High-fat diet alters the radiation tolerance of male and female mice and the modulatory effect of melatonin

Lu Lu[1,*], Jingming Ren[1], Tong Yuan[1], Xinran Lu[1], Yinping Dong[1], Wenxuan Li, Xinyue Wang[1], Qidong Huo[1], Junling Zhang, Deguan Li[1], Saijun Fan[1,*]

Institute of Radiation Medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin Key Laboratory of Radiation Medicine and Molecular Nuclear Medicine, Tianjin 300192, China

***Corresponding authors:**

Lu Lu, Institute of Radiation Medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin Key Laboratory of Radiation Medicine and Molecular Nuclear Medicine, Tianjin 300192, China. Email: lulu@irm-cams.ac.cn

Saijun Fan, Institute of Radiation Medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin Key Laboratory of Radiation Medicine and Molecular Nuclear Medicine, Tianjin 300192, China. Email: fansaijun@irm-cams.ac.cn

Supplementary information

Supplementary Methods

Animal experiment grouping

The specific experimental groups were established as follows. For experiments assessing the survival of mice exposed to lethal dose irradiation, mice were divided into two groups (10 mice per group for each sex): (1) ND+TBI and (2) HFD+TBI. For ionizing radiation-induced hematopoietic system injury experiments, mice were divided into six groups (6 mice per group for each sex): (1) ND, (2) ND+TBI, (3) ND+TBI+MLT, (4) HFD, (5) HFD+TBI, and (6) HFD+TBI+MLT. For ionizing radiation-induced intestinal injury experiments, mice were divided into six groups (10 mice per group for each sex): (1) ND, (2) ND+WAI, (3) ND+WAI+MLT, (4) HFD, (5) HFD+WAI, and (6) HFD+WAI+MLT.

Oral glucose tolerance test and organ weights

At 2 weeks following TBI, glucose tolerance tests were conducted following the gavage of glucose. Specifically, mice were fasted for 16 h overnight prior an oral glucose tolerance test (OGTT). A fasting (T0) blood sample from the tail tip was initially collected (Accu-chek Aviva, Roche, Indianapolis, IN, USA), after which, the mice were then given a gavage of 2 g kg^{-1}glucose (Sigma-Aldrich Co., St. Louis, MO, USA). Further blood samples were collected at 15, 30, 60, and 120 min post-oral glucose administration.

Biochemical analyses

Peripheral blood was collected from the orbital sinus of mice, and serum was obtained by centrifugation at 3000 × *g* for 10 min. The contents of lipase and total cholesterol (TC) in the serum of male and female mice in each group were determined using an SMT-120VP auto dry biochemical analyzer and corresponding commercial kits (Seamaty, Chengdu, China).

Peripheral blood cell and bone marrow nucleated cell counts

Peripheral blood was obtained from the orbital sinus and bone marrow nucleated cells **(**BMNCs) were harvested from a femur of each mouse as previously described[13]. Cell counts were performed using a Celltac E hemocytometer (Nihon kohden, Japan).

Induction of differentiation and Oil Red O staining

Cell differentiation was induced using one of two differentiation media. Medium A comprised DMEM supplemented with 10% FBS, 10 μM insulin (P0005; Bioss, Beijing, China), 0.5 mmol/L 3-isobutyl-1-methylxanthine (IBMX; I7018; Sigma-Aldrich, Zwijndrecht, The Netherlands), and 1 μmol/L dexamethasone (Dex; D4902; Sigma), whereas medium B comprised DMEM supplemented with 10% FBS and 10 μM insulin. 3T3-L1 cells were placed in 6-well plates, and after the cells had fully grown, contact inhibition was maintained for 48 h to induce cell cycle arrest. Thereafter, the medium was replaced with differentiation medium A followed by incubation for 48 h, after which, the medium was replaced with differentiation medium B, which was changes at 2-day interval until the cells had completely differentiated on day 14.

