Histidine ammonia-lyase knockout alters bioenergetics of *Trypanosoma cruzi* without affecting its infection capability in the insect vector

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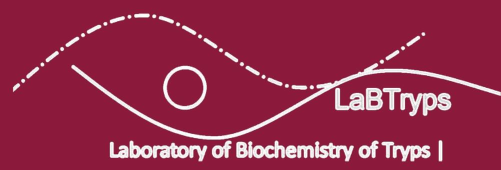

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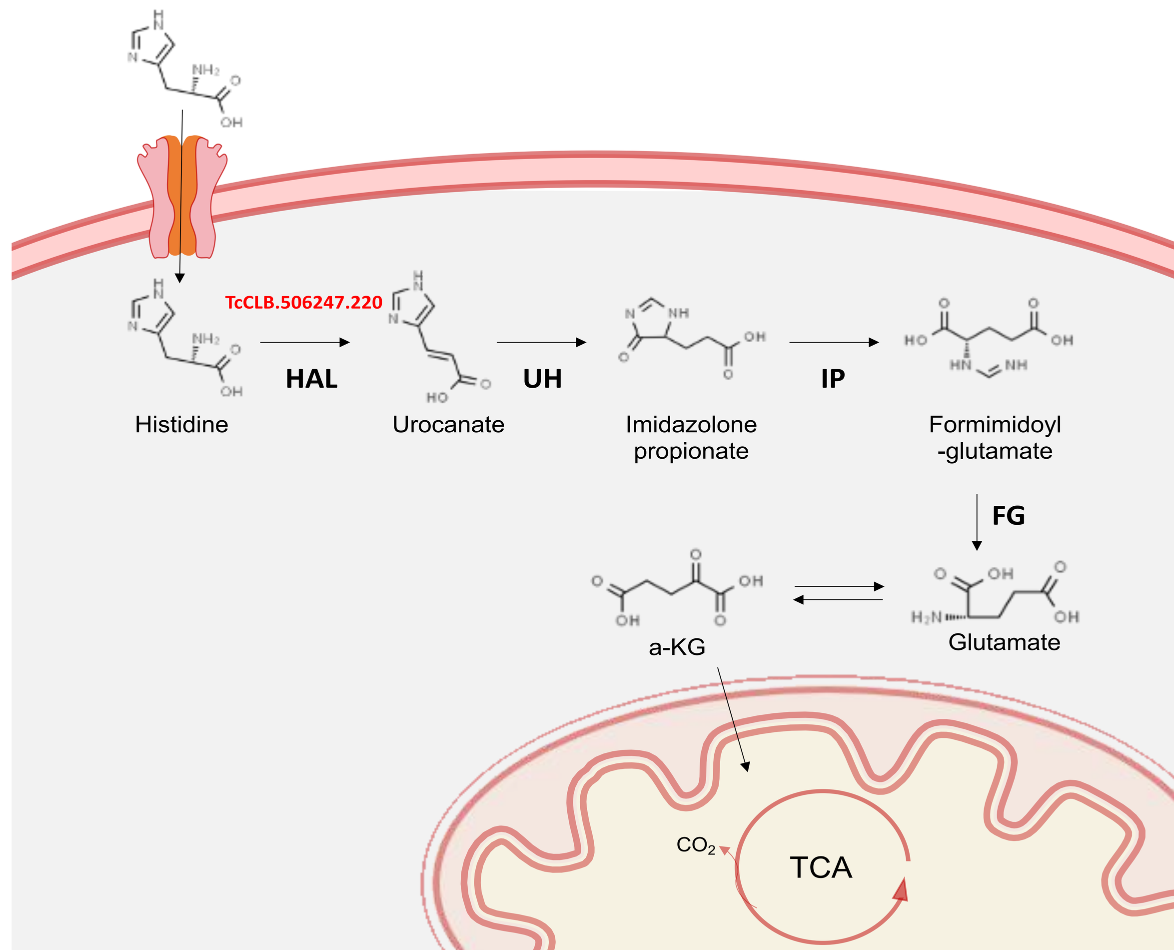

Figure 1. The histidine degradation pathway leads to the formation of glutamate through four enzymatic steps. *Trypanosoma cruzi* has putative coding sequences for the four enzymes involved in His degradation. Previous data from our lab has shown that *T. cruzi* can transport and fully oxidize His to CO_2 via the tricarboxylic acid cycle. HAL: histidine ammonia-lyase; UH: urocanate hydratase; IP: imidazolonepropionase; FG: formiminoglutamase.

