Supplementary information

Maternal gut microbial legacy shapes intestinal health and colitis susceptibility of offspring

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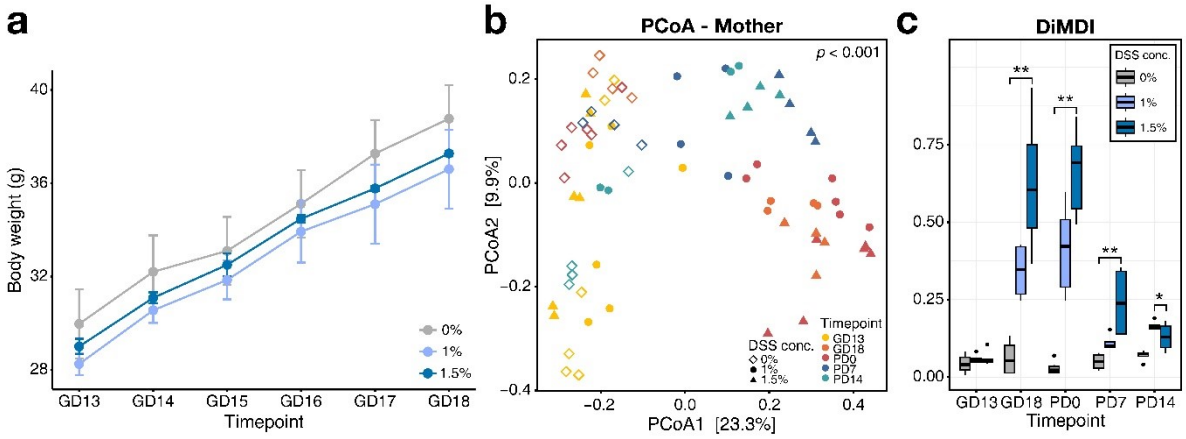
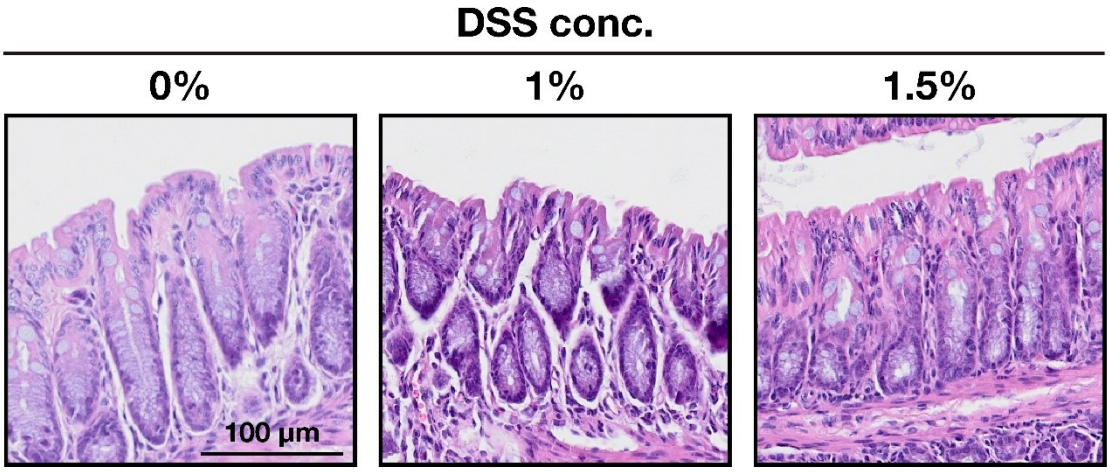
Supplementary Table 1. List of primers used in this study.

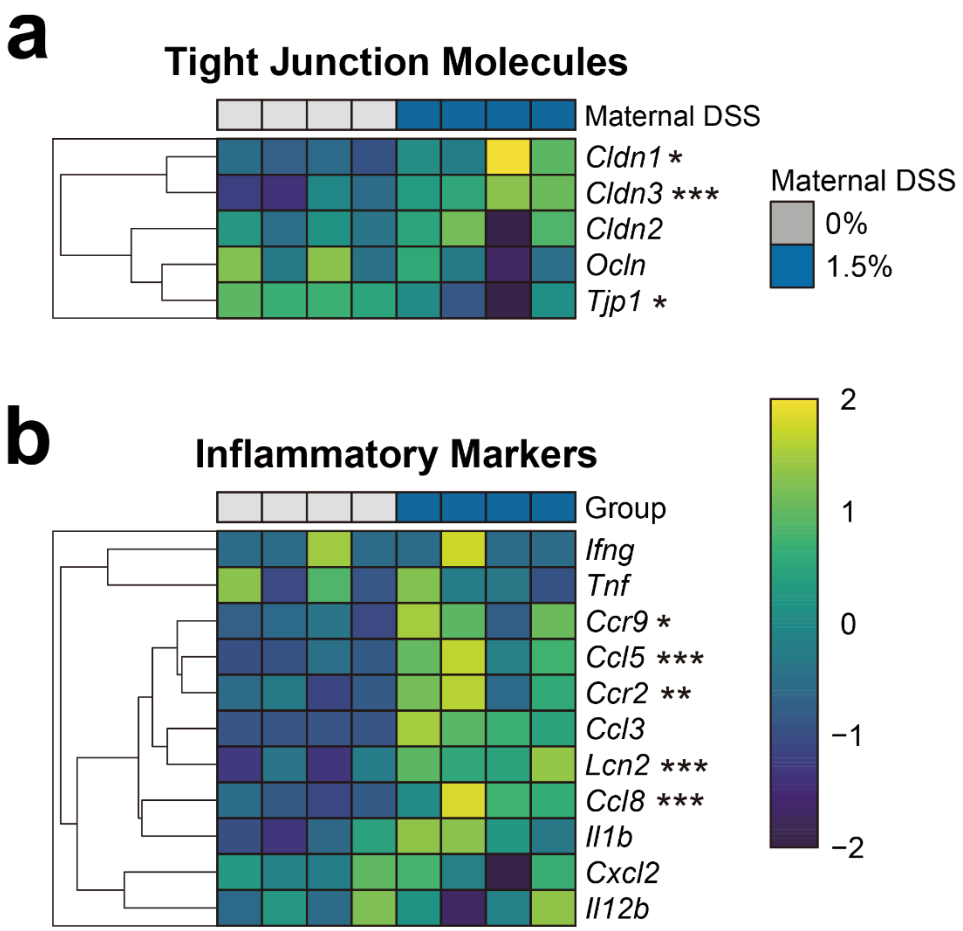
Gene	Sense Sequence	Antisense Sequence
36b4	5′-ACCTCCTTCTTCCAGGCTTT-3′	5′-CTCCAGTCTTTATCAGCTGC-3′
Tjp1	5′-TCATCCCAAATAAGAACA-3′	5′-GAAGAACAACCCTTTCATAAGC-3′
Ocln	5′-AAGCAAGTGAAGGGATCTGC-3′	5′-GGGGTTATGGTCCAAAGTCA-3′
Cldn1	5′-GTGACCGCTCAGGCCATCT-3′	5′-ACTGTATCTGCCCGGTGCTT-3′
Cldn2	5′-ACCAGCGGCGAGTAGAAGTC-3′	5′-GGCATCCTGGGCTTTATCC-3′
Cldn3	5′-GGAGGGCCTGTGGAAC-3′	5′-CAGCAGCGAGTCGTACATTTT-3′
Lcn2	5′-CCTCAAGGACGACAACATCATCT-3′	5′-ACCACCCATTCAGTTGTCAATG-3′
Ifng	5′-CAGCAACAGCAAGGCGAAA-3′	5′-CTGGACTGGGTTGTTGAC-3′
Il12	5′-GAAGCTGGTGCTGTAGTTCTCATATTT-3′	5′-AGACCCTGCCCATTGAACTG-3′
Tnf	5′-GACTTTCTCCTGGTATGAGATAGCAAA-3′	5′-AGGCTGCCCCGACTACGT-3′



