Supplemental Figure 1.

Acetylation of histone H3 in Jurkat cells following t-BHQ treatment.

5 x10^6 Jurkat cells were treated with 50 uM t-BHQ for 1, 6 or 24 hrs. 50 ug of whole cell lysates were subjected to SDS-PAGE and Western blotting with anti-ferritin H, anti-acetyl histone H3 lysine 9 (H3K9), anti-acetyl histone H3 lysine 18 (H3K18), or anti-acetyl histone H3 antibody that detects acetyl H3K9 and H3K14. Jurkat cells were also treated with 5 mM sodium butyrate (NaBu) for 24 hrs as a positive control for histone acetylations. Western blotting for β-actin was shown for loading control of each cell lysate.

Supplemental Figure 2.

t-BHQ-mediated Nrf2 nuclear accumulation and the effect of the PI3K inhibitor or PTEN induction.

A) 5x10^6 Jurkat cells were pretreated with 0.1% DMSO or 50 uM LY294002 for 1 hr, followed by treatment with 0, 10 or 50 uM t-BHQ for 6 hrs. Nuclear and cytoplasmic fractions were prepared and 30 ug of each fraction was subjected to Western blot using anti-Nrf2, anti-phospho-Ser473 AKT, anti-AKT, anti-Lamin B (as a nuclear fraction marker) or anti-LDH (as a cytoplasmic fraction marker) antibody. K562 cell lysates transiently transfected with pCMVNrf2 was loaded as a positive control of Nrf2 Western blotting. Representative results from five independent similar experiments are shown.

B) 5x10^6 tet-inducible PTEN Jurkat (PIJ17) cells were pretreated with or without 1 ug/ml Doxycycline for 24 hrs, followed by treatment with 2 or 10 uM t-BHQ for 6 hrs. Nuclear and cytoplasmic fractions were isolated and 30 ug of each fraction was subjected to

Western blot with anti-Nrf2 antibody. Lamin B and LDH western blots are for nuclear and cytoplasmic fraction markers, respectively. Representative results from six independent experiments are shown.

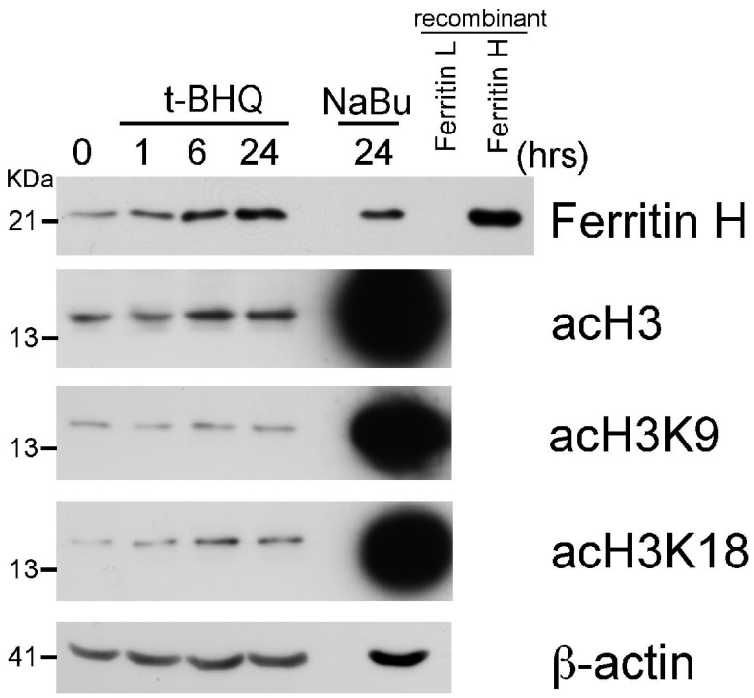
Supplemental Figure 3.

Effect of N-acetyl cysteine (NAC) pretreatment on t-BHQ-mediated ferritin H and NQO1 expression.

