

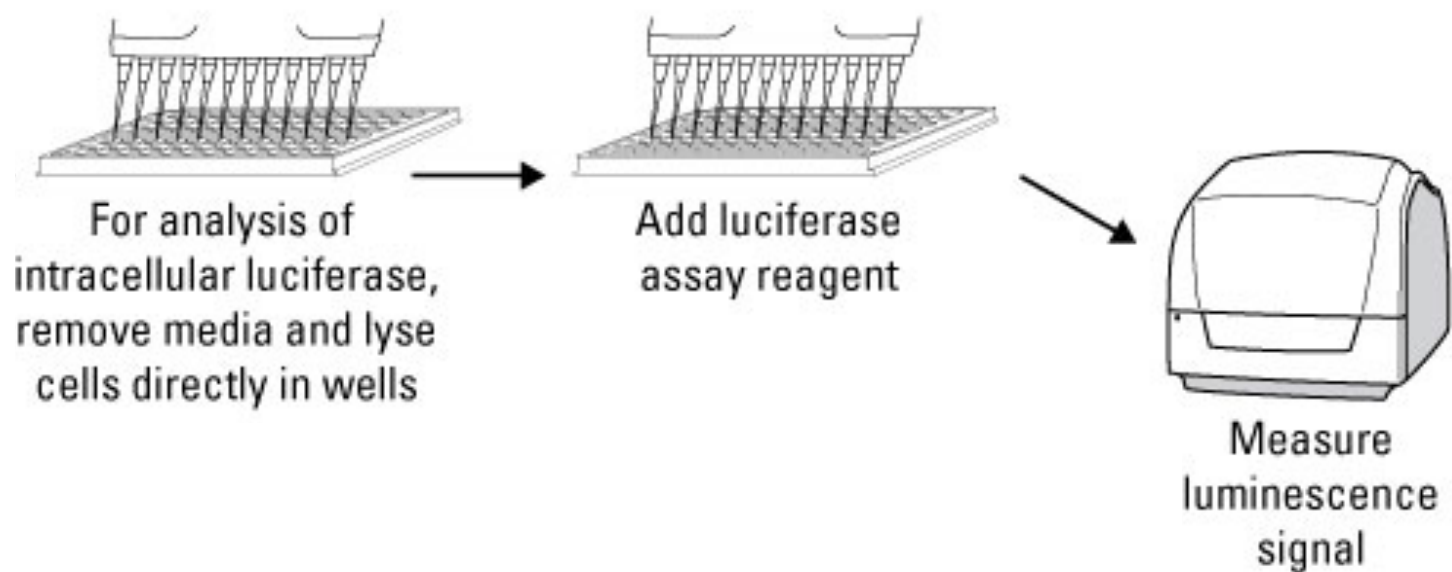
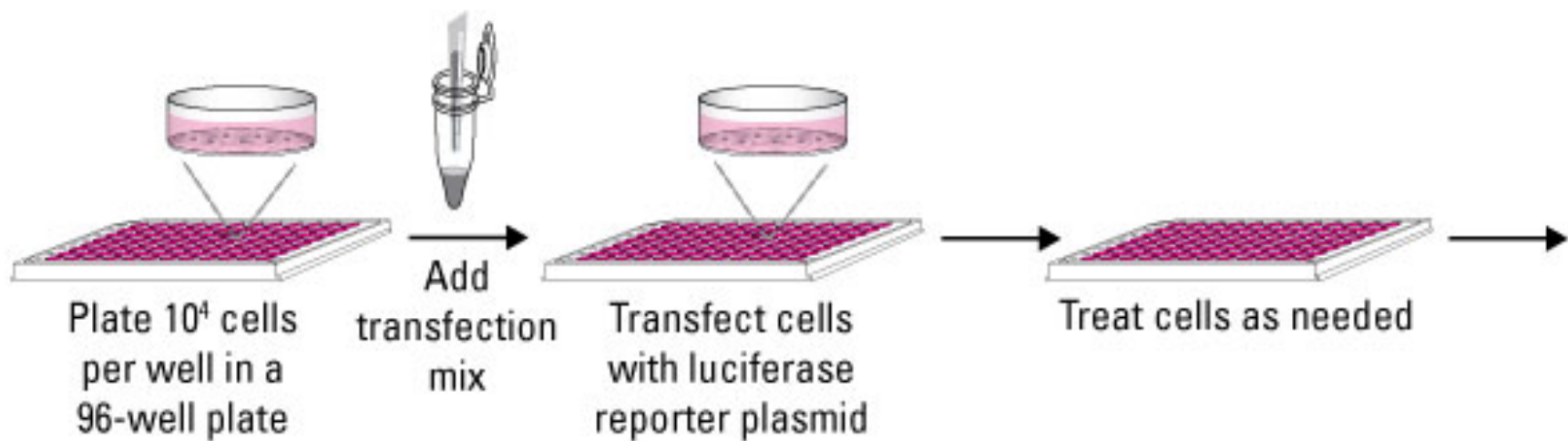
Drug Assays, Organelles

