A novel EPAC specific inhibitor suppresses pancreatic cancer cell migration and invasion

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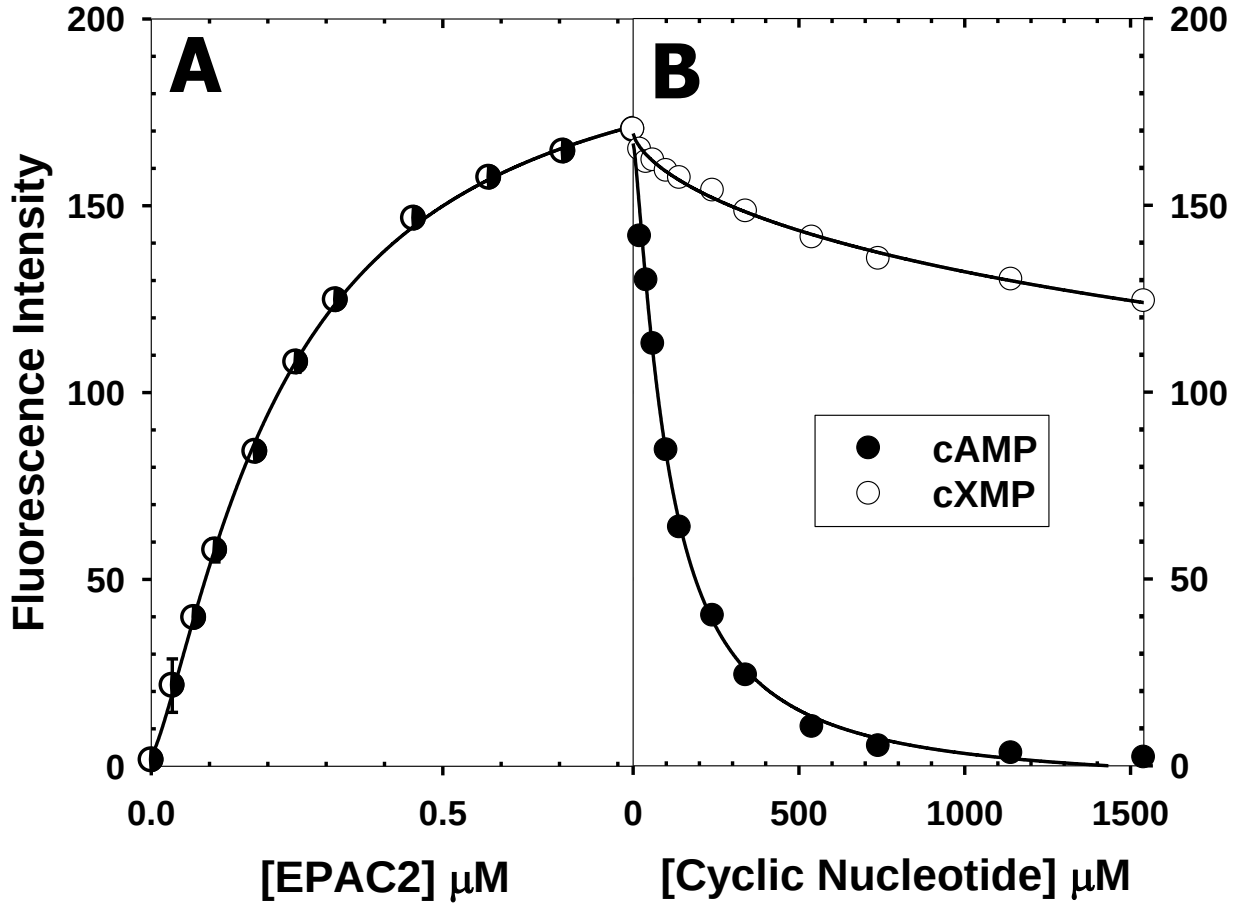
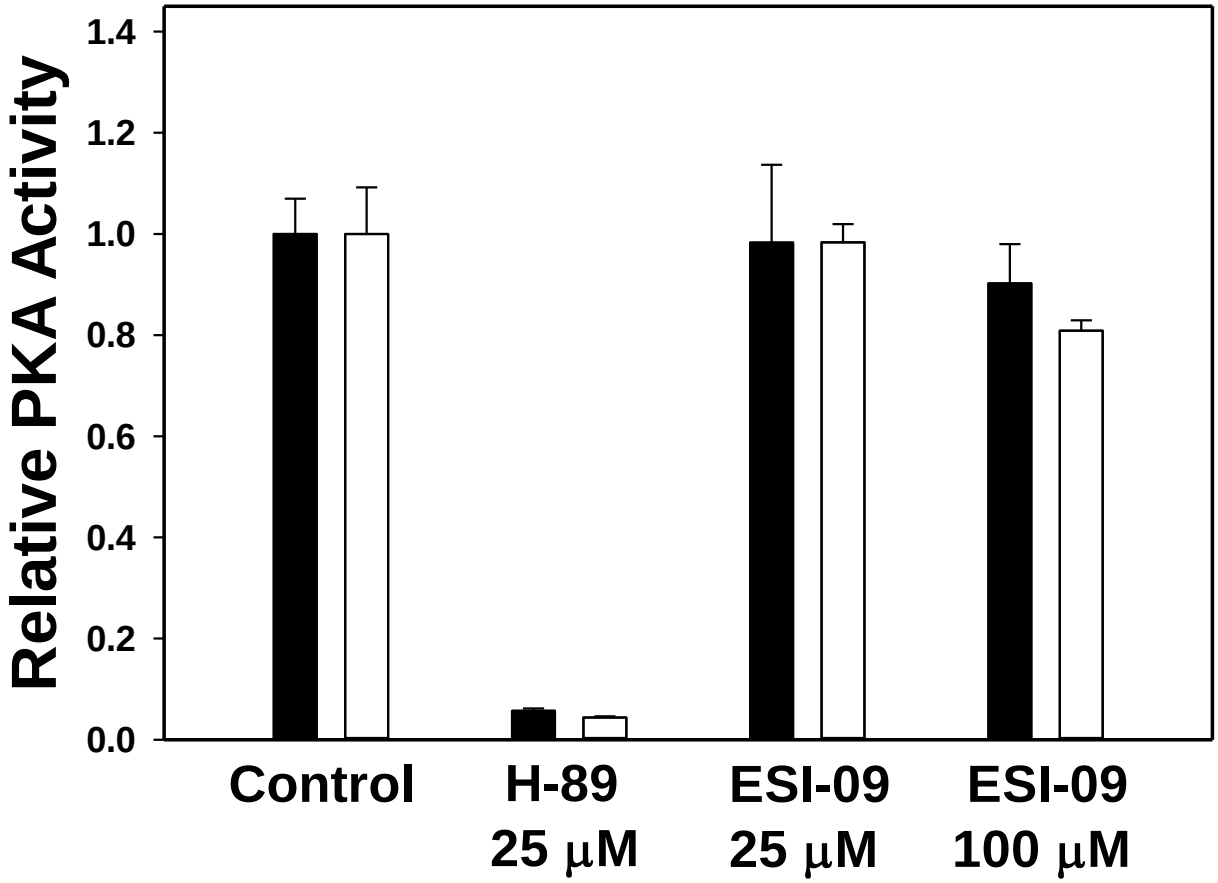
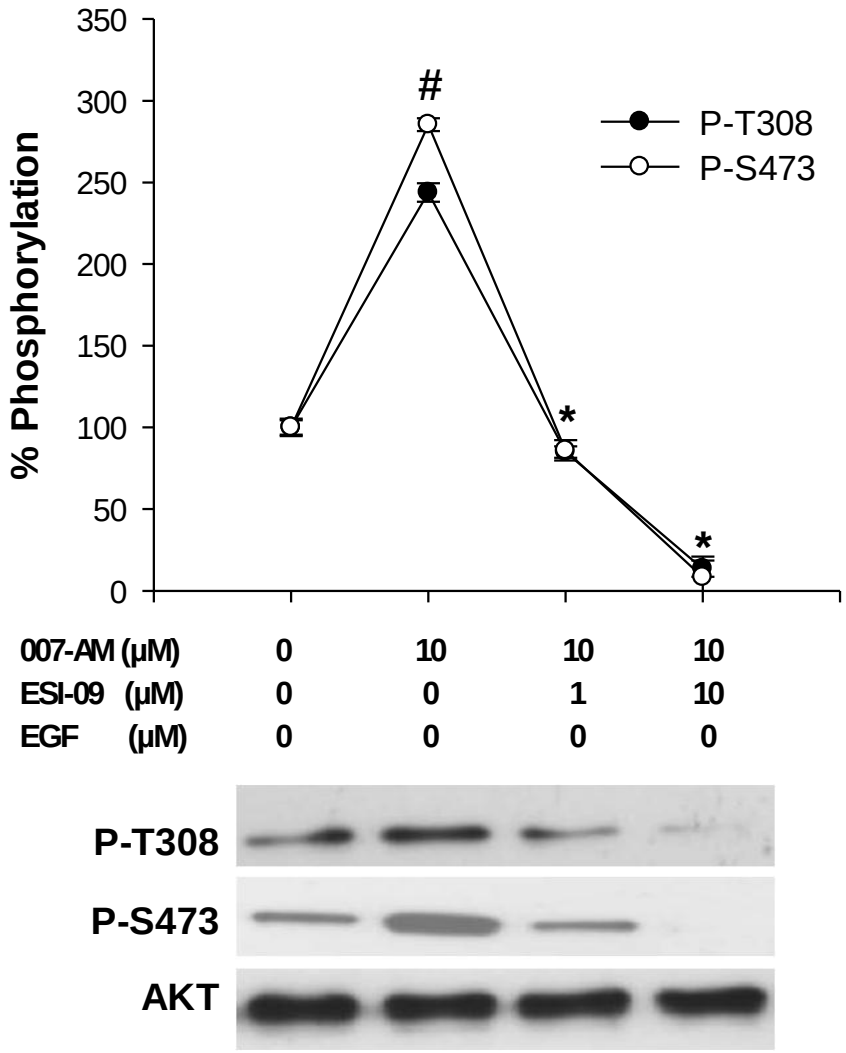
Supplemental Figures for Molecular Pharmacology MOL #80689



