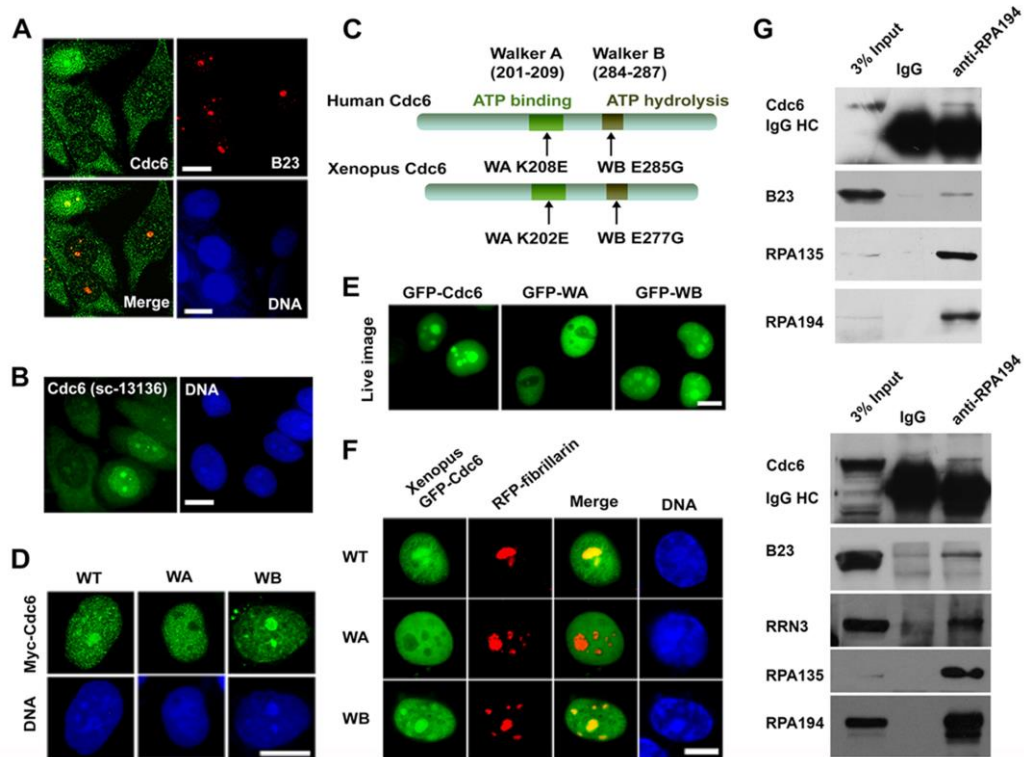
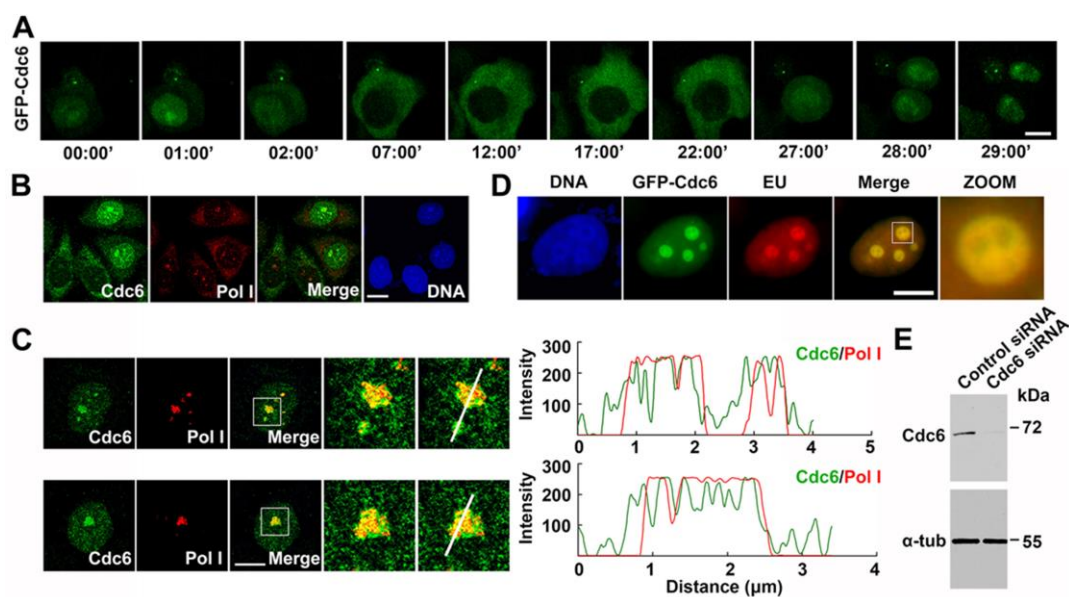


