

Cell Reports, Volume 25

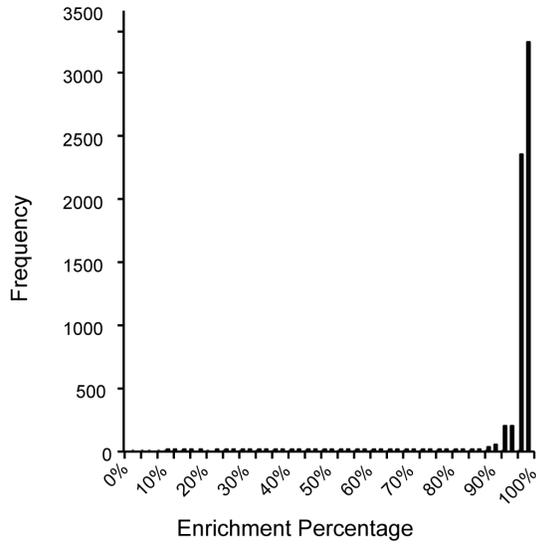
Supplemental Information

Integrated *In Vivo* Quantitative Proteomics and Nutrient Tracing Reveals Age-Related Metabolic Rewiring of Pancreatic β Cell Function

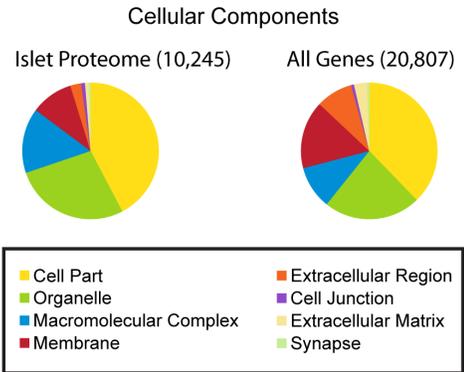
Matthew Wortham, Jacqueline R. Benthuisen, Martina Wallace, Jeffrey N. Savas, Francesca Mulas, Ajit S. Divakaruni, Fefen Liu, Verena Albert, Brandon L. Taylor, Yinghui Sui, Enrique Saez, Anne N. Murphy, John R. Yates III, Christian M. Metallo, and Maike Sander

SUPPLEMENTAL FIGURES

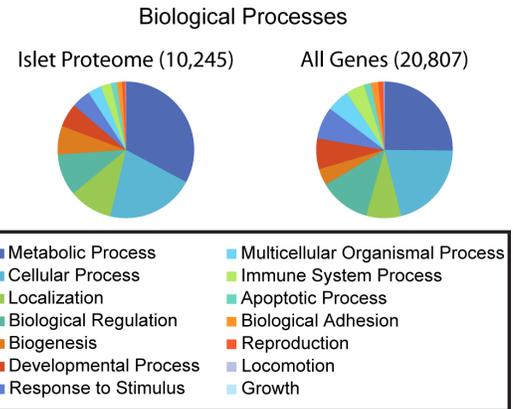
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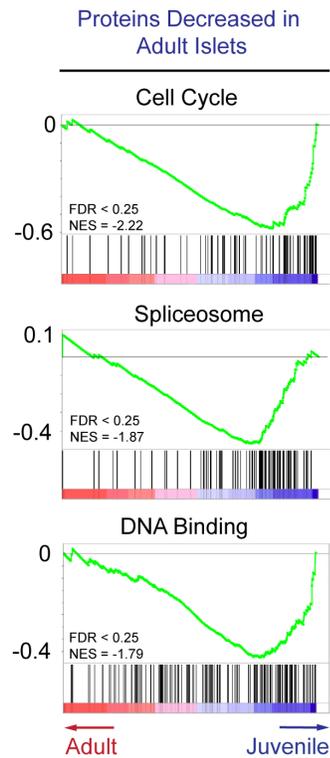
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E

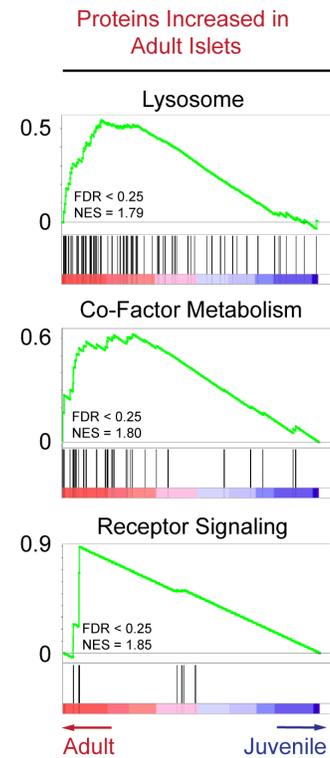


Figure S1 – Related to Figure 1. SILAM Mud-PIT MS captures a diverse set of islet proteins.