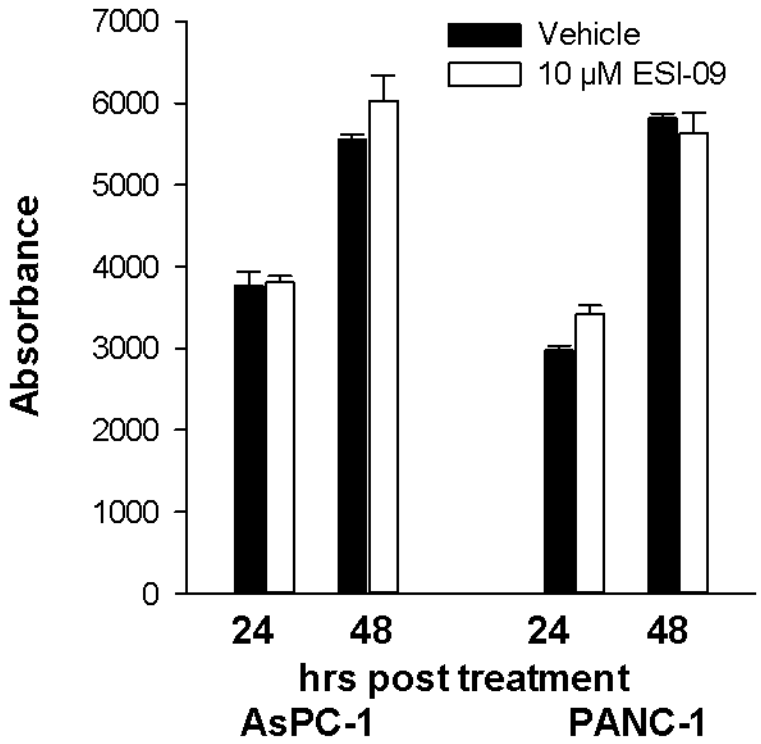
Supplemental Figure 1. Changes in 8-NBD-cAMP fluorescence as a function of EPAC2 and cyclic nucleotide concentrations. (A) Change in fluorescence when 8-NBD-cAMP (0.1 μM) is titrated with EPAC2. Fitting of the titration curve led to an apparent dissociation constant of 0.27 μM for binding of 8-NBD-cAMP to EPAC2. (B) The fluorescence change can be reversed by the addition of excess cAMP (closed circles) but not by Xanthosine- 3', 5'- cyclic monophosphate (cXMP, open circles) purchased from Biolog Life Science Institute (Bremen, Germany).



