Supporting Information

Induction of the Antioxidant Response by the Transcription Factor NRF2 Increases Bioactivation of the Mutagenic Air Pollutant 3-Nitrobenzanthrone in Human Lung Cells

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Table of Contents:

Table S1. Primer Sequences Designed for Each Gene

Gene	Forward qPCR Primer	Reverse qPCR Primer	Amplicon Length (bp)
AKR1C1	GTAAAGCTTTAGAGGCCAC	ATAAGGTAGAGGTCAACATAA	249
AKR1C2	GTAAAGCTCTAGAGGCCGT	CTGGTCGATGGGAATTGCT	179
AKR1C3	AAGTAAAGCTTTGGAGGTCACA	GGACCAACTCTGGTCGATGAA	185
NQO1	TCCCCCTGCAGTGGTTTGGAGT	ACTGCCTTCTTACTCCGGAAGGGT	127
GAPDH	CATCTCTGCCCCCTCTGCTGA	GGATGACCTTGCCCACAGCCT	305

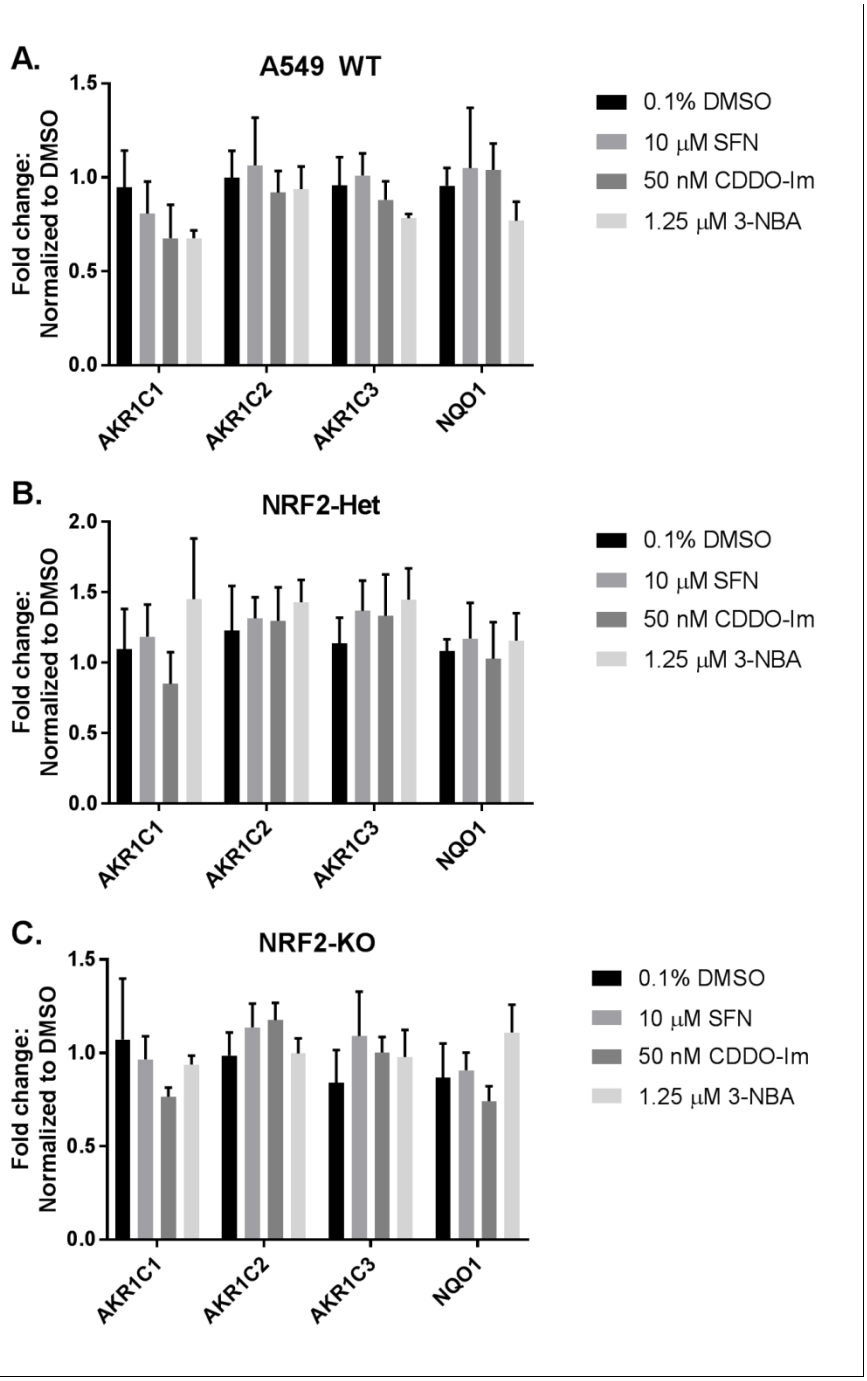


Figure S1. NRF2 activators (CDDO-Im and SFN) did not alter *AKR1C1-1C3* or *NQO1* transcription in A549 wt (A), A549 NRF2-Het (B), or A549 NRF2-KO cells (C). Treatment with 1.25 μM 3-NBA also failed to significantly change *AKR1C1-1C3* or *NQO1* transcript levels, indicating that A549 