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Microbiota from young mice counteracts susceptibility to age-related gout through modulating butyric acid levels in aged mice

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eLife Assessment

This is an **important** study showing that age-related gut microbiota modulate uric acid metabolism through the NLRP3 inflammasome pathway and thereby regulate susceptibility to age-related gout. Several experimental approaches (mechanistic insights) and methods (data quality) remain **incomplete**. This paper should be of interest to researchers working on gout and microbiota.

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Abstract

Gout is a prevalent form of inflammatory arthritis that occurs due to high levels of uric acid in the blood leading to the formation of urate crystals in and around the joints, particularly affecting the elderly. Recent research has provided evidence of distinct differences in the gut microbiota of patients with gout and hyperuricemia when compared to healthy individuals. However, the link between gut microbiota and age-related gout remained underexplored. Our study found that gut microbiota plays a crucial role in determining susceptibility to age-related gout. Specifically, we observed that age-related gut microbiota regulated the activation of the NLRP3 inflammasome pathway and modulated uric acid metabolism. More scrutiny highlighted the positive impact of "younger" microbiota on the gut microbiota structure of old or aged mice, enhancing butanoate metabolism and butyric acid content. Experimentation with butyrate supplementation indicated that butyric acid exerts a dual effect, inhibiting inflammation in acute gout and reducing serum uric acid levels. These insights emphasize the potential of gut microbiome rejuvenation in mitigating senile gout, unraveling the intricate dynamics between microbiota, aging, and gout. It potentially serves as a therapeutic target for senile gout-related conditions.

Introduction

Gout, the most common inflammatory arthritis in elderly individuals, results from the deposition of monosodium urate crystals in articular and nonarticular structures(1), particularly among individuals aged 75-84 years, and the occurrence rate in this population can reach 4%(2). The development of gout is primarily attributed to the significant risk posed by a high serum urate concentration(2). Pathological hyperuricemia is defined by a serum urate concentration exceeding 408 μmol/L [6.8 mg/dL], which forms monosodium urate crystals in vitro at physiological pH and temperature(3). The reason why the elderly population commonly experiences gout is complicated by the fact that the population is also ageing, and the treatment of this disease is often intricate due to the presence of comorbidities and medications prescribed for concurrent conditions(2). Although the basic principles for the prevention and treatment for gout remain the same across different age groups, elderly individuals often exhibit lower tolerance for medication dosages, types, side effects, and surgical procedures due to their physiological factors. Moreover, despite the increasing severity of gout among elderly individuals, research on this issue remains scarce.

The gut microbiota plays essential roles in regulating energy and metabolism, as revealed by recent studies(4, 5), which have shown that individuals with hyperuricemia and gout exhibit dysbiosis of the gut microbiota(6, 7). Moreover, because the gut microbiota can directly participate in the metabolism of purines and uric acid(8), it may play a crucial role in gout and hyperuricemia development. Although numerous studies have examined the relationship between the microbiome and early life stages, the impact of the microbiome's on ageing and frailty in later life needs to be explored. Furthermore, studies have shown that the structure of the gut microbiota undergoes a gradual "ageing" process with advancing age, and this process is characterized by a decrease in the microbial diversity(9, 10). In addition, transplantation of a young microbiota can improve central nervous system inflammation and retinal inflammation in aged mice(11), and counteract age- related behavioral deficits(12). We hypothesize that the high prevalence of gout in the elderly population may be closely related to its "ageing" gut microbiota. The association between the "ageing" gut microbiota and gout in elderly individuals has not been reported. Hence, we conducted a study to elucidate the influence of the ageing gut microbiota on the occurrence and progression of gout in elderly individuals.

In this study, we first tested the sensitivity to monosodium urate (MSU) in different age groups and conducted a microbiota clearance (Abx) on mice of different age groups to assess their sensitivity to MSU again. At the same time, we also tested their serum uric acid levels. We found that sensitivity to MSU increased with age, but changed after clearing the gut microbiota in terms of sensitivity to MSU and serum uric acid levels. Then we performed cross-age fecal microbiota transplantation (FMT) and subsequently stimulated the mice with MSU with the aim of how mice belonging to different age groups exhibit sensitivity to MSU after undergoing FMT. Because hyperuricemia is a necessary physiological factor for gout, we also evaluated the expression levels of uric acid-producing enzymes and uric acid transport proteins in the mice. Surprisingly, transplantation of the gut microbiota from aged mice into young mice, significantly increased their sensitivity to MSU. Conversely, the transplantation of the gut microbiota from young mice into aged mice, significantly decreased their sensitivity to MSU. These findings suggest that the gut microbiota of older individuals plays a promoting role in the occurrence and progression of gout. Moreover, an analysis of the serum uric acid levels of the mice after cross-age FMT yielded similar results. To investigate the underlying mechanisms of how gut microbiota influences gout and hyperuricemia, we performed 16S rDNA sequencing and untargeted metabolomics analysis of fecal samples. We then, observed a significant increase in the abundance of Bifidobacterium and Akkermansia in the gut microbiota of young mice and old or aged mice after transplantation of the gut microbiota from young mice. To further investigate the potential biological pathways affected

by the gut microbiota, we performed functional metagenomic analysis using Tax4FUN software and found that butanoate metabolism was more robust in young mice than in aged mice. Furthermore, we also observed that transplantation of the gut microbiota from young mice to aged mice enhanced the butanoate metabolism of the recipient mice. Due to the limitations of untargeted metabolomics, we did not observe any differences in the levels of butyric acid among the different groups. However, the pathway data obtained by fecal untargeted metabolomics also yielded similar results. Based on the above- described results, we hypothesize that butyrate may play a significant role in these processes. Excitingly, the results from a short-chain fatty acid (SCFA) analysis support our hypothesis. Furthermore, a supplementation experiment using butyrate revealed, that the results aligned well with the cross-age FMT findings, which suggests that transplantation of the gut microbiota from young mice into aged mice can effectively prevent gout and hyperuricemia, and that butyrate is likely the critical factor playing a crucial role. Our research findings demonstrate the potential of young gut microbiota in preventing gout in elderly individuals and provide new insights and perspectives for the prevention and treatment of gout in elderly individuals.

Results

Gout susceptibility increases with age, related to gut microbiota

In this study, we used the male C57BL/6 mice belonging to three age groups: young (~3 months), old (~18 months) and aged (~24-months) mice (**Fig. 1a**). To investigate the impact of age on gout, we used a mouse model in which subcutaneous injections of MSU crystals were administered to the dorsal aspect of the hind paws (13–15). The result showed that the old group exhibited a significant increase in footpad swelling compared with the young group, and the levels of IL-1β was significantly elevated in the Old and Aged groups (**Fig. 1b, c**). The levels of IL-6 was significantly elevated in the Old and Aged groups, after MSU stimulated. While no significant difference in foot tissue TNF-α concentration was found between Aged and Young groups, an significant upward trend was observed in Old group(supplementary material 4 part one). A prominent correlation was found between the occurrence of gout and the concentration of serum uric acid, which exhibits an upwards trend with advancing age(2). Thus, we also measured the serum uric acid level, we found uric acid levels significantly increase with age (**Fig. 1d**). There are reports indicating a close relationship between gut microbiota and gout(6). Therefore, we simultaneously tested the gut microbiota of mice at different ages. The results showed that as age increases, the ASVs (Amplicon Sequence Variants) showed a declining trend (**Fig. 1e**). Moreover, the principal coordinates analysis (PCoA) results revealed distinctive differences in the phylogenetic community structures between these groups (**Fig. 1f**). Then we carried out antibiotics (ABX) cocktail on mice of different age groups, found changes in the footpad swelling, IL-1β and discovered no differences in the level of serum uric acid (**Fig. 1g-i**). There was no significant difference in the content of IL-1β in the foot tissues of mice of all ages without MSU stimulation, whether treated with antibiotics or untreated (supplementary material 4 part two). These data highlight indicates that aging-associated changes in the gut microbiota exacerbate gout attacks.

Aged-to-young FMT worsens acute gout, whereas young-to-aged FMT reduced this disease

Based on the above results, we conducted fecal microbiota exchanges between male C57BL/6 mice also belonging to three age groups: young (~3 months), old (~18 months), and aged (~24-months) mice. The experimental design and timeline are presented in **Figure 2a**. To investigate the impact of cross-age FMT on gout, we used a gout mouse model as aforementioned. The results of **supplementary figure 1a** showed that young mice transplanted with fecal microbiota from

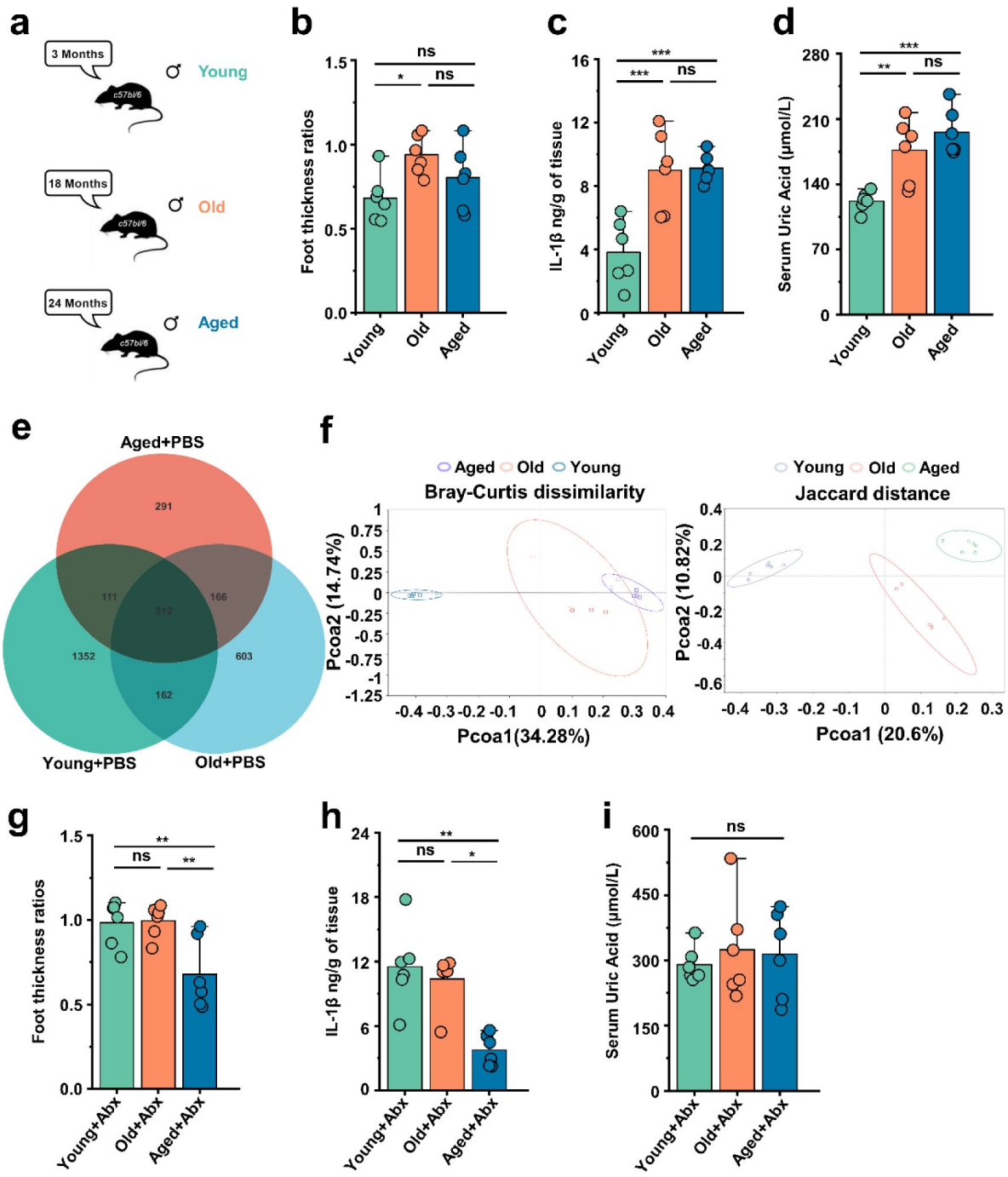

Fig 1

Gout susceptibility increases with age, related to gut microbiota

(a) Mice of different ages.
(b-d) The foot thickness ratios (b), foot tissue's IL-1β concentrations (c) and serum concentrations of uric acid of three different age ranges groups (d) were tested after MSU administration (n=6).
(e-f) The three different age ranges groups' ASVs (Amplicon Sequence Variants)
(e) and PcoA analysis (using Bray-Curtis dissimilarity and Jaccard distance) (f). (g-i) The foot thickness ratios (g), foot tissue's IL-1β concentrations (h) and serum concentrations of uric acid of three different age ranges groups (i) (treated with antibiotics (ABX) cocktail) were tested after MSU administration (n=6).
Values are presented as the mean ± SEM. Differences were assessed by One-Way ANOVA and denoted as follows: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, "ns" indicates no significant difference between groups.

mice in the old or aged group (Young+Old or Young+Aged) exhibited a significant increase in footpad swelling compared with the control group (Young+PBS). However, no significant difference in footpad swelling was found between old or aged mice transplanted with fecal microbiota from mice in the young groups (Old+Young and Aged+Young) and the control groups (Old+PBS and Aged+PBS) (**supplementary figure 1a**). Subsequently, we conducted a comparative analysis of haematoxylin & eosin (H&E)-stained tissue sections (scar, 1000μm), derived from mice with cross-age FMT and MSU-induced acute gout. We found that the Young+Old and Young+Aged group showed more inflammatory cell infiltration in the subcutaneous soft tissues than the Young+PBS group, whereas the Old+Young and Aged+Young groups showed less inflammatory cell infiltration (**Fig. 2b**). The inflammatory factors in the foot tissue of the mice with cross-age FMT were then examined. The levels of IL-1β, IL-6, and TNFα were significantly elevated in the Young+Aged group and significantly lower in the Aged+Young group compared with the Aged+PBS group (**Fig. 2c-e**). Meanwhile, to investigate the impact of cross-age FMT on MSU-induced inflammation, we utilized an animal model of C57BL/6 mice administered an intraperitoneal injection of 2.5 mg of MSU (16). Six hours after MSU intraperitoneal injection, we washed the mouse peritoneal cavity with sterile PBS and collected the peritoneal fluid and peritoneal cells separately. Simultaneously, we also examined the levels of inflammatory cytokines in mouse serum. Consistent with the acute gout model, the Young+Old or Young+Aged group exhibited more pronounced activation of IL- 1β, IL-6, and TNF-α in peritoneal fluid (**Fig. 2f-h**) and serum (**Supplementary figure 1b-c**). Although no differences in the IL-6 levels of serum and peritoneal fluid was found between the Old+PBS and Old+Young groups or between the Aged+Young and Aged+PBS groups (**supplementary figure 1c** and **Fig. 2g**), a significant reduction in IL-1β and TNF-α was observed (**supplementary figure 1b**, d and **Fig. 2f, h**). These findings suggest that the increased susceptibility to gout in the elderly may closely related to “aging” gut microbiota.

“Younger” gut microbiota suppresses NLRP3 inflammasome pathway, “aging” gut microbiota promotes

The pathogenesis of acute gout has been primarily linked to the activation of proinflammatory pathways, notably NLRP3, and this activation instigates a surge in the production of the inflammatory cytokines, which further underscores their pivotal roles in the progression of the disease’s (17). We primarily examined proteins of foot tissue and peritoneal cells associated with the NLRP3 inflammasome pathway. Interestingly, we discovered that the Young+Aged group demonstrated more pronounced activation of NLRP3, Pro- Caspase-1, Caspase-1, and IL-1β compared with the control group (**Fig. 3a-b**). However, the Old+Young and Aged+Young groups showed lower protein levels of Caspase-1 and IL-1β compared with the control group (**Fig. 3c-f**), which suggests that the gut microbiota of elderly mice may make them more sensitive to MSU stimulation, whereas the gut microbiota of young mice can effectively resist the MSU stimulation, and inhibit Caspase-1 cleavage, and IL-1β secretion. Meanwhile, the NLRP3 inflammasome pathway also was investigated in peritoneal cells, and similar results were obtained in the gout model. The fecal microbiota from mice in the old or aged group exacerbated the activation of Pro-Caspase -1, Caspase-1, Pro-IL-1β, and IL-1β in peritoneal cells, increasing the production of IL-1β (**Supplementary figure 2a-b**). In contrast, the fecal microbiota from mice in the young group inhibited the cleavage of Caspase-1 and the secretion of IL-1β (**Supplementary figure 2c-f**).

Beneficial effects of FMT from young to aged mice on uric acid metabolism

Serum samples were collected from the the different groups, and their serum uric acid levels were initially assessed. Surprisingly, we observed an elevation in the average serum uric acid levels in the Young+Old or Young+Aged group, whereas the Old+Young or Aged+Young group exhibited decreases in the serum uric acid levels compared with the same-age control group (**Fig. 4a**). Our

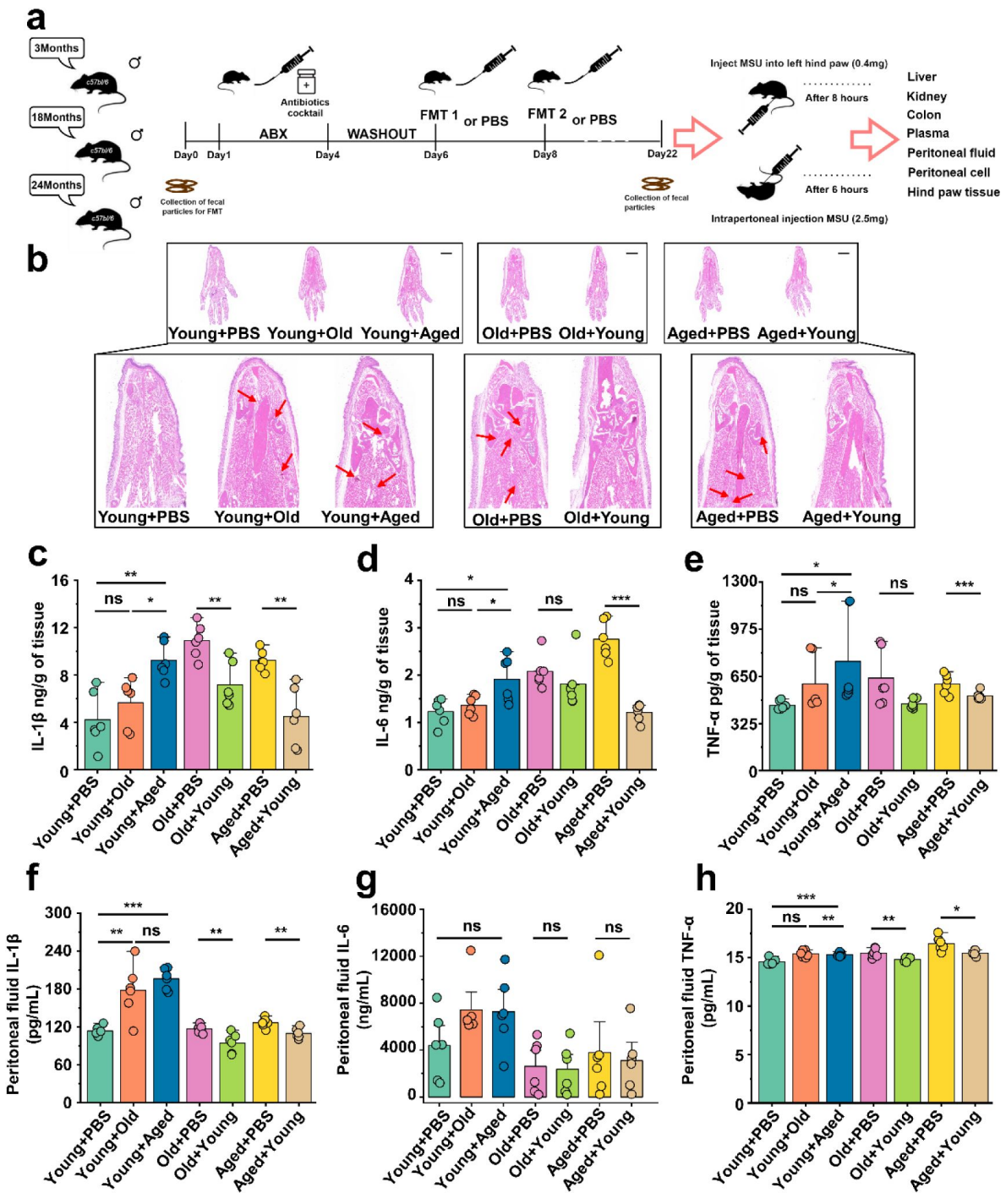

Fig 2

Aged-to-young FMT worsens acute gout, whereas young-to-aged FMT reduced this disease.

(a) Overall experimental design and timeline for experiments.
(b) Representative H&E-stained images of left foot tissues. Scale bars 1000 μm and 3x magnification.
(c-e) Foot tissue inflammatory parameters, including IL-1β (c), IL-6 (d) and TNF-α (e) concentrations, from the indicated mice are shown (n=6).
(f-h) The peritoneal fluid concentrations of IL-1β (f), IL-6 (g) and TNF-α (h) inflammatory parameters were measured in the indicated mice(n=6).
Values are presented as the mean ± SEM. Differences were assessed by t-test or One-Way ANOVA and denoted as follows: *p < 0.05, **p < 0.01, and ***p < 0.001, “ns” indicates no significant difference between groups.

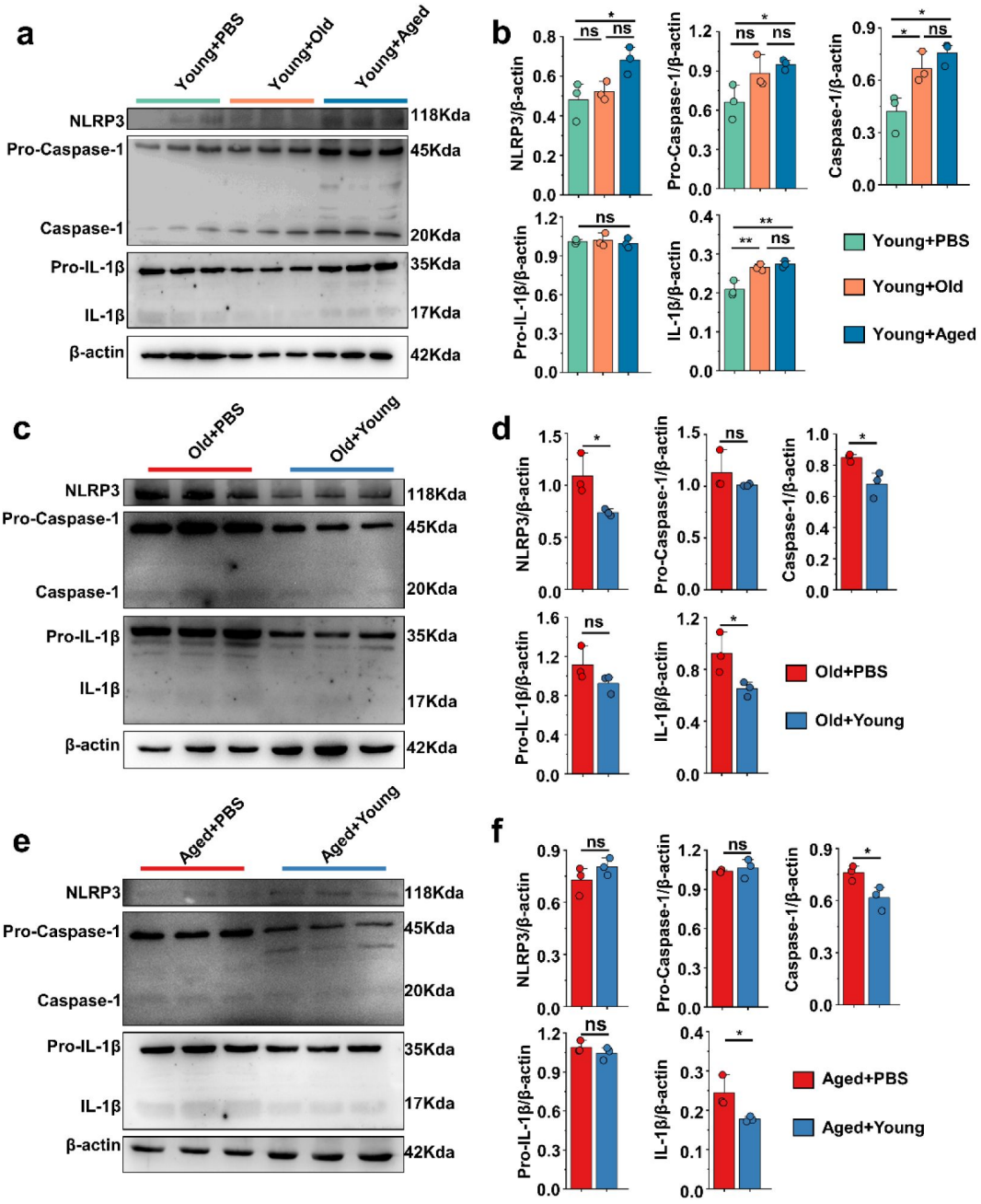

Fig 3

"Younger" gut microbiota suppresses NLRP3 inflammasome pathway, "aging" gut microbiota promotes

(a-b) Representative western blot images and band density (Young+PBS, Young+Old and Young+Aged) of foot tissue NLRP3 pathways proteins (n=3).
(c-d) Representative western blot images and band density (Old+PBS and Old+Young) of foot tissue NLRP3 pathways proteins (n=3).
(e-f) Representative western blot images and band density (Aged+PBS and Aged+Young) of foot tissue NLRP3 pathways proteins (n=3).
Values are presented as the mean ± SEM. Differences were assessed by t-test or One-Way ANOVA and denoted as follows: *p < 0.05, **p < 0.01, and ***p < 0.001, "ns" indicates no significant difference between groups.

study, showed that compared with those of the same-age control group, the serum levels of AST and ALT were elevated in the Young+Old and Young+Aged groups, although the differences were not significant, whereas the Aged+Young groups exhibited modest decreases in these levels (**Supplementary figure 3a-b**). Furthermore, similar trends were found for indicators (Crea and BUN) of renal function (**Supplementary figure 3c-d**). Hyperuricemia is attributed to increased uric acid synthesis and decreased uric acid excretion in the body. We first measured the activity of enzymes involved in uric acid synthesis, namely adenosine deaminase (ADA), guanine deaminase (GDA), and xanthine dehydrogenase (XOD), in the liver and that of (XOD) in the kidney. The activity of ADA, GDA, and XOD in the liver, and the activity of XOD in the kidney of the Young+Old and Young+Aged groups did not significantly differ from those of the same-age control groups (**Fig. 4b-e**). The fecal microbiota from mice in the young group induced a notable decrease in the activity of enzymes related to uric acid synthesis, and the most prominent reductions were found for ADA and XOD activity in the liver and XOD activity in the kidney (**Fig. 4b, d and e**). We then examined the mRNA levels of relevant proteins involved in uric acid transport. We initially measured the renal injury marker KIM-1, and observed that the fecal microbiota from mice in the young group contributed to attenuating the mRNA expression levels of this marker compared with the same-age control group (**Fig. 4f**). The urate transporter URAT1, which is responsible for uric acid reabsorption, exhibited a similar trend (**Fig. 4g**). However, cross-age FMT did not significantly impact the mRNA expression of another uric acid reabsorption protein, GLUT9 (**Fig. 4h**). The Young+Aged group showed lower mRNA expression levels of the uric acid excretion proteins OAT1 and OAT3 compared with the Young+PBS group (**Fig. 4i-j**). Although the mRNA expression levels of OAT1 and OAT3 did not show significant differences, a significant increasing trend was found for the other uric acid excretion protein, ABCG2, in aged mice transplanted with fecal microbiota from mice in the young group exhibited a significant increasing trend (**Fig. 4k**).

