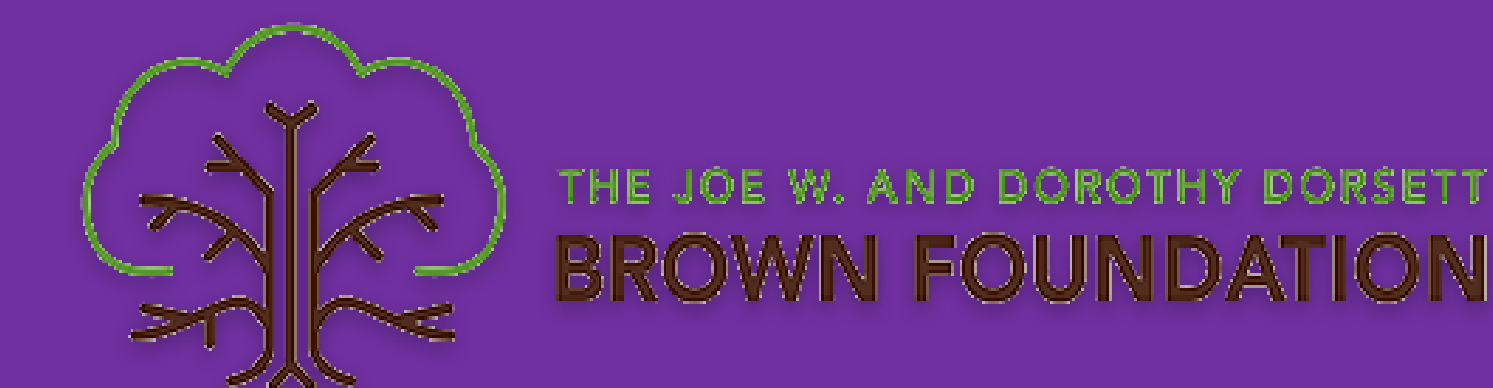


Temperature-dependent regulation of cytochrome c oxidase subunit expression in *Leishmania*

Kaleah J. Smith, Isabel Stephany-Brassesso and Ben L. Kelly

Department of Microbiology, Immunology and Parasitology, LSU Health Science Center
New Orleans, LA



Introduction

Leishmaniasis is a chronic, persistent, and often fatal infection caused by various species of the protozoan parasite, *Leishmania*. Transmission occurs via the bite of female phlebotomine sandflies (see Fig. 1.) and >12 million people, living primarily in tropical and subtropical regions, are infected.

Current treatments against leishmaniasis are toxic and drug resistant parasites are more incident. Therefore, there is an urgent demand for better, low-toxicity drugs against *Leishmania*.

To meet this demand, a better understanding of essential parasite-specific gene expression and metabolism is expected to guide the development of effective anti-leishmanials.

Our laboratory has discovered a novel gene regulatory pathway, whereby a subunit of *Leishmania* mitochondrial cytochrome c oxidase subunit 4 (LmCOX4) is transiently lost upon exposure to mammalian temperature (35°C) (see Fig. 2.).

Significantly, LmCOX4 is critically important for *Leishmania* energy metabolism, including production of ATP; thus, LmCOX4 represents a validated drug target. Further, the initial loss of LmCOX4 upon entering the mammalian host has important implications for understanding how *Leishmania* maintains mitochondrial fitness to survive in the mammalian host.

