Supplemental Methods:

Swine Model of Myocardial Ischemia and Reperfusion Injury

The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Tufts Medical Center. All experiments were performed according to the committee's guidelines. Animals were pre-medicated with Telazol (0.8 ml/kg, intramuscular). General anesthesia was induced and maintained with isoflurane (1-2%). All animals were intubated and mechanically ventilated (Harvard Apparatus Inc) with room air and supplemented oxygen to maintain physiologic pH and oxygen saturation. Surface electrocardiography leads, an orogastric tube, peripheral 18 G venous catheters and a rectal thermistor were placed in all animals. Heating pads were used as needed to maintain a core body temperature $>$ 99°F Vascular access sheaths were then deployed into the right internal jugular vein (10 Fr), left carotid artery (7 Fr), and both femoral arteries (7 Fr) and veins (10Fr). Unfractionated heparin boluses with a goal activated clotting time of 300-400 seconds, continuous lidocaine infusion (1 mg/kg), and norepinephrine (0.16 mcg/min) were initiated in all animals. A 6Fr Judkins right coronary catheter (Boston Scientific) engaged the left coronary artery via the right femoral artery and baseline angiograms were recorded. A coronary wire was delivered into the LAD and a 3.0 x 8 mm angioplasty balloon (Boston Scientific) was deployed in the mid-LAD after the first diagonal branch with angiographic confirmation of LAD occlusion. Coronary angiography performed immediately after reperfusion and again after the end of the study protocol confirmed patency of the LAD. Following reperfusion, the LAD balloon was left in position for repeat balloon occlusion during Evans-blue counterstaining.

Mitochondrial Isolation

Heart tissue samples were collected from the center of the infarct zone and in a remote non-infarct zone as defined by Evans blue and TTC staining systematically across all cardiac samples. Tissues were minced in a buffer containing 10mmol/L HEPES, 220mmol/L Mannitol, 70mmol/L Sucrose, 1mmol/L EGTA, pH 7.4 and 0.5 mmol/L phenylmethylsulfonyl fluoride freshly added, then homogenized in a Dounce tissue grinder. Debris were spun down at 700g for 10 minutes at 4°C. The supernatant was stored (homogenate 1) and the process was repeated with the pellet (homogenate 2). Homogenates 1 and 2 were pooled and centrifuged at 700g for 10 minutes at 4°C to sediment residual debris. The supernatant was transferred into fresh tubes, and the mitochondria were sedimented at 10,000g for 10 minutes at 4°C. After an additional washing step, the mitochondrial pellet was resuspended in a buffer containing 70 mmol/L sucrose, 220 mmol/L mannitol, 10 mM KH_2PO_4, 5mM $MgCl_2$, 1 mmol/L EGTA, 2 mmol/L HEPES pH 7.4, and mitochondrial protein concentration was determined using the Bradford assay.

Succinate Assay

Succinate accumulation in the homogenate of cardiac tissues isolated from sham and infarct zone was quantified using a Succinate assay kit (ab204718). The assay detects succinate which is utilized by an enzyme Succinyl-CoA Synthetase provided by the kit to form an intermediate that reduces a colorless probe in the kit to colored product with absorbance at OD 450 nm. The detected signal was the measured and analyzed as per the manufacture's protocol.

Mitochondrial membrane potential

Mitochondrial membrane potential was quantified in the freshly isolated mitochondria from infarct and non-infarct zone and was determined using JC-1 dye kit as per manufacture's instruction (T4069, Sigma). The cyanine dye JC-1 a dual-emission potential-sensitive probe was used to determine mitochondrial transmembrane potential. The probe is a green fluorescent (Ex 520 nm) at low membrane potential but higher potentials, the probe forms aggregates changing emission to (Em 596 nm) The ratio of red to green fluorescence of the probe suggests the membrane potential.

Cardiolipin Measurement

The cardiolipin and MLCL content in the mitochondria was quantified in the isolated and frozen mitochondria from the infarct and non-infarct. To extract the lipid 200 µg of mitochondria as slowly pipetted on to a Whatman paper in a spot. The sample spots from the dried filter paper were cut out and placed in a glass vial containing 1:1 mixture of chloroform and methanol. Then vortexed and place on a sonicator bath for 10 minutes at room temperature. The filter paper was removed and 5 µl of internal control was added to each sample. The samples were then dried down under nitrogen gas at 60°C. The samples were reconstituted before LCMS screen for cardiolipin analysis.

