Supplementary Figure Legends

Supplementary Figure 1. Effects of antibiotic treatment on the gut microbiome in HFD-fed B6J, 129T and 129J mice. Related to Figure 2. (A) Visualization of relative abundance of phyla for each individual mouse in each experimental group. Mice were housed at 4 per cage with two cages for each experimental group. (B) PCA of fecal sample 16S rRNA sequencing data of the three mouse strains with or without antibiotic treatment (n=8 per group).

Supplementary Figure 2. Effects of antibiotic treatment on body weight and food intake. Related to Figure 3. (A-C) Body weight during the antibiotic intervention. (A) = B6J, (B) = 129T and (C) = 129J. (D-F) Food intake of mice was measured by housing in CLAMS apparatus. (D) = B6J, (E) = 129T and (F) = 129J. Data were normalized to lean body mass as determined by DEXA. Blue: placebo, Pink: vancomycin, Green: metronidazole treatment. Data are plotted as means ± SEM. *$p<0.05$, **$p<0.01$ (n=8 per group in all panels)

Supplementary Figure 3. Insulin signaling in adipose tissue and muscle of B6J mice with or without antibiotics and effect of antibiotic treatment on glucose metabolism of 129T or 129J mice. Related to Figure 3. (A-B) Western blot for insulin signaling in epididymal

adipose tissue (A) and muscle (B) of B6J mice. Quantitation of pAKT is shown in Figures 3G and 3H. Blood glucose levels (fasted for 4 hours) of 129T (C) and 129J (E) mice at 15 weeks of age. Insulin tolerance test of 129T (D) and 129J (F) mice (14 week old; 7 weeks on diet; 8 weeks on antibiotics). Serum insulin levels of 3 mice strains at fasted (A) or fed (B) conditions (n=8 per group) (15 week old; 8 weeks on diet; 9 weeks on antibiotics). Blue: placebo, Pink: vancomycin, Green: metronidazole group. Means ± SEM are plotted. #$p<0.05$ vs. placebo group. (n=8 per group in panels C-H)

Supplementary Figure 4. Effects of gut microbiota transfer from antibiotic-treated mice on body weight and glucose tolerance and proportion of DC cells and CD4 positive lymphocytes in mesenteric lymph node of 3 mice strains on chow diet, HFD or HFD + antibiotic treatment. Related to Figure 4 and 5. (A) Study design for bacterial transfer from HFD-fed B6J mice with or without antibiotics to B6J mice. The recipient mice were treated with a mixture of antibiotics (ampicillin (1 g/L), vancomycin (0.5 g/L), neomycin (1 g/L) and metronidazole (1 g/L)) in drinking water for 3 days prior to bacterial transfer. (B) Body weight of the recipient mice pre- and post-bacterial transfer. (C-E) Intraperitoneal glucose tolerance test (IPGTT) of the HFD-fed B6J recipient mice performed pre (open circle) and post (solid circle) bacterial transfer (at day 9) from mice treated with placebo (C), vancomycin (D) or metronidazole (E) (n=6 per group). Means ± SEM are plotted. *$p<0.05$.

(F) Study design for bacterial transfer from HFD-fed B6J mice with or without antibiotics to HFD-fed GF- B6J mice. (G) Percentage of CD11b+ F4/80+ CD11c + cells and (H) CD4+ cells in mesenteric lymph node. Mice were 11 weeks old; 4 weeks on diet; and 5 weeks of antibiotics. Analysis was performed by BD LSRII (BD Biosciences). (n=5 per group). Means ± SEM are plotted.

Supplementary Figure 5. Effect of antibiotic treatment on adipose tissue and colon inflammation in HF-fed B6J mice. Related to Figure 5. (A-B) qPCR analysis for inflammatory markers of mesenteric adipose tissue (A) and epididymal adipose tissue (B) of HFD–fed B6J mice. (C) Quantification of crown-like structures in adipose tissue of B6J mice treated with placebo, vancomycin or metronidazole. (D) qPCR analysis for inflammatory markers in the colon of B6J mice (n=8 per group; 16 weeks old; 9 weeks on diet; 10 weeks of antibiotics for all panels). Data are shown as means ± SEM. *$p<0.05$, **$p<0.01$

Supplementary Figure 6. Effect of antibiotic treatment on inflammatory markers in adipose tissue, liver and colon in HFD-fed 129T mice. Related to Figure 5. qPCR analysis for inflammatory markers in (A) epididymal adipose tissue, (B) mesenteric adipose tissue, (C) liver and (D) colon of HFD-fed 129T mice (n=8 per group; 16 weeks old; 9 weeks on diet; 10 weeks of antibiotics). Data are shown as means ± SEM. *$p<0.05$, **$p<0.01$

Supplementary Figure 7. Effect of antibiotic treatment on HF-fed B6J mice on inflammatory markers in adipose tissue, liver and colon in HFD-fed 129J mice. Related to Figure 5. qPCR analysis for inflammatory markers in (A) epididymal adipose tissue, (B) mesenteric adipose tissue, (C) liver and (D) colon of HFD-fed 129J mice (n=8 per group; 16 weeks old; 9 weeks on diet; 10 weeks of antibiotics). Data are shown as means ± SEM. *p<0.05, **p<0.01

Supplementary Figure 8. Gene expression profile of the liver, epididymal adipose tissue of B6J mice treated with or without antibiotics for 5 weeks and evaluation of the gut permeability and TG content in the liver of B6J mice. Related to Figure 5. (A-C) QPCR analysis for inflammatory markers (A) and ER stress markers (B) in the liver and inflammatory markers in epididymal adipose tissue (C) of B6J mice treated with placebo, vancomycin or metronidazole. (n=4 per group; 11 weeks old; 4 weeks on diet; 5 weeks of antibiotics). (D) Plasma endotoxin level of HFD-fed B6J mice treated with or without antibiotic treatment for 9 weeks. (E) qPCR analysis for lipopolysaccharide binding protein in the liver of HFD-fed B6J mice treated with or without antibiotics, (F) qPCR analysis for OCC1 and ZO-1 in the colon of HFD-fed B6J mice treated with or without antibiotics. (G) TG content in the liver of HFD-fed B6J mice treated with or without antibiotics for 10 weeks.

(n=8 per group; 16 weeks old; 9 weeks on diet; 10 weeks of antibiotics bot panels D-G). Data are shown as means ± SEM. *p<0.05, **p<0.01

Supplementary Figure 9. Relative abundance of total bile acids in plasma of individual mouse. Related to Figure 6. Mice were subjected to placebo, vancomycin or metronidazole at 6 weeks old. HFD was started at 7 weeks old. At 11 weeks of age, plasma was collected and bile acid levels were analyzed by LC-MS (n=4 per group).

Supplementary Figure 10. Downregulation of TGR5 by HFD in the liver of 129 mice, the effects of RG-239 on body weight and blood glucose in B6J mice. Related to Figure 7. (A) qPCR analysis for BaiE gene in the feces collected from 129T and 129J mice treated for 8 weeks (n=8 per group). (B) Western blot for TGR5 in the liver of 129T and 129J mice on chow or HFD for 22 weeks (upper panel). Quantification of TGR5 normalized by actin (lower panel) (n=4 per sample). (C) Body weight of B6J mice treated with or without RG-239 (10 mg/kg/day) for 2 weeks (n=3 per group). (D) Blood glucose levels (fed) of B6J mice with or without RG-239 (10mg/kg/day) for 3 days (n=5 per group). Data are shown as means ± SEM. *p<0.05, **p<0.01

Supplemental Experimental Procedures.

Insulin and glucose tolerance test

Oral glucose tolerance test (2 g/kg weight) and intraperitoneal insulin tolerance test (1.0 unit/kg) were performed after a 4-hour and 2-hour fasting period, respectively. Blood was collected from the tail at specific time intervals and glucose was measured using a glucometer (Infinity, US Diagnostics Inc.).

