Supplemental Methods

Mice. University of Chicago (UC) and Cincinnati Children's Hospital Medical Center (CCHMC) colonies were derived from the C57BL/6J and C57BL/6NCrl respectively and maintained by in-house breeding for the past 10 years. UC colony mice were housed on pine shaving bedding and provided with an autoclaved Teklad-2918 diet and chlorinated, reverse-osmosis (RO) water through automated watering valves *ad libitum*. Cages were individually ventilated; cage supplies and bedding were autoclaved prior to use. The mice were kept on a 12-hour light/dark cycle at a room temperature of 20-24 °C and identified using unique 5-digit metal ear tags. The CCHMC colony was maintained on Bed o'Cobs, 1/4-inch corncob autoclaved bedding, fed autoclaved Lab diet 5010, and received UV-sterilized and Ultra-filtered RO water delivered using an auto-water system on an alternate design, ventilated caging system.

Gnotobiotic mouse husbandry. All mice were bred and housed in the Gnotobiotic Research Animal Facility (GRAF) at UC. GRAF is an operational facility of the UC Animal Resource Center. The sterility of the isolators was checked weekly by both cultivation and 16S rRNA analysis of fecal samples by quantitative PCR. Mice were maintained in Trexler-style, flexible-film isolator housing units (Class Biologically Clean) with Ancare polycarbonate mouse cages (catalog #N10HT) and Teklad pine shavings (7088; sterilized by autoclave) on a 12-hour light/dark cycle at a room temperature of 20-24 °C. Mice were provided with autoclaved sterile water, United States Pharmacopeia (USP) grade, at pH 5.2 *ad libitum*. Bedding was changed weekly. All mice were fed autoclaved Purina Lab Diet® 5K67 that was stored in a temperature-controlled environment in accordance with The Guide for the Care and Use of Laboratory Animals (8th Edition, 2013).

Tissue collection. To examine bacterial colonization or gene expression, tissues were collected from C57BL/6 littermates at the indicated timepoints. Mice were euthanized, and tissues samples were collected from the oropharynx (proximal to esophagus site), ear (represents skin; non–ear-

tagged ear), esophagus (whole; distal and proximal ends), colon pellets, and distal colon. Tissues were stored in DNA/RNAshield at -80 °C[32] after collection until DNA and RNA were isolated as indicated below.

DNA and RNA isolation. Tissues were extracted from DNA/RNAshield, submerged in liquid nitrogen, and crushed with a pre-chilled sterile glass rod. The tissue powder was resuspended in 200 μL quantitative real time (qRT) PCR–grade, sterile double-distilled water (DDW; Teknova; W3335) and split into two portions with 100 μL per portion. Alternatively, 5-mm stainless steel beads (Qiagen, 69989) and a 10-minute cycle of 30 bps on a Retsch MM 400 machine was used to homogenize the samples in DNA/RNAshield, before splitting them into two portions of equal volume. One portion (100 μL) was used to extract both the host DNA and bacterial DNA together with the Quick-DNA extraction kit (Zymo Research; D3020) according to the manufacturer's recommendations. The other portion (100 μL) was used to extract both the host RNA and bacterial RNA together via homogenization in 700 μL Trizol. After spin-down, 140 μL of chloroform was added to the lysates. RNA was extracted with the Quick-RNA extraction kit (Zymo Research; R1050) according to the manufacturer's recommendations.

qRT-PCR and bacterial load assessment. The bacterial load was assessed by qRT-PCR by comparing bacterial 16S amplicons with host genomic DNA amplicons. Relative quantification was performed via $2^{-\Delta\Delta Ct}$ method. The 319/806 V3/V4 Illumina primers (319F Primer Sequence: ACTCCTACGGGAGGCAGCAG; 806R Primer Sequence: GGACTACCGGGGTATCTAAT) were used for 16S amplification. The beta 2 microglobulin primers (forward: CGTAACACAGTTCCACCCG; reverse CTACCTGGCATGCCCTTTG) were used for the host genomic DNA amplification.

16S rRNA gene amplicon library preparation. The concentration of DNA was measured on a Nanodrop-nd1000 instrument. The V4 region of the 16S rRNA gene was PCR amplified with

region-specific primers (515F [Parada] Primer Sequence: GTGYCAGCMGCCGCGGTAA; 806R [Apprill] Primer Sequence: GGACTACNVGGGTWTCTAAT) modified from the original pair.[1, 2] Sequencing libraries were prepared following a previously published protocol in[3] at the Environmental Sample Preparation and Sequencing Facility (ESPSF) at Argonne National Laboratory. The 16S rRNA gene amplicon sequencing was performed on an Illumina MiSeq instrument to generate 151-bp, paired-end reads with 12-bp barcodes.

References

1. Caporaso JG, Lauber CL, Walters WA, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 2012;6:1621-4.
2. Caporaso JG, Lauber CL, Walters WA, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci U S A 2011;108 Suppl 1:4516-22.
3. Matson V, Fessler J, Bao R, et al. The commensal microbiome is associated with anti–PD-1 efficacy in metastatic melanoma patients. Science 2018;359:104-108.