Untargeted FTICR-MS Based Plasma Metabolomic Analysis and Translation to Type 2 Diabetes

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Overview

- ❖ We introduced a high-throughput, highly reproducible FTICR-MS based platform for plasma metabolomics.
- ❖ Rapid untargeted plasma metabolic phenotyping identifies both Type 2 diabetes fingerprint and specific metabolic changes.
- ❖ Mouse plasma samples were used for development of FTICR-MS based platform for metabolomics.
- ❖ Plasma samples from diabetes-susceptible human were analyzed via developed FTICR-MS based platform.

Introduction

Type 2 diabetes (T2D) is an extremely prevalent metabolic disorder that negatively affects the health of hundreds of millions of people worldwide. A number of circulating metabolites have been positively associated with T2D status in both pre-clinical models and human populations. However, it is unclear whether the observed metabolic changes are a consequence of high glucose levels and therefore of the T2D or if the metabolic changes are causative and lead to the development of T2D. Here, we report a high-throughput ultra-high resolution magnetic resonance mass spectrometry (MRMS) based platform for plasma metabolite fingerprinting, which provides broad metabolic profiles in minutes. This new platform was used as a first-pass tool to investigate the fundamental changes in metabolism associated with T2D.

Methods

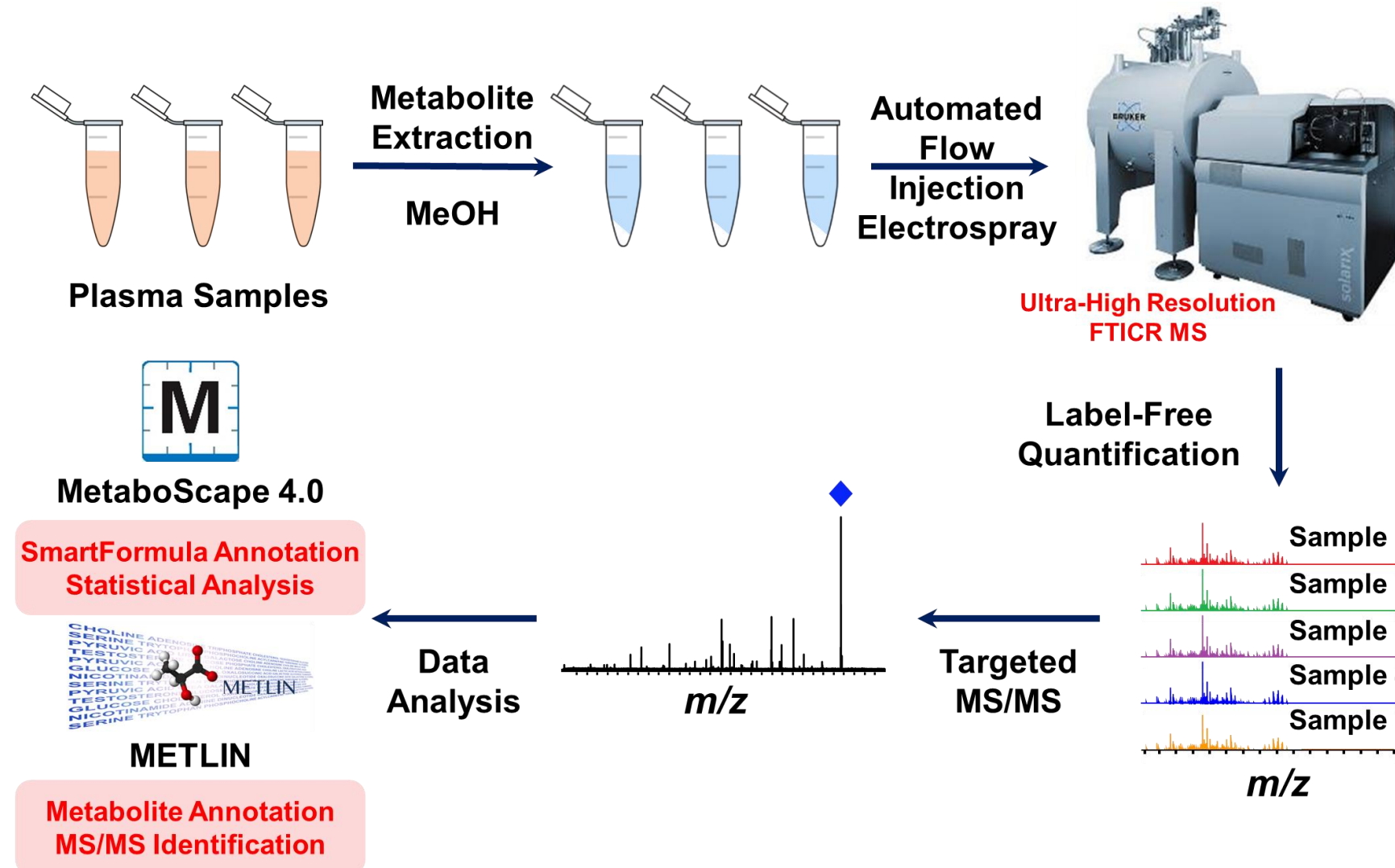### FTICR-MS Based Plasma Metabolomic Analysis

Figure 1. Workflow of FTICR-MS based platform for plasma metabolomics. Metabolite extracts were directly injected into FTICR-MS without LC separation. MS: Bruker solariX 12T FTICR mass spectrometer.

