

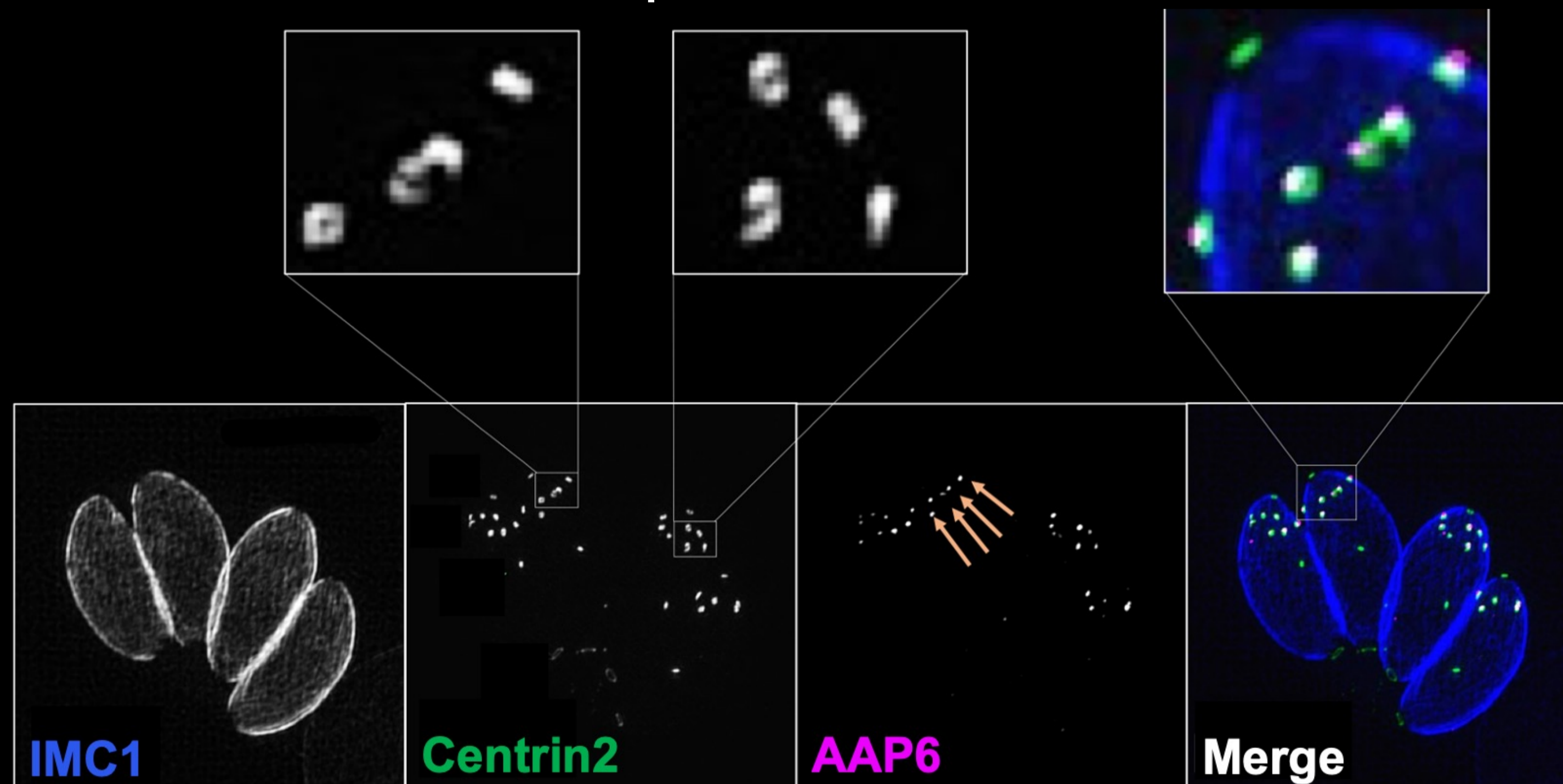
# Apical Annuli are essential exocytic sites in *Toxoplasma*

