

Metabolism in a 3D environment: chemical cartography of Chagas disease

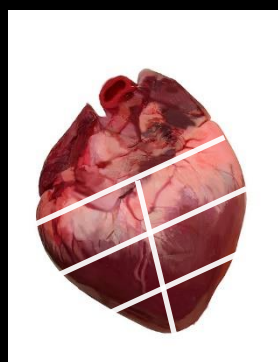
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Department of Chemistry and Biochemistry

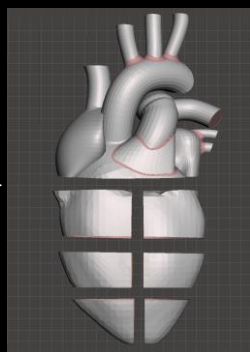
San Diego State University

twitter: @LabMccall

Chemical cartography: understanding *where* molecules are, to define *what* they do



Section samples



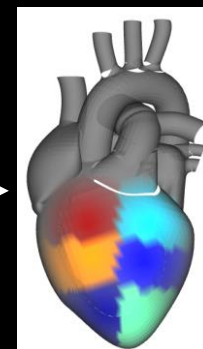
Register sample position in 3D space



Extract metabolites



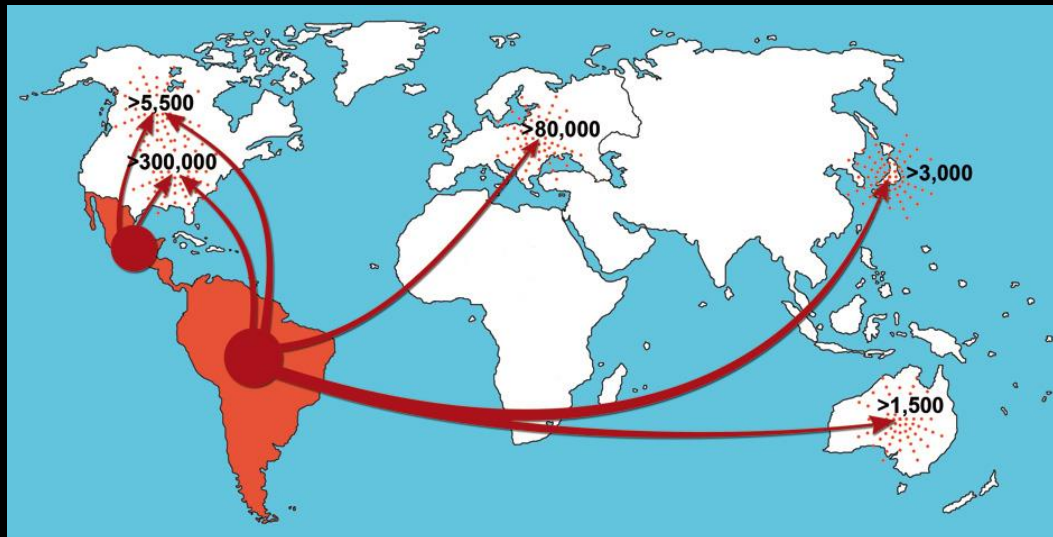
LC-MS/MS



Map metabolite position in 3D

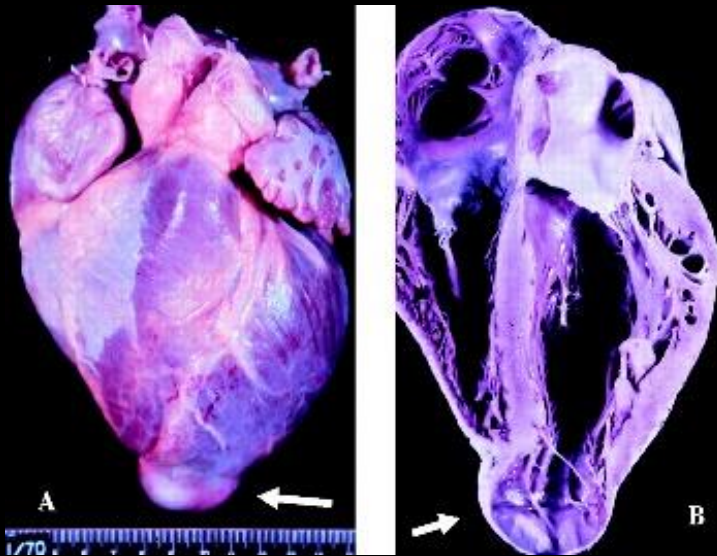
Chagas disease

- Parasitic disease, caused by unicellular *Trypanosoma cruzi* protozoan parasites
- Endemic from central USA to South America
 - 13% of Latin American population at risk
- >5 million people currently infected
- In the USA: 300,000-1 million infected
- 12,000 deaths/year
- Current treatments show limited efficacy in late-stage disease
 - need for host-targeted therapy?



Characteristic Chagas disease pathological findings

apical aneurysm



megacolon



megaesophagus



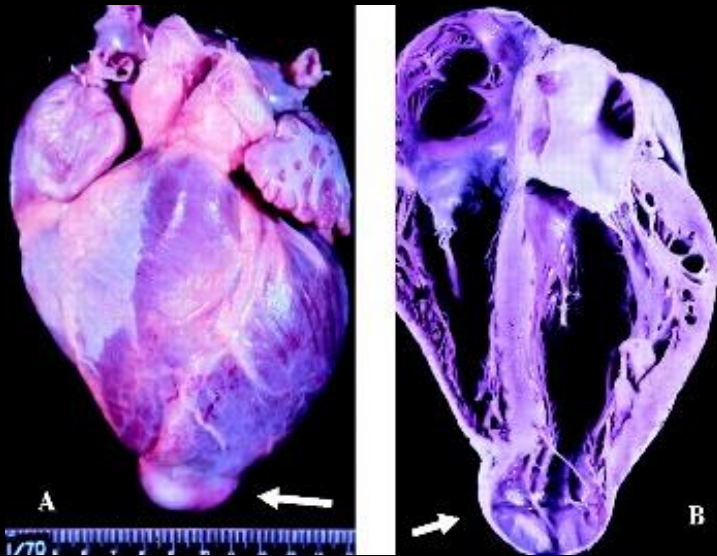
Marin-Neto *et al*, *Circulation* 2007

https://www.cdc.gov/parasites/cme/chagas/lesson_2/19.html

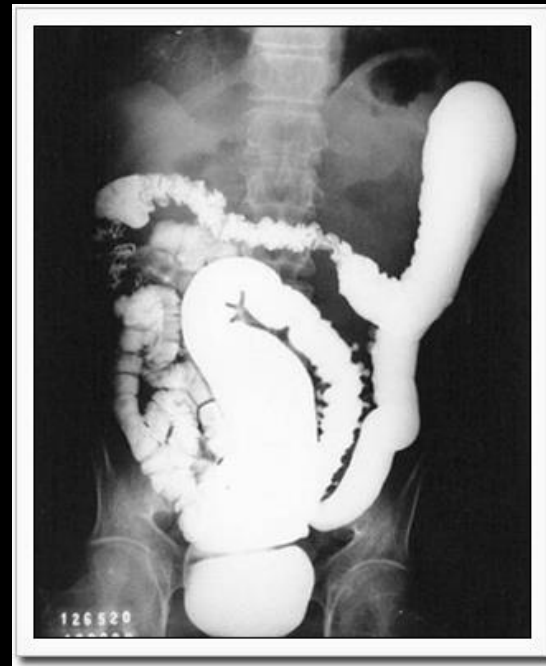
Abud *et al*, *Radiol Bras* 2016

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Marin-Neto *et al*, *Circulation* 2007

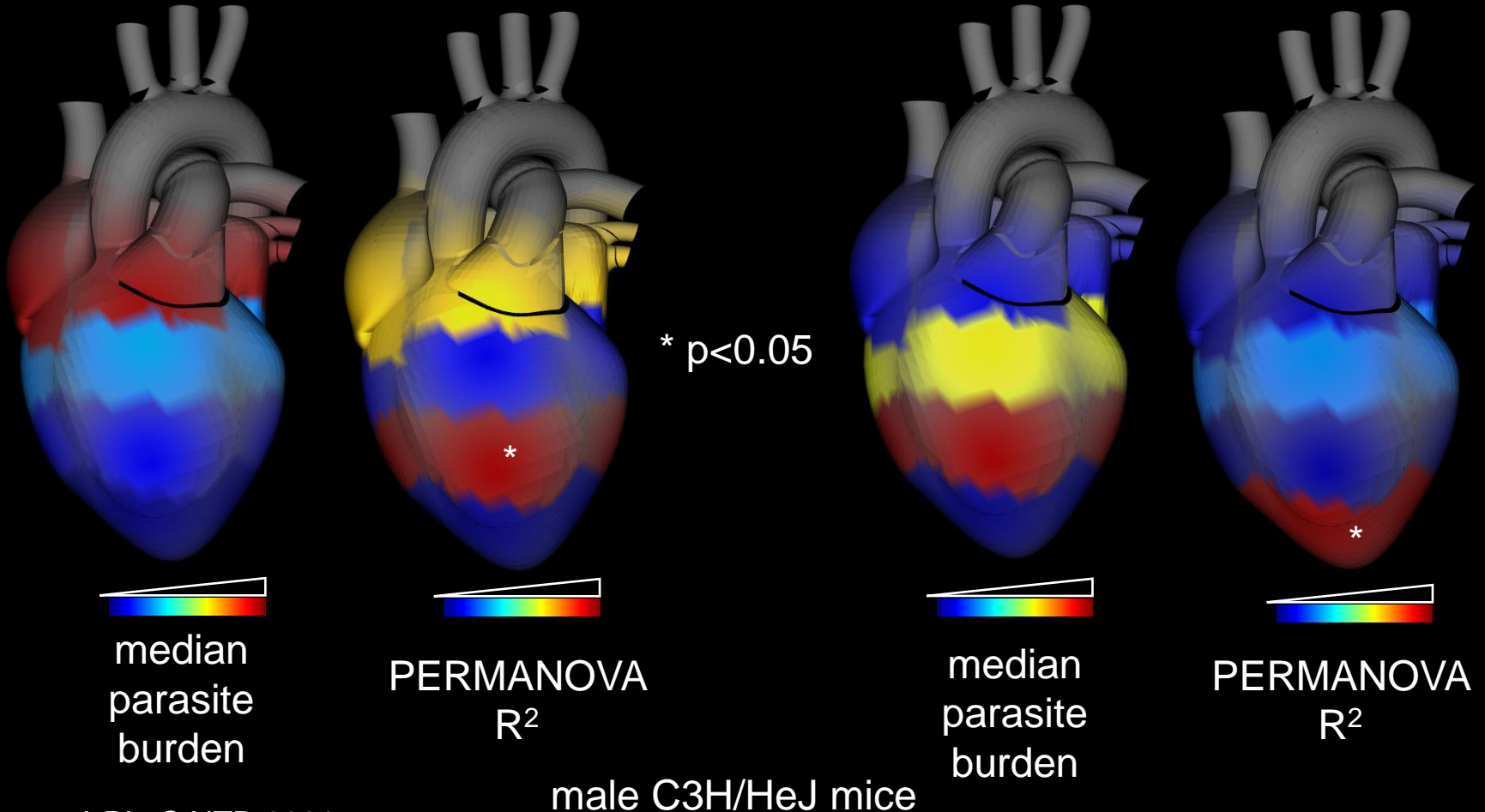
https://www.cdc.gov/parasites/cme/chagas/lesson_2/19.html

Abud *et al*, *Radiol Bras* 2016

Disconnect between parasite persistence and metabolic alterations in chronic cardiac CD

