Supplemental Legends

Figure S1, Related to Figure 2: Food consumption and body weight during NeuCode labeling and effects of label incorporation quantitation using NeuQuant software.

- **A)** Food consumption during the course of the NeuCode labeling experiment in Figure 2.
- **B)** Mouse body weights at the same labeling times as in A).
- **C)** The percentage of proteins able to be quantified by 'area under the curve' analysis out of all proteins identified plotted against the average % incorporation of NeuCode label for all proteins in the MS run.

Figure S2, Related to Figure 3: Food consumption in NeuCode experiment Day 7

- **A)** Amount of food eaten by each mouse during the first 2 weeks of NeuCode labeling. The last 5 days of this period are daily tamoxifen injections.
- **B)** Food consumption during the last 7 days of NeuCode labeling. KO2 became lethargic and was sacrificed 1.5 days earlier than other mice.

Figure S3, Related to Figure 3: Bap1 mRNA expression in various tissues following tamoxifen treatment.

qPCR measurements of Bap1 mRNA in all tissues resected 7 days after the fifth and final daily tamoxifen injection in the NeuCode labeling experiment shown in Figure 3. n=6 Wt and n=6 Ko.

Figure S4, Related to Figure 3: Independent cohort hypoglycemia data

An independent cohort of unlabeled Bap1 homozygous Wt and Ko mice measured for glucose at day 7 post-deletion. N=6 for each genotype. Error bars = SEM.

Figure S5, Related to Figure 4: Food consumption in NeuCode experiment Day 90.

Food consumption during the 21 day labeling period for BMC-Bap1 Wt and Ko mice at 90 days post-tamoxifen injection.

Figure S6, Related to Figure 4: Ribosome and mitochondrial gene sets changing in the pancreas day 90 dataset

Volcano plot of pancreas proteomic data for BMC-Bap1 Ko/Wt mice at 12 wks post-tamoxifen injection. Nominal p-values are plotted on the y-axis and log2 fold-change of Ko/Wt

on the x-axis. Ribosomal structural subunits, mitochondrial proteins (from MitoCarta), and the Reg pancreatic inflammation markers are annotated.

Figure S7, Related to Figures 6 and 7: Fed and Fasted mRNA expression of gluconeogenic markers

(A, B, C) mRNA expression of A) Pgc1a and gluconeogenic targets B) Pck1 and C) G6pc in BMC-Bap1 Wt and Ko mice fed ad libitum or fasted 12 hr. n=3 mice in each group. Error = s.d. * $p<.05$ compared to Wt-Fed group using two-tailed student T-test.

(D) Glycogen (dark pink) stained by PAS and counterstained with hematoxylin. Frozen liver sections were from $Bap1^{fl/fl}$,Alfp-cre^{-} and $Bap1^{fl/fl}$,Alfp-cre^{+} P0 neonates sacrificed approximately 12 hr post-natal.

Table S1: Labeling kinetics data, Related to Figure 1.

Table S2: NeuCode control ratio analysis, Related to Figure 2.

Table S3: Day 7 NeuCode results, Related to Figure 3.

Table S4: Day 90 NeuCode results, Related to Figure 4.

Table S5: NeuCode day 7 K-GG liver data, Related to Figure 4.

Supplemental Experimental Procedures

NeuCode labeling efficiency studies. Male C57BL/6J mice (6 weeks; the Jackson Laboratory) were fed laboratory control diet for 2 weeks before being fed a customized lysine-free diet (Harlan, Madison, WI) combined with 1% natural light lysine (K000). Starting at 10 weeks of age, mice were fed *ad libitum* lysine-free diet containing 1% K602 (n = 12), 1% K080 (n = 12), or 1% K000 (n=3) (Cambridge Isotopes, Boston, MA). Food consumption and body weight were monitored throughout the experiment. At four time points (3, 10, 20, 30 days) mice from K602 (n = 3) and K080 (n = 3) were sacrificed. The control group fed K000 (n = 3) was sacrificed at 30 days. After sacrificing animals by cervical dislocation, tissues were dissected, washed in phosphate-buffered saline (PBS), and frozen in liquid nitrogen. Pancreatic islets of Langerhans were isolated by collagenase digestion and a Ficoll gradient separation as previously described (Rebaglia *et al.* 2005).

