

Supplemental Material

Selenoprotein P regulates 1-(4-Chlorophenyl)-benzo-2,5-quinone induced oxidative stress and toxicity in human keratinocytes

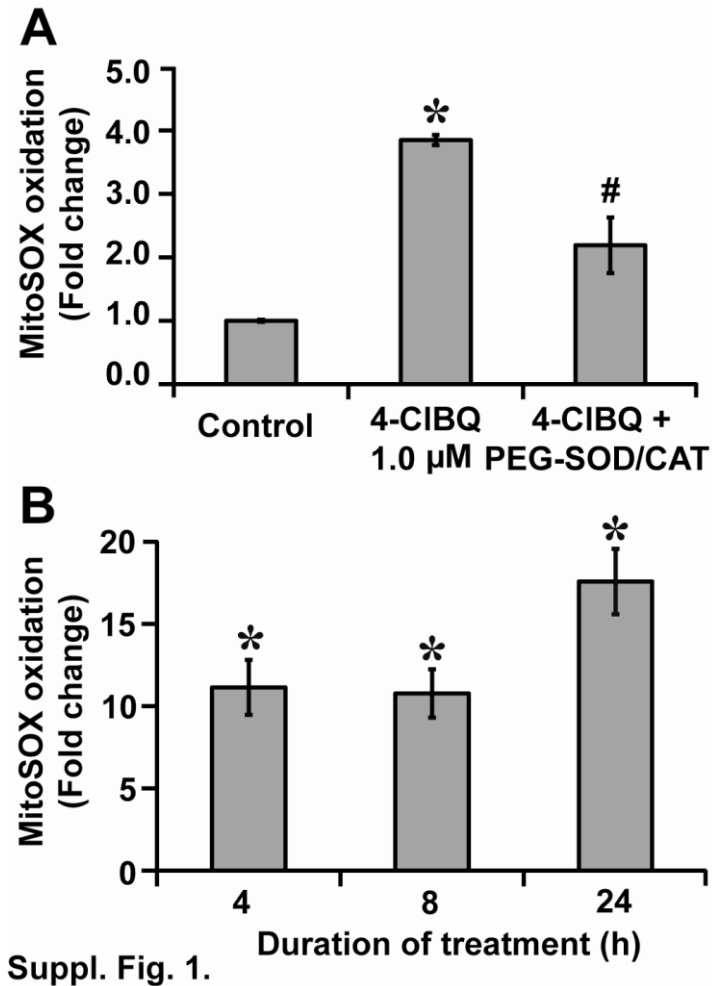
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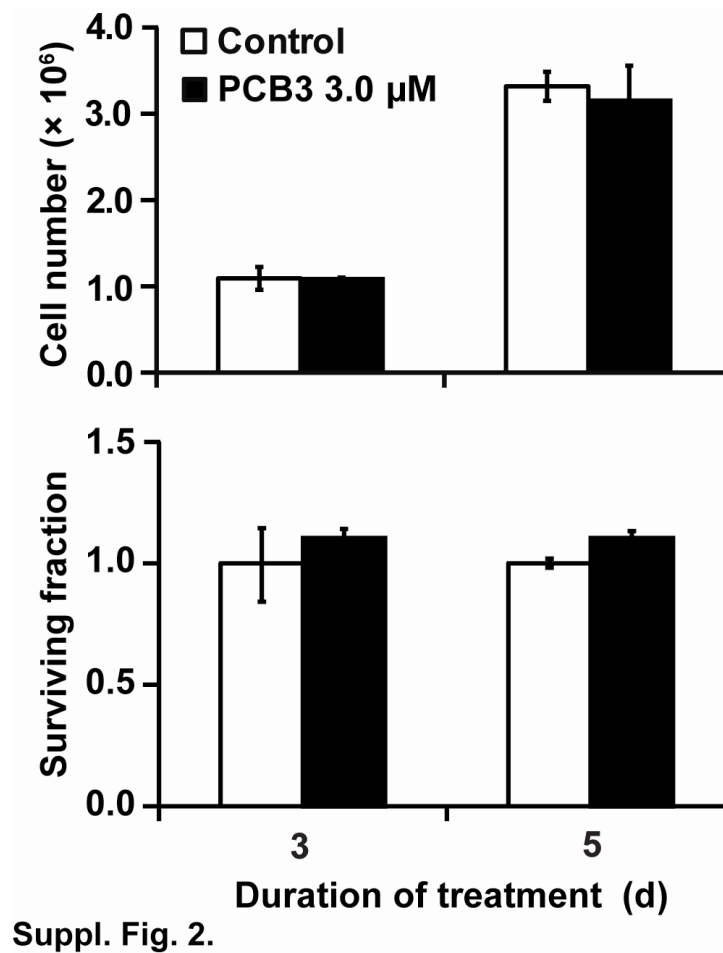
Suppl. Table 1: Primer sequence used in the PCR assay

