

**Supplementary data for:**

**Evidence for a shared nuclear pore complex architecture that is conserved from the last common eukaryotic ancestor**

Jeffrey A. DeGrasse,<sup>1</sup> Kelly N. DuBois,<sup>2</sup> Damien Devos,<sup>3</sup> T. Nicolai Siegel,<sup>4</sup> Andrej Sali,<sup>5</sup> Mark C. Field,<sup>2</sup> Michael P. Rout,<sup>6</sup> and Brian T. Chait<sup>1,\*</sup>

<sup>1</sup>Laboratory of Mass Spectrometry and Gaseous Ion Chemistry, The Rockefeller University, 1230 York Ave, New York, NY 10065, USA, <sup>2</sup>Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QP, UK, <sup>3</sup>Structural Bioinformatics, European Molecular Biology Laboratory, Meyerhofstrasse 1, D-69117 Heidelberg, Germany, <sup>4</sup>Laboratory of Molecular Parasitology, The Rockefeller University, 1230 York Ave, New York, NY 10065, USA, <sup>5</sup>Department of Biopharmaceutical Sciences, University of California, San Francisco, 1700 4th Street, San Francisco, CA 94158, USA and <sup>6</sup>Laboratory of Cellular and Structural Biology, The Rockefeller University, 1230 York Ave, New York, NY 10065, USA

## Methods

### Biochemical separation and proteomic strategies

#### Strategy 1

*Methanol Protein Precipitation:* To one volume of the *Trypanosoma brucei* nuclear pore complex enriched preparation (TbNEP) (1-5 ml), 5 volumes of HPLC-grade methanol were added and left to incubate for 4 hours at 4°C. The exact amount of sample material depends on the individual preparation and is empirically determined by pilot precipitations. Generally, one wants to use the maximum amount of material without saturating the SDS-PAGE gel or chromatography column. The precipitate was recovered by centrifugation (3300 (g) in a Beckman GH-3.8 for 15 minutes at 4°C). The pellet was resuspended with 500 µl of 90% methanol, transferred to a microcentrifuge vial, and then left to incubate for 1 hour at 4°C. The suspension was spun one final time in a microcentrifuge (16,000 (g), 15 minutes, 4°C). The supernatant was removed to leave the protein sample pellet.

*SDS-PAGE:* The protein sample pellet was resuspended in 20 µl LDS sample buffer (Invitrogen, Carlsbad, CA), 8 µl 10x sample reducing agent (Invitrogen) and 52 µl water. After mixing, the solution was heated to 70°C for 10 minutes and allowed to cool to room temperature. To alkylate the reduced cysteines, 1 M iodoacetamide was added to a final concentration of 100 mM and the reaction was allowed to proceed for 30 minutes in the dark. NuPAGE® 10% and 4-12% bis-Tris gels (with MOPS running buffer) and Novex® 8% Tris-glycine gels (Tris-glycine running buffer) were used to increase resolution at specific mass ranges. For example, a Novex® 8% Tris-glycine gel offers high mass resolution while NuPAGE® 10% bis-Tris gels offer superior low mass resolution. Twenty (20) µl of alkylated sample was loaded to each gel. Electrophoresis was set at a constant 125 V for 5 minutes followed by a constant 200 V for 45 minutes. The gel was fixed in 50% methanol and 7% acetic acid for 15 minutes and then washed extensively. The proteins were visualized with GelCode® Blue colloidal Coomassie stain (Pierce, Rockford, IL) and documented by photography or digital flatbed scanning.

*In-Gel Digestion:* On a white shallow plate or glass pane, the entire gel lane was cut into

2 mm bands using a MicroScapel (Electron Microscopy Sciences, Hatfield, PA). Roughly thirty 2 mm bands were excised from a 10 cm gel after staining. Using fine point tweezers (Electron Microscopy Sciences) or MicroScapel, the excised gel band was diced into 1 mm<sup>3</sup> cubes and transferred to a microcentrifuge vial. The gel pieces were completely destained to remove all traces of stain and detergent. To the gel pieces, 500 µl of destain solution (25 mM ammonium bicarbonate in 50% acetonitrile) was added and the vials were agitated (medium setting) at 4°C with a vertical vortexer (Tomy Mixer, Tomy Seiko Co., Ltd., Tokyo, Japan). After destaining, 100 µl acetonitrile was added and then aspirated after 10 minutes. A trypsin (bovine, modified, sequencing grade, Roche Applied Science, Indianapolis, IN) aliquot was diluted in digestion buffer to a final concentration of 50 ng/µl. To the dehydrated gel pieces, ≥ 100 ng trypsin was added as well as 40 µl of 50 mM ammonium bicarbonate. The digestion mixture was incubated at 37°C for 4 hours. POROS® R2 (Applied Biosystems, Foster City, CA) C18 resin was used to extract and recover the peptides from the gel pieces and digestion buffer. To prepare the resin, sequentially wash 500 mg of POROS R2 beads with 10 ml of: (1) methanol, (2) 80% acetonitrile, and then (3) 20% ethanol. The washed beads are resuspended in 20% ethanol to a final concentration of 50 mg/ml. Nine volumes of 2% trifluoroacetic acid, 5% formic acid (*aq*) was added to 1 volume of POROS R2 bead slurry. Forty (40) µl of diluted POROS beads was mixed with the digest. The peptide/bead mixture was agitated (medium setting) in a vertical vortexer at 4°C for 4 hours. The POROS beads are separated from the gel pieces with the use of ZipTips® (C18, Size P10, Millipore, Billerica, MA), which have been washed with elution solution (20% acetonitrile, 50% methanol, and 0.1% trifluoroacetic acid). The 80 µl peptide/bead mixture was transferred into a washed and conditioned ZipTip from the top and, using a syringe, the supernatant was discarded. 20 µl of 0.1% trifluoroacetic acid was added to the gel pieces and then transferred to the ZipTip from the top. The wash solution was expelled using a syringe. The POROS beads on the ZipTip were washed two more times using 20 µl of 0.1% trifluoroacetic acid. A saturated solution of the matrix, 2,5-dihydroxy benzoic acid (DHB) (Lancaster, Pelham, NH), was prepared in elution solution at room temperature and then diluted to 40% (v/v) saturated just prior to use. The peptides were slowly eluted onto a MALDI plate with 2.5 µl 40% DHB in elution

solution to produce the “sample spot” [1-3].

*MALDI MS & MS/MS:* Using the MALDI QqTOF mass spectrometer [4], a mass spectrum was acquired for each sample spot. The spectra were then filtered with BackgroundFinder (written by Markus Kalkum) to remove background ions (such as trypsin autolysis peptides) and the resulting parent masses were transferred to method files that are compatible with the MALDI ion trap mass spectrometer for MS/MS analysis (modified LCQ series, Thermo Fisher) [5]. We generated an in-house assembled *T. brucei* proteome database based on the raw data that was released from the *T. brucei* genome sequencing project [6]. We identified the proteins by comparing our MS data against this database with previously established proteomic bioinformatic tools, such as ProFound, Sonar, and X!Tandem [7-9]. The confidence of the identification is related to the quantity and quality of the mass data [5, 10].

## Strategy 2

*Methanol Protein Precipitation:* As described in Strategy 1.

*Hydroxyapatite Chromatography:* In a 50 ml centrifuge tube, 7.5 ml Macro-Prep ceramic hydroxyapatite (HA) type I, 40  $\mu\text{m}$  (Bio-Rad Laboratories, Hercules, CA), was washed with 20 ml of 200 mM  $\text{Na}_2\text{HPO}_4$  (do not adjust pH). The HA was allowed to settle and the wash solution and suspended fine particles were aspirated. The wash was repeated 3 times with 20 ml loading buffer (10 mM  $\text{NaH}_2\text{PO}_4$ , pH 6.8, and 0.1 mM  $\text{CaCl}_2$  in water), aspirating the loading buffer and suspended fine particles after each wash. To the final volume of HA, 4 volumes of loading buffer, supplemented with 0.1% SDS, was added. Following methanol precipitation, the protein sample pellet was resuspended in HA sample buffer (10 mM Tris, 10 mM DTT and 2% SDS) and heated at 60°C for ten minutes. One volume of sample was diluted with 19 volumes of HA loading buffer. To the diluted sample, conditioned HA beads were added. Roughly 2 ml of bead slurry is required for less than 0.5 ml of sample. The mixture was incubated for 30 minutes under gentle agitation. The mixture was poured into a Poly-Prep® Chromatography Column (Bio-Rad, Hercules, CA) and the flowthrough was collected. The beads were washed with 4 ml of 0.1% SDS in loading buffer. The wash was collected to monitor protein binding and combined with the flowthrough. The proteins were then eluted from the HA.



All elution buffers are prepared from appropriate volumes of mobile phase A (1 mM DTT, 0.1 mM CaCl<sub>2</sub>) and mobile phase B (1 M NaH<sub>2</sub>PO<sub>4</sub>, pH 6.8, 1 mM DTT). SDS is added, just before use, at a final concentration of 0.1%. The mobile phase was added in successive order to the column (300, 325, 350, 375, 400 and 500 mM NaH<sub>2</sub>PO<sub>4</sub>) and the eluate was collected. Generally, 4 ml of elution buffer is sufficient.

*Trichloroacetic Acid Precipitation:* The final volume of eluate was adjusted to 10 ml with water and the proteins were precipitated by sodium deoxycholic acid/trichloroacetic acid, as follows. The protein suspension was diluted with water to 1 ml (small scale) or 10 ml (large scale). Respectively, 100 µl or 1 ml of 0.3% sodium deoxycholic acid was added along with an equivalent volume of 72% trichloroacetic acid. After mixing, the solution was left to incubate at 4°C for 1 hour and then spun at maximum rotor speed (1 hour, 4°C). The pellet was washed in 1.2 ml acetone and transferred to a fresh microcentrifuge vial and stored overnight at -20°C. The pellet was recovered by centrifugation in a microcentrifuge (16,000 (g), 4°C, 1 hour).

*SDS-PAGE:* The same as described in Strategy 1, except in Strategy 2, 4-12% bis-Tris gels were used and the entire pellet from each fraction was loaded onto a lane within the gel (see Figure 1).

*In-Gel Digestion:* As described in Strategy 1. All seven lanes of each gel were analyzed in their entirety by in-gel digestion and MALDI mass spectrometry.

*MALDI-MS and MS/MS:* As described in Strategy 1.

### Strategy 3

*Reversed Phase C4 Clean-Up Cartridge:* To avoid the potential losses of methanol precipitation, a clean-up cartridge (Michrom Bioresources, Auburn, CA.) packed with C4 resin (Grace-Vydac, Hesperia, CA.) was used to concentrate the protein mixture. The C4 cartridge was prepared by washing 3x with 500 µl methanol, 3x with 500 µl 95% acetonitrile and 0.1% trifluoroacetic acid, and, finally, 3x with 500 µl 0.1% trifluoroacetic acid. 100 µl of the TbNEP was diluted with 500 µl of 0.1% trifluoroacetic acid and loaded onto the cartridge. The cartridge was then washed extensively with 0.5% acetic acid and 0.1% trifluoroacetic acid. After washing, the protein was eluted with 500 µl 95% acetonitrile and 0.1% trifluoroacetic acid and then dried in a speedvac.

*High Performance Liquid Chromatography:* The protein sample pellet was resuspended in 50  $\mu$ l of 50 mM ammonium bicarbonate. A total of 500 ng of trypsin was added and the solution was left to incubate at 37°C for 24 hours. The digestion was quenched by acidification with 0.1% acetic acid (final concentration). 10  $\mu$ l (20%) of the digest was loaded onto a C18 reversed phase column (0.18 mm x 250 mm, 1.8  $\mu$ l/min) and eluted under the following conditions: 25% solvent B (95% acetonitrile, 0.1% trifluoroacetic acid) for 5 minutes; then, 25-100% solvent B in 40 minutes. After the analytical run, the column was cleaned and conditioned with 100% solvent B for 5 minutes and then 25% solvent B for 20 minutes. For this strategy, an Ultimate HPLC system (LC Packings-Dionex, Sunnyvale, CA) was used. Solvent A is 5% acetonitrile, 0.1% trifluoroacetic acid in water.

*Electrospray ionization (ESI)-MS/MS:* To ensure maximum coverage of the TbNEP, LC-MS/MS was employed as a complementary technique to MALDI MS/MS. The HPLC analytical run was online with the Finnigan LCQ series (ThermoElectron Corp., San Jose CA.) mass spectrometer. One data dependent MS/MS scan was acquired after each full (MS) scan. The data was analyzed as above in Strategy 1.

#### Strategy 4

*Chemical Extraction:* Three separate separations were conducted on the TbNEP to reduce the complexity of the sample by enriching for transmembrane proteins (base extraction), integral proteins which are closely associated with the lamina (salt and detergent) and proteins that are peripheral to the NE (heparin) [11].

