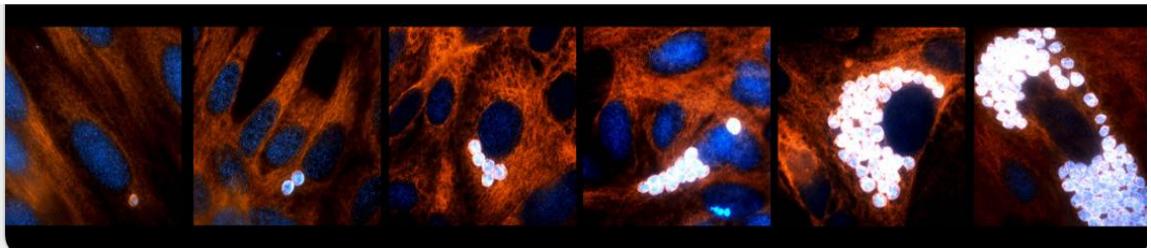
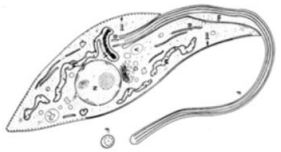
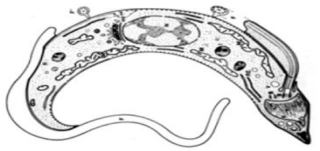


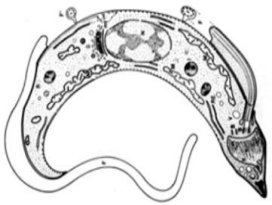
From gene profiling to inferences about metabolic changes in *Trypanosoma cruzi* infection

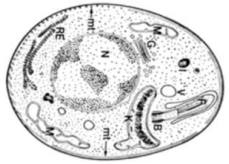
Workshop “Metabolism and mathematical models: Two for a tango” 2nd Edition, 2022

Carlos Robello

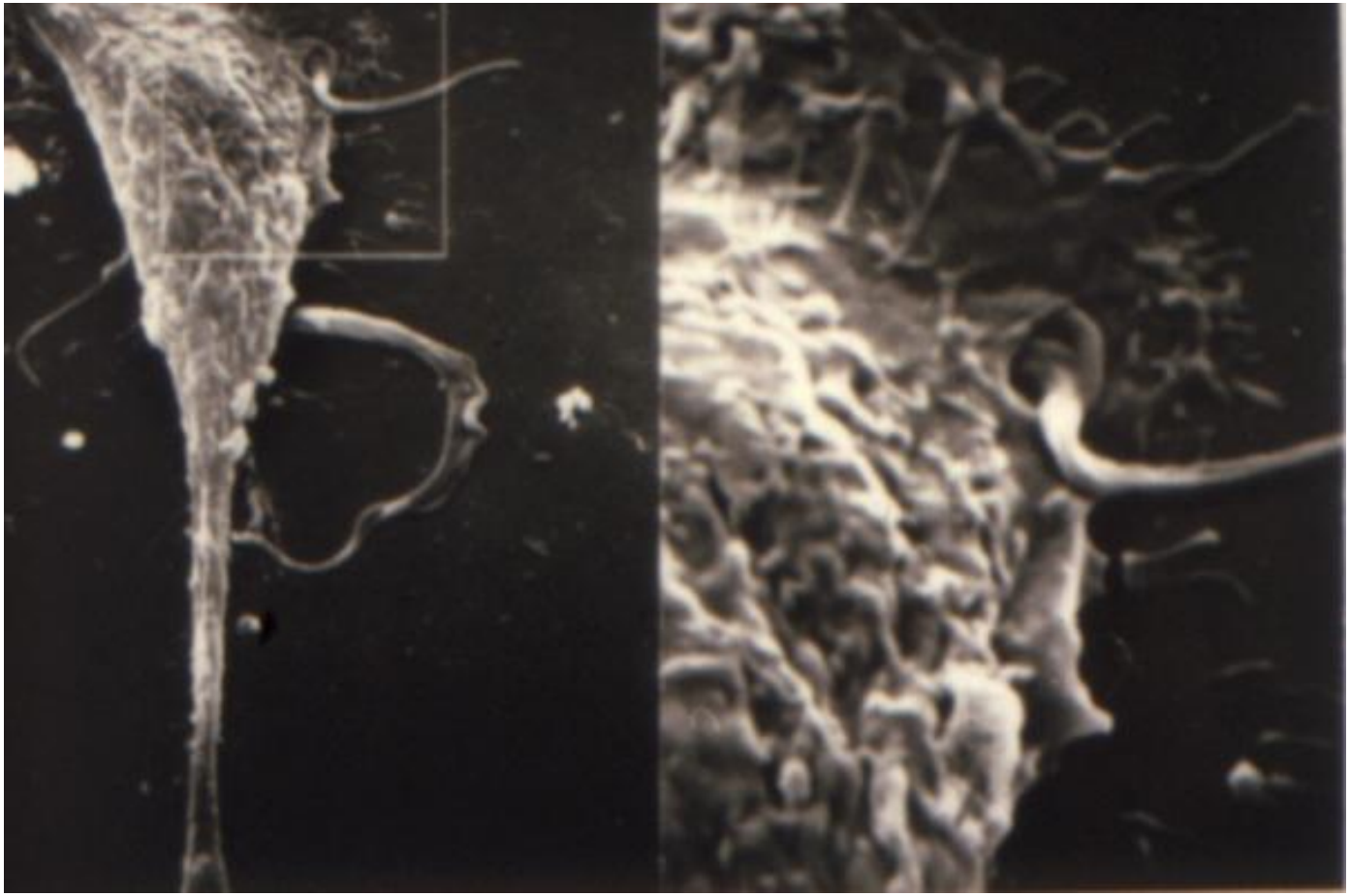








T cruzi can invade almost any cell type



Osuna A., Rodriguez N., Boy M., Castanys S., Gamarro F. Biol. Res. 26:19-26. 1993

Fibroblasts
Macrophages
Epithelial cells

Adipocytes
Cardiomyocytes
Etc

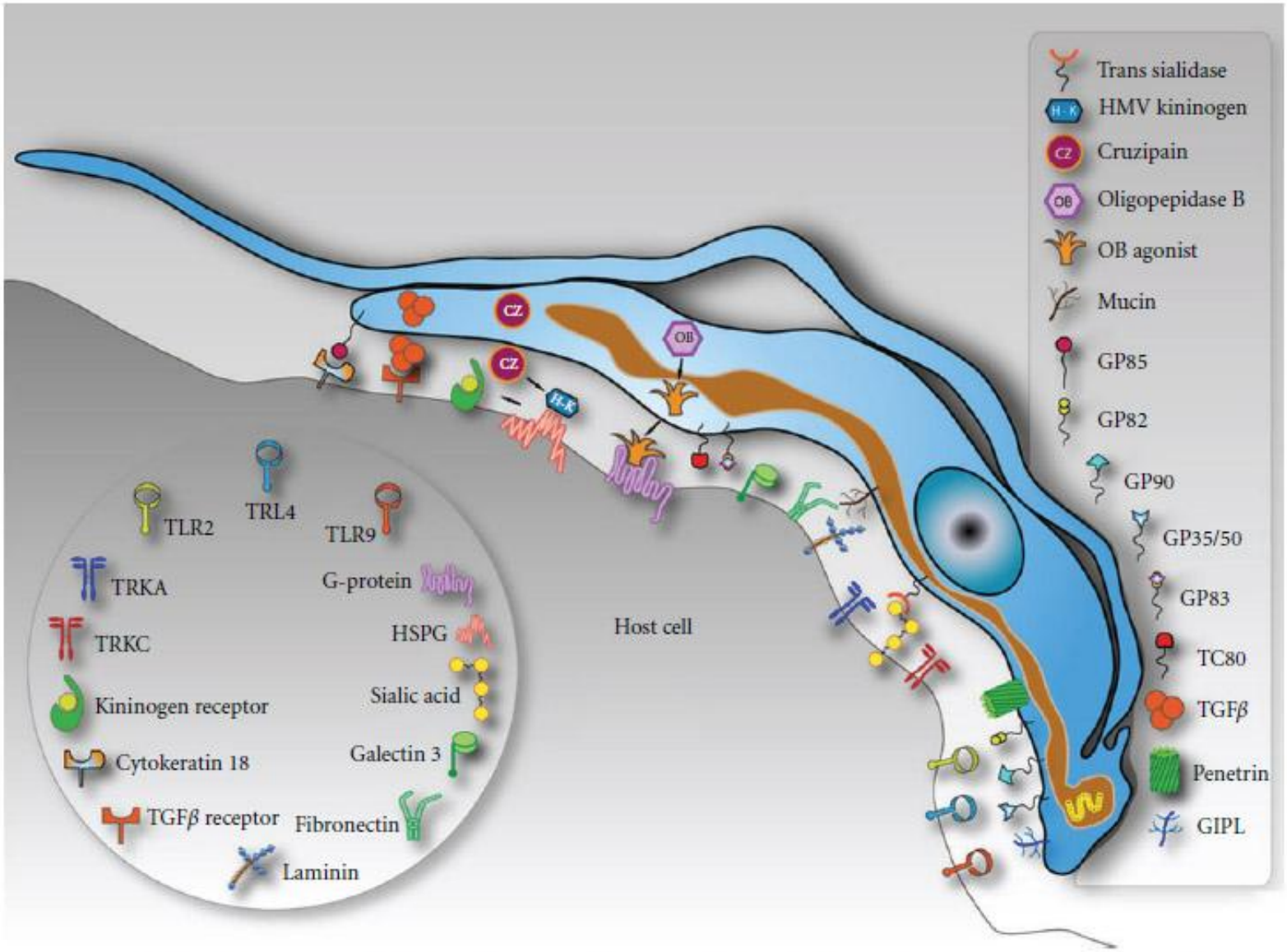
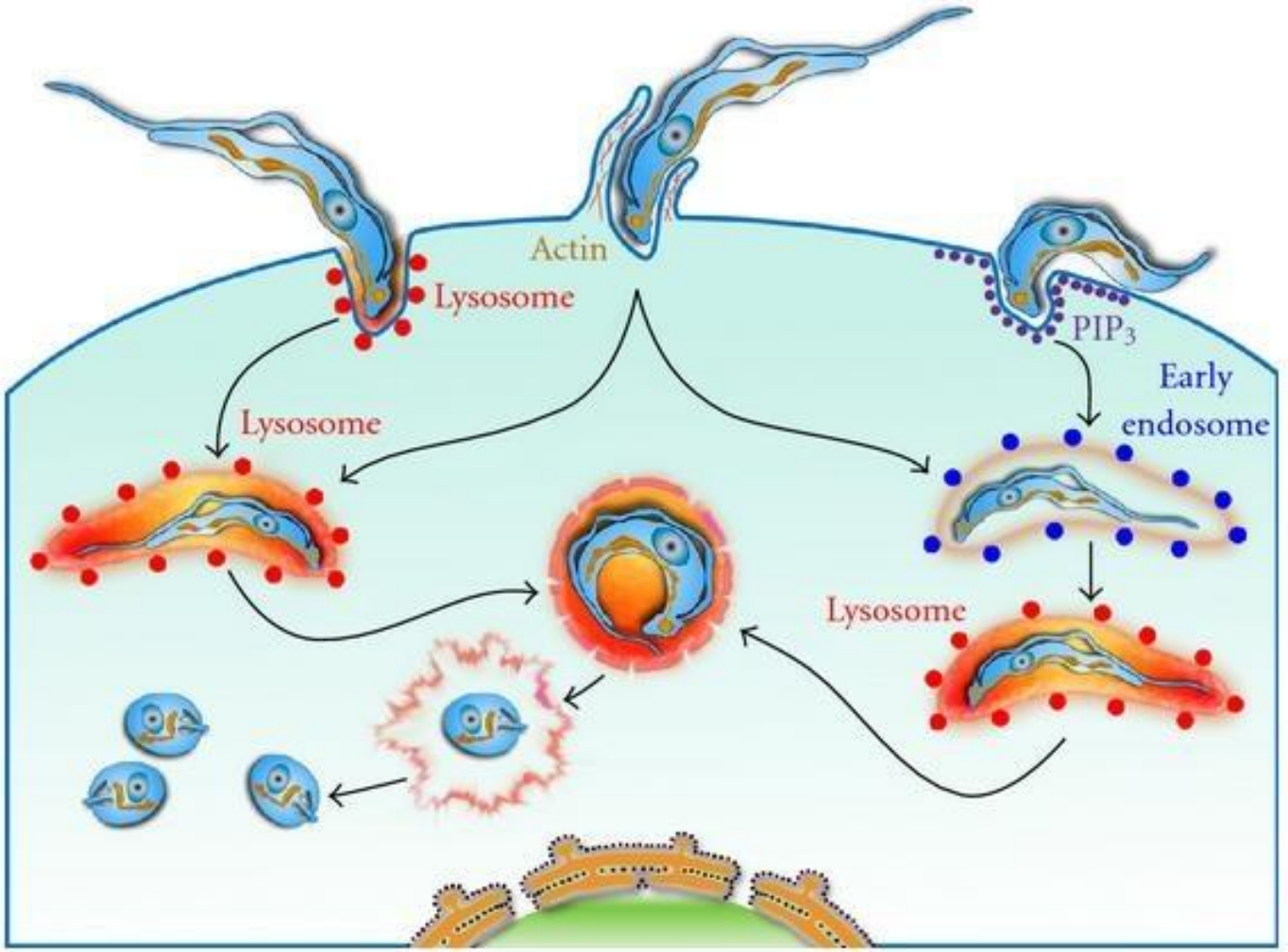


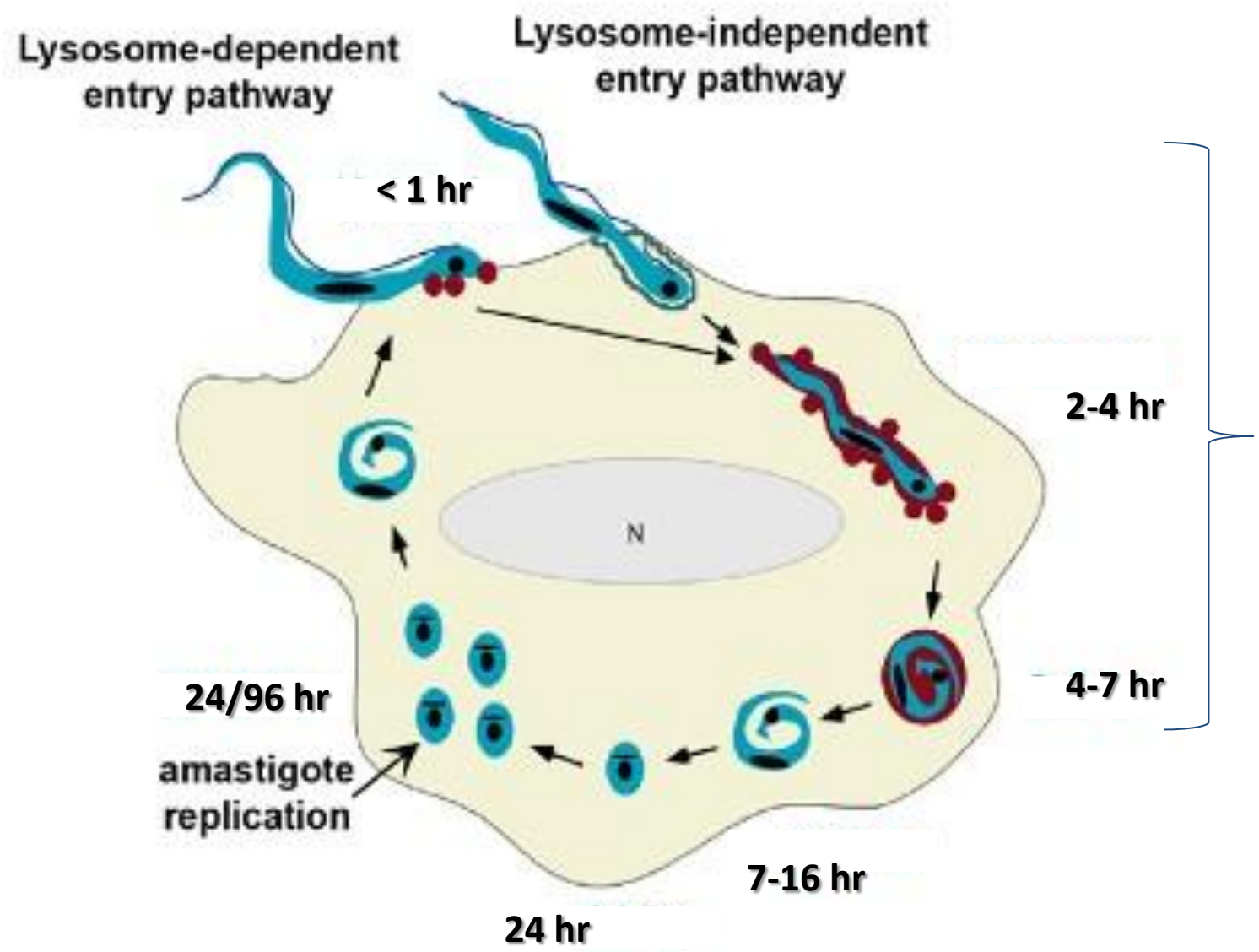
FIGURE 2: Schematic model summarizing the molecules involved on parasite-host cell interaction process and exposed on the surface of a hypothetical host cell and in trypomastigotes of *Trypanosoma cruzi*.

Barrias, E.S. et al. Front Immunol., 2013.

Invasion



De Souza, W. et.al. Int. J. Cell Biol., 2010.



Modified from http://search.sph.harvard.edu/faculty/barbara-burleigh/

Working hypothesis

During this host-pathogen interaction, *Trypanosoma cruzi* induces changes in gene expression profiles, reprogramming the host cell.

By interrogating the response of different cell types to infection with *T. cruzi*, we intend to approach the molecular bases of this interaction, which will allow knowing those host factors necessary for the infection and persistence of *Trypanosoma cruzi*.

These factors can lead to new molecular targets => Host targeted therapies.

