Supplementary Information for

TbSmee1 regulates hook complex morphology and the rate of flagellar pocket uptake in *Trypanosoma brucei*

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Running Title: Hook complex biogenesis and function in *T. brucei*

Key Words: *Trypanosoma brucei*, cytoskeleton, cell cycle, flagellum, polo-like kinase, Flagellar pocket

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Figure S1

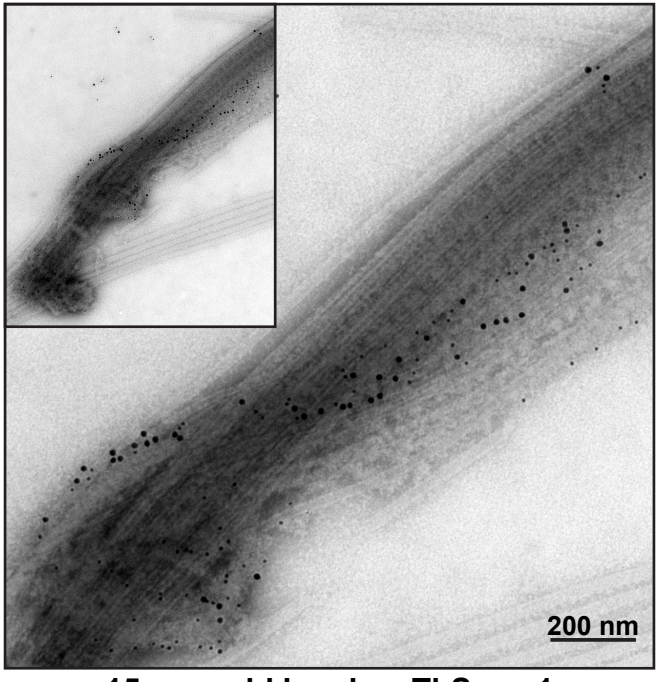

Figure S1 Dual immuno-electron microscopy with TbMORN1 confirms TbSmee1 localization to the HC shank. Flagella were isolated from 3×HA-TbSmee1 cells and then labeled with antibodies against HA, a biotin-conjugated secondary antibody, and 15 nm gold-conjugated streptavidin. TbMORN1 was labeled with anti-TbMORN1 followed by a 10 nm gold-conjugated secondary antibody. The labeled isolated flagella were negatively stained and examined by transmission electron microscopy.

Figure S2

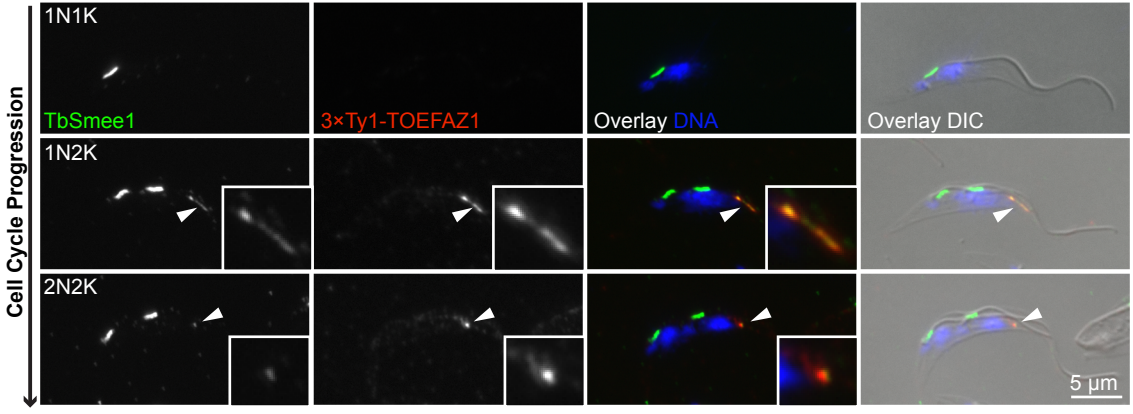

Figure S2 TbSmee1 colocalizes with TOEFAZ1 at the new FAZ tip. Cells expressing endogenously tagged 3×Ty1- TOEFAZ1 were fixed and labeled with antibodies against TbSmee1 (TbSmee1; green) and Ty1 (3×Ty1- TOEFAZ1; red), along with DAPI to label the DNA (DNA; blue). The cells were visualized using fluorescence microscopy and DIC. The filled arrowheads depict the location of the new FAZ tip in a dividing cell and insets are three-fold magnification of the area surrounding new FAZ tip

