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Supplemental Information

Mitochondrial Preprotein Translocase of Trypanosomatids Has a Bacterial Origin

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Supplemental Inventory

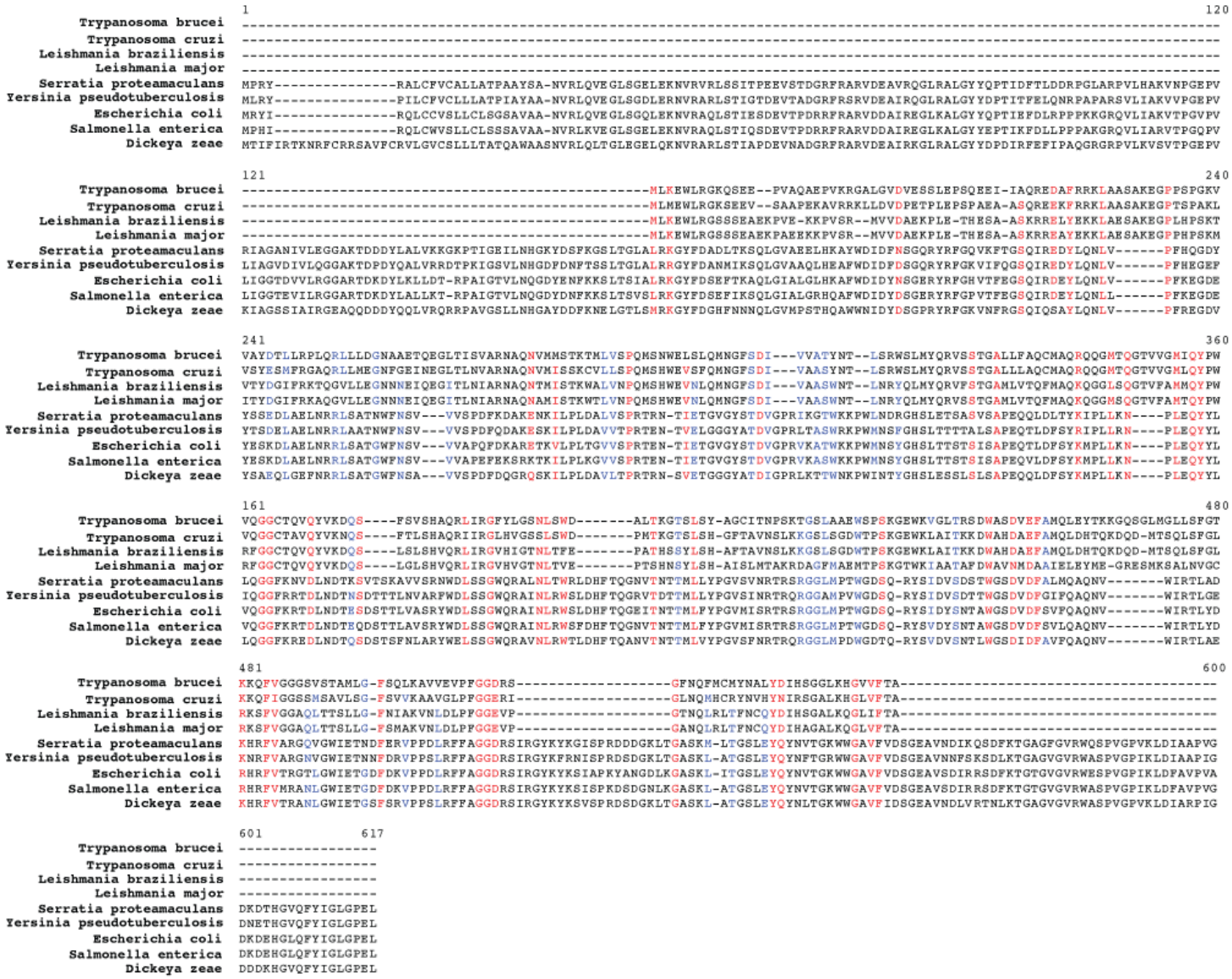
1 120
Trypanosoma brucei
Trypanosoma cruzi
Leishmania braziliensis
Leishmania major
Serratia proteamaculans
Yersinia pseudotuberculosis
Escherichia coli
Salmonella enterica
Dickeya zeae
121 240
241 360
161 480
481 600
601 617

Figure S1.

