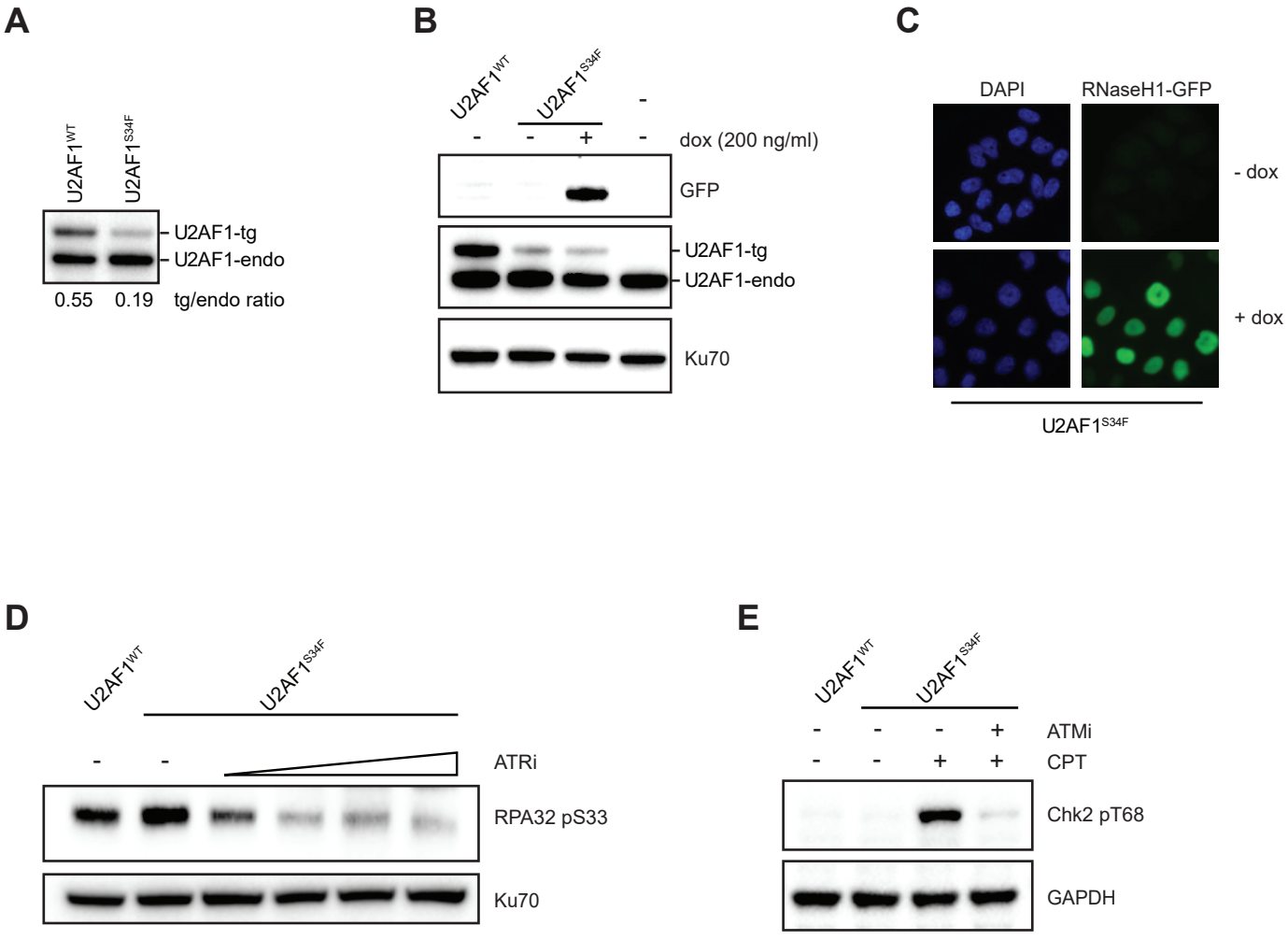
Supplementary Fig. S1



Supplementary Figure S1. U2AF1^{S34F} expressing cells induce R loop-associated ATR-mediated RPA32 phosphorylation. (**A**) HeLa cells stably expressing U2AF1^{WT} or U2AF1^{S34F} mutant were grown asynchronously. Levels of transgenic (tg) and endogenous (endo) U2AF1 proteins were analyzed by western blot. Indicated band intensities were quantified using BioRad Image Lab to calculate their ratios. (**B** and **C**) HeLa cells stably expressing U2AF1^{WT} or U2AF1^{S34F} mutant were grown asynchronously. Expression of RNaseH1 in U2AF1^{S34F} cells was induced by addition of doxycycline (200 ng/ml) for 24h. Levels of indicated proteins were analyzed by western blot in **B**. In **C**, nuclear version of GFP-tagged RNaseH1 in U2AF1^{S34F} cells was analyzed by immunofluorescence using an anti-GFP antibody. **(D** and **E)** HeLa cells expressing U2AF1^{WT} or U2AF1^{S34F} were grown for 48h. In **D**, U2AF1^{S34F}-expressing cells were treated with two-fold dilution ATRi (1.25 -10 µM) for 1h. In **E**, U2AF1^{S34F}-expressing cells pre-treated with either DMSO or 10 µM ATMi for 15 minutes prior to treatment with 1 µM CPT for an additional 1h.