PGC-1 β promotes enterocyte lifespan and tumorigenesis in the intestine

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The mucosa of the small intestine is renewed completely every 3–5 d throughout the entire lifetime by small populations of adult stem cells that are believed to reside in the bottom of the crypts and to migrate and differentiate into all the different populations of intestinal cells. When the cells reach the apex of the villi and are fully differentiated, they undergo cell death and are shed into the lumen. Reactive oxygen species (ROS) production is proportional to the electron transfer activity of the mitochondrial respiration chain. ROS homeostasis is maintained to control cell death and is finely tuned by an inducible antioxidant program. Here we show that peroxisome proliferator-activated receptor- γ coactivator-1 β (PGC-1 β) is highly expressed in the intestinal epithelium and possesses dual activity, stimulating mitochondrial biogenesis and oxygen consumption while inducing antioxidant enzymes. To study the role of PGC-1 β gain and loss of function in the gut, we generated both intestinal-specific PGC-1 β transgenic and PGC-1 β knockout mice. Mice overexpressing PGC-1 β present a peculiar intestinal morphology with very long villi resulting from increased enterocyte lifespan and also demonstrate greater tumor susceptibility, with increased tumor number and size when exposed to carcinogens. PGC-1 β knockout mice are protected from carcinogenesis. We show that PGC-1 β triggers mitochondrial respiration while protecting enterocytes from ROS-driven macromolecule damage and consequent apoptosis in both normal and dysplastic mucosa. Therefore, PGC-1 β in the gut acts as an adaptive self-point regulator, capable of providing a balance between enhanced mitochondrial activity and protection from increased ROS production.

nuclear receptors | gene expression | molecular pathology | colon cancer

The intestine represents the interface between the organism and its luminal environment and is constantly challenged by mechanical stress, diet-derived toxins and oxidants, and endogenously generated reactive oxygen species (ROS), which can induce serious damage to all biological molecules and cell structures (1). To preserve cellular integrity and tissue homeostasis, the intestine possesses self-renewing capacity via the continuous migration of new enterocytes that undergo differentiation from the crypt to the apical compartment of the villus, where they become competent to apoptosis and are shed into the lumen. ROS accumulation within intestinal epithelial cells promotes apoptotic cell death in the differentiated compartment (2). The mitochondrial electron transport chain is a major site of ROS production in the cells. Under physiological conditions, the balance between ROS generation and detoxification is controlled by a set of cellular enzymes including superoxide dismutase and catalase. Important components of the ROS-scavenging pathways are linked to mitochondrial oxidative metabolism via the peroxisome proliferator-activated receptor- γ coactivators 1 α and 1 β (PGC-1 α and PGC-1 β), apparently enabling cells to maintain normal redox status in response to changing oxidative capacity (3). PGC-1 α and PGC-1 β are master regulators of mitochondrial biogenesis and oxidative metabolism as well

as antioxidant defense. Both PGC-1 α and PGC-1 β are preferentially expressed in tissues with high oxidative capacity where they participate, through mitochondrial biogenesis, in the metabolic response to high energy demand (4), such as cold-adapted thermogenesis in brown adipose tissue (5), fiber-type switching in striated muscle (6), and fatty acid β oxidation and gluconeogenesis in liver during a fasting state (7, 8). The increase in mitochondrial biogenesis and activity stimulated by PGC-1 proteins may cause an increase in the production of ROS. However, PGC-1 α also has been shown to increase the expression of the major mitochondrial antioxidant enzyme superoxide dismutase 2 (Sod2) (3, 9). Therefore, PGC-1 α is able to upgrade aerobic energy metabolism while preserving ROS homeostasis, by simultaneously promoting ROS formation and detoxification. Recently, it has been shown in *Drosophila* that the PGC-1 α homolog spargel is able to induce mitochondrial function and oxygen consumption, which is coupled to the induction of scavenger systems and ROS reduction, finally leading to increased longevity (10). On the other hand, in the differentiated intestinal epithelium of mice, PGC-1 α induces mitochondrial biogenesis and oxygen consumption, but it is not able to induce the ROS-scavenging apparatus, thus promoting ROS-dependent apoptotic cell death (2).