Bap1 Mice. Mutant mouse strains were generated using C57BL/6 ES cells. Mice with *loxP* sites flanking (fl) *Bap1* exons 4 and 5 were generated by genOway (Dey et al. 2012). *Bap1*$^{fl/fl}$ mice were crossed to the inducible general deleter C57BL/6 NTac-Gt(ROSA)26Sortm9(Cre/ESR1)Arte (denoted as Rosa26.creERT2 in text). To delete Bap1, *Bap1*$^{fl/fl}$*;Rosa26.creERT2*$^{+}$ mice were injected intraperitoneally with 60 mg/kg tamoxifen dissolved in sunflower oil daily once daily for 5 days. Day 0 is defined as the day of the last tamoxifen injection in figures. The liver specific deletion of BAP1 was generated by crossing Bap1$^{fl/fl}$ mice to the C57/BL6N Alfp.cre deleter strain obtained from the German Cancer Research Center. For high fat diet feeding, 6 week-old male mice were fed with an adjusted calories diet (HFD, containing 60% fat, Harlan Teklad TD.06414) starting 2 weeks post Bap1 deletion. For NeuCode labeling, mice were fed a customized lysine-free diet (Harlan, Madison, WI) combined with 1% K602, K521, K440, or K080 for 3 weeks before sacrificing, resecting and snap-freezing tissues. Small pieces of frozen tissue were cut and DNA was extracted using Qiagen's DNeasy and RNA extracted using RNeasy kits. The Genentech Institutional Animal Care and Use Committee approved these protocols.

Genotyping and quantitative PCR. *Bap1* genotyping primers: 5' CCATCAGTGACTACTGGGGAGCAAC, 5' ACAGATGGCTGGGCACATCTG, and 5' GAACCCTCCGTTGCATAGTGTTG amplified 234 bp WT, 350bp floxed, and 503 bp KO DNA fragments. Cre primers 5′-GCTAAACATGCTTCATCGTCGGTC and 5′-CCAGACCAGGCCAGGTATCTCTG amplified a 582 bp DNA fragment. Cre genotyping primers

for Alp-Cre: 5’ GATTTCCGTCTCTGGTGTAGC, 5’ GCCATCTTCCAGCAGG. Genotyping was performed as previously described (Dey et al, 2012). qPCR was performed using an Applied Biosystems QuantStudio 7 and Fast 2X Mastermix reagent as per manufacturer’s recommendations. dCt’s were calculated using by subtracting the average Ct value of Actb and Hprt. ABI Taqman 20X reagents used were: Actb:Mm00607939_s1, G6pc:Mm00839363_m1,Pck1:Mm01247058_m1,Ppargc1a:Mm01208835_m1,Hprt:Mm00446968_m1,Bap1:Mm01219723_g1,Bap1:Mm00550845_m1.

Protein preparation and Digestion. Tissue samples were pulverized using a Qiagen TissueLyzer II in 1-2ml of 9M urea in 20mM Hepes with ½X tablet of Complete protease inhibitor and 1 mM sodium orthovanadate, 2.5 mM sodium pyrophosphate, and 1 mM ß-glycerophosphate. Lysate was cleared by centrifugation at 20000 g. Following protein concentration estimation by BCA assay, a small amount (<1 mg) of each lysate was mixed according to the experiment. For labeling efficiency studies, samples were left unmixed to assess the amount of unlabeled protein in each sample. For multiplexing Bap1 mouse tissues, lysates were mixed in equal amounts to provide two samples (4-plex and 2-plex) per tissue. Protein disulfide bonds were reduced by addition of 5 mM dithiothreitol (DTT) and incubation for 45 min at 37°C. Free thiols were alkylated by the addition of 15 mM iodoacetamide and incubation in the dark at room temperature for 30 min. The alkylation reaction was quenched by addition of 5 mM DTT. Proteolytic digestion was performed by addition of Lys-C (Wako) at a 1:100 enzyme-to-protein ratio and incubation at 37°C for 2 hr. The urea concentration was then diluted to 4 M using 50 mM Tris, 3 mM $CaCl_2$, and another bolus of Lys-C was added at a 1:100 enzyme-to-protein ratio. The sample was then incubated overnight at room temperature while rocking. The digestion was quenched by the addition of TFA to 1% and then desalted with tC18 Sep-Pak cartridges (Waters). This test sample was analyzed and, if necessary, lysate mixing ratios were adjusted to ensure total protein input was the same across all channels. The newly mixed lysate was then prepped in an identical manner prior to fractionation.

High-pH Reverse Phase Separation. Peptides were fractionated across a Gemini C18 reversed phase column (4.6 mm x 250 mm; Phenomenex) with mobile phases A: 20 mM ammonium formate pH 10; B: 20 mM ammonium formate pH 10 and 80% acetonitrile. The gradient was generated by a Surveyor LC quaternary pump (Thermo) at 0.8 ml/min flow rate. Fractions were collected every minute and pooled into 16 samples which were dried for protein analysis.

