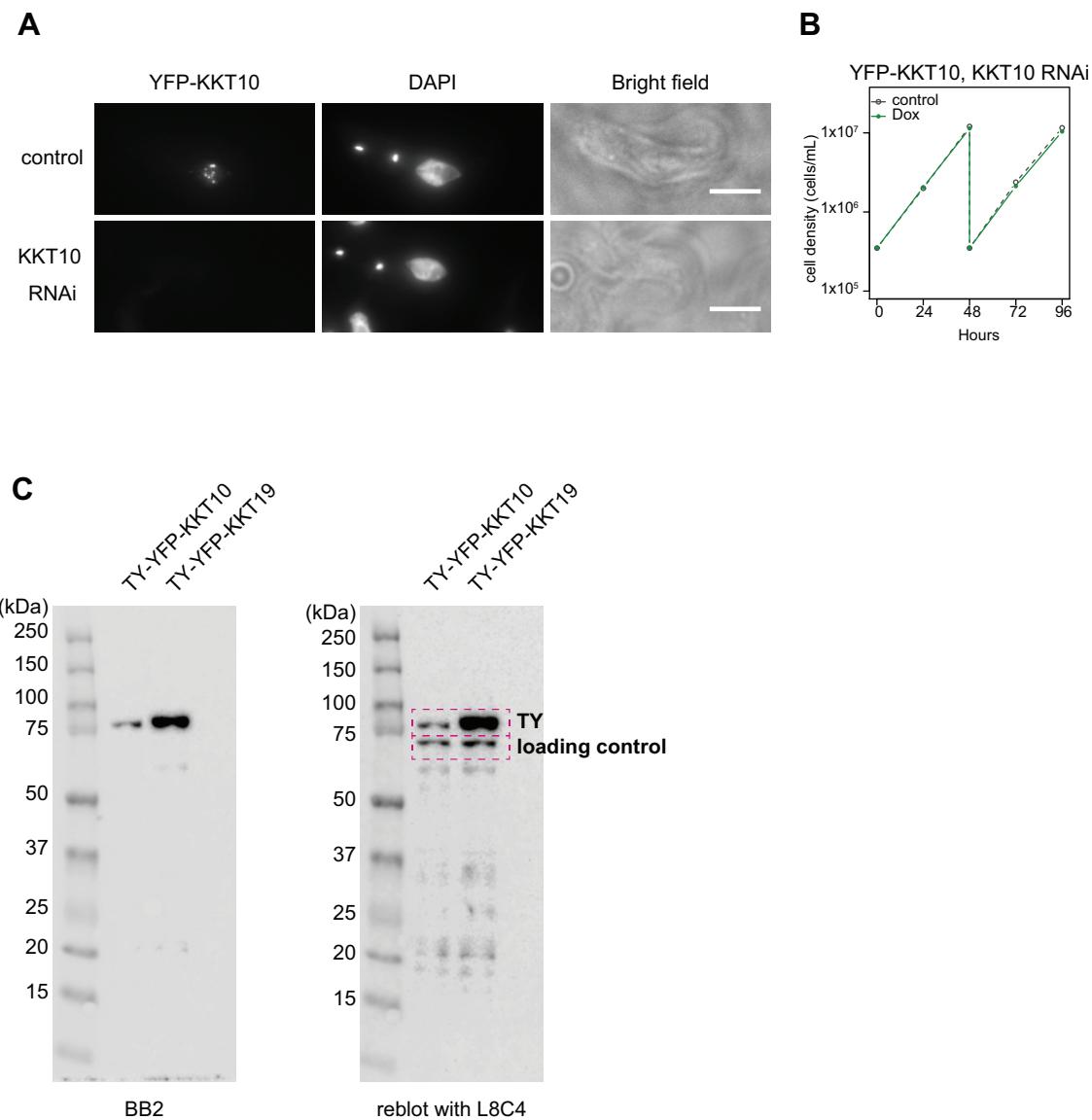
