## S1 File. Supplementary methods.

## Measurement of Pol β dRP lyase activity using dRP lyase activity assay

Pol  $\beta$  dRP lyase activity was examined in 10  $\mu$ l-reaction mixture containing 30 mM HEPES, pH 7.8, 50 mM KCl, 0.5% inositol, 0.1 mg/ml BSA, 5 mM Mg<sup>2+</sup> and 25 nM preincised P<sup>32</sup>-labeled AP site containing substrates. An AP site was generated by pre-incubating the uracil-containing substrate (S1 Table) with 5 U UDG for 30 minutes. The photolyzed DOB-containing substrate was subjected to photolysis at 365 nm UV light for 20 minutes to remove the protecting group for creating the DOB lesion immediately prior to incubation with pol  $\beta$  at 37 °C for 30 min. The dRP lyase activity assay was initiated by adding 2.5 nM of pol  $\beta$  that was pre-crosslined with DOB-containing substrates and incubated at 37 °C for 30 min. The reaction was terminated by transferring reaction mixture on ice, and the dRP lyase products were stabilized by addition of 340 mM NaBH<sub>4</sub> and incubation on ice for 30 min. The dRP lyase activity of pol  $\beta$  that was incubated with the unphootylzed substrate was measured as the control of the experiments. The dRP lyase products were recovered by ethanol precipitation and resuspended in 10  $\mu$ l of 2×stopping buffer. Substrates and products were separated by 18% urea-denaturing PAGE.