Supplemental Material:

Animals

All animals (Charles River Laboratory) were treated in accordance with *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, 1996). All protocols were approved by the Institutional Animal Care and Use Committee. Adult male and female Sprague-Dawley rats were sexually mature (11-13 weeks old). Ovariectomized female rats were obtained from Charles River Laboratory.

In vivo myocardial ischemia

The left coronary artery occlusion model has been described previously[1]. Briefly, male and female rats were anesthetized with ketamine (95 mg/kg) plus xylazine (5.2 mg/kg) injected intramuscularly, and administered glycopyrrolate (0.02 mg/kg) intramuscularly. The animals were intubated under direct visualization, passing an endotrachael tube into the trachea. Anesthesia was maintained with 1-3% isoflurane in oxygen, administered through a precision vaporizer and a positive pressure ventilator. Body temperature was carefully monitored with a rectal probe and was maintained at 37.0 °C throughout the experiment by using circulating heated water blankets. After respiratory stabilization, a skin incision was made, and the ribs were gently spread to expose the left side of the heart and visualize the left anterior descending coronary artery (LAD). An 8-0 Vicryl suture (Ethicon, Inc.) was placed around the LAD and the ends were threaded through a silicone tube to form a snare. Coronary artery occlusion was produced by tightening the snare and was verified by the presence of epicardial cyanosis in the ischemic zone. Typical ECG changes immediately after LAD occlusion were used as signs of successful induction of anterior wall ischemia. After 45 min occlusion, reperfusion was achieved by loosening the snare. All rats were subjected to a 45 min LAD occlusion and 2 h reperfusion.

After 2 hours the LAD was re-occluded in the same location as before. Evans blue dye (5 ml of a 7.0% solution; Sigma-Aldrich) was injected through the carotid artery catheter into the heart for delineation of the ischemic zone from the non-ischemic zone. The heart was rapidly excised and was cut into 3 or 4 cross-sectional pieces then incubated in 1.0% 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich) in 0.1 M phosphate buffer adjusted to pH 7.4 for 15 minutes at 37°C for demarcation of the viable and nonviable myocardium within the risk zone. The areas of infarction, area at risk (AAR), and non-ischemic left ventricle were assessed using NIH Image J.

Langendorff Perfused Hearts

After intraperitoneal injection of sodium pentobarbital, a transverse abdominal incision was made, the vena cava was exposed, and heparin sodium was administered intravenously. The heart was quickly excised, placed in ice cold Krebs-Henseleit (K-H) buffer (120 nM NaCl, 4.6 mM KCl, 1.2 mM MgSO4, 1.2 mM KH2PO4, 1.25 mM CaCl2, 25 mM NaHCO3, 11 mM glucose), and connected to the perfusion cannula via the aorta. A water filled latex balloon was inserted into the left ventricle to measure hemodynamic parameters using a Powerlab 4/25 and Chart v5.5 software (AD Instruments, Colorado Springs, CO). Hearts were stabilized for 20 minutes with K-H buffer gassed with 95% O2 and 5% CO2, followed by 30 minutes of global ischemia and 1.5 hours of reperfusion. Rate pressure product (left ventricular developed pressure, LVDP × heart rate) was used as a measure of function. Hearts were maintained at 37ºC for the duration of the protocol. The hearts that received wortmannin (WM) were stabilized for 10 minutes with K-H buffer then perfused for 10 minutes with WM (100nM) and subjected to 30 minutes of global ischemia followed by reperfusion as previously published[2]. The hearts that received Alda-1 (aldehyde dehydrogenase activator) were stabilized for 10 minutes with K-H buffer then perfused for 10 minutes with Alda-1 (20μM) prior to 30 minutes of global ischemia and for 10 min immediately at the onset of reperfusion as previously published[3]. The hearts that

received DOG (1,2-dioctanoyl-*sn*-glycerol) were stabilized for 15 minutes with K-H buffer then perfused for 5 minutes with DOG (3μM) followed by 10 min of washout prior to 30 minutes of global ischemia as previously published[4]. The hearts that received Ro-31-7549 (PKC inhibitor) were stabilized for 10 minutes with K-H buffer then perfused for 10 minutes with (875nM) prior to 30 minutes of global ischemia. At the end of reperfusion infarct size was measured with 2,3,5-triphenyltetrazolium chloride (TTC) as described previously[5,6]. Hearts were initially perfused with TTC and then incubated in TTC for an additional 15 minutes at 37ºC. The hearts were subsequently fixed in formalin and 4-6 cross sectional slices were taken. These slices were imaged on a Leica Stereoscope and the percentage of infarct (white area) to viable tissue (red area) was analyzed using ImageJ software. Area of infarct was expressed as a percentage of total ventricles.

Cardiomyocyte isolation

Adult cardiomyocytes were isolated from the left ventricle of male and female Sprague-Dawley rats. Rats were anesthetized with pentobarbital sodium and heparinized; hearts were excised, mounted on a Langendorff apparatus and perfused withCa^{2+} free normal Tyrode solution. Blendzyme Type IV (0.042 mg/ml) (Roche Applied Science, Indianapolis, IN) was then added to the perfusate at 37°C. After 5–8 min, the ventricles were removed, cut, and shaken gently at 34°C in the blendzyme IV solution. This digestion was repeated 4-6 times as needed.

