Supplemental Data

Expanded methods:

Coronary artery ligation (CAL)

Mice were anesthetized with tribromoethanol (125mg/kg i.p.), intubated, ventilated, and a 0.7cm incision made on the left chest through the fourth intercostal space. The pericardium was opened and the left anterior descending coronary artery was either completely tied off at its mid segment with 7/0 prolene to experimentally induce myocardial infarction (MI), or loosely tied, to generate sham animals.

Genotyping

DNA was extracted from tissue using the tissue Extract-N-Amp kit (Sigma Aldrich, USA) per manufacturer's instructions. Primers and PCR conditions used to detect wildtype and floxed leptin receptor alleles were as described by Cohen *et al*[1] and are 5' GTCACCTAGGTTAATGTATTC (forward) and 5' TCTAGCCCTCCAGCACTGGAC (reverse). To detect the excised gene, the listed forward primer was used with the following 'deletion-specific' reverse primer which binds downstream of the 3' lox P sequence: 5' GCAATTCATATCAAAACGCC. PCR cycling conditions consisted of 94ºC for 5 minutes, followed by 40 cycles of 94ºC for 1 minute, 55ºC for 1 minute, and 68ºC for 2 minutes. Using these reaction conditions, PCR products of ~150bp (endogenous leptin receptor) and ~200bp (floxed leptin receptor) will be seen. A deletion product of ~300bp will only be formed if the sequence between lox P sites has been removed and brings the reverse 'deletion-specific' primer close enough to the forward primer to allow for DNA amplification. To detect the cre gene, the following primers were used 5'ATGGATTTCCGTCTCTGGTG and reverse 5' TGCATGATCTCCGGTATTGA. PCR cycling conditions for cre amplification consisted if 94ºC for 5 minutes, followed by 40 cycles of 94ºC for 1 minute, 60ºC for 1 minute, and 72ºC for 1 minute. Under these reaction conditions, a PCR product of ~175bp will be seen. All PCR products were separated on a 2% agarose gel and visualized with ethidium bromide staining under ultraviolet light.

Echocardiography

Under 2% isoflurane, parasternal short axis m-mode images of the murine left ventricle post-CAL/sham surgeries were obtained using a VisualSonics 770 machine with a 25-MHz linear transducer to determine heart rate (HR), end-diastolic dimension (EDD), end-systolic dimension (ESD), anterior and posterior wall thickness, average wall thickness (anterior + posterior wall thickness/2), and % fractional shortening (%FS), which was calculated as %FS=100%*(EDD-ESD)/EDD. These parameters were averaged over 10-20 cardiac cycles.

Pressure volume acquisition and analysis

Mice were anesthetized with 1-2% isoflurane provided in room air via face mask and placed on a warming pad at 37º Celsius. The right carotid artery was dissected and exposed, and a 1.4 french Mikro-Tip conductance catheter was introduced into the artery and advanced into the left vetricle via the aortic valve. Once steady state hemodynamics were obtained, pressure volume loops were recorded using Chart and Scope software v 5.4.2 (AD Instruments, USA) and a MPVS400 system (Millar Instruments, USA). For all animals, parallel conductance was determined in each mouse in triplicate using a 10-12 μl bolus of hypertonic (15%) saline given through a left jugular venous catheter.[2] The blood-filled cuvette calibration method was used to calculate absolute volume data. Data recorded in Chart and Scope software was subsequently analyzed using pressure-volume analysis software (PVAN) v3.6 (Millar Instruments, USA). Between 4-10 loops were selected and analyzed at 5 distinct timepoints throughout steady state (prior to saline boluses) for each mouse, and subsequently averaged to obtain final hemodynamic measurements.

***Ex vivo* cardiac perfusion**

At 1 month post CAL/sham surgeries, mice were administered anesthesia and anticoagulated with 100 units of heparin given subcutaneously. Hearts were quickly excised, aortas cannulated, and retrograde perfusion begun at a constant 37º Celsius and 70-80 mmHg with a modified Krebs-Henseleit bicarbonate (KHB) solution (118.5mM NaCl, 25mM $NaHCO_3$, 4.7mM KCl,1.2mM $MgSO_4$, 1.2mM KH_2PO_4, 2.5mM

$CaCl_2$, 0.5mM EDTA, 11mM glucose and 0.4mM palmitate bound to 3% BSA) containing 0.2 μCi/ml 5-[^{3}H]glucose (specific activity 13.5 Ci/mmol). Oxygen was continuously provided by gassing the solution with 95% O_2:5% CO_2 (pH 7.4). Heart rate was determined by EKG electrodes attached to the spontaneously beating heart. After 5 minutes of stabilization, steady state glycolytic rate was determined by averaging the rate of ^3H_2O production from 5 perfusate samples (collected every 5 minutes) from the beating heart over a 15 second collection period, normalized to dry heart weight. ^{3}H water was separated from ^{3}H glucose by ion exchange as described by Lopaschuk and Barr,[3] and calculations were adjusted for the volume of perfusate collected per unit time to determine glycolytic rate.

