Supporting Information

Kim et al. 10.1073/pnas.1207718109

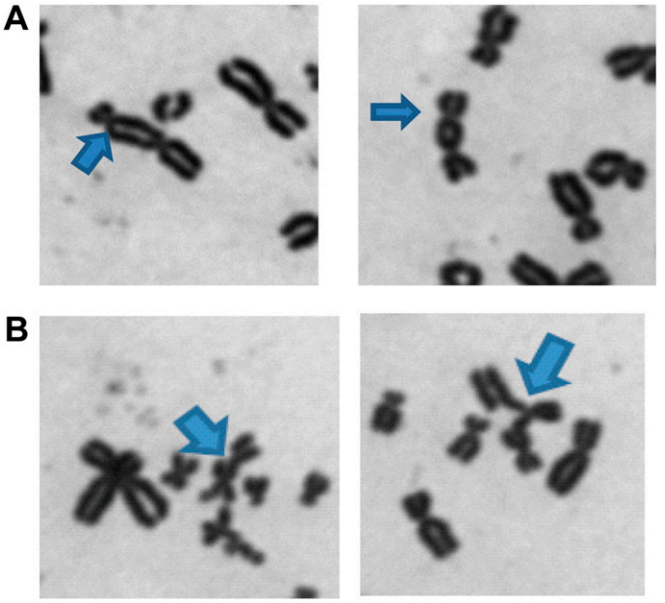


Fig. S1. BARD reduces chromosome aberrations after IR. (*A*) Sectorial metaphases showing G1-type chromosome aberrations, such as dicentrics, after 5 Gy of IR exposure. The arrows indicate dicentrics metaphase. (*B*) Sectorial metaphases showing S- or G2-type chromosomal aberrations, such as tri- or quadriradials, after 4 Gy of IR exposure. The arrows indicate chromatid breaks and triradials. Magnification: 600×.

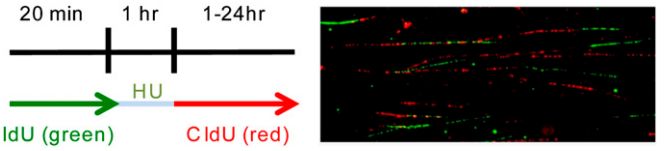


Fig. S2. Schematic of single DNA fiber analysis. Green tracts illustrate 5-iododeoxyuridine (IdU), and red tracts illustrate 5-chlorodeoxyuridine (CIdU). An example image of DNA labeled with IdU and CIdU is shown. Hydroxyurea (HU) does result in DNA double-strand breaks (DSBs) after stalled replication forks, which is close to the DSBs induced by other agents.

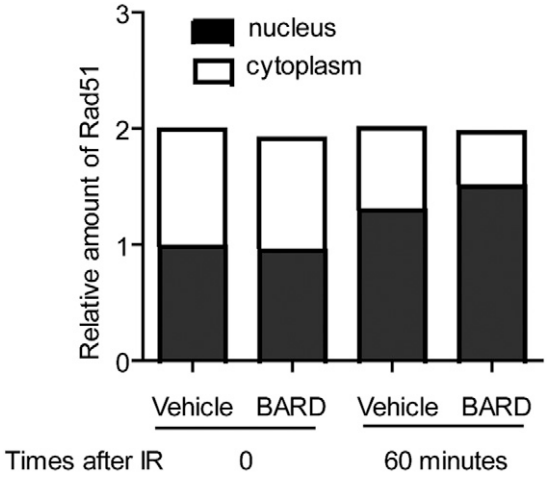
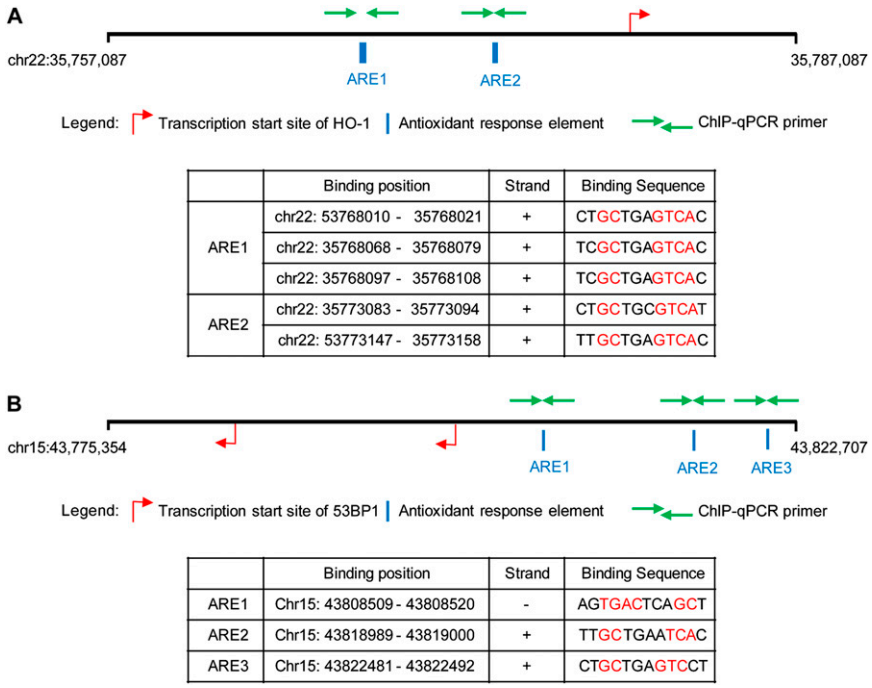


