Elucidating host and parasite factors involved in *Leishmania* quiescence

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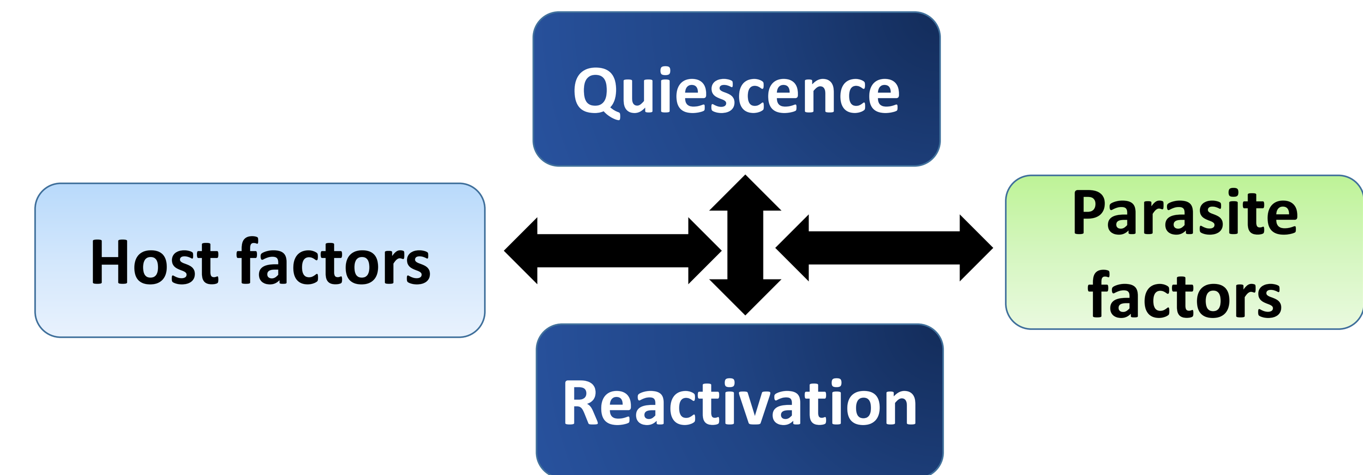
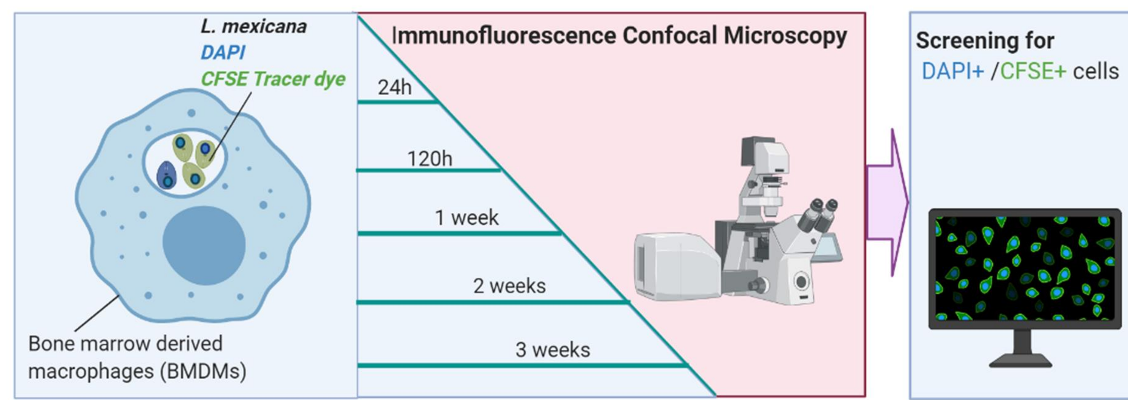
Introduction

Macrophages play a significant role in the immune response including pathogen clearance and presentation of antigen to activate T cells. Despite these antimicrobial properties, macrophages serve as favourable niches for long-term persistence of numerous pathogens, including *Leishmania mexicana*. Quiescent, non-dividing *Leishmania* are hypothesized to persist despite immunological stressors and anti-leishmanial chemotherapy and may contribute to relapses of infection month to years after treatment. The later often is associated with immunosuppression. We aim to understand **(1) which host factors contribute to *Leishmania* quiescence** and **(2) understand parasite factors underlying this quiescent phenotype.**



