Analytical Platform 1 (ceramide profiling) was conducted by the Nikolau group at the W.M. Keck Metabolomics Research Laboratory, Iowa State University. Plant material (approximately 50 mg) was extracted according to the protocol of (Sullards and Merrill, 2001). Tissue was homogenized with 0.5 mL phosphate buffer (PBS) and 0.5 mL methanol, spiked with 0.5 nmol N-Heptadecanoyl-D-*erythro*-Sphingosine (Avanti Polar Lipids Inc., Alabaster, AL, USA), and the mixture was incubated at 48 ºC for 16 h. After cooling to room temperature, 100 μL of 1 M KOH in methanol was added, the sample was shaken vigorously for 1 min, and incubated at 37 ºC for 2 h. Mixture was then neutralized with glacial acetic acid, and 1.5 mL chloroform and 2 mL water were added, and the mixture was shaken vigorously for 1 min. Following centrifugation (3,400 *g*, 10 min) to achieve phase separation, the lower organic layer was recovered and evaporated to dryness in a 2 mL glass vial using a gentle stream of nitrogen. The recovered metabolites were dissolved in 500 μL acetonitrile, and 35 μL of BSTFA + TMCS were added. Following vigorous shaking for 30 sec, the samples were heated at 100 ºC for 2 h. Mixture was evaporated to dryness using a gentle stream of nitrogen, dissolved in 200 μL chloroform and transferred to a 2 mL autosampler glass vial, fitted with a 200 μL conical glass insert. Following evaporation, the sample was dissolved in an appropriate volume of chloroform for GC-MS analysis.

GC-MS analyses were performed on an Agilent 6890N GC coupled to an Agilent 5973 inert MSD detector. Samples (1 μL per injection) were loaded with an Agilent 7683 G2613A auto-sampler onto a HP-5MS fused silica column (30 m x 250 μm; 0.25 μm film thickness). The initial temperature of the injector and MSD interface were set at 310ºC and 260ºC, respectively. Analytes were separated at a flow rate of 0.7 mL/min using He as carrier gas, using a thermal gradient that started at 70 ºC (2 min), and ramped first to 230ºC at 5 ºC/min, where it was held for 5 min, then to 270ºC at 5 ºC/min, where it was held for 5 min, and finally ramped to 320 ºC at 5ºC/min, where it was held for 15 min. Data were acquired using MSD Chemstation (D.00.01) software. Ceramides were identified and analyzed by their characteristic mass-spectrum as described in (Raith et al., 2000). Peak areas were obtained from the total ion chromatogram, raw data were exported to Microsoft Excel and peak areas were normalized to tissue mass and the internal standard.

Raith K, Darius J, Neubert RH (2000) Ceramide analysis utilizing gas chromatography-mass spectrometry. J Chromatogr A **876:** 229-233

Sullards MC, Merrill AH, Jr. (2001) Analysis of sphingosine 1-phosphate, ceramides, and other bioactive sphingolipids by high-performance liquid chromatography-tandem mass spectrometry. Sci STKE **2001:** PL1