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Resveratrol Derivatives Alleviate Colorectal Adenoma via Gut Microbiota-Mediated ADA-Ado-A2AR Pathway in *Apc*^{Min/+} Mice

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ABSTRACT: Derivatives of the natural polyphenol resveratrol, particularly MR-4 (a methoxylated derivative with potent chemopreventive effects in liver, colorectal, and ovarian cancer models), show stronger antiproliferative properties in human colorectal cancer (CRC) cells and are more bioavailable than resveratrol. However, the role and mechanism of MR-4 against colorectal adenoma (CRA), the most common precancerous lesion of CRC, remain elusive. The current study investigates the impact of MR-4 on the interactions between CRA-associated gut microbiota, metabolites, and microbial enzymes by performing metagenome and metabolome analyses. MR-4 intervention significantly decreases the number of adenomas, ameliorates gut barrier disruption, and inhibits inflammation and related oncogenic pathways. Notably, the anti-adenoma effects of MR-4 are reduced in *Apc*^{Min/+} mice with gut microbiota depletion. Furthermore, MR-4 reduces gut dysbiosis and reinstates beneficial bacteria, including *Roseburia intestinalis* (*R. intestinalis*). In addition, metabolomics analysis reveals significant alterations in purine metabolism during CRA progression in *Apc*^{Min/+} mice, which are notably inhibited by MR-4, especially for adenosine (Ado). Multi-omics analysis shows that MR-4 modulates *R. intestinalis*, to upregulate Ado deaminase (ADA), thereby reducing Ado accumulation and A2AR signaling, which in turn decreases VEGF expression in CRA, highlighting a novel mechanism where MR-4 reduces tumor angiogenesis by restoring gut microbiota and purine metabolism, with *R. intestinalis*–ADA–Ado–A2AR signaling as potential targets. This study reveals gut microbiota-dependent mechanisms of a structurally optimized food compound, advancing strategies for dietary polyphenol-based CRA prevention.

Keywords: Resveratrol derivatives; Colorectal adenoma; Purine metabolism; Gut microbiota; Adenosine; Adenosine deaminase

1. Introduction

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Worldwide, colorectal cancer (CRC) is the leading cause of cancer-related morbidity and mortality, and more than 90% of CRCs develop from colorectal adenoma (CRA) as the “adenoma-carcinoma” sequence^[1]. CRA occurs in about half of the patients by the age of 70, and 10% of these tumors progress to malignant CRC^[2]. Therefore, further explanations of the mechanisms underlying CRA progression and effective chemoprevention are crucial to reducing the incidence of CRC. Normal colonic epithelium to adenoma to carcinoma progresses relatively slowly, providing a window of opportunity for intervention. Chemoprevention is the process of using a synthetic or natural substance to prevent cancer, delay cancer onset, reverse cancer progression, or delay the process of carcinogenesis^[3]. In spite of the fact that nonsteroidal anti-inflammatory drugs, COX-2 inhibitors, and calcium show promise for chemoprevention, they are currently not proven to be very effective or have adverse effects^[3]. Current research therefore focuses on the exploration of chemopreventive agents with multiple targets and multiple pathways.

The profound influence of gut microbiota on health and disease risks^[4,5], particularly cancer, has garnered significant attention^[6]. Research highlights the strong correlation between gastrointestinal malignancies, such as CRC^[7], and the gut microbiota due to their spatial proximity^[8]. Altered gut microbiota compositions, including an increase in pathogens like *Escherichia coli* *pks*⁺, *Fusobacterium nucleatum*, and Enterotoxigenic *Bacteroides fragilis*, and a decrease in beneficial bacteria like *Lactobacillus*, *Bifidobacterium*, and *Faecalibaculum rodentium*, have been observed in patients and animals with CRA or CRC^[9]. These changes are indirectly reflected in the gut microbiota's metabolites and co-metabolites, which play crucial roles in tumor initiation, progression, and deterioration^[10]. Metabolites like SCFAs^[11], tryptophan^[12], and microbial bile acid metabolites^[13] are implicated in CRA and CRC, and could serve as potential biomarkers and therapeutic targets. Hence, emphasis on reinstating the gut microbiota dysregulation and restoring the associated altered microbial metabolites with the selected chemopreventive agents can be a promising new strategy for CRA intervention.

As a natural polyphenol, resveratrol has the potential for the chemoprevention of CRC. However, resveratrol, like other polyphenols, has poor absorption, low bioavailability, and short retention times, which limits its full chemopreventive potential^[14]. To optimize resveratrol in these respects, Trans- 3,4,5,4'-tetramethoxystilbene (MR-4, Figure 1A), an O-methylated congener of it, has been synthesized, whose accumulation is apparently high in the gastrointestinal tract^[15] and exhibits preferential growth inhibition and proapoptotic properties compared to resveratrol^[16]. It is noteworthy that MR-4 reduced the number of adenomas in the small intestine and suppressed tumor formation in the colon of *Apc*^{Min/+} mice, significantly accumulating in the intestine, suggesting that the gastrointestinal tract is the main site of action of MR-4 preventing CRA^[17]. Therefore, it is reasonable to expect that gut microbiota and their metabolites are supposed to resume the essential role to enable the chemopreventive efficacy of MR-4 against CRA. However, the previous studies have not yet demonstrated that MR-4 intervention can alter gut microbiota composition, and it is unclear how it regulates gut microbiota in *Apc*^{Min/+} mice to coordinate bacterial metabolites.

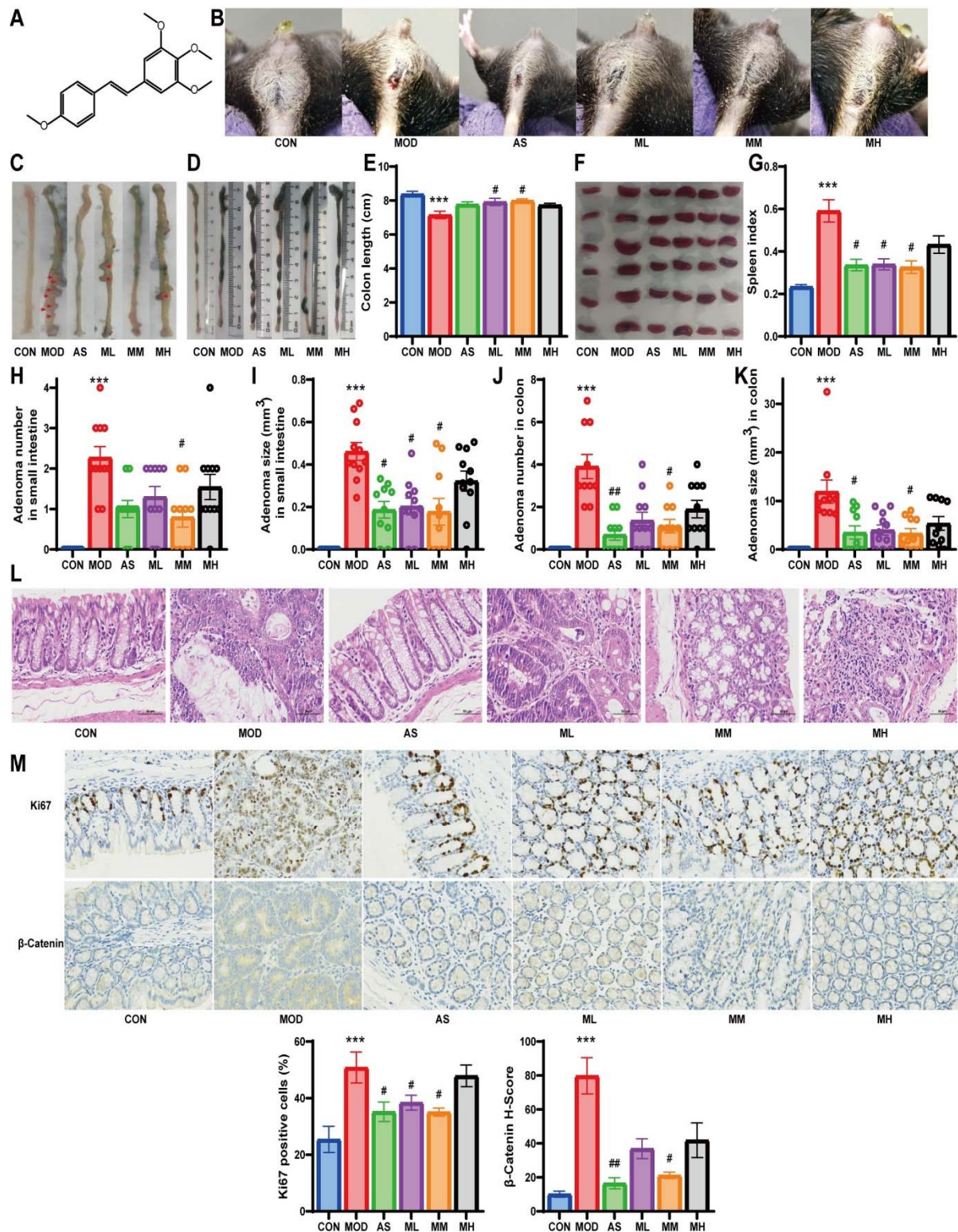


Figure 1 MR-4 alleviates the progression of CRA in $Apc^{Min/+}$ mice. (A) Chemical structure of MR-4. (B) Feces bleeding. (C, D) Representative images of colon tissues. (E) Length of colons. (F) Representative images of spleen tissues. (G) Index of the spleen. (H) Adenoma number in the small intestine. (I) Adenoma size in the small intestine. (J) Adenoma number in the colon. (K) Adenoma size in the colon. (L) H&E staining of the colon tissues (400 ×). (M) Immunohistochemical staining for CRA-related proteins in $Apc^{Min/+}$ mice from different groups. All data are expressed as mean ± S.E.M (n = 3–11 per group) (***) $P < 0.001$, vs CON group, # $P < 0.05$, ## $P < 0.01$, vs MOD group.), CON: wild-type group, MOD: model group, AS: aspirin group, ML: low- dose group, MM: middle- dose group, MH: high- dose group.

Here, the MR-4 preventative effect on the CRA was explored using the adenomatous polyposis coli mutant ($Apc^{Min/+}$) mice CRA model from a gut microbiota-associated system metabolism perspective. We

initially confirmed the inhibitory effect of MR-4 on CRA development, and further conducted experiments with gut microbiome-depleted model mice to verify the crucial roles of the gut microbiota in MR-4 intervention for CRA. Furthermore, using metagenomic sequence-based microbiota analysis, we investigated the regulatory effect of MR-4 on gut dysbiosis in *Apc*^{Min/+} mice. In addition, we utilized untargeted metabolomics to identify and characterize the differential metabolites that were significantly altered by MR-4 to regulate the gut microbiota in mice to improve CRA. Further multi-omics comprehensive analysis was conducted to explore the impact of MR-4 on the interactions among CRA-related intestinal microbiota, metabolites, and microbial enzymes. Through our research, we will provide new insights into the understanding of how MR-4 may improve CRA by affecting gut microbiota and its metabolites.

2. Materials and methods

2.1 Reagents and Materials

MR-4 was designed and synthesized at the National Chinmedomics Research Center of the Heilongjiang University of Chinese Medicine. Its purity (> 98%) (Figure S1-2) and structure were confirmed by nuclear magnetic resonance spectroscopy (Figure S3-4, Table S1). Formic acid, chromatographic-grade methanol and acetonitrile were obtained from Thermo Fisher Scientific, Inc. (Waltham, MA, USA).

2.2 Animal Experiments and Experiment Design

All animal experiments were performed in compliance with animal welfare guidelines and approved by the Animal Care and Ethics Committee of the Heilongjiang University of Chinese Medicine (2023070301), and all experiments were carried out according to the Declaration of Helsinki. Four-week-old male *Apc*^{Min/+} mice and C57BL/6 J mice (18–20 g) were purchased from GemPharmatech Co. Ltd. (Nanjing, China) and under SPF condition with (23 ± 2) °C and a 12-h light/dark cycle. All mice had *ad libitum* access to high-fat diet (Research Diets, D12492; 60% fat by calories) and water. At 7 weeks of age, a group of C57BL/6 J mice was included as a control (CON, n = 11), and the *Apc*^{Min/+} mice were randomly divided into five groups (n = 10 or 11 in each group): unprevented CRA model group (MOD); CRA with aspirin (25 mg/kg BW) group (AS); CRA with low-dose (60 mg/kg BW) MR-4 group (ML); CRA with medium-dose (120 mg/kg BW) MR-4 group (MM); CRA with high-dose (240 mg/kg BW) MR-4 group (MH). The compound was suspended in 0.5% sodium carboxymethyl cellulose (CMC-Na). During 3 weeks of the intervention, administration of MR-4 by gastric gavage was conducted once daily. Simultaneously, rats in the CON group and MOD group were given 0.5% CMC-Na as vehicle control.

