

**Supplementary Figure 1. *Engyodontium* colonization impacts intestinal neutrophils and Treg cells at steady state.**

**(A)** Flow cytometry analysis of mouse fecal commensal microbiome binding to Dectin-1-Fc protein.

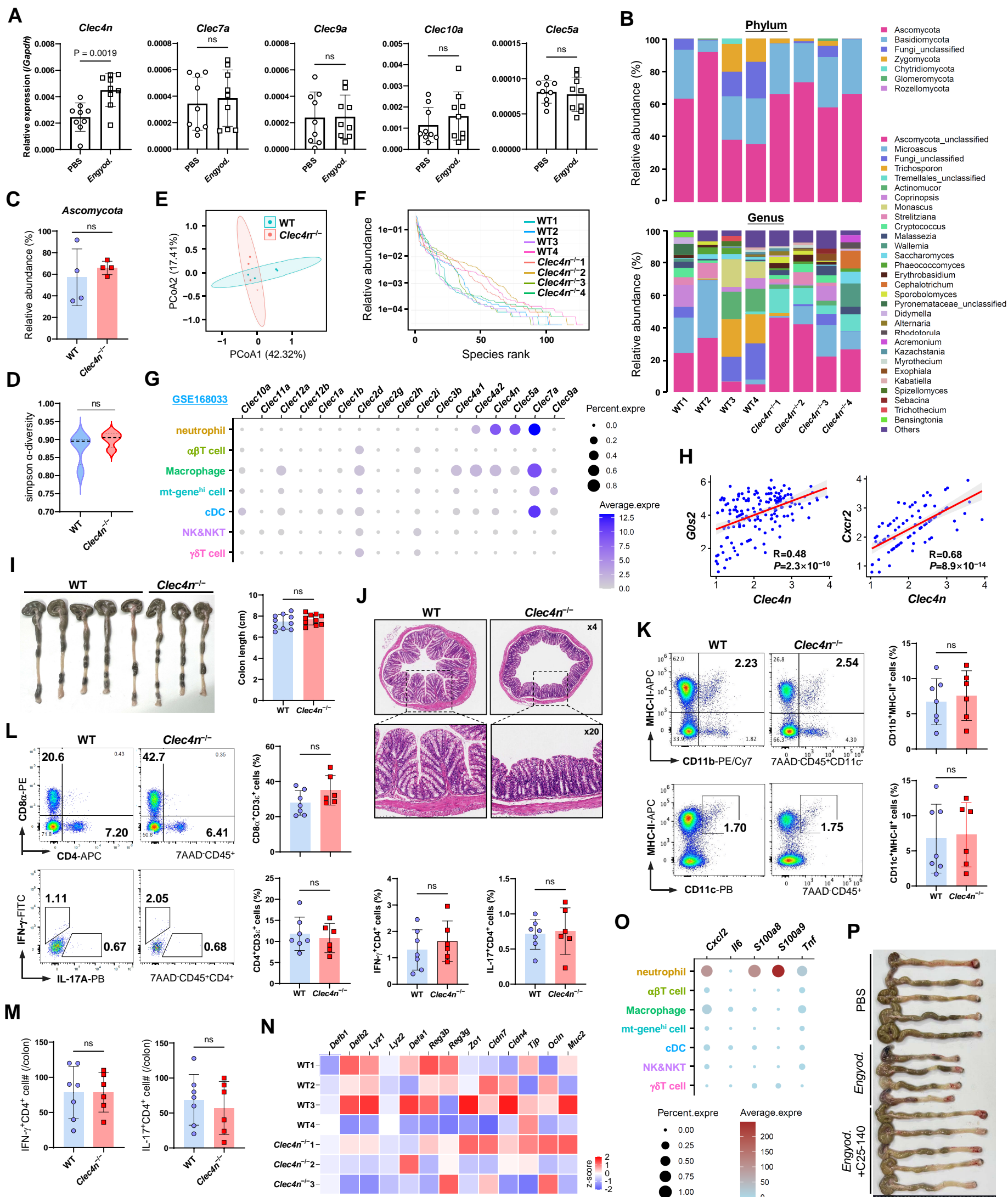
**(B)** Protocol for intestinal fungus isolation and identification.

**(C)** Phylum and genus composition of mouse fecal commensal fungi bound by Dectin-1-Fc, identified by ITS1-2 sequencing.

**(D)** FACS analysis of Dectin-2-Fc-positive fecal microorganisms.

**(E-I)** In parallel with the experiment described in Figure 1I-1L, the following were evaluated: **(E)** gross colonic appearance and colon length (WT n=13, *Clec4n*<sup>-/-</sup> n=12), **(F)** proportions of monocytes (Ly6C<sup>+</sup>CD11b<sup>+</sup>), dendritic cells (MHC-II<sup>+</sup>CD11c<sup>+</sup>), M1 macrophages (MHC-II<sup>+</sup>CD11b<sup>+</sup> in CD11c<sup>-</sup> cells), B cells (MHC-II<sup>+</sup>CD11b<sup>-</sup> in CD11c<sup>-</sup> cells), **(G)** IL-17<sup>+</sup> CD4<sup>+</sup> T cells, and IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> T cells by FACS analysis (WT n=10, *Clec4n*<sup>-/-</sup> n=9), **(H)** mRNA expression of antimicrobial peptides and epithelial tight junction genes in colon tissues by qPCR (heatmap) (n=5/group), and **(I)** protein levels of IL-6, TNF- $\alpha$ , IL-1 $\beta$  and S100A8/9 calprotectin in the colon tissue lysates by ELISA (n=8/group).

Data in panels **(E-G)** are pooled from three and in **(I)** are pooled from two independent experiments. Data in panels **(E-G, I)** are shown as mean  $\pm$  SD. Statistical analysis: two-tailed unpaired Student's t-test **(E-I)**.



**Supplementary Figure 2. *Engyodontium* colonization up-regulates Dectin-2 expression in mouse colon, while Dectin-2 deficiency rarely affects most colonic immune cell populations.**

**(A)** In parallel with the experiment described in Figure 1I-1L, the mRNA expression levels of genes encoding CLRs in colon tissues were measured by qPCR (n=9 /group).

**(B-F)** The relative abundance of the entire intestinal fungal community **(B)** and *Ascomycota* **(C)**,  $\alpha$ -diversity **(D)**,  $\beta$ -diversity (PCoA, **E**) and the species rank of the intestinal fungi **(F)** in WT and *Clec4n*<sup>-/-</sup> mouse feces were assessed using fungal ITS1-2 sequencing analysis (n=4 /group).

**(G)** Dot plots illustrating the proportion of cells expressing specific CLR-encoding genes and the average expression level of each gene within distinct cell clusters of colonic immune cells from C57BL/6 mice under steady-state conditions, derived from the reanalysis of a public single-cell RNA-sequencing (scRNA-seq) dataset (GSE168033).

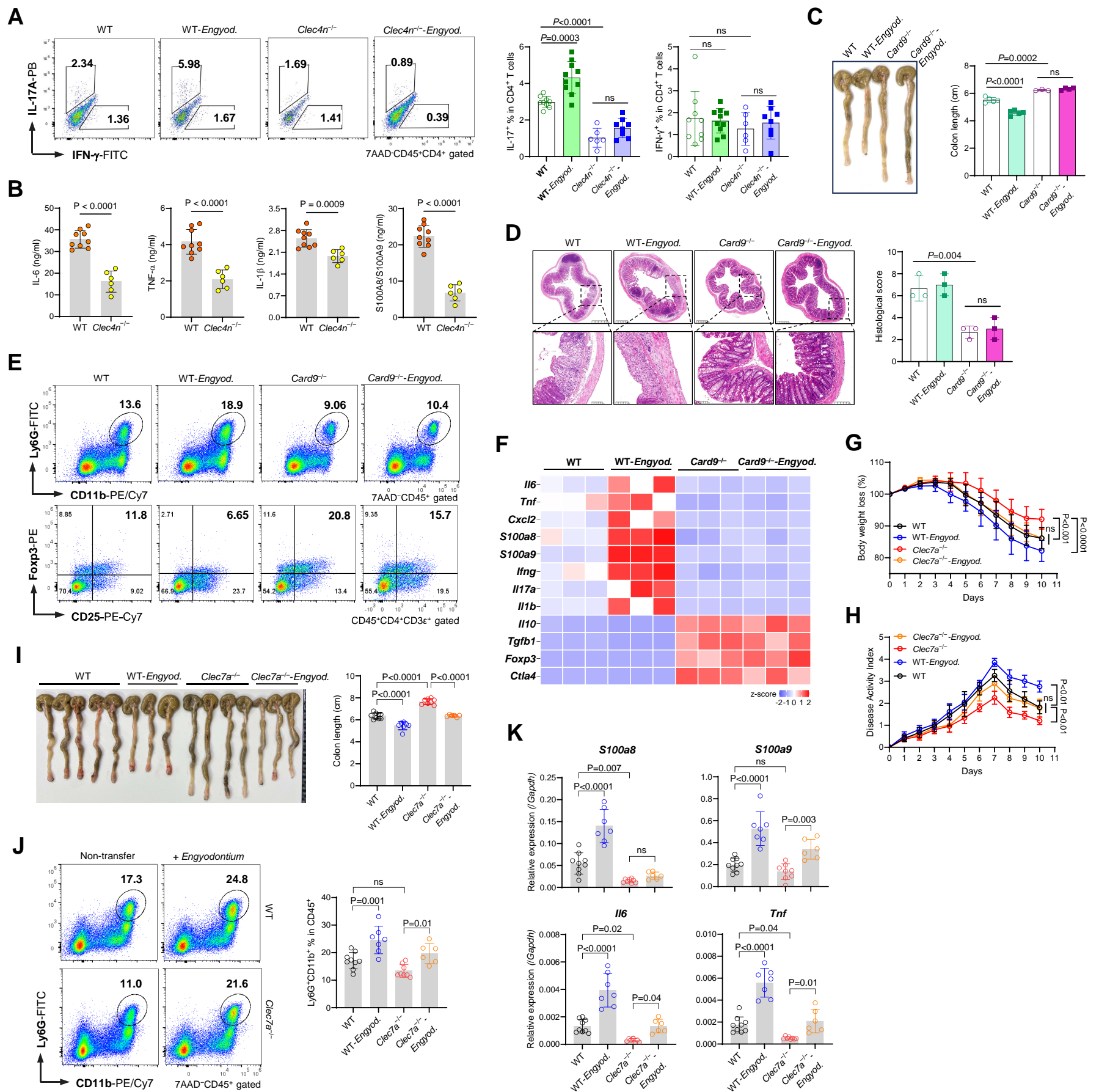
**(H)** Point plot depicting the correlation of expression levels between *Clec4n* and *G0S2* or *Cxcr2* in cells double-positive for *Clec4n* and *G0S2* or *Cxcr2*, using scRNA-seq datasets (GSE168033).

