Supplementary Materials and Methods

Pancreatic histological evaluation

Pancreatic histological changes were evaluated according to the Schmidt's standard (Schmidt J et al., Ann Surg 215:44-56, 1992) as follows (Table S1). The total histopathological score of the pancreas included scores of edema, acinar necrosis, hemorrhage and inflammatory infiltrate.

Table S1. Histopathologic Scoring Criteria

Edema	
0	Absent
0.5	Focal expansion of interlobar septae
1	Diffuse expansion of interlobar septae
1.5	Same as 1 + focal expansion of interlobular septae
2	Same as 1 + diffuse expansion of interlobular septae
2.5	Same as 2 + focal expansion of interacinar septae
3	Same as 2 + diffuse expansion of interacinar septae
3.5	Same as 3 + focal expansion of intercellular spaces
4	Same as 3 + diffuse expansion of intercellular spaces
Acinar necrosis	
0	Absent
0.5	Focal occurrence of 1 - 4 necrotic cells/HPF
1	Diffuse occurrence of 1 - 4 necrotic cells/HPF
1.5	Same as 1 + focal occurrence of 5 - 10 necrotic cells/HPF
2	Diffuse occurrence of 5 - 10 necrotic cells/HPF
2.5	Same as 2 + focal occurrence of 11 - 16 necrotic cells/HPF
3	Diffuse occurrence of 11 - 16 necrotic cells/HPF (foci of confluent necrosis)
3.5	Same as 3 + focal occurrence of >16 necrotic cells/HPF
4	>16 necrotic cells/HPF (Extensive confluent necrosis)
Hemorrhage	
0	Absent
0.5	1 focus
1	2 foci
1.5	3 foci
2	4 foci
2.5	5 foci
3	6 foci
3.5	7 foci
4	≥ 8 foci
Inflammatory infiltrate	
0	0 - 1 intralobular or perivascular leukocytes/HPF
0.5	2 - 5 intralobular or perivascular leukocytes/HPF
1	6 - 10 intralobular or perivascular leukocytes/HPF
1.5	11 - 15 intralobular or perivascular leukocytes/HPF

2	16 - 20 intralobular or perivascular leukocytes/HPF
2.5	21 - 25 intralobular or perivascular leukocytes/HPF
3	26 - 30 intralobular or perivascular leukocytes/HPF
3.5	> 30 leukocytes/HPF or focal microabscesses
4	> 35 leukocytes/HPF or confluent microabscesses

HPF represents " high-power field ".

Immunohistochemistry

Ileal paraffin sections were subjected to deparaffinization, rehydration and heat-mediated antigen retrieval using citrate buffer. Endogenous peroxidase activity was blocked by 3% H_2O_2. After incubation with goat serum, the sections were then incubated with primary antibodies against TLR4 (1:150, ABclonal, Wuhan, Hubei, China), p-IRE1α (1:150, Cell Signalling Technology, Denver, MA, USA), p-eIF2α (1:150, ABclonal), ATF6 (1:150, ABclonal) overnight at 4℃. After washing three times with PBS, the sections were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 30 minutes at room temperature. Finally, diaminobenzidine (DAB) was added for visualization.

Fecal DNA extraction

Bacterial DNA was extracted from fecal samples with the E.Z.N.A. Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's protocols (https://www.omegabiotek.com/product/e-z-n-a-soil-dna-kit/).

