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IκBζ is an essential mediator of immunity to oropharyngeal candidiasis

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SUMMARY

Fungal infections are a global threat, yet there are no licensed vaccines to any fungal pathogens. Th17 cells mediate immunity to *Candida albicans*, particularly oropharyngeal candidiasis (OPC), but essential downstream mechanisms remain unclear. In murine model of OPC, IκBζ (*Nfkbiz*, a noncanonical NF-κB transcription factor) was upregulated in an IL-17-dependent manner and was essential to prevent candidiasis. Deletion of *Nfkbiz* rendered mice highly susceptible to OPC. IκBζ was dispensable in hematopoietic cells and acted partially in the suprabasal oral epithelium to control OPC. One prominent IκBζ-dependent gene target was β-defensin (BD)-3 (*Defb3*), an essential antimicrobial peptide. Human oral epithelial cells required IκBζ for IL-17-mediated induction of BD2 (*DEFB4A*, human orthologue of mouse *Defb3*) through binding to the *DEFB4A* promoter. Unexpectedly, IκBζ regulated the transcription factor EGR3, which was essential for *C. albicans* induction of BD2/*IDEFB4A*. Accordingly, IκBζ and EGR3 comprise an antifungal signaling hub mediating mucosal defense against oral candidiasis.

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AUTHOR CONTRIBUTIONS

Conceptualization – SLG, TCT
Methodology – DMS, ACP, NOP, JTD
Investigation – TCT, BMC, SPA, ID, JTD, NOP, DMS
Writing – original draft – TCT, SLG
Writing – review and editing – SLG, TCT, MJM, HRC, NOP
Visualization – TCT, SLG
Supervision – SLG, HRC, MJM, ACP
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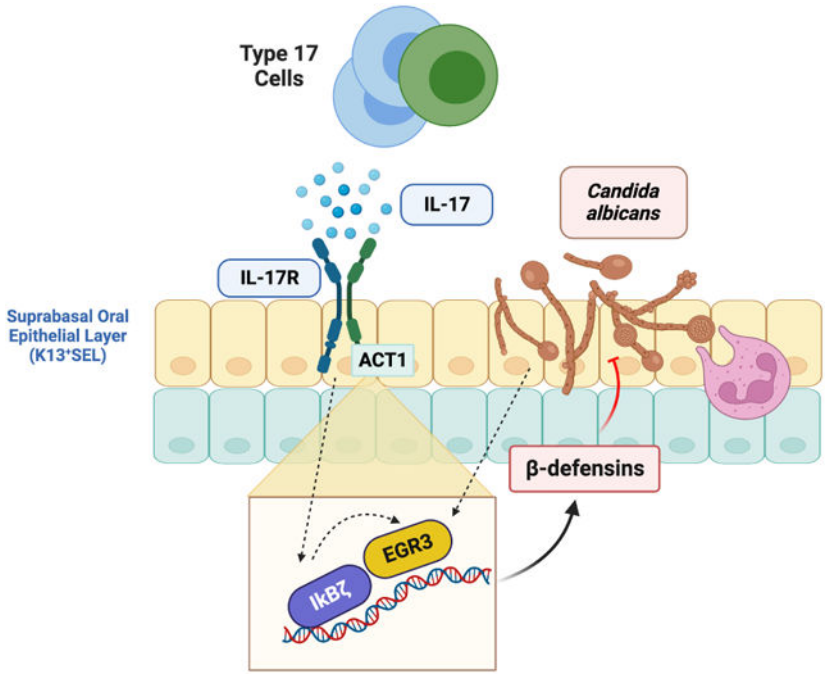
DECLARATION OF INTERESTS

The authors declare no competing interests.

Graphical Abstract



eTOC blurb

Fungal pathogens are of increasing medical concern. Oropharyngeal candidiasis (OPC) is the most common fungal infection in humans, but tissue-specific correlates of oral immunity are poorly understood. Taylor et al show that IL-17 prevents OPC through the non-canonical transcription factor, IκBζ, that regulates essential antimicrobial peptide effectors.

Keywords

Candida albicans ; IL-17 signaling; oral epithelium; defensins; signal transduction; cytokines

INTRODUCTION

Fungal infections are of increasing medical concern, especially among the immunocompromised. To date, there are only limited effective anti-fungal therapies and no licensed vaccines to any fungal species. *Candida albicans* is the most common causative agent of fungal infections in humans, with multiple manifestations including oral, vaginal and systemic disease [1]. Oropharyngeal candidiasis (OPC, oral thrush) can range from a mild, self-limiting condition to a severe superficial infection linked to oral cancer, nutritional deficits, and failure to thrive in infants [2,3]. OPC occurs as a result of systemic immune defects that occur in HIV infection, chemotherapy, antibiotic use or congenital immune defects, as well conditions that negatively impact oral-specific immunity such as head-neck irradiation and Sjögren's syndrome [4].

Compared to many other tissues, site-specific immunity within the oral mucosa is still poorly understood [5,6]. The cytokine IL-17 (IL-17A) has emerged as a vital mediator of immunity to candidiasis, especially in the oropharynx [7]. Although produced predominantly by lymphocytes, IL-17 acts upon nonhematopoietic cells, particularly epithelial cells lining the gut, lung and mouth. *C. albicans* is dimorphic, and the conversion from a yeast form to an invasive hyphal morphotype is a key feature of pathogenesis. Oral epithelial cells (OECs) respond to pathogenic hyphae by upregulating an innate immune defense program composed of cytokines, chemokines, antimicrobial peptides (AMPs), and other antifungal effectors [8,9].

The stratified oral epithelium is comprised of multilayered cell subsets that provide the first line of pathogen recognition [10-12]. Upon infection and damage caused by *C. albicans* hyphal invasion, the suprabasal epithelial layer (SEL) of the tongue and buccal mucosa is sloughed and swallowed, aiding clearance. The basal epithelial layer (BEL) responds to *C. albicans*-induced injury through a wound healing process that replaces the exfoliated SEL [13]. OEC layers are characterized by distinct cytokeratin pairs, with the SEL expressing keratin 13 (K13) and K4, and the BEL is marked by K5 and K14 [14]. We showed that IL-17RA signaling is required in $K13^+$ cells, whereas IL-22R acts dominantly in the $K14^+$ cell layer [15]. Thus, specific cellular subsets within the oral epithelium respond preferentially to inflammatory stimuli.

IL-17 signals through IL-17RA and IL-17RC via the essential adaptor Act1 [16]. Loss of either subunit or Act1 in mice or humans predisposes to oral candidiasis [17-23]. IL-17 orchestrates essential antifungal events via complex downstream signaling pathways that converge on activation of transcription factors (TFs). Prominent IL-17-activated TFs include CCAAT/enhancer binding proteins (C/EBP)-β and -δ, MAPK-associated TFs (c-Fos, c-Jun), AT-rich interacting protein 5a (Arid5a), CUX1, and NF-κB family members [16,24,25]. In addition, IL-17 promotes post-transcriptional pathways that stabilize or otherwise control target mRNAs, many of which include these TFs [16]. Prior studies surprisingly ruled out roles for many IL-17-induced TFs in OPC, including C/EBPβ, c-Fos and Arid5a [26-28] and thus the essential signaling activators that underlie IL-17-driven immunity to OPC have been enigmatic.

Another IL-17-activated TF is IκBζ, encoded by *Nfkbiz*. IκBζ is an atypical member of the IκB family that, despite its name, usually acts as a transcriptional activator [29,30]. Originally identified as a mediator of IL-1/TLR responses, IκBξ regulates genes characteristic of the IL-17 pathway through promoter activation and chromatin remodeling [25,31-35]. In T cells, IκBζ promotes Th17 differentiation. Consequently, mice with a global *Nfkbiz* gene deletion are resistant to IL-17-driven conditions such as experimental autoimmune encephalomyelitis (EAE) and psoriasis-like skin inflammation [34,36]. Despite such impairments, IκBζ-deficient mice develop spontaneous dermatitis and lacrimal inflammation that bears similarities to Sjögren's syndrome [37].

Given these dichotomous physiologic impacts of IκBζ, it was not obvious whether IκBζ loss would enhance IL-17-driven inflammation and thus protect against OPC, or instead impair IL-17 signaling and thereby promote candidiasis. Here we report that mice lacking *Nfkbiz* in all tissues are highly sensitive to OPC. A portion of this susceptibility was

mediated through suprabasal OECs, particularly via antifungal β-defensins. Moreover, IκBζ regulates the TF early growth factor 3 (Egr3) in OECs. Although not a direct target of IL-17, Egr3 drives production of β-defensins in response to *C. albicans* infection in a manner distinct from IκBζ. These findings revealing a regulatory TF circuit that coordinates the fungal- and host-driven pathways controlling oral mucosal candidiasis.

RESULTS

IκBζ is induced by IL-17 in oral candidiasis

IL-17 and IL-22 are nonredundant mediators of host defense against OPC. Whereas IL-17 acts dominantly on a $K13^+$ suprabasal layer of the oral epithelium (SEL), IL-22 acts on the stem-like basal $K14^+$ epithelial cell layer (BEL) [13,15] (Fig 1a). To probe mechanisms that drive effective antifungal immunity, we evaluated a previously-published RNASeq dataset [15] for TFs induced in the oral mucosa in an IL-17-dependent manner [38]. We specifically focused on genes induced at day 2, as this coincides with peak expression of Type 17 cytokines (*Il17a, Il22*) and essential antifungal genes (*Defb3*, CXC chemokines) (Fig 1b) [15,17].

