

## Supplementary Figures

Jiménez-Sánchez et al.

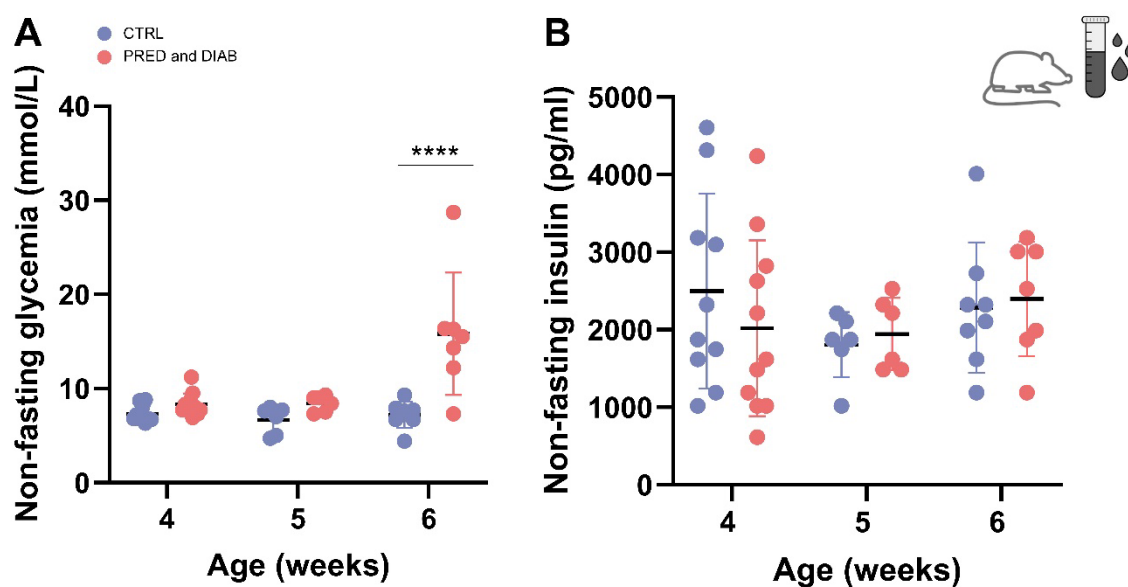


Figure S1.  $Phb2^{-/-}$  mice are asymptomatic at 5 weeks of age. (A) Random blood glucose levels measured from the ages of 4–6 weeks in  $\beta$ - $Phb2^{-/-}$  (pre- and diabetic mice, PRED and DIABETIC) and control (CTRL) ( $Phb2^{fl/fl}$  floxed) mice ( $n = 6–10$  mice). (B) Random insulin levels measured from the ages of 4–6 weeks in  $\beta$ - $Phb2^{-/-}$  and control ( $Phb2^{fl/fl}$  floxed) mice ( $n = 6–10$  mice). The 2-way analysis of variance (ANOVA) was used for multiple comparisons, \*\*\*  $p < 0.001$ .

