Structure of the full kinetoplastids mitoribosome and insight on its large subunit maturation

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Supplementary Figure 1. Purification of *L. tarentolae* and *T. cruzi* mitoribosome

(A) Overview of the kinetoplastids mitoribosome purification protocol. (B) Representative sucrose gradient profiles resulting from mitoribosome purification are shown, absorbance at 260 and 280 nm are displayed. The composition of these fractions was assessed by mass-spectrometry analyses and direct cryo-EM inspection. For both organisms, fraction 1 contained non-ribosomal complexes (mainly respiratory complexes) and fraction 5 contained matured monosomes, fractions 5 were used for cryo-EM data collection and reconstruction presented in Fig. S3 and S4. In the case of *L. tarentolae*, fractions 2, 3 and 4 all contained SSU-like particles (mature and assembly intermediates). Fraction 3 also contained LSU assembly intermediate, which was used for cryo-EM data collection and reconstruction presented in Fig. S2. In the case of *T. cruzi* fractions 2, 3 and 4 also contained a mixture of SSU and LSU, but assembly intermediate did not accumulate to the same extend as observed with *L. tarentolae*. 
Supplementary Figure 2. Data processing workflow of *L. tarentolae* data sets
Graphical summary of the processing workflow described in Methods for the *L. tarentolae* samples, resulting in the LSU assembly intermediate for Data set 1 and the full mitoribosome for Data set 2. 2D classes are presented in (A), with selected classes for further processing highlighted either in blue or orange, and 3D processing and refinement presented in (B).
Supplementary Figure 3. Local resolution of *L. tarentolae* final reconstructions

Local resolutions of the different reconstructions are presented (A). The maps are colored by resolution, generated using ResMap (41), and a cut view is provided. (B) For both reconstructions FSC plots are displayed for resolution estimation (output from RELION (42)) as well as map-to-model FSC (out from PHENIX (43) validation). The map resolution is calculated at the 0.143 threshold.
Supplementary Figure 4. Data processing and cryo-EM reconstructions of T. cruzi mitoribosome

Graphical summary of the processing workflow T. cruzi full mitoribosome. 2D classification and 3D processing are presented in (A) with respective ResMap (41) representation and FSC plots presented in (B). Second round of 3D classification lead to the identification of two rotational states presented in (C). Cryo-EM reconstructions of T. cruzi full mitoribosome (D) as well as the mature LSU, seen from the solvent view (E) and the interface view (F). Compared with the L. tarentolae reconstructions presented in Figure 1, the ribosomes are nearly identical.
Supplementary Figure 5. Data processing and model of the mt-IF3 SSU of *T. cruzi*

Cryo-EM map reconstruction of the *T. cruzi* in presence of mt-IF3 is shown in (A), with r-proteins colored in orange, 9S rRNA in yellow, mt-IF3 in red and uS12m in green. Local resolution generated using ResMap (41) are shown in (B), and the processing workflow with the respective FSC plots for initial and focused 3D refinement are displayed in (C). The resulting atomic model is presented in (D), each r-proteins are individually annotated, 9S rRNA is colored in orange. (E) mt-IF3 is shown in its density (cyan) and compared with the recent human SSU+mt-IF3 complex (PDB:6RW4). *T.*
*cruzi* mt-IF3 contacts h44 and the C-terminal part of the protein contacts uS12, contributing to the SSU stabilization. In human, no contact is made with uS12 and the N-terminal part of the protein contacts mS37 and uS7m (28) which is not the case in Trypanosoma.
Supplementary Figure 6. Local resolution of the assembly factors
Each density for all the identified assembly factors of the LSU are presented, with each maps colored by resolution, generated using ResMap (41). Side chains in their density are also shown.
Supplementary Figure 7. Model in their respective densities

(A-B) Densities of the immature LSU rRNA extremities as shown in Fig. 3, mL67 and mL81 surrounding densities are also shown. (C) Pseudouridine synthase mt-LAF3 acting on a uridine residue. (D) Model of the mature LSU in its density showing the matured peptide channel. (E) mt-LAF2 and mt-LAF4 involved in CP maturation in their respective densities. (F) Anti association complex formed by mt-Rsfs, L0R8F8 and mt-SAF32.
Supplementary Figure 8. Intersubunit bridges

List of the intersubunit bridges observed in the mature and full kinetoplastid mitoribosomes. Both the SSU and LSU are presented on the intersubunit side and bridge positions are indicated in green. Most of the bridges involve protein:protein interactions, except for the highly conserved rRNA:rRNA bridge between h24 and H68. Overall, the other bridge positions seem to be common with other mitoribosomes and bacterial ribosomes but involve mitochondria and kinetoplastid-specific r-proteins, for example, bL31m is the only bacterial-type r-protein involved in the bridges between the SSU's head and LSU's CP. One truly kinetoplastid-specific bridge is observed with mS63 and mL85, which involves kinetoplastid only r-proteins.
Supplementary Figure 9. Central protuberance maturation in Leishmania LSU
Comparison of the assembly intermediate and mature CP area of the LSU. In the assembly intermediate, CP proteins are prevented from binding by mt-LAF2 and mt-LAF4. Upon release of the maturation factors the whole CP block can dock the LSU and additional r-proteins contacting the CP, like bL33m and the C-terminus of mL59/64 can lock the structure. 12S rRNA is displayed in cyan.
Supplementary Figure 10. Structural conservation and sequence comparison of the DEAD-box RNA helicase mt-LAF2

mt-LAF1 is shown in (C) with its DEAD and helicase domains colored in green and its unmodeled flexible insertion shown in grey. Aside the insertion, the helicase appears to be conserved with enzymes of the DEAD-box family, highlighted by the superimpositions (D) and sequence alignments (E) of mt-LAF2 with yeast mitochondrial Mss116 enzyme (13, 14), or cytosolic Has1 and eIF4-A1. The enzyme is in closed state (D) and the interaction with RNA (A) as well as with the ATP ligand (B) is highly conserved.
Supplementary Figure 11. Analysis of the pseudouridine synthases mt-LAF3 and mt-LAF4

Structural conservation and sequence comparison of the two pseudouridine synthases mt-LAF3 and mt-LAF4 are shown. In (A) the pseudouridine synthase mt-LAF4, homolog of the bacterial enzyme RluD (20, 21), is shown with its rRNA target (blue). The enzyme is particularly conserved with enzymes of the Rlu family, as highlighted by the superimpositions (C) and sequence alignments (D) of mt-LAF3 with *E. coli* Rlu enzymes. Its mode of action is also conserved, as shown in (B). In (E) the analysis of mt-LAF6 shows the presence of an insertion domain (pink) and sequence alignment with other pseudouridine synthases highlight that none of the catalytic sites of mt-LAF4 are conserved, mt-LAF4 is thus inactive.
Supplementary Figure 12. Conservation of the three GTPases with their bacterial homologs

The three GTPases mt-RbgA, mt-EngA and mt-EngB are compared with their bacterial homologs. In (A) mt-EngA is compared with *B. subtilis* EngA crystal structure obtained with GDP, and *E. coli* EngA structure obtained by cryo-EM in complex with the LSU with excess of GMP-PNP, a non-hydrolysable analog of GTP (PDB: 3J8G and 5M7H). The *B. subtilis* one has its first G-domain shifted compared to *E. coli* EngA and mt-EngA which represent one of the alternative structural state of the GTPase. Thus, mt-EngA is in GTP state. (B) shows mt-RbgA superimposed with *S. aureus* RbgA (PDB: 6G14). In (c) mt-EngB is superimposed with two crystal structures of its bacterial homolog YihA/EngB (PDB: 3PQC and 1SVW). This protein was never observed in native assembly, but our reconstruction appears to be quasi-identical to the crystal structures previously obtained. In context of the LSU assembly intermediate, it interacts with H54 and appears here to block uL2m association.
Supplementary Figure 13. Analysis of the RNA 2’O-methyltransferase mt-LAF7

