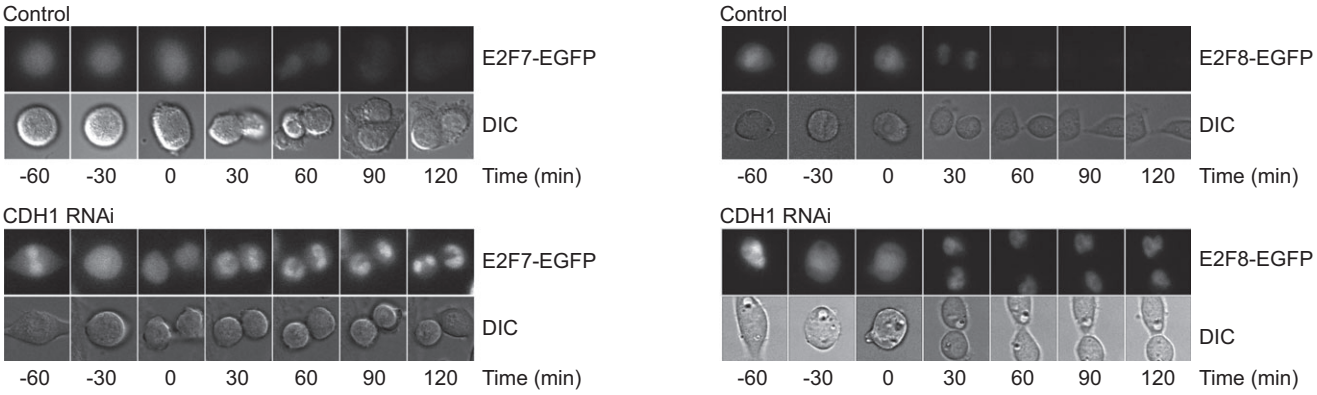
Expanded View Figures

A



B

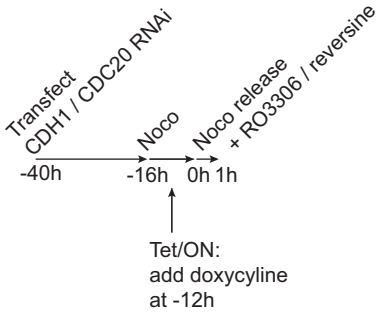


Figure EV1. Detection of E2F7/8 during mitosis and mitotic exit.

A Montages of representative cells from HeLa cells with inducible expression of E2F7/8-EGFP. Time (min) indicates time from onset of anaphase.

B Schematic overview of mitotic release experiments shown in Fig 2G and H.

