Supplementary Data

Chemoprotective effects of dietary grape powder on ultraviolet B radiation-mediated skin carcinogenesis in SKH-1 hairless mice

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Materials and Methods

Preparation of epidermal gDNA

Skin from 5 randomly selected animals in the control and 5% GP groups were heated for 15 seconds at 60°C followed immediately by incubation for 30 seconds in ice water. Excess water was removed by placing tissue between two paper towels and the epidermis was gently separated from the remaining tissue, treated with Proteinase K and RNase A, and then genomic DNA (gDNA) was isolated using the QIAamp DNA Mini Kit (Qiagen) according to manufacturer's protocol.

Immuno-slot blot analysis

The quality and quantity of gDNA samples were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) before measurement of photoproducts as previously reported (Gaddameedhi *et al.*, 2010b). Briefly, 200 ng and 300 ng of gDNA was used per slot for CPDs and (6-4) PPs detection, respectively. gDNA was denatured by heating for 10 minutes at 100°C, and neutralized immediately by placing on ice and adding cold ammonium acetate to a final 1 M concentration. Prepared gDNA samples were bound to a nitrocellulose membrane (pre-wet with 6x saline-sodium citrate (SSC) buffer) using a Bio-Dot SF slot-blot apparatus (BioRad Laboratories, Hercules, CA) and crosslinked by baking for 2 hours at 80°C in a vacuum oven (Shel Lab, Cornelius, OR). Blots were subsequently blocked in PBS-T and 5% nonfat dry milk for 1 hour at room temperature (RT). The membrane was incubated with anti-CPD or anti-(6-4) PP antibodies (see Supplementary Table S3) in ice-cold PBS-T for 12-16 hours at 4°C with gentle shaking. A secondary antibody (anti-mouse conjugated with HRP) was detected using Clarity Western ECL chemiluminescent substrate (#170-5061, Bio-Rad Laboratories) or SuperSignal West Femto (#34096, Thermo Fisher) reagents using Bio-Rad Imager. Afterward, the membrane was stained with SYBR gold (#S11494, 1:5000 dilution, Thermo Fisher) as an internal control, by incubating for 1 hour at RT under light protection. Signals were quantified using Adobe Photoshop CS6.

Tissue embedding and epidermal thickness evaluation

Harvested tumors, adjacent (involved) skin, and non-UV treated (un-involved) stomach skin were formalin fixed, paraffin embedded, sectioned, and mounted on serial slides at the University of Wisconsin Carbone Cancer Center Experimental Pathology Core Laboratory. One slide from each mouse sample was stained with hematoxylin and eosin. Epidermal thicknesses were evaluated by analyzing 5 fields of view from each mouse section using an EVOS XL Core Cell Imaging System (Thermo Fisher Scientific) and measured using ImageJ software (NIH, Rockville, MD). Sections of measurement were obtained if the following criteria were present, 1) the hair follicle was in the correct orientation and 2) a basal layer was present. Sample measurements were averaged together, graphed and subjected to statistical analysis.

Tissue lysate preparation and immunoblot analysis

Liquid nitrogen flash-frozen tumors were ground into powder using a mortar and pestle in liquid nitrogen before being divided for protein and RNA analysis. For protein analysis, the powdered tissue was lysed in 1X RIPA lysis buffer (Millipore, Billerica, MA) with freshly added PMSF (Amresco, Solon, OH) and protease inhibitor cocktail (Thermo Scientific, Waltham, MA). Protein concentration was determined by BCA Protein Assay (Thermo Scientific), and used for immunoblot analysis. Equal amounts of protein from 3 mice from each experimental group were pooled for subsequent studies, with 3 separate groupings made to represent averages of the entire cohort. These pooled protein samples were subjected to SDS-PAGE, transferred to a 0.2 μm nitrocellulose membrane (Bio-Rad, Hercules, CA), and blocked with 5% non-fat dry milk in TBS-T. Membranes were probed with primary antibody (see Supplementary Table S3) and appropriate secondary antibody conjugated with horseradish peroxidase (HRP) (Cell Signaling Technology, Danvers, MA) before chemiluminescence detection using the Kodak ImageStation 4000MM (Carestream Health, Inc, Rochester, NY).

