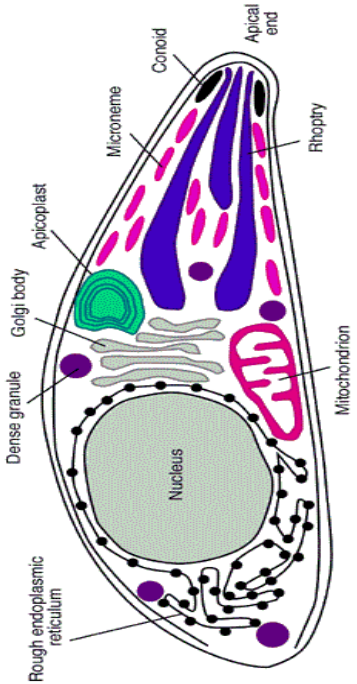
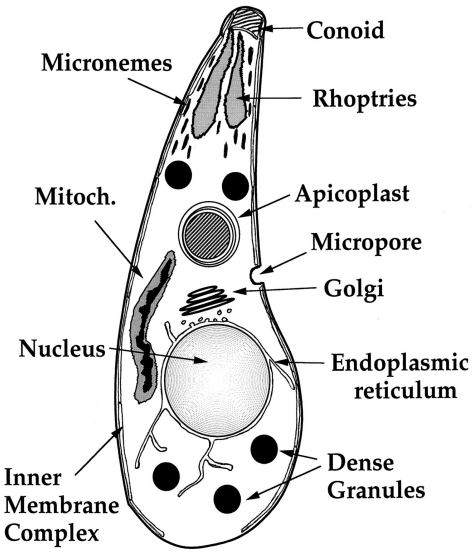
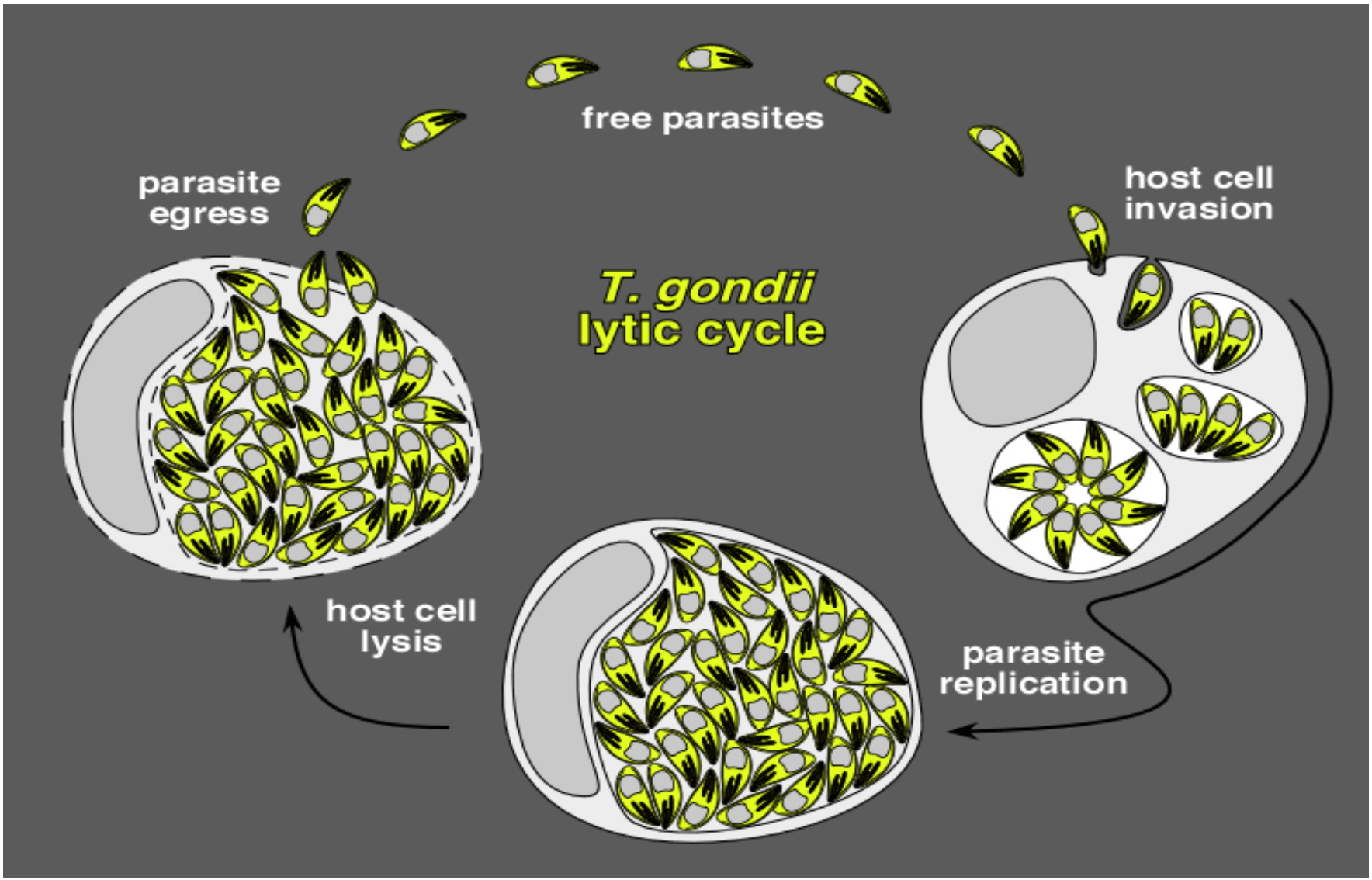
Immunofluorescence assays (IFA) to quantify intracellular invasion and visualize organelles

