Supplementary Materials and Methods

Redoxome quantitative analysis

Cells were plated in 6-well plates at density of 350 000 cells per well (2 ml volume of medium) and allowed to attach. After different treatments cells were washed 3 times with 1 ml ice-cold PBS, 50 mM NEM. One ml/well 20% TCA was added and the 6-well plates were incubated on ice. Cellular extracts were centrifuged for 20 min at 14000 rpm at 4°C and the pellet was washed 3 times with cold acetone and dried using an Eppendorf Vacufuge Concentrator. Dried pellet was resuspended in 70 µl TES (100 mM Tris-HCl, 1% SDS, 10 mM EDTA, pH 8.8) containing 100 mM NEM, and protein concentration was determined using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific).

Purification of proteins containing oxidized thiols was performed according a published protocol with slight modifications [1]. (A) Labeling oxidized thiols with EZ-Link Biotin-HPDP: 3 mg proteins were precipitated with 10% TCA, washed twice with cold acetone, dried and resuspended in 1200 µl TES + 20 DTT to reduce the oxidized thiols. Non-bond DTT was removed by repeating the precipitation and wash steps. Dried Pellet was resuspended in 300 µl TES + 0.4 mM EZ-Link Biotin-HPDP (Thermo Fisher Scientific) to allow labeling of newly reduced thiols. Non-bond biotin is eliminated as done for non-bound DTT. Dried pellet was resuspended in 500 µl TES and protein concentration was determined. (B) Purification of biotinylated proteins: For purification of the biotin-tagged proteins, 3 mg of proteins are incubated with NeutrAvidin Agarose Resins (Thermo Fisher Scientific). Prior to incubation, resin equilibration was done according to manufacturer instruction using TES. The non-bond fraction is collected and stored at –80°C. After 5 washes with TES, the biotynilated fraction is eluted by adding 500 µl TES + DTT 20 mM. The fraction is concentrated by precipitation, washed and resuspended in TES and protein concentration is determined using Pierce BCA Protein Assay Kit.

20 µg of proteins prepared from cells in the various conditions investigated were low migrated on a NuPAGE Novex 4–12% Bis-Tris Protein Gels (Thermo Fisher Scientific), stained in Coomassie blue (SimplyBlue Safestain, Thermo Fisher Scientific) and fixed with a solution containing 50% ethanol, 10% MeCOOH and 40% H_2O. In-gel digestion was performed using sequencing grade trypsin (12.5 µg/ml; Promega) overnight in 25 mM NH_4HCO_3 at 37°C. Protein digests were analyzed in triplicate with an Orbitrap Fusion Tribrid coupled to a Nano-LC

Proxeon 1000 equipped with an EASY-Spray ion source (all from Thermo Fisher Scientific). Label-free liquid-chromatography tandem mass spectrometry (LC-MS/MS) acquisition was performed with a 2-h gradient. Peptides were separated by chromatography with the following parameters: Acclaim PepMap100 C18 pre-column, Pepmap-RSLC Proxeon C18 column, 300 nl/min flow rate, gradient from 95% solvent A (H_2O, 0.1% HCO_2H) to 35% solvent B (100% MeCN, 0.1% HCO_2H) over a period of 97 minutes, followed by column regeneration for 23 min. Peptides were analyzed in the Orbitrap cell, in full ion scan mode, at a resolution of 120,000, with a mass range of m/z 350-1550 and an AGC target of 4×10^5. Fragments were obtained by HCD with a collisional energy of 30, and a quadrupole isolation window of 1.6 Da. MS/MS data were acquired in the Orbitrap cell in the top-speed mode, with a total cycle of 3 s at a resolution of 30,000, with an AGC target of 1×10^4. Peptides with charge states from 2 to 8 were selected for MS/MS acquisition. MS/MS data were processed with an in-house Proteome Discoverer (v 2.1) using Sequest search node. The following variable modifications (2 maximum per peptide) were allowed: oxidation (Met), phosphorylation (Ser, Thr, Tyr), acetylation (Protein N-term), carbamidomethylation (Cys) N-Ethylmaleimide (C), biotin-HPDP (Cys). The mass tolerance was set to 7 ppm for precursor ions and 0.5 Da for fragments. Label-free quantification in between subject analysis was performed on raw data with Progenesis-Qi software 3.0 (Nonlinear Dynamics Ltd, Newcastle, UK) using the following procedure: (i) chromatograms alignment, (ii) peptide abundances normalization, (iii) statistical analyses of features, and (iv) peptides identification using the Proteome discoverer (v 2.1) software (Thermo Fisher Scientific). All results were 1% FDR filtered before exporting. The resulting files were imported into Progenesis-Qi software for report edition. Variations of protein abundance were considered as validated if their Anova *P* values were lower than 0.05.

References

1. Liu P, Zhang H, Wang H, Xia Y. Identification of redox-sensitive cysteines in the Arabidopsis proteome using OxiTRAQ, a quantitative redox proteomics method. Proteomics 2014, 14(6):750-762.