



HAL
open science

Investigating the interplay between PIKfyve and ClC-7 in lysosomal acidification and trafficking

Xavier Leray, Anowarul Amin, Mary Weston, Joseph Mindell

► To cite this version:

Xavier Leray, Anowarul Amin, Mary Weston, Joseph Mindell. Investigating the interplay between PIKfyve and ClC-7 in lysosomal acidification and trafficking. Gordon Research Conference on Organellar Channels and Transporters, Aug 2019, West Dover, VT, United States. . hal-04653194

HAL Id: hal-04653194

<https://hal.inrae.fr/hal-04653194v1>

Submitted on 18 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Investigating the interplay between PIKfyve and CIC-7 in lysosomal acidification and trafficking.

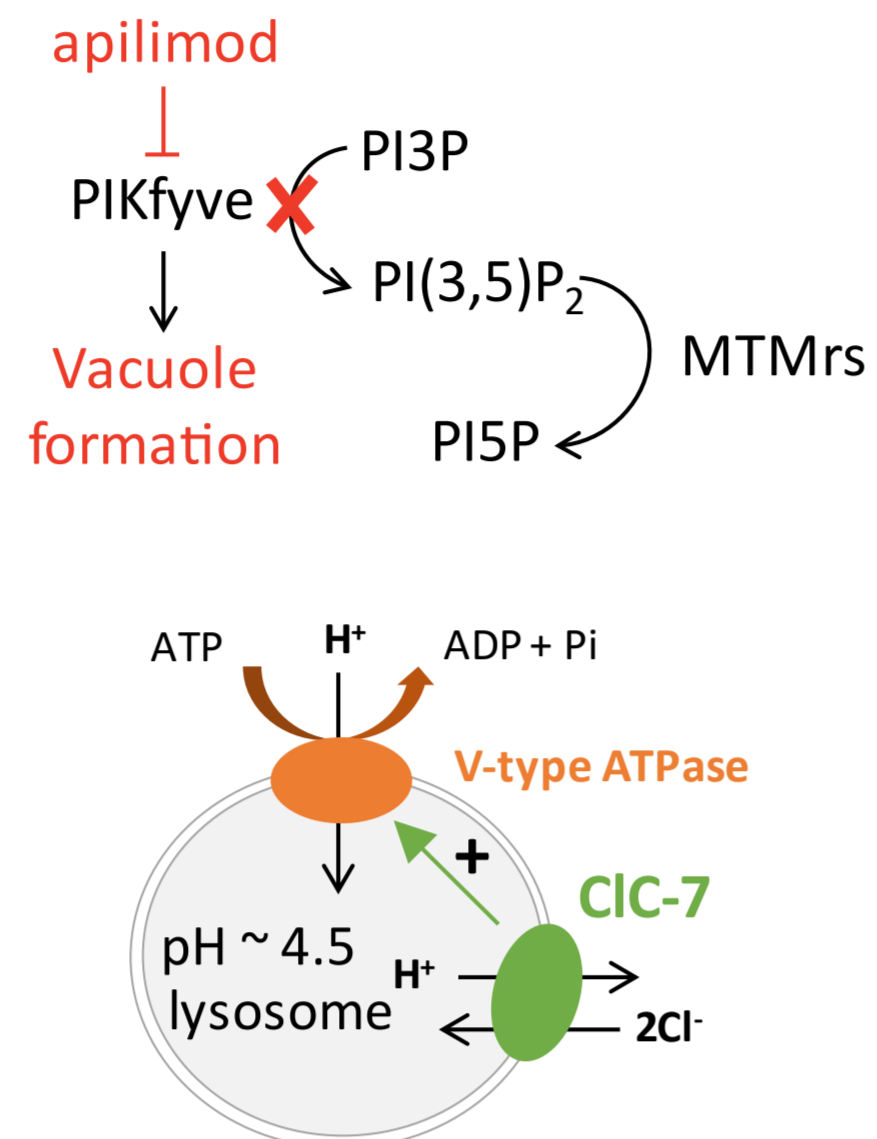


Xavier Leray, Anowarul Amin, Mary Weston, Joseph Mindell

Membrane Transport Biophysics Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda



PIKfyve is a lipid kinase found in the membrane of late endosomes and lysosomes. It is responsible for the synthesis of the phospholipid PI(3,5)P₂. PIKfyve inhibition quickly and drastically impairs late endosomal/lysosomal formation, leading to the generation of big vacuoles. Recently, knocking out lysosomal Cl⁻/H⁺ exchanger CIC-7 has been proposed to provide substantial resistance against this vacuole phenotype. Given that CIC-7 is suggested to play a role in lysosomal acidification, it raises the possibility that PIKfyve may regulate CIC-7 activity, in turn modulating lysosomal pH, and that endosomal/lysosomal pH could tune endocytic trafficking. In this new study, we are investigating the interplay between PIKfyve and CIC-7 in lysosomal acidification and vacuole formation.

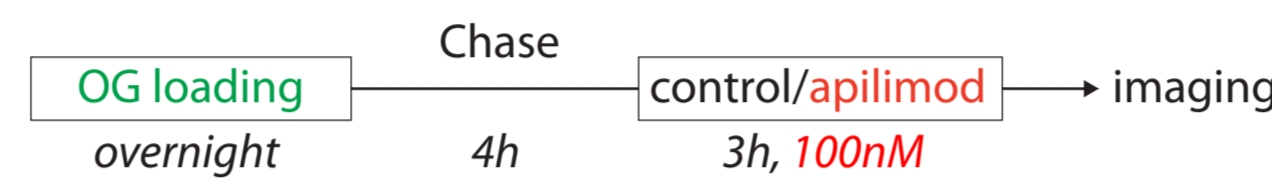


Open Questions

- Is lysosomal pH altered under PIKfyve inhibition?
- Is CIC-7 critical for this process?
- Is lysosomal pH critical for vacuole formation?