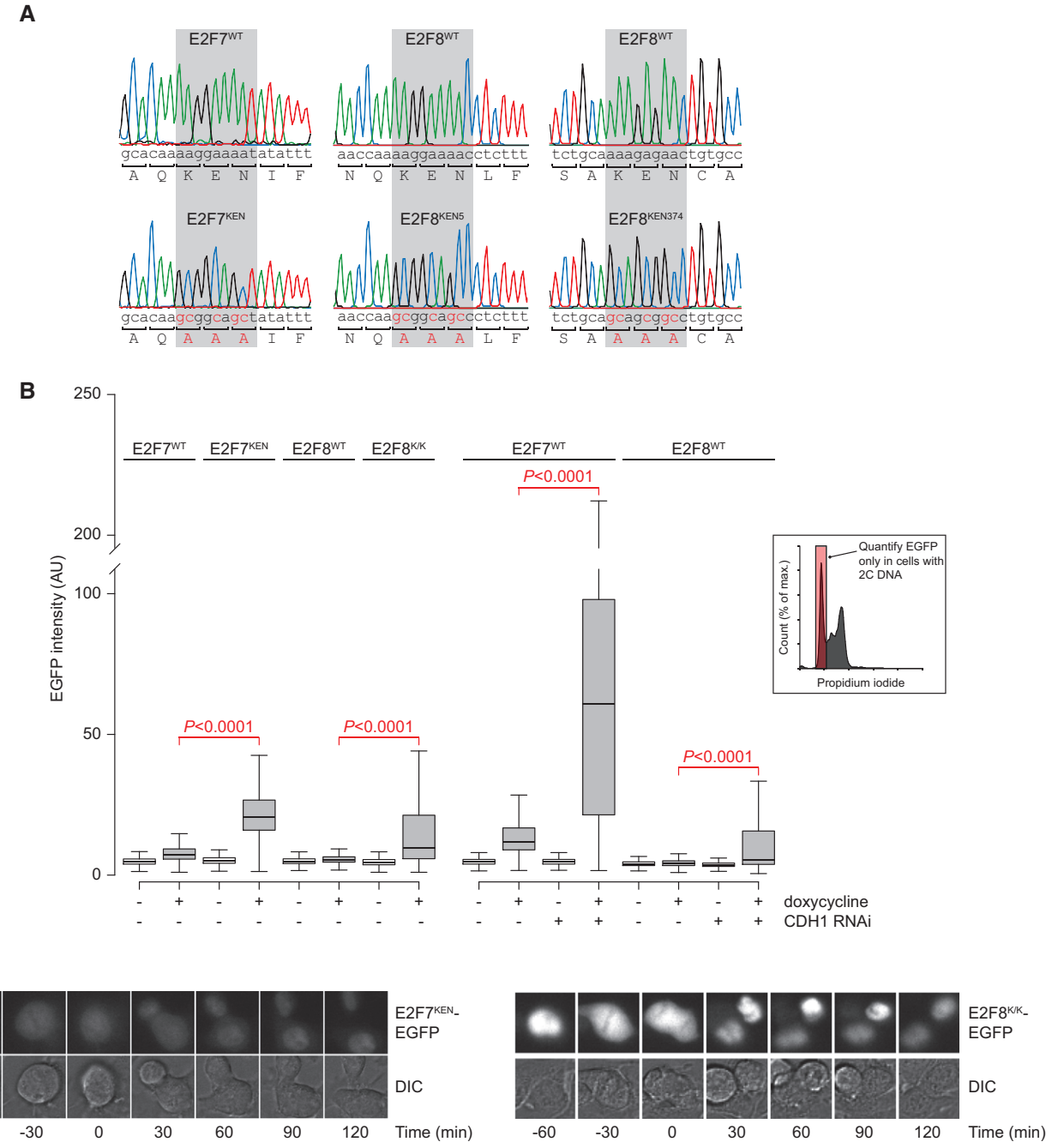


Figure EV2. Generation of KEN mutant E2F7 and E2F8.

A Sanger sequencing result after site-directed mutagenesis of the nucleotides encoding the KEN domains in E2F7 and E2F8. The middle panel shows the N-terminal KEN sequence starting at amino acid 5, the right panel shows the KEN domain starting at amino acid 374 in E2F8.

B Quantification of EGFP levels determined with FACS analysis in HeLa cell lines with stable inducible expression of indicated constructs, with or without CDH1 RNAi transfection. Boxes indicate 25th and 75th percentiles, whiskers indicate 5th and 95th percentiles. For every condition, 10,000 cells were measured. Statistics were performed with Kruskal-Wallis tests followed by Dunn's individual group comparison method. The inlay shows how we first gated the G1 cells (2C DNA content) prior to EGFP measurement.

C Montages of representative HeLa cells expressing KEN mutant versions of E2F7/8. Time (min) indicates time from onset of anaphase.

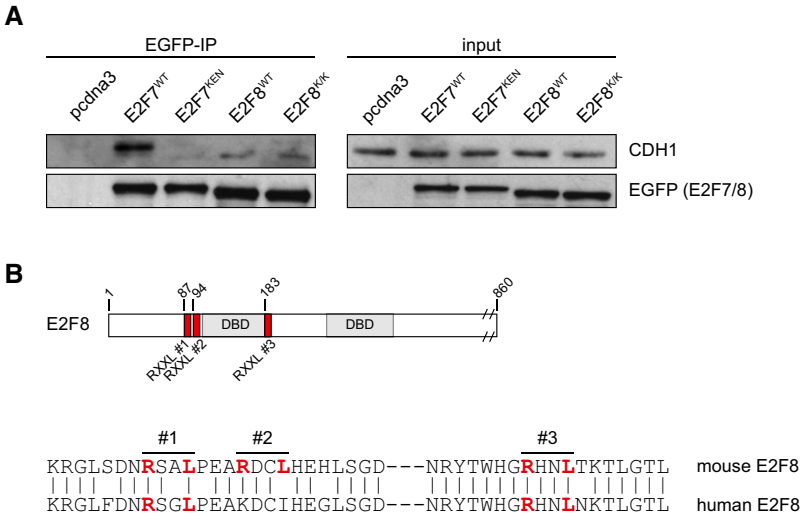


Figure EV3. E2F8 binds CDC20.

