Lim et al., http://www.jcb.org/cgi/content/full/jcb.201412068/DC1

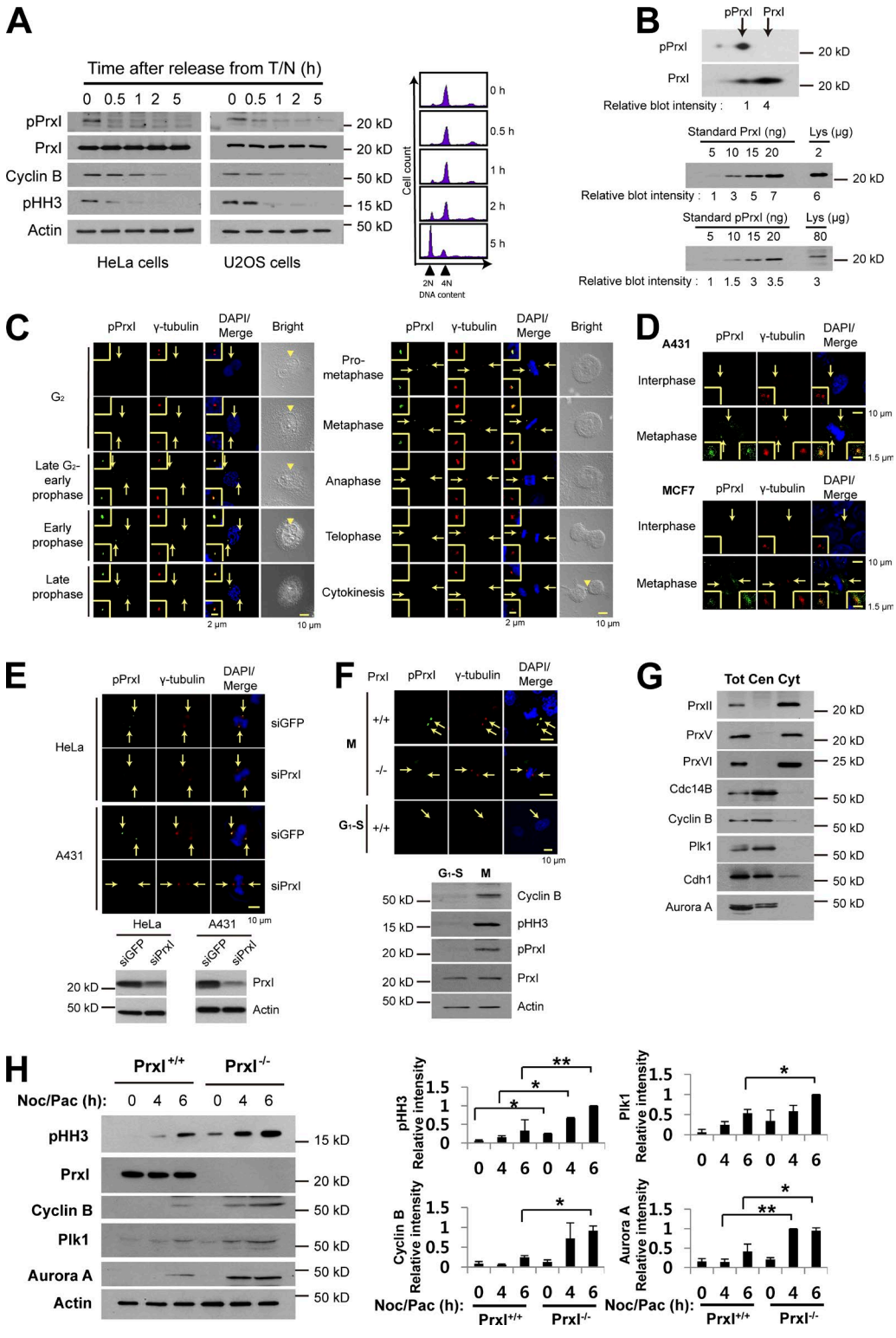


Figure S1. **Phosphorylation of PrxI at Thr^{90}.** (A) Phosphorylation of PrxI at Thr^{90} during cell cycle progression. HeLa and U2OS cells arrested at prometaphase by treatment with thymidine and nocodazole (T/N) were collected at the indicated times after release in fresh medium (at 0 h) for immunoblot analysis with antibodies to the indicated proteins. U2OS cells collected at the indicated times after the release were also analyzed for DNA content by flow cytometric analysis with propidium iodide (right). (B) Estimation of the proportion of the Thr^{90}-phosphorylated form of PrxI in prometaphase HeLa cells. A standard sample of PrxI phosphorylated at Thr^{90} (pPrxI) was prepared by incubation of purified recombinant PrxI with Cdk1–cyclin B. 2D-PAGE followed by immunoblot analysis with antibodies to phospho-PrxI and to PrxI indicated that ∼20% of the recombinant PrxI was phosphorylated (top). Lysates prepared from HeLa cells synchronized at prometaphase by treatment with thymidine and nocodazole, as well as standard PrxI and phospho-PrxI preparations, were subjected to immunoblot analysis with antibodies to PrxI or to phospho-PrxI. Comparison of the immunoblot intensities obtained for cell lysates (Lys) with antibodies to PrxI with those of purified PrxI indicated that 2 µg of total cell lysate contain ∼18 ng PrxI (middle), whereas comparison of the immunoblot intensities obtained for cell lysates with antibodies to phospho-PrxI with those of standard phospho-PrxI indicated that 80 µg cell lysates contain ∼15 ng of 20% phosphorylated PrxI (bottom). From these results, pPrxI was estimated to account for ∼0.4% of total cellular PrxI. (C) Phosphorylation of PrxI at the centrosome of HeLa cells during early mitosis. HeLa cells that had been arrested at the G_1–S border with a double thymidine block were released in fresh medium and collected during G_2 or mitosis at 8–9 h after release. Cells in the indicated phases were examined by confocal immunofluorescence microscopy with antibodies to phospho-PrxI (green) and to γ-tubulin (red). Cell cycle stage was monitored by staining of DNA with DAPI (blue) and bright-field imaging. The cell at late G_2–early prophase shows an intact nuclear membrane (yellow arrowhead in bright-field image), partially condensed chromosomes in the nucleus, two separated centrosomes, and a flattened cell morphology. Early and late prophases were estimated on the basis of the time before or after breakdown of the nuclear envelope. The areas indicated by the arrows are shown at higher magnification in the insets. Arrowheads