Supplemental Information File

The Human Transient Receptor Potential Melastatin 2 Ion Channel Modulates ROS through Nrf2

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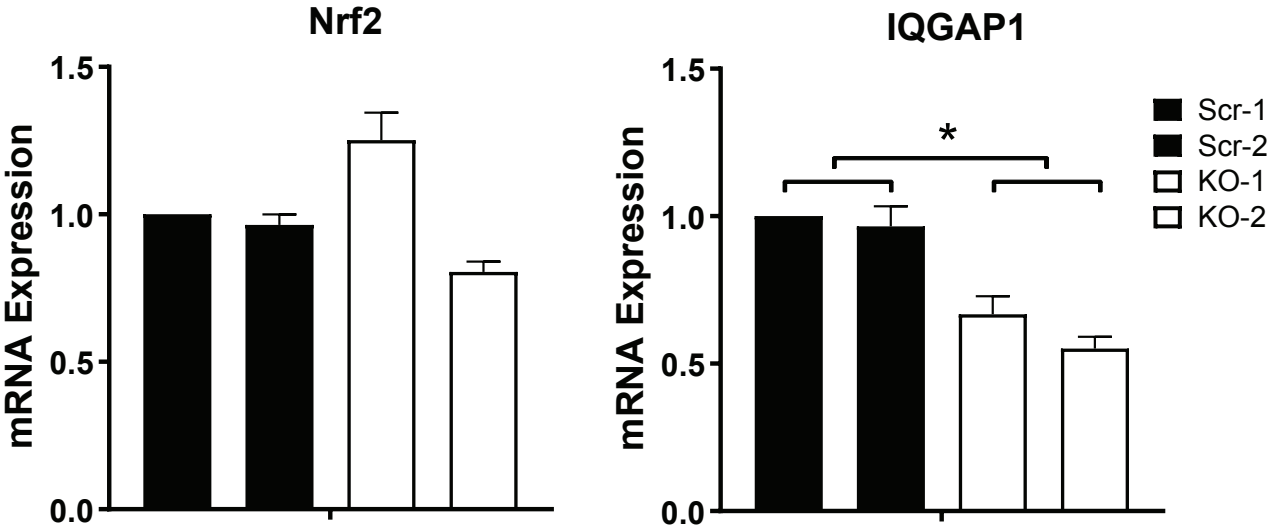
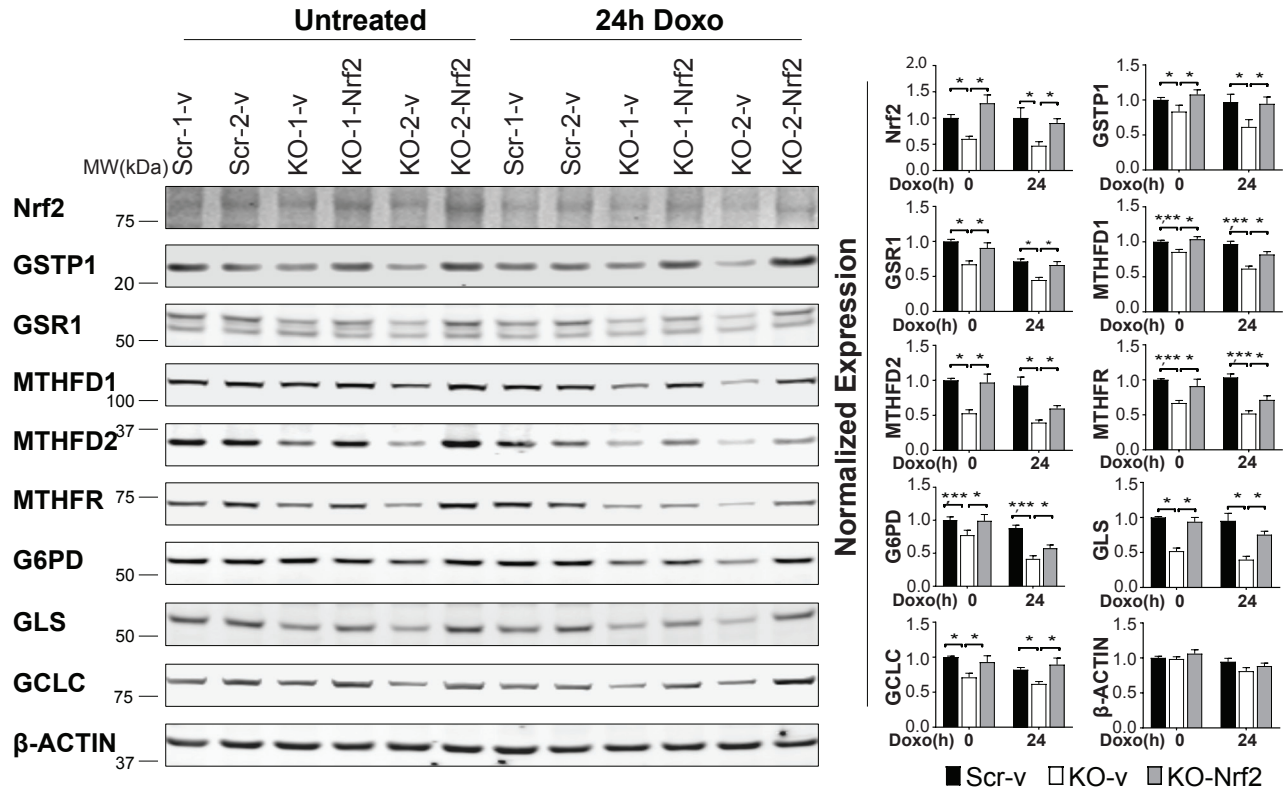
Supplementary Figure S1



Supplementary Figure S1. RT-PCR of Nrf2 and IQGAP1. RT-PCR was used to measure Nrf2 and IQGAP1 mRNA in TRPM2 depleted SH-SY5Y cells. Results in the KOs were normalized to the average of the scrambled controls in each experiment, and the means $\pm$ SEM for five experiments are shown. *$p<0.0001$, Student's T-test.