1) PIKfyve inhibition hyperacidifies lysosomes

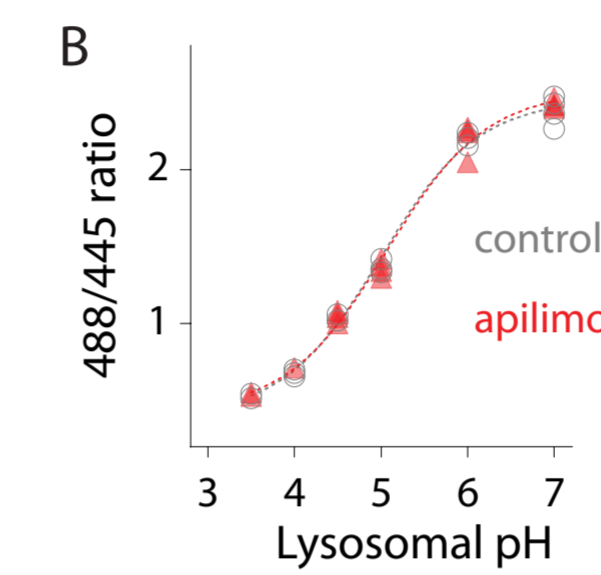
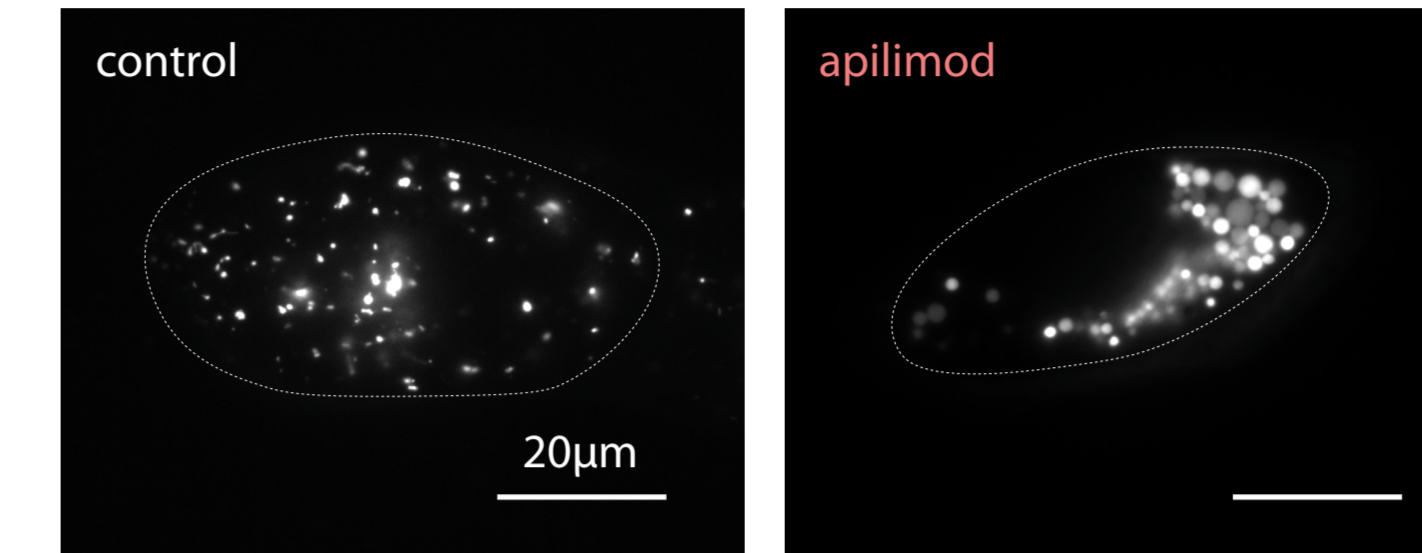
Apilimod is a PIKfyve inhibitor.
OG is a pH-sensitive ratiometric dye.



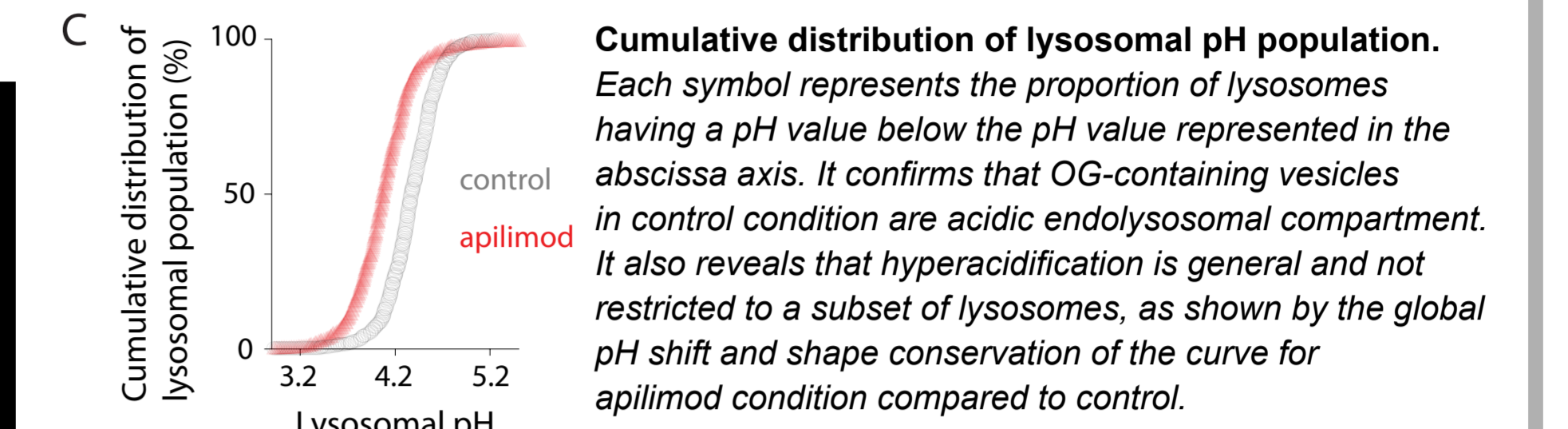
For method explanations, see the bottom center section of this poster.

First, Oregon Green 488 dextran (OG) is loaded into the endocytic pathway by fluid phase endocytosis. Then, OG is washed out from the media and a 4h incubation period let OG-containing endosomes to mature and eventually fuse with lysosomes. Finally cells are incubated for 3h with the PIKfyve inhibitor apilimod before performing pH-ratiometric live cell imaging with an epifluorescence microscope.

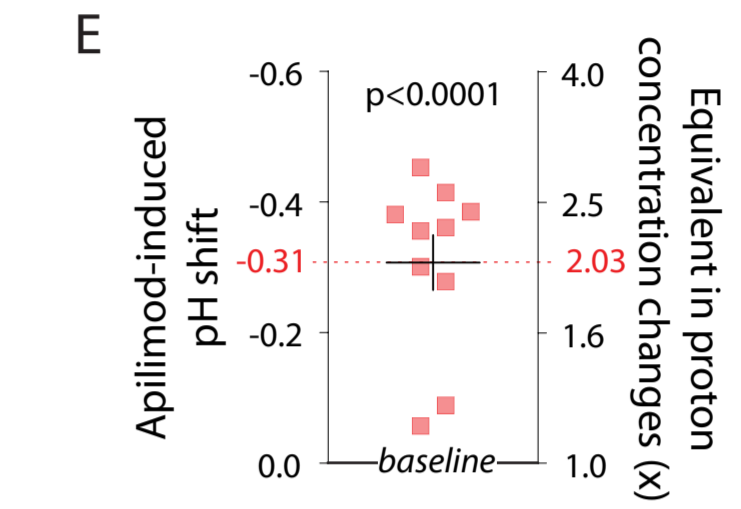
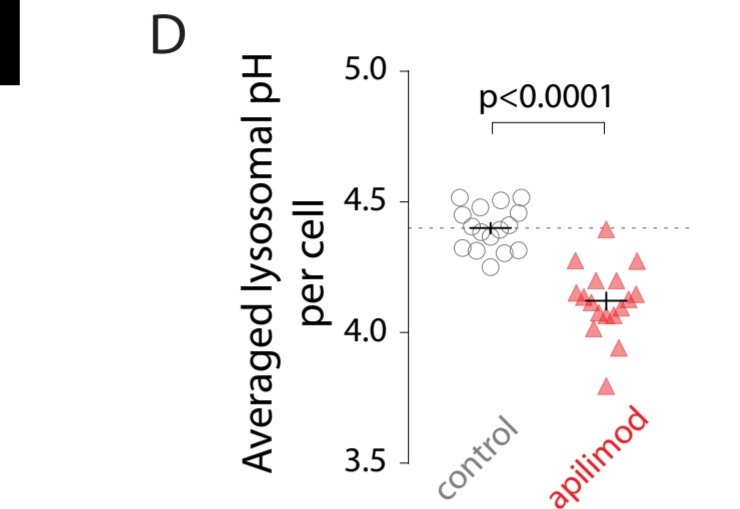
A Images of live U2OS cells lysosome-loaded with OG (bright spots).



pH calibration curves for control and apilimod treated cells are similar. This control experiment indicates that pH measurements is not altered when done on big vacuoles instead of small lysosomes. pH measured in both conditions can thus be compared without bias.