## 1. Supplemental Figures



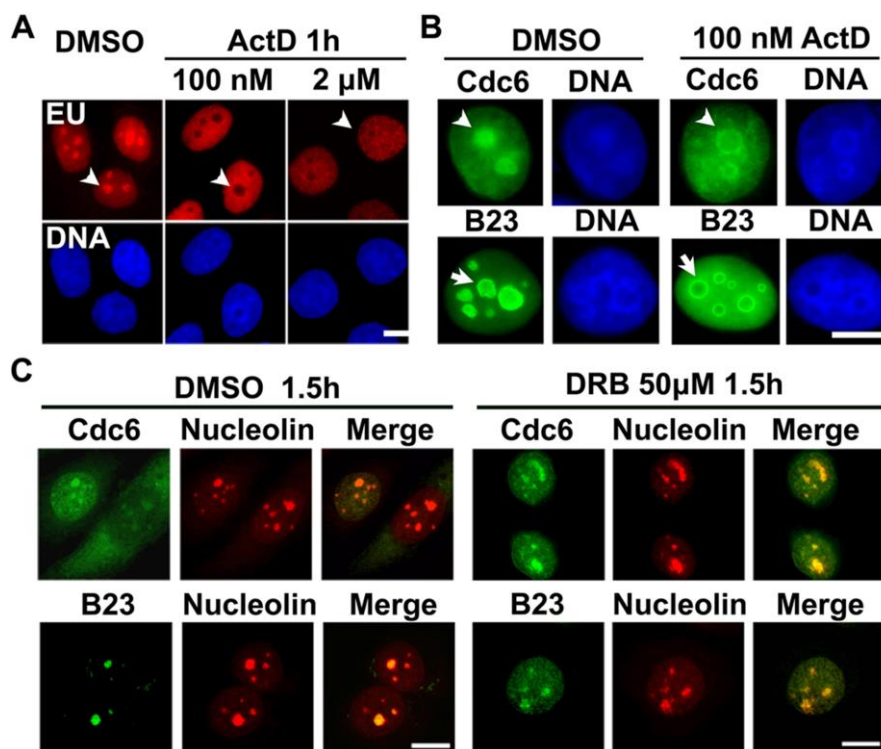
**Figure S1. Cdc6 localizes in the nucleolus dependent on its ATP-binding domain.**

(A) Cdc6 colocalizes with B23. Representative confocal immunofluorescence images of HeLa cells stained for Cdc6 and B23. Scale bar, 10  $\mu$ m. (B) Cdc6 localizes in the nucleolus. Representative immunofluorescence images of HeLa cells stained for Cdc6 by a second monoclonal antibody against Cdc6 (sc-13136, Santa Cruz). Scale bar, 10  $\mu$ m. (C) Schematic representation of human and Xenopus Cdc6 with Walker A (WA) and Walker B (WB) motifs shown, and with the mutation sites of WA and WB mutants indicated. (D) Representative immunofluorescence images of HeLa cells transfected with Myc-Cdc6 WT, WA or WB and stained for Myc-Cdc6. Scale bar, 10  $\mu$ m. (E) Live cell imaging of HeLa cells transfected with GFP-Cdc6 WT, WA mutant or WB mutant. Scale bar, 10  $\mu$ m. (F) Representative immunofluorescence images of XTC cells co-transfected with Xenopus GFP-Cdc6 WT, GFP-WA or GFP-WB and RFP-fibrillarin. Scale bar, 10  $\mu$ m. (G) Cdc6 interacts with RPA194. HEK 293T cell nuclear extracts were immunoprecipitated with RPA194 antibody and followed by Western blotting. Two repeats of the experiments are shown.



