

1 **SUPPLEMENTAL FIGURE LEGENDS**

2

3 FIG S1. Sequence alignment of Erv1 orthologs from a taxonomically diverse range of
4 eukaryotes. Sequences were aligned in CLUSTALW; residues encompassing the core, flavin-
5 binding five-helix bundle are boxed; proximal cysteine pairs are highlighted in blue; distal
6 cysteine pairs are highlighted in red; the cysteine pair that contributes thermostability in Erv1
7 from yeast, and likely other Erv1 proteins, is yellow. H66 and Y70 in TbERV1 which could be
8 predicted to form an 'O₂ channel' are in bold; where H, rather than D, is present at the analogous
9 position in other Erv1 proteins this is also shown in bold. The alignment also reveals that
10 although it is not unprecedented for the distal cysteine pair to be C-terminal, the C-terminal
11 extension downstream of the flavin-binding domain is uniquely long in trypanosomatid Erv1.
12 Cm, *Cyanidioschyzon merolae*; Dd, *Dictyostelium discoideum*; Ec, *Encephalitozoon cuniculi*;
13 Lm, *Leishmania major*; Ot, *Ostreococcus tauri*; Ps, *Phytophthora sojae*; Sc, *Saccharomyces*
14 *cerevisiae*; Tb, *Trypanosoma brucei*; Tt, *Tetrahymena thermophila*.

15

16 FIG S2. Circular dichroism analysis of *in vitro*-folded recombinant TbERV1 variants.

17 Circular dichroism spectra reveal a similar overall fold for *in vitro*-folded recombinant TbERV1
18 and the site-directed mutant proteins that were subjected to kinetic analysis (Tables 1 and 2).
19 Spectra are normalized for protein concentration. Solid line – wild type *T. brucei* Erv1; Short
20 dashes – proximal variant (C60S/C63S) *T. brucei* Erv1; Long dashes – 'O₂ channel' variant
21 (H66D/Y70R) *T. brucei* Erv1; Mixed dashes - distal variant (C261S/C265S) *T. brucei* Erv1.

22

23 FIG S3. Protein stained SDS-PAGE (left) and anti-Erv1 Western Blot (right) of purified *T.*
24 *brucei* Erv1 and variants

25 In each analysis, lane M is molecular weight markers, lane 1 is wild type TbERV1, lane 2 is the
26 proximal (C60S/C63S) variant, lane 3 is the distal (C261S/C265S) variant and lane 4 is the
27 channel (H66D/Y70R) variant. The proportion of protein present as full length and degradation
28 products is different in each ERV1 variant. Lane 2-4 were each loaded with the same amount of
29 protein (75 pmol for the protein stained SDS-PAGE, 25 pmol for the Western Blot). Molecular
30 weights of the markers (in kDa) are indicated.

31

32 FIG S4. Mitochondrial swelling is not seen following RNAi knockdown of components of the
33 mitochondrial and cytosolic Fe-S cluster assembly pathways.

34 For each mutant analyzed RNAi induction is lethal. Thus, cells were fixed and processed for
35 TEM in the same way as the *TbERV1* RNAi mutants shown in FIG 5., 24 h before cultures
36 ceased growth. Longitudinal sections through representative individual cells induced for RNAi
37 against *TbNBP35* (A), *TbFRAT* (B), *TbATMI* (C), *TbCFD1* (D) or *TbISA1+TbISA2* (E). Scale
38 bars represent 1 μ m.

39

Sc -----MKAIDKMTDNPPEGLSGRKIIYDEDGK 28
 Dd MAFGNKINQILDEKSHITTPATTENENKKNKNVEITTIKKASFLSKDITNEDEDDCGT 60
 Tt -----MSYSEQEKKKDCPEVV 17
 Ps -----
 Tb -----
 Lm -----
 Ec -----M 1
 Cm ----MSRSVWFEKLRAGIGTVATPLPLPNTAIRRSQSWRRFVARTIVAFITVAGLATIA 56
 Ot -----

Sc P**CRS**CNTLLDFQYVTGKISNGLKNLSSNGKLAGTGALTGEASELMPGSRTYRKVDPDVE 88
 Dd EL**LG**VTEKKNQMFQ**MIS**INNNNNNNNNNGNNDNKNNE**DSKY**WEPMPSPPTTI 120
 Tt **CE**KSDPLGTYR**KA**Q**QPK**TM**FK**DE**DD**DDHSN**SS**GELEK**SE**DKVA**QDD**--PYKYCPNNKQ 75
 Ps -----MVATKSDPN**C**VEPA**C**ADKMDFFKSSMGKKATKPA**Q**PK--PATD**C**PLDRQ 48
 Tb -----MSKQ**E**PLQ**KI**PG**E**CP-TPR 18
 Lm -----MSDDDVHERLTTIP**G**EC**P**-TP**L** 21
 Ec KREQSTGRILVVLAAAWV**TY**RCYGLMVRDREGK**V**SEL**GG**SGKSENPRTEKLS**K**EIRE 61
 Cm WHSSSWREAVFP**T**K**V**FSQASTEAAAA**AP**D**T**ARA**AP**GLPR**T**MLTK**AP**G**KI**PFQ**DE**K**P**PT**R**A 116
 Ot MSSNERRHTVHTG**PL**AG**L**R**D**A**F**AR**L**NR**G**Q**R**GETSG**K**V**T**DS**K**TSS**K**R**N**AS**D**K**A**D**T**Q**R**TS**R**E 60

