Supplemental Materials
Molecular Biology of the Cell

Figure S1

Krüger *et al.*

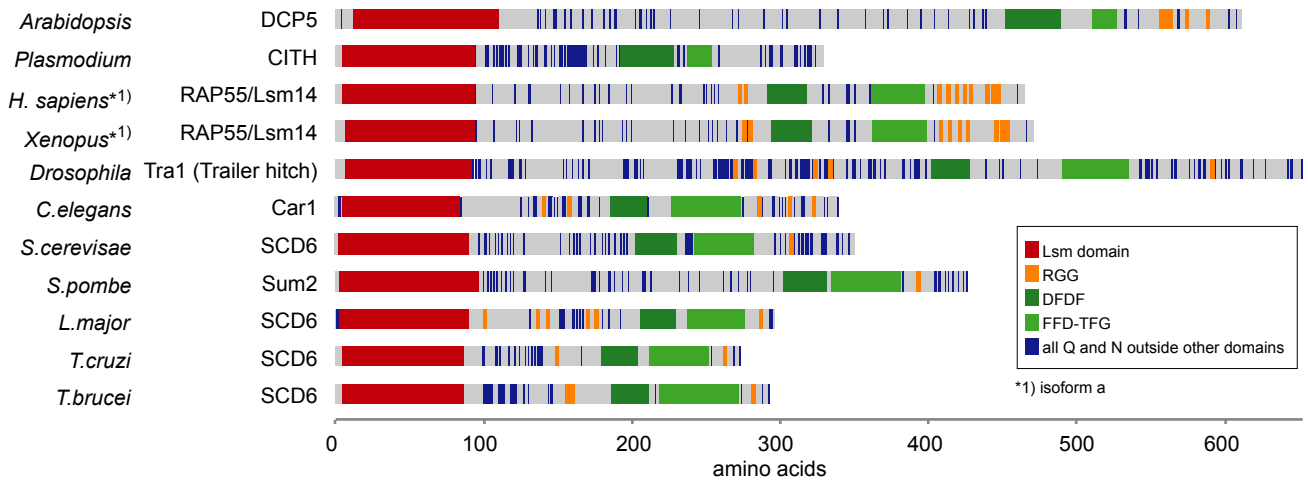


Figure S1: Schematics of orthologues to SCD6/RAP55 from various organisms
Domains, RGG motifs and Q or N residues outside of other domains are marked.

Figure S2

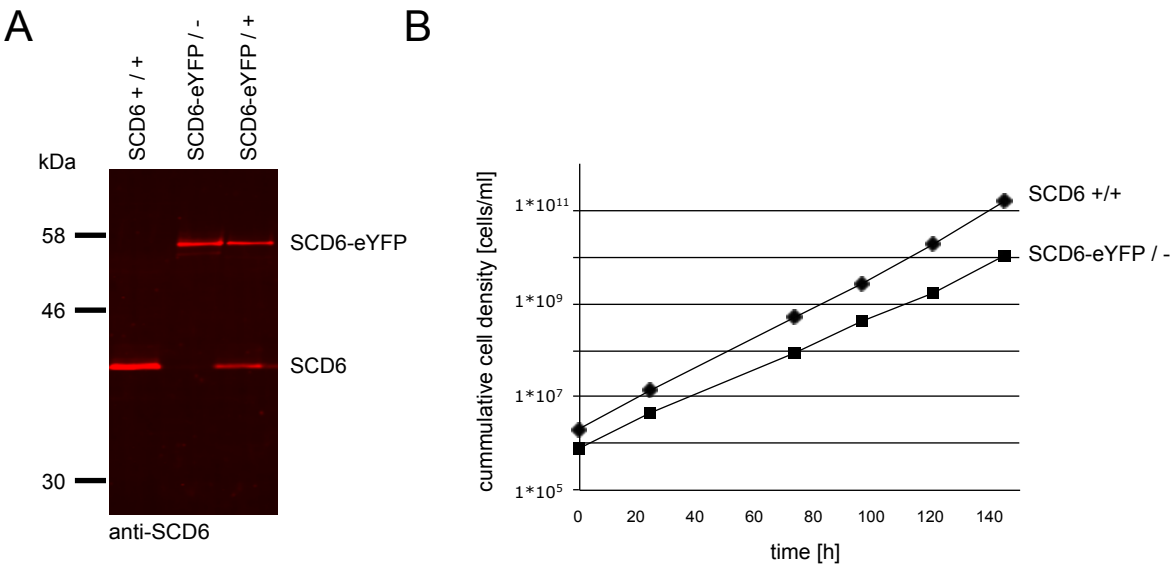
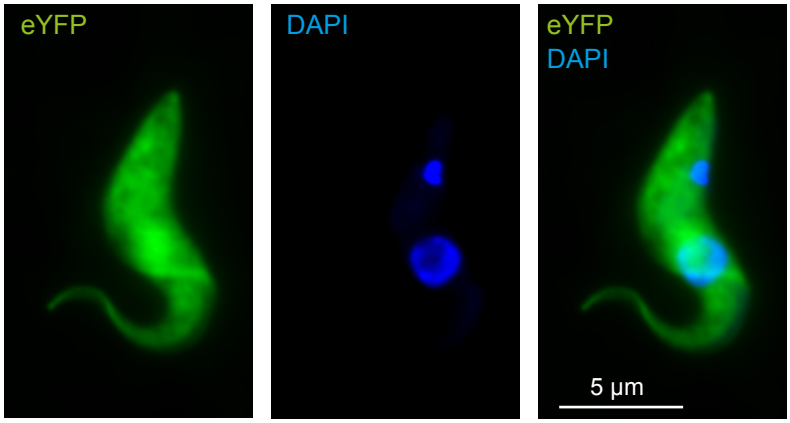


