Supplemental material

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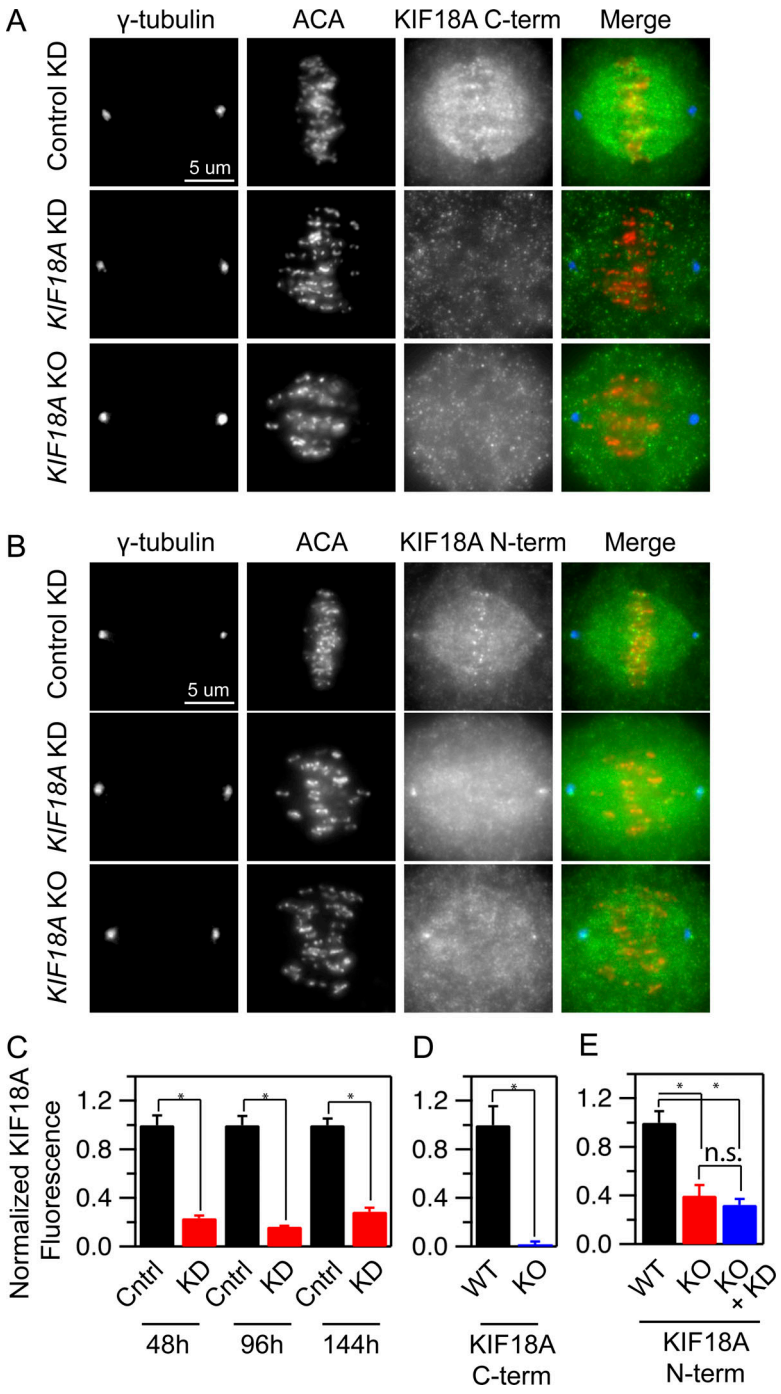


Figure S1. **KIF18A protein is undetectable in *KIF18A* KO hTERT-RPE1 cells. (A and B)** Representative images of metaphase hTERT-RPE1 cells from the indicated conditions labeled for spindle poles (γ-tubulin), kinetochores (ACA), and KIF18A, using antibodies against either the KIF18A C terminus (A) or N terminus (B). Some nonspecific spindle and spindle pole staining from KIF18A N-terminal antibodies is observed in *KIF18A* KO cells. **(C–E)** Quantification of KIF18A immunofluorescence from *KIF18A* KD cells (C) and *KIF18A* KO cells (D and E). Note that treating *KIF18A* KO cells with *KIF18A* siRNAs (KO + KD) does not reduce KIF18A immunofluorescence levels. All data were derived from at least three independent experiments; error bars indicate SD. Related to Fig. 1.

