Exploring the activity and essentiality of the putative Δ6-desaturase in the procyclic and bloodstream forms of *Trypanosoma brucei*

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1. Our method to investigate the activity of Tb-Δ6 desaturase

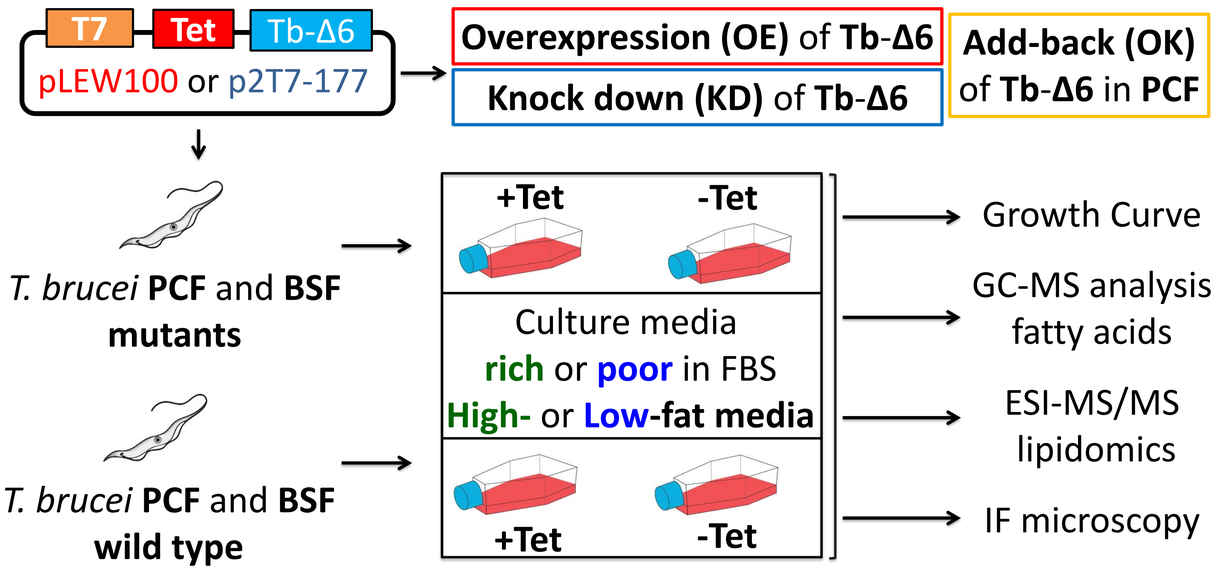


Figure 1. Schematic representation of the method used in this project

2. Tb-Δ6 is essential for parasites growth in low-fat media

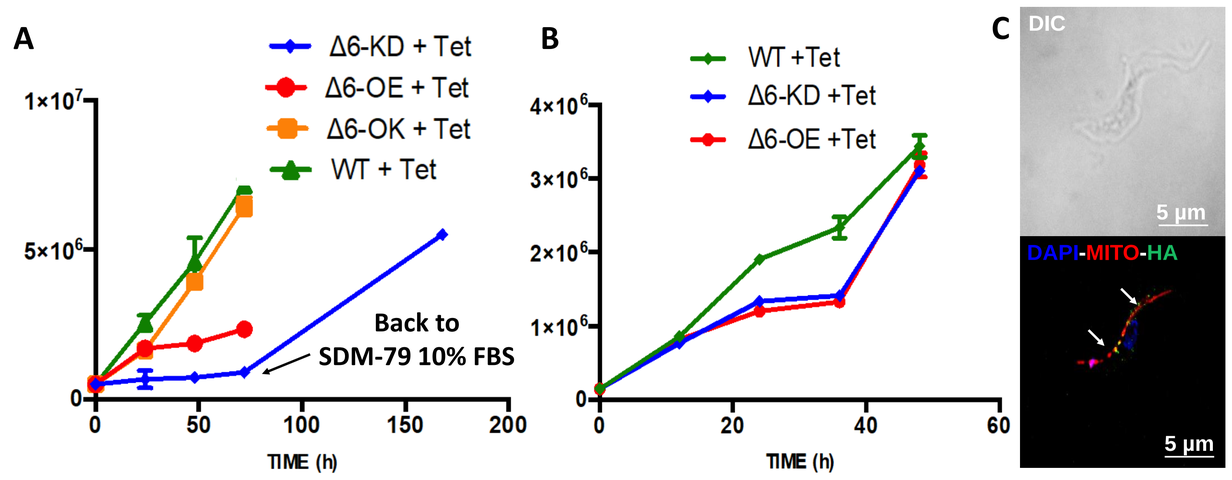


Figure 2. A, B) Growth curves of *T. brucei* PCF (**A**) and BSF (**B**) wild type (WT), overexpression (OE) and knock down (KD) of Tb-Δ6 cultured in low-fat media**. C)** Immunofluorescence microscopy of BSF *T. brucei* OE-Δ6 shows that Tb-Δ6 (white arrows) is a mitochondrial associated enzyme.