Multiple TF classes were upregulated in the tongue during OPC. CCAAT/Enhancer binding proteins C/EBPβ and C/EBPδ are instrumental in regulating many downstream IL-17 target genes [39,40] and were upregulated in OPC, though surprisingly expression was not strongly IL-17-dependent in this setting (Fig 1c). C/EBPβ contributes to OPC immunity in only a limited way [27], but the contribution of C/EBPd is unknown. *Cebpd*$^{-/-}$ mice [41] were infected orally with *C. albicans* and fungal loads assessed at 5 days p.i., the time point at which control WT mice efficiently cleared the infection. Mice lacking C/EBPδ cleared the fungus completely, with no weight changes or detectable fungal loads after 5 days of infection (Supplementary Fig S1a, b). Therefore, IL-17-associated C/EBPβ and C/EBPδ are dispensable to control OPC.

In addition to C/EBPs, several members of the NF-κB family were elevated during OPC, including *Nfkbiz*, encoding IκBζ (Fig 1c, d). IκBζ protein was significantly increased in WT tongue homogenates following oral *C. albicans* infection and was largely absent in tongues from *Il17ra*$^{-/-}$ mice (Fig 1e). In keeping with findings in mouse, a prominent band corresponding to IκBζ was evident in immunoblots of lysates from a human OEC line (TR146) following IL-17 treatment, though densitometric measurements showed quite a bit of variablity (Fig 1f). Induction of IκBζ was cyclical, with prominent induction consistently seen at 1 hour, reduced expression at 1-3 hours and then rebound at 6 hours. This likely reflects the dependence of IκBζ on the canonical NF-κB pathway, which undergoes dynamic oscillations due to a complex network of transcriptional and post-transcriptional feedback regulators [29,42]. Upon IL-17 stimulation, a prominent, slower-migrating band was observed in immunoblots, which likely corresponds to a post-transcriptionally modified form of IκBζ (e.g., a phosphorylated isoform [43]). Hence, IκBζ is elevated in OPC in a manner requiring IL-17 signaling.

IκBζ in the nonhematopoietic compartment is required for immunity to OPC

Although numerous TFs are upregulated during OPC in an IL-17-dependent manner, surprisingly few evaluated thus far are needed for fungal clearance [26-28]. Studies of IκBζ in oral settings have been confounded by the fact that *Nfkbiz*$^{-/-}$ mice show major baseline defects, including a Sjögren's-like syndrome with oral manifestations [35,37]. Therefore, to avoid developmental or early-life influences of IκBζ deficiency, we crossed *Nfkbiz*$^{fl/fl}$ to *Rosa26*^{CreERT2} mice, which express Cre under control of a ubiquitous but tamoxifen (TAM)-inducible promoter, permitting timed but permanent deletion of the *Nfkbiz* gene in all tissues. *Nfkbiz*^{R26ERT2} mice were administered TAM for 5 days and rested for 7-9 days (Fig 1b). Unlike mice lacking IκBξ from birth [37], *Nfkbiz*^{R26ERT2} mice given TAM and subject to sham infections (PBS) did not show apparent inflammatory lesions or other deficits. However, *Nfkbiz*^{R26ERT2} mice subjected to OPC exhibited high fungal loads after 5 days, at levels similar to mice lacking the IL-17 receptor (*Il17ra*$^{-/-}$) or its essential adaptor Act1 (*Act1*$^{-/-}$) (Fig 2a). All infected *Nfkbiz*^{R26ERT2} mice had detectable fungal loads and showed progressive weight loss throughout the infection (Fig 2a). Therefore, IκBζ is vital for immunity to OPC.

IL-17 mediates immunity to oral *C. albicans* through antimicrobial peptides (AMPs) such as β-defensin 3 (BD3, encoded by *Defb3*) and through neutrophil recruitment, mediated indirectly by epithelial expression of CXC chemokines [15,17,44]. Loss of *Nfkbiz* impaired *Defb3* expression in tongue during OPC (Fig 2b). Expression of *Cxcl1* and *Cxcl5* both trended downward in *Nfkbiz*^{R26ERT2} mice, and neutrophil recruitment to the oral mucosa was concomitantly reduced (Fig 2c, d). These defects likely explain the susceptibility of IκBζ-deficient mice to oral candidiasis.

IL-17 and IL-22 act dominantly in nonhematopoietic cells due to restricted expression of their respective receptors, but *Nfkbiz* is ubiquitously expressed in oral cell types in both hematopoietic and stromal cell types (Supplementary Fig S2) [45]. To determine whether IκBζ functions in hematopoietic cells to during OPC, WT recipient mice were lethally irradiated and adoptively transferred with bone marrow from WT or *Nfkbiz*^{R26ERT2} donors. After 6 weeks, chimeric mice and controls were treated with TAM (to delete *Nfkbiz*), subjected to OPC, and fungal loads evaluated at day 5. WT recipients cleared the fungus, regardless of whether they were given WT or *Nfkbiz*^{R26ERT2} BM (Fig 2e), indicating that IκBζ is, rather surprisingly, not required in hematopoietic cells to drive immunity to OPC.

IκBζ functions in the oral epithelium

The above data pointed to essential IκBζ activities within the nonhematopoietic compartment, and we have shown that IL-17 antifungal signaling occurs dominantly in the SEL (see Fig 1a). To determine if IκBζ was similarly required for antifungal activities in the suprabasal oral epithelia, we crossed *Nfkbiz*$^{fl/fl}$ to mice bearing a K13^{Cre} cassette [15]. At 5 days p.i. *Nfkbiz*^{K13} mice consistently had detectable fungal loads, whereas WT and *Nfkbiz*$^{fl/fl}$ littermate controls cleared the infection (Fig 3a). Nearly all mice showed detectable fungal loads, whereas only 2/10 control mice showed any evidence of colonization. Moreover, fungal burdens in *Nfkbiz*^{K13} mice were reproducibly lower than in mice with a complete *Nfkbiz* deletion (compare to Fig 2a). *Nfkbiz*^{K13} mice fully regained

body weight, commensurate with the modest oral fungal loads. At 10 days p.i., half the *Nfkbiz*^{K13} mice still bore fungal loads (Fig 3b). Thus, IκBζ exerts some of its antifungal activities in K13^{+}SELs, notably the cell type that is most responsive to IL-17, yet also clearly acts in additional stromal cell types.

Since only part of the nonhematopoietic IκBζ-dependent response could be attributed to K13^{+} cells, we interrogated its role in the K14^{+} BEL (see Fig 1a) [13]. *Nfkbiz*$^{fl/fl}$ mice were crossed to mice bearing a TAM-inducible K14^{CreERT2} cassette, administered TAM as described above, and subjected to OPC. *Nfkbiz*^{K14ERT2} mice showed a very modest susceptibility to OPC, with only 35% (9/26) mice having detectable fungal burdens (Fig 3c). Even when considering only mice with a detectable infectious load, the mean fungal burden in *Nfkbiz*^{K14ERT2} mice was low, averaging 196 CFU/g. Collectively, these data suggest that IκBζ is an essential mediator of immunity to OPC, acting partially in OECs but evidently in additional cell types as well.

IκBζ regulates oral β-defensins

To understand mechanisms by which IκBζ drives host defense within oral epithelium, we performed transcriptomic profiling of tongues from *Nfkbiz*^{K13} mice or *Nfkbiz*$^{fl/fl}$ (WT) littermates. This was assessed at day 2 p.i., when fungal loads were not yet different (Fig 4a). Partek Pathway analysis identified ‘Cytokine-cytokine receptor interactions’ and the ‘IL-17 signaling pathway’ as the top pathways altered by an SEL-specific *Nfkbiz* deletion (Fig 4b). Consistent with this, characteristic IL-17-target genes known to be required for immunity to OPC were impaired in *Nfkbiz*^{K13} tongue tissue, including *Defb3* and neutrophil-attracting chemokines (*Cxcl1, Cxcl2, Cxcl5*) (Fig 4c). Verification by qPCR confirmed that *Defb3* mRNA was reduced in *Nfkbiz*^{K13} mice (Fig 4d).

The human orthologue of murine BD3 is human β-defensin 2 (BD2, encoded by *DEFB4A*) [46]. BD2 is upregulated in the OEC cell line TR146 upon IL-17 stimulation or *C. albicans* infection *in vitro* [15,47]. RNA silencing of *NFKBIZ* in TR146 cells abrogated IL-17 induction of *DEFB4A* mRNA and BD2 secretion (Fig 4e, f). To ascertain whether IκBζ occupied the *DEFB4A* proximal promoter, we performed ChIP-qPCR using primers spanning four predicted TF binding site regions upstream of the *DEFB4A* transcriptional start site (TSS) (Fig 4g). There was strong enrichment of *DEFB4A* in IκBξ-pulldowns within region 1, a distal site encompassing a C/EBP recognition element (also known as NFIL6) and region 4, a proximal site encompassing a C/EBP and NF-κB sites. Thus, IκBζ regulates *DEFB4A*/BD2 but not via canonical NF-κB binding sites.

