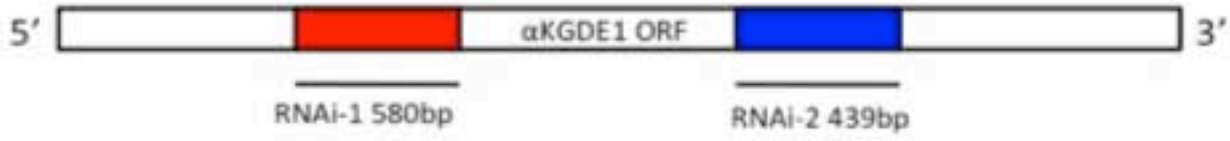
A

E1 RNAi 1 Forward Primer	ccctcgagtggcgcagagtcacttattg
E1 RNAi 1 Reverse Primer	ccaagcttaatgggacactgaaaggcac
E1 RNAi 2 Forward Primer	ctcgaggcccaccgtgtaaatatgga
E1 RNAi 2 Reverse Primer	aagcttacacgcgattcaacgtgata

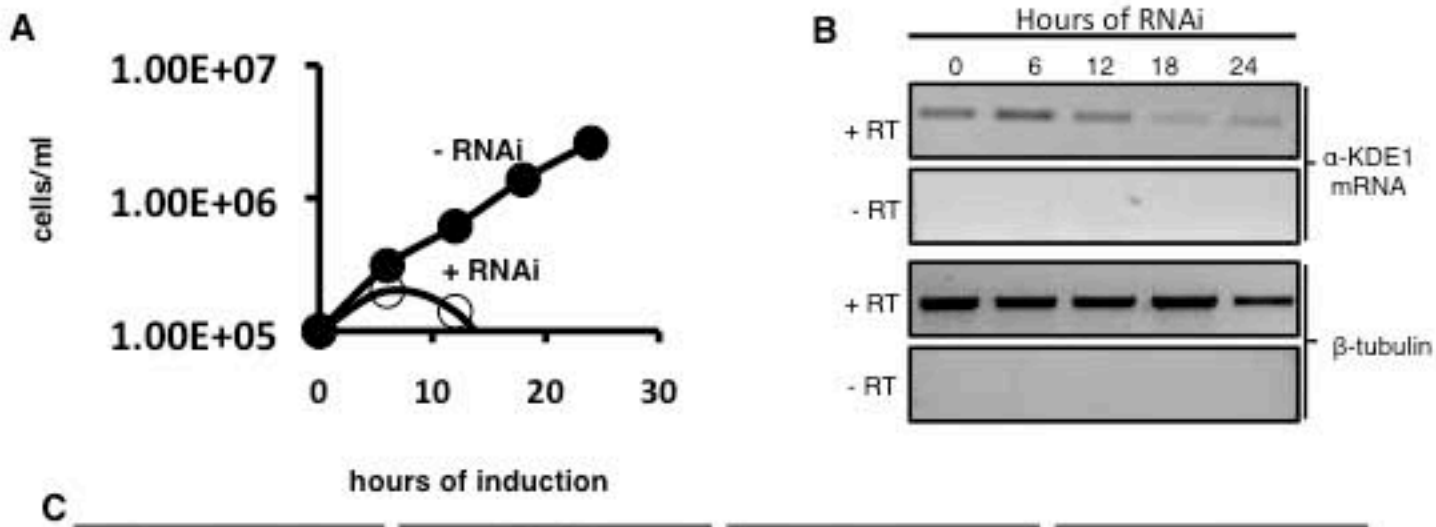
B



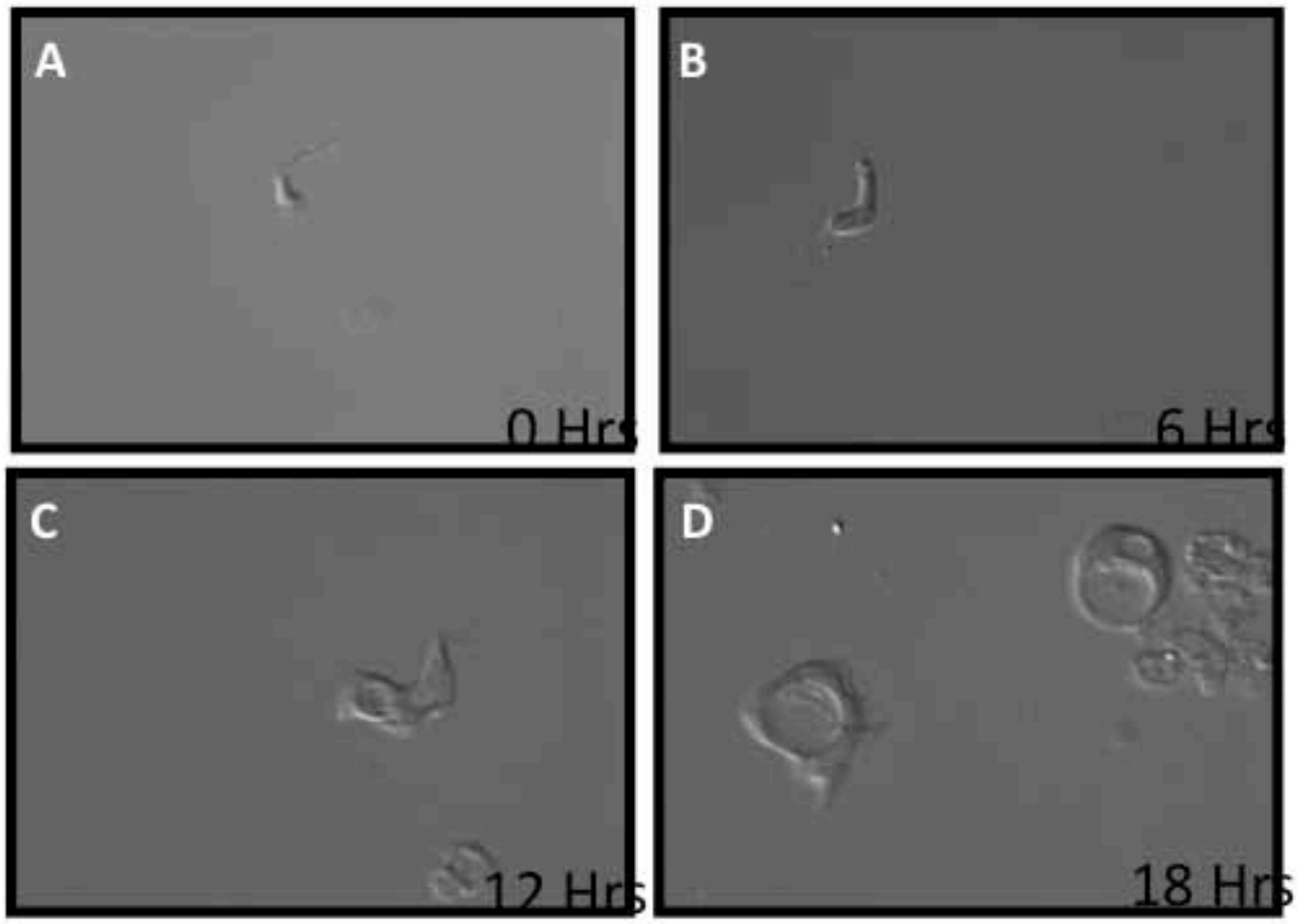
C

GAAGTTAACCTACGCAAAACTTTTGTTGACGCCGACCACGGATCTTGCGATCGCACAAACGAGCAGACACGGATTCGCACTGCACTT
GGGTATTAGCATTGATAACGGAACAACTGTCAGTGTGGGGACAGTTAGAAAAAATTAATTAAGGAAGCGAAAAAGAAAGAAGAGA
CGAGGTGACTGAATTCTGAACGATGATGCGAAGGCTCAGTCCTGTGAACGGTTCGGTGGTTTCGCCCAATGTCGTAGCAGCGGTGTC
ATCGGTGCTTCAGCGGCAGCAACGCCATCAGTCCGACGCCCCACTCAGGCGACTGTTGTATGAAAACGACAGTTTTCTCAGCGGCAC
CTCGGCTAGGTACATCGAATCGATGTACGAAAATTGGAAGGCAGATAAAAGTTCCGTGAACGAATCGTGGAATGAGGTATTCTCCTC
AAGGGACCTGCAGTCATTTGAGAGACCACTCCTAACTTCACCCATCCGTGTTGTACCAGCTTCAGCTGAGGACAAGATGGAGGTGGC
ACAATCGCAGGATATCTGCGCGCGACTGACGTTAATGATCCAAGCGTTTGAGGACTACGGGCATCTTGCAGCTCGAACTGACCCTCT
GGATGCTGAGGATGACTCTCTTCACCGTTCCCCGCAGGTTAAACCCAGAGAGAGGGTACACCTTGGTCTTGAAAGCTTTGGTTTTTCG
