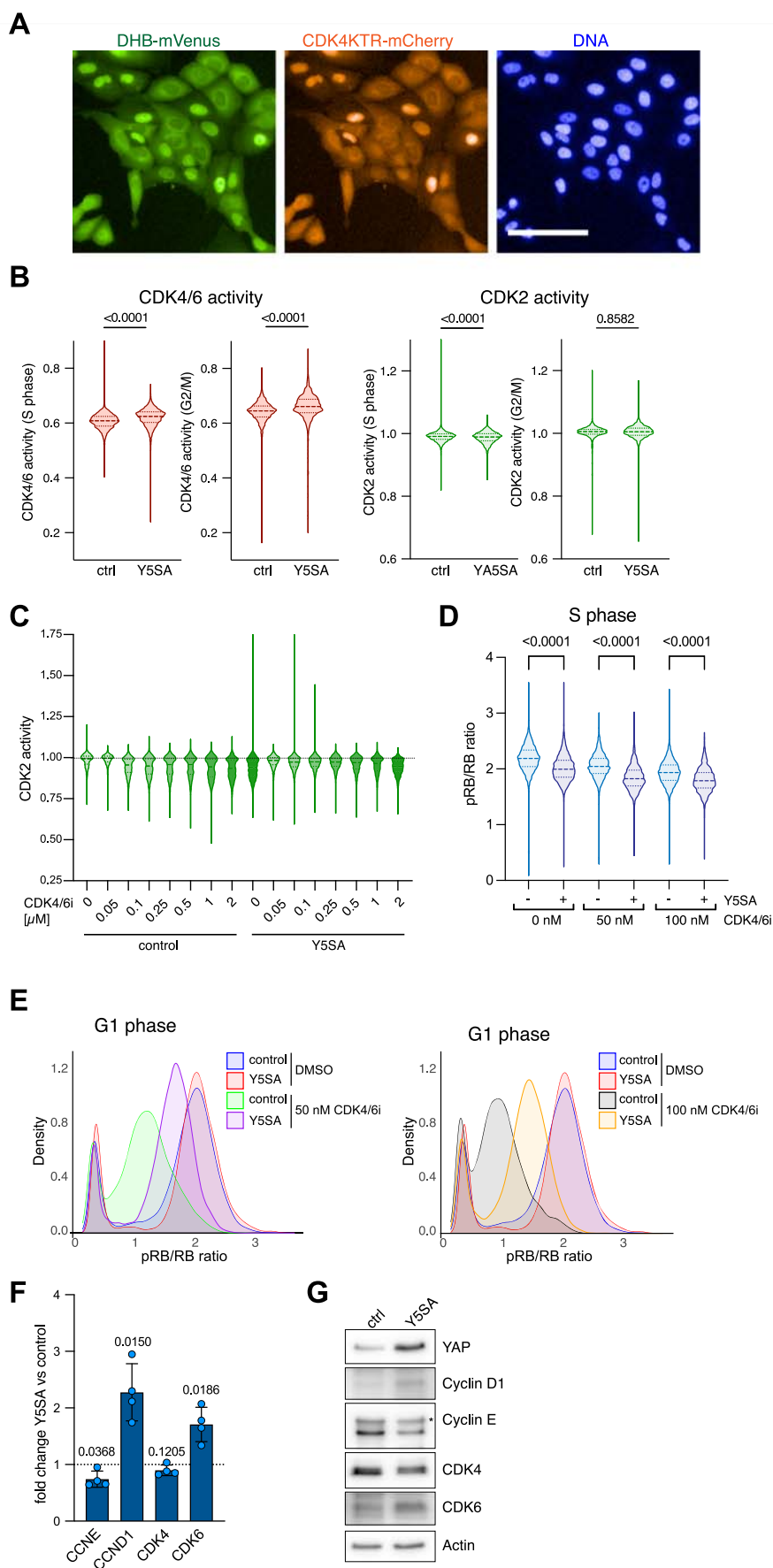


Expanded View Figures

Figure EV1. Oncogenic YAP activates CDK4/CDK6 in G1, but does not lead to CDK2 activation.

