

**Supplemental Information**

**N-acyl Taurines and Acylcarnitines Cause  
an Imbalance in Insulin Synthesis and Secretion  
Provoking  $\beta$  Cell Dysfunction in Type 2 Diabetes**

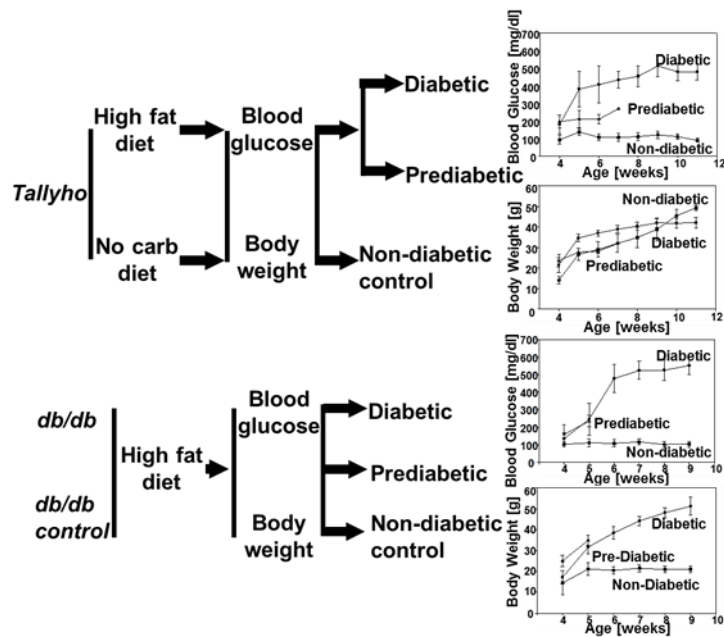
**Michaela Aichler, Daniela Borgmann, Jan Krumsiek, Achim Buck, Patrick E. MacDonald, Jocelyn E. Manning Fox, James Lyon, Peter E. Light, Susanne Keipert, Martin Jastroch, Annette Feuchtinger, Nikola S. Mueller, Na Sun, Andrew Palmer, Theodore Alexandrov, Martin Hrabe de Angelis, Susanne Neschen, Matthias H. Tschöp, and Axel Walch**

## **Supplemental Information**

### **N-acyl Taurines and Acylcarnitines Cause an Imbalance in Insulin Synthesis and Secretion Provoking $\beta$ -Cell Dysfunction in Type 2 Diabetes**

