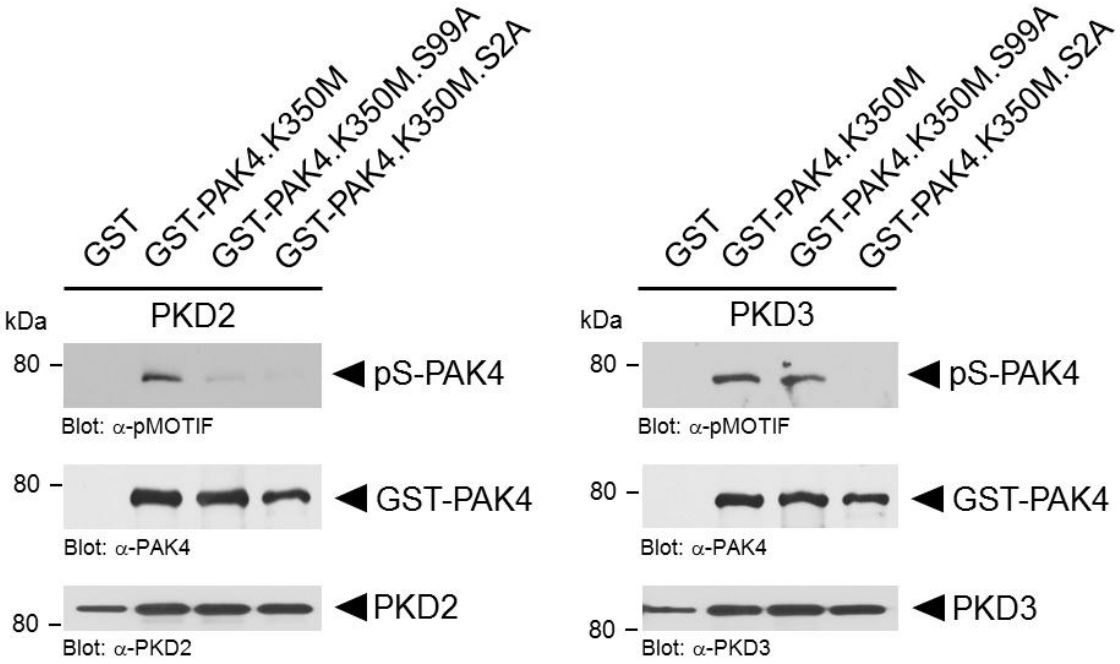
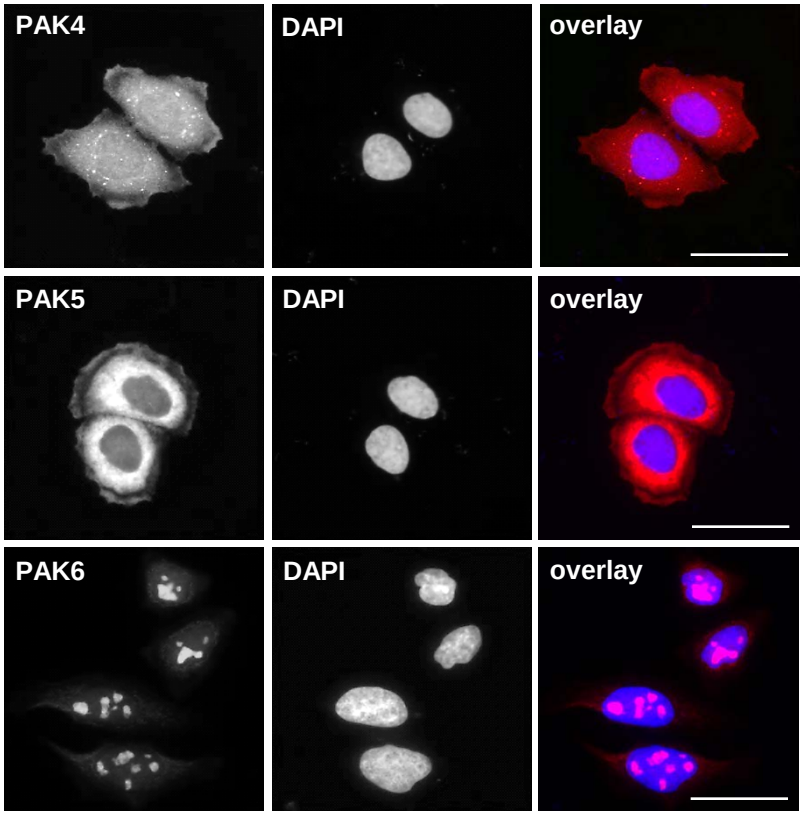
Supplemental Figure S1



