Activation of HIF-1α and LL-37 by commensal bacteria inhibits Candida albicans colonization

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Candida albicans colonization is required for invasive disease1–3. Unlike humans, adult mice with mature intact gut microbiota are resistant to C. albicans gastrointestinal (GI) colonization2,4, but the factors that promote C. albicans colonization resistance are unknown. Here we demonstrate that commensal anaerobic bacteria—specifically clostridial Firmicutes (clusters IV and XIVa) and Bacteroidetes—are critical for maintaining C. albicans colonization resistance in mice. Using Bacteroides thetaiotamicron as a model organism, we find that hypoxia-inducible factor-1α (HIF-1α), a transcription factor important for activating innate immune effectors, and the antimicrobial peptide LL-37 (CRAMP in mice) are key determinants of C. albicans colonization resistance. Although antibiotic treatment enables C. albicans colonization, pharmacologic activation of colonic HIF1α induces CRAMP expression and results in a significant reduction of C. albicans GI colonization and a 50% decrease in mortality from invasive disease. In the setting of antibiotics, Hif1α and Camp (which encodes CRAMP) are required for B. thetaiotamicron–induced protection against C. albicans colonization of the gut. Thus, modulating C. albicans GI colonization by activation of gut mucosal immune effectors may represent a novel therapeutic approach for preventing invasive fungal disease in humans.

Commensal fungi, mostly Candida spp., have been detected in the GI tracts of various mammals5. Although reportedly 40–60% of humans are colonized with C. albicans (CA) in the GI tract, adult mice are resistant to GI colonization by CA2,4. Because colonization is a prerequisite for CA invasive disease1–3 and CA colonization resistance is abrogated by antibiotic treatment4, understanding of the factors that modulate CA colonization could lead to novel methods for preventing CA dissemination.

Commensal anaerobic bacteria in the GI tract provide an important defense mechanism against infections by inhibiting the growth of potentially pathogenic bacteria6,7. One mechanism for GI colonization resistance involves stimulation of the mucosal immune system by members of ‘beneficial’ microbiota8. Yet there have been no studies to date examining a commensal bacteria– or host-mediated immune response that modulates fungal colonization. Thus, we asked whether identifying a single bacterial species that promotes CA colonization resistance could help unveil host immune effectors critical for maintaining CA colonization resistance in the mouse GI tract.

To determine the effect of specific antibiotics on CA colonization resistance, we treated mice with various antibiotics for 5 d, orally challenged them with CA, and then assessed their susceptibility to CA colonization. CA was unable to establish sustained GI colonization in adult control (no antibiotic treatment) mice (Fig. 1a), regardless of the mouse strain tested (Supplementary Table 1). In the treatment groups, CA colonization levels were directly proportional to how effective the specific antibiotic was in depleting anaerobic bacteria: penicillin G (PCN) > clindamycin > metronidazole > streptomycin (STR) (Fig. 1a and Supplementary Table 2). In fact, CA GI colonization levels in PCN-treated mice were comparable to colonization levels in non-antibiotic-treated, germ-free mice (Fig. 1b). Even CA strains that had been serially passaged through the GI tract of an antibiotic-treated mouse could not persistently colonize a mouse GI tract with an intact gut microbiome (Supplementary Fig. 1a,b).

The gut microbiota in infant humans and mice have substantially fewer commensal anaerobes than those in adults10,11. Hence, CA established persistent GI colonization in postnatal day (P)14 and P28 mice but not in adolescent (P42) animals (Fig. 1c and Supplementary Table 2). Other CA strains, including two clinical isolates (Fig. 1d and Supplementary Table 3), and other Candida spp. that infect humans (Fig. 1e) were also unable to colonize mice with intact gut microbiota (Supplementary Table 2). Together, these findings indicate that a mature adult bacterial microbiota, particularly comprising commensal anaerobes, is essential for maintaining CA colonization resistance.

To identify specific members of the gut microbiota essential for maintaining CA colonization resistance, we profiled the gut microbiomes (using 16S rRNA sequencing and bacterial group quantitative PCR (qPCR)) of CA colonization–resistant (no antibiotic treatment or STR-treated) or CA colonization–susceptible (treated with PCN)
or PCN and STR (PS) mice (Fig. 2a). The bacterial phyla Firmicutes and Bacteroidetes account for >95% of the bacteria in the distal guts of healthy adult mice and humans\(^1\). The abundance of Firmicutes-Bacteroidetes markedly decreased in CA-colonized (PCN- or PS-treated) mice compared to colonization-resistant (sterile water– or STR-treated) mice (Fig. 2b). PCN-treated mice exhibited the most significant decrease (3–4 log fold) in total gut bacteria (eubacteria, EUBAC) by qPCR compared to the more modest 0.5 log fold reduction seen in the STR-treated group (Fig. 2c). Streptomycin is an antibiotic that is effective against Gram-negative bacteria and completely ineffective against obligate anaerobic bacteria\(^12\). PCN-treated mice had marked decreases in all bacterial groups, with the exception of Proteobacteria and Enterobacteriaceae (ENTERO). Penicillin G has activity against Gram-positive bacteria and anaerobes, but it is ineffective against Gram-negative bacteria\(^13\) (Fig. 2c). Notably, endogenous gut fungi (which include some Candida spp., but not CA) increased significantly (\(P = 0.029\), Mann-Whitney) in mice treated with antibiotics (e.g., PCN) active against Bacteroidetes (mouse intestinal Bacteroides, MIB) and clostridial Firmicutes (clostridial cluster XIVa, EREC, and clostridial cluster IV, CLEPT) (Supplementary Fig. 2). Thus, only antibiotic treatment that sufficiently depleted anaerobic bacteria (e.g., PCN) was sufficient to overcome CA colonization resistance in the mouse GI tract.

