APPENDIX

TITLE

Cezanne/OTUD7B is a cell cycle-regulated deubiquitinase that antagonizes the degradation of APC/C substrates

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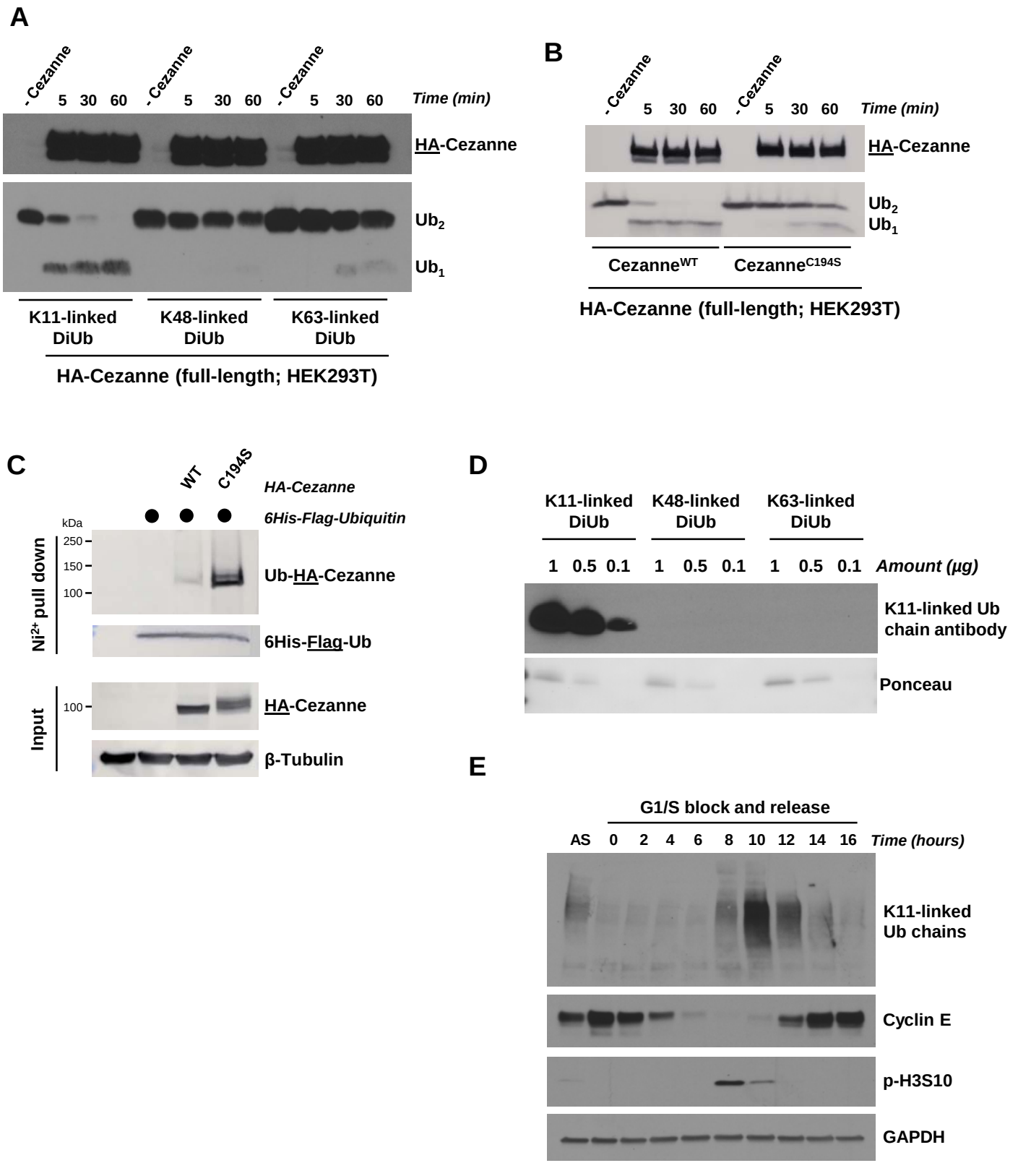
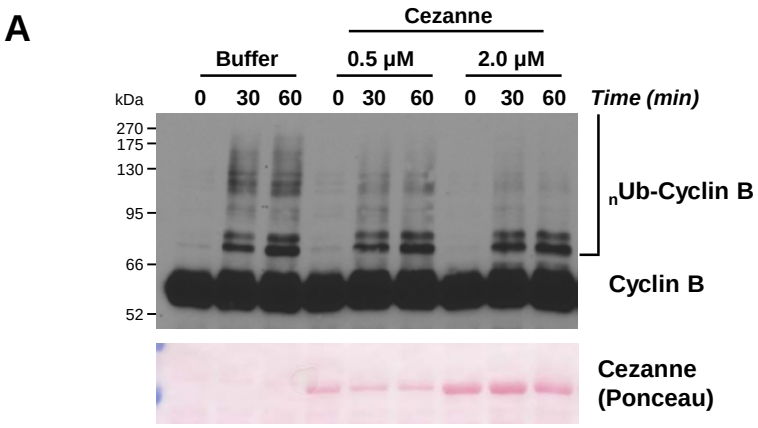
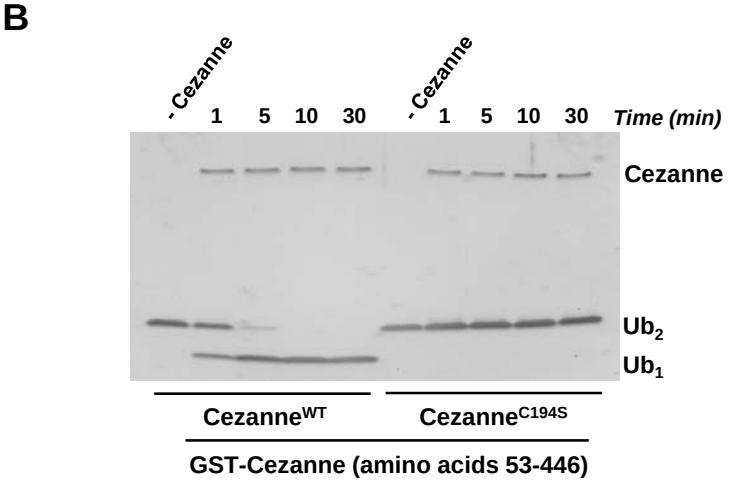