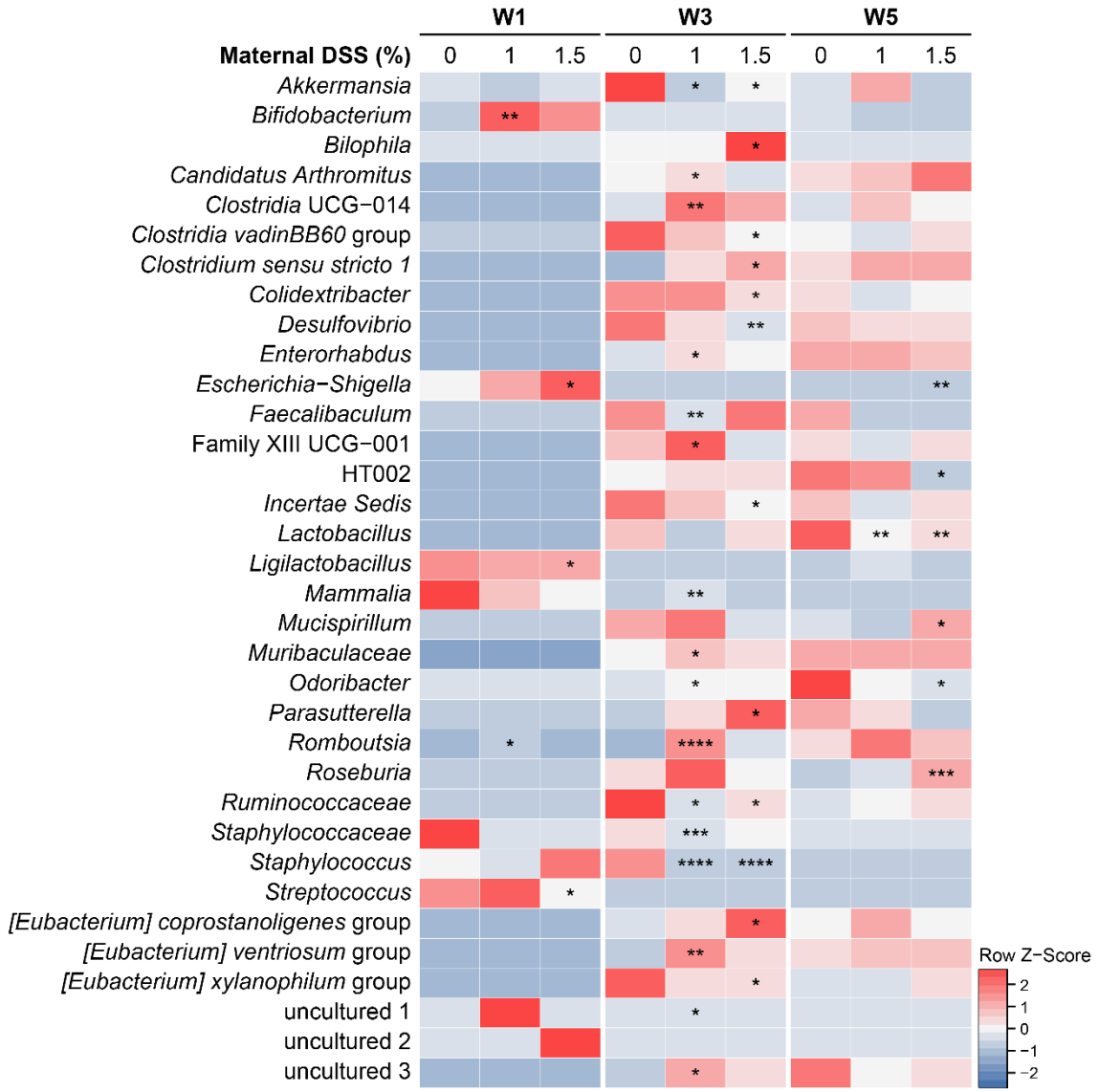
Supplementary Figure 1. Intestinal conditions in the offspring are influenced by maternal conditions during pregnancy. a Body weight changes of mother during DSS treatment in pregnancy (n=4 per group). **b** Principal coordinate analysis (PCoA) of gut microbiota in mother (n=4 per group). GD13: gestation day 13, GD18: gestation day 18, PD0: postpartum day 0, PD7: postpartum day 7, PD14, postpartum day 14 (n=4 per group). **c** Changes of DSS-induced microbial dysbiosis index (DiMDI) of maternal gut microbiota during pregnancy (n=4 per group). Statistical significance is indicated as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. A Kruskal-Wallis test was used to compare three groups.



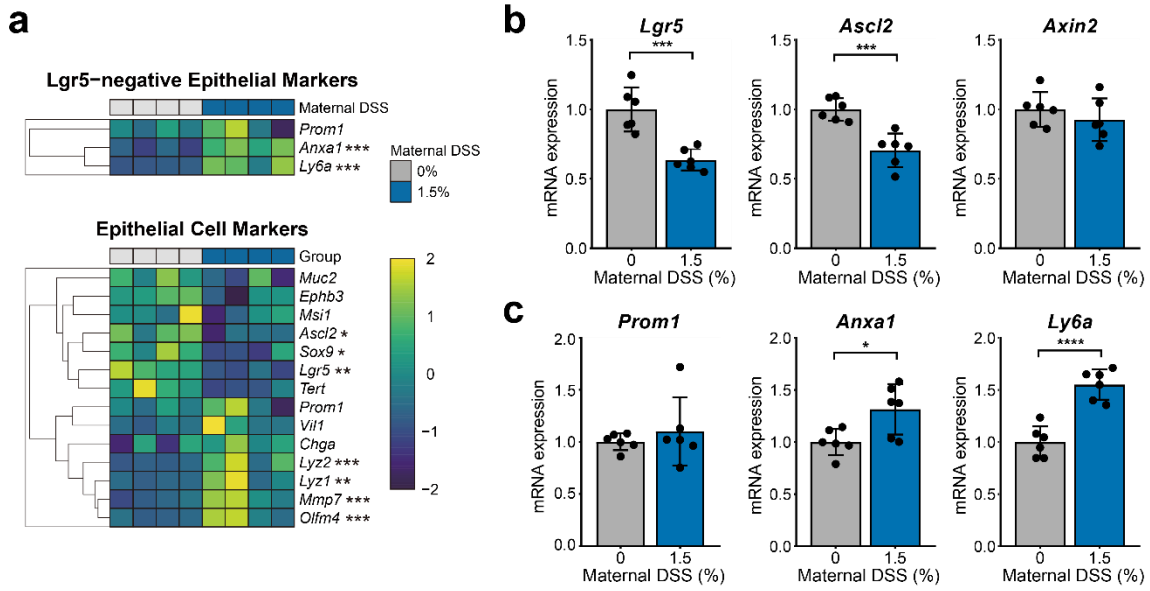
Supplementary Figure 2. Sections of colon from offspring (at 3 weeks-of-age) were analyzed by hematoxylin and eosin (H&E) staining.



