Supplemental Figures

MCB 2301-04

Nutrients Suppress PI3-Kinase/Akt Signaling via Raptor-Dependent mTOR-Mediated IRS-1 Phosphorylation

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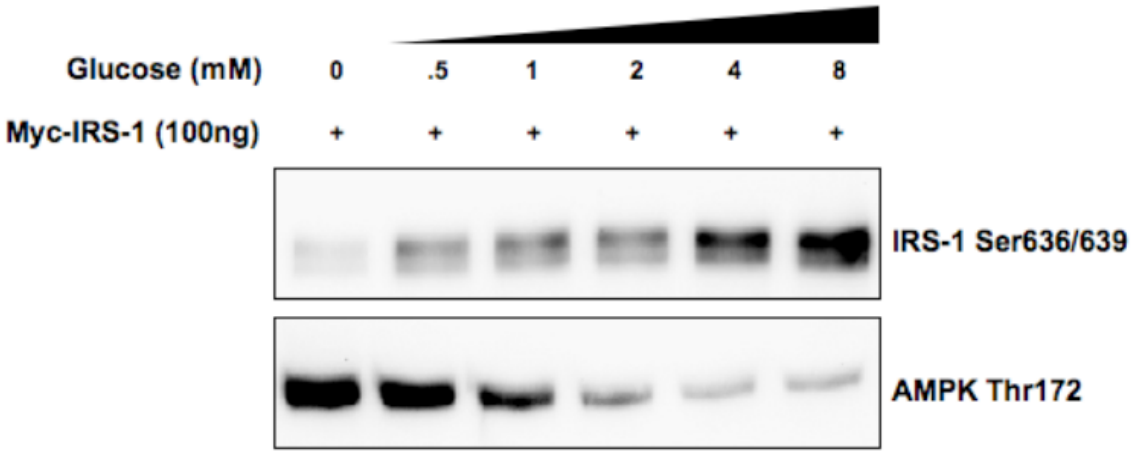

Figure S1. Glucose stimulates phosphorylation of IRS-1 on Ser636/639 in an insulin-independent manner. 293HEK cells were transfected with Myc-IRS-1 and starved for six hours in glucose-free KRP followed by stimulation with increasing concentration of glucose for another 10 min as indicated in the figure. Total cell lysates were analyzed by Western blotting.

