Supporting Information

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Plasmids and Reagents

CUE domain-containing protein 2 (CUEDC2) full-length or truncated mutants were subcloned into pCDNA3.0-Flag, pXJ40-Myc, pMSCV-IRES-GFP, and pET28A vectors or as described before (1, 2). Site-directed mutagenesis of CUEDC2 [CUEDC2–KEN-box mutant (KM) and CUEDC2–D-box mutant (DM)] was done by standard methods. Myc-Cdh1 and Cdh1 deletion mutants were kindly provided by Marc W. Kirschner, Harvard Medical School, Boston, MA (3) and T. B. Kang, Sun Yat-Sen University Cancer Center, Guangzhou, China (4). Myc-Skp2 was a gift from Meloche Sylvain, Université de Montréal, Montreal, Canada (5), and pcDNA3.0-Cyclin A and GFP-Emi1 were from Peter K. Jackson, Department of Cell Regulation, Genentech Inc., South San Francisco, CA (6). Antibodies recognizing the following targets were obtained from commercial sources: HA (sc-7392), Myc (sc-40), Cyclin A (sc-751), Geminin (sc-13015), Skp2 (sc-7164), Cyclin-dependent kinase 2 (CDK2) (sc-163), p21 (sc-397), and p53 (sc-126) antibodies were purchased from Santa Cruz Biotechnology; Flag (F-3165), Tubulin (T5168), and BrdU (B3434) antibodies were from Sigma; Emi1 (38-5000) antibody was from Invitrogen; Cdh1 antibody (MS-1116-P0) was from Oncogene and Cdc27 antibody (610455) was from BD Biosciences. Mouse monoclonal antibody against CUEDC2 was prepared in our laboratory. Ubiquitin was from Boston Biochem; ATP, nocodazole, Taxol, thymidine, MG132, and cycloheximide were purchased from Sigma.

Cell Culture and Transfection

HEK293T, HeLa, and U2OS cells were cultured in DMEM containing 10% FBS. MCF-10A cells were cultured in DMEM-F12 containing 5% horse serum (GIBCO), 10 μg/mL insulin, 10 ng/mL EGF, 100 ng/mL cholera toxin, and 0.5 μg/mL hydrocortisone. Plasmids were transfected by Lipofectamine 2000 (Invitrogen), and cells were harvested between 24 and 48 h after transfection. siRNAs from Invitrogen or Dharmacon were transfected by Lipofectamine RNAiMax (Invitrogen). The CUEDC2 siRNA (target sequence: 5′-CCAAGAUGAGGCAACUGGCGCUGAG-3′) was from Invitrogen (no. HSS149051), and control siRNA was described as before. CUEDC2 shRNAs (#1 and #2) were inserted into the pSuper-retro-GFP vector, target sequences were as follows: 5′-GAAGCTGATCCGATACATC-3′ and 5′- GTACATGATGG-TGGATAGC-3′, respectively. The Cdh1 siRNA was from Dharmacon and targeted to 5′-GAAGGGTCTGTTCACGTATTCCC-TT-3′. Retroviral shRNA virus packaging and subsequent infection were performed as described previously.

Immunoprecipitation and Immunoblotting

For CUEDC2 immunoprecipitation experiments, extracts from HeLa cells were prepared as described previously (1, 7), with slight modifications. Briefly, G_1-phase cells were synchronized as described before and were harvested in SB buffer [25 mM Hepes, pH 7.5, 1.5 mM $MgCl_2$, 5 mM KCl, 1 mM DTT, 1× Complete protease inhibitors (Roche), 15 mM creatine phosphate, 2 mM ATP] and homogenized by freeze-thawing and passage through a needle. Extracts were cleared by subsequent centrifugations. Immunoprecipitate was performed with monoclonal anti-CUEDC2 antibody. Proteins were separated by SDS/PAGE and analyzed by Western blotting with the indicated antibodies. For other immunoprecipitation experiments, cells were harvested in a Cdc20 lysis buffer (50 mM Tris·HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM $MgCl_2$, and 0.5% Nonidet P-40) supplemented with Complete protease inhibitor mixture, 1 mM NaF, 1 mM Na_3VO_4, and 1 mM DTT. Immunoprecipitate was performed with mouse anti-Flag M2 affinity gel, and proteins were separated by SDS/PAGE and analyzed by Western blotting with the indicated antibodies.

