Pharmacological Inhibition and Genetic Knockdown of EPAC1 Reduce Pancreatic Cancer Metastasis in vivo

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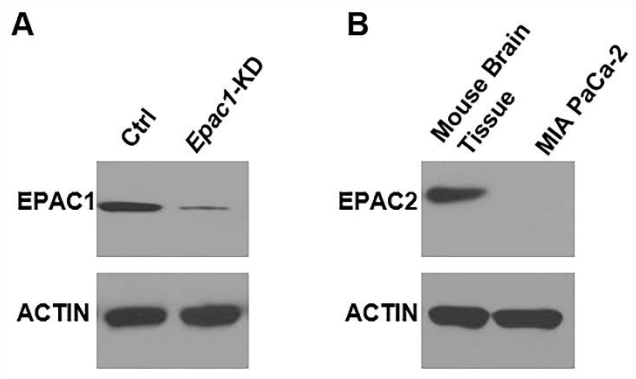
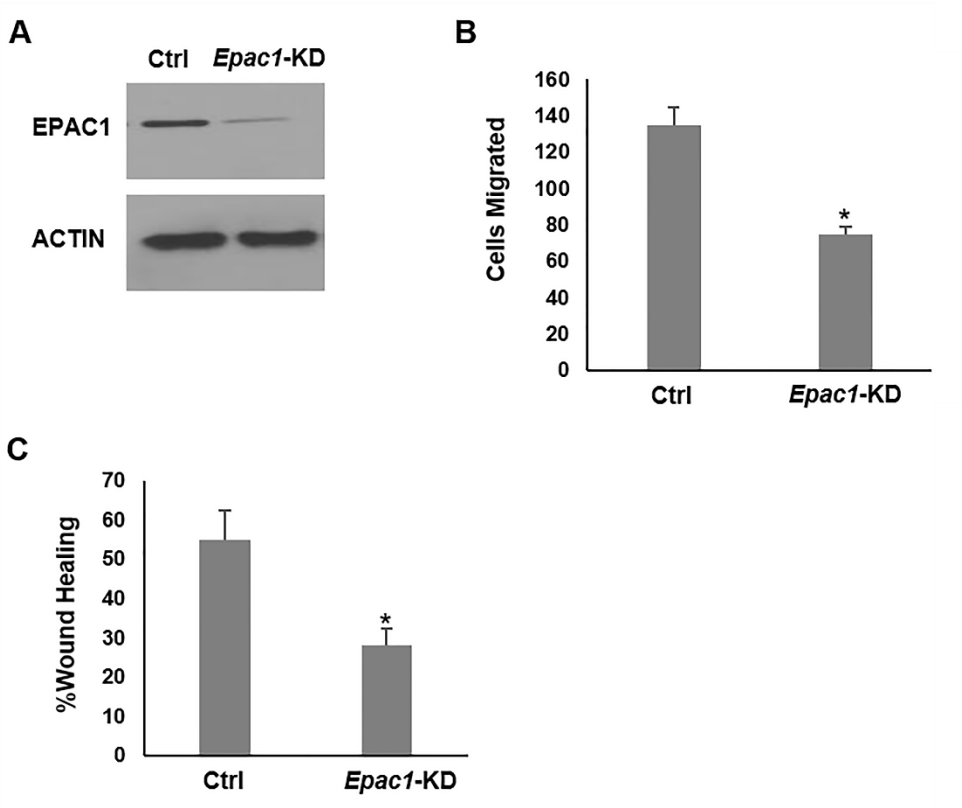
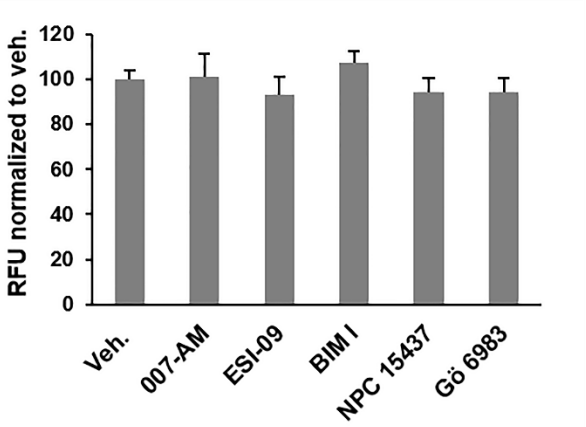


