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Supplemental information

Gut microbe-derived milnacipran enhances tolerance to gut ischemia/reperfusion injury

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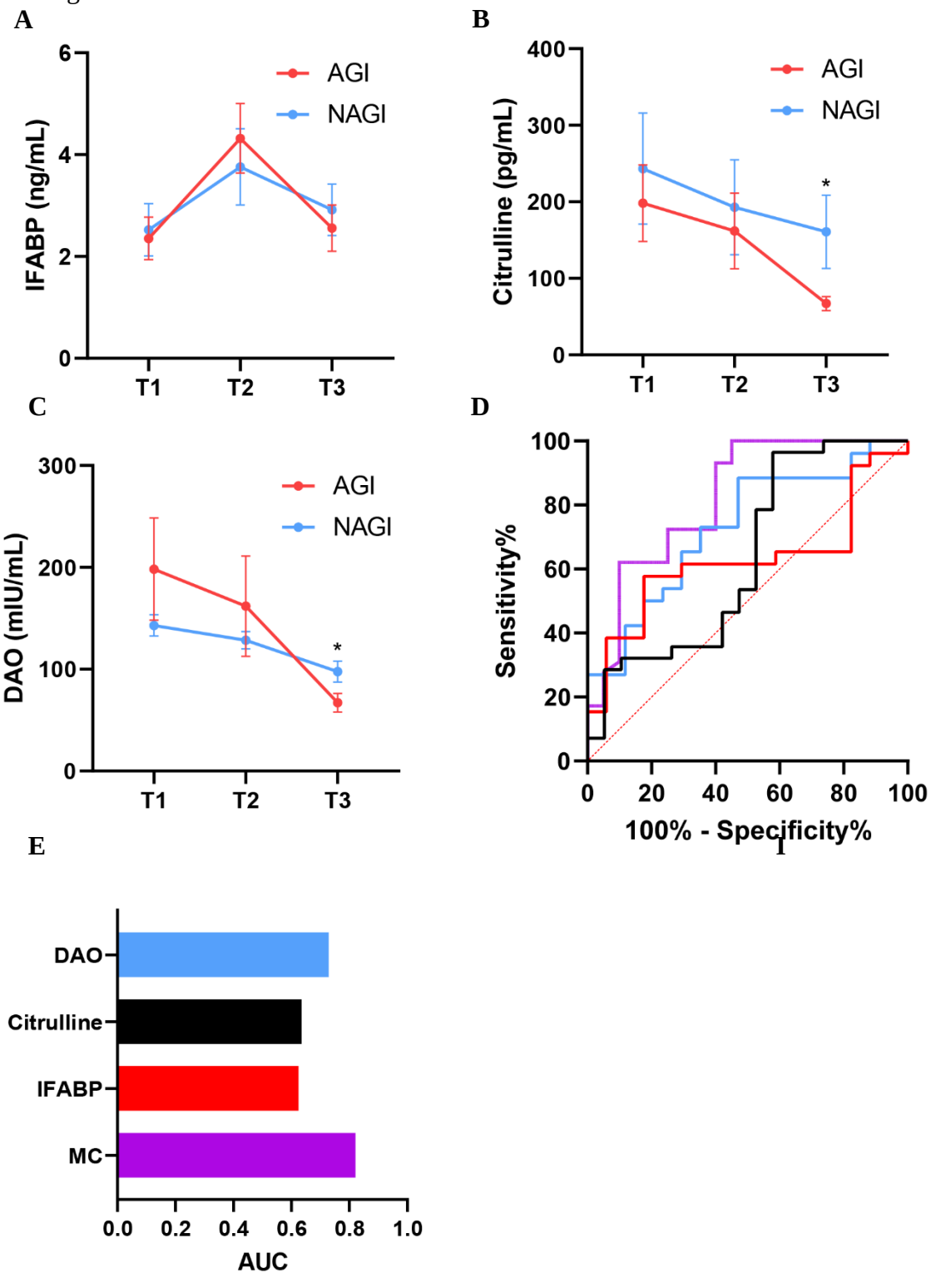
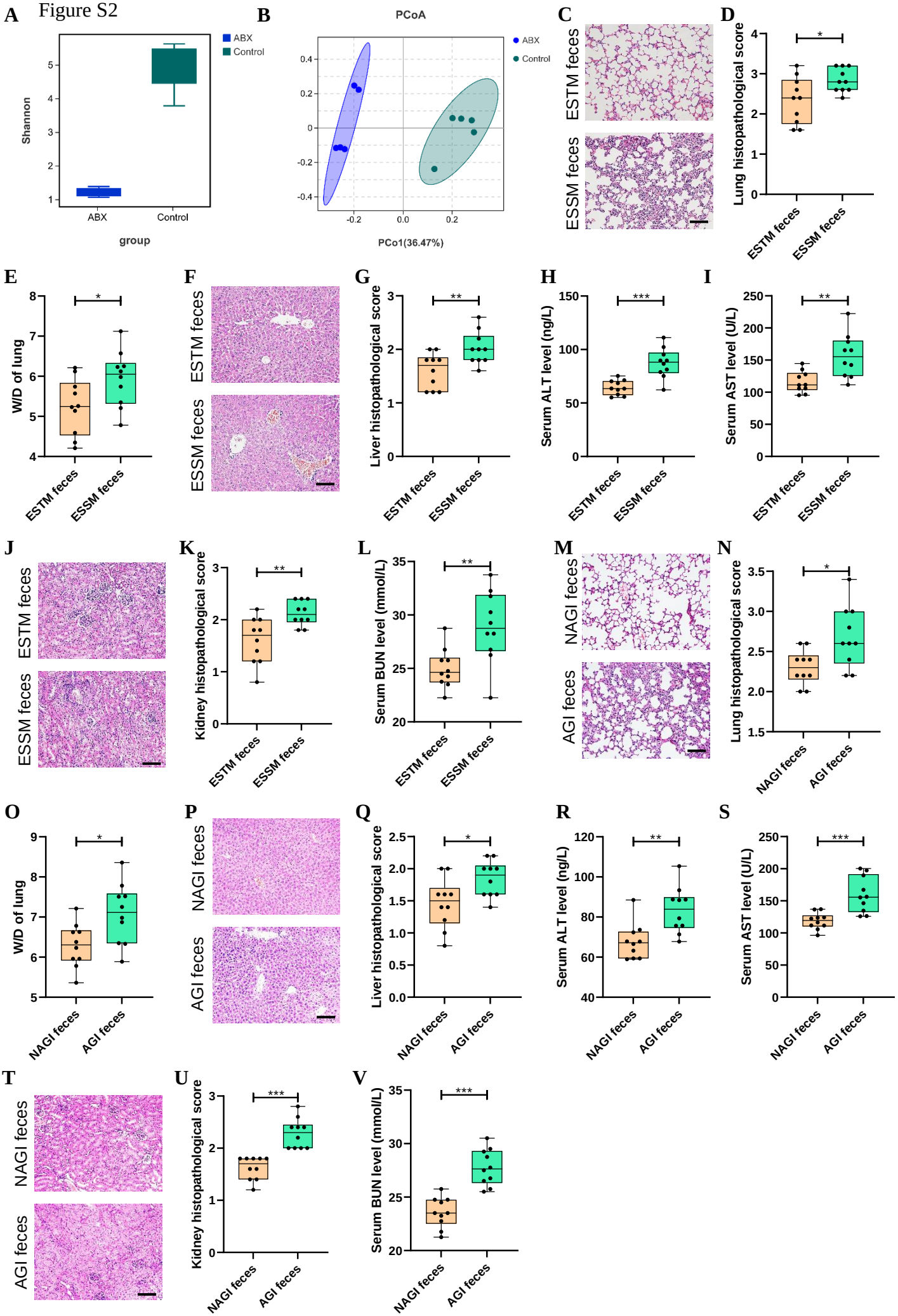
Figure S1