3. Polyunsaturated fatty acid profile defines Tb-Δ6 activity

20:4 —ELO→ 22:4 ---Tb-Δ6---→ 22:5 ---DES---→ 22:6

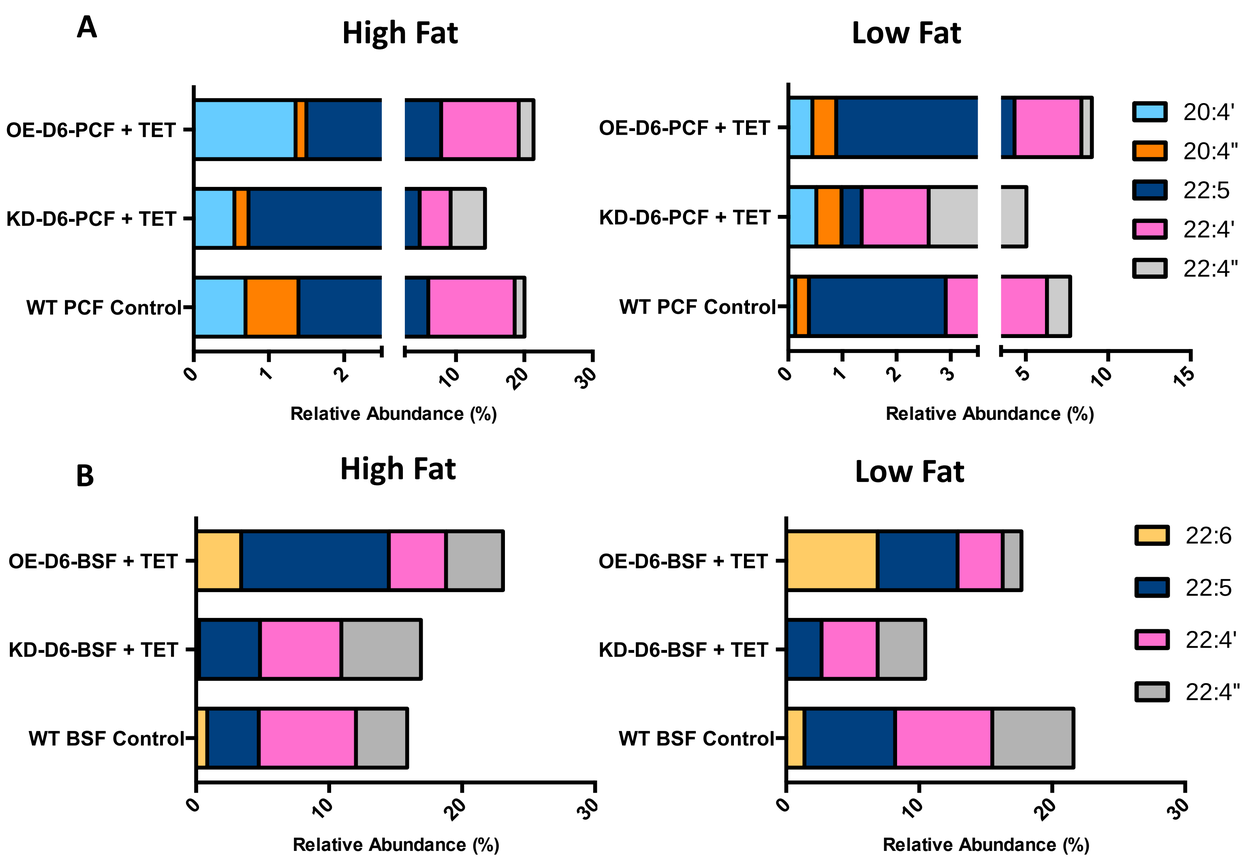


Figure 3. A) Bar charts of the PUFA