We postulated that cessation of antibiotics in CA-colonized mice would allow suppressed bacterial groups to regrow and restore CA colonization resistance. Indeed, CA colonization levels steadily decreased and were undetectable 35 d after antibiotic cessation (Fig. 2d). Overall bacterial levels (EUBAC) returned to pre-antibiotic-treated levels 14 d after stopping antibiotics (Fig. 2e). Notably, Bacteroidetes and clostridial Firmicutes increased significantly (although they still remained lower than baseline), whereas Lactobacillus (LACT), segmented filamentous bacteria (SFB) and ENTERO remained suppressed for the duration of the experiment (Fig. 2c). In total, these findings suggested that Bacteroidetes and clostridial Firmicutes may be the bacterial groups most effective in promoting CA colonization resistance.

To test this hypothesis, we performed oral bacterial add-back experiments in antibiotic-treated CA-colonized mice. Notably, among Bacteroidetes, the addition of Bacteroides fragilis did not significantly change CA colonization levels compared to a no-bacteria control group, whereas the presence of Bacteroides thetaiotamicron (B. theta) resulted in undetectable CA levels in all mice 14 d after bacterial inoculation (Fig. 2f and Supplementary Table 3). Similarly, among Firmicutes, Blautia producta promoted complete elimination of CA, whereas the Lactobacillus spp. (L. acidophilus and L. reuteri) did not consistently eliminate CA colonization (Fig. 2f). Both members of the phylum Proteobacteria (Escherichia coli and Pseudomonas aeruginosa), however, had no significant effect on CA (Fig. 2f). Collectively, these experiments showed that individual Firmicutes and Bacteroidetes species are sufficient to promote CA colonization resistance in the gut.

Because B. theta and B. producta had the most significant (\(P = 0.0002\) for both B. theta and B. producta, Mann-Whitney) effect in our model, we replicated these experiments in germ-free mice that can be colonized with CA without antibiotic pretreatment. Although B. producta and B. theta GI levels remained consistent throughout the duration of the experiment, CA levels steadily decreased and were undetectable by day 20 in the B. producta group (Fig. 2g), and they significantly decreased (nearly 5 log fold) but remained detectable in the B. theta group (Fig. 2b).

Overall, we found that only antibiotics that depleted anaerobic bacteria were sufficient to overcome CA colonization resistance. Among the bacteria we tested, clostridial Firmicutes and Bacteroidetes most effectively fostered colonization resistance, although individual bacterial species varied greatly in this ability. Ultimately, we identified two genetically distinct bacterial species, B. theta and B. producta, that individually promoted CA colonization resistance.

B. theta and B. producta induces host immune effectors critical for maintaining colonization resistance, and we focused on the transcription factor HIF-1\(\alpha\) and the antimicrobial peptide LL-37 (CRAMP). HIF-1\(\alpha\) is an essential regulator of mammalian innate defense\(^14\), and it increases expression of antimicrobial cathelicidin
Figure 2 Clostridial Firmicutes and Bacteroidetes promote C. albicans GI colonization resistance. (a) CA colonization levels of mice pretreated with antibiotics and orally gavaged with CA. CA levels measured 7 d after oral gavage; n = 8 per group. (b) Relative abundance of bacterial phyla as determined by 16S rRNA sequencing of fecal specimens collected from C3H/HeN mice treated with sterile water, STR, PCN or PS; n = 3 per group. (c) Bacterial group qPCR (copies per gram of feces) performed on fecal genomic DNA collected from mice treated with sterile water or oral antibiotics. n = 4 per group. (d,e) CA colonization levels (d) and bacterial group qPCR (e) in PS-treated CA-colonized mice after cessation of the oral antibiotic (discontinued on day 0). CA levels and bacterial group qPCR measured every 7 d; n = 4–5 per group. (f) Bacterial (black circles) and CA (red triangles) levels in antibiotic-treated mice colonized with CA and gavaged with one bacterial commensal species. Colonization levels measured 14 d after bacterial gavage; n = 8 per group. (g,h) Bacterial (black circles) and CA (red triangles) levels in mice colonized with CA and then gavaged with B. producta (g) or B. thetaiotamicron (h). Colonization levels measured at specified time points after bacterial gavage; n = 4 per group. For all experiments, points represent results from individual animals. Horizontal lines represent the median with interquartile range (a,d,f,h). Bars represent the mean ± s.e.m. (c,e); statistical analysis by Mann-Whitney test, *P < 0.05, **P < 0.01, ***P < 0.001. NS, not significant.
peptides in myeloid cells. Cathelicidin-related antimicrobial peptides are a family of polypeptides that serve a critical role in mammalian innate immune defense against bacterial infection. The human cathelicidin LL-37 has been shown to have anti-Candida activity and to inhibit CA adhesion to epithelial surfaces. Notably, mRNA expression of Hif1α and Camp (cathelicidin-related antimicrobial peptide, the LL-37 ortholog) significantly increased in colonization-resistant mice compared to CA-colonized mice. Both HIF1A (or Hi1α) and CAMP (or Camp) expression markedly increased in human colonocytes exposed to CA-colonized mice, suggesting that Hif1α and Camp may be critical immune effectors for maintaining CA colonization resistance. In addition to indirect host effects, B. theta may have a direct inhibitory effect on CA. Both B. producta and B. theta produce short-chain fatty acids (SCFAs) that have numerous immunomodulatory properties. In fact, bacterially produced SCFAs at physiologically relevant doses inhibited CA growth in vitro and diminished CA colonization in mice, although not to the degree seen with B. theta, suggesting that the host response is important in order to completely suppress CA colonization.

