

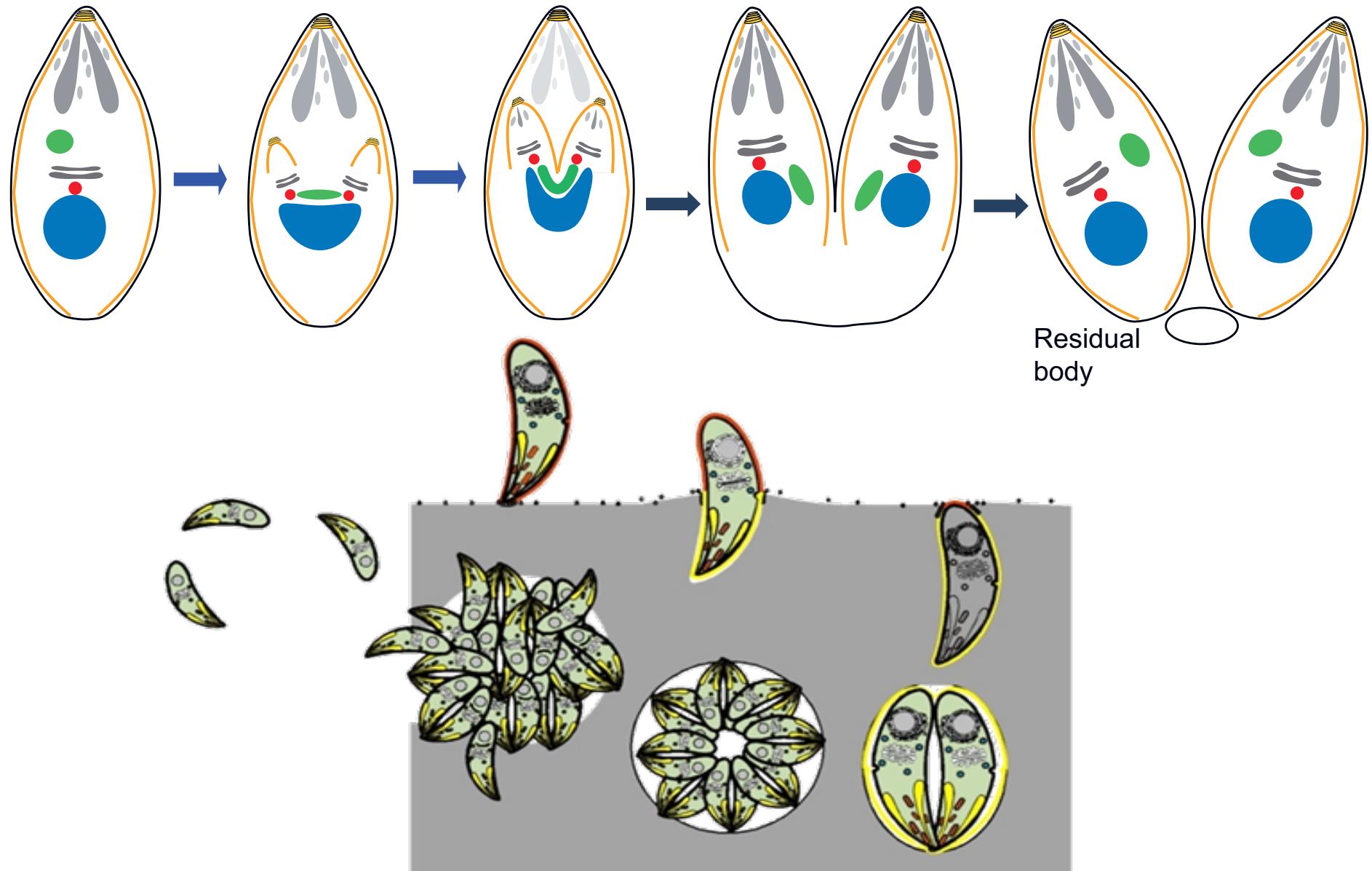
# **The effect of Aspartyl protease (ASP3- *Toxoplasma gondii*) knock down on intracellular multiplication and the integrity and position of organelles**

**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

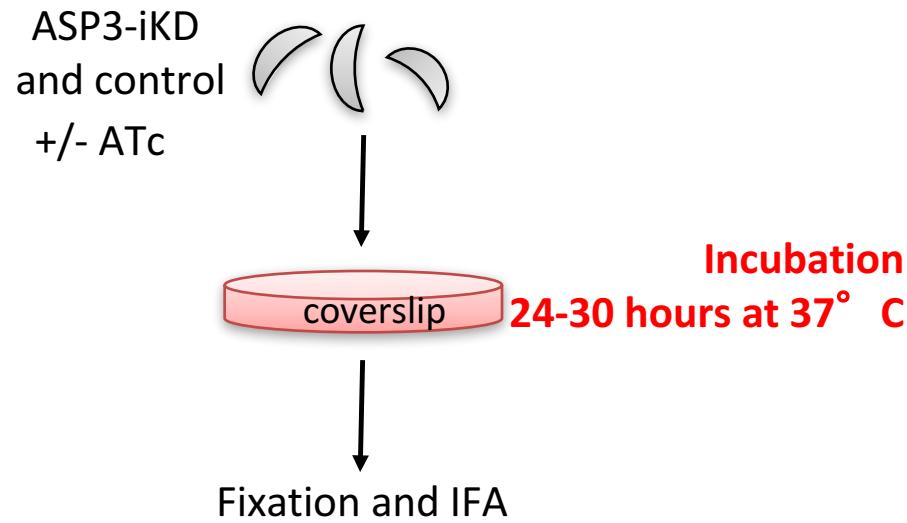
Intracellular growth assay by IFA to count the number of parasite inside vacuoles