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Arwa Maqboul

Luciferase-Based Parasite Growth Inhibition Assay for Measurement of Drug Susceptibility & IC50

Methodology

- Fibroblasts monolayer
- 400 *T. gondii* parasites per well were added.
- (WT + 49c), (WT+ 49b), (Mutant + 49c), (Mutant+ 49b), Control??
- 4 days incubation
- Lyse cells and Parasites in the plate with 150 μL of 2x lysis buffer
- Transfer 20 μL to the reading plates, + 20 μL of substrate buffer
- Chemiluminescence reader



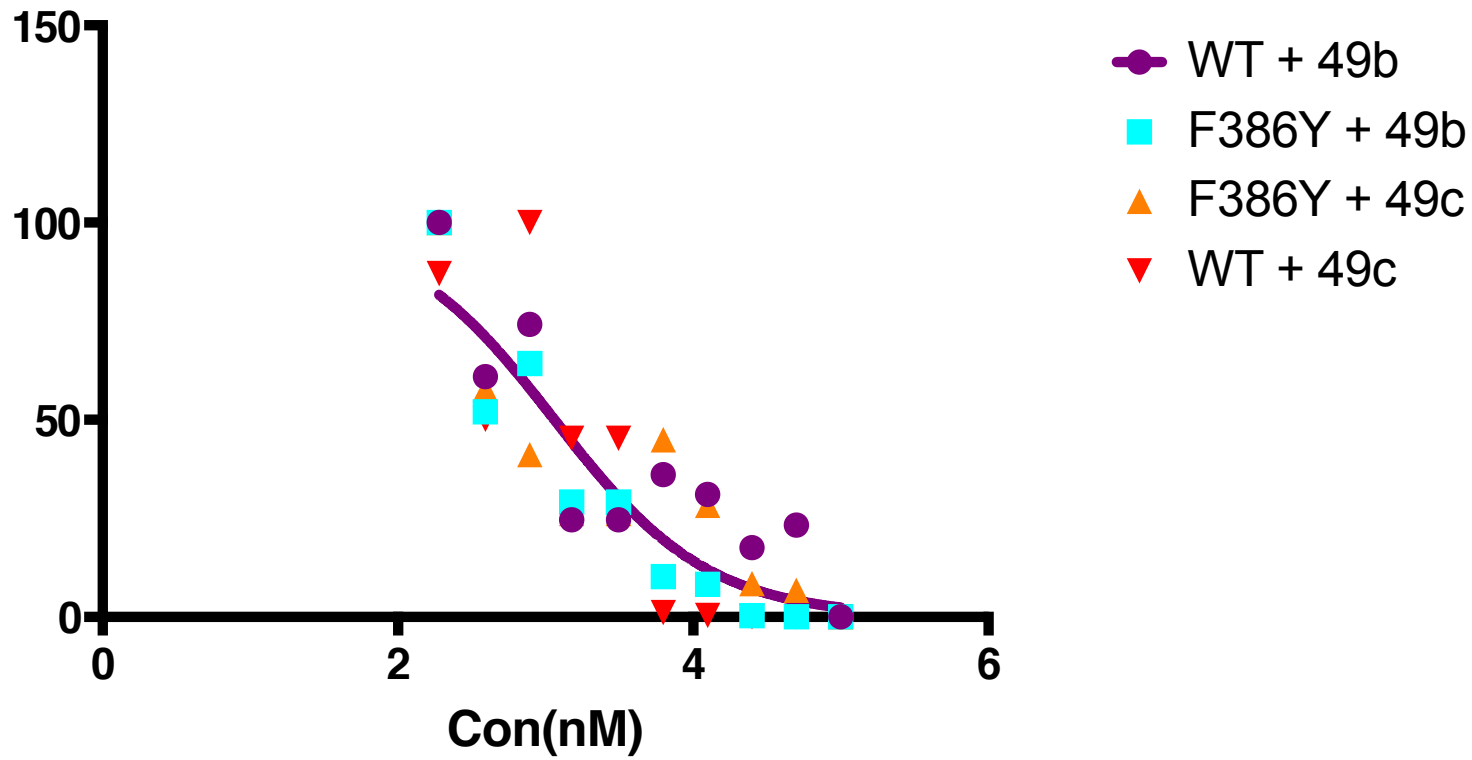
	1	2	3	4	5	6	7	8	9	10	11	12	
A	0		90 nM		180 nM		360 nM		720 nM		1.5 μ M		} WT + 49b