While β-defensins exert direct candidacidal activity and are critical to prevent OPC [15,48], they are also reported to have additional immune properties. For example, many defensins have chemotactic properties, engaging the chemokine receptor CCR6 that is expressed on Type 17 cells and thus serving to recruit cells to the inflammatory milieu [49-52]. To explore the possibility that BD3 might more broadly influence the antifungal immune landscape, we subjected *Defb3*$^{-/-}$ mice to OPC or sham infections and analyzed the transcriptome from tongues harvested at 24 h p.i. by RNA-Seq (Fig 4h). Remarkably few genes apart from *Defb3* itself were differentially expressed in *Defb3*$^{-/-}$ mice upon OPC compared to sham-treated controls. The absence of any genes implicated in immune cell mobilization or

immune pathways contrasts with the marked differential gene expression changes seen in the absence of the IL-17 signaling system or IκBζ [15,17]. Hence, β-defensins appear to be a functional endpoint of antifungal signals, rather than amplifying any kind of feed-forward inflammatory circuit through chemotactic recruitment of immune cells.

We next evaluated the impact of IκBζ in the SEL on neutrophil recruitment. *Nfkbiz*^{K13} mice showed reduced expression of the chemokines *Cxcl1* and *Cxcl5* (Fig 5a). However, neutrophil recruitment to tongue was not impaired in *Nfkbiz*^{K13} mice (Fig 5b). *Nfkbiz*^{K14ERT2} mice did not show defects in *Defb3* or neutrophil recruitment (Supplementary Fig S3), commensurate with their mild susceptibility to OPC. This observation is reminiscent of other knockout settings where disease is modest (e.g., IL-17F-deficiency [53,54]), and explains why the oral fungal loads in *Nfkbiz*^{K13} mice are less than those with a complete IL-17 signaling defect.

Egr3 regulates β-defensins in OECs

RNA-Seq analysis of *Nfkbiz*^{K13} tongue showed that IκBζ-deficiency in K13^{+} OECs reduced expression of multiple TFs during OPC (Fig 6a). Egr3 (early growth factor 3) stood out because it has been previously shown to be upregulated in response to *C. albicans* hyphae in macrophages and OECs [55-57], though *Egr3* was not IL-17-inducible. Unlike IκBζ, total Egr3 protein levels were not altered in tongue upon OPC (Fig 6b), nor was *EGR3* mRNA induced in TR146 cells in response to IL-17 (Fig 6c). However, *C. albicans* infection of TR146 cells in culture upregulated *EGR3* mRNA, which was blocked by *NFKBIZ* siRNA (Fig 6d). Though not IL-17-inducible, *EGR3* silencing in TR146 cells diminished IL-17 induction of *DEFB4A* mRNA and BD2 secretion (Fig 6e, f). Although there were no predicted Egr3 recognition elements in the *DEFB4A* promoter, Egr3 can form complexes with NF-κB p50 and thereby bind DNA indirectly [58]. ChIP indicated that Egr3 occupancy of the *DEFB4A* promoter was elevated in the vicinity of regions 2, 3 and 4 (Fig 6g), which notably differs from the occupancy site identified for IκBζ (see Fig 4g). These data suggest that Egr3 participates in the oral host response to *C. albicans* by regulating key β-defensins.

Egr3 is induced by live *C. albicans* or zymosan *in vitro*, but how this occurs is not well understood [55,56]. Multiple fungal properties are required for virulence in OECs (Fig 7a). To determine requirements for *EGR3* induction, TR146 cells were infected with mutant, avirulent *C. albicans* strains defective in (i) cellular adhesion (*als3Δ*), (ii) hyphal formation (a 'yeast-locked' strain, *efgΔ/cph1Δ)*, and (iii) candidalysin, a secreted pore-forming peptide needed to drive innate gene expression in OECs (*ClysΔ*) [12,59]. As controls, we used *C. albicans* strains CAF2-1, SC5314, and the autotrophic strain BWP17 + CIp30 (parental control for *ClysΔ*). After infection *in vitro, EGR3* was induced at 4 h p.i by the virulent controls but not by the avirulent *efgΔ/cph1Δ, als3Δ* or the *ClysΔ* strains (Fig 7a). Although the yeast-locked *efgΔ/cph1Δ* strain failed to induce *EGR3*, hyphal formation *per se* appears not to be a prerequisite for this response, as *als3Δ* and *ClysΔ* can form filaments normally. Rather, upregulation of *EGR3* requires adherence to target cells and secretion of the candidalysin peptide is required.

We next investigated how IκBζ and EGR3 regulate anti-fungal cytokine and chemokine genes in oral epithelial cells in response to *C.albicans*. Accordingly, we silenced *NFKBIZ* or

EGR3 in TR146 cells, which impaired induction of *IL1F9* (IL-36γ, required for immunity to OPC [60]) and the chemokine *CCL20* (Fig 7b). Silencing EGR3 IL1F9 and IL6 with a trend to impaired IL8 and CCL20 (Fig 7c). Hence, *NFKBIZ* and *EGR3* both promote OEC expression of genes involved in immunity to *C. albicans*.

DISCUSSION

The data described here reveal that IκBζ is a central TF mediating oral defense to *C. albicans*. IκBζ works jointly with other TFs such as Egr3 to regulate production of β-defensins, essential for effective clearance of *C. albicans* from the oral mucosa. IκBζ is induced by many cytokines that participate in immunity to candidiasis, so it is likely that IκBζ is a convergence point downstream of many cytokine receptors that function to limit fungal colonization.

Despite high microbial loads, infections of the oral mucosa are uncommon, implying unique oral immune defense mechanisms [5,6,45]. IL-17 and IL-22 are potent antifungal cytokines operative in the oral cavity, in large part because of their capacity to drive expression of β-defensins [13,15]. Though produced by the same Type 17 cells, IL-17 and IL-22 function in distinct layers of the stratified oral epithelium, with each cell subset mounting separate and distinctive responses to *C. albicans* infection. Even so, their actions are interconnected, because IL-22 drives proliferation of BELs that replenish the SEL, thus restoring expression of IL-17R and licensing responsiveness to this cytokine [13]. Both IL-17 and IL-22 upregulate IκBζ, in OECs, though this is just one of many TFs known to be IL-17-inducible. Here we show empirically that IκBζ, but not C/EBPδ, is required for immunity to oral candidiasis, and that in the oral epithelium IκBζ acts within the post-mitotic SEL. This is consistent with a model in which IκBζ acts downstream of the IL-17R within the SEL. Still, IκBζ likely acts in other cytokine pathways (e.g., IL-1 or IL-36) that also participate in OPC immunity [60-62]. Related to this, the relatively modest phenotype observed in mice lacking IκBζ in the K14^{+} BEL (*Nfkbiz*^{K14}) implies that IκBζ is not a major component of IL-22 antifungal signaling, which rather is the province of STAT3 [13].

In the context of oral candidiasis, antimicrobial peptides (AMPs) are a particularly important yet relative understudied component of immune defense [63]. According to the APD3 database, there are 3569 AMPs, including 152 human host defense peptides, 1288 antifungal peptides, and 737 anti-*Candida* peptides [64]. In the mouth, AMPs are produced by OECs and neutrophils and found in high concentrations in saliva [65-68]. AMPs function in diverse ways, by targeting and disrupting microbial cell membranes, mediating apoptosis, or by inhibiting protein and nucleic acid biosynthesis, protease activity and cell division [69]. AMPs can also serve as chemoattractants to recruit immune cells to the local microenvironment and promote wound healing [49,70]. β-defensins are primarily expressed in mucosal and epithelial cells [63]. In humans, BD1 is constitutively expressed, whereas BD2 and BD3 are induced by inflammatory stimuli, including *C. albicans*, IL-17 and IL-22 [8,13,17]. Human BD1, BD2 and BD3 exert direct antifungal activity against *C. albicans* [65,71,72]. Though evolutionarily divergent, murine β-defensins have similar activities and are nonredundant for immunity to OPC [15,48]. Human BD2 and mouse BD3 were reported to bind to CCR6, a receptor for CCL20 found on Th17 cells [49,51,52], though this relationship is not universally accepted [50].

Loss of BD3 in mice surprisingly did not result in detectable gene disruption during the peak stages of immunity to OPC, contrasting with the substantial changes in mice lacking IL-17RA or IκBζ. The only altered gene in *Defb3*$^{-/-}$ mice was *Sycp1*, which is not part of the classic IL-17 or *Candida albicans* gene signatures. Consequently, these findings imply that BD3 acts at the "end of the line" to clear the fungus without further amplifying the immune response.

In the acute OPC infection model used here, IκBζ is not essential in hematopoietic cells to prevent infection. However, IκBζ can play T cell- and DC-intrinsic roles in driving Th17 cell differentiation [36,73]. In this regard, the 5-day OPC model represents an innate response to *C. albicans*, since this fungus is not part of the commensal flora in mice [2,7,61,74,77]. IL-17 and IL-22 in are produced mainly in γδ-T cells and CD4^{+}TCRβ^{+} 'natural' Th17 cells [44,61,74,78,79]; only after exposure to *C. albicans* do mice mount an antigen-specific conventional Th17 adaptive response [61,75,77,80]. Hence, it will be informative to determine the role of IκBζ in recall settings.

