$PI(3,5)P_2$ Controls Membrane Traffic by Direct Activation of Mucolipin Ca^{2+} Release Channels in the Endolysosome

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Running head: TRPML1 is an endolysosomal Ca^{2+} release channel specifically activated by $PI(3,5)P_2$

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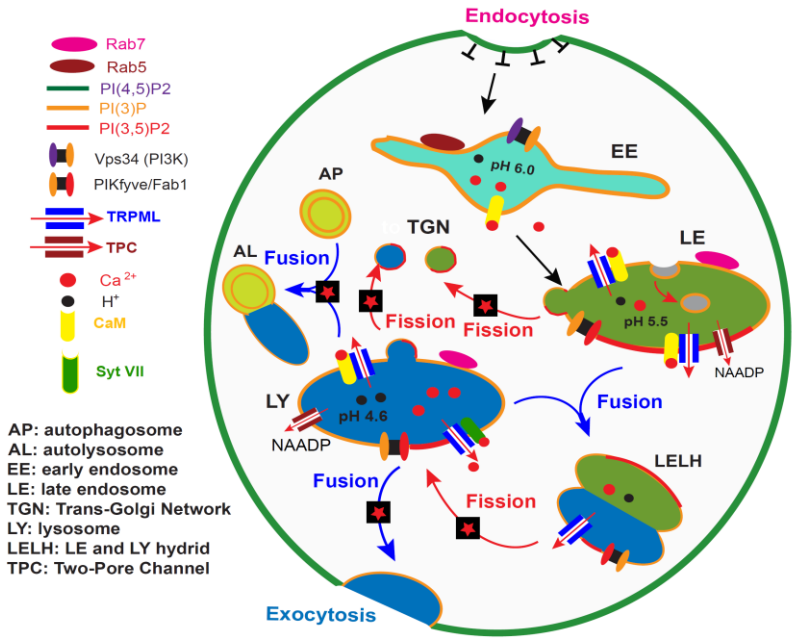
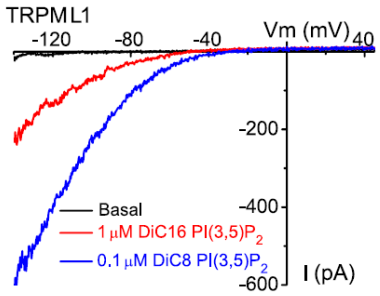
Supplementary Figures S1-S6



Supplementary Figure 1. TRPMLs and PIPs in the endocytic pathway.

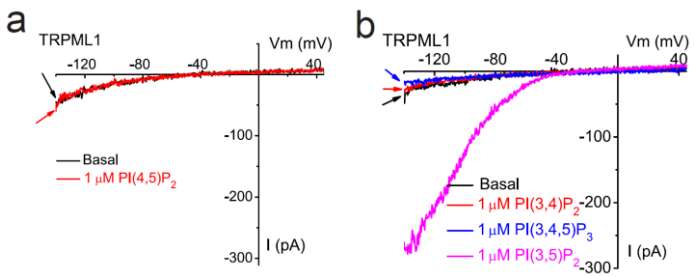
Intracellular compartments in the endocytic pathways undergo cargo-dependent maturation (indicated by black arrows), membrane fusion (blue arrows), and fission/budding (red arrows). The molecular identities of intracellular compartments were defined by specific recruitment of Rab GTPases, and phosphoinositide (PIP) composition. PI(3)P is synthesized by Vps34 PI 3-kinase in early endosomes (EEs). $PI(3,5)P_2$ is presumed to be produced in late endosomes and lysosomes (LELs) by PIKfyve/Fab1 PI 5-kinase using PI(3)P as substrate. Endolysosomes are Ca^{2+} stores, with a luminal Ca^{2+} concentration estimated to be approximately 0.5 mM. The pH of each organelle is indicated. In endolysosomes, TRPML-mediated intra-endosomal Ca^{2+} release may activate Ca^{2+}-sensor proteins such as synaptotagmin (Syt) and calmodulin (CaM) to trigger homotypic and heterotypic fusion. Early endosomes (EEs; pH 6.0; PI(3)P;) are derived from the primary endocytic vesicles after endocytosis. EEs can undergo maturation through membrane trafficking to become late endosomes (LEs; pH 5.5; PI(3)P + $PI(3,5)P_2$). LEs can fuse with lysosomes (LYs; pH 4.5; PI(3)P+$PI(3,5)P_2$) to form LE-LY hybrids. LYs can be reformed from LE-LY hybrids (LELH) in a fission-dependent mechanism. Besides fusion with LEs, LYs also fuse with autophagosomes (APs) to form autolysosomes (ALs), or fuse with the plasma membrane in exocytosis. TRPML1-3 channels are predominantly localized in LEs and LYs. Activation of TRPML channels by $PI(3,5)P_2$ may induce intralysosomal Ca^{2+} release. Alternatively, activation of Two-pore Channels (TPCs) by NAADP may also induce intralysosomal Ca^{2+} release. LEs,

LYs, or LE-LY hybrids undergo CaM- or Syt- dependent membrane fusion or fission and budding. Retrograde transport vesicles, derived from LEs or LYs upon membrane fission, transport lipids and proteins in a retrograde direction to the trans-Golgi Network (TGN). Stars indicate membrane fusion and fission processes that are reportedly defective in both TRPML1-deficient and $PI(3,5)P_2$-deficent cells.



