Out with the bad, in with the good! How do we harness commensal fungi in early life without risking dangerous pathogenic infection?

In early life, commensal microbes play a crucial role in shaping multiple aspects of host development. However, because the immune system is also maturing during this period, there's an increased risk of opportunistic infections from certain members of the microbiota. Despite extensive research focusing on how the host regulates commensal microbes, we have limited understanding of the microbial characteristics that distinguish between commensalism and pathogenesis, particularly in early life. This knowledge gap is significant because the microbes that provide beneficial developmental stimuli often have close genetic relatives with those posing the greatest risk for infections(Wiles and Guillemin 2019). Our collaboration will focus on one such group of fungal commensals to elucidate microbial mechanisms differentiating beneficial and pathogenic activities in the early life microbiota.

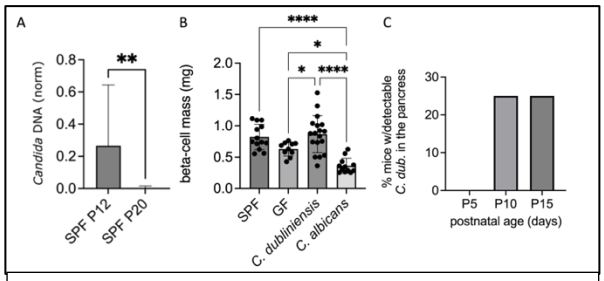

Fig. 1. (A) *Candida* specific ITS gene copies detected in the neonatal mouse gut of SPF (specific pathogen free) mice at postnatal (P) age day 12 and 20. Abundance is high initially, and lowers with age, similarly to humans. (B) Pancreatic beta-cell mass of individual mouse pups (P20) mono-associated (MA) with the indicated fungi. (C) % mice per MA litter w/ detectable *C. dubliniensis* in the pancreas.

Candida are polymorphic fungi that colonize mucosal niches shortly after birth and are particularly abundant during early human and mouse development (Fig. 1A)(Ward Tonya, Dominguez-Bello Maria et al. 2018, Rao, Coyte et al. 2021). Certain *Candida* species, pose a significant threat during these early stages. For example, 1-10% of infants born prematurely develop fungal sepsis, with *Candida albicans* being the primary cause of infection(Manzoni, Jacqz-Aigrain et al. 2011). Yet, recent findings published by Dr. Hill show that a species closely related to *C. albicans, C. dubliniensis,* plays a beneficial role in promoting immune pathways(Hill, Bell et al. 2025). Specifically, *C. dubliniensis* colonization in neonatal mice enhances the development of insulin-producing beta-cells in the pancreas via resident macrophages (Fig. 1B), leading to long-term benefits for metabolic health[6]. This in contrast with the effect of *C. albicans,* which causes a significant *reduction* in beta-cell mass (Fig. 1B)(Hill, Bell et al. 2025). Paradoxically, the beneficial impact of *C. dubliniensis* correlates with a behavior normally associated with pathogens, namely, escape from the gut and dissemination. Indeed, in disseminated infection models, *C. albicans* is far more virulent than *C. dubliniensis*. Nevertheless, *C. dubliniensis* disseminates to the pancreas during the developmental time period that co-incides with greatest beta-cell expansion (Fig. 1C), indicating its disseminatory capacity may be required for its beneficial effect. This dichotomy between pathogenicity and commensalism at disseminated sites highlights the need to better understand the natural interplay between different *Candida* species and the host during early life development.

One possibility is that the widely diverse polymorphic characteristics of *Candida spp*. result in differential immune recognition and downstream outcomes at systemic sites. Both *C. dubliniensis* and *C. albicans* form multiple semi-stable cellular morphotypes in vivo, including yeast and hyphae(Moran, Coleman et al. 2012, Noble, Gianetti et al. 2017). However, little is known about how these dynamic morphotypes are regulated in vivo. Cell wall composition and expression of effectors and virulence factors differs dramatically between morphotypes(Noble, Gianetti et al. 2017). Hyphae are classically considered the most pathogenic, as they express adhesins and cytolytic toxins that facilitate adherence and invasion of host tissue. Indeed, Dr. Ost previously found that *C. albicans* hyphae exacerbated damaging inflammation associated with colitis. Futhermore, alterations in *C. dubliniensis* cell wall glycan abundances, which are also dynamically regulated during morphological switches, appear to mediate the effect of *C. dubliniensis* in the host pancreas(Hill, Bell et al. 2025). Thus, differing impacts of *C. albicans* and *C. dubliniensis* may depend on the representation of distinct morphotypes within the developing gut. Appreciating the nuances of these host-microbe dynamics could allow us to harness beneficial effects of early life commensal *Candida* species, while circumventing the harms of pathogenic Candidiasis. We hypothesize that the difference between beneficial and pathogenic outcomes in early life are the result of differential colonization and dissemination capacities, as well as on host sensing of unique varitaions in fungal cell structure.