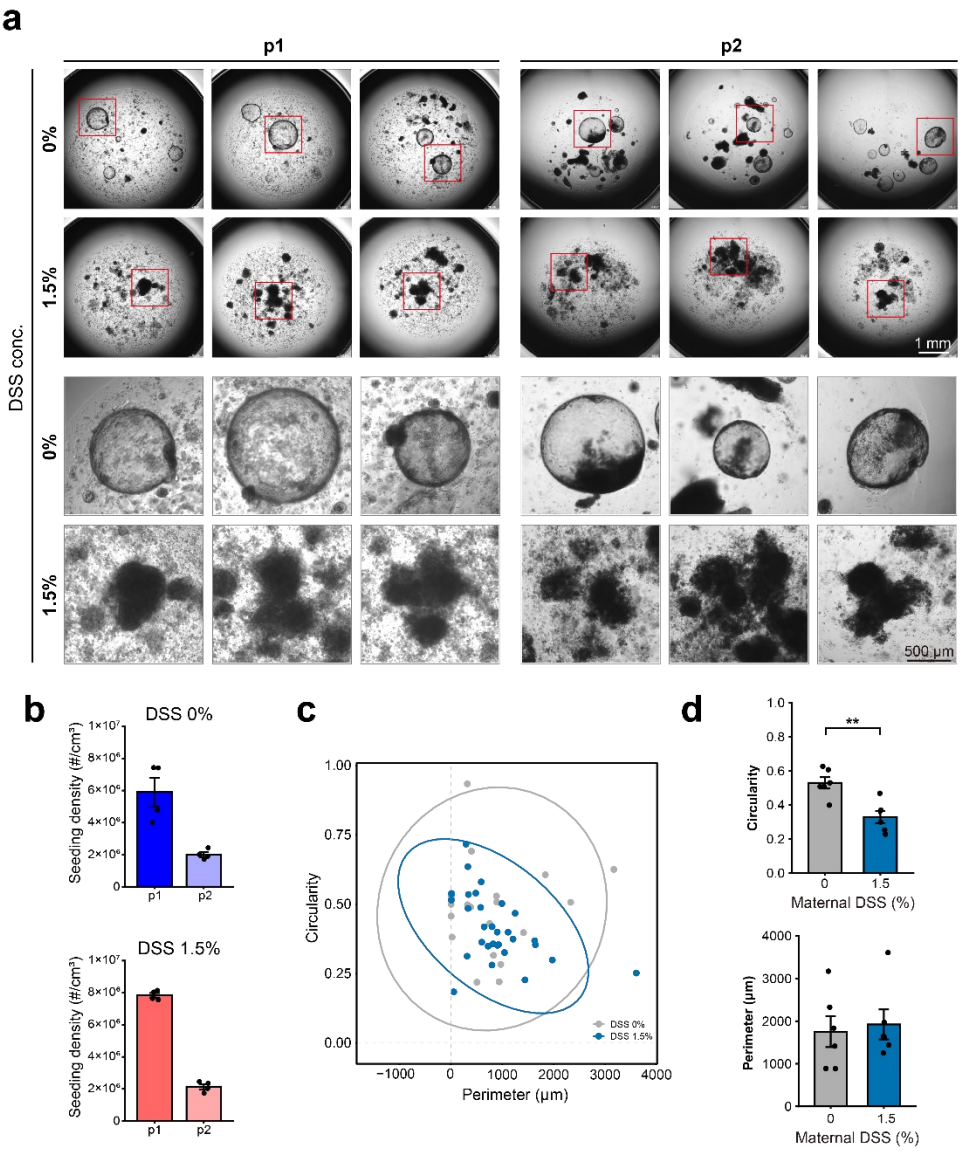
Supplementary Figure 3. Expression of tight junction and inflammatory genes in the colonic tissue of offspring. a,b Heatmaps displaying the differential expression profiles of genes associated with tight junction integrity **(a)** and inflammatory and cytokine responses **(b)** in colonic tissues of offspring. The color scale represents Z-score–normalized expression values (blue, low; yellow, high). Statistical significance is indicated as $*p < 0.05$, and $**p < 0.01$. For significance testing, DESeq2 uses a Wald test: the shrunken estimate of LFC is divided by its standard error, resulting in a z-statistic, which is compared with a standard normal distribution. (See Materials and methods for details.)



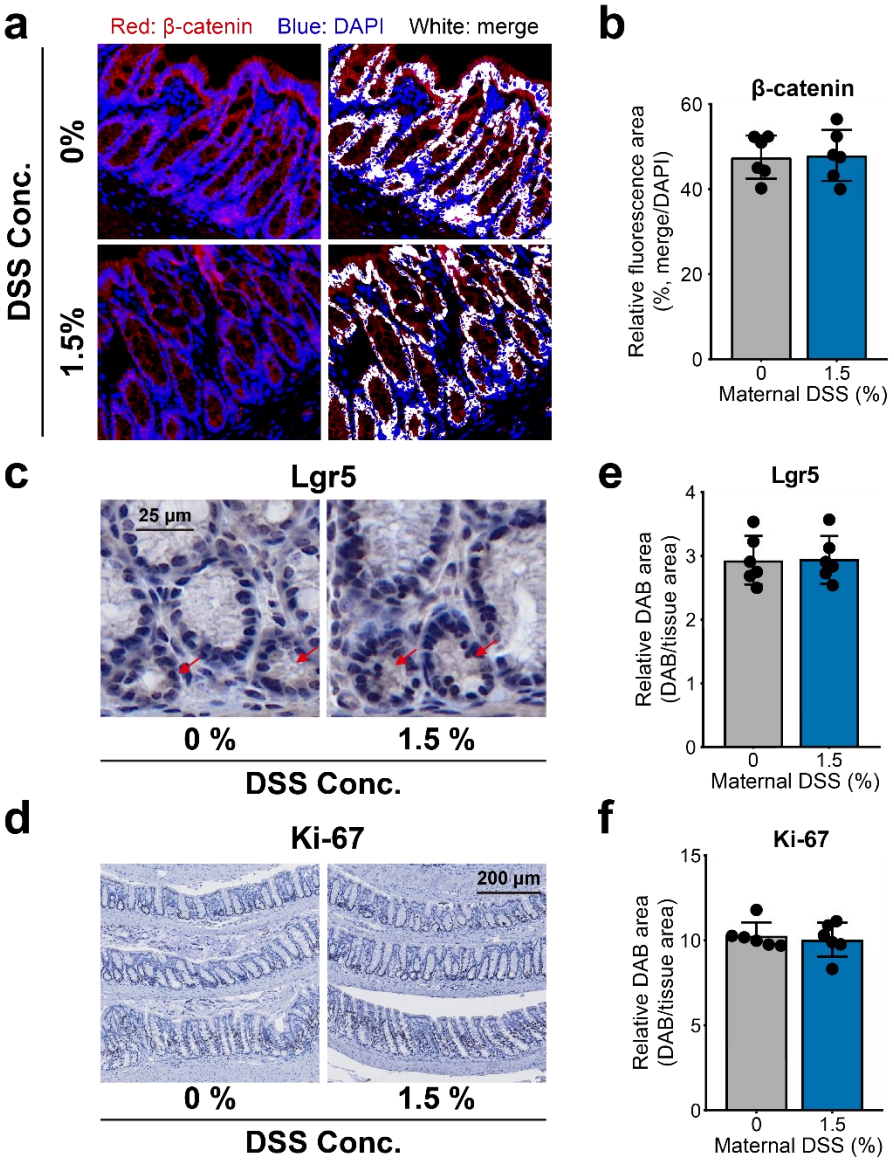
Supplementary Figure 4. Heatmap showing the relative abundance of the differential genera identified by MaAsLin2. Statistical significance is indicated as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.



