Supporting Information

Watkins *et al.* 10.1073/pnas.0809661105

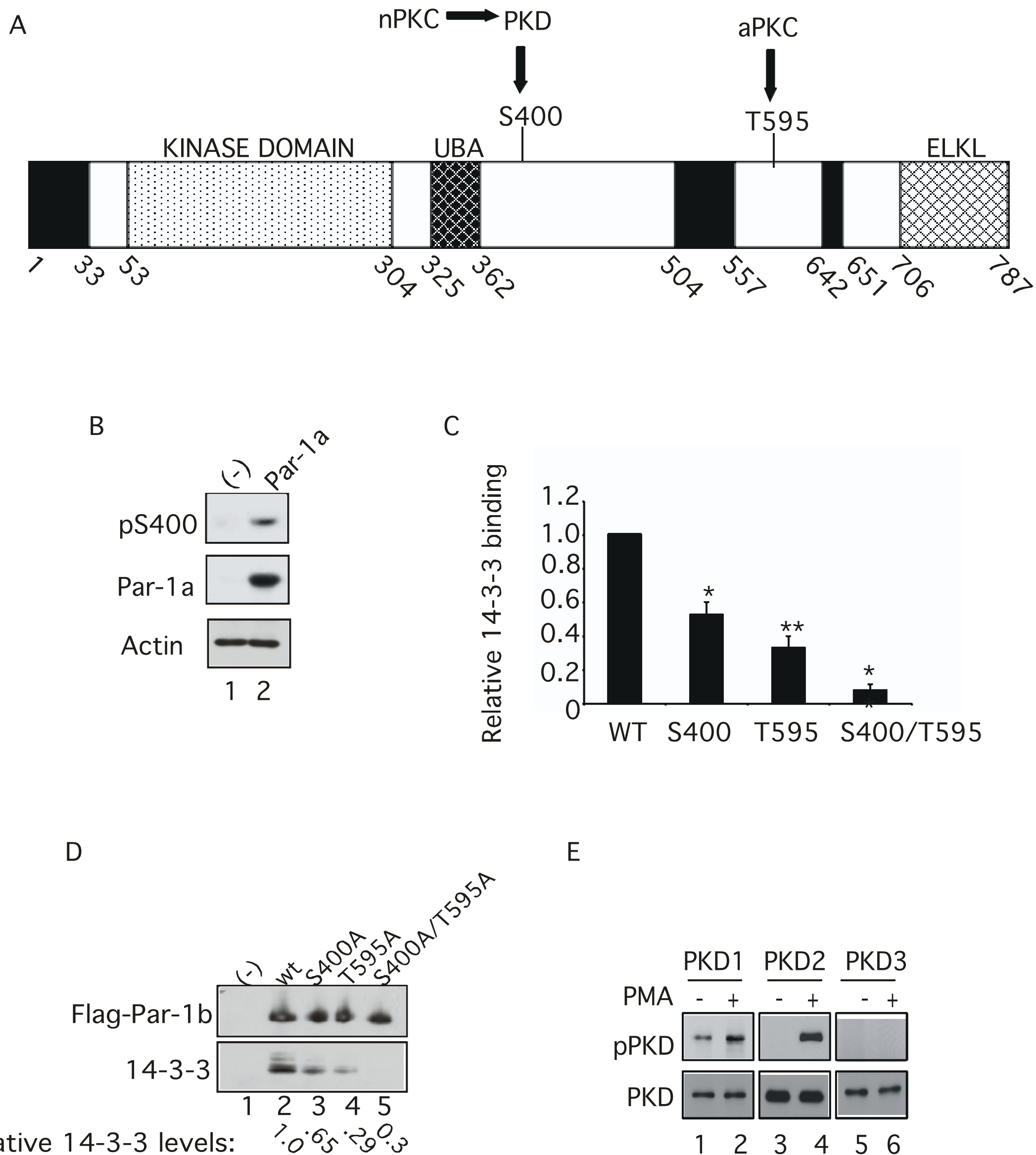

Fig. S1. Par-1a is phosphorylated on serine (S)400 in vivo. (*A*) The kinase-, ubiquitin-associated (UBA)-, and ELKL (Glutamic acid, Leucine, Lysine, Leucine)-domains (ELKL)-domains of Par-1b are indicated. Black boxes represent regions that are absent in spliced variants of human Par-1b. The signaling pathways regulating phosphorylation of S400 and T595 are indicated. (*B*) HeLa cells were transfected with plasmid encoding flag-tagged Par-1a by using Lipofectamine 2000 for 24 h. Lysates from control cells (lane 1) or cells expressing flag-tagged Par-1a (lane 2) were resolved directly by SDS/PAGE. Western blotting was performed with indicated antibodies. (*C*) HeLa cells were transfected with plasmids encoding the indicated flag-tagged proteins by using Lipofectamine 2000 for 24 h. Lysates were incubated with flag agarose. Precipitates were resolved by SDS/PAGE and analyzed by Western blotting by using the indicated antibodies. Relative levels of 14-3-3 in each precipitate were determined from Western blotting by using the ImageJ program and are indicated below the blot. The mean ± SEM for 5 independent experiments is shown. Student's *t* test was performed for comparisons between groups. A representative Western blot is shown in Fig. 2*C*. *, $P < 0.005$; **, $P < 0.001$. (*D*) HeLa cells were transfected with plasmids encoding the indicated flag-tagged proteins by using Lipofectamine 2000 for 24 h and then incubated with 200 ng/mL of PMA for 1 min. Lysates were incubated with flag agarose and precipitates were resolved by SDS/PAGE and analyzed by Western blotting for the indicated proteins. Relative levels of 14-3-3 in each precipitate were determined from Western blotting by using the ImageJ program and are indicated below the blot. (*E*) HeLa cells were cultured in the absence of serum for 16 h and treated with vehicle (lanes 1, 3, and 5) or with 400 ng/mL PMA for 10 min (lanes 2, 4, and 6). PKD1, PKD2, and PKD3 were immunoprecipitated and samples were resolved by SDS/PAGE. Immunoblotting was performed with a phospho-specific antibody that recognizes activated PKD1. Blots were stripped and reprobed with PKD1-, PKD2-, or PKD3-specific antibodies (*Lower*).