cells with CRISPR-Cas9 knockout of NRF2 possess stable expression of ARE-genes that are not further induced through NRF2-dependent or independent mechanisms

during exposures to 3-NBA. Bar graphs show mean ± SD of $n = 2$/group. The effect of NRF2 treatment on the expression levels of each gene was analyzed by a one-way ANOVA with a post-hoc Dunnett's multiple comparison test.

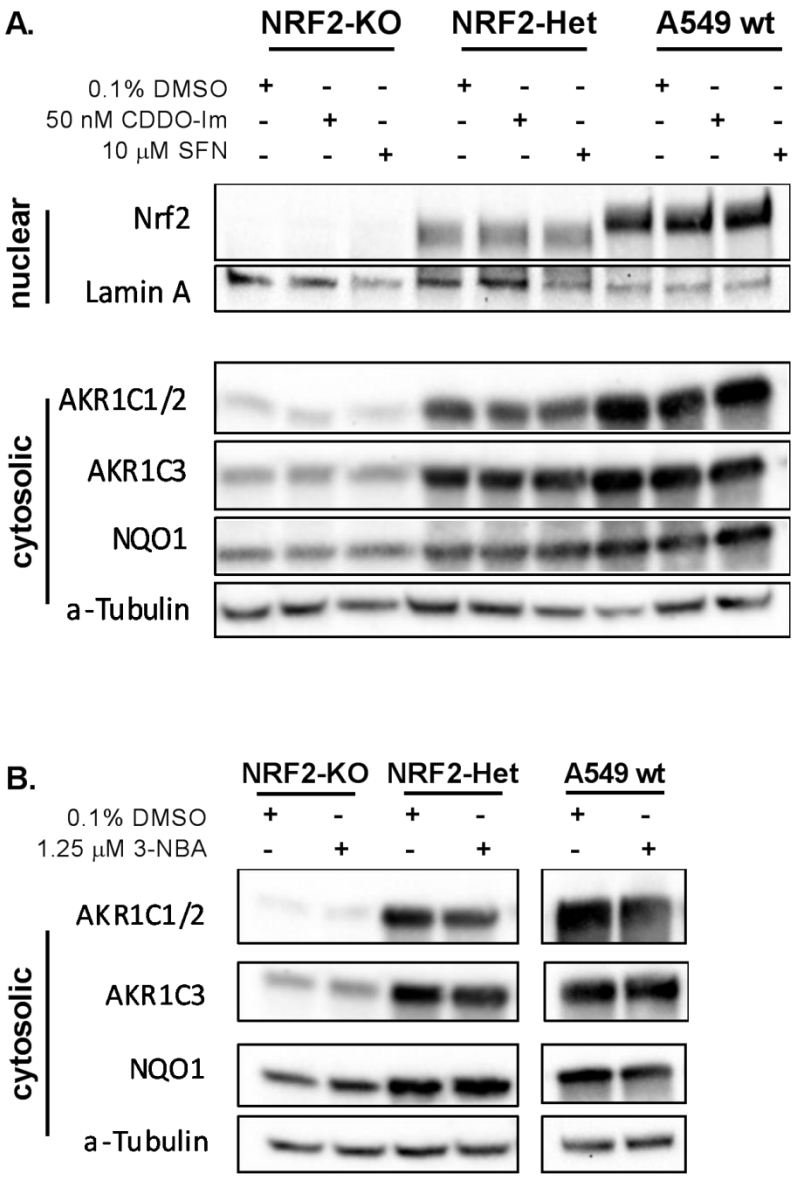


Figure S2. NRF2 activators (CDDO-Im and SFN) did not lead to NRF2 recruitment to the nucleus and did not alter AKR1C1-1C3 or NQO1 protein levels in A549 cell line variants (A). Western blot is representative of two independent experiments. Treatment with 1.25 μM 3-NBA also failed to significantly change AKR1C1-1C3 or NQO1 protein levels (B), indicating that A549 cells with CRISPR-Cas9 knockout of NRF2 possess stable protein expression of ARE-genes that are not further induced through NRF2-dependent or independent mechanisms during exposures to 3-NBA.