Check the integrity and position of organelles by IFA

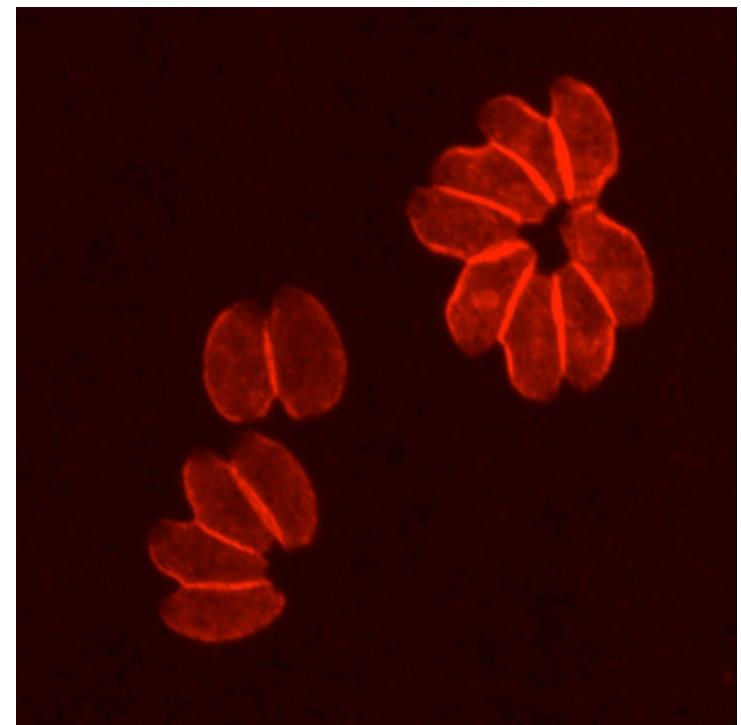
# The used parasites

- 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.

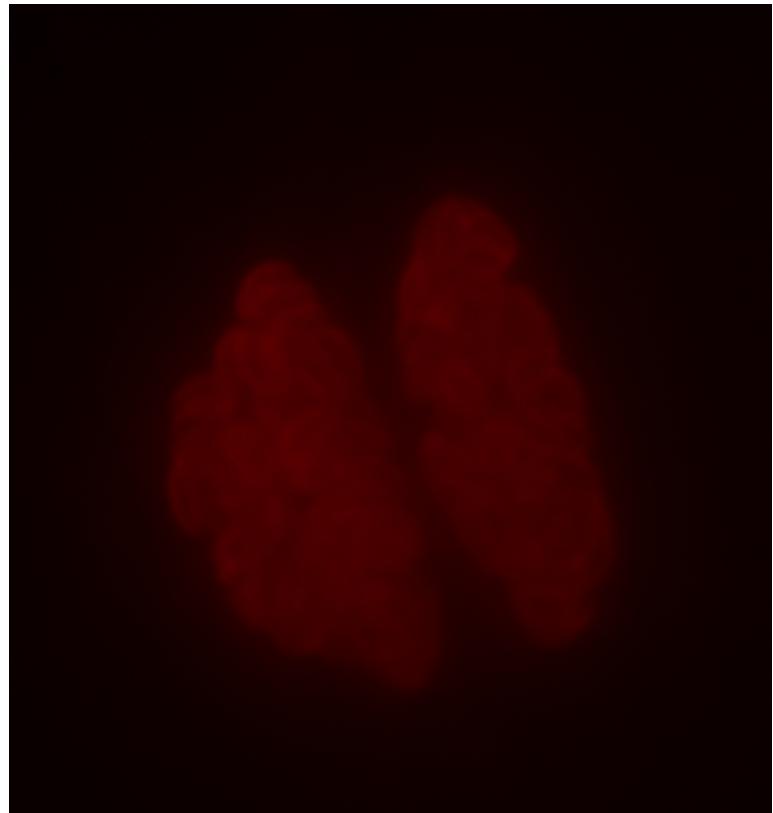
# I-Intracellular growth assay (IFA)



