

Supplementary data for:

RAB11 FUNCTION IN *TRYPANOSOMA BRUCEI*; IDENTIFICATION OF CONSERVED AND NOVEL INTERACTION PARTNERS

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Running title: Conservation and novelty of Rab11 functions

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Supplementary figure legends

Table S1: Accession numbers for members of the family of Rab11-interacting proteins (FIPs) as retrieved by BLAST analysis and HMM searches of selected genomes. See materials and methods for details of sources of data.

Table S2: Accession numbers for TbAZI1 homologues retrieved from selected genomes. See materials and methods for details of sources of data.

Figure S1: Costaining of trypanosomes for Sec15 and Rab5A. Bloodstream form parasites expressing YFP tagged TbSec15 were fixed and prepared for immunofluorescence analysis using anti-GFP antibody. Location of YFP-TbSec15 compared to location of early endosomes by immunostaining of Rab5A. Left panels, YFP-TbSec15 (green, white arrows), center panels, rabbit polyclonal anti-Rab5A (red). DNA was visualized with DAPI (blue, right panels, merge). Scale bar 2µm.

Figure S2: BD-Rab11QL is expressed in *S. cerevisiae*. AH109 yeast cells transformed with pGBKT7-Rab11QL were grown to mid-log phase. Yeast total cell lysates were fractionated by SDS-PAGE and analysed by Western blotting for expression of Rab11Q66L fused to the binding domain in the yeast two hybrid system, Rab11 antibody was used at a dilution of 1:2000.

Figure S3: Identification of two novel Rab11-interacting proteins by yeast two hybrid screen of a *T. brucei* genomic library. Panel A and B: Multiple sequence alignments of RBP74 and TbAZI1 against other kinetoplastid sequences and against the human AZI1 sequence were generated by ClustalW and drawn with ESPRIPT (90). Amino acidic sequences were obtained from geneDB and ncbi. Accession numbers are Tb927.5.1640 and Tc00.1047053506315.80 for RBP74 in *T. brucei* and the *T. cruzi* orthologue; Tb09.211.4830, Tc00.1047053510761.4 and LmF35.1650 for TbAZI1 in *T. brucei* and the orthologues in *T. cruzi* and *L. major*. Strictly conserved residues are in black, boxed residues designate 75% in conservation levels. Underlined are the library fragments of RBP74 and TbAZI1 as returned from the yeast two-hybrid screen.

Figure S4: RBP74 RNAi. Panel A: Growth curves of uninduced and induced RBP74 RNAi in triplicate cultures. Panel B: Quantitative real time PCR assay for RBP74 mRNA levels in uninduced and induced RBP74 RNAi cultures. Tubulin was used as a standard. Panel C: ConA and transferrin uptake upon RBP74 knockdown as measured by FACS. Closed bars indicate uninduced cells, open bars indicate induced cells. Panel D: Transferrin recycling in the RBP74 RNAi cell line.

Figure S5: Rab11 is not affected upon TbAZI1 knockdown. Rab11 localization and levels in induced RBP74 RNAi cells (upper panels) and TbAZI1 RNAi cells (lower panels) as measured by immunofluorescence (left panels) and Western blotting (right panels). Scale bars are 2µm.

Figure S6: ConA and transferrin uptake and recycling are not affected by RNAi of TbAZI1. Endocytosis was analysed by measuring ConA (mainly VSG) and transferrin (ESAG6/7-mediated) uptake

by FACS in uninduced (closed bars) and induced (open bars) TbAZI1 RNAi cells (upper histogram). Transferrin recycling was measured by FACS in TbAZI1 uninduced (solid line) and induced cells (dotted line) (lower graph).

Figure S7: TbAZI1 knockdown does not produce any defects in the staining of the flagellum attachment zone nor in the structure of the paraflagellar rod as visualized by immunofluorescence analysis. Uninduced and induced TbAZI1 RNAi cells were stained for the component A of the paraflagellar rod (PFRA) with L8C4 antibody (upper panels) or the flagellum attachment zone antibody L3B2 (bottom panels). Left, merge of PFR-A or FAZ (red) and DAPI fluorescence (blue); right, phase contrast. Scale bars are 2 μ m.

