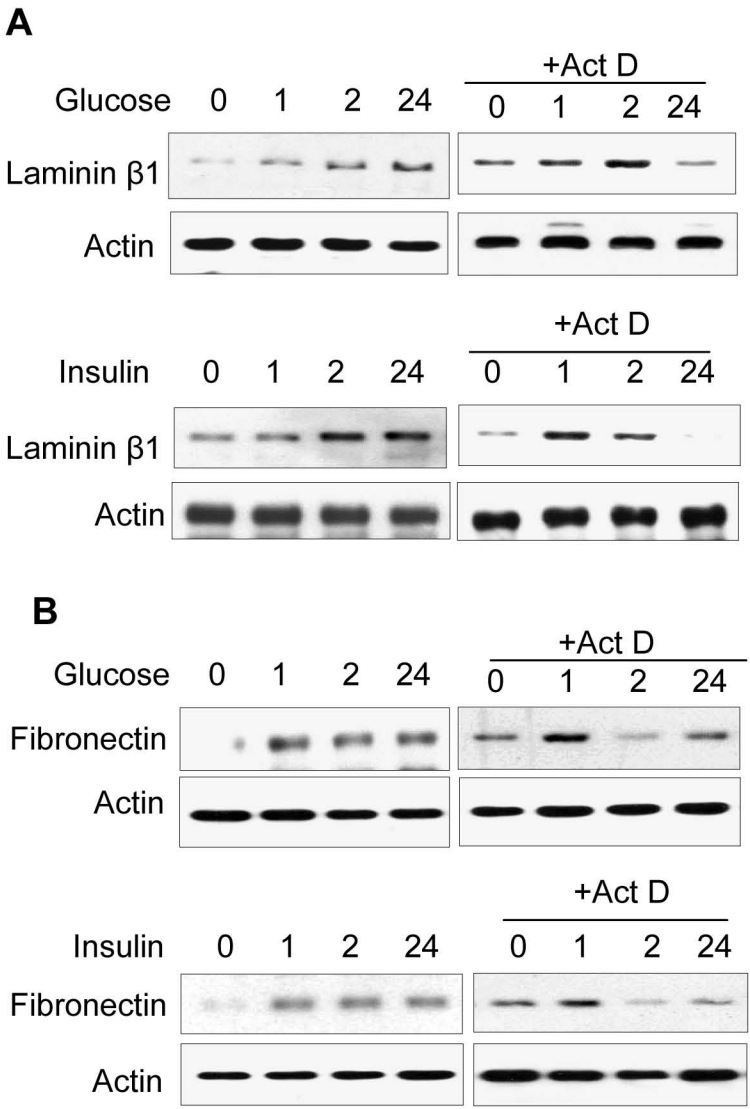
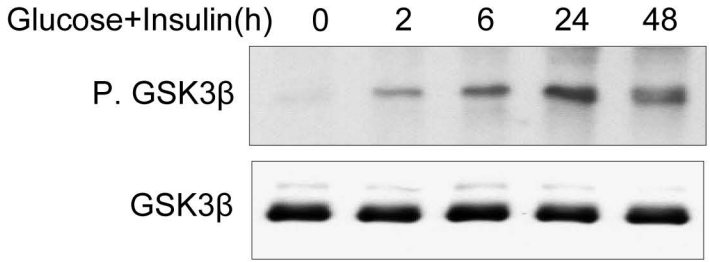
Fig S1.



Supplementary Figure S1. Actinomycin D, a transcriptional inhibitor, blocked Laminin β1 (A) and fibronectin (B) synthesis induced by high glucose and high insulin at 24 and 48 h. Quiescent MCT cells were pre-treated with or without actinomycin D (1microg/ml) prior to incubation with or without high glucose or high insulin for 1h, 24h and 48h. Equal amounts of protein from cell lysates were immunoblotted with laminin β1 (A) and fibronectin (B) antibodies. The lower panels show immunoblots for actin antibody to assess loading.