A
IFABP (ng/mL)
AGI
NAGI
T1
T2
T3
B
Citrulline (pg/mL)
C
DAO (mIU/mL)
D
Sensitivity%
100% - Specificity%
E
DAO
Citrulline
IFABP
MC
AUC

Supplemental Figure S1. Preoperative fecal MC content in CPB patients is associated with susceptibility to postoperative intestinal injury. Related to Figures 2.

(A-C) The levels of intestinal fatty-acid binding protein (IFABP) (**A**), citrulline (**B**) and diamine oxidase (DAO) (**C**) in patient serum preoperatively (T1), at the end of the operation (T2), and at 6 h after surgery (T3). **(D-E)** The receiver operating characteristic (ROC) analysis (**D**) and the area under the ROC curve (AUC) (**E**) to evaluate the ability of preoperative fecal MC content, plasma IFABP, citrulline, and DAO levels at T3 to distinguish postoperative acute gastrointestinal function injury (AGI≥1) in patients with CPB. (**A-C**) The results are shown as mean±SEM, "*" indicates $p<0.05$.

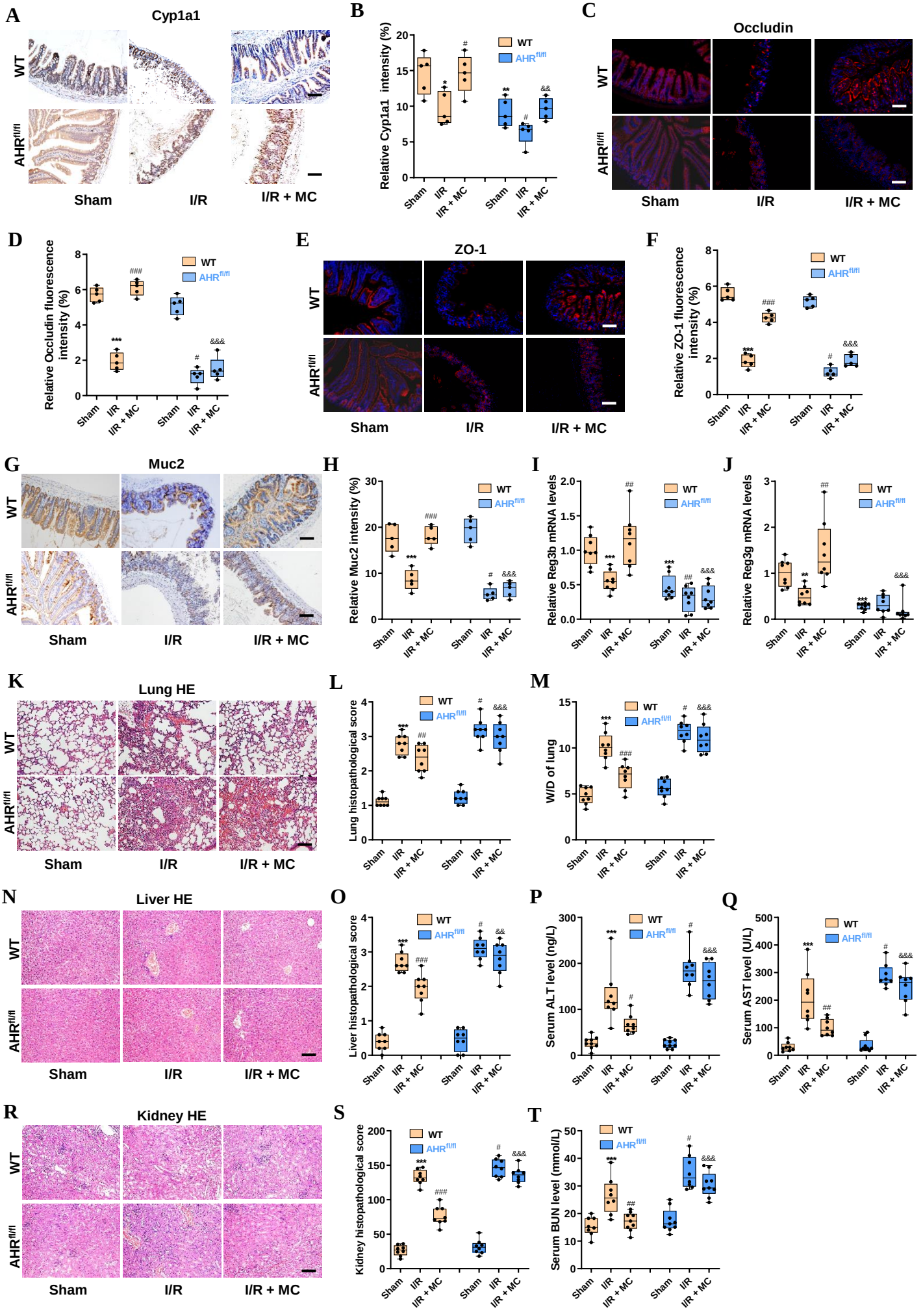
Figure S2


A
ABX
Control
Shannon
group
B
PCoA
ABX
Control
PCo1(36.47%)
C
ESTM feces
ESSM feces
D
Lung histopathological score
ESTM feces
ESSM feces
E
W/D of lung
F
G
Liver histopathological score
H
Serum ALT level (ng/L)
I
Serum AST level (U/L)
J
K
Kidney histopathological score
L
Serum BUN level (mmol/L)
M
NAGI feces
AGI feces
N
Lung histopathological score
O
P
Q
Liver histopathological score
R
Serum ALT level (ng/L)
S
Serum AST level (U/L)
T
U
Kidney histopathological score
V
Serum BUN level (mmol/L)

Supplemental Figure S2. Gut microbiota affects susceptibility to enterogenic sepsis induced by intestinal ischemia/reperfusion (I/R). Related to Figures 3.

(A, B) Alpha diversity indices (Simpson) of the control and ABX-treated mouse groups (n = 5). **(B)** Principal coordinate analysis (PCoA) of the control and ABX-treated mouse groups (n = 5). (**C-E)** Mouse lung tissue hematoxylin-eosin (HE) staining (**C**); the pathological damage scores (**D**) and wet/dry weight ratios (**E**); the scale bar is 100 μm (n = 8). **(F-I)** Mouse liver tissue HE staining (**F**); the pathological damage scores (**G**) and plasma ALT (**H**) and AST levels (**I**); the scale bar is 100 μm (n = 8). **(J-L)** Mouse kidney tissue HE staining (**J**); the pathological damage scores (**K**) and plasma BUN levels (**L**); the scale bar is 100 μm (n = 8). **(M-O)** Mouse lung tissue HE staining (**M**); the pathological damage scores (**N**) and wet/dry weight ratios (**O**); the scale bar is 100 μm (n = 8). **(P-S)** Mouse liver tissue HE staining (**P**); the pathological damage scores (**Q**) and plasma ALT (**R**) and AST levels (**R**); the scale bar is 100 μm (n = 8). **(T-V)** Mouse kidney tissue HE staining (**T**); the pathological damage scores (**U**) and plasma BUN levels (**V**); the scale bar is 100 μm (n = 8). The results are expressed as the median and quartile, and are representative of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by were determined by Mann Whitney test in (A), and adonis analysis and anosim analysis in (**B)**.