Gene	Gene bank No.	Sequence (5'--> 3')	Amplicon size (bp)
<i>β-actin</i>	NM_001101.3	Forward: TCACCATTGGCAATGAGCGGTT Reverse: AGTTTCGTGGATGCCACAGGACT	89
<i>catalase</i>	NM_001752.3	Forward: CATCCAGAAGAAAGCGGTCAA Reverse: CCAAGTGAGATCCGGACTGC	140
<i>cygb</i>	NM_134268.4	Forward: CAAGGTGGAACCGGTGTACT Reverse: TCACGTGGCTGTAGATGAGG	137
<i>dusp1</i>	NM_004417.3	Forward: CGCGCAAGTCTTCTTCCTCAAA Reverse: AAGGGCAGGATTTCCACCGG	195
<i>mnsod</i>	NM_000636.2	Forward: GGCCTACGTGAACAACCTGAA Reverse: CTGTAACATCTCCCTTGGCCA	71
<i>sepp1</i>	NM_005410.2	Forward: AACAGAGAGCCAGGACCAAA Reverse: GCCTGCAGTATGCACAGGTA	144

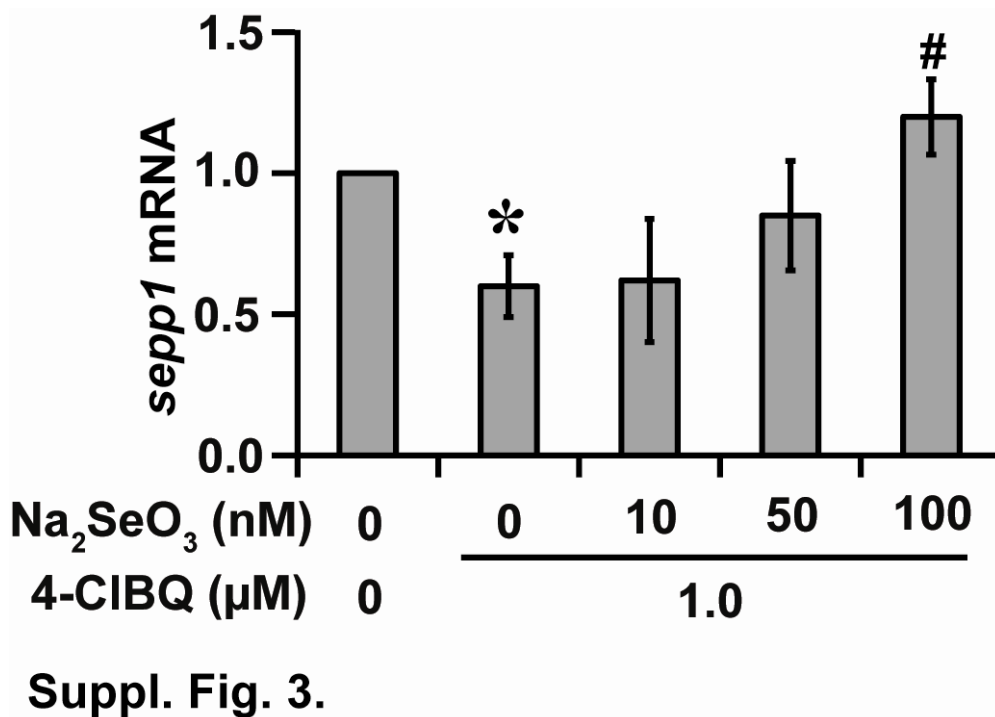


Suppl. Fig. 1.

