(lanes 5-8) and products were imaged using a PhosphorImager. (B) Reactions were performed similar to those in panel A except the ^{32}P-label was on the DNA strand complementary to the damaged strand. A schematic of DNA substrates below each gel is presented with the cisplatin indicated by the carat and the position of the ^{32}P-label by the asterisk.

Figure 5: Immunoprecipitation and Western Blot Analysis of Photocrosslinked XPC in the Absence or Presence of Damaged DNA. (A) XPC-Rad23B and the indicated DNA substrates were bound, crosslinked for the indicated time, and immunoprecipitated using the anti-Rad23B antibody. The IP was then separated by SDS-PAGE, transferred to PVDF and probed with anti-XPC antibody. Antibody reactivity was visualized by chemiluminescence detection using a HRP-conjugated secondary antibody. Bracket 1 denotes a covalent protein-protein crosslink that occurs between XPC and Rad23B subunits that is independent of DNA. Bracket 2 indicates a covalent protein-DNA crosslink. Free, uncrosslinked XPC is indicated by the arrow. (B) Identical reactions were performed as described in panel A except the samples were heat denatured prior to immunoprecipitation.

Figure 6: Model of XPC-Rad23B Binding to Cisplatin Damaged DNA. XPC is presented as a yellow complex, Rad23B as a mauve complex, XPA as a brown complex and RPA as a green complex. XPC-Rad23B recognizes and binds to cisplatin damaged DNA with placement of the XPC subunit both 5′ and 3′ of the cisplatin lesion. The Rad23B subunit interacts with the undamaged strand of the cisplatin damaged duplex 3′ of the cisplatin lesion. After damage recognition, RPA-XPA binds to the damaged duplex and XPC-Rad23B prepares to dissociate from the damaged DNA duplex.