Kinase Assay

For in vitro kinase assays of CDK2, harvested cells were lysed in Lysis buffer A, containing 50 mM Tris·HCl (pH 8.0), 150 mM NaCl, 1% Nonidet P-40, 10 mM *para*-nitrophenylphosphate (PNPP), 1 mM DTT, 1 mM Na_3VO_4, and protease inhibitors (Roche). Active Cdk2 complex was immunoprecipitated with antibodies to Cdk2 (Santa Cruz) coupling with protein A-Sepharose beads (GE Healthcare). Histone H1 (New England Biolabs), as the substrate, were incubated with active Cdk2 complex in Kinase buffer. The Kinase assays were performed in 20-μL reactions, containing 50 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, 2 mM DTT, 20 mM β-glycerolphosphate, 20 mM PNPP, 10 mM NaF, 0.1 mM Na_3VO_4, 10 μM cold ATP, 10 μCi of [γ-^{32}P]ATP, protease inhibitors, 10 μL of Cdk2 coupling beads, and 1 μg Histone H1. The reactions were incubated at 30 °C for 30 min, quenched with SDS sample buffer, and analyzed by SDS/PAGE followed by autoradiography.

For in vitro kinase assays of ERK1/2, GST, GST-CUEDC2 (wild type), or GST-CUEDC2 (S110A) purified recombinant proteins were incubated with immunoprecipitated human ERK1/2 complex using MAP Kinase/ERK Immunoprecipitation Kinase Assay Kit (Millipore). The kinase assays were carried out in 30-μL reactions, containing 50 μM cold ATP, 5 μCi of [γ-^{32}P]ATP, 10 μL of ERK1/2 complex, and 1 μg of purified recombinant proteins in kinase buffer. The reactions were incubated at 30 °C for 30 min, quenched with SDS sample buffer, and analyzed by SDS/PAGE followed by autoradiography.

Protein Purification

All His-fusion proteins were expressed in *Escherichia coli* BL21 cells. His-CUEDC2 (WT) and His-CUEDC2 (KM) proteins were purified by Ni-NTA agarose (QIAGEN) resins. Cells were lysed in Lysis buffer, containing 50 mM Na_2HPO_4, 300 mM NaCl, 20 mM imidazole, and 1 mM PMSF, and the supernatants were incubated with Ni-NTA agarose resins for 4 h at 4 °C. The proteins were eluted by Elution buffer, containing 50 mM Na_2HPO_4, 300 mM NaCl, and 250 mM imidazole, and concentrated by Microcon centrifugal filter devices (Millipore).

In Vitro Binding Assays

For in vitro binding assays, Flag-Cdh1 was translated in vitro with TNT T7 Quick Coupled Transcription/Translation System (Promega). The system was lysed in CDC20 buffer, lysates were incubated with Ni-NTA agarose (QIAGEN) resins-bound purified His-CUEDC2 (WT) or His-CUEDC2 (KM) for 2 h at 4 °C. The resins were washed three times with CDC20 buffer, and bound proteins were analyzed by SDS/PAGE.

1. Gao YF, et al. (2011) Cdk1-phosphorylated CUEDC2 promotes spindle checkpoint inactivation and chromosomal instability. *Nat Cell Biol* 13(8):924–933.
2. Zhang WN, et al. (2012) CUEDC2 (CUE domain-containing 2) and SOCS3 (suppressors of cytokine signaling 3) cooperate to negatively regulate Janus kinase 1/signal transducers and activators of transcription 3 signaling. *J Biol Chem* 287(1): 382–392.
3. Wei W, et al. (2004) Degradation of the SCF component Skp2 in cell-cycle phase G1 by the anaphase-promoting complex. *Nature* 428(6979):194–198.
4. Lv XB, et al. (2010) Damaged DNA-binding protein 1 (DDB1) interacts with Cdh1 and modulates the function of APC/CCdh1. *J Biol Chem* 285(24):18234–18240.
5. Rodier G, et al. (2005) p107 inhibits G1 to S phase progression by down-regulating expression of the F-box protein Skp2. *J Cell Biol* 168(1):55–66.