Base extraction: To one volume of nuclear envelopes (in sucrose solution), 9 volumes of 100 mM NaOH with solution P (1:100) was added and mixed completely by vortexing. Note: Solution P is made by dissolving 0.04% (w/v) pepstatin A (Sigma-Aldrich, St. Louis, MO) and 1.8% PMSF (Sigma-Aldrich, St. Louis, MO) in absolute (anhydrous) ethanol.

Salt and detergent extraction: To one volume of nuclear envelopes (in sucrose solution), 9 volumes of salt and detergent extraction buffer (400 mM NaCl and 1% (w/v)  $\beta$ -octylglucoside in 25 mM Hepes, pH 7.5) with solution P (1:100) was added and mixed completely by vortexing.

Heparin extraction: To one volume of nuclear envelopes (in sucrose solution), 9 volumes of 10 mg/ml heparin in bis-Tris/Mg buffer with solution P (1:100) was added and mixed completely by vortexing.

The extractions were left to incubate on ice for 1 hour. The extractions were then underlaid with 1 M sucrose in bis-Tris/Mg buffer with solution P (1:100) and spun at 103,460 (g) for 35 minutes. The extracted proteins are retained in the supernatant. The supernatant was carefully transferred to a fresh vial and precipitate the proteins with trichloroacetic acid/deoxycholate precipitation (Strategy 2). The enriched pellet was washed with 1 ml acetone, transferred to a microcentrifuge vial, and then stored overnight at -20°C. Recover the pellet by centrifugation in a microcentrifuge (16,000 (g), 1 hour, 4°C). The pellets from both the supernatant and the precipitate were checked on a gel (Figure 1). The extraction was repeated and the pellets were prepared for HPLC analysis. For HPLC analysis, the protein sample pellet was resuspended in 50 µl of 50 mM ammonium bicarbonate. A total of 500 ng of trypsin was added and the solution was left to incubate at 37°C for 24 hours. The digestion was quenched by acidification with 0.1% acetic acid (final concentration).

*Reversed Phase C18 "Push-Off" cartridge:* 5 µl of the peptide digest mixture was diluted 1 into 3 and passed through a ZipTip packed with POROS C18 resin, in a fashion analogous to Strategy 1. The amount of resin present was such that the binding capacity of the column was less than the total amount of peptide present in the digest. The flowthrough is passed over a second ZipTip/POROS column with less resin than the first. The peptides bound to the first and second columns are eluted with 20 µl 95% acetonitrile, 0.1% trifluoroacetic acid. Both the eluates from columns 1 and 2 (enriched in the abundant peptides) and the flowthrough (enriched in less abundant peptides) are analyzed by HPLC. This protocol is credited to Júlio C. Padovan.

*High Performance Liquid Chromatography:* 10 µl of the peptide mixture was loaded onto a C18 reversed phase column (0.2 mm x 50 mm, 2.2 µl/min) and eluted under the following conditions: 30% solvent B (95% acetonitrile, 0.1% trifluoroacetic acid) for 2 minutes, 30-100% B in 14 minutes. After the analytical run, the column is cleaned and conditioned at 100% solvent B for 7 minutes and then 5% solvent B for 20 minutes. For this work, an Ultimate HPLC system (LC Packings-Dionex, Sunnyvale, CA) was used.

Solvent A is 5% acetonitrile, 0.1% trifluoroacetic acid.

*ESI-MS/MS*: As described in Strategy 3.

## Strategy 5

*Methanol Protein Precipitation*: As described in Strategy 1.

*Hydroxyapatite Chromatography*: As described in Strategy 2.

*Trichloroacetic acid precipitation*: As described in Strategy 2.

*High Performance Liquid Chromatography*: From each fraction, the protein sample pellet was resuspended in 50  $\mu$ l of 50 mM ammonium bicarbonate. A total of 500 ng of trypsin was added and the solution was left to incubate at 37°C for 24 hours. The digestion was quenched by acidification with 0.1% acetic acid (final concentration). 10  $\mu$ l of the peptide solution was loaded onto a C18 column (BioBasic® PicoFrit C18 column 75  $\mu$ m ID, (PFC7515-BI-5, New Objective, Woburn, MA)) and, after extensive washing with 0.1% acetic acid (solvent A), eluted with 70% acetonitrile and 0.1% acetic acid (solvent B) in a linear gradient (0 - 100% solvent B) in 60 minutes at 20  $\mu$ l/min. After the analytical run, the column was cleaned and conditioned with a blank run and 20 minutes of 100% solvent A.

*ESI-MS/MS*: The HPLC analytical run was online with a Finnigan LTQ XL (ThermoElectron Corp., San Jose CA.) mass spectrometer. The method was designed to acquire one MS scan to determine parent masses, and then acquire 10 MS/MS scans which are dependent on the first MS scan. Dynamic exclusion was used so that any given parent mass is analyzed and fragmented only once in a 30 second time window. The data was analyzed as above in Strategy 1.

*Pattern recognition*: We scanned both the trypanosome protein database and the TbNEP dataset for the presence of FG repeat domains by using a pattern recognition algorithm written by David Fenyö (ProteinInfo. <http://prowl.rockefeller.edu>). Using a wildcard string as input (e.g. FGxFGxFGxFGxFG), we analyzed the returned matches by hand for clusters of repeated FG dipeptides with the assumption that the spacer regions in *T. brucei* would be of similar composition to yeast and vertebrates. In order to be considered as a putative FG repeat domain, two major factors were considered. First, at least 5 FG dipeptides must be present. Second, the domain must be enriched in

prolines and depleted in arginine. Later, we interrogated each putative FG repeat domain to ensure that it was natively disordered. Ultimately, many putative FG nups were confirmed by GFP localization. We did not find any recognizable FG-repeat domain in the trypanosome database that was not also present in the TbNEP dataset.

*GFP tagging and localization: Primers used in this study.*

Accession	Mass (kDa)	MW+GFP (kDa)	Annotation	Forward Primer	Reverse Primer	NE localization?
Tb11.03.0140N	158.3	185.8	TbNup158 N837-GFP	CCGCTGCCAGAAAGAAGGTAACAG ATTTGAGCTTACGATGTGAACACG GGGACGTGGGTTTACGTGATGAAT GTTGGCggtaccgggccccccctcgag	CCTTTTCATTACCAACCCTCGTCA CCTTCGCATCACCCATATTTCCAA CCTCTGTTTGCCTTCCACTGTTCTt ggcgccgctctagaactagtgat	Foci at the NE
Tb927.4.1310	47.4	74.9	Hypothetical	GAGATCCGTGGCACTGAGGCGTCA TTAGGATTATCACCAGACGAGGAG GGTAATCTCGTGTCTATGACAAAAG AGTTTggtaccgggccccccctcgag	AGCAACATATGTCCCGACAAAACC GTAACCCACACCTTCTCCCTGGT CACACGGCACACGGAACGCGTCA Ctggcgccgctctagaactagtgat	Inconclusive
Tb10.61.0160	42.9	70.4	Hypothetical	CAAAGAGGGGCTATTAGCCTGCGC ATCCTTCGATCAAAGTATTCGAGTT ATGCTCGTGACCAAGTTGTCGCTC GTACAAGgtaccgggccccccctcgag	TCTCATGGCTCCACTCCATTTTGT CATCACTTGCAAATTTGGTAACC TACTGTGCTACACCTGTTTCACC ACtggcgccgctctagaactagtgat	Inconclusive
Tb10.70.1110	59.9	87.4	Hypothetical	GGAGTGCCACAAATGTGGAGCAAC GAAGACGGTAGCGGTTGCCCGCGG CGGTGCTTTTGTGCGGAAGCCCGT CAAATggtaccgggccccccctcgag	ACAACGTCTATCCAGCGTGATAGT TATTTGCCGCTGTTATAGCAAGCA AAGTTAAATAAACGAATAGGAATG Tctggcgccgctctagaactagtgat	Inconclusive
Tb10.70.1120	109.2	136.7	Hypothetical	ATTTGCGAGTAAGTGAAGCAAAGA ATCTTGACGAAGCCGATGTTTGTGT TGATTGTGGCATTGCAAATGCGAC AAAAGgtaccgggccccccctcgag	AAAAACGACCCACACGACTGACAAC ACGGTTAGAAGCTTCGGACGCTGG ACTTAATAGATAGATTTAATGAAATt ggcgccgctctagaactagtgat	Inconclusive
Tb10.70.1130	48.2	75.7	Hypothetical	TGTTGGCAGAGTGGAGAGGCAGAT GACATTGGATCTTGACACAGTTTG CGCAACGACATTCTTGATGAAACAA AGAAAggtaccgggccccccctcgag	CTGCTGGGAGATCTTGTTTGGTAA GTTTCAGATTAGAAACGACATAATA CTCCACATTTGATTTGCAAAGG AAtggcgccgctctagaactagtgat	Inconclusive
Tb927.4.2070	511.3	538.8	Hypothetical	AAAGTGTAATGACTTAGTTATAAAG AGACTAGAGGATGAGGTTAAAGCT CTTCGTGAAGAAGTGGTGGAAAT GAGGCggtaccgggccccccctcgag	GTAGCAATAATACGATTTAAAAAT GTCAAAATTGTCAGCAACAAAGAT GCTTACACGAACGAAAAAAG Atggcgccgctctagaactagtgat	Inconclusive
Tb927.4.590	88.0	115.5	Hypothetical	CTTACATGCAGCTTTGGTGTCCCTT CCCTTGTTGGCAGCTACTTTGCCAT GCGTAAAGGTGTGAGACAAGTGTG GCGAggtaccgggccccccctcgag	AAAACGTACACATACACACATCGC AACCAAGGCGACGACATGTTAATC CCGAGAAGTCAATATCTCTCGGGA tggcgccgctctagaactagtgat	Inconclusive
Tb927.6.1830	49.9	77.4	Hypothetical	GCACCGCAACATCCCGGCGCCAT GGAAGAGAAGGAAAGTGGTGAAGA GGATGATGTTTTGAGATGAATAGG CAACAAGgtaccgggccccccctcgag	TTTTGCCTTTCCCTTCGGGTGTTA CTGTCGTTTACCCTTTTCCAGAC TTACCTTCTTCATACGCACTACT Gtggcgccgctctagaactagtgat	Inconclusive
Tb927.7.3330	502.6	530.1	Hypothetical	GGAAGGTGAAGACGATGAGGACGA GGGCGACGCAACCGGTTGCCAAC AACGCATTTGGGAGGCCATGGGC GCACCATggtaccgggccccccctcgag	TACATTAGACATCATTATCGACTG TAACCTAGGTAGTGTATGAGATAC CGTATCAATTACACTGAGTGTGTC Atggcgccgctctagaactagtgat	Inconclusive
Tb11.01.0420	35.1	62.6	sec13,putative	GTCTTGTGCGAACGGAACGCAAG CATGTGGCAGGAGTACGACGGGG GACAGGAGTGGGAACGTTCTGTGA AGCTACCTggtaccgggccccccctcgag	AAGCAGACGGTATTACCTCAGTTC TCATGTCCCCCTTCTTCTCCCG GGAGGCAACACACATGTGCACAA CAAtggcgccgctctagaactagtgat	Proximal to golgi

