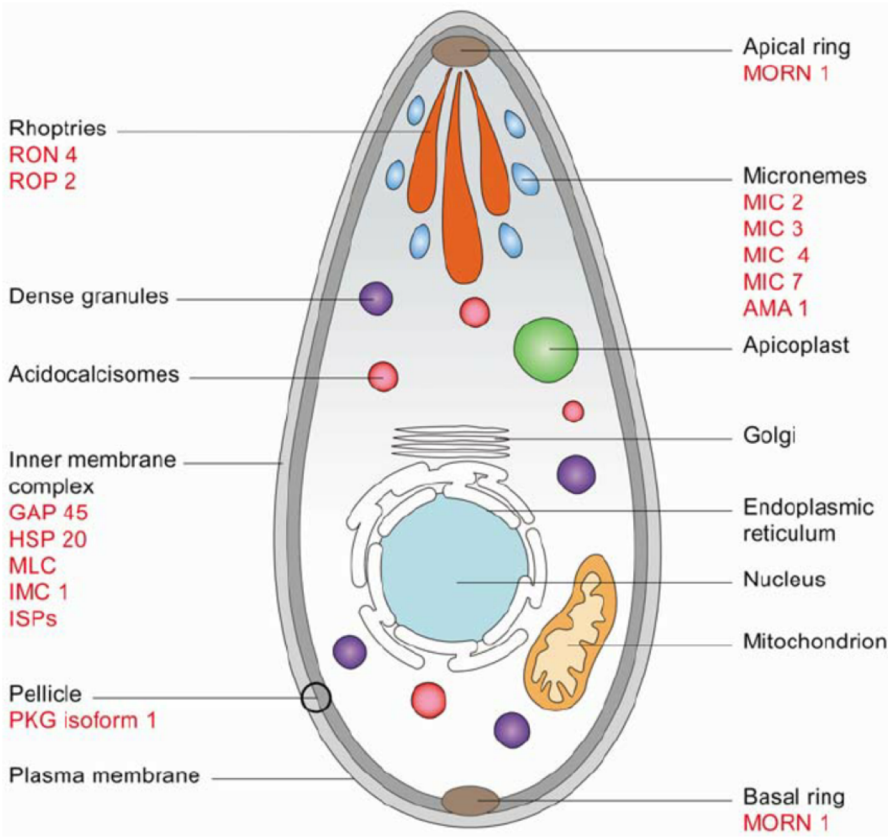
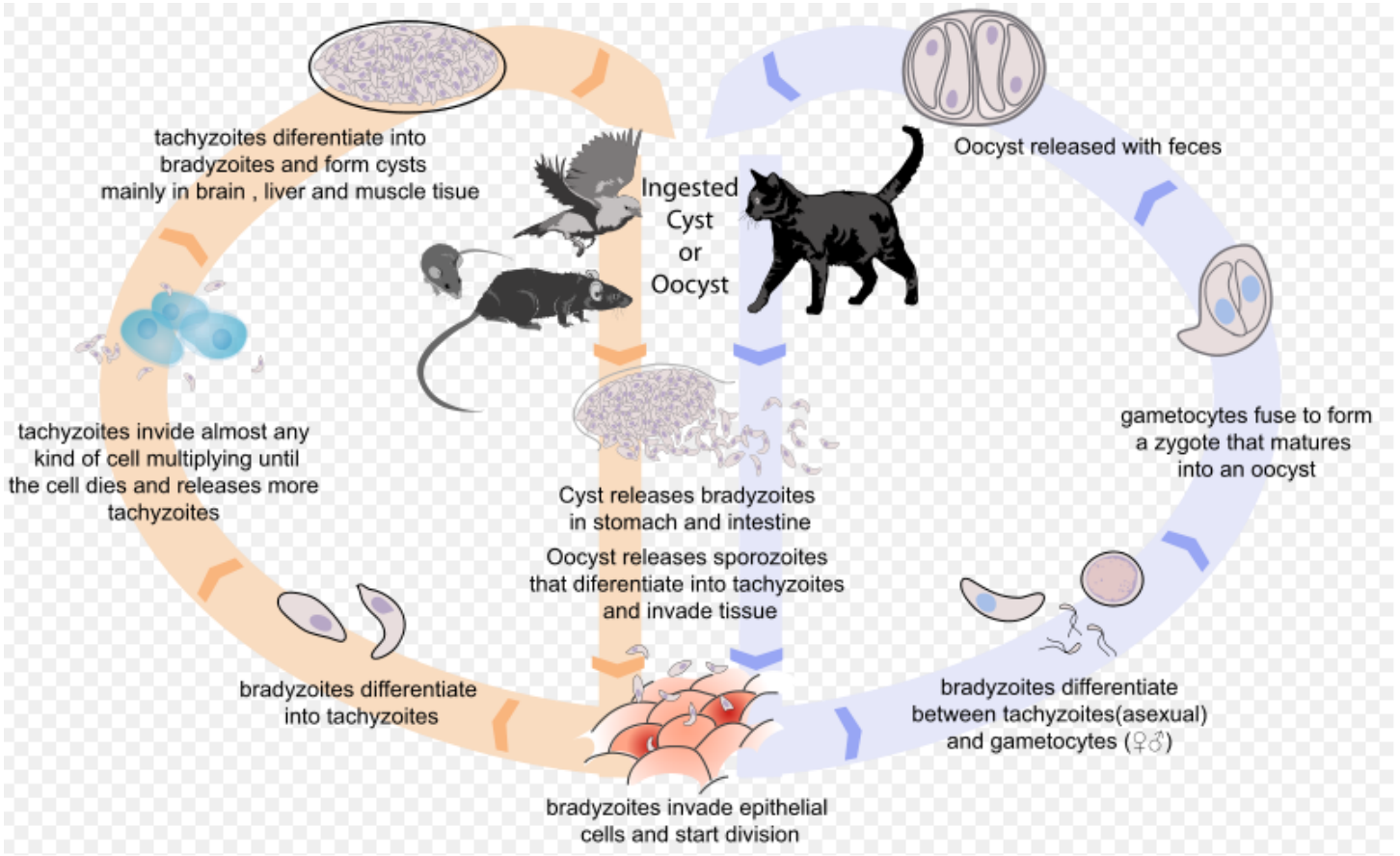
ROLE OF GUANYLATE CYCLASE IN THE LYTIC CYCLE OF TOXOPLASMA GONDII