To further test whether HIF-1α or LL-37 is required to prevent CA colonization, we first used a well-established HIF-1α agonist, l-mimosine (mimosine), in an in vitro fungicidal assay using cultured human colonocytes. mRNA and protein expression of both HIF1A and CAMP increased in colonocytes exposed to mimosine. Mimosine stabilizes HIF-1α through inhibition of prolyl hydroxylases and inhibits HIF-1α degradation. This probably explains the modest increase in HIF1A gene expression compared to the more pronounced increase in LL-37 protein expression. We observed that the candidacidal activity of LL-37, confirmed using a spot assay, is enhanced at lower pH values, as has been previously reported with LL-37 and other antifungal agents (Supplementary Fig. 6d). Of note, the pH in the more-distal segments of the intestine is acidic, ranging from 2.0 to 4.5. The murine cathelicidin CRAMP (CRAMP (AA 135–173)) and its human homolog CRAMP NS (AA 135–173) are highly conserved antibacterial peptides that are produced in response to infection and inflammation. CRAMP is a member of the cathelicidin family of host-defense peptides that are induced in response to inflammation. The human cathelicidin LL-37 has been shown to have anti-Candida activity and to inhibit CA adhesion to epithelial surfaces. Notably, mRNA expression of Hif1α and Camp (cathelicidin-related antimicrobial peptide, the LL-37 ortholog) significantly increased in colonization-resistant mice compared to CA-colonized mice. Both HIF1A (or Hi1α) and CAMP (or Camp) expression markedly increased in human colonocytes (HT-29 cells) exposed to CA (or in the colon of CA-colonized, antibiotic-treated mice). The colon had the highest concentration of fungi in colonization-resistant mice compared to CA-colonized mice. Interestingly, B. theta induced a significantly greater degree of mouse colonic Hi1α and Camp expression compared to monocolonization with other CA (Fig. 3a–h). Co-colonization with B. theta and CA induced greater colonic Hi1α and Camp expression (Fig. 3i) compared to monocolonization with either B. theta or CA. We concluded that this additive mucosal immune stimulatory effect might explain how B. theta facilitates CA colonization reduction in germ-free mice. Our findings suggest that Hif1α and Camp may be critical immune effectors for maintaining CA colonization resistance.
GI colonization levels in colonocytes is required for mimosine-dependent killing of CA. (a) GI tract colonization levels in Hif1a KO and Hif1a WT/Vil-Cre (a), or Camp knockout (KO) (b) mice treated with antibiotics, colonized with CA and then treated or untreated with l-mimosine; n = 8 per group. Bars represent the mean ± s.e.m. (c,d) Survival curves of Hif1a KO and Hif1a WT/Vil-Cre (c) or Camp-KO (d) mice treated with antibiotic water, colonized with CA, treated or untreated with l-mimosine for 5 d, and then given cyclophosphamide (Cy). L-Mimosine treatment continued for an additional 7 d after the first cyclophosphamide dose, n = 8 per group. (e,f) Hif1a and Camp mRNA levels in colons of Hif1a KO, Hif1a WT/Vil-Cre and Camp-KO mice treated or untreated with l-mimosine; n = 4. All data shown are means ± s.e.m. Assays were performed in triplicate. (g) C. albicans (red triangles) and B. theta (black circles) GI colonization levels in antibiotic-treated Hif1a KO, Hif1a WT/Vil-Cre and Camp-KO mice; n = 6 for Hif1a KO and Camp-KO mice; n = 5 for Hif1a WT/Vil-Cre mice. Black circles (B. theta) and red triangles (CA) represent results from individual animals. Horizontal lines represent the median with interquartile range. Statistical analysis by Mann-Whitney test (a,b,e–g) or log-rank test (c,d). *P < 0.05; **P < 0.01; NS, not significant.

from pH 6.4–7.5 in the small intestine to pH 6.4–7.0 in the colon. Furthermore, the distal intestine has the lowest oxygen tension in the GI tract, and, in principle, could directly impact HIF-1α activation and LL-37 expression. HT-29 colonocytes pretreated with mimosine significantly reduced fungal levels in a dose-dependent manner (Supplementary Fig. 6e), but this effect was diminished when we knocked down HIF1A mRNA expression (Supplementary Fig. 6f–h). Also, mimosine did not inhibit the growth of CA in the absence of cocultured colonocytes (Supplementary Fig. 6i). Thus, pharmacological activation of HIF-1α in colonocytes is required for mimosine-dependent killing of CA.