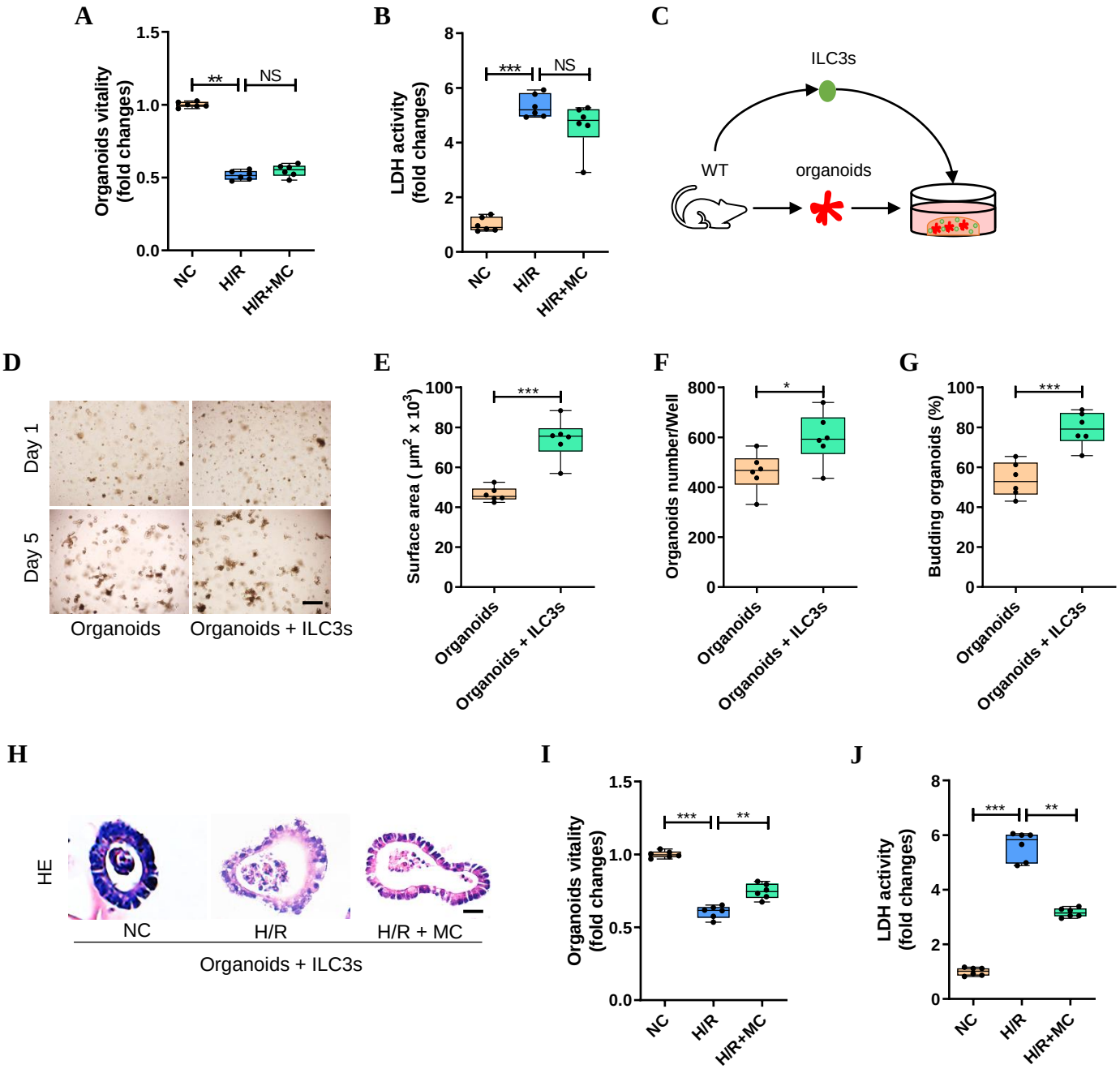
Figure S3


A
Cyp1a1
WT
AHRfl/fl
Sham
I/R
I/R + MC
B
Relative Cyp1a1 intensity (%)
C
Occludin
D
Relative Occludin fluorescence intensity (%)
E
ZO-1
F
Relative ZO-1 fluorescence intensity (%)
G
Muc2
H
Relative Muc2 intensity (%)
I
Relative Reg3b mRNA levels
J
Relative Reg3g mRNA levels
K
Lung HE
L
Lung histopathological score
M
W/D of lung
N
Liver HE
O
Liver histopathological score
P
Serum ALT level (ng/L)
Q
Serum AST level (U/L)
R
Kidney HE
S
Kidney histopathological score
T
Serum BUN level (mmol/L)

Supplemental Figure S3. Milnacipran attenuates intestinal I/R injury by activating the intestinal epithelial AHR. Related to Figures 4.

(A-B) Immunohistochemical results (**A**) and relative quantitative analysis (**B**) of cyp1a1 in intestinal tissue; the scale bar is 100 μm (n = 5). **(C-D)** Immunofluorescence results (**C**) and relative quantitative analysis (**D**) of Occludin in intestinal tissue; the scale bar is 100 μm (n = 5). **(E-F)** Immunofluorescence results (**E**) and relative quantitative analysis (**F**) of ZO-1 in intestinal tissue; the scale bar is 100 μm (n = 5). **(G-H)** Immunohistochemical results (**G**) and relative quantitative analysis (**H**) of Muc2 in intestinal tissue; the scale bar is 100 μm (n = 5). **(I-J)** The mRNA levels of the intestinal immune barrier markers Reg3b (**I**) and Reg3g (**J**) (n = 8). **(K-M)** Mouse lung tissue HE staining (**K**); the pathological damage scores (**L**) and wet/dry weight ratios (**M**); the scale bar is 100 μm (n = 8). **(N-Q)** Mouse liver tissue HE staining (**N**); the pathological damage scores (**O**) and plasma ALT (**P**) and AST levels (**Q**); the scale bar is 100 μm (n = 8). **(R-T)** Mouse kidney tissue HE staining (**R**); the pathological damage scores (**S**) and plasma BUN levels (**T**); the scale bar is 100 μm (n = 8). The results are expressed as the median and quartile, and are representative of three independent experiments. *, # and & $p < 0.05$, **, ## and && $p < 0.01$, ***, ### and &&& $p < 0.001$ were determined by two-way ANOVA and Tukey's post hoc test. "*" indicates $p<0.05$ compared with wild-type mouse Sham group; "#" indicates $p<0.05$ compared with wild-type mouse I/R group; "&" indicates $p<0.05$ compared with wild-type mouse I/R+MC group. I/R + MC group: WT mice or $AHR^{flox/flox}$ mice were injected intraperitoneally with 10 mg/kg MC 1 hour before intestinal I/R.

