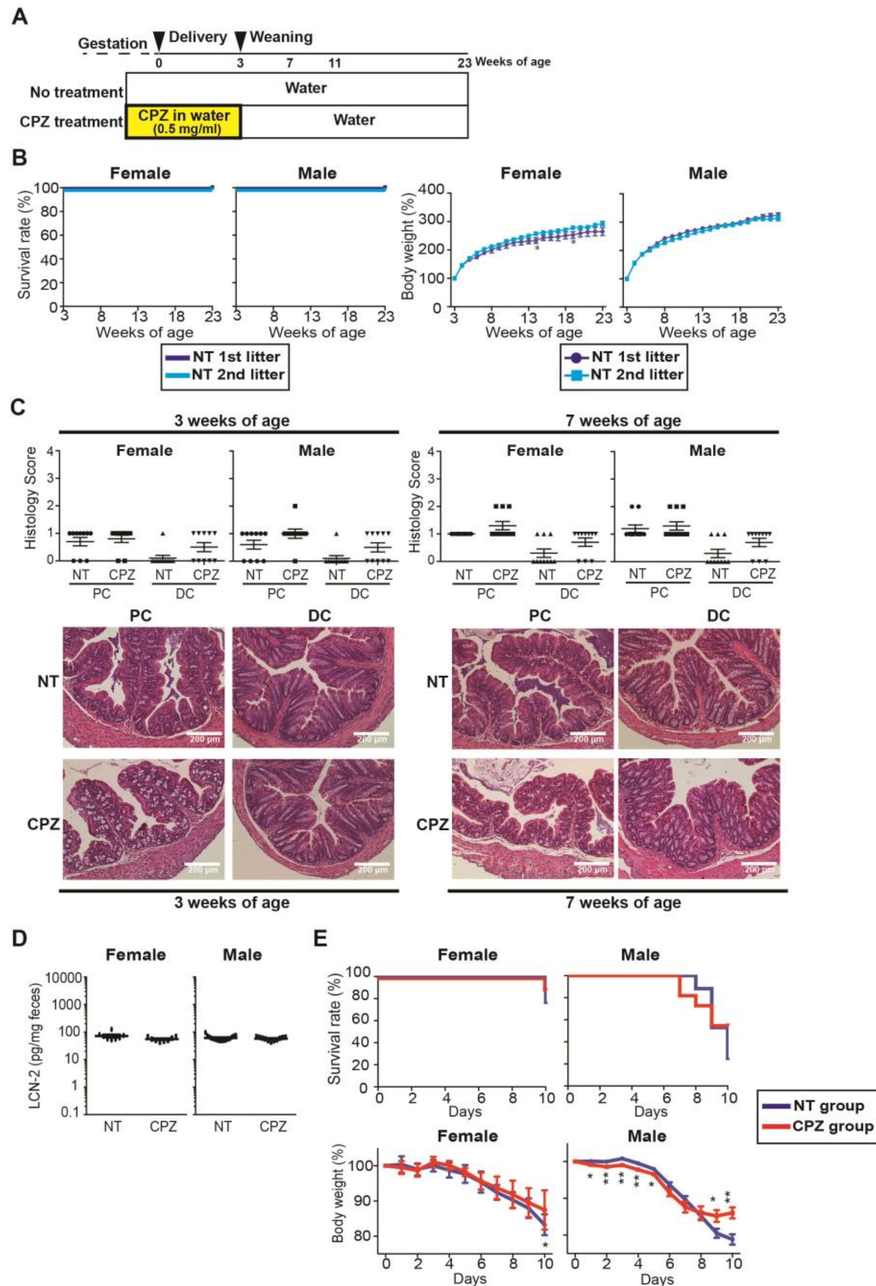

Figure S1. No significant differences were observed in colitis outcome between litters of cohort 2 NT IL-10 KO mice and between NT versus CPZ WT mice following DSS-challenge, Related to Figure 1.
(A) Cefoperazone (CPZ) treatment protocol. Dams in cohort 1 were treated with CPZ [(0.5 mg/ml) administration in drinking water] during the second pregnancy from day 14 of gestation until the end of the weaning period (an additional 21 days). (B) Survival curves and weight changes for 1st and 2nd litters (NT) in cohort 2 that were never exposed to antibiotics (n=7 females in 1st litter, n=14 in 2nd litter; n=15 males in 1st litter, n=7 in 2nd litter). (C) Histological assessment of proximal colon (PC) and distal colon (DC) at 3 and 7 weeks of age. No significant differences in histological scores in PC and DC were observed between NT and CPZ groups at 3 and 7 weeks of age in both genders. Representative pictures are presented for PC and DC at each age. (D) Fecal lipocalin-2 (LCN-2) levels were measured in all wild type (WT) subjects at 23 weeks of age (NT group n=8 females; 17 males. CPZ group n=8 females, 11 males). (E) Survival curves and weight changes for NT and CPZ WT mice that were treated with 2.5% dextran sulfate sodium (DSS) at 23 weeks of age. Mice showing more than 20% body weight loss were euthanized. *$p < 0.05$, **$p < 0.01$. Data are represented as mean ± SEM for (B)-(E).