6. Miller JJ, et al. (2006) Emi1 stably binds and inhibits the anaphase-promoting complex/cyclosome as a pseudosubstrate inhibitor. *Genes Dev* 20(17):2410–2420.

7. Frescas D, Pagano M (2008) Deregulated proteolysis by the F-box proteins SKP2 and beta-TrCP: Tipping the scales of cancer. *Nat Rev Cancer* 8(6):438–449.

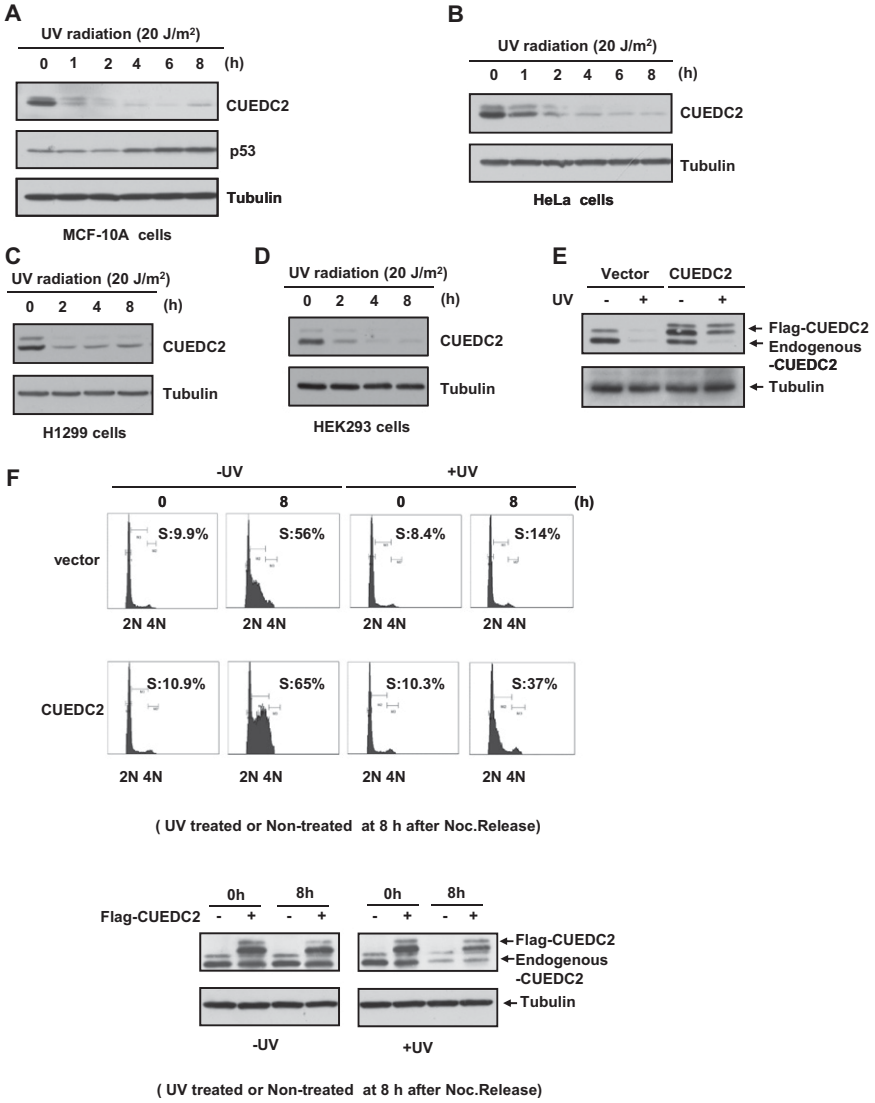

Fig. S1. CUEDC2 is degraded during UV-induced G_1 arrest and its overexpression overcomes such an arrest. (*A–D*) U2OS, HeLa, H1299, and HEK293 cells were treated with UV-C (20 J/m^2) irradiation and harvested at indicated times, and CUEDC2 protein levels were determined by immunoblotting. (*E*) CUEDC2 protein levels were detected in Fig. 1*D*. (*F*) FACS analysis and S-phase percentage of cells in Fig. 1*E*. The exogenous and endogenous CUEDC2 protein levels are shown at *Bottom*.