Figure S2: SCD6-eYFP is functional
A cell line was made that had one SCD6 allele replaced by a blasticidin resistence gene using 5' and 3' UTR sequences for homologous recombination. The second allele was endogenously tagged with a C-terminal eYFP tag, as described in Kelly et al., 2007. (A) Western blot with cell lysates of the cell lines SCD6 + / +, SCD6-eYFP/- and SCD6-eYFP / -. (B) Growth of the SCD6-eYFP/- cells in comparison to wild type cells (SCD6 +/+)

Kelly, S., Reed, J., Kramer, S., Ellis, L., Webb, H., Sunter, J., Salje, J., Marinsek, N., Gull, K., Wickstead, B. et al. (2007). Functional genomics in *Trypanosoma brucei*: a collection of vectors for the expression of tagged proteins from endogenous and ectopic gene loci. Mol Biochem Parasitol 154, 103-109.

Figure S3

A



B

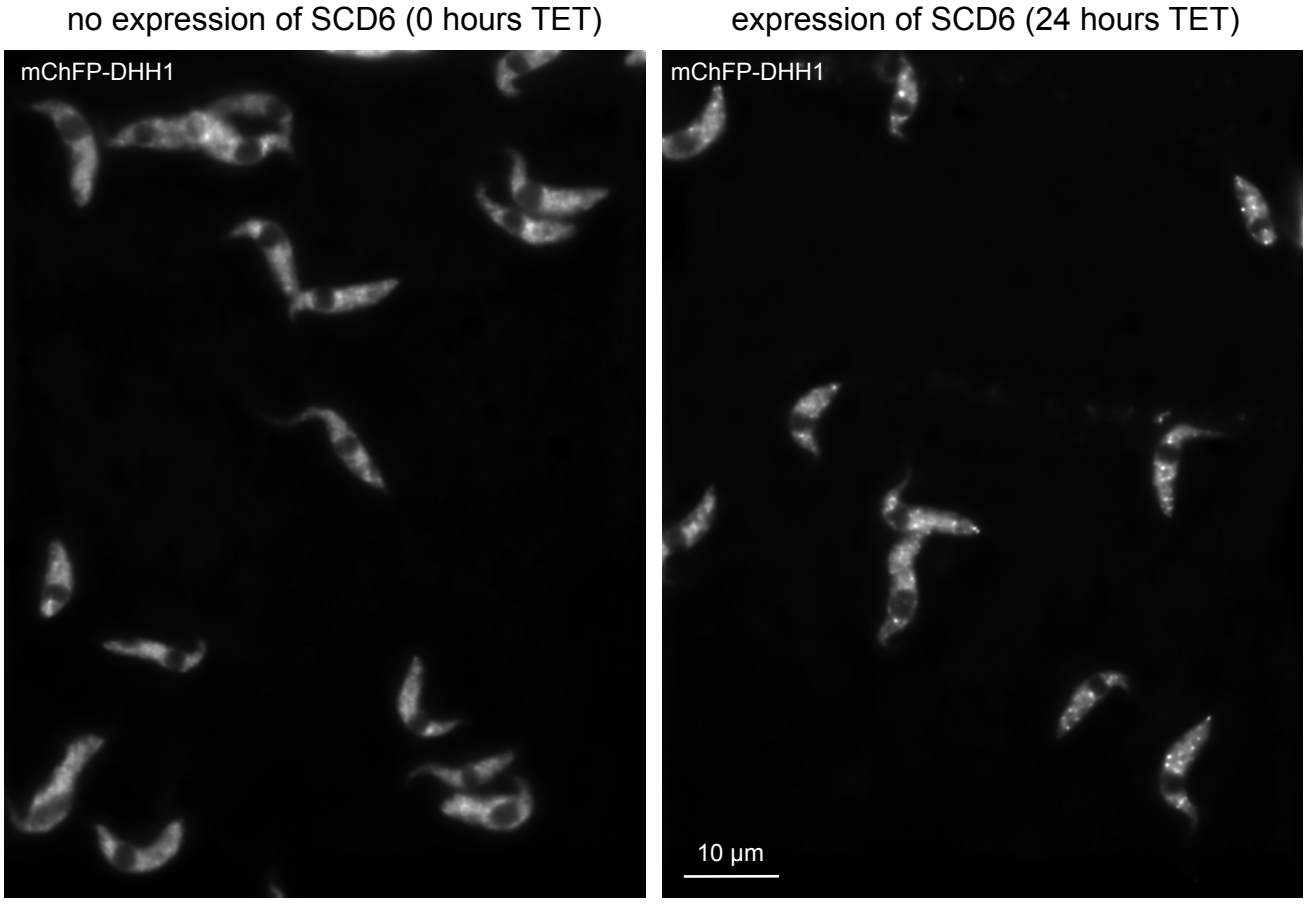


Figure S3:
A) Inducible expression of eYFP in procyclic trypanosomes does not result in the formation of granules. The total fluorescence intensity of this particular cell was 349207 and therefore well above the treshhold expression level required for granule formation at inducible SCD6-eYFP overexpression.
B) Inducible expression of SCD6 (without eYFP tag) causes recruitment of mChFP-DHH1 to granules in a similar way as does the expression of SCD6-eYFP.

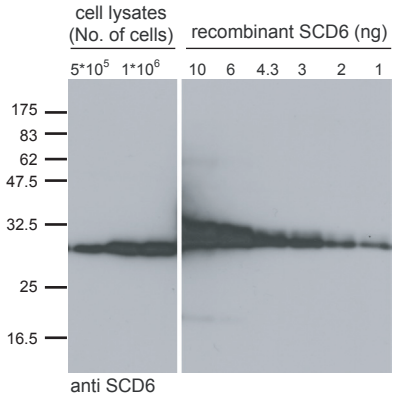


Figure S4:
A titration of recombinant SCD6 protein purified from *E. coli* was used to estimate the amount of SCD6 molecules in a procyclic trypanosome cell. There are about 3 ng in $1x10^6$ cells, equivalent to ~60,000 SCD6 molecules per cell.

