Single-cell RNA-sequencing analysis of life and cell-cycle progression in *Leishmania mexicana*.

A crucial life-cycle development stage of Leishmania takes place in the macrophage vacuole, where metacyclic promastigotes differentiate into amastigotes. Dynamic changes in transcription underlying these complex cellular transitions through life cycle stages have not yet been described in detail. To evaluate these changes and identify putative differentiation regulators, we employed single-cell RNA sequencing (scRNA-seq) at five time-points as *L. mexicana* differentiated from promastigotes to axenic-amastigotes in culture. Clustering analysis of over 16,500 parasites across three experiments revealed five developmental stages, including promastigote and amastigote forms, as well as populations transitioning between these. From these data, we identified over 800 differentially expressed markers for a metacyclic-like cluster. Our analysis additionally suggests we can transcriptionally distinguish different replicative promastigote forms going through cell-cycle stages, which have previously been associated with changes in morphology. We also explored one transitional group of cells between the metacyclic and axenic amastigotes, revealing genes possibly associated with survival strategies required during the switch from the insect vector to mammalian host. The many novel genes detected include some that have been found to be essential for life-cycle progression in *Trypanosoma brucei* but have not yet been investigated in *L. mexicana*, as well as cell-cycle progression markers. Trajectory inference analysis further revealed phasic expression of transcripts of interest within the transitional and cell cycling clusters. To explore this transitional population in more detail, we performed scRNA-seq of *L. mexicana*-infected human macrophages, allowing us to ask if the transcriptional processes seen during differentiation to axenic amastigotes in culture are also seen as amastigotes form in a host cell. Ultimately, our aim is to find and validate potential new targets for halting the development of the parasite within the mammal, and thereby limiting infection and disease.