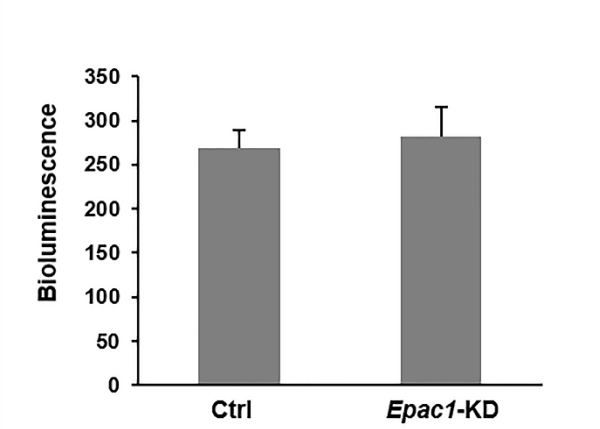
Supplementary Fig. S1. EPAC1 and EPAC2 expression in MIA PaCa-2. (A) Expression and knockdown by shRNA (clone 32) of EPAC1 in MIA PaCa-2 cells were confirmed by Western blotting. (B) EPAC2 expression in MIA PaCa-2 was probed. Mouse brain tissue was used as a positive control for EPAC2 expression.



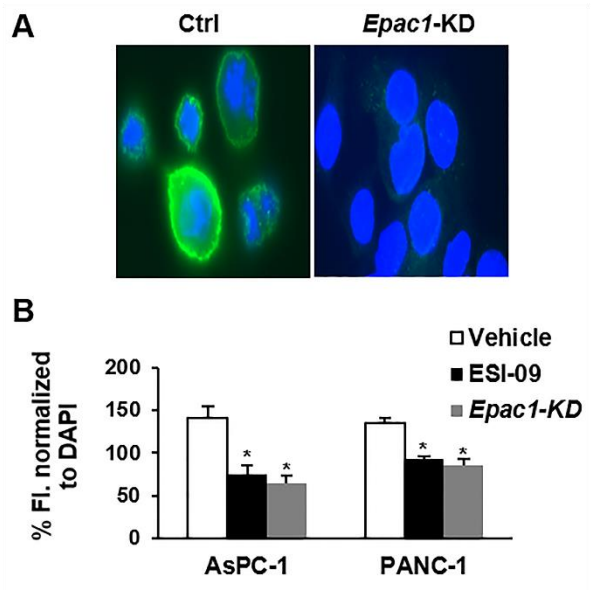
Supplementary Fig. S2. EPAC1 knockdown decreases invasion and migration of MIA PaCa-2. (A) Cells were transfected with non-targeting shRNA (Ctrl) or an *Epac1* specific shRNA (clone 28) and EPAC1 expression was probed by Western blotting. Knockdown of EPAC1 reduced invasion/migration of MIA PaCa-2 cells in invasion/migration and wound-healing assays. (B) Quantification of the number of migrating cells. (C) Quantification of wound closure presented as the distance traveled by the edge of the wound relative to the wound's initial size. * Significantly lower than Ctrl cells ($P < 0.02$). Bars represent mean ± S.D. (n=3).



