Supplemental Materials

Sestrin2 Promotes LKB1-Mediated AMPK Activation in the Ischemic Heart

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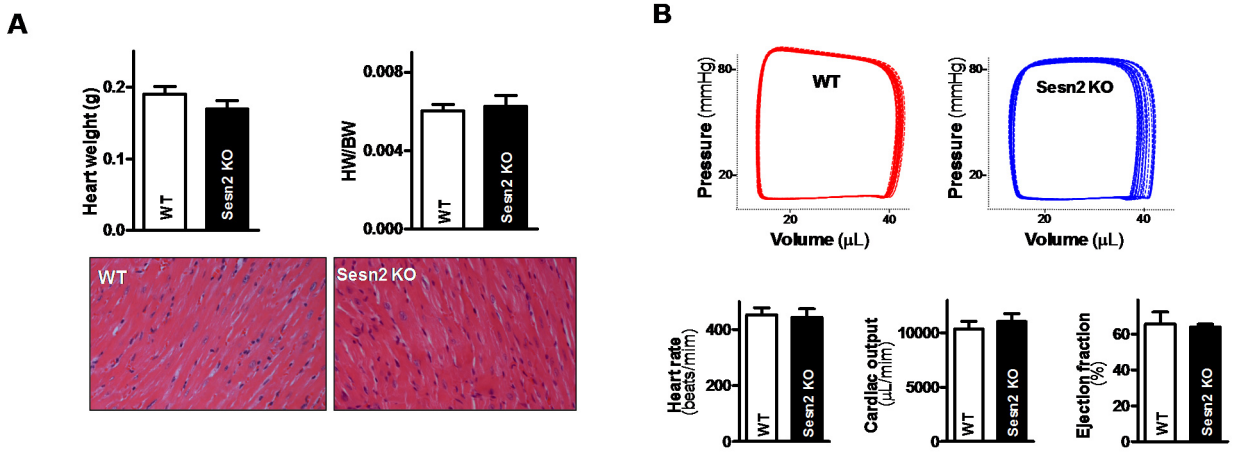
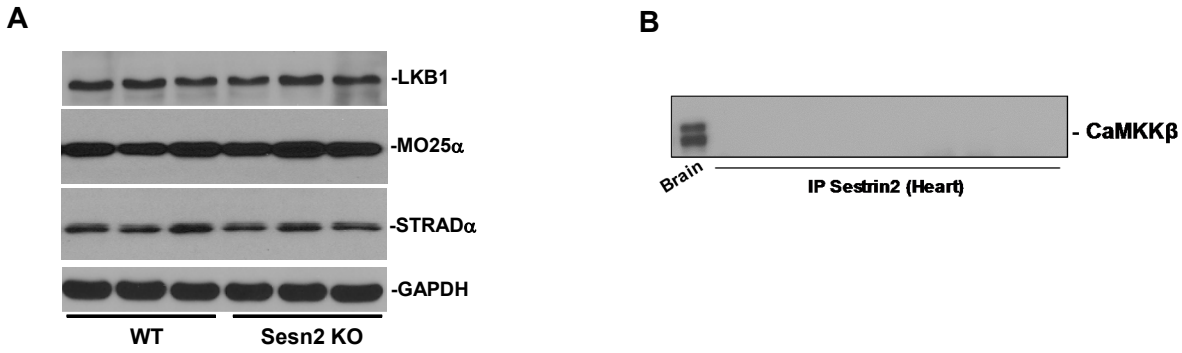
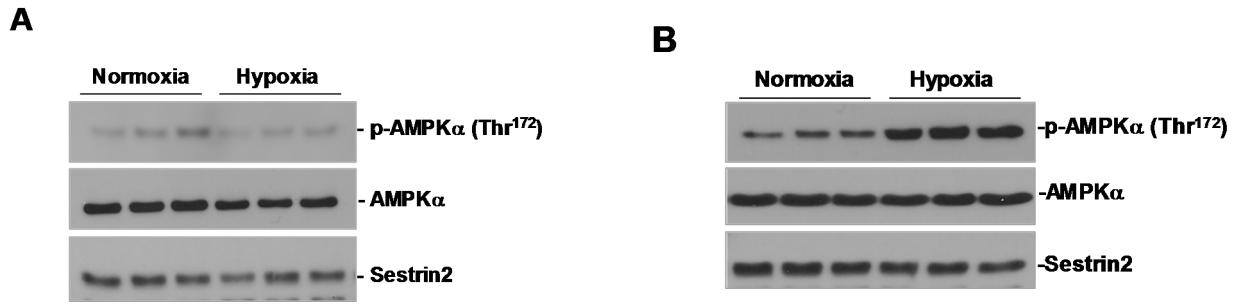