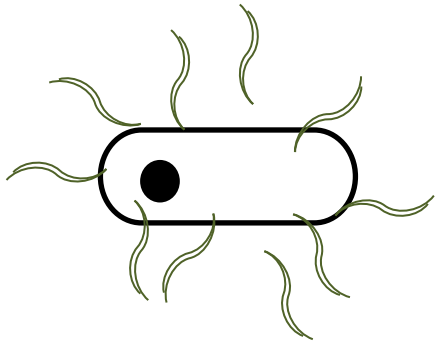
Outline

We aim to know the main changes (genes/pathways/processes) during the early/immediate response to *Trypanosoma cruzi* infection in human cells

- Epithelial cells
- Cardiomyocytes
- Macrophages

Experimental Design

Human cells + Dm28c (1:10)



Early Response

T = 0hs | T = 3 hs | T = 6 hs | T = 12 hs

RNA

Transcriptomics

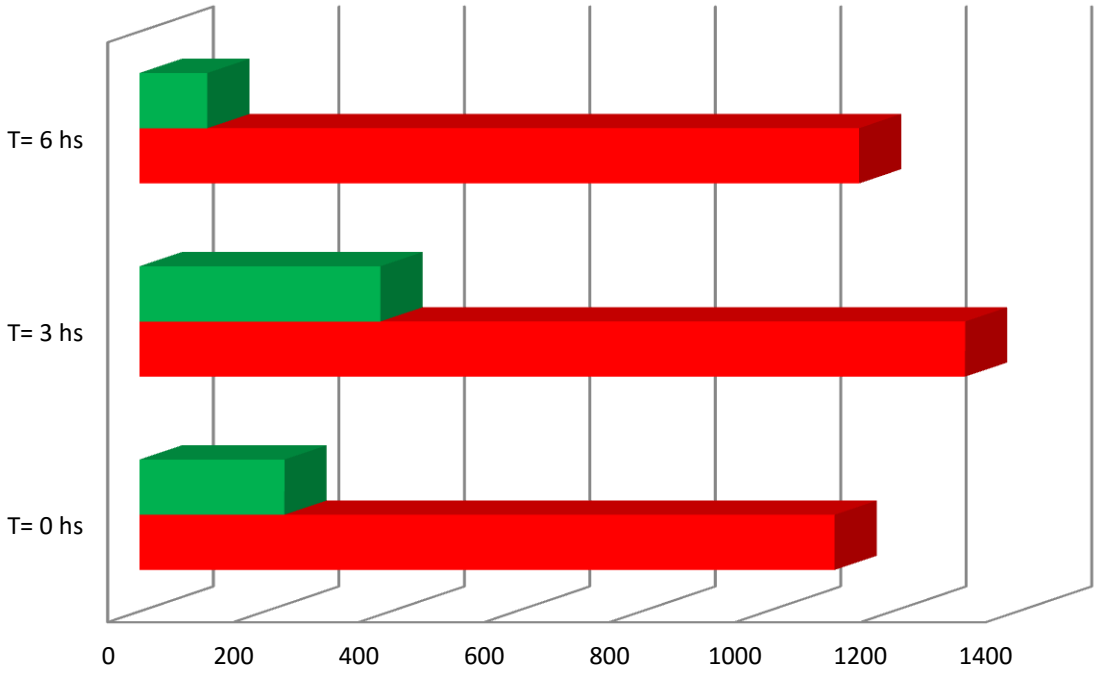
Outline

We aim to know the main changes (genes/pathways/processes) during the early/immediate response to *Trypanosoma cruzi* infection in human cells

- **Epithelial cells**
- Cardiomyocytes
- Macrophages

Drastic gene expression changes in the first hours after invasion

Diferentially expressed genes

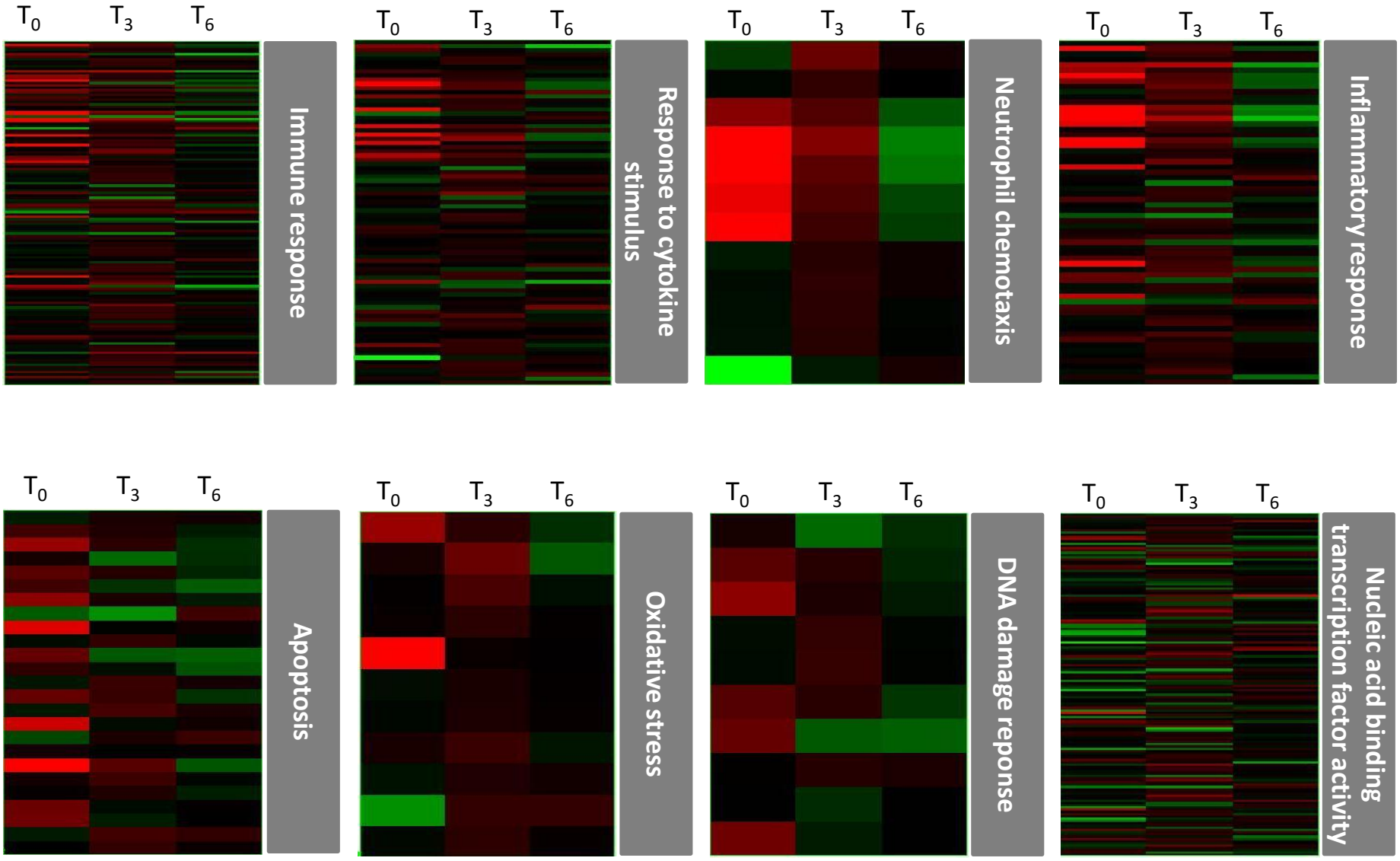


	T= 0 hs	T= 3 hs	T= 6 hs
Down regulated	231	384	108
Up regulated	1108	1316	1147

Chiribao et al., Biomed Res Int. 2014;2014:439501.

$p \leq 0.05$
Fold change ≥ 2

Gene Ontology and Pathways Analysis



Chiribao et al., Biomed Res Int. 2014;2014:439501.

Most of the up regulated genes remain in this condition in the early response to *T. cruzi*

Time	Up regulated	Down regulated
0hs vs control	1108	231
3hs vs 0hs	289	271
6 hsvs 3hs	52	35

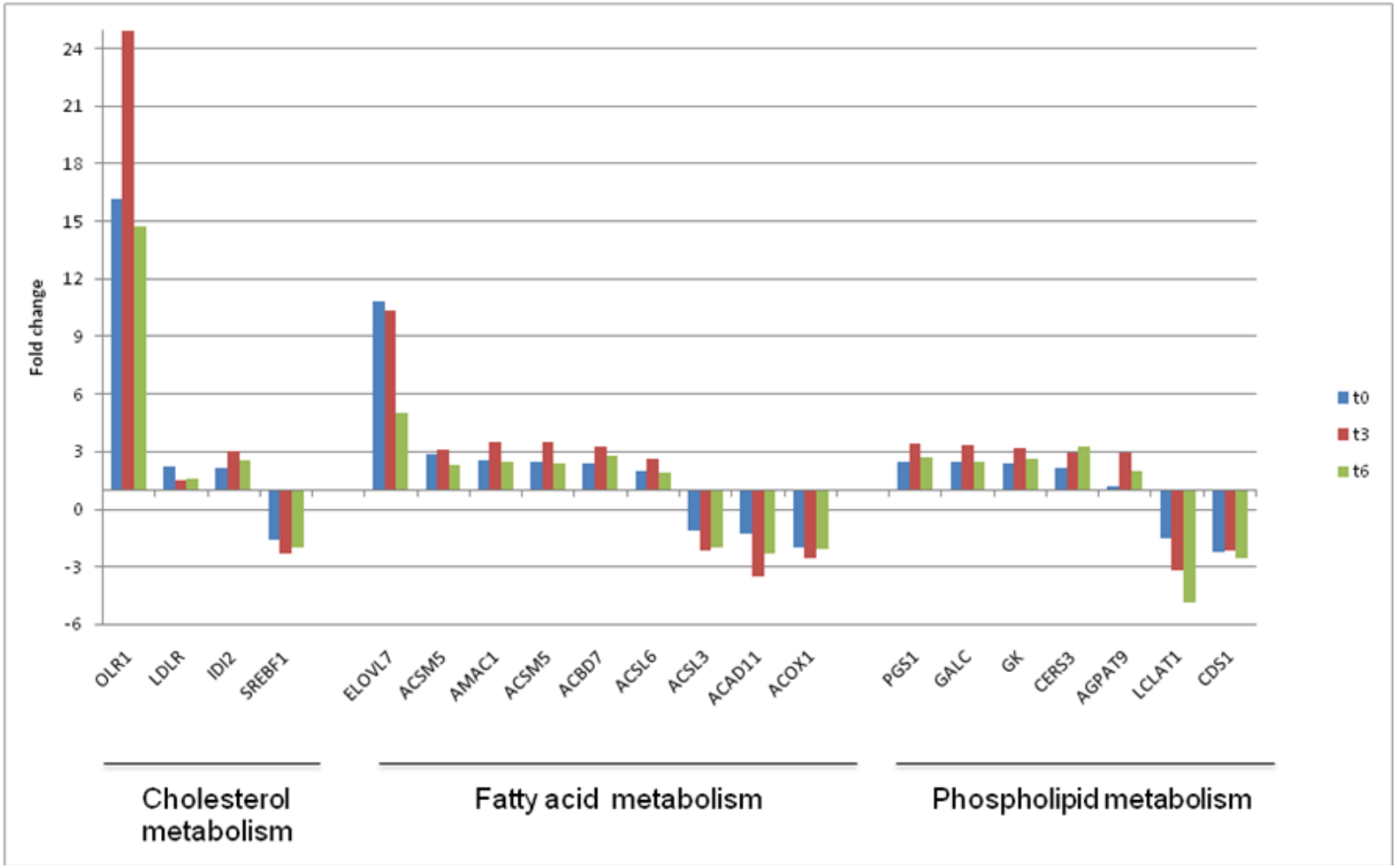
Chiribao et al., Biomed Res Int. 2014;2014:439501.

In contrast only 28 genes remain down regulated during early response to *T. cruzi*

Chiribao et al., Biomed Res Int. 2014;2014:439501.

Some remarks

Temporal regulation of host genes related to **lipid metabolism** after infection with *T. cruzi*



Chiribao et al., Biomed Res Int. 2014;2014:439501.

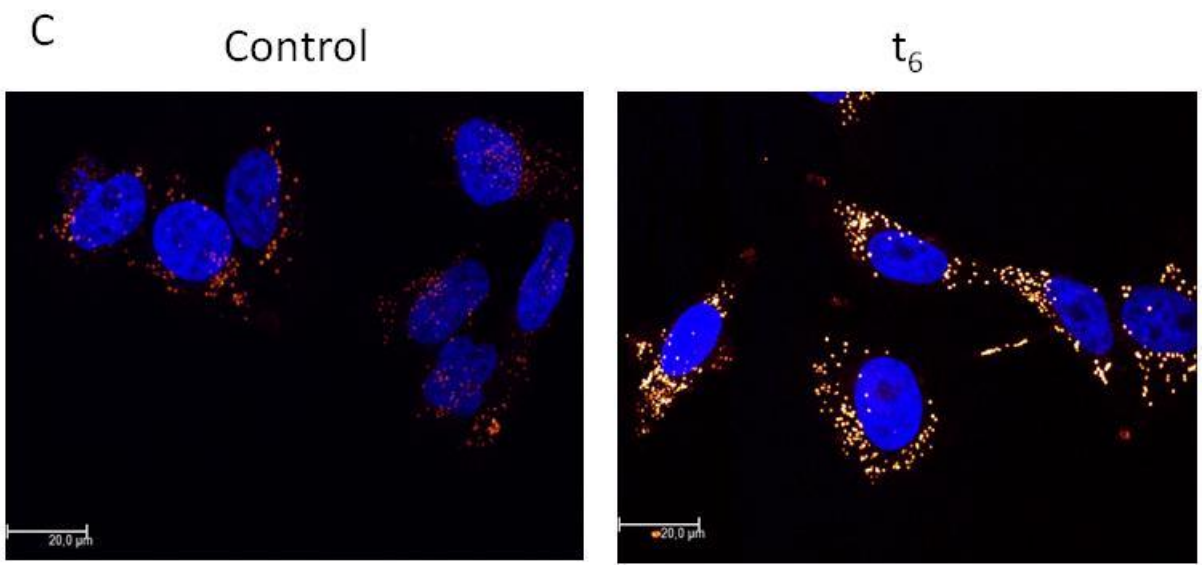
Some remarks

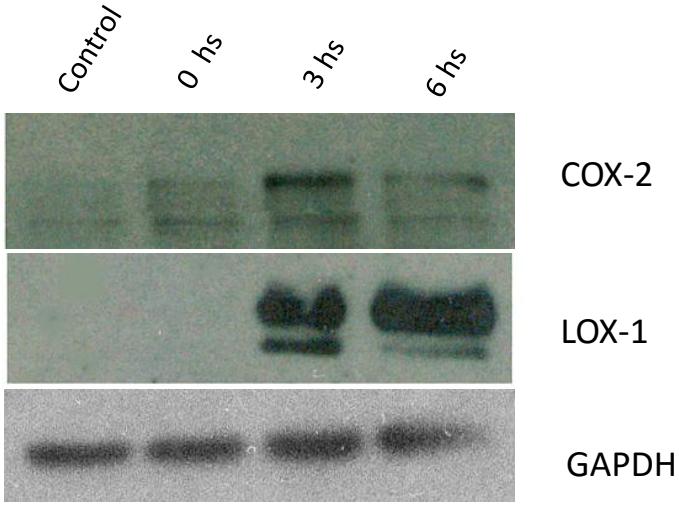
Cholesterol metabolism: the most remarkable changes occur in cholesterol transport related genes: low density lipoprotein receptor (rLDL) and oxidized low density lipoprotein receptor (OLR1), responsible for the entry of cholesterol and oxidized cholesterol, respectively, are overexpressed immediately after infection.

Fatty acids: Several genes from fatty acid metabolism are affected, in particular members of the acyl-CoA synthetase family (ACSL6, ACSM5, and AMAC1) and fatty acids transport (SLC27A1) that participate in fatty acid activation and uptake

One of the most up-regulated genes: very long chain fatty acid elongase 7 (ELOVL7). Very long fatty acids are essential precursors of signaling molecules related to the arachidonic acid and prostaglandin metabolism, suggestinga role in modulation of infalammatory responses

Some remarks





Interleukin 8 and DNA Damage Response

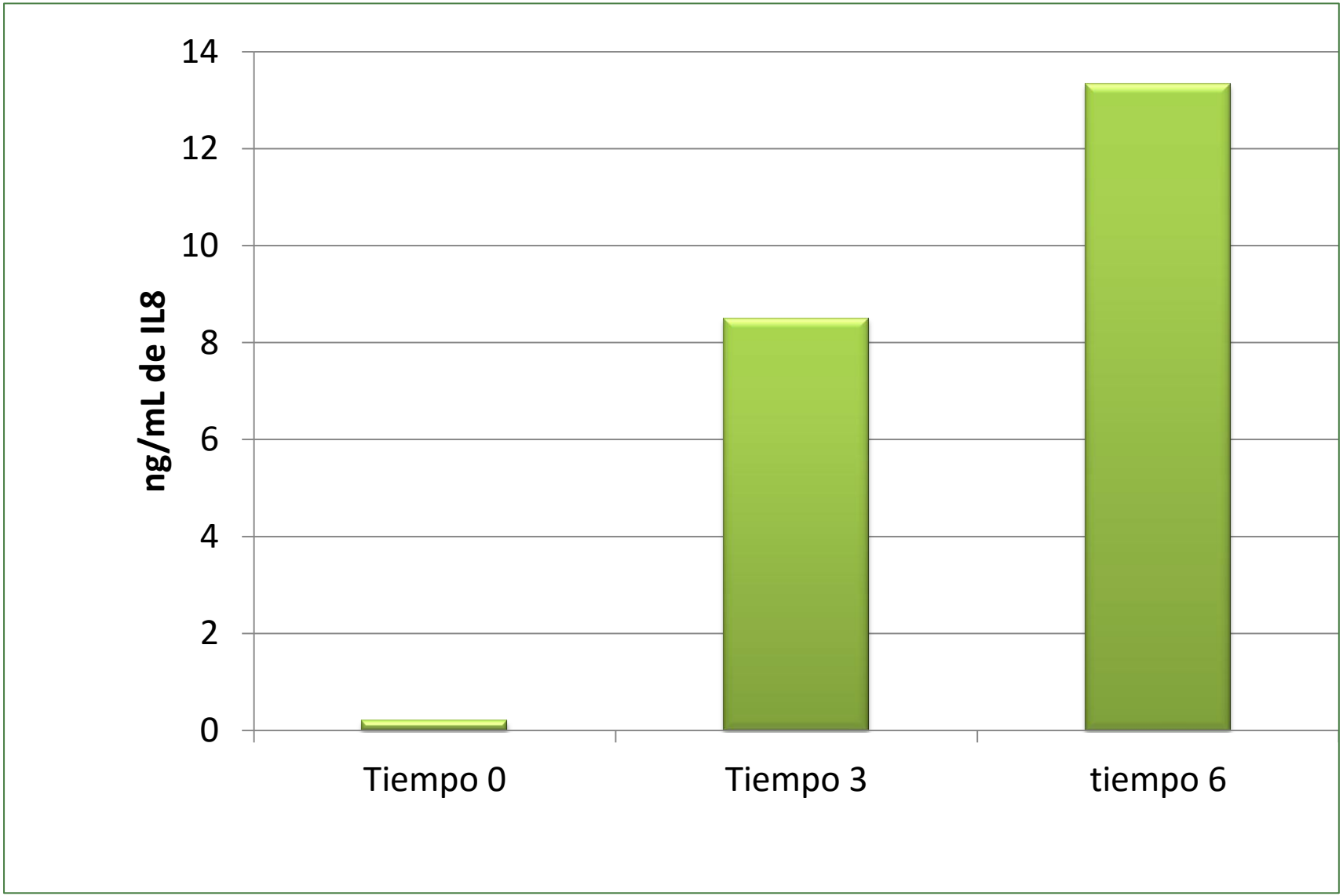
A- IL8 is not an interleukin, but a cytokine discovered as “neutrophil chemotactic factor”. It is also a potent angiogenic factor.

