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Supporting Information

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Supporting Information

for

Photoaffinity Isolation and Identification of Proteins in Cancer Cell Extracts that Bind to Platinum-Modified DNA

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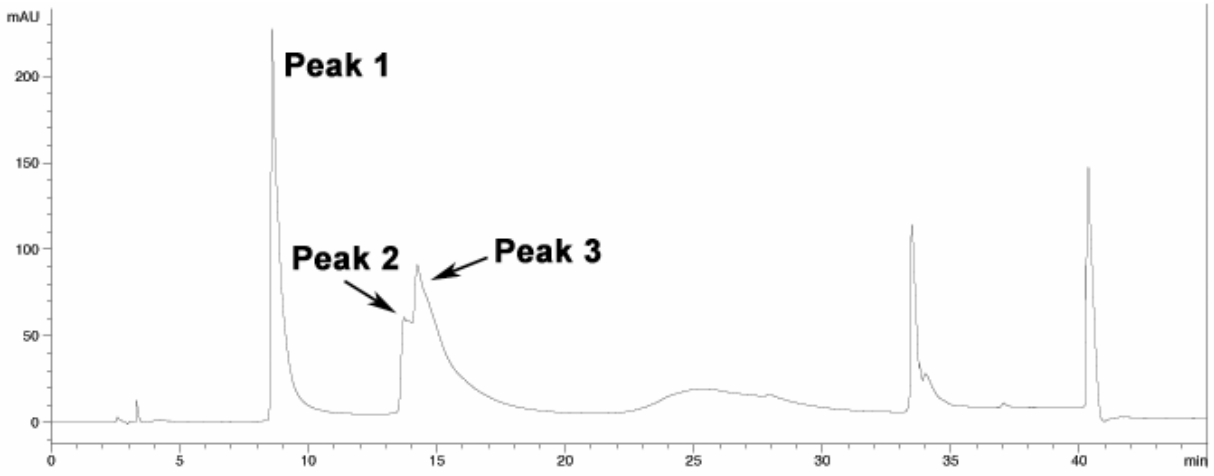


Figure S1. RP-HPLC purification of the reaction between a 25-base DNA fragment containing a 1,3-d(GpTpG) site and the activated form of PtBP6 yields three major peaks. Peak 1 is the starting material, and peaks 2 and 3 are platinated DNA.

