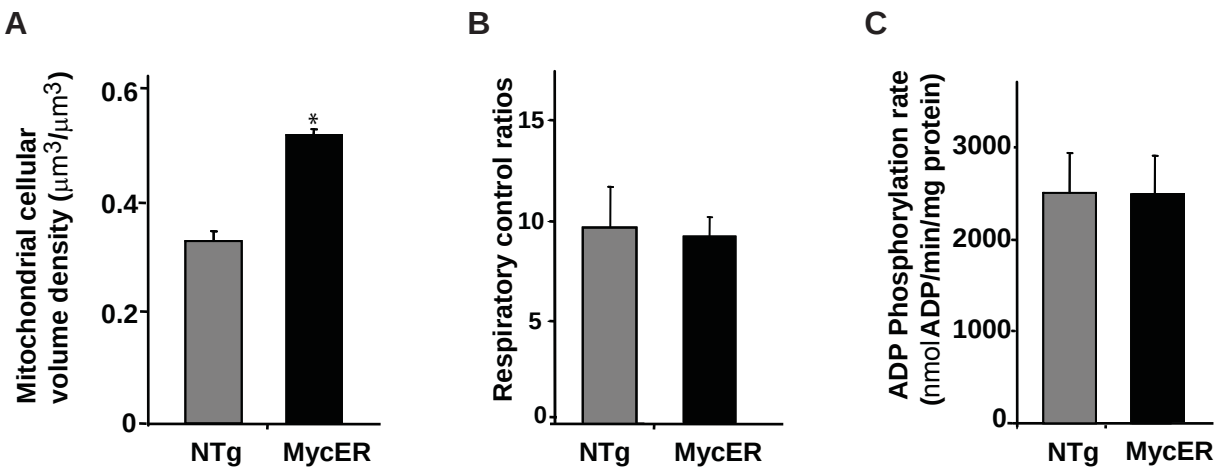
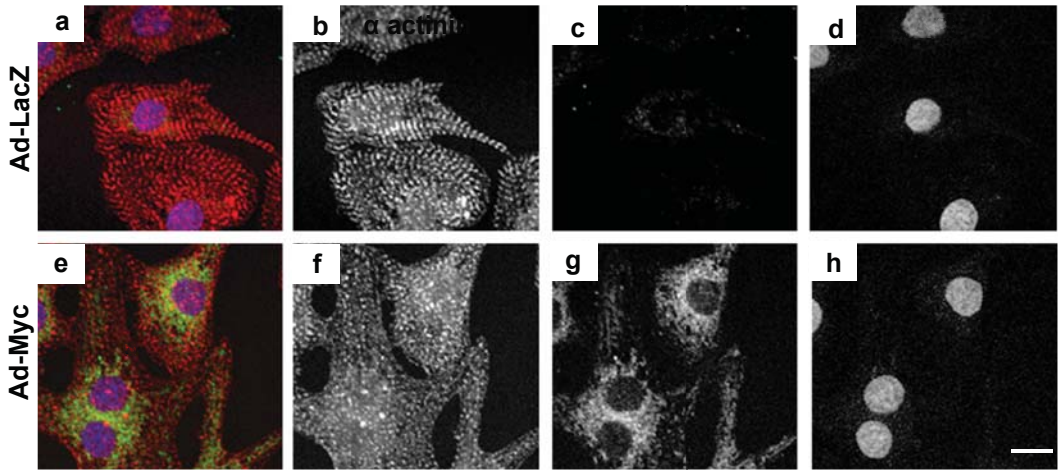
Supplemental Figure S1



Supplemental Figure S1: Myc stimulates mitochondrial biogenesis. (A) Quantitative morphometric measurement of mitochondrial cellular volume density ($\mu m^3/\mu m^3$) based on analysis of electron micrographs from treated NTg and MycER ventricles (**P*<0.001 vs treated NTg littermates, n=3). Effects of Myc activation on (B) respiratory control ratio (RCR) and (C) rate of ADP phosphorylation from NTg and MycER mice after 4-OHT treatment.