TcHAL knockout generated by CRISPR-Cas9 does not affect epimastigotes proliferation

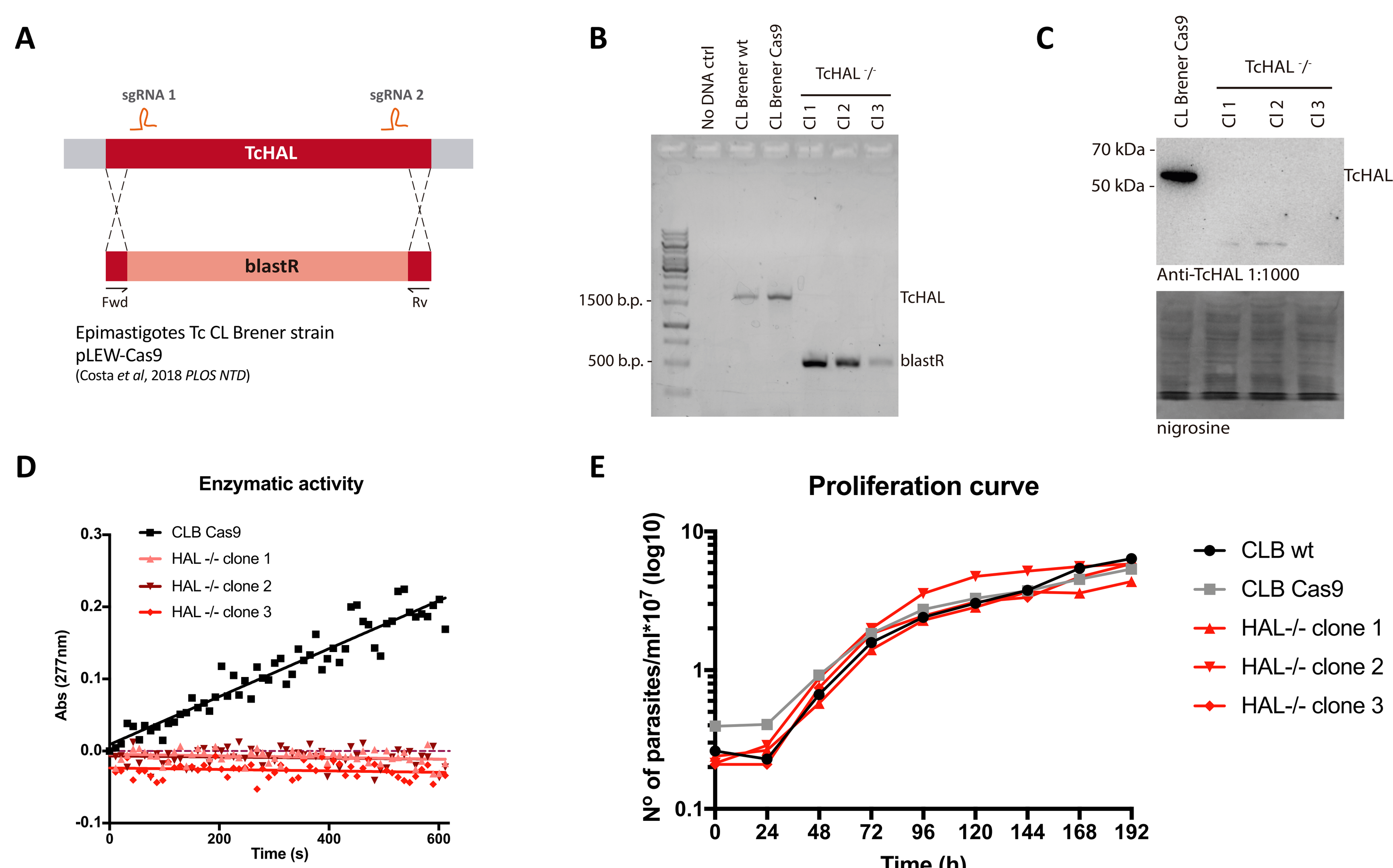

Figure 2. A. Knockout strategy. Double-knockout of histidine ammonia-lyase (TcHAL) was generated by CRISPR-Cas9 using the CL Brener pLEW-Cas9 cell line (Costa et al, 2018), two single-guide RNAs, and a DNA donor containing blastR CDS. Three independent clones were obtained by nucleofection, antibiotic selection, and cloning by limiting dilution. Confirmation of TcHAL double-knockout was performed by **B. PCR** using specific primers shown in A, **C. Western blotting** using polyclonal anti-TcHAL (1:1000) and **D. Enzymatic activity** measuring urocanate formation at 277nm. E. The proliferation curve shows no difference when $TcHAL^{-/-}$ clones are compared to the control cell lines CL Brener wild type (CLB wt) and CL Brener pLEW-Cas9 (CLB Cas9).