A Co-immunoprecipitation of EGFP-tagged E2F7 and E2F8 with endogenous CDH1. GFP-binding agarose beads were used to pull down the fusion proteins. Cells were treated with 10 μM MG132 for 5 h prior to harvesting to prevent potential immediate proteasomal degradation of E2F7/8 after binding to CDH1.

B Schematic overview of putative D-boxes (RXXL) in mouse E2F8, and alignment of human and mouse E2F8 sequences.

C Co-immunoprecipitation of EGFP-tagged E2F8^{WT} or E2F8$^{K/Kmut}$ with CDC20-Flag after 48 h of co-expression in 293T cells. Cells were treated with 10 μM MG132 for 5 h prior to harvesting to prevent potential immediate proteasomal degradation of E2F7/8 after binding to CDC20.

D Co-immunoprecipitation as in (B), performed with GFP-binding agarose beads. Note that CDC20 only interacted with E2F8, in a KEN-independent manner.

Figure EV4. Stabilization of KEN mutant E2F7/8 inhibits DNA replication and triggers cell death.

A FACS data showing scatterplots of DNA synthesis (anti-BrdU-FITC) versus DNA content (propidium iodide) in cells with stable expression of indicated inducible constructs. Gates were used to calculate the percentage of BrdU-positive cells shown in Fig 4A.

B Montages of PCNA dot formation and cell death in cells with inducible expression of indicated constructs, and effect of CDH1 RNAi. Arrowheads indicate a cell that enters S phase, but undergoes cell death. Time in hh:mm from the onset of imaging and doxycycline induction.