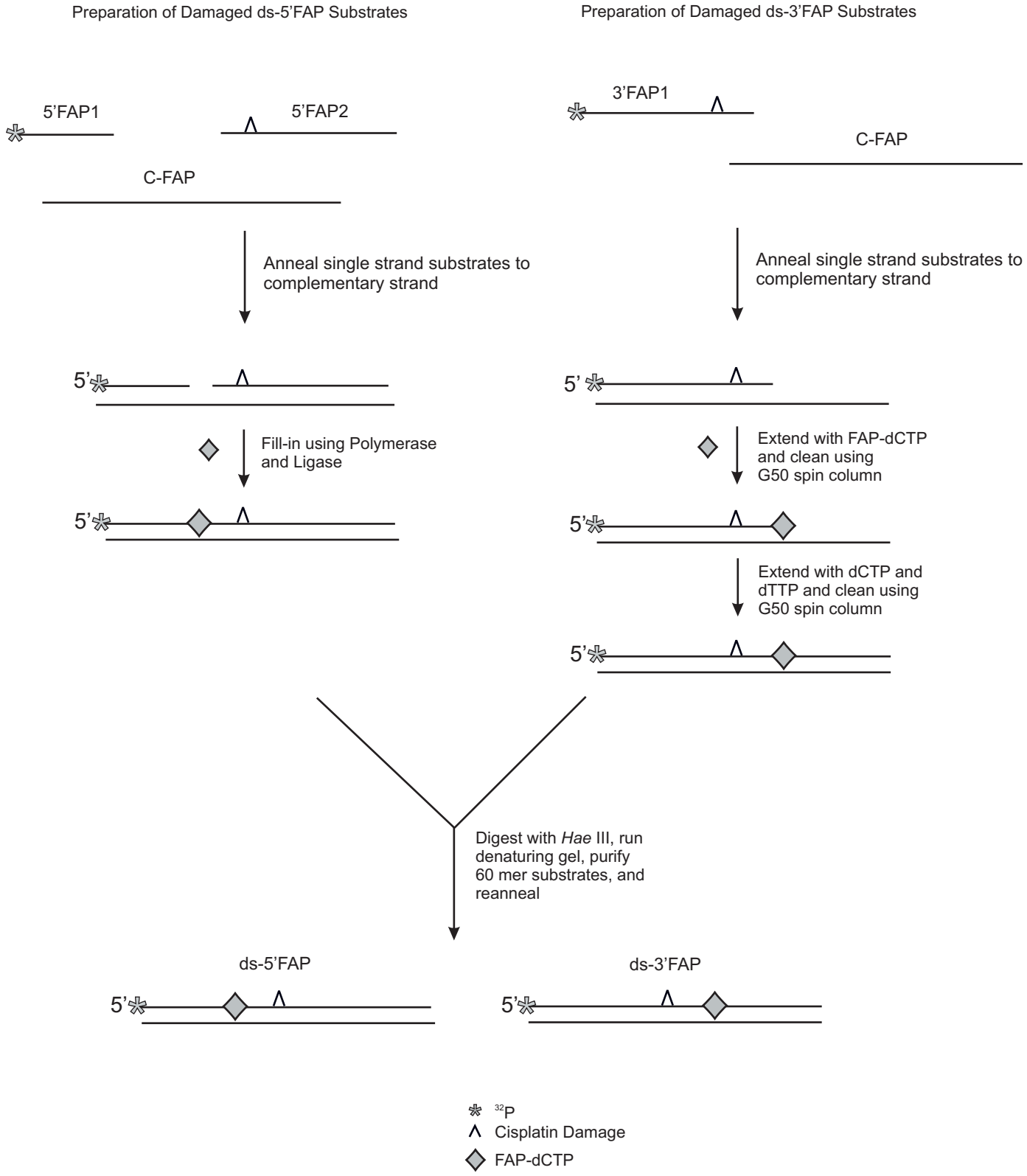
Supplementary Figure 1: Preparation of Cisplatin Damaged Double Strand DNA Substrates Containing a Photoreactive Analogue. The carat represents cisplatin damage, while the asterisk represents the radio-label and the diamond represents the photoreactive analogue (FAP-dCTP). Single-strand damaged substrates were radio-labeled, phosphorylated, and annealed to the complement strand. 5′FAP duplex DNA substrates

were filled in with the FAP-dCTP analogue. 3´FAP duplex DNA substrates were extended with the FAP-dCTP analogue and excess FAP-dCTP was removed using Sephadex G50-spin column chromatography. Extension of the 3´FAP duplex DNA substrate was completed by adding dCTP and dTTP nucleotides and Klenow DNA polymerase. Damaged substrates were then digested with *Hae*III and 60-mer substrates were purified via denaturing polyacrylamide gel electrophoresis and reannealed.