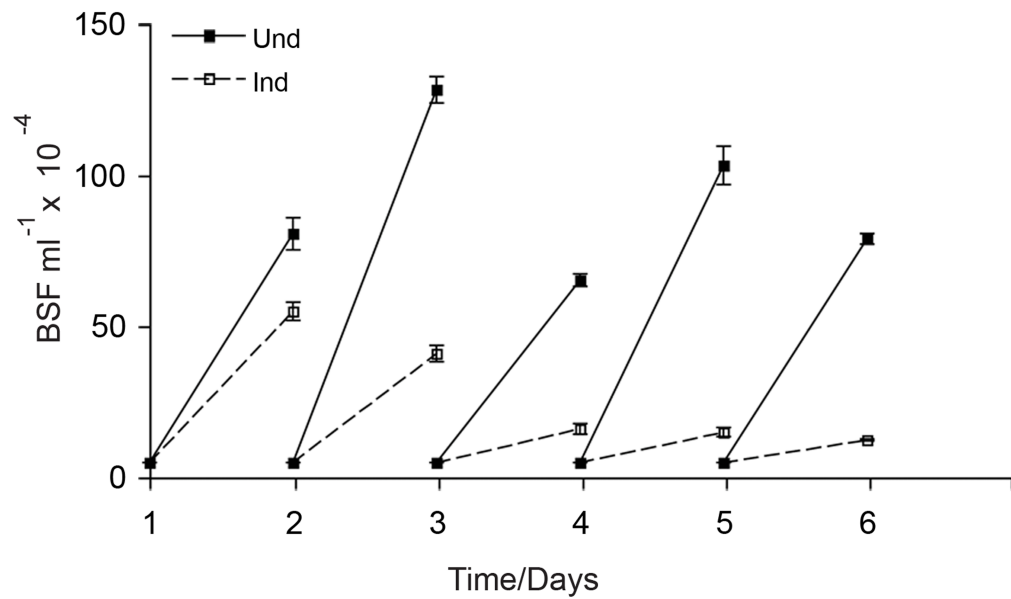
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32kDa —