(1) Add 200 mg feces sample to a Disruptor Tube.
(2) Add 725 μl SLX-Mlus Buffer. Vortex at maximum speed for 3-5 minutes to lyse samples.
(3) Centrifuge at 500 g for 5 seconds to remove drops of liquid from the lid.
(4) Add 72 μl DS Buffer. Vortex to mix thoroughly.
(5) Incubate at 70 ℃ for 10 minutes. Briefly vortex the tube once during incubation.
(6) Centrifuge at 10,000 g for 5 minutes at room temperature.
(7) Transfer 400 μl supernatant into a new 1.5 mL microcentrifuge tube.
(8) Add 135 μl chilled P2 Buffer. Vortex to mix thoroughly.
(9) Let sit on ice for 3 minutes.
(10) Centrifuge at maximum speed (≥ 13,000 g) for 1 minute.
(11) Carefully transfer the supernatant to a new 1.5 mL microcentrifuge tube.
(12) Add 200 μl cHTR Reagent. Vortex to mix thoroughly.
(13) Let sit at room temperature for 2 minutes.
(14) Centrifuge at maximum speed for 1 minute.
(15) Transfer cleared supernatant (~ 500 μl) to a new 1.5 mL microcentrifuge tube.
(16) Add an equal volume XP1 Buffer. Vortex to mix thoroughly.
(17) Insert a HiBind DNA Mini Column into a 2 mL Collection Tube.
(18) Tranfer up to 700 μl sample from Step 16 to the HiBind DNA Mini Column.
(19) Centrifuge at 10,000 g for 1 minute at room temperature.
(20) Discard the filtrate and reuse the Collection Tube.
(21) Repeat Steps 18-20 until all the lysate from Step 16 has passed through the

HiBind DNA Mini Column.
(22) Add 500 µl HBC Buffer.
(23) Centrifuge at 10,000 g for 1 minute.
(24) Discard the filtrate and reuse the Collection Tube.
(25) Tranfer the HiBind DNA Mini Column into a new 2 mL Collection Tube.
(26) Add 700 µl DNA Wash Buffer.
(27) Centrifuge at 10,000 g for 1 minute.
(28) Discard the filtrate and reuse the Collection Tube.
(29) Repeat Steps 26-28 for a second DNA Wash Buffer wash step.
(30) Centrifuge the empty HiBind DNA Mini Column at maximum speed for 2 minutes at room temperature.
(31) Tranfer the HiBind DNA Mini Column into a new 1.5 mL microcentrifuge tube.
(32) Add 50-100 µl Elution Buffer heated to 70 ℃ directly onto the center of HiBind matrix.
(33) Let sit at room temperature for 1-2 minutes.
(34) Centrifuge at maximum speed for 1 minute.
(35) Take the filtrate from Step 34 and place onto the center of the same HiBind DNA Mini Column used in the procedure.
(36) Let sit at room temperature for 1 minute.
(37) Centrifuge at maximum speed for 1 minute.
(38) Store eluted DNA at -20 ℃ for future use.

