SUPPORTING INFORMATION

Figure S1. ESI mass spectra of untreated native full-length APE1 (A) and denatured full-length APE1 treated with NEM (B). To denature APE1, 2 M guandinium hydrochloride was added to 10 μM APE1, 70 μM NEM, and 10 mM Tris pH 7.5. The guanidium hydrochloride was dialyzed from the sample in two steps reducing the concentration by 1 M in each step. The samples were then subjected to ESI analysis using an Agilent 6520 Q-TOF instrument. Deconvolution was done using Mass Hunter software that is part of that system. In (A), the only major peak is the parent peak for full-length APE1 labeled P with an observed mass of 35,641.3 (expected 35,641.5). Treatment of denatured APE1 (B) resulted in 4 major peaks including the parent peak labeled P, along with +5 (36,266.6), +6 (36,392.4), and +7 (36,517.3) NEM modifications to the parent molecule. No higher mass peaks for modification with NEM beyond +7NEM were observed.

Figure S2. ESI mass spectra of C65A Δ40APE1 without (A) and with incubation with E3330 (B) in the presence of NEM for 12 h. (C) ESI mass spectra of C99A/C138A Δ40APE1 incubated with E3330 and NEM for 12 h. Samples were incubated in 10 mM HEPES at pH 7.5([protein] = 100 μM; [E3330] = [NEM] = 500 μM). The symbol * denotes water adducts. Mass spectra were collected on a Waters Micromass Q-TOF instrument and deconvolution was done with MaxEnt1 algorithm that was part of that system.

Figure S3. HDX kinetics of Δ40APE1. The curve was fitted with a custom program operating in MathCAD. It used the D uptakes of Δ40APE1 after 1 h incubation. The protein concentration was 12.5 μM. The sample was incubated in 10 mM HEPES with 150 mM KCl at 25 ºC for 1 h. HDX was done in a 90% D_2O medium (pH 7.5) with 10 mM HEPES and 150 mM KCl at 25 ºC. The HDX reaction was quenched by adding sufficient 1.0 M cold HCl to give a pH of 2.5.

Figure S4. Product-ion spectrum (MS/MS) showing disulfide bond formation between (A) C65 and C93, (B) C65 and C99, (C) C93 and C99 (D) C65 and C138 and (E) C93 and C138. Product ions generated from peptide A are labeled in red and those from peptide B in green. The symbol ++ denotes doubly charged ions; the symbol +++ denotes triply charged ions.