NeuCode Mass spectrometry and high performance liquid chromatography. Online reverse-phase chromatography was performed using a nanoAcquity UPLC (Waters, Milford, MA) or Easy-nanoLC 1000 (Thermo Fisher Scientific, San Jose, CA). Peptides were eluted over an analytical column (75 μm ID) heated to 60°C and packed with 30 cm of 1.7 μm diameter, 130 Å pore size, Bridged Ethylene Hybrid C18 particles (Waters). Mobile phase A was composed of water, 0.2% formic acid, and 5% DMSO. Mobile phase B was composed of acetonitrile and 0.2% formic acid. The gradient was optimized to ensure even elution of peptides over a 70 min period. Eluted peptide cations were converted to gas-phase ions by electrospray ionization and analyzed on an Orbitrap Elite mass spectrometer (Thermo Scientific). A survey scan was performed in the Orbitrap at 30,000 resolving power to identify precursors to sample for data-dependent, top 20 ion trap CAD MS/MS (rapid scan analysis). An additional quantitative 480,000 resolving power scan immediately followed the survey scan. Ion trap MS/MS scans were performed while the FT transient collected, by enabling "Preview Mode". Monoisotopic precursor selection was on and precursors with unknown charge or charge of +1 were excluded from MS/MS. MS1 and MS/MS target-ion accumulation values were set to $1x10^6$ and $5x10^3$, respectively. Dynamic exclusion was set to 45 s for -25 ppm and +15 ppm around the selected precursor.

NeuCode data processing. Data reduction was performed with COMPASS (Wenger et al., 2011), a program that converts output files to searchable text files. These text files were then searched against a target-decoy database containing mouse protein entries from UniProt using the Open Mass Spectrometry Search Algorithm (OMSSA, 2.1.8). Cysteine carbamidomethylation was set as a fixed modification, while methionine oxidation was set as a variable modification. To search NeuCode labeled data a variable modification was considered with the average lysine isotopologue mass (+8.0322 Da), as the precursor mass was determined from a medium resolution scan. Precursor mass tolerance was defined as 150 ppm and fragment ion mass tolerance was set to 0.35 Da. Search results were filtered based on precursor mass error and e-value using FDR-Optimizer within COMPASS.

Peptides were quantified using NeuQuant, a previously described software implementation (Merrill et al., 2014). Quantified peptides from all samples were then grouped into proteins using Protein Hoarder within COMPASS. Data from each 4-plex and 2-plex experiment for each tissue was then combined using Procyon within COMPASS. Procyon was developed as a tool to combine quantitative data from multiple experiments and uses a unique identifier (e.g., protein group number or protein group number/modification site) to quickly (3 to 30 sec) create an

output file that contains quantitative data from each experiment for each protein group. Procyon also executes significance testing by enabling users to add unique samples to analysis groups and create comparisons between these groups. If multiple experiments are loaded (as in the case with the 4-plex and 2-plex experiments) Procyon will calculate the mean normalized value for each protein measurement prior to combining the data from both experiments. This enables Procyon to perform significance testing using either a fold change, p-value, or corrected p-value (P-value, Benjamini-Hochberg correction). Procyon outputs contain Log2 transformations of the normalized intensities, the mean normalized intensities, the ratios of user defined comparisons, the significance of those comparisons based on the p-value and adjusted P-value. For gene set enrichment analysis we used only proteins that were quantified in all six mice (3 KO, 3 WT). We input as a list into the DAVID software the protein set for each tissue with greater than 1.5-fold change (2-fold for spleen) and with a nominal P-value of <.05 (Huang et al., 2009). Other settings were left as default. For plotting mitochondrial proteins in figure 3F, we mapped protein IDs from the pancreas data set to MitoCarta (Pagliarini et al., 2008), a more comprehensive list of mouse mitochondrial proteins than currently annotated by gene ontology.

Ubiquitination analysis. Immuno-affinity enrichment and mass spectrometry of diglycine-containing peptides was performed as in Cunningham et al., 2015, with the following modifications. NeuCode liver protein samples were mixed prior at 15mg each sample for 4-plex analysis and 30mg/sample for 2-plex analysis (60mg total). Samples were reduced, alkylated, trypsin digested, immuno-affinity enriched, and run in LC-MS as previously described except a 480,000 resolution scan was set to immediately follow the primary 30,000 resolution scan to resolve NeuCode peaks. Data was searched with GFY as described (Cunningham et al., 2015) except adding a variable mass on lysines of 154.13773 to account for NeuCode labeling. Data was analyzed as above using NeuQuant and median-normalized in each NeuCode channel. Ubiquitinated peptides quantified in 2 or more NeuCode channels were aligned with their matching protein quantifications from non-enriched whole cell lysate. For histone H2A isoforms, the peptides used for non-ubiquitinated total protein quantification were manually curated as the software aggregating peptides to protein-level measurement algorithms did not accurately distinguish isoforms .For the canonical H2a isoform we used the ratio from the peptide VTIAQGGVLPNIQAVLLPKK. For H2ay (macro-H2A), we averaged ratios from: NCLALADDRK, FVIHCNSPVWGADKCEELLEK, GkLEAIITPPPAKK, GkLEAIITPPPAK, EFVEAVLELRK, EFVEAVLELRKK. For H2ax we averaged ratios from KSSATVGPKAPAVGK and SSATVGPKAPAVGK.