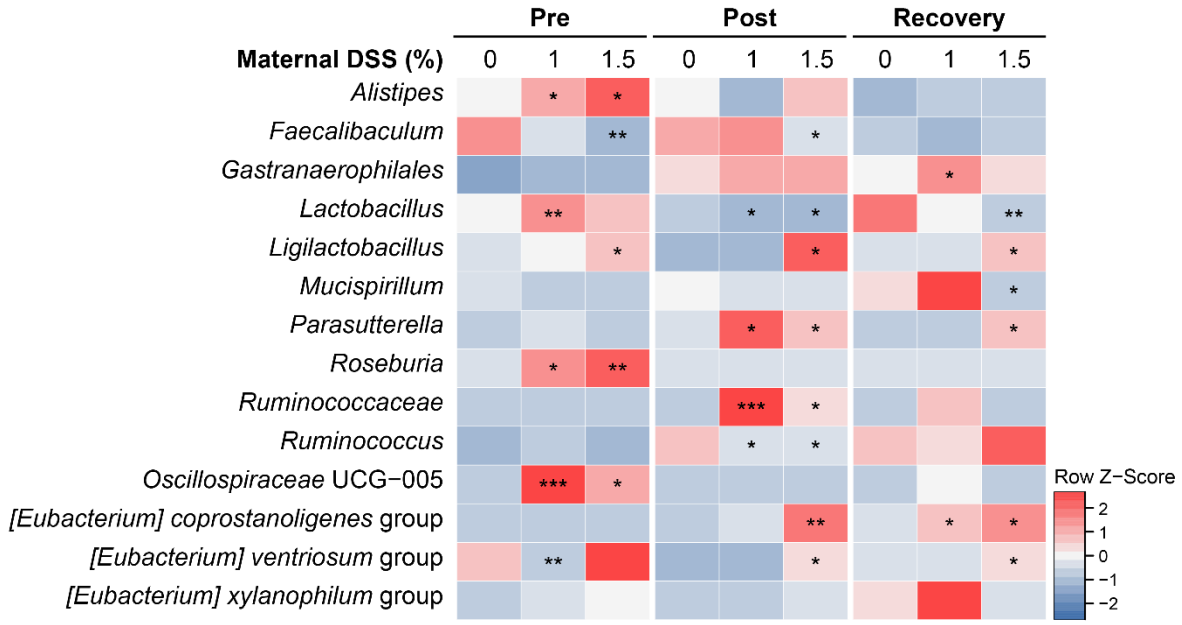
Supplementary Figure 5. Additional validation of Wnt/ISC-related gene expression in offspring. a Heatmaps analysis of specific functional gene sets: Epithelial and Non-Lgr5 epithelial cell markers (n=4 per group). **b** mRNA expression of canonical Wnt/ISC-related genes (*Lgr5, Ascl2, Axin2*) in colonic tissue from offspring of control and DSS-treated dams (n=6 per group). **c** mRNA expression of Lgr5-negative regenerative stem cell–associated markers (*Prom1, Anxa1, Ly6a*) (n=6 per group). All box plots show the median (center line), interquartile range (box limits), and whiskers extending to 1.5× the IQR. Error bars represent mean ± SEM unless otherwise indicated. Statistical significance is indicated as $*p < 0.05$, and $**p < 0.01$. For significance testing, DESeq2 uses a Wald test: the shrunken estimate of LFC is divided by its standard error, resulting in a z-statistic, which is compared with a standard normal distribution (a). (See Materials and methods for details.) Statistical differences among groups for (b,c) were assessed using Student's t-test.



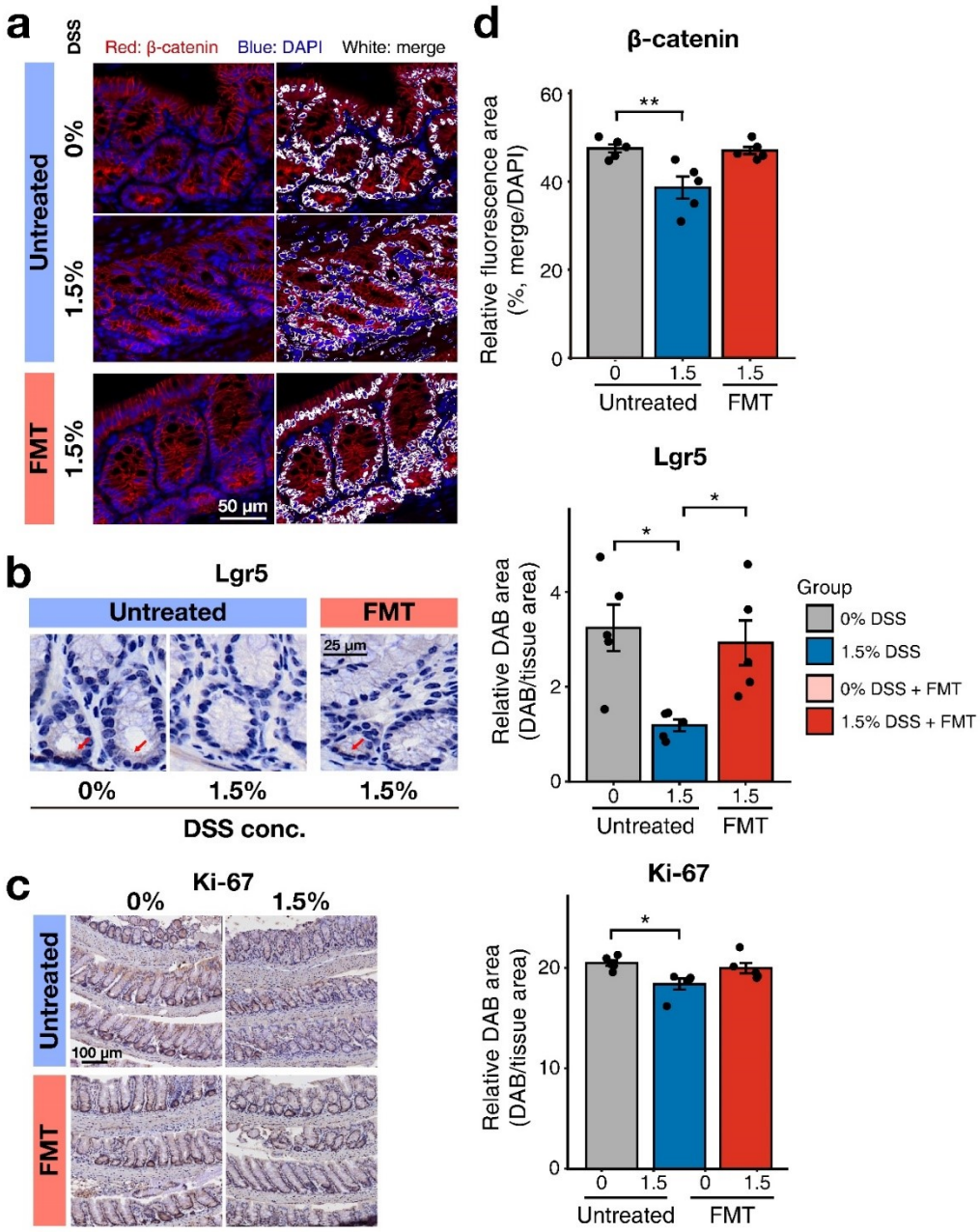
Supplementary Figure 6. Impaired organoid-forming capacity in offspring of DSS-treated dams. **a** Representative bright-field images of colon organoid cultures derived from offspring of control and DSS-treated dams (n=6 per group). **b** Quantification of organoid seeding density in each condition (n=6 per group). **c** Quantification of organoid morphology based on circularity and perimeter measurements (n=6 per group). **d** Correlation analysis between organoid circularity and perimeter, illustrating differences in organoid structural organization between groups (n=6 per group). Data are presented as mean ± SEM, with each dot representing an individual animal. Statistical significance is indicated as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. Statistical differences among groups were assessed using one-way or two-way ANOVA with appropriate post-hoc tests, as indicated.



