**Functional Profiling of Amino Acid Metabolism in Trypanosoma cruzi Using Bar-seq CRISPR-Cas9 Screening**

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In its complex life cycle, *Trypanosoma cruzi*, the causative agent of Chagas disease, faces drastic changes in temperature, nutrient availability, and osmotic pressure. One of the many tools that these parasites have evolved to cope with these challenges is their flexible metabolism of amino acids. Beyond the role in translation, amino acids participate in the control of cell volume, responses to different types of stress, and support parasite differentiation and infection. Aiming to identify which steps of the amino acid metabolic network are essential for *T. cruzi* to complete its life cycle, we generated 44 mutant knockout cell lines targeting enzymes putatively involved in amino acid metabolism, comprising 16 homozygous and 28 heterozygous lines. Using bar-seq CRISPR-Cas9 genome editing, each cell line was assigned a unique 17-nt barcode sequence to enable parallel phenotyping via next-generation sequencing. The mutant cell lines, along with a cell line expressing the thermostable red-shifted firefly luciferase PpyRE9h, were pooled in equal numbers to assess epimastigote proliferation and differentiation into metacyclic trypomastigotes *in vitro*. These infective forms were then purified and used to infect two mouse strains with distinct genetic backgrounds, BALB/c and immunodeficient SCID mice. Parasite load was monitored using blood parasitemia counts and bioluminescence. Approximately half of the infected mice showed a positive bioluminescence signal over the course of infection. At 25 days post-infection, all mice were euthanised, and bioluminescence analysis confirmed the presence of parasites in multiple tissues, particularly in the spleen, visceral fat, and intestinal regions. Samples for gDNA isolation and library preparation were collected throughout the parasite proliferation and differentiation *in vitro*, and from both blood and infected organs in the infection experiments *in vivo.* The relative fitness throughout the various stages of the life cycle will be assessed by quantifying each barcode abundance. Together, these approaches will allow the identification of amino acid metabolic steps that are vital for parasite development and infectivity, and that may represent novel therapeutic targets against *T. cruzi*.