MeBoP 2018

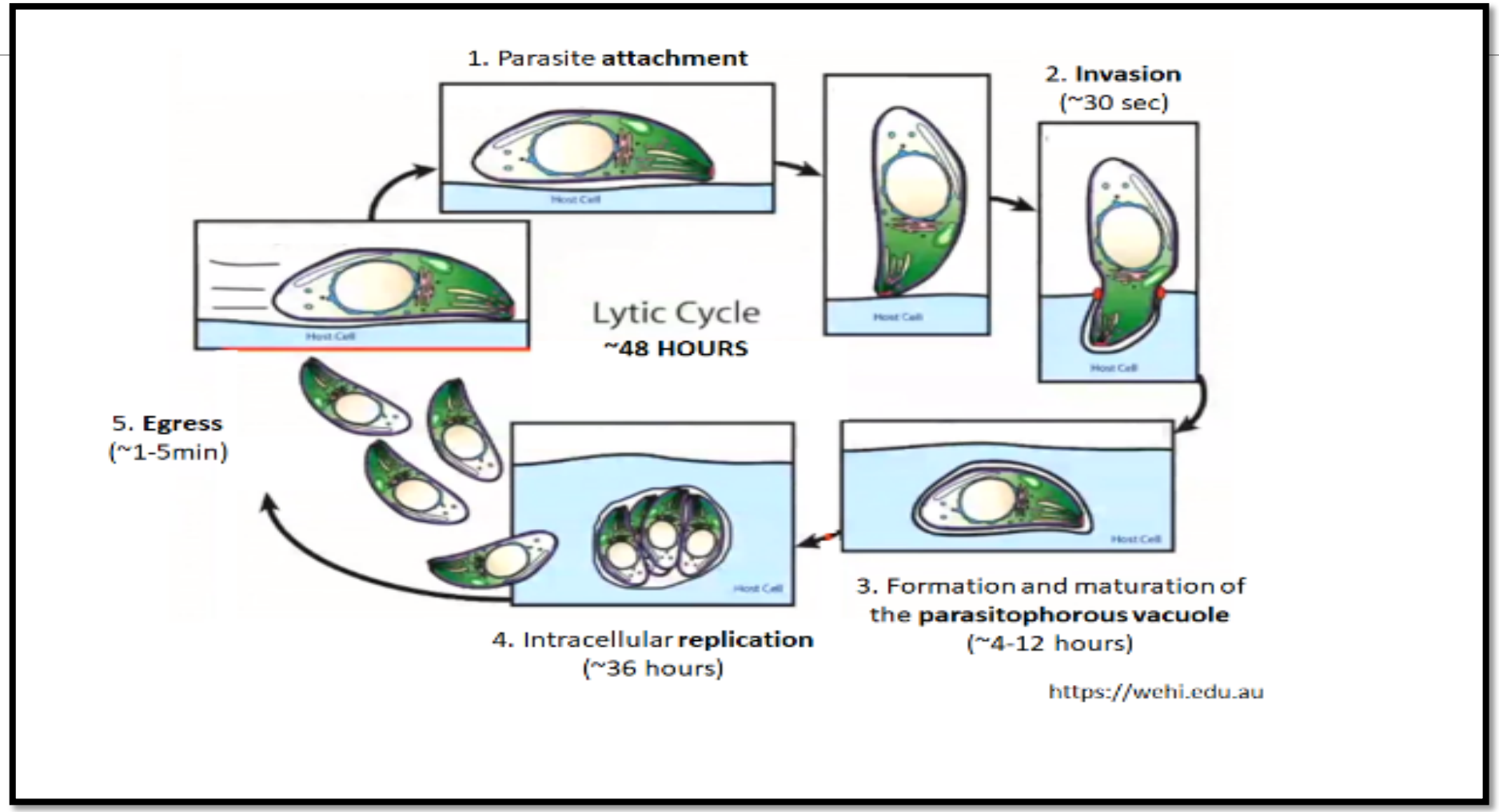
28th July, 2018

LIFE CYCLE MORPHOLOGY





LYTIC CYCLE

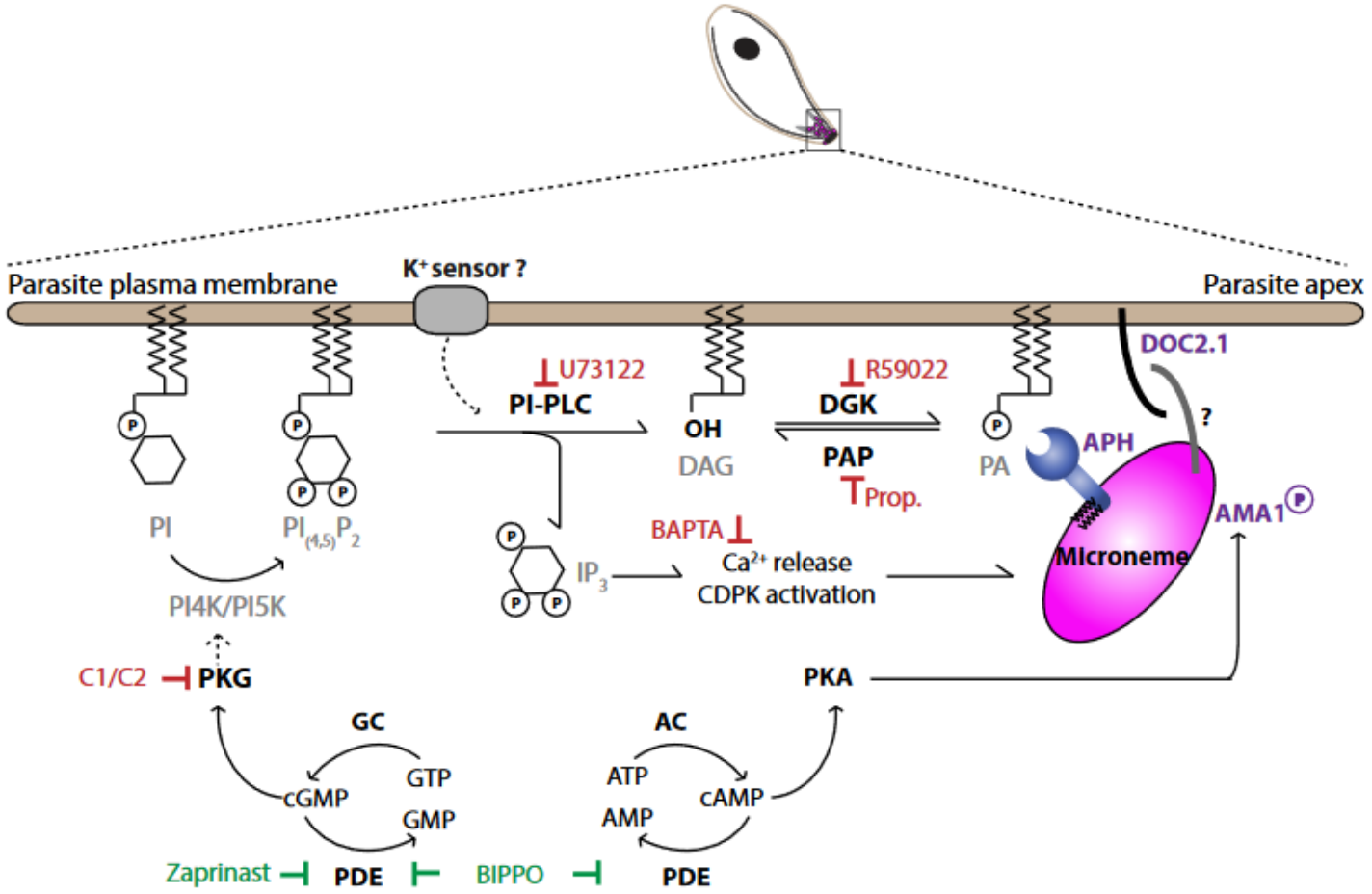

1. Parasite **attachment**
2. **Invasion**
(~30 sec)
Lytic Cycle
~48 HOURS
5. **Egress**
(~1-5min)
3. Formation and maturation of the **parasitophorous vacuole**
(~4-12 hours)
4. Intracellular **replication**
(~36 hours)
https://wehi.edu.au

