Supplemental Information

Supplemental Materials and Methods

Cell lines and Cell Culture. The DLD1 colon tumor cell line contains mutations in the *hMSH6* gene (44). A one bp deletion results in a termination codon at residue 222 and a second mutation results in a termination codon at 1103. This cell line is defective in mismatch repair and exhibits microsatellite instability. The DLD1 chromosome 2 transfer line restores the mismatch repair deficiency (44). HCT116 cells harbor a homozygous nonsense mutation in the *hMLH1* gene at residue 252 (45). The HCT116 chromosome 3 transfer line restores the mismatch repair deficiency (46). XP20S cells were derived from a patient with XP and harbored a homozygous mutation at the 3'-splice acceptor site of intron 3 of *hXPA* (22).

NCI Approved Oncology Drugs Set II Screen. Cells were plated in 384 well plates at a density of 1000 cells/well and allowed to attach overnight. The following day, cells were treated with the drugs at a final concentration of 10 μM. After treatment times of 24 or 72 hours, cell viability was measured using CellTiter-Glo® (Promega) according to manufacturer's instructions. Cells were treated with vehicle (DMSO) or 10 μg/ml puromycin as negative and positive controls, respectively. The percent viability was calculated: $100 - ((\frac{average\ reading\ negative\ control - sample\ reading}{average\ reading\ negative\ control - average\ reading\ positive\ control}) * 100)$. Dose-response curves were performed in 96 well plates with cells plated at a density of 3000 cells/well. The percent viability was calculated as described above. The differences in cell viability were confirmed by clonogenic survival assays.

Protein Purification. Pellets were resuspended in buffer B (40 mM TrisCl, pH 8.0, 0.5 M NaCl, 5 mM imidazole) supplemented with 1 mM phenylmethanesulfonyl fluoride solution (PMSF) (Sigma-Aldrich) and complete EDTA-free protease inhibitor cocktail tablet (Roche) and lysed via sonication. The lysate was cleared by centrifugation and applied to a 5 ml HiTrap Chelating HP column (GE Healthcare) charged with $NiSO_4$ using a linear imidazole gradient (from 5 to 500 mM) in buffer B. Pol β was eluted at about 275 mM imidazole. The fractions were combined, concentrated to about 1 ml and diluted to 10 ml in buffer D (50 mM Tris-HCl pH 8.0, 1 mM EDTA, 10% glycerol, 100 mM NaCl). The diluted protein applied to a HiTrap SP HP column (GE Healthcare) using a linear NaCl gradient from 100 mM to 2000 mM. Purified protein fractions eluted at approximately 1000-1200 mM NaCl. The protein was concentrated, glycerol was added to a final concentration of 15%, and aliquots were flash frozen in liquid nitrogen and

stored at -80°C. Final protein concentration was determine using the absorbance at 280 nm and the extinction coefficient for Pol β (21200 M^{-1} cm^{-1}).

Preparation of DNA Substrates. The template strand of DNA was platinated on the two guanines by incubating 250 μM template DNA with 350 μM cisplatin (water was used in mock reaction) in buffer P (1 mM Na_2PO_4, pH 7.4, 3 mM NaCl) overnight at 37°C. Platinated and mock template DNA was purified by PAGE.. The platinated DNA migrated slower and was able to be distinguished from the mock DNA. The primer oligo was radiolabelled with γ-^{32}P ATP using T4 polynucleotide kinase (New England Biolabs) and were purified using Microspin columns (Biorad). Primer and template oligos were annealed by denaturing at 95°C for 5 minutes, slow cooling to 50°C for 30 minutes, holding at 50°C for 20 minutes, and then resting on ice. Complete substrate annealing was confirmed using 12% native PAGE and visualized using autoradiography.

Supplemental Figure Legends

Figure S1. Dose-response curve of MCF7 cells. MCF7 cells were plated at a density of 3,000 cells/well in a 96 well plate. Cells were treated with various concentrations of cisplatin (0-25 μM) and cell viability was measured after 24 h using CellTiter-Glo®. Data are presented as mean ± SEM of the percent viability (n=6).

Figure S2. Western blotting analysis of Pol β in cell lines. Representative western blots show expression of exogenous HA-tagged WT P242R, and E295K Pol β. The specific cell lines are shown above the blot. Endogenous Pol β was used as a loading control. The ratio of exogenous HA-tagged Pol β to endogenous Pol β was quantified and is depicted below the blots.

Figure S3. Pol β and XPA interact *in vitro*. Pulldown using whole cell extract from Pol $\beta^{-/-}$ MEFs and his-tagged purified Pol β proteins. Lane 1: WT Pol β protein; Lane 2: P242R Pol β protein; Lane 3: no protein; Lane 4: 25% input.

Figure S4. The effect of E295K Pol β expression on survival following cisplatin treatment. A. A549 cells expressing WT, P242R, or E295K Pol β or containing empty vector were treated with varying concentrations of cisplatin (0-12.5 μM). Clonogenic survival assays were performed, and data are presented as mean ± SEM of the percent survival (n = 3-6). B. A549 cells expressing WT, P242R, E295K, the P242R/E295K double mutant Pol β or containing empty vector were treated with varying concentrations of cisplatin (0-6.25 μM). Clonogenic

survival assays were performed, and data are presented as mean ± SEM of the percent survival (n = 3). C. DLD1 cells deficient in MSH6 (-MSH6; open bars) or complemented with MSH6 (+MSH6; filled bars) were treated with 6.25 μM cisplatin and allowed to recover for 0 and 96 h. Apoptotic cells were measured by staining with Annexin V antibody. Data are presented as the ratio of apoptotic cells in cisplatin-treated cells vs untreated cells for each cell line. WT and P242R data also shown in Figure 5D.

Supplemental Tables

Table S1. Difference in viability of crosslinking agents from high-throughput screen.

Drug Name	Difference in viability at 24 h (WT-P242R)[a]	Difference in viability at 72 h (WT-P242R)[a]
cisplatin	-31.3	-14.4
carboplatin	-23.9	-26
oxaliplatin	-2.5	-10.7
mitomycin C	11.6	1.7
cyclophosphamide	3.1	7.4

[a] The difference in viability was determined by subtracting the viability of P242R-expressing cells from WT-expressing cells for the individual drugs. Positive numbers and negative numbers indicate the drugs where P242R-expressing cells were more sensitive or resistant to, respectively, compared to WT-expressing cells.