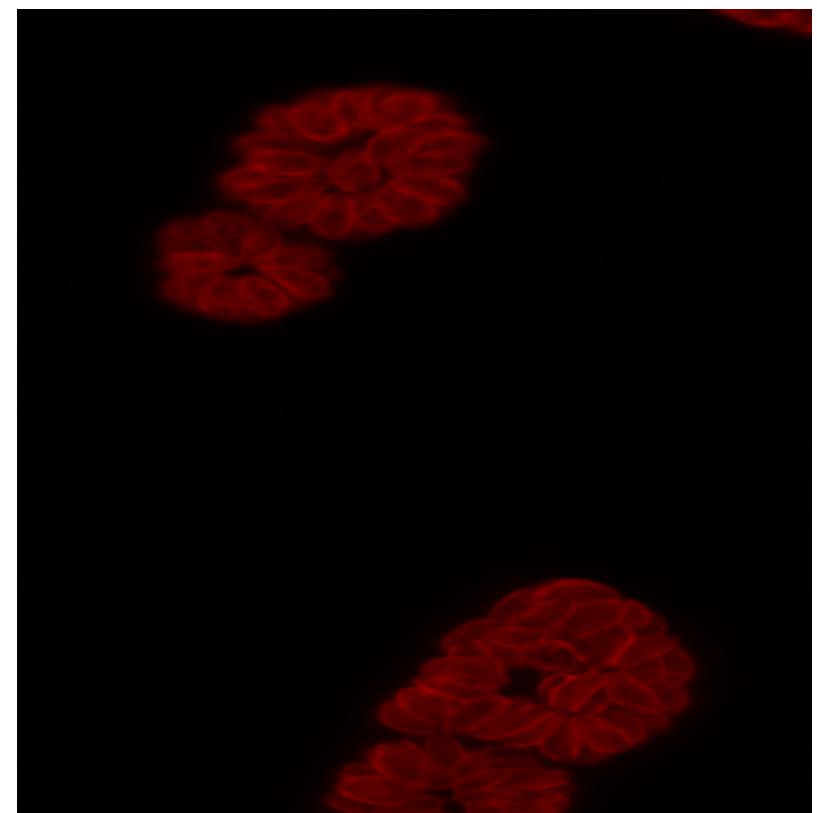
- with **permeabilization**
- primary Ab: anti-GAP45
- secondary Ab: anti-rabbit-Alexa594)