Cumulative distribution of lysosomal pH population. Each symbol represents the proportion of lysosomes having a pH value below the pH value represented in the abscissa axis. It confirms that OG-containing vesicles in control condition are acidic endolysosomal compartment. It also reveals that hyperacidification is general and not restricted to a subset of lysosomes, as shown by the global pH shift and shape conservation of the curve for apilimod condition compared to control.

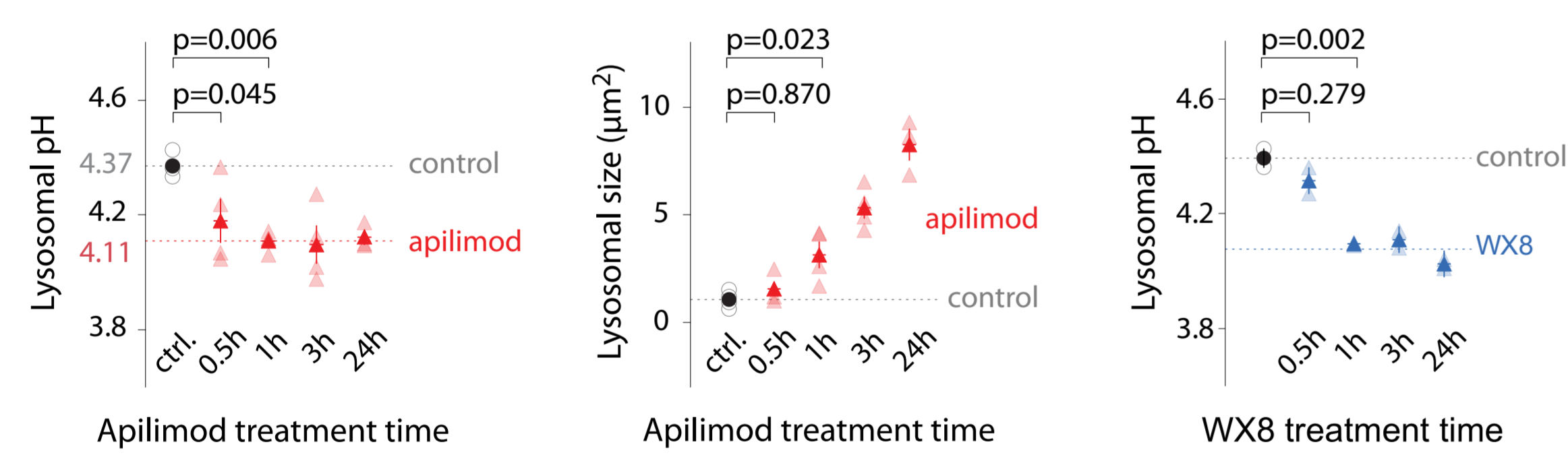
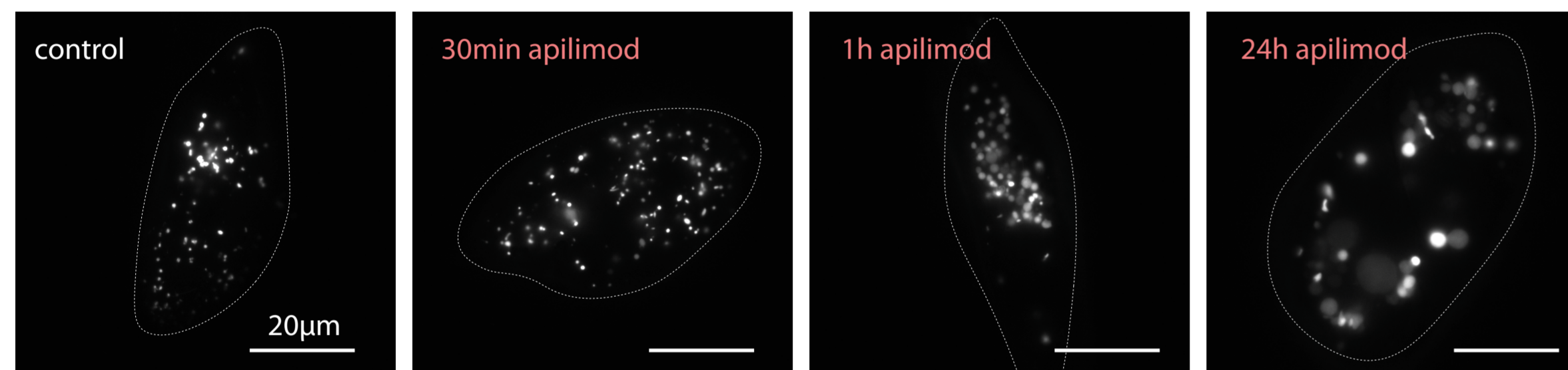
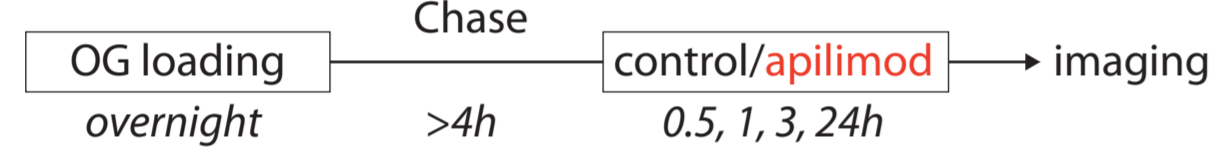


(E) **Lysosomal pH.** Representative experiment. Each dot represents the averaged lysosomal pH of one cell (n=16-17). (F) **pH-shift.** Each dot represents the apilimod-induced lysosomal pH shift measured in one experiment (n=10).