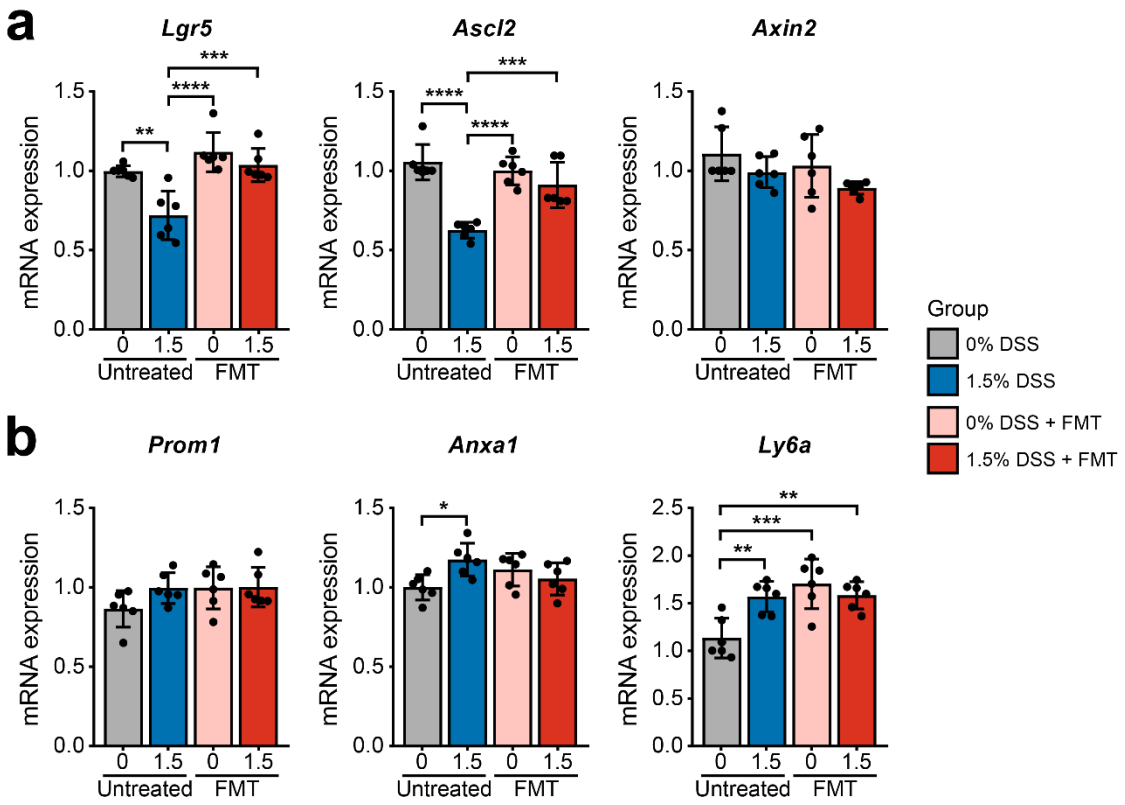
Supplementary Figure 7. No significant differences in epithelial Wnt/ISC-associated markers at 5 weeks of age. a Immunofluorescence staining showing β-catenin localization (red) with DAPI nuclear counterstaining (blue) in colonic crypts of offspring **b,c** Representative immunohistochemical staining for Lgr5 and Ki-67 in colonic crypts of offspring. Crypt orientation may vary due to tissue sectioning. **d** Quantification of the β-catenin–positive nuclear area in merged images, as well as Lgr5-positive and Ki-67–positive areas, normalized to total tissue area (n=6 per group). All box plots show the median (center line), interquartile range (box limits), and whiskers extending to 1.5× the IQR. Error bars represent mean ± SEM unless otherwise indicated. Statistical significance is indicated as $*p < 0.05$, and $**p < 0.01$. Statistical differences among groups were assessed using Student's t-test.



Supplementary Figure 8. Heatmap displaying the relative abundance of differentially abundant genera identified using multivariate analysis by linear models (MaAsLin2). The analysis compares microbial composition across three time points—pre-DSS (Pre), post-DSS (Post), and recovery (Recovery)—in offspring born to mothers treated with 0%, 1%, or 1.5% DSS. Statistical significance compared to the 0% DSS control group is indicated as *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.



