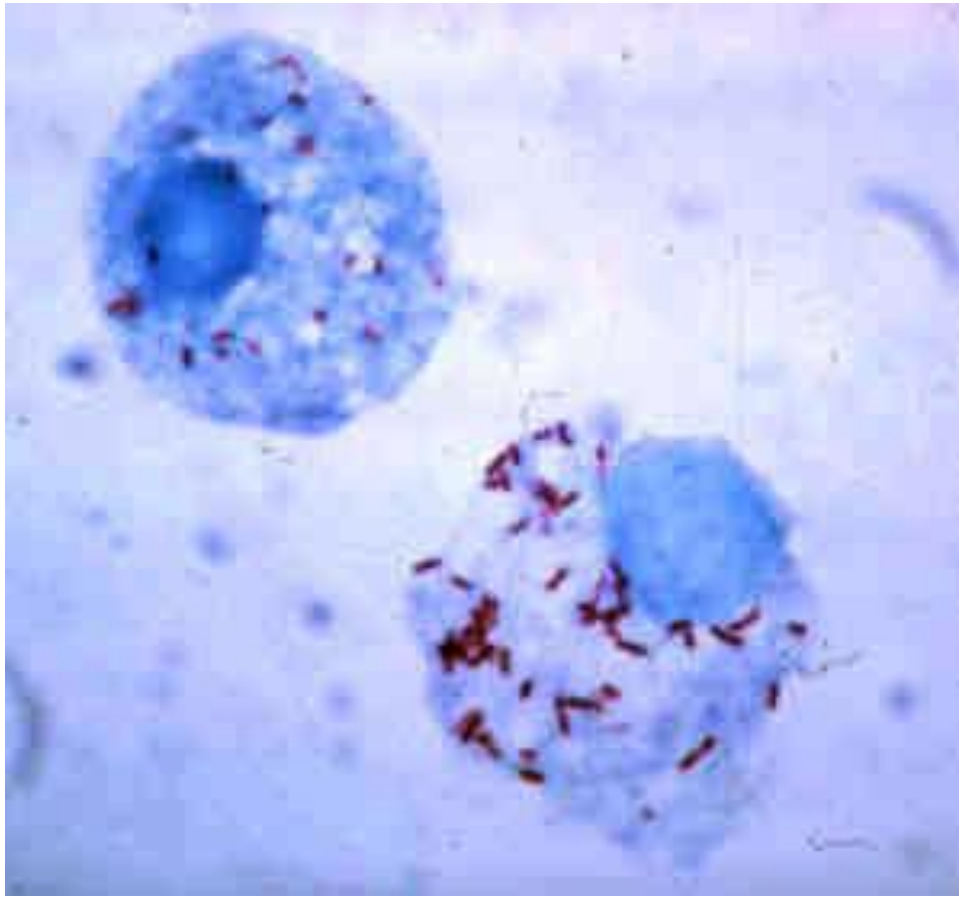
Subversion of Cell Signaling by Pathogens



Subversion of Cell Signaling by Pathogens

"Pathogens exploit several eukaryotic signaling pathways during an infection".

They have evolved specific effectors and toxins to hijack host cell machinery for their own benefit.

Signaling molecules are preferentially targeted by pathogens because they globally regulate many cellular processes.

Both viruses and bacteria manipulate and control pathways that regulate host cell survival and shape, including MAPK signaling, G-protein signaling, signals controlling cytoskeletal dynamics, and innate immune responses".

Neal M. Alto and Kim Orth

Subversion of Cell Signaling by Pathogens

-Escape from macrophage, phagolysosome

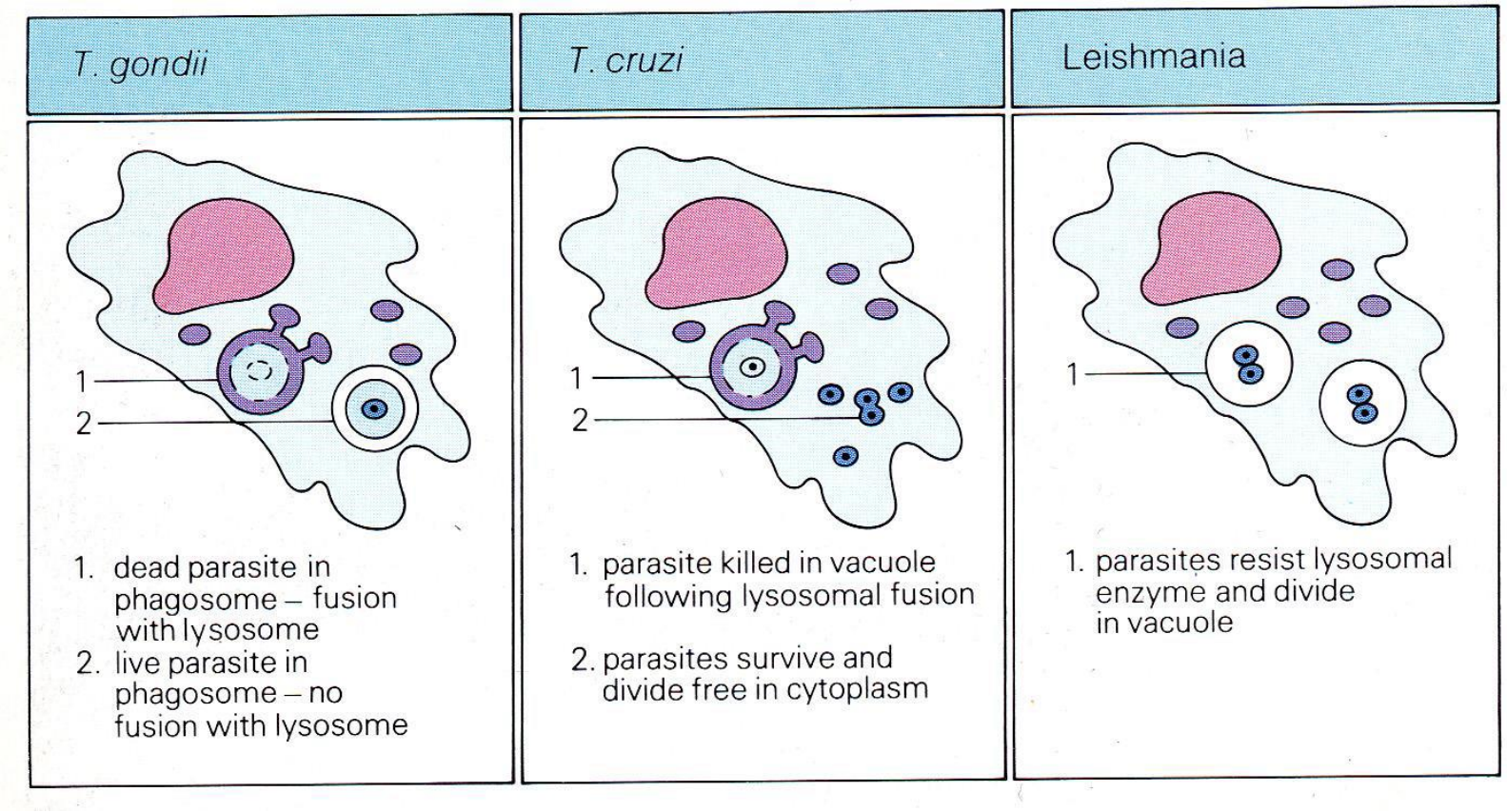
-Resistance to Humoral and/or Cellular Immune Response : Antigen Variation (*T. brucei*).

-Host cell signaling pathways alterations.

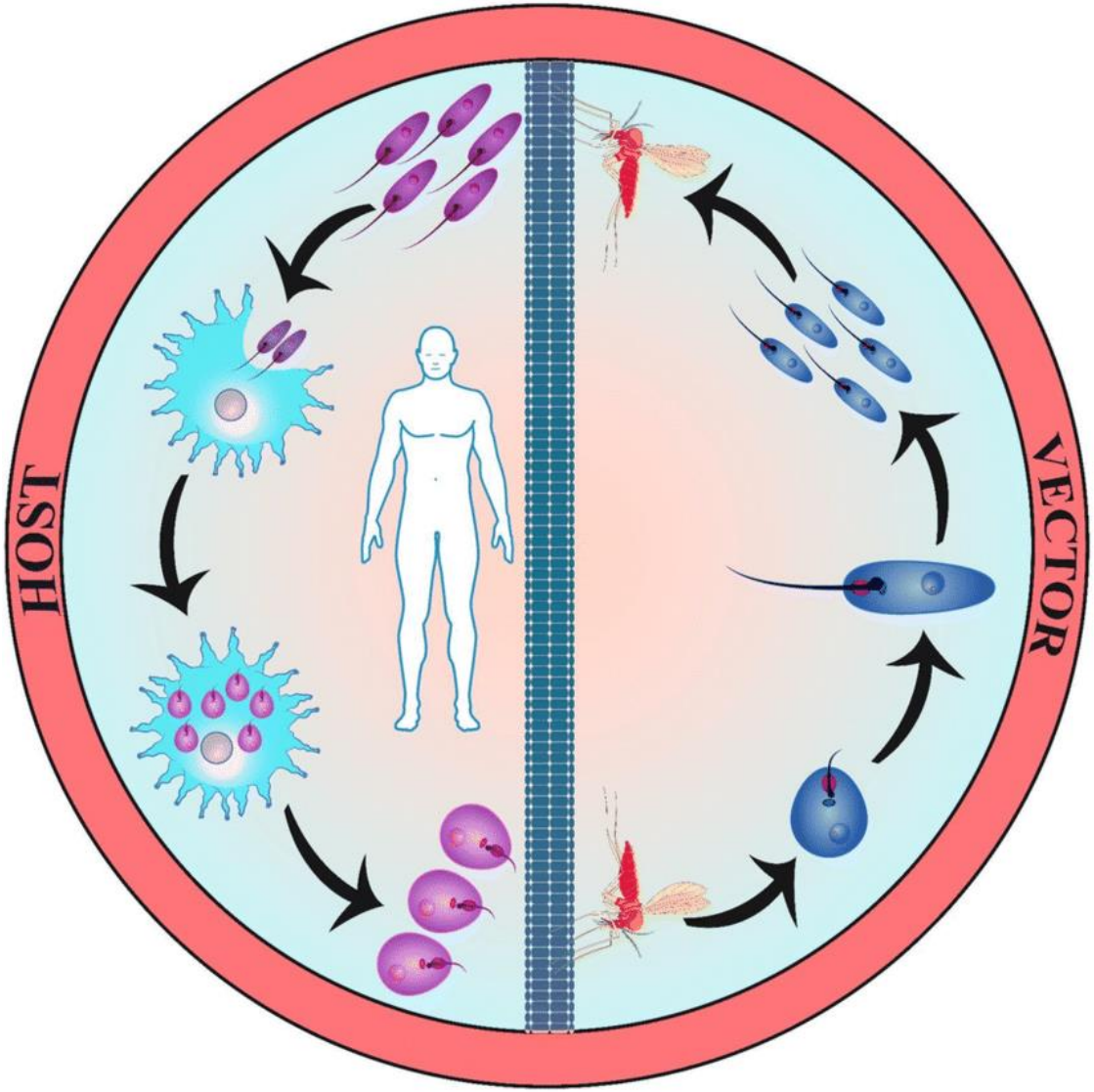
-Modulation of host cell functions responsible for pathology development (imune response).

Escape from macrophage, phagolysosome

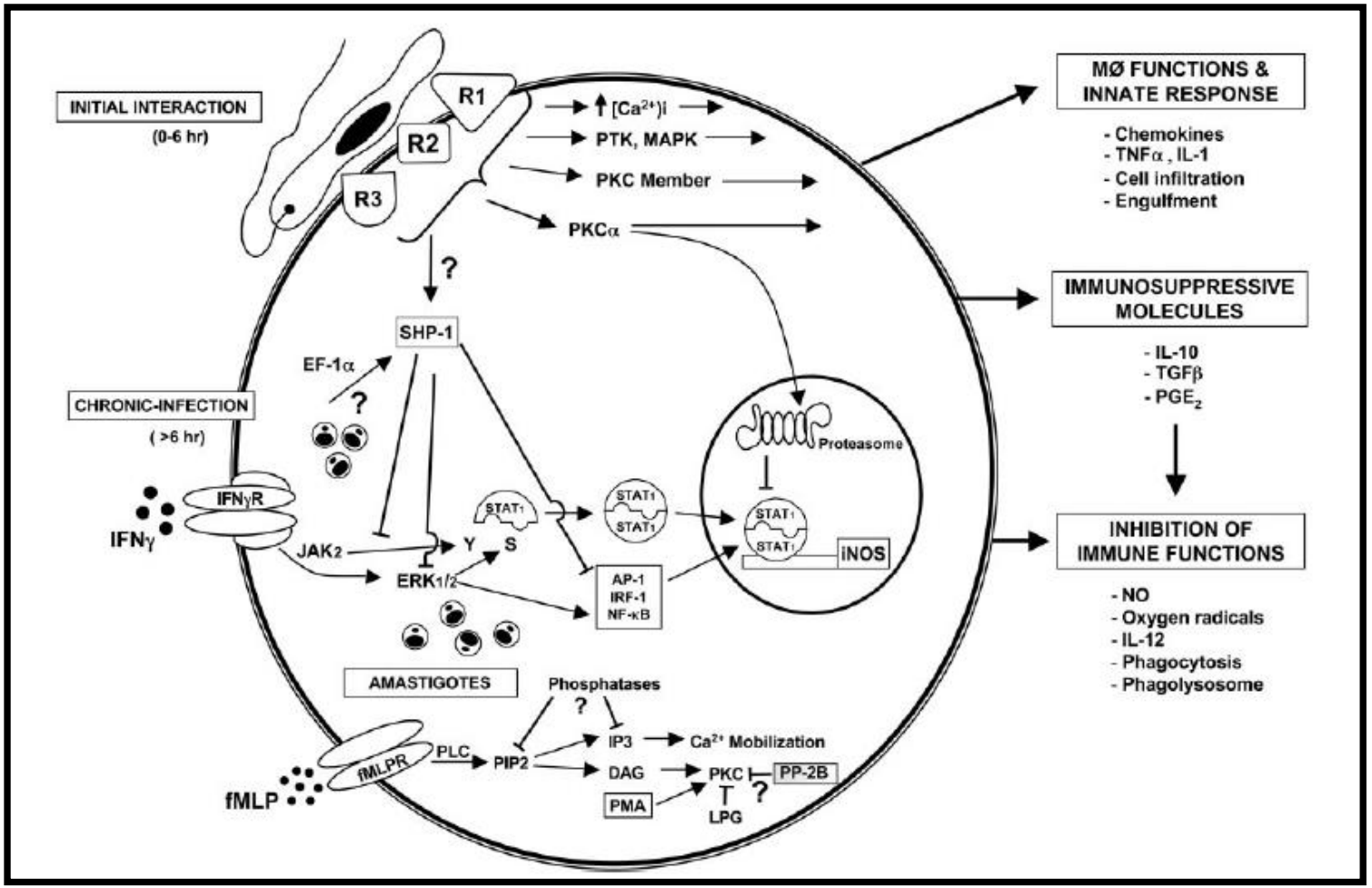
Formation of phagolysosomes is essential for the intracellular destruction of microorganisms and pathogens