Simulated ischemia model

The cardiac myocytes were subjected to simulated ischemia by pelleting the myocytes as described previously[7]. Briefly, freshly isolated myocytes from male or female rat heart ($2x10^4$ cells) were divided into experimental groups and treated with or without 100nM of wortmannin (WM) for 10 min and then covered with mineral oil and incubated for 30 min.

Mitochondria isolation

For mitochondria isolation, hearts were cannulated and perfused with K-H buffer for 5 minutes to washout the blood. The buffer was aerated with 95% O2 and 5% CO2, at pH of 7.4 at 37°C. Freshly isolated mitochondria were prepared from hearts by differential centrifugation as described previously[8]. After separation of the fractions, mitochondrial and cytosolic proteins were submitted to a western blotting protocol to verify the purity of the isolation procedure using prohibitin and LDH antibody as markers for mitochondria and cytosolic compartments respectively.

Mitochondrial oxygen consumption

Measurement of mitochondrial respiration was performed at 25°C in a chamber (600 μL) connected to a Clark-type O2 electrode (Instech) and O2 monitor (Model 5300, YSI, Inc). The mitochondria were incubated in respiration buffer containing (in mmol/L) KCl 120, MOPS 5, EGTA 1, KH2PO4 5, and 0.2% BSA. After addition of glutamate/malate (10/2 mmol/L), state 3 respiration was measured by addition of ADP (0.5 mmol/L). On depletion of ADP, state 4 respiration was determined. [8].

H_2O_2 production

Hydrogen peroxide (H_2O_2) production from isolated heart mitochondria or cardiac myocytes was measured fluorimetrically by measurement of oxidation of Amplex Red to fluorescent resorufin (Invitrogen kit). After ischemia, myocytes ($2x10^4$ cells) were resuspended in a Media 199. Mitochondria were incubated at 1.75 mg/ml mitochondrial protein in buffer containing 120 mMKCl, 5 mM MOPS, 10 mM Tris and 5mM KH2PO4 (pH 7.25). All incubations also contained 50 μM Amplex Red, 5 U/mL of horseradish peroxidase. The increase in

fluorescence at an excitation of 544 nm and an emission of 590 nm was monitored. Standard curves were generated using known amounts of hydrogen peroxide [9; 10]. Hydrogen peroxide was measured in mitochondria (10 mM glutamate + 2 mM malate + ADP) during normoxia. The mitochondria were incubated until the oxygen was consumed and were left anoxic for 30 minutes followed by reoxygenation.

In another series of studies we measured H_2O_2 generation from α-ketoglutarate dehydrogenase. Permeabilized mitochondria (1% Triton) were incubated at a concentration of 1.0 mg/ml in buffer containing 120 mM KCl, 5 mM MOPS, 10 mM Tris and 5 mM KH_2PO_4 (pH 7.25). 50 µM Amplex Red, 5 U/mL of horseradish peroxidase, and 0.12mM HS-CoA were added. The reaction was started with the addition of 5mM of α-ketoglutarate. The increase in fluorescence (544nm excitation, 590nm emission) was monitored, and after 5 minutes, 2 µM of NADH was added.

ALDH activity

Enzymatic activity of mitochondrial ALDH was determined spectrophotometrically by monitoring the reductive reaction of NAD+ to NADH at 340 nm as previously described[11]. ALDH2 assays were carried out at 25 °C in 60 mM Na-pyrophosphate buffer (pH 8.5) with 5 mM pyrazole. To this volume, 200 µM propionyl aldehyde and 250 µg of mitochondrial protein lysate were added. To start the reaction, 2 mM NAD was added and the accumulation of NADH was monitored for 3 min. ALDH activity were expressed as µmol/min/mg protein.

Western blotting

Hearts from male and female rats were perfused with K-H buffer for 5 minutes to washout the blood and freeze clamped for western blot analysis. Frozen tissue was pulverized in liquid nitrogen using mortar and pestle until completely powdered. Samples were suspended standard lysis buffer. Protein concentrations were determined using Bradford assay and bovine serum albumin as a standard. Equal amounts of proteins of each sample (20 µg) were separated by 4-12% Bis-Tris Gel (Invitrogen, USA). The resolved proteins were electrophoretically transferred to nitrocellulose membranes at 30 V for 1 hour using a NuPAGE transfer buffer with 10% methanol added. Gel transfer efficiency and equal load was verified using reversible Ponceau staining. After blocking for 1 hour at room temperature with SuperBlock blocking buffer (Thermo scientific, Rockford, Illinois) membranes were incubated for 1 hour at room temperature with anti-phospho Serine/Threonine PKC substrate (rabbit, Cell signaling,1:1,000 dilution). The membrane was washed and a secondary antibody rabbit-HRP (Cell signaling,1:5,000 dilution) was added for 1 hour. After the final washes, the membranes were incubated with Western lightning Plus-ECL (PerkinElmer, Shelton, CT) and exposed to X-ray films. After exposure, membranes were stripped with Re-Blot Plus Mild Solution (Chemicon, Temecula, CA). The same protocol was followed as above with the primary antibody being aldehyde dehydrogenase-2 (1:1000, Santa Cruz Biotech, Santa Cruz, CA) and secondary being a donkey anti goat-HRP antibody (1:5000, Santa Cruz Biotech, Santa Cruz, CA). Optical densities for each band were obtained using ImageJ.

