LC-MS Analysis. The LC-MS analyses were performed by strictly following the SOP in both positive and negative ionization with extraction duplicates. A total of 12 sample injections (2 samples with extraction duplicates and injection triplicates per genotype) and 10 quality control injections (a pooled mixture of the extracts of both samples) were performed in each ionization polarity. MS/MS spectra were acquired for all samples for identification. Parameters used for data acquisition are described below.

Instrument:	Thermo Vanquish UHPLC linked to Bruker Impact II QTOF Mass Spectrometer
Column:	Waters Acquity CSH C18 column, 1.7 µm.
MPA:	NovaMT MixA
MPB:	NovaMT MixB
Gradient:	NovaMT 26-min-gradient
Flow Rate:	250 µL/min.
Injection Volume:	5.0 µL for positive ionization and 10.0 µL for negative ionization
Column Oven Temperature:	42 °C
Mass Range:	m/z 150-1500
MS/MS Collision Energies:	10-80 eV

Data Processing. LC-MS data from 22 injections were independently processed in positive and negative ionization. Lipid features were extracted and aligned using NovaMT LipidScreener. The data acquired in positive and negative ionization from each sample extraction were combined, i.e. the detected features from all samples were merged into one feature-intensity table. Missing values were substituted by the average intensity of the sample group for features detected in at least 50% of injections within the group (Nestin Cre, SR Flox and QC); or by the global minimum for all sample and QC injections for features detected in less than 50% of injections within the group. Parameters used for data processing are below.

Minimum Intensity Cut-off:	3000 cts for negative ionization; 3000 cts for positive ionization
Minimum Peak Length:	6 spectra
Retention Time Tolerance	5 seconds
Mass Tolerance:	5 mDa
Feature Filtering:	Detection for ≥80% of injections in at least one sample group (Nes Cre, SRFlox and QC)

Lipid Identification. A three-tier identification approach based on MS/MS identification and MS match was employed for lipid identification. The parameters used for identification are described below.

Tier 1 (MS/MS identification):	MS/MS match score ≥ 500; precursor m/z error ≤ 5 mDa
Tier 2 (MS/MS identification):	MS/MS match score ≥ 100; precursor m/z error ≤ 5 mDa
Tier 3 (MS match):	Mass match with m/z error ≤ 5 mDa

After tier 3 identification, a six-tier filtering and scoring approach embedded in NovaMT LipidScreener was employed to restrict the number of matches and select the best identification option to determine the lipid sub-classes for normalization. Data normalization was performed by using a set of 14 deuterated internal standards belonging to different lipid classes. The positively and putatively identified lipids were matched to one of the 14 internal standards according to lipid class similarity and expected retention time range for each class. Intensity ratios, i.e., intensity of each lipid divided by intensity of the matched internal standard, were calculated for normalization. Statistical analysis was performed with MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/). Non-informative features (i.e., internal standards) and features with low repeatability (RSD >20% for QC injections) were filtered out. The dataset was further normalized by auto-scaling and to the median intensity. Finally, the normalized and auto-scaled features were used for statistical analysis.