We next tested the importance of HIF-1α and CRAMP in vivo using genetically engineered mice. We demonstrated that CA GI colonization levels significantly decreased in mice treated with mimosine, but that this effect was nullified in mimosine-treated mice that had Hif1a specifically deleted from their intestinal epithelium (Hif1a WT/Vil-Cre mice, Fig. 4). In prior observations, mortality from invasive disease significantly decreased in mice with CA GI colonization levels < 10^7 CFU/g feces (data not shown). Thus, we administered cyclophosphamide to induce disseminated disease. Strikingly, mice treated with mimosine had a 50% reduction in overall mortality (P = 0.038 by Fisher’s exact test) and significantly increased length of survival (P < 0.001 by log-rank test) compared to their untreated counterparts (Fig. 4c). Further, mimosine treatment had no measurable effect on survival in Hif1a KO/Vil-Cre mice (Fig. 4c). We confirmed a reduction in colonization levels and increased length of survival (P = 0.0082 by log-rank test, Supplementary Fig. 7) when using a second CA strain, CAF2-1.

We next examined whether CRAMP was necessary for the mimosine-induced antifungal effects that we observed. First, we verified that colonic Hif1α and Camp mRNA expression significantly increased in mimosine-treated mice (Fig. 4e). Second, Camp-knockout mice treated with mimosine showed neither a significant decrease in C. albicans GI colonization levels (Fig. 4b) nor an increase in mortality after administration of cyclophosphamide (Fig. 4d) compared to untreated controls. As expected, colonic Hif1α mRNA expression significantly increased, and Camp expression was negligible in mimosine-treated Camp-knockout mice (Fig. 4e,f).
In mice pretreated with antibiotics (PS) and then co-colonized with CA and B. theta, HIF-1α and CRAMP were required for B. theta–induced protection against CA colonization of the gut (Fig. 4g). In the absence of antibiotics, HIF-1α or CRAMP was not necessary to maintain CA colonization resistance in mice, which we attribute to the presence of other redundant immune pathways that may aid in maintaining CA colonization resistance when a mature and intact gut microbiota is present (Supplementary Fig. 8).

Humans are considered the main reservoir of CA. Reportedly 40–80% of humans living in westernized societies are colonized with CA, but more recent studies of humans living in remote and traditional societies exhibit a CA GI carriage rate of less than 10%7,28. An ongoing study of human subjects suggests that the CA carriage rate in humans may be lower than previously reported (data not shown). Thus, CA might not be a “normal” commensal of the human gut, but a more recently acquired commensal resulting from medical advances (for example, antibiotic use) and adoption of Western diets.

Commensal anaerobes, which account for >99% of all gut bacteria29–31, are needed to maintain CA colonization resistance in mice. Notably, we show that two anaerobic species belonging to the same genus, Bacteroides fragilis and Bacteroides thetaiotaomicron, had markedly different effects on CA colonization: B. theta reduced CA colonization but B. fragilis did not. This discrepancy is probably explained by differing commensal bacteria–host immune response effects (Fig. 3e–h). Furthermore, B. theta has been shown to stimulate production of other antimicrobial peptides (RegIIIγ ref. 32), Ang4 (ref. 33)) that may result in fungal killing. In contrast, B. fragilis has been shown to control activation of T cell–dependent immune responses34, which we have previously shown to have no effect on CA GI colonization in mice4. Similarly, L. acidophilus reduces Candida colonization in the stomachs of mice35, but we did not see this effect of L. acidophilus in the distal gut. In contrast, L. reuteri significantly (P = 0.0025, Mann-Whitney) decreased CA colonization and merits further study. Identifying distinct bacterial species may allow us to uncover other host immune effectors that promote CA colonization resistance.

In a stressed system (after exposure to antibiotics and the onset of the resultant gut dysbiosis), the magnitude of the microbiota stimulus that activates the gut’s redundant systems for maintaining CA colonization is markedly diminished, and we find that the elimination of select immune effectors (HIF-1α, CRAMP) is enough to nullify the CA-protective effect mediated by single bacterial species such as B. theta. These findings underline the importance of an intact gut microbiota in maintaining effective gut mucosal defenses against microbial pathogens, and they may have major clinical ramifications for patients (e.g., cancer and transplant patients) who receive broad-spectrum antibiotics and are at high risk for developing invasive fungal infections.