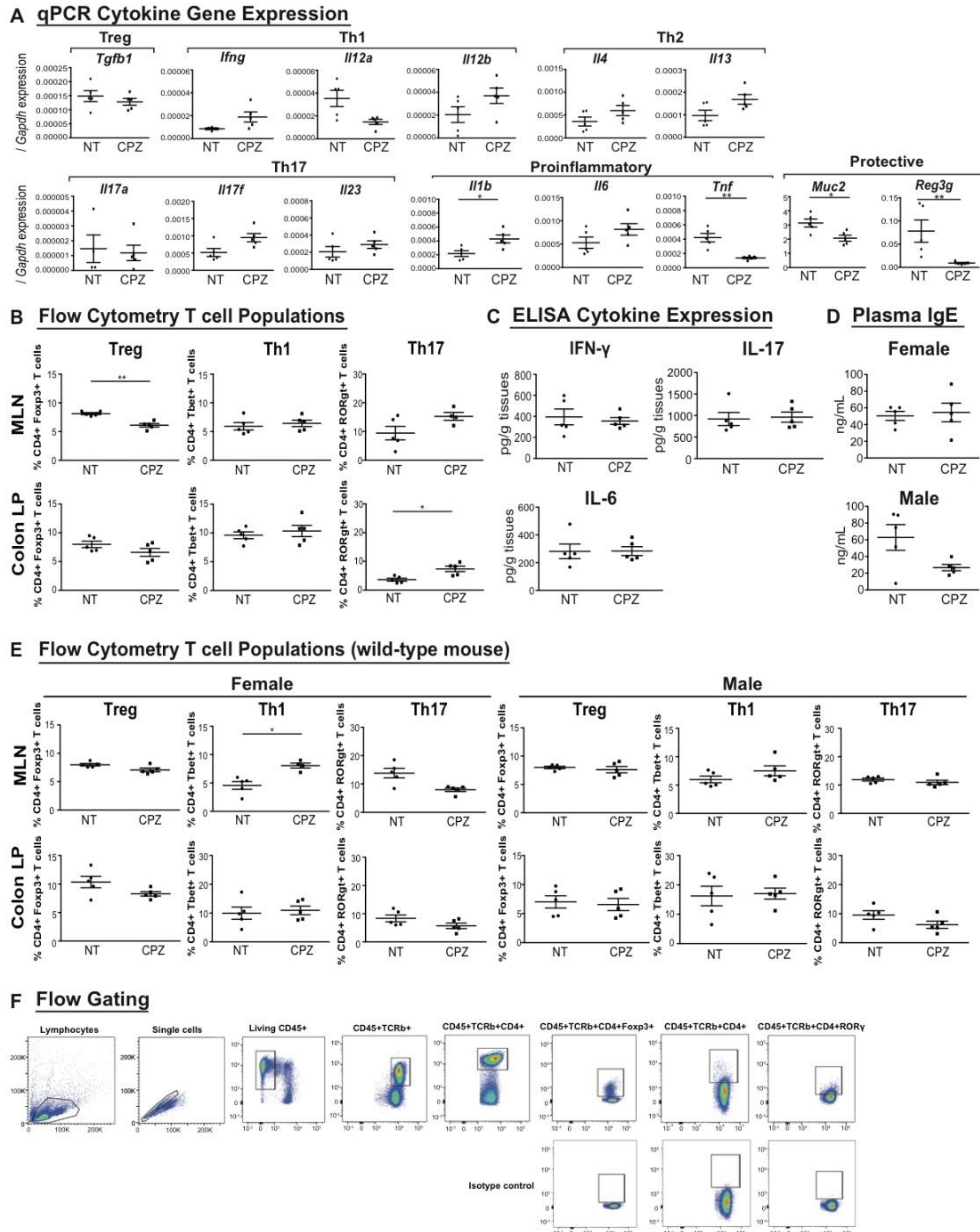

Figure S2. Female IL-10 KO offspring from CPZ-exposed dams exhibit aberrant immunity similar to male offspring, but WT offspring from WT CPZ-exposed dams do not regardless of gender, Related to Figure 2. (A) Real-Time qPCR mRNA levels of pro- vs. anti-inflammatory cytokine genes involved in colonic inflammation from colonic mucosal scrapings in female IL-10 KO mice of non-treated (NT; black circles) versus cefoperazone (CPZ; black squares) exposed dams at 3 weeks of age. mRNA levels are expressed as ΔΔCT relative to the housekeeper gene *Gapdh*. (B) Flow cytometric analyses of live $CD45^{+}TCR\beta^{+}CD4^{+}$ T cells expressing $Foxp3^{+}$ (Treg), $T\text{-}bet^{+}$ (Th1) or $ROR\gamma t^{+}$ (Th17) in MLNs and colonic LPs of NT versus CPZ females at 3 weeks of age. Data represents percentage of live $CD4^{+}$ cells. (C) Protein levels of inflammatory cytokines in MLNs of NT versus CPZ IL-10 KO females at 23 weeks of age determined via ELISA. (D) Flow cytometric analyses in MLNs and colonic LPs of NT versus CPZ WT female and male offspring at 3 weeks of age. (E) Representative images of flow cytometry gating strategy for analyzing MLN and colonic LP T cell populations with representative isotype controls. *n*=4-5/group. **p* < 0.05, ***p* < 0.01 via Mann-Whitney *U*-test. Data are represented as mean ± SEM.