IκBζ was originally termed 'IL-1-inducible nuclear ankyrin-repeat protein' (INAP) or 'molecule-possessing ankyrin repeats induced by lipopolysaccharide,' (MAIL) [81-83]. Unlike canonical NF-kB, IκBζ is regulated by expression rather than subcellular localization [30], and its expression is quite dynamic. The oscillatory nature of IκBξ expression is not a unique observation [29,43], and the underlying mechanisms governing this are likely to be a result of intersecting feedback regulatory systems affecting transcription, mRNA stability and protein degradation[84]. IκBζ is transcriptionally controlled by classical NF-κB, which itself exhibits cyclical expression due to feedback inhibitors such as IκB and the ubiquitin editing enzyme A20 [42]. The 3' untranslated region (UTR) of the *Nfkbiz* gene harbors AU-rich elements recognized by RNA binding proteins (RBP) that control translation efficiency and/or mRNA stability [85-87], including the IL-17-induced inhibitor/endoribonuclease Regnase-1 (MCPIP1)that degrades *Nfkbiz*. Consequently, loss of Regnase-1 improves systemic responses to candidiasis by releasing restraints on IL-17 signaling [87]. Another IL-17-activated RBP, Arid5a, stabilizes *Nfkbiz* mRNA and augments RNA translation of IκBζ [86], although surprisingly Arid5a is dispensable for immunity to oral and systemic candidiasis [28]. The ability of *Nfkbiz* to be regulated at both transcriptional and post-transcriptional levels indicates complex nodes of IκBζ regulation, the nuances of which have yet to be elucidated in full.

IκBζ contains 7 ankyrin repeat motifs that facilitate association with other TFs, such as NF-κB p65 or p50 subunits or ROR nuclear receptors, interactions important for its capacity to transactivate gene expression [25,88,94]. ChIP demonstrated association of IκBζ with NF-κB and C/EBP (NFIL6) recognition elements. Since we have ruled out essential roles for C/EBPβ and C/EBPδ in immunity to OPC, the nature of other proteins interacting with IκBζ in this context remains unknown and will be interesting to pursue.

Egr3 is a zinc-finger TF important for cell growth and development [95]. Egr3 is linked to IL-17 production in γδ-T cells and directs expression of IL-17 target genes such as *IL6* and *IL8* [96,97]. *C. albicans* induces Egr3 in reconstituted human oral epithelium [55]. Likewise, Egr3 is induced in a human OEC line by zymosan and live pathogenic *C. albicans*,

dependent on the Dectin-1 receptor [56]. Here we connect IκBζ to Egr3 *in vivo* based on altered expression in *Nfkbiz*^{K13} mice, and show that this TF regulates IL-17 induction of BD2 in an oral epithelial cell line. An acknowledged limitation of this study is that we were not able to evaluate OPC in Egr3-deficient mice. Exactly how EGR3 participates in the response to OPC will require further study but is likely to be revealing.

IκBζ has emerged as a central signaling mediator in several IL-17-mediated inflammatory diseases. We first connected IκBξ to IL-17 in mesenchymal cells [49,103], and multiple studies have since linked this TF to downstream IL-17 signaling [27,37]. IκBζ also mediates signals by IL-1 [89-91] and IL-36 [40,104], both of which help control candidiasis [60-62]. There is no information regarding IκBζ in other fungal infections, to our knowledge, nor are there genetic associations with *NFKBIZ* and human candidiasis. GWAS studies link this factor to psoriasis vulgaris, psoriasis arthritis, and ulcerative colitis [98-100]. Furthermore, *Nfkbiz*-deficient mice are resistant to experimental autoimmune encephalomyelitis (EAE), an IL-17-dependent model of multiple sclerosis [36]. IκBζ in intestinal epithelial cells is essential for promoting hemostasis in the gut and skin [35]. Blockade of IL-17 has been very successful clinically, and therefore IκBζ has potential as a therapeutic target for similar conditions. However, the present studies suggest that targeting IκBζ is likely to be come with similar risks for mucosal candidiasis [101].

Mucosal candidiasis is associated with clinical IL-17 blockade [101]. IL-17 integrates multiple TFs in its downstream signaling cascades [16,24], but the majority evaluated so far are surprisingly dispensable for immunity to OPC, even though many drive IL-17-dependent autoimmunity [26,28,102,103]. Accordingly, the pathways vital for host defense are not always those that promote pathogenic autoimmunity, and therefore defining the relevant molecular players in each will be valuable for pursuing clinical strategies to spare host defense while intervening in autoimmune disease.

STAR METHODS

RESOURCE AVAILABILITY

Lead contact—Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Sarah Gaffen (sarah.gaffen@pitt.edu).

Materials availability—All unique/stable reagents generated in this study will be available from the Lead Contact with a completed Materials Transfer Agreement. The following mice are under MTA from other institutions: *Nfkbiz*$^{fl/fl}$ (RIKEN #RBRC06410) [104], *Act1*$^{-/-}$ (NCH). *Cebpd*$^{-/-}$ (NCI) [105,106], *Il17ra*$^{-/-}$ (Amgen) and *Il22*$^{-/-}$ (Genentech). *K14*^{CreERT2}, *Rosa26*^{CreERT2}, *Defb3*$^{-/-}$ mice were from the MMMRC (stock # 011694). *K13*^{Cre} [15] were from The Jackson Laboratory. *Candida albicans strain* SC5314 is available at ATCC. Other fungal strains (CAF2-1, BWP17+CIp30, *als3Δ, efgΔ/cph1Δ, ClysΔ)* will be made available upon request.

Data and Code Availability—RNA-seq data have been deposited at GEO and will be publicly available as of the date of publication. Accession numbers are listed in the

key resources table. This paper analyzes existing, publicly available data (GSE164241, SRP075350, also listed in the key resources table. Uncropped original immunoblot images are available at the Mendeley Data repository (https://data.mendeley.com/datasets/jxpw9krpz2/1). Diagrams were created on Biorender.com.

This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper will be made available from the lead contact upon request.

EXPERIMENTAL MODEL DETAILS

Mice—WT mice were from The Jackson Laboratory, Taconic Farms or generated in-house as littermates from breeding. All mice were on the C57BL/6 background and housed in groups in SPF conditions. Both sexes were used and were assigned randomly to experimental groups.

Cell lines—TR146 cells (female) were purchased from ECACC (European Collection of Authenticated Cell Cultures) and cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM-F12; Gibco) with Pen/Strep and 15% FBS. Aliquots from original purchase were stored as early passage stocks. Aliquots were used for a limited number of passages. Cells were authenticated by visual assessment and distinctive culture characteristics.

Candida albicans—*C. albicans* were stored as frozen aliquots, streaked on YPD/Amp to generate single colonies, and cultured to log phase in YPD at 30°C.

METHOD DETAILS

Oropharyngeal candidiasis—Mice were maintained in specific pathogen free conditions with food and water ad libitum and a 12 hour dark-light cycle. Mice of both sexes ages 6-12 weeks were used. OPC was induced by sublingual inoculation with 0.0025g cotton balls saturated with 10^7 CFU *C. albicans* yeast (strain CAF2-1, cultured to log phase at 30°C) or PBS (uninfected controls) for 75 mins under general anesthesia [38]. Weight was tracked daily. Tongue homogenates were prepared using gentleMACS (Miltenyi Biotec) with C-tubes. Fungal burdens were determined by plating serial dilutions in PBS plated on YPD agar with ampicillin and cultured for 48 h at 30°C. Limit of detection is ~30 CFU/g. Where indicated, tamoxifen (TAM, 20 mg/ml in sesame or corn oil) was administered i.p. prior to infection starting on day −12 for 5 days, then rested for 7-9 days. Mice were euthanized if they lost more than 25% weight. Animalsexperiments were conducted according to national and international guidelines and approved by the University of Pittsburgh IACUC.

Cell Culture and RNA silencing—Recombinant mouse or human IL-17 (PeproTech) was used at 100 ng/mL. SiRNAs were from Dharmacon (SMARTpool ON-TARGET plus). See Table S1 for detailed information. TR146 cells were seeded overnight in antibiotic-free media and transfected 18-24 h later with 50 nM siRNA in DharmaFect Reagent. Culture media was replaced after 24 h and stimulations or infections occurred 24 h later. TR146 cells were infected with an MOI of 10 *C. albicans* yeast [15].

Radiation Chimeras—On day −1, recipient mice (CD45.1 wild type) were given sulfamethoxazole and trimethoprim in the drinking water for 10 days. On day 0, mice were irradiated (900 rad). One day post irradiation, 7-9 x10^6 cells donor femoral BM cells from WT or *Nfkbiz*^{R26ERT2} were injected i.v. into recipients. After 6 weeks, reconstitution was assessed by flow cytometry for CD45 markers. Reconstituted mice were treated with TAM (20 mg/ml in sesame or corn oil) to induce Cre-mediated deletion, then mice were subjected to OPC.

Flow Cytometry—Tongue tissue was digested with collagenase IV (0.7 mg/mL) in HBSS. Filtered cell suspensions were separated by Percoll gradient centrifugation. Antibodies: anti-CD45 (Invitrogen; #48-0451-82), anti-CD11b (BioLegend; #101222) and anti-Ly6G (BD Biosciences; #551461). Dead cells were excluded using Ghost Dye (Tonbo Biosciences; #13-0870-T100). Data were acquired with an LSRFortessa and analyzed using FlowJo software (Tree Star).

qPCR, RNA Seq—RNA from tongue homogenates was extracted with RNeasy kits (Qiagen). cDNA was synthesized by superscript III Frist Strand Kit (Thermo Fisher) or iScript cDNA synthesis kit (Biorad). Real time PCR was performed using SYBER Green FastMix ROX (Quanta Biosciences) or iTaq Universal Syber Supermix (BioRad) on a CFX Real time Detection System (Biorad). Data were normalized to *Gapdh*. Primers were from QuantiTect Primer Assays (Qiagen) and see Table S1 for detailed information. For RNA Seq, cDNA libraries were prepared from tongue RNA (Nextera XT Kit) harvested at day 2 p.i. and RNASeq was performed on the Illumina NextSeq 500 platform by the Health Sciences Sequencing Core at the University of Pittsburgh. For *Defb3*$^{-/-}$ RNASeq reads were annotated and aligned to the UCSC mouse refence genome (mm10, GRCm38.75) using HISAT2. Read counts were generated using subreads FeatureCounts and differential gene expression was performed using De-Seq2 package. Statistical analysis was calculated using R. *Il17ra*$^{-/-}$ RNASeq data was downloaded from Ref [15] and analyzed using CLC genomics Workbench. *Nfkbiz*^{K13} RNASeq data was analyzed using Partek Flow Software. Sequencing reads were annotated and aligned to UCSC mm10 using STAR. STAR alignment files were used to generate read counts for each gene and differentially expressed genes was performed using Gene Specific Analysis (GSA). RNA-seq raw data files are available at the NCBI Sequence Read Archive (BioProject ID PRJNA955230 and SUB13072303).