Supplementary Figure S2

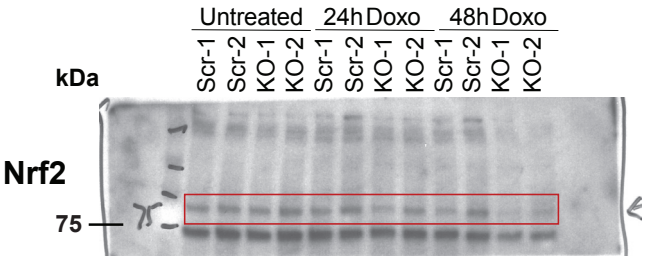


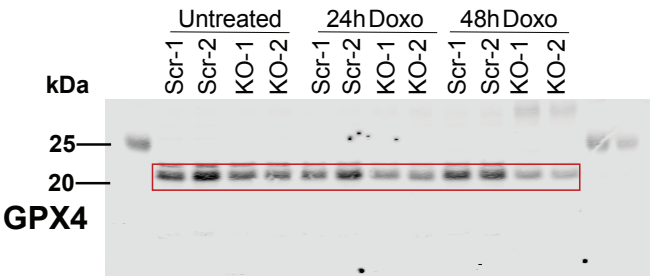
Supplementary Figure S2. Expression of Nrf2 fully restores GLS and GCLC and partially restores expression of other antioxidant enzymes in TRPM2 depleted cells. SH-SY5Y cells in which TRPM2 was depleted were stably transfected with empty vector (2 clones, KO-1-v and KO-2-v) or Nrf2 (2 clones, KO-1-Nrf2 and KO-2-Nrf2). Scrambled control cells were transfected with empty vector (2 clones, Scr-1-v and Scr-2-v). Cells were untreated or treated with 0.3 μM doxorubicin for 24 hours. Western blotting was performed with antibodies to Nrf2, GSTP1, GSR1, MTHFD1, MTHFD2, MTHFR, G6PD, GLS, GCLC, and actin. Representative blots from three experiments are shown. Intensity of bands was quantitated with Li-Cor technology. Blots were normalized by comparing bands to each protein's time 0 average scrambled control. Normalized means $\pm$ SEM for Scr and KO cells at each time point from three experiments are shown on the right. *$p \leq 0.02$ group effect; **$p \leq 0.05$, group x doxorubicin exposure time interaction effect; two-way ANOVA.

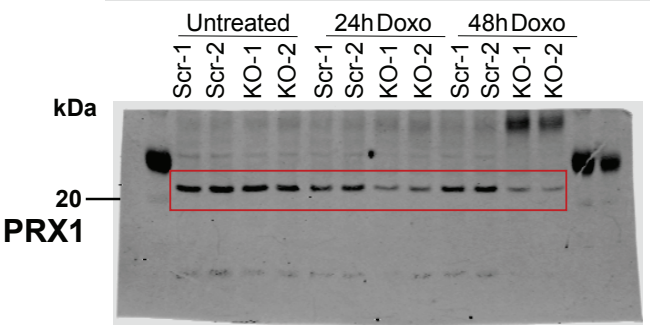
Supplementary Figure S3

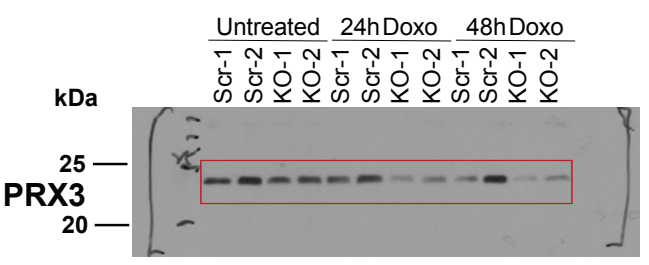
These figures show non-cropped Western blot images used to create Figures 2,3,4,6, and Supplementary Figure S2. Blots were cut around the expected molecular weights for each protein before probing, and the probed blots are shown. Red rectangles mark the bands used.