#### **Authors**

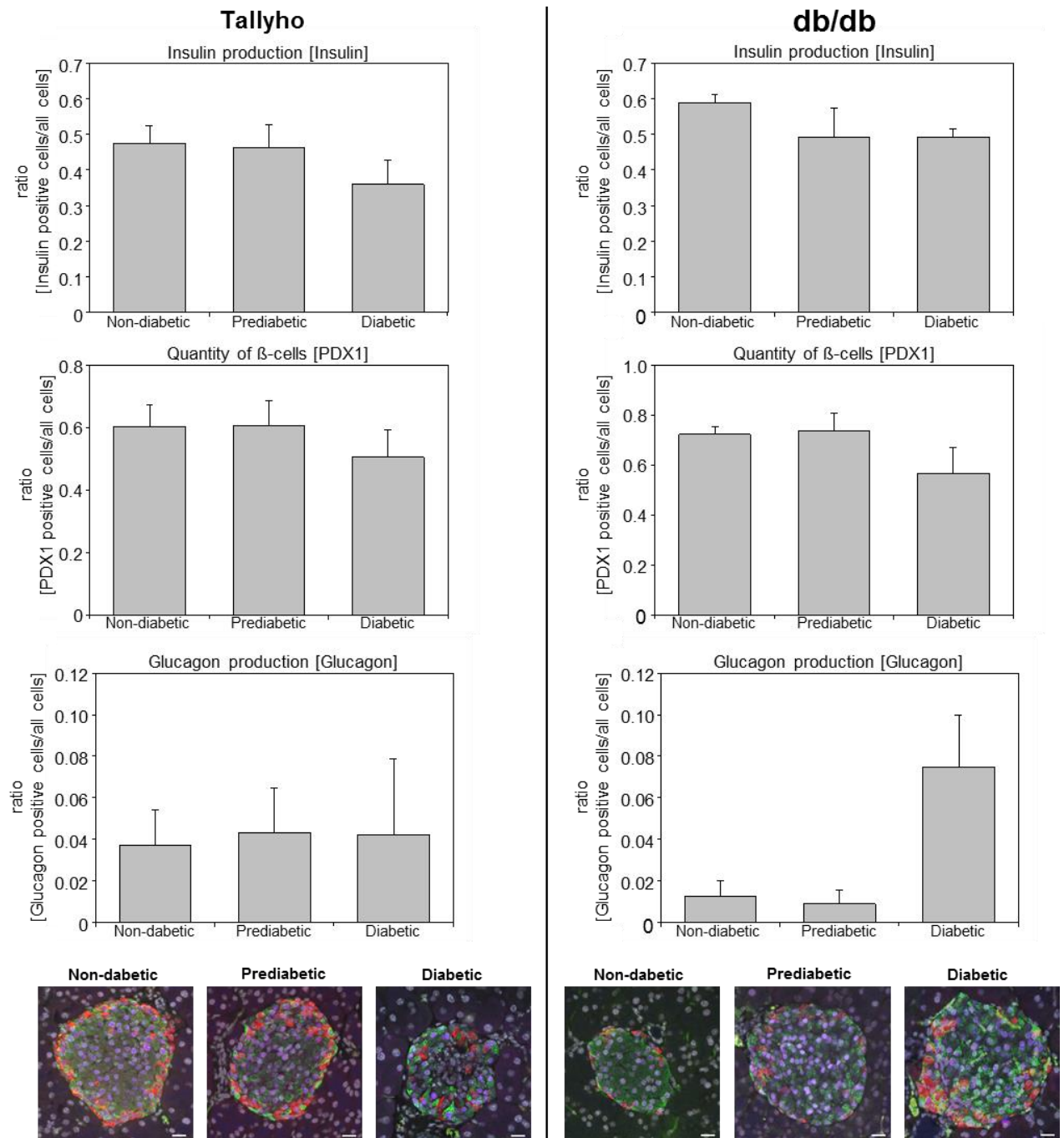
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**Figure S1. Experimental setup of study groups, Related to STAR Methods, Figure 1 and Figure 2.**

We used two different diabetes mouse models to determine the metabolic alterations of  $\beta$ -cells during the progression of T2D that are not specifically representative of a particular genetic background. The BKS.Cg-Cock7m+/+Leprdb/J (*db/db*) mouse represents a common model widely used for antidiabetes drug testing, however it develops monogenic obesity due to disruption of leptin-receptor signaling. In contrast *TallyHo/JngJ* (*TallyHo*) mice recapitulate broader aspects of polygenic human “diabesity”. Based on their phenotypic parameters, animals were included that either presented a prediabetic or overtly diabetic disease state in addition to non-diabetic controls.

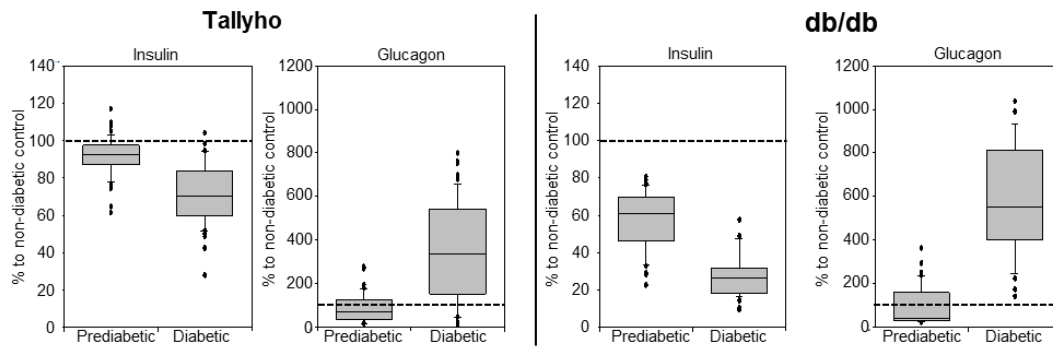
## Immunofluorescence



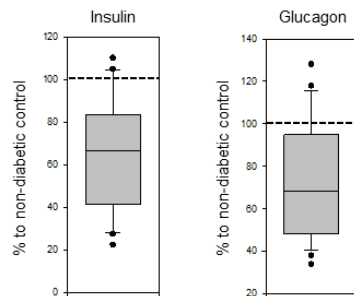
**Figure S2. Determination of overall quality and composition of LHI by immunofluorescence, Related to Figure 1.**

