Supporting Information

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SI Materials and Methods

Glucose Tolerance Tests (GTT). After an overnight fast, GTTs were performed by i.p. injection of 1.5 g/kg body weight of dextrose (Abbott Laboratories). Blood samples were drawn at 0-, 15-, 30-, 60-, 90-, and 120-min time points and glucose levels measured with a Bayer Elite glucometer. Insulin levels were measured at 0-, 15-, 30-, 60-, and 90-min time points during GTT.

Bomb Calorimetry. The energy content of food and feces were determined using a calorimeter system (IKA C7000, Staufen, Germany). Following dehydration, food and feces samples were homogenized, squeezed into a pill, and placed inside a decomposition vessel (bomb) and combusted with O_2. The temperature measurement took place directly in the bomb and caloric value was calculated from the heat released during the combustion process.

Indirect Calorimetry. Animals were monitored automatically for food consumption and metabolic measurements. For O_2 consumption and CO_2 production mice were housed in metabolic cages placed inside a climate chamber. Gas concentrations were measured by sucking compressed air through custom-made metabolic chambers (flow rate of 50 L/h). The O_2 and CO_2 content of the individual animals were recorded by using O_2 and CO_2 analyzers (Magnos106, Uras14, ABB, Germany). Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were calculated accordingly: VO_2 [mL O_2/h] = (dVol% O_2) × flow rate [L/h] × 10 and VCO_2 [mL CO_2/h] = (dVol% CO_2) × flow rate [L/h] × 10. The respiratory exchange rate was calculated as follows: RER = VCO_2/VO_2. Heat production (HP) was calculated accordingly: HP [mW] = MR × (4.44 + 1.43 × RQ). Data were collected every 30 min over 12-h dark and 12-h light cycles.

Transmission Electron Microscopy (TEM). The tissue specimens were immediately fixed in 3% glutaraldehyde in cacodylate buffer, postfixed in osmium tetroxide, dehydrated in graded series of ethanol, and routinely embedded in epon. Ultrathin sections were examined with a Zeiss EM 10 CR transmission electron microscope after staining with uranyl acetate and lead citrate.

Positron Emission Tomography (PET). All mice were anesthesized with 4% isoflurane (Abbott GmbH, Wiesbaden, Germany) before and 1.5% isoflurane during PET imaging using a veterinary anesthesia system (Vetland Medical Sales and Services, Louisville, KY). [^{18}F]FDG-PET was performed using a dedicated small animal PET system (MicroPET Focus 120, SIEMENS Preclinical Solutions, Knoxville, TN). 3,7–7,4 MBq [^{18}F]FDG was administered, and data acquisition was started immediately after the tracer injection. Data were acquired for 60 min. All acquisition was done in list mode format and histogrammed into a frame of sinogram. The sinogram was reconstructed into a 128 × 128 × 95 voxel image with filtered back projection method with a cut-off at the Nyquist frequency. The voxel size equals 0.433 × 0.433 × 0.796 mm^3. Data were normalized and corrected for randoms, dead time, and decay.

Lipid Determination of Fecal Specimen. Freshly collected stool specimen was treated with 36% acetic acid and directly homogenized on glass slides. Following incubation with 1% Sudan III, the sample was boiled on a hot plate, and the process was repeated 2 times. Finally, the samples were covered with cover slides for microscopic evaluation.

Determination of Fibrosis. Fibrosis was quantified with Sirius Red using Direct red 80 and Fast green FCF (color index 42053) (Sigma). Following deparaffinization and rehydration, sections were incubated for 2 h with an aqueous solution of saturated picric acid containing 0.1% Direct red 80 and 0.1% Fast green FCF. Red-stained collagen fibers were visualized under a microscope.

Determination of Mucin Production. Mucins were detected by Alcian Blue staining. Following deparaffinization and rehydration, sections were incubated for 30 min in Alcian Blue solution (1g Alcian Blue/3% acetic acid, pH 2.5) (Sigma), washed, and counterstained in 0.1% Nuclear Fast Red solution (Sigma). Strongly acidic sulfated mucosubstances were visualized as blue under the microscope.