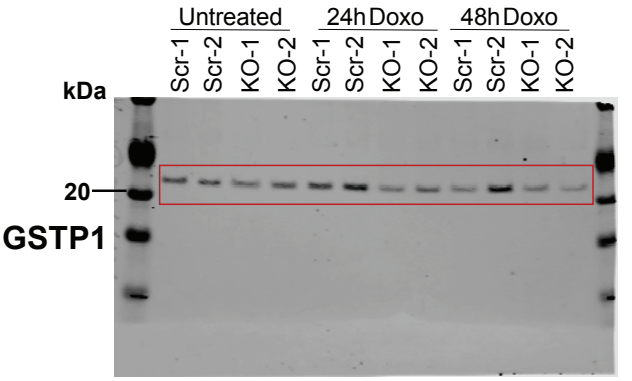
Full-Length Blots Figure 2a

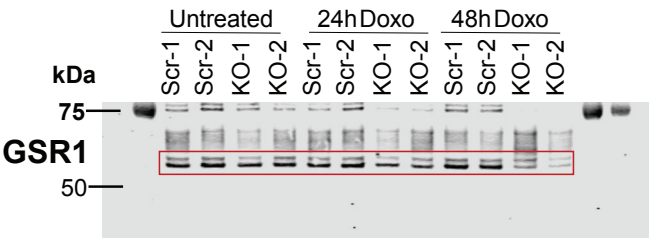


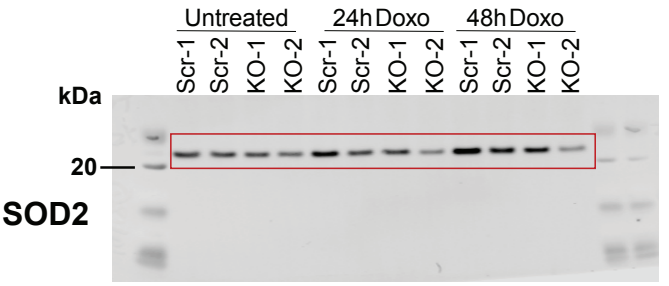


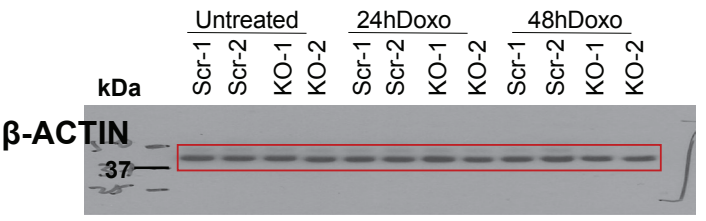






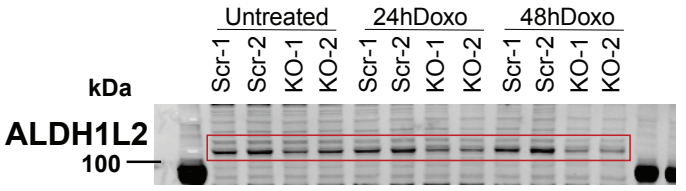


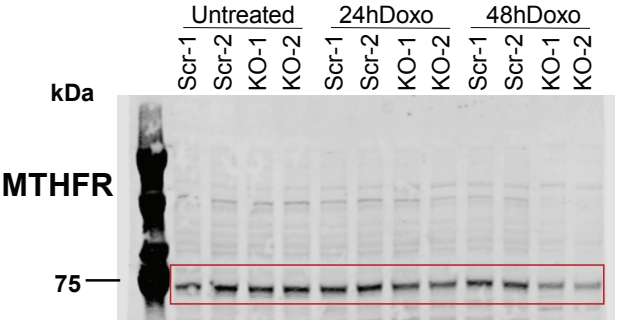


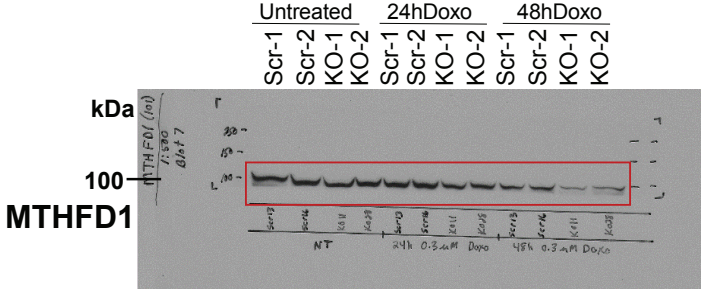


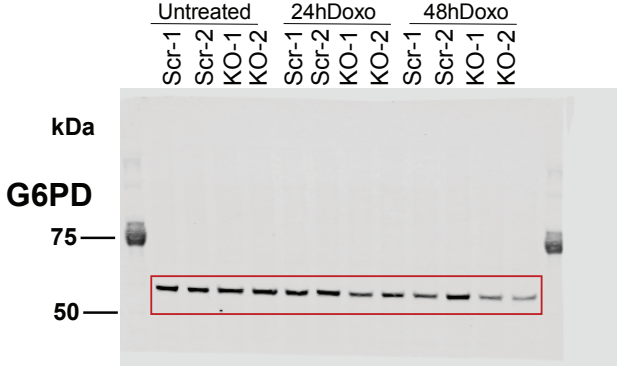
Supplementary Figure S3 (continued)

