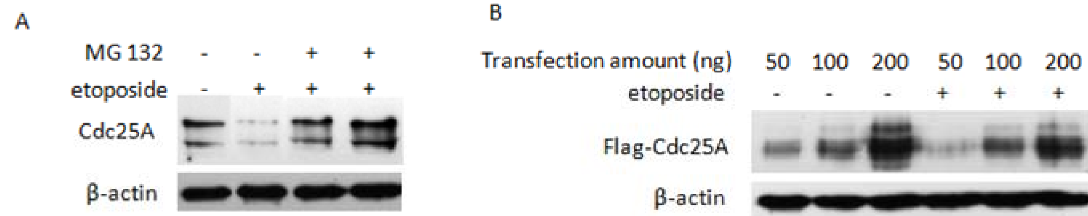
Appendix A. Supplementary data

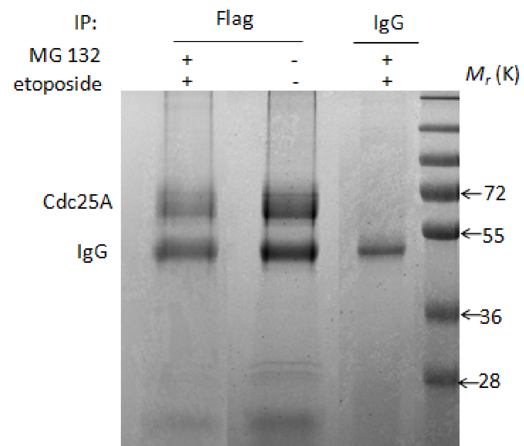
Supplementary Fig. 1 Degradation of Cdc25A *in vivo* following etoposide-induced DNA damage. (A) Etoposide-induced degradation of endogenous Cdc25A and its recovery after MG 132 incubation. U2OS cells were treated with 40 μM etoposide for 12 h, or incubated with etoposide for 12 h and then 10 μM MG 132 was added for an additional 2 h or 4 h, respectively. The indicated proteins were analyzed by Western blotting. (B) Degradation of ectopic Cdc25A after etoposide treatment. HEK293 cells were transfected with increasing amount of flag-tagged Cdc25A, and were then cultured in the absence or presence of 40 μM etoposide for 12 h. Cell lysates were harvested and analyzed for Cdc25A expression profiles by Western blotting, β-actin was immunoblotted to control for protein loading.
Supplementary Fig. 2 Immunoprecipitation of Cdc25A. HEK293 cells transfected with 50 μg flag-tagged Cdc25A cDNA and were untreated or treated with 40 μM etoposide for 12 h followed by incubation with 10 μM MG 132 for an additional 4 h. Cell lysates were immunoprecipitated with mouse anti-Cdc25A antibody or normal mouse IgG. Immunoprecipitates were analyzed by SDS–PAGE and Coomassie brilliant blue staining.
Supplementary Fig. 3 Phosphorylation profiling of cdc25A by trypsin digestion. cdc25A from control cells and from cells subjected to DNA damage were digested with trypsin and the resulting peptides were profiled by MALDI-TOF MS as described in the experimental section. Panels A (control cdc25A) and B (DNA damaged cdc25A) represent the low mass region of the MALDI spectra for each sample corresponding to the expected mass for the tryptic peptide containing S519 (EMYSR). The peptide mass detected at $m/z = 685.31$ was subjected to MALDI-TOF/TOF fragmentation to confirm the sequence as EMYSR (Panel C). The inset shows the sequence coverage for the b and y ions. The blue + 80 Da arrow in panel A represents the expected mass shift for the phosphorylated form of EMYSR containing S519.
Supplementary Fig. 4 Phosphorylation profiling of cdc25A by chymotrypsin digestion. Cdc25A from control cells and from cells subjected to DNA damage were digested with chymotrypsin and the resulting peptides were profiled by MALDI-TOF MS as described in the experimental section. Panels A (control cdc25A) and B (DNA damaged cdc25A) represent the mid-mass region of the MALDI spectra for each sample corresponding to the expected mass for the chymotryptic peptide containing S513 (AGEKSKREMY). The peptide mass detected at $m/z = 1198.5$ (boxed in blue) was subjected to MALDI-TOF/TOF fragmentation to confirm the sequence as AGEKSKREMY (Panel C). The inset shows the sequence coverage for the b and y ions. The