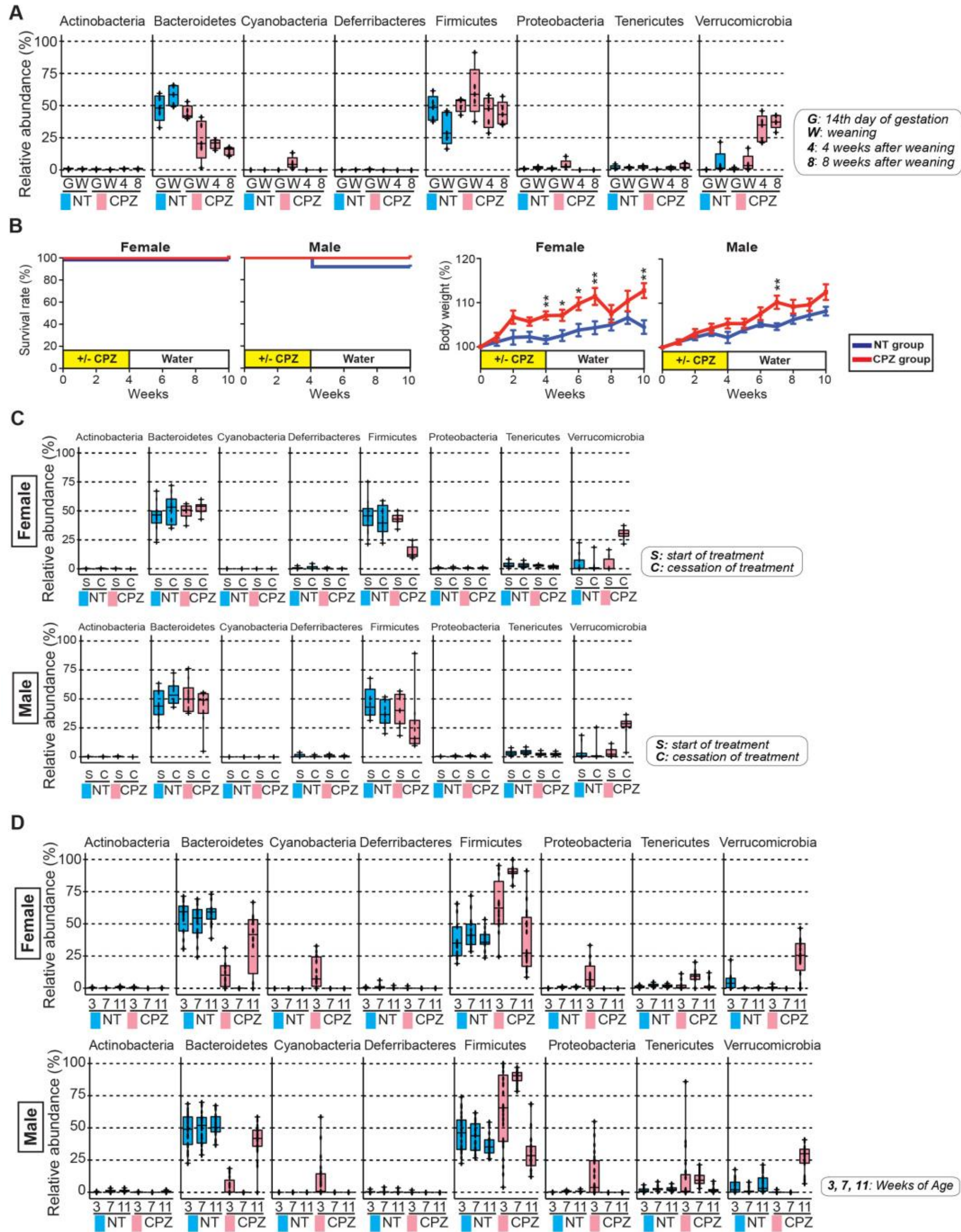

Figure S3. Maternal peripartum exposure to CPZ induces a persistent gut dysbiosis in dams and offspring, but CPZ-induced dysbiosis in adulthood does not lead to colitis outcome, Related to Figure 3.
(A) The gut microbiota of IL-10 KO dams before and after CPZ treatment. Relative abundance (%) of oligotype phyla taxa in non-treated (NT; light blue bars) versus CPZ-exposed (CPZ; pink bars) dams at the 14th day of gestation (G; before treatment), weaning (W; at the end of treatment), 4 weeks and 8 weeks after CPZ cessation (4 and 8; 4 and 8 weeks after weaning). Dots represent individual samples. (B) Survival curves and weight changes in NT and CPZ-treated adult IL-10 KO mice (NT n=15 females, 12 males. CPZ n=8 females, 10 males). (C) Adult NT versus CPZ IL-10 KO female (top panel) and male (bottom panel) microbiota at the start (S, week 0) and the end (C, week 4) of treatment. Relative abundance (%) of oligotype phyla taxa in NT versus CPZ adult mice. (D) IL-10 KO female pup (top panel) and male pup (bottom panel) microbiota over time. Relative abundance (%) of oligotype phyla taxa of NT versus CPZ pups 3, 7, and 11 weeks of age. Dots represent individual samples.