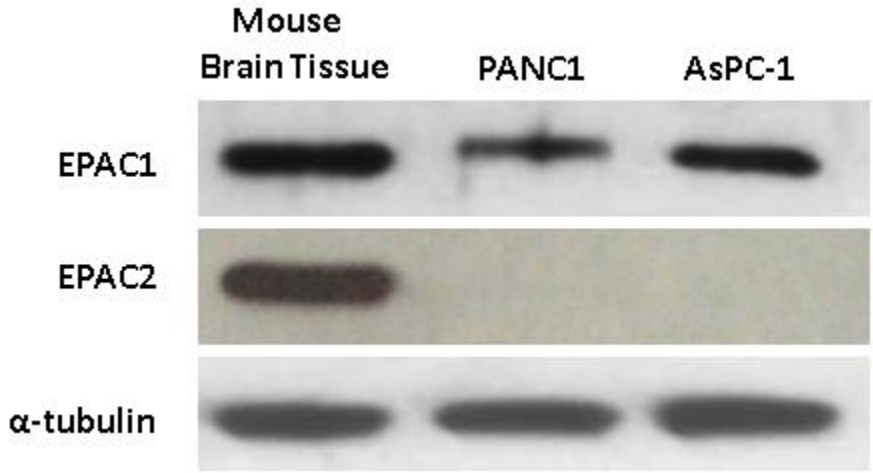
Supplemental Figure 2. Effects of ESI-09 on type I and II PKA activities. Relative Type I (filled bars) and II (open bars) PKA holoenzyme activities in the presence of 100 μM cAMP plus vehicle control, 25 μM H-89, 25 μM or 100 μM of ESI-09. Data presented as means ± standard deviations (n=3).



Supplemental Fig. 3. ESI-09 inhibits EPAC-mediated Akt phosphorylation in INS-1 cells. Serum-starved INS-1 cells were stimulated with vehicle or 10 µM 007-AM after pretreatment with the indicated concentrations of ESI-09. Cell lysates were subjected to Western blot analyses using anti-phospho-Akt T308 and S473antibodies (representative blot shown). Data points represent mean ± s.d. (n=3). $^{\#}$Significantly higher than vehicle group ($P < 0.02$). *Significantly lower that 007-AM stimulated group ($P < 0.02$).



Supplemental Figure 4. Effects of ESI-09 treatment on pancreatic cancer cell viability. AsPC-1 and PANC-1 cells were seeded into triplicate wells of a 96-well plate (2×10^3 cells/well) in 100 µl of RPMI 1640 medium containing 10% FBS. Cells were treated with vehicle (open bars) or 10 µM ESI-09 (filled bars) and incubated at 37 °C in 5% CO_2. Cell viability was measured at 24 and 48 hrs by an Almar Blue assay. Briefly, 10 µl of Alamar Blue was added to each well and the plate was incubated for another 4 hrs. Following the incubation, fluorescence was measured using a SpectraMax M2 microplate reader (Molecular Devices) with excitation and emission wavelengths set at 530 and 590 nm, respectively. Bars represent mean ± s.d. (n=3).



Supplemental Figure 5. Expression levels of EPAC1 and EPAC2 in the PDA cell lines PANC-1 and AsPC-1. Relative cellular levels of EPAC1 and EPAC2 as compared to those of mouse brain tissue probed by EPAC1 or EPAC2 specific antibodies.