Supporting Information

Wang et al., Aging Cell, "The oxidative DNA lesions 8,5′-cyclopurines accumulate with aging in a tissue-specific manner".

Experimental procedures

Materials and mice.

Erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) hydrochloride was purchased from Tocris Bioscience (Ellisville, MO, USA). Nuclease P1 and phosphodiesterases 1 and 2 were from Sigma-Aldrich (St. Louis, MO, USA). Alkaline phosphatase and proteinase K were from New England Biolabs (Ipswich, MA, USA).

The ERCC1-deficient (*Ercc1*$^{-/\Delta}$) mice and DNA repair-proficient littermates (WT, *Ercc1*$^{+/-}$ and *Ercc1*$^{+/\Delta}$) were bred and genotyped as previously described (Goss *et al.*, 2011). Experiments involving mice were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and in accordance with the NIH guidelines for humane care of animals.

Extraction of nuclear DNA from mouse tissues.

Nuclear DNA was isolated from mouse tissues using a high-salt method (Miller *et al.*, 1988). Tissues were ground under liquid nitrogen into fine powders in a mortar. A nuclei lysis buffer containing 20 mM Tris (pH 8.3), 20 mM EDTA, 400 mM NaCl, 1% SDS (w/v) and 0.02% proteinase K (w/v) was added to the tissue and incubated in a water bath at 55˚C overnight. Half volume of saturated NaCl solution was added to the digestion mixture, incubated at 55˚C for 15 min then centrifuged at ~10,000 rpm at room temperature for 30 min. The supernatant was collected and centrifuged again. The nucleic acids in the supernatant were precipitated with cold ethanol, dissolved in water and incubated in the presence of 0.03% RNase A (w/v) and 0.25 U/μL of RNase T1 at 37˚C for 6 h, and subsequently extracted with an equal volume of chloroform/isoamyl alcohol (24:1, v/v) twice. The DNA was then precipitated from the aqueous

layer by cold ethanol, centrifuged at 7,000 rpm at 4˚C for 15 min, washed twice with 70% cold ethanol and dried under vacuum. The pellet was dissolved in water and quantified by using ultraviolet spectrophotometry.

Enzymatic digestion of nuclear DNA.

Nuclease P1 (0.1 U/μg DNA), phosphodiesterase 2 (0.000125 U/μg DNA), 20 nmol of EHNA and a 20-μL solution containing 300 mM sodium acetate (pH 5.6) and 10 mM zinc chloride were added to 30-120 μg of DNA. In this context, EHNA served as an inhibitor for deamination of dA to 2'-deoxyinosine (dI) induced by adenine deaminase (Wang *et al.*, 2011). The above digestion was continued at 37˚C for 48 h. To the digestion mixture were then added alkaline phosphatase (0.05 U/μg DNA), phosphodiesterase 1 (0.00025 U/μg DNA) and 40 μL of 0.5 M Tris-HCl buffer (pH 8.9). The digestion was continued at 37˚C for 2 h and subsequently neutralized by addition of formic acid. To the mixture were then added uniformly ^{15}N-labeled standard lesions, which included 200 fmol of *R*-cdG, 150 fmol of *S*-cdG, 100 fmol of *R*-cdA and 60 fmol of *S*-cdA. The enzymes in the digestion mixture were subsequently removed by chloroform extraction twice. The resulting aqueous layer was subjected to off-line high performance liquid chromatography (HPLC) separation for the enrichment of the lesions under study, following our previously described procedures (Wang *et al.*, 2011).

LC-MS/MS/MS Analysis.

The LC-MS/MS/MS experiments were conducted using an LTQ linear ion trap mass spectrometer using our recently described conditions (Wang *et al.*, 2011).

References

Goss JR, Stolz DB, Robinson AR, Zhang M, Arbujas N, Robbins PD, Glorioso JC, Niedernhofer LJ (2011) Premature aging-related peripheral neuropathy in a mouse model of progeria. *Mech. Ageing Dev.* **132**, 437-442.

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16**, 1215.

Wang J, Yuan B, Guerrero C, Bahde R, Gupta S, Wang Y (2011) Quantification of oxidative DNA lesions in tissues of Long-Evans Cinnamon rats by capillary high-performance liquid chromatography-tandem mass spectrometry coupled with stable isotope-dilution method. *Anal. Chem.* **83**, 2201-2209.

Table S1 Levels of 8,5'-cyclo-2'-deoxyguanosine (cdG) and 8,5'-cyclo-2'-deoxyadenosine (cdA) in nuclear DNA of tissues of *Ercc1*$^{-/\Delta}$ mice and age-matched littermates (n = 3).