Quantitative analysis of immunofluorescence for insulin, PDX1 and glucagon. Insulin production was reduced as the disease progressed. The quantity of β-cells was comparable between prediabetic mice and non-diabetic controls and was only slightly, but not significantly, reduced in overtly diabetic mice. Glucagon production changed as expected with progression of the disease. Data are represented as the mean ± SEM. Examples of immunofluorescence stained LHI are presented (green: insulin, red: glucagon, blue: PDX1, grey: DAPI; scale bar 20 μm). Data are presented as mean ± SEM.

## **A** MALDI-Hormone Imaging – mouse Langerhans Islets in intact immediated environment

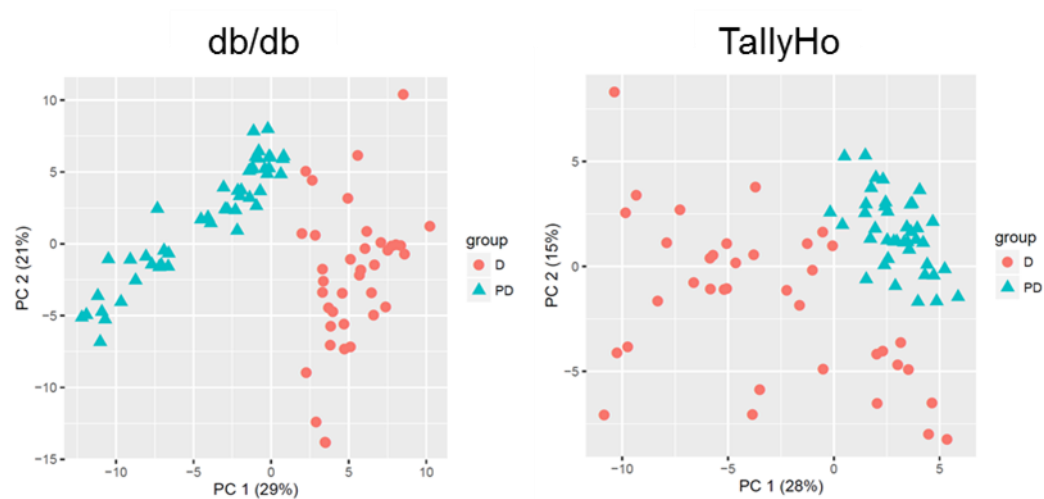


## **B** MALDI-Hormone Imaging – isolated human Langerhans islets



**Figure S3. Determination of the overall quality and composition of LHI by MALDI-Hormone imaging, Related to Figure 1 and Figure 2.**

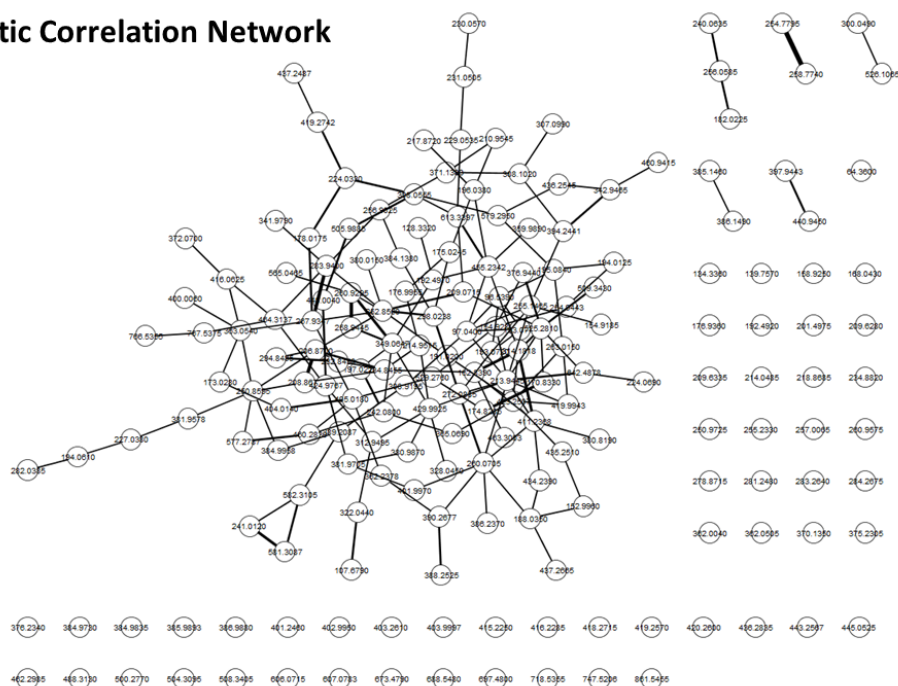
(A) MALDI-TOF analyses of insulin and glucagon contents confirmed the results for LHI from mice. (B) The insulin and glucagon contents of isolated human LHI were both reduced in patients with T2D compared with healthy controls.