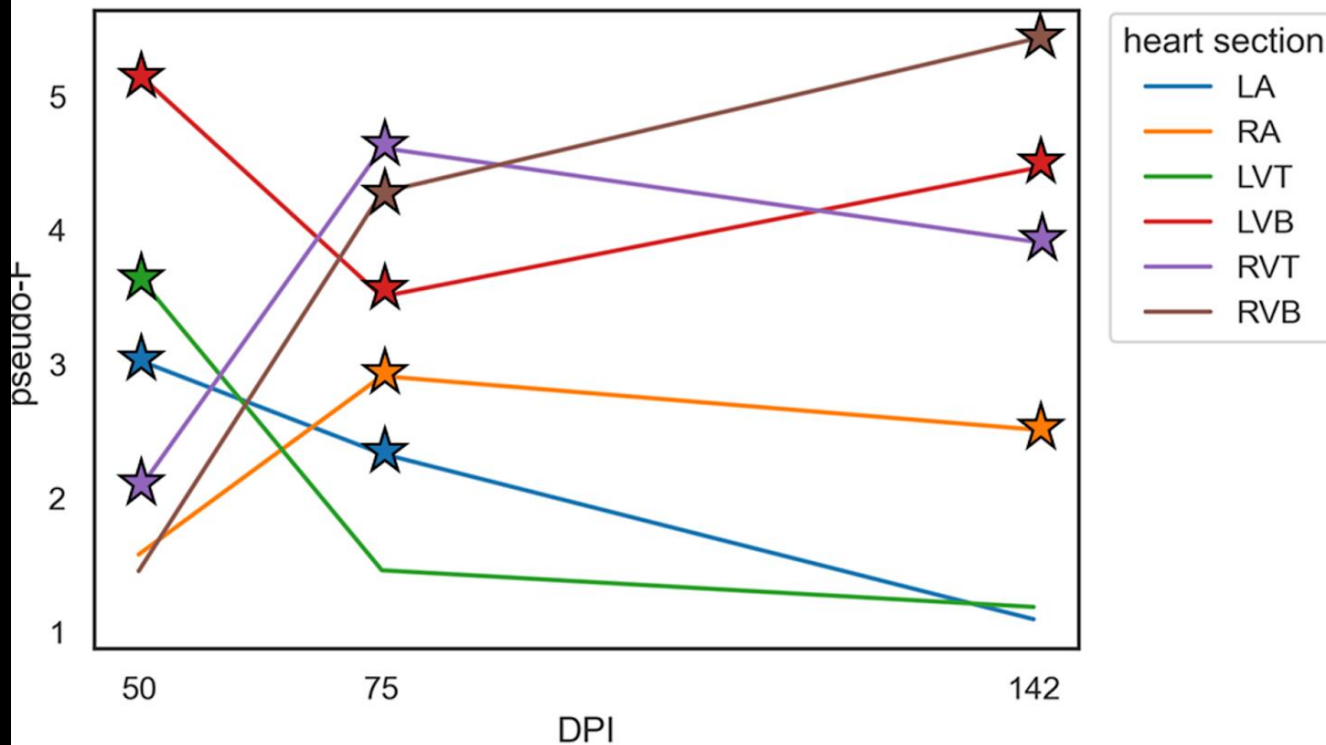
T. cruzi strain CL

T. cruzi strain Sylvio X10/4



Worsening metabolic alterations at the heart apex

PERMANOVA pseudo-F trajectories over time



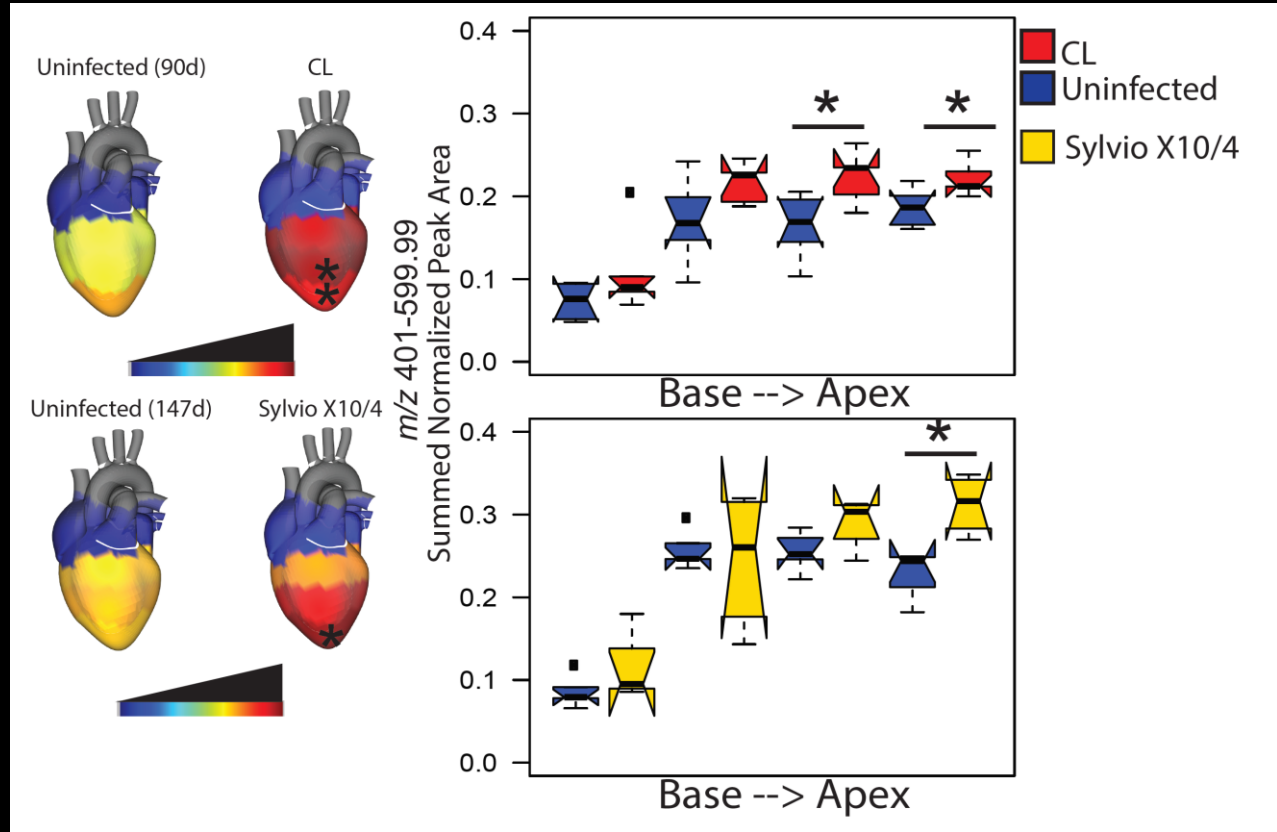
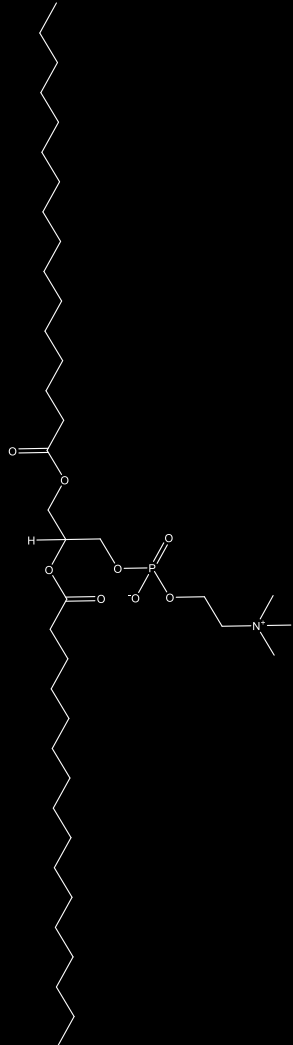
142 DPI



6
PERMANOVA pseudo-F
0

female BALB/c mice + *T. cruzi* strain H1 (Tcl)

Locally-increased glycerophosphocholines in chronic cardiac Chagas disease



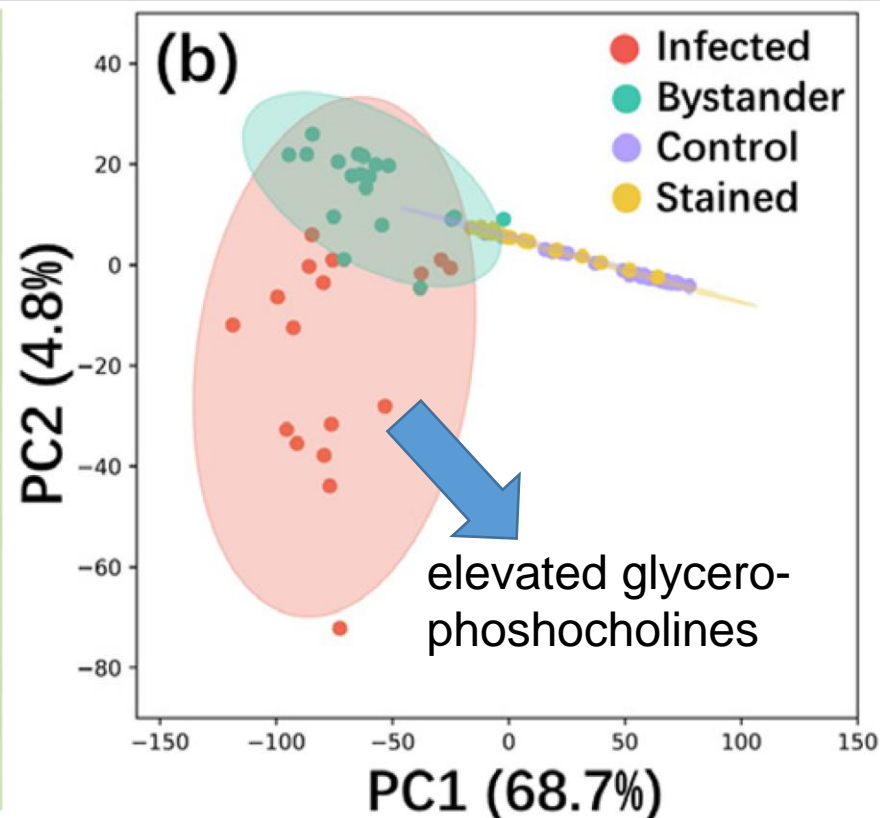
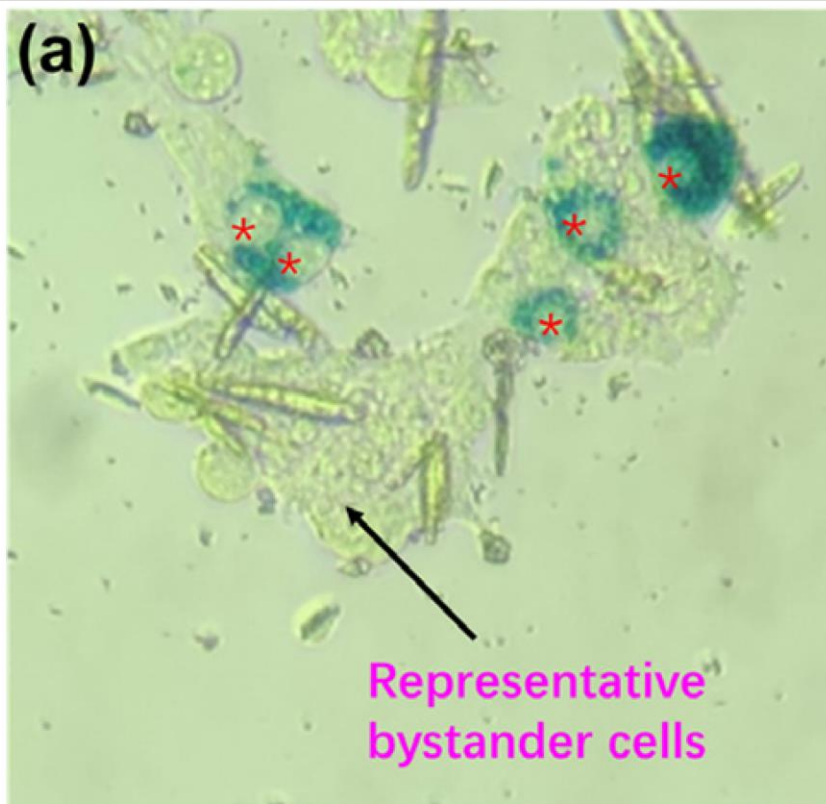
male C3H/HeJ mice

External validation: Magnitude of glycerophosphocholine perturbation is proportional to disease severity

m/z range	Correlated Pathology Indicator	Correlation Coefficient	p values	Corrected p values
400-499	Inflammation	0.470	0.005	0.033
	Fibrosis	0.556	6.41E-4	0.009
	CTGF	0.644	4.03E-5	2.94E-4
	TGF- β	0.533	0.001	0.009
500-599	Fibrosis	0.517	0.002	0.012
	CTGF	0.669	1.51E-5	2.94E-4
	TGF- β	0.532	0.001	0.009
All	Inflammation	0.423	0.013	0.057
	CTGF	0.525	0.001	0.007

- Independent chronic *T. cruzi* infection model
- Female BALB/c mice + *T. cruzi* strain H1
- 209 days post-infection

Validation at the single-cell level



Chemical cartography of chronic cardiac Chagas disease: take-home messages

- Disconnect between metabolic perturbation and parasite localization but concurrence between sites of metabolic perturbation and sites of clinical symptoms
 - bystander effect of infection
- Major alterations in:
 - acylcarnitine levels
 - glycerophosphocholine levels
 - purine levels
- Alterations are proportional to disease severity