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## Summary

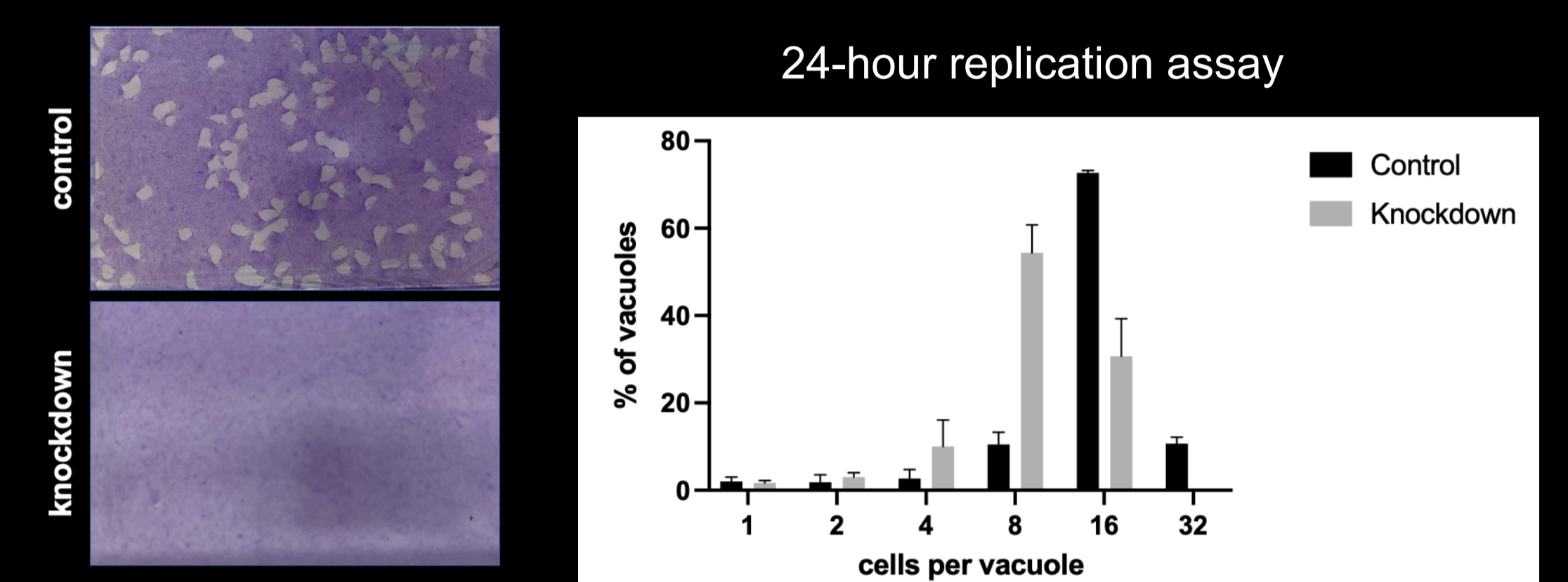
- *Toxoplasma* secretes GRA proteins packaged inside dense granules from an unknown site to establish and maintain a parasitophorous vacuole essential for its growth and proliferation and to intervene in host responses to its presence.
- Apical annuli are 5-7 ring structures found in the apical region of the cell. All the proteins previously localised to these structures are embedded in the Inner Membrane Complex.
- We identified 4 proteins that localise to the annuli that are associated with the plasma membrane.
- Depletion of each of these proteins causes a severe growth phenotype, as well as defects in GRA secretion.
- Furthermore, we observed a failure of delivery of a GPI-anchored protein (Sag1), to the plasma membrane, but not HP03 (a polytopic protein).
- Our data suggest that the apical annuli are exocytic sites that mediate secretion through *Toxoplasma*'s complex pellicle.