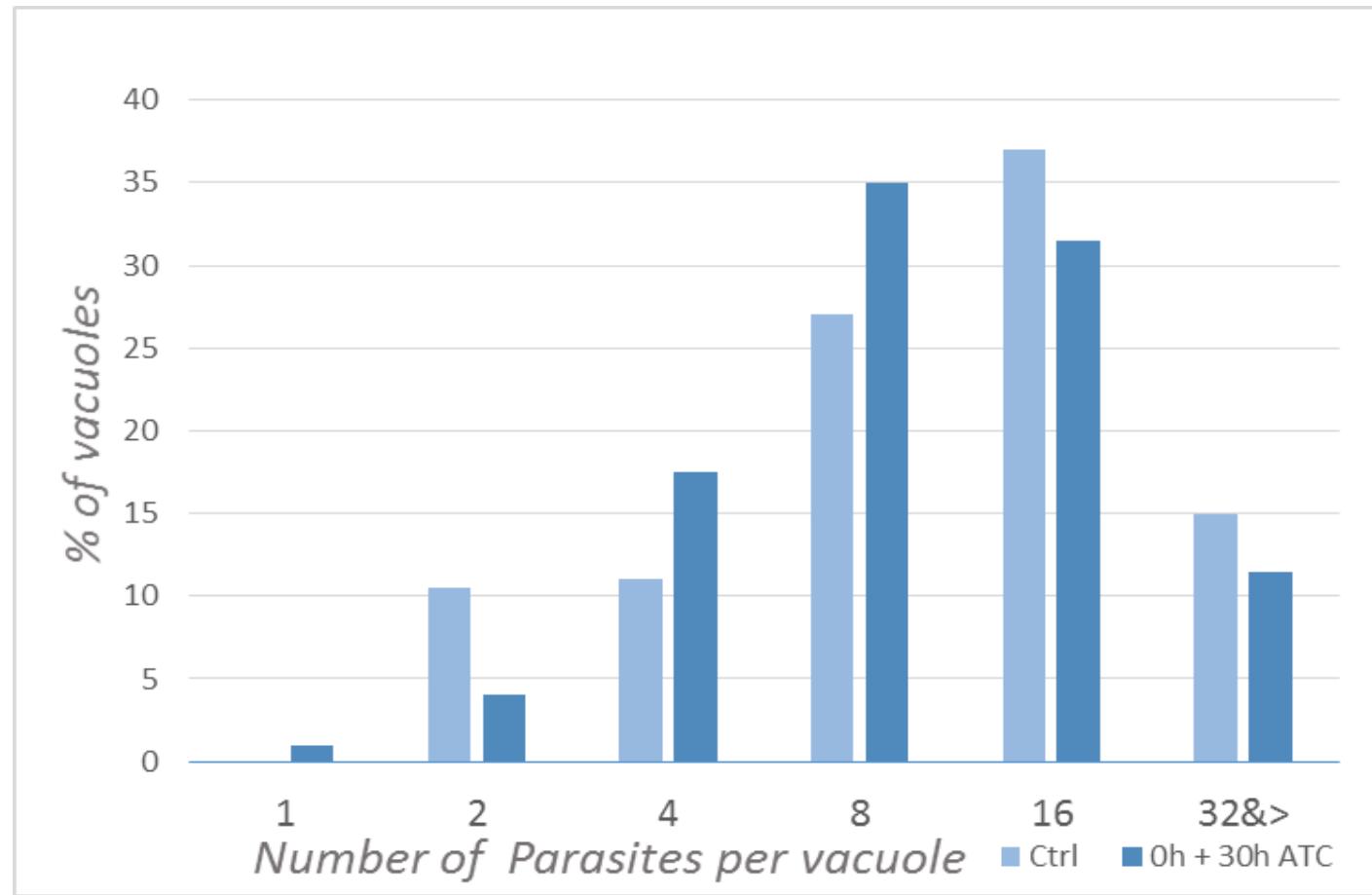
**ASP3-iKD**  
**-ATC**



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



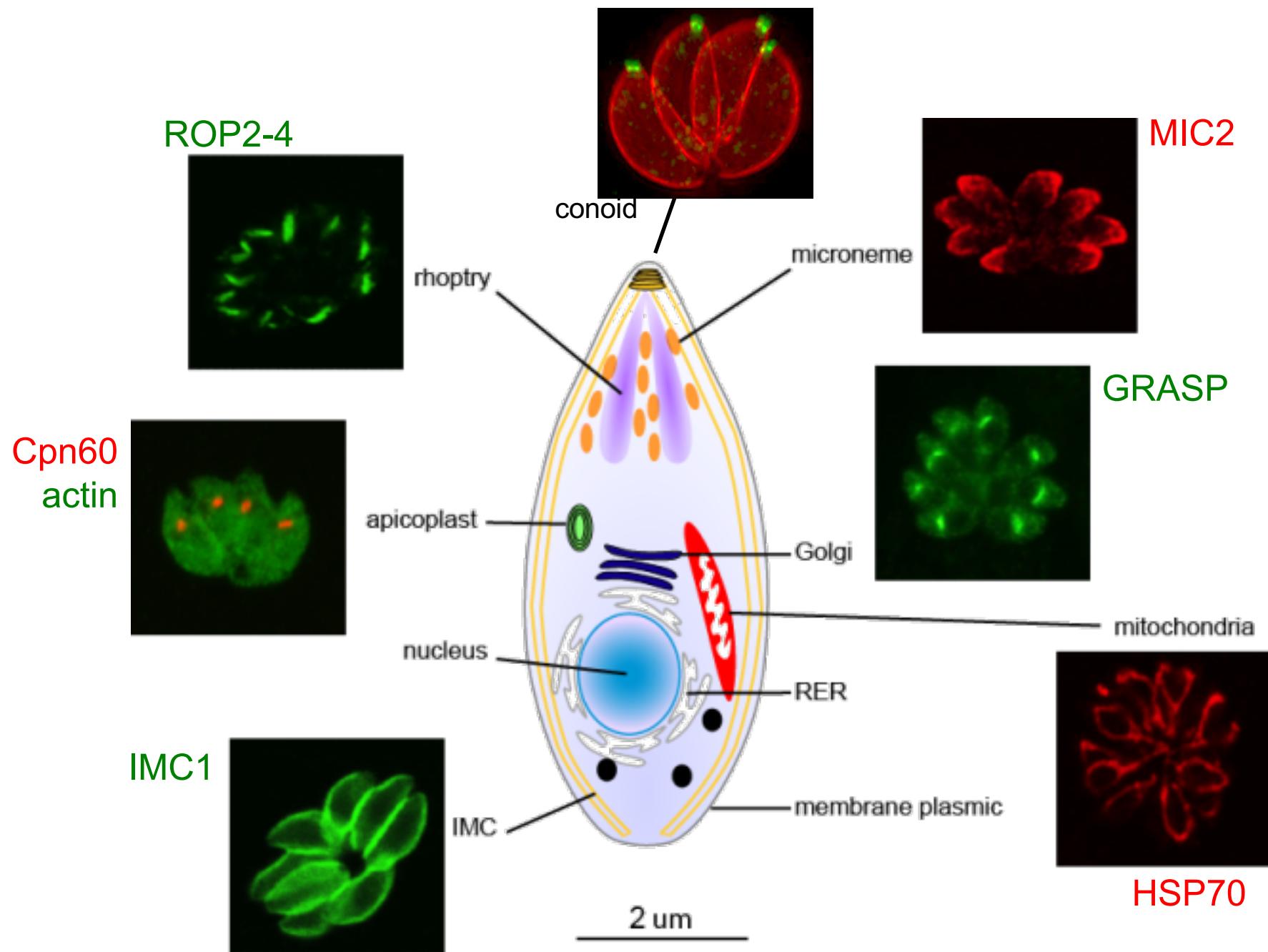
Counted the number of parasites per vacuole  
over 100 vacuoles detected



