# Supporting Information

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### SI Materials and Methods

**RT-PCR.** RNA was isolated from whole mouse kidney or from microdissected glomeruli with attached juxtraglomerular apparatus (JGA) using a RNeasy kit (Qiagen) and then treated with DNase. Isolated RNA was subjected to reverse transcription (RT) (SuperScript III; Invitrogen) or, to generate control samples, was subjected to mock RT (MRT) where water was substituted for SuperScript III. PCR was then performed using HotStarTaq Plus Master Mix (Qiagen) according to standard cycling protocols. MRT and RT were always run simultaneously and, as expected, MRT reactions failed to produce bands. All PCR products were sequenced to confirm their identity. PCR primers used were: G protein-coupled receptor 41 (GPR41) (ACGGCGGTGAGCATCGAACG, TTCCACCCCCTCCTGCGGTC; 685 bp), G protein-coupled receptor 43 (GPR43) (CGTTGGGGCTCAGAGGCGAC, TGCTCGGGAAGATCCGGGGG; 386 bp) Olfr78 (also known as MOL2.3 and MOR18-2: ACTGCGTCACGCTGCTGTCC, GCC\ATGTAGGACAAGGGTGATAGGA; 614 bp), and β-actin (GCTCGTCGTCGACAACGGCTC, CAAACATGATCTGGGTCATCTTCTC; 353 bp).

Full-length expression constructs were prepared for Olfactory Receptor 78 (Olfr78) and it's human homolog, OR51E2. The mOR-EG (mouse olfactory receptor EG) full-length construct was a kind gift from Kazushige Touhara (University of Toyko, Tokyo) (1). To clone the full-length sequence of Olfr78 and OR51E2, the sequence encoding mOR-EG was excised from its parent vector (pME18S) and PCR products containing the full-length sequence of OR51E2 and Olfr78 were ligated into the corresponding sites in this vector. Olfactory receptors (ORs) were ligated in frame with an upstream start site, such that they incorporated sequences encoding N-terminal Flag and Rho tags. Full-length human OR51E2 was amplified by PCR from human DNA, taking advantage of the fact that ORs do not contain introns. Full-length Olfr78 was amplified from kidney RNA after performing RT. For both Olfr78 and OR51E2, the PCR product was ligated into the pME18S vector between the EcoRI and XhoI restriction sites.

**In Vivo Studies.** For measurement of blood pressure (BP) during i.v. administration of propionate, mice were anesthetized with ketamine (50 mg/kg) and inactin (130 mg/kg) and then a venous cannula (for drug delivery) and an arterial cannula [for BP measurement, using an analog single-channel transducer signal conditioner model BP-1 (World Precision Instruments)] were inserted. Mouse plasma volume was calculated by determining the total blood volume of mice based on body weight. Propionate was administered as a 100-μL injection volume at a dose calculated to yield the desired final plasma concentration. The renin release assay (isolated perfused juxtaglomerular apparatus, JGA) was performed as described previously (2, 3). Plasma renin activity (PRA) was measured as previously described using a fluorescence resonance energy transfer-quenched, 5-(2-aminoethylamino)naphthalene-1-sulfonic acid (EDANS)-conjugated renin substrate. PRA was determined as the slope of the increase in EDANS fluorescence as a function of time (3, 4). Tail cuff BP measurements were obtained using a BP-2000 BP Analysis System (Visitech).

Gut microbiota biomass was depleted using an antibiotic treatment protocol modified from Rakoff-Nahoum et al. (5). Baseline BP measurements were obtained while mice had ad libitum access to water containing 1.5% Equal sweetener. Mice were then given access only to water containing 1.5% Equal (to mask the taste of the antibiotics and ensure normal drinking behavior) along with 1 g/L ampicillin, 500 mg/L vancomycin, and 1 g/L neomycin. Both the mice and the water bottles were weighed daily (Monday through Friday) to monitor water intake.

**Multiplex Sequencing of Amplicons Generated from Bacterial 16S rRNA Genes.** Genomic DNA was extracted from feces using a bead-beating protocol (6). Briefly, fecal samples were resuspended in a solution containing 500 μL of extraction buffer [200 mM Tris (pH 8.0), 200 mM NaCl, 20 mM EDTA], 210 μL of 20% SDS, 500 μL of phenol:chloroform:isoamyl alcohol (pH 7.9, 25:24:1; Ambion), and 500 μL of 0.1-mm diameter zirconia/silica beads. Cells were then mechanically disrupted using a bead beater (BioSpec Products; 5 min at room temperature), followed by extraction with phenol:chloroform:isoamyl alcohol and subsequent cleanup using the Qiagen PCR kit. Methods for generating amplicons from variable region 4 of bacterial 16S rRNA genes and multiplex barcoded sequencing with an Illumina instrument have been described in early publications (7). Data analysis (taxonomic assignment, and principal coordinates analysis of phylogenetic measurements of community similarity scores defined by unweighted and weighted UniFrac) was performed using the QIIME v1.3 pipeline (7, 8).