(A) Representative immunofluorescence images showing the activity-sensors for CDK2 (DHB-mVenus) and CDK4 (mCherry-CDK4KTR). Scale bar: 100 μ m. (B) CDK4/6 and CDK2 activity during S and G2 phase in control and YAP5SA-expressing MCF10A cells. Violin plots display single-cell measurements from at least 2064 cells per condition. *P* values were calculated using unpaired Student's *t* test ($n = 3$ independent replicates). (C) CDK2 activity of control and YAP5SA-expressing MCF10A cells treated with increasing concentrations of palbociclib, measured by high-content microscopy using the CDK2 reporter construct. Data represent single-cell analysis from at least 1038 cells per condition from a representative experiment ($n = 3$ independent replicates). (D) Distribution of the pRB/RB ratio in S-phase. Violin plots display single-cell measurements from a representative experiment, with at least 1441 cells analyzed per condition. *P* values were calculated using ordinary one-way ANOVA ($n = 3$ independent replicates). (E) Density plots of the pRB/RB ratio for the conditions indicated in the legend. Kernel density estimation (KDE) was used to visualize the distribution of the pRB/RB ratios without binning. The density is normalized such that the area under each curve equals 1. (F) RT-qPCR analysis of the indicated G1 cyclins and CDKs. Data are shown as mean $\pm$ SD. Statistical significance was assessed using Student's *t* test ($n = 4$ independent replicates)). (G) Western blot analysis of the indicated G1 cyclins and CDKs. Actin served as a loading control. Source data are available online for this figure.



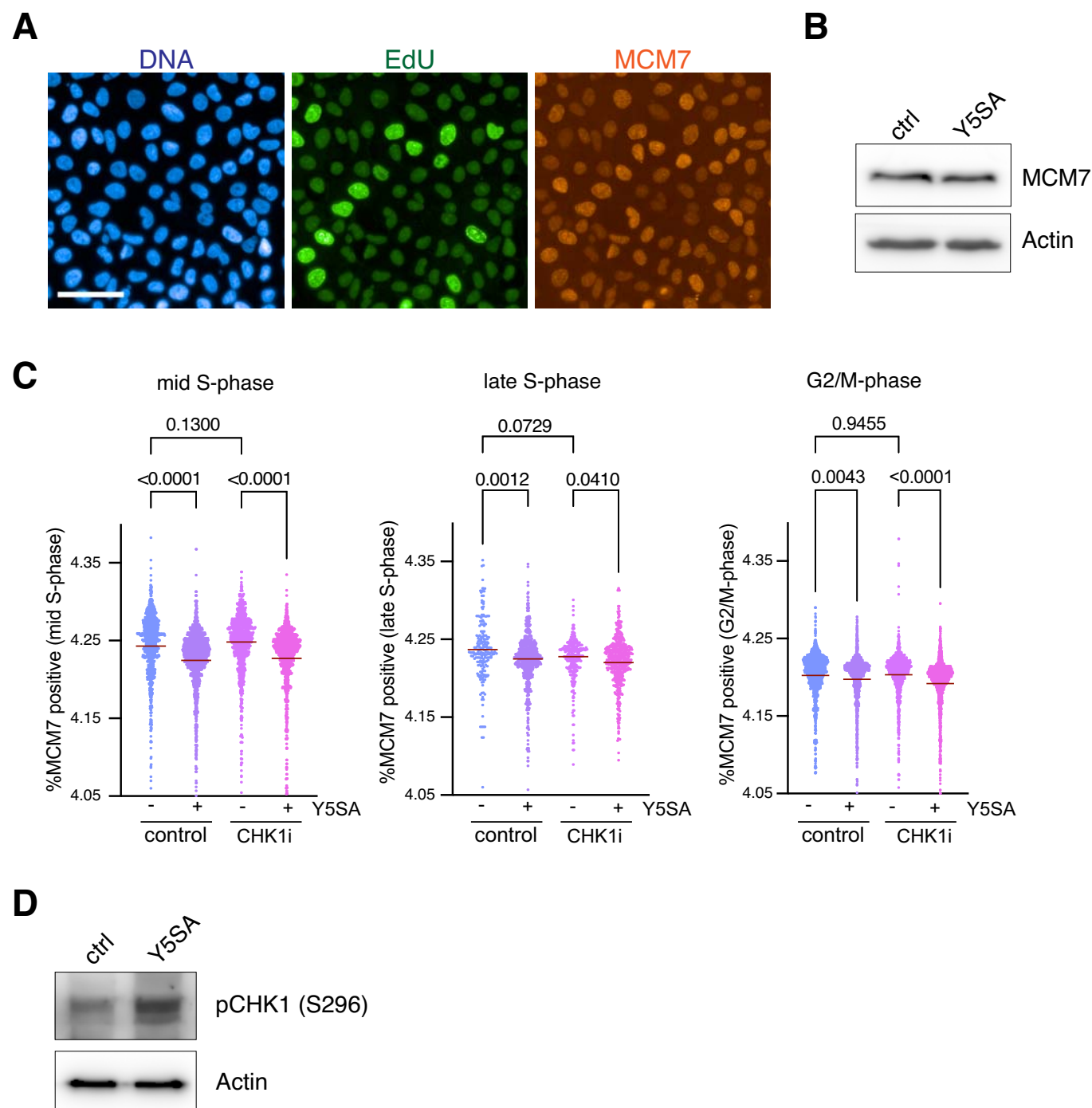


Figure EV2. YAP5SA does not alter MCM7 protein levels.