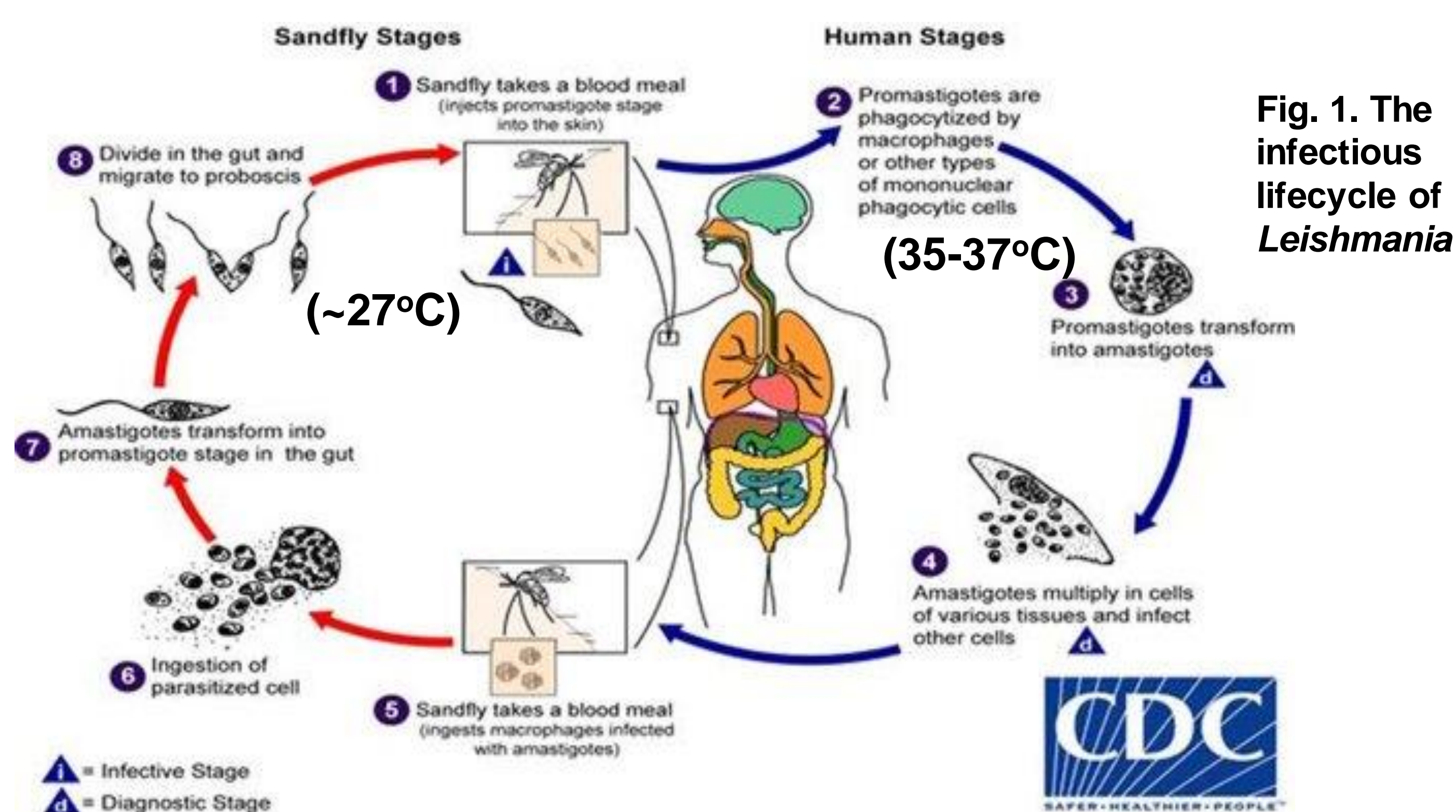