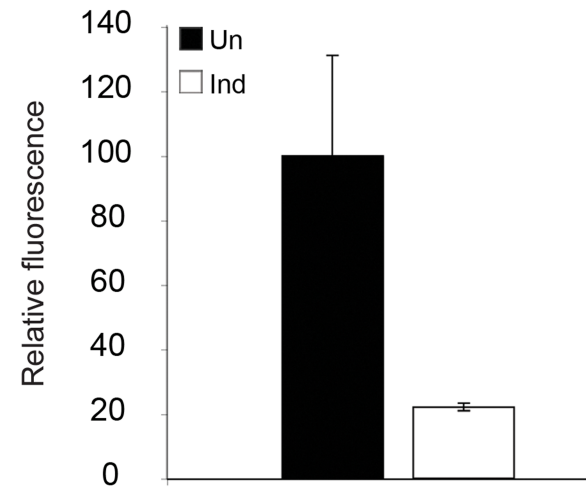
BD-Rab11QL

Figure S3

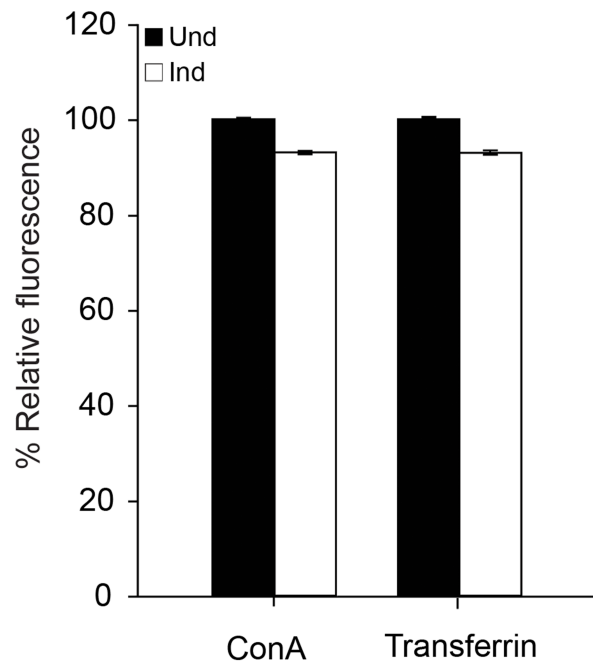
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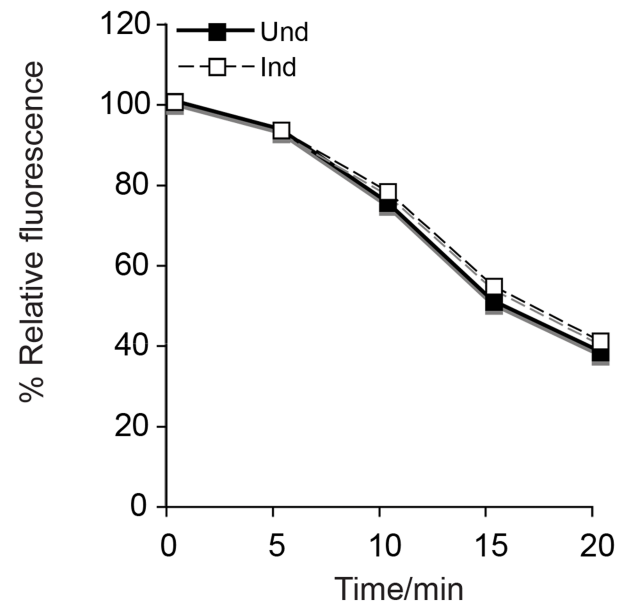
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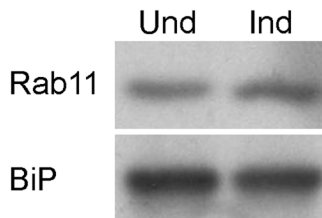
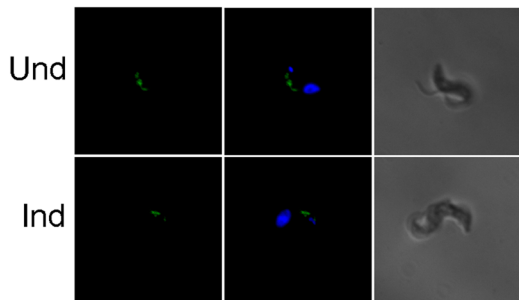


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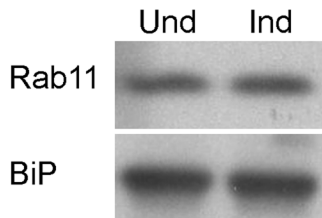
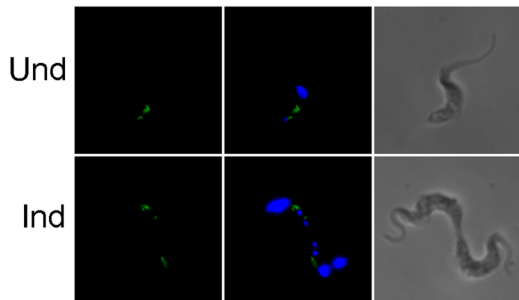
RBP74 knock down

