

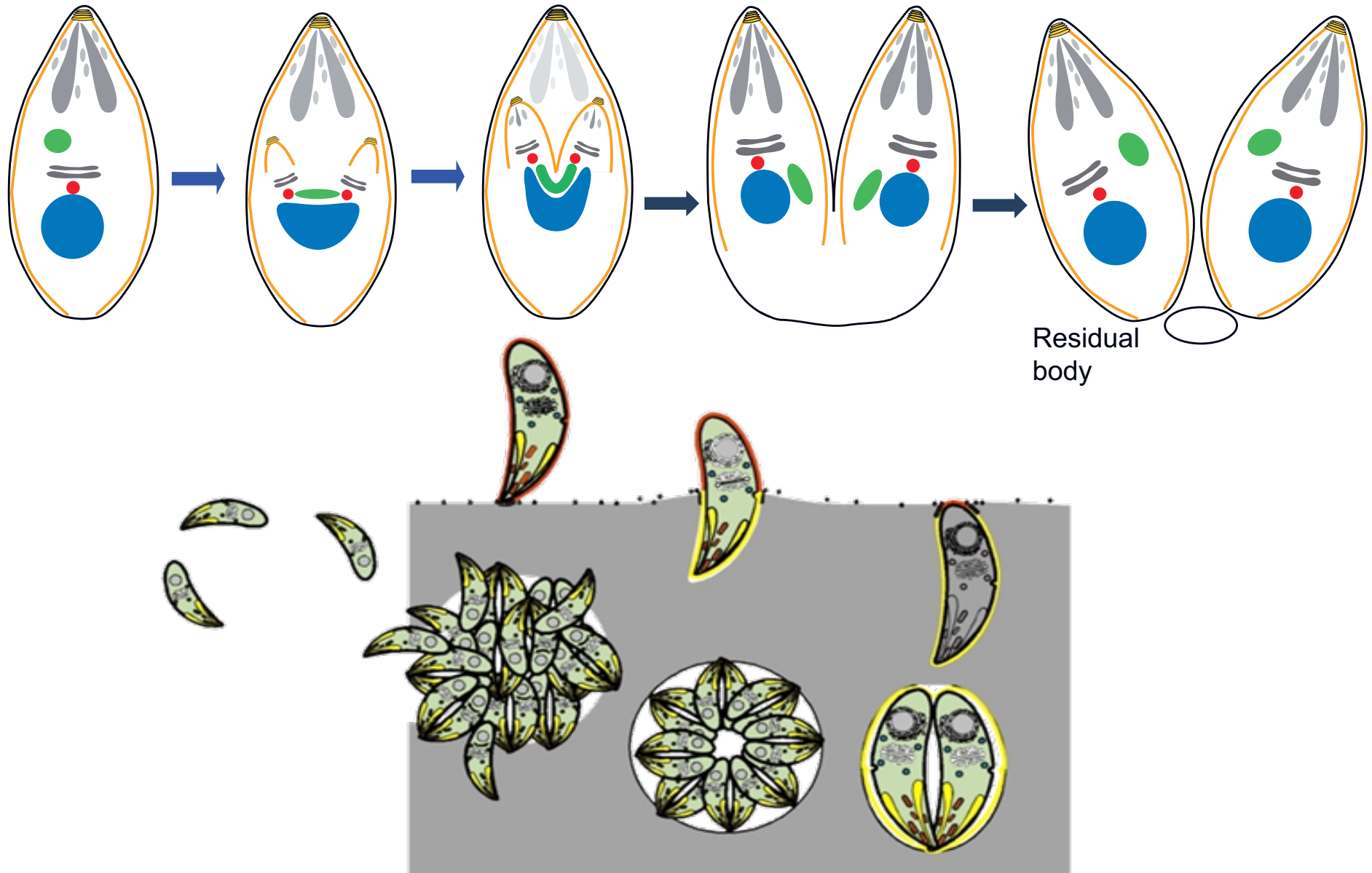
**The effect of Aspartyl protease  
(ASP3- *Toxoplasma gondii*) knock  
down on intracellular multiplication  
and the integrity and position of  
organelles**

**BY**

**Awa & Fatima**

# *Toxoplasma gondii* intracellular replication

- Replication by endodyogeny and organization in rosettes



# Aspartyl protease *ASP3- Toxoplasma gondii*

- It is a post golgi resident protease
- It has endopeptidase activity
- It is implicated in protein transport outside the cells

# Assessment of ASP3 knock down on intracellular growth

Intracellular growth assay by IFA to count the number of parasite inside vacuoles