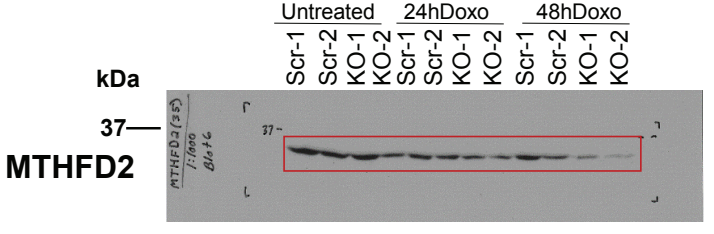
Full-Length Blots Figure 2b

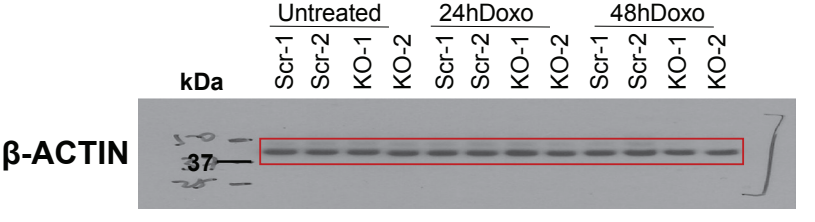






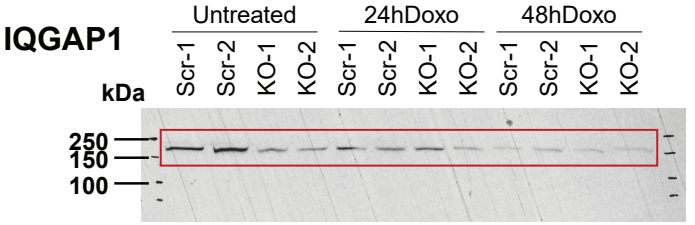


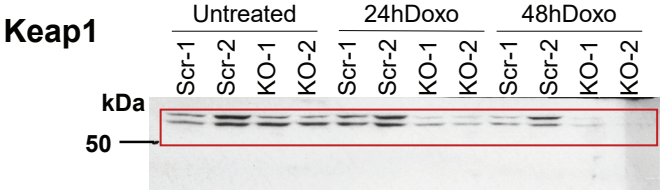


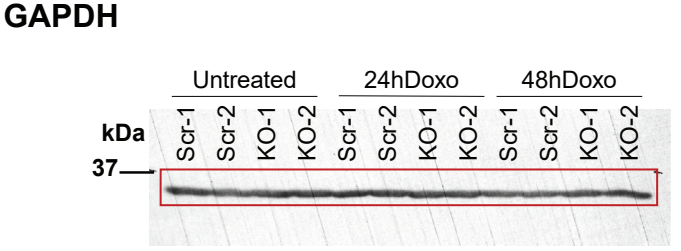


Supplementary Figure S3 (continued)

Full-Length Blots Figure 2c

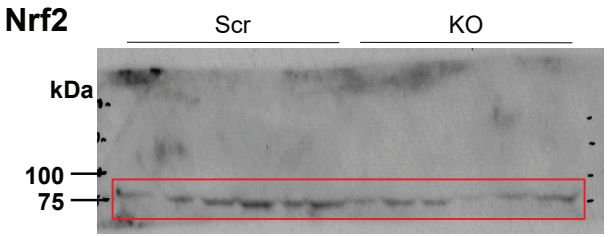


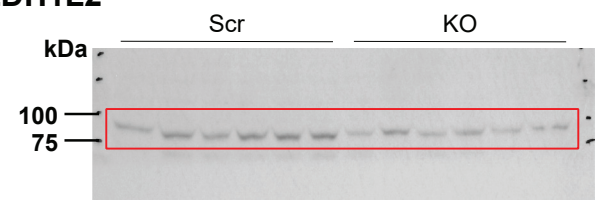


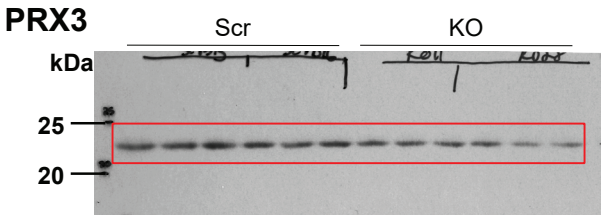


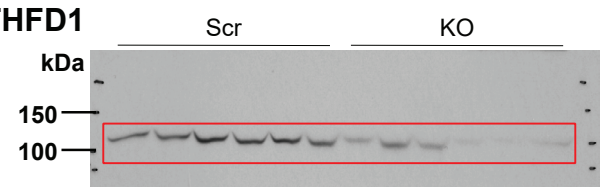
Supplementary Figure S3 (continued)

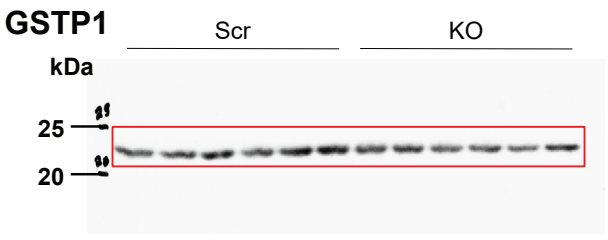
Full-Length Blots Figure 3a

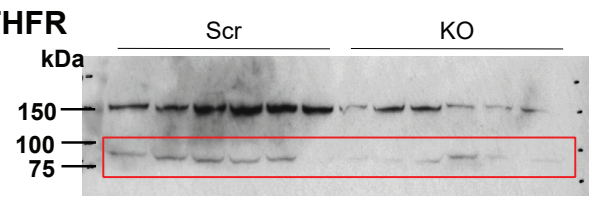


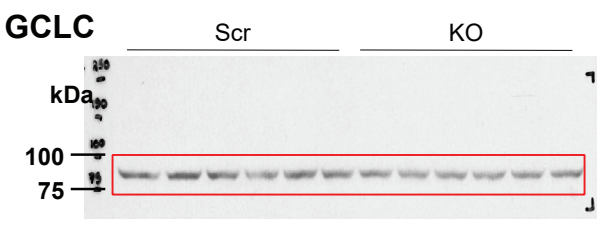


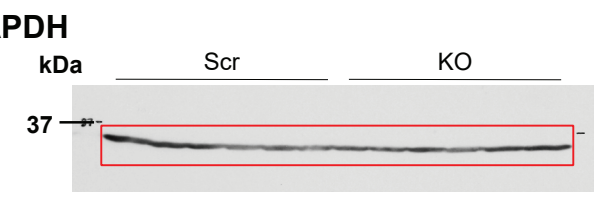








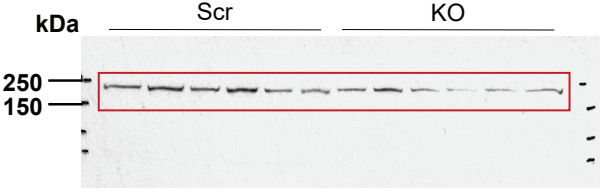




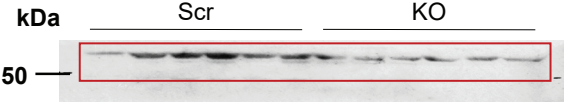
Supplementary Figure S3 (continued)

