Supplemental Figure Legends

Supplemental Figure S1. Focal radiation (RT) inhibits tumor growth in a dose-dependent manner and alternative antibiotic regimens can reduce the efficacy of RT. Related to Figure 1.

Orthotopic E0771 mammary tumors were grown to a median diameter of 1.0 cm. Mice were then treated with 8 or 16 Gy of localized kV irradiation using a small animal irradiator. A sample dose distribution map for the radiation is shown **(A)**. Total tumor burden/animal assessed every 3 days until endpoint for each of the two doses **(B)**. Mice were then started with antibiotics (Abx) for one week prior to being treated with localized kV irradiation (16 Gy). Both individual tumors **(A, B)** and mean tumor burden ± SEM **(C)** are displayed with their indicated treatment. Antibiotics (Abx) were vancomycin, neomycin and streptomycin. Significance was determined by two-way ANOVA with post-hoc testing for tumor growth and Log-Rank test for the Kaplan-Meier survival curves. Numbers (n) for each experiment are listed on the figure and are pooled data from two independent experiments. For all figures, significance is shown as *$p < 0.05$, ***$p < 0.001$.

Supplemental Figure S2. Different antifungal regimens enhance the efficacy of radiation. Related to Figure 2.

Orthotopic E0771 mammary tumors were grown to a median diameter of 1.0 cm and mice were then started with the antifungal 5-fluorocytosine (5-FC) for one week prior to being treated with localized kV radiation (RT) (16 Gy). Total tumor burden/animal assessed every 3 days until endpoint. Both individual tumors **(A, B)**, mean tumor burden ± SEM **(C)** and survival curves **(D)** are displayed with their indicated treatment. Significance was determined by two-way ANOVA with post-hoc testing for tumor growth and Log-Rank test

for the Kaplan-Meier survival curves. Numbers (n) for each experiment are listed on the figure and are pooled data from two independent experiments. *p < 0.05.

Supplemental Figure S3. FACS gating strategy and numbers of specific immune populations. Related to Figure 3.

Single-cell suspensions were made from E0771 mammary tumors. CD45+ leukocytes were then isolated using magnetic beads (Miltenyi Biotech). The resulting leukocytes were then stained with fluorescently-labeled antibodies and analyzed on a flow cytometer (LSR II, BD Biosciences). The resulting data was then analyzed using FlowJo (Treestar) using the gating strategy as indicated **(A)**. Total cell numbers per million cells was then elucidated for the indicated cell population **(B-K)**. Significance was determined by Student's t-test with Welch's correction. Numbers (n) for each experiment are listed on the figure and are pooled data from all experiments. For all figures, significance is shown as *p < 0.05, **p < 0.01, ***p < 0.001.

Supplemental Figure S4. CD8 and CCL2 depletion reduce the efficacy of combining fungal depletion with radiation. Related to Figure 3.

E0771 tumors grown to approximately 0.5 cm and then started on the antifungal (AF) fluconazole. One week after starting AF mice were injected with either anti-CD8 depleting antibody **(A, B)** or anti-CCL2 depleting antibody **(C, D)** two days prior to treatment with localized kV radiation (RT) (16 Gy). Total tumor burden/animal assessed every 3 days until endpoint. Significance was determined by two-way ANOVA with post-hoc testing for tumor growth and Log-Rank test for the Kaplan-Meier survival curves. Numbers (n) for each experiment are listed on the figure and are pooled data from at least two

independent experiments. For all figures, significance is shown as * p<0.05, ** p<0.01, ***p < 0.001.

Supplemental Figure S5. *Candida* efficiently colonizes SPF mice, Altered Schaedler Flora (ASF) mice are a fungi-free system similar to germ-free mice and germ-free mice colonized with *Candida* have reduced tumor growth and survival time following radiation. Related to Figure 5.

DNA was isolated from stool collected from control or *Candida* colonized mice and assessed by qPCR using primers specific for *Candida albicans* **(A).** Stool from *Candida* colonized mice subsequently treated with fluconazole (F) was also assessed for *Candida* levels by quantitative PCR (qPCR) (**B**). ASF and germ-free (GF) mice were kept and propagated in a gnotobiotic facility at Cedars-Sinai Medical Center prior to initiating tumor experiments. Specific-pathogen free (SPF) stools were collected at the same time from the general vivarium. Bacterial and fungal levels were assessed by qPCR with primers specific for bacterial 16S ribosome **(C)** and fungal 18S rRNA (Fungiquant) **(D)**. Individual mice were compared to the average germ-free mouse which is indicated by the red line. E0771 tumors were grown in either germ-free mice (GF) or germ-free mice monocolonized with *Candida*. Tumors were grown to a median diameter of 1.0 cm and then treated with localized kV irradiation (16 Gy). Total tumor burden/animal assessed every 3 days until endpoint **(E)** and mice were also monitored for survival **(F)**. Significance was determined by two-way ANOVA with post-hoc testing for tumor growth and qPCR quantification of bacteria and fungi and Log-Rank test for the Kaplan-Meier survival curves. Numbers (n) for each experiment are listed on the figure and are pooled data

from at least two independent experiments. * p<0.05, ** p<0.01, *** p < 0.001, **** p<0.0001