(A) Labeling efficiency of islet proteins shown as the number of peptides with the indicated percent incorporation of ^{15}N .

(B and C) Gene Ontology analysis of the entire quantified islet proteome as compared to all genes shows the distribution of proteins categorized by cellular component and biological process.

(D and E) Gene Set Enrichment Analysis of islet proteins differentially regulated in islets from 4-week-old and 1-year-old mice.

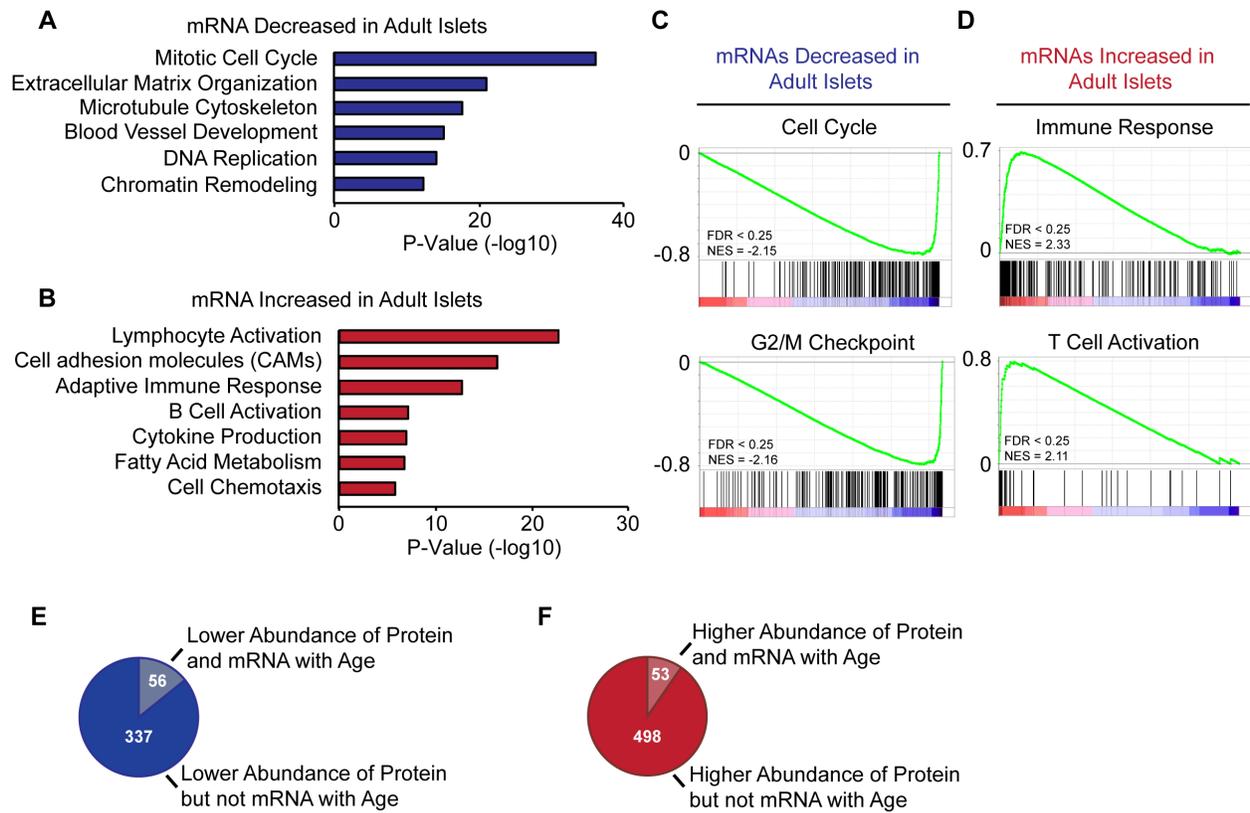


Figure S2 – Related to Figure 2. RNA-sequencing reveals mRNAs regulated in islets with age.

