Supplementary

Appendix 1

Untargeted Lipidomics (Material and Methods Section)

1. Lipid Extraction

Tissue sample was grounded by liquid nitrogen. Then samples (Plasma and Urea sample can be prepared with buffer directly.) were firstly bath sonicated for 15 min with 400 uL ice-cold 75% Methanol to break up the cells. Next, 1 mL MTBE was added and the samples were shaken for 1 h at room temperature. Next, phase separation was induced by adding 250 uL water, letting sit for 10 min at room temperature and centrifuging for 15 min at 14,000g, 4 ℃. Because of the low density and high hydrophobicity of MTBE, lipids and lipophilic metabolites are mainly extracted to the upper MTBE-rich phase. The lipid was transferred to fresh tubes and dried with air Nitrogen.

Additionally, to ensure data quality for metabolic profiling, Quality control (QC) samples were prepared by pooling aliquots of all samples that were representative of the all samples under analysis, and used for data normalization. QC samples were prepared and analyzed with the same procedure as that for the experiment samples in each batch. Dried extracts were then dissolved in 50% acetonitrile. Each sample was filtered with a disposable 0.22 μm cellulose acetate and transferred into 2 mL HPLC vials and stored at -80°C until analysis.

2. UHPLC-MS/MS analysis

Lipids analysis was performed on Q Exactive orbitrap mass spectrometer (Thermo, CA) coupled with UHPLC system Ultimate 3000 (Thermo Scientific).

Samples were separated using a Hypersil GOLD C18 (100×2.1mm, 1.9μm) (Thermo Scientific). Mobile phase A is prepared by dissolving 0.77g of ammonium acetate to 400ml of HPLC-grade water, followed by adding 600ml of HPLC-grade acetonitrile. Mobile phase B is prepared by mixing 100ml of acetonitrile with 900ml isopropanol. The flow rate was set as 0.3 mL/min. The gradient was 30% B for 0.5 min and was linearly increased to 100% in 10.5 min, and then maintained to 100% in 2 min, and then reduced to 30% in 0.1 min, with 4.5 min re-equilibration period employed. Both electrospray ionization (ESI) positive-mode and negative mode were applied for MS data acquisition. The positive mode of spray voltage was 3.0 kV and the negative mode 2.5 kV. The ESI source conditions were set as follows: Heater Temp 300 ℃, Sheath Gas Flow rate, 45arb, Aux Gas Flow Rate, 15 arb, Sweep Gas Flow Rate, 1arb, Capillary Temp, 350 ℃, S-Lens RF Level, 50%. The full MS scans were acquired at a resolution of 70,000 at m/z 200, and 17,500 at m/z 200 for MS/MS scan. The maximum injection time was set to for 50 ms for MS and 50 ms for MS/MS. MS data was acquired using a data-dependent Top10 method dynamically choosing the most abundant precursor ions from the survey scan (200–1500 m/z) for HCD fragmentation. Stepped Normalized collision energy was set as 15, 25, 35 and the isolation window was set to 1.6 Th. The duty

Quality control (QC) samples were prepared by pooling aliquots of all samples that were representative of the samples under analysis, and used for data normalization. Blank samples (75 %ACN in water) and QC samples were injected every six samples during acquisition.

3. Data preprocessing and filtering

Lipids were identified and quantified using LipidSearch 4.1.30 (Thermo, CA). Mass tolerance of 5ppm and 10ppm were applied for precursor and product ions. Retention time shift of 0.25min was performed in “alignment”. M-score and chromatographic areas were used to reduce false positives. The lipids with less than 30% RSD of MS peak area in QC samples were kept for further data analysis.

4. Multivariate statistical analysis

SIMCAP software (Version 14.0, Umetrics, Umeå, Sweden) was used for all multivariate data analyses and modeling. Data

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were mean-centered using Pareto scaling. Models were built on principal component analysis (PCA), orthogonal partial least-square discriminant analysis (PLS-DA) and partial least-square discriminant analysis (OPLS-DA). All the models evaluated were tested for over fitting with methods of permutation tests. The descriptive performance of the models was determined by R2X (cumulative) (perfect model: R2X (cum) = 1) and R2Y (cumulative) (perfect model: R2Y (cum) = 1) values while their prediction performance was measured by Q2 (cumulative) (perfect model: Q2 (cum) = 1) and a permutation test (n = 200). The permuted model should not be able to predict classes: R2 and Q2 values at the Y-axis intercept must be lower than those of Q2 and the R2 of the non-permuted model. OPLS-DA allowed the determination of discriminating metabolites using the variable importance on projection (VIP). The VIP score value indicates the contribution of a variable to the discrimination between all the classes of samples. Mathematically, these scores are calculated for each variable as a weighted sum of squares of PLS weights. The mean VIP value is 1, and usually VIP values over 1 are considered as significant. A high score is in agreement with a strong discriminatory ability and thus constitutes a criterion for the selection of biomarkers.

The discriminating metabolites were obtained using a statistically significant threshold of variable influence on projection (VIP) values obtained from the OPLS-DA model and two-tailed Student's t test (p value) on the normalized raw data at univariate analysis level. The p value was calculated by one-way analysis of variance (ANOVA) for multiple groups analysis. Metabolites with VIP values greater than 1.0 and p value less than 0.05 were considered to be statistically significant metabolites. Fold change was calculated as the logarithm of the average mass response (area) ratio between two arbitrary classes. On the other side, the identified differential metabolites were used to perform cluster analyses with R package.

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Appendix 2

1. RNA extraction and qualification

1.1 RNA extraction

Total RNA of each sample was extracted using Trizol Reagen (Invitrogen) or RNeasy Mini Kit (Qiagen).

1.2 RNA qualification

1) RNA degradation and contamination was monitored on 1% agarose gels.
2) RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). (OD260/280 =1.6~1.8)

2. Library preparation for Transcriptome sequencing

A total amount of 1 µg RNA per sample was used as input material for the RNA sample preparations.

2.1 Library construction

Sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer's recommendations and index codes were added to attribute sequences to each sample.

1) mRNA purification: Briefly, mRNA was purified from total RNA using poly-Toligo-attached magnetic beads.
2) Fragmentation: Fragmentation was carried out using divalent cations under elevated temperature in NEBNext First StrandSynthesis Reaction Buffer (5X).
3) First strand cDNA synthesis: First strand cDNA was synthesized using randomhexamer primer and M-MuLV Reverse Transcriptase (RNase H-).
4) Second-strand cDNA synthesis: Second-strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H.
5) adaptor ligation: Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of 3' ends of DNA fragments, NEBNext Adaptor with hairpin loop structure were ligated to prepare for hybridization.
6) Library purification: In order to select cDNA fragments of preferentially 350 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA).
7) Library amplification : Then 3 µl USER Enzyme (NEB, USA) was used with size-selected, adaptor-ligated cDNA at 37 ℃ for 15 min followed by 5 min at 95 ℃ before PCR. Then PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer.

2.2 Library pure and quality control

At last, PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system, and quantified by Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA) .

3. Clustering and sequencing (Novogene Experimental Department)

The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumia) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina Hiseq platform and paired-end reads were generated.

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