Probiotic therapy would seem to be an obvious approach for modulating CA colonization, and it has had some efficacy in both mice36 and humans37,38. In cancer patients, the introduction of probiotics raises the concern that the probiotic itself may cause infection. In fact, when using B. theta or B. producta as probiotic therapy in anti-biotic-treated mice, we were able to prevent CA dissemination only to find occasional evidence of B. theta or B. producta dissemination (data not shown).

Pharmacologic modulation of HIF-1α levels has been explored extensively in the context of cancer therapy and angiogenesis19,20. HIF-1α agonists boost the bactericidal capacity of phagocytes, and we show that they may augment the fungicidal capacity of GI epithelial cells. As noted above, another potential approach could use commensal anaerobe metabolites, such as SCFAs. Finally, because SCFAs are naturally occurring metabolites of the gut, adverse effects typically associated with pharmacologic agents may not be a concern.

In conclusion, the emergence of invasive fungal disease in humans correlates with advances in medical therapy, particularly antibiotic use and invasive surgical procedures. When augmenting innate cellular function or mucosal integrity is difficult, if not impossible (e.g., in immunocompromised patients), boosting GI mucosal immune effectors to reduce fungal burden may be the key to tipping the balance back toward homeostasis and preventing invasive fungal disease.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. 16S rRNA data (analyzed data and trimmed FASTA sequences for each sample) were deposited at Figshare (http://dx.doi.org/10.6084/m9.figshare.950989).

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

A.Y.K. and L.V.H. conceived and designed the experiments. L.V.H. provided gnotobiotic mice and mucosal immunology instruction and support. A.Y.K., D.E., L.A.C., T.R.S.-W. and M.M.N. performed the experiments. A.Y.K., J.K., M.K., X.Z. and Y.X. conducted microbial profiling and statistical analysis. A.Y.K. and L.V.H. analyzed the data. A.Y.K. wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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**ONLINE METHODS**

**Fungal and bacterial strains.** The *Candida* spp. and bacterial strains used are listed in Supplementary Table 3. *C. albicans* strain SC5314 was originally obtained from W.A. Fonzi (Georgetown University Medical Center, Washington, D.C.). All other CA strain sources are cited in the Acknowledgments section. Unless otherwise noted, *C. albicans* refers to strain SC5314.

**Mouse studies.** Mouse strains used are listed in **Supplementary Table 1**. All animal experiments were done in accordance with US National Institutes of Health (NIH) guidelines, the Animal Welfare Act and US federal law. The University of Texas Southwestern Medical Center’s Institutional Animal Care and Use Committee approved the experimental protocol “2009-0243” that was used for this study. All animals were housed in a centralized and Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) –accredited research animal facility that is fully staffed with trained husbandry, technical and veterinary personnel. Unless otherwise noted, mice used for experiments were C57/BL6 (Harlan), sex-matched and 6–8 weeks of age. Mice within an experiment were littermates that remained cohoused in the same cage to ensure a shared microbiota. Germ-free C57BL/6 mice were maintained in isolators as described.

For germ-free and neonatal/infant mouse experiments, a sample size of four per group was chosen as the best compromise between providing an adequate sample size for assessing differences in colonization balanced with the limited availability of germ-free and neonatal mice. With the antibiotic-treated mice, we used a sample size of 5–8. Inclusion/exclusion criteria were not established. No animals were excluded from analysis. Randomization was not used. For the murine studies, age and sex-matched mice were arbitrarily assigned to a treatment group (i.e., antibiotics, bacteria or mimosine). No blinding was done for mouse studies.

**Conditional knockout of Hif1a in the murine intestine.** *Hif1afl/fl* (B6.129-Hif1aαtm3Rsjo/J, C57/BL background, Jackson Laboratories, floxed at exon 2) were bred with a transgenic strain expressing Cre recombinase under the control of the murine villin promoter (B6.SJL-Tg(Vil-cre)997Gum/J, C57/BL background, Jackson Laboratories). *Hif1a* deletion in intestinal tissues (versus non-intestinal tissues) was confirmed by PCR on mouse genomic DNA (Supplementary Fig. 9). *Hif1a* mRNA levels were markedly decreased in the ileums and colons, but not in the spleens, of *Hif1afl/flVil-Cre* mice compared with their wild-type littermates (Supplementary Fig. 9).

**Antibiotic experiments.** Mice were fed sterile or antibiotic-supplemented water and colonized with *C. albicans* as previously described. As for oral antibiotic treatment, mice were fed sterile water with 1 mg/ml streptomycin (STR), 2) 1500 U/ml penicillin G (PCN), 3) PCN/STR, 4) 0.5 mg/ml clindamycin (C) or 5) 1 mg/ml metronidazole (M) for 5 d before *C. albicans* administration. For oral antibiotic treatment, mice were treated with PS water (starting on day −14). *C. albicans* GI colonization levels were quantified every 7 d thereafter. Bacterial add-back in antibiotic-treated mice. PS-treated adult mice (C3H/HeN, female, 6–8 weeks, n = 8 per group) were colonized with *C. albicans*. Mice were then gavaged with a single bacterial strain (2 × 10^6 CFU). A no-bacteria control was included. All mice were then transitioned to sterile water. Bacterial and *C. albicans* GI colonization levels were measured after 14 d of co-colonization.