B- A surprising number of genes related with DDR transduction pathway were altered, suggesting early DNA damage on host cells

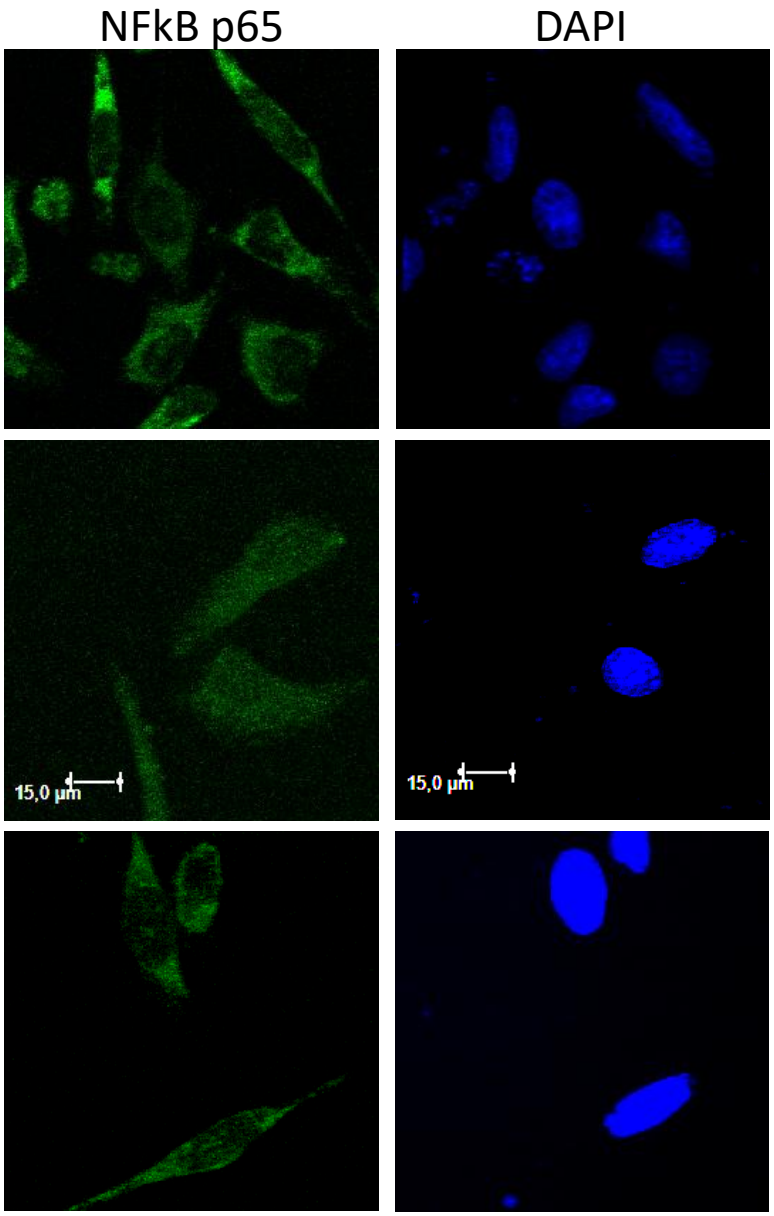
C- IL8 is regulated by several transcription factors, including NFkB

D- It was recently described that DDR activation through ATM phosphorylation activates in turn NFkB

T. cruzi induces IL8 secretion



NFkB is activated in the *T cruzi* early response



Conclusions: epithelial cells

- Lipid metabolism coding genes are altered, firstly at the level of very long chain fatty acids, uptake of cholesterol, phospholipids. Overexpression of COX-2 and LOX

- The most upregulated chemokines (CXCL1, CXCL2, IL8) have similar functions; chemotaxis (recruitment of professional phagocytic cells, in particular neutrophils)

- Interleukin 8 is the most up regulated gene (FC > 300): angiogenesis and recruitment of PMN

- *T. cruzi* infection up regulates DNA Damage Response genes, probably in response to DNA damage

- Alteration of apoptosis and proliferation pathways

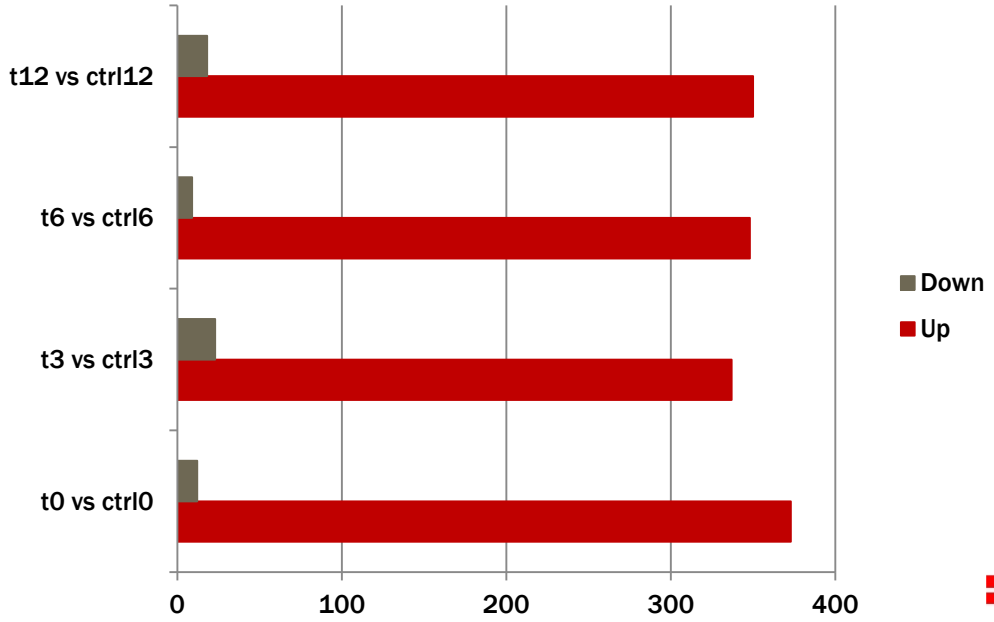
- A group of lncRNAs of unknown function are up regulated, with high fold change values

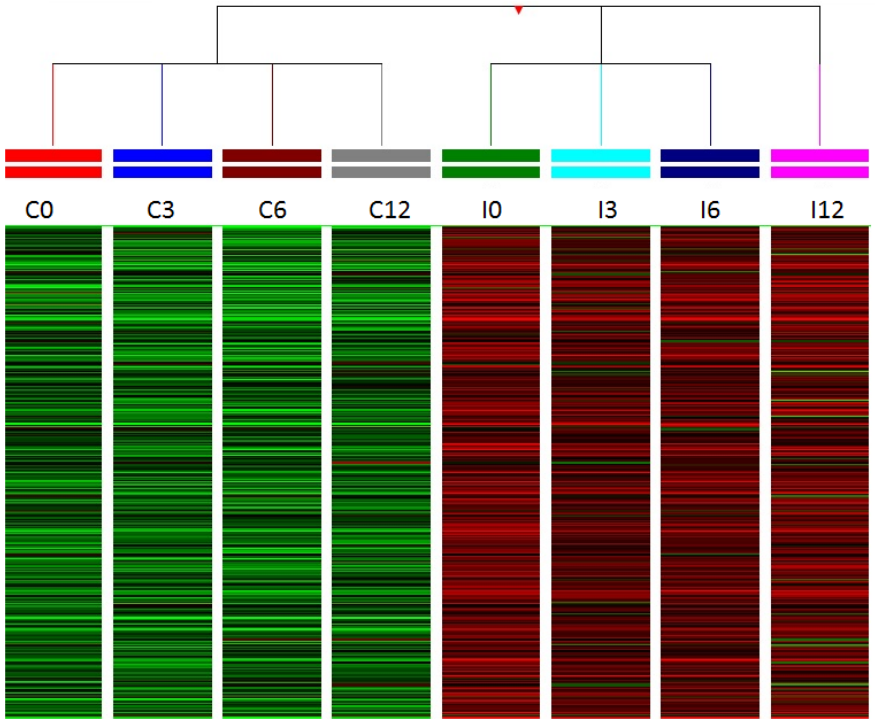
Outline

We aim to know the main changes (genes/pathways/processes) during the early/immediate response to *Trypanosoma cruzi* infection in human cells

- Epithelial cells
- **Cardiomyocytes**
- Macrophages

Cardiomyocytes / mRNA





p≤0.01, Fc≥2

	T=0	T=3	T=6	T=12
Upregulated	373	337	348	350
Downregulated	12	23	9	18
Total	385	360	357	368

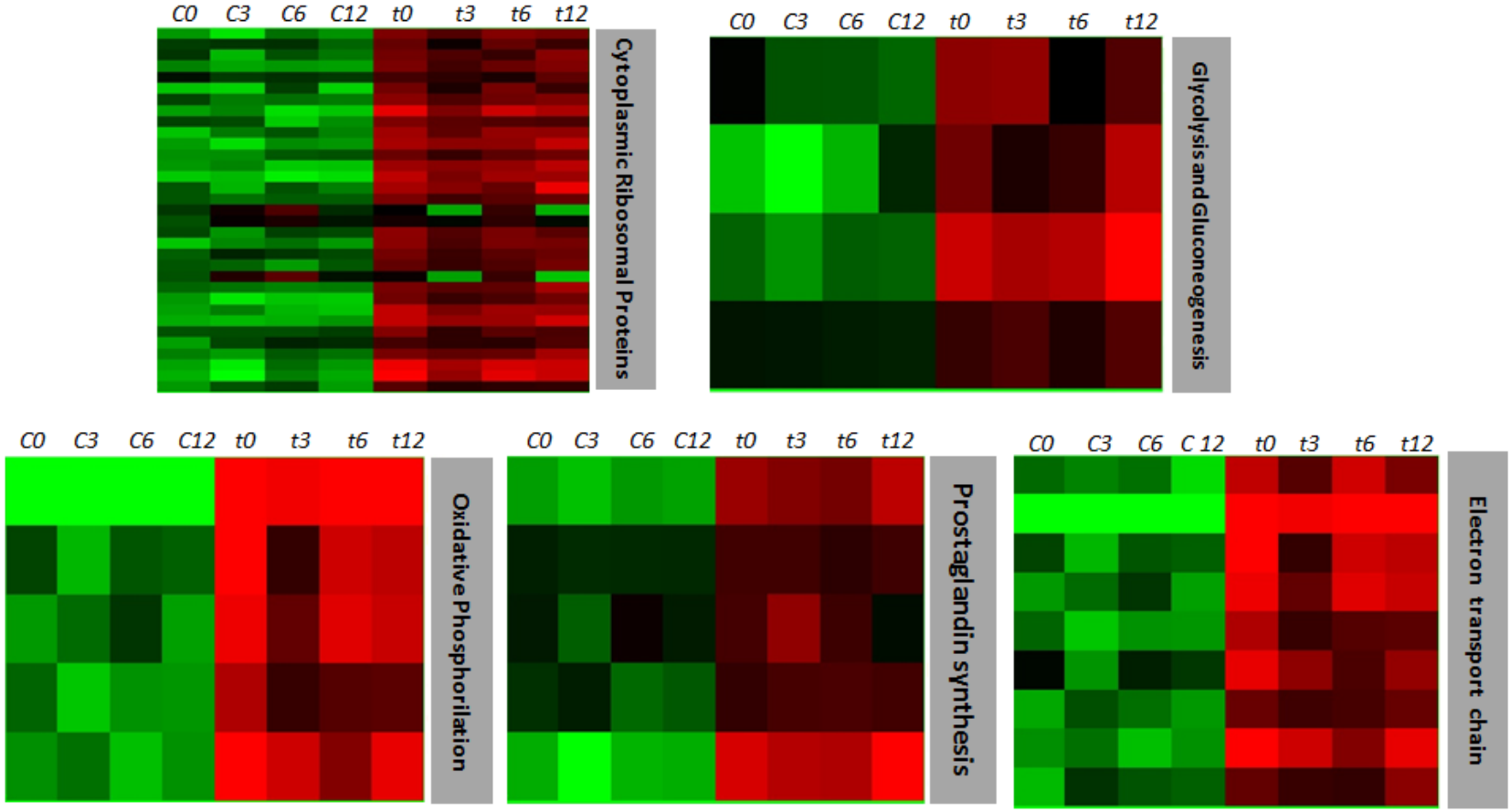
Libisch et al., Front Microbiol. 2018; 9:1889

Cardiomyocytes / mRNA

Pathway	p-value	Matched Entities	Pathway Entities of Experiment Type
Cytoplasmic Ribosomal Proteins	4.7E-10	23	88
Pathogenic escherichia coli infection	8.6E-8	8	64
Electron_Transport_Chain	8.2E-6	8	104
Prostaglandin_Synthesis_and_Regulation	1.3E-5	5	31
Oxidative phosphorylation	3.1E-4	5	62
Proteasome Degradation	4.8E-4	5	65
Glycolysis_and_Gluconeogenesis	0.0012	4	47
cytochrome_P450	0.0025	4	63
Focal_Adhesion_	0.0026	7	188

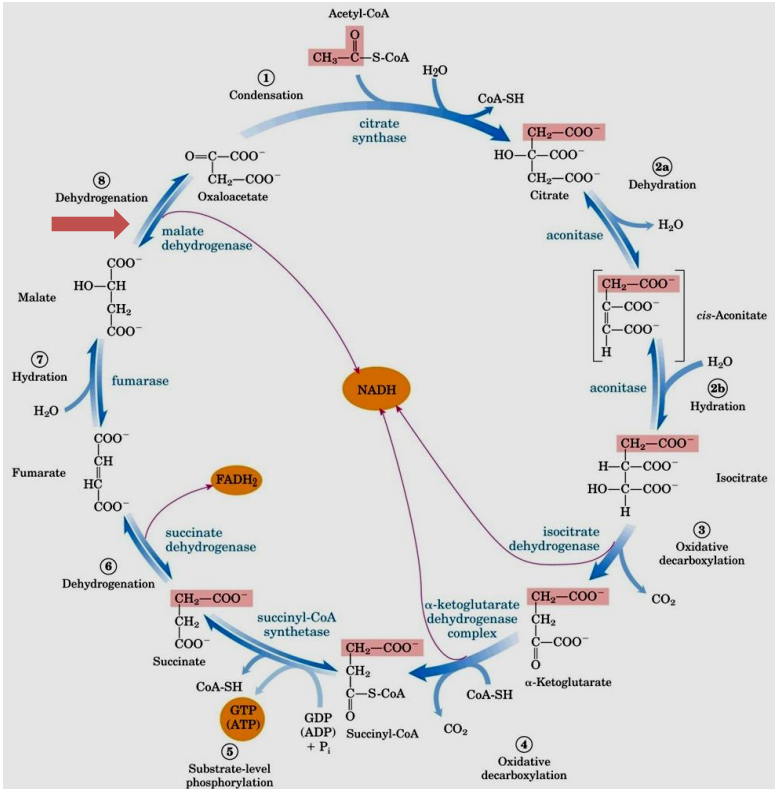
Libisch et al., Front Microbiol. 2018; 9:1889

