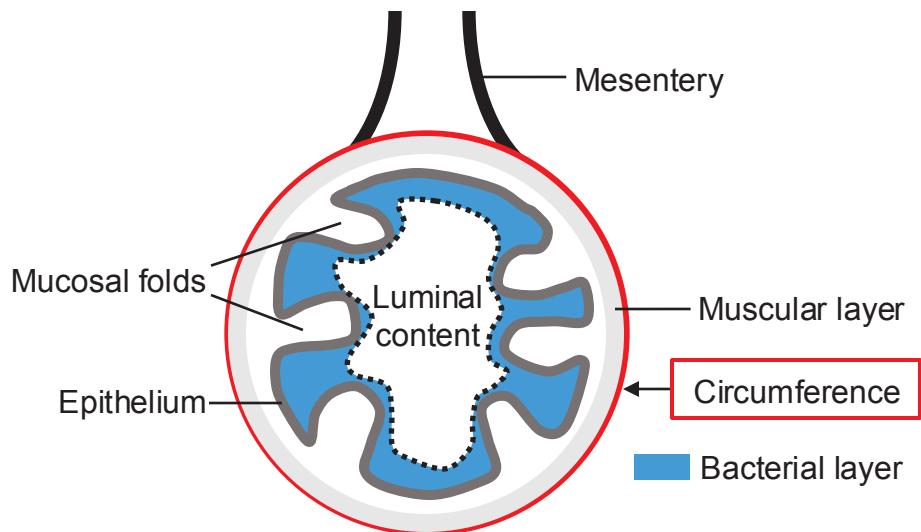


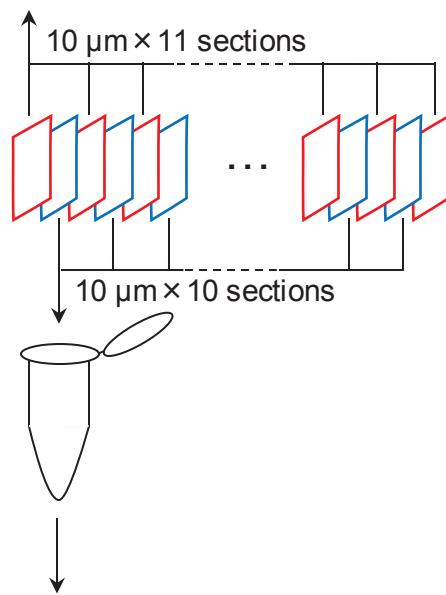
Supplementary Fig. 1: The regions dissected from the rat ascending colon in this study. The region including aggregated lymphoid tissue (arrow, red box) and the adjacent area on the cecal side of the aggregated lymphoid tissue (blue box) was dissected for histological analysis.



$$\text{Settlement level of indigenous bacteria} = \frac{\text{Total area of the bacterial layer (blue)}(\text{mm}^2)}{\text{Circumference of the intestine (red circle)}(\text{mm})}$$

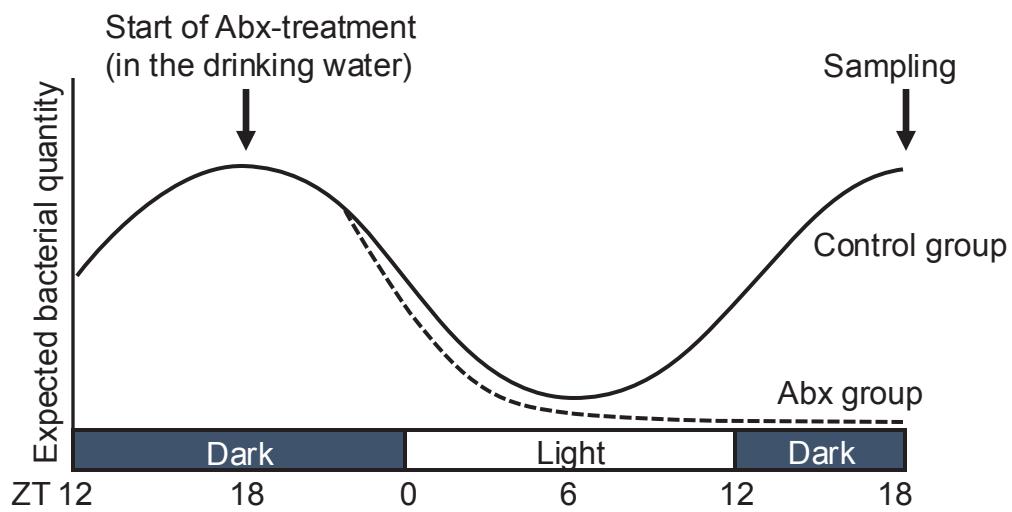
Supplementary Fig. 2: Method of evaluating the settlement level of the indigenous bacteria in the ascending colon. After measuring the total area of the bacterial layer (blue) and the outer circumference of the intestinal tract (red circle), the total area of the bacterial layer (mm^2) / the length of the outer circumference (mm) was calculated to evaluate the settlement level of the indigenous bacteria.

