

Supplementary data for:

Chaperone requirements for biosynthesis of the trypanosome variant surface glycoprotein

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Supplementary figure legends

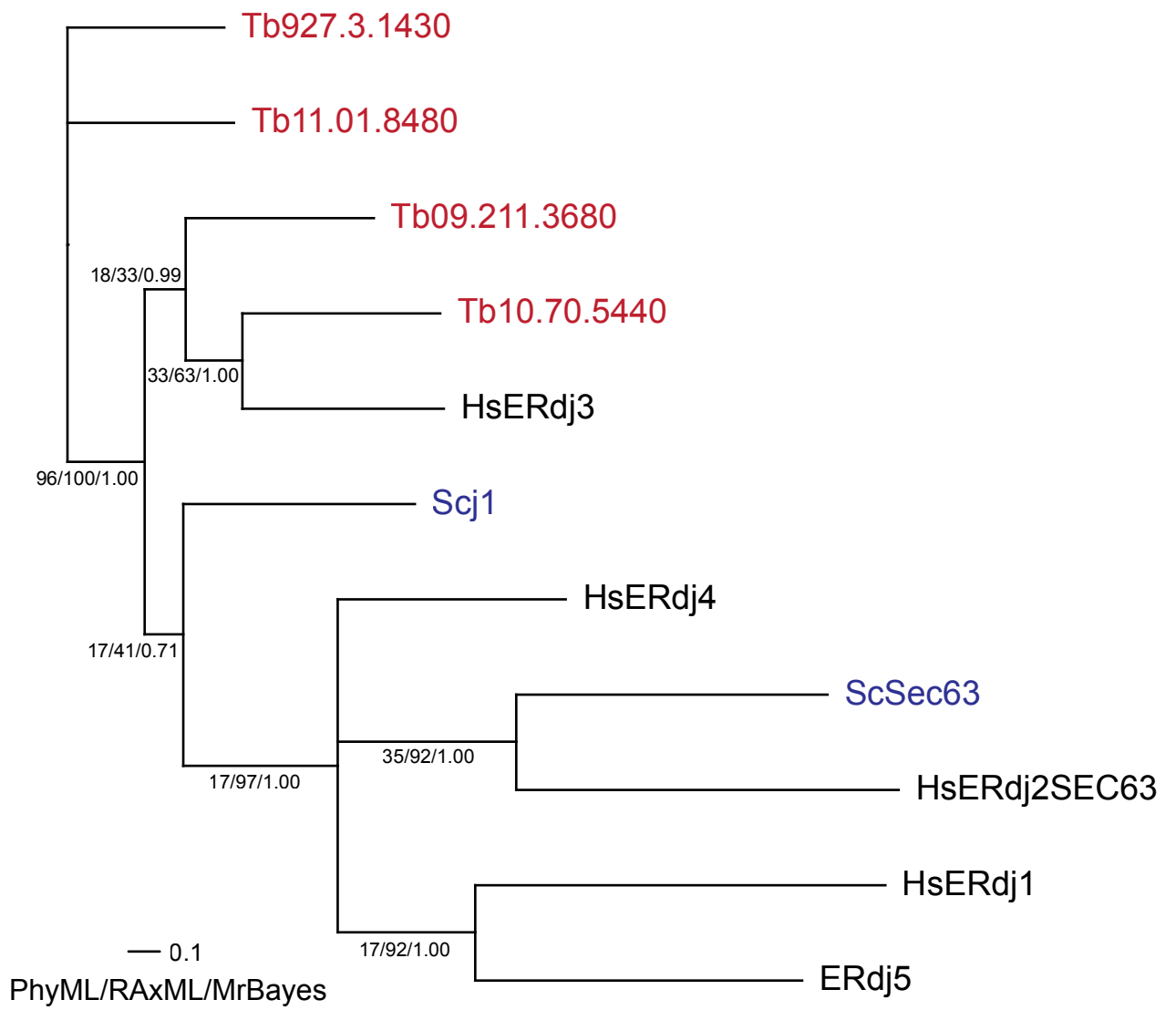
Figure S1: Phylogenetic reconstruction of part of the DNAj family. Sequences for representative members of the DNAj family were retrieved from the NCBI nr database to represent the major Opisthokonta (animals and fungi) ER chaperone families plus Sec63 (*H. sapiens*, black, *S. cerevisiae*, blue). Searches of the *T. brucei* genome database using BLAST using the higher eukaryote sequences returned as most significant the four sequences shown here in by geneDB accession number (red) as well as Tb09.211.1550 (data not shown). All other sequences were rejected based on significantly lower expect values, excessive or very small predicted polypeptide size or reverse BLAST failure (frequently demonstrating orthology to mitochondrial DNAj proteins). Sequences were aligned in Clustal, manually edited in MacClade and subjected to phylogenetic analysis. Initial rounds demonstrated that Tb09.211.1550 was highly divergent and was removed. Further rounds of reconstruction resulted in the tree shown. Values at the internodes are bootstrap/bootstrap/posterior probability for RaXML, PhyML and Mr Bayes reconstructions. Data suggest that Tb09.211.3680 and Tb10.70.5440 are orthologs of ERdj3. The remaining two sequences are either trypanosome-specific or orthologs to DNAj proteins not included in the present analysis.