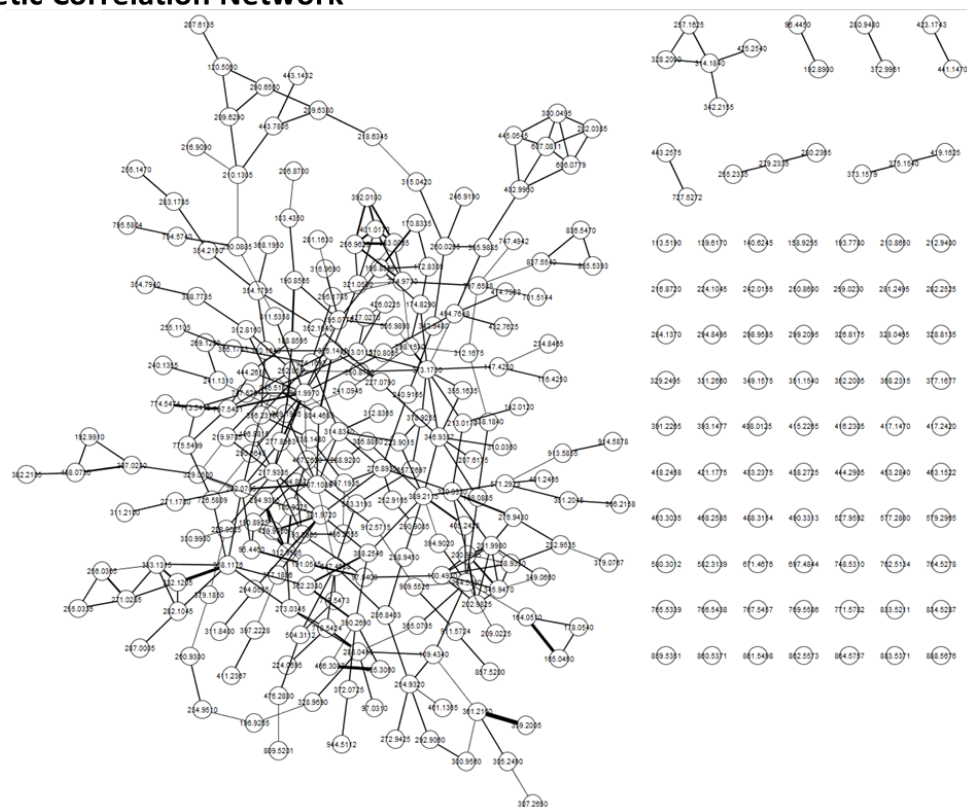
**Figure S4. Clear molecular separation between the disease states in mice, Related to Figure 2.**

Principal component analysis of the molecular individuality of the prediabetic and overtly diabetic groups in each mouse model revealed a clear molecular separation between the disease states.