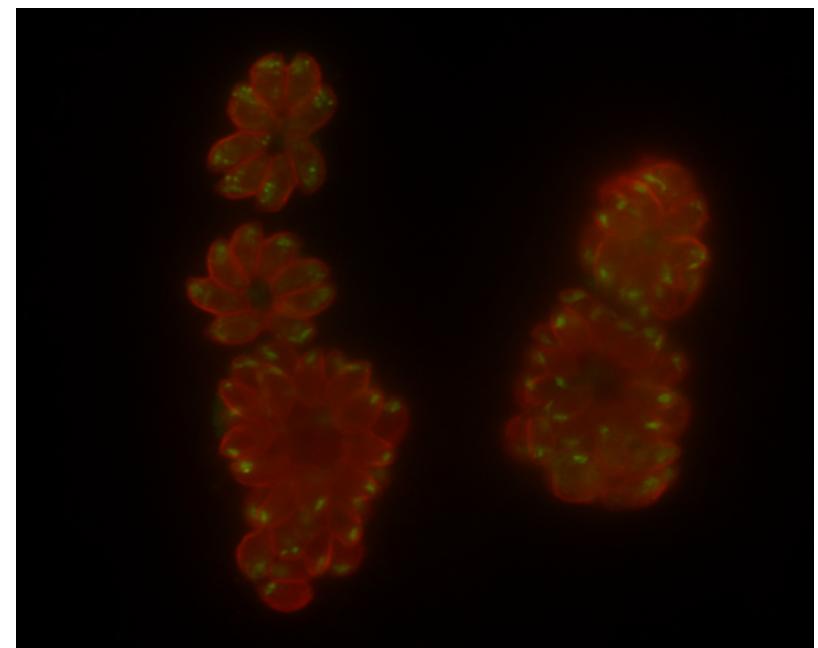
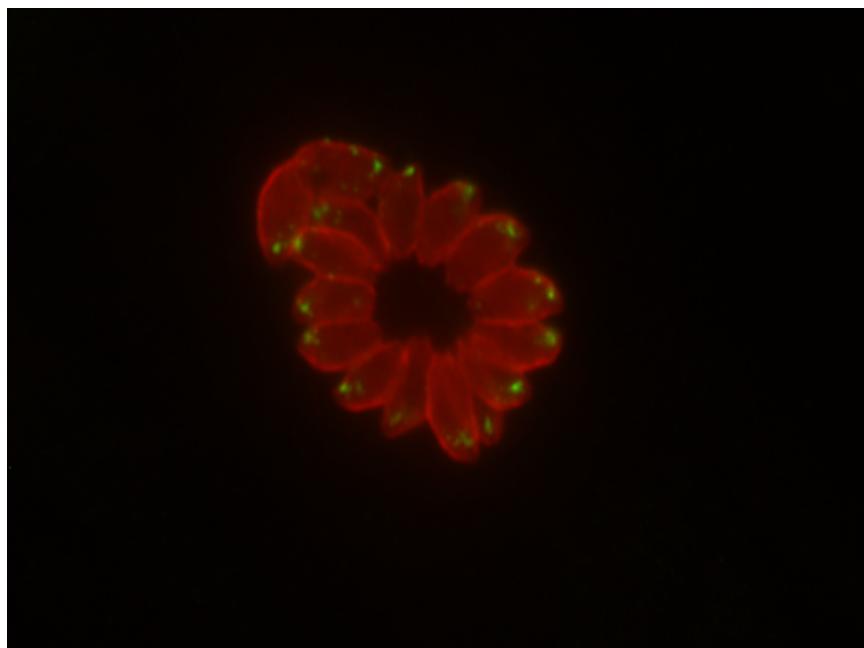
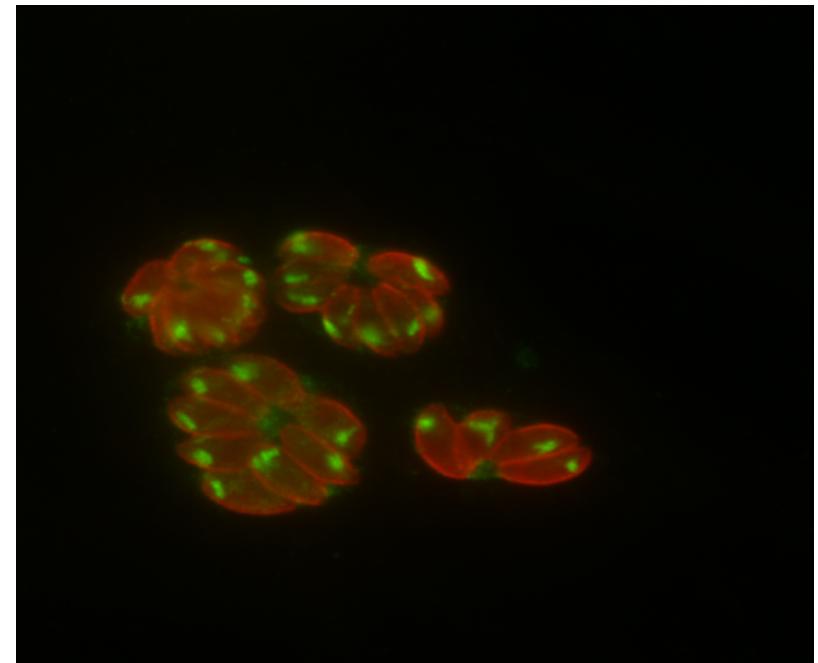
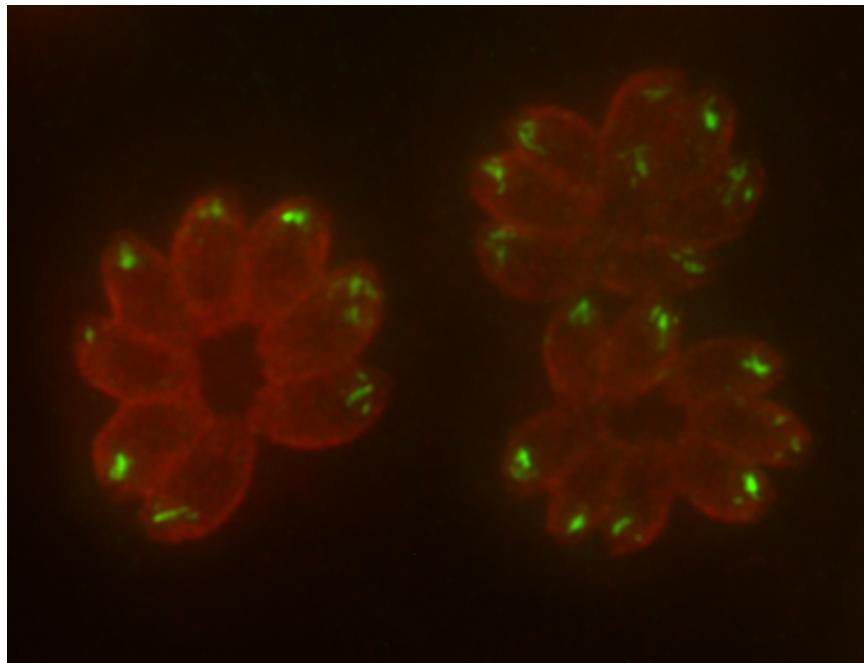
*ASP3 knockdown has no effect on intracellular growth*