Aim 1: To establish an infection-model in which we can observe and study quiescent cells

Figure 1: ***Experimental strategy - Long-term infection to trace quiescent L.mexicana.*** Bone marrow derived macrophages (BMDMs) were infected with CFSE Cell tracer- labelled *L.mexicana* for 24h, 120h, 1 week, 2 weeks or 3 weeks. Media was changed ever 1-2 days as the BMDMs were continuously stimulated with M-CSF. DAPI was used to detect the parasite nuclei. CFSE cell tracer dye was used to detect non-dividing or slow- dividing *L.mexicana*.



L.mexicana amastigotes retain Cell tracer dye 3 weeks after infection

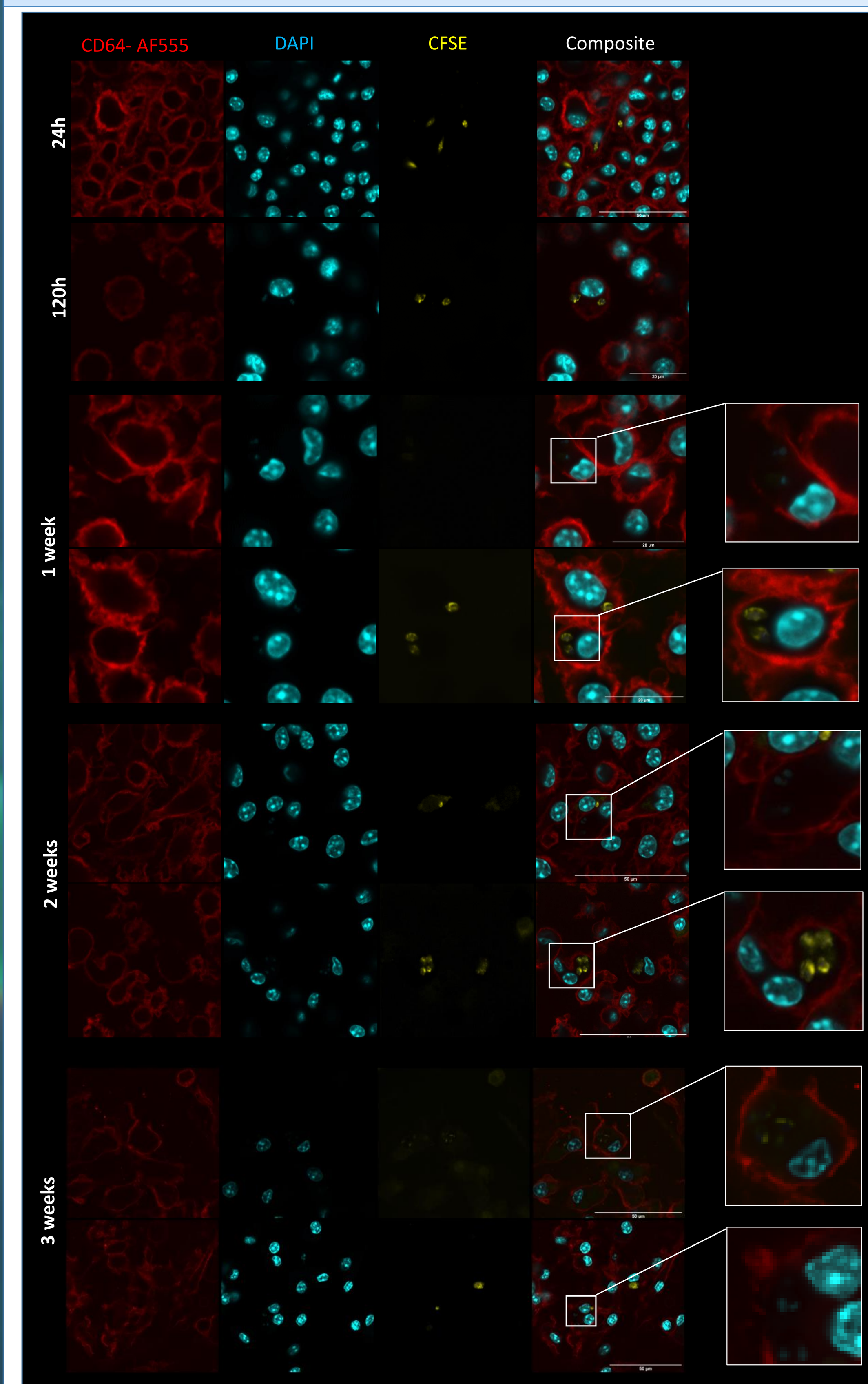
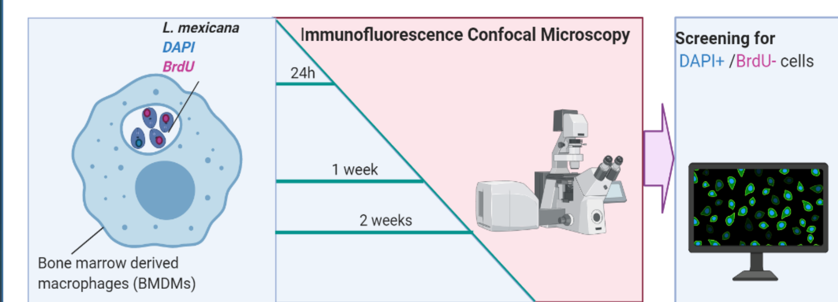