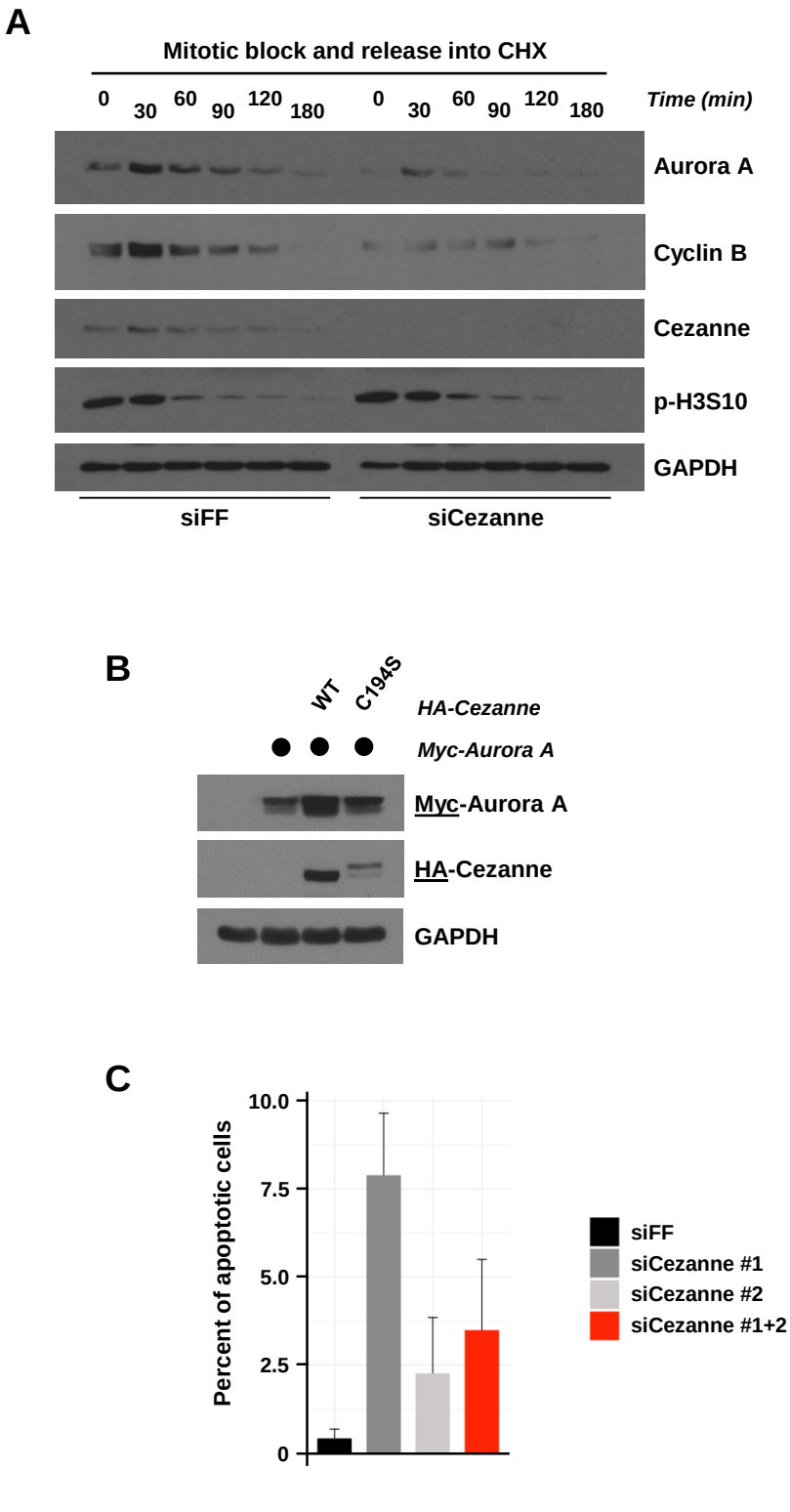
Appendix Figure S2



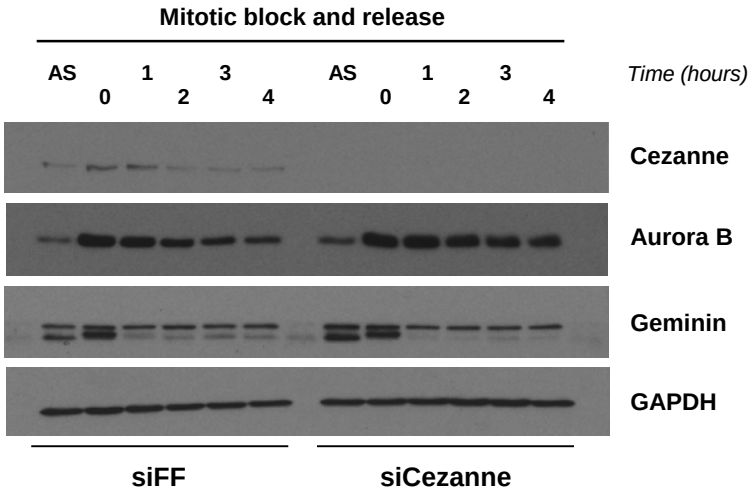
α-Cdc27 IP, APC/C ubiquitination assay



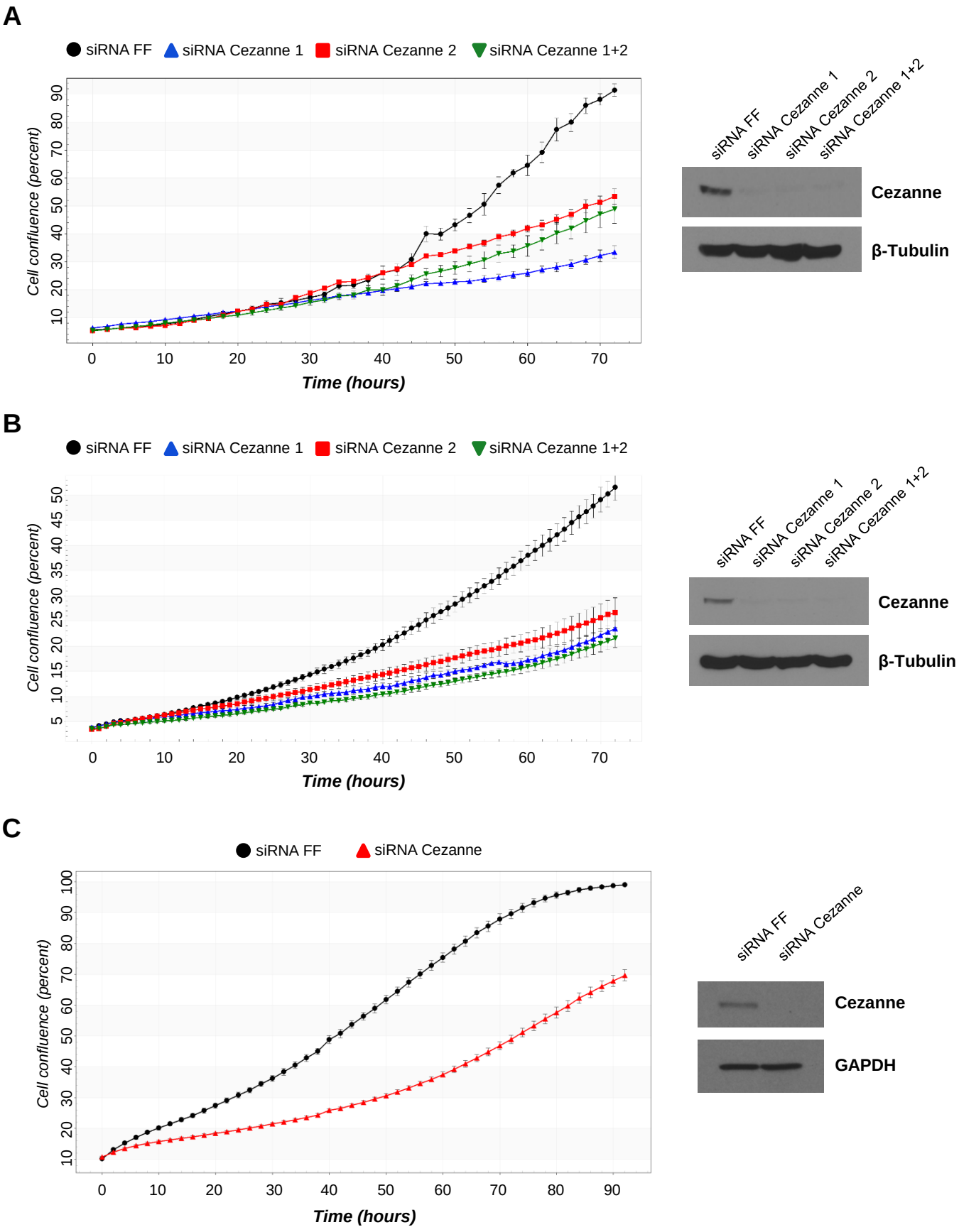
Appendix Figure S3



Appendix Figure S4



Appendix Figure S5



APPENDIX FIGURE LEGENDS

Appendix Fig S1. Cezanne is a K11-specific DUB and K11 chains are cell cycle regulated.

(A) HA-tagged version of Cezanne was transfected in HEK-293T cells and isolated on HA beads. Beads were split in 3 different tubes and incubated with 1 µM of the indicated diubiquitin probes in DUB reaction buffer. Reaction products were analyzed by immunoblot.

(B) HA-tagged versions of WT or catalytically dead (C194S) Cezanne transfected in HEK-293T cells and isolated on HA beads were incubated with 1 µM of K11 diubiquitin probes and analyzed as in (A).

(C) HA-tagged WT or C194S versions of Cezanne were transfected in HEK-293T cells with a 6His-Flag-ubiquitin construct. Ubiquitination was analyzed by immunoblot after denaturing Ni^{2+} pull down with the indicated antibodies.