Supplemental Figure S6. Melanomas with high expression of Dectin-1 exhibit worse survival and a premature stop codon at (SNP rs169105) correlates with lower expression levels in breast tissue. Related to Figure 6.

Analysis of Dectin-1 (CLEC7A) expression in melanomas from The Cancer Genome Atlas (TCGA) was performed. Dectin-1 expression was normalized to the expression level of the macrophage marker CSF1R. Levels that were above the mean were considered high risk while those below the mean were considered low risk. Survival (**A**) was then plotted for normalized Dectin-1 expression above and below the mean. Significance was determined by Log-Rank test for the Kaplan-Meier survival curves. The table on bottom shows the hazard ratio, confidence interval and p-value for the comparison. Genotype-Tissue Expression (GTEx) Database was examined for the Dectin-1 polymorphism Y238X (minor allele associated with single-nucleotide polymorphism (SNP) rs16910526), which creates a premature stop codon in Dectin-1. mRNA expression levels of Dectin-1 in breast tissue was then plotted based on the presence of the major (AA) or minor allele (CC) at SNP rs16910526 (**B**). Significance was determined by two-way ANOVA with post-hoc testing for mRNA expression and Log-Rank test for the Kaplan-Meier survival curves. Numbers (n) for each experiment are listed on the figure.

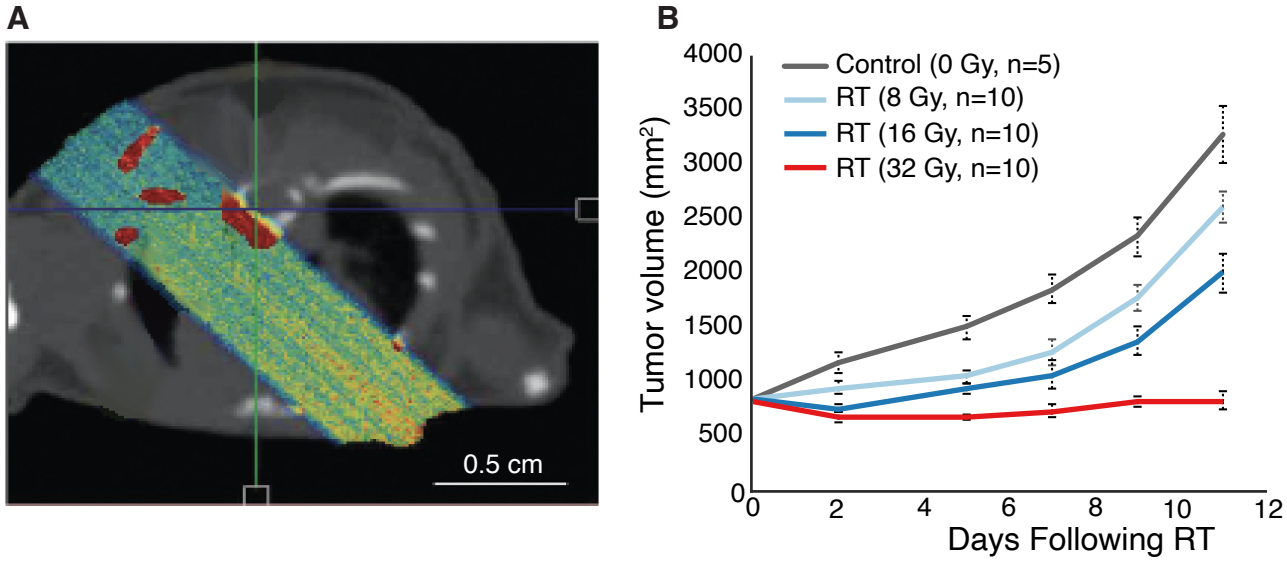
Supplemental Data Table S1. Bacteria and fungi families found in fecal samples of specific-pathogen free (SPF) mice, Related to Figures 1, 2 and 4.