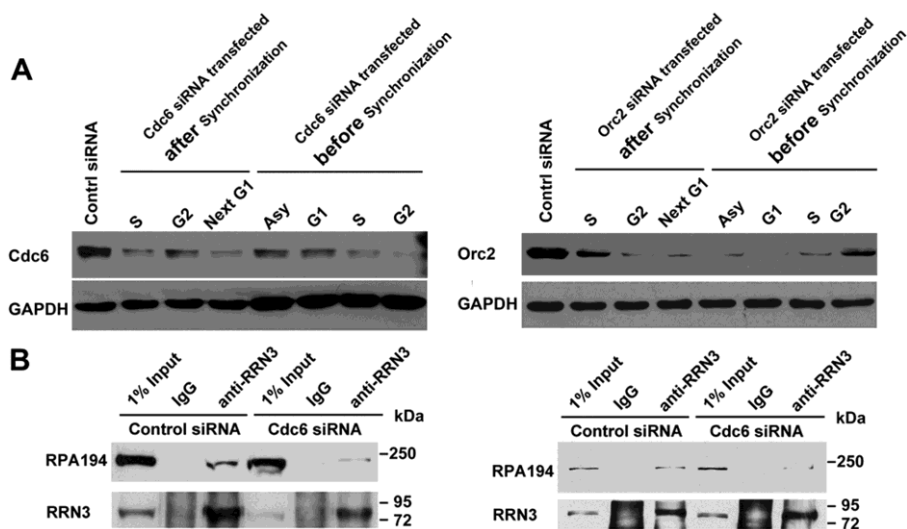
**Figure S2. Cdc6 colocalizes with Pol I and rDNA transcription sites.**

(A) Cellular location of Cdc6 in a whole cell cycle. Time-lapse live cell imaging of HeLa cells transfected with GFP-Cdc6 progressing from nucleolar localized G1 phase to the next G1 phase. Scale bar, 10  $\mu$ m. (B) Cdc6 colocalizes with Pol I. Representative confocal immunofluorescence images of asynchronous HeLa cells stained for Cdc6 and Pol I. Scale bar, 10  $\mu$ m. (C) The nucleolar Cdc6 and RPA194 signals are overlapping by line scan bisecting analysis. Representative confocal immunofluorescence images of HeLa cells stained with Cdc6 and RPA194. Line scan bisecting the nucleolus and intensity were analyzed by ImageJ showing overlap of the Cdc6 and Pol I signals in the nucleolus. (D) Cdc6 colocalizes with rRNA transcription sites in the nucleolus. Representative immunofluorescence images of EU incorporation in HeLa cells transfected with GFP-Cdc6. Scale bar, 10  $\mu$ m. (E) The Cdc6 depletion efficiency shown by Western blot for Cdc6 and  $\alpha$ -tubulin.



**Figure S3. The nucleolar localization of Cdc6 is related to rDNA transcription, but not Pol II transcription.**

(A) 100 nM Actinomycin D (ActD) treatment inhibits rRNA transcription. Representative immunofluorescence images of HeLa cells treated with DMSO or ActD for 1 h. Note that rRNA transcription visualized by nucleolar EU incorporation was inhibited in ActD treated cells (arrowheads). Scale bar, 10 μm. (B) Cdc6 nucleolar localization is decreased by ActD treatment. Representative immunofluorescence images of HeLa cells treated with DMSO or ActD for 1 h and stained for Cdc6 or B23. Note that Cdc6 (arrowhead) or B23 (arrow) nucleolar localization was decreased in ActD treated cells. Scale bar, 10 μm. (C) Cdc6 nucleolar localization is not affected by 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) treatment. Representative immunofluorescence images of HeLa treated with DMSO or 50 μM DRB for 1.5 h and stained for Cdc6, Nucleolin and B23. Scale bar, 10 μm.



**Figure S4. The interaction between RRN3 and Pol I was impaired by Cdc6 depletion.**

(A) Cdc6 or Orc2 depletion efficiency by “Cdc6 depletion **after** synchronization” or “Cdc6 depletion **before** synchronization” shown by Western blot for Cdc6 or Orc2 and  $\alpha$ -tubulin. (B) The interaction between RRN3 and RPA194 is impaired by Cdc6 depletion. The nuclear extracts of HEK 293T cells transfected with control siRNA or Cdc6 siRNA were immunoprecipitated with RRN3 antibody and Western blot for RPA194 and RRN3. Two repeats of the experiments are shown.

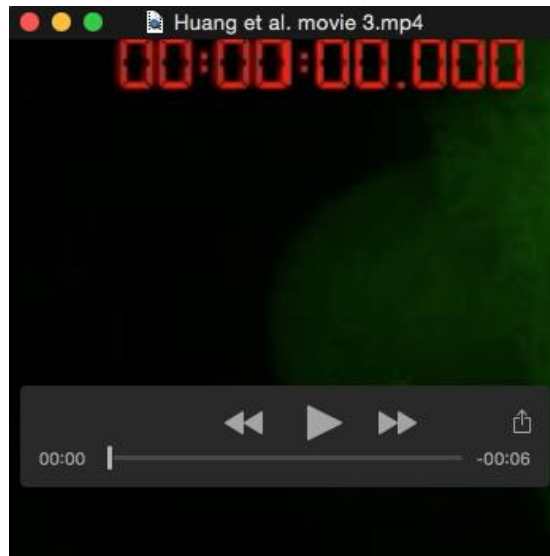
## 2. Supplemental movies



**Movie 1. Time-lapse live cell imaging of HeLa cells transfected with GFP-Cdc6 WT.** Note that Cdc6 WT left the nucleus and appeared in the cytoplasm in S phase. Scale bar, 10  $\mu$ m.