To elucidate the specific gut microbiota responsible for the inhibitory effect of MR-4 on CRA, the following experimental design was implemented. At 4 weeks of age, a group of C57BL/6 J mice was included as a control (CON, n = 11), and the *Apc*^{Min/+} mice were randomly divided into three groups (n = 10 or 11 in each group): MOD group, Antibiotic (Abx) group, and Abx+MM group. Abx and Abx+MM group mice were given drinking water with an antibiotics cocktail (0.2 g/L ampicillin, neomycin and

metronidazole, and 0.1 g/L vancomycin) to deplete the gut microbiota, and every other 1 weeks throughout the entire experiment^[18]. At 7 weeks of age, Abx+MM group mice were treated with MR-4 (120 mg/kg BW) by oral gavage once daily for 3 weeks. CON, MOD and Abx group mice were gavaged with 0.5% CMC-Na.

2.3 Sample Collection

The fresh feces were collected 21 days after administration and stored at -80°C until used for metagenomic sequencing. Feces samples were collected on day 22 for metabolomic analysis. At the end of the experiment, all mice were fasted for 12 h. On day 22, the blood, spleen and colorectal tissues were collected upon sacrifice at 1 hour after dosing, then the blood was allowed to clot followed by centrifugation and serum harvesting, the serum was stored at -80°C for further analysis, spleen weight and colon length were measured, and the number of polyps was recorded. Then, the colon was divided into two parts. One part was snap frozen in liquid nitrogen and then stored at -80°C for further analysis, and the other was fixed in 4% paraformaldehyde for the tissue section.

2.4 Enzyme-Linked Immunosorbent Assay (ELISA) Analysis

Colonic cytokines (IL-1 β , IL-6, TNF- α , IL-17, and IL-22) and serum LPS were quantified using ELISA kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), while VEGF in cell supernatants and ADA and adenosine in bacterial culture media were measured using ELISA kits from Aviva Systems Biology (San Diego, California, USA), all following the respective manufacturers' protocols.

2.5 Hematoxylin and Eosin (HE) Staining

Colon tissue was fixed overnight in 4% paraformaldehyde. The fixed tissues were embedded in paraffin for a section and then stained with hematoxylin and eosin (H&E) for histological analysis.

2.6 Immunohistochemistry Staining

β -Catenin (diluted 1:1000, Servicebio, China) and Ki67(diluted 1:500, Servicebio, China) protein expression was identified by immunostaining of paraffin-embedded colon tissue. Immunohistochemistry was performed by deparaffinization, antigen retrieval, permeabilization, and blocking in bovine serum albumin. The software APathwell (Servicebio, Wuhan, China) was used for image analysis.

2.7 Western Blotting Analysis

The total protein of colon tissues was extracted, and Western blotting analysis was conducted as described previously^[19] using the following antibodies: IL-6 (1:1000, GB11117, Servicebio), STAT3 (1:1000, GB111176, Servicebio), p- STAT3 (1:1000, AF3293, Affinity), ZO-1 (1:1000, GB111402, Servicebio), Occludin (1:1000, GB11149-2, Servicebio), CD73 (1:1000, GB111807, Servicebio), ADA (1:1000, 100833-T36, Sinobiological), A2AR (1:1000, AB3461, Abcam), VEGF (1:1000, GB11034B, Servicebio), and β -actin (1:2000, GB12001, Servicebio). The band intensity on the Western blots was quantified by densitometry with AIWBwellTM software (Servicebio, Wuhan, China).

2.8 Immunofluorescence

For immunocytofluorescence, cells were fixed with 4% paraformaldehyde, then blocked and incubated with primary antibodies (Occludin, ZO-1, all diluted 1:500, Servicebio, China) at 4 °C overnight, followed by secondary antibody (diluted 1:300, Servicebio, China) at room temperature for 1 hour. Nuclei were stained with DAPI (Servicebio, China). Samples were analyzed using confocal microscopy (Nikon Eclipse C1).

2.9 Transmission Electron Microscopy

Colonic tissue specimens from mice of CON, MOD, MM, Abx, and Abx+MM groups were carefully collected and fixed in a 2.5% glutaraldehyde solution within 100 mmol/L phosphate buffer (Servicebio, Wuhan, China). Subsequently, ultrathin sections were precisely prepared using a Leica UC7 ultramicrotome. The ultrastructure of these tissues was then meticulously examined with a HITACHI HT7800 transmission electron microscope, providing high-resolution insights into cellular and subcellular details.

2.10 Metagenomic Sequencing and Analysis

The extraction of genomic DNAs from fecal samples, preparation of DNA libraries, and subsequent metagenomic sequencing were conducted by Biomarker Technologies Corporation in Beijing, China. After quality testing, the genomic DNA was fragmented, processed, and ligated with adaptors to form a sequencing library for Illumina sequencing. The sequencing reads were filtered, assembled using MEGAHIT, and coding regions were identified by MetaGeneMark to construct non-redundant gene sets with cd-hit software. Alpha diversity was measured by Simpson, Chao, ACE and Shannon statistics. Beta diversity was accessed by UniFrac distance, and the principal coordinates analysis (PCoA) was used for ordination analysis. Unclassified differentially abundant species-level genome bins were taxonomically annotated against the Genome Taxonomy Database. The encoded proteins from these bins were predicted and annotated against the KEGG (<http://www.genome.jp/kegg/>) database to screen enzymes involved in metabolism.

2.11 Metabolomics Analysis

Fecal sample preparation: 50 mg fecal sample and 0.5 mL of 75% (v/v) methanol extract solution were added to an Eppendorf tube. After three rounds of homogenization and sonication, a 1 h incubation at -20 °C was followed by centrifugation of the samples for 15 min at 4 °C at 13,000 r/min. Finally, the supernatant was collected for analysis. QC samples were prepared by mixing equal aliquots from each sample.

UPLC-Orbitrap ID-X Tribrid-MS analysis and data preprocessing: A UPLC system was performed using Vanquish (Thermo Fisher Scientific), fitted with a Waters Corporation ACQUITY UPLC™ BEH C18 column (100 mm x 2.1 mm, i.d., 1.7 microns), and coupled to a high-resolution accurate mass Orbitrap ID-X Tribrid mass spectrometer system (Thermo Fisher Scientific). The mobile phase was eluted with 0.1% formic acid water (A) to 0.1% acetonitrile (B) by gradient elution. The elution conditions were shown in Table S2. The flow rate was 0.3 mL·min⁻¹, the column temperature was 40 °C, and the injection volume was 4 µL. An electrospray ion source (ESI) was used for positive ion detection. The spray voltage was 3.5 kV,

the ion source temperature was 350 °C, the sheath gas flow rate was 40 arb, the auxiliary gas flow rate was 15 arb, and the ion transport tube temperature was 350 °C. The scanning mode was full MS/DD-MS² based on the AcquireXTM intelligent data acquisition technology (Thermo Fisher Scientific), with an Orbitrap resolution of 120000. The scanning range was m/z 50–1,200, and the impact energy gradient was 20, 40, and 60 eV. Raw data were imported into Compound Discoverer TM software 3.3 (Thermo Fisher Scientific) for peak picking, deconvolution, and identification of the metabolites. Databases, including KEGG (<http://www.genome.jp/kegg/>) and MetaboAnalyst (<http://www.metaboanalyst.ca/>), were used for pathway enrichment analysis.

2.12 Microbial DNA Extraction and Specific Bacteria Quantification

Fecal DNA from mice was extracted utilizing the DNeasy PowerSoil Kit (QIAGEN) following the protocol provided by the manufacturer. Subsequently, quantitative polymerase chain reaction (qPCR) was conducted to quantify the levels of *Olsenella uli* and *R. intestinalis*. This was achieved by incorporating 20 ng of genomic DNA into a 20 µL reaction mixture of universal SYBR Green PCR Master Mix (Takara), and executing the qPCR on an ABI QuantStudio 7 Flex Real-Time PCR System. The quantitation of specific bacterial strains was normalized relative to the universal 16S gene. The primer sequences for the detection of these targeted bacteria are detailed in Table S5.

2.13 Integrated Analysis of Metagenome and Metabolome Based on MetOrigin

We used Metorigin (<http://metorigin.met-bioinformatics.cn/>) for the traceability analysis of differential metabolites, where origin analysis and function analysis were performed based on the simple Metorigin analysis mode in its official website.

2.14 Cell culture

CRC cell lines SW480 (RRID: CVCL_0546) and HCT116 (RRID: CVCL_0291) were obtained from Pricella (Wuhan, China) and FENGHUIHENGWU (Changsha, Hunan, China), respectively. Human normal colon epithelial cell line NCM460 (RRID:CVCL_0460) was obtained from BeNa Culture Collection (Beijing, China). All cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin in a humidified atmosphere containing 5% carbon dioxide.

2.15 Metabolites treatment

Ado (purity ≥ 98%) was purchased from the Biomedicine Institute of Northwest University (Xi'an, China). Cells were seeded (1,000 cells/well) into a 96-well plate, and maintained in medium with or without supplementation of Ado for up to 48 h.

2.16 Viability assay of *R. intestinalis*

R. intestinalis was purchased from BeNa Culture Collection (Beijing, China). *R. intestinalis* was cultivated in sterilized reinforced clostridial medium and treated with MR-4 at final concentrations of 2

µg/mL and 4 µg/mL. Then, the OD 600 value was recorded after co-cultivation at 12 h using microplate reader.

2.17 Colony formation

Cells (500 cells/well) were seeded into a 6-well plate. Subsequently, they were treated with either *R. intestinalis* conditioned medium, *Escherichia coli* conditioned medium, fecal extracts conditioned medium (all at 20% in DMEM), or adenosine. After 24 hours of culture, cells were fixed with 4% paraformaldehyde, stained with 0.5% crystal violet solution, and colonies containing more than 50 cells were counted. The experiment was performed in triplicate for each treatment condition.

2.18 Cell proliferation

For cell proliferation analysis, cells in a 96-well plate were subjected to various treatments and then assessed using CCK-8 (ZETA Life, Menlo Park, CA, USA) according to the manufacturer's instructions.

2.19 Cell Migration Assay

Cells were seeded in a 6-well plate and grown to confluence. A scratch was made in the cell monolayer using a sterile pipette tip. Following the specified interventions, cell migration into the wounded area was monitored and photographed at different time points. The wound closure was quantified by measuring the wound area using image analysis software.

2.20 Cell Apoptosis Analysis

Cells were treated with *R. intestinalis* conditioned medium (20% in DMEM). *Escherichia coli* conditioned medium (20% in DMEM) was used as a control. Following treatment, cells were stained with Annexin V-FITC and PI according to the manufacturer's instructions. Apoptosis was analyzed by flow cytometry, and the percentages of early and late apoptotic cells were calculated.

2.21 A2AR siRNA Transfection

HCT116 and SW480 cells were transfected with siRNA targeting A2AR or β -actin siRNA (negative control) using HiperFect transfection reagent (Qiagen, Hilden, Germany). After 72 hours of incubation, with Ado (1 mmol/L) added during the final 48 hours, supernatants were collected. Cells were subsequently assessed for cell proliferation, colony formation, and migration. The primer sequences for the detection are detailed in Table S5.

2.22 Statistical Analysis

The experimental data were analyzed using the statistical software SPSS 22.0 and GraphPad Prism (version 7.0). Experimental data are presented as mean $\pm$ standard error of the mean (S.E.M.). Normality of distribution was first assessed for all datasets. Data following a normal distribution were subjected to one-way analysis of variance (ANOVA) to determine the statistical significance of differences between groups. For data not conforming to normality, the non-parametric Kruskal-Wallis test was applied to independent samples to evaluate significant intergroup disparities. Limma was used for the differential analyses of metagenomic data. Comparisons with the CON group were indicated as * $P < 0.05$, ** $P < 0.01$,

*** $P < 0.001$, while comparisons with the MOD group were indicated as # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$.

