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Supplementary Information

Novel selenium-riched *Pichia kudriavzevii* as a dietary supplement to alleviate dextran sulfate sodium-induced colitis in mice by modulating gut microbiota and host metabolism

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Experimental

Selection of selenium doses in animal experiments

The doses of selenium in *Pichia kudriavzevi* were chosen to be < 0.01, 0.15, and 0.4 mg/kg, mirroring variations in human dietary intakes as previously reported [1, 2]. In mice, a dietary selenium concentration of 0.15 mg/kg is recognized as the threshold required for maximal expression of GPX1, equivalent to a daily selenium intake of 55 μg/day in humans. Similarly, the 0.4 mg/kg selenium diet in mice aligns with supplementing the human diet with 200 μg/day, a dosage commonly used in clinical trials.

Results and discussion

LSeY and Y intervention regulated DSS-induced host metabolism

The volcano plot analysis revealed that compared to the DSS group, LSeY treatment significantly upregulated 205 metabolites and downregulated 473 metabolites (Fig. S1A). Further KEGG enrichment analysis indicated that LSeY administration primarily enriched pathways related to amino acid metabolisms, such as arginine and proline metabolism, lysine degradation, and tryptophan metabolism. Among these, arginine and proline metabolism were most influenced by LSeY treatment (Fig. S1B). In the DSS group, several metabolites within the arginine and proline metabolism pathway were significantly altered. Specifically, levels of N-acetyl-L-glutamate 5-semialdehyde were markedly decreased ($p < 0.001$), while creatinine ($p < 0.001$) and trans-3-hydroxy-L-proline ($p < 0.01$) were significantly increased (Fig. S1C). LSeY

treatment led to a significant increase in N-acetyl-L-glutamate 5-semialdehyde levels ($p < 0.01$) and a decrease in creatinine ($p < 0.01$) and trans-3-hydroxy-L-proline levels ($p < 0.001$) (Fig. S1C). Previous research suggests that supplementation with N-acetyl-L-glutamate 5-semialdehyde enhances intestinal immunity [3]. Additionally, elevated levels of this metabolite have been observed following cranberry juice consumption, potentially contributing to the bioactive effects of cranberries in combating urinary tract infections [4]. Creatinine levels are known to rise in mice with colitis, serving as a potential marker for disease severity and a management tool for ischemic colitis [5]. Administration of *Lactobacillus plantarum* NK151, *Bifidobacterium longum* NK173, and *Bifidobacterium bifidum* NK175 administration has been shown to alleviate colitis and depression in mice by reducing creatinine levels [4]. Furthermore, lysine degradation is an essential aspect of central metabolism and is relevant in IBD [6]. Following DSS treatment, levels of key products of lysine degradation, including, pipecolic acid ($p < 0.01$), 5-hydroxylysine ($p < 0.001$) and 5-acetamidovalerate ($p < 0.001$), were significantly increased. However, LSeY treatment significantly decreased these metabolites, highlighting the regulation of lysine degradation by LSeY treatment. Han et al. also reported that after administering *Bifidobacterium bifidum* in response to DSS exposure, there were alterations in pipecolic acid levels, which are involved in lysine degradation [6].

Further analysis was conducted on the metabolomic profile of mouse feces following the strain Y intervention. The intervention with strain Y led to a significant upregulation of 142 metabolites and the downregulation of 240 metabolites (Fig. S2A).