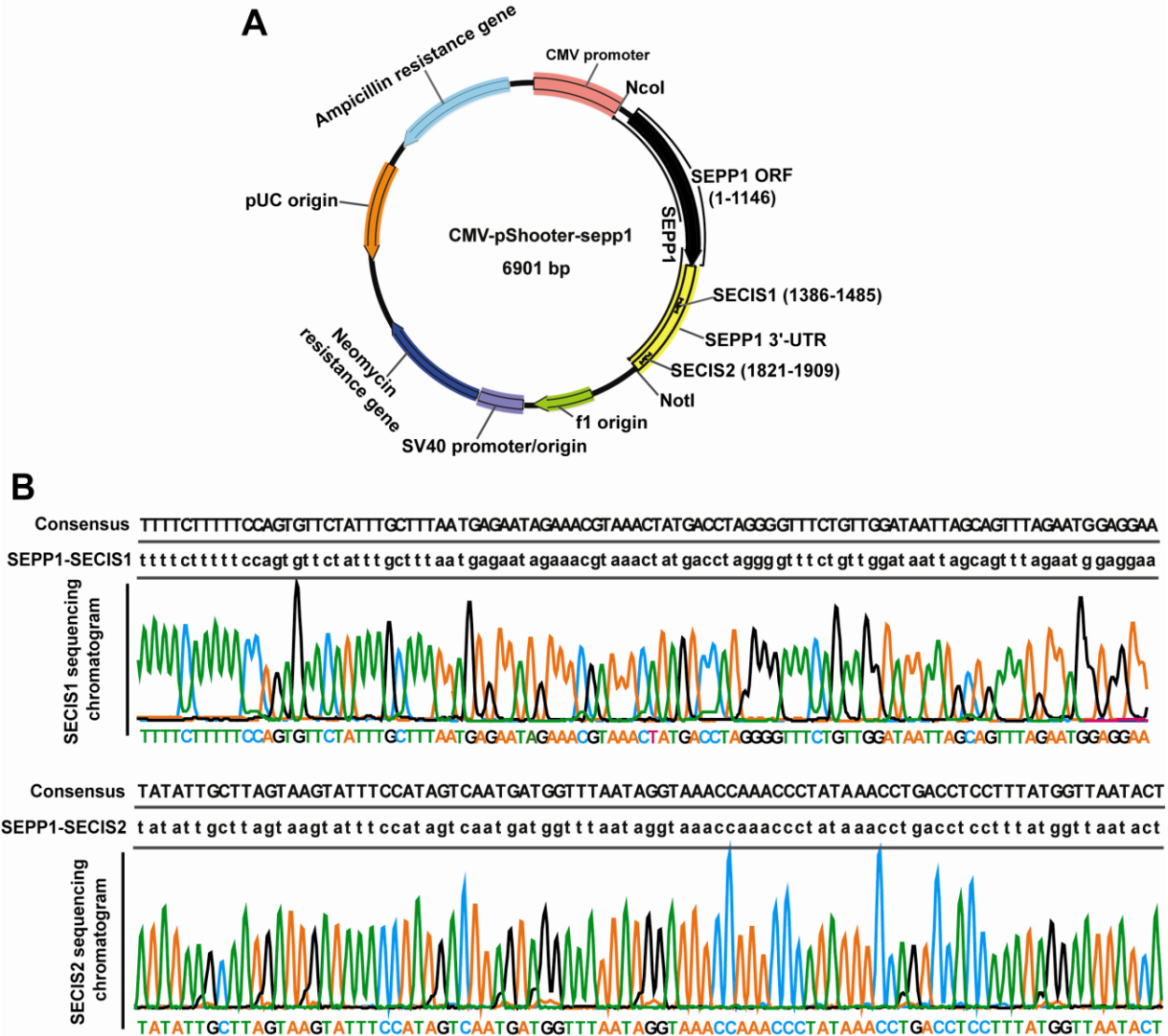
Suppl. Fig. 1. 4-CIBQ treatment increases mitochondrial ROS levels. (A) The specificity of MitoSOX Red oxidation for measurements of superoxide and hydrogen peroxide levels was determined by incubating cells with PEG-SOD and PEG-CAT. (B) MitoSox Red oxidation in 3.0 μM 4-CIBQ treated cells at indicated times. Fold change in MFI was calculated relative to time-matched untreated cells. Asterisks represent statistical significance compared to untreated cells; # represents statistical significance compared to 4-CIBQ treated cells; $P < 0.05$, $n = 3$.