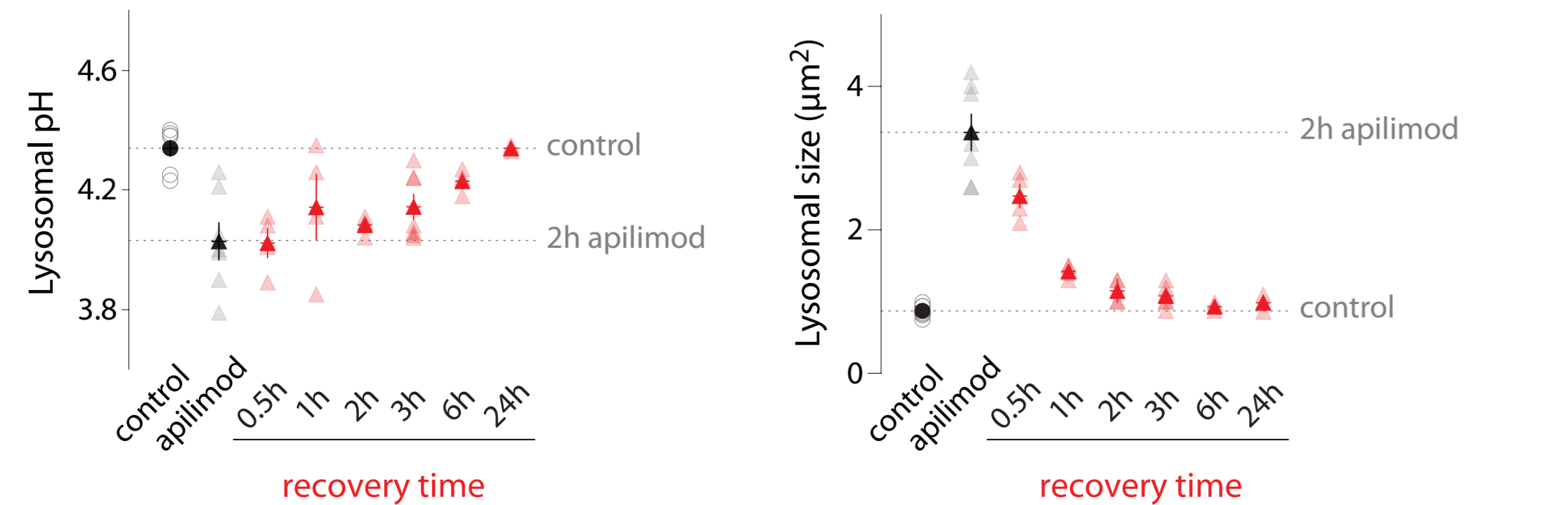
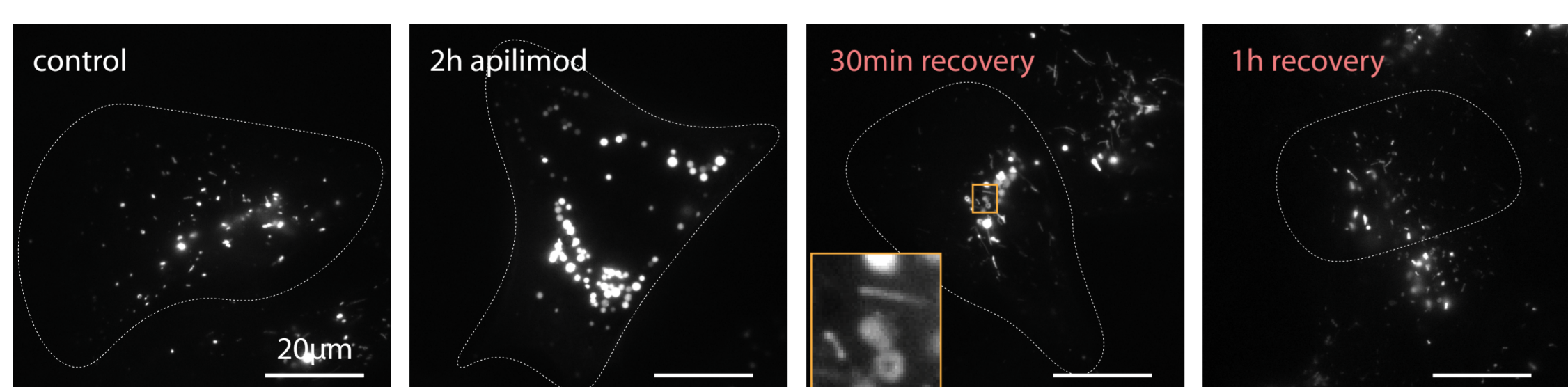
2) Does lysosomal pH alteration and increase in size correlate?

Apilimod and WX8 are PIKfyve inhibitors.

A Evolution of lysosomal pH and size during PIKfyve inhibition.



