Supplemental materials

Table-S1: List of Primers used in this study

Name	Sequence[1]
Tim17-TAP Forward	5′–GATCAAGCTTATGACAACACTTCTCGAC-3′
Tim17-TAP Reverse	5′–GATCCTCGAGGCGTTGAGCCAACCC-3′
1310-RNAi Forward	5'-GATCGGATCCTGGAGGCTGAAGTGGAGTATGC-3'
1310-RNAi Reverse	5'-GATCAAGCTTGGCGAAAACCAGGGATAAACG-3',
2470-RNAi Forward	5'-GATCGGATCCTGTAATGCGTAATGCCTGCTTG-3'
2470-RNAi Reverse	5'-GATCAAGCTTTGTGGAAGAGGGTAATGCCGTC-3'
3620-RNAi Forward	5'-GATCGGATCCTCCTCCAAAGTGCTCTACGACG-3'
3620-RNAi Reverse	5'-GATCAAGCTTCCTGTGCCAACTGTTTTCCCTC-3'
1740-RNAi Forward	5'-GATCGGATCCTCTCAGGAGTGCGGAAAGGAAG-3'
1740-RNAi Reverse	5'-GATCAAGCTTCAACACATTCTGCCAACGGTG-3'
COIV Forward	5'-AGAAGCTTATGTTTGCTCGCCGCT-3'
COIV Reverse	5'-AAGAATTCCTAAATCTTGTTTGA-3'
AAC Forward	5'-AGAAGCTTGCTATGACGGATAAAAAGCGGG-3'
AAC Reverse	5'-AAGAATTCCTAATTCGATCTGGCGCC-3'
TAO Forward	5'-GATCAAGCTTATGTTTCGTAACCACGC-3'
TAO Reverse	5'-GATCGGATCCCACGTGTTTGTTAC-3'
DHFR Forward	5'-GATCGGATCCATGGTTCGACCATTG-3'
DHFR Reverse	5'-GATCGAATTCTTATTTCTTCTCGTAGACTTC-3'
1740-TAP Forward	5'-GATCAAGCTTATGCGCCGAGGTTTC-3'
1740-TAP Reverse	5'-GATCCTCGAGGTGTGTGGGAGTCTTCG-3'
2470-TAP Forward	5'-GATCAAGCTTATGTTTCGTCTAGACGGC-3'
2470-TAP Reverse	5'-GATCCTCGAGGCACGCTGCTCGCATCCG-3'
MRP2 Forward	5'-GATCAAGCTTATGCTCCGACGTATC-3'
MRP2-2Xmyc Reverse	5'-GATCGGATCCCTACAGGTCTTCTTCAGAGATCAGTT TCTGTTCCAGGTCTTCTTCAGAGATCAGTTTCTGTTC CACAGATGTGCGAGC

[1]Restriction enzyme sites are underlined
[2]Gene ID for RNAi targets: Tb927.1.1310 for 1310-RNAi, Tb927.6.2470 for 2470-RNAi, Tb927.10.3620 for 3620-RNAi, and Tb927.8.1740 for 1740-RNAi.

Table- S2. Mass-Spectrometry results

Proteins identified by at least 2 peptides were listed. Gene ID numbers are from Gene DB database for *T. brucei*. A brief description of the proteins that has been previously characterized is included. Hypothetical proteins are trypanosome specific and no match has been found in other eukaryotes. The number of peptides and respective sequence coverage for each protein identified are included.

Gene ID	Description	# of peptide	Sequence coverage
Tb927.1.1310	Hypothetical	2	10
Tb11.01.3290	Hypothetical	4	8
Tb927.8.1740	Hypothetical	6	18
Tb11.02.5660	Hypothetical	9	27
Tb927.6.2470	Hypothetical	6	27
Tb09.160.0670	Hypothetical	2	1.1
Tb927.5.2330	Hypothetical	2	1
Tb927.3.1380	ATP synthase β	8	14
Tb927.5.1060	MPP	3	10
Tb10.70.0430	Hsp60	6	13
Tb927.6.3740	Hsp70	6	10
Tb11.01.3550	Oxoglutarate DH E2	2	3
Tb927.10.2900	Importin β	2	6
Tb11.47.0004	Oxoglutarate DH E1	2	3
Tb927.8.1420	Acyl CoA DH	7	13
Tb10.70.5820	Hexokinase	5	16
Tb09.211.3540	Glycerol kinase	5	9
Tb927.8.1900	Tryparedoxin peroxidase	2	12