Plasma analysis and liver TG content

Fasting plasma was obtained from a tail vein after a 4-hour fast. Samples for random fed conditions were obtained between 9:00 and 11:00 am. Insulin, TNFα levels were measured by the Specialized Assay Core at the Joslin Diabetes Center. Plasma for endotoxin measurement was obtained by cardiac puncture and measured by Limulus Amebocyte Lysate (Lonza, Basel, Switzerland). Liver TG content was measured by Triglycerides Gpo Trinder (CATACHEM, Oxford, CT) according to the manufacturer's protocol.

Body Composition and Metabolic analysis

Body composition was measured with a Lunar PIXImus2 densitometer (GEMedical Systems). Food intake was measured on the same mice with an open-circuit Oxymax system

(Columbus Instruments).

Quantitative Real-time PCR

Total RNA was isolated with an RNeasy mini kit (QIAGEN), and complementary DNA (cDNA) was synthesized with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's protocol. The PCR amplification reaction was performed using gene-specific primers and IQ SYBR Green Supermix (Bio-Rad). Fluorescence was monitored and analyzed in a CFX384 Real-Time PCR Detection System (Bio-Rad) with an initial denaturation at 95℃ for 3 min, followed by 40 cycles of 10 s at 95℃ and 45 s at 60℃. Each sample was run in duplicate or triplicate and the relative mRNA levels are normalized to Tata-binding protein (TBP) mRNA. The data were analyzed according to the $2^{\Delta\Delta CT}$ method. Primer sequences are listed in Supplementary Table.

Western Blotting

To assess insulin signaling *in vivo*, 5 U insulin (Sigma-Aldrich) was injected via the inferior vena cava. Five minutes later, samples of liver, skeletal muscle and adipose tissue were dissected and immediately frozen in liquid nitrogen. Proteins are extracted with 1x RIPA buffer containing 0.1% SDS. 20 μg of protein was subjected to SDS-PAGE and transferred to polyvinylidene fluoride membranes. Primary antibodies for pIR/IGF-1R

(#3024), pAKT (Ser437) (#9271), total AKT (#9272), pERK (#9101), ERK (#9102), GAPDH (#5174) were from Cell Signaling Technology (Danvers, MA) (1:1000) and for TGR5 (1:1000) was from Santa Cruz Biotechnology (Santa Cruz, CA) (#sc98888). The appropriate secondary antibodies conjugated to horseradish peroxidase (HRP) were from Santa Cruz Biotechnology (1:10000). Membranes were visualized with SuperSignal West Pico substrate (Pierce Biotechnologies, Rockford, IL) or Immobilon Western HRP Substrate (Millipore, Billerica, MA). Quantification was performed by using ImageJ software.

Fecal Energy Content

Fecal samples were collected from individual mouse for 3 days and dried at 60℃ for 3 days. Energy content was measured with Bomb Calorimeter at Central Analytical Laboratory in University of Arkansas (Fayetteville, AR).

FITC-Dextran experiment

Mice were fasted for 2 hours. FITC-Dextran (Sigma-Aldrich) was administered to the mice by oral gavage (100 μl/ 10 g body weight). Four hours after gavage, the mice were anesthetized with isoflurane, blood samples were collected by cardiac puncture at the time of sacrifice (400~500 μl). Following centrifugation (13000 rpm, 10 min), the serum was diluted 1:1 (vol/ vol) in PBS. Fluorescence intensity of each sample was measured (excitation: 485

nm, emission: 528 nm) with fluorescence spectrometer. and FITC- dextran concentrations were determined from a standard curve.

Isolation of Peritoneal Macrophages

Peritoneal macrophages of mice were harvested by washing the peritoneal cavity with 6 ml of RPMI supplemented with 10% FBS. Cells were incubated at 37°C to adhere for 2 hours. Then, non-adherent cells were removed by washing with PBS and the medium was replaced. Macrophage cultures were maintained in RPMI with 10% FBS in a humidified atmosphere with 5% CO_2 at 37°C. After 16 hours of culture, cells were stimulated with 10 ng/ml LPS (Sigma-Aldrich) or vehicle for 6 hours for qPCR analysis.

Flow Cytometry Analysis

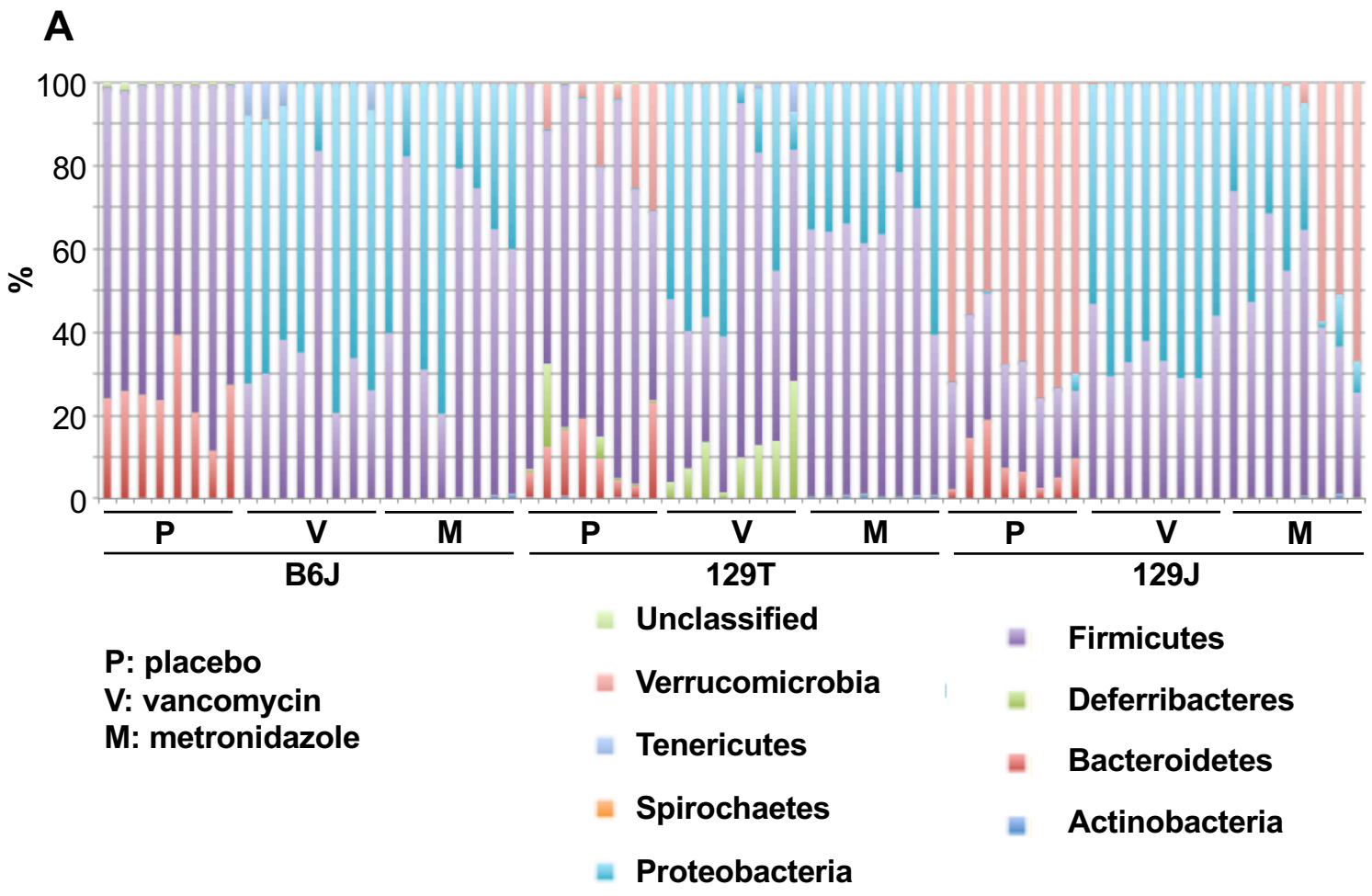
For isolation of lamina propria cells, the entire colon was washed in PBS, opened longitudinally, Peyer's patches excised, and remaining tissue cut into 1 cm pieces. The colons were incubated in HBSS (pH 7.4) supplemented with 5 mM EDTA and 8 mM dithiothreitol (Sigma-Aldrich) using a shaker for 30 min at 37 °C. The tissue was filtered and transferred to RPMI supplemented with 10% FBS, collagenase type II (1 mg/ml) (Gibco), DNase I (1 mg/ml) (Sigma-Aldrich) and Dispase II (3 mg/ml) (Roche, Indianapolis, IN), and incubated while shaking for 30 min at 37°C. The digested pieces were filtered through a 100-μm cell

