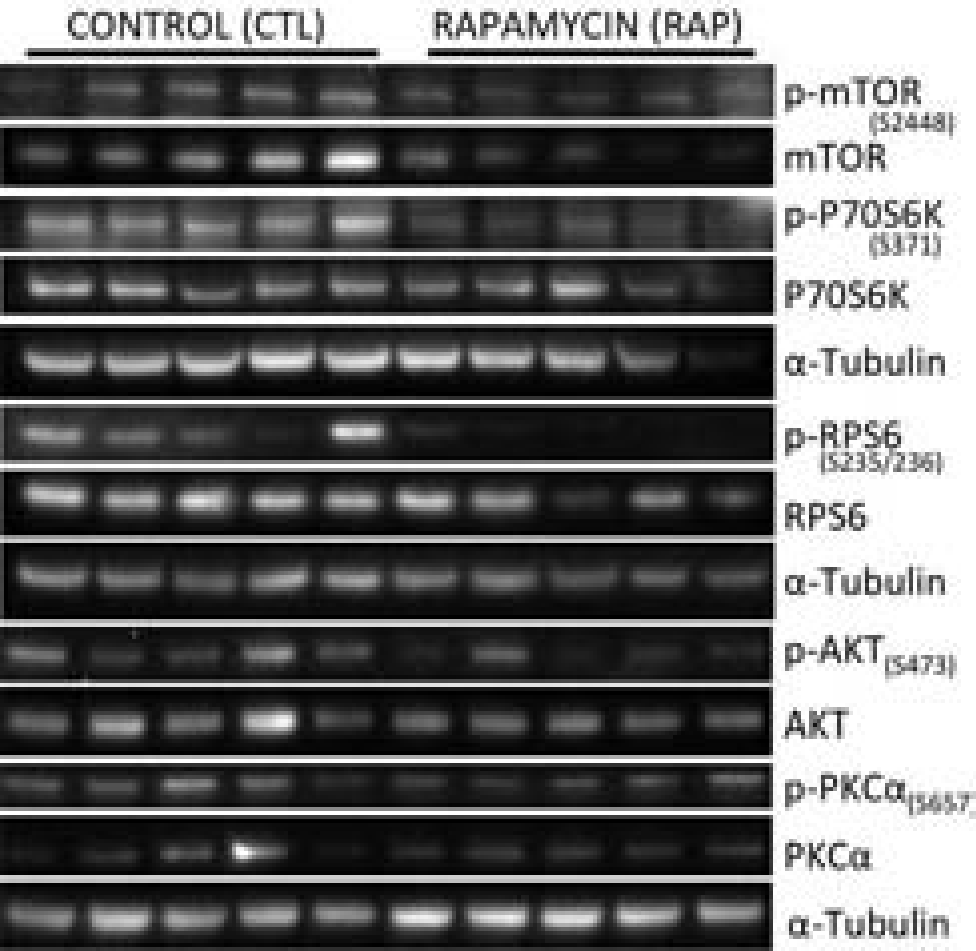
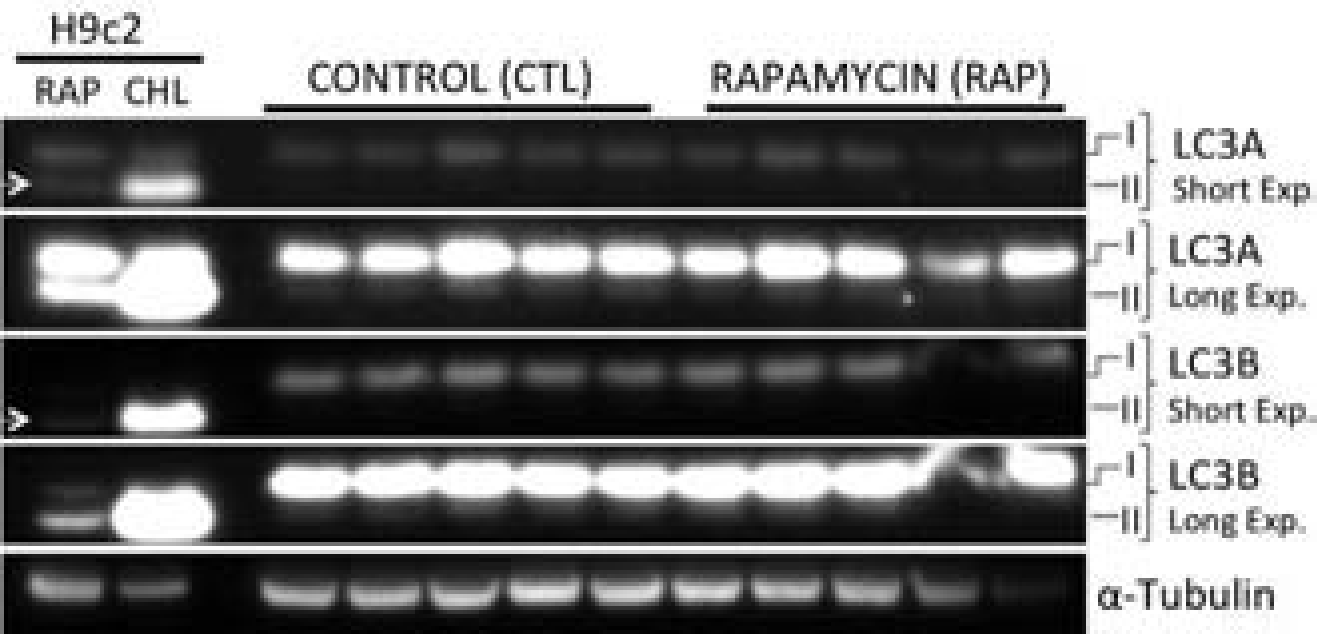
Supplemental Material