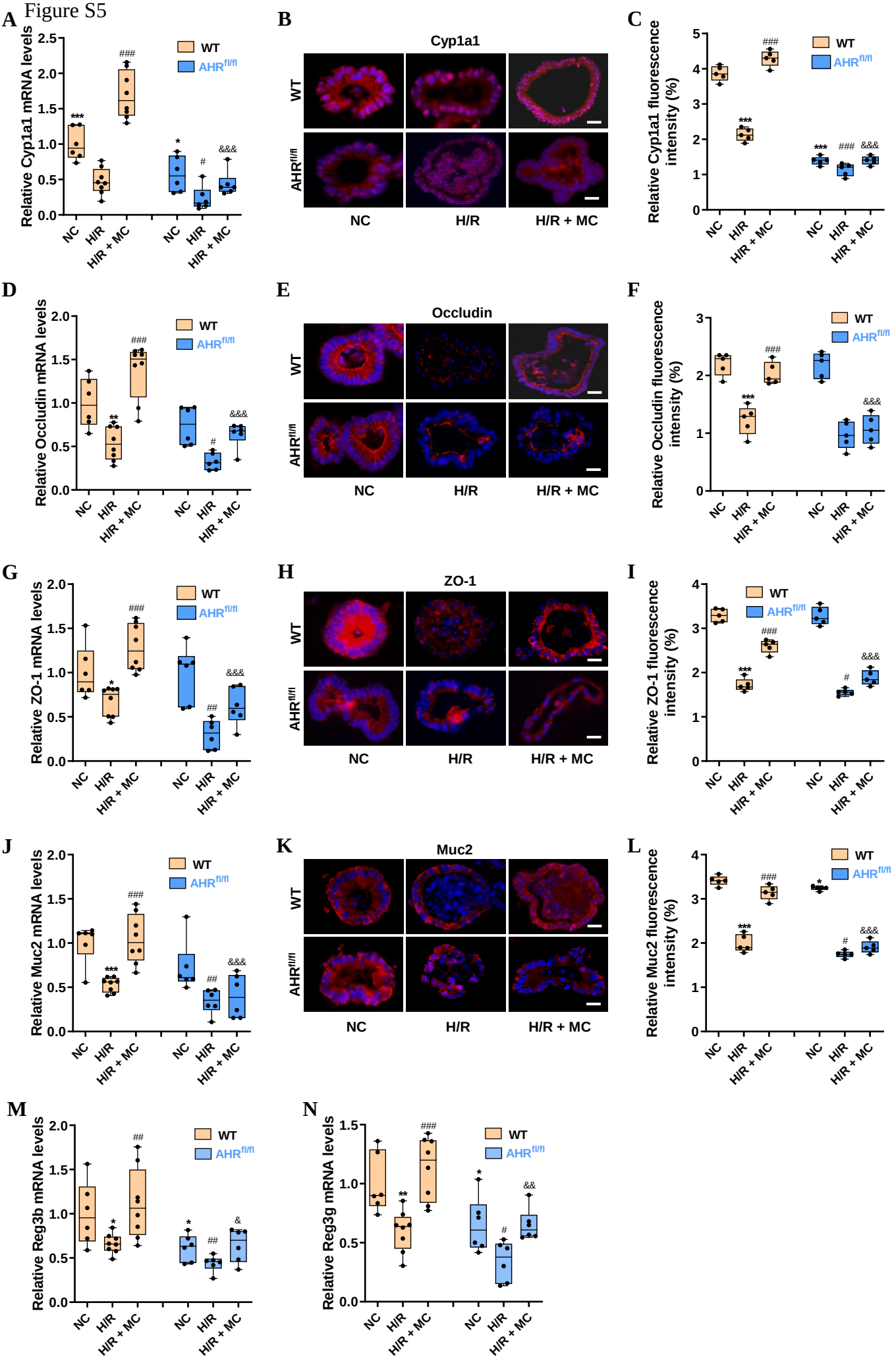
Figure S4


A
Organoids vitality (fold changes)
1.5
1.0
0.5
0.0
**
NS
NC
H/R
H/R+MC
B
LDH activity (fold changes)
8
6
4
2
0
***
NS
NC
H/R
H/R+MC
C
ILC3s
WT
organoids
D
Day 1
Day 5
Organoids
Organoids + ILC3s
E
Surface area (μm² x 10³)
100
80
60
40
20
0
***
Organoids
Organoids + ILC3s
F
Organoids number/Well
800
600
400
200
0
*
Organoids
Organoids + ILC3s
G
Budding organoids (%)
100
80
60
40
20
0
***
Organoids
Organoids + ILC3s
H
HE
NC
H/R
H/R + MC
Organoids + ILC3s
I
Organoids vitality (fold changes)
1.5
1.0
0.5
0.0
***
**
NC
H/R
H/R+MC
J
LDH activity (fold changes)
8
6
4
2
0
***
**
NC
H/R
H/R+MC

Supplemental Figure S4. Supplementation with ILC3s restores the protective effect of milnacipran against H/R injury in intestinal organoids. Related to Figures 4.

(A, B) Intestinal organoid viability (**A**) and lactate dehydrogenase (LDH) levels (**B**) in culture medium were detected after H/R in intestinal organoids cultured alone (n = 6). **(C)** Schematic diagram of the coculture system of intestinal organoids and ILC3s. **(D)** Brightfield images of organoids without or with ILC3s on day 1 (scale bar is 400 μm) and day 5 (scale bar is 50 μm) (n = 6). **(E-G)** Microscopic tracing of organoids to measure surface area (**E**), organoid numbers (**F**) and the percentage of budding organoids (**G**) on day 5 (n = 6). **(H)** Hematoxylin-eosin (HE) staining of intestinal organoids; the scale bar is 20 μm (n = 6). **(I)** Intestinal organoid viability (n = 6). **(J)** LDH levels in the medium (n = 6). The results are expressed as the median and quartile, and are representative of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ were determined by one-way ANOVA and Tukey's post hoc test in A, B, I, J, and Mann Whitney test in E-G.

