S3 Fig. Acquisition of amastigote traits by *L. major* cultured at 37⁰ C in the absence of host cells.

*L. major* promastigotes were cultured until the third day of stationary phase at 26⁰ C, then placed in fresh media and cultured at 37⁰ C for 24 hours prior to harvest and analysis. (A) Parasites were fixed and stained with the indicated markers prior to confocal microscopy. Micrographs were visually scored for PFR negativity or T17/T18 positivity. N > 200 parasites. (B) Flow cytometric analysis of YFP fluorescence of the starting culture of stationary phase parasites (green, 26⁰ C) and parasites following 24 hours of culture at 37⁰ C (purple). (C) Representative electron micrograph of a parasite following culture at 37⁰ C that has typical amastigote morphology including a round shape and a spacious flagellar pocket.