Supplemental Data Table S2. 16S OTU Counts, Related to Figure 4 (.xls)

Supplemental Data Table S3. ITS OTU Counts, Related to Figure 4 (.xls)

Supplemental Data Table S4. Patient characteristics for Triple-Negative Breast Cancer cohort, Related to Figure 6

Fig. S1.



Alternative Abx: Vancomycin 500mg/L, Neomycin 1g/L, Streptomycin 5mg/mL

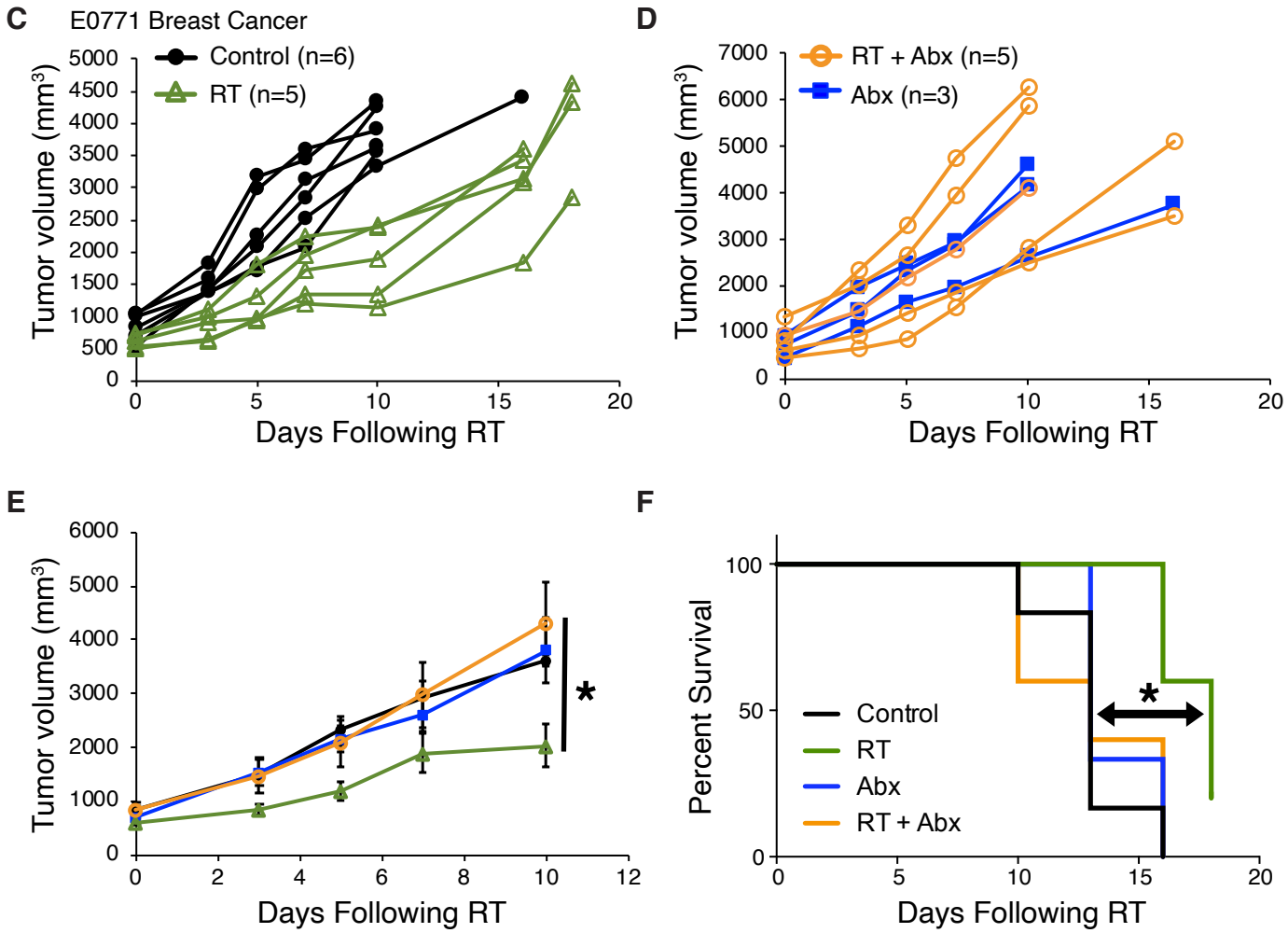


Fig S2.

