Figure S1. Disassembly of MCC complex in Cdc20ΔC-Box/ΔIR

A) Quantification of endogenous and exogenous Cdc20 using the outlined synchronization protocol in Figure 1C and inducing with 10 ng/ml of doxycycline for 24 hours. The boxes and numbers indicate the corresponding values of endogenous and exogenous Cdc20. B) Similar to A) but also probing for Cdc20 levels in the Cdc20 mutant cell lines. Degradation products from the YFP-tagged Cdc20 constructs are indicated by the line and star. B) Purification of endogenous p31 from nocodazole arrested cells and probed for the association with the indicated proteins. C) Cdc20 wt or Cdc20 ΔC-Box/R499E expressing cells were released from a thymidine block into taxol for 11 hours. Cells were collected by mitotic shake-off and reseeded into ZM447439 for the indicated times before harvesting and purifying Cdc20 complexes using a GFP-binder affinity resin. The levels of APC/C and MCC components were analyzed by western blot. D) Quantification of the experiments shown in B) with the mean and standard deviation from 3 independent experiments shown. Relative fluorescence intensity was measured and normalized to YFP-Cdc20 and time point 0.