AAGGAAGATCATGACCGTGTCGTACGTGTGGGTTTCATGGATCAATTGGGAGGCGCGATGAGCTCGAGCAGCAGGGCCATGACTAT
CAAGGAGCTGCACGACCATTTGACGAAGTGTTTCTGTGGGCGCGTGGGATGCGAATTAGGACACGTTGATGATGCAAATGTCGTCT
GTTTCGTCCGTGAAGTTGTGGAGGGTTATAACACTGAGCACAATCCACTGCGGCGCCCGTTGTCCAAGGAGGAGAAACTGTGGGTG
TGGGACCTTGTTGCCTCCGCCGTGCACTTTGAAGATTTCTTCAAGCGTAAGTATACCACACAGAAGAGGTTCGGTTGCGATGGCGCA
GAGTCACTTATTGTTGGTTTGCGTTCACTCATGGAGTCAGCCTCTGATCACGGTGTTGAGAAGGTTAATTTCGGTATGGCACATCGTG
GTCGTTTGAATACACTGTATAACGTTATCGGTAAGTCATTCCCAGTGATATTAAAAGAGTTTGTAGGTATCACGGCCCCTGAACTGGA
ACCATTTAAGGTACAATCCGATGTGAAGTACCATCTTGGGGCCAAATCTACGGTGAAGATGCGCAATGGAAAGTTGATGTTTACAGA
ATTGCTCGCCAACCCATCACATCTGGAGGCAGTCAACCCGGTCTTGCAAGGGTACACCAGGGCAGCACAACTCAAGCACGGAGACGC
TGGGCAGTCGAAGGTACTCCCCGTGGAGATACACGGCGATGCTGCTTTTGCTGGGCAAGGTGTCACATTCGAGACGATGTGCATCA
GTGAGGTTCCCAAGTTTACGACAGGTGGTACTATTCACGTTGTGGTTAACAACCAGATTGGCTTCACTACGGACCCCCGGTGCTCCCG
AAGCAGTCCGTACTGCTCTGACCTGGGCCGTGCCTTTCAGTGTCCCATTTTCCATGTAAATGGTGATTGTCCTGAGGATGTCGCTCGT