**(I-N)** In parallel with the experiment described in Figure 2D-2F, the following were evaluated: **(I)** gross colonic appearance and colon length (WT n=10, *Clec4n*<sup>-/-</sup> n=9), **(J)** histological analyses of the distal colon (H&E staining), **(K)** proportions of dendritic cells (MHC-II<sup>+</sup>CD11c<sup>+</sup>) and M1 macrophages (MHC-II<sup>+</sup>CD11b<sup>+</sup>CD11c<sup>-</sup>) (WT n=7, *Clec4n*<sup>-/-</sup> n=6), **(L, M)** proportions of CD8<sup>+</sup> and CD4<sup>+</sup> T cells, proportions and absolute numbers of IL-17<sup>+</sup>CD4<sup>+</sup> T cells and IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> T cells by FACS analysis (WT n=7, *Clec4n*<sup>-/-</sup> n=6), and **(N)** mRNA expression of antimicrobial peptides and epithelial tight junction genes in colon tissues by qPCR, presented in a heatmap (WT n=4, *Clec4n*<sup>-/-</sup> n=3).

**(O)** Dot plots showing the proportion of cells expressing indicated genes and the average expression level of each gene within distinct cell clusters of colonic immune cells from C57BL/6 mice under steady-state conditions, derived from the reanalysis of the scRNA-seq dataset GSE168033.

**(P)** In parallel with the experiment described in Figure 2I-2L, gross colonic appearance in the indicated groups is exhibited.

Data in panels **(A, C, I, K-M)** are pooled from two independent experiments and are presented as mean  $\pm$  SD. Statistical analysis: two-tailed unpaired Student's t-test **(A, C, D, I, K-N)**.



**Supplementary Figure 3. *Engyodontium* exacerbates colitis primarily through Dectin-2–CARD9 signaling, not Dectin-1.**

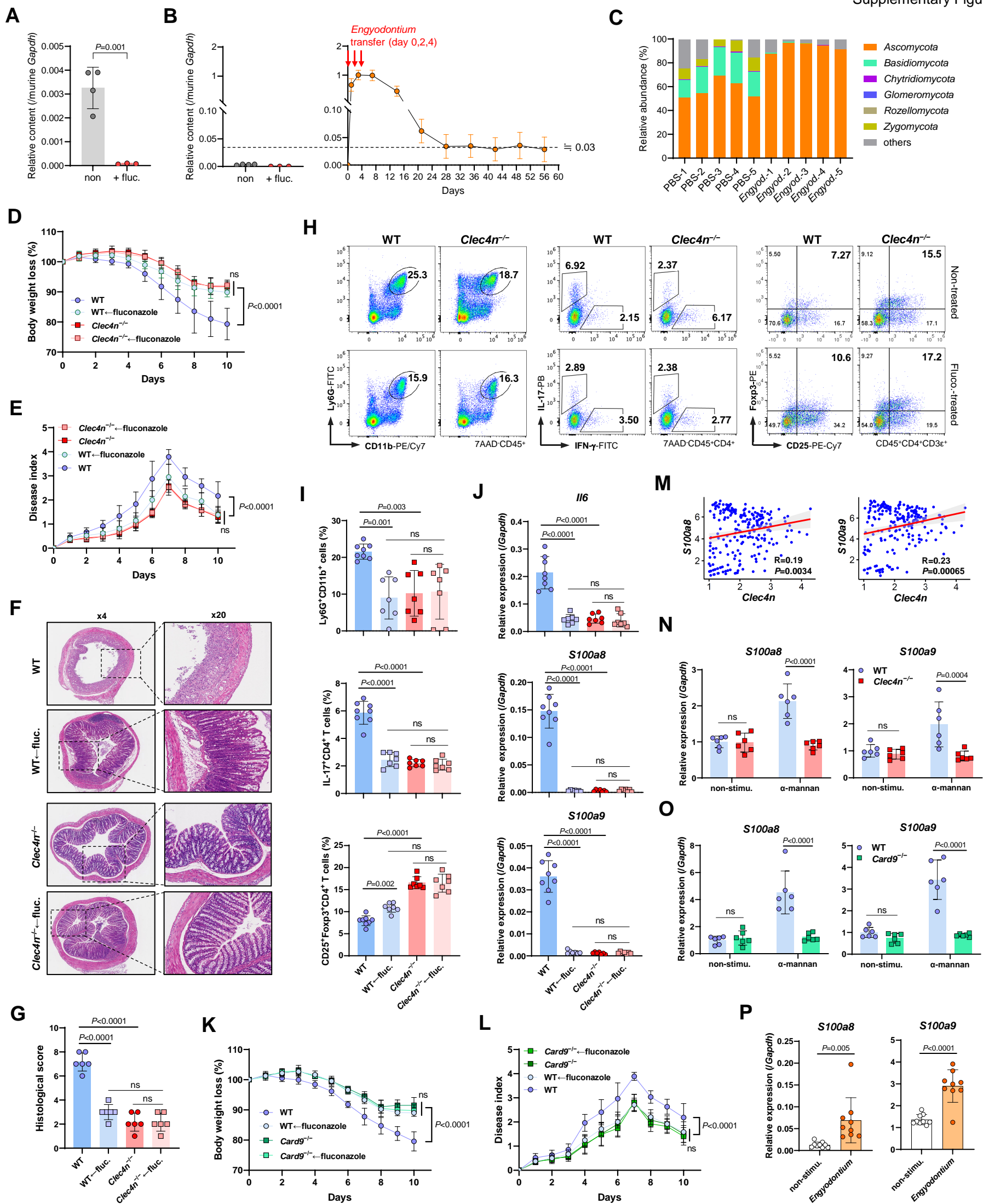
**(A)** In parallel with the experiment described in Figure 3E, IFN- $\gamma$ /IL-17-producing CD4<sup>+</sup> T cells were quantified using FACS (WT n=9, WT-*Engyodontium* n=10, *Clec4n*<sup>-/-</sup> n=6, *Clec4n*<sup>-/-</sup>-*Engyodontium* n=8).

**(B)** In parallel with the experiment described in Figure 3G, protein levels of IL-6, TNF- $\alpha$ , IL-1 $\beta$  and S100A8/9 calprotectin in the colon tissue lysates from WT and *Clec4n*<sup>-/-</sup> mice were examined by ELISA (WT n=9, *Clec4n*<sup>-/-</sup> n=6).

**(C-F)** In parallel with the experiment described in Figure 3H-3J, on day 10 post-DSS treatment, mice were sacrificed, and gross colon observations, colon length measurements **(C)**, and histological analyses of the distal colon (H&E staining) **(D)** were conducted. Following leukocyte isolation from the cLP, the proportions of neutrophils (Ly6G<sup>+</sup>CD11b<sup>+</sup> in CD45<sup>+</sup> cells) and Tregs (CD25<sup>+</sup>Foxp3<sup>+</sup> in CD4<sup>+</sup> T cells) were quantified using FACS **(E)**. The mRNA expression levels of immune-related genes in colon tissues were determined by qPCR and presented in a heatmap **(F)** (**C, E**, WT groups n=5, *Card9*<sup>-/-</sup> group n=3, *Card9*<sup>-/-</sup>-*Engyodontium* group n=4; **D, F**, n=3/group).

**(G-K)** WT and *Clec7a*<sup>-/-</sup> mice were orally administrated with PBS or *Engyodontium* sp. every other day for three times, followed by administration of 1.5% DSS in drinking water from the second fungal colonization for 7 days. Body weight loss **(G)** and disease activity index **(H)** were monitored daily during colitis induction. On day 10 post-DSS treatment, mice were sacrificed, and gross colon observations and colon length measurements **(I)** were conducted. Following leukocyte isolation from the cLP, the proportions of neutrophils (Ly6G<sup>+</sup>CD11b<sup>+</sup> in CD45<sup>+</sup> cells) were quantified using FACS **(J)** (WT n=9, WT-*Engyodontium* n=7, *Clec7a*<sup>-/-</sup> n=8, *Clec7a*<sup>-/-</sup>-*Engyodontium* n=6). The mRNA expression levels of indicated genes encoding cytokines and calprotectin in colon tissues were determined by qPCR **(K)** (WT n=9, WT-*Engyodontium* n=7, *Clec7a*<sup>-/-</sup> n=8, *Clec7a*<sup>-/-</sup>-*Engyodontium* group n=6).

Data in panels **(A, B, G-K)** are pooled from two independent experiments. Data in panels **(A -D, G-K)** are presented as mean  $\pm$  SD. Statistical analysis: one-way ANOVA with Tukey's multiple comparisons test (**A, C, D, F-K**), two-tailed unpaired Student's t-test (**B**).



**Supplementary Figure 4. Intestinal commensal fungi aggravate colitis through the Dectin-2-CARD9 pathway.**

**(A, B)** C57BL/6 mice were treated with fluconazole for 1 week, and were then administrated with  $5 \times 10^8$  CFU *Engyodontium* sp. every other day for 3 doses. Mouse feces were then harvested once a week until Day 56 after the initial *Engyodontium* colonization, and relative content of *Engyodontium* was examined by qPCR. **(A)** The content of *Engyodontium* in the feces of normal mice and mice pretreated with fluconazole (murine *Gapdh* as reference control). **(B)** Kinetics of *Engyodontium* content in mouse feces during and after the administration (n=3-4/group).

