Expanded View Figures

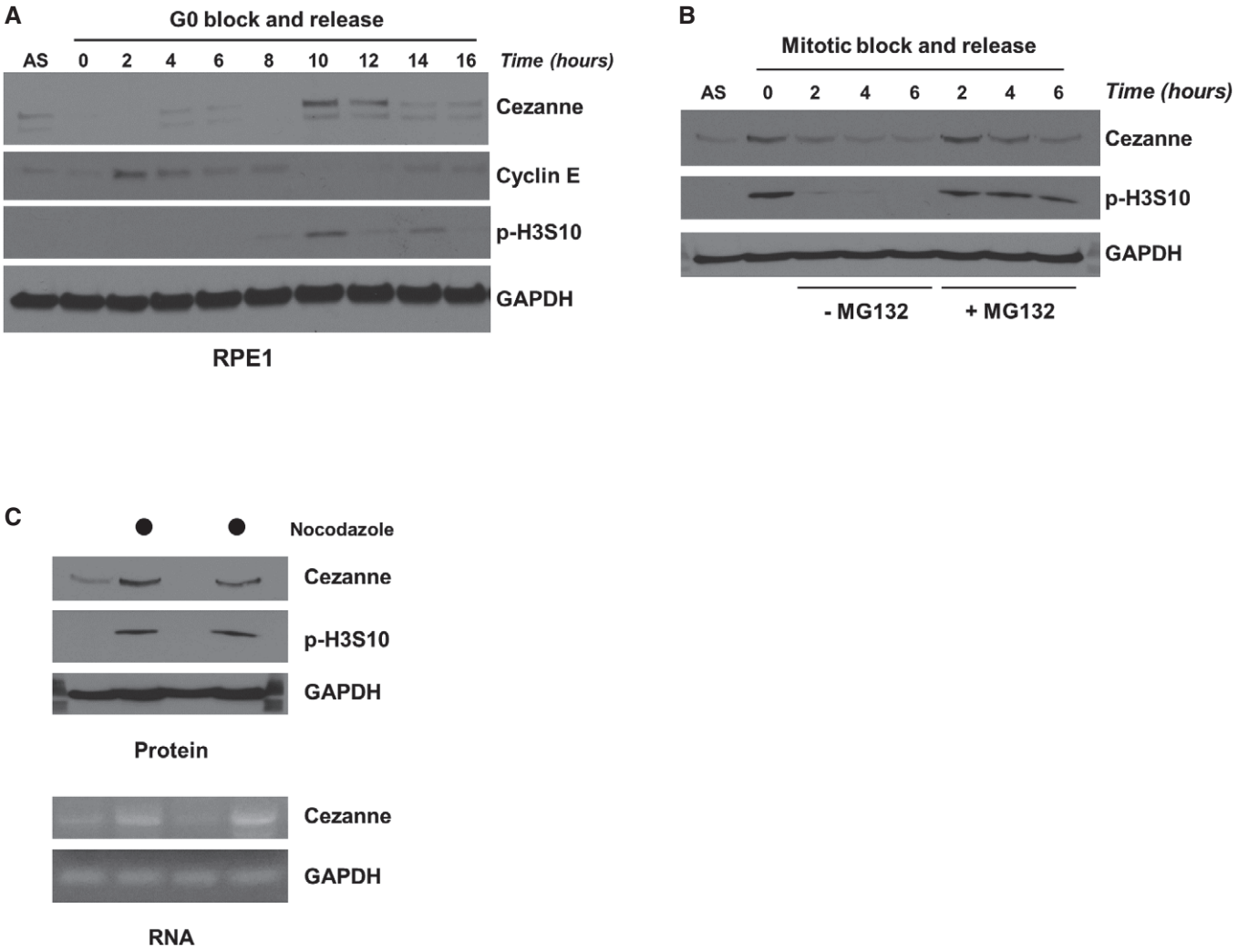

Figure EV1. Cezanne is cell cycle-regulated.

A RPE1 cells were synchronized by serum deprivation and then released into the cell cycle and analyzed by immunoblot.

B U2OS cells were synchronized in mitosis with nocodazole, isolated by "shake-off", and released into the cell cycle with or without the proteasomal inhibitor MG132. Samples were analyzed by immunoblot.

C U2OS cells grown asynchronously or synchronized in mitosis with nocodazole and isolated by "shake-off" were analyzed by immunoblot and RT–PCR.