RNA Analysis. Real-Time PCR was performed as previously described in Arkan *et al.* [(2005) IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* 11:191–198]. For staining, tissues were immediately fixed in 4% paraformaldehyde and processed according to standard procedure. Immunohistochemistry and immunofluorescent staining were performed using α-insulin (Zymed), α-amylase (Sigma), and α-Gr1 (BD PharMingen), α-F4/80 (BD PharMingen), respectively.

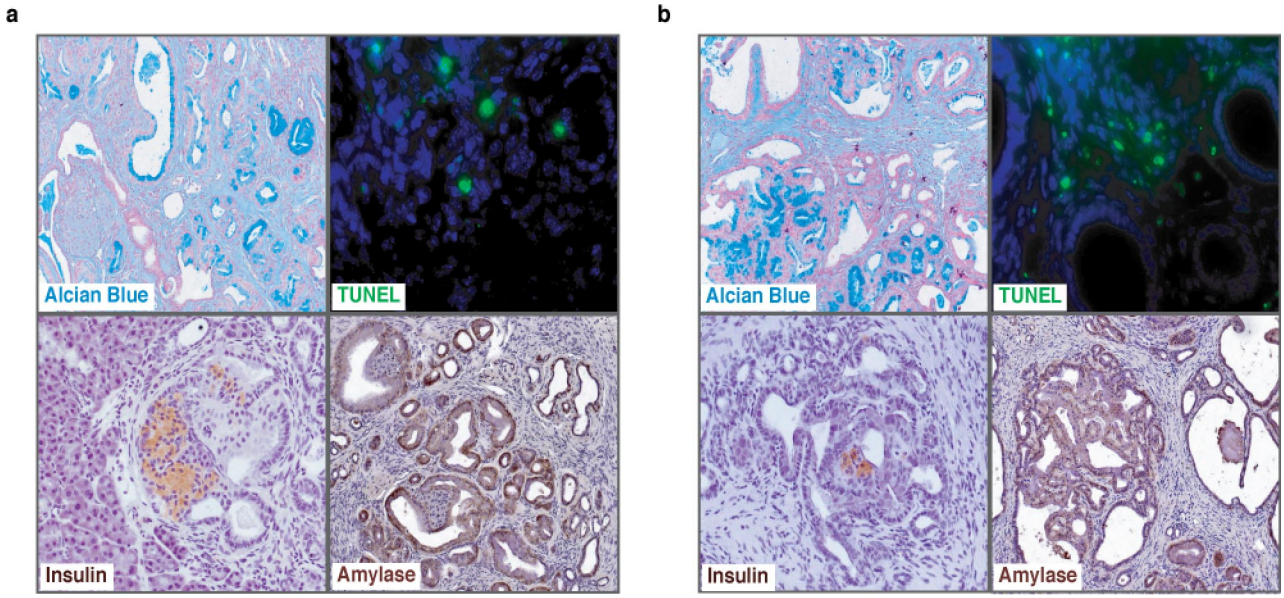

Fig. S1. Oncogenic *K-ras* activation leads to PanIN lesions and a HFD accelerates PanIN development. (*a*) Histological analysis of p48-Kras pancreata on a ND. Apoptotic index was measured by TUNEL. Partial-to-complete loss of acini was shown by decreased α-amylase staining and mucin production was analyzed by Alcian Blue staining. β-Cell compartment of the endocrine pancreas was checked by immunoreactivity to α-insulin. (*b*) Histological analysis of p48-Kras pancreata on a HFD. Apoptotic index was measured by TUNEL. Complete loss of acini was shown by decreased α-amylase staining and mucin production was analyzed by Alcian Blue staining. β-Cell compartment of the endocrine pancreas was checked by immunoreactivity to α-insulin.

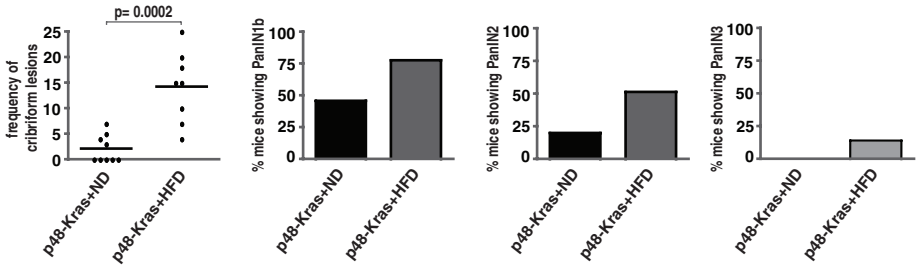

Fig. S2. HFD increases PanIN grade at advanced ages. Histological analysis of pancreata from 30-week-old p48-Kras fed on either a ND ($n = 9$) or a HFD ($n = 8$) showed increasingly advanced PanIN lesions on a high caloric diet. *, $P \leq 0.05$.

