Key Leishmania trans-regulators are essential for parasite surveillance and infectivity

Ewan Parry* (University of York), Natalia M. Monteiro-Teles* (University of York), Rachel Neish (University of York), Fabiano Pais (University of York), Adam Dowle (University of York), Katherine Newling (University of York), Jeremy C. Mottram (University of York), Pegine B. Walrad (University of York)

*Co authorship

Like other Kinetoplastids, gene expression in *Leishmania* species is overwhelmingly post-transcriptionally controlled. This elevates the importance of RNA binding proteins (RBPs) in these systems as the primary gene regulators. Building upon the *L. mexicana* RBPome we isolated previously from the 3 main parasite lifecycle stages (Pablos et al. MCP, 2019), 70 non-basal RBPs were selected toward further investigation. An *L. mexicana* barcoded *trans*-regulator knockout clone library was created using CRISPR-cas9 (Baker et al. Nat Comms, 2021) and screened through lifecycle progression and macrophage or mouse infections. Remarkably, 60% of the RBPs screened are essential for cell viability and 26% contribute to lifecycle progression to human-infectious stages, infectivity and/or virulence. Examination of individual knockout lines verify the screen outcomes of specific RBPs essential for parasite growth, viability and infectivity. 13 RBPs were endogenously tagged, immunoprecipitated and submitted to transcriptomic and proteomic analyses to identify all RNP components. Discrete complexes have been identified that may represent novel virulence factors. Further analyses are underway to map interaction dynamics of these key RNP regulators that drive differentiation and virulence capacity in *Leishmania*.

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