RESEARCH QUESTION

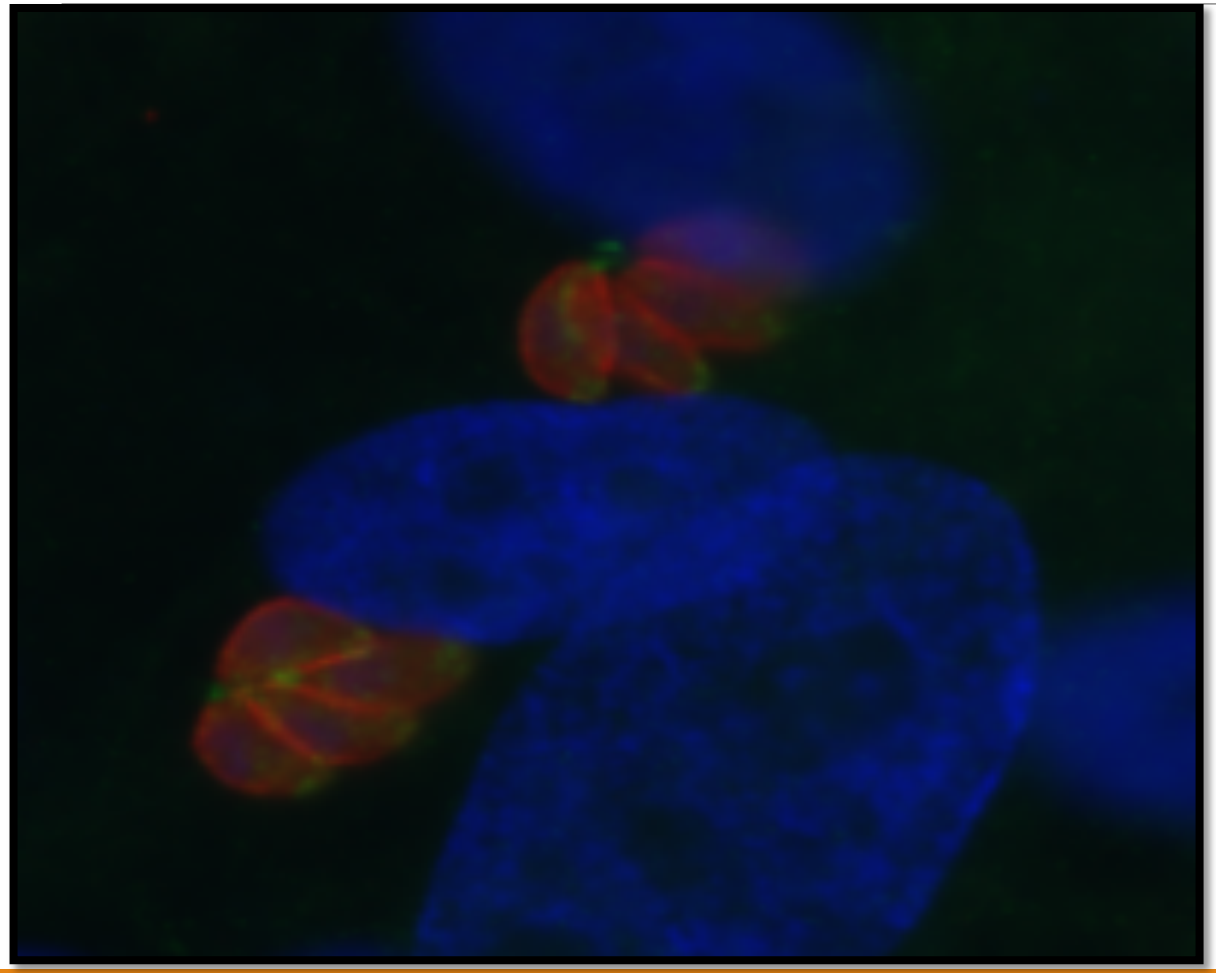
What is the role of GC?

- Function in toxoplasma gondii
- Localization

FUNCTION OF GC



LOCALISATION OF GC

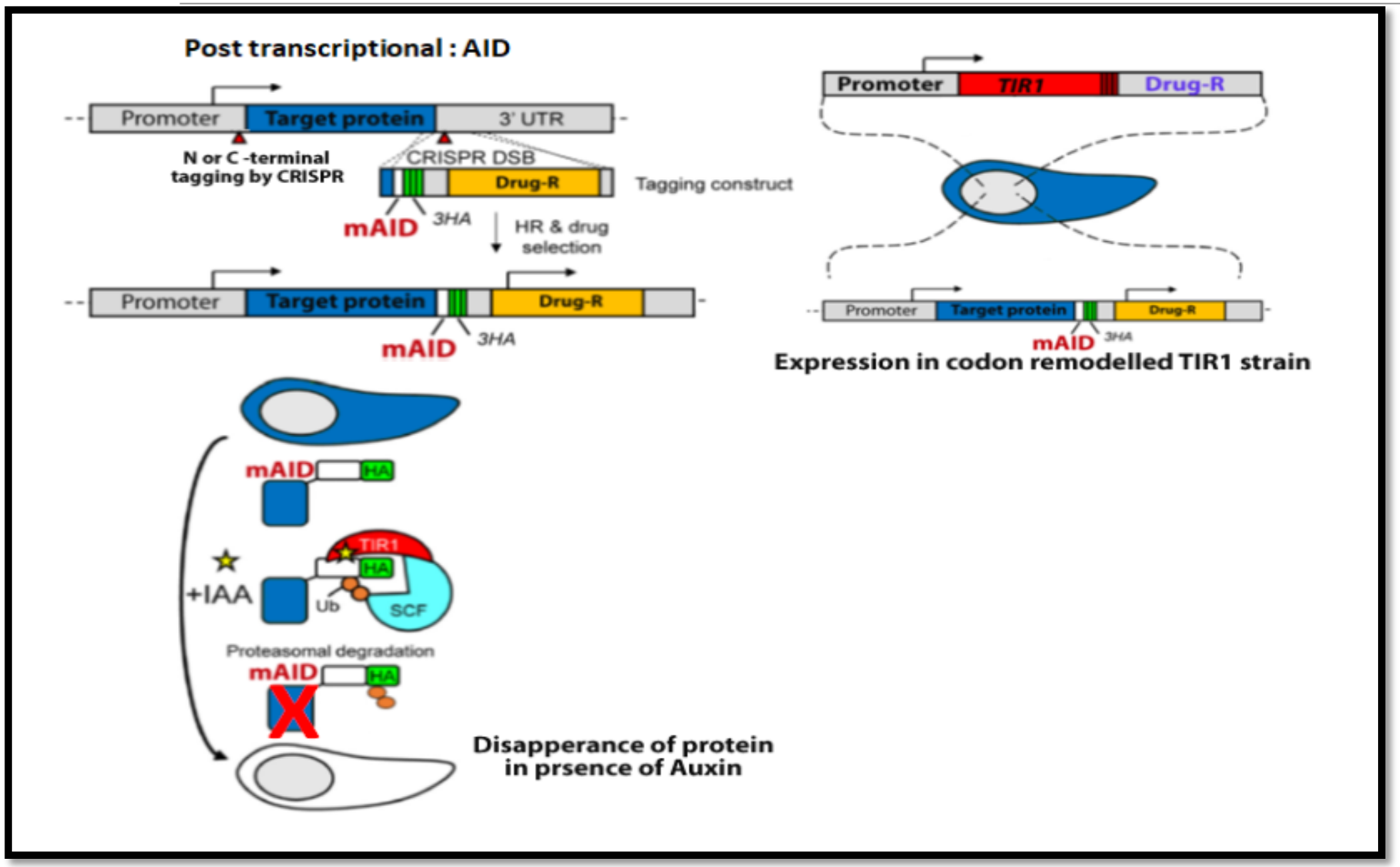


- Using IFA
- GC = HA tag
- GAP45 (anti rabbit) = Inter membrane complex (red)
- Located in : APICAL TIP

GC IS ESSENTIAL FOR LYTIC PROCESS

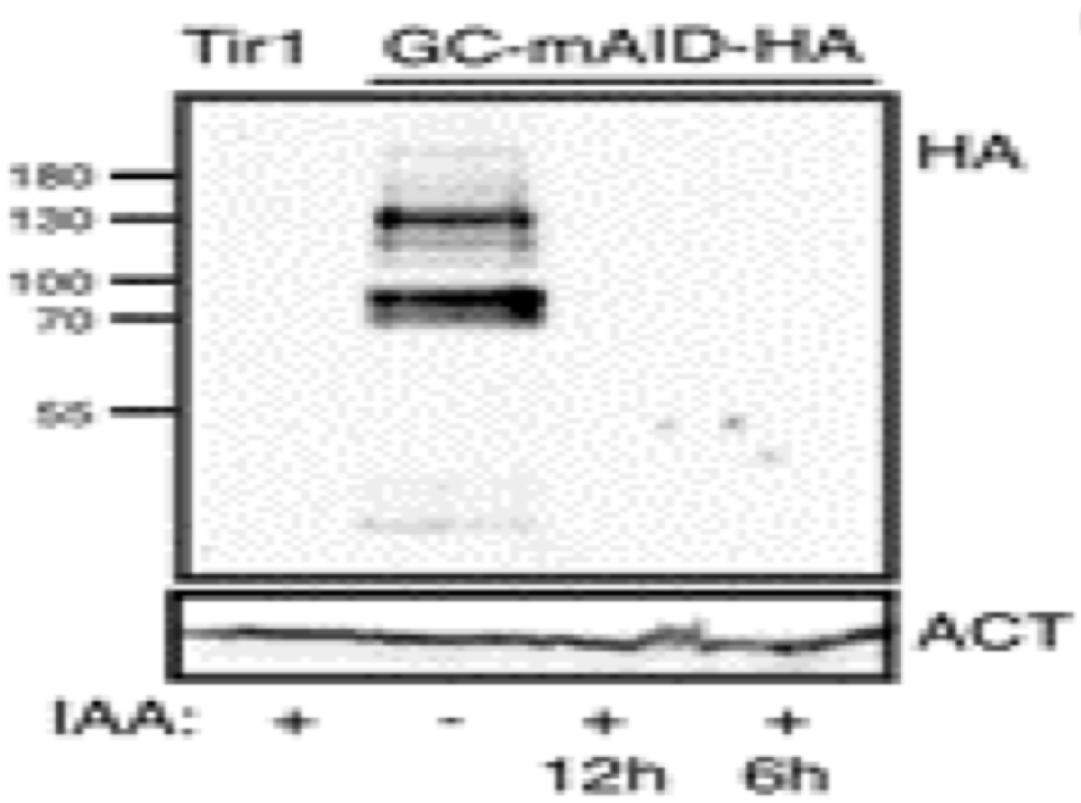
- Knock down
- Western blot
- Plaque assay