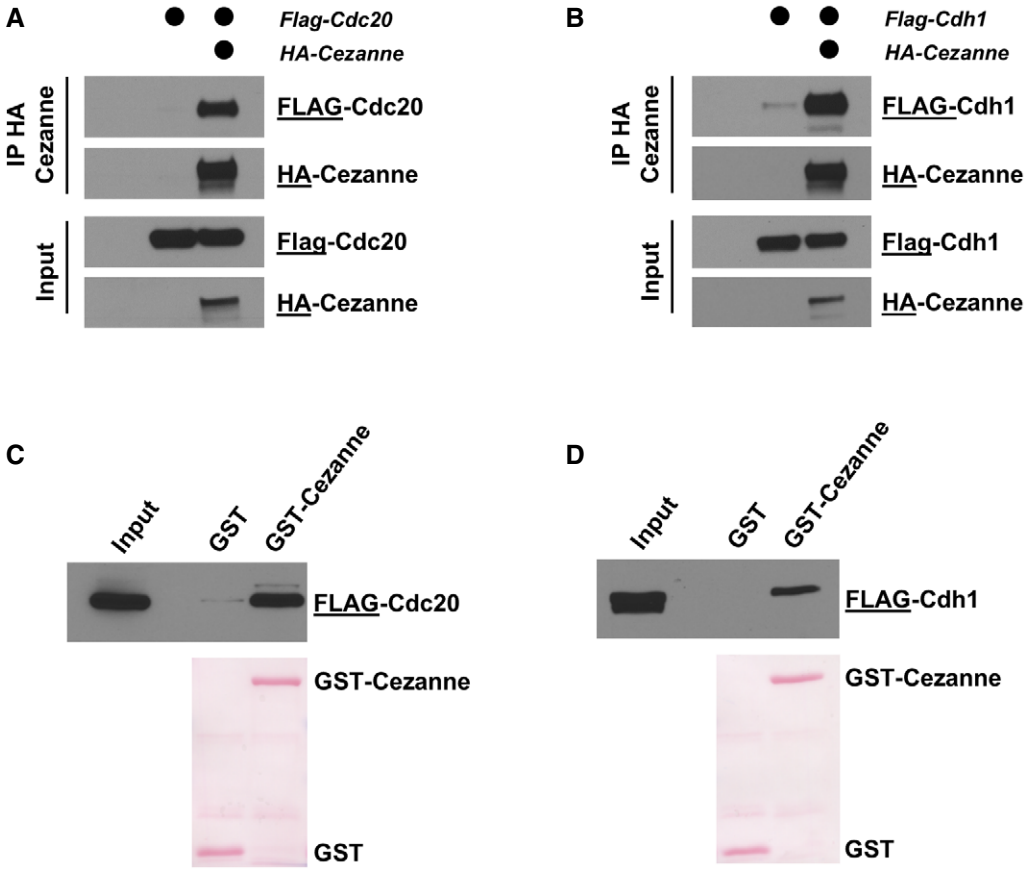

Figure EV2. Cezanne binds APC/C co-activators *in vivo* and in vitro.

A HA-Cezanne and FLAG-Cdc20 were ectopically expressed in HEK-293T cells, and Cezanne was immunoprecipitated on anti-HA beads. Samples were analyzed by immunoblot with the indicated antibodies.

B HA-Cezanne and FLAG-Cdh1 interaction was analyzed as in (A).

C 5 μg of GST-Cezanne coated on GSH beads was incubated with lysate of HEK-293T cells expressing a FLAG-tagged version of Cdc20. *In vitro* binding was analyzed by immunoblot using the indicated antibodies. GST was used as a negative control.

D Interaction of GST-Cezanne with Cdh1 was analyzed as in (C).

