Title Page for Supplementary Information

Phosphorylation Regulates CAP1 (Cyclase-Associated Protein 1) Functions in the Motility and Invasion of Pancreatic Cancer Cells

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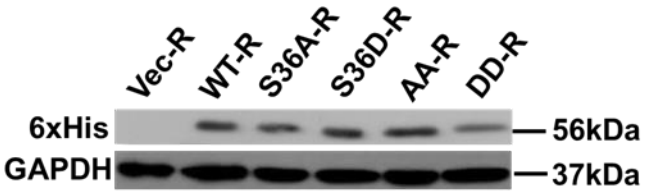
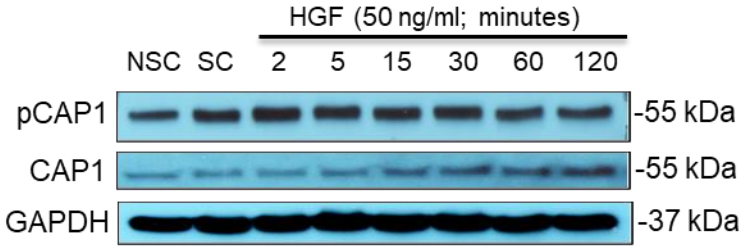
Supplementary Figure 1



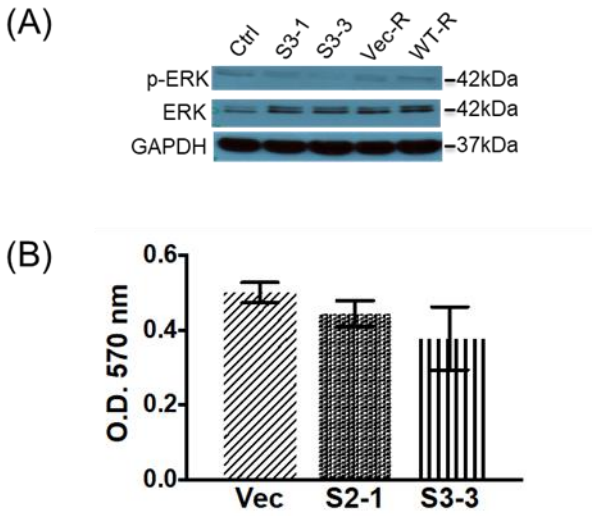
Supplementary Figure 1: Confirmation of re-expression of WTCAP1 and the AA and DD phosphor mutants, along with other two phosphor-mutants S36A and S36D in the CAP1-knockdown S3-3 stable PANC-1 cells. The expression was confirmed in Western blotting using an antibody against the 6xHis tag of the re-expressed protein. Two irrelevant lanes (with samples of S36A and S36D) were removed from this Western blot image, and presented in Fig. 4A.

Supplementary Figure 2



Supplementary Figure 2: Hepatocyte Growth Factor (HGF) did not have an obvious effect in inducing CAP1 dephosphorylation. PANC-1 cells were serum-starved for 22 hrs and treated with 50ng/ml HGF for the indicated durations, and phosphorylation at S307/S309 on CAP1 was detected in Western blotting. CAP1 and GAPDH were also detected, with the latter serving as a loading control. Non-starved control cells (NSC) and starved control cells (SC), which were serum-starved but without HGF stimulation, were also included.

Supplementary Figure 3



Supplementary Figure 3: Depletion of CAP1 in PANC-1 cells did not cause significant alterations in ERK or cell proliferation. **(A)** Knockdown of CAP1 in PANC-1 cells, or re-expression of CAP1 in the knockdown cells, did not cause remarkable changes in ERK activity. The control cells and two knockdown stable clones (S3-1 and S3-3), along with S3-3 cells harboring an empty re-expression vector (Vec-R) or re-expressing WTCAP1 (WT-R) were used in testing ERK expression and phosphorylation. **(B)** MTT assays show that the CAP1-knockdown cells that derive from both S2 and S3 shRNA constructs did not have significantly reduced proliferation. The p values comparing the control (Vec) cells to S2-1 cells was at 0.209, and that between control cells and S3-3 cells was at 0.223, respectively.