Zinc-binding triggers a conformational-switch in the cullin-3 substrate adaptor protein KEAP1 that controls transcription factor NRF2

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SUPPORTING INFORMATION:

SUPPLEMENTAL FIGURE LEGENDS:

Figure S1: **A series of cartoons modelling the Keap1 homodimer, either alone or complexed with Cul3-Rbx1. A** and **B**), side-on and top-down views of the Keap1 homodimer. The three major domains of Keap1, the BTB- (amino-acids 60-176), BACK- (in gold; amino-acids 177-327) and Kelch (amino-acids 328-624), are indicated. Residues that are important for recognizing 'danger' signals are depicted as sticks; the NO Sensor present in the BTB domain comprises five basic residues (H129, K131, R135, K150 and H154; all shown in blue) and C151 (yellow). H225, C226 and C613 (all in red) contribute to the Zn^{2+} Sensor and, C288 (green) is involved in sensing alkenals. Finally, a triad of space-filled arginine residues, R380, R415 and R483 (purple), are present on the top face of the Kelch domain, where they make important contacts with transcription factor Nrf2. **C** and **D**), model of the $CRL3^{Keap1/Keap1}$ complex (side-on and top-down representations). Cul3 is in black; Rbx1 in red. $CRL3^{Keap1/Keap1}$ can recruit a Ub-charged E2 enzyme through Rbx1; to represent this, a model of Ub~UbcH7 has been docked upon $CRL3^{Keap1/Keap1}$. UbcH7 is coloured light-teal; Ub is surface-rendered in dark-teal, excepting residue G76, which is in orange. In order for Nrf2 to become ubiquitylated, it must bind simultaneously to the two arginine triads present upon the two Kelch domains in the complex. For this purpose, the Neh2 domain of Nrf2 is equipped with two Keap1-binding sites (ETGE and DLG motifs; written in purple). See text for further details. Modelling was carried out as described in the Materials and Methods; all images were rendered in PyMOL.

Figure S2: Validating *in vivo*-cross-linking assay for Keap1-Cul3 interaction. A & **B**, The indicated combinations of proteins were transiently expressed in COS1 cells. *In vivo* formaldehyde cross-linking of proteins was performed, followed by purification of his-tagged proteins. Input and pull-down samples were blotted with the indicated antibodies.

Figure S3: Validation of the Keap1-based FRET reporter. **A** & **B**, The indicated combinations of proteins were transiently expressed in COS1 cells. The next day, EGFP-tagged proteins were immunoprecipitated. Input and IP samples were blotted with the specified antibodies. **C**, COS1 cells were transiently transfected with plasmid(s) encoding: a Keap1FRET reporter molecule, a derivative thereof bearing a non-fluorescent mutant EYFP, or a combination of the mutant reporter plus EYFP. For each case, the FRET efficiency was measured in 40 cells. The graph displays the mean ± SEM. **D** & **E**, The FRET efficiency displayed by Keap1FRET, or variants thereof, was measured in COS1 cells before (control) or 30 min post-treatment with 100 μM Acro. In each case, data were collected over three independent experiments and are presented as mean ± SEM (n = 117 – 120 cells). Data was analysed by two-way ANOVA with a Bonferroni post-test. *, $P < 0.001$.

Figure S4: A model for the operation of the Keap1-Nrf2 system in healthy and stressed cells. A) A Molecular Interaction Map depicting the Nrf2-Keap1 system based on the data presented in this paper and elsewhere in the literature. Nrf2 is assumed to shuttle rapidly backwards and forwards between the nucleus and the cytoplasm, in which compartment it constitutively engages with the BCR^{Keap1} ubiquitin ligase complex (the

Nrf2:BCR^{Keap1} complex is represented by node *x*). **B**) In healthy cells, Nrf2 is ubiquitylated as a consequence of this interaction, and rapidly proteolyzed by the 26S proteasome (null symbol). **C**) However, in stressed cells, endogenous 'danger' signals are unavoidably produced and modify the Keap1 adaptor protein. 'Danger' signals include free Zn^{2+} liberated from damaged proteins, and α,β-unsaturated aldehydes, such as 4-hydroxy-2-nonenal and acrolein, that commonly arise from lipid peroxidation. These modifications to Keap1 suffice to inhibit ubiquityation of Nrf2, causing it to rapidly accumulate and induce numerous Antioxidant Response Elements (ARE)-bearing genes. The ensuing broad-based adaptive response boosts the cells resilience and ability to survive injurious conditions. Note that some of the products of Nrf2 target genes, such as GstA4 and MT-I, might have evolved specifically to moderate the 'danger' signals, which are themselves potentially toxic. Note moreover that in addition to α,β-unsaturated aldehydes, which covalently bind to Keap1 through Michael addition-type reactions with Cys-151, Cys-273, and Cys-288, other types of electrophiles, both endogenous and exogenous, are also recognized by Keap1, and additional residues, including Cys-226, Cys-613, and Cys-434, have also been implicated on these processes. **D**) Finally, unlike the small-molecule 'danger' signals, the autophagy adaptor protein p62 actually competes with Nrf2 for binding to Keap1. Therefore, conditions that lead to p62 accumulation also stabilizes Nrf2 and initiate a cytoprotective response.