in the bright-field images indicate an intact nuclear membrane at G_2 and early prophase or the midbody during cytokinesis. (D) Phosphorylation of PrxI at the centrosome in mitotic A431 and MCF7 cells. Asynchronous A431 (top) or MCF7 (bottom) cells were subjected to confocal immunofluorescence microscopy with antibodies to phospho-PrxI (green) and to γ-tubulin (red). Cell cycle stage was monitored by staining of DNA with DAPI (blue). The areas indicated by the arrows are shown at higher magnification in the insets. (E) Demonstration of the specificity of the antibodies to phospho-PrxI with the use of cells depleted of PrxI. HeLa or A431 cells that had been transfected with control (siGFP) or PrxI-specific (siPrxI) siRNAs were subjected to confocal immunofluorescence microscopy with antibodies to phospho-PrxI (green) and to γ-tubulin (red; top). Arrows indicate centrosomes. HeLa or A431 cells transfected as in the top were subjected to immunoblot analysis with antibodies to PrxI and to β-actin (bottom). (F) Demonstration of the specificity of the antibodies to phospho-PrxI with the use of MEFs derived from PrxI-deficient mice. Wild-type (+/+) or PrxI knockout (−/−) MEFs were synchronized in G_1–S or mitosis as described in Materials and methods. Cells in G_1–S or mitotic (M) phases were subjected to confocal immunofluorescence microscopy with antibodies to phospho-PrxI (green) and to γ-tubulin (red; top). Cell cycle stage was monitored by staining of DNA with DAPI (blue). Arrows indicate centrosomes. The cell cycle stage of wild-type MEFs treated as in the top was verified by immunoblot analysis with antibodies to cyclin B1 and to pHH3 (bottom). (G) Immunoblot detection of Prx isoforms and mitotic regulatory proteins in subcellular fractions of HeLa cells. Total cell lysates without nuclei (Tot) as well as cytosolic (Cyt) and centrosome (Cen) fractions prepared from HeLa cells arrested at prometaphase as described in Fig. 1 C were subjected to immunoblot analysis with antibodies to the indicated proteins. (H) The effect of PrxI ablation on mitotic entry was evaluated with the use of MEFs derived from PrxI-deficient mice. PrxI wild-type (Prxl$^{+/+}$) or PrxI-deficient (Prxl$^{-/-}$) MEFs were partially synchronized at mitosis by treatment with nocodazole (Noc; 500 ng/ml) and paclitaxel (Pac; 5 µM) for 4 or 6 h. Cell lysates were then subjected to immunoblot analysis with antibodies to the indicated proteins (left). The relative immunoblot intensities of pHH3, cyclin B, Plk1, and Aurora A that were normalized by those of actin were also determined as means ± SD from three independent experiments (right). *, $P < 0.05$; **, $P < 0.005$ (Student's *t* test).