(A) Representative immunofluorescence image of MCM7 staining. Scale bar: 50 μ m. (B) Immunoblot analysis to determine MCM7 levels. Actin served as a loading control. (C) Chromatin-bound MCM7 in mid and late S-phase and in G2. Violin plots display single-cell measurements from a representative experiment, with ≥ 204 cells analyzed per condition. *P* values were calculated using ordinary one-way ANOVA ($n = 3$ independent replicates). (D) Immunoblot analysis to determine pCHK1 (S296). Actin served as loading control. Source data are available online for this figure.

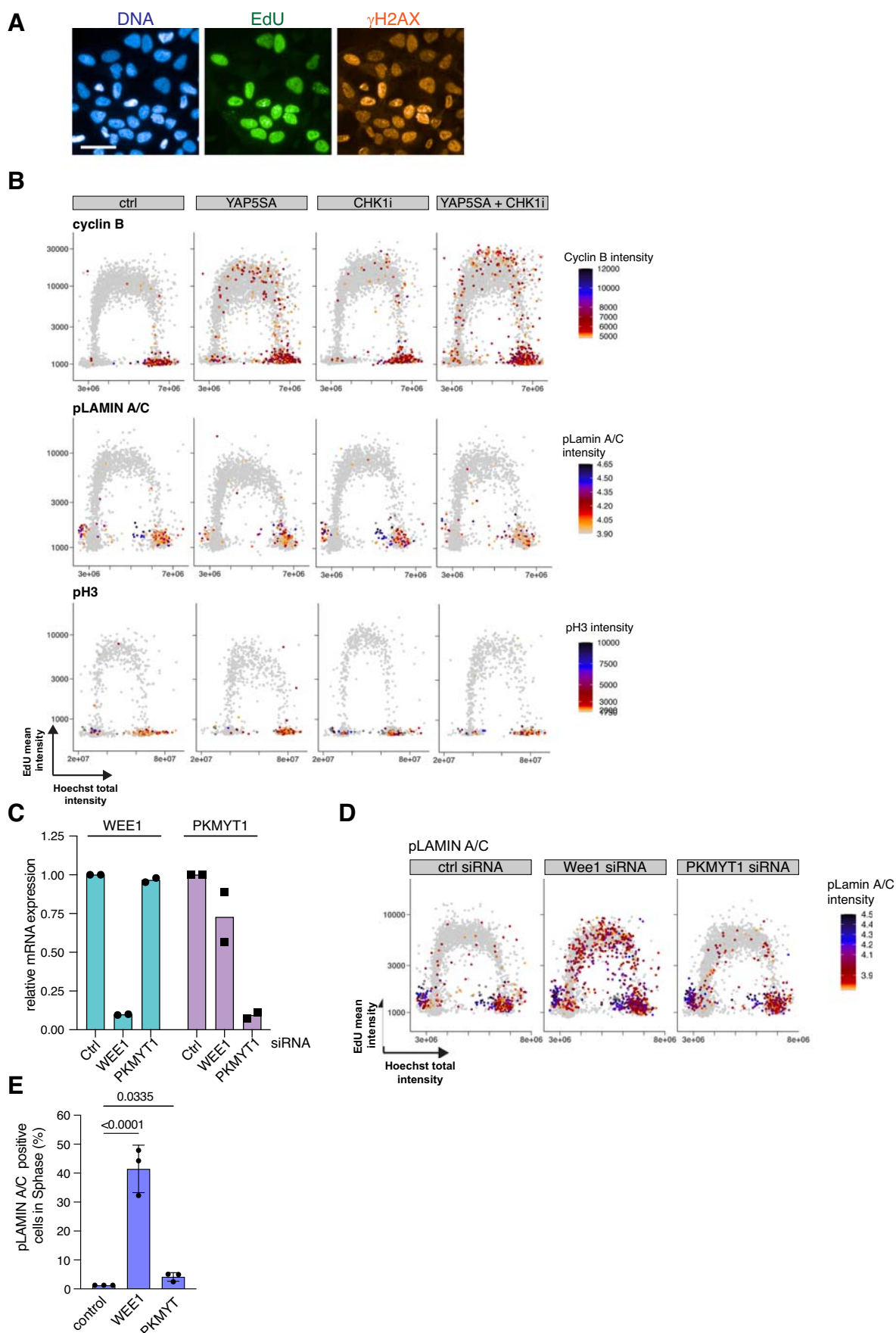


Figure EV3. CHK1 inhibition in YAP5SA-expressing cells does not lead to premature mitosis in S-phase.