(A) Gene ontology (GO) analysis of islet mRNAs decreased in 1-year-old compared to 4-week-old mice (n=3 per group).

(B) GO analysis of islet mRNAs increased in 1-year-old compared to 4-week-old mice.

(C) Gene Set Enrichment Analysis (GSEA) of islet mRNAs decreased with age.

(D) GSEA of islet mRNAs increased with age.

(E and F) Pie graphs representing the number of uniquely named proteins found differentially expressed in adult and juvenile islets with and without coordinate mRNA regulation.

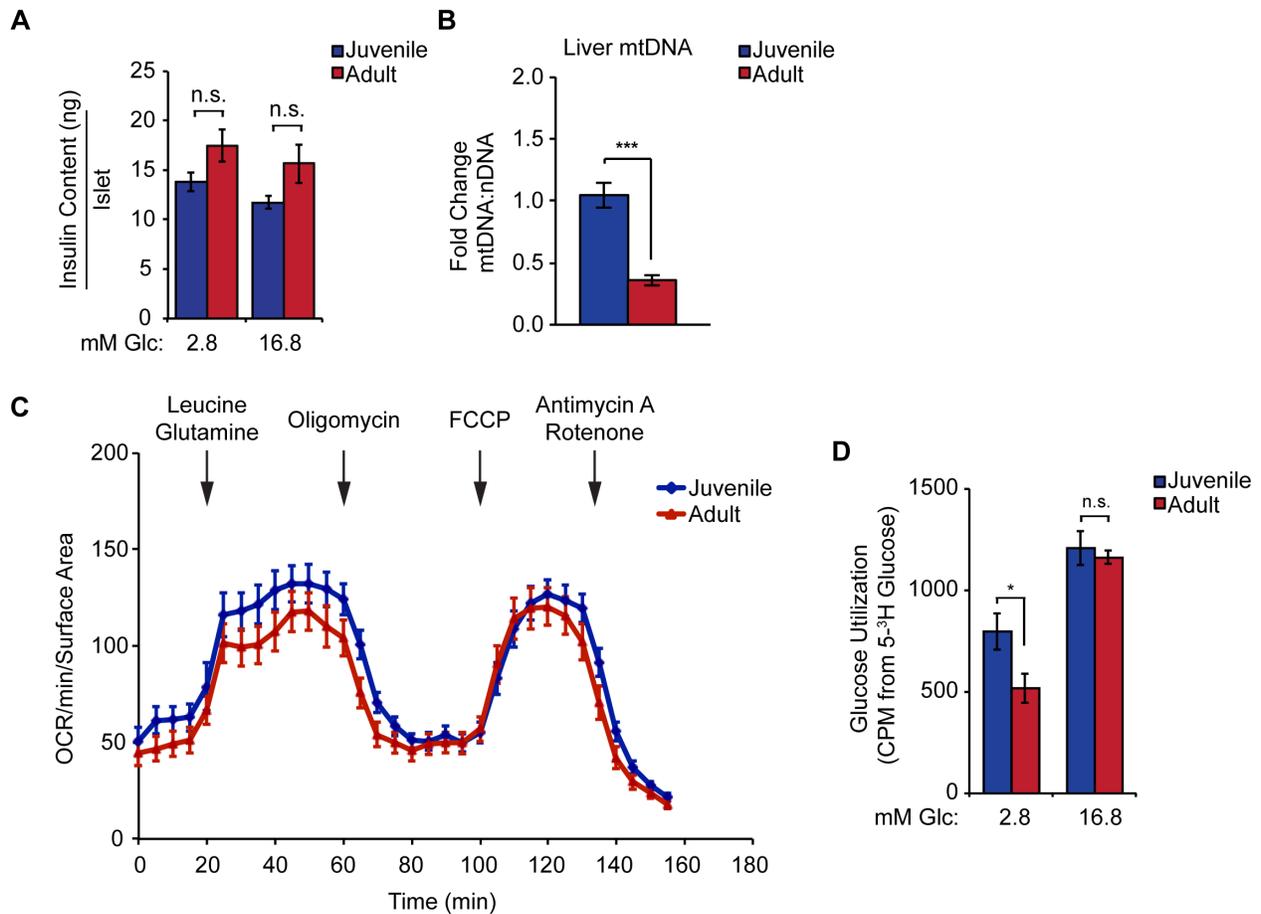


Figure S3 – Related to Figure 3. Age-associated changes in islet insulin content, liver mitochondrial DNA content and islet function.