The homodimeric mt-LAF5.a/mt-LAF5.b 2’O-methyltransferases are shown in the assembly intermediate context (A), with nearby possible rRNA targets -immature L7/12 stalk and H95-highlighted with a red star. Structural conservation is highlighted by structural superimpositions with several bacterial homologs, notably with *S. actuosus* NHR involved in L7/12 stalk methylation (44) (B), and crucial residues for the activity involved in S-adenosyl-l-methionine (SAM) binding are shown in (C). In (D-G) an analysis of the RNA binding N-terminal domain is shown. The N-terminal domain of mt-LAF5 (D) is superimposed (E) and the sequences are compared (G) with two eukaryotic proteins harbouring a similar dsRNA binding domain, which were structurally characterized in complex with their double stranded RNA target (F) as well as with *S. actuosus* NHR domain.
| Uniprot ID | Description | LSU Intermediate | Full ribosome | Mass (Da) | Name |
|-----------|-------------|------------------|---------------|-----------|-------|
| Q0QA0D    | Uncharacterized protein | 111            | 116           | 94,405    | m551  |
| E9AF15    | Uncharacterized protein | 145            | 92            | 73,032    | m522  |
| Q0QEF4    | Uncharacterized protein | 138            | 136           | 199,549   | m648  |
| Q0QC7Y9   | Uncharacterized protein | 118            | 90            | 50,291    | u615m |
| Q0QLN8    | Ribosomal S5_C domain-containing protein | 118          | 85            | 49,269    | u65m  |
| Q0QFA7    | Uncharacterized protein | 108            | 87            | 72,061    | m553  |
| E9AK9     | 3DS Ribosomal protein S17-like protein | 107          | 60            | 35,960    | u517m |
| Q0QL90    | Uncharacterized protein | 105            | 109           | 131,518   | m547  |
| E9ADG8    | Uncharacterized protein | 104            | 85            | 132,979   | m649  |
| Q0QB37    | Uncharacterized protein | 104            | 74            | 39,525    | u59m  |
| E9ADN4    | Uncharacterized protein | 99             | 100           | 65,047    | m555  |
| Q0QL10    | Uncharacterized protein | 93             | 72            | 37,729    | b518m |
| E9ACG9    | Uncharacterized protein | 83             | 53            | 35,718    | m559  |
| Q0QAU8    | Uncharacterized protein | 78             | 13            | 118,433   | mt-SA1 |
| Q0QSU8    | Uncharacterized protein | 75             | 2             | 88,279    | m55AF5|
| Q0QM2     | Uncharacterized protein | 70             | 43            | 92,398    | u510m |
| Q0QH6     | Uncharacterized protein | 66             | 46            | 36,753    | m623  |
| Q0BM5     | Uncharacterized protein | 66             | 38            | 19,451    | m572  |
| Q0QTR6    | Uncharacterized protein | 65             | 55            | 75,360    | m652  |
| E9A4O0    | Uncharacterized protein | 62             | 44            | 99,438    | m635  |
| E9AFH3    | Uncharacterized protein | 61             | 48            | 49,540    | m526  |
| Q0QBM1    | Putative translation initiation factor IF-2 | 61             | 1             | 85,695    |       |
| Q0SG4D    | Uncharacterized protein | 59             | 53            | 54,177    | m629  |
| E9ACZ5    | Uncharacterized protein | 58             | 59            | 20,859    | m533  |
| Q0QEX9    | Uncharacterized protein | 58             | 3             | 76,209    | m55AF7|
| Q0QWV4    | Uncharacterized protein | 56             | 43            | 19,837    | m571  |
| Q0QH9     | Putative DNA photolyase (EC 4.1.99.3) | 56           | 1             | 102,527   |       |
| Q0QF04    | Putative 50S ribosomal protein L17 | 55             | 33            | 35,768    | b17m  |
| Q0QL03    | Uncharacterized protein | 52             | 34            | 21,484    | m570  |
| Q0QW8     | Uncharacterized protein | 49             | 42            | 45,656    | m567  |
| Q0QW2     | Uncharacterized protein | 48             | 32            | 35,046    | u58m  |
| E9AXS5    | Ribosomal protein L3-like protein | 48            | 37            | 53,941    | u3m   |
| E9ACT9    | Putative ribosomal protein L11 | 47             | 43            | 40,208    | u11m  |
| Q0QD57    | Uncharacterized protein | 46             |               | 87,867    |       |
| Q0Q84     | Uncharacterized protein | 45             | 46            | 35,098    | m560  |
| Q0Q7N2    | Uncharacterized protein | 45             | 39            | 19,577    | u519m |
| Q0Q66     | G3R domain-containing protein | 45            | 30            | 22,207    | m641  |
| Q0QA7     | LSU_S5_G8 domain-containing protein | 45          | 35            | 31,181    | m643  |
| Q0Q57     | Uncharacterized protein | 45             | 31            | 53,552    | u24m  |
| E9A4T4    | G domain-containing protein | 45            | 1             | 51,585    | mt-RgsA|
| Q0Q8F4    | Uncharacterized protein | 44             | 34            | 129,063   | m550  |
| Q0Q8H5    | Uncharacterized protein | 43             | 25            | 22,427    | b516m |
| Q0Q68     | Uncharacterized protein | 42             | 38            | 40,909    | u511m |
| Q0Q98     | Pyruvate dehydrogenase E1 component subunit beta (EC 1.3.1.1) | 41          | 178           | 37,898    |       |
| Q0Q5S4    | Uncharacterized protein | 41             | 36            | 49,937    | u4m   |
| E9AD0     | Uncharacterized protein | 41             | 6             | 97,582    | m67L  |
| Q0QBP3    | Uncharacterized protein | 41             | 3             | 82,977    | m55AF9|
| Q0QH1     | DEAD/DEAH box helicase-like protein (Placêee!!) | 41        | 2             | 87,259    | mt-LAF2|
| Q0Q2S1    | Dehydrogenase-like protein | 40            | 5             | 45,565    | m55AF21|
| Q0Q45     | Uncharacterized protein | 40             | 36            | 65,691    | m68   |
| Q0Q2E1    | Putative ribosomal protein L2 | 40            | 47            | 43,310    | u2m   |
| Q4Q8I8 | Uncharacterized protein | 40 | 2 | 77,058 |
| Q4QCM3 | Uncharacterized protein | 39 | 4 | 135,363 | mt.SAF21 |
| Q4Q06 | Uncharacterized protein | 39 | 10 | 18,356 | b.35n |
| Q4QDG1 | Putative pyruvate dehydrogenase E1 component alpha subunit | 37 | 204 | 42,874 |
| E9AGG2 | Uncharacterized protein | 37 | 31 | 35,422 | mL53 |
| Q4Q22 | Uncharacterized protein | 37 | 26 | 39,591 | mL75 |
| Q4QFF1 | Uncharacterized protein | 37 | 2 | 86,657 |
| E9A0X8 | Uncharacterized protein | 36 | 41 | 36,015 | mL58 |
| Q4Q71 | UIM zinc-binding domain-containing protein | 36 | 36 | 25,129 | mL90 |
| Q4QF5 | Uncharacterized protein | 34 | 25 | 29,238 | mL34 |
| Q4Q5W8 | Uncharacterized protein | 34 | 23 | 43,526 | mL55 |
| Q4QCG8 | Uncharacterized protein | 34 | 28 | 24,276 | mL37 |
| Q4Q740 | Putative heat shock 70-related protein 1, mitochondrial | 34 | 1 | 70,609 |
| E9AFB1 | SpolI methylase domain-containing protein | 33 | 1 | 63,776 | mt.LAF5 |
| Q4Q5U3 | Methylcrotonoyl-CoA carboxylase biotinylated subunit protein-like | 31 | - | 75,913 |
| Q4Q847 | Uncharacterized protein | 31 | 25 | 27,437 | mL62 |
| Q4Q9M1 | Ubiquitin-like domain-containing protein | 31 | 24 | 27,308 | mL68 |
| Q4Q7F8 | Uncharacterized protein | 31 | 19 | 56,475 | mL72 |
| Q4QBM1 | Uncharacterized protein | 31 | 3 | 43,369 | mt.SAF18 |
| Q4Q0K4 | DUF4460 domain-containing protein | 30 | 1 | 82,621 | mt.SAF6 |
| Q4QE10 | Uncharacterized protein | 29 | 13 | 44,293 | mL66 |
| Q4QCY7 | Uncharacterized protein | 29 | 20 | 16,666 | b.19n |
| Q4QB6 | Uncharacterized protein | 29 | 16 | 18,070 | mL63 |
| Q4Q710 | G domain-containing protein | 28 | 4 | 100,895 |
| E9AF47 | Uncharacterized protein | 28 | 28 | 33,355 | mL61 |
| Q4Q1C8 | Uncharacterized protein | 28 | 25 | 39,338 | mL74 |
| Q4Q1U2 | Uncharacterized protein | 28 | 20 | 22,405 | b.20m |
| Q4QF0S | Uncharacterized protein | 28 | 29 | 21,066 | b.21n |
| Q4QD09 | Uncharacterized protein | 28 | 24 | 18,822 | mL93 |
| Q4Q4U3 | Dihydrolipoate dehydrogenase | 27 | 70 | 50,578 |
| E9AC77 | Pseudouridylate synthase-like protein | 27 | - | 47,376 | mt.LAF3 |
| MVP | Major vault protein | 27 | - | 93,301 |
| Q4Q8P8 | Uncharacterized protein | 27 | 20 | 19,525 | u.614n |
| Q4Q8K0 | Uncharacterized protein | 27 | 2 | 49,309 | mL71 |
| Q4Q5G4 | GTPase Der | 27 | 1 | 56,468 | mt.EngB |
| Q4Q8T5 | Rhodanese domain-containing protein | 26 | 19 | 30,098 | mL67 |
| Q4Q0G0 | Uncharacterized protein | 26 | 7 | 21,319 | mL52 |
| Q4Q0F0 | Uncharacterized protein | 25 | 15 | 33,787 | mL63 |
| Q4Q760 | DNAJ-like protein | 25 | 2 | 45,324 | mL37 |
| Q4Q701 | Uncharacterized protein | 25 | 15 | 50,687 | mL70 |
| Q4Q3H1 | Putative 3-methylcrotonyl-CoA carboxylase beta subunit | 24 | 1 | 58,353 |
| Q4QBK0 | Uncharacterized protein | 24 | 19 | 32,229 | u.22m |
| Q4Q2Y7 | Uncharacterized protein | 24 | 24 | 54,161 | u.29m |
| Q4QCO4 | Uncharacterized protein | 24 | 18 | 28,912 | mL80 |
| Q4QDF5 | Uncharacterized protein | 24 | 16 | 38,596 | mL76 |
| Q4Q8B1 | Uncharacterized protein | 24 | 12 | 26,176 | mL49 |
| Q4Q5C2 | 2-oxoisovalerate dehydrogenase subunit alpha | 23 | 2 | 53,314 |
| Q4Q5G8 | Uncharacterized protein | 23 | 17 | 18,799 | b.66m |
| Q4Q8B9 | Uncharacterized protein | 23 | 12 | 28,558 | mL59/64 |
| Q4Q716 | Putative superoxide dismutase (EC 1.15.1.1) | 22 | 11 | 30,329 | mL42 |
| ACT | Actin | 21 | 2 | 41,991 |
| Q4Q7V3 | Uncharacterized protein | 21 | 22 | 27,904 | mL80 |
| Accession | Description                                      | Length | Mass (kDa) | Function |
|-----------|--------------------------------------------------|--------|------------|----------|
| Q0Q4L5    | Uncharacterized protein                          | 21     | 21,040     | mL86     |
| Q0QF3N    | Uncharacterized protein                          | 21     | 86,153     | mS73     |
| Q0QD47    | Putative potassium voltage-gated channel        | 21     | 34,694     | mt.SAF22 |
| Q0Q1E3    | Uncharacterized protein                          | 21     | 11,851     | mS4      |
| Q0Q6W6    | Uncharacterized protein                          | 20     | 11,489     | mL98     |
| Q0Q469    | Uncharacterized protein                          | 20     | 39,233     | mL86     |
| Q0QB7     | Uncharacterized protein                          | 19     | 55,433     | mL98     |
| Q1X7F7    | Excreted/secreted protein 15                     | 19     | 92,282     | mS54     |
| Q0QF19    | Ribosomal L18e/L15P domain-containing protein    | 19     | 43,501     | mL98     |
| Q0Q8D3    | tRNA pseudouridine synthase A-like protein (EC 4.2.1.70) | 19     | 58,367     | mL98     |