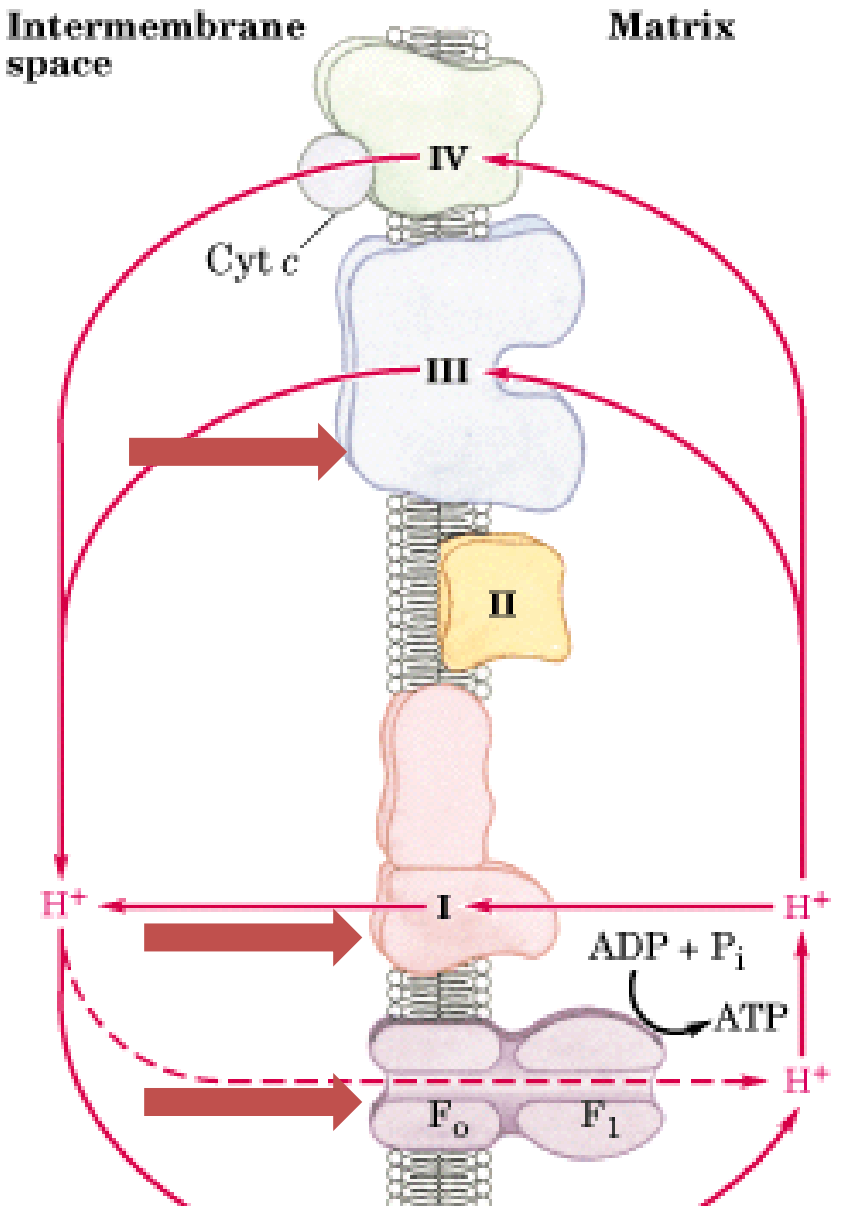
Cardiomyocytes / mRNA



Libisch et al., Front Microbiol. 2018; 9:1889

Cardiomyocytes





Cardiomyocytes / mRNA

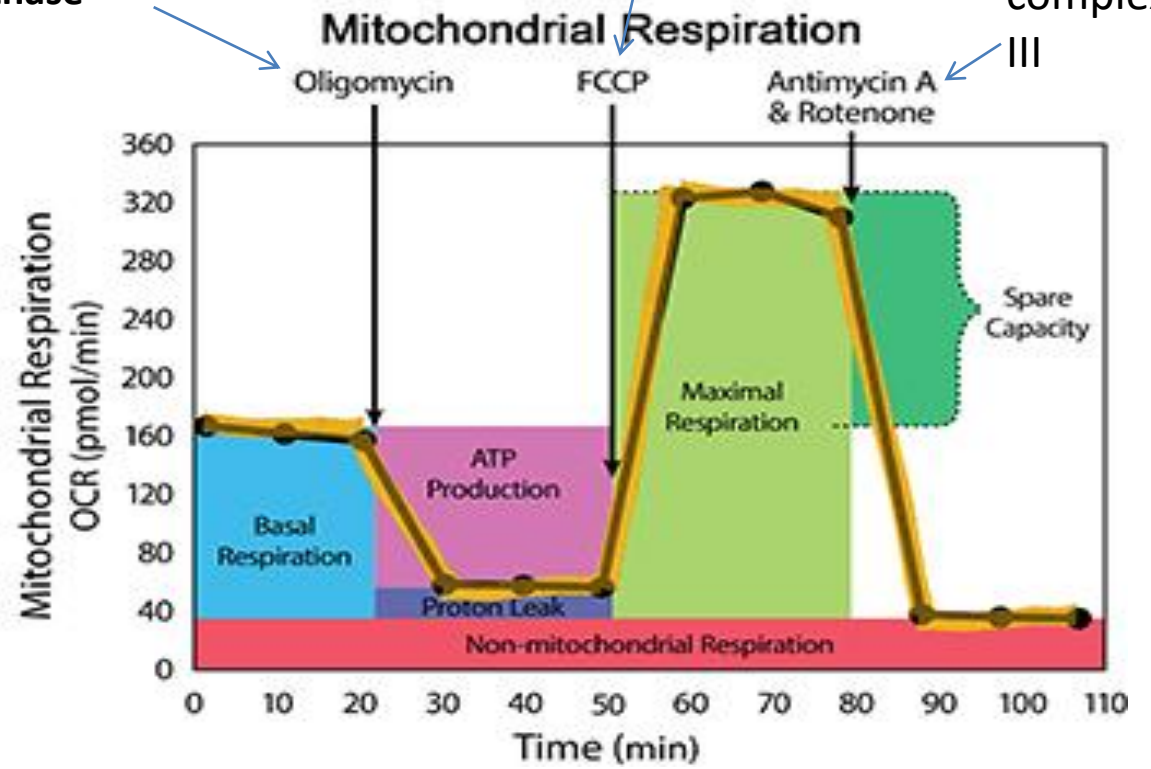
Electron Transport Chain Oxidative Phosphorilation *					
Gene ID	**Description**	**FcT0**	**FcT3**	**FcT6**	**FcT12**
ATP5E*	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit (ATP5E)	40.09	31.41	31.07	24.12
ATP6*	Mitochondrially encoded ATP synthase 6	13.38	6.08	6.16	8.54
COX6C	Cytochrome c oxidase subunit VIc (COX6C)	5.36	3.42	6.13	6.96
NDUFB4*	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 4, 15kDa (NDUFB4)	9.22	3.19	4.79	7.70
NDUFB8*	Homo sapiens NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8, 19kDa (NDUFB8)	4.75	4.19	3.68	3.89
CYTB	Mitochondrially encoded cytochrome b	3.86	5.22	1.81	3.15
UQCR11	Ubiquinol-cytochrome c reductase, complex III subunit XI (UQCR11).	4.66	2.20	2.79	4.20
UQCRFS1	Ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1 (UQCRFS1).	5.01	1.80	2.13	3.74
Glycolysis and Gluconeogenesis					
ALDOC	Aldolase C, fructose-bisphosphate (ALDOC)	2.27	4.95	2.17	4.18
LDHB	Lactate dehydrogenase B (LDHB), transcript variant 1	11.65	12.99	7.45	4.01
MDH2	Malate dehydrogenase 2, NAD (mitochondrial) (MDH2).	7.86	10.21	6.54	10.65
PFKP	Phosphofructokinase, platelet (PFKP), transcript variant 1.	1.61	1.85	1.55	2.14

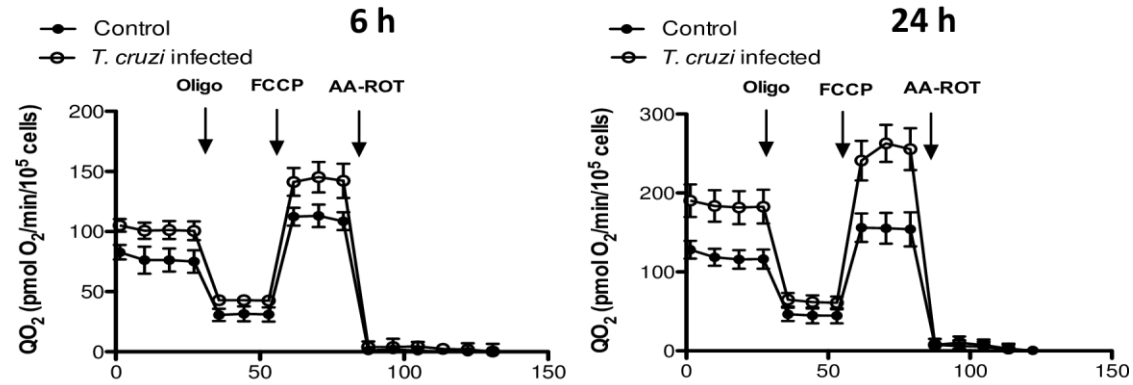
Cardiomyocytes / mRNA

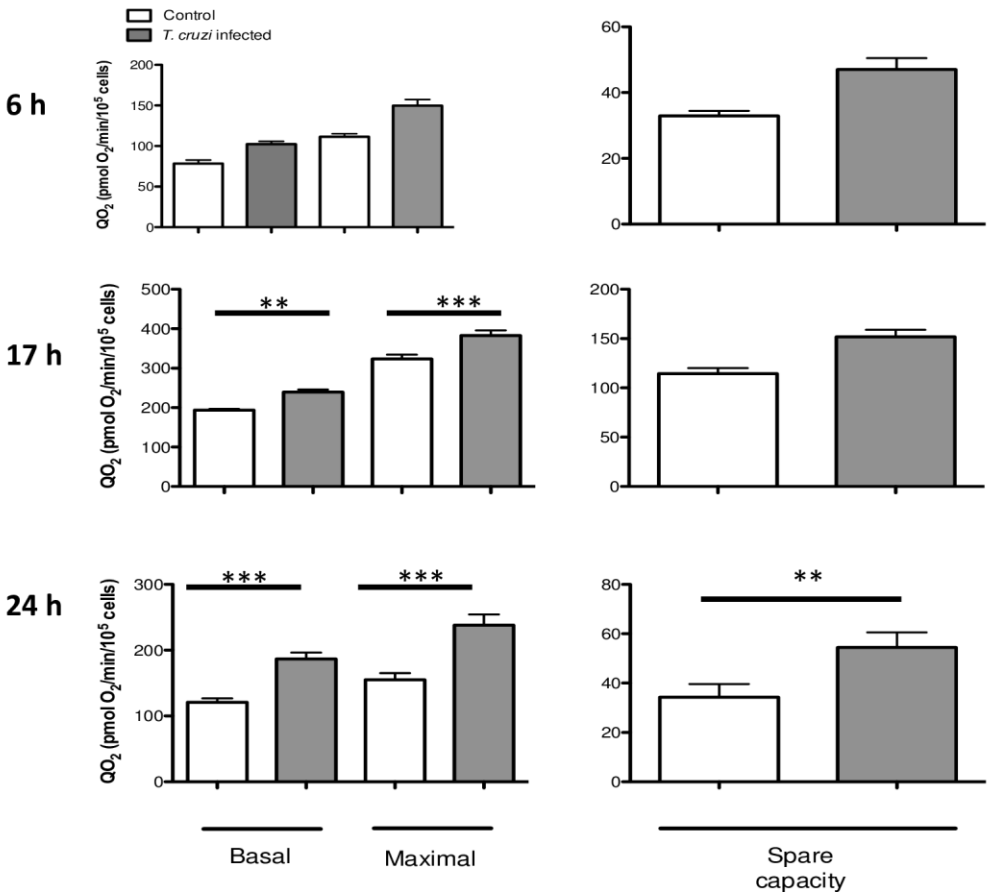
Inhibitor of ATP synthase

Uncoupler of oxidative phosphorylation

Inhibit complex III

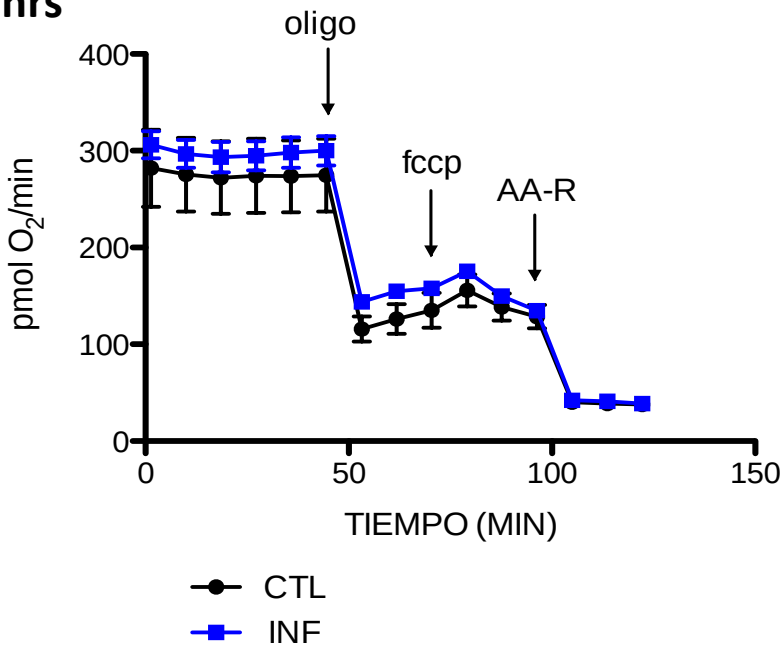


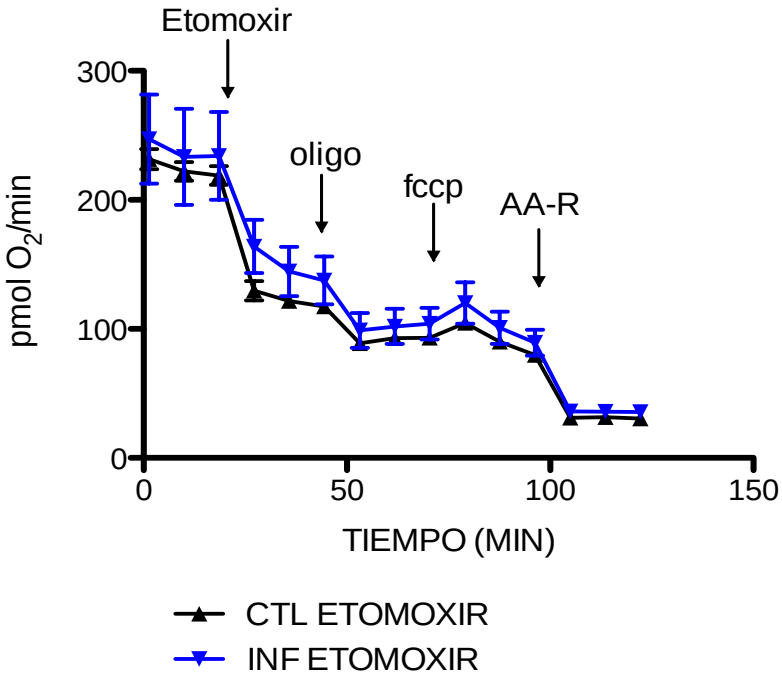




No significant differences in QO_2 were observed between infected and control cells at 6 hpi. However, at 17 hpi, the cardiomyocytes showed an increase observed in basal and maximum respiration, and at 24h, respiratory reserve capacity also increased significantly