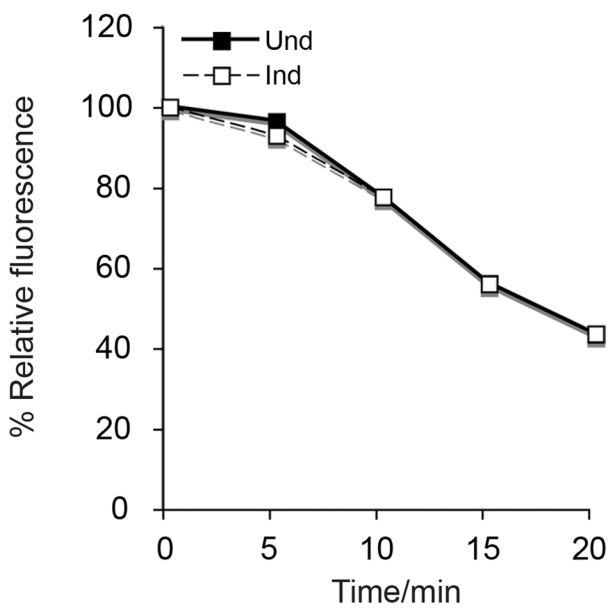
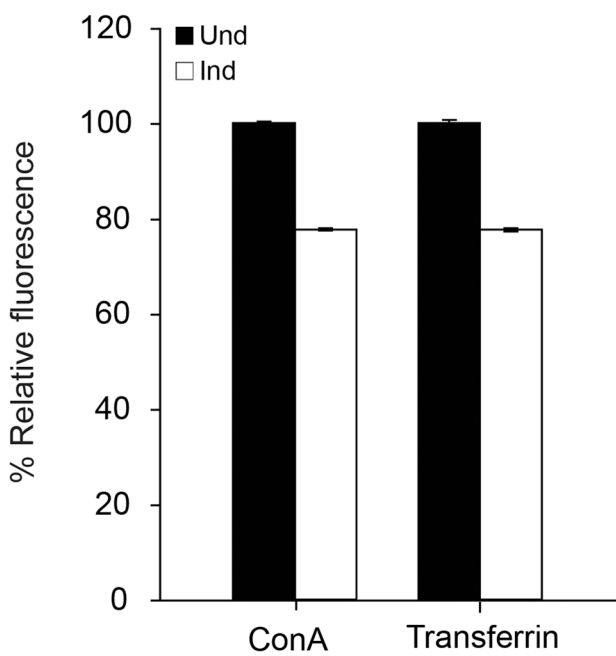
Rab11



TbAZI1 knock down

Rab11



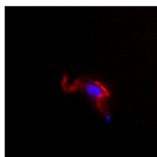


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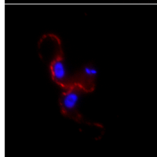
PFR

Phase

Und



Ind

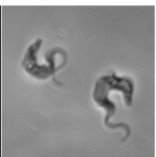
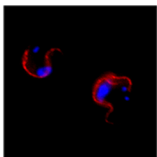


TbAZI1 knock down

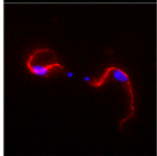
FAZ

Phase

Und



Ind



on numbers	
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Table S2: Organisms and accession numbers for AZ11 homologues

Lineage	Taxon	Accession number
Metazoa	<i>Homo sapiens</i>	NP_055799.2
	<i>Drosophila melanogaster</i>	CG1625-PA
	<i>Caenorhabditis elegans</i>	-
Fungi	<i>Nematostella vectensis</i>	jgi Nemve1 245298 estExt_fggenes1_pg.C_1540001
	<i>Batrachochytrium dendrobatidis</i>	BDEG_03563.1
	<i>Saccharomyces cerevisiae</i>	-
Amoebozoa	<i>Cryptococcus neoformans</i>	-
	<i>Dictyostelium discoideum</i>	-
Plantae	<i>Entamoeba histolytica</i>	-
	<i>Arabidopsis thaliana</i>	-
	<i>Chlamydomonas reinhardtii</i>	jgi Chlre3 148926 Chlre2_kg.scaffold_23000076
Chromalveolata	<i>Ostreococcus tauri</i>	-
	<i>Cyanidioschyzon merolae</i>	-
	<i>Thalassiosira pseudonana</i>	-
	<i>Phytophthora ramorum</i>	>jgi Phyra1_1 80449 fgenes1_pg.C_scaffold_49000055
	<i>Paramecium tetraurelia</i>	>GSPATP00027365001
	<i>Plasmodium falciparum</i>	-
	<i>Toxoplasma gondii</i>	20.m03921
Kinetoplastida	<i>Eimeria tenella</i>	GLIMMERHMM190_V3.0.1_PHASES00000215796
	<i>Cryptosporidium parvum</i>	-
	<i>Theileria parva</i>	-
	<i>Tetrahymena thermophila</i>	3720.m00056
	<i>Trypanosoma brucei</i>	Tb09.211.4830
	<i>Trypanosoma cruzi</i>	Tc00.1047053510761.4
	<i>Leishmania major</i>	LmjF35.1650
	<i>Naegleria gruberi</i>	-
	<i>Trichomonas vaginalis</i>	-