3. Results

3.1 MR-4 Significantly Prevents CRA Formation in $Apc^{Min/+}$ Mice

In order to determine whether MR-4 inhibited CRA progression, we administered it to $Apc^{Min/+}$ mice. There was no significant difference in body weight change among the groups (data not shown). In Figure 1B, D, it was shown that $Apc^{Min/+}$ mice exhibited significant rectal bleeding and shortened colon length ($P < 0.001$), whereas oral lower and medium dose MR-4 administration obviously reduced the bleeding and colon shortening ($P < 0.05$) (Figure 1E). Additionally, $Apc^{Min/+}$ mice had significant spleen swelling ($P < 0.001$) (Figure 1F and G), which is a crucial indicator of phenotyping systemic inflammation. Fortunately, administration of lower and medium dose MR-4 both prominently decreased the spleen index ($P < 0.05$ at least). Figure 1C shows a representative image of the colon. $Apc^{Min/+}$ mice displayed a significant rise in intestinal and colonic polyp number and volume ($P < 0.001$, Figures 1H-K). Mid-dose MR-4 treatment markedly reduced these parameters ($P < 0.05$). Histopathological examination showed increased inflammatory infiltration and epithelial disruption in the MOD group (Figure 1L). Notably, all MR-4 doses mitigated colonic lesions in $Apc^{Min/+}$ mice, with the mid-range dose showing the most pronounced improvement. Furthermore, immunohistochemistry revealed significantly increased expression of CRA markers Ki67 and β -Catenin in the MOD group versus the CON group (Figure 1M), with notable reductions in the MM groups. In comparison with AS, a recognized non-steroidal anti-inflammatory drug (NSAID) for CRA prevention, MR-4 demonstrated comparable anti-CRA efficacy (Figure 1).

3.2 MR-4 Suppresses Inflammatory Cytokines and Repairs Gut barrier Function in $Apc^{Min/+}$ Mice

To investigate the effects of MR-4 on the inflammatory response, we measured the levels of inflammatory cytokines in the colon. In $Apc^{Min/+}$ mice, the levels of IL-6, IL-1 β , IL-17, and IL-22 were significantly higher than those in normal mice ($P < 0.001$), as was TNF- α ($P < 0.01$). However, following intervention with varying doses of MR-4, these inflammatory cytokines were reduced to varying extents. Overall, the moderate dose demonstrated the most pronounced effect (at least $P < 0.05$) (Figure 2A). Previous research has documented the pivotal role of IL-6/STAT3 signaling pathway activation in the pathogenesis of CRC^[20]. Consequently, we conducted an investigation into the expression levels of STAT3 and its phosphorylated form, phospho-STAT3 (p-STAT3), within the colonic epithelium. Significantly elevated p-STAT3 levels were evident in $Apc^{Min/+}$ mice compared to the control group. Notably, MR-4 intervention effectively suppressed p-STAT3 expression in colonic tissue ($P < 0.01$), as illustrated in Figure 2B. We also assessed the anti-inflammatory effects of AS, which were comparable to those of MR-4, as shown in Figure 2A, B.

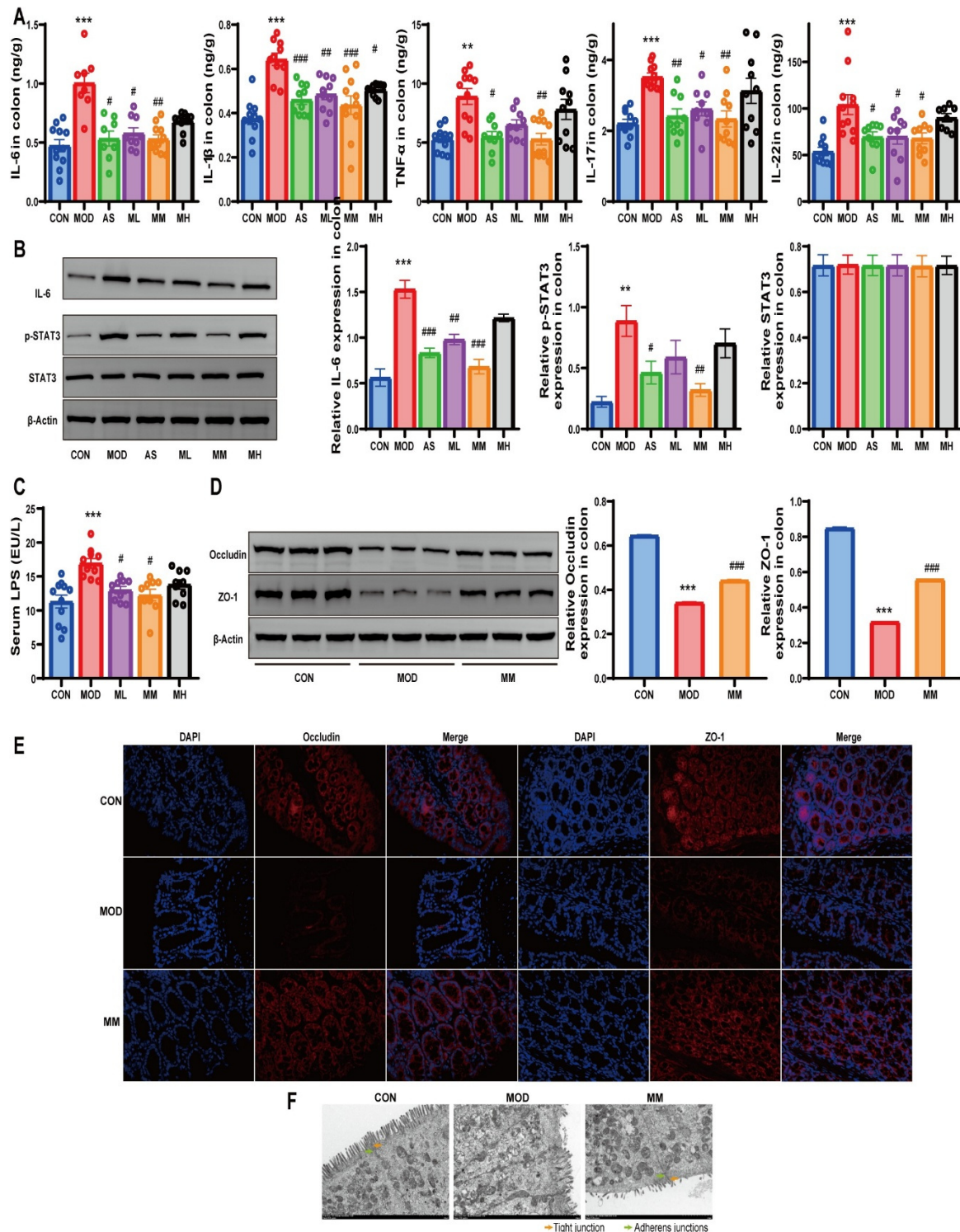


Figure 2 MR-4 suppresses inflammatory cytokines and repairs gut barrier function in *Apc^{Min/+}* mice. (A) Levels of inflammatory cytokines (IL-6, IL-1β, TNF-α, IL-17 and IL-22) in the colon tissues. (B) Expression of IL-6, STAT3 and p-STAT3 in the colon tissues. (C) Mouse serum LPS concentrations. (D, E) Expression of the gut barrier-associated proteins occludin and ZO-1 in colon tissues by Western blotting and immunofluorescence staining. (F) Representative images of intercellular junctions of the three group mice by TEM. All data are expressed as mean ± S.E.M (n = 3-11 per group) (***P* < 0.01, ****P* < 0.001, vs CON group, #*P* < 0.05, ###*P* < 0.01, ####*P* < 0.001, vs MOD group.), CON: wild-type group, MOD: model group, AS: aspirin group, ML: low-dose group, MM: middle-dose group, MH: high-dose group.

To determine the influence of MR-4 on intestinal barrier function in *Apc^{Min/+}* mice, we quantified the expression of occludin and ZO-1, key proteins of colonic tight junctions, as well as serum lipopolysaccharide (LPS) levels. We observed a significant increase in serum LPS levels in *Apc^{Min/+}* mice compared to normal mice (Figure 2C), which were markedly reduced following intervention with low or moderate doses of MR-4.

Meanwhile, compared to normal mice, $Apc^{Min/+}$ mice exhibited a significant reduction in the levels of occludin and ZO-1, as evidenced by western blot analysis (Figure 2D) and immunofluorescence staining (Figure 2E), which were substantially increased following MR-4 intervention. In keeping with this, MR-4 restored the gut barrier structure by TEM (Figure 2F). These results suggest that MR-4 suppresses the inflammatory response by reducing the levels of key pro-inflammatory cytokines, and simultaneously ameliorates gut barrier dysfunction by upregulating the expression of occludin and ZO-1, which may collectively contribute to its chemopreventive effects against CRA.

3.3 Antibiotic-mediated Microbiota Depletion Attenuates the Inhibitory Effect of MR-4 on CRA in $Apc^{Min/+}$ Mice

To determine gut microbiota's role in MR-4's anti-CRA effects, we treated $Apc^{Min/+}$ mice with antibiotics (Abx) to deplete the microbiota and evaluated tumor progression. Depletion was verified by metagenome sequencing (Figure S5). As expected, antibiotics reduced polyp incidence in $Apc^{Min/+}$ mice, and ameliorated colon pathology (Figure 3A-F). Importantly, the combination therapy (Abx+MM) showed no statistically significant enhancement over antibiotics alone in these parameters. In colonic tissue, Ki67 protein levels were significantly lower in both Abx and Abx+MR-4 mice compared to the MOD group ($P < 0.01$ at least), with Ki67 significantly further reduced by MR-4 ($P < 0.01$, Figure 3G).

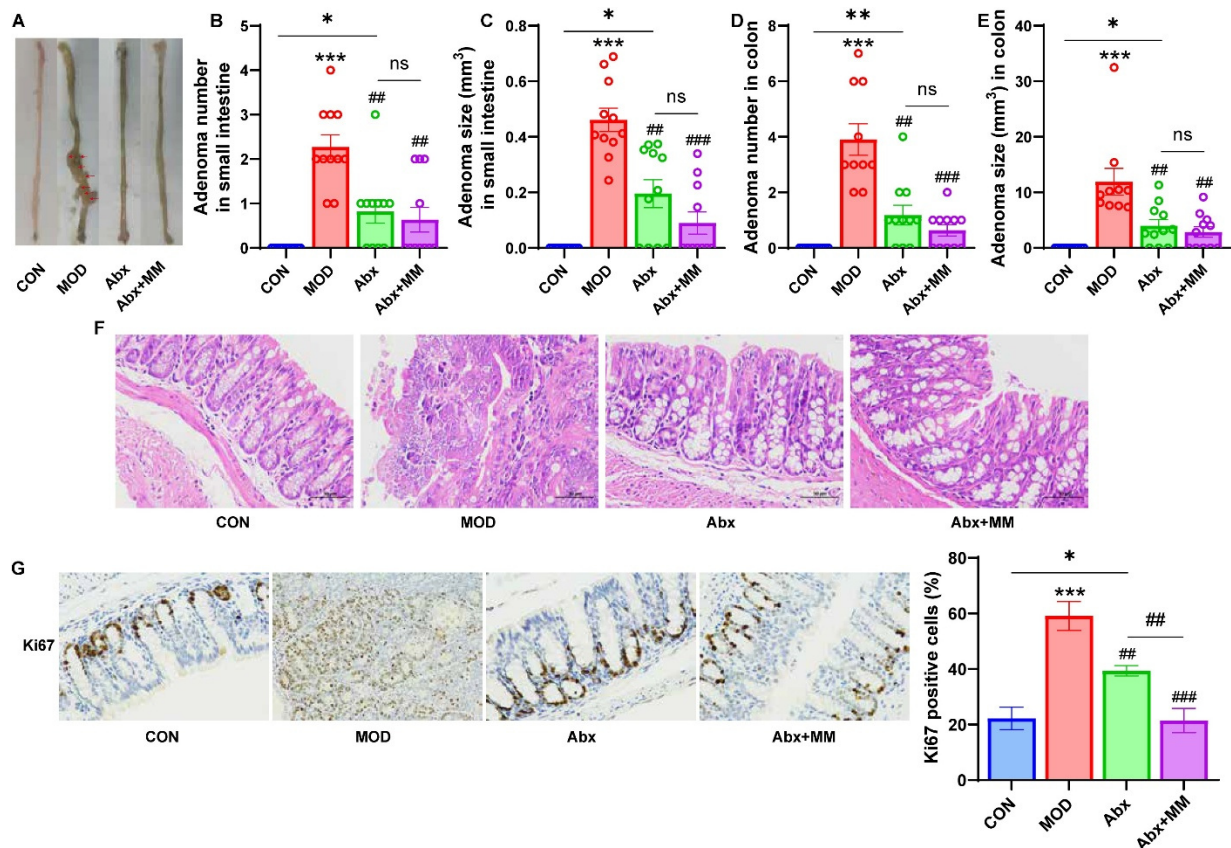


Figure 3 Antibiotics diminish the inhibitory effect of MR-4 on colorectal adenomas in $Apc^{Min/+}$ mice. (A) Representative images of colon tissues. (B) Adenoma number in the small intestine. (C) Adenoma size in the small intestine. (D) Adenoma number in the colon. (E) Adenoma size in the colon. (F) H&E staining of the colon tissues (400 $\times$). (G) Immunohistochemical staining for CRA-related proteins in $Apc^{Min/+}$. All data are expressed as mean $\pm$ S.E.M (n = 3-11 per group) (** $P < 0.01$, *** $P < 0.001$, vs CON group, # $P < 0.05$, ### $P < 0.01$, #### $P < 0.001$, vs MOD group or vs Abx group.), CON: wild-type group, MOD: model group, Abx: antibiotic group, Abx+MM: antibiotic + middle-dose group.