Accession	Mass (kDa)	MW+GFP (kDa)	Annotation	Forward Primer	Reverse Primer	NE localization?
Tb11.03.0810	109.7	137.2	TbNup110	ATGCGACTACTGCACGTCACAAAG CAACTTGTGGAGAGAGTCAAACC AGTCGAAGTAAAGGAGAAATCCAG TCCAGTgggtaccgggccccctcgag	TACACGAATTGTATACAAACCTGA CTAGCAGACGTAAAGCCGCTACGA ACCTTTACTGTGTTCAAACAAAAA Tggcgccgctctagaactagtgat	Yes
Tb09.160.0340	92.3	119.8	TbNup92	GTAACCTGGGATAAGTTGGTTGAG CTCATTACCCTGTTAAAATCGGTG TCGTGGCAGAGGAAGTAAAGCAGC CACAGgttaccgggccccctcgag	TCTCATCCTTTAGAGCCTCTCTCC CCCTCTTCCCTCCCCCCCCCCA ATTGCAGAGGGAGCATAAATATATT ggcgccgctctagaactagtgat	Yes
Tb10.61.2630	41.6	69.1	TbSec13	CCGTGAAGGCAATGAGTTTTGCCA ACCATGGTTAAAACACCTCTCAGC GAATACGAAAACAAAATCAGCGG GTTACGgttaccgggccccctcgag	TTCTCCTCCACCCATCCAACCGA CCTCCCGTTGTAATATCTCTCCA CCATATCTTGTCAAACAAAGTTGTC Tggcgccgctctagaactagtgat	Yes
Tb11.02.2120	48.4	75.9	TbNup48	GATGGCTGCACGATGGGTCTGTTG CTTCTGTTGTGGAAGCGGACCAG TCTGTGCTATTGTTCTGCGTATC ATGCAggtaccgggccccctcgag	TCTGTGCAAAAAAGGGGTGTGGG CATCGTTCAATCTTCCCCATCCTT CGCCCCGAATATCATCGTGGCCTC Tggcgccgctctagaactagtgat	Yes
Tb09.211.4780	82.4	109.9	TbNup82	GTTCCGGCTCACTCGGGAATCT AGCCAGCTACAACGCTAAAAGCGGC ACCCATTCTGTGAGAGCTGATGAG GCTTCTAggtaccgggccccctcgag	ATGTATATAGGTGTTTTCTCCAC TCTATACAAGACATCACACAAGGG TCTAGACATGCAGCCACACTTATC CTggcgccgctctagaactagtgat	Yes
Tb11.02.0460	89.1	116.6	TbNup89	GCATGATCTTTTAGTGAAGAGGC GAAAAAGTTGCTGAACACAACAG CAGCTTTTTGTGACATCAAAACCGA AATGggtaccgggccccctcgag	TAAGGCCACCCACACTCTTGAG CTACATCAAGCGTACGAGCACCTC TTACCGTCTTATCTCCACCCACACA Cgtggcgccgctctagaactagtgat	Yes
Tb10.6k15.3670	96.5	124.0	TbNup96	GGGTGCGTCTGGAAGAGGCATA TGAACCGCTCTCTTAGATGAGCT AATCGTTCTAGAGCGGTTGTTGTC CAGggtaccgggccccctcgag	TCCCCTATTCCGTCATTCAACTCA CTAGAGAAGGGGGAGGGAGTGT TCACCAGTATCGAAATATAACGCT ggcgccgctctagaactagtgat	Yes
Tb10.6K15.1530	181.5	209.0	TbNup181	GGTACAGGAAGAGTTGCAGGGACT TGCCGAAAGACTCCGCACTGTGAGC GACTTCATGGCGGACGACAGGAGC CAGCTTggtaccgggccccctcgag	ACCTCTTTTTTTTTTTGCTTTCAC CGTTTCTCCTCCACATTCAAAAAC CAAAAAGTTTCATCCACAACAATA Tggcgccgctctagaactagtgat	Yes
Tb927.4.2880	225.5	253.0	TbNup225	TCAGGTAGTCTTGTGAGTGCATTC TTCGCGCGGTGAAGTGCAATC TCCAATCGTGTGCGGGAGGCCCTCC CGTATggtaccgggccccctcgag	AGCATCACTTCCATCACTACCGC TATTCCTCAAAGTTTGTATTCTGTT TGATACACCATTTCCCTTACCCTT Tggcgccgctctagaactagtgat	Yes
Tb11.01.7630	108.7	136.2	TbNup109	CTGTGAGGCAATTTGAAACGTAGC AAGAAGCCGTGCGGGCGTGTGAGA CCATGTGAGTATCATGACAGGGTG AGGAAAggtaccgggccccctcgag	CTTATGTCAGCTGTTTTCTATTA ACTTCTCCTCTACCTCCACAAC CCAGTGATGTGCGACCAAAACCGG CAtggcgccgctctagaactagtgat	Yes
Tb927.7.2300	132.3	159.8	TbNup132	GTGTGCCGATATTTTACAAATGCT GAGCAACAGTTGTGATGTGCTGG CTTCGAGCTCGCTCCGCTGGACTC GTGTCAggtaccgggccccctcgag	AATGAGTAAATGTCGGTGGCTTGA AAATATACGGAATGCAAGTTATT TTAACGGTTTCCGTCACGGCTGG Tggcgccgctctagaactagtgat	Yes
Tb10.6k15.2350	144.3	171.8	TbNup144	CGGGCATTGAGCGCTGCGGAGGG ATTGCTCGAAACAGTCAACCTTGCC CTGAATCGTGCCAGTTCAGGTTTA CTTTGgtaccgggccccctcgag	CAGTTTTAGTCAATTAATATTCGCT GTCGACGAGACAGGTCAACGCA GTGAAATAATCTATACCGTACAC ggcgccgctctagaactagtgat	Yes
Tb11.01.7200	52.7	80.2	TbNup53a	TGCGATGGAAGTGTGCGCAGCTCA GGCATCCGAGTTGGAGCGAGAGGT TGATTTGTTGCTAGGGAAGTCAACG ATCGCTggtaccgggccccctcgag	GGGCGCAGAAGAAAGAAATGTGC AAATGCATCTTACCTGCGCCCTC TACTTTTACCTATCTTGCCAATCG AAtggcgccgctctagaactagtgat	Yes
Tb927.3.3540	52.8	80.3	TbNup53b	AATCGGAGGCGCCACCATATTTGG TGCGGGGACCTCTGCTGTGACCC AAGGAAGACCTGAACAAAACCGG TCGCTCggtaccgggccccctcgag	CAGGGGACGCGCGGGCGGAA TCAAAGCAACCTGCACACATGCA CCGTTCAATACCTTGCACGCGGA GGTAtggcgccgctctagaactagtgat	Yes
Tb927.4.4310	64.2	91.7	TbNup64	TGGAAGCGGCGCGCTTAAACCAGC TGACCCCAAGAAAGGAGTCACTTC AAGTGTGCGAAAAAGGCCGTGAG GAGGTACggtaccgggccccctcgag	TGTGGCCGAGGATTTCTCCGGA AGCCCGAAAGTGCACACCCAAAG TGAGCAGACAAGCAGTAAAGAAAA AAAtggcgccgctctagaactagtgat	Yes
Tb927.8.8050	74.8	102.3	TbNup75	AGCCAGCCGCGATCGGTTTCACT GCACCCAGCTAAACGCCAATTTG GTTGCCCGTAGGAGGACACCGAAG CGGTTGgtaccgggccccctcgag	AAACAACAACACACACACACAC ACACACATATATATATATATATA TATATATGCGGCAATTACATAAAG cgcgccgctctagaactagtgat	Yes
Tb927.3.3180	98.1	125.6	TbNup98	TGGGAATGCTTCAGCAAGTGTGA AAAGAACAATGCTCCACGGAATCCC TTCTCATTGTTGCTCTTCTGGGA ATGCTggtaccgggccccctcgag	ACTAAAGAAAGGTAGAAAAACAAG AAAAACCAAAATAGGTACCTGAC GCAGCGGCAACACCACGTCGACT TGCtggcgccgctctagaactagtgat	Yes
Tb11.01.2880	149.2	176.7	TbNup149	GGAGAATTATAGGGTAGAAATAA GTCCGGTGATAAAAATATGTTGGC TCATTTGGAGAATGCTTCACTAAA CCCTATggtaccgggccccctcgag	AAACCCTAACAAATTTATACACC AGCGCTTGCAACAGAAAGGTGATTA GCATCAAAGTAACTTCAATAAAT Tggcgccgctctagaactagtgat	Yes
Tb11.01.2885	140.3	167.8	TbNup140	TGTTTTTCACTTCTTCTTTTTCTA GTATTGCCGGCAATATAAGGCCAC ACCTGATTTTAAAGTGTCTTTTCG CAGgtaccgggccccctcgag	TTAAGTATAATAACAATAATTTA AGCACTTATCATGATTTTTTTA GTTACCTCCATAACCAATAAACTT ggcgccgctctagaactagtgat	Yes
Tb11.03.0140	158.3	185.8	TbNup158	TGTGAATGCGCCAAGCATTGCGAC TCACCTGCTCTGAGGAAGCGCT TCACCGTTTCACTGCGGGTTTACA AAGAGAggtaccgggccccctcgag	TCCTTTTCACTTCAACCACTCTGTC ACCTTCCGATCACCTTATATTTCCA ACCTCTGTTTGCCTCCACTGTTCT ggcgccgctctagaactagtgat	Yes
Tb927.2.4230	406.8	434.3	TbNUP-1	CACAGCGACAGGTACGGCAAGTCA TGGACATACGTAGCACAAGAAAA GGTCTCGTTACGCCAATGCGGTCT CGgtaccgggccccctcgag	TCTAGTGCATGTGTAGTGAAGT GCACACTTTATGCACTAATAACAG GTTTGAAGTACTTACCTGGCATCT CTggcgccgctctagaactagtgat	Yes

*Northern blotting:* Total RNA was isolated from procyclic form cells (Qiagen RNeasy Mini Kit used according to manufacturer's instructions). For each sample 3 µg RNA in 4 µl water was incubated at 50°C for 40 minutes with 3.88 µl DMSO, 2.33 µl glyoxyl, and 0.32 µl 0.5M sodium phosphate pH 6.9. Four µg Millenium Marker (Ambion, Inc.) was prepared in the same way. Samples were separated on a 1.4% agarose gel made with 10mM sodium phosphate, pH 6.9 and blotted onto a Hybond-N+ nitrocellulose membrane (Amersham Biosciences). Probe sequences were amplified from genomic DNA using the following primers: 10.70.1110F: 5'-ACGTGGATGTGTGTGAAGGA-3'; 10.70.1110R: 5'-AACACGGCAATTAAAGCACC-3'; 10.70.1120F: 5'-TTGCCTAGCAGTTCGCCTAT-3'; 10.70.1120R: 5'-ACAACAACCCTCGGTTTCAG-3'; 10.70.1130F: 5'-ATATCGAGGATTTCCCACC-3'; 10.70.1130R: 5'-GCAACTTCGAGAAGCCAAAC-3'; and cloned into the pCR® 2.1TOPO vector® (Invitrogen). Inserts were excised with EcoRI (NEB) and used as templates for probe labeling with <sup>32</sup>P-dCTP using the DecaPrime™II Random Prime DNA Labeling Kit (Ambion, Inc.) Blots were hybridized overnight.

## Results

Tb10.70.1120 was identified in the TbNEP dataset and happens to be one of three closely arranged hypothetical proteins on chromosome 10 (Tb10.70.1130, Tb10.70.1120, and Tb10.70.1110). The FG nups Tb11.01.2800 and Tb11.01.2885 are also closely arranged together on chromosome 11. It was postulated that these two groups of ORFs could represent a fusion gene for three reasons. First, the intergenic regions are relatively small (427 and 486 bp, respectively, for Tb10.70.1130, Tb10.70.1120, and Tb10.70.1110, and 152 bp between Tb11.01.2800 and Tb11.01.2885. The mean length of *T. brucei* intergenic regions is 1279 bp (Berriman *et al.* 2005)). Second, the two orthologous regions in the *L. major* genome contains one gene rather than three or two. Lastly, genomic tagging proved unsuccessful for Tb10.70.1130, Tb10.70.1120, and Tb10.70.1110. In order to determine the transcript size of these predicted genes, and thus if these genes are separate or fused in *T. brucei*, northern blots were performed against procyclic RNA with probes complementary to each of the genes. As seen in Figure S6, the results of these

northern blots were not consistent with a model of either separated genes or fused genes. The localization of these genes will be further investigated to determine if they are indeed associated with the NPC.

### Supplemental references

1. Cristea, I.M., Williams, R., Chait, B.T., and Rout, M.P. (2005). Fluorescent proteins as proteomic probes. *Mol. Cell. Proteomics* 4, 1933-1941.
2. Tackett, A.J., Dilworth, D.J., Davey, M.J., O'Donnell, M., Aitchison, J.D., Rout, M.P., and Chait, B.T. (2005). Proteomic and genomic characterization of chromatin complexes at a boundary. *J Cell Biol* 169, 35-47.
3. Archambault, V., Li, C.H.X., Tackett, A.J., Wasch, R., Chait, B.T., Rout, M.P., and Cross, F.R. (2003). Genetic and biochemical evaluation of the importance of Cdc6 in regulating mitotic exit. *Mol Biol Cell* 14, 4592-4604.
4. Krutchinsky, A.N., Zhang, W.Z., and Chait, B.T. (2000). Rapidly switchable matrix-assisted laser desorption/ionization and electrospray quadrupole-time-of-flight mass spectrometry for protein identification. *J Am Soc Mass Spectr* 11, 493-504.
5. Krutchinsky, A.N., Kalkum, M., and Chait, B.T. (2001). Automatic identification of proteins with a MALDI-quadrupole ion trap mass spectrometer. *Anal Chem* 73, 5066-5077.
6. Berriman, M., Ghedin, E., Hertz-Fowler, C., Blandin, G., Renaud, H., Bartholomeu, D.C., Lennard, N.J., Caler, E., Hamlin, N.E., Haas, B., et al. (2005). The genome of the African trypanosome *Trypanosoma brucei*. *Science* 309, 416-422.
7. Craig, R., and Beavis, R.C. (2004). TANDEM: matching proteins with tandem mass spectra. *Bioinformatics* 20, 1466-1467.
8. Field, H.I., Fenyo, D., and Beavis, R.C. (2002). RADARS, a bioinformatics solution that automates proteome mass spectral analysis, optimises protein identification, and archives data in a relational database. *Proteomics* 2, 36-47.
9. Zhang, W.Z., and Chait, B.T. (2000). Profound: An expert system for protein identification using mass spectrometric peptide mapping information. *Anal Chem* 72, 2482-2489.



