bind multiple mitochondrial genome targets are indicated in blue with black lines. MitomiR-378 predicted interaction with ATP6 is indicated with an orange line. **(b)** Conserved sequence specific tri-nucleotide motifs and their location within the identified mitomiRs.

Supplementary Figure 2. Dicer and GW182 protein contents. Western blot analyses of Dicer and GW182 in cytosol, crude mitochondrial subpopulations and percoll gradient purified mitochondria. COXIV is used as a mitochondrial protein marker.

Supplementary Figure 3. Crosslinked immunoprecipitation (CLIP) in cardiac mitochondrial subpopulations. Schematic representation of CLIP-Ago2 and the CLIP-FXR1 experimental designs.

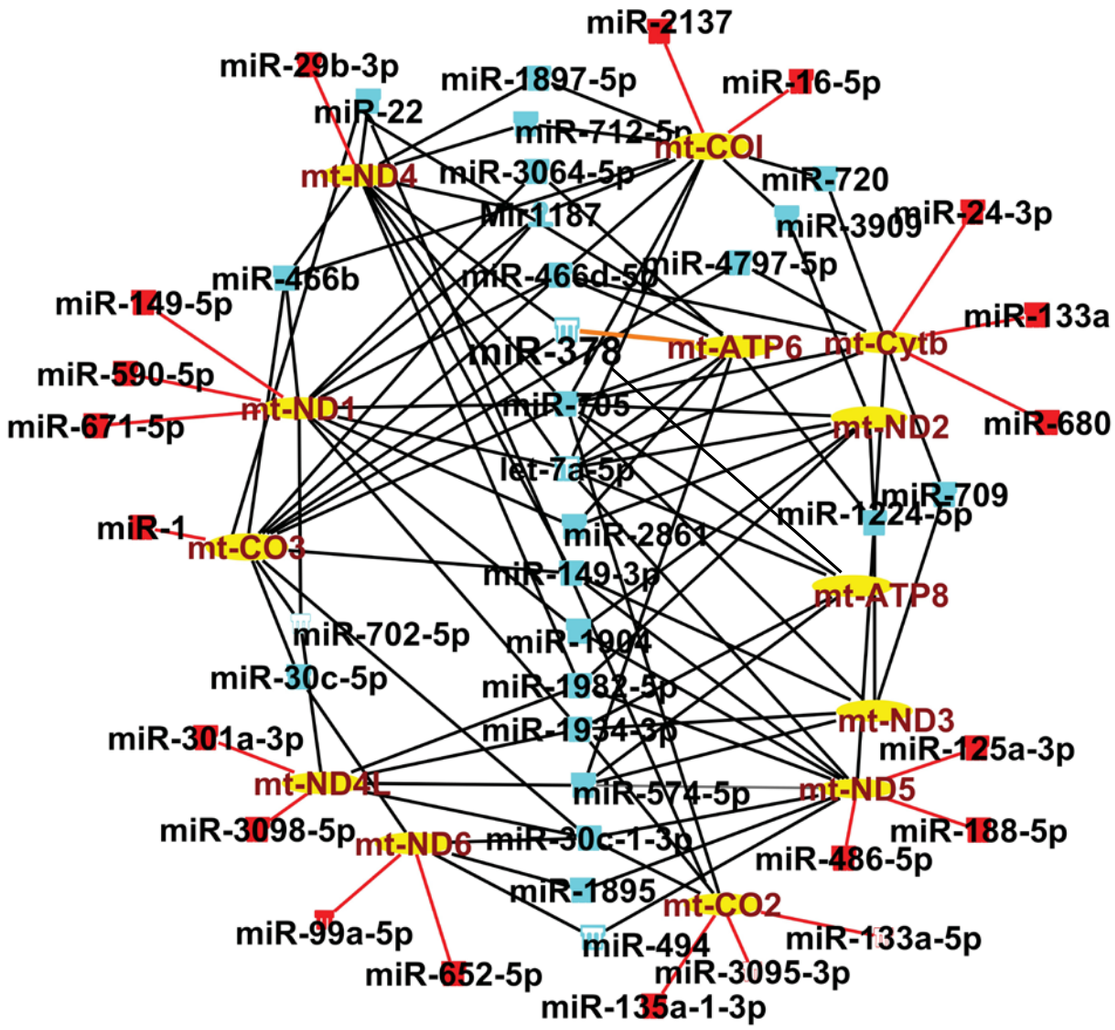
Supplementary Figure 4. **Genomic sequencing analyses of mitochondrial RISCome association with mitomiR.** MitomiR heat map derived from next generation sequencing of small RNAs identifying enrichment and depletion patterns within the mitoRISCome of diabetic SSM and IFM, relative to controls.