Figure 2: ***Confocal images of BMDMs infected with CFSE-labelled L.mexicana.*** After 1, 2 and 3 weeks both *L.mexicana,* that had lost the dye, and *L.mexicana,* that had retained the dye, were detected by confocal microscopy, suggesting that quiescent amastigotes can be detected *in vitro* inside of macrophages.

Figure 3: ***Experimental strategy to detect non-proliferating L. mexicana over the course of 2 weeks.*** BMDMs were infected with *L .mexicana* for 24h, 1 week or 2 weeks. Infected BMDMs were pulse labelled with BrdU 24h (24h timepoint) or 5 days prior to collection (1 week and 2 weeks timepoints). BrdU integration was detected with Anti-BrdU antibodies conjugated to AF647.



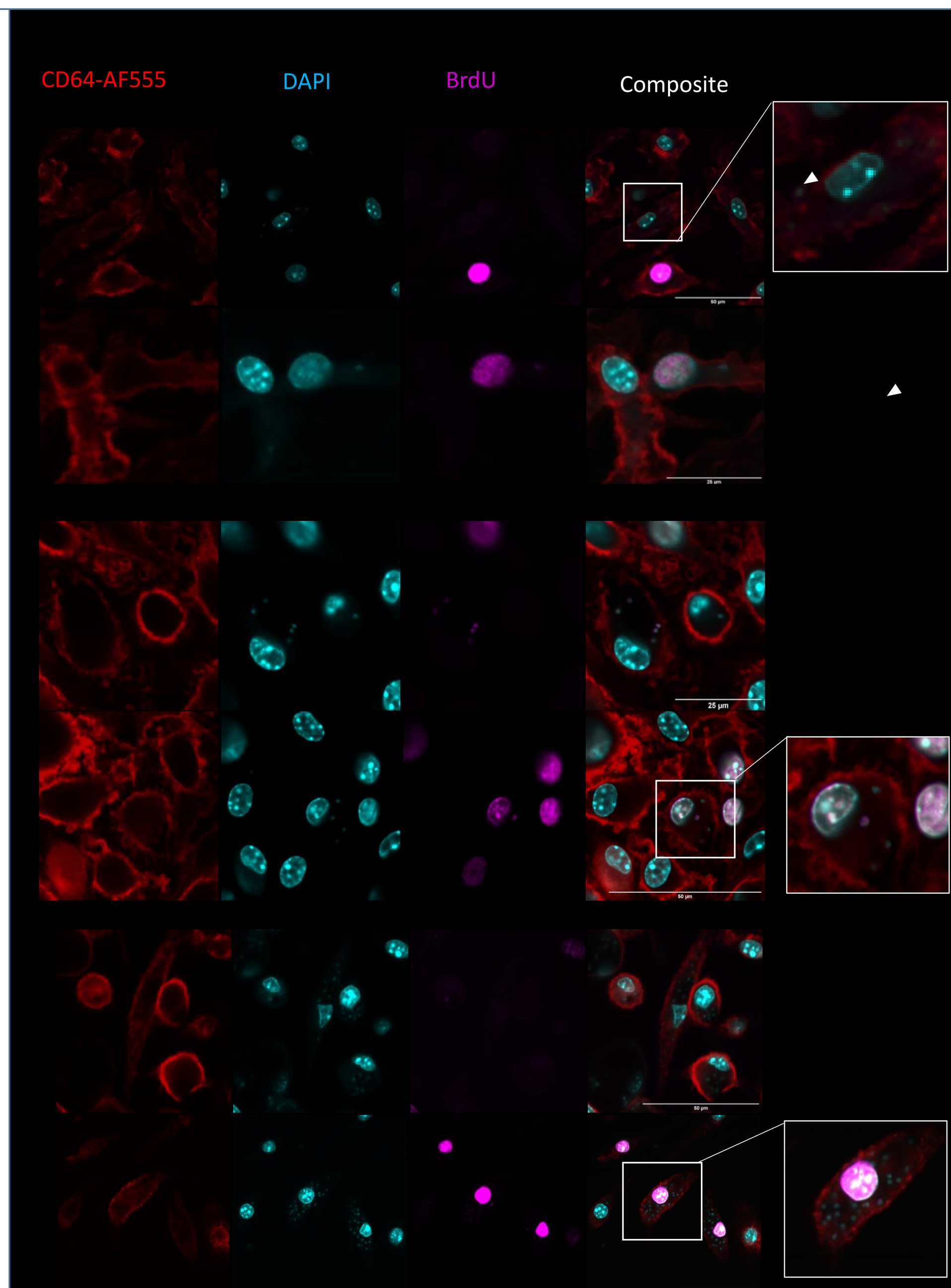
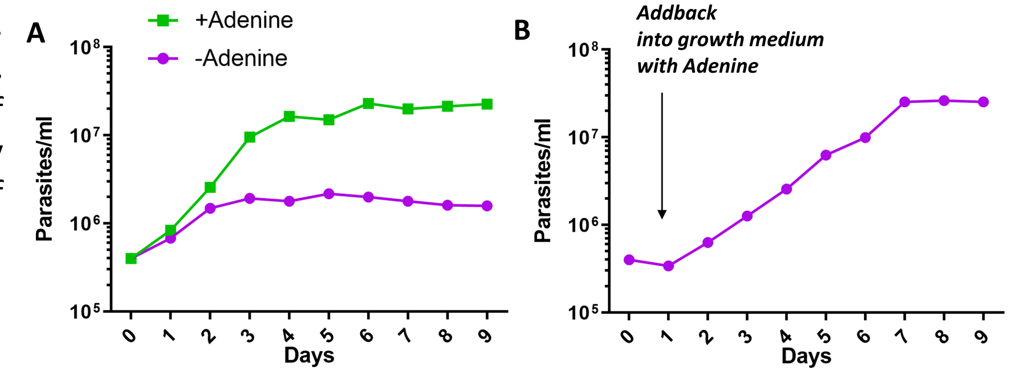


Figure 4: ***Confocal images of BMDMs infected with CFSE-labelled L.mexicana.*** After 1 and 2 weeks two populations, BrdU+ and BrdU- population were detectable *in vitro,* confirming that some *intracellular L. mexicana* are non- proliferative *in vitro.*

Aim 2: To identify markers of "quiescence" by studying purine-starvation induced growth arrest

Figure 5: ***Purine-starvation model of quiescence.*** Method developed after Carter et al. 2010. *L.mexicana* promastigotes that are starved of purines (i.e. Adenine) undergo a temporary growth-arrest (A), that is reversible by addition of a purine source (Adenine) (B).