Because ABCG2 is also expressed in the intestine, we examined its mRNA expression levels in the colon and found that the fecal microbiota from mice in the aged group could inhibit its expression (**supplementary figure 3e**). Similar trends were found for the mRNA expression levels of mice in ZO-1 and JAMA in the colon of the Young+Aged group (**Supplementary figure 3g-h**). No significant alterations in the colonic Occludin mRNA expression levels were observed among all the groups (**supplementary figure 3f**). However, the mRNA expression levels of JAMA in the Old+Young and Aged+Young groups were significantly different from those in the same-age control groups (**supplementary figure 3h**). Our study, showed that compared with those of the same-age control group, the colon levels of ZO-1 and Occludin protein were significantly decreases in the Young+Old and Young+Aged groups, whereas the Old+Young and Aged+Young groups exhibited modest elevated in these levels (supplementary material 4 part three).These results suggests that the fecal microbiota from mice in the old or aged group leads to insufficient uric acid excretion, whereas the fecal microbiota from mice in the young group promotes uric acid elimination, inhibits reabsorption, and may contribute to the integrity of the intestinal barrier structure and the maintenance of normal physiological function.

Modifications in the gut microbiota composition following cross-age FMT

To characterize the age-related alterations in the gut microbiota and assess changes following transplantation, we conducted 16S rDNA amplicon sequencing of all the mice at the endpoint. We focused on investigating the gut microbiota of mice after cross-age FMT. We detected the relative abundance of the top 10 phylum levels in each group (**Fig. 5a**) and found no significant differences in the abundance of Bacteroidetes and Firmicutes in the transplanted groups compared with that of their respective control groups (**Supplementary figure 4a-b**). No significant differences were found in the the ratio of Firmicutes to Bacteroidetes (**supplementary figure 4c**). The species richness, evenness, and rarity are fundamental components of biodiversity and are commonly quantified using indices such as Chao1, observed otus, Shannon, and Simpson indices. Although no significant differences in the Simpson index were found among

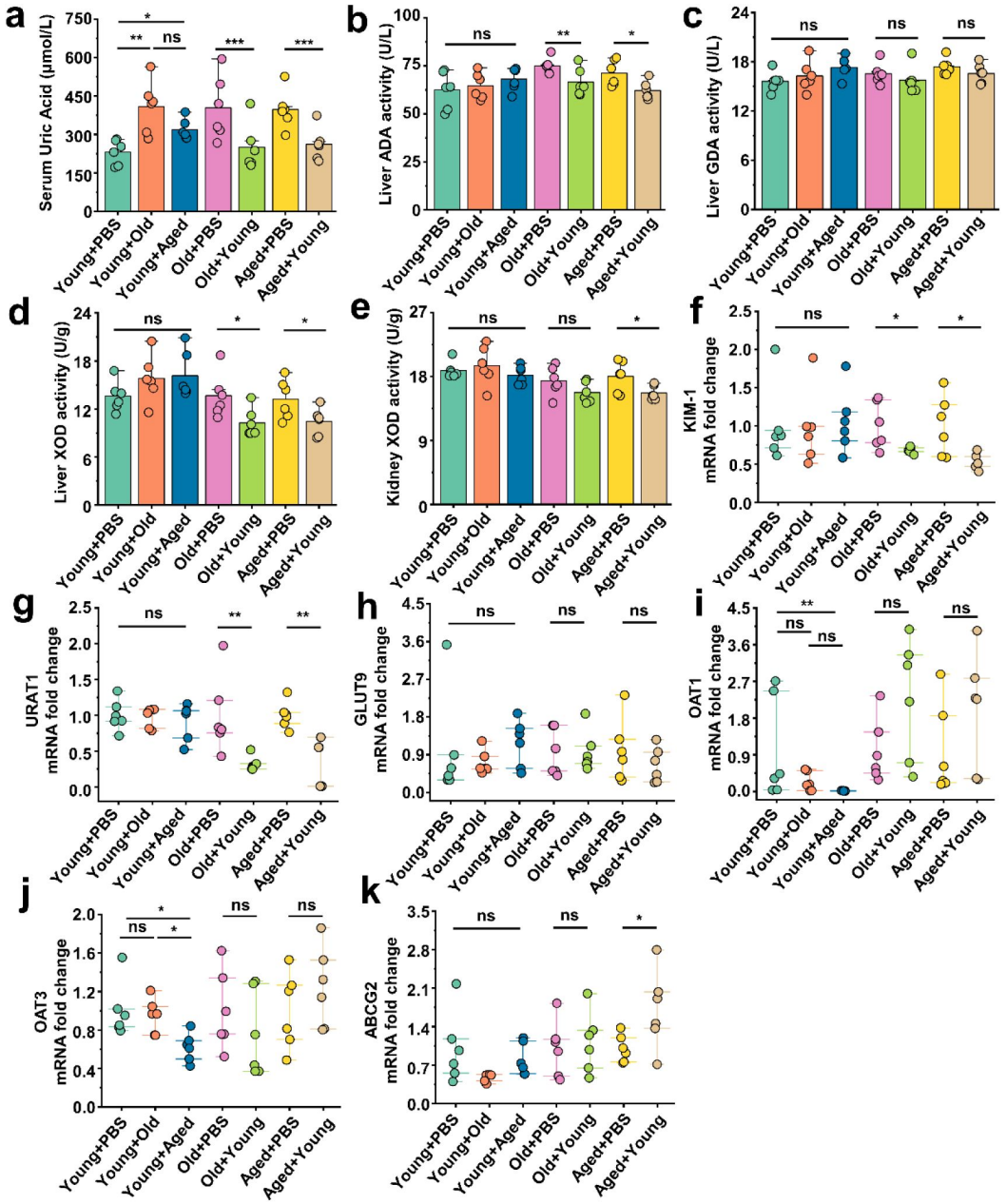

Fig 4

Beneficial effects of FMT from young to aged mice on uric acid metabolism.

(a) All groups' serum concentrations of uric acid (n=6).
(b-d) The activity of uric acid-producing enzymes of liver in the cross-age fecal microbiota transplantation group and its control group (n=6), including ADA (b), GDA (c) and XOD (d).
(e) The activity of XOD of kidney in the cross-age fecal microbiota transplantation group and its control group (n=6).
(f) Relative kidney injury molecule-1 (KIM-1) expression in the indicated groups by qPCR (n = 6).
(g and h) Relative renal genes for uric acid reabsorption expression in the indicated groups by qPCR (n = 6), including URAT1 (g) and GLUT9 (h).
(i-k) Relative renal genes for uric acid excretion expression in the indicated groups by qPCR (n = 6), including OAT1 (i), OAT3 (j) and ABCG2 (k).
Values are presented as the mean ± SEM. Differences were assessed by t-test or One-Way ANOVA and denoted as follows: *p < 0.05, **p < 0.01, and ***p < 0.001, "ns" indicates no significant difference between groups.

these groups (**supplementary figure 4e**), and the indices of the Old+Young and Aged+Young groups were not significantly different from those of the Old+PBS and Aged+PBS groups, respectively, the Chao 1 (**Fig. 5b**), observed_otus (**Fig. 5c**), and Shannon (**supplementary figure 4d**) indices of the Young+Aged group were significantly lower than those of the Young+PBS group. These findings are in line with other studies(18), indicating declines in the richness and diversity of the gut microbiota during aging. Moreover, the principal coordinates analysis (PCoA) results revealed distinctive differences in the phylogenetic community structures between these groups. We showed that the Young+Old and Young+Aged groups tended to be closer to the Old+PBS and Aged+PBS groups, and the Old+Young and Aged+Young groups tended to be closer to the Young+PBS group (**Fig. 5d**). We then compared the abundance of the top 15 genus level among all the groups (**supplementary figure 4f**). A Metastats analysis, found that the abundance of Bifidobacterium significantly differed between the Young+PBS group and the Young+Old and Young+Aged groups, and that the abundance of Lachnoclostridium showed an increasing trend in the Old+Young and Aged+Young groups (**Fig. 5e**). The ternary plot showed that Akkermansia appears to be the dominant species in the Young+PBS, Old+Young and Aged+Young group (**Fig. 5f**). According to previous reports(19–22) on Bifidobacterium and Akkermansia, we hypothesize that these genera or their metabolites may play a key role in resistance to gout and hyperuricemia. Based on recent research findings(23) and a KEGG analysis of bacterial community functions, we discovered that butanoate metabolism was more robust in the Young+PBS group than in the Old+PBS and Aged+PBS groups (**Table 2a-b**). However, no similar phenomenon was observed in the Young+Old and Young+Aged groups compared with the Young+PBS group (**Table 2c-d**). More interestingly, the Old+Young and Aged+Young groups showed stronger butanoate metabolism than the Old+PBS and Aged+PBS groups, respectively (**Table 2e-f**). Considering the results from the analysis modifications in the gut microbiota composition after cross-age FMT, both Bifidobacterium and Akkermansia metabolites include SCFAs. These findings indicate that butyric acid derived from the young microbiome may be the critical element responsible for controlling gout. Numerous studies have reported the beneficial effects of butyric acid, but further research on the relationship between butyric acid and gout is needed.

Changes in fecal microbiota metabolism and pathways after cross-age FMT

We also performed an untargeted metabolomics analysis of samples collected after cross-age FMT to investigate the research question. Chemical classification of the metabolites identified in this study was performed, and a pie chart was generated to reflect the distribution and numbers of metabolites in each category. The pie chart for Class I metabolite classification and KEGG pathway annotation is shown in **supplementary figure 5a**. Among the 42 samples analysed in this study, a total of 1009 metabolites were identified in the positive ion mode, and 522 metabolites were identified in the negative ion mode among the 42 samples analyzed in this project. The volcano plot provides an overview of the distribution of differentially expressed metabolites (**supplementary figure 5b**). We also used the KEGG database for metabolic analysis and network research of the identified biological entities. The enrichment results are based on KEGG pathway units, and hypergeometric testing was performed to identify pathways enriched in differentially abundant metabolites compared with all specified metabolite backgrounds. Through pathway enrichment, we discerned and elucidated the principal biochemical metabolic pathways and signal transduction pathways in which the differentialy abundant metabolites actively participate. The metabolites showing differential abundance between the Young+PBS and Old+PBS groups were enriched in the secondary bile acid biosynthesis pathway (**supplementary figure 6a**). Interestingly, the metabolites showing differential abundance between the Young+PBS and Aged+PBS groups were also enriched in the butanoate metabolism pathway (**supplementary figure 6b**). The same phenomenon was found from the comparisons of the Young+PBS and Young+Old groups, Young+PBS and Young+Aged groups, Old+PBS and Old+Young groups, and

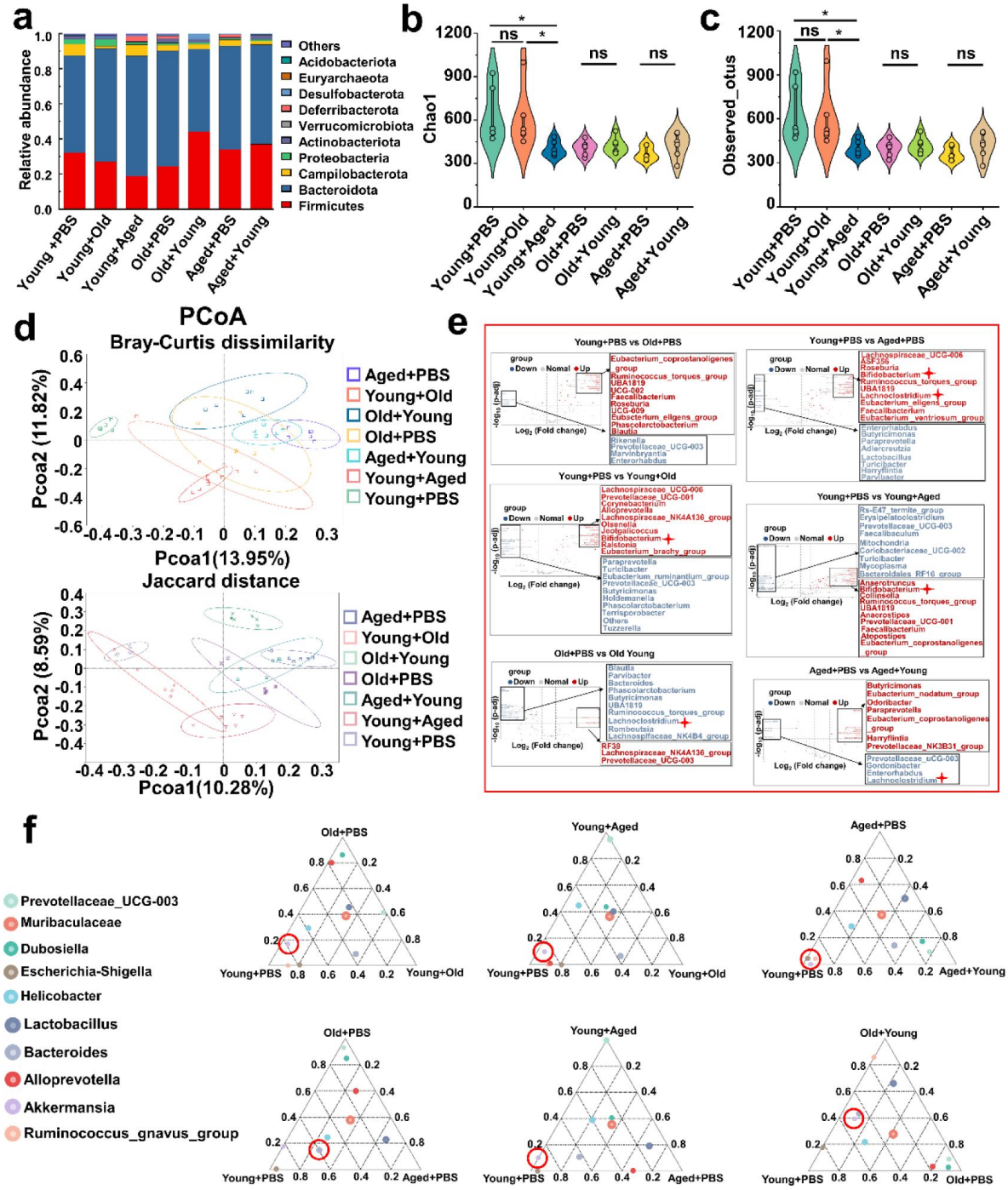

Fig 5

Modifications in the gut microbiota composition following cross-age FMT.

(a) Bacterial composition at the phylum levels (top 10) of the indicated groups (n=6).
(b-c) Alpha diversity indices including Chao1 (b) and observed_otus (c) index in the indicated groups (n=6).
(d) β-diversity difference among the seven groups analyzed by the PcoA using Bray-Curtis dissimilarity and Jaccard distance.
(e) Volcano Plot of inter-group significance analysis using metastat (t-test, $p < 0.05$).
(f) The ternary plot of three different groups among the seven groups at genus levels (top 10).

Aged+Young and Aged+PBS groups: the metabolites identified as showing differential abundance between the groups metabolites were enriched in the butanoate metabolism pathway (**Fig. 6a-d**). Furthermore, we found that the metabolites showing differential abundance between the Old+PBS and Old+Young groups and between the Aged+Young and Aged+PBS groups were enriched in the secondary bile acid biosynthesis pathway (**Fig. 6c-d**). Due to the limitations of untargeted metabolomics, we did not observe any differences in butyric acid. However, the results were consistent with the predicted functional capabilities of microbial communities and corresponded to butanoate metabolism. Based on the abovementioned results, butyrate may play an important role.

Butyrate supplementation inhibits gout and MSU-induced peritonitis

Based on the predictions of the functional capabilities of microbial communities and untargeted metabolomics results, we hypothesized that butyrate might play a key role. Because butyric acid is a short-chain fatty acid, we measured the levels of SCFAs (nk/g) in the Young+PBS, Aged+PBS, and Aged+Young groups. Subsequently, we observed that the butyric acid content in the faeces of Young+PBS group was significantly higher than that in the Aged+PBS group. Simultaneously, the butyric acid content in the faeces of mice in the Aged+Young was also significantly higher than that in the Aged+PBS (**Fig. 7a**). 2-Methylvalerate and 3-Methylvalerate were not detected, and 4-Methylvaleric was detected in only a subset of samples, thus, these results are not presented. The difference analysis of the detected short chain fatty acids is shown in **Supplementary figure 7a-h**. Therefore, mice were supplemented with butyrate for 14 days and then subjected to intraperitoneal injections of MSU into the dorsal aspect of the hind paws to observe its anti-inflammatory effects in acute gout. The experimental results showed that butyrate exerted significant anti-inflammatory effects in the acute gout model with inflammation induced by intraperitoneal injection of MSU. Mice with gout model, supplemented with butyrate showed significant reductions in the foot thickness ratio (**Fig. 7b**) and the levels of inflammatory factors (IL-1β, IL-6, and TNF-α) in their foot tissue (**Fig. 7c-e**). Pathological sections revealed that the foot tissue of mice supplemented with butyrate exhibited less inflammatory cell infiltration than that of the control group (**Fig. 7f**). Moreover, after supplementation with butyrate, mice that received an intraperitoneal injection of MSU showed significant decreases in the levels of inflammatory factors (IL-1β, IL-6, and TNF-α) in both serum (**Supplementary figure 7i-k**) and peritoneal fluid (**Fig. 7g-i**) compared with those in the control group. Subsequently, we also analyzed the expression of proteins associated with the NLRP3 inflammasome pathway. Although no significant change in the expression of NLRP3 after supplementation with butyrate was observed in both the gout and peritonitis models, supplementation with butyrate effectively suppressed the production of Caspase-1 and IL-1β (**Fig. 7j and supplementary figure 7l**). The anti-inflammatory effects of butyrate have been widely reported, but limited studies have investigated its specific on gout. Here we demonstrate that butyrate effectively inhibits inflammation induced by MSU stimulation.

Serum uric acid-lowering effect of butyrate in old or aged mice

This has been proved in **figure 1d**, that the serum uric acid levels in mice of different age groups exhibited increase in their serum uric acid levels with increase age. There is scarce research on the impact of butyrate on serum uric acid levels, and the effects of butyrate on hepatic uric acid production or renal uric acid excretion have not been investigated. Additionally, the impact of butyrate on the serum uric acid levels in aged mice remains unexplored. Aged mice were supplemented with butyrate for 14 days, and we found that butyrate supplementation significantly reduced the serum uric acid levels in 18-month- old and 24-month-old mice (**Fig. 8a**). In this regard, we also assessed the activity of enzymes implicated in uric acid synthesis, namely, ADA, GDA, and XOD in the liver, as well as that of XOD in the kidney. In addition to

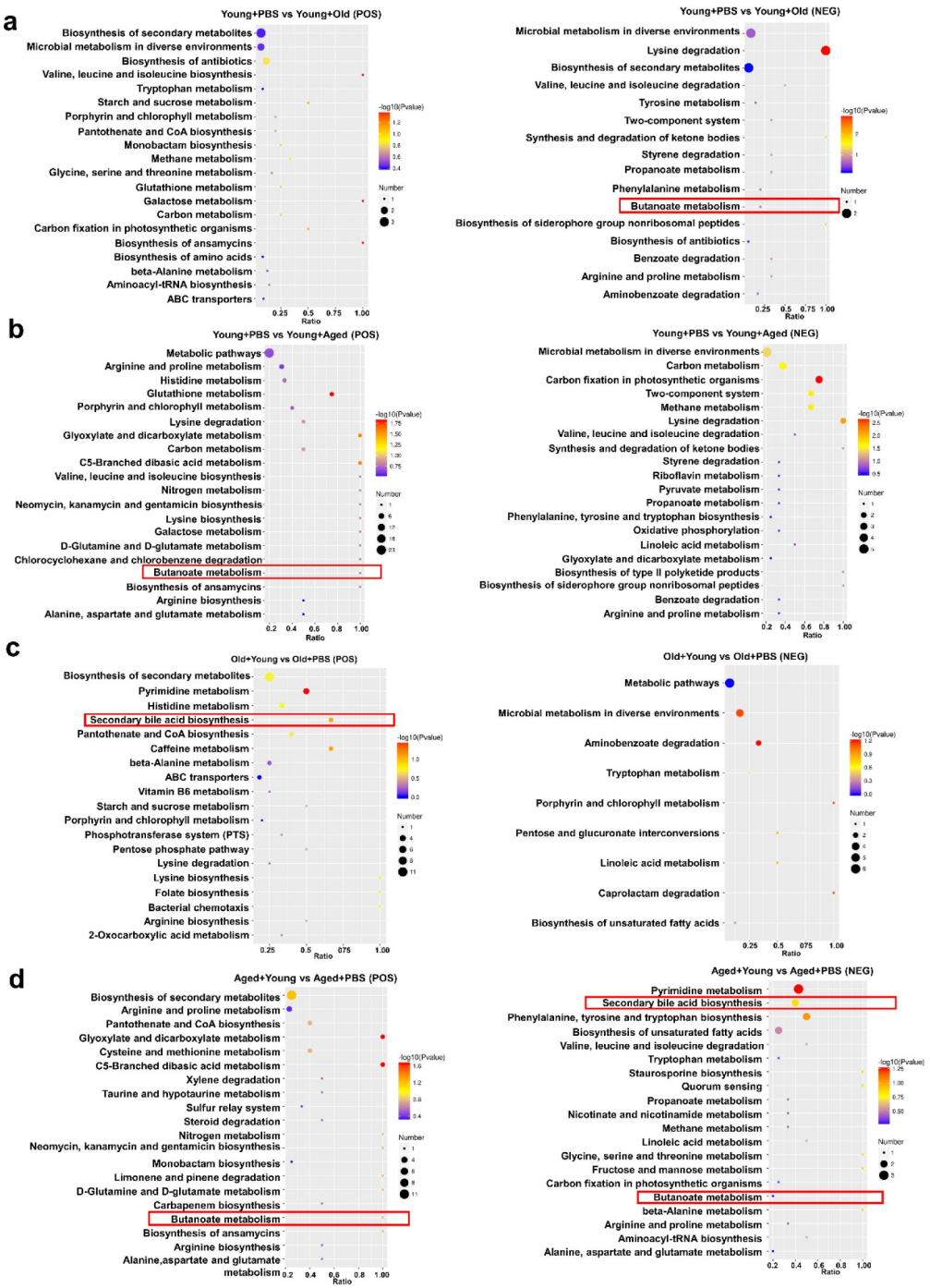

Fig 6

Changes in fecal microbiota metabolism and pathways after cross-age FMT.

(a) Comparison of differential KEGG enrichment bubble plots between Young+PBS and Young+Old.
(b) Comparison of differential KEGG enrichment bubble plots between Young+PBS and Young+Aged.
(c) Comparison of differential KEGG enrichment bubble plots between Old+PBS and Old+Young.
(d) Comparison of differential KEGG enrichment bubble plots between Aged+PBS and Aged+Young.
The enrichment analysis was performed at the KEGG Pathway level using a hypergeometric test, as shown in the figure below. The pathways that were significantly enriched in the differential metabolites compared to the background of all identified metabolites. Pathway enrichment analysis enables us to determine the major biochemical metabolic pathways and signaling transduction pathways that are implicated by the differential metabolites.

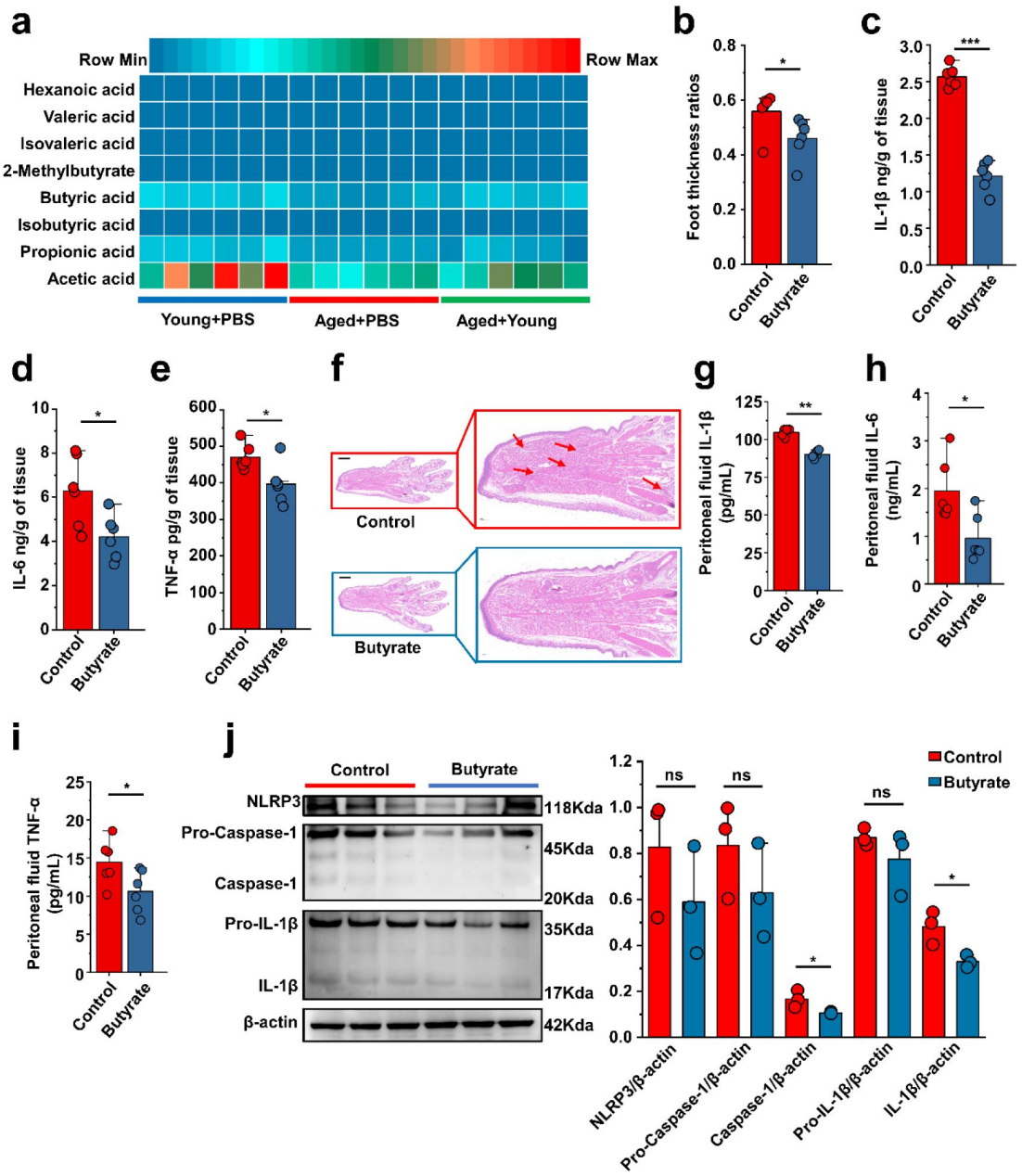

Fig 7

Butyrate supplementation inhibits gout and MSU-induced peritonitis.