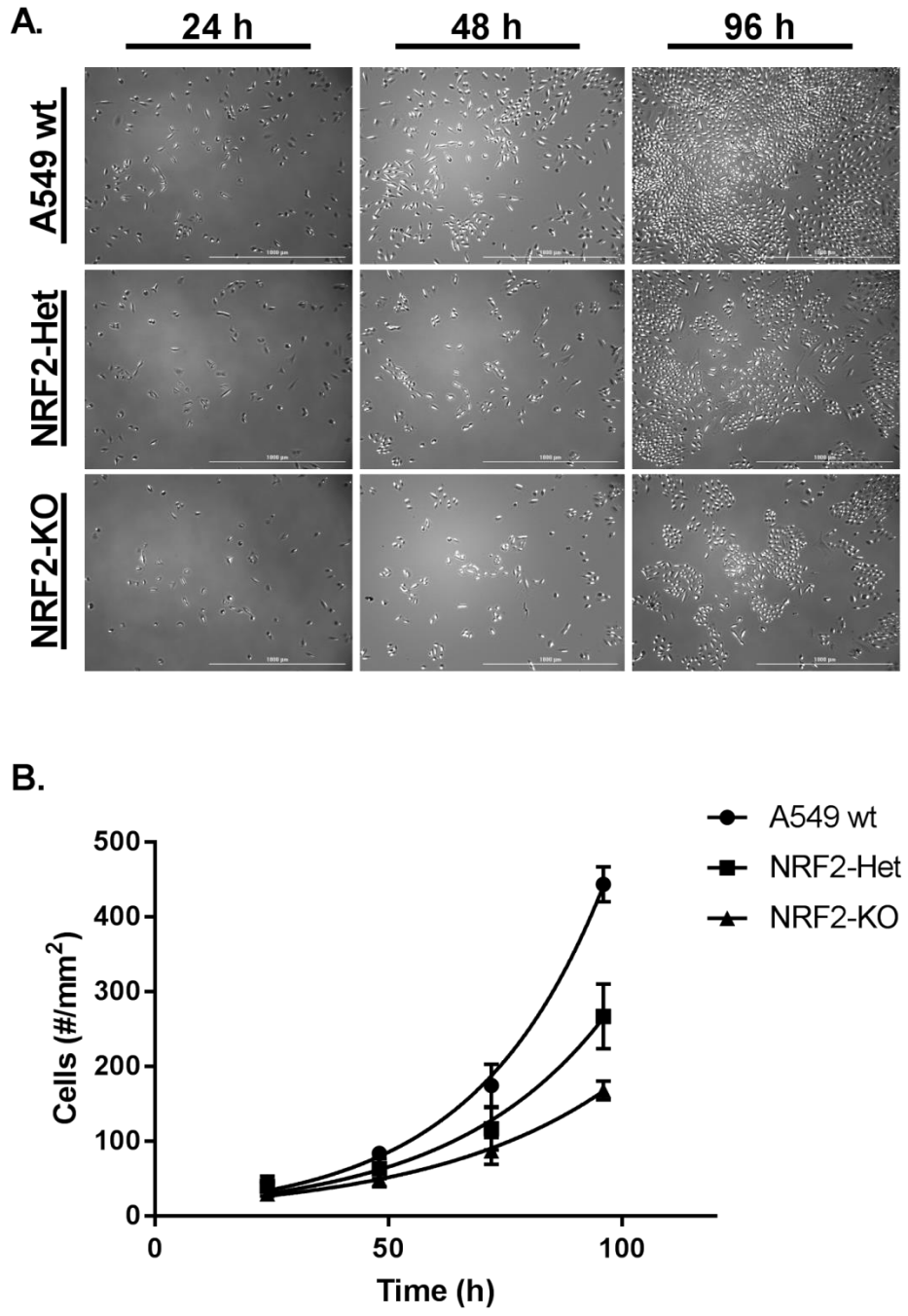
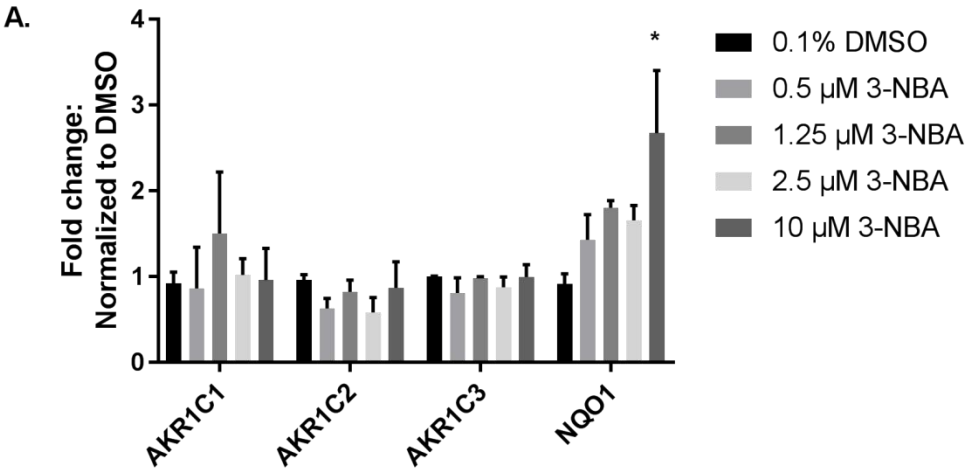
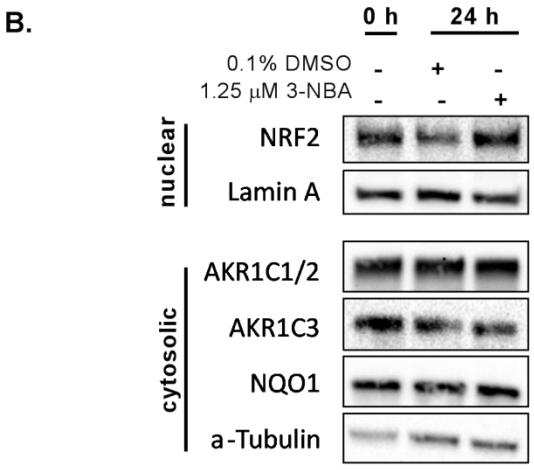