KEGG enrichment analysis revealed that these metabolites are involved in pathways related to cyanoamino acid metabolism, D-amino acid metabolism, and glycine, serine and threonine metabolism, etc (Fig. S2B). The expression of lotaustralin, a compound in the cyanoamino acid metabolism pathway, was elevated ($p < 0.001$), but its levels were reduced following treatment with strain Y ($p < 0.05$) (Fig. S2C). Lotaustralin can release cyanide, which is toxic to both humans and animals, particularly when consumed in large amounts [7]. Furthermore, DSS induction can cause disturbances in D-amino acid metabolism, as supported by the increased levels of 1-pyrroline-4-hydroxy-2-carboxylate ($p < 0.01$). Treatment with strain Y notably restored the expression of metabolite 1-pyrroline-4-hydroxy-2-carboxylate in DSS-induced colitis mice ($p < 0.01$), aligning their levels more closely with those of healthy controls (Fig. S2C). The results highlighted that strain Y treatment played a role in the regulation of D-amino acid metabolism. Additionally, the levels of betaine, which is involved in glycine, serine and threonine metabolism, were significantly reduced following DSS treatment ($p < 0.001$) (Fig. S2C). However, strain Y treatment effectively restored betaine levels in DSS-induced colitis mice ($p < 0.05$). Betaine, a natural product with well-known anti-inflammatory properties, is widely found in plants and animals [8]. It holds the potential to be a candidate drug for strengthening intestinal barrier dysfunction and ameliorating colitis by inhibiting oxidative stress-induced inflammatory pyroptosis, modulating inflammatory responses, strengthening the intestinal barrier, and altering gut microbiota composition [9, 10]. In conclusion, this study revealed that *P. kudriavzevii* enriching or not enriching selenium could alleviate IBD

by regulating gut microbiota and metabolites. Selenium enrichment further enhanced the therapeutic effect, and a high selenium intake may be advisable for improved treatment outcomes.

Figure Captions

Fig. S1. LSeY treatment regulated the metabolites of colitis mice. (A) Volcano plots between LSeY-DSS and DSS groups. (B) Pathway enrichment analysis of differential metabolites (LSeY-DSS *vs* DSS). (C) The abundance of the differential metabolites of N-acetyl-L-glutamate 5-semialdehyde, creatinine, trans-3-hydroxy-L-proline, pipecolic acid, 5-hydroxylysine and 5-acetamidovalerate, respectively. $p < 0.05$, *; $p < 0.01$, **; and $p < 0.001$, ***.

Fig. S2. Y treatment regulated the metabolites of colitis mice. (A) Volcano plots between Y-DSS and DSS groups. (B) Pathway enrichment analysis of differential metabolites (Y-DSS *vs* DSS). (C) The abundance of the differential metabolites of lotaustralin, 1-Pyrroline-4-hydroxy-2-carboxylate, and betaine, respectively. $p < 0.05$, *; $p < 0.01$, **; and $p < 0.001$, ***.