GC IS ESSENTIAL FOR LYTIC PROCESS



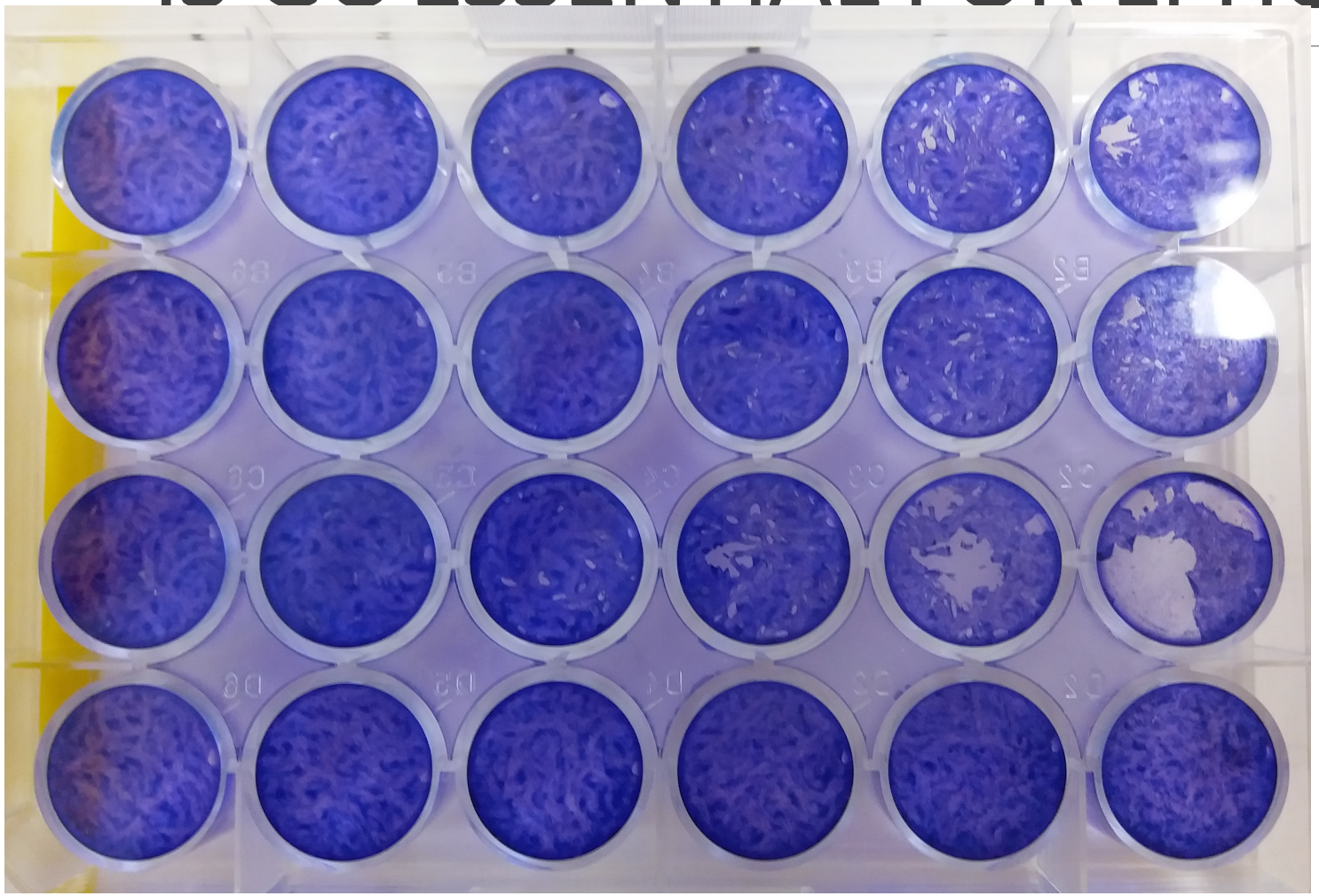
- Knock down
- AID Degron
- Auxin

GC IS ESSENTIAL FOR LYTIC PROCESS



- 6 hours
- 12hours
- Knock down confirmed

IS GC ESSENTIAL FOR LYTIC PROCESS?



Tir1 - IAA

Tir1 + IAA

GC mAID – IAA

GC mAID + IAA

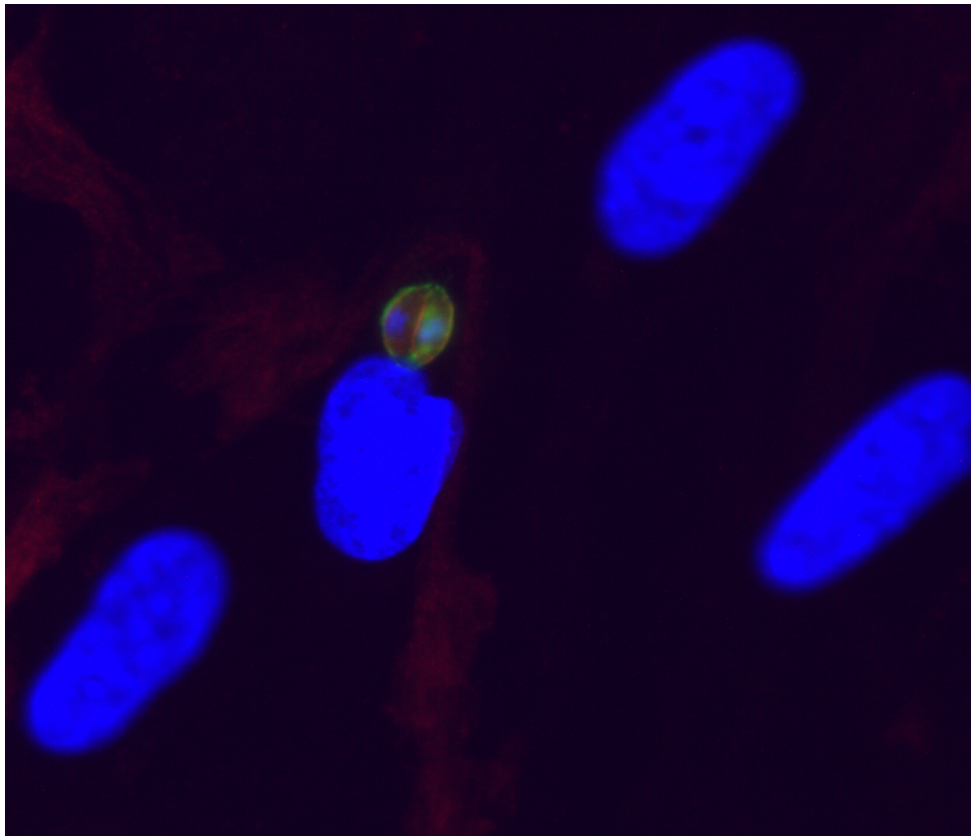
WHERE IN THE LYTIC PROCESS IS GC INVOLVED?