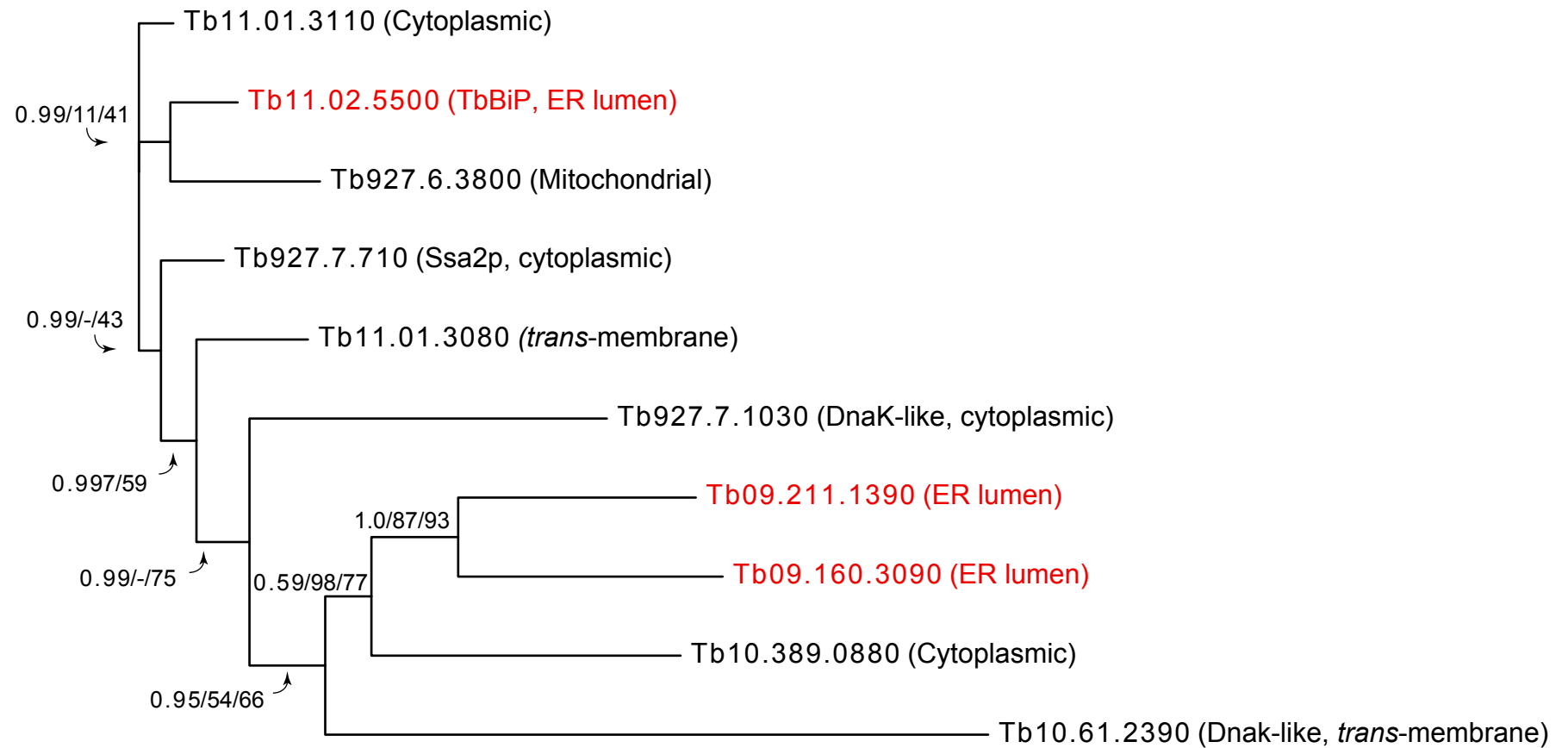
Figure S2: Phylogenetic reconstruction of part of the trypanosome Hsp70 family. Sequences for representative members of the trypanosome Hsp70 family were retrieved from geneDB. Sequences were aligned in Clustal, manually edited in MacClade and subjected to phylogenetic analysis. Gene products in red were analysed. Values at the internodes are bootstrap/bootstrap/

