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Supplemental Figure 1

1 60
MSYNYVVTAQKPTAVNGCVTGHFTSAEDLNLLIAK**NTRLEIYVVTAEGLRPVK**EVGMYGK

120
IAVMELFRPKGESKDLLFILTAKYNACILEYKQSGESIDIITRAHGNVQDRI**GRPSETGI**

180
IGIIDPECRMIGLRLYDGLFKVIPLDRDNKELKAFNIRLEELHVIDVKFLYGCQAPTICF

240
VYQDPQGRHVKTYEVSLREKEFNKGPWKQENVEAEASMVIAVPEPFGGAIIIGQESITYH

300
NGDKYLAIAPPIIKQSTIVCHNRVDPNGSRYLLGDMEGR**LFMLLLEKEEQMDGTVTLK**DL

360
RVELLGETSIAECLTYLDNGVVFVGSRLGDSQLVKLNVDSNEQGSYVVAMETFTNLGPIV

420
DMCVVDLERQGQGQLVTCSGAFKEGSLRIIR**NGIGIHEHASIDLPGIK**GLWPLR**SDPNRE**

480
TDDTLVLSFVGQTRVLMLNGEEVEETELMGFVDDQQTFFCGNVAHQQLIQITSASVRLVS

540
QEPKALVSEWKEPQAKNISVASCNSSQVVVAVGRALYYLQIHPQELRQISHTEMEHEVAC

600
LDITPLGDSNGLSPLCAIGLWTDISARILKLPSFELLHKEMLGGEIIPRSILMTTFESSH

660
YLLCALGDGALFYFGLNIETGLLSDRKKVTLGTQPTVLRTFR**SLSTTNVFACSDRPTVIY**

720
SSNHKLVFSNVNLKEVNYMCPLNSDGYPDSLALANNSTLTIGTIDEIQKLHIRTVPLYES

780
PRKICYQEVSQCFGVLSSR**IEVQDTSGGTTALRPSASTQALSSSVSSSK**LFSSSTAPHET

840
SFGEEVEVHNLLIIDQHTFEVLHAHQFLQNEYALSLVSCK**LGKDPNTYFIVGTAMVYPEE**

900
AEPKQGRIVVFQYSDGKLQTVAEKEVKGAVYSMVEFNGKLLASINSTVRLYEWTTEKELR

960
TECNHYNNIMALYLKTKGDFILVGDLMR**SVLLLAYKPMEGNFEEIAR**DFNPNWMSAVEIL

1020
DDDNFLGAENAFNLFVCQKDSAATTDEERQHLQEVGLFHLGEFVNVFCHGSLVMQNLGET

1080
STPTQGSVLFGTVNGMIGLVTSLSESWYNLLLDMQNRLNKVIKSVGKIEHSFWRSFHTER

1140
KTEPATGFIDGDLIESFLDISRPKMQEVVANLQYDDGSGMKREATADDLIKVVEELTRIH

Supplemental Figure 1. DDB1 Protein Sequence.
Peptides identified by mass-spectrometry are in bold and underlined.