Significance

The mucosa of the small intestine is renewed completely every 3–5 d during the entire lifetime through the continuous steps of proliferation, migration, and differentiation of the cells of the mucosa from the crypt site on the bottom to the villus site on the top of the mucosa. The factors that regulate enterocyte lifespan and aging are of special interest as related to colon cancer susceptibility. Here, using genetically modified gain- and loss-of-function models, we present the importance of the mitochondrial respiration chain and reactive oxygen species homeostasis in the gut and identify the protein peroxisome proliferator-activated receptor- γ coactivator-1 β as a gene-expression modulator of enterocyte lifespan in both normal and tumoral conditions.

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isolated from the crypt–villus axis of the intestinal epithelium (Fig. S1A). Although PGC-1 β is expressed along the entire crypt–villus axis, its expression seems to be higher in the fractions (V2–C1) that correspond to the middle region of the villus down to the crypt (Figs. S1B and S2C). Although PGC-1 α is finely regulated at transcriptional and posttranslational levels in various tissues, the overall expression of PGC-1 β is constant, thus indicating that PGC-1 β serves as a rheostat of mitochondrial function. To investigate the role of PGC-1 β in the intestinal epithelium in a gain-of-function fashion, we generated iPGC-1 β mouse model in which human PGC-1 β (hPGC-1 β) is overexpressed selectively in the epithelial cells of the intestine. To this end, we subcloned the hPGC-1 β coding sequence downstream of the villin promoter. The highest levels of hPGC-1 β mRNA were found in the jejunum, where it was 90 times higher as compared with mouse PGC-1 β (mPGC-1 β); the lowest expression was found in the colon, where we observed 23 times higher levels of hPGC-1 β vs mPGC-1 β (Fig. S1C). These mice express the hPGC-1 β transgene along the entire crypt–villus axis as demonstrated by mRNA and protein analysis on different fractions collected from the ilea of transgenic mice (Figs. S1D and S2C). The mRNA analysis shows that the expression of the human coactivator is driven by villin promoter in all the fractions considered but is higher in the fraction corresponding to the most differentiated enterocytes. However, immunohistochemical analysis for PGC-1 β protein in ileum specimens from wild-type and iPGC-1 β mice reveals an overexpression of the coactivator through the entire axis of transgenic mice, without showing significant differences along the axis (Figs. S1E and S2C).

The overexpression of hPGC-1 β protein in the intestinal epithelium leads to a striking difference in the morphology of the intestine, specifically in the dimensions of villi and crypts in the small and large intestine, respectively. The epithelium is visibly longer in transgenic than in wild-type mice (Fig. 1C), and quantification of the length shows that the epithelium of transgenic mice is 30% longer than that of wild-type mice (Fig. 1D). This significant variation in the length of intestinal epithelium in the small and large intestine theoretically could be explained either by a higher rate of proliferation forcing the migration of the enterocytes from the crypt along the crypt–villus axis or by a lower rate of apoptosis at the apex of the villi. To address this point, we decided to examine the proliferative state of the enterocytes in the intestinal epithelium of wild-type and transgenic mice using bromodeoxyuridine (BrdU). Mice were killed at 2 h and 72 h after intraperitoneal (i.p.) injection of BrdU to examine BrdU-positive cell migration (Fig. 1E). At the first time point (2 h), BrdU-stained cells were found in the stem cell niches of the crypts and in the transit-amplifying compartment in both wild-type and iPGC-1 β mice (Fig. 1E). Strikingly, at 72 h, the BrdU-labeled enterocytes had migrated to the tips of the villi in control mice, but in the transgenic mice the labeled cells reached only halfway up the villus, likely because of the greater length of the villi. Notably, 72 h after BrdU injection, although most of the BrdU staining was lost in the wild-type villi because of apoptotic shedding of mature enterocytes, the iPGC-1 β mice retained the majority of the BrdU-positive cells (Fig. 1F). Thus, the overexpression of PGC-1 β in the enterocytes leads to significant changes in the length of the intestinal epithelium without affecting enterocyte proliferation. Indeed, these results suggest that the cycling stem cells of the transgenic crypts proliferate at the same rate as those of the wild-type crypts, and the longer villi are the result of increased enterocyte lifespan arising from the longer migrating time to the apical apoptotic compartment.

