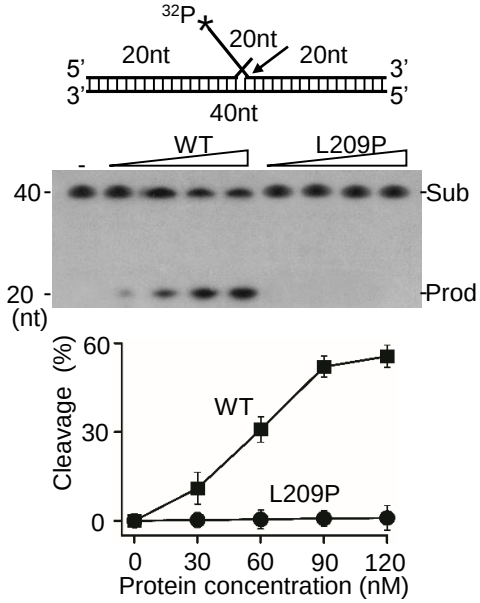

Figure S1. Activities of WT and mutant FEN1 on double-flap substrates at 22℃. DNA substrates were labeled with ^{32}P as indicated. Labeled substrates (1 pmol) were incubated with various amounts of purified WT or L209P FEN1 at 22℃ for 30 min. The FEN1 concentration is 0-120 nM. The top panel shows the schematic structure of the corresponding DNA substrates. The middle panel shows the PAGE gel separating the DNA substrates (Sub) and the cleavage products (Prod). The graph on the bottom represents the quantification of the PAGE image.

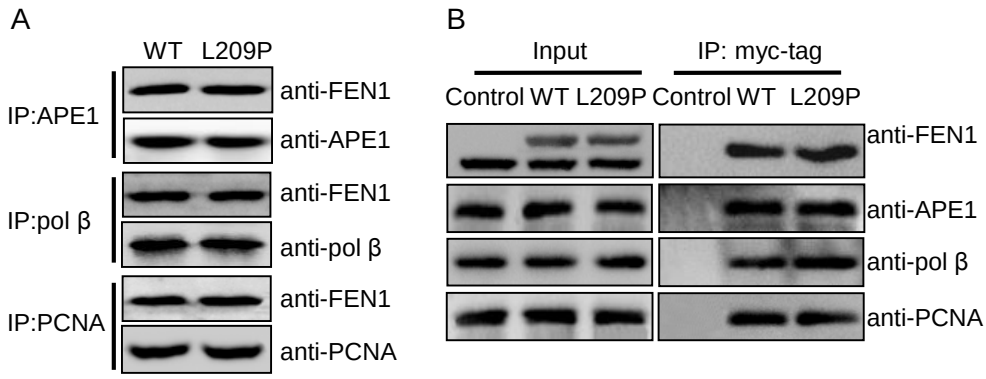

Figure S2. L209P mutation does not affect the FEN1 interaction with APE1, pol β and PCNA. (**A**) Pull-down assay. Purified APE1, pol β and PCNA were mixed in Tris buffer. The interaction between two proteins was determined by pull-down assay and western blotting. (**B**) Co-immunoprecipitation assay. C-myc-tagged WT or L209P FEN1 was expressed in SW480 cells. C-myc-tagged proteins were precipitated using anti-c-myc antibody, followed by westerrn blot analysis using FEN1, APE1, pol β and PCNA antibodies.

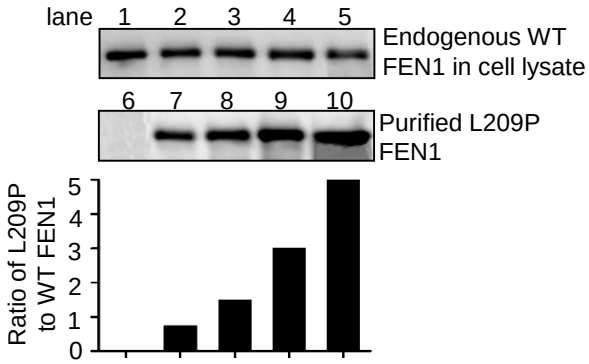

Figure S3. Western blotting assay of the amount of L209P FEN1 added to the LP-BER assay, relative to the WT FEN1 within the whole cell extract. Western blot analysis using FEN1 antibody. Lane 1-5, the whole cell extract of SW480 cells. Lane 6-10, the different amounts of purified L209P FEN1. The graph on the bottom represents the quantification of the western blotting image.