Next, we assessed the impact of antibiotics on MR-4 regulation of the IL-6/STAT3 pathway and gut barrier integrity. Notably, the implementation of MR-4 further reduced IL-6 expression in colonic tissue ($P < 0.001$) without further diminishing p-STAT3 expression (Figure 4A). Additionally, antibiotics had no significant effect on MR-4's protection of the gut barrier, as evidenced by similar colon occludin, and ZO-1 expression with or without MR-4 treatment (Figure 4B-C), which was consistent with transmission electron microscopy results (Figure 4D).

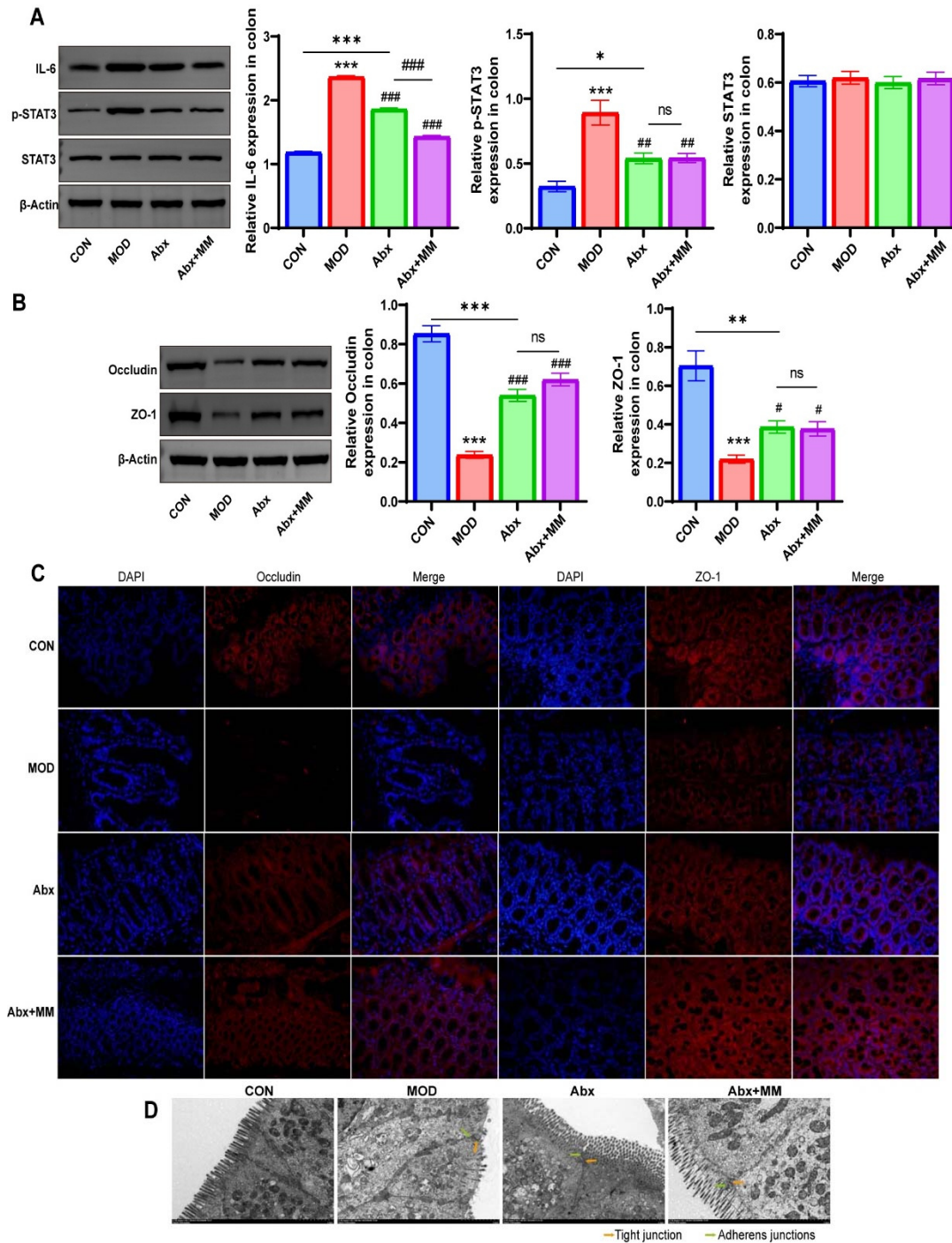


Figure 4 Antibiotics diminish MR-4's inhibition of inflammatory pathways and gut barrier repair in *Apc^{Min/+}* mice. (A) Expression of IL-6, STAT3 and p-STAT3 in the colon tissues. (B, C) Expression of the gut barrier-associated proteins occludin and ZO-1 in colon tissues by Western blotting and immunofluorescence staining. (D) Representative images of intercellular junctions of the four group mice by TEM. All data are expressed as mean $\pm$ S.E.M (n = 3-11 per group) (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, vs CON group, # $P < 0.05$, ### $P < 0.001$, vs MOD group or vs Abx group.), CON: wild-type group, MOD: model group, Abx: antibiotic group, Abx+MM: antibiotic + middle- dose group.

Furthermore, fecal extracts from the MOD group promoted cancer cell proliferation, clonogenicity, and migration, while inhibiting apoptosis. Conversely, MR-4-treated mouse fecal extracts inhibited these processes and promoted apoptosis (Figure S6). Additionally, direct treatment of cancer cells with MR-4 in vitro also demonstrated direct anti-tumor effects (Figure S7). These results further support that gut microbiota at least partially mediates the inhibitory effect of MR-4 on CRA development.

3.4 MR-4 Promotes the Growth of the Beneficial Gut Bacterium *R. intestinalis* and Modulates Purine Metabolism in *Apc*^{Min/+} Mice

To determine the impact of MR-4 on gut microbiota, shotgun metagenomic sequencing analyses were performed across all experimental groups post oral MR-4 administration. As shown in **Figure 5A**, Compared to wild-type mice, *Apc*^{Min/+} mice showed decreased gut microbiota richness, as indicated by significantly reduced Chao 1 and ACE indices ($P < 0.05$), and lower Shannon ($P < 0.01$) and Simpson ($P < 0.05$) indices, suggesting diminished diversity and evenness. MR-4 treatment significantly improved Shannon and Simpson indices ($P < 0.05$), without impacting Chao 1 and ACE, indicating selective enhancement of microbiota diversity and evenness without affecting richness. Furthermore, beta diversity by Principal Coordinates Analysis (PCoA) showed the gut microbiota was reshaped by MR-4 ($P < 0.001$, Figure 5B). Besides, at the phylum level, Firmicutes and Bacteroidota predominated across groups, while at the genus level, *Chlamydia*, *Mucispirillum*, and *Bacteroides* were relatively abundant (Figure 5C, D). MR-4 treatment significantly altered the composition of four phyla and eleven genera in the MM group compared to the MOD group (at least $P < 0.05$, Figure S8A, B). Notably, at the species level, our analysis highlighted significant alterations in the abundance of specific bacterial species that are potentially critical to the chemopreventive effects of MR-4. *Apc*^{Min/+} mice displayed 127 upregulated and 195 downregulated bacterial species compared to wild-type mice ($P < 0.05$, $FC > 1.5$, Figure 5E). Among these, MR-4 treatment particularly enriched probiotics such as *R. intestinalis*, *Eubacterium plexicaudatum*, and *Lachnospiraceae* bacterium A2, while depleting pathogenic species *Olsenella uli* and *Coriobacteriaceae* bacterium (Figure 5F). Co-occurrence analysis revealed that probiotics are strongly positively correlated with each other and strongly negatively correlated with pathogens. For instance, the probiotic *R. intestinalis* is positively correlated with *Roseburia faecis* and negatively correlated with *Olsenella uli*, suggesting that *R. intestinalis* may play a beneficial role in slowing down the progression of CRA (Figure 5G). Based on differential analysis at the bacterial species level, Mantel tests revealed varying degrees of correlation between bacterial genera and serum biochemical parameters (Figure S8C).

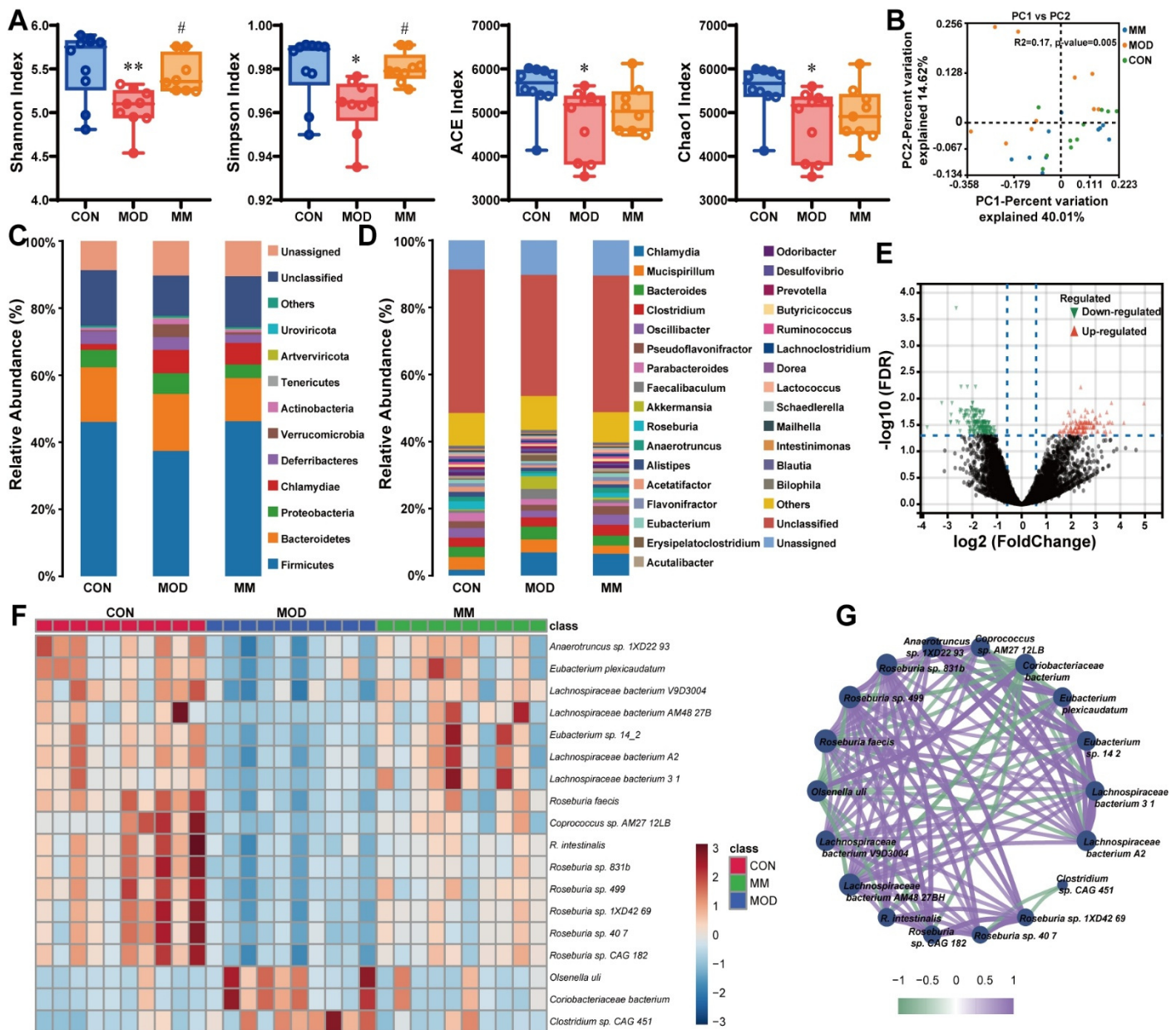


Figure 5 MR-4 effects on gut microbiota diversity and distribution in *Apc*^{Min/+} mice. (A) Species diversity differences between groups were estimated by the Shannon, Simpson, ACE and Chao1 indices. (B) Principal coordinates analysis (PCoA) at the species level comparing microbial beta-diversity based on Bray–Curtis distance. (C, D) Alterations in microbiota at the phylum and genus level. (E) Volcano plots was constructed using Limma. (F) Heatmap for differential bacteria among stool samples of different groups. (G) Co-occurrence analysis: Spearman correlation coefficients between microbes ($R > 0.65$). Purple lines indicate positive correlations, green lines indicate negative correlations. All data are expressed as mean $\pm$ S.E.M ($n = 8$ –10 per group) (* $P < 0.05$, ** $P < 0.01$, vs CON group, # $P < 0.05$, vs MOD group.), CON: wild-type group, MOD: model group, MM: middle- dose group.