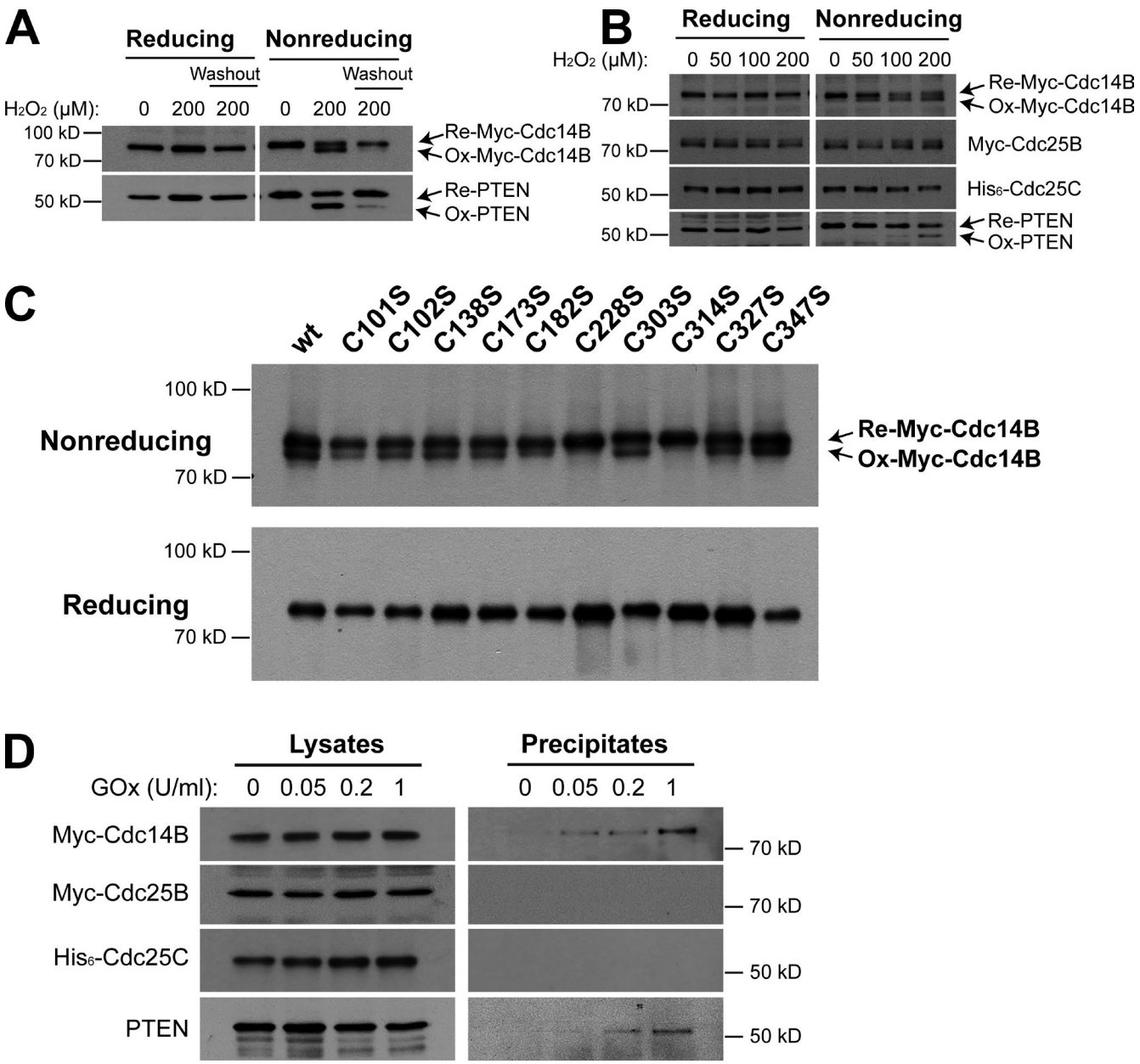


Figure S2. **Sensitivity of Cdc14B and insensitivity of Cdc25B and Cdc25C to oxidation by H_2O_2.** (A) HeLa cells expressing Myc epitope–tagged Cdc14B were incubated for 5 min with or without exogenous H_2O_2 (200 μM) and then lysed. Some cells treated with H_2O_2 were also subsequently incubated in the absence of H_2O_2 for 30 min before lysis (Washout). Cell lysates were subjected to alkylation with *N*-ethylmaleimide (NEM) and either treated with 1 mM DTT (Reducing) or left untreated (Nonreducing) before immunoblot analysis. Reduced (Re-) and oxidized (Ox-) forms of Myc-Cdc14B and endogenous PTEN were detected with antibodies to Myc and to PTEN, respectively. (B) HeLa cells expressing Myc-Cdc25B, His_6-tagged Cdc25C, or Myc-Cdc14B were treated with the indicated concentrations of H_2O_2 for 10 min, after which cell lysates were prepared, processed as in A and subjected to immunoblot analysis. Reduced and oxidized forms of Myc-Cdc14B and endogenous PTEN were detected with antibodies to Myc and to PTEN, respectively. Myc-Cdc25B and His_6-Cdc25C were detected with antibodies to Myc and to Cdc25C, respectively. (C) HeLa cells expressing Myc epitope–tagged wild-type (wt) or the indicated mutant forms of Cdc14B were incubated with 200 μM H_2O_2 for 5 min, after which cell lysates were prepared, processed as in A and subjected to immunoblot analysis with antibodies to Myc. (D) HeLa cells expressing Myc-Cdc14B, Myc-Cdc25B, or His_6-Cdc25C were treated with the indicated concentrations of glucose