- Invasion
- Replication
- Egress

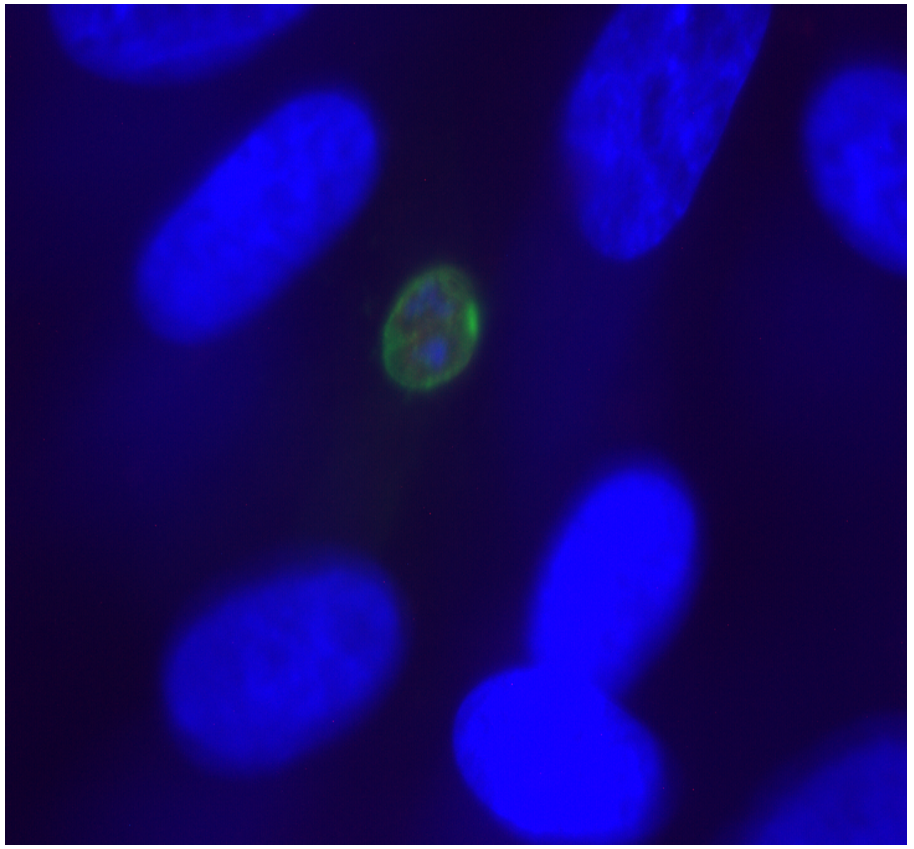
- IFA
- Primary antibodies
- GRA3 (anti mouse) = Vacuole (green)
- GAP45 (anti rabbit) = Inter membrane complex (red)
- Secondary antibodies

WHERE IN THE LYTIC PROCESS IS GC INVOLVED?

Host post infection (HPI) = 12 hours



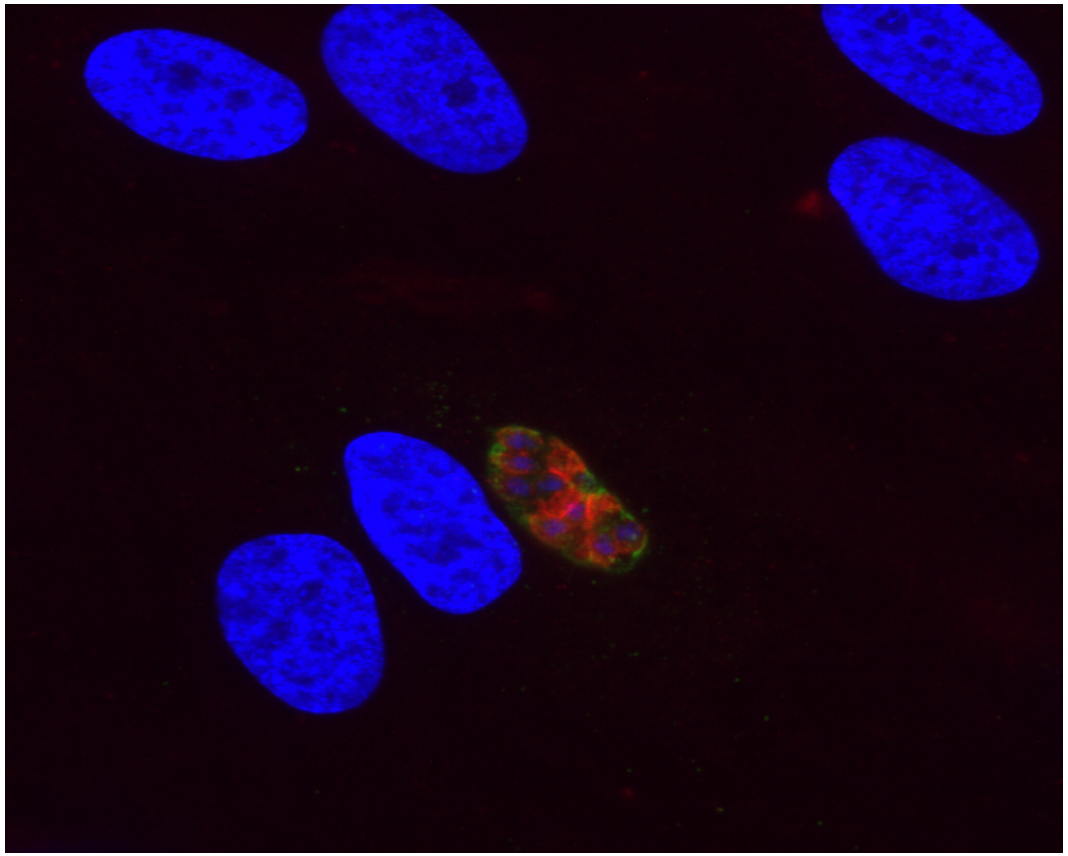
Tir 1 (+IAA)



GC (+ IAA)

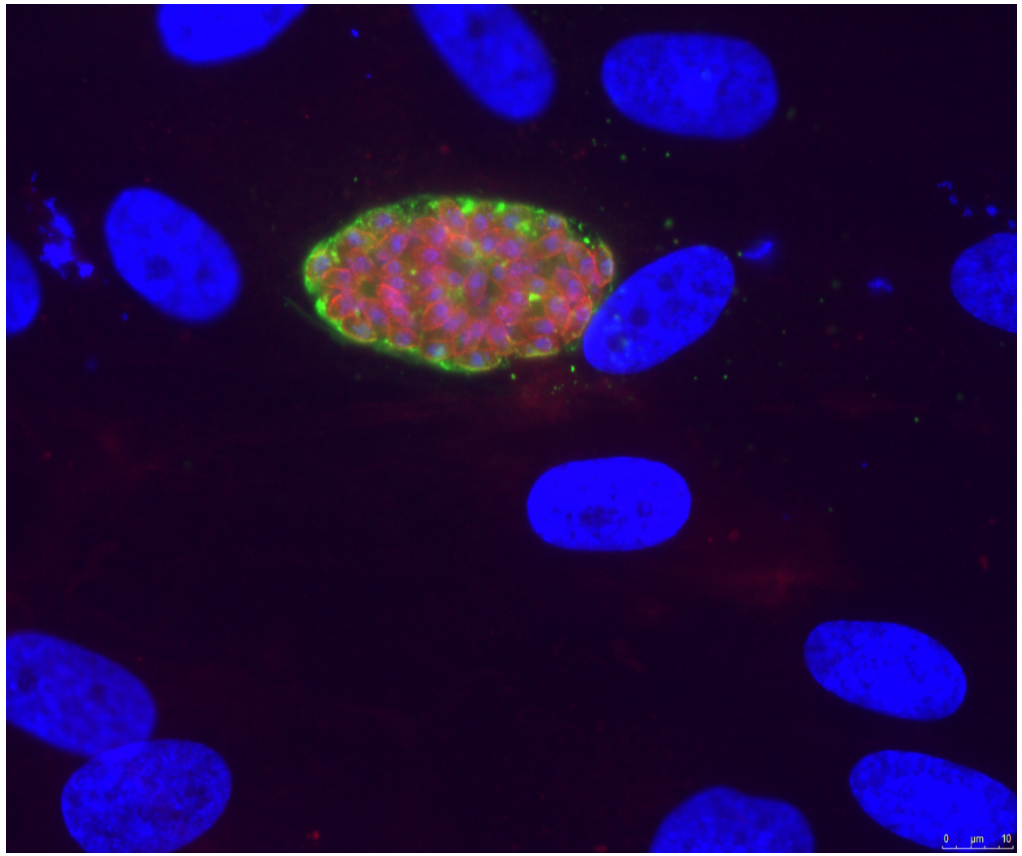
WHERE IN THE LYTIC PROCESS IS GC INVOLVED?