Levels (Lesions per 10^7 nucleotides)					
	Control			*Ercc1*$^{-/\Delta}$	
Lesions in the *liver*	**10 wk**	**21 wk**	**3 yr**	**10 wk**	**21 wk**
***R*-cdG**	2.1±1.1	10.1±2.3*	1.4±0.2†	24.3±2.6‡	73.1±24.3$^{\S\|}$
***S*-cdG**	3.2±2.2	10.5±3.6*	6.9±1.3	20.3±2.6‡	56.4±2.7$^{\S\|}$
***R*-cdA**	2.0±0.8	9.5±4.3*	1.7±0.2†	25.4±6.8‡	83.7±32.4$^{\S\|}$
***S*-cdA**	2.1±1.1	5.8±1.9*	4.2±1.0	9.6±3.3‡	40.9±6.8$^{\S\|}$
	Control			*Ercc1*$^{-/\Delta}$	
Lesions in the *kidney*	**10 wk**	**21 wk**	**3 yr**	**10 wk**	**21 wk**
***R*-cdG**	3.5±0.2	4.4±0.9	7.3±3.5	2.8±0.8	7.3±1.1$^{\S\|}$
***S*-cdG**	9.1±2.2	19.8±2.2*	28.6±8.0¶	5.3±0.8	28.1±3.3$^{\S\|}$
***R*-cdA**	2.0±0.5	4.7±1.1*	6.5±2.5¶	2.7±0.5	7.2±0.4$^{\S\|}$
***S*-cdA**	2.6±0.8	12.5±1.5*	12.2±3.5¶	1.9±0.2	18.1±1.4$^{\S\|}$
	Control			*Ercc1*$^{-/\Delta}$	
Lesions in the *brain*	**10 wk**	**21 wk**	**3 yr**	**10 wk**	**21 wk**
***R*-cdG**	1.7±0.6	1.1±0.2	1.6±0.3†	2.1±0.4	1.1±0.2$^{\|}$
***S*-cdG**	3.7±1.0	3.8±1.0	5.3±0.3	6.6±1.3‡	2.5±0.5$^{\|}$
***R*-cdA**	1.4±0.4	0.8±0.5	1.6±0.1†	1.7±0.5	0.9±0.2
***S*-cdA**	2.2±0.9	1.2±0.4	2.0±0.1†	2.1±0.3	1.0±0.2$^{\|}$

*Significant difference between 10 week control and 21 week control
¶Significant difference between 10 week control and 3 year control
†Significant difference between 21 week control and 3 year control
$^{\|}$Significant difference between 10 week *Ercc1*$^{-/\Delta}$ and 21 week *Ercc1*$^{-/\Delta}$
‡Significant difference between 10 week control and 10 week *Ercc1*$^{-/\Delta}$
§Significant difference between 21 week control and 21 week *Ercc1*$^{-/\Delta}$

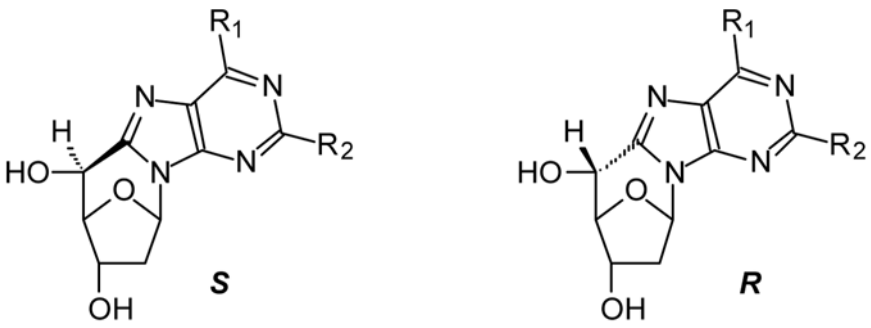

Fig. S1 Structures of cdA and cdG. For 8,5'-cyclo-2'-deoxyadenosine (cdA), R_1=NH_2, R_2=H; for 8,5'-cyclo-2'-deoxyguanosine (cdG), R_1=OH, R_2=NH_2.