Quantitative real time RT-PCR analysis

All real time PCR experiments were repeated at least 3 times to assure reproducibility of results. Real-time RT-PCR was performed using the DNA binding dye SYBR Green[4] and previously validated primers and conditions for the real-time RT-PCR detection of mouse leptin receptor,[5] mouse atrial naturetic peptide (ANP),[6] mouse brain naturetic peptide (BNP),[6] mouse β-myosin heavy chain (βMHC),[6] interleukin (IL)-1β,[7] IL-10,[7] tumor necrosis factor alpha (TNFα),[7] and mouse GAPDH.[5] Specific PCR products were confirmed by demonstrating the presence of a single first derivative melting peak,[8] and by agarose gel electrophoresis.[9] Equal amplification kinetics of the target and the reference genes (GAPDH) were confirmed by serial dilutions as described.[10] Quantification was then performed using the comparative Ct method ($2^{-\Delta\Delta Ct}$).[11]

Western Blotting

All Western Blot experiments were repeated at least 3 times to assure reproducibility of results. Protein was extracted from cardiac tissue in RIPA buffer, subjected to SDS-PAGE, and electro-transferred to PVDF membranes in equal amounts as previously described.[5] To determine molecular weight, a broad molecular weight protein marker was run on all gels (Fermentas USA, cat #SM1841). Antibodies that

were utilized detected leptin receptor (Santa Cruz Biotechnology, USA; cat#8325), Y703-phosphorylated STAT-3 (Cell Signaling Technology, USA; cat #9131), total STAT-3 (Cell Signaling Technology, USA; cat #9132), Thr172-phosphorylated AMPK (Cell Signaling Technology, USA; cat #2531), total AMPK (Cell Signaling Technology, USA; cat #2532) and GAPDH (Research Diagnostics, USA; cat #RDI-TRK5G4-6C5). Chemiluminescent methods were used to visualize antibody deposition on X-ray films which were subsequently digitized using a Visioneer OneTouch Scanner model 9220 and imported into Paperport SE software version 8.0 (Scansoft Inc.). Quantification of bands on x-ray film was performed using Image J software (NIH, USA) with normalization of total protein amounts to GAPDH detected on the same blot. The ratio of the integrated density of the target band to that of GAPDH was obtained and used for comparisons between the various treated and untreated groups, with results expressed as a fold (for increases) or percent (for decreases) change relative to it.

Determination of infarct area and cardiomyocyte area and width

Mouse cardiac tissue was harvested and fixed in 2% paraformaldehyde and cryoprotected in 30% sucrose, as previously described.[5] Short-axis cryotome sections (6 micron) taken at the level of the mid-left ventricle were collected on slides, and stained with hematoxylin and eosin (H&E). A duplicate set of slides were stained with wheat germ agglutinin linked to FITC (1:1000 dilution) for 30 minutes at room temperature (Invitrogen, USA, catalog #W6748) to visualize cell membranes. All wheat germ agglutinin stained sections were further characterized with cy3-linked phalloidin (1:250 dilution) for 30 minutes at room temperature (Invitrogen USA, catalog #R415) to visualize sacromeric actin, and DAPI (Sigma, USA, catalog #32670) to detect nuclei, as previously described.[5] Images were acquired using an Olympus (Melville, USA) Provis AX70 microscope at appropriate magnifications, digitized using a cooled charge-coupled device camera (Optronics Magnifier, USA) at 12-bit grey depth, assembled in Adobe Photoshop 8.0 (Adobe Systems,USA) and imported into Image-J software (NIH) for analysis. Calibration of the software was to a known measured distance at equal objective power. For determination of infarct areas, as well as myocyte width and areas, the "freehand selection" tool in Image J was used to select the region

of interest. Further, for myocyte dimensions, at least 50 measurements were taken per slide for each surviving mouse group member in both long and short axes by a sample-blinded reviewer.

Determination of collagen fractional area

Mouse cardiac tissue was harvested and fixed in 2% paraformaldehyde and cryoprotected in 30% sucrose, as previously described.[5] Short-axis cryotome sections (6 micron) taken at the level of the mid-left ventricle were collected on slides, and stained with picrosirius red F3BA (Polysciences USA, cat #24901C) per manufacturer's instructions. Images were acquired using an Olympus (Melville, USA) Provis AX70 microscope at appropriate magnifications, digitized using a cooled charge-coupled device camera (Optronics Magnifier, USA) at 12-bit grey depth, assembled in Adobe Photoshop 8.0 (Adobe Systems, USA) and imported into Metamorph v7.5 (Molecular Devices, USA) image analysis software. Images were examined using equal threshold settings, and areas calculated using the integrated morphometry analysis feature of the software. Final results are expressed as a mean percent collagen area relative to the total area of imaged tissue.