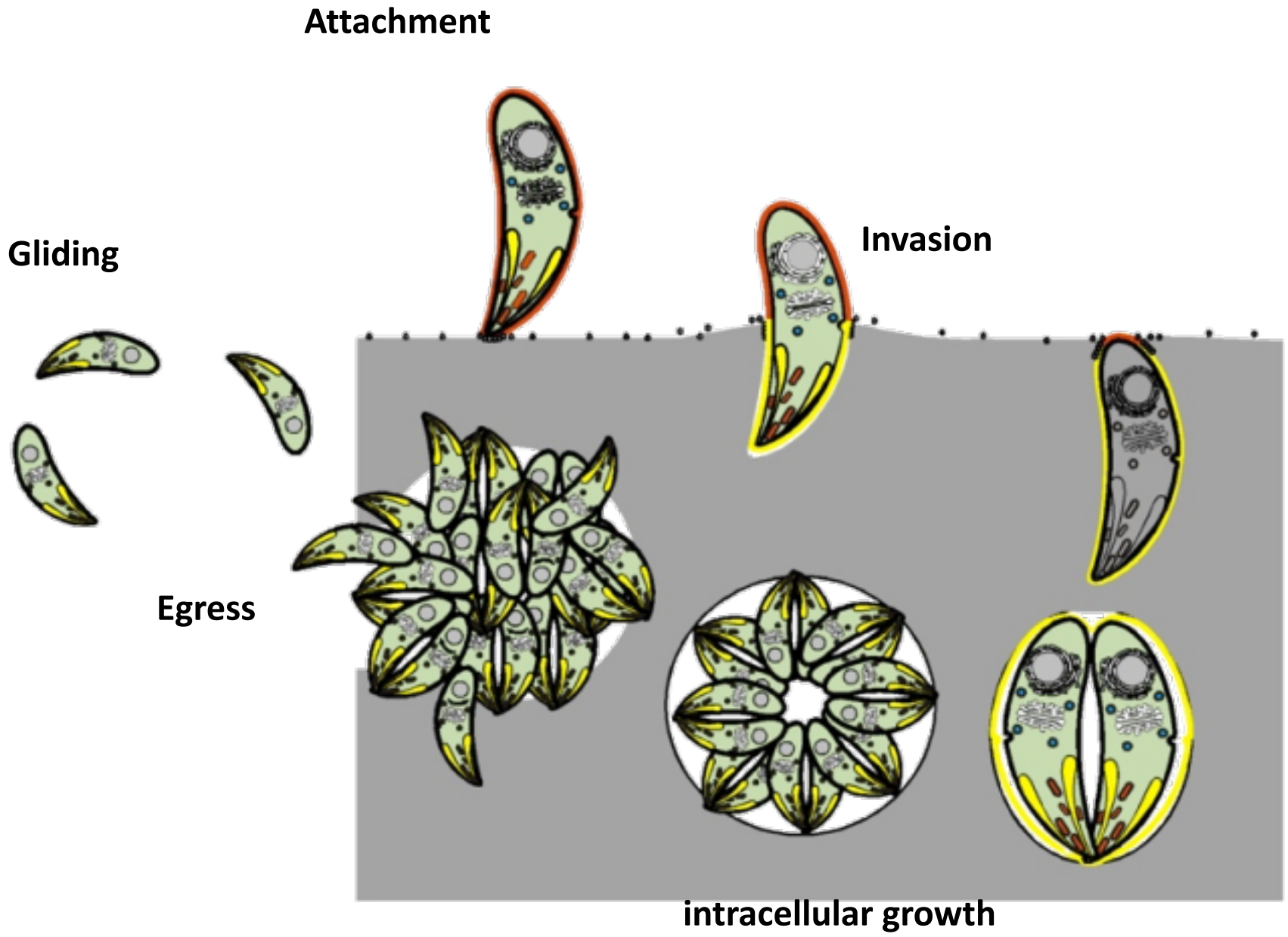


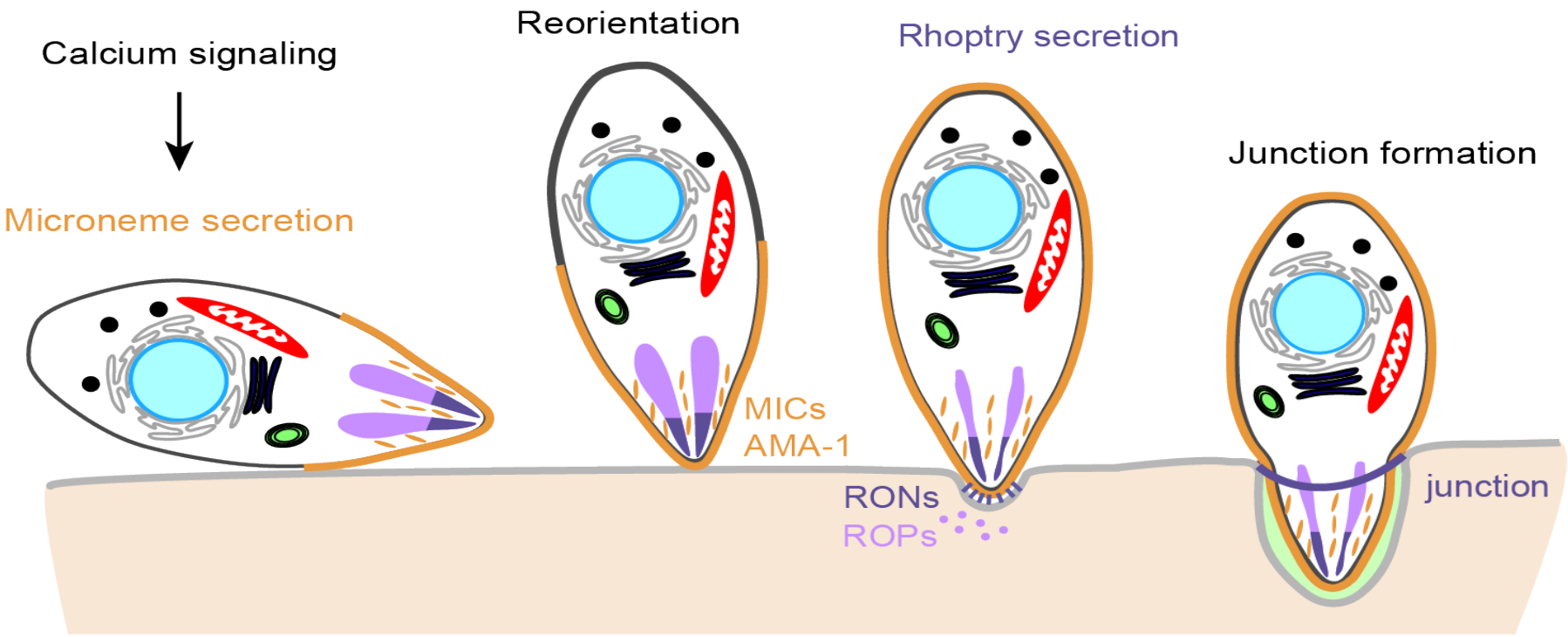
By:
Alireza
Amjed

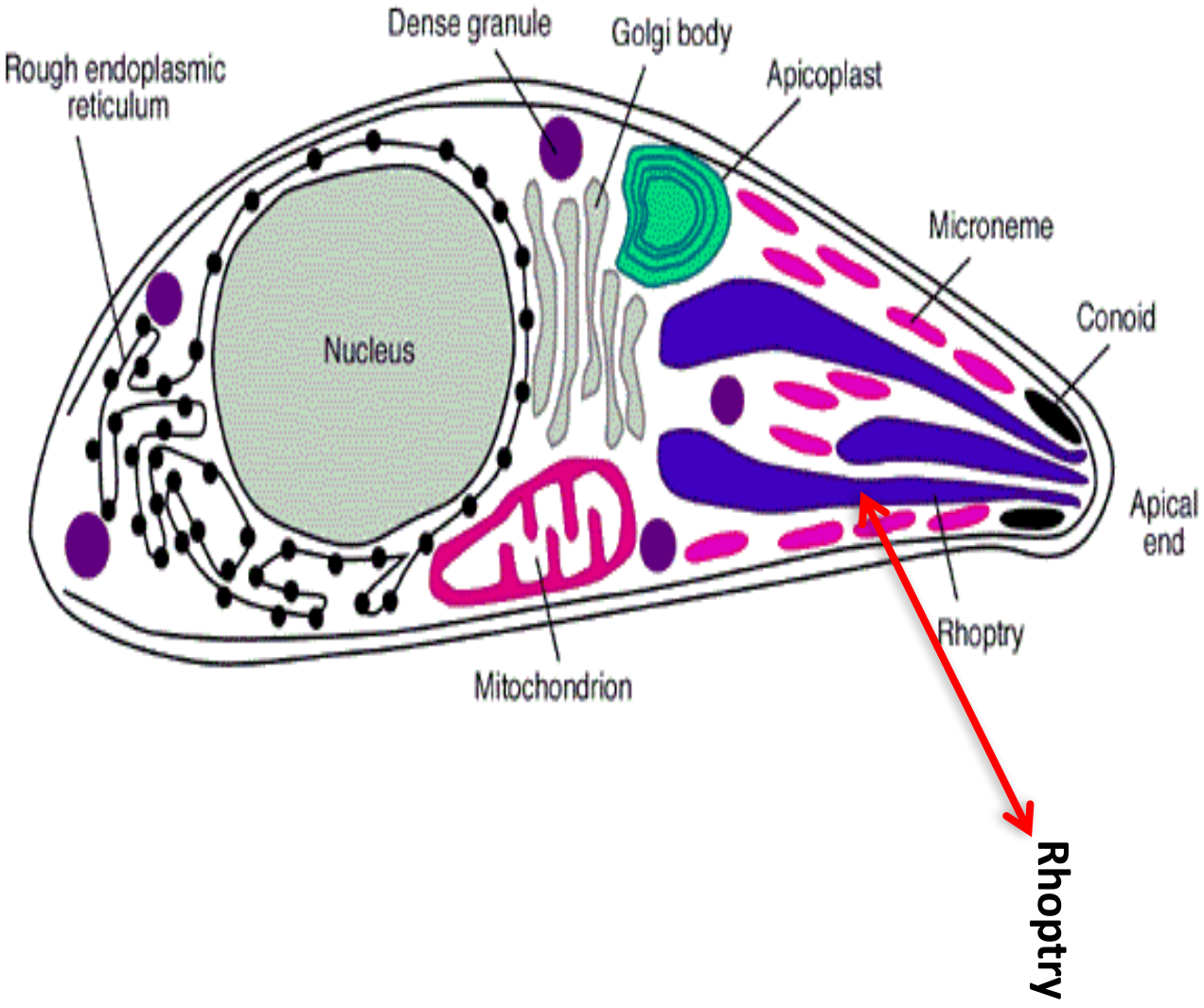


T. gondii Lytic Cycle




Attachment
Gliding
Invasion
Egress
intracellular growth


Calcium signaling
Microneme secretion
Reorientation
MICs
AMA-1
Rhoptry secretion
RONs
ROPs
Junction formation
junction


Dense granule
Golgi body
Rough endoplasmic reticulum
Apicoplast
Microneme
Conoid
Nucleus
Apical end
Rhoptry
Mitochondrion
Rhoptry

Methods

According to the protocol in Page 12:

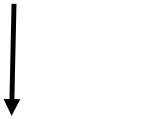
- specific primary Ab (alpha –SAG1)
- secondary (Rop2)

Invasion assay (red/green)