GTGTTTGCGTTCGCTGTGGACCTCCGTTCAAGGTTCAACAAGTCTGTTGTTATTGATCTGGTGTGCTATCGCCGGTATGGACACAACG
AAAATGACGATCCCACTATCACGCAGCCCCTCTTATACAAGCGAGTTCACGCGACACCCGATGTGTTTGCCCGCTACTCAGAAAAGCT
TGTTGAAGAGGGAGTCGTGACCAAAGAGCAACAGACGGCCAAGGCCAAGGAGCAGAAGGATCACTACGGAAAATATCAGAGTGAG
GTCGGGAATATACGGTACAGCGATTACCTCAAGTCCGGCATACCTGAGCTGTGGAGAGGCATGAAGTACTCAGATGAACTCGGAGA
AGTGACATTGGAGCCCACCGCTGTTGCGCGGGAAAGTCTCCAACCGGTGATTGATTCGCTTAAAAAGGTGCCGGAGGGGTTTGTGG
TTAATTCCAAACTCAAGGCGGTGCTCGAGCAGCGAGTCCAGTCTCTGGAGAAGGGTGAAAAGATTGATTGGGGCGCTGCTGAAGCG
CTTGCCTTTGGTTCACTATTACTGGAGGGCACTCATGTACGGGTGATGGGTCAGGACGTGGAGCGCGGCACATTTTCGCACAGACAC
GCAGTTTTACATGACCAGAACAAAGTTGACACGTACACGCCGCTCGCCCATATAAGGAAGGACCAGGCACCGTTCATAATCACTAAC
AGCCCTCTCAATGAGTATGGGGTACTGGGTTATGCCAGCGGTTACGCCATTTACGACCCCAATGCGCTTGTTTTGTGGGAAGCACAAT
TCGGTGACTTTGCTAACGGAGCCGCCATTATTTTTGATCAATTCCTTTCCAGTGGAGAAACCAAATGGAATCAGCAGCAGGCGGTCGT
CGTTTCTCTTCCCCATGGATTCGATGGGCGCGGAGCGGAACACAGCAGCGGCCGCATTGAGAGGTTCTTGCAAGCCGTCGCAGAAG
ACGTGGACACTCCGGCGTACTCCCCTGAGGAGCGCGCCCACCGTGTAAATATGGAGGTTGTGTTCCCCAGCACACCGGCGCAGTACT