HPI = 24 hours



GC (+IAA)

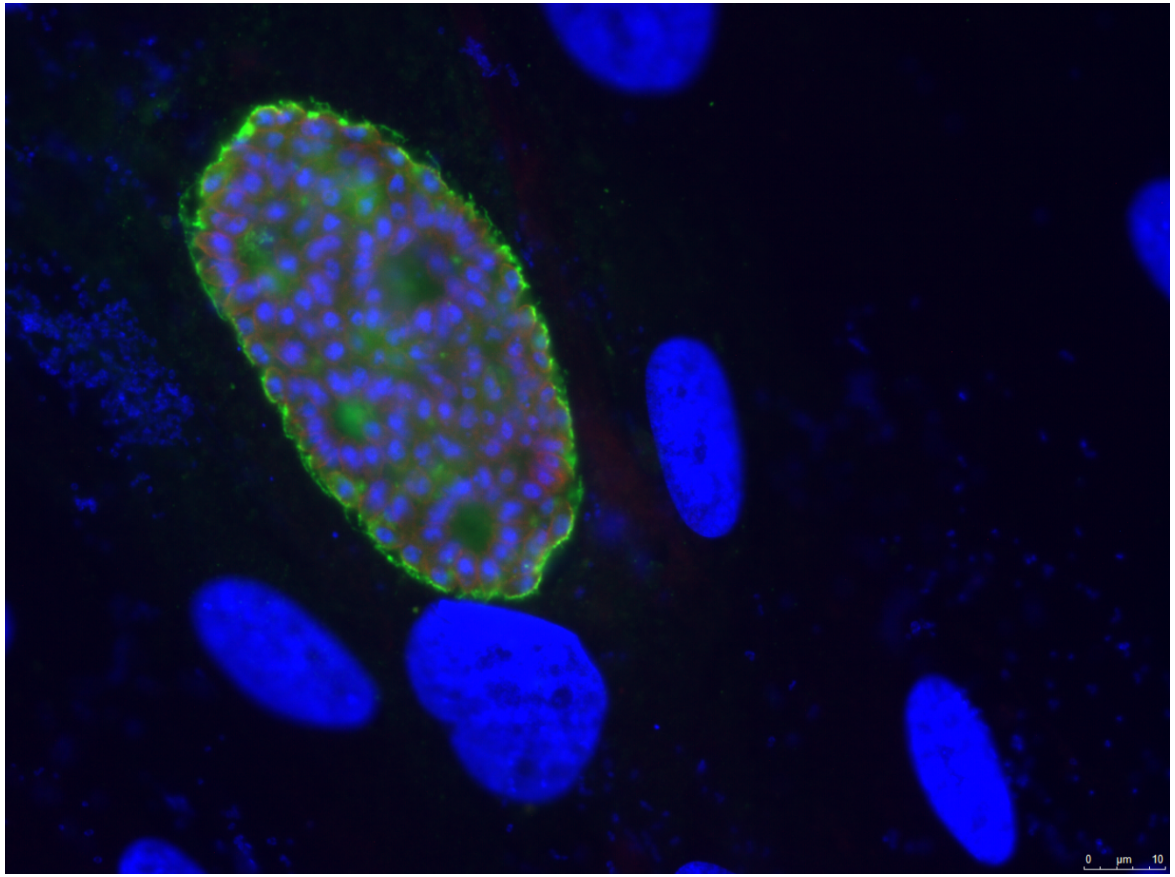
HPI = 48 hours



GC (+IAA)

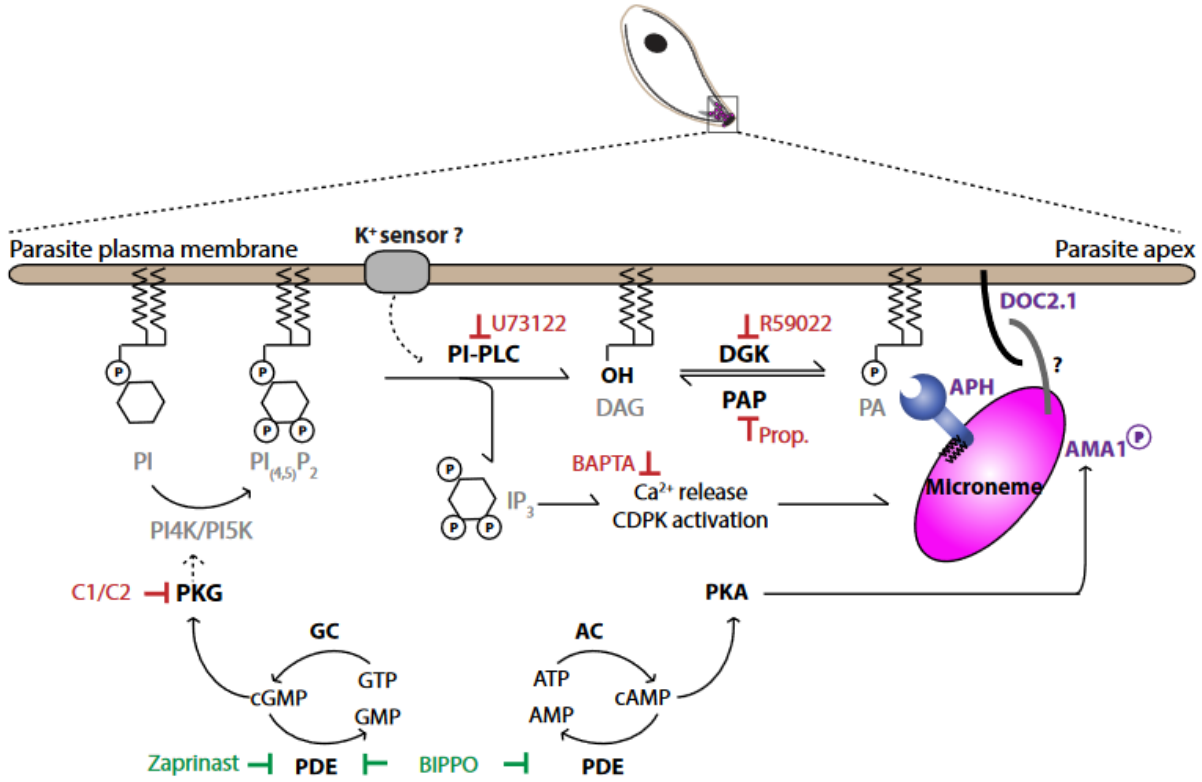
WHERE IN THE LYTIC PROCESS IS GC INVOLVED?