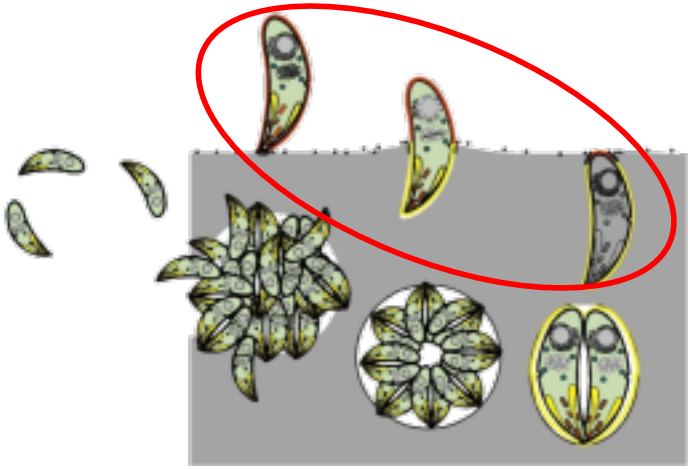
ASP3-iKD
and control
+/- ATc

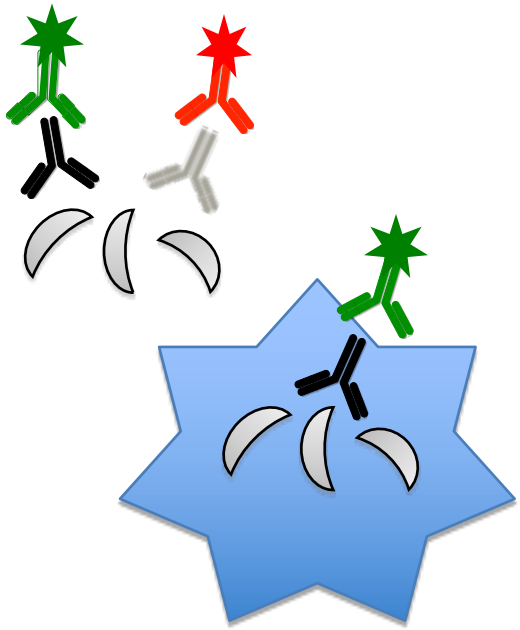
coverslip

Invasion
30 minutes at 37°C



Fixation and IFA

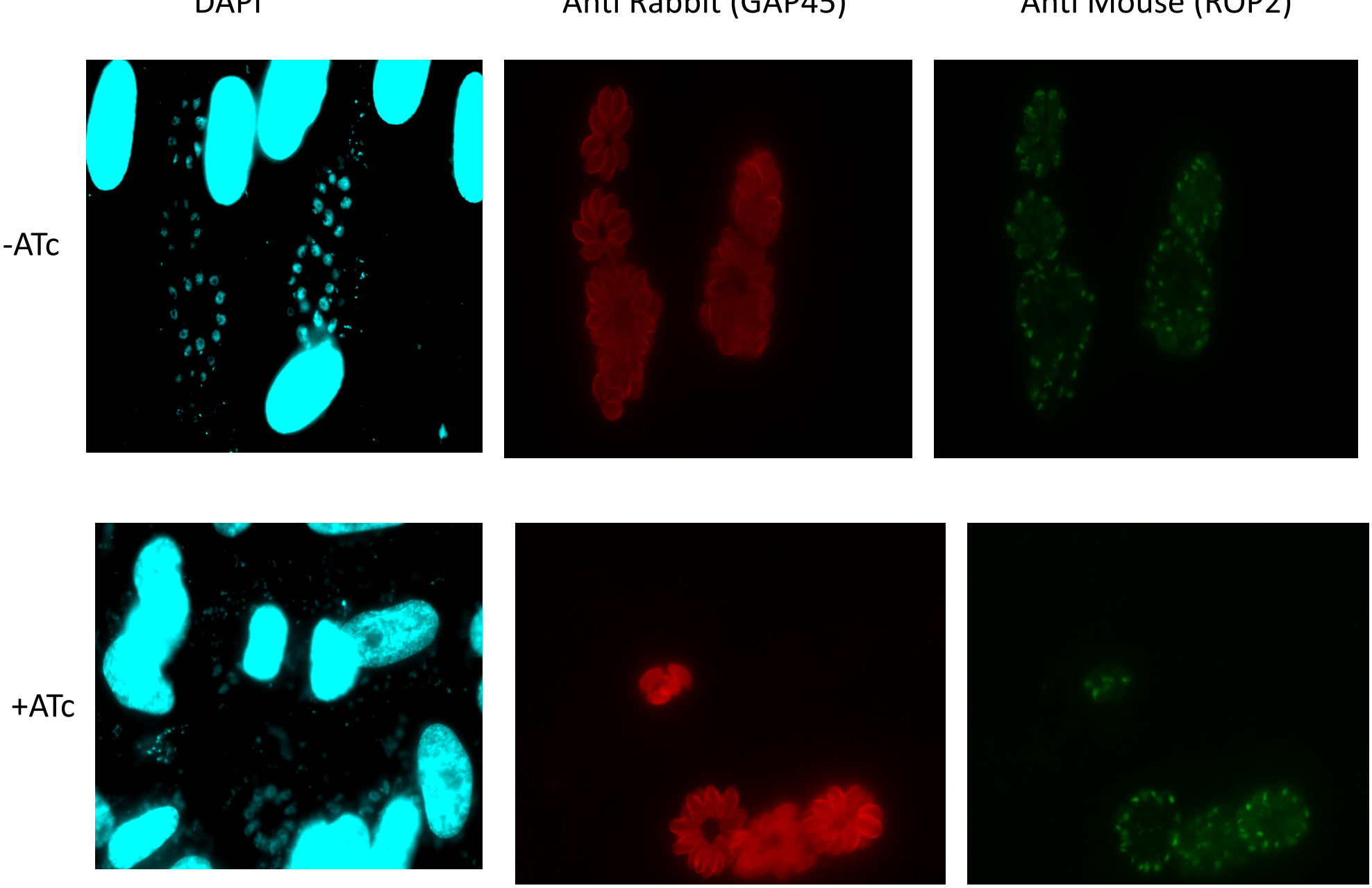


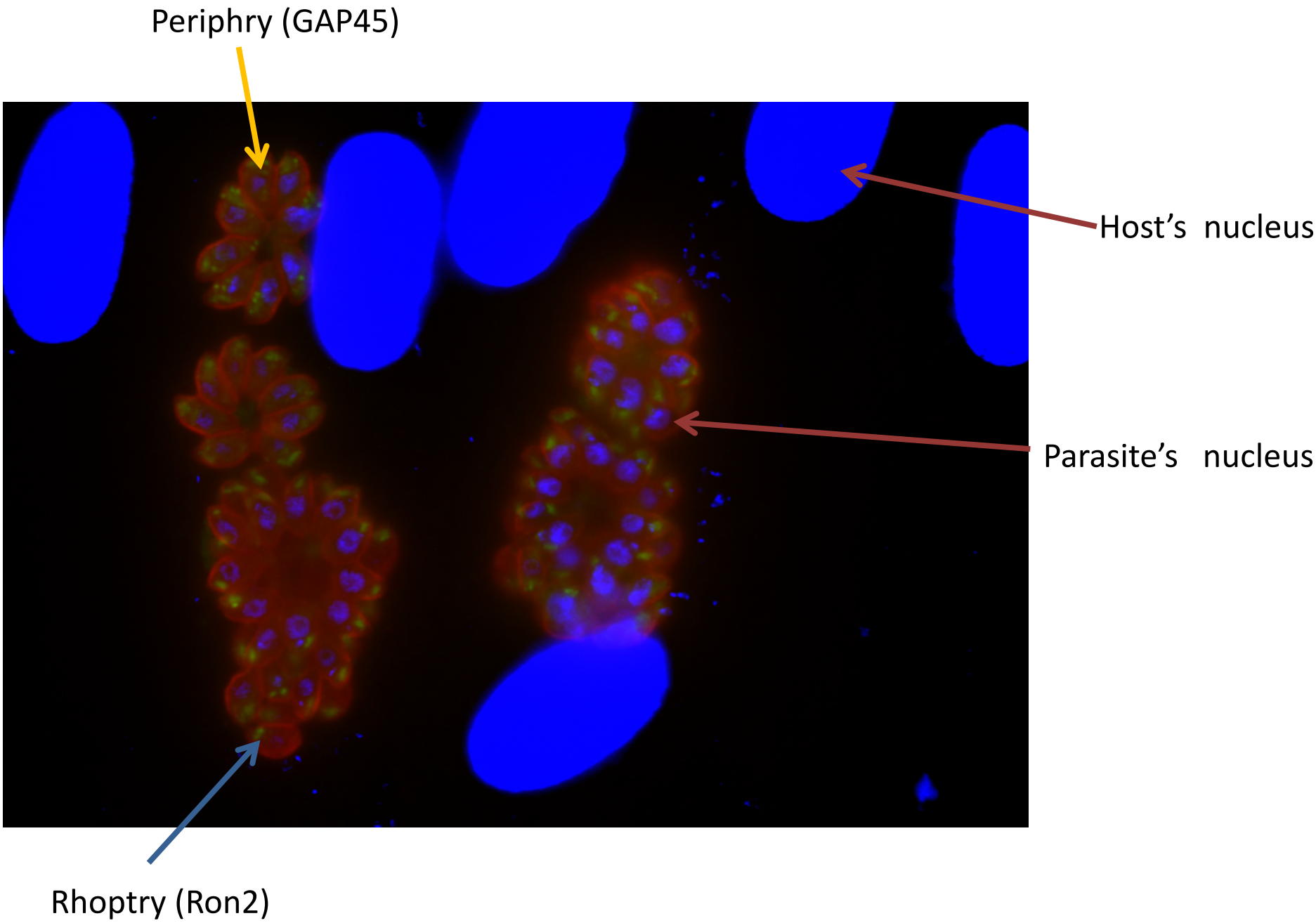