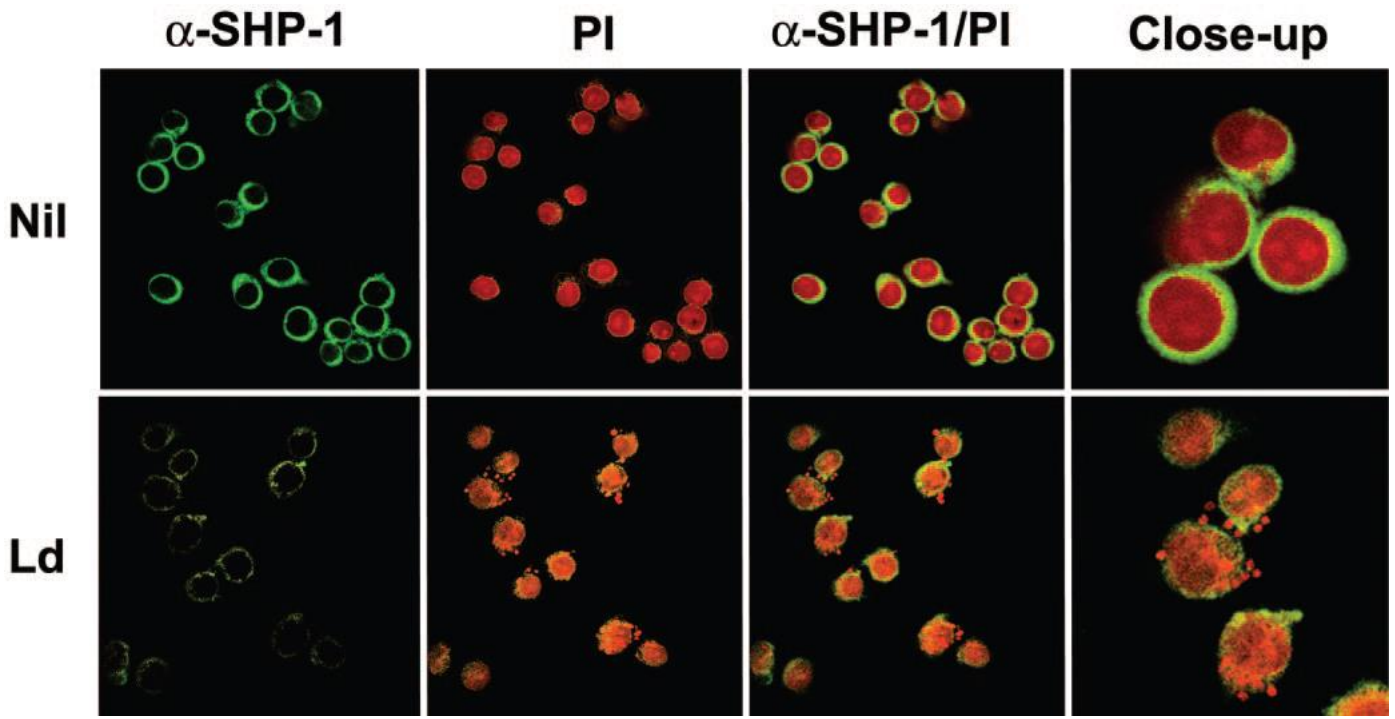
Life cycle of Leishmania



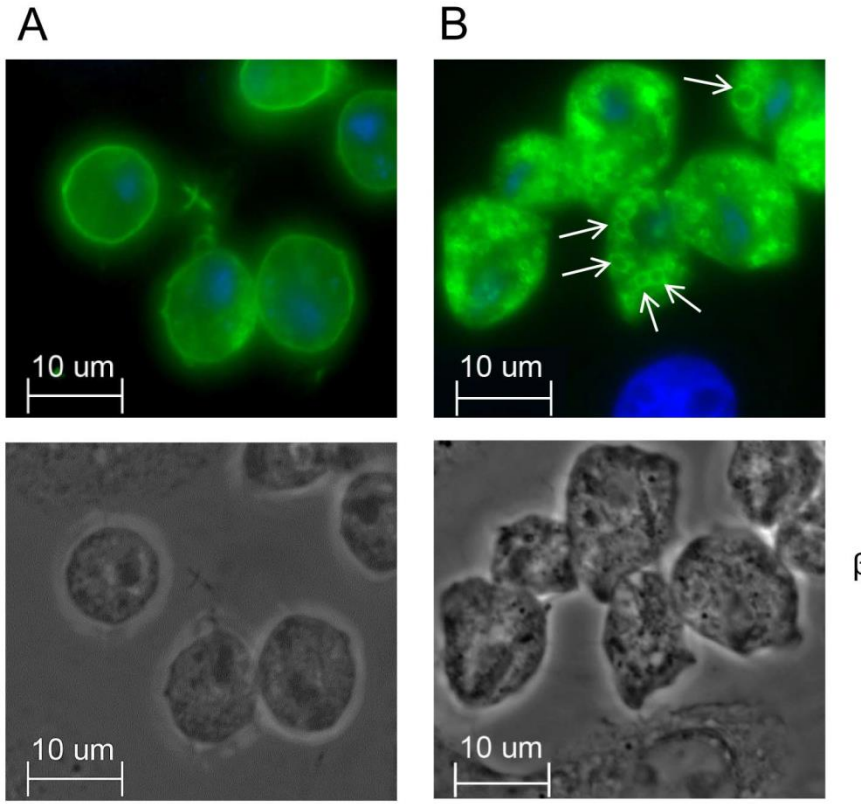
Leishmania inhibition of macrophage function



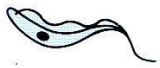
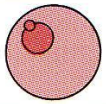
Leishmania inhibition of macrophage function (redistribution of phosphatase SHP-1)



Trichomonas vaginalis Exosomes Deliver Cargo to Host Cells and inhibits host response



Resistance to Humoral and/or Cellular Immune Response: Antigen Variation (T. brucei).

parasite and habitat		antibody			cell-mediated immunity	
		importance	mechanism	means of evasion	importance	mechanism
T. brucei free in blood		++++	lysis with complement which opsonizes for phagocytosis	antigenic variation	–	
Plasmodium inside red cell		+++	blocks invasion opsonizes for phagocytosis	intracellular habitat	?+	macrophage activation
T. cruzi inside macrophage		++	limits spread in acute infection	intracellular habitat	+++ (chronic phase)	macrophage activation by lymphokines and killing by metabolites of O_2
Leishmania inside macrophage		+	limits spread	intracellular habitat	++++	

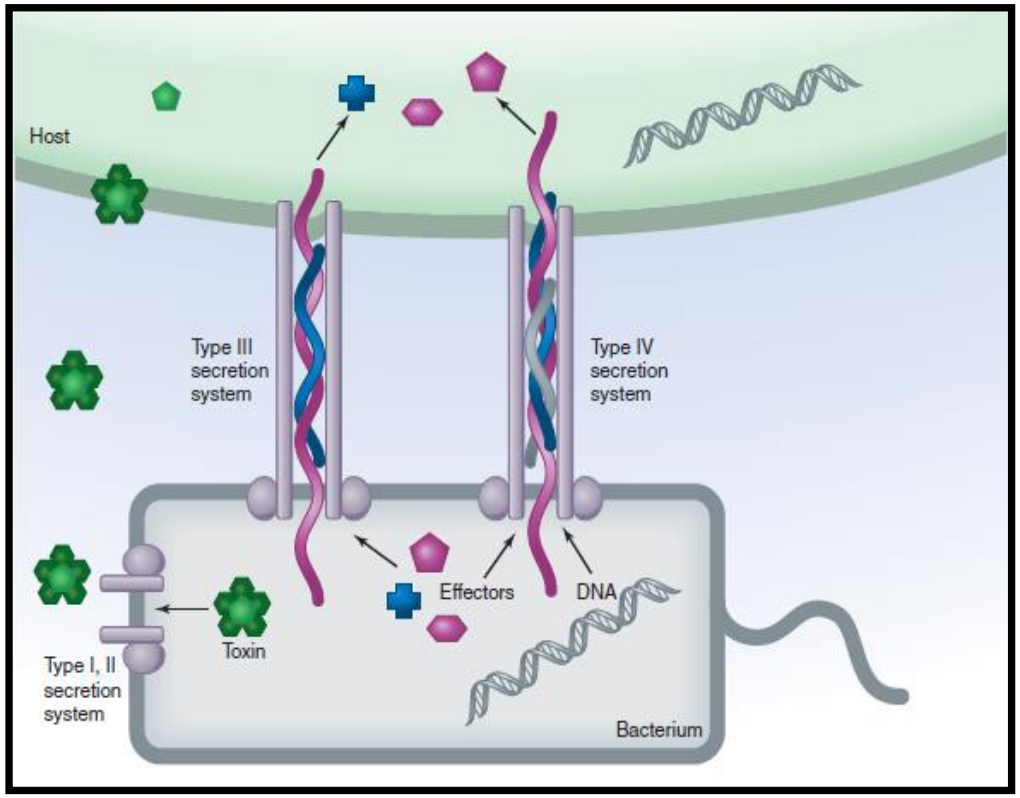
Host cell signaling pathways alterations

Host cell signaling pathways alteration by bacteria

Pathogen	Toxin	Effector	Target	Activity
Vibrio cholerae	Cholera toxin		$G\alpha_s$	ADP ribosylation
Vibrio cholerae	EF edema factor		Calmodulin	Adenylate cyclase
Vibrio cholerae	LF lethal factor		MKK1,2	Metalloprotease
Bordetella pertussis	Pertussus toxin		$G\alpha_i$	ADP ribosylation
Clostridium botulinum	C3 botulin toxin		Rho GTPases	ADP ribosylation
Escherichia coli	CNF1		Rho GTPases	Deamination
EPEC/EHEC O157:H7		Tir	Actin	Recruits NCK adaptor
EPEC/EHEC O157:H7		Map	Rho GTPases	GEF
EPEC/EHEC O157:H7		EspFu	N-WASP	Activator of N-WASP
EPEC/EHEC O157:H7		EspG	p21-activated kinase (PAK)	Activator of PAK
EPEC/*Burkholderia* spp.		Cif/CBHP	Ubiquitin, Nedd8	Ubiquitylation inhibitor
Yersinia spp.		YopH	p130Cas	Tyrosine phosphatase
Yersinia spp.		YopE	Rho-like GTPases	GAP
Yersinia spp.		YopT	Rho GTPase	Cysteine protease
Yersinia spp.		YpkA	$G\alpha_q$, Rho GTPases	Ser/Thr kinase, GDI
Yersinia spp.		YopJ	MAPKKs, IKK-β	Ser/Thr acetyltransferase
Vibrio parahaemolyticus		VopA/p	MAPKKs	Ser/Thr/Lys acetyltransferase
Vibrio parahaemolyticus		VopS	Rho-GTPases	AMPylation
Vibrio parahaemolyticus		VPA0450	Phosphatidylinositol 4,5-bisphosphate	Lipid phosphatase
Vibrio parahaemolyticus		VopL	Actin	Actin nucleator
Histophilus somni		IbpA	Rho GTPases	Ampylation
Legionella pneumophila		DrrA/SidM	Rab1b	AMPylation, GEF
Legionella pneumophila		SidD	Rab1b	DeAMPylation
Legionella pneumophila		AnkX	Rab1b	Phosphocholination
Shigella spp.		OspF	MAPK	Phosphothreonine lyase
Shigella spp.		IpgD	Phosphatidylinositol 4,5-bisphophate	Lipid phosphatase
Shigella spp.		IpaH9.8	Ste7 MAPK	E3 ubiquitin ligase
Shigella spp.		IpgB2	Rac1 and RhoA	GEF
Salmonella spp.		SopB	Phosphatidylinositol 4,5-bisphophate	Lipid phosphatase
Salmonella spp.		SopE	Cdc42 and Rac GTPases	GEF
Salmonella spp.		SptP	Small GTPase	GAP, tyrosine phosphatase
Pseudomonas aeruginosa		ExoS	Small GTPases	ADP ribosylation, GAP
Listeria monocytogenes		ActA	Arp2/3, actin	Activator of Arp2/3
Viral effector				
Vaccinia virus		A36R	Actin	Adaptor recruits Nck and Grb2
Adenovirus		E1B-55K	p53, Mre11, BLM helicase	Ubiquitin ligase adaptor
Adenovirus		E4orf6	p53, Mre11, BLM helicase	Ubiquitin ligase adaptor
Papillomavirus		E6	E6-AP, p53	E3 ubiquitin ligase
KSHV		RTA	IRF-7	E3 ubiquitin ligase
Gammaherpesviruses		K3 and K5	MHC class 1	E3 ubiquitin ligase
Herpes simplex virus 1		ICP0	PML	E3 ubiquitin ligase

Cold Spring Harbor Perspectives in Biology

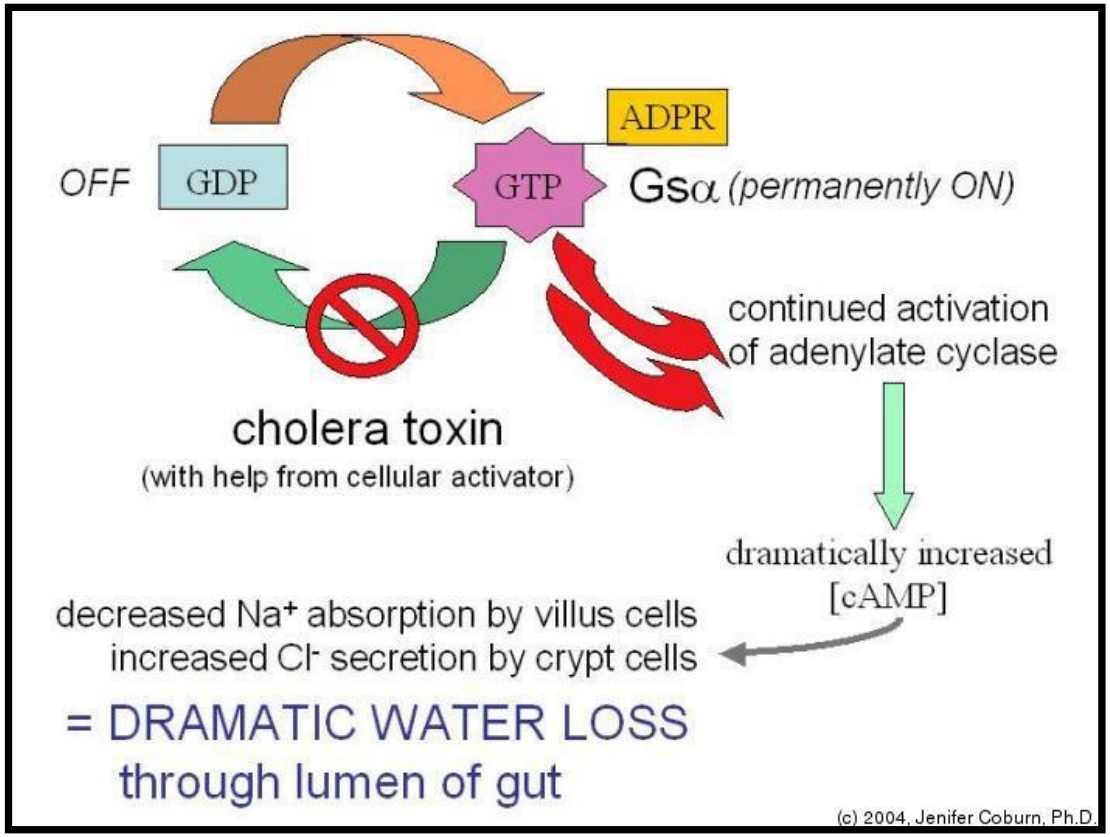
Host cell signaling pathways alteration by bacteria
Transport of toxins and effector proteins



Prototypical bacterial mechanisms for altering host innate immunity are the specialized secretion systems (particularly types III and IV and, in some instances, type VI) of Gram negative bacteria, which enable infecting bacteria to inject effector proteins directly into the cytoplasm of host cells.