Quantitative PCR confirmed MR-4's ability to restore the imbalance between *Olsenella uli* and *R. intestinalis* abundance in *Apc*^{Min/+} mice (**Figure 6A**). This observation underscores the importance of these specific microbial species in the context of MR-4's therapeutic effects. Interestingly, our results showed that MR-4 significantly increased the viability of *R. intestinalis* in vitro (Figure 6B). As shown in Figure 6C, *R. intestinalis* culture medium significantly inhibited the growth of 2 CRC cell lines HCT116 and SW480 ($P < 0.001$), but not the normal colon epithelial cell line NCM460. Moreover, *R. intestinalis* culture medium not only significantly reduced colony formation and migration in HCT116 and SW480 ($P < 0.05$) but also promoted apoptosis (Figure 6D–F).

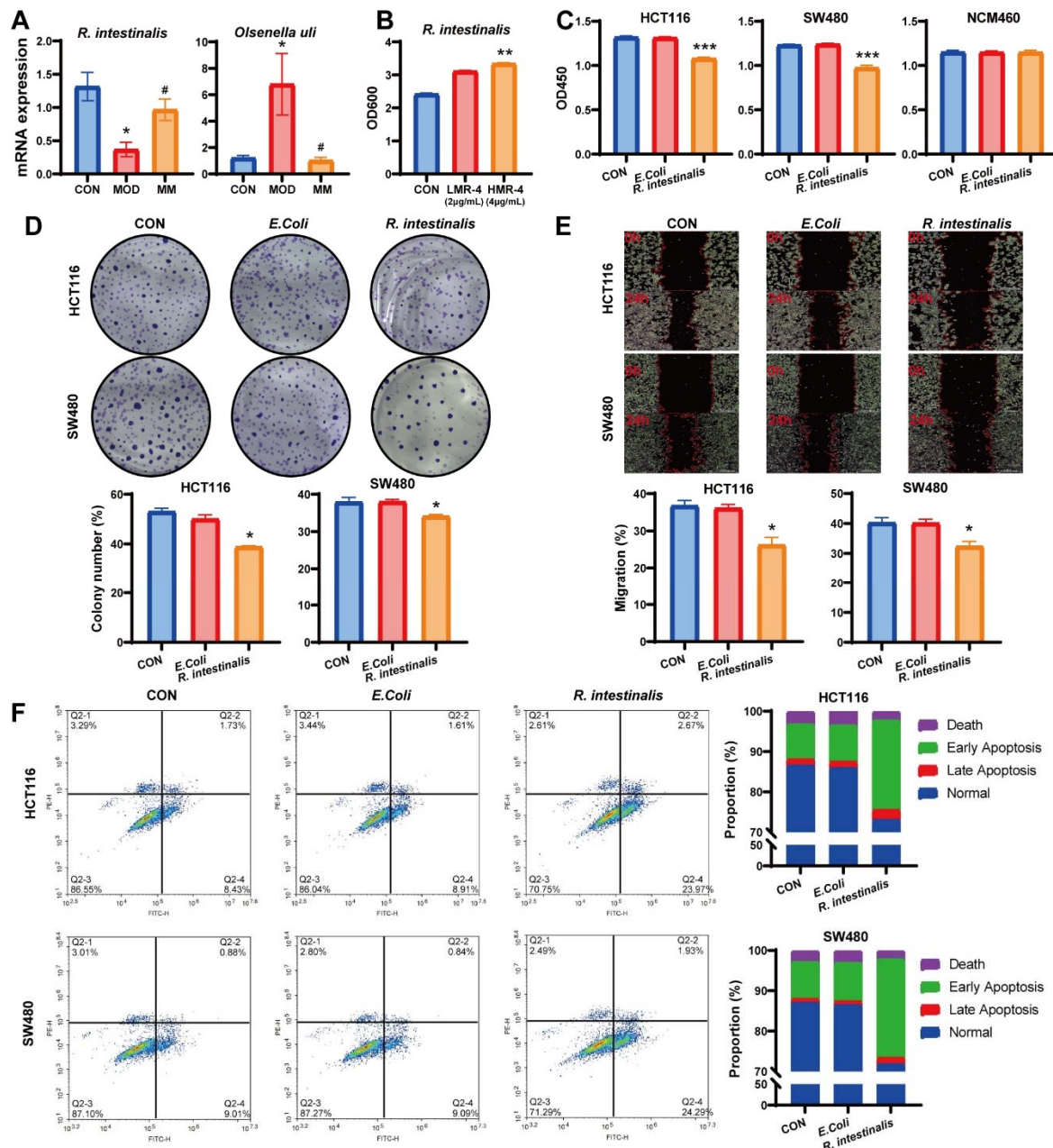
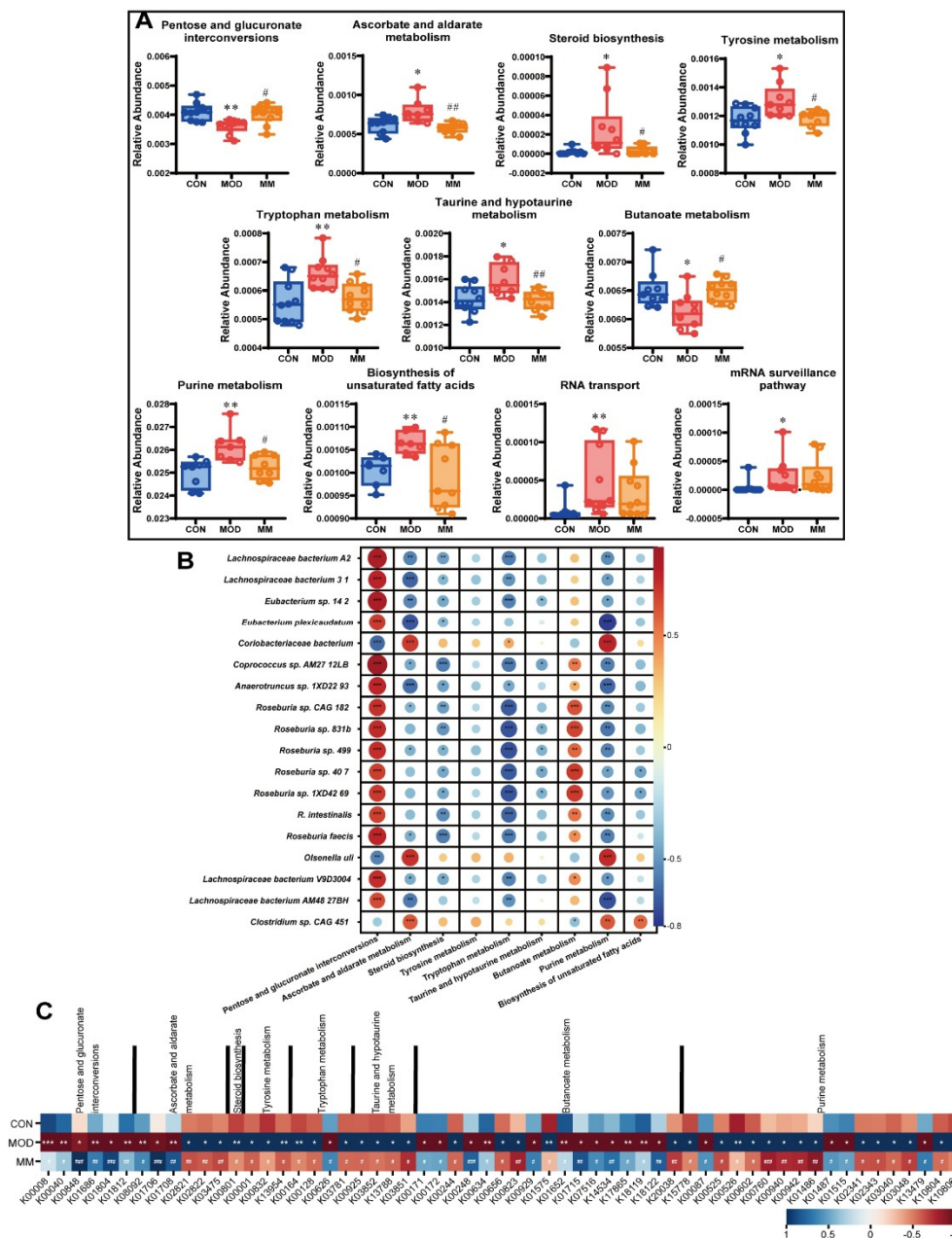


Figure 6 *R. intestinalis* is a key functional strain in the gut microbiota modified by MR-4. (A) The abundance of species *R. intestinalis* and *Olsenella uli* among the CON, MOD, and MM groups was validated by quantitative PCR. (B) *R. intestinalis* was treated with MR-4 under anaerobic conditions for 12 h, then bacterial growth was analyzed by measuring the turbidity at 600 nm. (C) Coincubation with *R. intestinalis* conditioned medium inhibited proliferation of HCT116 and SW480 cells but not NCM460 cells. (D-F) Representative images and quantitative analysis of colony formation, migration and apoptosis of HCT116 and SW480 cells treated with blank control, *Escherichia coli* (*E. coli*) culture medium (as negative control) and *R. intestinalis* culture medium, respectively. All data are expressed as mean $\pm$ S.E.M (n = 3 per group) (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, vs CON group, # $P < 0.05$, vs MOD group.), CON: wild-type group or control, MOD: model group, MM: middle- dose group.

After annotating metagenome-analyzed microbial genes in the KEGG Orthology (KO) database, PCA analysis revealed a significant difference in KO gene distribution between the CON and MOD groups, with the MM group aligning with the CON group (Figure S9). Our statistical analysis identified 11 KEGG metabolic pathways associated with CRA, among which nine were significantly reprogrammed by MR-4. Among them, the purine metabolism pathway, which plays a central role in various biological processes, was particularly affected, along with pathways for steroid biosynthesis and unsaturated fatty acids (Figure

7A). Of the 18 species exhibited varying degrees of association with the 9 key metabolic pathways, *R. intestinalis* and *Olsenella uli* reflected substantial correlations to purine metabolism, a pathway crucial for the microbiota's interaction with host physiology (Figure 7B). The significant modulation of purine metabolism by MR-4 underscores its potential as a therapeutic intervention, highlighting the importance of *R. intestinalis* and *Olsenella uli* in the microbiota-host interactions related to CRA. Additionally, MR-4 effectively reversed differences in KO genes between the CON and MOD groups (Figure 7C). Taken together, these data suggest that MR-4 might reverse microbial dysbiosis in CRA by specifically targeting key metabolic pathways, such as purine metabolism, which could have profound implications for the treatment and management of CRA.



3.5 MR-4 Regulates Gut Microbiota-Associated Metabolites, Highlighting Purine Metabolism and Adenosine in *Apc*^{Min/+} Mice

The metabolites secreted by the gut microbiota plays a crucial role in maintaining physiological homeostasis, with specific focus on purine metabolism and its key metabolite, Ado^[21,22]. We next further aimed to investigate the subsequent changes in systemic metabolites that help us to better understand the biochemical mechanism of MR-4 improved CRA in *Apc*^{Min/+} mice via targeting the gut microbiota. PCA and PLS-DA revealed significant metabolic differentiation between the CON and MOD groups, with MR-4 restoring these to normal levels (**Figure 8A**). Notably, among the 47 metabolites with significantly altered levels in the feces of *Apc*^{Min/+} mice compared to healthy controls, Ado and other purine metabolites stood out as critical targets of MR-4 intervention (Table S3, Figure 8B). Steroid biosynthesis, purine metabolism, and the biosynthesis of phenylalanine, tyrosine, and tryptophan, along with phenylalanine metabolism and primary bile acid biosynthesis, were identified as the main pathways influenced by MR-4, accounting for both the development of CRA and the chemopreventive effects of MR-4 (Figure 8C). In particular, the modulation of purine metabolism, with Ado as a central node, appears to be a key mechanism by which MR-4 influences CRA progression. Our further investigation into the distribution of fecal metabolites mapped to the purine metabolic pathway revealed significant elevation in the levels of adenine, Ado, hypoxanthine, 2'-deoxyadenosine, xanthosine, and xanthine in the model group compared to the control group ($P < 0.01$). As shown in Figure 8D, MR-4 intermediate-dose prophylaxis significantly reversed these levels. To clarify the effect of antibiotics on MR-4's regulation of purine metabolism, we determined the content of purine metabolites in the intestines with or without MR-4 treatment in *Apc*^{Min/+} mice under antibiotics intervention, and observed no significant differences (Figure S10), suggesting that MR-4 exerts its chemopreventive effects against CRA in *Apc*^{Min/+} mice, at least in part, by modulating gut microbiota-associated purine metabolism. Subsequently, the results of co-culture between Ado and CRC cells demonstrated that Ado significantly promoted proliferation, colony formation, and migration in HCT116 and SW480 cells in vitro (Figure 8E-G). This sets the stage for a more detailed exploration of the role of purine metabolism and Ado in the chemopreventive effects of MR-4.