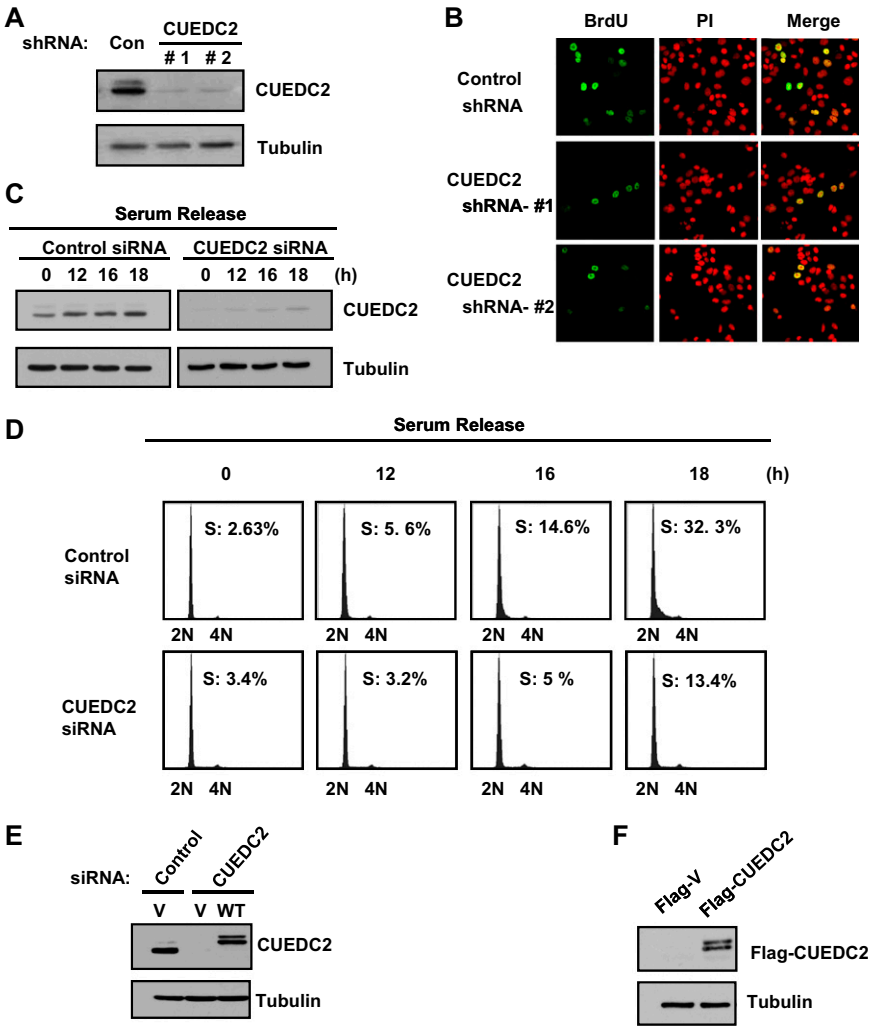

Fig. S2. CUEDC2 promotes G_1–S transition during the normal cell cycle. (*A* and *B*) HeLa cells stably expressing control or CUEDC2 shRNA were synchronized at G_1 phase by nocodazole releasing for 8 h, and then cells were pulsed with BrdU (10 μM) for 2 h and fixed for immunofluorescence analysis as in Fig. 2*B*. Knockdown effect of CUEDC2 and representative merges of the three images visualized by microscopy are shown in *A* and *B*, respectively. (*C* and *D*) Western blot of CUEDC2 expression and S-phase percentage of MCF-10A transfected with control or CUEDC2 siRNA released from serum starvation in Fig. 2*C* are shown, respectively. (*E*) Immunoassay of MCF-10A cells in Fig. 2*D*. (*F*) Western blot of CUEDC2 expression in MCF-10A cells in Fig. 2*E*.

