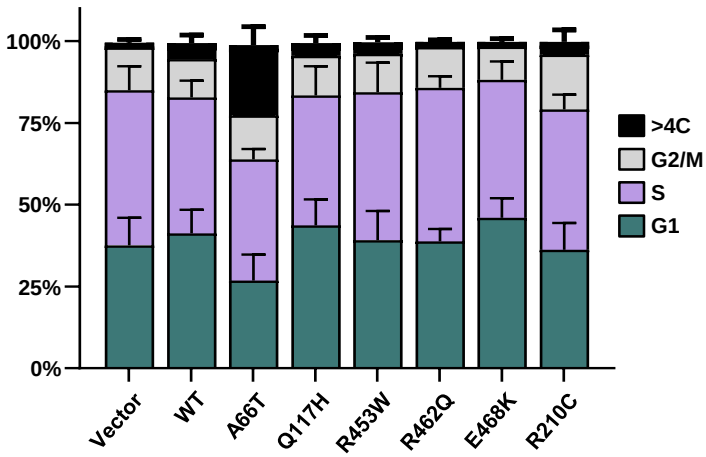
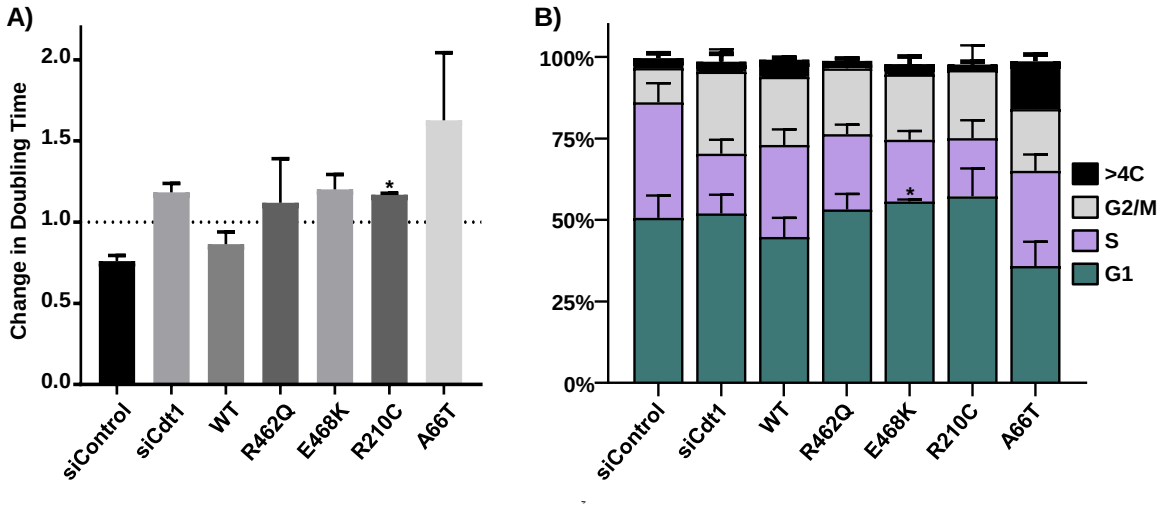
Supplemental Materials