(A) Representative immunofluorescence images of γ H2AX immunostaining. Scale bar: 50 μ m. See also Fig. 4B. (B) High-content microscopy-based analysis of cytoplasmic cyclin B, pLAMIN A/C(S22) and pH3(S10). S-phase cells were labeled with EdU. For each panel, the following number of cells were randomly selected: cyclin B: 5000 cells, pLamin: 3500 cells, pH3: 1600 cells ($n = 3$ independent replicates). (C) RT-qPCR was used to validate the siRNA-mediated knockdown of WEE1 and PKMYT1 ($n = 2$ independent replicates). (D) High-content microscopy-based analysis of pLAMIN A/C(S22) in cells transfected with WEE1 and PKYT1 specific siRNAs or with a control siRNA. For each sample, 4000 cells were randomly selected ($n = 3$). (E) Quantification of the percentage of cells positive for pLAMIN A/C(S22) in S-Phase in the experiment shown in (D). Mean $\pm$ SD. P values were calculated using ordinary one-way ANOVA ($n = 3$ independent replicates). Source data are available online for this figure.

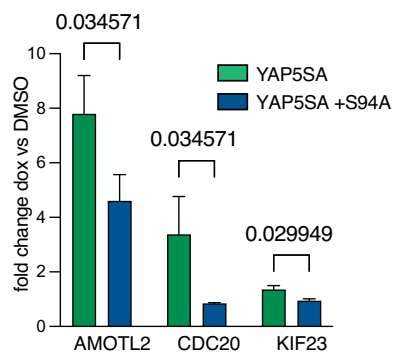
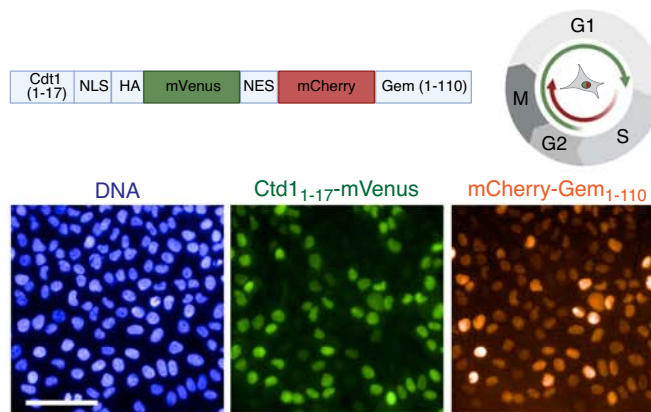
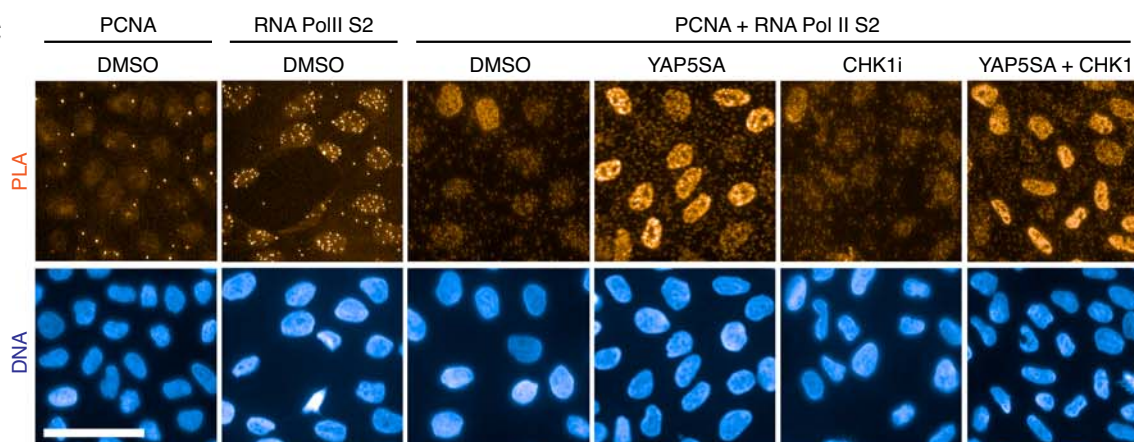
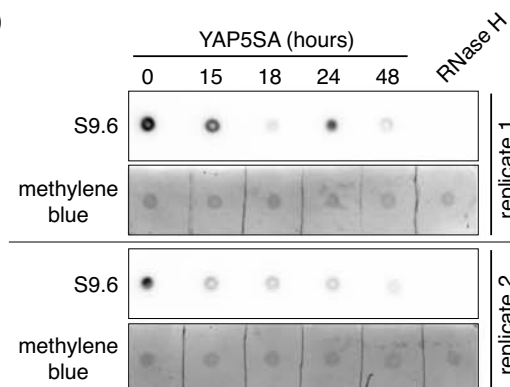
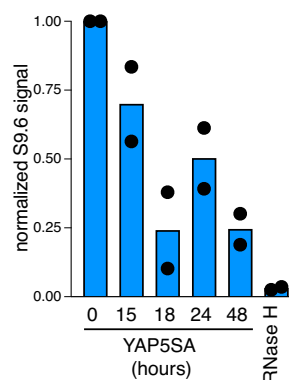
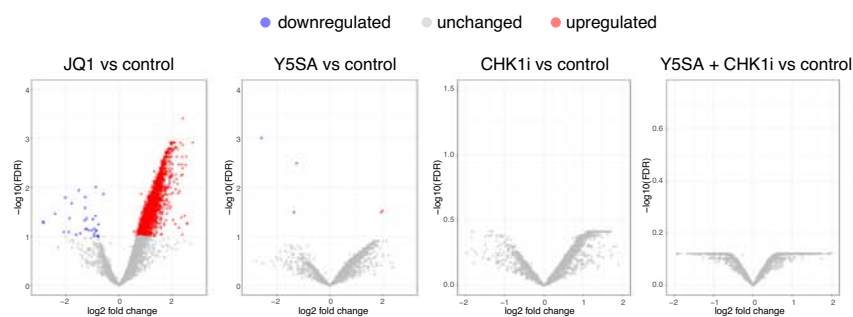
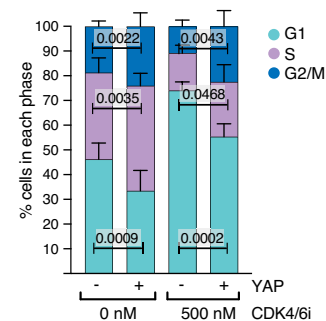
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Figure EV4. YAP5SA enhances the proximity between the transcription and replication machinery but does not lead to increased R-loop levels.