**(C)** C57BL/6 mice were administrated with  $5 \times 10^8$  CFU *Engyodontium* sp. every other day for 3 doses, and on Day 3 after the last administration, fecal DNA was isolated and relative abundance of commensal fungi was determined by qPCR (n=5/group).

**(D-J)** WT and *Clec4n*<sup>-/-</sup> mice were administered fluconazole for 2 weeks, followed by 1.5% DSS in drinking water for 7 days. Body weight loss **(D)** and disease activity index **(E)** were evaluated daily. On day 10 post-DSS treatment, mice were sacrificed. Histological analyses of the distal colon (H&E staining) **(F, G)** were conducted. Leukocytes from the cLP were harvested, and the proportions of neutrophils (Ly6G<sup>+</sup>CD11b<sup>+</sup> in CD45<sup>+</sup> cells), IL-17<sup>+</sup>CD4<sup>+</sup> T cells, and Tregs (CD25<sup>+</sup>Foxp3<sup>+</sup> in CD4<sup>+</sup> T cells) were determined by FACS **(H, I)**. The mRNA expression levels of *Il6*, *S100a8*, and *S100a9* in colon tissues were assessed by qPCR **(J)**. **(D-F, H-J)**, WT n=8, WT fluconazole treated n=7, *Clec4n*<sup>-/-</sup> n=7, *Clec4n*<sup>-/-</sup> fluconazole n=7; **G**, n=6/group).

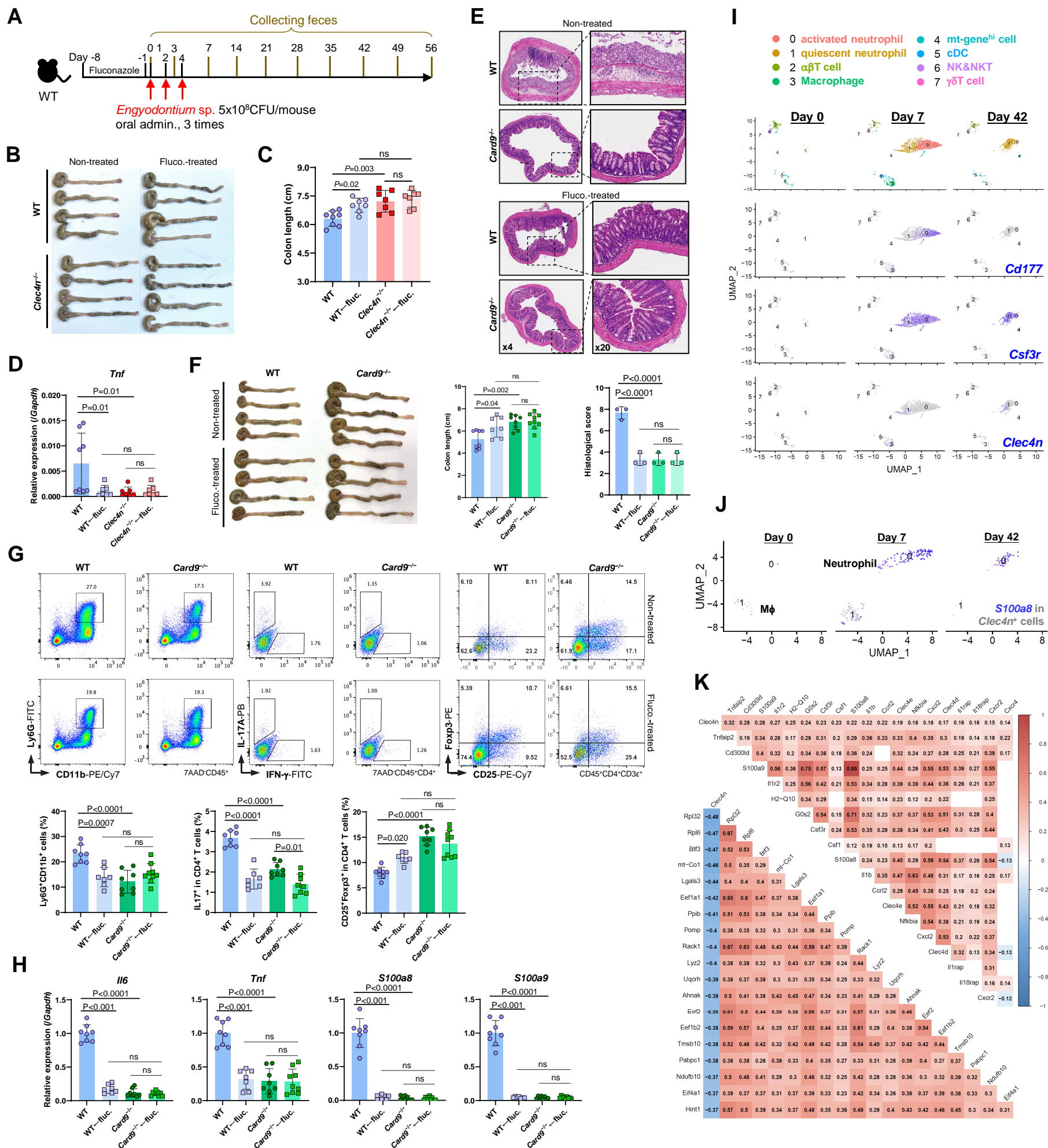
**(K, L)** WT and *Card9*<sup>-/-</sup> mice were administered fluconazole for 2 weeks, followed by 1.5% DSS in drinking water for 7 days. Body weight loss **(K)** and disease activity index **(L)** were evaluated daily (WT n=8, WT fluconazole treated n=7, *Card9*<sup>-/-</sup> n=8, *Card9*<sup>-/-</sup> fluconazole n=9).

**(M)** Correlation of expression levels between *Clec4n* and *S100a8* or *S100a9* in *Clec4n* and *S100a8/S100a9* double-positive cells, analyzed from pooled scRNA-seq datasets (GSE168033) derived from mouse cLP cells on Day 0 to 42 post-DSS treatment.

**(N, O)** Bone marrow-derived neutrophils from WT and *Clec4n*<sup>-/-</sup> mice **(N)** or *Card9*<sup>-/-</sup> mice **(O)** were induced in vitro and stimulated with  $\alpha$ -mannan for 24 hours, followed by qPCR analysis of *S100a8* and *S100a9* expression (n=6 technical replicates/group).

**(P)** C57BL/6 mice were administered 1.5% DSS for 7 days. On day 10 after DSS treatment, mouse colon tissues were obtained, and cLP CD11b<sup>+</sup> cells were isolated and stimulated with *Engyodontium* sp. for 24 hours. The relative expression of *S100a8* and *S100a9* in harvested cells after culture was determined by qPCR (n=9/group).

Data in panels **(D-L, N-P)** are pooled from two independent experiments. Data in panels **(A, B, D-G, I-L, N-P)** are presented as mean  $\pm$  SD. Statistical analysis: two-tailed unpaired Student's t-test **(A, B, N, P)**, one-way ANOVA test with Bonferroni's multiple comparisons: **(D, E, K, L)**, one-way ANOVA with Tukey's multiple comparisons test **(F, I, J)**, Spearman's correlation test **(M)**.



## Supplementary Figure 5. Intestinal commensal fungi exacerbate colitis in a Dectin-2-CARD9 signaling dependent manner

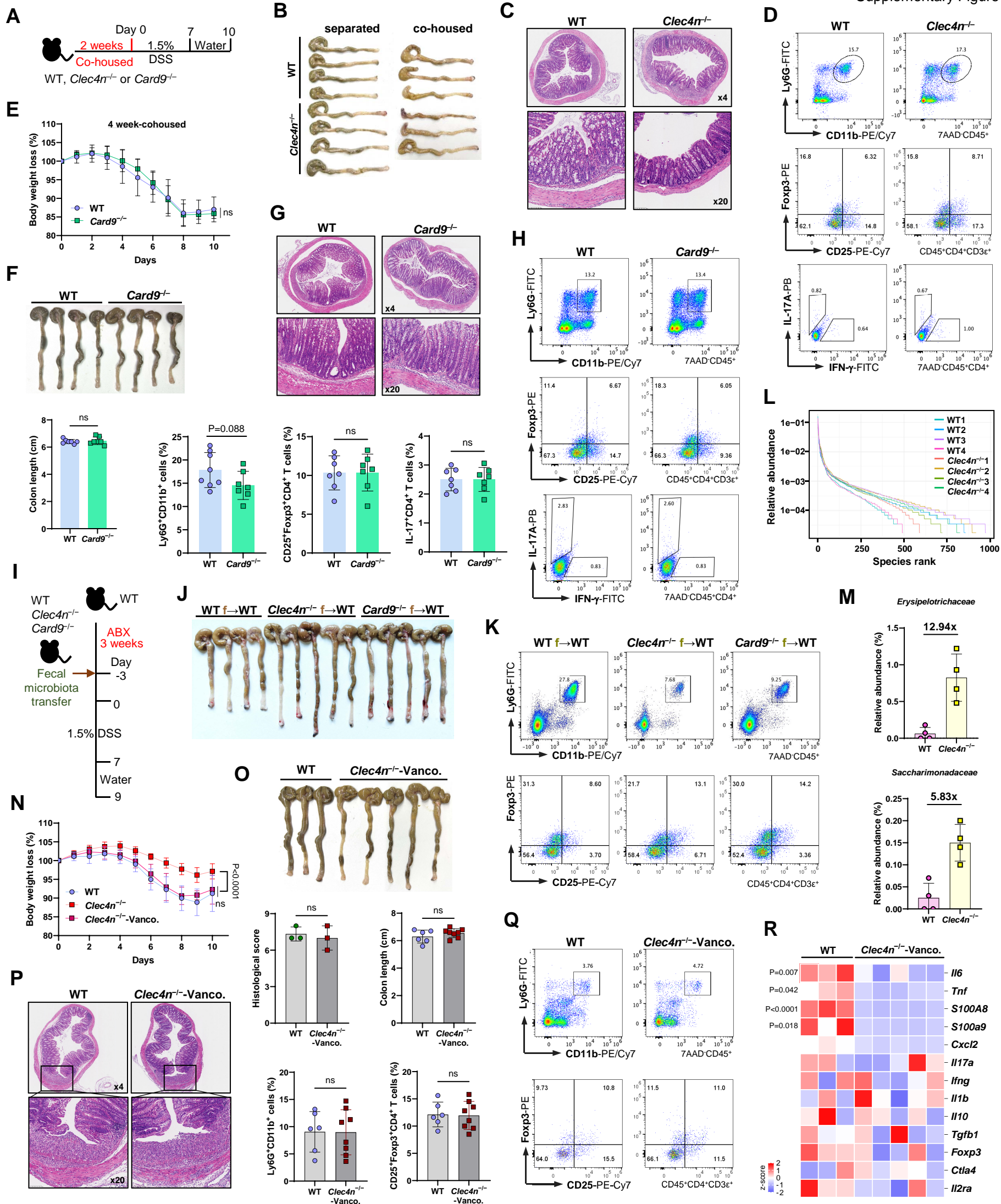
(A) The protocol of the experiment described in Supplementary Figure 4A-B.

