

SUPPLEMENTARY ONLINE DATA

Immunocytochemical techniques reveal multiple, distinct cellular pools of PtdIns4P and PtdIns(4,5)P₂

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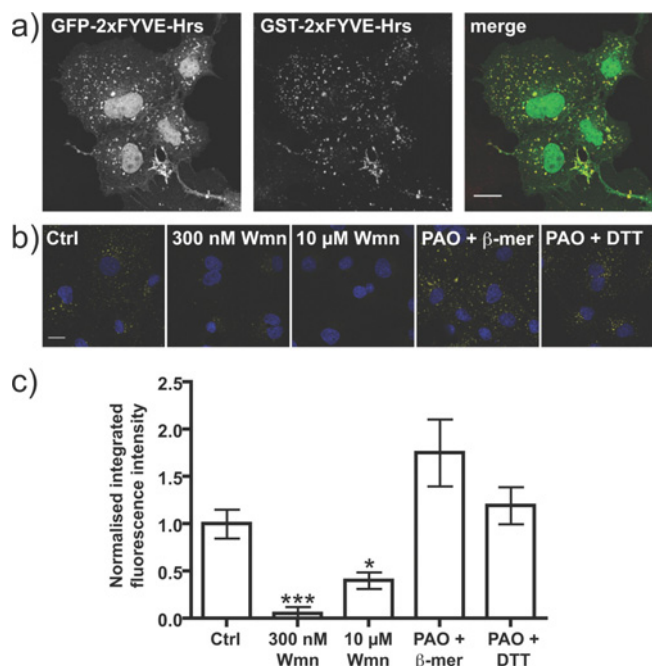


Figure S1 PtdIns3P staining with GST-2x FYVE-Hrs

(a) COS-7 cells expressing GFP-tagged 2x FYVE-Hrs were fixed and were co-stained using an anti-GST antibody against GST-2x FYVE-Hrs as described in Figure 3. (b) Cells were pre-treated with the indicated inhibitor as described in Figure 6. Scale bars = 20 μm. (c) Quantification of results shown in (b) (n = 30 cells). See the Experimental section for details. *P < 0.05, ***P < 0.001.

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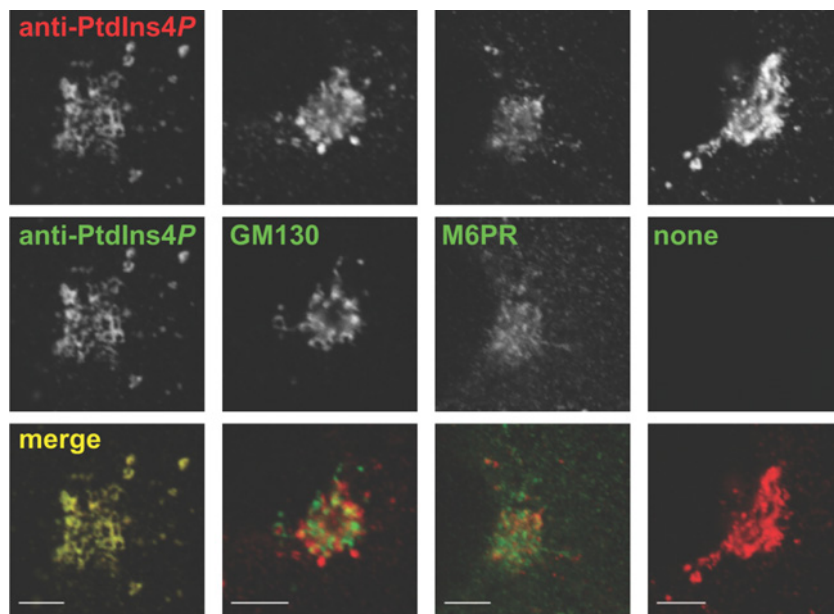


Figure S2 Anti-PtdIns4P antibody co-labelling with Golgi markers

COS-7 cells were fixed and stained with the indicated antibodies as described in Figure 3(a).

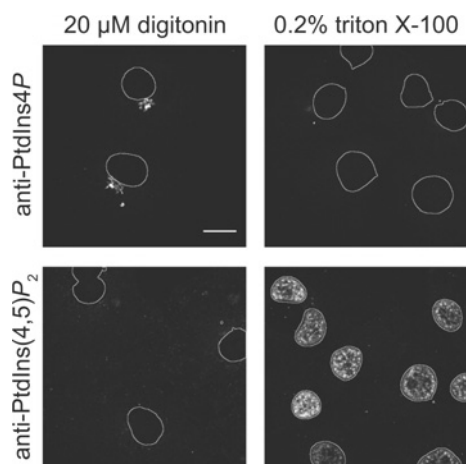


Figure S3 Effect of detergent on PtdIns4P/PtdIns(4,5)P₂ staining after FA fixation and room temperature staining

COS-7 cells were fixed and processed as in Figure 3, except cells were permeabilised with either 20 μ M digitonin or 0.2 % Triton X-100 as indicated, then stained with anti-PtdIns4P or anti-PtdIns(4,5)P₂ antibodies. DAPI stained nuclei are outlined. Scale bar = 20 μ m.

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