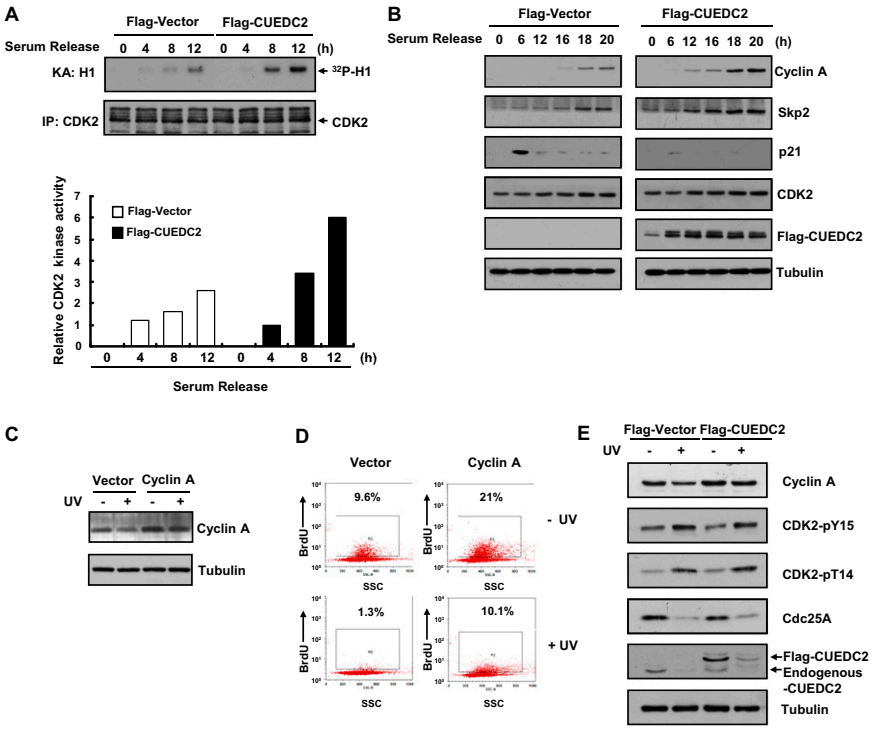

Fig. S3. CUEDC2 regulates Cyclin A level and CDK2 activity. (*A*) MCF-10A/Flag-Vector or MCF-10A/Flag-CUEDC2 were synchronized in G_0/G_1 phase by serum starvation and released into fresh medium for the indicated times. CDK2 kinase activity was detected with histone H1 as substrate, and the corresponding quantity of CDK2 activity is shown by the bar chart at *Bottom*, respectively. (*B*) MCF-10A/Flag-Vector or MCF-10A/Flag-CUEDC2 were treated as in *A* and harvested at indicated time. Whole-cell lysates were analyzed by Western blot with indicated antibodies. (*C*) Whole-cell lysates from HeLa cells transfected with vector or Cyclin A in Fig. 3*D* were analyzed by Western blot. (*D*) FACS distribution of BrdU-positive cells in an experiment of Fig. 3*D* is shown. (*E*) MCF-10A/Flag-Vector or MCF-10A/Flag-CUEDC2 stable cell lines were treated as in *D*. Whole-cell lysates were analyzed by Western blot with indicated antibodies.