1. Carefully wash the ascending colon to extract luminal contents
2. Fix with 4% PFA and embed as frozen blocks
3. Make serial tissue sections
4. HE stain to observe the indigenous bacteria and confirm the absence of luminal content

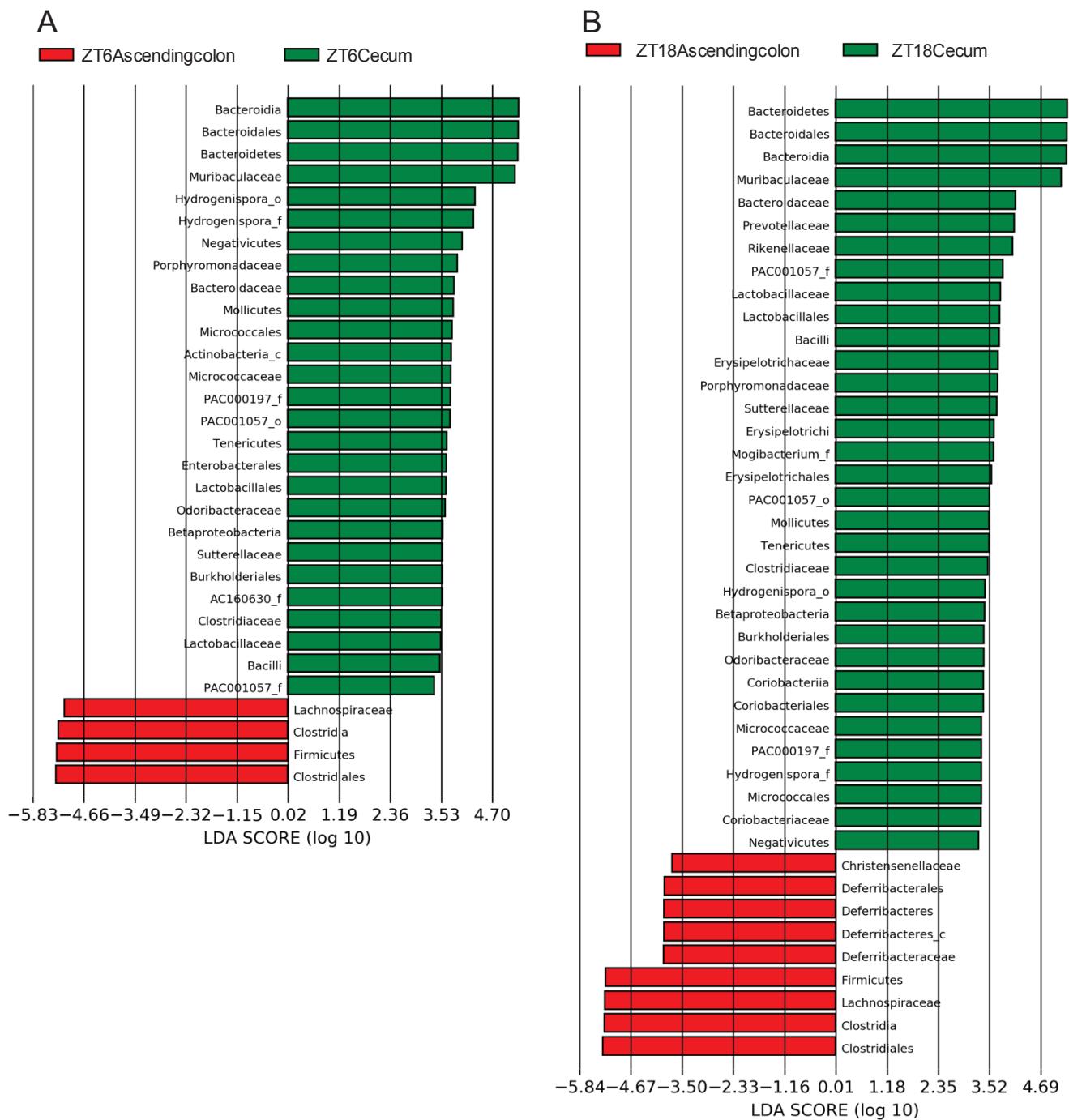


5. DNA extraction, PCR and 16S rRNA amplicon sequencing

Supplementary Fig. 3: Workflow in tissue section 16S rRNA amplicon sequencing.