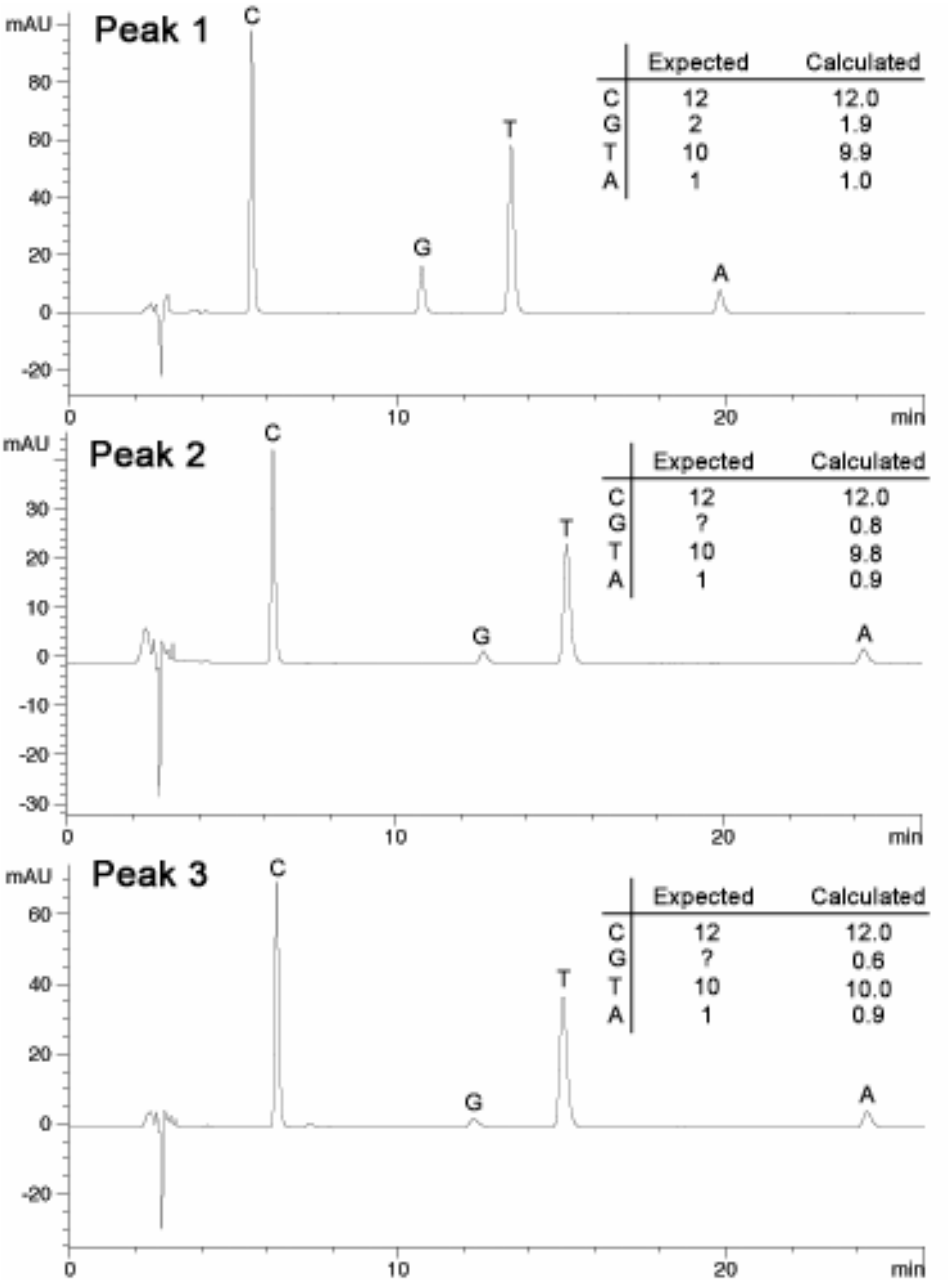


Figure S2. Nuclease digestion analysis of the products of the reaction between PtBP6 and a 25-base DNA fragment containing a single 1,3-d(GpTpG) site indicate that the platinum atom is bound to guanosine. The signal for this base is diminished after the platination reaction (Peaks 2 and 3) compared to starting material (peak 1).

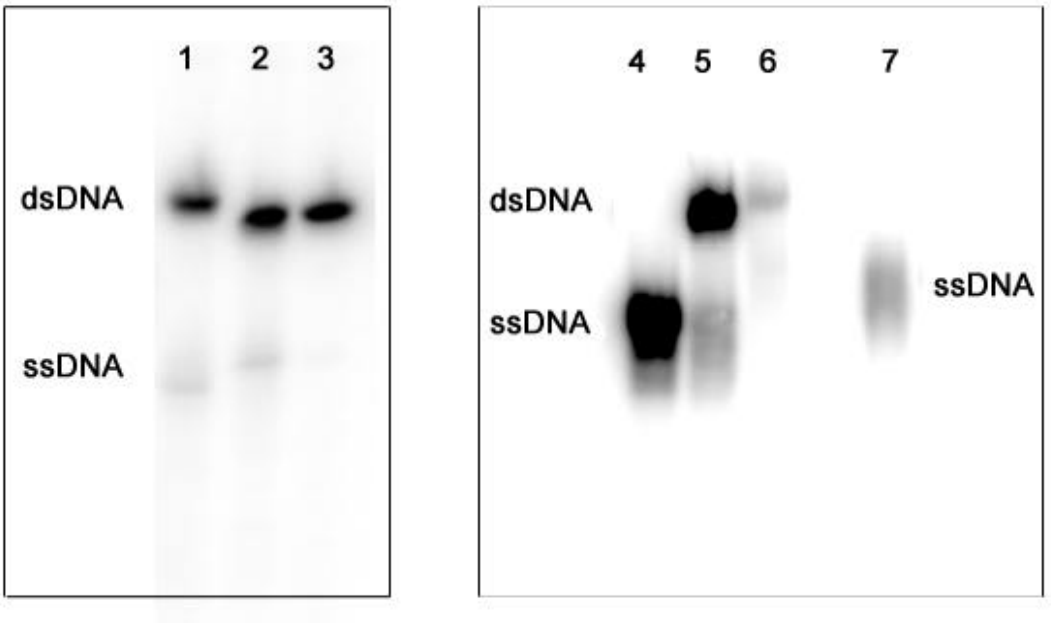


Figure S3. Analysis of annealed 25-bp duplex probes by native PAGE indicates that the purified duplexes were isolated from single-stranded starting material. Lane 1: DNA duplex containing d(GpTpG)-PtBP6 peak 3. Lane 2: DNA duplex containing d(GpTpG)-PtBP6 peak 2. Lane 3: DNA duplex containing d(GpTpG)-PtBP6 peak 1. Lane 4: single-stranded DNA. Lane 5: DNA duplex containing d(GpG) site. Lane 6: DNA duplex containing d(GpG)-PtBP6. Lane 7: single-stranded DNA containing d(GpG)-PtBP6.