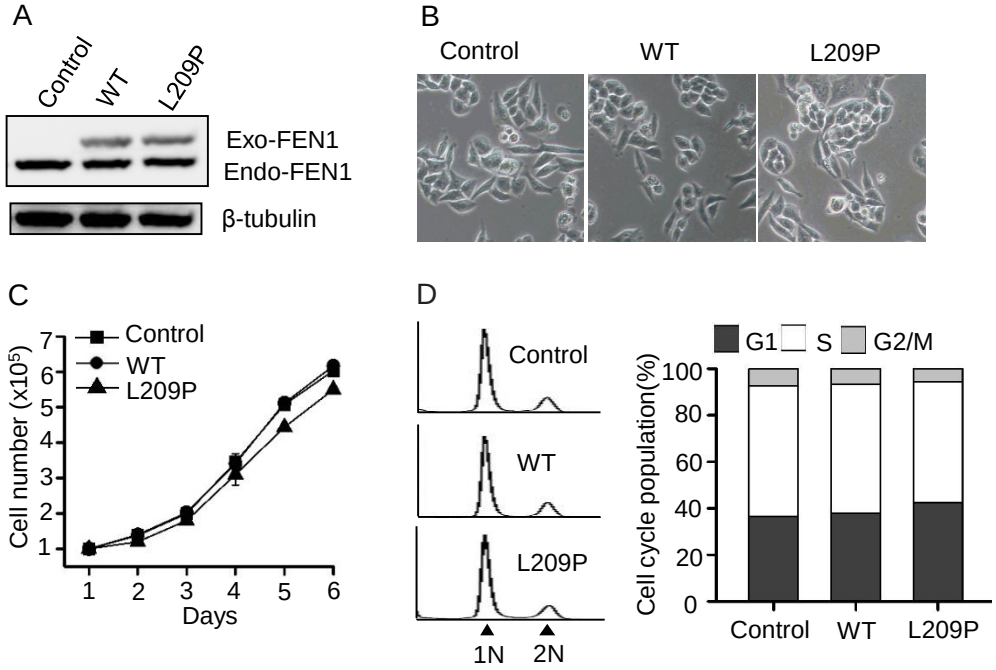

Figure S4. Expression of L209P hinders cell growth slightly. (**A**) Western blotting shows the establishment of cell lines that express the WT or L209P FEN1. (**B**) Microscope image of SW480 cells and SW480 cells expressing WT or L209P FEN1. (**C**) Growth curve of SW480 and WT or L209P FEN1 SW480 cells. (**D**) Cell cycle profile of SW480 and WT and L209P cells. Right panel is the quantification result of left panel.

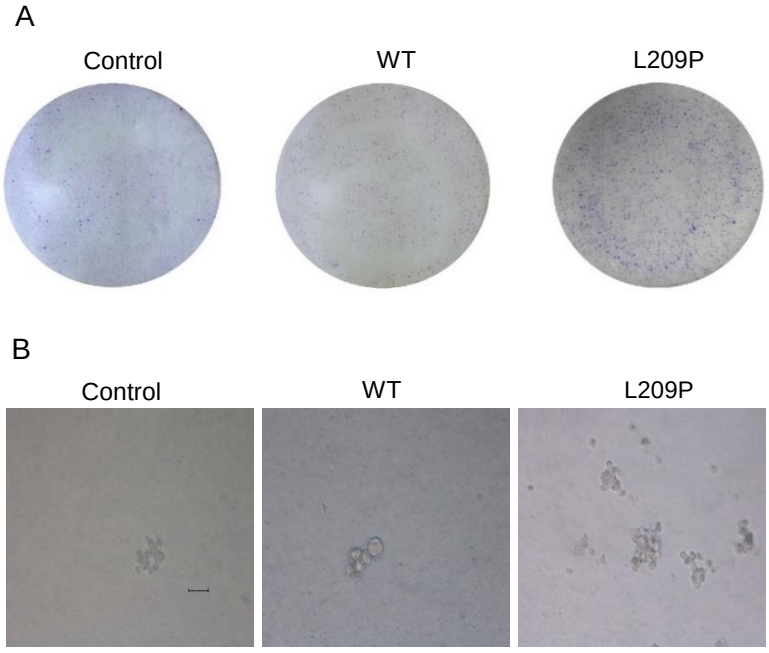

Figure S5. L209P mutation induces cellular transformation by (**A**) focus formation and (**B**) Soft-agar colony formation assay. (**A**) WT, L209P and parental control SW480 cells. At various passages, 1×10^4 cells were seeded into 6-cm dishes and after 10 days they were stained with crystal violet to visualize foci. The presence of foci was also monitored by microscopic examination. (**B**) WT, L209P and parental control SW480 cells was grown in soft agar. Scale bars, 140 μm .