Supplemental material

Bass and Cortez, https://doi.org/10.1083/jcb.201810058

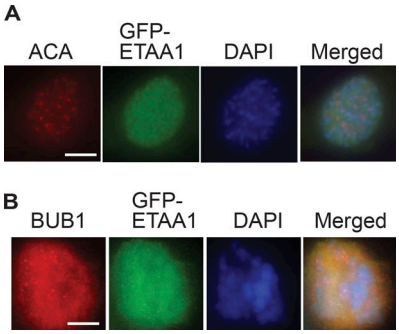


Figure S1. **Localization of ETAA1 in mitotic cells.** Localization of GFP-ETAA1 in mitotic U2OS cells was examined by immunofluorescence. **(A and B)** Kinetochores were stained with ACAs (A) or BUB1 (B) antibody. Bars, 5 µm.

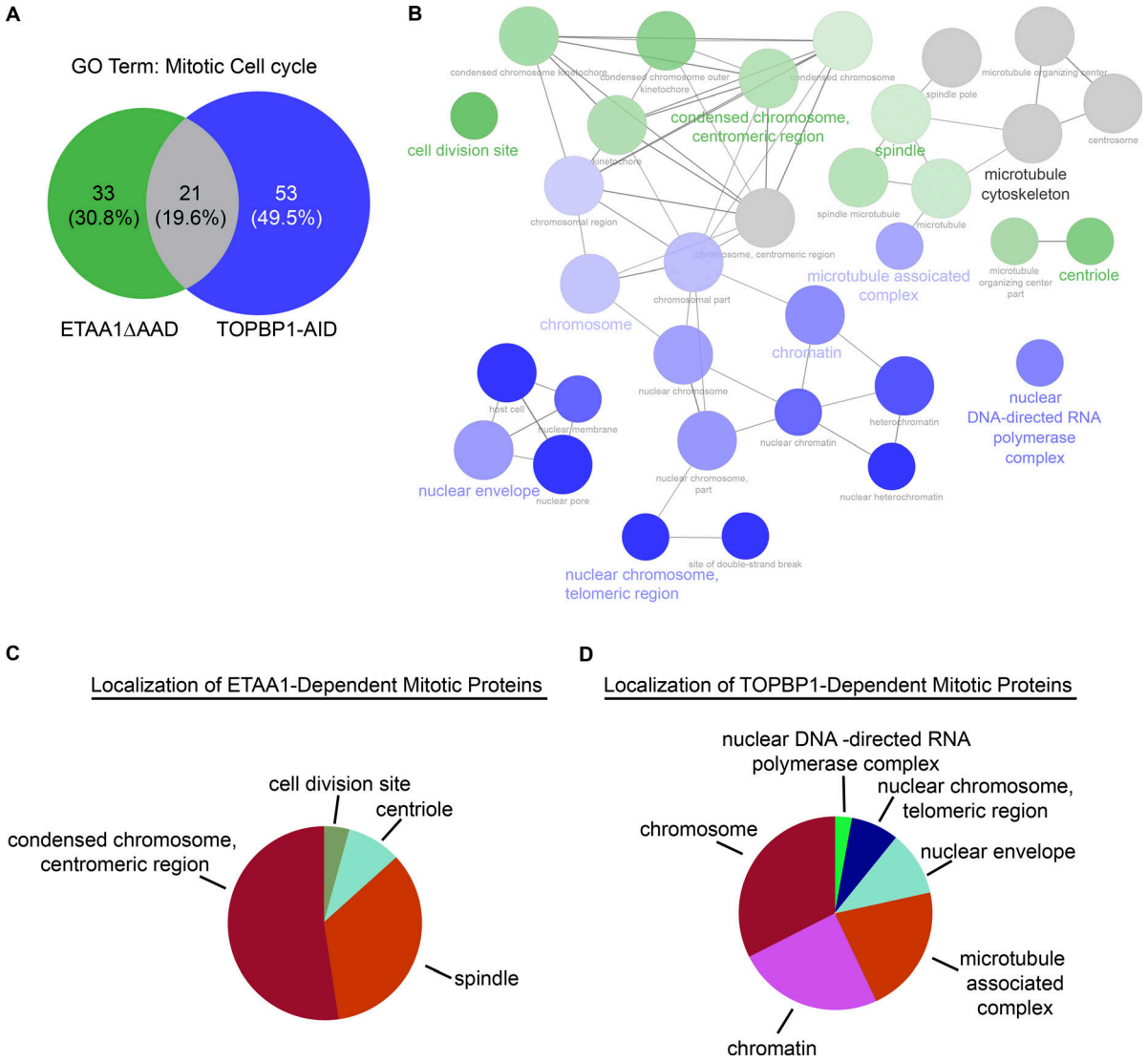


Figure S2. **Analysis of ETAA1- and TOPBP1-dependent phosphoproteins. (A)** Comparison of mitotic cell cycle phosphoproteins regulated by ETAA1 or TOPBP1. **(B)** GO network comparing localization of mitotic cell cycle proteins regulated by ETAA1 or TOPBP1. Each node in the network represents a localization term. Larger node size corresponds with a more significant enrichment. Node color denotes enrichment in ETAA1 or TOPBP1 clusters; darker green corresponds with more enrichment in ETAA1 cluster, while darker blue corresponds to more enrichment in TOPBP1 cluster. Gray nodes are not specific to either ETAA1 or TOPBP1. Similar localization GO terms were placed into functional groups with most significantly enriched term indicated by large colored text. **(C)** Overview chart displaying localization of ETAA1-dependent mitotic proteins. **(D)** Overview chart displaying localization of TOPBP1-dependent mitotic proteins.