B	3 μ M		6 μ M		12 μ M		24 μ M		50 μ M		10 μ M		
C	0		90 nM		180 nM		360 nM		720 nM		1.5 μ M		} F386Y+ 49b
D	3 μ M		6 μ M		12 μ M		24 μ M		50 μ M		10 μ M		
E	0		90 nM		180 nM		360 nM		720 nM		1.5 μ M		} F386Y+ 49c
F	3 μ M		6 μ M		12 μ M		24 μ M		50 μ M		10 μ M		
G	0		90 nM		180 nM		360 nM		720 nM		1.5 μ M		} WT + 49c
H	3 μ M		6 μ M		12 μ M		24 μ M		50 μ M		10 μ M		

Control??

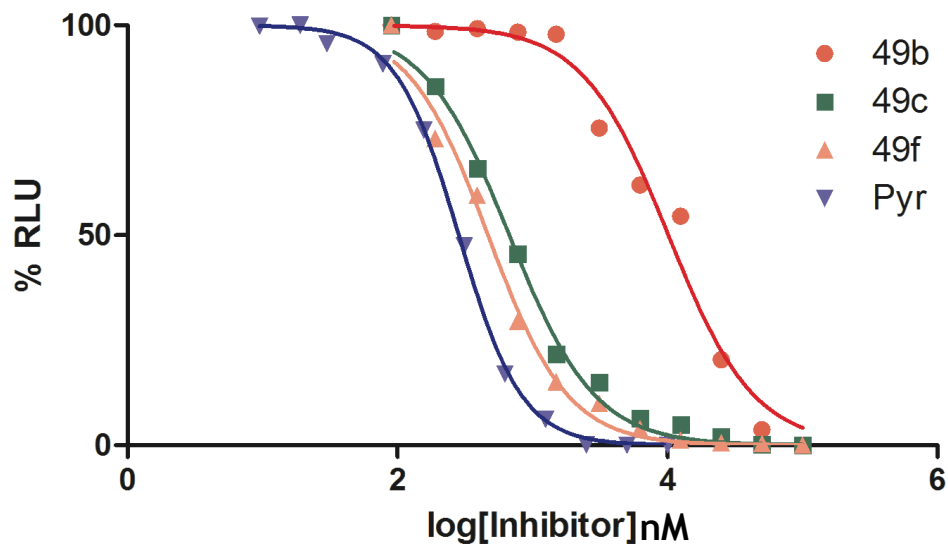
Results

- No well control no drug
- No first 2 wells, mathematically we need 12 points
- Percentage relative to non treated control
- Log, then normalize nonlinear regression fitting
log50