Bone marrow transplantation experiments. Recipient animals, *Bap1*$^{fl/fl}$*;Rosa26.creERT2*$^{+}$ or *Bap1*$^{wt/wt}$*;Rosa26.creERT2*$^{+}$, received 2 doses of 525 Rads from a ^{137}Cs source separated by a 4 h interval. Donor bone marrow cells from Bap1$^{wt/wt}$ animals were injected into the tail vein. Reconstituted mice were given water containing 0.11 mg/mL polymyxin B and 1.1 mg/mL neomycin for two weeks and then switched to regular water.

Serum chemistry and Hematology. Blood was obtained from the retro-orbital sinus under anesthesia and collected in tubes containing K3-EDTA (hematology samples) or containing no additive and spun to collect serum (clinical chemistry samples). Total peripheral blood cell counts and red blood cell indices were measured using a Sysmex XT-2000iV hematology analyzer (Sysmex Corporation, Kobe, Japan). For blood glucose, blood samples were obtained by tail nick (5ul) and glucose concentration measured immediately using a glucometer (OneTouch Glucometer made by Lifescan, USA). Serum samples were analyzed using a Beckman AU480 automated clinical chemistry analyzer (Beckman Coulter, Inc., Brea, CA) using the supplied assays (total triglycerides, ALT, AP, total protein). Cholesterol was measured using a Cholestech LDX analyzer (Alere, Inc). Serum insulin was measured with ultra-sensitive mouse insulin ELISA kit (Crystal Chem, Inc.). Serum ketone bodies were measured on the Beckman AU480 using a 3rd party assay (Ranbut) manufactured by Randox Laboratories (Crumlin, County Antrim, UK). All measurements were done as per manufacturer's instructions. Fed glucose was measured at 9–10 am in the morning, and fasted glucose was measured after 16hr overnight fasting unless otherwise specified. For glucose tolerance test (GTT) assay, mice were fasted for ~6hr, and injected intraperitoneally (i.p.) with glucose solution at 2 g/kg. For pyruvate tolerance tests (PTT), mice were fasted overnight (~16 hr). Blood glucose was measured using OneTouch Glucose meter (Life Scan) on samples obtained from tail nick bleeds at the indicated times after glucose administration.

Histology and Immunohistochemistry. For histologic evaluation of the pancreas and liver, tissue samples were formalin-fixed, paraffin embedded, sectioned at 4 μm, and routinely stained with hematoxylin and eosin. Additional sections of liver were OCT embedded and frozen at the time of necropsy. Frozen liver samples were sectioned at *5* μm on a cryostat and fixed in neutral buffered formalin, soaked in propylene glycol for 2 minutes, and then transferred to oil red O pre-heated to 60C. Tissues were then transferred to 85% propylene glycol for 2 minutes, rinsed with water, and counterstained with Modified Mayer's hematoxylin. Insulin immunohistochemistry was performed on 4 μm sections of formalin-fixed, paraffin-embedded mouse pancreas using the Dako polyclonal guinea pig anti-insulin antibody (Dako, Carpentaria,

CA) at a working concentration of 5.5 µg/ml. Non-specific protein binding was blocked with goat serum and endogenous peroxidases were blocked with hydrogen peroxide. Immunolabeling was detected with an ABC Peroxidase Elite detection system (Vector Laboratories, Burlingame, CA) and 3,3'-diaminobenzidine chromogen. Sections were counterstained with Mayer's hematoxylin. Cleaved caspase 3 immunohistochemistry was performed on a Ventana (Tucson, AZ) autostainer using rabbit polyclonal anti-cleaved caspase 3 antibody (Cell Signaling Technologies, Danvers, MA) and CC1 standard antigen retrieval (Ventana). Approximately 5 µm thick tissue sections were incubated with primary antibodies at 0.06 µg/ml concentration. Primary antibodies were detected with biotinylated goat anti-rabbit antibodies (Vector Laboratories, Burlingame, CA) and the reaction was visualized with DAB Map detection kit (Ventana). Slides were counterstained with hematoxylin. Tissues were evaluated for glycogen using the Periodic Acid Schiff (PAS) reaction. Tissue sections were incubated for 5 minutes in 0.5% periodic acid, rinsed and then incubated for in Schiff's reagent (American MasterTech, Lodi, CA) for 15 minutes. Slides were then rinsed with water for 10 minutes and counterstained with hematoxylin.

In-gel proteomics of neonate livers.

