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Supplementary Materials for

Phosphatidylinositol 4-phosphate is a major source of GPCR-stimulated phosphoinositide production

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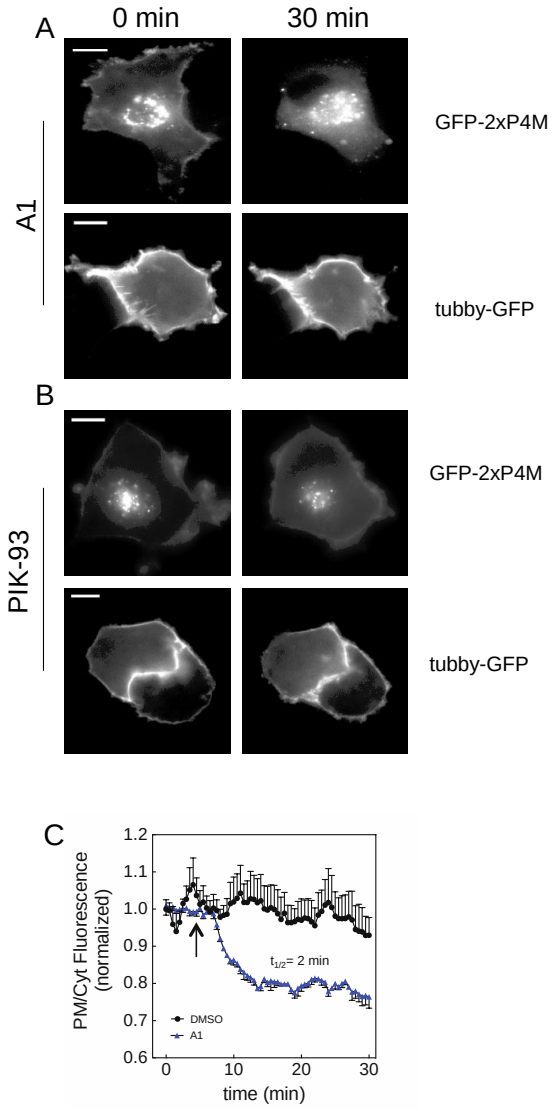

Fig. S1. Effects of A1 and PIK93 on PM PI4,5P$_2$ abundance. (**A** to **C**) PANC-1 cells were transduced with an adenovirus expressing either GFP-2xP4M or tubby-GFP and then were imaged by epifluorescence video microscopy. Shown are representative images before and after a 30-min treatment with (A) 100 nM A1 or (B) 300 nM PIK93. (C) Pooled data from the analysis of videos from the experiments shown in (A) from three independent experiments. The $t_{1/2}$ values were calculated by fitting the data with a single exponential decay function using Graph Pad Prism software.