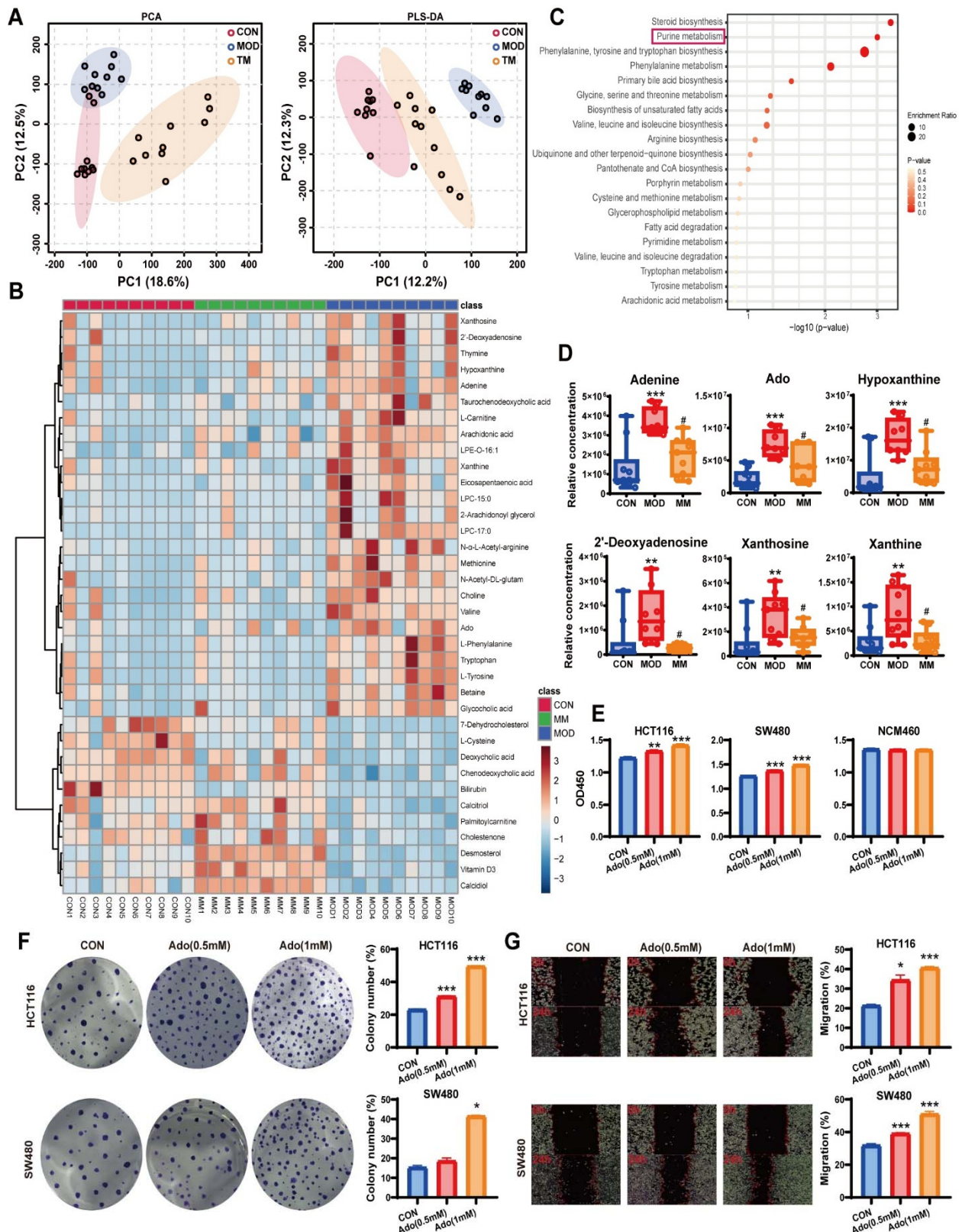


Figure 8 MR-4 alters gut microbiota-related metabolites in the stool. (A) Untargeted feces metabolomes changes were induced by the development of CRA and MR-4 prevention of the *Apc^{Min/+}* mice, which were visualized with the Principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA) assay; (B) Heatmap overview of differential metabolites in feces throughout different groups. (C) Enrichment analysis of differential metabolites between MOD and MM group from the *Apc^{Min/+}* mice. Enrichment Ratio>1.5 was included. (D) The statistical scatter plots of fecal levels of purine metabolites in different groups. (E) Coincubation with Ado culture medium promoted proliferation of HCT116 and SW480 cells. (F, G) Representative images and quantitative analysis of colony formation and migration in HCT116 and SW480 cells treated with control vehicle and Ado, respectively. All data are expressed as mean $\pm$ S.E.M (n = 3-10 per group) (* P < 0.05, ** P < 0.01, *** P < 0.001, vs CON group, # P < 0.05, vs MOD group), CON: wild-type group or control, MOD: model group, MM: middle- dose group.

3.6 MR-4 Upregulates ADA by Regulating *R. Intestinalis*, Thereby Reducing Ado Accumulation and A2AR Signaling Pathway

We conducted an integrated analysis utilizing metagenomic and metabolomic data to explore mechanisms linking gut microbes and metabolites in MR-4's suppression of CRA progression. Initially, we categorized the origins of differential metabolites, revealing that most are microbiota-host co-metabolites (**Figure 9A**). Subsequent functional metabolite analysis confirmed that the majority of these pathways are consistent with co-metabolism database entries (Figure 9B, Figure S11). To explore more accurate evidence of unique metabolic characteristics and microbial enzyme–metabolite interactions, based on the existing metabolite reactions in the KEGG database, we associated the metabolite markers with the KO gene enzyme markers found in *Apc*^{Min/+} mice (Table S4). In *Apc*^{Min/+} mice, enzymes linked to Ado production (CD73-EC:3.1.3.5, and EC:3.1.4.16 3.1.3.6) were upregulated in reactions R00185 and R01562 (Figure S12), while Ado substrate increased in R01560 (Figure 9C), with metabolizing enzymes EC:3.5.4.4 (Ado deaminase, ADA) and EC:2.4.2.1 downregulated. MR-4 significantly reversed these changes compared to the MOD group. Western blot analysis shows MR-4 reverses the upregulation of CD73 (Figure S13) and downregulation of ADA (Figure 9D) in the MOD group. Docking and molecular dynamics simulations demonstrate direct interactions between MR-4 and ADA and CD73 proteins (Figure S14). Next, we specifically explored the relationship between the key functional bacterium and the Ado (Figure S15). *R. intestinalis* demonstrated a stronger negative correlation with Ado (Figure 9E). When cultivated in a culture medium containing 10 mmol/L Ado, *R. intestinalis* promoted Ado degradation by increasing the activity of ADA. Moreover, the addition of MR-4 further enhanced the activity of ADA, facilitating the conversion of Ado (Figure. 9F, G). These findings collectively confirm the significant role of Ado for MR-4 in inhibiting CRA by modulating *R. intestinalis*.

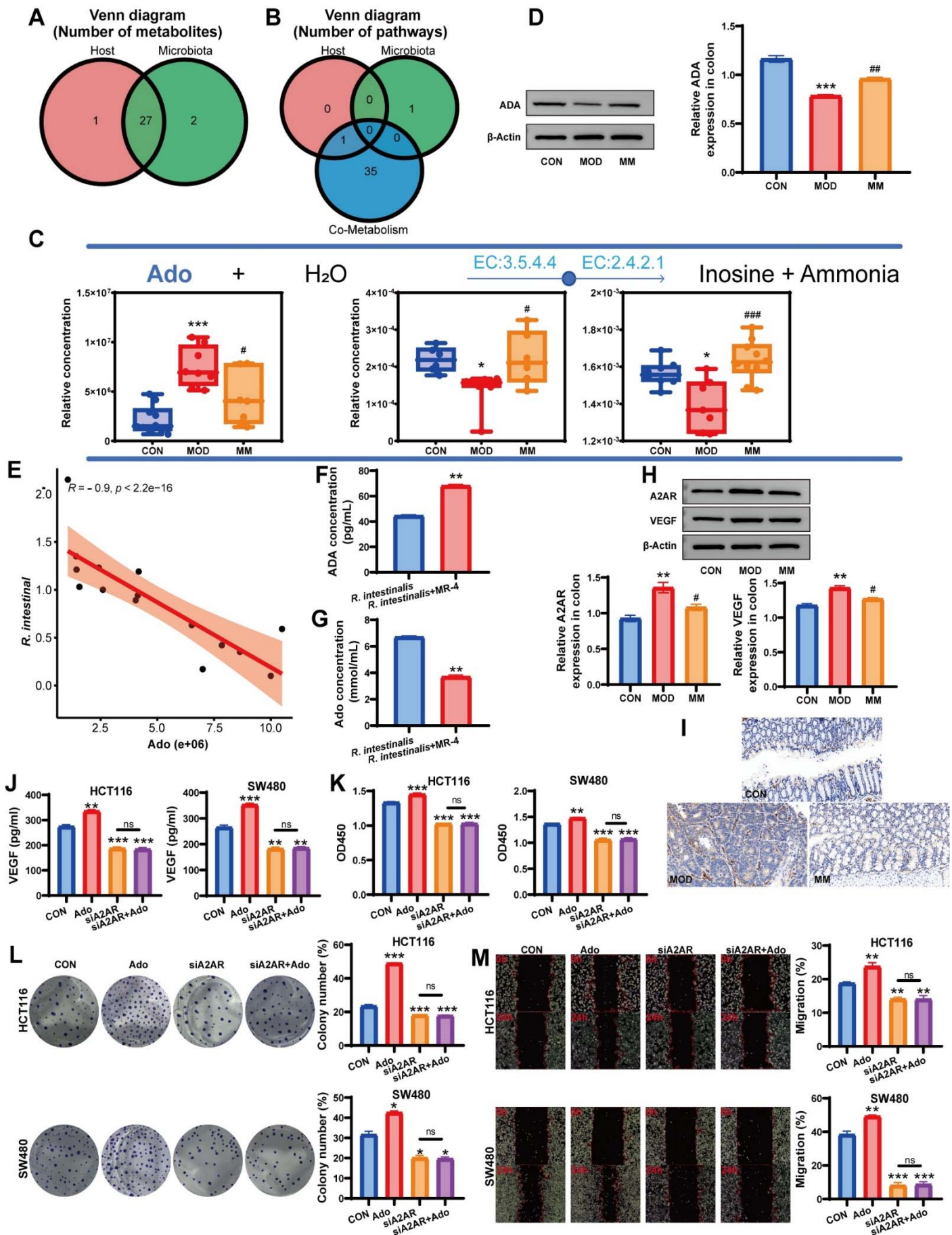


Figure 9 MR-4 inhibits CRA through the *R. intestinalis*-ADA-Ado-A2AR pathway. (A) Metorigin metabolite traceability analysis of differential metabolite. (B) Venn diagram of enrichment analysis of differential metabolic pathway. (C) The R01560 reaction formulas with enzymes and metabolites markers. (D) Expression of the ADA protein in colon tissues by Western blotting. (E) Correlation between *R. intestinalis* and Ado. (F, G) MR-4 promotes the production of ADA by *R.*

intestinalis, which promotes Ado degradation. (H) Expression of the A2AR and VEGF protein in colon tissues by Western blotting. (I) Immunohistochemical expression of CD31 in colorectal tissues of *Apc*^{Min/+} mice from different groups (400×). (J) Suppression of A2AR eliminates Ado-induced upregulation of VEGF. (K-M) Silencing of A2AR inhibits Ado-driven cell proliferation, colony formation, and migration. All data are expressed as mean ± S.E.M (n = 3-10 per group) (**P* < 0.05, ***P* < 0.01, ****P* < 0.001, vs CON group, #*P* < 0.05, ##*P* < 0.01, ###*P* < 0.001, vs MOD group), CON: wild-type group or control, MOD: model group, MM: middle- dose group.