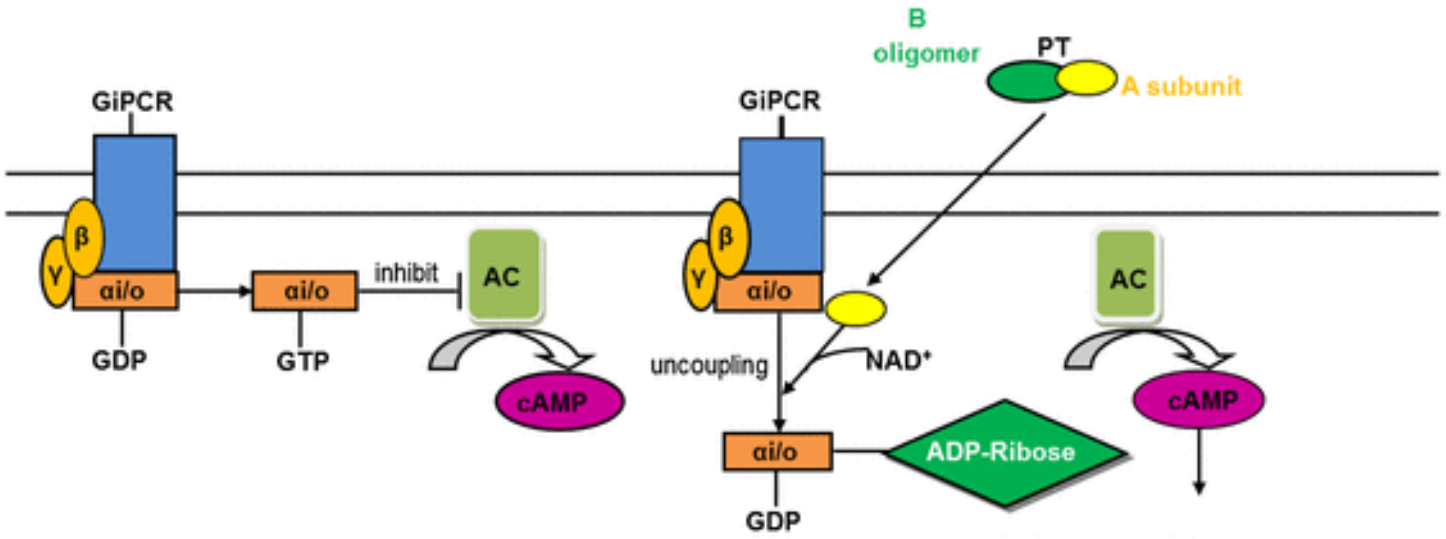
The effector proteins directly modify the function of host factors engaged in innate immune signaling, cytoskeletal dynamics, membrane trafficking, phosphoinositide lipid metabolism, host cell signaling, ubiquitin modification pathways, transcription, and protein modification, among others.

Cholera toxin



The CTA1 fragment catalyses ADP-ribosylation of the Gs alpha subunit (Ga_s) proteins using NAD. The ADP-ribosylation causes the Ga_s subunit to lose its catalytic activity in hydrolyzing GTP to GDP + P_i so it remains activated longer than normal

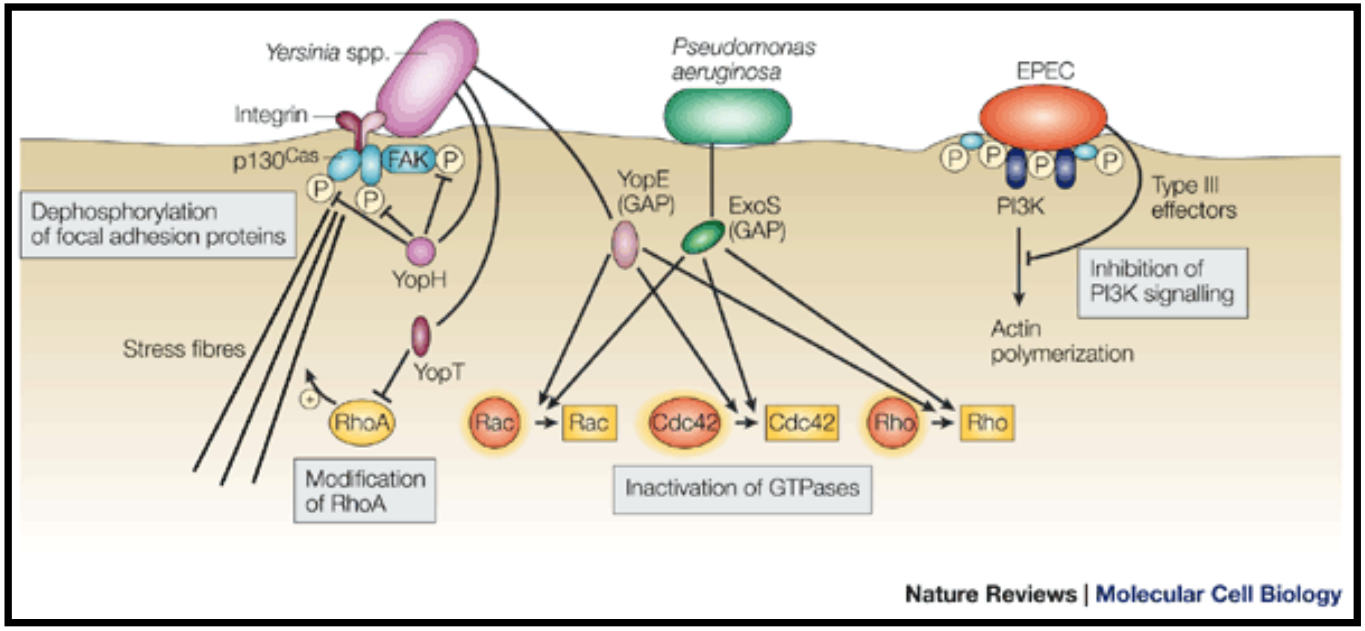
Pertussis Toxins



Inhibition of G_i proteins results in constitutive expression of AC activity that leads to accumulation of cAMP level and stimulation of cAMP-mediated signalling like protein kinase activity

PT catalyzes the ADP-ribosylation of the α_i subunits of the heterotrimeric G protein. This prevents the G proteins from interacting with G protein-coupled receptors on the cell membrane.

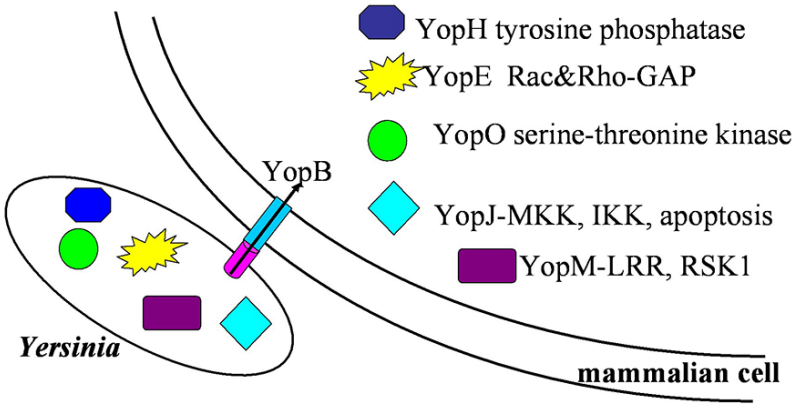
Effector proteins that interfere with adhesion complexes

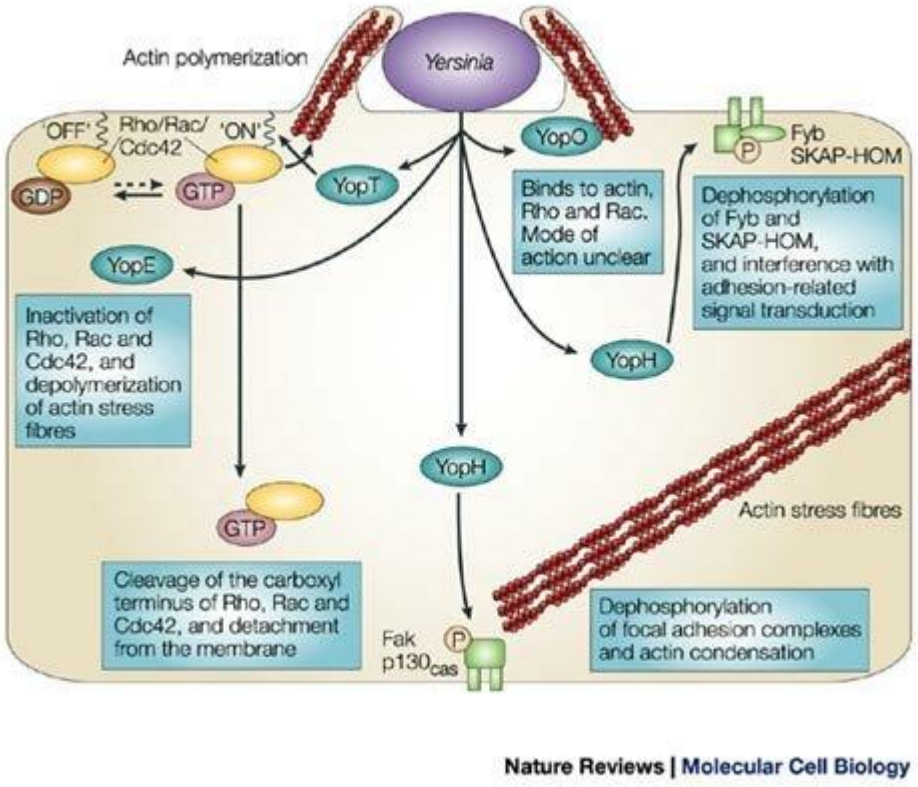


YopH contains an unregulated, highly active tyrosine phosphatase domain linked to a leader sequence that both guides its translocation from the bacterium into the host cell and determines its localization after delivery.

YopH is translocated into eukaryotic cells through the Yersinia T3SS and proceeds to focal adhesions, where it dephosphorylates critical phosphorylated tyrosine residues (see Devreotes and Horwitz 2012).

Yersinia inhibition of adhesion and phagocytosis





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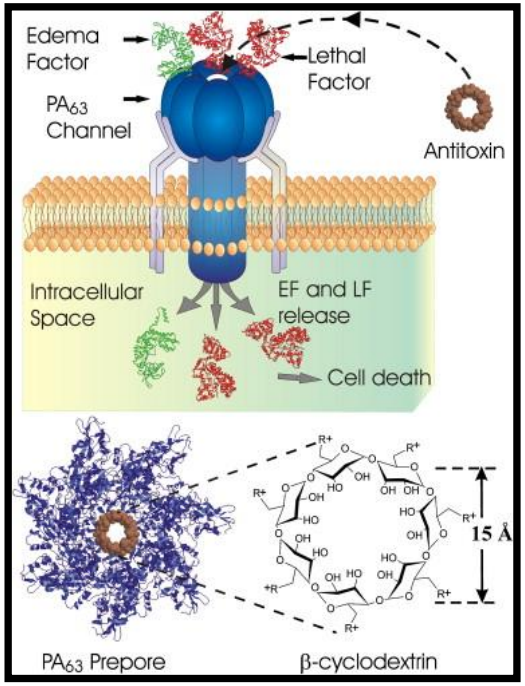
https://gsbs.tufts.edu/faculty-research/joan-mecsas-lab

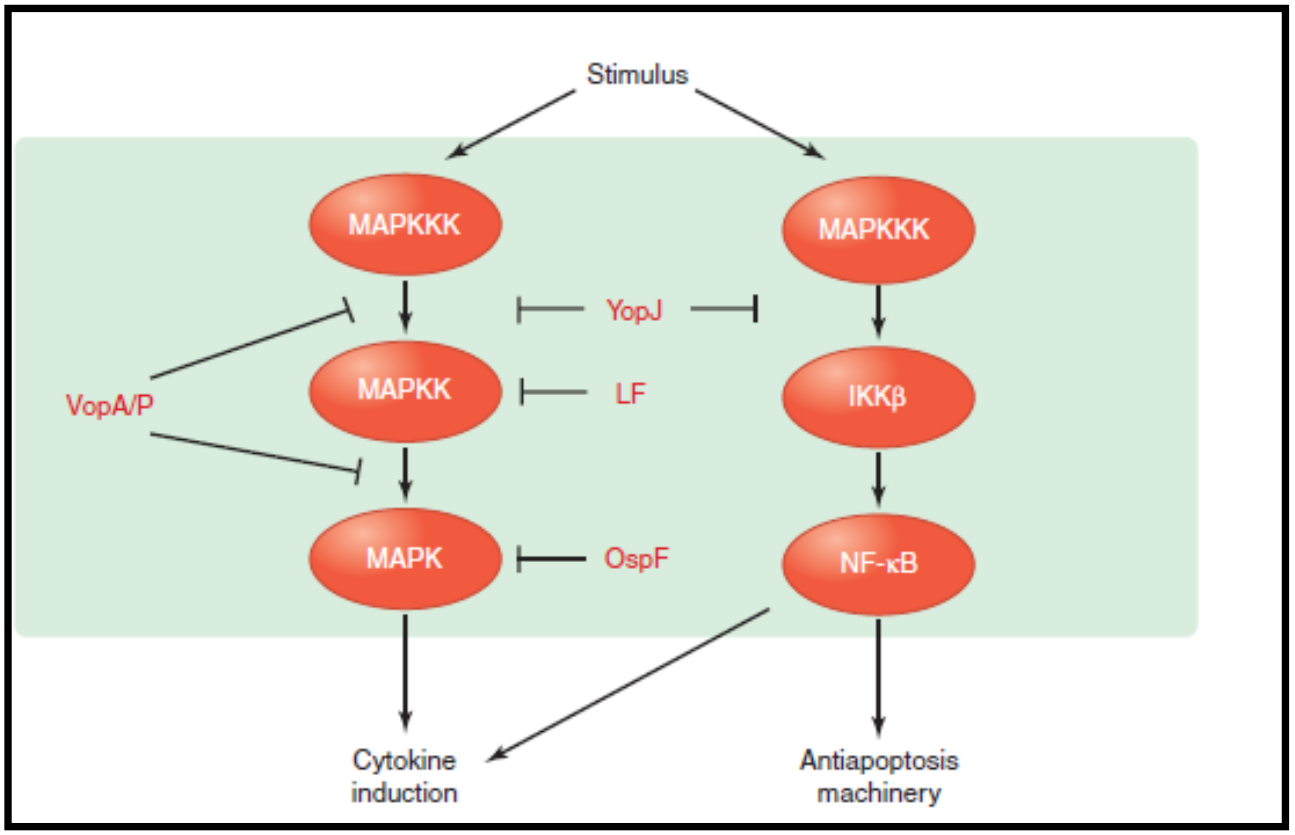
Interfering with host signaling cascade

Bacillus anthracis, the causal agent of Anthrax, releases a multi-subunit complex called anthrax toxin, composed of protective antigen (PA), edema factor (EF), and lethal factor (LF) (Collier 2009).