(B-D) In parallel with the experiment described in Supplementary Figure 4D-J, the following were evaluated: (B) gross colonic appearance, (C) colon length measurement, and (D) mRNA expression of *Tnf* in colon tissues by qPCR (D, WT n=8, WT fluconazole treated n=7, *Clec4n*<sup>-/-</sup> n=7, *Clec4n*<sup>-/-</sup> fluconazole n=7).

(E-H) In parallel with the experiment described in Supplementary Figure 4K-L, the following were evaluated: (E) histological analyses of the distal colon (H&E staining), (F) gross colon observations and colon length. (G) Leukocytes from the cLP were harvested, and the proportions of neutrophils (Ly6G<sup>+</sup>CD11b<sup>+</sup> in CD45<sup>+</sup> cells), IL-17<sup>+</sup>CD4<sup>+</sup> T cells, and Tregs (CD25<sup>+</sup>Foxp3<sup>+</sup> in CD4<sup>+</sup> T cells) were determined by FACS. (H) The mRNA expression levels of *Il6*, *S100a8*, and *S100a9* in colon tissues were assessed by qPCR (F-H, WT n=8, WT fluconazole n=7, *Card9*<sup>-/-</sup> n=8, *Card9*<sup>-/-</sup> fluconazole n=9; E, n=3/group).

(I-K) UMAPs illustrating the distribution of cells expressing indicated genes in each cell cluster of colonic immune cells (I), and the distribution of *S100a8* expression in each *Clec4n*<sup>+</sup> colonic cell cluster (J) before and after 7 days of DSS treatment, as well as after 35 days of recovery (Day 42), by reanalyzing the scRNA-seq dataset (GSE168033). (K) Heatmap showing the correlation among the indicated gene expressions in *Clec4n*<sup>+</sup> colonic cells using Spearman's correlation analysis.

Data in panels (D, F-H) are pooled from two independent experiments, and in panel (B, C) are from one of two independent experiments. Data in panels (D-H) and are presented as mean ± SD. Statistical analysis: one-way ANOVA with Tukey's multiple comparisons test (D-H), Spearman's correlation test (K).



## Supplementary Figure 6. Attenuation of intestinal fungus-promoted colitis in Dectin-2/CARD9 signaling-deficient mice is dependent on commensal microbiota

**(A-D)** In parallel with the experiment described in Figure 4A-F, the following were evaluated: **(A)** the protocol of the co-housing experiments, **(B)** gross colonic appearance, **(C)** histological analyses of the distal colon (H&E staining) in co-housed groups, and **(D)** proportions of neutrophils (Ly6G<sup>+</sup>CD11b<sup>+</sup> in CD45<sup>+</sup> cells), Treg cells (CD25<sup>+</sup>Foxp3<sup>+</sup> in CD4<sup>+</sup> T cells), IL-17<sup>+</sup>/IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> T cells in co-housed groups by FACS analysis (**D**, n=6 /co-housed group, WT separated n=10, *Clec4n*<sup>-/-</sup> separated n=7).

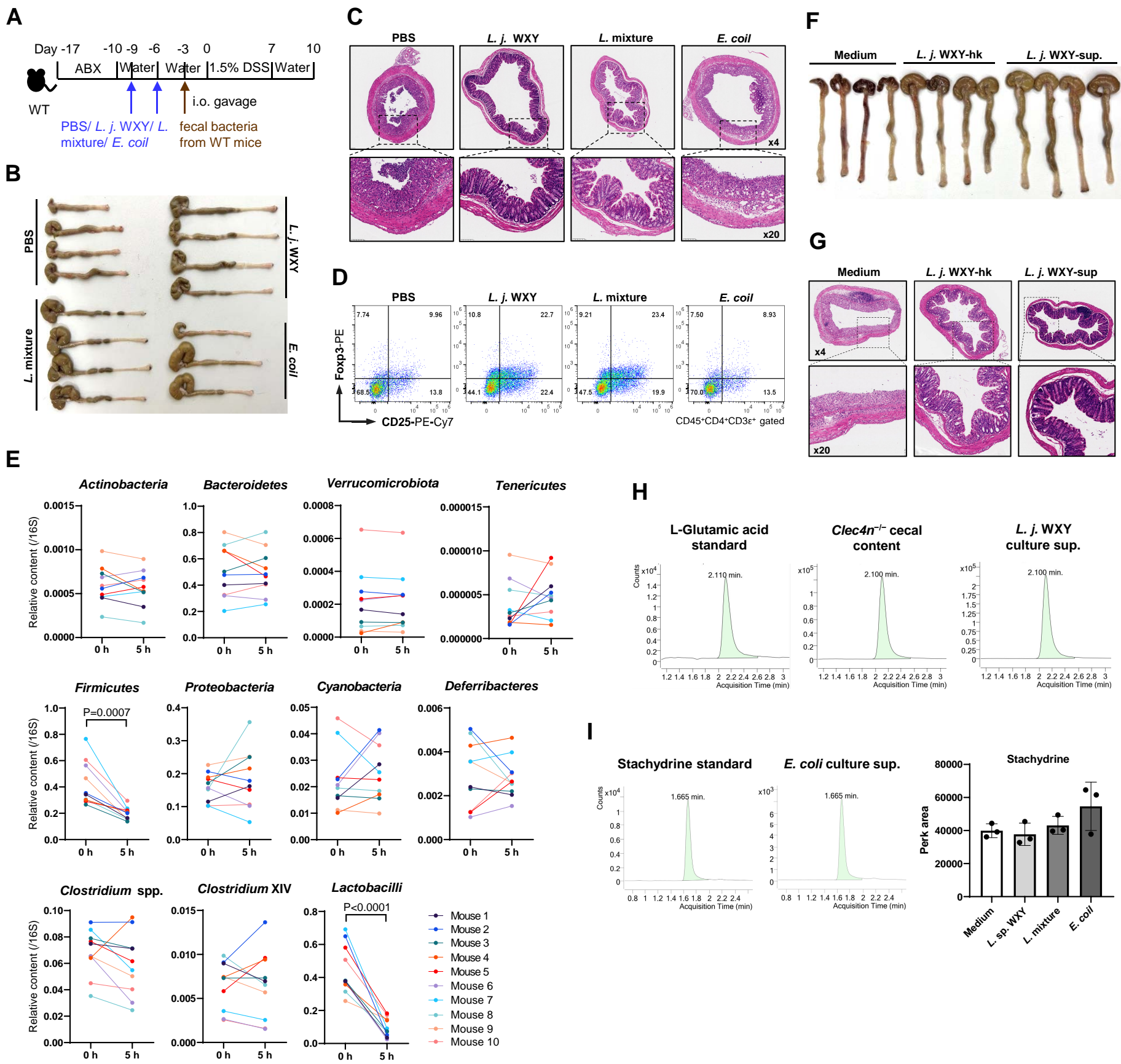
**(E-H)** In parallel with the experiment described in Figure 4G-I, the following were evaluated: **(E)** daily body weight loss post-DSS treatment, **(F)** gross colonic appearance and colon length, **(G)** histological analysis of the distal colon (H&E staining), and **(H)** proportions of neutrophils (Ly6G<sup>+</sup>CD11b<sup>+</sup> in CD45<sup>+</sup> cells), Treg cells (CD25<sup>+</sup>Foxp3<sup>+</sup> in CD4<sup>+</sup> T cells), and IL-17<sup>+</sup>/IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> T cells by FACS analysis (**E, F, H** n=7 /group; **G**, n=5 /group).

**(I-K)** In parallel with the experiment described in Figure 4J-N, the following were evaluated: **(I)** the protocol of the fecal microbiota-transfer experiments, **(J)** gross colonic appearance, and **(K)** proportions of neutrophils (Ly6G<sup>+</sup>CD11b<sup>+</sup> in CD45<sup>+</sup> cells) and Treg cells (CD25<sup>+</sup>Foxp3<sup>+</sup> in CD4<sup>+</sup> T cells) by FACS analysis (WT-f n=9, *Clec4n*<sup>-/-</sup>-f n=10, *Card9*<sup>-/-</sup>-f n=10).

**(L, M)** In parallel with the experiment described in Figure 4O-S, the following were evaluated: **(L)** species rank of the whole fecal microflora, and **(M)** relative abundance of *Erysipelotrichaceae* and *Saccharimonadaceae* in fecal bacteria from WT and *Clec4n*<sup>-/-</sup> mice, determined by 16S rDNA sequencing analysis (n=4 /group).

**(N-R)** In parallel with the experiment described in Figure 4T, the following were evaluated: **(N)** daily body weight loss post-DSS treatment, **(O)** gross colonic appearance and colon length, **(P)** histological analysis of the distal colon (H&E staining), **(Q)** proportions of neutrophils (Ly6G<sup>+</sup>CD11b<sup>+</sup> in CD45<sup>+</sup> cells) and Treg cells (CD25<sup>+</sup>Foxp3<sup>+</sup> in CD4<sup>+</sup> T cells) by FACS analysis, and **(R)** mRNA expression of genes encoding cytokines and antimicrobial peptides in colon tissues, shown in a heatmap (WT n=9, *Clec4n*<sup>-/-</sup> n=8, *Clec4n*<sup>-/-</sup>-Vanco. n=8).