10. Zhang, W.Z., Krutchinsky, A.N., and Chait, B.T. (2003). "De novo" peptide sequencing by MALDI-quadrupole-ion trap mass spectrometry: A preliminary study. *J Am Soc Mass Spectr* *14*, 1012-1021.
11. Schirmer, E.C., Florens, L., Guan, T.L., Yates, J.R., and Gerace, L. (2003). Nuclear membrane proteins with potential disease links found by subtractive proteomics. *Science* *301*, 1380-1382.
12. Fontoura, B.M.A., Blobel, G., and Matunis, M.J. (1999). A conserved biogenesis pathway for nucleoporins: Proteolytic processing of a 186-kilodalton precursor generates Nup98 and the novel nucleoporin, Nup96. *J Cell Biol* *144*, 1097-1112.
13. Bridges, D.J., Pitt, A.R., Hanrahan, O., Brennan, K., Voorheis, H.P., Herzyk, P., de Koning, H.P., and Burchmore, R.J.S. (2008). Characterisation of the plasma membrane subproteome of bloodstream form *Trypanosoma brucei*. *Proteomics* *8*, 83-99.
14. Broadhead, R., Dawe, H.R., Farr, H., Griffiths, S., Hart, S.R., Portman, N., Shaw, M.K., Ginger, M.L., Gaskell, S.J., McKean, P.G., et al. (2006). Flagellar motility is required for the viability of the bloodstream trypanosome. *Nature* *440*, 224-227.
15. Jones, A., Faldas, A., Foucher, A., Hunt, E., Tait, A., Wastling, J.M., and Turner, C.M. (2006). Visualisation and analysis of proteomic data from the procyclic form of *Trypanosoma brucei*. *Proteomics* *6*, 259-267.

## Supplemental figure legends

**Figure S1: Western blotting was used to verify the proper insertion of the GFP or 3x HA epitope in the transgenic cell lines.** The red dots indicate bands that migrate to the proper apparent molecular weight.

**Figure S2: (A) Fluorescent microscopy images of COOH-terminal GFP labeled *T. brucei* proteins identified in TbNEP that do not localize to the nuclear periphery.** Also shown is the corresponding DAPI fluorescence DNA marker in blue. Scale bar, 2  $\mu\text{m}$ . (B) Following autoproteolytic cleavage, the NH<sub>3</sub>-terminal daughter protein of HsNup98-96 localizes to the nuclear interior [12]. To investigate localization pattern of the NH<sub>3</sub>-terminus of TbNup158 a mutant (N-832-GFP) was generated. Interestingly, this mutant localized to discreet foci at the NE and not to the nuclear interior. This phenotype highlights the functional difference between TbNup158 and HsNup98-96. Scale bar, 2  $\mu\text{m}$ . (C) Although Tb11.01.0420 was not identified in the TbNEP dataset, it exhibits sequence similarity to Sec13, which is a Nup. This protein was tagged with HA on its COOH-terminus to check its localization. Immunofluorescence microscopy was accomplished with a rabbit anti-HA antibody (Santa Cruz Biotech) at 1:800 as a primary antibody. Goat anti-rabbit IgG, conjugated with Alexa 568, (Molecular Probes) at 1:4000 served as a secondary antibody. To highlight the golgi apparatus, anti-GRASP (a gift from Graham Warren) was diluted to 1:500 and used goat anti-mouse IgG oregon green (Molecular Probes) at 1:4000.

**Figure S3: Pair-wise alignment matrices of the structural scaffold Nups.** (A)  $\beta$ -propeller Nups. (B)  $\alpha$ -solenoid Nups. (C) NH<sub>3</sub>-terminal  $\beta$ -propeller followed by an  $\alpha$ -solenoid. (D) Nuclear basket Nups. The Nups are aligned using the pair-wise FASTA alignment algorithm with default settings. The Smith-Waterman score, a measure of the similarity between two aligned sequences, is recorded at the intersection of each pair of Nups. The scores are normalized to the highest score between two homologous (but not paralogous) Nups within each matrix. The normalized score is then visualized with a

grayscale gradient. Paralogous pairs are indicated in black but have a normalized score greater than 1. Sc, *S. cerevisiae*; Hs, *H. sapiens*; At, *A. thaliana*; Tb, *T. brucei*.

**Figure S4: Each *H. sapiens*, *S. cerevisiae* and *T. brucei* FG Nup is graphically depicted.** Glycine residues are colored in gray. Acidic residues (Asp and Glu) are colored in red, and the basic residues (Lys and Arg) are colored in blue. Clustering of charged residues is observed in regions that are depleted of glycine which is consistent with the correlation of glycine and charged residues (see Figure 6).

**Figure S5: The Kap family is less divergent in primary structure compared to the Nups.** The *Trypanosoma brucei* 927 genome database at geneDB (<http://www.genedb.org>) was searched using the complete set of *S. cerevisiae* Kap sequences (14 in total) together with the majority of validated Kaps from *H. sapiens* (18 sequences) using pBLAST, with the BLOSUM45 amino acid identity matrix. Up to five hits were retained from each search, and the resulting dataset was initially parsed for redundancies, and then for clear non-Kap annotation by reverse BLAST against the *S. cerevisiae* genome. Sequences not providing a clear hit against a Kap sequence were removed, all other sequences were retained. Significantly, there is no clear BLAST expect score cutoff, and hence the inclusion of potentially non-Kap sequences with low significance, which are rejected by the reverse BLAST, is essential to ensure completeness. A total of 14 sequences were retained following this procedure, which compares well with *S. cerevisiae*. Sequences were then aligned using ClustalW and the alignment manually edited to remove data-poor regions using MacClade, and the resulting alignments subjected to phylogenetic reconstruction using both MrBayes for Bayesian analysis and PhyML for maximum likelihood. The phylogeny shown here is a Bayesian representation, but all robust features were also retained with the maximum likelihood method. Sequences in purple are *T. brucei*, in red *H. sapiens* and in green *S. cerevisiae*, while bold sequences are to highlight entries that do not have conserved topology in the maximum likelihood reconstruction. Numbers designate posterior probabilities and values above 0.80 are shown only for clarity. The Kaps can be grouped into seven subclades, of which five (I, II, IV, V and VII) have good statistical support and

are stable regardless of reconstruction method. Clades III and VI are not well supported and most of the Kap sequences comprising these groupings are elsewhere on the PhyML topology (data not shown). The status and assignment of these sequences (in bold) is unsure at this time and likely requires experimental validation. Most significantly 9 out of 14 (64%) *T. brucei* putative Kap sequences demonstrate robust sequence orthology with higher eukaryote KAPs, in clear contrast to the low level of sequence retention in the nucleoporin class.

**Figure S6: Tb11.01.5410 is not present in the TbNEP dataset, but is a potential homolog to the opisthokont  $\beta$ -propeller Seh1.** The Bayesian representation was generated similar to the phylogeny in Figure S5. Three *S. cerevisiae* sequences (ScSec13, ScSeh1 and ScYtm1) were used to initially search the *T. brucei* and several other genomes.

**Figure S7: (A) Schematic of chromosome 10, location 1440000-1450000 (<http://www.genedb.org>) containing the genes Tb10.70.1130 (1130), Tb10.70.1120 (1120), and Tb10.70.1110 (1110).** The size of each predicted gene is shown below it. (B) Northern blotting using probes against 1130, 1120, and 1110. Sizes are indicated in base pairs (bp). (C) Schematic of chromosome 11, location 2907500-2920000 (<http://www.genedb.org>) containing the genes Tb11.01.2880 (2880) and Tb11.01.2885 (2885). The size of each predicted gene is shown below it. (D) Northern blotting using probes against 2880 and 2885. Sizes are indicated in base pairs (bp). See section 4 for details.