Reverse transcription and quantitative real-time PCR (RT-qPCR) analysis

RNA was isolated from the tumor tissue powder (described above) was subjected to RNA isolation using the RNeasy Plus Mini Kit (Qiagen, Germantown, MD) according to manufacturer's protocol, followed by RNA quantification. Equal amounts of RNA from 3 mice from each experimental group were pooled for subsequent studies, with 3 separate groupings made to represent averages of the entire cohort. RNA was transcribed using random primers and M-MLV reverse transcriptase (Promega, Madison, WI). RT-qPCR was then performed with SYBR Premix Ex Taq II (TaKaRa, Mountain View, CA) and the appropriate primer set (see Supplementary Table S4). Relative target mRNA levels were calculated using the ΔΔCT comparative method using *Gapdh* and *Actb* as endogenous controls.

Oxidative stress PCR array and Ingenuity Pathway Analysis (IPA)

The effect of GP feeding on oxidative stress and antioxidant response genes was assessed using the Qiagen Mouse Oxidative Stress RT2 Profiler™ PCR Array (#PAMM-065Z) as per the manufacturer's instructions. Ct values for the genes were uploaded to the Qiagen GeneGlobe Data Analysis Center and analyzed using *Gapdh* and *Actb* reference genes. The data analysis web portal calculated fold-change using ΔΔCT method (Supplementary Table S1 and S2), and also presented data as heat map (Supplementary Figure S2). In addition, selected genes from the PCR array results were validated using RT-qPCR analysis. Primers pairs detailed in Supplementary Table S4 were retrieved from Primer Bank (Wang *et al.*, 2012). Further, to understand the pathways controlled by GP supplementation, differentially expressed genes from the PCR array were analyzed using Qiagen's IPA software. The predicted gene-gene interaction and functional networks were generated and analyzed using the inputs of gene identifiers and fold-change regulation (≥1.75-fold change in 5% GP with statistical significance as well as with minimum 1.4-fold change in 3% GP).

Immunohistochemistry

For immunostaining, slides were deparaffinized using xylenes and rehydrated via graded ethanol (100-70%). Using heat-induced epitope retrieval, slides were steamed for 45 minutes in IHC-Tek Epitope

Retrieval Solution (#IW-1100, IHC World, LLC, Ellicott City, MD), followed by a 20 minute RT incubation. Slides were immunostained using the Vectastain ABC Kit (#AK-5001, Vector Labs, Burlingame, CA) per manufacturer's protocol, with an overnight incubation at 4°C with primary antibody (see Supplementary Table S3) in a humidifying chamber. After a final wash, slides were exposed to Vector Red Alkaline Phosphatase Substrate Kit (#SK-5100, Vector Labs) until desired staining intensity was observed (approximately 5 minutes) then washed and counterstained with Harris modified hematoxylin (#SH30-500D, Fisher Scientific), dehydrated with graded ethanol (70-100%) and xylenes, then mounted with Permount mounting medium (Fisher Scientific) diluted in xylene (1:2). Images for Ki-67 and NRF2 were obtained at 20x using a Nuance Multispectral Tissue Imaging System (Perkin Elmer, Waltham, MA) and 4-HNE imaging was captured at 20x using an EVOS® XL Core Cell Imaging System (Thermo Fisher Scientific).

Caspase Glo 3/7 Assay

Caspase-Glo 3/7 Assay (Promega) was used according to the manufacturer's protocol. Protein lysates from 5 tumors from each group (control and 5% GP) were prepared by grinding with mortar and pestle on liquid nitrogen, then resuspended in PBS + 1% NP40 with protease (Thermo Fisher) and phosphatase (CalBioChem, Millipore Sigma) inhibitors and sonicated on ice. Protein was quantified using a BCA Assay (Thermo Fisher), normalized to 100 μg/mL, and loaded with an equal volume of Caspase-Glo reagent. Luminescence was read using a Biotek Synergy H1 plate reader (Biotek, Winooski, VT).

Immuno-slot blot: cyclobutane pyrimidine dimers (CPDs)