Figure S1. Multiple Sequence Alignment of ATOM Homologs from Trypanosomatides and YtfM-like Proteins from Bacteria

Analysis was performed using CLUSTAL W (1.83). GI of sequences are 70831434 (*Trypanosoma brucei*), 71659630 (*Trypanosoma cruzi* strain CL Brener), 154345277 (*Leishmania braziliensis*), 72549827 (*Leishmania major* strain Friedlin), 157368706 (*Serratia proteamaculans*), 153948759 (*Yersinia pseudotuberculosis*), 209398307 (*Escherichia coli*), 198243763 (*Salmonella enterica*), 251788425 (*Dickeya zeae*). Colours indicate regions of high (red) and low (blue) homology.

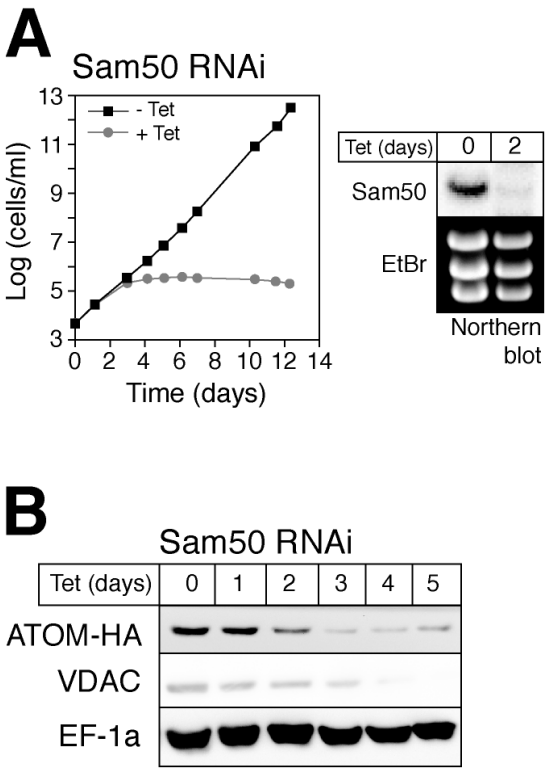

Figure S2. In Vivo Assembly of ATOM Requires Sam50

(A) Growth curve of uninduced and tet-induced Sam50 RNAi cell line. Northern blot indicates efficient ablation of Sam50 mRNA in induced cells. The ethidium bromide (EtBr) stained region of the gel depicting the rRNAs serves as a loading control.

(B) Levels of HA-tagged ATOM (ATOM-HA) and VDAC in total cellular extracts rapidly decline during tet-induction of Sam50 RNAi cells, whereas the levels of elongation factor 1a (EF-1a) are not affected.

