AMPKα2 deficiency exacerbates pressure-overload induced left ventricular hypertrophy and dysfunction in mice

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Running Title: AMPKα2 regulates pressure overload hypertrophy

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Myocardial AMPK activity: Isoform-specific activities of AMPK were measured as previously described [1]. Left ventricular lysates were immunoprecipitated with specific antibodies against the AMPKα1 or AMPKα2 catalytic subunit and protein A/G beads (Santa Cruz Biotechnologies). Immunoprecipitates were washed in lysis buffer for 4 times and wash buffer (240 mmol/l HEPES and 480 mmol/l NaCl) for 2 times. Kinase reactions were performed in 40 mmol/l HEPES (pH 7.0), 0.2 mmol/l AMP, 80 mmol/l NaCl, 0.8 mmol/l DTT, 5 mmol/l MgCl2, 0.2 mmol/l ATP containing 2 μCi [32P]-ATP (MP Biomedicals), and 0.2 mmol/l synthetic SAMS peptide (Upstate) in a final volume of 40 μl for 20 min at 30°C. At the end of the reaction, a 20-μl aliquot was removed and spotted on Whatman P81 paper. The papers were washed six times in 1% phosphoric acid and one time with acetone. Radioactivity was quantitated with a scintillation counter. Activity was expressed as incorporated ATP (picomoles) per milligram of protein per minute.

Neonatal rat cardiomyocyte (NVM) isolation and culture, cell area determination and protein synthesis. NVM were isolated from 2-day-old Sprague-Dawley rats by enzymatic digestion[2] and separated from non-muscle cells on a discontinuous Percoll gradient according to a modified protocol from Dr. U. Mende [2] with minor modification as previously described [3]. Briefly, viable myocytes were plated in DMEM (2 X 10^5cells/well of 24 well dish) containing 10% FCS, and incubated for 48-72 hrs to allow attachment and spreading. The medium was replaced with serum free media for 24 hours prior to treatment. For adenovirus transduction, cardiomyocytes were infected with 1, 10. or 25 plaque-forming units/cell of purified adenovirus expressing constitutively active AMPK1(CA AMPK) or GFP 24 hours before treatment with phenylephrine. To induce hypertrophy, cells were treated with 50μM phenylephrine (Sigma) for 48 hours. AMPK activators, AICAR (0.2mM) (5-aminoimidazole-4-carboxy-amide-1-d-ribofuranoside)(Sigma), or Metformin (5mM)(sigma) was added 15 minutes prior to treatment with Phenylephrine. After 48 hours treatment, cells were fixed with

4% paraformaldehyde/PBS pH 7.4, permeabilized with 0.1% triton x-100 for 5 minutes, and immunostained for myosin heavy chain(MHC)(Abcam; Cambridge, MA), followed by detection with alexa fluor 555 conjugated anti-mouse (Invitrogen). In some experiments, ANP a hypertrophy marker, was also stained using rabbit anti-anf(Peninsula Biolabs;San Carlos, CA) and Rhodamine-phalloidin(5 units/ml in PBS Ph 7.4; Invitrogen) was used to stain actin. DAPI (300nM) (Invitrogen) was used to stain nucleii. Cell area was analyzed using Image J 1.34s (NIH, USA). At least 100 individual cells were measured per experiment. An additional set of cells were washed once with PBS, then collected for determining p-ACC^{Ser79} and total ACC by Western blot. Cardiomyocyte protein synthesis was measured by [H^3]-Leucine incorporation(2μCi/well during 48 hours treatment) as previously described [3].

Western Blots were performed as previously described [4]. Primary antibodies against ANP, total-Akt, p-Akt^{Ser473}, p-S6$^{Ser235/236}$, total p70S6K and p-p70S6K^{Thr389}, total-elf4e, p-elf4e^{Ser209}, and p-4EBP1^{Thr46} p-AMPK$^{Thr\ 172}$ were purchased from Transduction Laboratories, Santa Cruz Inc, Sigma, Upstate or Cell Signaling Technology, respectively.