**Bacterial add-back in germ-free mice.** Germ-free mice (no antibiotic treatment) were first colonized with *C. albicans* by oral gavage (2 × 10^6 CFU). *B. theta* or *B. producta* was then administered by oral gavage (2 × 10^6 CFU). Bacterial and *Candida* colonization levels were checked every 2–3 d, up to 20 d after bacterial oral gavage.

**SCFA experiments.** PS-treated adult mice (C3H/HeN, female, 6–8 weeks, n = 4 per group) were colonized with *C. albicans* by oral gavage. Mice were then treated with PS water ± 50 mM SCFAs (butyric, acetic or propionic acid). The no-treatment control group received pH-adjusted (with HCl) PS water. Colonization levels were checked after 14 d of SCFA water treatment.

1-**Mimosine experiments.** *Hif1afl/fl* and *Hif1afl/flVil-Cre* mice were bred, genotyped, and functionally confirmed as noted above. Congenic *Camp*-knockout mice (B6.129X1-Campmin1J, Jackson Laboratories) were obtained. Decreased *Camp* mRNA expression in intestinal tissues was confirmed (Supplementary Fig. 9c). Mice were treated with PS and then colonized with *C. albicans* strains SC5314 or CAFA-2. Mice were treated with PS water or PS water and 500 µM 1-mimosine ad libitum. PS and PS-mimosine water was changed every 2–3 days for the duration of the experiment. *C. albicans* GI colonization levels were checked daily for the first 5 d of mimosine treatment. Cyclophosphamide was administered to all groups on days 1, 3 and 5 of mimosine treatment to induce disseminated disease.

**Creation of mouse-adapted *C. albicans* strains.** *C. albicans* strains SC5314 and CAFA-2 were orally gavaged to adult mice (C3H/HeN, female, 6–8 weeks, Harlan) pretreated with penicillin-streptomycin, recovered from fecal cultures, and archived (18% glycerol frozen stock). Archived *C. albicans* strains were grown and administered to a new set of antibiotic-treated mice, recovered and archived again. This process was carried out for a third time. Final archived cultures were used for mouse experiments.

**Isolation of bacterial and fungal genomic DNA.** Fecal specimens or intestinal tract segments were collected, flash frozen with liquid nitrogen, weighed and immediately resuspended in extraction buffer (200 mM NaCl, 200 mM Tris, 20 mM EDTA, 6% SDS) and 0.5 ml of phenol–chloroform–isoamyl alcohol, pH 7.9 (Ambion). Cells were lysed by bead-beating (0.1 mm zirconia/silica beads for bacterial gDNA (BioSpec), 0.6 mm acid-washed glass beads for fungal gDNA (Sigma)) and subjected to additional phenol–chloroform extractions. Crude DNA extracts were treated with RNaseA (Qiagen) and column-purified (PCR Purification Kit, Qiagen). DNA concentrations were quantified by a fluorescence-based assay (Quant-IT PicoGreen dsDNA, Life Technologies).

**Isolation of ileal and colonic intestinal epithelial cells (IECs).** To confirm deletion of *Hif1a* (Exon 2) in intestinal epithelial cells of *Hif1afl/flVil-Cre* mice, colons and ileums were isolated, opened longitudinally and rinsed with PBS. The epithelial integrity was disrupted by treatment with 1 mM diethythero (DTT) for 30 min at 37 °C on a shaker, followed by vortexing for 1 min. The liberated IECs were collected, resuspended in 5 ml of 20% Percoll and overlaid on 2.5 ml of 40% Percoll in a 15-ml Falcon tube. Percoll gradient separation was performed by centrifugation at 780g for 20 min at 25 °C. The interface cells were collected and used as IECs. gDNA was immediately isolated from IECs with the protocol detailed above.

