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Melatonin promotes gut anti-oxidative status in perinatal rat by remodeling the gut microbiome

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ABSTRACT

Gut health is important for nutrition absorption, reproduction, and lactation in perinatal and early weaned mammals. Although melatonin functions in maintaining circadian rhythms and preventing obesity, neurodegenerative diseases, and viral infections, its impact on the gut microbiome and its function in mediating gut health through gut microbiota remain largely unexplored. In the present study, the microbiome of rats was monitoring after fecal microbiota transplantation (FMT) and foster care (FC). The results showed that FMT and FC increased intestinal villus height/crypt depth in perinatal rats. Mechanistically, the melatonin-mediated remodeling of gut microbiota inhibited oxidative stress, which led to attenuation of autophagy and inflammation. In addition, FMT and FC encouraged the growth of more beneficial intestinal bacteria, such as *Allobaculum, Bifidobacterium,* and *Faecalibaculum*, which produce more short-chain fatty acids to strengthen intestinal antioxidation. These findings suggest that melatonin-treated gut microbiota increase the production of SCFAs, which improve gut health by reducing oxidative stress, autophagy and inflammation. The transfer of melatonin-treated gut microbiota may be a new and effective method by which to ameliorate gut health in perinatal and weaned mammals.

1. Introduction

Gut health directly determines nutrition absorption and is vital to animal growth and development [1]. There are high incidences of intestinal diseases worldwide that are characterized by chronicity and recurrent attacks, which makes them difficult to treat; therefore, the incidences of intestinal diseases have surpassed some chronic conditions, such as diabetes and cardiovascular diseases, and have become the most important global public health concern today. The gut microbiota play a central role in the pathogenesis of these diseases because they mediates environmental changes in the intestinal immune system [2]. It has been implicated in mammalian gut health that remodeling gut dysbiosis with healthy microbiota is beneficial to mammalian gut health. Pregnancy is a physiological state characterized by oxidative stress disturbance that leads to the complications relevant with pregnancy [3]. A previous study has shown that disruption of material and placental redox status predisposes to premature childbirth, leading to a worse physiological state in the weaning rat [4]. Besides, oxidative stress in Utero can be a determining factor in the mortality and morbidity of premature newborns. Additional exploration of the composition and function of the gut microbiota and the potential for their manipulation to cure disease is an area of rapid growth and great promise.

The mammalian gut microbiota comprise trillions of microbes that especially facilitate host gut health and prevent diarrhea, inflammatory bowel disease, and irritable bowel syndrome [5,6]. They are considered important partners of both human and nonhuman cells because they interact with virtually all cells. In fact, there are numerous metabolites,

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including short-chain fatty acids (SCFAs), secondary bile acids, indoleacrylic acid, skatole, tryptamine, and histamine, produced by the gut microbiome that can influence mammal metabolism [7–11]. Increasing studies have indicated that changes in gut microbiota composition are associated with intestinal diseases and obesity and their associated metabolic disorders [12–14]. Both baseline microbiota and microbial richness are inversely associated with insulin resistance and diarrhea [5,15]. Interestingly, fecal microbiota transplantation (FMT) is an important method by which to establish or remodel functional gut microbiota to improve gut health or cure some conditions, such as diarrhea and obesity [5,16]. Moreover, studies have shown that early weaned animals maintained intestinal health possibly through reprogrammed gut microbiota by eating or licking the dung from healthy adult animals; therefore, foster care (FC) or co-cage feeding may be a new and promising method by which to establish or reprogram functional gut microbiota to ameliorate intestinal health.

The use of pro- and prebiotics, instead of antibiotics, has increased in popularity for both the prevention and treatment of various gut-related diseases [17]. There is much to learn in this field, and new gut microbial therapeutics are likely to rapidly evolve over the next decade. Finding probiotic and prebiotic alternatives to traditional treatments to prevent gastrointestinal disorders is crucial for gut health in human and non-human animals. Melatonin is a highly effective antioxidant and the most versatile and ubiquitous hormonal molecule. It is produced not only in the pineal gland but also in various other tissues of mammals, particularly in the gastrointestinal tract [18]. Melatonin plays a vital role in the regulation of other hormones to maintain circadian rhythms and prevent obesity, neurodegenerative diseases, and viral infections [19–22]. Recent studies have shown that melatonin is implicated as an antioxidant to prevent autophagy and inflammation, which harm the gut microbiota [23–25]; however, few clinical or experimental studies have evaluated the effects and regulatory mechanisms of melatonin-treated gut microbiota on gut health in perinatal and early weaned animals.

In the present study, we investigated the effect and underlying mechanism of melatonin-treated gut microbiota on gut health in perinatal female rats and early weaned rats to alleviate oxidative stress during pregnancy or postnatal period in the hope of achieving the goal of intestinal health in pregnant, postnatal and neonatal rats.

2. Materials and methods

2.1. Animals

Male and female Sprague-Dawley rats (10-12 weeks) were purchased from The Fourth Military Medical University (Xian, Shaanxi, China). The rats were housed in a pathogen-free rat colony (temperature, 25 ± 2 °C; relative humidity, 45%–60%; lighting cycle, 12 h/d, 08:00–20:00 for light) and had free access to food and drinking water. The normal chow diet was purchased from Beijing HFK Bioscience Co. Ltd. (Beijing, China), and the feed formula is in Table S1. After superovulation of rats by an intraperitoneal injection of 10 IU PMSG (Sigma, St Louis, MO, USA), the female rats were mated. Pregnancy is inspected by detecting vaginal suppositories and vaginal smears in every morning and evening. The perinatal rats, newborn rats and male rats were selected for experiments. The animal experiments were all approved by the Research Ethics Committee of Northwest A&F University (2011-31101684).

2.2. Experiment design

For the pregnant rat experiments, a total of 120 pregnant rats were randomly divided into six groups, including control (Ctr), melatonin (Mel), fecal microbiota transplantation (FMT), antibiotic (Ant), Mel + Ant and FMT + Ant groups. Ctr group: pregnant rats received a basal diet and normal drinking water. Mel group: pregnant rats received a basal diet, normal drinking water and melatonin by oral gavage (50 mg/kg/day [26–28]; M5250, Sigma, MO, USA). FMT group: pregnant rats received a basal diet, normal drinking water and fecal suspension by oral gavage (0.005 ml/g/day [29]; see 2.3 FMT for production of fecal suspension). Ant group: pregnant rats were treated with antibiotic cocktails composed of vancomycin (100 mg/kg, V871983, MACKLIN, China), neomycin sulfate (200 mg/kg, N6063, MACKLIN, China), metronidazole (200 mg/kg, M813525, MACKLIN, China), and ampicillin (200 mg/kg, A910962. MACKLIN, China) by oral gavage once daily. Mel + Ant group: pregnant rats were treated with compound antibiotics on the second day of pregnancy once a day for 5 days to deplete the gut microbiota, then received a basal diet, normal drinking water and melatonin by oral gavage (50 mg/kg/day) until day 5 before delivery (A5 group) and day 3 after delivery (P3 group). FMT + Ant group: antibiotic cocktails (vancomycin, 100 mg/kg; neomycin sulfate, 200 mg/kg; metronidazole, 200 mg/kg; ampicillin, 200 mg/kg) was added to fecal suspension. The pregnant rats received a basal diet, normal drinking water and fecal suspension by oral gavage.

For the weaned and lactation rat experiments, a total of 80 suckling rats at 11 d of age (weaned at 21 days of age) were randomly selected for four groups including Ctr, Mel, FMT, and FC groups. Ctr group: suckling rats fed on breast milk; Mel group: suckling rats fed on breast milk and melatonin by oral gavage (50 mg/kg/day); FMT group: suckling rats fed on breast milk and fecal contents dilution (0.005 ml/g/rat); FC group: suckling rats fed on breast milk of female rats which received a basal diet and melatonin by oral gavage (50 mg/kg/day). FC is a way by caging lactating rats treated with melatonin and suckling rats, and normal suckling rats could obtain beneficial bacterial communities through feces and urine in the environment and breast milk of melatonin-treated rats.

For the male rat experiments, a total of 20 male rats were randomly selected for two groups, including Ctr and Mel group. Ctr group: rats received a basal diet and normal drinking water; Mel group: male rats received a basal diet, normal drinking water and melatonin by oral gavage.

Rats were sacrificed to collect serum, duodenum, jejunum, ileum, and colon in each group. Five rats were sacrificed to collect duodenum, jejunum, ileum, and colon in each group. These intestinal tissues were fixed in 2.5% glutaraldehyde solution and 2.5% paraformaldehyde solution and saved at −80 °C.

2.3. FMT

FMT is an approach through oral administration the feces of melatonin-treated pregnant rats into normal pregnant rats. After 3 weeks of melatonin supplementation, rats were placed in sterile cages and feces were collected. The fecal was collected and diluted with phosphate buffer saline (PBS) through a modified procedure (100 mg/ml). Then, fecal suspension was filtered by 200 mesh, 400 mesh and 800 mesh sterile mesh filter to remove impurities. The suspension was homogenized by vortex for 5 min and centrifuged at 1000 g for 3 min. The fecal suspension was collected into new sterile tubes and 10% glycerol was added for store at −80 °C. Finally, fecal suspension was thawing in a 37°C-water bath before oral gavage [5,30,31].

2.4. Hematoxylin and eosin staining

Hematoxylin and eosin (H&E) staining was performed as previously described [32]. Briefly, the rat intestinal tissue, including duodenum, jejunum, ileum, and colon, were fixed with 4% paraformaldehyde-PBS overnight, and then dehydrated and embedded in paraffin blocks. The 5 μM sections were deparaffinized, hydrated, and stained with H&E. Intestinal villus height and crypt depth were measured using ImageJ software.