Suppl. Fig. 2. PCB3 is non-toxic to HaCaT cells. Cell numbers (*Top panel*) and cell survival (*Bottom panel*) were determined at the end of 3 and 5 d of 3.0 μM PCB3 treatments.

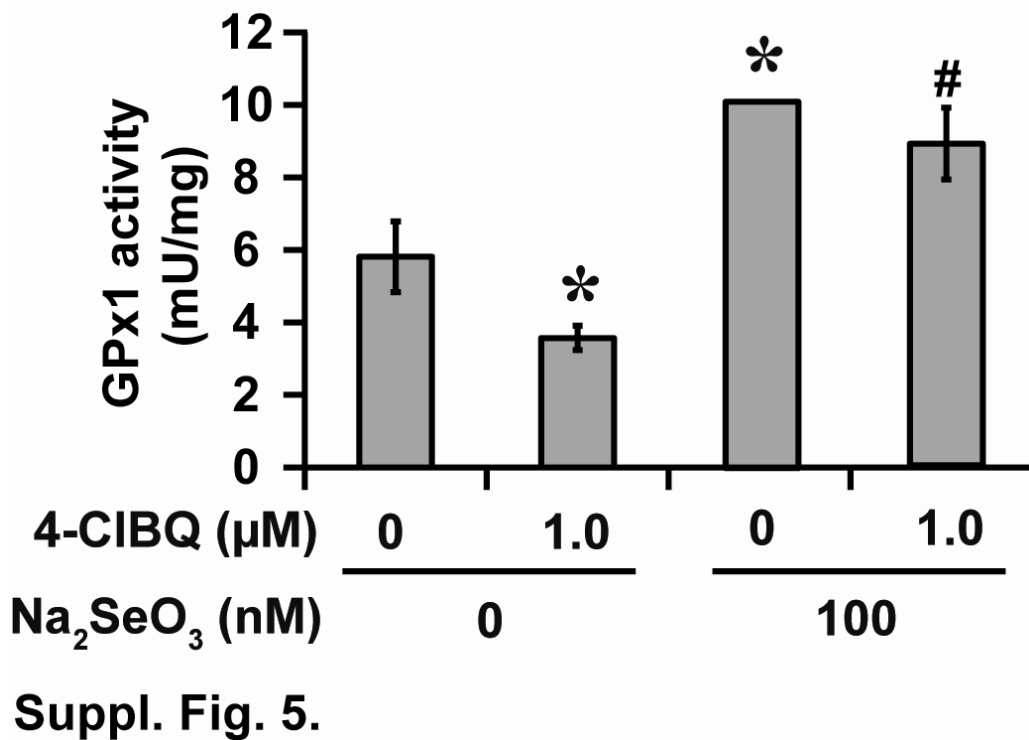


Suppl. Fig. 3. Sodium-selenite supplementation suppressed 4-CIBQ induced decrease in *sepp1* expression. A quantitative RT-PCR assay was used to measure *sepp1* mRNA levels in sodium-selenite (10-100 nM) supplemented and 4-CIBQ treated cells. Asterisk represents statistical significance compared to untreated cells; # represents statistical significance compared to 1.0 µM 4-CIBQ treated cells; $P < 0.05$, $n = 3$.



Suppl. Fig. 4.

Suppl. Fig. 4. (A) Illustration of the engineered CMV-pShooter-sepp1 plasmid DNA containing human *sepp1* cDNA that includes the ORF and 2 SECIS. (B) Insert sequence was verified by sequencing; chromatograms of the two SECIS sequences are shown.



Suppl. Fig. 5. Sodium-selenite supplementation suppressed 4-CIBQ induced decrease in GPx1 activity. GPx1 activity was measured using the resources of the University of Iowa Holden Comprehensive Cancer Center Radiation and Free Radical Research Core. Asterisk represents statistical significance compared to untreated cells; # represents statistical significance compared to 1.0 μM 4-CIBQ treated cells; $P < 0.05$, $n = 3$.