Supporting Information

CRISPR-generated Nrf2a loss- and gain-of-function mutants facilitate mechanistic analysis of chemical oxidative stress-mediated toxicity in zebrafish

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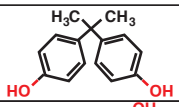
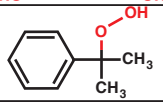
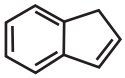
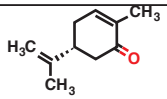
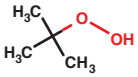
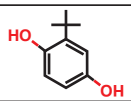
Figure S1.

Chemical	CAS	Structure	LC_{50}	DMSO conc.	40% LC_{50}	20% LC_{50}	Gene Expression: *hmox1a*	*gclc*	*gstp*	*prdx1*	*gpx1a*	*nqo1*	*sod1*	*sod2*
Bisphenol A	80-05-7		12.8	0.003%	5.11	--								
R-(-)-Carvone	6485-40-1		58.2	--	23.3	11.6		*	*	*			*	*
Cumene hydroperoxide	80-15-9		23.4	0.00009%	9.35	4.68		*	*	*	*	*		
Hydroquinone	123-31-9		0.24*	--	0.095	--								
Indene	95-13-6		50.9	0.05%	20.4	--	*	*				*		*
tert-Butyl hydroperoxide	75-91-2		176	--	70.3	35.2		*	*	*	*	*	*	*
tert-Butyl hydroquinone	1948-33-0		0.35*	0.000002%	0.142	--			*	*		*		

- Fold Change in Expression

0.4 0.6 0.8 1 2 4 6 8 10

Figure S1. All chemicals used in the present study. All concentrations are mg/L. LC_{50} concentrations marked with * were determined for this work whereas all others were identified in ref 1. DMSO concentrations (in % v/v where indicated) are final concentrations in all exposures including the 0 mg/L control. Heat map represents expression changes from exposure to 40% LC_{50} for each chemical from 5 hpf to 96 hpf with daily renewal. Asterisks in boxes indicate significantly different expression ($p < 0.05$) relative to untreated controls. Control (untreated) expression in all cases is set to 1.0. All data represent the mean ± SEM (n = 5 biological replicates, 10 larvae per replicate).

Note. While researchers have used *t*BHQ as a strong activator of Nrf2a in zebrafish,[2-5] we found that effects of *t*BHQ on the Nrf2a-responsive genes *gstp* and *prdx1* were only moderate at 96 hpf relative to other compounds tested. It is likely that the length of exposure used in this study accounted for this difference relative to previous work. For example, *t*BHQ strongly activates Nrf2a under brief 3-6 hr exposures especially at 2 dpf.[5,6] In addition, an *in vivo* study using rat embryos indicated that NRF2 activation by *t*BHQ was greater after acute exposures than subacute exposures.[7] Because our group was examining the effects of several compounds on Nrf2a-dependent gene expression, it was beyond the scope of the study to test the effects of the test agents at multiple time points.

Figure S2.

A.

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WT      GTCTGTTTTCTCTGCAGGACATGGATCTGATCGATATCCTGTGGCGGCAGGATGTGGATC    60
w210    GTCTGTTTTCTCTGCAGGACATGGATCTGATCGATATCCTGTGGCGGCAGGATGTGGATC    60
w211    GTCTGTTTTCTCTGCAGGACATGGATCTGATCGATATCCTGTGGCGGCAGGATGTGGATC    60
w212    GTCTGTTTTCTCTGCAGGACATGGATCTGATCGATATCCTGTGGCGGCAGGATGTGGATC    60
dw213   GTCTGTTTTCTCT-------------------------------------------------    13
dw214   GTCTGTTTTCTCTGCAGGACATGGATCTGATCGATATCCTGTGGCGGCAGGATGTGGATC    60

WT      TGGGCGCGGGCCGTGAGGTGTTCGACTTCAGCTACCGGCAGAAGGAGGTGGAGCTGCGCA   120
w210    TGGGC-----TCGTGAGGTGTTCGACTTCAGCTACCGGCAGAAGGAGGTGGAGCTGCGCA   115
w211    TGGGCGCGGGCCGTGAGGTGTTCGACTTCAGCTACCGGCAGAAGGAGGTGGAGCTGCGCA   120
w212    TGGGCGC---------------------------------------------------     67
dw213   -----------------------------------------------------------     13
dw214   TGGGCGCGGGC-----------------------------------------------     71