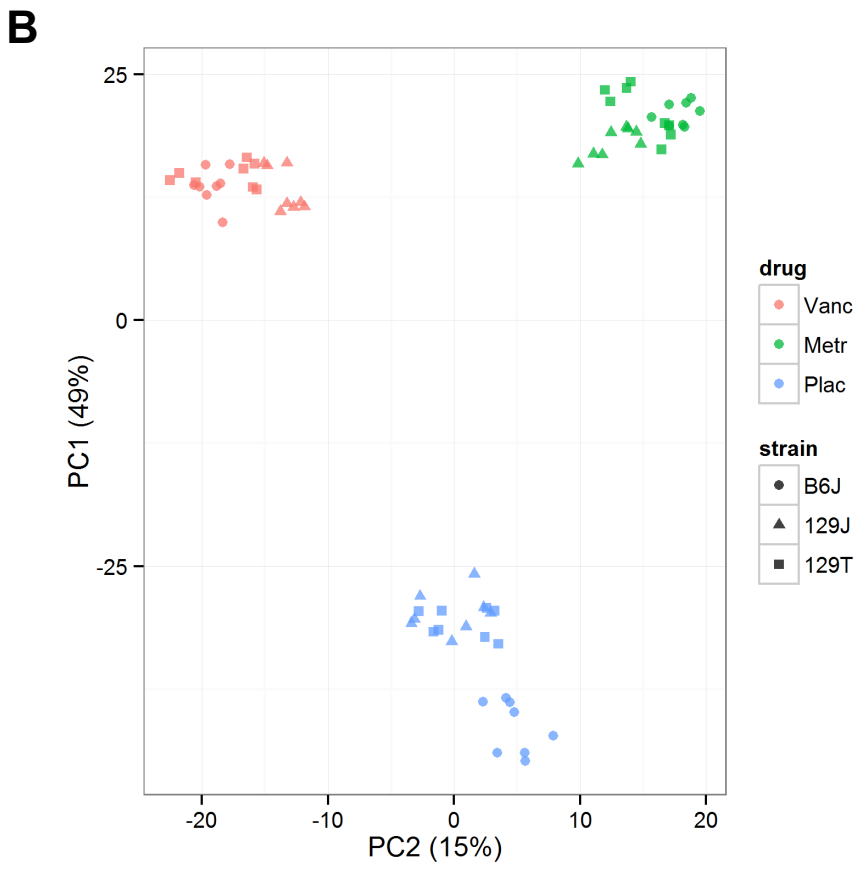
strainer and washed with RPMI with 10% FBS. Mesenteric lymph nodes were isolated, mechanically digested and filtered through a 40-μm cell strainer. Dead cells were stained with LIVE/DEAD stain (Thermo Fisher, Waltham, MA), according to the manufacturer's protocol. Cells were incubated with Fc block, stained with antibodies for CD45 (Pacific Blue) (#103125/ 30F11), MHC class II (PerCP-Cy5.5) (#107625/ M5/114.15.2), CD11b (Alexa Fluor 700) (#101222/ M1/70), CD11c (Brilliant Violet 711) (#117349/ N418), F4/80 (PeCy7) (#123113/ BM8), CD4 (Alexa Fluor 488) (#100425/ GK1.5), and dump (PerCpCy5.5). All antibodies were purchased from BioLegend (San Diego, CA). Mature macrophages, dendritic cells and CD4+lymphocytes were identified as $CD45^+$/ MHC class II^+/ $CD11c^+$/$CD11b^+$/ $F4/80^+$; or $CD45^+$/ MHC class II^+/ $CD11c^+$/ $CD11b^+$ $F4/80^-$; or $CD45^+$/ $CD4^+$ cells, respectively. Analysis was performed using a BD LSRII (BD Biosciences). Flow cytometry for Kupffer cell sorting was performed as previously described (Kohli et al., 2010). Cell staining was performed with 7AAD (BD Biosciences, Franklin Lakes, NJ) and antibodies for CD45 conjugated with PE (eBioscience, San Diego, CA) (#12-0451/30-F11), CD11b conjugated with Alexa Fluor 700 (#101222/ M1/70), F4/80 conjugated with APC/Cy7 (#13117/ BM8) and Gr-1 conjugated with FITC (#108405/RB6-8C5) (BioLegend). Activated Kupffer cells were identified as $7AAD^-$ / $CD45^+$/ $CD11b^+$/ $F4/80^+$/ $Gr\text{-}1^+$ cells. Cell sorting was performed with FACSAria (BD Biosciences).

Gnotobiotic transfer studies

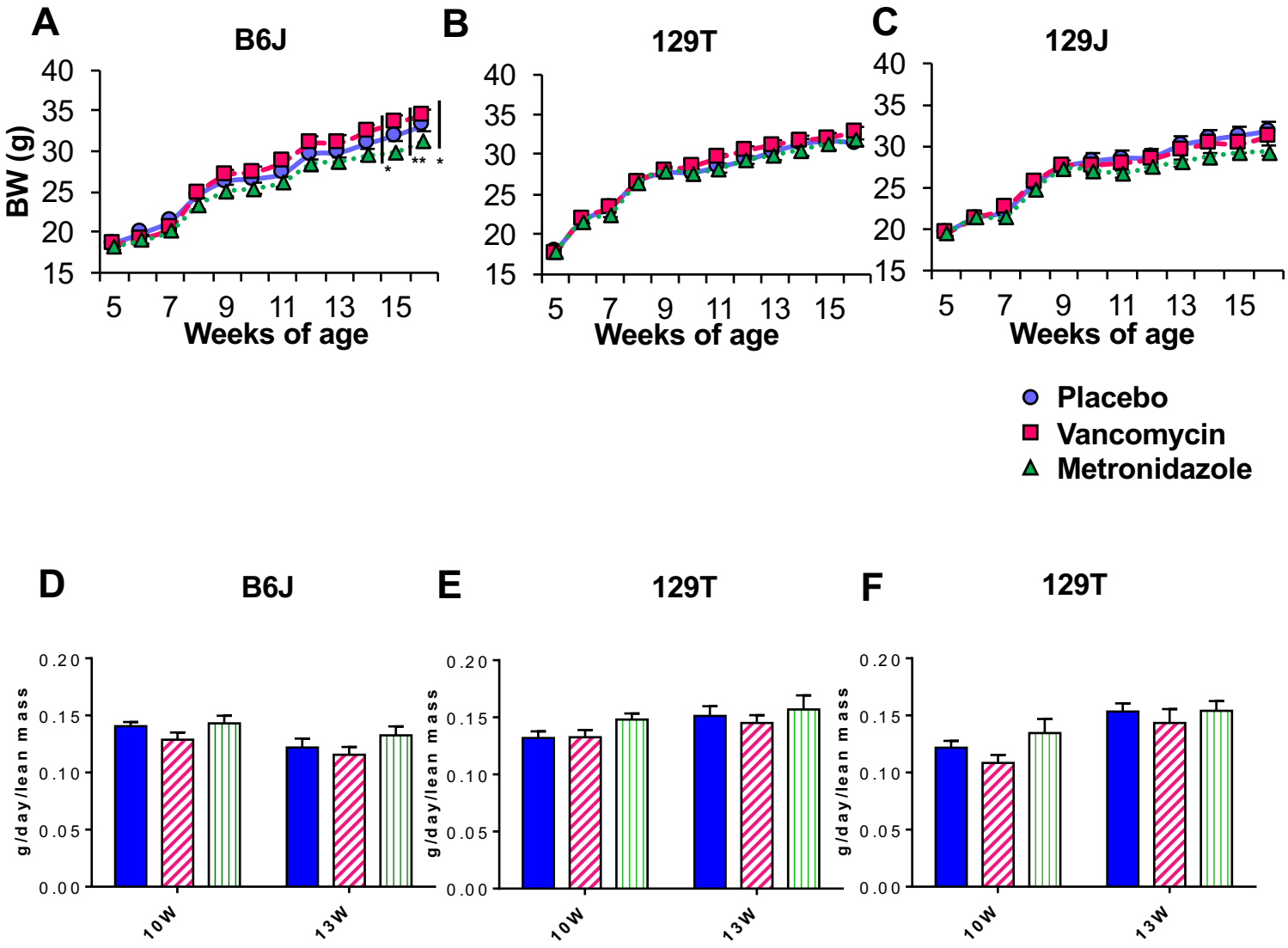
Five week-old male GF mice were given HFD. After one week, transfer of the cecum contents from 3 strains of donor mice was performed on the day of their arrival from vendors. The colonized mice were continuously fed the HFD for 8 more weeks. Female GF mice were maintained under HFD for 8 weeks prior to receiving a transfer from antibiotic-treated donor mice. The age-matched HFD fed donor mice were given antibiotics for one week prior to transfer.