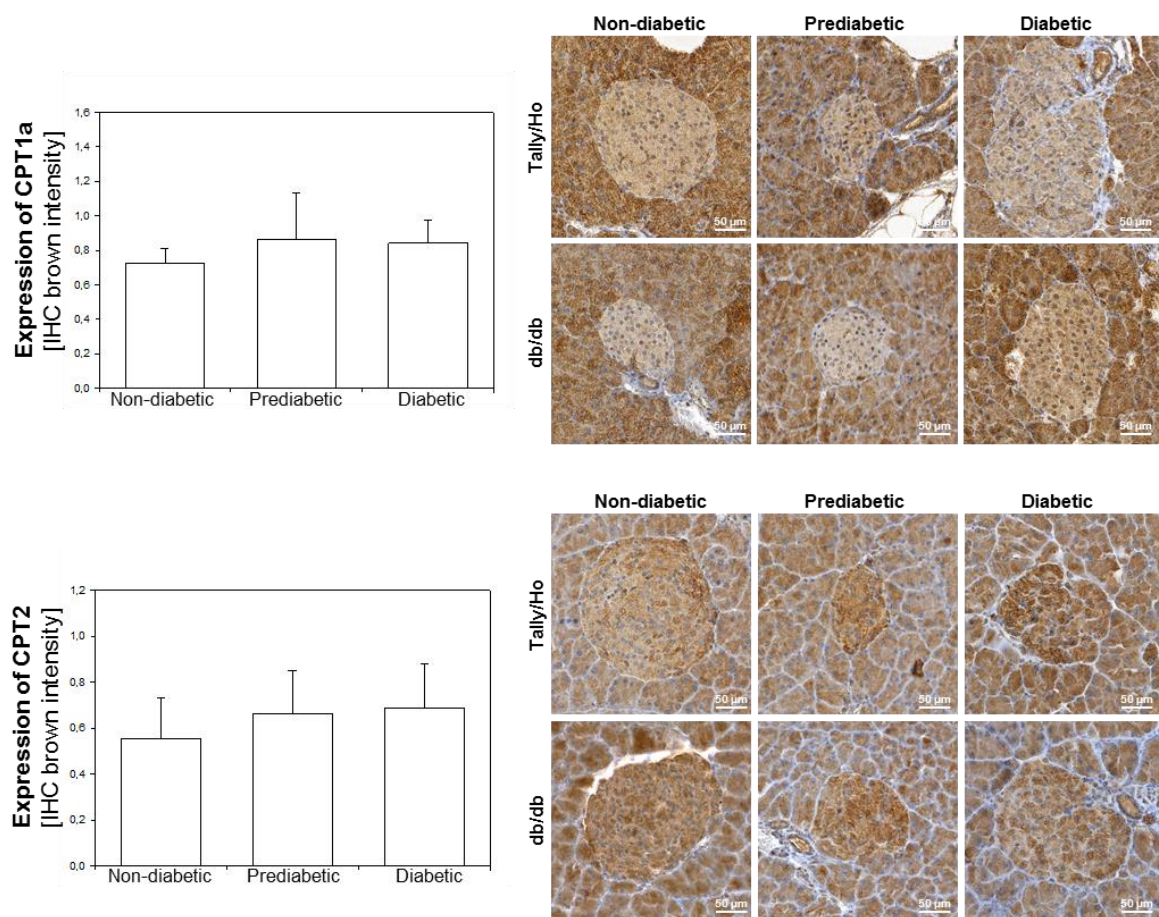
## Prediabetic Correlation Network



## Diabetic Correlation Network

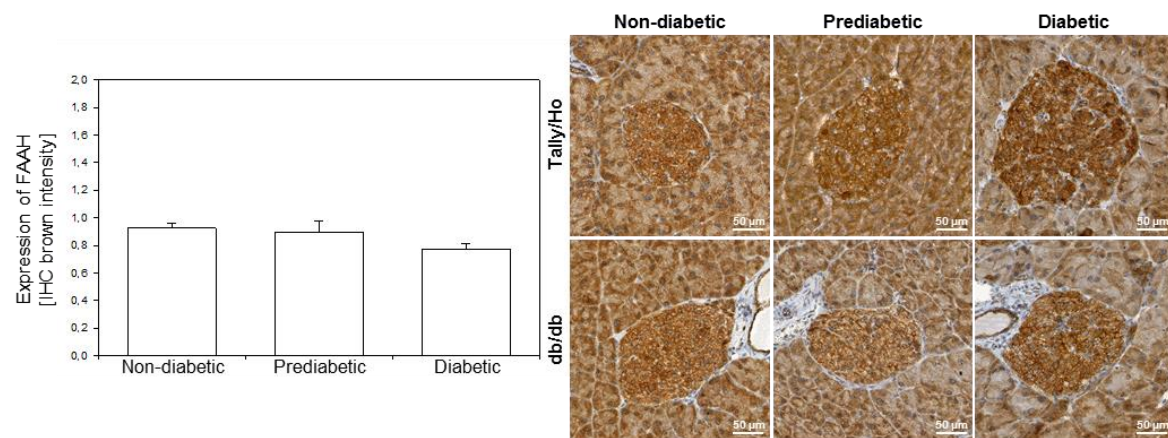


**Figure S5. Correlation network analysis of metabolites, Related to Figure 3.** (A) Row data of the correlation network analysis of metabolites associated with the prediabetic disease state. (B) Row data of the correlation network analysis of metabolites associated with the overtly diabetic disease state.