Oxygen Consumption Rate

Oxygen consumption rate (OCR) of isolated mitochondria from infarct and non-infarct zone was analyzed in the Seahorse XF96 extracellular flux analyzer (Seahorse Bioscience, Billerica, MA). Isolated mitochondria (600 ng for Complex I substrate) were given into a chilled XF cell culture plate. The plate was centrifuged at 2000g for 20 minutes at 4°C to sediment the mitochondria. The volume of each well was filled up to a final volume of 175 µl per well with mitochondrial assay buffer (70 mmol/L sucrose, 220 mmol/L mannitol, 10 mM KH_2PO_4, 5 mM $MgCl_2$, 1 mmol/L EGTA, 2 mmol/L HEPES pH 7.4). The plate was incubated for 10 minutes at 37°C without CO_2. Oxygen consumption was then analyzed using two well-established Seahorse assays, namely, the Mitostress test and the Electron flow assay. For the Mitostress test the basal oxygen consumption was measured in the presence of Complex I substrate (5.5 mM Malate, 5.5 mM Pyruvate and 2.2 mM ADP) followed by sequential addition of the mitochondrial assay solution (MAS) (control), 25 µl of 45 µmol/L Oligomycin, 10 µmol/L FCCP, 55 µmol/L Antimycin and 55 µmol/L Rotenone. As an additional assessment of electron transport chain function, the electron flow assay was performed and measured basal oxygen consumption in the presence of Complex I substrate (5.5 mM Malate, 5.5 mM Pyruvate and 2.2 mM ADP) followed by sequential addition of 25 µl of 2 µmol/L, Rotenone, 5 mmol/L, Succinate, 55 µmol/L Antimycin, 10 mmol/L N1,N1,N1,N1-tetramethyl-1,4-phenylenediamine (TMPD) with 1 Mmol/L Ascorbate.

Mitochondrial CI Activity Assay

Mitochondrial CI activity in the homogenate of cardiac tissues isolated from infarct zone was quantified using a CI Enzyme Activity assay that measures CI NADH dehydrogenase activity by quantifying the oxidation of NADH to NAD+ and the simultaneous reduction of a provided dye that increases absorbance at 450 nm.

A/D state of Mitochondrial Complex I

To analyze the activated (A) and deactivated (D) state of mitochondrial CI, oxidation of NADH was determined spectrophotometrically as a decrease in absorption at 340nm. To quantify NADH oxidation, 25µg of isolated mitochondria from the infarct and non-infarct zones were treated with

20μM NADH in 10 μl of assay buffer (0.25M sucrose, 50mM Tris HCl, 0.2mM EDTA pH 7.0) for 30 seconds followed by the treatment with an additional 90μl of 165μM NADH and 90μl of assay buffer. To quantify total CI activity, 25μg of isolated mitochondria from infarct and non-infarct zones were treated with 1mM NEM in 10μl of assay buffer, followed by 90μl of additional NADH (165μM) and assay buffer. Absorbance was measured at 340 nm for 30 minutes at 30 second intervals.

Metabolomic screen

Cardiac tissue samples were inventoried and stored at and maintained at -80°C until processed. The metabolites in the cardiac tissue lysates were quantified using Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC MS) at Metabolon. Samples were prepared using the automated MicroLab STAR® system from Hamilton Company. To remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills GenoGrinder 2000) followed by centrifugation. The resulting extract was divided into five fractions: two for analysis by two separate reverse phase (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), one for analysis by RP/UPLC-MS/MS with negative ion mode ESI, one for analysis by HILIC/UPLC-MS/MS with negative ion mode ESI, and one sample was reserved for backup. Samples were placed briefly on a TurboVap® (Zymark) to remove the organic solvent. The sample extracts were stored overnight under nitrogen before preparation for analysis. All sample analysis utilized a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. The sample extract was dried then reconstituted in solvents compatible with the analyzer methods.

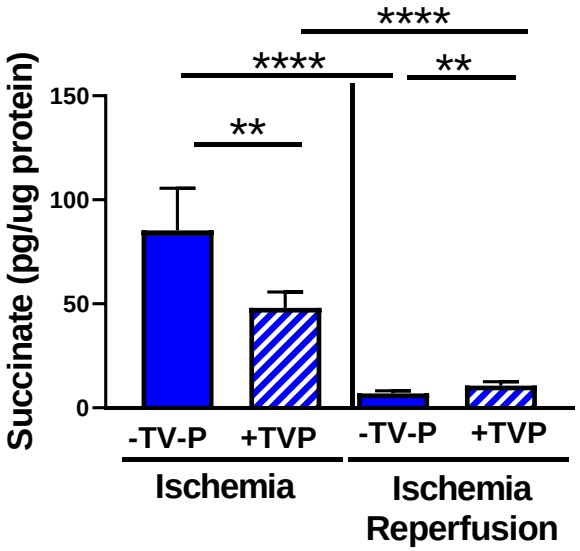
Supplemental Table 1:

		Forward	Reverse
1.	SUS_NDUFA8	5’-AGGCAAAGTTTGATGAGTG	5’-GTAAAGGCCGATCTGTTTTC
2.	SUS_CoX 7C	5’-AGAGTATTCGGAGGTTCAC	5’-AAATGGCAAATTCTTCCCTG
3.	SUS_CoQ3	5’-AAAGAATCAGCTCAGTTGG	5’-TCATGCTACAGGATTTGAAC
4.	SUS_CoQ6	5’-GACCAAATTGTTGATTGGTG	5’-GACCAAATTGTTGATTGGTG
4.	SUS_Gapdh	5’-CACGATGGTGAAGGTCGGAG	5’-TTGACTGTGCCGTGGAACTT

Supplemental Table 2:

		Cat. No	Source
1.	Anti-PHD2	NB100-2219	Novus
2.	Anti-PHD3	NB100-303	Novus
3.	Anti- HIF-1α	NB100-449	Novus
4.	Anti-Rabbit Mab	A6154	Sigma
4.	Anti-Mouse Mab	NXA931	GE healthcare

Supplemental Figure 1. Succinate levels before and after reperfusion with and without trans-valvular pump activation.



Supplemental Figure 2: Heatmaps show significant changes in metabolite levels for glycolysis and fatty acid oxidation from tissue samples obtained from the infarct zone after reperfusion across the four groups. Compared to IR, TV-P metabolite levels are significantly different with a pattern that more closely approximates sham control samples. Values indicate relative levels normalized to total protein.