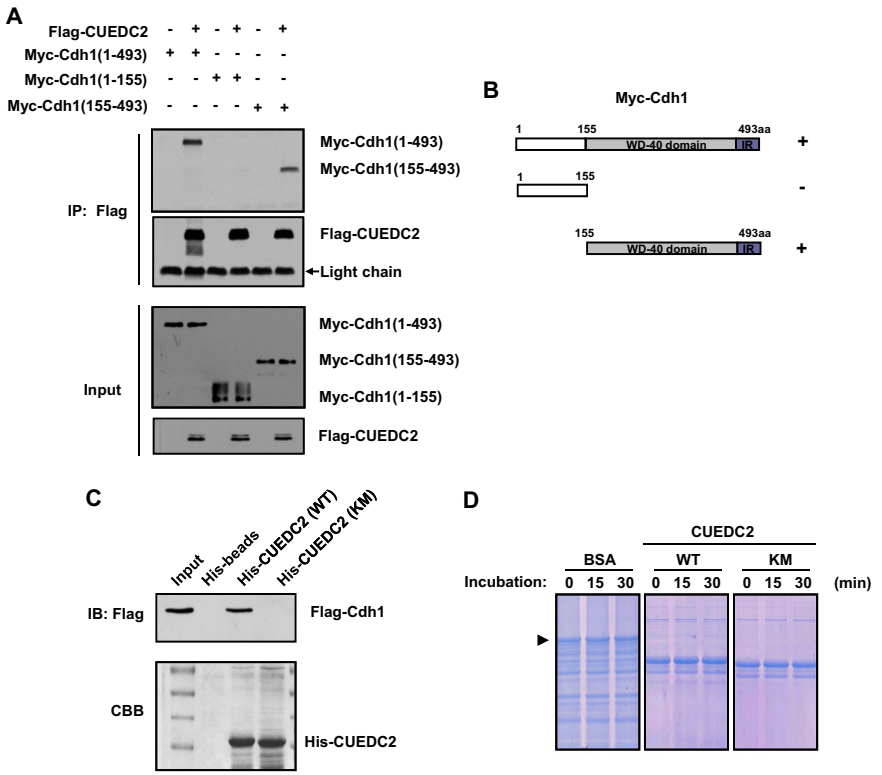

Fig. S4. CUEDC2 interacts with Cdh1 and inhibits APC/C^{Cdh1} activity. (*A*) Immunoassay of HEK293T cells cotransfected with vectors expressing Flag-tagged CUEDC2 and Myc-tagged Cdh1 or various Cdh1 mutants. Cell lysates were immunoprecipitated with anti-Flag M2 beads, and immunoprecipitates were probed with an anti-Myc or anti-Flag antibody. (*B*) A schematic representation of the various Cdh1 deletion mutants. (*C*) His pull-down assay of interaction between CUEDC2 and Cdh1. His-CUEDC2 and Flag-Cdh1 were obtained by protein purification and in vitro translation, respectively. (*D*) His-CUEDC2 (WT) and His-CUEDC2 (KM) were purified as described in *Materials and Methods*. BSA and the purified proteins used in Fig. 4*F* were separated on SDS/PAGE and stained with Coomassie blue.