Figure S3. Effects of NRF2 on proliferation of A549 cell lines. To monitor proliferation of A549 NRF2-KO, A549 NRF2-Het, and A549 wt cell lines, 1 x 10^3 cells were plated per well and monitored over a 96 h time course (A). Images are representative of four independent experiments (n=4). Cell counts were then normalized to area and expressed as number of cells per mm^2 (B). Plotted time course shows mean cell counts ± SD of $n = 4$/cell line.


A.
Fold change: Normalized to DMSO
0
1
2
3
4
*
AKR1C1
AKR1C2
AKR1C3
NQO1
0.1% DMSO
0.5 μM 3-NBA
1.25 μM 3-NBA
2.5 μM 3-NBA
10 μM 3-NBA


B.
0 h
24 h
0.1% DMSO - + -
1.25 μM 3-NBA - - +
nuclear
NRF2
Lamin A
cytosolic
AKR1C1/2
AKR1C3
NQO1
a-Tubulin

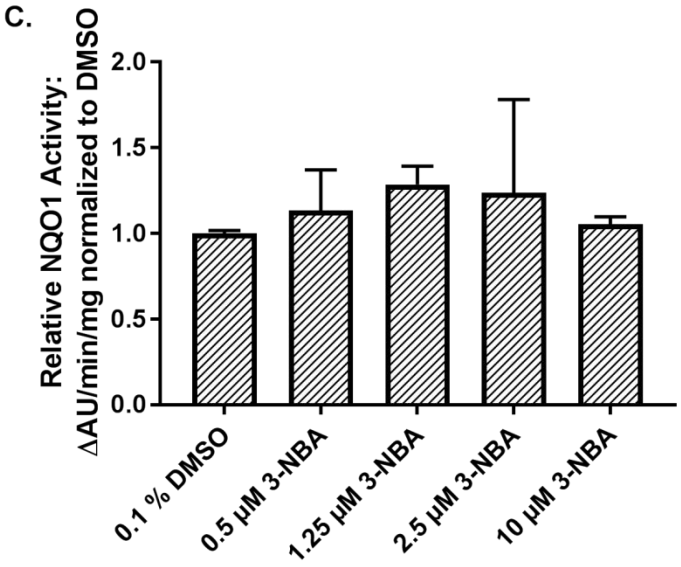

C.
Relative NQO1 Activity: ΔAU/min/mg normalized to DMSO
0.0
0.5
1.0
1.5
2.0
0.1 % DMSO
0.5 μM 3-NBA
1.25 μM 3-NBA
2.5 μM 3-NBA
10 μM 3-NBA