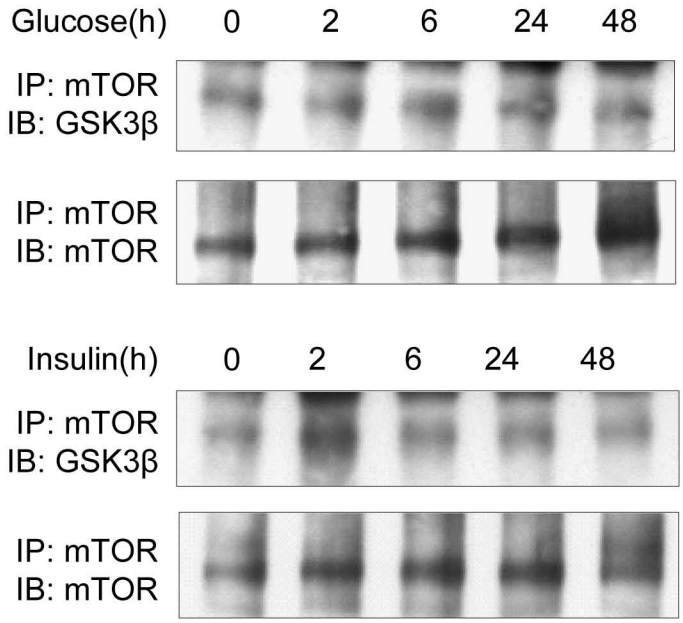
Fig S2.



Supplementary Figure S2. Incubation of MCT cells with high glucose + high insulin stimulates GSK3β phosphorylation.

Equal amounts of protein from lysates of MCT cells treated with high glucose (30mM) and high insulin (1nM) together were subjected to immunoblotting with phospho-specific and total protein antibodies for GSK3β. Representative blots from 3 experiments are shown.

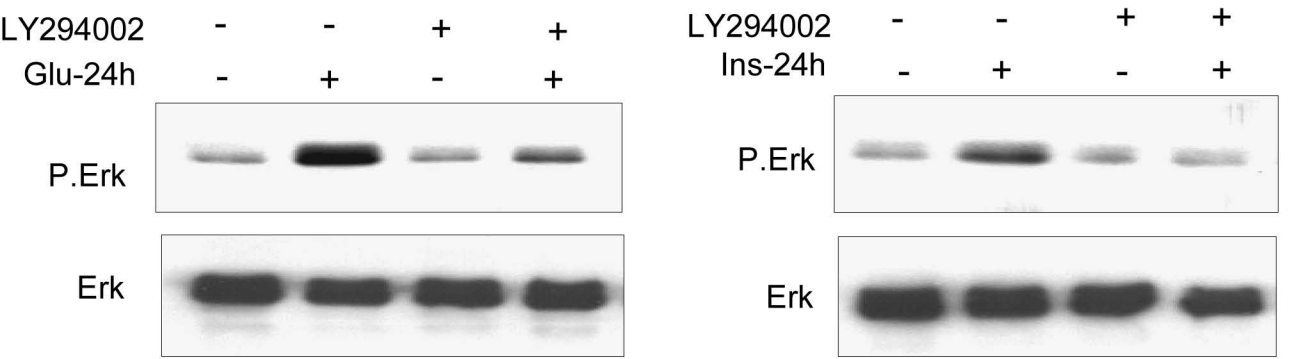
Fig S3.



Supplementary Figure S3. High glucose and high insulin do not alter association of GSK3β with mTOR.

Equal amounts of protein (500 microg) immunoprecipitated (IP) with mTOR antibody were separated by SDS-PAGE and immunoblotted with an anti-GSK3β antibody. Immunoblotting with mTOR antibody was done to assess loading (*bottom*). Representative blots from 2 independent experiments are shown. Note that the two proteins exist in a complex at basal state; the intensity of association did not change with incubation with high glucose or high insulin.

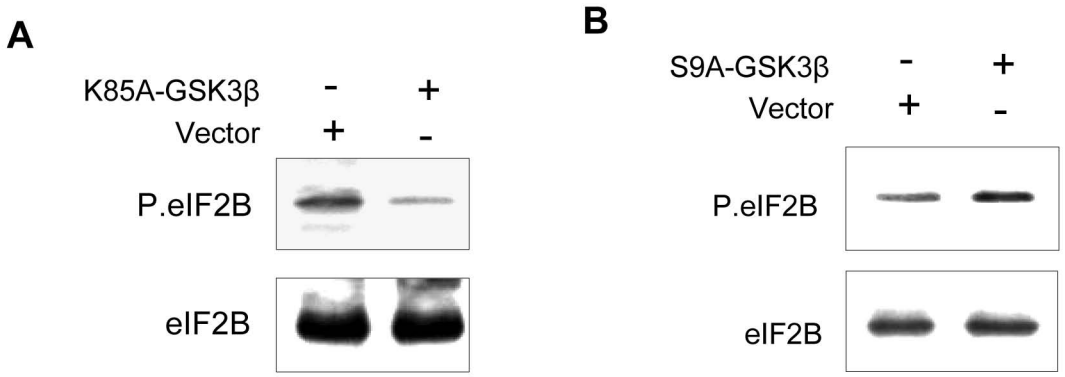
Fig S4.