TCCACTTGTTGCGCCGGCACGTCCGTCGCAATTTCCGCAAGCCGCTTATGTTATTCTTTTCGAAACAGTTCTTGCGCACACCGAACGAG
TCATCCATGGAACAAATAACCAGTGGCGGTTTCCAACCTGTTATCGGTGACCCATCCGTTCCACCCGAGAAGGCGCGTCGCCTCGTGA
TGTGCACTGGGCAAATATACCACATCCTCAATAGGTACCGTGAATCCAACAACTGTGGACGGGACGTGGCGCTTGTGCGGATCGAGG
AGCTCTCACCATTCCCAGTTGCTGAGGTGCAGAAGTTGTTGGCGGATTACAGTGGTGTCGAGCTGATGTGGGCACAGGAAGAGCCA
CGCAATCAAGGCGCCTTTTATCACGTTGAATCGCGTGTGGAGTACTACACGGGAGGAGCCCGAGAGCTGCGATATGCTGGGCGTGC
GATCAGTGCTGCCCCTTCTACGGCCTACAAGAGCACTCACGACGCCGAGGAGAGGTACATTTGCGAATGCGTATTTTCATAAACTCCG
AACGGCTGATGCGAATGCGCCGTGGAAAAGAAAATGGGCAGATGACTATAGGCGGAGCAAGATGAAAGACTAAGTAAAGTGAATG
TCGGTTGATGAGAGGGGAATGGTTGCGAGCACAGTATACAGAAAAGGAGGCAATAGGAAGAAAATGACAGCTTCGAGGTGGCAAA
TTACGAGGTTGAAGACACGTGGCGAGGCGAGGTGTATTAACATTTCGGCTGAGTGGGGGTTTCAGTTAAAGTGGCGCGTTACTGCG
ACGGGTTCCCTTGTGCTTGTTGTAGACGTCTAAGATAGATGAATTGATACAAGGAGAATTTCTATTCATAAAACTGTCGTGTGTGCAC
GCGAACGAATGGAAGCAACTGCGTAATCCAAGCACAAGCACAGATGCACCTAAAAACACGACATCATTAGCCGAGTGAAAAAGCTG
CACAAACCCCTGTGGGTATGTAAAAGAGCGCTTGTAAATG