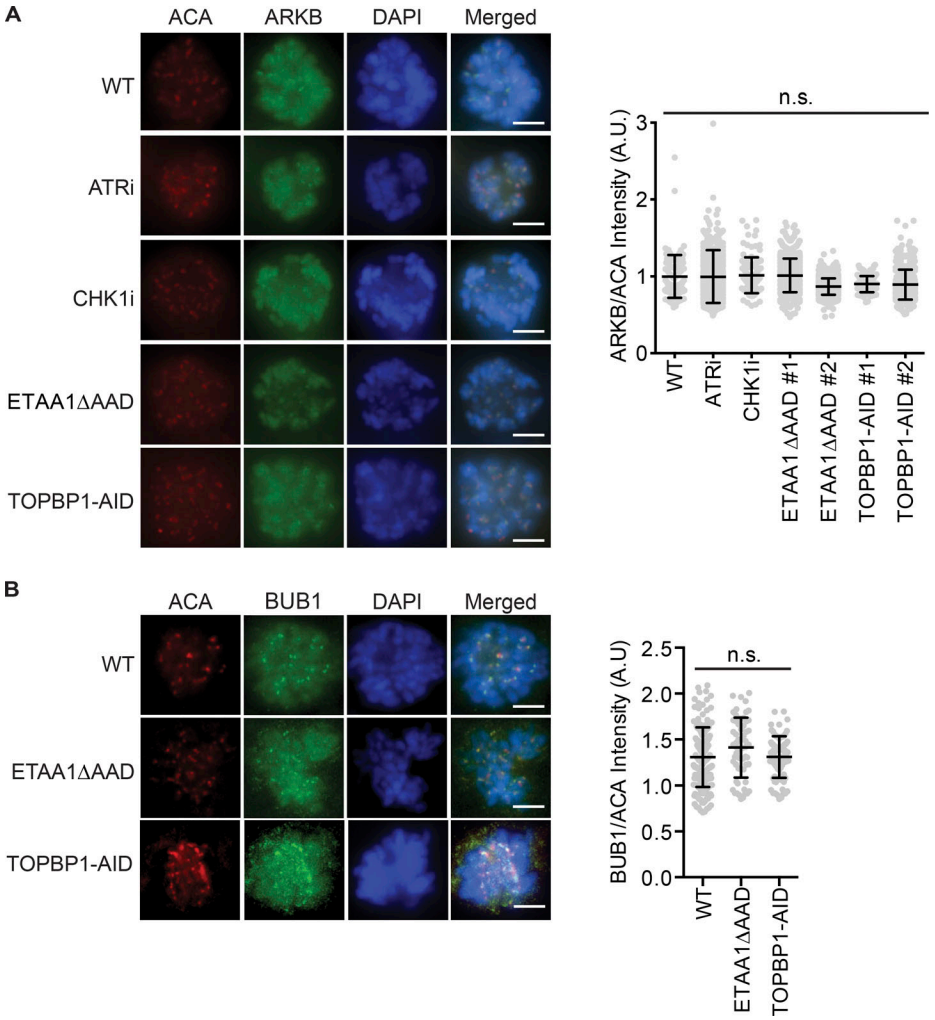


Figure S3. **Localization and expression of Aurora B and Bub1 are unaltered by ATR inactivation.** HCT116 (WT) and two clones of ETAA1ΔAAD and TOPBP1-AID cells were arrested in G_2 by addition of a CDK1 inhibitor (RO-3306; 10 µm) for 16 h to avoid replication stress induced by ATRi or TOPBP1 degradation. Cells were treated with IAA or ATRi for 2 h then released into fresh media containing taxol, IAA, or ATRi. Cells were fixed 1 h after release. **(A and B)** Shown are representative immunofluorescent images and quantification of Aurora B (A) or BUB1 (B) from two independent experiments in which at least 100 cells were measured per condition. Cells were costained with ACAs