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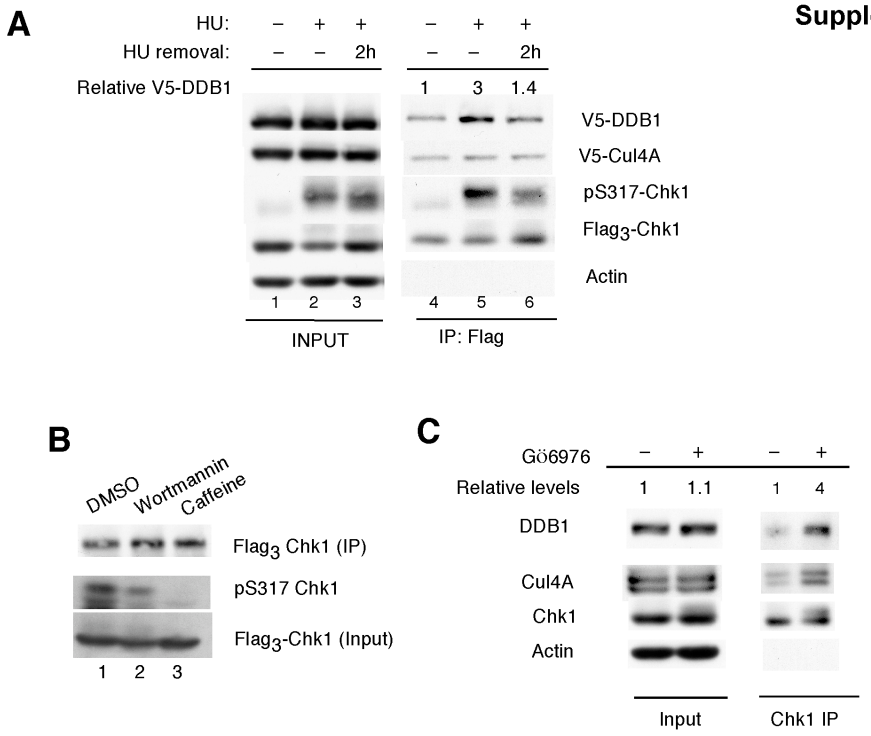
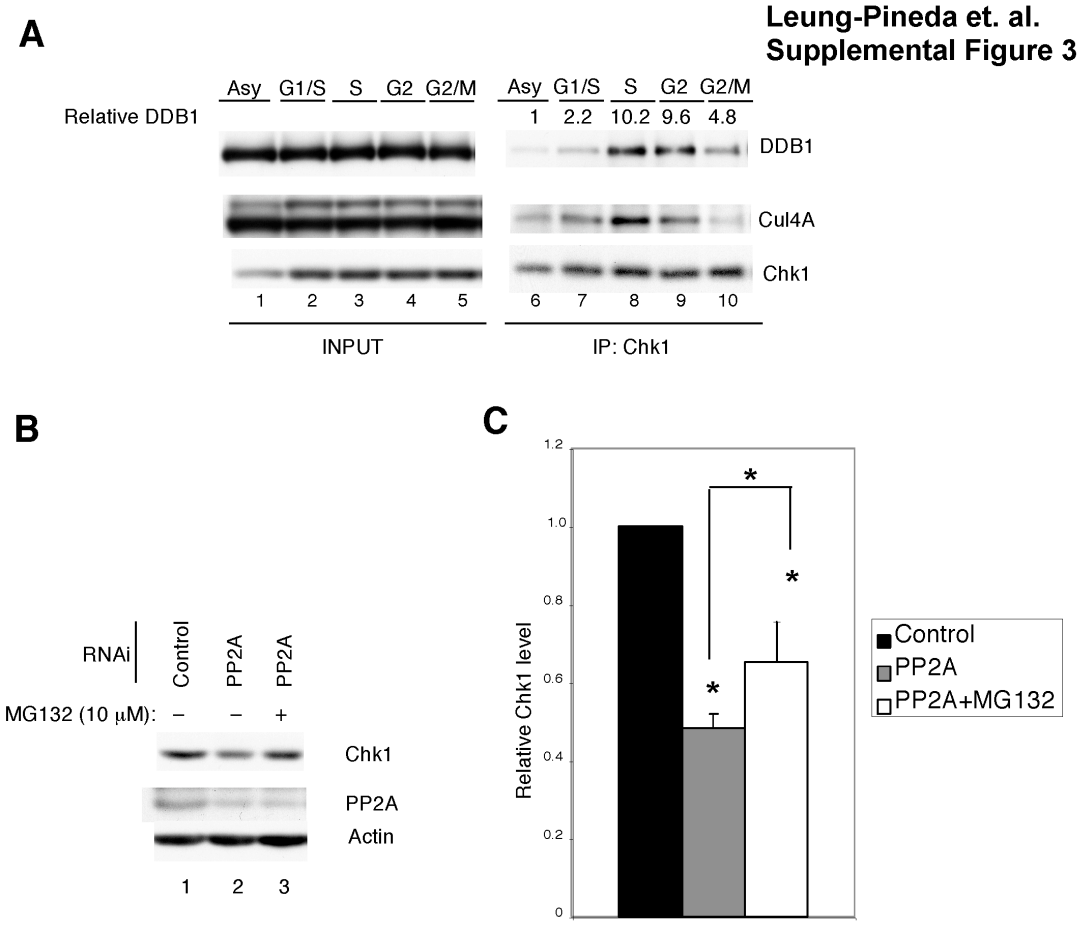
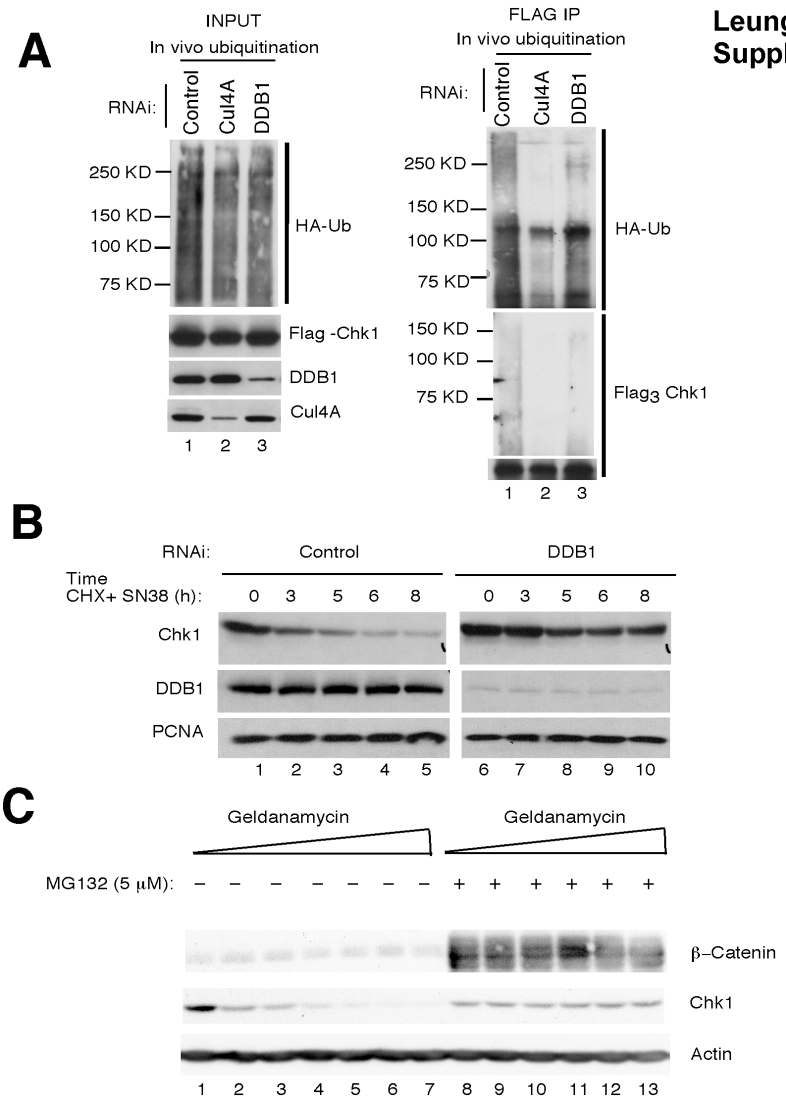
Supplemental Figure 2