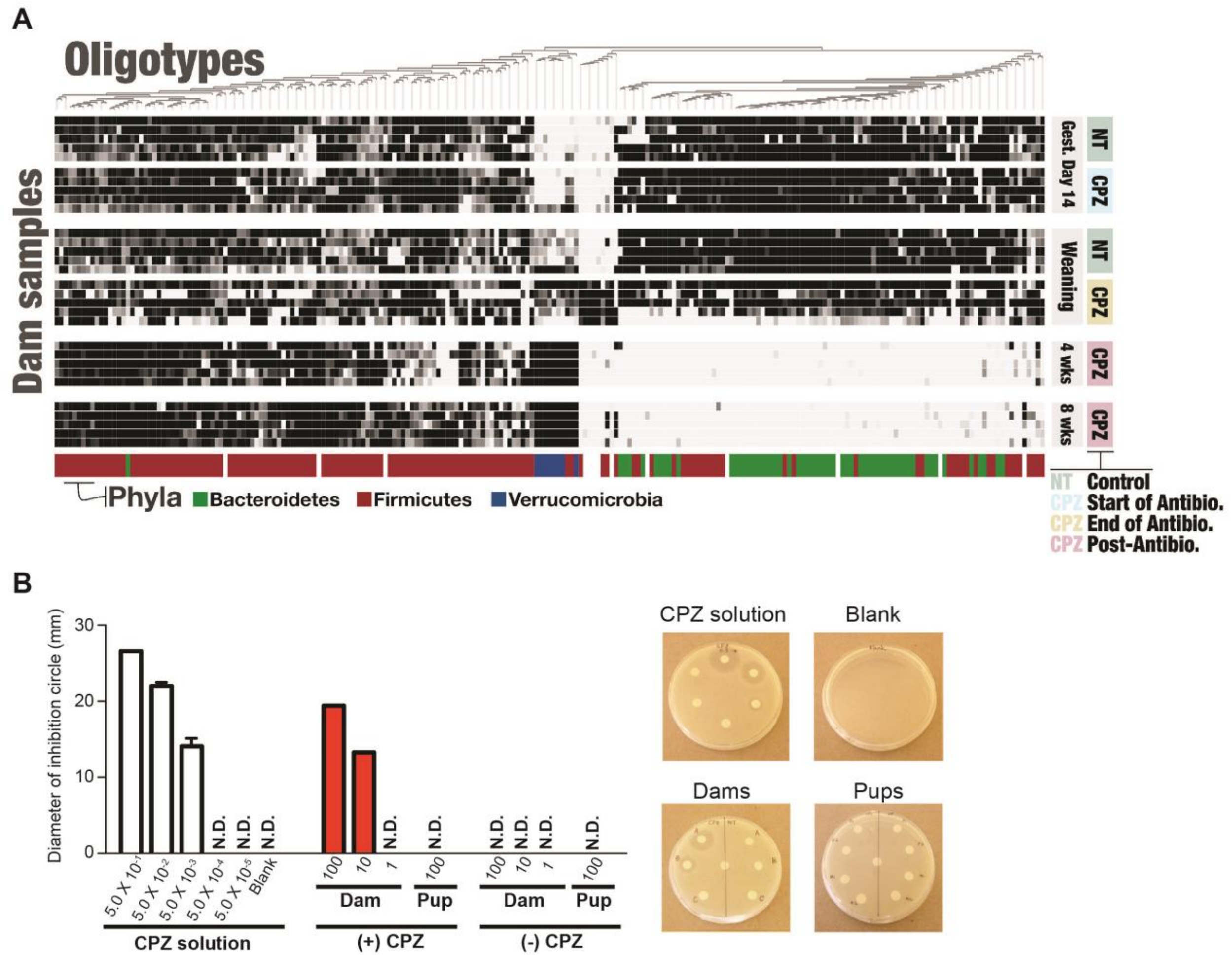

Figure S4. Dams exposed to peripartum CPZ exhibit persistent changes in specific microbial oligotypes with minimal to no CPZ transfer between dams and pups during the peripartum CPZ exposure period, Related to Figure 4.