Intestinal PGC-1 β Overexpression Induces Genes Involved in Mitochondrial Function. To study the transcriptional scenario that PGC-1 β eventually activates in this intestinal context, we performed microarray analysis of ileum samples from wild-type

and iPGC-1 β mice. The data showed that overexpression of the PGC-1 β coactivator can induce a plethora of genes involved in several metabolic pathways (Fig. 2A). The majority of target genes whose expression is enhanced 1.3-fold or more by PGC-1 β encode for proteins that play an active role in oxidative phosphorylation (Fig. 2A). Other pathways influenced by the overexpression of PGC-1 β are the tricarboxylic acid (TCA) cycle, the nuclear factor erythroid 2 (NF-E2)-related-like 2 (NRF2)-mediated oxidative stress response, the glycolysis and gluconeogenesis pathways, and fatty acid, glutathione, and ubiquinone metabolism. These results were confirmed by real-time qPCR analysis of the expression levels of medium-chain acyl-CoA dehydrogenase (Mcad), cytochrome *c* (cytC), ATP β -synthase (ATP β synt), and mitochondrial transcription factor A (Tfam), which were increased 1.4-, 2.3-, 1.5-, and 1.7-fold, respectively,

Fig. 2. Intestinal PGC-1 β induces genes involved in mitochondrial function. (A) The gene-expression profiles of ileum samples from wild-type and iPGC-1 β mice were analyzed by microarray analysis. The metabolic pathways differentially expressed in wild-type and iPGC-1 β mice were identified using DAVID software available on the DAVID Bioinformatics Resources website (david.abcc.ncifcrf.gov). The number of genes up-regulated by 1.5 fold in the iPGC-1 β mice is indicated for each pathway. (B) Mcad, cytC, ATP β synt, and mitochondrial Tfam mRNAs were measured in ileum specimens from wild-type and iPGC-1 β mice by real-time qPCR. Wild-type and transgenic mice ($n = 6$) were compared using a Student *t* test followed by a Mann–Whitney *u* test. Results are expressed as mean $\pm$ SEM; * $P < 0.05$. (C) Western blot analysis demonstrates increased COX I and porin protein in enterocytes isolated from iPGC-1 β mice as compared with enterocytes from wild-type mice. (D) PGC-1 β overexpression determines the increase in both the endogenous and COX respiratory capacities of intact enterocytes. A-T, ascorbate/TMPD-dependent oxygen consumption; ER, basal endogenous respiration; UR, DNP-uncoupled respiration. * $P < 0.05$. (E) Enterocytes isolated from iPGC-1 β mice present higher enzymatic activity of both Complex IV and citrate synthase. * $P < 0.05$.

sirtuin family, representing the major mitochondrial deacetylase. Therefore, it would be involved in ROS detoxification indirectly, by regulating Sod2 activity (13). Interestingly, the antioxidant enzymes analyzed here were induced in the iPGC-1 β mouse mucosa along the entire crypt–villus axis with the highest mRNA expression in the differentiated apical enterocytes (Fig. S24). The increase in Sod2 expression in the iPGC-1 β mice also was confirmed by immunohistochemical analysis in the differentiated compartment of the villus as well as in the crypts (Fig. S2B). Western blot analysis confirmed highest overexpression of Sod2 in the most differentiated fractions of iPGC-1 β villi (Fig. S2C). Overall, PGC-1 β overexpression in the intestine decreases ROS-driven macromolecule damage, thus leading to a reduction in apoptotic cell death at the apex of the villi. Together with a higher respiratory rate and more efficient mitochondrial activity, this coactivator can induce the whole battery of mitochondrial antioxidant enzymes, thus prolonging enterocyte survival and conferring the peculiar morphology of the epithelium of the iPGC-1 β mice compared with wild-type mice.

Intestinal PGC-1 β Overexpression Promotes Intestinal Carcinogenesis.