Supplementary Figure 1 **Fujisaka et al**



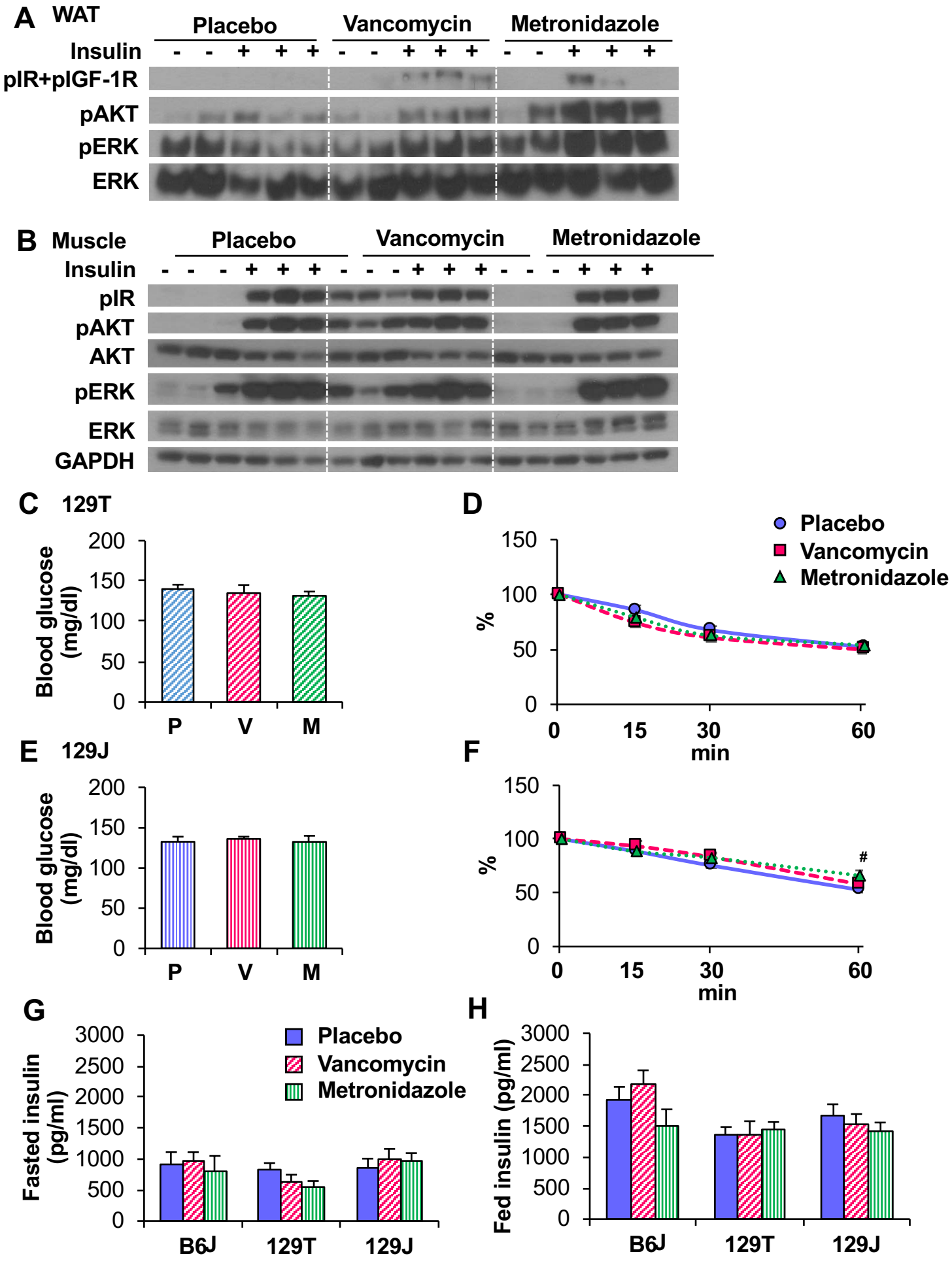


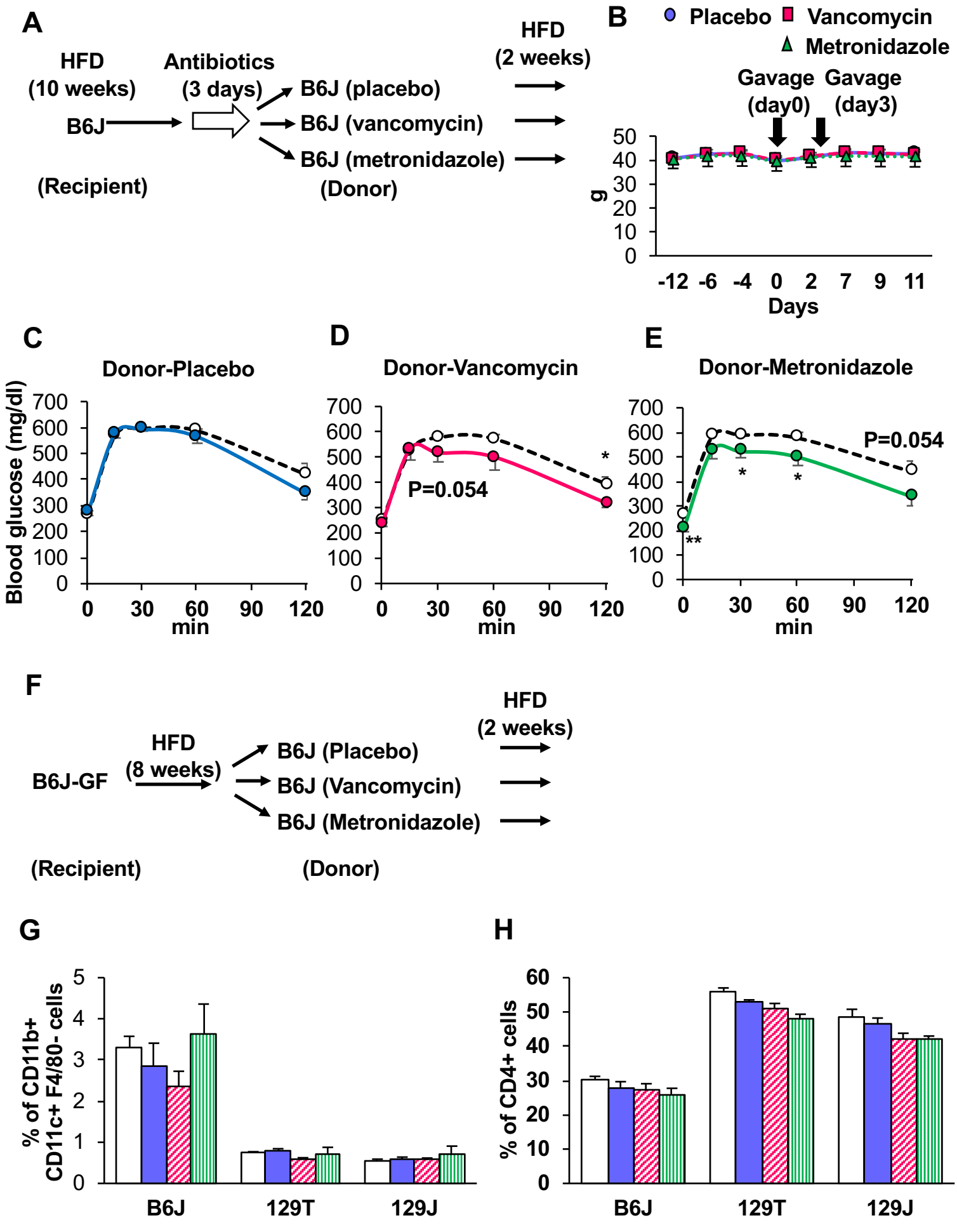
Supplementary Figure 2 **Fujisaka et al**