2.5. ROS generation assay

The fresh intestinal tissues were collected and frozen at −80 °C. The samples were sliced into 5-10 μM pieces and stained with DHE at 37 °C for 30 min. The sections were washed on time with PBS for 10 min and stained with DAPI. Likewise, intestinal epithelial cells were washed 3 times with PBS and incubated with 10 μM DCFH-DA (Invitrogen, Eugene, OR, USA) for 30 min, and then washed with PBS and stained with DAPI. The sections and cells were captured with a Zeiss 710 laser scanning confocal microscope. The ROS assay kit (Beyotime Institute of Biotechnology, Shanghai, China) was used to measure ROS concentration according to the manufacturer's instructions. Briefly, the crushed intestinal tissues and IEC-6 cells were incubated with 10 μM DCFH-DA for 30 min and then were measured by UV/visible spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan) and flow cytometry (BD FACSAriaTM III, USA).

2.6. Transmission electron microscopy

The fresh ileum tissues were fixed in 2.5% glutaraldehyde solution at 4 °C overnight and incubated with PBS containing 2% OsO4 for 4 h. The ileum tissues then were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, 95%, and 100%) for 10 min at each step for 2 times and then transferred into a mixture of alcohol and Spurr resin (v:v = 1:1, v:v = 1:2, v:v = 1:3) for 12 h and Spurr resin for 24 h for 2 times. Ultrathin sections were obtained using a diamond knife and stained with uranyl acetate, and then specimens were observed by TEM (JEM-1011, JEOL, Japan).

2.7. Immunohistochemistry

Immunohistochemistry stains were detected using IHC kit (MaiXin, China). Briefly, the tissues were cut into 3-10 μM long pieces and blocked with 1% w/v BSA for 1 h, and then anti-NF-κB antibodies were incubated overnight at 4 °C. Next, the samples were dehydrated in an ethanol (30-100%) gradient and treated with xylene to increase the transparency of slides.

2.8. Cytokine analysis

The levels of IL-6, IL-1β, IL-10, and TNF-α in rat serum were determined using ELISA kits (Raybiotech, GA, USA) according to the manufacturer's instructions.

2.9. Antioxidant capacity detection

The levels of malondialdehyde (MDA) and total antioxidant capacity (T-AOC) in serum were measured by spectrophotometric methods according to manufacturer instructions of assay kits (Nanjing Jiancheng, Nanjing, China). All samples were measured by a UV/visible spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan). Reduced glutathione (GSH) and oxidized glutathione (GSSG) in serum were determined by ELISA kits (mlbio, China) according to the manufacturer's instructions. Briefly, serum GSH is determined directly by DTNB, and GSSG are measured after reduction with glutathione reductase enzyme to GSH molecules. Meanwhile, the expression levels of antioxidant enzymes in tissues and cells were detected.

2.10. Western blotting analysis

Total protein was isolated from intestinal tissues and cells by incubating in RIPA buffer containing protease inhibitor cocktail for 30 min and then centrifuged at 12,000 g for 10 min at 4 °C to remove the precipitate. The total protein content was determined using the BCA Protein Assay Kit (Pierce), and 30 μg proteins were separated by a reducing SDS-PAGE electrophoresis on 10% or 15% Bis-Tris gels, transblotted onto nitrocellulose membranes and probed with different primary antibodies: Table S2. The gray values of the bands were measured by ImageJ software and then normalized to β-actin content.

2.11. Cell culture and treatment

The rat intestinal epithelial cell line IEC-6 was purchased from the American Type Culture Collection (ATCC, USA). The cells were cultured in DMEM-F12 medium supplemented with 10% FBS (Life Technologies) in a humidified 5% CO_2 incubator at 37 °C. For SCFAs stimulation, cells were treated with different concentrations of SCFAs or combined with H_2O_2 (50 μM) for 12 h. For H_2O_2 stimulation, cells were treated with H_2O_2 (200 μM) or combined with different concentrations of N-acetylcysteine (NAC) for 12 h. For melatonin stimulation, cells were treated with different concentrations of melatonin for 12 h.

2.12. Evaluation of autophagosome

IEC-6 were seeded in 24-well plates with 1 cm coverslips, and the cells were grown to 80% confluence before transient transfection with the GFP-MAP1LC3B (Beyotime Institute of Biotechnology, Shanghai, China). After 24 h cultured, the cells were treated with 200 μM H_2O_2 for 2 h. Finally, the cells were washed with PBS 3 times and observed under a laser-scanning confocal microscope (Zeiss LSM 710 META, Oberkochen, Germany).

2.13. Immunofluorescence

For immunofluorescence analysis, IEC-6 cells were fixed in 4% paraformaldehyde for 10 min and were permeabilized in 0.5% Triton X-100 for 20 min at room temperature and blocked in 1% BSA for 30 min. Next, the cells were incubated with primary antibodies overnight at 4 °C. After that, the samples were washed three times with PBS for 10 min each time and incubated with secondary antibodies for 2 h. The nuclei were stained with DAPI for 10 min and then washed one time with PBS for 15 min. Immunofluorescent images were captured with a Zeiss 710 laser scanning confocal microscope.

2.14. Gut microbiota analysis

Total genomic bacterial DNA of the fecal mixture of the duodenum, jejunum, ileum, and colon was extracted using an E.Z.N.A.® Stool DNA Kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions. PCR amplification of bacteria DNA in the present study was performed. Briefly, the V3-V4 regions of the bacteria 16S ribosomal RNA gene were amplified by PCR as following: 95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. The primers used are 338F 5′-ACTCCTACGGGAGGCAGCA-3′ and 806R 5′-GGACTACHVGGGTWTCTAAT-3′. PCRs were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer 5 μM, 0.4 μL of FastPfu DNA polymerase, and 10 ng of template DNA.

Amplicons were extracted from 2% agarose gel, purified by the AxyPrep DNA gel extraction kit (Axygen Biosciences, Union City, CA, USA), and quantified by QuantiFluorTM-ST (Promega, Madison, WI, USA) according to the manufacturer's protocols. Then purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq platform according to the standard protocols by a commercial company (Novogene, Beijing, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SUB2623584).

Raw data were firstly conducted by using the FLASH analysis tool (V1.2.7, http://ccb.jhu.edu/software/FLASH/), and then filtered with the Quality Control software package (V1.7.0, http://qiime.org/scripts/split_libraries_fastq.html). To obtain the effective tags, the UCHIME algorithm (http://www.drive5.com/usearch/manual/uchime_algo.

html) was used to remove the chimera sequences. The reads were picked to form distinct OTUs using Uparse software (http://drive5.com/uparse/) at 97% of sequence similarity and then were classified to different levels by comparing to GreenGenes database using PyNAST software (V1.2). We appreciate Novogene for providing 16s amplicon sequences service.

2.15. SCFAs

For detection of SCFAs, fecal samples were measured using the previous method [25,33]. Briefly, the fecal mixture samples were treated as follows: 0.3 g of feces was added to 1.2 mL sterile saline, evenly dispersed by stirring with a glass rod, and left to stand for 5 min; 600 μL 50% sulfuric acid (Sigma Aldrich) and 1.2 mL ether were added, standing for 10 min with intermittent shaking. The supernatant was obtained by centrifugation at 5000 rpm for 30 min and used for chromatographic analysis. The content of SCFA was measured by GC-MS (Agilent, USA).

2.16. Statistical analysis

Statistical analyses were performed using the SPSS 23.0 statistical software package (SPSS Inc, Chicago, IL, USA). The variables were first tested for normality (Shapiro –Wilk test) and homoscedasticity (Levene's test) and followed with Student's t-test. The data were compared by analysis of variance (ANOVA) and differences located with Duncan's multiple range test. Correlation analysis between microbiota and SCFAs was conducted by Pearson correlation analysis. All data of each group were presented as mean ± SEM and statistical significance was set at $P < 0.05$.

3. Results

3.1. Melatonin-treated gut microbiota improve the gut health

Gut health in perinatal animals, which is affected by intestinal oxidative stress and microbiota, is important for absorbing nutrients, reproducing, and lactating. To investigate the effects of melatonin and melatonin-remodeled FMT on gut health, perinatal female rats were treated with melatonin and FMT. The timeline of treatments and sample collection is showed in Fig. 1A. We noticed that the concentration of melatonin in feces (81 pg/ml) was extremely lower than in oral solution (5×10^9 pg/ml) (Supplementary Fig. 1A), thus the small function of melatonin in fecal suspension can be largely ignored. Besides, the melatonin concentration in the rat serum treated with melatonin is 190 pg/ml, which is 4 times higher than the physiological concentration in normal rat serum (Supplementary Fig. 1B) and also higher than melatonin concentration in rats at night (nearly 100 pg/ml [34]), thus the small fluctuations of melatonin caused by circadian rhythms can be largely ignored. Moreover, the administration of melatonin has no impact in the liver, kidney function and body weight of rat (Supplementary Figs. 1C–V). Interestingly, the results showed that FMT and melatonin increased the villus height/crypt depth (V/C) of the duodenum, jejunum and ileum on antenatal day 5 (A5) and postnatal day 3 (P3) rats (Fig. 1B–H), and the phenotype induced by FMT was similar to that in the melatonin group, which indicated that melatonin improved gut health, possibly by reprogramming the gut microbiota.

To further explore the effects of reprogramming gut microbiota using melatonin on gut health, lactating rats were treated with melatonin and co-cage feeding (FC) with non melatonin–treated neonatal rats. The timeline of treatments and sample collection are shown in Fig. 1I. The results showed that V/C increased on day 3 after weaning (Fig. 1J–M). Besides, we noticed FMT and FC also improve the gut health by protecting intestinal barrier integrity (Fig. 1N). We also examined the expression of zonula occludens-1 (ZO-1), occludin, and claudin in the gut tissue to assess the effect of FMT or FC on gut health and found that their expression was increased by >200% in the FC group (Fig. 1O-R).

