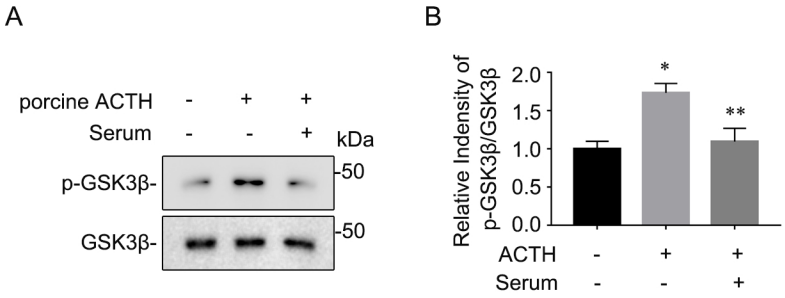
Supplementary Materials

Supplementary Figure Legends:

Supplementary Figure 1. The patient's serum is sufficient to block the biological functionality of porcine ACTH. (A) B16 melanoma cells were treated for 24 hours with vehicle, porcine corticotropin (100 mIU/mL), or porcine corticotropin (100 mIU/mL) premixed with an equal volume of the patient's serum. Cell lysates were collected after different treatments and processed for immunoblot analysis for phosphorylated GSK3β (p-GSK3β) and total GSK3β, which is a downstream signaling transducer of the melanocortin signaling pathway. (B) Arbitrary units of p-GSK3β/GSK3β ratios expressed as immunoblot densitometric ratios of the molecules as folds of the vehicle control group. The cell culture experiment was repeated 3 times (n = 3); *$P < 0.01$ versus control group; **$P < 0.01$ versus ACTH alone-treated group.

Supplementary Figure 2. Immunoblot-based antibody assay reveals no human ACTH-reactive antibodies in the patient's serum. Standard human ACTH was fractionated on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels, followed by Coomassie Brilliant Blue (CBB) staining (leftist panel), or by membrane transfer and immunoblotting overnight with a commercial rabbit anti-human corticotropin antibody (Ab) as positive control or with the patient's sera (1:100, 1:500 and 1:1,000 dilution). The blots were then developed by using an anti-rabbit secondary antibody or anti-human IgG antibody. Bands for human corticotropin were indicated.

Supplementary Figure 1



Supplementary Figure 2