posterior probability for RaXML, PhyML and Mr Bayes reconstructions. Annotations based on BLAST similarity to sequences at NCBI nr database and PSORT II are also provided. Note that most of these annotations should be considered tentative.

Figure S3: Immunofluorescence microscopy data archive. Data are shown for cells at one or two days post induction for RNAi for the indicated open reading frame. Cells were fixed, stained for either BiP or VSG (green) and counterstained for DNA using DAPI. Example images are binned according to frequency of the morphology observed. Frequent; >70%, common; 10-25%, rare; <5%. In all instances several hundred cells were analysed per gene product and representative images are shown for each category and time. Inductions were performed at least twice for each gene product with similar results.

Figure S4: Clustal alignments for predicted amino acid and DNA sequences of EDEM ORFs from *T. brucei*. Sequences corresponding to geneDB accessions Tb927.8.2910, Tb927.8.2920, Tb927.8.2930 and Tb927.8.2940 were aligned with Clustal X using default parameters.





0.3

MrBayes/RAxML/PhyML

Predicted amino acid

Tb927.8.2940 MKGAQFLKMPQVRLVLLVLRVFFVHLPFAFGDAFPVNGARGGNSQGYNTDGMHPHQAEM
Tb927.8.2930 MKGAQFLKMPQVRLVLLVLRVFFVHLPFAFGDAFPVNGARGGNSQGYNTDGMHPHQAEM
Tb927.8.2920 MKGAQFLKMPQVRLVLLVLRVFFVHLPFAFGDAFPVNGARGGNSQGYNTDGMHPHQAEM
Tb927.8.2910 -----MPVWRALLVLTVTFVRLPSVMREERFHQYGN-GESRKLHNVSDMHPHQAEM
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Tb927.8.2930 LPYVRDMIDHAFGSYIKYAFPKDELCPVSGTGKNTMGGYGWTLLIDSLDTLAIAGFHKEFR
Tb927.8.2920 LPYVRDMIDHAFGSYIKYAFPKDELCPVSGTGKNTMGGYGWTLLIDSLDTLAIAGFHKEFR
Tb927.8.2910 LPYVRDMIDHAFGSYIKYAFPKDELCPVSGTGKNTMGGYGWTLLIDSLDTLAIAGFHKEFR