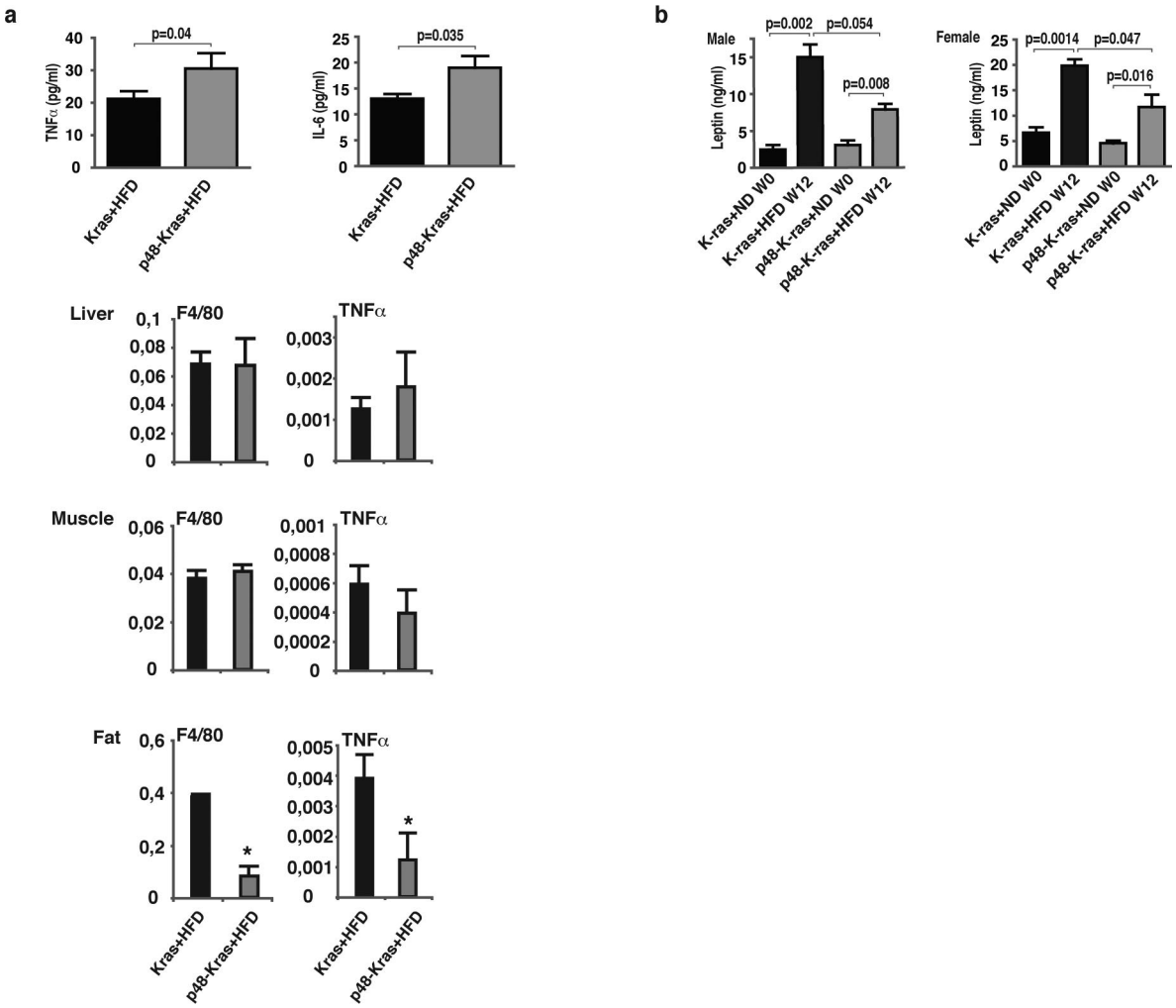

Fig. S3. Increased circulating cytokine and decreased adipokine levels in p48-Kras mice on HFD. (*a*) Analysis of plasma samples from p48-Kras mice showed significantly increased circulating levels of proinflammatory cytokines: TNFα and IL-6. This increase correlated more with the pancreas-specific production of these cytokines because expression levels for F4/80 cell surface marker and TNFα remained unchanged in liver or muscle whereas they were decreased in fat tissue in accordance with (*b*) leptin levels detected in these animals. Data are mean values $\pm$ SEM. $n = 4–6$ mice per genotype. *, $P \leq 0.05$.

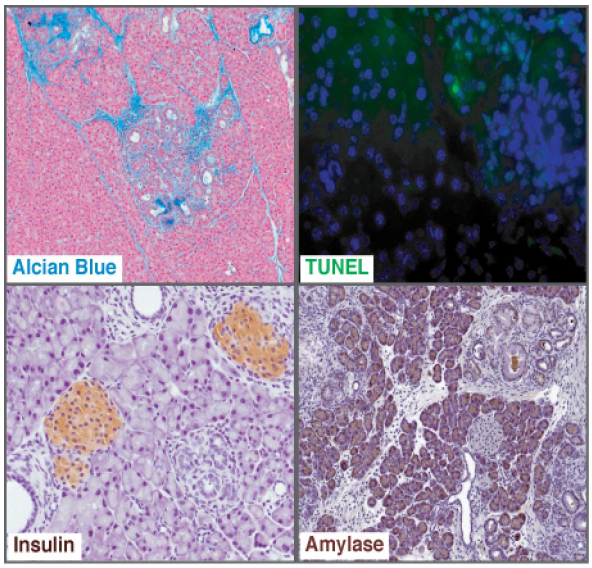