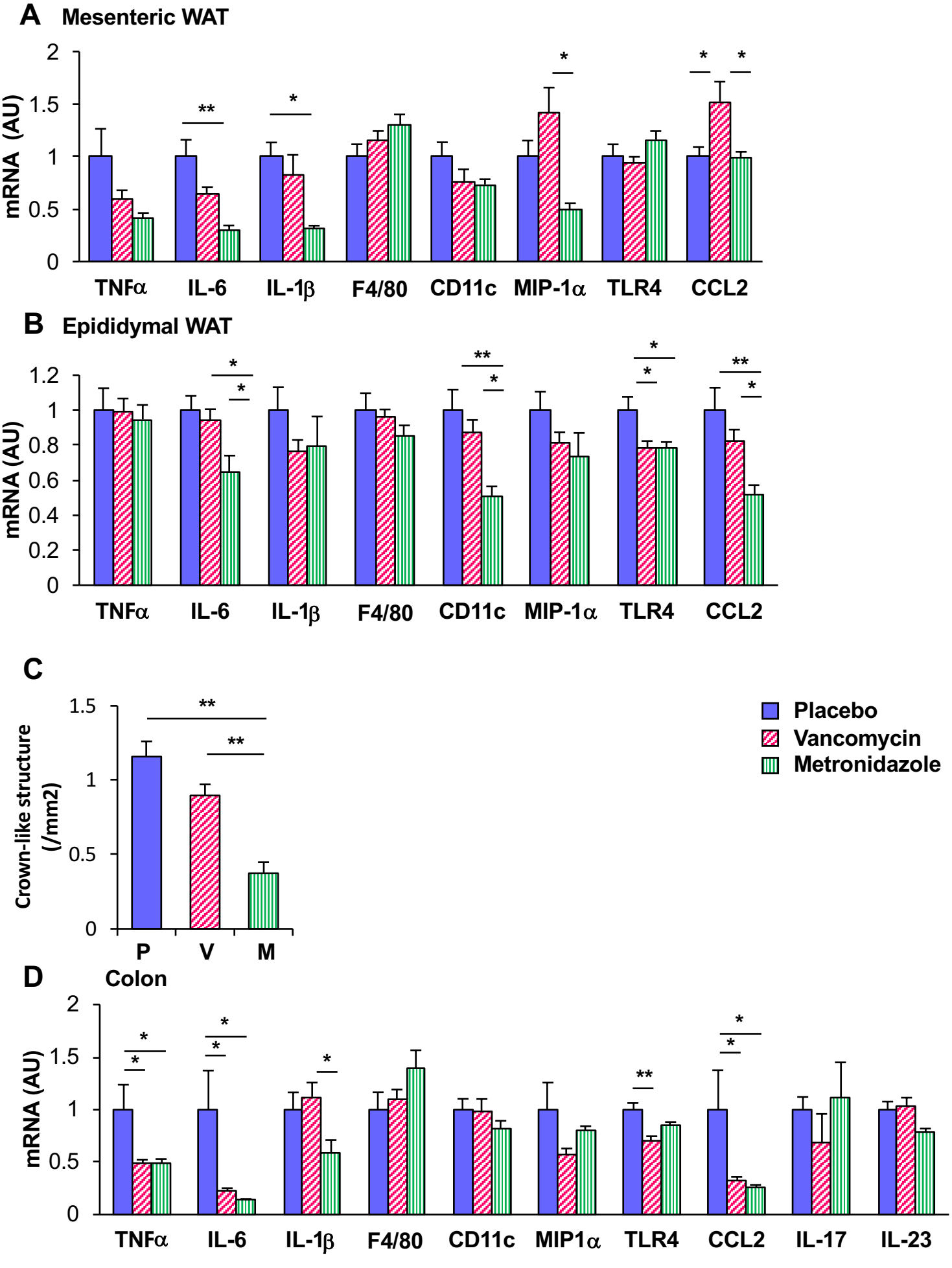
Supplementary Figure 3

Fujisaka et al



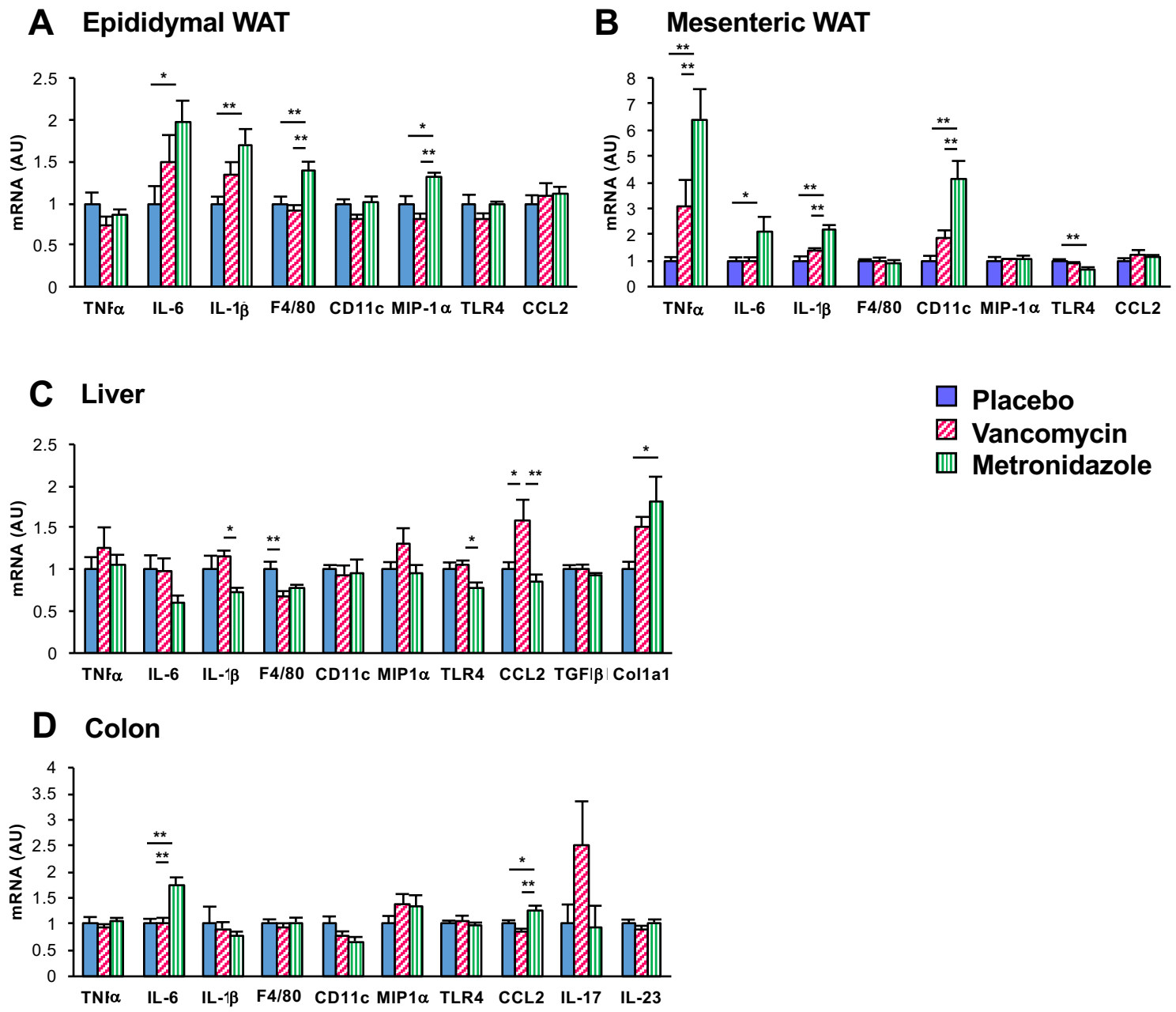

A
HFD
(10 weeks)
B6J
(Recipient)
Antibiotics
(3 days)
B6J (placebo)
B6J (vancomycin)
B6J (metronidazole)
(Donor)
HFD
(2 weeks)
B
Placebo
Vancomycin
Metronidazole
Gavage (day0)
Gavage (day3)
g
Days
C
Donor-Placebo
Blood glucose (mg/dl)
min
D
Donor-Vancomycin
P=0.054
E
Donor-Metronidazole
P=0.054
F
B6J-GF
HFD
(8 weeks)
B6J (Placebo)
B6J (Vancomycin)
B6J (Metronidazole)
HFD
(2 weeks)
(Recipient)
(Donor)
G
% of CD11b+ CD11c+ F4/80- cells
B6J
129T
129J
H
% of CD4+ cells
B6J
129T
129J

