Supplemental Materials

Molecular Biology of the Cell

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Supplemental Figure Legends

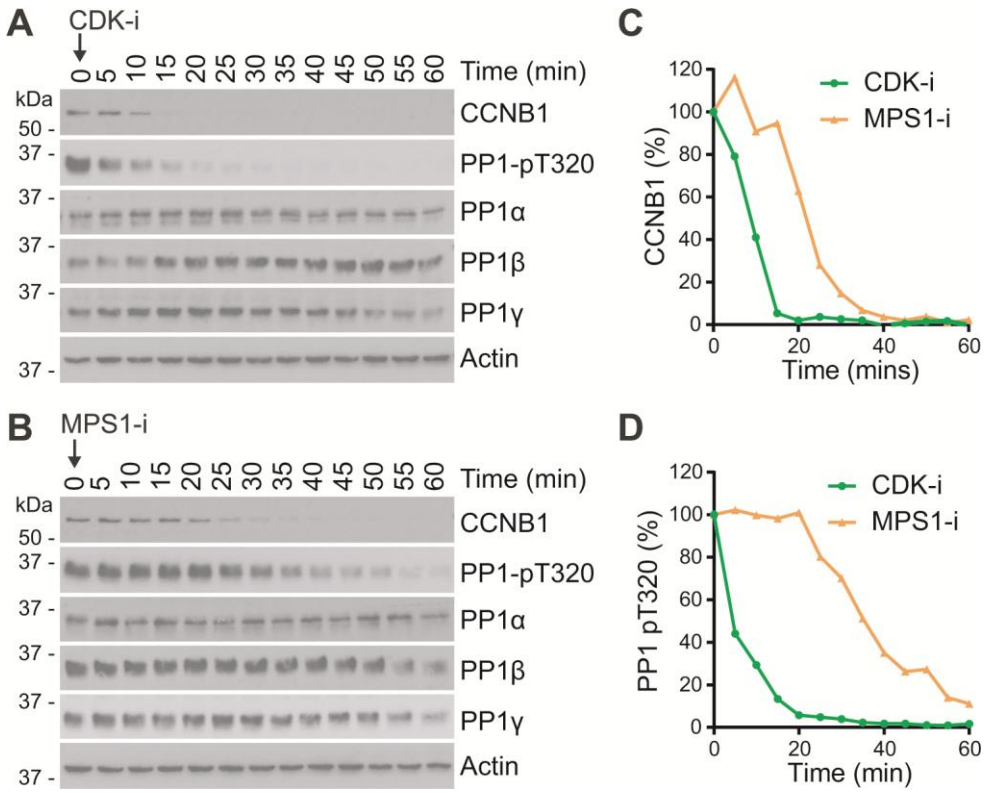

Figure S1. CDK1 inhibition accelerates cyclin B destruction at the metaphase-to-anaphase transition. **(A)** Synchronous progression of mitotic HeLa cells into anaphase was triggered with CDK1 inhibitor (CDK-i, 5 µM flavopiridol) or **(B)** MPS1 inhibitor (MPS1-i, 2 µM AZ3146), and samples were collected every 5 min and blotted with the indicated antibodies. **(C)** Cyclin B1 or **(D)** PP1 pT320 levels are plotted as a function of time from Western blot analysis of mitotic exit following CDK or MPS1 inhibition.