Figure S4. 3-NBA does not function as an NRF2 activator in HBEC3-KT cells. HBEC3-KT cells were exposed to multiple doses of 3-NBA (0.5 – 10 μM) for 16 h (A). Quantitative RT-PCR was utilized to quantify mRNA levels of *AKR1C1-1C3* and *NQO1* expressed as copy number and normalized to cells that were treated with vehicle control, 0.1% DMSO. Bar graphs show mean fold change ± SD of $n = 2$/group. The effect of 3-NBA treatment on the expression levels of each gene was analyzed by a one-way ANOVA with a post-hoc Dunnett's multiple comparison test. Asterisks indicate a statistically significant difference from the vehicle control (*$p \leq 0.05$). HBEC3-KT cells were exposed to 1.25 μM 3-NBA to assess whether exposure to 3-NBA led to recruitment of NRF2 to the nucleus or increased protein levels of ARE genes. Immunoblots revealed that 3-NBA had little effect on NRF2 localization in the nucleus and protein levels of AKR1C1-1C3 and NQO1 (B). A NQO1 activity assay was conducted in HBEC3-KT cells after a 24 h of exposure to 3-NBA (C). Bar graphs show mean ± SD of $n = 2$-3/group. These changes were not statistically significant when tested by a one-way ANOVA with a post-hoc Dunnett's multiple comparison test.

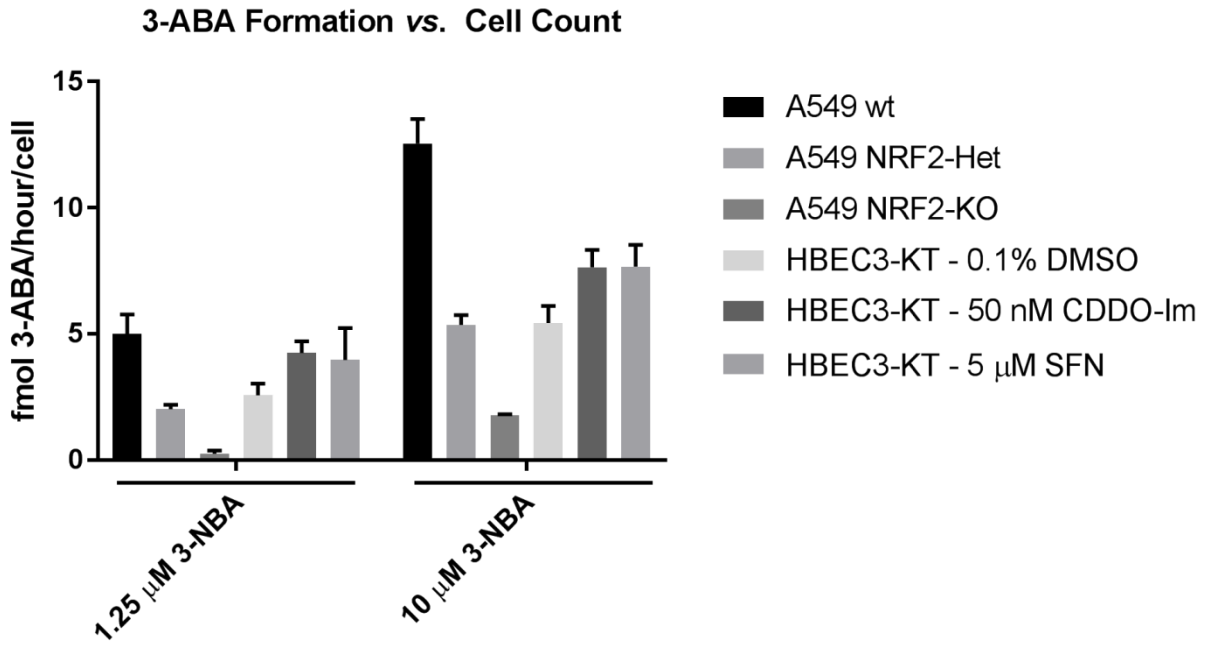


Figure S5. Determination of the metabolic activation of 3-NBA to 3-ABA in A549 cell variants (A549 wt, NRF2-Het, and NRF2-KO) and HBEC3-KT cells ± NRF2 activators. The intrinsic fluorescence of 3-ABA (λ_{ex} 520 nm, λ_{em} 650 nm) was used to detect the final reduction product, 3-ABA. Formation of 3-ABA was normalized to cell count from duplicate plates that underwent identical treatment conditions. Values are expressed as mean ± SD and show the relative formation of fmol 3-ABA per hour per cell. A549 wt cells are able to metabolize 3-NBA to a much greater extent than HBEC3-KT which is likely due to constitutive NRF2 activity and subsequent upregulation of *AKR1C1-1C3* and *NQO1*. Experiments were repeated 4 independent times (n = 4).