HeLa 24 hrs


oligo
fccp
AA-R
400
300
200
100
0
pmol O_2/min
0
50
100
150
TIEMPO (MIN)
CTL
INF


Etomoxir
oligo
fccp
AA-R
300
200
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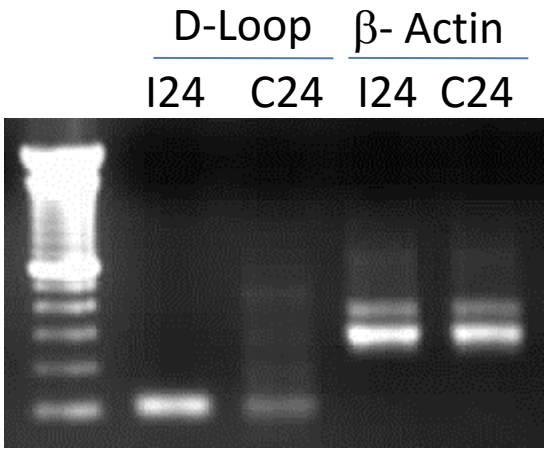
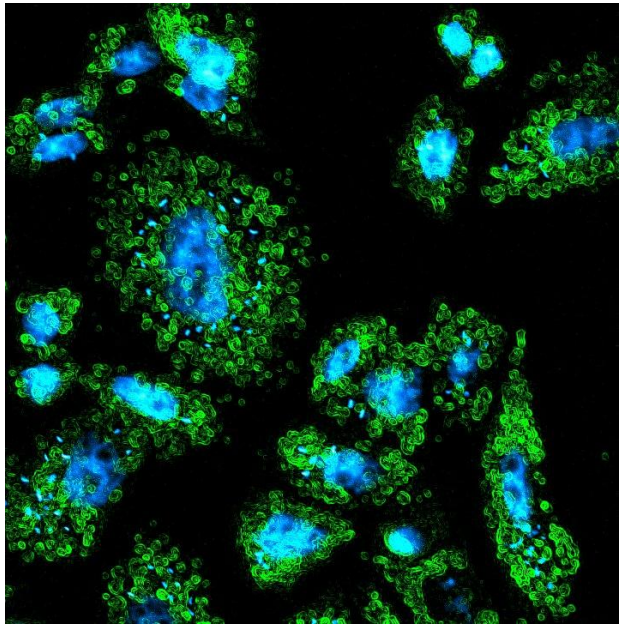
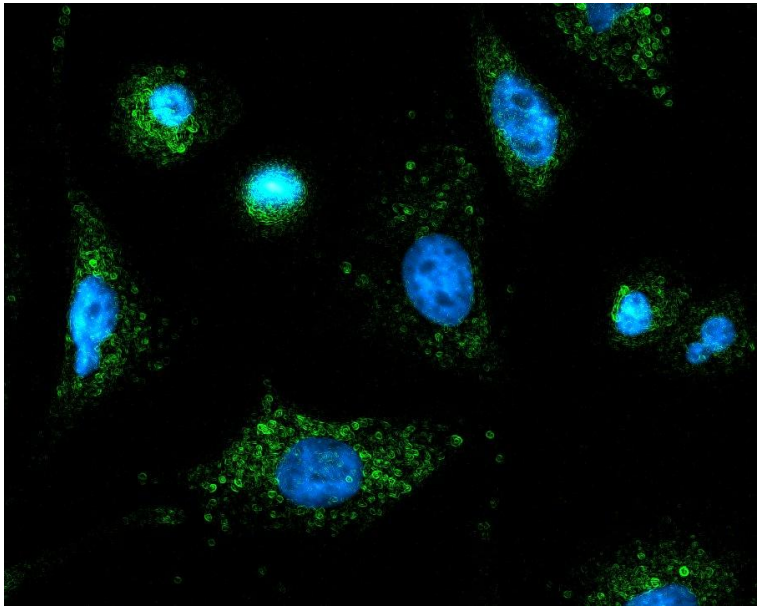
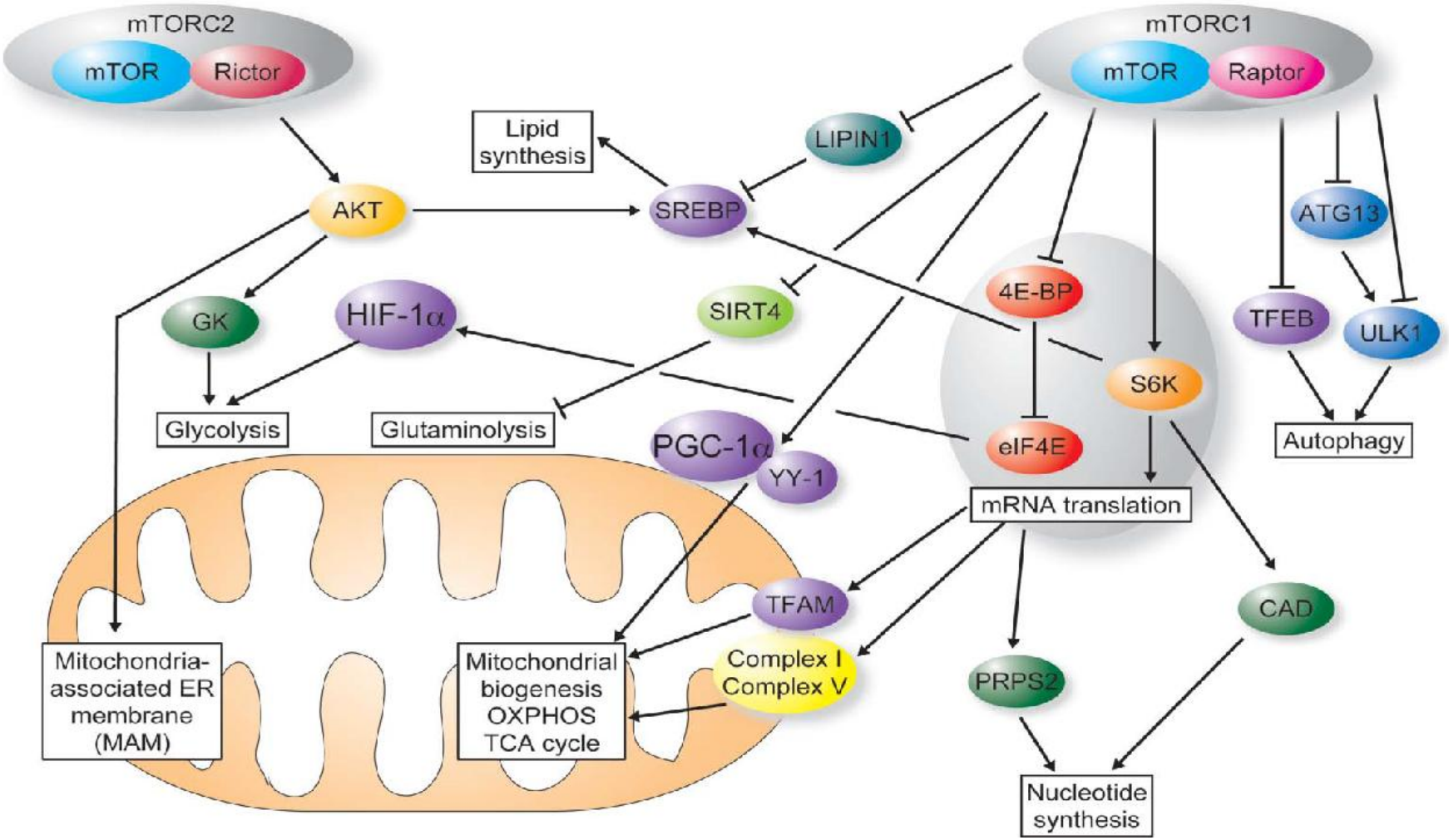
pmol O_2/min
0
50
100
150
TIEMPO (MIN)
CTL ETOMOXIR
INF ETOMOXIR





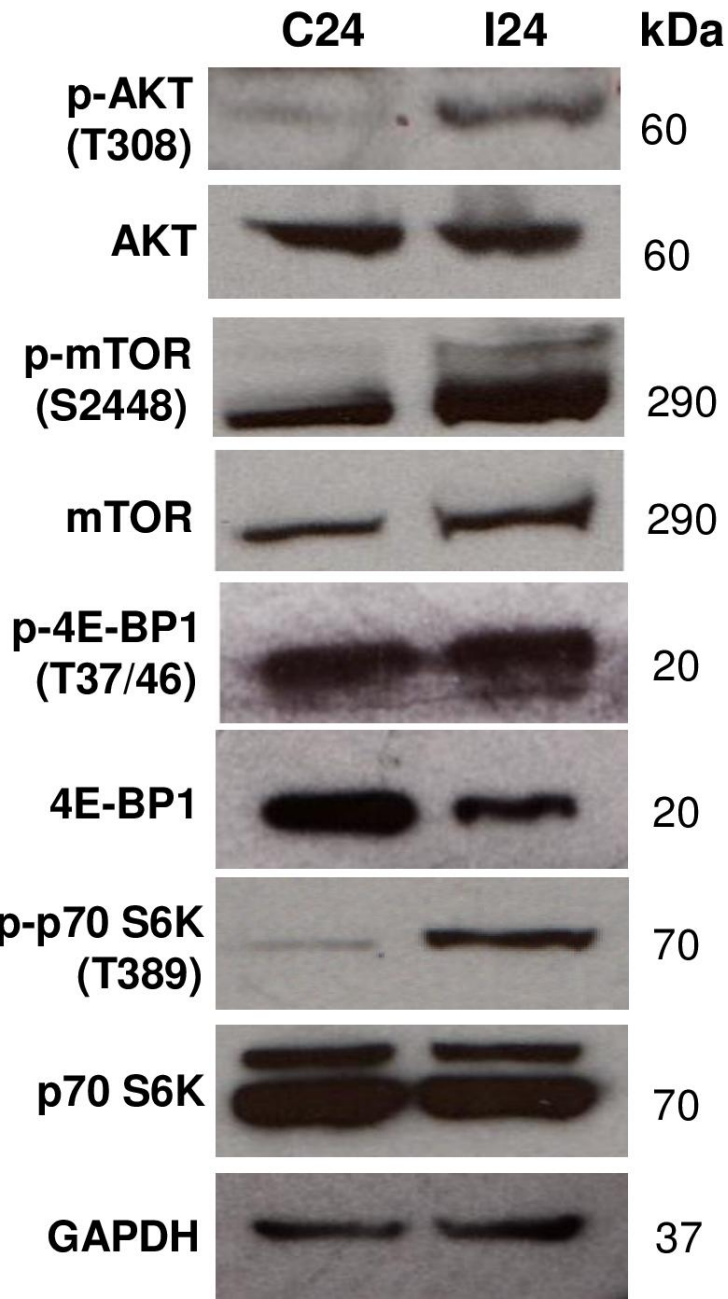


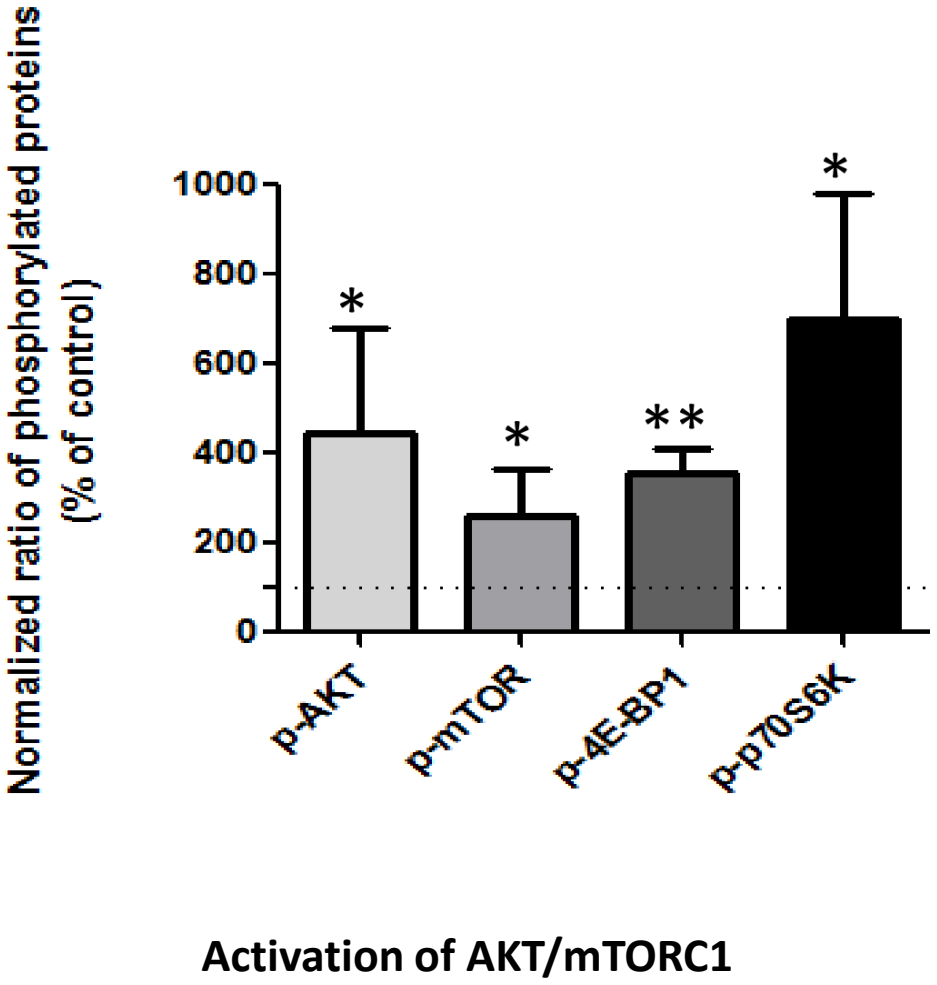
Libisch et al., Front Microbiol. 2018; 9:1889



Morita et al. Cell Cycle, 2015.

AKT / mTORC1

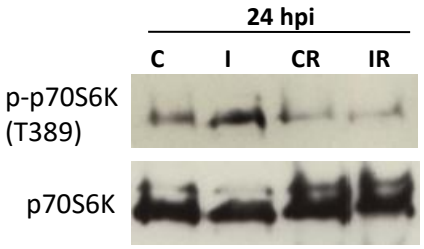


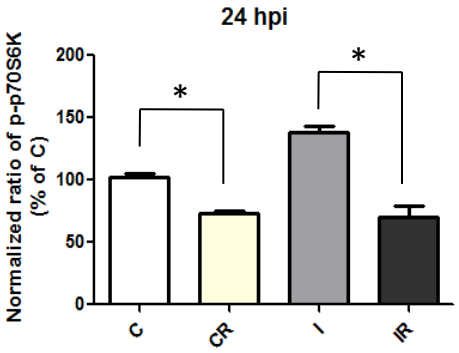


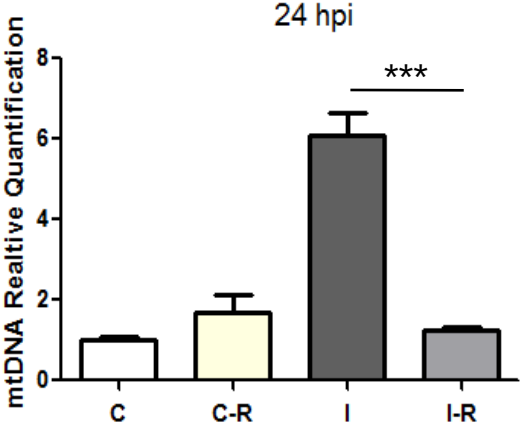
Activation of AKT/mTORC1

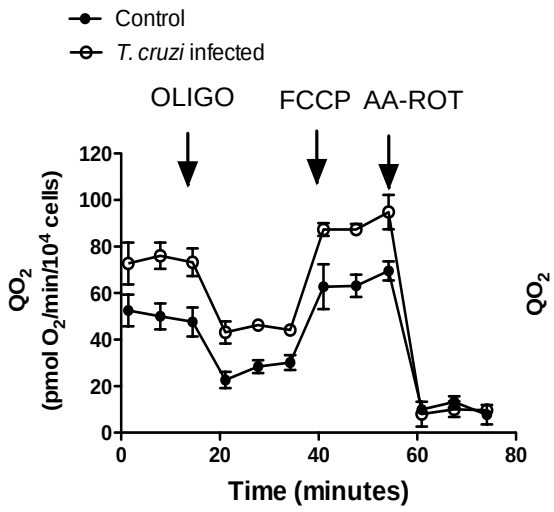
Libisch et al., Front Microbiol. 2018; 9:1889

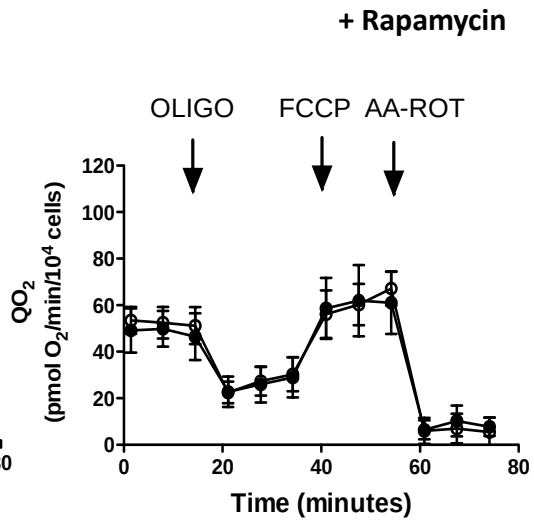
Rapamycin

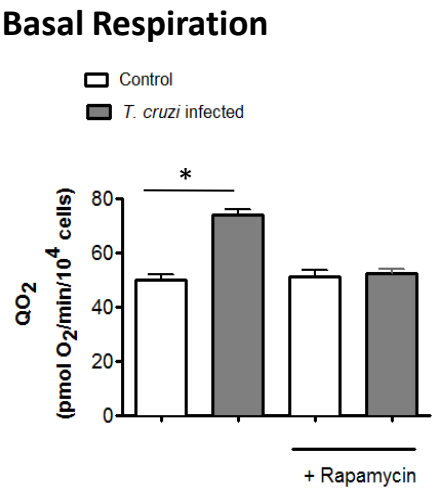


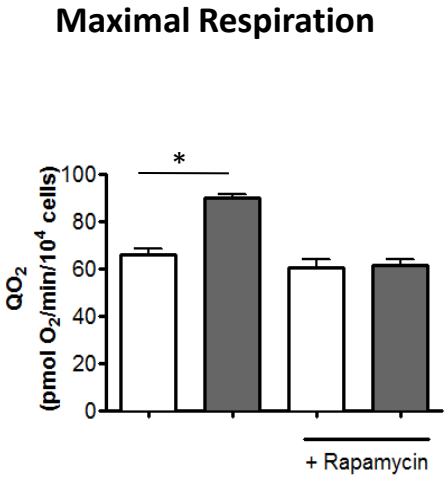












Libisch et al., Front Microbiol. 2018; 9:1889

Conclusions: Cardiomyocytes

- The early response to *T. cruzi* implies significant expression changes on hundred of genes, mainly related to **energy metabolism** and **protein synthesis**

- At the phenotypic level a) infected cells immediately increase their **basal** an **maximal respiratory** rates, and the **spare respiratory capacity** in the fist 24 hours of infection, related to b) **mitochondrial biogenesis**

- Increase in respiratory chain generate ROS that can explain the donuts shape of mitochondria

- Activation of mTORC1 through AKT

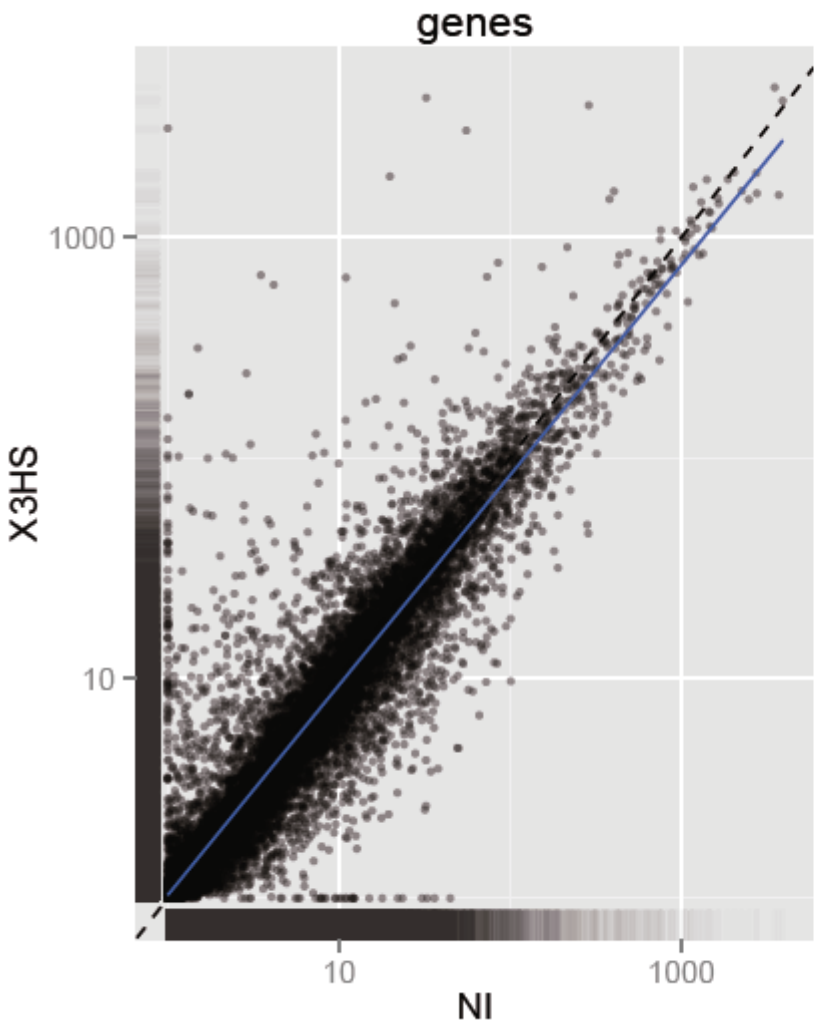
- These phenotypic changes resemble molecular mechanisms OF hypertrophic cardiomyopathy

