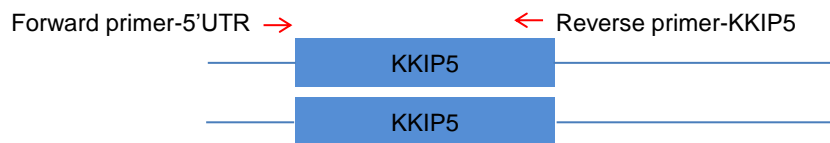


Figure S1

A

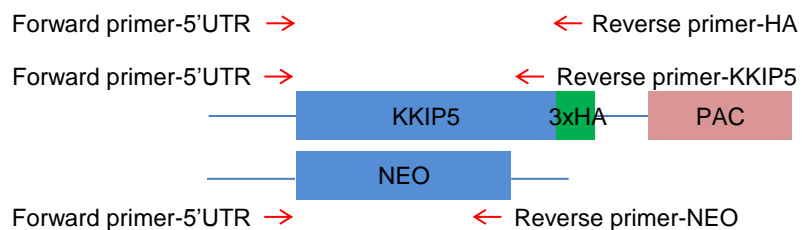
Lister427 cell line (KKIP5/KKIP5^{+/+})



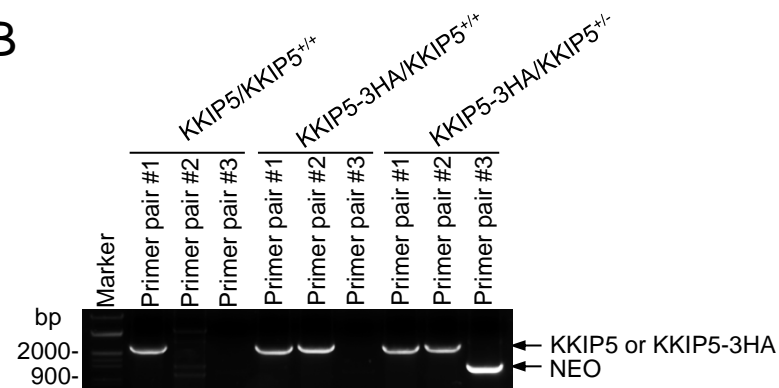
KKIP5-3HA cell line (KKIP5-3HA/KKIP5^{+/+})



KKIP5-3HA+KKIP5 KO cell line (KKIP5-3HA/KKIP5^{+/-})



B



Primer pair #1: Forward primer-5'UTR + Reverse primer-KKIP5

Primer pair #2: Forward primer-5'UTR + Reverse primer-HA

Primer pair #3: Forward primer-5'UTR + Reverse primer-NEO

C

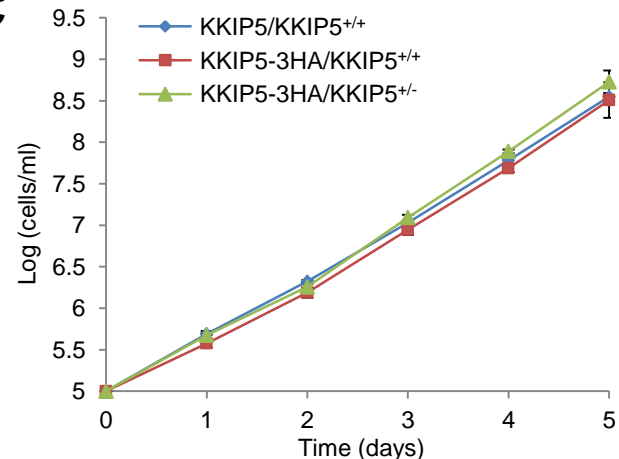


Figure S1. Endogenous tagging of KKIP5 with a triple HA epitope at its C-terminus does not affect KKIP5 function. (A). Schematic representation of the genotype of the wild-type Lister427 cell line, KKIP5-3HA cell line, and the KKIP5-3HA-KKIP5 knockout (KO) cell line. The positions of the primers used for PCR are indicated by red arrows. Note that the size of the genes and the HA tag is not on the same scale. (B). PCR amplification of KKIP5 sequence, KKIP5-3HA sequence, and neomycin-resistance gene. (C). Growth curves of the wild-type Lister427 cell line, the KKIP5-3HA cell line, and the KKIP5-3HA-KKIP5 KO cell line. Error bars indicate S.D. from three independent experiments.

