Characterization of a highly diverged mitochondrial ATP synthase F₀ subunit in *Trypanosoma brucei*

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Running title: Highly diverged F₀ subunit of *T. brucei*

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Fig. S1. Further proteins that interact with Tb927.8.3070

A volcano plot depicting of the SILAC-IP analysis of crude mitochondrial extracts from Tb927.8.3070-myc expressing cells as shown in Fig 1C. Proteins more than 10-fold enriched are labelled with either their name or accession numbers.
**Fig. S2. In silico analysis of Tb927.8.3070**

(A) List of HHpred results using Tb927.8.3070 as the input sequence. The hits related to ATP synthase subunit b are highlighted in blue. (B) The sequence of Tb927.8.3070 that displays secondary structure homology to regions in the ATP synthase subunit b of spinach (*Spinacia oleracea*) chloroplasts, yeast (*S. cerevisiae*) and *Bacillus* species using HHpred. (C) Sequence alignment between Tb927.8.3070 and its orthologs in Kinetoplastid species using Clustal Omega (84). TcCLB *T. cruzi*, TM *T. theileri*, TcIL *T. congolense*, Baya *B. ayalai*, EMOLV *E. monterogeii*, LENLEM *L. enriettii*, Lbr *L. braziliensis*, Lta *L. tarentolae*, LAMA *L. amazonensis*, Lmx *L. mexicana*, LARLEM *L. arabica*, Ld *L. donovani*, LINF *L. infantum*, LAEL *L. aethiopica*, Lmj *L. major*, Lsey *L. seymouri*, CFAC *C. fasciculata*. 
Table S3. List of proteins found more than 5-fold enriched in the Tb927.8.3070-myc SILAC CoIP and those found more than 1.5-fold downregulated in Tb927.8.3070 SILAC RNAi experiment.

<table>
<thead>
<tr>
<th>ORF</th>
<th>MW (kDa)</th>
<th>Predicted TMD</th>
<th>Tryptag localisation</th>
<th>Enrichment in Tb927.8.3070-myc SILAC CoIP</th>
<th>Downregulation in Tb927.8.3070 SILAC RNAi</th>
<th>Associated with MCU</th>
<th>Importome IM protein</th>
<th>PSI-BLAST PSI-BLAST</th>
<th>HHpred hit</th>
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<td>Tb927.2.5930</td>
<td>60.5</td>
<td>N</td>
<td>Mito</td>
<td>21.4x</td>
<td>1.73x</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>-</td>
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<td>Y</td>
<td>Mito</td>
<td>18.4x</td>
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<td>N</td>
<td>Mito</td>
<td>16.8x</td>
<td>1.61x</td>
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<td>Y</td>
<td>nd</td>
<td>-</td>
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<td>1.07x</td>
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<td>1.04x</td>
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<td>Non mito</td>
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<td>nd</td>
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</table>

1 Functional predictions were performed using InterPro and BLAST analysis.
2 TMD were predicted using TMHMM.
3 Localisation as assessed from images in the Tryptag database [85], C terminal tag only, na = image not available
4 In this work, nd = protein not detected in this analysis.
5 Proteins found in associated with TbMCU in this publication. Y= protein found associated, nd = protein not detected in this analysis.
6 Proteins listed in the mitochondrial importome defined in this publication. Y= protein listed, nd = protein not detected in this analysis.
7 Proteins found in IM fraction in this publication. Y= protein found in IM, N= protein not in IM, nd = protein not detected in this analysis.
8 Protein sequence similarity assessed by PSI-BLAST against sequences in S. cerevisiae databases
9 The top HHpred hit was recorded, unless one of the hits was a known F1F0 ATP synthase subunit. Also recorded was the hit number (#), the number of amino acids covered by the structural homology and the total number of amino acids of the protein hit in question (x/y), the probability of the hit in % and the p-value of the hit.
REFERENCES