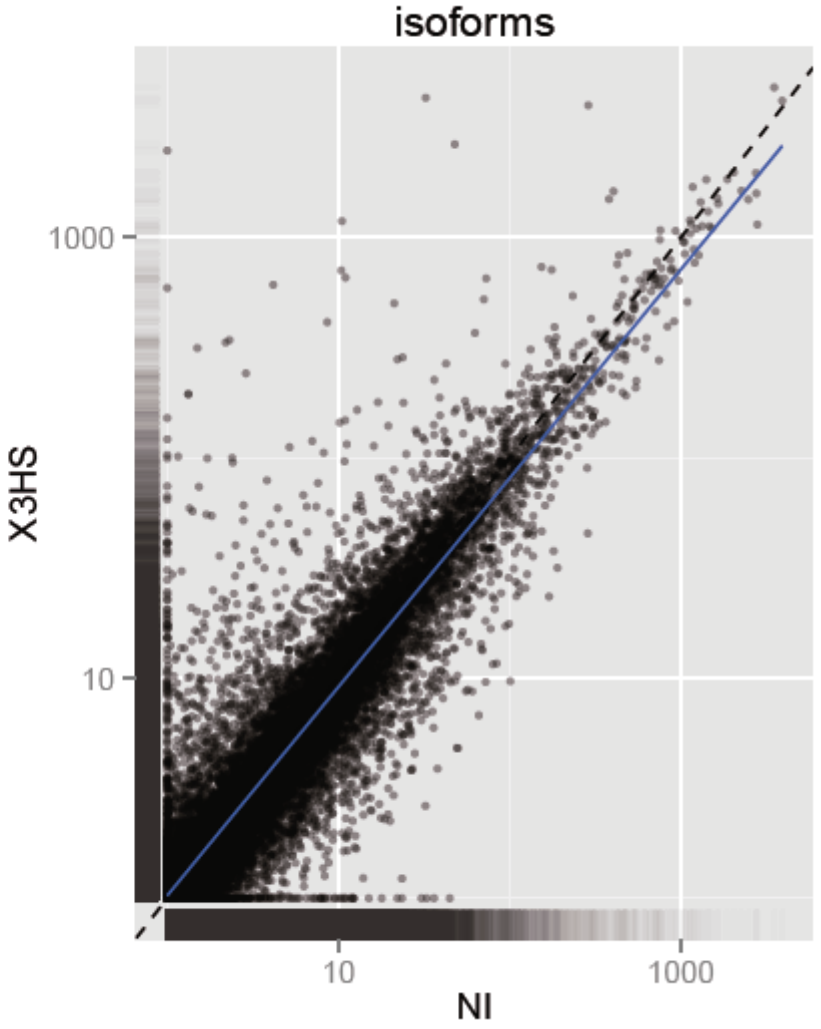
Outline

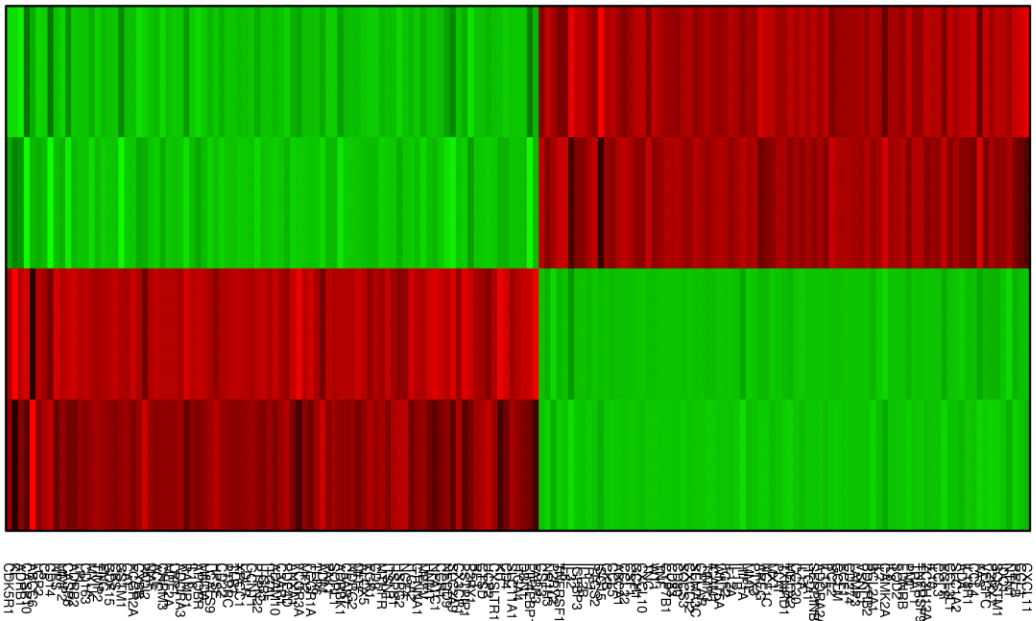
We aim to know the main changes (genes/pathways/processes) during the early/immediate response to *Trypanosoma cruzi* infection in human cells

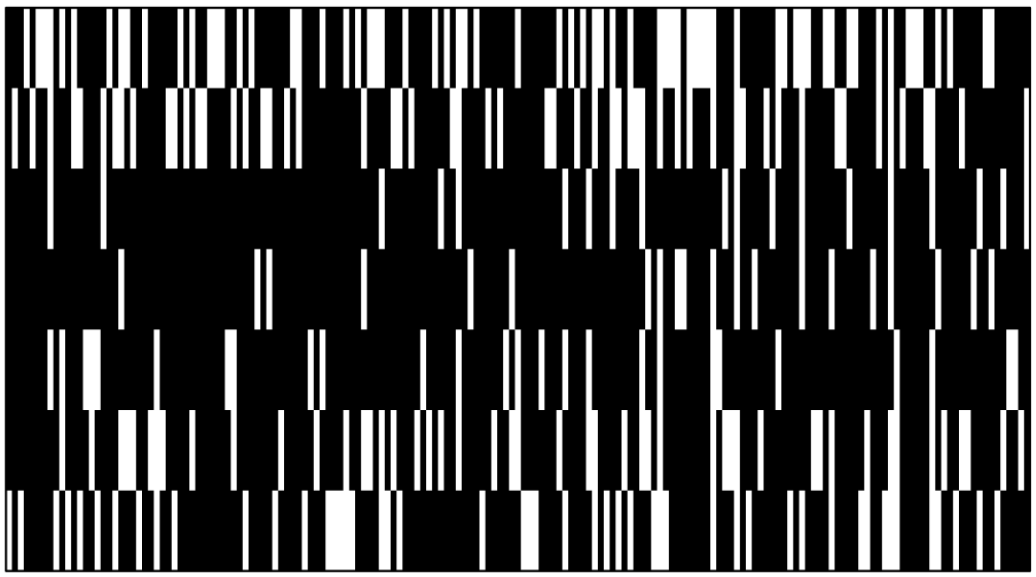
- Epithelial cells
- Cardiomyocytes
- **Macrophages**

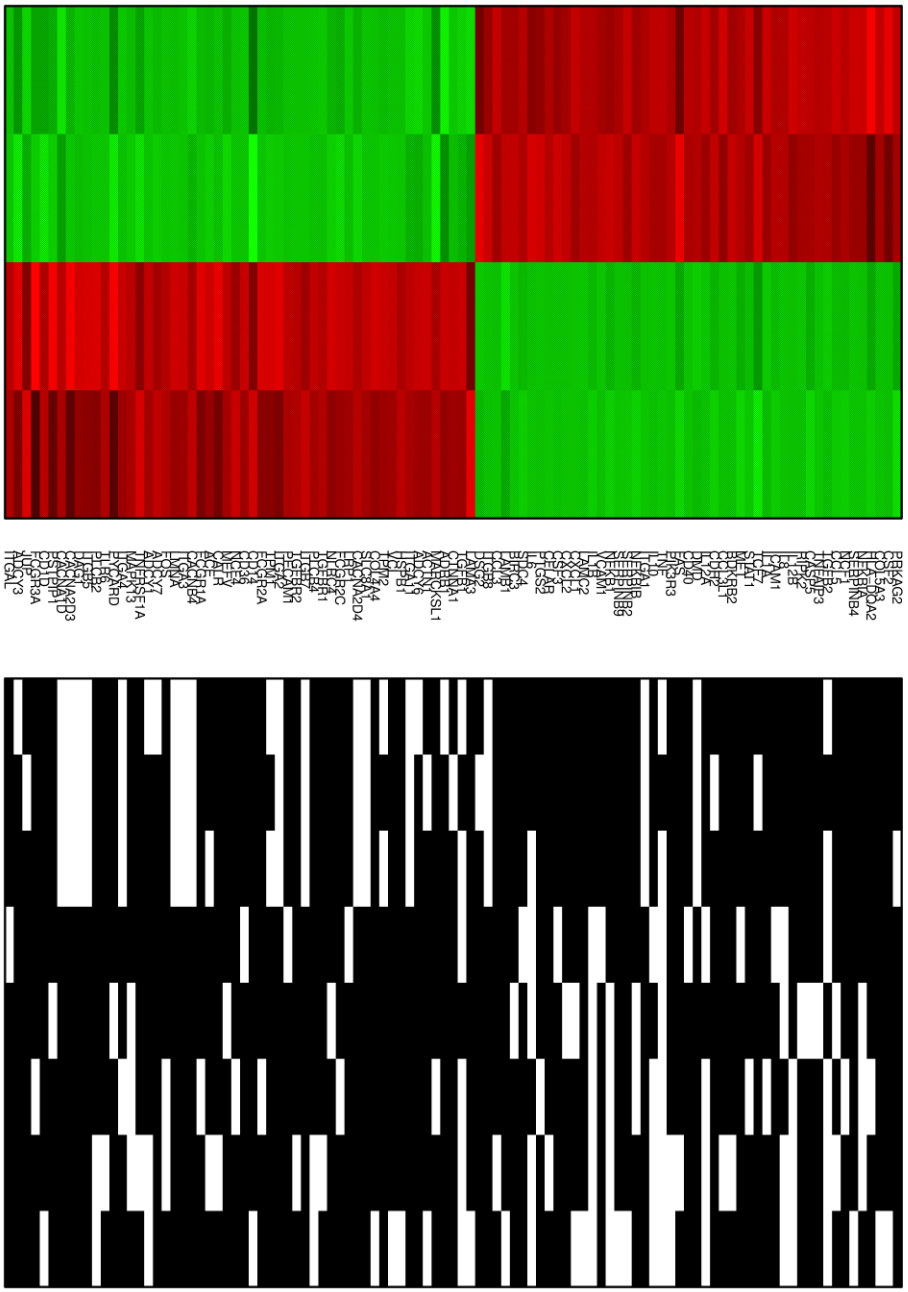
By RNA-seq we could identify also differential gene splicing as an effect of infection






X3HS_0
X3HS_1
NI_0
NI_1


Rheumatoid arthritis
Atherosclerosis
Hepatitis C
Tuberculosis
Systemic infection
Asthma
Alzheimer's disease


X3HS_0
X3HS_1
NI_0
NI_1
Dilated cardiomyopathy
Arrhythmogenic right ventricul...
Hypertrophic cardiomyopathy (H...
Malaria
NOD−like receptor signaling pa...
Leishmaniasis
Chagas disease (American trypa...
Amoebiasis

Conclusions: Macrophages

T. cruzi induce drastic changes not only at the gene expression level but also alters alternative splicing patterns of genes related to apoptosis, immune response, autoimmune and infectious diseases

General Conclusion

- *Trypanosoma cruzi* induces cellular reprogramming through changes in gene expression patterns.

- Each cell type has different responses, and this phenomena is probably related to dissemination and persistence of the infection

- The study of these responses can give clues on new strategies of treatment of Chagas Disease

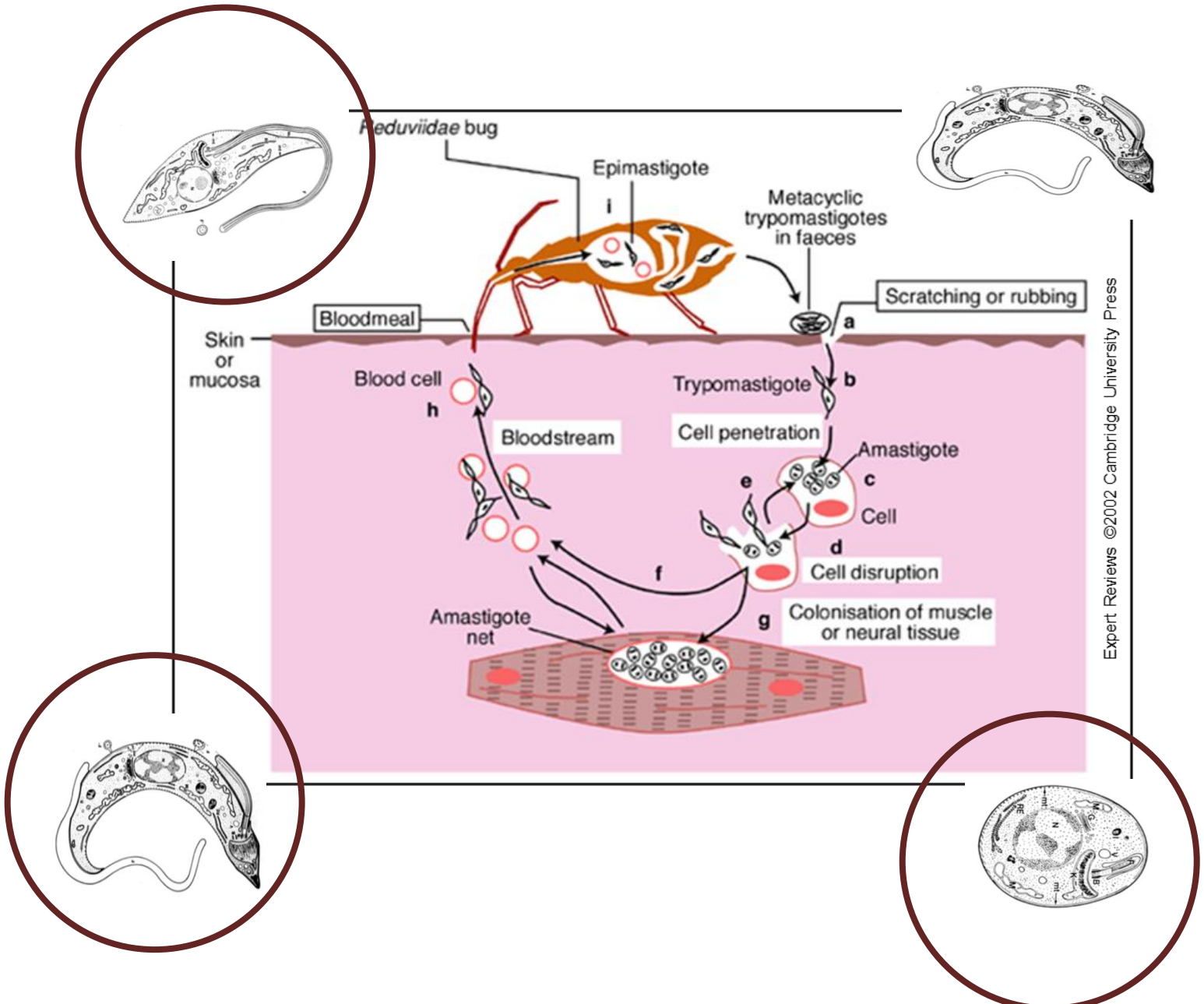
Is there a common pathway?