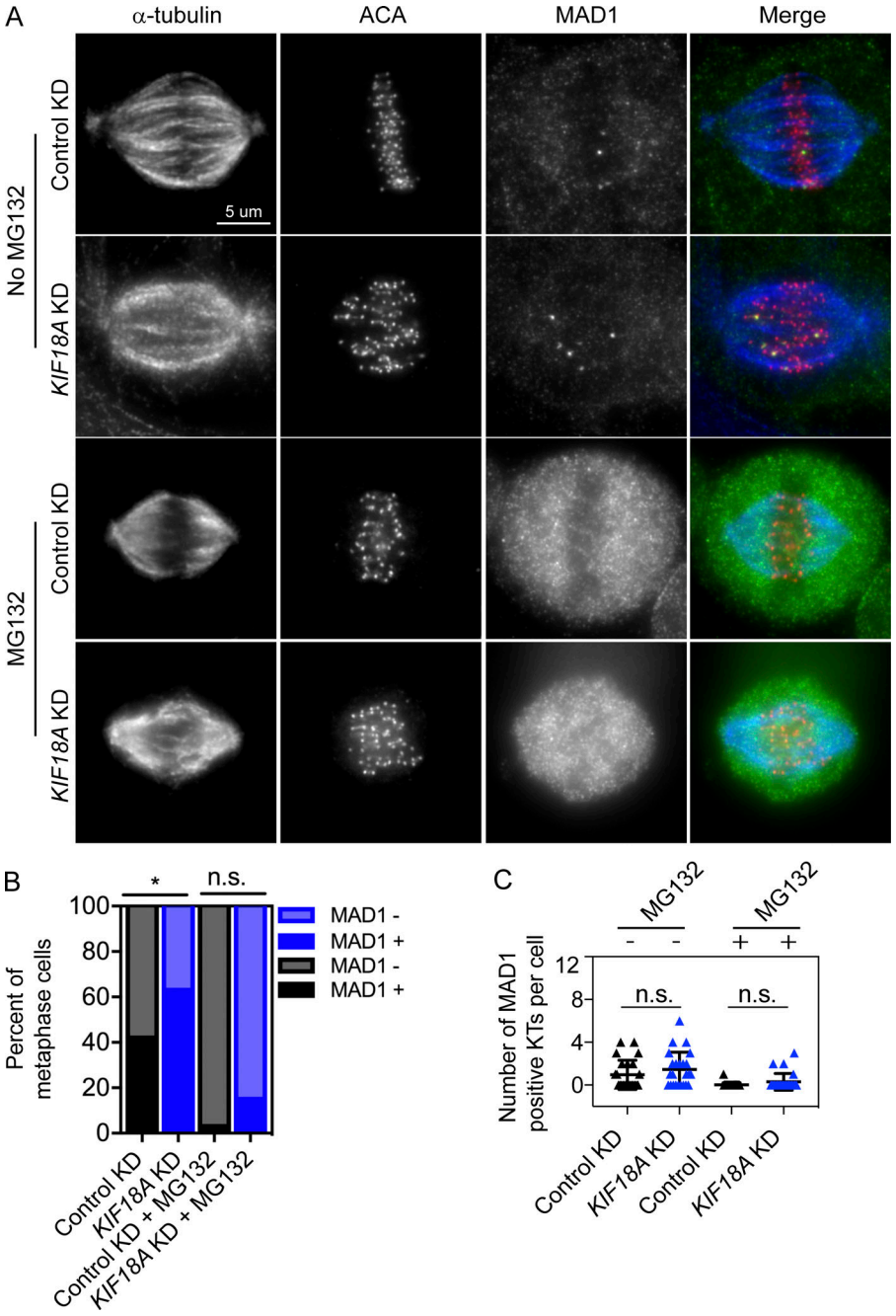


Figure S2. **KIF18A depleted hTERT-RPE1 cells show a decrease in MAD1-positive kinetochores when arrested in MG132. (A)** Representative images of fixed control and *KIF18A* siRNA-treated cells labeled with anti-MAD1 antibodies in the absence or presence of MG132. Scale bar is 5 µm. **(B)** Quantification of the percentage of cells containing MAD1-positive kinetochores. **(C)** The number of MAD1-positive kinetochores per cell. Error bars indicate SD. Control no MG132, *n* = 24 cells; *KIF18A* KD no MG132, *n* = 24 cells; control with MG132, *n* = 33 cells; *KIF18A* KD with MG132, *n* = 27 cells. Data in B were compared via χ^2 test. Data in C were compared by one-way Anova using Tukey's multiple comparison test. *, P < 0.01. Data were collected from three independent experiments. Related to Fig. 1.