Equal amounts of α-KGDH from porcine hearts (cat # K1502, lot 075K7501; Sigma, St. Louis, MO) with and without phosphorylation by PKCε (PKCε kinase cat # 7492, Cell Signaling Technologies, Beverly, MA) were separated by 4-12% Bis-Tris Gel (Invitrogen, USA). The resolved proteins were electrophoretically transferred to nitrocellulose membranes at 10 V overnight using a NuPAGE transfer buffer with addition of 10% methanol. Gel transfer efficiency and equal load was verified using reversible Ponceau staining. After blocking for 2 hour at room temperature with BSA, membranes were incubated for 3 hours at room temperature with anti-phospho serine PKC substrate (Cell Signaling Technologies, Beverly, MA,1:1000 dilution). After washing, membranes were subsequently incubated with anti-rabbit horseradish peroxidase conjugated secondary antibody (1:5000, Cell Signaling Technologies, Beverly, MA) for 1 h at

room temperature. Proteins were detected using SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology, Rockford, IL) and visualized by autoradiography. The same protocol was used to analyze and quantify PKCε (1:1000, Santa Cruz Biotech, Santa Cruz, CA) in mitochondria from male and female samples. Optical densities for each band were obtained using ImageJ.

2D Gel Electrophoresis-DIGE (Two-dimensional difference in gel electrophoresis) in 24cm gels

Mitochondrial proteins isolated from rat heart were resuspended in 50 µl lysis buffer containing 7 M urea, 2 M thiourea, 4% CHAPS and 15 mM Tris-HCl, at pH to 8.5. The total amount of protein concentration in each sample was determined using a modified Bradford assay, USB protein determination reagent (USB Corporation, OH, USA). Four hundred picomoles of CyDye (Cy3, Cy5 and Cy2) were used to label 50 µg of male, female and standard samples as suggested in the manufacturer's protocol. The samples were brought up to a 440 µL volume using rehydration solution (7 M urea, 2 M thiourea, 4% CHAPS (w/v), 10 µl De-streak reagent (v/v) (GE Healthcare), and 1% (pH 3-10 NL) Pharmalyte (v/v), 13 mM DTT and trace amounts of bromophenol blue and then loaded onto an immobilized gradient strip (24 cm Immobiline DryStrip, pH 3-10NL, GE Healthcare/Amersham Biosciences, Piscataway, New Jersey).

Isoelectric focusing was achieved by active rehydration for ~10 h at 30 V followed by stepwise application of 250, 500, 1000, and 8000 V for a total of ~70000 Vh (Ettan IPG Phor, GE Healthcare/Amersham Biosciences, Piscataway, New Jersey). Immobiline DryStrip gel strips were equilibrated in 10 mL of SDS equilibration solution containing 50 mM Tris-HCl (pH 8.8), 6 M urea, 30% glycerol, 2% SDS, trace amounts of bromophenol blue and 1.0% DTT for 10 min followed by a second 10 min incubation in a solution containing 50 mM Tris-HCl (pH 8.8), 6 M urea, 30% glycerol, 2% SDS, trace amounts of bromophenol blue 2.5% iodoacetemide. The strips were briefly washed in electrophoresis buffer (25 mM Tris (pH 8.3), 192 mM glycine, and 0.2% SDS), applied to 10-15% SDS-PAGE gels (Nextgen gels, Ann Arbor, Michigan) and sealed with 0.5% agarose containing bromophenol blue.

Electrophoresis was performed in an Ettan DALT-12 tank (GE Healthcare/Amersham Biosciences, Piscataway, New Jersey) in electrophoresis buffer until the dye front advanced completely (~2500 Vh). The gels were imaged using the Typhoon 9400 Variable Mode Imager (GE Healthcare/Amersham Biosciences, Piscataway, New Jersey) and were then placed in 500 mL of a fixative solution containing 30% ethanol and 7.5% acetic acid. After two short water rinses the gels were placed in 500 mL of Coomassie Blue protein gel stain for total protein visualization (EZBlue gel staining reagent, Sigma, Saint Louis, Missouri) for 48 hours. Finally, the gels were washed in de-staining solution for 3 hours, changed every hour, before scanning for total proteins [12-14].

2D-DIGE image analysis and mass spectrometry.

Cy2, Cy3, Cy5, Pro-Q and Coomassie Blue images were collected using a Typhoon scanner in fluorescence mode (GE Healthcare/Amersham Biosciences, Piscataway, New Jersey). The Cy2 images were scanned at an excitation wavelength of 520/40 (maximal/bandwidth) using a blue laser while the Cy3 images were scanned with an excitation wavelength of 580/30 using a green laser. The Cy5 images were scanned using a 670/30 excitation wavelength and a red laser. Pro-Q images were collected using the excitation wavelength set at 580/30 using a green laser. For total protein staining we used Coomassie blue and the images were scanned at an excitation wavelength at 610/30. Analysis for the differences between male and female proteins was performed using Progenesis SameSpots software (Nonlinear Inc, Durham, North Carolina). Spots of interest were matched to the Coomasie stained image. Spots of interest were processed by the fully automated Spot Handling Workstation (GE Healthcare/Amersham Biosciences, Piscataway, New Jersey).