The PI3K/ AKT pathway is a common response to *T. cruzi* infection

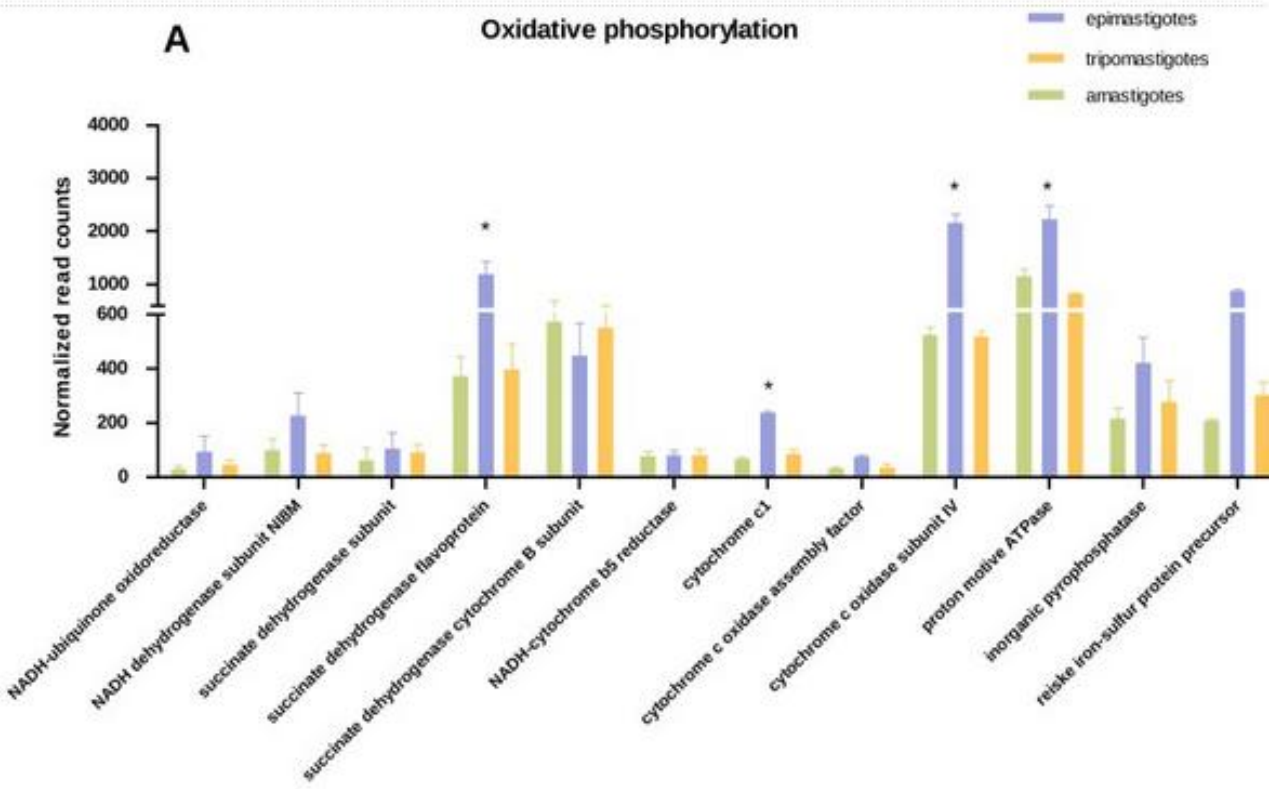
Different drugs targeting PI3K have been developed and employed in clinical trials evaluations, bringing the option of considering them as repurposed drugs for host-directed therapies in Chagas and others infectious diseases

Drug	taget
Rapamicina	mTORC1 Inhibition
Metformina	complex I Inhibition
Imatinib	BCR/Abl Inhibition
Galloflavin	LDHA/LDHB Inhibition
Atovaquone	complex III Inhibition (plasmodium)
Visnagin	MDH2 Inhibition
AICAR	mTORC1 Inhibition
Curcuma	Glycolysis ? Respiration?

The parasite also readjusts its gene expression during the life cycle

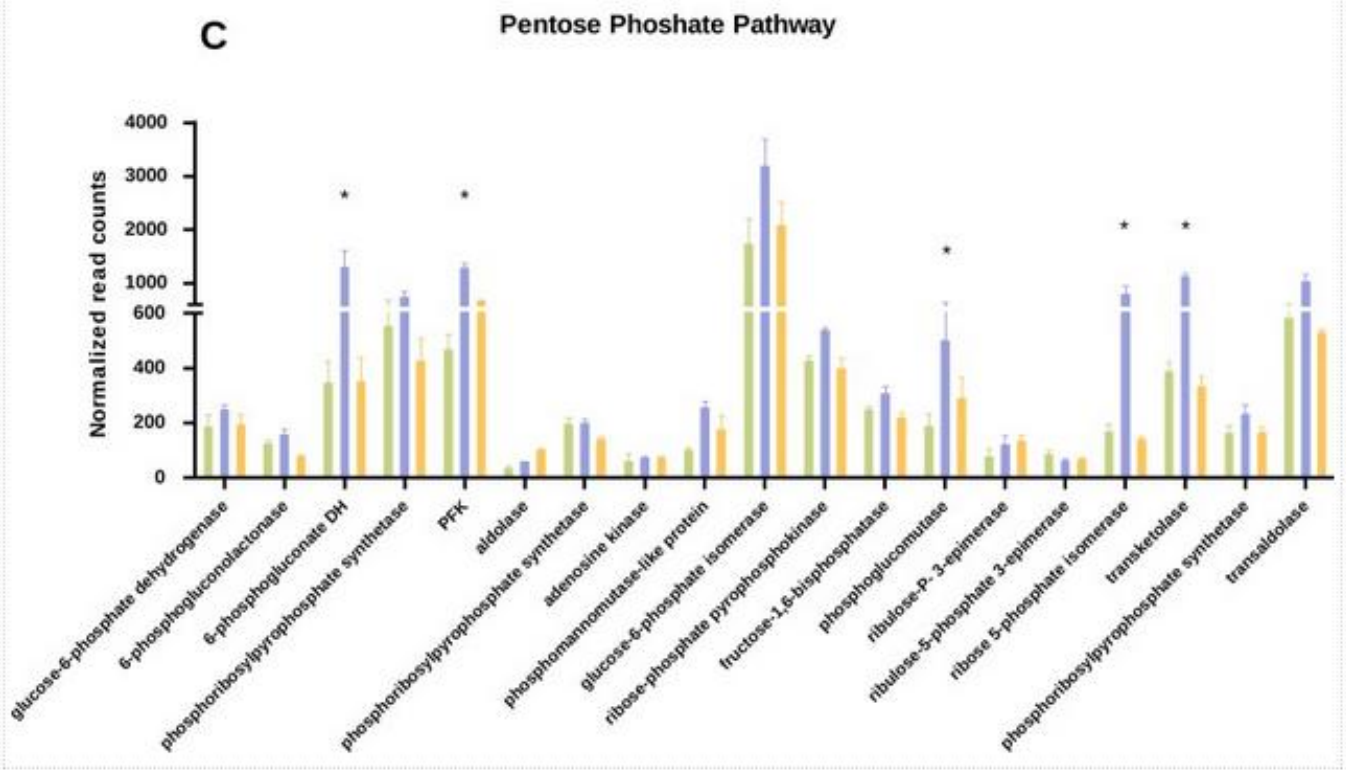


Gene Expression



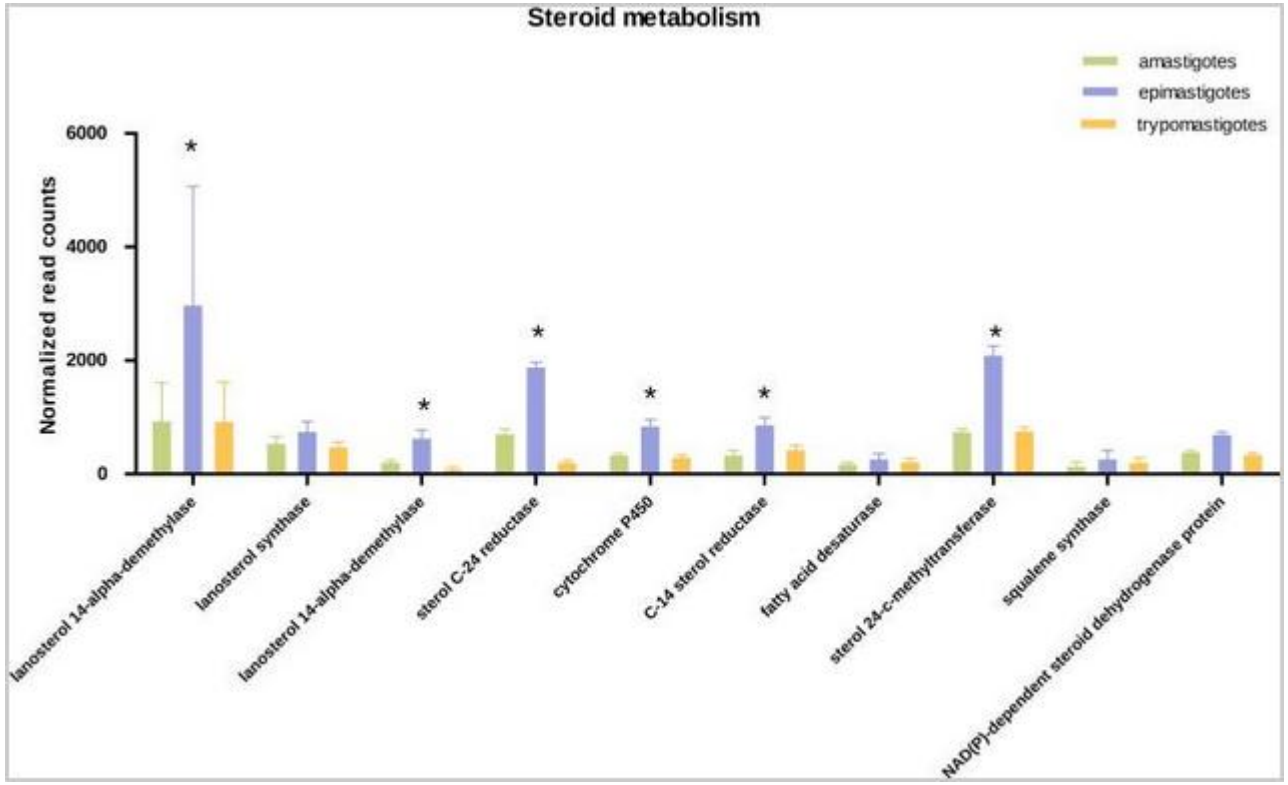
Berná L, Chiribao ML y col. PeerJ. 2017;5,

Gene Expression



Berná L, Chiribao ML y col. PeerJ. 2017;5,

Gene Expression



Berná L, Chiribao ML y col. PeerJ. 2017;5,

Gene Expression

PeerJ

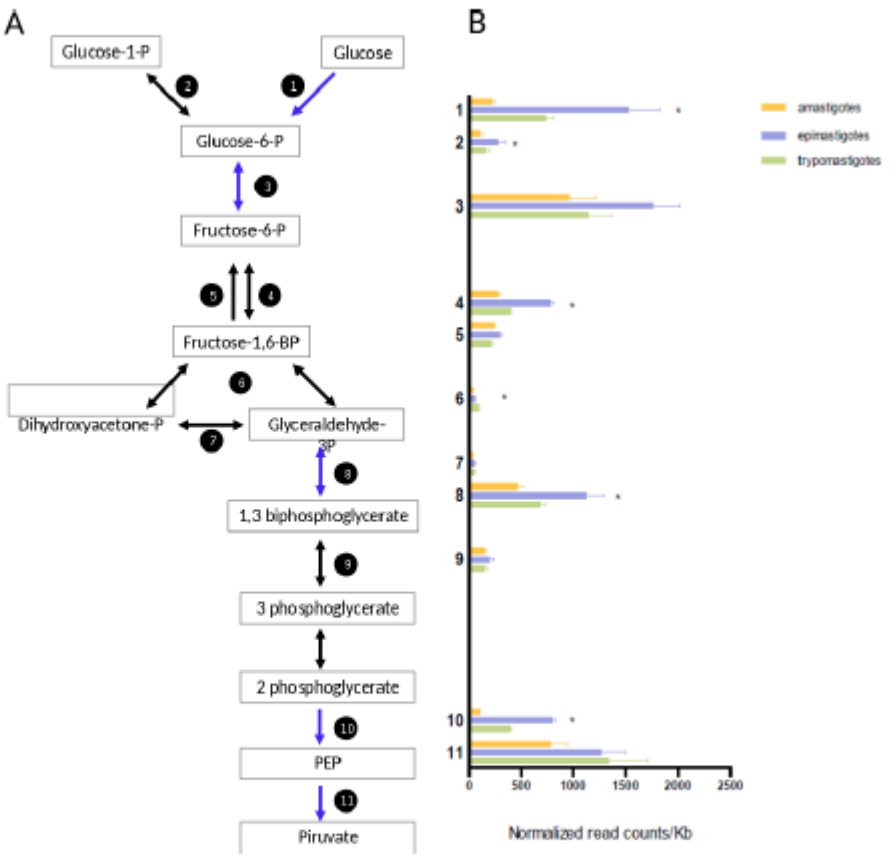
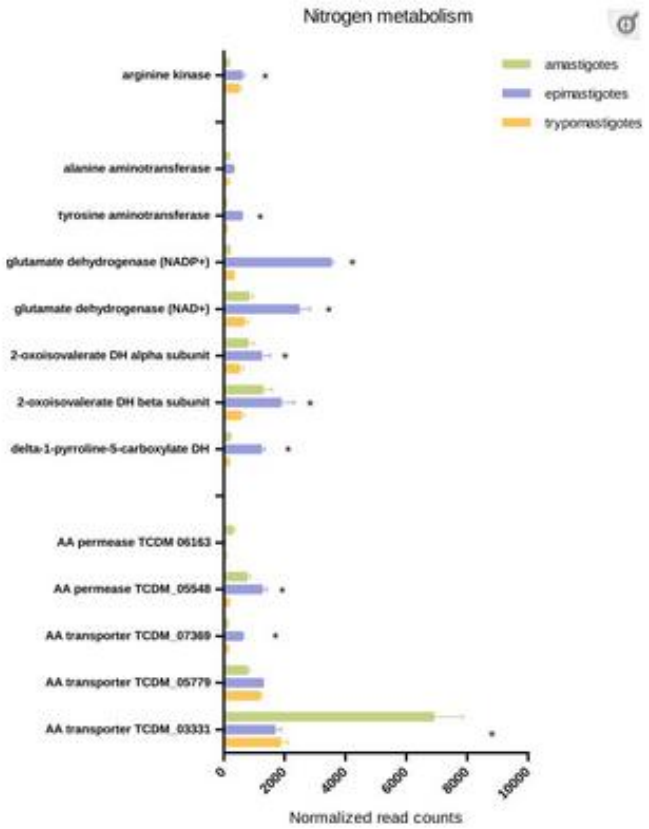


Figure 5 Glucose metabolism. (A) Schematic diagram of glucose catabolism. Each reaction is assigned with a number (1: hexokinase, 2: phosphoglucomutase, 3: glucose-6-phosphate isomerase, 4: phosphofructokinase, 5: fructose-1,6-biphosphatase, 6: aldolase, 7: triosephosphate isomerase, 8: glyceraldehyde 3-phosphate dehydrogenase, 9: phosphoglycerate kinase, 10: enolase and 10: pyruvate kinase 2). (B) Expression of glucose metabolism genes of each reaction is shown as normalized count per gene size in kilobases. The three cycle stages are represented: amastigotes (green), epimastigotes (blue) and trypomastigotes (orange). (*) Denotes differentially expressed genes.

Berná L, Chiribao ML y col. PeerJ. 2017;5,

Gene Expression



Berná L, Chiribao ML y col. PeerJ. 2017;5,

Gene Expression

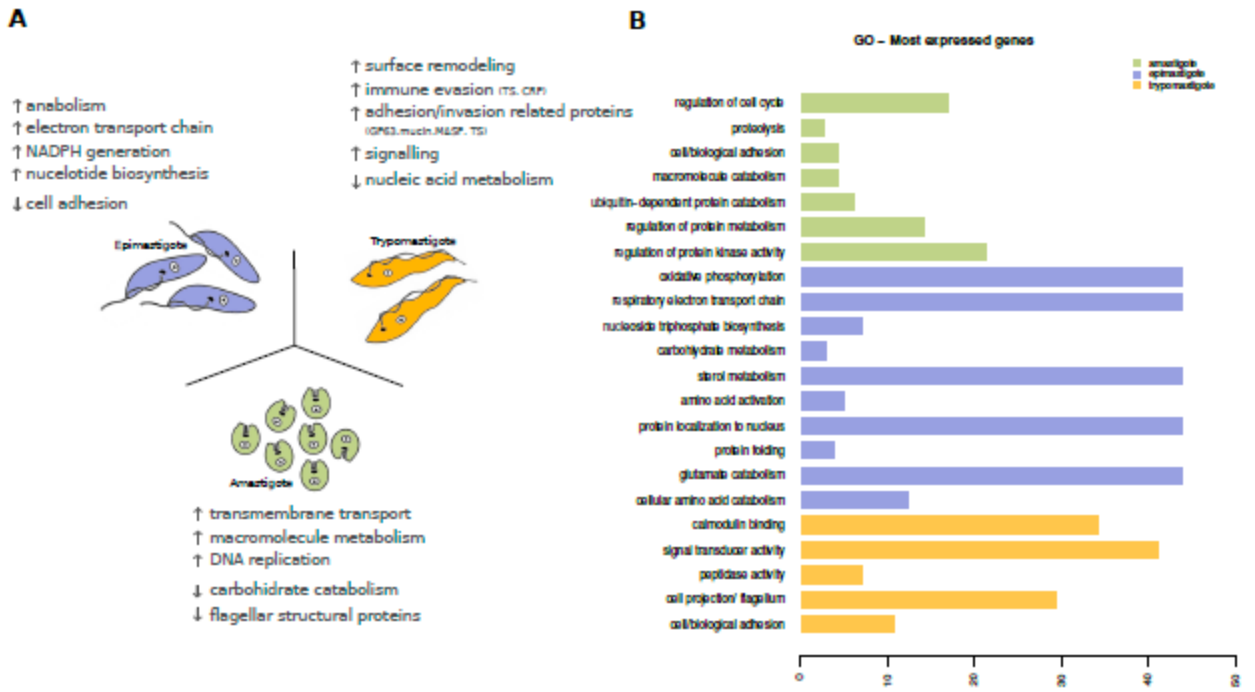
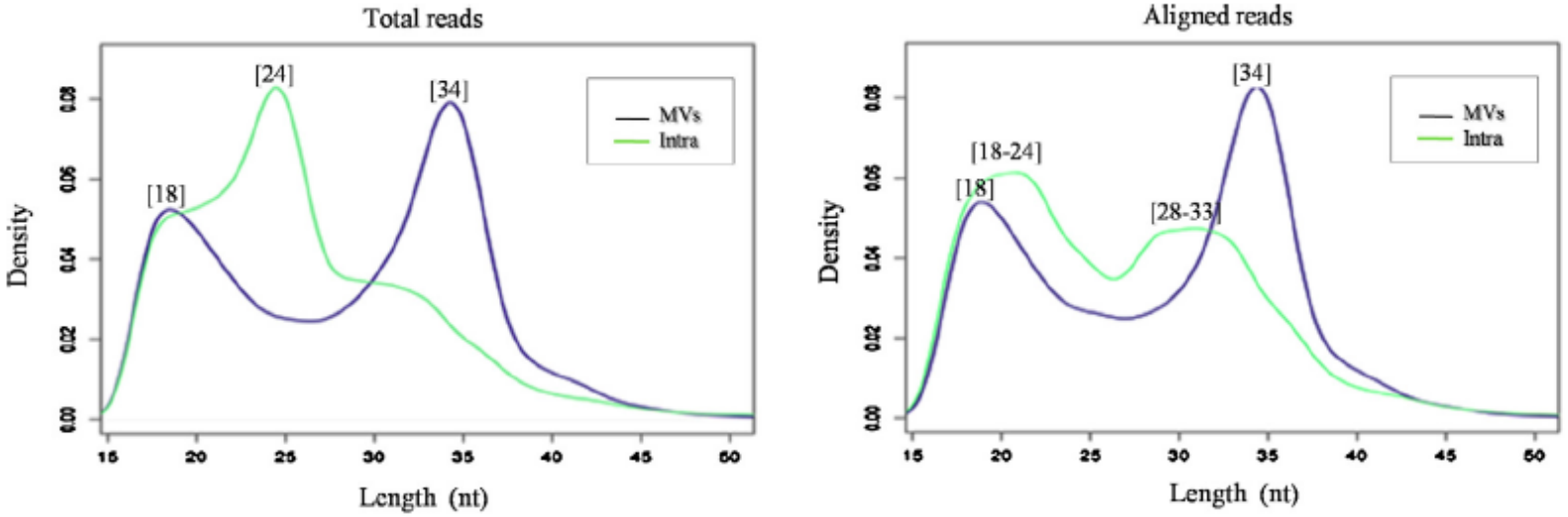


Figure 8 Expression levels overview of Trypanosoma cruzi. (A) Diagram of the *Trypanosoma cruzi* stages and the major findings of transcriptoma analysis. (B) Gene ontology (GO) enrichment analysis, showing GO terms exhibiting statistical significant differences (Fisher Exact Test, filtering *p*-values for multiple testing using False Discovery Rate) for the most expressed genes specific to amastigote (green), epimastigote (blue) and trypomastigote (orange).

