Supplemental Methods

Western blot

Protein levels of NER relevant components in the panel of WM793 cells (XPC, XPA, XPE, XPF, p21, β-tubulin (Santa Cruz), RPA70, PCNA (GenScript), p53 (cell signaling)) and NM23 expression in melanoma and breast cancer cells (cell signaling) were determined from whole cell lysates separated on 4-20% polyacrylamide gel and transferred onto a nitrocellulose membrane. Primary antibodies were incubated overnight in 5% milk in PBS-T, washed, then incubated with secondary antibodies conjugated to horseradish peroxidase which were revealed with chemiluminescence (GE-healthcare).

Determination of Mutation Spectra

6-TG-resistant colonies were cloned by limiting dilution and expansion ($\geq$ 6 passages) in culture media containing 6-TG (40 μM). mRNA was isolated from clones using a RNeasy Plus Mini Kit (Qiagen, USA). The *hprt* gene (NM_000194.2) was amplified by using primers that amplified three overlapping fragments that covered coding regions between 296 bp – 1051 bp of the *hprt* gene. Primer set 1 (296bp – 507bp; encompassing exons 2 – 4) GGACAGGACTGAACGTCTTGCTCG (+) and TGTCCCCTGTTG ACTGGTCATTACA (-). Primer set 2 (338bp – 655bp; encompassing exons 3 – 7); TGCTGGTGAAAAGGACCCACG (+) and ACAATCCGCCCAAAGGGAACTGA (-). Primer set 3 (625bp – 1051 bp; encompassing exons 7 – 9); TGCTGGTGAAAAGGACCCCACG (+) and ACAACAATCCGCCC AAAGGGAACT (-). PCR cycling conditions for all primer sets were as follows: initial

melting step (94 ^{o}C, 2 min), 30 cycles of denaturation (94 ^{o}C, 40s), annealing (60 ^{o}C; 30s) and extension (72 ^{o}C, 1 min), followed by a final extension step at 72 ^{o}C for 10 min. PCR products were analyzed on a 1% agarose gel and purified for sequencing for QIAquick PCR Purification Kit (Qiagen, USA). All PCR products were sequenced at the University of Kentucky Advanced Genetics Technologies Center using a ABI prism 3100 DNA sequencer and sequences aligned with BLAST software to identify mutations.

Selection of WM793 melanoma cells for growth factor-independence

WM793 and stably-transfected lines expressing wild-type and mutant variants of NM23-H1 were seeded at high density (10^4 cells/well) in a 24-well plate pre-coated with 0.1% gelatin. Cells were adapted to growth for 7 days in serum-free melanocyte growth medium supplemented with 5 μg/ml of bovine insulin in a humidified atmosphere with 5% CO_2 at 37 °C. Surviving cells were trypsinized, plated at low density (10^3 cells/well) in a 6-well plate containing protein-free melanocyte medium (pH 6.8), and grown for 11 weeks. After colonies of 1-3 mm were formed, cells from five wells of the 6-well plate were pooled and re-seeded at 10^3 cells/well for subsequent cycles of selection. Colonies were visualized by crystal violet staining or used for DNA damage analysis.

SKY analysis

Cell cultures received nocodazole (0.5 μg/ml) for 2 hours before harvesting. Chromosome spreads were prepared using air-drying methods. After sequential digestion with RNase and pepsin according to the procedure recommended by Applied Spectral Imaging, Inc. (Vista, CA 92081), the chromosomal DNA on slides was denatured in 70%

formamide and then hybridized with a cocktail of human SKY paint probes tagged with various nucleotide analogues (i.e., a mixture of individual chromosome DNA prepared by flow-sorting and PCR amplification). Thirty to fifty mitoses were chosen at random and the images developed by combinations of five different flouorophores such as Rhodamine, Texas-Red, Cy5, FITC and Cy5.5 were captured with a Spectral cube and Interferometer module installed on a Nikon microscope. Spectra-Karyotypes were carried out using SKY View software (Version 1.62).

Microsatellite instability analysis

WM793-derived cell lines that were selected for growth factor-independent proliferation were grown to 75% confluence in MCDB media on 150 mm culture plates. Cells were washed and harvested after treatment with 0.05% Trypsin:EDTA for 2 min, then pelleted by centrifugation for 5 minutes. The pellets were processed according to manufacturer's protocol with DNeasy Blood and Tissue Kit (Qiagen). Genomic DNA (1.5 ng) was amplified in a multiplex PCR reaction with MSI analysis primer mix (Promega) and Gold Star Taq polymerase (ABI) according to the MSI Analysis kit protocol. Each amplified sample was combined an formamide/internal lane standard, denatured for 3 minutes at 95^{o}C and snap-cooled on ice. The denatured samples were analyzed in triplicate on Applied Biosystems ABI 3730 genetic analyzer according to the manufacturer's instructions. The raw data was analyzed with Peak Scanner Software v1.0 (ABI).

8-hydroxy-2-deoxy Guanosine (8-OH-dG)

WM793-derived cell lines that were selected for growth factor-independent proliferation were grown to 75% confluence in MCDB media on 150 mm culture plates (~5 x 10^6 cells). Cells were washed and harvested after treatment with 0.05% Trypsin:EDTA for 2 min, then pelleted by centrifugation for 5 minutes. The pellets were processed according to manufacturer's protocol with DNeasy Blood and Tissue Kit, with 1mM glutathione supplemented in all extraction buffers to reduce DNA oxidation during the extraction procedure (Qiagen, USA). The levels of 8-OH-dG from total genomic DNA were measured using an enzyme immunoassay (Cayman, USA).