to show kinetochore localization. Bars, 5 µm. Error bars represent standard deviation. n.s., not significant.

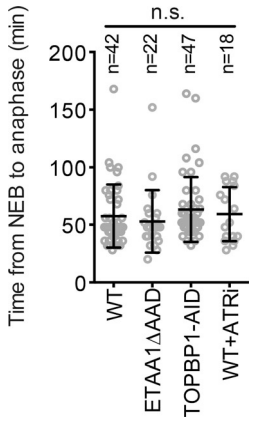


Figure S4. **Length of mitosis is unchanged by loss of ETAA1, TOPBP1, or ATR activity**. HCT116 WT, ETAA1ΔAAD, and TOPBP1-AID cells expressing GFP-H2B for chromatin visualization were examined by live cell imaging and time between NEB and onset of anaphase was measured. The number of cells examined is indicated in parentheses. Significance was determined by ANOVA with a Dunnett multiple comparison post-test. n.s., not significant, $P > 0.05$.

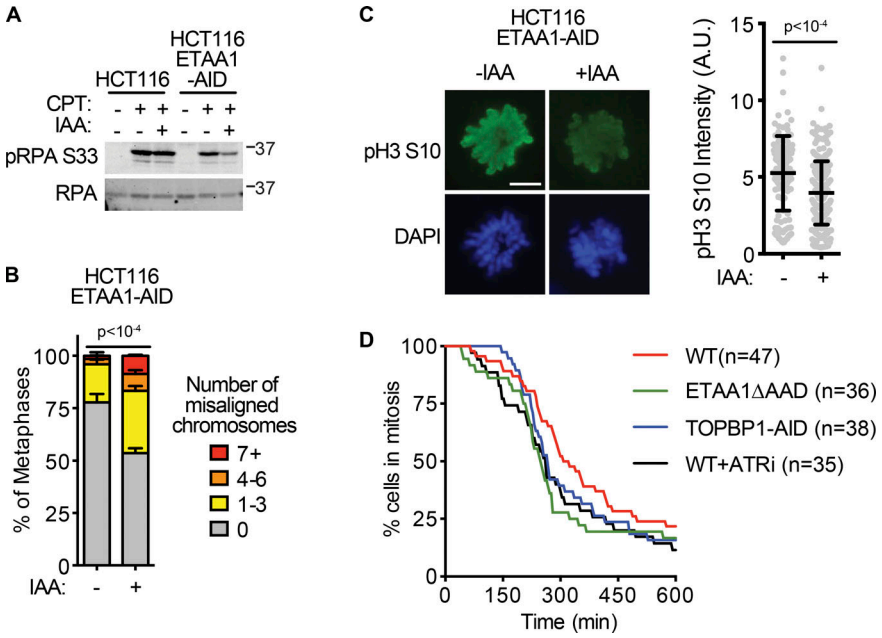
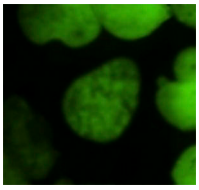
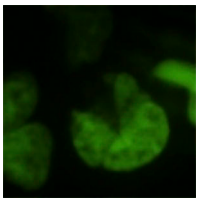