Sample Information

1. Mouse Plasma Samples:
Obese mice with Purina diet and severely insulin resistant were used for development of FTICR-MS based platform for plasma metabolomics.

2. Human Plasma Samples:
Control (n=14); T2D-DL (n=6); T2D-M (n=9)
Diet/Lifestyle; Metformin

Data Analysis Parameters

MetaboScape 4.0 Bucket list: T-ReX 2D Algorithm
mzDelta: 0.5 mDa
Max. Charge: 3
Intensity Threshold: 0
Minimum # Features for Results: 5
Positive Mode: +H, +NH$_4$, +Na, +H-H$_2$O
Negative Mode: -H, +Cl

MetaboScape 4.0 SmartFormula Annotation:
Mass Accuracy: $\Delta m/z < 2$ ppm; $\Delta m/z < 5$ ppm
mSigma* ($\Delta m/z < 2$ ppm): < 20; < 50

*Isotopic pattern fit score

METLIN Annotation:
$\Delta m/z < 2$ ppm

Results and Discussion

FTICR-MS Based Platform for Metabolomics
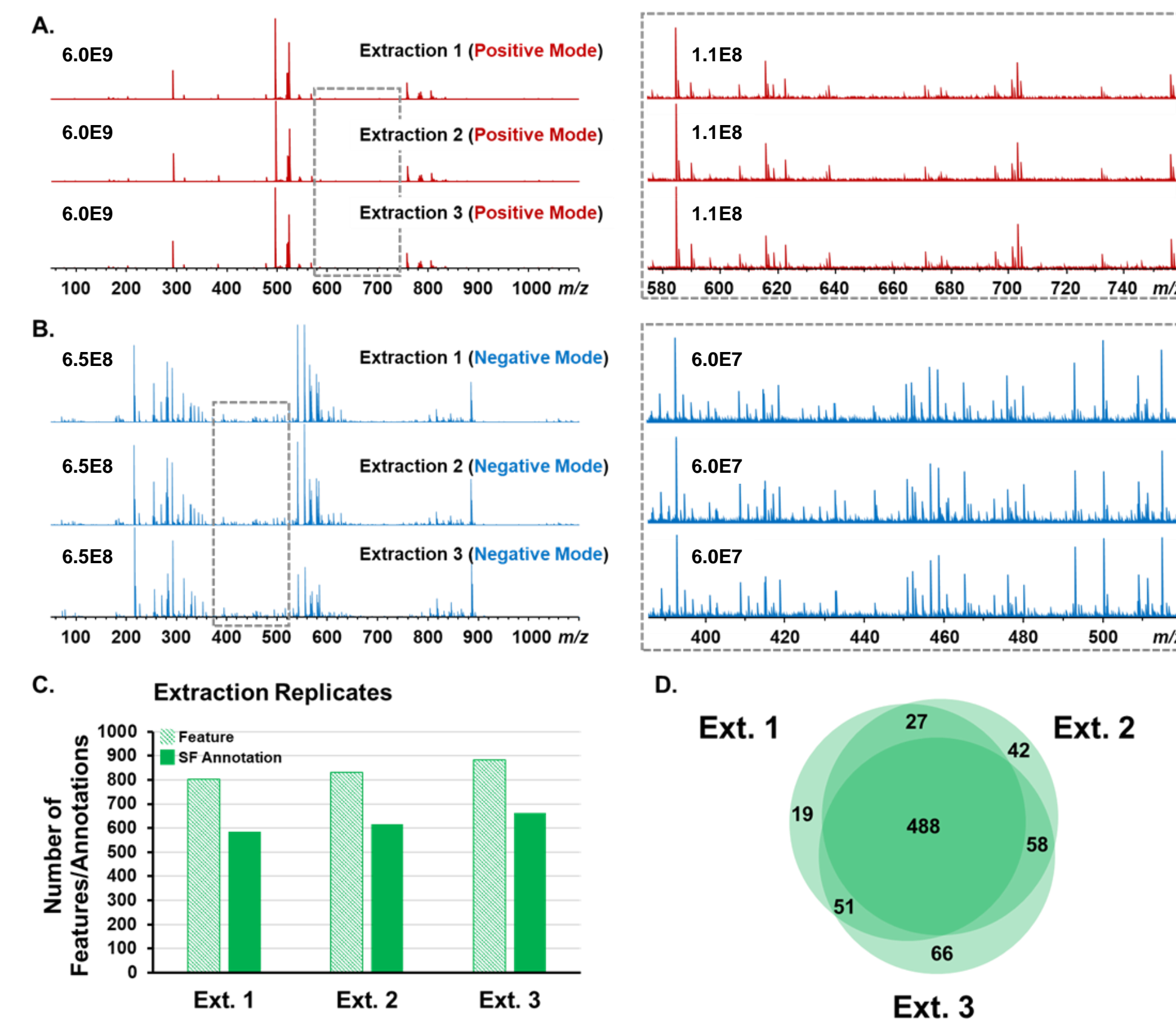
1. High Reproducibility