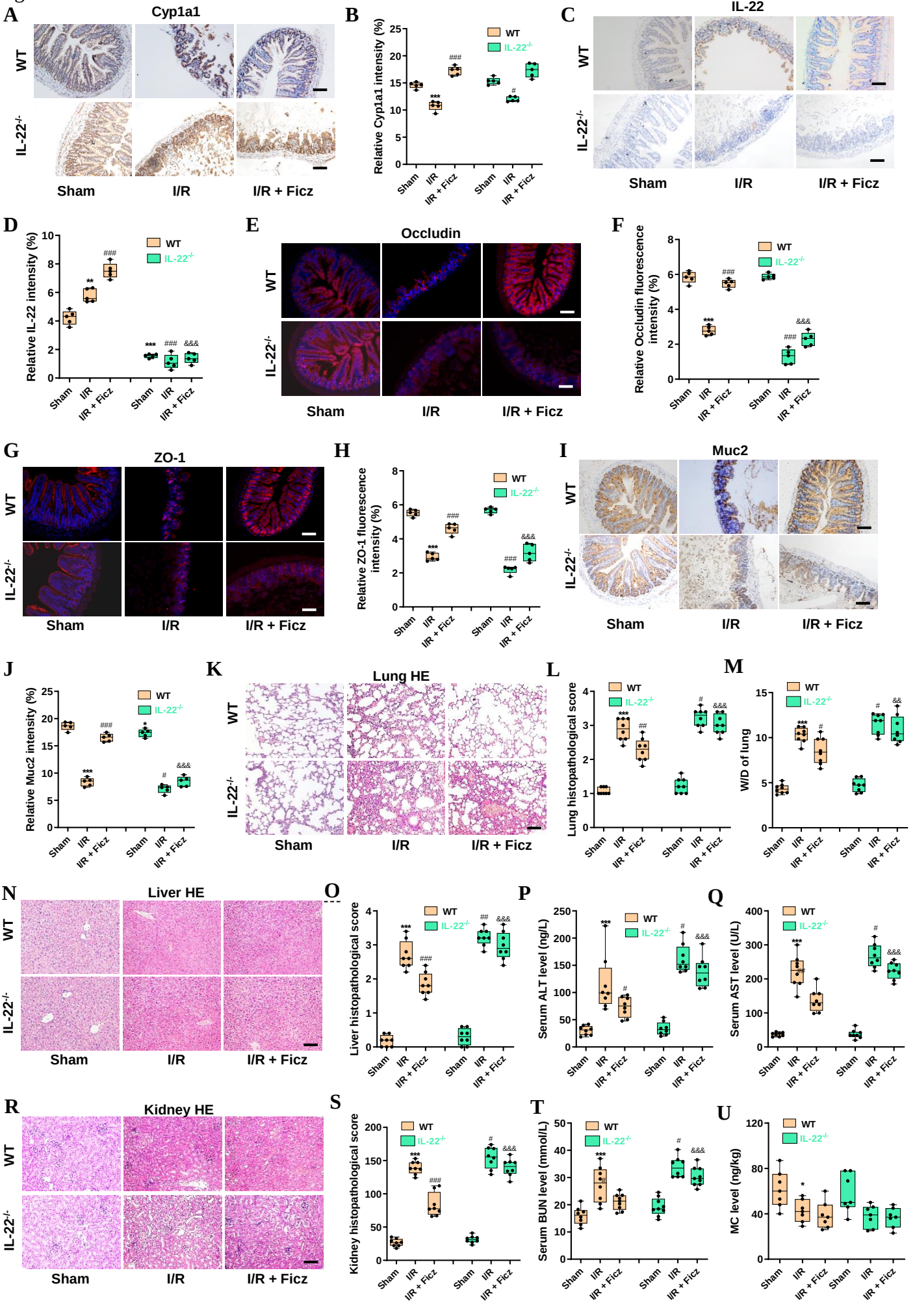
Figure S5


A
Relative Cyp1a1 mRNA levels
WT
AHR fl/fl
NC
H/R
H/R + MC
B
Cyp1a1
WT
AHR fl/fl
NC
H/R
H/R + MC
C
Relative Cyp1a1 fluorescence intensity (%)
D
Relative Occludin mRNA levels
E
Occludin
F
Relative Occludin fluorescence intensity (%)
G
Relative ZO-1 mRNA levels
H
ZO-1
I
Relative ZO-1 fluorescence intensity (%)
J
Relative Muc2 mRNA levels
K
Muc2
L
Relative Muc2 fluorescence intensity (%)
M
Relative Reg3b mRNA levels
N
Relative Reg3g mRNA levels

Supplemental Figure S5. Milnacipran (MC) maintains intestinal organoid barrier homeostasis by activating the intestinal epithelial AHR. Related to Figures 4.

(A) The mRNA levels of cyp1a1 in intestinal organoids (n = 6). **(B-C)** Immunofluorescence (**B**) and relative quantitative analysis (**C**) of cyp1a1 in intestinal organoids; the scale bar is 20 μm (n = 5). **(D)** The mRNA levels of Occludin in intestinal organoids (n = 6). **(E-F)** Immunofluorescence (**E**) and relative quantitative analysis (**F**) of Occludin in intestinal organoids; the scale bar is 20 μm (n = 5). **(G)** The mRNA levels of ZO-1 in intestinal organoids (n = 6). **(H-I)** Immunofluorescence (**H**) and relative quantitative analysis (**I**) of ZO-1 in intestinal organoids; the scale bar is 20 μm (n = 5). **(J)** The mRNA levels of Muc2 in intestinal organoids (n = 6). **(K-L)** Immunofluorescence (**K**) and relative quantitative analysis (**L**) of Muc2 in intestinal organoids; the scale bar is 20 μm (n = 5). **(M-N)** The mRNA levels of Reg3b (**M**) and Reg3g (**N**) (n = 6). The results are expressed as the median and quartile, and are representative of three independent experiments. *, # and & $p < 0.05$, **, ## and && $p < 0.01$, ***, ### and &&& $p < 0.001$ were determined by two-way ANOVA and Tukey's post hoc test. "*" indicates $p<0.05$ compared with wild-type mouse NC group; "#" indicates $p<0.05$ compared with wild-type mouse H/R group; "&" indicates $p<0.05$ compared with wild-type mouse H/R+MC group. H/R + MC group: 10 μmol/L MC was added to intestinal organoids 1 h before H/R.