Supplementary Fig. 4: Schematic diagram of the 1-day treatment with antibiotics (Abx). The solid line represents the predicted bacterial quantities in the control group, based on the findings of the histological analysis. The dashed line represents the expected bacterial reduction in the antibiotic-treated (Abx) group.

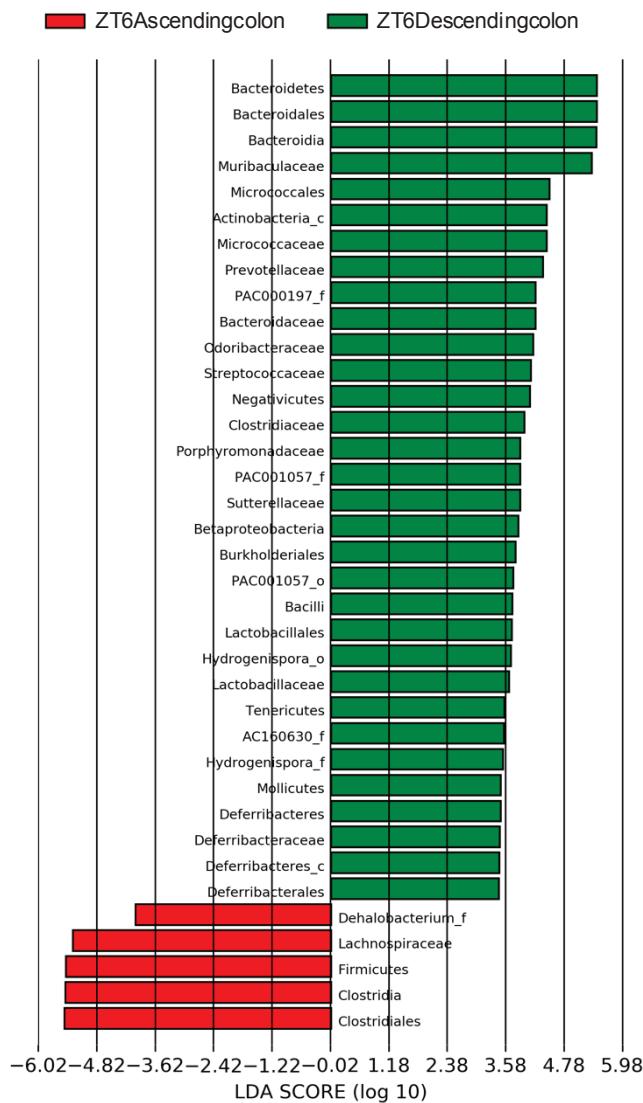


Supplementary Fig. 5: Comparison of the relative abundance between the indigenous bacteria of the ascending colon including the ALT-AC and the bacteria in the cecal contents at each taxonomic level (phylum ~ family) by LEfSe analysis.

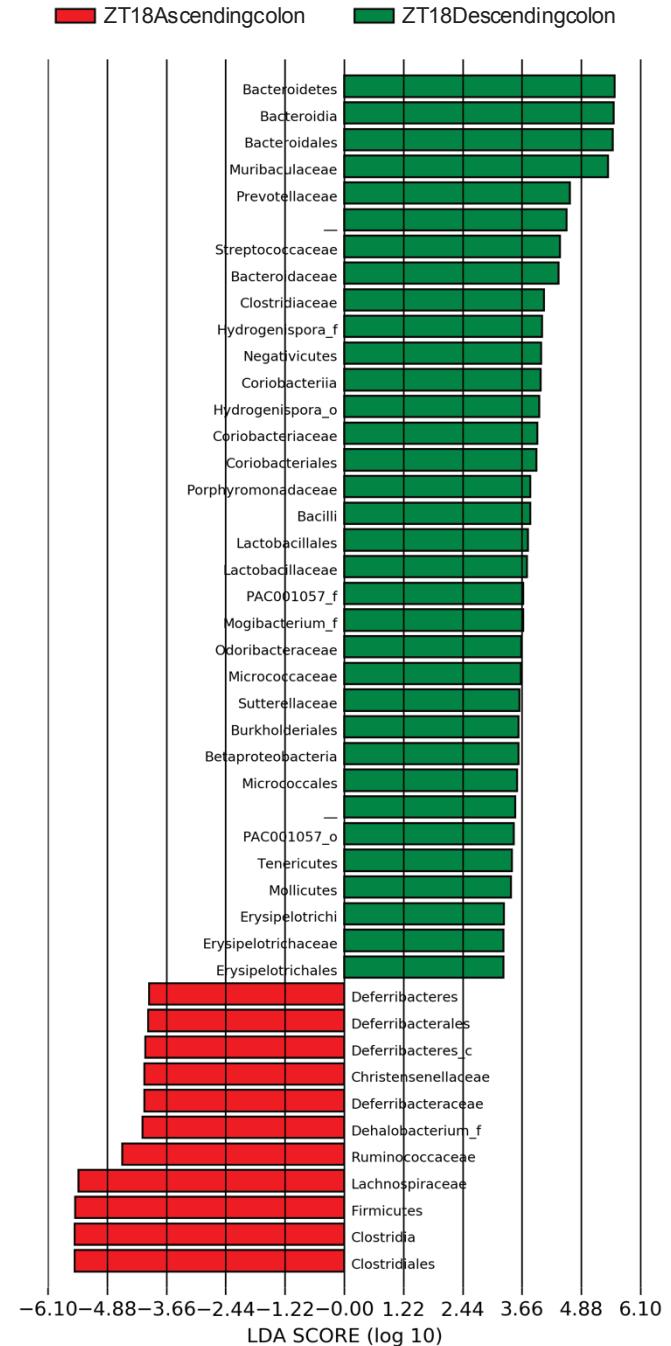
A) Comparison of the relative abundance between the indigenous bacteria of the ascending colon (red) and the bacteria in the cecal contents (green) at ZT6.