Supplementary Figure 5 **Fujisaka et al**

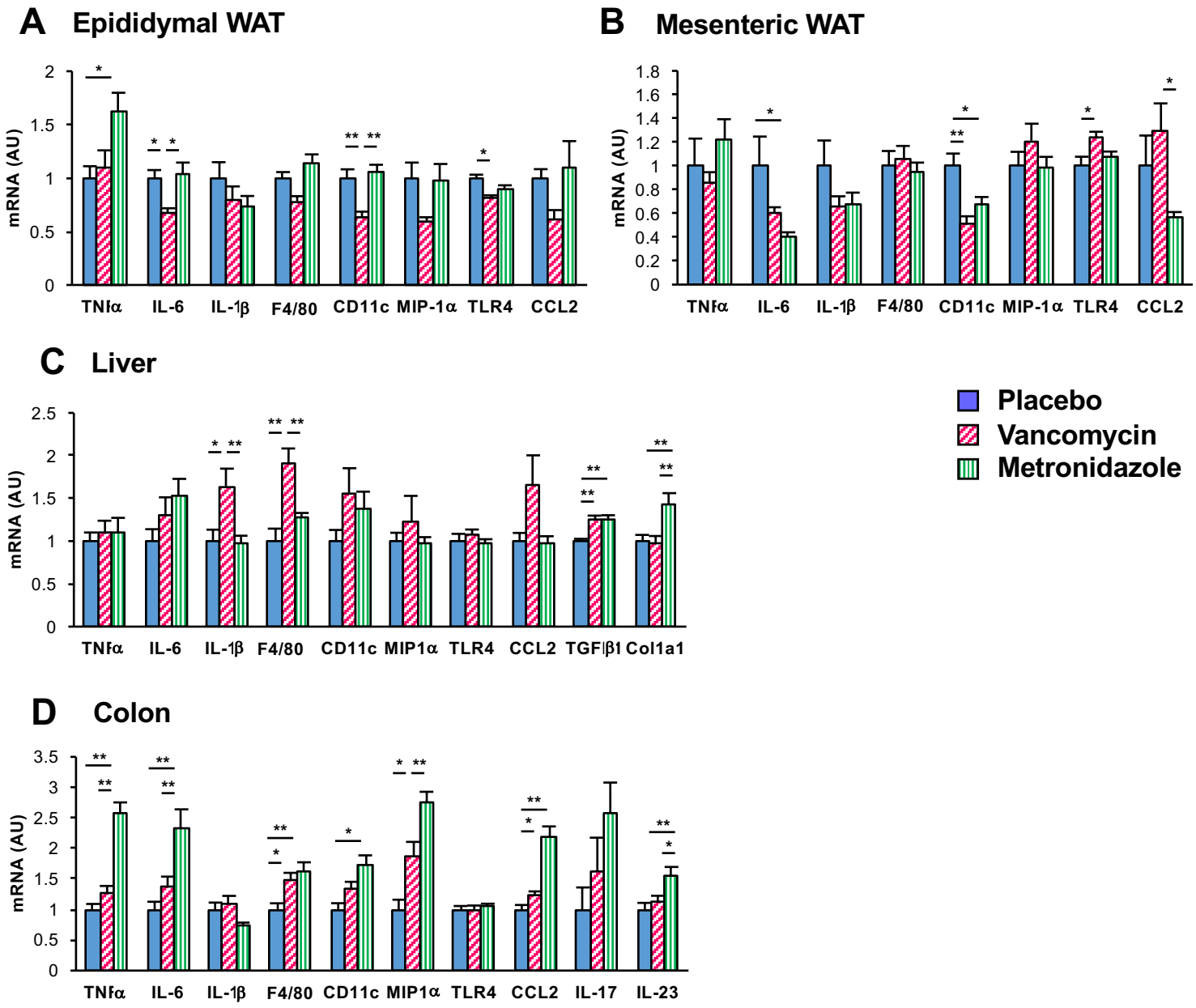


Supplementary Figure 6

Fujisaka et al



Supplementary Figure 7



Supplementary Figure 8

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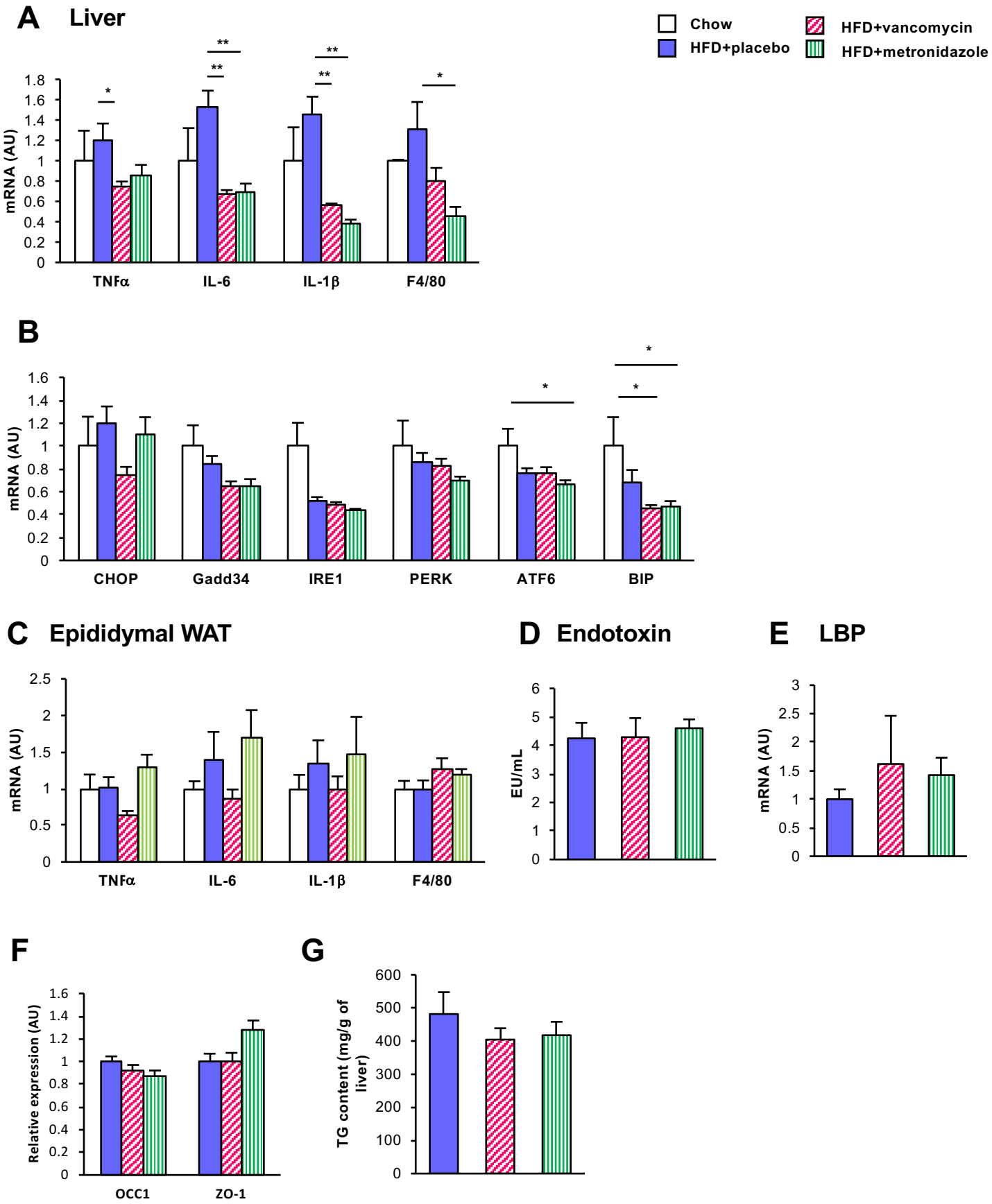