(a) The concentration of primary short-chain fatty acids (SCFAs) in fecal samples from both young (3 months), aged (24 months) mice and Aged+Young (FMT from young to aged). Graphs were generated to illustrate the changes in individual SCFAs, with a sample size of 6 in the Young+PBS, Aged+PBS and Aged+Young group(n=6).
(b-e) The foot thickness ratios (b) were tested after MSU administration, foot tissue inflammatory parameters, including IL-1β (c), IL-6 (d) and TNF-α (e) concentrations, from the indicated mice are shown(n=6).
(f) Representative H&E-stained images of left foot tissues. Scale bars 1000 μm and 3x magnification.
(g-i) The peritoneal fluid concentrations of IL-1β (g), IL-6 (h) and TNF-α (i) inflammatory parameters were measured in the indicated mice (n=6).
(j) Representative western blot images of foot tissue NLRP3 pathways proteins and band density (n=3).
Values are presented as the mean ± SEM. Differences were assessed by t-test or One-Way ANOVA and denoted as follows: *p < 0.05, **p < 0.01, and ***p < 0.001, “ns” indicates no significant difference between groups.

identifying that butyrate can reduce the activity of ADA in 24-month-old mice (Aged) (**Fig. 8b**), and supplementation with butyrate did not have significant effects on the activities of enzymes involved in uric acid production in both 18-month-old and 24-month-old mice (**Fig. 8c-e**). Butyrate may not necessarily lower the serum uric acid levels by inhibiting uric acid production. Because butyrate might exert its influence on serum uric acid levels by affecting uric acid transport, we subsequently examined the expression of relevant transporter mRNA in mouse kidneys and the expression of the colonic ABCG2 transporter that facilitates uric acid excretion. The expression of the renal injury marker KIM-1 was significantly decreased in mice supplemented with butyrate (**Fig. 8f**). In contrast to the results obtained cross-age FMT, no significant difference in the expression of URAT1 was observed (**Fig. 8g**). However, the expression of GLUT9 was significantly decreased in 24-month-old mice after butyrate supplementation, whereas no significant difference was observed in the 18- month-old mice (**Fig. 8h**). We also found that the supplementation of old or aged mice with butyrate resulted in an increased mRNA expression level of uric acid transporters involved in uric acid excretion and significantly increased the expression of OAT1 and OAT3 (**Fig. 8i-j**). Although no significant difference in the expression of ABCG2 was detected in old mice, a significant increased in its expression was observed in aged mice after supplementation with butyrate (**Fig. 8k**). Meanwhile, the mRNA expression of ABCG2 in the colons was significantly increased in old or aged mice after supplementation with butyrate (**Fig. 8l**). Moreover, supplementation with butyrate significantly increased the mRNA expression of ZO-1, Occludin, and JAMA in the colons of mice (**Supplementary figure 8a-c**). Based on the abovementioned results, we found that supplementation with butyrate promotes uric acid excretion and improves the intestinal tight junction integrity.

Discussion

The incidence of gout and hyperuricemia continually to increases as individuals. However, research on gout among the elderly population is relatively limited. Previous studies have confirmed a close relationship between gout and the gut microbiota(24, 25), but the specific connection between the gut microbiota of older individuals and gout remains unexplored. FMT from young to old mice also reportedly alleviates age-related stroke(26)and other benefits(11, 27). And there are also studies indicating Older patients demonstrate heightened inflammatory reactions during gout attacks(28). Based on this finding, we hypothesize that the gut microbiota of different age groups may exert varying effects on gout. Therefore, we conducted cross-age FMT to investigate the interaction between the gut microbiota in different age groups and gout.

This study demonstrated that FMT from young to aged mice effectively alleviated the inflammatory response caused by MSU and improved uric acid metabolism in elderly mice, reducing the symptoms of gout. In contrast, FMT from old or aged to young mice exacerbated gout, indicating the importance of the gut microbiota composition in gout development. Activation of the NLRP3 inflammasome has been implicated in the pathogenesis of gout, leading to the production of inflammatory cytokines such as IL-1β, IL-6, and TNF-α. The beneficial effects of FMT from young to aged mice could be attributed to inhibition of the NLRP3 inflammasome pathway. A “younger” gut microbiota could suppress the activation of NLRP3, Caspase-1 and IL-1β, thereby reduce the inflammatory response in gout. Furthermore, the modulation of uric acid metabolism in old or aged mice played a role in the effects of young gut microbiota transplantation. Additionally, the improvement in the intestinal tight junction integrity observed after transplantation of the gut microbiota from young to old or aged mice may contribute to enhanced elimination of uric acid. Recent studies have also shown a direct relationship between hyperuricemia and gut microbiota. Certain anaerobic microbial communities are capable of effectively degrading purines and uric acid and thereby regulate the abundance of purines in the body to maintain the body’s uric acid balance(29–31). Our study also showed that the gut microbiota plays a role in maintaining the balance of serum uric acid in the body. The “ageing” gut

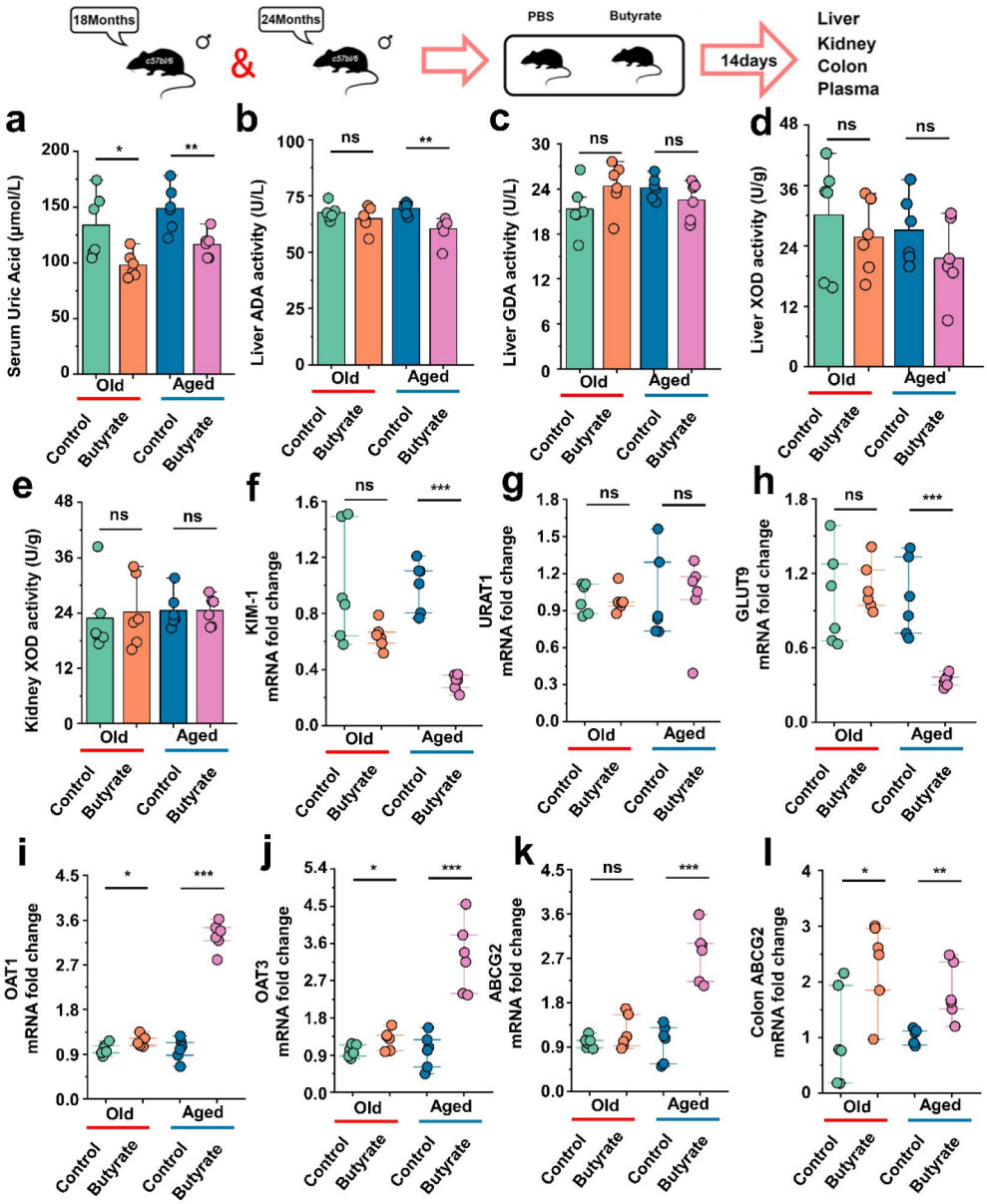

Fig 8

Serum uric acid-lowering effect of butyrate in old or aged mice.

(a) The serum uric acid concentrations in Old+PBS and Old+Butyrate, Aged+PBS and Aged+Butyrate (n=6).
(b-d) The activity of uric acid-producing enzymes of liver in the Old or Aged control group and the old or aged group supplemented with butyrate (n=6), including ADA (b), GDA (c) and XOD (d).
(e) The activity of XOD of kidney in the Old or Aged control group and the old or aged group supplemented with butyrate (n=6).
(f) Relative kidney injury molecule-1 (KIM-1) expression in the indicated groups by qPCR (n = 6).
(g and h) Relative renal genes for uric acid reabsorption expression in the indicated groups by qPCR (n = 6), including URAT1 (g) and GLUT9 (h).
(i-k) Relative renal genes for uric acid excretion expression in the indicated groups by qPCR (n = 6), including OAT1 (i), OAT3 (j) and ABCG2 (k).
(l) Relative colonic genes for uric acid expression in the indicated groups by qPCR, including ABCG2 (n = 6).
Values are presented as the mean ± SEM. Differences were assessed by t-test or One-Way ANOVA and denoted as follows: *p < 0.05, **p < 0.01, and ***p < 0.001, “ns” indicates no significant difference between groups.

microbiota is more likely to elevate the levels of serum uric acid in the body by suppressing the expression of uric acid excretion transporter (OAT1, OAT3). The “younger” gut microbiota helps to restrain the increase of serum uric acid levels in old or aged mice by suppressing the expression of uric acid reabsorption transporters (URAT1) and the activity of uric acid-producing enzymes (mainly XOD) and also promotes the expression of uric acid excretion transporters (ABCG2) in aged mice. We also found that old or aged mice exhibited stronger purine metabolism (**Table 2a, b**). These results may explain the reason for the high levels of uric acid detected in elderly individuals.

Moreover, through 16S rDNA sequencing analysis, we found that the abundance of Bifidobacterium and Akkermansia was higher in young mice. Additionally, FMT from young to old or aged mice increased the abundance of the Akkermansia genus. There are existing reports regarding the therapeutic effects of Bifidobacterium(32) and Akkermansia(33) on gout and hyperuricemia. Therefore, we performed further investigation, and using microbiome-predicted functional and untargeted metabolomics data, we observed a significant enhancement in butanoate metabolism in young mice. Surprisingly, using untargeted metabolomics data, comparisons of the results after FMT from young to old or aged mice with those of old or aged mice also identified the secondary bile acid biosynthesis pathways. Studies have indicated that transplantation of the gut microbiota from healthy mice to progeroid mice can enhance the enrichment of secondary bile acid biosynthesis pathways, and the restoration of secondary bile acids may potentially contribute to extension of the healthspan and lifespan in progeroid mice(34). The current investigation into the impact of secondary bile acids on gout and hyperuricemia is still in its incipient phase, with the intrinsic mechanisms remaining largely unelucidated, thereby rendering this domain a pivotal subject of profound scientific inquiry.

Moreover, old or aged mice transplanted with gut microbiota from young mice also exhibited an increase in butanoate metabolism. Upon comparison, we discovered other bacteria genera that produce butyrate, such as Lachnoclostridium. Additionally, literature(35, 36) reports have indicated that Bifidobacteria combined with other genera can enhance the production of butyrate. Meanwhile, Akkermansia, particularly the species Akkermansia muciniphila, has been found to confer several beneficial traits, as evidenced by preclinical studies. These traits include promoting the growth of butyrate- producing bacteria through the production of acetate, which leads to a decrease in the loss of the colonic bilayer and subsequent reduction in inflammation(37). These findings led us to hypothesize that butyric acid might play a role in this phenomenon. Although, **Figure 7** does show a similar trend for acetic and propionic acids as for butyric acid. However, considering the predictive data of microbial function and the non-targeted metabolomic data, there is an enhancement of Butanoate_metabolism in both young mice and elderly mice receiving young mouse intestinal microbiota transplants. Therefore, we prioritized butyrate as the subject of our study. Due to the scarcity of elderly mice, we are unable to conduct subsequent experiments with acetic and propionic acids, which is one of the limitations of this study. This work will be addressed in our follow-up research. Subsequently, we selected the Young+PBS, Aged+PBS, and Aged+Young groups for analysis of the short- chain fatty acid levels and discovered that the fecal butyric acid content in young mice was higher than that in aged mice, and that FMT from young mice led to an increase in fecal butyric acid content in aged mice. Consistent with previous reports, the content of SCFAs in the faeces of elderly mice was lower than that in the faeces of young mice(38). This result shifted our focus to butyric acid. Butyric acid, a SCFA derived from the gut microbiota, has been found to play a crucial role in host health(39). Emerging evidence indicates that among SCFAs, butyrate plays a pivotal role as a regulator in mediating metabolic control of the microbiota(40). The anti-inflammatory effects of butyric acid have been extensively reported,(41, 42) but only a few studies have investigated its impact on gout. Similarly, research on the anti-hyperuricemia effects of butyric acid is relatively scarce. We then conducted experiments using gout and peritonitis models, and found that mice supplemented with

butyrate exhibited relief in symptoms of gout and inflammation. Butyrate could also reduce the serum uric acid levels in old or aged mice by promoting the expression of uric acid excretion transporters.

The findings of this study demonstrate the positive effects of gut microbiota transplantation from young to aged mice in mitigating gout symptoms. Inhibition of the NLRP3 inflammasome pathway was found to modulate uric acid metabolism, and increase butyric acid levels. The gut microbiota of young mice effectively alleviated the inflammatory response and improved uric acid metabolism in aged mice. We enhanced Butanoate metabolism in the gut microbiota of young mice, while the opposite was observed in aged mice. Furthermore, we observed higher levels of butyrate in the feces of young mice. Moreover, butyrate has excellent anti-inflammatory and uric acid-lowering effects. These findings highlight the potential of targeting the gut microbiota as a therapeutic approach for the prevention and treatment of senile gout-related conditions.

Materials and Methods

Preparation of MSU crystals

A solution consisting of 10 mM uric acid and 154 mM NaCl (both from Sigma) was adjusted to pH 7.2 and agitated for 7 days to produce MSU crystals. For sterilization the needle-shaped crystals were washed with ethanol, dried under sterile conditions and heated at 180°C for 2 h. The crystals were stored at 20 mg/mL in sterile PBS for the experimental model.

Animals

All the mice in the different age groups were purchased from Longde Biotechnology Co., LTD (Changchun, Jilin, China). The mice were housed in a controlled environment with a temperature of 24±2°C and a 12-hour light/12- hour dark cycle, in a pathogen-free facility. These mice were provided ad libitum access to food and water. All animal experiments were conducted in compliance with the regulations set forth by the Administration of Affairs Concerning Experimental Animals in China. The Institutional Animal Care and Use Committee at Jilin University approved the entire protocol, including the mouse autopsy and sample collection (20170318).

Animal and FMT

Young (3 months), old (18 months) and aged (24 months) male mice were randomly reassigned to experimental cages one week prior to the experiment. The mice in the FMT groups were subjected to antibiotic interventions and depletion of the host gut microbiota by via oral gavage of a 3-day broad- spectrum antibiotic cocktail regimen (vancomycin, 100 mg/kg; metronidazole, 200 mg/kg; ampicillin 200 mg/kg, neomycin 200 mg/kg) to maximize potential engraftment of the donor microbiota. Those in the PBS groups were orally gavaged with isopycnic PBS.

Before antibiotic interventions, fecal slurries from the donor mice were prepared by pooling fecal pellet material from 12-15 mice per cage (chosen randomly) for each age group. At the time of pellet collection, at least three pellets from each mouse were collected into sterile centrifuge tubes, and the mice were then returned to their cage. The fecal pellets from each age group were mixed, and sterile PBS (five times the weight of the fecal pellets) was added. The homogenate was centrifuged at 500 × g and 4°C for 10 min and fecal slurry was collected. After addition of 10% glycerol (v/v) and the slurry was stored at –80°C until use. After antibiotic washout, recipient mice were reassigned to different groups and received heterochronic donor microbiota twice 3 days apart by oral gavage of the fecal slurry preparation. Each mouse received 200 μL the fecal slurry preparation during each gavage.

Control groups for 3-month-old, 18-month-old and 24-month-old receiving heterochronic transfer were gavaged with PBS only, revealed antibiotic treatment/PBS gavage only and were administered control microbiota (obtained from a donor pool of the same age group).

Before MSU injection, fecal pellets were collected for sequencing and untargeted metabolomics analysis on day 22, and were then stored at −80°C. Blood and tissues were collected at the end of the experimental period. All FMT interventions were performed at the same time of day across all the cages to control for the circadian rhythm variabilities in feeding and the microbiota/metabolite composition.

Experimental model details

Gout model

To establish this model, we administered 0.4 mg of preformed MSU crystals, resuspended in 20 μL of sterile PBS via subcutaneous injection into the left hind paws of the animals. We measured the hind paw thickness using calipers (the minimum accuracy of which was 0.01 mm) before and 8 hours after MSU injection. Eight hours after injection, mouse dorsal foot tissues were collected for histopathological observation and protein extraction.

Peritonitis model

Peritonitis was induced in the mice by intraperitoneal injection of MSU crystals at a dose of 2.5 mg in PBS. Six hours later, the peritoneal cells were collected by lavage (3 mL of PBS), and the cell count was determined using a hemocytometer to ensure consistent cell numbers. The lavage fluid was used to detect cytokines, and proteins were cells extracted from the cells.

Butyrate supplementation experiment

In this experiment, 3-month-old mice received a supplemental dose of butyrate (administered via gavage at a concentration of 200 mg/kg) for 14 days. Subsequently, these mice were subjected to monosodium urate (MSU) stimulation. The investigation was conducted utilizing both gout and peritonitis models to assess the effects of butyrate supplementation. Simultaneously, mice aged 18 months and 24 months were given the same dosage of supplemental butyrate as the 3-month-old mice for 14 days. Subsequently, their liver, kidneys, intestines, and blood samples were collected for analysis.

Histological analysis

The left hind paws were collected and immediately fixed with 4% paraformaldehyde for more than 48 h. The fixed tissues were prepared after paraffin embedding. At least three slices from each tissue were prepared. The prepared sections were then dewaxed with xylene and hydrated via different gradients of alcohol. Furthermore, all hydrated sections were stained with hematoxylin &eosin (H&E), detected under an optical microscope (Olympus, Tokyo, Japan) and analyzed with Caseviewer2.0 software.

Western blotting

The total proteins from the peritoneal cells and foot tissue samples were extracted using a protein extraction kit (Thermo Fisher Scientific, USA). The targeted proteins were separated by 10% or 15% SDS–PAGE based on the molecular weight and then bonded to 0.22 μm PVDF membranes. After blocking with 5% skim milk for three hours at RT, specific antibodies, including NLRP3, Caspace-1, IL-1β and β-actin, were used to detected target proteins at an appropriate final concentration according to the manufacturer's instructions. The PVDF membranes were then

incubated with goat anti-rabbit IgG (1:20,000), wash with TBST, and determined using the ECL plus western blotting detection system (Tanon, China). The grey values of the western blotting bands were procured using Image-Pro Plus 6.0 software.

RNA extraction and qPCR

Tissue samples were collected and total RNA was extracted using Trizol (Takara, CA, JPN) as previously described, cDNA was reverse transcribed using a TransScript Uni All-in-One (TransGen Biotech, Beijing, China) and reacted with specific primers using a FastStart Universal SYBR Green Master Mix (ROX) (Roche, Switzerland, Basel) and Bio-Rad CFX96 (Bio-Rad Laboratories, CA, USA). The specific primers used in this study are shown in **Table 1**. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression levels of genes using the control group as the calibrator.

Gene	Primer	Sequence(5' to 3')
KIM-1	sense	TGGTTGCCTTCCGTGTCTCT
KIM-1	antisense	TCAGCTCGGGAATGCACAA
URAT1	sense	GGCTCATCACAAAGACCCCA
URAT1	antisense	CAGCATGTTCTGGGTGGTGA
OAT1	sense	GCAGCCTATGCACCCAACTA
OAT1	antisense	ACAGCATAGGCGATACCAGC
OAT3	sense	GGGCTCGAGTGGGAAGTATG
OAT3	antisense	CTGGTAAGGGCCGATTGAGG
GLUT9	sense	GAAGGGTGTTCCTGGCTACC
GLUT9	antisense	TGAACACGGACCCAAACCAG
ABCG2	sense	TTGGACTCAAGCACAGCGAATG
ABCG2	antisense	TCCGCAGGGTTGTTGTAGGG
ADA	sense	CATGCGGTTGTTCGCTTCAA
ADA	antisense	CTGCGTTGATGTTCAGTCGC
XDH	sense	ATGACGAGGACAACGGTAGATG
XDH	antisense	GAAGGCGGTCATACTTGGAGA
GDA	sense	CGACAGCGGCAAAATAGTGTT
GDA	antisense	GGGGCATGGATGTGGGTATC
ZO-1	sense	TTTCAGAGTGGGGAAACCTCC
ZO-1	antisense	CACTCTTCCTTAGCTGCTGAAC
JAMA	sense	TTGGCGTCTGGTTTGCCTAT
JAMA	antisense	CACTTCGAGTACTGGGCTGG
OCCLUDIN	sense	ATGCTCTCTCAGCCAGCGTA
OCCLUDIN	antisense	CCACACAGGCAAATATGGCG
GAPDH	sense	AGGTCGGTGTGAACGGATTTG
GAPDH	antisense	GGGGTCGTTGATGGCAACA

Table 1.