Chagasic megacolon and megaoesophagus

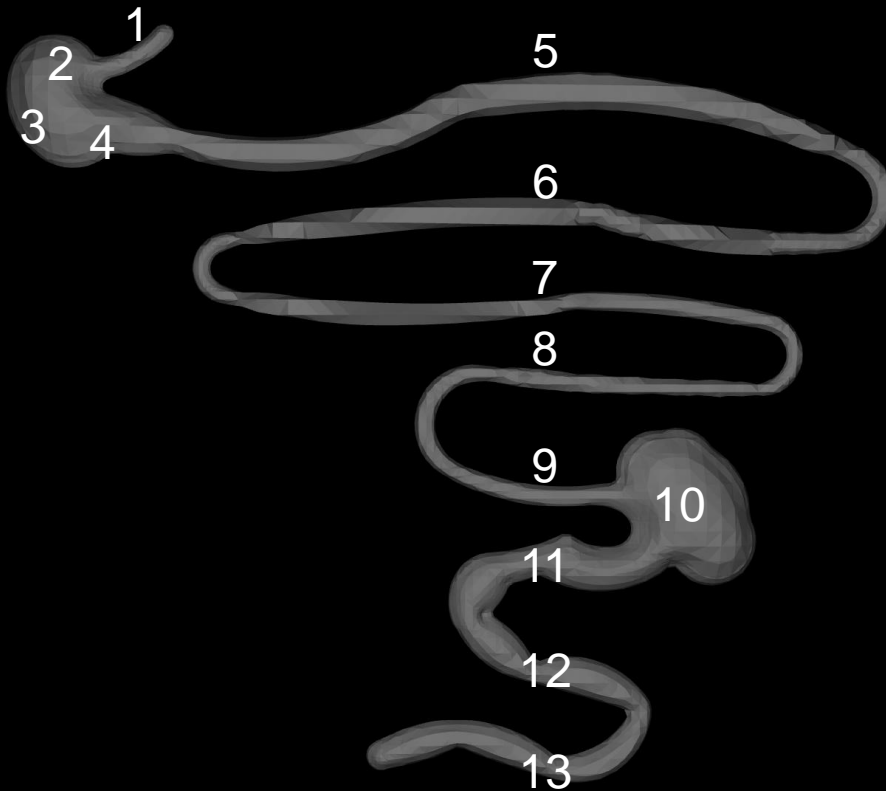
megacolon



megaoesophagus

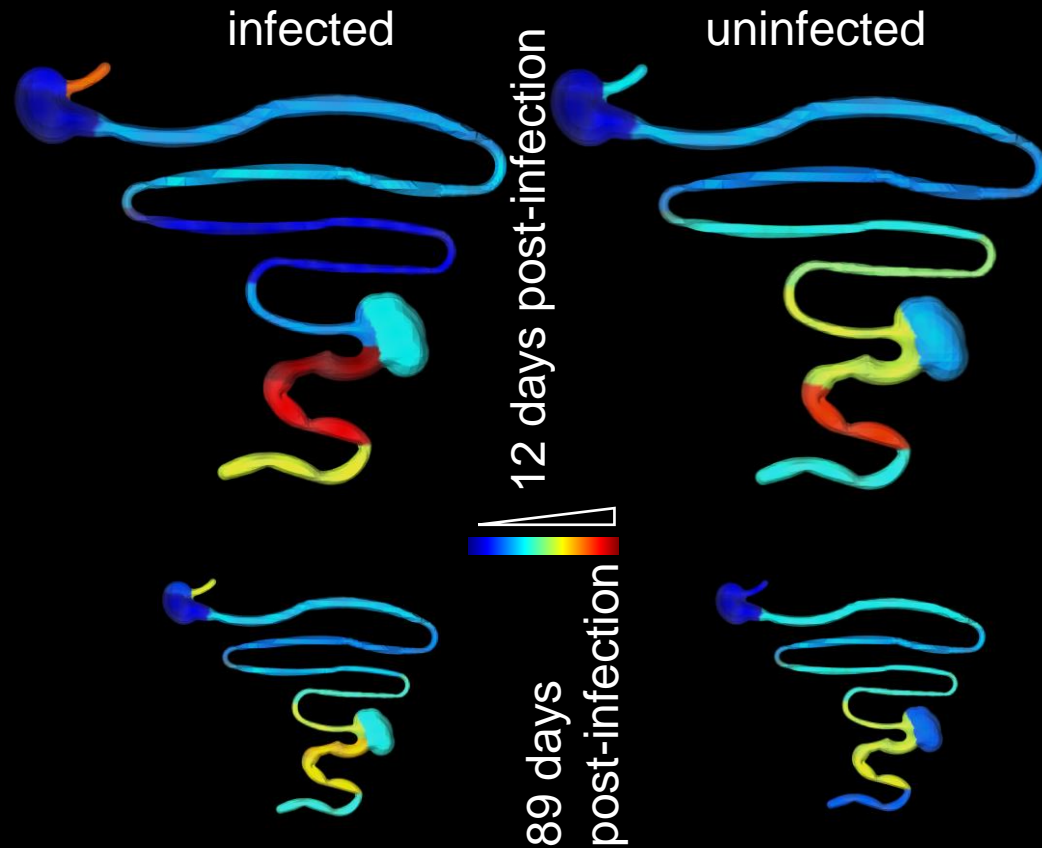
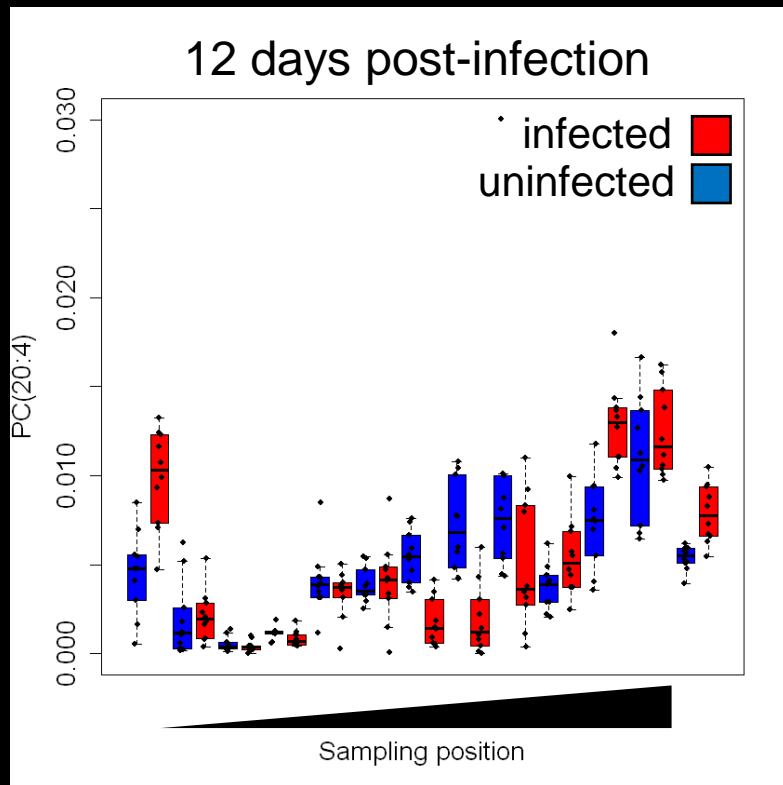


Sampling locations

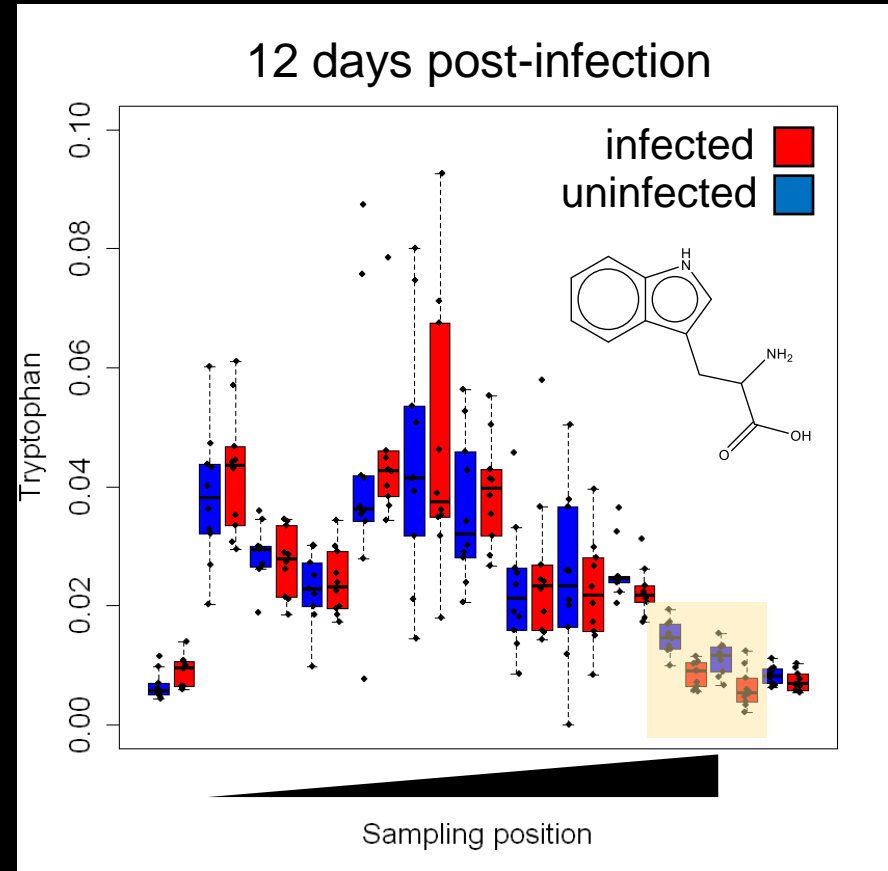
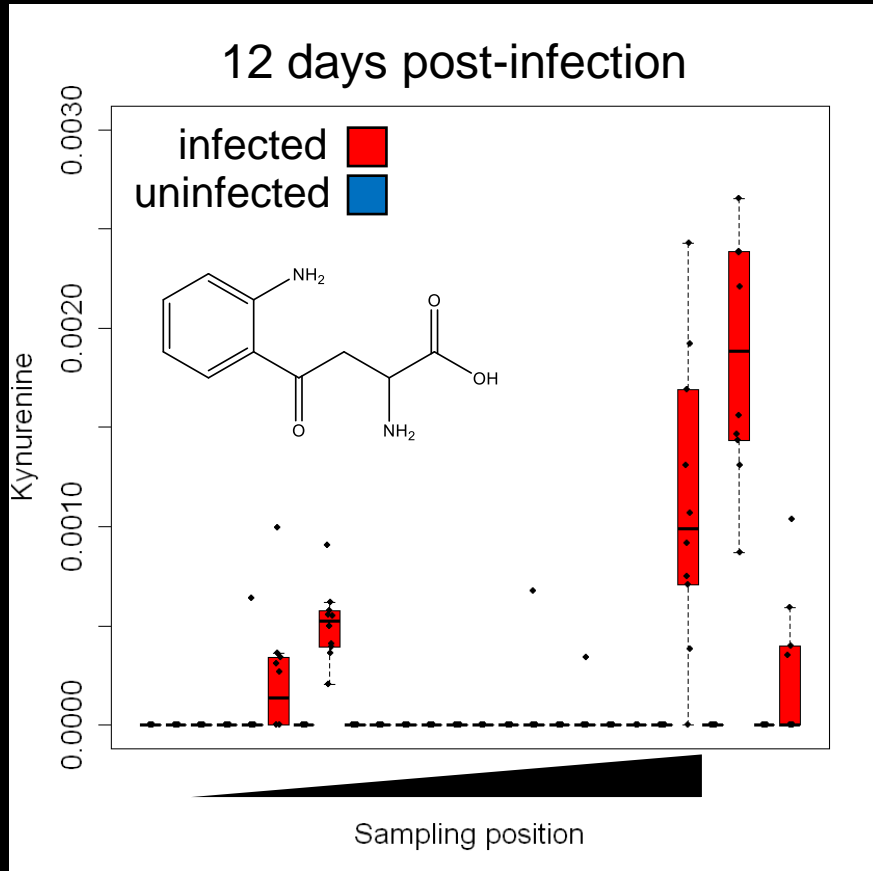


- as well as stomach, SI and LI contents
- mouse model
 - acute stage
 - chronic stage
- 2 biological replicates
- positive and negative mode MS
- 16S sequencing
- total:
 - 637 samples
 - 9 days of instrument time

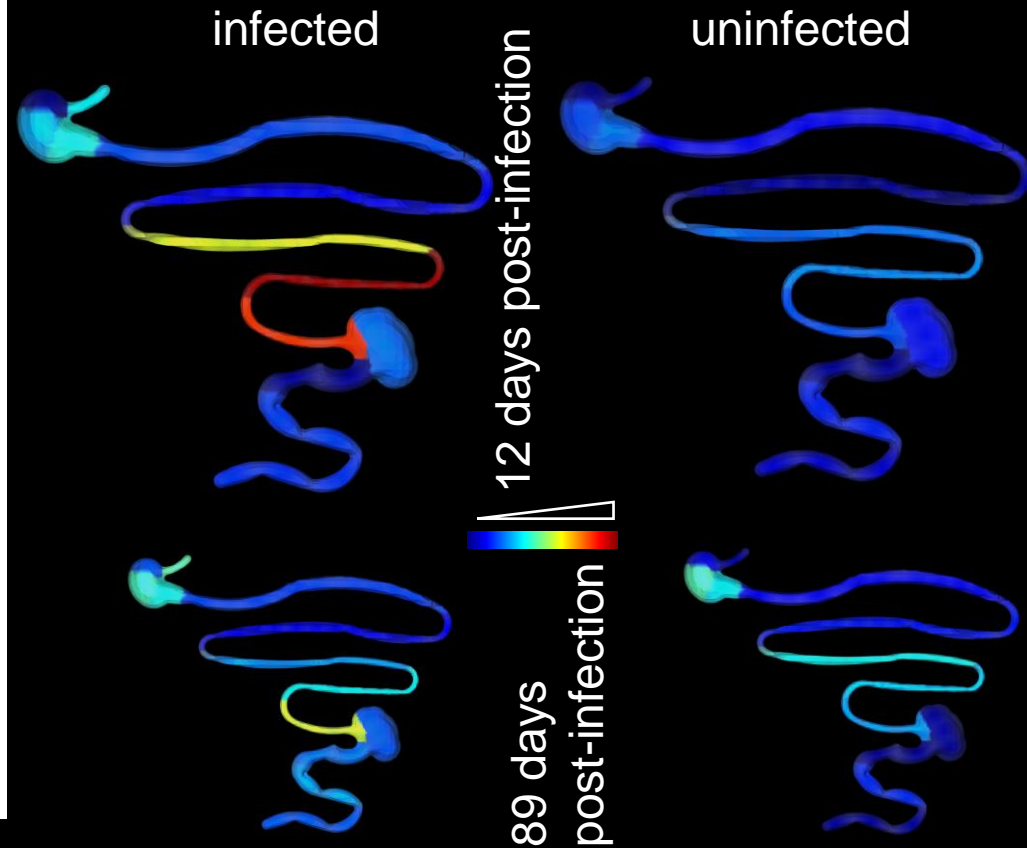
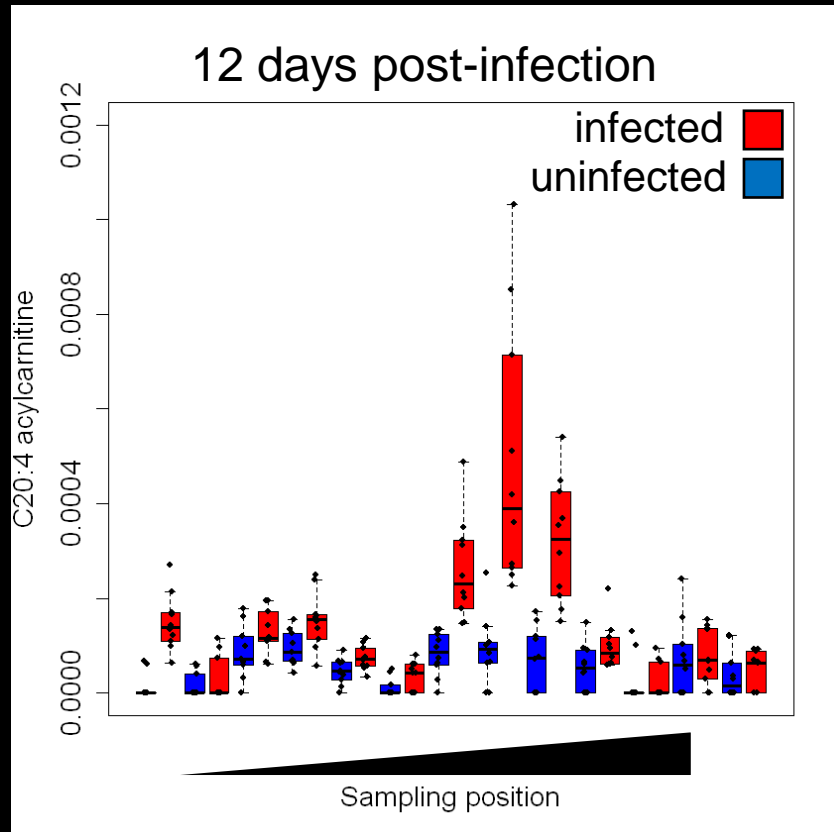
Specific chemical changes associated with infection: PC(20:4)