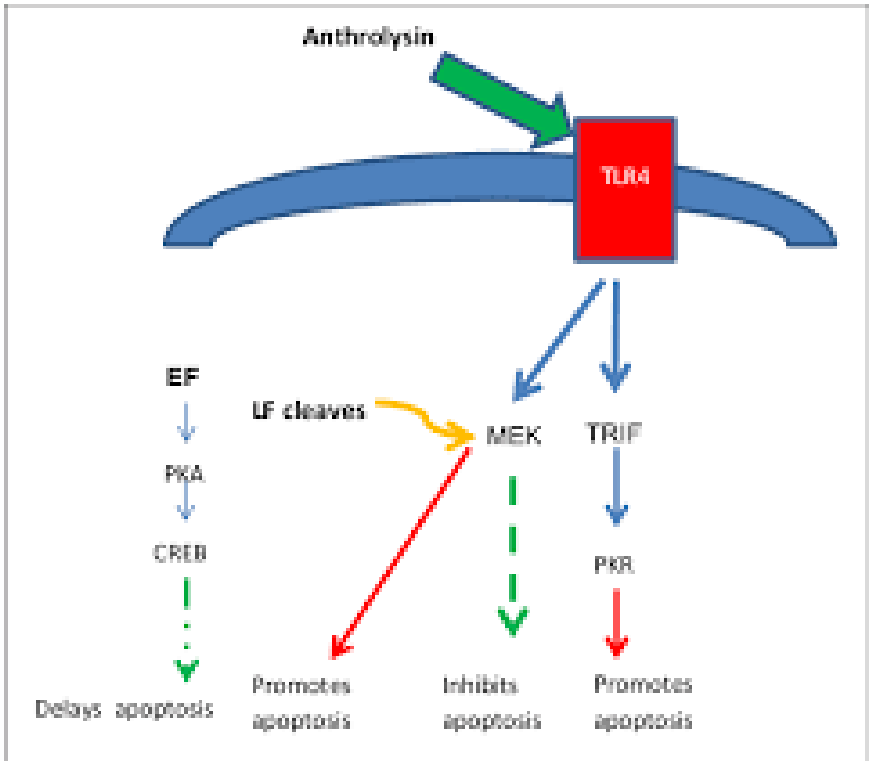
The **calcium-binding protein calmodulin** binds to cytoplasmic EF, causing a change in conformation that generates an active enzyme that produces **cAMP** from cellular ATP. The excess cAMP globally disrupts signaling by activating downstream effectors, such as **PKA**.

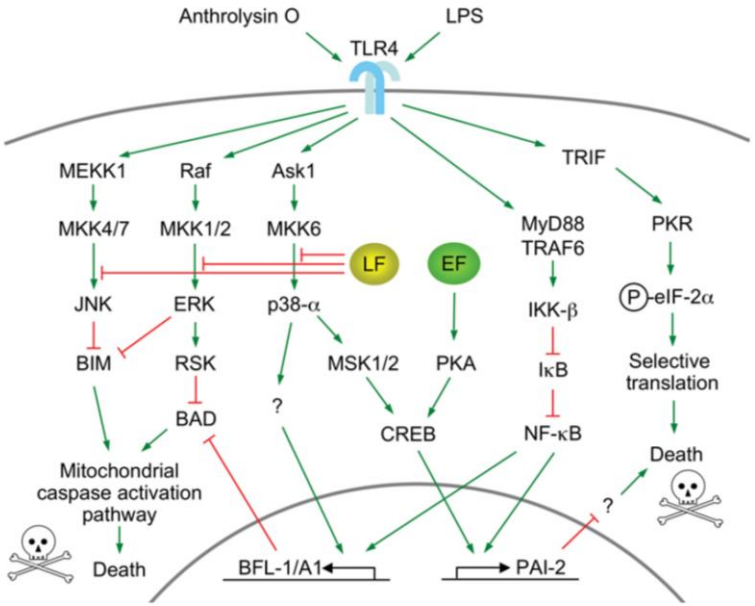


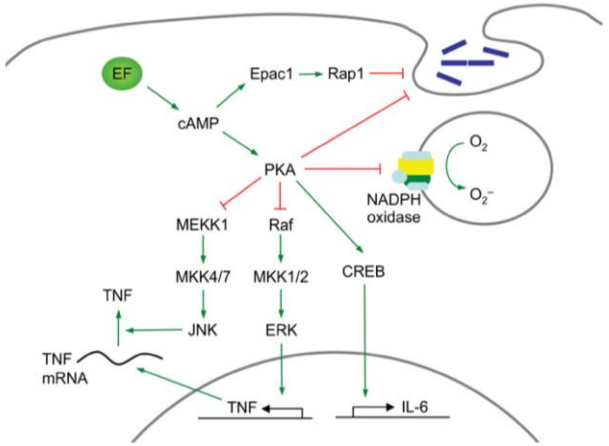
Interfering with the MAPK signaling cascade



Cytoplasmic LF (*Bacillus anthracis*) is an **active metalloprotease that cleaves the amino-terminal extensions from MAPKKs MKK1 and MKK2,** producing kinases that can no longer interact with their substrates to activate a proliferative response. Both of the toxins have an irreversible toxic effect on the infected cell.


Anthrolysin
TLR4
EF
PKA
CREB
Delays apoptosis
LF cleaves
MEK
TRIF
PKR
Promotes apoptosis
Inhibits apoptosis
Promotes apoptosis

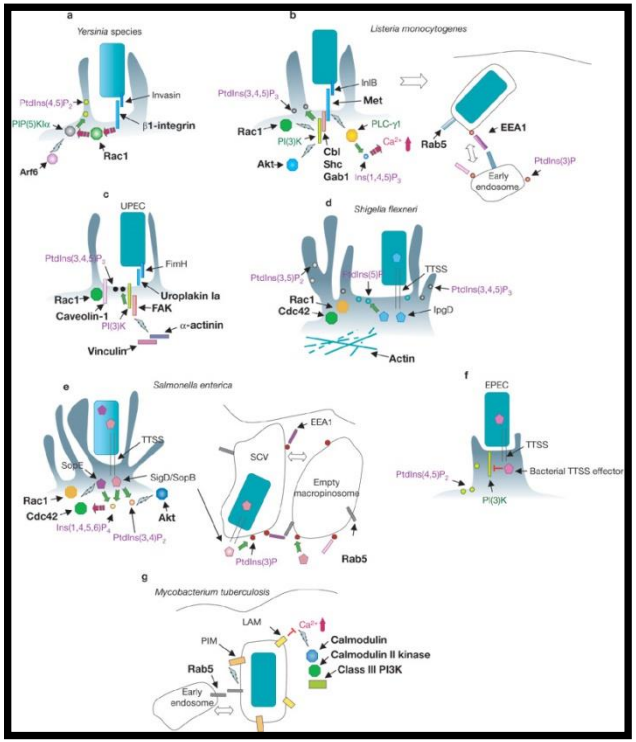

Anthrolysin O
LPS
TLR4
MEKK1
Raf
Ask1
TRIF
MKK4/7
MKK1/2
MKK6
LF
EF
MyD88
TRAF6
PKR
JNK
ERK
p38-α
IKK-β
P-eIF-2α
BIM
RSK
MSK1/2
PKA
IκB
Selective translation
BAD
?
CREB
NF-κB
Death
Mitochondrial caspase activation pathway
?
Death
BFL-1/A1
PAI-2


EF
Epac1
Rap1
cAMP
PKA
NADPH oxidase
O_2
O_2^-
MEKK1
Raf
MKK4/7
MKK1/2
CREB
TNF
JNK
ERK
TNF mRNA
TNF
IL-6

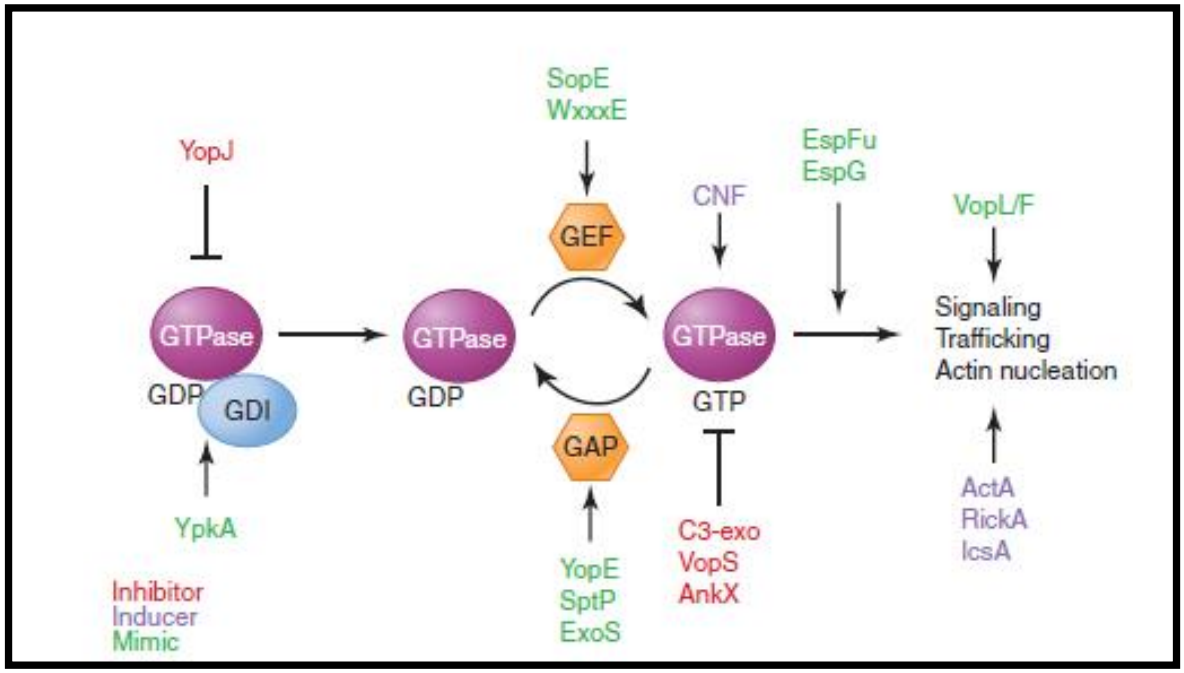
Highjacking Lipid signaling

Phosphoinositides, particularly phosphatidylinositol 4,5- bisphosphate (PIP2), regulate the actin cytoskeleton beneath the plasma membrane, functioning in signaling as well as trafficking by targeting vesicles around the cell.

Disruption of phosphoinositide homeostasis at the plasma membrane by bacterial effectors can destabilize actin dynamics and alter the morphology of the membrane. This facilitates the entry of intracellular pathogens or, in the case of extracellular pathogens, can disrupt membrane integrity, which leads to rapid cell lysis in the subsequent stage of infection to facilitate pathogen spreading (Hamet al. 2011).



Bacterial Guanine-Nucleotide Exchange Factor (GEF) Mimics

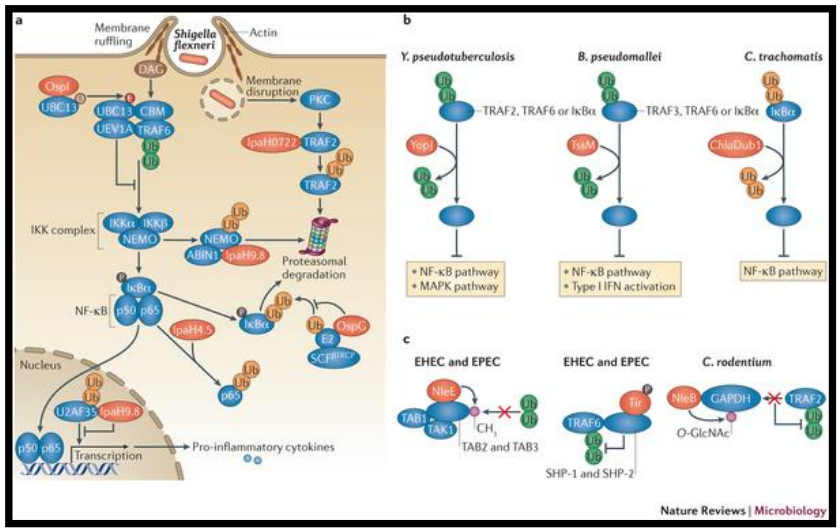


Targeting ubiquitin-mediated signal transduction

Many viruses, including baculoviruses, poxviruses, and herpes simplex virus, encode their own ubiquitin molecules but have significantly altered the ubiquitin gene (Haas et al. 1996).

Many bacteria also secrete enzymes that modify host ubiquitin or ubiquitin-likemolecules (UBLs) ex: ubiquitin deamidation on Q40 by the bacterial type III effector Cif (Cui et al. 2010).

These posttranslational modifications potently inhibit polyubiquitin chain synthesis, resulting in accumulation of host substrates and severe cytopathic effects.



Pathogenic Actin Nucleation Factors

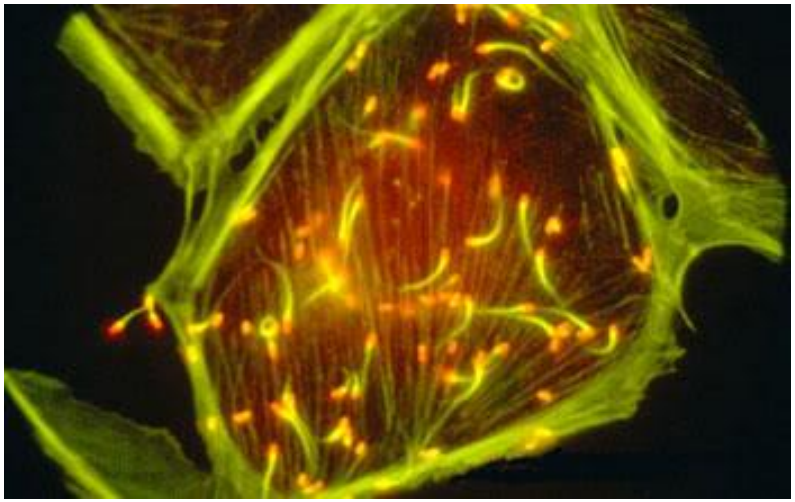
ActA directly recruits and activates the Arp2/3 complex at the surface of Listeria monocytogenes (Welch et al. 1998). It has, in fact, been an essential tool for studies of various biological processes including cell motility and provided the first physiological evidence for the nucleating activity of the Arp2/3 complex (Welch et al. 1998).

Like ActA, Shigella VirG/IcsA induces formation of actin comet tails, but this pathogen uses a distinct mechanism (Egile et al. 1999).