Aim 1: *Determine whether there is differential colonization or dissemination dynamics between pathogenic vs beneficial Candida that are unique during early life*.
Aim 2: *Compare Candida morphological, cell wall, and single-cell transcriptional changes that occur along the timeline of host colonization in pathogenic vs beneficial Candida.*

Research Strategy: We will mono-associate germ-free (GF) dams and their pups with wild-type cultures of either *C. albicans* or *C. dubliniensis.* Mouse experiments will be done using standard gnotobiotic techniques in Dr. Hill's established germ-free mouse facility at Boulder, using GF Swiss Webster mice (which have large litters) . As illustrated in Figure 2, we will sample litters from each treatment group (*C. dubliniensis*, *C. albicans*, or GF) at five-day intervals across postnatal life to capture any changes in phenotype that mirror the progressive development of the pups. Importantly, our studies analyzing the effect of *Candida* on pancreatic tissue show that these phenotypes manifest between postnatal day P10 and P15 (Hill, Bell et al. 2025). Finally, to test whether fungal-host phenotypes are unique to early-life, we will also sample adult mice from each treatment group. Biological Replicates and Mice: To power this project statistically, we will collect three biological replicates at each time point for each of the 3 treatment groups and appropriate statistical testing will be used to determine significant differences between groups.

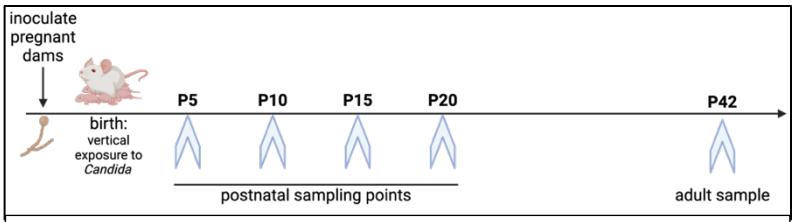

Fig. 2. Schematic of longitudinal sampling plan. P = postnatal day age

Aim 1 – Hill Lab: *Determine whether there is differential colonization or dissemination dynamics between pathogenic vs beneficial Candida that are unique during early life*: To compile a comprehensive, longitudinal snapshot of *Candida* colonization and dissemination, we will sample tissues from intestinal and clinically relevant systemic sites, from pups across treatment groups, including: intestinal and colonic contents, pancreas, spleen, liver, & kidneys. Each sample will be collected and processed aseptically, weighed, homogenized, and resuspended in sterile PBS. Fungal load will be enumerated by quantitative culture on fungal selective media, along with qPCR using species-specific rDNA primers. This approach has been successful in capturing even small biomasses of fungi within mammalian tissue (Fig. 1A, C, (Dohlman, Klug et al. 2022)). This will comprehensively profile the disseminatory capacity of each *Candida* species during early life development.

Aim 2 – Ost Lab: *Compare Candida morphological, cell wall, and single-cell transcriptional changes that occur along the timeline of host colonization in pathogenic vs beneficial Candida:* Using intestinal contents collected in Fig. 2, we will profile *C. albicans* and *C. dubliniensis* morphology, expression of immune-stimulatory cell wall carbohydrates, and transcriptional profiles at each developmental time point. Fungal morphology will be assessed using immunohistochemistry and imaging flow cytometry, techniques previously employed by Dr. Ost for *C. albicans*(Ost, O'Meara et al. 2021). We will use fluorescent stains and flow cytometry (also established techniques in Ost lab) to quantify the levels and architecture of fungal cell wall components important for activating anti-fungal innate immune receptors, including β-glucan, mannan, and exposed chitin(Avelar, Dambuza et al. 2022, Hill, Bell et al. 2025). Special attention will be given to cell wall mannan, as Dr. Hill established a correlation between *C. dubliniensis* mannan levels and ability to stimulate beta cell expansion(Hill, Bell et al. 2025). Finally, we will employ single-cell RNA sequencing on intestinal *C. albicans* and *C. dubliniensis* to define distinct transcriptional states within the gut. The Ost lab has optimized a droplet-based scRNA sequencing for *C. albicans* (10x genomics platform), revealing significant transcriptional differences between this fungus from culture compared to sorted from mouse intestinal contents, and three distinct transcriptional states within the gut (Fig. 3). We will use this technique to profile both *C. albicans* and *C. dubliniensis* from mice (pooled for each group) at P15, a critical time point for stimulating beta cell expansion, and in adulthood. This will be used to compare the transcriptional states represented for both *C. albicans* and *C. dubliniensis* and identify cellular processes and effectors that differentiate these species.

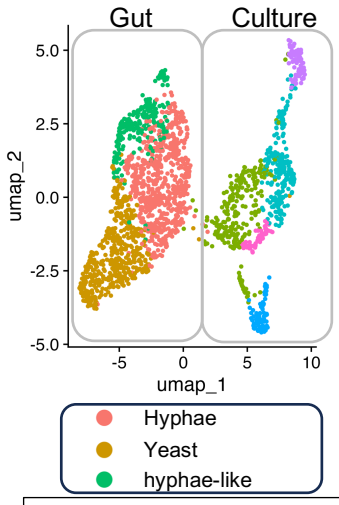

Fig. 3. scRNA-sequencing umap of *C. albicans* sorted from the mouse intestinal contents and from culture. Gut-associated cell types noted.

Impact & Outlook: This study aims to provide the first comprehensive understanding of *Candida-host* interaction dynamics during early life development. These foundational insights will pave the way for ongoing collaboration between the Ost and Hill labs, elucidating the 1) immune pathways regulating gut escape in pathogenesis vs commensalism, 2) health implications of perturbing *Candida* colonization early in life, and 4) how the microbiota regulates both dissemination and pathogenic potential of *Candida* species. These are primary themes of both the Hill and Ost labs and will provide the necessary preliminary data for new grant applications, which we envision at NIAID and/or NIDDK. The immediate-term plan is to submit an application for an mPI R21 for NIAID (already discussed these aims with NIAID PO Dona Love).