Besides, to further prove the beneficial effects are indeed coming from gut microbiota, sterile rats (antibiotic treated) were treated with melatonin and FMT. The results showed antibiotic treatment significantly inhibited the improvement of melatonin on gut health (Supplementary Figs. 2A–I). Moreover, fecal suspension treated with antibiotic significantly reduced the effect of FMT on rat V/C ratio and internal barrier integrity (Supplementary Figs. 2J–S).

Taken together, the results showed that melatonin-treated gut microbiota improved gut health of antenatal and postnatal rats as well as weaning offspring, including control of V/C and intestinal barrier integrity.

3.2. Melatonin-treated gut microbiota inhibit gut oxidative stress

Melatonin acts to reduce ulcer formation seemingly by directly scavenging toxic oxygen-based reactants and possibly promoting antioxidative enzyme activities. The effect of FMT or FC on gut health was investigated to determine whether it was an antioxidant-dependent process. As expected, the results indicated that FMT markedly decreased reaction oxygen species (ROS) production in the intestinal tissue of A5 and P3 rats (Fig. 2A-B). Meanwhile, the levels of T-AOC were increased (Fig. 2C) and MDA were reduced (Fig. 2D). Moreover, compared with the control group, FMT upregulated the protein levels of glutathione peroxidase 5 (GPX5), superoxide dismutase type 1 (SOD1), SOD2, and catalase (CAT) in the intestinal tissues of A5 and P3 rats (Fig. 2E–I).

Besides, similar results were obtained for FC. FC decreased ROS production (Fig. 2J, K), increased the production of T-AOC, decreased MDA concentration (Fig. 2L, M), and upregulated the protein levels of GPX5, SOD1, SOD2, and CAT (Fig. 2N–R) in the intestinal tissues 3 d after weaning. Similarly, to clarify the alleviation of oxidative stress is due to melatonin remodeled gut microbiota. Sterile rats were treated with FMT and melatonin and the results suggested that antibiotic treatment significantly inhibited the improvement of melatonin on gut antioxidant (Supplementary Figs. 3A–H), and fecal suspension treated with antibiotic significantly reduced the effect of FMT on rat GSH/GSSG ratio (Supplementary Fig. 3I).

In summary, these data suggested that melatonin-treated gut microbiota promote the production of gut antioxidants to ameliorate gut health, which is beneficial to both perinatal and early weaned animals.

3.3. Melatonin-treated gut microbiota suppresses oxidation-induced intestinal autophagy

Oxidative stress damages intestinal epithelial cells and causes cell autophagy [35]. To examine whether oxidative stress promotes epithelial cell autophagy, we treated IEC-6 cells with hydrogen peroxide (H_2O_2), which mimics oxidative stress. The results showed that the application of H_2O_2 downregulated the expression of NRF2 and CAT (Fig. 3A–C), also led to the promotion of autophagy, as evidenced by an increase in the number of punctate green fluorescent protein (GFP)-LC3 foci (Fig. 3F, G). Moreover, there was a sharp increase in LC3II, autophagy related 7 (ATG7), Beclin1 (BECN1) and a reduction in P62 in IEC-6 cells (Fig. 3A, D, E, H-J). However, N-acetylcysteine (NAC, a ROS scavenger) treatment was found to restore the effect of H_2O_2 on protein expression (Fig. 3A–E). These results suggested that oxidative stress amplified epithelial cell autophagy.

Next, we used transmission electron microscopy to determine whether melatonin-treated gut microbiota affect autophagy in the intestine *in vivo*. Gut autophagy significantly inhibited cellular autophagic vacuoles following FMT treatment (Fig. 3K, L). Moreover, the data showed that FMT treatment resulted in a sharp decrease in LC3II and an increase in P62 in the intestine, and the autophagy-related proteins ATG7 and BECN1 were obviously downregulated (Fig. 3M-Q). To further understand the regulatory mechanism of melatonin-treated gut

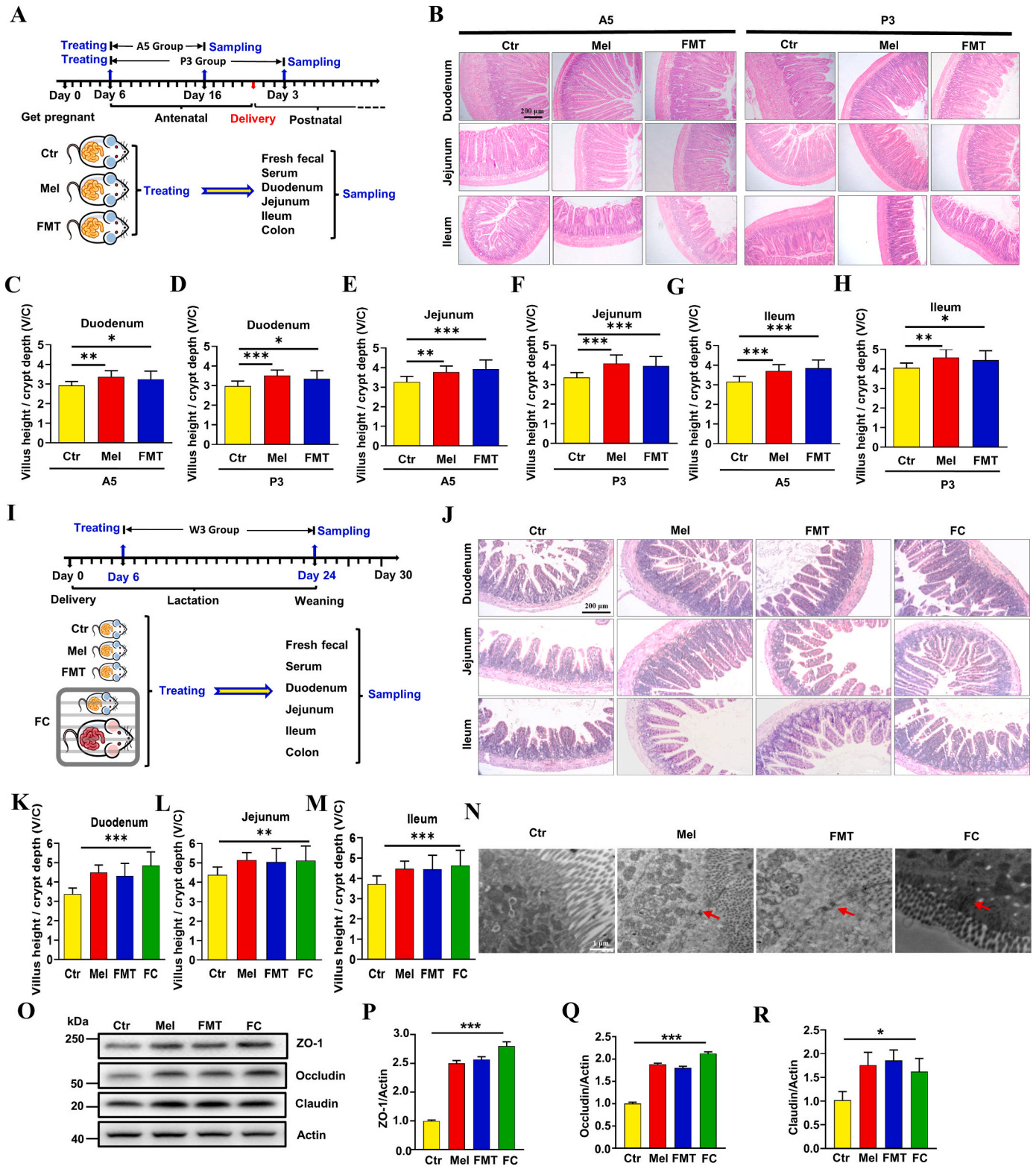

Fig. 1. Melatonin-treated gut microbiota reprograms the gut to improve gut health

The timeline of treatments and sample collection in this study **(A)**. Intestinal histological morphology indicated by H&E staining in A5 and P3 rats (n = 5 rats/group) **(B)**. The ratio of intestinal villus height/crypt depth of duodenum **(C, D)**, jejunum **(E, F)** and ileum **(G, H)**. A minimum of 10 tissue sections were cut from each sample. Five longest villi in each tissue section and a total of 250 longest villi were analyzed in each group. The timeline of treatments and sample collection **(I)**. Intestinal histological morphology indicated by H&E staining in early-weaned rats on day 3 after weaning (n = 20 rats/group) **(J)**. The V/C ratio of duodenum **(K)**, jejunum **(L)** and ileum **(M)**. A minimum of 10 tissue sections were cut from each sample (randomly selected 10 rats/group). Five longest villi in each tissue section and a total of 500 longest villi were analyzed in each group. Intestinal histological images using TEM, the red arrow indicates intestinal barrier **(N)**. Intestinal tissue expression of ZO-1, Occludin, and Claudin proteins (n = 3) **(O)**. The protein relative levels of Occludin **(P)**, ZO-1 **(Q)**, and Claudin **(R)**. Mel, melatonin; FMT, fecal microbiota transplantation; A5, antenatal female rat on day 5; P3, postnatal female rat on day 3. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