WT      GGCGGAGGGAGCAGGAGGAGCAGGAGCTGCAGGAGCGTCTGCAGGAGCAGGAGAAGACAC   180
w210    GGCGGAGGGAGCAGGAGGAGCAGGAGCTGCAGGAGCGTCTGCAGGAGCAGGAGAAGACAC   175
w211    GGCGGAGGGAGCAGGAGGAGCAGGAGCTGCAGGAGCGTCTGCAGGAGCAGGAGAAGACAC   180
w212    -----------------------------------------------------------     67
dw213   ---------------------------------GCGTCTGCAGGAGCAGGAGAAGACAC     39
dw214   -----------------------------------------------------------     71

WT      TGCTGGCTCAGCTGCAGCTCGACGAGGAGACCGGAGAGTTCCTGCCGCGCAGCACACCGC   240
w210    TGCTGGCTCAGCTGCAGCTCGACGAGGAGACCGGAGAGTTCCTGCCGCGCAGCACACCGC   235
w211    TGCTGGCTCAGCTGCAGCTCGACGAGGAGACCGG----------TCGGCACACCGC       226
w212    -----------------------------------------------------------     67
dw213   TGCTGGCTCAGCTGCAGCTCGACGAGGAGACCGGAGAGTTCCTGCCGCGCAGCACACCGC    99
dw214   -------------------------------------------------ACACCGC     78

WT      TCACACACACACCTGAAGCAGACGGAGGAGGAGCGGGAGAAATCACACAGGTACACATGC   300
w210    TCACACACACACCTGAAGCAGACGGAGGAGGAGCGGGAGAAATCACACAGGTACACATGC   295
w211    TCACACACACACCTGAAGCAGACGGAGGAGGAGCGGGAGAAATCACACAGGTACACATGC   286
w212    TCACACACACACCTGAAGCAGACGGAGGAGGAGCGGGAGAAATCACACAGGTACACATGC   127
dw213   TCACACACACACCTGAAGCAGACGGAGGAGGAGCGGGAGAAATCACACAGGTACACATGC   159
dw214   TCACACACACACCTGAAGCAGACGGAGGAGGAGCGGGAGAAATCACACAGGTACACATGC   138
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B.

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WT      15  DMDLIDILWRQDVDLGAGREVFDFSYRQKEVELRRRREQEEQELQERLQEQEKTLLAQLQ   75
w210    15  DMDLIDILWRQDVDLGS*                                           32
w211    15  DMDLIDILWRQDVDLGAGREVFDFSYRQKEVELRRRREQEEQELQERLQEQEKTLLAQLQ
w212    15  DMDLIDILWRQDVDLGAHTHT*                                       36
dw213   15  ----------------------------------------------EQEKTLLAQLQ   26
dw214   15  DMDLIDILWRQDVDLGAG-------------------------------------------   33
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WT          LDEETGEFLPRSTPLTHTPEADGGGAGEITQ     106
w210        ---                                  32
w211        LDEETGRHTAHTHT*                      89
w212        ---                                  36
dw213       LDEETGEFLPRSTPLTHTPEADGGGAGEITQ      57
dw214       ------------TPLTHTPEADGGGAGEITQ      52
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Figure S2. Independently-isolated *nfe2l2a* mutant lines encode null (LOF) or hyperactive (GOF) alleles. (a) Genomic sequences of Intron 1 (grey letters) and Exon 2 (black letters) for wild type and five mutant alleles show deletions (dashes) and insertions (bold text) near CRISPR guide sites (underlined in wild type, with PAM sites double underlined). (b) Protein sequences predicted from Exon 2 cDNA show that three out-of-frame deletions result in putative LOF alleles with altered amino acids (bold) and novel stop codons (*), while the two in-frame deletions result in putative GOF alleles by removing of some or all of the DLG and ETGE motifs bound by Keap1 (underlined) and lysine residues that are targets of ubiquitination (double underline).

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