After discarding the culture medium, the cells were fixed with 4% paraformaldehyde and then stained for 15 min with Oil Red O, which binds to triglycerides in cellular lipid droplets. Photographs were taken under an EVosFLAuto2 fluorescence microscope (AMAFD2000; Thermo Fisher).

Cell proliferation assays

HCT116 and Me180 cells were placed in 96-well plates at a concentration of 8×10^3 cells per well and left for 24 h to attach to the well walls. Having attached, the cells were treated with different concentrations (0 to 4 mM) of MLT. At 24, 48, and 72 h, 20 μL of 5 mg/mL MTT was added to each well followed by incubation for 4 h, after which dimethyl sulfoxide was added to the wells and absorbance was measured at 490 nm using a multimode reader.

Colony formation assay

HCT116 and Me180 cells were placed in the wells of six-well plates (900 cells per well) and treated with 0.5 mM and 0.25 mM MLT, respectively, 30 min prior to exposure to 4 Gy ionizing radiation.

The irradiated cells were incubated at 37°C for 14 days, changing the medium at 2-day intervals, until colonies were visible. The colonies were fixed with methanol, stained with 0.1% crystal violet, and then dried and photographed, followed by counting using image J software.

Immunofluorescence staining

HCT116 and Me180 cells were plated on slides, cultured overnight, and then fixed with 4% paraformaldehyde at room temperature for 15 min prior to permeabilization with 0.2% Triton X-100 for 20 min. The treated cells were incubated with Bodipy 493/503 Neutral Lipid Droplet Fluorescent Probe for 30 min at 37°C, and then observed and imaged using an EVosFLAuto2 fluorescence microscope (AMAFD2000; Thermo Fisher).

Triglyceride assay

HCT116 and Me180 cells (4×10^6) were collected and ultrasonically disrupted for 1 min. Having extracted triglycerides with isopropyl alcohol, these hydrolyzed to glycerol and fatty acids. The glycerol thus obtained was treated with periodic acid to generate formaldehyde, the absorbance of which at 420 nm was measured using a multimode reader.

Supplementary figures and figure legends

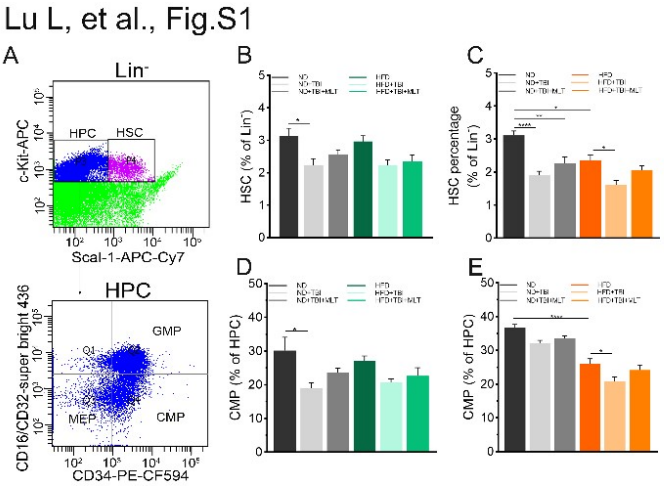

Fig. S1. Melatonin ameliorates radiation-induced hematopoietic injury in a sex-dependent manner in high-fat diet-fed mice.

Male and female C57BL/6J mice were fed a normal diet (ND) or a 35% high-fat diet (HFD) starting at 6 weeks of age and continuing for 8 weeks, treated with or without melatonin (MLT), and then given total body irradiation. (A) Representative image of FACS analyses. (B-E) FACS analyses of HSC (B and C) and CMP cells (D and E) in tibiae 2 weeks after irradiation. Mean ± SEM (n = 6); *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, between the two cohorts.