Figure 2. A. Mass spectra and zoomed-in mass spectra of 3 extraction replicates of mouse plasma sample in positive mode. **B.** Mass spectra and zoomed-in mass spectra of 3 extraction replicates of mouse plasma sample in negative mode. **C.** The numbers of features after blank reduction and SF annotations of 3 extraction replicates. **D.** Venn diagram of SF annotations from 3 extraction replicates (3 injection replicates in each extraction).
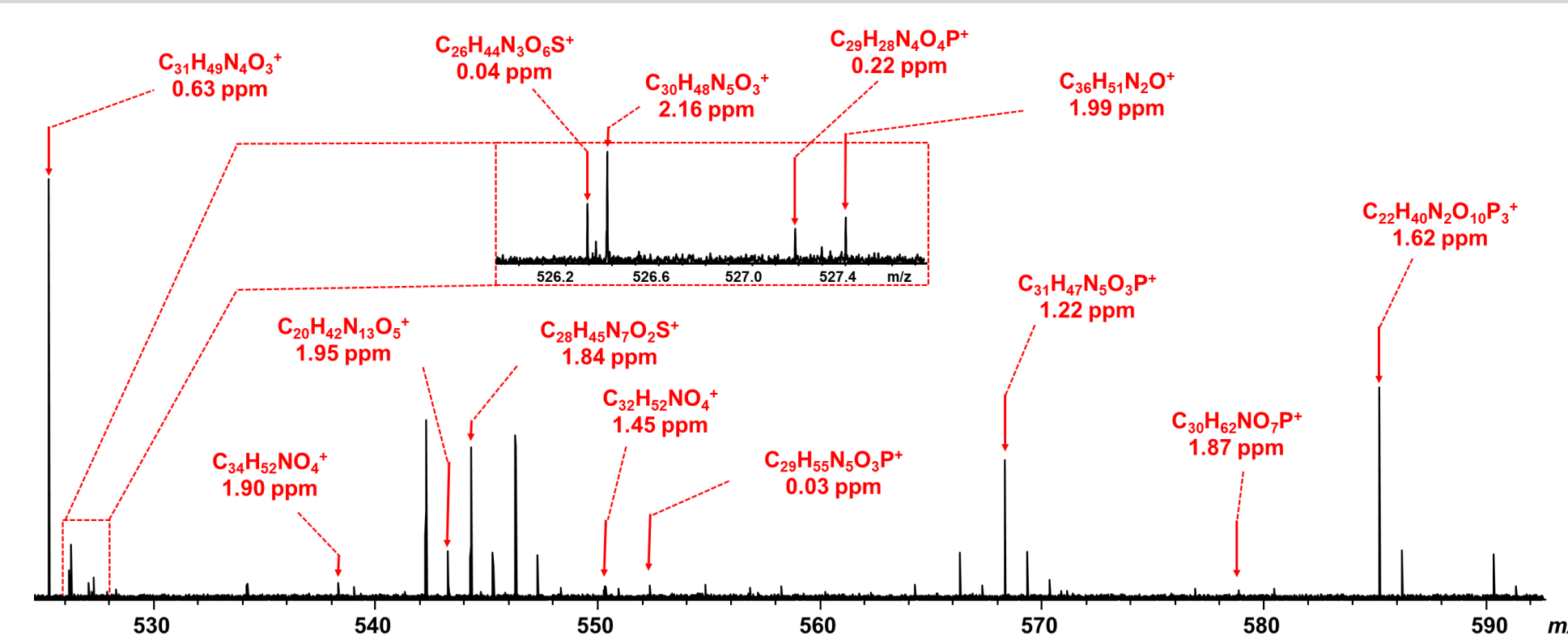
2. Ultra-High Resolution FTICR MS

Figure 3. Representative ultra-high resolution FTICR mass spectrum. 13 unique SmartFormula annotations are identified in the 70 m/z window.