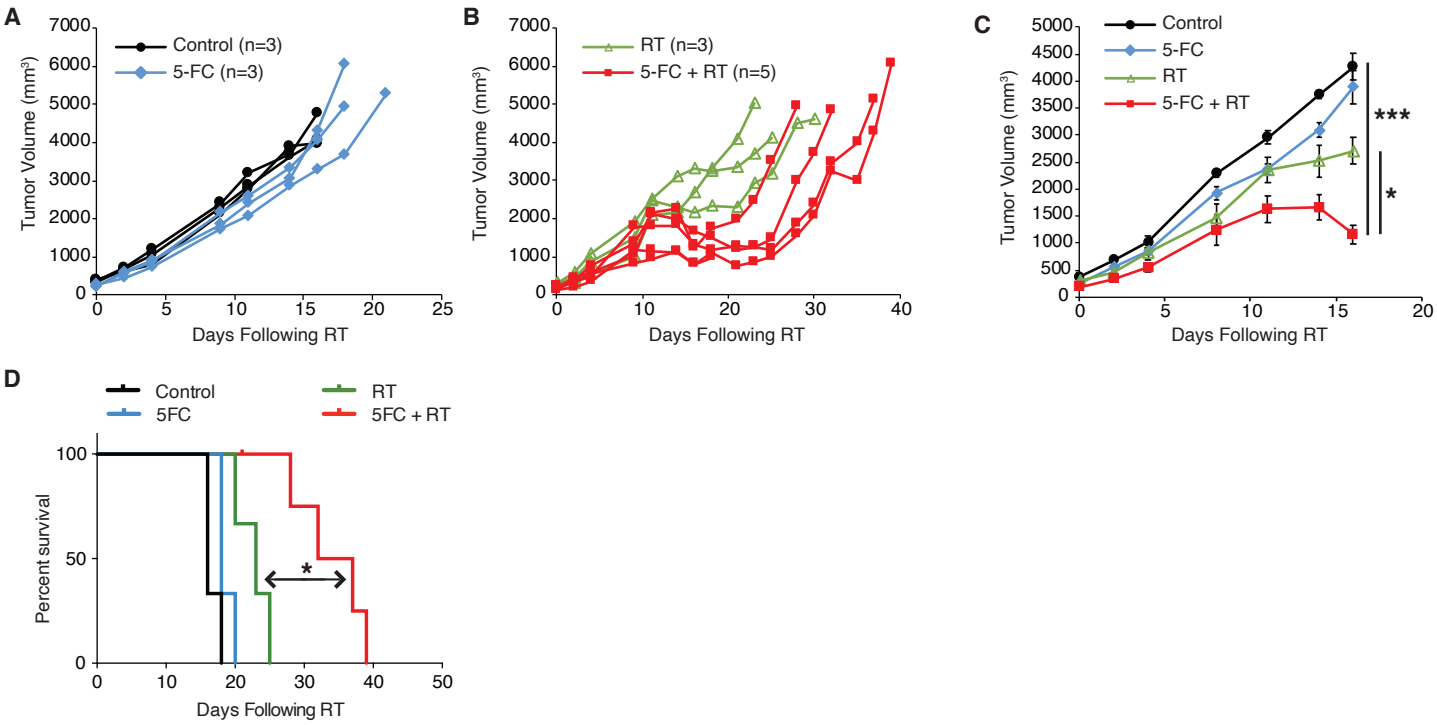

A
Control (n=3)
5-FC (n=3)
Tumor Volume (mm3)
Days Following RT
B
RT (n=3)
5-FC + RT (n=5)
Tumor Volume (mm3)
Days Following RT
C
Control
5-FC
RT
5-FC + RT
***
*
Tumor Volume (mm3)
Days Following RT
D
Control
5FC
RT
5FC + RT
Percent survival
*
Days Following RT

Fig. S3.