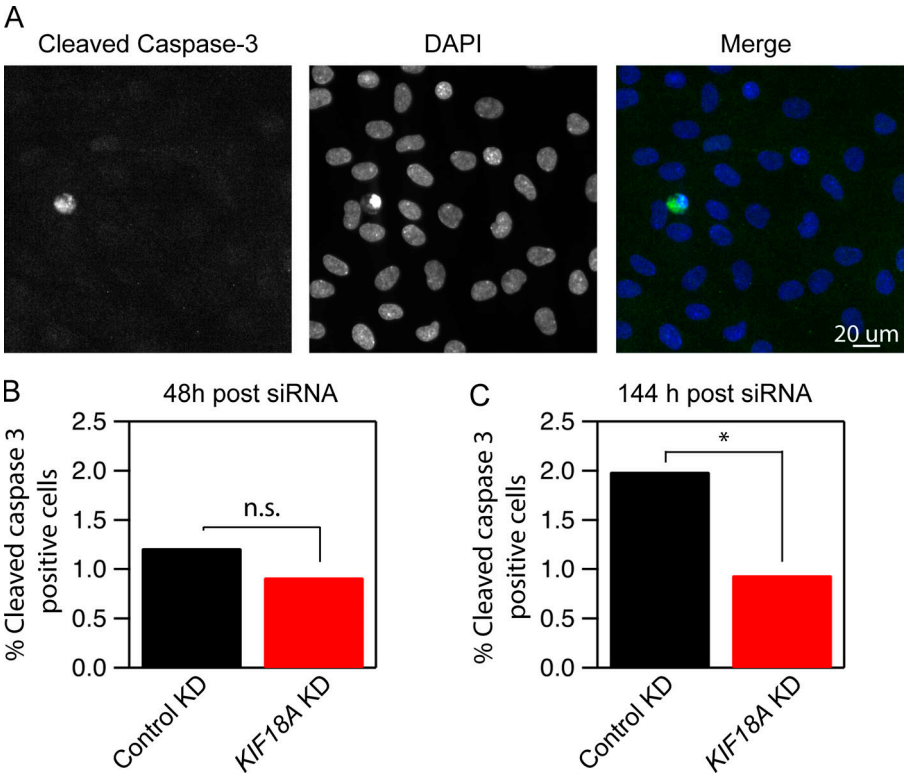


Figure S3. **Loss of KIF18A function does not lead to increased apoptosis. (A)** Representative images of *KIF18A* KD cells stained for the apoptotic marker cleaved caspase-3. **(B and C)** Plots indicating the percentage of cleaved caspase-3–positive cells in control and *KIF18A* KD cells 48 h (B) and 144 h (C) following siRNA treatment. Data from three independent experiments were compared via χ^2 test. *, $P < 0.05$. Related to Fig. 2.