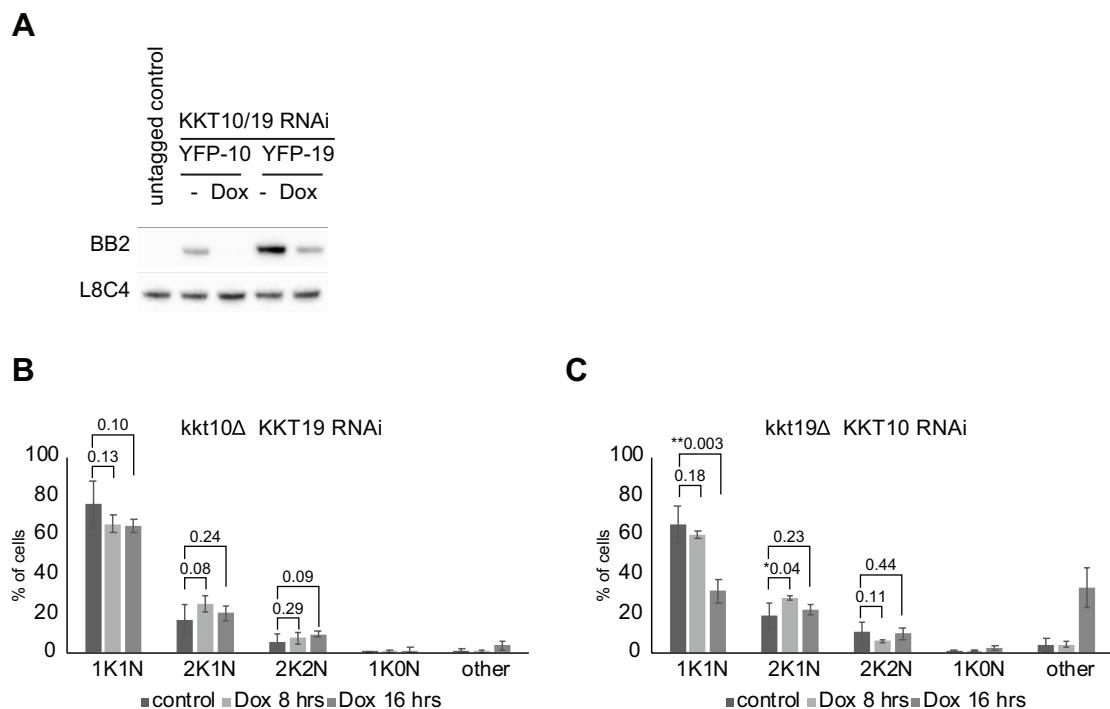