## Do plasma membrane proteins associate with the apical annuli?



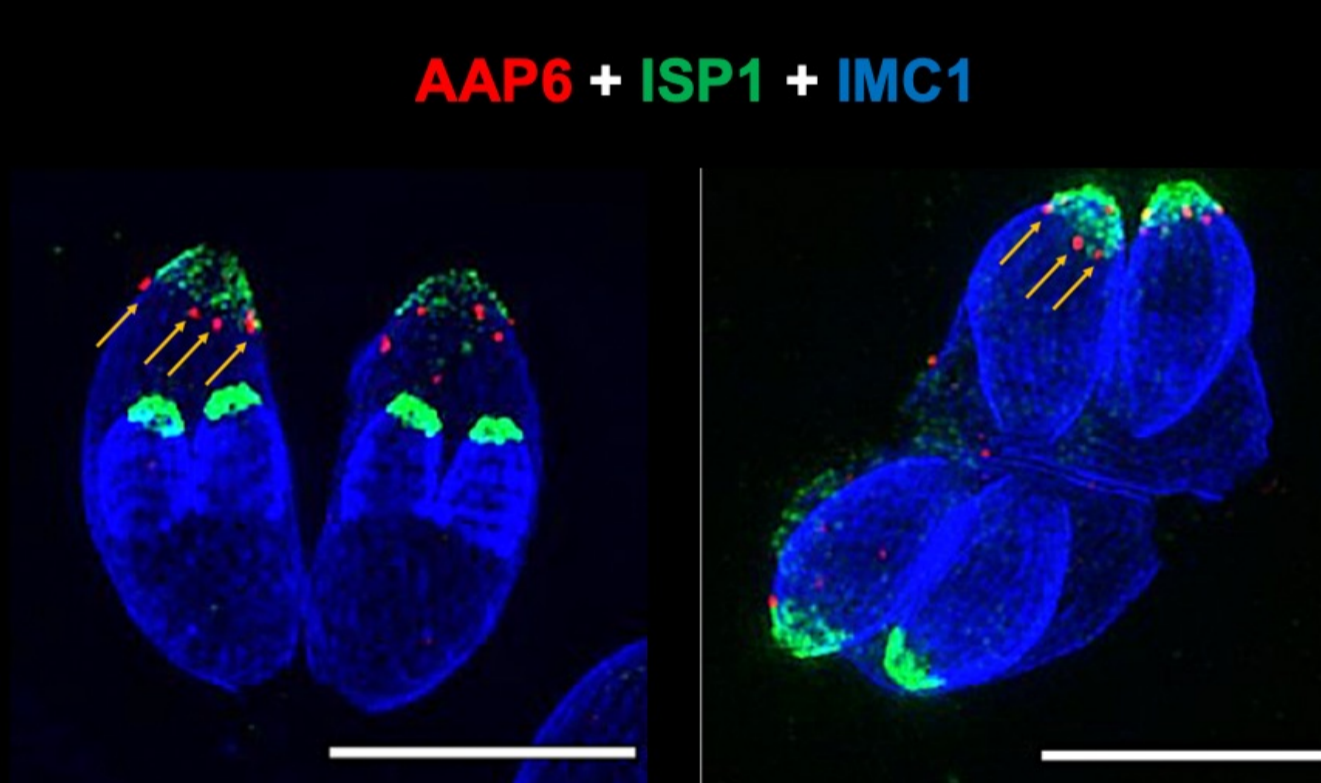
- Using HyperLOPIT spatial proteomics, we identified AAP6 as a novel component of the annuli and the first one to associate with the plasma membrane.
- *Centrin2* is used in this study as an apical annuli marker.