(D) Indicated amounts of diubiquitin probes were separated by SDS-PAGE and immunobloted with K11 specific antibodies. Ponceau staining was used to control that same amount of all 3 different probes was used.

(E) HeLaS3 cells were synchronized at the G1/S boundary using a double thymidine block protocol, then analyzed by immunoblot after release into the cell cycle.

Appendix Fig S2. Cezanne activity regulates the ubiquitination of Cyclin B.

(A) APC/C immuno-purified from mitotic HeLaS3 cell extracts using anti-Cdc27 beads was mixed with in vitro translated Cyclin B, E1, E2, ATP, ubiquitin, and indicated amounts of Cezanne. Aliquots were collected at indicated time points and analyzed by immunoblot using Cyclin B antibodies.

(B) The activity of wild type and catalytically dead Cezanne was analyzed in vitro using K11-linked diubiquitin probes and analyzed by silver stain.

Appendix Fig S3. Cezanne depletion increases apoptosis.

(A) U2OS cells transfected with firefly control or Cezanne siRNA (siFF and siCezanne, respectively) were synchronized in mitosis then released in medium containing cycloheximide. Protein levels were analyzed at the indicated time points by immunoblot.

(B) HEK-293T cells were transfected for 24 hours as indicated and analyzed by immunoblot.

(C) Plot showing the frequency of apoptotic cells observed by confocal microscopy in control or Cezanne depleted U2OS cells (error bars show standard deviation, duplicate experiments, n>1000 cells per condition).

Appendix Fig S4. Cezanne depletion does not affect every APC/C substrate

U2OS cells transfected with firefly control or Cezanne siRNA (siFF and siCezanne, respectively) and synchronized in mitosis were released in fresh medium and analyzed by immunoblot with the indicated antibodies at the indicated time points. (Additional immunoblotting of these samples is shown in Fig 6A, and the same Cezanne and GAPDH controls are shown for both).

Appendix Fig S5. Cezanne depletion decreases cell proliferation.

(A) U2OS cells were transfected with the indicated siRNAs in 60 mm plates, and proliferation was analyzed by live imaging using the IncuCyte system (left) and by immunoblot to confirm efficient knock down of Cezanne (right).

(B) Proliferation of control of Cezanne depleted HCT116 cells was analyzed as in (A).

(C) Proliferation of control or Cezanne depleted HeLaS3 cells was analyzed as in (A) and (B).