**Figure S6. Expression of CPT1a and CPT2 in Langerhans islets, Related to Figure 5.**

There was no significant difference in the expression of CPT1a or CPT2 between non-diabetic, prediabetic, and diabetic groups.



**Figure S7. Expression of FAAH, Related to Figure 7.**

The expression of FAAH was not significantly different between non-diabetic, prediabetic, and diabetic groups.

**A**

|                       | Tallyho          |                  |                 | db/db            |                  |                 |
|-----------------------|------------------|------------------|-----------------|------------------|------------------|-----------------|
|                       | Prediabetic      | Diabetic         | Contr.          | Prediabetic      | Diabetic         | Contr.          |
| Mice [n=x]            | 4                | 6                | 4               | 5                | 5                | 5               |
| Body weight [g]       | 29.1 $\pm$ 0.7   | 41.0 $\pm$ 0.7   | 37.8 $\pm$ 2.4  | 34.9 $\pm$ 0.9   | 48.9 $\pm$ 1.2   | 22.7 $\pm$ 0.6  |
| Blood glucose [mg/dl] | 237.7 $\pm$ 15.8 | 504.1 $\pm$ 17.8 | 110.2 $\pm$ 6.0 | 233.3 $\pm$ 12.3 | 542.3 $\pm$ 19.2 | 107.4 $\pm$ 6.2 |

**B**

|                                       | Tallyho     |          |        | db/db       |          |        |
|---------------------------------------|-------------|----------|--------|-------------|----------|--------|
|                                       | Prediabetic | Diabetic | Contr. | Prediabetic | Diabetic | Contr. |
| Langerhans Islets [n=x]               | 40          | 37       | 66     | 49          | 39       | 32     |
| Differential masses vs. Control [n=x] | 495         | 653      |        | 785         | 651      |        |

**Table S1. Overview of experimental groups, physiological parameters and detected metabolites, Related to STAR Methods, Figure S1 and Figure 2.**

(A) Study groups and physiological parameters. (B) 263 LHIs were analyzed for in situ-metabolomics.

A

## Annotated molecules correlating with blood glucose

### Prediabetic condition

| annotated molecule      | Succinate  | Pyrophosphate                            | Leucine/Isoleucine | Cytidine                | Deoxy-ribose-phosphate | Palmitic acid       |
|-------------------------|------------|--|--------------------|-------------------------|------------------------|---------------------|
| accuracy [ppm]          | 0          | 1  | 1                  | 7                       | 1                      | 0                   |
| ion                     | [M+Cl]-    | [M-H <sub>2</sub> O-H]-                  | [M+K-2H]-          | [M-H <sub>2</sub> O-H]- | [M+K-2H]-              | [M-H]-              |
| m/z                     | 152.996    | 158.925                                  | 168.043            | 224.069                 | 250.9725               | 255.233             |
| Correlation Coefficient | -0.339     | -0.33                                    | 0.293              | 0.321                   | -0.298                 | 0.399               |
| P Value                 | 0.00652    | 0.00826                                  | 0.0197             | 0.0103                  | 0.0175                 | 0.0012              |
| annotated molecule      | Oleic acid | Stearic acid                             | AMP/cAMP           | GMP                     | UDP                    | Palmitoyl-carnitine |
| accuracy [ppm]          | 2          | 0  | 0                  | 1                       | 0                      | 0                   |
| ion                     | [M-H]-     | [M-H]-                                   | [M-H]-             | [M+K-2H]-               | [M+Na-2H]-             | [M+K-2H]-           |
| m/z                     | 281.248    | 283.264                                  | 328.045            | 400.006                 | 424.9767               | 436.2835            |
| Correlation Coefficient | 0.476      | 0.434                                    | -0.331             | 0.324                   | -0.357                 | 0.503               |
| P Value                 | 0.0000815  | 0.000386                                 | 0.00798            | 0.00954                 | 0.00409                | 0.0000309           |
| annotated molecule      | ADP        | Linoleyl carnitine/Linoleaidyl carnitine | Stearyl carnitine  | ATP                     |                        |                     |
| accuracy [ppm]          | 0          |  | 2                  | 0                       |                        |                     |
| ion                     | [M+Na-2H]- | [M+K-2H]-                                | [M+K-2H]-          | [M-H]-                  |                        |                     |
| m/z                     | 448.004    | 460.283                                  | 464.3137           | 505.9885                |                        |                     |
| Correlation Coefficient | -0.53      | 0.391                                    | 0.397              | -0.321                  |                        |                     |
| P Value                 | 0.0000805  | 0.00183                                  | 0.00186            | 0.0111                  |                        |                     |