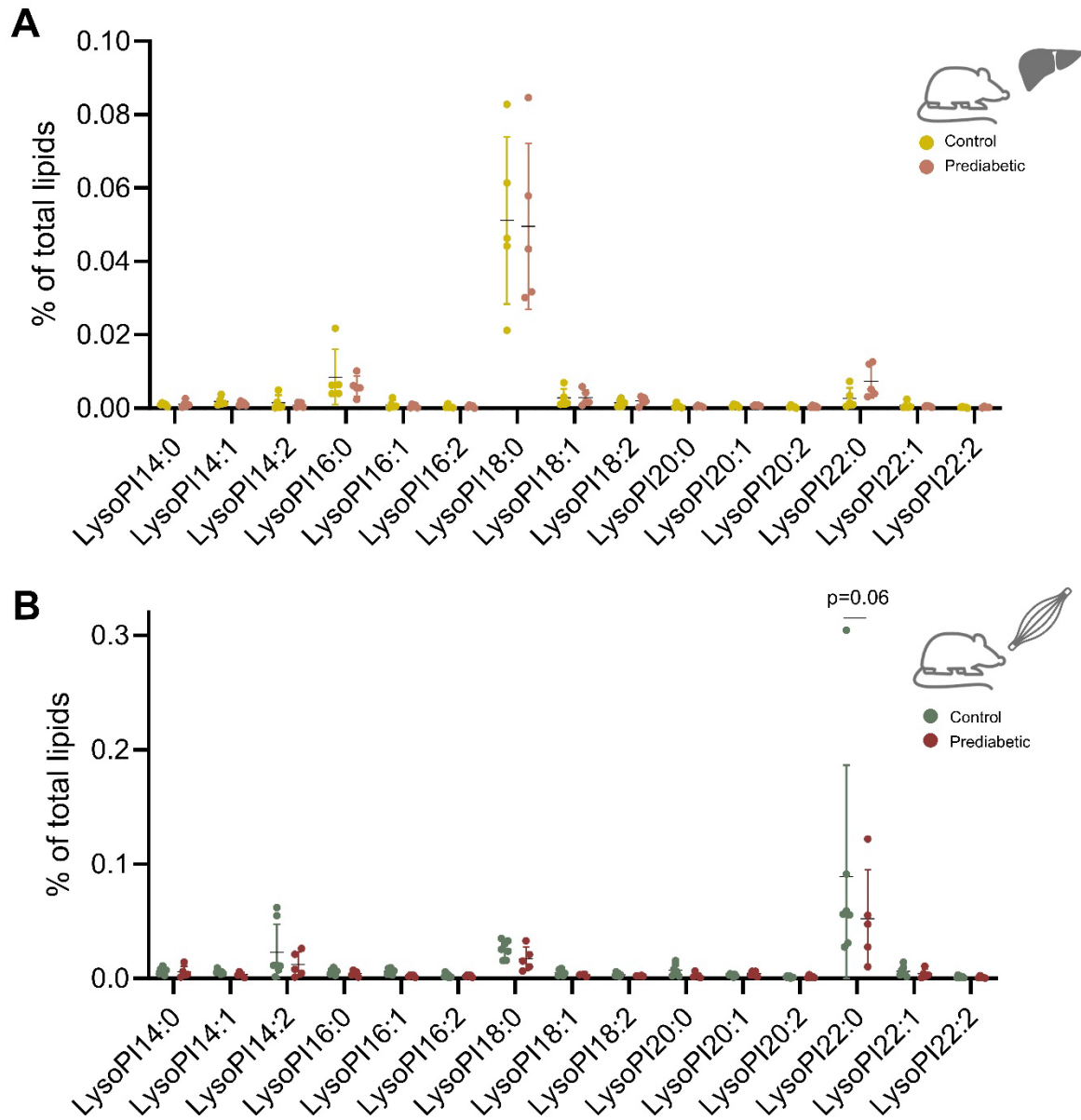


Figure S2. Single Lysophosphatidylinositol species (Lyso PI) in the liver (A) and muscle (B) of the  $\beta$ -cell  $Phb2^{-/-}$  prediabetic (n=5 for liver and muscle) vs. control (n=5 for liver, n=7 for muscle) mice. Data are expressed as percentage of total lipid amount detected. The 2-way analysis of variance (ANOVA) was used for multiple comparisons, \*  $p<0.05$ , \*\*  $p<0.01$ , \*\*\*  $p<0.001$ , \*\*\*\* $<0.0001$ .

