SUPPLEMENTARY INFORMATION

Oligo	Sequence (5' to 3')
QRshRNA top strand	gatccccGGCCATCTGAGCCCAGATAttcaagagaTATCTGGGCTCAGATGGCCtttttggaaa
QRshRNA bottom strand	agcttttccaaaaa GGCCATCTGAGCCCAGATA tctcttgaTATCTGGGCTCAGATGGCCggg
hPMC2shRNA top strand	gatcccc AGGACAAATGGTGATATTGttcaagagaCAATATCACCATTTGTCCTtttttggaaa
hPMC2shRNA bottom strand	agcttttccaaaaaAGGACAAATGGTGATATTGtctcttgaaCAATATCACCATTTGTCCTggg
hPMC2 569miR top strand	tgctgCAAACCAGATGTCTTCCTCGGgttttggccactgactgacCCGAGGAACATCTGGTTTG
hPMC2 569miR bottom strand	cctgCAAACCAGATGTTCCTCGGgtcagtcagtggccaaaaCCCGAGGAAGACATCTGGTTTGc

Table 1. Sequence of DNA oligos cloned into pSuper-QRshRNA, pSuper-hPMC2shRNA and PCDNA-hPMC2 569miR plasmids respectively.

Primer	Sequence (5' to 3')	Application
GAPDH RT for	TCTGGTAAAGTGGATATTGTTG	RT-PCR
GAPDH RT rev	GATGGTGATGGGATTTCC	RT-PCR
NQO1 RT for	AACTATGCCATGAAGGAGGCTG	RT-PCR
NQO1RT rev	GCTATATGTCAGTTGAGGTTC	RT-PCR
GSTP-1 RT for	TACCATCCTGCGTCACCT	RT-PCR
GSTP-1 RT rev	CGTCATTCACCATGTCCA	RT-PCR
GCSH RT for	CACCATCATCAATGGGAAG	RT-PCR
GCSH RT rev	GCGATAAACTCCCTCATCC	RT-PCR
NQO1ups f	GCCTGTAATCCCAGCACTTC	ChIP
NQO1 ups r	GGGAGGTGAGGATGACACAG	ChIP
NQO1 EpRE f	CATGCACCCAGGGAAGTGTGTTGT	ChIP
NQO1 EpRE r	GCACGAAATGGAGCAGAAAAAGAG	ChIP
NQO1 pro f	GTAGGCTGTCCACCTCCAAC	ChIP
NQO1 pro r	CACCAGTGCTCGAGAGAC	ChIP
pS2 ERE f	GGCCATCTCTCACTATGAATCACTTCTGC	ChIP
pS2 ERE r	GGCAGGCTCTGTTTGCTTAAAGAGCG	ChIP

Table 2. Primer sequences used in the various PCR reactions described

Antibody	Source	Application
Anti-Acetyl Histone H3	Upstate	ChIP
Anti-FLAG M2	Sigma	WB
Anti-GST-pi (MSA-102)	Stressgen	WB
ER alpha (H-184)	Santa Cruz Biotechnology	WB
ER alpha (HC-20)	Santa Cruz Biotechnology	ChIP
ER beta (H-150)	Santa Cruz Biotechnology	WB, ChIP, IP
GCS Ab-1	Neomarkers	WB
hPMC2	Custom antigen purified (12)	WB, ChIP, IP
Nrf2 (C-20)X	Santa Cruz Biotechnology	ChIP
Normal rabbit IgG	Santa Cruz Biotechnology	ChIP, IP
PARP-1 (H-300)	Santa Cruz Biotechnology	WB, ChIP
QR	Custom antigen purified (17)	WB
SRC-1 (M-341)	Santa Cruz Biotechnology	ChIP
Topo IIbeta (H-286)	Santa Cruz Biotechnology	ChIP