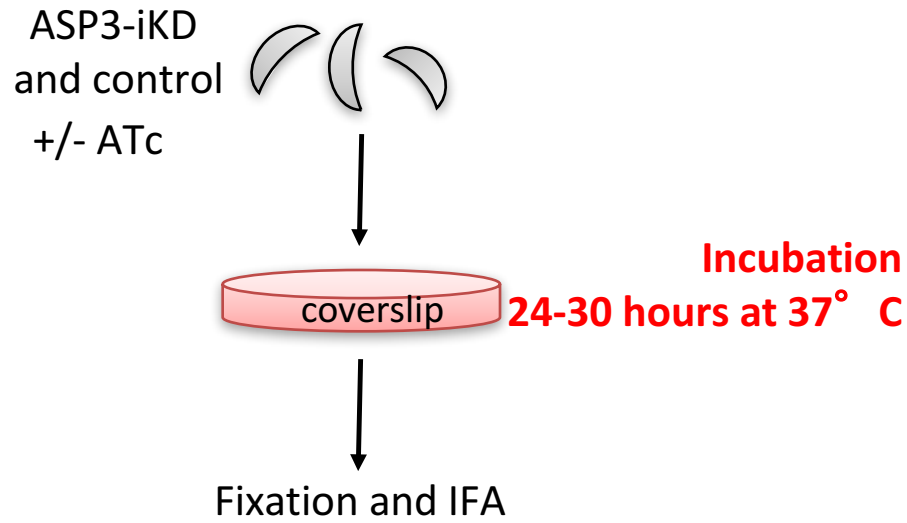
Check the integrity and position of organelles by IFA



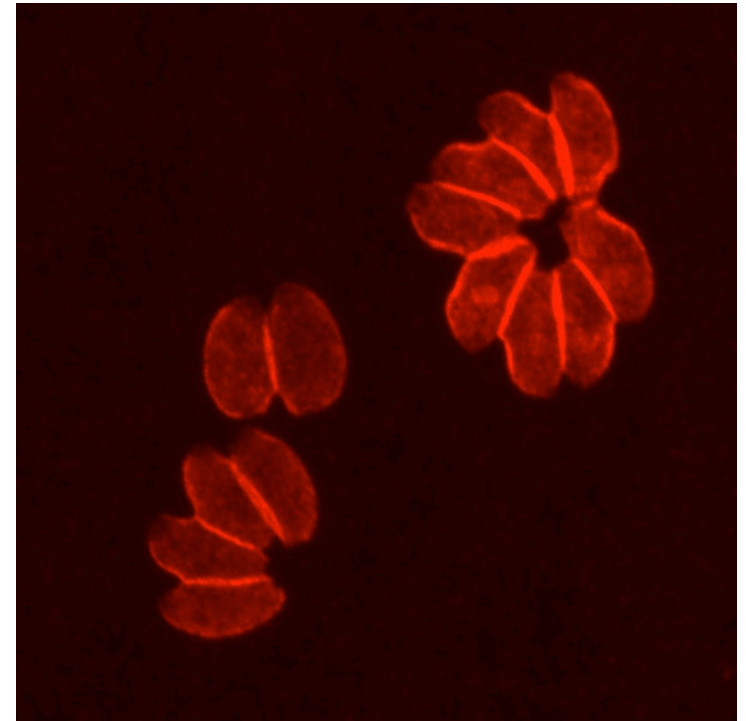
# The used parasites

- A native parasite (as a control)
- A genetic modified parasite (Tg Asp3) though Tet-inducible knock-down of Asp3.
  - ATC**: modified parasite but without adding tetracyclin, so the gene is on.
  - +**ATC**: modified parasite with tetracyclin, so the gene is knocked down.

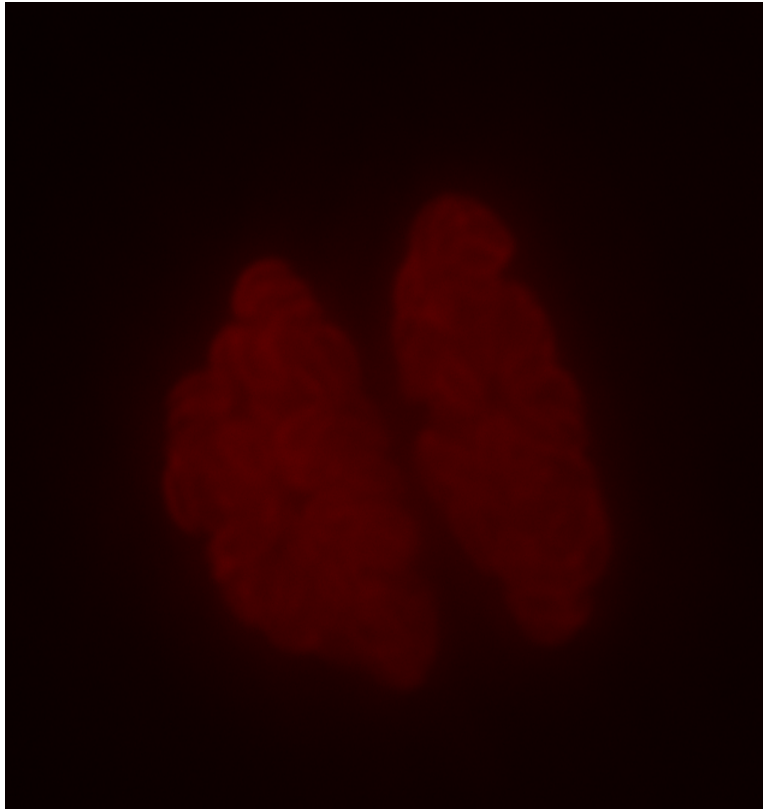
# I-Intracellular growth assay (IFA)