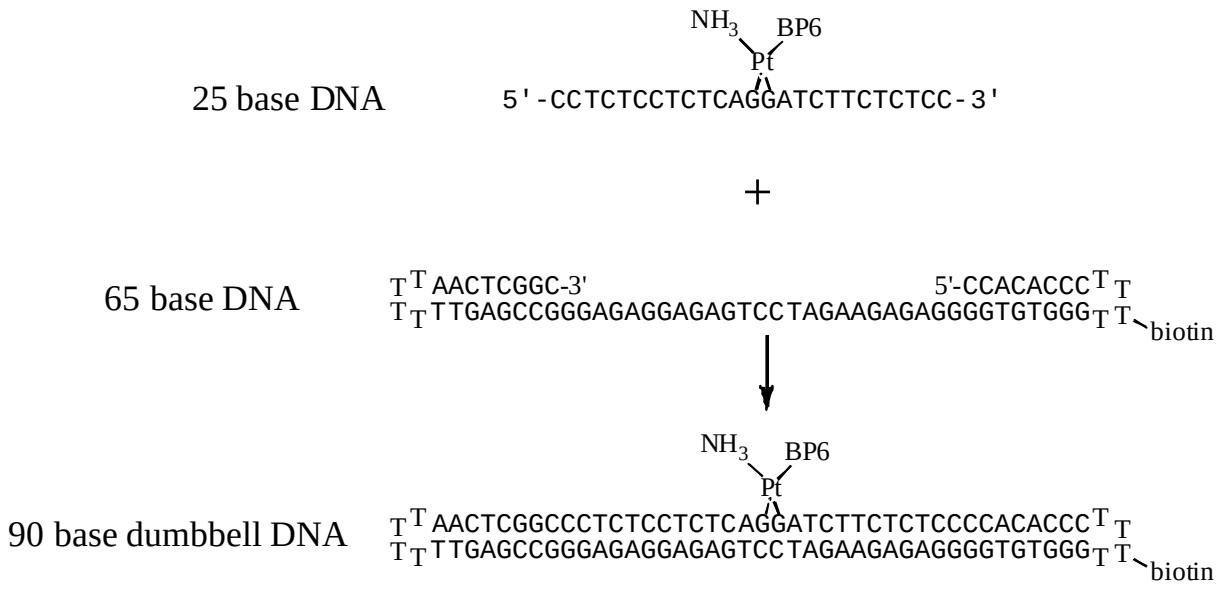


Figure S4. Synthesis of a 90-base dumbbell DNA probe from a PtBP6-modified 25-base DNA and a biotinylated 65-base DNA. The product of this reaction was characterized in the text.

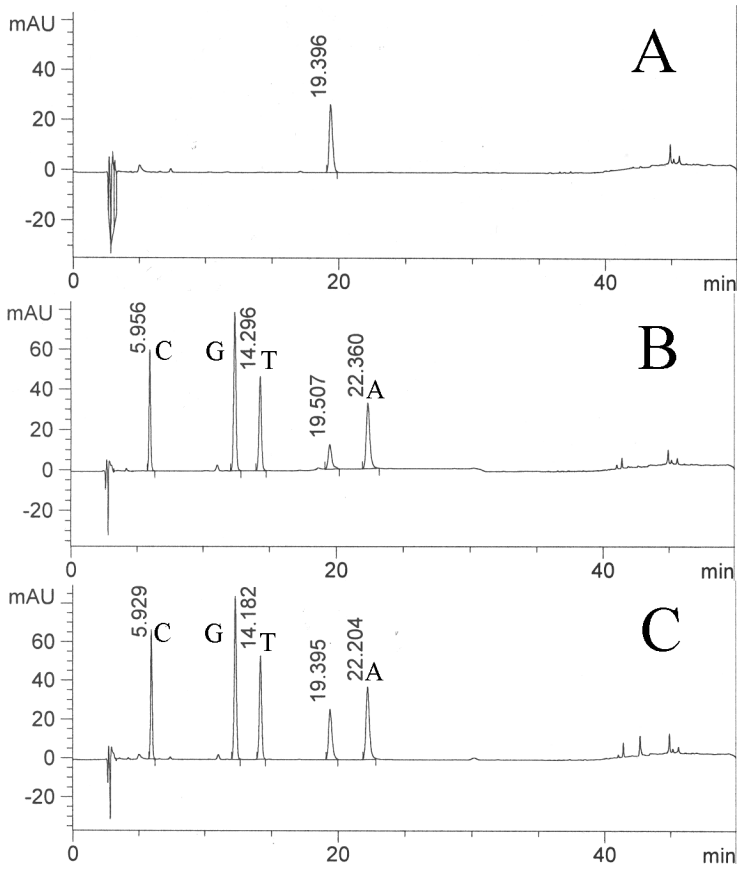
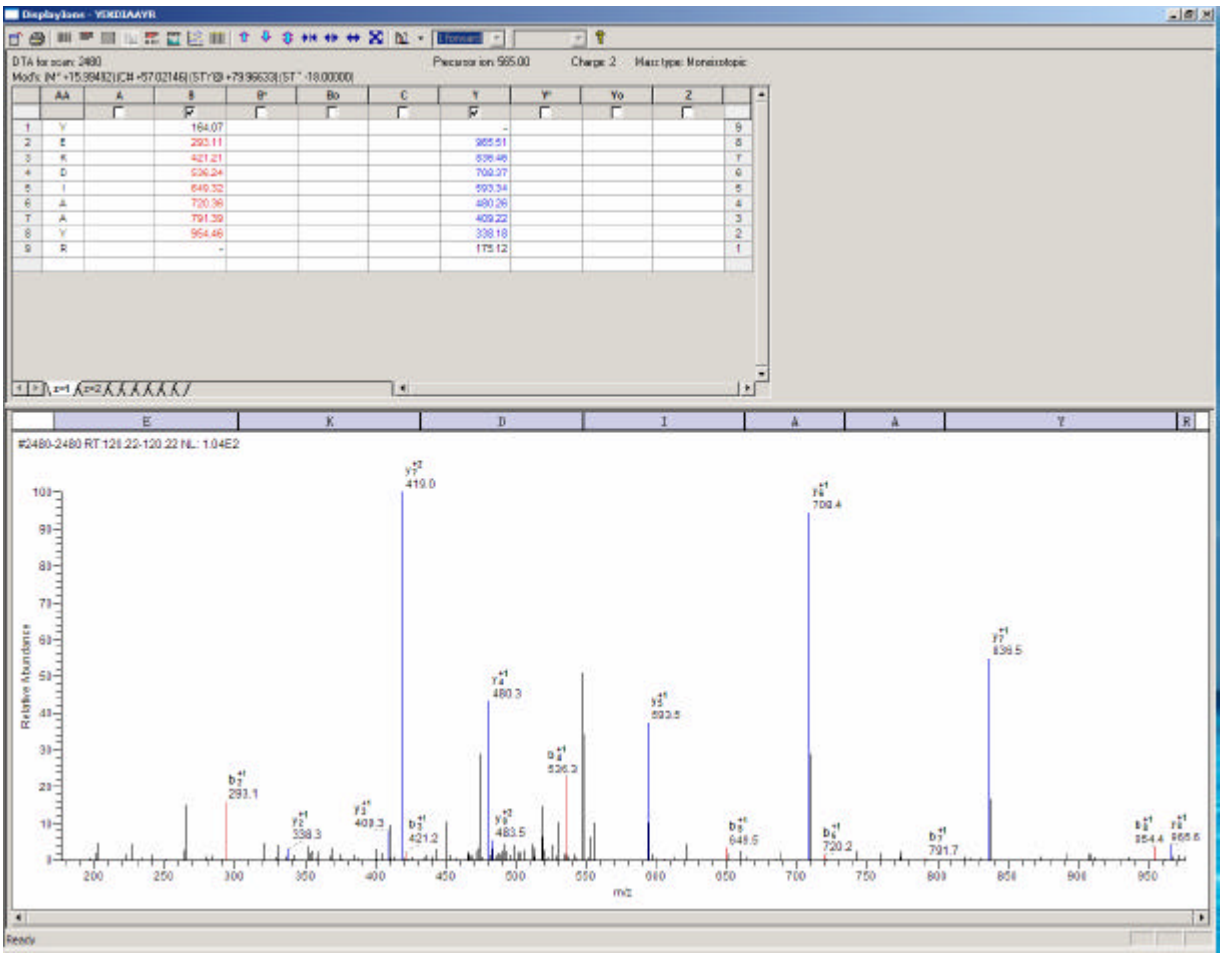