Table 3. Antibodies and their sources

SUPPLEMENTARY METHODS

RT-PCR assays

Cells were washed with PBS and total mRNA extracted using Trizol® reagent from Invitrogen (Carlsbad, CA) as per the manufacturer's protocol. Three micrograms of mRNA was reverse transcribed using the M-MLV Reverse Transcriptase kit (Invitrogen) following the recommended protocol. One micro liter of the cDNA was PCR-amplified for varying cycle numbers using primers listed in table 2. The amplified products were run on a 2% agarose gel and visualized by ethidium bromide staining. Fluorescence was captured by an eight-bit digital camera, and signal intensities were quantitated using GeneTools software from Syngene (Frederick, MD). Signals in each case were normalized to their respective GAPDH values to calculate the relative expression levels.

Western blotting and quantitation

Whole cell lysates were prepared using mammalian protein extraction reagent from Pierce. Fifty micro grams of the total protein extract was separated on a 12% SDS-polyacrylamide gel and electrophoretically transferred onto nitrocellulose membrane (Pall Corporation, Pensacola, FL). Membranes were blocked with 5% BSA and probed with the indicated primary antibodies overnight. The membranes were probed with HRP-conjugated anti-rabbit IgG or anti-mouse IgG secondary antibodies (1:5000) for detection with Super Femto reagents (Pierce Chemicals). Signals were visualized by exposure to X-ray films and the chemiluminescence was quantified using Syngene software as before. The signal intensities in each case were normalized to their respective GAPDH loading controls. Fold change was calculated as the ratio of

normalized protein expression in TOT-treated samples to that of the ethanol-treated samples.

Endogenous Immunoprecipitation

Cells were lysed with the IP buffer (50 mM Tris–HCl pH 8, 150 mM NaCl, 0.5% NP-40 and mM EDTA) and sonicated using a Branson 450 sonicator with a 3-mm tapered micro tip at power setting 2 and 70% duty for 3 cycles of 15 seconds each. The supernatant was precleared with protein A sepharose beads from Pierce (Rockford, IL) for 1 h. The precleared lysates were split into three fractions of equal volume and each fraction was incubated for 3 hours at 4^0 C with 5 μg of the indicated antibodies preadsorbed to protein A beads. Precleared lysate was used as the input. The beads were washed 4 times with the IP buffer and resuspended in 50 μl of SDS loading buffer (Biorad) for western blot analysis.

8-OHdG immunostaining and quantitation

Cells grown on coverslips were immunostained for 8-OHdG as described previously (Bianco NR *et al.*, 2003). Briefly, methacarn fixed cells were treated with 3% H_2O_2 to remove peroxidase activity. Cells were blocked with normal goat serum and permeabilized with proteinase K treatment. Cells were immunostained with anti-8-oxo-dG monoclonal antibody 1F7 (1:100; Trevigen, Gaithersburg, MD). Immunostaining was developed by the peroxidase-antiperoxidase procedure and staining intensity (OD) measured as before. The OD of randomly selected fields of cells was measured and the background OD was subtracted from each. Each experiment was performed four times,