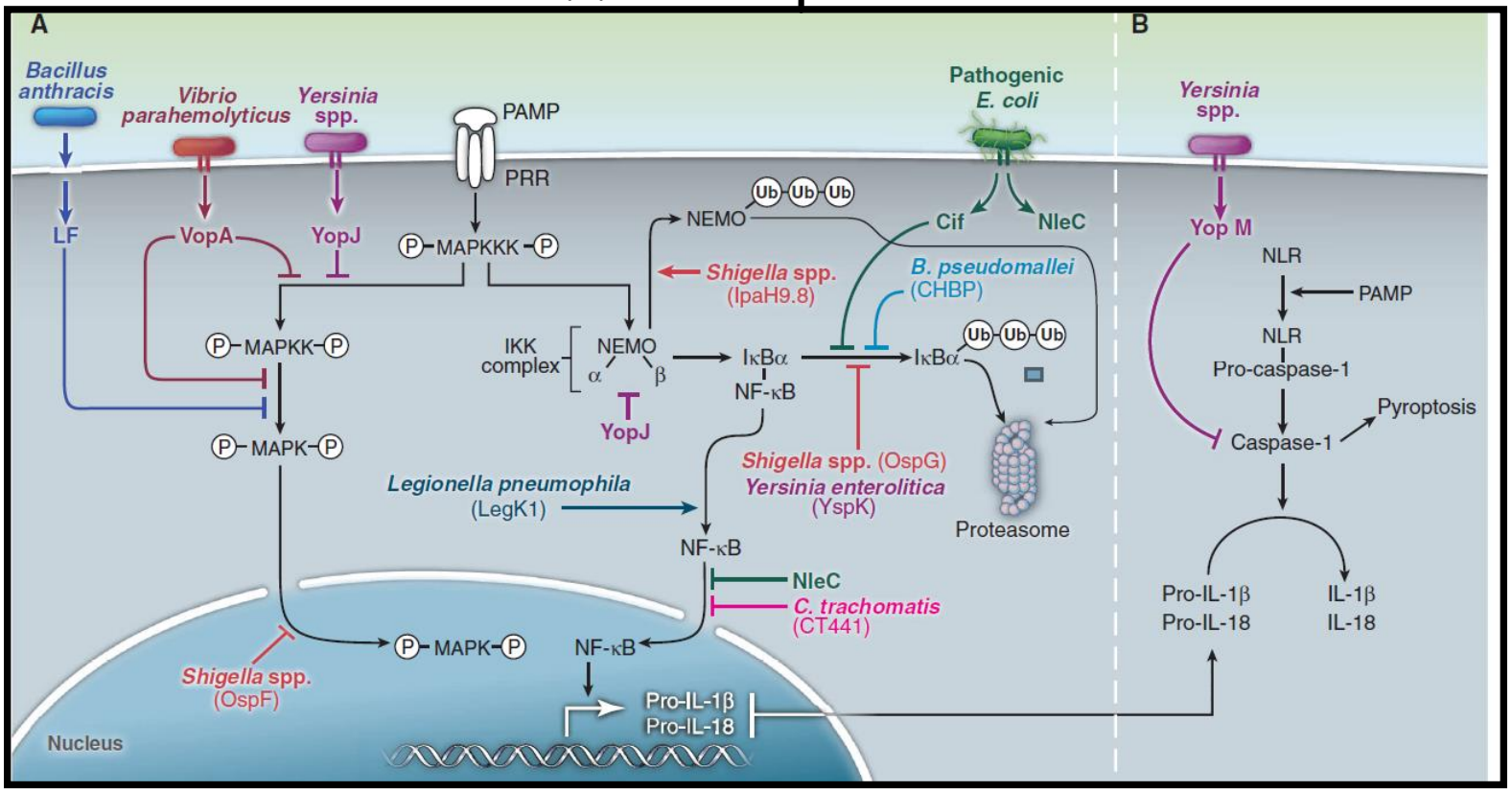
Finally, Vaccinia virus uses the membrane-anchored protein A36R to facilitate intracellular movement that is strikingly similar. A large domain of A36R on the viral surface is phosphorylated by Src-family tyrosine kinases and then directly interacts with the adaptor protein Nck and subsequently recruits N-WASP.

Pathogenic Actin Nucleation Factors

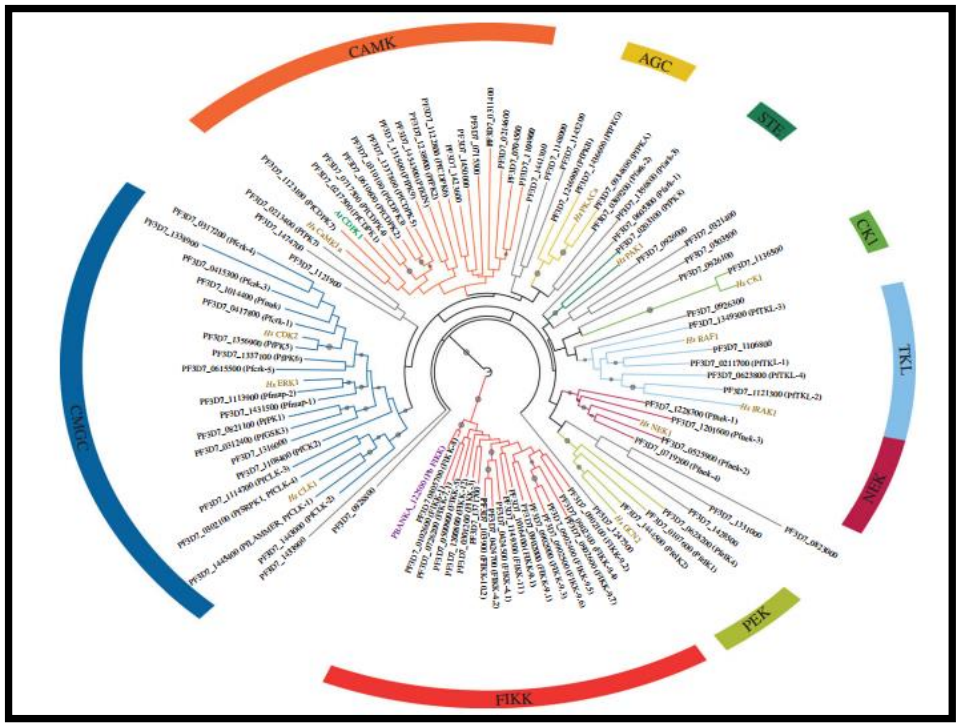
https://br.video.search.yahoo.com/search/video;_ylt=Awrih0btBFxl6RgAjyse6gt.;_ylu=c2VjA3NlYXJjaAR2dGlkAw--;_ylc=X1MDMjExNDcxMDA0NgRfcgMyBGZyA21jYWZlZQRmcjIDcDpzLHY6dixtOnNiLHJnbjp0b3AEZ3ByaWQDcENRQV9hR2pTRFcwVDI3cnZxM0JjQQRuX3JzbHQDMARuX3N1Z2cDMARvcmlnaW4DYnludmlkZW8uc2VhcmNoLnlhaG9vLmNvbQRwb3MDMARwcXN0cgMEcHFzdHJsAzAEcXN0cmwDNDQEcXVlcnkDUGF0aG9nZW5pYyUyMEFjdGluJTIwTnVjbGVhdGlvbiUyMEZhY3RvcnMlMjBsaXN0ZXJpYQR0X3N0bXADMTcwMDUyOTQyOQ--?p=Pathogenic+Actin+Nucleation+Factors+listeria&ei=UTF-8&fr2=p%3As%2Cv%3Av%2Cm%3Asb%2Crgn%3Atop&fr=mcafee&type=E210BR885G0#id=8&vid=c53b1fd237cec1995f602e3b66bcad32&action=view



Manipulation of the proinflammatory transcriptional response by bacterial effector proteins.



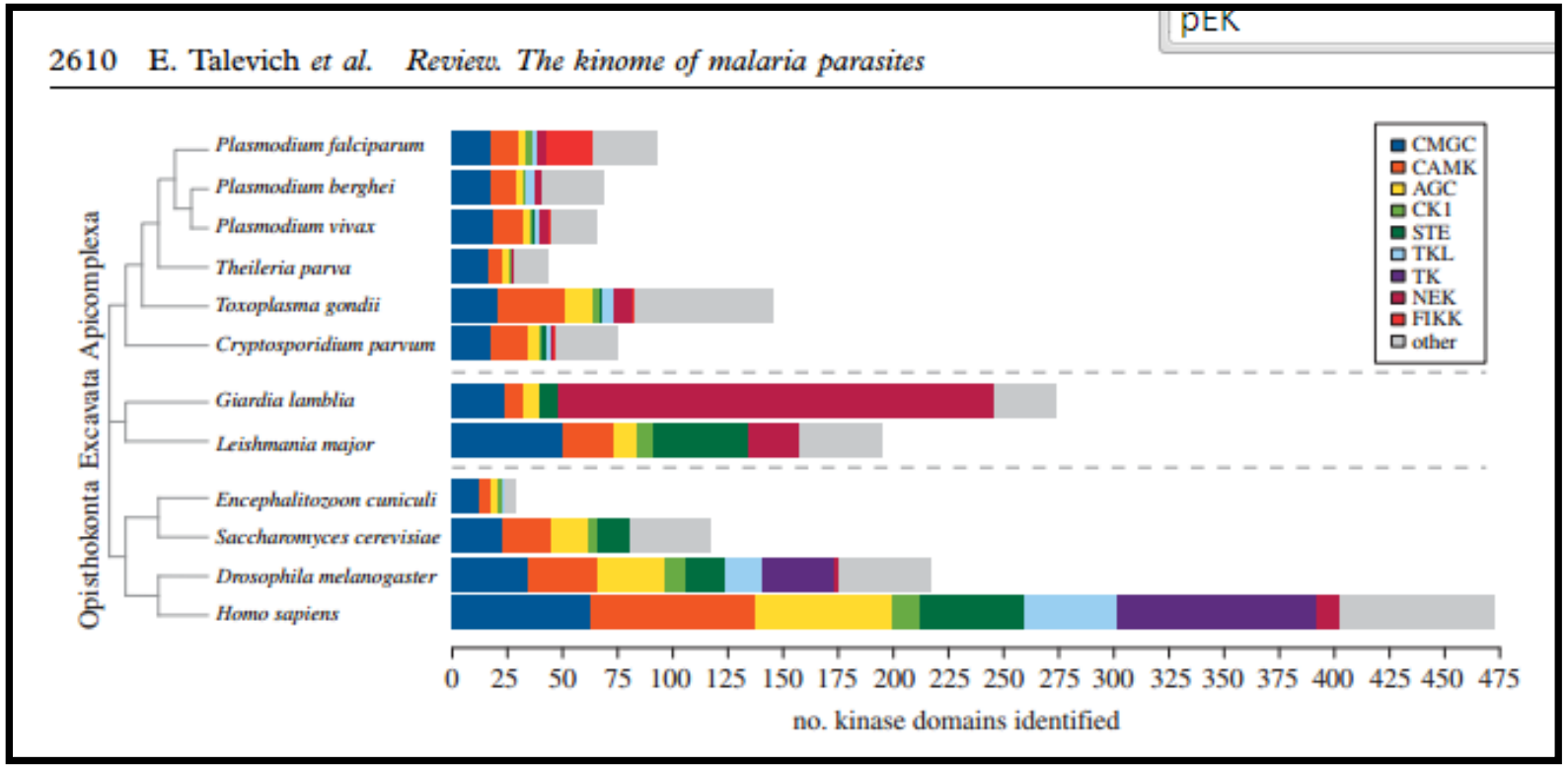
Malaria parasite Kinome



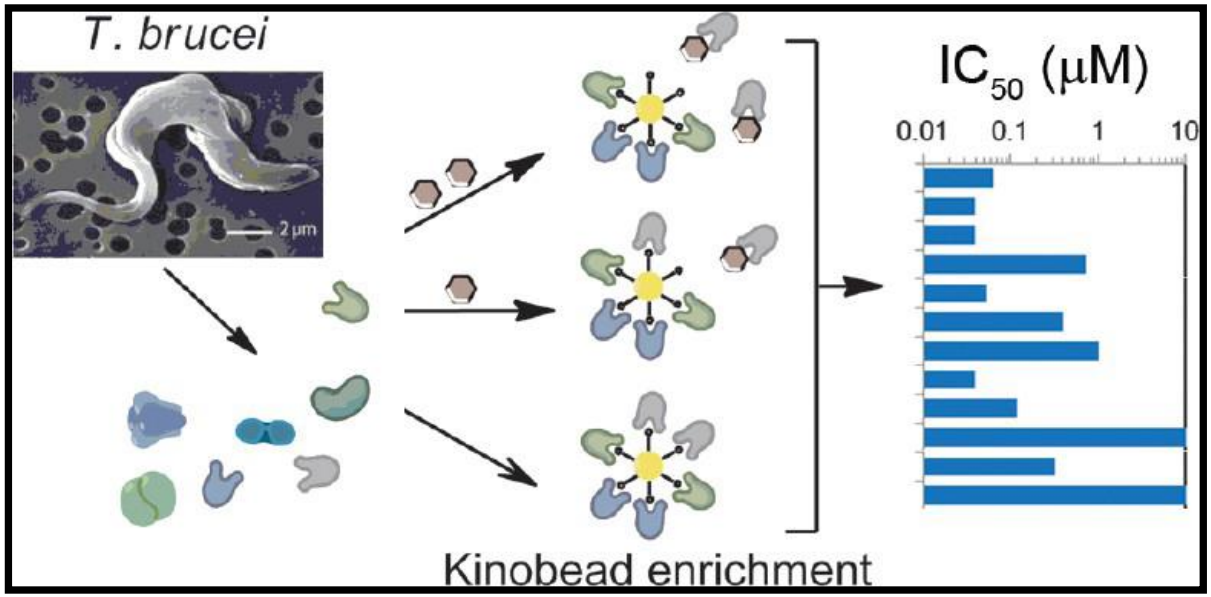
FIKKs, a novel family of atypical protein kinase-like enzymes named after a conserved Phe–Ile–Lys–Lys motif, and a family of CDPKs similar to calcium-regulated protein kinases found in plants but not in metazoans.

The CMGC group (named after the initials of some members) includes key kinases: the MAPK growth- and stress-response kinases, the cell cycle CDK (cyclin dependent kinases) and kinases involved in splicing and metabolic control.