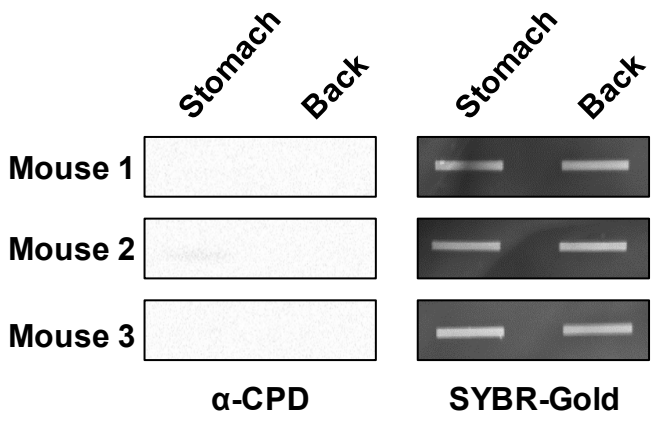
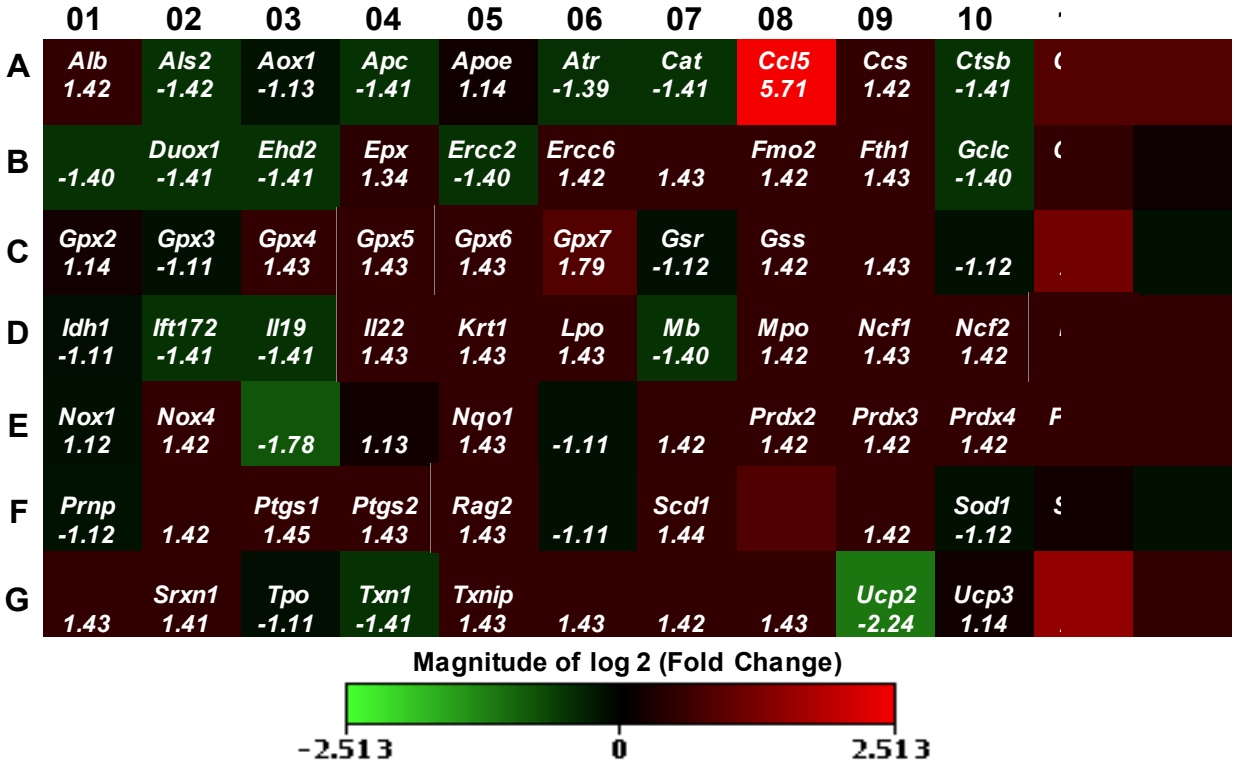


Figure S1. Immuno-slot blot analysis for CPDs in normal skin samples from a separate cohort of untreated mice that had no UV exposure. A separate cohort of 12 female retired breeder mice were aged to 36 weeks while on the control diet. At the termination of the experiment, mice were euthanized and tissues were collected for further analysis of DNA damage. Stomach and back skin from 3 representative animals is displayed above. SYBR gold, which stains total nucleic acid was used as an internal control. No CPDs were detected in normal skin samples.