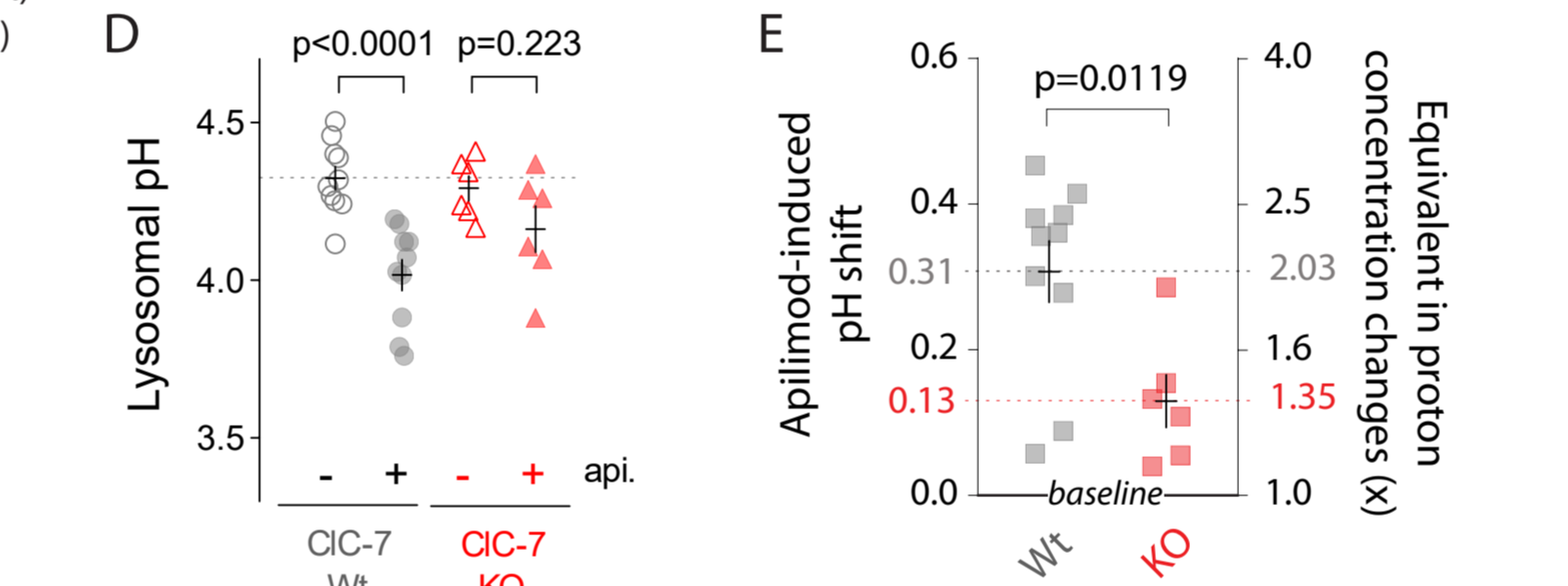
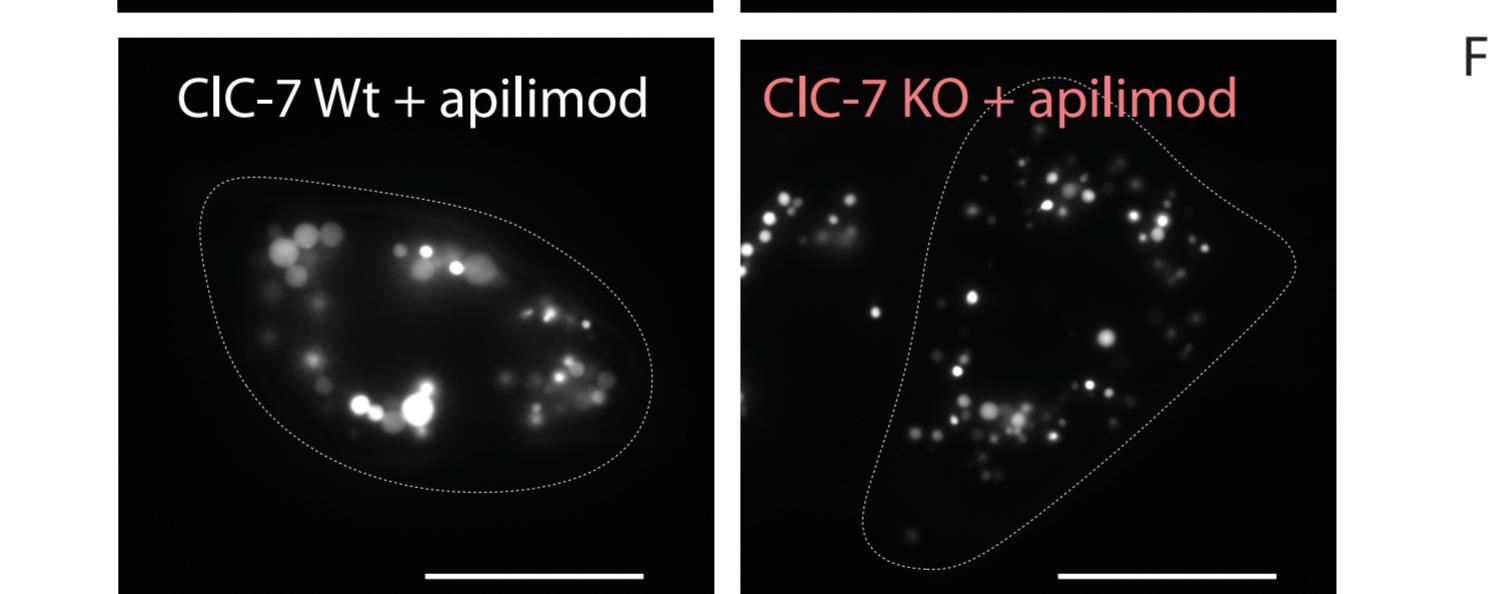
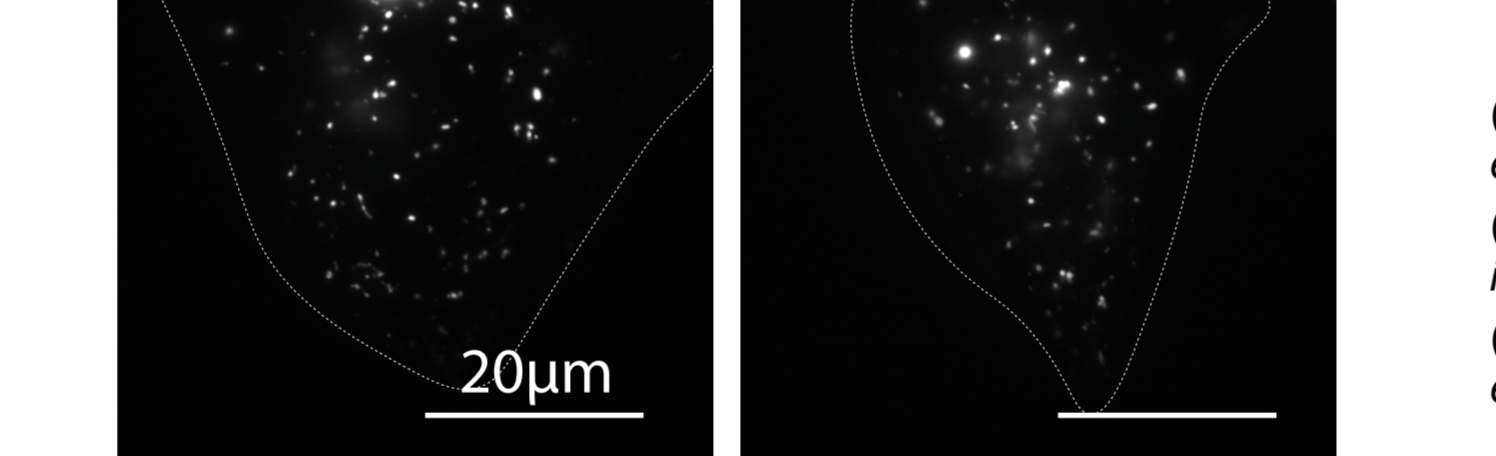
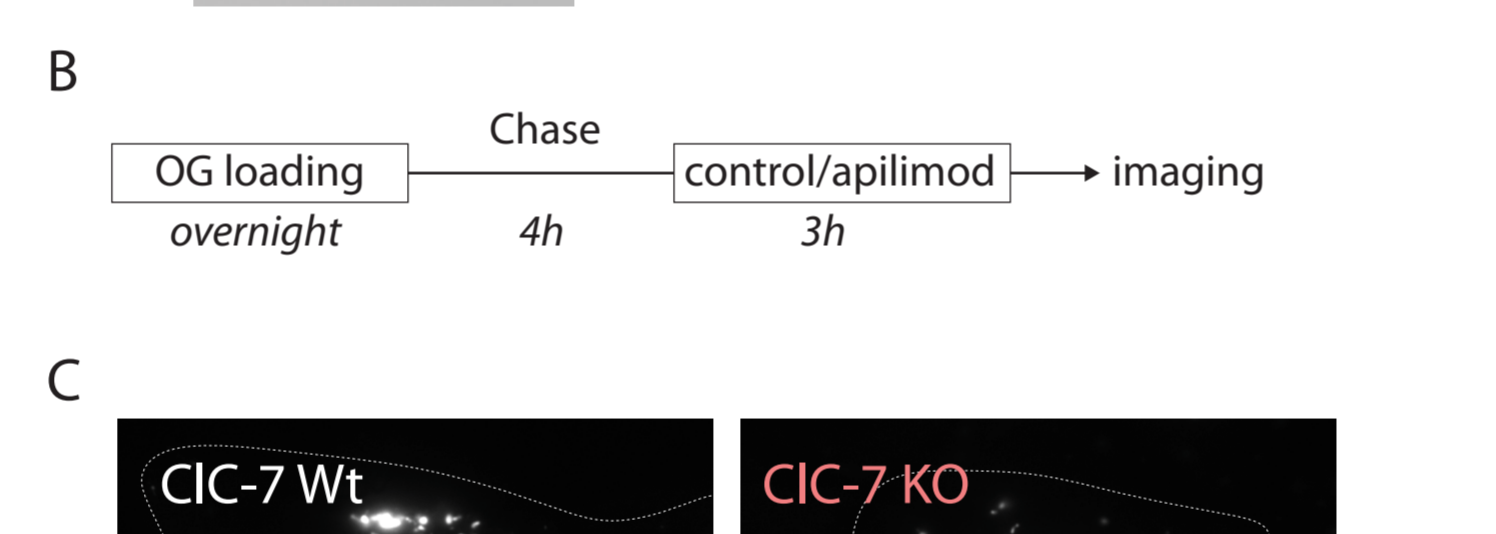
B Evolution of lysosomal pH and size after PIKfyve removal.



