Supplementary Data

Supplementary Materials and Methods

Nanospray ionization liquid chromatography tandem mass spectrometry analysis

For nanospray ionization liquid chromatography tandem mass spectrometry (nLC-MS/MS) analysis, proteins were digested in a solution as described previously, with minor modifications (14). Briefly, samples were 1:1 (v/v) diluted with 8.0 *M* urea in 10 m*M* Tris pH 8.0 (final volume < 100 μL). Proteins were reduced and alkylated with 1 m*M* dithiothreitol and 1 m*M* iodoacetamide for 1 h in two successive steps at room temperature (RT). A two-step enzymatic digestion was performed. First, proteins were digested by addition of 1 μg lysyl endopeptidase (Lys-C) (Wako Chemicals) for 3 h at RT. After dilution of the sample with 50 m*M* ammonium bicarbonate to a final concentration of 2 *M* urea, proteins were further digested overnight at 37°C by 1 μg trypsin (Promega). Reactions were terminated by adding trifluoroacetic acid to a final concentration of 1%. Samples were desalted and concentrated using Stage tips (13). nLC-MS/MS analysis was performed as described previously (18). Chromatographic separations were performed using an in-house packed reversed-phase C18 column on an Agilent nanoflow 1100 liquid chromatograph. The nano-LC was coupled on-line *via* a nanoelectrospray ion source (Thermo Fisher Scientific) to a 7T linear ion trap Fourier transform ion cyclotron resonance mass spectrometer (LTQ FT Ultra, Thermo Fisher Scientific). Mass spectrometric data files were searched using the database search program Mascot (Matrix Science Inc., version 2.2). The database used for the searches consisted of the human RefSeq database (release 33) with addition of the sequences of the TAP proteins used in this study and known contaminants such as trypsin and Lys-C. An in-house developed script was used to parse first-ranked peptides from the Mascot search HTML files, and evaluate peptide and protein identifications.

Digital-imaging microscopy of roGFP fluorescence

Digital-imaging microscopy of roGFP fluorescence was performed as described previously (15). Pairs of images were captured at 535 nm emission after alternate excitation at 400 and 480 nm every 5 s. After 3 min, cells were successively treated with 1 m*M* H_2O_2 and 10 m*M* dithiothreitol. For each image of a mito-roGFP pair, a mitochondrial mask was generated and applied to the corresponding background corrected image to calculate the mean fluorescence intensity per mitochondrial pixel (6). Similarly, the mean fluorescence intensity per cytosolic pixel was calculated for each background corrected image of the cyto-roGFP pair. Finally, for each image pair, the 400 nm (oxidized state)/480 nm (reduced state) fluorescence intensity ratio was calculated and the values obtained with H_2O_2 (100% oxidation) and dithiothreitol (100% reduction *i.e.*, 0% oxidation) were used to determine the percentage oxidation. Image processing and analysis were performed using Image Pro Plus 5.1 (Media Cybernetics).

Cell lines for inducible expression of AcGFP1- and TAP-tagged BOLA1 and TAP-tagged GLRX5

HEK293 cells stably expressing AcGFP1-tagged GLRX5 (GLRX5-GFP) and BOLA1 (BOLA1-GFP) under an inducible promoter were generated using the Gateway system (Invitrogen). GLRX5 (NG_021217) was cloned from a human heart cDNA bank (Clontech) with in frame 5′ and 3′ Gateway AttB-sites. The primers used contained partial AttB-sites (underlined) and gene specific nucleotides (forward: 5′-AA AAA GCA GGC TTC GCC ACC ATG AGC GGG TCC CTC GGC CGA GC-3′; reverse: 5′-A GAA AGC TGG GTG CTT GGA GTC TTG GTC TTT C-3′. AttB-sites were completed using AttB-adapter primers (forward: 5′-G GGG ACA AGT TTG TAC AAA AAA GCA GGC T-3′; reverse: 5′-GGG GAC CAC TTT GTA CAA GAA AGC TGG GT-3′). The PCR product was recombined with pDONR201 to yield the GLRX5 entry vector. The BOLA1 entry vector (BC_063405; Clone 5190565) was purchased from Open Biosystems. Entry vectors were recombined with the AcGFP1-Destination vector and used for stable expression of C-terminally-tagged protein in T-REx™ Flp-In™ HEK293 cells (3). Similarly, entry vectors were recombined with a TAP-Destination vector to produce inducible TAP expression vectors (16). All constructs were verified by sequence analysis. Expression was induced by addition of doxycycline (1 μg/mg, 24h).