Fig. S4. TNFR1 deletion attenuates PanIN development. Histological analysis of TNFR1$^{-/-}$-p48-Kras pancreata on a HFD. Apoptotic index was measured by TUNEL. Acini integrity was shown by decreased α-amylase staining and mucin production was analyzed by Alcian Blue staining. β-Cell compartment of the endocrine pancreas was checked by immunoreactivity to α-insulin.

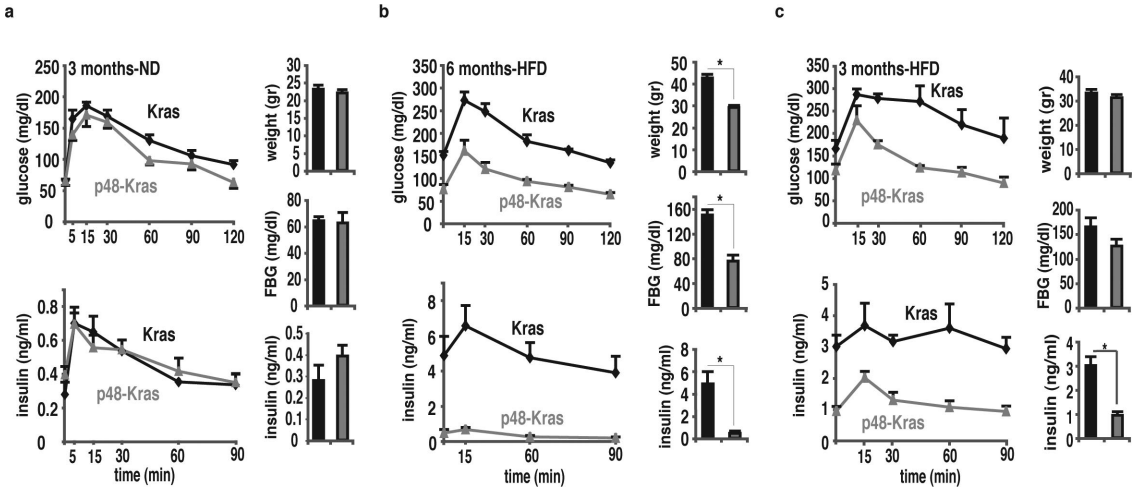

Fig. S5. p48-Kras mice remain insulin sensitive and glucose tolerant on a HFD regardless of gender. (*a*) During GTTs, male Kras and p48-Kras mice did not show any difference in their glucose curves when fed a ND. (*b* and *c*) However, when kept on the HFD, p48-Kras males at both 3 and 6 months of age, regardless of weight changes, remained significantly glucose tolerant in contrast to their littermate controls. Data are mean values ± SEM. $n = 5–6$ mice per genotype. *, $P \leq 0.05$.