| Q0Q719    | Uncharacterized protein                          | 19     | 27,177     | mL98     |
| Q0Q9X5    | ATP synthase subunit beta (EC 7.1.2.2)           | 18     | 56,321     | mL98     |
| Q0QE16    | Uncharacterized protein                          | 18     | 13,242     | mL98     |
| E9AF38    | Uncharacterized protein                          | 18     | 60,195     | mL98     |
| Q0QA05    | G domain-containing protein                      | 17     | 28,797     | mL98     |
| Q0Q152    | Uncharacterized protein                          | 17     | 6,123      | mL98     |
| E9AE12    | Putative 2-oxoisovalerate dehydrogenase beta subunit, mitochondrial | 16     | 39,499     | mL98     |
| Q0QE5S    | Ribosomal L18e/L15P domain-containing protein    | 16     | 30,464     | mL98     |
| Q0Q7F8    | SAM_MT_RSMB NOP domain-containing protein        | 15     | 128,336    | mL98     |
| Q0QP9     | Uncharacterized protein                          | 15     | 54,117     | mL98     |
| Q0Q709    | Uncharacterized protein                          | 15     | 16,693     | mL98     |
| Q0Q4K0    | G domain-containing protein                      | 15     | 86,805     | mL98     |
| E9AE83    | Uncharacterized protein                          | 14     | 58,252     | mL98     |
| Q0Q1F5    | Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase | 14     | 48,751     | mL98     |
| Q0Q9E8    | Uncharacterized protein                          | 14     | 21,224     | mL98     |
| E9AFH1    | PKS_AT domain-containing protein                 | 14     | 59,053     | mL98     |
| Q0Q1F1    | ATPase alpha subunit (EC 3.6.3.14)               | 13     | 62,549     | mL98     |
| E9AD06    | Asl carrier protein                              | 13     | 16,607     | mL98     |
| Q0QBK2    | Peptidyl-prolyl cis-trans isomerase (PPIase) (EC 5.2.1.8) | 13     | 25,288     | mL98     |
| Q0Q2H6    | SDS ribosomal protein L13-like protein           | 13     | 20,470     | mL98     |
| Q0Q6A3    | Uncharacterized protein                          | 13     | 21,815     | mL98     |
| Q0QB16    | Uncharacterized protein                          | 13     | 22,223     | mL98     |
| E9AJ8     | Uncharacterized protein                          | 12     | 223,610    | mL98     |
| Q0Q5S1    | Putative ribosomal protein L14                   | 12     | 21,591     | mL98     |
| Q0Q4D6    | Uncharacterized protein                          | 12     | 18,660     | mL98     |
| Q0QAP7    | Uncharacterized protein                          | 12     | 37,692     | mL98     |
| Q0QA80    | Uncharacterized protein                          | 12     | 27,479     | mL98     |
| Q0QA46    | Uncharacterized protein                          | 12     | 35,448     | mL98     |
| E9AFX0    | Uncharacterized protein                          | 12     | 96,809     | mL98     |
| Q0QC51    | Major vault protein-like protein                 | 11     | 109,068    | mL98     |
| E9AE13    | Uncharacterized protein                          | 11     | 22,797     | mL98     |
| Q0Q712    | Uncharacterized protein                          | 11     | 24,585     | mL98     |
| B6SBL6    | Mitochondrial guide RNA binding complex subunit 2 | 11     | 60,624     | mL98     |
| E9AFQ9    | Uncharacterized protein                          | 11     | 60,738     | mL98     |
| Q0QB62    | Uncharacterized protein                          | 10     | 30,378     | mL98     |
| Q0Q1M0    | Chaperonin HSP60, mitochondrial                 | 10     | 59,318     | mL98     |
| Q0Q9F0    | Ribosomal L18e/L15P domain-containing protein    | 10     | 19,101     | mL98     |
| E9AB15    | Uncharacterized protein                          | 10     | 27,480     | mL98     |
| Q0Q6S5    | TRU domain-containing protein                    | 10     | 68,300     | mL98     |
| Q0QB85    | EngB-type G domain-containing protein            | 10     | 104,946    | mL98     |
| Q0Q1D4    | Uncharacterized protein                          | 9      | 51,414     | mL98     |
| Protein ID | Description                                      | Accession | Mass q | m/Δm  |
|------------|--------------------------------------------------|-----------|--------|--------|
| Q0QFT7     | Uncharacterized protein                          |           | 9      | 11     | 30,257 | mS65  |
| Q0Q62A     | Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) |           | 9      | -      | 39,194 | mL82  |
| Q0Q992     | Uncharacterized protein                          |           | 9      | 15     | 37,845 | mL82  |
| Q0Q1E3     | Uncharacterized protein                          |           | 9      | 9      | 22,135 | mL82  |
| Q0Q1T7     | Uncharacterized protein                          |           | 9      | 6      | 22,303 | mL88  |
| Q0Q1A6     | Peptidyl-prolyl cis-trans isomerase (PPIase) (EC 5.2.1.8) |           | 9      | 5      | 28,895 | mL82  |
| E9ACP5     | Uncharacterized protein                          |           | 9      | 4      | 17,863 | mL84  |
| Q0Q315     | Tubulin beta chain                               |           | 9      | 3      | 49,707 | mL88  |
| Q0Q8J2     | Uncharacterized protein                          |           | 9      | -      | 37,207 | mL84  |
| Q0Q0C4     | Uncharacterized protein                          |           | 9      | -      | 20,976 | mt-SAF29 |
| Q0Q0G0     | G domain-containing protein                      |           | 8      | 1      | 44,291 | mL64  |
| Q0Q518     | AKAP7_NLS domain-containing protein              |           | 8      | 1      | 31,417 | mt-SAF23 |
| Q0Q8P2     | Uncharacterized protein                          |           | 8      | -      | 97,115 | mL79  |
| Q0Q478     | Uncharacterized protein                          |           | 8      | 14     | 33,336 | mL79  |
| Q0Q564     | Uncharacterized protein                          |           | 8      | 9      | 21,571 | mL72m |
| E9A000     | Uncharacterized protein                          |           | 8      | 5      | 21,523 | mL89  |
| Q0Q1E3     | Uncharacterized protein                          |           | 8      | -      | 62,282 | mL79  |
| Q0Q27I     | DUF4379 domain-containing protein                |           | 8      | -      | 20,241 | mt-SAF33 |
| Q0Q4V1     | Uncharacterized protein                          |           | 7      | -      | 117,808 | mL79  |
| Q0Q4V5     | Uncharacterized protein                          |           | 7      | -      | 56,030 | mL79  |
| E9A029     | LMWPc-domain-containing protein                  |           | 7      | 1      | 33,277 | mtSAF26 |
| Q0Q4J5     | Uncharacterized protein                          |           | 7      | -      | 53,871 | mt-SAF15 |
| Q0Q1S4     | Putative 40S ribosomal protein S18               |           | 6      | -      | 17,394 | mL79  |
| E9A4A0     | G domain-containing protein                      |           | 6      | -      | 69,132 | mL79  |
| Q0Q3S0     | CCHC-type domain-containing protein              |           | 6      | 1      | 123,835 | mL79  |
| E9A4A8     | Uncharacterized protein                          |           | 6      | -      | 24,907 | mL79  |
| E9A4C8     | Uncharacterized protein                          |           | 6      | -      | 67,613 | mL79  |
| Q0Q4A2     | Uncharacterized protein                          |           | 6      | 3      | 16,272 | mL69  |
| Q0Q595     | Uncharacterized protein                          |           | 6      | -      | 23,326 | mt-SAF15 |
| Q0Q4E8     | Uncharacterized protein                          |           | 6      | 4      | 36,029 | mL73  |
| Q0Q559     | Putative GTP-binding protein                     |           | 6      | -      | 53,369 | mL73  |
| Q0Q5F7     | Uncharacterized protein                          |           | 6      | -      | 14,757 | mL73  |
| Q0Q408     | Uncharacterized protein                          |           | 6      | -      | 85,407 | mL73  |
| Q0Q4E9     | Uncharacterized protein                          |           | 6      | -      | 19,653 | mL73  |
| Q0Q4G5     | SpoU_methylase domain-containing protein         |           | 6      | -      | 54,837 | mL73  |
| E9A4D6     | Uncharacterized protein                          |           | 6      | -      | 46,851 | mt-SAF17 |
| Q0Q4S1     | 405 ribosomal protein S4                        |           | 5      | -      | 30,682 | mL73  |
| Q0Q1C2     | 405 ribosomal protein S24                       |           | 5      | 1      | 15,770 | mL73  |
| Q0Q0Q0     | Uncharacterized protein                          |           | 5      | 2      | 44,859 | mL73  |
| Q0Q4M4     | Uncharacterized protein                          |           | 5      | 1      | 118,855 | mL73  |
| Q0Q1N7     | Uncharacterized protein                          |           | 5      | -      | 96,956 | mL73  |
| Q0Q4E1     | Uncharacterized protein                          |           | 5      | -      | 113,481 | mL73  |
| Q0Q5A3     | Uncharacterized protein                          |           | 5      | 10     | 58,044 | mL38  |
| Q0Q8T2     | Mitochondrial guide RNA binding complex subunit 1 |           | 5      | 2      | 54,654 | mL38  |
| B6SB7      | Mitochondrial edited mRNA stability factor 1     |           | 5      | -      | 51,539 | mL38  |
| E9A4D3     | Uncharacterized protein                          |           | 5      | -      | 79,288 | mt-SAF10 |
| E9A72      | DUF4139 domain-containing protein                |           | 5      | -      | 69,358 | mL73  |
| E9A5K7     | SET domain-containing protein                    |           | 5      | -      | 34,786 | mL73  |
| Q0QFF2     | Putative ribonucleoprotein p18, mitochondrial    |           | 4      | -      | 21,300 | mL73  |
| Q0QCO6     | Putative A TP synthase F1 subunit gamma protein (EC 3.6.3.1) |           | 4      | -      | 34,420 | mL73  |
| Q0Q5E6     | Histone H2A                                      |           | 4      | -      | 18,696 | mL73  |
| Accession    | Description                                                      | Start | End  | Length |
|--------------|------------------------------------------------------------------|-------|------|--------|
| Q4Q12        | Uncharacterized protein                                          | 4     | 13   | 13,946 |
| Q4Q27        | Uncharacterized protein                                          | 4     | 1    | 17,496 |
| Q4Q31        | Uncharacterized protein                                          | 4     | -    | 37,766 |