Supplemental Figure S2



Supplemental Figure S2: Overexpression of Myc induces TFAM expression in NRVMs. Confocal micrographs of NRVMs illustrating upregulation of TFAM in cardiomyocytes following Myc activation. Cardiomyocytes were stained for TFAM (panels c, g and green in overlay), sarcomeric α-actinin, (panels b, f and red in overlay) and DAPI (panels d, h and blue in overlay). Bar represents 20 μm.

Supplemental Experimental Procedures

Morphometric Analysis

Cardiac mitochondrial volume densities were determined from electron micrographs as described previously (1, 2). Data were expressed as volume density (volume of mitochondria [µm3] per cytoplasmic volume [µm3]).

Metabolic and Mitochondrial studies

Nuclear Magnetic Resonance (NMR) Measurements

For ^{13}C NMR, hearts were isolated and connected to a working heart perfusion system. After a period of stabilization, ^{13}C labeled substrates were given in murine physiological concentrations which included labeled acetoacetate (as a surrogate for ketones), labeled mixed free fatty acids, and labeled lactate as well as unlabeled glucose. Labeling continued for 30 minutes. The myocardial tissue was then freeze clamped. Carbon labeling of glutamate from the myocardium was determined by ^{13}C NMR and isotopomer analyses. All of the labeled carbon resonances of glutamate were integrated with the Lorentzian peak fitting subroutine in the acquisition program (NUTS; Acorn NMR, Livermore, CA).

For ^{31}P NMR, spectra were obtained from using a 14.1-T Varian Inova spectrometer (Varian Instruments, Palo Alto, CA). The perfused heart was mounted in a 10-mm broadband probe tuned to 243 MHz. A glass capillary containing methylene diphosphonate (MDP) placed close to the heart was used as an intensity reference. Fully-relaxed spectra consisting of 256 transients were collected using a 90-degree pulse every 17 seconds with a 37,879 Hz sweep width and 75,758 points. Two spectra were collected, one before the onset of 30 minutes ischemia, the other begun after 1 hour of reperfusion recovery. Spectra were analyzed by zero filling, applying a 20 Hz

exponential filter, fourier transformed and peak areas determined by fitting Lorenzian lines to the peaks corresponding to MDP, PCr and beta-ATP using custom software.

Ventricular tissues from NTg and MycER Tg mice after OHT treatment were sent to Analytical Core of Mouse Metabolic Phenotyping Center (MMPC) at the Yale University school of Medicine (http://mouse.yale.edu/index.html) for the analysis of glycogen levels. Mitochondrial DNA from equivalent masses of ventricular tissue from NTg and MycER mice after vehicle or 4-OHT treatment was purified with Wako mtDNA Extractor Kit (Wako, Richmond, VA) and an aliquot of isolated mtDNA was loaded on ethidium bromide-stained agarose gel (1.2%).

To analyze mitochondrial oxidative capacity measurements, mitochondria were isolated from hearts by homogenization and differential centrifugation as described previously (3). All measurements were carried out using a customized Fiber Optic Spectrofluorometer (Ocean Optics) with continuously stirred cuvette at room temperature. Mitochondria were added to incubation buffer containing 100 mM KCl, 10 mM HEPES, pH 7.4 with tris, followed by addition of 2.5 mM Pi and pyruvate, malate, glutamate, all 1.5 mM. Buffer O2 content was continuously monitored via fiber optic O2 sensor FOXY-AL300 (Ocean Optics) inserted through the hole in the cuvette cover. Membrane potential (Dy) was determined by change in tetramethylrhodamine methyl ester (TMRM) fluorescence at 580 nm. Dy is expressed as a precentage of the TMRM fluorescence in the presence of coupled mitochondria and substrates (100%), relative to that after addition of FCCP or alamethicine to fully depolarize mitochondria (0%). Changes in mitochondria volume were evaluated by light scattering as described previously (3).

State 3 respiration was determined by measuring mitochondrial oxygen uptake after the addition of ADP to a final concentration of 400 μM. State 4 respiration was determined by measuring mitochondrial oxygen uptake on complete phosphorylation of

ADP to ATP. Mitochondria were considered viable where the respiratory control ratio (RCR) (state 3/state 4) was greater than 4. The rate of ADP phosphorylation was calculated indirectly based on time interval required to recover membrane potential (and state 4 respiration) after ADP addition. Membrane potential recovery signals that at least 90% of ADP is phosphorylated (4).