**Quantitative PCR for microbiota analysis.** Bacterial and fungal loads were quantified by qPCR analysis (SsoAdvanced SYBR Green Supermix, Bio-Rad) of microbial gDNA using universal 16S rRNA gene or fungal internal transcribed spacer (ITS1-2) primers (Supplementary Table 4). The abundance was given by oral gavage on day −7. PS water was replaced with sterile water on day 0. *C. albicans* GI colonization levels were quantified every 7 d thereafter. Bacterial add-back in antibiotic-treated mice. PS-treated adult mice (C3H/HeN, female, 6–8 weeks, n = 8 per group) were colonized with *C. albicans*. Mice were then gavaged with a single bacterial strain (2 × 10^6 CFU). A no-bacteria control was included. All mice were then transitioned to sterile water. Bacterial and *C. albicans* GI colonization levels were measured after 14 d of co-colonization.
Preparation of mouse colon protein extracts and western blot analysis. Protein extracts for western blot analysis were generated from mouse distal colon. A 2 cm piece of freshly isolated intestinal tissue was flushed, flash frozen and pulverized under liquid N2. The pulverized tissue was resuspended in 1 ml of ice-cold extraction buffer (8 M urea, 0.15 M Tris-HCl pH 7.5) with protease inhibitors (Complete Protease Inhibitor Cocktail, Roche) and lysed by homogenization, sonication and passing the suspension through an 18-gauge needle 3–5 times followed by 3–5 passages through a 21-gauge needle. Total protein was precipitated with trichloroacetic acid and resuspended in electrophoresis sample buffer (50 mM Tris-HCl pH 8.8, 2% SDS, 10% glycerol, 2 mM EDTA, 100 mM DTT). Equal amounts of protein (25 µg), as quantified using the Micro BCA Protein Assay (Pierce), were loaded onto a 10–20% Tris-Tricine gel (Bio-Rad), subjected to electrophoresis and transferred to a 0.45 µm PVDF membrane (Bio-Rad). Membranes were blocked in Odyssey Blocking Buffer (Li-Cor) for 1 h at room temperature and incubated overnight at 4 °C with a polyclonal rabbit anti-CRAMP antibody (against amino acids 135–173, PA-CRCL-100, Innovagen). Of note, mouse CRAMP (amino acids 28–173) has a molecular mass of 16.422 kDa. Actin (pan-actin rabbit monoclonal antibody, D181C1, Cell Signaling) was used as an internal control to confirm equal protein loading. To calculate relative protein levels, the density of specific bands was quantified using a densitometer imaging system (ImageJ software version 1.43 s, NIH, Bethesda, MD); http://imagej.nih.gov. Values obtained for CRAMP immunoblots were normalized to the optical density of corresponding immunoblots for actin.

16S rRNA gene PCR amplification and sequencing. 16S rRNA genes (variable region 4, V4) were amplified from each sample using a composite forward primer and a reverse primer containing a unique 10-base barcode that was used to tag PCR products from respective samples. We used the forward primer 5′-CCTACCTCCTACGGGAGGCAGCAG-TAGTCAG-NNNNNNNNNNNYTGGYDFAAGNG-3′: the italicized sequence is 454 Life Sciences primer A; NNNNNNNNNN designates the unique 10-base barcode used to tag each PCR product; and the bold sequence is the broad-range bacterial primer 536F. The reverse primer used was 5′-CCATATGCTTCCTGGCTTGCAGCTTCAAT-3′; the italicized sequence is 454 Life Sciences’ primer B, and the bold sequence is the broad-range bacterial primer 926B5R. PCR reactions consisted of 2.5 U FastStart High Fidelity Taq and 1× buffer (Roche), 400 nM of each primer and 50 ng template. Reaction conditions were 3 min at 95 °C, followed by 15 cycles of 30 s at 95 °C, 45 s at 65–50 °C (decreasing by 1 °C per cycle) and 60 s at 72 °C; then 20 cycles of 30 s at 95 °C, 45 s at 57 °C and 60 s at 72 °C on an Eppendorf Mastercycler. Four independent PCRs were performed for each sample, combined and purified with Ampure magnetic purification beads (Agencourt), and products visualized by gel electrophoresis. No-template extraction controls were analyzed for absence of visible PCR products. Products were quantified using Quant-IT PicoGreen dsDNA Assay Kit (Life Technologies). A master DNA pool was generated from the purified products in equimolar ratios to a final concentration of 10 ng/ml. The pooled products were sequenced using the Roche 454 Titanium platform (University of Texas at Austin Genome Sequencing and Analysis Facility) using Roche/454 Titanium chemistry.

16S rRNA gene sequence analysis. Sequences generated from pyrosequencing-barcoded 16S rRNA gene PCR amplicons were quality filtered. Sequences shorter than 200 nucleotides or longer than 1000 nucleotides were removed. Sequences containing ambiguous bases, primer mismatches, homopolymer runs in excess of six bases and uncorrectable barcodes were also removed. Sequences that passed the quality filtration were denoised and analyzed using the open source software package Quantitative Insights Into Microbial Ecology (QIIME)46. 16S rRNA gene sequences were assigned to operational taxonomic units (OTUs) using UCLUST (http://www.drive5.com/usearch/), with a threshold of 97% pairwise identity, then classified taxonomically using Greengenes (http://greengenes.lbl.gov). 16S rRNA data (analyzed data and trimmed fasta sequences for each sample) was deposited at Figshare (http://figshare.com/articles/Fan_et_al_NMED_A69544_16S_rRNA_Data/950989).

qPCR for quantifying gene expression. Total RNA was isolated from the distal small intestine or colon using the Qiagen RNeasy RNA isolation kit and from HT-29 human colonocytes with Trizol reagent (Life Technologies). Total RNA was used to synthesize cDNA (Script, Bio-Rad). qPCR analysis was performed using the SsoAdvanced SYBR Green Supermix (Bio-Rad) and specific primers (Supplementary Table 4). Signals were normalized to 18S rRNA levels within each sample and normalized data were used to calculate relative levels of gene expression using ΔΔCt analysis.