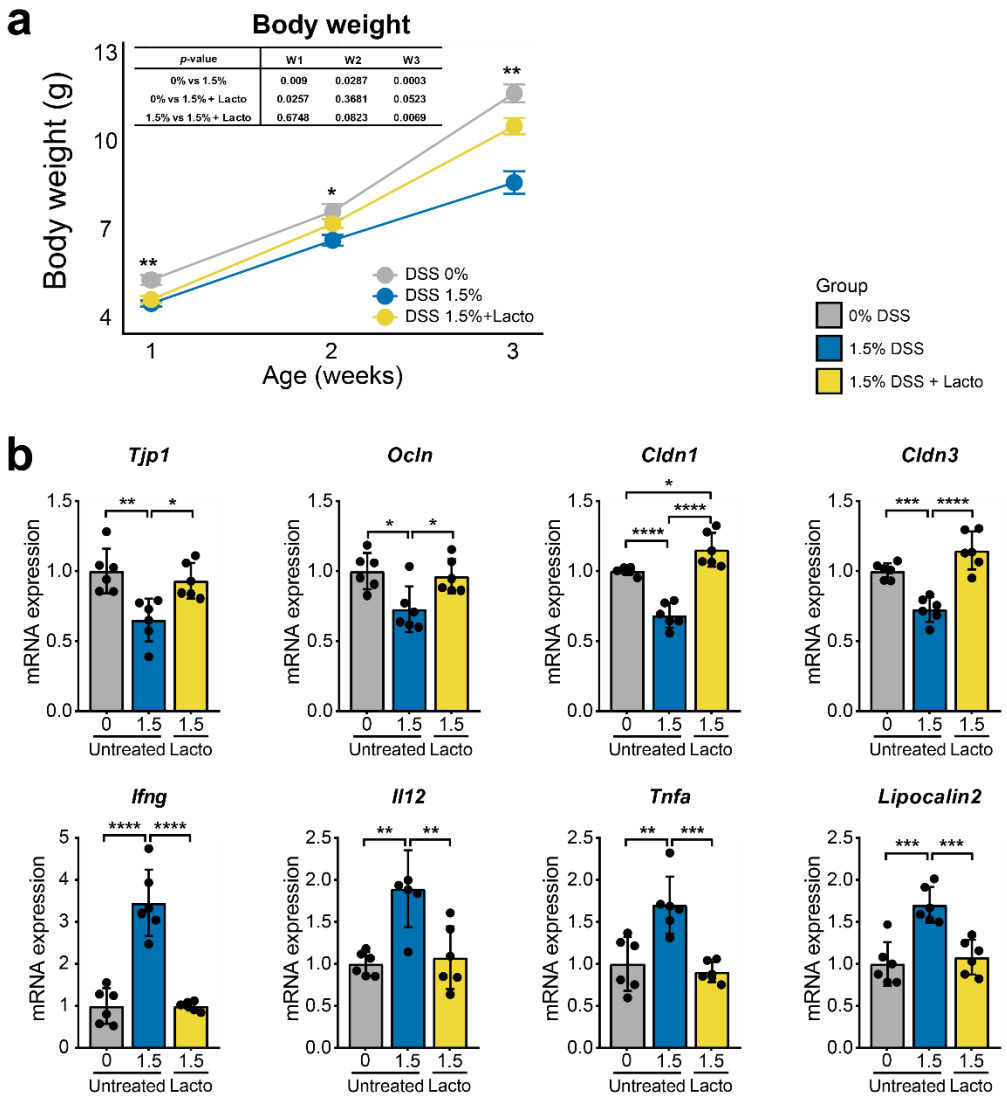
Supplementary Figure 9. FMT during early life improves proliferation of intestinal epithelial cells. a-c Immunofluorescence staining analysis image of localization of β-catenin, Lgr5 (crypt orientation may vary due to tissue sectioning), and Ki-67 in the crypt cells of offspring. **d** Relative fluorescence area of β-catenin, Lgr5, and Ki-67 (n=5 per group). Quantification was performed based on tissue area to assess overall epithelial changes across the colon. All box plots show the median (center line), interquartile range (box limits), and whiskers extending to 1.5× the IQR. Error bars represent mean ± SEM unless otherwise indicated. Statistical significance is indicated as $*p < 0.05$ and $**p < 0.01$. A two-way ANOVA with Tukey's post-hoc test was used to compare the different groups.



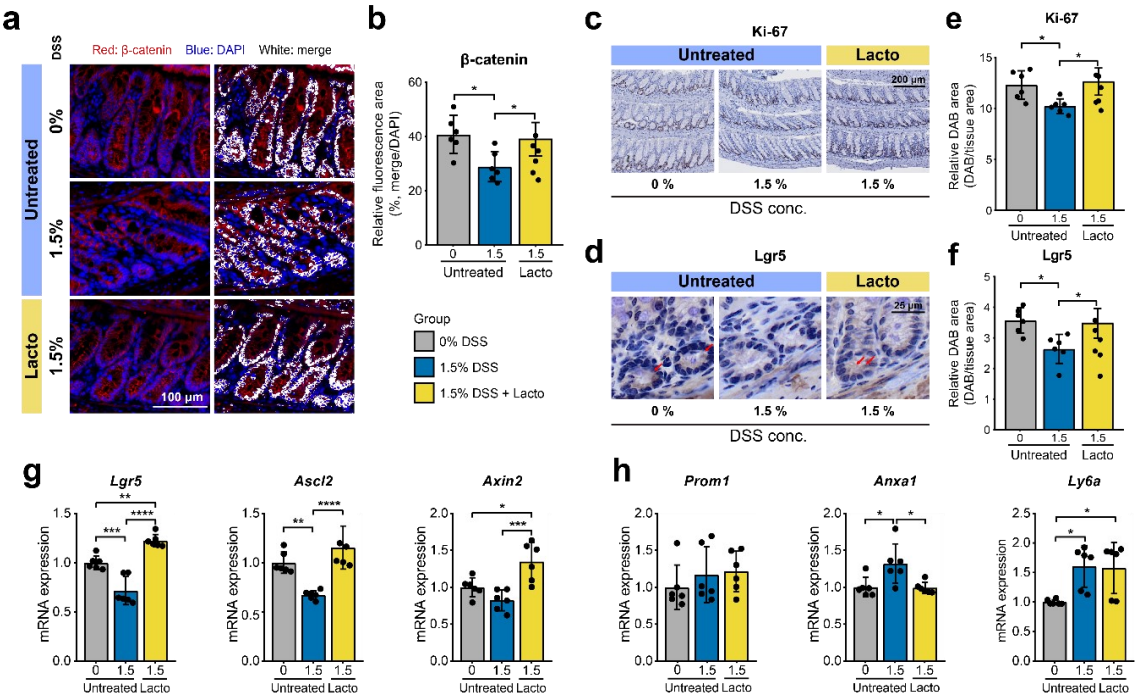
Supplementary Figure 10. Early-life FMT partially restores Wnt/ISC-related gene expression in offspring. **a** mRNA expression of canonical Wnt/intestinal stem cell (ISC)–associated genes (Lgr5, Ascl2, Axin2) in colonic tissue from offspring born to control or DSS-treated dams, with or without early-life fecal microbiota transplantation (FMT) (n = 6 per group). **b** mRNA expression of Lgr5-negative regenerative stem cell–associated markers (Prom1, Anxa1, Ly6a) in the same groups (n = 6 per group). Data are presented as mean ± SEM, with each dot representing an individual animal. Statistical significance is indicated as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. Statistical differences among groups were assessed using one-way or two-way ANOVA with appropriate post-hoc tests, as indicated.