Specific chemical changes associated with infection: kynurenine vs tryptophan



Specific chemical changes associated with infection: arachidonyl-carnitine



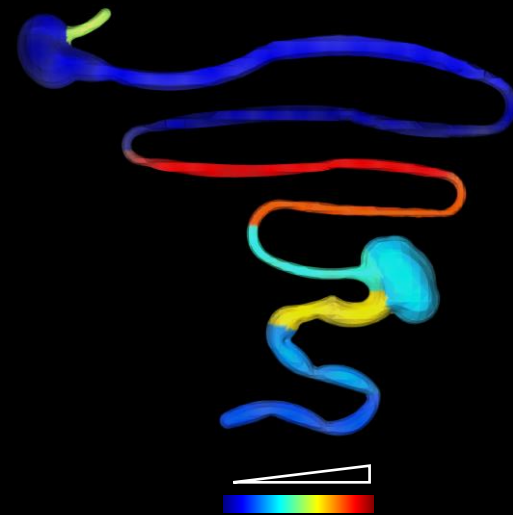
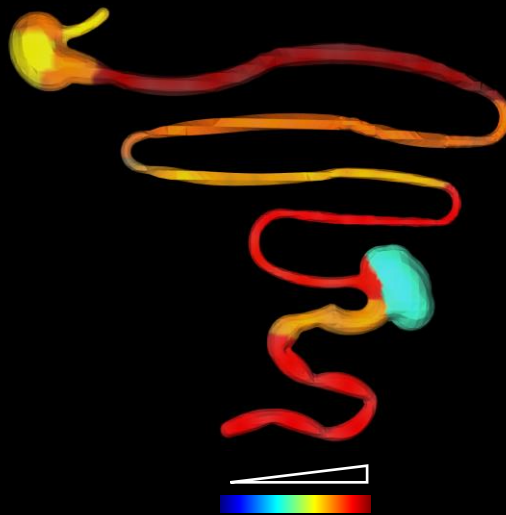
GI tract metabolic trajectories

male C3H/HeJ mice + *T. cruzi* strain CL+luc

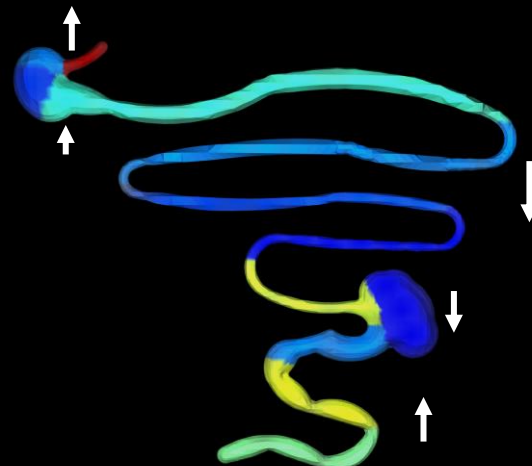
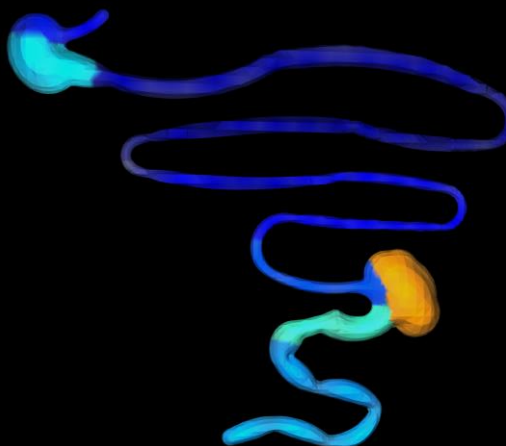
parasite burden

PERMANOVA R²

12 days



89 days



Chemical cartography of GI Chagas disease: take-home messages

- Sites of metabolic perturbation match with sites of disease symptoms
 - low metabolic resilience in the oesophagus and the colon
- Effects of infection on the microbiome community composition
- Specific localized metabolic alterations, including:
 - glycerophosphocholines
 - kynurenine
 - acylcarnitines

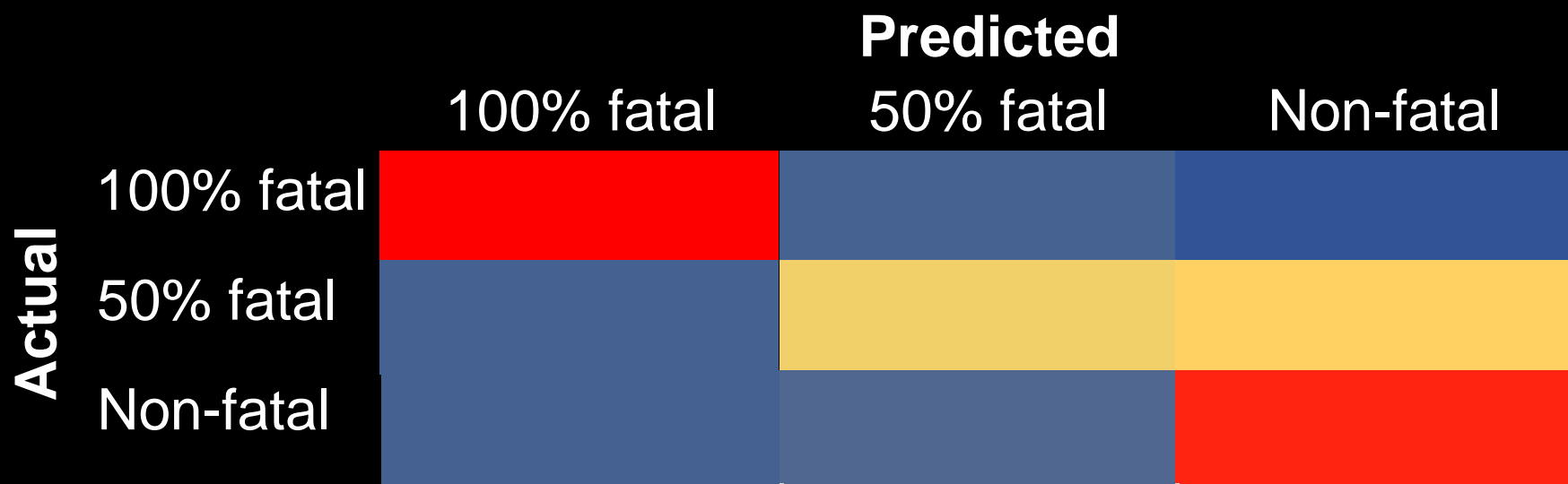
Why do we care?

Translational applications

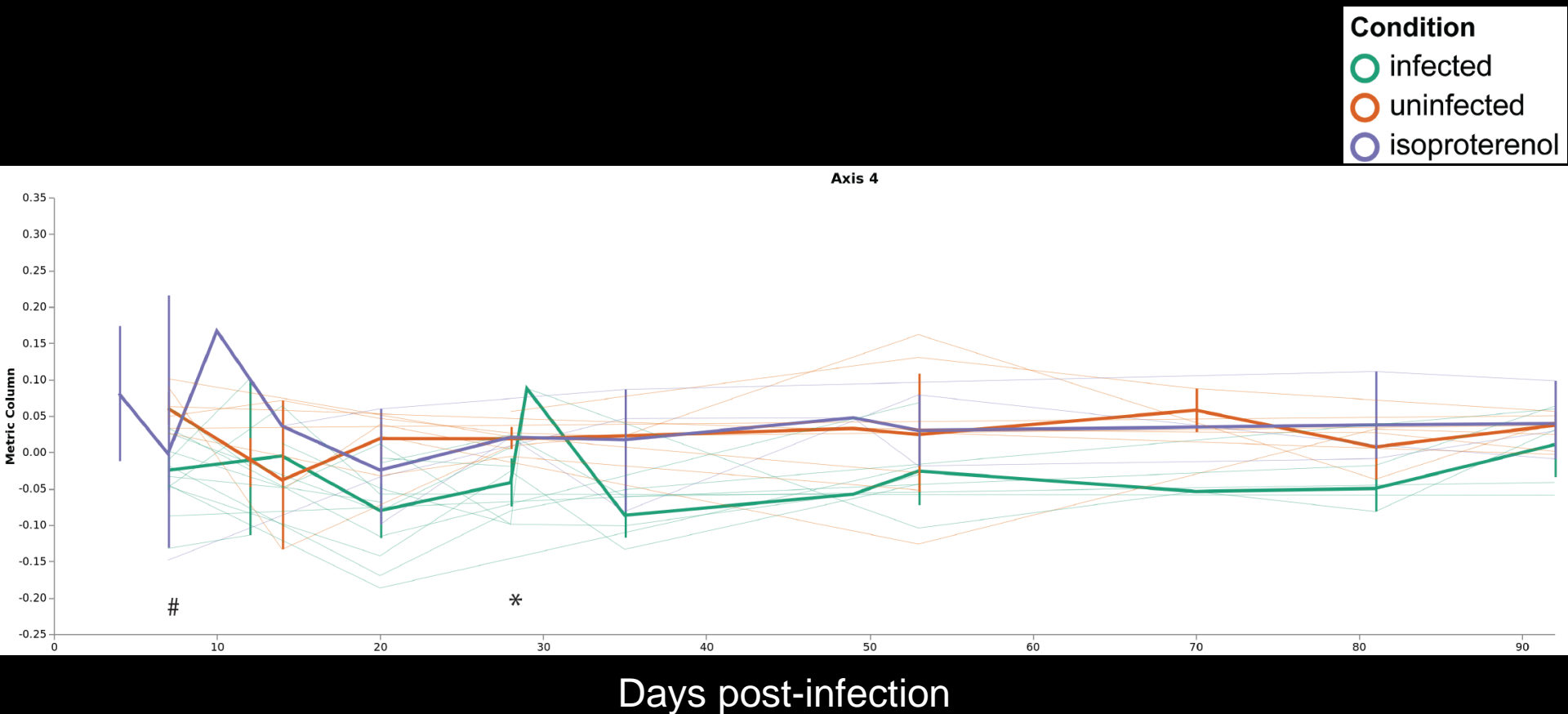
- biomarker discovery
- drug development

Can we use metabolomics to monitor disease progression?

Can we use metabolomics to monitor disease progression?