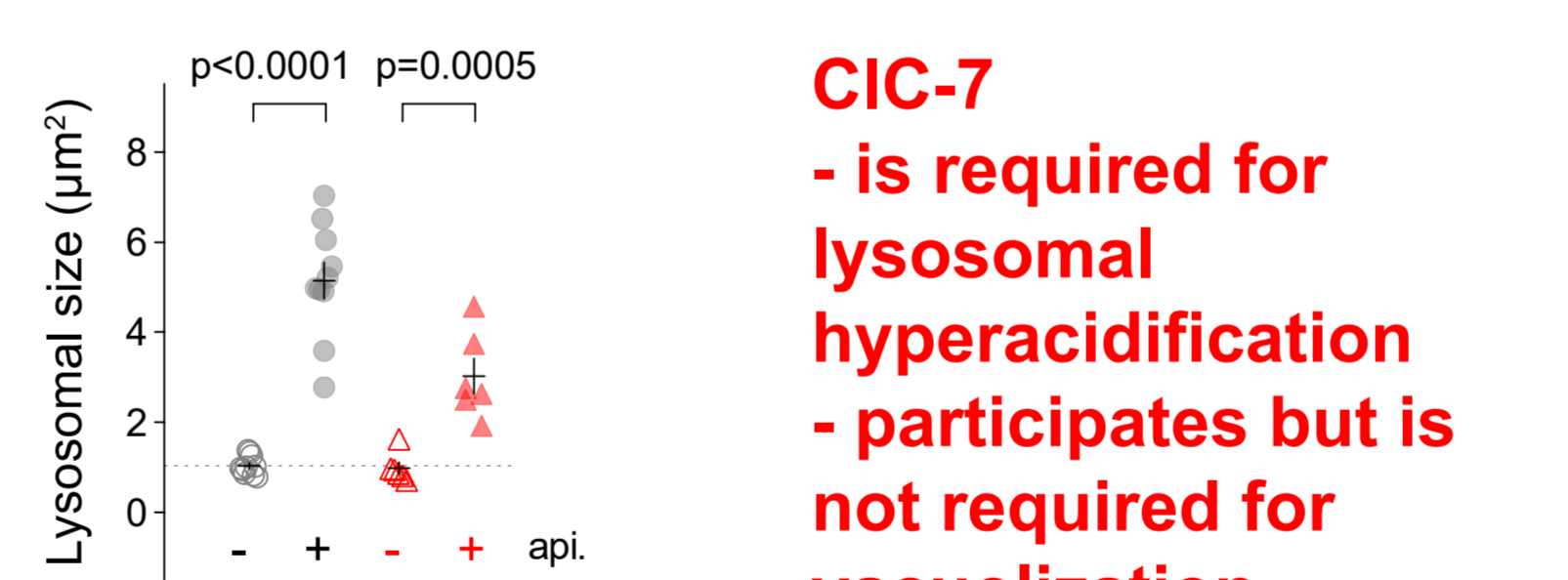
Evolutions of lysosomal pH and size show different kinetics.

3) Is CIC-7 involved in hyperacidification?

CRISPR-Cas9 CIC-7 KO in U2OS cells



(D) **Lysosomal pH.** Each dot represents average of cells lysosomal pH in one experiment (n=10 and n=6 for CIC-7 Wt and KO, respectively). (E) **pH-shift.** Each dot represents the apilimod-induced lysosomal pH-shift measured in one experiment.

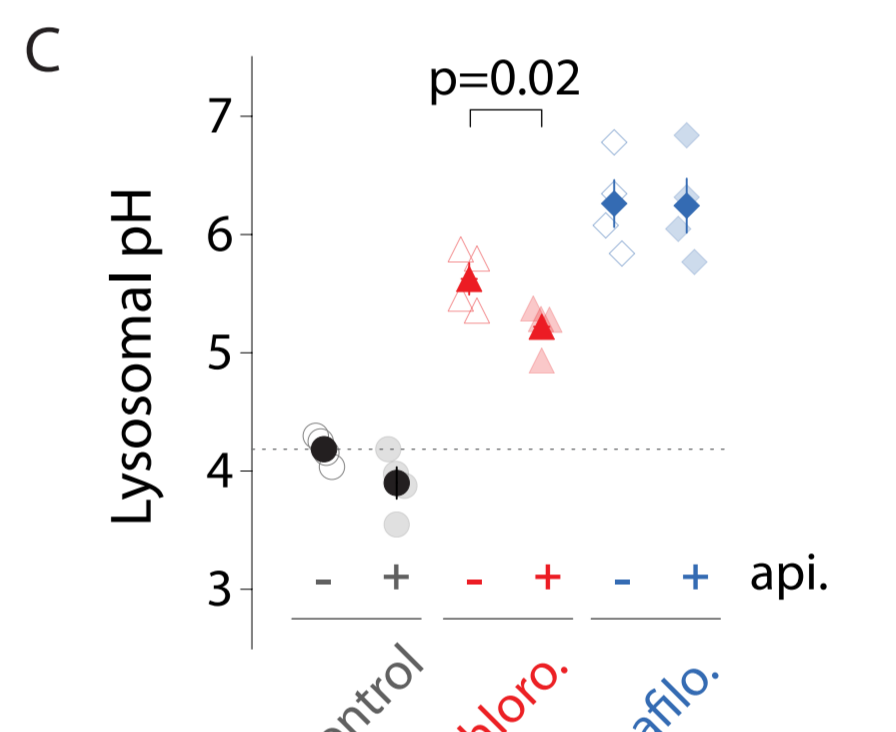
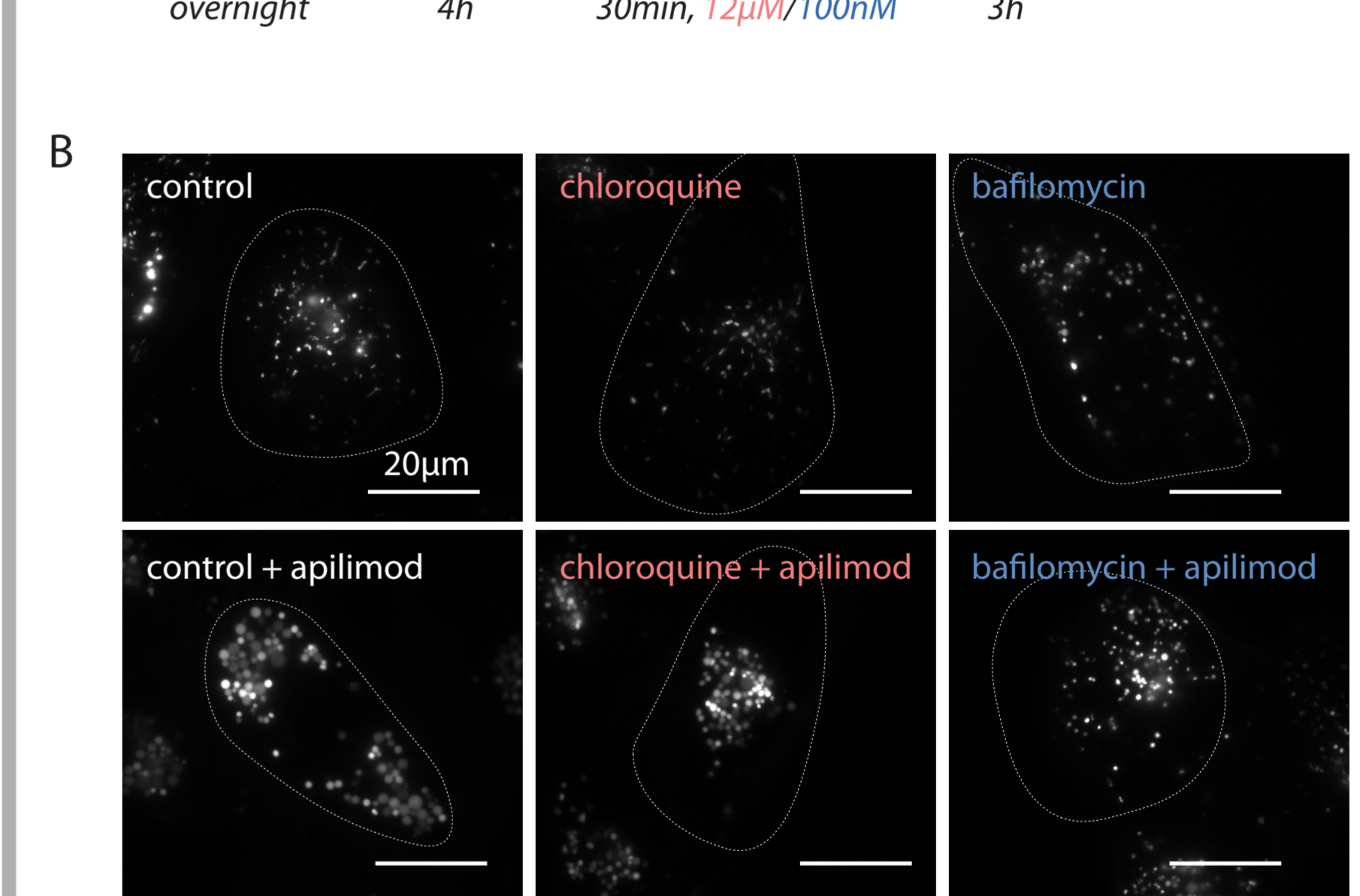
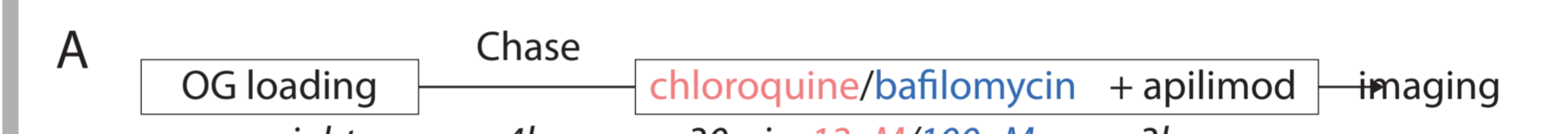


