

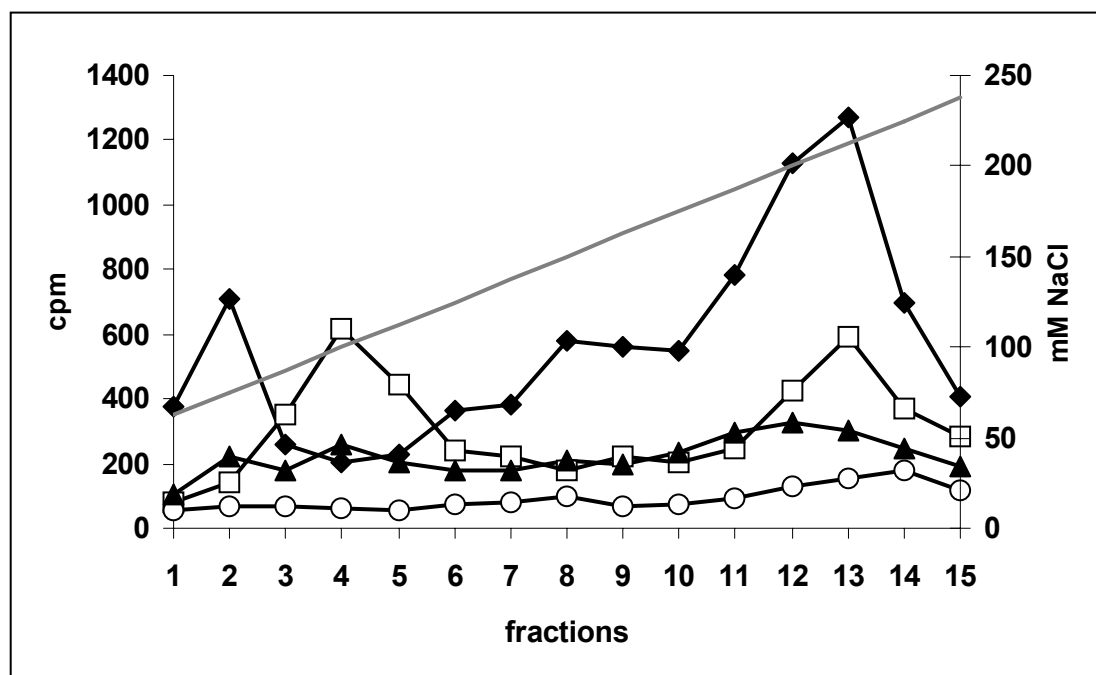


Figure 1S CK1δ kinase activity in established pancreatic tumor cell lines.

Equal protein amounts (3 mg) of MiaPaCa-2, BxPc3, PancTu1 and Colo357 cell extracts were loaded onto a 1 ml Resource Q column and eluted with a linear gradient of increasing NaCl concentration (solid diagonal line). Fractions (0.2 ml) were collected and the CK1δ/ε specific kinase activity was determined using the GST-p53¹⁻⁶⁴ (FP267) fusion protein as substrate. In each fractionation, the kinase activity was eluted from the column between 220 and 240 mM NaCl. Phosphorylated proteins were separated on SDS-PAGE, Coomassie stained and isolated. Phosphate incorporation was measured by Cerenkov counting. Differences in the kinase activity up to 6 fold were detected. The highest CK1δ activity was found in extracts of MiaPaCa2 cells, a moderate CK1δ activity was observed in BxPc3 and PancTu1 cell extracts, and a very low CK1δ activity was seen in Colo357 extracts.