Figure S5. Purified platinated and unplatinated 90-base dumbbell DNA probes were digested with nucleases to each individual base. The ratios of these bases were analyzed to determine that the purified products were 90-base DNA. These results are discussed in the text. A) Solvents alone. B) Unplatinated 90-base DNA. C) PtBP6-modified 90-base DNA.

Figure S6. Mass spectra of single peptides used to identify three of the proteins from the preparative-scale photo-cross-linking of the duplex containing a 1,3-d(GpTpG) adduct of PtBP6 and two proteins from the 90-base dumbbell probe. From 25-bp probe: A) HMGB1, B) HMGB3, RPA1 from band 2 (C) and band 3 (D); from 90-base probe: E) YB-1, and F) Msh6. The location of bands 2 and 3 are depicted in Figure 2B in the main text.

A)

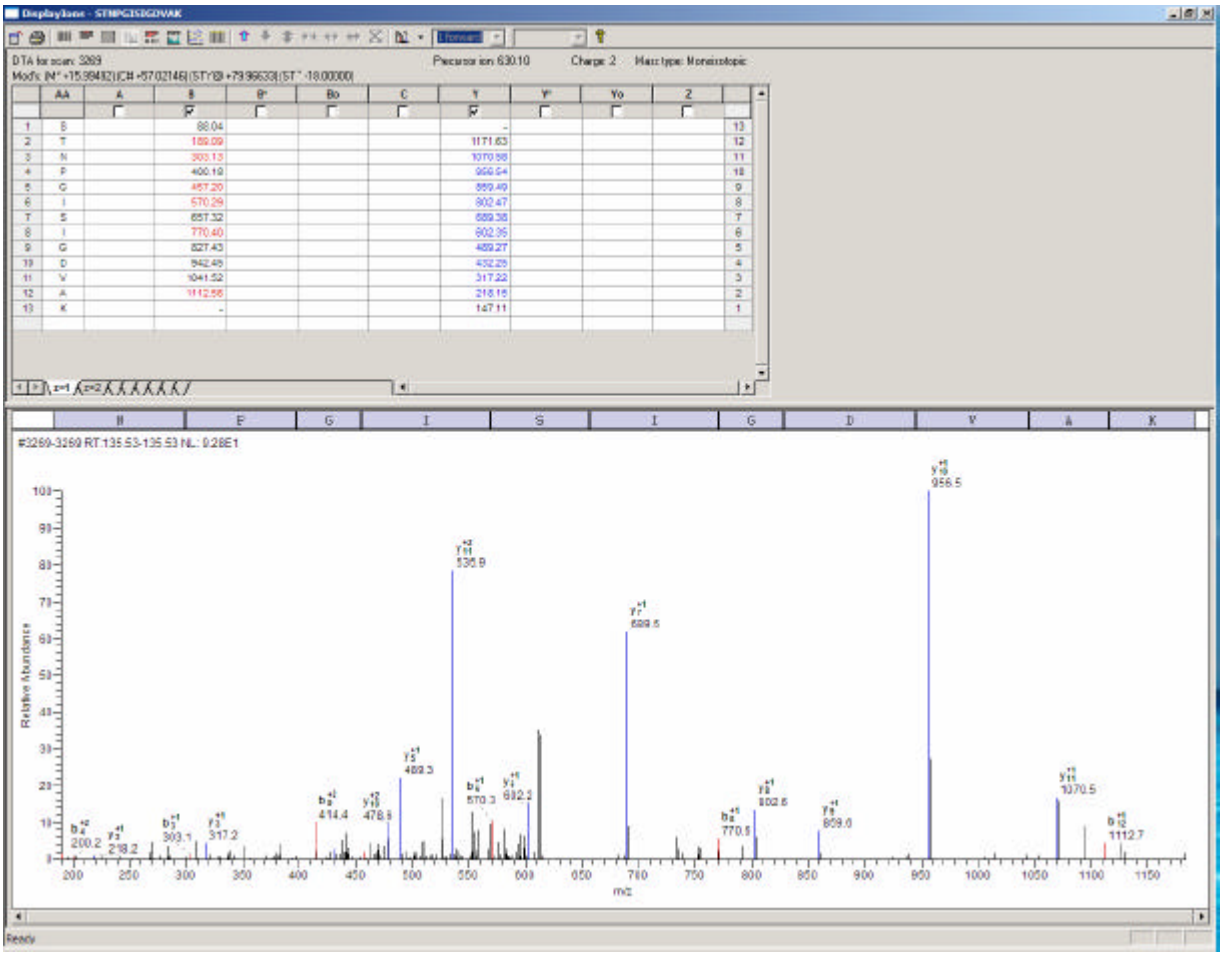


Protein: HMGB1
Parent peptide: YEKDIAAYR
MH^+ parent peptide: 1128.56836
Ions found/ions expected: 13/16