Supplementary Table 2. Bray-Curtis distances from groups to donor microbiota across timepoints

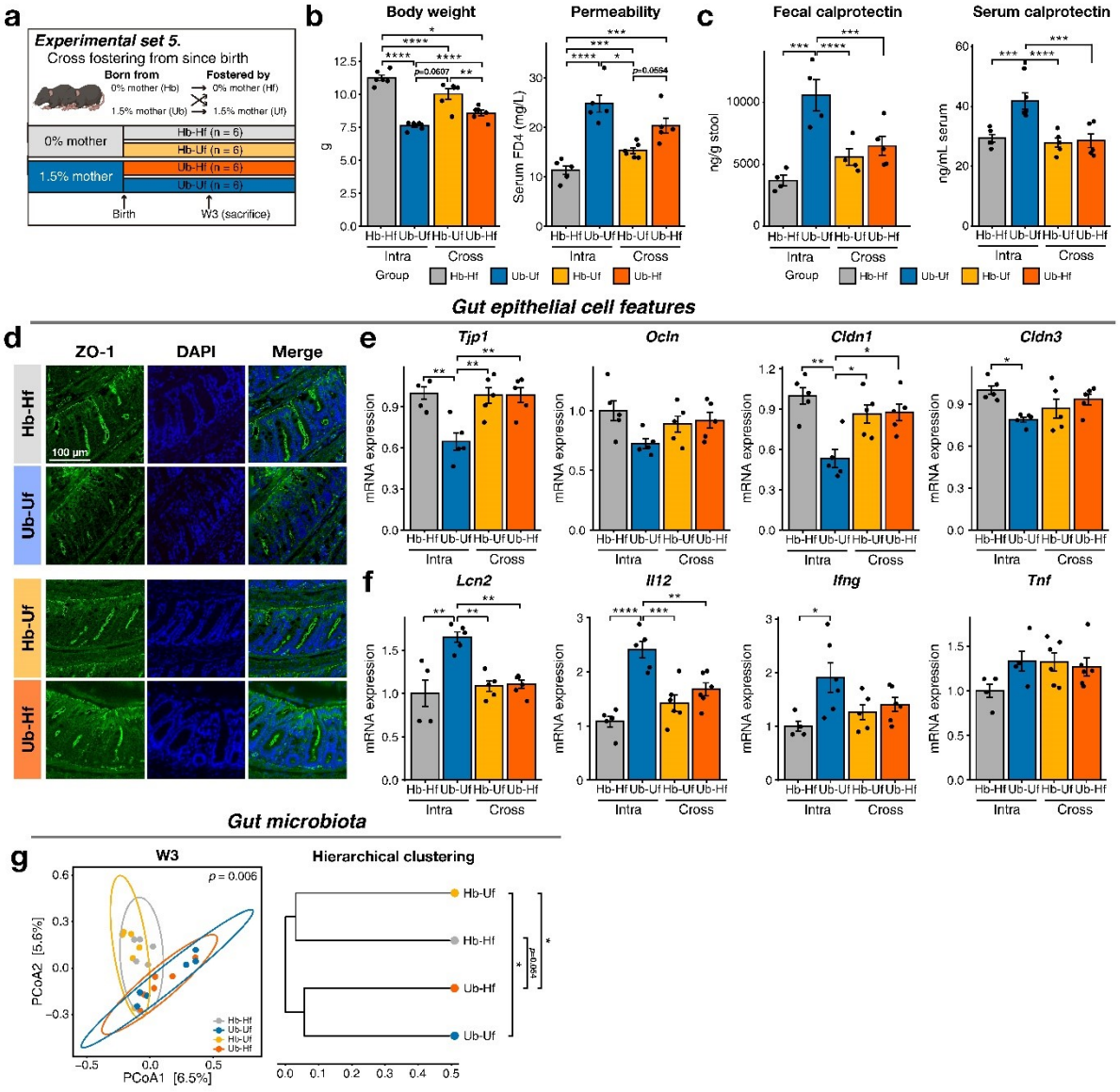
Group	Age	
	W3	W5
0% DSS-Untreated	0.049	0.240
1% DSS-Untreated	0.151	0.138
1.5% DSS-Untreated	0.178	0.228
0% DSS-FMT	0.117	0.085
1% DSS-FMT	0.280	0.109
1.5% DSS-FMT	0.217	0.174



Supplementary Figure 11. Early-life Lactobacillus supplementation improves growth, epithelial barrier markers, and inflammatory responses in offspring. **a** Changes in body weight of offspring from 1 to 3 weeks of age following early-life oral supplementation with *Lactobacillus (*n = 6 per group). **b** mRNA expression of genes encoding tight junction–associated proteins in colon tissue from offspring at 2 weeks of age (n = 6 per group). **c** mRNA expression of pro-inflammatory markers in colon tissue from offspring at 3 weeks of age (n = 6 per group). Data are presented as mean ± SEM, with each dot representing an individual animal. Statistical significance is indicated as *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. Statistical differences among groups were assessed using one-way or two-way ANOVA with appropriate post-hoc test.