Supplementary Figure 5. Translational regulation of mitochondrial genome-encoded ATP6 protein via miR-378 interaction. Schematic diagram of miR-378 mechanism of action in the translational regulation of mitochondrially-encoded ATP6 and function of the ATP synthase activity during diabetic insult, impacting cardiac function.

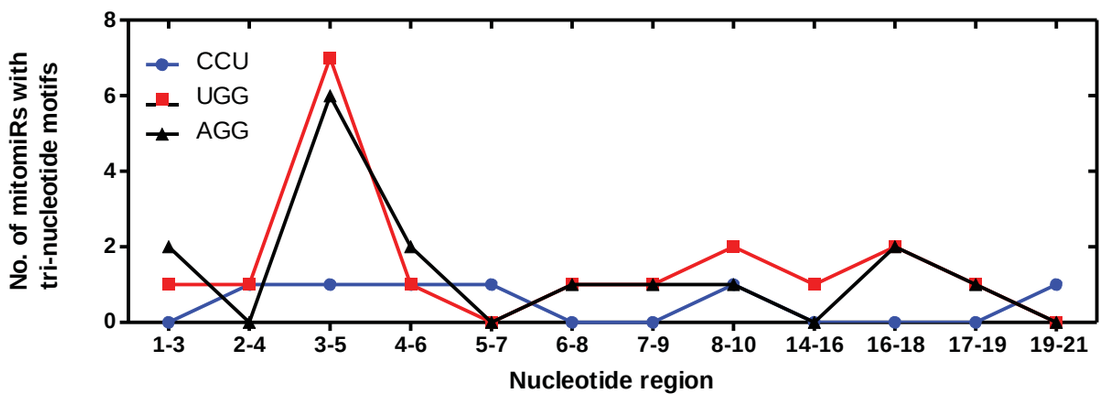
Supplementary Table 1. **Statistics of Ago2 CLIP-seq data generated from isolated mitochondrial subpopulations following diabetic insult.** Total, aligned and uniquely matched Ago2 CLIP-seq tags. Maximum and average read lengths are included.