Malaria parasite Kinome



Chemical Proteomic Analysis Reveals the Drug ability of the Kinome of *Trypanosoma brucei*



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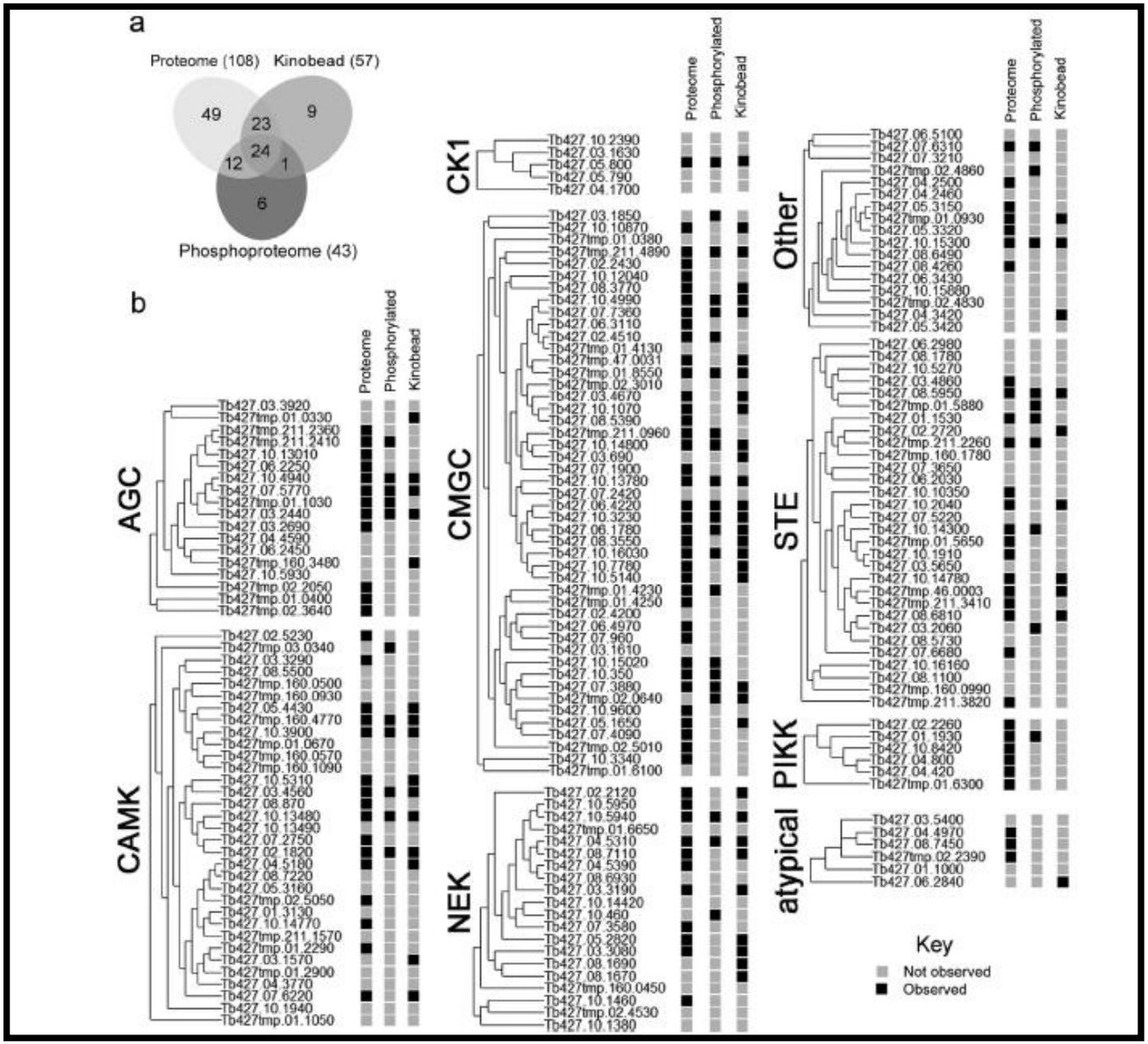

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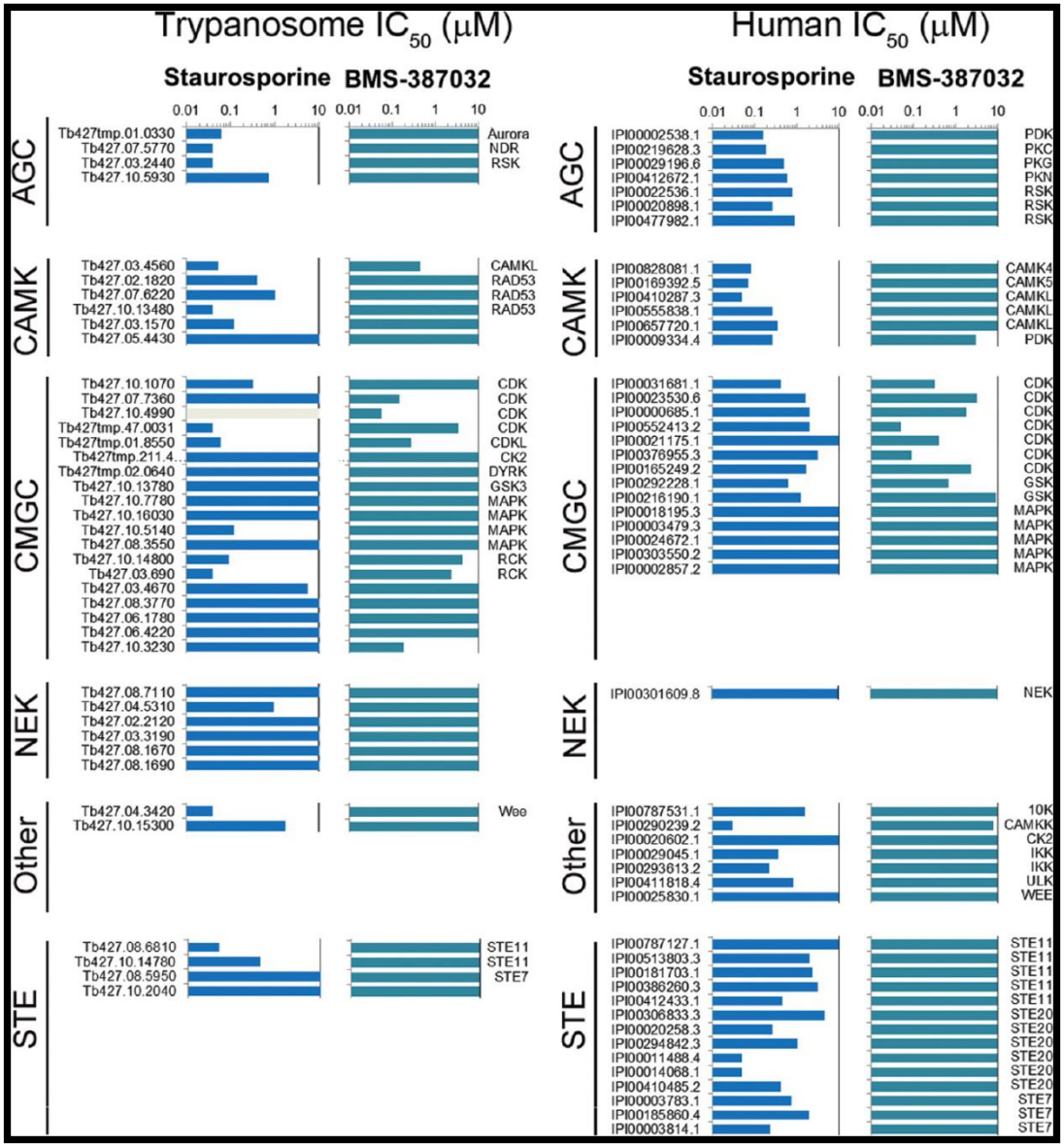
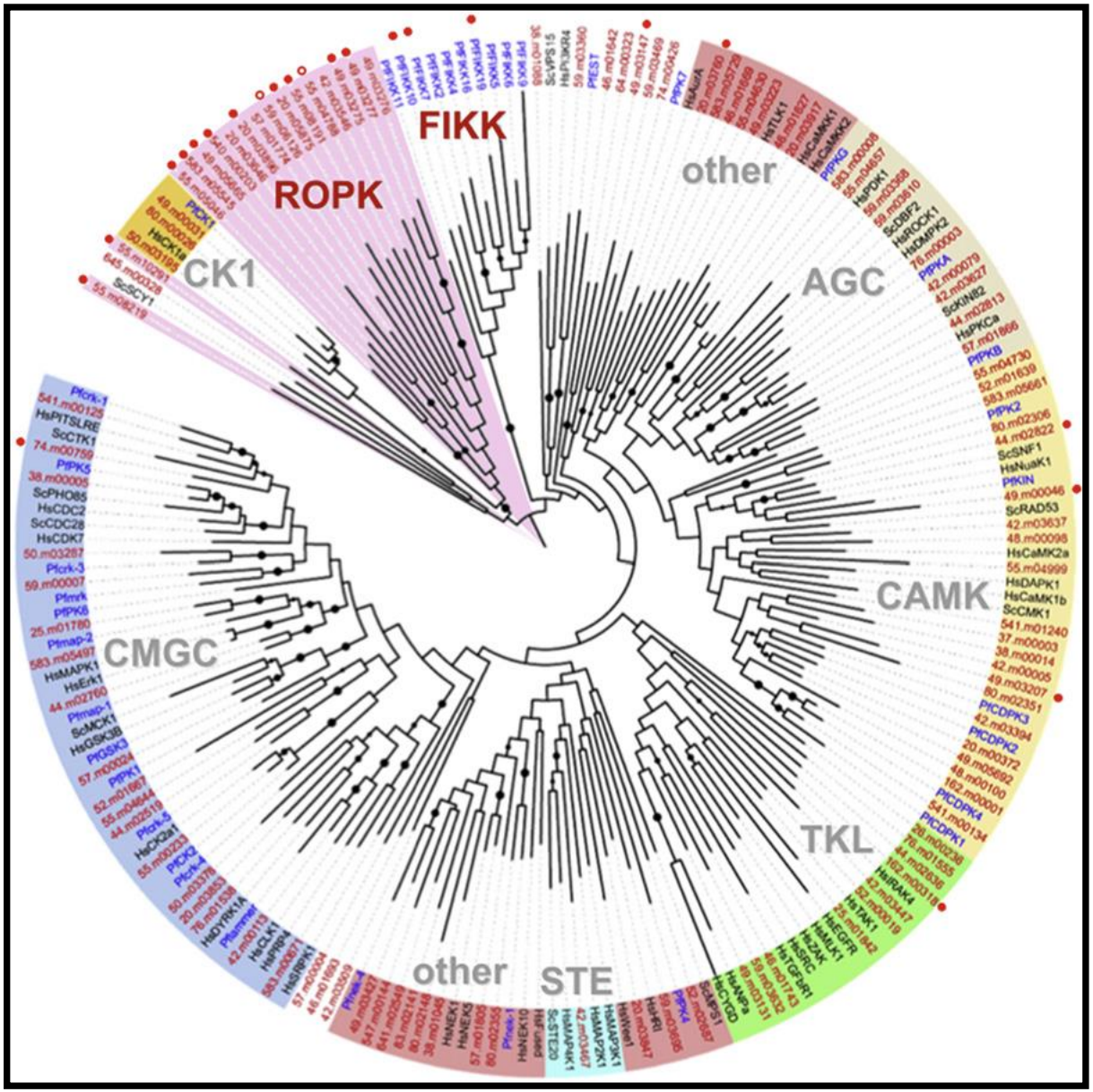


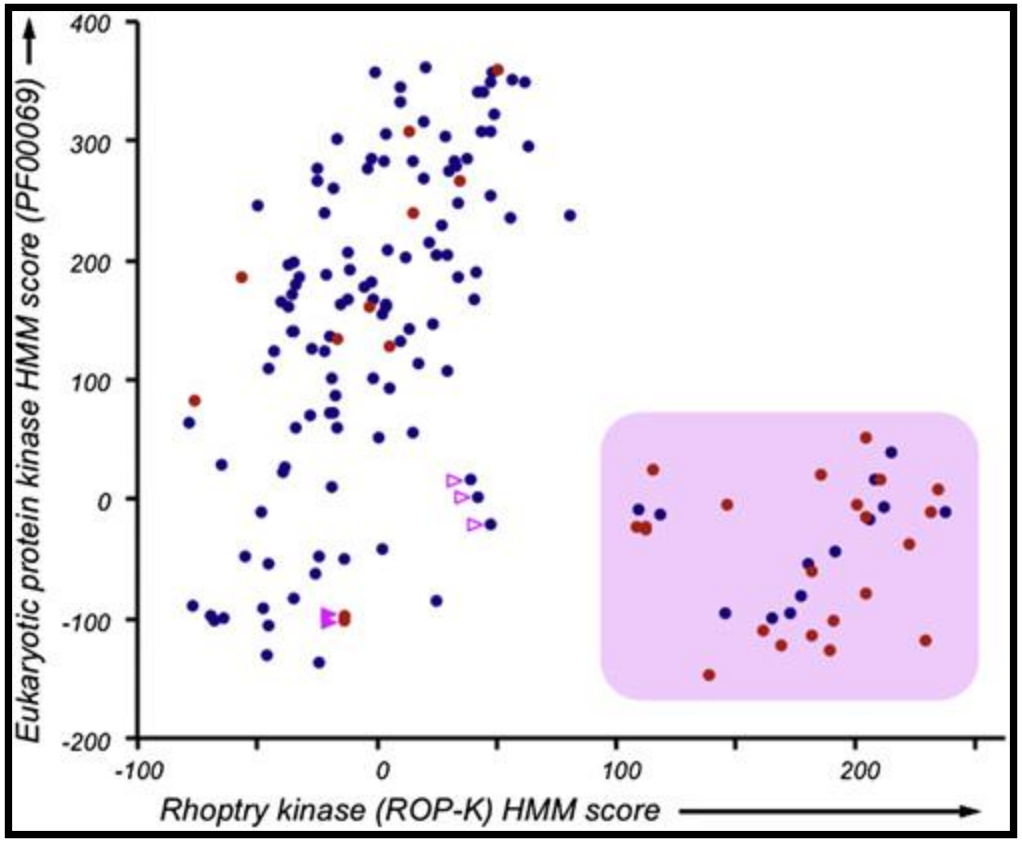
Chemical Proteomic Analysis Reveals the Drugability of the Kinome of *Trypanosoma brucei*

	GSK3	PK50	PK53
compound ID	DDD85893	DDD34425	DDD88213
T. brucei enzyme IC_{50} (μM)[a]	<0.002	0.013 ± 0.006	0.73 ± 0.14
T. brucei kinobead IC_{50} (μM)[b]	<0.039	not observed	5.7
T. brucei EC_{50} (μM)[c]	1.3 ± 1.2	0.86 ± 0.52	45 ± 4
H. sapiens MRC5 EC_{50} (μM)[c]	28 ± 9.7	>50	>50
H. sapiens kinobead IC_{50} (μM)[b]	<0.06	not observed	not observed