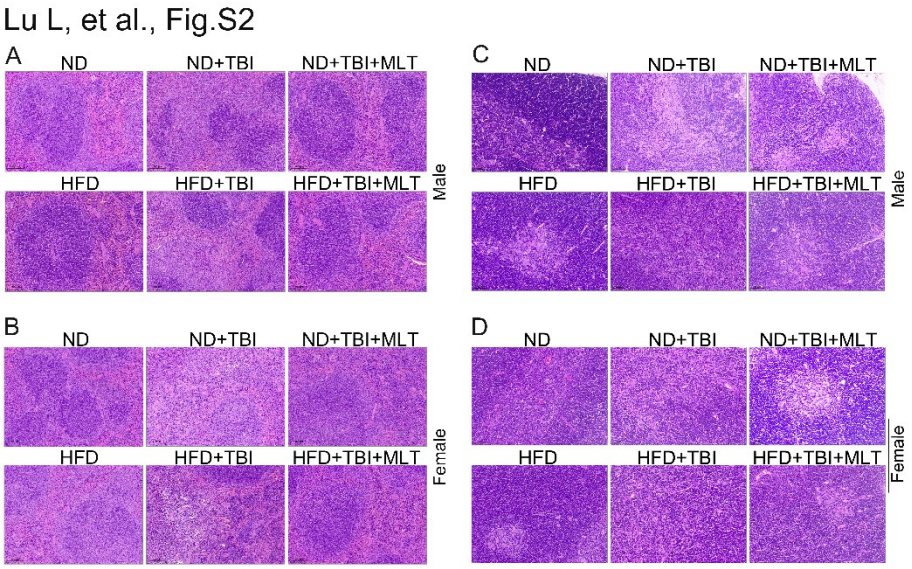

Fig. S2. Melatonin alleviates pathological damage to the spleen and thymus caused by ionizing radiation and a high-fat diet.

(A-D) Representative hematoxylin and eosin staining of spleen and thymus from male (A and C) and female (B and D) mice (scale bar, 50 μm).

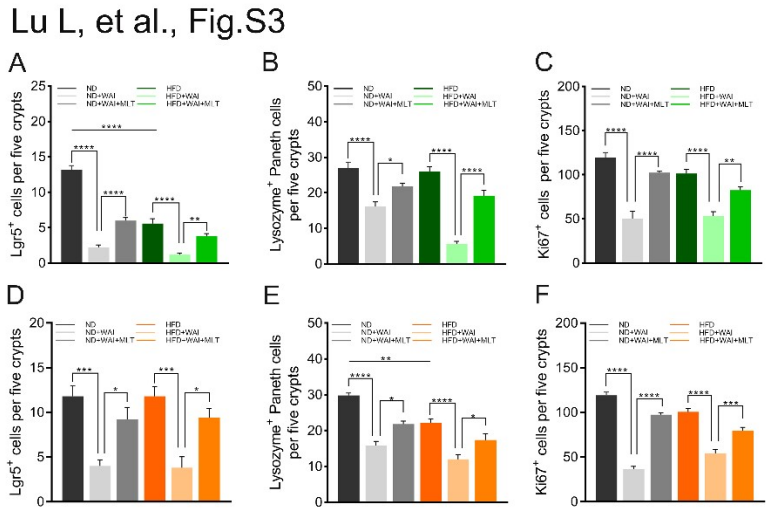

Fig. S3. Melatonin increases the number of Lgr5$^+$ intestinal stem cells and their progeny in irradiated mice.

Male and female C57BL/6J mice were fed a normal diet (ND) or a 35% high-fat diet (HFD) starting at 6 weeks of age and continuing for 8 weeks, treated with or without melatonin (MLT), and then given whole abdominal irradiation. (A-F) Quantification of Lgr5$^+$, lysozyme$^+$, and Ki67$^+$ in male (A-C) and female (D-F) mice. Data are presented as the means ± SEM, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