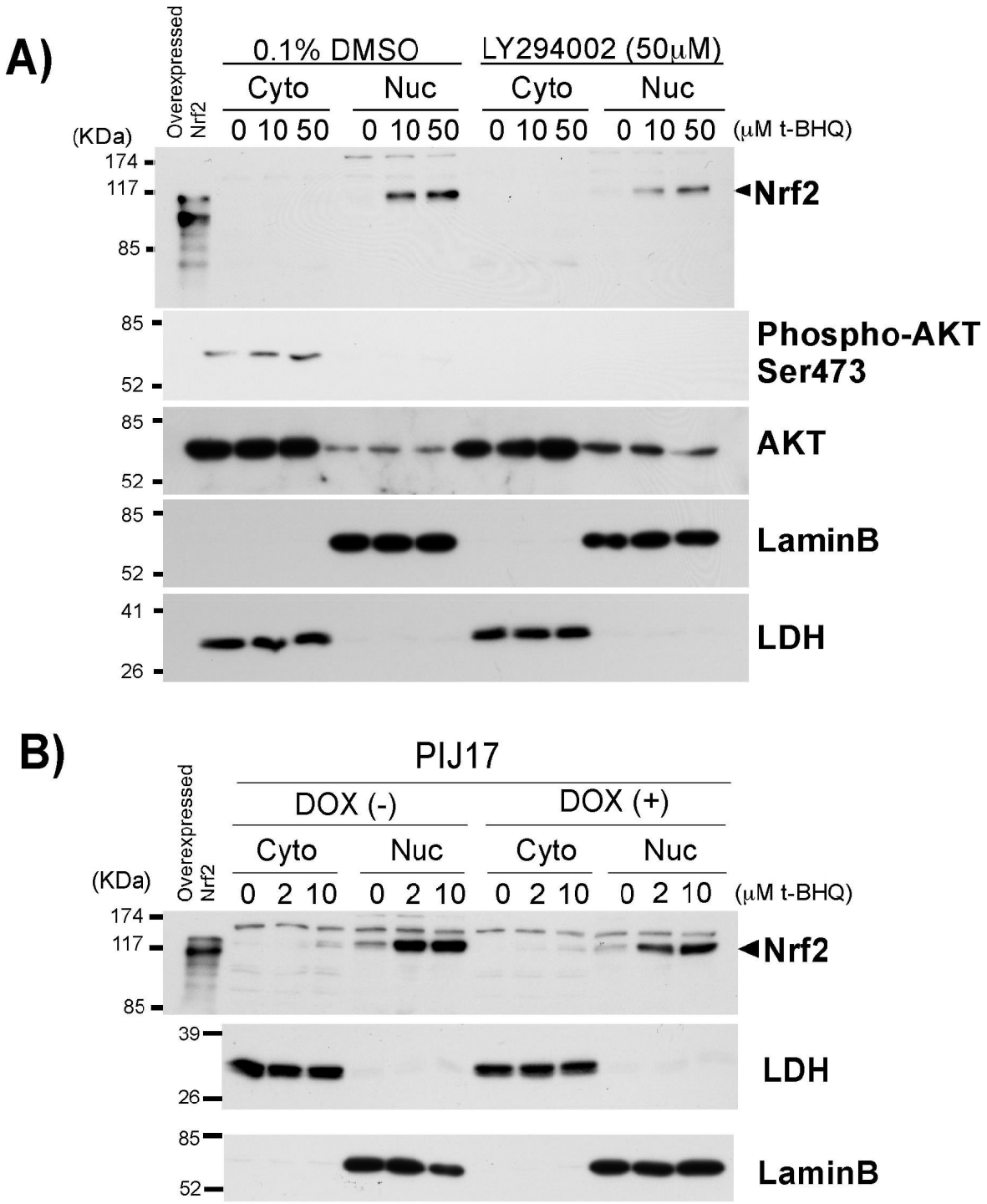
Jurkat cells were pretreated with none, Tris-HCl (pH7.4, final 4 mM), or 2 mM N-acetyl cysteine (NAC, in final 4 mM Tris-HCl, pH 7.4) for 2 hrs and then treated with 10 or 30 uM t-BHQ for 24 hrs in the continuous presence of 2 mM NAC. Total RNA was isolated and subjected to Northern blotting for ferritin H and NQO1 mRNA expression.

Supplemental Table 1.