Figure S1

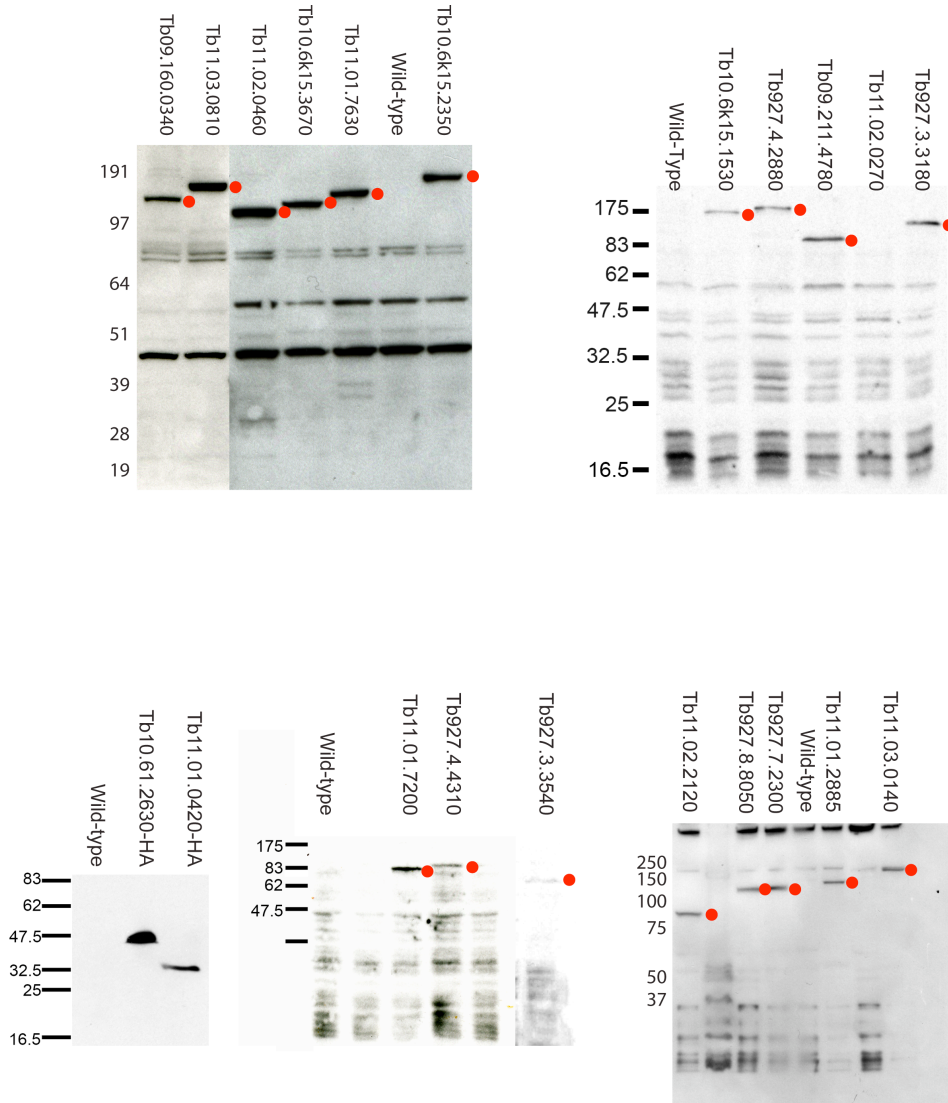


Figure S2

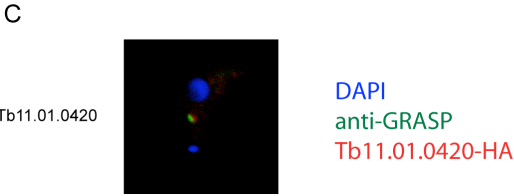
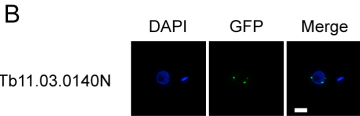
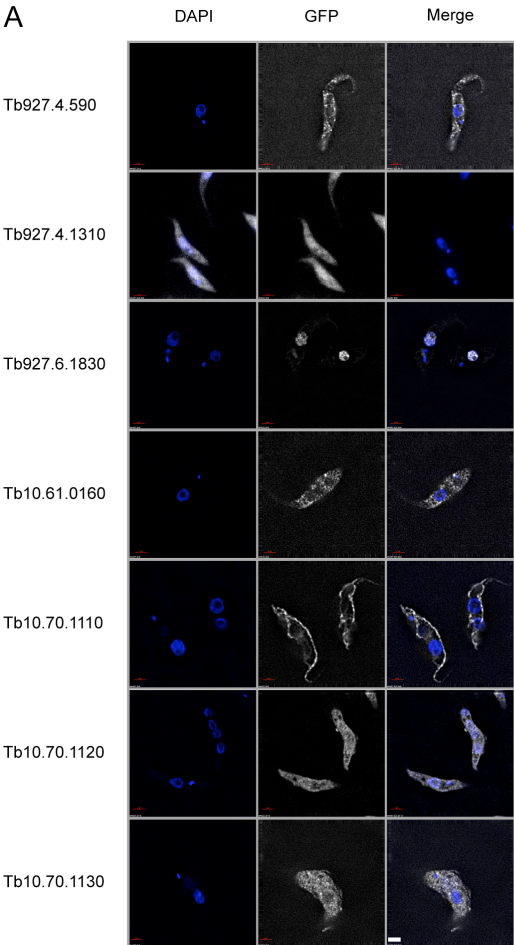
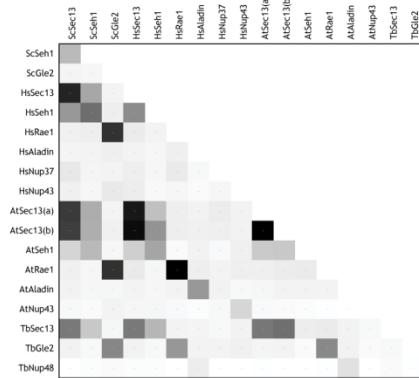
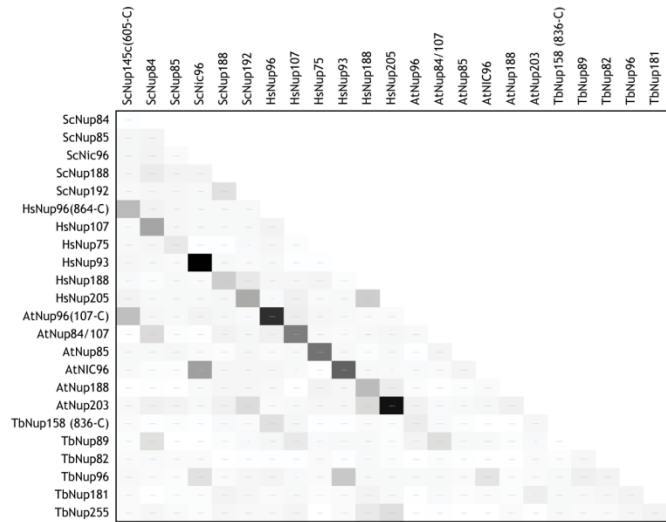


Figure S3

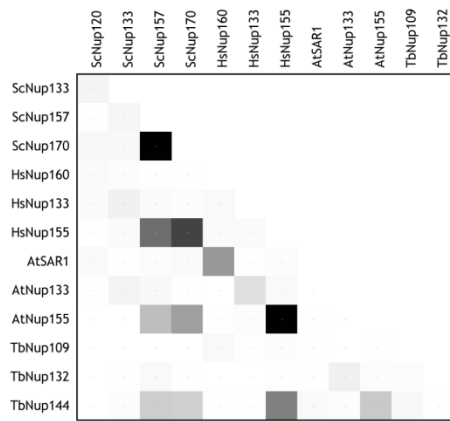
A



B



C



D

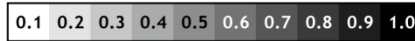
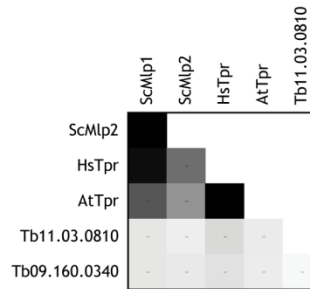