Sc Q**L**GRSSW**TL**LLHSVAASYP**AQ**PTD**Q**Q**G**EM**K**Q**FL**NI**F**SHI**Y**PC**NW****C**AKDFEKYI**R**EN**A**P**Q**V 148
 Dd ELGNSG**W**TL**L**H**T**IAAY**Y**PE**K**P**S**E**K**K**K**Q**D**IK**E**FL**Q**S**F**SK**V**Y**P**CK**V****C**AKDFREIM**K**ET**P**PI**L** 180
 Tt SLG**F**FT**W**N**F**L**H**T**M**AI**Y**Y**P**K**N**P**T**EE**E**Q**Q**K**M**K**N**F**D**S**F**AT**F**Y**P**CK**P****C**AL**H**F**Q**K**D**IL**K**T**P**PA**V** 135
 Ps ELGNAT**W**GL**L**HS**M**GI**Y**Y**P**DK**P**SE**Y**Q**A**K**A**K**T**F**I**E**A**L**A**L**M**Y**P**CV**H****C**AD**D**F**Q**KEI**A**K**S**PP**R**V 108
 Tb ELG**K**AG**W**I**L**HS**A**AA**V**F**P**Y**N**P**T**Q**Q**E**A**FR**N**FL**H**GW**S**H**A**Y**A**CS**H****C**AY**H**M**R**R**Y**F**H**Q**N**PP**V**V 78
 Lm ELG**M**SG**W**N**L**HS**A**AV**Y**Y**P**K**P**SA**V**Q**T**AM**K**N**F**IES**W**A**H**V**Y**AC**SW****C**AY**H**M**R**E**Y**VR**D**H**S**PD**V** 81
 Ec RLGR**S**T**W**TL**L**H**T**M**G**AR**Y**PA**F**PT**Y**Q**Q**K**D**TL**S**F**I**HL**L**SS**V**F**P**CG**E****C**T**K**H**F**Q**K**LL**S**D**Y**PP**R**V 121
 Cm ELGRAG**W**TL**L**HS**I**AAN**Y**PE**V**AT**P**EM**Q**T**H**AR**Q**F**I**AS**F**AAL**Y**PC**P**T**C**RE**H**F**Q**GY**V**R**T**HP**P**AL 176
 Ot DLGRAT**W**P**F**L**H**T**L**AA**Q**F**P**EE**P**TR**Q**ER**D**ARE**L**IG**M**TR**L**Y**P**CG**E****C**AR**H**F**E**E**I**VR**K**N**P**PD**C** 120
 ** * :*: . :* .: : : : :.* * .:. . .*

Sc ESREELGR**W**MC**E**AHN**K**V**N**K**L**R**K**PK**F**DC**N**---FWEKRWKDGWDE----- 189
 Dd DSQ**N**DFAL**W**LC**N**AH**N**N**V**N**L**Q**L**G**K**PT**F**DC**N**---LINKRWKINN**N**EFH----- 224
 Tt ES**N**ESLS**I**W**L**CE**R**H**N**L**V**N**K**W**L**G**K**Q**F**DC**S**FE-NLEK**R**W**R**T**G**Y**D**H**C**K---DQ**V**T**K**L**Q**--- 187
 Ps ES**R**T**T**F**S**M**W**LC**E**Q**H**N**I**V**N**R**K**I**H**K**P**LF**E**CT**M**E-KLEER**W**R**K**G**K**P**S**W**G**E**D**G**E**ES**A**Q**D**AL**G** 167
 Tb TD**K**L**A**L**N**R**Y**LC**E**F**H**NA**V**NER**V**G**N**K**I**Y**D**CP**M**-N**V**L**R**R**W**H**P**T**F**P-D**M**E**D**Q**P**T**I**E**E**Q**V**K**S**L**E** 136
 Lm RD**K**L**T**V**S**R**Y**VC**E**M**H**ND**V**N**R**L**G**K**D**V**F**DC**S**PS-V**V**L**R**R**W**H**P**G**Y**PN**K**M**E**D**T**P**T**I**E**E**Q**L**A**S**D** 140
 Ec GS**N**E**E**F**K**T**W**LC**E**V**H**N**V**N**R**R**L**G**K**T**V**V**D**CR---TV**D**E**I**W**D**CG**C**E**A**----- 162
 Cm ES**R**E**Q**F**V**K**W**CB**R**AH**N**AV**N**L**R**L**G**K**P**T**I**PC**T**DL**Q**L**L**D**K**R**W**R**D**CH**C**DE**Q**EP**Q**N**M**I**R**AAR**L**L**I**E 236
 Ot TS**G**L**E**L**Q**R**W**MC**E**V**H**NE**V**NT**S**L**G**K**P**MF**D**CA**K**TS**Q**R**S**R**L**DC**D**G**D**G**E**L**T**GC**S**L**E**DR**R**R**R**L**T**R 180
 . . : * . ** * : : *