Immunofluorescence assay or IFA

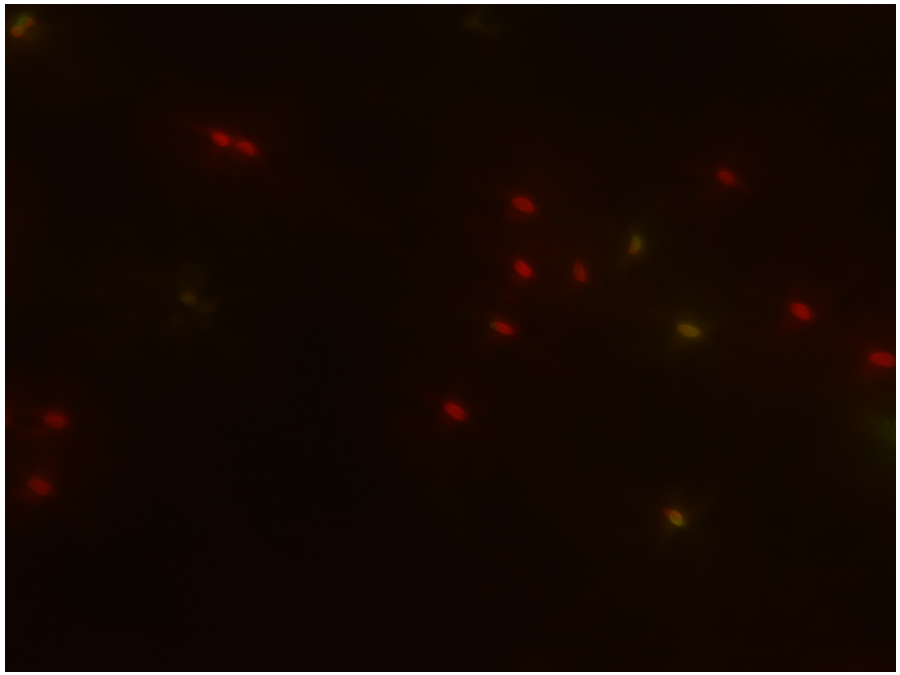
Step 1: **No permeabilization**!!
Staining of the extracellular parasites
(primary Ab: surface marker, ex: anti-SAG1)

Step 2: **after permeabilization**!!