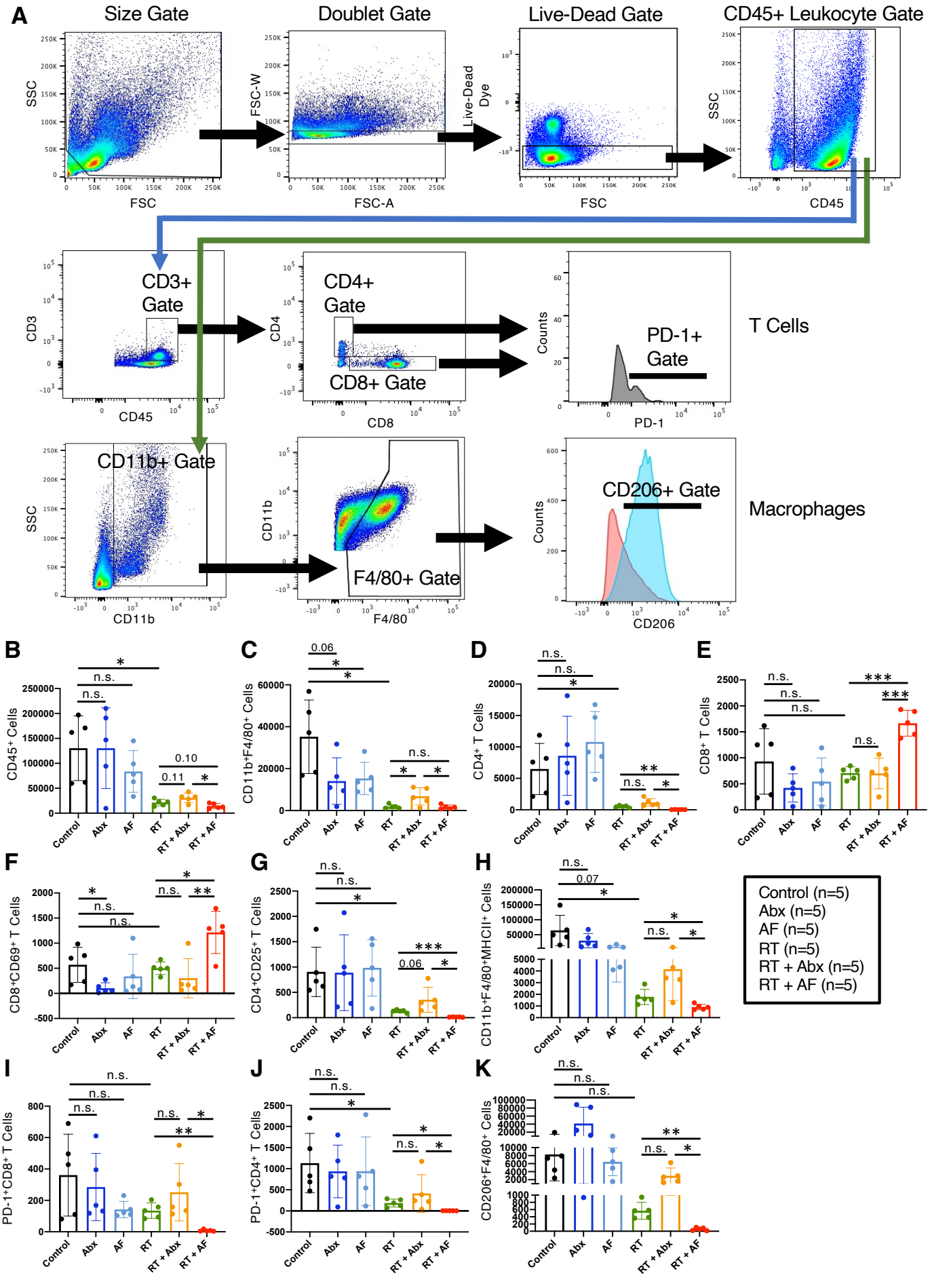

A
Size Gate
Doublet Gate
Live-Dead Gate
CD45+ Leukocyte Gate
SSC
FSC
FSC-W
FSC-A
Live-Dead Dye
CD45
CD3+ Gate
CD4+ Gate
CD8+ Gate
PD-1+ Gate
CD3
CD4
CD8
PD-1
Counts
T Cells
CD11b+ Gate
F4/80+ Gate
CD206+ Gate
CD11b
F4/80
CD206
Macrophages
B
CD45⁺ Cells
C
CD11b⁺F4/80⁺ Cells
D
CD4⁺ T Cells
E
CD8⁺ T Cells
F
CD8⁺CD69⁺ T Cells
G
CD4⁺CD25⁺ T Cells
H
CD11b⁺F4/80⁺MHCII⁺ Cells
I
PD-1⁺CD8⁺ T Cells
J
PD-1⁺CD4⁺ T Cells
K
CD206⁺F4/80⁺ Cells
Control
Abx
AF
RT
RT + Abx
RT + AF
n.s.
0.06
0.07
0.10
0.11
Control (n=5)
Abx (n=5)
AF (n=5)
RT (n=5)
RT + Abx (n=5)
RT + AF (n=5)

Fig. S4.