References

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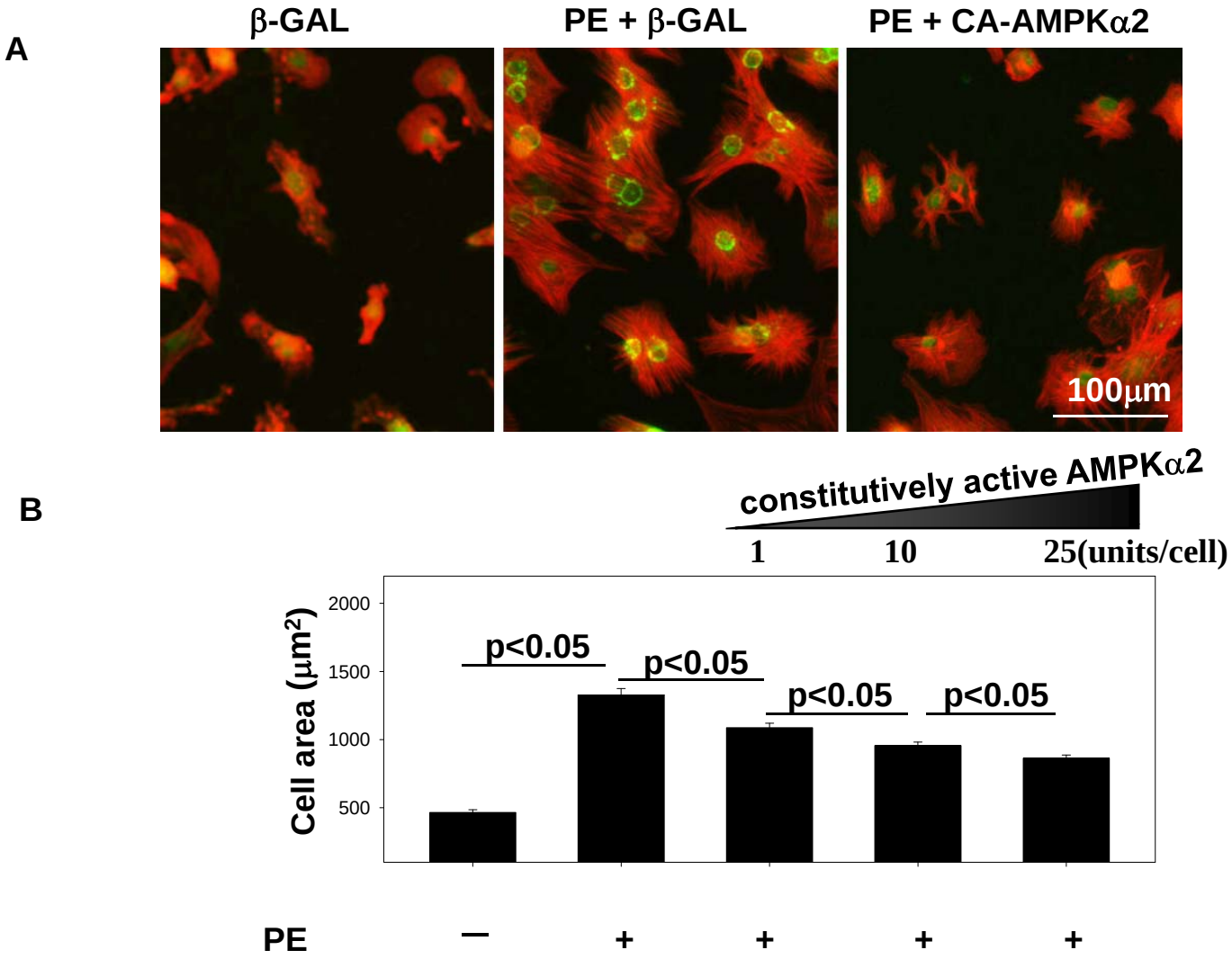

Figure S1. Expression of constitutively active AMPKα2(CA-AMPK) significantly attenuated phenylephrine (PE) induced hypertrophy in cultured rat cardiac myocytes. Red: phalloidin; green: ANP.