HPI = 55 hours



GC might be involved in parasite egress

GC COULD INTERACT WITH DGK2

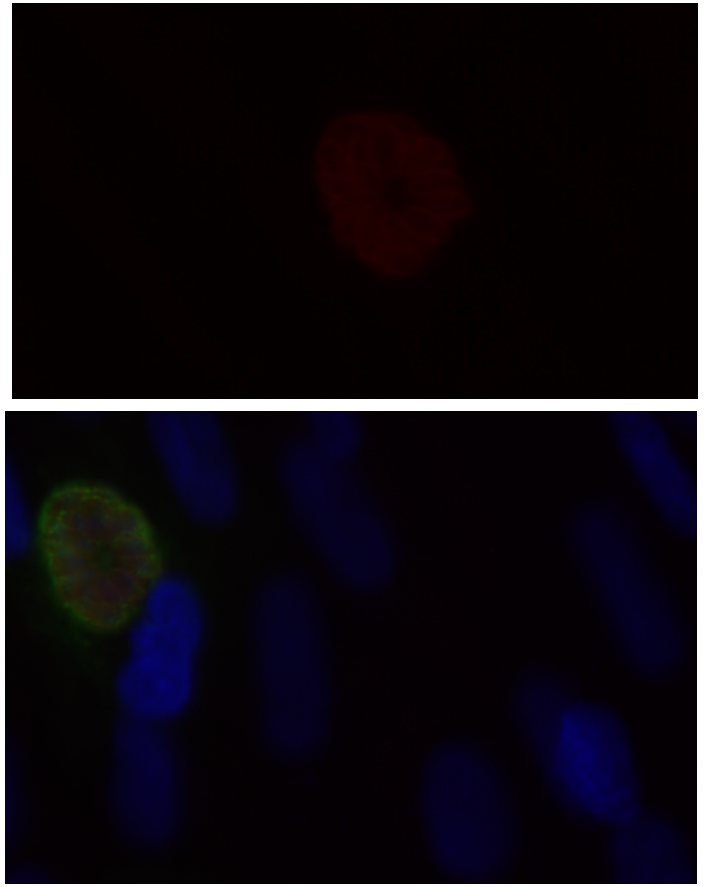


GC MIGHT BE INVOLVED IN PARASITE EGRESS

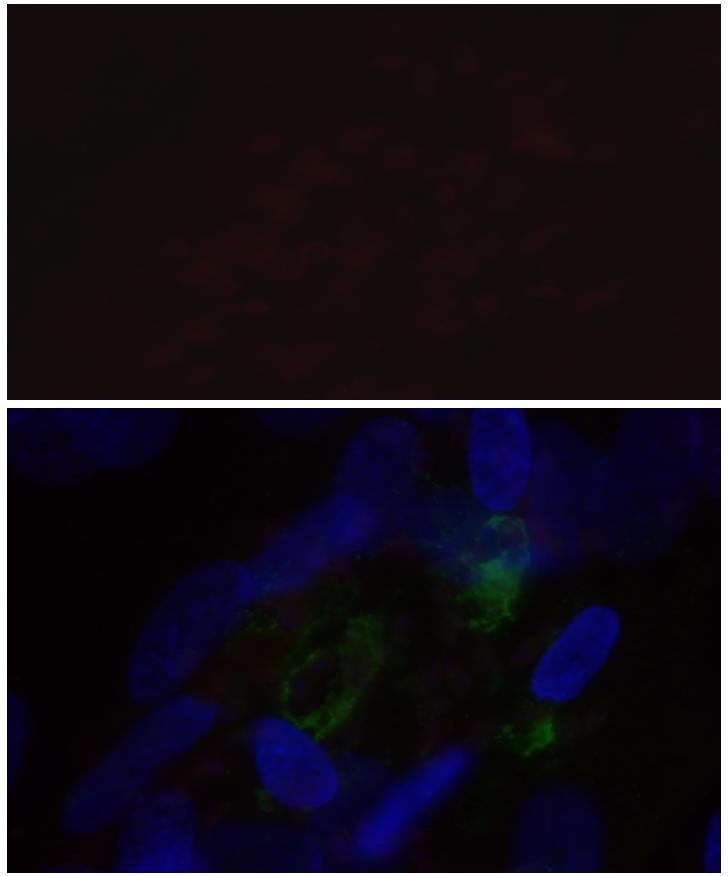
WESTERN BLOT

- PA
- BIPPO
- NO MICRONEME SCRETION IN THE ABSENCE OF GC

GC MIGHT BE INVOLVED IN PARASITE EGRESS



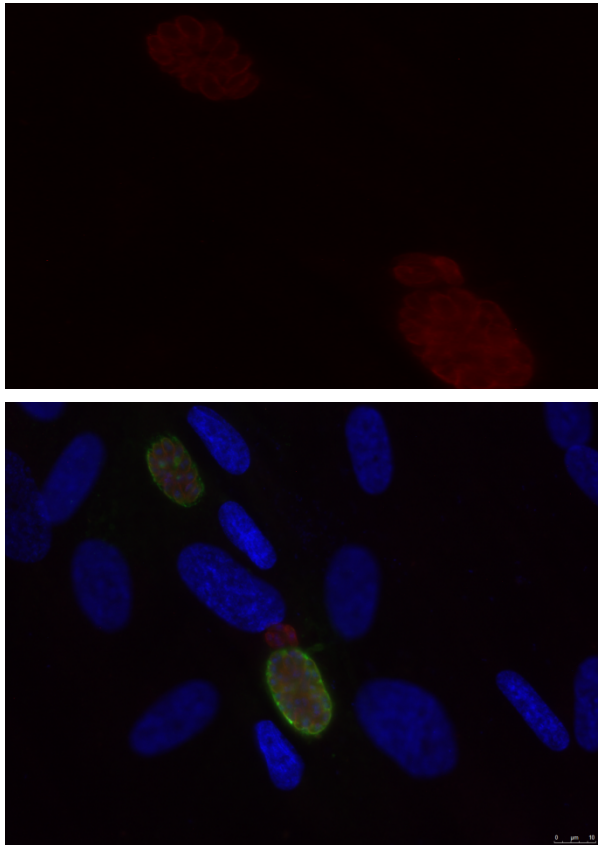
Tir 1 (-IAA) DMSO- No Egress



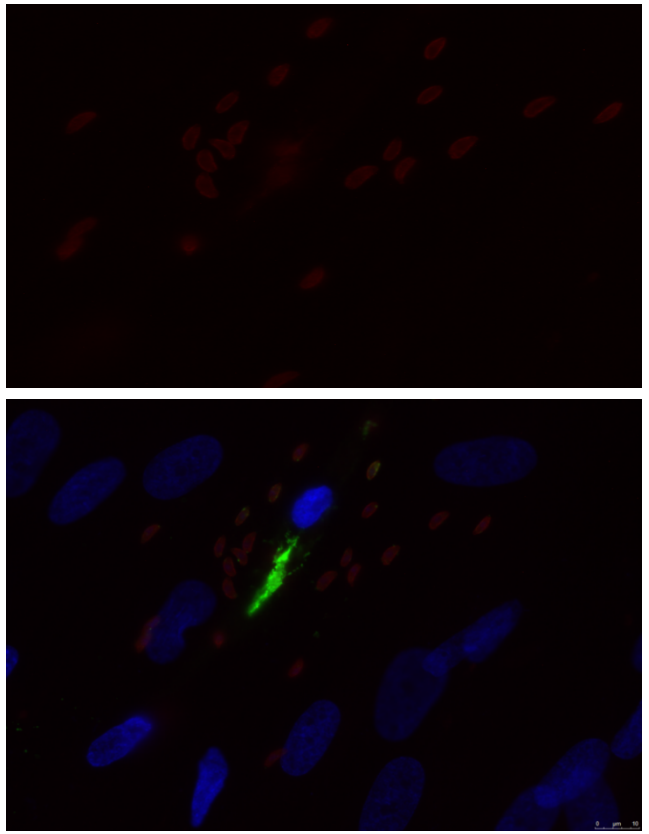
Tir 1 (-IAA) BIPO-Egress

- 5-Benzyl-3-isopropyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (BIPPO)
  - potent inhibitor of phosphodiesterases triggered egress
  - promotes cAMP-dependent phosphorylation
  - useful tool for the dissection of signal transduction pathways in apicomplexan parasites.