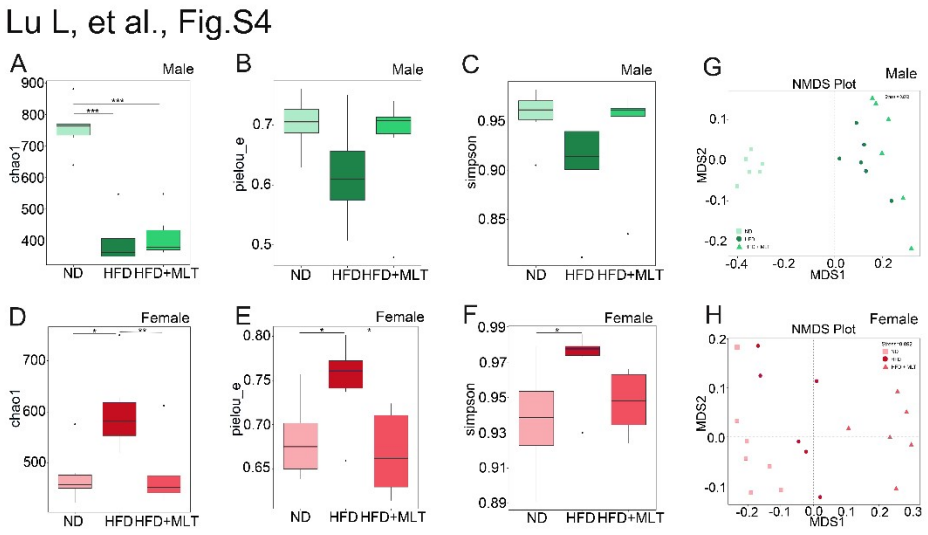

Fig. S4. Sex-specific changes in the intestinal flora in high-fat diet-fed mice following melatonin therapy.

After treating mice with a high-fat diet (HFD) and melatonin (MLT) for 8 weeks, feces were collected and evaluated based on 16S rRNA high-throughput sequencing. (A-F) The Chao1, Pielou_e, and Simpson indices of enteric bacteria in male (A-C) and female (D-F) mice were measured. The Wilcoxon rank-sum test revealed statistical significance. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, n = 6. (G and H) Non-metric multidimensional scale analysis was used to assess the gut microbiome taxonomic profiles of male (G) and female (H) mice (n = 6 mice per group).