Molecular Biology of the Cell

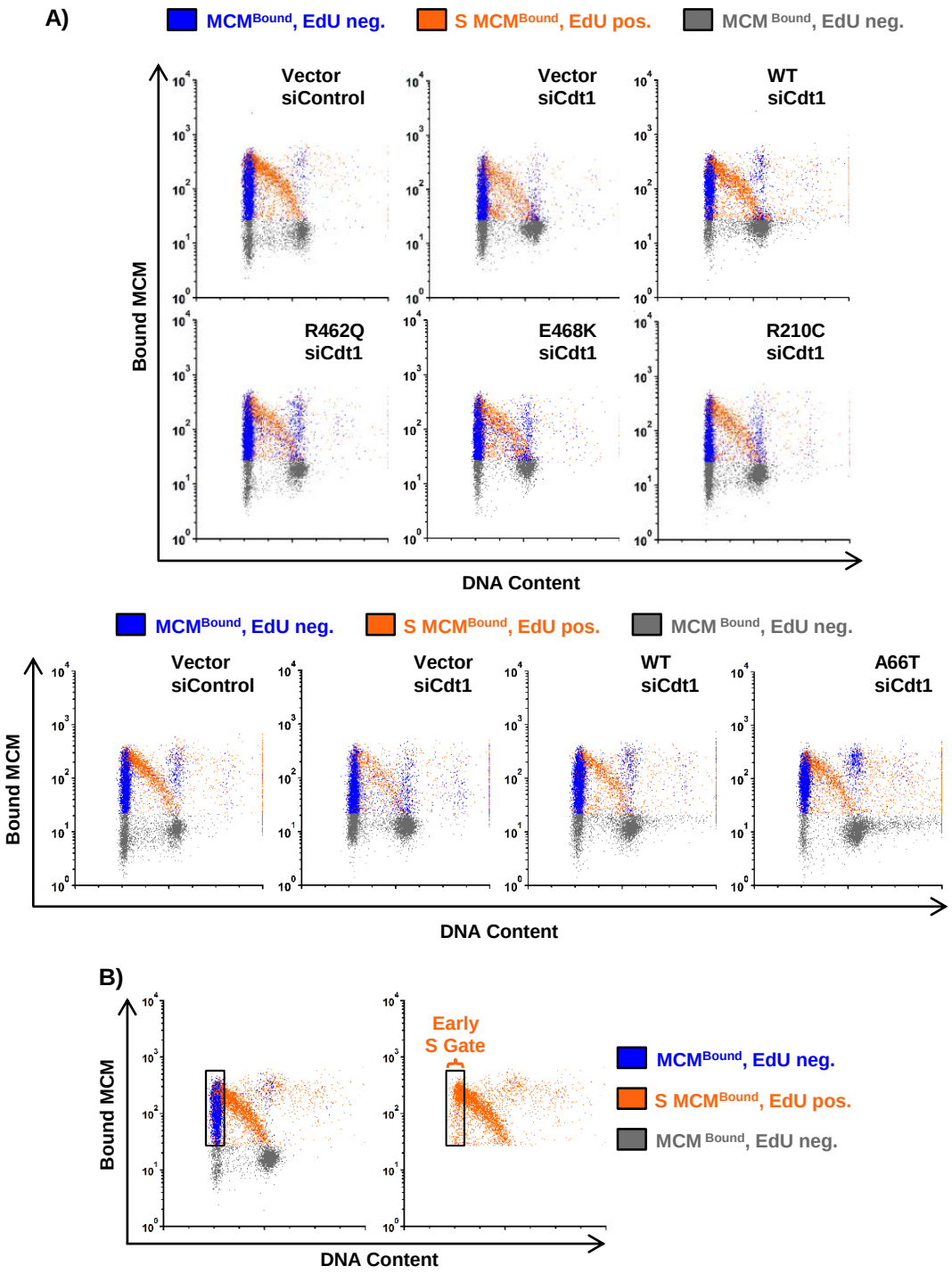
Pozo et al.



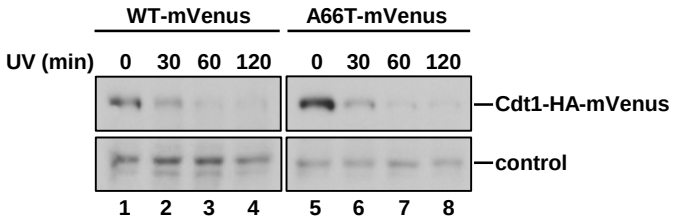
Supplemental Figure 1. Stacked bar graphs of cell cycle phase distribution of cells overproducing WT or variant Cdt1 from Figure 1. The percentage of U2OS cells in each cell cycle phase and percentage of re-replicating cells from at least three biological replicates is graphed. Bars represent mean with error ± SD.



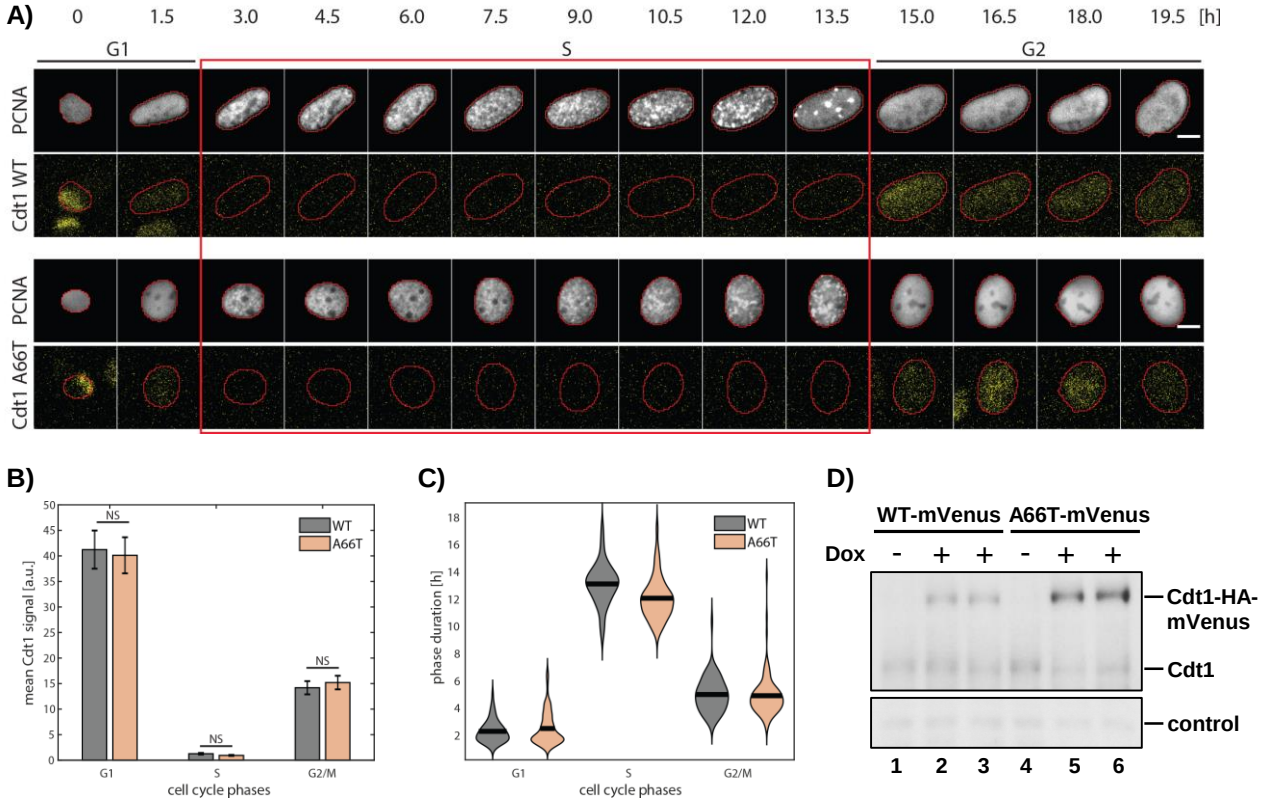
Supplemental Figure 2. Change in population doubling times and cell cycle phase distributions of cells expressing WT or variant Cdt1 used in complementation experiments. (A) The change in doubling time was calculated by dividing the doubling time of siRNA + doxycycline treated-cells by the doubling time of the corresponding untreated U2OS cells. A doubling time value of 1 signifies no change in doubling time. Bars represent mean with error ± SD. * = p value <0.05 where indicated, otherwise differences between WT Cdt1 and the variants was not significantly different. (B) Stacked bar graphs of cell cycle distribution of U2OS cells from Figure 3. The percentage of cells in each cell cycle phase and percentage of re-replicating cells from at least three biological replicates are graphed. Bars represent mean with error ± SD. * = p value <0.05 where indicated, otherwise differences between WT Cdt1 and the variants was not significantly different.