Figure S5

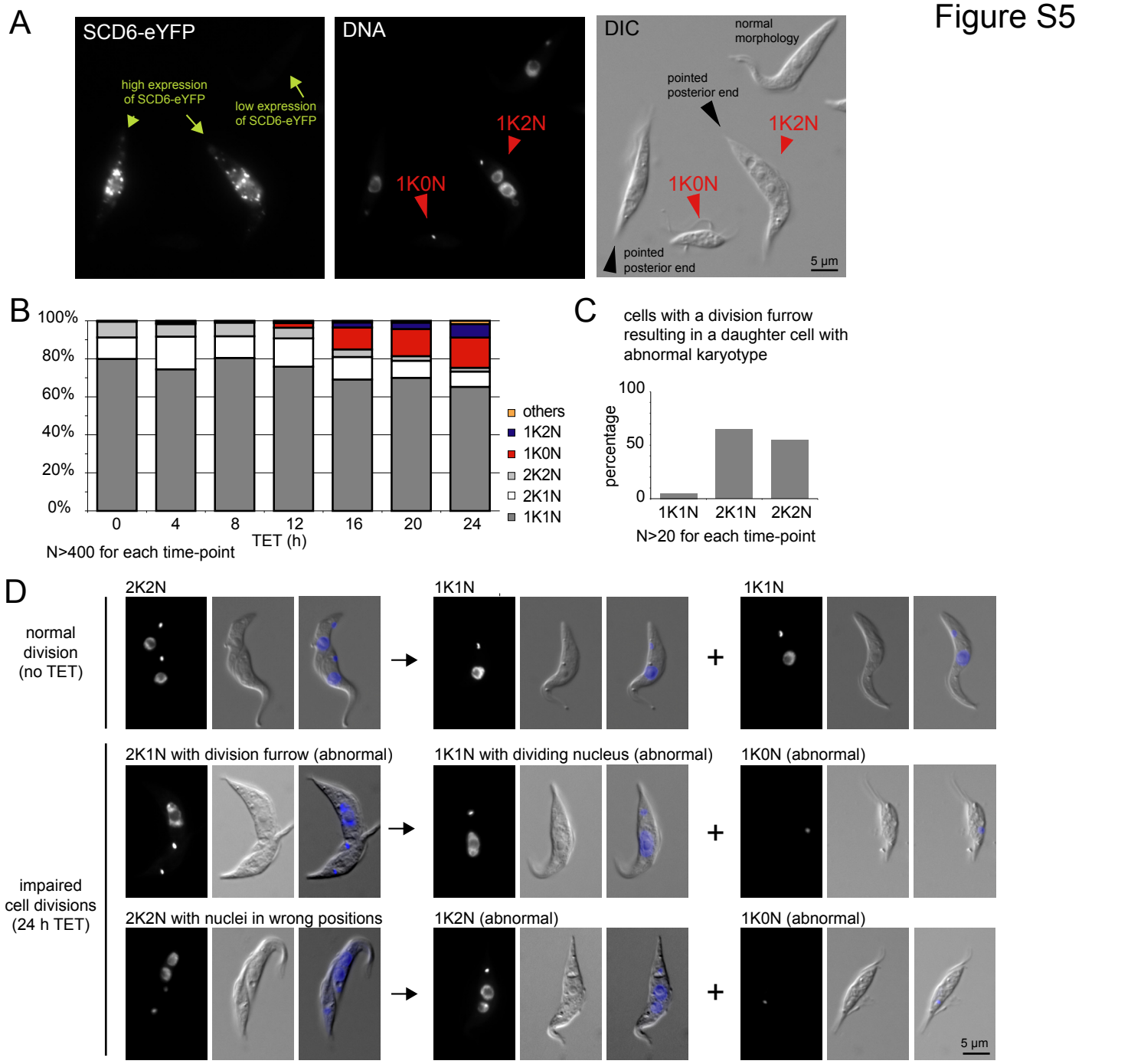


Figure S5: Over-expression of SCD6-eYFP results in a block in mitosis

Over-expression of SCD6-eYFP causes a change in cell morphology and an increase in cells with abnormal karyotypes. Cells become pointed at both ends (A) and an increasing number of cells has abnormal karyotypes (A and B). After mitosis, procyclic trypanosomes have one nucleus and one kinetoplast (1K1N), then first divide the kinetoplast (2K1N) and subsequently the nucleus (2K2N) that will move in between the two kinetoplasts to allow longitudal cell division. On over-expression of SCD6-eYFP, abnormal 1K0N and 1K2N cells increased (A and B). In addition, the majority of cells with normal karyotypes were abnormal in size and shape of the nuclei and position/presence of the division furrow: 1K1N cells often had a large and bilobed nucleus and sometimes a division furrow; the majority of 2K1N cells had large nuclei and a division furrow in a position to produce a 1K0N cell as well as a 1K1N cells with a bilobed nucleus; many of the 2K2N were about to divide into a 1K2N and 1K0N cell and often nuclei did not become separated and appeared different in size. In fact, more than half of all 2K1N and 2K2N cells with a division furrow present had the division furrow wrongly positioned (C). Taken together, the data can be explained with untimed cell division prior to either the division of the nucleus or the correct positioning of the two daughter nuclei: 2K1N cells divide to a 1K0N cell and a 1K1N cell with a dividing nucleus and 2K2N cells give rise to 1K0N and 1K2N cells (D).