Normalize of Transform of Data 1

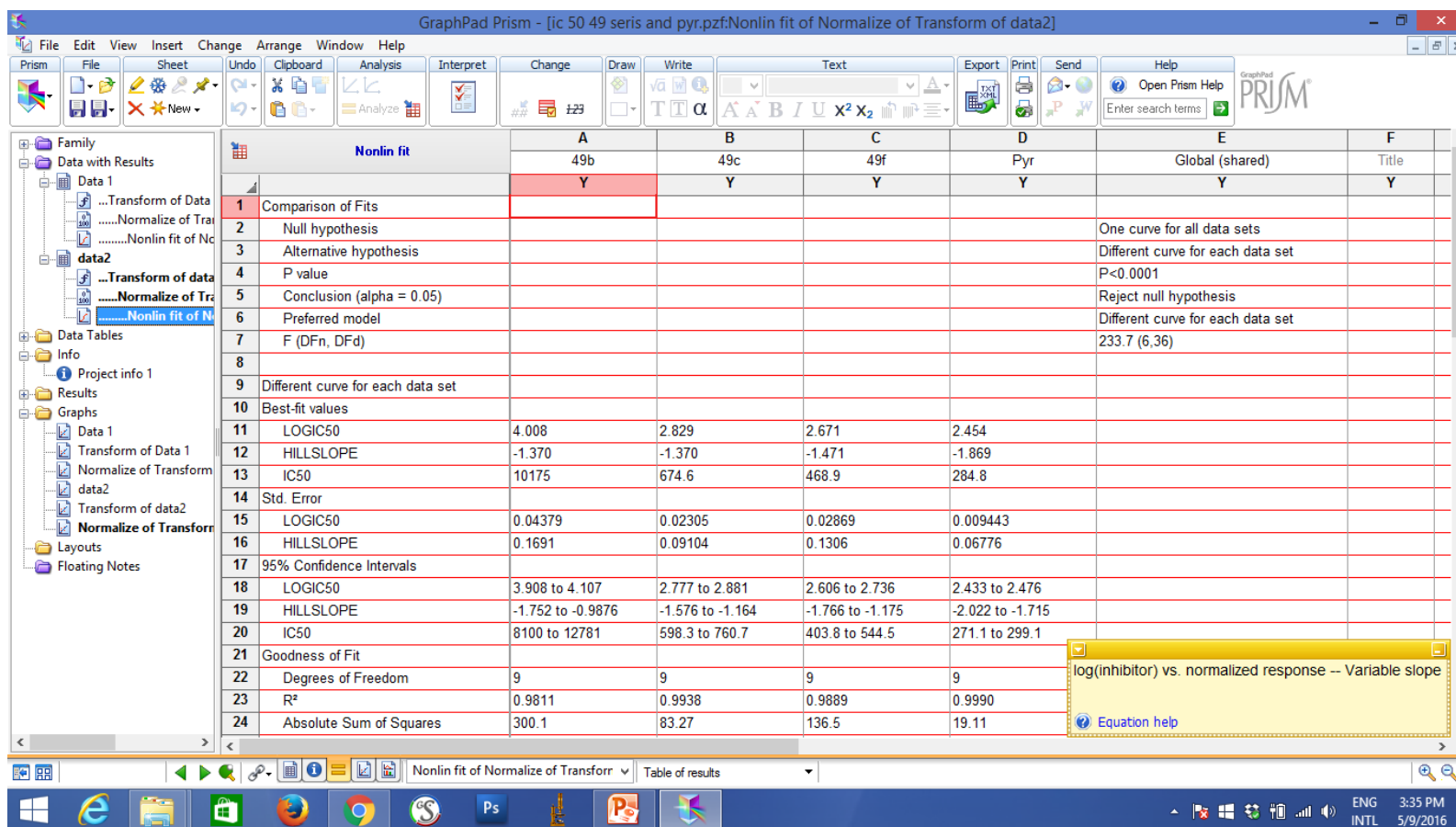


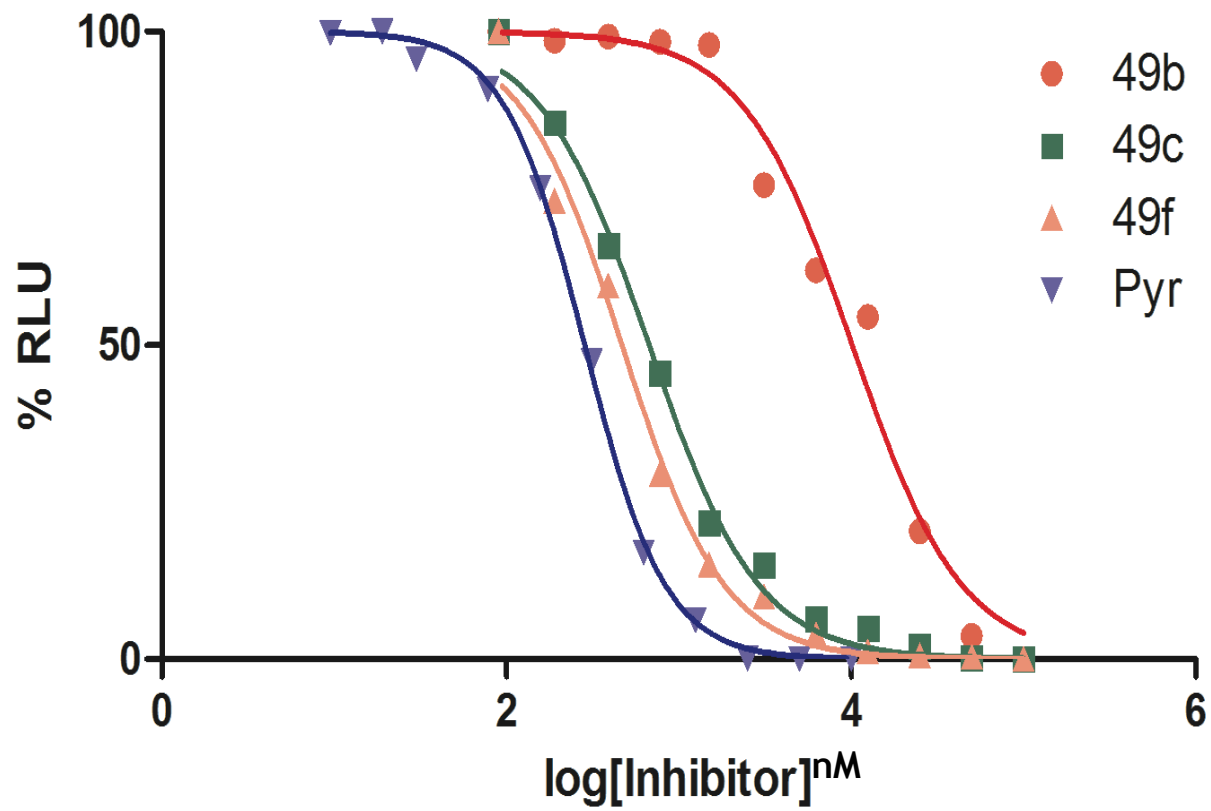
- mutant is more resistant
- data with no sense
- experiment fails
- technical issue (no enough substrate for first 2 wells)



