Supplemental material

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Figure S1. ATAD5 knockdown generates abnormally large PCNA foci. In all experiments, cells were transfected with ATAD5 or control siRNA and analyzed after 72 h incubation unless otherwise specified. (A, B, and D) RPE cell. (A) After extracting soluble proteins, cells were fixed, stained with anti-PCNA antibody, and analyzed by confocal microscopy. (B) Box blot showing the quantitation of chromatin-bound PCNA signal intensity using Image-Pro Plus 7.0 software (Media Cybernetics). The data shown are from a single representative experiment out of three repeats. For the experiment shown, n > 200 for each condition. A.U., arbitrary unit; red bars in the graph, mean value; P, significance by t test. (C) HeLa cells were allowed to bind to different sized microbeads at 48 h after transfection; large one for control and small one for ATAD5 knockdown. Cells were replated in a single slide chamber and incubated before fixation. Cells were stained with anti-PCNA antibody and analyzed by confocal microscopy. Asterisks mark control knockdown cells bound to large beads. (D) PCNA foci in early S-phase nuclei were quantitatively analyzed for foci signal intensity. The data shown are from a single representative experiment out of three repeats. For the experiment shown, n =5 for each condition. Red bars in the graph, mean value. A t test was performed between the control knockdown cell with maximum mean foci signal intensity and the ATAD5 knockdown cell with minimum. Bars (all images): 5 µm.





Figure S2. The consecutive images of a GFP-PCNA-positive cell. The representative consecutive images of a GFP-PCNA-positive cell with an interval of 60 min were displayed. (A) Control knockdown. (B) ATAD5 knockdown. Bars, 5 µm.



Figure S3. **ATAD5 knockdown generates inactive replication factories.** RPE cells were transfected with *ATAD5* or control siRNAs. 72 h after transfection, cells were pulse-labeled with BrdU for 3 min, fixed, and stained with both anti-PCNA and anti-BrdU antibodies and analyzed by confocal microscopy. Bars, 5 µm.



Figure S4. **ATAD5 knockdown delays S-phase cell cycle progression.** 48 h after transfection, HEK293T cells were arrested with a double thymidine block. At indicated times after release from the G1/S block, cells were pulse-labeled with BrdU for 30 min and collected for cell cycle analysis. The data shown are from a single representative experiment out of four repeats. Numbers are the relative percentage of cell cycle stage.



Figure S5. **ATAD5 knockdown increases cellular size**. (A) HeLa cells were transfected with ATAD5 or control siRNA. The FSC (forward scatter)/SSC (side scatter) plots were obtained with flow cytometry at 72 h after transfection. Three independent experiments were performed and a represented image is presented. The data shown are from a single representative experiment out of five repeats. (B) HeLa cells were cotransfected with a combination of ATAD5 siRNA and DNA vector expressing FLAG-tagged ATAD5 (F-ATAD5) or empty vector. The FSC/SSC plots were obtained with flow cytometry at 72 h after transfection. The data shown are from a single representative experiment out of three repeats.