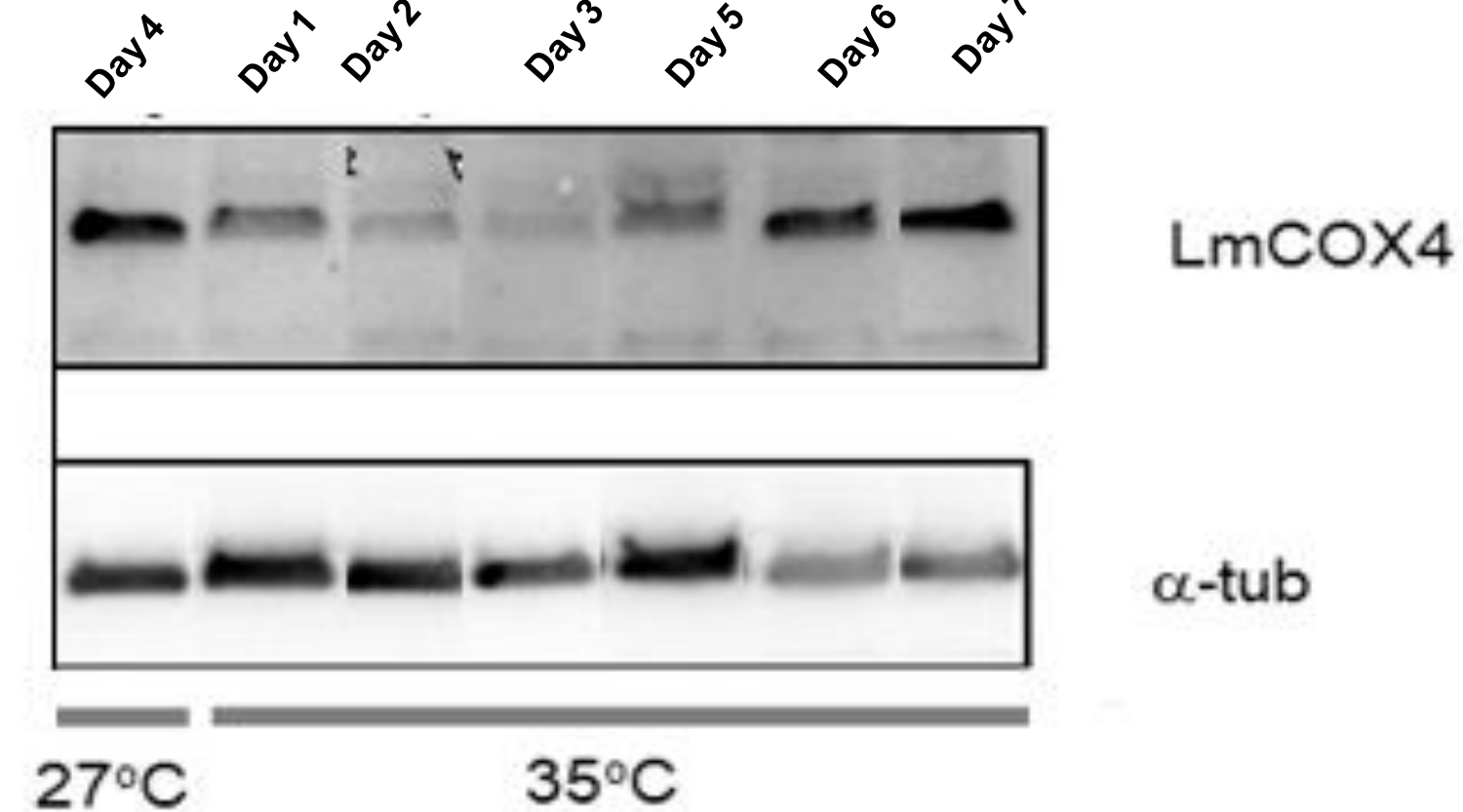