Full-Length Blots Figure 3b

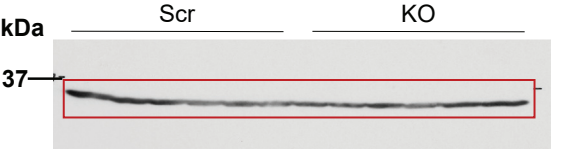
IQGAP1



Keap1

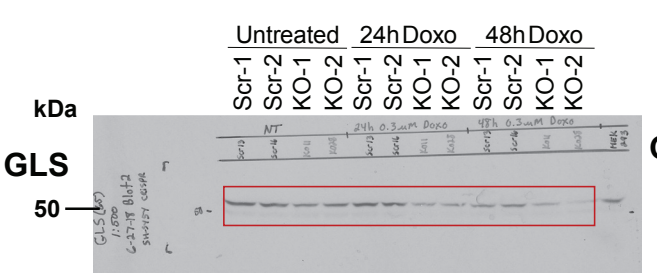


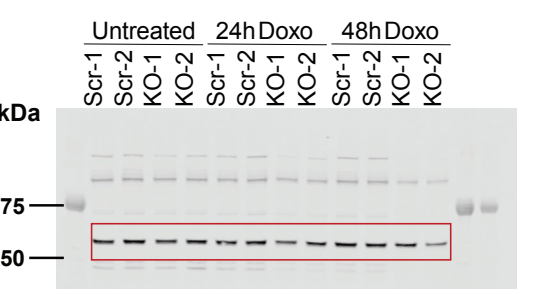
GAPDH

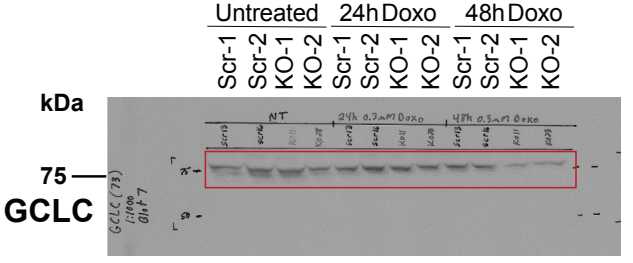


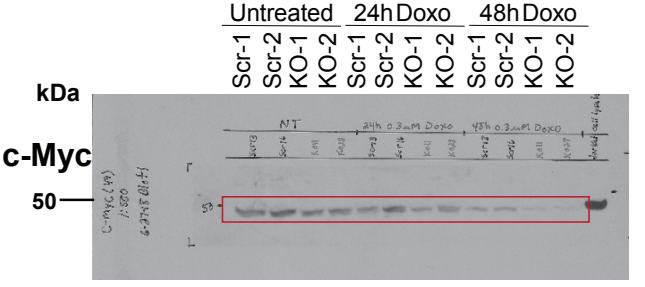
Supplementary Figure S3 (continued)