Gelatin Zymography

All gel zymography experiments were repeated at least 3 times to assure reproducibility of results. Protein extracts used in Western Blotting described above were subjected to SDS-PAGE in equal amounts using 10% gels containing gelatin (Biorad USA; cat#161-1131). To determine molecular weight, a broad molecular weight protein marker was run on all gels (Fermentas USA, cat #SM1841). Purified mouse MMP2 (cat#ab39303) and MMP9 (cat#39309) proteins were purchased from Abcam USA, and run in lanes next to the samples as positive controls. Gels were subsequently placed in a renaturing solution (2.5% triton-X100) for 30 minutes, followed by overnight incubation in a development solution (50mM Tris, 200mM NaCl, 5mM $CaCl_2$, 0.02% Brij-35, pH7.5) at 37ºC. Gels were then stained for 1 hour with shaking at room temperature (in 40% methanol, 10% acetic acid and 0.5% Commassie Blue R-250), and destained for 1 hour with shaking at room temperature (in 40%

methanol and 10% acetic acid). Gels were then imaged using a Visioneer OneTouch Scanner model 9220 and imported into Paperport SE software version 8.0 (Scansoft, USA). Quantification of zymographic bands was performed using Image J software (NIH) and used for comparisons between the various treated and untreated groups, with results expressed as a fold change relative to the oil treated sham ObRKO group which was arbitrarily assigned a value of 1.

Immunofluorescence/TUNEL staining

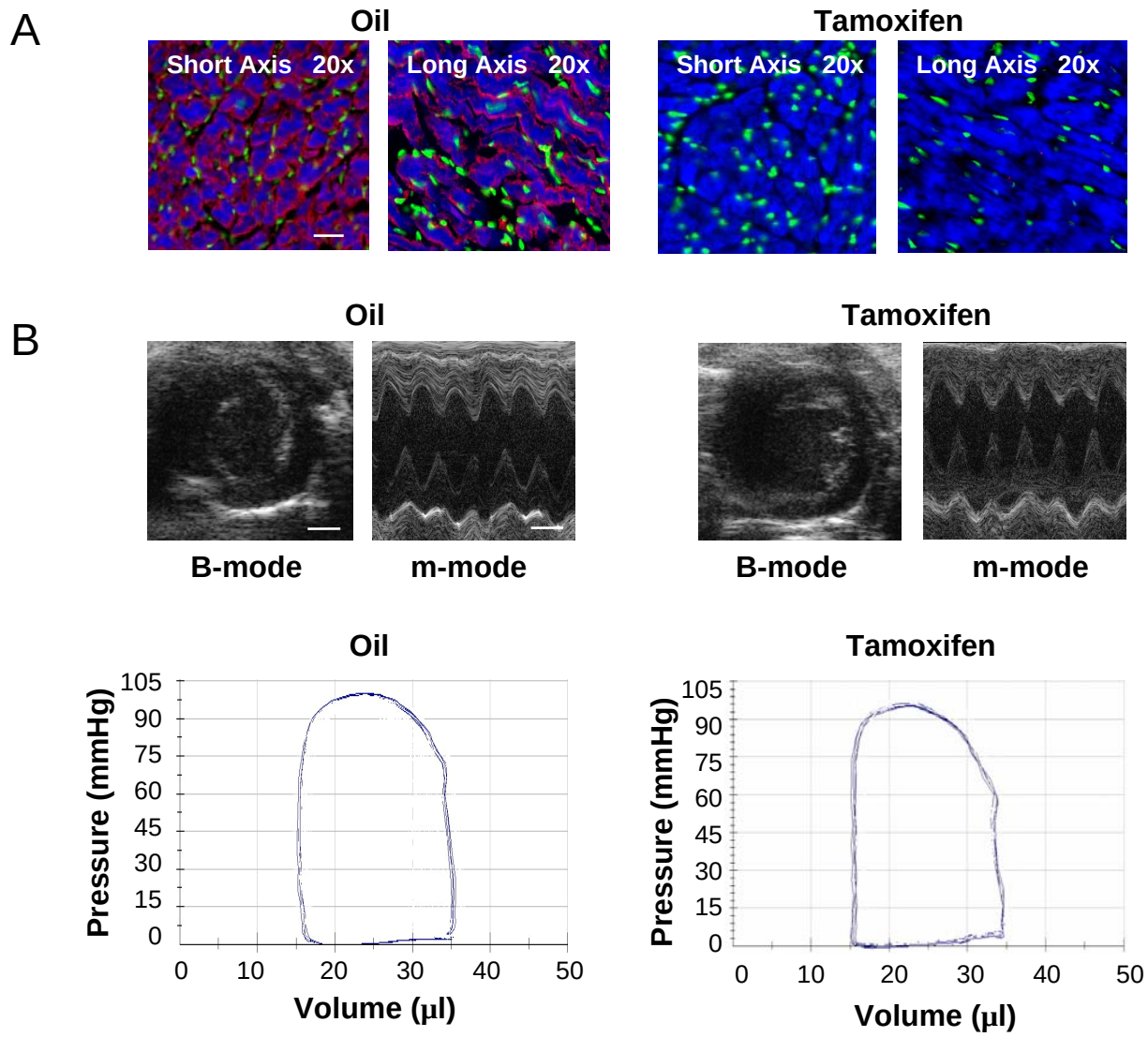
Mouse cardiac tissue was harvested and fixed in 2% paraformaldehyde and cryoprotected in 30% sucrose, as previously described.[5] Short-axis cryotome sections (6 micron) taken at the level of the mid-left ventricle were collected on slides, treated with ice cold acetic acid:ethanol (1:2) for 10 minutes, rinsed in 1x phosphate buffered saline (PBS) pH7.4, and processed using the Apoptag Plus Fluorescein *In Situ* Apoptosis Detection Kit (Chemicon, USA; cat#S7111) per manufacturer's directions. For subsequent CD45 staining, slides were probed with a rat anti-mouse CD45 antibody diluted 1:50 (PharMingen USA; cat#553076), and a cy3 linked goat anti-rat secondary antibody diluted 1:1000 (Invitrogen USA; cat#A10522). For alpha-actinin staining, slides were first blocked using the reagents supplied in a mouse on mouse (MOM) immunodetection kit (Vector Labs USA; cat#BMK-2202), then probed with a mouse anti-mouse alpha-actinin antibody diluted 1:50 (Sigma USA; cat#A7732) and a biotin linked anti-mouse secondary antibody supplied within the kit per manufacturer's directions. Detection was via a rhodamine-avidin tertiary antibody (Vector Labs USA; cat# A-2012) diluted 1:500. All TUNEL, CD45, and alpha-actinin stained sections were further characterized with DAPI staining to detect nuclei as described.[5] Images were acquired using an Olympus (Melville, USA) Provis AX70 fluorescent microscope at 20x magnification, digitized using a cooled charge-coupled device camera (Optronics Magnifier, USA) at 12-bit grey depth, and finally assembled in Adobe Photoshop 8.0 (Adobe Systems, USA). Quantification of CD45 and TUNEL positive cell numbers was performed on at least ten 20x fields per mouse (~3000-4000 cells/field with a minimum of 10 mice in each experimental group) in both infarcted and non-infarcted tissue using the image analysis software Metamorph v7.5 (Molecular Devices, USA). Results are