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

# Integrity and positioning of organelles by immunofluorescence



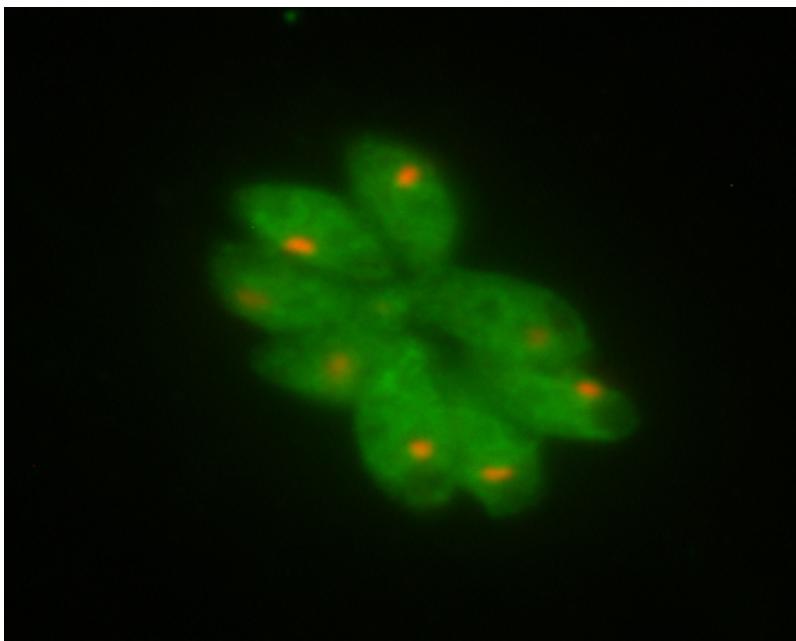
**RON9-GAP45 for Rhopty apical part    ROP2-GAP45 for Rhopty bulb**