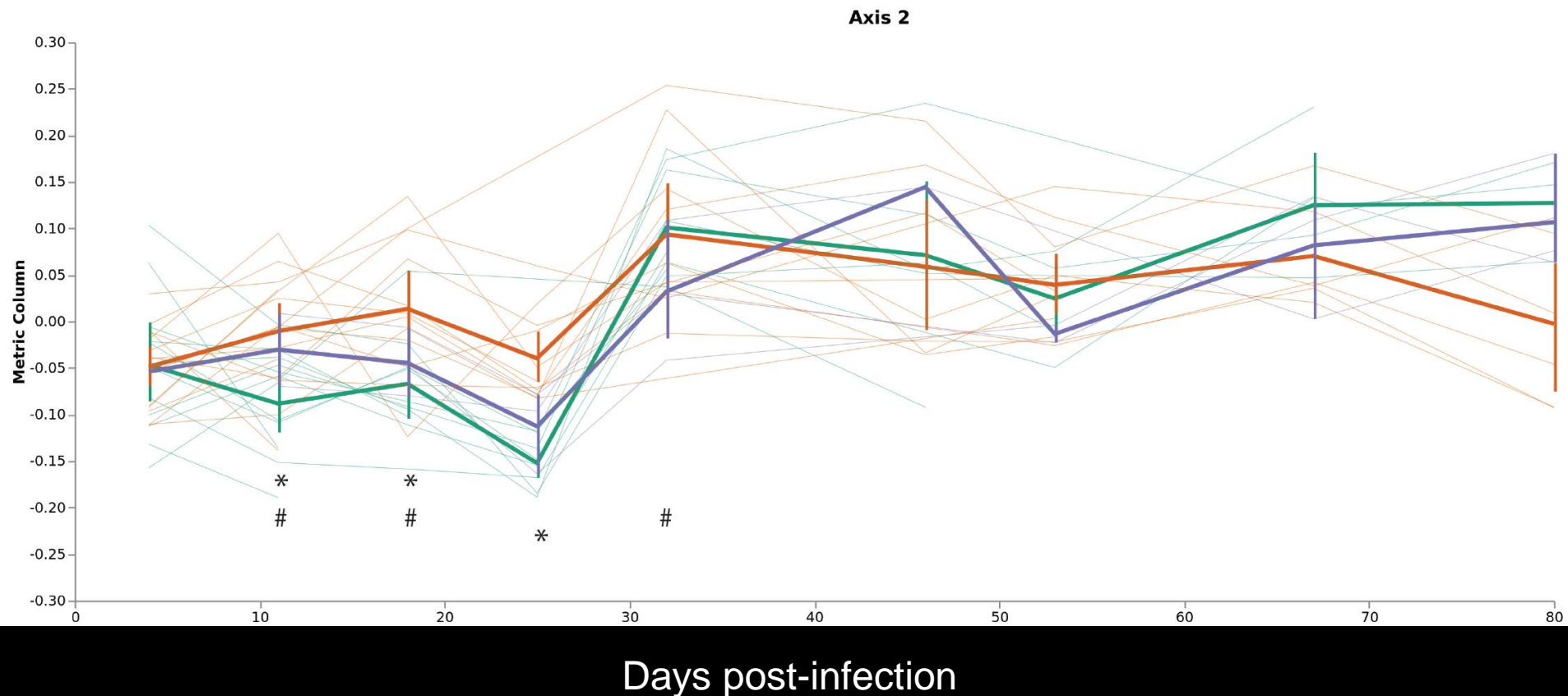


Temporal impact of *T. cruzi* infection on biofluids: saliva



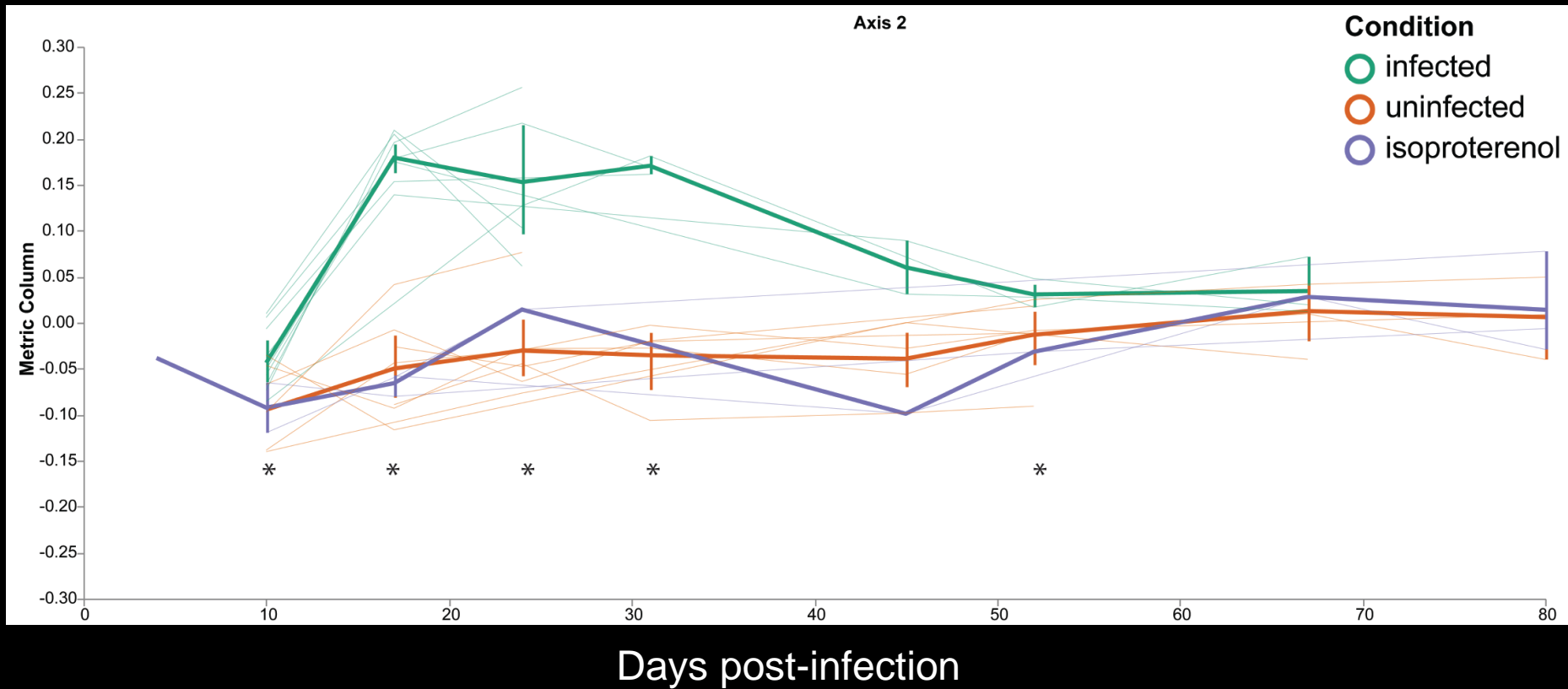
male Swiss Webster mice + *T. cruzi* strain Sylvio X10/4

Temporal impact of *T. cruzi* infection on biofluids: plasma



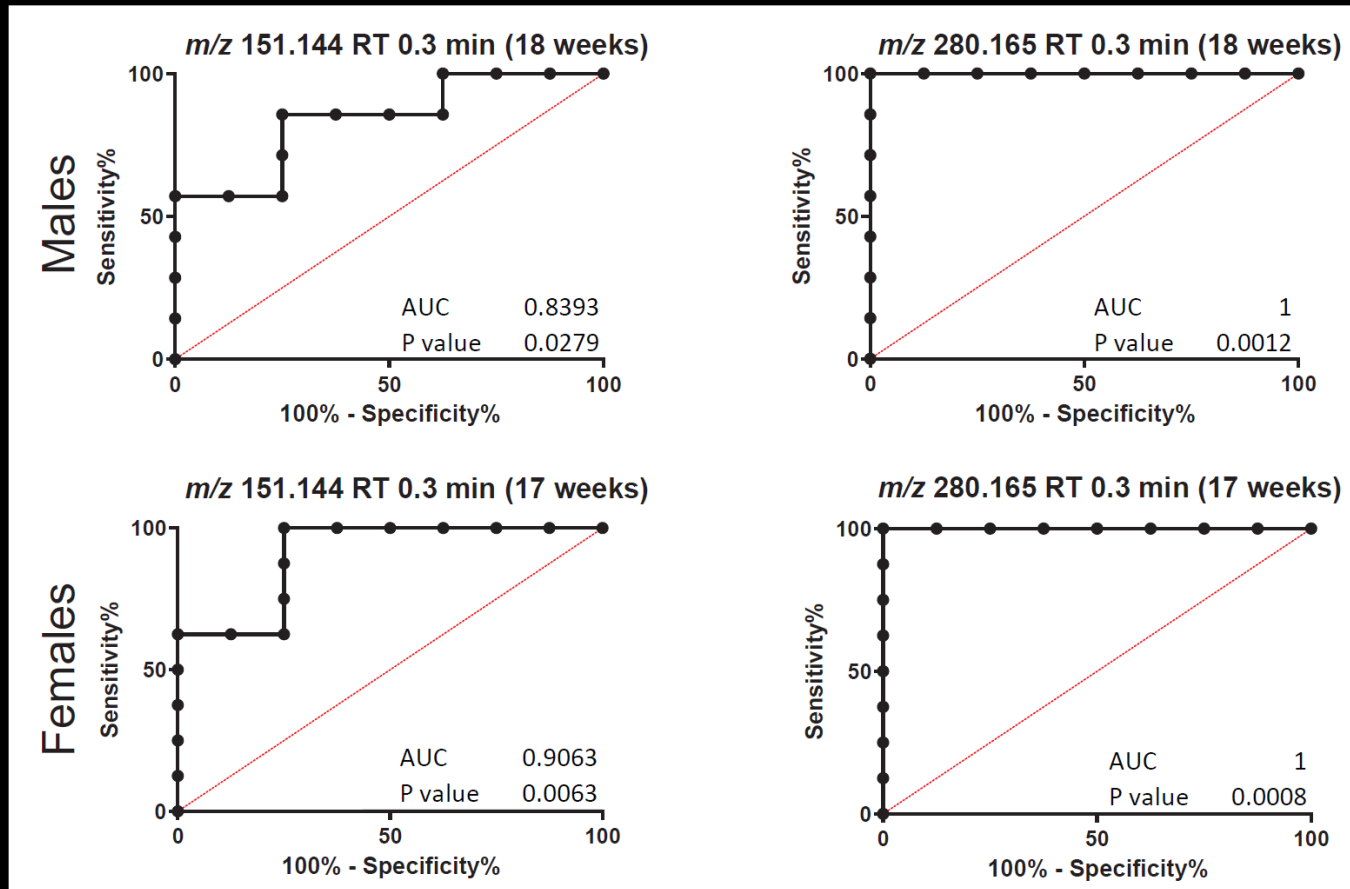
male Swiss Webster mice + *T. cruzi* strain Sylvio X10/4

Temporal impact of *T. cruzi* infection on biofluids: urine



male Swiss Webster mice + *T. cruzi* strain Sylvio X10/4

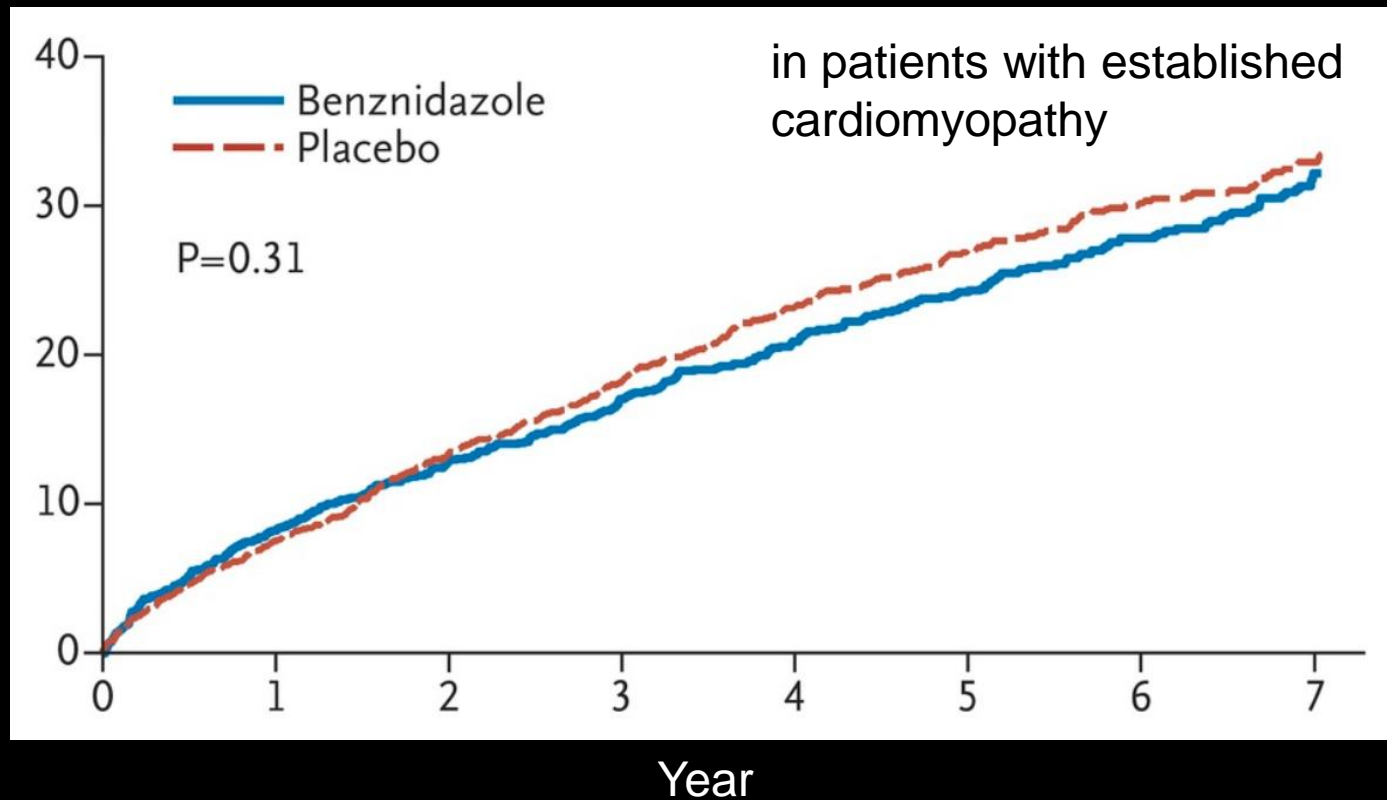
Reproducible urinary biomarkers of infection status



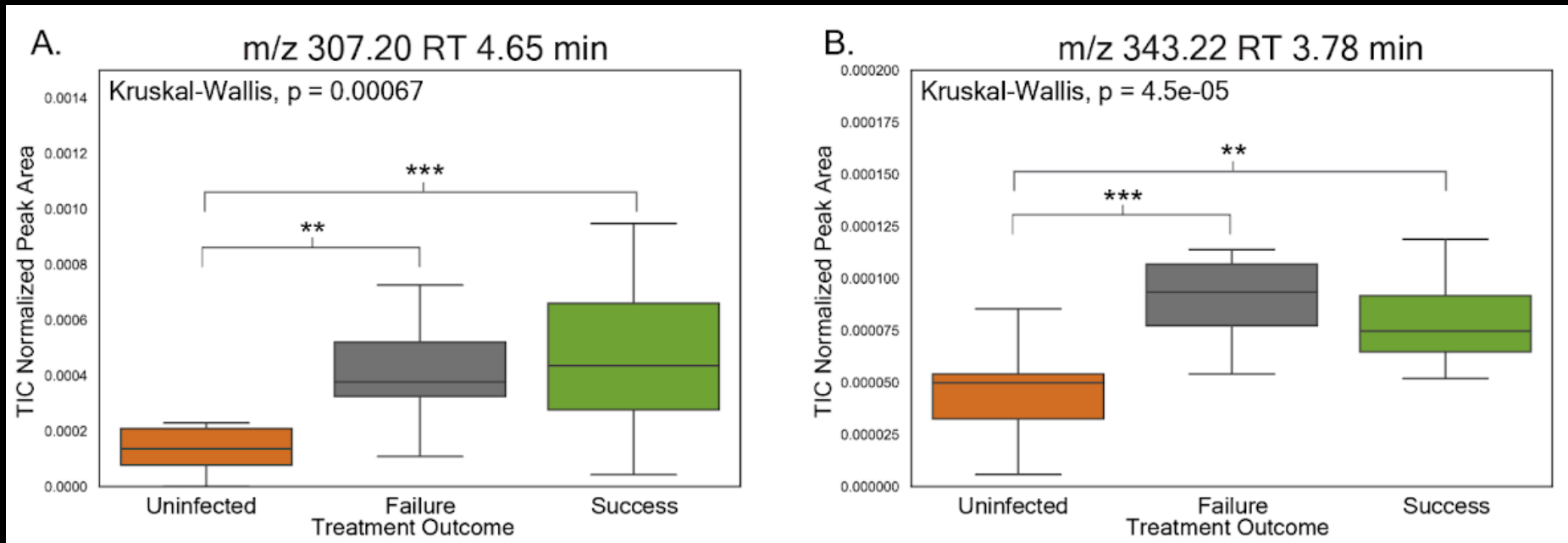
Swiss Webster mice + *T. cruzi* strain Sylvio X10/4