CIC-7 - is required for lysosomal hyperacidification - participates but is not required for vacuolization.

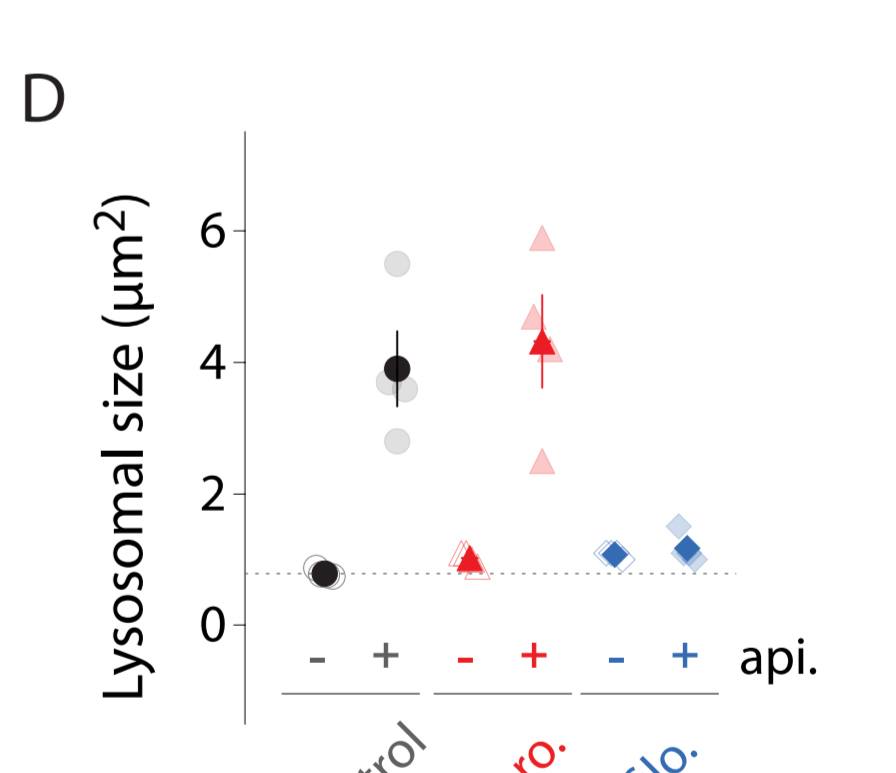
4) Is lysosomal hyperacidification involved in vacuole formation?

Chloroquine is a lysosomotropic agent. Bafilomycin-A1 is a v-ATPase inhibitor.

Effect of lysosomes pre-alkalinization on apilimod treatment.

