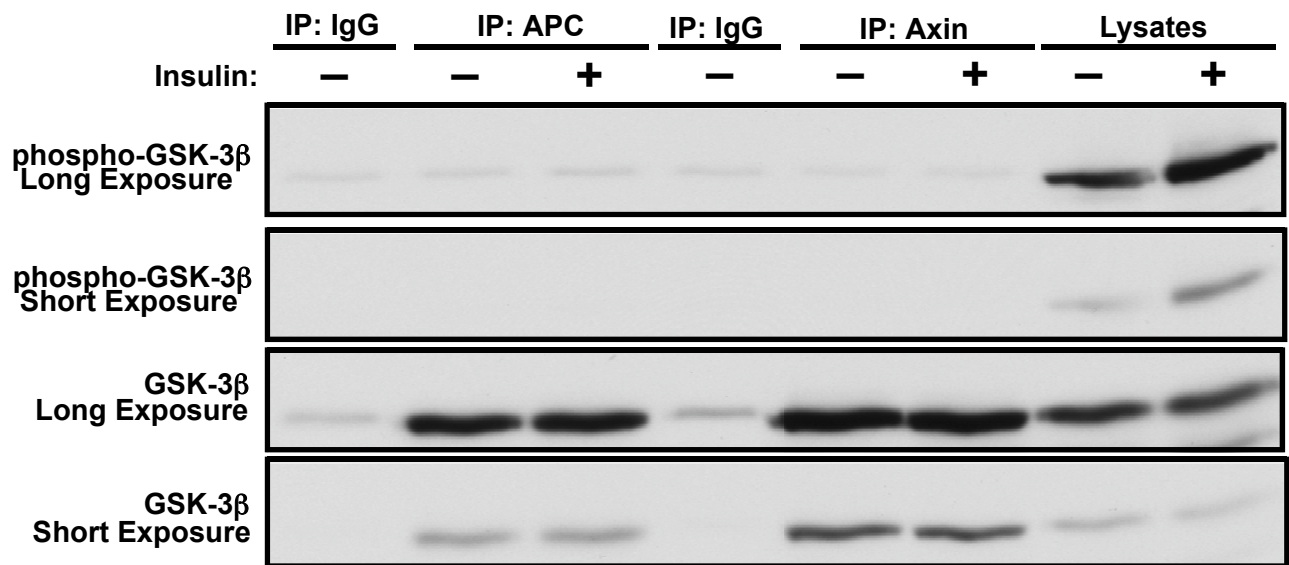
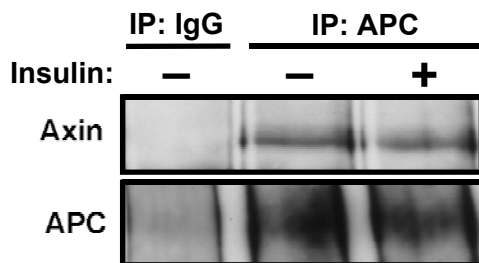


Supplemental Figure 5

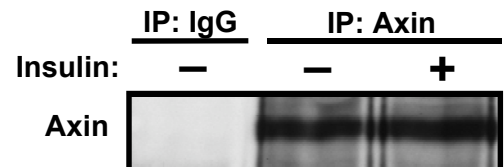
A



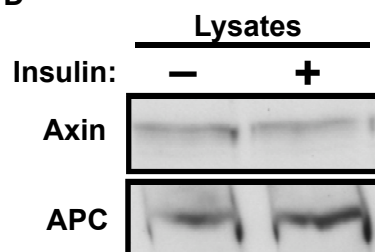
B



C



D



Supplemental Figure S5. Insulin does not induce phosphorylation of GSK-3 associated with the Axin/APC complex. 293T cells were treated with 2 μ M insulin for 30 min followed by immunoprecipitation of endogenous protein using control IgG, APC, or Axin antibodies. (A) Insulin induced serine-9 phosphorylation of GSK-3 β in whole cell lysates but not GSK-3 β associated with immunoprecipitated APC or Axin, which detects primarily the Axin/APC complex. The longer exposures show background GSK-3 β or phospho-GSK-3 β present in immunoprecipitates to make it clear that, at the limit of detection, there is no change in phosphorylation of GSK-3 β associated with this complex, as described previously (12). (B) Immunoblot of APC and Axin present in APC IPs. (C) Immunoblot of Axin in Axin IPs. (D) Insulin does not affect Axin or APC levels in the input lysates.