



Figure S8. Tyrosine-phosphorylated AKT binds to phosphatidic acid. Protein-lipid overlay assay was performed using nitrocellulose membranes spotted with 100 pmol of different phospholipids. **(A-C, F-G)** Cells transfected with vector or activated Ack1 and AKT or Y176F were lysed and immunoprecipitated with pTyr-beads followed by elution with phenylphosphate. The eluted Tyr-phosphorylated proteins were incubated with phospholipid blots overnight at 4°C. Blots were extensively washed and bound proteins were detected with **(A, B and F)** pTyr176-AKT and **(C and G)** AKT antibodies. **(D and E)** Cells expressing HA-tagged **(D)** AKT and **(E)** Y176F mutant AKT were lysed and immunoprecipitated with HA-beads followed by elution with HA peptide. The eluate was incubated with phospholipid blots and bound proteins were detected with AKT antibodies. The pTyr176-AKT bound to phosphatidic acid, in contrast, AKT and Y176F mutant proteins bound primarily to phosphatidyl-inositol 3,4,5-triphosphate. **(H)** HA peptide and phenylphosphate eluate was immunoblotted with antibodies shown to confirm presence of desired proteins.