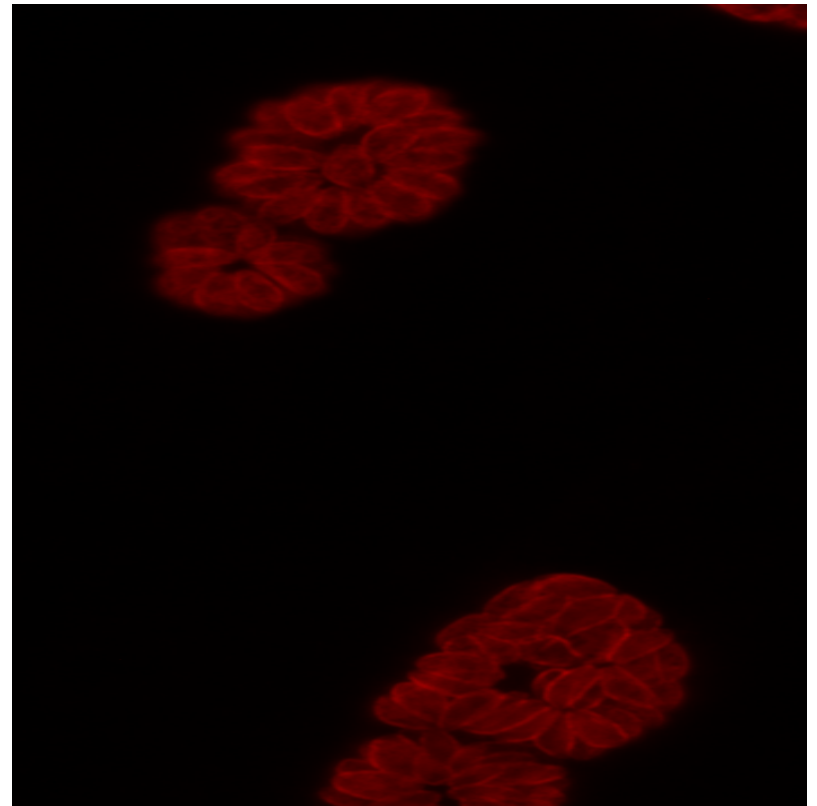
- with **permeabilization**
- primary Ab: anti-GAP45
- secondary Ab: anti-rabbit-Alexa594)