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Tb927.8.2930 RHAKWVEEHLTFDIDESVSVFETTIRALGGLLAAHFMYEEGIVPIIPSEHDYNGGFLRLA
Tb927.8.2920 RHAKWVEEHLTFDIDESVSVFETTIRALGGLLAAHFMYEEGIVPIIPSEHDYNGGFLRLA
Tb927.8.2910 RHAKWVEEHLTFDIDESVSVFETTIRALGGLLAAHFMYEEGIVPIIPSEHDYNGGFLRLA

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Tb927.8.2920 VDLADRLMPCFDTPGTGIPYGVNLRGVSQGGESQLANTAGSGTLLVEMTVLSRITGDGKY
Tb927.8.2910 VDLADRLMPCFDTPGTGIPYGAJNLRGVSQGGESQLANTAGAGTLLMEMTVLSRITGDEKY
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Tb927.8.2920 ERAARRASEALFAARDSQTELMGTYVSVSSGGFSSSESSVAGSGLDSAIEYFIKSHSMSGD
Tb927.8.2910 ERAARRASEALFAARDSQTELMGNHHTHTGIWRHGESSVGGNIDSVIEYFIKSHSMSGD
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Tb927.8.2920 IGDWERFERTARAVNRYVRKGGMLLAASMYSGRRLQTSQESLSSFFPGNLVLGGHLHEAV
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Tb927.8.2920 ESSWPIHTFFKHFGVLPEIFSLESGEPSWRSHDYIGRPEHIESLYMLYRATRDPTYLLMG
Tb927.8.2910 ESSWPIHTFFKHFGVLPEIFSLESGEPSWMSHDYVGRPEHIESLYMLYRATRDPTYLLMG
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DNA

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Tb927.8.2910 -----ATGCCGTGGGTTCGTGCTTTACTAGTTCTCGTGACG
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Tb927.8.2940 -----
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Tb927.8.2930 GAGTGGTGGGCGCCTACTTCCCTGGAGACCTTGGACAAGGAGGCTGAGGATCCCGCTGCT
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Tb927.8.2940 -----
Tb927.8.2920 GACGGTGAGGTTGTTGGGGCGAATGATAAGGGTGGTGCAGCCCTGTACCAGTCCACTGT
Tb927.8.2910 GACGGTGAGGTTGTTGGGGCGAATGATAAGGGTGGTGCAGCCCTGTACCAGTCCACTGT

Tb927.8.2930 GCCAATCACGCCCTGAGTGATGTAGGGAGGCTGTCGAAGTCTGTGTTTCGATAA
Tb927.8.2940 -----
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Tb927.8.2910 GCCAATCACGCCCTGAGTGATATAGGGAGGCTGTCGAAGTCTGTGTTTCGATAA

Table S1. Primers for p2T7 RNAi constructs. Primer sequences are given 5' to 3', and the corresponding gene is designated by the geneDB accession number, except for VSG MITat1.2 where the NCBI accession is given.