Supplemental Figure legends

Fig. S1. Standard protein-run on Superose 12 column. Standard molecular marker protein mixture (GE Health Care 17-0441-01) (200 µl) were loaded on Superose 12 column, run at 0.2 ml/min flow rate. Fractions (250 µl each) were collected on FRAC200, analyzed on a SDS-PAGE (4-12%) and stained with Sypro ruby. Fractions 46-66 were shown. 1 µg of each standard protein was also loaded on the gel as indicated. Peak fractions were indicated by arrows. The peak fractions for thyroglobuline (669 kDa), ferritin (440 kDa), catalase (232 kDa), and aldolase were 50, 54, 56, and 57 fractions, respectively. BM: Bench Mark Ladder from Invitrogen.

Fig. S2. Effect of TbTim17 RNAi and VDAC RNAi on the level of mitochondrial proteins. *A and B,* TbTim17 RNAi and VDAC RNAi cell lines were grown in absence (- Tim17 RNAi and – VDAC RNAi, respectively) or in the presence (+ Tim17RNAi and +VDAC RNAi, respectively) of doxycycline for 48 h. Total mitochondrial proteins were analyzed by SDS-PAGE and immunoblot using Tim17, VDAC and TAO antibodies as probes. Amount of proteins loaded in each lane are indicated.

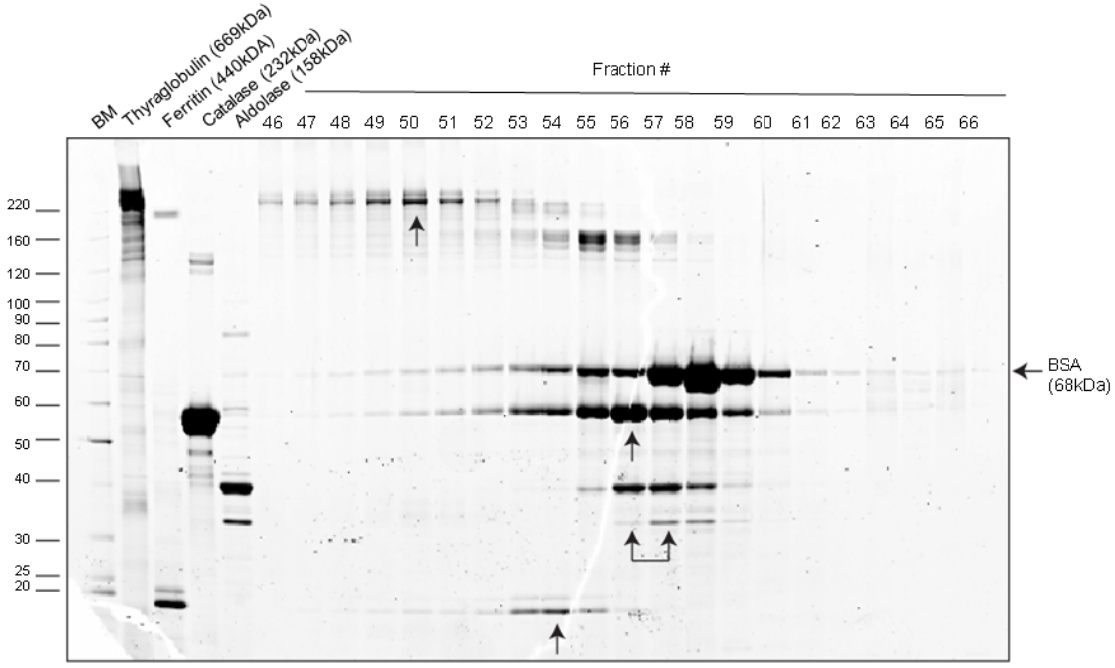
Fig. S3. Effect of RNAi on cell growth. The inducible *T. brucei* RNAi cell lines for Tb927.1.1310 (Tb1310 RNAi), Tb927.6.2470 (Tb2470 RNAi), Tb927.10.3620 (Tb3620 RNAi), Tb927.8.1740 (Tb1740 RNAi), and TbTim17 RNAi were developed as described. Cells were allowed to grow in the absence (Uninduced) or presence (induced) of doxycycline (1 µg/ml). Cells were counted at different time points as indicated. The log of the cumulative cell number was plotted versus time in days. Each growth curve was repeated three times and similar results were observed. Total cellular RNA was isolated from the uninduced (-) and induced (+) cells followed by northern blot analysis, using specific probes indicated at the side of the blots. Ethidium bromide-stained ribosomal RNAs (rRNA) were used as loading controls.

