Sample Preparation

Whole *Arabidopsis thaliana* leaves were immediately shock frozen in liquid nitrogen after harvesting and weighing. A Retsch MM 200 ball mill (Verder, Haan, Germany) was applied for pulverization of the plant leaves. The cells were dissolved in 1% sodium dodecyl sulfat (BioXtra, ≥99.0% (GC), Sigma Aldrich Chemie (Steinheim, Germany)) containing lysis buffer (150 mM sodium chloride (Fluka Chemie AG (Buchs, Switzerland)), 50 mM Tris(hydroxymethyl)aminomethane (research grade, Serva Electrophoresis (Heidelberg, Germany)), pH 7.8). Whole cell lysates were prepared by ultrasonic trifluoroethanol-solubilization and (Fluka Analytical, Buchs, Schweiz) in a Bioruptor (Diagenode Inc, New Jersey, USA), followed by 50 mM tris-(2-carboxyethyl)phosphine (Sigma Aldrich Chemie (Steinheim, Germany) reduction at 60 °C for one hour in a Thermomixer comfort (Eppendorf, Hamburg, Germany). The alkylation of cystein residues was carried out in 200 mM iodoacetamide (Bioultra , Sigma Aldrich) at RT for 30 min under protection against light. To extract the proteins, an ethanol (Chromasolv plus, for HPLC, ≥99.9%, Sigma Aldrich) precipitation was performed overnight at -20 °C. The samples were centrifuged at 4 °C and 14,000 rpm for 5 min in a Hermle zentrifuge (Hermle Labortechnik GmbH, Wehringen, Germany). After discarding the supernatant, the airdried pellets were solved in 100 µl 0.5 M triethylammoniabicarbonate (TEAB) (Sigma Aldrich).

Before digestion, the amount of protein in the extracts was determined via Bradford assay using the Infinite 200Pro (Tecan Group Ltd, Männedorf, Switzerland) instrument. The samples were digested with Sequencing Grade Modified Trypsin, Porcine (Promega) in a protein:enzyme ratio of 100:1 at 37 °C over night. The obtained peptides were purified using Pierce C-18 Spin Columns (Thermo Scientific, Rockford, USA) according to the manufacturer's protocol. The eluted peptides were completely dried in a Vacuum Concentrator and resolved in 0.050% aqueous trifluoroacetic acid to a final concentration of 0.5 µg/µl.

Liquid Chromatography

Peptides were separated via IP-RP-HPLC on an UltiMate 3000 system (Dionex, Germering, Germany) using a poly-(styrene/divenylbenzene) separation column (150 mm x 0.2 mm, prepared in house[17]) after partially injecting of 5 µL sample. The separations were performed at 45 °C at a flow rate of 1 µL/min and a linear gradient of 0 – 40 % acetonitrile (Chromasolv Gradient grade, for HPLC, 99.9 %) in 0.050 % aqueous TFA in 300 min. The chromatographic measurements were performed using the software Chromeleon® Chromatography Data System, version 7.1.0.898 from Dionex Corporation

Mass Spectrometry

The HPLC system was hyphenated to a LTQ Orbitrap XL mass spectrometer (Thermo Scientific) via an electrospray source (Thermo Scientific) and a PicoTip (10 µm I.D., New Objective) emitter. The measurements were performed in positive ionization mode and a heated capillary temperature of 250 °C and the voltage was set to 1.4 kV. During the data acquisition of 300 min, MS^1 FTMS full scans were acquired from 450-2000 m/z at a resolution of 60,000 with an automated gain control (AGC) of 1E6 and a maximum ion injection time of 100 ms. Peptides were identified using the five most intense precursor ions for fragmentation by Collision Induced Dissociation (CID) with a normalized collision energy of 35.00 % using an isolation window of 2 Da, AGC 10,000 with 100 ms maximum injection time and a dynamic exclusion window +/-10 ppm of 30 s duration. Singly charged ions were excluded. For analysis of the massspectrometric measurements the software Xcalibur®, version 2.0.7 SP1 from Thermo Fisher Scientific Inc. was used.