$TcHAL^{-/-}$ epimastigotes are not able to oxidise His

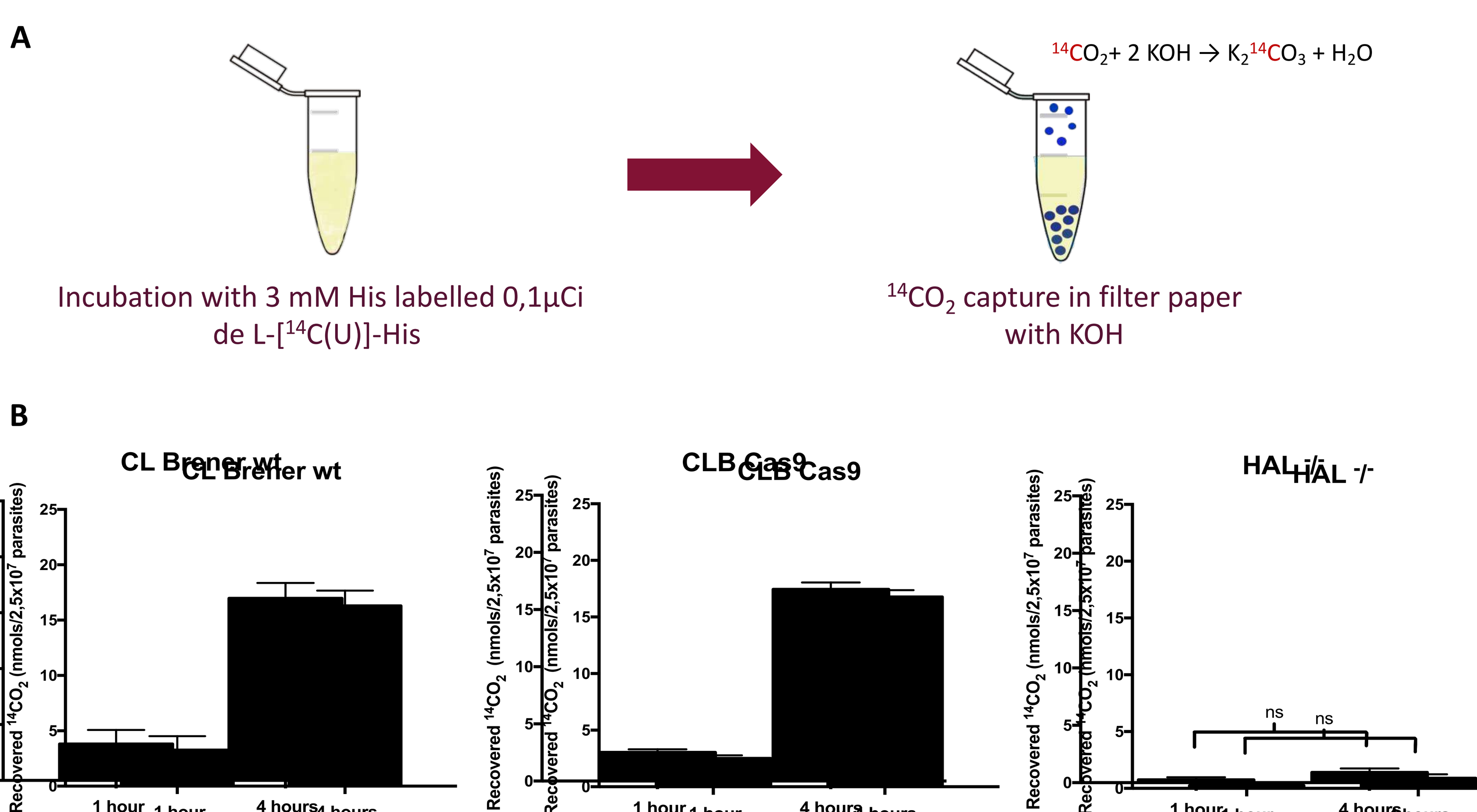

Figure 3. A. Experimental design for CO_2 trapping. Epimastigotes were incubated in PBS containing 0,1µCi de L-[^{14}C(U)]-His for 1 and 4 hours. Radiolabeled CO_2 produced by the complete oxidation of His is captured using filter paper embedded with KOH. **B. Graphs show no accumulation of CO_2 formation in $TcHAL^{-/-}$ mutants** when compared to the control cell lines CL Brener wild type (wt) and CL Brener pLEW-Cas9 (CLB Cas9). Representative graphs of three independent biological replicates are shown. Error bars show the standard deviation of three technical replicates.

$TcHAL^{-/-}$ mutants are not able to use His to maintain cell viability during nutritional stress