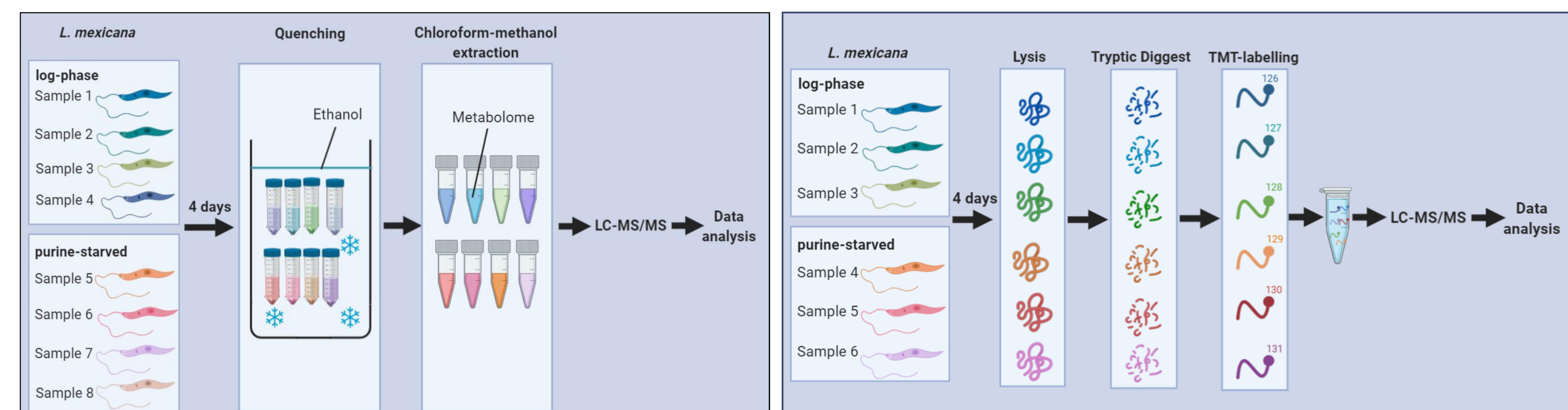


Figure 6: Experimental strategy - Metabolomics and proteomics of *L. mexicana,* that had been starved for 4 days.