Figure S4

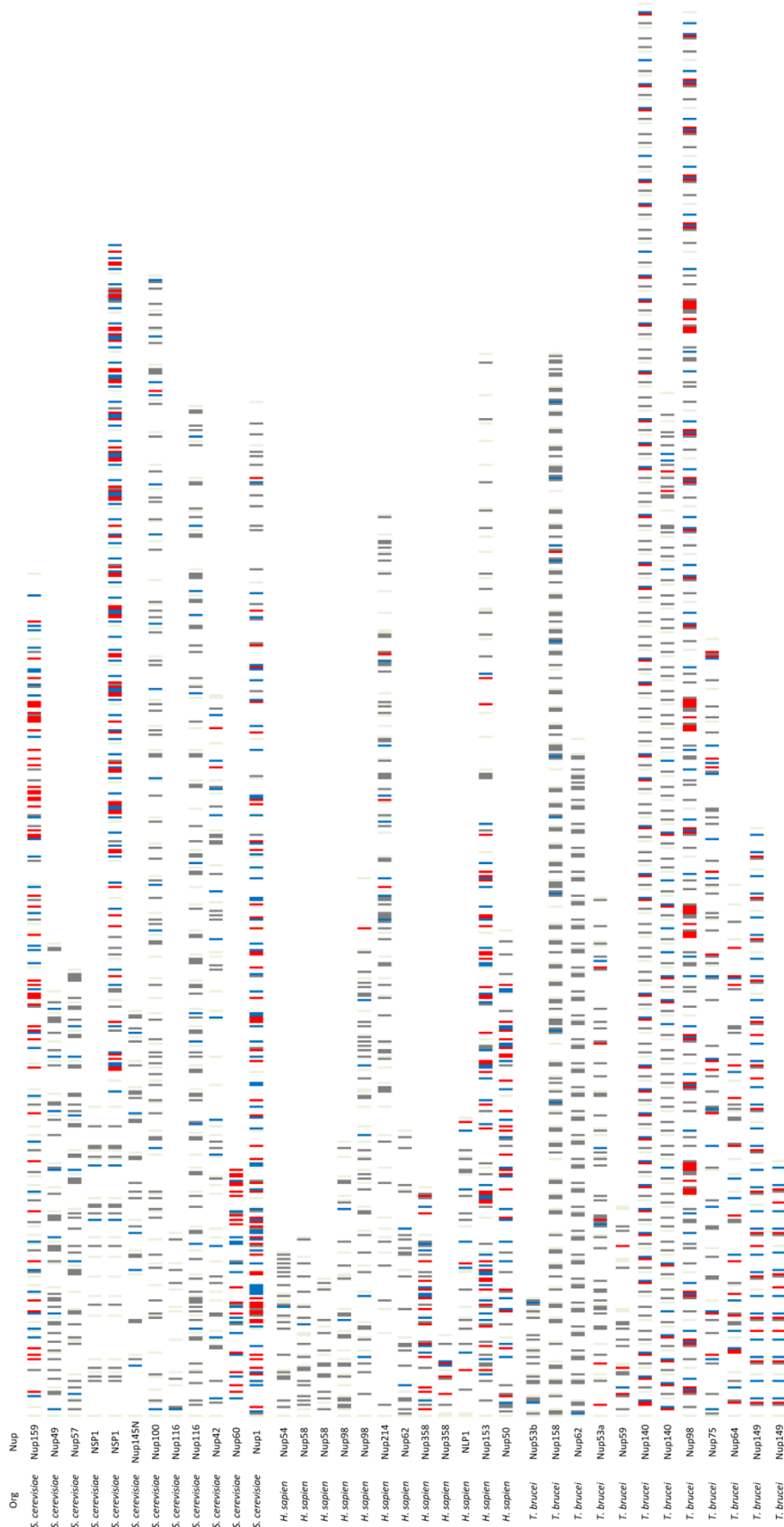




Figure S5

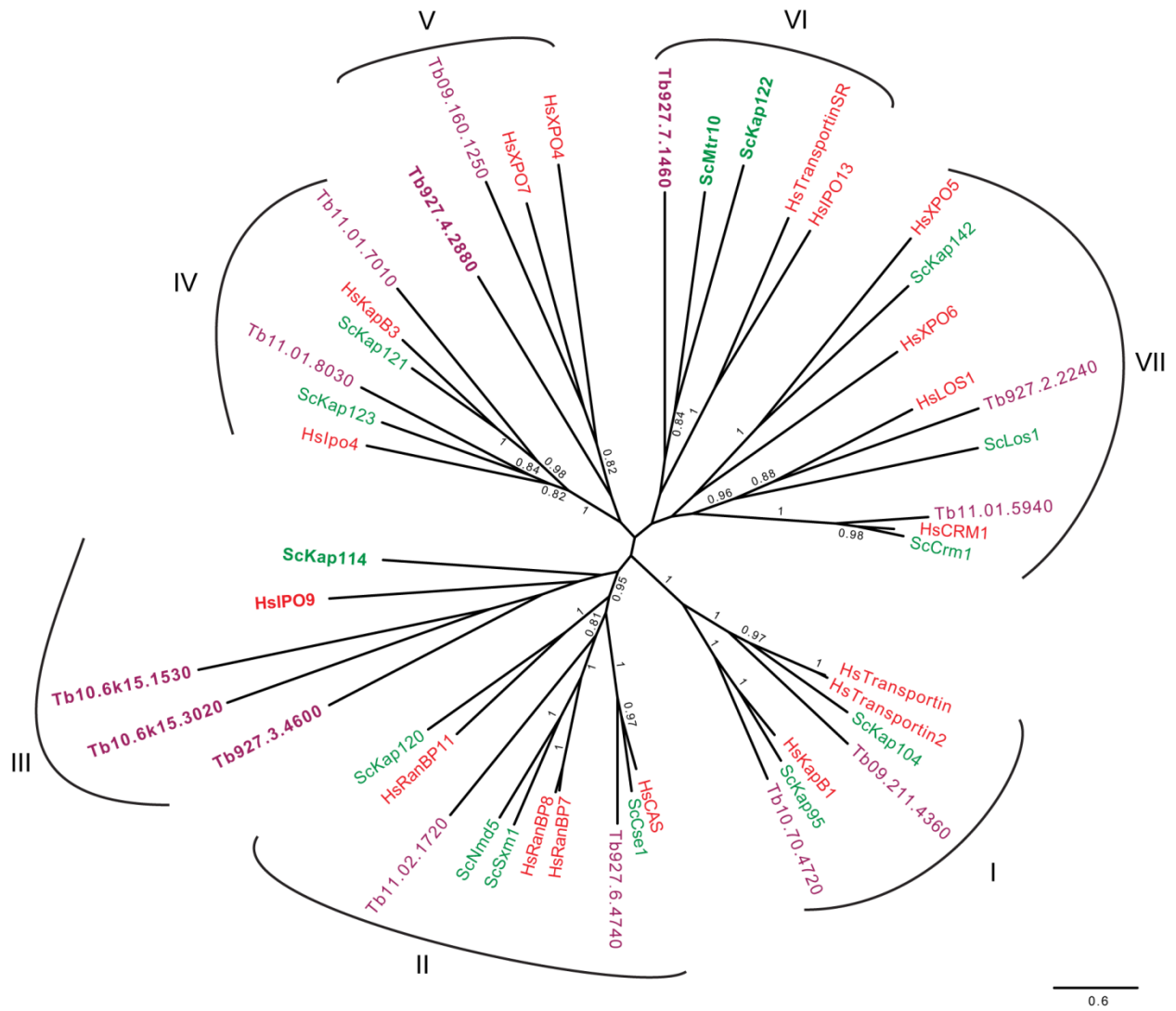


Figure S6

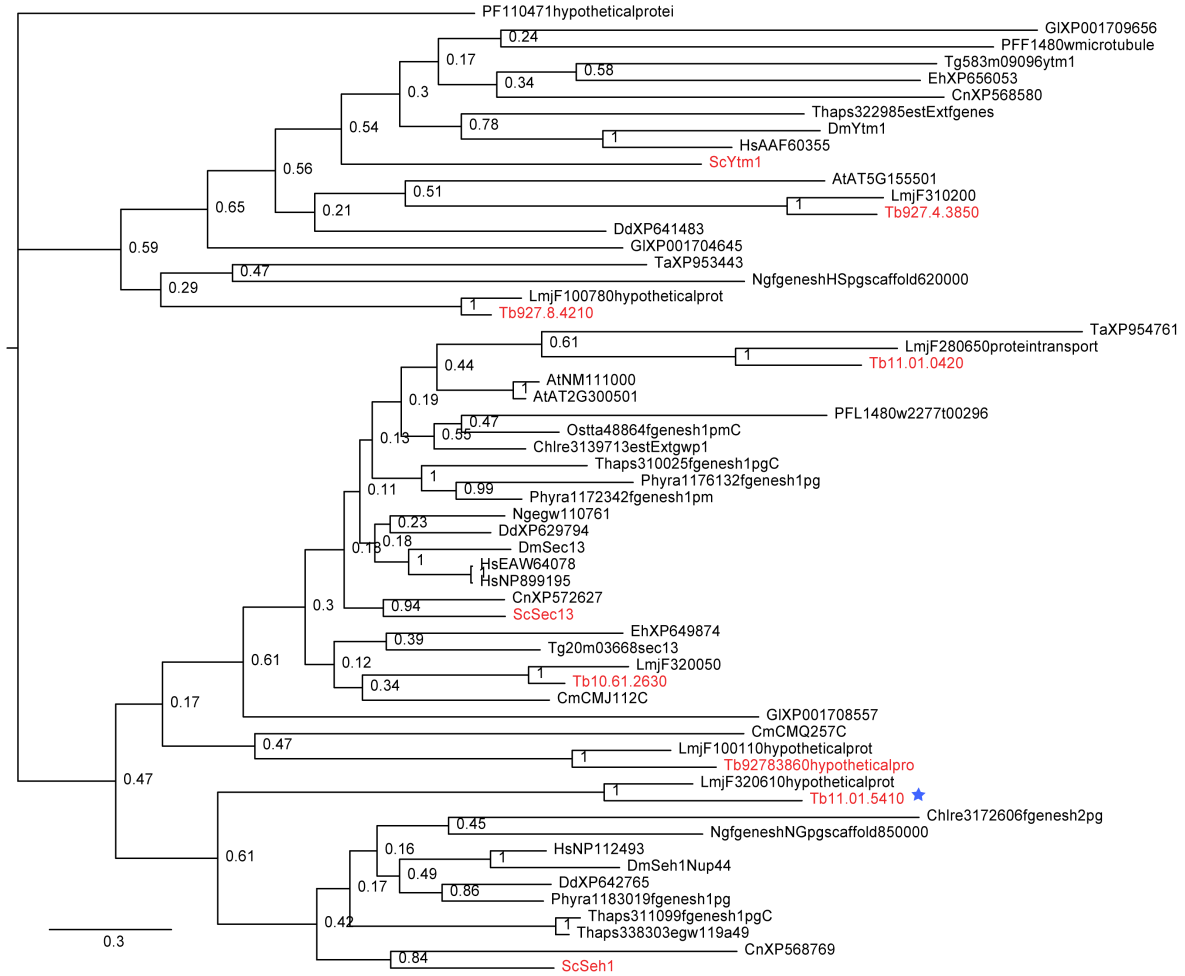
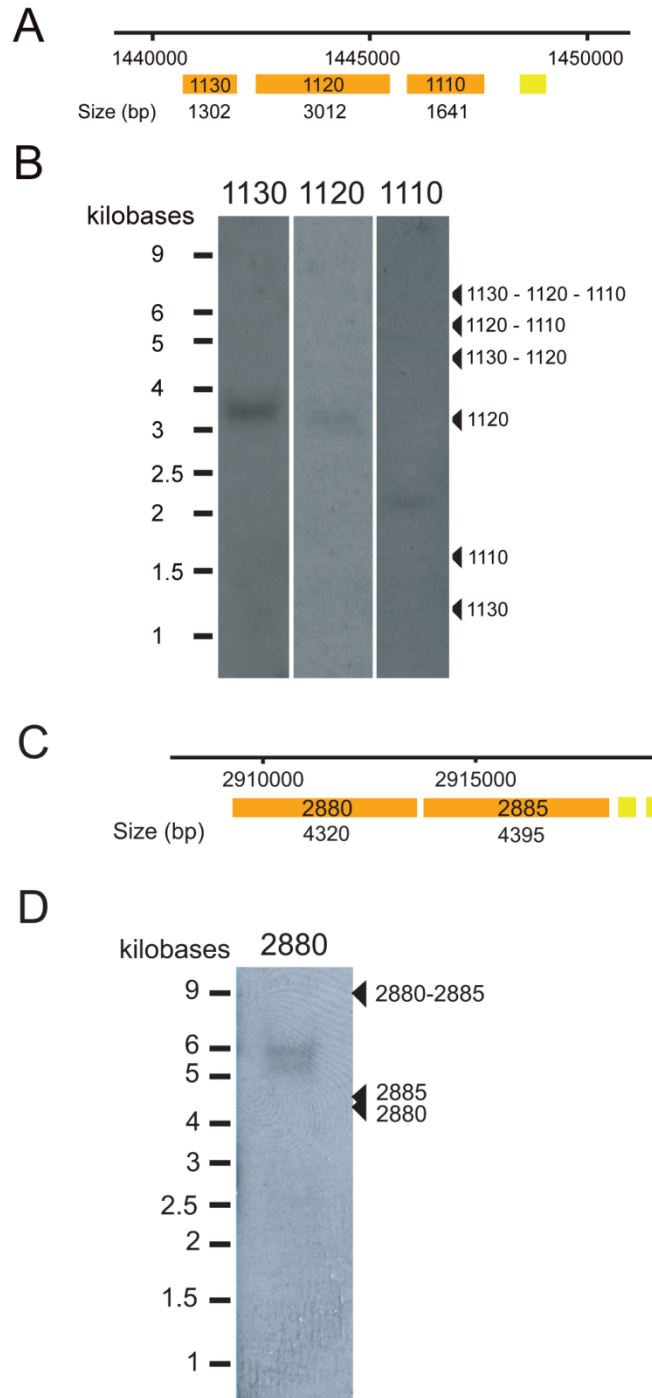


Figure S7



## Tables (MS Excel format)

**Table S1: The unannotated members of the TbNEP dataset.** Notations are as follows: (a) as determined with Nucleo: \* probability  $\geq 0.70$ , \*\* probability  $\geq 0.80$ , \*\*\* probability  $\geq 0.90$ ; (b) TMH and signal peptides were simultaneously predicted with Phobius; (c) as predicted with PEPCOIL, window=28: \* probability  $\geq 0.70$ , \*\* probability  $\geq 0.80$ , \*\*\* probability  $\geq 0.90$ ; (d) HMMer against the Pfam profile databases, expectation  $< 0.1$ ; (e) present in *T. brucei* bloodstream plasma membrane fraction [13]; (f) present in *T. brucei* bloodstream cytoskeletal fraction [13]); (g) present in *T. brucei* flagellum proteome [14]; (h) expressed in procyclic form [15]; and, (i) the residue boundaries of the domains are listed along with the domain identifier: CC, coiled coil; FG, FG repeat, the plurality motif is listed within parans.

**Table S2: The annotated proteins within the TbNEP dataset.**



Accession Number	GeneDB Annotation	Mass (kDa)	Protein Expect	# of Identified peptides	NLS (a)	Signal Peptide (b)	TMH domains (c)	Coiled Coils (c)	Plam Matches (d)	Bioinformatic Notes	GFP localized?
Tb10.61.0160	Conserved hypothetical	42.9	-4.1	2				***	WD domain, G-beta repeat	Similarity to Monad	Inconclusive
Tb927.4.1310	Conserved hypothetical	47.3	-1.1	2				***	Zn-finger in Ran binding protein	ZFP family member	Inconclusive
Tb10.10.1130	Conserved hypothetical	48.2	not found								Inconclusive
Tb927.8.1830	Conserved hypothetical	49.9	-1.1	2	*				WD domain, G-beta repeat	Similarity to ScPnt2	Inconclusive
Tb10.70.1110	Conserved hypothetical	59.9	not found		**				Zn-finger in Ran binding protein	Zinc finger, RanBP2-type	Inconclusive
Tb927.4.590	Conserved hypothetical	87.9	-64.9	21		Yes	1		DUF1620	(e), GFP labeling shows a speckled cytosolic pattern	Inconclusive
Tb10.70.1120	Conserved hypothetical	109.2	-1.1	2	*				Zn-finger in Ran binding protein	Zinc finger, RanBP2-type (f), paralogous to NUP-1	Inconclusive
Tb927.7.3330	Conserved hypothetical	502.3	-15.2	7	**			***		(f), large beta sheet presence, however it is very large protein	Inconclusive
Tb927.3.5370	Conserved hypothetical	34.2	-71.7	10							Inconclusive
Tb927.7.6670	Conserved hypothetical	333.6	-71.5	27	**	Yes	3	***			Inconclusive
Tb927.8.2670	Conserved hypothetical	30.6	-62.7	14		Yes	4				Inconclusive
Tb10.61.0690	Conserved hypothetical	153.8	-65.7	24						(e,f,g), possible cysteine protease	Inconclusive
Tb927.4.2650	Conserved hypothetical	72.5	-61.4	15	**						Inconclusive
Tb09.160.4710	Conserved hypothetical	170.2	-50.1	13							Inconclusive
Tb927.6.2930	Conserved hypothetical	43.2	-48.3	11						(e,f)	Inconclusive
Tb927.5.4200	Hypothetical protein	29.1	-46.2	7		Yes					Inconclusive
Tb11.02.4120	Conserved hypothetical	27.5	-45.2	9		Yes				(e,f,g)	Inconclusive
Tb927.2.2510	Conserved hypothetical	29.1	-44.3	13							Inconclusive
Tb927.7.5940	Conserved hypothetical	67.1	-43.7	11					14	Major Facilitator Superfamily	Inconclusive
Tb11.01.4740	Conserved hypothetical	61.1	-42.9	12		Yes					Inconclusive
Tb11.02.5660	Conserved hypothetical	46.2	-42.9	8					1	***	Inconclusive
Tb09.211.1240	Conserved hypothetical	38.2	-42.7	10							Inconclusive
Tb10.61.5210	Conserved hypothetical	28.8	-40.8	11							Inconclusive
Tb927.5.440	Conserved hypothetical	84.5	-40.5	2		Yes	1		1		Inconclusive
Tb927.7.900	Conserved hypothetical	64.7	-39.0	13		Yes	1				Inconclusive
Tb927.6.1500	Conserved hypothetical	45.3	-38.6	7							Inconclusive
Tb927.2.2240	Conserved hypothetical	59.6	-31.4	11							Inconclusive
Tb11.01.0480	Conserved hypothetical	63.5	-31.0	7					9	EF hand, SNARE associated Golgi protein	Vacuole membrane protein 1 (mouse)-like
Tb11.04.000	Hypothetical protein	27.6	-29.9	11							Inconclusive
Tb09.211.1800	Conserved hypothetical	53.6	-29.2	7	**					LisH	(e,f), Nuclear - Confirmed by mAb
Tb927.6.4320	Conserved hypothetical	44.3	-28.9	7					1	Eukaryotic translation initiation factor 3 subunit 7 (UPF0172)	Inconclusive
Tb927.8.1570	Conserved hypothetical	28.8	-28.6	9							Inconclusive
Tb927.3.5620	Conserved hypothetical	112.5	-27.9	13					***	SPT16, Rtt108	(e,f), Probable transcription factor (FACT complex)
Tb927.3.5350	Conserved hypothetical	11.5	-26.9	5					1		(e)
Tb09.211.2530	Conserved hypothetical	41.5	-26.8	5					1		(e)
Tb10.70.3750	Conserved hypothetical	31.5	-26.6	6					7		(e,f)
Tb927.1.860	Conserved hypothetical	29.5	-26.6	6		Yes					(e,f)
Tb927.3.3890	Conserved hypothetical	36.6	-26.0	5					3		(e)
Tb927.4.2530	Conserved hypothetical	16.8	-26.0	7							(e)
Tb927.5.1790	Conserved hypothetical	48.1	-25.8	5							(e)
Tb927.7.6290	Conserved hypothetical	36.4	-25.4	5					***	Tetrapeptide repeat	(e), Tetrapeptide repeat protein
Tb10.61.0540	Conserved hypothetical	36.3	-23.8	5	**				***		(e,f,g)
Tb927.5.2530	Conserved hypothetical	114.8	-23.1	13						DUF699, DUF1728	(e), SSR Alpha
Tb927.2.1910	Conserved hypothetical	29.2	-22.5	6		Yes	1				(e,f,g,h)
Tb927.6.4500	Conserved hypothetical	22.6	-22.2	2		Yes	1				(e), SSRI Alpha
Tb927.4.1300	Conserved hypothetical	41.9	-22.1	7							(e,f,g,h)
Tb927.3.1740	Conserved hypothetical	62.1	-20.9	9							(e)
Tb10.70.7770	Conserved hypothetical	37.0	-20.2	2							(e)