Data in panels **(E, F, H, N, O, Q)** are pooled from two independent experiments, and in panels **(B, C, D, G, J, K, R)** are from one of two independent experiments. Statistical analysis: two-way ANOVA test with repeated measures (**E**), two-tailed unpaired Student's t-test (**F, H, O-R**), one-way ANOVA test with Bonferroni's multiple comparisons (**N**). Data in panels **(E, F, H, M-Q)** are presented as mean  $\pm$  SD.



## Supplementary Figure 7. Dectin-2 deficiency-facilitated colonic *Lactobacillus johnsonii* WXY attenuates DSS-colitis

**(A-D)** In parallel with the experiment described in Figure 5B-F, WT mice were treated with a CEGKMV-mixed antibiotic cocktail in drinking water for 1 week. Subsequently, the indicated bacterial strains were administered orally twice over 7 days, followed by the transfer of the whole fecal microbiota from normal WT mice for 3 days and 1.5% DSS treatment for 7 days **(A)**. On day 10 post-DSS treatment, mice were sacrificed for gross colon observation **(B)** and histological analysis of the distal colon (H&E staining) **(C)**. Proportions of Tregs (CD25<sup>+</sup>Foxp3<sup>+</sup> in CD4<sup>+</sup> T cells) were determined by FACS **(D)** (PBS n=7, *L. j.* WXY n=7, *L.* mixture n=8, *E. coli* n=7).

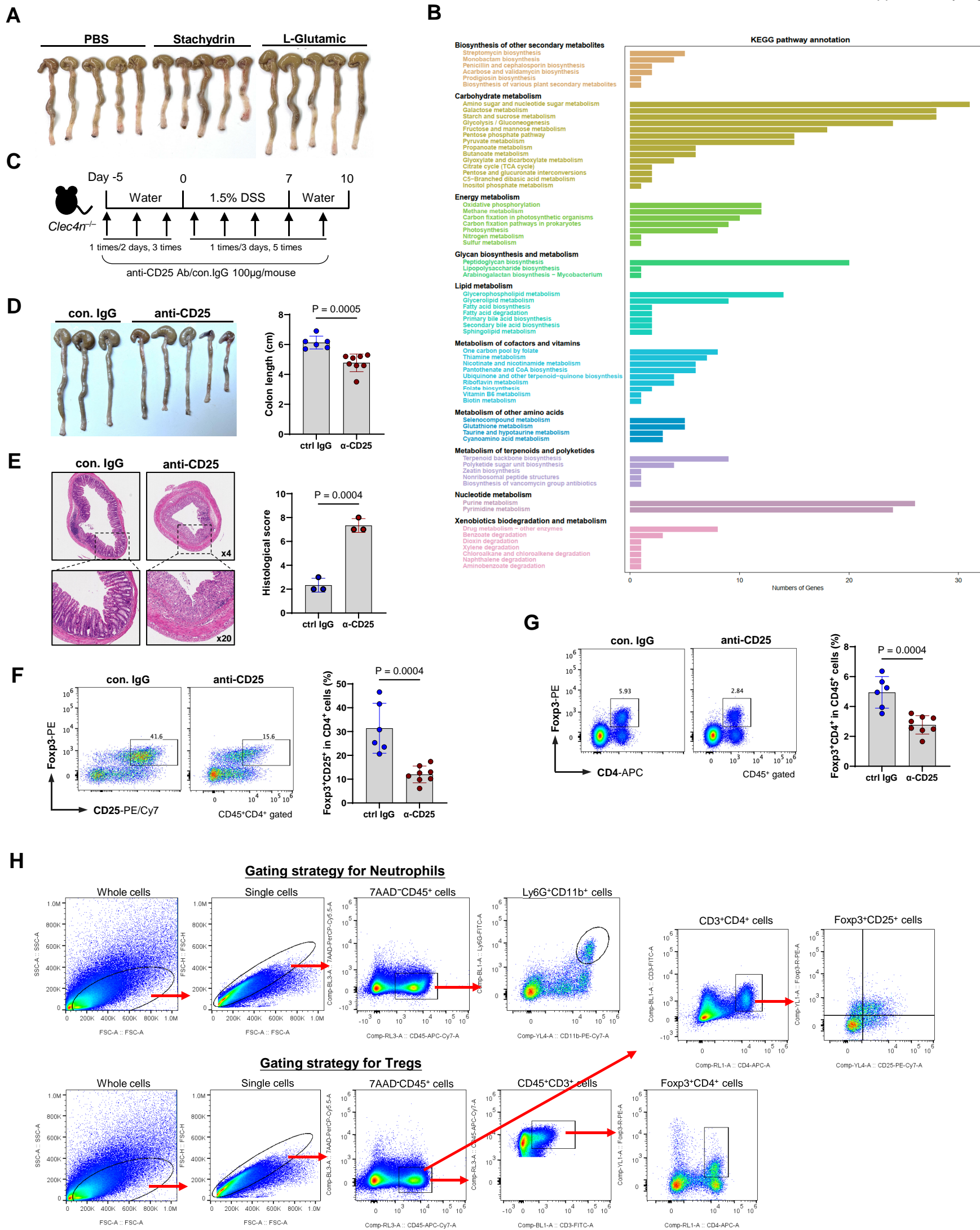
**(E)** In parallel with the experiment described in Figure 5I, relative contents of other commensal fecal bacteria were determined by qPCR (n=10).

**(F, G)** In parallel with the experiment described in Figure 5O-S, gross colonic appearance and colon length **(F)** and histological analysis of the distal colon (H&E staining) **(G)** were carried out.

**(H)** Histograms show the retention time of the L-glutamic acid standard and that of L-glutamic acid detected in the cecal contents from *Clec4n*<sup>-/-</sup> mice or in the culture supernatant of *L. j.* WXY, in parallel with the experiment described in Figure 6X and 6Y.

**(I)** Histograms show the retention time of the stachydrine standard and that of stachydrine detected in the culture supernatant of *E. coli*. The bar graph displays the amount of stachydrine detected in the culture supernatants of each indicated bacterial strain using liquid chromatography for targeted metabolomics (n=3 technical replicates/group).

Data in panels **(E)** are pooled from two independent experiments, and in panels **(B, C, D, F, G)** are from one of two independent experiments. Statistical analysis: paired Student's t-test **(E)**, one-way ANOVA with Tukey's multiple comparisons test **(I)**. Data in panels **(I)** are presented as mean  $\pm$  SD.



**Supplementary Figure 8. Glutamic acid secreted by *Lactobacillus johnsonii* WXY inhibits DSS-colitis by promoting Treg cell expansion**

**(A)** Gross observation of the colon was performed in parallel with the experiment described in Figure 6A-E.

**(B)** Other physiological metabolic functions of *L. j.* WXY were exhibited from complete genome sequencing analysis using KEGG annotation, in parallel with the experiment described in Figure 6P.

**(C-G)** The experimental protocol is shown in **(C)** in parallel with the experiment described in Figure 6T and 6U. On day 10 post-DSS treatment, gross observation of the colon tissues, colon length measurement **(D)**, and histological analysis of the distal colon (H&E staining) **(E)** were performed. The proportions of Foxp3<sup>+</sup>CD25<sup>+</sup> in CD4<sup>+</sup> T cells **(F)** and Foxp3<sup>+</sup>CD4<sup>+</sup> in CD45<sup>+</sup> cells **(G)** were examined by FACS **(D, F, G, control IgG n=6, anti-CD25 n=8, E, n=3/group)**.

**(H)** Gating strategy for cLP neutrophils and Tregs of mice by flow cytometry. Whole cells were firstly gated for the cluster located on the left bottom of FSC-SSC dot panel (region from 100K~1M of FSC axis and 0K~900K of SSC axis) with the elliptic gate, and then was gated on FSC-H × FSC-A to get the single cells. live cells were then selected by gating on 7AAD-negative cells with rectangle gate. To analyze neutrophils within the leukocytes, CD45<sup>+</sup> population was further gated for showing CD11b<sup>+</sup>Ly6G<sup>+</sup> cell cluster. To analyze Treg cells, CD45<sup>+</sup> population was further gated for showing Foxp3<sup>+</sup>CD4<sup>+</sup> cell cluster in CD3ε<sup>+</sup> T cell population or CD25<sup>+</sup>Foxp3<sup>+</sup> cell cluster in CD3ε<sup>+</sup>CD4<sup>+</sup> T cell population.

Data in panels **(D, F, G)** are pooled from two independent experiments, and in panel **(A)** are from one of two independent experiments. Statistical analysis: two-tailed unpaired Student's t-test **(D-G)**. Data in panels **(D-G)** are presented as mean ± SD.



## Supplementary Figure 9. Constitutive expression of Glutamic acid receptors on colonic T cells

(A) The relative abundance of the whole *Lactobacilli* and indicated *Lactobacillus* species in fecal bacteria from CD patients and healthy individuals was determined by 16S rDNA sequencing analysis (HC, n=95; CD, n=65).

(B) Point plots show the correlation between the expression levels of *CLEC6A* and *S100A8*, *S100A9*, *CSF3R*, or *G0S2* in the colon tissues from all pooled human samples in the public RNA-seq dataset (GSE117993).