**Cpn60-Actin for Apicoplast**

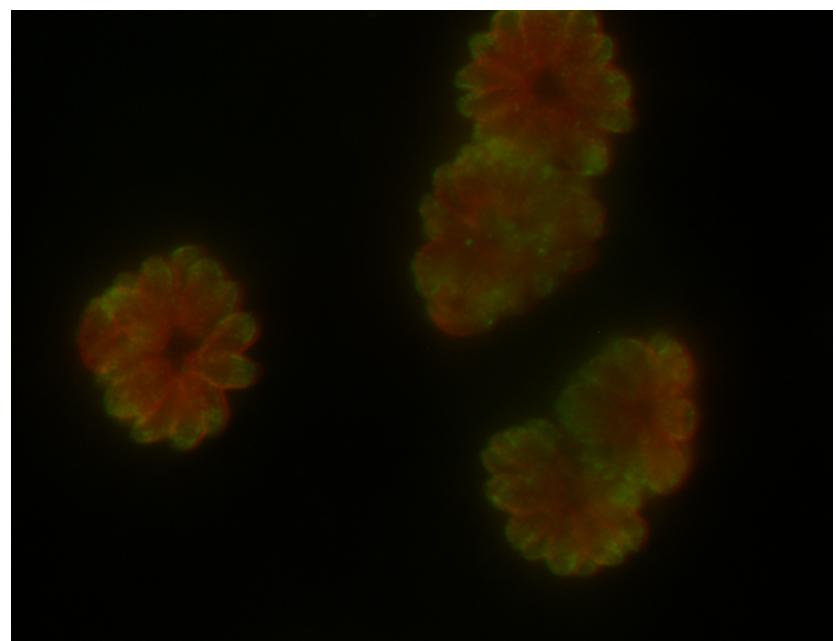
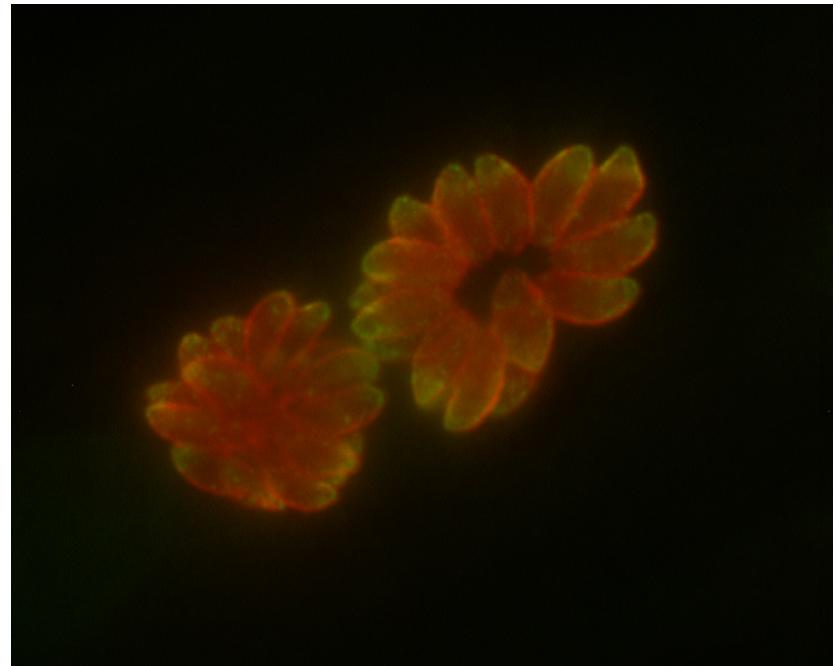