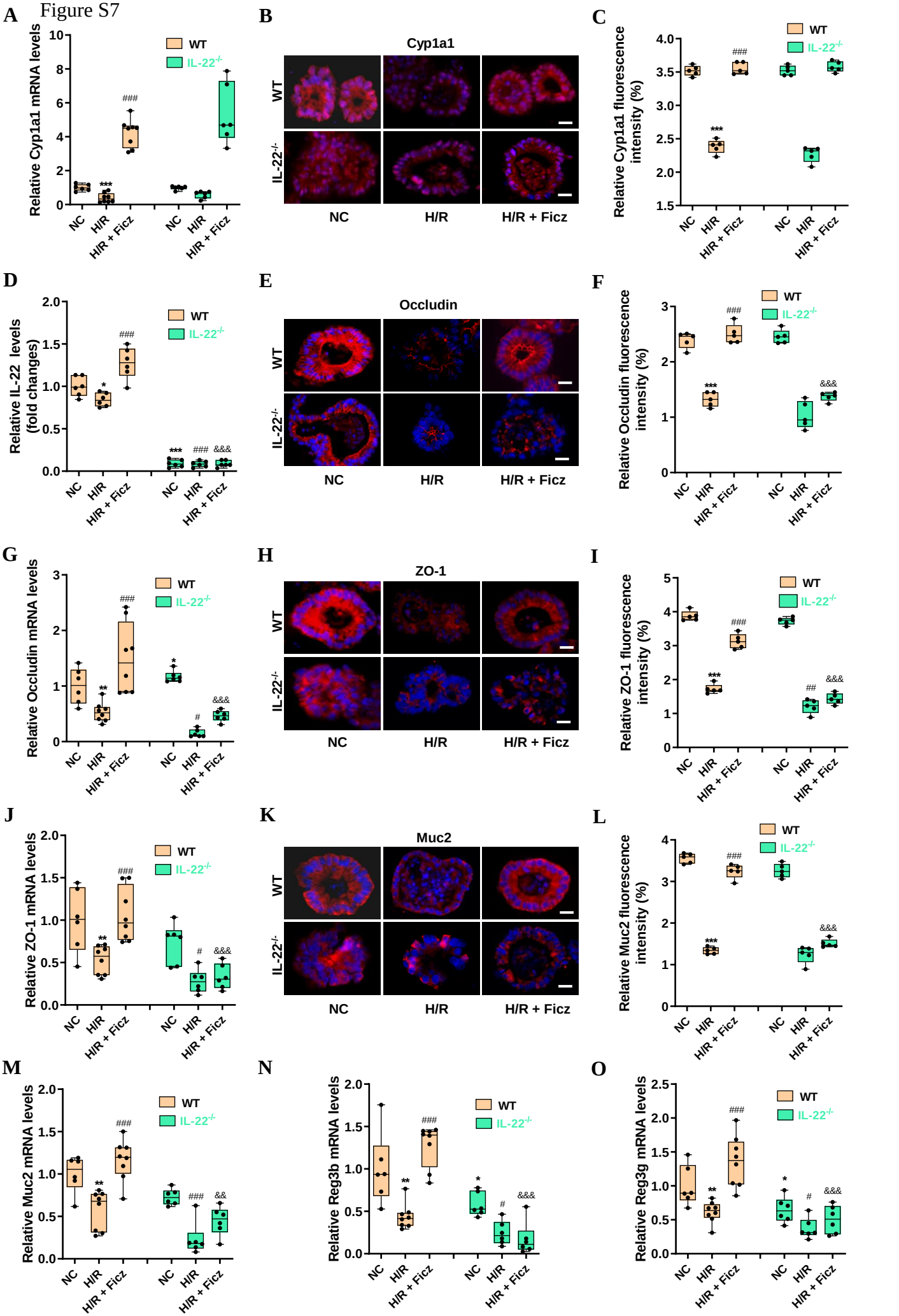
Figure S6


A
Cyp1a1
WT
IL-22-/-
Sham
I/R
I/R + Ficz
B
Relative Cyp1a1 intensity (%)
WT
IL-22-/-
C
IL-22
D
Relative IL-22 intensity (%)
E
Occludin
F
Relative Occludin fluorescence intensity (%)
G
ZO-1
H
Relative ZO-1 fluorescence intensity (%)
I
Muc2
J
Relative Muc2 intensity (%)
K
Lung HE
L
Lung histopathological score
M
W/D of lung
N
Liver HE
O
Liver histopathological score
P
Serum ALT level (ng/L)
Q
Serum AST level (U/L)
R
Kidney HE
S
Kidney histopathological score
T
Serum BUN level (mmol/L)
U
MC level (ng/kg)

Supplemental Figure S6. AHR activator Ficz attenuates intestinal I/R injury in mice by releasing IL-22. Related to Figures 6.

(A-B) Immunohistochemical results (**A**) and relative quantitative analysis (**B**) of cyp1a1 in intestinal tissue; the scale bar is 100 μm (n = 5). **(C-D)** Immunohistochemical results (**C**) and relative quantitative analysis (**D**) of IL-22 in intestinal tissue; the scale bar is 100 μm (n = 5). **(E-F)** Immunofluorescence results (**E**) and relative quantitative analysis (**F**) of Occludin in intestinal tissue; the scale bar is 100 μm (n = 5). **(G-H)** Immunofluorescence results (**G**) and relative quantitative analysis (**H**) of ZO-1 in intestinal tissue; the scale bar is 100 μm (n = 5). **(I-J)** Immunohistochemical results (**I**) and relative quantitative analysis (**J**) of Muc2 in intestinal tissue; the scale bar is 100 μm (n = 5). **(K-M)** Mouse lung tissue hematoxylin-eosin (HE) staining (**K**); the pathological damage scores (**L**) and wet/dry weight ratios (**M**); the scale bar is 100 μm (n = 8). **(N-Q)** Mouse liver tissue HE staining (**N**); the pathological damage scores (**O**) and plasma ALT (**P**) and AST levels (**Q**); the scale bar is 100 μm (n = 8). **(R-T)** Mouse kidney tissue HE staining (**R**); the pathological damage scores (**S**) and plasma BUN levels (**T**); the scale bar is 100 μm (n = 8). (**U**) LC–MS/MS-targeted detection of the MC content in the feces. The results are expressed as the median and quartile, and are representative of three independent experiments. *, # and & $p < 0.05$, **, ## and && $p < 0.01$, ***, ### and &&& $p < 0.001$ by were determined were determined by two-way ANOVA and Tukey's post hoc test. "*" indicates $p<0.05$ compared with wild-type mouse Sham group; "#" indicates $p<0.05$ compared with wild-type mouse I/R group; "&" indicates $p<0.05$ compared with wild-type mouse I/R+Ficz group. I/R + Ficz group: mice were treated with daily intraperitoneal injection of 50 μg/kg Ficz for 7 consecutive days prior to induction of intestinal I/R.