(A) RT-qPCR was used to analyze expression of the indicated genes upon expression of YAP5SA or YAP5SA-S94A. Mean $\pm$ SD. *P* values were calculated using an unpaired Student's *t* test ($n = 3$ independent replicates). (B) Scheme of the PIP-FUCCI cell cycle sensors and representative images of MCF10A-YAP5SA cells expressing the sensors. Scale bar: 100 μ m. (C) Representative images of Proximity Ligation assay (PLA) between PCNA and RNA Pol II phosphorylated at Serine 2. Scale bar: 50 μ m. See Fig. 5I. (D) Dot blot assay to detect R-loops in control MCF10A cells and cells expressing YAP5SA for the indicated times. Total DNA was analyzed with the S9.6 monoclonal antibody, which recognizes DNA:RNA hybrids. RNase H1 treatment served as a control. Methylene blue staining was used as a control for equal loading ($n = 2$ independent replicates). (E) Quantification of the S9.6 dot blot signal in (D) normalized to total DNA detected by methylene blue staining ($n = 2$ independent replicates). (F) MapR was used to assess R-loop levels in control MCF10A cells and YAP5SA-expressing cells control treated or after treatment with CHK1i. Treatment with the BRD4 inhibitor JQ1, which has been shown to increase R-loop levels, served as positive control. MapR peaks were called by MACS2. The volcano plot shows differential R-loops as identified by DiffBind ($n = 2$ independent replicates). (G) Fraction of cells in each phase of the cell cycle with and without treatment with 500 nM palbociclib for 24 h. Mean $\pm$ SD. *P* values were calculated using ordinary one-way ANOVA ($n = 5$ independent replicates). Source data are available online for this figure.