Listovsky and Sale et al., http://www.jcb.org/cgi/content/full/jcb.201302060/DC1

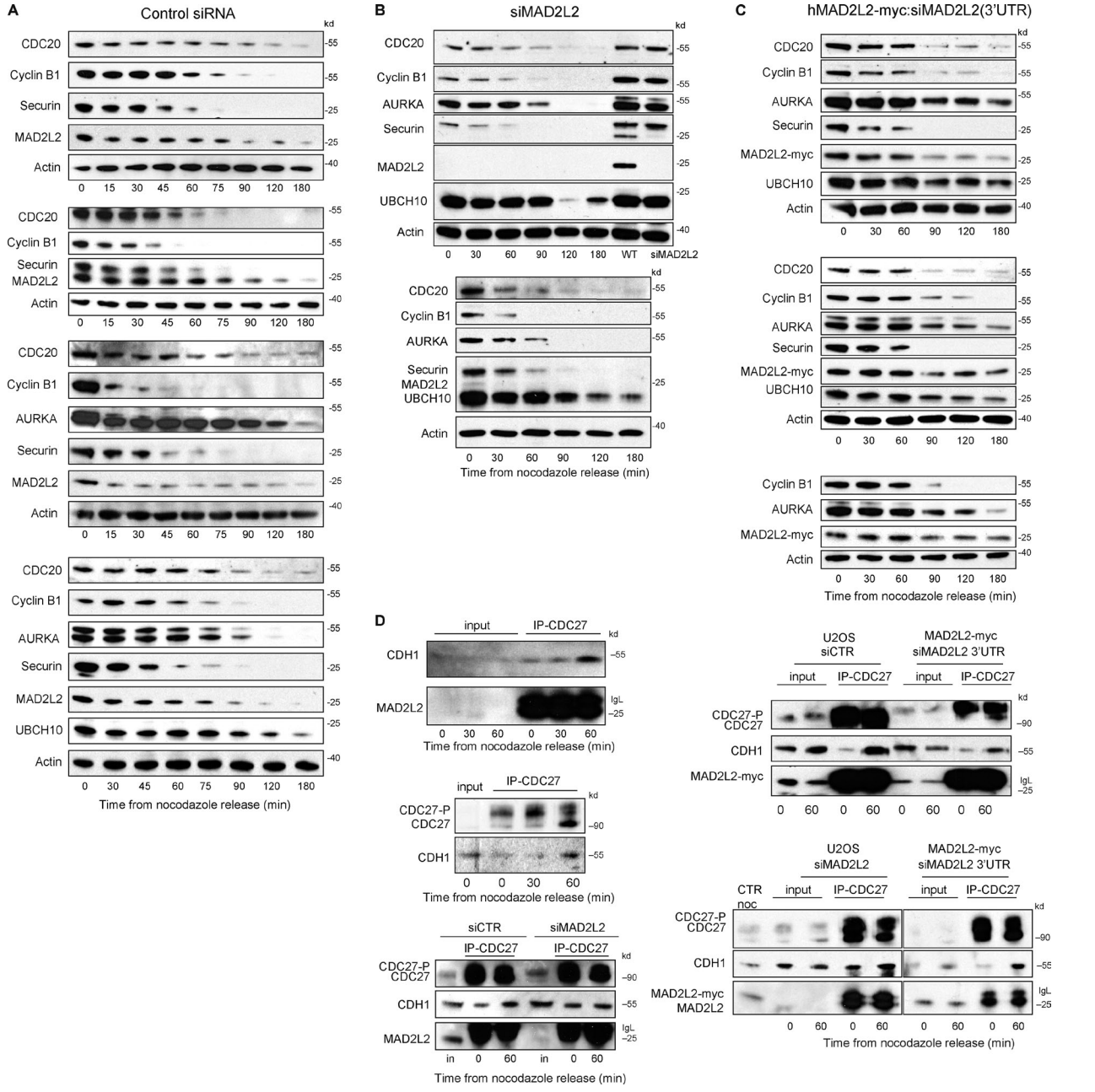


Figure S1. **Premature degradation of APC/C substrates in cells depleted of MAD2L2 due to premature association of CDH1 with the APC/C.** (A–C) Additional examples of blots showing substrate degradation in cells treated with control siRNA (A), MAD2L2 siRNA (B), and MAD2L2 siRNA complemented with hMAD2L2-myc (C). These blots contribute to Fig. 2 E and Tables 1 and 2. (D) Additional examples of CDH1 immunoprecipitations that contribute to Fig. 6 D.