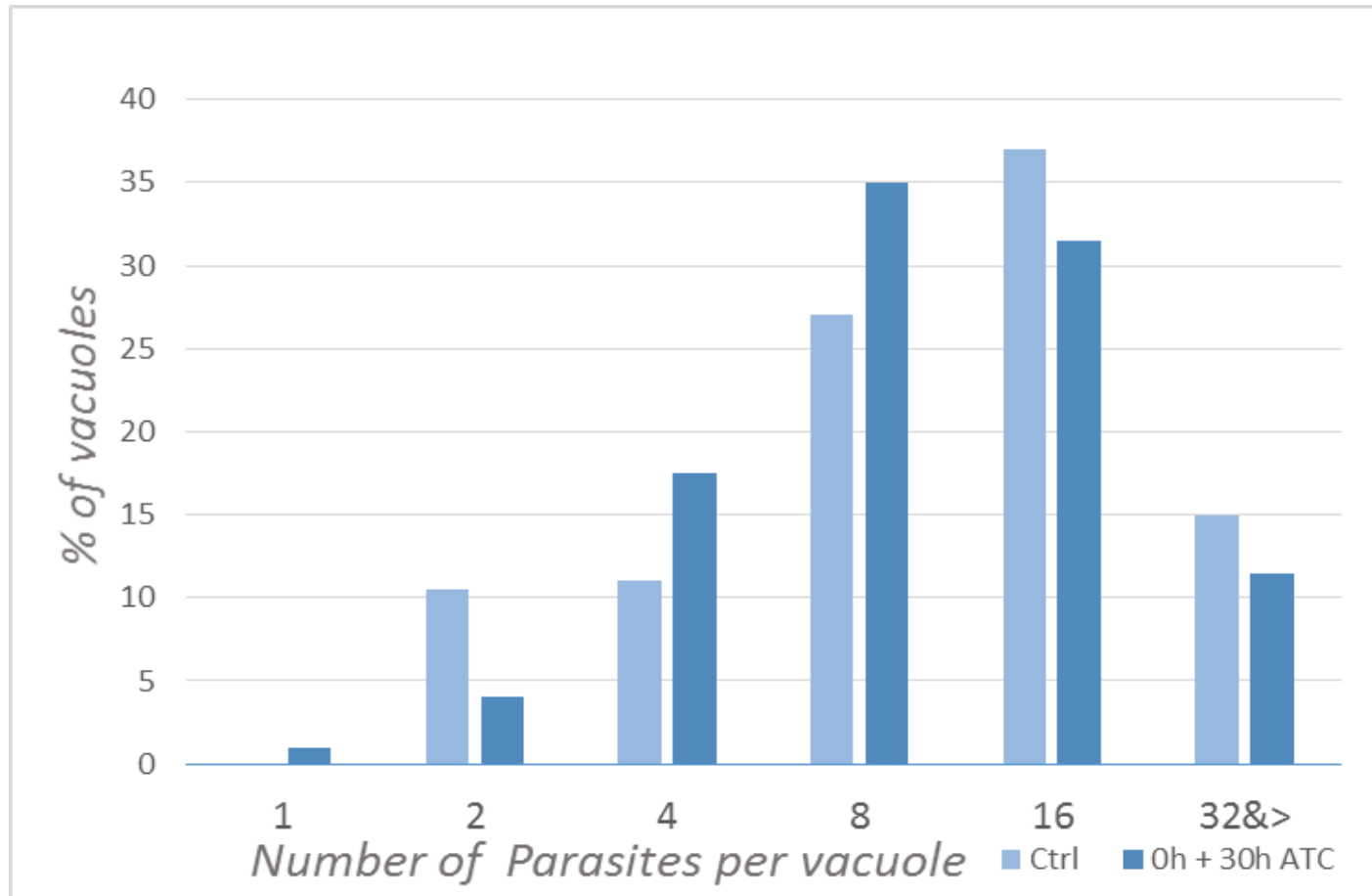
**ASP3-iKD**  
**-ATC**



**ASP3-iKD**  
**+ATC**



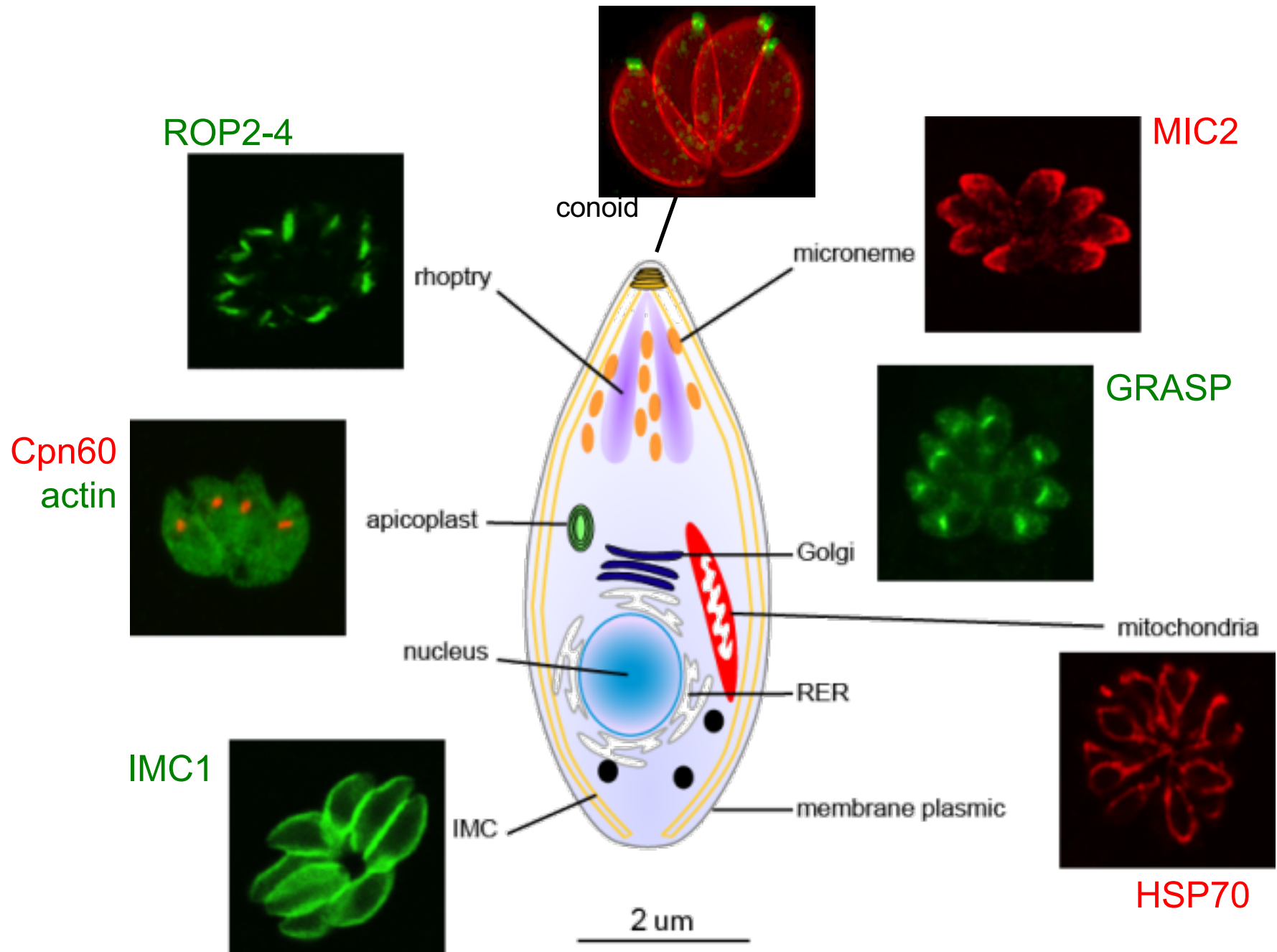
Counted the number of parasites per vacuole  
over 100 vacuoles detected