Chromatin Immunoprecipitation—ChIPs were performed with the SimpleChIP Plus sonication Kit (Cell Signaling Technology). Cells were crosslinked using disuccinimidyl glutarate (ThermoFisher), followed by formaldehyde cross-linking. Proteins were immunoprecipitated using the following Abs: anti- IκBζ (Cell Signaling; #9244), anti-Egr3 (Cell Signaling; #2559) or IgG (Cell Signaling; #2729). The following primer pairs spanning NF-kB and C/EBP (NFIL6) sites of the *DEFB4A* proximal promoter were used for PCR: Region 1: (5'-CAGCCCCTCACTCCATTCAC-3'; 5'-TGGTGAGTCAGAGAATGGTCC-3') Region 2: (5'-GAGGAAGGAAGTGGGCATCC-3'; 5'-ATACAGGGCTGGCTCAAACC-3'), Region 3: (5'-CCATCACCAACAGGGAGACC-3'; 5'-CTACCACCCGCACTTGAGTT-3') Region

4: (5'-CAGCCCCTCACTCCATTCAC-3'; 5'-TGGTGAGTCAGAGAATGGTCC-3'). Delta Ct value was calculated for each sample from the Ct value obtained for the input.

Immunoblotting and ELISA—TR146 cells were seeded in DMEM-F12 18-24 hours before transfection, infection, or cytokine treatment. Standard denaturing SDS-PAGE and wet transfer were performed. Abs used for immunoblotting: IκBζ (Cell Signaling; #9244, #76041), Egr3 (Cell Signaling; #2559), β-actin (Abcam; #49900). Human BD2 (hBD2) ELISA: plates were coated with 100 uL of anti-hBD2 antibody (Peprotech; #6500P16) overnight. Standard curves were generated with recombinant hBD2 (Peprotech; #300-49). Biotinylated goat anti-hBD2 (Peprotech; #500-P161Gbt) was secondary Ab followed by detection with streptavidin-HRP and absorbance at 450 and 570 nm.

QUANTIFICATION AND STATISTICAL ANALYSIS

Fungal loads were analyzed by ANOVA with Dunn's multiple comparisons test. Weight loss was analyzed by two-way ANOVA. qPCR was analyzed by one-way ANOVA, Student's t-test and indicated post hoc analyses described in Figure Legends. Normality and lognormality tests were performed for each dataset. Data were analyzed on GraphPad Prism and a P value of <0.05 was considered significant. Each symbol represents one mouse or sample. *P <0.05, ** <0.01, ***<0.001, and ****< 0.0001.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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INCLUSION AND DIVERSITY

One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in science. We unequivocally support inclusive, diverse and equitable conduct of research.

ABBREVIATIONS

AMP	antimicrobial peptide
ARID	AT-rich interacting domain
BD	β-defensin
BEL	basal epithelial layer
C/EBP	CCAAT Enhancer binding protein

ChIP	chromatin immunoprecipitation
Clys	candidalysin
IκBζ	Inhibitor of NF-κB zeta
MOI	multiplicity of infection
OEC	oral epithelial cell
OPC	oropharyngeal candidiasis
RBP	RNA binding protein
SEL	suprabasal epithelial layer
TAM	tamoxifen

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Highlights

- During candidiasis, IκBζ (*Nfkbiz*) is upregulated in the oral mucosa via IL-17
- Deletion of *Nfkbiz* renders mice highly susceptible to oropharyngeal candidiasis
- IκBζ acts partially in the suprabasal oral epithelium
- IκBζ and EGR3 regulate β-defensins, nonredundant antifungal effectors

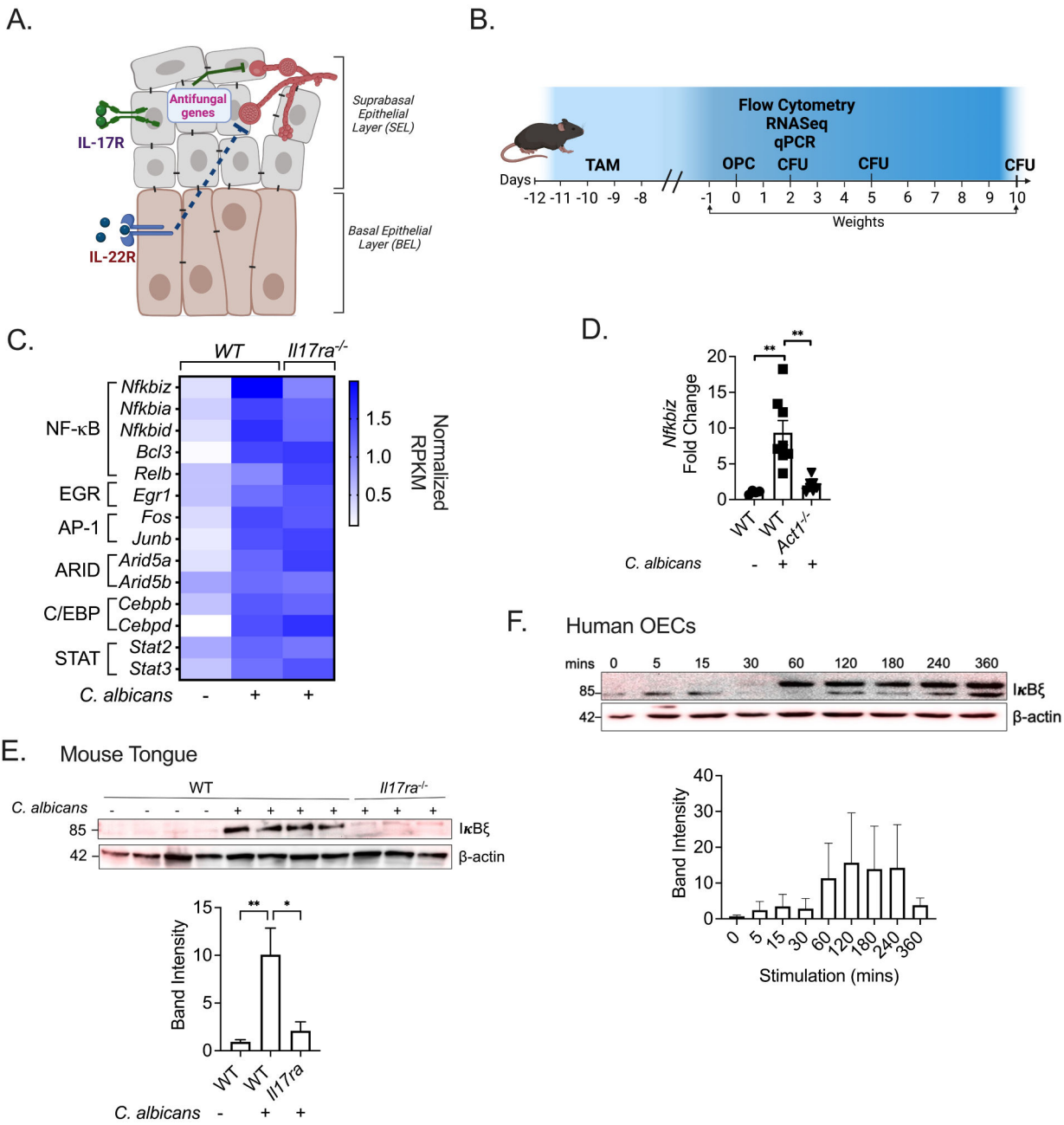

Figure 1. IκBζ is induced by IL-17 in OPC

(A) The stratified oral epithelium and cytokine receptor localization. (B) Timeline of oral *C. albicans* infection procedure. (C) Heat map of TF mRNAs upregulated in tongues from WT mice (*Nfkbiz*$^{+/+}$ animals obtained from breeding colonies) or *Il17ra*$^{-/-}$ mice on day 2 p.i. Data derived from published RNA-Seq data [15] (n= 2 or 3) (D) The indicated mice were subjected to OPC and RNA extracted from tongue at day 2. *Nfkbiz* levels were assessed by qPCR normalized to *Gapdh*. Data are mean ± SEM, analyzed by ANOVA with Tukey's multiple comparisons test. (E) Top: Tongue homogenates from WT and *Il17ra*$^{-/-}$ mice isolated 2 days p.i. were immunoblotted for IκBζ and β-actin. Size markers are shown. Bottom: Densitometry analysis normalized to β-actin (n=3). Data are mean ± SEM,

analyzed by 1-way ANOVA with Tukey's multiple comparisons test. (F) Top: TR146 cells were treated ± IL-17 (100 ng/ml) for the indicated times and immunoblotted for IκBξ and β-actin. Bottom: Densitometry analysis normalized to β-actin (n=3). Data are from 3 independent experiments. Data are mean ± SEM, analyzed by 1-way ANOVA with Tukey's multiple comparisons test.