Figure S3

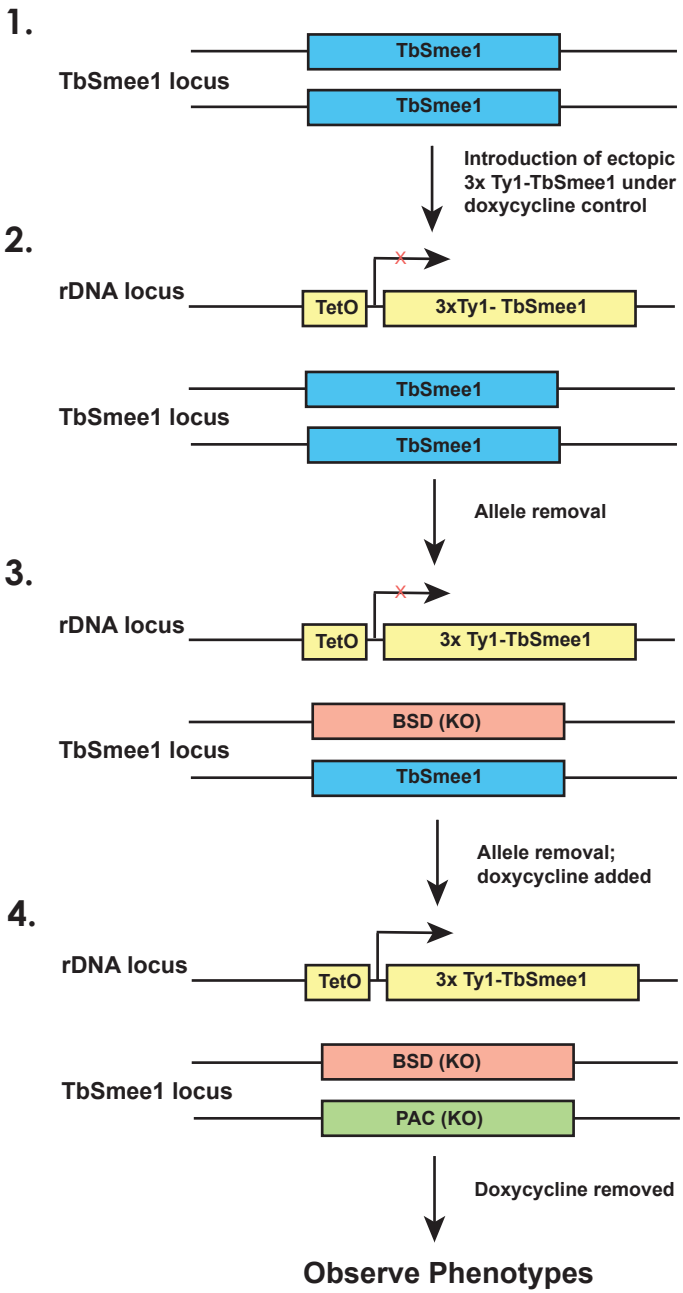

Figure S3 Schematic of TbSmee1 conditional knockout creation. Steps 1,2: 3×Ty1-TbSmee1 was integrated into an ectopic rDNA locus and is under doxycycline control. Step 3: At the endogenous locus, one allele of TbSmee1 was replaced with a blasticidin resistance cassette. Step 4: Expression of the ectopic copy of 3×Ty1-TbSmee1 was induced through the addition of doxycycline and the remaining endogenous allele of TbSmee1 was replaced with a puromycin resistance cassette. To observe the resultant phenotypes of TbSmee1 depletion, doxycycline is removed from the culture medium, which turns off the expression of the ectopic 3×Ty1-TbSmee1.