(A) Insulin content of juvenile and adult islets following a one-hour incubation in the indicated glucose concentrations (n=7-9 replicates per group, calculated from 10 islets per replicate).

(B) Mitochondrial DNA (mtDNA) compared to nuclear DNA (nDNA) in liver from juvenile and adult mice (n=3 mice per group).

(C) Oxygen consumption rates (OCR) of islets from juvenile and adult mice treated with 10 mM Leucine and 10 mM Glutamine, 5 μ M Oligomycin, 2 μ M FCCP, 0.5 μ M Rotenone and 2 μ M Antimycin A (n=7-10 per group).

(D) Glucose utilization rates of juvenile and adult islets as determined by release of ³H₂O from islets incubated with 5-³H glucose. Islets were incubated with the indicated concentrations of unlabeled glucose (Glc) spiked with 5-³H glucose (n=5-6 per group).

Glc, glucose; error bars represent \pm S.E.M.; *p < 0.05, ***p < 0.001 by unpaired two tailed t-test.

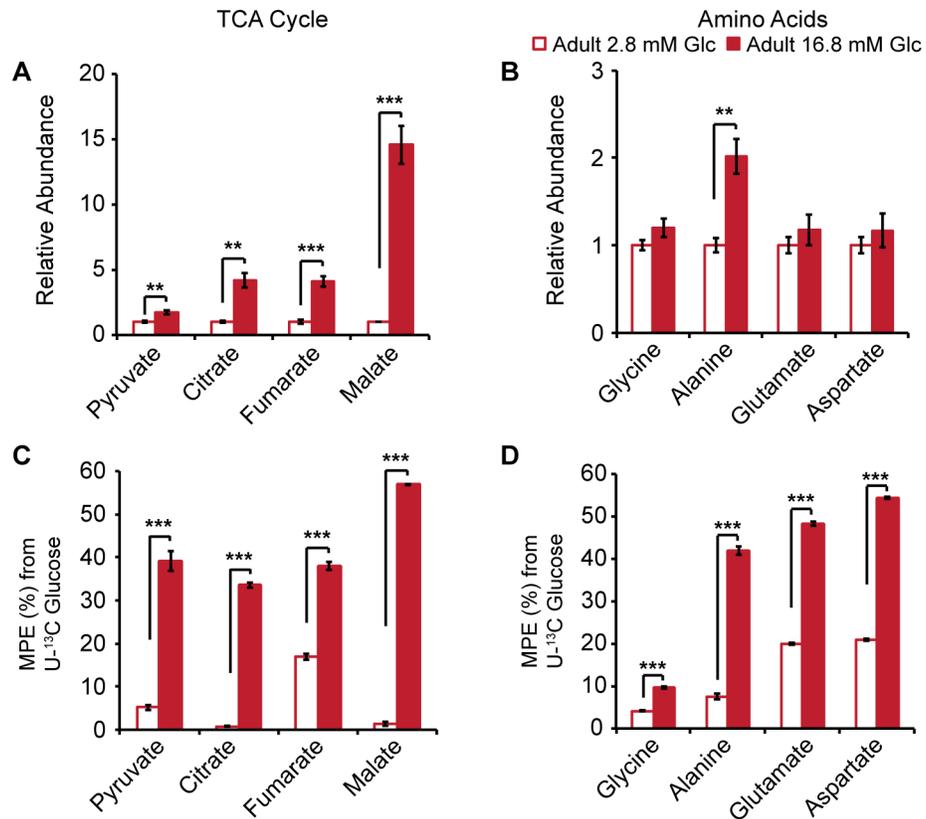


Figure S4 – Related to Figure 4. U-¹³C glucose tracing and targeted metabolomics in islets from adult mice.

(**A** and **B**) Abundances of the indicated metabolites following a one-hour trace with 2.8 mM or 16.8 mM U-¹³C glucose in adult islets, expressed relative to metabolite levels of islets incubated in 2.8 mM U-¹³C glucose.