ROS are mutagenic and may stimulate tumorigenesis through the oxidation of DNA and the subsequent accumulation of mutations in key genes involved in cell cycle and proliferation as well as in carcinogenesis. Recently, however, it was demonstrated that activating a ROS-detoxification program promotes tumorigenesis (14). To investigate if the effect of PGC-1 β on enterocyte survival also might affect the ROS antioxidant response and the life span in transformed enterocytes, we tested the effects of PGC-1 β overexpression in two different models of intestinal tumorigenesis. The first chemical model consisted of a single i.p. injection with azoxymethane (AOM) a DNA-alkylating agent facilitating base mispairings (15), followed by three cycles of oral dextran sodium sulfate (DSS) to sustain intestinal tumor progression via induction of colitis (16). The second, a genetic model, was generated by crossing *Apc^{min/+}* mice (17) with iPGC-1 β mice. In the chemical model, gross morphology (Fig. 4A) and histological analysis of the intestine revealed worse dysplasia of the tumors in iPGC-1 β mice (Fig. 4B), in which both the average number of tumors and average tumor size were increased (Fig. 4C), as compared with their littermate controls. In the genetic model of intestinal tumorigenesis, the number and size of the tumors were quantified in 7-mo-old mice, because 7 mo is the age of onset of tumor formation in the wild-type mice (FVB/N) crossed with *Apc^{min/+}* mice (C57BL/6J strain). Notably, in a group of 20 iPGC-1 β /*Apc^{min/+}* mice, approximately 40% died before reaching the age of 7 mo, whereas there was no mortality at this age in the control littermates. Consistently, the surviving iPGC-1 β /*Apc^{min/+}* mice showed a significant increase in the number and size of tumors in the ileum compared with FVBN/*Apc^{min/+}* mice (Fig. 4D). Surprisingly, the total number of colon tumors did not differ between the two mouse groups. However, the size of the colon tumors was significantly greater in iPGC-1 β /*Apc^{min/+}* mice than in their age-matched control (FVBN/*Apc^{min/+}*) (Fig. 4D).

Interestingly, the tumors from iPGC-1 β /*Apc^{min/+}* mice maintain the overexpression of the PGC-1 β transgene (Fig. 5B) as shown in the immunohistochemistry, confirming that the villin promoter also is able to drive the expression of the hPGC-1 β during tumor development. In accordance, microarray analysis performed on tumor specimens from wild-type and iPGC-1 β mice revealed that the overexpression of the coactivator still is able to induce expression of genes involved in oxidative phosphorylation, the TCA cycle, glycolysis, and fatty acid metabolism (Fig. S34). Nevertheless, microarray analysis showed that several pathways involved in tumorigenesis, such as ephrin receptor, mTor, and ERK/MAPK signaling, are up-regulated in tumors of iPGC-1 β mice (Fig. S34). Interestingly, several genes involved in molecular mechanisms of cancer were affected by the overexpression of PGC-1 β : The protumorigenic proteins *Fzd10*,

Fig. 4. Intestinal PGC-1 β promotes chemically and genetically induced intestinal carcinogenesis. (A) Gross morphology of colon samples from wild-type and iPGC-1 β mice. (B) Paraffin-embedded ileum and colon specimens from wild-type mice and iPGC-1 β mice were stained with H&E and observed by light microscopy. (Magnification: 25 $\times$) (C) Number of tumors (Left) and number of tumors categorized by size (Right) per mouse from colons of wild-type and iPGC-1 β mice at the end of the AOM-DSS treatment. The comparison of wild-type and transgenic mice ($n = 10$) was performed using a Student *t* test followed by a Mann–Whitney *u* test. Results are expressed as mean $\pm$ SEM; * $P < 0.05$. (D) Intestinal PGC-1 β promotes genetically induced intestinal carcinogenesis. Surviving 7-mo-old iPGC-1 β /*Apc^{min/+}* mice presented more tumors in the ileum than FVBN/*Apc^{min/+}* mice. Tumors in both the ileum and colon were larger in iPGC-1 β /*Apc^{min/+}* mice than in FVBN/*Apc^{min/+}* mice. The comparison of FVBN/*Apc^{min/+}* ($n = 19$) and iPGC-1 β /*Apc^{min/+}* mice ($n = 12$) was performed using a Student *t* test followed by a Mann–Whitney *u* test. Results are expressed as mean $\pm$ SEM; * $P < 0.05$.

Jun, and Wnt5a were overexpressed, and, conversely, proapoptotic genes *Bad*, *Bid*, *Fas*, *Apaf1*, and *Casp8* were reduced at transcriptional levels. Furthermore, microarray analysis highlighted potential activated transcription factors (TFs), among which were estrogen receptor-related α (*Err α*) and the two PGC-1 coactivators, because of their similar transcriptional activity, and TFs involved in cell-cycle and proliferation, such as β -catenin and c-myc (Fig. S4B). However, c-myc levels were similar in the two mouse groups, but cyclin D1 mRNA levels were decreased in tumors from iPGC-1 β mice as compared with tumors from wild-type mice, suggesting that the observed phenotype is the result not of further activation of the β -catenin/Tcf4 complex but rather of a putative metabolic advantage that PGC-1 β overexpression confers to neoplastic cells (Fig. 5A and Fig. S34). In this respect, although tumors from iPGC-1 β /*Apc^{min/+}* mice showed a significant increase in mitochondrial and oxidative phosphorylation genes, the antioxidant enzymes, such as Sod2, as confirmed by histochemical analysis (Fig. 5B), and Gpx4, Prdx5, Prdx3, Txn2, and Sirt3 were increased as compared with tumors from wild-type mice (Fig. 5A).