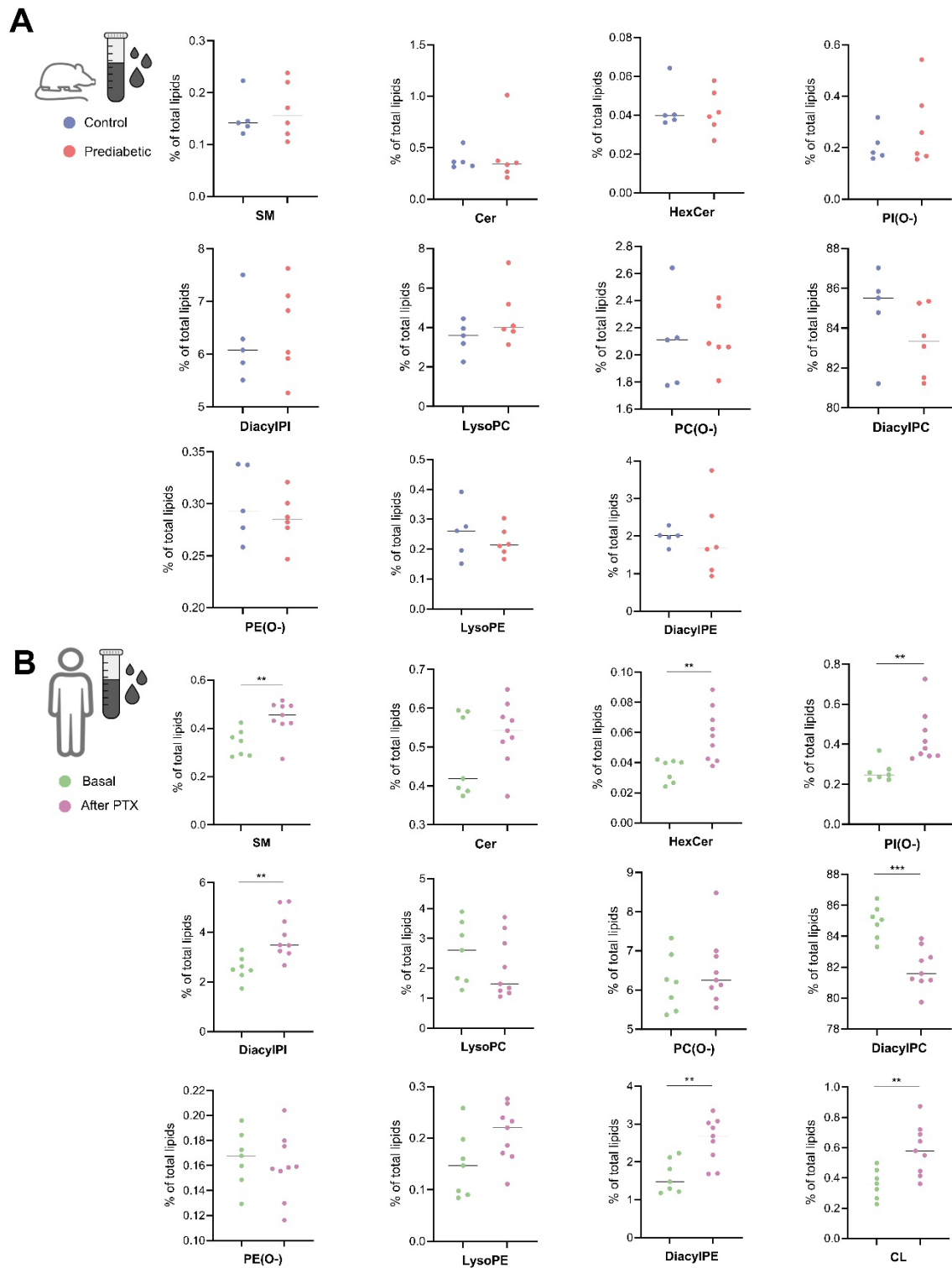


Figure S3. Circulating lipid amount representing the sum of all lipids detected in the specified lipid class, expressed as percentage of total lipid amount detected in the prediabetic mouse  $\beta$ -cell  $Phb2^{-/-}$  (n=6) *versus* control (n=5) and in patients after pancreaticoduodenectomy (n=9) *versus* the basal state (n=7). Unpaired Student's t-test was used to establish significance, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