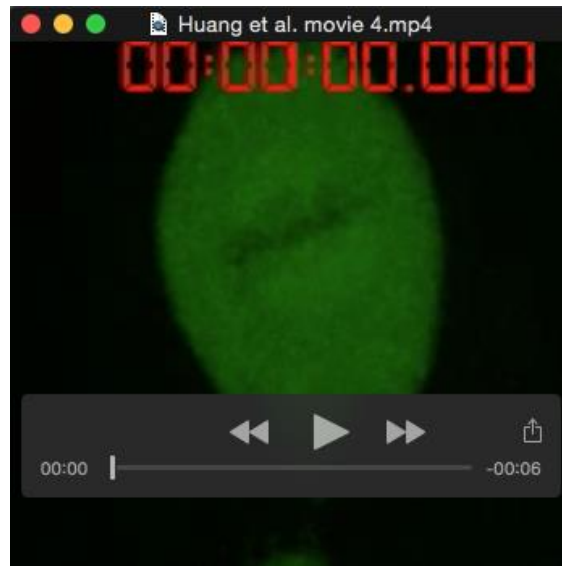


**Movie 2. Time-lapse live cell imaging of HeLa cells transfected with GFP-Cdc6 WT.** Note that Cdc6 WT localized in the nucleus and the nucleolus in late mitosis and G1 phase. Scale bar, 10  $\mu$ m.



**Movie 3. Time-lapse live cell imaging of HeLa cells transfected with GFP-Cdc6**

**WB.** Note that Cdc6 WB localized in the nucleus and the nucleolus in late mitosis and G1 phase. Scale bar, 10  $\mu$ m.



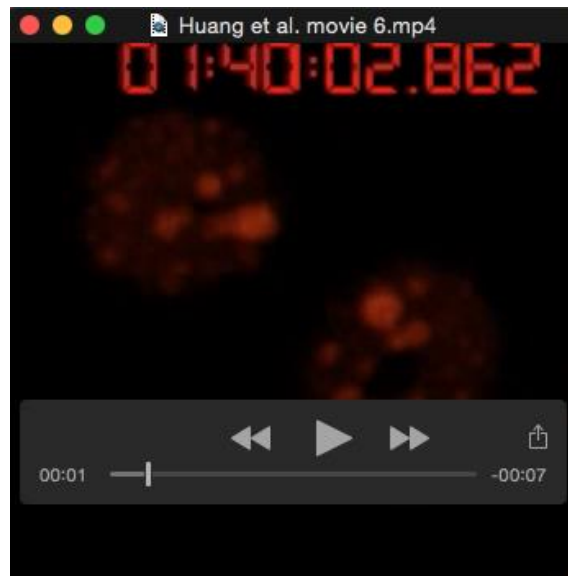
**Movie 4. Time-lapse live cell imaging of HeLa cells transfected with GFP-Cdc6**

**WA.** Note that Cdc6 WA localized in the nucleus but was excluded from the nucleolus in late mitosis and G1 phase. Scale bar, 10  $\mu\text{m}$ .





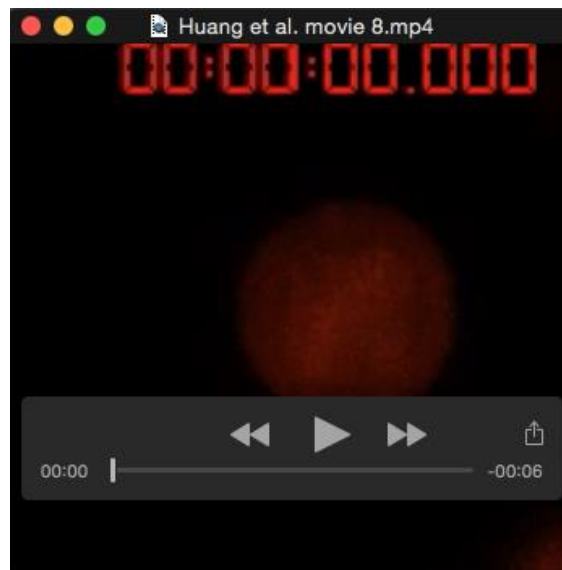
**Movie 5. Time-lapse live cell imaging of HeLa cells co-transfected with GFP-Cdc6 and mCherry-B23.** The channel of GFP-Cdc6 is shown. Scale bar, 10  $\mu\text{m}$ .