Supplementary Figure 9

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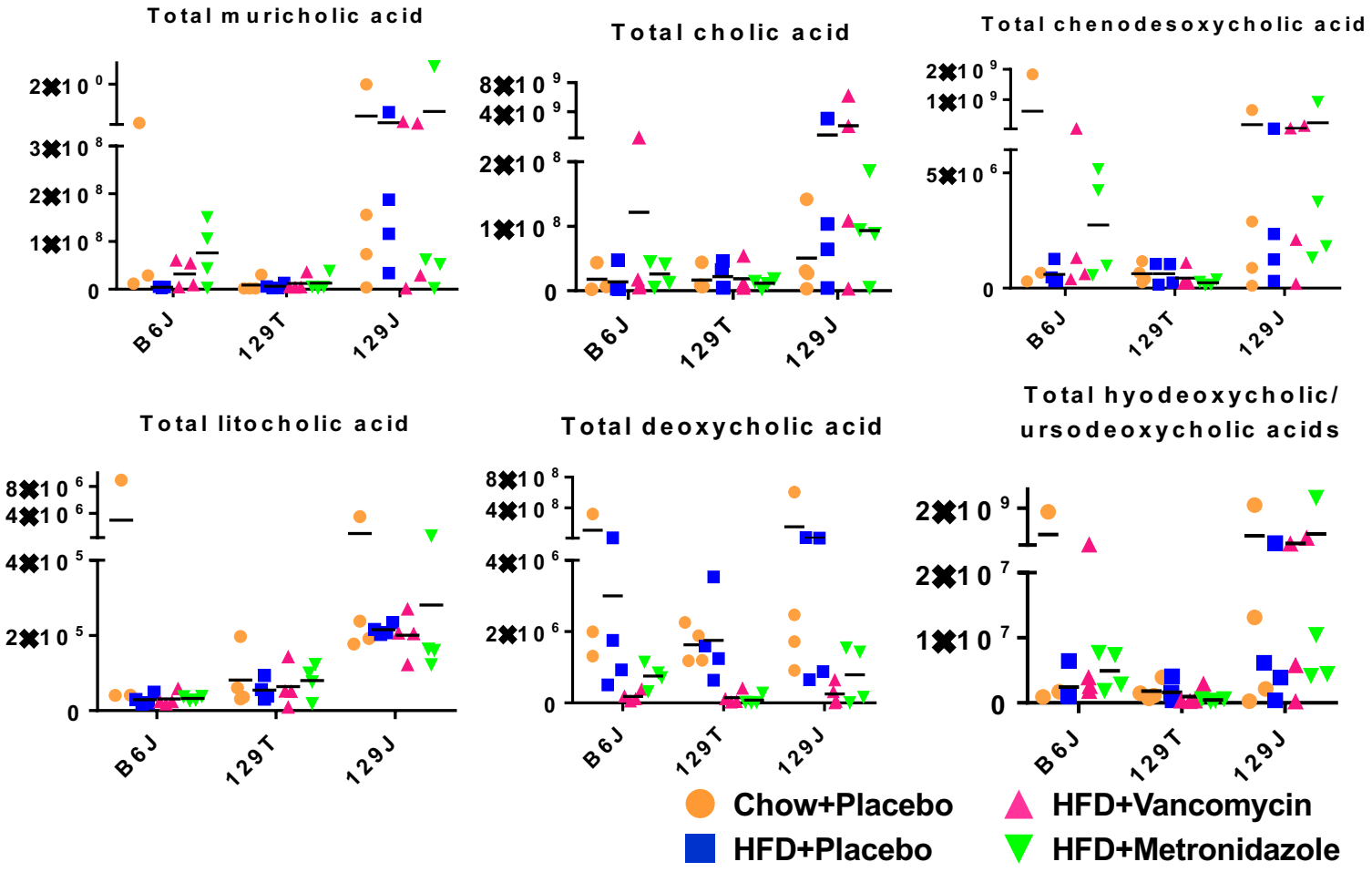
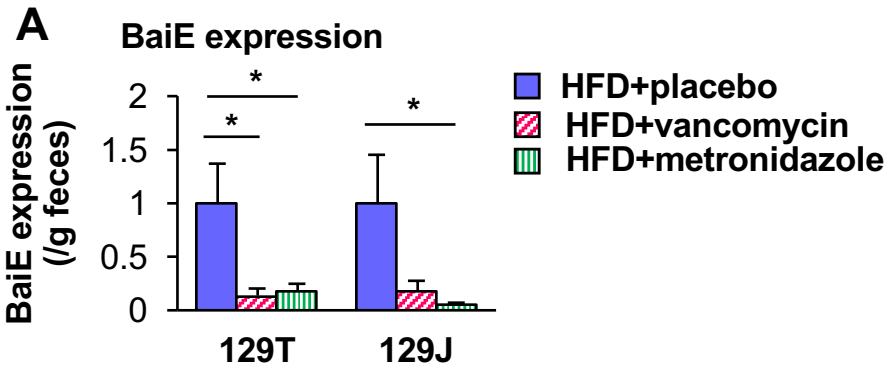
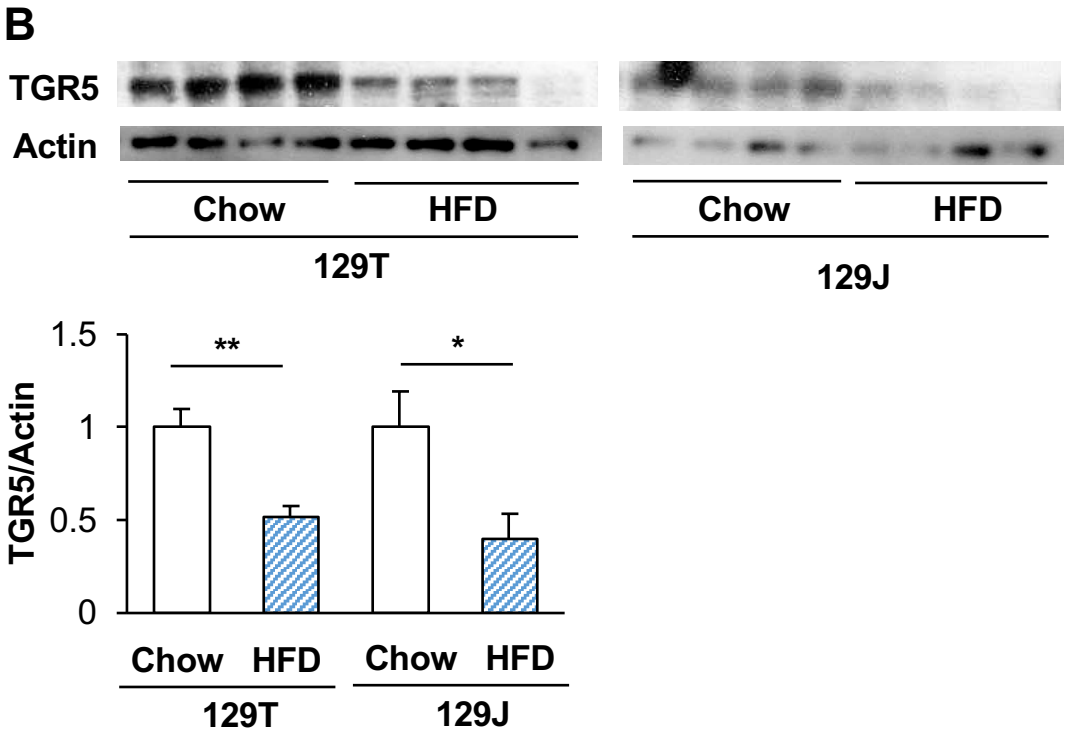
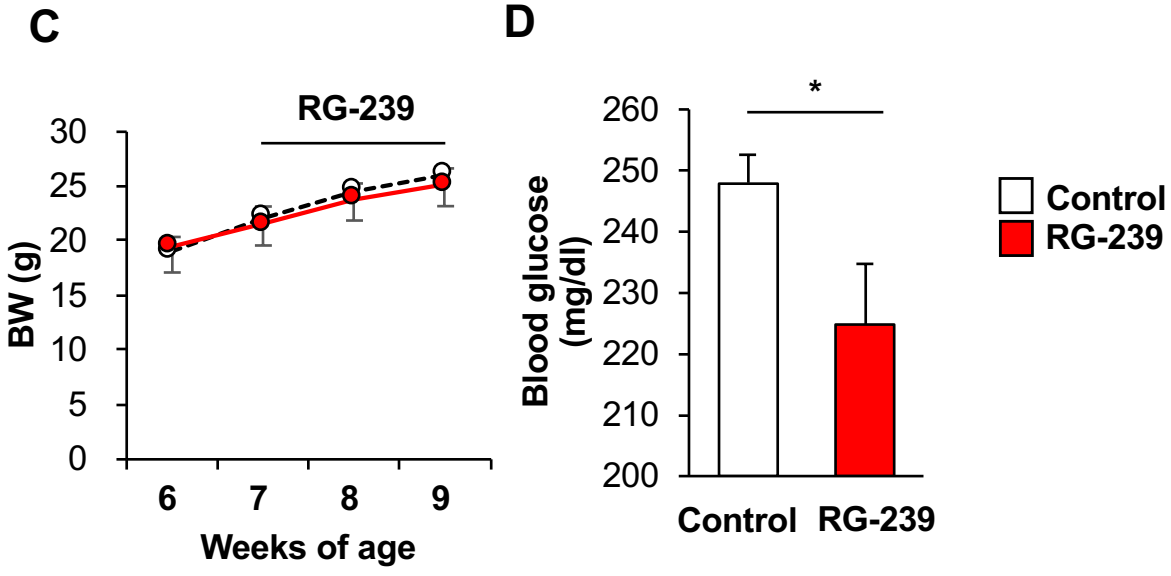