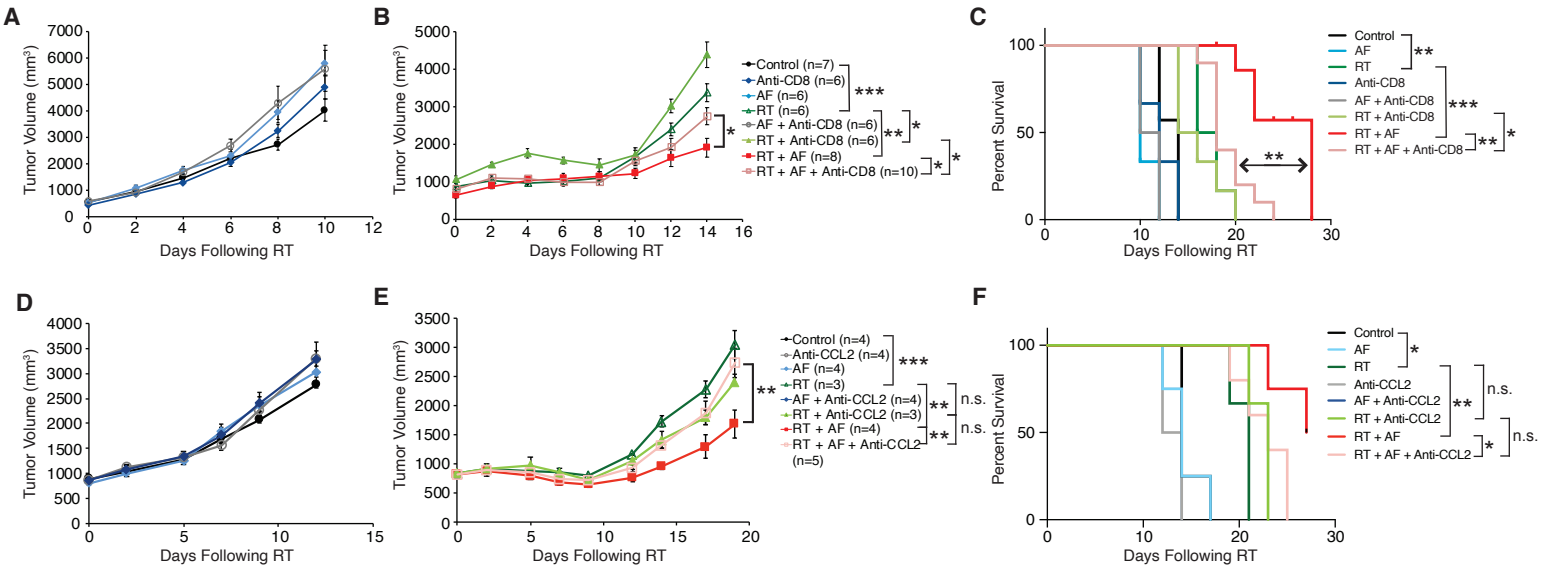
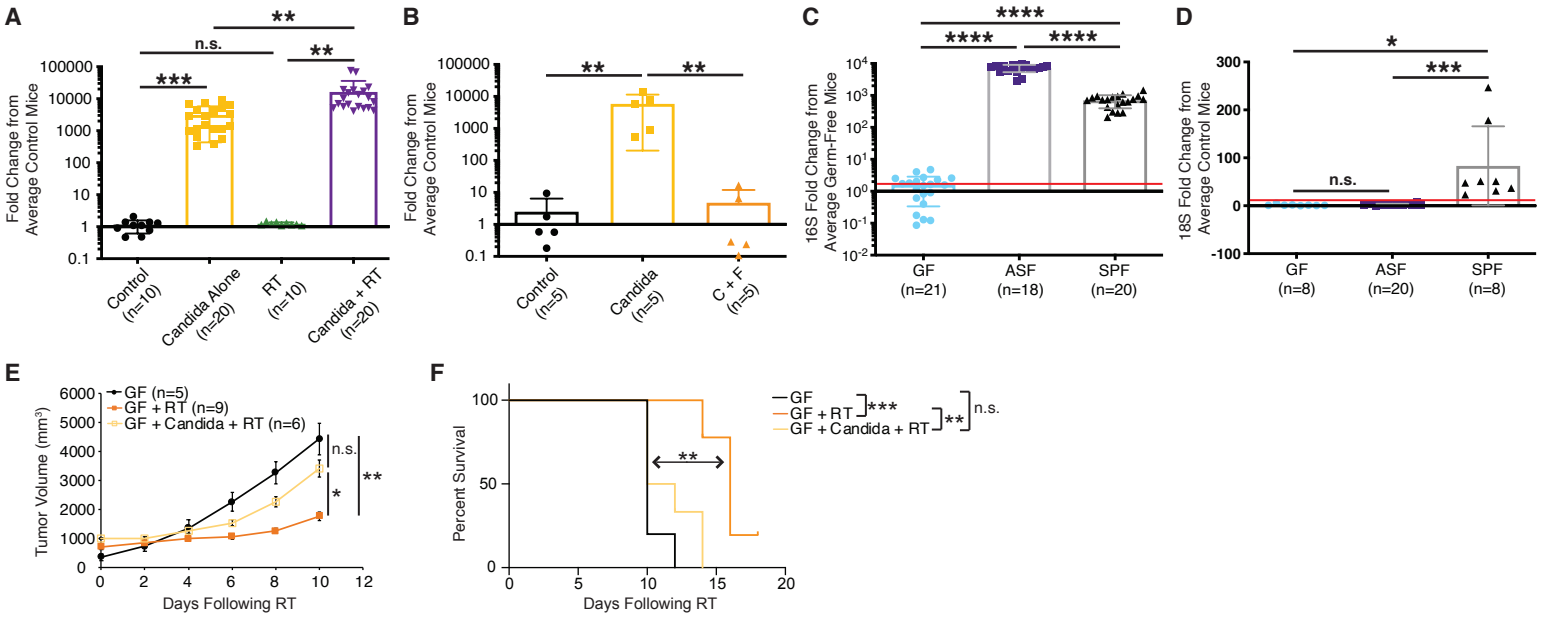
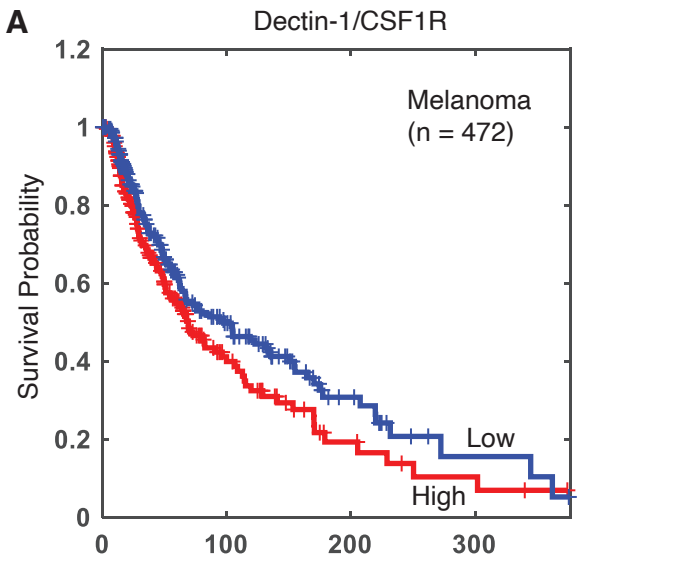