Staining of the intracellular <u>and</u> extracellular parasites
(primary Ab: inside marker, ex: anti-GAP45 then secondary Ab)

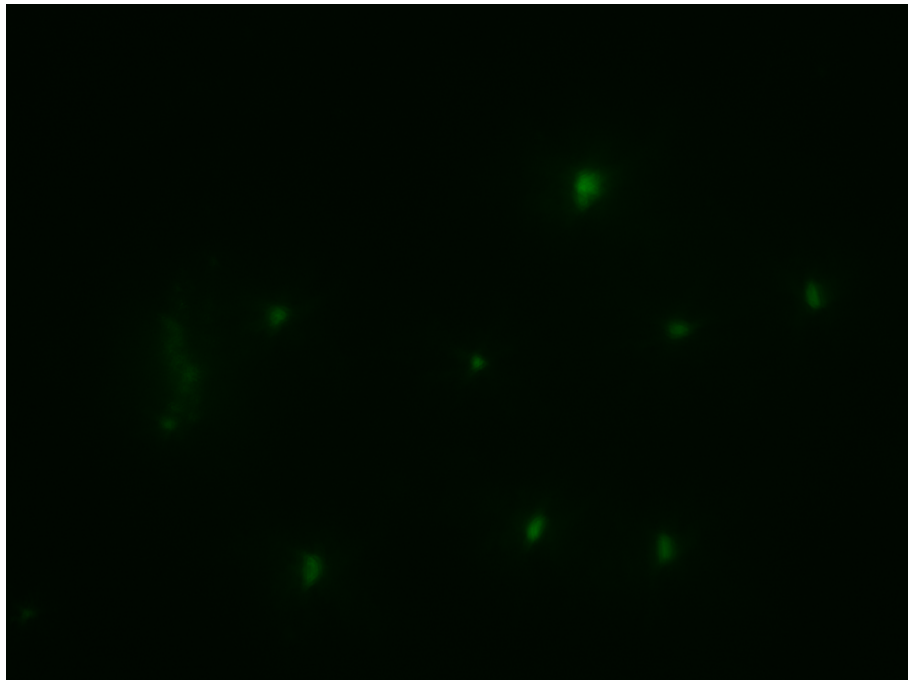

DAPI
Anti Rabbit (GAP45)
Anti Mouse (ROP2)
-ATc
+ATc



Organelles (Rhoptery)