Oligonucleotides

Table 2a Young+PBS VS Old+PBS								
Taxa	avg(Young+PBS)	sd(Young+PBS)	avg(Old+PBS)	sd(Old+PBS)	p.value	q.values	interval lower	interval upper
Genetic Information Processing;Translation;Transfer_RNA_biogenesis	0.025388	0.000319	0.026282	0.000752	0.032527	0.095267	-0.00169	-1E-04
Metabolism;Amino acid metabolism;Amino_acid_related_enzymes	0.018844	0.000148	0.019137	0.000258	0.042118	0.102535	-0.00057	-1.3E-05
Genetic Information Processing;Translation;Ribosome	0.015723	0.000269	0.016458	0.000594	0.028278	0.090633	-0.00137	-0.00001
Genetic Information Processing;Translation;Aminoacyl-tRNA_biosynthesis	0.015499	0.000262	0.0164	0.000581	0.0106	0.060087	-0.00152	-0.00029
Cellular Processes;Transport and catabolism;Exosome	0.015496	0.000151	0.014721	0.000475	0.008904	0.057414	0.000277	0.001273
Genetic Information Processing;Replication and repair;DNA_replication_proteins	0.014807	0.000271	0.015186	0.000287	0.040656	0.101038	-0.00074	-2E-05
Genetic Information Processing;Replication and repair;Chromosome_and_associated_proteins	0.0131	0.000238	0.013673	0.000459	0.028126	0.090633	-0.00107	-8E-05
Metabolism;Amino acid metabolism;Alanine,_aspartate_and_glutamate_metabolism	0.011744	0.000114	0.011268	0.000326	0.014186	0.066361	0.000134	0.000819
Metabolism;Amino acid metabolism;Cysteine_and_methionine_metabolism	0.009602	7.4E-05	0.009046	0.000372	0.013822	0.065951	0.000166	0.000946
Genetic Information Processing;Replication and repair;Nucleotide_excision_repair	0.008149	0.000239	0.008575	0.000252	0.013177	0.065517	-0.00074	-0.00011
Genetic Information Processing;Replication and repair;DNA_replication	0.007972	0.000183	0.008423	0.000317	0.016667	0.072413	-0.00008	-0.00011
Genetic Information Processing;Translation;Messenger_RNA_Biogenesis	0.007849	5.8E-05	0.008027	0.000156	0.037877	0.100003	-0.00034	-1.4E-05

Metabolism;Amino acid metabolism;Glycine,_serine_and_threonine_metabolism	0.007893	0.000123	0.007344	0.000264	0.002338	0.042225	0.000268	0.000083
Metabolism;Carbohydrate metabolism;Pentose_phosphate_pathway	0.007552	0.000179	0.007998	0.000372	0.003220 8	0.095267	-0.000084	-5E-05
Metabolism;Carbohydrate metabolism;Butanoate_metabolism	0.007496	0.000432	0.006856	0.000234	0.013454	0.065517	0.000174	0.001105
Metabolism;Carbohydrate metabolism;Glyoxylate_and_dicarboxylate_metabolism	0.007261	0.000272	0.006593	0.000058	0.037471	0.100003	5.11E-05	0.001284
Metabolism;Amino acid metabolism;Lysine_biosynthesis	0.006277	0.000198	0.006577	0.000222	0.033542	0.095267	-0.000057	-2.9E-05
Metabolism;Amino acid metabolism;Arginine_biosynthesis	0.005337	0.000206	0.004818	0.000269	0.004248	0.042225	0.000208	0.000083
Metabolism;Lipid metabolism;Lipid_biosynthesis_proteins	0.004748	0.000253	0.004195	0.000254	0.003621	0.042225	0.000227	0.000088
Metabolism;Metabolism of terpenoids and polyketides;Terpenoid_backbone_biosynthesis	0.004268	0.000118	0.004511	0.000132	0.007384	0.057414	-0.000404	-8.1E-05
Metabolism;Lipid metabolism;Glycerophospholipid_metabolism	0.004116	0.00012	0.00449	0.00018	0.002372	0.042225	-0.000058	-0.000017
Metabolism;Energy metabolism;Nitrogen_metabolism	0.004843	0.000271	0.004057	0.000366	0.002089	0.042225	0.000368	0.001206
Metabolism;Metabolism of cofactors and vitamins;One_carbon_pool_by_folate	0.004295	4.61E-05	0.003931	0.000257	0.017267	0.073561	9.46E-05	0.000634
Genetic Information Processing;Replication and repair;Base_excision_repair	0.003915	6.37E-05	0.004159	0.000206	0.003302 1	0.095267	-0.000046	-2.7E-05
Metabolism;Metabolism of cofactors and vitamins;Pantothenate_and_CoA_biosynthesis	0.004248	0.000113	0.003948	0.000163	0.004954	0.045425	0.000117	0.000483
Metabolism;Glycan biosynthesis and metabolism;Glycosyltransferases	0.003709	0.000154	0.003904	0.000119	0.003521 7	0.098192	-0.000037	-1.7E-05
Metabolism;Amino acid metabolism;Valine,_leucine_and_isoleucine_biosynt	0.003878	0.000305	0.00317	0.000297	0.022	0.422	0.000321	0.001096

hesis					48	25		
Genetic Information Processing;Transcription;RNA_polymerase	0.003305	7E-05	0.003533	0.000182	0.026747	0.090633	-0.00042	-3.6E-05
Metabolism;Carbohydrate metabolism;Pentose_and_glucuronate_interconversions	0.003491	7.67E-05	0.002991	0.000471	0.048036	0.108091	6.23E-06	0.000993
Metabolism;Glycan biosynthesis and metabolism;Other_glycan_degradation	0.002999	0.000399	0.002122	0.000652	0.022059	0.080739	0.000162	0.001592
Metabolism;Energy metabolism;Photosynthesis_proteins	0.00274	0.000139	0.002997	8.72E-05	0.008279	0.057414	-0.00038	-7.7E-05
Metabolism;Energy metabolism;Photosynthesis	0.002731	0.000139	0.002958	8.61E-05	0.008761	0.057414	-0.00038	-7.4E-05
Metabolism;Amino acid metabolism;Valine,_leucine_and_isoleucine_degradation	0.00267	0.000206	0.002337	0.000011	0.008698	0.057414	0.000112	0.000556
Cellular Processes;Cell motility;Cytoskeleton_proteins	0.002762	6.51E-05	0.002926	6.67E-05	0.001549	0.042225	-0.00025	-7.9E-05
Cellular Processes;Cellular community - prokaryotes;Biofilm_formation-Escherichia_coli	0.002944	0.000017	0.002446	0.000315	0.009901	0.060087	0.000158	0.000837
Metabolism;Metabolism of other amino acids;Glutathione_metabolism	0.002308	0.000124	0.002655	0.000202	0.006697	0.057064	-0.00057	-0.000013
Metabolism;Amino acid metabolism;Histidine_metabolism	0.002726	0.000184	0.002115	0.000282	0.00178	0.042225	0.000299	0.000924
Metabolism;Xenobiotics biodegradation and metabolism;Drug_metabolism-other_enzymes	0.002595	4.74E-05	0.002414	0.000157	0.035973	0.098192	1.66E-05	0.000346
Metabolism;Metabolism of other amino acids;Cyanoamino_acid_metabolism	0.002675	7.72E-05	0.002356	0.000246	0.023199	0.082607	6.12E-05	0.000577
Unclassified;Cellular processes and signaling;Cell_growth	0.002913	0.000282	0.002442	0.000367	0.033195	0.095267	4.65E-05	0.000897
Cellular Processes;Cell growth and death;Necroptosis	0.002668	0.000129	0.002208	0.000181	0.00066	0.042225	0.000255	0.000665
Genetic Information Processing;Folding, sorting	0.0020	6.24E	0.002	0.00	0.0	0.0	-	-

and degradation;Sulfur_relay_system	33	-05	222	0115	08311	57414	0.00031	6.4E-05
Metabolism;Biosynthesis of other secondary metabolites;Monobactam_biosynthesis	0.002121	3.47E-05	0.001984	8.96E-05	0.011592	0.061459	4.23E-05	0.000231
Metabolism;Carbohydrate metabolism;C5-Branched_dibasic_acid_metabolism	0.002032	0.000102	0.001717	0.000241	0.022336	0.080739	6.07E-05	0.000057
Cellular Processes;Cellular community - prokaryotes;Biofilm_formation-Vibrio_cholerae	0.001887	0.0001	0.001714	0.00012	0.022317	0.080739	3.04E-05	0.000315
Metabolism;Amino acid metabolism;Phenylalanine_metabolism	0.001845	0.000192	0.001557	9.34E-05	0.015359	0.070468	7.01E-05	0.000479
Human Diseases;Endocrine and metabolic diseases;Insulin_resistance	0.001786	0.000103	0.001495	0.000116	0.010145	0.042225	0.000149	0.000433
Metabolism;Metabolism of terpenoids and polyketides;Prenyltransferases	0.001408	5.91E-05	0.001516	8.12E-05	0.026094	0.090224	-0.00002	-1.6E-05
Metabolism;Metabolism of other amino acids;D-Glutamine_and_D-glutamate_metabolism	0.001416	4.8E-05	0.001524	5.98E-05	0.006665	0.057064	-0.000018	-3.8E-05
Environmental Information Processing;Signal transduction;HIF-1_signaling_pathway	0.001241	3.17E-05	0.001343	9.34E-05	0.043249	0.103127	-0.00002	-4.3E-06
Metabolism;Glycan biosynthesis and metabolism;Glycosphingolipid_biosynthesis-globo_and_isoglobo_series	0.001423	0.000143	0.001106	0.000281	0.041764	0.102535	1.54E-05	0.000618
Metabolism;Biosynthesis of other secondary metabolites;Phenylpropanoid_biosynthesis	0.001572	6.81E-05	0.001159	0.000289	0.016311	0.072413	0.000011	0.000715
Organismal Systems;Nervous system;GABAergic_synapse	0.001346	3.61E-05	0.001224	7.85E-05	0.010521	0.060087	3.86E-05	0.000205
Metabolism;Metabolism of cofactors and vitamins;Vitamin_B6_metabolism	0.00127	5.5E-05	0.001084	0.000119	0.010047	0.060087	6E-05	0.000312
Organismal Systems;Nervous system;Glutamatergic_synapse	0.001287	3.41E-05	0.001179	7.72E-05	0.016694	0.072413	2.65E-05	0.000019
Metabolism;Enzyme families;Protein_phosphatase_and_associated_proteins	0.001301	7.07E-05	0.001114	0.000012	0.010832	0.060087	5.64E-05	0.000319

Unclassified;Metabolism;Carbohydrate_metabolism	0.001315	0.000147	0.001019	0.000118	0.003551	0.042225	0.000123	0.000468
Organismal Systems;Endocrine system;Insulin_signaling_pathway	0.001273	8.54E-05	0.00102	0.000129	0.003262	0.042225	0.00011	0.000397
Organismal Systems;Endocrine system;PPAR_signaling_pathway	0.001209	6.31E-05	0.000931	0.000165	0.007484	0.057414	0.000104	0.000452
Metabolism;Metabolism of other amino acids;beta-Alanine_metabolism	0.001119	0.000151	0.000946	6.77E-05	0.038154	0.100003	1.25E-05	0.000333
Unclassified;Viral protein family;Viral_proteins	0.00087	4.86E-05	0.000995	5.84E-05	0.002547	0.042225	-0.000019	-5.6E-05
Metabolism;Glycan biosynthesis and metabolism;Glycosaminoglycan_degradation	0.000925	0.000151	0.000591	0.000218	0.013149	0.065517	8.89E-05	0.000579
Metabolism;Biosynthesis of other secondary metabolites;Novobiocin_biosynthesis	0.001016	5.43E-05	0.00082	0.000101	0.003339	0.042225	8.73E-05	0.000305
Metabolism;Biosynthesis of other secondary metabolites;Tropane,_piperidine_and_pyridine_alkaloid_biosynthesis	0.001043	6.63E-05	0.000805	7.65E-05	0.000202	0.042225	0.000145	0.000033
Cellular Processes;Transport and catabolism;Lysosome	0.00084	0.0002	0.000516	0.000241	0.030551	0.093446	3.74E-05	0.000061
Metabolism;Carbohydrate metabolism;Ascorbate_and_aldarate_metabolism	0.000764	6.21E-05	0.000622	8.97E-05	0.011082	0.060087	4.14E-05	0.000243
Metabolism;Biosynthesis of other secondary metabolites;Acarbose_and_validamycin_biosynthesis	0.000751	2.85E-05	0.000653	9.16E-05	0.045896	0.107335	2.48E-06	0.000194
Metabolism;Lipid metabolism;Synthesis_and_degradation_of_ketone_bodies	0.000626	3.57E-05	0.000706	7.54E-05	0.049832	0.108091	-0.000016	-7.7E-08
Environmental Information Processing;Signal transduction;AMPK_signaling_pathway	0.000694	4.69E-05	0.000623	3.94E-05	0.018262	0.076435	1.49E-05	0.000127
Unclassified;Metabolism;Glycan_biosynthesis_and_metabolism	0.000642	4.08E-05	0.000512	6.35E-05	0.002595	0.042225	5.91E-05	0.00002

Environmental Information Processing;Signal transduction;Phosphatidylinositol_signaling_system	0.000471	3.16E-05	0.000509	1.46E-05	0.030116	0.09331	-7.2E-05	-4.9E-06
Cellular Processes;Cell growth and death;Ferroptosis	0.000572	3.2E-05	0.00033	0.000121	0.003812	0.042225	0.000114	0.000368
Genetic Information Processing;Folding, sorting and degradation;Proteasome	0.000566	5.42E-05	0.000444	0.000112	0.047251	0.108059	1.93E-06	0.000241
Organismal Systems;Endocrine system;Adipocytokine_signaling_pathway	0.000557	4.04E-05	0.000303	0.00013	0.038	0.042225	0.000118	0.00039
Metabolism;Biosynthesis of other secondary metabolites;Isoquinoline_alkaloid_biosynthesis	0.000477	1.95E-05	0.000386	6.85E-05	0.020656	0.078274	1.99E-05	0.000163
Metabolism;Lipid metabolism;Secondary_bile_acid_biosynthesis	0.000335	2.13E-05	0.000417	6.43E-05	0.025372	0.089018	-0.00015	-1.4E-05
Metabolism;Biosynthesis of other secondary metabolites;Glucosinolate_biosynthesis	0.000341	3.28E-05	0.000265	2.64E-05	0.001454	0.042225	3.73E-05	0.000114
Metabolism;Glycan biosynthesis and metabolism;Glycosylphosphatidylinositol_(GPI)-anchored_proteins	0.000296	4.79E-05	0.000188	5.55E-05	0.004804	0.045425	4.17E-05	0.000175
Metabolism;Glycan biosynthesis and metabolism;N-Glycan_biosynthesis	0.000331	9.81E-06	0.000253	4.94E-05	0.010931	0.060087	2.64E-05	0.00013
Metabolism;Metabolism of terpenoids and polyketides;Biosynthesis_of_siderophore_group_nonribosomal_peptides	0.000249	6.71E-05	0.000164	3.97E-05	0.028492	0.090633	1.14E-05	0.000158
Human Diseases;Cancers;Renal_cell_carcinoma	0.000173	2.1E-05	0.000284	5.25E-05	0.002352	0.042225	-0.00017	-5.5E-05
Metabolism;Glycan biosynthesis and metabolism;Glycosaminoglycan_binding_proteins	0.000171	1.89E-05	0.000106	2.46E-05	0.000544	0.042225	3.65E-05	9.35E-05
Organismal Systems;Digestive system;Protein_digestion_and_absorption	0.000241	5.39E-05	0.000103	0.00010	0.019433	0.078274	2.92E-05	0.000247
Metabolism;Lipid metabolism;Steroid_hormone_biosynthesis	0.000174	6.14E-05	8.44E-05	7.21E-05	0.043251	0.103127	3.33E-06	0.000176
Environmental Information Processing;Signaling molecules and interaction;CD_Molecules	0.000238	5.41E-05	9.98E-05	0.00010	0.0204	0.0782	2.82E-05	0.000249

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Organismal Systems;Digestive system;Carbohydrate_digestion_and_absorption	0.000114	3.23E-05	7.38E-05	1.73E-05	0.02846	0.090633	5.52E-06	7.5E-05
Metabolism;Biosynthesis of other secondary metabolites;Flavone_and_flavonol_biosynthesis	0.000118	8.7E-06	9.64E-05	1.63E-05	0.02043	0.078274	4.45E-06	3.95E-05
Human Diseases;Cancers;Choline_metabolism_in_cancer	9.26E-05	7.18E-06	0.000109	1.08E-05	0.01345	0.065517	-2.8E-05	-4.3E-06
Environmental Information Processing;Signal transduction;Phospholipase_D_signaling_pathway	7.63E-05	6.87E-06	9.25E-05	1.34E-05	0.03126	0.094411	-3.1E-05	-1.9E-06
Genetic Information Processing;Transcription;Basal_transcription_factors	7.48E-05	9.1E-06	6.1E-05	1.11E-05	0.04049	0.101038	7.35E-07	2.69E-05
Organismal Systems;Endocrine system;Renin-angiotensin_system	6.03E-05	1.93E-05	2.88E-05	2.59E-05	0.03952	0.101038	1.87E-06	6.12E-05
Human Diseases;Infectious diseases;African_trypanosomiasis	3.1E-05	1.36E-05	7.93E-06	3.38E-06	0.00787	0.057414	8.83E-06	3.73E-05
Human Diseases;Infectious diseases;Chagas_disease_(American_trypanosomiasis)	2.83E-05	1.31E-05	5.73E-06	3.61E-06	0.00716 9	0.057414	8.87E-06	3.63E-05
Human Diseases;Neurodegenerative diseases;Prion_diseases	2.71E-05	3.06E-06	2.21E-05	4.03E-06	0.03621 8	0.098192	4.01E-07	9.7E-06
Organismal Systems;Digestive system;Mineral_absorption	1.16E-05	1.93E-06	8.81E-06	1.93E-06	0.02998 8	0.093331	3.34E-07	5.3E-06
Metabolism;Metabolism of terpenoids and polyketides;Type_I_polyketide_structures	9.57E-06	5.92E-06	3.27E-06	1.48E-06	0.04755 8	0.108059	9.56E-08	1.25E-05
Metabolism;Biosynthesis of other secondary metabolites;Stilbenoid,_diarylheptanoid_and_gingerol_biosynthesis	3.46E-06	5.49E-07	1.76E-06	9.04E-07	0.04074	0.042225	7.08E-07	2.69E-06
Metabolism;Biosynthesis of other secondary metabolites;Flavonoid_biosynthesis	3.46E-06	5.49E-07	1.76E-06	9.04E-07	0.04074	0.042225	7.08E-07	2.69E-06
Metabolism;Metabolism of terpenoids and polyketides;Biosynthesis_of_12-,_14-_and_16-membered_macrolides	3.33E-06	2.17E-06	1.02E-06	4.86E-07	0.04745 6	0.108059	3.67E-08	4.57E-06

Metabolism;Metabolism of terpenoids and polyketides;Biosynthesis_of_enediyne_antibiotics	2.58E-06	1.55E-06	8.51E-07	4.94E-07	0.040537	0.101038	1.03E-07	3.35E-06
Human Diseases;Immune diseases;Systemic_lupus_erythematosus	5.61E-07	2.55E-07	2.46E-07	9.81E-08	0.028032	0.090633	4.64E-08	5.83E-07
Human Diseases;Infectious diseases;Vibrio_cholerae_infection	7.42E-07	4.06E-07	2.66E-07	2.15E-07	0.036006	0.098192	4.04E-08	9.12E-07
Human Diseases;Infectious diseases;Pathogenic_Escherichia_coli_infection	6E-07	3.16E-07	1.71E-07	8.14E-08	0.019688	0.078274	9.83E-08	7.61E-07
Organismal Systems;Endocrine system;Ovarian_Steroidogenesis	3.18E-07	2.43E-07	6.29E-08	5.25E-08	0.049664	0.108091	5.32E-10	5.09E-07
Environmental Information Processing;Signal transduction;Calcium_signaling_pathway	7.35E-08	4.47E-08	1.4E-08	1.79E-08	0.02067	0.078274	1.24E-08	1.07E-07
Environmental Information Processing;Signaling molecules and interaction;G_protein-coupled_receptors	2.34E-08	1.07E-08	2.05E-09	3.26E-09	0.003543	0.042225	1.02E-08	3.26E-08
Environmental Information Processing;Signaling molecules and interaction;Neuroactive_ligand-receptor_interaction	5.88E-09	4.51E-09	1.14E-09	1.32E-09	0.049187	0.108091	2.35E-11	9.47E-09
Metabolism;Glycan biosynthesis and metabolism;Glycosaminoglycan_biosynthesis-chondroitin_sulfate/dermatan_sulfate	2.39E-09	1.68E-09	5.21E-10	8.36E-10	0.043658	0.103127	6.93E-11	3.66E-09
Metabolism;Biosynthesis of other secondary metabolites;Clavulanic_acid_biosynthesis	7.9E-10	6.83E-10	1.4E-11	1.12E-11	0.038774	0.100551	5.91E-11	1.49E-09

Table 2b Young+PBS VS Aged+PBS								
Taxa	avg(Young+PBS)	sd(Young+PBS)	avg(Aged+PBS)	sd(Aged+PBS)	p.value	q.values	interval lower	interval upper
Metabolism;Nucleotide metabolism;Purine_metabolism	0.025653	0.000514	0.026306	0.00031	0.027754	0.018099	-0.00122	-9.1E-05
Genetic Information Processing;Translation;Transfer_RNA_biogenesis	0.025388	0.000319	0.025764	0.000192	0.037679	0.020034	-0.00072	-2.7E-05
Metabolism;Nucleotide metabolism;Pyrimidine_metabolism	0.020031	0.000437	0.020588	0.000135	0.024899	0.016912	-0.00101	-9.9E-05
Genetic Information Processing;Translation;Ribosome	0.015723	0.000269	0.016031	0.000179	0.045392	0.02079	-0.00061	-7.9E-06
Genetic Information Processing;Translation;Aminoacyl-tRNA_biosynthesis	0.015499	0.000262	0.015904	0.000183	0.012754	0.011118	-0.00007	-0.00011
Cellular Processes;Transport and catabolism;Exosome	0.015496	0.000151	0.01467	0.000299	0.000423	0.001644	0.000506	0.001146
Genetic Information Processing;Replication and repair;DNA_replication_proteins	0.014807	0.000271	0.015109	0.000133	0.042432	0.020702	-0.00059	-1.4E-05
Genetic Information Processing;Replication and repair;Chromosome_and_associated_proteins	0.0131	0.000238	0.013723	0.000145	0.000519	0.0017	-0.00088	-0.00036
Metabolism;Amino acid metabolism;Alanine,_aspartate_and_glutamate_metabolism	0.011744	0.000114	0.011119	0.000336	0.004746	0.006652	0.000273	0.000978
Genetic Information Processing;Replication and repair;Mismatch_repair	0.010868	0.000292	0.011191	0.000174	0.04767	0.02099	-0.00064	-4.2E-06

					2	3		
Genetic Information Processing;Replication and repair;Homologous_recombination	0.010494	0.000212	0.010894	0.000219	0.009155	0.009537	-0.00068	-0.00012
Metabolism;Glycan biosynthesis and metabolism;Peptidoglycan_biosynthesis_and_degradation_proteins	0.010172	0.000138	0.010573	0.000247	0.008688	0.009515	-0.00067	-0.00013
Metabolism;Amino acid metabolism;Cysteine_and_methionine_metabolism	0.009602	7.4E-05	0.009323	5.47E-05	3.49E-05	0.000528	0.000195	0.000364
Metabolism;Energy metabolism;Carbon_fixation_pathways_in_prokaryotes	0.009118	0.000366	0.008631	0.000453	0.004429 2	0.002070 2	1.72E-05	0.001082
Metabolism;Glycan biosynthesis and metabolism;Peptidoglycan_biosynthesis	0.008308	0.000207	0.008721	0.000328	0.002989 5	0.001864 2	-0.00077	-5.1E-05
Genetic Information Processing;Replication and repair;DNA_replication	0.007972	0.000183	0.00835	0.000115	0.002382	0.000418 6	-0.00058	-0.00018
Genetic Information Processing;Translation;Messenger_RNA_Biogenesis	0.007849	5.8E-05	0.007653	0.000128	0.011143	0.001023 6	6.06E-05	0.000332
Metabolism;Amino acid metabolism;Glycine,_serine_and_threonine_metabolism	0.007893	0.000123	0.0077	0.000166	0.004723 4	0.002099 3	2.91E-06	0.000383
Metabolism;Carbohydrate metabolism;Pentose_phosphate_pathway	0.007552	0.000179	0.008037	0.000253	0.000404 3	0.000616 8	-0.00077	-0.00002
Genetic Information Processing;Folding, sorting and degradation;RNA_degradation	0.007475	8.88E-05	0.007297	0.000143	0.003041 9	0.001873 3	2.15E-05	0.000336
Metabolism;Carbohydrate metabolism;Butanoate_metabolism	0.007496	0.000432	0.006828	0.000131	0.011301	0.001027	0.000216	0.000112

Genetic Information Processing;Transcription;Transcription_machinery	0.007439	9.11E-05	0.006994	0.00041	0.044514	0.020702	1.55E-05	0.000874
Metabolism;Carbohydrate metabolism;Propanoate_metabolism	0.006937	0.000132	0.007178	9.95E-05	0.005804	0.007512	-0.00039	-8.8E-05
Metabolism;Carbohydrate metabolism;Glyoxylate_and_dicarboxylate_metabolism	0.007261	0.000272	0.006487	0.000322	0.001233	0.003019	0.000389	0.001159
Metabolism;Carbohydrate metabolism;Citrate_cycle_(TCA_cycle)	0.006205	0.000286	0.005779	0.000294	0.002945	0.018636	5.2E-05	0.000798
Unclassified;Metabolism;Energy_metabolism	0.006315	0.000289	0.005571	0.00032	0.001779	0.003455	0.000352	0.001137
Unclassified;Poorly characterized;General_function_prediction_only	0.005257	0.000243	0.006104	0.00068	0.027117	0.017958	-0.000156	-0.00013
Metabolism;Energy metabolism;Carbon_fixation_in_photosynthetic_organisms	0.005094	4.39E-05	0.004982	4.4E-05	0.001278	0.003032	5.58E-05	0.000169
Metabolism;Amino acid metabolism;Arginine_biosynthesis	0.005337	0.000206	0.004855	0.000135	0.001101	0.003019	0.000253	0.000711
Metabolism;Amino acid metabolism;Phenylalanine,_tyrosine_and_tryptophan_biosynthesis	0.004979	0.00031	0.004465	0.000266	0.011991	0.001067	0.000141	0.000886
Genetic Information Processing;Folding, sorting and degradation;Protein_export	0.004501	8.55E-05	0.00462	8.29E-05	0.00345	0.002034	-0.000023	-1.1E-05
Metabolism;Lipid metabolism;Lipid_biosynthesis_proteins	0.004748	0.000253	0.004339	8.86E-05	0.009127	0.009537	0.000143	0.000675
Metabolism;Metabolism of terpenoids and	0.0042	0.000	0.004	4.29E	0.0	0.0	-	-

polyketides;Terpenoid_backbone_biosynthesis	68	118	426	-05	20507	15782	0.00028	3.4E-05
Metabolism;Metabolism of cofactors and vitamins;Thiamine_metabolism	0.004472	6.82E-05	0.004227	0.000161	0.011682	0.0105	7.47E-05	0.000415
Metabolism;Lipid metabolism;Glycerophospholipid_metabolism	0.004116	0.00012	0.004402	0.00011	0.001578	0.003306	-0.00043	-0.00014
Metabolism;Energy metabolism;Nitrogen_metabolism	0.004843	0.000271	0.004069	0.000223	0.000336	0.001553	0.000454	0.001095
Metabolism;Metabolism of cofactors and vitamins;One_carbon_pool_by_folate	0.004295	4.61E-05	0.003978	7.5E-05	1.69E-05	0.000528	0.000235	0.0004
Genetic Information Processing;Replication and repair;Base_excision_repair	0.003915	6.37E-05	0.004133	8.09E-05	0.000485	0.001658	-0.00031	-0.00012
Metabolism;Metabolism of other amino acids;Selenocompound_metabolism	0.004118	5.86E-05	0.004009	5.9E-05	0.009112	0.009537	3.38E-05	0.000185
Metabolism;Metabolism of cofactors and vitamins;Pantothenate_and_CoA_biosynthesis	0.004248	0.000113	0.003982	4.2E-05	0.001355	0.003046	0.000147	0.000384
Metabolism;Glycan biosynthesis and metabolism;Glycosyltransferases	0.003709	0.000154	0.003989	0.000158	0.011138	0.010236	-0.00048	-7.9E-05
Metabolism;Amino acid metabolism;Valine,_leucine_and_isoleucine_biosynthesis	0.003878	0.000305	0.003302	0.000124	0.004186	0.006251	0.000254	0.000898
Genetic Information Processing;Transcription;RNA_polymerase	0.003305	7E-05	0.003427	4.68E-05	0.0065	0.007932	-0.00002	-4.4E-05
Metabolism;Carbohydrate	0.0034	7.67E	0.003	0.000	0.0	0.0	0.00	0.00

metabolism;Pentose_and_glucuronate_interconversions	91	-05	163	104	0014	0098	0209	0446
Metabolism;Lipid metabolism;Sphingolipid_metabolism	0.003031	0.000248	0.00258	0.000251	0.01058	0.0100433	0.000131	0.000773
Metabolism;Metabolism of cofactors and vitamins;Folate_biosynthesis	0.002563	5.22E-05	0.002807	0.000132	0.0046744	0.0065652	-0.00038	-0.000011
Metabolism;Biosynthesis of other secondary metabolites;Streptomycin_biosynthesis	0.002693	4.16E-05	0.002542	4.81E-05	0.000182	0.0010149	9.31E-05	0.000209
Cellular Processes;Cellular community - prokaryotes;Biofilm_formation-Escherichia_coli	0.002944	0.00017	0.002448	6.37E-05	0.000042	0.0016444	0.000317	0.000675
Metabolism;Metabolism of other amino acids;Glutathione_metabolism	0.002308	0.000124	0.002741	0.000188	0.001237	0.0030119	-0.00064	-0.000022
Metabolism;Energy metabolism;Sulfur_metabolism	0.0026	9.62E-05	0.00237	6.26E-05	0.000966	0.0029447	0.000123	0.000337
Metabolism;Amino acid metabolism;Histidine_metabolism	0.002726	0.000184	0.002265	6.44E-05	0.001014	0.0029887	0.000269	0.000654
Metabolism;Xenobiotics biodegradation and metabolism;Drug_metabolism-other_enzymes	0.002595	4.74E-05	0.002421	5.19E-05	0.000123	0.0000098	0.000011	0.000238
Metabolism;Metabolism of other amino acids;Cyanoamino_acid_metabolism	0.002675	7.72E-05	0.002357	0.000201	0.009879	0.0090706	0.000106	0.000053
Unclassified;Cellular processes and signaling;Cell_growth	0.002913	0.000282	0.002427	0.000246	0.009998	0.0090706	0.000145	0.000827
Cellular Processes;Cell growth and death;Necroptosis	0.002668	0.000129	0.002126	0.000197	0.000	0.001	0.000323	0.000761