**Table S2B**

### Diabetic condition

| annotated molecule      | Pyrophosphate           | Cytidine | N-Acetyl-glucosamine phosphate | N-Acetyl-glucosamine phosphate | AMP/cAMP | N-Acetylmuramic acid phosphate |
|-------------------------|-------------------------|----------|--------------------------------|--------------------------------|----------|--------------------------------|
| accuracy [ppm]          | 4                       | 1        | 2                              | 1                              | 3        | 6                              |
| ion                     | [M-H <sub>2</sub> O-H]- | [M-H]-   | [M-H <sub>2</sub> O-H]-        | [M-H]-                         | [M-H]-   | [M-H]-                         |
| m/z                     | 158.9255                | 242.0785 | 282.0385                       | 300.0495                       | 328.0465 | 372.0725                       |
| Correlation Coefficient | -0.57                   | 0.663    | -0.456                         | -0.469                         | -0.59    | -0.564                         |
| P Value                 | 0.00102                 | 0.000563 | 0.0114                         | 0.00888                        | 0.000606 | 0.00116                        |
| annotated molecule      | UDP                     |          |                                |                                |          |                                |
| accuracy [ppm]          | 2                       |          |                                |                                |          |                                |
| ion                     | [M-H]-                  |          |                                |                                |          |                                |
| m/z                     | 402.996                 |          |                                |                                |          |                                |
| Correlation Coefficient | -0.444                  |          |                                |                                |          |                                |
| P Value                 | 0.0139                  |          |                                |                                |          |                                |

**Table S2: Correlation of metabolites with blood glucose, Related to Figure 2.** (A) Correlation of annotated metabolites with blood glucose in prediabetes. (B) Correlation of annotated metabolites with blood glucose in diabetes.