Perfused mouse heart model

For isolated perfused heart studies, the heart was rapidly excised and placed into ice-cold modified Krebs-Heseleit perfusion buffer containing (in mmol/L): NaCl, 118; NaHCO3, 25; KCl, 4.7; KH2PO4, 1.2; CaCl2, 1.75; MgSO4, 1.2; glucose, 11; Na-pyruvate, 1; and EDTA, 0.6. The aorta was cannulated and perfused at a constant pressure of 80 mmHg by gravity. The perfusion fluid was equilibrated with 95% O2, 5% CO2 to maintain pH 7.4. A fluid-filled balloon was inserted into the left ventricle via the mitral valve and connected to a pressure transducer for continuous recording of left ventricular pressure. The balloon was inflated to an end-diastolic pressure of 4 to 8 mmHg. The left ventricular pressure signal was recorded by a Powerlab (ADInstruments) data acquisition system and processed to yield heart rate and left ventricular dP/dT by Chart 5 software. After 30 minutes of stabilization, hearts were subjected to 30 minutes of global normothermic (37ºC) ischemia by stopping the perfusion followed by 60 minutes of reperfusion. After the ischemic insult there was no contractile activity. Thus, the first time LV function was measured was after 30 minutes of ischemia followed by 15 minutes of reperfusion.

RNA analysis

Primer sequences were designed with the OligoPerfect Designer software using DNA sequences obtained from GenBank database. For semi-quantitative analysis, all

primers were cycled appropriately and resolved on 1% agarose gels. Real-time quantitative PCR was conducted using ABI PRISM 7700 Sequence Detection System; Taqman (ABI, Foster City, CA). PCR amplicons were detected by fluorescent detection of SYBR Green (QuantiTect SYBR Green PCR Kit, Qiagen).

Immunostaining

For cultured cells, NRVMs were fixed in 4% paraformaldehyde (Sigma) and permeabilized using 1% Triton-X 100 (Sigma). Immunostaining was performed as described (5). Images were acquired using a confocal TCS SP2 AOBS laser-scanning microscope system (Leica Microsystems Inc., Exton, PA, www.leica.com) and processed with Adobe Photoshop 7.0 (Adobe Systems Inc., San Jose, CA). Antibodies and fluorescent reagents. The polyclonal rabbit antibody against ENO-1α was generously provided by Angelica Keller (University of Paris, France) (6). Antibodies were purchased against sarcomeric α-actinin (clone EA53; Sigma), α-cardiac actin (Progen; Heidelberg, Germany), MCAD (Cayman Chemicals), HK-2 (Chemicon International), tubulin (BD Biosciences), ATP5A & B (Abcam), ATP5C (Proteintech Group, Inc), Lamin B and proliferating cell nuclear antigen (PCNA), PGC-1α, CPT-1, TFAM and c-Myc (Santa Cruz Biotechnology, Santa Cruz, CA). Secondary antibodies and fluorescent reagent combinations for the triple immunofluorescence have been described previously (7)**.**

Table S1. Primer sequences used in ChIP assay

Gene Symbol	Forward	Reverse
ENO-1α	GCTCTCCTACTAGCTTCGCTTG	CAACCCTGAAACTCGGTGAT
PFK-1	GATGTCAACCTCTGGCCTCT	GCTTACTTTGTGTGCCATGC
LDHA	TGGGTCTAAGGGAAGGGAAG	CAGGCCAGGTTCATCTGCTA
HK-2	GAGCCCCTAATTTCTTGATGG	CCCCTAAGCAGGATTCTTCC

TFAM	TTGGCTGGCTAAGCTCATCT	AAGGCTGAGAAGCGATAGCA
POLG	AGCATCATTGTCCGGGATAG	TGTCAGGGTGACACATTGCT
POLG2	AAATGATTGTGGGGCTTGG	GAGGCCTCTACGCGAATAAA

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6. Keller, A., Rouzeau, J.D., Farhadian, F., Wisnewsky, C., Marotte, F., Lamande, N., Samuel, J.L., Schwartz, K., Lazar, M., and Lucas, M. 1995. Differential expression of alpha- and beta-enolase genes during rat heart development and hypertrophy. *Am. J. Physiol.* **269**:H1843-1851.
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