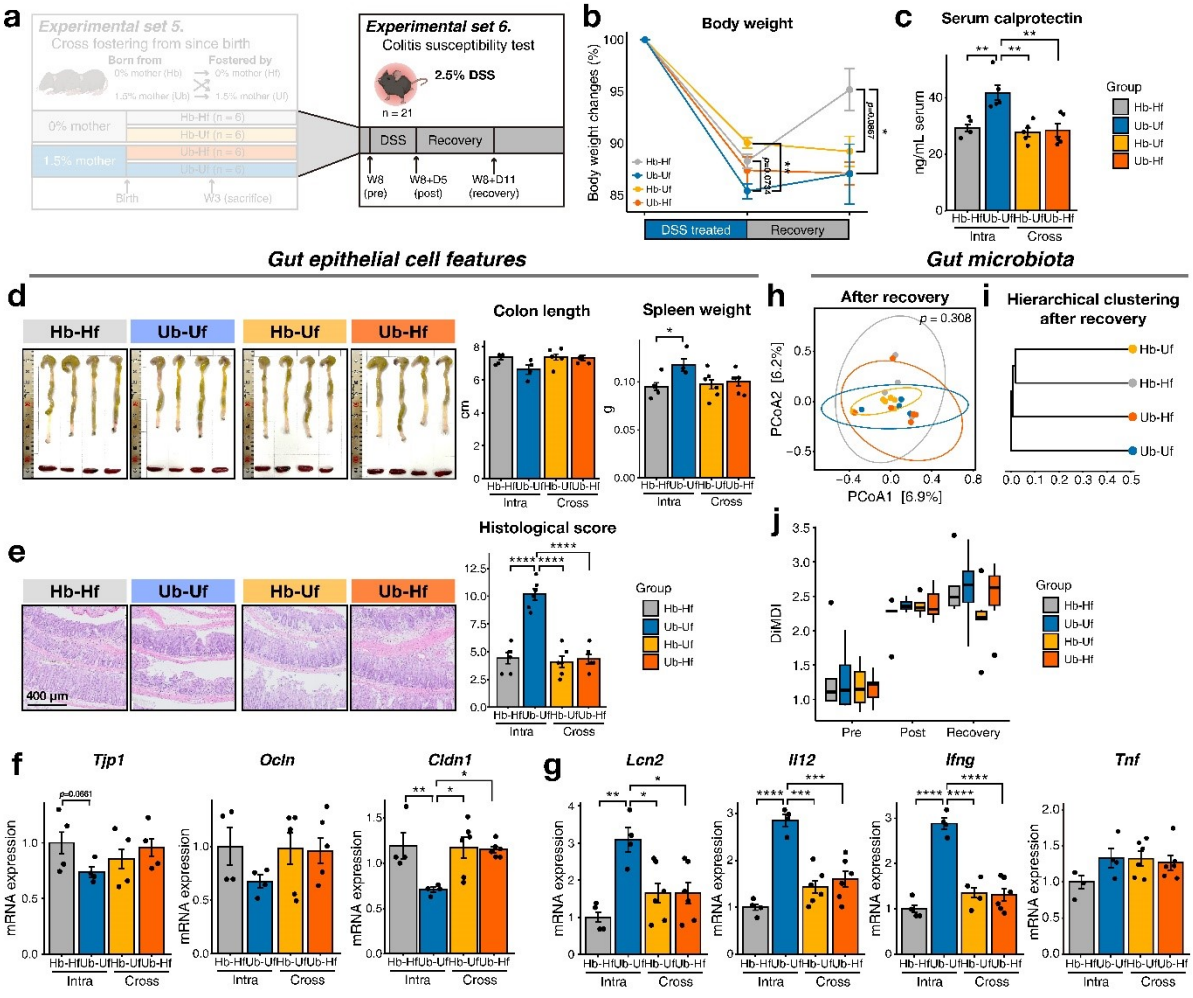


Supplementary Figure 12. Early-life Lactobacillus supplementation restores epithelial proliferation and Wnt/ISC-associated features in offspring. **a** Immunofluorescence staining showing β-catenin localization (red) with DAPI nuclear counterstaining (blue) in colonic crypts of offspring. **b** Quantification of the β-catenin–positive nuclear area in merged images. **c,d** Representative immunohistochemical staining for Lgr5 and Ki-67 in colonic crypts of offspring. Crypt orientation may vary due to tissue sectioning. **e,f** Quantification of Lgr5-positive and Ki-67–positive areas, normalized to total tissue area (n=6 per group). **f** mRNA expression of canonical Wnt/intestinal stem cell (ISC)–associated genes (Lgr5, Ascl2, Axin2) in colonic tissue from offspring born to control or DSS-treated dams, with or without early-life fecal microbiota transplantation (FMT) (n = 6 per group). **g** mRNA expression of Lgr5-negative regenerative stem cell–associated markers (Prom1, Anxa1, Ly6a) in the same groups (n = 6 per group). Data are presented as mean ± SEM, with each dot representing an individual animal. Statistical significance is indicated as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. Statistical differences among groups were assessed using one-way or two-way ANOVA with appropriate post-hoc tests, as indicated. All box plots show the median (center line), interquartile range (box limits), and whiskers extending to 1.5× the IQR. Error bars represent mean ± SEM unless otherwise indicated. Statistical significance is indicated as $*p < 0.05$, and $**p < 0.01$. Statistical differences among groups were assessed using two-way ANOVA with Tukey's post-hoc test.

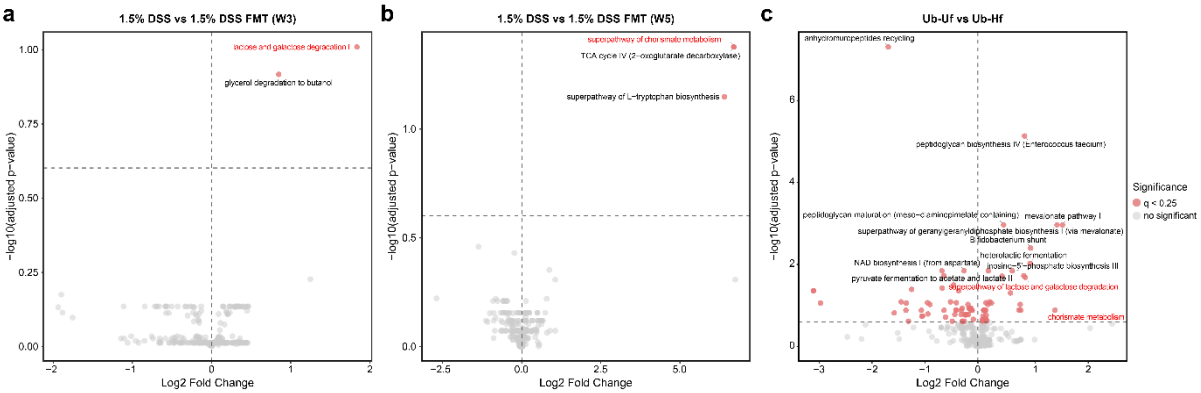


Supplementary Figure 13. Cross-fostering alters the gut microbiota and alleviates intestinal conditions in offspring of mothers with colitis. a Schematic graphic of study design for experiment set 5. The offspring born to mothers treated with 0% DSS (Healthy birth, Hb) and 1.5% DSS (Unhealthy birth, Ub) were subjected to intra-fostering (Healthy fostering, Hf (n=6); Unhealthy fostering, Uf (n=6)) and cross-fostering (Unhealthy fostering, Uf (n=6); Healthy fostering, Hf (n=6)). The intestinal conditions of the offspring were analyzed at 3 weeks. **b** Body weight and gut permeability (serum FD4 levels) of offspring at 3 weeks. **c** Fecal and serum calprotectin level of each group at 3 weeks of life (n=5 per group). **d** Immunofluorescence staining image of localization of zonula occludens-1 (ZO-1) and Claudin-3 in the gut epithelial cells of offspring. **e,f** mRNA gene expression levels related to (e) gut tight junction and **(f)** inflammatory response in the gut epithelial cells (n=4-5 per group). **g** Principal coordinate analysis (PCoA) and hierarchical cluster of gut microbiota in the offspring. Adonis was performed to compare the gut microbiota between the groups (n=6 per group). All box plots show the median (center line), interquartile range (box limits), and whiskers extending to 1.5× the IQR. Error bars represent mean ± SEM unless otherwise indicated. Statistical significance is indicated as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and

****$p < 0.0001$. Statistical analysis for the phenotypic data was performed using two-way ANOVA with Tukey's post-hoc test.



Supplementary Figure 14. Maternal conditions during fostering affect susceptibility of adult mice to colitis. **a** Schematic graphic of study design for experiment set 6. Offspring (n=21) after intra/cross fostering were subjected to 2.5% DSS treatment at week 8 of life. **b** Body weight changes of offspring before/after DSS treatment and after 5 days of the recovery period (Hb-Hf, n=4; Ub-Uf, n=5; Hb-Uf, n=6; Ub-Hf, n=4). **c** Serum calprotectin level of offspring after recovery (n=6 per group). **d** Colon length and spleen weight of offspring after recovery (n=5-6 per group). **e** Hematoxylin and eosin (H&E) staining of colon tissue, and histological score of offspring after recovery (n=5 per group). **f,g** mRNA gene expression levels related to **(f)** gut tight junction and **(g)** immune response in the gut epithelial cells (n=4-6 per group). **h-i** Principal coordinate analysis (PCoA) and hierarchical cluster of gut microbiota in the offspring. **j** Changes of DSS-induced microbial dysbiosis index (DiMDI) of gut microbiota in the offspring during DSS treatment. Adonis was performed to compare the gut microbiota between the groups (n=4-6 per group). All box plots show the median (center line), interquartile range (box limits), and whiskers extending to 1.5× the IQR. Error bars represent mean ± SEM unless otherwise indicated. Statistical significance is indicated as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. Statistical analysis for comparing the four groups was performed using two-way ANOVA with Tukey's post-hoc test.



Supplementary Figure 15. Volcano plots showing differential microbial pathways between (a) 1.5% DSS and 1.5% DSS-FMT treated groups at 3 weeks, (b) at 5 weeks, and (c) Ub-Uf and Ub-Hf groups. Volcano plots illustrating differential metabolic pathways identified via PICRUSt2 analysis based on 16S rRNA gene sequencing data. The x-axis represents the log2 fold change, and the y-axis represents the -log10 (adjusted p-value). Red dots indicate metabolic pathways with significant differential abundance (False Discovery Rate $q < 0.25$).