## Is AAP6 required for parasite growth?



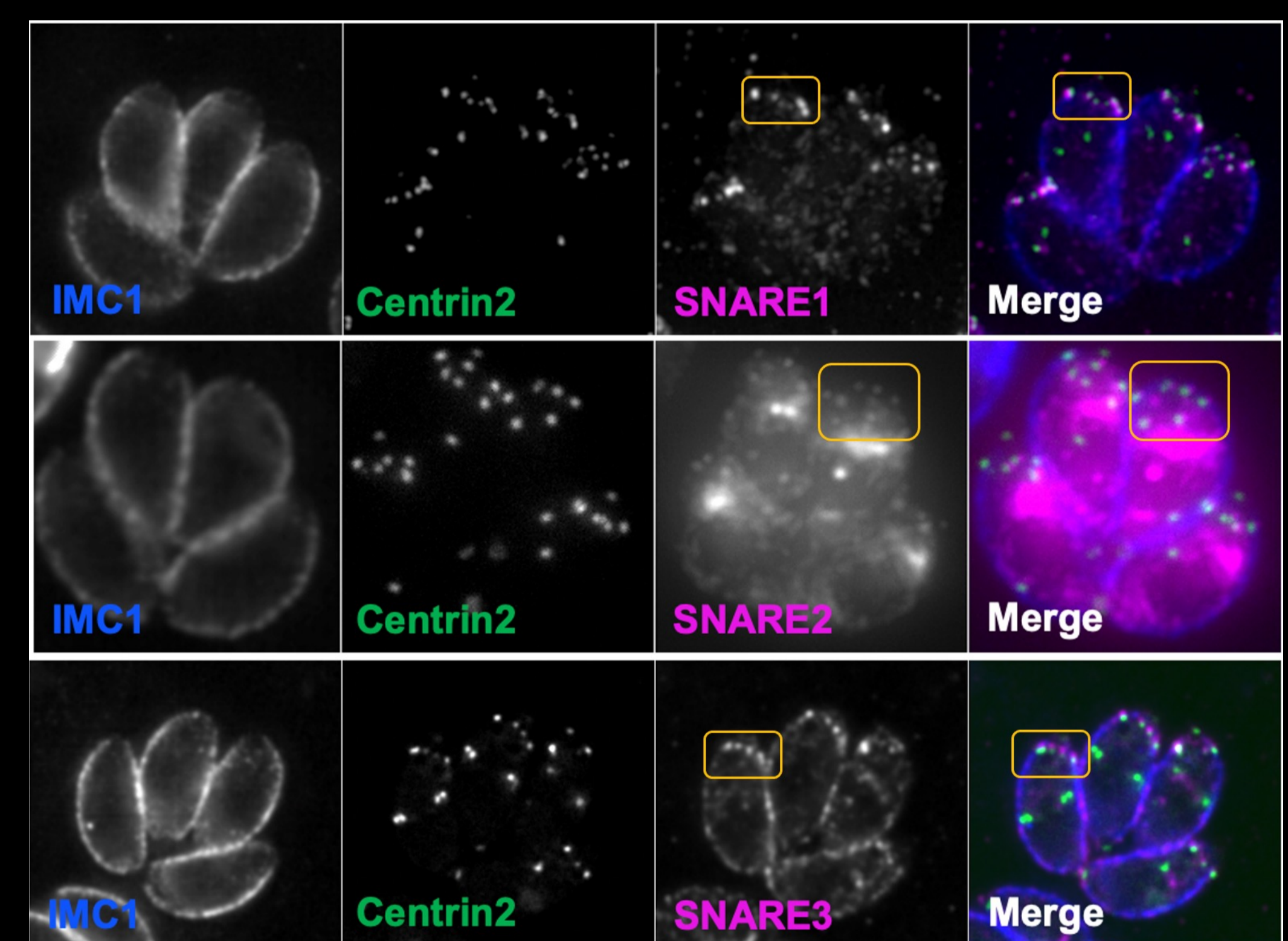
- AAP6-depleted cells fail to form growth plaques over 7 days, indicating that AAP6 is important for the lytic cycle.
- Furthermore, knockdown of AAP6 causes a replication defect.