Figure S4

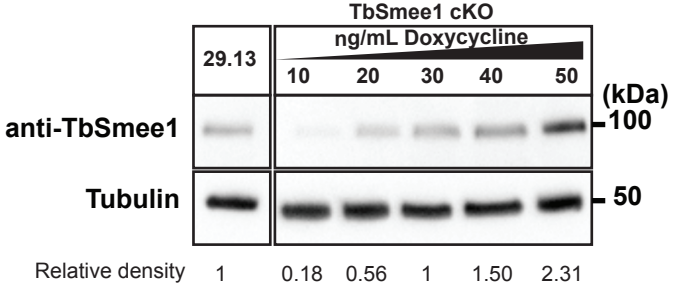

Figure S4 Titration of doxycycline to match expression of ectopic 3×Ty1-TbSmee1 to endogenous levels. The TbSmee1 cKO cell line was grown in a variety of doxycycline concentrations before being collected for western blot analysis. The TbSmee1 cKO lysates and control T. brucei 29-13 lysates were separated by SDS-PAGE, transferred to nitrocellulose, and probed with rabbit anti-TbSmee1 and anti-tubulin as a loading control. The blot was analyzed semi-quantitatively to determine that 30 ng mL^{-1} of doxycycline approximated endogenous levels of expresion, so 35 ng mL^{-1} was used in all following experiments to slightly overexpress TbSmee1 to ensure normal growth.

Figure S5

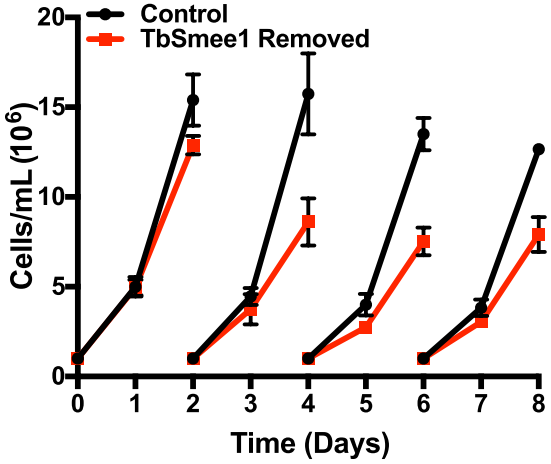

Figure S5 TbSmee1-depletion leads to a 40% decrease in cell growth. TbSmee1 cKO cells were grown for 8 days in either the presence (Control) or absence (TbSmee1 Removed) of doxycycline. Cells from each culture were monitored by cell count, and cultures were re-seeded to starting densities every two days using either doxycycline- or vehicle- containing media. T0 represents the culture at the start of each experiment.