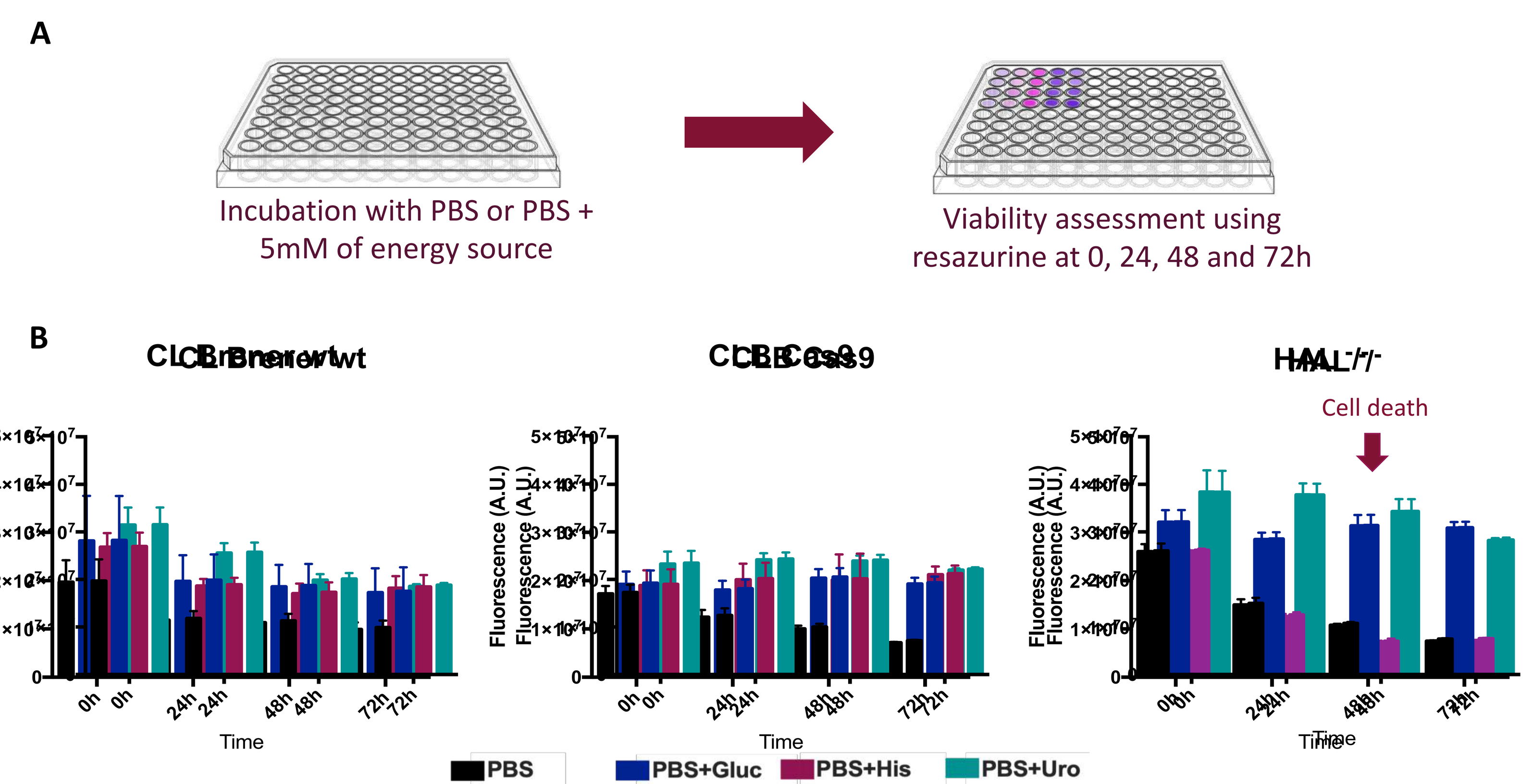

Figure 4. A. Experimental design for viability assessment during nutritional stress. Epimastigotes were incubated in PBS containing or not an energy source (glucose, histidine, or urocanate) for up to 72h. Cell viability was assessed using resazurin at the time points of 0, 24, 48, and 72h. **B. Graphs show that $TcHAL^{-/-}$ mutants are not able to maintain cell viability when kept in the presence of His as the only energy source.** At 48h, microscope inspection revealed cell death of the $TcHAL^{-/-}$ in PBS+His (arrow), indicating His toxicity. Graphs of three independent biological replicates are shown. Error bars show the standard deviation of three biological replicates.