Toxoplasma Kinome



Toxoplasma Kinome

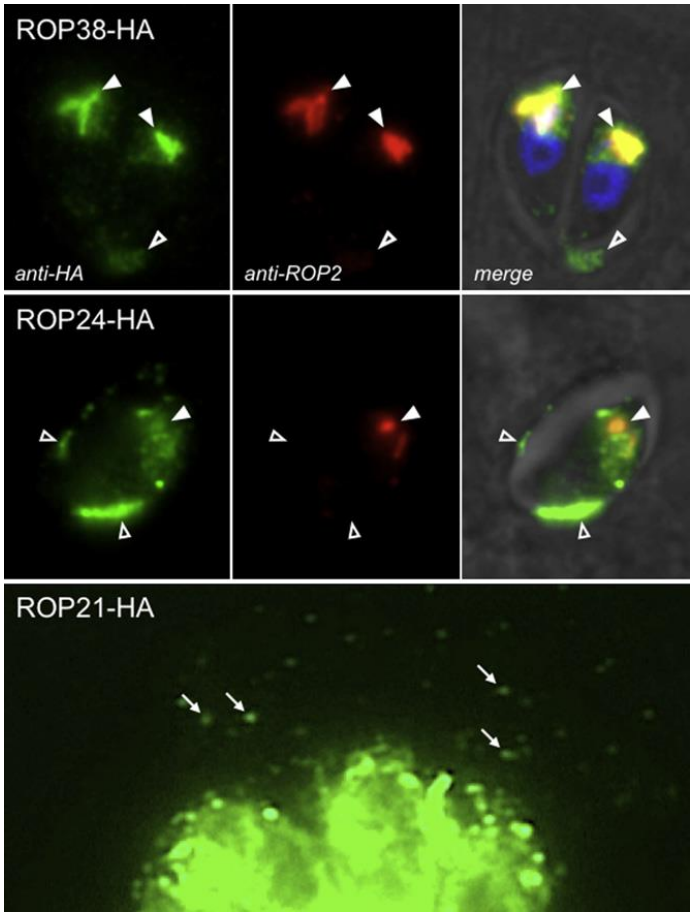


Toxoplasma rhoptries

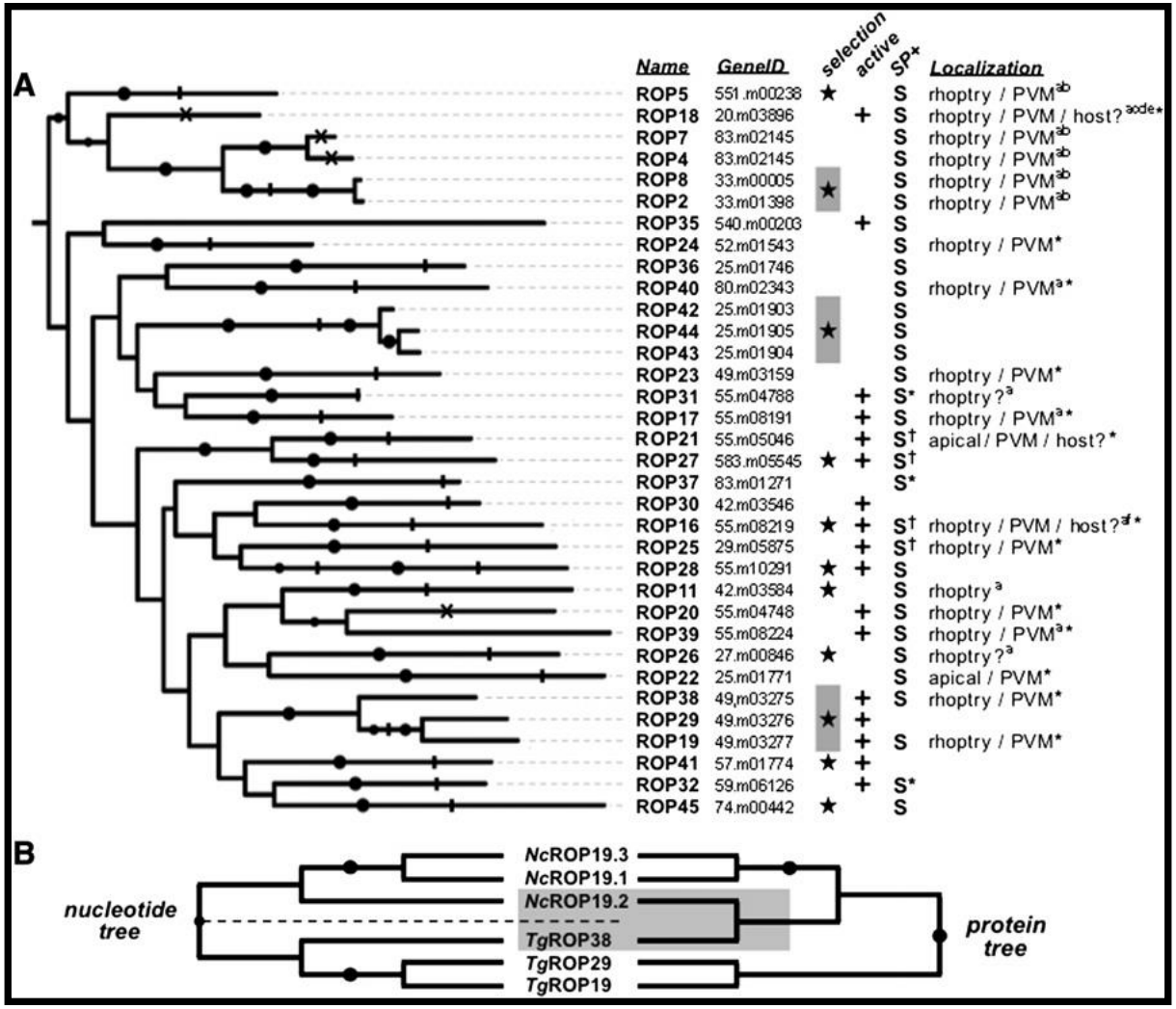
Current findings suggest that rhoptries are most analogous to secretory lysosomal granules because they receive material from both biosynthetic and endocytic cell pathways.

In the exit site of endoplasmic reticulum, the rhoptry proteins (in a form of proproteins) are loaded into coated vesicles and then travel to the Golgi apparatus where they are sorted to an immature rhoptry using the specific sorting signals.

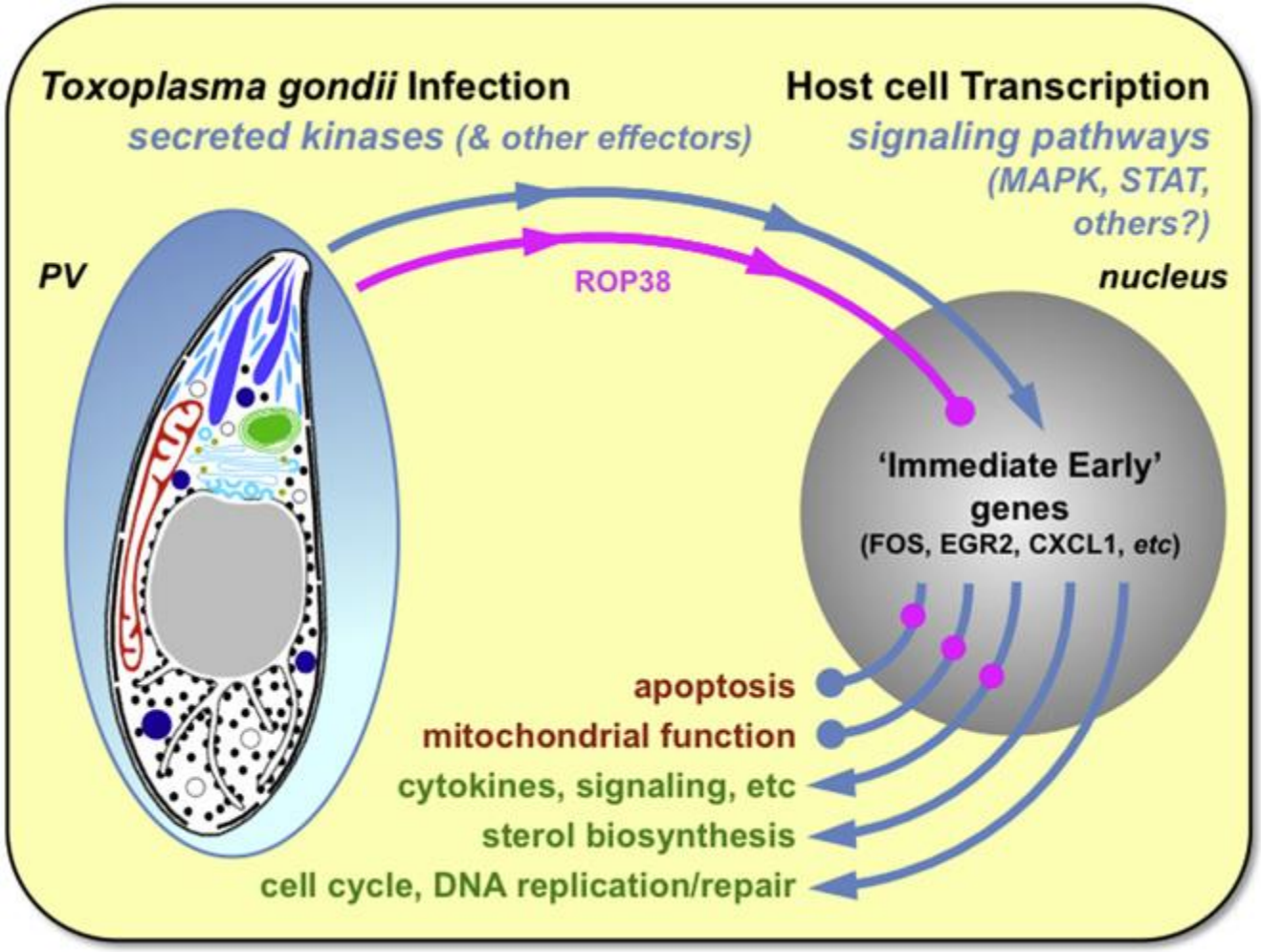
Both tyrosine-based and dileucine sorting motifs were detected within cytoplasmic tails of the predominant ROP2 family proteins. Proproteins undergo proteolytic cleavage of an N-terminal prodomain,



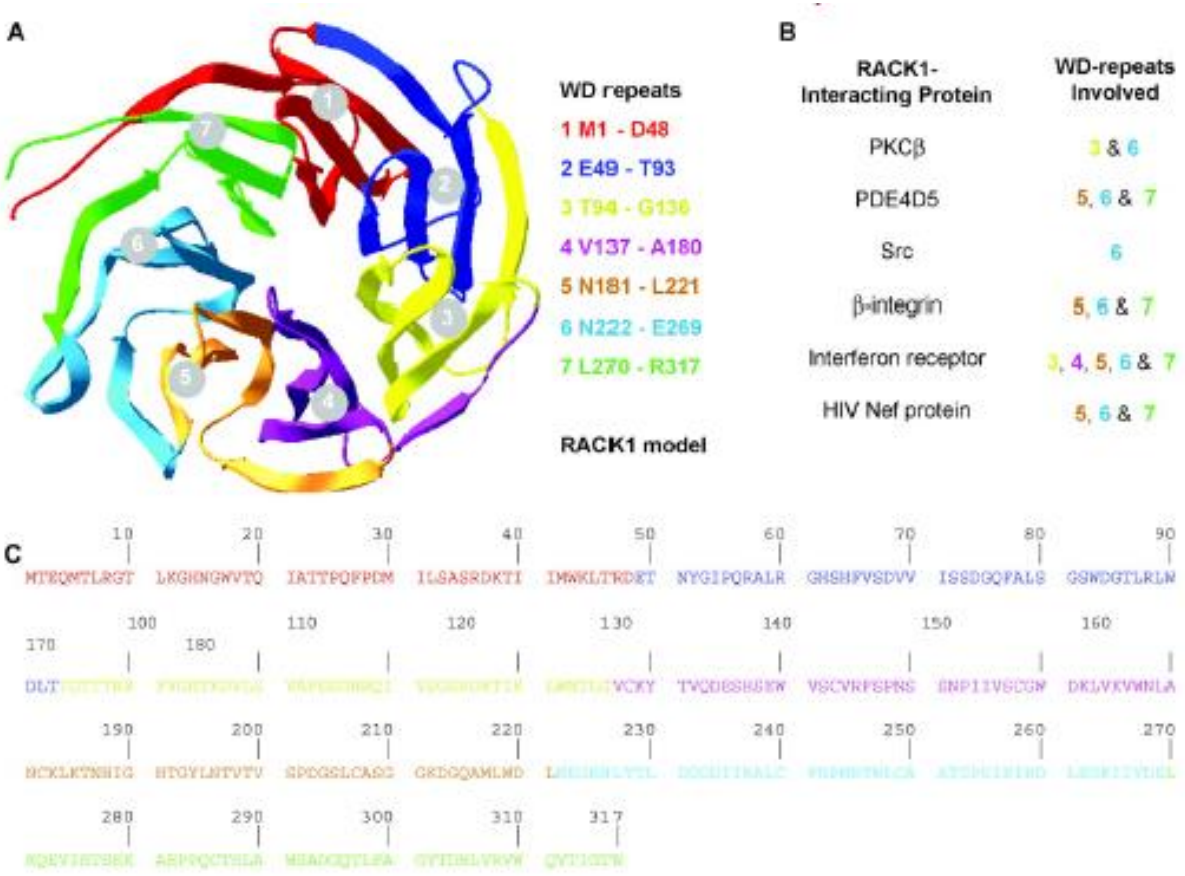
Toxoplasma Kinome



Toxoplasma Subversion of the host



Conserved scaffold proteins
RACK, TRACK and LACK



Yarwood Mol. Pharmacol. 2002

Figure 1 A

```
                         L01                    L02                          L03
LACK     MNYEGHLKGHRGWVTSLACPQQAGSYIKVVSTSRDGTAISWKANPDRHSVDSDYGLPSHRLEGHTGFVSCVSLAHATDYA
         :  .: :::: :::::..:  :  ..:  .:.::: : : :: . :    ...::.:.. :.::. ::: : ..   ..:
RACK     MTLRGTLKGHNGWVTQIATTPQFPDMI--LSASRDKTIIMWKLTRD----ETNYGIPQRALRGHSHFVSDVVISSDGQFA

                L04                                        L05
LACK     LTASWDRSIRMWDLRNGQCQRKFLKHTKDVLAVAFSPDDRLIVSAGRDNVIRVWNVAGECMHEFLRDSHEDWVSSICFSP
         :..::: ..:.::: .:   :.:. ::::::.:::: :.: :::..::..:..::. : : .    .:: .::: . :::
RACK     LSGSWDGTLRLWDLTTGTTTRRFVGHTKDVLSVAFSSDNRQIVSGSRDKTIKLWNTLGVCKYTVQDESHSEWVSCVRFSP

                         L06                                                L07
LACK     SLEHPIVVSGSWDNTIKVWNVNGGKCERTLKGHSNYVSTVTVSPDGSLCASGGKDGAALLWDLSTGEQLFKINVESPINQ
         .  .::.:: .::. .::::. . : . .  ::..:..::::::::::::::::::: :.::::. :..:. ..  . ::
RACK     NSSNPIIVSCGWDKLVKVWNLANCKLKTNHIGHTGYLNTVTVSPDGSLCASGGKDGQAMLWDLNEGKHLYTLDGGDIINA

                   L08                    L09
LACK     IAFSPNRFWMCVATERSLSVYDLESKAVIAEL------TPDGAKPSECISIAWSADGNTLYSGHKDNLIRVWSIS
         . ::::::.:.:.::  :....:::.: .. ::      : . :.: .: :.::::::.::..:. :::.:::...
RACK     LCFSPNRYWLCAATGPSIKIWDLEGKIIVDELKQEVISTSSKAEPPQCTSLAWSADGQTLFAGYTDNLVRVWQVT
```

B

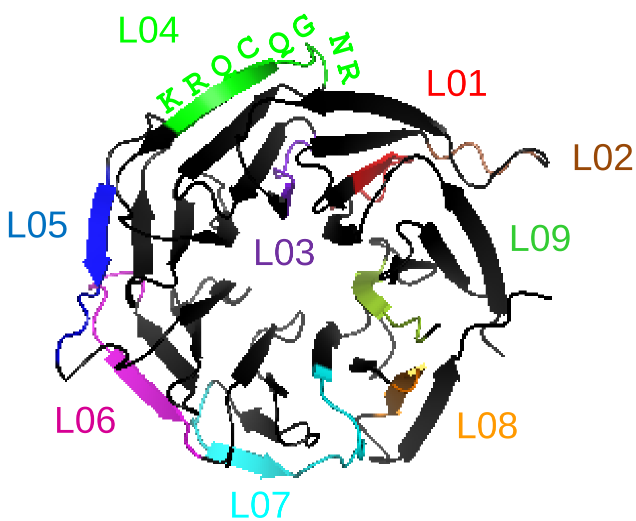
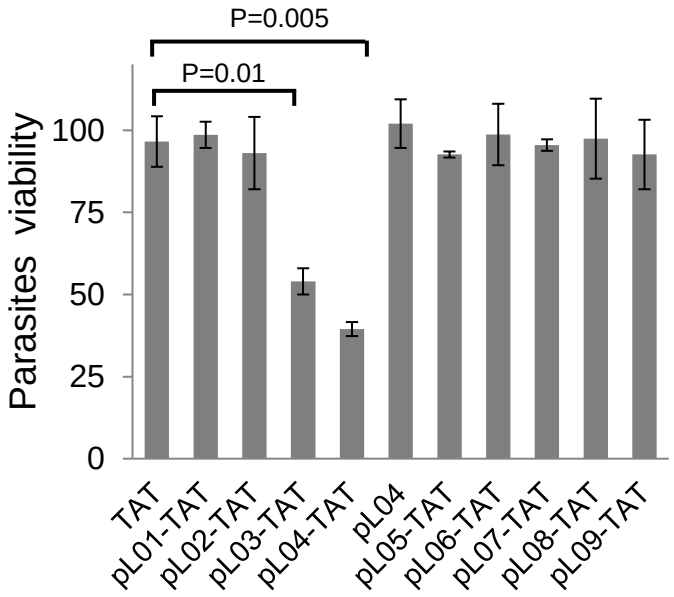