Sc -----
 Dd -----
 Tt -----
 Ps -----
 Tb LKE-----KN**E**TP**Q**GV**S**DR**W**R**Q**NS**S**AS**P**D**G**N**V**GR**S**VG**D**AR 173
 Lm RE**K**ST**A**KE**A**TH**H**Q**Q**E**E**R**R**F**S**R**L**GL**H**ARE**A**AR**N**E**A**V**A**WR**D**AS**E**T**A**SK**K**AD**G**L**W**SW**R**I**F**R 200
 Ec -----
 Cm SR**R**EP**N**AG----- 243
 Ot -----

Sc -----
 Dd -----
 Tt -----
 Ps -----
 Tb **W**T**D**T**S**ES**R**RT**N**--V**G**E**I**S**A**G-----**W**G**T**A**G**E**K**M**Q**R**N**S**A**G**D**GV**S**D**A**G**A**SE**K**----**K**W**W** 221
 Lm **K**P**V**L**G**T**D**A**A**K**S**N**A**L**P**G**T**N**S**SP**P**PT**T**Q**Y**K**P**S**I**SS**S**D**A**L**N**T**P**ST**G**A**V**A**T**A**A**S**D**V**G**A**A**H**P**R**H** 260
 Ec -----
 Cm -----
 Ot -----

Sc -----
 Dd -----
 Tt -----
 Ps -----
 Tb **R**W**G**N**S**T**S**S**T**T**A**T**I**AT**P**SA**A**E**P**A**E**D**V**E**A**S**V**T**S**I**L**S**K**L**R**A**C**M**V**Y**C**P**D**D**K**K**S**A 273
 Lm **S**W**S**T**P**T**S**E**A**H**N**S**F**L**M**P**T**S**S**H**G**A**A**D**A**D**T**D**I**D**A**V**L**K**R**L**K**Q**C**Q**V**Y**C**P**E**D**E**L**K**L 312
 Ec -----
 Cm -----
 Ot -----