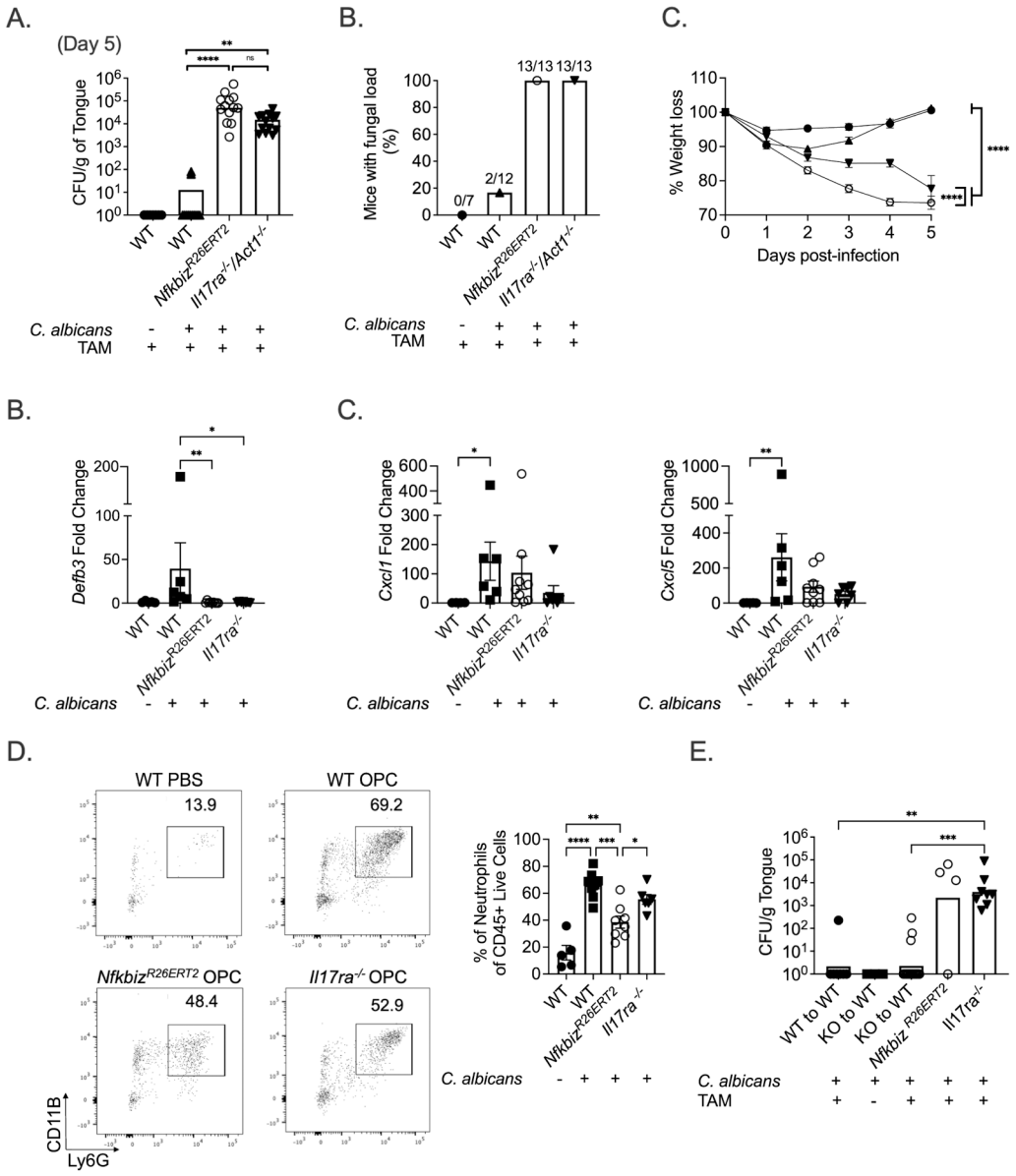

Figure 2. IκBζ in non-hematopoietic cells protects against OPC

(A) The indicated mice were treated with TAM on days −12 to −8, rested for 7-9 d, and infected orally with *C. albicans*. Fungal loads were assessed on days 4-5. Data show geometric mean of CFU/g tongue homogenates, analyzed by ANOVA with Dunn's multiple comparisons test. The percentage of mice per cohort with a detectable fungal load is shown. Weight loss is shown as percentage of starting weight, analyzed by 2-way ANOVA with Tukey's multiple comparisons test. Pooled from 2 independent experiments. (B, C) Expression of indicated genes in tongue was assessed by qPCR normalized to *Gapdh*. Data are ± SEM relative to WT untreated mice, analyzed by ANOVA with Tukey's multiple comparisons test. (D) The indicated mice were subjected to OPC and cells from tongue harvested at day 2 were analyzed by flow cytometry. Left: Representative plot showing percentage of $CD11b^+$ $Ly6G^+$ cells in neutrophils (gated on live, $CD45^+$ cells). Right: Data

compiled from two independent experiments. (E) Bone marrow from indicated donors was transferred into irradiated recipients (KO = *Nfkbiz*^{R26ERT2}). After 6 weeks, recipients were given TAM on days −12 to −8, subjected to OPC, and fungal burden assessed on day 5. Data show geometric mean, analyzed by ANOVA with Dunn's multiple comparisons test. Data pooled from two independent experiments, analyzed by ANOVA test with Dunn's multiple comparison test.

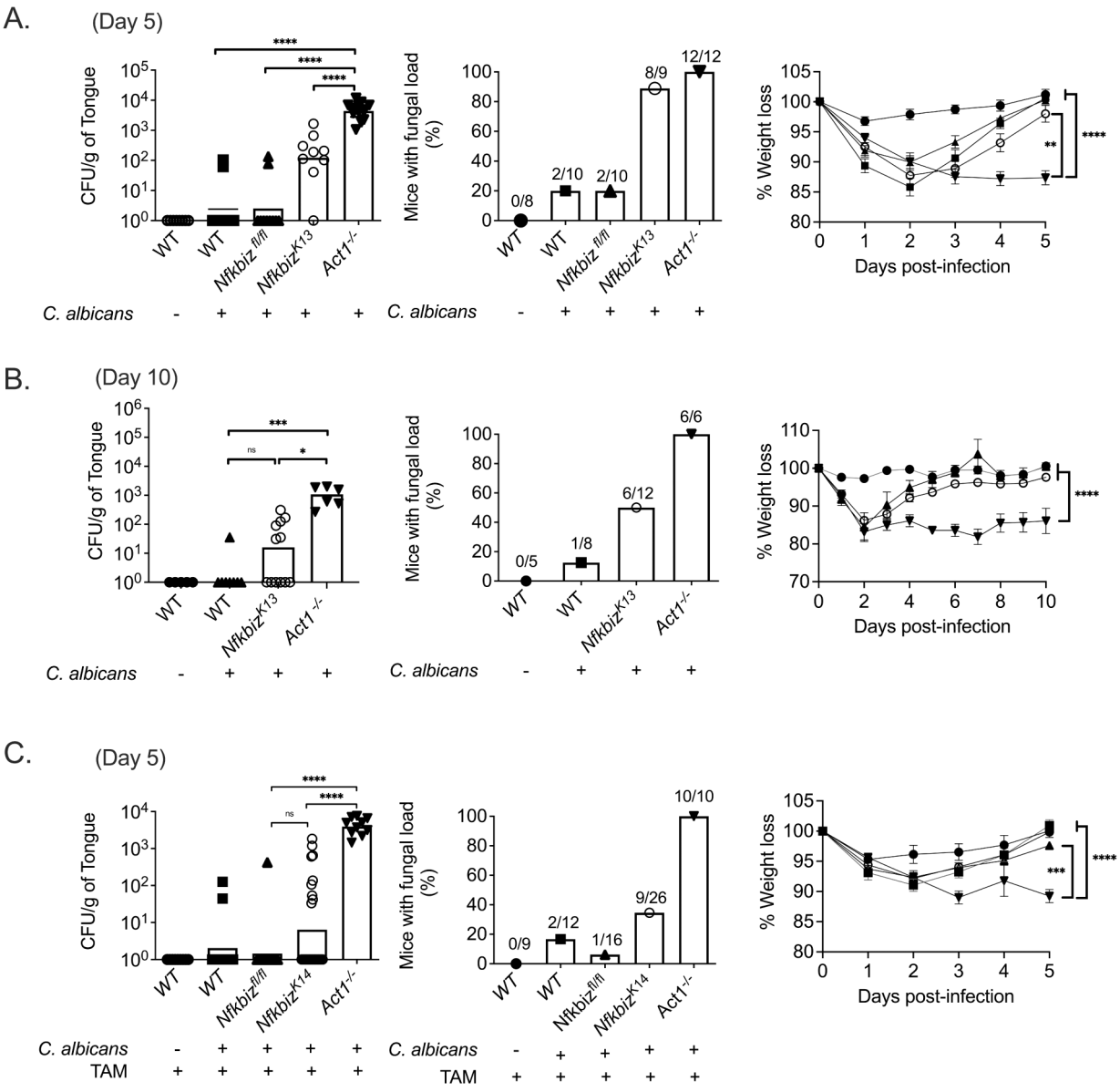

Figure 3. IκBζ functions within the oral epithelium

(A, B) The indicated mice were infected orally with *C. albicans*. Fungal loads, percentage of mice with detectable fungal loads, and weight loss were assessed on day 5 (A) or day 10 (B). Data were pooled from 3 independent experiments. (C) The indicated mice were treated with TAM on days −12 to −8, rested for 7 d, and infected orally with *C. albicans*. Fungal loads, percent of mice with detectable fungal loads, and weight loss were assessed on day 5. Throughout: Bars show geometric mean. Data were analyzed by ANOVA with Dunn's multiple comparison test. Weight loss was analyzed by 2-way ANOVA with Tukey's multiple comparison test.