a. Heat map of control vs. 3% GP



b. Heat map of control vs. 5% GP

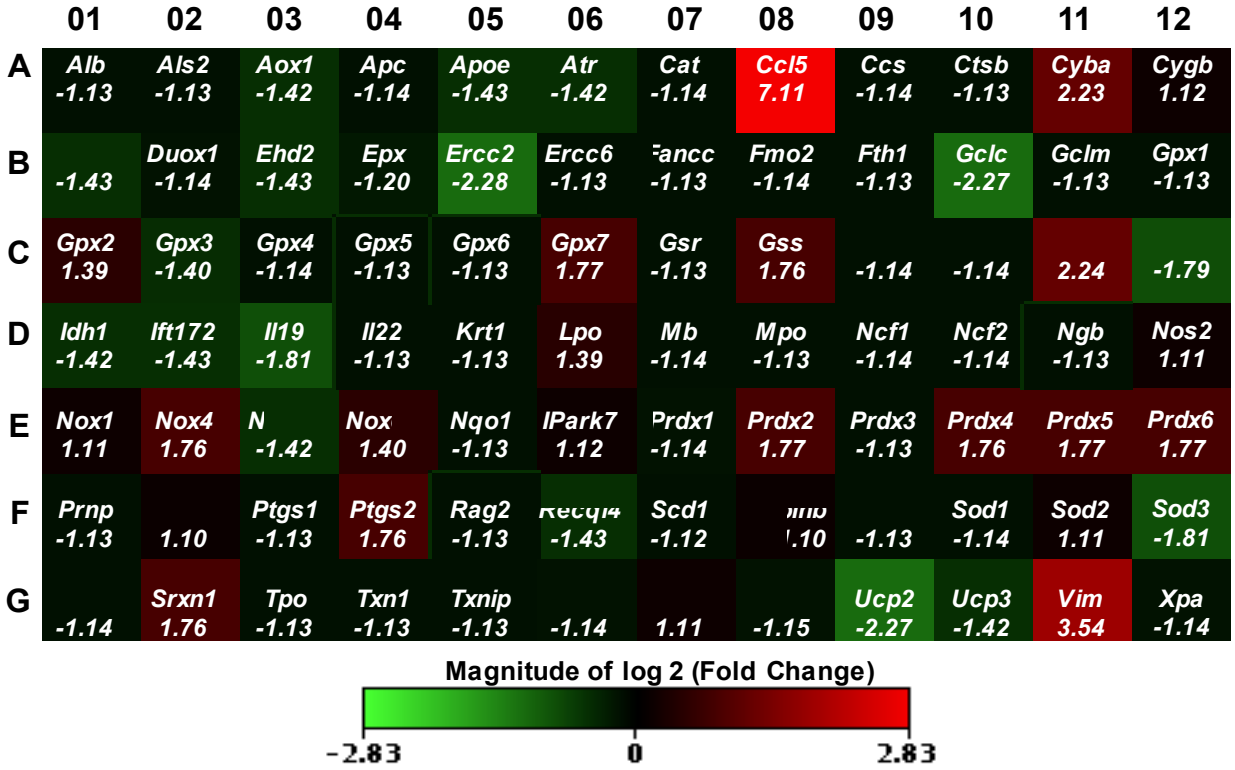


Figure S2. Effects of dietary grape on oxidative stress genes. Heat maps of control vs. 3% and 5% GP were generated to display gene fold changes. Upregulated genes are displayed in red and downregulated genes are shown in green, black boxes indicate no/negligible fold change in those particular genes.

Table S1: List of key altered genes in response to grape powder (GP) treatments. 18 genes were identified with ≥1.75-fold change and statistical significance (P<0.05) in mouse samples treated with 5% GP. The data from 3% GP group has also been shown for comparative analysis. Genes showing ≥1.75-fold change in 5% GP and ≥1.4-fold in 3% GP, resulting total 16 genes were used for subsequent RT-qPCR validation and IPA analysis.

			3% GP		5% GP	
Gene Symbol	**Gene Name**	**Profiler Category**	**Fold Regulation**	**p-value**	**Fold Regulation**	**p-value**
Ccl5	Chemokine (C-C motif) ligand 5	Oxidative Stress Responsive Gene	5.71	0.014645	7.11	0.000002
Cyba	Cytochrome b-245, alpha polypeptide	Superoxide Metabolism Gene	1.8	0.091247	2.23	0.0004
Ercc2	Excision repair cross-complementation group 2	Oxidative Stress Responsive Gene	-1.4	0.24914	-2.28	0.010589
Gclc	Glutamate-cysteine ligase, catalytic subunit	Oxidative Stress Responsive Gene	-1.4	0.247225	-2.27	0.009965
Gpx7	Glutathione peroxidase 7	Oxidative Stress Responsive Gene	1.79	0.003654	1.77	0.004132
Gss	Glutathione synthetase	Oxidative Stress Responsive Gene	1.42	0.215394	1.76	0.004271
Hmox1	Heme oxygenase (decycling) 1	Oxidative Stress Responsive Gene	2.27	0.000352	2.24	0.000424
Hspa1a	Heat shock protein 1A	Oxidative Stress Responsive Gene	-1.11	0.773925	-1.79	0.014468
Nox4	NADPH oxidase 4	Superoxide Metabolism Gene	1.42	0.221724	1.76	0.004844
Prdx2	Peroxiredoxin 2	Oxidative Stress Responsive Gene/ Peroxiredoxin	1.42	0.213154	1.77	0.004229
Prdx4	Peroxiredoxin 4	Peroxiredoxin (TPx)	1.42	0.215395	1.76	0.004488
Prdx5	Peroxiredoxin 5	Peroxiredoxin (TPx)	1.42	0.213666	1.77	0.004204
Prdx6	Peroxiredoxin 6	Peroxiredoxin (TPx)	1.43	0.210228	1.77	0.003882
Ptgs2	Prostaglandin-endoperoxide synthase 2	Peroxidase	1.43	0.210952	1.76	0.003908
Sod3	Superoxide dismutase 3, extracellular	Antioxidant	-1.12	0.7442	-1.81	0.010659
Srxn1	Sulfiredoxin 1 homolog	Antioxidant	1.41	0.225001	1.76	0.005224
Ucp2	Uncoupling protein 2	Superoxide Metabolism Gene	-2.24	0.011074	-2.27	0.01068
Vim	Vimentin	Oxygen Transporter	2.85	0.032417	3.54	0.000045