Fig. 2. LmCOX4 is transiently lost, following exposure to mammalian temperature¹

Western blot showing expression of LmCOX4 under normal insect temperature (27°C) versus exposure to mammalian temperature (35°C). Expression of α -tubulin (α -tub) as a protein loading control is indicated.



¹Adapted from Shaheen *et al.*, Dynamic modulation of *Leishmania* cytochrome c oxidase subunit IV (LmCOX4) expression in response to mammalian temperature. *Molecular and Biochemical Parasitology* 244 (2021) 111391.

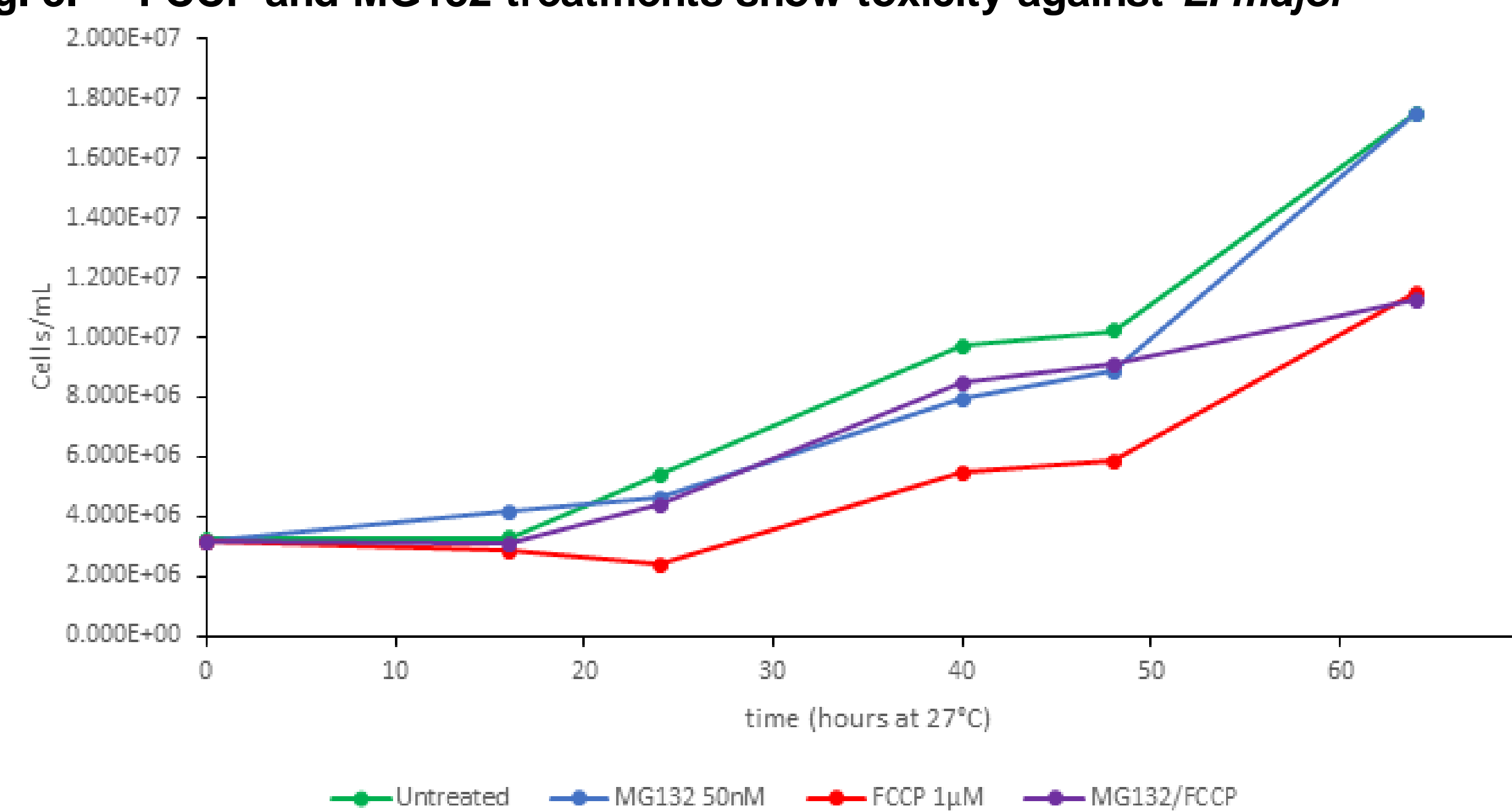
Objectives

To understand molecular mechanisms that underlie transient loss of LmCOX4 at 35°C, experiments to examine the relationship between LmCOX4 cellular abundance, heat stress (35°C) and mitochondrial import are in progress.

Currently, we are using the pharmacologic agent Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), a disrupter of mitochondrial membrane potential, to inhibit translocation of LmCOX4 from the cytosol into the mitochondria. Once optimized, these experiments will allow us to establish the importance of mitochondrial protein localization and mitochondrial function in control of LmCOX4 abundance. Such preliminary studies may then allow us to determine whether the ~35°C heat stress encountered by *Leishmania*, following entry into mammalian host, disrupts LmCOX4 translocation, as a possible mechanism for LmCOX4 loss.

