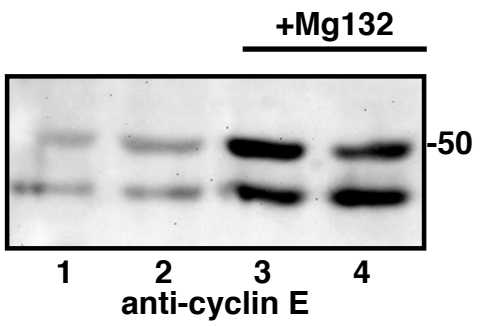

Figure S1: Transfected cyclin E is stabilized by MG132. WT 293 cells were transfected with wild-type myc-tagged cyclin E. MG132 was added 18 hours before harvest to the indicated samples. Cells were harvested, lysed and analyzed by western blot. Migration of size markers indicated to the right of gel image (kDa).

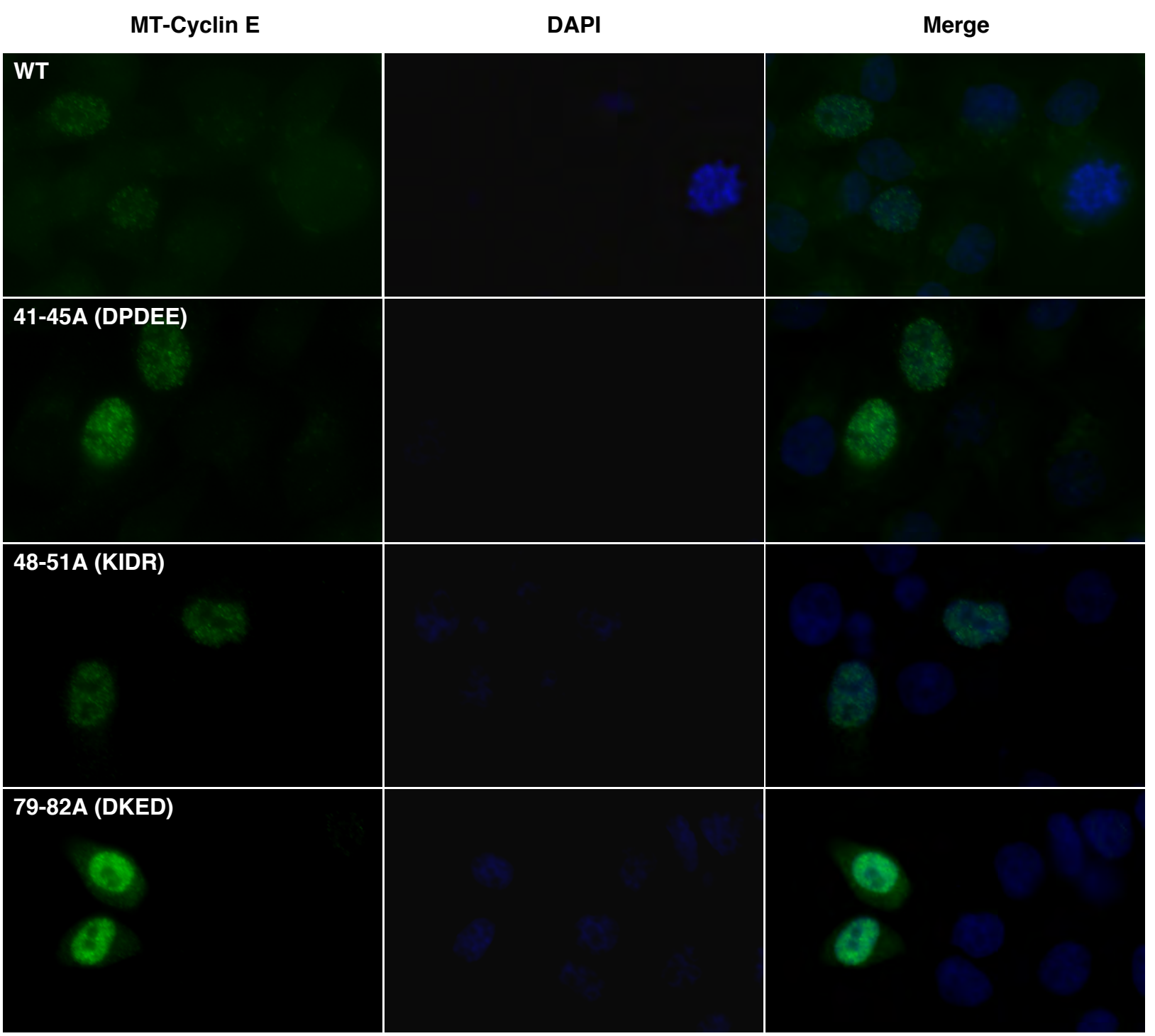

Figure S2: Localization of cyclin E mutants are the same as wild-type. Myc-tagged cyclin E mutants were transfected into HeLa cells and localization was determined using immunofluorescence. The localization of several mutants is shown: DPDEE (41-45)→AAAAA (row 2), KIDR (48-51)→AIAA (row 3), and DKED (97-82)→ AAAA (row 4). The top row shows the localization of wild-type cyclin E.