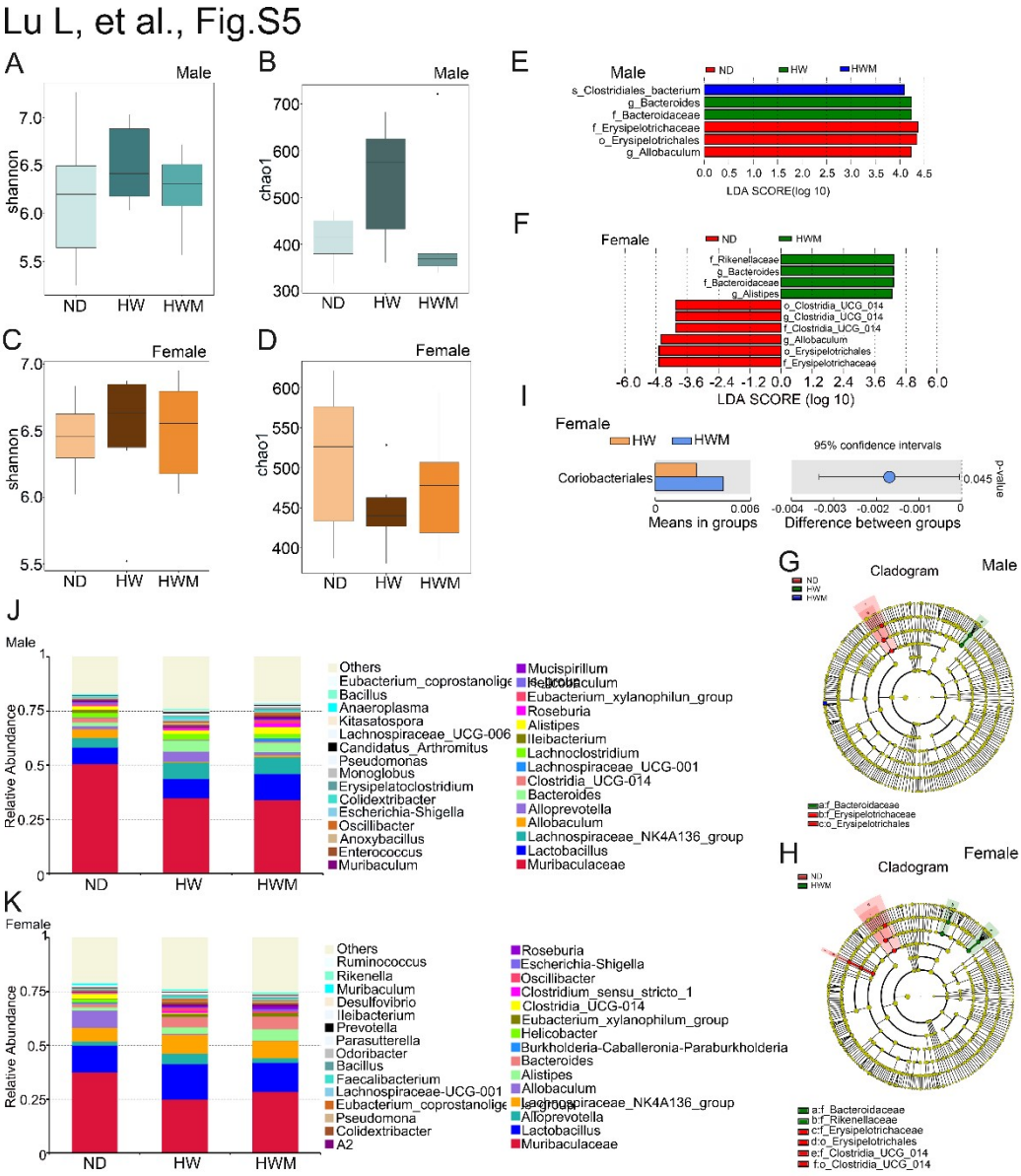

Fig. S5. Sex-distinct radioprotective effects of melatonin on gut microbiota in irradiated mice fed a high-fat diet.

Mice were fed a high-fat diet (HFD) with or without melatonin (MLT) for 8 weeks, and then given whole abdominal irradiation. Fecal samples were collected and examined based on untargeted metabolomics. (A-D) The Shannon and Chao1 indices of gut bacteria in male (A and B) and female (C and D) mice. The top and bottom edges of boxes represent the 75th and 25th percentile values, respectively, and the lines within boxes represent the 50th quartile (median) values. In each case, the lowest and highest diversity values are indicated by the ends of the whiskers. (E-H) The linear discriminant effect size analysis of the intestinal microbiota of male (E and G) and female (F and H) mice. (J and K) The relative abundance of intestinal bacterial flora at the genus level in male (J) and female (K) mice. (I) The abundance of most altered strains in HFD-fed female mice was examined based on 16S rRNA high-throughput sequencing. Statistically significant differences are shown: *$p < 0.05$; Wilcoxon rank-sum, n = 6. (HW, HFD+WAI; HWM: HFD+WAI+MLT).