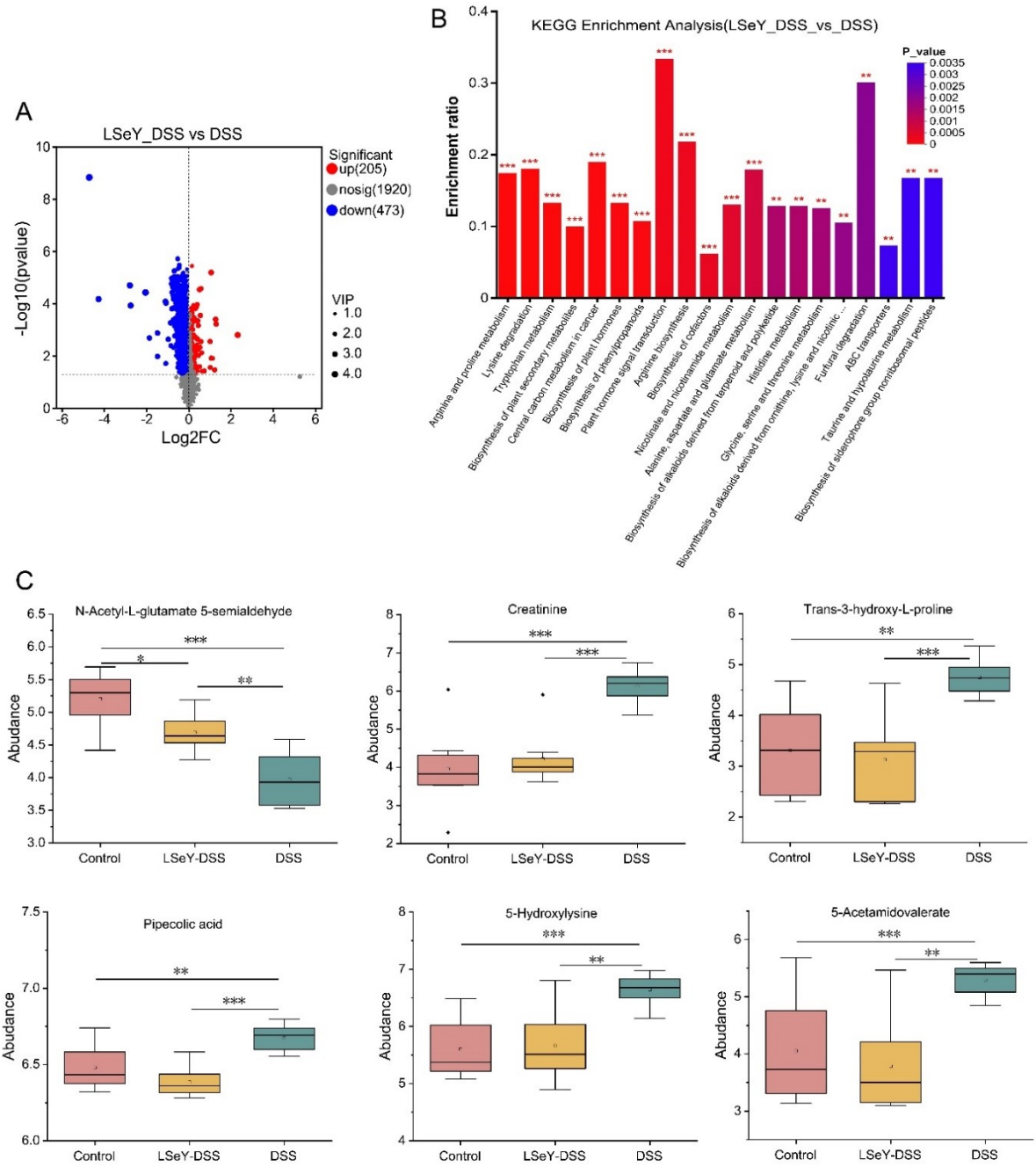
Fig. S3. Heat map analysis of the correlation between metabolites and gut microbiota.

Fig. S4. Changes in body weight of mice during antibiotic removal of intestinal microbiota.

Fig. S5. Fecal microbiota transplantation alleviated colon and serum inflammatory disturbance in DSS-induced colitis mice. The levels of (A) TNF-a, (B) IL-1β, (C) IL-17, (D) IL-6, and (E) IL-10 in colonic tissue. The levels of (F) TNF-a, (G) IL-1β, (H) IL-17, (I) IL-6, and (J) IL-10 in serum. $p < 0.05$, *; $p < 0.01$, **; and $p < 0.001$, ***.

Fig. S6. Fecal microbiota transplantation decreased colon and serum oxidative stress in DSS-induced colitis mice. The expression of (A) MPO, (B) MDA, (C) CAT, (D) SOD, and (E) GPX in colonic tissue. The expression of (F) MPO, (G) MDA, (H) CAT, (I)

SOD, and (J) GPX in serum. $p < 0.05$, *; $p < 0.01$, **; and $p < 0.001$, ***.