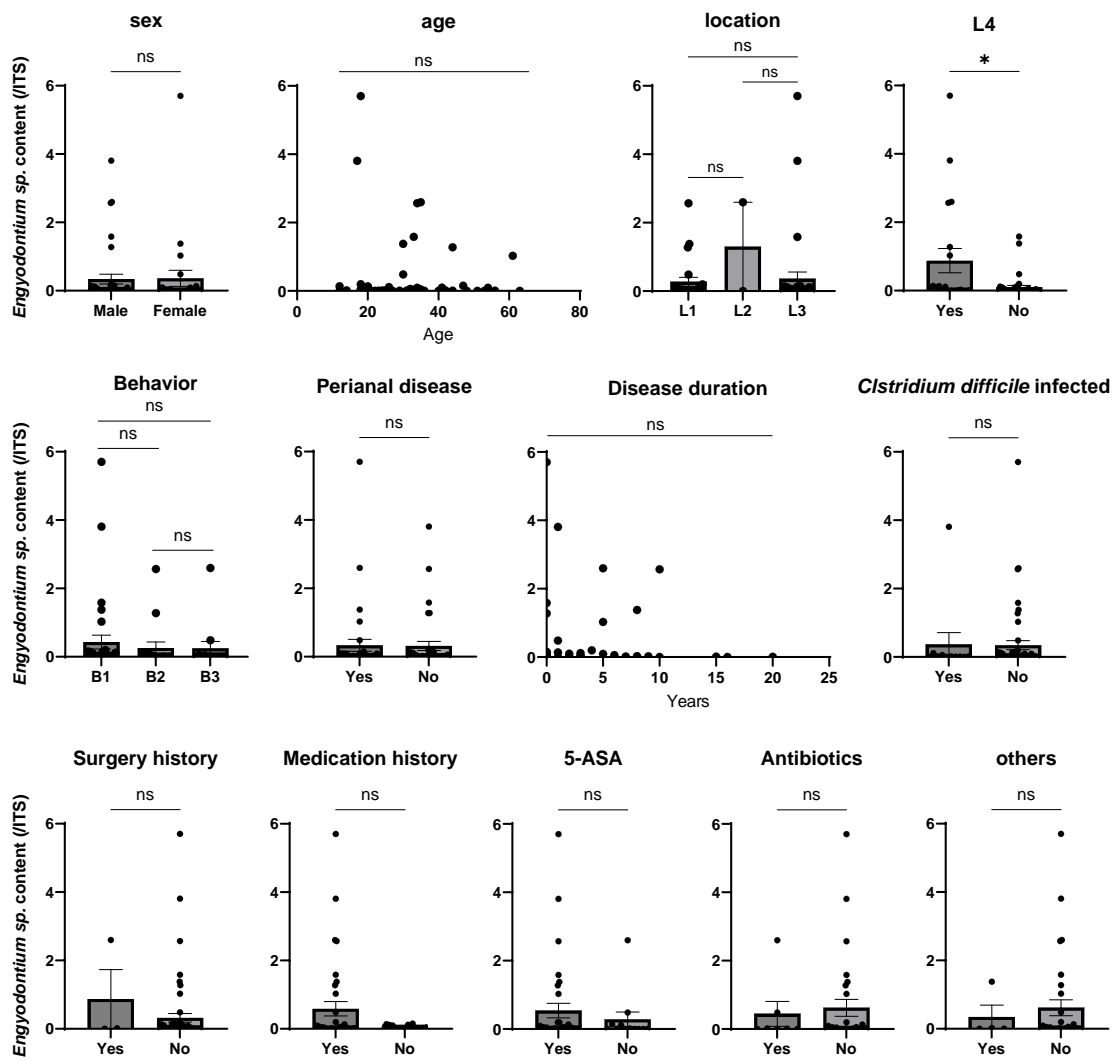
(C-E) A public scRNA-seq dataset of colonic cells from IBD patients (GSE202052) was reanalyzed. A UMAP plot identifies cell type clusters in inflammatory colon tissues (C), violin plots illustrate the expression of indicated genes in each identified cell cluster (D), and a point plot shows the correlation between the expression levels of *S100A8* and *CLEC6A* in *CLEC6A<sup>+</sup>S100A8<sup>+</sup>* cells (E).

(F) In parallel with the experiment described in Figure 7S, the relative amount of glutamine in the feces harvested from CD patients and healthy individuals was assessed by liquid chromatography for untargeted metabolomics (HC, n=80; CD, n=65).

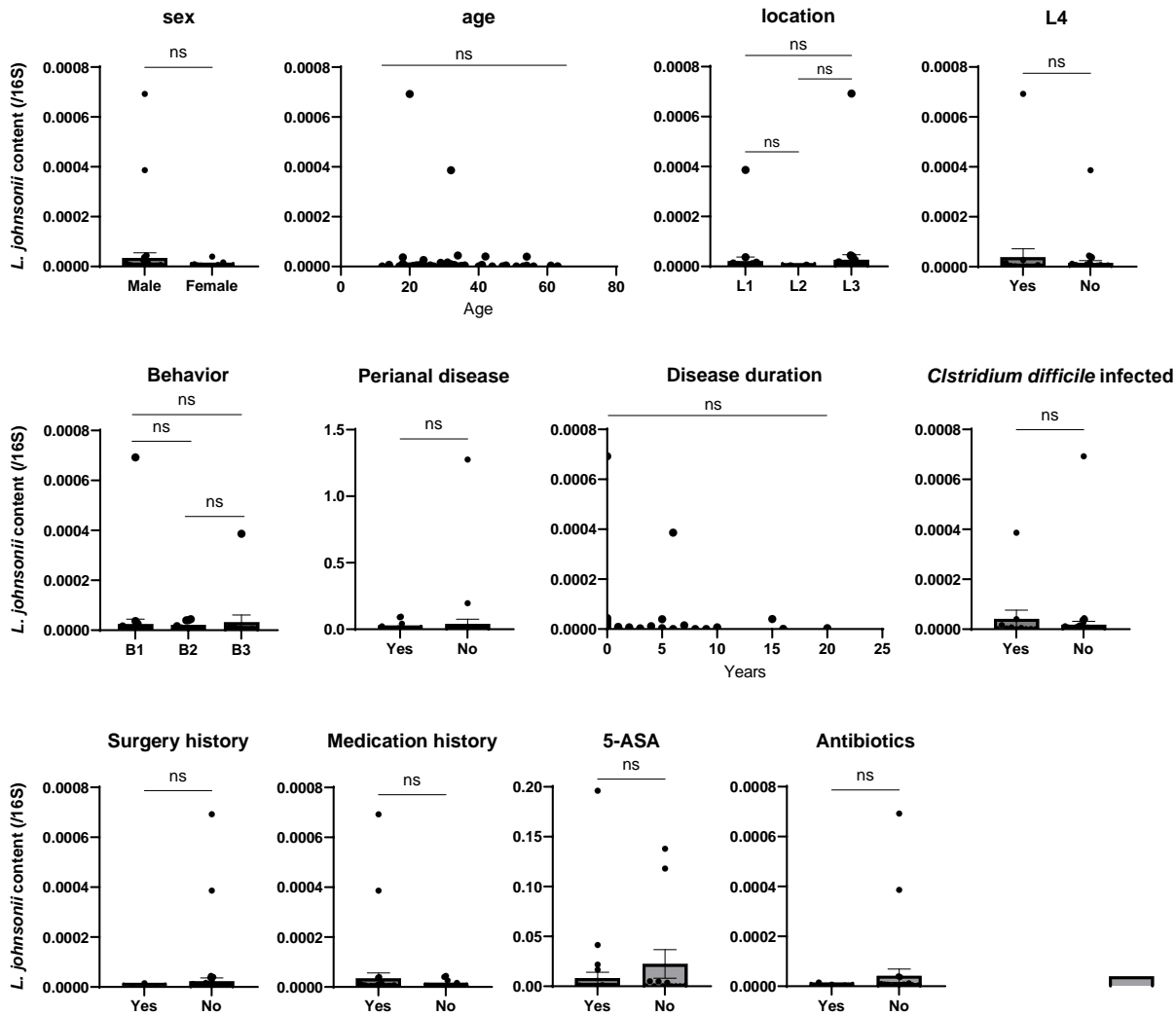
(G-I) Another scRNA-seq public dataset of colonic cells from IBD patients (GSE202052) was reanalyzed. A UMAP plot displays each T cell cluster (subsets) in the colon of IBD patients (G), a heatmap shows the gene profiles of identified T cell subsets (H), and violin plots illustrate the expression of indicated T-cell associated genes and glutamic acid receptors *SLC7A5*, *SLC3A2*, and *GRM3* in each identified cell cluster (I).

Data in panel (A) are presented as mean  $\pm$  SEM, and in (F) are presented as mean  $\pm$  SD. Statistical analysis: two-tailed Mann-Whitney test (A), two-tailed unpaired Student's t-test (F).

A



B



**Supplementary Figure 10. The abundances of *Engyodontium* sp. and *L. johnsonii* were not associated with clinical parameters of CD patients.**

The potential correlation between the content of *Engyodontium* sp. (**A**) or *L. johnsonii* (**B**) and sex, age, disease location (L1: ileal, L2: colonic, L3: ileocolonic, L4: upper-isolated gastrointestinal), disease behavior (B1: non-stricturing and non-penetrating, B2: stricturing, B3: penetrating), disease duration, *Clostridium difficile* infection, or medication use (surgery history, 5-aminosalicylates (ASA), antibiotics and others) in CD patients (n=63). For categorical variables (e.g., disease location, medication use), comparisons were made using Mann-Whitney U or Kruskal-Wallis tests; for continuous variables (e.g., disease duration), Spearman correlation analysis was employed. Details in clinical parameters of individuals are listed in Supplementary Table 2.