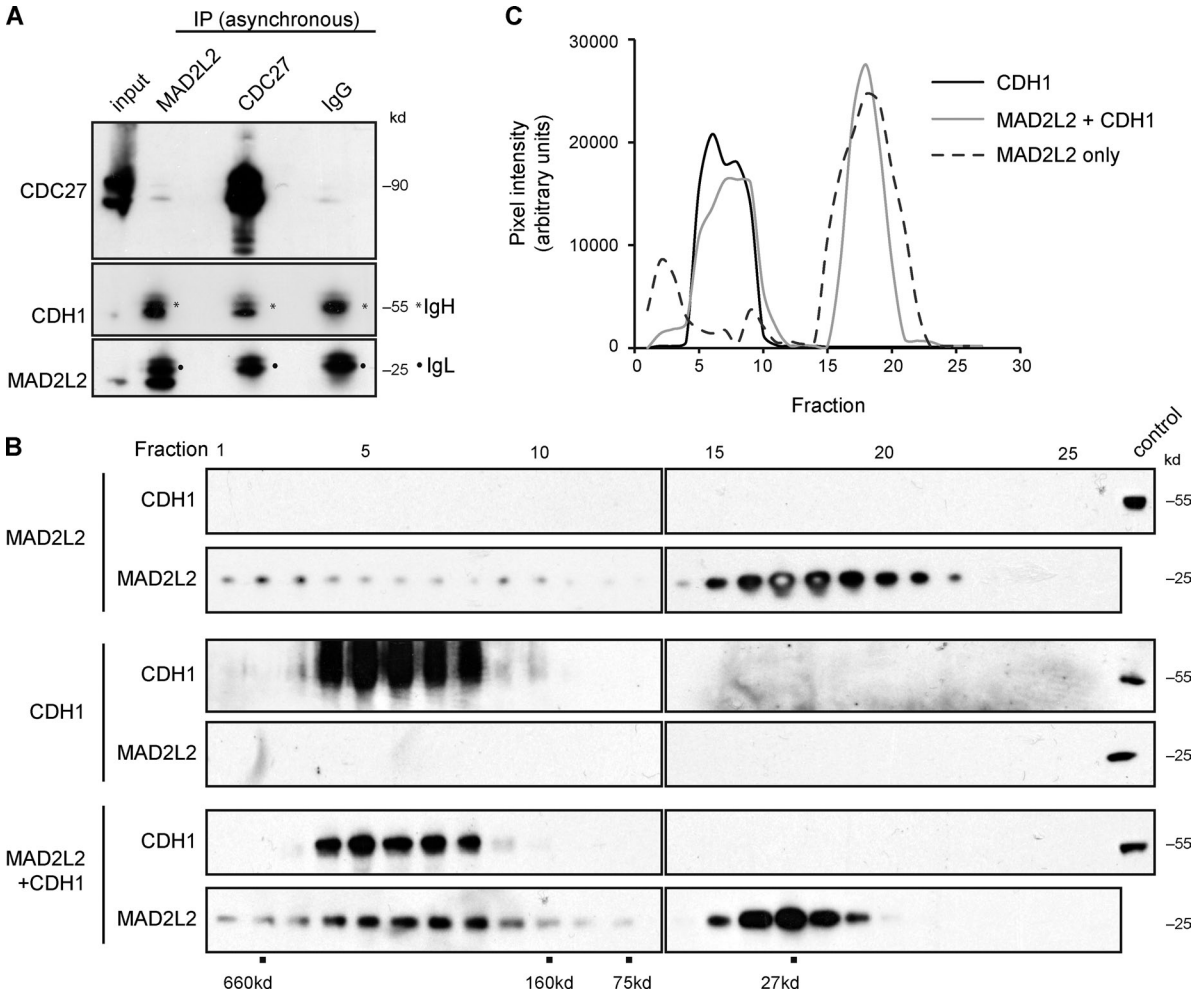


Figure S2. **Interaction of MAD2L2 with CDH1 in vivo and in vitro.** (A) In asynchronous 293 cells, MAD2L2 can been seen either interacting with CDH1 or with CDC27. (B) Size-exclusion chromatography of purified CDH1 and MAD2L2. Once mixed with CDH1, MAD2L2 shifts into the higher molecular weight fractions that contain CDH1. The elution volume of a control protein, the ε exonuclease subunit of *E. coli* DNA polymerase III, was not affected by addition of either MAD2L2 or CDH1. (C) Quantification of gel filtration experiments in B. B and C are representative of two experiments.