Neonate livers were lysed in RIPA buffer, extracted for 15min on ice, and spun for 20min at 4C at 16000g. Soluble protein lysate was normalized by BCA assay and 50ug of protein was electrophoresed 4-5cm into a Bis-Tris gel. Each sample lane was cut into 4 equal pieces, diced into 1mm cubes, and destained in 50% methanol/100mM Ammonium bicarbonate (Ambic). Gel pieces were dehydrated in 100% acetonitrile (ACN), rehydrated with trypsin (Promega) at 1ug/50ul in 100mM Ambic (~100ul volume), incubated on ice 1hr and digested overnight at 37C. 100ul extraction buffer (50%ACN/5% formic acid) was added to each digest and allowed to extract 5min by shaking. The extract was saved and gel pieces further extracted with 100ul 100% ACN. Samples were dried and resuspended in .1% formic acid and 2% ACN for mass spectrometry. Approximately 1ug of digested peptide from each gel band sample was separated over a 60min 98% to 75% acetonitrile gradient on a Pepmap C18 column from Thermo (100A, 2um, 75umx15cm). Peptides were ionized into a Thermo Fusion Orbitrap instrument with an MS1 scan at 120,000 resolution and accepting TopN peptides over a 2s cycle for MS2 sequencing. MS1 and MS/MS target-ion accumulation values were set to $1x10^5$ and $5x10^3$, respectively. Data was searched with Mascot (50ppm) and filtered to 1% FDR at the peptide level and 2% FDR at the protein level using the GFY software suite (Bakalarski et al. 2008). Area under the curve analysis quantification was performed using the Vista module in the GFY

suite. To collapse multiple peptide quantifications to a single protein quantification we used a published linear mixed effect model (Bingol et al. 2014). Biological replicate data were then averaged, the standard error and p-values (Student T-Test) calculated for selected proteins in Figure 7.

Statistical Analyses. Mean values measured from animal groups were compared using unpaired two-way student's T-tests for all metabolic, proteomic, and qPCR mRNA measurements. NeuCode proteomic data was analyzed at the protein-level using T-test nominal p-values and corrected by Benjamini-Hochberg for multiple hypothesis testing where indicated. DAVID software was used with default settings for protein set enrichment analysis. Proteins were selected as input if they exhibited a greater than 1.5-fold change in the liver or pancreas, (2-fold for spleen) and a nominal p-value of <.05 (Huang et al., 2009).

Supplemental References

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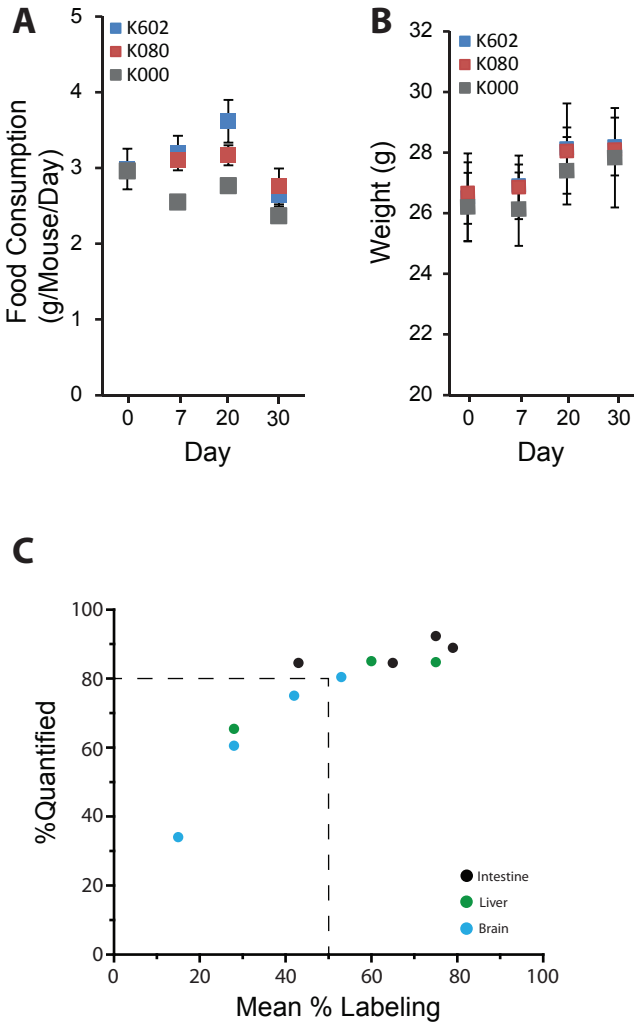
Figure S1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K080
K000
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2
3
4
5
0
7
20
30
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K602
K080
K000
Weight (g)
20
22
24
26
28
30
32
0
7
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Mean % Labeling
Intestine
Liver
Brain