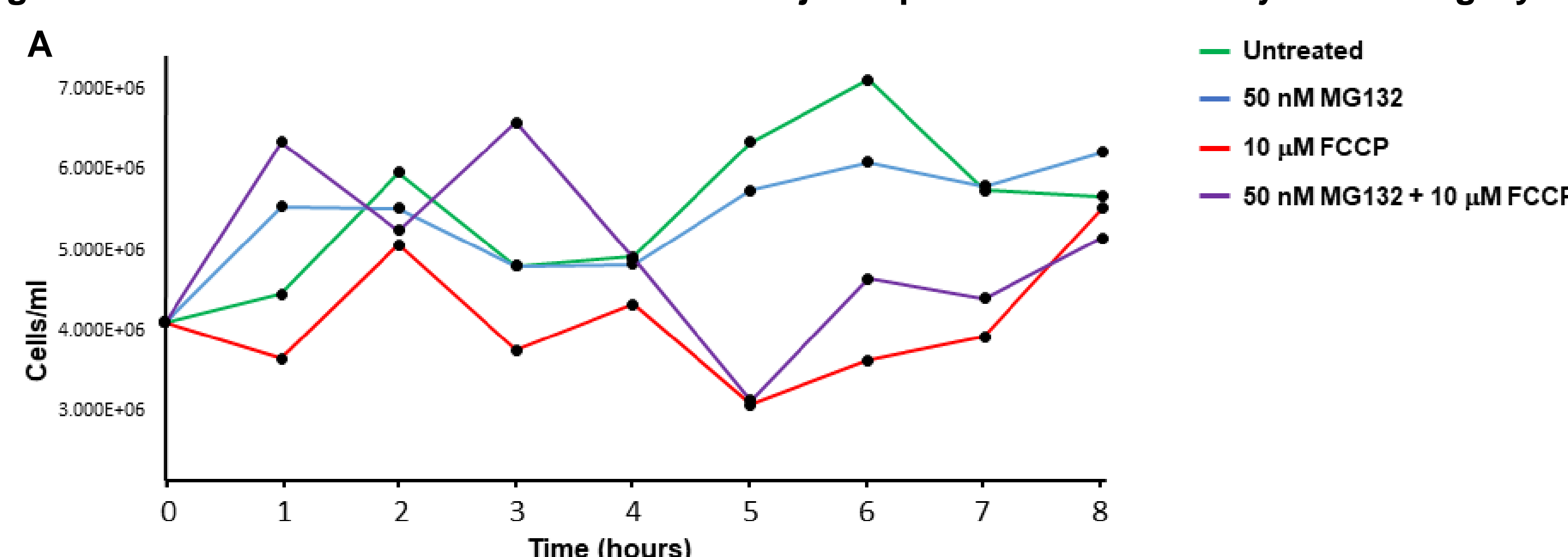
Results

Fig. 3. FCCP and MG132 treatments show toxicity against *L. major*



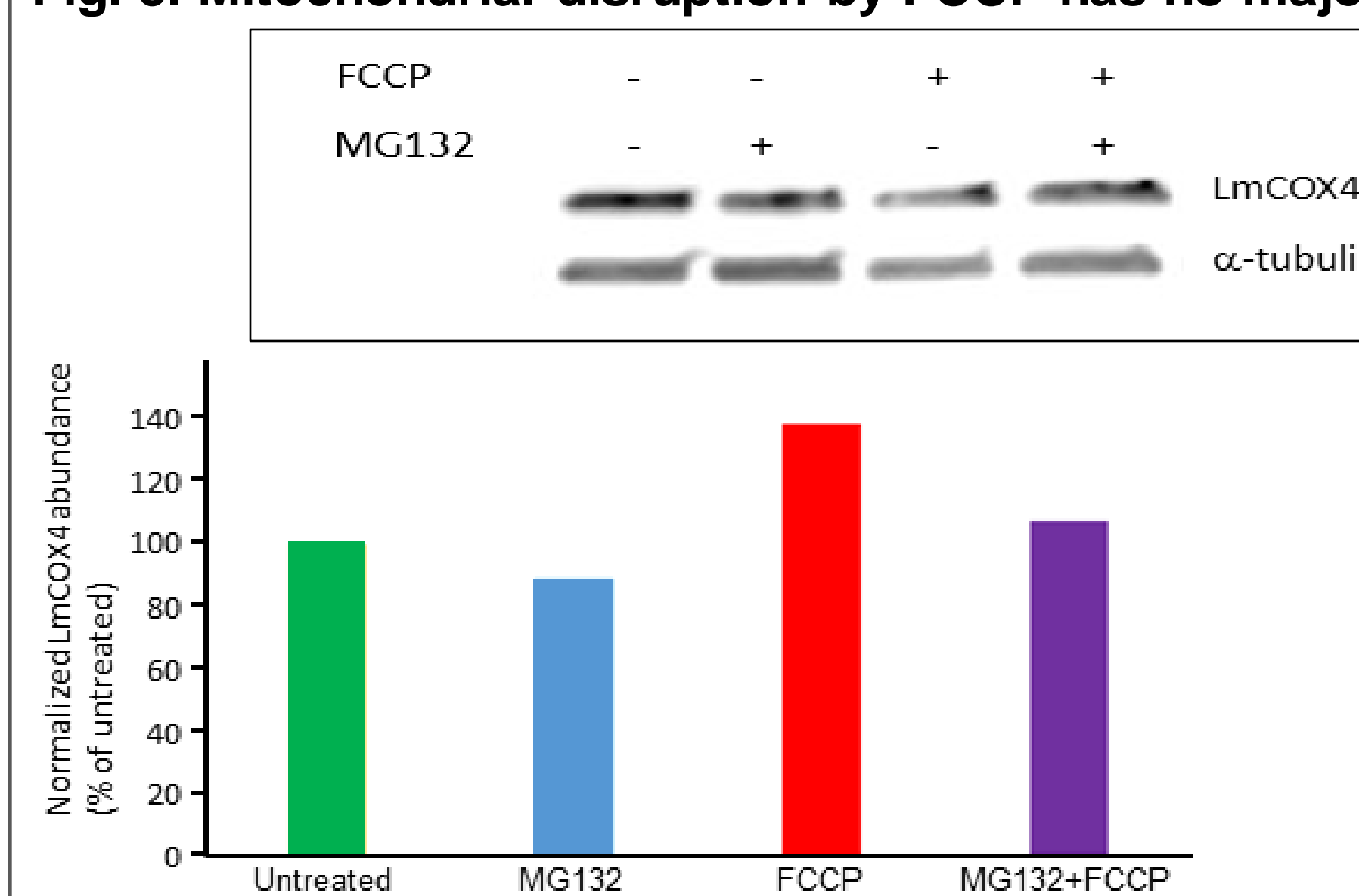
L. major cells were incubated *in vitro* in the presence of MG132 (a proteasomal protein degradation inhibitor), FCCP, or both inhibitors as indicated for up to 64 hr. The parasites were counted using a hemocytometer. Both FCCP and MG132 show toxicity against *L. major*. FCCP shows greater toxicity against *L. major* at the indicated time points.