A
B
C
Tumor Volume (mm³)
Days Following RT
Control (n=7)
Anti-CD8 (n=6)
AF (n=6)
RT (n=6)
AF + Anti-CD8 (n=6)
RT + Anti-CD8 (n=6)
RT + AF (n=8)
RT + AF + Anti-CD8 (n=10)
Percent Survival
Control
AF
RT
Anti-CD8
AF + Anti-CD8
RT + Anti-CD8
RT + AF
RT + AF + Anti-CD8
D
E
F
Control (n=4)
Anti-CCL2 (n=4)
AF (n=4)
RT (n=3)
AF + Anti-CCL2 (n=4)
RT + Anti-CCL2 (n=3)
RT + AF (n=4)
RT + AF + Anti-CCL2 (n=5)
Anti-CCL2
AF + Anti-CCL2
RT + Anti-CCL2
RT + AF + Anti-CCL2
n.s.

Fig. S5.

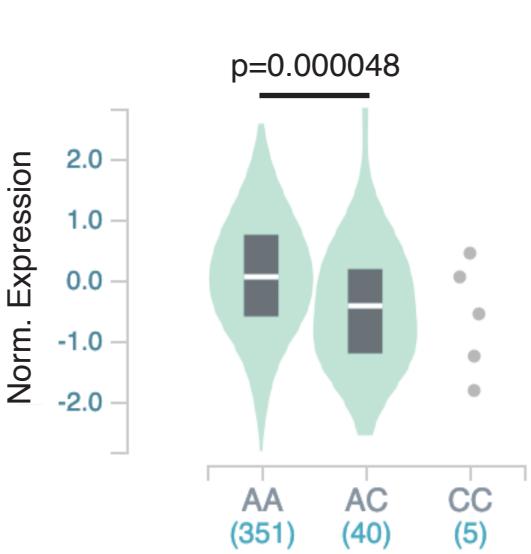

A
B
C
D
Fold Change from Average Control Mice
16S Fold Change from Average Germ-Free Mice
18S Fold Change from Average Control Mice
Control (n=10)
Candida Alone (n=20)
RT (n=10)
Candida + RT (n=20)
Control (n=5)
Candida (n=5)
C + F (n=5)
GF (n=21)
ASF (n=18)
SPF (n=20)
GF (n=8)
ASF (n=20)
SPF (n=8)
n.s.