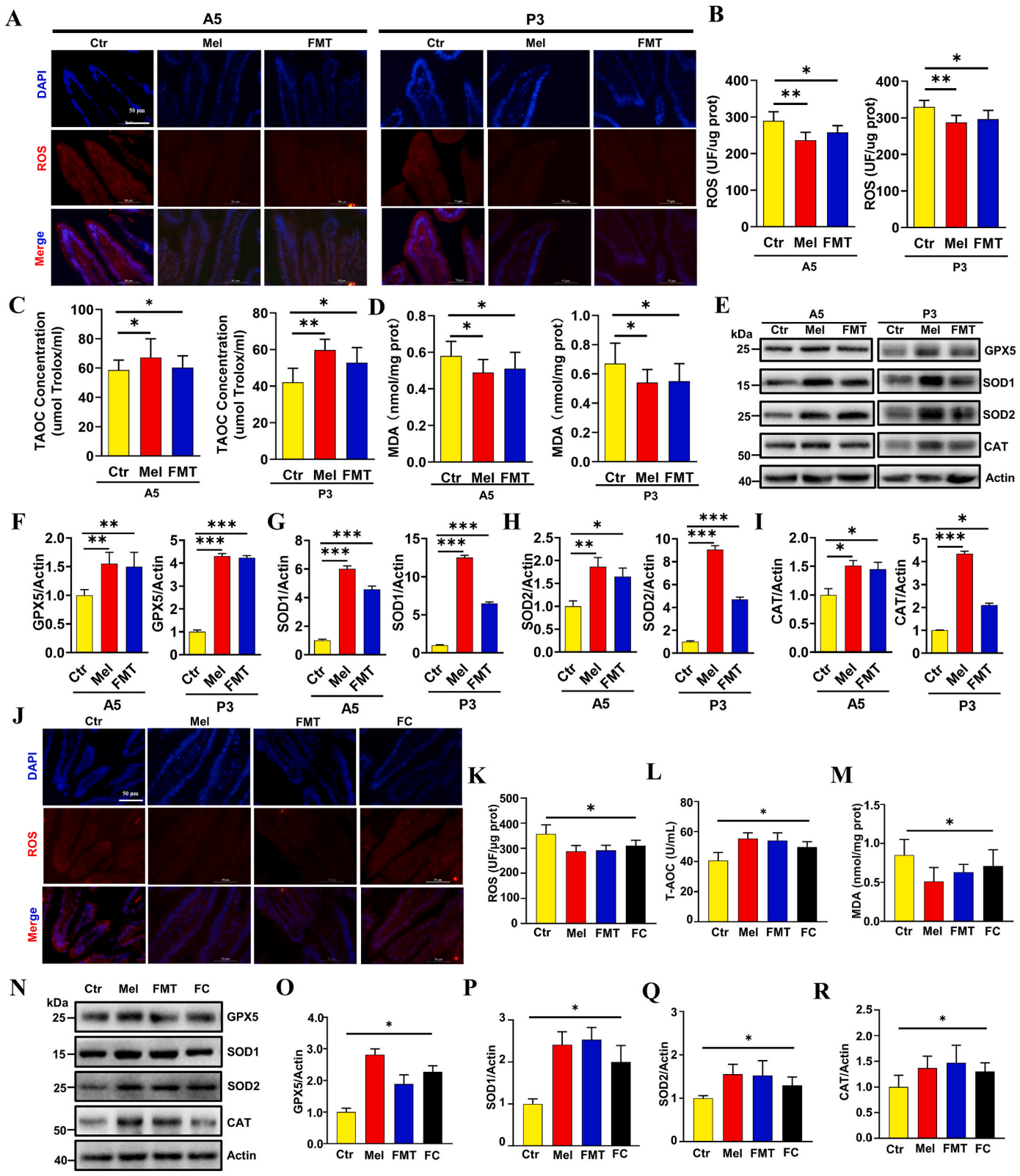

Fig. 2. Melatonin-treated gut microbiota inhibits gut oxidative stress

Ileum histological immunofluorescent imagines showed ROS level after being treated with melatonin and FMT in A5 and P3 rats **(A)**. The detections of ROS **(B)**, T-AOC **(C),** and MDA in serum **(D)**. Intestinal tissue expression of GPX5, SOD1, SOD2 and CAT proteins (n = 3) **(E)**. The protein relative level analysis of GPX5 **(F)**, SOD1 **(G)**, SOD2 **(H),** and CAT **(I)**. Ileum histological immunofluorescent imagines showed ROS level after being treated with melatonin, FMT, and FC in early-weaned rats **(J)**. The detections of ROS **(K)**, T-AOC **(L),** and MDA **(M)** in intestinal tissue or serum (n = 10). Intestinal tissue expression of GPX5, SOD1, SOD2 and CAT proteins (n = 3) **(N)**. The protein relative levels of GPX5 **(O)**, SOD1 **(P)**, SOD2 **(Q)**, and CAT **(R)**. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

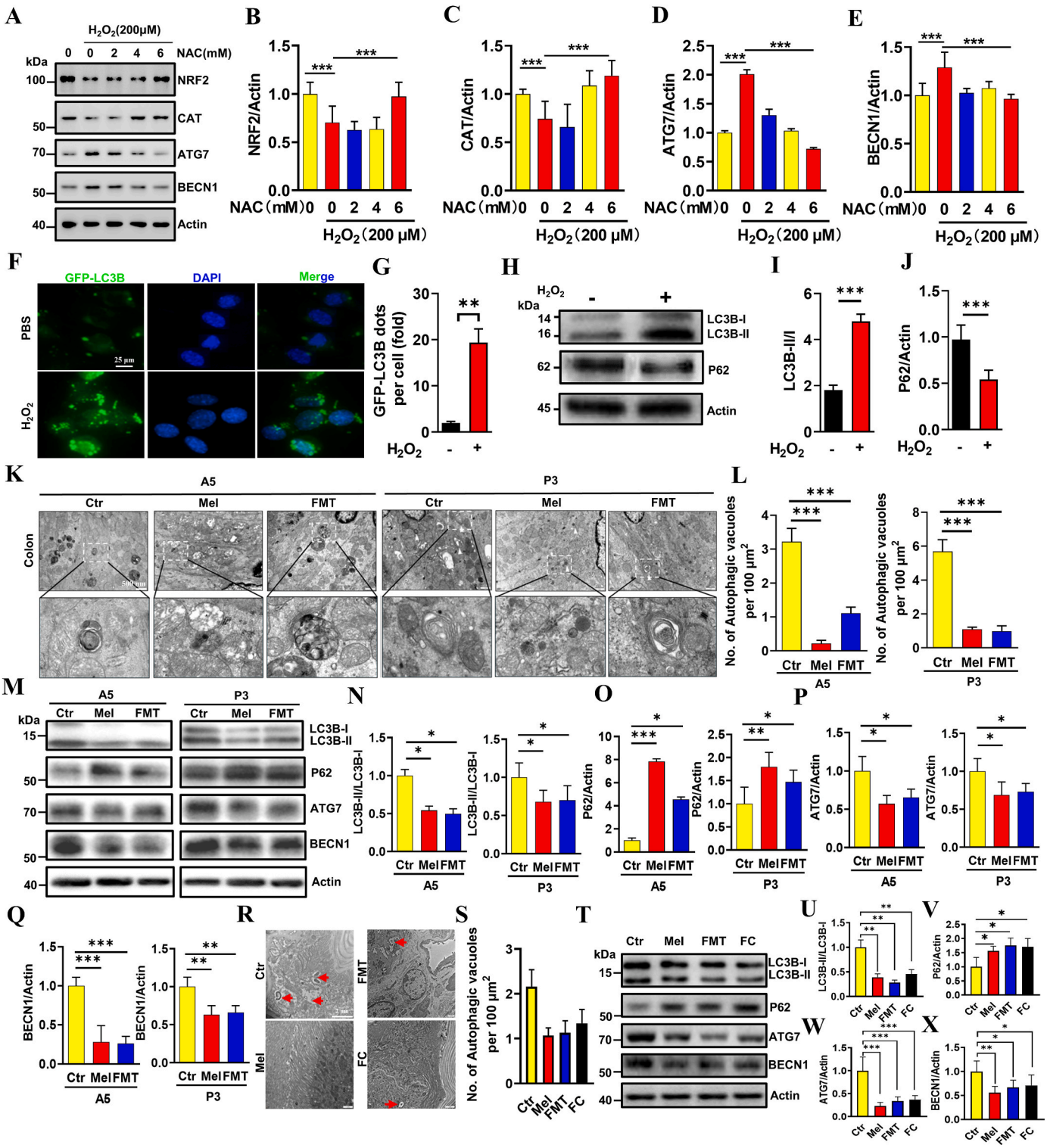

Fig. 3. Melatonin-treated gut microbiota suppresses oxidation-induced intestinal autophagy

The IEC-6 cells were treated with 200 μM H_2O_2 alone or in combination with indicated concentrations of N-acetylcysteine (NAC) for 12 h, and then the levels of NRF2, CAT, ATG7 and BECN1 were evaluated by western blot **(A)**. The protein relative levels of NRF2 (**B**), CAT (**C**), ATG7 (**D**) and BECN1 (**E**). The IEC-6 cells were treated with 200 μM H_2O_2 for 2 h, and then analyzed GFP-LC3B dots per cell **(F, G)**. Cell expression of LC3B-I, LC3B-II, and P62 proteins (n = 3) **(H)**. The protein relative levels of LC3B-II/LC3B-I **(I)** and P62 **(J)**. Intestinal histological images using transmission electron microscopy (TEM) in A5 and P3 rats **(K)**, white square indicates autophagic vacuole. Analysis of autophagic vacuoles in the colon. For counting autophagic vacuoles including autophagosomes and autolysosomes, 10 randomly selected images in each sample and data from 5 rats in each group were compared **(L)**. Intestinal tissue expression of LC3B-I, LC3B-II, P62, ATG7, and BECN1 proteins (n = 3) **(M)**. The protein relative level analysis of LC3B-II/LC3B-I **(N)**, P62 **(O)**, ATG7 **(P),** and BECN1 **(Q)**. Intestinal histological images using TEM and red arrows indicate autophagic vacuoles **(R)**. Analysis of autophagic vacuoles in intestinal cells. For counting autophagic vacuoles, 5 randomly selected images in each sample and data from 10 rats in each group were compared **(S)**. Intestinal tissue expression of LC3B-I, LC3B-II, P62, ATG7, and BECN1 proteins (n = 3) **(T)**. The protein levels of LC3B-II/LC3B-I **(U),** P62 **(V)**, ATG7 **(W),** and BECN1 **(X)**. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

microbiota in autophagy, we used the weaning rats and sterile rat model. The results showed that, as with melatonin and FMT, FC also markedly restrained gut autophagy (Fig. 3R, S) and downregulated the protein levels of LC3II, ATG7, and BECN1 but upregulated the protein level of P62 (Fig. 3T–X). More importantly, antibiotic treatment significantly inhibited the alleviative effect of melatonin and FMT on gut autophagy (Supplementary Figs. 4A–I).