Fig. 4. FCCP and MG132 treatments have no major impact on overall *L. major* cell integrity



A: *L. major* cells were incubated *in vitro* in the presence of MG132 (a proteasomal protein degradation inhibitor), FCCP, or both inhibitors as indicated for up to 8 hr. The parasites were counted using a hemocytometer. Although these treatments are known to be toxic to *Leishmania* cell growth, they have less than 2-fold impact on parasite cell numbers and integrity during the 8 hr assay period used for Western blot analysis of LmCOX4 expression (Fig. 5). B: Phase contrast microscope image (400X magnification) showing *L. major* promastigotes cultured *in vitro*.

Fig. 5. Mitochondrial disruption by FCCP has no major effect upon LmCOX4 abundance



L. major cells were incubated in the presence of MG132, FCCP, or both inhibitors as indicated in Fig. 3. for 8 hr at 27°C. Although a slight increase in LmCOX4 abundance was observed, this finding is not conclusive since this experiment has only been performed once. MG132 was used to stabilize any mis-localized LmCOX4 precursor (containing the mitochondrial targeting sequence potentially expected under FCCP treatment) to be otherwise degraded by the *Leishmania* proteasome. The LmCOX4 precursor, predicted to be 28 amino acids larger than mature mitochondrial LmCOX4 was not observed, however. Bar graph (lower panel) indicates quantitation of α -tubulin-normalized LmCOX4 expression from the Western blot shown in the upper panel.

Summary, Conclusions and Future Studies

Our preliminary experiments to establish FCCP as a useful reagent to examine the importance of mitochondrial function and protein translocation upon LmCOX4 import and abundance have demonstrated the following:

FCCP is toxic to *Leishmania*, consistent with its known ability to disrupt mitochondrial function and mitochondrial protein translocation in other eukaryotes

Despite this toxicity, and consistent with its predicted specific effects on mitochondria, FCCP does not disrupt the general cellular integrity during the 8 hr assay period used.

During this 8 hr assay period, no major FCCP effects upon LmCOX4 mitochondrial translocation (indicated by absence of the predicted larger nuclear-encoded LmCOX4 precursor protein) nor its abundance were observed.

We conclude that either, despite its toxicity, FCCP does not disrupt mitochondrial transport in *Leishmania*, or that we need to further optimize FCCP treatment conditions to observe this effect. With respect to LmCOX4 abundance, we conclude that either FCCP has no effect on LmCOX4 abundance, or further optimization is needed.

In our future studies we will optimize these experiments by testing different doses of FCCP and include longer exposure times. We will also perform replicate sets of experiments for statistical significance.