reported as an apoptotic index or inflammation index, representing the percentage of nuclei that are associated with either TUNEL or CD45 staining, respectively.

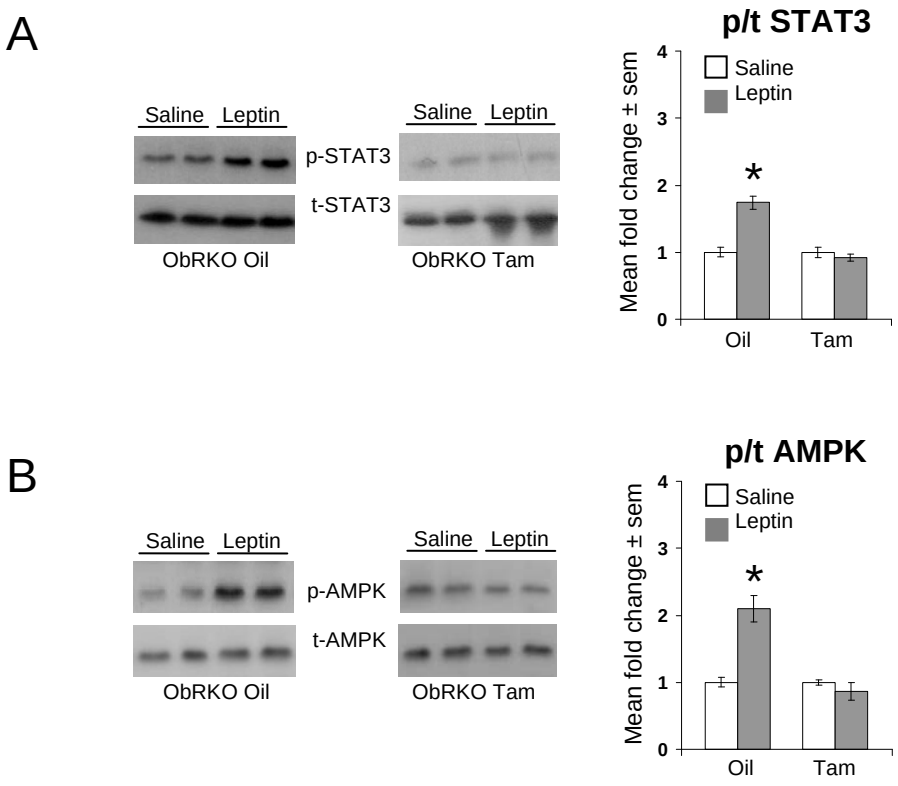
***In vitro* caspase-3 activity assay**

All *in vitro* caspase-3 experiments were repeated at least 3 times to assure reproducibility of results. Caspase-3 activity was determined using the Caspase-3/CPP32 Colorimetric Assay kit (Biovision USA; cat# K106-100). Briefly, protein was extracted from whole heart homogenates in the cell lysis buffer provided by the manufacturer. Equal amounts (100μg) were then incubated with a DEVD-*p*NA substrate at 37º Celsius for 2 hours in a 96 well plate. The plate was then read at 405nm on a Hewlett-Packard SpectraCount plate reader. Activity was then calculated and expressed as a fold change by comparison of optical density readings of various groups after subtraction of background wells from the entire plate.