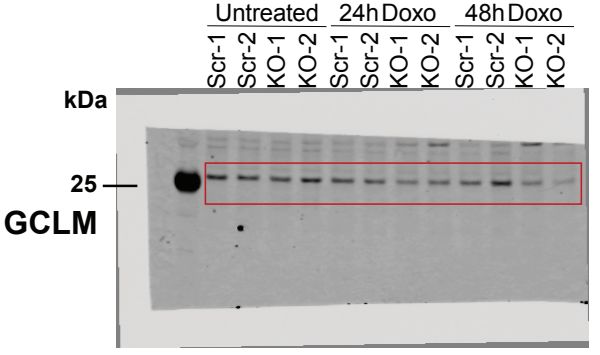
Full-Length Blots Figure 4e

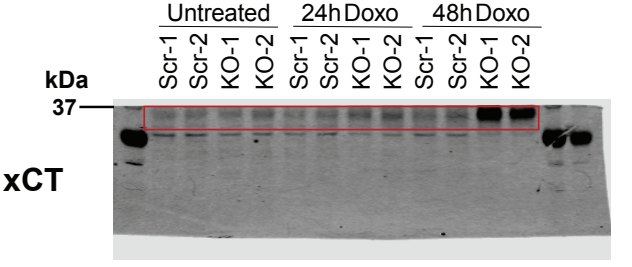


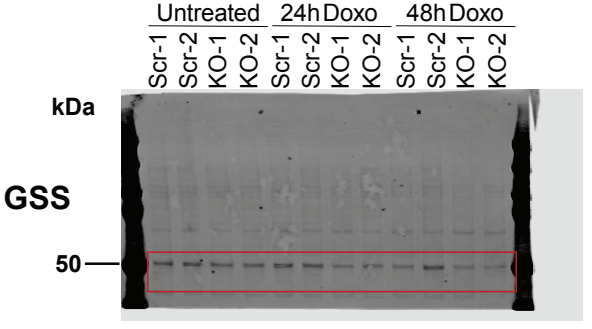


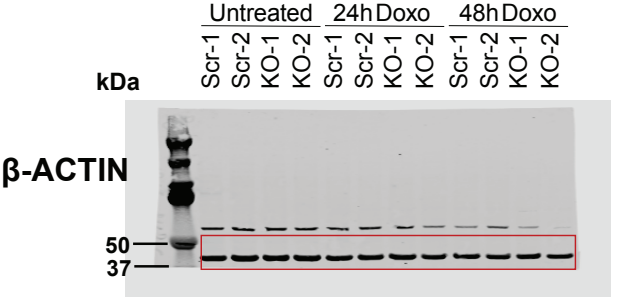






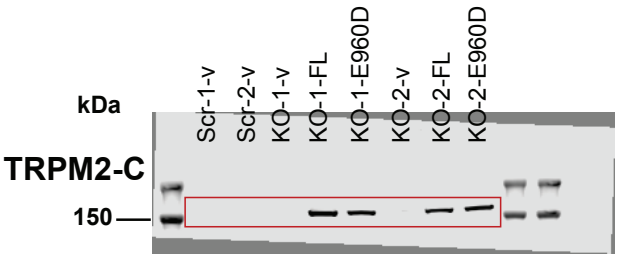


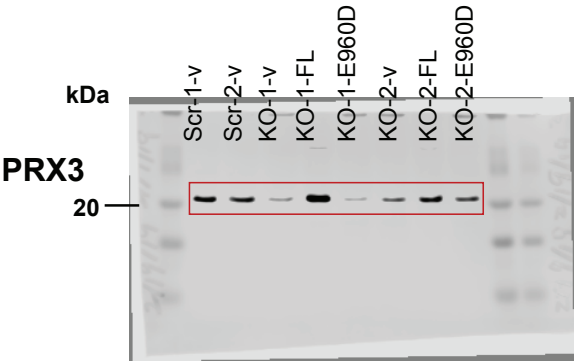


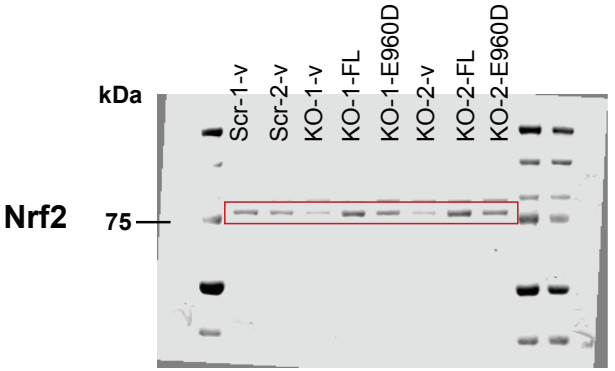


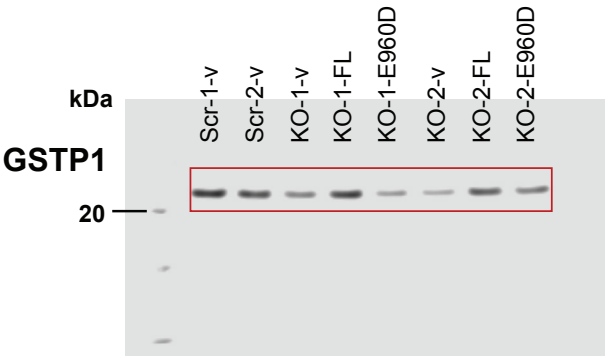
Supplementary Figure S3 (continued)