Figure S6

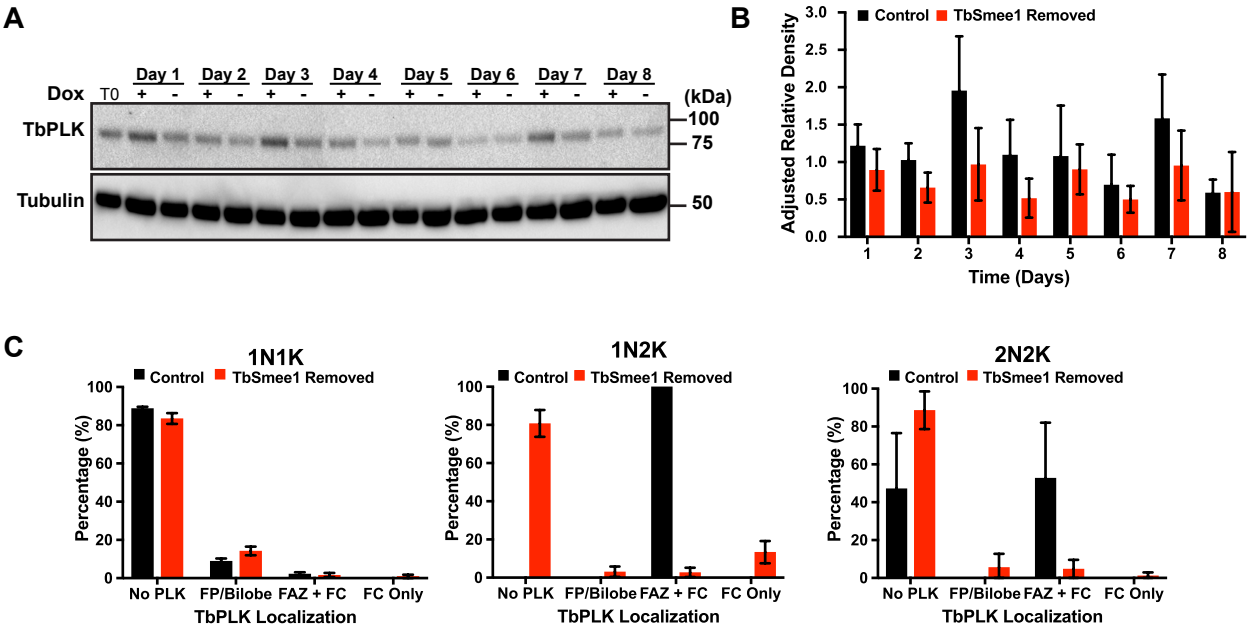

Figure S6 TbPLK mislocalization is not due to change in protein expression (A) TbSmee1 cKO cells were grown for 8 days in either the presence (+) or absence (-) of doxycycline. Cells from each culture were monitored by cell count and collected daily to monitor for TbPLK expression by anti-TbPLK western blotting, using tubulin as a loading control. T0 represents the culture at the start of each experiment.(B) Semi-quantitative analysis of western blot for TbPLK expression in TbSmee1 cKOs. Values are normalized against anti-tubulin loading control and are relative to TbPLK expression at T0. Data are means ± SD of three independent experiments. (C) Quantitation of TbPLK localization at 48 hours of TbSmee1 depletion. Data are means ± SD of three independent experiments.

Figure S7

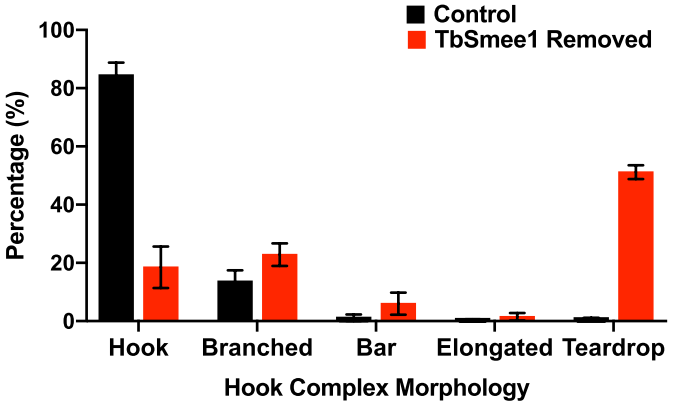

Figure S7 TbSmee1 depletion for 2 days leads to altered HC morphology. Quantitation of HC morphology in non-dividing 1N1K control (Control) and TbSmee1-depleted cells (TbSmee1 Removed) for 2 days. Data are means ± SD of three independent experiments.