(A) Comprehensive comparison of gut microbes in fecal samples of IL-10 KO dams collected over time. Oligotypes were determined in samples obtained from non-treated (NT) and cefoperazone (CPZ)-exposed dams at the 14th day of gestation (Gest. D. 14; just before the start of antibiotic exposure) and weaning (Weaning; end of antibiotic exposure), as well as 4 and 8 weeks (4, 8 wks; post-antibiotic exposure) after CPZ cessation in CPZ-exposed dams. Each column presents an individual oligotype and each row represents an individual sample. Dominant phyla are represented by the colored bars at the bottom. (B) Antibiotic sensitivity patterns of E. *coli* K12 to CPZ solution, cecal contents from NT and CPZ-exposed dams, and cecal contents from their respective pups at weaning [(-) CPZ: NT dams and their pups, (+) CPZ: CPZ dams and their pups]. For examining E. *coli* sensitivity to CPZ water solution, six paper discs were impregnated with 5.0×10^{-1} (concentration in dams' drinking water) 5.0×10^{-2}, 5.0×10^{-3}, 5.0×10^{-4}, 5.0×10^{-5} mg/ml of CPZ and water (blank), respectively. For examining E. *coli* sensitivity to dam intestinal contents, paper discs were impregnated with sterile-filtered cecal supernatant (100 mg/1 ml of water) obtained and diluted 10-fold sequentially (100, 10 and 1) from NT and CPZ dams. For examining E. *coli* sensitivity to pup intestinal contents, testing pup samples, paper discs were impregnated with sterile-filtered cecal supernatant (100mg/1 ml of water) from NT and CPZ pups. Representative pictures of plates are shown. Plates with blank LB broth were prepared to rule out the possibility of contamination (plate labeled Blank). Data are represented as mean ± SEM for CPZ solution plates.