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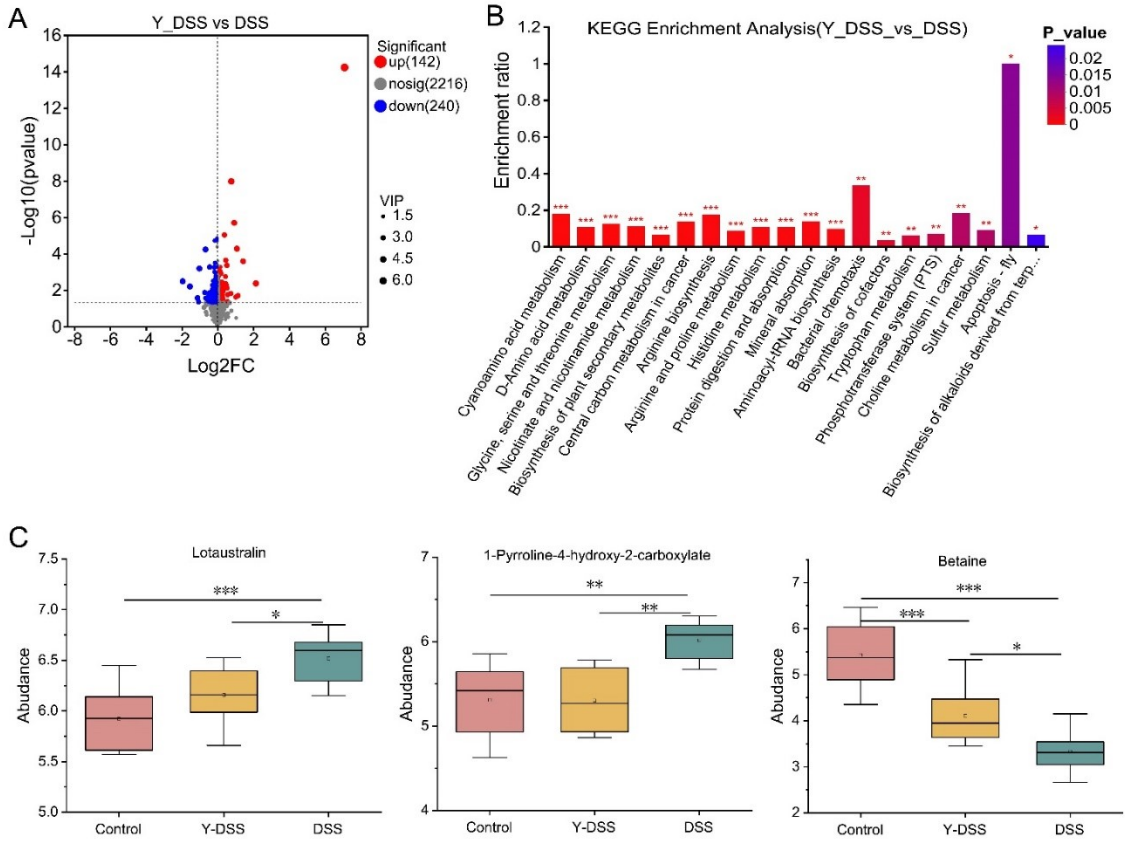
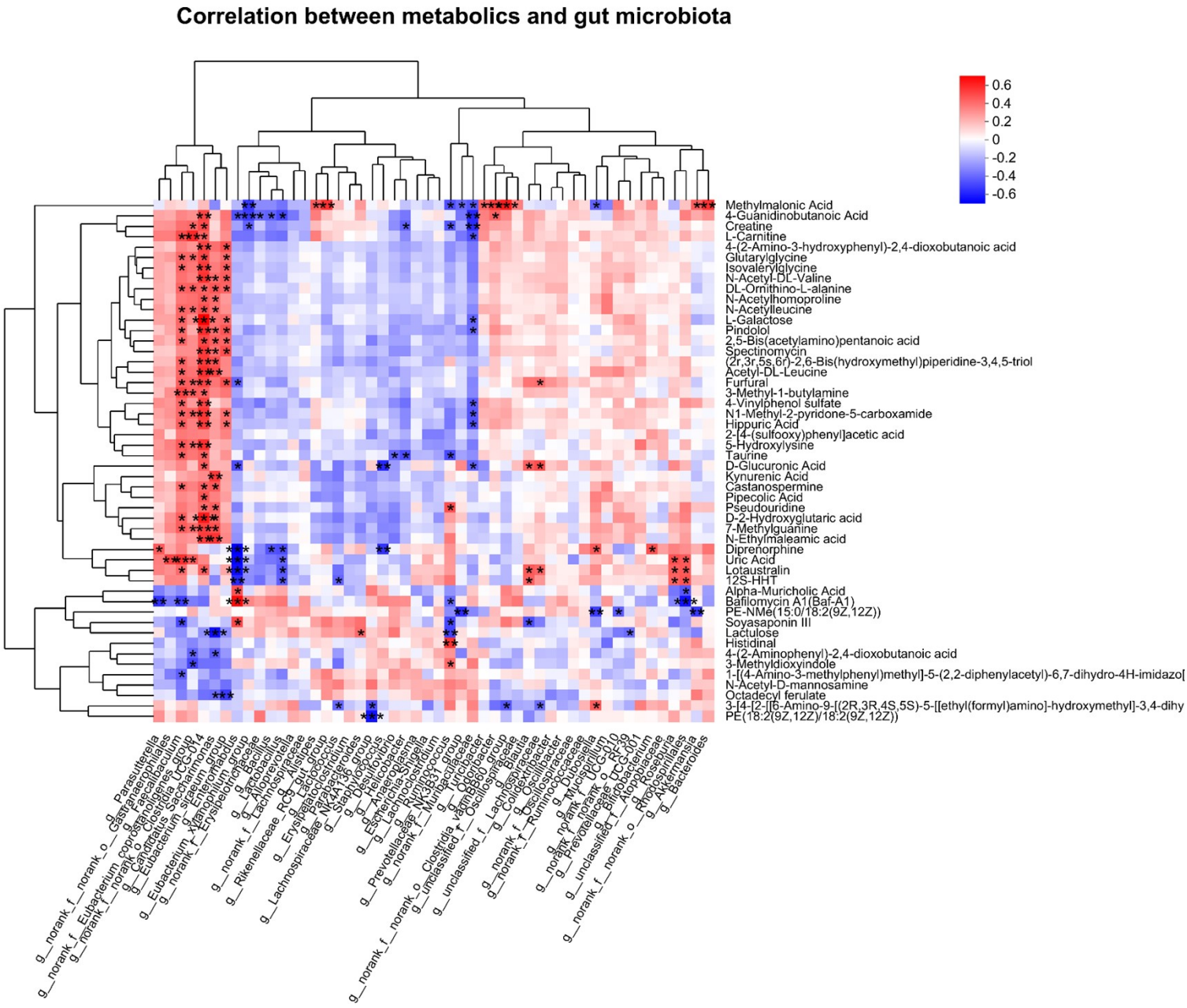


