SUPPORTING INFORMATION

Genomic DNA isolation. To reduce the adventitious oxidation of DNA, we have utilized an optimized salt-based DNA isolation methodology as described earlier (1, 2). Briefly, the harvested cells were washed with pre-warmed PBS and lyzed with 4 ml of a solution containing 0.5 M Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 10 mM NaCl, 1% SDS, and 0.5 mg/ml proteinase K at 37°C overnight. After addition of 2 ml of saturated NaCl (~6 M) to each sample, incubation was performed at 56°C for 10 minutes. Subsequently, centrifugation was done at 5000 x*g* for 30 minutes, and the supernatant containing DNA was mixed with 2 volumes of pre-chilled absolute ethanol. The DNA was spooled by gently inverting the mix, washed with 70% ethanol, air-dried, and dissolved in TE buffer (1 mM EDTA, 10 mM Tris-HCl pH 7.5). All DNA samples were preserved at –80°C until further analysis.

Terminal transferase-dependent polymerase chain reaction. TD-PCR was used for DNA footprinting of polymerase-blocking lesions, including dipyrimidine photolesions on the entire non-coding strand of the *cII* transgene (3). Briefly, genomic DNA was subjected to nine cycles of primer extension using a custom-made biotinylated primer (*cII*.P_1: 5'-CAACAGCATAAATAACCCCGCTCTTAC-3'; T_m = 59.8°C) in a Vent $^{(exo-)}$ DNA polymerase mix reaction (New England Biolabs, Beverly, MA). The thermocycler settings were as follows: 3 minutes at 95°C, 5 minutes at 60°C, and 10 minutes at 72°C. The extension products were mixed with streptavidin-coupled magnetic beads (Dynal Biotech ASA, Oslo, Norway), and gently rotated for 45 minutes at room temperature to allow binding of the products to the beads. The beads were washed thoroughly with 1x TE, pH

7.5 in a magnetic particle concentrator (Dynal Biotech ASA), and the bead-bound DNA was resuspended in 0.1x TE, pH 7.5. After denaturation with 0.15 M NaOH, the single-stranded DNA underwent 3'- ribotailing by terminal deoxynucleotidyl transferase (4). The ribotailed products were ligated overnight at 17°C, and afterward were exponentially amplified by PCR using a nested primer (*cII*.P_2: 5'-CCGCTCTTACACATTCCAGCCCTG-3'; T_m = 63.2°C) and the LP25 linker primer (4) in an Expand Long Polymerase mix reaction (Roche, Indianapolis, IN). The thermocycler settings were as follows: 2 minutes at 95°C, 2 minutes at 61°C, 3 minutes at 72°C, twenty-one cycles of (45 seconds at 95°C, 2 minutes at 61°C, and 3 minutes at 72°C), 45 seconds at 95°C, 2 minutes at 61°C, and 10 minutes at 72°C. The amplified products were labeled using a fluorescence infrared dye-labeled primer (*cII*.P_3: 5'-CCGCTCTTACACATTCCAGCCCTG-3'; T_m = 63.2°C; IRD-700; LI-COR, Lincoln, NE) in a mixture of Expand Long Polymerase. The thermocycler settings were as follows: 2 minutes at 95°C, 2 minutes at 65°C, 3 minutes at 72°C, four cycles of (45 seconds at 95°C, 2 minutes at 65°C, and 3 minutes at 72°C), 1 minute at 95°C, 2 minutes at 60°C, and 10 minutes at 72°C. The labeled products were subjected to polyacrylamide–urea gel electrophoresis using an IR^2 Long Ranger 4200 system with simultaneous detection (LI-COR) (3).

Ligation-mediated polymerase chain reaction. LM-PCR was used for DNA footprinting of CPDs and oxidized (ring-opened) purines on the entire non-coding strand of the *cII* transgene (3). The principles of LM-PCR are essentially similar to TD-PCR with the only differences being the primer extension and ligation steps. Unlike TD-PCR, LM-PCR

requires conversion of original DNA templates to single strand breaks with 5'- phosphate groups at the lesion formation sites. To convert CPDs or oxidized (ring-opened) purines to single strand breaks with 5'- phosphate termini, we pre-treated the genomic DNA with T4 Endo V digestion plus CPD photolyase reactivation or Fpg digestion, respectively. The pre-treated DNA underwent a single round of primer extension using the same protocol as described for TD-PCR. The captured and purified extension products were ligated overnight, and then amplified exponentially by PCR using the same procedures as described for TD-PCR. Subsequently, labeling, gel electrophoresis, and visualization were performed using standardized protocols as described for TD-PCR (3).

cII Mutation detection system. Genomic DNA of transgenic Big Blue® rodents contains multiple copies of the chromosomally integrated λLIZ shuttle vector, which carries two reporter genes, namely the *cII* and *lacI* (5). The mutation detection system is based on the recovery of this coliphage vector from the genomic DNA, followed by bacterial phenotypic expression assay (6). Briefly, the recovered vector is packaged into viable bacteriophages, and the infective phage particles are introduced into an indicator host *Escherichia coli* (*E.coli*). The lambda phages can multiply either lytically or lysogenically in the host *E.coli* depending on the status of cII transcription (7). The cII protein is indispensable for activating the cI repressor and lambda integrase, both of which are essential for lysogenization (7). The *E. coli* indicators that carry phages with a mutated *cII* undergo lysis, thereby forming visible plaques on special agar lawn (6). The lambda LIZ shuttle vector, however, harbors a *cI857* temperature sensitive (*ts*) mutation, which makes the $cI_{(ts)}$ protein labile at temperature exceeding 32°C (8). Consequently, all

vector-bearing phages, irrespective of the status of *cII* mutation, multiply lytically in the host *E. coli* at incubating temperatures greater than 32°C (6). This temperature sensitivity is the basis for the *cII* selection assay in which phenotypic expression of the *cII* mutants is achieved under selective incubation condition, *i.e.*, 24°C. Under non-selective incubation condition, *i.e.*, 37°C, however, both wild type and mutant *cII* are expressed (6). The ratio of plaques formed under the selective condition to those arisen under the non-selective condition is commonly referred to as the "*cII* mutant frequency", which denotes the mutation rate in the *cII* transgene (3). In addition, phenotypically expressed *cII* mutants can be subjected to DNA sequencing, thereby providing information on the types of mutation, *i.e.*, mutation spectrum (3).

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