Figure S2

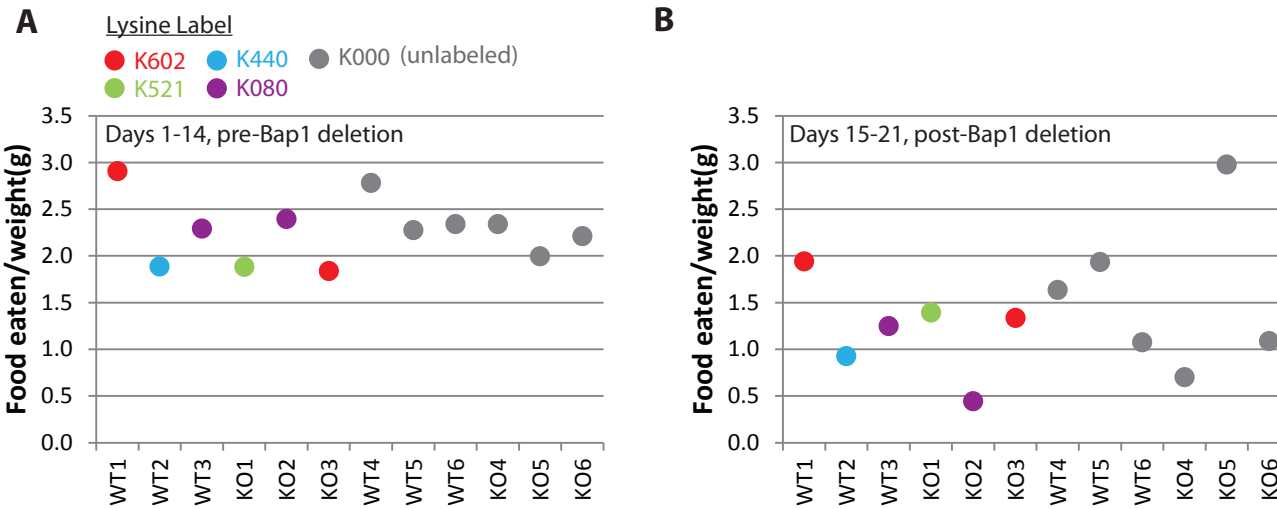
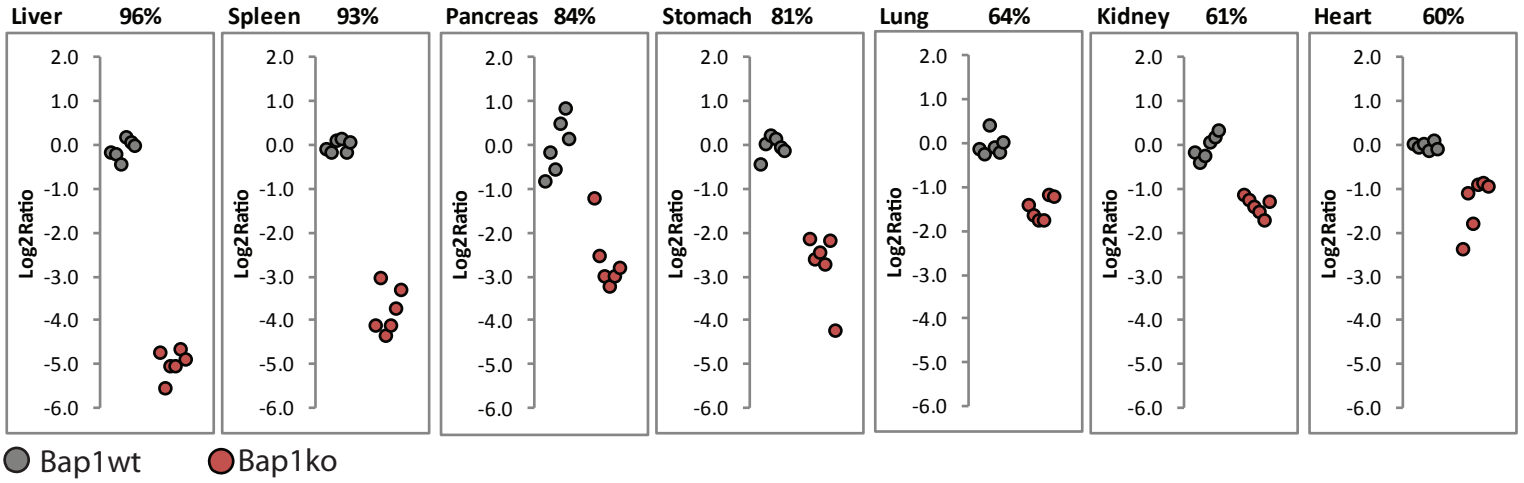


Figure S3



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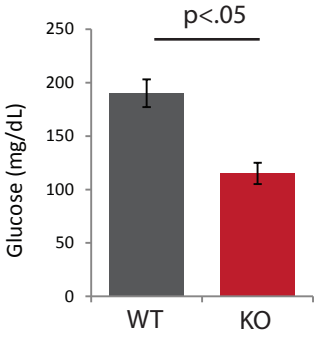