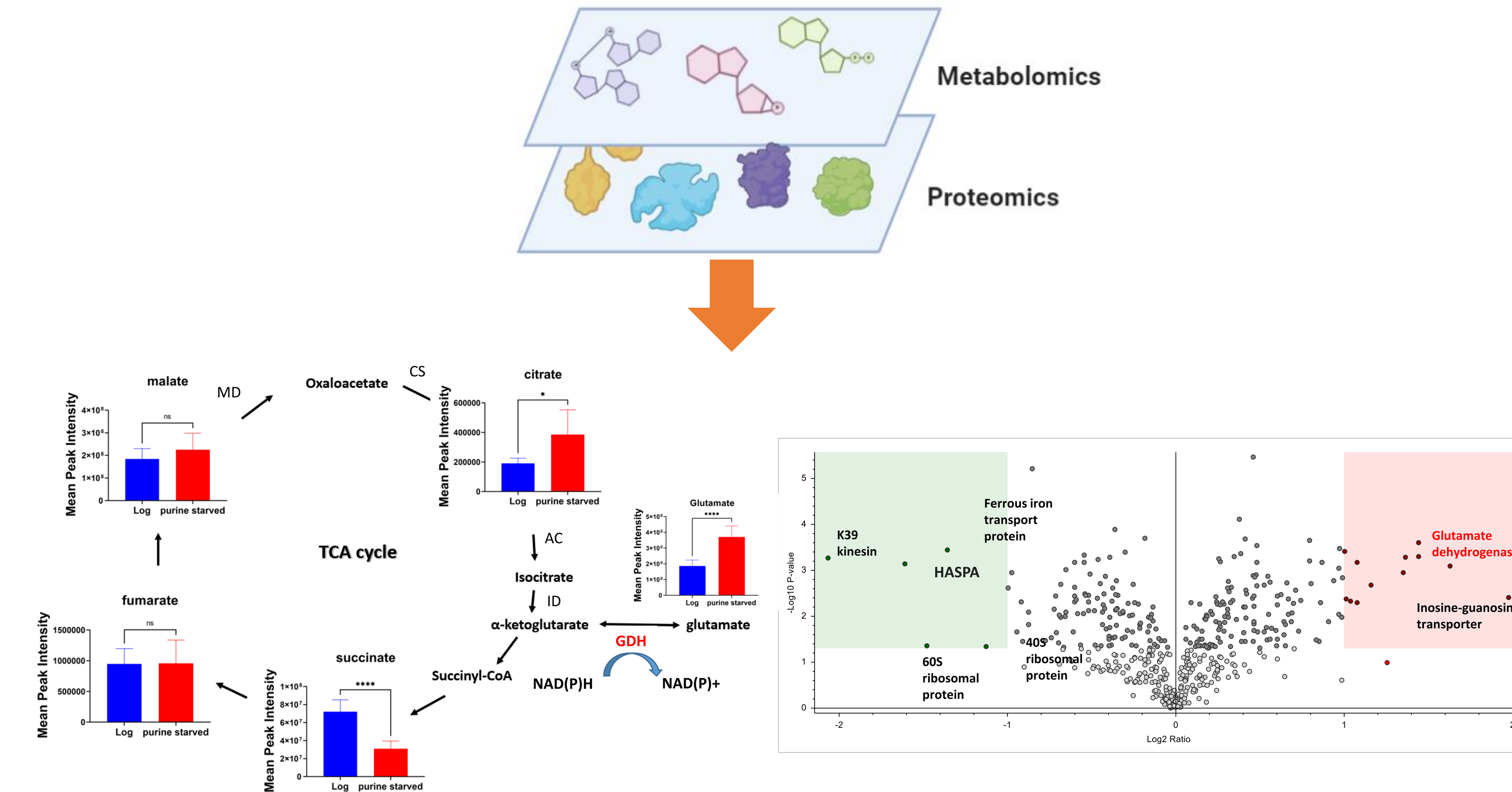


Figure 7: TCA-Cycle metabolites changed in purine-starved *L. mexicana.* *GDH: Glutamate dehydrogenase, CS: Citrate synthase, AC: aconitase, ID: isocitrate dehydrogenase, MD: malate dehydrogenase* were also identified and upregulated in the proteomic data set.

Figure 8: Volkano plot showing up- and downregulated proteins in purine-starved *L. mexicana* compared to Log-phase *L . mexicana.* Glutamate dehydrogenase is one of the most differentially up-regulated proteins in purine-starved *Leishmania.*