Tb10.70.2320	Conserved hypothetical	25.9	-20.0	5		Yes				ABC1 family	(g), BOP1-like WD-40, Erb1p homolog
Tb10.61.5160	Conserved hypothetical	60.5	-19.2	3					3		(e,f)
Tb10.70.3010	Conserved hypothetical	14.7	-19.2	6		Yes	1				(e)
Tb11.02.4620	Conserved hypothetical	88.9	-17.6	7	*					BOP1NT, WD domain, G-beta repeat	(g), BOP1-like WD-40, Erb1p homolog
Tb10.70.1570	Conserved hypothetical	107.2	-17.5	10					***		(e,f)
Tb927.5.1930	Conserved hypothetical	24.1	-17.4	4		Yes					(e)
Tb927.5.3190	Conserved hypothetical	23.1	-17.4	5	**						(e)
Tb927.3.4100	Conserved hypothetical	63.2	-17.3	7		Yes	13			Major Facilitator Superfamily	(h), mitochondrial
Tb927.4.4920	Conserved hypothetical	42.7	-16.8	3						LETM1-like protein	(h), mitochondrial
Tb10.70.2450	Conserved hypothetical	37.1	-15.9	9						DUF650	(e)
Tb927.7.4790	Conserved hypothetical	58.7	-15.9	5							(e)
Tb11.02.3310	Conserved hypothetical	13.5	-15.7	4							(e)
Tb11.01.3860	Conserved hypothetical	18.3	-15.6	6		Yes					(e)
Tb927.7.5840	Conserved hypothetical	26.1	-15.6	4					5	***	(e)
Tb09.160.1070	Conserved hypothetical	41.4	-15.2	5		Yes			2	Methyltransferase domain	(h), BAP28
Tb09.160.1640	Conserved hypothetical	287.0	-14.8	6					2	HEAT repeat (expect=48)	(h), BAP28
Tb11.02.1320	Conserved hypothetical	39.0	-14.6	4							Inconclusive
Tb11.01.1090	Conserved hypothetical	23.7	-14.4	4							Inconclusive
Tb11.01.0490	Conserved hypothetical	60.0	-12.8	7	*				***	Sas10/Up3/C10 family	Ribonucleoprotein, SAS10 homolog
Tb11.01.1900	Conserved hypothetical	30.2	-12.8	6						Macro domain (ADP-ribose binding module)	(h)
Tb11.02.2040	Conserved hypothetical	14.0	-12.3	2					***	Alba, DNA/RNA-binding protein	(h)
Tb927.5.3250	Conserved hypothetical	172.0	-12.2	5							(e), possible member of cohesin complex
Tb10.70.0870	Conserved hypothetical	127.8	-12.0	5					***	STAG domain	(e), possible member of cohesin complex
Tb11.01.2490	Conserved hypothetical	450.0	-12.0	10					***	Leucine Rich Repeat	(e,f,g,h)
Tb11.01.8770	Conserved hypothetical	110.0	-12.0	5							(e,f,g,h)
Tb10.70.7760	Conserved hypothetical	46.7	-11.6	6					1		(e)
Tb927.8.5440	Conserved hypothetical	37.0	-11.6	6							(e)
Tb09.211.4520	Conserved hypothetical	36.7	-11.4	6							(e)
Tb927.6.2320	Conserved hypothetical	20.1	-11.3	5		Yes	1				(e)
Tb927.4.310	Conserved hypothetical	73.0	-10.9	10					2	SPRY domain, HECT domain	(e)
Tb10.61.2050	Conserved hypothetical	44.1	-10.7	3					2	**	Probable DnaJ
Tb11.02.1550	Conserved hypothetical	50.3	-10.6	5		Yes					(h)
Tb11.01.8225	Conserved hypothetical	16.3	-10.5	3							(h)
Tb927.7.2700	Conserved hypothetical	249.9	-10.4	8						DUF1309	(h), Possible FG - discounted after examination of amino acid composition, Prediction
Tb11.01.1540	Conserved hypothetical	118.7	-10.2	6					2		(e)
Tb11.01.0022	Conserved hypothetical	201.2	-10.1	21					2		(e)
Tb11.01.1830	Conserved hypothetical	25.3	-10.0	4					4	Bromodomain	(e)
Tb11.01.5480	Conserved hypothetical	13.5	-10.0	5					4	DUF423	(e,h)
Tb11.01.3020	Conserved hypothetical	68.9	-9.8	4					***		(e,h)
Tb927.2.3800	Conserved hypothetical	55.4	-9.8	1		Yes					(e,h)
Tb927.5.5980	Conserved hypothetical	526.0	-9.8	7					**		(e)
Tb10.61.0520	Conserved hypothetical	33.3	-9.7	5					**	Tetrapeptide repeat	(e)
Tb11.01.5780	Conserved hypothetical	67.1	-9.7	4		Yes				Pyridine nucleotide-disulphide oxidoreductase	(e)
Tb10.61.5230	Conserved hypothetical	30.8	-9.5	4						RNA recognition motif (e.g. a.k.a. RHM, RBD, or RNP domain)	(e), Probable C-terminal Protein, CEP250
Tb11.02.0840	Conserved hypothetical	26.5	-9.4	3							(e)
Tb927.8.3950	Conserved hypothetical	102.0	-9.4	5					***		(e), Probable C-terminal Protein, CEP250
Tb927.8.590	Conserved hypothetical	12.2	-9.1	1							(e)
Tb927.7.4880	Conserved hypothetical	23.2	-9.0	3					6		(e)
Tb927.8.9680	Conserved hypothetical	69.5	-9.0	1					***		(e)
Tb11.02.0880	Conserved hypothetical	486.2	-8.9	2					2	SPRY domain, HECT domain	(e)
Tb927.6.1920	Conserved hypothetical	42.3	-8.9	4	**				**	PPAK motif (double check)	(e)
Tb927.7.4580	Conserved hypothetical	60.5	-8.9	8	**				**	WD domain, G-beta repeat	(h), Prp19/Pso4-like (spliceosomal)
Tb927.2.5240	Conserved hypothetical	54.2	-8.8	3							(e)
Tb927.6.4130	Conserved hypothetical	11.7	-8.7	2					***	C2 domain	(e,f,g,h), signal transduction
Tb927.4.2090	Conserved hypothetical	104.8	-8.5	5							(e)
Tb927.8.2470	Conserved hypothetical	11.2	-8.4	1							(e)
Tb10.70.2970	Conserved hypothetical	24.0	-8.3	5					1	***	(e)
Tb11.02.0800	Conserved hypothetical	166.1	-8.1	5					1	***	(e)
Tb927.5.5560	Conserved hypothetical	59.9	-8.1	4					1		Spermidine synthase-like
Tb927.3.1690	Conserved hypothetical	17.1	-8.0	3							(e)
Tb927.2.2610	Conserved hypothetical	16.0	-7.9	2		Yes					(e)
Tb927.7.840	Conserved hypothetical	14.5	-7.9	3					1	***	(e)
Tb10.70.7440	Conserved hypothetical	69.0	-7.7	2							(e)
Tb927.3.3130	Conserved hypothetical	177.8	-7.7	6		Yes			**	SAM domain (Sterile alpha motif)	(e)
Tb927.7.6980	Conserved hypothetical	104.8	-7.7	5					1		Probable SAP 130 homolog
Tb927.5.1630	Conserved hypothetical	17.6	-7.5	4					1		Cytochrome b5-like Heme/Steroid binding domain
Tb927.8.940	Conserved hypothetical	237.3	-7.5	6					2		Large protein. 1 TMH on each terminus. Large amount of beta sheets. Possible candidate for a membrane nup
Tb10.70.5220	Conserved hypothetical	64.2	-7.4	1					7		Cleft lip and palate transmembrane protein 1 (CLPTM1)
Tb11.01.8500	Conserved hypothetical	25.8	-7.4	2					1		(e), ClpTm1 homolog