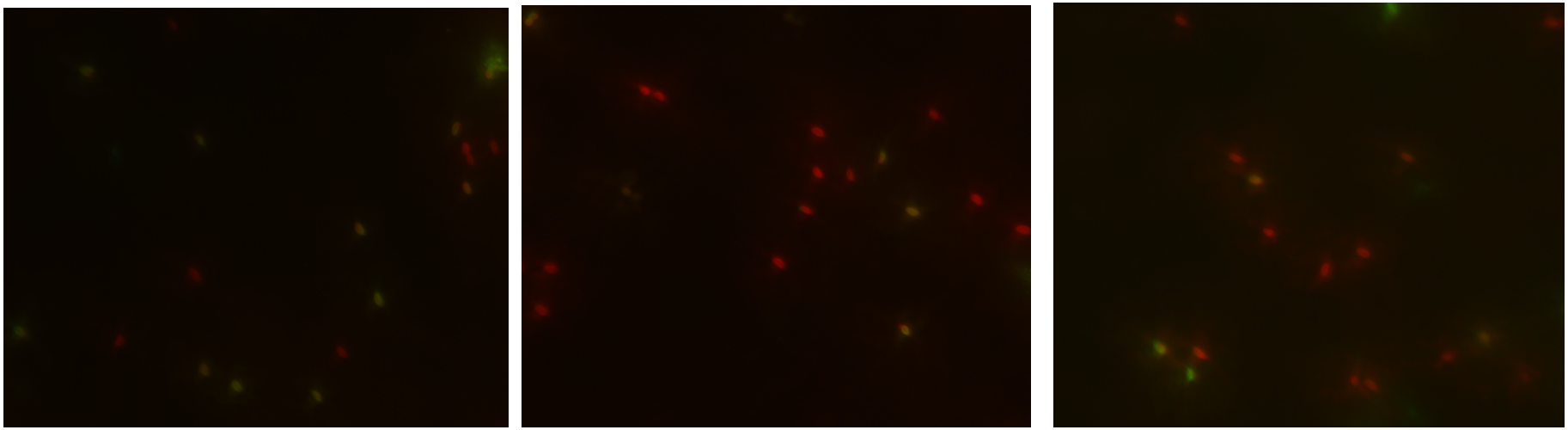
Invasion assay - Atc group



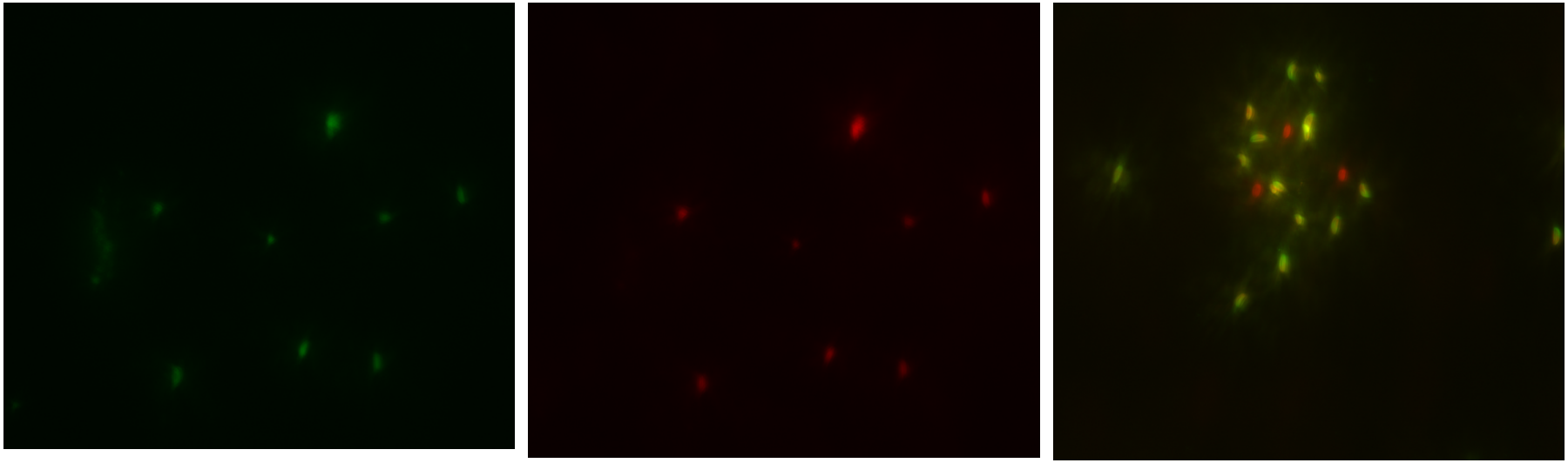
Invasion assay + Atc group

Green: Extracellular
Red: Intracellular

-ATc



+ATc



Green: Extracellular
Red: Intracellular

ASP3 knockdown severely impacts invasion

Invasion Assay