Supplementary Figures

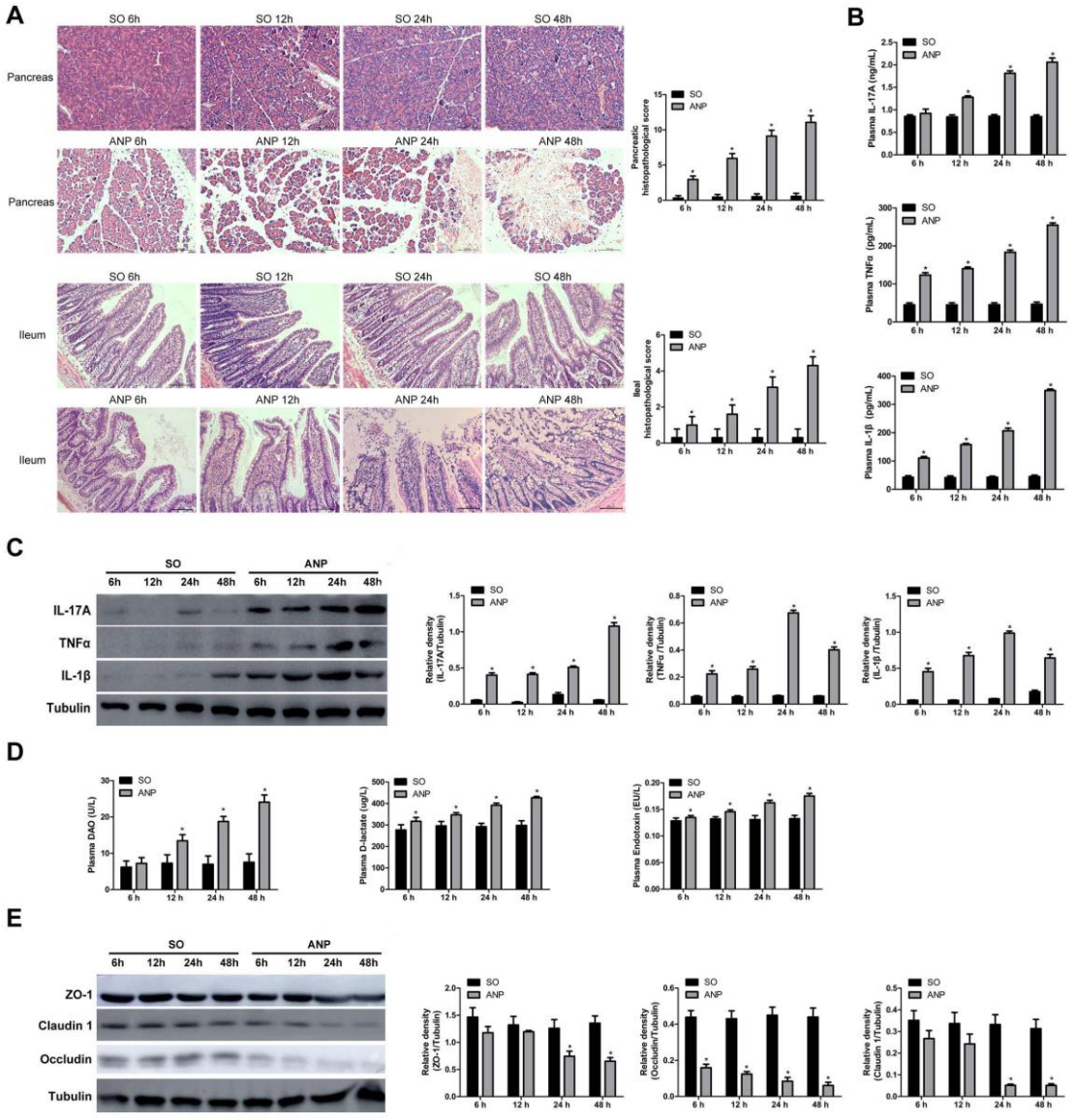

Figure S1. ANP induced pancreatic and intestinal injury in conventional rats. (A) Histological analysis of the pancreas and ileum (HE, × 200). (B) IL-17A, TNFα and IL-1β levels in the plasma by ELISA. (C) IL-17A, TNFα and IL-1β expression in the ileum by Western Blot. (D) DAO, D-lactate and endotoxin levels in the plasma. (E) ZO-1, claudin 1 and occludin expression in the ileum by Western Blot at 6, 12, 24 and 48 h after ANP induction in conventional rats. n = 10 per group. Three independent experiments were performed. Comparison between two groups (SO *vs.* ANP at the same timepoint) was performed by Student's *t*-test. **P* < 0.05 *vs.* SO group. SO represents sham-operated.