Table S2: Effect of grape powder treatments on 84 oxiodative stress PCR array genes.

Gene	Gene Description	Profiler Category	3% GP		5% GP	
			Fold Change	p-value	Fold Change	p-value
Alb	Albumin	Antioxidant	1.42	0.218858	-1.13	0.364246
Als2	Amyotrophic lateral sclerosis 2 (juvenile) homolog (human)	Oxidative Stress Responsive Gene	-1.42	0.236393	-1.13	0.360353
Aox1	Aldehyde oxidase 1	Involved in Reactive Oxygen Species (ROS) Metabolism	-1.13	0.751417	-1.42	0.592941
Apc	Adenomatosis polyposis coli	Antioxidant- Peroxidase	-1.41	0.251594	-1.14	0.363309
Apoe	Apolipoprotein E	Oxidative Stress Responsive Gene	1.14	0.654409	-1.43	0.172057
Atr	Ataxia telangiectasia and rad3 related	Oxygen Tansporter	-1.39	0.386002	-1.42	0.366689
Cat	Catalase	Antioxidant- Peroxidase, Oxidative Stress Responsive Gene	-1.41	0.240764	-1.14	0.339531
Ccl5	Chemokine (C-C motif) ligand 5	Oxidative Stress Responsive Gene	5.71	0.014645	7.11	0.000002
Ccs	Copper chaperone for superoxide dismutase	Involved in Superoxide Metabolism	1.42	0.215228	-1.14	0.345722
Ctsb	Cathepsin B	Antioxidant- Peroxidase, Oxidative Stress Responsive Gene	-1.41	0.240381	-1.13	0.354946
Cyba	Cytochrome b-245, alpha polypeptide	Involved in Superoxide Metabolism	1.8	0.091247	2.23	0.0004
Cygb	Cytoglobin	Oxygen Tansporter	1.8	0.08987	1.12	0.405717
Dnm2	Dynamin 2	Oxygen Tansporter	-1.4	0.242862	-1.43	0.220371
Duox1	Dual oxidase 1	Antioxidant- Peroxidase, Oxidative Stress Responsive Gene	-1.41	0.243269	-1.14	0.350213
Ehd2	EH-domain containing 2	Antioxidant- Peroxiredoxin	-1.41	0.203231	-1.43	0.190148
Epx	Eosinophil peroxidase	Antioxidant- Peroxidase, Oxidative Stress Responsive Gene	1.34	0.273282	-1.2	0.230678
Ercc2	Excision repair cross-complementing rodent repair deficiency, complementation group 2	Oxidative Stress Responsive Gene	-1.4	0.24914	-2.28	0.010589
Ercc6	Excision repair cross-complementing rodent repair deficiency, complementation group 6	Oxidative Stress Responsive Gene	1.42	0.213211	-1.13	0.360697
Fancc	Fanconi anemia, complementation group C	Oxygen Tansporter	1.43	0.21034	-1.13	0.355737
Fmo2	Flavin containing monooxygenase 2	Involved in ROS Metabolism	1.42	0.212799	-1.14	0.345907
Fth1	Ferritin heavy chain 1	Oxidative Stress Responsive Gene	1.43	0.210781	-1.13	0.353059
Gclc	Glutamate-cysteine ligase, catalytic subunit	Oxidative Stress Responsive Gene	-1.4	0.247225	-2.27	0.009965
Gclm	Glutamate-cysteine ligase, modifier subunit	Oxidative Stress Responsive Gene	1.43	0.205741	-1.13	0.345013
Gpx1	Glutathione peroxidase 1	Antioxidant- Glutathione Peroxidase, Oxidative Stress Responsive Gene	1.11	0.634835	-1.13	0.336807
Gpx2	Glutathione peroxidase 2	Antioxidant- Glutathione Peroxidase, Oxidative Stress Responsive Gene	1.14	0.88457	1.39	0.469283
Gpx3	Glutathione peroxidase 3	Antioxidant- Glutathione Peroxidase, Oxidative Stress Responsive Gene	-1.11	0.380624	-1.4	0.225606

