Supplemental data

Pre-existing commensal dysbiosis is a host-intrinsic regulator of tissue inflammation and tumor cell dissemination in hormone receptor-positive breast cancer

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Figure S1

Antibiotic treatment results in gut dysbiosis.

A-C. 16S rDNA analysis of the fecal microbiome from vehicle (non-dysbiosis) and antibiotic-treated mice (dysbiosis). **A.** Phylum and genus level comparisons of changes within the microbiome of vehicle- or antibiotic-treated groups. Proportions calculated based upon total reads. **B.** Beta diversity – diversity of commensal microorganisms between non-dysbiotic and dysbiotic groups. A Permanova test was performed to assess significance. **C.** Shannon diversity index – alpha diversity or species richness of commensal microorganisms within each treatment group. A Kruskal-Wallis test was used to measure significance. **D.** Cecal weights comparing mice without commensal dysbiosis to mice with commensal dysbiosis.

Figure S2

Commensal dysbiosis does not alter mammary tissue pathology.

A. C57BL/6 mice were treated as described in Fig. 1A. At day 0, before tumor initiation, mammary tissue was harvested, formalin-fixed, and paraffin-embedded. Sections were deidentified, stained with hematoxylin and eosin, and blindly scored by a pathologist. **B**. Representative images of mammary tissue from dysbiotic and non-dysbiotic mice. Top images are 4X magnification. Arrows designate areas of leukocytic infiltration. Bottom images are 20X magnification and demonstrate representative mammary glands with multiple layers of epithelium. **C-D**. Principal component analysis of metabolomics of feces (**C**) and mammary tissue (**D**) from dysbiotic and non-dysbiotic mice, with or without BRPKp110 mammary tumors. Samples were annotated and grouped using principal

component analysis according to dysbiosis or tumor status. Significance was calculated using a one-way ANOVA. A significant association suggests that the variability explained by a principal component is driven by a corresponding sample annotation, such as dysbiosis.

Figure S3

Flow cytometry gating strategies for quantitating disseminated GFP$^+$ tumor cells.

A. Gating strategy for quantitating GFP$^+$ disseminated tumor cells. Events are gated on cells and counting beads by FSC-H/SSC-H, singlets by FSC-A/FSC-H, live cells by SSC-H/Zombie aqua negative, CD45$^+$ cells, endogenous GFP$^+$ cells, and anti-GFP$^+$ cells. **B.** Representative plots for quantitating circulating tumor cells in peripheral blood in dysbiotic and non-dysbiotic mice. Gates were drawn in part using GFP$^+$ BRPKp110 tumor cells as a positive control. **C.** Representative plots for quantitating tumor cell dissemination to tumor-draining axillary lymph nodes in dysbiotic and non-dysbiotic mice. Gates were drawn in part using a sample spiked with tumor cells as a positive control.

Figure S4

Commensal dysbiosis does not alter estrus cycling.

A. C57BL/6 mice were orally gavaged for 14 days with a broad-spectrum cocktail of antibiotics or water as a vehicle control. Gavage was ceased four days prior to tumor initiation. During this four-day period, estrus cycle tracking was performed daily by collecting vaginal lavage. Vaginal lavage smears were stained with crystal violet to

examine vaginal cytology. **B.** Compiled data representing the frequency of mice cycling as defined by progression from proestrus to estrus.

Figure S5

Commensal dysbiosis enhances breast tumor cell dissemination in an autochthonous model of HR$^+$ mammary cancer.

A. Genetic, autochthonous HR$^+$ breast tumors were initiated using adenovirus-Cre injection into the fourth mammary fat pad in L-Stop-L-*KRas*^{G12D}*p53*$^{flx/flx}$L-Stop-L-*Myristoylated p110α*−GFP$^+$ mice on a C57BL/6 background. Three days after adenovirus-Cre injection, mice were gavaged with antibiotics for a total of 14 days, and tumors were measured by calipers every 2-3 days after reaching a palpable size. **B-D.** Genetic, autochthonous HR+ breast tumors were initiated as described in A. Once tumors reached approximately 1cm in diameter, they were sterilely excised, and 10mg pieces were taken from the same tumor region, sliced into two equal 5mg pieces, and surgically implanted into the mammary tissue of C57BL/6 recipient mice that had been orally gavaged as described in Fig. 1A, with one 5mg fragment implanted into a non-dysbiotic mouse and the other half implanted into a dysbiotic mouse. 27 days after this paired tumor transplantation, GFP$^+$ tumor cell dissemination was quantified in lung tissue (**C**) and tumor-draining axillary lymph nodes (**D**) by flow cytometry. The gating strategy is shown in **B**, with the GFP$^+$ tumor gate chosen based upon FMOs and a stained lung sample spiked with GFP$^+$ BRPKp110 tumor cells as a positive control. Data is represented as percent GFP$^+$CD45$^-$ cells of live, singlet cells.

Figure S6

Representative plots quantitating disseminated GFP+ mammary tumor cells in mice treated with absorbable vs. non-absorbable antibiotics.

Representative plots of data from Fig. 6 quantifying GFP^+ tumor cells circulating in peripheral blood (**A**) and in the lungs (**B**). The anti-GFP gate was chosen based upon an anti-GFP FMO and a stained lung sample spiked with GFP^+ BRPKp110 tumor cells. Each plot is representative of an individual experimental animal.

Figure S7

Fecal microbiota transplantation from dysbiotic mice does not alter tumor progression but enhances tumor dissemination to distal sites.

A. Tumor growth kinetics from Fig. 7. **B-D**. Representative plots of data from Fig. 7 quantifying GFP^+ tumor cells circulating in peripheral blood (**B**), within the lungs (**C**), and in the axillary lymph nodes (**D**). The anti-GFP gate was chosen based upon an anti-GFP FMO and a stained lung sample spiked with GFP^+ BRPKp110 tumor cells.