Growth Rates of Miltefosine-Resistant *Leishmania* Mutants

Espada, C. R. et al. (2021). Ros3 (Lem3p/CDC50) Gene Dosage Is Implicated in Miltefosine Susceptibility in Leishmania (Viannia) braziliensis Clinical Isolates and in Leishmania (Leishmania) major. *ACS Infectious Diseases*, 7(4), 849-858. DOI: 10.1021/acsinfecdis.0c00531.

Leishmania mutants; *major/ braziliensis*; drug resistance	
Index	BP2
Standardization	
Counting	X
Viability	
Volume	

The Challenge:

Characterizing how *ros3* gene dosage variations influence the susceptibility and cellular uptake of miltefosine in *Leishmania* parasites to understand clinical drug resistance mechanisms.

CASY's Contribution:

CASY automated doubling time characterization for wild-type and *ros3* knockout strains. The data confirmed that while complete *ros3* removal increased miltefosine resistance 20-fold, it caused no significant growth impairment. This validated that resistance stemmed from transport defects rather than metabolic bias.

Key Benefits to Researchers:

Precise Characterization: CASY's 60µm capillary enabled exact cell density measurements to accurately determine the doubling time of multiple *Leishmania* clones.

Bias Mitigation: Standardized growth rate analysis ensured that drug susceptibility assays were not skewed by variations in cell proliferation across different mutant lines.

Robust Validation: Provided the critical quantitative evidence that *ros3* gene dosage is a primary determinant of drug uptake, independent of parasite fitness or kinetics.

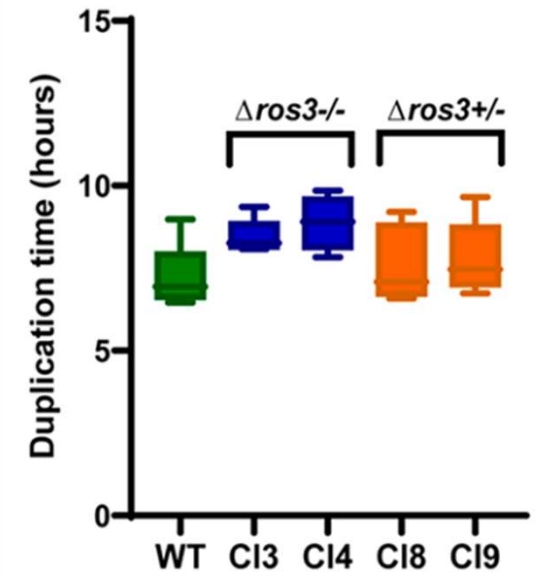

Figure 3B: (Parasite doubling time). This graph illustrates the duplication times for WT and various *ros3* knockout clones, utilizing cell density data generated by the CASY system to confirm growth consistency.