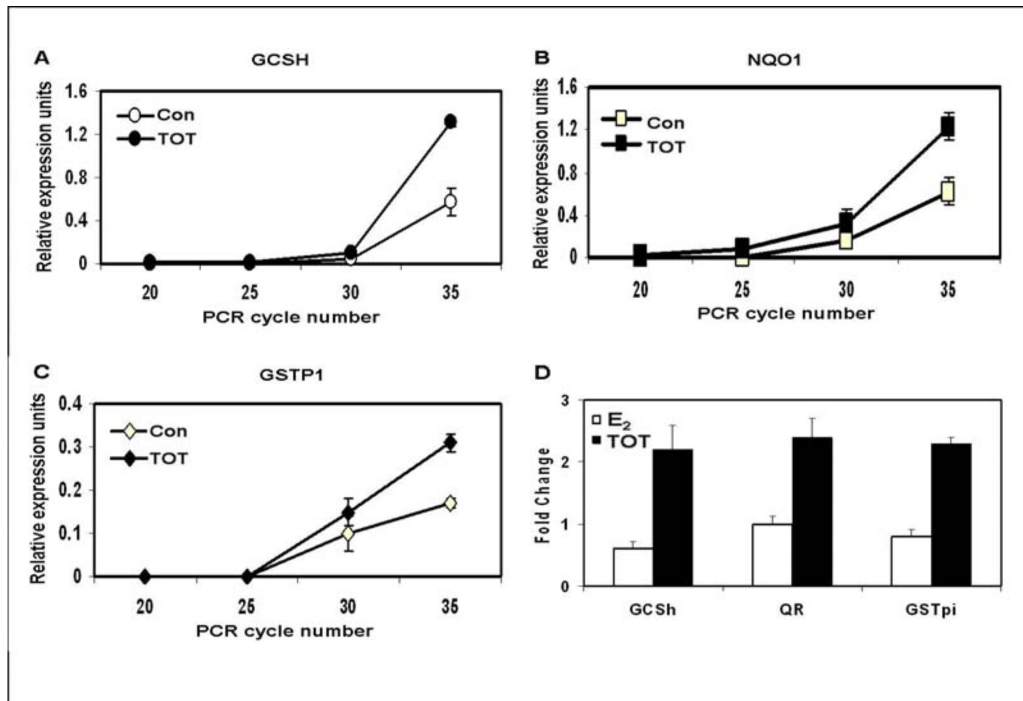
and results were measured under the same optical and light conditions. An electronic shading correction was used to compensate for any unevenness that might be present in the illumination. Statistical analysis was performed using two tailed Student's *t* test.

Chromatin Immunoprecipitation (ChIP)

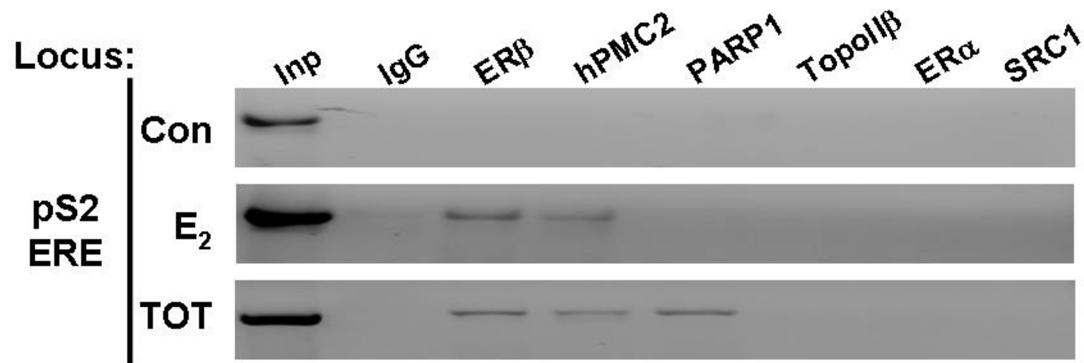
Cells were grown in 100-mm dishes and processed for ChIP analyses as previously described (Sripathy *et al.*, 2006). Briefly, cells were fixed with 1% formaldehyde and lysed in SDS-lysis buffer with protease inhibitors. Lysed cells were sonicated using a Branson 450 sonicator with a 3-mm tapered microtip at power setting 3 and 70% duty for 10 pulses/cycle and nine cycles (~5-W output for 8 to 10 seconds). Clarified, sonicated chromatin was diluted 10-fold in chromatin immunoprecipitation (ChIP) dilution buffer. One milliliter of the diluted chromatin was used for overnight immunoprecipitation with a given antibody. The antibody-chromatin complexes were pulled down using protein A beads. The beads were subjected to a series of washes as described and the antigen-DNA complexes eluted. The eluates were reverse cross linked overnight at 65^0 C and the DNA was purified by phenol: chloroform extraction. Ethanol precipitated pellets were resuspended in 50 μl of water and 2 to 4 μl of the suspension was used as template for PCR analysis. PCR-amplified products were run on a 2% agarose gel and visualized by ethidium bromide staining. The fluorescence was captured by an eight-bit digital camera and the images optimized using Adobe Photoshop. Fluorescence was captured and quantified as before and the IP signals were normalized to their respective inputs. Relative recruitment levels of TOT-treated

samples were calculated as the ratio of normalized IP signals from TOT-treated cells to that of the vehicle-treated cells.