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4. Kang JJ, et al. (2008) The collecting duct is the major source of prorenin in diabetes. *Hypertension* 51(6):1597–1604.
5. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R (2004) Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 118(2):229–241.
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7. Yatsunenko T, et al. (2012) Human gut microbiome viewed across age and geography. *Nature* 486(7402):222–227.
8. Caporaso JG, et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 7(5):335–336.

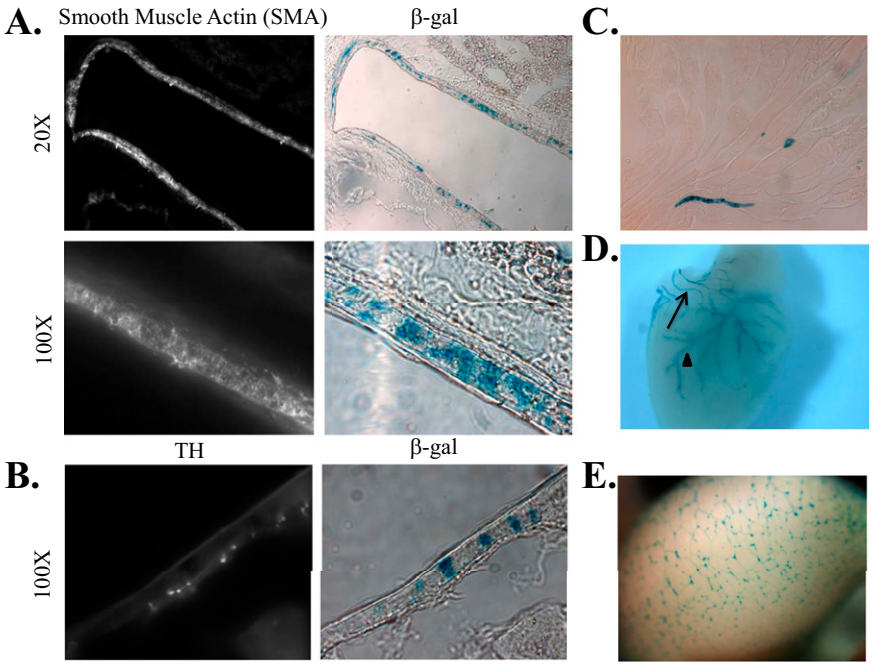


**Fig. S1.** Localization of Olfr78 expression (as identified by β-galactosidase activity). β-Galactosidase in renal vessels colocalizes with a smooth muscle marker (*A*) but not a neuronal marker (*B*). β-Galactosidase is also found in small vessels in skeletal muscle (*C*) and in the heart (*D*, arrowhead), as well as in axons of neurons in the heart (*D*, arrow) and the enteric plexus (*E*).