B) Comparison of the relative abundance between the indigenous bacteria of the ascending colon (red) and the bacteria in the cecal contents (green) at ZT18.

A



B



Supplementary Fig. 6: Comparison of the relative abundance between the indigenous bacteria of the ascending colon including the ALT-AC and the bacteria in the feces from the descending colon at each taxonomic level (phylum ~ family) by LEfSe analysis.

A) Comparison of the relative abundance between the indigenous bacteria of the ascending colon (red) and the bacteria in the feces (green) at ZT6.

B) Comparison of the relative abundance between the indigenous bacteria of the ascending colon (red) and the bacteria in the feces (green) at ZT18.

Supplementary Table 1: All down-regulated differentially expressed genes (DEGs) in the Abx treatment group compared with the control group.

Mat2a	C2cd4b	Rab37	Aqp8	Rgs16	LOC102550225
Rem2	Crym	Serpine1	Fitm2	Pcsk9	Spdef
Crybb1	Celf3	LOC103692885	LOC100912538	NEWGENE_1310847	Sgk1
Mettl11b	Plk3	LOC102547718	Mfsd2a	Fabp4	Pim3
Slfn4	Hbegf	Cbarp	Col11a2	Atoh1	Nup210l
Map3k1	RGD1311946	Mast4	Bhlha15	Ctgf	Esco2
LOC100912537	Clec2d2	LOC103690018			

Supplementary Table 2: All up-regulated DEGs in the Abx treatment group compared with the control group.

Cyp2b1	Mt2A	Capns1	Agmo	Efcab6	Efna1
Cyp3a9	Oas3	Drc3	Irf7	Apoa1	Gdpd2
Acer1	Cideb	Cyp3a62	Oas1a	Ube2l6	Slc51b
Mt1	Cyp8b1	LOC102555576	Smim24	Cebpb	Slc5a9
LOC100364027	Apoc3	LOC501266	LOC108350522	Dhrs9	Tmem140
Casr	Tldc2	Espn	LOC103690142	LOC100360476	Oas2
Pkrl	Lgals5	LOC108348140	Dio1	Gzma	Amn
LOC102554372	Wfdc3	Isg15	Fabp2	Myo1a	LOC103691958
LOC691320	LOC689766	Ckmt1b	Tubal3	Tmem246	Igsf23
Gal3st1	Mkm2os	Slc51a	Dnah11	Cox7a1	Apoa4
Ces2	Mcpt1	Prl2a1	Mcpt8l3	Pink1	Mcpt8l2
Adtrp	Prrx1	Sectm1b	Cyp4x1	Bmp3	Aldh1l1
Zbtb16	Ephx1	Bpi	LOC100360218	Mall	Abca12
Cyp27a1	Gzmf	Nxf2	Prlr	Tcerg1l	Serpina3n
Slc2a12	Ascl4	Frmd1	Bmp8a	Mep1b	Aadat
LOC102546833	Mep1a	Spats2l	C1qtnf5	RGD1308544	LOC100912599
LOC108348048	Best2	Cck	Ggt6	Guca2a	Rnase12
LOC102550965	LOC108348093	Cyp4b1	Atp8b3	Gstm7	