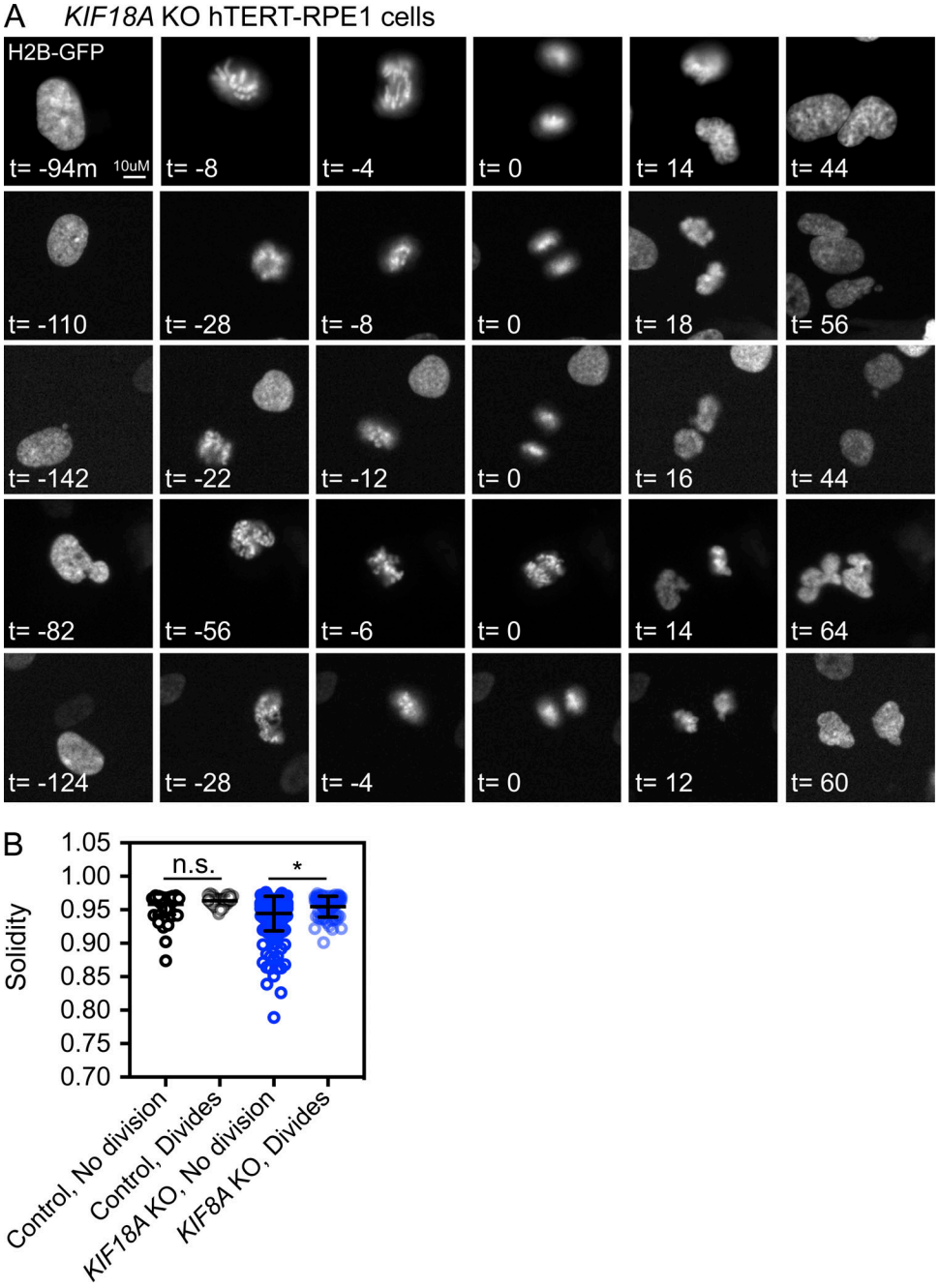
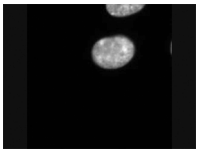
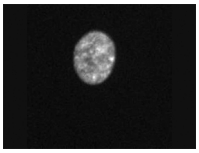


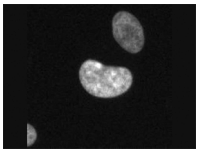
Figure S4. **Interphase nuclear defects occur as *KIF18A* KO cells exit mitosis. (A)** Representative stills from time-lapse imaging of *KIF18A* KO cells transfected with histone-2B-GFP (H2B-GFP). Abnormal nuclear shapes and MN are apparent within 1 h of anaphase (time = 0). **(B)** Plot of nuclear solidity measurements from selected control and *KIF18A* KO hTERT-RPE1 cells. All measurements were made from the first frame taken during the three independent time-lapse imaging experiments analyzed for Fig. 4. Cells were categorized as "divides" if they entered mitosis during the live cell imaging time period. Cells categorized as "no division" remained in the field of view for at least 10 h but did not divide. Data from three independent experiments were compared using a Kruskal–Wallis test with post hoc Dunn's multiple comparison tests. *, $P < 0.001$. Error bars indicate SD. WT RPE1, no division $n = 74$; WT RPE1, divides $n = 32$; KIF18A KO, no division $n = 279$; KIF8A KO, divides $n = 68$. Related to Fig. 4.



Video 1. **Time-lapse fluorescence imaging of normal chromosome division in an hTERT-RPE1 cell expressing H2B-GFP.** Single focal plane images were collected every 2 min and are played back at 10 frames per second. Related to Fig. 4.



Video 2. **Time-lapse fluorescence imaging of MN formation after division of a *KIF18A* KO hTERT-RPE1 cell expressing H2B-GFP.** Single focal plane images were collected every 2 min and are played back at 10 frames per second. Related to Fig. 4.



Video 3. **Time-lapse fluorescence imaging of abnormal nuclear shape formation after division of a *KIF18A* KO hTERT-RPE1 cell expressing H2B-GFP.** Single focal plane images were collected every 2 min and are played back at 10 frames per second. Related to Fig. 4.

Provided online are two tables in Excel. Table S1 summarizes data from FISH experiments and Table S2 summarizes measurements from live cell analyses of H2B-GFP–expressing hTERT-RPE1 cells.