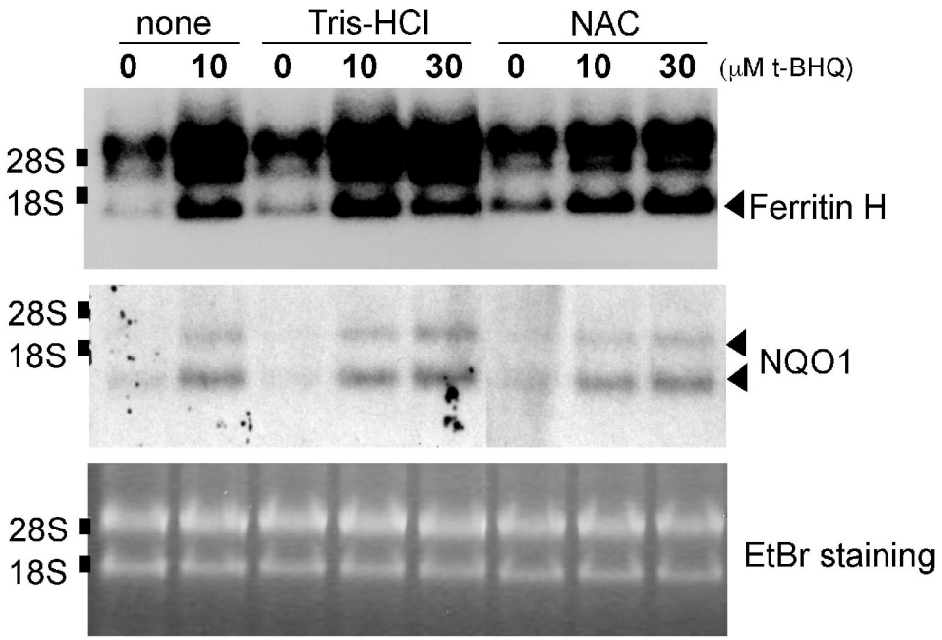
Antibodies, primers and siRNAs used in this study.



Supplemental Fig. 1



Supplemental Fig. 2



Supplemental Fig. 3

Antibodies	*Vender, Catalog number*	*applications*
Anti-HA	Covance, MMS-101R	Western
Anti-ferritin H	Santa Cruz Biotechnology sc-25617	Western
Anti-AKT	Cell Signaling, 9272	Western
Anti-phosphoAKT Ser473	Cell Signaling, 9271	Western
Anti-PTEN	Cell Signaling, 9552/9556	Western/ChIP
Anti-Nrf2	Santa Cruz Biotechnology 13032x	Western, ChIP
Anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Chemicon/Millipore, MAB374	Western
Anti-β-actin	Sigma, A5441	Western
Anti-lamin B	Oncogene, NA12	Western
Anti-lactate dehydrogenase (LDH)	Chemicon/Millipore, AB1222	Western
Anti-caspase 3	Cell Signaling, 9665	Western
Anti-Bach1	Santa Cruz Biotechnology sc-14700x	ChIP
Anti-acetyl histone H3	Upstate, 06-599	Western
Anti-acetyl histone H3 Lys9	Cell Signaling, 9671	Western
Anti-acetyl histone H3 Lys9	Upstate, 07-352	ChIP
Anti-acetyl histone H3 Lys14	Upstate, 07-353	ChIP
Anti-acetyl histone H3 Lys18	Cell Signaling, 9675	Western, ChIP
Anti-p300	Santa Cruz Biotechnology sc-585x	ChIP
Anti-CBP	Santa Cruz Biotechnology sc-369x	ChIP

ChIP assay primer sequences	*PCR amplification target*
5'-CCCTCCAGGTCTTATGACTGCTC-3'	Ferritin H ARE forward
5'-GTTTCTGGAGGTTCAGCACGTC-3'	Ferritin H ARE reverse
5'-CACACTGACTCCTCCAAATGAACTTTAG-3'	Ferritin H non-ARE forward
5'-GTACCATATTCCCAAATGGTCGGTC-3'	Ferritin H non-ARE reverse
5'-TAGGAGGCTCAGAGCGACCA-3'	Rad51 forward
5'-GTCCGCCAGCGGCTTTCAGAA-3'	Rad51 reverse

siRNA sequences	*RNA interference target*
5'-GUGAAGAUCUUGACCAAUGUU-3'	siPTEN (sense D-003023-05, Dharmacon)
5'-CAUUGGUCAAGAUCUUCACUU-3'	siPTEN (antisense D-003023-05, Dharmacon)

Supplemental Table 1