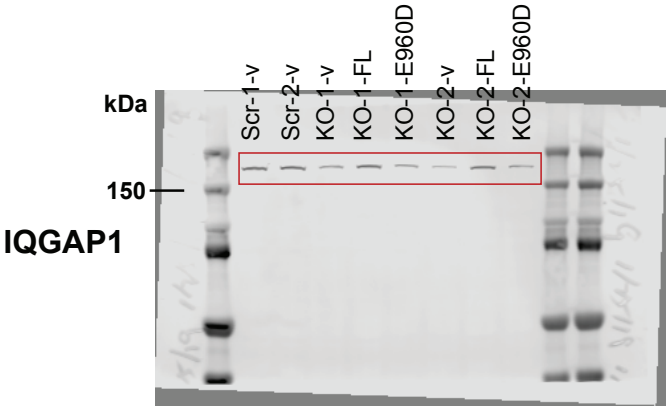
Full-Length Blots Figure 6a

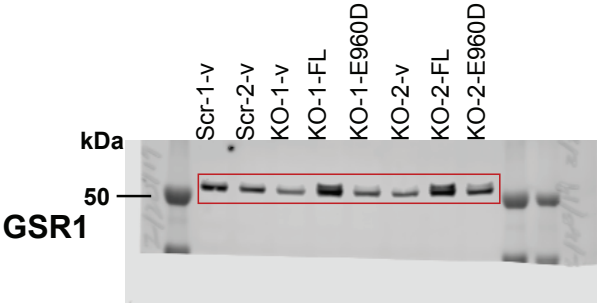


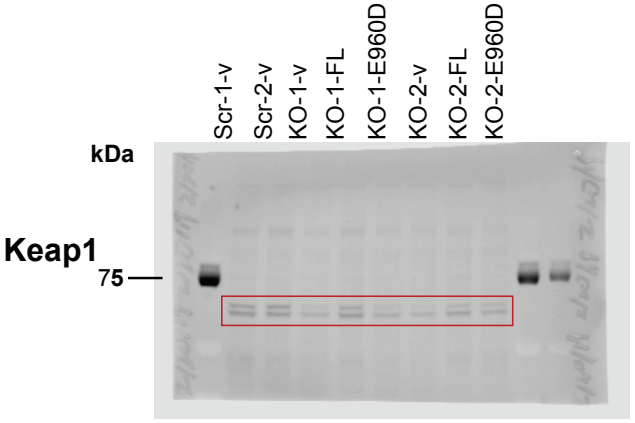


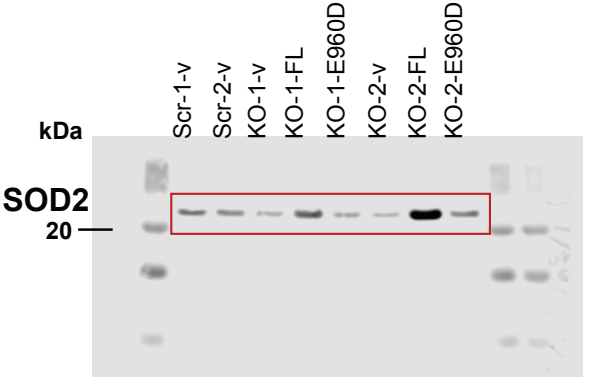






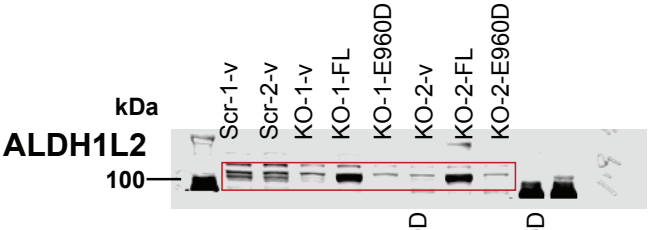


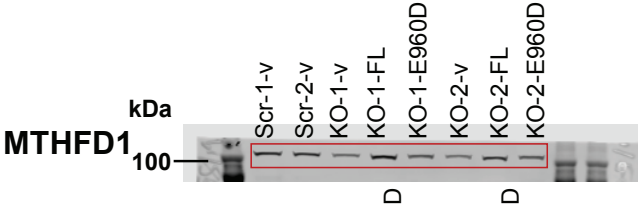


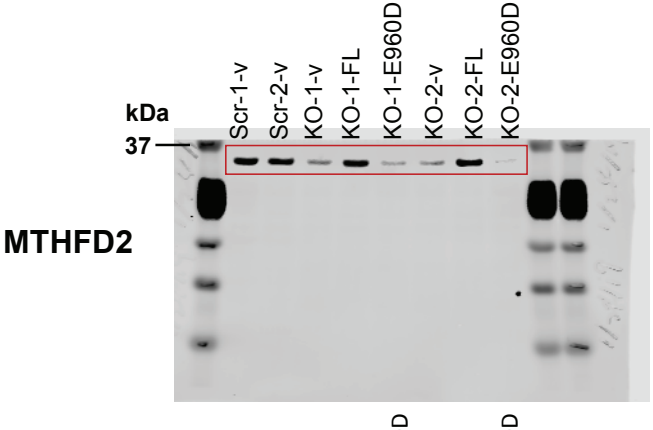


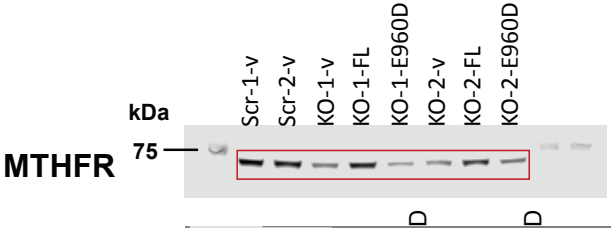
Supplementary Figure S3 (continued)

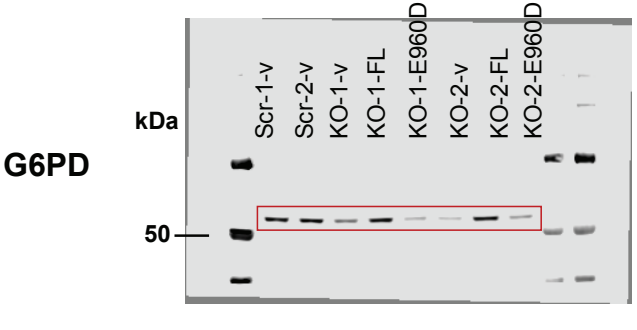
Full-Length Blots Figure 6a (continued)