Fig. S2. Y treatment regulated the metabolites of colitis mice. (A) Volcano plots between Y-DSS and DSS groups. (B) Pathway enrichment analysis of differential metabolites (Y-DSS *vs* DSS). (C) The abundance of the differential metabolites of lotaustralin, 1-Pyrroline-4-hydroxy-2-carboxylate, and betaine, respectively. $p < 0.05$, *; $p < 0.01$, **; and $p < 0.001$, ***.



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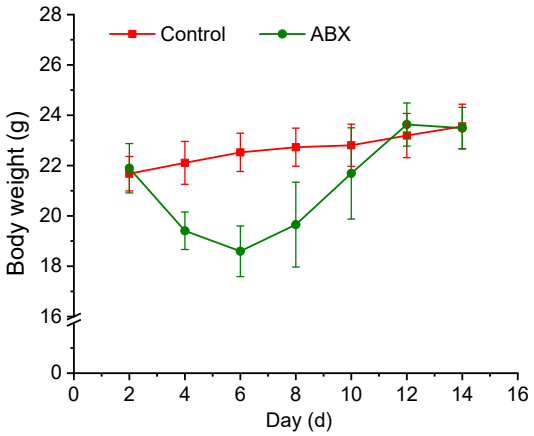
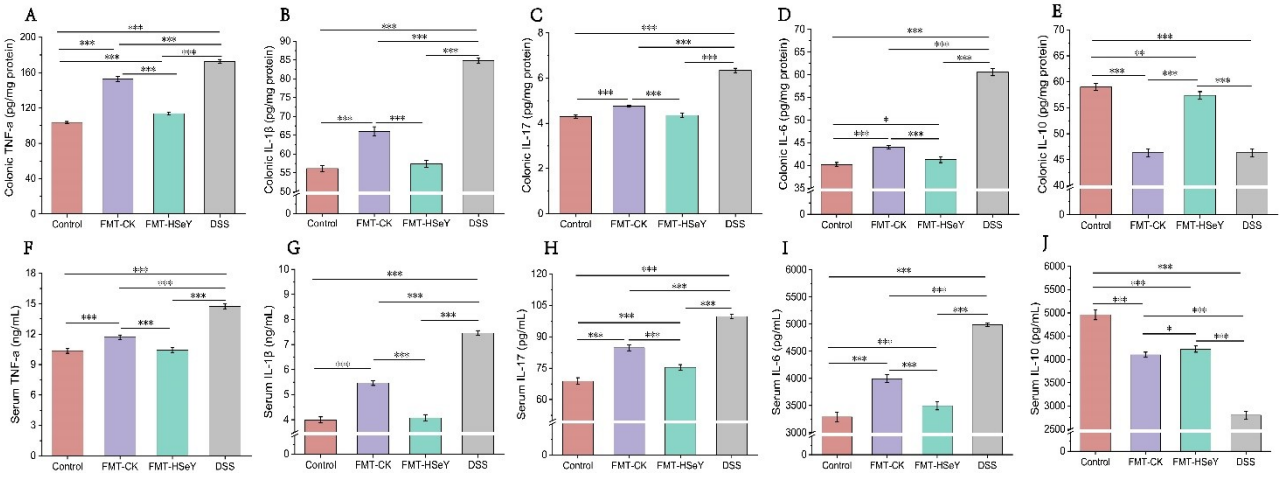


Fig. S4 Changes in body weight of mice during antibiotic removal of intestinal microbiota.



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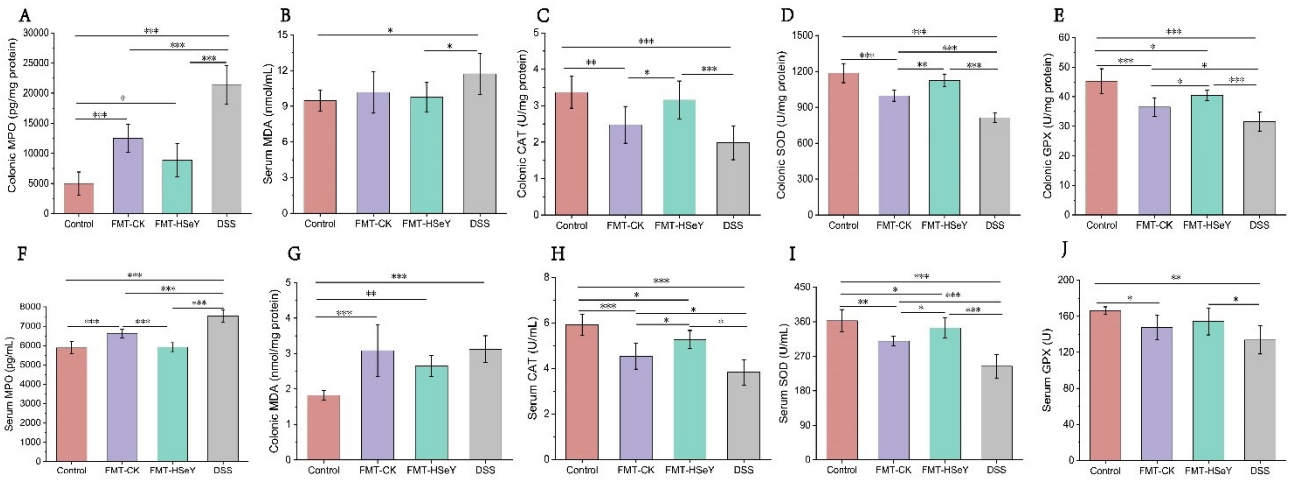

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