## Supplemental materials



**Figure S1. KKT10-specific RNAi does not affect cell growth.**

- (A) Example of 2K1N cells expressing YFP-KKT10 under KKT10-specific RNAi. Cells were fixed at 24 hours postinduction of RNAi. Control is an uninduced cell culture. Maximum intensity projections are shown. Bars, 5  $\mu$ m.
- (B) Growth curve of YFP-KKT10 with KKT10-specific RNAi. Control is an uninduced cell culture.
- (C) Top-bottom gels of cropped immunoblots shown in Fig. 1C.

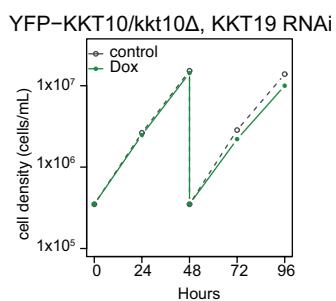


**Figure S2. Cell cycle profiles of KKT10/19 depletion.**

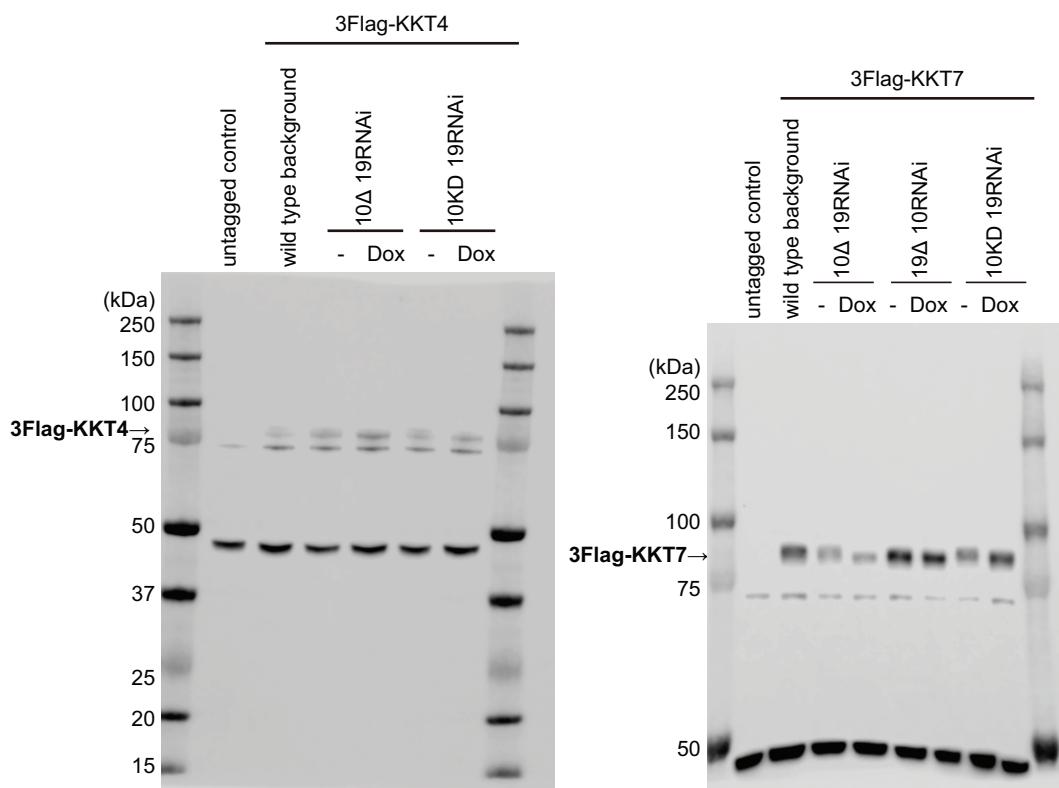
(A) TY-YFP-tagged KKT10 and KKT19 after KKT10/19 double knockdown RNAi for 24 hours were detected by immunoblotting against the TY tag using BB2 antibodies. PFR2 detected by L8C4 antibodies was used as a loading control. SmOxP9 was used as an untagged control.

(B and C) Quantification of cells with indicated DNA contents. Control is an uninduced cell culture. Error bars represent standard deviation from three independent experiments ( $n \geq 314$ ).