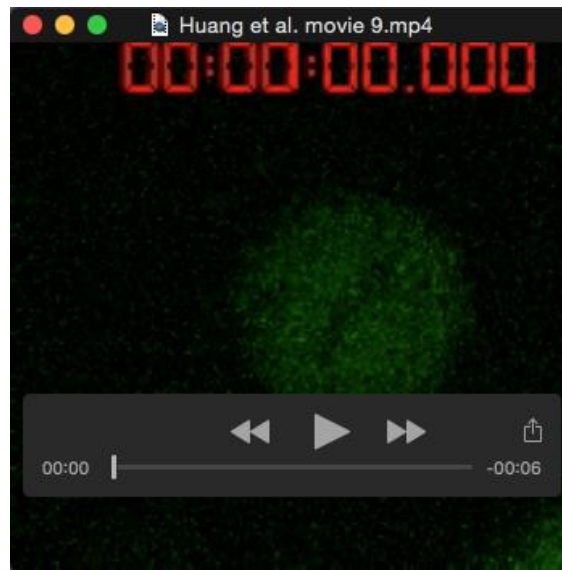
**Movie 6. Time-lapse live cell imaging of HeLa cells co-transfected with GFP-Cdc6 and mCherry-B23.** The channel of mCherry-B23 is shown. Scale bar, 10  $\mu\text{m}$ .



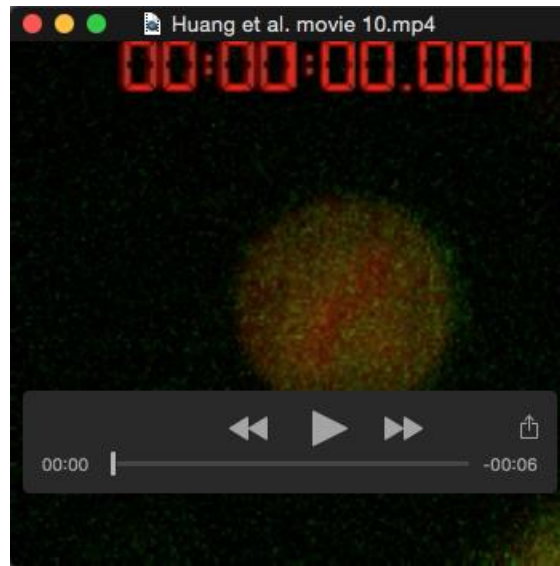
**Movie 7. Time-lapse live cell imaging of HeLa cells co-transfected with GFP-Cdc6 and mCherry-B23.** The combined channels of movies 5 and 6 are shown. Scale bar, 10  $\mu\text{m}$ .



**Movies 8. Time-lapse live cell imaging of HeLa cells co-transfected with mCherry-Cdc6 and GFP-RPA194.** The channel of mCherry-Cdc6 is shown. Scale bar, 10  $\mu\text{m}$ .



**Movie 9. Time-lapse live cell imaging of HeLa cells co-transfected with mCherry-Cdc6 and GFP-RPA194.** The channel of GFP-RPA194 is shown. Scale bar, 10  $\mu\text{m}$ .



**Movie 10. Time-lapse live cell imaging of HeLa cells co-transfected with mCherry-Cdc6 and GFP-RPA194.** The combined channels of Movies 8 and 9 are shown. Scale bar, 10  $\mu\text{m}$ .

### **3. Supplemental Tables**

**Supplemental Table 1. Mass spectrometry analysis of immunoprecipitates of RRN3.** HEK 293T cells were transfected with GFP-RRN3 and synchronized to G1 phase by double-thymidine block and release. By proteomic analysis of the immunoprecipitates of GFP-RRN3, Cdc6 was identified as a protein that interacts with RRN3.

[Click here to Download Table S1](#)

**Supplemental Table 2. Mass spectrometry analysis of the immunoprecipitates of Pol I.** HEK 293T cells were transfected with GFP-RPA194 and synchronized to G1 phase by double-thymidine block and release. By proteomic analysis of the immunoprecipitates of the GFP-RPA194, Cdc6 was identified as a protein that interacts with RPA194.

[Click here to Download Table S2](#)