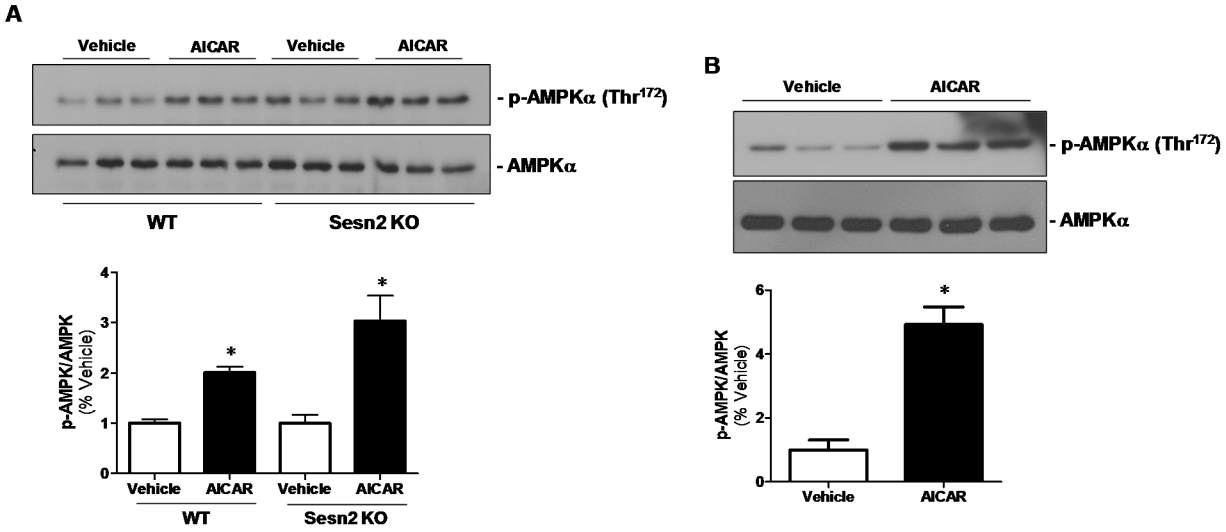
Supplemental Figure 1. Basal Cardiac Morphology and Hemodynamics in WT and Sestrin2 KO (Sesn2 KO) Hearts. (A) Heart weights and heart weight as a percentage of body weight comparing WT and Sestrin2 KO hearts (upper). Light microscopy of H&E stained heart tissue sections demonstrating no apparent structural differences in WT and Sestrin2 KO hearts (lower). (B) *In vivo* pressure-volume relationships of WT and Sestrin2 KO hearts demonstrating similar hemodynamic properties (upper). Heart rate, cardiac output, and ejection fraction measurements from WT and Sestrin2 KO hearts indicate no major differences in cardiac function (lower). n=4-6 per group



Supplemental Figure 2. (A) Immunoblots of LKB1, MO25α, STRADα and GAPDH in WT and Sesn2 KO hearts under basal conditions. (B) Immunoblot of CaMKKβ from Sestrin2 immune-complexes. Brain tissue was used as a positive control for CaMKKβ detection.



Supplemental Figure 3. (A) Immunoblots of p-AMPKα (Thr^{172}), AMPKα, and Sestrin2 in HeLa cells exposed to normoxic (95% air, 5% CO_2) or hypoxic (95% N_2, 5% CO_2) conditions. (B) Immunoblots of p-AMPKα (Thr^{172}), AMPKα, and Sestrin2 in HL-1 cardiomyocytes cells exposed to normoxic or hypoxic conditions.



Supplemental Figure 4. Effects of AMP on Sestrin2-induced AMPK Phosphorylation. (A) Immunoblots of p-AMPKα (Thr^{172}) and AMPKα from cardiomyocytes isolated from WT and Sesn2 KO hearts treated with either vehicle or AICAR (1 mM). Bar graphs show the quantification of AMPK phosphorylation relative to the amount of total AMPKα and is expressed as a percentage of vehicle treatment. (B) Immunoblots of p-AMPKα (Thr^{172}) and AMPKα from HeLa cells treated with either vehicle or AICAR (1 mM). Bar graphs show the quantification of AMPK phosphorylation relative to the amount of total AMPK and is expressed as a percentage of vehicle treatment. Values are means ± SEM; **p*<0.05 *vs.* vehicle; n=3 per group.