Fig. S4. Effect of RNAi on mitochondrial membrane potential. The *T. brucei* RNAi cell lines for *A,* Tb927.8.1740 (1740 RNAi), *B*, Tb927.1.1310 (1310 RNAi), *C,* Tb927.6.2470 (2470 RNAi), and *D,* the parental *T. brucei* 427 (Wt Con) cells were grown for 3 days in the presence (+ Dox, grey and green lines) and absence (- Dox, orange and blue lines) of doxycycline were stained with MitoTracker Red and FACS analysis was performed as described in the materials and methods. Wt Con cells were also pre-treated (dark and light magenta lines) with CCCP to disrupt mitochondrial membrane potential. The y-axis represents the total cell count and the X-axis represents the relative fluorescence intensity. The red line is representing the unstained cells.

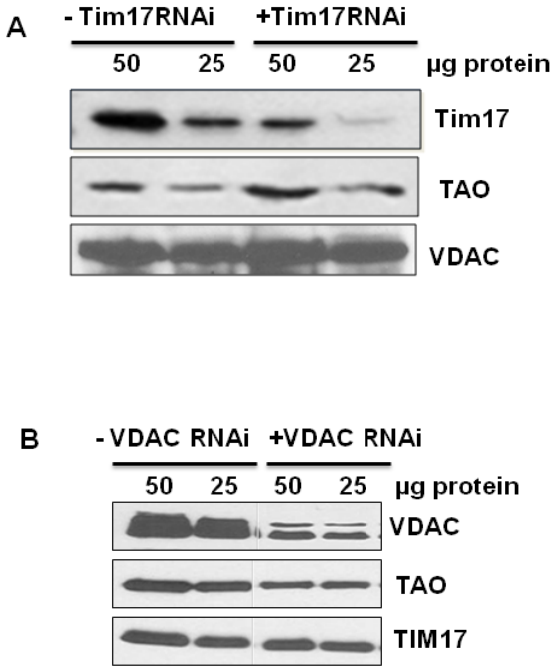
Fig. S5. Effect of RNAi on steady state levels of cellular proteins. *T. brucei* 1310 RNAi, 2470 RNAi, 1740 RNAi, and Tim17 RNAi cells were grown in the absence (-) or presence (+) of doxycycline for 48 h. Total cellular proteins were harvested and analyzed by immunoblot using anti-β tubulin, anti-VDAC, and anti-TbTim17 as probes. Equal amount of cells (5×10^6) were loaded per lane. Gene IDs for RNAi targets: Tb927.1.1310 for 1310-RNAi, Tb927.6.2470 for 2470-RNAi, and Tb927.8.1740 for 1740-RNAi.

Fig. S6. The steady-state level of mitochondrial proteins at 96 h after induction of RNAi. *T. brucei* 427 (parental), 1310 RNAi, 2470 RNAi, 1740 RNAi, and 3620 RNAi

cells were grown in the presence of doxycycline for 96 h and mitochondria were isolated. Mitochondrial proteins (50 and 25 μg) were analyzed by immunoblot using anti-TbTim17, anti VDAC, and anti-Hsp70 as probes. Gene IDs for RNAi targets: Tb927.1.1310 for 1310-RNAi, Tb927.6.2470 for 2470-RNAi, and Tb927.8.1740 for 1740-RNAi.