E
F
GF (n=5)
GF + RT (n=9)
GF + Candida + RT (n=6)
Tumor Volume (mm³)
Days Following RT
Percent Survival
GF
GF + RT
GF + Candida + RT
n.s.

Fig. S6.



	HR	P-value
Dectin-1/CSF1R	1.300166	0.051968



Supplemental Table S1. Bacteria and fungi families found in fecal samples of specific-pathogen free (SPF) mice, Related to Figures 1, 2 and 4*

	Family (in SPF mice)	Bacterial Classification	Abx Sensitivity
Bacteria	Lactobacillaceae	Gram Positive, Aerobic	Ampicillin, Vancomycin
	Clostridiaceae	Gram Positive, Anaerobic	Metronidazole, Imipenem
	Muribaculaceae (S24-7)	Gram Negative, Anaerobic	Ampicillin, Imipenem
	Lachnospiraceae	Gram Negative, Anaerobic	Ampicillin, Imipenem
	Turicibacteraceae	Gram Positive, Anaerobic	Streptomycin, Vancomycin, Ampicillin
	Streptococcaceae	Gram Positive, Facultative Anaerobe	Streptomycin, Vancomycin, Ampicillin
	Erysipelotrichaceae	Gram Positive, Aerobic	Streptomycin, Vancomycin, Ampicillin
	Paraprevotellaceae	Gram Negative, Aerobic	Ampicillin, Imipenem
	Ruminococcaceae	Gram Positive, Anaerobic	Ampicillin, Imipenem
	Rikenellaceae	Gram Negative, Anaerobic	Ampicillin, Imipenem
	Coriobacteriaceae	Gram Positive, Anaerobic	Ampicillin, Imipenem
	Desulfovibrionaceae	Gram Negative, Anaerobic	Ampicillin, Imipenem
	Porphyromonadaceae	Gram Negative, Anaerobic	Ampicillin, Imipenem
	Leuconostocaceae	Gram Positive, Facultative Anaerobe	Streptomycin, Vancomycin, Ampicillin
	Alcaligenaceae	Gram Negative	Ampicillin, Imipenem
	Verrucomicrobiaceae	Gram Negative, Aerobic	Ampicillin, Imipenem
	Bacteroidaceae	Gram Negative, Anaerobic	Ampicillin, Imipenem
	Enterococcaceae	Gram Positive, Facultative Anaerobe	Streptomycin, Vancomycin, Ampicillin
Fungi**	Aspergillaceae		
	Schizophyllaceae		
	Davidiellaceae		
	Trichocomaceae		
	Sporidiobolaceae		
	Didymellaceae		
	Clavicipitaceae		
	Saccharomycetaceae		
	Cystofilobasidiaceae		
	Nectriaceae		
	Pleosporaceae		
	Tremellaceae		

***Reference:** *The Johns Hopkins POC-IT ABX Guide*, ed. P.G. Auwaerter and J.G. Bartlett. 2020, Baltimore, MD: Unbound Medicine, Inc.

****Fungal sensitivities are much less well-characterized due in part to the challenge of culturing some fungi and nearly all fungi exhibit some sensitivity to broad-spectrum antifungals including fluconazole, amphotericin B and/or 5-fluorocytosine

Supplemental Data Table S4. Patient characteristics for Triple-Negative Breast Cancer cohort, Related to Figure 6

Patient Characteristics (n = 50)	
Age - Median (Range)	56 (29-89)
Female Gender, %	50/50 (100%)
Triple-Negative (ER-, PR-, Her2-)	50/50 (100%)
Clinical Stage	
1	17/50 (34%)
2	26/50 (52%)
3	7/50 (14%)
Grade	
1	1/50 (2%)
2	5/50 (10%)
3	44/50 (88%)
T Stage	
1	14/50 (28%)
2	29/50 (58%)
3	4/50 (8%)
4	3/50 (6%)
N Stage	
0	4/50 (8%)
1	12/50 (24%)
2	27/50 (54%)
3	7/50 (14%)
Treatment	
Chemotherapy	37/50 (74%)
Radiation	28/50 (56%)
RCB Class	
Mastectomy	18/50 (36%)
Lumpectomy	32/50 (64%)
Ethnicity	
Asian	3/50 (6%)
Black/African-American	7/50 (14%)
Non-Hispanic White	40/50 (80%)