Supplementary References

1. Beyer TA, *et al.* Impaired liver regeneration in Nrf2 knockout mice: role of ROS-mediated insulin/IGF-1 resistance. *EMBO J* 27: 212–223, 2008.
2. Cheng NH, *et al.* AtGRXcp, an Arabidopsis chloroplastic glutaredoxin, is critical for protection against protein oxidative damage. *J Biol Chem* 281: 26280–26288, 2006.
3. Dieteren CE, Koopman WJ, and Nijtmans LG. Chapter 7 tracing human mitochondrial complex I assembly by use of GFP-tagged subunits. *Methods Enzymol* 456: 133–151, 2009.
4. Goldberg AV, *et al.* Localization and functionality of microsporidian iron-sulphur cluster assembly proteins. *Nature* 452: 624–628, 2008.
5. Koopman WJ, Verkaart S, Visch HJ, van der Westhuizen FH, Murphy MP, van den Heuvel LW, Smeitink JA, and Willems PH. Inhibition of complex I of the electron transport chain causes $O_2^{-\bullet}$-mediated mitochondrial outgrowth. *Am J Physiol Cell Physiol* 288: C1440–C1450, 2005.
6. Koopman WJ, *et al.* Computer-assisted live cell analysis of mitochondrial membrane potential, morphology and calcium handling. *Methods* 46: 304–311, 2008.
7. Li H, *et al.* Histidine 103 in Fra2 is an iron-sulfur cluster ligand in the [2Fe-2S] Fra2-Grx3 complex and is required for *in vivo* iron signaling in yeast. *J Biol Chem* 286: 867–876, 2010.
8. Li J, *et al.* Proteomic analysis of mitochondria from *Caenorhabditis elegans. Proteomics* 9: 4539–4553, 2009.
9. Meja KK, *et al.* Curcumin restores corticosteroid function in monocytes exposed to oxidants by maintaining HDAC2. *Am J Respir Cell Mol Biol* 39: 312–323, 2008.
10. Nojiri H, *et al.* Oxidative stress causes heart failure with impaired mitochondrial respiration. *J Biol Chem* 281: 33789–33801, 2006.
11. Pagliarini DJ, *et al.* A mitochondrial protein compendium elucidates complex I disease biology. *Cell* 134: 112–123, 2008.
12. Rada P, *et al.* The monothiol single-domain glutaredoxin is conserved in the highly reduced mitochondria of *Giardia intestinalis. Eukaryot Cell* 8: 1584–1591, 2009.
13. Rappsilber J, *et al.* Detection of arginine dimethylated peptides by parallel precursor ion scanning mass

spectrometry in positive ion mode. *Anal Chem* 75: 3107–3114, 2003.
14. Tjalsma H, *et al.* Proteomic inventory of "anchorless" proteins on the colon adenocarcinoma cell surface. *Biochim Biophys Acta* 1764: 1607–1617, 2006.
15. Verkaart S, Koopman WJ, Cheek J, van Emst-de Vries SE, van den Heuvel LW, Smeitink JA, and Willems PH. Mitochondrial and cytosolic thiol redox state are not detectably altered in isolated human NADH: ubiquinone oxidoreductase deficiency. *Biochim Biophys Acta* 1772: 1041–1051, 2007.
16. Vogel RO, *et al.* Cytosolic signaling protein Ecsit also localizes to mitochondria where it interacts with chaperone NDUFAF1 and functions in complex I assembly. *Genes Dev* 21: 615–624, 2007.
17. Wang X, *et al.* Genome-wide transcriptome profiling of region-specific vulnerability to oxidative stress in the hippocampus. *Genomics* 90: 201–212, 2007.
18. Wessels HJ, *et al.* LC-MS/MS as an alternative for SDS-PAGE in blue native analysis of protein complexes. *Proteomics* 9: 4221–4228, 2009.