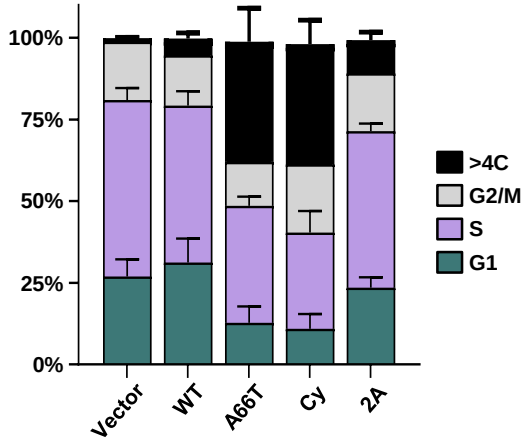
Supplemental Figure 3. Analytical flow cytometry profiles of siRNA-treated cells used for MCM binding analysis in Figure 3. (A) Cells were treated with 100 nM siRNA for 72 hours in 0.002 µg/mL – 0.006 µg/mL doxycycline then labeled with EdU for 30 minutes prior to harvesting and extraction of soluble MCM. Samples were stained with anti-MCM2 antibody and DAPI to measure chromatin-bound MCM and overall DNA content, respectively. (B) Representative example (Vector plus siControl) treated as in A showing the gating scheme used to quantify chromatin-bound MCM specifically in early S phase.



Supplemental Figure 4. Cdt1-A66T variant is targeted for CRL4^{Cdt2}-dependent degradation. U2OS cells were treated with 100 µg/mL doxycycline for 16 hours to induce ectopic expression of Cdt1-WT-mVenus and Cdt1-A66T-mVenus. Cells were then subjected to 20 J/m2 UV and harvested at the indicated time points post-irradiation for subsequent immunoblot analysis with anti-Cdt1 antibody. A non-specific band serves as loading control.



Supplemental Figure 5. Cdt1 WT- and A66T-mVenus dynamics in asynchronously proliferating cells. (A) Selected images from a typical time lapse experiment showing progression through the cell cycle of a U2OS cell expressing Cdt1 WT-Venus (upper rows) and Cdt1 A66T-Venus (lower rows). Cells stably co-expressed PCNA-mCherry. Division into cell cycle phases (G1, S, G2+M) was based on the variance of PCNA distribution as indicated by the red box; scale bar 10 µm. (B) Quantification of Cdt1 WT and A66T Venus intensities in different cell cycle phases (n=70 Cdt1 WT and n=110 A66T, error bars are s.e.m.). No significant differences were observed between populations according to Wilcoxon rank sum test. (C) Distribution of durations of cell cycle phases in populations expressing Cdt1 WT and A66T. Black lines indicate the mean. (D) Representative anti-Cdt1 immunoblot of U2OS cells treated with 5 µg/mL doxycycline (lanes 2 and 5) and 10 µg/mL doxycycline (lanes 3 and 6) for 72 hours. A non-specific band serves as a loading control.



Supplemental Figure 6. Stacked bar graphs of cell cycle distribution of U2OS cells overproducing WT or variant Cdt1 from Figure 6. The percentage of cells in each cell cycle phase and percentage of re-replicating cells from at least three biological replicates is graphed. Bars represent mean with error ± SD.