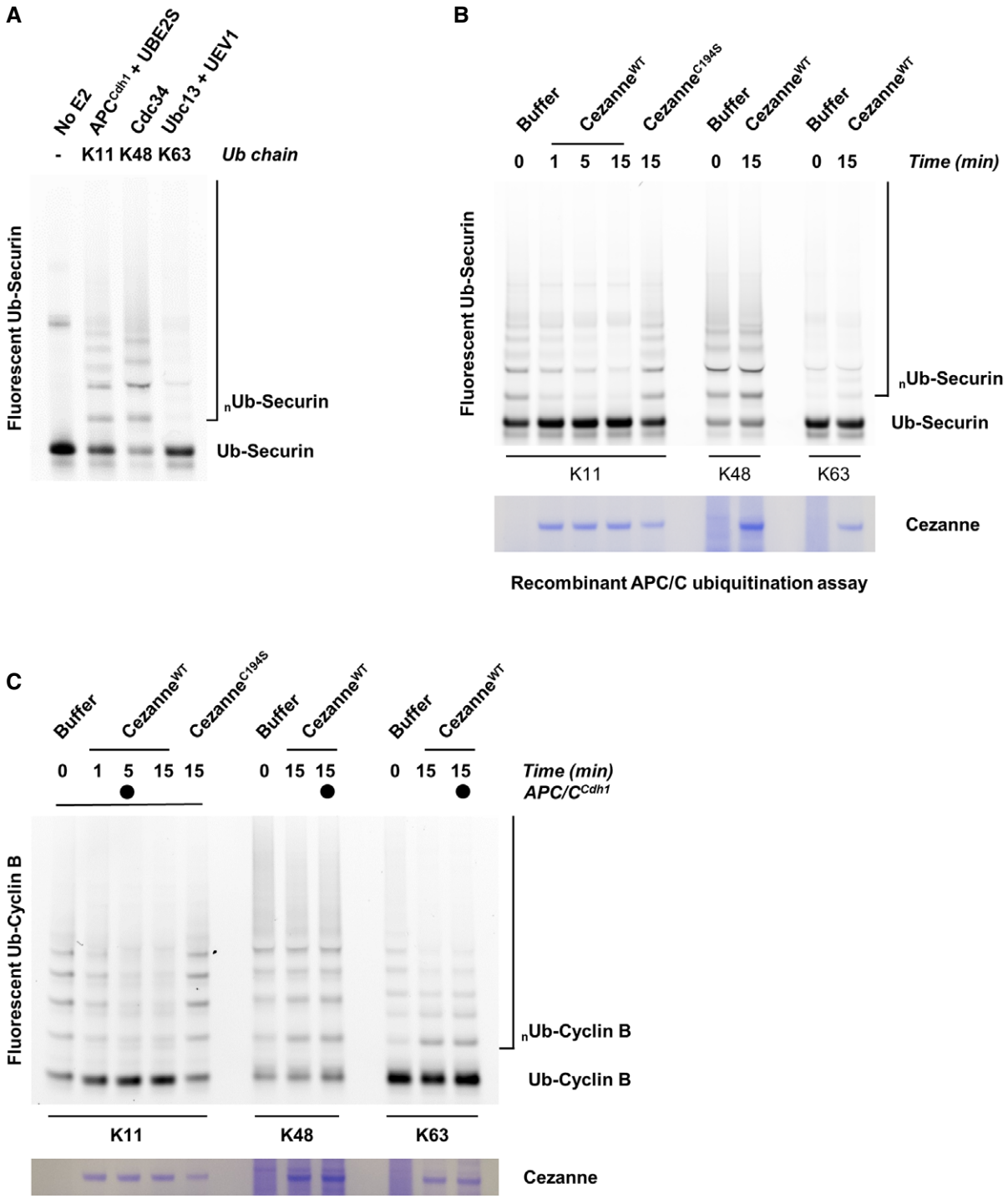

Figure EV3. Cezanne regulates the ubiquitination of APC/C substrates.

A Ubiquitination reactions of a fluorescein-labeled Securin fused to ubiquitin (Ub-Securin) were carried out in the presence of either recombinant APC/C^{Cdh1}, Cdc34, or Ubc13 and UEV1 to generate the indicated ubiquitin chain topologies. Reactions were quenched with EDTA and then analyzed by SDS–PAGE and fluorescence scanning.

B Fluorescein-labeled Ub-Securin conjugated to K11, K48, or K63 ubiquitin chains was incubated with recombinant Cezanne for the indicated times and then analyzed by SDS–PAGE, fluorescence scanning, and Coomassie staining.

C Fluorescein-labeled Ub-Cyclin conjugated to K48 or K63 ubiquitin chains was incubated with recombinant Cezanne in the absence or presence of APC^{Cdh1} for the indicated times and then analyzed by SDS–PAGE, fluorescence scanning, and Coomassie staining.