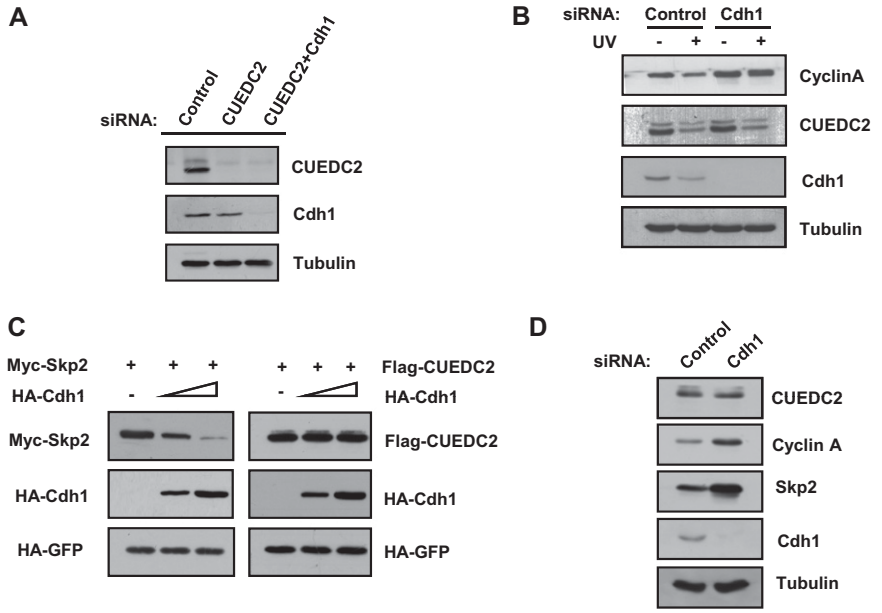

Fig. S5. CUEDC2 regulates G_1–S transition through Cdh1. (*A*) Knockdown effect of Fig. 4*F* was analyzed by Western blot with indicated antibodies. (*B*) U2OS cells transfected with either control or Cdh1 siRNA were treated with or without UV-C (20 J/m^2) irradiation. Whole-cell lysates were analyzed by Western blot with indicated antibodies. (*C*) HEK293T cells were cotransfected increasing amounts of HA-tagged Cdh1 with Myc-tagged Skp2 (*Left*) or Flag-tagged CUEDC2 (*Right*), and HA-GFP was transfected to indicate the transfection efficiency. Cell lysates were analyzed by immunoblot with indicated antibodies. (*D*) Protein levels of U2OS cells transfected with control or Cdh1 siRNA.

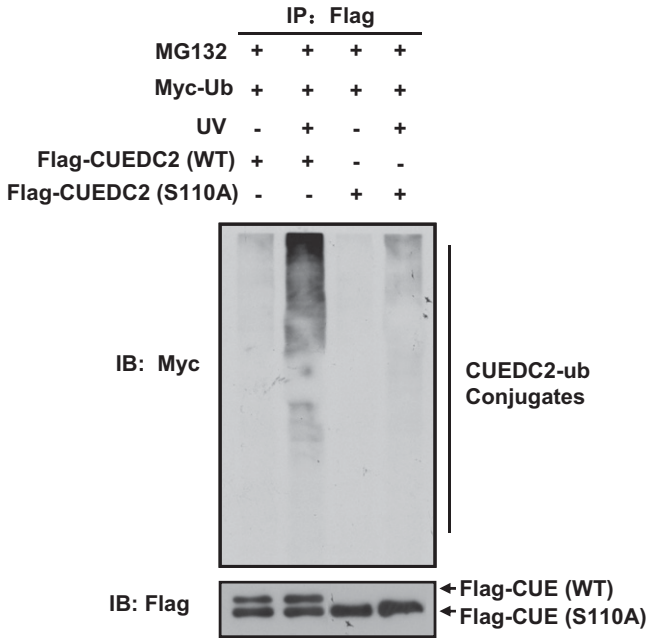

Fig. S6. The ubiquitination of CUEDC2 S110A mutant was decreased upon UV irradiation. Myc-tagged ubiquitin and Flag-tagged CUEDC2 (WT) or CUEDC2 (S110A) was cotransfected into HEK293T cells. Cells were exposed to UV-C (20 J/m^2) irradiation at 24 h after transfection and harvested at 4 h after UV treatment. Flag-tagged proteins were pulled down by anti-Flag M2 beads, and then detected by immunoblotting using anti-Myc antibody.

