Characterization of a highly diverged mitochondrial ATP synthase $F_0$ subunit in *Trypanosoma brucei*

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Running title: Highly diverged $F_0$ 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.
A

| No | Hit                          | Score | Template Hit | Matched Hits |
|----|------------------------------|-------|--------------|--------------|
| 1  | 408_D DNA polymerase process5 | 51.6  | 37.47        | 51.6         |
| 2  | 688_H RSA-10: Polymyeloma f-1 | 51.1  | 6.06         | 51.6         |
| 3  | 3CN_A HaeIII-recognize prot-3 | 48.0  | 3.88         | 3.88         |
| 4  | 468D_D RrI1 complex subunit 9 | 46.1  | 103-155      | 103-155      |
| 5  | 610D_C LrI-like protease C-55 | 45.3  | 35-119       | 35-119       |
| 6  | 20QG_A H4B4 designed peptide | 43.7  | 22-101       | 22-101       |
| 7  | 3CV_B Cernovin 4, 7x bundle 41 | 41.7  | 103-155      | 103-155      |
| 8  | 3SNW_D Oppax-tongue-containing 40 | 40.2  | 30-119       | 30-119       |
| 9  | 769D_D Protein transport protein 36.9 | 39.6  | 103-155      | 103-155      |
| 10 | 3S0S_D DNA damage checkpoint p | 38.8  | 35-119       | 35-119       |
| 11 | 6KPL_F Cannabinoid receptor 1 | 34.2  | 35-119       | 35-119       |
| 12 | 6KTY_F High temperature lethal 33.9 | 34.6  | 35-119       | 35-119       |
| 13 | 6KQO_D ATP synthase subunit 3 | 33.2  | 35-119       | 35-119       |
| 14 | 3MPL_A Proteosome-associated 32.8 | 32.1  | 35-119       | 35-119       |
| 15 | 6888_D ATP synthase subunit 9 | 32.6  | 35-119       | 35-119       |
| 16 | 3MPZ_F Uncharacterized protein 32.3 | 32.2  | 35-119       | 35-119       |
| 17 | 592F_C Leptin alpha; Cytokins 30.9 | 32.0  | 35-119       | 35-119       |
| 18 | 610H_A Hypothyroid protein SP 20.6 | 21.9  | 35-119       | 35-119       |
| 19 | 100D_A LPHI-CMTHY; four-hel 30.5 | 21.8  | 35-119       | 35-119       |
| 20 | 605G_D Tall-arched protein I 25.6 | 21.2  | 35-119       | 35-119       |
| 21 | 4K5A_B Receptor-interacting sp 28.2 | 20.2  | 35-119       | 35-119       |
| 22 | 2N1S_B Fast-contracting protein 20.0 | 19.4  | 35-119       | 35-119       |
| 23 | 6H5C_F RrI1 complex subunit 10 | 20.0  | 35-119       | 35-119       |
| 24 | 2C9P_A Unconventional myosin-X 26.1 | 18.9  | 35-119       | 35-119       |
| 25 | 306E_B Calcium load-activated 26.0 | 17.7  | 35-119       | 35-119       |
| 26 | 266A_C Calcium unoproteor 26.0 | 17.7  | 35-119       | 35-119       |
| 27 | 27SA_D Alpha-carotene-like prot 25.8 | 17.7  | 35-119       | 35-119       |
| 28 | 4CAW_D Protein Y90875; Metal 25.7 | 17.7  | 35-119       | 35-119       |
| 29 | 6K2Q_F Virusence protein prot 25.6 | 17.6  | 35-119       | 35-119       |
| 30 | 358C_C DnaA-like single; Co 25.5 | 17.6  | 35-119       | 35-119       |
| 31 | 73G_A ATP synthase subunit b 25.4 | 17.6  | 35-119       | 35-119       |
| 32 | 3ZS1_b General control protein 25.2 | 17.6  | 35-119       | 35-119       |
| 33 | 3N92_F Mitotic transcription 24.1 | 17.3  | 35-119       | 35-119       |
| 34 | 1092_F Mitotic checkpoint sp 23.1 | 17.3  | 35-119       | 35-119       |
| 35 | 3599_F Intraconal protein 24.3 | 17.3  | 35-119       | 35-119       |
| 36 | 2N0A_B Trimmer collin coll 23.1 | 17.3  | 35-119       | 35-119       |
| 37 | 4H9A_A GrpE chaperonin 24.1 | 17.3  | 35-119       | 35-119       |
| 38 | 6K3D_B Viscosia transport CMD 23.0 | 17.3  | 35-119       | 35-119       |
| 39 | 6V9L_F Mitotyly peptide L 22.8 | 17.3  | 35-119       | 35-119       |
| 40 | 346S_F Signal peptide 22.8 | 17.3  | 35-119       | 35-119       |
| 41 | 4G22_F Spc45; SSM1_2, Dmp (pres 22.5 | 17.3  | 35-119       | 35-119       |
| 42 | 267A_D alpha antigen-like prot 22.5 | 17.3  | 35-119       | 35-119       |
| 43 | 4N6O_F Uncharacterized protein 21.0 | 17.3  | 35-119       | 35-119       |
| 44 | 13O_A Early Endosmal Antiun 20.7 | 17.2  | 35-119       | 35-119       |
| 45 | 462B_D ATP synthase subunit b 20.7 | 17.2  | 35-119       | 35-119       |