*ASP3 knockdown has no effect on intracellular growth*

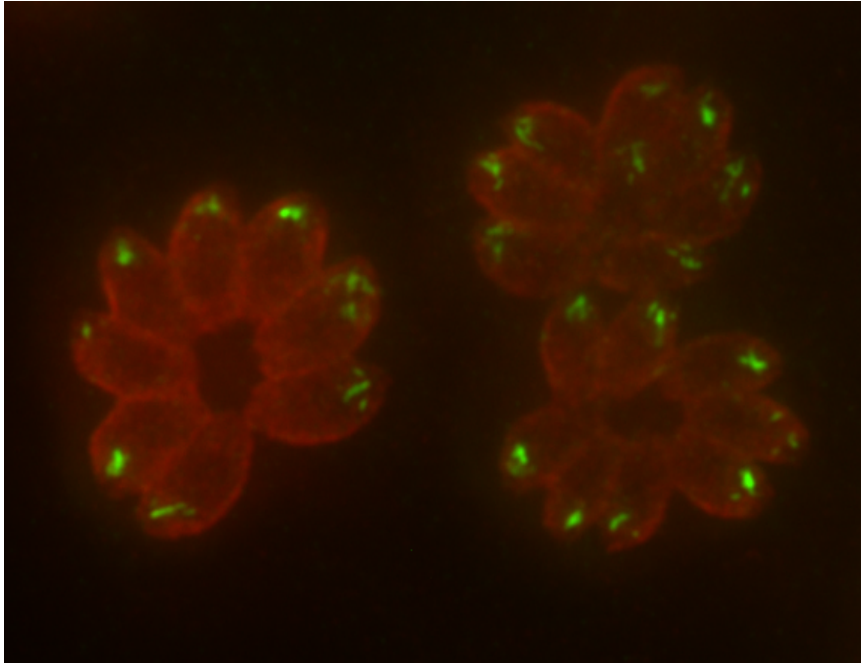
## **II-The integrity and position of different organelles by immunofluorescence**

# Integrity and positioning of organelles by immunofluorescence

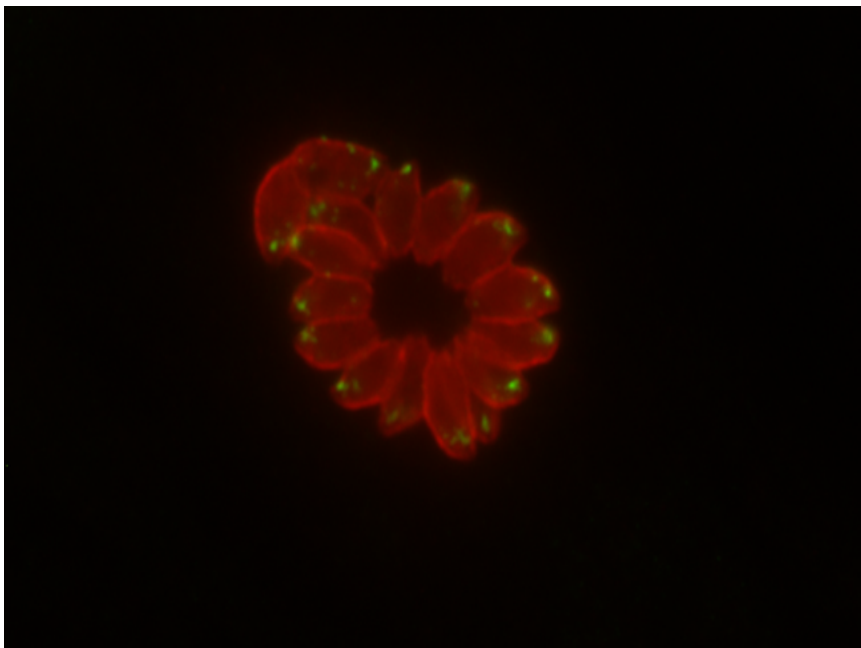
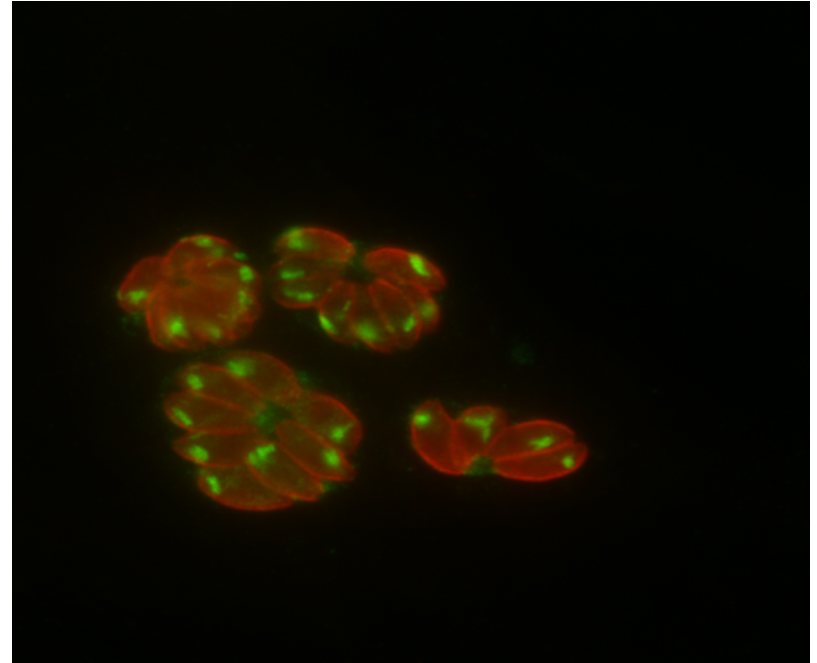