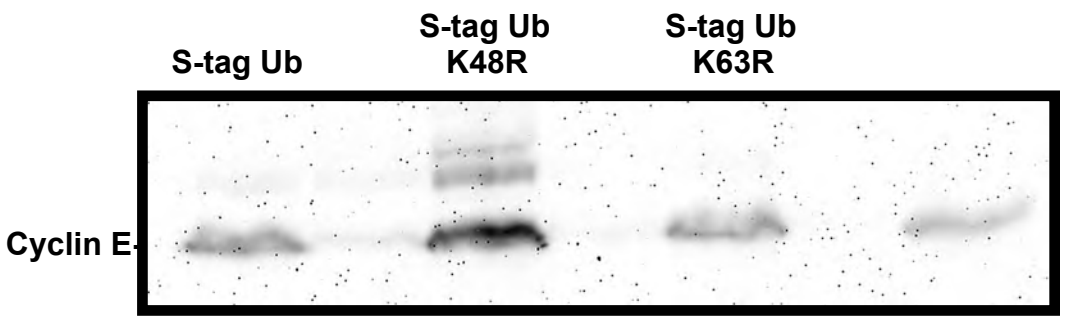

Figure S3: Ubiquitin mutants and cyclin E. Myc-cyclin E was transfected in the presence or absence of S-tagged ubiquitin mutants. The Ubiquitinmutant K48R, which cannot form degradative linkages is shown in lane 2. Ubiquitin mutant K63R, which cannot form non-degradative K63 linkages,is shown in lane 3. Cyclin E alone is shown in lanes 1 and 4.