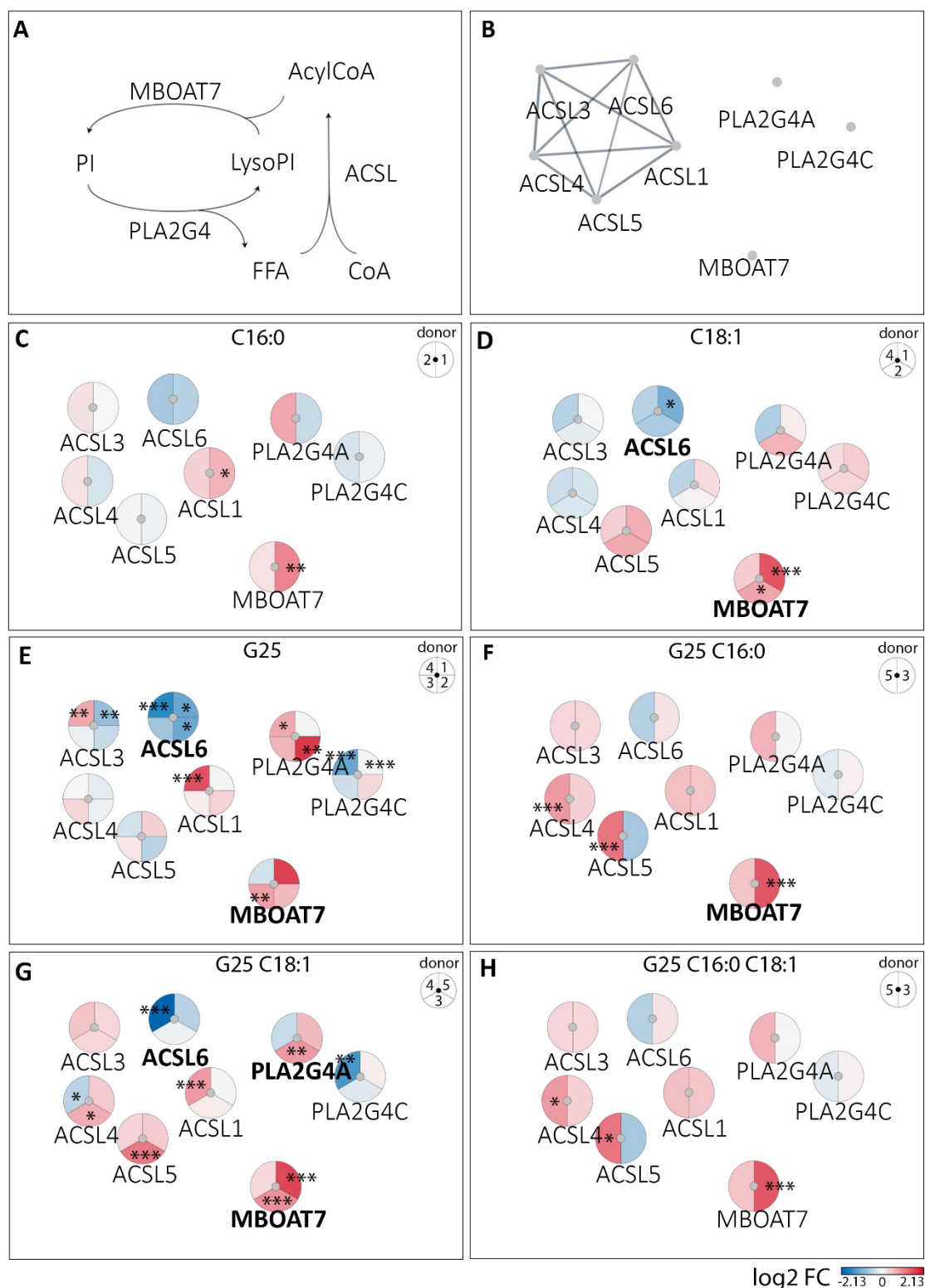


Figure S4. LysoPI metabolism-related transcriptomic regulation in human islets under metabolic stress conditions. (A) Land's cycle, where LysoPi is produced by cytosolic iPLA2 (calcium-independent PLA2), which removes fatty acids from the sn-1 and sn-2 position of phosphatidylinositol (PI). Then, MBOAT7 specifically esterifies arachidonyl-CoA to lysoPI to re-generate PI. (B) Functional interaction network of genes involved in lysoPI pathways.

Node connections were established according to the STRING interaction knowledgebase (using the Cytoscape StringApp) with a confidence score  $>0.4$ . (C–H) Effects of high 25 mmol/l glucose (G25) and 0.4 mmol/l oleate (C18:1) or palmitate (C16:0) on the transcriptional regulation of genes involved in LysoPI. Human islets were exposed to (C) C16:0 at G5.5, (D) C18:1 at G5.5, (E) G25, (F) G25 + C16:0, (G) G25 + C18:1 and (H) G25 + C16:0 + C18:1 for 72h before RNA-Sequencing analysis. Effects of culture conditions on transcript levels are compared with standard G5.5 medium and shown as upregulated (red), downregulated (blue), or unchanged (white). Missing values are represented in grey. Each disk is split into individual changes for the different donors. The colour code reflects the transcriptional changes in log<sub>2</sub> fold changes (log<sub>2</sub> FC) for that particular gene in individual donors. Significant changes were considered when two or more independent islet batches (donors) exhibited down- or upregulation with a log<sub>2</sub> FC threshold of 0.5 associated with at least one or more  $p < 0.05$ . Genes significantly regulated are highlighted in bold. \*adjusted  $p < 0.05$ , \*\*adjusted  $p < 0.01$ , \*\*\*adjusted  $p < 0.001$  between control G5.5 and the specific culture condition.