Supplemental Figure 2. Phosphorylation regulates interactions between Cul4/DDB1 and Chk1. (A) Asynchronously growing HeLa cells were co-transfected with plasmids encoding $Flag_3$-Chk1, V5-DDB1 and V5-Cul4A. Cells were incubated in the absence or presence of HU (20 mM) for 4 h. In some cases, the HU-containing media was removed and replaced with fresh media and the cells were incubated for an additional 2 h. Flag-tagged Chk1 was isolated and precipitates were analyzed for the presence of DDB1 and Cul4A by Western blotting using anti-V5 antibodies. In addition, Chk1 phosphorylation on S317 was monitored. Relative levels of DDB1 are indicated. (B) HeLa cells expressing $Flag_3$-Chk1 and incubated with DMSO, wortmannin or caffeine for 2 h followed by HU and MG132 for 4 h. Lysates were either analyzed directly by Western blotting (input) or were first incubated with Anti-Flag M2-Agarose to isolate flag-tagged Chk1 prior to Western blotting. (C) Asynchronously growing HeLa cells were incubated with DMSO or Gö6976 for 2 h followed by HU and MG132 for 4 h. Lysates were incubated with affinity purified anti-Chk1 antibodies and analyzed by Western blotting with the indicated antibodies. Relative levels of DDB1 are indicated.



Supplemental Figure 3. Interactions between Chk1 and Cul4A/DDB1 throughout the cell cycle. (A) HeLa cells were synchronized by a double thymidine block and release protocol. Cells were collected at 0 h (G1/S), 2 h (S), 4 h (G2) and 6 h (G2/M) after the second release. A fraction of the cells were subjected to flow cytometry to determine cell cycle position (data not shown). The remaining cells were lysed and endogenous Chk1 was immunoprecipitated using affinity-purified Chk1 antibodies. Precipitates were resolved by SDS-PAGE and subjected to Western blotting with antibodies specific for DDB1 and Cul4A. (B) Asynchronously growing HeLa cells were treated with control siRNAs or PP2A-specific siRNAs for 48 h. Cells were then incubated with DMSO or MG132 (10 uM) for 6 h. Cells were harvested and analyzed by Western blotting (B). The data from 4 independent experiments is presented graphically in panel C. The data is presented as mean +/- standard deviation. The asterisk (*) indicates $p < 0.05$.



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Supplemental Figure 4

Supplemental Figure 4. DDB1 knockdown stabilizes Chk1 and geldanamycin promotes Chk1 degradation. (A) Asynchronously growing HeLa cells were co-transfected with plasmids encoding $Flag_3$-Chk1 and HA-Ub together with the indicated siRNAs for 48 h and then treated with HU (20 mM) and MG132 (10 μM) for 4 h. Cell lysates were prepared and analyzed directly by Western blotting (right panel) or flag-tagged Chk1 precipitates were isolated prior to SDS-PAGE and subjected to Western blotting with antibodies specific for HA (top right panel) or Flag (bottom right panel). (B) Asynchronously growing HeLa cells were transfected with control- or DDB1-specific siRNAs for 48 h followed by incubation with cycloheximide (CHX) and SN38 (1 μM) for the indicated times. Lysates were prepared and analyzed by Western blotting. (C) Asynchronously growing HeLa cells were incubated with increasing concentrations of geldanamycin [50 nM (lane 8), 100 nM (lane 9), 250 nM (lane 10), 350 nM (lane 11), 500 nM (lane 12), 1 μM (lane 13) with or without MG132 (5 μM)] for 27 h. Cells lysates were subjected to Western blotting with the indicated antibodies.