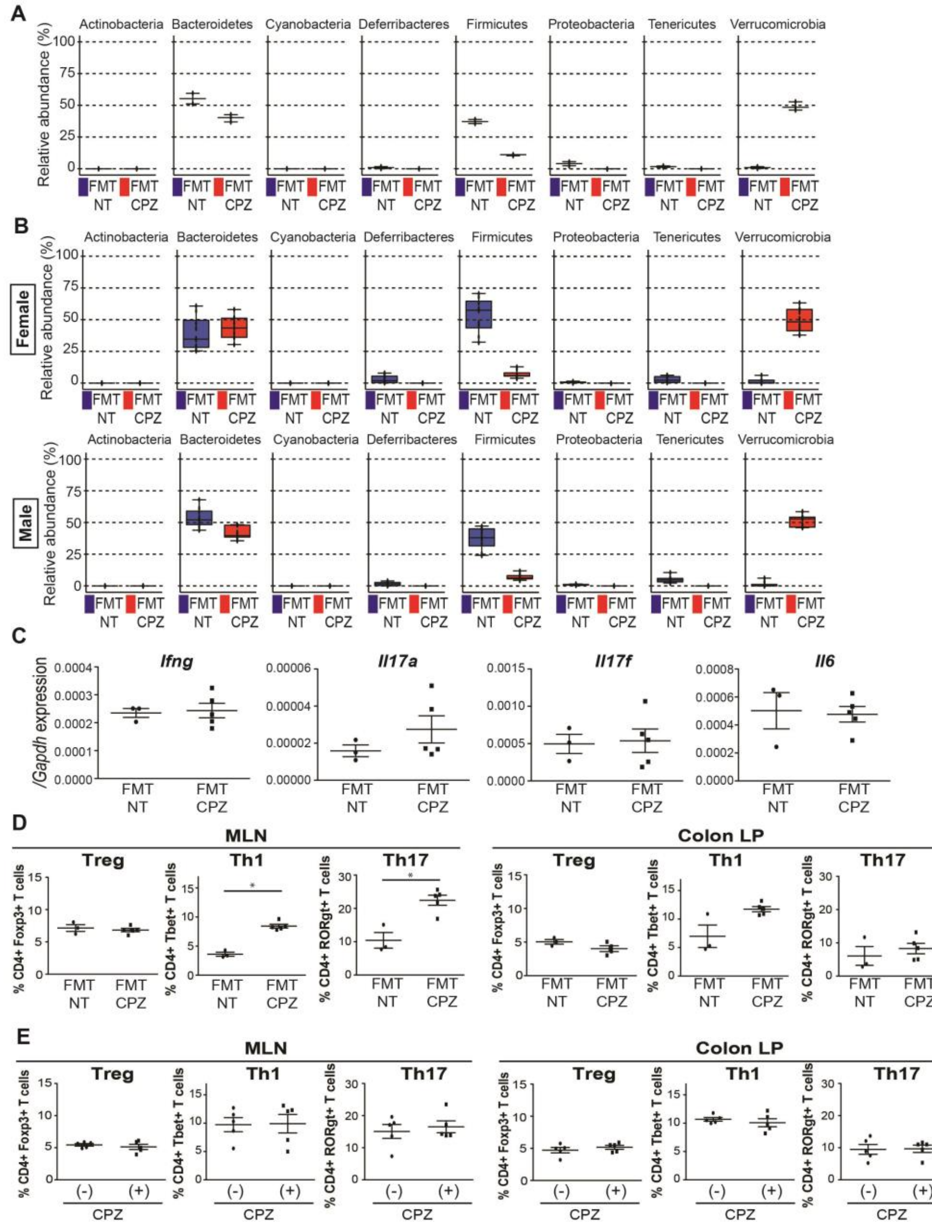

Figure S5. Fecal transplantation of CPZ-induced maternal dysbiosis recapitulates changes in the host immune system of offspring from FMT-dams, Related to Figure 5.

(A) Bacterial community composition of fecal-microbiota-transplanted (FMT) IL-10 KO dams (FMT-NT: FMT with non-treated fecal sample, FMT-CPZ: FMT with CPZ-exposed fecal sample). (B) IL-10 KO female pups (top panel) and male pups (bottom panel) microbiota at 3 weeks of age from FMT-NT (blue bars) versus FMT-CPZ (red bars) dams. Oligotype phyla taxa are presented as relative abundance and represented as box plots. Dots represent individual samples. (C) Real-Time qPCR mRNA levels of cytokines from colonic mucosal scrapings of FMT-NT (black circles) versus FMT-CPZ (black squares) female IL-10 KO mice at 3 weeks of age (*n*=3-5/group). mRNA levels are expressed as ΔΔCT relative to the housekeeper gene *Gapdh*. (D) Flow cytometric analyses of live $CD45^+TCR\beta^+CD4^+$ T cells expressing $Foxp3^+$ (Treg), $T\text{-}bet^+$ (Th1) or ROR γ t^+ (Th17) in MLNs and colonic LPs of FMT-NT versus FMT-CPZ female offspring. (E) Flow cytometric analyses of live $CD45^+TCR\beta^+CD4^+$ T cells expressing $Foxp3^+$ (Treg), $T\text{-}bet^+$ (Th1) or ROR γ t^+ (Th17) in MLNs and colonic LPs of germ-free male pups with/without maternal peripartum exposure to CPZ (*n*=5/group). Data represents percentage of live $CD4^+$ cells.

Supplemental Experimental Procedures

Antibiotic sensitivity assay

Kirby Bauer disk diffusion assays were performed with E. *coli* K12 lawns grown on LB agar plates with slight modifications(Bauer et al., 1966; Chait et al., 2010). To test whether the presence of CPZ in intestinal contents would inhibit *E. coli* growth, lawns were incubated overnight with circular discs impregnated with either: (1) CPZ solution starting at the treatment concentration given to pregnant dams (0.5 mg/ml; diluted 10-fold 4x); (2) cecal contents from NT or CPZ-exposed dams (sterile-filtered (0.22μM) supernatant of 100 mg of cecal content/1 ml of water; diluted 1, 10, 100-fold), and (3) cecal contents of offspring from NT or CPZ-exposed dams immediately prior to weaning (sterile-filtered (0.22μM) supernatant of 100 mg of cecal content/1 ml of water). The diameter of the zone of inhibition circle (mm) was measured as an indicator of the level sensitivity of *E. coli* to the presence of CPZ.