Figure S2

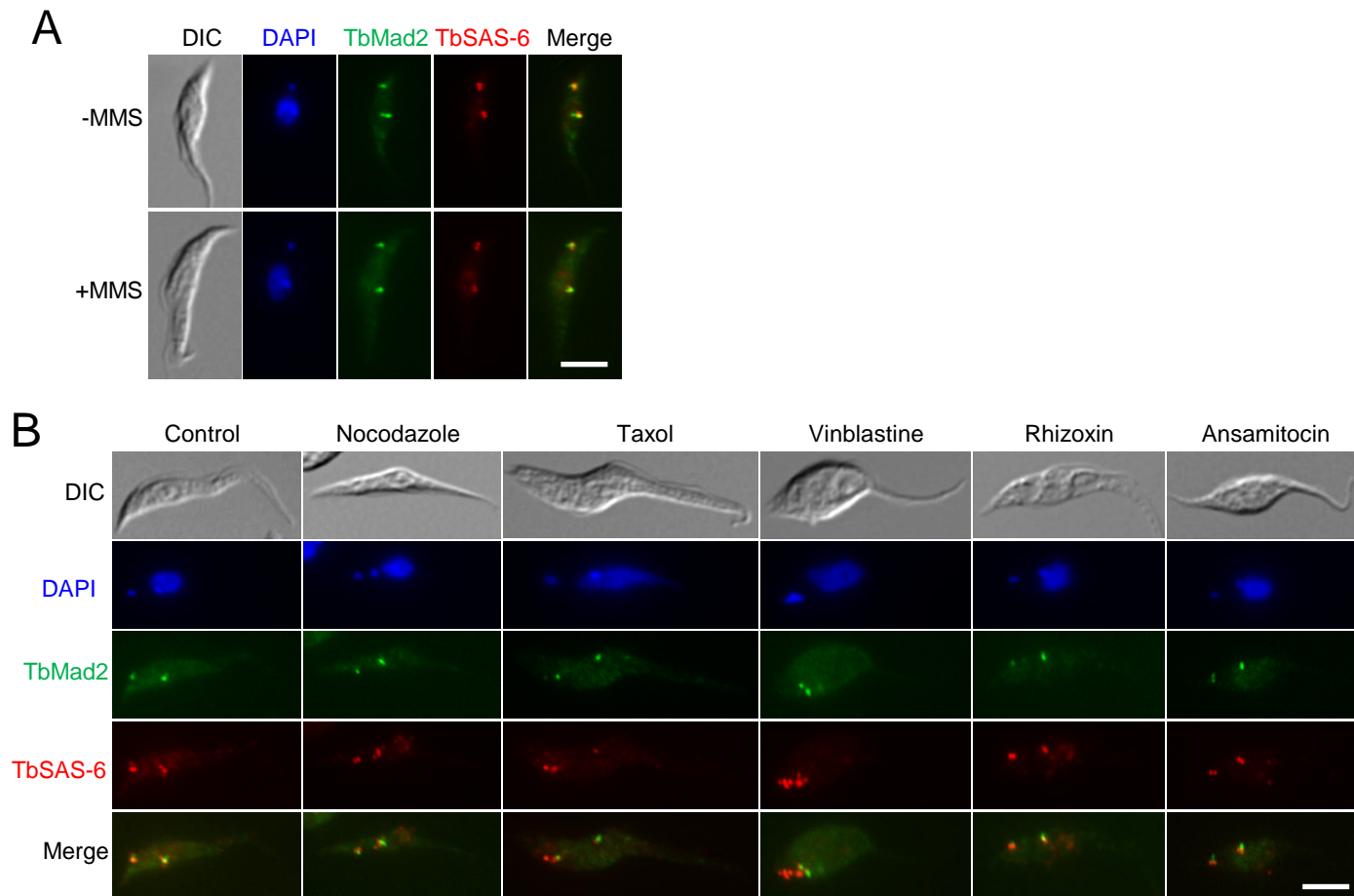


Figure S2. Effect of DNA damage and microtubule perturbation on TbMad2 localization. (A). Effect of MMS treatment on TbMad2 localization. TbMad2 was endogenously tagged with a triple HA epitope and immunostained with FITC-conjugated anti-HA antibody. Scale bar: 5 μ m. (B). Effect of microtubule perturbation on TbMad2 localization. Scale bar: 5 μ m.