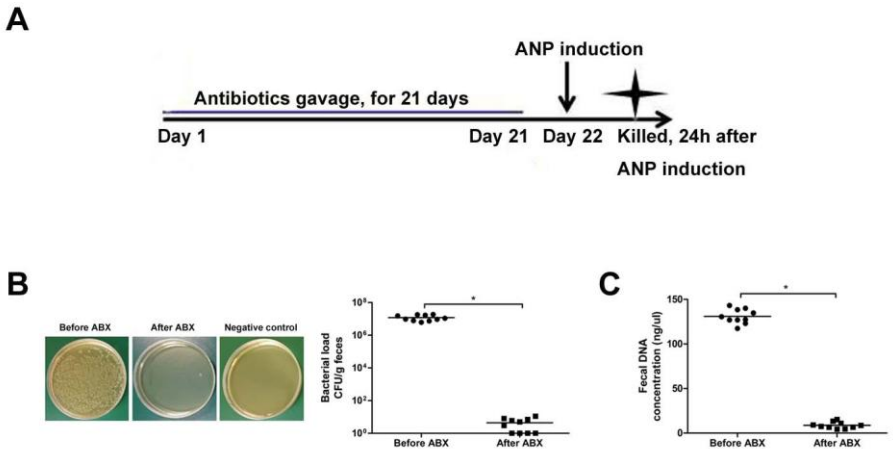

Figure S2. Gut microbiota-depleted rats were obtained by using a cocktail of antibiotics (ABX). (A) The time axis of antibiotic treatment and ANP induction. (B) Bacterial quantity by fecal cultivation. (C) Fecal bacterial DNA concentration before and after ABX treatment. n = 10 per group. Three independent experiments were performed. Comparison between two groups (Before ABX *vs.* After ABX) was performed by Student's *t*-test. **P* < 0.05.