Figure S8

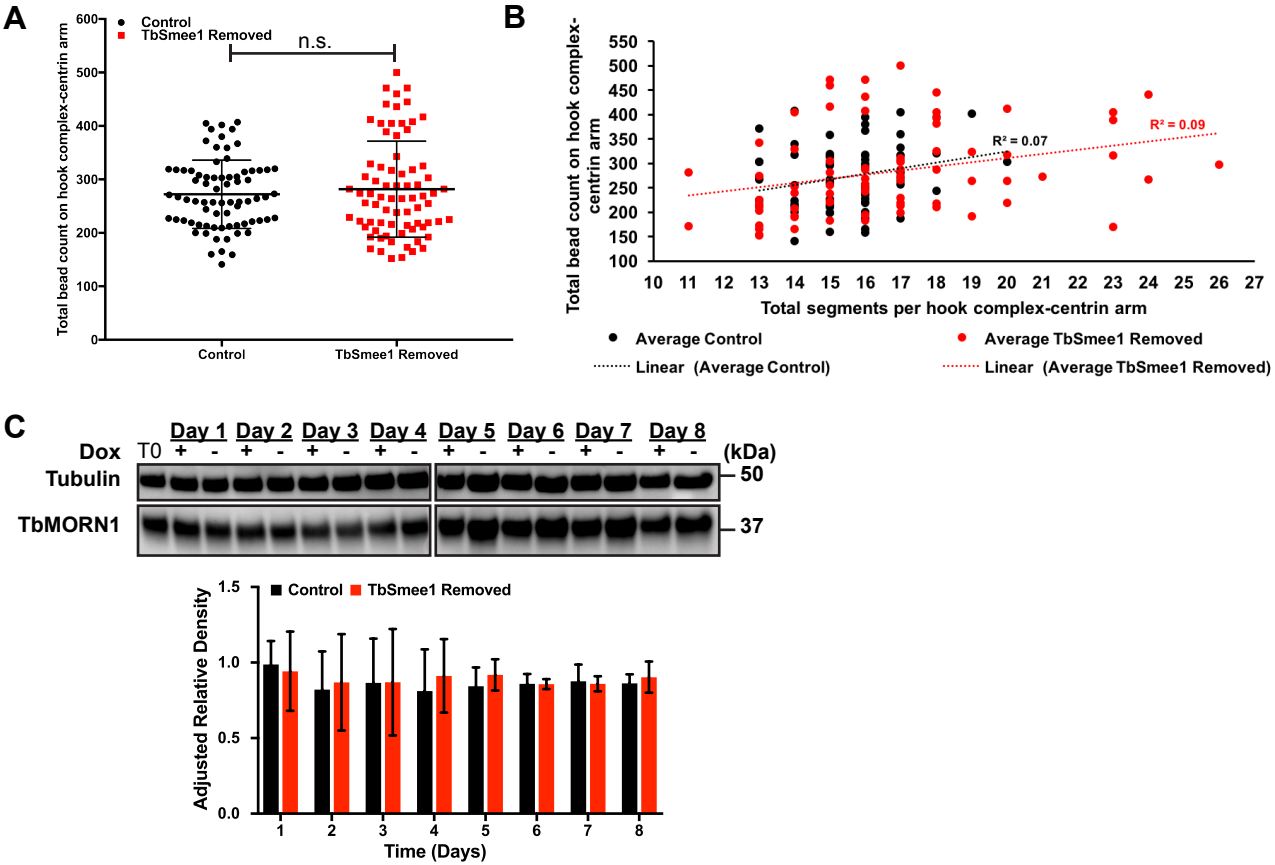

Figure S8 Amount of TbMORN1 immunogold particles remains the same between control and TbSmee1-depleted cells independent of HC-centrin arm size. (A) Quantitation of total number of TbMORN1 immunogold particles on HC-centrin arms of control and TbSmee1-removed cells. Each marker represents one HC-centrin arm and the error bars indicate quartiles. n.s; not significant (two-tailed unpaired Student's t test). (B) Correlation of total TbMORN1 immunogold particles on HC-centrin arm to total number of HC-centrin arm segments. Dotted lines indicate linear regressions. (C) TbSmee1 cKO cells were grown for 8 days in either the presence (+) or absence (-) of doxycycline. Cells from each culture were monitored by cell count and collected daily to monitor for TbMORN1 expression by anti-TbMORN1 western blotting, using tubulin as a loading control. T0 represents the culture at the start of each experiment. The TbMORN1 western blot was semi-quantitatively analyzed with the TbMORN1 values normalized against the anti-tubulin loading control and are relative to TbMORN1 expression at T0. Data are means ± SD of three independent experiments.