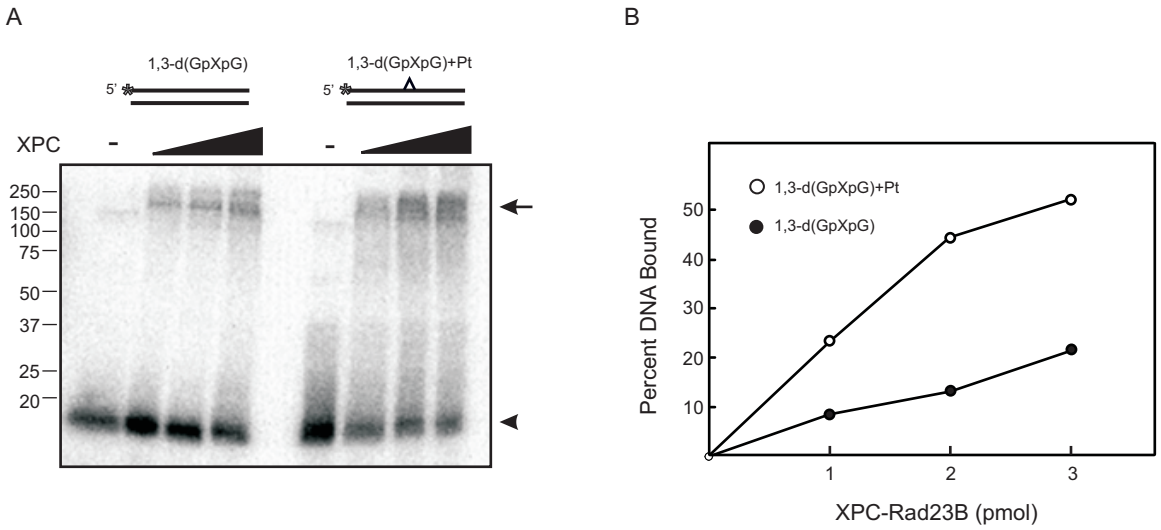
Supplementary Figure 2: XPC-Rad23B Photocrosslinks to 1,3-Cisplatin-Damaged DNA. (A) Increasing amounts of XPC-Rad23B were incubated with undamaged or cisplatin damaged ds-DNA with the ^{32}P-label on the damaged strand and photocrosslinked for 60 minutes. Samples were heat denatured, separated via SDS-PAGE and radioactivity detected by PhosporImager analysis. (B) Quantification of XPC-Rad23B photocrosslinked to ds-undamaged or 1,3-cisplatin cisplatin-damaged substrates presented in A.

Supplementary Figure 3: Photocrosslinking XPC-Rad23B to Control and Cisplatin-Damaged Substrate Containing 3´ Photoreactive FAP-dCTP Analogue. (A) XPC-Rad23B (6 pmol) was incubated and bound to FAP-dCMP modified control DNA (lane 1) or cisplatin-damaged DNA (lane 2) and photocrosslinked for 10 minutes. Samples were heat denatured, separated via SDS-PAGE and radioactivity detected by PhosporImager analysis. The covalent protein-DNA complex is indicated by the arrow and the free DNA by the arrow head. (B) Quantification of the binding data from three independent crosslinking experiments. A paired T-test was utilized to determine the statistical significance (P-value <0.11). (C) A schematic of each DNA substrate is depicted above the gels with the cisplatin indicated by the carat, the position of the ^{32}P-label by the asterisk and the FAP-dCMP analogue indicated by the diamond.