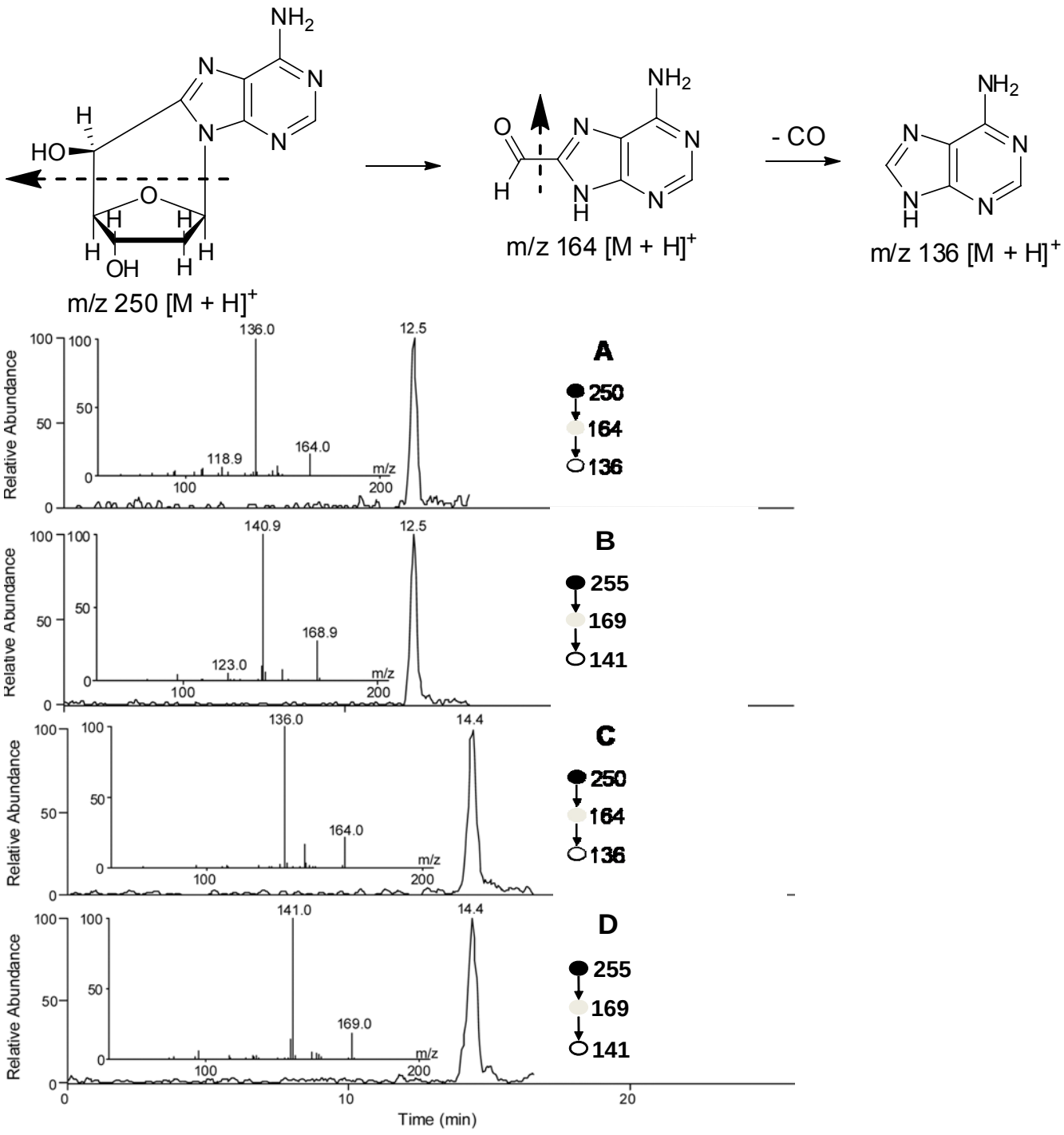

Fig. S2 Representative LC-MS/MS/MS data. Selected-ion chromatograms (SICs) for monitoring the *m/z* 250→164→136 [**A**, for unlabeled *R*-cdA], *m/z* 255→169→141 [**B**, for uniformly ^{15}N-labeled *R*-cdA], 250→164→136 [**C**, for unlabeled *S*-cdA], and *m/z* 255→169→141 [**D**, for uniformly ^{15}N-labeled *S*-cdA] transitions of the *R*-cdA- and *S*-cdA-containing fractions from HPLC enrichment for the digestion mixture of nuclear DNA from the brain tissue of a 21 week-old DNA repair-proficient mouse. Shown in the insets are the positive-ion MS^3 spectra for the unlabeled and labeled *R*-cdA and unlabeled and labeled *S*-cdA. Top is a scheme showing the proposed fragmentation pathways for the production of *m/z* 164 and 136 fragment ions from the $[M+H]^+$ ion of unlabeled cdA (8,5'-cyclo-2'-deoxyadenosine).