Figure S5

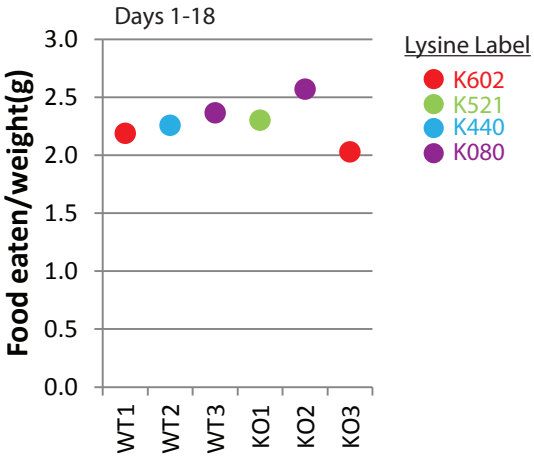
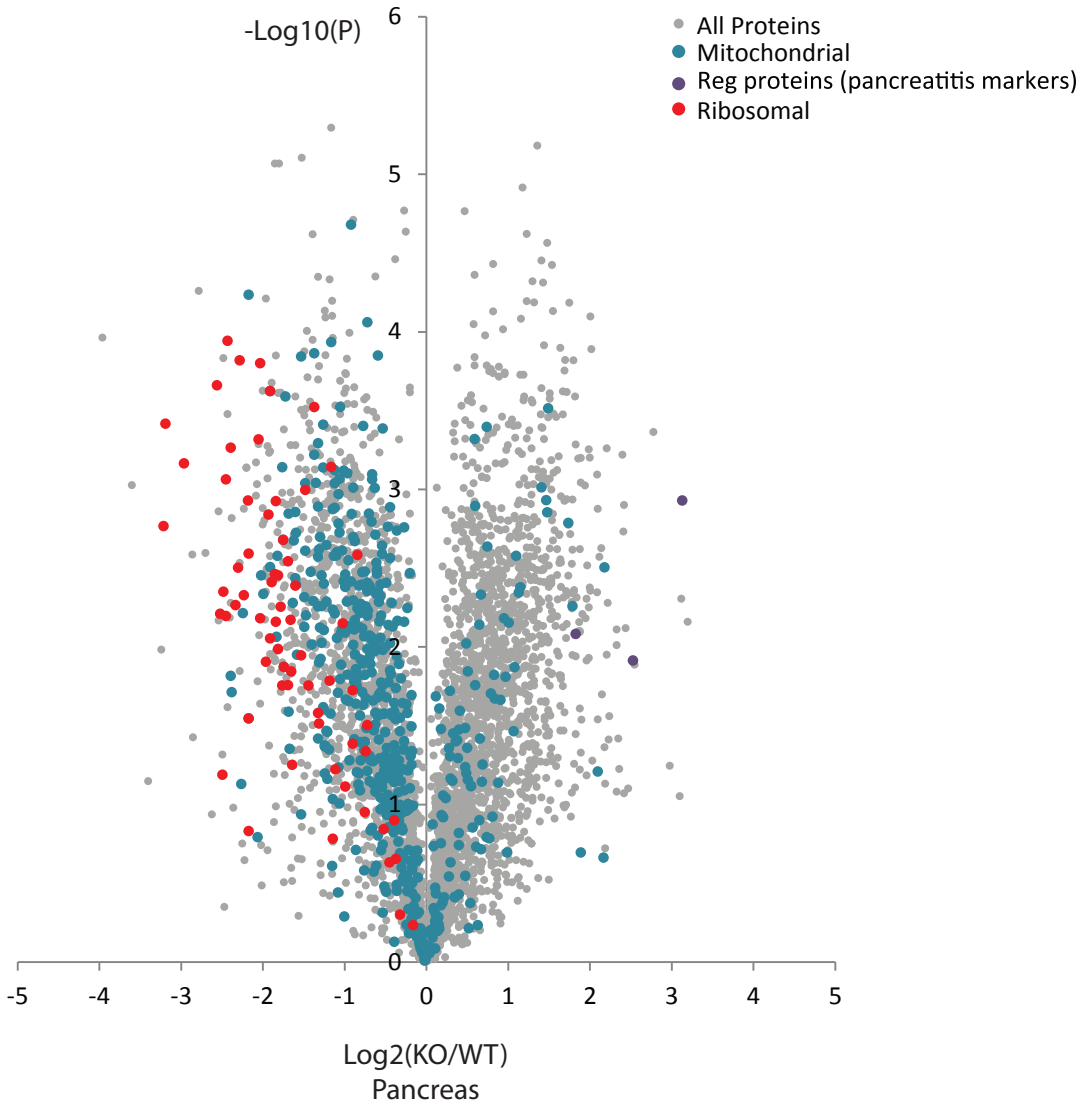