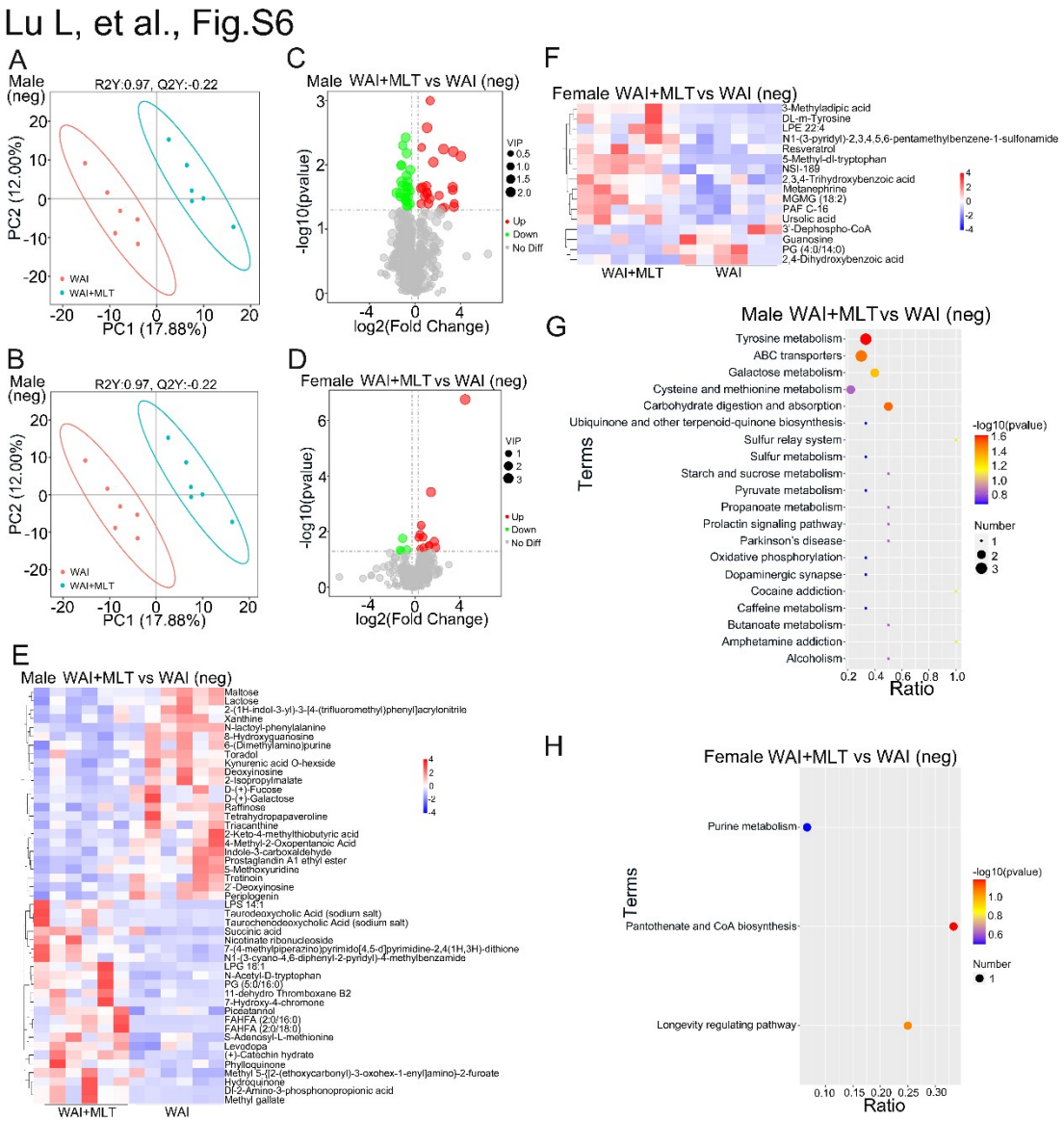

Fig. S6. Significant sex-dependent effects of melatonin on fecal metabolites in irradiated mice fed a high-fat diet.

Mice were fed a high-fat diet (HFD) with or without melatonin (MLT) for 8 weeks, and then given whole abdominal irradiation. Fecal samples were collected and examined based on untargeted metabolomics. (A and B) The partial least-sums discriminate analysis of fecal metabolites of male (A) and female (B) mice under negative ion mode. (C and D) A volcano plot of differential metabolites in male (C) and female (D) mice under negative ion mode. (E and F) A heatmap of differential metabolites (VIP > 1.0, FC > 1.2 or FC < 0.833 and *P*-value < 0.05) between groups of males (E) and female (F) mice. (G-H) The KEGG enrichment of differential metabolites in HFD-fed male (G) and female (H) mice under negative ion mode.