MS/MS spectrum for the peptide YEKDIAAYR, which was identified during LC-MS analysis of the preparative-scale photocrosslinking of the 25-bp duplex containing a 1,3-d(GpTpG) intrastrand adduct of PtBP6. The MS/MS data show all fragments of the parent peptide that were found. Peptides fragment at amide bonds between amino acids, producing two peptides, a “b” peptide that includes the N-terminus up to the fragmented bond, and the “y” pep-

tide that extends from the fragmented bond to the C terminus. For example, if this peptide fragments at the fifth amino acid, between isoleucine and alanine residues, the "b_5" peptide will be YEKDI, molecular weight 649.32, and the "y_5" peptide will be AAYR, molecular weight 480.26. Both of theses fragments are present in the spectrum. Taking into account all possible fragments, which are listed in the table above the spectrum, this spectrum includes signals for 13 of 16 expected ions, confirming that the parent peptide is YEKDIAAYR.

B)

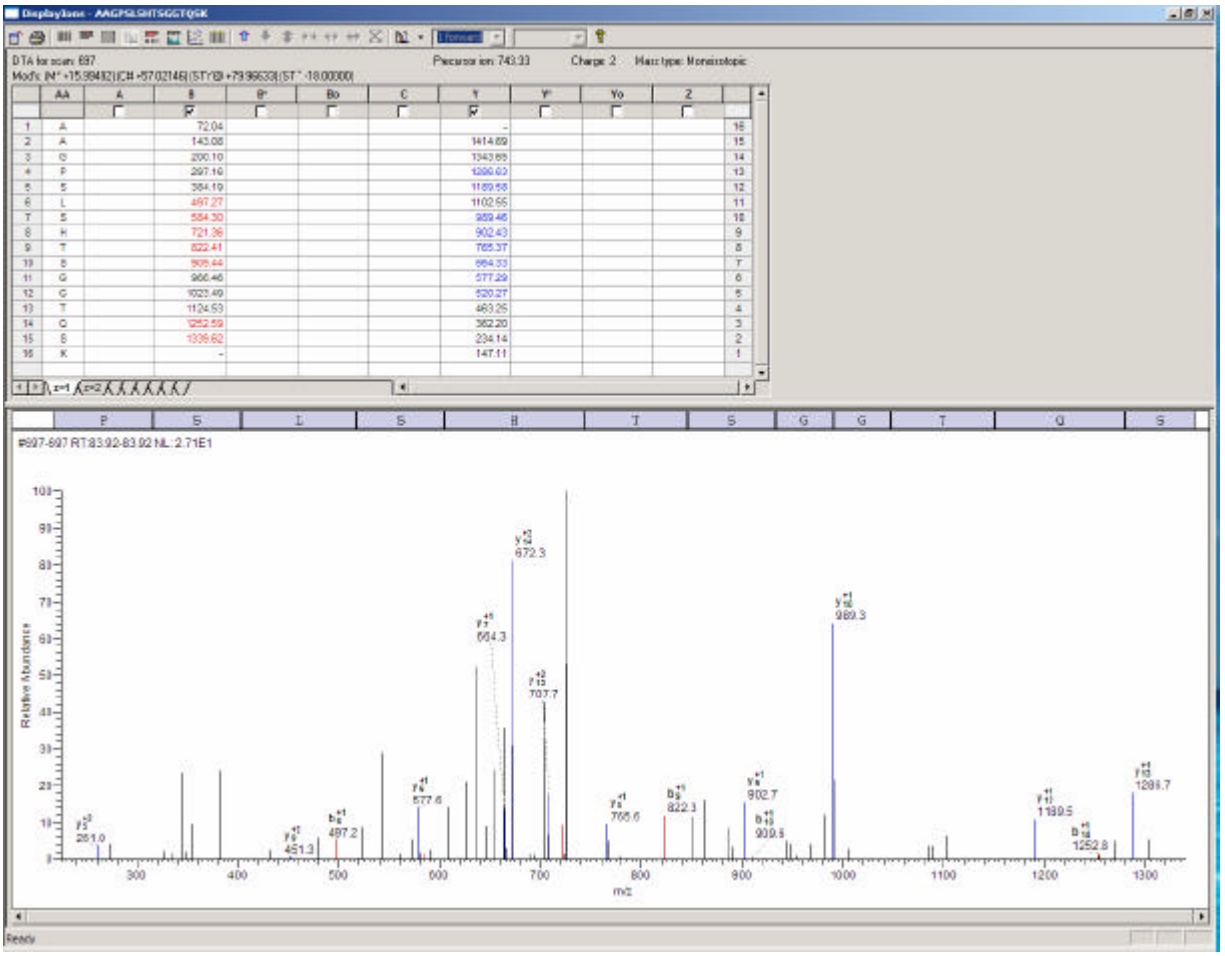


Protein: HMGB1
Parent peptide: YEKDIAAYR
MH^+ parent peptide: 1128.56836
Ions found/ions expected: 13/16