(A) Typical fluorescent microscopy image (single plane) at 24 hours tetracyclin induction showing two cells with high SCD6-eYFP expression (including one with the abnormal 1K2N karyotype), one cell with low SCD6-eYFP expression and one 1K0N cell. Black arrows in the DIC image point to the spindle-shaped cell poles that are absent in the cell with low expression level. (B) Karyotype analysis over a time-course of induction of SCD6-eYFP over-expression. Normal karyotypes are shown in three different shades of grey, abnormal karyotypes in colour. (C) Percentage of cells with normal karyotypes that had a division furrow resulting in daughter cells with abnormal karyotypes. The analysis was done after 24 hours induction with tetracyclin. (D) Two models of an asymmetric cell division caused by SCD6-eYFP overexpression that are in agreement with the data. A normal cell division is shown on top. All images were taken using a Zeiss Axioskop microscope equipped with a Plan-Apochromat 100x/1.4 Oil DIC objective and a monochrome CCD camera AxioCam MR.

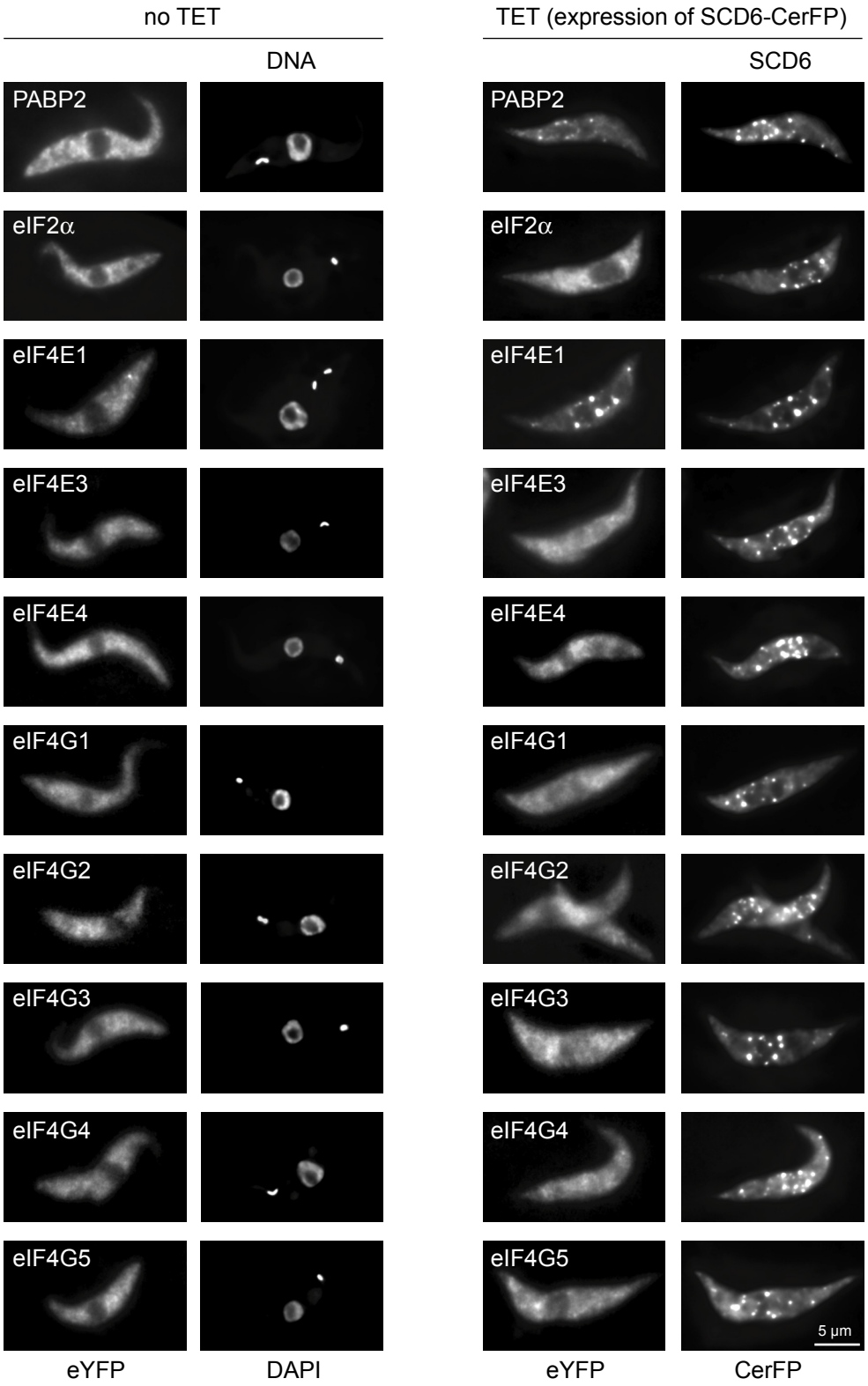


Figure S6: Localization of other proteins to SCD6 induced granules
Trypanosome homologues to translation initiation factors were expressed as eYFP fusion proteins from their endogenous loci in a cell line engineered to over-express SCD6-CerFP at induction with tetracyclin (TET). Fluorescent microscopy images (Z-stack projection of deconvolved Z-stacks, sum slices) are shown for cells not expressing SCD6-CerFP (no TET) and after 24 hours of induction.