Peptide extracts were analyzed using the Proteomics Analyzer (ABI 4700 MALDI TOF/TOF). Peptides were analyzed using the ABI GPS Explorer software (Applied Biosystems, Foster City, California) and full scan mass spectrometry spectra were obtained first followed by MS/MS spectra. Protein identification was carried out using the search engine MASCOT[12-14].

Pro-Q Diamond

To analyze phosphoproteins in 24 cm gel we used Pro-Q Diamond stain (Molecular Probes, Eugene, OR) which selectively stains phosphate groups attached to tyrosine, serine, or threonine residues. After the second dimension the gels were placed in a fixative solution containing 30% methanol and 7.5% acetic acid. The gels were rocked in this solution for 8 hours and the solution was replaced for overnight rocking. The gels were then washed 4 times with 500 ml of warm water for 30 min. The gels were stained with 500 mL of Pro-Q-Diamond stain, for 2 hours while shaking. The gels were then de-stained in 500 mL Pro-Q-Diamond Destain solution (Molecular Probes, Eugene, Oregon) every hour for a total of 3 hours. Finally, the gels were washed twice in water, (ten minutes each) and were Imaged using the Typhoon 9400 (GE Healthcare/Amersham Biosciences, Piscataway, New Jersey) in fluorescence mode. After imaging, the gels were placed in Coomassie blue (EZBlue gel staining) for 48 hours rocking[12, 13].

2D Gel Electrophoresis, Western blotting in 11 cm gels and Peptide Identification

Whole heart extracts were suspended in standard lysis buffer (7 M urea, 2 M thiourea and 4% CHAPS) and then cleaned using acetone precipitation. The samples (400 µg) were brought to 200 µL in rehydration solution (7 M urea, 2 M thiourea, 4% CHAPS (w/v), 0.2% Bio-Lyte (pH 3-10 Bio-Rad, Hercules, California), 4 mM DTT and trace amounts of bromophenol blue) and then loaded onto an immobilized gradient strip (11 cm ReadyStrip IPG, pH 4-7, Bio-Rad, Hercules, California).

Isoelectric focusing was performed as previously described for the large 2D gels except that the total voltage hours were ~28000 Vh. ReadyStrip IPG strips were equilibrated as detailed previously. After equilibration the strips were briefly washed in electrophoresis buffer and applied to 8-16% Criterion Precast Tris-HCL gels (Bio-Rad, Hercules, California), sealed with 0.5% agarose containing bromophenol blue and run for 2 hours at 150V.

The proteins were electrophoretically transferred to nitrocellulose membranes at 10 V during 10 hours at 4°C. Gel transfer efficiency and equal loading was verified using MemCode reversible protein stain (Thermo scientific, Rockford, Illinois). After blocking for 1 hour at room temperature with SuperBlock blocking buffer (Thermo scientific, Rockford, Illinois) membranes were incubated for 3 hours at room temperature with anti-phospho serine PKC substrate (rabbit, Cell signaling,1:1,000 dilution). The membrane was washed and a secondary antibody conjugated with infrared Dye (LI-COR, at 1:10,000) was added for 1 hour. After the final washes, the membranes were scanned and analyzed using the Odyssey™ Infrared Imager (LI-COR, USA).

The nitrocellulose membrane was placed in water overnight. Spots of interest were cut from the membrane, placed in new tubes and were destained with water until the MemCode stain was removed. To digest the peptides 20 µl of trypsin solution (Promega, Wisconsin) was added to each vial containing the membranes and 80 µl of 25 mM ammonium bicarbonate was added to cover. The vials were placed in a 37°C water bath for overnight digestion. The trypsin digests were transferred to new sterile tubes and the vials were placed in a speed vac until completely dried. The digests were resuspended in 20 µl of 0.1% formic acid. Each digest was concentrated and cleaned using C18 resin in a tip (Millipore, Massachusetts) following manufacture guidelines for LC MS/MS analysis.

The trypsinized samples were analyzed using nanoLC-ESI-MS/MS with LTQ Orbitrap XL. An Eksigent® NanoLC-2D™ HPLC with a C18 column 75 µm i.d. x 10 cm was used to separate peptides at 300 nL/min with 42 min gradients from 2 to 95% acetonitrile in 0.1% formic acid. MS scans were performed on the linear trap and up to six of the most intense ions per cycle were fragmented and analyzed in the linear trap.

Database searching was performed using the Mascot search engine (Matrix Science, v.2.2). All the MS/MS datasets were searched against the Swiss Prot (Swiss Institute of Bioinformatics) *Rattus norvegicus* database (v14.6; 7254 sequences). Protein modifications were selected as carbamidomethyl (C) (fixed) and oxidation (M) (variable). Up to one missed cleavage was allowed. The following criteria were used to generate a high-confidence data set: at least two peptides must be identified for each protein, with an ion expectation less than 1, corresponding to a significance level of $p<0.05$.