**RON9-GAP45 for Rhoptry apical part**

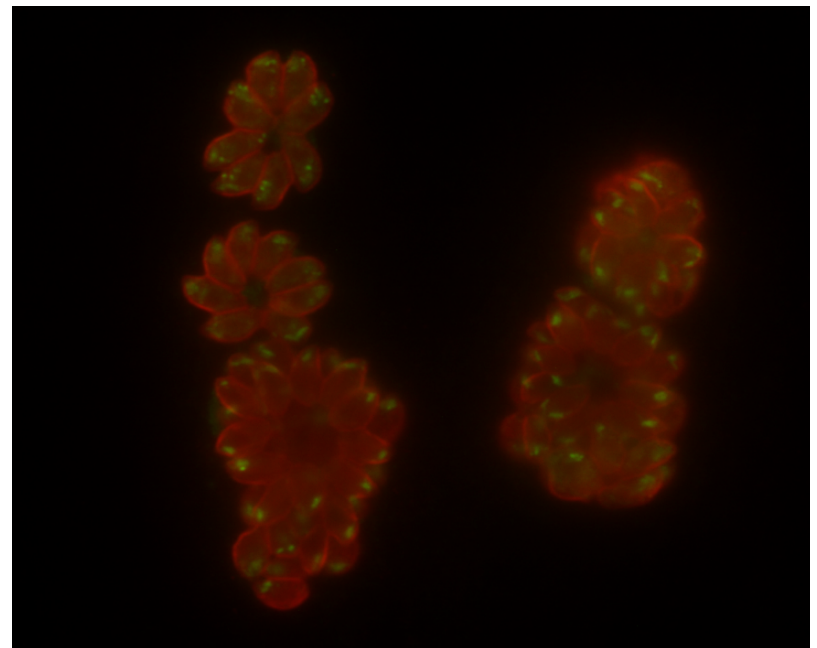
**ROP2-GAP45 for Rhoptry bulb**



**-ATC**

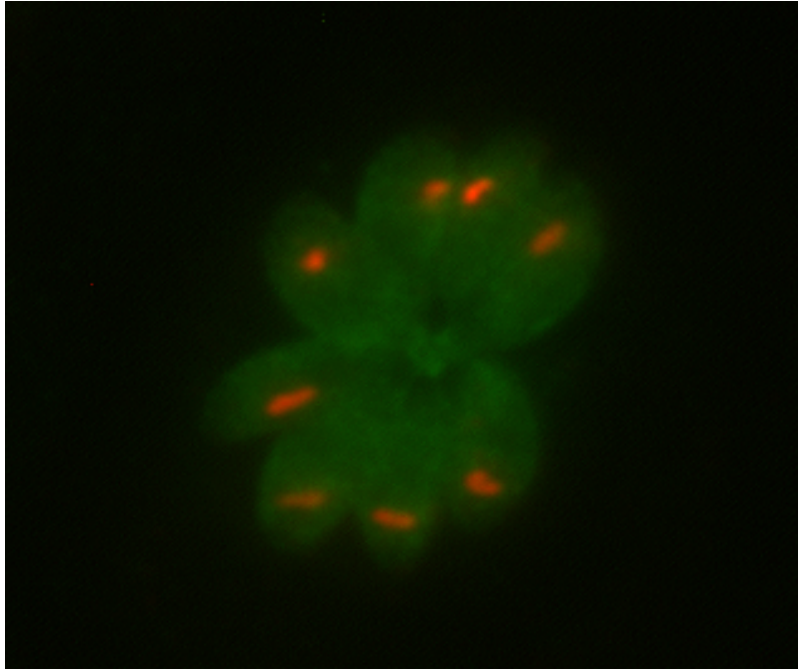