MS/MS spectrum for the peptide YEKDIAAYR, which was identified during LC-MS analysis of the preparative-scale photo-cross-linking of the 25-bp duplex containing a 1,3-d(GpTpG) intrastrand adduct of PtBP6. The MS/MS data show all fragments of the parent peptide that were found. Peptides fragment at amide bonds between amino acids, producing two pep-

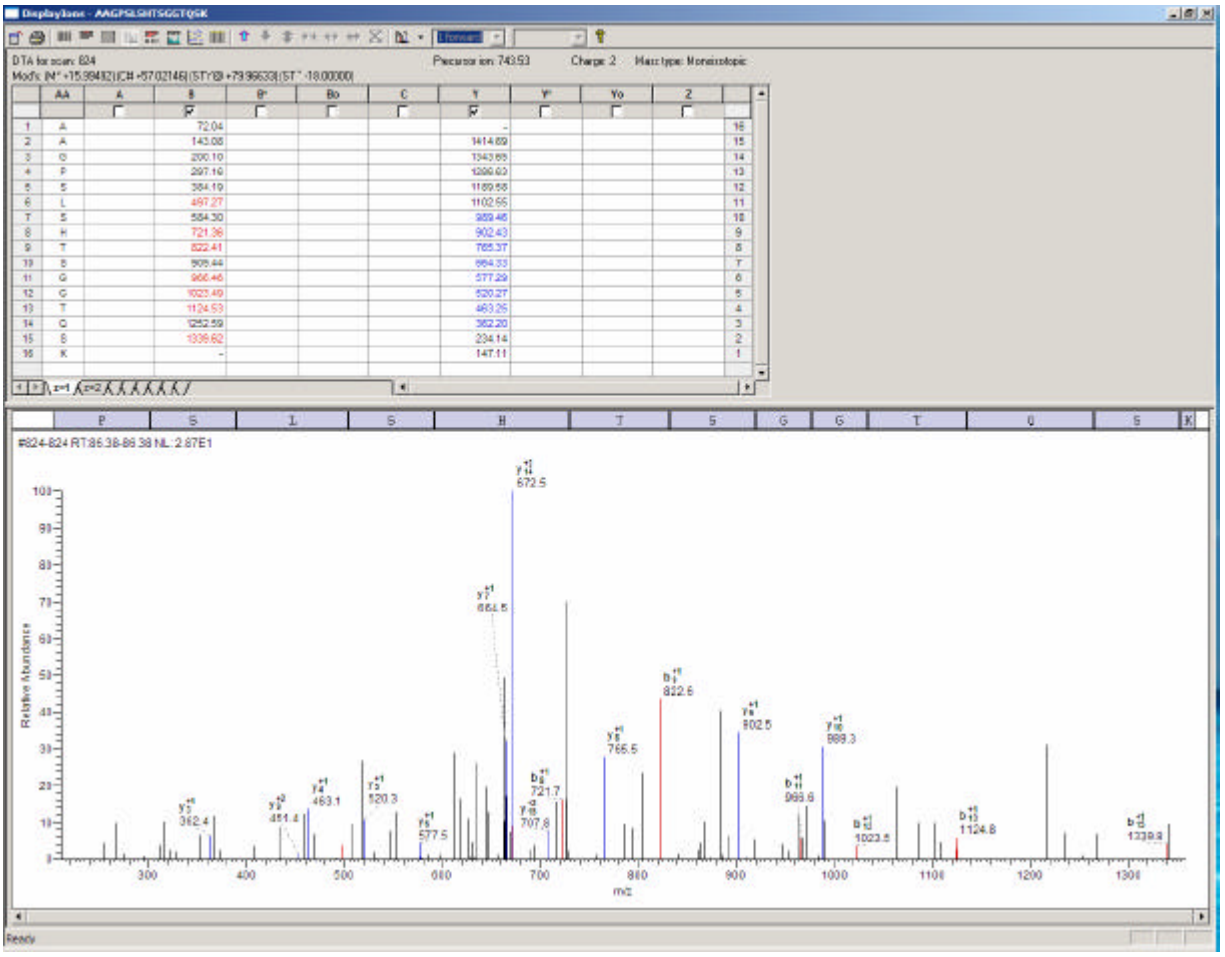
tides, a “b” peptide that includes the N-terminus up to the fragmented bond, and the “y” peptide that extends from the fragmented bond to the C-terminus. For example, if this peptide fragments at the fifth amino acid, between isoleucine and alanine residues, the “b_5” peptide will be YEKDI, molecular weight 649.32, and the “y_5” peptide will be AAYR, molecular weight 480.26. Both of theses fragments are present in the spectrum. Taking into account all possible fragments, which are listed in the table above the spectrum, this spectrum includes signals for 13 of 16 expected ions, confirming that the parent peptide is YEKDIAAYR.