RESULTS

Purity of the mitochondria

To assess the purity of the mitochondria we measured the enrichment of mitochondrial proteins. As shown in Supplemental Figure I, the mitochondria show enrichment of mitochondrial markers and lack of cytosolic markers. Additionally, we verified the integrity of the isolated mitochondria by measuring mitochondrial state 3 and state 4 O_2 consumption.

Phosphorylation of PDH-E1α

As shown in Figure 2, for PDH-E1α, females show an increase in the spot at the higher pI and a decrease in the spot at a lower pI value consistent with less of an acidifying post translational modification such as phosphorylation in females. We confirmed the increase in phosphorylation of PDH-E1-α using ProQ staining (Suppl. Fig II and Suppl. Table I). Because phosphorylation of the E1 subunit of PDH decreases the activity of PDH, this increase in phosphorylation in this subunit in males would account for the relative reduction in glucose metabolism that we observed previously in males compared to females[6].

2D DIGE comparing females to ovex females

Hearts from bilaterally ovariectomized females show more post-ischemic contractile dysfunction and have larger infarcts size than hearts from intact female (see Figure 1D and E). To examine the mechanism involved in the cardioprotection observed in females compared with ovariectomized females we performed 2D-DIGE. As shown in Supplemental Figure III and Supplemental Table II, we found that ALDH2 from intact females was shifted to a more acidic pI compared to Ovx. The more basic pI for ALDH2 in Ovx hearts was similar to the pI value observed in males. These data suggest that the increase in phosphorylation in ALDH2 in intact females is related to estrogen.

2D DIGE Male vs Female after ischemia

We were interested in examining whether the male-female differences observed at baseline were maintained during ischemia and reperfusion. We therefore performed a set of experiments using a proteomic approach to examined gender-specific differences after 30 minutes of ischemia and 10 minutes of reperfusion. As shown in Suppl Fig. IV and Suppl. Table III, after ischemia and reperfusion the increase in ALDH2 in females at the more acidic (phosphorylated) pI was still present, suggesting that the phosphorylation in this enzyme in female is maintain after ischemia. We also found that the decreases in fatty acid binding protein and glyceraldehydes-3-phosphate dehydrogenase observed in females at baseline were preserved following ischemia and reperfusion.

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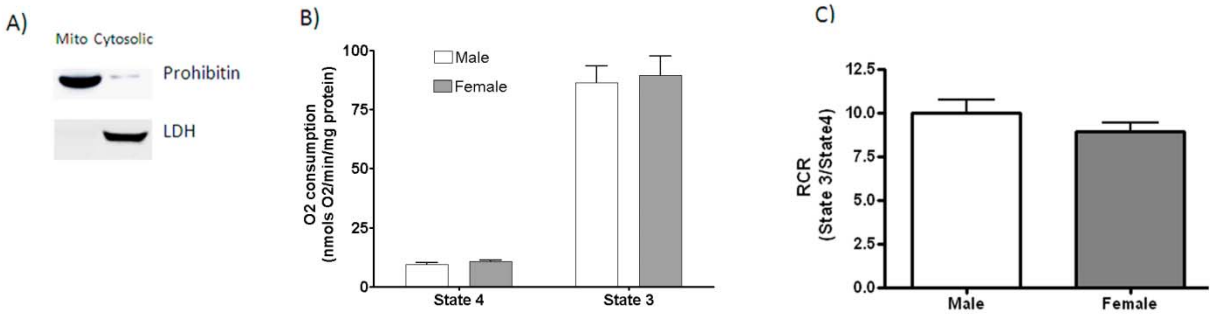
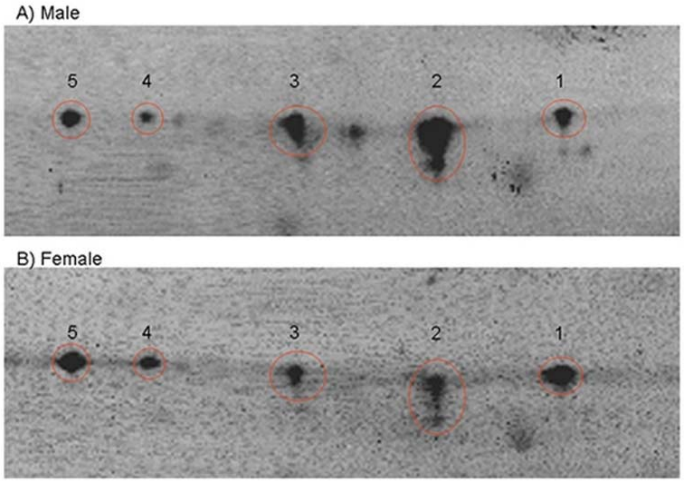
Supplemental Figure I