Figure S3

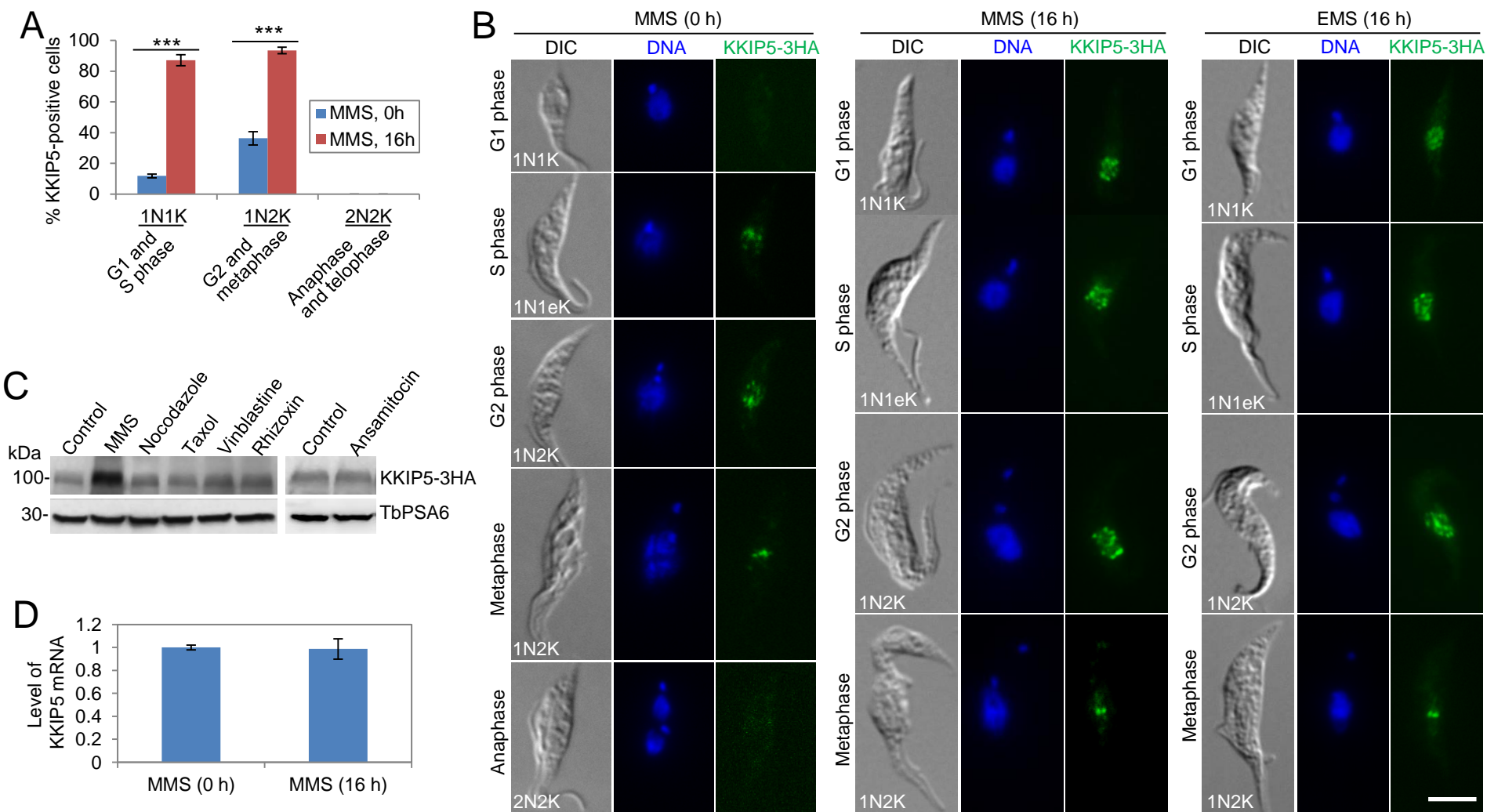


Figure S3. Effect of DNA damage-inducing agent MMS on cell cycle progression and KKIP5 localization. (A). Effect of MMS treatment on KKIP5 localization to kinetochores. 200 cells were counted for each time point. Error bars indicate S.D. from three independent experiments. ***, $p < 0.001$. **(B).** Immunofluorescence microscopy of KKIP5 localization in control, MMS-treated cells, and EMS-treated cells. KKIP5 was endogenously tagged with a triple HA epitope and immunostained with FITC-conjugated anti-HA antibody. **(C).** Effect of microtubule perturbation on KKIP5 protein level. KKIP5 was endogenously tagged with a triple HA epitope and detected by anti-HA antibody. MMS treatment was included as a control. **(D).** KKIP5 mRNA level in control and MMS-treated cells determined by quantitative RT-PCR. Error bars indicate S.D. from three independent experiments.