Figure S7

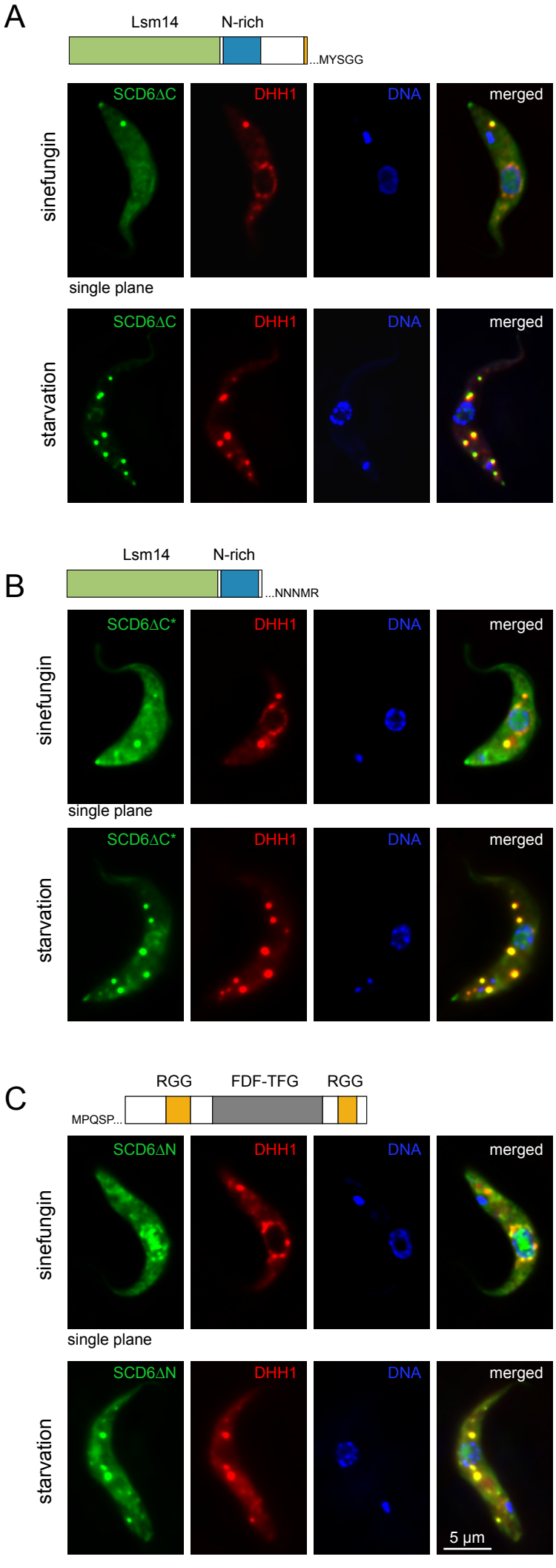


Figure S7:
Cells expressing a C-terminally or N-terminally truncated SCD6 mutant as indicated for 24 hours were treated with sinefungin (60 min) or starvation (120 min PBS). Projections of deconvolved Z-stacks of one representative cell, or single plane images are shown.