| E9ADV9       | PKS_ER domain-containing protein                                 | 4     | 4    | 37,762 |
| Q4Q5A0       | Uncharacterized protein                                          | 4     | 8    | 16,876 |
| Q4Q28        | Uncharacterized protein                                          | 4     | 13   | 13,444 |
| Q4Q77        | Uncharacterized protein                                          | 4     | 10   | 22,140 |
| O97016       | Putative chaperone protein DNAj                                  | 4     | -    | 51,472 |
| Q4Q63        | Putative DEAD-Box ATP-dependent (RNA) helicase                   | 4     | -    | 66,051 |
| Q4QCN7       | Putative 40S ribosomal protein S11 (Ribosomal protein S11)       | 3     | -    | 16,312 |
| Q4Q59        | Uncharacterized protein                                          | 3     | 1    | 42,045 |
| Q4QW3        | Putative 40S ribosomal protein S15A                              | 3     | 1    | 14,696 |
| Q4Q64        | Ribosomal protein S25                                            | 3     | -    | 13,147 |
| Q4Q66        | Histone H2B                                                      | 3     | -    | 11,847 |
| Q4Q9A4       | Uncharacterized protein                                          | 3     | -    | 56,366 |
| Q4Q9A2       | Glutaredoxin domain-containing protein                            | 3     | -    | 19,586 |
| Q4Q8U4       | Uncharacterized protein                                          | 3     | -    | 14,421 |
| Q4Q9A2       | Uncharacterized protein                                          | 3     | -    | 27,500 |
| Q4Q9A2       | Uncharacterized protein                                          | 3     | -    | 21,298 |
| Q4Q5P0       | 40S ribosomal protein S2                                          | 2     | -    | 28,658 |
| Q4Q9A2       | Uncharacterized protein                                          | 2     | -    | 18,120 |
| Q4Q9A2       | p36 LACK protein                                                 | 2     | -    | 34,429 |
| Q4Q1V3       | Putative 40S ribosomal protein S9 (Ribosomal protein S9)          | 2     | 1    | 22,112 |
| Q4Q5P0       | 40S ribosomal protein S5                                          | 2     | -    | 30,008 |
| Q4Q9A2       | 40S ribosomal protein S12                                        | 2     | -    | 15,608 |
| Q4Q9A2       | Uncharacterized protein                                          | 2     | -    | 107,067|
| Q4Q9A2       | Small nuclear ribonucleoprotein component-like protein            | 2     | -    | 110,261|
| Q4Q9A2       | Uncharacterized protein                                          | 2     | -    | 46,449 |
| Q4Q9A2       | Uncharacterized protein                                          | 2     | -    | 92,869 |
| Q4Q9A2       | Putative 60S ribosomal protein                                    | 2     | 10   | 19,515 |
| Q4Q9A2       | Carbonic anhydrase (EC 4.2.1.1) (Carbonate dehydratase)          | 2     | 3    | 34,533 |
| Q4Q9A2       | Uncharacterized protein                                          | 2     | -    | 34,711 |
| Q4Q9A2       | Uncharacterized protein                                          | 2     | -    | 17,424 |
| Q4Q9A2       | MRP-L46 domain-containing protein                                 | 2     | 9    | 33,299 |
| Q4Q9A2       | Uncharacterized protein                                          | 2     | 15   | 33,161 |
| Q4Q9A2       | Putative spliced leader RNA PSE-promoter transcription factor     | 2     | 1    | 77,394 |
| Q4Q9A2       | Polyadenylate-binding protein (PAPB)                             | 2     | -    | 65,351 |
| Q4Q9A2       | Putative cytochrome c oxidase VII (EC 1.9.3.1)                    | 2     | -    | 19,070 |
| Q4Q9A2       | S-adenosyl-methyltransferase mraW-like protein (EC 2.1.1.1)      | 2     | -    | 54,542 |
| Q4Q9A2       | Uncharacterized protein                                          | 2     | -    | 45,498 |
| Q4Q9A2       | Uncharacterized protein                                          | 2     | -    | 13,023 |
| Q4Q9A2       | Uncharacterized protein                                          | 2     | -    | 57,197 |
| Q4Q9A2       | Uncharacterized protein                                          | 2     | -    | 79,926 |
| Accession | Description                                                                 | Uncharacterized | Mass (Da) |
|-----------|-----------------------------------------------------------------------------|----------------|-----------|
| Q0464     | Uncharacterized protein                                                      | 2              | 36,023    |
| RS6       | 40S ribosomal protein 5S                                                      | 1              | 28,304    |
| Q0216     | Putative ubiquitin/ribosomal protein S27a                                    | 1              | 16,997    |
| Q0440     | Putative 40S ribosomal protein 5S                                            | 1              | 24,472    |
| Q0806     | Putative 40S ribosomal protein S17                                            | 1              | 16,512    |
| Q0403     | Putative 40S ribosomal protein S21                                            | 1              | 17,003    |
| Q0489     | Putative 40S ribosomal protein S23                                            | 1              | 15,936    |
| Q0117     | Putative 40S ribosomal protein S10                                            | 1              | 18,650    |
| Q0959     | Uncharacterized protein                                                       | 1              | 16,713    |
| Q0177     | Putative DEAD/h helicase                                                      | 1              | 123,202   |
| Q0338     | Histone H4                                                                   | 1              | 11,439    |
| Q0036     | Uncharacterized protein                                                       | 1              | 260,220   |
| EA5F3     | Putative sm-f rNFP core complex protein                                       | 1              | 27,541    |
| EA6D1     | Tryparaedol-like protein                                                      |                | 1         |
| EA5F2     | Putative 60S ribosomal protein L17                                            | 1              | 19,113    |
| Q0837     | Uncharacterized protein                                                       | 1              | 59,267    |
| Q0136     | Uncharacterized protein                                                       | 1              | 42,620    |
| Q0032     | Uncharacterized protein                                                       | 1              | 61,688    |
| Q0329     | Uncharacterized protein                                                       | 1              | 9,846     |
| Q0035     | Chaperone protein DNA-like protein                                            | 1              | 58,661    |
| Q0147     | Isocitrate dehydrogenase (NADP) [EC 1.1.1.42]                                | 1              | 48,490    |
| Q0980     | Metallo-beta-lactamase family proteinlike protein                            | 1              | 42,570    |
| Q0146     | Probable citrate synthase, mitochondrial [EC 2.3.3.16]                       | 1              | 52,329    |
| Q04A8     | Putative 2,4-dienoyl-coa reductase fadh1 [EC 1.3.1.34]                       | 1              | 78,693    |
| Q0658     | Putative ATP synthase, epsilon chain [EC 3.6.3.14]                          | 1              | 20,100    |
| Q0837     | Putative cytochrome c oxidase subunit 10 [EC 1.9.3.1]                       | 1              | 14,120    |
| Q0148     | Putative DEAD/DEAH box helicase                                              | 1              | 77,583    |
| Q0034     | Putative small nuclear ribonucleoprotein                                     | 1              | 76,688    |
| Q0148     | Splicing factor pter1-like protein                                           | 1              | 41,893    |
| Q0141     | Uncharacterized protein                                                       | 1              | 16,860    |
| Q0148     | Uncharacterized protein                                                       | 1              | 75,893    |
| Q0559     | Uncharacterized protein                                                       | 1              | 14,999    |
| Q0989     | Uncharacterized protein                                                       | 1              | 63,489    |
| Q0710     | DUF4139 domain-containing protein                                            | 1              | 62,682    |
| Q0764     | Uncharacterized protein                                                       | 1              | 52,514    |
| Q04A8     | Uncharacterized protein                                                       | 1              | 78,474    |
| Q0356     | Uncharacterized protein                                                       | 1              | 18,757    |
| Q0316     | Uncharacterized protein                                                       | 1              | 83,946    |
| EA9F18    | d(3)com domain-containing protein                                            | 1              | 13,514    |
| EA9F21    | Uncharacterized protein                                                       | 1              | 66,953    |
| Q0854     | Putative ribosomal protein 520                                               | 1              | 13,011    |
| Q0994     | Putative 60S ribosomal protein L7                                             | 1              | 28,845    |
| Q04FX5    | Putative 60S ribosomal protein L2                                             | 1              | 28,273    |
| Q0889     | Putative 60S ribosomal protein L13a                                           | 1              | 25,435    |
| Q02H9     | Putative 60S ribosomal protein L21                                            | 1              | 17,988    |
| Q01P8     | Putative 60S ribosomal protein L7a (Ribosomal protein L7a)                   | 1              | 29,764    |
| EA9D6     | Uncharacterized protein                                                       | 1              | 33,647    |
| Q0986     | Uncharacterized protein                                                       | 1              | 99,707    |
| EA9F18    | d(3)com domain-containing protein                                            | 1              | 50,291    |