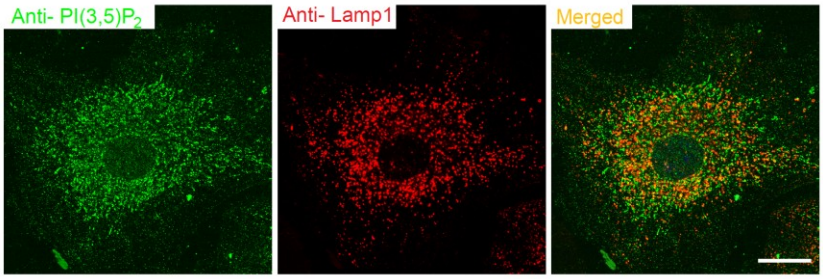
Supplementary Figure 2. Activation of TRPML1 by full length diC16 $PI(3,5)P_2$.

Long-chain phosphoinositide diC16 $PI(3,5)P_2$ activated whole-endolysosome I_{TRPML1}. I_{TRPML1} was elicited by voltage ramps (-140 to +140 mV; 400 ms).



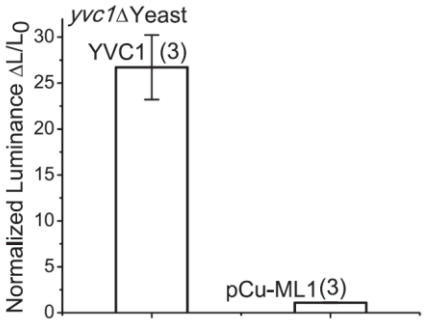
Supplementary Figure 3. Specific activation of TRPML1 by $PI(3,5)P_2$.

a) Whole-endolysosome I_{TRPML1} was insensitive to $PI(4,5)P_2$ (1 μM). **b)** Insensitivity of I_{TRPML1} to $PI(4,5)P_2$ (1 μM) or $PI(3,4,5)P_3$ (1 μM). $PI(3,5)P_2$ readily activated I_{TRPML1} in the same vacuole.

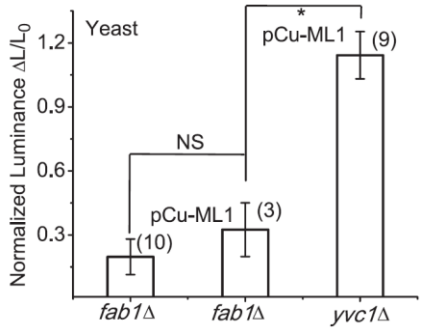


Supplementary Figure 4. Immuofluorescence of $PI(3,5)P_2$ in Lamp1-positive compartments.

Mouse fibroblast cells were immunostained with anti-$PI(3,5)P_2$ (green) and anti-Lamp1 (red). The majority of Lamp1-positive compartments were immunostained by anti-$PI(3,5)P_2$, which also recognized some Lamp1-negative compartments. Scale Bar = 10 μm.



Supplementary Figure 5. Overexpression of TRPML1 in *fab1*Δ cells failed to restore hyperosmolarity-induced Ca^{2+} response. TRPML1 is expressed using the pCu expression vector (pCu-ML1). Hyperosmolarity-induced Ca^{2+} responses were measured using the aequorin-mediated luminescence assay. Hyperosmotic shock was induced by addition of 0.9 M NaCl. Hyperosmolarity-induced Ca^{2+} responses (normalized with baseline luminescence before hyperosmotic shock) from *fab1*Δ cells, and pCu-ML1-transformed *yvc1*Δ cells were re-plotted from Fig. 6 for comparison.



Supplementary Figure 6. Overexpression of YVC1 in *yvc1*Δ cells restored hyperosmolarity-induced Ca^{2+} response. YVC1 is expressed using the pVT expression vector. Hyperosmolarity-induced Ca^{2+} responses were measured using the aequorin-mediated luminescence assay. Hyperosmotic shock was induced by addition of 0.9 M NaCl. Hyperosmolarity-induced Ca^{2+} responses from pCu-ML1-transformed *yvc1*Δ cells were re-plotted from Fig. 6 for comparison.