Figure S9

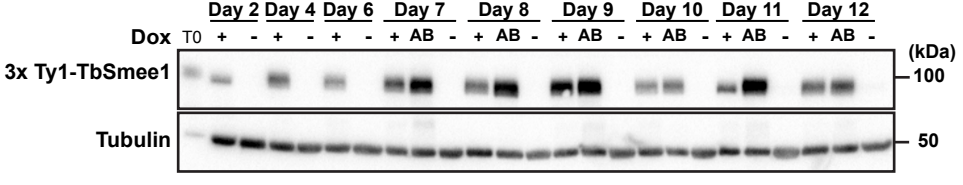

Figure S9 Addition of doxycycline to TbSmee1- depleted cells restores expression of the ectopic 3×Ty1- TbSmee1 allele and leads to restored cell growth. (A)TbSmee1 cKO cells were treated with either doxycycline (Control; +) or vehicle control (TbSmee1 Removed; -) for 6 days before treating TbSmee1-depleted cells with doxycycline (AB; TbSmee1 Rescue) for an additional 6 days. Cells were monitored for cell growth, collected at the indicated intervals, and lysed for western blotting. The lysates were separated by SDS-PAGE, transferred to nitrocellulose, and blotted with anti-Ty1 and anti-tubulin (loading control) antibodies. Molecular weights in kDa are indicated on the right.