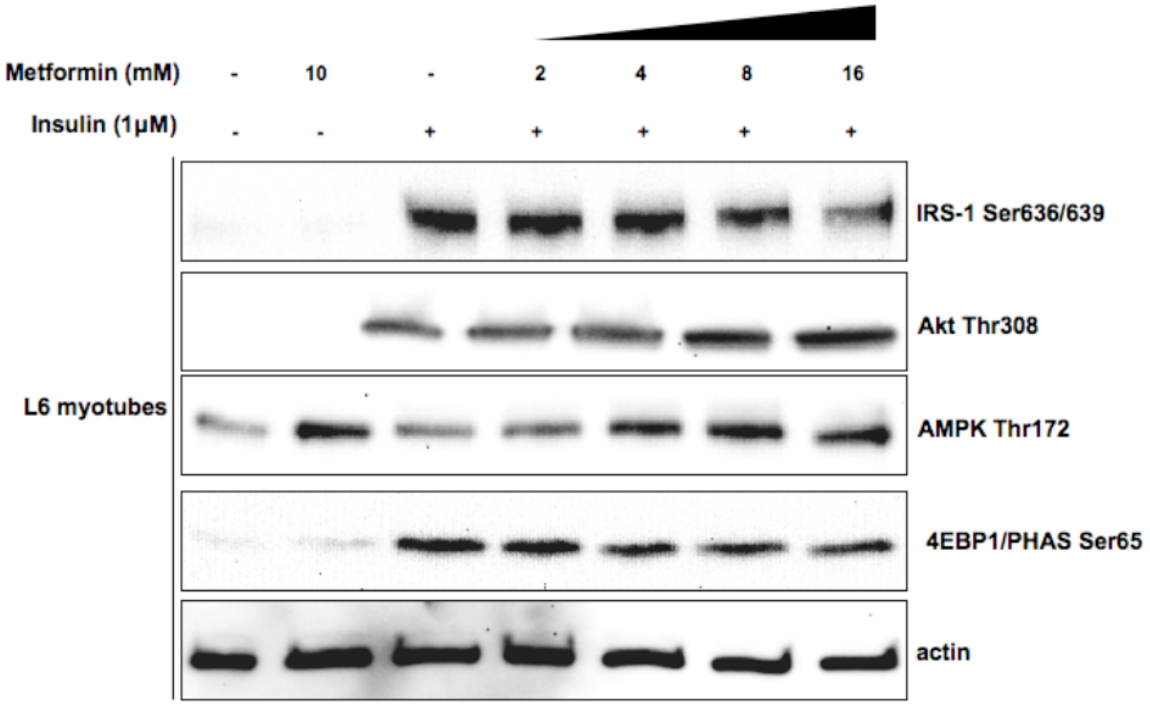

Figure S2. Metformin suppresses mTOR signaling and enhances Akt phosphorylation in L6 myotubes. Cells were serum starved for six hours, pre-treated for 60 minutes with indicated doses of metformin and stimulated with insulin for another 30 min. Total cell lysates were analyzed by Western blotting.