**Supplementary Table 1. Metabolite selection list (associated with Figure 5V, 5W)**

| Name   | Formula           | control         | <i>E. coli</i>  | <i>L. j.</i> WXY | <i>L. mix</i>    | <i>L. j.</i> WXY/control | <i>L. mix</i> /control | <i>L. j.</i> WXY/ <i>E. coli</i> | <i>L. mix</i> / <i>E. coli</i> |
|--|-------------------|-----------------|-----------------|------------------|------------------|--------------------------|------------------------|----------------------------------|--------------------------------|
| 2-Dimethylamino-5,6-dimethyl-4-hydroxypyrimidine   | C8 H13 N3 O       | 253469372       | 1175946072      | 3261509068       | 3459282889       | 12.9                     | 13.6                   | 2.8                              | 2.9                            |
| heptaminol   | C8 H19 N O        | 28757875        | 27700481        | 357889526        | 131842686        | 12.4                     | 4.6                    | 12.9                             | 4.8                            |
| (2E)-N-(4-Aminobutyl)-3-(2,4-dihydroxy-3-methoxyphenyl)acrylamide  | C14 H20 N2 O4     | 21666963        | 50476733        | 194883658        | 56619281         | 9.0                      | 2.6                    | 3.9                              | 1.1                            |
| <b><i>L-Glutamic acid</i></b>  | <b>C5 H9 N O4</b> | <b>72089773</b> | <b>56804073</b> | <b>143125045</b> | <b>120048310</b> | <b>2.0</b>               | <b>1.7</b>             | <b>2.5</b>                       | <b>2.1</b>                     |
| <i>Stachydrine</i>   | C7 H13 N O2       | 15697395        | 5439419         | 124803756        | 16881595         | 8.0                      | 1.1                    | 22.9                             | 3.1                            |
| 4-Imidazolemethanol  | C4 H6 N2 O        | 26179223        | 15653941        | 57388260         | 30380565         | 2.2                      | 1.2                    | 3.7                              | 1.9                            |
| 6-Methyl-2-pyridinemethanol  | C7 H9 N O         | 16954306        | 11142222        | 51650154         | 31312847         | 3.0                      | 1.8                    | 4.6                              | 2.8                            |
| 7,8-Dihydroneopterin   | C9 H13 N5 O4      | 565651          | 8012504         | 32538846         | 24311107         | 57.5                     | 43.0                   | 4.1                              | 3.0                            |
| Acetylcholine  | C7 H15 N O2       | 15270799        | 10604730        | 30966336         | 32835640         | 2.0                      | 2.2                    | 2.9                              | 3.1                            |
| Zearalanone  | C18 H24 O5        | 9998116         | 12378296        | 30549159         | 30097020         | 3.1                      | 3.0                    | 2.5                              | 2.4                            |
| Histamine  | C5 H9 N3          | 1942021         | 9248372         | 25150695         | 25966999         | 13.0                     | 13.4                   | 2.7                              | 2.8                            |
| 7-Methyladenine  | C6 H7 N5          | 8981524         | 10911963        | 21584737         | 33604089         | 2.4                      | 3.7                    | 2.0                              | 3.1                            |
| [(4Z,6E,10Z,13Z)-1-Carboxy-4,6,10,13-nonadecatetraen-8-yl]dioxanyl   | C20 H31 O4        | 308084          | 78682           | 21406764         | 417001           | 69.5                     | 1.4                    | 272.1                            | 5.3                            |
| 3- $\beta$ -Mycarosylerythronolide B   | C28 H50 O10       | 9603667         | 62351           | 20453351         | 17130117         | 2.1                      | 1.8                    | 328.0                            | 274.7                          |
| n-Hexanamide   | C6 H13 N O        | 9115584         | 7347416         | 18664506         | 9614562          | 2.0                      | 1.1                    | 2.5                              | 1.3                            |
| Asparaginyl-4-hydroxyproline   | C9 H15 N3 O5      | 1726198         | 7279201         | 16900300         | 7152294          | 9.8                      | 4.1                    | 2.3                              | 1.0                            |
| 3-[2-[(E)-[3-(2-carboxyethyl)-5-[(4-ethyl-3-methyl-5-oxo-pyrrolidin-2-yl)methyl]-4-methyl-pyrrol-2-ylidene)methyl]-5-[(3-ethyl-4-methyl-5-oxo-pyrrolidin-2-yl)methyl]-4-methyl-1H-pyrrol-3-yl]propanoic acid | C33 H46 N4 O6     | 6704247         | 64978           | 13367408         | 9803907          | 2.0                      | 1.5                    | 205.7                            | 150.9                          |
| Oleamide   | C18 H35 N O       | 5832914         | 1494861         | 11853679         | 7142491          | 2.0                      | 1.2                    | 7.9                              | 4.8                            |
| 1-(Methylnitrosoamino)-4-(3-pyridinyl)-1,4-butanediol  | C10 H15 N3 O3     | 669519          | 3588897         | 10465084         | 2179408          | 15.6                     | 3.3                    | 2.9                              | 0.6                            |
| Levetiracetam  | C8 H14 N2 O2      | 3233985         | 1857144         | 7256745          | 4025374          | 2.2                      | 1.2                    | 3.9                              | 2.2                            |
| 4-Methylhistamine  | C6 H11 N3         | 824202          | 200801          | 4826235          | 4308272          | 5.9                      | 5.2                    | 24.0                             | 21.5                           |
| 2,6-Diamino-4-hexenoic acid  | C6 H12 N2 O2      | 1114791         | 1353556         | 2780151          | 12366240         | 2.5                      | 11.1                   | 2.1                              | 9.1                            |



### Supplementary Table 3. Sequences and Primers

#### For qPCR (mouse)

|               |       |   |                           |
|---------------|-------|---|---------------------------|
| <b>Gapdh</b>  | mouse | F | TTCACCACCATGGAGAAGGC      |
|               |       | R | GGCATGGACTGTGGTCATGA      |
| <b>Il6</b>    | mouse | F | GAGGATACCACTCCCAACAGACC   |
|               |       | R | AAGTGCATCATCGTTGTTTCATACA |
| <b>Tnf</b>    | mouse | F | GAGGATACCACTCCCAACAGACC   |
|               |       | R | AAGTGCATCATCGTTGTTTCATACA |
| <b>S100a9</b> | mouse | F | GAGGATACCACTCCCAACAGACC   |
|               |       | R | AAGTGCATCATCGTTGTTTCATACA |
| <b>S100a8</b> | mouse | F | GAGGATACCACTCCCAACAGACC   |
|               |       | R | AAGTGCATCATCGTTGTTTCATACA |
| <b>Cxcl2</b>  | mouse | F | CCAACCACCAGGCTACAGG       |
|               |       | R | GCGTCACACTCAAGCTCTG       |
| <b>Il17a</b>  | mouse | F | TCCCACGAAATCCAGGATGC      |
|               |       | R | GGATGTTCAAGTTGACCATCAC    |
| <b>Ifng</b>   | mouse | F | GAACTGGCAAAGGATGGTGA      |
|               |       | R | TGTGGGTTGTTGACCTCAAAC     |
| <b>Il10</b>   | mouse | F | GCTCTTACTGACTGGCATGAG     |
|               |       | R | CGCAGCTCTAGGAGCATGTG      |
| <b>Tgfb1</b>  | mouse | F | TGACGTCACCTGGAGTTGTACGG   |
|               |       | R | GGTTCATGTCATGGATGGTGC     |
| <b>Foxp3</b>  | mouse | F | CCCATCCCCAGGAGTCTTG       |
|               |       | R | ACCATGACTAGGGGCACTGTA     |
| <b>Il2ra</b>  | mouse | F | GGGAAAACGGGGTGGACTC       |
|               |       | R | CTGTGGTGGTTATGGGGCAG      |
| <b>Il2</b>    | mouse | F | TGAGCAGGATGGAGAATTACAGG   |
|               |       | R | GTCCAAGTTCATCTTCTAGGCAC   |
| <b>Defb1</b>  | mouse | F | AGGTGTTGGCATTCTACAAG      |
|               |       | R | GCTTATCTGGTTTACAGGTTCCC   |
| <b>Defb2</b>  | mouse | F | TATGCTGCCTCCTTTTCTCA      |
|               |       | R | GACTTCCATGTGCTTCCTTC      |
| <b>Lyz1</b>   | mouse | F | GAGACCGAAGCACCGACTATG     |
|               |       | R | CGGTTTTGACATTGTGTTCCG     |
| <b>Lyz2</b>   | mouse | F | ATGGAATGGCTGGCTACTATGG    |
|               |       | R | ACCAGTATCGGCTATTGATCTGA   |
| <b>Defa1</b>  | mouse | F | TCAAGAGGCTGCAAAGGAAGAGAAC |
|               |       | R | TGGTCTCCATGTTCAAGCAGACG   |
| <b>Reg3b</b>  | mouse | F | CTCTCCTGCCTGATGCTCTT      |
|               |       | R | GTAGGAGCCATAAGCCTGGG      |
| <b>Reg3g</b>  | mouse | F | TCAGGTGCAAGGTGAAGTTG      |
|               |       | R | GGCCACTGTTACCACTGCTT      |
| <b>Zo1</b>    | mouse | F | GCCGCTAAGAGCACAGCAA       |
|               |       | R | GCCCTCCTTTAACACATCAGA     |
| <b>Cldn7</b>  | mouse | F | GGCCTGATAGCGAGCACTG       |
|               |       | R | GTGACGCACTCCATCCAGA       |
| <b>Cldn4</b>  | mouse | F | GTCCTGGGAATCTCCTTGGC      |
|               |       | R | TCTGTGCCGTGACGATGTTG      |
| <b>Tjp</b>    | mouse | F | GCCGCTAAGAGCACAGCAA       |
|               |       | R | TCCCCACTCTGAAAATGAGGA     |
| <b>Ocln</b>   | mouse | F | TTGAAAGTCCACCTCCTTACAGA   |
|               |       | R | CCGGATAAAAAGAGTACGCTGG    |
| <b>Muc2</b>   | mouse | F | GCTGACGAGTGGTTGGTGAATG    |
|               |       | R | GATGAGGTGGCAGACAGGAGAC    |
| <b>Ctla4</b>  | mouse | F | GCTTCTAGATTACCCCTTCTGC    |
|               |       | R | CGGGCATGGTTCTGGATCA       |
| <b>Clec5a</b> | mouse | F | TCGGGGCTTATCGTAGTAGTG     |
|               |       | R | TGTAGGCATGGTACTTTCTGCAT   |
| <b>Il2ra</b>  | mouse | F | GGGAAAACGGGGTGGACTC       |
|               |       | R | CTGTGGTGGTTATGGGGCAG      |
| <b>Il1b</b>   | mouse | F | CAACCAACAAGTGATATTCTCCATG |
|               |       | R | GATCCACACTCTCCAGCTGCA     |