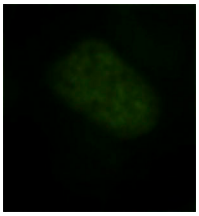
Figure S5. **ETAA1-AID cells exhibit mitotic defects**. **(A)** HCT116 WT and HCT116 ETAA1-AID cells were treated with 100 nM CPT for 4 h after a 2-h pretreatment with IAA. Cells were then lysed and immunoblotted with the indicated antibodies. **(B)** HCT116 WT and HCT116 ETAA1-AID cells were arrested for 16 h with CDK1i, mock-treated or treated with IAA for 2 h, released from CDK1i, and fixed. Metaphase cells were scored for the number of misaligned chromosomes. Displayed is the mean and standard deviation of three independent experiments in which at least 100 metaphases were scored per condition. **(C)** pH3 S10 was measured in HCT116 WT and HCT116 ETAA1-AID cells. Cells were arrested with CDK1i, mock- or IAA-treated for 2 h, released from CDK1i, and fixed. Bar, 5 µm. Mean and standard deviation is shown in representative quantification from two independent experiments. Significance was determined by a Dunnett multiple comparison post-test. **(D)** Synchronized HCT116 WT, ETAA1ΔAAD, and TOPBP1-AID cells were examined for ability to sustain mitotic arrest during nocodazole treatment. Cells expressing H2B-GFP were released from a double thymidine block, and 1 µM nocodazole, 250 µM reversine, and ATRi were added 1.5 h before starting imaging. The number of cells scored is indicated in parentheses. Molecular weight is given in kilodaltons.



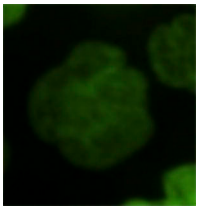
Video 1. **HCT116-WT progression through mitosis.** Representative video of a HCT116 cell expressing H2B-GFP used, for quantification in Fig. 6 B and Fig. S4. Images were acquired every 4 min.



Video 2. **HCT116-ATRi–treated cells progression through mitosis.** Representative video of an ATRi-treated cell expressing H2B-GFP, used for quantification in Fig. 6 B and Fig. S4. Images were acquired every 4 min.



Video 3. **HCT116-ETAA1ΔAAD progression through mitosis.** Representative video of an ETAA1ΔAAD cell expressing H2B-GFP, used for quantification in Fig. 6 B and Fig. S4. Images were acquired every 4 min.



Video 4. **HCT116-TOPBP1-AID progression through mitosis.** Representative video of a TOPBP1-AID cell expressing H2B-GFP, used for quantification in Fig. 6 B and Fig. S4. Images were acquired every 4 min.

Tables S1, S2, and S3 are provided as separate Microsoft Excel files. Table S1 shows phosphoproteomic data from ETAA1ΔAAD and TOPBP1-AID cells Table S2 shows ETAA1- and TOPBP1-dependent phosphorylation sites. Table S3 shows GO analysis of ETAA1- and TOPBP1-dependent phosphoproteomes