ORF (geneDB accession)	Forward	Reverse
11.02.5450/11.02.5500 ¹	CACCTTGGTGGTGAGGACTT	GGGGATCTGTGTGTTACGCT
09.160.3090	GCAGCTTGACACCTCAATA	TGGCACCACCAATTAGTTCA
09.211.1390	CAATTCGTCTGACTTTGCGA	CCAGTGCTCTACCGCCTAAG
11.01.3110	CGATGCACTCTTCGAAAACA	AGCGTCTAGGTCTGAAGGTCA
927.3.3580	TTCTGAACGAGAACCAGCCT	CCTCTGCGTTTACTTCCTCG
927.3.1430	TTTATTGGCGGAGAATCAGG	CTGGTCAGCCTCCATTTTCAT
11.01.8480	TGCAAATGAGGTGCTTTCTG	GGGGATGTTTCTTCGTCTGA
09.211.3680	GCAAAGCGTAAGCATTGACA	CGCTGTCTGGGTCTTTCTTC
10.70.5440	TGTGCTCATCATTCTAGCGG	CTGTTTGACCTCCACCGAAT
927.4.5010/927.8.7410 ¹	GACTTACGGAAGATGCTCGC	CTCCGGCTTTTGTCTTCTG
10.05.0080	TTACGTGGGACAAGGACACA	TGGCTGAATAACGCTGTGAG
927.8.2910/2920/2930 ¹	GTATGAGTGGGGACATTGGG	CATAACACAATGCGGTGAGG
927.8.2940	GCATCTGAGGCTCTTTTTGTC	CAAGGAAAATATCTCCGGCA
927.3.4630	AACACGACTGACGTTTGCTG	CCATACCGACAAAAGTGGCT
11.01.2470	GAAGAAGACACTTCAGGCCG	TGTTTCGTGGGTTTTGTTTCA
11.02.1680	GAGCAACATGACGCAGAAAA	TCCTCTTGGAGGAAGCTGAA
10.20.0130	TACGTTTGACGCTGTTTTCG	ACCGTGTGCTTACGTGTCA
927.7.1300	ATGGTGTGGACACTGCAAAA	CTCCACCTTATAGCGCTTCG
10.6k15.2290	CCCTGAAGCTCACCAAAGAG	TGAAGTGCTATTGCTGGCTG
927.8.4890	TGGGATGTACATTTAGCCA	ATATCCGGTGTTCCTCTG
927.7.5790	GGAACTCATTTGCACTTCTGC	CACATGTTGCTTTTAACAAATTC
927.4.4740	CCTGATGGGGGATTAATGTG	GGCACACCAACCTGAAGAAT
11.01.2640	GGTTGTGTTTCAGGCTTGTT	TAAAATACGGGAAATGCCCA
927.7.3870	AACCCAAAACACGAGGAGTG	TTCTGCTTTGTTCTCCGCTT
927.2.5140	AGCTCAGAGTGCCCTTATCG	TTACCCCATGACTGATTCCG
11.01.8120	CTTCATGGCTGTCCTTCGAG	CGCATCTTTTACCCCAAGAA
11.02.4100	AGTTGCCAAACCTGACCAAC	AACACATAGGCCACCAAAGC
VSG MITat 1.2 (P26332; NCBI)	TTTCTGCAGCGGTCACATG	TGCTTGTGTTGGCTTTCAG

1: 11.02.5450 and 11.02.5500 are identical, 927.4.5010 (chromosome 4) and 927.8.7410 (chromosome 8) are identical, and 927.8.2910, 927.8.2920 and 927.8.2930 are identical.

Table S2. Primers for qRT-PCR transcription analysis. Primer sequences are given 5' to 3', and the corresponding gene is designated by the geneDB accession number.

geneDB accession	Forward	Reverse
09.160.3090	AGTCCCTTGATGCCTGGTC	GCTTCTGCAACTCTGTCACG
11.01.3110	CGTGTGTGCTCCCATCTTAT	ATCCCACCACCCATTCCT
927.4.5010	CCCCAATCCAGCGTATAAGA	TCGCCGATGATAATGTCCTT
10.05.0080	TATGAGTCGGGGGCATACAT	CTTCCACCCTGTTCTTTGC
11.01.2640	GAAAACGAAGGGATGCGTTA	AGGAGATGAGCAGCGTGTTT
927.7.3870	TCAAAGCTATGTCACCACAGG	TTTCGCTCTGCCGTAAAAAT
927.2.5140	AACGCGCTAAGGATGAGGAT	CTTGATCTTCATTACCCCATGA
11.01.8120	GCTGTGCCTATTGAGGAGGTT	TGCTCATCTGTTCGCATCTTT

1: 11.02.5450 and 11.02.5500 are identical, 927.4.5010 (chromosome 4) and 927.8.7410 (chromosome 8) are identical, and 927.8.2910, 927.8.2920 and 927.8.2930 are identical.