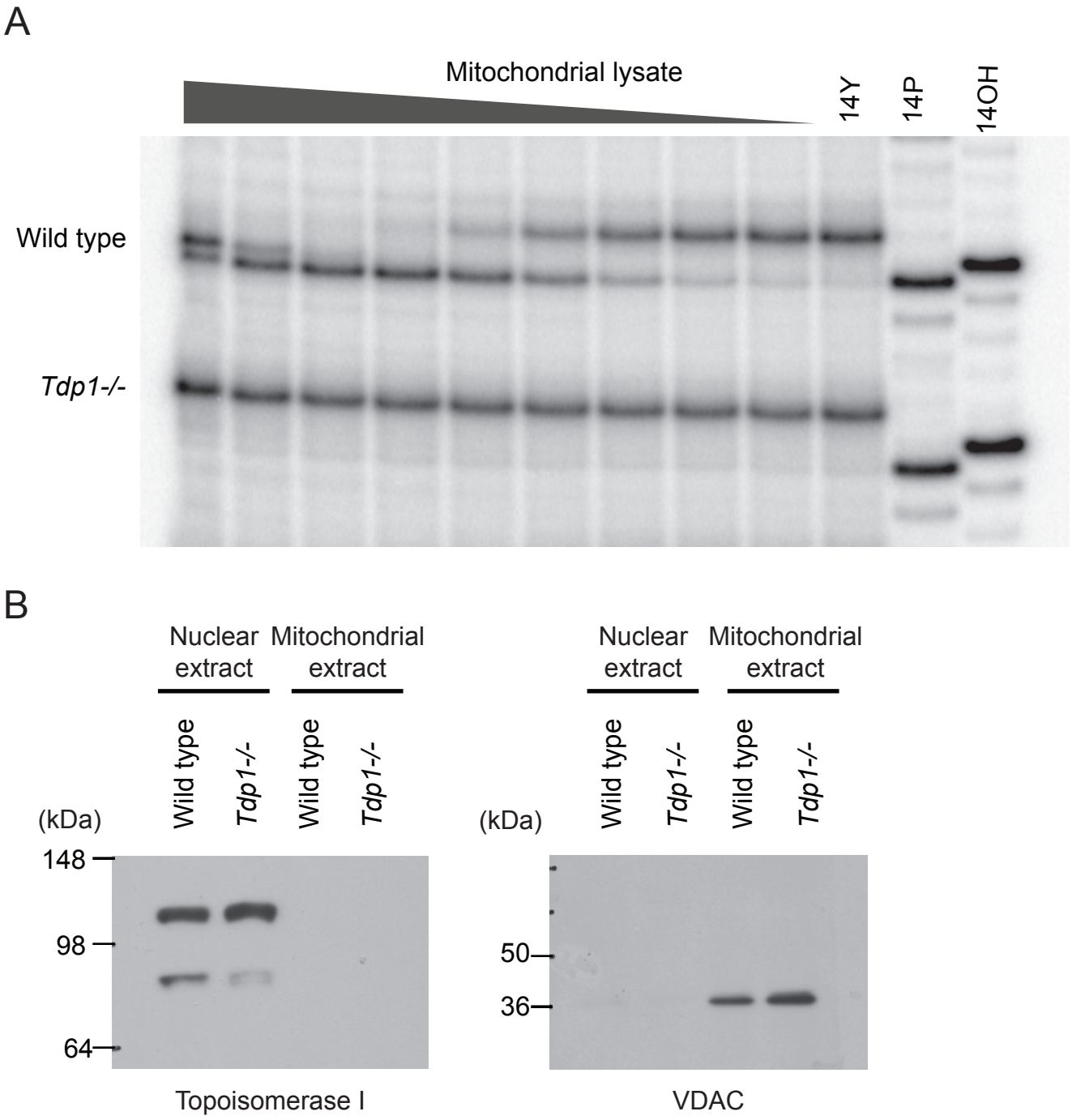