Furthermore, A2A receptor (A2AR), abundant in tumor cells, enhances Ado signaling, stimulating VEGF production for angiogenesis and tumor progression. Notably, MR-4 mitigates the upregulation of A2AR, VEGF, and CD31 in colorectal tumors (Figure 9H-I). To validate the pivotal role of A2AR in the gut microbiota-mediated inhibition of colorectal cancer cell proliferation by MR-4, we conducted experiments using two colorectal cancer cell lines in vitro. Specifically, the siRNA for A2AR was selected. The expression of A2AR was decreased by ~ 80% after transfection with A2AR -siRNA (Figure S16). Consistent with our previous findings, Ado stimulation led to increased VEGF levels. However, silencing A2AR not only abrogated Ado-induced VEGF upregulation (Figure 9I) but also inhibited Ado-driven cancer cell proliferation, colony formation, and migration (Figure 9J-L, Figure S17). Thus, MR-4 interrupts the ADA-Ado pathway, hinders A2AR-mediated VEGF surge, thereby suppressing angiogenesis and tumor growth.

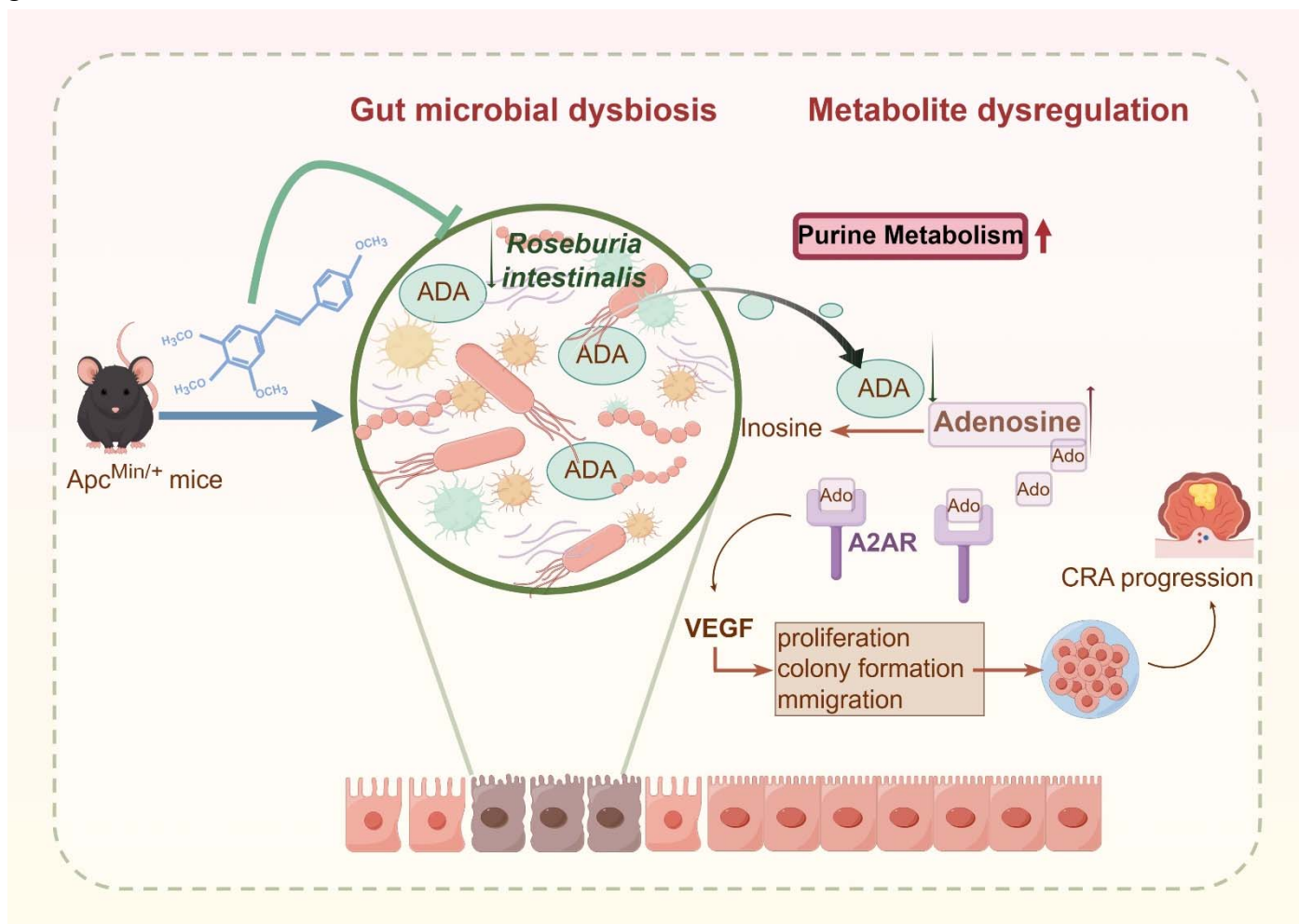


Figure 10 Illustration of the molecular mechanism of MR-4 in preventing CRA in mice (By Figdraw).

4. Discussion

CRC is one of the most common malignancies worldwide, and CRA is the most prevalent precancerous lesion associated with CRC development. Although resveratrol derivatives, particularly MR-4, have demonstrated potent antiproliferative properties in human CRC cells and exhibit higher bioavailability than resveratrol, the role and mechanism of MR-4 in CRA remain largely unexplored. This study aims to fill this gap by investigating the impact of MR-4 on the interactions between CRA-associated gut microbiota, metabolites, and microbial enzymes through comprehensive metagenome and metabolome analyses. Our findings reveal that MR-4 significantly reduces the number of adenomas, ameliorates gut barrier disruption, and inhibits inflammation and related oncogenic pathways. Notably, the anti-adenoma effects of MR-4 are diminished in *Apc*^{Min/+} mice with depleted gut microbiota, highlighting the critical role of the gut microbiota in mediating these effects. The core discovery of this study is that MR-4 modulates the gut microbiota, particularly by reinstating beneficial bacteria such as *R. intestinalis*, and metabolically targets purine metabolism to reduce Ado accumulation and A2AR signaling. This, in turn, decreases VEGF expression and tumor angiogenesis in CRA. Our study thus uncovers a novel mechanism by which MR-4 exerts its anti-adenoma effects through the *R. intestinalis*–ADA–Ado–A2AR signaling axis, offering new insights into the potential therapeutic targets for CRA.

The significance of the gut microbiota in colorectal carcinogenesis is well-established^[23]. While the natural polyphenol resveratrol is known to modulate the microbiota in gut barrier-related diseases^[24], the role of its more potent derivative, MR-4, remained elusive. Previous studies on MR-4 focused on its direct cytotoxic effects, such as activating mitochondrial apoptosis and modulating signaling pathways in various cancers^[25–31]. Crucially, its unique pharmacokinetic profile—high accumulation specifically within gastrointestinal tissue^[15,17]—provides a strategic advantage for targeting CRA. This tissue-specific bioavailability allows MR-4 to achieve high local concentrations at the host-microbiota interface, enabling it to: (a) directly remodel the mucosa-associated microbiota (e.g., enriching *R. intestinalis*); and (b) exert potent local effects on epithelial and immune cells (e.g., suppressing IL-6/STAT3, enhancing barrier integrity) in concert with the microbial community. Moving beyond prior research on direct cytotoxicity, our study employed a multi-omics approach to dissect MR-4's mechanism in CRA. We not only confirm its antiproliferative effects but also propose a novel paradigm: MR-4 inhibits CRA by synergistically regulating the gut microecology and metabolic environment, an efficacy fundamentally underpinned by its targeted delivery to the gastrointestinal tissue. This offers new insights and therapeutic targets for CRC prevention.

This study demonstrates that MR-4 effectively reverses CRA by modulating gut microbiota. Among the microbiota, *R. intestinalis*, a beneficial butyrate-producing bacterium, exhibits anti-inflammatory and anti-tumor effects^[32], enhances anti-PD-1 therapy in CRC by activating CD8⁺ T cells, and is involved in maintaining intestinal epithelial barrier function^[33], thereby reducing the incidence of CRC^[33]. Our findings indicate that *R. intestinalis* directly inhibits CRC cells, while MR-4 promotes its activity to suppress cell proliferation, restore intestinal barrier function, and inhibit CRA progression. The gut microbiota significantly influences the host's purine balance through the breakdown and metabolism of purine compounds,

representing a potential mechanism by which intestinal bacteria impact health^[21]. By analyzing metabolite profiles in fecal samples, we assessed CRA's impact on microbial metabolism and MR-4's regulatory role during intervention. Screening identified 36 fecal metabolites significantly altered by MR-4 intervention, with purine metabolism as a key affected pathway, reflecting MR-4's impact on microbial function. Purine metabolism, a fundamental process in cellular energy transfer and nucleotide synthesis, is closely associated with microbial imbalances and the pathogenesis of adenomas^[34]. Notably, CRA fecal samples exhibited a marked increase in purine-related metabolites, particularly Ado, a purine nucleoside with pro-tumorigenic effects^[35].

The interplay between purine metabolism and gut microbiota may be mediated by microbial enzymes. Studies have shown that ribonucleoside hydrolase in *Lactobacillus* can hydrolyze nucleosides into nucleobases, reducing serum uric acid level in mice by degrading purine nucleosides and decreasing purine absorption in the intestine^[36]. Our study first demonstrates that the accumulation of Ado in CRA mouse feces is associated with a reduction in *R. intestinalis*-related ADA levels, a change effectively reversed by MR-4. Reports indicate that Ado facilitates tumor survival through various mechanisms. The downregulation of CD26-bound extracellular soluble ADA on tumor cell surfaces contributes to Ado accumulation in solid tumors^[37,38]. Ado, by activating A2AR, stimulates the production of VEGF, thereby promoting angiogenesis and driving tumor progression^[39,40]. Additionally, gene chip analysis shows MR-4 regulates multiple genes in endothelial cells, impacting cytokines, transport, metabolism, enzymes, apoptosis, and anti-angiogenesis^[41]. MR-4 significantly inhibits HUVEC proliferation, migration, and VEGF-induced angiogenesis by blocking VEGFR2 phosphorylation and downstream signaling^[42]. Our results demonstrate that MR-4 exerts an anti-CRA effect by modulating gut microbiota-related ADA activity to inhibit Ado accumulation, thereby reducing A2AR activation, decreasing VEGF expression, and suppressing tumor angiogenesis (Figure 10). This finding not only provides a new strategy for CRA treatment but also uncovers the complex relationships among gut microbiota, purine metabolism, and tumor angiogenesis, shedding light on the role of intestinal microbes in cancer development.

Notably, MR-4 has previously been reported to attenuate adenoma formation by down-regulating mucosal PGE-2^[17]. The present study now demonstrates that MR-4 additionally suppresses tumor angiogenesis via the ADA–Ado–A2AR axis, revealing a coordinated microbiota-metabolic mechanism potentiated by its distinctive pharmacokinetic profile^[15,17]. While recent studies highlight impressive microbiota-mediated effects of epigallocatechin (ameliorating colitis via microbial remodeling-induced GPR43 activation)^[43] and curcumin (reshaping microbiota to enhance CD8⁺ T-cell infiltration)^[44], their mechanisms remain largely immune-centric or broad-spectrum. In contrast, MR-4's O-methylation confers metabolic stability and unique tissue distribution, enabling sustained mucosal exposure that facilitates precision-targeted enrichment of keystone symbionts (e.g., *R. intestinalis*) and efficient manipulation of specific metabolic axes (adenosine-purine metabolism). Collectively, these findings indicate that MR-4 exerts multi-target chemopreventive activity against CRA through two synergistic pathways—PGE-2-mediated

attenuation of inflammation and purine-metabolism-mediated inhibition of angiogenesis—thereby representing a distinct strategy of microbiota-precision metabolic intervention against CRA.

Notwithstanding our findings, this study has limitations. While antibiotic depletion evidences microbiota dependency, fecal microbiota transplantation (FMT) from MR-4-treated donors would more definitively establish causality and represents a crucial future direction. Similarly, although *Escherichia coli* control indicates species-specificity, a heat-killed *R. intestinalis* control would better confirm the requirement for live bacterial metabolic activity. Addressing these aspects in subsequent research will further strengthen the mechanistic insights into MR-4's mode of action.