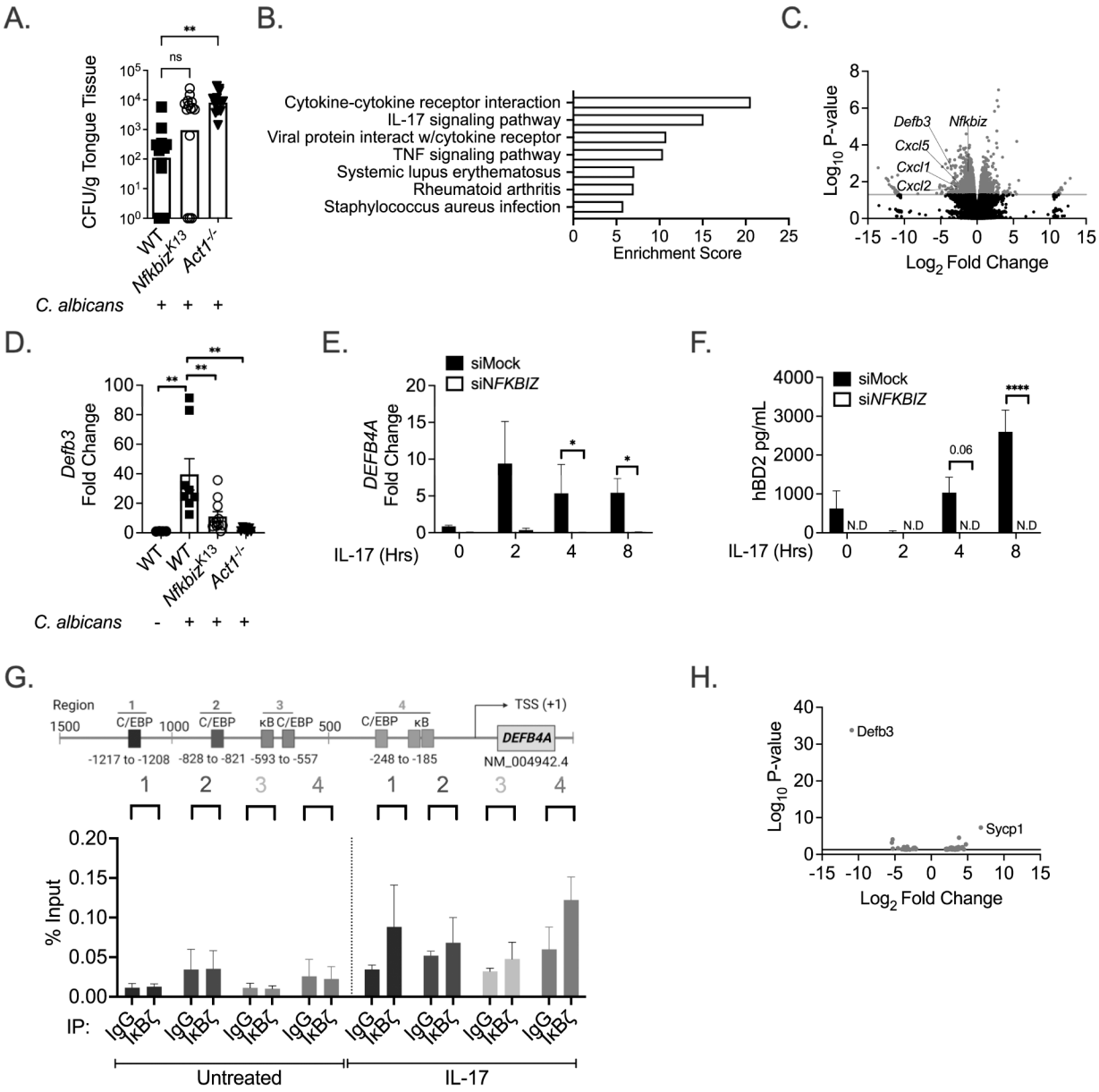

Figure 4. IκBζ in the SEL regulates β-defensins

(A) WT and *Nfkbiz*^{K13} were infected orally with *C. albicans*, and fungal loads assessed on day 2. Data show geometric mean, analyzed by ANOVA with Tukey's multiple comparison test. Data pooled from 3 independent experiments. (B) RNA-seq data (n=3) from *C. albicans*-infected tongues of WT and *Nfkbiz*^{K13} mice on day 2 was analyzed by Partek pathway analysis. (C) Comparison of transcriptional responses induced in tongue during OPC from WT and *Nfkbiz*^{K13} mice on day 2. (D) *Defb3* was assessed by qPCR normalized to *Gapdh*. Data are mean ± SEM relative to WT untreated mice, analyzed by ANOVA with Tukey's multiple comparisons test. (E) TR146 cells transfected with indicated siRNAs targeting were treated with IL-17 and *DEFB4A* was assessed by qPCR normalized to *GAPDH*. Data are as fold-increase compared to untreated (time 0), analyzed by ANOVA with Dunn's multiple comparison test. (F) Supernatants from TR146 samples from panel D

were analyzed for human BD2 by ELISA. Data are mean ± SEM, analyzed by ANOVA with Holm-Sidak's multiple comparisons test. (G) Top: Diagram of predicted TF binding sites in *DEFB4A* proximal promoter. Bottom: TR146 cells were treated ± IL-17 (100 ng/ml) for 4 h and subjected to ChIP with anti-IκBξ Abs or IgG control. Indicated promoter regions were analyzed by PCR, normalized to input. Data are mean ± SEM of three independent experiments. Data analyzed pairwise by Student's t test. (H) Comparison of transcriptional responses induced in tongue during OPC from WT and *Defb3*$^{-/-}$ mice on day 1. Data are from 3 mice.

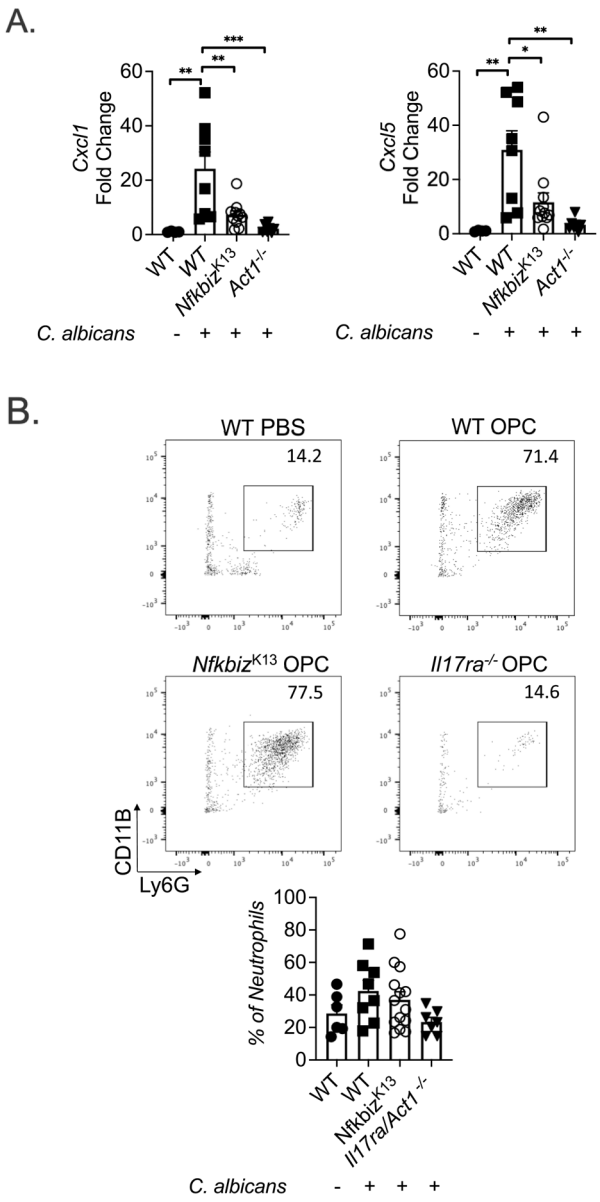

Figure 5. IκBζ in the SEL does is not required for neutrophil recruitment in OPC

(A) WT and *Nfkbiz*^{K13} mice were subjected to OPC. On day 2, *Cxcl1* and *Cxcl5* were assessed by qPCR normalized to *Gapdh*. Data are presented as mean ± SEM normalized to WT untreated mice, analyzed by ANOVA with Tukey's multiple comparisons test. (B) Cells from tongue harvested at day 2 p.i. were stained with the indicated Abs and analyzed by flow cytometry. Left: Representative plot showing percentage of CD11b^{+} Ly6G^{+} cells in neutrophils (gated on live, CD45^{+} cells). Right: Data pooled from two independent experiments, analyzed by ANOVA with Tukey's multiple comparisons test.