## Does AAP6 span the plasma membrane?



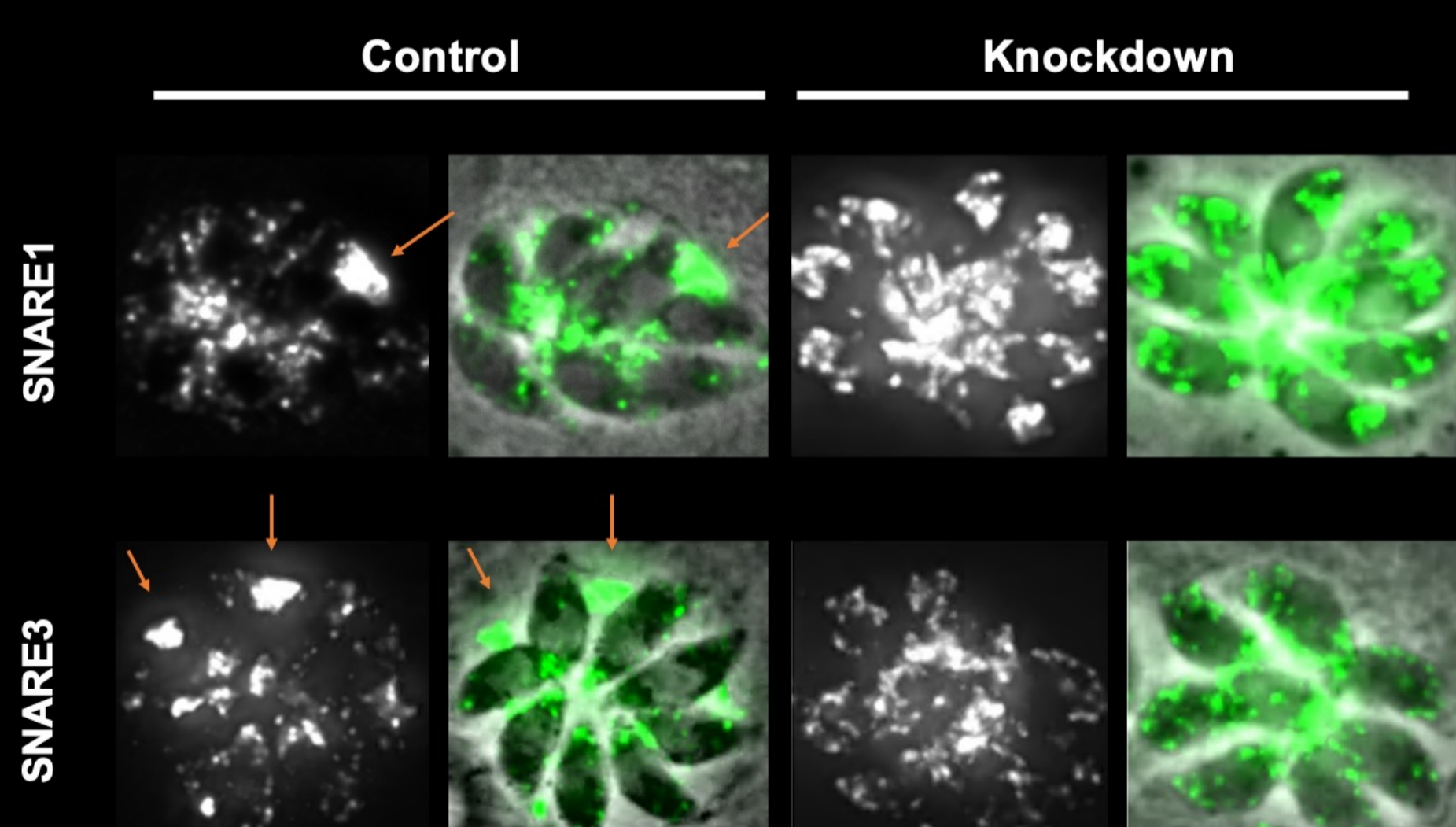
- AAP6 has 9 transmembrane domains and is sensitive to trypsin shaving consistent with spanning the plasma membrane.
- Its recruitment to the annuli in forming daughter cells occurs at the same time as their acquisition of the plasma membrane from the mother.