Supplemental Fig. S1



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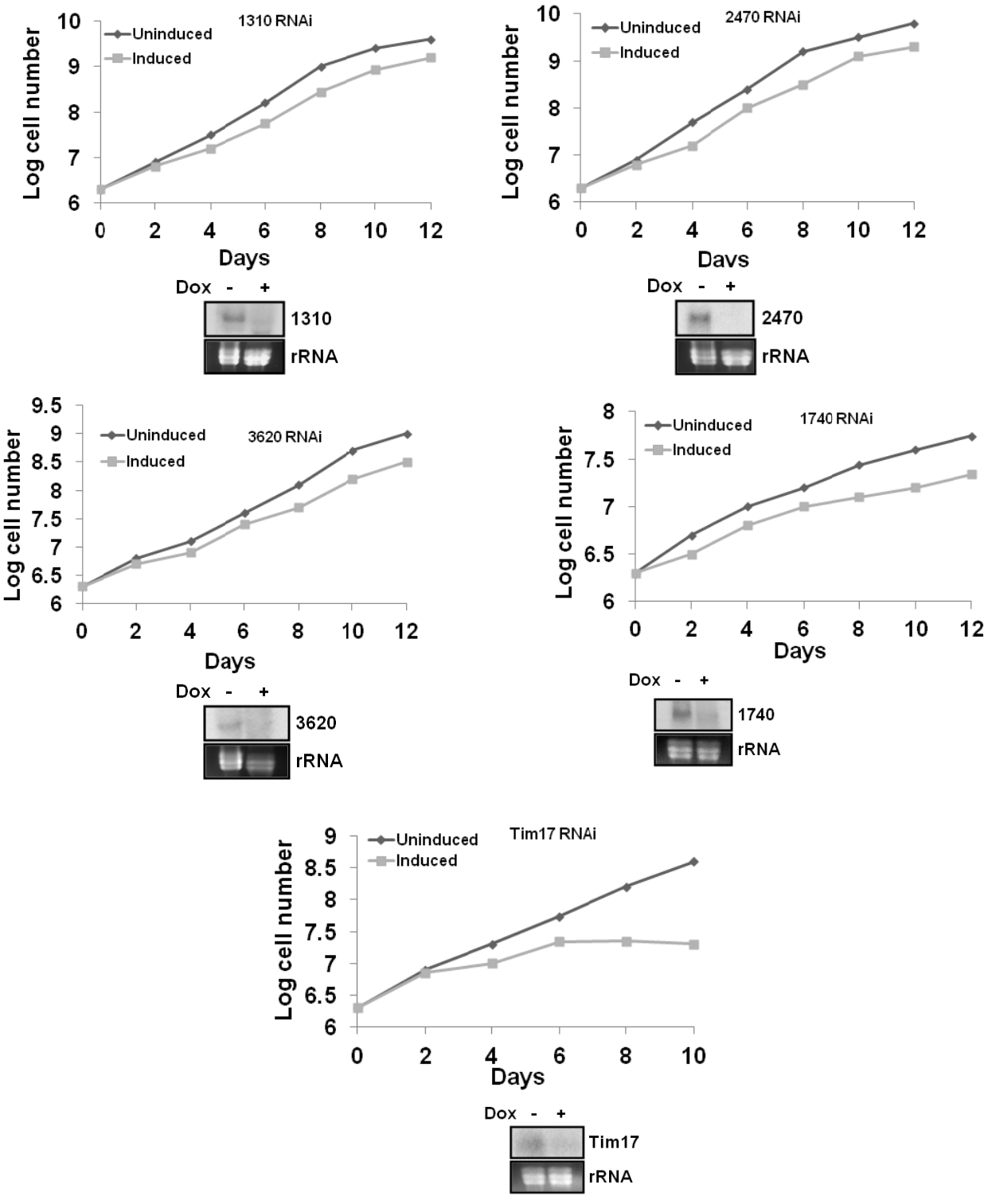