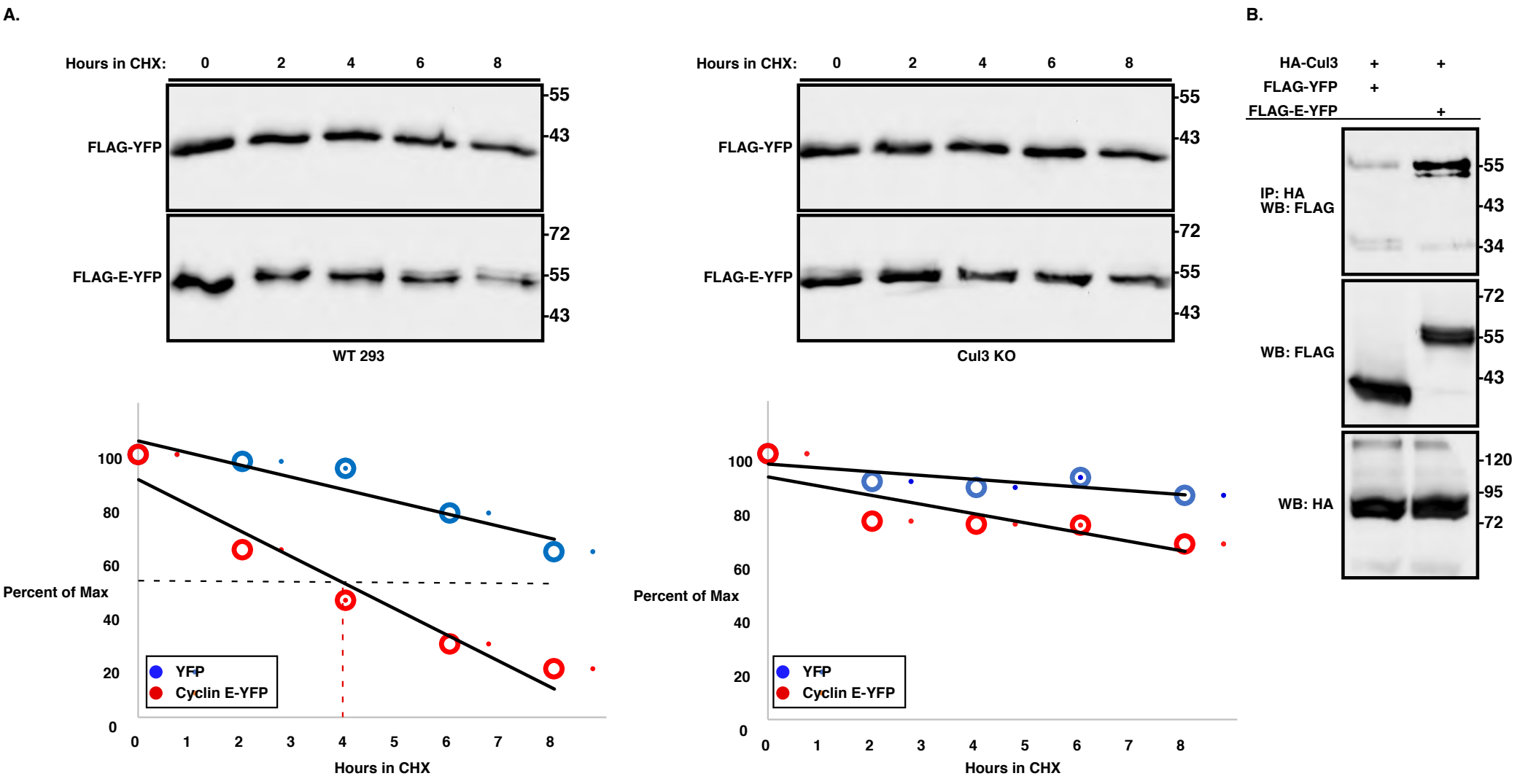

Figure S4: The cyclin E N-terminus induces degradation of a YFP fusion protein. A: Cyclin E residues 2 through 86 were fused to a Flag-tagged YFP construct. Half-lives of the resulting cyclin E-YFP fusion protein were then measured in 293 cells and compared to a Flag-tagged YFP control. The resulting western blots (top panel) were quantified and the half-life of the control YFP is shown in blue and cyclin E-YFP fusion protein is shown in red (graph, bottom panel). B: YFP (lane 1) or the cyclin E-YFP fusion protein (lane 2) were co-transfected with HA-tagged Cul3 and immunoprecipitated using HA antibody. IP results are shown in the top panel. The two lower panels show relative amounts of protein in each of the original samples. Migration of size markers indicated to the right of gel images (kDa).

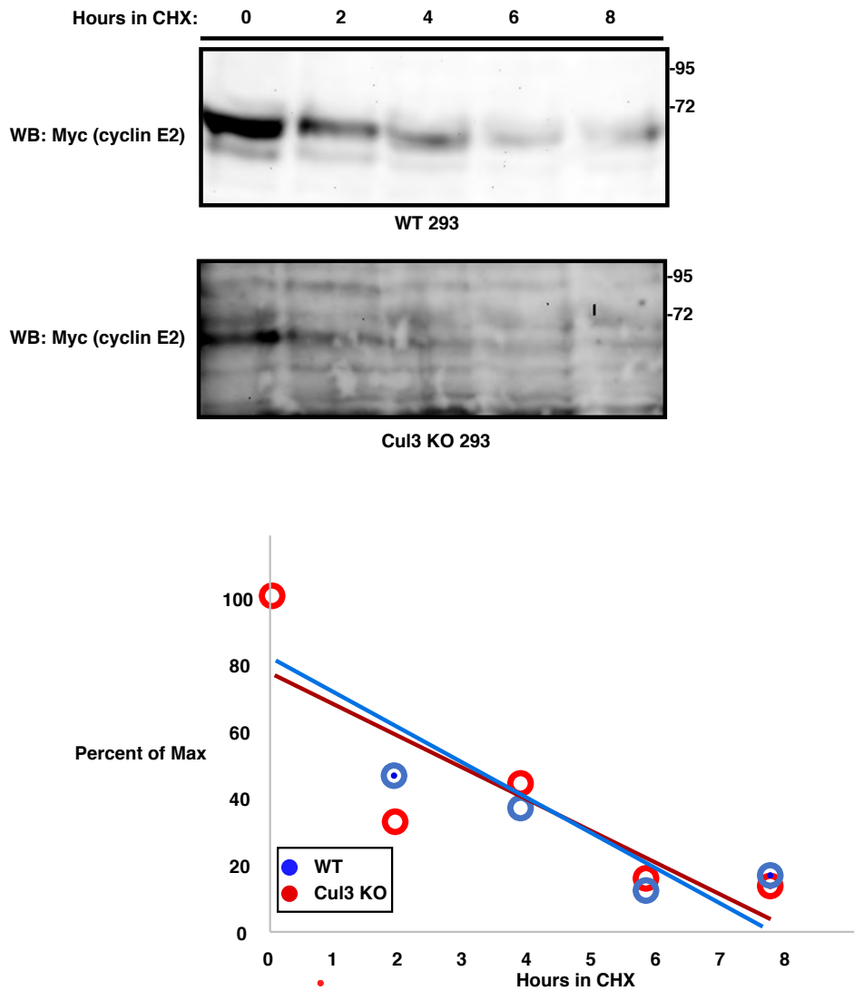

Figure S5: Cul3 has no effect on the half-life of cyclin E2. WT and Cul3KO 293 cells were transfected with Myc-cyclin E2. Cycloheximide was added 24 hours post-transfection and cells were harvested every two hours following cycloheximide addition. Top panel: Western blots showing protein expression levels. Migration of size markers indicated to the right of gel images (kDa). Lower panel: plot of half lives as percent of maximum signal.