Figure S2

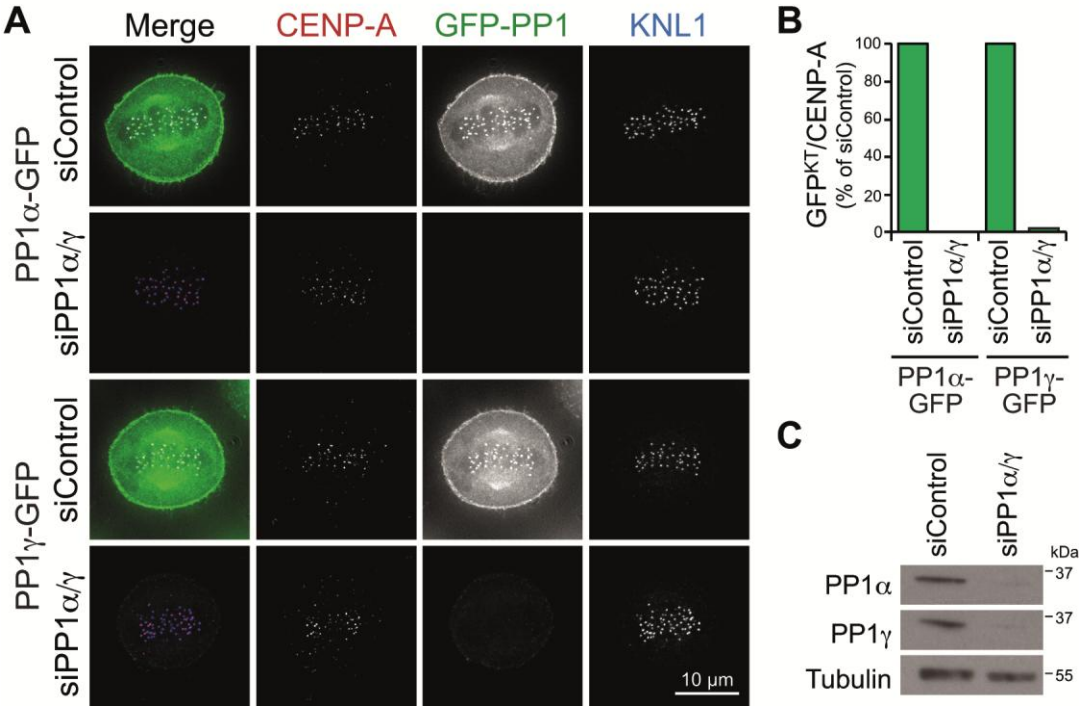

Figure S2. Characterisation of PP1α and PP1γ localisation and depletion efficiency. **(A)** HeLa Flp-In TRex cells induced to express PP1α-GFP or PP1γ-GFP were depleted of PP1α/γ and processed for immunofluorescence analysis. CENP-A and KNL1 were stained as reference markers for the kinetochores. **(B)** PP1α-GFP or PP1γ-GFP kinetochore intensity in siControl or siPP1α/γ co-depleted cells was quantified relative to CENP-A. **(C)** Western blot analysis of siControl and siPP1α/γ co-depleted cells. Tubulin was used as a loading control.