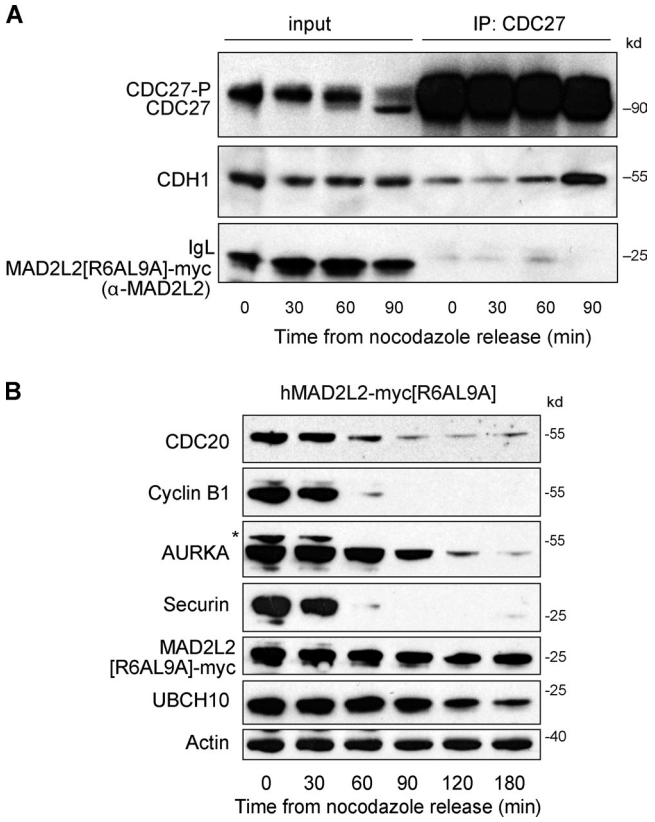


Figure S3. **Effect of overexpression of hMAD2L2[R6AL9A].** (A) Overexpression of stable hMAD2L2[R6AL9A] induces a modest delay in association of CDH1 with the APC/C. (B) Overexpression of stable hMAD2L2[R6AL9A] does not significantly delay the degradation of mitotic substrates on release from nocodazole.

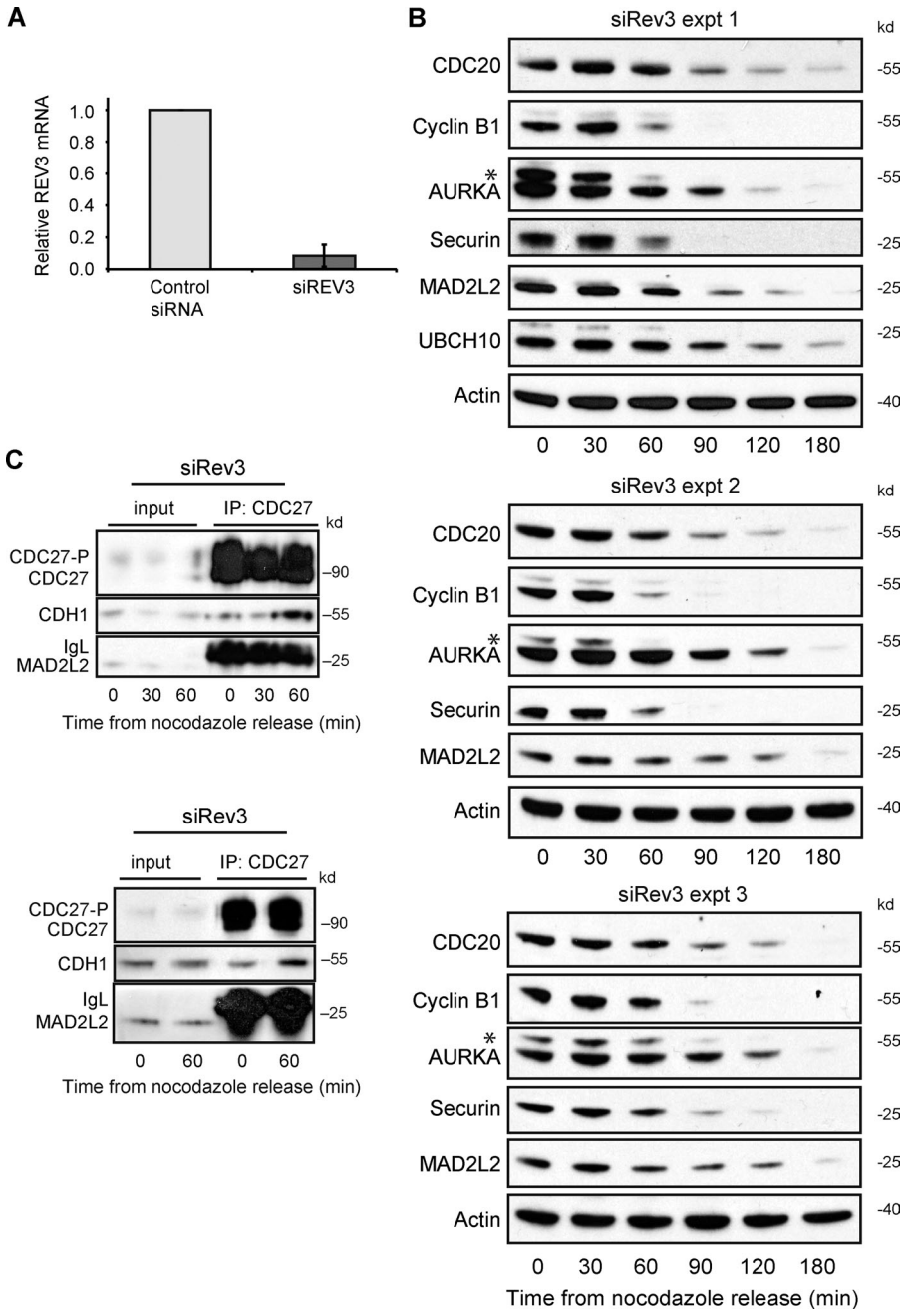
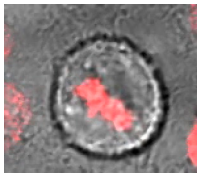
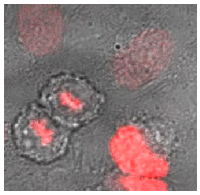