**+ATC**

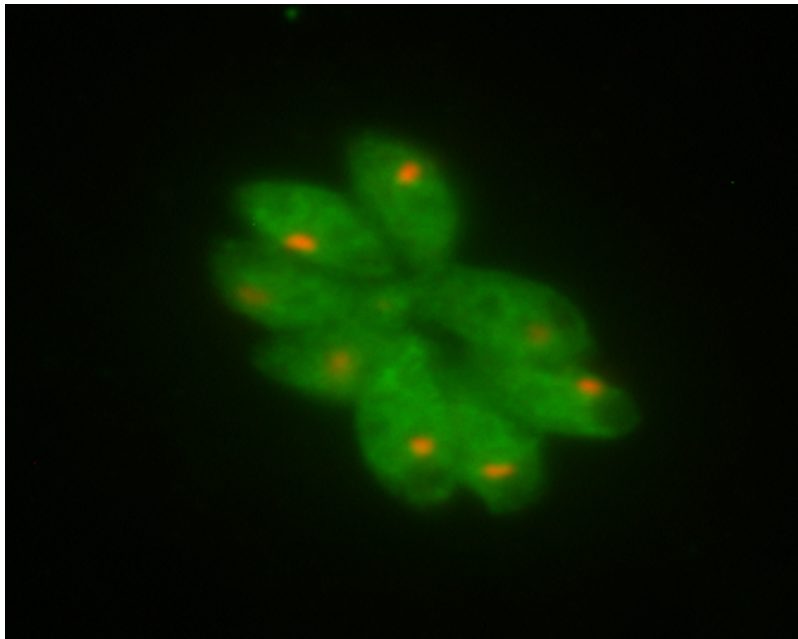




## Cpn60-Actin for Apicoplast

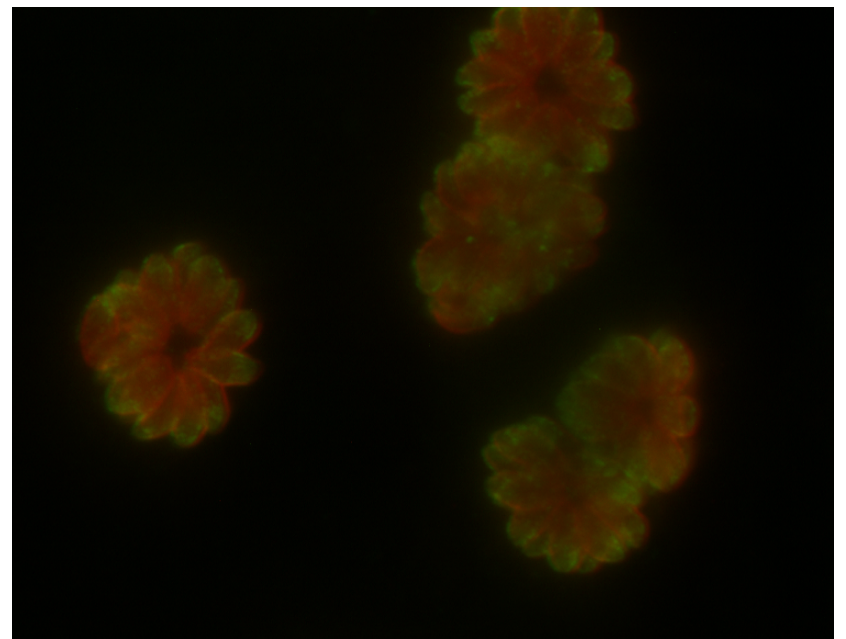
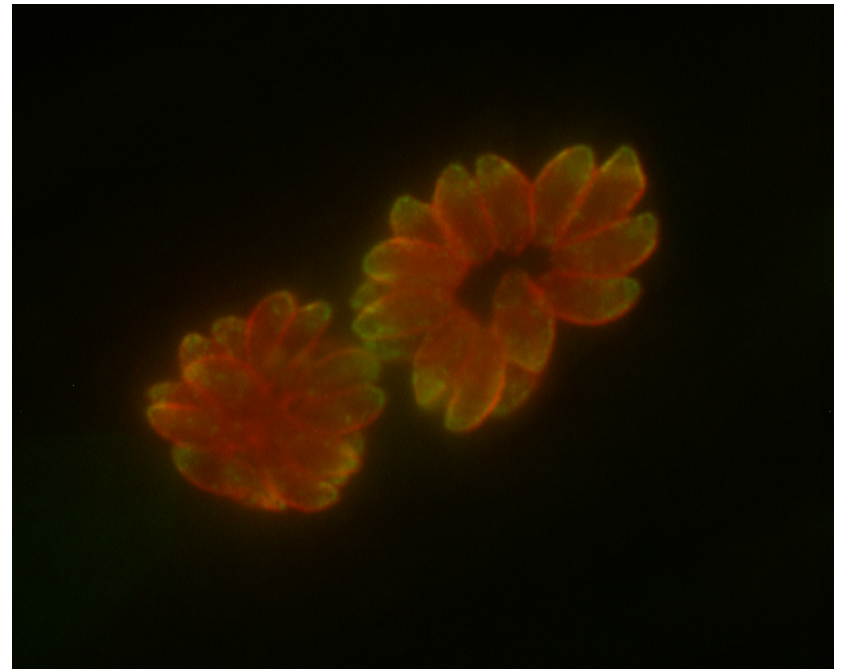