Table S2 continued…

Gpx4	Glutathione peroxidase 4	Antioxidant- Glutathione Peroxidase, Oxidative Stress Responsive Gene	1.43	0.212575	-1.14	0.352836
Gpx5	Glutathione peroxidase 5	Antioxidant- Glutathione Peroxidase, Oxidative Stress Responsive Gene	1.43	0.213375	-1.13	0.356473
Gpx6	Glutathione peroxidase 6	Antioxidant- Glutathione Peroxidase, Oxidative Stress Responsive Gene	1.43	0.213375	-1.13	0.356473
Gpx7	Glutathione peroxidase 7	Antioxidant- Glutathione Peroxidase, Oxidative Stress Responsive Gene	1.79	0.003654	1.77	0.004132
Gsr	Glutathione reductase	Oxidative Stress Responsive Gene	-1.12	0.397303	-1.13	0.360304
Gss	Glutathione synthetase	Oxidative Stress Responsive Gene	1.42	0.215394	1.76	0.004271
Gstk1	Glutathione S-transferase kappa 1	Antioxidant- Glutathione Peroxidase	1.43	0.212967	-1.14	0.354319
Gstp1	Glutathione S-transferase, pi 1	Antioxidant- Glutathione Peroxidase	-1.12	0.389275	-1.14	0.349131
Hmox1	Heme oxygenase (decycling) 1	Oxidative Stress Responsive Gene	2.27	0.000352	2.24	0.000424
Hspa1a	Heat shock protein 1A	Oxidative Stress Responsive Gene	-1.11	0.773925	-1.79	0.014468
Idh1	Isocitrate dehydrogenase 1 (NADP+), soluble	Oxidative Stress Responsive Gene	-1.11	0.807066	-1.42	0.18914
Ift172	Intraflagellar transport 172 homolog (Chlamydomonas)	Oxygen Tansporter	-1.41	0.2433	-1.43	0.21774
Il19	Interleukin 19	Involved in ROS Metabolism	-1.41	0.248498	-1.81	0.083693
Il22	Interleukin 22	Involved in ROS Metabolism	1.43	0.213375	-1.13	0.356473
Krt1	Keratin 1	Oxidative Stress Responsive Gene	1.43	0.211246	-1.13	0.358458
Lpo	Lactoperoxidase	Antioxidant- Peroxidase	1.43	0.210268	1.39	0.231326
Mb	Myoglobin	Oxygen Tansporter	-1.4	0.259115	-1.14	0.359846
Mpo	Myeloperoxidase	Antioxidant- Peroxidase, Oxidative Stress Responsive Gene	1.42	0.215271	-1.13	0.356085
Ncf1	Neutrophil cytosolic factor 1	Involved in Superoxide Metabolism	1.43	0.212503	-1.14	0.350568
Ncf2	Neutrophil cytosolic factor 2	Involved in Superoxide Metabolism	1.42	0.216312	-1.14	0.351757
Ngb	Neuroglobin	Oxygen Tansporter	1.43	0.213375	-1.13	0.356473
Nos2	Nitric oxide synthase 2, inducible	Involved in Superoxide Metabolism	1.43	0.275414	1.11	0.406513
Nox1	NADPH oxidase 1	Involved in Superoxide Metabolism	-1.12	0.944014	1.11	0.633415
Nox4	NADPH oxidase 4	Involved in Superoxide Metabolism	1.42	0.221724	1.76	0.004844
Noxa1	NADPH oxidase activator 1	Involved in Superoxide Metabolism	-1.78	0.132915	-1.42	0.177651
Noxo1	NADPH oxidase organizer 1	Involved in Superoxide Metabolism	1.13	0.67067	1.4	0.159773
Nqo1	NAD(P)H dehydrogenase, quinone 1	Oxidative Stress Responsive Gene	1.43	0.213773	-1.13	0.372423
Park7	Parkinson disease (autosomal recessive, early onset) 7	Oxidative Stress Responsive Gene	-1.11	0.783525	1.12	0.399985
Prdx1	Peroxiredoxin 1	Antioxidant- Peroxiredoxin, Oxidative Stress Responsive Gene	1.42	0.220131	-1.14	0.33925
Prdx2	Peroxiredoxin 2	Antioxidant- Peroxiredoxin, Oxidative Stress Responsive Gene	1.42	0.213154	1.77	0.004229
Prdx3	Peroxiredoxin 3	Antioxidant- Peroxiredoxin	1.42	0.213458	-1.13	0.354926
Prdx4	Peroxiredoxin 4	Antioxidant- Peroxiredoxin	1.42	0.215395	1.76	0.004488

