Investigating the interplay between PIKfyve/PI(3,5)P2 and ClC-7 in lysosomal acidification and trafficking

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PI(3,5)P2 is a low abundant phospholipid synthesized by the lipid kinase PIKfyve and found in the membrane of late endosomes and lysosomes. Following pharmacological inhibiton of PIKfyve, PI(3,5)P2 depletion quickly and drastically impairs late endosomal/lysosomal formation, leading to the generation of big vacuoles. In a recent study, Gayle et al. observed that the deletion of ClC-7, a lysosomal transporter thought to facilitate lysosomal acidification, abolished the effects of PIKfyve inhibition, including vacuole formation (Gayle et al., Blood, 2017).
In this new study, we are investigating the interplay between PIKfyve/PI(3,5)P2 and ClC-7 in lysosomal acidification and vacuole formation.

Open Questions

Does PI(3,5)P2 level impact lysosomal/vacuole pH?
Is ClC-7 a target of PI(3,5)P2?
Is the lysosomal pH essential for the vacuole formation?

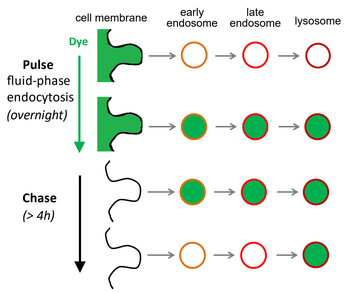
Main Results

PI(3,5)P2 depletion leads to lysosomal/vacuole hyperacidification.
Knocking out ClC-7 protects against lysosomal hyperacidification, but not vacuolization.
Lysosomal alkalanizing agent (chloroquine) does not prevent vacuolization.

Conclusions

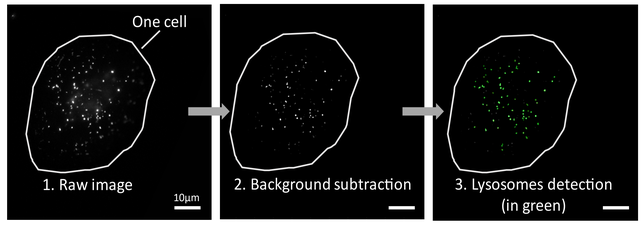
PI(3,5)P2/PIKfyve activity inhibits lysosomal acidification through ClC-7.
Vacuolization is not link to lysosomal hyperacidification.

How to "dye-load" lysosomes



How to extract lysosomal fluorescence

We use the software Slicer2D to detect fluorescent lysosomes and extract through image processing their size and fluorescent intensity at 488nm and 445nm.

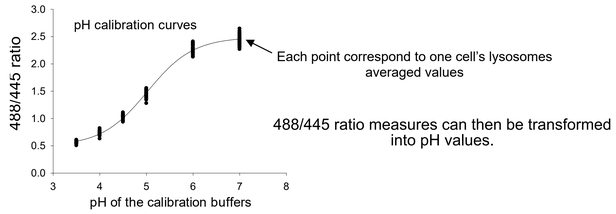


For each cell, the 488/445 ratio of each individual fluorescent lysosome is measured.

How to measure lysosomal pH

Oregon Green 488 Dextran is a pH ratiometric fluorescent dye.
The 488nm/445nm ratio will predictibly be affected by pH.

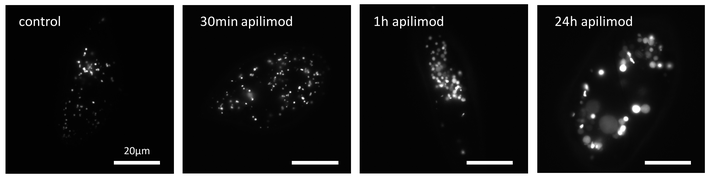
Monensin and Nigericin are ionophores that equilibrates the pH between media, cytoplasm and lysosomes. Measuring 488/445 lysosomal ratio in pH calibrated buffers in presence of Monensin and Nigericin generates a pH calibration curves.

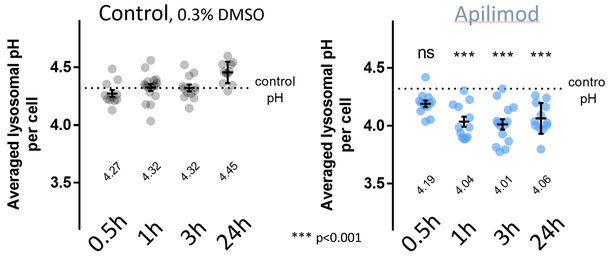


1) Does PI(3,5)P2 level impact lysosomal pH?

Apilimod is a PIKfyve inhibitor.

Timecourse effect of apilimod 100nM on lysosomal pH of U2OS cells.