					376	607		
Genetic Information Processing;Folding, sorting and degradation;Sulfur_relay_system	0.002033	6.24E-05	0.002168	1.8E-05	0.002388	0.004186	-0.0002	-7E-05
Organismal Systems;Aging;Longevity_regulating_pathway-worm	0.001962	1.51E-05	0.002049	4.11E-05	0.002488	0.004186	-0.013	-4.3E-05
Metabolism;Biosynthesis of other secondary metabolites;Monobactam_biosynthesis	0.002121	3.47E-05	0.001968	8.39E-05	0.004892	0.006741	6.45E-05	0.000242
Metabolism;Carbohydrate metabolism;C5-Branched_dibasic_acid_metabolism	0.002032	0.000102	0.001784	8.8E-05	0.011146	0.003019	0.0126	0.000371
Unclassified;Cellular processes and signaling;Membrane_and_intracellular_structural_molecules	0.001747	7.54E-05	0.001925	0.000136	0.023341	0.016912	-0.032	-3.1E-05
Cellular Processes;Cellular community - prokaryotes;Biofilm_formation-Vibrio_cholerae	0.001887	0.0001	0.001767	6.28E-05	0.035597	0.020034	1.03E-05	0.000231
Human Diseases;Drug resistance;Antimicrobial_resistance_genes	0.001562	0.00013	0.001938	0.000318	0.033363	0.019522	-0.071	-4E-05
Human Diseases;Drug resistance;Cationic_antimicrobial_peptide_(CAMP)_resistance	0.001578	0.000133	0.001938	0.000162	0.001951	0.003704	-0.055	-0.000017
Metabolism;Amino acid metabolism;Phenylalanine_metabolism	0.001845	0.000192	0.001538	7.37E-05	0.0093	0.009572	0.0105	0.000509
Human Diseases;Endocrine and metabolic diseases;Insulin_resistance	0.001786	0.000103	0.001469	4.21E-05	0.000286	0.001439	0.0208	0.000426
Metabolism;Metabolism of terpenoids and polyketides;Prenyltransferases	0.001408	5.91E-05	0.001515	2.26E-05	0.005	0.006	-0.00	-4.6E-

					042	837	017	05
Metabolism;Metabolism of other amino acids;D-Glutamine_and_D-glutamate_metabolism	0.001416	4.8E-05	0.001472	3.06E-05	0.041377	0.020702	-0.00011	-2.8E-06
Environmental Information Processing;Signal transduction;HIF-1_signaling_pathway	0.001241	3.17E-05	0.001378	8.59E-05	0.009558	0.009606	-0.00023	-4.7E-05
Metabolism;Glycan biosynthesis and metabolism;Glycosphingolipid_biosynthesis-globo_and_isoglobo_series	0.001423	0.000143	0.001238	7.89E-05	0.024677	0.016912	3.06E-05	0.000339
Metabolism;Metabolism of other amino acids;Taurine_and_hypotaurine_metabolism	0.001248	2.41E-05	0.001392	6.39E-05	0.001772	0.003455	-0.00021	-7.7E-05
Metabolism;Biosynthesis of other secondary metabolites;Phenylpropanoid_biosynthesis	0.001572	6.81E-05	0.001167	0.000172	0.001339	0.003046	0.000223	0.000587
Metabolism;Metabolism of other amino acids;D-Alanine_metabolism	0.001168	4.12E-05	0.001297	8.82E-05	0.013815	0.011577	-0.00022	-3.5E-05
Cellular Processes;Transport and catabolism;Peroxisome	0.001301	6.36E-05	0.001189	8.51E-05	0.029136	0.018575	1.41E-05	0.000021
Organismal Systems;Nervous system;GABAergic_synapse	0.001346	3.61E-05	0.001162	0.000101	0.005527	0.007034	7.72E-05	0.000029
Human Diseases;Infectious diseases;Staphylococcus_aureus_infection	0.000788	0.000143	0.001567	0.000581	0.020721	0.015805	-0.00139	-0.000017
Metabolism;Metabolism of terpenoids and polyketides;Polyketide_sugar_unit_biosynthesis	0.001273	3.92E-05	0.001102	4.39E-05	3.58E-05	0.000528	0.000117	0.000224
Metabolism;Metabolism of cofactors and vitamins;Vitamin_B6_metabolism	0.00127	5.5E-05	0.001147	5.2E-05	0.002	0.004	5.39E-05	0.000192

					645	345		
Organismal Systems;Nervous system;Glutamatergic_synapse	0.001287	3.41E-05	0.001097	0.000116	0.008547	0.009483	6.98E-05	0.000312
Metabolism;Enzyme families;Protein_phosphatase_and_associated_proteins	0.001301	7.07E-05	0.001026	0.000148	0.004244	0.006251	0.000118	0.000433
Unclassified;Metabolism;Carbohydrate_metabolism	0.001315	0.000147	0.001103	3.74E-05	0.015668	0.012748	5.76E-05	0.000365
Unclassified;Metabolism;Amino_acid_metabolism	0.001195	5.78E-05	0.001107	4E-05	0.013495	0.011414	2.31E-05	0.000153
Organismal Systems;Endocrine system;Insulin_signaling_pathway	0.001273	8.54E-05	0.001	6.37E-05	0.000126	0.000098	0.000176	0.000372
Metabolism;Metabolism of cofactors and vitamins;Ubiquinone_and_other_terpenoid-quinone_biosynthesis	0.000957	9.74E-05	0.001156	6.87E-05	0.002744	0.004423	-0.000031	-8.9E-05
Human Diseases;Cardiovascular diseases;Fluid_shear_stress_and_atherosclerosis	0.001166	2.7E-05	0.001026	9.88E-05	0.016498	0.013297	3.66E-05	0.000243
Organismal Systems;Endocrine system;PPAR_signaling_pathway	0.001209	6.31E-05	0.000967	3.82E-05	3.71E-05	0.000528	0.000172	0.000031
Metabolism;Metabolism of other amino acids;beta-Alanine_metabolism	0.001119	0.000151	0.000955	4.08E-05	0.004471	0.002070 2	5.42E-06	0.000322
Environmental Information Processing;Signaling molecules and interaction;Bacterial_toxins	0.000828	8.51E-05	0.001118	0.000234	0.027702	0.018099	-0.000054	-4.4E-05
Unclassified;Viral protein family;Viral_proteins	0.00087	4.86E-05	0.001018	4.82E-05	0.000	0.001	-0.00	-8.6E-

					345	553	021	05
Metabolism;Biosynthesis of other secondary metabolites;Novobiocin_biosynthesis	0.001016	5.43E-05	0.000857	5.38E-05	0.000468	0.001658	8.94E-05	0.000229
Metabolism;Carbohydrate metabolism;Inositol_phosphate_metabolism	0.000854	5.25E-05	0.000953	8.36E-05	0.038817	0.020034	-0.00019	-6.4E-06
Metabolism;Biosynthesis of other secondary metabolites;Tropane,_piperidine_and_pyridine_alkaloid_biosynthesis	0.001043	6.63E-05	0.000836	5.62E-05	0.000184	0.001049	0.000127	0.000286
Cellular Processes;Cellular community - prokaryotes;Biofilm_formation-Pseudomonas_aeruginosa	0.000931	9.62E-05	0.000813	5E-05	0.030701	0.018733	1.43E-05	0.000221
Metabolism;Biosynthesis of other secondary metabolites;Acarbose_and_validamycin_biosynthesis	0.000751	2.85E-05	0.000637	2.76E-05	3.4E-05	0.000528	7.86E-05	0.000151
Metabolism;Lipid metabolism;Synthesis_and_degradation_of_ketone_bodies	0.000626	3.57E-05	0.000714	6.61E-05	0.021124	0.001583	-0.00016	-1.7E-05
Metabolism;Biosynthesis of other secondary metabolites;Carbapenem_biosynthesis	0.000704	1.62E-05	0.000657	3.2E-05	0.014556	0.012073	1.22E-05	8.07E-05
Environmental Information Processing;Signal transduction;AMPK_signaling_pathway	0.000694	4.69E-05	0.000558	5.71E-05	0.001228	0.003019	6.87E-05	0.000204
Metabolism;Xenobiotics biodegradation and metabolism;Drug_metabolism-cytochrome_P450	0.000515	3.98E-05	0.000629	0.000103	0.042406	0.020702	-0.00022	-5.3E-06
Genetic Information Processing;Folding, sorting and degradation;Protein_processing_in_endoplasmic_reticulum	0.000645	2.96E-05	0.000512	0.000115	0.036719	0.020034	1.16E-05	0.000253
Metabolism;Xenobiotics biodegradation and metabolism;Metabolism_of_xenobiotics_by_cytochrome_P450	0.000478	4.11E-05	0.00059	0.000101	0.041763	0.020770	-0.00022	-5.7E-06

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Environmental Information Processing;Signal transduction;Phosphatidylinositol_signaling_system	0.000471	3.16E-05	0.000551	3.83E-05	0.002862	0.004527	-0.00013	-3.5E-05
Metabolism;Metabolism of cofactors and vitamins;Retinol_metabolism	0.000448	4.19E-05	0.000565	9.17E-05	0.024724	0.016912	-0.00021	-2E-05
Unclassified;Metabolism;Nucleotide_metabolism	0.000424	6.16E-05	0.000559	7.62E-05	0.007571	0.00874	-0.00022	-4.5E-05
Unclassified;Metabolism;Biosynthesis_and_biodegradation_of_secondary_metabolites	0.000552	8.08E-05	0.000446	4.36E-05	0.022652	0.016682	1.95E-05	0.000194
Cellular Processes;Cell growth and death;Ferroptosis	0.000572	3.2E-05	0.000384	4.92E-05	3.43E-05	0.000528	0.000133	0.000242
Environmental Information Processing;Signal transduction;PI3K-Akt_signaling_pathway	0.000531	3.76E-05	0.000404	0.000105	0.029838	0.018642	1.7E-05	0.000237
Metabolism;Metabolism of terpenoids and polyketides;Biosynthesis_of_vancomycin_group_antibiotics	0.000483	1.79E-05	0.000414	2.67E-05	0.000537	0.0017	3.98E-05	9.93E-05
Genetic Information Processing;Folding, sorting and degradation;Proteasome	0.000566	5.42E-05	0.000391	0.000103	0.006859	0.008253	6.44E-05	0.000286
Organismal Systems;Endocrine system;Adipocytokine_signaling_pathway	0.000557	4.04E-05	0.000034	6.34E-05	7.79E-05	0.000832	0.000147	0.000287
Metabolism;Biosynthesis of other secondary metabolites;Isoquinoline_alkaloid_biosynthesis	0.000477	1.95E-05	0.000398	4.93E-05	0.008874	0.009537	2.78E-05	0.000132
Human Diseases;Cancers;Prostate_cancer	0.000502	2.81E-05	0.000392	0.000103	0.046448	0.002079	2.4E-06	0.000217

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Organismal Systems;Immune system;IL-17_signaling_pathway	0.000497	2.85E-05	0.000387	0.000101	0.044528	0.020702	3.75E-06	0.000216
Organismal Systems;Endocrine system;Estrogen_signaling_pathway	0.000497	2.84E-05	0.000387	0.000101	0.044832	0.020702	3.53E-06	0.000216
Organismal Systems;Endocrine system;Progesterone-mediated_oocyte_maturation	0.000497	2.84E-05	0.000387	0.000101	0.044832	0.020702	3.53E-06	0.000216
Organismal Systems;Immune system;Th17_cell_differentiation	0.000497	2.84E-05	0.000387	0.000101	0.044832	0.020702	3.53E-06	0.000216
Organismal Systems;Immune system;Antigen_processing_and_presentation	0.000497	2.84E-05	0.000387	0.000101	0.044832	0.020702	3.53E-06	0.000216
Metabolism;Metabolism of other amino acids;Phosphonate_and_phosphinate_metabolism	0.000382	2.97E-05	0.000323	2.78E-05	0.005649	0.007424	2.13E-05	9.54E-05
Metabolism;Biosynthesis of other secondary metabolites;Glucosinolate_biosynthesis	0.000341	3.28E-05	0.000272	1.21E-05	0.002427	0.004186	3.48E-05	0.000104
Unclassified;Genetic information processing;Translation_proteins	0.000322	2.64E-05	0.000271	1.91E-05	0.003657	0.005568	2.15E-05	8.15E-05
Genetic Information Processing;Translation;Ribosome_biogenesis_in_eukaryotes	0.000305	7.95E-06	0.000294	8E-06	0.003188	0.001918	1.21E-06	2.17E-05
Environmental Information Processing;Signal transduction;FoxO_signaling_pathway	0.0002	3.42E-05	0.000294	5.37E-05	0.006194	0.007669	-0.000015	-3.5E-05
Metabolism;Glycan biosynthesis and metabolism;N-Glycan_biosynthesis	0.000331	9.81E-06	0.000253	2.52E-05	0.000028	0.001443	5.17E-05	0.000105

					3	9		
Organismal Systems;Aging;Longevity_regulating_pathway-mammal	0.00017	2.6E-05	0.000284	5.38E-05	0.002025	0.004094	-0.00017	-5.6E-05
Human Diseases;Cancers;Renal_cell_carcinoma	0.00017 3	2.1E-05	0.000251	5.73E-06	0.000149	0.000098	-0.0001	-5.6E-05
Environmental Information Processing;Signal transduction;MAPK_signaling_pathway-plant	0.00014 4	2.46E-05	0.000255	4.18E-05	0.000468	0.001658	-0.00016	-6.6E-05
Human Diseases;Neurodegenerative diseases;Huntingtons_disease	0.00015 1	1.32E-05	0.000223	5.85E-05	0.002842 9	0.001826	-0.00013	-1.1E-05
Metabolism;Metabolism of terpenoids and polyketides;Nonribosomal_peptide_structures	0.00015 1	7.28E-06	0.000191	2.38E-05	0.000776	0.000857	-6.5E-05	-1.5E-05
Metabolism;Glycan biosynthesis and metabolism;Glycosaminoglycan_binding_proteins	0.00017 1	1.89E-05	0.000129	2.28E-05	0.000595 6	0.000759 4	1.53E-05	6.95E-05
Organismal Systems;Digestive system;Protein_digestion_and_absorption	0.00024 1	5.39E-05	0.000134	5.61E-05	0.000749 5	0.000874	3.53E-05	0.000177
Environmental Information Processing;Signaling molecules and interaction;CD_Molecules	0.00023 8	5.41E-05	0.000129	5.88E-05	0.000732 6	0.000869 3	3.68E-05	0.000182
Human Diseases;Neurodegenerative diseases;Amyotrophic_lateral_sclerosis_(ALS)	9.5E-05	1.98E-05	0.000171	3.73E-05	0.002499	0.004186	-0.00012	-3.6E-05
Genetic Information Processing;Folding, sorting and degradation;Ubiquitin_system	0.00015 3	3.86E-06	0.00016	4.01E-06	0.000942 5	0.000958 5	-1.2E-05	-2.2E-06
Environmental Information Processing;Signal transduction;MAPK_signaling_pathway-yeast	6.68E-05	1.96E-05	0.000151	3.85E-05	0.001174	0.003445	-0.00013	-4.3E-05

					6	5		
Environmental Information Processing;Signal transduction;MAPK_signaling_pathwayfly	0.000104	8.44E-06	0.000133	1.86E-05	0.009917	0.009706	-4.9E-05	-9.5E-06
Metabolism;Biosynthesis of other secondary metabolites;Flavone_and_flavonol_biosynthesis	0.000118	8.7E-06	0.000105	5.92E-06	0.012997	0.011216	3.58E-06	2.31E-05
Human Diseases;Cancers;Choline_metabolism_in_cancer	9.26E-05	7.18E-06	0.000117	5.01E-06	9.04E-05	0.000858	-3.2E-05	-1.6E-05
Genetic Information Processing;Replication and repair;Non-homologous_end-joining	9.66E-05	1.61E-05	7.88E-05	7.5E-06	0.004381 6	0.002070 2	6.49E-07	3.49E-05
Environmental Information Processing;Signal transduction;Phospholipase_D_signaling_pathway	7.63E-05	6.87E-06	0.000102	6.17E-06	4.38E-05	0.000535	-3.4E-05	-1.8E-05
Genetic Information Processing;Transcription;Basal_transcription_factors	7.48E-05	9.1E-06	5.9E-05	4.71E-06	0.006124	0.007669	6.02E-06	2.55E-05
Metabolism;Lipid metabolism;alpha-Linolenic_acid_metabolism	3.57E-05	1.21E-05	5.48E-05	1.65E-05	0.004747	0.002099 3	-3.8E-05	-2.6E-07
Organismal Systems;Endocrine system;Renin-angiotensin_system	6.03E-05	1.93E-05	2.79E-05	1.29E-05	0.007966	0.008954	1.09E-05	5.39E-05
Cellular Processes;Cell growth and death;Meiosis-yeast	3.84E-05	1.22E-05	1.95E-05	1.4E-06	0.012394	0.010916	6.11E-06	3.16E-05
Metabolism;Xenobiotics biodegradation and metabolism;Steroid_degradation	4.6E-05	2.1E-05	2.09E-05	2.37E-06	0.003226	0.001934	3.06E-06	4.71E-05
Human Diseases;Infectious diseases;African_trypanosomiasis	3.1E-05	1.36E-05	1.27E-05	5.55E-06	0.001918	0.001546 9	3.94E-06	3.26E-05

Human Diseases;Infectious diseases;Chagas_disease_(American_trypanosomiasis)	2.83E-05	1.31E-05	9.67E-06	5.34E-06	0.015649	0.012748	4.83E-06	3.25E-05
Human Diseases;Neurodegenerative diseases;Prion_diseases	2.71E-05	3.06E-06	1.99E-05	2.06E-06	0.001095	0.003019	3.76E-06	1.06E-05
Metabolism;Metabolism of terpenoids and polyketides;Type_I_polyketide_structures	9.57E-06	5.92E-06	1.81E-06	4.14E-07	0.002367	0.001691 2	1.54E-06	1.4E-05
Metabolism;Lipid metabolism;Steroid_biosynthesis	4.22E-06	2.71E-06	1.11E-06	4.34E-07	0.003743 6	0.002003 4	2.62E-07	5.95E-06
Genetic Information Processing;Replication and repair;Fanconi_anemia_pathway	1.83E-06	3.84E-07	1.22E-06	2.53E-07	0.001032 4	0.000991	1.85E-07	1.04E-06
Human Diseases;Cancers;Bladder_cancer	6.04E-06	3.41E-06	8.4E-07	2.57E-07	0.001335 1	0.011405	1.63E-06	8.78E-06
Metabolism;Biosynthesis of other secondary metabolites;Stilbenoid,_diarylheptanoid_and_gingerol_biosynthesis	3.46E-06	5.49E-07	2.61E-06	4.99E-07	0.001905 1	0.001504 2	1.71E-07	1.52E-06
Metabolism;Biosynthesis of other secondary metabolites;Flavonoid_biosynthesis	3.46E-06	5.49E-07	2.61E-06	4.99E-07	0.001905 1	0.001504 2	1.71E-07	1.52E-06
Organismal Systems;Digestive system;Bile_secretion	2.19E-06	1.48E-06	5.04E-07	1.75E-07	0.003805 4	0.002003 4	1.36E-07	3.24E-06
Organismal Systems;Endocrine system;Oxytocin_signaling_pathway	1.42E-06	9.88E-07	2.69E-07	1.46E-07	0.003561 3	0.002003 4	1.13E-07	2.18E-06
Metabolism;Metabolism of terpenoids and polyketides;Biosynthesis_of_12-,_14-_and_16-membered_macrolides	3.33E-06	2.17E-06	5.4E-07	1.34E-07	0.002520 8	0.001695 6	5.16E-07	5.06E-06

Genetic Information Processing;Translation;mRNA_surveillance_pathway	6.58E-07	4.11E-07	2.08E-07	5.96E-08	0.043396	0.020702	1.94E-08	8.8E-07
Metabolism;Metabolism of terpenoids and polyketides;Biosynthesis_of_enediyne_antibiotics	2.58E-06	1.55E-06	4.91E-07	2.05E-07	0.021067	0.01583	4.64E-07	3.71E-06
Environmental Information Processing;Signal transduction;cAMP_signaling_pathway	3.15E-06	2.21E-06	3.89E-07	1.16E-07	0.027999	0.018121	4.44E-07	5.08E-06
Metabolism;Xenobiotics biodegradation and metabolism;Bisphenol_degradation	2.34E-06	1.44E-06	5.92E-07	3.24E-07	0.030626	0.018733	2.34E-07	3.25E-06
Metabolism;Biosynthesis of other secondary metabolites;Isoflavonoid_biosynthesis	4.08E-07	1.61E-07	1.97E-06	1.41E-06	0.041675	0.020702	-3E-06	-8.6E-08
Organismal Systems;Nervous system;Retrograde_endocannabinoid_signaling	1.11E-06	7.26E-07	2.69E-07	1.64E-07	0.035054	0.020034	8.47E-08	1.6E-06
Organismal Systems;Endocrine system;Regulation_of_lipolysis_in_adipocyte	1.11E-06	7.26E-07	2.69E-07	1.64E-07	0.035054	0.020034	8.47E-08	1.6E-06
Human Diseases;Cardiovascular diseases;Hypertrophic_cardiomyopathy_(HCM)	2.38E-07	9.33E-08	3.92E-09	8.5E-09	0.001587	0.003306	1.36E-07	3.32E-07
Organismal Systems;Endocrine system;Renin_secretion	2.38E-07	9.33E-08	3.92E-09	8.5E-09	0.001587	0.003306	1.36E-07	3.32E-07
Environmental Information Processing;Signal transduction;Sphingolipid_signaling_pathway	7.27E-07	4.34E-07	1.8E-07	8.06E-08	0.026428	0.017638	9.32E-08	1E-06
Human Diseases;Infectious diseases;Vibrio_cholerae_infection	7.42E-07	4.06E-07	2.59E-07	4.56E-08	0.032847	0.019352	5.79E-08	9.08E-07

Organismal Systems;Immune system;Fc_gamma_R-mediated_phagocytosis	7.14E-07	4.29E-07	1.63E-07	8.71E-08	0.024663	0.0169122	1.02E-07	1E-06
Cellular Processes;Transport and catabolism;Endocytosis	7.14E-07	4.29E-07	1.63E-07	8.71E-08	0.024663	0.0169122	1.02E-07	1E-06
Organismal Systems;Endocrine system;GnRH_signaling_pathway	7.14E-07	4.29E-07	1.63E-07	8.71E-08	0.024663	0.0169122	1.02E-07	1E-06
Environmental Information Processing;Signal transduction;Ras_signaling_pathway	7.14E-07	4.29E-07	1.63E-07	8.71E-08	0.024663	0.0169122	1.02E-07	1E-06
Human Diseases;Infectious diseases;Pathogenic_Escherichia_coli_infection	6E-07	3.16E-07	2.38E-07	1.56E-07	0.038692	0.0200034	2.47E-08	7E-07
Metabolism;Glycan biosynthesis and metabolism;Glycosylphosphatidylinositol(GPI)-anchor_biosynthesis	8.38E-07	7.9E-07	8.43E-09	6.81E-09	0.049832	0.0218331	8.95E-10	1.66E-06
Organismal Systems;Endocrine system;Ovarian_Steroidogenesis	3.18E-07	2.43E-07	2.55E-08	1.25E-08	0.031973	0.001918	3.73E-08	5.47E-07
Organismal Systems;Digestive system;Pancreatic_secretion	2.51E-07	1.78E-07	4.81E-08	3.38E-08	0.037784	0.0200034	1.65E-08	3.89E-07
Environmental Information Processing;Signal transduction;cGMP-PKG_signaling_pathway	2.45E-07	1.74E-07	4.81E-08	3.38E-08	0.038398	0.0200034	1.52E-08	3.79E-07
Organismal Systems;Circulatory system;Adrenergic_signaling_in_cardiomyocytes	2.45E-07	1.74E-07	4.81E-08	3.38E-08	0.038398	0.0200034	1.52E-08	3.79E-07
Organismal Systems;Endocrine system;Insulin_secretion	2.45E-07	1.74E-07	4.81E-08	3.38E-08	0.038398	0.0200034	1.52E-08	3.79E-07

Organismal Systems;Digestive system;Salivary_secretion	2.45E-07	1.74E-07	4.81E-08	3.38E-08	0.038398	0.020034	1.52E-08	3.79E-07
Organismal Systems;Excretory system;Endocrine_and_other_factor-regulated_calcium_reabsorption	2.45E-07	1.74E-07	4.81E-08	3.38E-08	0.038398	0.020034	1.52E-08	3.79E-07
Organismal Systems;Digestive system;Gastric_acid_secretion	2.45E-07	1.74E-07	4.81E-08	3.38E-08	0.038398	0.020034	1.52E-08	3.79E-07
Organismal Systems;Excretory system;Aldosterone-regulated_sodium_reabsorption	2.45E-07	1.74E-07	4.81E-08	3.38E-08	0.038398	0.020034	1.52E-08	3.79E-07
Organismal Systems;Nervous system;Cholinergic_synapse	4.28E-08	1.78E-08	2.62E-07	1.94E-07	0.038928	0.020034	-4.2E-07	-1.6E-08
Environmental Information Processing;Signal transduction;Calcium_signaling_pathway	7.35E-08	4.47E-08	1.74E-09	1.24E-09	0.011002	0.010236	2.49E-08	1.19E-07
Cellular Processes;Transport and catabolism;Phagosome	2.94E-08	2.02E-08	6.79E-09	5.38E-09	0.040009	0.020467	1.45E-09	4.37E-08
Environmental Information Processing;Signal transduction;TNF_signaling_pathway	2.23E-07	2.06E-07	1.38E-09	9.89E-10	0.046451	0.02079	5.12E-09	4.37E-07
Human Diseases;Infectious diseases;Leishmaniasis	2.23E-07	2.06E-07	1.38E-09	9.89E-10	0.046451	0.02079	5.12E-09	4.37E-07
Environmental Information Processing;Signal transduction;VEGF_signaling_pathway	2.23E-07	2.06E-07	1.38E-09	9.89E-10	0.046451	0.02079	5.12E-09	4.37E-07
Environmental Information Processing;Signal transduction;NF-kappa_B_signaling_pathway	2.23E-07	2.06E-07	1.38E-09	9.89E-10	0.046451	0.02079	5.12E-09	4.37E-07