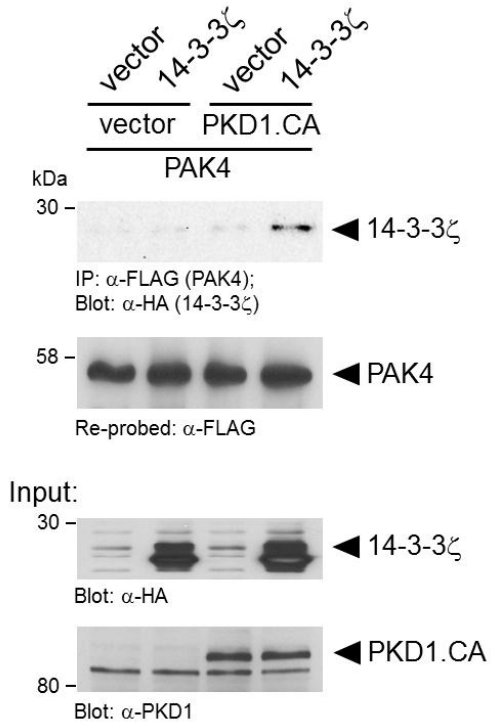
Suppl. Fig. S1: **S99 is phosphorylated by PKD2, but not PKD3.** *In vitro* kinase assays were performed with purified recombinant PKD2 or PKD3 and either purified GST-tagged PAK4 with only an inactivating K350M mutation (GST-PAK4.K350M), additional mutation at S99 (GST-PAK4.K350M.S99A) or additional mutation at S99 and S474 (GST-PAK4.K350M.S99A.S474A). To analyze substrate phosphorylation, Western blots of resolved proteins were probed with α-pMOTIF (recognizes a phosphorylated PKD substrate motif). Additional blots (α-PAK4 and α-PKD2 or α-PKD3) were performed to control input of purified proteins.

Supplemental Figure S2



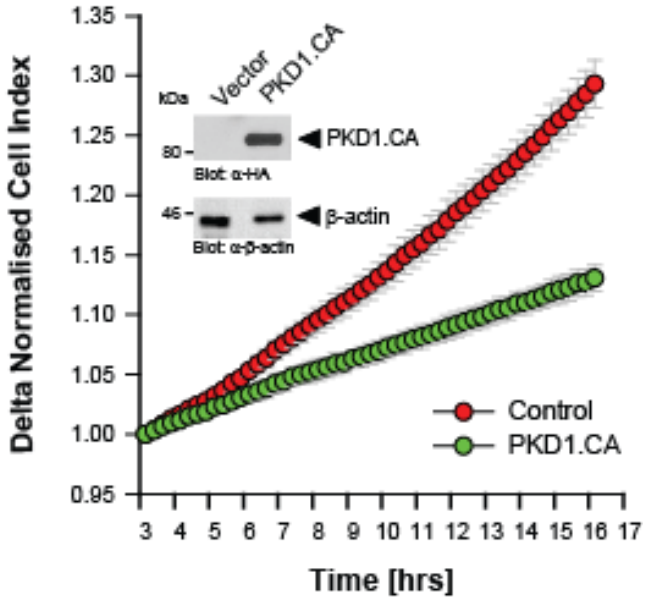
Suppl. Fig. S2: **Localization of endogenous PAK4, PAK5 and PAK6.** HeLa cells ($1x10^4$ cells, ibiTreat μ-slide) were subjected to indirect immunofluorescence analysis. Samples were labeled with α-PAK4 (Abcam ab62509; dilution 1:200), α-PAK5 (Abcam ab62510; dilution 1:200) or α-PAK6 (Abcam ab62511; dilution 1:200) and secondary Alexa Fluor 546 anti-rabbit antibodies (dilution 1:800). Additionally, nuclei of cells were stained with DAPI. The bar indicates 50 μm.

Supplemental Figure S3



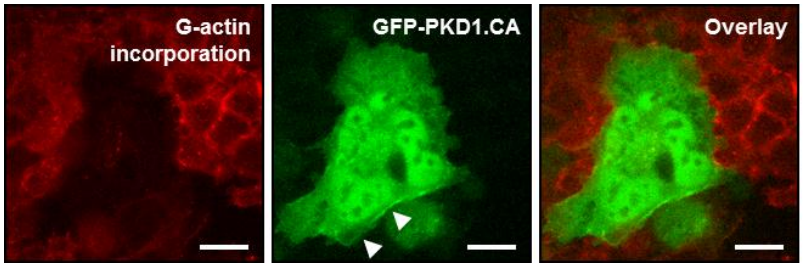
Suppl. Fig. S3: **14-3-3ζ interacts with PAK4 in presence of active PKD1.** Cells were co-transfected with Flag-tagged PAK4, vector control or active PKD1 (PKD1.CA) and HA-tagged vector control or 14-3-3ζ as indicated. PAK4 was immunoprecipitated (α-FLAG) and samples were analyzed for co-immunoprecipitated 14-3-3ζ (Blot: α-HA). Blots were stripped and analyzed for total PAK4 (α-FLAG). Control blots were performed on lysates to determine expression of active PKD1 (α-PKD1 staining) and 14-3-3ζ (α-HA staining).

Supplemental Figure S4



Suppl. Fig. S4: **Active PKD1 blocks directed cell migration.** HeLa cells (5 x 10^5 cells, 6 cm dish) were transfected with control vector or constitutively-active PKD1. After three hours of attachment, cell migration towards NIH-3T3 conditioned media over 14 hours was continuously monitored in real-time using Transwell CIM-plate 16 and the xCELLigence RTCA DP instrument. Error bars (grey) represent four experiments. Inset shows control blots of cell lysates probed for expression of active PKD1 (Blot: α-HA). Staining for β-actin (Blot: α-β-actin) served as loading control.

Supplemental Figure S5



Suppl. Fig. S5: **Active PKD1 blocks free barbed-end induced actin incorporation.** HeLa cells (5 x 10^4 cells, 8 well ibiTreat µ-slide) were transfected with control vector or GFP-tagged constitutively-active PKD1. To analyze cofilin-mediated actin incorporation F-actin free barbed ends were performed as previously described (Spratley et al.). Bar is 20 µm.