Treatment success is not just about clearing the pathogen

Cumulative Percentage of Patients with Death or Cardiac Events

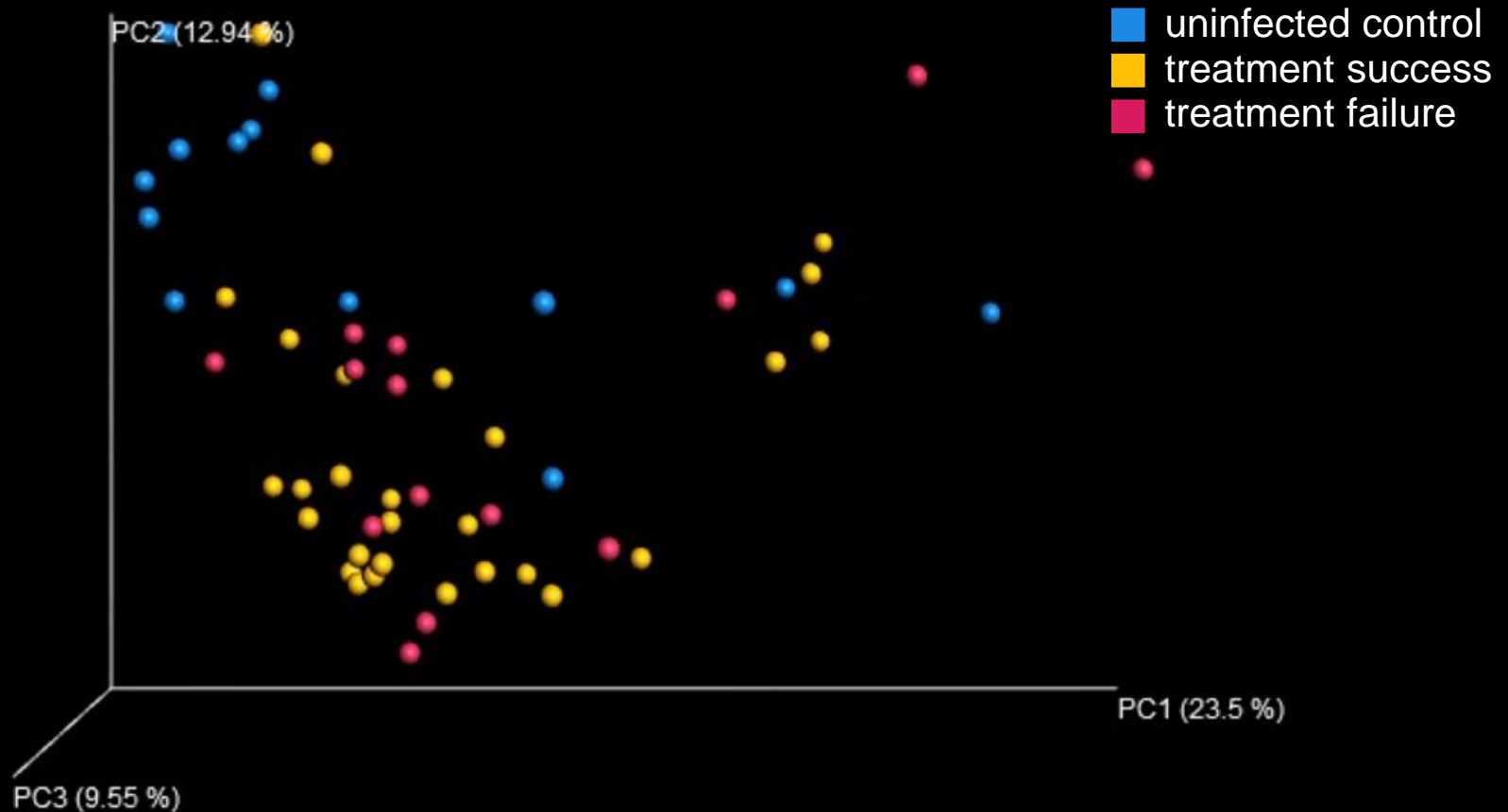


Benznidazole treatment does not restore biomarkers of infection status



BALB/c + *T. cruzi* strain CL+luc

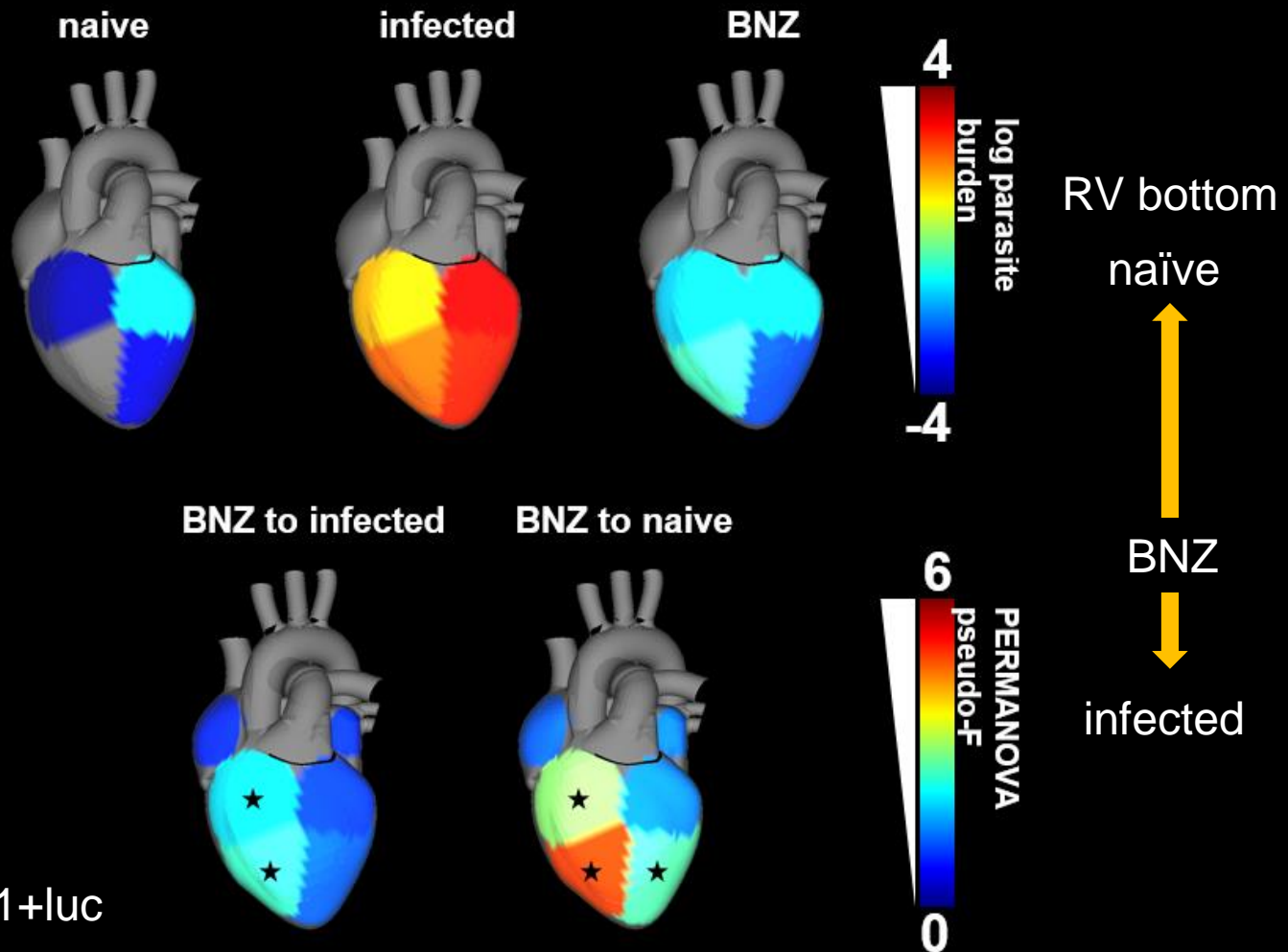
Parasite clearance is insufficient to enable metabolic restoration in urine



PERMANOVA $p < 0.05$ for infected vs uninfected mice

PERMANOVA $p > 0.05$ for successfully treated vs unsuccessfully treated mice

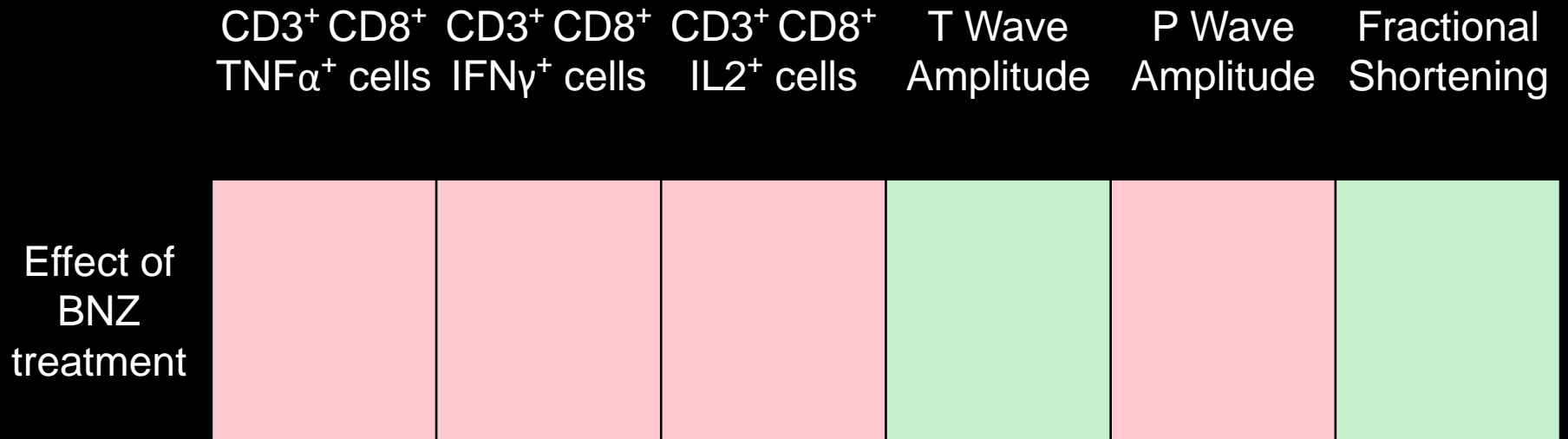
Incomplete cardiac metabolic restoration following benznidazole treatment