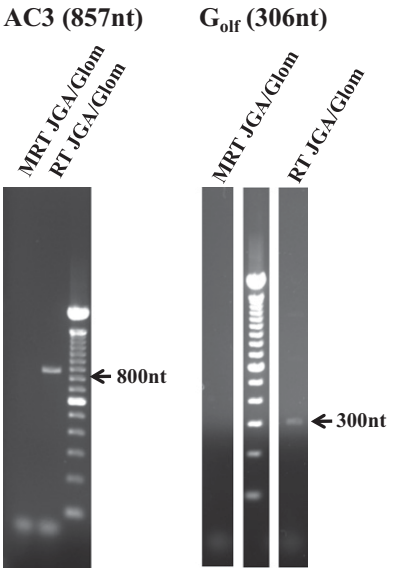


**Fig. S2.** PCR reveals that AC3 and $G_{olf}$ are expressed in microdissected JGA/glomeruli preparations.

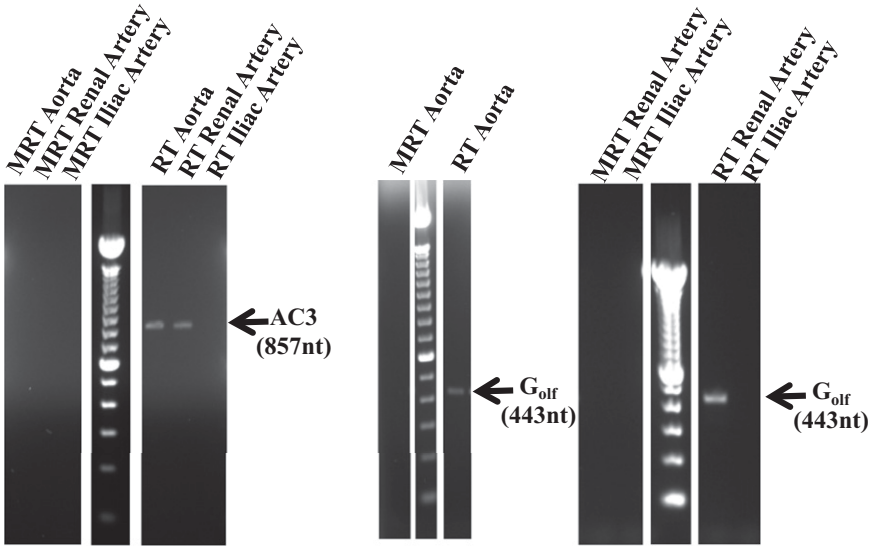


**Fig. S3.** PCR reveals that AC3 and $G_{olf}$ are found in aorta and renal artery, but are not detectable in iliac artery.

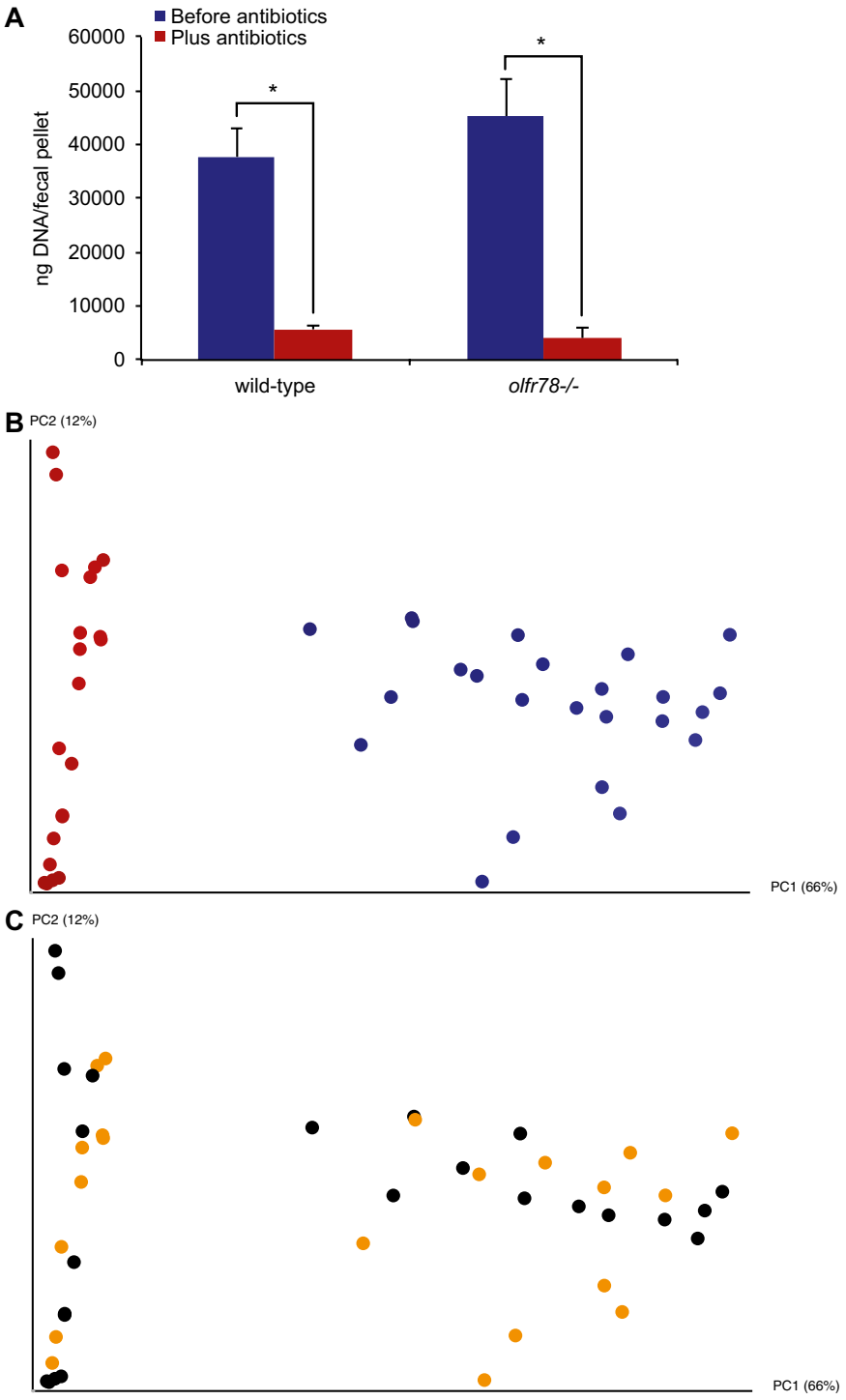


**Fig. S4.** Treatment with antibiotics effectively reduced biomass of the gut microbiota (*A*). *B* shows the change in microbiota structure before (blue) and after (red) antibiotic treatment. This response was not different between genotypes (*C*) clustered by treatment and colored by genotype: WT (black) KO (orange).