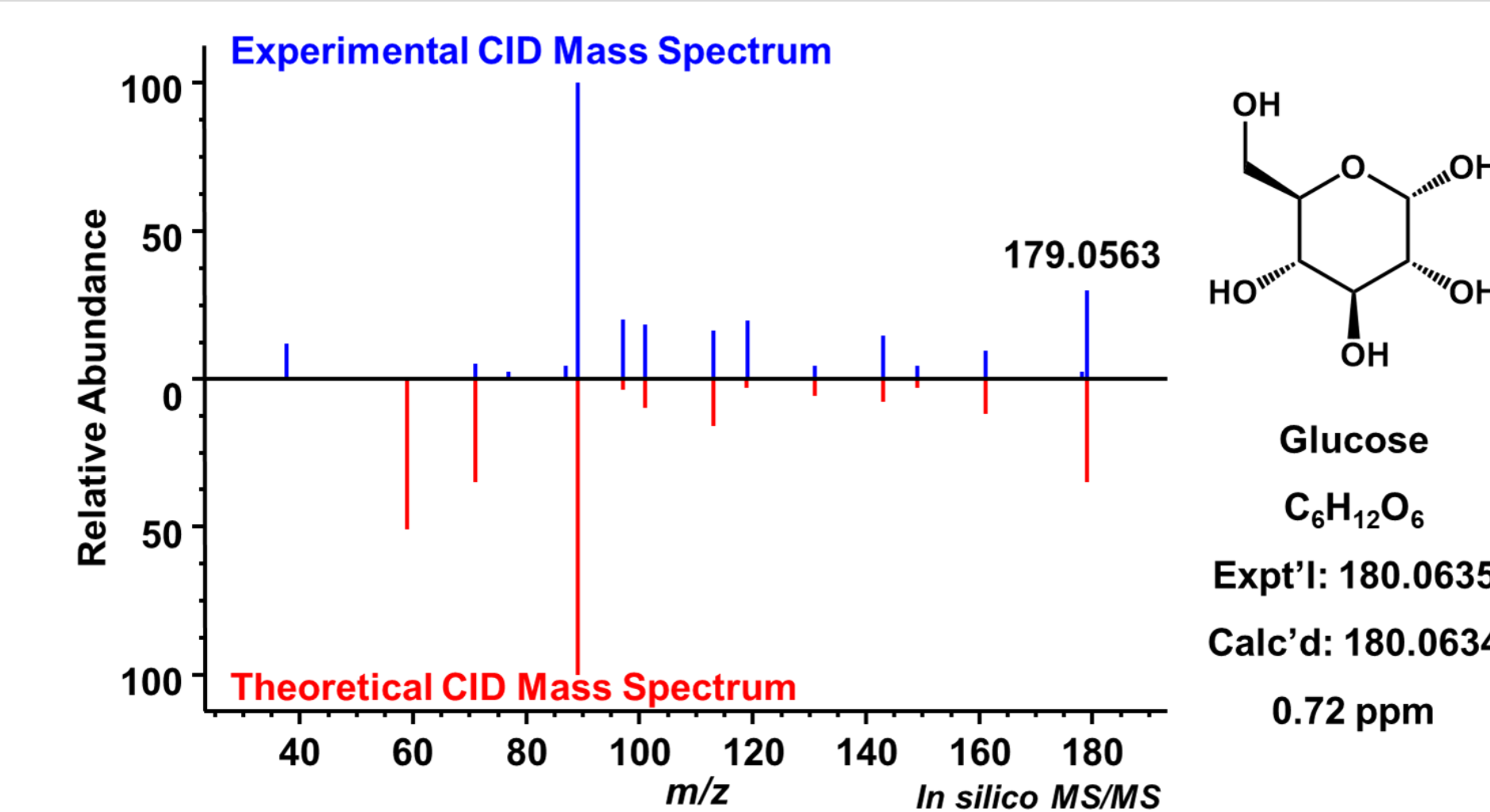#### 3. MS/MS Capability for Targeted Metabolite Identification

Figure 4. MS/MS analysis of metabolite at 179.0563 m/z. The experimental CID mass spectrum is compared with the theoretical CID mass spectrum of glucose.