Supplementary Figure 1

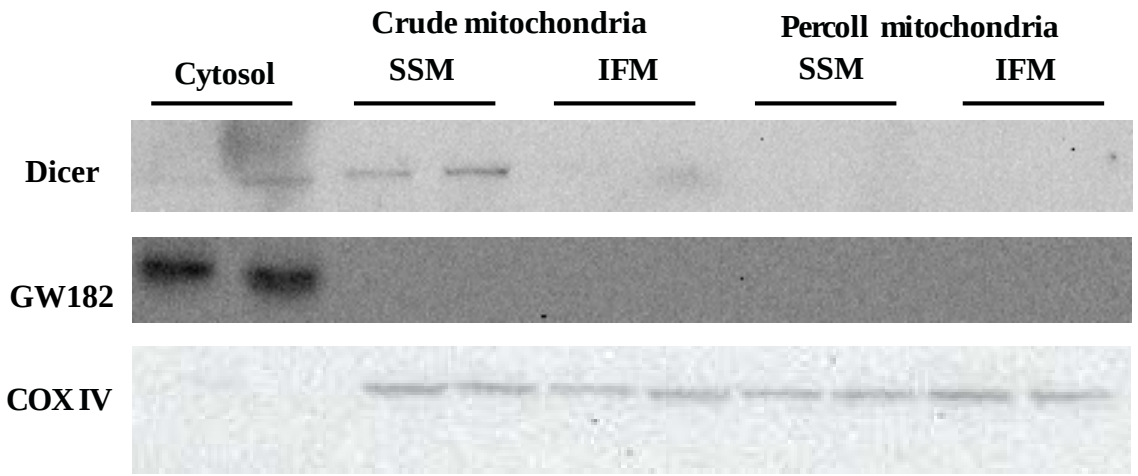
a



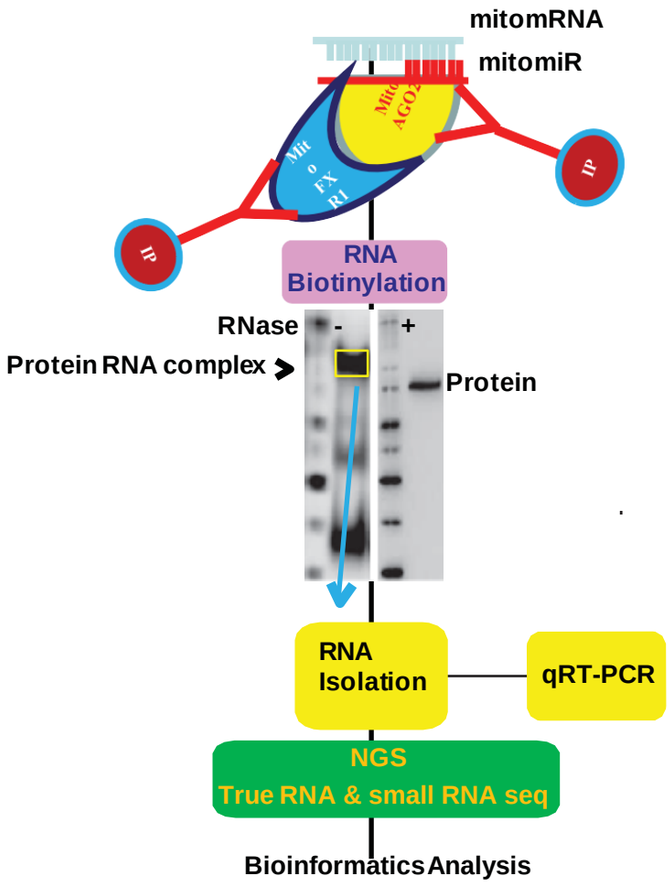
b



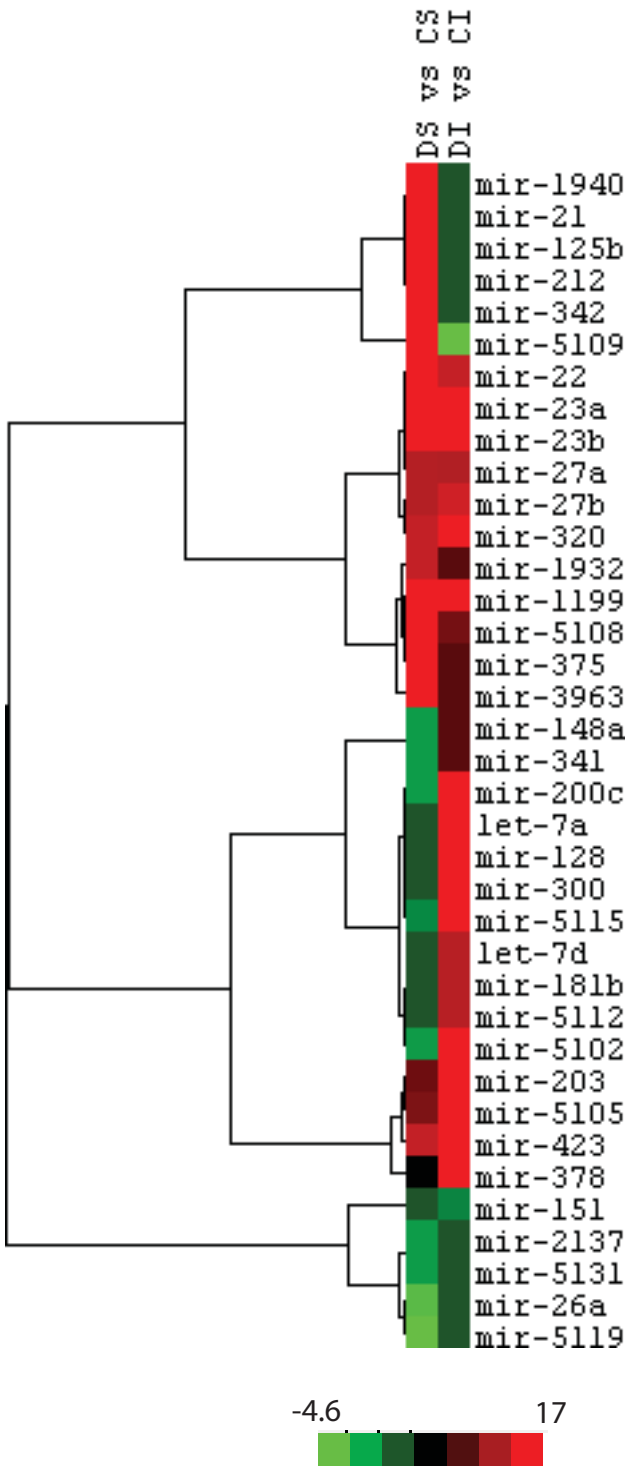
Supplementary Figure 2



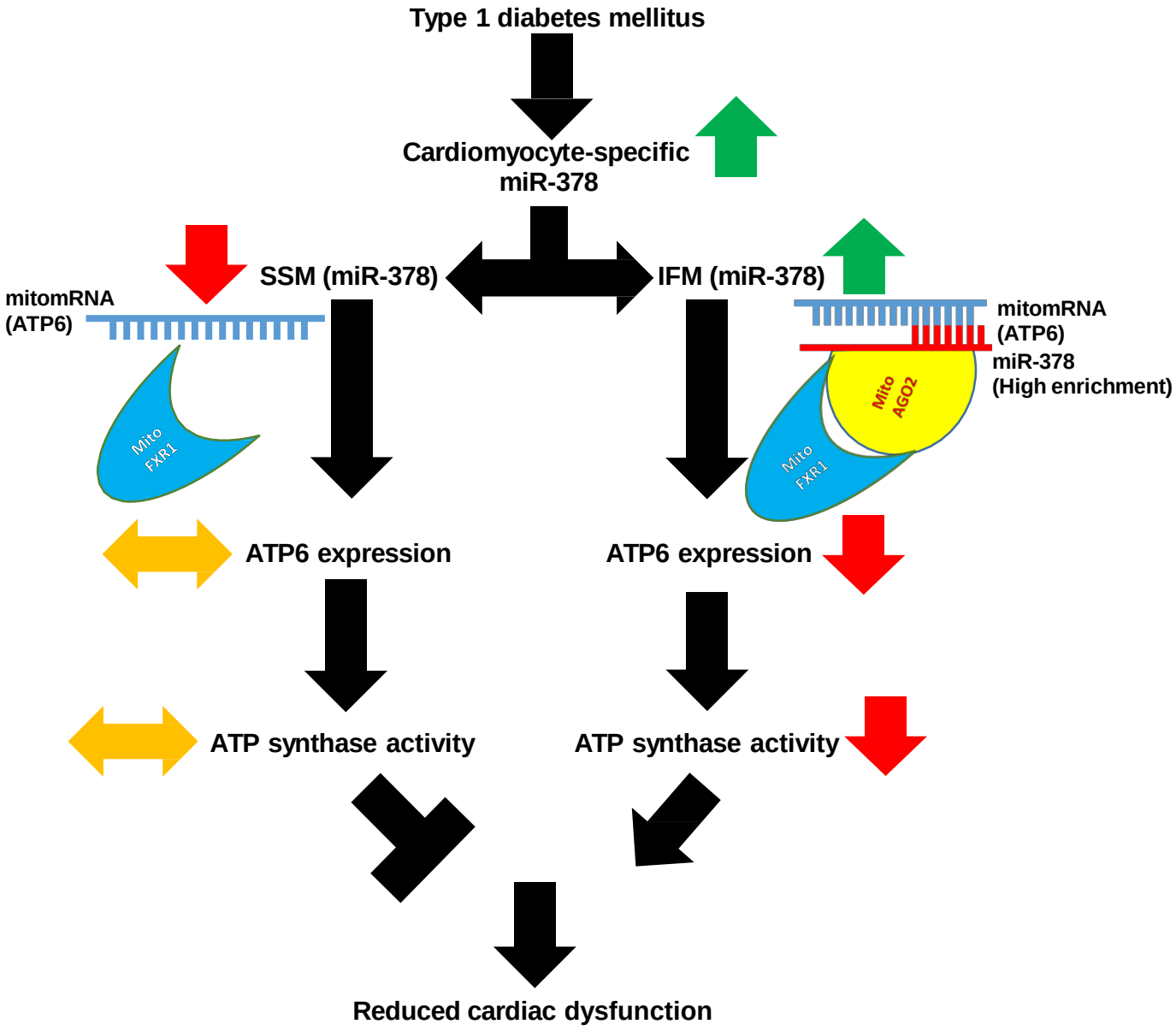
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Table 1

Alignment Statistics	SSM Control	SSM Diabetic	IFM Control	IFM Diabetic
Total number of reads	3802312	1357854	870888	878354
Aligned reads	1284	1567	565	1514
Uniquely matched reads	1043	1159	417	1122
Maximum read length	151	151	151	151
Average read length	151	150	151	151