Supplementary file 1. Detailed methodology for the proteome analysis.

Protein extraction and digestion

Homogenized root tissue (200 mg)was suspended in extraction buffer (50 mM HEPES-KOH pH 8.0, 100 mM NaCl, and 4 % (w/v) SDS at a 1:2 (w/v) ratio and protein was extracted by agitation at 1000 RPM at 95^{o}C for 10 min using an Eppendorf tabletop shaker. Samples were then centrifuged at 20,000 x g for 5 min at room temperature and the supernatants were transferred to new tubes. The protein extracts were then quantified by bicinchoninic acid (BCA) assay (23225; Thermo Scientific, ON, Canada). For each sample, 200 µg of extracted protein was reduced with 10 mM dithiothreitol (DTT), followed by alkylation with 30 mM Iodoacetamide (IA) for 30 min in the dark at room temperature. Total proteome peptide fractions were then generated using a KingFisher APEX (Thermo Scientific, ON, Canada) automated sample processing system as outlined by Leutert et al [1]. Proteins were then digested using sequencing grade trypsin (V5113; Promega, WI, USA) that was suspended in 50 mM triethylammonium bicarbonate buffer (TEAB, Sigma, ON, Canada) pH 8.5. Following digestion, samples were acidified with trifluoroacetic acid to a final concentration of 0.5 % (v/v). Peptides were desalted as previously described [2] using an Opentrons (OT-2) liquid handling robot mounted with OMIX C18 pipette tips (A5700310; Agilent, CA, USA). Desalted peptides were dried and stored at -80^{o}C prior to re-suspension in 3.0 % (v/v) acetonitrile (ACN) / 0.1 % (v/v) FA prior to MS injection.

Nanoflow liquid chromatography with tandem mass spectrometry analysis

Peptides were measured using a Fusion Lumos Tribrid Orbitrap mass-spectrometer (Thermo Scientific) utilizing a BoxCarDIA data acquisition scheme as previously described [3,4]. Using an Easy-nLC 1200 system, 1 µg of digested peptide was injected for each sample (LC140; Thermo Scientific) and separated using a 25 cm Easy-Spray PepMap C18 column (ES902; Thermo Scientific). The column was equilibrated using 100% of solvent A (0.1%), and segmoidal solvent B gradient (80% ACN/0.1% FA) from 4 to 41% B (0–65 min) was utilized for sample elution as previously described [4]. To perform MS^1 acquisition, two sets of ten BoxCar windows were used for tSIM scanning and these scans covered a range of 350–1400 *m/z*. The precursor acquisition was conducted at a resolution of 120,000 with a normalized automatic gain control (AGC) target of 100% per isolation window. Furthermore, MS^2 detection was accomplished by utilizing twenty-eight 38.5 *m/z* windows with an overlay of 1 *m/z* and a minimum *m/z* of 200. A resolution of 30,000 was used in conjunction with a dynamic maximum injection time and a minimum of six desired points across each peak.

Data processing

BoxCarDIA raw files were analyzed using Spectronaut v16 (Biognosys AG, Schlieren, Switzerland) using a directDIA analysis mode with the default search parameters without the inclusion of an N-acetyl variable modification [3,4]. Spectra were searched against the *B. napus* Westar proteome [5]. The trypsin enzyme specificity was programmed to allow two missed cuts while ensuring a false discovery rate (FDR) for both protein and peptide spectrum matches (PSM) at 1%. The data was filtered by setting a threshold of *q*-value ≤ 0.01, and global normalization was conducted for MS^2-level quantification. To determine the difference in

protein abundance, a significance threshold was set using a Bonferroni correction with a *q*-value < 0.05 and $\log_2$ fold change > 0.50 or < - 0.50.

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