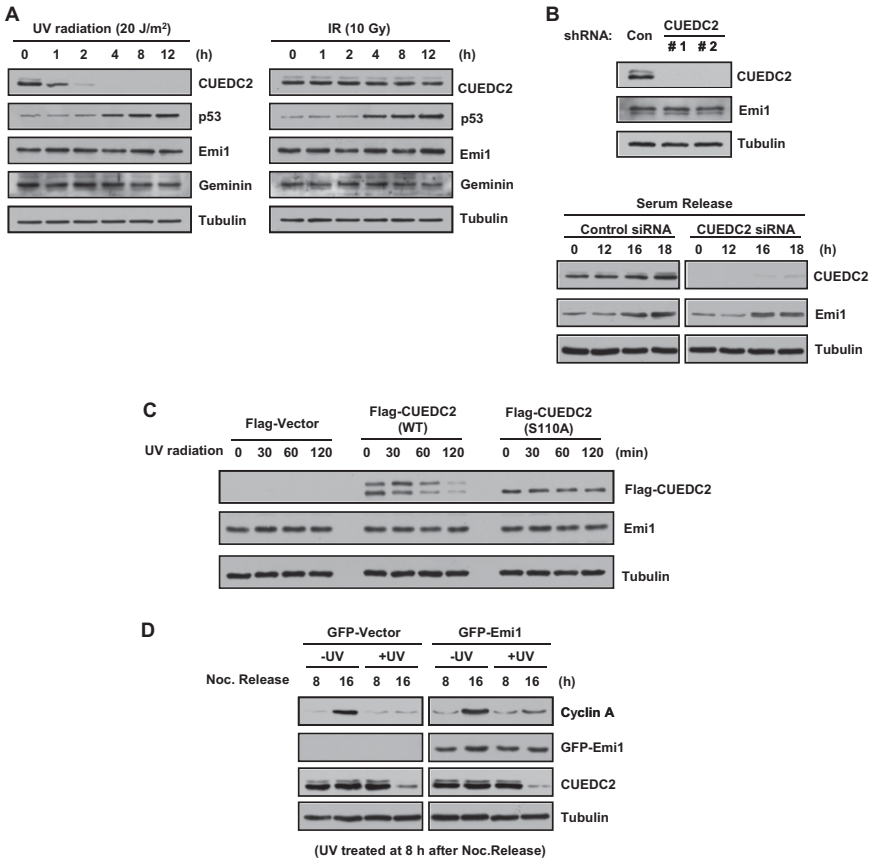

Fig. S7. CUEDC2 does not affect Emi1 protein levels with or without UV treatment. (*A*) Immunoblot analysis of Emi1 and other proteins in U2OS cells treated with UV-C (20 J/m^2) or IR (10 Gy) irradiation. (*B*) Western blot of CUEDC2 and Emi1 proteins levels in HeLa cells stably expressing control or CUEDC2 shRNA or in MCF-10A cells transfected with control or CUEDC2 siRNA released from serum starvation. (*C*) MCF-10A cells stably expressing Flag-Vector, Flag-CUEDC2 (WT), and Flag-CUEDC2 (S110A) were treated with UV-C (20 J/m^2) irradiation. The CUEDC2 protein and Emi1 protein levels were detected at the indicated time. (*D*) Immunoassay of HeLa cells transfected with control or Emi1 expression vectors and treated or untreated by UV-C (20 J/m^2) irradiation at 8 h after nocodazole release.

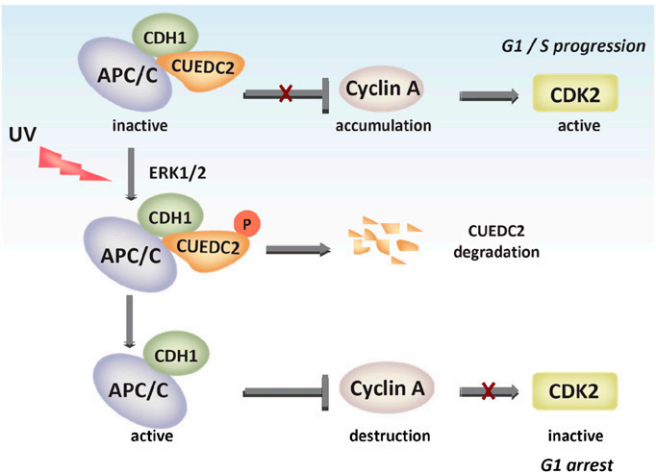

Fig. S8. Schematic presentation roles of CUEDC2 in APC/C^{Cdh1}-regulated G_1–S progression and UV-induced G_1 arrest.