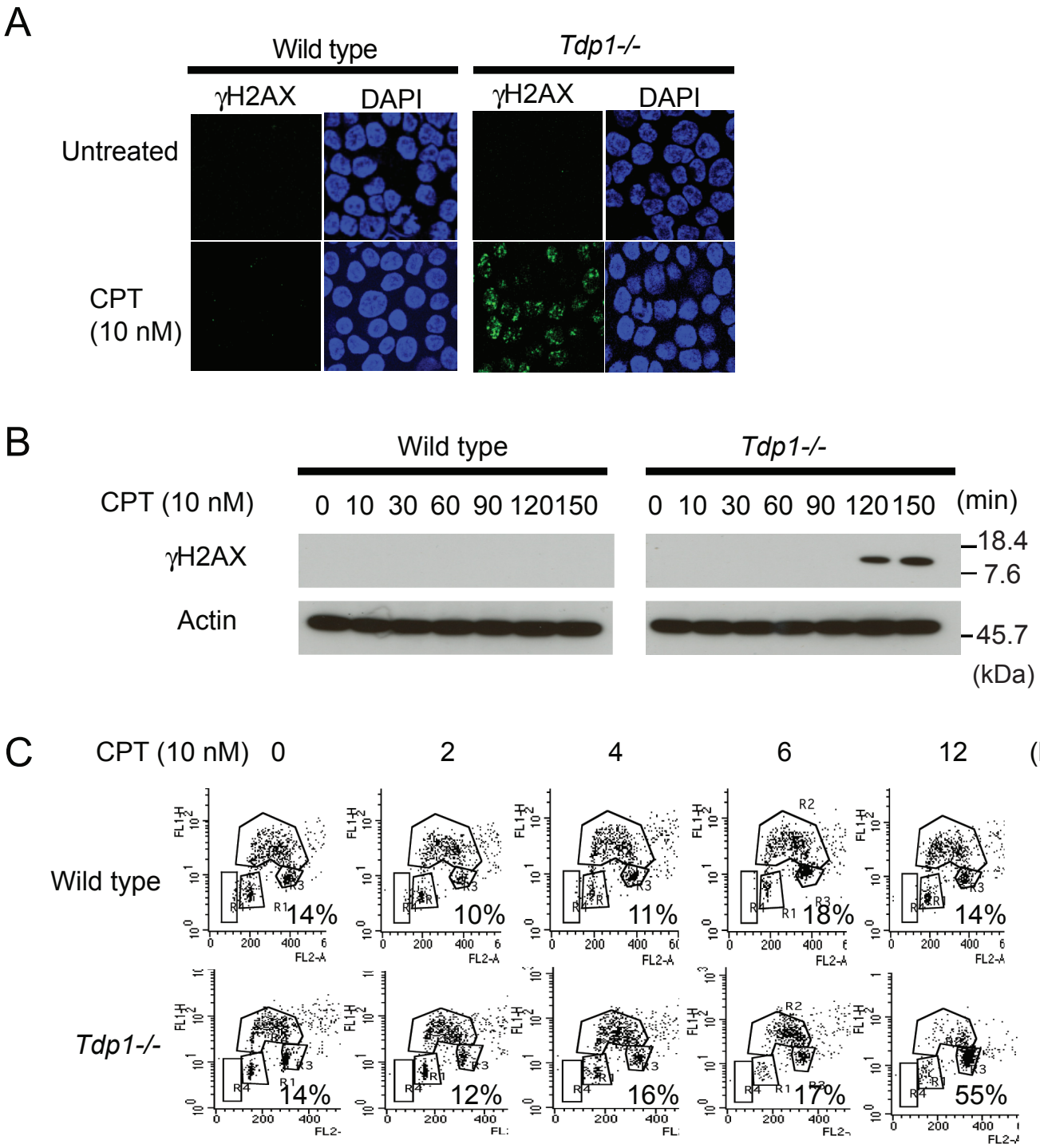
Supplemental figure 1