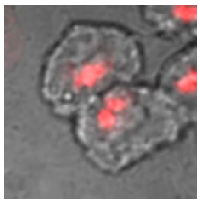
Figure S4. **The role of MAD2L2 in control of APC/C^{CDH1} activation is independent of REV3.** (A) qPCR for REV3 transcript after knockdown. The level of REV3 transcript was normalized to that of GAPDH and the result for cells treated with REV3 siRNA is presented relative to cells treated with control siRNA. Error bars = 1 SD of four experiments. (B) Depletion of REV3 has no significant impact on the kinetics of APC/C substrate degradation. Blots of the three independent siRNA experiments that comprise the siREV3 degradation curves in Fig. 2 E. The asterisk indicates remnant Cyclin B1 signal in the AURKA blot. (C) Depletion of REV3 does not lead to premature association of CDH1 with CDC27. Blots for the two experiments that contribute to Fig. 6 D. Control blots can be seen in Fig. 6 and Fig. S1.



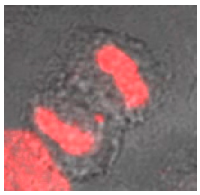
Video 1. **Mitosis in a U2OS cell transfected with control siRNA, in which the chromosomes are visualized by expression of mCherry-tagged histone H2B (red).** The red channel is superimposed on the transmission image. Confocal images were collected with a laser-scanning confocal microscope (C1-si; Nikon) with 1 frame every 5 min. Total length of movie, 20 frames (100 min).



Video 2. **Mitosis in a U2OS cell transfected with control siRNA, in which the chromosomes are visualized by expression of mCherry-tagged histone H2B (red).** The red channel is superimposed on the transmission image. Confocal images were collected with a laser-scanning confocal microscope (C1-si; Nikon) with 1 frame every 5 min. Total length of movie, 45 frames (225 min).



Video 3. **Mitosis in a U2OS cell transfected with siRNA against MAD2L2, in which the chromosomes are visualized by expression of mCherry-tagged histone H2B (red).** The red channel is superimposed on the transmission image. Confocal images were collected with a laser-scanning confocal microscope (C1-si; Nikon) with 1 frame every 5 min. Total length of movie, 20 frames (100 min).



Video 4. **Mitosis in a U2OS cell transfected with siRNA against MAD2L2, in which the chromosomes are visualized by expression of mCherry-tagged histone H2B (red).** The red channel is superimposed on the transmission image. Confocal images were collected with a laser-scanning confocal microscope (C1-si; Nikon) with 1 frame every 5 min. Total length of movie, 20 frames (100 min).