**Supplementary Table 1. List of proteins identified by mass spectrometry analysis in the Leishmania samples**
The total list of proteins identified by quantitative nano LC-ESI-MS/MS in the fractions that were used to generate the reconstructions of the LSU assembly intermediate and the full mitoribosome of Leishmania are presented. UNIPROT IDs correspond to *Leishmania major*. Columns C and D provide the number of spectra for each identified protein. Maturation factors are highlighted in blue in column F.
| Uniprot ID | Description | SSU + mt-IF3 | Full ribosome | Mass (kDa) | Name |
|-----------|-------------|--------------|---------------|-----------|------|
| Q4DN9A    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 121 | 102 | 135,998 | mS49 |
| Q4CTE8    | 2-oxoglutarate dehydrogenase E1 component, putative OS=Trypanosoma cruzi | 169 | 24 | 112,242 | mS49 |
| V5BN8B    | Uncharacterized protein OS=Trypanosoma cruzi Dm28c | 143 | 124 | 135,938 | mS49 |
| Q4DB95    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 142 | 119 | 135,845 | mS49 |
| Q4CYI3    | 2-oxoglutarate dehydrogenase subunit, putative OS=Trypanosoma cruzi | 138 | 16 | 113,643 | mS48 |
| Q4DV2     | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 123 | 132 | 206,251 | mS48 |
| K2N7T3    | 2-oxoglutarate dehydrogenase subunit, putative OS=Trypanosoma cruzi | 112 | 17 | 113,779 | mS48 |
| V5BMW3    | 2-oxoglutarate dehydrogenase E1 component OS=Trypanosoma cruzi | 112 | 20 | 112,404 | mS48 |
| Q4DEM5    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 108 | 89 | 94,164 | uS10m |
| V5B2KO    | Uncharacterized protein OS=Trypanosoma cruzi Dm28c | 100 | 110 | 194,279 | mS48 |
| K2MVD1    | 2-oxoglutarate dehydrogenase E1 component, putative OS=Trypanosoma cruzi | 94 | 16 | 112,898 | mS26 |
| K2NAM2    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 91 | 101 | 201,004 | mS48 |
| Q4DVS1    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 85 | 69 | 76,127 | mS53 |
| Q4OUA8    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 83 | 44 | 51,629 | mS26 |
| Q4DLY9    | 2-oxoglutarate dehydrogenase, E2 component, dihydrolipoamide | 83 | 11 | 44,032 | mS48 |
| Q4CVW1    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 82 | 62 | 77,862 | mS54 |
| K2M5S1    | 2-oxoglutarate dehydrogenase, E2 component, dihydrolipoamide | 82 | 11 | 43,989 | mS54 |
| Q4CM44    | 2-oxoglutarate dehydrogenase subunit, putative OS=Trypanosoma cruzi | 80 | 13 | 38,021 | mS47 |
| Q4DIK2    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 78 | 54 | 134,647 | mS47 |
| Q4DGO5    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 77 | 52 | 134,550 | mS47 |
| Q4E2AK    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 75 | 59 | 81,302 | mS52 |
| Q4CJW0    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 75 | 60 | 128,849 | mS50 |
| K2MOF5    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 68 | 55 | 76,848 | uS10m |
| Q4D9W9    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 68 | 75 | 113,325 | mS48 |
| Q4DIY1    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 67 | 69 | 99,100 | mS35 |
| Q4E4EO    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 66 | 49 | 37,149 | bS19m |
| K2N5XL    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 63 | 48 | 37,093 | bS19m |
| K2MX46    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 50 | 23 | 51,568 | mS26 |
| Q4DTP7    | ATP synthase subunit beta OS=Trypanosoma cruzi (strain CL Brener) | 47 | 10 | 55,731 | mS48 |
| Q4E2O1    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 47 | 41 | 28,832 | mS34 |
| Q4D9W9    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 43 | 31 | 50,264 | uS9m |
| Q4E4R7    | 30S ribosomal protein S17, putative OS=Trypanosoma cruzi | 43 | 32 | 35,826 | uS17m |
| K2NKW7    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 40 | 33 | 94,888 | mS51 |
| K2NW49    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 39 | 35 | 66,909 | mS55 |
| Q4D0U2    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 39 | 33 | 35,578 | mS59 |
| Q4CS70    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 37 | 29 | 48,970 | mS43 |
| Q4CT44    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 37 | 36 | 37,664 | uS11m |
| Q4DFP3    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 37 | 37 | 34,817 | mS23 |
| K2MV19    | ATP synthase subunit alpha OS=Trypanosoma cruzi marinkellei | 36 | 0 | 62,951 | mS59 |
| K2M5F5    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 35 | 29 | 35,580 | mS59 |
| Q4E46E    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 34 | 45 | 73,008 | mS22 |
| Q4D913    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 32 | 2 | 42,844 | mS41 |
| Q4CO3C    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 32 | 22 | 24,722 | mS55 |
| Q4DY17    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 30 | 24 | 33,161 | uS9m |
| Q4K9R2    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 29 | 38 | 20,946 | mS33 |
| Q4AC7     | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 28 | 25 | 47,797 | mS66 |
| Q4DE3     | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 28 | 2 | 42,700 | mS31 |
| Q4O6V2    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 28 | 27 | 34,302 | mS60 |
| Q4D7Y5    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 28 | 22 | 32,872 | mS61 |
| Q4CT7     | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 28 | 4 | 23,295 | mS69 |
| Q4DFQ8    | Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) | 28 | 27 | 21,638 | mS69 |
| Accession | Name | Organism | Mw | >1s | >2s |
|-----------|------|----------|----|-----|-----|
| Q0E219 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 26 | 26 | 27,653 |
| Q0D2A7 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 26 | 22 | 21,686 |
| Q0D4X4 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 25 | 20 | 19,369 |
| Q0D7F6 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 24 | 24 | 47,739 |
| Q0D9A1 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 24 | 26 | 46,202 |
| Q0WU84 | Dihydrolipoamide dehydrogenase | OS=Trypanosoma cruzi (strain CL Brener) | 23 | 132 | 50,512 |
| Q0CP38 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 23 | 77 | 27,872 |
| Q0DG66 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 23 | 14 | 25,894 |
| Q0DC33 | Dihydrolipoamide dehydrogenase | OS=Trypanosoma cruzi (strain CL Brener) | 22 | 158 | 50,588 |
| Q0DT77 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 22 | 26 | 34,964 |
| K2MTK9 | Uncharacterized protein | OS=Trypanosoma cruzi marinkellei | 20 | 15 | 57,754 |
| Q0E1JX | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 20 | 14 | 29,786 |
| Q0D0I4 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 19 | 31 | 31,580 |
| K5NM10 | Uncharacterized protein | OS=Trypanosoma cruzi marinkellei | 19 | 11 | 26,783 |
| Q0E566 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 18 | 9 | 29,682 |
| Q0D583 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 18 | 15 | 19,835 |
| Q0DT78 | Acetyltransferase component of pyruvate dehydrogenase complex | OS=Trypanosoma cruzi (strain CL Brener) | 17 | 792 | 49,690 |
| Q0DY70 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 17 | - | 42,967 |
| K5MF15 | Uncharacterized protein | OS=Trypanosoma cruzi marinkellei | 17 | 8 | 24,888 |
| Q0D7J1 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 17 | 8 | 22,982 |
| Q0DT73 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 16 | 10 | 29,624 |
| Q0DO03 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 16 | 17 | 27,460 |
| Q0F1E1 | Cyclophilin, putative | OS=Trypanosoma cruzi (strain CL Brener) | 16 | 26 | 25,587 |
| K5MY51 | Uncharacterized protein | OS=Trypanosoma cruzi marinkellei | 15 | - | 42,905 |
| K5PA71 | Uncharacterized protein | OS=Trypanosoma cruzi marinkellei | 15 | 18 | 24,554 |
| Q0DU99 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 14 | 31 | 53,091 |
| Q0UX09 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 14 | 21 | 21,625 |
| Q0D2A7 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 14 | 10 | 19,162 |
| Q4CS81 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 14 | 28 | 15,822 |
| Q0E5Y6 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 13 | 19 | 35,044 |
| Q0D3U1 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 13 | 49 | 31,630 |
| Q0DIY0 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 13 | 10 | 22,380 |
| VS8FP9 | Dihydrolipoamide dehydrogenase | OS=Trypanosoma cruzi (strain CL Brener) | 12 | 553 | 49,719 |
| Q0MG69 | Acetyltransferase component of pyruvate dehydrogenase complex | OS=Trypanosoma cruzi (strain CL Brener) | 12 | 544 | 49,659 |
| K5MQ09 | Dihydrolipoamide dehydrogenase, putative | OS=Trypanosoma cruzi (strain CL Brener) | 12 | 439 | 49,617 |
| Q0OH10 | ATP synthase F1 subunit gamma protein, putative | OS=Trypanosoma cruzi (strain CL Brener) | 11 | - | 34,612 |
| Q0DR63 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 10 | 28 | 50,187 |
| Q0DE13 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 10 | 23 | 40,053 |
| Q4CG2B | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 10 | 41 | 32,773 |
| Q0D533 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 10 | 10 | 22,254 |
| Q0C4W2 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 9 | 24 | 36,064 |
| Q0E9X6 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 9 | 9 | 34,292 |
| K5MX05 | Uncharacterized protein | OS=Trypanosoma cruzi marinkellei | 9 | 17 | 31,950 |
| Q0DV7 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 9 | 26 | 21,306 |
| Q0RG02 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 9 | 4 | 19,771 |
| Q0VF14 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 9 | 15 | 13,944 |
| Q4CS45 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 8 | 21 | 62,718 |
| Q0D757 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 8 | 28 | 58,478 |
| V58764 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 8 | 23 | 56,385 |
| K5MN17 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 8 | 44 | 48,797 |
| K5NB85 | Uncharacterized protein | OS=Trypanosoma cruzi marinkellei | 8 | 27 | 44,214 |
| Q0WI44 | Uncharacterized protein | OS=Trypanosoma cruzi (strain CL Brener) | 8 | 34 | 39,285 |
| Accession | Description                                      | OS          | Length | Molecular weight |
|-----------|--------------------------------------------------|-------------|--------|------------------|
| Q4DB71    | Pyruvate dehydrogenase E1 beta subunit, putative | OS=Trypanosoma cruzi | 8      | 638              | 37,868 |
| Q4NC63    | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 8      | 26               | 32,842 |
| Q4E134     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 8      | -                | 26,627 |
| Q4E3P9     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 8      | -                | 27,644 |
| Q4S3X9     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 8      | 30               | 26,859 |
| Q4S5P6     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 8      | 29               | 21,890 |