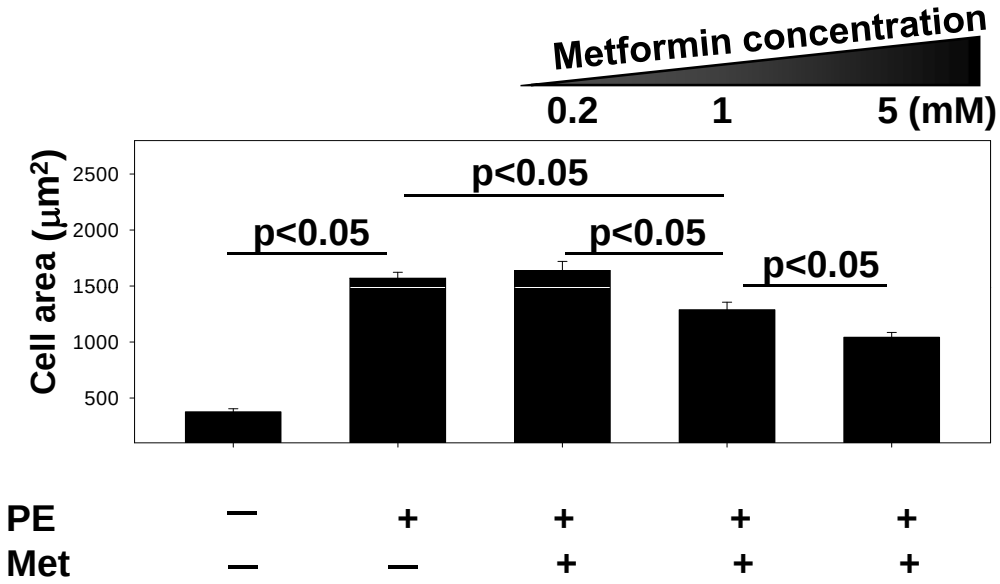

Figure S2. Activation of AMPK by AMP activator metformin dose dependently attenuated phenylephrine (PE, 50 μM) induced hypertrophy in cultured rat cardiac myocytes.

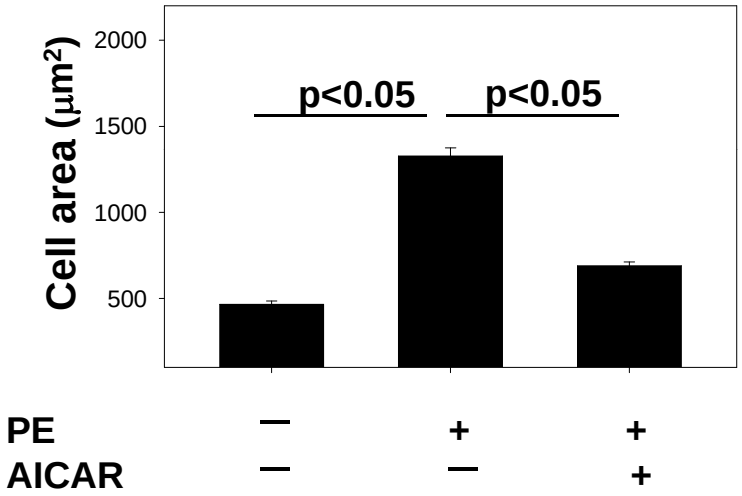

Figure S3. Activation of AMPK by AMP analogue AICAR (0.2 mM) significantly attenuated phenylephrine (PE, 50 μM) induced hypertrophy in cultured rat cardiac myocytes (staining for α-myosin heavy chain).