(**C** and **D**) Molar percent enrichment (MPE) of ¹³C for the indicated metabolites.

Glc, glucose; n=4-6 per group; error bars represent ± S.E.M.; **p < 0.01, ***p < 0.001 by unpaired two tailed t-test.

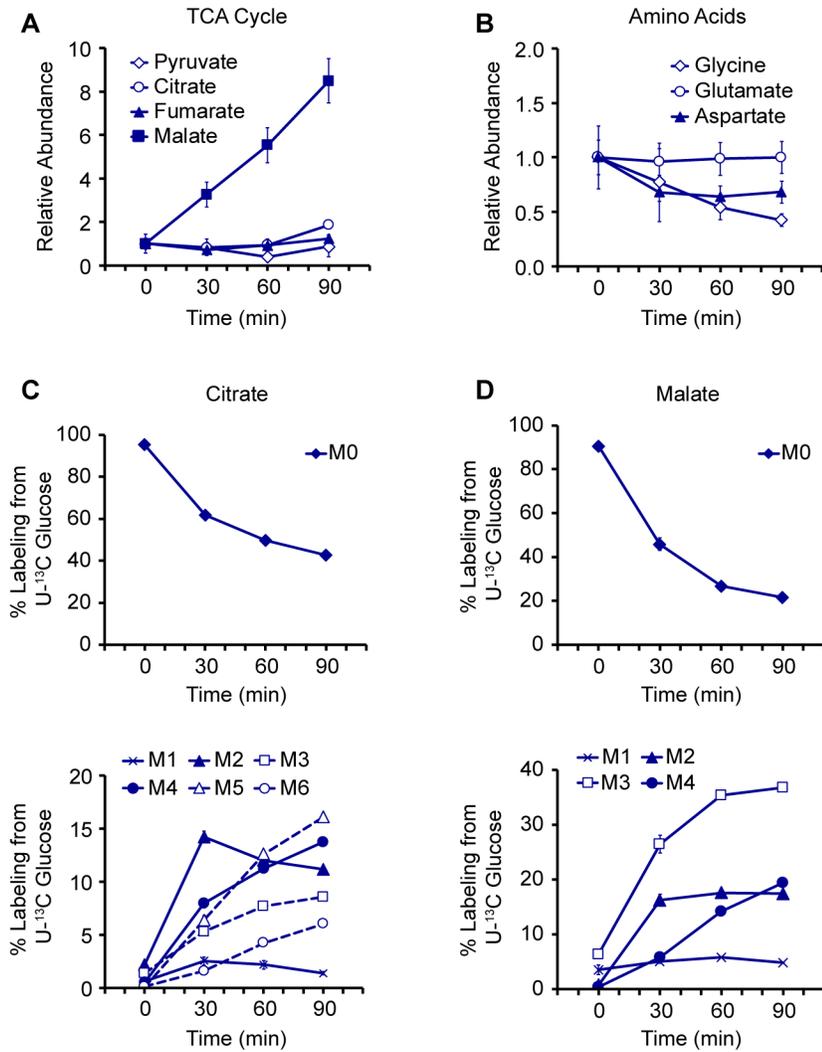


Figure S5 – Related to Figure 5. Isotopologue distributions and metabolite abundances over a time course of U-¹³C glucose tracing.

(A and B) Abundances of the indicated metabolites over a time course of 16.8 mM U-¹³C glucose tracing in islets from 4-week-old mice, expressed relative to metabolite levels at 0 minutes.

(C and D) Isotopologue distributions of ¹³C for citrate (C) and malate (D) over a time course of 16.8 mM U-¹³C glucose tracing in islets from 4-week-old mice. Isotopologue distributions are expressed as % of the total metabolite pool containing the indicated number of labeled atoms.

n=6-7 per group; error bars represent S.E.M.