Taken together, the data indicated that melatonin-treated gut microbiota improved gut health by inhibiting oxidative stress–induced autophagy.

3.4. Melatonin-treated gut microbiota inhibit oxidation-related intestinal inflammation

Extensive research has revealed the mechanism by which continued oxidative stress can lead to chronic inflammation [36]. To this end, we treated IEC-6 cells with H_2O_2, which led to a significant increase in the levels of NRF2, GCLM and GCLC (Fig. 4A–D), also promoted cell inflammation, as evidenced by an increase in NF-κB and tumor necrosis factor (TNF)α (Fig. 4A, E, F), but treatment with NAC significantly reversed the changes in these gene expression (Fig. 4A–F). These results suggested that oxidative stress caused epithelial cell inflammation.

Next, we sought to determine whether melatonin-treated gut microbiota is involved in intestinal inflammation in rats. The results showed that FMT significantly inhibited gut inflammation, as indicated by the decreased levels of NF-κB (Fig. 4G), TNF-α (Fig. 4H), interleukin (IL)-1β (Fig. 4I), IL-6 (Fig. 4J), and IL-10 (Fig. 4K). Similarly, FMT upregulated the protein levels of HSP72 but downregulated the protein levels of NF-κB (Fig. 4L-N), and antibiotic treatment significantly inhibited the improvement in inhibiting oxidation-related intestinal inflammatory (Supplementary Figs. 5A–C). Further, FC also significantly inhibited gut inflammation (Fig. 4O–S). Meanwhile, FC upregulated the protein levels of HSP72 but downregulated the protein levels of NF-κB (Fig. 4T–V). Besides, Mel and FMT markedly inhibited p38 MAPK signaling pathway (Supplementary Figs. 5D–H).

In conclusion, by inhibiting oxidative stress, melatonin-treated gut microbiota attenuated inflammation to improve gut health of rats.

Considering that melatonin can impact the secretion of progesterone which modulate the pregnancy associated gut microbiota, we used the male rat model (which do not produce progesterone). Our results showed that melatonin improve the gut health in male rat (Supplementary Figs. 6A–E), as well as gut oxidative stress (Supplementary Figs. 6F–J), intestinal autophagy (Supplementary Figs. 6K and L) and inflammation (Supplementary Fig. 6M), ruling out the effect of progesterone.

3.5. Melatonin reprograms gut microbiota in perinatal and weaning rats

SCFAs have been recognized as potential mediators of the effects of gut microbiota on intestinal antioxidant capacity. Thus, we next explored the effect of melatonin on gut microbiota, which produce SCFAs. We treated perinatal and weaning rats with melatonin (Mel), FMT (fecal samples collected from melatonin-treated pregnant rats), and FC (co-cage feeding with melatonin-treated rats). The composition of fecal microbiota was determined by sequencing fecal bacterial 16S rRNA in A5 and P3 rats, as well as rats on 3 d after weaning (W3). The experimental design for feces bacterial 16S rDNA gene amplicon sequencing analysis is shown in Fig. 5A. In this study, total tags of 64,734 raw reads were generated from each sample (Supplementary Fig. 7A). After removing the low-quality sequences, more than 50,000 clean tags were clustered into operational taxonomic units (OTUs) (Supplementary Figs. 7B and C). Perinatal rats as well as early weaned rats treated with melatonin, FMT, and FC, exhibited a greater diversity of microbiota, as evidenced by the higher Shannon and Simpson indices compared with those of the controls (Fig. 5B, C). We analyzed the overall differences in β-diversity using the PCoA plot and weighted and unweighted UniFrac distances. The controls and treated animals showed a distinct clustering of the microbial community and structure, and the FMT and FC groups had a structure similar to that of the Mel groups (Fig. 5D, E; Supplementary Figs. 7D–G), which indicated that the microbial transplantation was successful. The overall microbial composition in 10 groups differed at the phylum, order, and genus–species levels (Fig. 5F–H). At the genus level, compared with the control groups, the treatment groups showed a higher relative abundance of *Allobaculum* and *Faecalibaculum*, and the antenatal melatonin (AMel), antenatal FMT (AFMT), weaned melatonin (WMel), weaned FMT (WFMT), and weaned FC (WFC) groups showed a higher relative abundance of *Bifidobacterium* (Fig. 5I–L).

Together, the findings suggested that melatonin reprograms gut microbiota in perinatal and weaning rats, and that the FMT and FC models enable melatonin-treated gut microbiota to colonize rats.

3.6. Melatonin-treated gut microbiota increases SCFA production

Based on the analysis presented above, changes in the abundance of *Allobaculum*, *Faecalibaculum*, and *Bifidobacterium* may be involved in SCFA production. SCFAs, including acetic, propionic, and butyric acids, are the main metabolic products of anaerobic bacterial fermentation in the intestine [37]. To further determine whether FMT and FC reproduce the microbial phenotype of melatonin treatment, especially in terms of SCFA production, we collected feces from treatment and control groups and determined the SCFA concentration. The results indicated that both Mel and FMT increased the concentrations of total SCFAs, acetic acid, and butyric acid in A5 and P3 rats (Fig. 6A, B, D) but did not change the concentrations of propionic acid (Fig. 6C). The results were similar among early weaned rats and A5 and P3 rats (Fig. 6E–H). These data indicated that production of SCFAs, especially acetic and butyric acids, by remodeling gut microbiota might be a potential effect of melatonin-mediated improvements in gut health, although we cannot exclude other factors caused by altered microbiota that affect gut health, such as some antioxidants, toxins, and other metabolites.

Furthermore, the change in the abundance of *Lactobacillus* was not affected by fecal acetic acid concentration (Fig. 6I), but the change in the abundance of *Allobaculum*, *Bifidobacterium,* and *Faecalibaculum* was (Fig. 6J–L). Similarly, the change in the abundance of *Lactobacillus* and *Allobaculum* was not affected by fecal butyric acid concentrations (Fig. 6M, N), but the change in the abundance of *Bifidobacterium* and *Faecalibaculum* was (Fig. 6O, P). The results indicated that *Allobaculum*, *Bifidobacterium,* and *Faecalibaculum* contributed to acetic and butyric acid production to protect gut health.

3.7. SCFAs inhibit oxidation-induced intestinal autophagy and inflammatory

In order to understand the mechanism by which SCFAs protect gut health, based on the ratio of fecal acetic acid and butyric acid in perinatal female rats and early-weaned rats (Fig. 6A–H), we next treated IEC-6 cells using the ratio of acetic acid: butyric acid = 4:1 to measure the autophagy, inflammation and antioxidant levels of the cells. The results showed that the combination of acetate and butyrate inhibited intestinal autophagy (Fig. 7A), inflammation (Fig. 7B) and intracellular ROS levels (Fig. 7C), and increased cellular antioxidant enzyme levels (NRF2, CAT, HO-1, NQO1, GCLC, GCLM, SOD1 and SOD2) (Fig. 7D, E). Furthermore, we demonstrate the effect of melatonin on gut health. Interestingly, we found that different concentrations of melatonin did not change the expression of autophagy, inflammation and oxidative stress-related genes (Supplementary Fig. 8A–R). However, melatonin treatment was found to restore the effect of H_2O_2 on autophagy and inflammation-related proteins expression in IEC-6 cells (Supplementary Figs. 8S–X). Overall, the results suggested that melatonin promotes a gut anti-oxidative status associated with autophagy and inflammation by remodeling the gut microbiome.