Figure S8

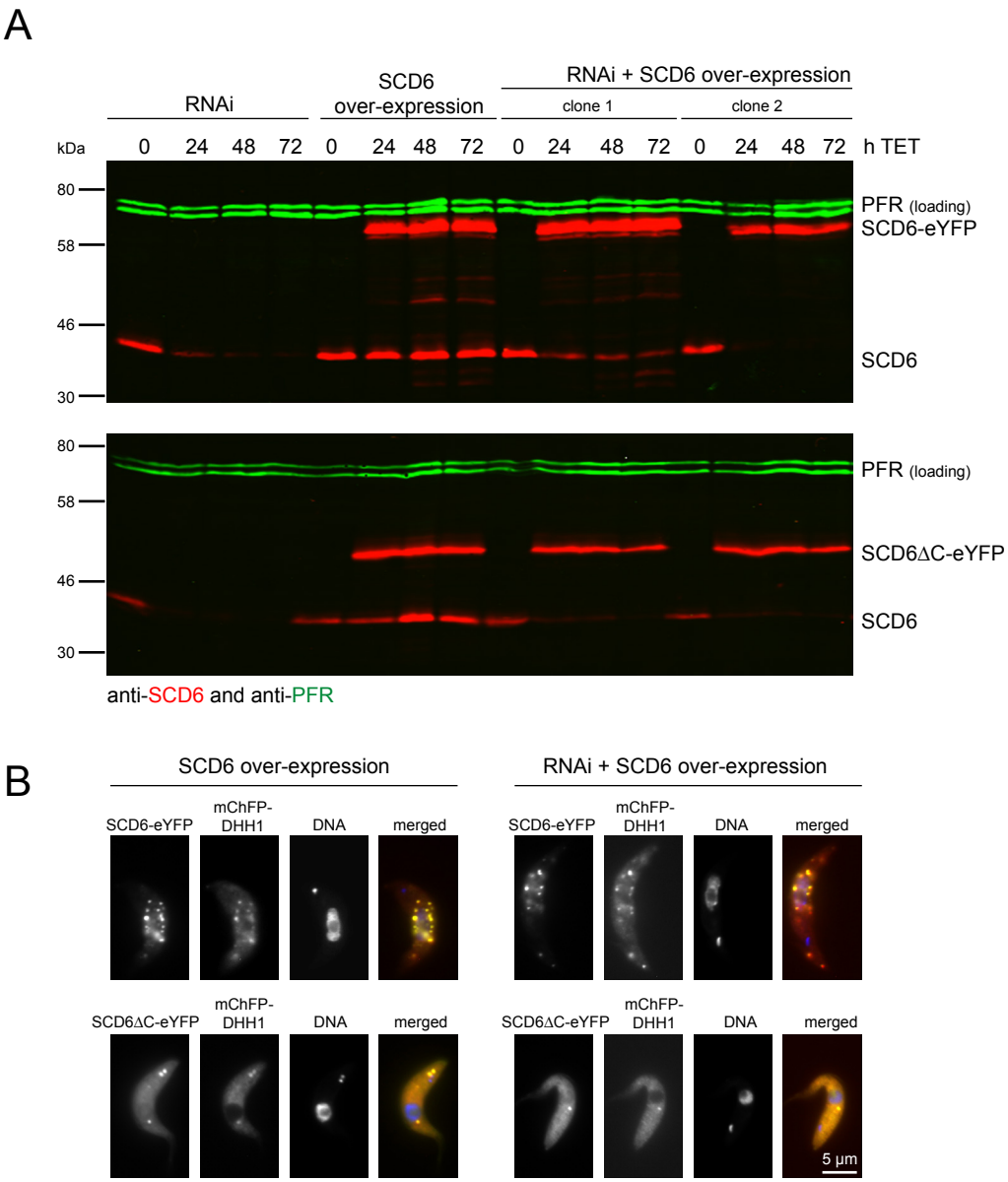


Figure S8: Simultanous RNAi knock-down of SCD6 and over-expression of SCD6-eYFP or SCD6ΔC-eYFP
Procyclic trypanosome cell lines were engineered for tetracyclin induced RNAi knock-down of SCD6, tetracyclin induced over-expression of either SCD6-eYFP or SCD6ΔC-eYFP as well as both RNAi and SCD6 over-expression together. Part of the 3' UTR sequence of SCD6 was used as RNAi target; the over-expression vectors contain the modified aldolase 3' UTR (Sunter et. al., 2012), and expression is therefore not affected by RNAi. (A) Western blot with cell lysates taken over a time-course of RNAi, over-expression or RNAi + over-expression probed with a polyclonal serum rised against SCD6 (Kramer et al., 2008); results from two different clones are shown. PFR served as loading control. The polyclonal SCD6 antiserume preferentially recognizes the C-terminal part of SCD6, thus, the apparent lower expression level of the SCD6ΔC-eYFP protein in comparison to the SCD6-eYFP protein is not real; when expression levels are compared using anti-GFP, expression of SCD6ΔC-eYFP is in fact higher than the expression of SCD6-eYFP (data not shown here). (B) Single plane fluorescence microscopy images of cells overexpressing SCD6-eYFP or SCD6 ΔC-eYFP in the absence or presence of RNAi knock-down of the endogenous SCD6.