Human Plasma Metabolomic Analysis

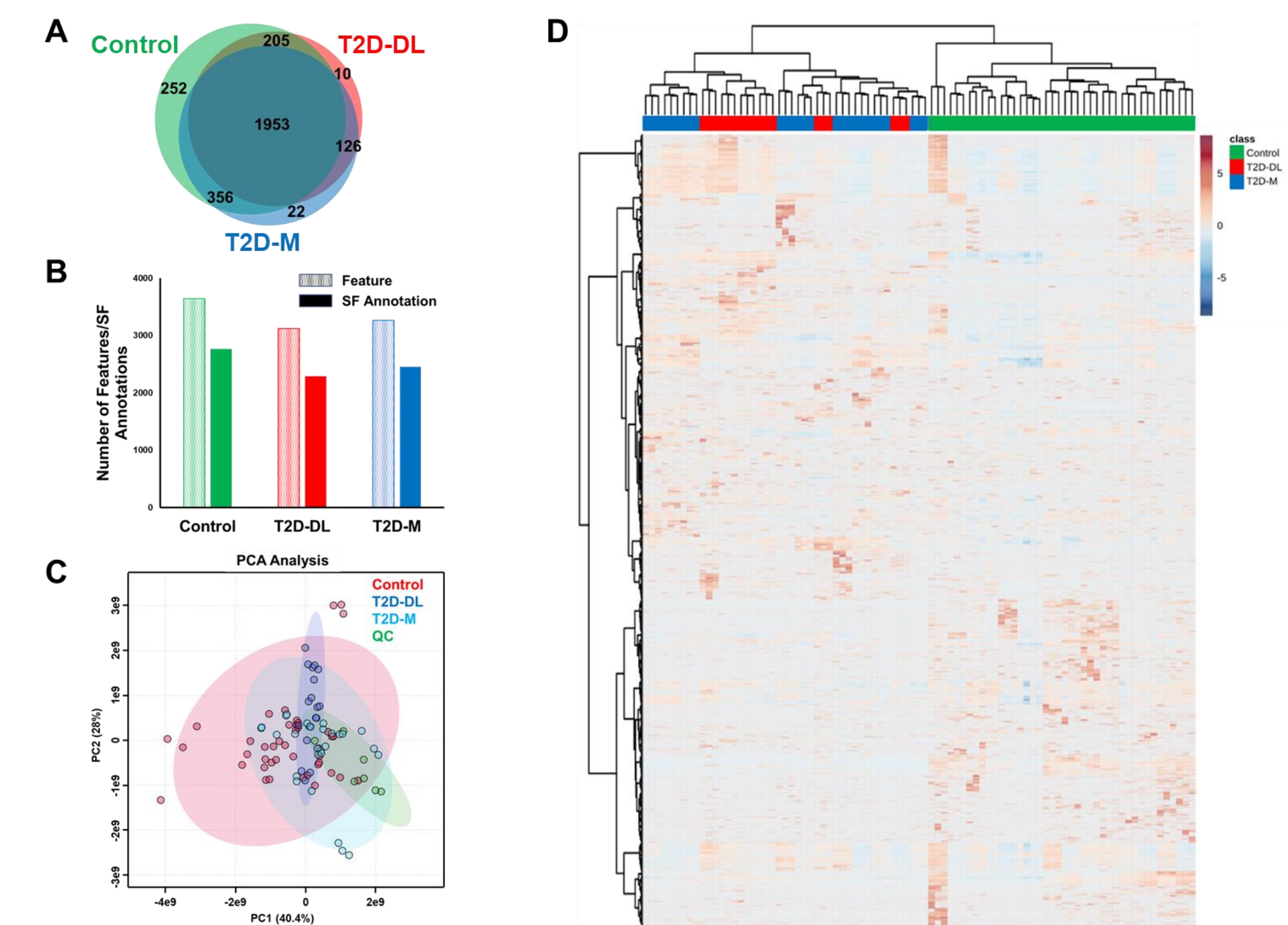#### 1. Overview of 3 Human Groups

Figure 5. A. Venn diagram of SF annotations from Control, T2D-DL, and T2D-M human plasma samples. **B.** The numbers of features (light shading) and SmartFormula annotations (solid shading) from MetaboScape 4.0 ($\Delta m < 5$ ppm) of 3 human groups. **C.** Principal component analysis (PCA) of the 3 human groups and quality control group (QC). **D.** Heat map represents all features in 3 groups.
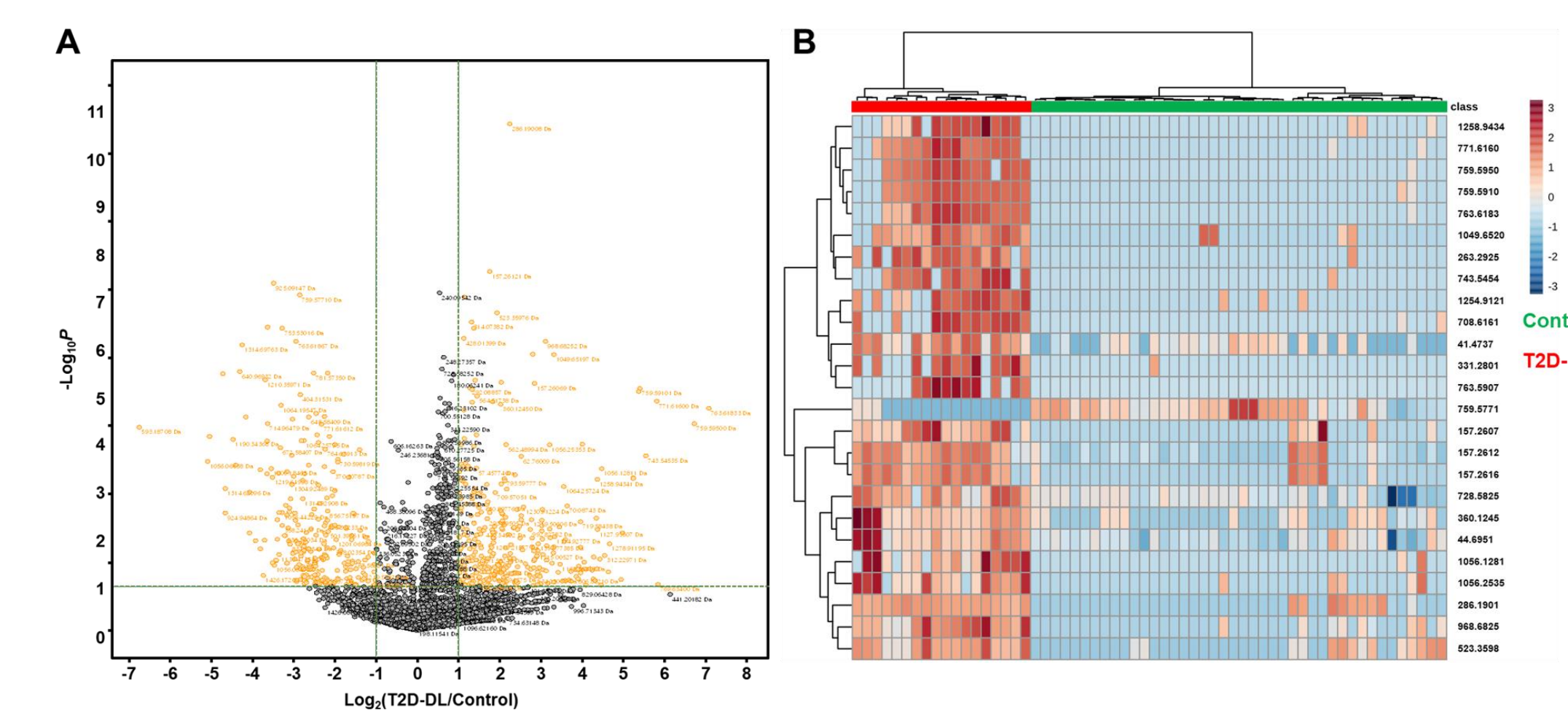
2. Control Group vs. T2D-DL Group