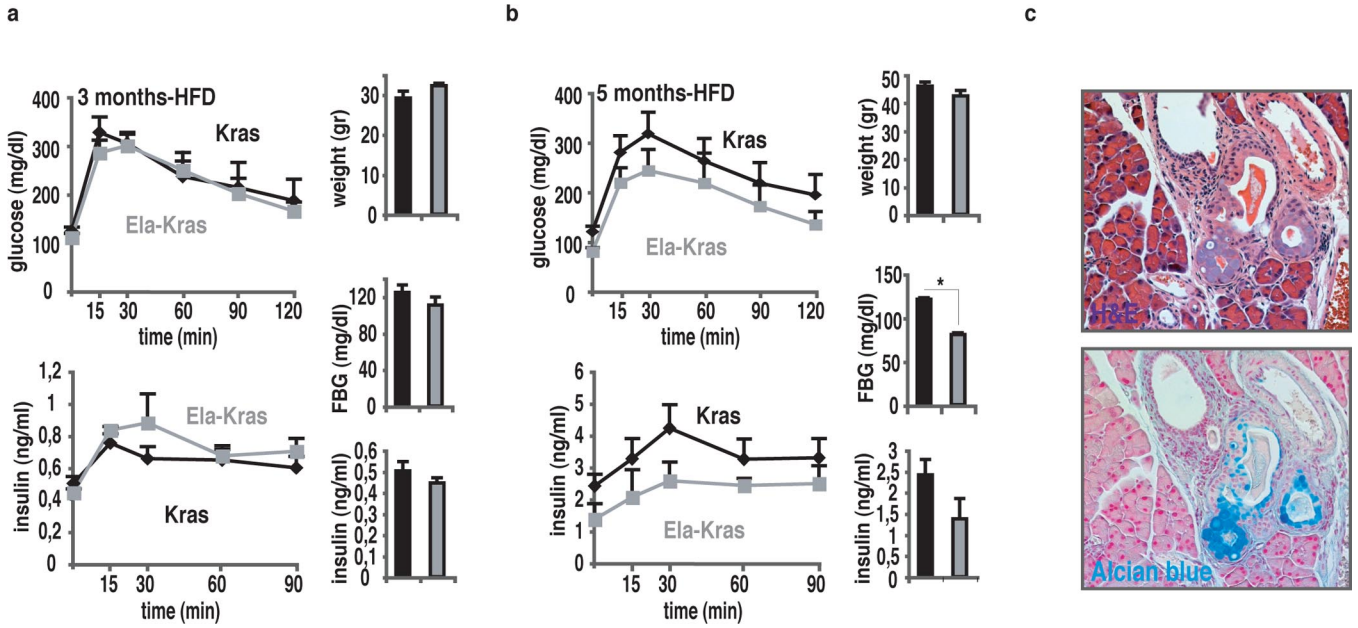

Fig. S6. Oncogenic K-ras expression in exocrine pancreas is responsible for the metabolic phenotype. (*a*) Female Kras controls and Ela-Kras mice did not show any difference in their response to a glucose challenge at 3 months of age. (*b*) Ela-Kras mice displayed significantly reduced fasting blood glucose levels at 5 months of age and an increasing trend toward improved glucose tolerance over age. (*c*) Induction of deletion took place around 4 weeks of age in Ela-Kras mice and histological analysis revealed mucin positive early PanIN 1a lesions. Data are mean values ± SEM. $n = 5–6$ mice per genotype. *, $P \leq 0.05$.