Table S2 continued…

Prdx5	Peroxiredoxin 5	Antioxidant- Peroxiredoxin	1.42	0.213666	1.77	0.004204
Prdx6	Peroxiredoxin 6	Antioxidant- Peroxiredoxin, Oxidative Stress Responsive Gene	1.43	0.210228	1.77	0.003882
Prnp	Prion protein	Oxidative Stress Responsive Gene	-1.12	0.39609	-1.13	0.370135
Psmb5	Proteasome (prosome, macropain) subunit, beta type 5	Oxidative Stress Responsive Gene	1.42	0.219841	1.1	0.663179
Ptgs1	Prostaglandin-endoperoxide synthase 1	Antioxidant- Peroxidase	1.45	0.19953	-1.13	0.363977
Ptgs2	Prostaglandin-endoperoxide synthase 2	Antioxidant- Peroxidase	1.43	0.210952	1.76	0.003908
Rag2	Recombination activating gene 2	Antioxidant- Peroxidase	1.43	0.213375	-1.13	0.356473
Recql4	RecQ protein-like 4	Involved in Superoxide Metabolism	-1.11	0.771828	-1.43	0.250213
Scd1	Stearoyl-Coenzyme A desaturase 1	Involved in Superoxide Metabolism	1.44	0.203015	-1.12	0.378903
Serpinb 1b	Serine (or cysteine) peptidase inhibitor, clade B, member 1b	Antioxidant- Peroxidase	1.78	0.087398	1.1	0.433564
Slc38a1	Solute carrier family 38, member 1	Oxygen Tansporter	1.42	0.213153	-1.13	0.353599
Sod1	Superoxide dismutase 1, soluble	Involved in ROS Metabolism, Oxidative Stress Responsive Gene	-1.12	0.390945	-1.14	0.348373
Sod2	Superoxide dismutase 2, mitochondrial	Involved in ROS Metabolism	1.13	0.335295	1.11	0.402898
Sod3	Superoxide dismutase 3, extracellular	Involved in ROS Metabolism	-1.12	0.7442	-1.81	0.010659
Sqstm1	Sequestosome 1	Oxidative Stress Responsive Gene	1.43	0.210373	-1.14	0.337401
Srxn1	Sulfiredoxin 1 homolog (S. cerevisiae)	Antioxidant	1.41	0.225001	1.76	0.005224
Tpo	Thyroid peroxidase	Antioxidant- Peroxidase, Oxidative Stress Responsive Gene	-1.11	0.797561	-1.13	0.722938
Txn1	Thioredoxin 1	Oxidative Stress Responsive Gene	-1.41	0.242038	-1.13	0.333495
Txnip	Thioredoxin interacting protein	Oxidative Stress Responsive Gene	1.43	0.211889	-1.13	0.353944
Txnrd1	Thioredoxin reductase 1	Antioxidant, Oxidative Stress Responsive Gene	1.43	0.2146	-1.14	0.34279
Txnrd2	Thioredoxin reductase 2	Antioxidant, Oxidative Stress Responsive Gene	1.42	0.211071	1.11	0.646497
Txnrd3	Thioredoxin reductase 3	Antioxidant	1.43	0.213614	-1.15	0.338762
Ucp2	Uncoupling protein 2 (mitochondrial, proton carrier)	Involved in Superoxide Metabolism	-2.24	0.011074	-2.27	0.01068
Ucp3	Uncoupling protein 3 (mitochondrial, proton carrier)	Oxidative Stress Responsive Gene	1.14	0.657215	-1.42	0.179396
Vim	Vimentin	Oxygen Tansporter	2.85	0.032417	3.54	0.000045
Xpa	Xeroderma pigmentosum, complementation group A	Oxidative Stress Responsive Gene	1.42	0.216064	-1.14	0.349442

Table S3: Antibodies used for western blotting (WB), immunohistochemistry (IHC), and/or slot blot (SB).