content in *T. brucei* PCF WT, OE and KD in high- (left) and low- (right) fat media. **B)** Bar charts of the PUFA content in *T. brucei* BSF WT, OE and KD in high- (left) and low- (right) fat media.

4. How to confirm product and substrate of Tb-Δ6?

20:4 —ELO→ 22:4 ---Tb-Δ6---→ 22:5 ---DES---→ 22:6

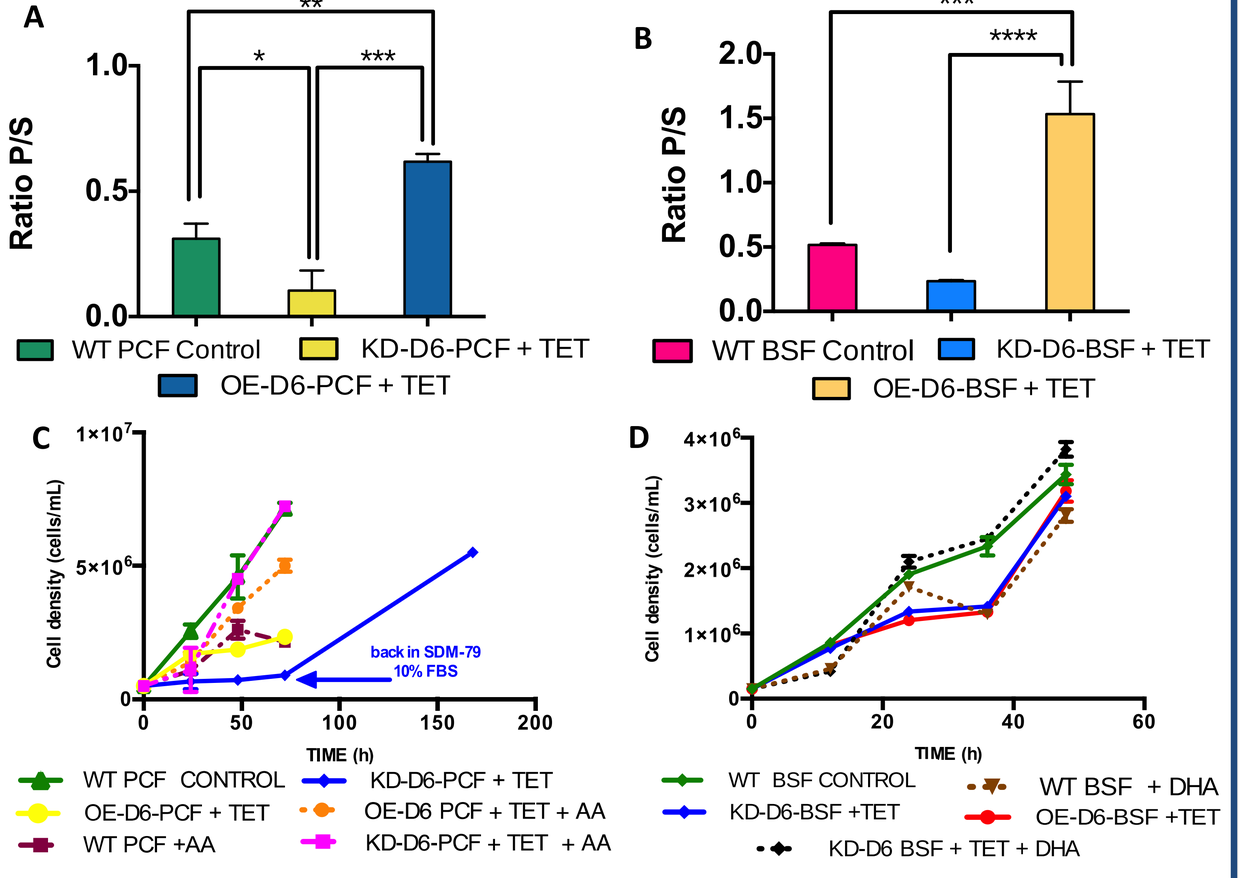
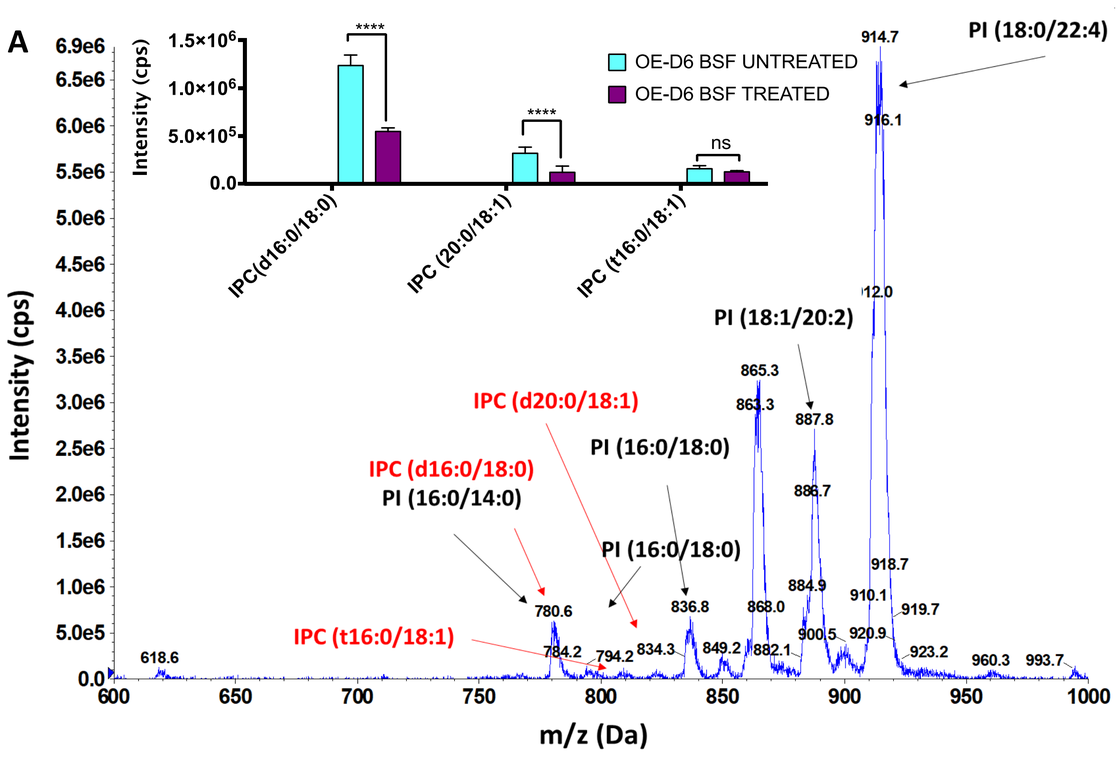