C)



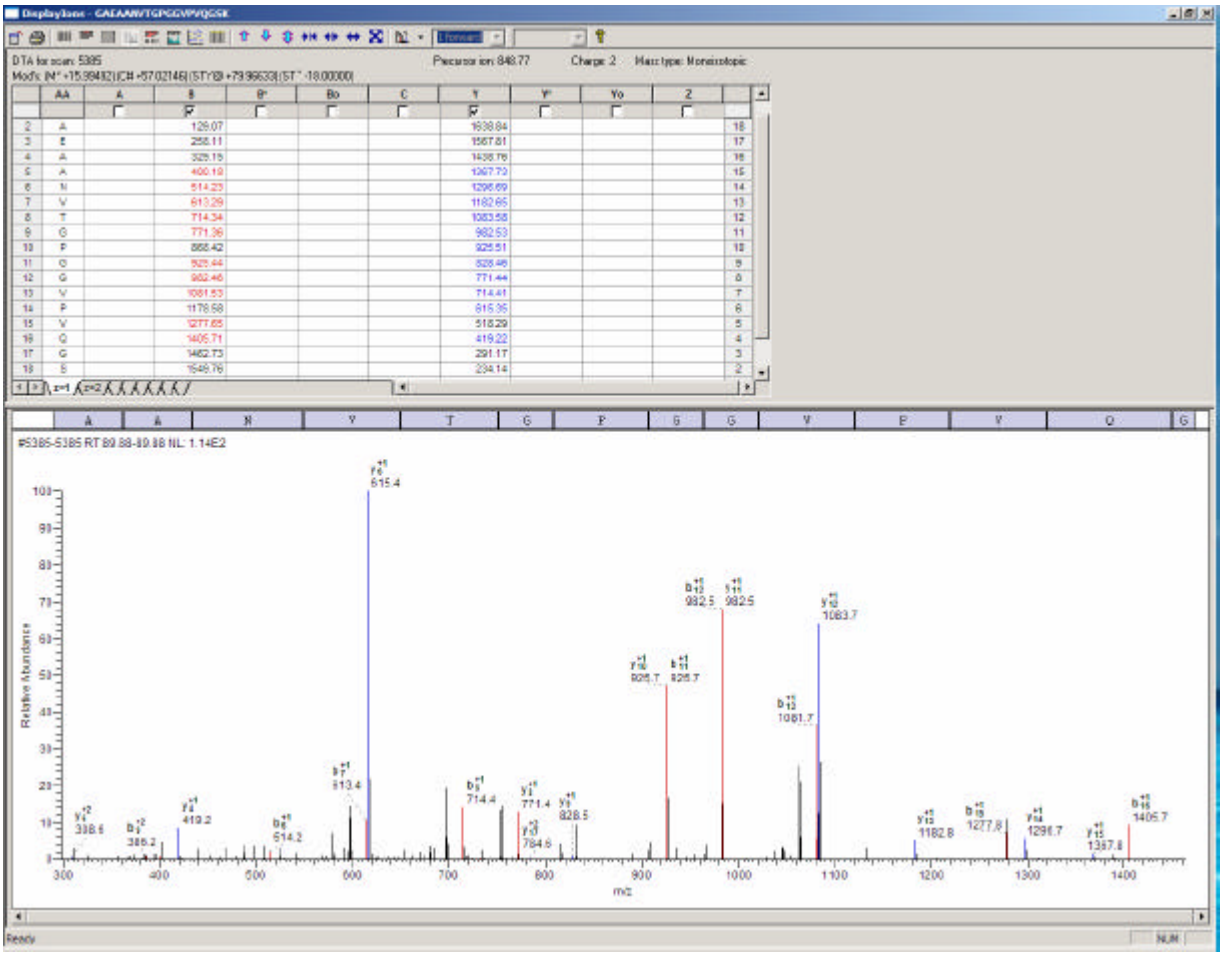
Protein: RPA1 (from band 2)
Parent peptide: AAGPSLSHTSGGTQSK
MH^+ parent peptide: 1485.729
Ions found/ions expected: 16/30

D)