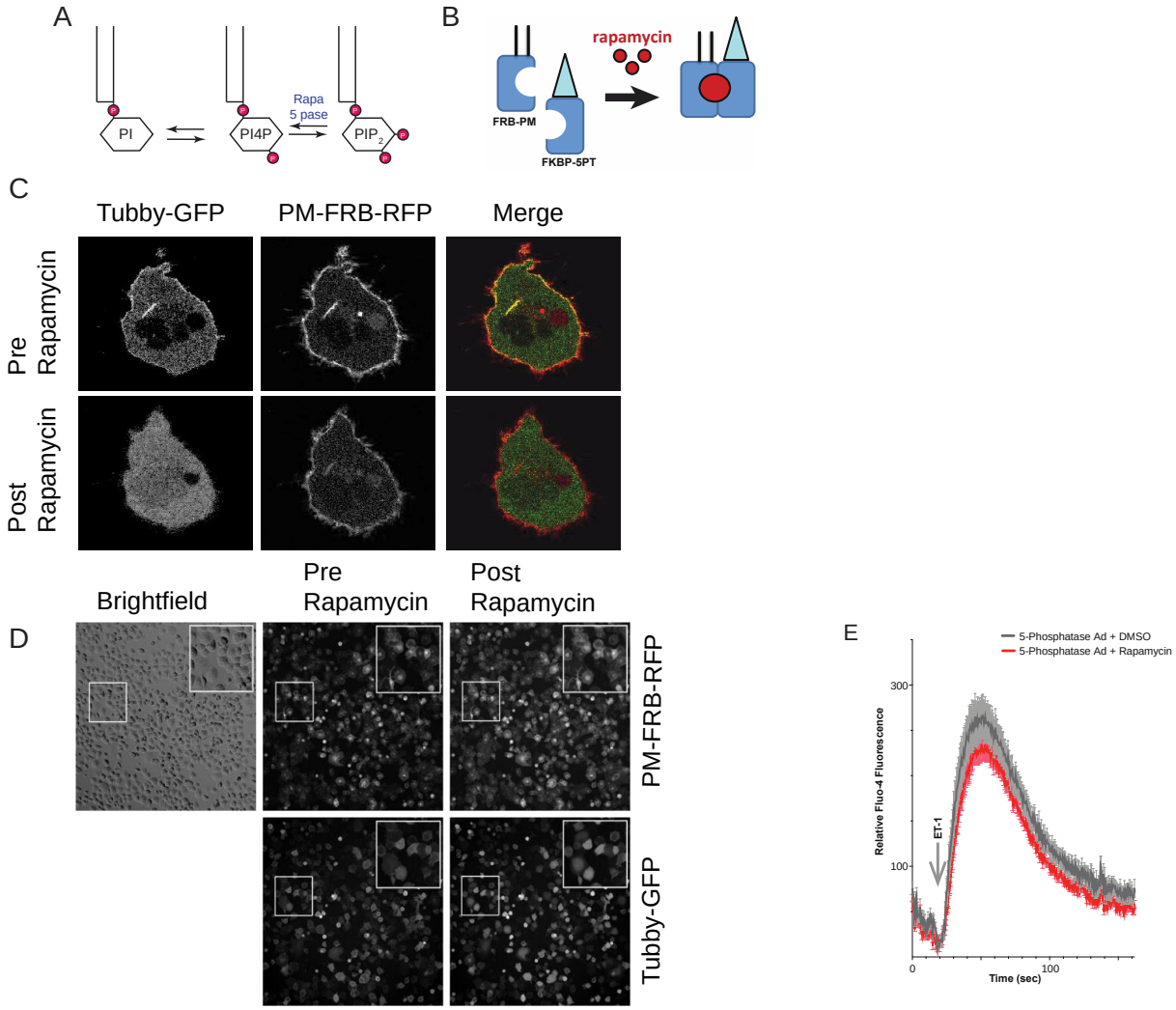

Fig. S2. PI4,5P$_2$ depletion in PANC-1 cells by RAPA-induced targeting of PI5 phosphatase to the PM. (**A** and **B**) Schema outlining (A) the PI4,5P$_2$ depletion strategy and (B) the RAPA-induced FKBP-5-phosphatase. (**C**) A PANC-1 cell transduced with an adenovirus expressing both the PM-FRB-mRFP and the FKBP-5-phosphatase and an adenovirus expressing tubby-GFP. Representative live-cell confocal images are shown of PM-FRB-mRFP in the red channel and tubby-GFP in the green channel before and after treatment with 1 μM RAPA. (**D**) PANC-1 cells were treated as described for (C) except that a 10× lens was used to visualize a wide field of cells to show the high efficiency of transduction and the depletion of PI4,5P$_2$. (**E**) MEFs were transduced with an adenovirus expressing both the PM-FRB-mRFP and the FKBP 5-phosphatase, loaded with Fluo-4, and then imaged in the wells of a 96-well plate before and after the addition of 100 nM ET-1 with and without the prior addition of RAPA for 20 min. All experiments in (C) to (E) were repeated at least three times and representative figures are shown.