Figure S1

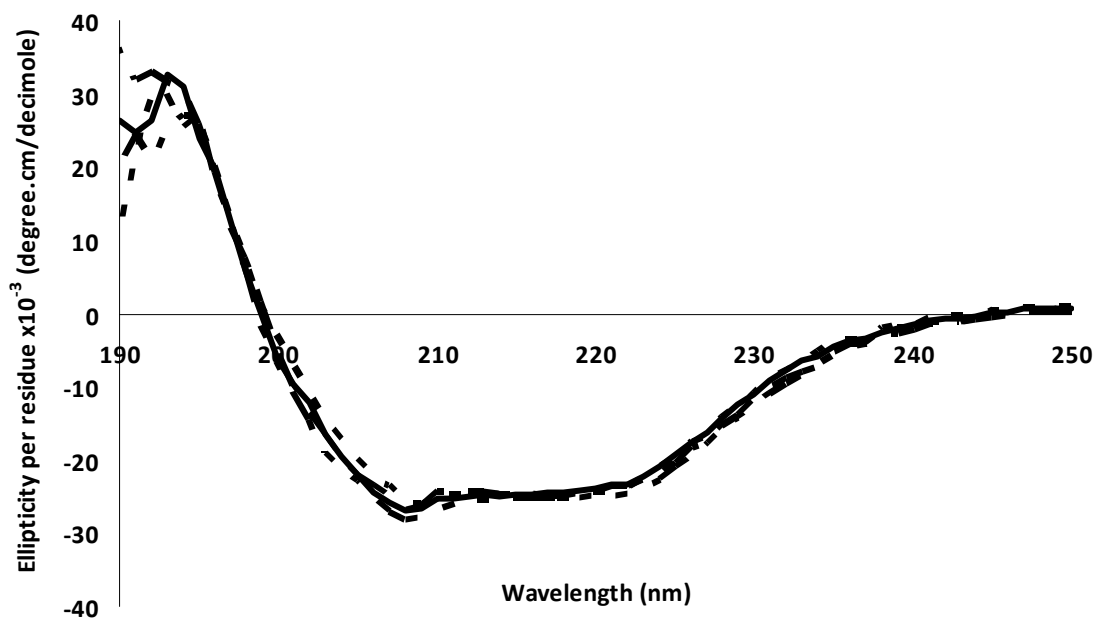


Figure S2

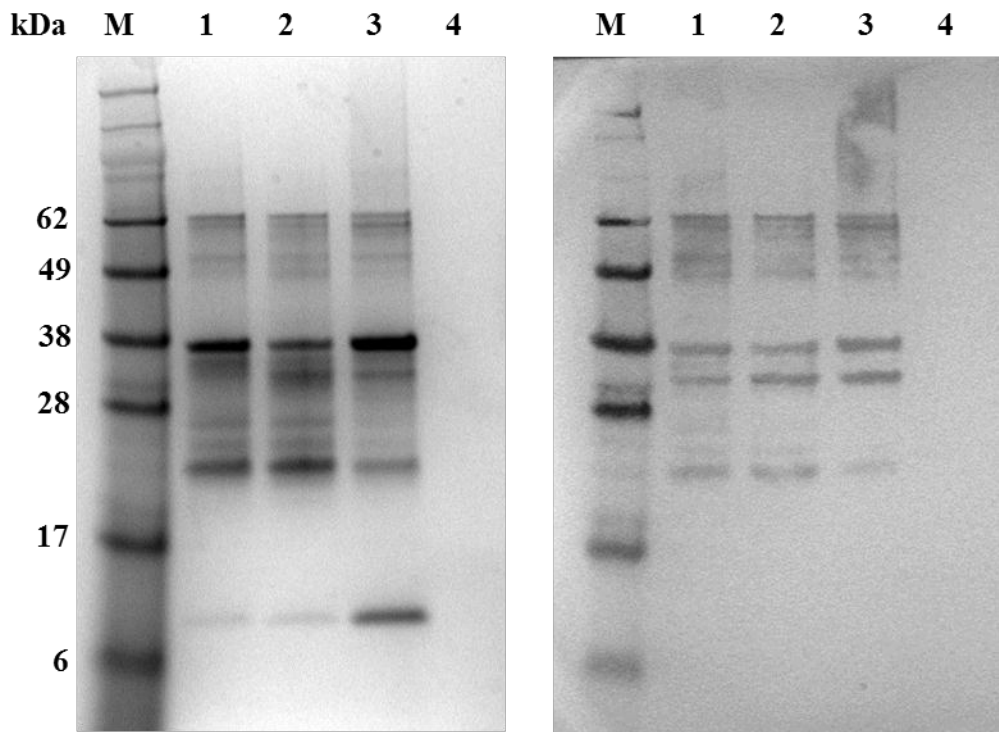


Figure S3

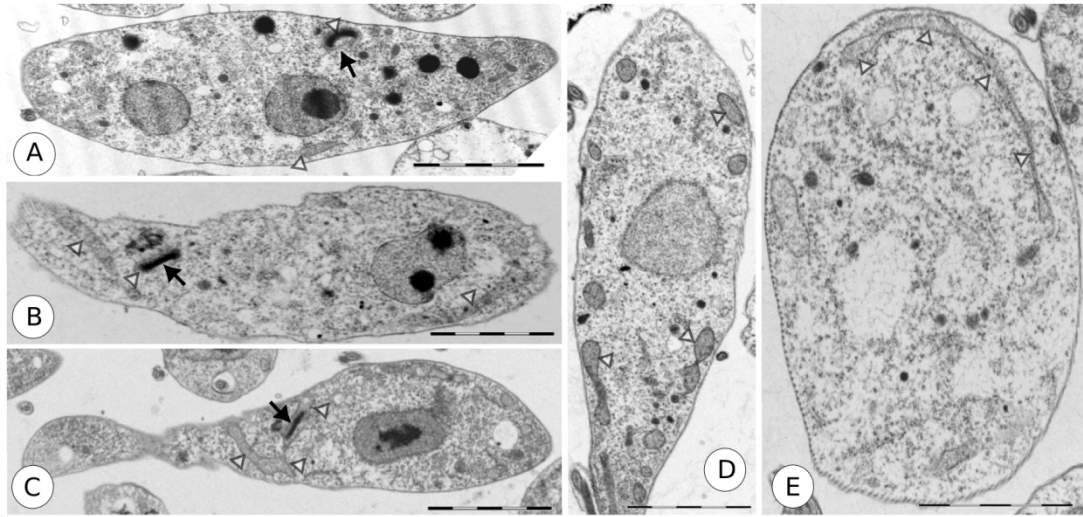


Figure S4