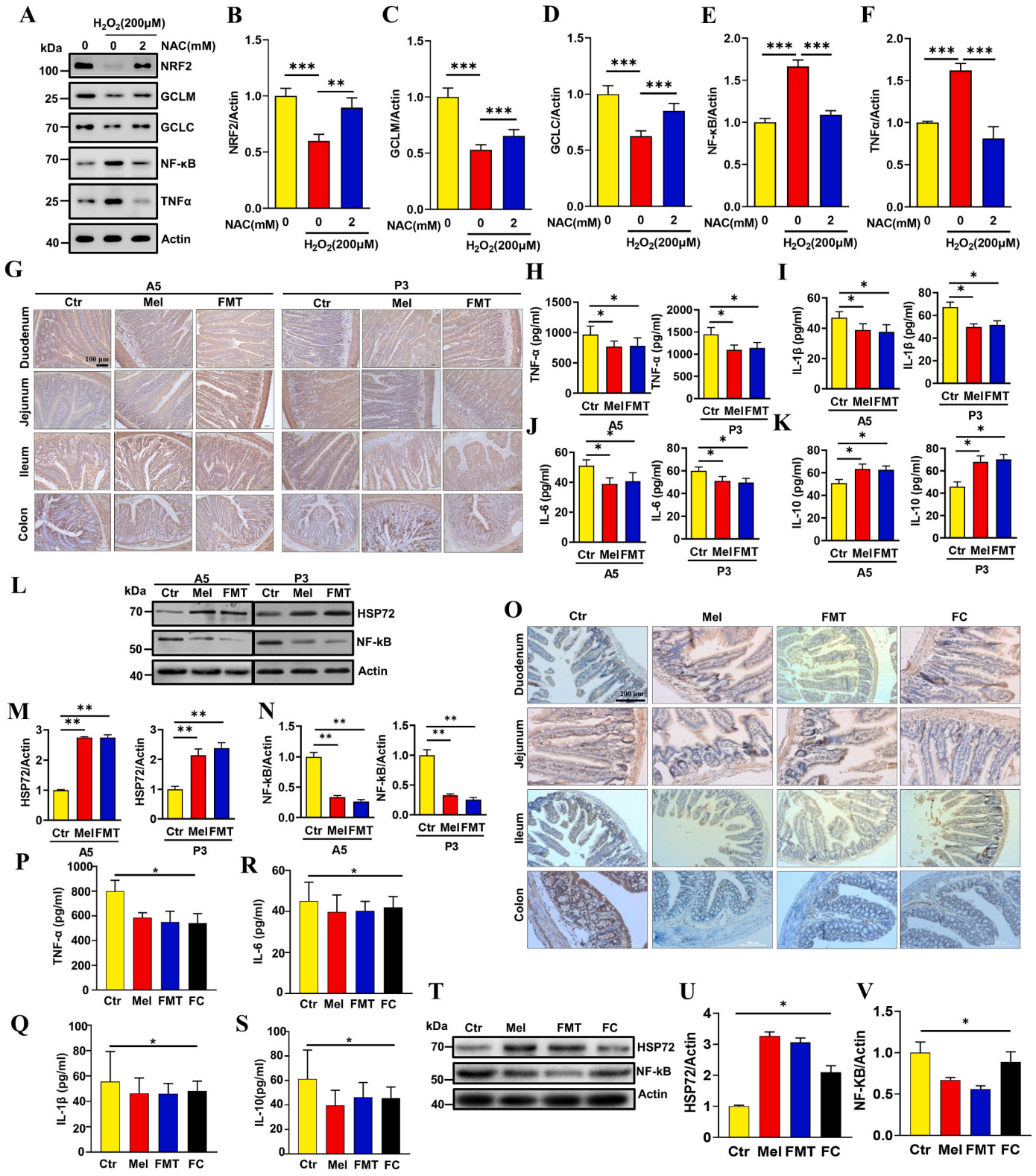

Fig. 4. Melatonin-treated gut microbiota inhibit oxidation-related intestinal inflammatory

The IEC-6 cells were treated with 200 μM H_2O_2 alone or in combination with 2 mM NAC for 12 h, and then the levels of NRF2, GCLM, GCLC, NF-κB and TNF-α were evaluated by western blot **(A)**. The protein relative level analysis of NRF2 **(B)**, GCLM **(C)**, GCLC **(D)**, NF-κB **(E)** and TNF-α **(F)**. Intestinal immunohistochemical images using antibody NF-κB **(G)**. Detections of TNF-α **(H)**, IL-1β **(I)**, IL-6 **(J)** and IL-10 **(K)** in serum (n = 5). Intestinal tissue expression of HSP72 and NF-κB proteins (n = 3) **(L)**. The protein relative levels of NF-κB **(M)** and HSP72 **(N)**. Intestinal immunohistochemical images using antibody NF-κB **(O)**. The detections of TNF-α **(P)**, IL-1β **(Q),** IL-6 **(R)**, and IL-10 **(S)** in serum. (n = 10). Intestinal tissue expression of HSP72 and NF-κB proteins (n = 3) **(T)**. The protein levels of NF-κB **(U)** and HSP72 **(V)** was showed, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

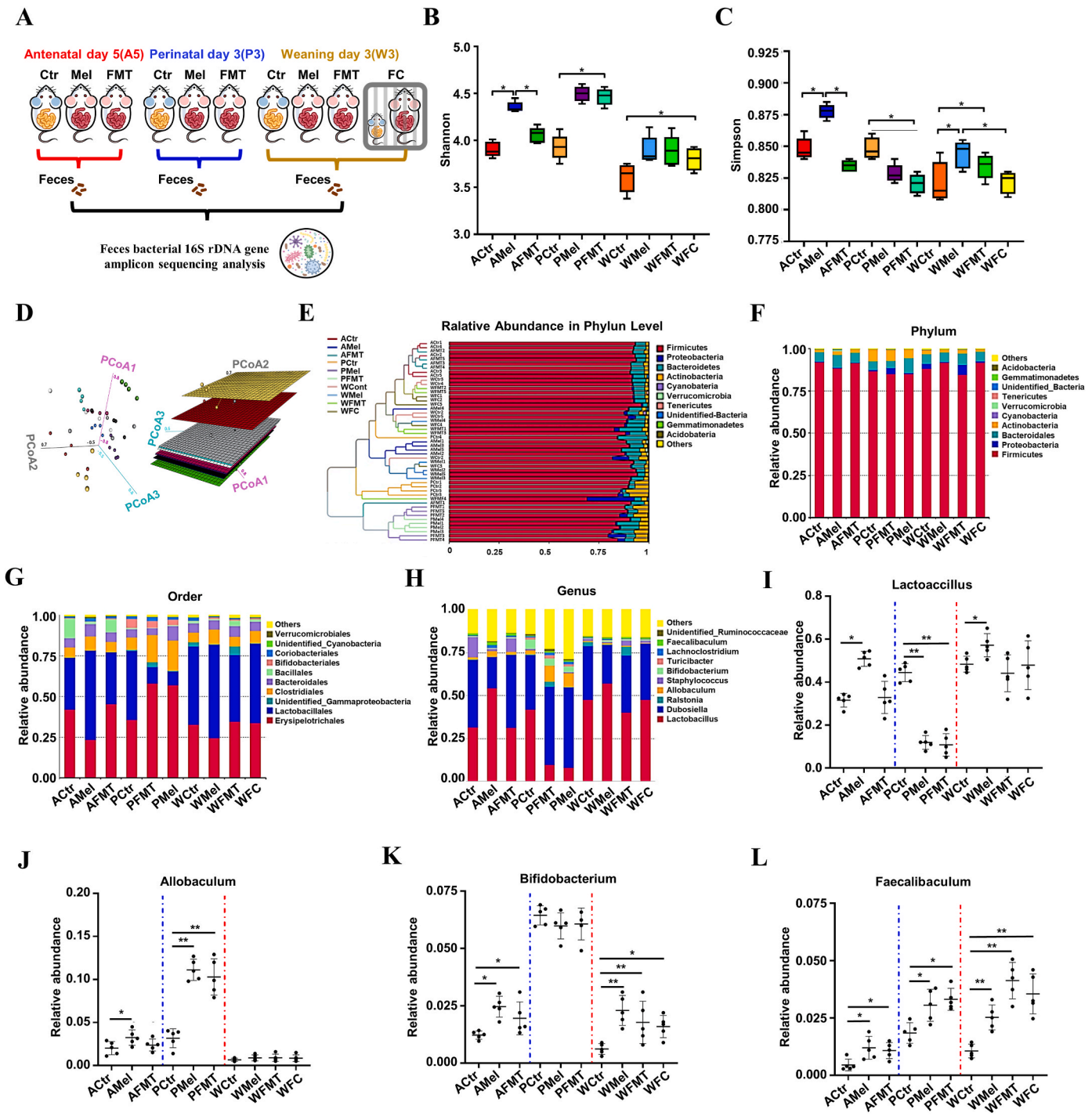

Fig. 5. Melatonin reprograms gut microbiota in perinatal and weaning rats

Experiment design for feces bacterial 16S rDNA gene amplicon sequencing analysis **(A)**. Shannon index in α-diversity analysis **(B)**. Simpson index in α-diversity analysis **(C)**. PCoA plot analysis from each sample (n = 5) **(D)**. Weighted UniFrac distance in each sample **(E)**. Microbiota compositions and abundances at the phylum **(F)**, order **(G)**, and genus **(H)** levels, respectively. Melatonin, FMT, and FC increase the abundances of *Lactobacillus* **(I)**, *Allobaculum* **(J)**, *Bifidobacterium* **(K)**, and *Faecalibaculum* **(L)** respectively. $^{*}P < 0.05$, $^{**}P < 0.01$.