Supplementary Figure S4. High glucose- and high insulin-induced Erk phosphorylation requires PI 3-kinase activation.

Serum-deprived MCT cells were pre-incubated with LY294002 (25 microM) prior to incubation with high glucose or high insulin for 24h. The lysates were fractionated by SDS PAGE and immunoblotted with phospho-specific antibody for Erk. High glucose- or high insulin-stimulated Erk phosphorylation in MCT cells was abrogated by pre-treatment of cells with LY294002, a PI 3-kinase inhibitor. Immunoblotting with total Erk antibody was done to assess loading. Representative blots from 4 experiments are shown.

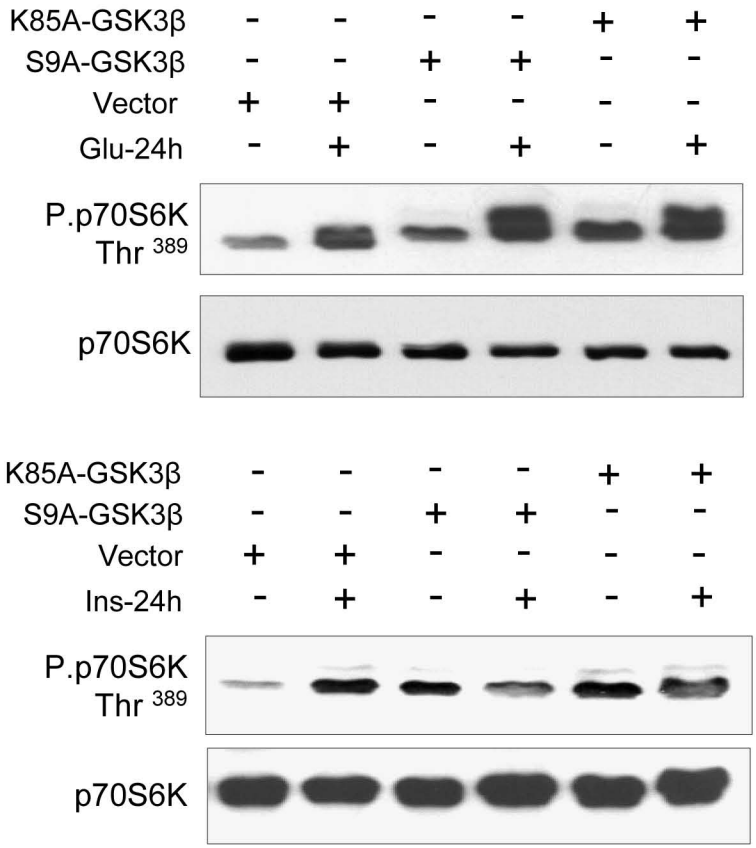
Fig S5.



Supplementary Figure S5.Regulation of eIF2Bε phosphorylation following expression of mutants of GSK3β.

MCT cells were transfected with control plasmid or a plasmid carrying kinase dead (K85A) construct of GSK3β (A) or constitutively active (S9A) construct of GSK3β (B). Equal amounts of lysate protein from cells were immunoblotted with antibody against phospho-eIF2B. Loading was assessed by immunoblotting with antibody against eIF2Bε. Representative blots from 3 experiments are shown. Note that overexpression of K85A mutant of GSK3β reduces eIF2Bε phosphorylation whereas it is stimulated with S9A mutant overexpression.

Fig S6.



Supplementary Figure S6. Overexpression of kinase inactive (K85A) and constitutively active (S9A) constructs of GSK3β does not regulate changes in p70S6 kinase phosphorylation induced by high glucose or high insulin.

Equal amounts of proteins from cells transfected with empty vector plasmid, kinase inactive (K85A) or constitutively active constructs (S9A) of GSK3β prior to treatment with or without high glucose or high insulin were immunoblotted with phospho-specific antibody for p70 S6kinase at Thr389. High glucose and high insulin stimulated p70 S6kinase phosphorylation at Thr389 in MCT cells transfected with vector plasmid. Transfection of the cells with kinase inactive (K85A) or constitutively active construct (S9A) of GSK3β did not affect phosphorylation of p70 S6kinase induced by high glucose or high insulin. Representative blots from 2 independent experiments are shown.