Figure 6. A. Volcano plot of Control vs. T2D-DL groups. 921 blank-reduced features show statistically significant differences (p(FDR) < 0.05). **B.** Heat map represents top 25 significant features from t-test analysis of Control vs. T2D-DL.
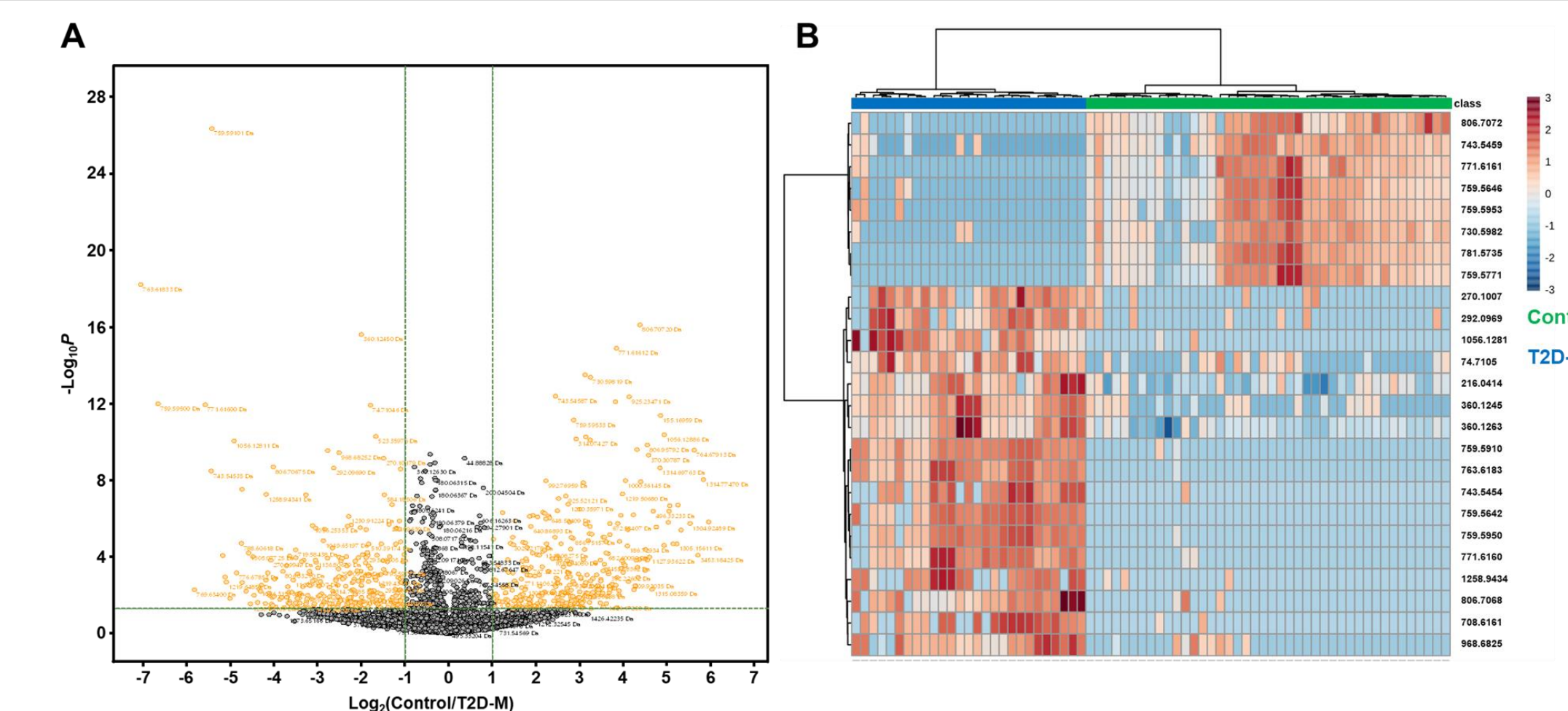
3. Control Group vs. T2D-M Group