Environmental Information Processing;Signaling molecules and interaction;G_protein-coupled_receptors	2.34E-08	1.07E-08	3.9E-09	5.34E-09	0.004475	0.00606652	8.08E-09	3.1E-08
Metabolism;Metabolism of terpenoids and polyketides;Monoterpenoid_biosynthesis	1.94E-08	1.38E-08	1.86E-10	1.57E-10	0.019193	0.01504 2	4.7E-09	3.37E-08
Organismal Systems;Excretory system;Vasopressin-regulated_water_reabsorption	4.89E-08	3.88E-08	5.81E-09	5.23E-09	0.0417	0.0020702	2.36E-09	8.37E-08
Organismal Systems;Nervous system;Synaptic_vesicle_cycle	4.89E-08	3.88E-08	5.81E-09	5.23E-09	0.0417	0.0020702	2.36E-09	8.37E-08
Organismal Systems;Immune system;Hematopoietic_cell_lineage	3.63E-08	2.98E-08	4.63E-10	3.75E-10	0.032105	0.001918	4.55E-09	6.71E-08
Environmental Information Processing;Signaling molecules and interaction;Neuroactive_ligand-receptor_interaction	5.88E-09	4.51E-09	5.53E-11	5.24E-11	0.02494 4	0.0016912	1.09E-09	1.06E-08
Metabolism;Glycan biosynthesis and metabolism;Glycosaminoglycan_biosynthesis-chondroitin_sulfate/dermatan_sulfate	2.39E-09	1.68E-09	1.75E-10	3.9E-10	0.022401	0.001664 1	4.52E-10	3.97E-09
Metabolism;Biosynthesis of other secondary metabolites;Clavulanic_acid_biosynthesis	7.9E-10	6.83E-10	3.61E-11	5.72E-11	0.042469	0.0020702	3.76E-11	1.47E-09

Table 2c Young+PBS VS Young+Old								
Taxa	avg(Young+PBS)	sd(Young+PBS)	avg(Young+Old)	sd(Young+Old)	p.value	q.values	interval lower	interval upper
Human Diseases;Endocrine and metabolic diseases;Insulin_resistance	0.001786	0.000103	0.001621	0.000144	0.048676	1	1.19E-06	0.000329
Organismal Systems;Nervous system;GABAergic_synapse	0.001346	3.61E-05	0.001278	5.28E-05	0.030139	1	8.12E-06	0.000127
Organismal Systems;Nervous system;Glutamatergic_synapse	0.001287	3.41E-05	0.00122	4.1E-05	0.019334	1	1.85E-05	0.000116
Human Diseases;Immune diseases;Primary_immunodeficiency	0.00037	1.16E-05	0.000352	1.56E-05	0.048671	1	1.3E-07	3.59E-05

Table 2d Young+PBS VS Young+Aged								
Taxa	avg(Young+PBS)	sd(Young+PBS)	avg(Young+Aged)	sd(Young+Aged)	p.value	q.values	interval lower	interval upper
Genetic Information Processing;Replication and repair;DNA_repair_and_recombination_proteins	0.035677	0.000782	0.036768	0.000855	0.043973	0.134546	-0.00215	########
Genetic Information Processing;Translation;Transfer_RNA_biogenesis	0.025388	0.000319	0.026138	0.000489	0.012424	0.090629	-0.00129	-0.00021
Metabolism;Nucleotide metabolism;Pyrimidine_metabolism	0.020031	0.000437	0.020771	0.000134	0.011107	0.090629	-0.00114	-0.00022
Genetic Information Processing;Translation;Ribosome	0.015723	0.000269	0.016378	0.000499	0.023175	0.119455	-0.00119	-0.00012
Genetic Information Processing;Translation;Aminoacyl-tRNA_biosynthesis	0.015499	0.000262	0.016244	0.000466	0.009445	0.087226	-0.00125	-0.00024
Genetic Information Processing;Replication and repair;DNA_replication_proteins	0.014807	0.000271	0.015335	0.000017	0.003	0.069	-0.00	-0.00

					349	354	083	023
Genetic Information Processing;Replication and repair;Chromosome_and_associated_proteins	0.0131	0.000238	0.013777	0.000364	0.004466	0.070819	-0.00108	-0.00027
Metabolism;Carbohydrate metabolism;Amino_sugar_and_nucleotide_sugar_metabolism	0.012636	0.000357	0.01204	0.000343	0.014709	0.094612	0.000145	0.001046
Metabolism;Amino acid metabolism;Alanine,_aspartate_and_glutamate_metabolism	0.011744	0.000114	0.011257	0.000426	0.037153	0.134546	4.10E-05	0.000934
Genetic Information Processing;Replication and repair;Mismatch_repair	0.010868	0.000292	0.011363	0.00034	0.022536	0.119455	-0.00009	######
Genetic Information Processing;Replication and repair;Homologous_recombination	0.010494	0.000212	0.010857	0.00027	0.027869	0.123562	-0.00068	######
Metabolism;Glycan biosynthesis and metabolism;Peptidoglycan_biosynthesis_and_degradation_proteins	0.010172	0.000138	0.010445	0.000238	0.041075	0.134546	-0.00053	######
Metabolism;Amino acid metabolism;Cysteine_and_methionine_metabolism	0.009602	7.40E-05	0.009104	0.000299	0.008535	0.087226	0.000185	0.000811
Genetic Information Processing;Replication and repair;Nucleotide_excision_repair	0.008149	0.000239	0.008565	0.00011	0.006039	0.075785	-0.00067	-0.00016
Genetic Information Processing;Replication and repair;DNA_replication	0.007972	0.000183	0.008364	0.000195	0.004922	0.072772	-0.00064	-0.00015
Metabolism;Carbohydrate metabolism;Glyoxylate_and_dicarboxylate_metabolism	0.007261	0.000272	0.006725	0.000464	0.040322	0.134546	3.01E-05	0.001041
Metabolism;Amino acid metabolism;Arginine_biosynthesis	0.005337	0.000206	0.004779	0.000157	0.000	0.069	0.000032	0.000796

					449	354		
Metabolism;Metabolism of terpenoids and polyketides;Terpenoid_backbone_biosynthesis	0.004268	0.000118	0.004527	0.000127	0.004455	0.0708819	-0.00042	-0.0001
Metabolism;Energy metabolism;Nitrogen_metabolism	0.004843	0.000271	0.004204	0.000351	0.005994	0.07585	0.000232	0.001047
Genetic Information Processing;Replication and repair;Base_excision_repair	0.003915	6.37E-05	0.0041	8.30E-05	0.001761	0.069354	-0.00028	########
Metabolism;Lipid metabolism;Fatty_acid_biosynthesis	0.004229	6.55E-05	0.004002	0.000185	0.028734	0.1248876	3.25E-05	0.000422
Metabolism;Metabolism of cofactors and vitamins;Pantothenate_and_CoA_biosynthesis	0.004248	0.000113	0.00396	0.000119	0.001548	0.069354	0.000139	0.000437
Metabolism;Glycan biosynthesis and metabolism;Glycosyltransferases	0.003709	0.000154	0.003935	0.000121	0.018867	0.107022	-0.00041	########
Metabolism;Amino acid metabolism;Valine,_leucine_and_isoleucine_biosynthesis	0.003878	0.000305	0.003116	0.000348	0.002468	0.069354	0.000034	0.001184
Genetic Information Processing;Transcription;RNA_polymerase	0.003305	7.00E-05	0.003506	0.000183	0.042956	0.1345466	-0.00039	########
Metabolism;Carbohydrate metabolism;Pentose_and_glucuronate_interconversions	0.003491	7.67E-05	0.003023	0.000435	0.004564 8	0.134546	1.30E-05	0.000923
Metabolism;Glycan biosynthesis and metabolism;Other_glycan_degradation	0.002999	0.000399	0.00215	0.000652	0.025313	0.1218166	0.000134	0.001565
Metabolism;Lipid metabolism;Sphingolipid_metabolism	0.003031	0.000248	0.002382	0.000423	0.0116	0.090	0.000189	0.001111

					33	629		
Cellular Processes;Cell motility;Cytoskeleton_proteins	0.002762	6.51E-05	0.002963	0.00012	0.007768	0.08604 7	-0.00033	######
Cellular Processes;Cellular community - prokaryotes;Biofilm_formation-Escherichia_coli	0.002944	0.00017	0.002471	0.00033	0.015333	0.09461 2	0.00012	0.000827
Metabolism;Metabolism of other amino acids;Glutathione_metabolism	0.002308	0.000124	0.002636	0.000165	0.003442	0.06935 4	-0.00052	-0.00014
Metabolism;Energy metabolism;Sulfur_metabolism	0.0026	9.62E-05	0.002303	0.000253	0.033449	0.13454 6	3.18E-05	0.000563
Metabolism;Amino acid metabolism;Histidine_metabolism	0.002726	0.000184	0.002175	0.000457	0.030885	0.13164 3	6.87E-05	0.001033
Metabolism;Metabolism of other amino acids;Cyanoamino_acid_metabolism	0.002675	7.72E-05	0.002398	0.000263	0.048672	0.13454 6	2.25E-06	0.000552
Cellular Processes;Cell growth and death;Necroptosis	0.002668	0.000129	0.00226	0.000271	0.012258	0.09062 9	0.000119	0.000696
Metabolism;Biosynthesis of other secondary metabolites;Monobactam_biosynthesis	0.002121	3.47E-05	0.002002	8.44E-05	0.016158	0.09461 2	3.02E-05	0.000208
Metabolism;Carbohydrate metabolism;C5-Branched_dibasic_acid_metabolism	0.002032	0.000102	0.001725	0.000275	0.040128	0.13454 6	1.89E-05	0.000597
Human Diseases;Endocrine and metabolic diseases;Insulin_resistance	0.001786	0.000103	0.001511	0.000136	0.003091	0.06935 4	0.000119	0.000432
Metabolism;Metabolism of terpenoids and polyketides;Prenyltransferases	0.001408	5.91E-05	0.001536	0.000108	0.035	0.134	-0.00	######

					041	546	024	
Metabolism;Metabolism of other amino acids;D-Glutamine_and_D-glutamate_metabolism	0.001416	4.80E-05	0.001513	1.80E-05	0.003163	0.069354	-0.00015	######
Metabolism;Glycan biosynthesis and metabolism;Glycosphingolipid_biosynthesis-globo_and_isoglobo_series	0.001423	0.000143	0.001089	0.000203	0.009381	0.087226	0.000104	0.000562
Metabolism;Biosynthesis of other secondary metabolites;Phenylpropanoid_biosynthesis	0.001572	6.81E-05	0.001168	0.000266	0.012665	0.090629	0.000125	0.000683
Metabolism;Metabolism of other amino acids;D-Alanine_metabolism	0.001168	4.12E-05	0.001231	5.04E-05	0.039106	0.134546	-0.00012	######
Metabolism;Enzyme families;Protein_phosphatase_and_associated_proteins	0.001301	7.07E-05	0.001132	0.000133	0.026147	0.121816	2.62E-05	0.000312
Unclassified;Metabolism;Carbohydrate_metabolism	0.001315	0.000147	0.001074	0.000171	0.026382	0.121816	3.48E-05	0.000446
Unclassified;Metabolism;Amino_acid_metabolism	0.001195	5.78E-05	0.000991	0.000177	0.036034	0.134546	1.84E-05	0.000039
Organismal Systems;Endocrine system;Insulin_signaling_pathway	0.001273	8.54E-05	0.001024	0.000108	0.001465	0.069354	0.000123	0.000375
Human Diseases;Cardiovascular diseases;Fluid_shear_stress_and_atherosclerosis	0.001166	2.70E-05	0.001097	5.46E-05	0.025061	0.121816	1.15E-05	0.000128
Unclassified;Viral protein family;Viral_proteins	0.000887	4.86E-05	0.000977	7.30E-05	0.016166	0.094612	-0.00019	######
Metabolism;Glycan biosynthesis and metabolism;Glycosaminoglycan_degradation	0.000925	0.000151	0.000613	0.000289	0.048	0.134	2.96E-06	0.000623

					334	546		
Metabolism;Biosynthesis of other secondary metabolites;Novobiocin_biosynthesis	0.001016	5.43E-05	0.000844	0.000111	0.010474	0.090629	5.42E-05	0.000291
Metabolism;Biosynthesis of other secondary metabolites;Tropane,_piperidine_and_pyridine_alkaloid_biosynthesis	0.001043	6.63E-05	0.000839	0.00011	0.004473	0.070819	8.29E-05	0.000324
Cellular Processes;Transport and catabolism;Lysosome	0.00084	0.0002	0.000481	0.000212	0.013085	0.090629	9.33E-05	0.000623
Metabolism;Carbohydrate metabolism;Ascorbate_and_aldarate_metabolism	0.000764	6.21E-05	0.000573	0.000134	0.015269	0.094612	4.95E-05	0.000334
Metabolism;Metabolism of terpenoids and polyketides;Biosynthesis_of_ansamycins	0.000844	2.70E-05	0.000795	3.53E-05	0.022916	0.119455	8.50E-06	9.01E-05
Environmental Information Processing;Signal transduction;AMPK_signaling_pathway	0.000694	4.69E-05	0.000612	4.03E-05	0.009321	0.087226	2.51E-05	0.000138
Unclassified;Metabolism;Glycan_biosynthesis_and_metabolism	0.000642	4.08E-05	0.000535	5.04E-05	0.002677	0.069354	4.70E-05	0.000166
Metabolism;Biosynthesis of other secondary metabolites;Neomycin,_kanamycin_and_gentamicin_biosynthesis	0.000529	2.51E-05	0.000498	1.48E-05	0.034166	0.134546	2.89E-06	5.76E-05
Metabolism;Biosynthesis of other secondary metabolites;Glucosinolate_biosynthesis	0.000341	3.28E-05	0.000253	3.57E-05	0.001223	0.069354	4.43E-05	0.000133
Human Diseases;Cancers;Renal_cell_carcinoma	0.000173	2.10E-05	0.000265	7.42E-05	0.027875	0.123562	-0.00017	########
Metabolism;Lipid metabolism;Steroid_hormone_biosynthesis	0.000174	6.14E-05	6.77E-05	3.33E-05	0.006	0.075	4.02E-05	0.000173

					16	85		
Organismal Systems;Digestive system;Carbohydrate_digestion_and_absorption	0.000114	3.23E-05	6.80E-05	2.74E-05	0.024285	0.121816	7.38E-06	8.47E-05
Metabolism;Biosynthesis of other secondary metabolites;Flavone_and_flavonol_biosynthesis	0.000118	8.70E-06	9.90E-05	1.79E-05	0.047375	0.134546	2.94E-07	3.85E-05
Metabolism;Enzyme families;Cytochrome_P450	7.32E-05	2.56E-05	4.52E-05	1.33E-05	0.047224	0.134546	4.43E-07	5.55E-05
Metabolism;Xenobiotics biodegradation and metabolism;Steroid_degradation	4.60E-05	2.10E-05	2.35E-05	6.47E-06	0.046449	0.134546	4.85E-07	4.46E-05
Human Diseases;Infectious diseases;African_trypanosomiasis	3.10E-05	1.36E-05	1.14E-05	8.24E-06	0.016221	0.009461 2	4.65E-06	3.44E-05
Human Diseases;Infectious diseases;Chagas_disease_(American_trypanosomiasis)	2.83E-05	1.31E-05	8.78E-06	7.11E-06	0.013033	0.009062 9	5.41E-06	3.37E-05
Human Diseases;Neurodegenerative diseases;Prion_diseases	2.71E-05	3.06E-06	2.13E-05	4.88E-06	0.037345	0.134546	4.31E-07	1.12E-05
Genetic Information Processing;Translation;mRNA_surveillance_pathway	6.58E-07	4.11E-07	1.73E-07	1.07E-07	0.033088	0.134546	5.52E-08	9.15E-07
Human Diseases;Immune diseases;Systemic_lupus_erythematosus	5.61E-07	2.55E-07	2.84E-07	1.41E-07	0.049627	0.134546	5.77E-10	5.52E-07
Human Diseases;Infectious diseases;Vibrio_cholerae_infection	7.42E-07	4.06E-07	1.88E-07	8.80E-08	0.019479	0.107933	1.29E-07	9.79E-07
Organismal Systems;Endocrine system;Ovarian_Steroidogenesis	3.18E-07	2.43E-07	5.52E-08	4.16E-08	0.045117	0.134 54	8.14E-09	5.17E-07

					5	6		
Environmental Information Processing;Signaling molecules and interaction;G_protein-coupled_receptors	2.34E-08	1.07E-08	4.33E-09	9.00E-09	0.007765	0.08604 7	6.32E-09	3.19E-08
Organismal Systems;Excretory system;Vasopressin-regulated_water_reabsorption	4.89E-08	3.88E-08	6.63E-09	6.05E-09	0.044322	0.134546	1.55E-09	8.29E-08
Organismal Systems;Nervous system;Synaptic_vesicle_cycle	4.89E-08	3.88E-08	6.63E-09	6.05E-09	0.044322	0.134546	1.55E-09	8.29E-08
Organismal Systems;Immune system;Hematopoietic_cell_lineage	3.63E-08	2.98E-08	3.89E-09	6.43E-09	0.044217	0.134546	1.20E-09	6.36E-08
Environmental Information Processing;Signaling molecules and interaction;Neuroactive_ligand-receptor_interaction	5.88E-09	4.51E-09	8.81E-10	1.21E-09	0.041204	0.134546	2.81E-10	9.72E-09
Metabolism;Glycan biosynthesis and metabolism;Glycosaminoglycan_biosynthesis-chondroitin_sulfate/dermatan_sulfate	2.39E-09	1.68E-09	5.30E-10	5.60E-10	0.041994	0.134546	9.26E-11	3.62E-09
Metabolism;Biosynthesis of other secondary metabolites;Clavulanic_acid_biosynthesis	7.90E-10	6.83E-10	1.51E-11	1.23E-11	0.038954	0.134546	5.80E-11	1.49E-09

Table 2e Old+PBS VS Old+Young								
Taxa	avg(Old+PBS)	sd(Old+PBS)	avg(Old+Young)	sd(Old+Young)	p.value	q.values	interval lower	interval upper
Genetic Information Processing;Replication and repair;DNA_replication_proteins	0.015186	0.000287	0.014866	9.1E-05	0.040875	0.279366	1.84E-05	0.000062
Metabolism;Carbohydrate metabolism;Pyruvate_metabolism	0.012992	0.000385	0.013656	0.000423	0.01754	0.279366	-0.00119	-0.00014
Genetic Information Processing;Replication and repair;Nucleotide_excision_repair	0.008575	0.000252	0.008078	0.000267	0.007815	0.279366	0.000163	0.000832
Metabolism;Amino acid metabolism;Glycine,_serine_and_threonine_metabolism	0.007344	0.000264	0.00785	0.000189	0.004085	0.279366	-0.00081	-0.00021
Metabolism;Carbohydrate metabolism;Butanoate_metabolism	0.006856	0.000234	0.00723	0.000214	0.016388	0.279366	-0.00066	-8.5E-05
Metabolism;Amino acid metabolism;Lysine_biosynthesis	0.006577	0.000222	0.006169	0.000258	0.015102	0.279366	9.81E-05	0.000072
Cellular Processes;Cell motility;Bacterial_chemotaxis	0.006541	0.000982	0.005025	0.001103	0.030986	0.279366	0.000017	0.002862
Metabolism;Carbohydrate metabolism;Fructose_and_mannose_metabolism	0.005034	0.000448	0.005551	0.000339	0.049861	0.298923	-0.00103	-3.9E-07
Cellular Processes;Cell growth and death;Cell_cycle-Caulobacter	0.005301	0.000205	0.005009	0.000152	0.020387	0.279366	5.65E-05	0.000527
Metabolism;Lipid metabolism;Lipid_biosynthesis_proteins	0.004195	0.000254	0.004486	8.15E-05	0.036704	0.279366	-0.00056	-2.5E-05
Metabolism;Metabolism of terpenoids and polyketides;Terpenoid_backbone_biosynthesis	0.004511	0.000132	0.004343	3.68E-05	0.024962	0.274426	2.99E-05	0.000306
Metabolism;Lipid metabolism;Glycerophospholipid_metabolism	0.00449	0.000018	0.004242	0.000119	0.020808	0.279366	4.8E-05	0.000449
Human Diseases;Drug resistance;beta-Lactam_resistance	0.003551	0.000255	0.004224	0.000625	0.037756	0.279366	-0.00137	-5.4E-05

Metabolism;Amino acid metabolism;Valine,_leucine_and_isoleucine_biosynthesis	0.00317	0.000297	0.00354	8.59E-05	0.026938	0.279366	-0.00068	-6E-05
Metabolism;Energy metabolism;Photosynthesis_proteins	0.00297	8.72E-05	0.002711	0.000103	0.000901	0.153405	0.000136	0.000382
Metabolism;Energy metabolism;Photosynthesis	0.002958	8.61E-05	0.002693	0.000108	0.000962	0.153405	0.000138	0.000391
Metabolism;Amino acid metabolism;Valine,_leucine_and_isoleucine_degradation	0.002337	0.00011	0.002675	0.000237	0.015654	0.248807	-0.00059	-8.6E-05
Cellular Processes;Cell motility;Cytoskeleton_proteins	0.002926	6.67E-05	0.002601	0.000202	0.009175	0.243757	0.000114	0.000537
Metabolism;Amino acid metabolism;Histidine_metabolism	0.002115	0.000282	0.002464	5.24E-05	0.028246	0.279366	-0.00064	-5.4E-05
Metabolism;Amino acid metabolism;Phenylalanine_metabolism	0.00157	9.34E-05	0.001676	4.86E-05	0.04133	0.279366	-0.00021	-5.4E-06
Metabolism;Metabolism of other amino acids;D-Glutamine_and_D-glutamate_metabolism	0.001524	5.98E-05	0.001445	3.28E-05	0.022738	0.274426	1.43E-05	0.000144
Metabolism;Metabolism of cofactors and vitamins;Vitamin_B6_metabolism	0.001084	0.000119	0.001216	4.67E-05	0.041152	0.279366	-0.00026	-7.2E-06
Unclassified;Metabolism;Carbohydrate_metabolism	0.001019	0.000118	0.001209	6.85E-05	0.008989	0.243757	-0.00032	-6.2E-05
Metabolism;Metabolism of other amino acids;beta-Alanine_metabolism	0.000946	6.77E-05	0.001021	3.03E-05	0.042008	0.279366	-0.00015	-3.6E-06
Environmental Information Processing;Signaling molecules and interaction;Bacterial_toxins	0.000835	0.000179	0.001174	0.000293	0.041303	0.279366	-0.00066	-1.7E-05
Metabolism;Glycan biosynthesis and metabolism;Glycosaminoglycan_degradation	0.000591	0.000218	0.000871	4.73E-05	0.02479	0.274426	-0.00051	-5.1E-05
Metabolism;Biosynthesis of other secondary metabolites;Tropane,_piperidine_and_pyridine_alkaloid_biosynthesis	0.000805	7.65E-05	0.0009	6.07E-05	0.039433	0.279366	-0.00018	-5.7E-06
Cellular Processes;Transport and catabolism;Lysosome	0.000516	0.000241	0.000789	5.37E-05	0.0382	0.2793	-0.00	-2.1E-

					6	66	053	05
Metabolism;Carbohydrate metabolism;Ascorbate_and_aldarate_metabolism	0.000622	8.97E-05	0.000961	0.000238	0.015635	0.248807	-0.00059	-8.9E-05
Metabolism;Metabolism of terpenoids and polyketides;Biosynthesis_of_ansamycins	0.00079	5.17E-05	0.000864	5.14E-05	0.031053	0.279366	-0.00014	-8.3E-06
Unclassified;Metabolism;Glycan_biosynthesis_and_metabolism	0.000512	6.35E-05	0.00064	4.16E-05	0.002866	0.217068	-0.0002	-5.7E-05
Metabolism;Lipid metabolism;Secondary_bile_acid_biosynthesis	0.000417	6.43E-05	0.000317	3.16E-05	0.010832	0.248807	3.07E-05	0.000168
Metabolism;Biosynthesis of other secondary metabolites;Glucosinolate_biosynthesis	0.000265	2.64E-05	0.000301	1.19E-05	0.019076	0.264435	-6.4E-05	-7.9E-06
Metabolism;Glycan biosynthesis and metabolism;Glycosylphosphatidylinositol_(GPI)-anchored_proteins	0.000188	5.55E-05	0.000272	2.39E-05	0.012006	0.248807	-0.00014	-2.5E-05
Human Diseases;Cancers;Proteoglycans_in_cancer	0.000297	3.5E-06	0.000286	9.84E-06	0.049327	0.29823	4.16E-08	2.07E-05
Metabolism;Metabolism of terpenoids and polyketides;Biosynthesis_of_siderophore_group_nonribosomal_peptides	0.000164	3.97E-05	0.000268	5.31E-05	0.003814	0.217068	-0.00016	-4.3E-05
Human Diseases;Cancers;Renal_cell_carcinoma	0.000284	5.25E-05	0.000224	9.02E-06	0.037456	0.279366	5.05E-06	0.000115
Metabolism;Glycan biosynthesis and metabolism;Glycosaminoglycan_binding_proteins	0.000106	2.46E-05	0.000166	2.76E-05	0.002884	0.217068	-9.3E-05	-2.6E-05
Organismal Systems;Digestive system;Carbohydrate_digestion_and_absorption	7.38E-05	1.73E-05	0.000145	4.15E-05	0.006776	0.243757	-0.00011	-2.7E-05
Human Diseases;Infectious diseases;African_trypanosomiasis	7.93E-06	3.38E-06	1.96E-05	6.83E-06	0.006524	0.243757	-1.9E-05	-4.4E-06
Human Diseases;Infectious diseases;Chagas_disease_(American_trypanosomiasis)	5.73E-06	3.61E-06	1.58E-05	7.11E-06	0.016372	0.248807	-1.8E-05	-2.4E-06
Organismal Systems;Digestive system;Mineral_absorption	8.81E-06	1.93E-06	1.57E-05	5.61E-06	0.028509	0.279366	-1.3E-05	-1E-06
Environmental Information Processing;Signaling	3.64E	2.05	8.03E-	4.03E	0.0	0.2	-	-

molecules and interaction;Cell_adhesion_molecules_and_their_ligands	-06	E-06	06	-06	46657	91674	8.7E-06	8.4E-08
Organismal Systems;Endocrine system;Relaxin_signaling_pathway	3.44E-06	1.9E-06	7.31E-06	3.46E-06	0.043812	0.279366	-7.6E-06	-1.4E-07
Environmental Information Processing;Signaling molecules and interaction;ECM-receptor_interaction	3.44E-06	1.9E-06	7.31E-06	3.46E-06	0.043812	0.279366	-7.6E-06	-1.4E-07
Cellular Processes;Cell growth and death;Focal_adhesion	3.44E-06	1.9E-06	7.31E-06	3.46E-06	0.043812	0.279366	-7.6E-06	-1.4E-07
Human Diseases;Endocrine and metabolic diseases;AGE-RAGE_signaling_pathway_in_diabetic_complications	3.44E-06	1.9E-06	7.31E-06	3.46E-06	0.043812	0.279366	-7.6E-06	-1.4E-07
Metabolism;Biosynthesis of other secondary metabolites;Stilbenoid,_diarylheptanoid_and_gingerol_biosynthesis	1.76E-06	9.04E-07	3.18E-06	7.56E-07	0.015052	0.248807	-2.5E-06	-3.4E-07
Metabolism;Biosynthesis of other secondary metabolites;Flavonoid_biosynthesis	1.76E-06	9.04E-07	3.18E-06	7.56E-07	0.015052	0.248807	-2.5E-06	-3.4E-07
Human Diseases;Immune diseases;Systemic_lupus_erythematosus	2.46E-07	9.81E-08	8.68E-07	3.66E-07	0.007701	0.243757	-1E-06	-2.4E-07
Human Diseases;Infectious diseases;Pathogenic_Escherichia_coli_infection	1.71E-07	8.14E-08	3.99E-07	1.87E-07	0.029627	0.279366	-4.3E-07	-3E-08
Organismal Systems;Endocrine system;Ovarian_Steroidogenesis	6.29E-08	5.25E-08	1.3E-07	4.22E-08	0.036164	0.279366	-1.3E-07	-5.3E-09
Environmental Information Processing;Signaling molecules and interaction;G_protein-coupled_receptors	2.05E-09	3.26E-09	3.22E-08	2.31E-08	0.023419	0.274426	-5.4E-08	-6E-09