4. Discussion

Gut health is vital for mammals, not only to improve nutrient absorption but also to protect against pathogens, toxins, and allergens. In perinatal and early weaned animals, intestinal health is also implicated in reproduction, lactation, and mortality rate after weaning; however, few clinical or experimental studies have evaluated the effects and regulatory mechanisms of melatonin on mammalian gut health. Therefore, we systematically investigated the effects and underlying mechanisms of melatonin on gut health in perinatal and early weaned rats. In our study, we have focused on the effect of melatonin on gut microbial remodeling and the ability of this effect to modulate gut health in rats. However, the rats we studied were untreated, and the implications of studying the effects of melatonin-regulated gut microbiota on environmental toxin-induced gut injury are also meaningful. The results indicated that melatonin improved gut health in these rats by

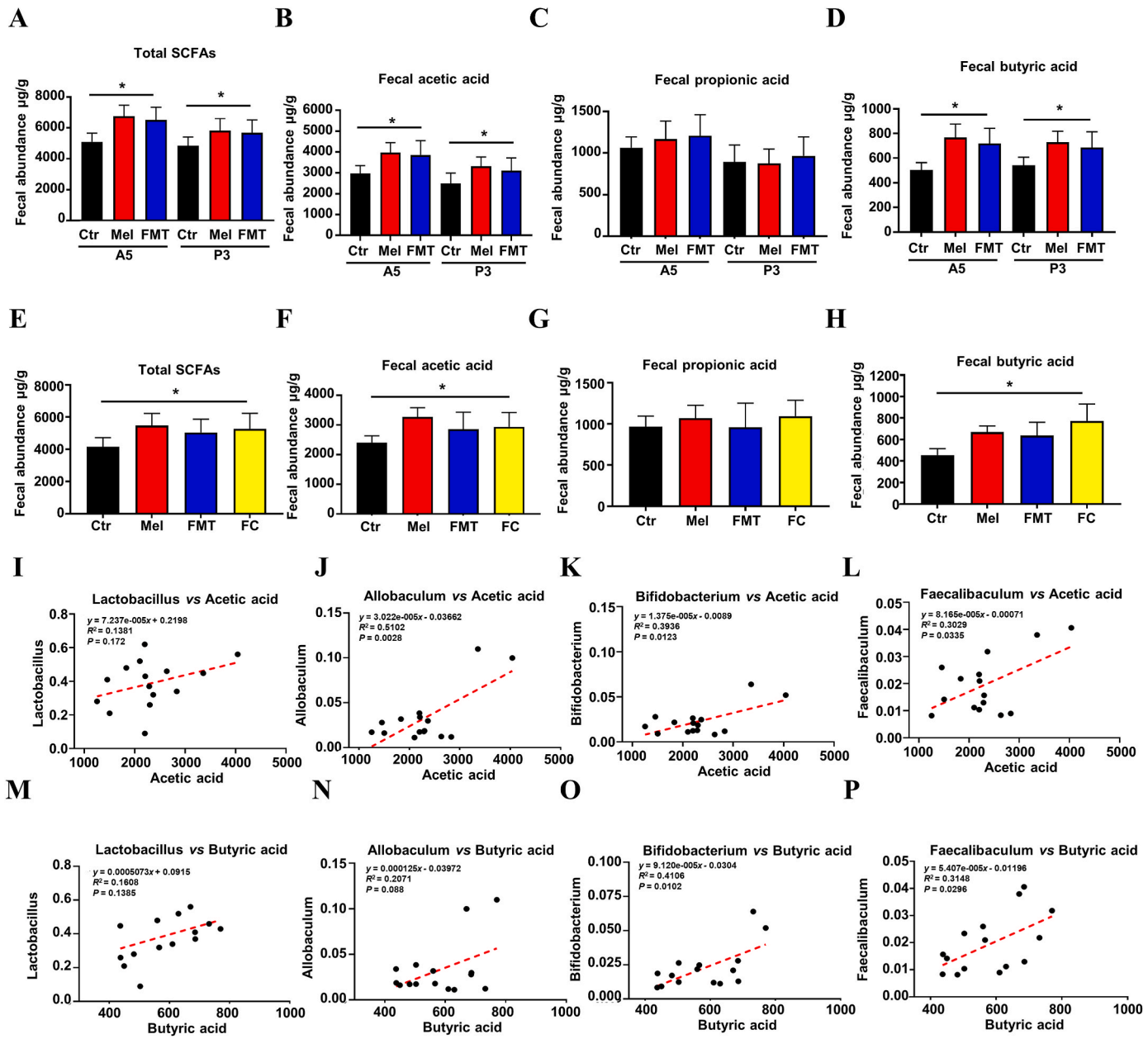

Fig. 6. Melatonin-treated gut microbiota increases SCFA production
The concentrations of fecal total SCFAs **(A)**, acetic acid **(B)**, propanoic acid **(C)**, and butyric acid **(D)** in perinatal female rats. The concentrations of fecal total SCFAs **(E)**, acetic acid **(F)**, propanoic acid **(G),** and butyric acid **(H)** in early-weaned rats. Correlation analyses between acetic acid and *Lactobacillus* **(I)**. Correlation analyses between acetic acid and *Allobaculum* **(J)**. Correlation analyses between acetic acid and *Bifidobacterium* **(K)**. Correlation analyses between propanoic acid and *Faecalibaculum* **(L)**. Correlation analyses between butyric acid and *Lactobacillus* **(M)**. Correlation analyses between butyric acid and *Allobaculum* **(N)**. Correlation analyses between butyric acid and *Bifidobacterium* **(O)**. Correlation analyses between butyric acid and *Faecalibaculum* **(P)**. Fecal SCFAs were determined by gas chromatography. Differences were assessed by Student's t-test, $*P < 0.05$. Correlation analysis between microbiota and acetic acid was conducted by Pearson correlation analysis.

remodeling the gut microbiome as follows: (1) melatonin reprogramed gut microbiota in perinatal and early weaned rats; (2) more importantly, melatonin-treated gut microbiota improved gut health; (3) specifically, melatonin-treated gut microbiota regulated gut antioxidation, autophagy, and inflammation; (4) SCFAs suppress oxidation-induced intestinal autophagy and inflammation.

Melatonin is a highly effective antioxidant that protects essential cell elements, such as the nucleus, mitochondria, and endoplasmic reticulum [38–40], and plays an important role in alleviating oxidative stress, inhibiting autophagy and inflammation [41,42]. Melatonin induces antioxidant gene NRF2-HO-1 reprogramming and leads to an improvement in NAFLD [43], prevents neuroinflammation and relieves depression by attenuating autophagy impairment through FOXO3a regulation [44]. Besides, melatonin inhibits mitochondrial dysfunction by suppressing NLRP3 inflammasome activation in chronic obstructive pulmonary disease mice [45]. It is now known to be present in the gastrointestinal tract, and its functions in the gut generally appear to be to protect the mucosa from erosion and ulcer formation [42,46]. In the present study, we investigated how melatonin-reprogrammed FMT affects the gut in perinatal and early weaned rats.

FMT is the infusion of liquid filtrate feces from a healthy donor into the gut of a recipient to cure a specific disease, including Parkinson's

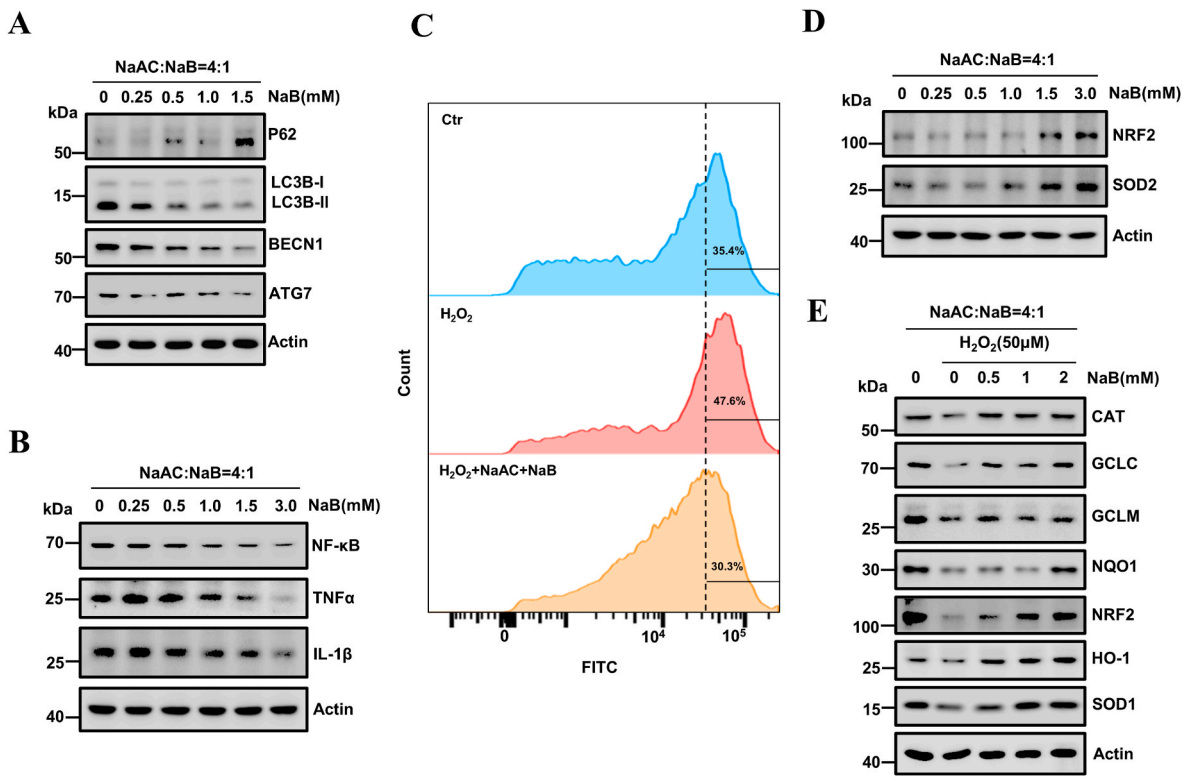

Fig. 7. SCFAs suppress oxidation-induced intestinal autophagy and inflammatory

The IEC-6 cells were treated with indicated concentrations of acetic acid (NaAC) and butyric acid (NaB) (NaAC: NaB = 4:1) for 12 h, and then the levels of P62, LC3II, BECN1 and ATG7 were evaluated by western blot **(A)**; the levels of NF-κB, TNF-α and IL-1β were evaluated by western blot **(B)**. The IEC-6 cells were treated with 200 μM H_2O_2 alone or in combination with 8 mM NaAC and 2 mM NaB for 12 h, and the ROS levels in the indicated cells were detected by FACS **(C)**. The IEC-6 cells were treated with indicated concentrations of acetic acid (NaAC) and butyric acid (NaB) (NaAC: NaB = 4:1) for 12 h, and then the levels of NRF2 and SOD2 were evaluated by western blot **(D)**. The IEC-6 cells were treated with 200 μM H_2O_2 alone or in combination with indicated concentrations of acetic acid (NaAC) and butyric acid (NaB) (NaAC: NaB = 4:1) for 12 h, and then the levels of indicated proteins were evaluated by western blot **(E)**.