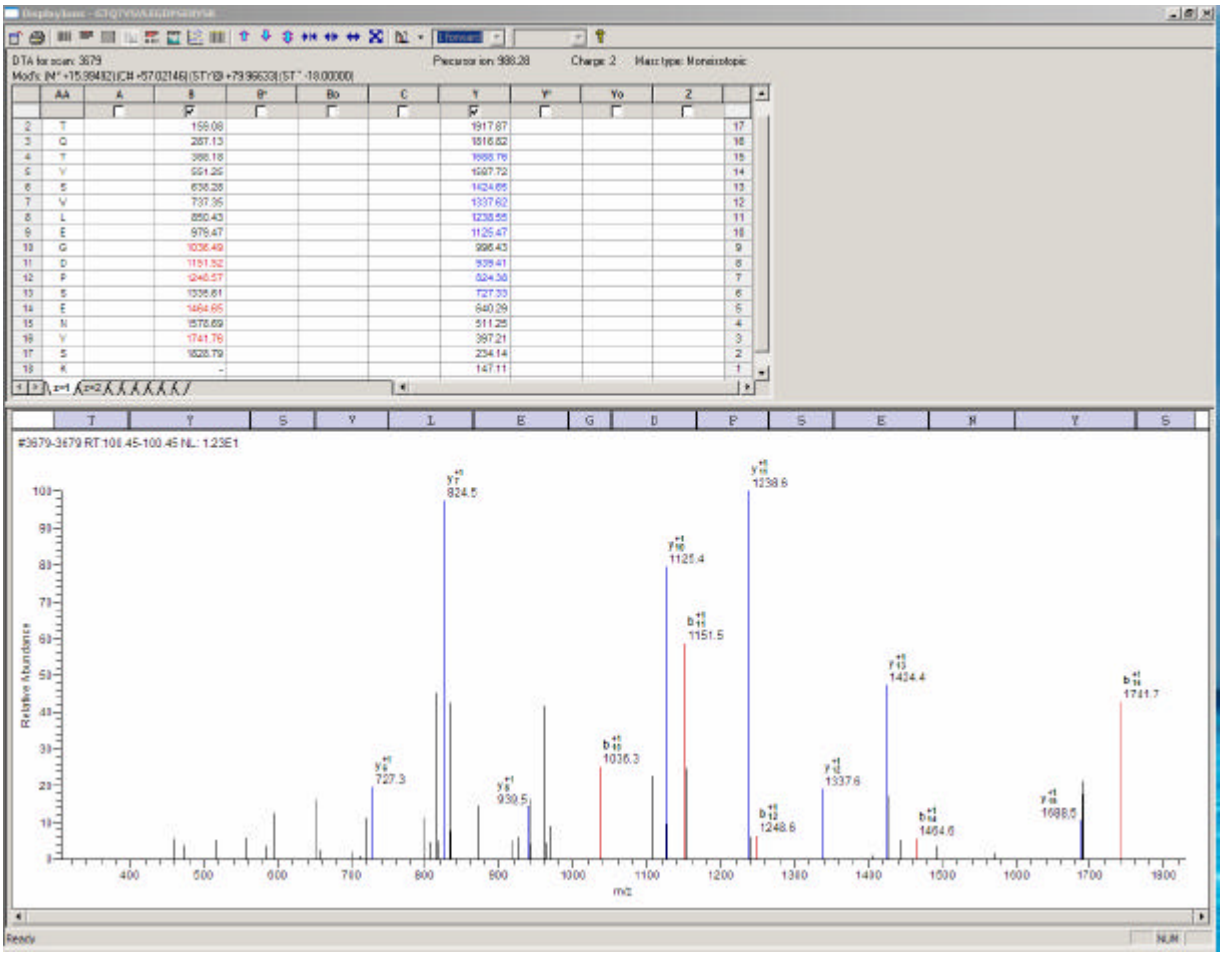
Protein: RPA1 (from band 3)
Parent peptide: AAGPSLSHTSGGTQSK
MH^+ parent peptide: 1485.729
Ions found/ions expected: 16/30

E)



Protein: YB1
Parent peptide: GAEAANVTGPGGVPQGSK
MH^+ parent peptide: 1697.52
Ions found/ions expected: 21/34

F)



Protein: Msh6
Parent peptide: GTQTYSVLEGDPSENYSK
MH^+ parent peptide: 1976.56
Ions found/ions expected: 13/34

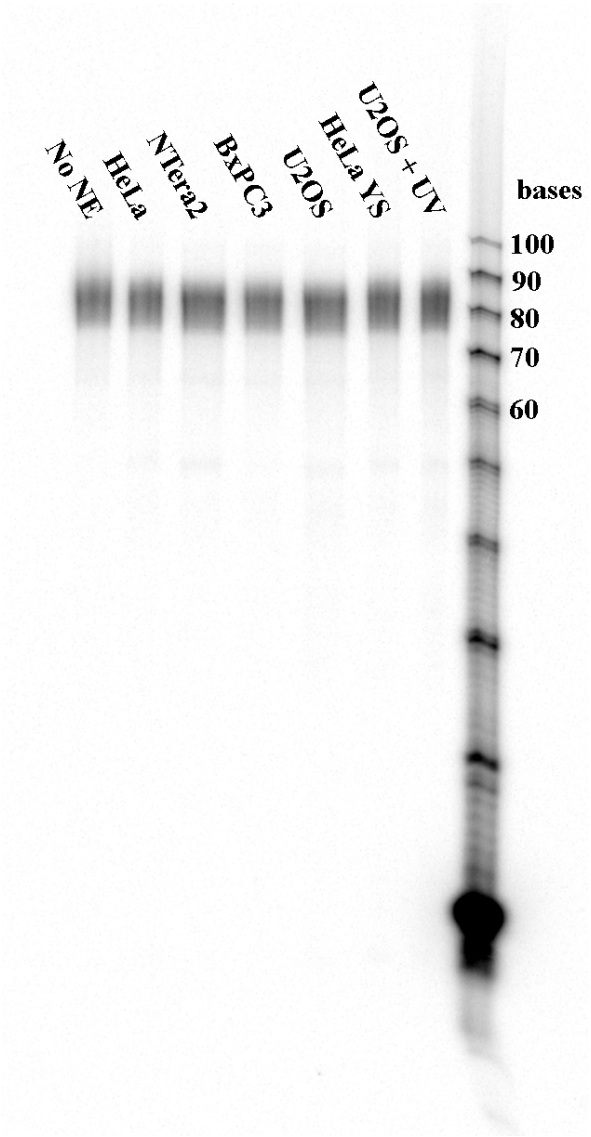


Figure S7. Repair assay of 90-base DNA. The platinated 90-base DNA probe was incubated with nuclear extracts from various cell lines with or without UV irradiation to determine whether the probe is cut by nucleases during the incubation period. These results indicate that the probe is not repaired or digested during the two-h incubation with nuclear extracts.