Table 2f Aged+PBS VS Aged+Young								
Taxa	avg(Aged+PBS)	sd(Aged+PBS)	avg(Aged+Young)	sd(Aged+Young)	p.value	q.values	interval lower	interval upper
Genetic Information Processing;Replication and repair;DNA_replication_proteins	0.015109	0.000133	0.014468	0.000277	0.001287	0.000830 73	0.000346	0.000937
Metabolism;Carbohydrate metabolism;Pyruvate_metabolism	0.013256	0.000309	0.013857	0.000478	0.003086 3	0.211941	-0.00113	######
Genetic Information Processing;Replication and repair;Mismatch_repair	0.011191	0.000174	0.010727	0.000429	0.004561 5	0.211941	1.22E-05	0.000917
Environmental Information Processing;Membrane transport;Secretion_system	0.010266	0.000857	0.009002	0.000808	0.002697 5	0.211941	0.000174	0.002318
Unclassified;Genetic information processing;Replication,_recombination_and_repair_proteins	0.009811	0.000476	0.008663	0.001048	0.040 3	0.211941	6.92E-05	0.002293
Metabolism;Energy metabolism;Carbon_fixation_pathways_in_prokaryotes	0.008631	0.000453	0.009592	0.000683	0.019043	0.185973	-0.00172	-0.00002
Genetic Information Processing;Translation;Messenger_RNA_Biogenesis	0.007653	0.000128	0.008007	0.000249	0.015963	0.185973	-0.00062	######
Genetic Information Processing;Folding, sorting and degradation;RNA_degradation	0.007297	0.000143	0.007737	0.000271	0.008643	0.164618	-0.00073	-0.00015
Metabolism;Energy metabolism;Methane_metabolism	0.007085	0.000104	0.00744	0.000299	0.032733	0.211941	-0.00067	######
Metabolism;Carbohydrate metabolism;Butanoate_metabolism	0.006828	0.000131	0.007411	0.000501	0.034917	0.211941	-0.00111	######
Metabolism;Carbohydrate metabolism;Citrate_cycle_(TCA_cycle)	0.005779	0.000294	0.006533	0.000662	0.038681	0.211941	-0.00146	######
Unclassified;Metabolism;Energy_metabolism	0.005571	0.00032	0.006071	0.000169	0.010204	0.164618	-0.00084	-0.00016
Metabolism;Energy metabolism;Carbon_fixation_in_photosynthetic_organisms	0.004982	4.40E-05	0.005252	0.000113	0.001201	0.000830 73	-0.00039	-0.00015

Metabolism;Amino acid metabolism;Arginine_biosynthesis	0.004855	0.000135	0.005175	0.000167	0.004703	0.13006	-0.00052	-0.00012
Metabolism;Lipid metabolism;Glycerophospholipid_metabolism	0.004402	0.00011	0.00409	0.000279	0.04047	0.211941	1.83E-05	0.000607
Metabolism;Glycan biosynthesis and metabolism;Glycosyltransferases	0.003989	0.000158	0.003766	0.000147	0.03004	0.211941	2.63E-05	0.000419
Metabolism;Amino acid metabolism;Valine,_leucine_and_isoleucine_biosynthesis	0.003302	0.000124	0.003687	0.000289	0.02058	0.185973	-0.00069	######
Metabolism;Energy metabolism;Sulfur_metabolism	0.00237	6.26E-05	0.002678	0.000107	0.00281	0.054485	-0.00042	-0.00019
Metabolism;Amino acid metabolism;Histidine_metabolism	0.002265	6.44E-05	0.002736	0.000321	0.01479	0.185973	-0.00081	-0.00014
Metabolism;Carbohydrate metabolism;C5-Branched_dibasic_acid_metabolism	0.001784	8.80E-05	0.001976	0.000135	0.01815	0.185973	-0.00034	######
Metabolism;Amino acid metabolism;Phenylalanine_metabolism	0.001538	7.37E-05	0.00177	0.000109	0.01977	0.095695	-0.00035	-0.00011
Unclassified;Metabolism;Amino_acid_metabolism	0.001107	4.00E-05	0.001305	0.000104	0.04041	0.13006	-0.00031	######
Unclassified;Viral protein family;Viral_proteins	0.001018	4.82E-05	0.000951	5.44E-05	0.04627	0.211941	1.36E-06	0.000134
Metabolism;Biosynthesis of other secondary metabolites;Novobiocin_biosynthesis	0.000857	5.38E-05	0.000986	9.59E-05	0.02113	0.185973	-0.00023	######
Metabolism;Biosynthesis of other secondary metabolites;Tropane,_piperidine_and_pyridine_alkaloid_biosynthesis	0.000836	5.62E-05	0.000952	9.70E-05	0.03409	0.211941	-0.00022	######
Human Diseases;Endocrine and metabolic diseases;Type_I_diabetes_mellitus	0.000693	8.78E-06	0.000723	2.70E-05	0.03861	0.211941	######	######
Metabolism;Biosynthesis of other secondary metabolites;Carbapenem_biosynthesis	0.000657	3.20E-05	0.000715	3.61E-05	0.01554	0.185973	-0.00101	######
Human Diseases;Immune	0.000	1.44E	0.0003	2.31E-	0.0	0.1	6.82	5.76

diseases;Primary_immunodeficiency	376	-05	44	05	1887	85973	E-06	E-05
Metabolism;Lipid metabolism;Secondary_bile_acid_biosynthesis	0.000359	2.94E-05	0.000324	2.35E-05	0.045604	0.211941	8.46E-07	6.97E-05
Metabolism;Biosynthesis of other secondary metabolites;Glucosinolate_biosynthesis	0.000272	1.21E-05	0.000331	3.73E-05	0.009543	0.164618	######	######
Metabolism;Xenobiotics biodegradation and metabolism;Atrazine_degradation	0.000234	5.77E-05	0.000163	5.24E-05	0.048173	0.211941	7.01E-07	0.000143
Human Diseases;Neurodegenerative diseases;Huntingtons_disease	0.000223	5.85E-05	0.000161	1.05E-05	0.047924	0.211941	8.15E-07	0.000123
Metabolism;Xenobiotics biodegradation and metabolism;Xylene_degradation	0.00019	1.18E-05	0.000164	1.55E-05	0.009188	0.164618	8.12E-06	4.38E-05
Genetic Information Processing;Folding, sorting and degradation;Ubiquitin_system	0.00016	4.01E-06	0.000146	8.65E-06	0.008999	0.164618	4.70E-06	2.31E-05
Metabolism;Xenobiotics biodegradation and metabolism;Toluene_degradation	0.00011	1.13E-05	8.93E-05	1.90E-05	0.04628	0.211941	4.43E-07	4.19E-05
Metabolism;Xenobiotics biodegradation and metabolism;Caprolactam_degradation	6.02E-05	1.83E-05	9.04E-05	2.37E-05	0.034398	0.211941	######	######
Metabolism;Lipid metabolism;Ether_lipid_metabolism	6.40E-05	2.06E-05	4.06E-05	7.48E-06	0.038507	0.211941	1.69E-06	4.50E-05
Metabolism;Lipid metabolism;alpha-Linolenic_acid_metabolism	5.48E-05	1.65E-05	3.19E-05	7.59E-06	0.017643	0.185973	5.35E-06	4.04E-05
Human Diseases;Cancers;Small_cell_lung_cancer	4.15E-06	1.57E-06	6.28E-06	9.43E-07	0.020795	0.185973	######	######
Genetic Information Processing;Replication and repair;Fanconi_anemia_pathway	1.22E-06	2.53E-07	9.36E-07	1.65E-07	0.047393	0.211941	4.11E-09	5.66E-07
Human Diseases;Cancers;Bladder_cancer	8.40E-07	2.57E-07	1.27E-06	3.32E-07	0.032744	0.211941	######	######
Metabolism;Metabolism of terpenoids and polyketides;Biosynthesis_of_type_II_polyketide_backbone	2.91E-07	4.49E-08	6.86E-07	1.91E-07	0.003227	0.124925	######	######

Organismal Systems;Excretory system;Vasopressin-regulated_water_reabsorption	5.81E-09	5.23E-09	2.11E-08	1.43E-08	0.048109	0.211941	########	########
Organismal Systems;Nervous system;Synaptic_vesicle_cycle	5.81E-09	5.23E-09	2.11E-08	1.43E-08	0.048109	0.211941	########	########

Table 2.

Changes in fecal microbiota metabolism and pathways after cross-age FMT.

Cytokine determination

For detection of the IL-β, IL-6 and TNF-α concentrations in mouse foot tissue, foot tissue was harvested from different treatment groups, and 10% tissue homogenates were prepared using RIPA buffer. The prepared samples were then centrifuged at 3,000 × g and 4°C for 10 min, and supernatants were collected. All prepared foot tissue supernatants were determined using IL-β, IL-6 and TNF-α ELISA kits at proper dilutions according to the manufacturer's instructions (BioLegend, CA, USA). For the detection of cytokines in serum and peritoneal fluid, samples were separated by centrifugation (500 × g, 4°C, 10 min) and detected using IL-β, IL-6 and TNF-α ELISA kits according to the manufacturer's instructions (BioLegend, CA, USA).

Serum biochemistry analysis

The levels of serum uric acid (SUA), serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum creatinine (Cr) and blood urea nitrogen (BUN) were measured using enzymatic kits from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

Assay of uric acid synthesis enzymes

To detect the activity of Adenosine deaminase (ADA) and Guanine Deaminase (GDA) in the mouse liver, the liver was collected from mice in the various treatment groups and homogenized in sterile physiological saline solution (10-fold weight ratio of tissue). Following preparation, the samples were subjected to centrifugation at 3,000 × g and 4°C for 10 minutes, and the supernatants were collected. The activity of ADA and GDA in the liver supernatants was determined using ELISA kits (FEIYA BIOTECHNOLOGY, China) according to the manufacturer's instructions with appropriate dilutions. To measure the activity of xanthine oxidase (XOD) in the liver and kidney of mice, tissues were collected from mice in the different treatment groups and homogenized in the buffer provided in the kit (10-fold weight ratio of tissue). The samples were then centrifuged at 8,000 × g and 4°C for 10 minutes, and the supernatants were collected. The activity of XOD in the liver and kidney supernatants was assessed using an ELISA kit (Beijing Solarbio Science & Technology Co., Ltd, China) according to the manufacturer's instructions.

Fecal microbiota composition analysis

For specific details, please refer to Supplementary material 1.

Untargeted metabolomic analysis

For specific details, please refer to Supplementary Material 2.

Analysis of short-chain fatty acids in faeces

For specific details, please refer to Supplementary Material 3.

Statistical analysis

All values were expressed as the means ± SEM. Raw data were subjected to one-way ANOVA to evaluate statistical significance between at least three groups, and pairwise comparison was conducted using Student's t-test. The results were considered statistically significant at $p < 0.05$.

Data and code availability

The 16S rDNA sequencing data and untargeted metabolomic data presented in this study can be found in online repositories. The findings of this study have been deposited into the CNGB Sequence Archive (CNSA) of China National GenBank Database (CNGBdb) under the accession number CNP0004751. The names of the repository/repositories and accession number(s) can be found below: *https://db.cngb.org/cnsa/project/CNP0004751_46c178da/reviewlink/*.

The lead contact will provide original western blot images and microscopy data from this paper upon request.

No unique code was generated in this study.

For any further information needed to reanalyze the data presented in this paper, it can be obtained from the lead contact upon request.

Declaration of interests

The authors declare no competing interests.

Acknowledgements

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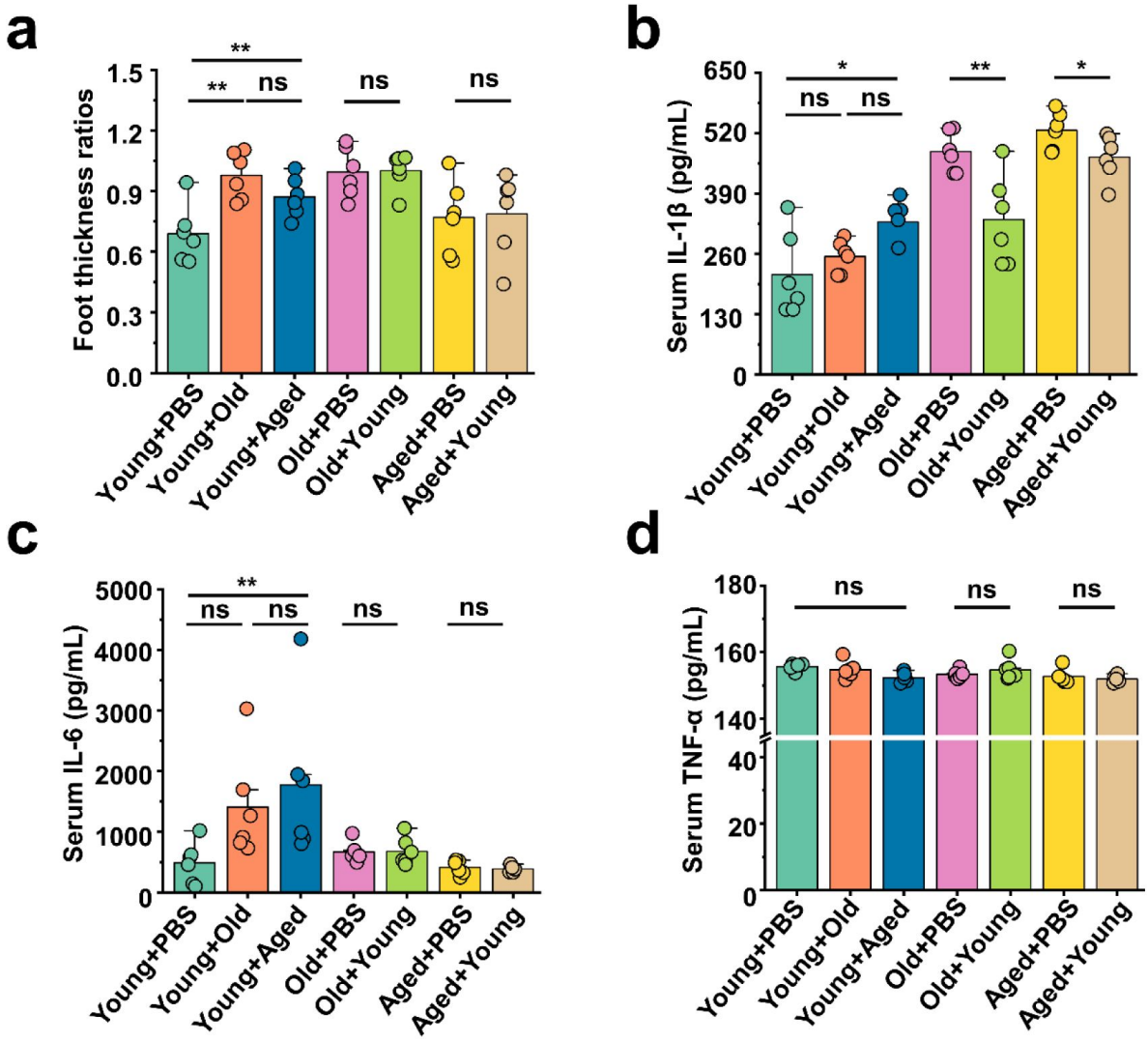
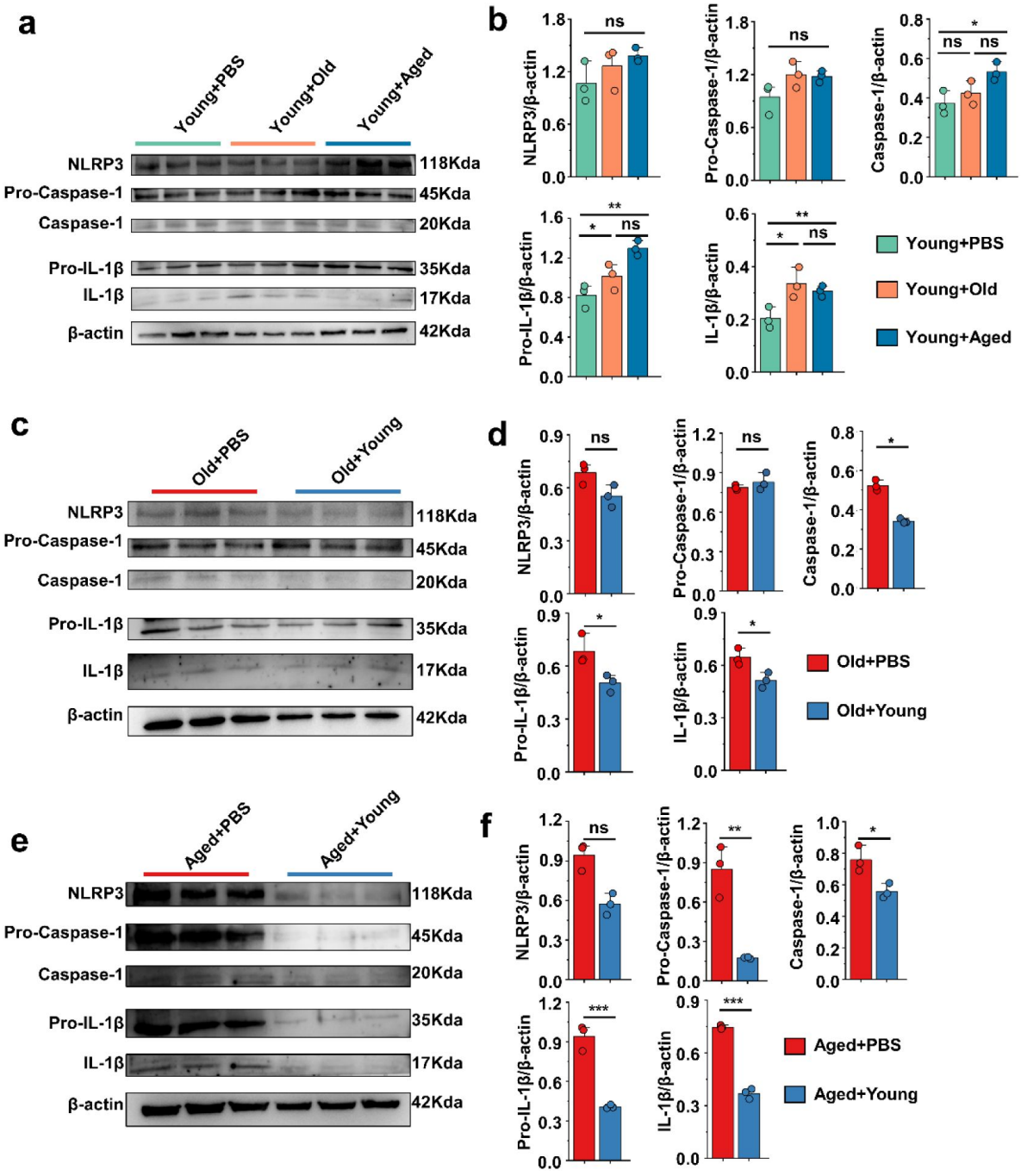
Author contributions

The study was designed by Naisheng Zhang, Wenlong Zhang, and Hang Gao. Ning Song conducted all mouse experiments and performed the statistical analysis. Mice sample collection was carried out by Jianhao Li, Mingze Wang and Yi Liu. Ning Song was responsible for software applications, investigation, and writing the original draft. Jianhao Li and Mingze Wang helped with validation and analysis. Ning Song and Yi Liu were responsible for resources and data curation. Wenlong Zhang and Zhiming Ma wrote and revised the final draft of the manuscript, and all authors participated in its revision and gave their approval.



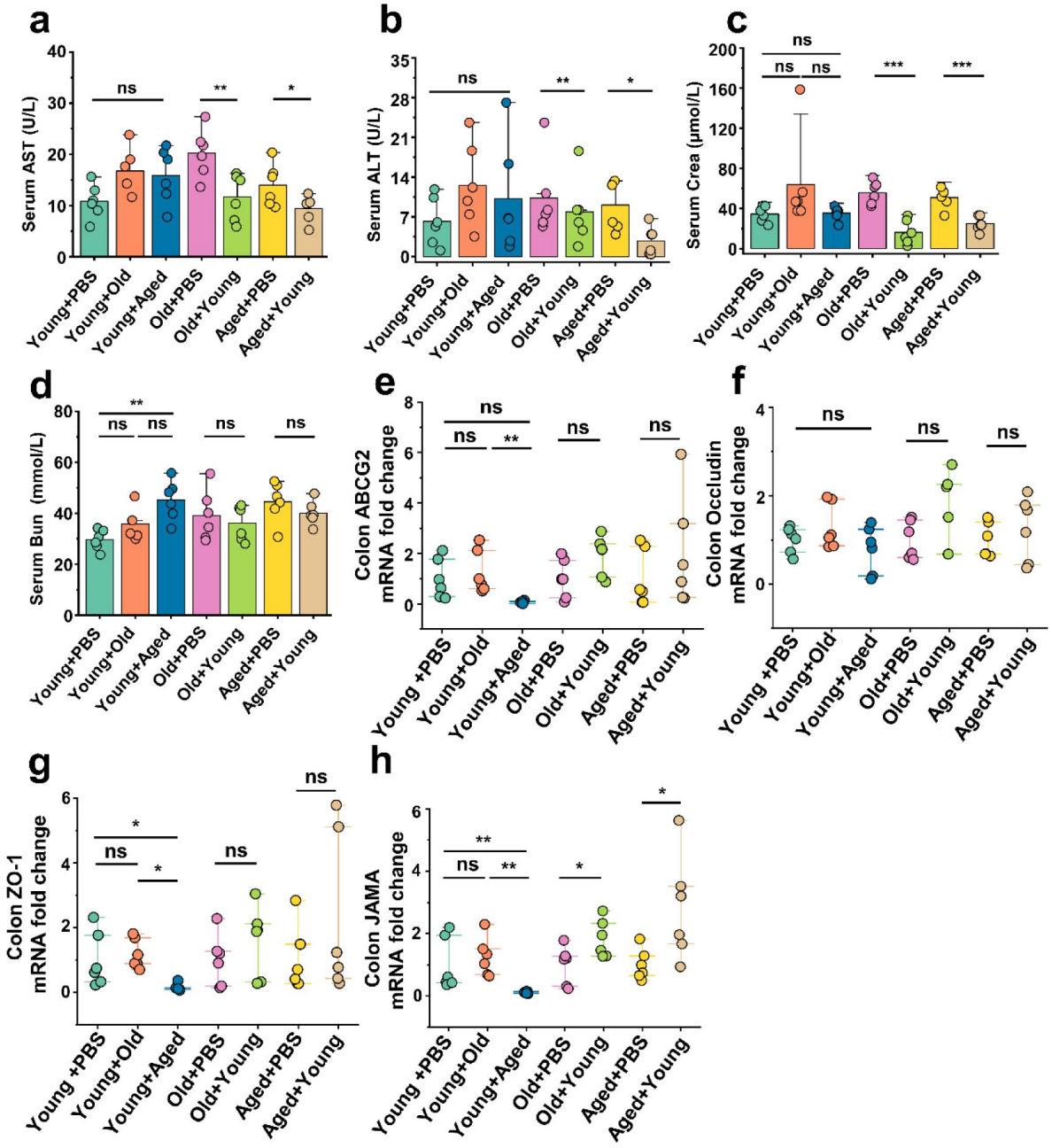
Supplementary Fig 1.

(a) All groups' foot thickness ratios were tested after MSU administration (n=6). (b-d) The serum concentrations of IL-1β (b), IL-6 (c) and TNF-α (d) inflammatory parameters were measured in the indicated mice(n=6).
Values are presented as the mean ± SEM. Differences were assessed by t-test or One-Way ANOVA and denoted as follows: *p < 0.05, **p < 0.01, and ***p < 0.001, "ns" indicates no significant difference between groups.