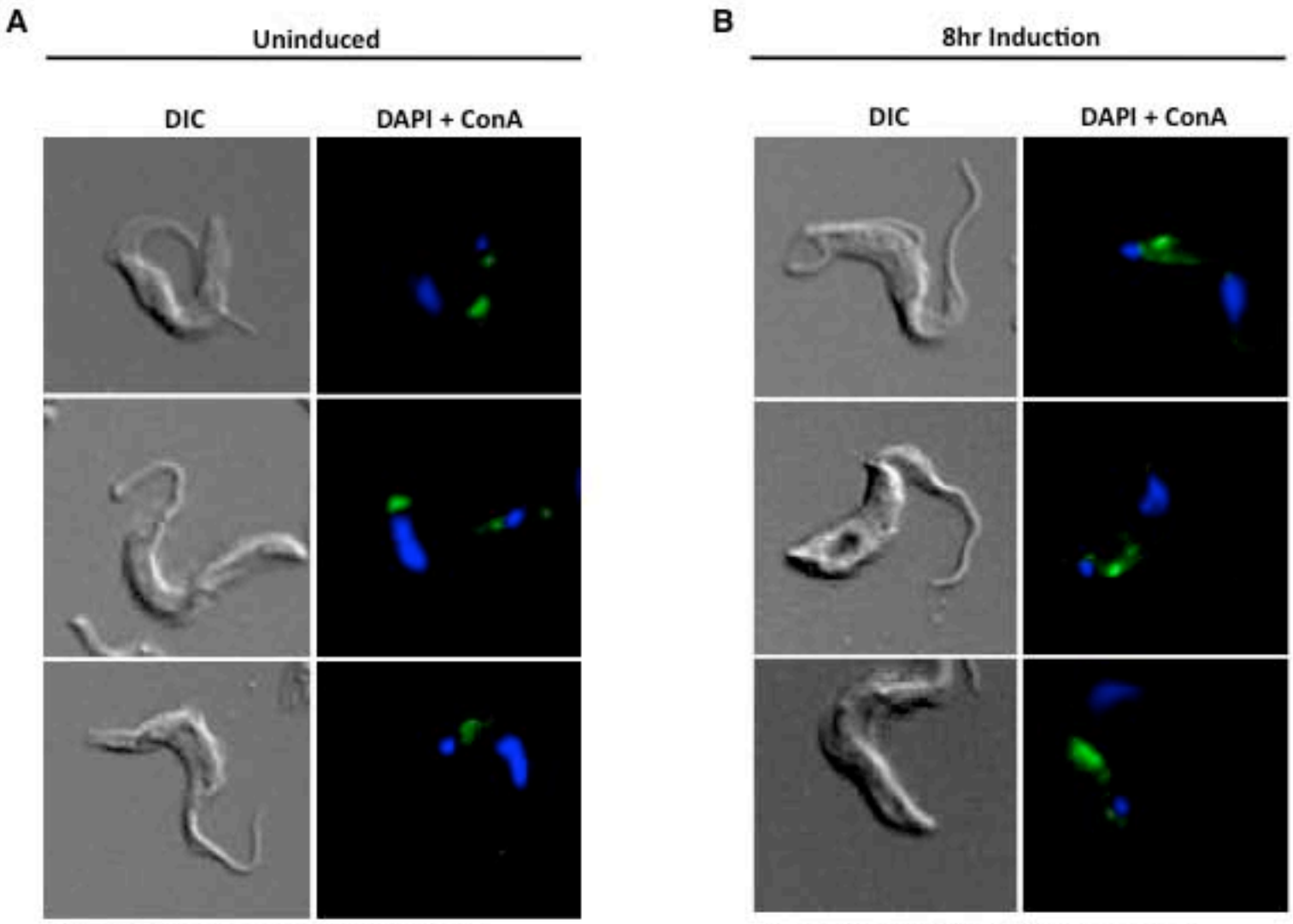
Supplemental Figure 1



Supplemental Figure 2



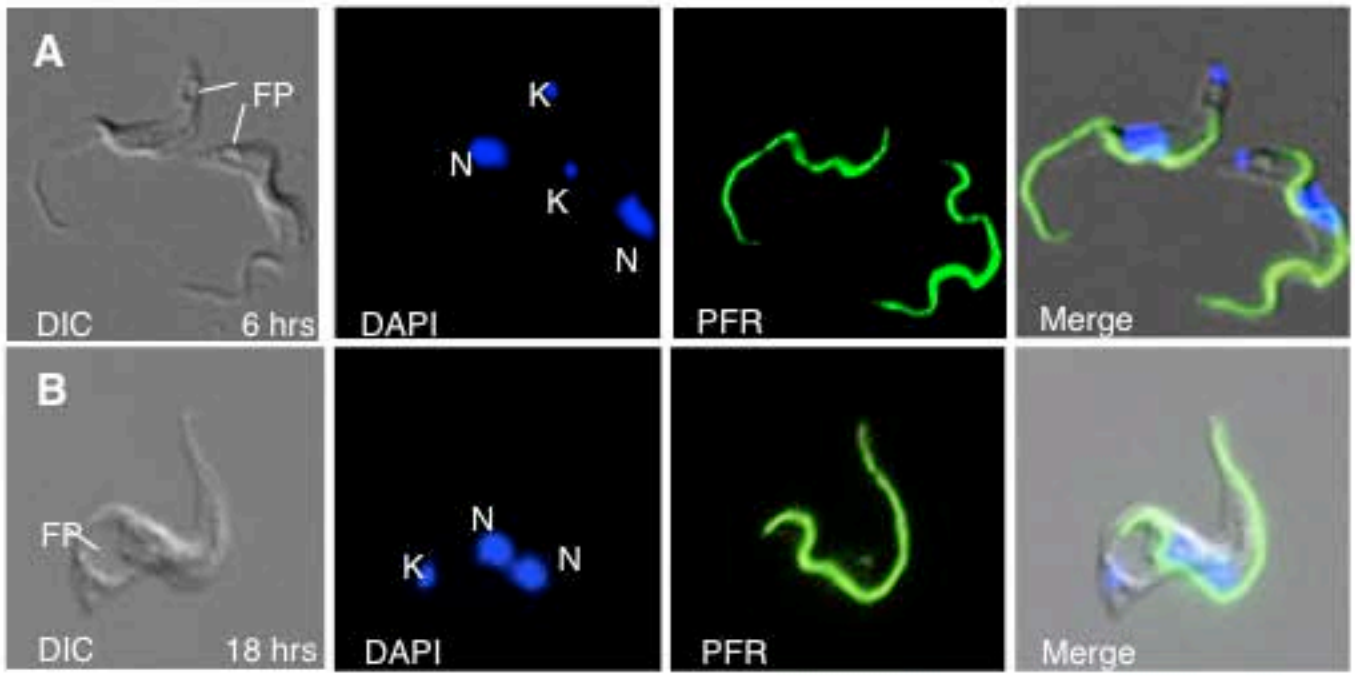