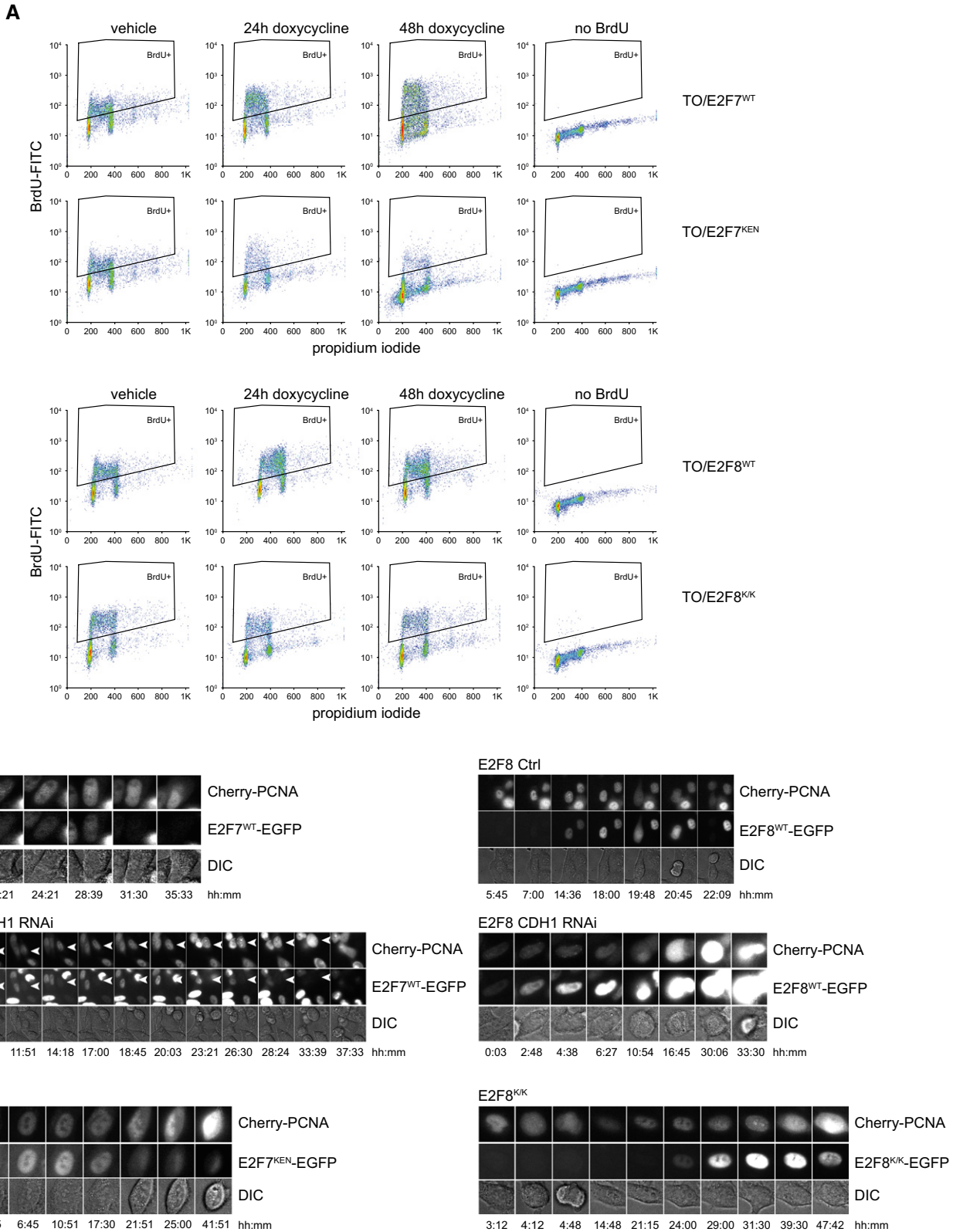


Figure EV4.

