Supplemental Materials and Methods

Fractionation of Nuclei and Cytoplasm

Human colon tissue and Caco-2 cells were fractionated into nuclei and cytoplasm essentially as described (1). For human tissue, excess OCT was removed from a ~2 cm x ~1 cm sample, and the tissue was homogenized in 1 ml buffer A (10 mM HEPES, pH 7.9 ; 1.5 mM $MgCl_2$; 10 mM KCl ; 0.5 mM DTT ; Sigma protease inhibitor cocktail) using a Power Gen 125 homogenizer with 7 x 65 mm generator (Fisher Scientific) for 3 x 5 s pulses at setting 3.5 with 1 min pauses on ice. After adding another 1 ml buffer A and swelling cells on ice for 5 min, nuclei were released by 20 strokes with a 2 ml Dounce homogenizer using pestle B (tight fitting). Homogenized tissue was centrifuged at 250 x g for 5 min at 4^{o}C, and supernatant was retained as the cytoplasmic fraction. Nuclei were resuspended in 1 ml buffer S1 (0.25 M sucrose ; 10 mM $MgCl_2$; Sigma protease inhibitor cocktail), filtered through 70 μ, layered over 1 ml buffer S3 (0.88 M sucrose ; 0.5 mM $MgCl_2$; Sigma protease inhibitor cocktail), and centrifuged at 2800 x g for 10 min at 4^{o}C. Nuclei were resuspended in 0.5 ml sonication buffer (50 mM HEPES pH 7.5 ; 150 mM NaCl ; 1 mM EDTA ; 2.5 mM EGTA ; 1 mM DTT ; 0.1% Tween 20 ; 10% glycerol ; Sigma protease inhibitors) and sonicated as described in *Materials and Methods*. Subconfluent, proliferating Caco-2 cells were trypsinized from a 150 mm dish, resuspended in 2 ml buffer A, Dounce homogenized after swelling on ice for 5 min, and further processed as above.

REFERENCES

1. **Boisvert FM, Lam YW, Lamont D, and Lamond AI.** A quantitative proteomics analysis of subcellular proteome localization and changes induced by DNA damage. *Mol Cell Proteomics* 9: 457-470.