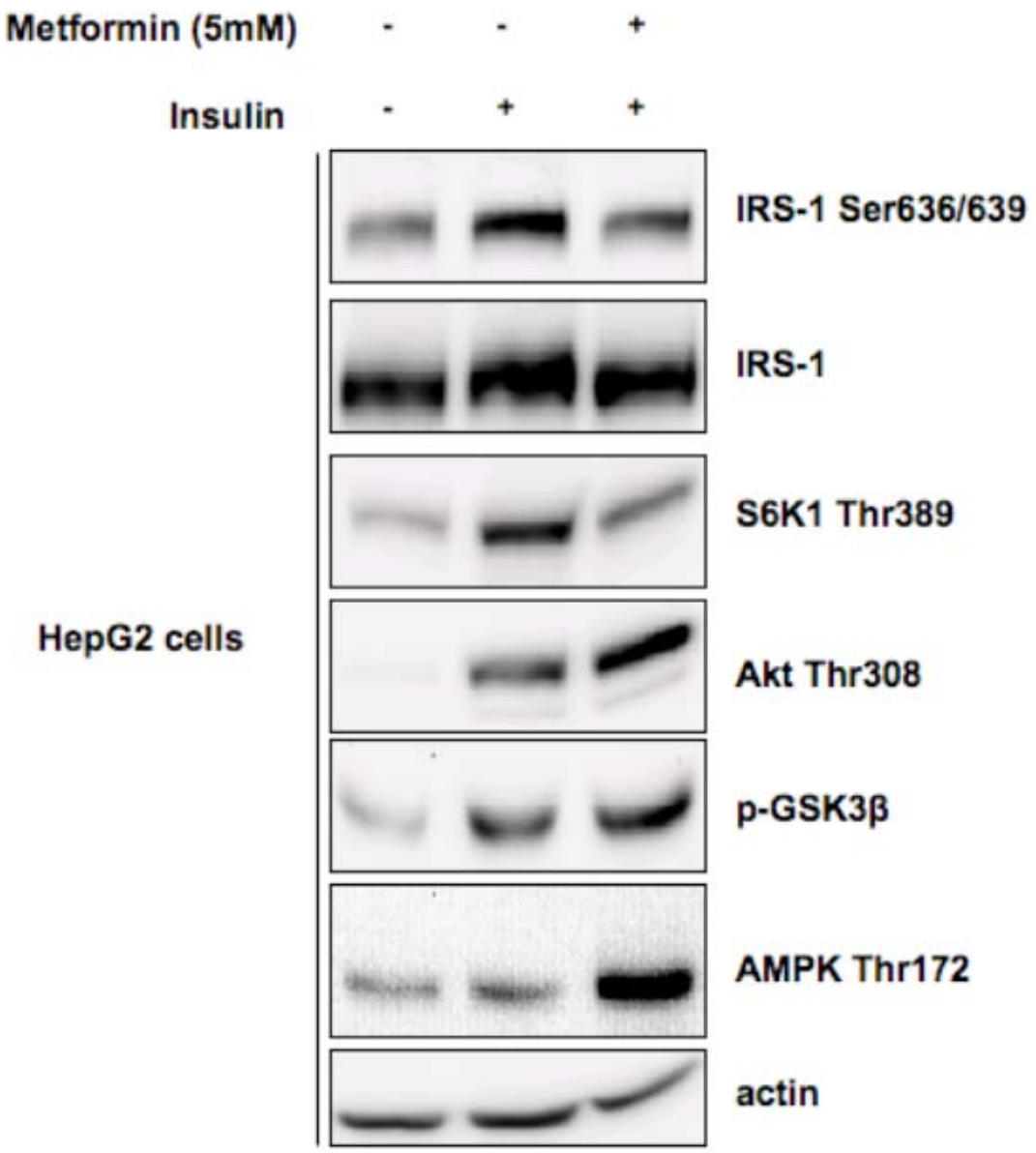

Figure S3. Metformin enhances Akt signaling. HepG2 cells were serum-starved for six hours, pre-treated for 60 minutes with metformin and stimulated with insulin for another 30 min. Total cell lysates were analyzed by Western blotting.

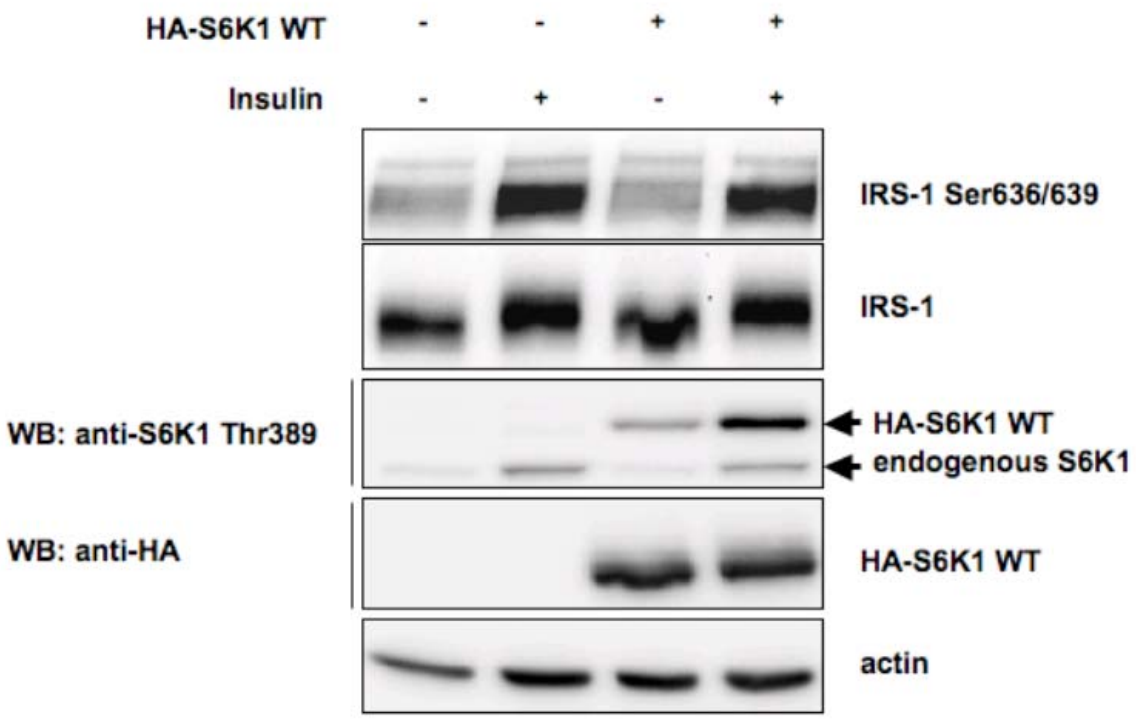

Figure S4. Over-expression of wild type S6K1 does not interfere with basal or insulin stimulated phosphorylation of IRS-1 on Ser636/639. NIH3T3 fibroblasts were transfected as indicated, serum-starved for six hours and stimulated with insulin for 30 min. Total cell lysates were analyzed by Western blotting.