Ischemia-Reperfusion Injury Surgical Protocol

After one week receiving either no drug or rapamycin, all animals were sedated with telazol (5 mg/kg IM) before endotracheal intubation and ventilation with a volume-cycled ventilator at 12 - 20 breaths/min. Anesthesia was maintained with a gas mixture of 1.5 - 2.0 L/min of O2 and 0.75 - 3.0% isoflurane. A right external jugular vein cutdown was performed for placement of a 7 Fr triple-lumen central venous catheter. The right common carotid artery was accessed and cannulated with a 5 Fr arterial sheath through the same skin incision. Each animal received a 1000 mL bolus of lactated ringers followed by a continuous infusion of 250 mL/hr. Under fluoroscopic guidance, the pressure-volume catheter was placed into the left ventricle (LV) via the right carotid sheath. At the time of median sternotomy, a phenylephrine drip (0.25 μg/kg/min) was started to prevent isoflurane-induced hypotension, and a heparin bolus of 80 U/kg was administered. The left anterior descending artery (LAD) was occluded 3 mm distal to the origin of the second diagonal branch by a Rummel tourniquet, or approximately one-third of the entire length of the LAD proximal to the apex in cases of atypical variation of the diagonals. After 60 min of ischemia, the tourniquet was released and the myocardium reperfused for 120 min. During the course of the operation, a single lidocaine dose of 1.5 mg/kg IV was given if three or more premature ventricular complexes were occurring over one min. Sustained ventricular tachycardia or fibrillation was managed with 50 J electric cardioversion with internal paddles until a perfusing rhythm was sustained. At the end of reperfusion, the LAD was re-occluded, the ascending aorta cross-clamped, and monastral blue pigment (Engelhard Corp., Louisville, KY) was injected into the aortic root to demarcate the area at risk (AAR). The heart was excised and then sectioned into four, 1 cm-thick axial slices perpendicular to the LAD from the apex to the point of LAD occlusion. Tissue from the second slice distal to the point of occlusion was separated into non-ischemic normal left ventricle (NV) as well as the left ventricular area at risk (AAR), as demarcated by the blue staining. A small, transmural myocardial sample from the AAR immediately adjacent to the NV was submerged in cold Krebs solution and placed on ice for microvessel studies to be conducted on the same day. The remaining tissue from the second circumferential slice was snap frozen in liquid nitrogen for molecular studies of protein expression of the NV. The remaining three axial sections were used for quantification of myocardial infarct size as described below. The surgeons performing the ischemia-reperfusion protocol were blinded from the animal treatment during the operation, and researchers were blinded during tissue processing and analysis.


CONTROL (CTL)
RAPAMYCIN (RAP)
p-mTOR (S2448)
mTOR
p-P70S6K (S371)
P70S6K
α-Tubulin
p-RPS6 (S235/236)
RPS6
α-Tubulin
p-AKT (S473)
AKT
p-PKCα (S657)
PKCα
α-Tubulin


H9c2
RAP CHL
CONTROL (CTL)
RAPAMYCIN (RAP)
I
II
LC3A
Short Exp.
I
II
LC3A
Long Exp.
I
II
LC3B
Short Exp.
I
II
LC3B
Long Exp.
α-Tubulin

Quantification and Analysis of the western blots from supplemental figures

	REMOTE LEFT VENTRICLE Arbitrary light intensity units (mean ± SEM) of 5 animals per group						
SPECIFIC FIRST ANTIBODY	CONTROL			RAPAMYCIN			p
p-mTOR (S2448)	1730	±	208.2	1197	±	90.18	**0.047**
mTOR	1817	±	276	880	±	46.66	**0.010**
p-mTOR / mTOR	0.9984	±	0.1367	1.386	±	0.1611	0.104
p-P70S6K (S371)	1688	±	55.6	1011	±	40.5	**< 0.0001**
P70S6K	1771	±	100.9	1572	±	155.1	0.300
p-P70S6K / P70S6K	9.641	±	0.5538	6.586	±	0.7308	**0.011**
LC3A - I	1462	±	125.5	1371	±	153.6	0.659
LC3A - II	1613	±	75.89	1566	±	67.03	0.651
LC3B - I	2431	±	91.42	2509	±	99.48	0.583
LC3B - II	2274	±	88.22	2333	±	97.4	0.664
p-RPS6 (S235/6)	1085	±	163.7	362.8	±	38.17	**0.003**
RPS6	1153	±	45.59	795.7	±	139.2	**0.041**
p-RPS6/RPS6	0.9471	±	0.1517	0.493	±	0.0621	**0.024**
p-PKCα (S657)	1345	±	130.7	1025	±	48.44	**0.050**
PKCα	1003	±	330.5	799	±	56.39	0.560
p-PKCα/PKCα	1.841	±	0.407	1.31	±	0.112	0.244
p-AKT (S473)	1124	±	111.8	789.5	±	72.59	**0.036**
AKT	969.6	±	132.5	719.5	±	25.19	0.101
p-AKT/AKT	0.6844	±	0.0983	0.6126	±	0.0576	0.546
α-Tubulin	1142	±	67.81	1311	±	56.59	0.091