5. Conclusions

MR-4 prevents CRA by restoring gut microbiota, especially *R. intestinalis*, and improving microbial purine metabolism to regulate Ado levels, thereby suppressing tumor angiogenesis via the ADA-Ado-A2AR pathway. This study offers new insights into the biochemical mechanism of MR-4's chemopreventive effects on CRA and adenoma-carcinoma progression.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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References

- [1] J.C. Sedlak, H. Yilmaz Ö, J. Roper, Metabolism and Colorectal Cancer, *Annu Rev Pathol* 18 (2023) 467-492. <https://doi.org/10.1146/annurev-pathmechdis-031521-041113>.
- [2] K.M. Miller, J.D. Waye, Approach to colon polyps in the elderly, *Am J Gastroenterol* 95 (2000) 1147-51. <https://doi.org/10.1111/j.1572-0241.2000.02001.x>.
- [3] B.W. Katona, J.M. Weiss, Chemoprevention of Colorectal Cancer, *Gastroenterology* 158 (2020) 368-388. <https://doi.org/10.1053/j.gastro.2019.06.047>.
- [4] L. Cao, Y. Wu, K.-Y. Liu, et al., *Cornus officinalis* vinegar alters the gut microbiota, regulating lipid droplet changes in nonalcoholic fatty liver disease model mice, *Food & Medicine Homology* 1 (2024) 9420002. <https://doi.org/10.26599/FMH.2024.9420002>.
- [5] Y. Gao, Inflammation and gut microbiota in the alcoholic liver disease, *Food & Medicine Homology* 1 (2024) 9420020. <https://doi.org/10.26599/FMH.2024.9420020>.
- [6] S.J. Blake, Y. Wolf, B. Boursi, et al., Role of the microbiota in response to and recovery from cancer therapy, *Nat Rev Immunol* 24 (2024) 308-325. <https://doi.org/10.1038/s41577-023-00951-0>.
- [7] X. Bai, B. Liu, D. Fan, et al., Modulating the gut microbiota: A novel perspective in colorectal cancer treatment, *Cancer Lett* 612 (2025) 217459. <https://doi.org/10.1016/j.canlet.2025.217459>.
- [8] M.T. White, C.L. Sears, The microbial landscape of colorectal cancer, *Nat Rev Microbiol* 22 (2024) 240-254. <https://doi.org/10.1038/s41579-023-00973-4>.
- [9] S. Li, J. Liu, X. Zheng, et al., Tumorigenic bacteria in colorectal cancer: mechanisms and treatments, *Cancer Biol Med* 19 (2021) 147-62. <https://doi.org/10.20892/j.issn.2095-3941.2020.0651>.

- [10] J. Li, A.H. Zhang, F.F. Wu, et al., Alterations in the Gut Microbiota and Their Metabolites in Colorectal Cancer: Recent Progress and Future Prospects, *Front Oncol* 12 (2022) 841552. <https://doi.org/10.3389/fonc.2022.841552>.
- [11] N. Li, L. Niu, Y. Liu, et al., Taking SCFAs produced by *Lactobacillus reuteri* orally reshapes gut microbiota and elicits antitumor responses, *J Nanobiotechnology* 22 (2024) 241. <https://doi.org/10.1186/s12951-024-02506-4>.
- [12] V. Damerell, N. Klaassen-Dekker, S. Brezina, et al., Circulating tryptophan-kynurenine pathway metabolites are associated with all-cause mortality among patients with stage I-III colorectal cancer, *Int J Cancer* 156 (2025) 552-565. <https://doi.org/10.1002/ijc.35183>.
- [13] J. Cong, P. Liu, Z. Han, et al., Bile acids modified by the intestinal microbiota promote colorectal cancer growth by suppressing CD8(+) T cell effector functions, *Immunity* 57 (2024) 876-889.e11. <https://doi.org/10.1016/j.immuni.2024.02.014>.
- [14] B. Ren, M.X. Kwah, C. Liu, et al., Resveratrol for cancer therapy: Challenges and future perspectives, *Cancer Lett* 515 (2021) 63-72. <https://doi.org/10.1016/j.canlet.2021.05.001>.
- [15] S. Sale, R.D. Verschoyle, D. Boocock, et al., Pharmacokinetics in mice and growth-inhibitory properties of the putative cancer chemopreventive agent resveratrol and the synthetic analogue trans 3,4,5,4'-tetramethoxystilbene, *Br J Cancer* 90 (2004) 736-44. <https://doi.org/10.1038/sj.bjc.6601568>.
- [16] M. Roberti, D. Pizzirani, D. Simoni, et al., Synthesis and biological evaluation of resveratrol and analogues as apoptosis-inducing agents, *J Med Chem* 46 (2003) 3546-54. <https://doi.org/10.1021/jm030785u>.
- [17] S. Sale, R.G. Tunstall, K.C. Ruparelia, et al., Comparison of the effects of the chemopreventive agent resveratrol and its synthetic analog trans 3,4,5,4'-tetramethoxystilbene (DMU-212) on adenoma development in the Apc(Min+) mouse and cyclooxygenase-2 in human-derived colon cancer cells, *Int J Cancer* 115 (2005) 194-201. <https://doi.org/10.1002/ijc.20884>.
- [18] J. Yang, H. Wei, Y. Lin, et al., High Soluble Fiber Promotes Colorectal Tumorigenesis Through Modulating Gut Microbiota and Metabolites in Mice, *Gastroenterology* 166 (2024) 323-337.e7. <https://doi.org/10.1053/j.gastro.2023.10.012>.
- [19] H. Li, C. Fan, C. Feng, et al., Inhibition of phosphodiesterase-4 attenuates murine ulcerative colitis through interference with mucosal immunity, *Br J Pharmacol* 176 (2019) 2209-2226. <https://doi.org/10.1111/bph.14667>.
- [20] A. Wu, D. Fang, Y. Liu, et al., Nuclear translocation of thioredoxin-1 promotes colorectal cancer development via modulation of the IL-6/STAT3 signaling axis through interaction with STAT3, *Theranostics* 13 (2023) 4730-4744. <https://doi.org/10.7150/thno.85460>.
- [21] K. Kasahara, R.L. Kerby, Q. Zhang, et al., Gut bacterial metabolism contributes to host global purine homeostasis, *Cell Host Microbe* 31 (2023) 1038-1053.e10. <https://doi.org/10.1016/j.chom.2023.05.011>.
- [22] B. He, T.K. Hoang, T. Wang, et al., Resetting microbiota by *Lactobacillus reuteri* inhibits T reg deficiency-induced autoimmunity via adenosine A2A receptors, *J Exp Med* 214 (2017) 107-123. <https://doi.org/10.1084/jem.20160961>.
- [23] B. Roje, B. Zhang, E. Mastorilli, et al., Gut microbiota carcinogen metabolism causes distal tissue tumours, *Nature* 632 (2024) 1137-1144. <https://doi.org/10.1038/s41586-024-07754-w>.
- [24] V. Prakash, C. Bose, D. Sunilkumar, et al., Resveratrol as a Promising Nutraceutical: Implications in Gut Microbiota Modulation, Inflammatory Disorders, and Colorectal Cancer, *Int J Mol Sci* 25 (2024). <https://doi.org/10.3390/ijms25063370>.
- [25] A. Gossiau, S. Pabbaraja, S. Knapp, et al., Trans- and cis-stilbene polyphenols induced rapid perinuclear mitochondrial clustering and p53-independent apoptosis in cancer cells but not normal cells, *Eur J Pharmacol* 587 (2008) 25-34. <https://doi.org/10.1016/j.ejphar.2008.03.027>.
- [26] A. Gossiau, M. Chen, C.T. Ho, et al., A methoxy derivative of resveratrol analogue selectively induced activation of the mitochondrial apoptotic pathway in transformed fibroblasts, *Br J Cancer* 92 (2005) 513-21. <https://doi.org/10.1038/sj.bjc.6602300>.
- [27] H. Piotrowska, K. Myszkowski, J. Abraszek, et al., DMU-212 inhibits tumor growth in xenograft model of human ovarian cancer, *Biomed Pharmacother* 68 (2014) 397-400. <https://doi.org/10.1016/j.biopha.2014.02.001>.
- [28] M. Cichocki, W. Baer-Dubowska, M. Wierzbowski, et al., 3,4,5,4'-trans-tetramethoxystilbene (DMU-212) modulates the activation of NF- κ B, AP-1, and STAT3 transcription factors in rat liver carcinogenesis induced by initiation-promotion regimen, *Mol Cell Biochem* 391 (2014) 27-35. <https://doi.org/10.1007/s11010-014-1983-9>.
- [29] X.P. Zhao, X.L. Zheng, M. Huang, et al., DMU-212 against EGFR-mutant non-small cell lung cancer via AMPK/PI3K/Erk signaling pathway, *Heliyon* 9 (2023) e15812. <https://doi.org/10.1016/j.heliyon.2023.e15812>.

- [30] H. Piotrowska, M. Kujawska, M. Nowicki, et al., Effect of resveratrol analogue, DMU-212, on antioxidant status and apoptosis-related genes in rat model of hepatocarcinogenesis, *Hum Exp Toxicol* 36 (2017) 160-175. <https://doi.org/10.1177/0960327116641734>.
- [31] H. Piotrowska, K. Myszkowski, R. Amarowicz, et al., Different susceptibility of colon cancer DLD-1 and LOVO cell lines to apoptosis induced by DMU-212, a synthetic resveratrol analogue, *Toxicol In Vitro* 27 (2013) 2127-34. <https://doi.org/10.1016/j.tiv.2013.09.012>.
- [32] J.A. Raygoza Garay, W. Turpin, S.H. Lee, et al., Gut Microbiome Composition Is Associated With Future Onset of Crohn's Disease in Healthy First-Degree Relatives, *Gastroenterology* 165 (2023) 670-681. <https://doi.org/10.1053/j.gastro.2023.05.032>.
- [33] X. Kang, C. Liu, Y. Ding, et al., *Roseburia intestinalis* generated butyrate boosts anti-PD-1 efficacy in colorectal cancer by activating cytotoxic CD8(+) T cells, *Gut* 72 (2023) 2112-2122. <https://doi.org/10.1136/gutjnl-2023-330291>.
- [34] W. Gao, X. Gao, L. Zhu, et al., Multimodal metagenomic analysis reveals microbial single nucleotide variants as superior biomarkers for early detection of colorectal cancer, *Gut Microbes* 15 (2023) 2245562. <https://doi.org/10.1080/19490976.2023.2245562>.
- [35] G.G. Yegutkin, D. Boison, ATP and Adenosine Metabolism in Cancer: Exploitation for Therapeutic Gain, *Pharmacol Rev* 74 (2022) 797-822. <https://doi.org/10.1124/pharmrev.121.000528>.
- [36] M. Li, X. Wu, Z. Guo, et al., Lactiplantibacillus plantarum enables blood urate control in mice through degradation of nucleosides in gastrointestinal tract, *Microbiome* 11 (2023) 153. <https://doi.org/10.1186/s40168-023-01605-y>.
- [37] C. Xia, S. Yin, K.K.W. To, et al., CD39/CD73/A2AR pathway and cancer immunotherapy, *Mol Cancer* 22 (2023) 44. <https://doi.org/10.1186/s12943-023-01733-x>.
- [38] J. Linden, Adenosine metabolism and cancer. Focus on "Adenosine downregulates DPPIV on HT-29 colon cancer cells by stimulating protein tyrosine phosphatases and reducing ERK1/2 activity via a novel pathway", *Am J Physiol Cell Physiol* 291 (2006) C405-6. <https://doi.org/10.1152/ajpcell.00242.2006>.
- [39] B. Allard, D. Allard, L. Buisseret, et al., The adenosine pathway in immuno-oncology, *Nat Rev Clin Oncol* 17 (2020) 611-629. <https://doi.org/10.1038/s41571-020-0382-2>.
- [40] L. Chen, M. Alabdullah, K. Mahnke, Adenosine, bridging chronic inflammation and tumor growth, *Front Immunol* 14 (2023) 1258637. <https://doi.org/10.3389/fimmu.2023.1258637>.
- [41] Y. Miao, L. Cui, Z. Chen, et al., Gene expression profiling of DMU-212-induced apoptosis and anti-angiogenesis in vascular endothelial cells, *Pharm Biol* 54 (2016) 660-6. <https://doi.org/10.3109/13880209.2015.1071414>.
- [42] L.K. Chen, P.F. Qiang, Q.P. Xu, et al., Trans-3,4,5,4'-tetramethoxystilbene, a resveratrol analog, potently inhibits angiogenesis in vitro and in vivo, *Acta Pharmacol Sin* 34 (2013) 1174-82. <https://doi.org/10.1038/aps.2013.60>.
- [43] S. Che, B. Qin, K. Wu, et al., EGCG drives gut microbial remodeling-induced epithelial GPR43 activation to lessen Th1 polarization in colitis, *Redox Biol* 75 (2024) 103291. <https://doi.org/10.1016/j.redox.2024.103291>.
- [44] H. Zhou, Y. Zhuang, Y. Liang, et al., Curcumin exerts anti-tumor activity in colorectal cancer via gut microbiota-mediated CD8(+) T Cell tumor infiltration and ferroptosis, *Food Funct* 16 (2025) 3671-3693. <https://doi.org/10.1039/d4fo04045g>.