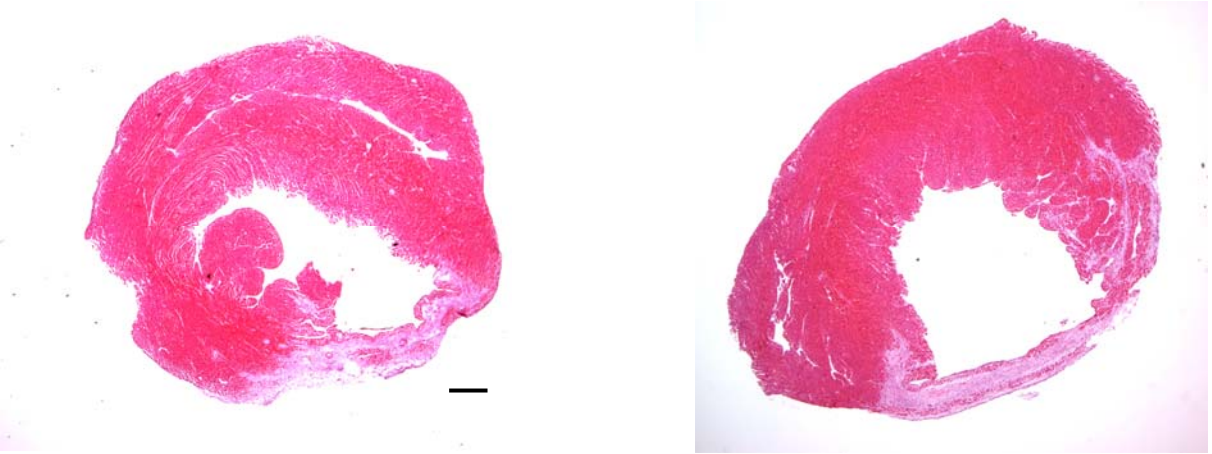
Supplemental Figures



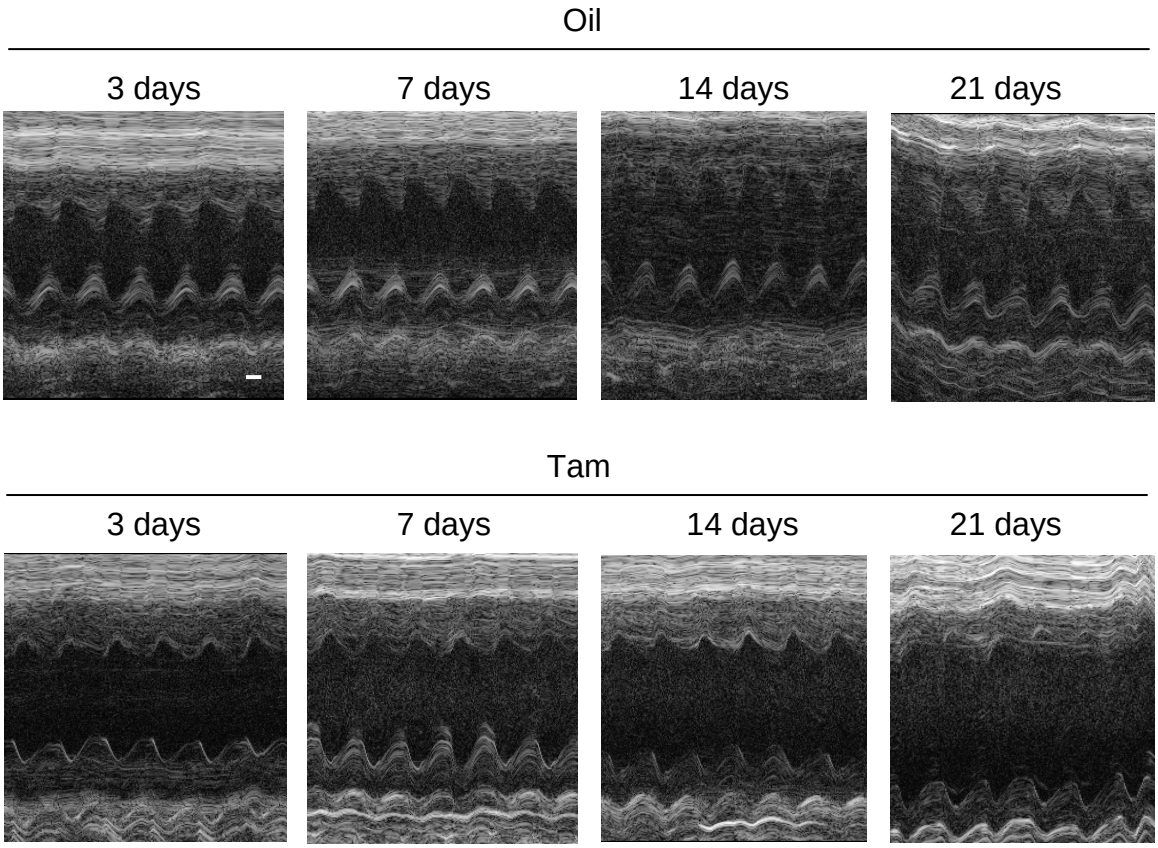
sFigure 1. Cardiac specific ObR deletion and reduction in ObR expression in ObRKO mice after tam treatment does not affect baseline cardiac structure or function at 1 month. *(A)* Representative 20x power immunoflourescent staining of cardiac sections from a oil-treated and tam-induced ObRKO mice showing cells in short (left images) and long (right images) axes. ObR staining is shown in red, nuclei (stained with DAPI) are shown in green, cells containing sarcomeric actin (stained with phalloidin) are shown in blue, and an accompanying scale bar (10µM) is shown in white. *(B)* Representative B and m-mode echocardiographic images with accompanying scale bars (1mm) in white (top panels) and pressure-volume loops (bottom panels) from an oil-treated and tam-induced ObRKO sham mouse.