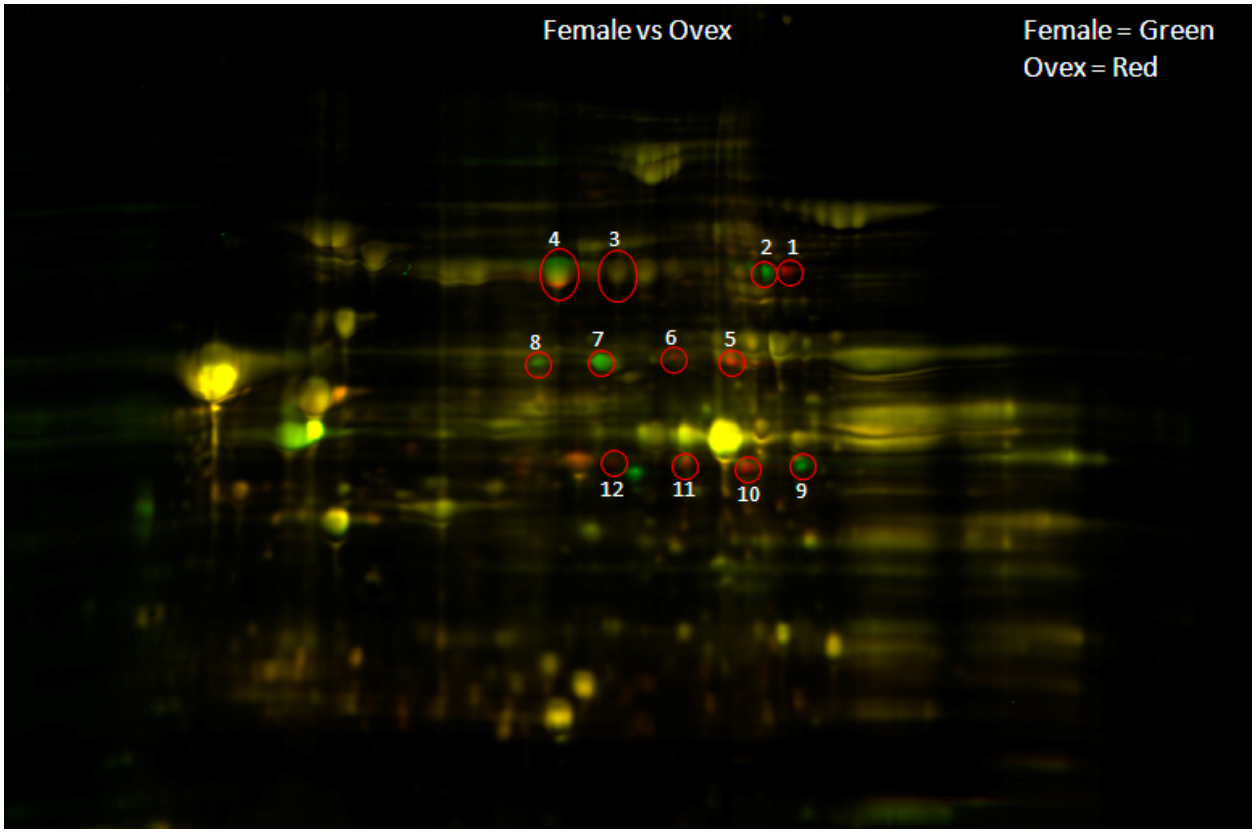
Supplement Figure I:
Evaluation of mitochondrial integrity. A) Western blot for mitochondria and cytosolic markers; B) Mitochondria oxygen consumption; C) Respiratory Control Ratio (RCR).

Supplemental Figure II



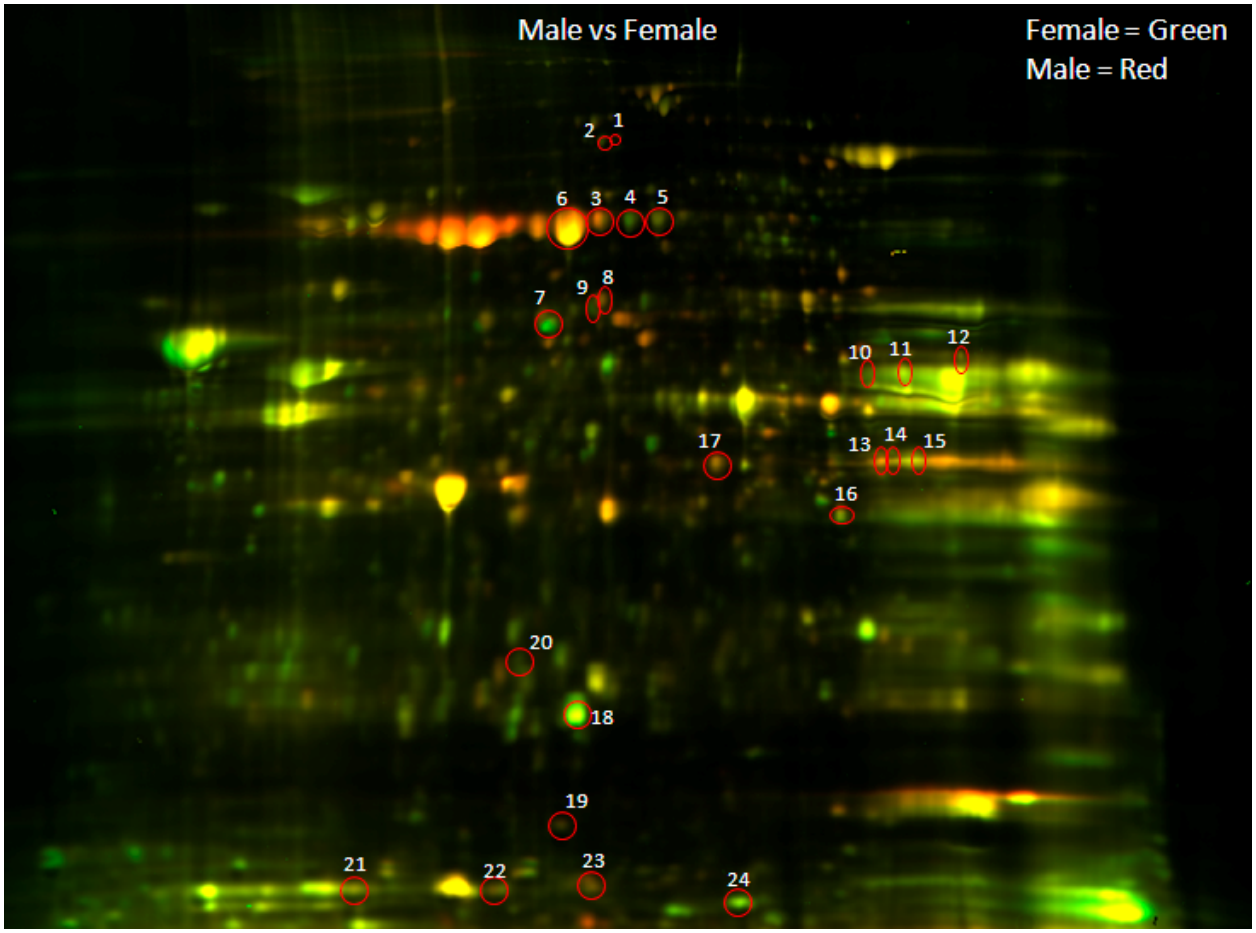
Supplement Figure II:
Mitochondrial phospho-proteomics: In gel phosphorylated proteins using ProQ Diamond staining. Spot numbers 1, 2 and 3 were identified as PDH-E1α. Spot numbers 4 and 5 were identified as branched-chain α-keto acid dehydrogenase E1α.