Supplemental Figure 3



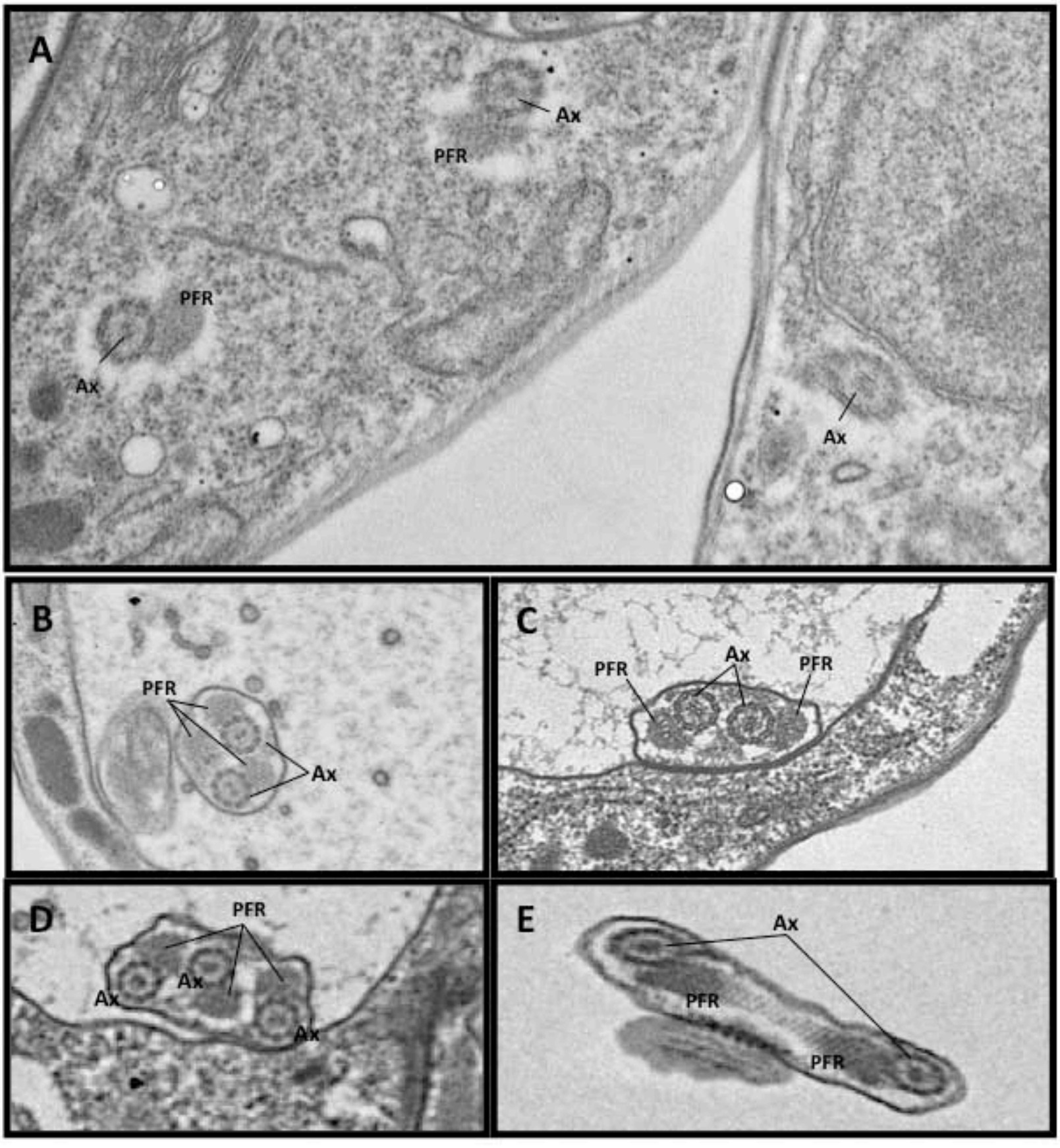
Average Mean Fluorescence = 20.14
Standard Deviation ± 3.80