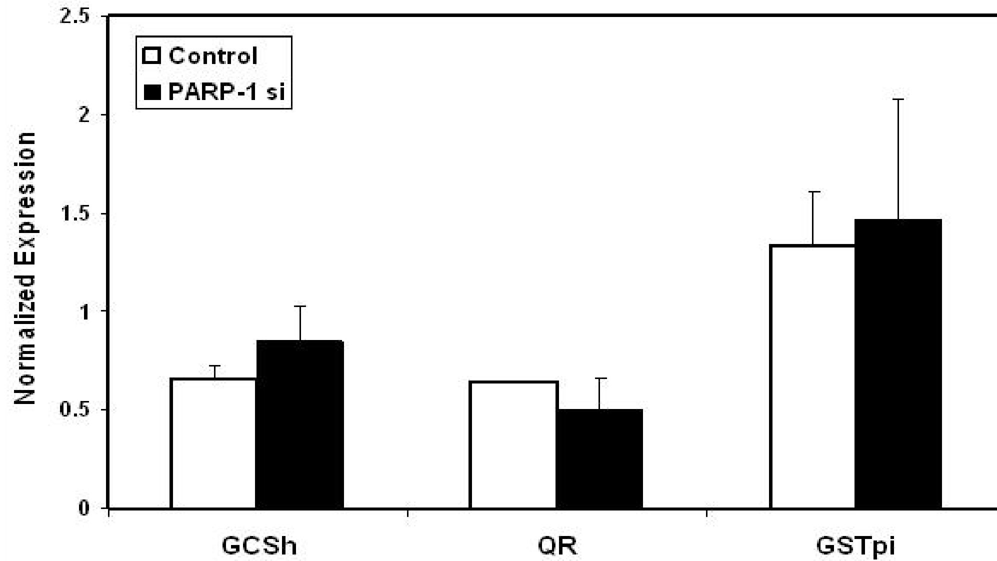
SUPPLEMENTARY DATA



Supplementary Figure 1. Tamoxifen treatment induces increased transcription of antioxidative enzyme levels. MCF7 cells were treated with either 0.01% ethanol (con) or TOT for 3 hours. (A, B and C) The change in mRNA levels of GCSH, NQO1 and GSTP-1 were determined using semi quantitative RT-PCR analysis. The quantified PCR signals in each case were normalized to their respective GAPDH controls. Error bars indicate standard error from 2 independent experiments. (D) Cell lysates from MCF7 cells treated with 10 nM E_2 or TOT for 3 hours, were subjected to Western blot analysis using the indicated antibodies. The quantified signals were normalized to their respective GAPDH controls and used to calculate the fold change in expression as described. Error bars indicate standard error of 3 independent experiments.



Supplementary figure 2. Ligand dependent recruitment of ERβ and hPMC2 to the ERE region. MCF10A FL-ERβ cells were treated with either 0.01% ethanol (Con), 10 nM E_2, or TOT for 3 hours, and were processed for ChIP analysis. Immunoprecipitated DNA was analyzed by PCR to determine the recruitment of the indicated proteins at the ERE region of the *pS2* gene.



Supplementary figure 3. PARP-1 down regulation does not significantly affect basal expression levels of antioxidative enzymes. MCF10A FL-ERβ (control and PARP-1 siRNA transfected) cells were treated with 0.01% ethanol for 3 hours after 24 hours post transfection. Whole cell lysates were analyzed for expression levels of QR, GCSh and GSTpi by western blotting. The data represent average of four independent experiments and error bars represent standard deviation.