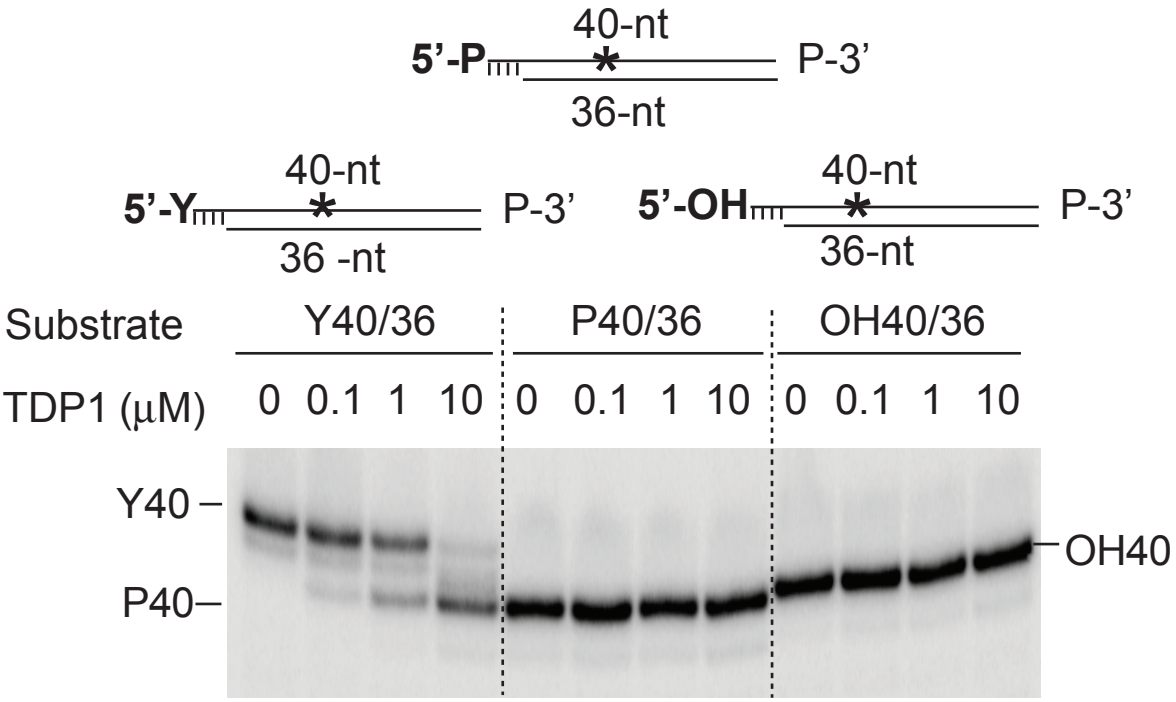
Supplemental figure S1. Tdp1 is localized and active in mitochondria. A.Representative gel images of cleavage assays from indicated cell lines. 5'-^{32}P-labeled 14Y (1nM) was incubated with serially diluted (1:3) mitochondrial lysates. Highest consentration was 3.0 μg in 10 μl reaction volume. B. Western blot analysis using nuclear extracts (8 μg/ lane) and mitochondrial extracts (6 μg /lane) prepared from indicated genotypes. Blots were probed with anti-human topoisomerase I (left) and anti-human VDAC (right). Topoisomerase I and VDAC were used as positive nuclear and mitochondrial markers, respectively.