**A**



**B**

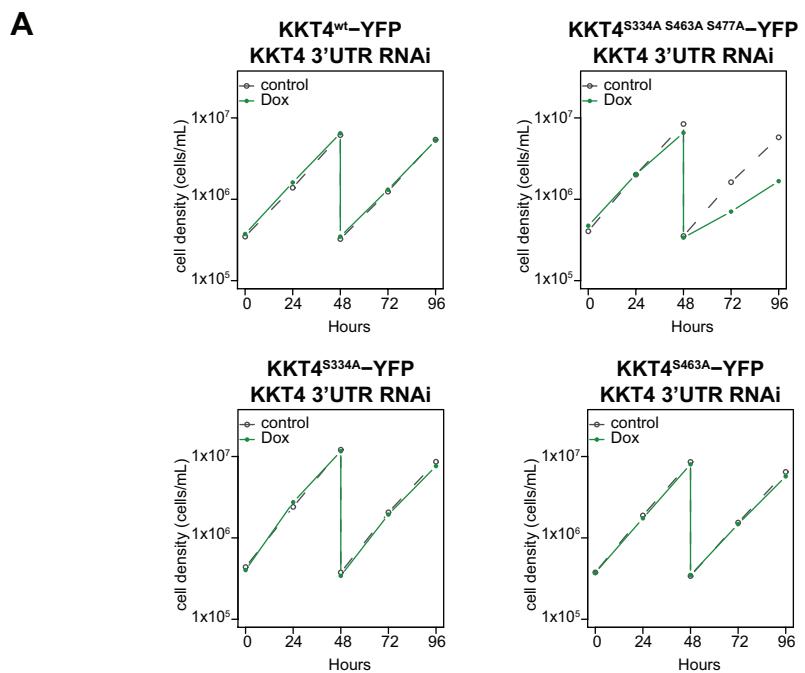


**Figure S3. YFP-KKT10<sup>WT</sup> is fully functional.**

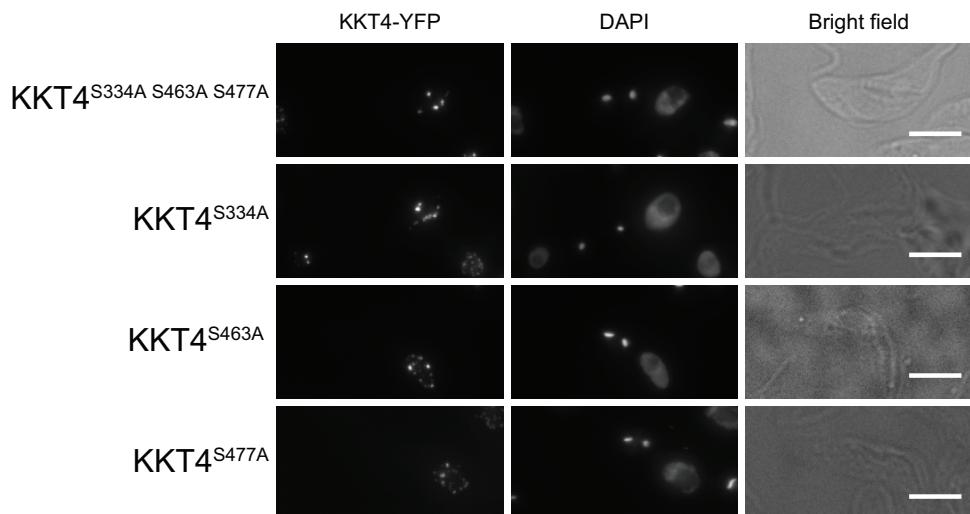
(A) Growth curve of YFP-KKT10/kkt10Δ KKT19 RNAi. Control is an uninduced cell culture.

Similar results were obtained from three independent experiments.

(B and C) Top-bottom gels of cropped immunoblots shown in Fig. 5B. SmOxP9 was used as an untagged control. 10KD is KKT10<sup>K158A</sup>.