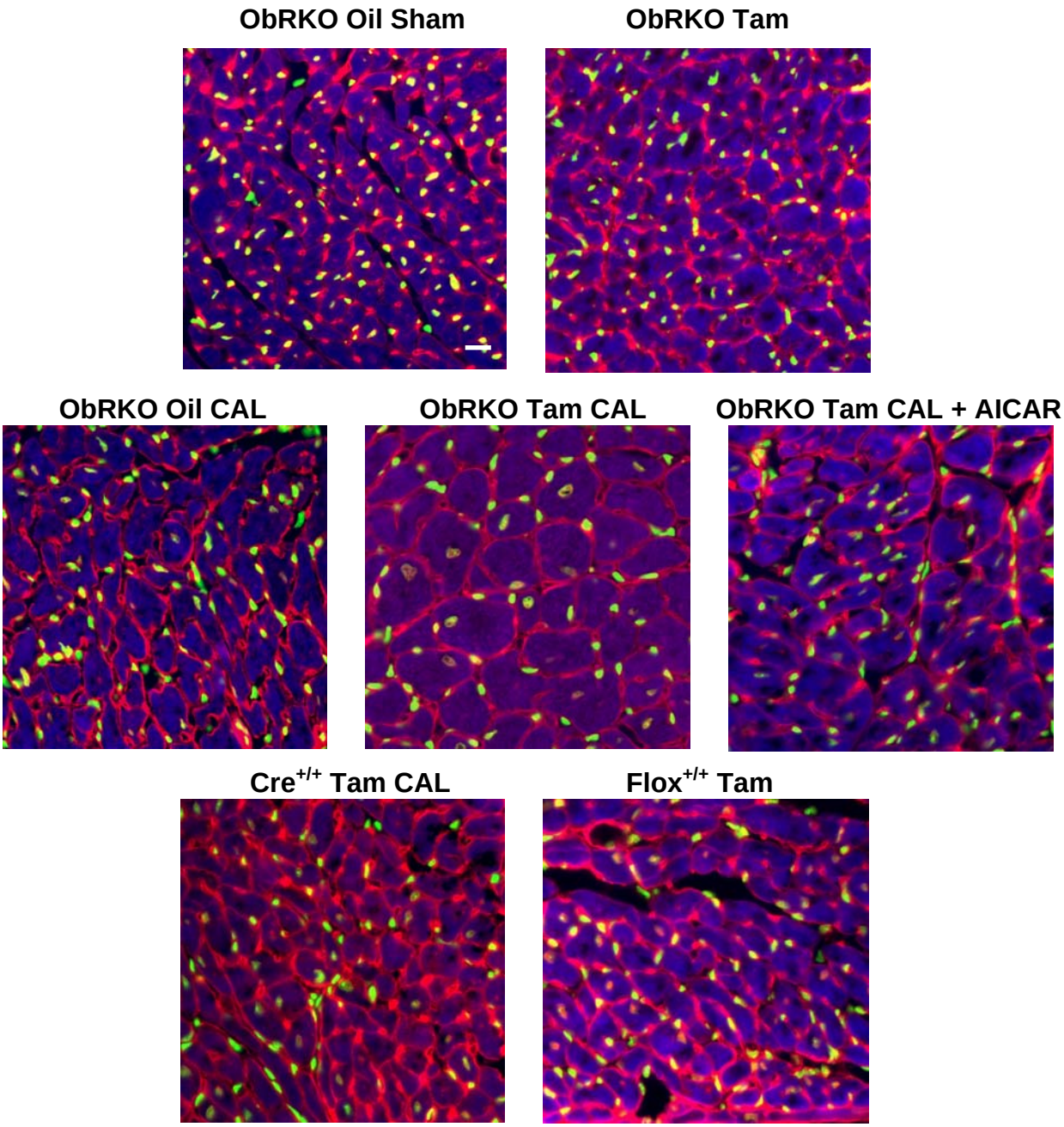
sFigure2. Tam-induced ObRKO mice demonstrate a loss of cardiac STAT3 and AMPK activation by acutely administered exogenous leptin post-MI. *p<0.05 versus saline. *(A)* Mean±sem fold change in cardiac p/t STAT3 in oil-treated and tam-induced ObRKO mice subjected to MI and acutely administered exogenous leptin or saline (right panel), along with representative Western blots showing two samples/group (left panels). *(B)* As in A, except that data for Thr172-p/t AMPK are shown**.**