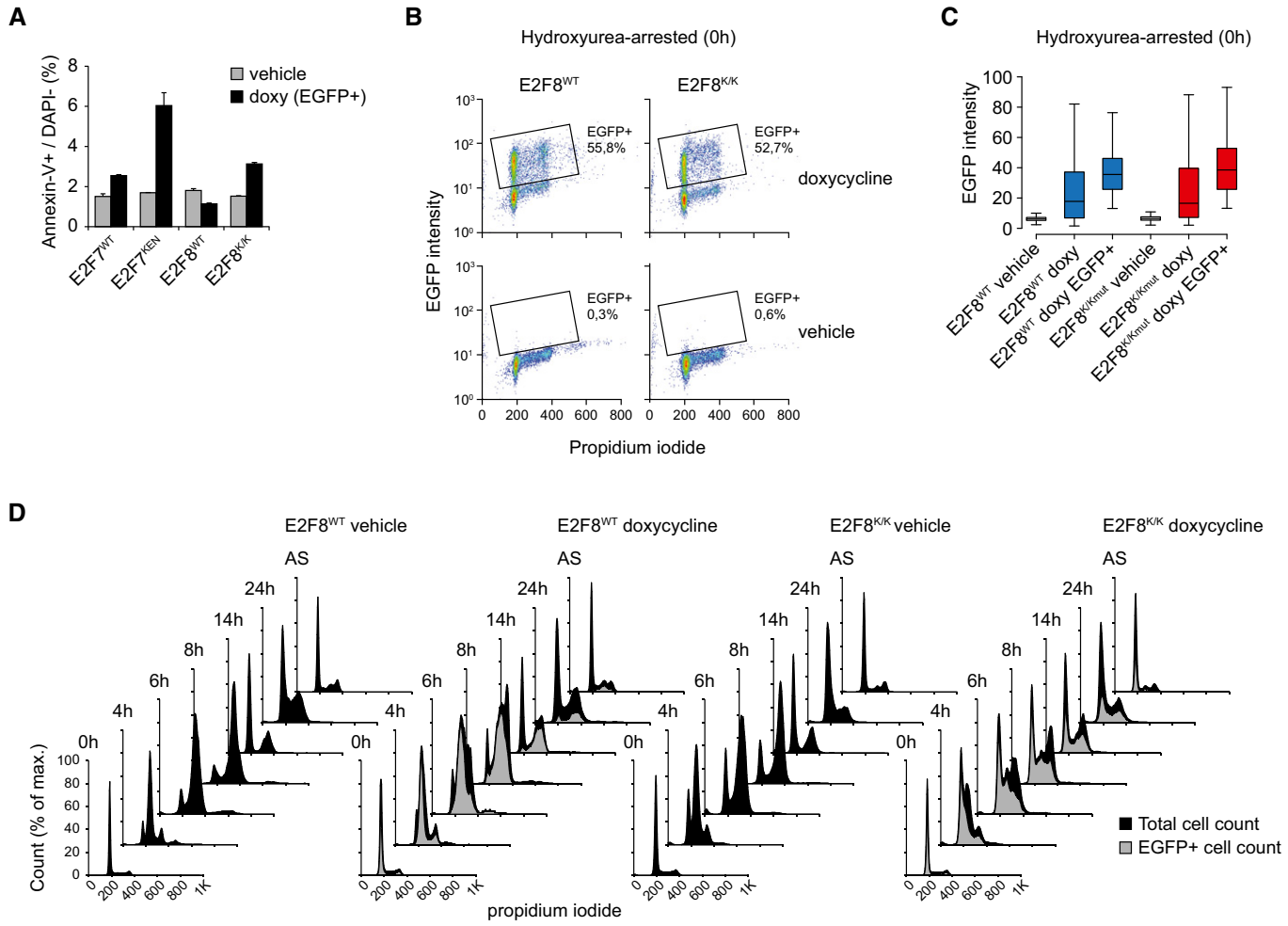


Figure EV5. Stabilization of KEN mutant E2F7/8 inhibits DNA replication and triggers cell death; continued.

A Apoptosis, measured by FACS analysis of annexin V-stained HeLa cells expressing the indicated inducible constructs. Apoptosis staining was done using the annexin V-APC detection kit (Thermo Scientific), according to the manufacturer's instructions. Doxycycline was added 24 h prior to harvesting, and only EGFP-positive cells were measured to avoid bias from differences in percentages of E2F-expressing cells. Bars represent average ± s.e.m. of four independent replicates.

B FACS plots of DNA content versus E2F8-EGFP intensity in HU-arrested cells, 12 h after doxycycline induction.

C Quantification of EGFP levels in cells with 2C DNA content, determined with FACS analysis in HeLa cell lines with stable inducible expression of indicated constructs. Boxes indicate 25th and 75th percentiles, whiskers indicate 5th and 95th percentiles. For every condition, 10,000 cells were measured.

D Representative FACS DNA content plots of HeLa cells with stable inducible expression of indicated E2F8 constructs. Gray overlays indicate the fraction of E2F8-EGFP-positive cells within each doxycycline-treated cell line.

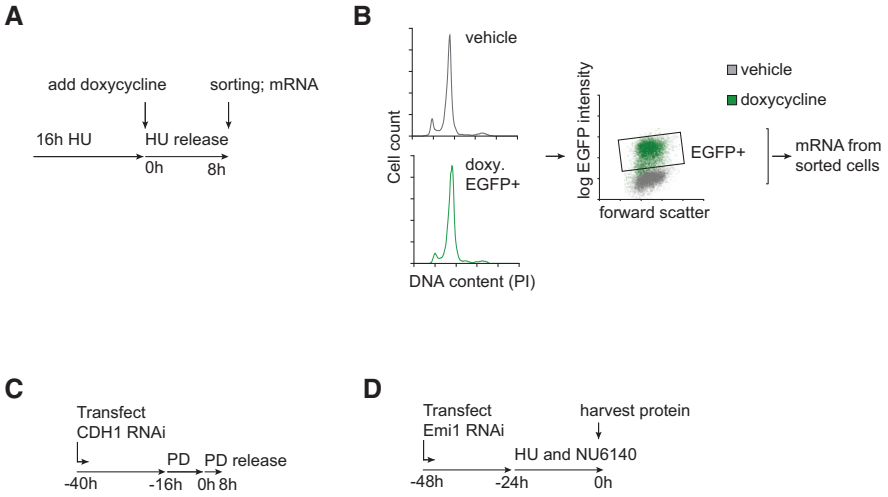


Figure EV6. Schematic overviews of experiments to show feedback between atypical E2Fs and APC/C^{Cdh1}.

A Experimental scheme of FACS sorting in synchronized cells with stable expression of E2F7/8 in the presence or absence of doxycycline induction.

B Example of gating strategy to sort late S/G2 cells with detectable E2F7/8-EGFP.

C Schematic overview of synchronization using the CDK4/6 inhibitor PD0332991. RPE cells were transfected with CDH1 RNAi, then treated overnight with 0.5 μM of the CDK4/6 inhibitor PD0332991, and released by washing away the drug.

D Schematic overview of Emi1 RNAi experiments in RPE cells. Hydroxyurea and the CDK2 inhibitor NU6140 were added simultaneously.