Figure S7


A
Relative Cyp1a1 mRNA levels
WT
IL-22-/-
B
Cyp1a1
WT
IL-22-/-
NC
H/R
H/R + Ficz
C
Relative Cyp1a1 fluorescence intensity (%)
D
Relative IL-22 levels (fold changes)
E
Occludin
F
Relative Occludin fluorescence intensity (%)
G
Relative Occludin mRNA levels
H
ZO-1
I
Relative ZO-1 fluorescence intensity (%)
J
Relative ZO-1 mRNA levels
K
Muc2
L
Relative Muc2 fluorescence intensity (%)
M
Relative Muc2 mRNA levels
N
Relative Reg3b mRNA levels
O
Relative Reg3g mRNA levels

Supplemental Figure S7. AHR activator Ficz maintains intestinal organoid barrier homeostasis by releasing IL-22. Related to Figures 6.

(A) The mRNA levels of cyp1a1 in intestinal organoids (n = 6). **(B-C)** Immunofluorescence (**B**) and relative quantitative analysis (**C**) of cyp1a1 in intestinal organoids; the scale bar is 20 μm (n = 5). **(D)** The IL-22 levels in the medium (n = 6). **(E-F)** Immunofluorescence (**E**) and relative quantitative analysis (**F**) of Occludin in intestinal organoids; the scale bar is 20 μm (n = 5). **(G)** The mRNA levels of Occludin in intestinal organoids (n = 6). **(H-I)** Immunofluorescence (**H**) and relative quantitative analysis (**I**) of ZO-1 in intestinal organoids; the scale bar is 20 μm (n = 5). **(J)** The mRNA levels of ZO-1 in intestinal organoids (n = 6). **(K-L)** Immunofluorescence (**K**) and relative quantitative analysis (**L**) of Muc2 in intestinal organoids; the scale bar is 20 μm (n = 5). **(M)** The mRNA levels of Muc2 in intestinal organoids (n = 6). **(N-O)** The mRNA levels of Reg3b (**N**) and Reg3g (**O**) (n = 6). The results are expressed as the median and quartile, and are representative of three independent experiments. *, # and & $p < 0.05$, **, ## and && $p < 0.01$, ***, ### and &&& $p < 0.001$ were determined by two-way ANOVA and Tukey's post hoc test. "*" indicates $p<0.05$ compared with wild-type mouse NC group; "#" indicates $p<0.05$ compared with wild-type mouse H/R group; "&" indicates $p<0.05$ compared with wild-type mouse H/R+Ficz group. H/R + Ficz group: 200 nM Ficz was added to ILC3s and the intestinal organoid coculture system 1 hour before H/R.

Supplemental Table S1. The characteristics of patients with cardiopulmonary bypass (CPB).
Related to Figures 2.

Parameter	**NAGI group (n=25)**	**AGI group (n=31)**	***P***
Age (years)	56.0 (45.0~62.0)	57.00 (49.5~63.5)	0.4839
Females (n)	15	19	>0.9999
BMI (Kg/m^2)	23.56 (16-33.78)	24.09 (18.75-26.78)	0.5859
Duration hospitalization (days)	19.0 (17.0-25.0)	19.0 (14.0-27.0)	0.565
Duration anesthesia (minutes)	356.0 (340.0-422.0)	305.3 (280.0-362.5)	0.3253
Duration surgery (minutes)	260.0 (225.0-297.0)	254.0 (237.0-324.5)	0.3176
Duration CPB (minutes)	132.0 (103.0-169.0)	112.0 (93.0-165.5)	0.5377
Bleeding volume (ml)	300 (300-400)	300 (300-400)	1
The time from the end of surgery to the first meal (hours)	24.10 (22.45-28.50)	30.71 (28.63-41.45)	<0.001
ICU care duration (hours)	48.56 (44.25-63.20)	85.95 (75.65-92.00)	<0.001
Preoperative MC content in feces (ng/kg)	241.11 (31.68-281.25)	25.32 (17.19-48.33)	<0.001

Supplemental Table S2. The quantitative RT-PCR primer sequence. Related to STAR Methods section

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
18S	CGATCCGAGGGCCTCACTA	AGTCCCTGCCCTTTGTACACA
ZO-1	AGAGACAAGATGTCCGCCAG	TGCAATTCCAAATCCAAACC
Occludin	CATTTATGATGAACAGCCCC	GGACTGTCAACTCTTTCCGC
Ki67	ATCATTGACCGCTCCTTTAGGT	GCTCGCCTTGATGGTTCCT
Lgr5	CCTACTCGAAGACTTACCCAGT	GCATTGGGGTGAATGATAGCA
Cyp1a1	CAATGAGTTTGGGGAGGTTACTG	CCCTTCTCAAATGTCCTGTAGTG
Reg3b	ACTCCCTGAAGAATATACCCTCC	CGCTATTGAGCACAGATACGAG
Reg3g	ATGCTTCCCCGTATAACCATCA	GGCCATATCTGCATCATACCAG
IL-22	GCTGCCTGCTTCTCATTGC	AAGGTGCGGTTGACGATGTA
Muc2	GGTGACACTGACGCTGGTTT	ACTGGTGAACACCGCGATAATA
16S	GTGSTGCAYGGYTGTCGTCA	ACGTCRTCCMCACCTTCCTC
Wnt3	TGGAACTGTACCACCATAGATGAC	ACACCAGCCGAGGCGATG
Notch1	GATGGCCTCAATGGGTACAAG	TCGTTGTTGTTGATGTCACAGT