Strain used RH/pTuB
CBG99 luciferase

49c IC50 96h 674.6 nM
49b IC50 96h 10175 nM





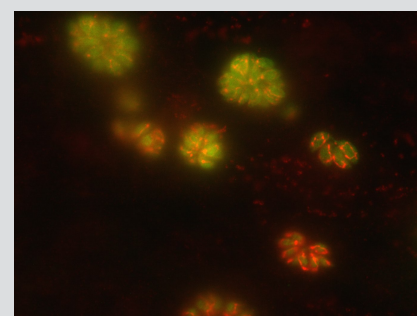
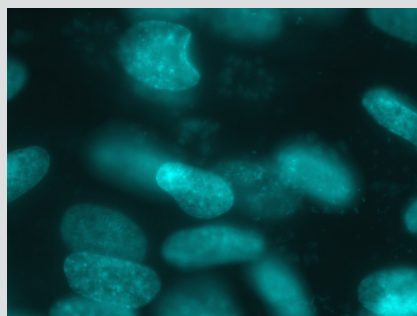
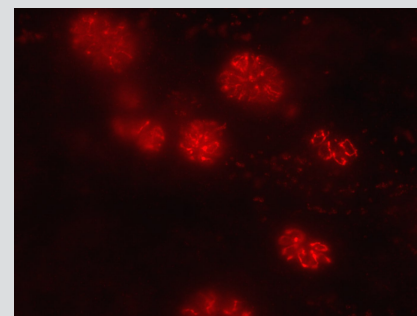
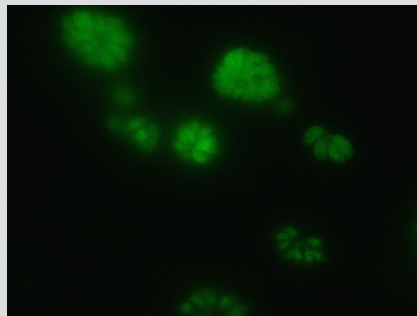
Strain used RH/CBG99 luciferase

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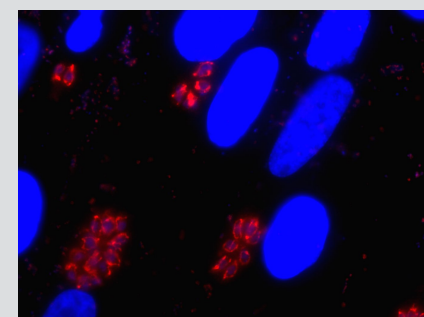
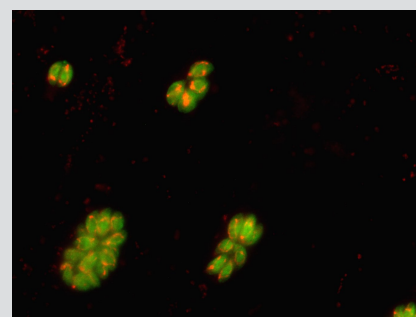
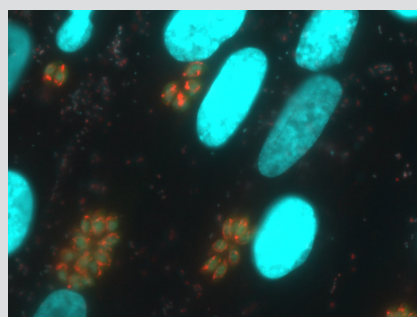
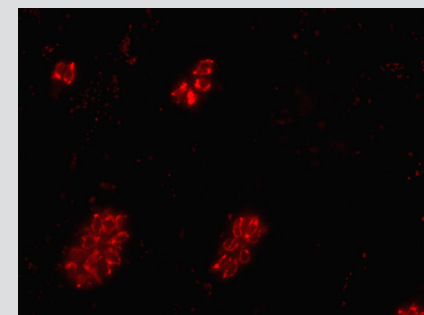
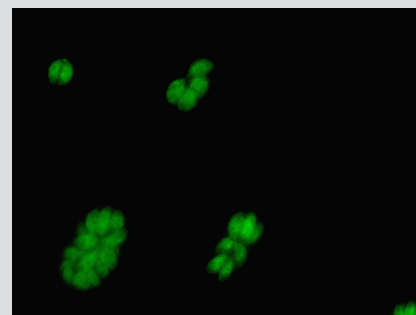
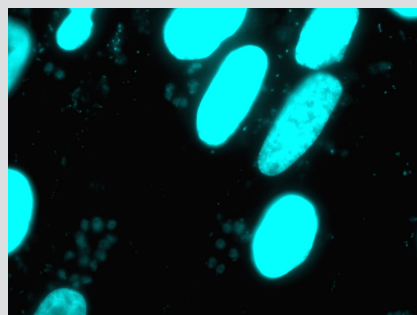
49b IC50 96h 10175 nM