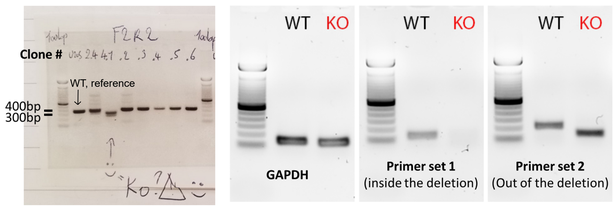
Representative data. Data replicated in two independent experiments.

PI(3,5)P2 depletion leads to hyperacidic lysosomes/vacuoles.

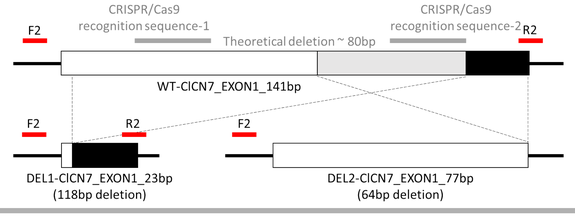
2) Is ClC-7 involved in hyperacidification?

A Construction of a U2OS ClC7-KO clone through CRISPR/Cas9 approach (LentiCRISPRv2-GeCKO)

Because no antibody tagerting ClC-7 are available, we selected and validated the ClC7-KO clone at the DNA/RNA level

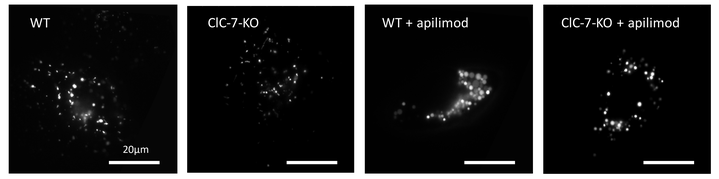


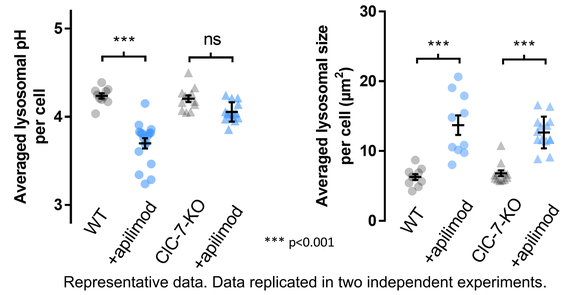
Model of the two DEL-ClCN7 alleles present in the ClC7-KO clone created after sequencing:



We have generated a ClC7-KO U2OS cell-line.

B Effect of apilimod (100nM, 3h) on lysosomal pH in ClC-7-KO U2OS cells.



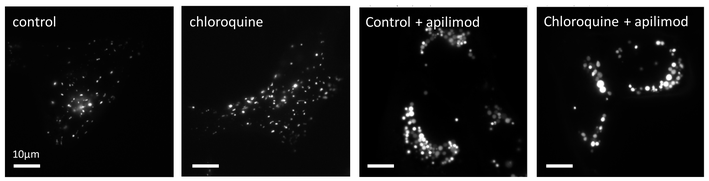


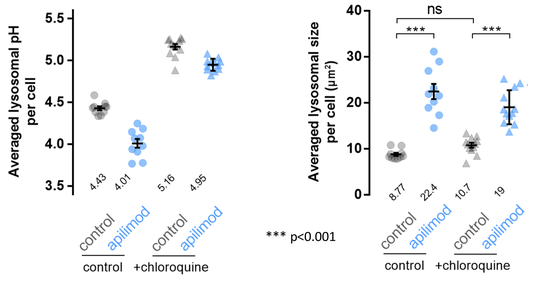
Representative data. Data replicated in two independent experiments.

ClC-7 is implicated in lysosomal hyperacidification, but not vacuolization.

3) Is lysosomal hyperacidification linked to vacuole formation?

Effect of choloroquine (5µM, overnight) on apilimod effect (100nM, 3h) in U2OS cells.

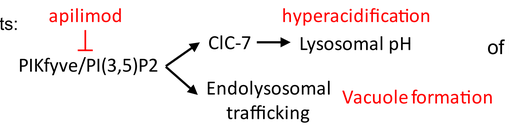




Lysosomal hyperacidification is not essential for vacuolization.

Results interpretation

Model supported by our results:



PIKFyve/PI(3,5)P2 regulation of lysosomal pH and endolysosomal trafficking are not connected.

Perspectives

Is the kinase activity of PIKfyve or its product, PI(3,5)P2, that regulates ClC-7?
Transfection of PI(3,5)P2; Inhibition of PI(3)P production (the substrate required to produce PI(3,5)P2).

Direct evidence for PI(3,5)P2 regulation/inhibition of ClC-7 activity?
Patch clamp of HEK cells expressing ClC-7 at the plasma membrane.

Is ClC-7 important for lysosomes reformation after releasing of PIKfyve inhibition?
PIKfyve inhibition by apilimod is reversible, and is associated with reformation of normal sized lysosomes in a timescale of hours.