| Q4S1J8     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 8      | 22               | 20,316 |
| Q4Q0T0     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 8      | 16               | 18,529 |
| Q4E1W9     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 8      | 26               | 12,761 |
| Q4C2Y2     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 7      | 25               | 93,811 |
| Q4N1M5     | Uncharacterized protein                           | OS=Trypanosoma cruzi marinkellei | 7      | 20               | 58,186 |
| Q4G1J1     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 7      | 25               | 52,750 |
| Q4G6V1     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 7      | 27               | 52,575 |
| Q4D2A1     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 7      | 27               | 16,637 |
| Q4D746     | Ribosomal protein L11, putative                  | OS=Trypanosoma cruzi (strain CL Brener) | 7      | 30               | 39,600 |
| Q4G223     | RNA-binding protein RGGm, putative               | OS=Trypanosoma cruzi (strain CL Brener) | 7      | 34               | 34,655 |
| Q4B8D7     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 7      | 13               | 30,734 |
| Q4S5V9     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 7      | 10               | 28,904 |
| Q4H989     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 7      | 26               | 25,032 |
| Q4A712     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 7      | 21               | 20,851 |
| Q4G9F7     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 7      | 13               | 16,836 |
| Q4C2C1     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 7      | 12               | 100,980 |
| Q4N598     | Ribosomal protein L3 mitochondrial, putative      | OS=Trypanosoma cruzi | 6      | 36               | 55,031 |
| Q96B8G     | Pyruvate dehydrogenase E1 component subunit alpha | OS=Trypanosoma cruzi | 6      | 933              | 42,770 |
| Q2N41      | Uncharacterized protein                           | OS=Trypanosoma cruzi marinkellei | 6      | 24               | 31,521 |
| Q4V44      | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 6      | 4                | 31,277 |
| Q2N1F5     | Uncharacterized protein                           | OS=Trypanosoma cruzi marinkellei | 6      | 2                | 30,145 |
| Q2S5E9     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 6      | 18               | 22,812 |
| Q4U58      | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 6      | 23               | 22,785 |
| Q4O4V7     | 60S ribosomal protein, putative                   | OS=Trypanosoma cruzi (strain CL Brener) | 6      | 54               | 19,644 |
| Q4C6N8     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 6      | 1                | 16,476 |
| Q4O49G     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 6      | 11               | 13,913 |
| Q4O1X4     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 6      | 15               | 13,008 |
| Q2N4E6     | Uncharacterized protein                           | OS=Trypanosoma cruzi marinkellei | 5      | 15               | 55,867 |
| Q4D961     | Ribosomal protein L2, putative                   | OS=Trypanosoma cruzi (strain CL Brener) | 5      | 30               | 48,809 |
| Q4O615     | Glutaminyl carboxypeptidase, putative             | OS=Trypanosoma cruzi (strain CL Brener) | 5      | 13               | 43,706 |
| Q4G5G7     | DnaJ chaperone protein, putative                 | OS=Trypanosoma cruzi (strain CL Brener) | 5      | 16               | 38,102 |
| Q4C3I3     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 5      | 16               | 33,585 |
| Q4D8B8     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 5      | 8                | 29,690 |
| Q4O1W1     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 5      | 8                | 24,087 |
| Q4C9Y1     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 5      | 15               | 23,583 |
| Q4E2Q1     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 5      | 14               | 22,459 |
| Q4O1X0     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 5      | 35               | 22,357 |
| Q4C9Q5     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 5      | 20               | 18,066 |
| Q4C2U9     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 5      | 13               | 16,579 |
| Q4O4C5     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 5      | 13               | 11,460 |
| Q4F1L1     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 4      | 13               | 51,062 |
| Q4D6S6     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 4      | 21               | 39,795 |
| K2N1L5     | Uncharacterized protein                           | OS=Trypanosoma cruzi marinkellei | 4      | 27               | 32,646 |
| V5D804     | Uncharacterized protein                           | OS=Trypanosoma cruzi Dm28c | 4      | 14               | 25,440 |
| Q4O1V6     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 4      | 32               | 17,575 |
| Q4FNN3     | Uncharacterized protein                           | OS=Trypanosoma cruzi (strain CL Brener) | 3      | 4                | 97,372 |
| Accession | Description                                                                 | Peptide Coverage | Start | End   | Score |
|-----------|------------------------------------------------------------------------------|------------------|-------|-------|-------|
| Q4V33     | Zn-finger protein, putative OS=Trypanosoma cruzi | 3                | 0     | 6     | 59,939 |
| Q4W012    | Uncharacterized protein OS=Trypanosoma cruzi (strain C) | 3                | 0     | 2     | 59,041 |
| K2MV12    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | -     | -     | 47,021 |
| Q4C66     | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 12    | -     | 42,299 |
| Q4DN00    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 10    | -     | 36,053 |
| Q4EM11    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 8     | -     | 35,506 |
| Q4DF99    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 16    | -     | 33,010 |
| Q4CV77    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 16    | -     | 26,230 |
| Q4CU00    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 18    | -     | 24,776 |
| Q4KO06    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 17    | -     | 21,933 |
| Q4CJ03    | Cyclophilin-like protein, putative OS=Trypanosoma cruzi marinkellei | 3                | 24    | -     | 21,633 |
| Q4OU02    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 10    | -     | 17,217 |
| Q4PJ3     | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 16    | -     | 11,340 |
| Q4OM54    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 15    | 5     | 87,265 |
| Q4OW02    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 25    | 5     | 70,553 |
| Q4 CYU3   | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 15    | 6     | 63,939 |
| K2UQ57    | Heat shock 70 kDa protein, mitochondrial, putative OS=Trypanosoma cruzi marinkellei | 3                | 15    | 6     | 56,909 |
| G8FT31    | Beta tubulin 1.9 OS=Trypanosoma cruzi PE=2 SV=1 | 3                | 15    | 10    | 49,700 |
| Q4EL15    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 15    | 10    | 42,289 |
| K2MJ55    | 2-oxosuccinate dehydrogenase beta subunit, mitochondrial OS=Trypanosoma cruzi marinkellei | 3                | 31    | 18    | 37,813 |
| K2UJ17    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 18    | 12    | 24,776 |
| Q4OM08    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 12    | 10    | 22,174 |
| Q4OV03    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 28    | 19    | 18,894 |
| Q4CJ18    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 4     | 2     | 12,313 |
| Q4OA4     | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 18    | 12    | 116,260 |
| Q4OU06    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 2     | -     | 11,770 |
| Q4D0T0    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 2     | 2     | 104,673 |
| Q4CW3     | Trifunctional enzyme alpha subunit, mitochondrial-like OS=Trypanosoma cruzi marinkellei | 3                | 1     | 4     | 75,687 |
| Q4Q04     | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 1     | 16    | 29,057 |
| Q4D69     | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 1     | 10    | 22,458 |
| K2NH19    | Cyclophilin-like protein, putative OS=Trypanosoma cruzi marinkellei | 3                | 1     | 14    | 21,682 |
| Q4D022    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 1     | 1     | 13,585 |
| Q4DT03    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 1     | -     | 12,484 |
| Q4DVX1    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | 1     | 1     | 12,279 |
| Q4D500    | Mitochondrial oligo_U binding protein TBRGG1, putative OS=Trypanosoma cruzi marinkellei | 3                | -     | 6     | 97,265 |
| Q4CN12    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | -     | 3     | 93,961 |
| Q4DG02    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | -     | 7     | 93,124 |
| Q4DFW2    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | -     | 7     | 93,091 |
| K2MN15    | Inorganic polyphosphate:ATP-NAD kinase, putative poly(P) OS=Trypanosoma cruzi marinkellei | 3                | -     | 8     | 88,728 |
| Q4NH09    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | -     | 7     | 78,508 |
| Q4BN28    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | -     | 3     | 77,093 |
| Q4HV00    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | -     | 2     | 71,442 |
| K2N86     | Glucose-regulated protein 78, putative OS=Trypanosoma cruzi marinkellei | 3                | -     | 1     | 71,396 |
| K2NU53    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | -     | 5     | 69,569 |
| Q4F08     | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | -     | 3     | 69,291 |
| Q4CX3     | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | -     | 4     | 69,087 |
| Q4DV13    | Small GTP-binding protein, putative OS=Trypanosoma cruzi marinkellei | 3                | -     | 3     | 64,673 |
| Q4OV06    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | -     | 2     | 64,110 |
| Q4CX1     | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | -     | 2     | 63,653 |
| K2ND60    | Polyadenylate-binding protein OS=Trypanosoma cruzi marinkellei | 3                | -     | 1     | 61,410 |
| Q4FRY7    | Uncharacterized protein OS=Trypanosoma cruzi marinkellei | 3                | -     | 6     | 60,962 |
| Q4CU66    | Inosine-5'-monophosphate dehydrogenase OS=Trypanosoma cruzi marinkellei | 3                | -     | 5     | 55,602 |
Supplementary Table 2. List of proteins identified by mass spectrometry analysis in the Trypanosoma samples.