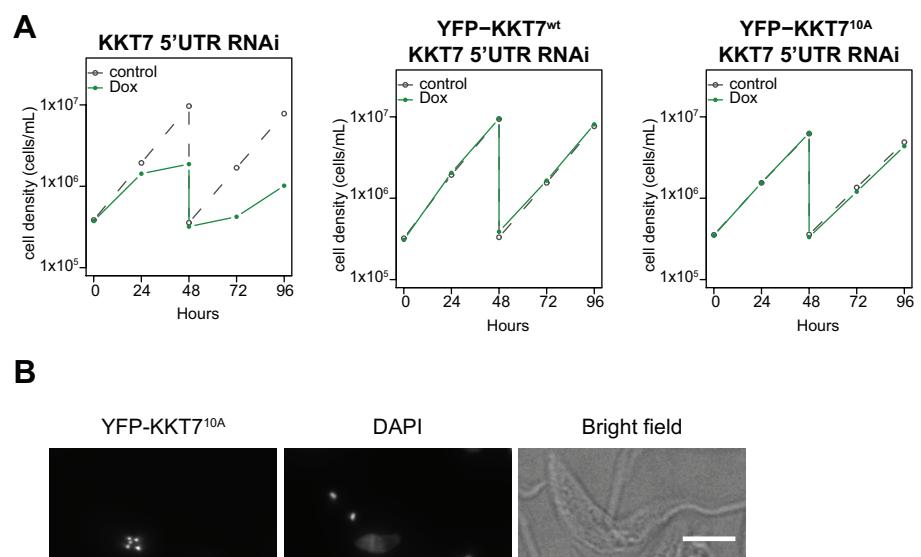
**B**



**Figure S4. Phospho-deficient KKT4<sup>S334A S463A S477A</sup> mutant cannot support cell growth.**

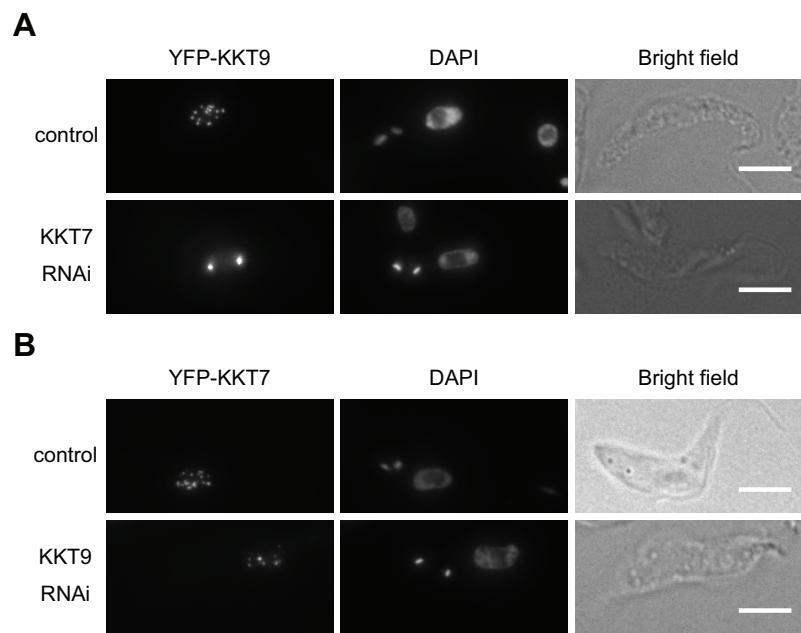
(A) Growth curve of KKT4<sup>WT</sup>-YFP with KKT4 3'UTR RNAi, KKT4<sup>S334A S463A S477A</sup>-YFP with KKT4 3'UTR RNAi, KKT4<sup>S334A</sup>-YFP KKT4 with 3'UTR RNAi, and KKT4<sup>S463A</sup>-YFP with KKT4 3'UTR RNAi. Control is an uninduced cell culture.

(B) KKT4<sup>S334A S463A S477A</sup>-YFP, KKT4<sup>S334A</sup>-YFP, KKT4<sup>S463A</sup>-YFP, and KKT4<sup>S477A</sup>-YFP localize normally at kinetochores. Bars, 5 μm.