Closed rombs: MiaPaCa-2 cell extracts, open square: BxPc3 cell extracts, closed triangle: PancTu1 cell extracts, open circle: Colo357 cell extracts, solid line: NaCl concentration

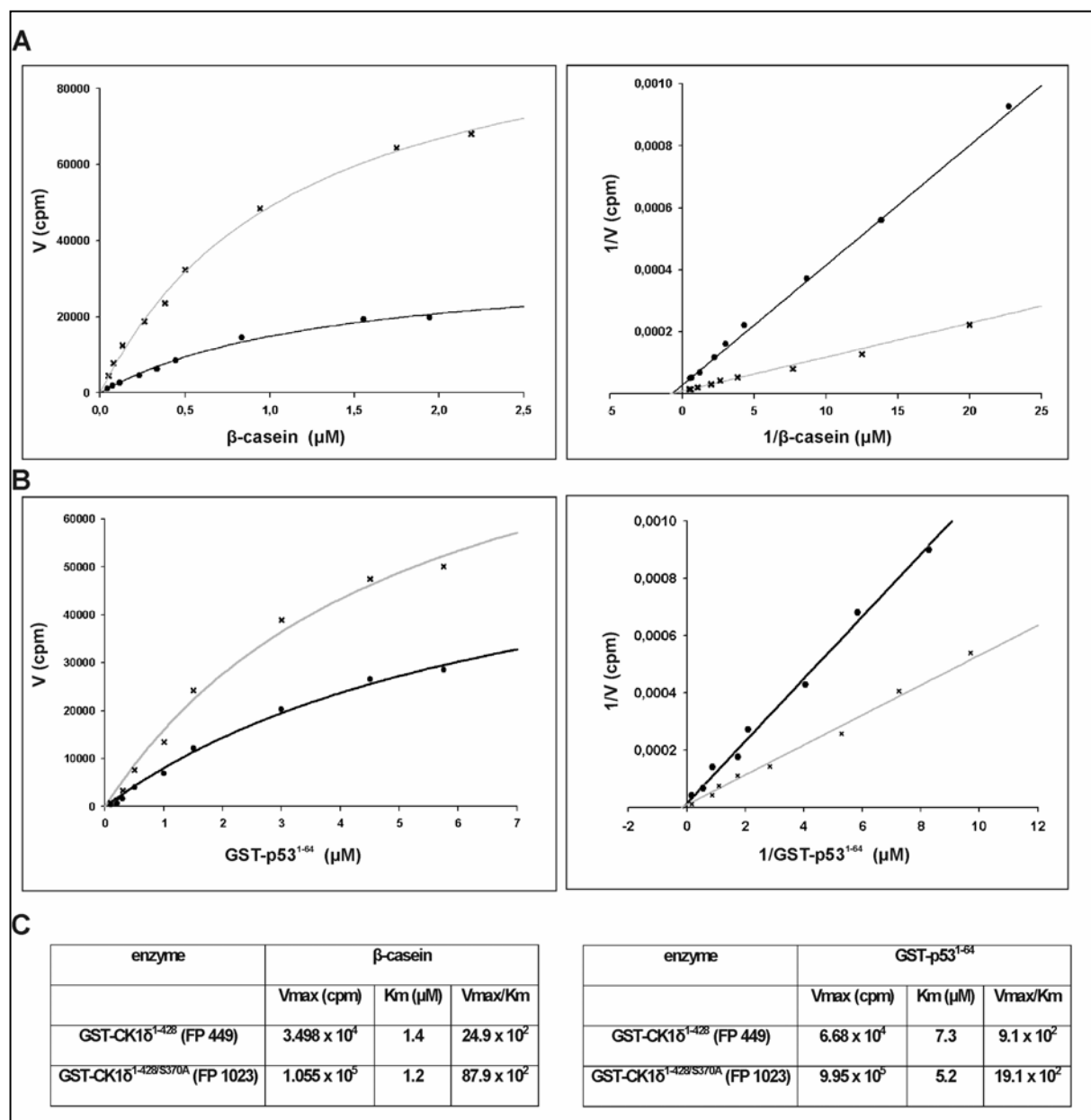


Figure 2S Comparison of the kinetic constants of GST-CK1δ¹⁻⁴²⁸ and GST-CK1δ^{1-428S370A}

The kinetic constants $V_{\max}$, K_m and $V_{\max}/K_m$ (enzyme efficiency ratio) of GST-CK1δ¹⁻⁴²⁸ (FP 449) (black) or GST-CK1δ^{1-428S370A} (FP 1023) (grey) for β-casein and GST-p53¹⁻⁶⁴ were experimentally calculated. Michaelis-Menten and Lineweaver-Burk plots are shown.