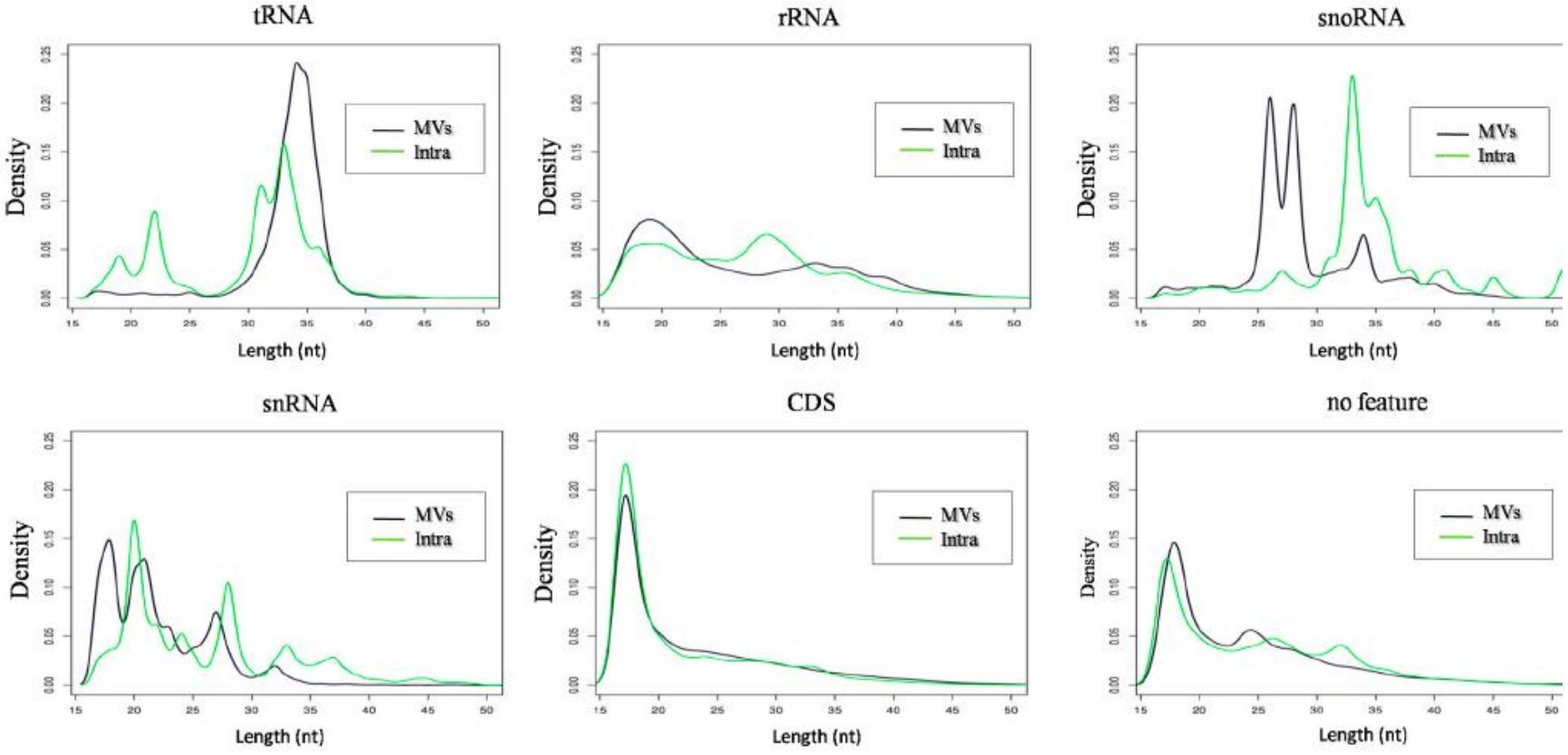
Berná L, Chiribao ML y col. PeerJ. 2017;5,

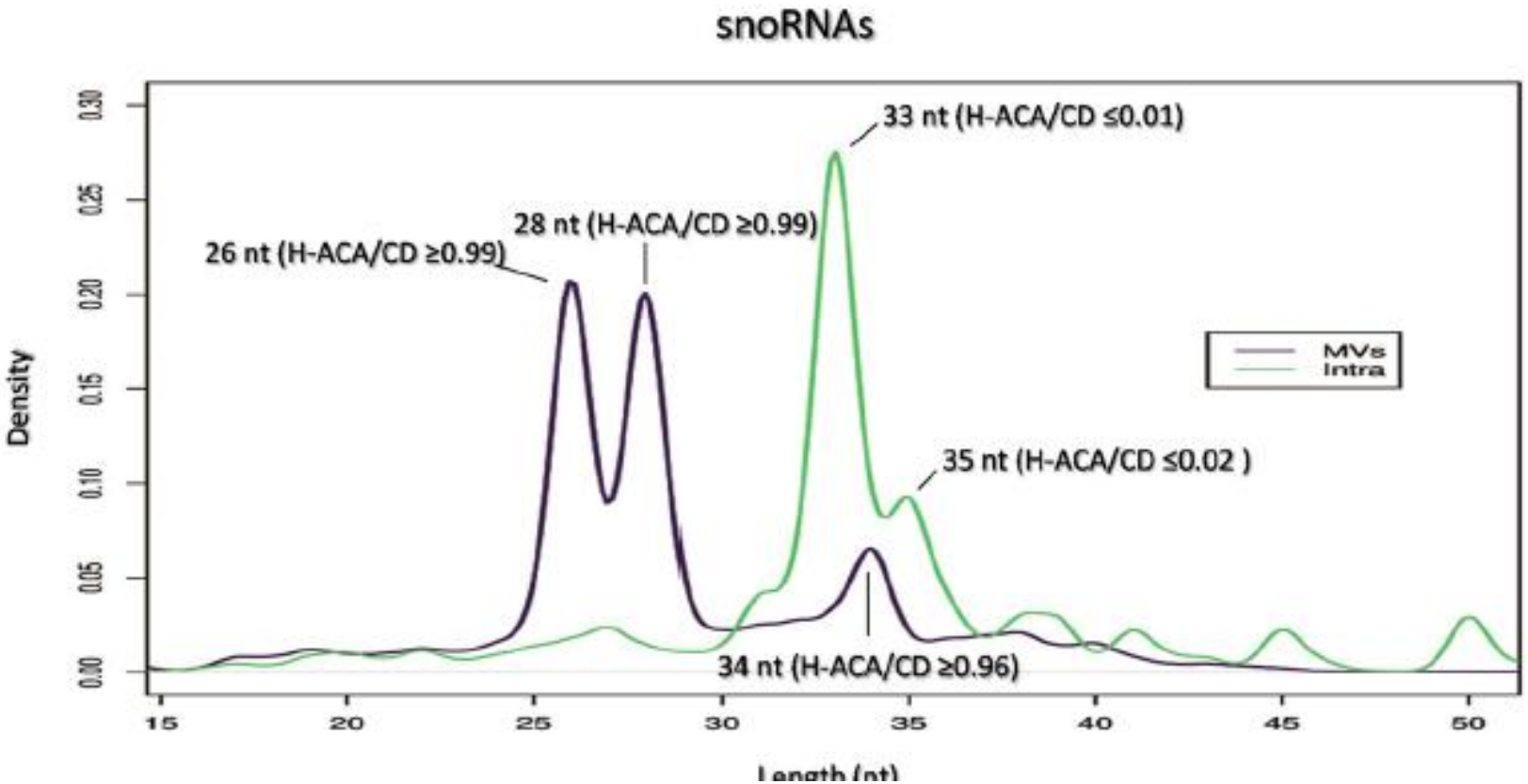
The parasite also readjusts its gene expression during the life cycle

Small RNAs



	Total reads	Aligned reads		Single mappers	
		Reads (% of total)	Unique sequences	Reads (% of aligned)	Unique sequences
MVs	11,851,968	10,107,119 (85)	684,211	6,100,251 (60)	435,131
Intra	4,413,742	2,385,885 (54)	212,383	1,402,678 (59)	131,409


tRNA
rRNA
snoRNA
snRNA
CDS
no feature
MVs
Intra
Density
Length (nt)


snoRNAs
33 nt (H-ACA/CD ≤0.01)
28 nt (H-ACA/CD ≥0.99)
26 nt (H-ACA/CD ≥0.99)
MVs
Intra
35 nt (H-ACA/CD ≤0.02)
34 nt (H-ACA/CD ≥0.96)
Density
0.30
0.25
0.20
0.15
0.10
0.05
0.00
15
20
25
30
35
40
45
50
Length (nt)

Transcriptional Studies on *Trypanosoma cruzi* – Host Cell Interactions: A Complex Puzzle of Variables

María Gabriela Libisch[1], *Natalia Rego*[2] *and Carlos Robello*[1,3*]

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TABLE 2 | Transcriptomic and functional results from different studies related to the host cell respiration response to *T. cruzi* infection.

N	Strain/DTU	PM	Infection model	MOI	IT	AT	Methodology	Effects on cellular respiration (reference, year)
***In vitro* transcriptomic experiments**								
1	Brazil/TcI	LIIS9	Cardiomyocytes from neonatal mice (C57BL/6)	5:1	24h	48hpi	Microarrays (Custom)	Repression of some OXPHOS related genes (Goldenberg et al., 2009)
2	Dm28c/TcI	Vero	Primary mouse cardiomyocytes from embryos	10:1	6h	1 to 48hpi	Microarrays (Affymetrix)	No significant changes of some OXPHOS (Marqus et al., 2011)
3	Tulahuen/TcII	Rat Heart myoblast	Primary human cardiomyocytes (PromoCell)	10:1	0	15min to 2hpi	Microarrays (Affymetrix)	No significant differences in pathways related to OXPHOS (Libisch et al., 2016)
4	Dm28c/TcI	Vero	Primary human cardiomyocytes (CellProgen)	10:1	2h	0, 3, 6, 12hpi	Microarrays (Agilent)	Up-regulation of OXPHOS related genes (Libisch et al., 2018)
***In vivo* transcriptomic experiments**								
5	Sylvio/TcI	C2C12	Male mice hearts(C3H/HeN)	10^6/mice	NA	3, 37, 110dpi	Microarrays (Clontech)	Down-regulation of OXPHOS related genes in cardiac tissue (Garg et al., 2003)
6	NA	NA	Myocardial tissues from CCC or DCM patients	NA	NA	NA	Microarrays (Affymetrix)	Up-regulation of OXPHOS related genes in CCC patients (Cunha-Neto et al., 2005)
7	Brazil/TcI Y/TcII	ND	Male mice heart (CD-1)	10^4/mice	NA	30 to 180dpi	Microarrays (Custom)	Repression of some OXPHOS related genes at the chronic stage (Mukherjee et al., 2009)
8	CpI1..7G2/ TcI JG/TcII	SWISS mice	Male mice heart (BALB/c)	50/Mice	NA	15dpi	Sequencing (Illumina)	Down-regulation of OXPHOS related genes when using the JG strain (De Castro et al., 2020)
***In vitro* functional experiments**								
9	Sylvio/TcI	C2C12	Cardiomyocytes (HL-1 and primary rat cardiomyocytes)	5:1	3h	48hpi	Histochemical staining	Decrease activities of complex I and II in HL-1 infected cardiomyocytes (Gupta et al., 2009)
10	Tulahuen/TcII Y/ TcII	LLCMK2	Normal human dermal fibroblasts	50:1	1h	48hpi	Seahorse	OCR increase in infected human fibroblast (Shah-Simpson et al., 2017)
11	Sylvio/TcI	C2C12	Human THP-1 macrophages	5:1	0	3 and 18hpi	Seahorse	OCR increase in infected human Macrophages (Koo et al., 2018)
12	Dm28c/TcI	Vero	Primary human cardiomyocytes (CellProgen)	10:1	2h	6, 17, 24hhpi	Seahorse	Up-regulation of OXPHOS related genes and OCR increase (Libisch et al., 2018)
14	CL/BrTcVI	Vero	Primary mouse cardiomyocytes (BALB/c)	5:1	0	24hpi	Seahorse	OCR decrease in infected mouse cardiomyocytes (Estrada et al., 2018)
***In vivo* functional experiments**								
15	Sylvio/TcI	C2C12	Cardiac mitochondria from male mice (C3H/HeN)	10^4/mice	NA	3-10dpi 14-40dpi >110dpi	Histochemical staining	Inhibition of the respiratory chain complexes (CI-CV) in the myocardium of infected mice (Aydinlioglu et al., 2004)
16	Sylvio/TcI	NA	6-8-week-old male C3H/HeN mice. (heart, stomach, skeletal muscle, colon)	10^4/mice	NA	20-35dpi 150-180dpi	Measure of antioxidant/oxidant status and mitochondrial function	Oxidative damage and mitochondria decay in acute infection in all tissues, and in heart and stomach in chronic infection. (Wen et al., 2008)
17	ND	NA	Myocardium homogenates from CCC patients	NA	NA	NA	Western blot	Decrease in components of the creatine kinase system and ATP synthase complex from CCC patients (Teixeira et al., 2011)
18	ND	NA	Cardiac biopsies from CCC patients	NA	NA	NA	Western blot and immunohistochemistry	Decrease in protein levels of subunits of the respiratory complexes (CI and CII) in chagasic hearts biopsies (Wen et al., 2012)

The blue and orange color show whether the study found a decrease or an increase in transcriptomic (light color) and functional (dark color) expression in T. cruzi infected cells, respectively. (PM, Parasite Propagation Mode; MOI, Multiplicity of Infection; IT, Interaction time; AT, Analyzed time; NA, Not Applicable; ND, No Data available; OXPHOS, Mitochondrial oxidative phosphorylation system; C2C12, mice myoblast cells; LLCMK2, Kidney cells from Macaca mulatta; Vero, Kidney cells from Cercopithecus aethiops; Seahorse, Seahorse extracellular flux analyzer).

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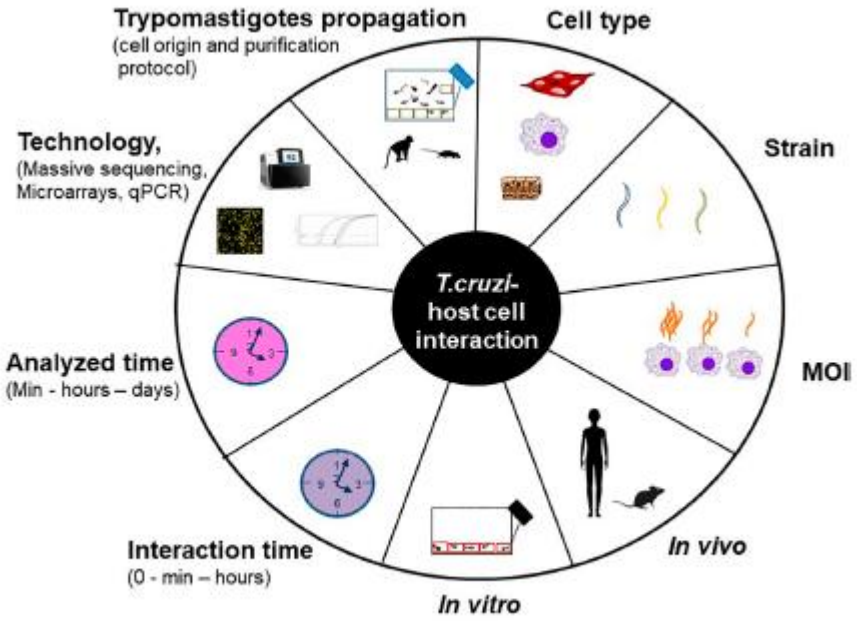

FIGURE 1 | Experimental variables that impact on *T. cruzi* – host cell interaction studies.

Perspectives: to generate tools that allow the integration of different "omics“/hos-pathogen crosstalk/targets

How to do it in networks.

This workshop is an initiative that goes in that direction.

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Fernando Alvarez-Valin

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GCRF Global Challenges Research Fund


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