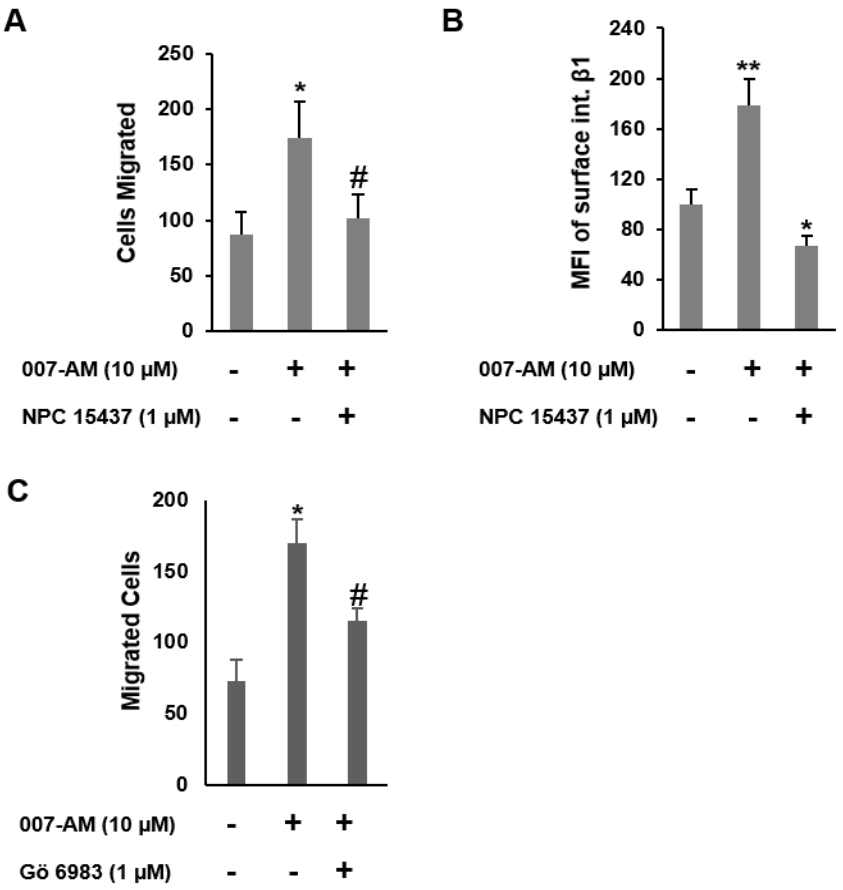
Supplementary Fig. S3. Effects of utilized compounds on viability of MIA PaCa-2. Cells were treated with vehicle DMSO, 10 μM 007-AM, 5 μM ESI-09, 1 μM BIM I, 1 μM NPC 15437, or 1 μM Gö 6983 for 24 hrs, and cell viability was determined by an almarBlue viability assay. RFU, relative fluorescence unit normalized to vehicle treated cells. Bars represent mean ± S.D. (n=3).



Supplementary Fig. S4. Luciferase activity in MIA PaCa-2 and MIA PaCa-2-*Epac1*-KD cells. MIA PaCa-2 cells were transduced with luciferase, followed by transfection with non-targeting shRNA (Ctrl) or an *Epac1* specific shRNA-clone 32 (*Epac1*-KD). An equal number of cells (2×10^4) was then used to examine luciferase activity in each cell line using an *in vitro* luciferase assay system. Bars represent mean ± S.D. (n=3)



Supplementary Fig. S5. Inhibition or suppression of EPAC1 decreases the level of surface integrin β1. Control or *Epac1*-KD AsPC-1 and PANC1 cells were treated with vehicle DMSO or the EPAC inhibitor ESI-09 (5 μM) for ~ 45 min and fixed with paraformaldehyde, followed by staining for total surface Itgβ1 with the FITC-conjugate antibody K20 (1:4) in non-permeabilizing buffer for 2 hr**.** Nuclei were stained with the dye DAPI and cells were mounted on a microscope slide. (A) Representative image showing surface Itgβ1 (green) and cell nuclei (blue) staining in AsPC-1. (B) Fluorescence intensity of Itgβ1 was normalized to that of the nuclei. * Significantly lower than vehicle-treated WT cells ($P < 0.01$). Bars represent mean ± S.D. (n=5).



Supplementary Fig. S6. PKC inhibition negates 007-AM's impact on MIA PaCa-2's migration and trafficking of integrin β1. (A) Cells were treated with 007-AM (10 μM) in the presence or absence of NPC 15437 (1μM) and invasion/migration was examined by a trans-well migration assay. (B) Cells were trypsinized, treated with 007-AM in the presence or absence of NPC 15437 in a non-permeabilizing buffer, and recovery of surface Itgβ1 was probed by FACS using the Itgβ1 antibody K20 by FACS. Mean fluorescence intensity (MFI) was normalized to vehicle-treated cells. (C) Cells were treated with 007-AM (10 μM) in the presence or absence of Gö 6983 (1μM) and invasion/migration was examined by a trans-well migration assay. ** Significantly higher than vehicle-treated WT cells ($P < 0.02$). * Significantly higher or lower than vehicle-treated WT cells ($P < 0.05$). # Significantly lower than 007-AM-treated cells ($P < 0.05$). Bars represent mean ± S.D. (n=3).