## Are there other plasma membrane proteins at the annuli?



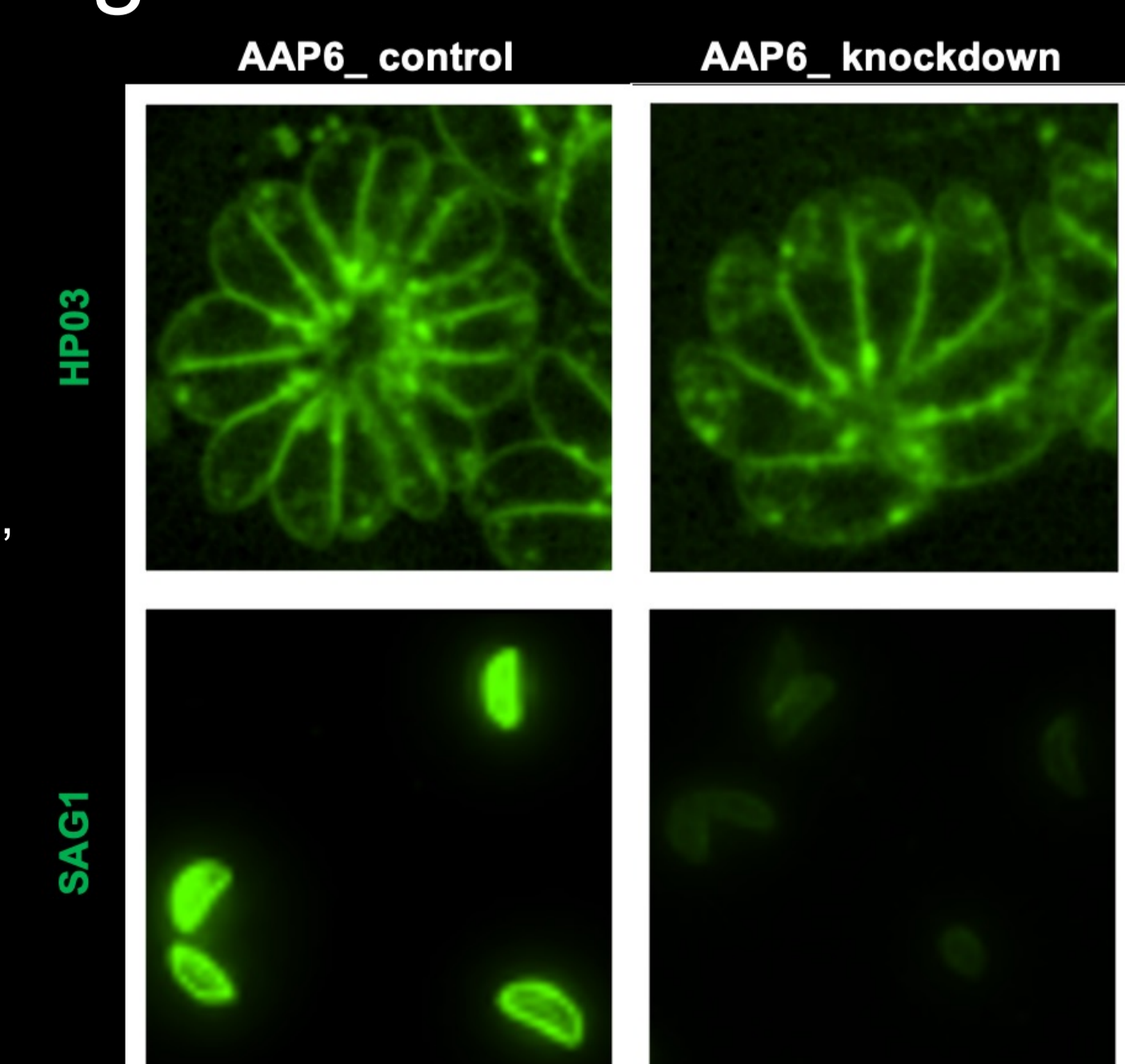
- Using AAP6 proximity labelling and proteomics, we detected three Q-SNAREs implicated in vesicle docking at the annuli.
- Depletion of each SNARE results in a replication defect.

## Are the SNAREs important for GRA secretion?



- Depletion of the annuli SNAREs causes defects in GRA secretion. A similar result was observed for SNARE2 (not shown).

## Are cell surface proteins secreted through the annuli?



- AAP6 and the SNAREs are required for normal rates of delivery of Sag1, a GPI-anchored cell surface protein, to the PM but not for HP03, a polytopic protein.