All images were taken using a Zeiss Axioskop microscope equipped with a Plan-Apochromat 100x/1.4 Oil DIC objective and a monochrome CCD camera AxioCam MR.

Sunter, J., Wickstead, B., Gull, K. and Carrington, M. (2012). A new generation of T7 RNA polymerase-independent inducible expression plasmids for *Trypanosoma brucei*. PLoS One 7, e35167.

Kramer, S., Queiroz, R., Ellis, L., Webb, H., Hoheisel, J. D., Clayton, C. and Carrington, M. (2008). Heat shock causes a decrease in polysomes and the appearance of stress granules in trypanosomes independently of eIF2(alpha) phosphorylation at Thr169. J Cell Sci 121, 3002-3014.

Table S1: Comprehensive list of plasmids used in this work (including supplementary figures)

Name of plasmid	ID of the trypanosome protein	Name of protein	Description	Based on mother plasmid	Reference for mother plasmid	Selection
3924	Tb11.03.0530	SCD6-eYFP	Inducible overexpression	3888	Sunter et al., 2012	BSD
3342	Tb09.211.2150	PABP2-mChFP	Endogenous expression	3086 (=2705 with Hygro)	/	Hygro
3925	Tb927.10.3990	mChFP-DHH1	Endogenous expression	2679	Kelly 2007	Puromycin
SK85	Tb11.18.0004	eIF4E1-mChFP	Endogenous expression	3086 (=2705 with Hygro)	/	Hygro
2792	Tb11.03.0530	SCD6-eYFP	Endogenous expression	2710	Kelly 2007	NEO
2913	Tb11.03.0530	ΔSCD6	Deletion of one SCD6 allele by replacing the ORF by BSD cassette	/	/	BSD
3351	Tb09.211.2150	PABP2-eYFP	Endogenous expression	3345	/	Puro
SK53	Tb927.3.2900	eYFP-eIF2a	Endogenous expression	/	/	Puro
SK54	Tb11.18.0004	eIF4E1-eYFP	Endogenous expression	2948	/	Hygro
SK56	Tb11.01.3630	eIF4E3-eYFP	Endogenous expression	2948	/	Hygro
SK57	Tb927.6.1870	eIF4E4-eYFP	Endogenous expression	2948	/	Hygro
SK60	Tb927.5.1490	eIF4G1-eYFP	Endogenous expression	2948	/	Hygro
SK62	Tb09.160.3980	eIF4G2-eYFP	Endogenous expression	2948	/	Hygro
SK58	Tb927.8.4820	eIF4G3-eYFP	Endogenous expression	2948	/	Hygro
SK64	Tb11.01.2330	eIF4G4-eYFP	Endogenous expression	2948	/	Hygro
SK66	Tb927.8.4500	eIF4G5-eYFP	Endogenous expression	2948	/	Hygro
SK69	Tb09.211.2150	SCD6-CerFP	Inducible overexpression	4256 (=3927 with CerFP)	Sunter et al., 2012	BSD
4209, 4191, 4009, SK29, SK23, SK27, SK25, 4260, 4175, 4007, 4008, 4176, 4010	Tb11.03.0530	SCD6Δ and ΔSCD6	Inducible overexpression of SCD6 truncations (see Figure 6 for details; plasmid numbers are in the same order as in Figure 6)	3888	Sunter et al., 2012	BSD
4296	Tb11.03.0530	SCD6-2NLS-eYFP	Inducible overexpression	4259 (=3888 with 2 NLS)	/	BSD
4297	Tb11.03.0530	SCD6ΔC-2NLS-eYFP	Inducible overexpression	4259 (=3888 with 2 NLS)	/	BSD
SK91	Tb11.18.0004	eIF4E1-NLS-CerFP	Inducible overexpression	SK89 (=4256 (=3927 with CerFP) with one NLS)	/	Puro
4286	Tb11.03.0530	SCD6 RNAi	RNAi, targeting the 3' UTR of SCD6	3666	Sunter et al., 2012	BSD
4287	Tb11.03.0530	SCD6-eYFP	Inducible overexpression	4275 (=Hygro in 3888)	/	Hygro
SK122		eYFP	Inducible overexpression	3383	Sunter et al., 2012	BSD
SK123	Tb11.03.0530	SCD6	Inducible overexpression	3383	Sunter et al., 2012	BSD