Mitochondria

-ATC



+ATC



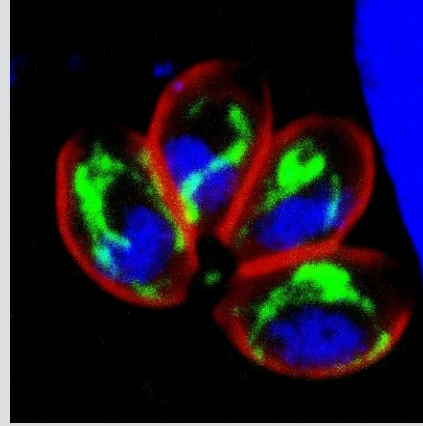
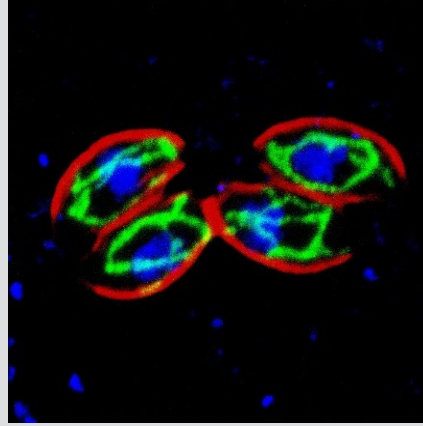
Mitochondria

Paco Pino

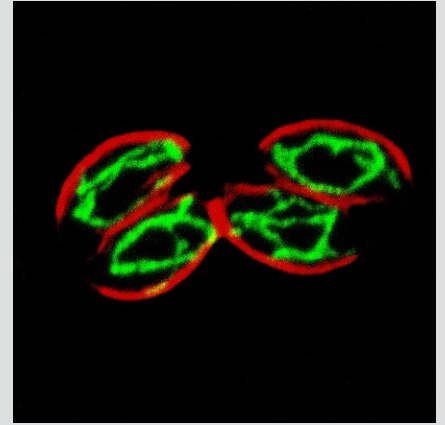
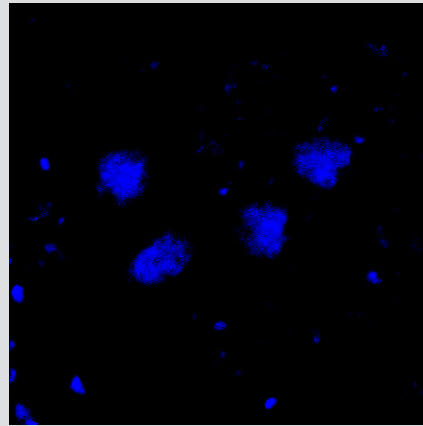
-ATC

+ATC 48h

Mito-mouse-green
GAP45-rabbit-red



-ATC



+ATC
48h