A

## Annotated molecules correlating with insulin content

### Prediabetic condition

| annotated molecule      | Pyrophosphate                    | Leucine/Isoleucine             | Pyrophosphate        | N2-Acetyl-L-ornithine  | Cytidine                | Acetylcarnitine       |
|-------------------------|----------------------------------|--------------------------------|----------------------|------------------------|-------------------------|-----------------------|
| accuracy [ppm]          | 1                                | 1                              | 0                    | 7                      | 7                       | 3                     |
| ion                     | [M-H <sub>2</sub> O-H]-          | [M+K-2H]-                      | [M-H]-               | [M+Cl]-                | [M-H <sub>2</sub> O-H]- | [M+K-2H]-             |
| m/z                     | 158.925                          | 168.043                        | 176.936              | 209.0715               | 224.069                 | 240.0635              |
| Correlation Coefficient | 0.277                            | -0.435                         | 0.357                | 0.26                   | -0.35                   | 0.66                  |
| P Value                 | 0.0278                           | 0.000369                       | 0.00412              | 0.0448                 | 0.00486                 | 5.67E-08              |
| annotated molecule      | Deoxy-ribose-phosphate           | Palmitic acid                  | N-Acetyl glucosamine | PPPi                   | Phospho-gluconic acid   | N-Ornithyl-L- taurine |
| accuracy [ppm]          | 1                                | 0                              | 3                    | 0                      | 0                       | 7                     |
| ion                     | [M+K-2H]-                        | [M-H]-                         | [M+Cl]-              | [M-H]-                 | [M-H <sub>2</sub> O-H]- | [M+Na-2H]-            |
| m/z                     | 250.9725                         | 255.233                        | 256.0585             | 256.9025               | 257.0065                | 260.0705              |
| Correlation Coefficient | 0.428                            | -0.311                         | 0.697                | 0.474                  | 0.457                   | -0.288                |
| P Value                 | 0.00047                          | 0.0131                         | 8.61E-10             | 0.000114               | 0.000166                | 0.0219                |
| annotated molecule      | Oleic acid                       | N-Acetyl glucosamine phosphate | Stearic acid         | CMP                    | AMP/cAMP                | GMP                   |
| accuracy [ppm]          | 2                                | 2                              | 0                    | 1                      | 0                       | 0                     |
| ion                     | [M-H]-                           | [M-H <sub>2</sub> O-H]-        | [M-H]-               | [M-H]-                 | [M-H]-                  | [M-H]-                |
| m/z                     | 281.248                          | 282.0385                       | 283.264              | 322.044                | 328.045                 | 362.0505              |
| Correlation Coefficient | -0.305                           | 0.288                          | -0.325               | -0.486                 | 0.259                   | -0.745                |
| P Value                 | 0.015                            | 0.0221                         | 0.00934              | 0.0000537              | 0.0404                  | 2.53E-12              |
| annotated molecule      | N-Acetylmuramic acid 6-phosphate | UDP                            | N-linoleoyl taurine  | N-oleoyl taurine       | N-stearoyl taurine      | GMP                   |
| accuracy [ppm]          | 0                                | 0                              | 0                    | 0                      | 1                       | 1                     |
| ion                     | [M-H]-                           | [M-H <sub>2</sub> O-H]-        | [M-H]-               | [M-H]-                 | [M-H]-                  | [M+K-2H]-             |
| m/z                     | 372.07                           | 384.9835                       | 386.237              | 388.2525               | 390.2677                | 400.006               |
| Correlation Coefficient | -0.466                           | 0.25                           | -0.607               | -0.453                 | -0.532                  | -0.423                |
| P Value                 | 0.000151                         | 0.0483                         | 1.31E-07             | 0.000191               | 0.00000709              | 0.000548              |
| annotated molecule      | ADP                              | Stearyl carnitine              | ATP                  | UDP-glucose/ galactose |                         |                       |
| accuracy [ppm]          | 0                                | 2                              | 0                    | 2                      |                         |                       |
| ion                     | [M+Na-2H]-                       | [M+K-2H]-                      | [M-H]-               | [M-H]-                 |                         |                       |
| m/z                     | 448.004                          | 464.3137                       | 505.9885             | 565.0465               |                         |                       |
| Correlation Coefficient | 0.262                            | -0.386                         | 0.309                | -0.618                 |                         |                       |
| P Value                 | 0.0381                           | 0.00252                        | 0.0144               | 1.82E-07               |                         |                       |

**Table S3: Correlation of metabolites with insulin content. Related to Figure 2, 3, 4, 5, 6, 7.**

(A) Correlation of annotated metabolites with insulin in prediabetes.

## B

### Annotated molecules correlating with insulin content

#### Diabetic condition

| annotated molecule      | Pyrophosphate           | Deoxyribose phosphate            | Cytidine | Glucose-phosphate | N-Acetyl glucosamine phosphate | Eicosadienoic acid |
|-------------------------|-------------------------|----------------------------------|----------|-------------------|--------------------------------|--------------------|
| accuracy [ppm]          | 4                       | 0                                | 1        | 2                 | 2                              | 2                  |
| ion                     | [M-H <sub>2</sub> O-H]- | [M-H]-                           | [M-H]-   | [M-H]-            | [M-H <sub>2</sub> O-H]-        | [M-H]-             |
| m/z                     | 158.9255                | 213.017                          | 242.0785 | 259.023           | 282.0385                       | 307.265            |
| Correlation Coefficient | 0.487                   | 0.397                            | -0.439   | 0.429             | 0.487                          | 0.555              |
| P Value                 | 0.00636                 | 0.0404                           | 0.0362   | 0.0179            | 0.00641                        | 0.00176            |
| annotated molecule      | AMP/cAMP                | N-Acetylmuramic acid 6-phosphate | UDP      |                   |                                |                    |
| accuracy [ppm]          | 3                       | 6                                | 2        |                   |                                |                    |
| ion                     | [M-H]-                  | [M-H]-                           | [M-H]-   |                   |                                |                    |
| m/z                     | 328.0465                | 372.0725                         | 402.996  |                   |                                |                    |
| Correlation Coefficient | 0.422                   | 0.457                            | 0.43     |                   |                                |                    |
| P Value                 | 0.0201                  | 0.0111                           | 0.0176   |                   |                                |                    |

**Table S3: Correlation of metabolites with insulin content. Related to Figure 2, 3, 4, 5, 6, 7.**

(B) Correlation of annotated metabolites with insulin in diabetes.