Fig. S3. Relative amount of Rad51 between the cytoplasm and nucleus. Rad51 protein levels in each fraction were normalized to unirradiated vehicle control. At 60 min after 5-Gy irradiation, nuclear localization of Rad51 is higher in bardoxolone methyl (BARD)-pretreated cells than in vehicle-pretreated control. There is no change in total Rad51 levels. qPCR, quantitative PCR.



	Binding position	Strand	Binding Sequence
ARE1	chr22: 53768010 - 35768021	+	CTGCTGAGTCAC
	chr22: 35768068 - 35768079	+	TCGCTGAGTCAC
	chr22: 35768097 - 35768108	+	TCGCTGAGTCAC
ARE2	chr22: 35773083 - 35773094	+	CTGCTGCGTCAT
	chr22: 53773147 - 35773158	+	TTGCTGAGTCAC

	Binding position	Strand	Binding Sequence
ARE1	Chr15: 43808509 - 43808520	-	AGTGACTCAGCT
ARE2	Chr15: 43818989 - 43819000	+	TTGCTGAATCAC
ARE3	Chr15: 43822481 - 43822492	+	CTGCTGAGTCCT

Fig. S4. Location of antioxidant response elements (AREs) in the promoter region of heme oxygenase-1 (HO-1) and p53 binding protein-1 (53BP1). Locations of nuclear factor-erythroid 2–related factor 2 (Nrf2) binding motifs are identified using search tools from SABiosciences in the promoter region of HO-1(*A*; chromosome 22) and 53BP1 (*B*; chromosome 15), including the direction of transcription (red arrow). "Core sequences" of AREs are marked in red. BARD, bardoxolone methyl.

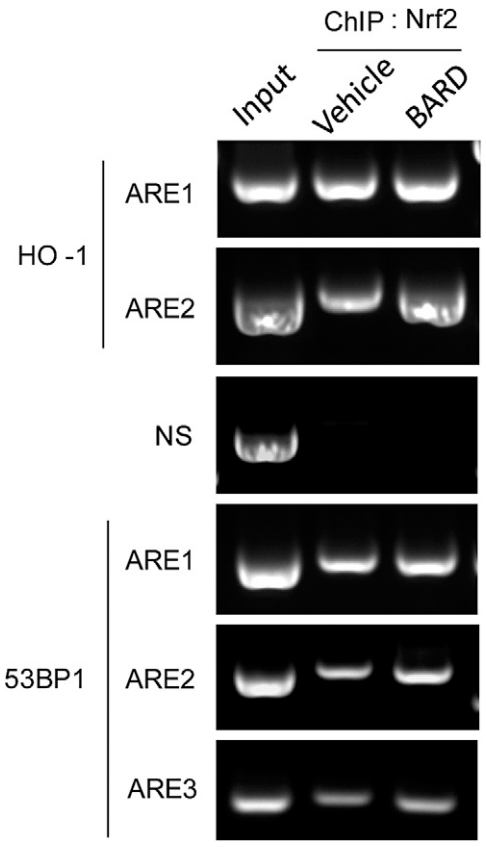


Fig. S5. Nuclear factor-erythroid 2–related factor 2 (Nrf2) binds to the p53 binding protein-1 (53BP1) promoter region harboring the antioxidant response elements (AREs) in human colonic epithelial cells (HCECs) after bardoxolone methyl (BARD) treatment. A ChIP assay was performed 18 h after vehicle or BARD treatment. Data shown are from a representative experiment.