Figure S3

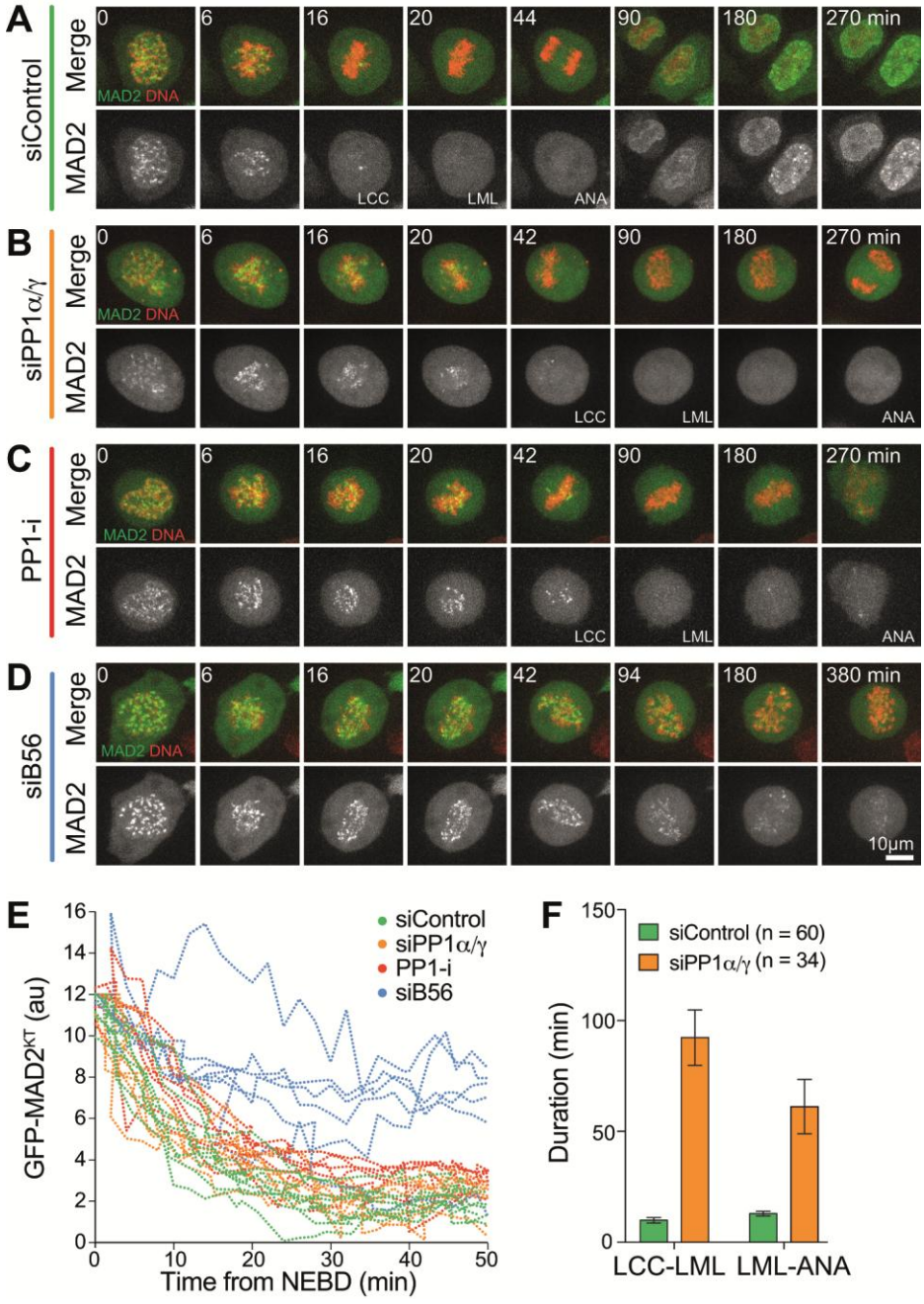

Figure S3. PP1 regulates cyclin B destruction downstream of checkpoint silencing. **(A)** HeLa cells stably expressing GFP-MAD2 were treated with control, **(B)** siPP1α/γ or **(D)** siPP2A-B56 for 60 h, or **(C)** 5 μM tautomycetin PP1-inhibitor for 30 min before imaging.

DNA was visualised with SiR-Hoechst DNA dye. Cells were imaged every 2 mins for a total of 10 hrs. **(E)** Total cellular intensity of kinetochore-localised MAD2 ($MAD2^{KT}$) is plotted as a function of time for siControl (n=7), siPP1α/γ (n=6), PP1-i (n=6), and siPP2A-B56 (n=5). **(F)** Bar graphs showing the mean time interval ± SD between the last chromosome congressed (LCC) and last MAD2 lost (LML), and last MAD2 lost and anaphase onset (ANA), in siControl (n=60) and siPP1α/γ cells (n=34) expressing GFP-MAD2.