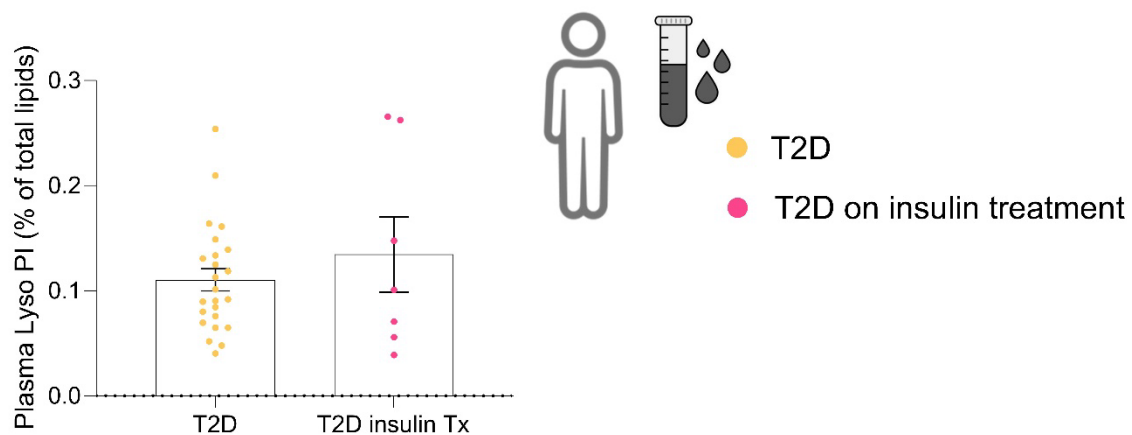


Figure S5. Total circulating Lysophosphatidylinositol (Lyso PI) levels expressed as percentage of total lipid amount detected in the plasma of patients from Geneva cohort with established T2D (Table S2), classified as per their insulin treatment (T2D insulin Tx,  $n=7$ ) versus non-insulin treated T2D ( $n=24$ ). Unpaired Student's t-test was used to establish significance between the two conditions.