To verify that the increased tumorigenesis in mice is further sustained by the action of antioxidant enzymes that promote longevity of transformed enterocytes, we analyzed 8-oxo-dG and NITT levels by immunohistochemical analysis (Fig. 5C).

(ileum) and colon (Fig. S4A). However, expression analysis did not succeed in showing complete knockout of PGC-1 β because of the presence of cells that do not express villin (i.e., muscle cells) in the intestinal tube (Fig. 6A). In line with the effect of PGC-1 β overexpression on the transcription of several metabolic pathways, microarray analysis of ileum samples from PGC-1 β fl/fl and PGC-1 β fl/+ mice (hereafter generically referred to as “PGC-1 β fl/? mice”) and iPGC-1 β KO mice revealed that PGC-1 β deletion in enterocytes led to the down-regulation of numerous pathways involved in metabolic cascades, such as oxidative phosphorylation (24 genes down-regulated in iPGC-1 β KO mice in comparison with the control group), the TCA cycle, glycolysis, gluconeogenesis, and fatty acid metabolism (Fig. S4B). To verify the effect of PGC-1 β on target genes in the large intestine, we performed real-time qPCR on colon samples from PGC-1 β fl/? and iPGC-1 β KO mice for genes implicated in oxidative phosphorylation (cytC), the TCA cycle (isocitrate dehydrogenase 3 subunit α , Idh3 α), and mitochondrial antioxidant enzymes. We found a 30–40% decrease for all genes except *Pdx5*, which showed only a slight reduction (Fig. 6B). Although the transcriptional activity of PGC-1 β in the intestine also was confirmed by its deletion within enterocytes, the morphological effect on intestinal homeostasis observed with its overexpression was not confirmed in the normal mucosa of iPGC-1 β KO mice. Indeed, the intestines of knockout mice

Fig. 6. Intestinal PGC-1 β ablation decreases antioxidant defense and intestinal carcinogenesis. (A) Paraffin-embedded ileum and colon specimens from PGC-1 β fl/? and iPGC-1 β KO mice were immunoassayed with PGC-1 β antibody to verify its deletion within enterocytes. (Magnification: 100 $\times$.) (B) CytC, ATP5synt, Idh3a, Sirt3, Sod2, Txn2, Prdx3, and Prdx5 mRNAs were measured in colon specimens from PGC-1 β fl/? and iPGC-1 β KO mice by real-time qPCR. The comparison of wild-type and transgenic mice ($n = 6$) was performed using a Student t test followed by a Mann-Whitney u test. Results are expressed as mean $\pm$ SEM; * $P < 0.05$. (C) Gross morphology of colon samples from PGC-1 β fl/? and iPGC-1 β KO mice. (D) Paraffin-embedded ileum and colon specimens from PGC-1 β fl/? and iPGC-1 β KO mice were stained with H&E and observed by light microscopy. (Magnification: 25 $\times$.) (E) Number of tumors (Left) and number of tumors categorized by size (Right) per mouse from colon of PGC-1 β fl/? and iPGC-1 β KO mice at the end of the AOM-DSS treatment. The comparison of PGC-1 β fl/? and iPGC-1 β KO mice ($n = 10$) was performed using a Student t test followed by a Mann-Whitney u test. Results are expressed as mean $\pm$ SEM; * $P < 0.05$. (F and G) Paraffin-embedded ileum specimens from PGC-1 β fl/? and iPGC-1 β KO mice were immunoassayed with 8-oxo-dG antibody (F) and with NITT antibody (G). Magnification: 100 $\times$.