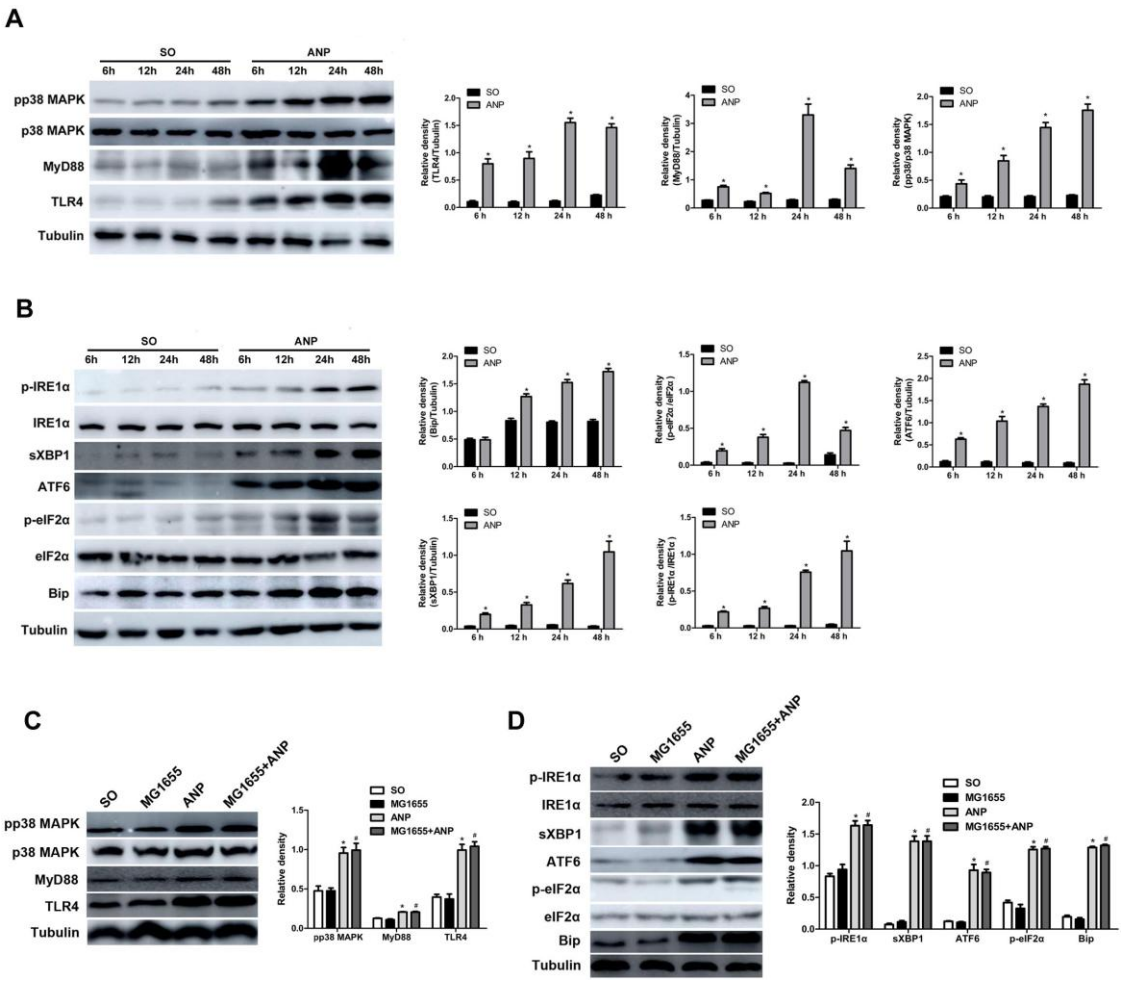

Figure S3. ANP induced TLR4 and ERS signalling in the ileum in conventional rats. (A-B) TLR4 (A) and ERS (B) signalling molecules expression in the ileum by Western Blot at 6, 12, 24 and 48 h after ANP induction in conventional rats. (C-D) Comparison of TLR4 (C) and ERS (D) signalling molecules after ANP induction between conventional rats and *E. coli* MG1655-gavaged conventional rats. n = 10 per group. Three independent experiments were performed. Data were analyzed by Student's *t*-test for two groups and one-way ANOVA followed by Bonferroni's *post* test for three or more groups. **P* < 0.05 *vs.* SO group, #*P* < 0.05 *vs.* MG1655 group. SO represents sham-operated.

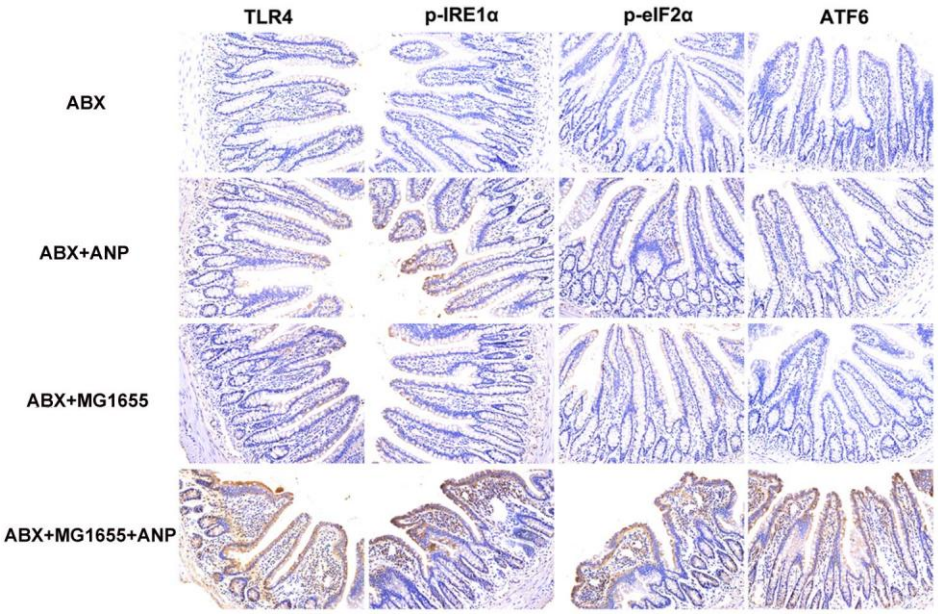

Figure S4. Immunohistochemistry analysis of TLR4, p-IRE1α, p-eIF2α, ATF6 in the ileum after ANP induction in *E. coli* MG1655-monocolonized rats. n = 10 per group. Three independent experiments were performed.