Supplemental figure 2



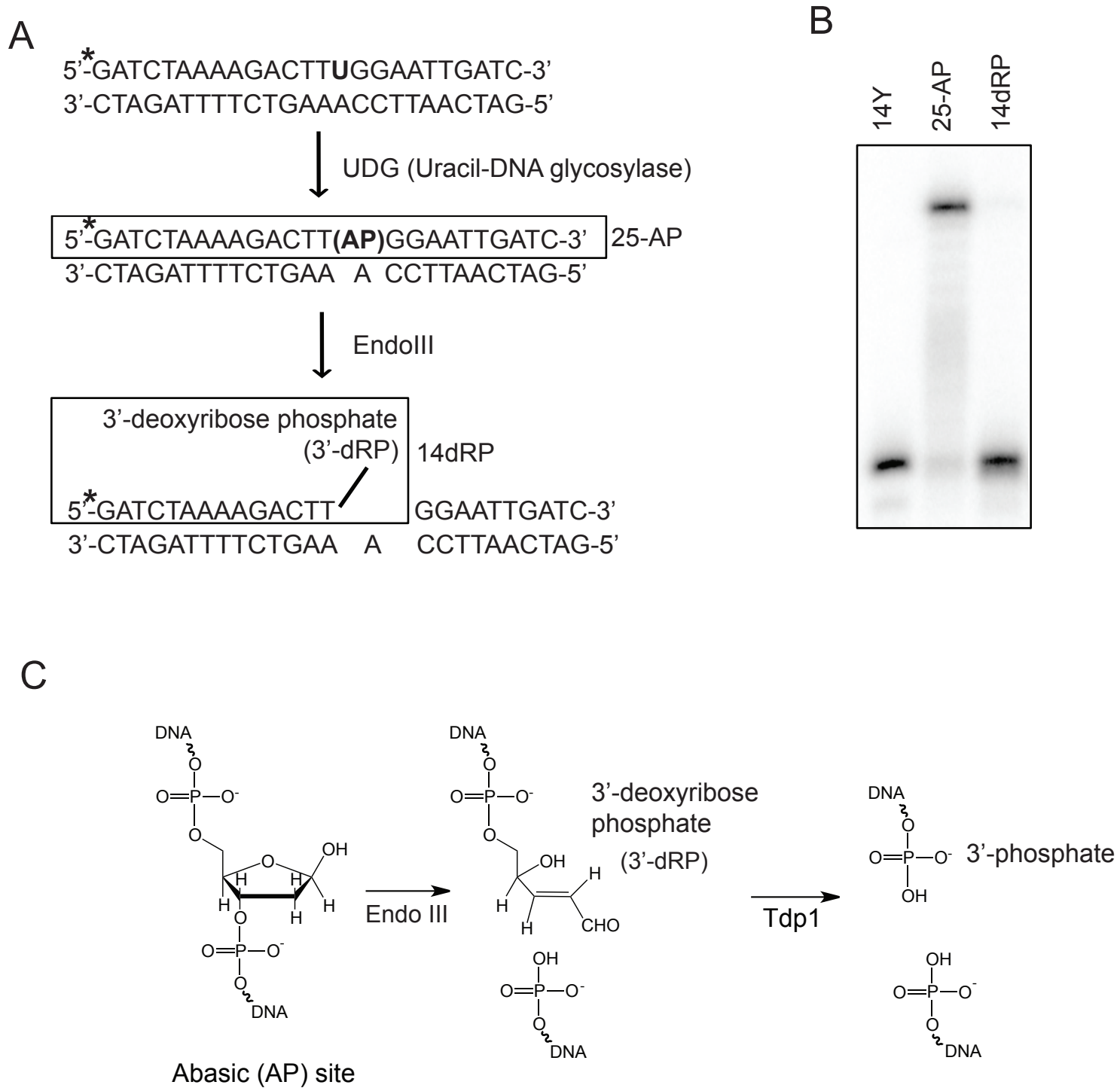
Supplemental figure S2. Impaired repair of CPT-induced lesion in Tdp1-/- cells. A. DNA damage measured by immunofluorescence microscopy with anti-γH2AX antibody. Cells were treated with or without 10 nM CPT for 2 hours. Nuclei were stained with DAPI. B. γH2AX induction measured by Western blotting in wild type and Tdp1-/- cells treated with 10 nM CPT for the indicated times. Actin was used as loading control. C. CPT-induced cell cycle arrest in the Tdp1-/- cells. Following 10 nM CPT treatment for 2, 4. 6, 12 hours, cells were labeled with BrdU for 10 min, and then analyzed by flow cytometry. Percent scores show the percent of cells accumulated in G2.

Supplemental figure 3



Supplemental figure S3. Preferential processing activity of recombinant human TDP1 for 5'-phosphotyrosyl linkage. Processing activity of recombinant human TDP1 on double-stranded substrates with 4-nt overhang harboring 5'-phosphotyrosyl linkage (Y40/36), 5'-phosphate (P40/36) or 5'-hydroxyl (OH40/36). Each substrate was incubated with the indicated concentrations of TDP1 for 30 min. Asterisk indicates the internal radiolabeled site.

Supplemental figure 4



Supplemental figure S4. Preparation of the 3'-deoxyribose phosphate (3'-dRP) substrate. A. Scheme for generating the 3'-dRP substrate. The DNA substrate harboring 25-nt with uracil in its intermediate site was incubated with UDG (Uracil-DNA glycosylase). The uracil site was converted into an AP site, resulting in a 25-nt DNA with the AP site at the 15th nucleotide from the 3'-end (25-AP). The AP site was cleaved with EndoIII resulting in 14-nt DNA substrate harboring the 3'-dRP (14dRP). Asterisk indicates the radiolabeled site at 5'-end. The boxed DNA indicates the substrates used in B. B. Gel image of DNA substrates shown in A. 14Y was used as a marker. C. The process of an AP site converted into 3'-phosphate and 5'-hydroxyl by serial cleavage of EndoIII and Tdp1 is shown with chemical structures. The AP site is hydrolysed by EndoIII, generating the 3'- dRP. Tdp1 converts the 3'- dRP into 3'-phosphate.