The total list of proteins identified by quantitative nano LC-ESI-MS/MS in the fractions that were used to generate the reconstructions of the SSU + mt-IF3 and the full mitoribosome of *Trypanosoma cruzi* are presented. UNIPROT IDs correspond to *Trypanosoma cruzi* (strain CL Brener) and *Trypanosoma cruzi marinkellei*, therefore the same protein may be listed multiple times. Columns C and D provide the number of spectra for each identified protein.
## Proteins of the Large subunit

| MRP | Uniprot ID | Chain ID | Size (aa) | Matured L SU | Immature LSU | Comments |
|-----|------------|----------|-----------|--------------|--------------|----------|
| mL38 | Q4Q211 | A | 180 | 73-179 | - | Absent in assembly intermediate, blocked by mt-L AF 1, part of the CP |
| mL39 | Q4Q5X5 | A | 807 | 14-401 | 14-389 | - |
| mL40 | Q4Q0U6 | B | 436 | 1-436 | 1-436 | - |
| mL41 | Q4Q0E1 | C | 262 | 9-220 | 9-220 | - |
| mL42 | Q4Q177 | D | 348 | 14-141 | - | Absent in assembly intermediate |
| mL43 | Q4QC79 | E | 346 | 14-339 | 14-238 | - |
| mL44 | Q4Q2H4 | F | 171 | 1-170 | 1-170 | - |
| mL45 | Q4Q2G1 | G | 374 | 10-374 | 10-374 | - |
| mL46 | Q4QF09 | H | 168 | 6-167 | - | Absent in assembly intermediate |
| mL47 | Q4Q0F4 | I | 305 | 10-266 | 10-266 | - |
| mL48 | Q4Q2C7 | J | 144 | 2-141 | 2-141 | - |
| mL49 | Q4Q162 | K | 194 | 10-188 | 10-188 | - |
| mL50 | Q4Q2Q5 | L | 186 | 2-179 | 2-179 | - |
| mL51 | Q4Q2BR0 | M | 348 | 34-389 | 34-389 | - |
| mL52 | Q4Q267 | N | 252 | 47-235 | 47-235 | - |
| mL53 | Q4Q234 | O | 234 | 10-226 | 10-226 | - |
| mL54 | Q4Q0M0 | P | 480 | 9-480 | 9-480 | - |
| mL55 | Q4Q137 | Q | 243 | 243-392 | 243-392 | - |
| mL56 | Q4Q0E1 | R | 185 | 21-185 | 21-185 | - |
| mL57 | Q4Q2C7 | S | 185 | 21-185 | 21-185 | - |
| mL58 | Q4Q234 | T | 185 | 21-185 | 21-185 | - |
| mL59 | Q4Q162 | U | 185 | 21-185 | 21-185 | - |
| mL60 | Q4Q234 | V | 185 | 21-185 | 21-185 | - |
| mL61 | Q4Q234 | W | 185 | 21-185 | 21-185 | - |
| mL62 | Q4Q234 | X | 185 | 21-185 | 21-185 | - |
| mL63 | Q4Q234 | Y | 185 | 21-185 | 21-185 | - |
| mL64 | Q4Q234 | Z | 185 | 21-185 | 21-185 | - |
| mL39 | Q4Q234 | A | 262 | 36-290 | 36-290 | - |
| mL40 | Q4Q234 | B | 262 | 36-290 | 36-290 | - |
| mL41 | Q4Q234 | C | 262 | 36-290 | 36-290 | - |
| mL42 | Q4Q234 | D | 262 | 36-290 | 36-290 | - |
| mL43 | Q4Q234 | E | 262 | 36-290 | 36-290 | - |
| mL44 | Q4Q234 | F | 262 | 36-290 | 36-290 | - |
| mL45 | Q4Q234 | G | 262 | 36-290 | 36-290 | - |
| mL46 | Q4Q234 | H | 262 | 36-290 | 36-290 | - |
| mL47 | Q4Q234 | I | 262 | 36-290 | 36-290 | - |
| mL48 | Q4Q234 | J | 262 | 36-290 | 36-290 | - |
| mL49 | Q4Q234 | K | 262 | 36-290 | 36-290 | - |
| mL50 | Q4Q234 | L | 262 | 36-290 | 36-290 | - |
| mL51 | Q4Q234 | M | 262 | 36-290 | 36-290 | - |
| mL52 | Q4Q234 | N | 262 | 36-290 | 36-290 | - |
| mL53 | Q4Q234 | O | 262 | 36-290 | 36-290 | - |
| mL54 | Q4Q234 | P | 262 | 36-290 | 36-290 | - |
| mL55 | Q4Q234 | Q | 262 | 36-290 | 36-290 | - |
| mL56 | Q4Q234 | R | 262 | 36-290 | 36-290 | - |
| mL57 | Q4Q234 | S | 262 | 36-290 | 36-290 | - |
| mL58 | Q4Q234 | T | 262 | 36-290 | 36-290 | - |
| mL59 | Q4Q234 | U | 262 | 36-290 | 36-290 | - |
| mL60 | Q4Q234 | V | 262 | 36-290 | 36-290 | - |
| mL61 | Q4Q234 | W | 262 | 36-290 | 36-290 | - |
| mL62 | Q4Q234 | X | 262 | 36-290 | 36-290 | - |
| mL63 | Q4Q234 | Y | 262 | 36-290 | 36-290 | - |
| mL64 | Q4Q234 | Z | 262 | 36-290 | 36-290 | - |
### Proteins of the Small subunit