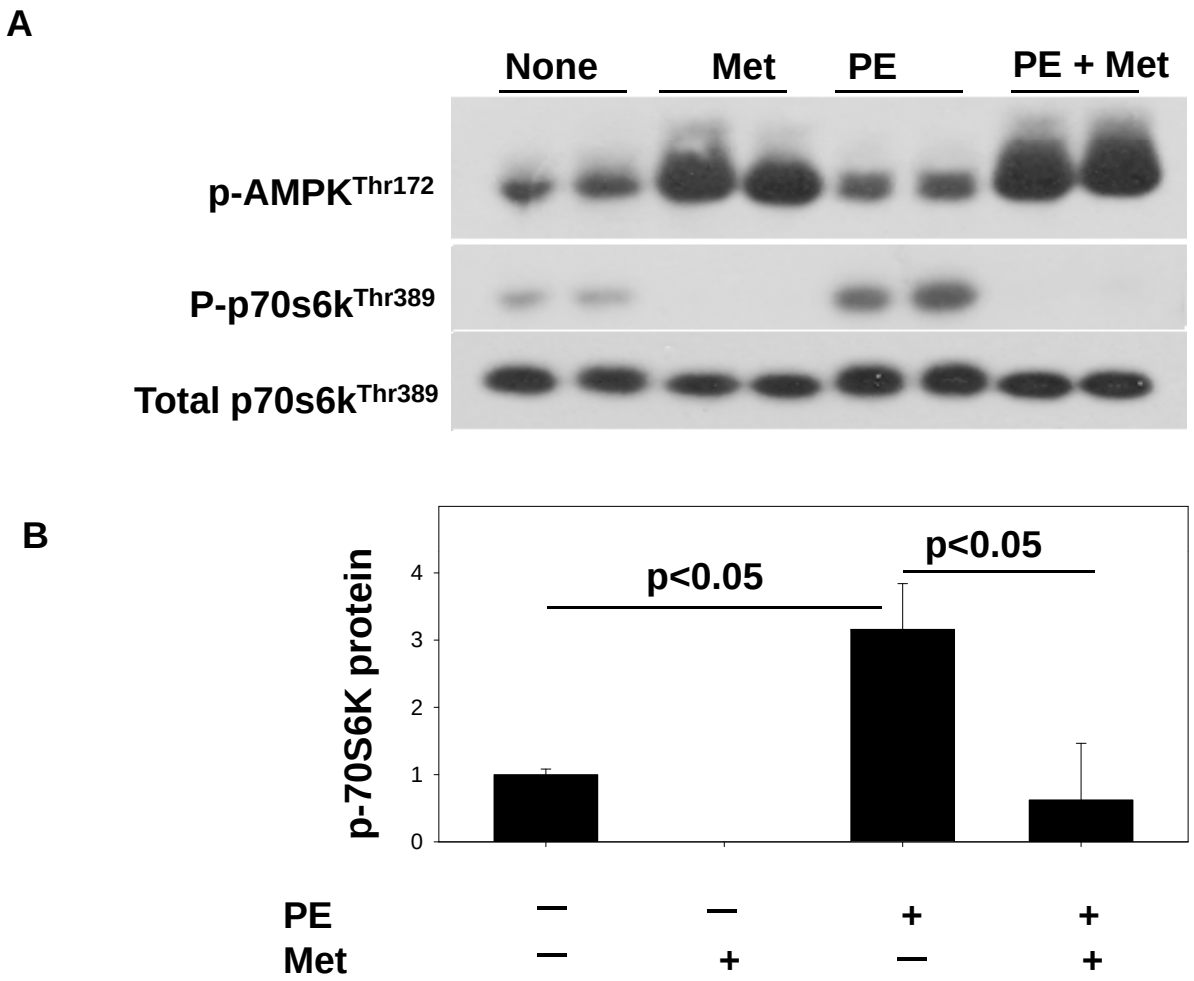

Figure S4. Activation of AMPK by metformin (Met) (5 mM) significantly attenuated phenylephrine (PE, 50 μM) induced increase of p-p70s6k.

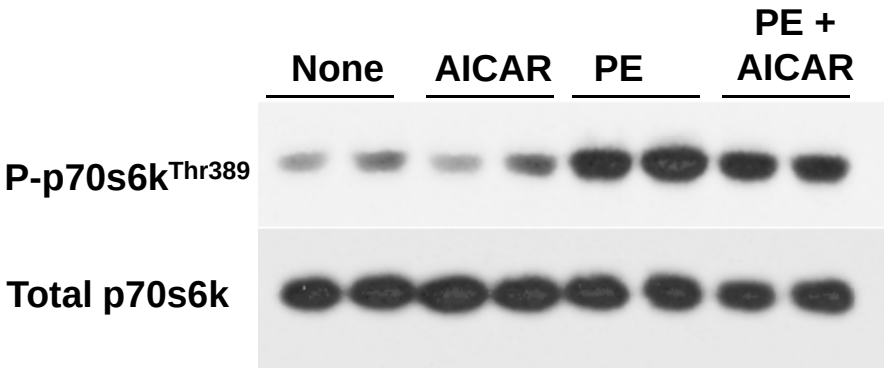

Figure S5. Activation of AMPK by AICAR (0.2 mM) also attenuated phenylephrine (PE, 50 μM) induced increase of p-p70s6k.