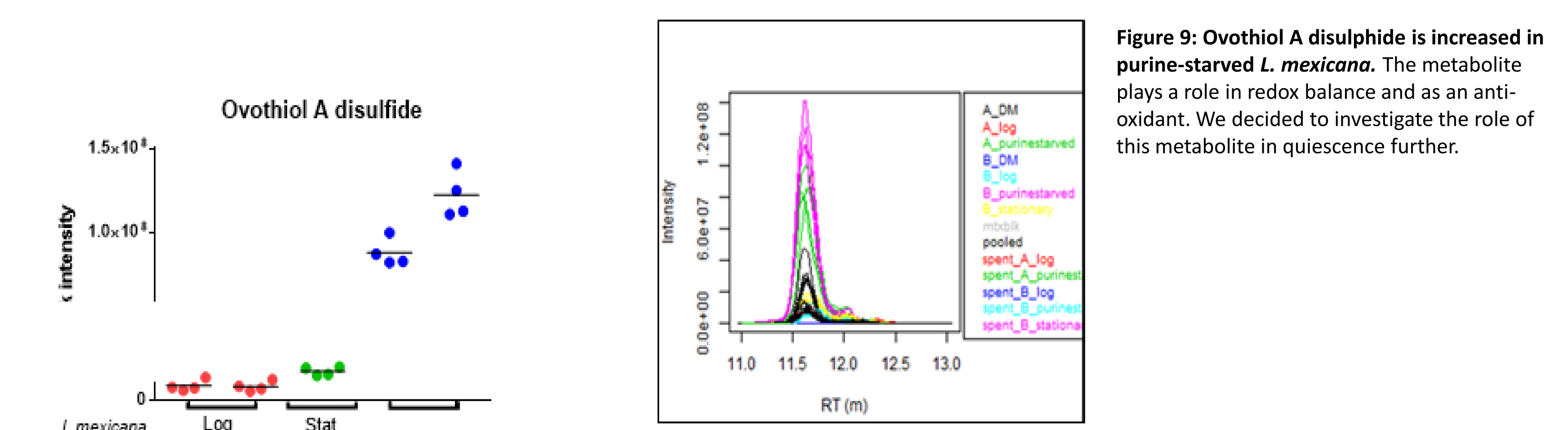


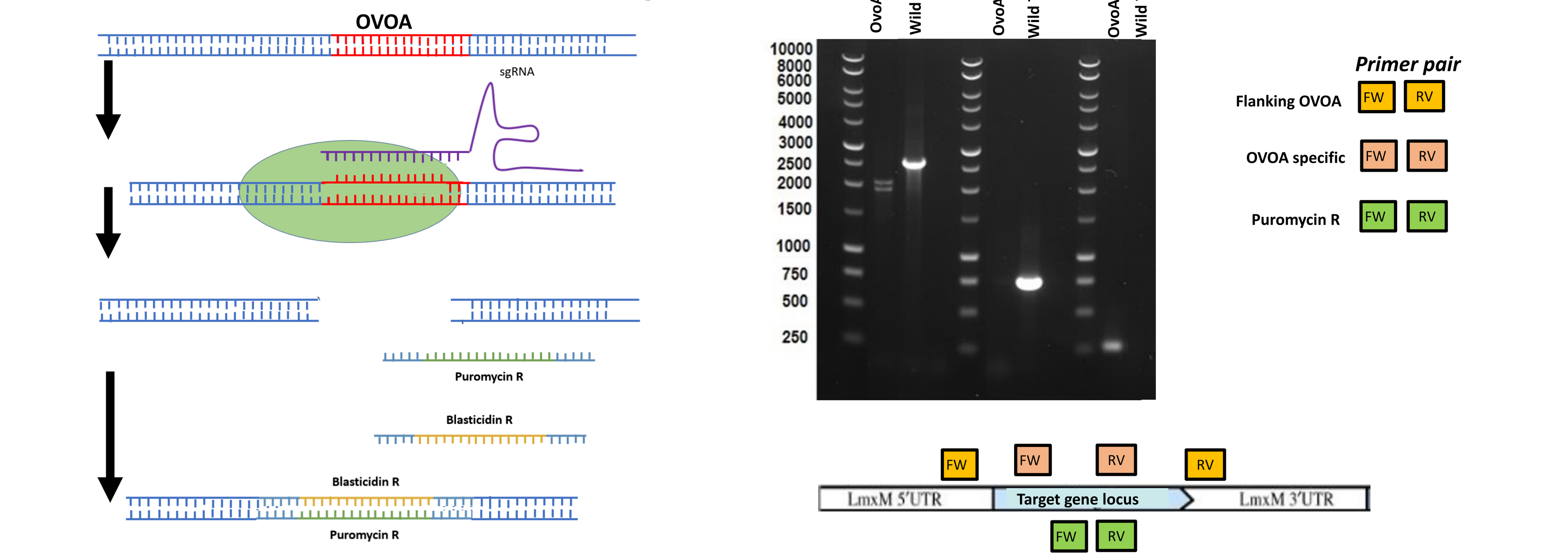
Figure 9: Ovothiol A disulphide is increased in purine-starved *L. mexicana.* The metabolite plays a role in redox balance and as an anti-oxidant. We decided to investigate the role of this metabolite in quiescence further.

Aim 3: To determine the importance of Ovothiol A in determining "quiescence"

The OVOA gene encodes the 5-Histidylcysteine sulfoxide synthase required for the generation of Ovothiol A. The target gene is replaced with two resistance cassettes facilitating Blasticidin or Puromycin resistance.

Method

CRISPR Cas9-mediated Knock out of OVOA gene



Future directions

We aim to use the intracellular model of "quiescence" to study the role of these parasites in drug treatment failure and determine if host factors influence this phenotype. We further would like to investigate the role of Ovothiol A further purine- starvation induced growth arrest.

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