| Chain ID | Comments |
|----------|----------|
| U6       | Unknown |

### Maturation factors

| MFCR   | Chain ID | Comments |
|--------|----------|----------|
| U6     | -        | -        |

### Identified factors

| Factor | Chain ID | Comments |
|--------|----------|----------|
| U6     | -        | -        |

### Modeled residue

| Modeled residue | Comments |
|-----------------|----------|
| mL80            | -        |
### Supplementary Table 3. List of proteins in the models of the Leishmania full ribosome and LSU assembly intermediate

| U3Am | uS3m | Q4Q1N8 | a | 431 | 24-431 |
|------|------|--------|---|-----|--------|
| U3Sm | Q4Q1G8 | b | 159 | 2-156 |
| U3Um | Q4Q9O2 | c | 311 | 61-311 |
| U3Vm | Q4Q67 | d | 351 | 1-351 |
| U3Wm | Q4Q8N0 | e | 822 | 9-822 |
| U3Xm | Q4Q6B8 | f | 371 | 10-230 |
| U3Ym | Q4Q3F8 | h | 166 | 10-166 |
| U3Zm | Q4QCV9 | i | 409 | 107-373 |
| U4Am | Q4Q8H5 | j | 189 | 10-189 |
| U4Bm | E4MY8 | k | 312 | 72-187 |
| U4Cm | Q4Q11O | l | 325 | 7-284 |
| U4Dm | Q4Q2N2 | n | 171 | 30-171 |
| U4Em | E4M13 | o | 192 | 67-165 |
| U4Fm | E4M19 | p | 321 | 21-201 |
| U4Gm | Q4QSG1 | q | 436 | 10-205 |
| U4Hm | Q4Q1U0 | r | 467 | 1-411 |
| U4Im | Q4QST5 | s | 179 | 9-273 |
| U4Jm | Q4Q9W5 | t | 357 | 10-225 |
| U4Km | E4MD40 | u | 800 | 26-859 |
| U4Lm | Q4QCB | v | 514 | 90-152 |
| U4Mm | Q4Q66 | w | 190 | 20-184 |
| U4Nm | Q4Q716 | x | 308 | 9-243 |
| U4Om | Q4Q2D4 | y | 485 | 5-316 |
| U4Pm | Q4Q19O | z | 1170 | 103-1113 |
| U4Qm | Q4QFP4 | aa | 1813 | 72-1670 |
| U4Rm | Q4Q6G8 | ab | 1177 | 27-1170 |
| U4Sm | Q4Q6F6 | ac | 1267 | 85-1266 |
| U4Tm | Q4Q4G0 | ad | 811 | 20-782 |
| U4Um | Q4Q2T6 | i | 677 | 1-474 |
| U4Vm | Q4QFA7 | ae | 601 | 2-545 |
| U4Wm | O4Q717 | af | 835 | 144-435 |
| U4Xm | Q4Q6P4 | ag | 564 | 3-559 |
| U4Ym | Q4QXW | ai | 379 | 1-379 |
| U4Vm | Q4Q7O8 | aj | 397 | 10-225 |
| U4Wm | E4ME8 | ak | 325 | 2-329 |
| U4Xm | E4MG9 | am | 313 | 21-312 |
| U4Um | Q4Q9W4 | an | 382 | 1-382 |
| U4Vm | E4ME7 | ac | 291 | 10-229 |
| U4Wm | Q4Q6K | ap | 645 | 1-245 |
| U4Xm | Q4Q6G5 | aq | 905 | 10-121 |
| U4Ym | Q4Q5L8 | ar | 282 | 16-279 |
| U4Zm | Q4Q1F7 | as | 270 | 24-270 |
| U5Ad | Q4Q2XO | at | 397 | 150-395 |
| U5Bd | Q4Q275 | au | 347 | 9-247 |
| U5Cd | Q4QWNO | av | 236 | 27-186 |
| U5Dd | Q4QHA2 | aw | 129 | 1-139 |
| U5Ed | Q4Q103 | ax | 186 | 10-189 |
| U5Fd | Q4Q7W | ay | 160 | 20-169 |
| U5Gd | Q4Q6W5 | az | 163 | 10-163 |
| U5Hd | Q4Q6W5 | ba | 35 | 66-91 |
Complete list of r-proteins and maturation factors identified in the kinetoplastids mitochondrial ribosomes. The proteins are colored by conservation with the bacterial ribosome (blue) other mitochondrial ribosomes (yellow) or specific to kinetoplastids (red). Proteins with gray cells indicate that they are absent in the LSU assembly intermediate. mS38 and uS12m, proteins located at the interface subunit were only visualised in the SSU/my-IF3 complex. m designate proteins encoded in the mitochondrial genome. Full list of proteins identified by mass spectrometry are provided in Supplementary Table 2 and 3.
| Structure                  | LSU mature | LSU intermediate | Lt-Full | Tc-SSU-if3 |
|---------------------------|------------|------------------|---------|-----------|
| Data collection           |            |                  |         |           |
| Microscope                | Fei Talos Arctica |                |         |           |
| Camera                    | Falcon3    |                  |         |           |
| Voltage                   | 200        |                  |         |           |
| Electron exposure (e/A²)  | 60         |                  |         |           |
| Pixel size                | 1.2        |                  |         |           |
| Magnification             | 120 000    |                  |         |           |
| Defocus range (μm)        | 0.6 – 2.7  |                  |         |           |
| Software                  | EPU        |                  |         |           |
| EM Reconstruction         |            |                  |         |           |
| Initial Particles         | 303 140    | 215 120          | 303 140 | 422 120   |
| Final Particles           | 82 060     | 59 200           | 82 060  | 148 180   |
| Accuracy of rotation      | 0.345      | 0.356            | 0.334   | 0.683     |
| Accuracy of translation   | 0.306      | 0.479            | 0.369   | 0.491     |
| Resolution                | 3.6        | 3.4              | 3.9     | 3.5       |
| B Factors                 | 121.6      | 116.1            | 137.9   | 131       |
| Real Space Refinement     |            |                  |         |           |
| Resolution (masked/unmasked) | 3.6/3.6    | 3.4/3.5          | 3.9/3.9 | 3.5       |
| CCmask                    | 0.80       | 0.76             | 0.76    | 0.67      |
| Resolution according to model versus map FSC = 0.143 / FSC = 0.5 (masked) criteria (Å⁻¹) | 3.5/3.6 | 3.4/3.5 | 3.9/4 | 3.4/3.6 |
| Resolution according to model versus map FSC = 0.143 / FSC = 0.5 (unmasked) criteria (Å⁻¹) | 3.6/4 | 3.4/4.2 | 3.9/5.7 | 3.5/4.1 |
| Model Composition         |            |                  |         |           |
| No. of chains             | 78         | 79               | 124     | 59        |
| Total atoms               | 143723     | 139604           | 303567  | 174007    |
| Protein residues          | 15004      | 15269            | 33249   | 19663     |
| RNA residues              | 1088       | 843              | 1692    | 621       |
| Ligands : Zn/Mg/NAD/UTP/GTP/ATP | 6/2/1/-/-/- | -/-/-/-/-/1/1 | -/-1/-/-/- | 4/18/-/-/1/1/- |
| Average B Factors (mean)  |            |                  |         |           |
| Protein                   | 48.5       | 48.2             | 74.4    | 30.3      |
| Nucleotide                | 52.1       | 67.8             | 90.6    | 36        |
| Ligands                   | 20.6       | 91.8             | 20.6    | 46.7      |
| Model Validation          |            |                  |         |           |
| RMSD bonds                | 0.009      | 0.008            | 0.007   | 0.012     |
| RMSD angles               | 1.01       | 0.858            | 0.772   | 1.05      |
| All-atom clashscore       | 24.5       | 21.4             | 21.27   | 32.11     |
| MolProbity                | 2.54       | 3.21             | 2.37    | 3.31      |
| Ramachandran Statistics   |            |                  |         |           |
|                  |     |     |     |     |
|------------------|-----|-----|-----|-----|
| Favored (%)      | 85.1| 83.65| 90.05| 87.75|
| Allowed (%)      | 14.2| 15.9| 9.71| 12.02|
| Outliers (%)     | 0.64| 0.45| 0.24| 0.24|

Supplementary Table 4. Cryo-EM data collection, refinement and validation statistics