Figure S6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6
5
4
3
2
1
0
All Proteins
Mitochondrial
Reg proteins (pancreatitis markers)
Ribosomal
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-4
-3
-2
-1
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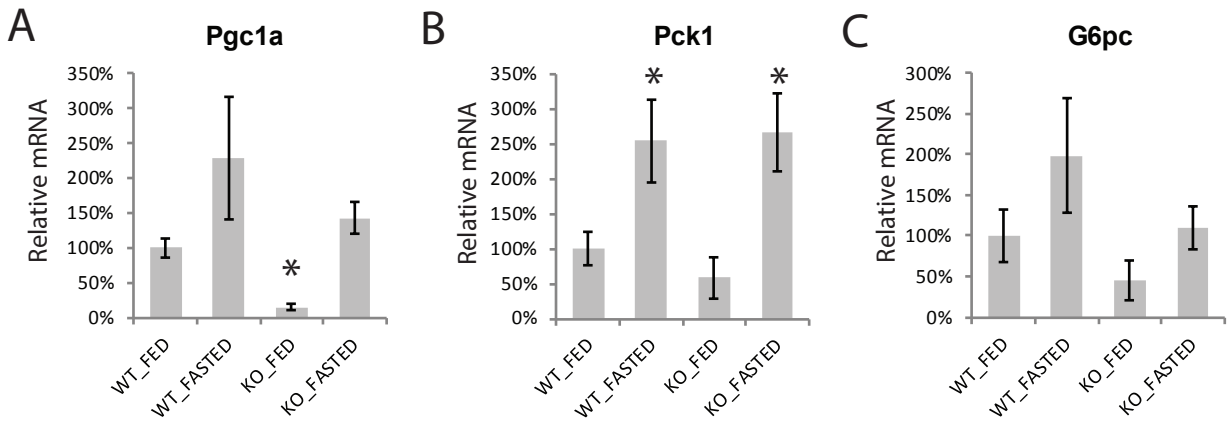
1
2
3
4
5
Log2(KO/WT)
Pancreas

Figure S7



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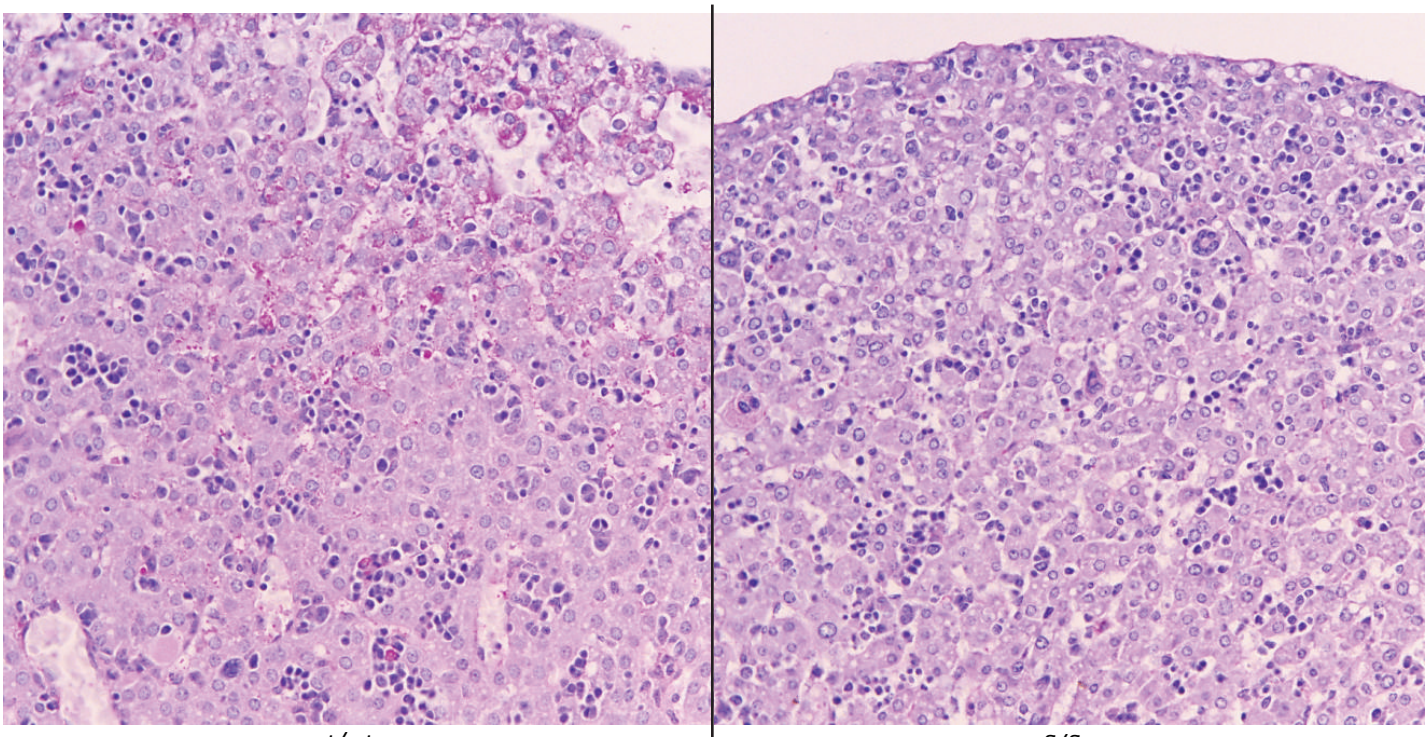

BAP1$^{wt/wt}$;Alfp.Cre^{+}

BAP1$^{fl/fl}$;Alfp.Cre^{+}