oxidase (GOx) in high-glucose medium for 30 min (to generate H_2O_2), after which cell lysates were prepared and processed for detection of oxidized Cys residues by alkylation with biotin-conjugated maleimide. Biotinylated proteins were precipitated with avidin-conjugated beads and were subjected, together with total cell lysates, to immunoblot analysis with antibodies to Cdc14B, to Myc (for Myc-Cdc25B), to Cdc25C, or to PTEN.

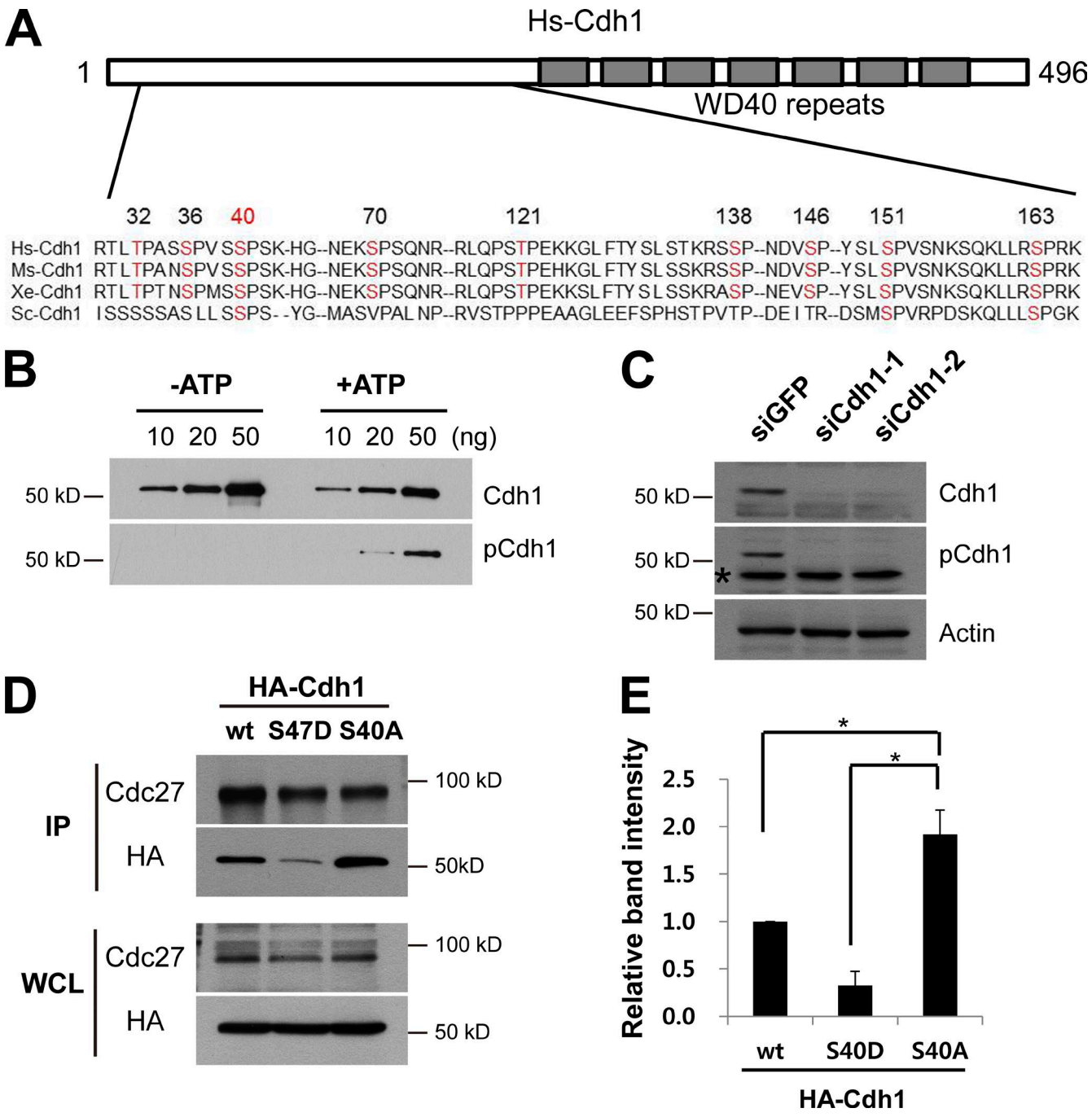
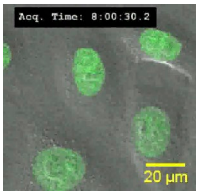
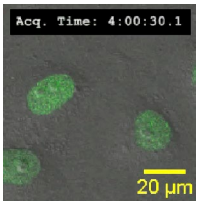


Figure S3. **Identification of a phosphorylation site of Cdh1 and characterization of antibodies to Ser^{40}-phosphorylated Cdh1.** (A) Amino acid sequence alignment of nine conserved sites of potential Cdk phosphorylation in Cdh1 of human (*Homo sapiens* [Hs]), mouse (Mus musculus [Ms]), *Xenopus* (Xe), and *Saccharomyces cerevisiae* (Sc). Sequence numbering refers to human Cdh1. (B) Recombinant human Cdh1 was incubated with Cdk1–cyclin B in the absence or presence of ATP, and the indicated amounts of reaction mixtures were then subjected to immunoblot analysis with antibodies to Cdh1 and to Ser^{40}-phosphorylated Cdh1 (pS-Cdh1). (C) HeLa cells that had been transfected with control (siGFP) or two different Cdh1-specific (siCdh1-1 or siCdh1-2) siRNAs for 48 h were synchronized at prometaphase by treatment with thymidine and nocodazole. Cell lysates were then subjected to immunoblot analysis with antibodies to Cdh1, to pS-Cdh1, and to β-actin. The asterisk indicates a nonspecific