Supplemental Figure S1. Wee1-Luciferase is an accurate surrogate for Wee1 degradation. Top Panel: Wee1-Luciferase is turned over faster than luciferase alone. HeLa cells were transfected with either Luciferase or Wee1-luciferase and the extent of degradation assayed after cycloheximide treatment for 0, 60, 90, or 120 minutes. Luminescence was detected after lysing cells with 'Brite-lite reagent. Middle Panel: The rate of Wee1-luciferase degradation is comparable to that of Myc-Wee1. HeLa cells were transfected with either Myc-Wee1 or Wee1-luciferase and the extent of degradation detected after cycloheximide treatment. Bottom panel: The rate of degradation of identified Wee1-Luc mutants is slower than that of Wild-type Wee1-luciferase.

Supplemental Figure S2. The Activation domain is required for Wee1 turnover. HeLa cells were synchronized at S phase and transfected with Wild-type Wee1 or the indicated mutants isolated from the Wee1-luciferase screen. Thymidine was added 4 hours after transfection. Cells were released from the thymidine block 24 hours later. Anti-myc Western Blot analysis was then performed. Skp-1 served as a loading control. Bottom Panel: Representative mutations isolated from Wee1 mutagenesis screen. G106/L483F mutations were on the same plasmid; the R167N/E273K and the C263Y/E306K mutations were also a double mutation. All other mutations were single mutations in the Wee1 coding region.

Supplemental Figure S3. Steady-state level of G106S/L483F mutant is higher than that of wild-type Wee1 or other mutants. HeLa cells were synchronized at S phase and transfected with wild-type Wee1 or the indicated mutants isolated from the Wee1-luciferase screen. Thymidine was added 4 hours after transfection. Cells were released from the thymidine block 24 hours later. Anti-myc Western blot analysis was then performed. Skp-1 served as a loading control. Bottom Panel: Pulse-chase analysis of Wild-type Myc-Wee1 or the Myc-G106/L483F-Wee1 mutant.

Supplemental Figure S4. Activation domain mutants increase phospho-Cdk1 phosphorylation to the same extent as Wild-type Wee1. HeLa cells were transfected with either WT-K328M-Wee1, K328M-S472A-Wee1, K328M-L483F-Wee1, or WT-Wee1, S472A-Wee1, L483F-Wee1. 48 hours after transfection, cells were isolated and processed for Western analysis using anti-phospho-Y15-Cdk1, Cdk1, or Flag antibodies.

Supplemental Figure S5. An N-terminal deletion of Wee1 is rapidly turned over in cells. HeLa cells were transfected with either Wild-type Wee1-luciferase or Δ214-Wee1-luciferase and incubated for 48 hours. Subsequently, cells were incubated with cycloheximide for the indicated times and the RLU detected.

Supplemental Figure S6. PEST-FIND analysis of human Wee1. + indicates region that contains strong PEST prediction while – is a region with a PEST prediction. N-terminal serines and activation domain sites we identified as increasing Wee1 steady state levels are shaded red. Serines either shown to be phosphorylated or required for Wee1 turnover are indicated as a large "S."

Supplemental Figure S7. R611 is required for Wee1 turnover. A. R611K-Wee1 is turned over in vitro slower than WT-Wee1. WT-Wee1 or R611K-Wee1 was incubated in somatic cell extracts as in Figure 2 and the extent of degradation detected after SDS-PAGE and autoradiography. B. R611K-Wee1 is turned over slower in cells than WT-Wee1. Pulse-chase chase analysis was performed on either Myc-WT-Wee1 or Myc-R611K-Wee1 and the extent of degradation detected after anti-myc immunoprecipitation and autoradiography.

Supplemental Figure S8. Cyclin A1/Cdk2 phosphorylates Wee1 in vitro and induces binding to GST-β-trcp and Tome-1. A. Cyclin A phosphorylates Wee1 in vitro. Flag-K328M-Wee1 or Flag-K328M-S472A Wee1 were incubated with either Cyclin A1/Cdk2, Cyclin B1, Cdk1, or Plk-1 along with ^{32}P-ATP, and the extent of phoshoryation detected by autoradiography. Vector alone was used as a control. B. GST-β-trcp/Wee1 interaction is stimulated by cyclin A1/cdk2. GST-β-trcp was incubated with ^{35}S-labeled Wee1 previously phosphorylated by either cyclin A1/Cdk2, cyclin B1/Cdk1, or Plk-1 (purchased from NEB) and the extent of association determined after extensive bead washing, radio. C. GST-Tome-1 association with Wee1 is stimulated by cyclin A1/Cdk2. Left Panel: GST-Tome-1 was incubated with in vitro translated Wee1 and the amount of associated Wee1 determined after performing a glutathione precipitation assay. Right Panel: quantification of Tome-1 binding to Wee1 in the presence of no kinase or cyclin A1/Cdk2. Tm1 indicates Tome-1 and cyc A indicates cyclin A1/cdk2