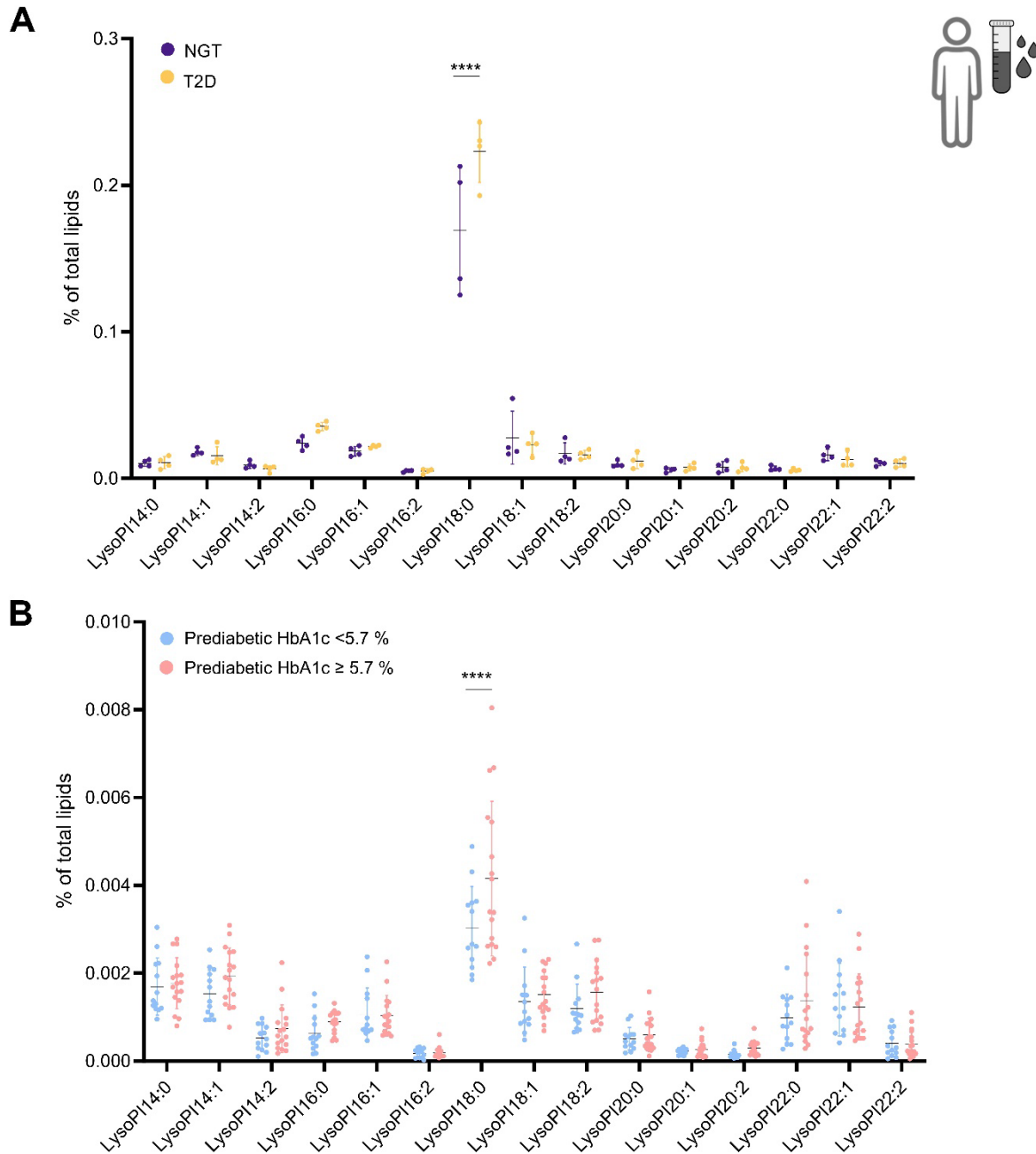


Figure S6. Circulating single Lysophosphatidylinositol species (Lyso PI) A) in patients from Amsterdam cohort with established T2D (n=4) vs. non-type 2 diabetic (NGT, n=4) (Table S5) (Amsterdam cohort, Table S5) and B) in patients at high risk of developing diabetes, as defined in the materials and methods section. These patients were stratified into two subgroups, according to the ADA criterion for prediabetes (American Diabetes Association Diagnosis and classification of diabetes mellitus) of HbA1c >5.7% (HbA1C ≥5.7% n=17, HbA1c <5.7% n=13, Table S4). Data are expressed as percentage of total lipid amount detected. The 2-way analysis of variance (ANOVA) was used for multiple comparisons, \*\*\*\*<0.0001.

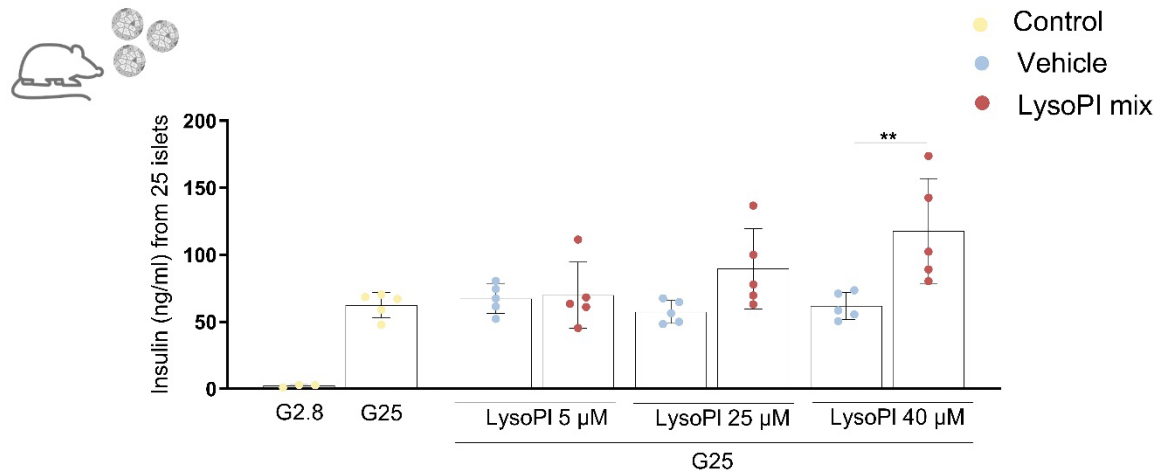


Figure S7. Dose-response effect of exogenous Lysophosphatidylinositol (Lyso PI) addition to isolated mouse islets on glucose-stimulated insulin secretion. Doses of 5, 25, and 40  $\mu$ M Lyso PI or vehicle (DMSO) were added to the islets isolated from control mice prior to glucose stimulation at 25 mmol/l glucose (G25) followed by insulin determination. Results are means  $\pm$  SD with single values representation. 1-way ANOVA was used for comparisons among groups, \*  $p < 0.05$ , \*\*\*\*  $p < 0.00001$ .