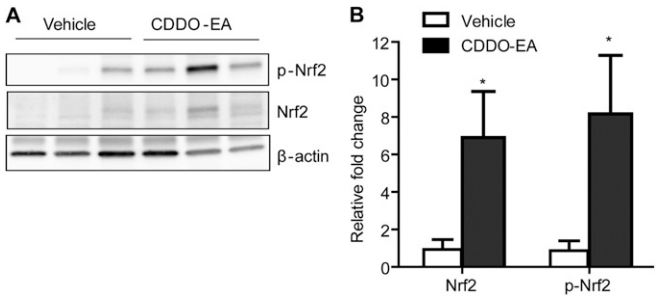


Fig. S6. 2-Cyano-3,12-dioxooleana-1,9 (11)-dien-28-oic acid (CDDO)–ethyl amide (EA) stabilizes Nuclear factor-erythroid 2–related factor 2 (Nrf2) and increases its phosphorylation in vivo. (*A*) Control or CDDO-EA diet was provided to unirradiated WT mice (n = 3 per group) for 3 d, and colon tissues were then lysed. Total Nrf2 and phospho-Nrf2 (p-Nrf2) were detected by Western blot analysis. (*B*) Quantitative data show the average intensity of total Nrf2 and p-Nrf2 levels from Western blots. *$P < 0.05$ in the unpaired Student t test (n = 3).

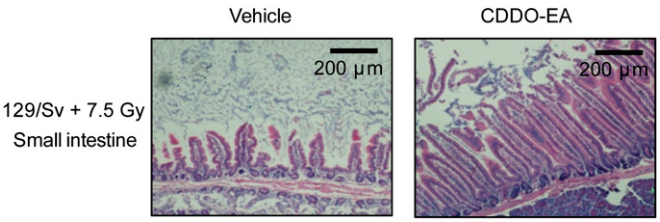


Fig. S7. 2-Cyano-3,12-dioxooleana-1,9 (11)-dien-28-oic acid (CDDO)–ethyl amide (EA) protects the small intestine from acute total body irradiation (TBI). Representative images of H&E staining of the small intestine in 129/Sv mice are shown at 5 d after TBI with or without prior feeding of CDDO-EA chow.

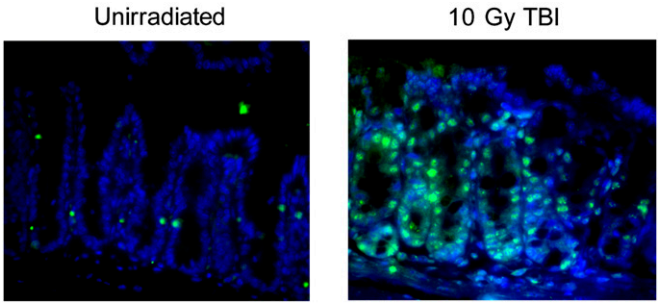


Fig. S8. Immunostaining of p53 binding protein-1 (53BP1) in mouse colon tissues. Representative images show 53BP1-positive cells (green) in colon tissues 3 d after 10-Gy doses of total body irradiation (TBI) exposure. DAPI was used for counterstaining (blue). Magnification: 400×.

Table S1. Calculation of dose-modifying factor from cell survival

Survival fraction	HCEC CT7			HCEC CT7/shNrf2		
	Dose, Gy			Dose, Gy		
	DMSO	BARD	DMF	DMSO	BARD	DMF
1	0	0		0	0	
0.9	0.48	0.78	1.61	0.22	0.23	1.05
0.8	0.91	1.30	1.42	0.45	0.47	1.05
0.7	1.32	1.75	1.32	0.70	0.73	1.05
0.6	1.73	2.18	1.26	1.00	1.02	1.05
0.5	2.16	2.62	1.21	1.27	1.33	1.05

BARD, bardoxolone methyl; CT7, trisomy 7; DMF, dose-modifying factor; HCEC, human colonic epithelial cell; shNrf2, short hairpin nuclear factor-erythroid 2–related factor 2.

Table S2. Primer sequence for ChIP-quantitative PCR

		Forward primer	Reverse primer
HO-1	ARE1	5′-CTGCCCAAACCACTTCTGTT-3′	5′-ATAAGAAGGCCTCGGTGGAT-3′
	ARE2	5′-CCCTGCTGAGTAATCCTTTCCCGA-3′	5′-ATGTCCCGACTCCAGACTCCA-3′
53BP1	ARE1	5′-CCGGATCTAAGCAAGGATTG-3′	5′-CAGTTTGGCCAGCTCCTAAG-3′
	ARE2	5′-CTAGATGAGGGCCCAGATGA-3′	5′-CTGAAGGAACCCTTTGTCCA-3′
	ARE3	5′-GAATGTCCTGGTCCTGGCTA-3′	5′-TCTAAAGGAGCTGGGGGAGT-3′
Nonspecific		5′-GCTATGTGGGAGGTTGAGGA-3′	5′-CCATGGTCAGCAGTTTGCTA-3′

ARE, antioxidant response element; 53BP1, p53 binding protein-1; HO-1, heme oxygenase-1.