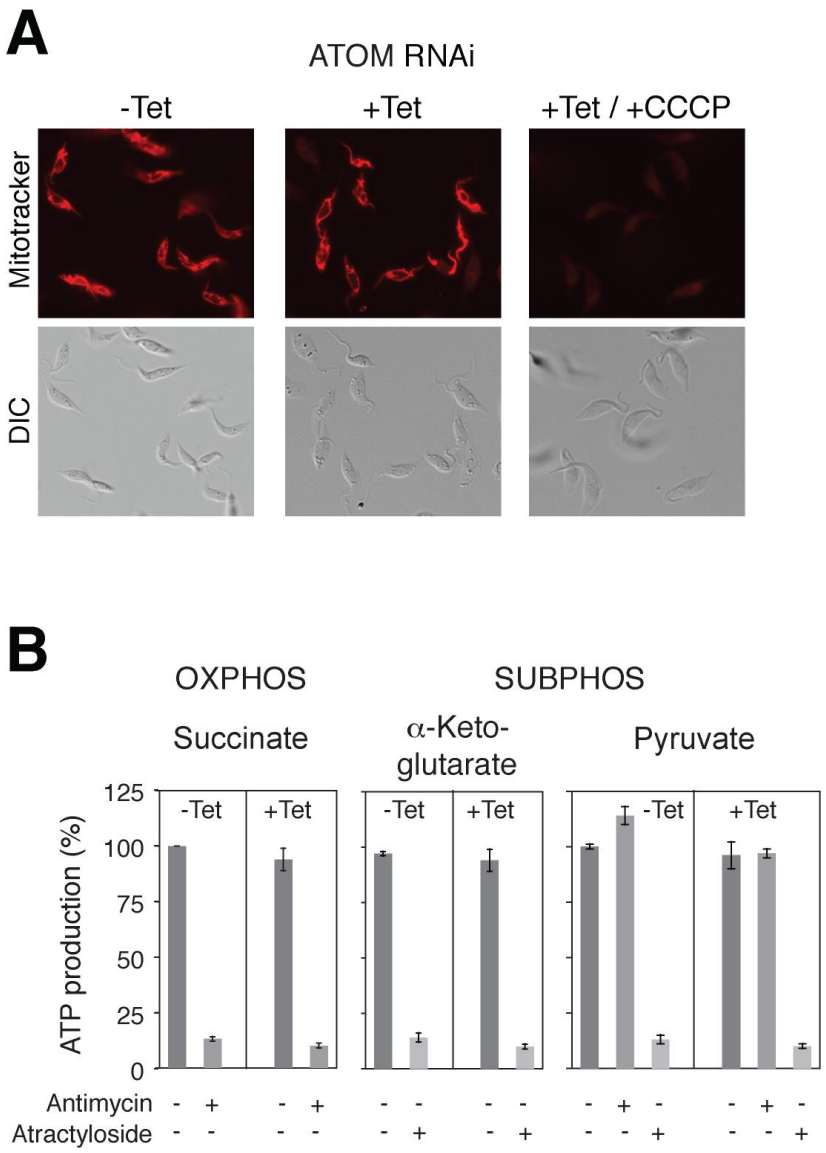

Figure S3. RNAi of ATOM Does Not Induce a General Mitochondrial Dysfunction

(A) Mitotracker staining shows that ATOM RNAi cells (+Tet, 48 hours) have an intact membrane potential. CCCP, carbonyl cyanide m-chlorophenylhydrazone; DIC, differential interference contrast microscopy.

(B) Mitochondrial oxidative (OXPHOS) and mitochondrial substrate level phosporylation (SUBPHOS) is not affected in induced ATOM RNAi cells (+Tet, 48 hours).

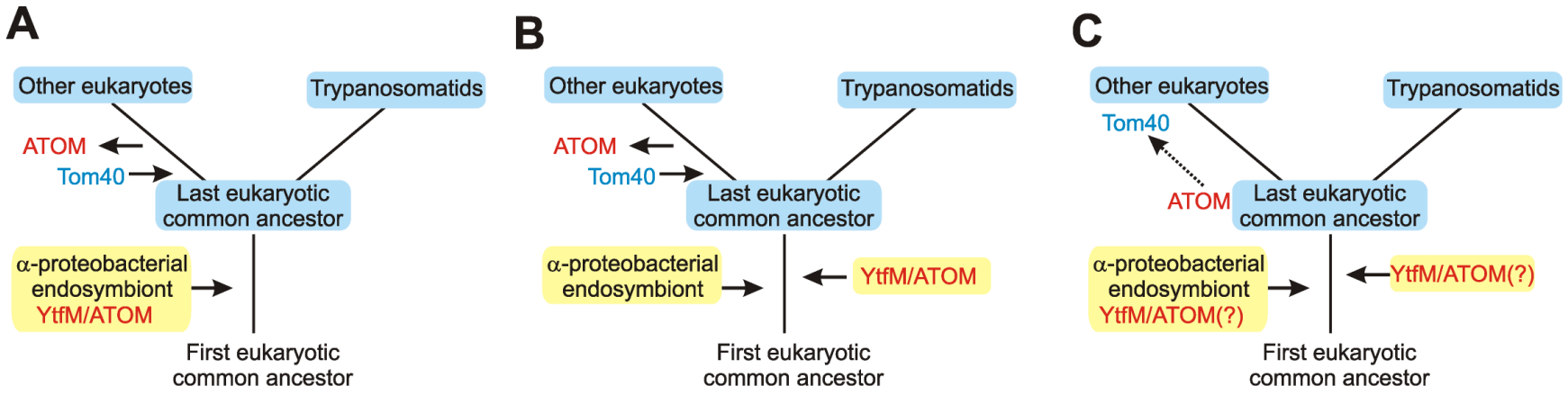

Figure S4. Models for the Evolution of the Mitochondrial Outer Membrane Protein Translocase

(A) YtfM/ATOM-type bacterial protein exporter but not Tom40 were present in the original endosymbiont. The bacterial exporter, adapted to function in reverse, was comandeered for mitochondrial protein import in the last eukaryotic common ancestor and has been retained in the line leading to the trypanosomatids. Tom40 evolved after the ancestor of trypanosomatids diverged from the rest of the eukaryotes. The ATOM in the main branch of eukaryotes was subsequently lost.

(B) As A but ATOM was aquired by horizontal gene transfer and not by the original endosymbiontic event.

(C) ATOM, either acquired by the endosymbiontic event or by horizontal gene transfer, evolved into Tom40 after the trypanosomatids split from all other eukaryotes. Yellow and blue indicate bacterial and eukaryotic evolutionary origins, respectively.