BALB/c
+ *T. cruzi* strain H1+luc

Liu *et al*, Nature Communications 2023

Incomplete immune restoration by benznidazole

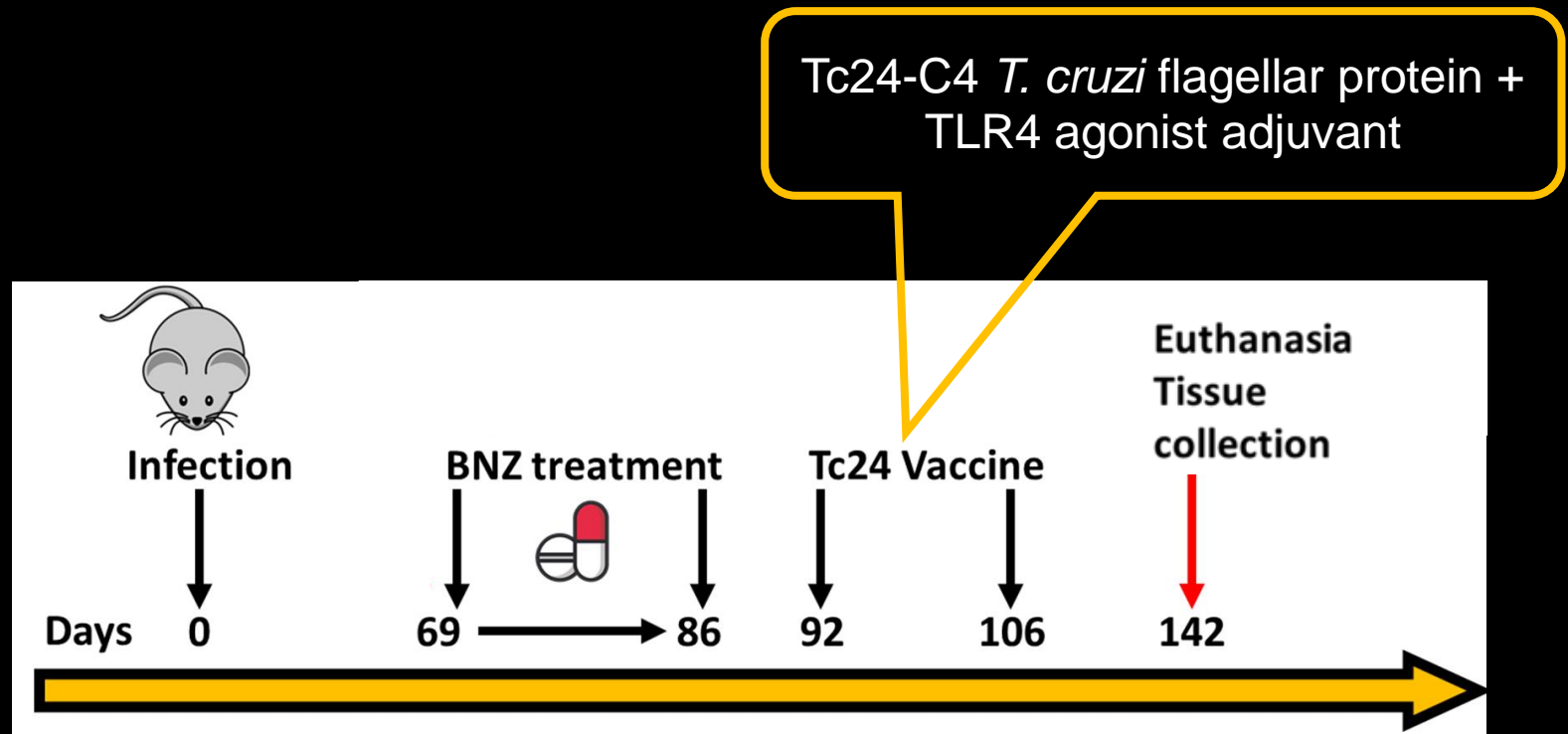


Not restored

Restored

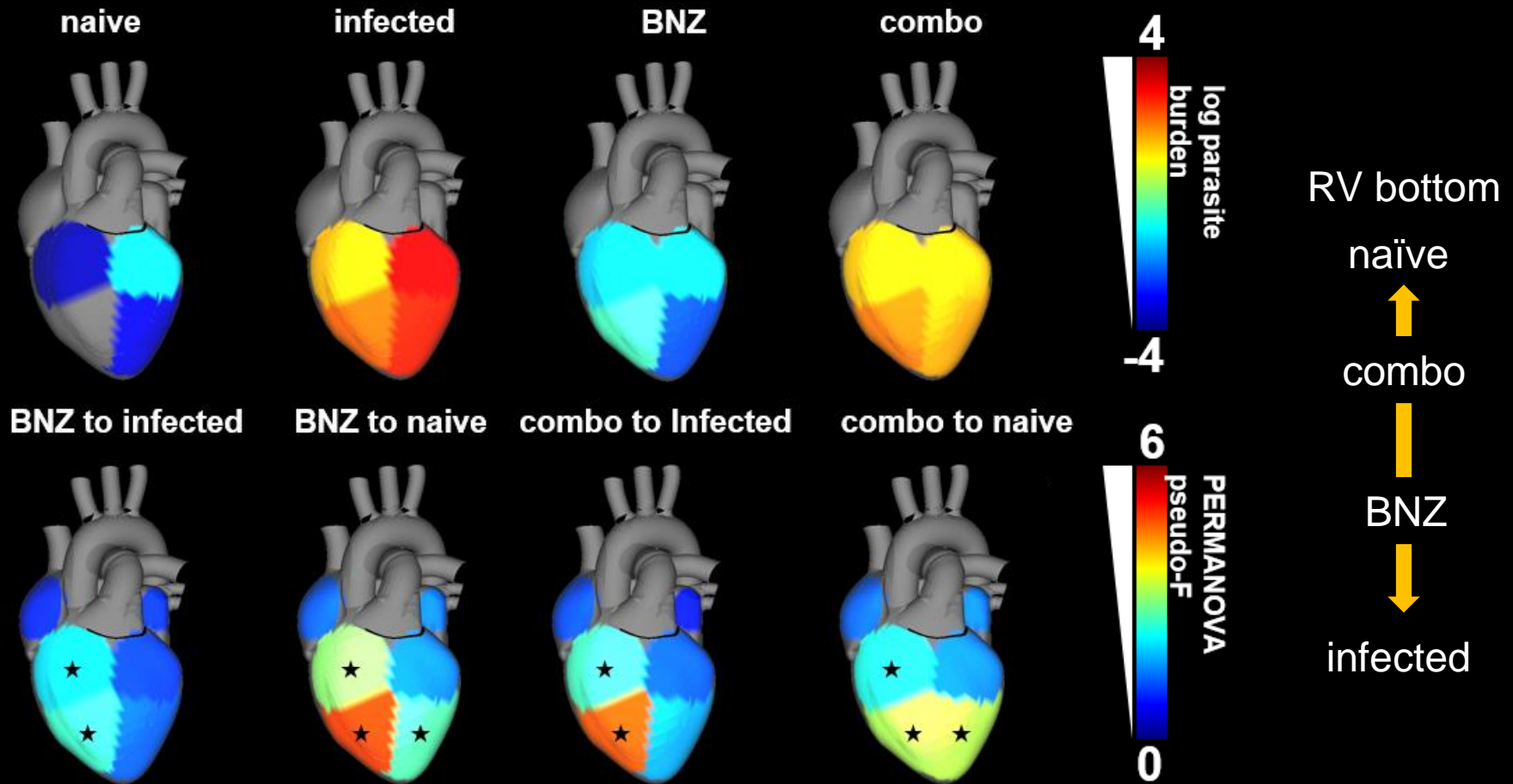
Unclear

New treatment strategies: vaccine-linked chemotherapy

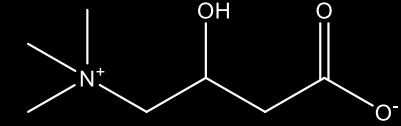


“combo treatment”

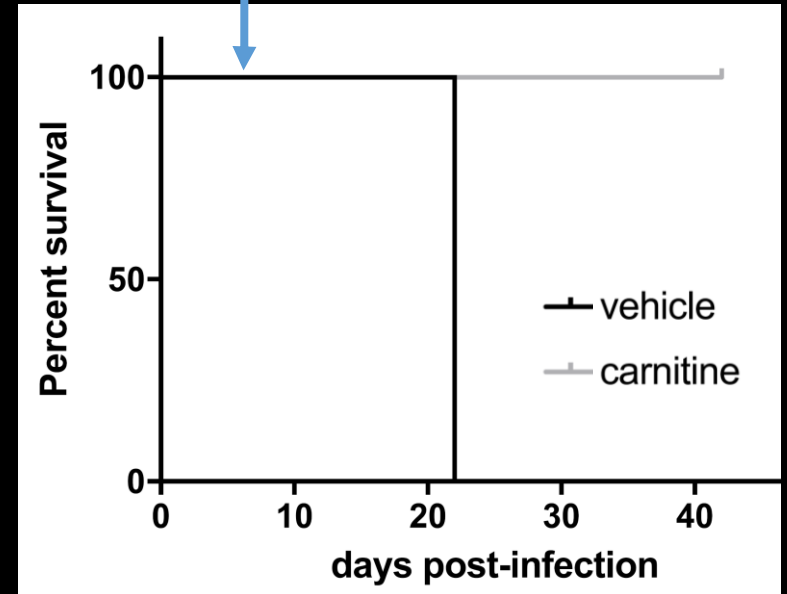
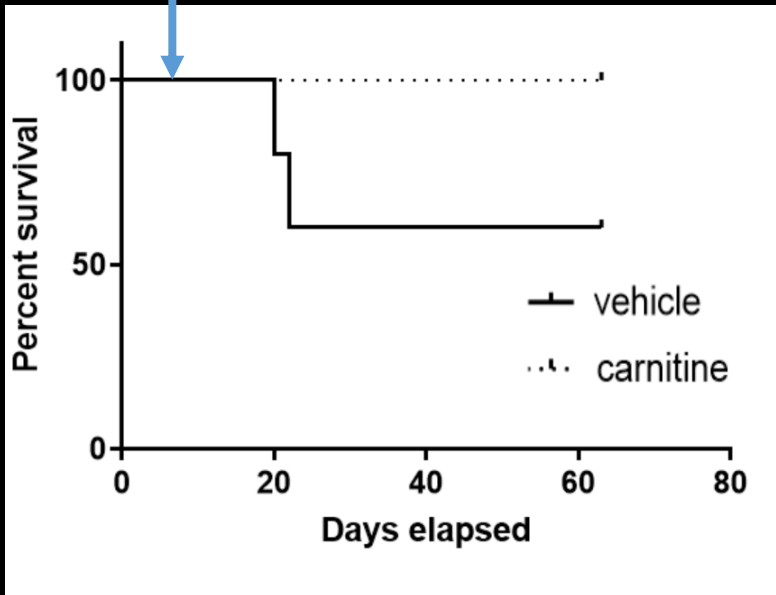
Superior cardiac metabolic restoration with combo treatment



New treatment strategy: carnitine supplementation



treatment start (day 7 post-infection)



5,000 CL+luc

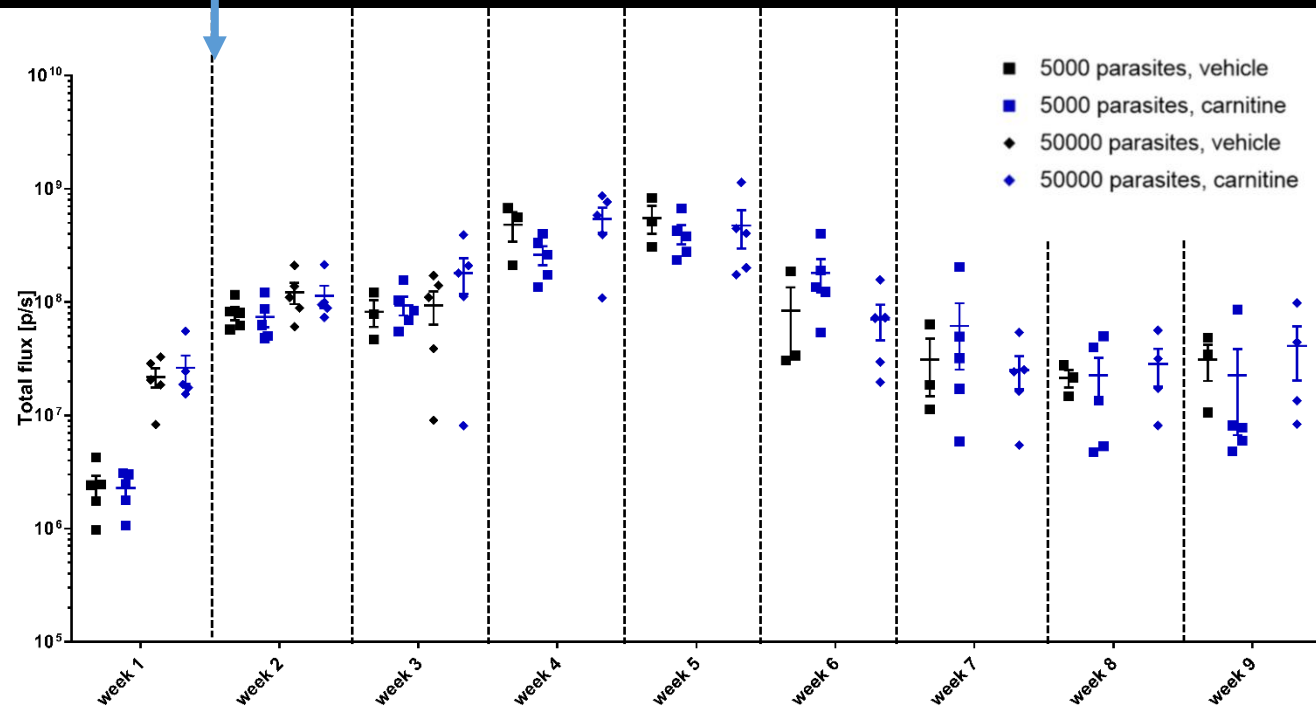
50,000 CL+luc

C3H/HeJ mice

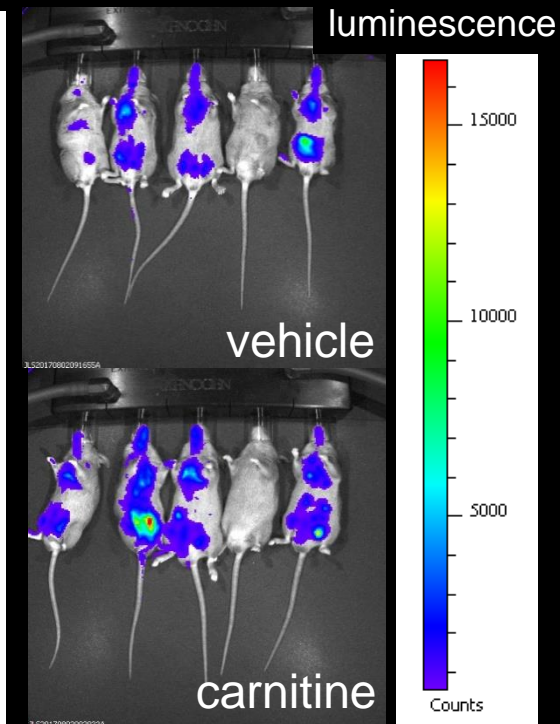
carnitine *ad libitum* in drinking water, equivalent to 100 mg/kg/day based on water consumption

Carnitine supplementation does not affect overall parasite burden

treatment start (day 7 post-infection)