Figure S10

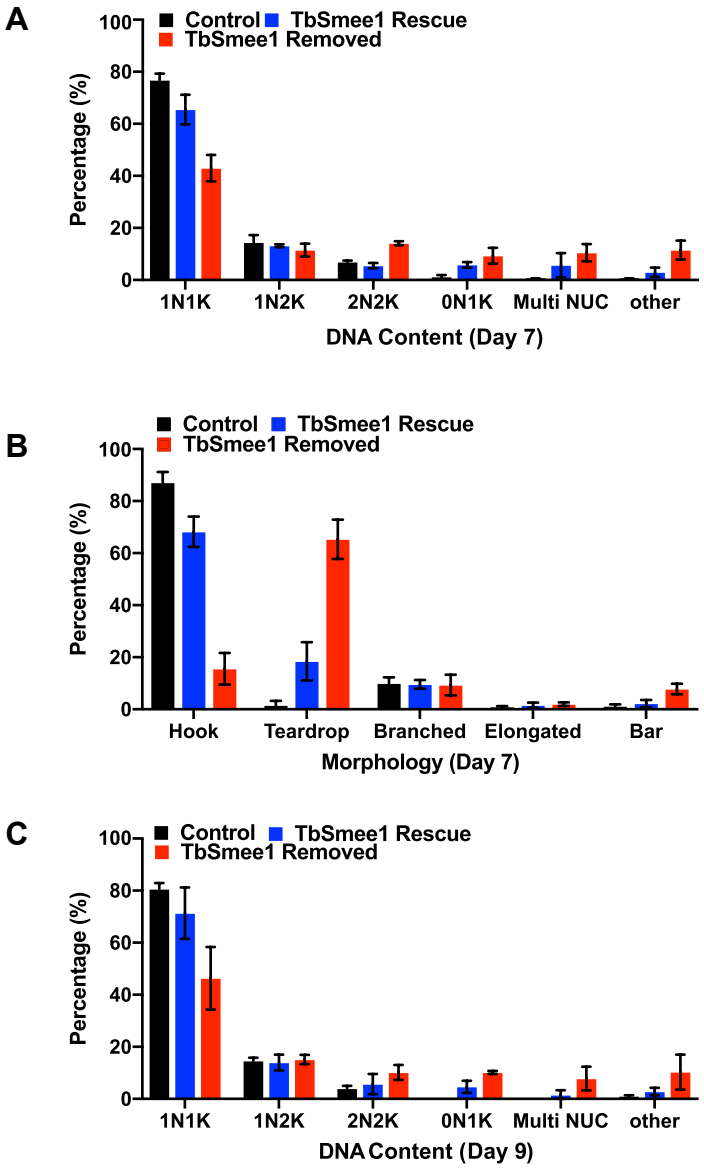

Figure S10 Restoring TbSmee1 expression leads to recovery of normal DNA content and hook complex morphology. (A) Quantitation of DNA content in control, TbSmee1-rescued, and TbSmee1 – depleted (TbSmee1 Removed) cells on day 7 (one day after doxycycline addition) of experiment. Data are means ± SD of three independent experiments. (B) Quantitation of hook complex morphologies in non-dividing 1N1K control, depleted TbSmee1 (TbSmee1 Removed) and rescued TbSmee1 (TbSmee1 Rescue) cells on day 7. Data are means ± SD of three independent experiments. (C) Quantitation of DNA content in control, TbSmee1-rescued, and TbSmee1-depleted (TbSmee1 Removed) cells on day 9 (three days post addition of doxycycline to rescue). Data are means ± SD of three independent experiments.

Figure S11

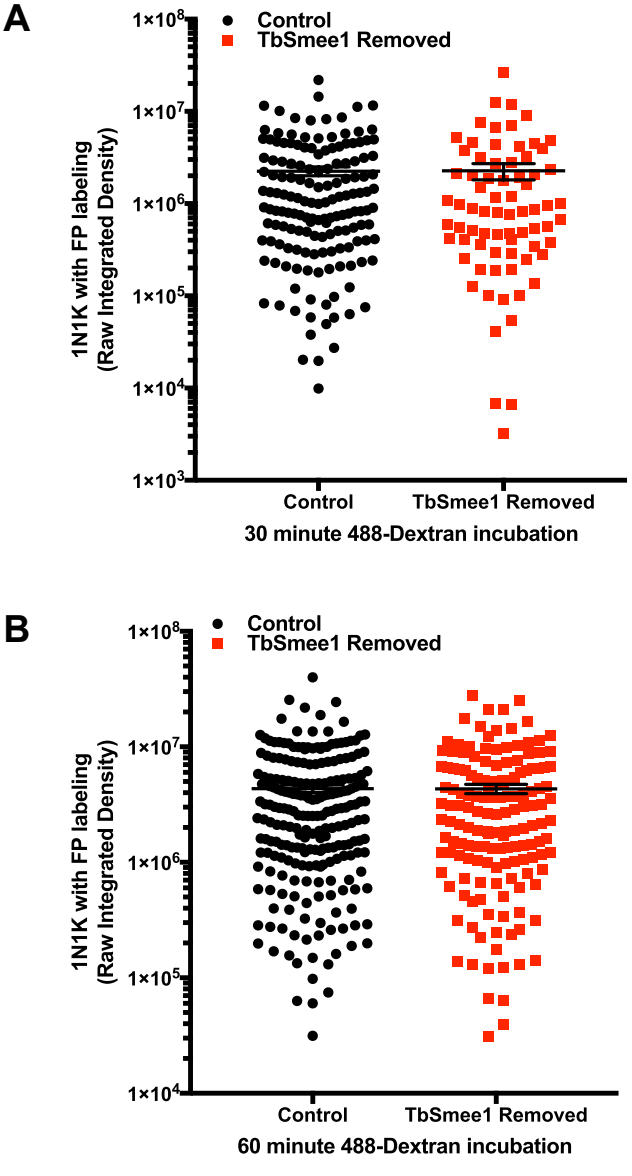

Figure S11 TbSmee1-depleted cells take up the same amount of fluorescent dextran as control cells. (A) Quantitation of raw integrated density of fluorescence intensity in non-dividing 1N1K control and TbSmee1-removed cells after 30 minutes of incubation with dextran. Fluorescence intensity is plotted on a logarithmic scale and each marker represents one cell. Error bars are mean ± SEM. (B) Quantitation of raw integrated density of fluorescence intensity in non-dividing 1N1K control and TbSmee1-removed cells after 60 minutes of incubation with dextran. Fluorescence intensity was plotted on a logarithmic scale and each marker represents one cell. Error bars are mean ± SEM.