B

C

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S-3
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.

| ORF and TritrypDB annotation1 | MW (kDa) | Predicted TMD2 | Tryptag localisation3 | Enrichment in Tb927.8.3070-myc SILAC CoIP4 | Downregulation in Tb927.8.3070 SILAC RNAI4 | Associated with MCU5 | Importome6 | IM protein7 | PSI-BLAST8 | HHpred hit9 |
|-------------------------------|---------|----------------|-----------------------|---------------------------------------------|------------------------------------------|---------------------|------------|------------|-----------|-----------|
| Tb927.2.5930                  | 60.5    | N              | Mito                  | 21.4x                                       | 1.73x                                    | Y                   | Y          | Y          | -         | Sec31 *S. cerevisiae* (#1) (137/1273AA) Probability 64.08%, p-value 0.0009 |
| Tb927.2.5140                  | 17.4    | Y              | Mito                  | 18.4x                                       | 1.48x                                    | Y                   | Y          | nd         | -         | Sestrin *H. sapiens* (#1) (26/415AA) Probability 34.92%, p-value 0.0013 |
| Tb927.10.9120                 | 27.3    | N              | Mito                  | 16.8x                                       | 1.61x                                    | nd                  | Y          | nd         | -         | Acetaldehyde dehydrogenase *Pseudomonas sp.* (#1) (84/312AA) Probability 48.0%, p-value 0.00015 |
| Tb927.5.2150                  | 60.1    | N              | Mito                  | 12.1x                                       | 1.07x                                    | Y                   | Y          | nd         | -         | Cyt c biogenesis protein *B. fragilis* (#1) (41/172AA) Probability 90.11%, p-value 1.5E-05 |
| Tb927.4.1760 CLDP17 [46]      | 17.4    | N              | Mito                  | 11.4x                                       | 1.04x                                    | nd                  | Y          | Y          | -         | ATPTG4 *T. gondii* (#14) (130/267AA) Probability 99.5%, p-value 2E-17 |
| Tb927.6.590                   | 12.3    | N              | Mito                  | nd                                          | 2.23x                                    | Y                   | Y          | Y          | -         | ATPEG3 *E. gracilis* (#1) (76/116AA) Probability 97.3%, p-value 1.6E-09 |
| Tb927.9.7980                  | 15.8    | N              | Non mito              | nd                                          | 2.01x                                    | nd                  | nd         | nd         | -         | ATPTG9 *T. gondii* (#7) (35/166AA) Probability 97.3%, p-value 1.6E-09 |
| Tb927.11.9940                 | 20.6    | N              | na                    | nd                                          | 1.69x                                    | nd                  | nd         | nd         | -         | Autophagy related protein 16 *H. sapiens* (#1) (125/301) Probability 82.66%, p-value 0.00037 |
| Tb927.10.1430                 | 27.3    | N              | na                    | nd                                          | 1.67x                                    | nd                  | nd         | N          | -         | Elongation factor P *N. meningitidis* (#1) (44/70) Probability 35.3%, p-value 0.0035 |

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 F1Fo 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.
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