GC MIGHT BE INVOLVED IN PARASITE EGRESS



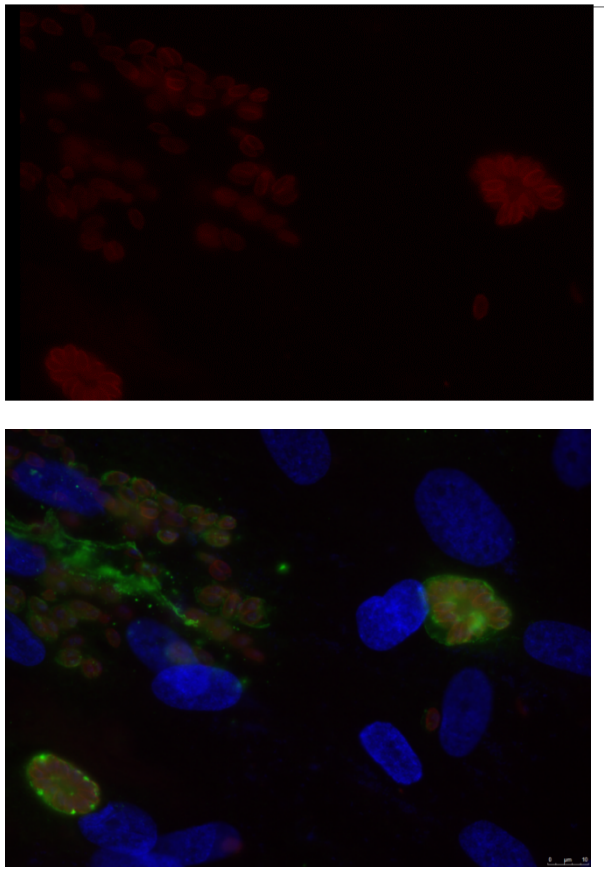
Tir 1 (+IAA) DMSO- No Egress



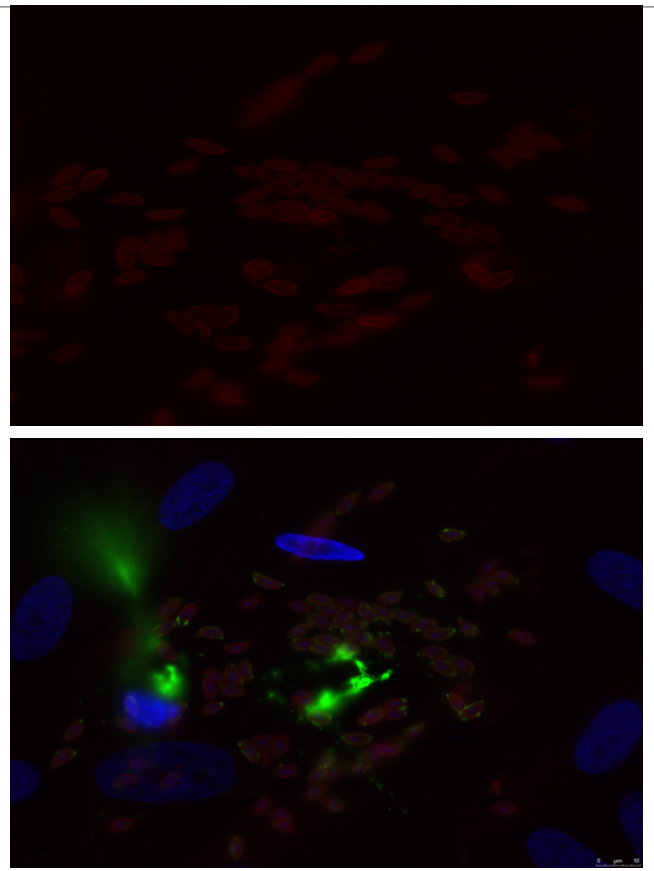
Tir 1 (+IAA) BIPO- Partial Egress

IAA has no effect on BIPPC

GC MIGHT BE INVOLVED IN PARASITE EGRESS



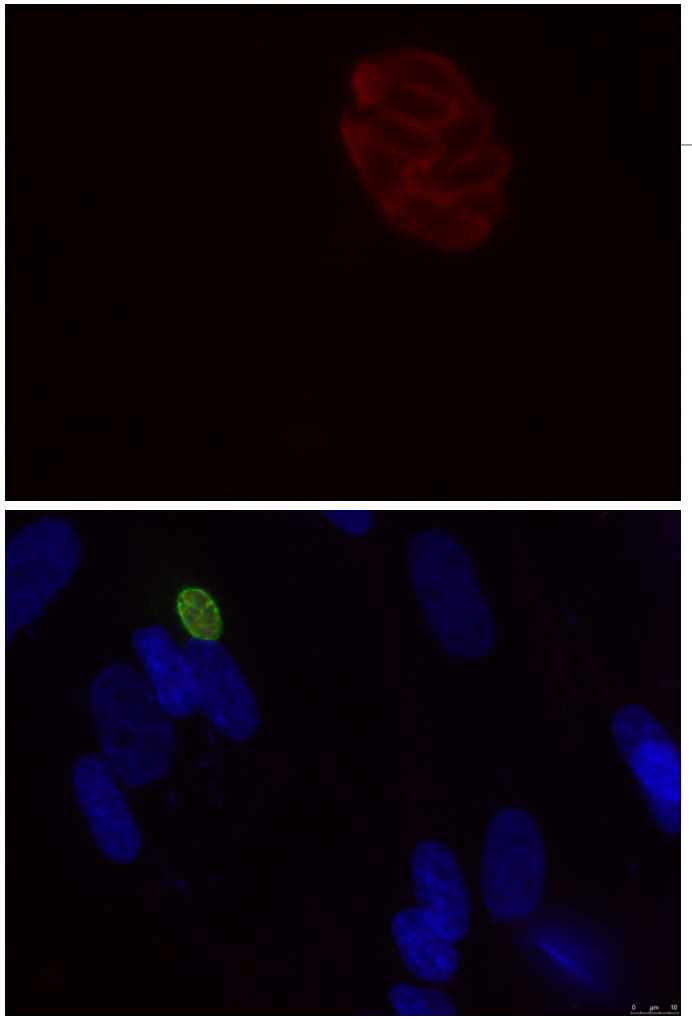
GC (-IAA) DMSO-Partial Egression (In= 8 and Out= 2)



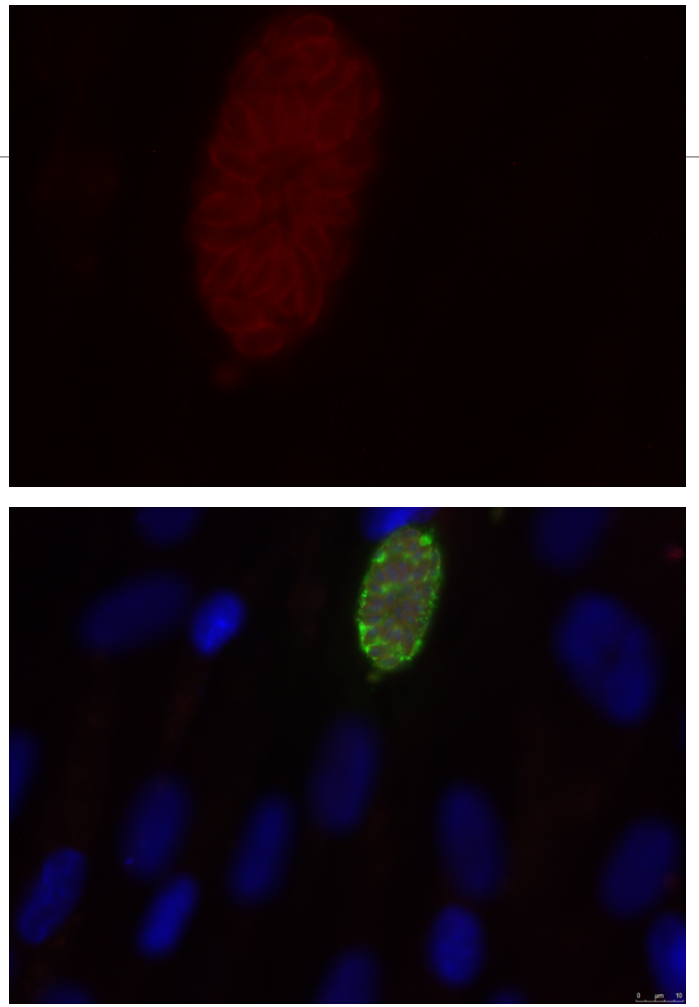
GC (-IAA) BIPPO: Massive egress (In: 5 and out: 6)

BIPPO potentiated to action of GC

GC MIGHT BE INVOLVED IN PARASITE EGRESS



GC (+IAA) DMSO: No Egress (In= 10, Out= 1)



GC (+IAA) –BIPPO: No Egress (In= 10, Out= 1)

- IAA- blocks the activity of GC
- BIPPO doesn't have a egression effect in the absence of GC

SUMMARY

- GC could be involved in the lytic cycle of the toxoplasma gondii
- GC could be involved in parasite egress
- GC could interact with DGK2


western.