disease, fibromyalgia, chronic fatigue syndrome, multiple sclerosis, obesity, and insulin resistance [6,47]. It is the transfer of minimally manipulated, prescreened donor stool into the gastrointestinal tract of a patient to ameliorate the dysbiotic state by increasing overall diversity and restoring the functionality of the microbiota [48]. Recent studies have shown that the gut microbiota are sensitive to melatonin exposure, and that melatonin treatment has been found to markedly affect the gut microbiota in both healthy and sick animal models [49–52]. The variation of specific bacteria among melatonin-reprogrammed gut microbiota affects obesity and insulin resistance [53,54]. A decrease in the *Firmicutes*-to-*Bacteroidetes* ratio and an increase in the abundance of the mucin-degrading bacteria *Akkermansia* are associated with a healthy mucosa [23]. Moreover, restoration of the gut microbiota to a premorbid state is a new therapeutic approach, and FMT is gaining increasing importance in both clinical and research settings. To date, although a large number of trials are ongoing worldwide to explore the potential therapeutic indications of FMT, it is currently recommended only for the treatment of recurrent *Clostridioides difficile* infection [55]. The findings of the present study have the potential to form the basis of screening for melatonin-reprogrammed gut bacterial strains, such as *Allobaculum*, *Bifidobacterium,* and *Faecalibaculum*, that can be used in the diagnosis or treatment of intestinal diseases.

In the present study, the composition and abundance of various bacteria in the gut microbiota differed among the AMel, AFMT, PMel, and PFMT groups, and more *Allobaculum*, *Bifidobacterium,* and *Faecalibaculum* were implicated in good gut health because they produce acetic and butyric acid. Here, it should be noted that feeding increases the amount of melatonin in the gastrointestinal tract, but pineal gland production of melatonin remains unaffected by feeding [56]. Moreover, the pineal gland and small bowel are highly effective in the synthesis and secretion of melatonin [57], but it needs to be further explored whether exogenous melatonin affects the synthesis of endogenous melatonin. As the classic antioxidant, melatonin increased the levels of SOD, CAT, and GSH, which indicated melatonin as the antioxidant reduce the ROS of intestine and improving intestinal health. Besides, in non-oxidative stress models, melatonin also reprogramed gut microbiota, increased butyrate levels, as well as inhibited intestinal inflammation [58]. Therefore, melatonin could reprogram intestinal microbiota through multiple pathways possibly due to the antioxidant effect of its and possibly due to the specific action of itself. In our study, melatonin and antibiotics co-treatment still showed antioxidant effect, suggest melatonin itself has obvious antioxidant effect. Our data also showed that antibiotic treatment significantly inhibited the antioxidant effect of melatonin compared to Ctr, suggesting that melatonin can also exert its antioxidant function on the gut through microorganisms. Moreover, our results showed that the melatonin concentration in the fecal suspension of FMT (81 pg/ml) was very weak compared to the melatonin concentration (5×10^9 pg/ml) in the melatonin suspension, and we found that antibiotics significantly eliminated the effect of FMT on the level of villus height/depth and GSH/GSSG ratio, which indirectly suggests, from another perspective, that it is the fecal microorganisms, but not melatonin, that is responsible for the antioxidant effect in FMT. Importantly, we also found that FMT + Ant (no microbes present, but trace melatonin present) did not affect the level of villus height/depth and GSH/GSSG ratio compared to Ctr, again suggesting that although trace melatonin is present in the feces of FMT, the role of this melatonin is extremely weak and can be largely ignored. Here, we have focused on the antioxidant effect of gut microbiota or secreted

secondary metabolites and the ability of this effect to modulate gut health in rats. As for how melatonin affects gut microbial remodeling, further studies are required.

Although some of the results of the present study are consistent with those of previous studies, some taxa and metabolites were different and even contrasting, which supports the need for more appropriate studies to ascertain the role of one or several specific bacteria as potentially beneficial to protect against specific intestinal diseases. In our current study, melatonin affects the relative abundance of *Firmicutes* and *Bacteroidetes* in rats, this is consistent with the conclusion in mice [59,60]. Interesting, our results suggest melatonin has weak effect on beneficial *Bacteroidetes*, but significantly increase the relative abundance of numerous beneficial *Firmicutes* in genus, including *Allobaculum* and *Faecalibaculum* which could produce SCFAs. The possible reason for this difference is the differences in fecal microbiota composition in rats and mice model itself, as reported by Ravinder et al. [61].

In this study, we focused on how to alleviate the oxidative stress challenges during reproduction of rats. Both antenatal and postnatal are subject to oxidative stress challenges, but pregnancy may involve hormones (e.g. progesterone). Comparatively, there is relatively little hormonal intervention in the postpartum period. Therefore, pregnant rats were categorised into antenatal and postnatal groups as mutually corroborative and complementary experiments. It is worth noting that our results indicated that oxidative stress and autophagy level of control group in antenatal and postnatal stage were different, suggesting there is a stage specific response to oxidative stress (as well as autophagy) during perinatal stage. In general, weaning in mammals results in a reduction in intestinal V/C and damages intestinal integrity [62]. The findings of the present study revealed that melatonin-treated gut microbiota (FTM and FC) could increase intestinal V/C and ameliorate gut antioxidation, which then inhibit autophagy and inflammation. Interestingly, FC also yielded the same effects, but it was not clear why animals subjected to oxidative stress exhibited a significant loss of crypt architecture, severe villous epithelial atrophy and degeneration, and shortening of villi [63–65]. Interestingly, melatonin is a double-edged sword in regulating autophagy because it can both promote and inhibit autophagy [66,67]. In the present study, the results indicated that melatonin-treated gut microbiota (FMT and FC) blocked autophagy by decreasing the number of autophagic vacuoles to exert its gut-protective role against intestinal oxidative damage. Thus, the results confirm and build on previous findings that intestinal melatonin is highly effective in protecting against oxidative stress–induced lesions [68,69].

FMT benefitted gut health by inhibiting oxidative stress in early weaned rats; therefore, we hypothesized that the early weaned rats in the FC group could gain beneficial microbiota to improve their gut health by eating or licking the dung of their mothers, who were treated with melatonin. In addition, the abundance of beneficial *Bacteroides* and *Lactobacillus* markedly increased upon melatonin treatment, FMT or FC, such as *Firmicutes,* including *Allobaculum*, *Faecalibaculum* and *Lactoaccillus*, which indicated that these important changes led to differences in intestinal microbial products, including total SCFAs and specifically acetic and butyric acid, in early weaned rats. Interestingly, *Allobaculum Faecalibaculum Lactoaccillus* and *Bifidobacterium* produces butyric acid, which protects gut health [70–72]. In addition, *Bifidobacterium* also produces moderate amounts of H_2O_2 to activate the body's production of catalase, which kills Gram-negative bacteria, such as *Shigella* and *Salmonella* [73]. Although the possibility that the altered microbiota did not produce these bacteriocins or that the changes in their abundance were the result of other factors cannot be excluded, the metabolic SCFAs from remodeled intestinal microbiota ameliorated gut antioxidation and inhibited autophagy and inflammation in early weaned rats in the FC group. More experimental studies are needed to further understand the composition and function of the reprogrammed microbiota and their bacteriocins. The findings of the present study suggested that FC improved the gut health of the foster rats by facilitating microbiota remodeling, especially by increasing the abundance of *Allobaculum*, *Bifidobacterium,* and *Faecalibaculum*. Lower levels of melatonin and SCFA concentration are correlated with increased intestinal permeability [74,75], and the results indicated that intestinal permeability decreased with the provision of exogenous melatonin or by increasing the levels of SCFAs. Previous studies have found that butyrate dramatically inhibited LPS-induced inflammatory and epithelium barrier dysfunction in Caco-2 cells [76]. And butyric acid could exert anti-ulcerative colitis effect in IEC-6 cells [77]. Furthermore, butyrate attenuates obesity-induced intestinal barrier dysfunction by induction of antimicrobial peptides [78]. In our study, we treated IEC-6 using the ratio of acetic acid: butyric acid (4:1) based on the ratio of fecal acetic acid and butyric acid (Fig. 6A–H) and literatures [77,79]. Our results showed that the combination of acetate and butyrate inhibited intestinal autophagy, inflammation and intracellular ROS levels, and increased cellular antioxidant levels. It should be noted that early weaned rats ate or licked the dung from female rats treated with melatonin, which might have contained undetected infinitesimal amounts of melatonin. Overall, although the regulation and effects on the gut microbiota were influenced by many factors and were very complex, the potential for FC manipulation to ameliorate gut health holds great promise.

5. Conclusion

Melatonin-treated gut microbiota increase the production of SCFAs, which may improve gut health by enhancing the host's antioxidant capacity. We suggest that precise manipulation of intestinal microbiota through melatonin-treated gut microbiota (FTM and FC) has a therapeutic potential against intestinal disorders caused by oxidative stress in perinatal and weaned mammals.

Ethics approval

The experimental protocols and mice handling procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, Northwest A&F University.

Author contributions

WP and LD provided financial support and conceived the study. XY, RC, WS, and GW performed most of the experiments, wrote the manuscript. WP, LD and GY revised the manuscript. XY, RC, WS, and GW conducted animal experimentation, histological analysis, and biochemical analysis. All authors read and approved the final manuscript.

Data availability statement

The 16S rRNA gene sequencing data of the discovery cohort were submitted to the SRA database in NCBI with the accession number PRJNA778474 on perinatal rats and PRJNA778499 on weaned rats, respectively. Moreover, the data supporting the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

All of the contributing authors declared no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2023.102829.

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