Supplementary Figure 3. Activation of Ack1 leads to pTyr176-AKT expression. A, HEK293 cells were transfected with caAck1 or kdAck and AKT constructs and cell lysates were immunoblotted using pTyr176-AKT (top panel), pTyr284-Ack1, AKT and Ack1 antibodies. B, HEK293 cells were transfected with Ack1 mutant E346K and Flag-tagged AKT or Y176F mutant construct; whole cell lysates were immunoprecipitated using FLAG antibodies, followed by immunoblotting with pTyr176-AKT antibodies (top panel). The lysates were also immunoblotted with FLAG (2nd panel) and Ack1 antibodies (bottom panel).