#### For qPCR (human)

|               |       |   |                          |
|---------------|-------|---|--------------------------|
| <b>GAPDH</b>  | human | F | TTCACCACCATGGAGAAGGC     |
|               |       | R | GGCATGGACTGTGGTCATGA     |
| <b>S100A8</b> | human | F | ATGCCGTCTACAGGGATGAC     |
|               |       | R | ACTGAGGACACTCGGTCTCTA    |
| <b>S100A9</b> | human | F | GGTCATAGAACACATCATGGAGG  |
|               |       | R | GGCCTGGCTTATGGTGGTG      |
| <b>CXCL1</b>  | human | F | CGCTACAGCGACGTGAAGAA     |
|               |       | R | GTTCCAGGCGTTGTAACCAC     |
| <b>CXCL2</b>  | human | F | CCGAAGTCATAGCCCACTCA     |
|               |       | R | TGGATTTGCCATTTTTCAGCATCT |

#### For mouse generating & typing

|   |                    |        |                              |
|---|--------------------|--------|------------------------------|
| <b>Clec4nKO<br/>CRISPR-Cpf1<br/>gene.</b> | target<br>sequence | Exon 1 | TTTCCCATGTCTTTAAATCAGTGGAGAG |
|   |                    | Exon 4 | TTTCTACCAAGGAGAAGTCTTGAGCAC  |
|   | typing<br>primers  | F      | CCACATTAGGAAGTGAAGAAAG       |
|   |                    | R      | ACAAGAAAGCACTACCTGTCTCC      |
| <b>Card9KO<br/>CRISPR-Cpf1<br/>gene.</b>  | target<br>sequence | Exon 2 | TTTCCGGTATAACTGAGGGTAGTAGGC  |
|   |                    |        |                              |
|   | typing<br>primers  | F      | TTGACTCTGTCTTCCACCTGAA       |
|   |                    | R      | TCACACTTAGTCACTCCAACGC       |

#### For qPCR (bacteria & fungi)

|   |          |   |                           |
|---|----------|---|---------------------------|
| <b>16S (8F&amp;R357)</b>                | bacteria | F | AGAGTTTGATCMTGGCTCAG      |
|   |          | R | CTGCTGCCTYCCGTA           |
| <b>Bacteroidetes</b>                    | bacteria | F | CRAACAGGATTAGATACCCT      |
|   |          | R | GGTAAGTTCTCTCGCGTAT       |
| <b>Firmicutes</b>                       | bacteria | F | TGAAACTYAAAGGAATTGACG     |
|   |          | R | ACCATGCACCACCTGTC         |
| <b>Actinobacteria</b>                   | bacteria | F | TACGGCCGCAAGGCTA          |
|   |          | R | TCRTCCCCACCTTCTCCG        |
| <b>aProteobacteria</b>                  | bacteria | F | CIAGTGTAGAGGTGAAATT       |
|   |          | R | CCCCGTCAATTCCTTTGAGTT     |
| <b>gProteobacteria</b>                  | bacteria | F | CGTAAGGGCCATGATG          |
|   |          | R | CTGCTGCCTYCCGTA           |
| <b>bProteobacteria</b>                  | bacteria | F | AACGCGAAAAACCTTACCTACC    |
|   |          | R | TGCCCTTTCGTAGCAACTAGTG    |
| <b>eProteobacteria</b>                  | bacteria | F | TAGGCTTGACATTGATAGAATC    |
|   |          | R | CTTACGAAGGCAGTCTCCTTA     |
| <b>Cyanobacteria</b>                    | bacteria | F | GGGGAATYTTCCGCAATGGG      |
|   |          | R | GACTACTGGGGTATCTAATCCCATT |
| <b>Tenericutes</b>                      | bacteria | F | ATGTGTAGCGGTAATAATGCGTAA  |
|   |          | R | CMTACTTGCGTACGTACTACT     |
| <b>Verrucomicrobia</b>                  | bacteria | F | TCAKGTCAAGTATGGCCCTTAT    |
|   |          | R | CAGTTTTYAGGATTTCTCCGCC    |
| <b>Deferribacteres</b>                  | bacteria | F | CTATTTCCAGTTGCTAACGG      |
|   |          | R | GAGHTGCTTCCCTCTGATTATG    |
| <b>Lactobacilli</b>                     | bacteria | F | TGGAACAGRTGCTAATACCG      |
|   |          | R | GTCCATTGTGGAAGATTCCC      |
| <b>Lactobacillus<br/>johnsonii</b>      | bacteria | F | TCGAGCGAGCTTGCTTAGATGA    |
|   |          | R | TCCGGACAACGCTTGCCACC      |
| <b>glsA</b>                             | bacteria | F | TGGTGAAGTAGGTGAAGCAGG     |
|   |          | R | AATGCGTTTGTGTCCCATG       |
| <b>ITS1-2</b>                           | fungi    | F | CTTGGTCATTTAGAGGAAGTAA    |
|   |          | R | GCTGCGTTCTTCATCGATGC      |
| <b>Engyodontium<br/>sp. SM14-25-6-1</b> | fungi    | F | GGAGGGATCATTACCGAGTTTACA  |
|   |          | R | TCCGTTGTTGAAAGTTTTGATTATT |
| <b>Ascomycota</b>                       | fungi    | F | GAATTGCAGMMWTCMGTGAATC    |
|   |          | R | GCCTGTYTGAGCGTCRTTTC      |
| <b>Basidiomycota</b>                    | fungi    | F | CGAATCTTTGAACGCAMCTTG     |
|   |          | R | GCCTGTTTGAGTATCATGA       |
| <b>Zygomycota</b>                       | fungi    | F | TTCAAAGAGTCAGGTTGTTTGG    |
|   |          | R | CAGTCTGGCTCCAAACGGTTC     |
| <b>Chytridiomycota</b>                  | fungi    | F | GTAACGGAGAATTAGGGTTCG     |
|   |          | R | CCTCCGCTTATTGATATGCT      |
| <b>Glomeromycota</b>                    | fungi    | F | GGAGATGCTGGAGAATTTG       |
|   |          | R | TGAAGGTGAGTCTGAAGAAG      |
| <b>Rozellomycota</b>                    | fungi    | F | CCTACGGGAGGCAGCAGT        |
|   |          | R | GACTACCAGGGTATCTAATCCTGTT |

**Supplementary Table 4. Antibodies used**

| Antibodies                            | Clone        | Cat#     | Source               |
|---------------------------------------|--------------|----------|----------------------|
| APC/Cyanine7 anti-mouse CD45          | 30-F11       | 103116   | BioLegend            |
| PE/Cyanine7 anti-mouse/human CD11b    | M1/70        | 101216   | BioLegend            |
| FITC anti-mouse Ly-6G                 | 1A8          | 127606   | BioLegend            |
| PE anti-mouse Ly-6C                   | HK1.4        | 128007   | BioLegend            |
| Pacific Blue™ anti-mouse CD11c        | N418         | 117322   | BioLegend            |
| APC anti-mouse F4/80                  | BM8          | 123115   | BioLegend            |
| FITC anti-mouse CD3ε                  | 145-2C11     | 100305   | BioLegend            |
| APC anti-mouse CD4                    | GK1.5        | 100411   | BioLegend            |
| PE anti-mouse CD8a                    | 53-6.7       | 100707   | BioLegend            |
| PE/Cyanine7 anti-mouse CD25           | PC61         | 102015   | BioLegend            |
| APC anti-mouse I-A/I-E                | M5/114.15.2  | 107614   | BioLegend            |
| Pacific Blue anti-mouse IL-17A        | TC11-18H10.1 | 506918   | BioLegend            |
| FITC anti-mouse IFN-γ                 | XMG1.2       | 505806   | BioLegend            |
| FITC anti-mouse IL-2                  | JES6-5H4     | 503805   | BioLegend            |
| PE anti-mouse FOXP3                   | MF-14        | 126403   | BioLegend            |
| Brilliant Violet 510 anti-human CD45  | 2D1          | 368526   | BioLegend            |
| FITC anti-human CD4                   | RPA-T4       | 300506   | BioLegend            |
| PE anti-human CD25                    | BC96         | 302606   | BioLegend            |
| PE/Dazzle 594 anti-human FOXP3        | 206D         | 320126   | BioLegend            |
| Rabbit polyclonal antibody to CLEC6A  | Polyclonal   | DF10182  | Affinity Biosciences |
| Rabbit polyclonal antibody to S100A8  | Polyclonal   | DF6556   | Affinity Biosciences |
| Rabbit polyclonal antibody to S100A9  | Polyclonal   | DF7596   | Affinity Biosciences |
| HRP- labeled goat anti-rabbit IgG     | Polyclonal   | GB23303  | ServiceBio           |
| Biotin anti-mouse/human CD11b         | M1/70        | 101203   | BioLegend            |
| Biotin anti-mouse CD11c               | N418         | 117303   | BioLegend            |
| Biotin anti-mouse I-A/I-E             | M5/114.15.2  | 107603   | BioLegend            |
| Biotin anti-mouse CD19                | 6D5          | 115504   | BioLegend            |
| Biotin anti-mouse TCR γ/δ             | GL3          | 118103   | BioLegend            |
| Biotin anti-mouse NK-1.1              | PK136        | 108703   | BioLegend            |
| Biotin anti-mouse CD8a                | 53-6.7       | 100704   | BioLegend            |
| Ultra-LEAF™ Purified anti-mouse IL-4  | 11B11        | 504135   | BioLegend            |
| Ultra-LEAF™ Purified anti-mouse IFN-γ | XMG1.2       | 505847   | BioLegend            |
| InVivoMAb anti-mouse CD25 (IL-2Rα)    | PC-61.5.3    | BE0012   | Bio X Cell           |
| InVivoMAb rat IgG1 isotype control    | HRPN-CP167   | BE0088   | Bio X Cell           |
| Goat Anti-Human IgG Fc (Biotin)       | Polyclonal   | Ab97223  | Abcam                |
| Anti-CXCL2 antibody                   | EPR28746-89  | ab317569 | Abcam                |
| Anti-Dectin-2 antibody                | D2.11E4      | GTX41453 | GeneTex              |
| Ly6g Rabbit Polyclonal Antibody       | Polyclonal   | 0809-11  | HUABIO               |