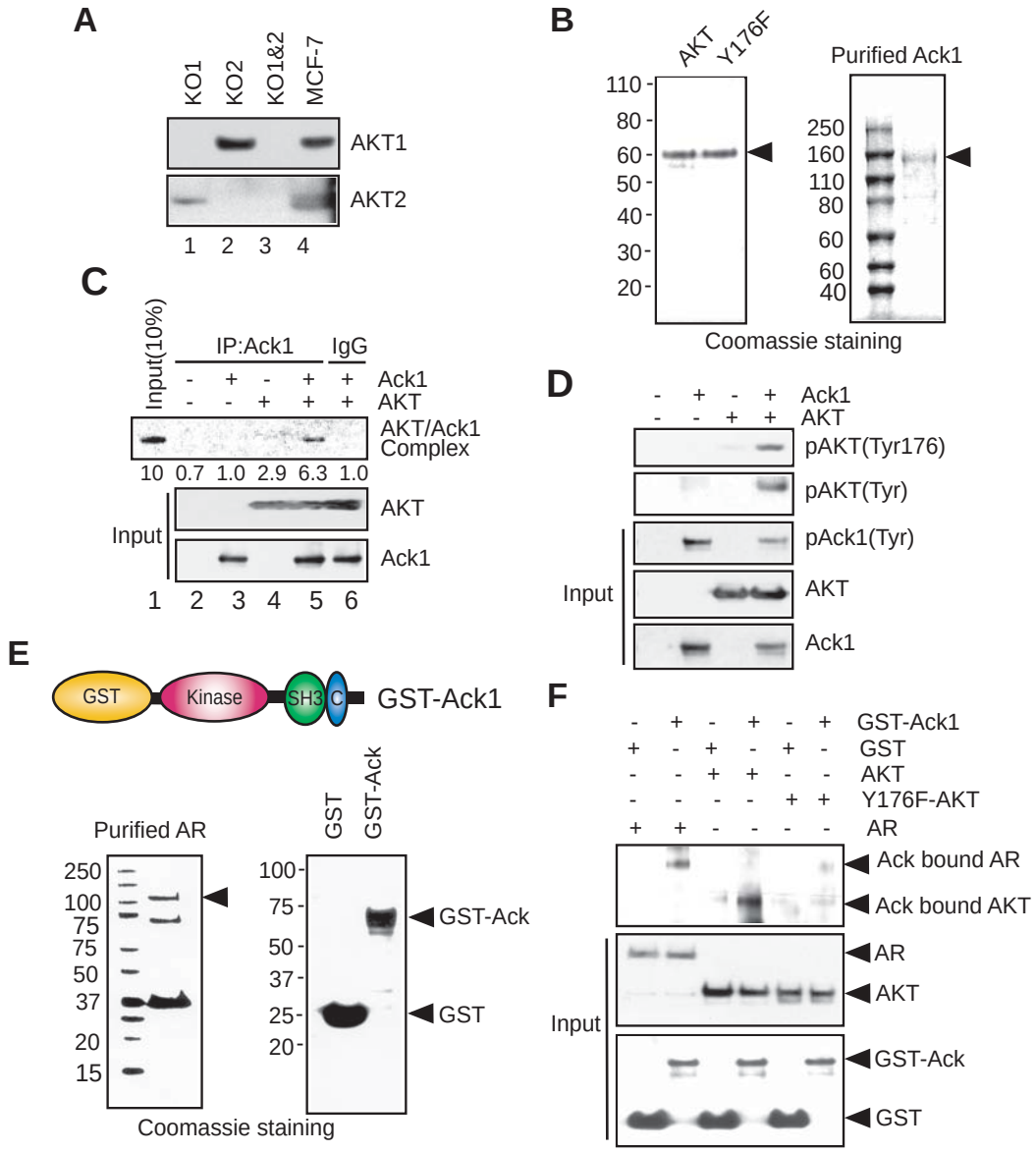

Figure S1. AKT is Tyr-phosphorylated by Ack1 *in vitro*. (A) AKT MEF KO1, KO2 and KO1&2s lack respective AKT isoforms. Equal amounts of MEFs protein lysates were subjected to IB as indicated. MCF-7 cell lysate was used as control. (**B**) Purification of Ack1 and AKT. HA-tagged Ack1 and AKT were expressed in HEK293T cells, lysed and incubated with HA-beads. Followed by extensive washing, proteins were eluted using HA-peptide (2nM, 1 hour) and assessed by SDS-PAGE and Coomassie Brilliant Blue-R250(BioRad) staining. **(C)** In vitro binding assay. Equimolar amounts of purified Ack1 and AKT proteins were incubated for 30 min, complex was immunoprecipitated with Ack1 (lanes 2-5) or IgG (lane#6) antibodies followed by IB with anti-AKT antibodies (top panel). About 6.35% of total AKT was in complex with Ack1. **(D)** In vitro phosphorylation of purified AKT by Ack1. Equimolar amounts of purified Ack1 and AKT proteins were incubated in kinase buffer for 1 hour at 37^0C and reaction mix was subjected to IB with pTyr176-AKT (top panel), pTyr (2^{nd} and 3^{rd} panels), AKT (4^{th} panel) and Ack1 (bottom panel) antibodies. (**E**) Schematic representation of GST-Ack1 construct. FLAG-tagged AR expressed in HEK293 cells and GST-tagged Ack1 was expressed in DH5 cells. Purified GST-Ack1 (right panel) and FLAG-AR (left panel) were assessed by SDS-PAGE followed by Coomassie staining. **(F)** *In vitro* binding assay. Equimolar amounts of purified HA-AKT or FLAG-AR proteins were incubated with GST-Ack1 bound to beads for overnight, beads were washed followed by IB with anti-FLAG/HA antibodies (top panel). Lower panels show IB with FLAG/HA (2^{nd} panel) and GST (bottom panel) antibodies.