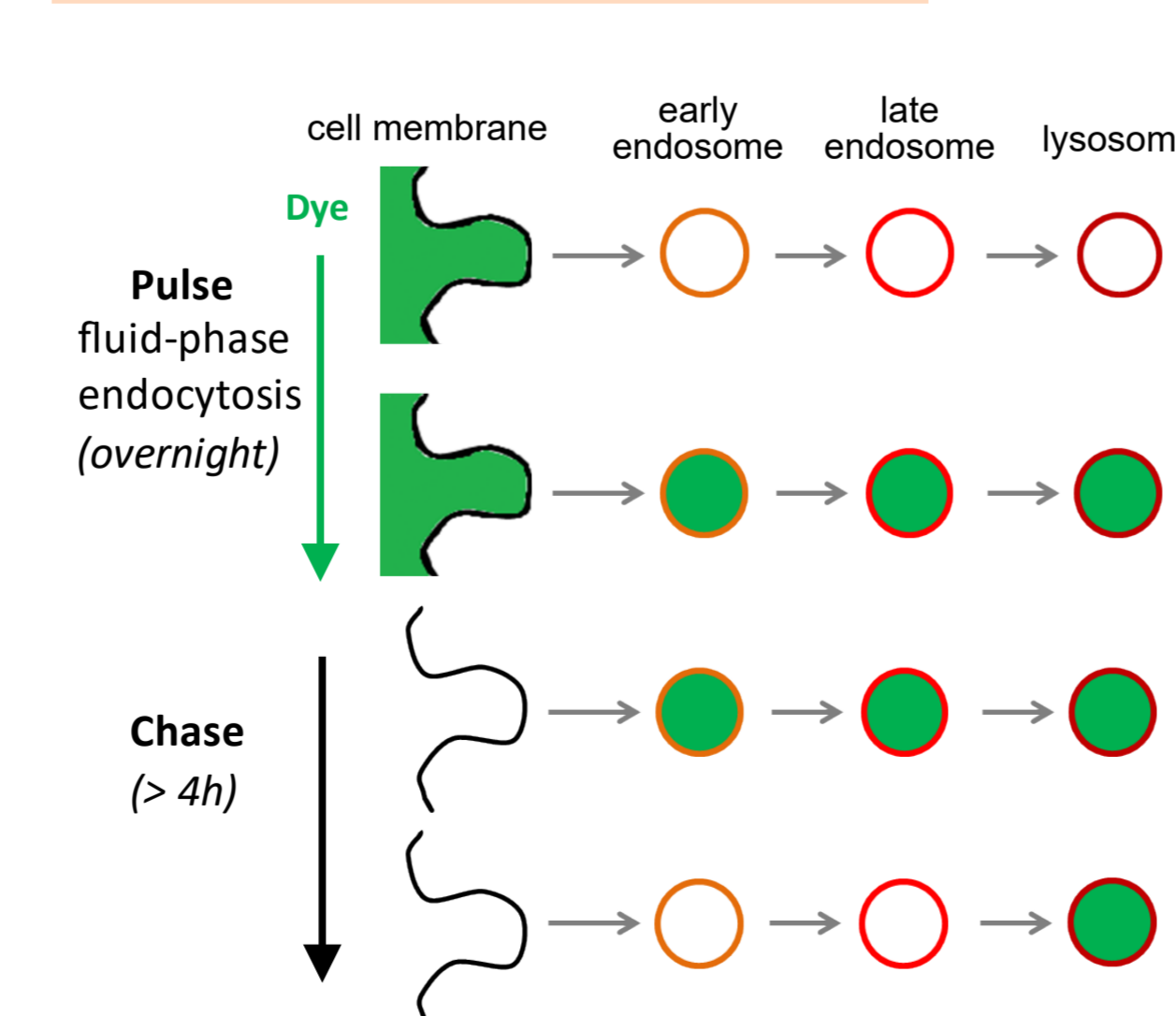


(C/D) **Lysosomal pH/size.** Each dot represents average of cells lysosomal pH/size in one experiment (n=4).



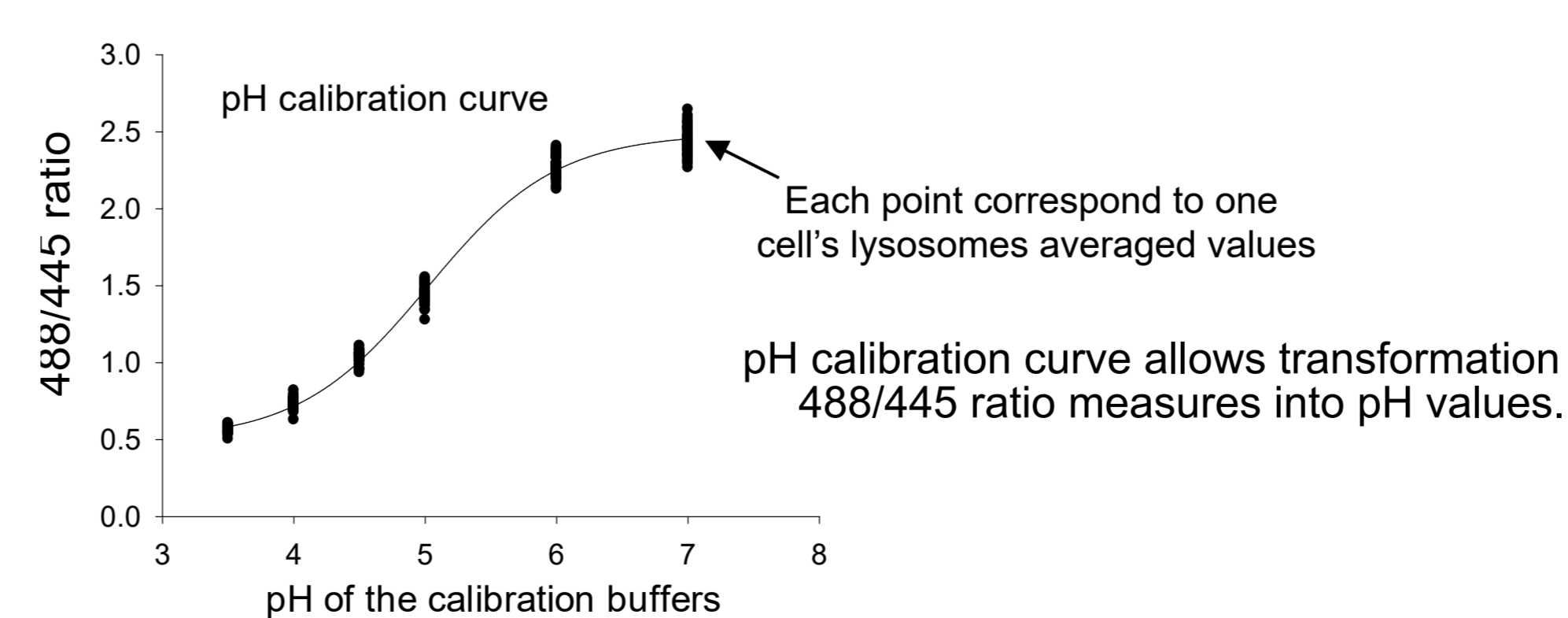
Lysosomal pH is not critical for lysosomal swelling.

How to "dye-load" lysosomes

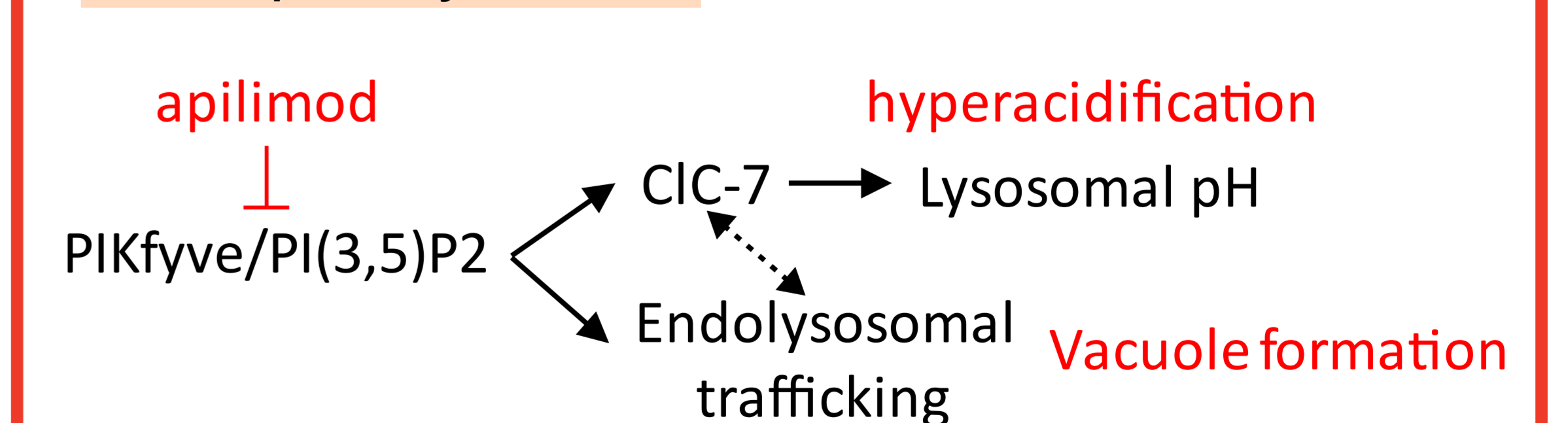


How to measure lysosomal pH

Oregon Green 488 Dextran is a pH ratiometric fluorescent dye. The 488nm/445nm ratio will predictably be affected by pH. Monensin and Nigericin are ionophores that equilibrate pH between media, cytoplasm and lumen of lysosomes. Measures of 488/445 lysosomal ratio in pH calibrated buffers in presence of Monensin and Nigericin can be plotted as a function of buffers pH, generating a pH calibration curve as depicted below.



Model supported by our results



Conclusions

- PIKfyve might regulate lysosomal pH through inhibition of CIC-7 activity.
- CIC-7 activity, but not lysosomal pH, might participate in endolysosomal trafficking.