appeared to be similar to those of PGC-1 β fl/? control mice, and the length of villi and crypts as well as migration along the crypt-villus axis (followed through BrdU injection) did not differ in iPGC-1 β KO and PGC-1 β fl/? mice (Fig. S4C). However, when we challenged PGC-1 β fl/? and iPGC-1 β KO mice with the AOM/DSS protocol, PGC-1 β ablation protected the intestine against tumorigenesis. Indeed, gross morphology of iPGC-1 β KO colon samples showed fewer tumors than in PGC-1 β fl/? mice (Fig. 6C). Histological analysis also revealed worse dysplasia (Fig. 6D), with a greater number and size of tumors in PGC-1 β fl/? mice than in knockout mice (Fig. 6E). In contrast with transgenic mice, in which, despite increased mitochondrial activity, the antioxidant machinery was able to cope with ROS-driven damage to macromolecules, the normal and dysplastic mucosa of iPGC-1 β KO mice were more susceptible to oxidative stress, as demonstrated by increased 8-oxo-dG and NITT staining in knockout as compared with control mice (Fig. 6F and G). In summary, intestinal-specific ablation of PGC-1 β led to decreased expression of target genes involved in several metabolic pathways, including

oxidative phosphorylation, glycolysis, and the Krebs cycle and of mitochondrial antioxidant enzymes affecting oxidative status without altering intestinal homeostasis in physiological conditions. Conversely, the absence of PGC-1 β protected mice against chemically induced tumorigenesis.

Transcriptional Regulatory Network of PGC-1 β . PGC-1 β can interact with several members of the nuclear receptor family as well as several non-nuclear receptor TFs to respond to diverse stimuli and regulate different pathways. To identify the TFs coregulated by this coactivator within the enterocytes, we sought to use next-generation DNA sequencing to determine the genome-wide binding sites for PGC-1 β . We performed ChIP with a PGC-1 β antibody in enterocytes isolated from wild-type, iPGC-1 β , PGC-1 β fl/?, and iPGC-1 β KO mice. PGC-1 β -associated DNA was isolated by immunoprecipitation, processed, and subjected to high-throughput DNA sequencing using the Illumina Genome Analyzer HiSeq 1000 platform. As control, input DNA isolated under the same procedure was sequenced. Genomic locations were obtained for

the use of antioxidant compounds would reduce cancer risk. However, although many cancer cell types have increased levels of ROS, they must restrict ROS levels and/or adjust the signaling pathways that are dependent on cellular redox status below a given threshold so as to escape cell death. Among the potential TFs that may collaborate with PGC-1 β , NRFs seem to be the most reliable of the TFs responsible for the increased enterocyte lifespan and tumorigenesis. Indeed, tumors may use adaptive mechanisms to keep their ROS burden within a range that permits growth and survival without affecting the mitochondrial competence that is important for cancer cell viability (20). In such contest, PGC-1 β acts as a gatekeeper of redox status, allowing enterocyte survival and thus increasing their lifespan and, in cancer-promoting conditions, tumor progression.

Materials and Methods

To generate the pSKvillin PGC-1 β , first the hPGC-1 β (3.1-kb) fragment with XhoI and KpnI restriction sites was generated by PCR from pcDNA3 PGC-1 β plasmid (kindly provided by Antonio Vidal-Puig, Department of Clinical Biochemistry, University of Cambridge, Cambridge, United Kingdom). Then the fragment was subcloned at the XhoI and KpnI restriction sites downstream of the villin promoter region of the pSKvillin plasmid (kindly provided by Deborah Gumucio, Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI). The iPGC-1 β transgenic mice were

generated by injecting the transgene plasmid digested with HpaI into the pronuclei of the fertilized eggs of FVB/N mice. Mice carrying the transgene were identified by PCR of genomic DNA to confirm the presence of the hPGC-1 β coding sequence. Stomach, liver, brain, kidney, jejunum, duodenum, ileum, and colon of transgenic mice were dissected and prepared for total RNA extraction and immunohistochemistry to evaluate the specific intestinal expression of transgene under the villin promoter control. To generate iPGC-1 β KO mice, we crossed villinCre transgenic mice (Jackson Laboratory) with PGC-1 β flox/flox (PGC-1 β fl/fl) mice. As control group we used both PGC-1 β fl/fl and PGC-1 β fl/+ mice (generally referred to as "PGC-1 β fl/? mice"). iPGC-1 β Apc^{Min/+} mice were generated by crossing iPGC-1 β transgenic mice with C57BL/6-ApcMin/+ mice (Jackson Laboratory). All the experiments presented in this study were carried out in male mice. The experimental protocol was approved by the Ethical Committee of the Consorzio Mario Negri Sud and also was certified by the Italian Ministry of Health according to internationally accepted guidelines for the animal care.

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