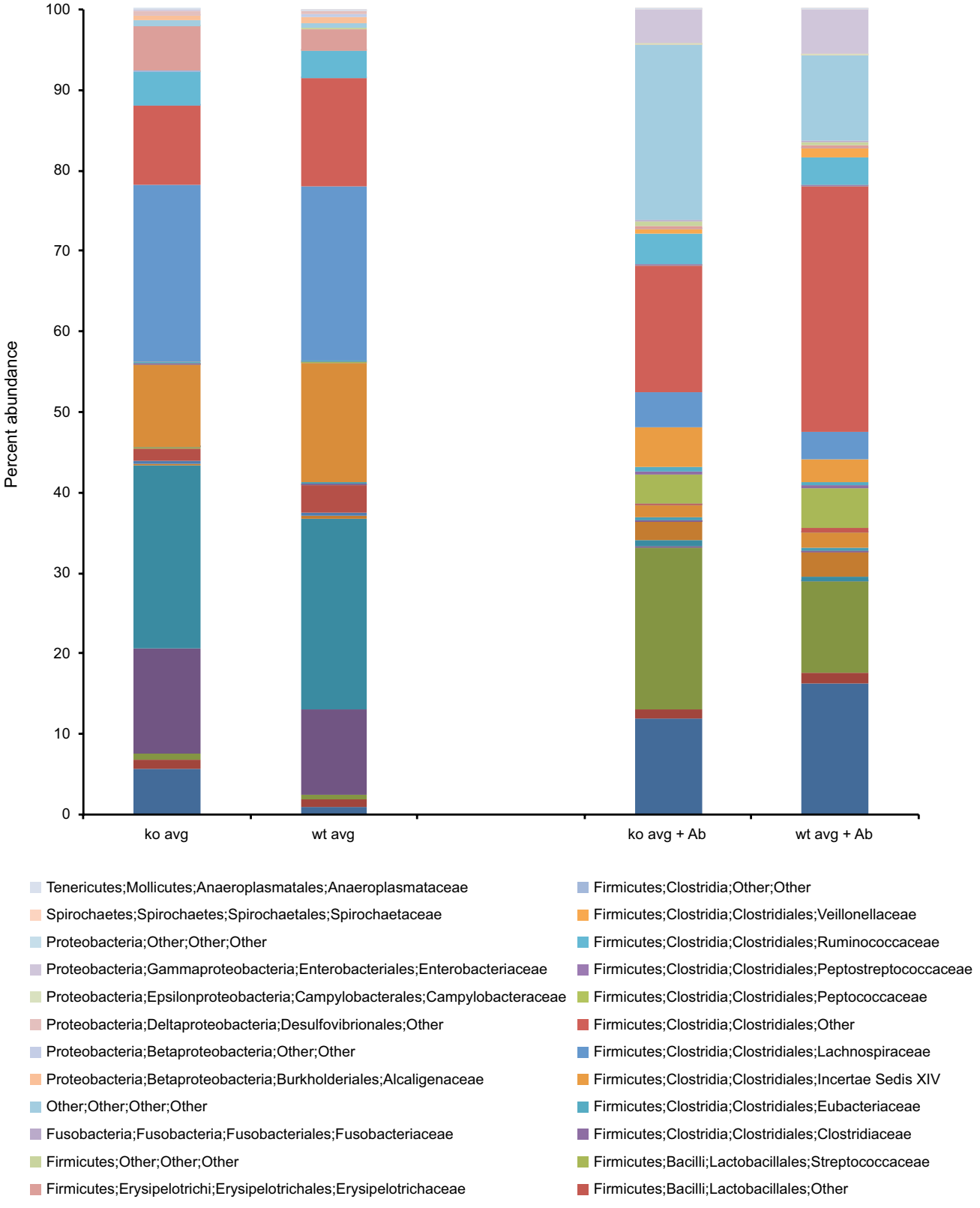


**Fig. S5.** Changes in the community composition (family level) are similar before and after antibiotics for WT and KO mice.