-ATC



+ATC

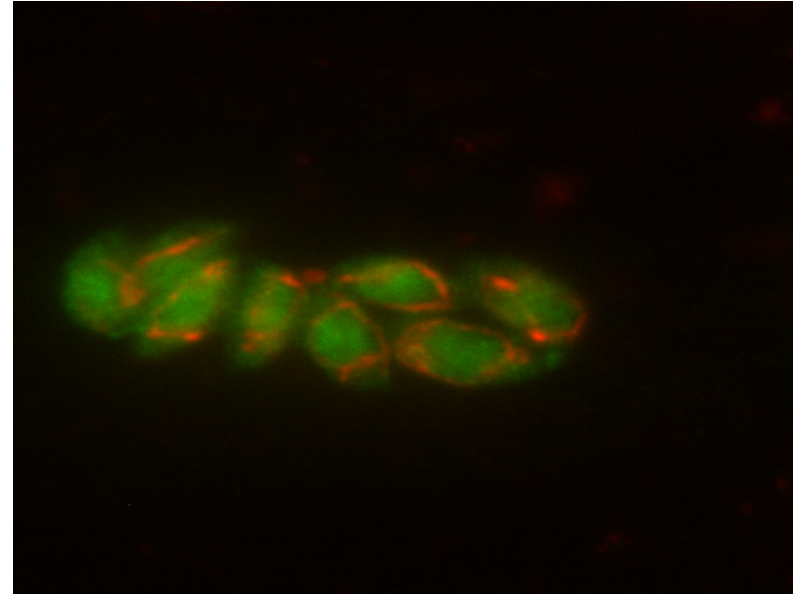
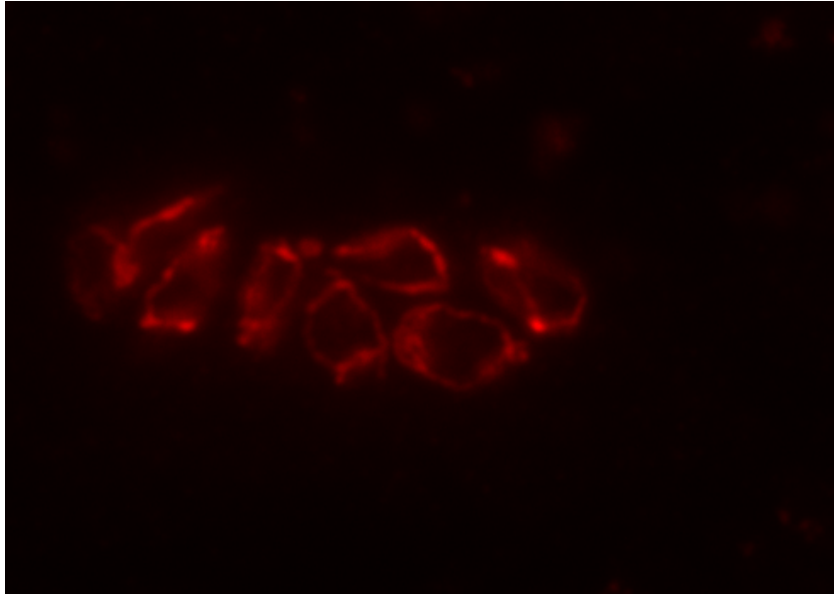
## MIC2-GAP45 for Microneme



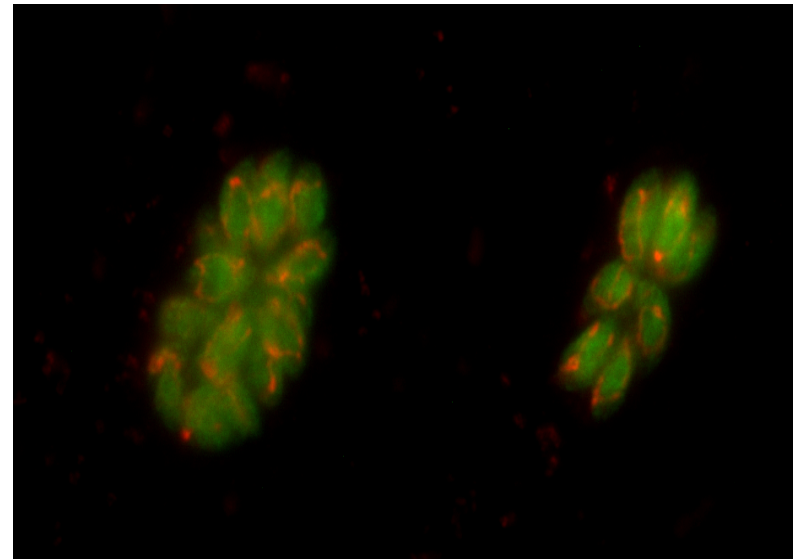
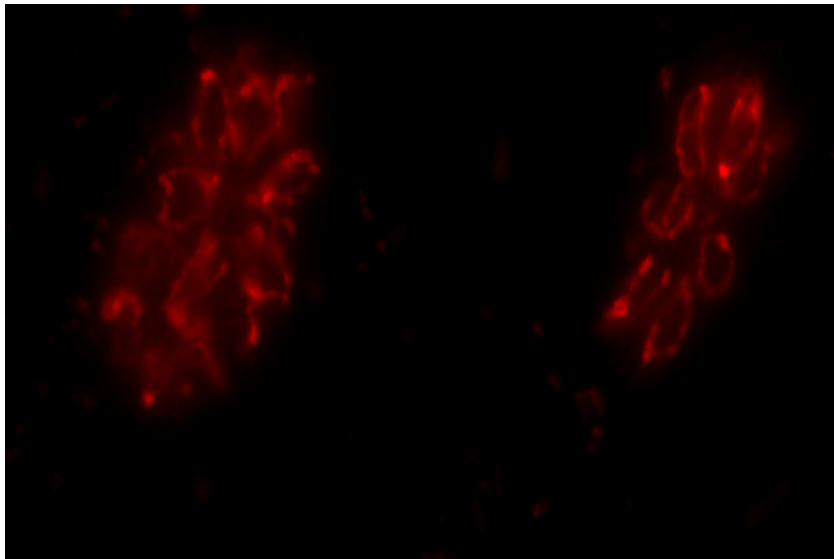


## HSP70-Actin for Mitochondria

**-ATC**



**+ATC**



# Conclusion

ASP3 knockdown has no effect on intracellular growth and integrity of *Toxoplasma* organelles

*Thank  
you*