blue + 80 Da arrow in panel A represents the expected mass shift for the phosphorylated form of AGEKSKREMY containing S513. The peptide and + 80 Da arrow highlighted in red (Panel A) represent a potential peptide/phosphopeptide pair for a predicted chymotryptic peptide containing serine 178. The 87 Da mass difference between y6 and y7 in panel D is highlighted as unphosphorylated S178, while the mass shift between y6 and y7 in panel E is consistent with S178 (87 Da) plus an additional 80 Da due to the phosphorylation as indicated. MALDI-TOF/TOF fragmentation of $m/z = 1388.62$ and 1468.59 are provided in Panel D and E, respectively. The insets show the sequence coverage for the b and y ions

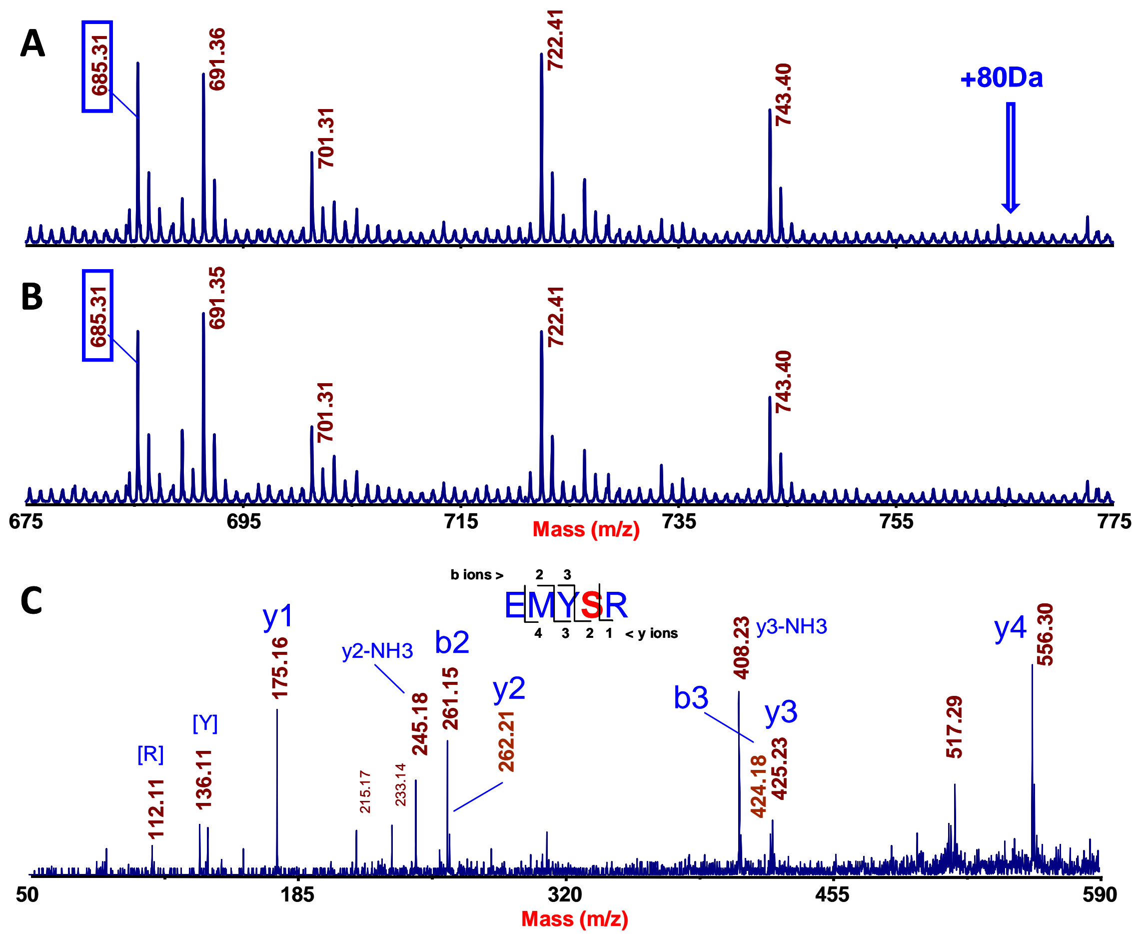
Supplementary figure 1



Supplementary figure 2



Supplemetary figure 3



Supplementary figure 4