Accession Number	GeneDB Annotation	Mass (kDa)	Protein Expect	Total # of peptides
Tb09.2.5.3400	Calcium-transporting ATPase (Calcium pump)	110.2	250.2	2446 51
Tb09.2.4.2320	NUP-1 protein	406.6	-222.9	47
Tb09.2.1.2330	beta tubulin	49.9	-215.6	43
Tb09.2.3.1890	ATPase beta subunit	55.3	-180.4	33
Tb11.02.5500	glucose-regulated protein 78	71.3	-171.3	35
Tb09.2.4.3380	vacuolar-type proton translocating pyrophosphatase 1	85.8	-162.3	26
Tb11.02.4170	TEF1, elongation factor 1B	64.4	-161.1	26
Tb09.2.7.420	ATP synthase alpha chain, mitochondrial precursor	63.4	-146.7	35
Tb11.01.3110	heat shock protein 70	75.3	-143.4	38
Tb11.02.0850	heat shock protein 70	75.3	-132.1	31
Tb09.2.5.1810	lysosomal/endosomal membrane protein p67	72.6	-120.8	21
Tb11.02.4150	Glycosomal Pyruvate Phosphate Kinase	100.3	-125.4	37
Tb09.2.4.1810	Glycosomal alpha-1,6-glucosyltransferase	45.3	-110.6	25
Tb09.2.6.3740	heat shock 70 kDa protein, mitochondrial precursor	71.4	-99.8	20
Tb09.160.3820	mgp5 protein	54.9	-96.8	21
Tb11.02.1480	mitochondrial processing peptidase alpha subunit, Clan ME, Family M16	57.9	-94.7	19
Tb11.02.0430	chaperonin Hsp60, mitochondrial precursor	59.4	-91.6	16
Tb09.2.5.1210	short-chain dehydrogenase	33.8	-89.7	21
Tb11.01.6120	mitochondrial carbonic dehydratase	34	-87.7	24
Tb09.2.2.4280	glycosomal phosphoenolpyruvate carboxylase	58.5	-87.4	18
Tb09.2.1.2010	RHS5-c	76.5	-86.5	21
Tb09.2.3.7550	Nucleolar Protein, SIK1	54.3	-83.5	25
H25N7.12	RHS4	97.7	-80.5	25
Tb11.01.4750	elongation factor 1 gamma	60.4	-76.5	20
Tb11.02.5800	RHS3	51.1	-76.1	16
Tb09.2.1.0980	glycosomal malate dehydrogenase	33.6	-75.3	25
Tb09.2.4.3590	translation elongation factor 1-beta	28.3	-75.2	16
Tb09.2.1.8370	IGL1, golgi/lysosome glycoprotein 1	67.5	-75.2	10
Tb11.02.0270	metalloproteinase, Clan ME, Family M17	63.7	-74.7	17
Tb11.02.1070	fructose-1,6-bisphosphate aldolase, glycosomal	41	-73.3	15
Tb09.2.1.4250	tyrosine phosphatase	22.4	-69.8	16
Tb11.02.0360	glt1, glycosomal kinase, glycosomal	56.3	-69.1	9
Tb09.2.3.3530	glycero-3-phosphate dehydrogenase	37.7	-66.4	18
Tb09.2.5.1060	mitochondrial processing peptidase, beta subunit	54	-65.6	17
Tb11.02.0490	glt2, glycosomal kinase, glycosomal	100.1	-65.1	10
Tb09.160.2840	ACS4, fatty acyl CoA synthetase 4	77.8	-62.8	17
Tb11.02.5280	glycerol-3-phosphate dehydrogenase	66.9	-62.4	15
Tb09.2.7.8550	TbT5, trans-sialidase	84.4	-61.3	25
Tb09.2.3.3580	LPG3, lipopolysaccharide biosynthetic protein	87.7	-60.2	13
Tb09.2.4.3400	PF191, 73 kDa paraflagellar rod protein	68.6	-59.7	15
Tb09.2.7.2330	histone H2A	14.2	-59.5	15
Tb11.04.0045	paraflagellar rod protein	32.1	-57.5	14
Tb11.02.4200	fatty acyl CoA synthetase	78.9	-56.5	17
Tb11.02.0370	ATP synthase F1 subunit gamma protein	34.3	-53.9	13
Tb09.2.1.1750	mitochondrial carrier protein	33.2	-53.5	13
Tb09.2.3.3270	TbPFK, ATP-dependent phosphofruktokinase	53.4	-50.0	19
Tb09.2.4.470	snRNP70 protein, GAR1	21.7	-50.0	16
Tb11.02.0880	mitochondrial carrier protein	33	-48.3	11
Tb11.01.3370	PEX11, glycosomal membrane protein	24	-47.0	11
Tb11.02.3290	ATP-dependent DEAD-box RNA helicase, DHH1	46.4	-46.7	16
Tb05.045.7.0	nucleolar RNA helicase II, Gu	63.9	-45.1	13
Tb09.160.3270	mDMH1, mitochondrial malate dehydrogenase	45.3	-45.9	12
Tb11.02.0170	RISP, release non-sulfur amino acid precursors	33.1	-45.7	8
Tb09.2.1.4310	Glutamate dehydrogenase	112	-44.2	19
Tb09.2.7.4180	long chain fatty acyl elongase	33.8	-44.2	9
Tb09.2.5.830	FRD3, NADH-dependent fumarylpyruvate reductase	123	-44.0	10
Tb09.2.2.4710	RNA-binding protein, RRM1	49.9	-43.3	8
Tb09.160.5480	adenosine transporter	50.6	-42.4	12
Tb11.02.0480	lanosterol 14-alpha-demethylase	54.3	-42.0	14
Tb09.2.1.0540	FBPase, fructose-1,6-bisphosphate, cytosolic	38.5	-42.0	8
Tb11.02.0230	zinc metalloproteinase	62.7	-41.0	7
Tb09.2.3.3530	heat shock protein 70	75.3	-39.1	9
Tb09.2.6.990	ATP synthase, epsilon chain	20.1	-39.0	7
Tb09.2.3.1790	pyruvate dehydrogenase E1 beta subunit	37.5	-38.4	8
Tb09.2.7.8360	histone H2A	18.6	-37.1	13
Tb09.2.1.2740	Gm53 protein	69.2	-36.9	19
Tb09.2.5.1710	ribonucleoprotein p18, mitochondrial precursor	21.2	-35.6	6
Tb11.02.1100	translation elongation factor 1-beta	21.9	-35.5	9
Tb09.2.4.410	prone oxidase	63.1	-35.1	10
Tb09.2.7.760	Nopp44/46	35	-35.1	7
Tb09.2.5.4190	histone H4	11.1	-34.8	9
Tb09.2.1.1511	kinetoplastid membrane protein KMP-11	11	-34.6	11
Tb11.04.0052	microtubule-associated protein 2, MAP2	560.8	-34.1	11
Tb11.02.406.0350	histone H2B	12.5	-33.7	10
Tb09.2.6.4210	aldehyde dehydrogenase	54.5	-33.5	11
Tb11.02.1130	ATP-dependent DEAD/II RNA helicase, DBP1	71.3	-33.1	13
Tb09.2.5.5050	60S ribosomal protein L4 (L1)	41.8	-31.9	11
Tb09.2.1.210	RHS1	63.1	-31.7	11
Tb09.2.5.900	glucosylacetyl transferase subunit	92.1	-31.2	11
Tb09.2.4.470	retrotransposon hot spot protein RHS4-c	98.2	-30.6	21
Tb11.02.0001	60S ribosomal protein	34.6	-30.4	10
Tb11.02.0615.0410	60S ribosomal protein L18	21.8	-30.4	21
Tb09.2.1.2530	Histone H3	14.7	-30.0	7
Tb11.02.0150	inosine-5-monophosphate dehydrogenase	48.4	-30.0	6
Tb11.02.0485	40S ribosomal protein S4	30	-29.1	6
Tb09.2.4.1330	type IB DNA topoisomerase large subunit	79.2	-29.0	10
Tb11.02.3210	thiose phosphatase isomerase	26.8	-28.8	9
Tb11.02.1350	perlecan transporter	69.2	-28.6	19
Tb09.2.8.1610	major surface protease gp63	62.9	-28.5	8
Tb11.04.0247	s-adenosyl-L-methionine-cc-24-delta-sterol-methyl transferase a	40.1	-28.3	6
Tb09.2.1.2120	RHS4	76.3	-28.2	19
Tb11.02.0560	60S ribosomal protein L6	21.1	-27.4	5
Tb09.2.6.1530	DHAP, alkyl-dihydroxycetone phosphate synthase	68	-27.0	16
Tb09.2.7.850	cation-transporting ATPase	140.3	-27.1	10
Tb09.2.3.1840	3-oxo-5-alpha-steroid 4-dehydrogenase	33.3	-27.1	8
Tb11.02.1080	heat shock protein 70	80.7	-26.5	16
Tb09.2.8.5010	PF192, 69 kDa paraflagellar rod protein	69.5	-26.4	7
Tb09.2.7.710	heat shock, 70 kDa	70.1	-26.1	13
Tb09.2.5.3510	SMC3	44.9	-25.6	16
Tb09.2.5.520	stomatol-like protein	136.2	-24.3	10
Tb09.160.3670	60S ribosomal protein S6	55.9	-24.3	8
Tb09.2.4.4.2630	40S ribosomal protein S6	13.9	-23.8	3
Tb09.2.5.2080	inosine-5-monophosphate dehydrogenase	28.7	-23.7	7
Tb09.2.2.440	RPN6, proteasome regulatory non-ATPase subunit 6	52.2	-23.4	6
Tb11.01.4940	AAA ATPase	57.2	-23.4	6
Tb09.2.8.1420	acyl-CoA dehydrogenase, mitochondrial precursor	141	-23.2	13
Tb09.2.4.1790	ribosomal protein L3	54.3	-22.5	5
Tb11.02.2880	40S ribosomal protein S15	20	-22.5	20
Tb09.2.1.620	DNAJ	84.6	-22.4	7
Tb11.02.1920	40S ribosomal protein S14	35	-22.2	6
Tb11.02.0890	RPS14, 40S ribosomal protein S14	15.5	-21.9	5
Tb09.160.2770	ACS1, fatty acyl CoA synthetase 1	78.9	-21.8	9
Tb09.2.1.4100	Nop96	85.8	-21.8	9
Tb09.2.5.5460	cytochrome C oxidase subunit IV	40.5	-21.7	4
Tb09.2.4.4620	flagellar calcium-binding protein TB-44A	45.6	-21.7	5
Tb11.02.0790	cytochrome c oxidase VIII (COX VIII)	18.7	-21.0	6
Tb09.2.6.2550	PSSA-2 procytic form surface glycoprotein	46	-20.9	46
Tb09.2.4.2760	RNA-binding protein, possible PAB1	79.7	-20.7	12
Tb11.02.0290	cytosolic coat protein	24.5	-20.7	7
Tb11.02.0290	short-chain dehydrogenase	36	-20.6	10
Tb09.2.3.960	protein transport protein Sncd1 gamma subunit	29.4	-20.5	4
Tb11.02.0330	cytoskeletal-associated protein CAP-5	7.6	-20.4	7
Tb11.02.0330	dihydroipoamide acetyltransferase precursor	34.5	-20.3	7
Tb11.02.0330	60S ribosomal protein L18a	48	-20.3	7
Tb11.02.0330	Tubulinin Fibrillin, F family M17	31.8	-19.9	6
Tb11.02.0250	heat shock protein, mitochondrial precursor	84.1	-19.6	6
Tb11.01.3590	vacuolar ATP synthase subunit B	55.5	-19.5	6
Tb09.2.7.4170	fatty acid elongase	30.4	-19.4	6
Tb11.02.0890	pyruvate dehydrogenase E1 component alpha subunit	40.4	-19.0	6
Tb09.2.3.490	TDPI1 high mobility group protein	32.8	-19.0	3
Tb09.2.4.2070	Antigenic protein, parasitoid NUP-1	51.1	-18.9	10
Tb09.2.8.2000	PP1ase cyclophilin type peptidyl-prolyl cis-trans isomerase	32.8	-18.9	7
Tb09.2.7.6660	hypoxanthine-guanine phosphoribosyltransferase	26.3	-18.7	2
Tb09.2.3.440	RHS4-c	97.7	-18.3	20
Tb09.2.11.0560	RNA-binding protein, DRDB3	36.9	-18.1	9
Tb11.02.0750	dynein light chain	10.4	-17.9	3
Tb09.2.6.2630	TCP-1-zeta 1-complex, protein 1, zeta subunit	59.5	-17.9	3
Tb09.2.7.990	chaperone protein DNAJ	85.1	-17.7	6
Tb11.02.0360	delta-6 fatty acid desaturase	47.9	-17.6	6
Tb09.2.5.1660	nucleoside diphosphate kinase	16.8	-17.6	6
Tb09.2.5.1660	DNAJ	44.7	-17.5	3
Tb09.2.1.1550	chaperone protein DNAJ	56.6	-17.1	9
Tb11.02.0450	universal nucleic acid binding protein (UMSBP)	63.9	-16.8	11
Tb11.02.0450	40S ribosomal protein S24E	15.6	-16.8	2
Tb11.01.3610	membrane-bound acid phosphatase	53.3	-16.7	4
H25N7.01	RHS2	31.6	-16.6	11
Tb11.02.2960	mitochondrial carrier protein	29.8	-16.5	5
Tb11.02.1920	fibrillarin	31.6	-16.3	10
Tb09.2.4.110	COX VII cytochrome c oxidase VII	13.5	-16.2	4
Tb09.2.1.2230	calpain-like protein fragment	13.5	-16.2	4
Tb09.2.4.2000	RuvB-like DNA helicase	52.5	-16.0	8
Tb09.160.4450	RPS3 40S ribosomal protein S3	31.9	-15.7	6
Tb09.2.1.0120	nascent polypeptide associated complex subunit	20.1	-16.0	3
Tb09.2.4.4210	ATP-dependent zinc metalloproteinase	96.1	-15.7	6
Tb11.01.3180	guanine nucleotide-binding protein beta subunit	34.4	-15.4	5
Tb09.2.4.1080	V-type ATPase, A subunit	67.7	-15.3	5
Tb09.2.7.3120	Sm-D1 small nuclear ribonucleoprotein SmD1	11.7	-15.1	3
Tb11.02.1680	mannose-specific lectin	62.2	-15.0	20
Tb09.2.1.1980	cytochrome c1, heme protein, mitochondrial precursor	30	-14.8	7
Tb09.2.3.2230	succinyl-CoA synthetase alpha subunit	31.4	-14.8	3
Tb09.2.7.2700	BSR NADH-cytochrome b5 reductase	31.8	-14.5	5
Tb11.02.0020	HTH2A glucose transferase	59.2	-14.4	15
Tb11.02.0020	TCP-1-beta 1-complex, protein 1, beta subunit	58	-14.4	6
Tb11.02.1770	Eukaryotic translation initiation factor 6 (eIF-6)	26.9	-14.4	4
Tb11.02.1770	TCP-1-theta 1-complex, protein 1, theta subunit	58	-14.4	4
Tb11.02.0290	protein tyrosine phosphatase	29.6	-14.4	3
Tb09.2.1.0680	CAAX prenyl transferase 1	48.6	-14.3	5
Tb09.2.4.2570	calcium phosphatase	113.9	-14.2	15
Tb09.2.5.3810	orotidine-5-phosphate decarboxylase/uracil phosphoribosyltransferase	49.9	-14.1	7
Tb09.2.1.120	60S ribosomal protein L13	26.6	-14.0	5
Tb09.2.1.120	RHS4	73.1	-13.7	6
Tb09.2.3.4700	dynamid	73.1	-13.7	6
Tb11.02.0011	ATP-dependent DEAD/II RNA helicase: NPC-associated RNA helicase (Allen et al., 2001)	82.6	-13.7	5
Tb11.02.0011	ATP-dependent DEAD/II RNA helicase	82.6	-13.7	5
Tb09.160.2910	tricarboxylate carrier	35.6	-13.3	3
Tb11.03.0090	ribokinase	35.2	-13.3	3
Tb09.2.3.1510	protein phosphatase 2C	31.9	-13.2	6
Tb09.160.4200	60S acidic ribosomal protein	11	-13.2	4
Tb09.2.4.2730	60S ribosomal protein L5	34.6	-13.1	6
Tb09.2.1.920	GPI transamidase component GP116	75.7	-13.1	4
Tb11.02.0890	60S ribosomal protein L5	17.3	-13.0	5
Tb09.2.8.2160	PGPA MRPA multidrug resistance protein	174.5	-13.0	6
Tb11.02.0870	Ran-binding protein	17.6	-13.0	3
Tb11.02.0110	ATP-dependent zinc metalloproteinase	62.2	-12.9	10
Tb11.01.5100	parafagellar rod component	68.3	-12.9	4
Tb09.2.4.3300	mitochondrial ATP-dependent zinc metalloproteinase	78.8	-12.9	3
Tb09.2.1.2940	60S ribosomal protein L23	14.5	-12.8	4
Tb09.2.5.1940	vacuolar proton translocating ATPase subunit A	89.5	-12.7	6
Tb11.02.0600	RPN9 proteasome regulatory non-ATPase subunit 9	45.9	-12.7	1
Tb09.2.3.3090	cytosolic leucine aminopeptidase	71.2	-12.5	5
Tb09.2.4.3550	60S ribosomal protein L13a	39.1	-12.5	4
Tb11.02.3240	short-chain dehydrogenase	38	-12.4	3
Tb11.02.4100	retrotransposon protein (Sncd1)	53.6	-12.3	5
Tb11.02.0780	squalene monooxygenase	63.7	-12.2	4
Tb09.2.7.440	I6 autoantigen	17	-12.2	4
Tb09.2.4.2180	60S ribosomal protein L5A	17	-12.2	3
Tb09.160.1820	cytochrome c oxidase subunit V	22.2	-12.1	4
Tb11.01.6360	metalloproteinase	74.4	-12.1	4
Tb09.160.3090	heat shock protein	90.8	-12.0	7
Tb09.2.6.3890	replication factor C, subunit 2	26.3	-12.0	6
Tb09.2.6.3160	splicing factor 3A, SAP61	61.3	-12.0	5
Tb09.2.2.970	mitochondrial carrier protein	33.9	-12.0	5
Tb11.01.3180	BRN NADH-cytochrome b5 reductase	31.8	-12.0	4
Tb11.01.0120	COP-coated vesicle membrane protein ev25 precursor	28.1	-12.0	4
Tb11.01.4720	cytochrome c oxidase subunit 10	13.6	-12.0	3
Tb09.2.3.5090	Cisplatin Factor 1-beta	28.5	-11.9	1