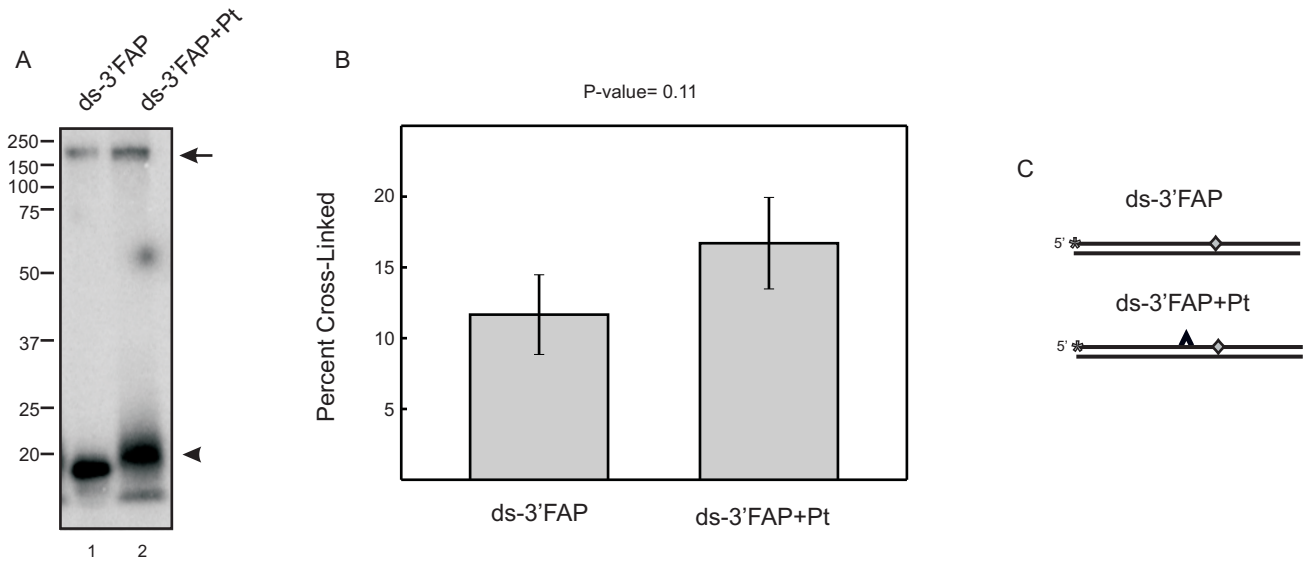
Supplementary Figure 4: XPC-Rad23B Photocrosslinks to Control and Cisplatin-Damaged DNA Containing a Photoreactive Analogue in the Complementary Strand. (A) XPC-Rad23B (6 pmol) was bound and photocrosslinked, for 10 minutes, to ds cisplatin-damaged DNA substrates containing the FAP-dCMP in the complementary strand, located either 5′ or 3′ of the site of cisplatin damage. The protein-DNA complexes were separated by SDS-PAGE and radioactivity was detected by PhosphorImager analysis. (B) Quantification of (A) in which XPC-Rad23B is photocrosslinked to a ds cisplatin-damaged DNA substrate which contains the photoreactive FAP-dCTP analogue in the complementary strand. (C) A schematic of each DNA substrate is depicted above the gels with the cisplatin indicated by the carat, the position of the ^{32}P-label by the asterisk and the FAP-dCMP analogue indicated by the diamond.



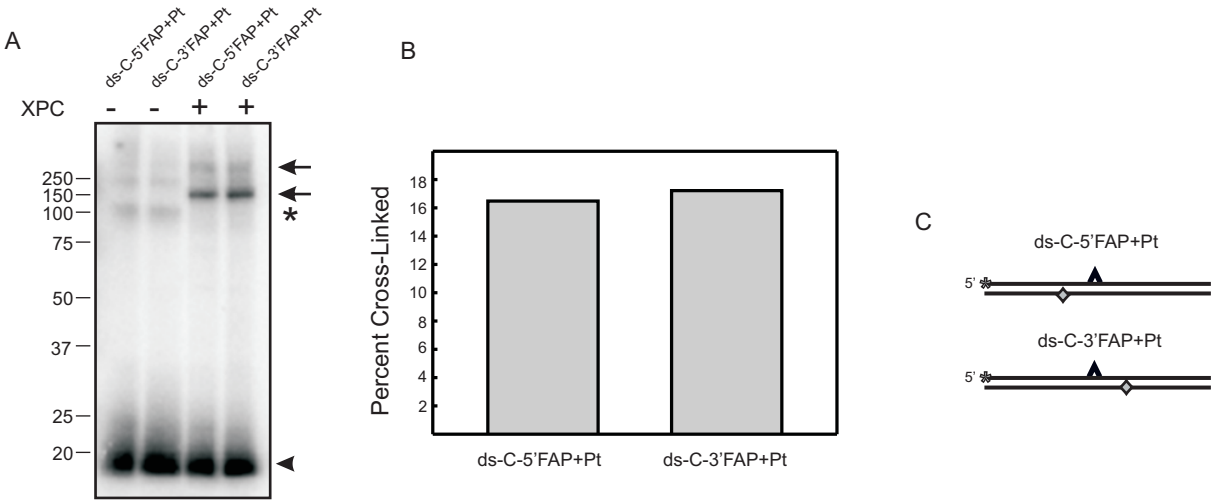
Supplementary Figure 1



Supplementary Figure 2



Su-pplementary Figure 3



Supplementary Figure 4