Figure S4

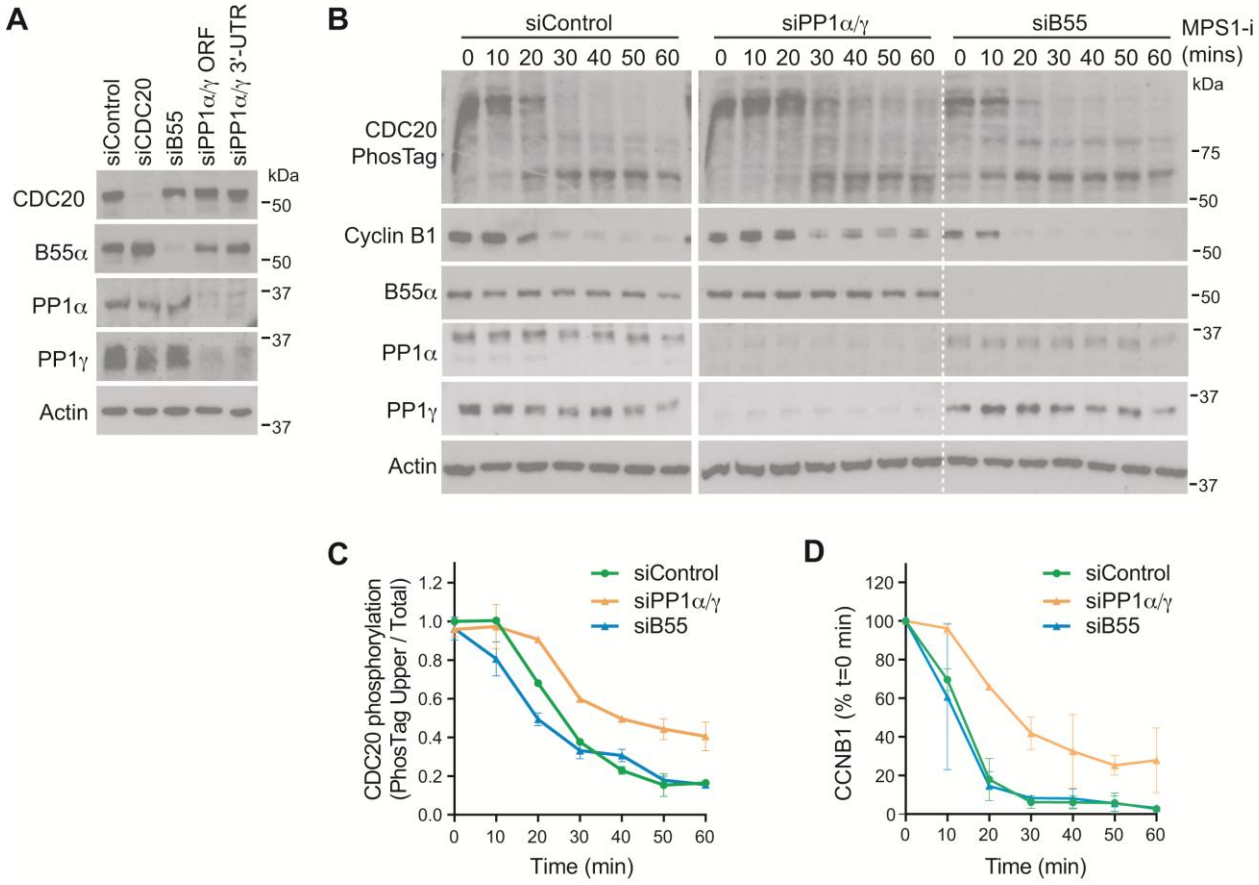

Figure S4. PP1α/γ depletion, but not PP2A-B55 depletion, impairs CDC20 dephosphorylation and cyclin B degradation. **(A)** Western blot of HeLa cells transfected with siControl, siCDC20, siPP1α/γ or siB55 duplexes to confirm specific depletion of the respective target proteins. **(B)** Synchronised HeLa cells transfected with siControl, siPP1α/γ or siB55 duplexes were treated with MPS1-i to initiate mitotic exit. Samples were collected every 10 min and analysed by immunoblotting using PhosTag gels for CDC20 and standard gels for all other proteins with the indicated antibodies. **(C)** Phospho-CDC20 (top band in the PhosTag blot) relative to total CDC20 is plotted in the

line graph (mean ± SD, n=2). **(D)** CCNB1 levels were measured as a function of time in siControl, siPP1α/γ and siB55 and plotted in the line graph (mean ± SD, n=2).

Figure S5

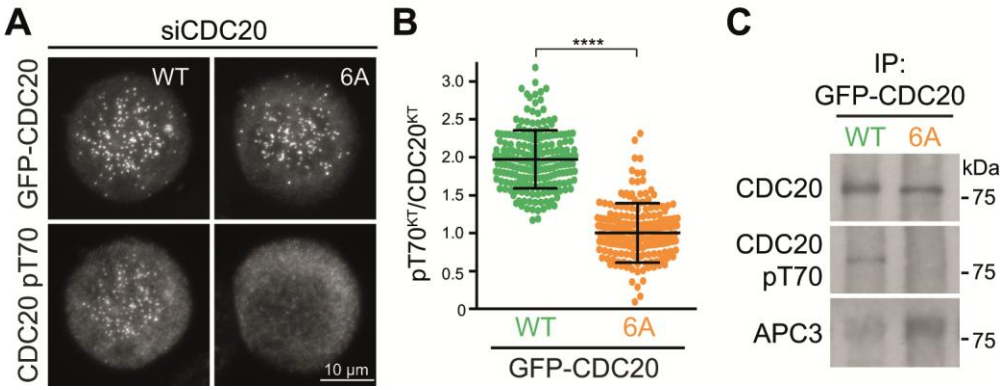

Figure S5. CDC20-pT70 antibodies specifically recognise phosphorylated CDC20. **(A)** HeLa Flp-In TRex cells depleted of the endogenous CDC20 and expressing GFP-CDC20^{WT} or GFP-CDC20^{6A} were treated with nocodazole for 20 min, then stained for CDC20 pT70. **(B)** CDC20 pT70 normalised for total GFP-CDC20 is plotted as mean ± SD. **(C)** HeLa Flp-In TRex cells depleted of the endogenous CDC20 were induced for GFP-CDC20^{WT} or GFP-CDC20^{6A}, then arrested in mitosis. GFP-CDC20 was immunoprecipitated using anti-GFP antibodies (IP), and the immunoprecipitated samples Western blotted for total CDC20, CDC20 pT70 and APC3.