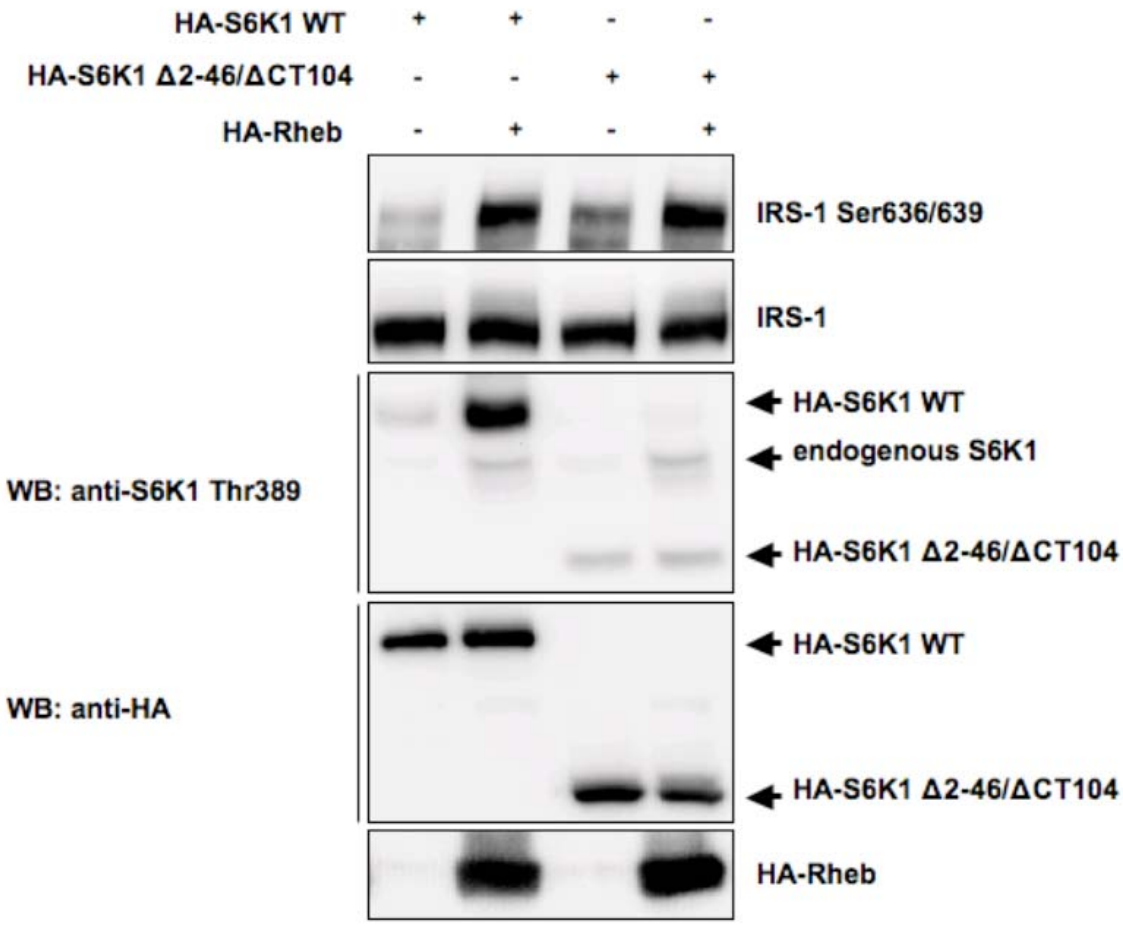

Figure S5. Over-expression of S6K1 Δ2-46/ΔCT104 mutant does not interfere with the ability of Rheb to stimulate phosphorylation of IRS-1 on Ser636/639. 293HEK cells were transfected as indicated in the figure and serum-starved for six hours. Total cell lysates were analyzed by Western blotting.