

Supplementary figure 1. Controls confirming the specificity of the anti-Keap1 antibody. (A). Western blot. 10 µg of NIH3T3 cell extract were loaded per lane and resolved on a 5-20% gradient gel. The blot was probed with an anti-Keap1 antibody (lane 1) or preimmune serum derived from the rabbit used to produce the anti-Keap1 antibody (lane 2). Molecular weight standards are shown on the left. As shown previously (40), the anti-Keap1 antibody recognizes a doublet with an apparent molecular weight of 67 kDa. (B). Immunofluorescence using preimmune serum. NIH3T3 cells were fixed with methanol and stained with preimmune serum followed by FITC-conjugated anti-rabbit antibody. No significant non-specific staining is observed. (C). Western blot of extracts derived from NIH3T3 cells expressing Keap1-GFP constructs. Please see Figure 4 for a schematic of the constructs. A blot probed with an anti-Keap1 antibody is shown on the left and the same blot probed with an anti-GFP antibody is shown on the right. The anti-Keap1 antibody is specific for the kelch repeats and therefore only recognizes constructs

containing this domain (NES-Kelch-GFP, Kelch-GFP and Keap1-GFP, marked with * in the right panel) in addition to endogenous Keap1. The specificity of the anti-Keap1 antibody for GFP-tagged Keap1 is also depicted using immunoflurescence in Figure 7.