$TcHAL^{-/-}$ epimastigotes are not able to use His to trigger O_2 consumption

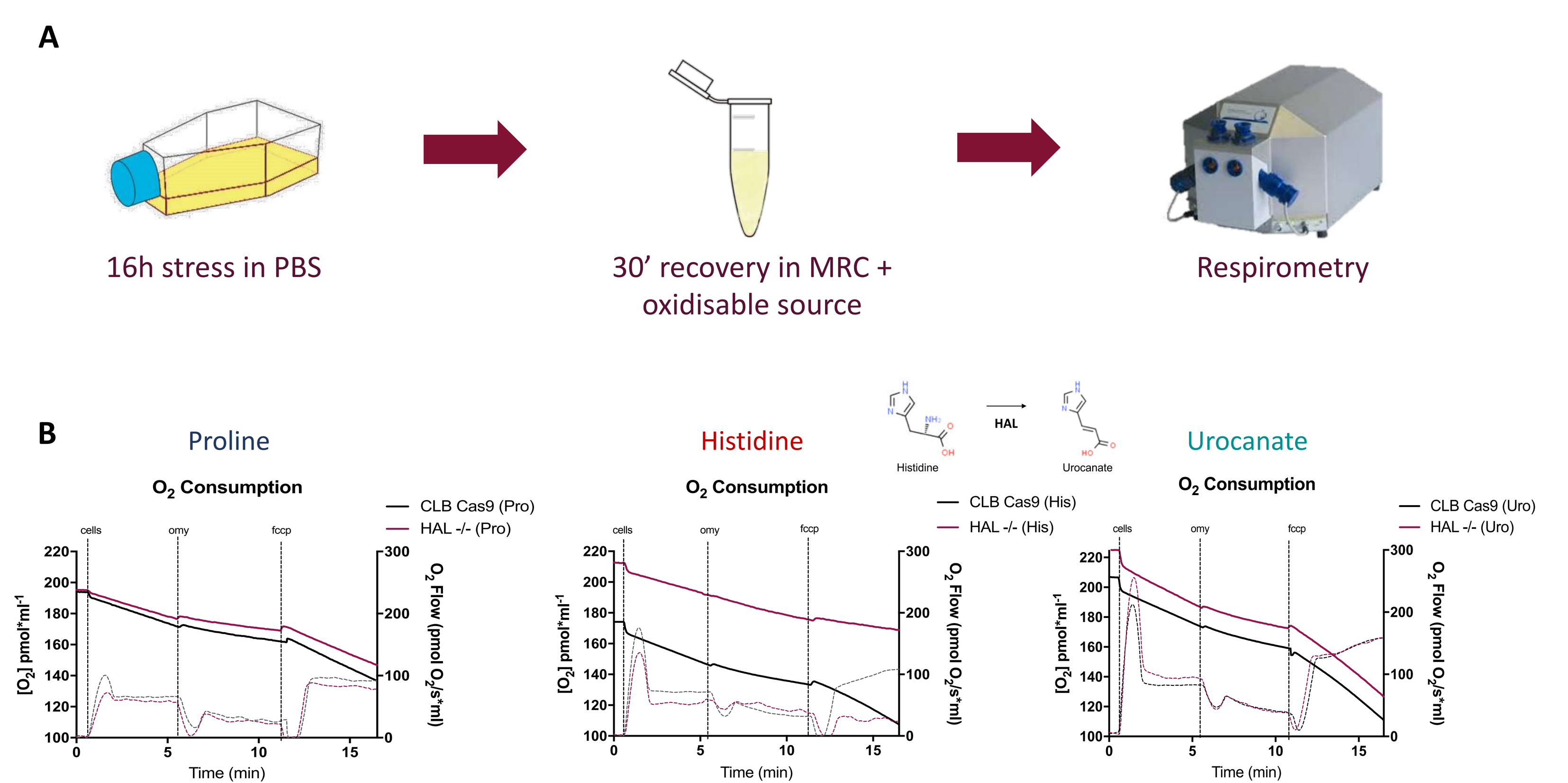

Figure 5. A. Experimental design for high-resolution respirometry. Epimastigotes were incubated in PBS for 16 hours for the depletion of intracellular metabolites. After, parasites were recovered for 30 minutes in respiration buffer (MRC) supplemented with an oxidizable source (proline, histidine, or urocanate). Respiration was measured using the O2k-Respirometer. **B. Graphs show that $TcHAL^{-/-}$ mutants are not able to use His to trigger O2 consumption** when compared to the control cell line CL Brener pLEW-Cas9 (CLB Cas9), but remain able to use proline and urocanate. Full respiratory capacity was measured after the addition of oligomycin (omy) and carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP). Representative graphs of three independent biological replicates are shown.

TcHAL knockout affects metacyclogenesis *in vitro* without altering the parasite's infection capability