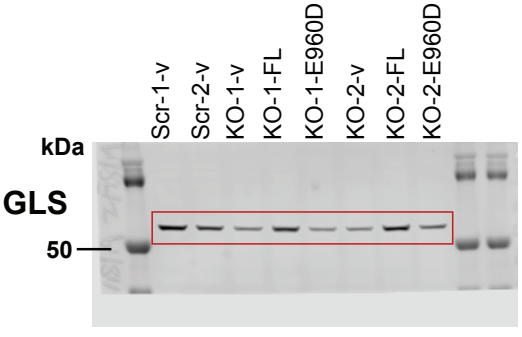


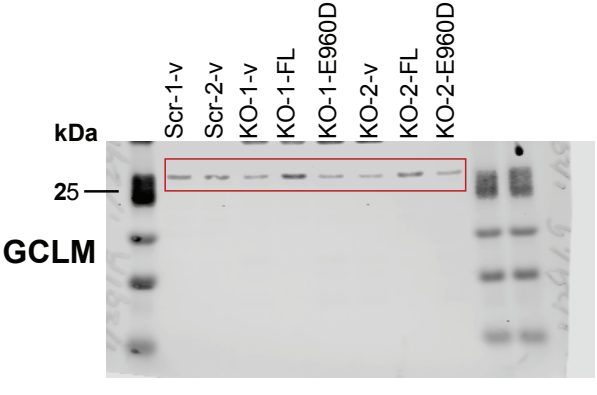


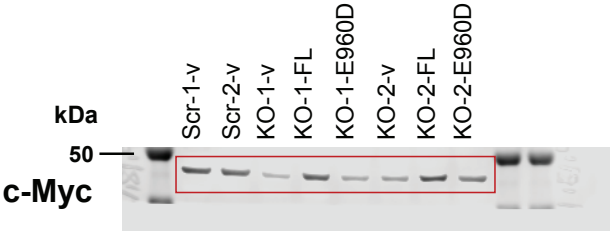


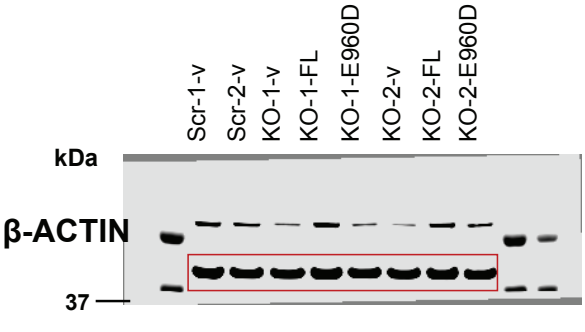






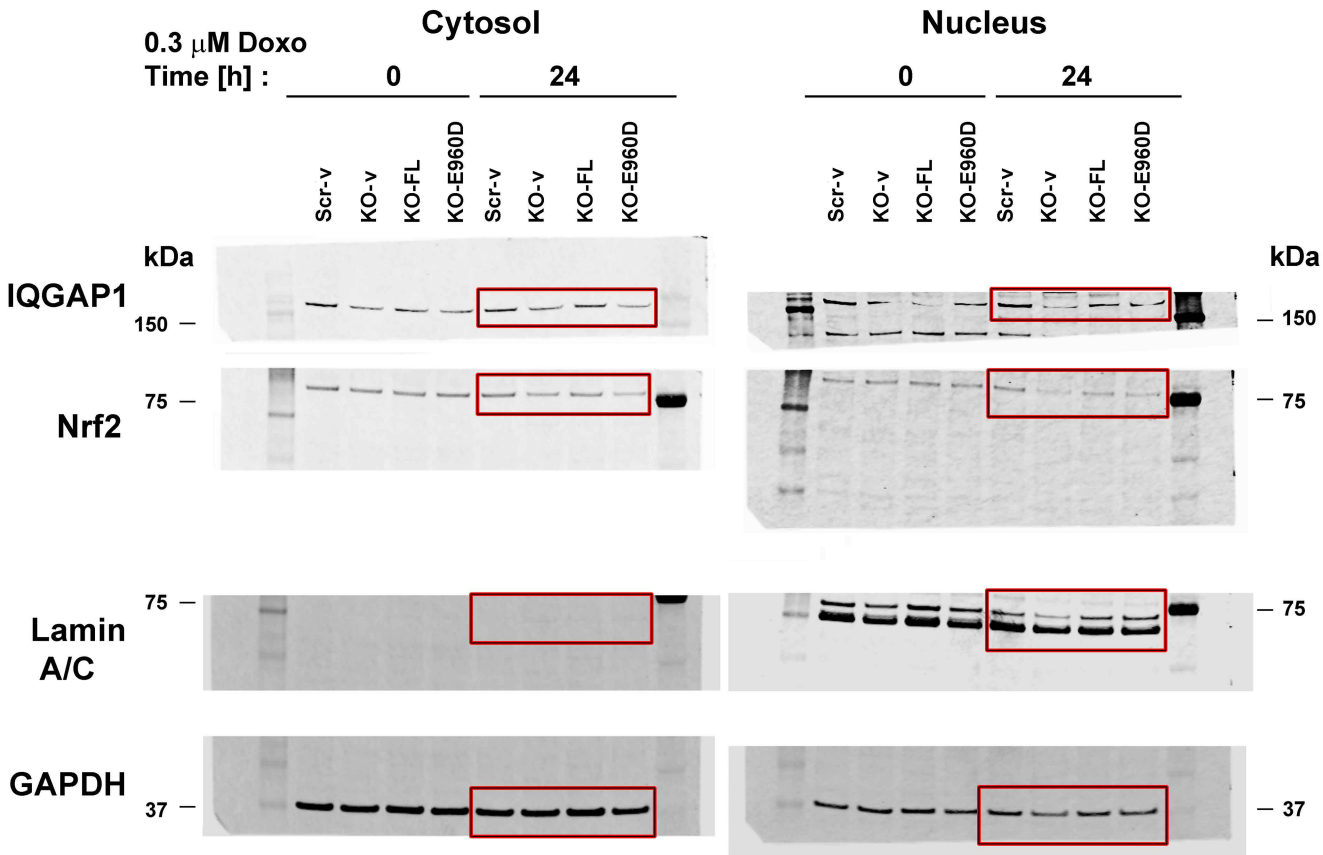






Supplementary Figure S3 (continued)

Full-Length Blots Figure 6b



Supplementary Figure S3 (continued)

Full-Length Blots Figure Supplementary S2