Supplemental Figure III



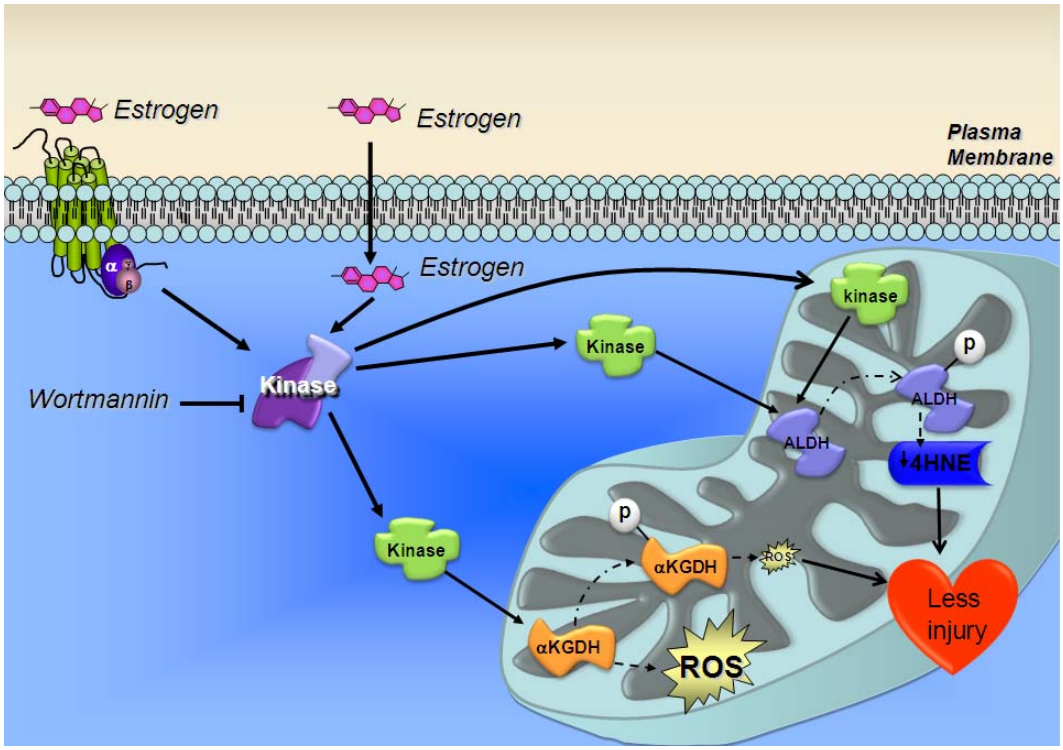
Supplemental Figure III:
2D DIGE of Ovx *vs* Female. Representative overlay of equal amounts of protein between Ovx (Cy5, red) and females (Cy3, green).

Supplemental Figure IV



Supplement Figure IV:
2D DIGE of Male *vs* Female mitochondrial extracts after ischemia. Representative overlay of equal amounts of protein between males (Cy5, red) and females (Cy3, green).

Supplemental Figure V



Supplement Figure V:
Proposed mechanism by which estrogen mediates cardioprotection.