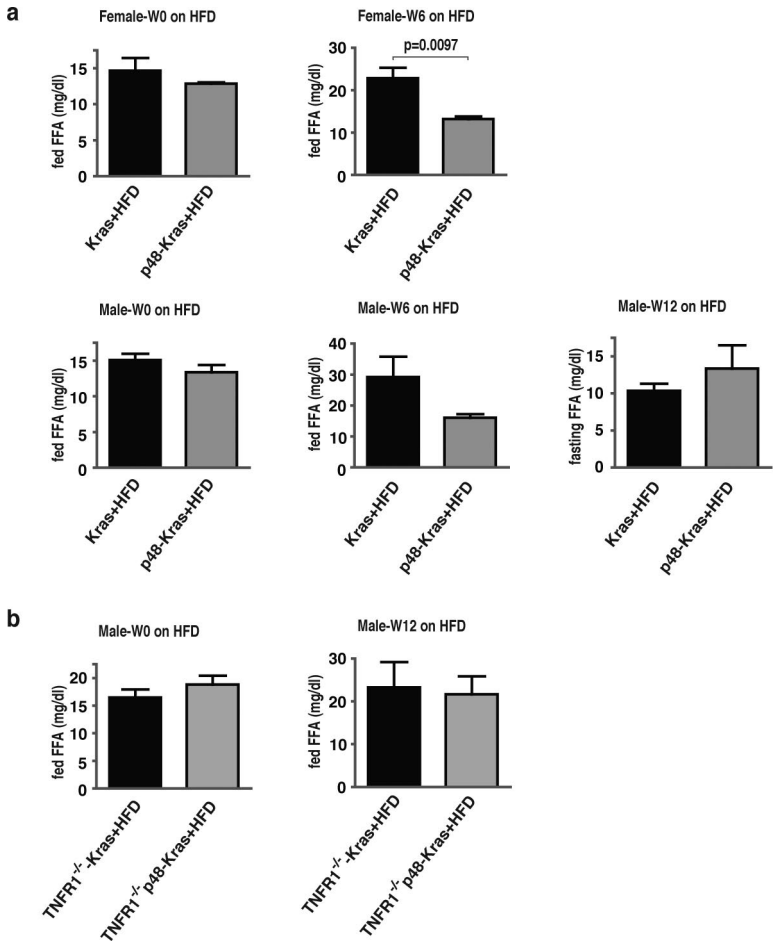

Fig. S7. Postprandial FFA levels are decreased in p48-Kras on a HFD. Circulating FFA levels were measured in p48-Kras, TNFR1$^{-/-}$-p48-Kras mice, and relative littermate controls, before and after a HFD regimen, under fed conditions. (*a*) Postprandial FFA levels remained significantly low in p48-Kras mice following high-fat feeding for 6 weeks compared to that of Kras controls. Fasting FFA in p48-Kras mice after 12 weeks on a HFD showed slightly higher levels than Kras controls but the difference remained insignificant. (*b*) Although very slightly raised after 12 weeks on the HFD, FFA levels stayed similar in both TNFR1$^{-/-}$-p48-Kras mice and littermates. Data are mean values ± SEM. $n = 5–6$ mice per genotype. *, $P \leq 0.05$.

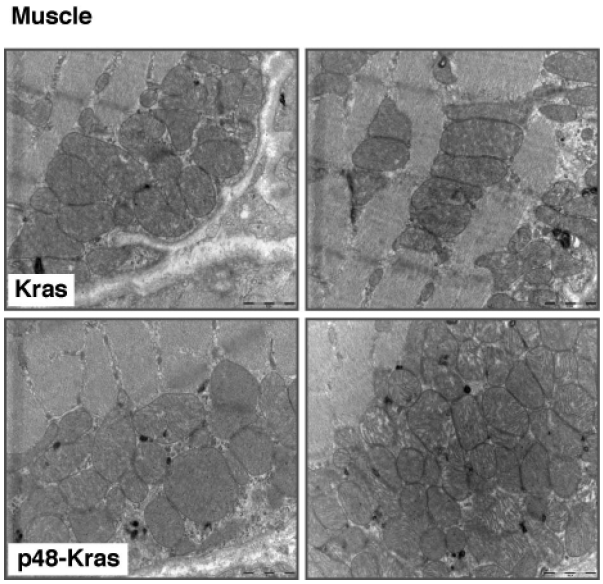

Fig. S8. Ultrastructural changes in the mitochondria during tumorigenesis is specific to liver. TEM pictures showing lack of ultrastructural changes in muscle mitochondria in p48-Kras mice and Kras control littermates on a HFD.