Figure 2 A

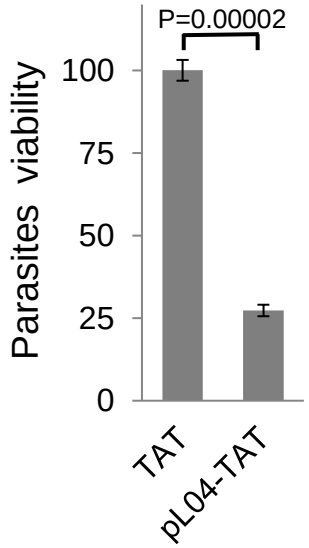


B

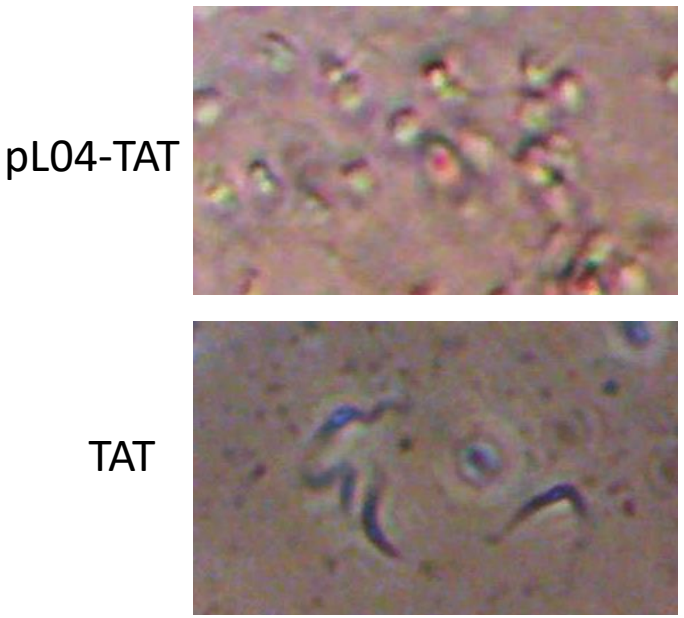
```
          L04
LACK      RNGQCQRK
            .:   :.
RACK      TTGTTTRR
            ::.  ..
TRACK     QTGVCQHK

          L04
LACK      RNGQCQRK
            ..: :::.:
TRACK     QTGVCQHK
```

C



D



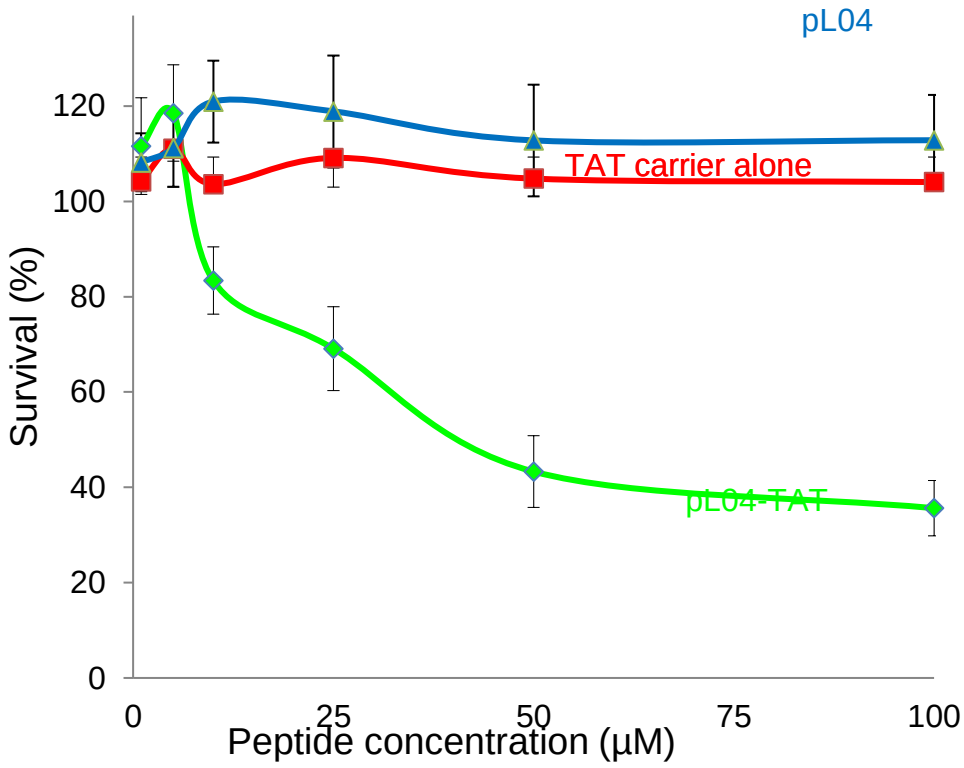

pL04
TAT carrier alone
pL04-TAT
Survival (%)
120
100
80
60
40
20
0
0
25
50
75
100
Peptide concentration (μM)

Figure 4

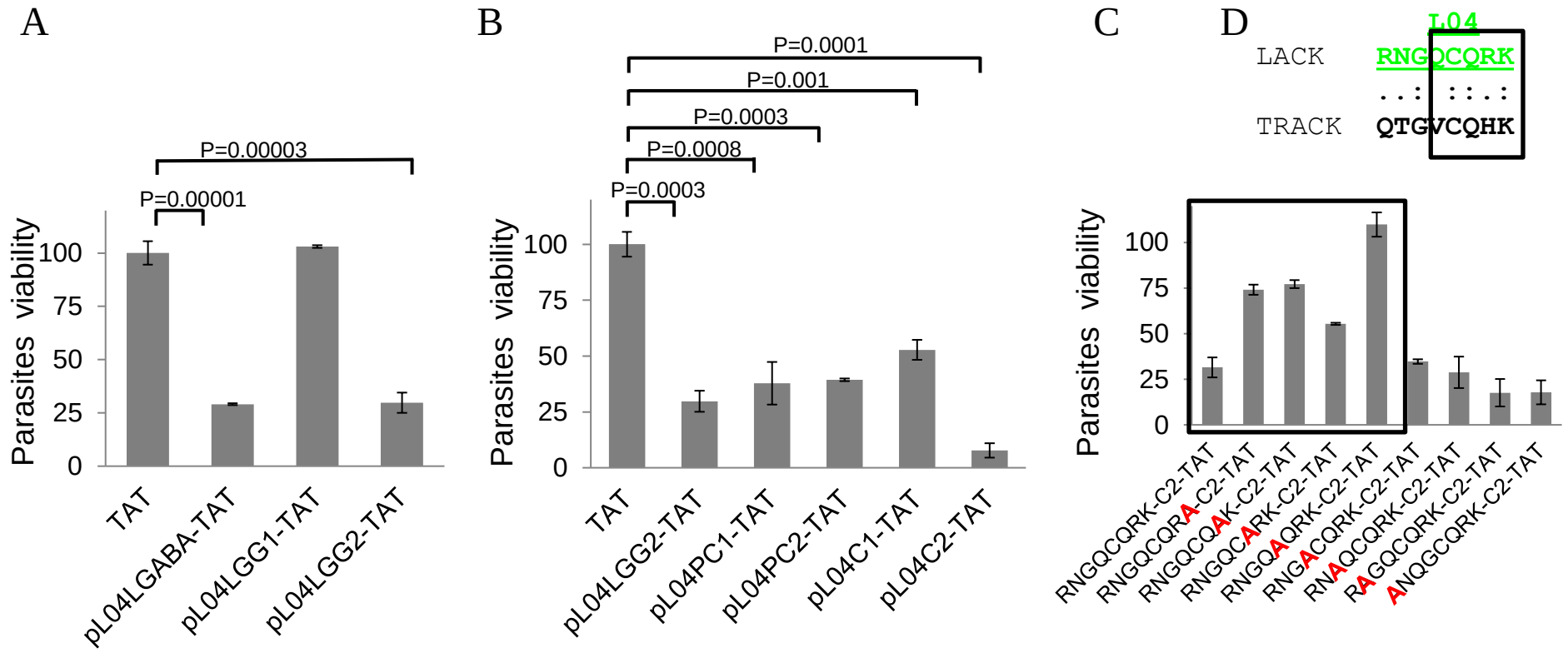
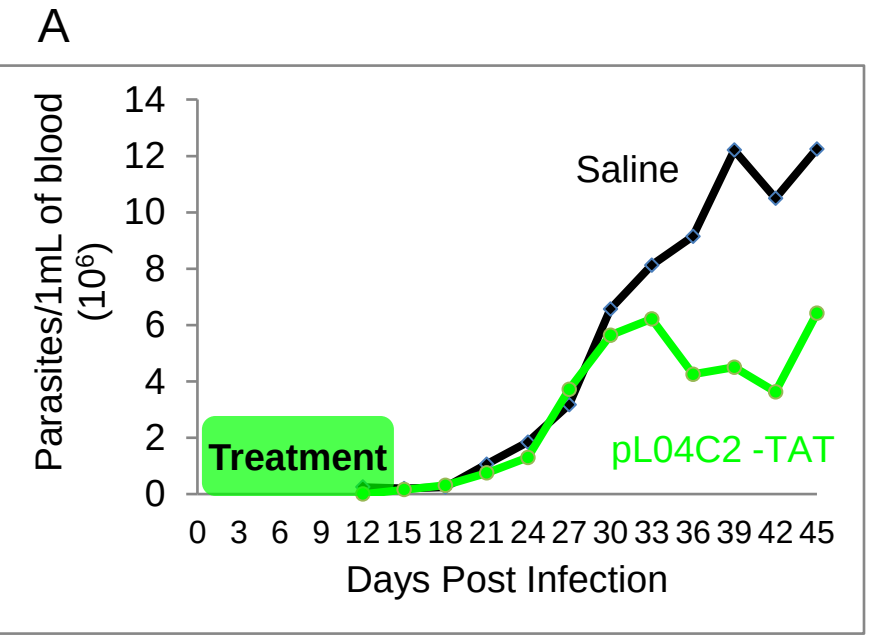
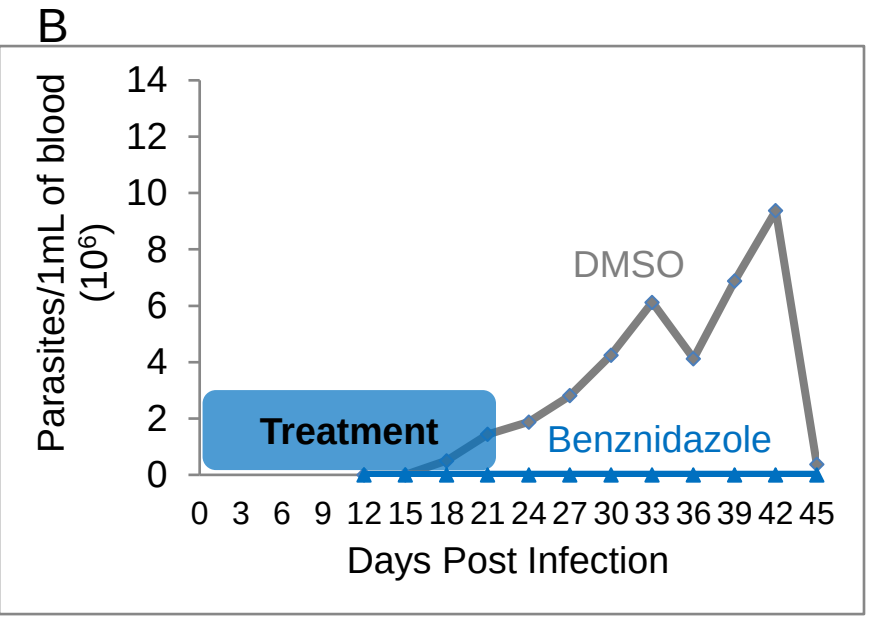
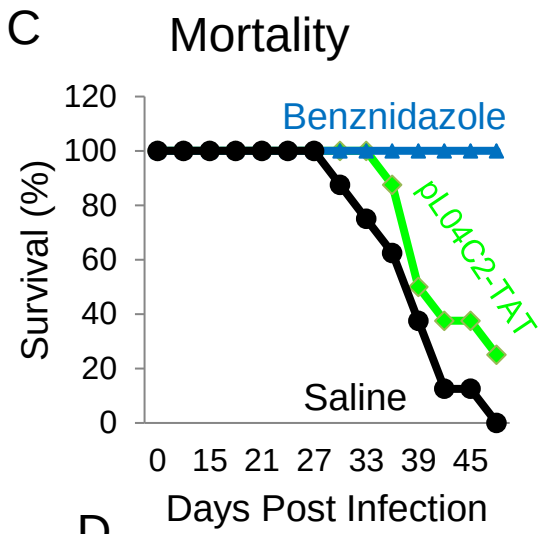
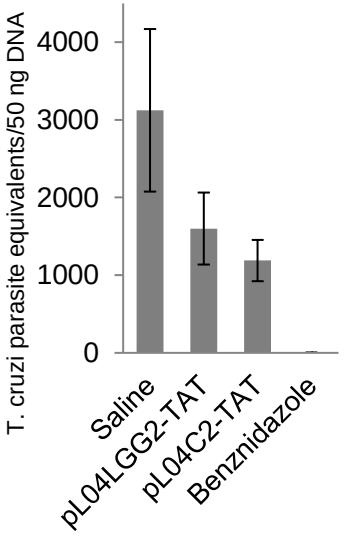

A
P=0.00003
P=0.00001
Parasites viability
100
75
50
25
0
TAT
pL04LGABA-TAT
pL04LGG1-TAT
pL04LGG2-TAT
B
P=0.0001
P=0.001
P=0.0003
P=0.0008
P=0.0003
Parasites viability
100
75
50
25
0
TAT
pL04LGG2-TAT
pL04PC1-TAT
pL04PC2-TAT
pL04C1-TAT
pL04C2-TAT
C
D
L04
LACK RNGQCQRK
TRACK QTGVCQHK
Parasites viability
100
75
50
25
0
RNGQCQRK-C2-TAT
RNGQCQRA-C2-TAT
RNGQCQAK-C2-TAT
RNGQCARK-C2-TAT
RNGQACQRK-C2-TAT
RNGACQRK-C2-TAT
RNAQCQRK-C2-TAT
RAGQCQRK-C2-TAT
ANQGCQRK-C2-TAT

Figure 6.


A
Parasites/1mL of blood (10⁶)
14
12
10
8
6
4
2
0
Saline
Treatment
pL04C2 -TAT
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45
Days Post Infection


B
Parasites/1mL of blood (10⁶)
14
12
10
8
6
4
2
0
DMSO
Treatment
Benznidazole
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45
Days Post Infection


C
Mortality
Survival (%)
120
100
80
60
40
20
0
Benznidazole
pL04C2-TAT
Saline
0 15 21 27 33 39 45
Days Post Infection


D
T. cruzi parasite equivalents/50 ng DNA
4000
3000
2000
1000
0
Saline
pL04LGG2-TAT
pL04C2-TAT
Benznidazole


E1
Saline
E2
Benznidazole
E3
pL04C2-TAT