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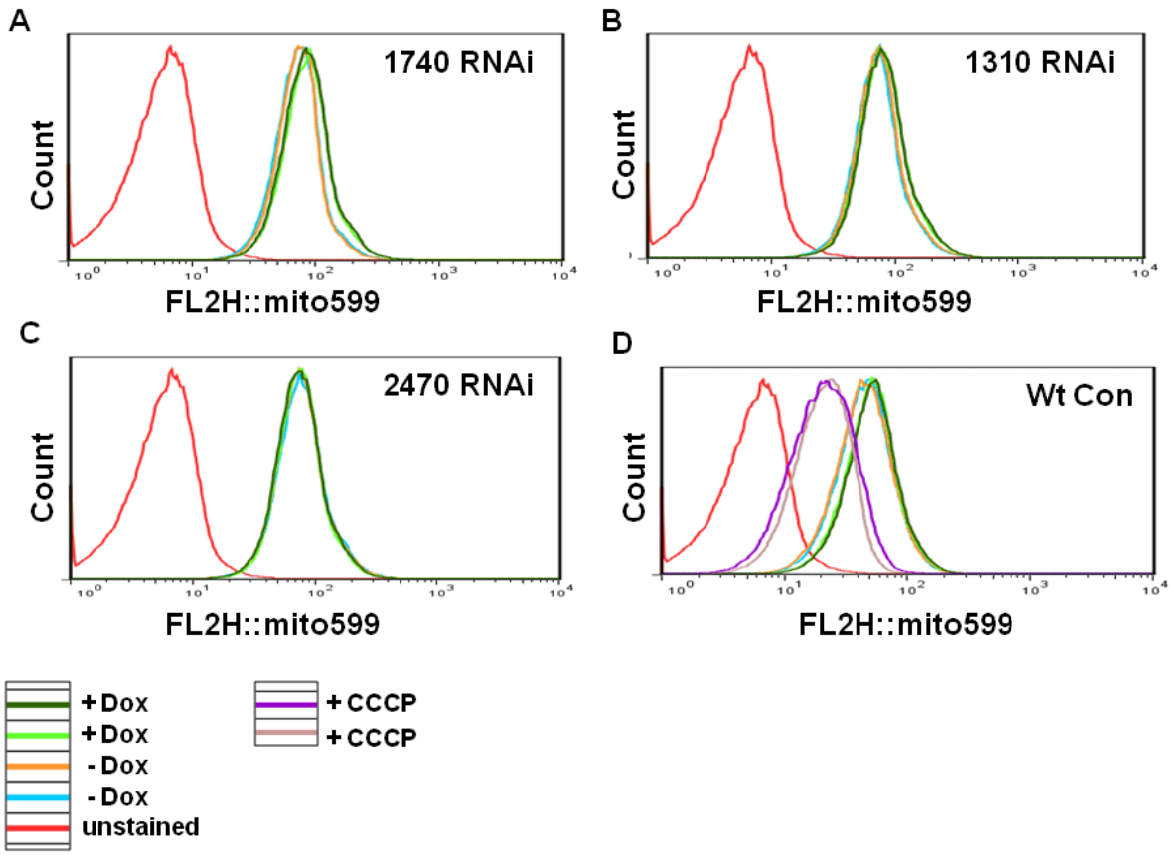

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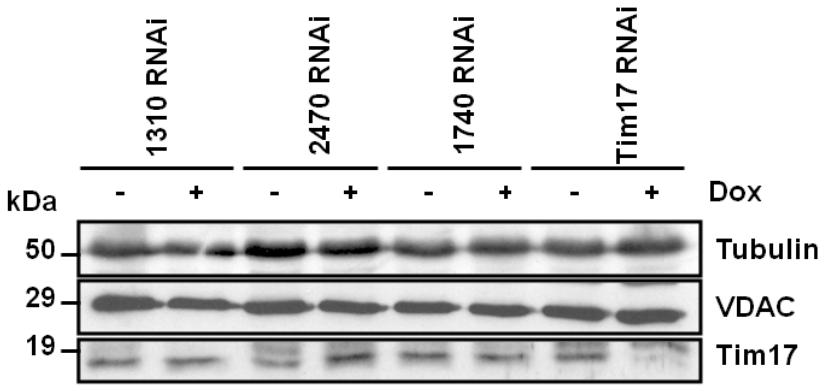
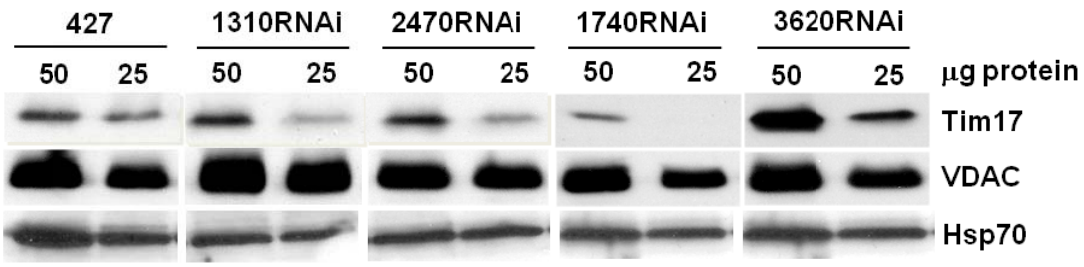


Supplemental Fig. S5.



Supplemental Fig. S6.