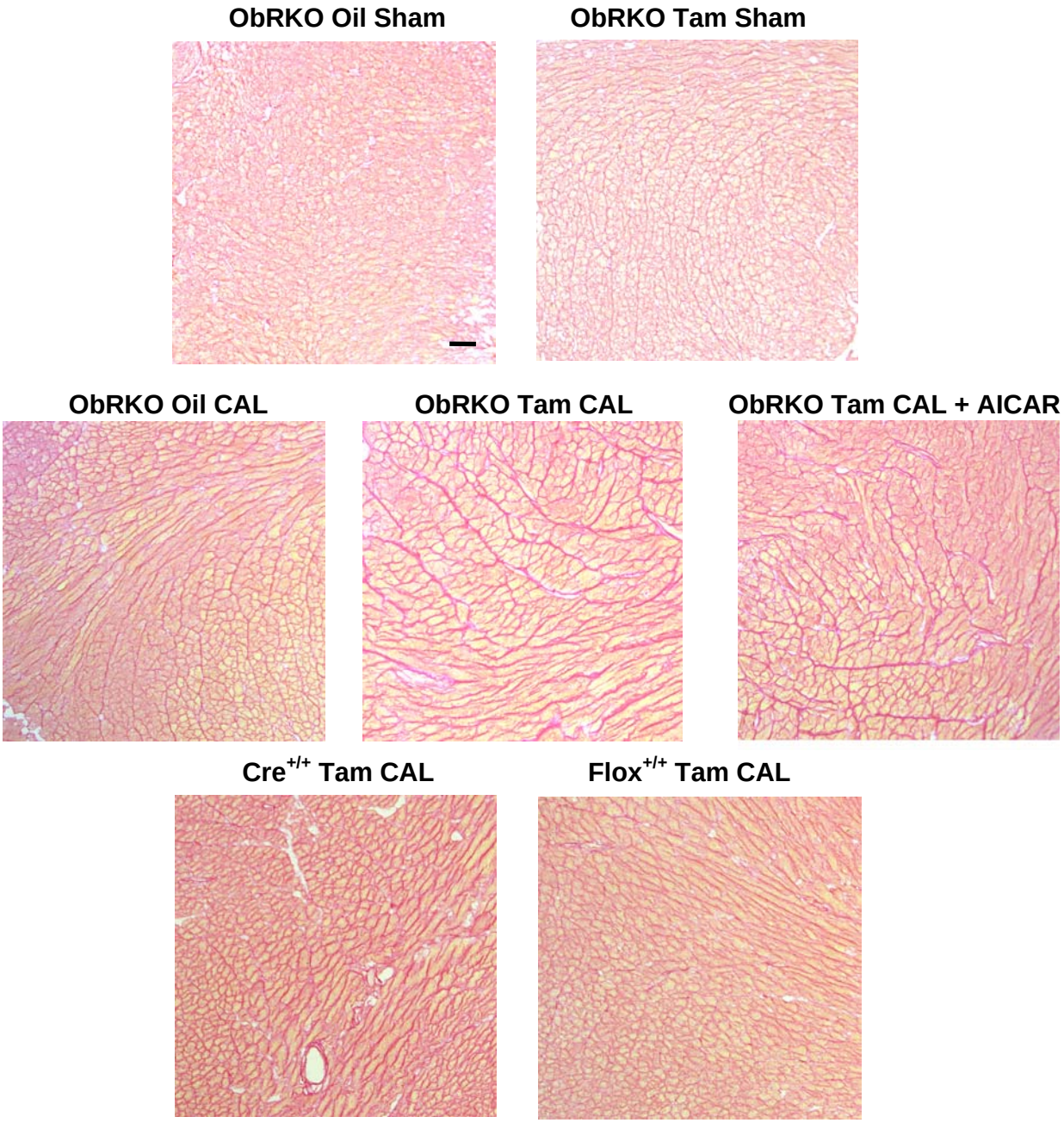
sFigure 3. Representative hematoxylin and eosin stained cardiac sections from an oil (left panel) and tam (right panel) treated ObRKO mouse at 1 month post-coronary artery ligation with scale bar (0.5 mm) shown in black.