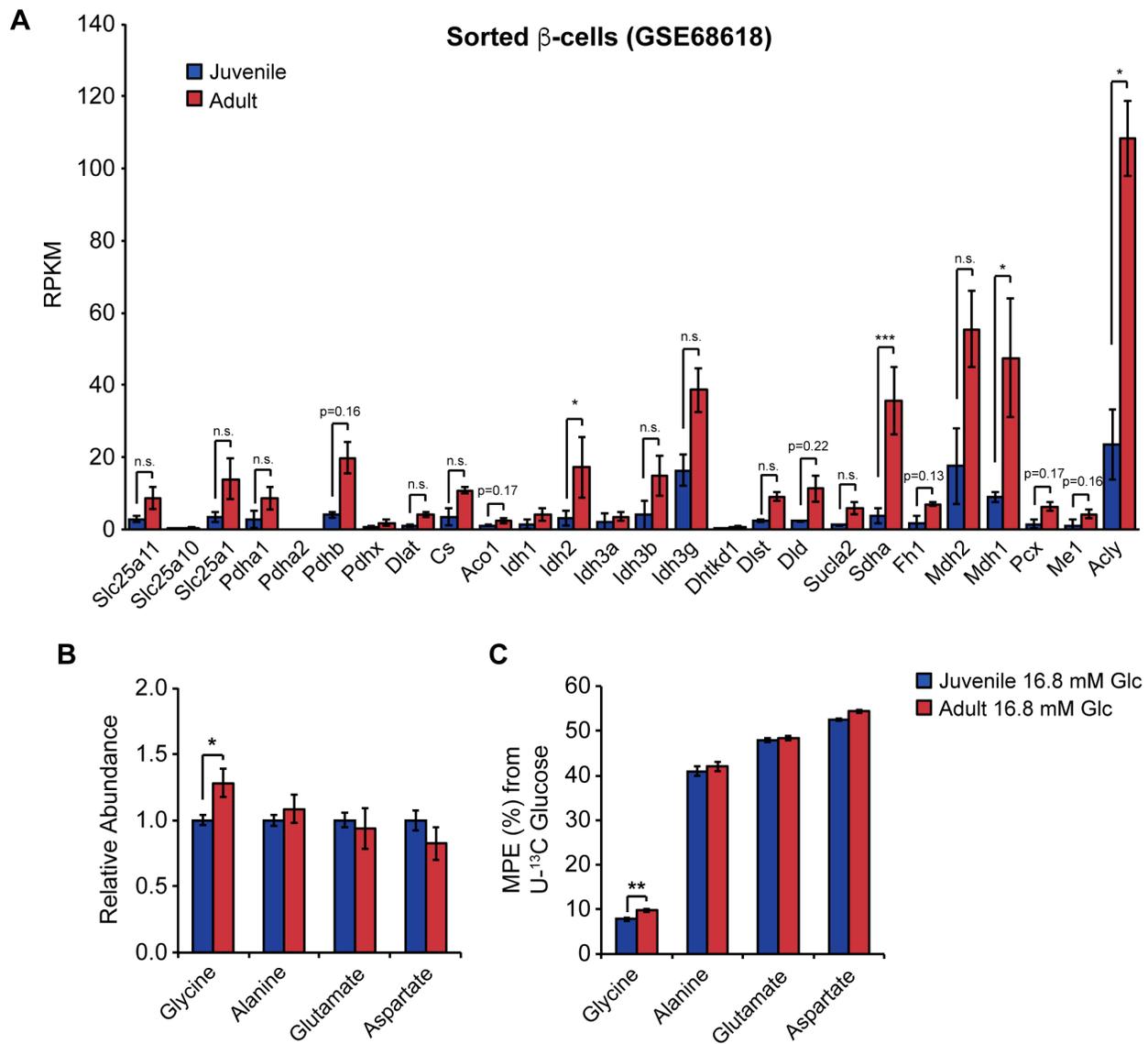


Figure S6 – Related to Figure 6. U-¹³C glucose tracing and targeted metabolomics of amino acids during glucose stimulation in juvenile and adult islets.

(A) RPKM values for mRNAs encoding the indicated enzymes and transporters in FACS-sorted β -cells from juvenile and adult mice (n=2-3 per group). Data from Avrahami et al., 2015. Error bars represent \pm S.E.M.; *p < 0.05, ***p < 0.001 by Cuffdiff.

(B) Abundances of the indicated amino acids following a one-hour trace with 16.8 mM U-¹³C glucose in juvenile and adult islets, expressed relative to metabolite levels of juvenile islets.

(C) Molar percent enrichment (MPE) of ¹³C for the indicated amino acids.

Glc, glucose; n=4-6 per group in B-D; error bars represent \pm S.E.M.; *p < 0.05, **p < 0.01, by unpaired two tailed t-test (B-C).