Supplemental Table I

Spot #	*Protein Name*	*MW (Da)*	*Theoretical Protein pI*	*Peptides Count*	*Changes in female*
1	(P35486) Pyruvate dehydrogenase E1α	43888	8.49	4	Increase
2	(P35486) Pyruvate dehydrogenase E1α	43888	8.49	7	Decrease
3	(P35486) Pyruvate dehydrogenase E1α	43888	8.49	3	Decrease
4	(P11960) Branched-chain α-keto acid dehydrogenase	50418	7.68	4	Increase
5	(P11960) Branched-chain α-keto acid dehydrogenase	50418	7.68	4	Increase

Supplement Table I:
Differences in phosphorylation identified by ProQ. Proteins identified with 2 or more different peptides by mass spectrometry using MALDI TOF/TOF. Three animals were in each group.

Supplemental Table II

Spot #	*Protein Name*	*MW (Da)*	*Theoretical Protein pI*	*Peptides Count*	*Changes in female*
1	(P18886) Carnitine O-palmitoyltransferase 2,	74633	6.89	7	Decrease
2	(P18886) Carnitine O-palmitoyltransferase 2,	74633	6.89	6	Increase
3	(Q920L2) Succinate dehydrogenase [ubiquinone] flavoprotein subunit,	72596	6.75	14	Decrease
4	(Q920L2) Succinate dehydrogenase [ubiquinone] flavoprotein subunit,	72596	6.75	3	Increase
5	(P11884) Aldehyde dehydrogenase,	56965	6.63	6	Decrease
6	(P11884) Aldehyde dehydrogenase,	56965	6.63	7	Decrease
7	(P11884) Aldehyde dehydrogenase,	56965	6.63	11	Increase
8	(Q01205) Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex,	49236	8.89	7	Increase
9	(P15651) Short-chain specific acyl-CoA dehydrogenase,	45022	8.47	5	Increase
10	(P15651) Short-chain specific acyl-CoA dehydrogenase,	45022	8.47	10	Decrease
11	(P15651) Short-chain specific acyl-CoA dehydrogenase,	45022	8.47	6	Decrease
12	(P15651) Short-chain specific acyl-CoA dehydrogenase,	45022	8.47	6	Decrease

Supplemental Table II:
Protein differences identified by 2D-DIGE and MS in Ovex *vs* Female. Proteins identified with 2 or more different peptides mass spectrometry using MALDI TOF/TOF. Three animals were in each group.

Supplemental Table III

Spot #	*Protein Name*	*M.W (Da)*	*Theoretical Protein pI*	*Peptides count*	*Changes in female*
1	(Q5XI78) 2-oxoglutarate dehydrogenase E1 component,	117418	6.30	5	Increase
2	(Q5XI78) 2-oxoglutarate dehydrogenase E1 component,	117418	6.30	6	Increase
3	(Q920L2) Succinate dehydrogenase [ubiquinone] flavoprotein subunit,	72596	6.75	7	Increase
4	(Q920L2) Succinate dehydrogenase [ubiquinone] flavoprotein subunit,	72596	6.75	6	Decrease
5	(Q920L2)Succinate dehydrogenase [ubiquinone] flavoprotein subunit,	72596	6.75	7	Increase
6	(Q920L2)Succinate dehydrogenase [ubiquinone] flavoprotein subunit,	72596	6.75	2	Decrease
7	(P11884) Aldehyde dehydrogenase, mitochondrial;	56966	6.63	4	Increase
8	(A0JPQ4) Tripartite motif-containing protein 72;	53767	5.88	5	Increase
9	(A0JPQ4) Tripartite motif-containing protein 72;	53767	5.88	4	Decrease
10	(P09605) Creatine kinase S-type,	47811	8.76	5	Decrease
11	(P09605) Creatine kinase S-type,	47811	8.76	5	Increase
12	(P56574) Isocitrate dehydrogenase [NADP],	51391	8.88	8	Decrease
13	(P04797) Glyceraldehyde-3-phosphate dehydrogenase;	36090	8.14	4	Decrease
14	(P04797) Glyceraldehyde-3-phosphate dehydrogenase;	36090	8.14	3	Decrease
15	(P04797) Glyceraldehyde-3-phosphate dehydrogenase;	36090	8.14	6	Increase
16	(Q9Z2L0) Voltage-dependent anion-selective channel protein 1;	30851	8.62	6	Decrease
17	(P29266) 3-hydroxyisobutyrate dehydrogenase,	35679	8.73	4	Decrease
18	(P31399) ATP synthase subunit d,	18809	6.17	7	Increase
19	(P07632) Superoxide dismutase [Cu-Zn];	16073	5.88	4	Decrease
20	(O35244) Peroxiredoxin-6;	24860	5.64	6	Increase
21	(P07483) Fatty acid-binding protein, heart;	14766	5.90	2	Decrease
22	(P07483) Fatty acid-binding protein, heart;	14766	5.90	2	Decrease
23	(P07483) Fatty acid-binding protein, heart;	14766	5.90	2	Decrease
24	(Q63362) NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5;	13460	6.84	4	Increase

Supplemental Table III:
Protein differences identified by 2D-DIGE in Male *vs* Female after ischemia. Proteins identified with 2 or more different peptides mass spectrometry using MALDI TOF/TOF. Three animals were in each group.