Fig 4. A, B) Ratio between products (22:5 and 22:6) and substrates (20:4 and 22:4) in *T. brucei* PCF (**A**) and BSF (**B**) in low-fat media. **C, D)** Growth curves of *T. brucei* PCF (**C**) and BSF (**D**) WT, OE and KD in low-fat media with or without supplementation of product 22:6 (DHA) (**D**) and the substrate (20:4) (AA) (**C**).

6. The overexpression of Δ6-desaturase causes production inositolphosphoryl-ceramide (IPC) in BSF



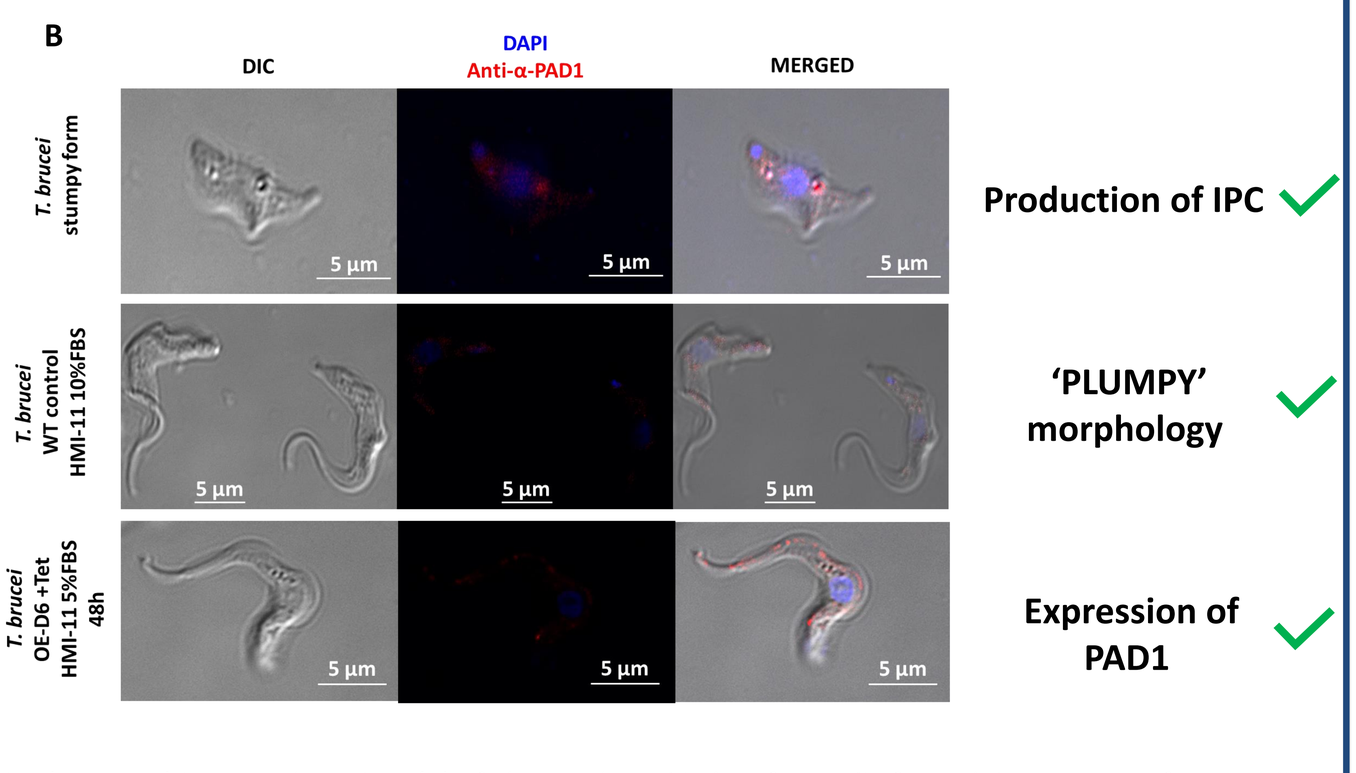


Figure 5. A) ESI-MS/MS spectra in negative mode for precursor of m/z 241 to detect IPC species (CE 60 eV) in BSF *T. brucei* OE (**B**) of Tb-Δ6 cultured in low-fat media for 48 h in presence of tetracycline. The red arrows highlight the formation of IPCs when Tb-Δ6 is overexpressed. The bar chart shows the amount of IPC produced by *T. brucei* OE in low-fat media which are untreated (light blue) or treated (purple) with the IPC-synthase inhibitor clemastine fumarate. **B)** Immunofluorescence microscopy images of samples of BSF *T. brucei* OE of Tb-Δ6 cultured in low-fat media for 48 h in presence of tetracycline show the presence of the protein associated with differentiation (PAD1) (red).

Reference:

Nat. Rev. Mol. Cell. Bio., **1**, 31-39 (2000)*; BMC Genom.,* **11**; 10:427 (2009), *Nat.,***459**, 213-217(2009),*Parasitol.,* **137**(9),1357-1392 (2010), *mSph.,* **3**, 1-14 (2018)

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