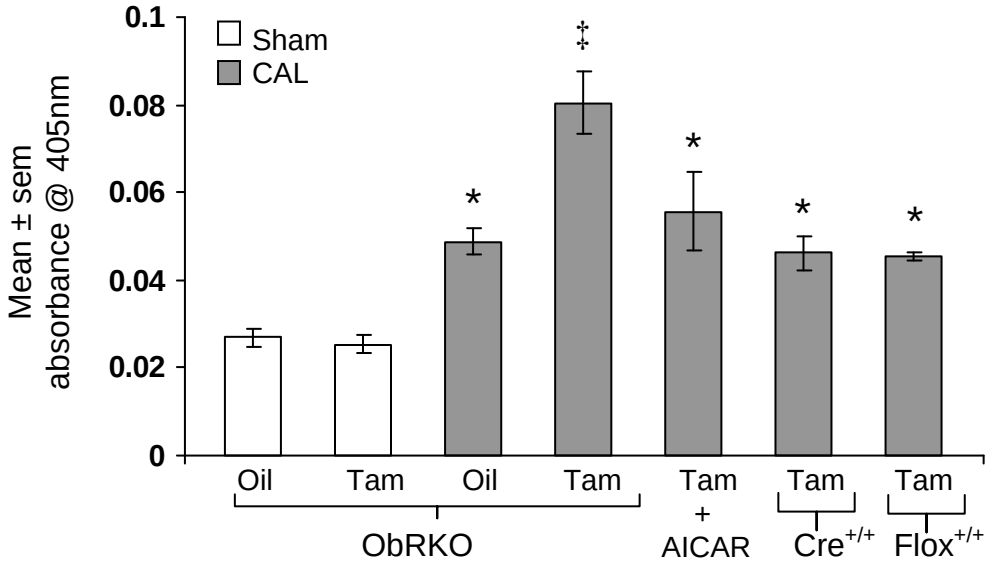
sFigure 4. Representative m-mode echocardographic images from an oil (top panels) and tam (bottom panels) treated ObRKO mouse at 3,7,14, and 21 days post-coronary artery ligation with scale bar (0.5 mm) shown in white.



sFigure 5. Representative 40x power immunoflourescent staining of cardiac sections from sham and CAL ObRKO mice treated with oil, tam, and tam + AICAR, along with tam-treated homozygous (+/+) parental MerCreMer ($Cre^{+/+}$) and floxed ($flox^{+/+}$) control CAL mice. Cardiomyocyte membranes (detected with wheat germ agglutinin) are shown in red, sarcomeric actin (detected with phalloidin) is shown in blue, nuclei (stained with DAPI) are shown in green, and accompanying scale bar (10μM) is shown in white.



sFigure 6. Representative 10x power brightfield images of picrosirius red staining of collagen in cardiac sections from sham and CAL ObRKO mice treated with oil, tam, and tam + AICAR, along with tam-treated homozygous (+/+) MerCreMer ($Cre^{+/+}$) and floxed ($flox^{+/+}$) parental control CAL mice. Scale bar (40µM) is shown in black.



sFigure 7. Tamoxifen induced ObRKO mice demonstrate increased cardiac caspase-3 activity post-MI that is rescued with AICAR. Mean±sem absorbance at 405nm after subtraction of background absorbance. *p<0.05 versus shams; †p<0.05 versus shams and all remote CAL groups, ‡p<0.05 versus all groups.

Group	Time post-surgery (Days)	Fractional Shortening (%)	End Diastolic Dimension (mm)	End Systolic Dimension (mm)	Average Wall Thickness (mm)	Heart Rate (bpm)
ObRKO Oil CAL (n=5)	3	32.0±1.0*	3.59±0.05*	2.44±0.08*	1.01±0.01*	527±8
	7	32.3±1.5*	3.75±0.08*	2.54±0.04*	1.04±0.01*	528±6
	14	27.4±0.4*	4.16±0.02*	3.02±0.02*	1.05±0.02*	514±20
	21	27.6±1.0*	4.42±0.03*	3.20±0.03*	1.07±0.02*	531±7
ObRKO Tam CAL (n=5)	3	25.4±1.2	4.02±0.09	3.00±0.08	1.07±0.01	519±9
	7	24.0±0.6	4.29±0.10	3.26±0.10	1.08±0.01	524±4
	14	23.2±0.9	4.47±0.08	3.43±0.08	1.11±0.01	518±6
	21	20.2±0.7	4.65±0.11	3.71±0.09	1.15±0.01	530±18

sTable 1. Mean±sem echocardiographic measurements on oil-treated and tam-induced ObRKO CAL mice, at 3, 7, 14, and 21 days post-surgery. $^{*}p<0.05$ versus Tam CAL on the same post-operative day.

Group	ANP	BNP	βMHC	IL1β	IL10	TNFα
ObRKO Oil Sham	2.19±0.67	4.50±0.06	16.23±0.53	11.80±0.24	6.59±0.22	15.51±0.20
ObRKO Tam Sham	1.98±0.36	4.41±0.24	16.19±0.81	11.71±0.53	6.54±0.10	15.79±0.84
ObRKO Oil CAL	0.78±0.12*	3.04±0.18*	13.50±0.50*	11.62±0.33	7.30±0.40*	15.09±0.29
ObRKO Tam CAL	-0.24±0.04†	2.52±0.15†	12.24±0.48†	7.96±1.80*†	9.48±1.32†	13.25±0.73*†
ObRKO Tam CAL + AICAR	0.71±0.13*	3.15±0.22*	13.56±.045*	11.72±0.17	7.23±0.17*	16.63±0.33†
MerCreMer$^{+/+}$ Tam CAL	0.74±0.11*	3.02±0.20*	13.40±0.46*	11.90±0.33	7.61±0.32*	15.21±0.32
ObR Floxed$^{+/+}$ Tam CAL	0.69±0.24*	3.10±0.04*	13.45±0.20*	11.64±0.39	7.51±0.29*	15.56±0.48

sTable 2. Mean±sem delta CT values for quantitative PCR data shown in Figure 4A. *$p<0.05$ versus shams; $^{\dagger}p<0.05$ versus all CAL groups.

Supplement References:

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