A
BaiE expression
BaiE expression (/g feces)
2
1.5
1
0.5
0
*
*
*
HFD+placebo
HFD+vancomycin
HFD+metronidazole
129T
129J


B
TGR5
Actin
Chow
HFD
Chow
HFD
129T
129J
TGR5/Actin
1.5
1
0.5
0
**
*
Chow
HFD
Chow
HFD
129T
129J


C
RG-239
BW (g)
30
25
20
15
10
5
0
6
7
8
9
Weeks of age
D
Blood glucose (mg/dl)
260
250
240
230
220
210
200
*
Control
RG-239
Control
RG-239

Supplementary Table

Gene	Sequence (Forward)	Sequence (Reverse)
Eubacteria	ACTCCTACGGGAGGCAGCAGT	ATTACCGCGGCTGCTGGC
TNFa	ACGGCATGGATCTCAAAGAC	AGATAGCAAATCGGCTGACG
IL-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
IL-1b	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
F4/80	CTGGGATCCTACAGCTGCTC	AGGAGCCTGGTACATTGGTG
CD11c	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTCA
MIP-1a	TTCTCTGTACCATGACACTCTGC	CGTGGAATCTTCCGGCTGTAG
TLR4	ACCAGGAAGCTTGAATCCCT	TCCAGCCACTGAAGTTCTGA
CCL2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
TGFb1	AAGTTGGCATGGTAGCCCTT	GCCCTGGATACCAACTATTGC
Col1a1	CCTCAGGGTATTGCTGGACAAC	TTGATCCAGAAGGACCTTGTTTG
CHOP	CTGCCTTTCACCTTGGAGAC	CGTTTCCTGGGGATGAGATA
Gadd34	GAGATTCCTCTAAAAGCTCGG	CAGGGACCTCGACGGCAGC
IRE1	CCCGTGGAGGTTCAGGAGA	CGGGAAGTGAAGTAGCGCAC
PERK	CGATCAAATGGAAGCCCTTA	TGCGGATGTTCTTGCTGTAG
ATF6	GAGTCGACGTTGTTTGCTGA	CAGGAACGTGCTGAGTTGAA
BIP	ATCTTTGGTTGCTTGTCGCT	ATGAAGGAGACTGCTGAGGC
BaiE	CAGGAGATGAAGGATATTGAGGC	ACGATATTTGGTGACAGGGTG
TGR5	GTCAGCTCCCTGTTCTTTGC	CAGGAGGCCATAAACTTCCA
IL-17A	GCCCTCAGACTACCTCAACC	ACACCCACCAGCATCTTCTC
IL-23	GGTGGCTCAGGGAAATGT	GACAGAGCAGGCAGGTACAG
OCC1	ACTATGCGGAAAGAGTTGACAG	GTCATCCACACTCAAGGTCAG
ZO-1	TTTTTGACAGGGGGAGTGG	TGCTGCAGAGGTCAAAGTTCAAG
LBP	GGCTCTGCAGAGAGAGCTGTACAA	TAGTTAAGGAATGCCTGGAACAGG
TBP	ACCCTTCACCAATGACTCCTATG	TGACTGCAGCAAATCGCTTGG