

An RBPome functional screen identifies a master regulator of *Leishmania* amastigote metabolism

E R.S. Parry¹; N M.M. Teles¹; RP Neish¹; K Newling³; FS Pais²; AA Dowle⁴; A Droop²; S James³; M Cayla¹; JC Mottram¹; PB Walrad¹;

¹ YBRI, Dept of Biology, University of York, UK; ² Data Science Lab, Bioscience Technical Facility, Dept of Biology, University of York, UK; ³ Genomics Lab, Bioscience Technical Facility, Dept of Biology, University of York, UK; ⁴ MAP Lab, Bioscience Technical Facility, Dept of Biology, University of York, UK.

Gene regulation in *Leishmania* species is overwhelmingly post-transcriptional. This elevates the importance of RNA binding proteins (RBPs) in gene expression control for these systems. Building upon the comparative *L. mexicana* RBPome we previously isolated (Pablos et al. MCP, 2019), 70 candidate *trans*-regulators were selected for further investigation. An *L. mexicana* barcoded *trans*-regulator knockout library was created using CRISPR-Cas9 and screened for lifecycle progression, including macrophage and mouse infections. Null mutants could not be generated for 60% of RBP genes screened; suggesting a high proportion of RBPs are essential for cell viability. Of the 27 viable RBP null mutants 64% exhibit loss of fitness during lifecycle progression to human-infectious stages, infectivity and/or virulence. Examination of individual null mutants verify competitive bar-seq screen outcomes that isolate specific RBPs implicit in parasite viability, replication and infectivity. Of these, 3 RBPs were endogenously tagged and immunoprecipitated. This includes the previously-unidentified *Leishmania* homolog of the Trypanosome respirome master regulator ZC3H40. In *Leishmania* we find ZC3H40 protein binds its essential cofactor ZC3H39 and associates and stabilises RNAs encoding essential mitochondrial proteins as well as both ZC3H40 and ZC3H39 mRNA, ensuring metabolic capacity in the amastigote human disease-causing form. This lends significant insight into the relatively uncharacterised *Leishmania* respirome requirements in human infectious stages and validates the biological relevance of our screen.

Pablos, LM, Ferreira, TR, Dowle, AA, Forrester, S, Parry, E, Newling, K, PB Walrad, 2019, The mRNA-bound Proteome of *Leishmania mexicana*: Novel Genetic Insight into an Ancient Parasite, *Molecular & Cellular Proteomics*, 18 (7): <https://doi.org/10.1074/mcp.RA118.001307>.