Gene Name	Supplier	Catalog Number	WB Dilution	IHC Dilution	SB Dilution	Molecular Weight (kDa)
PARP	Cell Signaling	9542	1:1000			116, 89
Caspase-7	Cell Signaling	12827	1:1000			35, 20
NRF2	Cell Signaling	12721	1:1000			97-100
B-Actin	Cell Signaling	4970, 3700	1:1000			45
PCNA	Santa Cruz	Sc-56	1:500			36
BCL-2	Santa Cruz	Sc-7382	1:500			26
4- Hydroxynonenal	Abcam	Ab46545	1:2000	1:50		n/a
Ki-67	Cell Signaling	12202		1:400		n/a
NRF2	Invitrogen	PA5-27882		1:100		n/a
CPD	Cosmo Bio	NM-DND-001			1:5000	n/a
(6-4) PP	Cosmo Bio	NM-DND-002			1:3000	n/a

Table S4: Primer sequences used for RT-qPCR validation.

Gene	Amplicon size (bp)	Orientation	Sequence (5'-3')	Length (bp)	Tm (°C)	Primer Bank ID
Ccl5	104	F	GCTGCTTTGCCTACCTCTCC	20	62.6	7305461a1
		R	TCGAGTGACAAACACGACTGC	21	62.8	
Cyba	127	F	TCACCAGGAATTACTACGTCCG	22	61.2	22094077a1
		R	GCTGCCAGCAGATAGATCACA	21	61.9	
Ercc2	152	F	ACCCGGAGCAGTTCTCCTAC	20	62.8	31542614a1
		R	GGTCACCTCCAGCGGATAAG	20	61.7	
Gclc	125	F	GGGGTGACGAGGTGGAGTA	19	62.3	33468897a1
		R	GTTGGGGTTTGTCCTCTCCC	20	62.1	
Gpx7	238	F	TCCGAGCAGGACTTCTACGAC	21	63	13195626a1
		R	TCTCCCTGTTGGTGTCTGGTT	21	62.8	
Gss	103	F	CAAAGCAGGCCATAGACAGGG	21	62.8	6680117a1
		R	AAAAGCGTGAATGGGGCATAC	21	61.3	
Hmox1	100	F	AAGCCGAGAATGCTGAGTTCA	21	61.7	6754212a1
		R	GCCGTGTAGATATGGTACAAGGA	23	61.2	
Nox4	145	F	GAAGGGGTTAAACACCTCTGC	21	60.6	7657389a1
		R	ATGCTCTGCTTAAACACAATCCT	23	60.2	
Prdx2	139	F	CACCTGGCGTGGATCAATACC	21	62.8	166235200c2
		R	GACCCCTGTAAGCAATGCCC	20	62.9	
Prdx4	101	F	CTCAAACTGACTGACTATCGTGG	23	60.4	7948999a1
		R	CGATCCCCAAAAGCGATGATTTC	23	62.1	
Prdx5	154	F	GGCTGTTCTAAGACCCACCTG	21	62.1	6755114a1
		R	GGAGCCGAACCTTGCCTTC	19	63	
Prdx6	115	F	CGCCAGAGTTTGCCAAGAG	19	61	6671549a1
		R	TCCGTGGGTGTTTCACCATTG	21	62.8	
Ptgs2	74	F	TGAGCAACTATTCCAAACCAGC	22	60.8	31981525a1
		R	GCACGTAGTCTTCGATCACTATC	23	60.3	
Srxn1	113	F	ATCGTGGTGCTGGATTGATTC	21	60.4	467583a1
		R	CACCCCAGAGATAAGATTACCCA	23	60.6	
Ucp2	109	F	ATGGTTGGTTTCAAGGCCACA	21	62.5	31543920a1
		R	CGGTATCCAGAGGGAAAGTGAT	22	61	
Vim	124	F	CGGCTGCGAGAGAAATTGC	19	61.8	31982755a1
		R	CCACTTTCCGTTCAAGGTCAAG	22	61.3	
Ki-67	104	F	ATCATTGACCGCTCCTTTAGGT	22	61.2	1177528a1
		R	GCTCGCCTTGATGGTTCCT	19	62	
Pcna	135	F	TTTGAGGCACGCCTGATCC	19	62.3	7242171a1
		R	GGAGACGTGAGACGAGTCCAT	21	63	
Gapdh	95	F	AGGTCGGTGTGAACGGATTTG	21	62.6	126012538c1
		R	GGGGTCGTTGATGGCAACA	19	62.6	
Actb	154	F	GGCTGTATTCCCCTCCATCG	20	61.8	6671509a1
		R	CCAGTTGGTAACAATGCCATGT	22	61.1	