Figure S4

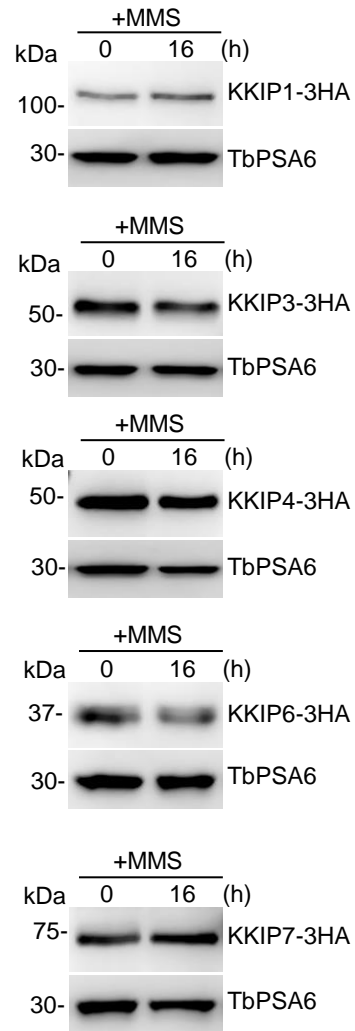


Figure S4. Effect of MMS treatment on the protein levels of outer kinetochore proteins. Five outer kinetochore proteins, KKIP1, KKIP3, KKIP4, KKIP6 and KKIP7 were each tagged with a triple HA epitope and their levels in control and MMS-treated cells were detected by anti-HA antibody. TbPSA6 served as the loading control.

Figure S5

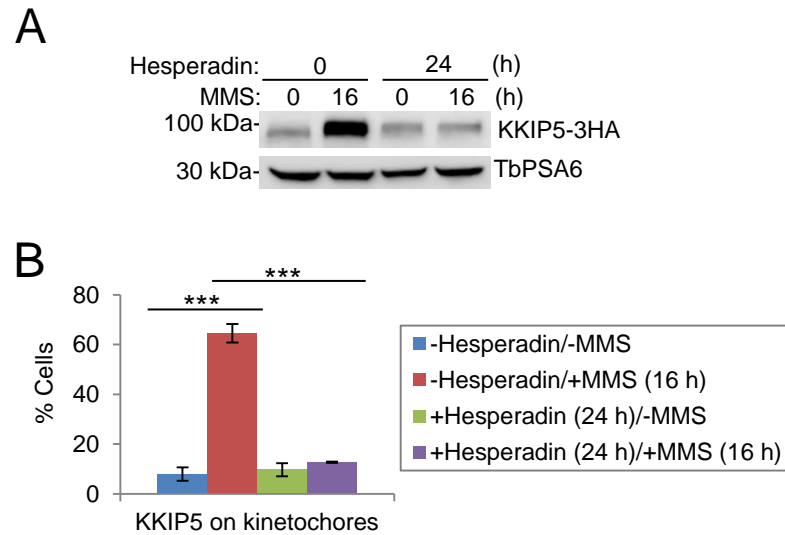


Figure S5. DNA damage-induced KKIP5 response requires TbAUK1 activity. (A). MMS-induced increase in KKIP5 protein level depends on TbAUK1 activity. Cells were treated with TbAUK1 inhibitor Hesperadin. **(B).** MMS-induced KKIP5-positive cells requires TbAUK1 activity. 200 cells were counted for each cell sample. Error bars indicate S.D. from three independent experiments. ***, $p < 0.001$.