Flow cytometry

MLN and colon LP were harvested for analyzing T cell populations with/without CPZ treatment using previously described methods(Davies and Parrott, 1981; Little et al., 2005). FcR blocking was performed for all samples with anti-mouse CD16/CD32 antibody (BD Biosciences, CA, San Jose). Cells were stained using the LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific, MA, Waltham) to assess viability. For surface stains, anti-mouse CD45 (Biolegend, San Diego, CA), anti-mouse TCRβ (eBioscience, San Diego, CA), and anti-mouse CD4 antibodies (Biolegend) were used. For intranuclear stains, the Foxp3/Transcription Factor Staining Buffer Set (eBioscience) was used followed by staining with the anti-mouse Foxp3 (eBioscience), anti-mouse T-bet (eBioscience) and anti-RORγt antibodies (eBioscience). The Rat IgG2a Kappa Isotype, Mouse IgG1 Kappa Isotype, and Rat IgG1 Kappa Isotype controls (eBioscience) were used. Samples were analyzed with a FACSCanto (BD Biosciences) and FlowJo v10.1 (FLOWJO, OR, Ashland).

Real-time qPCR

Messenger RNA was extracted from total colonic mucosal scrapings with TRIzol Reagent (Thermo Fischer Scientific). Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics Corporation, IN, Indianapolis) was used to obtain cDNA samples. Real-time qPCR was performed using iTaq Universal SYBR Green Supemix (Bio-Rad, CA, Hercules) with LightCycler 480II (Roche Diagnostics Corporation). The primers were designed based on the Primer Bank reference base (https://pga.mgh.harvard.edu/primerbank/). Forward (F) and reverse (R) primer sequences are as follows: *Tgfb1*, *F*-CTCCCGTGGCTTCTAGTGC; *R*-GCCTTAGTTTGGACAGGATCTG; *Ifng*, *F*-ATGAACGCTACACACTGCATC; *R*-CCATCCTTTTGCCAGTTCCTC; *Il12p35*, *F*-CTGTGCCTTGGTAGCATCTATG; *R*-GCAGAGTCTCGCCATTATGATTC; *Il12p40*, *F*-TGGTTTGCCATCGTTTTGCTG; *R*-ACAGGTGAGGTTCACTGTTTCT; *Il4*, *F*-GGTCTCAACCCCCAGCTAGT; *R*-GCCGATGATCTCTCTCAAGTGAT; *Il13*, *F*-CCTGGCTCTTGCTTGCCTT; *R*-GGTCTTGTGTGATGTTGCTCA; *Il17a*, *F*-GGCCCTCAGACTACCTCAAC; *R*-TCTCGACCCTGAAAGTGAAGG; *Il17f*, *F*-TGCTACTGTTGATGTTGGGAC; *R*-AATGCCCTGGTTTTGGTTGAA; *Il23*, *F*-ATGCTGGATTGCAGAGCAGTA; *R*-ACGGGGCACATTATTTTTAGTCT; *Il1b*, *F*-GCAACTGTTCCTGAACTCAACT; *R*-ATCTTTTGGGGTCCGTCAACT; *Il6*, *F*-TAGTCCTTCCTACCCCAATTTCC; *R*-TTGGTCCTTAGCCACTCCTTC; *Tnf*, *F*-CCCTCACACTCAGATCATCTTCT; *R*-GCTACGACGTGGGCTACAG; *Muc2*, *F*-ATGCCCACCTCCTCAAAGAC; *R*-GTAGTTTCCGTTGGAACAGTGAA; *Reg3g*, *F*-ATGCTTCCCCGTATAACCATCA; *R*-GGCCATATCTGCATCATACCAG; *Gapdh*, *F*-AGGTCGGTGTGAACGGATTTG; *R*-TGTAGACCATGTAGTTGAGGTCA.

ELISA

Frozen samples were resuspended in 1 ml of PBS per 100mg of feces or MLN with cOmplete, Mini Protease Inhibitor Tablets (Sigma-Aldrich). Mouse Lipocalin-2/NGAL DuoSet ELISA (R&D Systems, MN, Minneapolis) was used for fecal samples. Mouse ELISA Ready-SET-Go! for IFN-γ, IL-17 and IL-6 (eBioscience) was used for MLN samples. To analyze plasma IgE level, we perfrormed ELISA using goat anti-mouse IgE antibody (capture antibody) and goat anti-mouse IgE antibody labeled with HRP (secondary antibody) (SouthernBiotech, Brimingham, AL).

Supplemental References

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