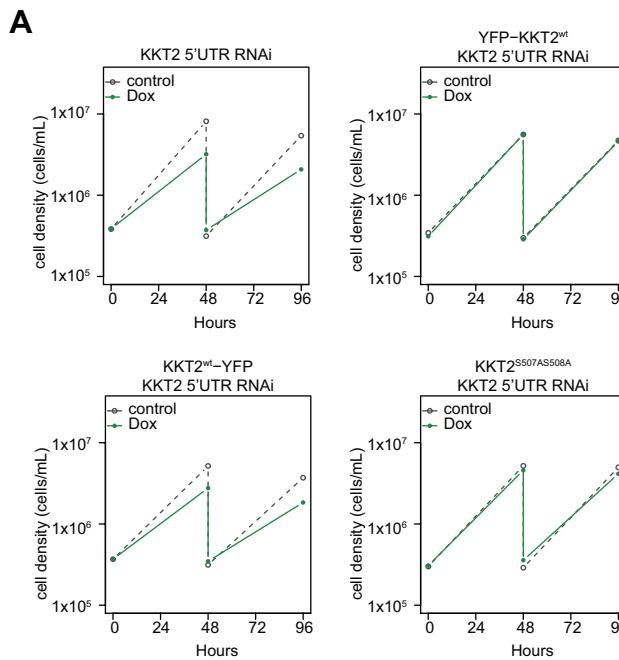
**Figure S5. KKT7<sup>10A</sup> is functional.**

- (A) Growth curve of KKT7 5'UTR RNAi, YFP-KKT7<sup>wt</sup> with KKT7 5'UTR RNAi, and YFP-KKT7<sup>10A</sup> with KKT7 5'UTR RNAi. Control is an uninduced cell culture.
- (B) YFP-KKT7<sup>10A</sup> localizes normally at kinetochores. Bar, 5  $\mu$ m.

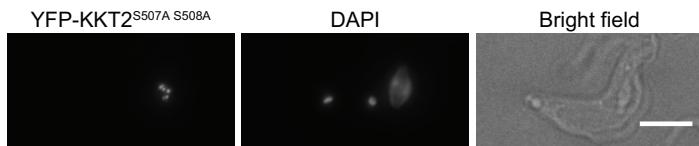


**Figure S6. Localization of KKT9 is affected in KKT7-knockdown cells.**

(A) YFP-KKT9 in KKT7 RNAi cells. (B) YFP-KKT7 in KKT9 RNAi cells. Cells were fixed at 24 hours postinduction of RNAi and stained with DAPI. Control is an uninduced cell culture. Maximum intensity projections are shown. Bars, 5 μm.



**B**



**Figure S7. YFP-KKT2<sup>S507A S508A</sup> is functional in procyclic cells.**

(A) Growth curve of KKT2 5'UTR RNAi, YFP-KKT2<sup>wt</sup> with KKT2 5'UTR RNAi, KKT2<sup>wt</sup>-YFP with KKT2 5'UTR RNAi, and YFP-KKT2<sup>S507AS508A</sup> with KKT2 5'UTR RNAi. Control is an uninduced cell culture.

(B) YFP-KKT2<sup>S507A S508A</sup> localizes normally at kinetochores. Example of cells expressing YFP-KKT2<sup>S507A S508A</sup>.

## Supplementary dataset

Table S1. Phosphorylation sites on kinetochore proteins identified in our previous immunoprecipitates of kinetochore proteins.

[Click here to Download Table S1](#)

Table S2. Raw data containing phosphorylation sites on all proteins identified in our previous immunoprecipitates of kinetochore proteins.

[Click here to Download Table S2](#)

Table S3. Phosphorylation sites on kinetochore proteins identified by proteomic studies of *T. brucei* cell extracts.

[Click here to Download Table S3](#)

Table S4. List of proteins identified in the immunoprecipitates of YFP-tagged KKT7N by mass spectrometry, Related to Figure 6.

[Click here to Download Table S4](#)

Table S5. List of proteins identified in the immunoprecipitates of YFP-tagged KKT7C by mass spectrometry, Related to Figure 6.

[Click here to Download Table S5](#)

Table S6. List of plasmids and bacmids used in this study.

[Click here to Download Table S6](#)

Table S7. List of primers used in this study.

[Click here to Download Table S7](#)

Table S8. List of synthetic DNA used in this study.

[Click here to Download Table S8](#)

Table S9. List of trypanosome cell lines used in this study.

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