Evidence for recycling of invariant surface *trans*-membrane domain proteins in African trypanosomes

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Supplementary material

Figure S1: Multiple sequence alignment of RME-8 orthologues identified in 19 species representing all eukaryotic linages. Sequences were aligned using ClustalW, and visualized in Jalview. Conserved residues are highlighted in color, based on amino acid characteristics. In the human sequence (Hs-NP_056083.3) the DnaJ domain is annotated in blue (starts at position ~1770 in the alignment), and the DUF4339 domain is annotated in red (starts at position ~1380 in the alignment). These two domain cover ~5% of the total sequence length, and hence are not the sole contributors to the extremely low e-values observed. For full sequence details, see Table S1.

Figure S2: Phylogenetic reconstruction of RME-8 orthologues identified in 19 species representing all eukaryotic linages. The tree shown is the best Bayesian topology. Numerical values at the nodes of the tree indicate statistical support (posterior probability) by MrBayes. For full sequence details, see Table S1. Alignments (available upon request) were created using MUSCLE. Only unambiguous homologous regions were retained for phylogenetic analysis; manual masking and trimming was performed in MacClade. ProtTest was used to estimate the appropriate model of sequence evolution (WAG+G). Phylogenetic analysis was performed using the program MrBayes version 3.1.2 with 1 million generations, removing all trees before a plateau established by graphical estimation; calculations were checked for convergence and had a splits frequency of <0.1.

Figure S3: TbRME-8 RNAi delays endocytosis in BSF. Control and tetracycline induced RNAi lines for TbRME-8 (24 hours post induction) were allowed to take up

FITC-conjugated ConA (green) at the indicated temperatures, fixed and co-stained for HA (red), and counterstained with DAPI for DNA (blue). ConA staining was assessed by immunofluorescence. Representative images show a delay in delivery of ConA to the lysosome at $37^{O}C$ in the knockdown cells. In control cells, ConA staining overlaps with the lysosomal marker p67 in the majority of cells within 30 minutes of exposure, while in the RNAi induced cell line, there is a marked delay to ConA arriving in the p67 compartment. Scale bar, bottom right, is 2 um.

Table S1: Database details and accession numbers of RME-8 protein homologues identified in this study. Databases used for BLAST analysis for the species studied are given, as well as accession numbers for RME-8 protein orthologs identified. Also shown are the E-values obtained by BLAST, as well as the percent identity and similarity of the identified orthologs to the human sequence.