Figure 7. A. Volcano plot of Control vs. T2D-M groups. 952 blank-reduced features show statistically significant differences (p(FDR) < 0.05). **B.** Heat map represents top 25 significant features from t-test analysis of Control vs. T2D-M.

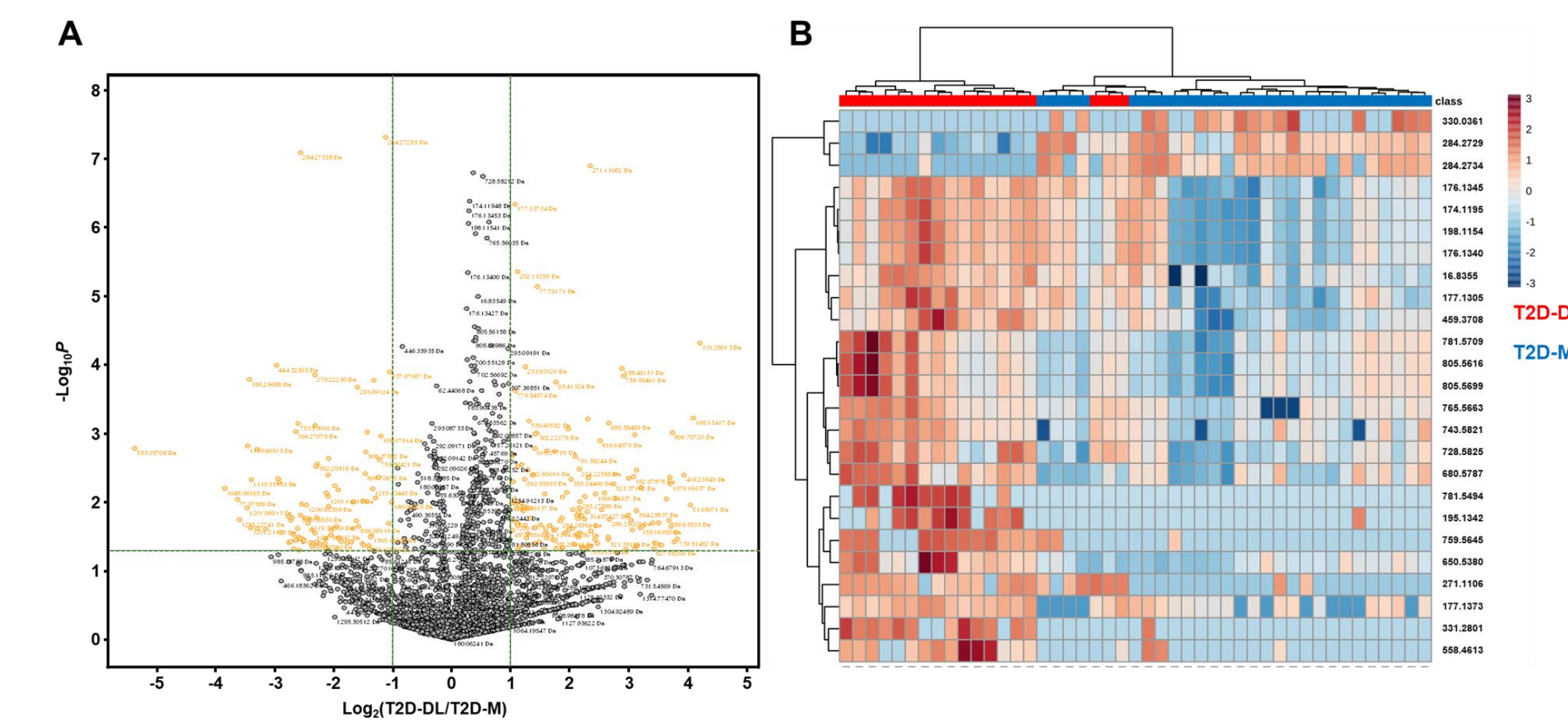#### 4. T2D-DL Group vs. T2D-M Group

Figure 8. A. Volcano plot of T2D-DL vs. T2D-M groups. 95 blank-reduced features show statistically significant differences (p(FDR) < 0.05). **B.** Heat map represents top 25 significant features from t-test analysis of T2D-DL vs. T2D-M groups.