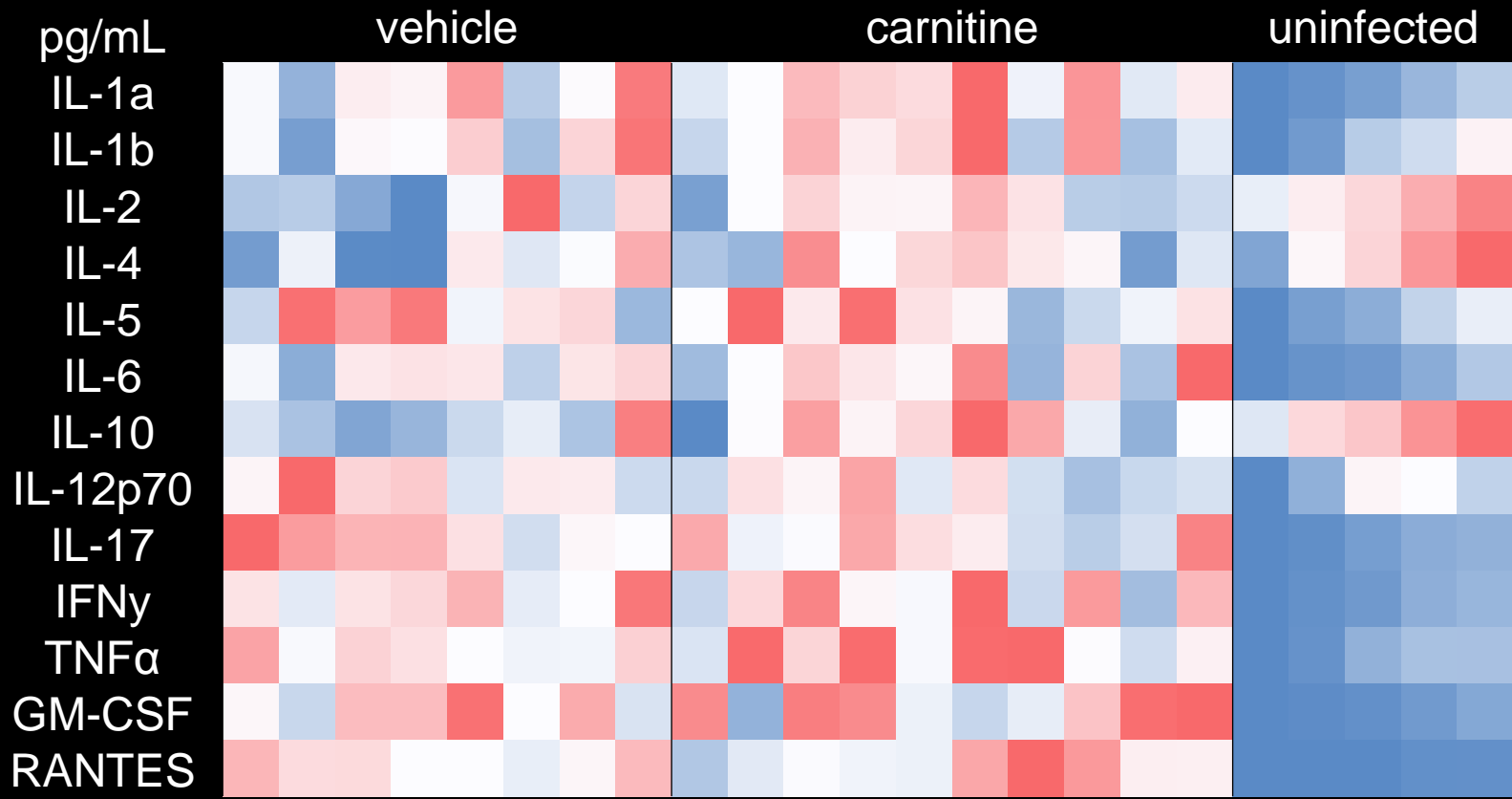
50,000 CL+luc



21 days post-infection
(14 days of treatment)

carnitine *ad libitum* in drinking water, equivalent to 100 mg/kg/day based on water consumption

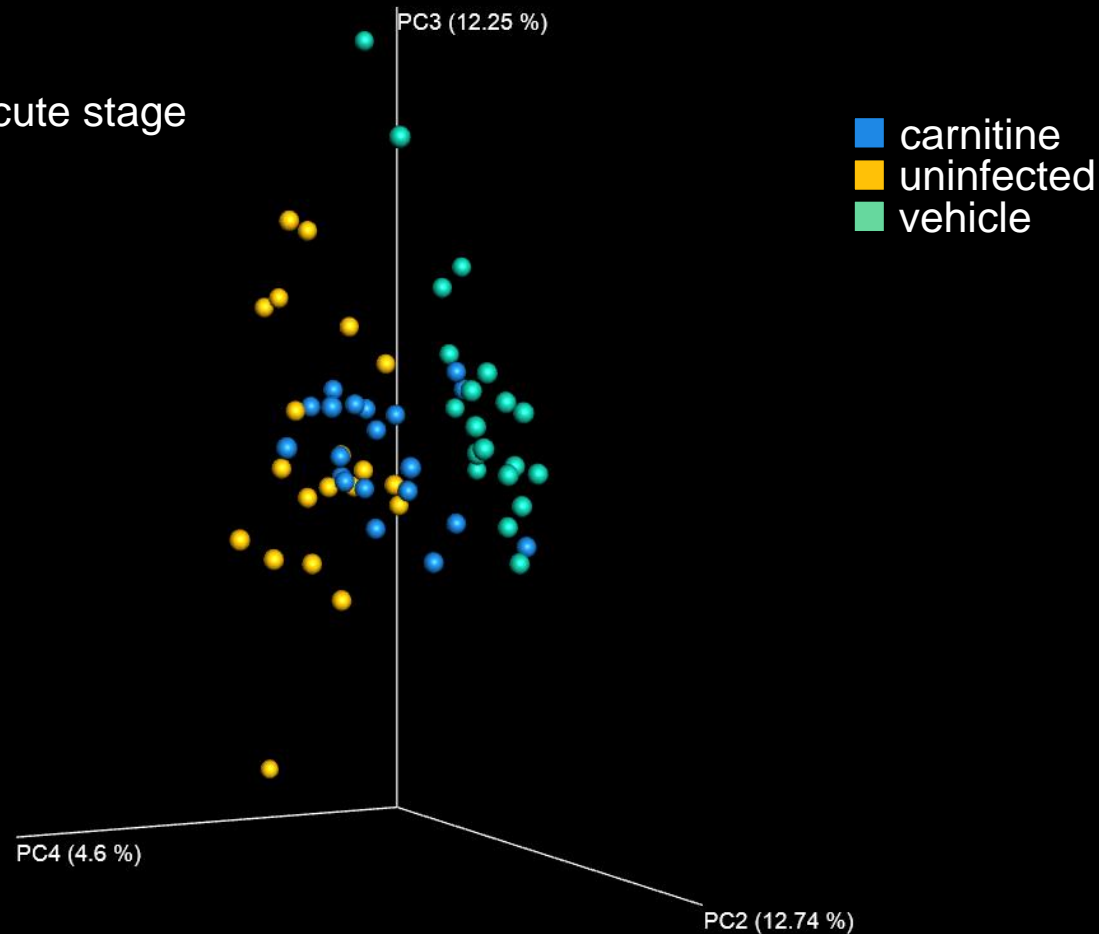
Carnitine supplementation does not affect the cardiac immune profile



p>0.05, Student's T-test carnitine vs vehicle

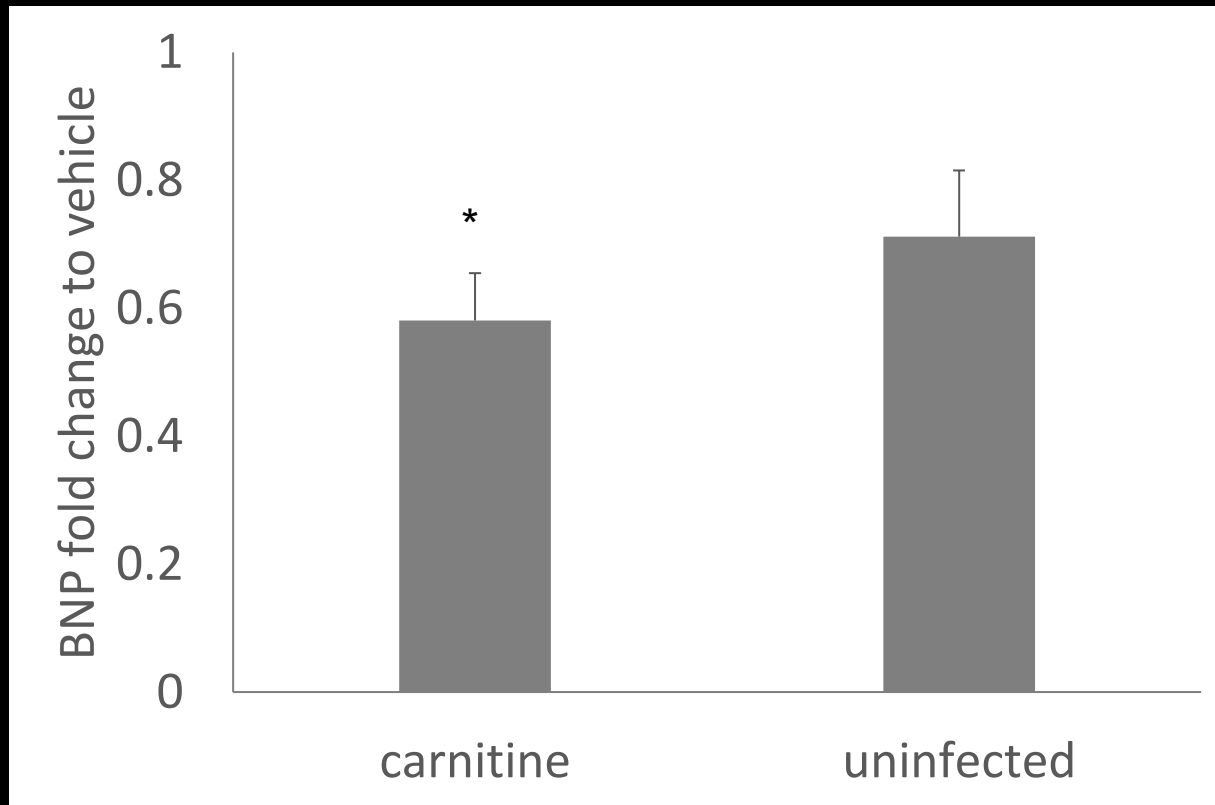
Carnitine supplementation “resets” cardiac metabolism

10 days of treatment, acute stage



$p < 0.05$ Mann-Whitney for reduced
distance carnitine-uninfected

Metabolic “resetting” reduces cardiac stress



* $p < 0.05$ treatment ΔCt to vehicle ΔCt

New targets: differential metabolic restoration depending on metabolite superclass

	Post-treatment status	Left ventricle top	Left ventricle bottom	Right ventricle top	Right ventricle bottom
Lipids and lipid-like molecules	Restored	80%	25%	100%	83%
	Not restored	20%	75%	0%	17%
Nucleosides, nucleotides, and analogues	Restored	/	33%	0%	33%
	Not restored	/	67%	100%	67%

New targets: differential metabolic restoration depending on metabolite superclass

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Chagas disease and chemical cartography: conclusions

- Metabolic explanation for Chagas disease tissue localization
- Identification of metabolic mechanisms of Chagas disease treatment failure
 - Novel LC-MS-based approaches to monitor treatment success
- Identification of a novel candidate for Chagas disease treatment
 - Novel metabolic mechanism of disease tolerance in Chagas disease

The **big** picture...

- Chemical cartography approach highlighted:
 - Novel insights into organ physiology
 - Identification of sites of tissue functional perturbation – these may not be the same as sites of pathogen persistence
 - Factors influencing disease pathogenesis
 - Integration with microbiome research
- New paths for patient monitoring and intervention
 - Identification of novel pathways that can be modulated for treatment
 - Predicting mild vs severe disease: determining who needs treatment the most
 - Assessment of treatment efficacy
- Spatio-metabolic approach to understand infectious diseases

Acknowledgements

OU – McCall lab

* = current members

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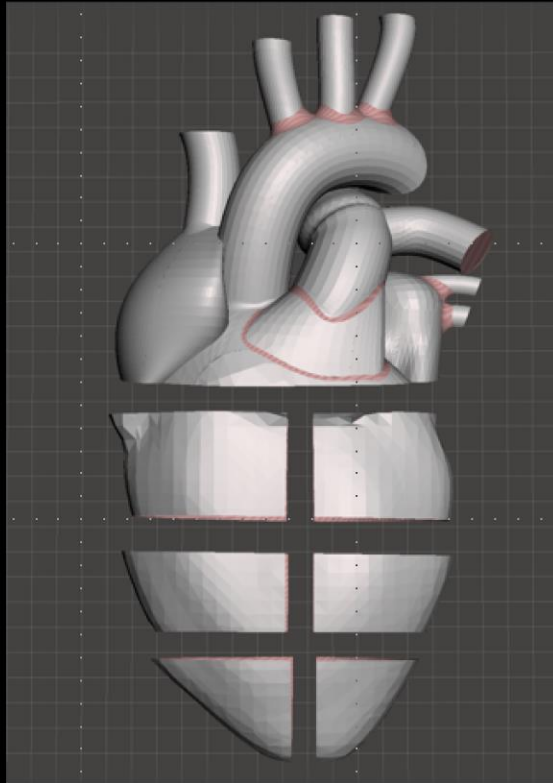
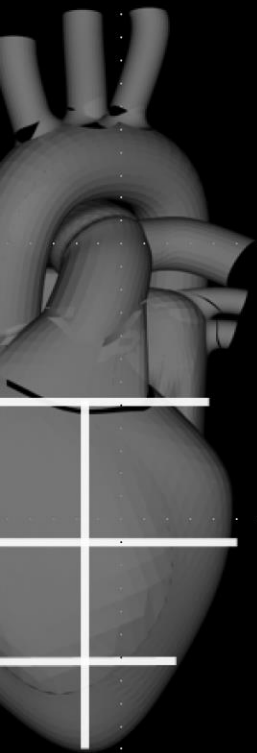
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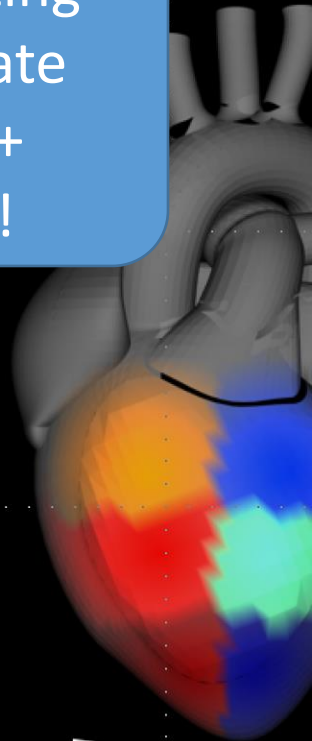
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R01AI177469,
R01AI170605,
R01GM145649,
R21AI148886 and
R21AI156669



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