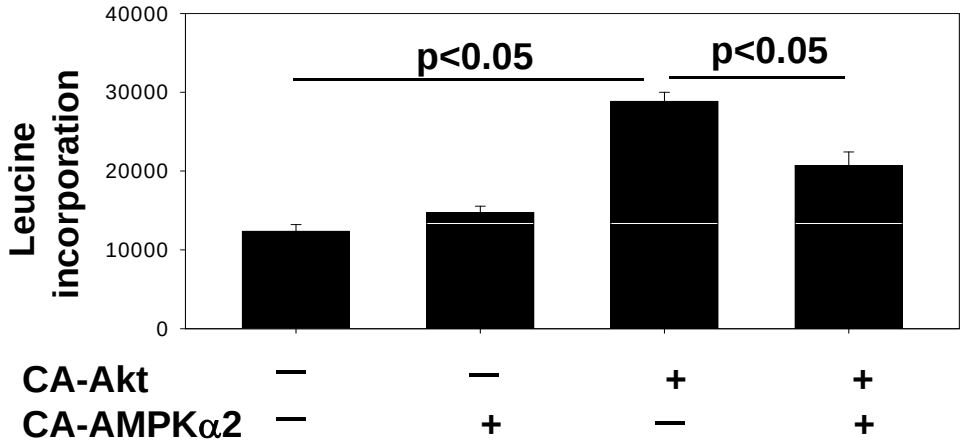

Figure S6. Overexpression of constitutively activate AMPKα2 (CA-AMPK, 5 pfus/cell) attenuated constitutively active Akt (CA-Akt, 5 pfus/cell) induced protein synthesis in cultured neonatal rat myocytes.

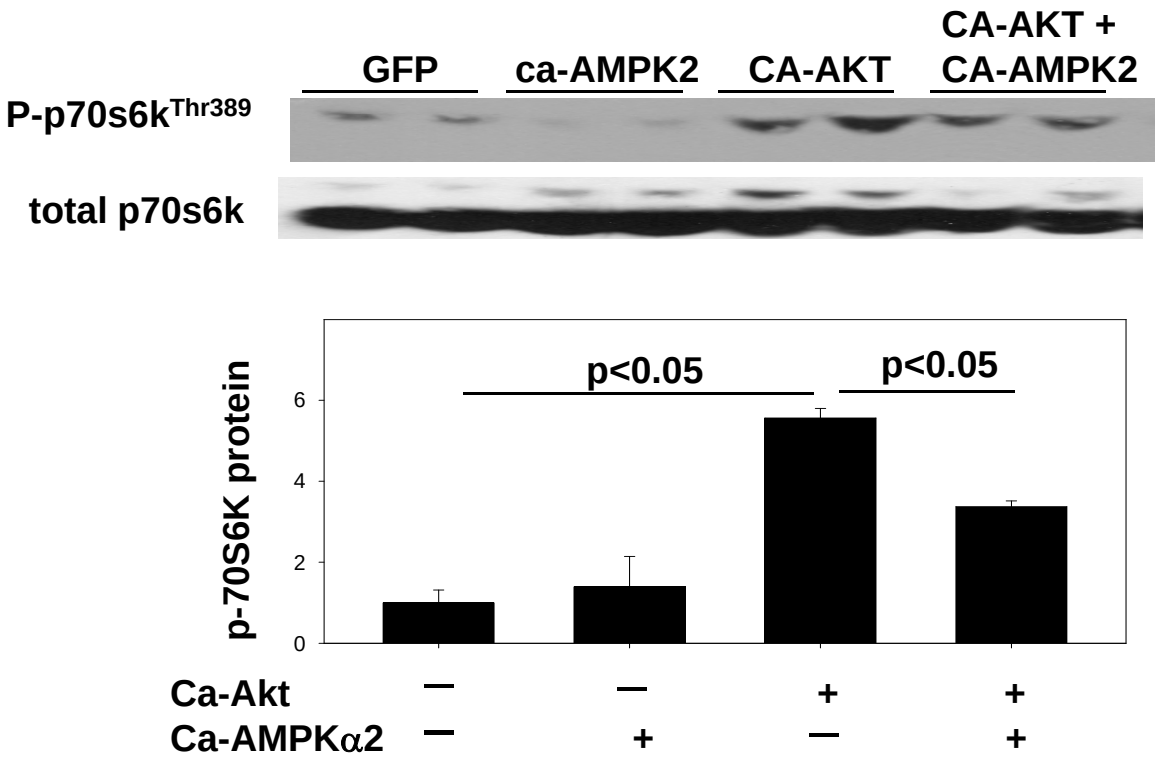

Figure S7. Overexpression of constitutive activated AMPKα2 attenuated CA-Akt-induced increase of p-p70s6k^{Thr389} in cultured neonatal rat myocytes.