5. Chemical Similarity Enrichment and Pathway Analysis
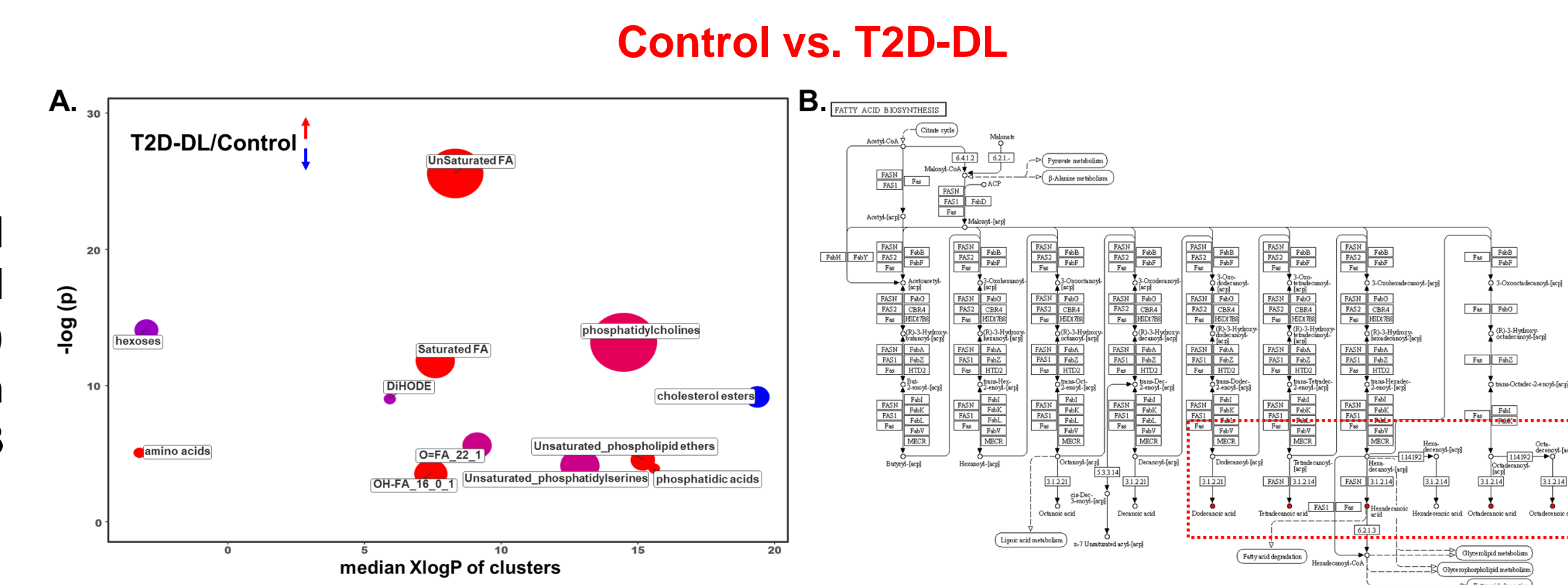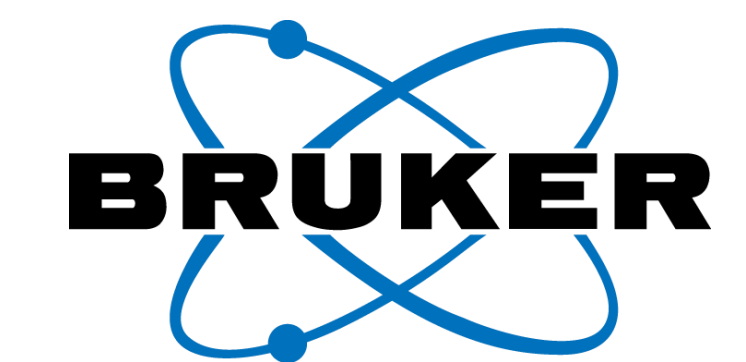
Control vs. T2D-DL

Figure 9. A. Chemical similarity enrichment analysis of Control vs. T2D-DL groups. The node sizes represent the total number of metabolites in each cluster set. The node color scale shows the proportion of increased (red) and decreased (blue) metabolites. **B.** Fatty acid biosynthesis pathway of Control vs. T2D-DL groups. All metabolites identified in this pathway increase in T2D-DL group. Pathway analysis was performed using KEGG Mapper.

Conclusions

- ❖ We introduced an integrated high-throughput FTICR-MS based platform for plasma metabolomic analysis.
- ❖ High injection and extraction reproducibilities were achieved.
- ❖ Thousands metabolic features were reproducibly detected and annotated in each human plasma group.
- ❖ MS/MS capability increases the confidence of targeted metabolite identification.
- ❖ This platform revealed significant metabolic changes in plasma from control vs. T2D human subjects, suggesting its place as a future tool for personalized T2D therapy.

Acknowledgements