Average Mean Fluorescence = 22.62
Standard Deviation ± 4.11

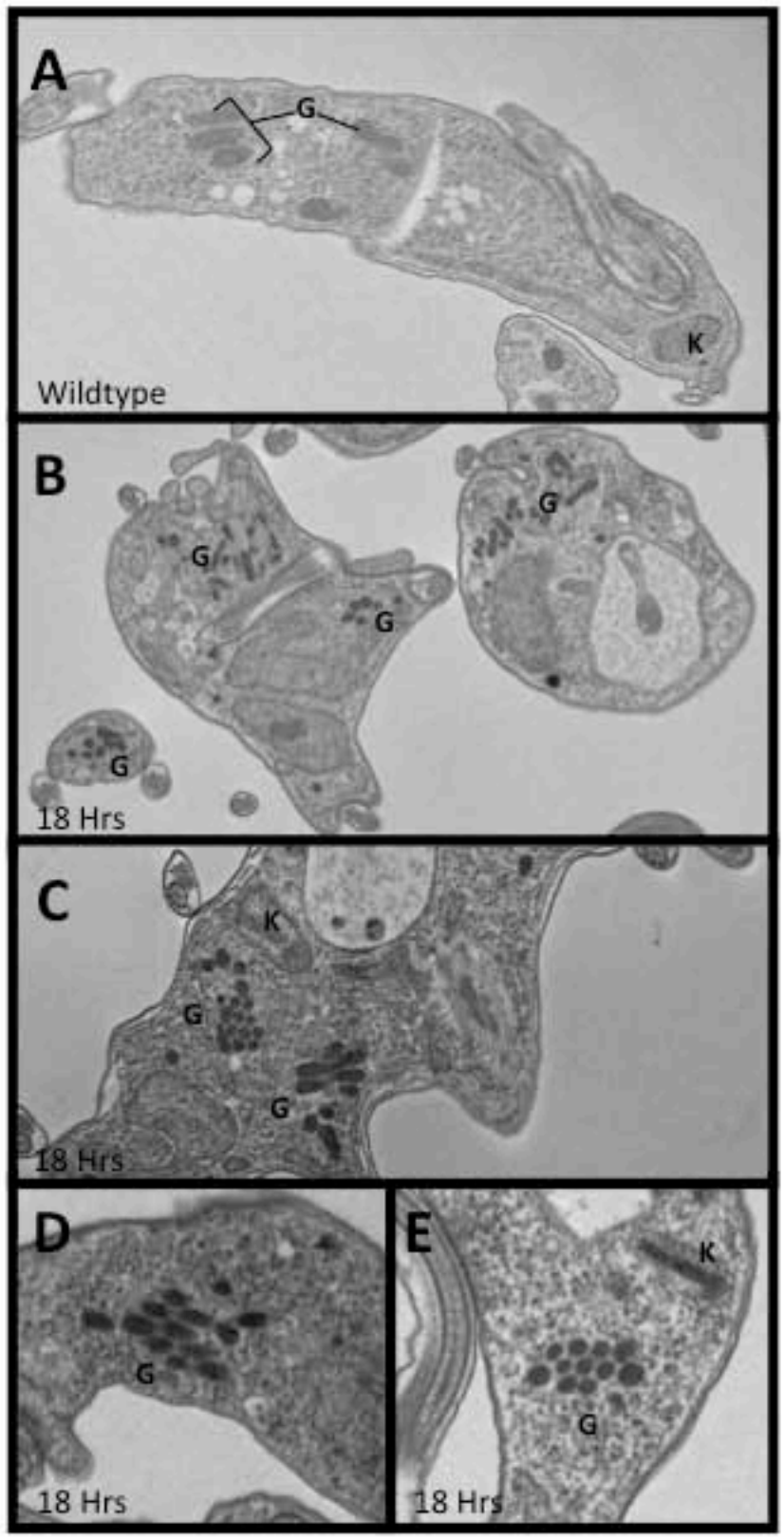
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7

SUPPLEMENTAL FIGURES

Supplemental Figures S1 to S7

Fig 1S Amplification of two α-KDE1 sequences for RNAi (A) Forward and reverse primer pairs for preparation of two α-KDE1 RNAi constructs. The complete primer sequence is shown. (B) Relative location and size of the two products from the α-KDE1 open reading frame (ORF). (C) Coding sequence of the α-KDE1. The sequences complementary to the PCR primers are indicated in red and blue.