HT-29 human colonocyte assays. HIF1A and CAMP expression in response to C. albicans. HT-29 cells were obtained from the American Type Culture Collection (ATCC HTB-38; human colorectal adenocarcinoma) and tested negative for mycoplasma contamination. HT-29 cells were propagated in RPMI-1640 with 10% FCS. HT-29 cells were exposed to C. albicans strain SC5314 at a multiplicity of infection (MOI) of 1 for 5 h. Cells were then washed with PBS three times, and total RNA was extracted with TRizol reagent (Life Technologies). HIF1a and CAMP gene expression was evaluated by qPCR as noted above. HIF1A and CAMP mRNA and protein expression in response to l-mimosine. HT-29 cells were propagated in RPMI-1640 with 10% FCS. HT-29 cells were exposed to l-mimosine (0-500 µmol/liter) for 5 h at 37 °C. Cells were then washed with PBS. For gene expression experiments, total RNA was extracted with RIPA buffer, and concentrations determined using BCA assay (Pierce). Proteins were separated by 4–12% Tris-tricine (Innventro) gel electrophoresis. Western blot analysis was performed using anti-HIF-1α (NB100-449, Novus) and anti-IL-37 (NBPI-76864, Novus) rabbit polyclonal antibodies. The secondary Ab was a horseradish peroxidase (HRP)-conjugated goat anti-rabbit (7074, Cell Signaling). Actin (pan-actin rabbit monoclonal antibody, D181C11, Cell Signaling) was used as an internal control to confirm equal protein loading. Immunoreactive proteins were detected using the ECL-chemiluminescent system (EMD Millipore Immobilon).

Effect of l-mimosine on C. albicans growth. C. albicans strain SC5314 cells were grown overnight, washed and resuspended in PBS. Cells were added to RPMI-1640 with 10% FCS at a concentration of 1 × 10^6 CFU/ml. l-mimosine (0–500 µM) was added, and samples were incubated at 37 °C for 8 h. 1 ml of culture was collected at 0, 2, 4, 6 and 8 h after addition of l-mimosine. Quantification of C. albicans was calculated by serial dilution and plating on YPD. HT-29 extracellular killing assays. C. albicans strain SC5314 was grown to mid-log phase (OD600 ~1.0–1.5) in YPD medium at 30 °C. Cells were harvested, washed with and resuspended in PBS, and enumerated with a hemocytometer. 1 × 10^5 CFU of C. albicans was placed in each well (24-well plate) containing confluent HT-29 colonocytes (MOI = 1.0). 24-well plates were quickly centrifuged and then incubated at 37 °C for 1, 3, or 5 h. Supernatants were collected, and cells were washed with PBS three times (and washes were pooled with the original supernatant). C. albicans levels (remaining CFU) were calculated by serial dilution and plating on YPD agar. For experiments with l-mimosine, HT-29 cells were treated with l-mimosine for 3 h before the addition of C. albicans.

HIF1A siRNA knockdown. HT-29 cells (1 × 10^6 cells) were freshly seeded into each well of a 24-well plate. After 24 h of incubation at 37 °C, HT-29 cells
were transfected with 5 nM of HIF1A siRNA (Qiagen) or a scrambled control using HiPerFect transfection reagent (Qiagen). Fungicidal assays were performed 48 h later.

**C. albicans spot assay.** *C. albicans* strain SC5314 cells were grown overnight, washed and resuspended. Cells were incubated with 30 µg of recombinant LL-37/ml (Anaspec) in sodium phosphate buffer at 37 °C for 1 h, serially diluted and spotted onto YPD-agar plates. Plates were incubated for 2–3 days at 30 °C.

**C. albicans SCFA in vitro growth experiments.** *C. albicans* strain SC5314 was grown overnight in YPD at 30 °C under aerobic conditions. Overnight cultures were then harvested, washed in PBS and then diluted to a starting OD$_{600}$ of ~0.2. SCFAs (butyric acid, acetic acid, or propionic acid) were added to the culture at the desired concentration resulting in a pH ranging from 3–6. pH-adjusted controls were created by titrating concentrated HCl into YPD. Cultures were grown at 30 °C at 250 r.p.m. under aerobic conditions for 12 h. Final OD$_{600}$ readings were then measured.

**Candida albicans and B. theta cocultured in vitro growth experiments.** *C. albicans* strain SC5314 and *B. theta* were first grown individually in TYG medium at 37 °C under anaerobic conditions. Overnight cultures were then diluted to an OD$_{600}$ = 0.2 for the initial time point (0 h). CA and *B. theta* cocultures were created by combining equivalent volumes (5 ml) of CA and *B. theta* individual diluted cultures. Cultures were sampled every 24 h (for a total of 96 h), and CA and *B. theta* (CFU) were enumerated by serial dilution and plating on YVG agar (aerobically) and BHI/blood agar (anaerobically), respectively.

**Statistical analyses.** A comparison of GI colonization levels was analyzed by Mann-Whitney tests, and when multiple comparisons or more than two groups were analyzed, Bonferroni's correction to the significance level $\alpha$ was invoked. Bacterial 16S rRNA gene copy numbers and qPCR gene expression analyses were compared using a Mann-Whitney test. Survival data were analyzed by both Fisher's exact test (overall survival) and log-rank test (length of survival, Kaplan-Meier curves). Statistical analyses were carried out using the GraphPad Prism Software (San Diego, CA).

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