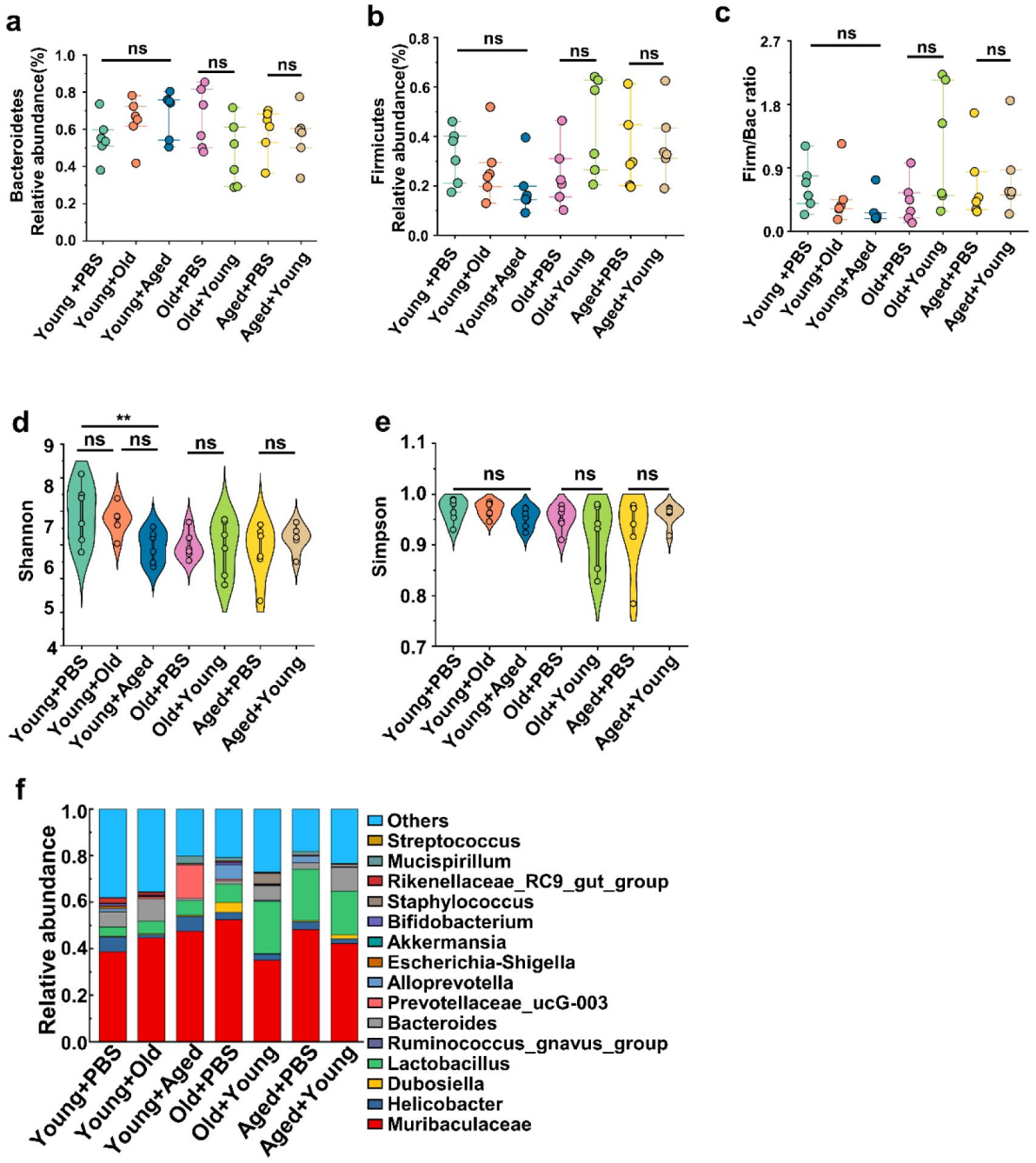
Supplementary Fig 2.

(a-b) Representative western blot images and band density (Young+PBS, Young+Old and Young+Aged) of peritoneal cells NLRP3 pathways proteins (n=3).
(c-d) Representative western blot images and band density (Old+PBS and Old+Young) of peritoneal cells NLRP3 pathways proteins (n=3).
(e-f) Representative western blot images and band density (Aged+PBS and Aged+Young) of peritoneal cells NLRP3 pathways proteins (n=3).
Values are presented as the mean ± SEM. Differences were assessed by t-test or One-Way ANOVA and denoted as follows: *p < 0.05, **p < 0.01, and ***p < 0.001, “ns” indicates no significant difference between groups.



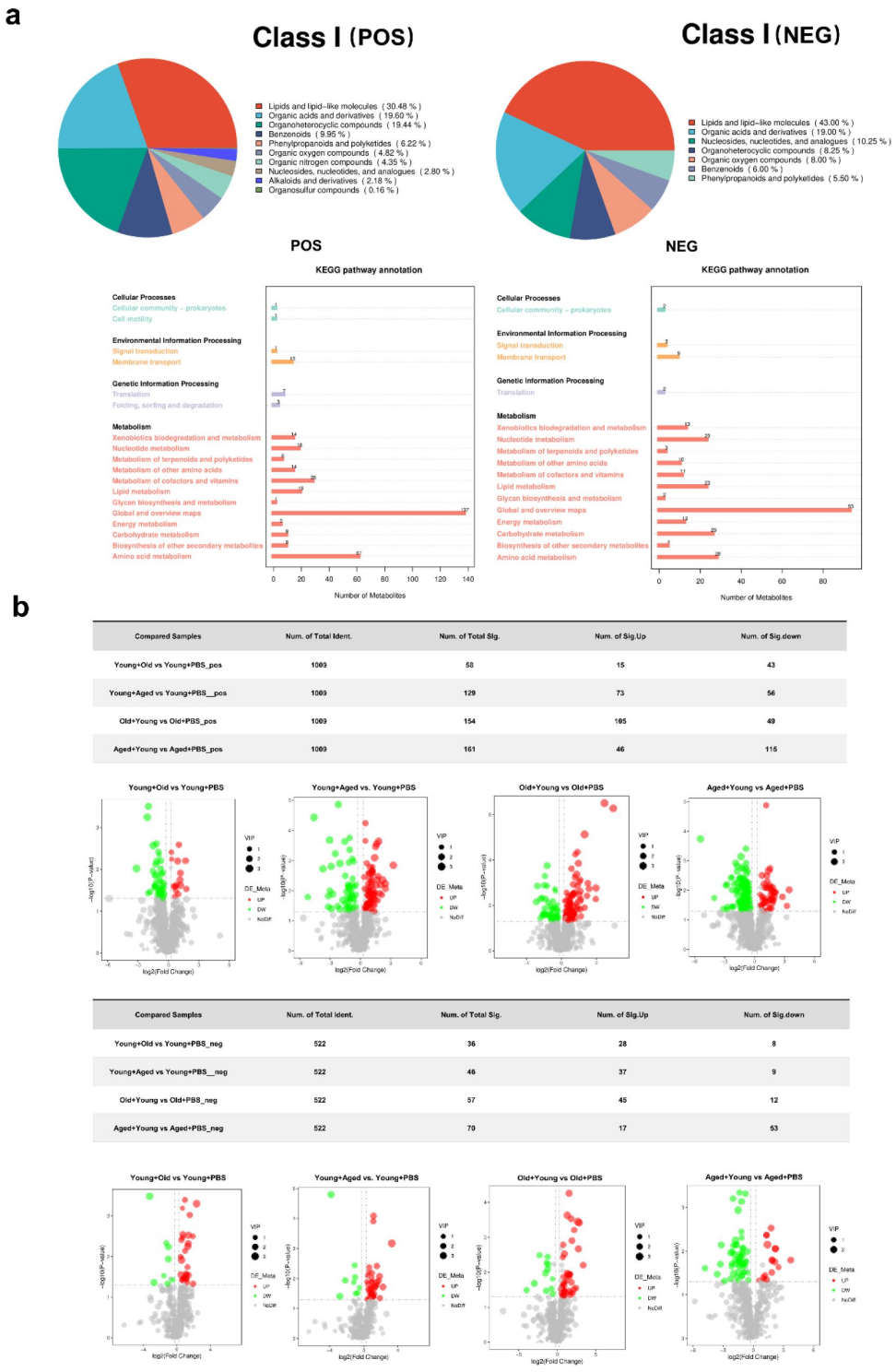
Supplementary Fig 3.

(a and b) Serum AST (a) and ALT (b) concentrations in the cross-age fecal microbiota transplantation group and its control group (n=6).
(c and d) Serum Crea (c) and BUN (d) concentrations in the cross-age fecal microbiota transplantation group and its control group (n=6).
(e-h) Relative colonic gene expression in the indicated groups by qPCR (n = 6), including ABCG2 (e), Occludin (f), ZO-1 (g) and JAMA (h).
Values are presented as the mean ± SEM. Differences were assessed by t-test or One-Way ANOVA and denoted as follows: *p < 0.05, **p < 0.01, and ***p < 0.001, “ns” indicates no significant difference between groups.



Supplementary Fig 4.

(a) Bacteroidetes relative abundance of the indicated groups (n=6).
(b) Firmicutes relative abundance of the indicated groups (n=6).
(c) The Firm/Bac ratio in the different groups (n=6).
(d-e) Alpha diversity indices including Shannon (d) and Simpson (e) index in the indicated groups (n=6).
(f) Bacterial composition at the genus levels (top 15) of the indicated groups(n=6).
Values are presented as the mean ± SEM. Differences were assessed by t-test or One-Way ANOVA and denoted as follows: *p < 0.05, **p < 0.01, and ***p < 0.001, “ns” indicates no significant difference between groups.



Compared Samples	Num. of Total Ident.	Num. of Total Sig.	Num. of Sig.Up	Num. of Sig.down
Young+Old vs Young+PBS_pos	1069	58	15	43
Young+Aged vs Young+PBS__pos	1069	129	73	56
Old+Young vs Old+PBS_pos	1069	154	105	49
Aged+Young vs Aged+PBS_pos	1069	161	46	115

Compared Samples	Num. of Total Ident.	Num. of Total Sig.	Num. of Sig.Up	Num. of Sig.down
Young+Old vs Young+PBS_neg	522	36	28	8
Young+Aged vs Young+PBS__neg	522	46	37	9
Old+Young vs Old+PBS_neg	522	57	45	12
Aged+Young vs Aged+PBS_neg	522	70	17	53

Supplementary Fig 5.

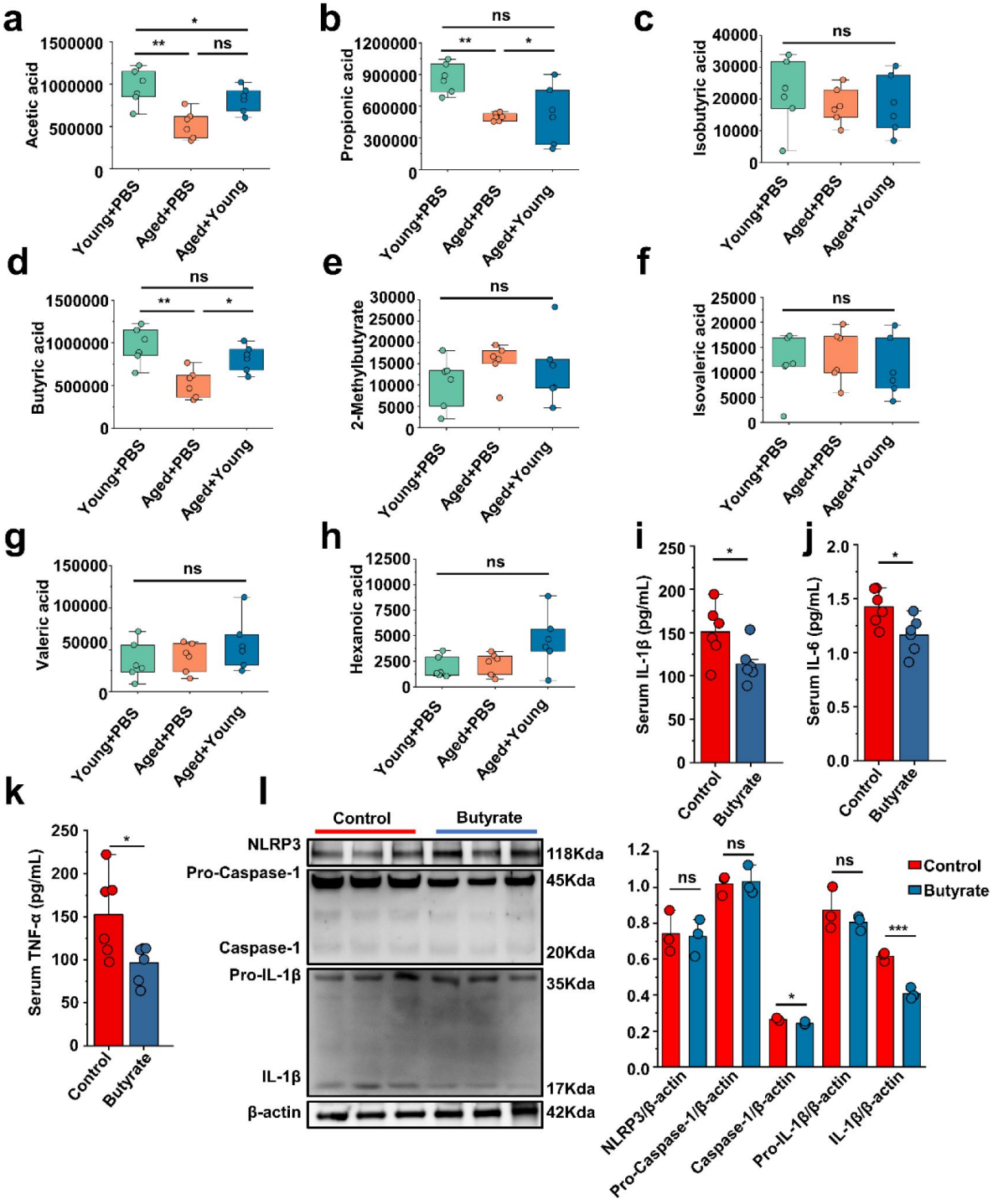
(a) Metabolite classification of various groups.
(b) Volcano plots of differential metabolites in each group.
The horizontal axis of volcano plot represents the $\log_2$ (Fold Change) in metabolite abundance between different groups, while the vertical axis of volcano plot represents the significance level ($-\log_{10}$ (P-value)) of the differences. Each point on the volcano plot represents a metabolite, with significantly upregulated metabolites depicted as red points and significantly downregulated metabolites depicted as green points. The size of the circles corresponds to the VIP (Variable Importance in Projection) value.

Supplementary Fig 6.

(a) Comparison of differential metabolite volcano plots and KEGG enrichment bubble plots between Young+PBS and Old+PBS.

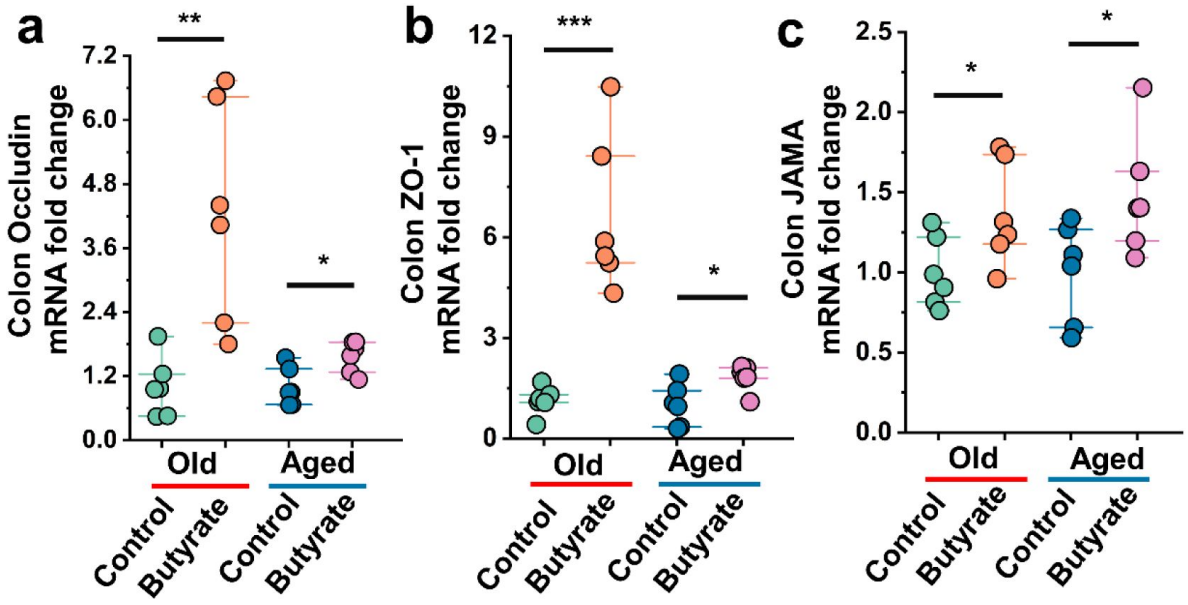
(b) Comparison of differential metabolite volcano plots and KEGG enrichment bubble plots between Young+PBS and Aged+PBS.

To visualize the differential metabolites resulting from pairwise comparisons, we generated volcano plots that provide a clear representation of the upregulation and downregulation of metabolites, especially those with significant fold change differences. The enrichment analysis was performed at the KEGG Pathway level using a hypergeometric test, as shown in the figure below. The pathways that were significantly enriched in the differential metabolites compared to the background of all identified metabolites. Pathway enrichment analysis enables us to determine the major biochemical metabolic pathways and signaling transduction pathways that are implicated by the differential metabolites.



Supplementary Fig 7.

(a-h) The difference analysis of the detected short chain fatty acids, including acetic acid (a), propionic acid (b), isobutyric acid (c), butyric acid (d), 2-merhylbutyrate (e), isovaleric acid (f), valeric acid (g), hexanoic acid (h).
(i-k) The serum concentrations of IL-1β (i), IL-6 (j) and TNF-α (k) inflammatory parameters were measured in the indicated mice (n=6).
(l) Representative western blot images and band density of peritoneal cells NLRP3 pathways proteins (n=3)
Values are presented as the mean ± SEM. Differences were assessed by t-test or One-Way ANOVA and denoted as follows: *p < 0.05, **p < 0.01, and ***p < 0.001, “ns” indicates no significant difference between groups.



Supplementary Fig 8.

(a-c) Relative colonic gene expression in the indicated groups by qPCR (n = 6), including Occludin (a), ZO-1 (b) and JAMA (c). Values are presented as the mean ± SEM. Differences were assessed by t-test or One-Way ANOVA and denoted as follows: *p < 0.05, **p < 0.01, and ***p < 0.001, “ns” indicates no significant difference between groups.

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Reviewer #2 (Public review):

Summary:

In their manuscript, the authors report that fecal transplantation from young mice into old mice alleviates susceptibility to gout. The gut microbiota in young mice is found to inhibit activation of the NLRP3 inflammasome pathway and reduce uric acid levels in the blood in the gout model.

Strengths:

The authors focused on the butanoate metabolism pathway based on the results of metabolomics analysis after fecal transplantation and identified butyrate as the key factor in mitigating gout susceptibility. In general, this is a well-performed study.

Weaknesses:

The discussion on the current results and previous studies regarding the effect of butyrate on gout symptoms is insufficient.

https://doi.org/10.7554/eLife.98714.3.sa2

Reviewer #3 (Public review):

The manuscript presents interesting findings on the role of gut microbiota in gout, focusing on the interplay between age-related changes, inflammation, and microbiota-derived metabolites, particularly butyrate. The study provides valuable insights into the therapeutic potential of microbiota interventions and metabolites for managing hyperuricemia and gout.

The manuscript has improved with the revisions made, particularly regarding clarifications on experimental design and the inclusion of supplementary data.

Comments on latest version:

The authors have addressed many previous concerns; however, some areas still require clarification and improvement to support more definitive conclusions.

(1) This study suggests that microbiota interventions, particularly butyrate, show promising therapeutic potential for hyperuricemia and gout. While the authors discuss the functions of certain butyrate-producing bacteria, I recommend further validating the gut microbiota-butyrate pathway by supplementing germ-free animal models with a single butyrate-producing strain, such as Clostridium butyricum. To strengthen the manuscript, I suggest the authors make further revisions to address these key issues.

(2) Additionally, I was unable to locate the full-length, uncropped Western blot images in the manuscript or supplementary materials. Could the authors please provide these?

https://doi.org/10.7554/eLife.98714.3.sa1

Author response:

The following is the authors' response to the previous reviews.

> ***Reviewer #2 (Public review):***
>
> *Summary:*

> *In their manuscript the authors report that fecal transplantation from young mice into old mice alleviates susceptibility to gout. The gut microbiota in young mice is found to inhibit activation of the NLRP3 inflammasome pathway and reduce uric acid levels in the blood in the gout model.*
>
> *Strengths:*
>
> *They focused on the butanoate metabolism pathway based on the results of metabolomics analysis after fecal transplantation and identified butyrate as the key factor in mitigating gout susceptibility. In general, this is a well-performed study.*
>
> *Weaknesses:*
>
> *The discussion on the current results and previous studies regarding the effect of butyrate on gout symptoms is insufficient. The authors need to provide a more thorough discussion of other possible mechanisms and relevant literature.*
>
> ***Reviewer #2 (Recommendations for the authors):***
>
> *General comments:*
>
> *I appreciate the authors' efforts to answer the comments raised in my previous review (as Reviewer#2). However, I still detect some issues that need to be fully addressed, with inadequate or even no answers for several comments.*

Thank you for your valuable feedback. Your previous suggestions have been incredibly helpful for our paper. Although we have strived to make the article as comprehensive as possible, there may still be some areas that are not perfectly refined.

> *The response to comment 1: The author's statement is not very convincing. What are the trends of inflammation factors? The data in Figure 1G-H suggest that butyrate may not be the only factor to explain this phenomenon. Authors should carefully interpret the data in Figure 1G-H.*

Sorry for the inadequate clarification on your question. We utilize antibiotics for treatment in order to establish the relationship between gut microbiota, age, and gout. Our research findings indicate that there is a trend for serum uric acid levels to increase with age, and we also observe that the older the age, the more pronounced the stimulation to MSU. We found that after clearing the gut microbiota and then stimulating with MSU, the trend of inflammation factors and serum uric acid level changing with age disappears. Thus, we can preliminarily draw the conclusion that the gut microbiota is closely associated with age, gout, and hyperuricemia.

> *The response to comment 2: I understand the importance of evaluating a range of indicators, but food thickness is the most crucial clinical marker for diagnosing goats. Please move the data from Supplemental Figure 1A to the main figure.*

Thank you for your suggestions. We have included the most significant results in the main figure, and the description of “foot thickness” has already been provided descriptively in the manuscript. Additionally, considering the layout and arrangement of the images, we have placed it in the supplementary figures 1.

> *The response to comment 3: The immunostaining for ZO-1 and Occludin is unclear. Please provide higher magnification images to confirm the specific staining.*

Thank you for your valuable feedback. We have enhanced the clarity of the images. In addition to adding immunohistochemical images in Supplementary Material 4, we have also submitted independent images.

> *The response to comment 4: The authors still haven't directly addressed my comment.*

Please accept our sincere apologies for not providing a clearer response to your question. The indicators related to uric acid-producing enzymes and uric acid transporters have been separately analyzed according to different age groups. The specific results are detailed in section " The expression of uric acid-producing enzymes activity and uric acid transporters at the mRNA level across different age groups" of Supplementary Material 4.

> *No response was given for comment 5. Please address it.*

In a PCoA plot, the distance between samples reflects the similarity in the structure of the microbial communities: the closer the distance, the more similar the composition of the communities; the greater the distance, the more pronounced the differences. We judge based on the relative distances of each group in the plot, observing their degree of proximity.

> *The response to comment 6: I understand the author's statement, and I suggest incorporating it into the discussion section of the revised manuscript.*

Thank you for your suggestions. We have incorporated the relevant content into our discussion.

> *The response to comment 7: Again, please incorporate this statement into the discussion section of the revised manuscript.*

Thank you for your suggestions. We have incorporated the relevant content into our discussion.

> ***Reviewer #3 (Public review):***
>
> *Summary:*
>
> *The revised manuscript presents interesting findings on the role of gut microbiota in gout, focusing on the interplay between age-related changes, inflammation, and microbiota-derived metabolites, particularly butyrate. The study provides valuable insights into the therapeutic potential of microbiota interventions and metabolites for managing hyperuricemia and gout. While the authors have addressed many of the previous concerns, a few areas still require clarification and improvements to strengthen the manuscript's clarity and overall impact.*
>
> *(1) While the authors mention that outliers in the data do not affect the conclusions, there remains a concern about the reliability of some figures (e.g., Figure 2D-G). It is recommended to provide a more detailed explanation of the statistical analysis used to handle outliers. Additionally, the clarity of the Western blot images, particularly IL-1β in Figure 3C, should be improved to ensure clear and supportive evidence for the conclusions.*

Thank you for your suggestion. We respond as follows: (1) Outliers can occasionally constitute intrinsic elements of the dataset, reflecting genuine occurrences within the experimental context. The elimination of such outliers has the potential to introduce bias into the results, thereby facilitating misconceptions regarding the underlying phenomenon under

investigation. In order to maintain the transparency and integrity of the dataset, we have elected to retain the outliers within our analysis. This decision is based on the recognition that these values may represent genuine experimental observations or unique conditions that are inherently meaningful to the phenomenon under investigation. By preserving these data points, we aim to provide a comprehensive and unbiased representation of the experimental results, allowing for a more nuanced interpretation of the findings. (2) Due to the scarcity of samples, we are unable to fulfill your request in the short term. Furthermore, we have noted that the band for IL-1β in Figure 3C is indeed visible and we consider it suitable for subsequent analysis.

> *(2) The manuscript raises a key question about why butyrate supplementation and FMT have different effects on uric acid metabolism and excretion. While the authors have addressed this by highlighting the involvement of multiple bacterial genera, it is still recommended to expand on the differences between these interventions in the discussion, providing more mechanistic insights based on available literature.*

Thank you for your suggestion. We have enriched the discussion in the manuscript and included additional comparisons

> *(3) It is noted that IL-6 and TNF-α results in foot tissue were requested and have been added to supplementary material. However, the main text should clearly reference these additions, and the supplementary figures should be thoroughly reviewed for consistency with the main findings. The use of abbreviations (e.g., ns for no significant difference) and labeling should also be carefully checked across all figures.*

Thank you for your valuable feedback. We have revised the manuscript in accordance with your suggestions.

> *(4) The manuscript presents butyrate as a key molecule in gout therapy, yet there are lingering concerns about its central role, especially given that other short-chain fatty acids (e.g., acetic and propionic acids) also follow similar trends. The authors should consider further acknowledging these other SCFAs and discussing their potential contribution to gout management. Additionally, the rationale for focusing primarily on butyrate in subsequent research should be made clearer.*

Thank you for your input. We have incorporated additional evidence into the discussion, explaining why we ultimately chose butyrate in subsequent research.

> *(5) The full-length uncropped Western blot images should be provided as requested, to ensure transparency and reproducibility of the data.*

Thank you for your suggestion. We have already included the relevant explanations in the manuscript.

> *(6) Despite the authors' revisions, several references still lack page numbers. Please ensure that all references are properly formatted, including complete page ranges.*

Thank you for your suggestions; we will make more detailed revisions to the references.

> *The manuscript has improved with the revisions made, particularly regarding clarifications on experimental design and the inclusion of supplementary data. However, some concerns about data quality, mechanistic insights, and clarity in the figures remain. Addressing these points will enhance the overall impact of the work and its potential contribution to the understanding of the gut microbiome in gout and*

> *hyperuricemia. A final revision, with careful attention to both major and minor points, is highly recommended before resubmission.*

Once again, we are grateful for your suggestions and recognition. Your input has been of immense help to our manuscript and has also provided us with a valuable learning opportunity.

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