band. (D and E) HeLa cells expressing HA-tagged wild-type (wt) or mutant (S40A or S40D) forms of human Cdh1were synchronized at prometaphase, and cell lysates were then subjected to immunoprecipitation with antibodies to the APC/C subunit Cdc27. The resulting immunoprecipitates (IP) as well as the whole cell lysates (WCL) were subjected to immunoblot analysis with antibodies to Cdc27 and to HA (D). The immunoblot intensities of the HA-Cdh1 bands in the immunoprecipitates were normalized by those of the corresponding Cdc27 bands and are presented as means ± SD from three independent experiments (E). *, $P < 0.05$ (Student's *t* test).



Video 1. **Cat-delPACT-H2B GFP-HeLa live-cell imaging of HeLa cells expressing histone H2B–GFP and Cat-delPACT.** HeLa cells expressing histone H2B–GFP (green) were infected with retrovirus containing the Cat-PACT gene, synchronized at G_1–S, and released in fresh medium. Live-cell images were obtained using a laser-scanning confocal microscope (Nikon A1R) and processed with NIS elements AR 3.0 software (Nikon). Frames were taken every 30 min for 13.5 h.



Video 2. **Cat-PACT-H2B GFP-HeLa live-cell imaging of HeLa cells expressing histone H2B–GFP and Cat-PACT.** HeLa cells expressing histone H2B–GFP (green) were infected with retrovirus containing the Cat-delPACT gene, synchronized at G_1–S, and released in fresh medium. Live-cell images were obtained using a laser-scanning confocal microscope (Nikon A1R) and processed with NIS elements AR 3.0 software (Nikon). Frames were taken every 30 min for 13.5 h.