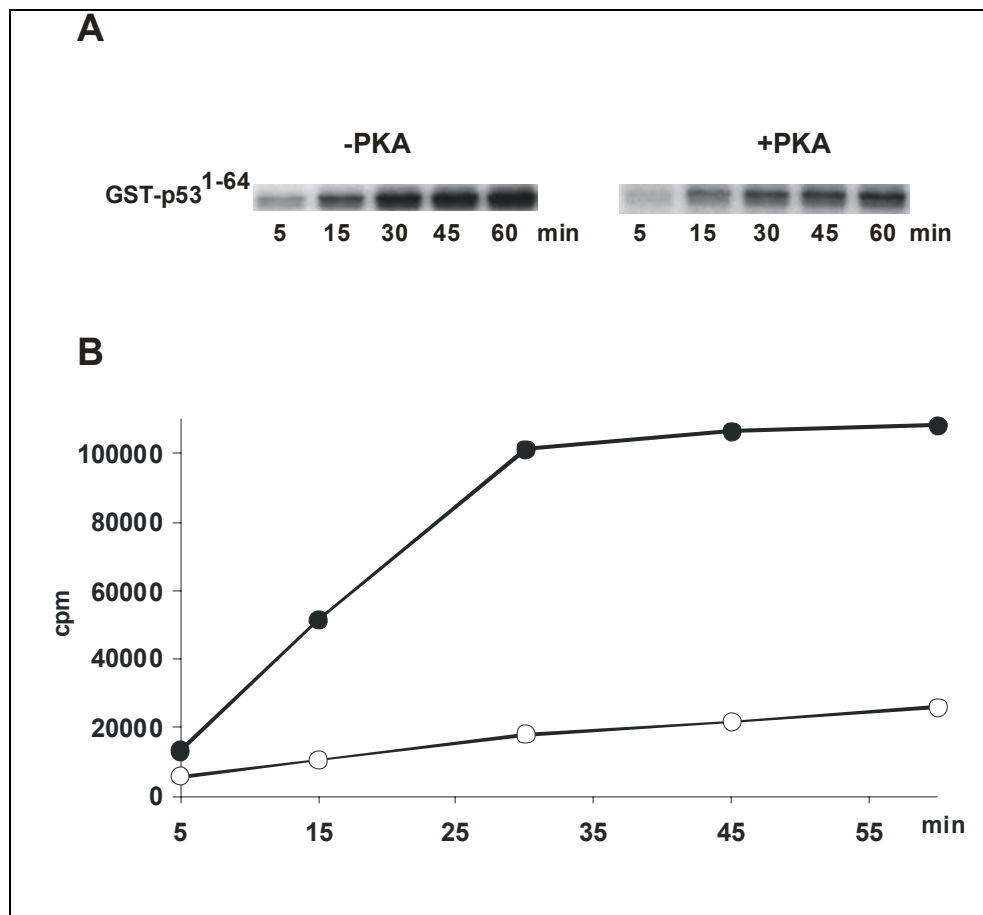


Figure 3S Phosphorylation of GST-CK1 δ by PKA affects its activity *in vitro*

GST-CK1 δ^{1-428} was incubated with [γ -³²P] ATP for 15 min at 30^o C, followed by the addition of the substrate (GST-p53¹⁻⁶⁴) alone or in combination with PKA. GST-p53¹⁻⁶⁴ was phosphorylated *in vitro* for the indicated periods of time. The incorporated phosphate was quantified by Cerenkov counting after separation of phosphorylated proteins by SDS-PAGE. **A:** Detection of substrate phosphorylation by GST-CK1 δ^{1-428} in the absence and presence of PKA by autoradiography. **B:** Quantification of phosphate incorporation. Closed circles: the degree of substrate phosphorylation in the absence of PKA, open circles: the degree of substrate phosphorylation in the presence of PKA.