**-ATC**



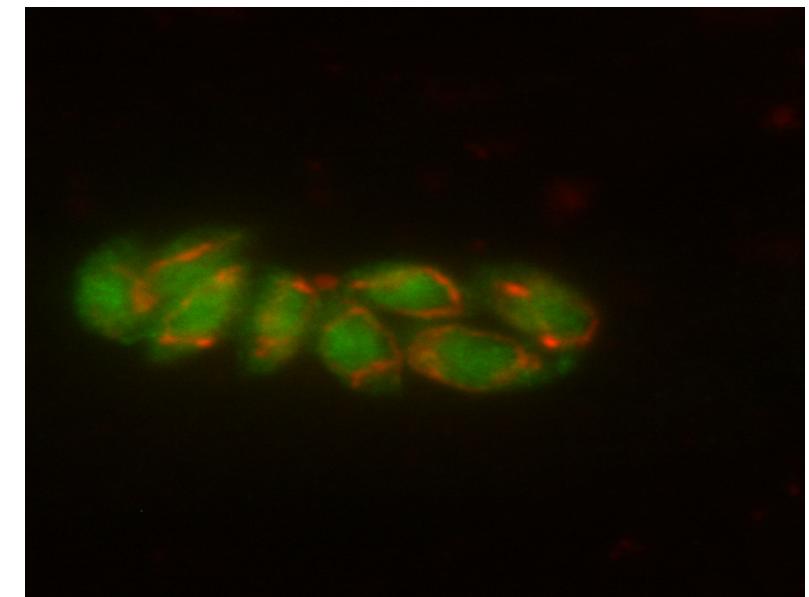
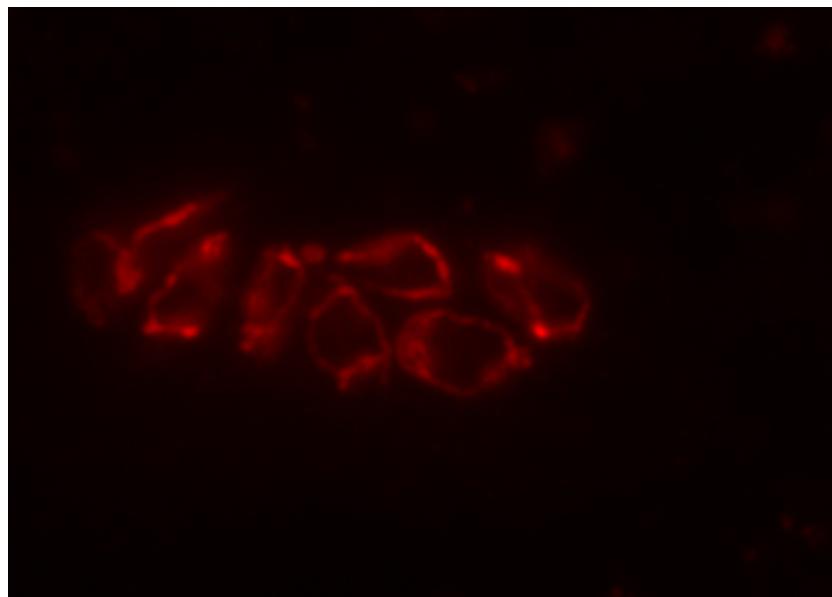
**+ATC**

**MIC2-GAP45 for Microneme**

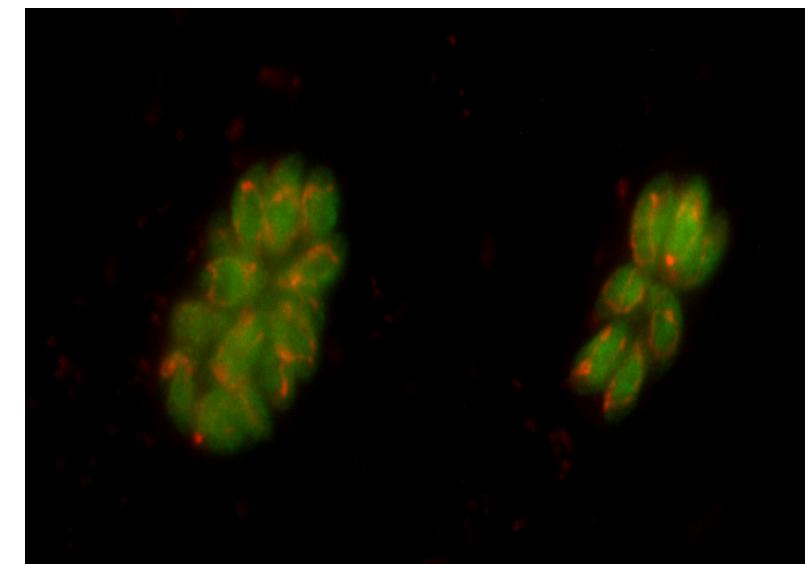
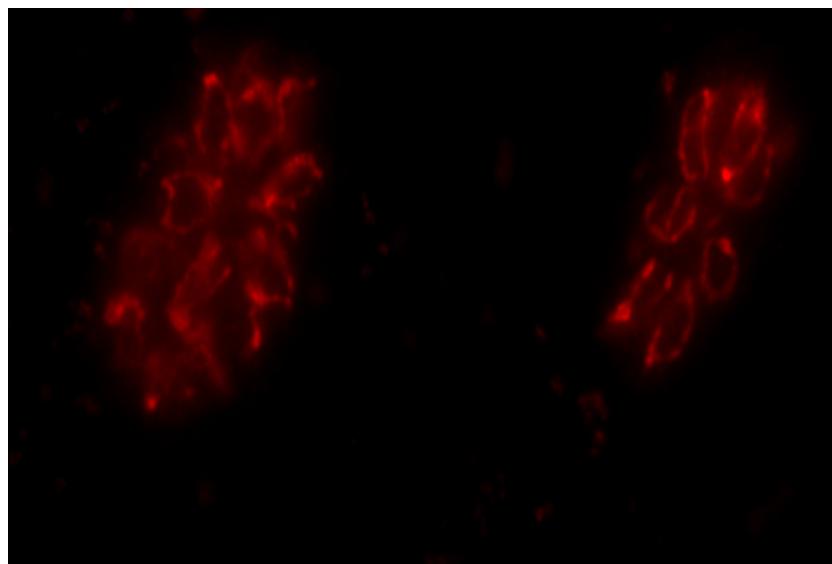


# HSP70-Actin for Mitochondria

-ATC



+ATC



# Conclusion

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

Thank  
you