Fig S2 Effects on BF *T. brucei* following α-KDE1RNAi knockdown with second RNAi sequence. (A) Growth of α-KDE1RNAi cells in culture at 37C in the presence (+) or absence (-) of doxycycline. (B) RT-PCR analysis of α-KDE1 and β-tubulin mRNA. Cellular RNA was extracted during time course induction with doxycycline, cDNA was prepared and used for PCR amplification, with (+) and without (-) reverse transcriptase (RT) to ensure no contaminating DNA was present from RNA preparation. PCR products were ran on agarose gels and imaged. (C) DIC images taken from videos of α-KDE1 RNAi cells following induction with doxycycline for 0, 6, 12, and 18 hours. The expanding posterior vacuole and internalized flagellum are visible.

Fig S3 Motility of BF *T. brucei* is altered by α-KDE1RNAi. (A-D)Videos of cells by DIC at the time of induction (0 hours) and 6, 12, 18 hours post induction.

Fig S4 Endocytosis during α-KDE1 RNAi induction. (A) Endocytosis of ConA was carried out for uninduced cells and internalized ConA signal was quantified using ImageJ softwear. (B) ConA endocytosis was also carried out after 8 hours of induction with doxycycline and internalized ConA signal was quantified with ImageJ softwear.

Fig S5 Localization of the posterior vacuole in α-KDE1RNAi *T. brucei*. Position of the vacuole relative to the kinetoplast (K) and paraflagella rod (PFR). (A) 6hours post induction. (B) 18 hours post induction.

Fig S6 Morphological changes to the flagellum in α-KDE1 RNAi *T. brucei.* Transmission electron microscopy of BF *T. brucei* following induction of α-KDE1 RNAi with doxycycline. (A-E) 18 hours after induction of α-KDE1 RNAi revealed flagellar axonemes (Ax) and paraflagellar rod (PFR) stripped of flagellar membrane in the cytoplasm. Flagella were also observed in the flagellar pocket and outside the cell that contained multiple Ax and PFR complexes.

Fig S7 Morphological changes to glycosomes in α-KDE1 RNAi *T. brucei.* Transmission electron microscopy of BF *T. brucei* following induction of α-KDE1 RNAi with doxycycline. (A) Representative untreated wild type BF *T. brucei*. (B-E) BF *T. brucei* 18 hours after induction of α-KDE1 RNAi revealed clusters of elongated glycosomes throughout the cytoplasm but predominately near the flagellar pocket.

The position of the flagellar pocket (FP), glycosomes (G), kinetoplast (K) and nucleus (N) are indicated.

Supplemental methods

Concanavalin A endocytosis assays. Uninduced and 8 hour induction E1 RNAi cells were prepared first treated for concanavalin A-FITC (Sigma) binding as described in main text methods. After washing to remove free Con A-FITC, cells were shifted from 3C to 37C for 30 minutes. These cells were then prepared for fluorescence microscopy following methods described in the main text. Uninduced and induced cells were imaged using a Zeiss Axio Observer inverted microscope equipped with an AxioCam HSm. During acquisition image exposure time was kept constant to allow for a relative comparison between samples. Raw TIF files were imported into ImageJ (1,2) for fluorescence quantification of the FITC signal. The signal of a fixed volume was quantified for the posterior region, from the kinetoplast to the nucleaus, for each cell. The average mean intensity and standard deviation was calculated for uninduced (n=12) and 8 hour induction (n=12) E1 RNAi cells.

RT-PCR analysis. Total RNA was extracted from cells using the TriPure Isolation Reagent (Roche, Indianapolis, IN, USA). From extracted RNAs, cDNA was prepared using Avian Myeloblastosis Virus Reverse Transcriptase (AMV RT) (Promega). Reactions were carried out with with and without RT to ensure no contaminating cellular DNA was carried over during RNA purification. RT-PCR reaction was carried out using cDNA derived from 20 ng of total cellular RNA. Reaction was carried out using 10 pmol of sense and antisense primer against β-Tubulin and the α-KDE1 coding sequence. RT-PCR cycle conditions: 95°C for 5 minutes, followed by 29 cycles of 95°C for 30s, 55°C for 30s and 72°C for 30s. PCR products were ran out on a 1% Agarose 1x TBE gel at 120v for 30 minutes and imaged on a Gel/Chemi Doc (Bio-Rad).

1. **Schneider, C.A., Rasband, W.S., Eliceiri, K.W.** "NIH Image to ImageJ: 25 years of image analysis". Nature Methods 9, 671-675, 2012.

2. **Abramoff, M.D., Magalhaes, P.J., Ram, S.J.** "Image Processing with ImageJ". Biophotonics International, volume 11, issue 7, pp. 36-42, 2004.