**SUPPLEMENTARY MATERIALS**

**Table S1** Microbes inhibited by sennoside A is in charge of SCFA production.

|  |  |  |
| --- | --- | --- |
| **Reduced genus by SenA** | **Pathways for Biosynthesis of SCFAs** | **SCFAs** |
| *Prevotella* | from pyruvate via acetyl-CoA | Acetate |
| *Ruminococcus* | from pyruvate via acetyl-CoA | Acetate |
| propanediol pathway | Propionate |
| *Coprcoccus*] | acrylate pathway | Propionate |
| phosphotransbutyrylase/butyrate kinase route | Butyrate |
| butyryl-CoA:acetate CoAtransferase route | Butyrate |
| *Oscillospira* | / | Butyrate |

**Table S2** Species clustering under corresponding treatment

Experiment 1

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ctrl-CM(%) | + | 100 | 50 | 25 | 12.5 | 6.25 | 3.125 | 1.5625 | 0.78125 | 0.390625 | 0 |
| SenA-CM(%) | + |
| SenA-CM(%) | - |

Experiment 2

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ctrl | + | 128 | 64 | 32 | 16 | 8 | 4 | 2 | 1 | 0.5 | 0 |
| SenA(μM) | + |
| SenA(μM) | - |

Experiment 3

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ctrl | + | 128 | 64 | 32 | 16 | 8 | 4 | 2 | 1 | 0.5 | 0 |
| Rhein(μM) | + |
| Rhein(μM) | - |

“+”, Supplement with bacterial strain; “-”, Asepticculture. The cultures for the inoculation were grown in their respective media at 37℃ under an anaerobic atmosphere (10% H2, 5% CO2 and 85% N2).



**Figure S1. Long-term administration of sennoside A did not induce intestinal tumorigenesis.** (A) Representative image of colon tissue in mice treated for 84 days with dietary sennoside A. (B) Haematoxylin and eosin (H&E) staining of the colon (magnification, ×100).



**Figure S2. Sennoside A and its glucosidase metabolites had no direct effect on tight junction proteins of colon epithelial cells.** (A) Effects of sennoside A on the viability of NCM-460 cells. (B) Images of NCM-460 cells treated with sennoside A (0, 32, and 128 μM) stained for claudin, E-cadherin and ZO-1. Magnification, ×400.

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**Figure S3. 16S rDNA sequencing revealed altered microbiota composition after sennoside A treatment.** (A) α-Diversity and (B) β-diversity (principal coordinate analysis (PCoA)) for microbial community similarity. (C) The relative abundance of microbial taxa was determined at the genus level, for which abundances >1% are shown. (D) Cladogram generated from LEfSe analysis showing the relationship between taxon (the levels represent, from the inner to outer rings, are the phylum, class, order, family, and genus).



**Figure S4 STAMP analysis uncovered the differences between the sennoside A-treated group and the control group.** (A) Genera with a significance of P<0.05, as determined by Welch’s t-test, are shown. (B) Heatmap showing the relative abundance of significantly altered bacterial genera.



**Figure S5. Measurement of SCFA concentrations by GC-MS in the caecal samples of mice treated with control or sennoside A, as also seen in Figure 3C.**

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**Figure S6. FMT protected the sennoside A-induced phenotype.** (A-E) Content determination of acetic acid, propionic acid, valeric acid, isobutyric acid and isovaleric acid in intestinal contents by GC-MS in the indicated groups. (F) Comparison of alpha-diversity indices (Shannon index) compared among different groups.



**Figure S7. Butyrate administration transformed sennoside A-induced gut dysbiosis.** Shannon index (A) and PCoA (B) comparing the bacterial community structure of faecal samples between the Sen A group and the Bt-SenA group. (C) Cladogram generated from LEfSe analysis, as seen in Figure 5B. (D) Bacterial taxonomic profiling at the order level of bacteria from different mouse groups.



**Figure S8.** **Butyrate protected the mice from sennoside A-induced intestinal mechanical barrier impairment.** (A) Representative images of in situ staining of the tight junction in the SenA *vs* Bt-SenA mouse colon (magnification, ×200). (B) Tight junction protein claudin-1, ZO−1, and E-cadherin protein levels in the colon were detected by Western blot.



**Figure S9. Sennoside A induced systemic low-grade inflammation**. (A) Relative body weight was measured throughout the 12-week period. (B) Colon length, as seen in Figure 6B. (C) Representative flow cytometric plots of Th-1, Th-2, and Th-17 cells. Numbers on the representative flow cytometry graph indicate the percentage of relevant cells in the CD4+ subset. (D) The absolute numbers of IFN-γ+CD4+ T cells (left), IL-4+CD4+ T cells (middle), and CIL-17+CD4+ T cells (right).

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**Figure S10. The proliferation of epithelial cells is already significantly increased in sennoside A-treated mice, which is ameliorated by sodium butyrate.** (A&B) Representative immunofluorescence images and quantitative analysis of Ki67-positive cells (red). (C&D) The percentage of Ki67-positive cells per view was calculated and presented as bar charts. \*P < 0.05, and \*\*\*P < 0.001.



**Figure S11.** **Key metabolites between the control and SenA groups.** The volcano plot (upper panel) and heatmaps (lower panel) indicate differential metabolites between the control and SenA groups, as determined by fold change>2 and p value<0.05.