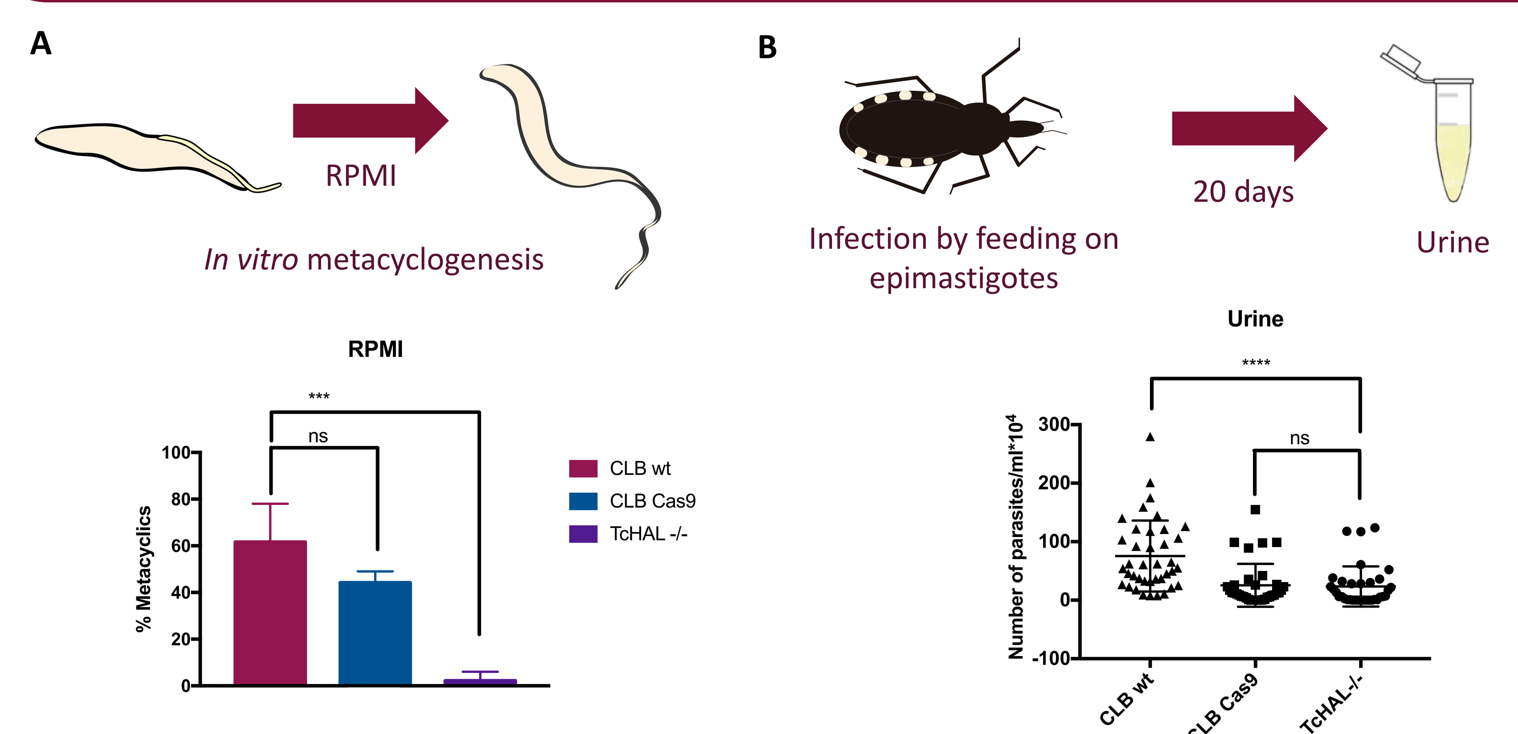

Figure 6. A. Experimental design for *in vitro* metacyclogenesis. Epimastigotes were incubated in RPMI for 8 days. After, metacyclic trypomastigotes (MTs) were counted in the supernatant of the cell culture. Graph shows the number of MTs in the supernatant of each cell line. Error bars show the standard deviation of three biological replicates. **B. Experimental design for *Rhodnius prolixus* infection.** Triatomines at 5th instar were fed on epimastigotes and after 20 days urine was collected after a new blood meal. Graph shows the number of parasites in the urine of insects infected with each cell line. CL Brener wild type (CLB wt); CL Brener pLEW-Cas9 (CLB Cas9); CL Brener pLEW-Cas9 $TcHAL^{-/-}$ ($TcHAL^{-/-}$).

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