		SHAM				IR					TV-P					ECMO					p-values IR v TV-P
Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	glucose 6-phosphate	4.7	0.8	0.7	3.58	0.22	0.16	0.062	0.19	0.3	1.1	0.68	0.5	0.5	0.4	0.25	0.28	0.14	0.3	0.13	0.0278
	fructose-6-phosphate	1	2.9	0.6	2.11	0.01	0.01	0.017	0.04	0	1.05	1.03	1.2	1.2	1.1	0.02	0.02	0.04	0.1	0.02	0.0108
	fructose 1,6-diphosphate/glucose 1,6-diphosphate	1.2	0.5	1.7	1	0.04	0.04	0.055	0.32	0.1	1.1	1.04	0.8	0.8	0.4	0.79	0.39	0.4	0.1	0.22	0.03
	3-phosphoglycerate	1.1	1.1	1	1.06	0.06	0.13	0.55	1.41	1.2	2.79	2.4	8.1	3.8	2.9	0.33	0.48	1.2	0.5	0.45	0.0393
	phosphoenolpyruvate (PEP)	2.3	1.1	2.2	1.17	0.1	0.11	1.371	2.52	2.5	0.76	0.38	11	6.6	3.6	0.43	0.64	0.92	0.2	0.49	0.0139
	pyruvate	3.3	0.8	1.4	0.75	0.04	0.06	0.242	0.23	0.1	0.48	0.94	2.8	2.7	1.3	0.1	0.14	0.12	0.7	0.9	0.0005
	lactate	1.4	0.9	2	1.16	0.36	0.36	0.168	0.33	0.2	0.41	0.52	0.8	0.8	0.7	0.4	0.38	0.34	0.5	0.43	0.0000
Fatty Acid Synthesis	malonylcarnitine	0.8	0.6	1.7	1.28	0.19	0.14	0.175	0.16	0.5	0.25	0.65	0.7	0.6	0.8	0.34	0.52	0.67	0.3	0.57	0.0011
	2-hydroxyglutarate	3.3	1	2.3	2.68	0.06	0.12	0.072	0.05	0.4	0.27	0.28	1.5	1.6	1.7	0.09	0.3	0.21	0.6	0.67	0.0001
	2-hydroxyadipate	1.2	0.7	1.2	1.07	0.99	0.73	0.326	0.31	0.8	0.57	0.86	2	1.1	2.1	0.43	0.51	0.44	0.4	0.71	0.0106
	2-methylmalonylcarnitine (C4-DC)	3	1.6	1.5	1.73	0.13	0.11	0.045	0.13	0.3	0.21	0.58	0.4	0.8	0.6	0.1	0.29	0.19	0.1	0.65	0.0002
Fatty Acid Metabolism (Acyl Carnitine)	acetylcarnitine (C2)	0.8	0.9	1.5	0.26	0.01	0.02	0.134	0.26	0.2	0.36	0.34	0.7	0.6	1.1	0.02	0.06	0.99	0.2	0.67	0.0008
	arachidoylcarnitine (C20)*	1.1	0.9	1.8	1.36	0.06	0.08	0.044	0.04	0.2	0.33	0.2	0.6	0.1	0.7	0.08	0.14	0.11	0.1	0.21	0.0044
	behenoylcarnitine (C22)*	7.3	0.7	1.9	1.86	0.43	0.51	0.21	0.31	0.4	1.08	1.03	1.2	1.3	1.5	0.39	0.81	0.6	0.6	0.67	0.059
	eicosenoylcarnitine (C20:1)*	0.4	0.9	2.6	1.06	0.03	0.05	0.027	0.03	0	0.32	0.96	0.6	0.1	0.7	0.06	0.09	0.1	0.1	0.37	0.0172
	erucoylcarnitine (C22:1)*	2.1	1	1.8	1.74	0.09	0.09	0.034	0.05	0.2	0.4	0.34	0.7	0.1	1.1	0.11	0.21	0.15	0.2	0.33	0.0022
	arachidonoylcarnitine (C20:4)	0.3	2.4	8.1	1	0.03	0.08	0.027	0.09	0.2	0.41	0.46	0.9	1.1	1.5	0.07	0.08	0.39	0.4	0.29	0.0043
	docosadienoylcarnitine (C22:2)*	1.1	1.1	2.8	1.35	0.04	0.06	0.018	0.03	0.1	0.59	0.41	0.3	0.1	0.5	0.05	0.06	0.09	0.1	0.16	0.0086
	docosatrienoylcarnitine (C22:3)*	0.4	1.6	3.9	1	0.04	0.07	0.035	0.08	0.1	0.4	0.89	0.4	0.2	0.7	0.09	0.07	0.12	0.1	0.26	0.0315
	3-hydroxydecanoylcarnitine	0.1	1.2	2.5	0.38	0.07	0.06	0.061	0.06	0	0.61	0.61	0.4	0.8	0.8	0.06	0.11	0.21	0.2	0.12	0.0772
	3-hydroxypalmitoylcarnitine	0.2	2.9	5.1	1.57	0.07	0.04	0.033	0.03	0.1	0.87	0.47	0.7	0.3	0.5	0.08	0.07	0.12	0.2	0.13	0.0573
	3-hydroxyoleoylcarnitine	0.1	2.9	4.2	1.59	0.04	0.03	0.029	0.03	0.1	0.78	0.51	1	0.3	0.7	0.07	0.08	0.18	0.2	0.23	0.0275
Carnitine Metabolism	deoxycarnitine	1.1	0.9	2.2	1.35	0.1	0.08	0.049	0.09	0.2	0.2	0.27	0.4	0.4	0.5	0.13	0.21	0.32	0.2	0.32	1.6936E-06
	carnitine	2.5	2	1.6	2.04	0.1	0.22	0.19	0.27	0.4	0.32	0.51	0.7	1	0.8	0.16	0.22	0.65	0.4	0.72	0.0017
Fatty Acid, Monohydroxy	3-hydroxydecanoate	0.9	3.7	4.4	2	0.17	0.38	0.387	0.17	0.5	0.57	0.59	0.7	0.8	0.5	0.48	0.35	0.47	0.2	0.44	0.0059
	3-hydroxylaurate	0.9	8.3	11	2.63	0.27	0.44	0.24	0.21	0.5	0.48	0.59	0.8	0.8	0.7	0.37	0.39	0.72	0.3	0.54	0.0068
	5-hydroxyhexanoate	1.8	1.2	2.3	2.41	0.16	0.16	0.158	0.16	0.2	0.2	0.39	0.3	0.8	0.5	0.16	0.16	0.42	0.2	0.16	0.0294

Supplemental Figure 3: Bar graphs showing A) mitochondrial membrane potential, B) total cardiolipin levels, C) glycerol-3-phosphate levels, D) ratio of monolysl-cardiolipin (MLCL) levels versus total cardiolipin levels in mitochondria isolated from the non-infarct (red) and infarct (blue) zones; and mRNA levels of E) NDUFA8; F) Coenzyme Q3, G) Coenzyme Q6; and H) Cox7c in tissue samples isolated from the non-infarct (red) and infarct (blue) zones after reperfusion.