Figure S12

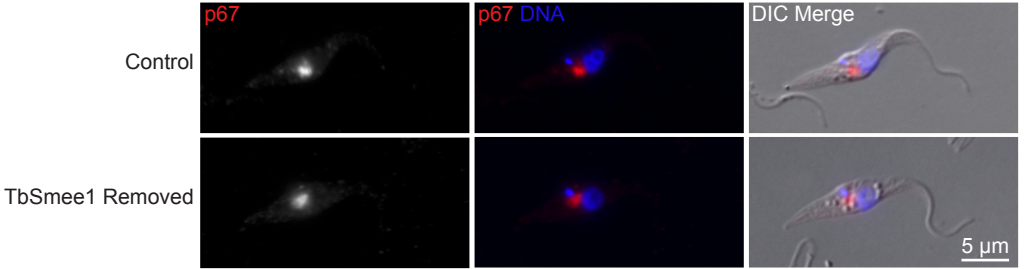

Figure S12 Lysosomal marker p67 is not altered upon depletion of TbSmee1. Control cells (Control) and cells depleted of TbSmee1 for 6 days were fixed and labeled with anti-p67 (p67; red). DAPI was used to visualize DNA (DNA; blue) and the samples were imaged using fluorescence and DIC microscopy.

Figure S13

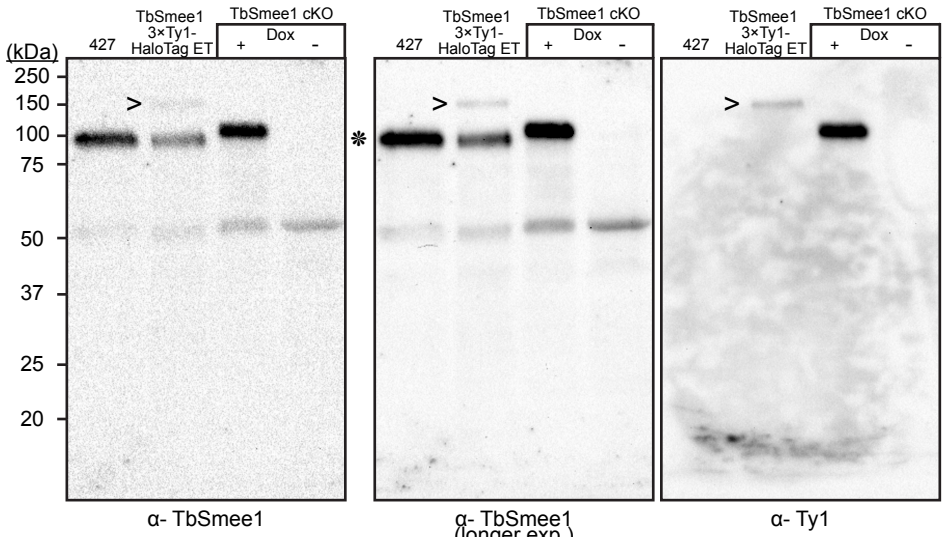

Figure S13 Specificity of the rabbit anti-TbSmee1 polyclonal. Three cell lines were used to validate the anti-TbSmee1 polyclonal: T. brucei Lister 427 (427), a cell line with TbSmee1 endogenously tagged with HaloTag® (TbSmee1 3×Ty1-HaloTag ET), and a cell line where the expression of TbSmee1 is under doxycycline control (TbSmee1 cKO). TbSmee1 cKO cell line was treated with either doxycycline (+) or vehicle control (-) for 6 days before lysates for western blotting were made. The lysates for all cell lines were separated using SDS-PAGE, transferred to nitrocellulose, then blotted with rabbit anti-TbSmee1. The membrane was then stripped and reprobed with anti-Ty1. Two exposures of the anti-TbSmee1 blot are shown to better display the HaloTagged protein (open arrowhead) and the endogenous protein (asterisk). Molecular weights are shown in kDa on the left.