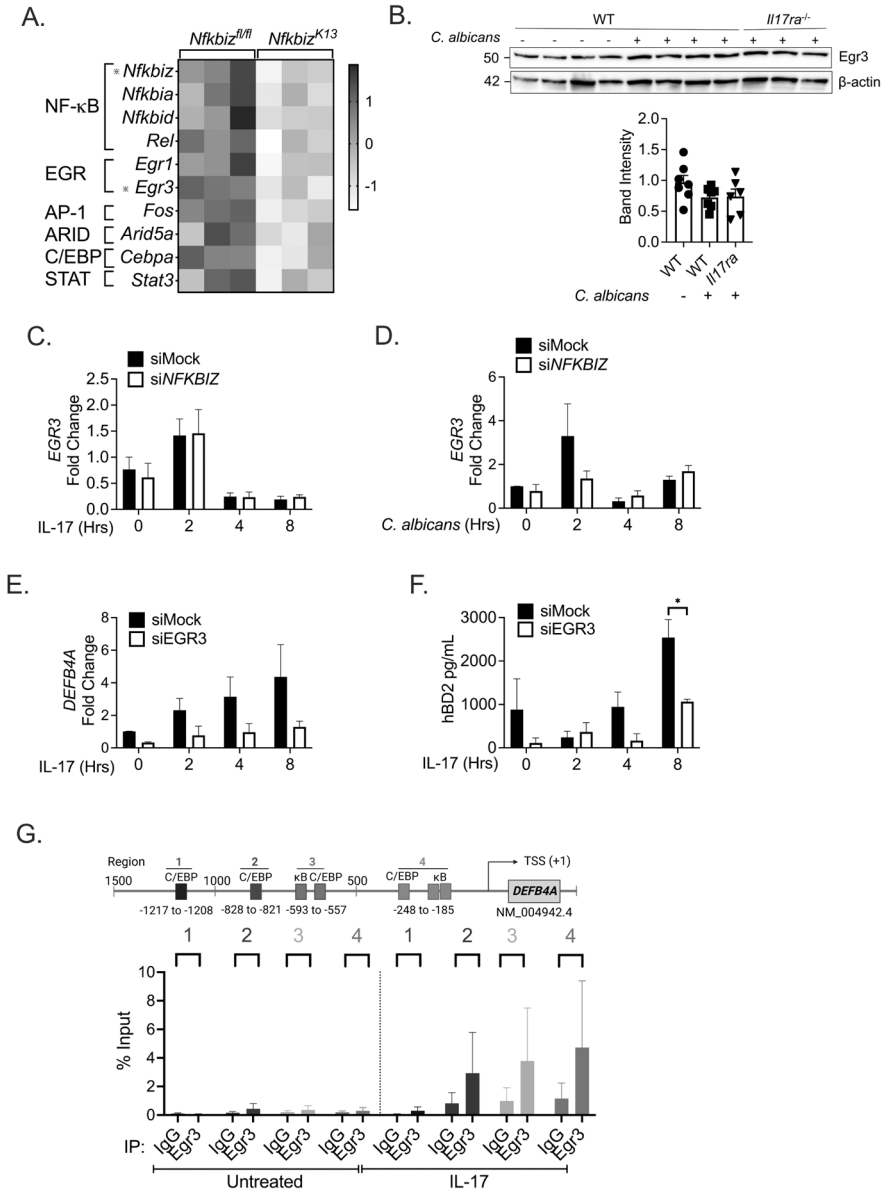

Figure 6. Egr3 regulates β-defensins in OECs

(A) Heatmap of differentially expressed transcription factor genes in WT (*Nfkbiz*$^{fl/fl}$ littermates) or *Nfkbiz*^{K13} mice (n=3) on day 2. Red asterisk highlights TFs examined in this study. (B) Top: Tongue homogenates from infected WT and *Il17ra*$^{-/-}$ mice on day 2 were probed for EGR3 or β-actin. Bottom: Densitometry analysis normalized to β-actin, analyzed using ANOVA with Tukey's multiple comparisons test (Note: these data are from same experiment as Figure 1e, so the β-actin loading control is repeated). (C) TR146 cells were transfected with the indicated siRNAs, treated ± IL-17 and *EGR3* assessed by qPCR normalized to *GAPDH*. Data are fold-increase compared to untreated (time 0), analyzed by ANOVA with Dunn's multiple comparisons test. (D) TR146 cells were co-cultured with PBS or *C. albicans* (MOI = 10) for the indicated times. *EGR3* was assessed by qPCR normalized to *GAPDH* and presented as fold-increase compared to untreated cells

(time 0) and analyzed by ANOVA. (E) TR146 cells were transfected with the indicated siRNAs, treated ± IL-17 and *DEFB4A* assessed by qPCR normalized to *GAPDH*. Data are fold-increase compared to untreated (time 0), analyzed by ANOVA with Dunn's multiple comparisons test. (F) Supernatants from samples in panel E were analyzed for human BD2 by ELISA and analyzed by ANOVA with Holm-Šídák's multiple comparisons test (G) Top: Diagram of predicted TF binding sites in *DEFB4A* proximal promoter. Bottom: TR146 cells were treated ± IL-17 (100 ng/ml) for 4 h and subjected to ChIP with anti-EGR3 Abs or IgG control. Indicated promoter regions were analyzed by PCR, normalized to input. Data are mean ± SEM of three independent experiments. Data analyzed pairwise by Student's t test.

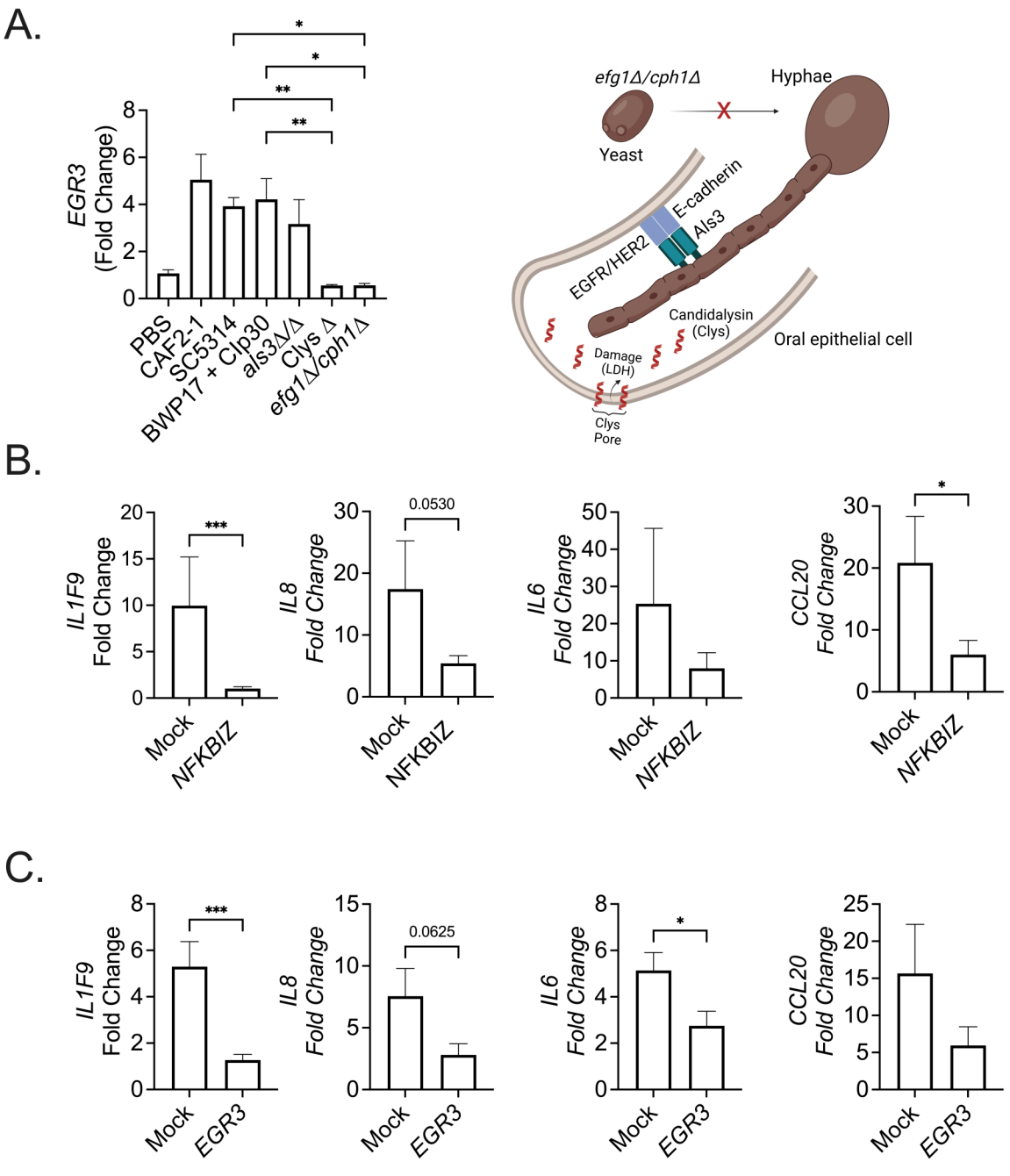

Figure 7. EGR3 responses in oral candidiasis

(A) TR146 cells were infected with the indicated strains of *C. albicans* (MOI = 10) for 4 h and *EGR3* levels were assessed by qPCR, normalized to *GAPDH*, analyzed by ANOVA with Tukey's multiple comparisons test. Representative of three independent experiments. Diagram of *C. albicans* virulence components and interactions with oral epithelium. (B, C) TR146 cells were transfected with indicated siRNAs and infected with *C. albicans* for 4 h. Indicated mRNAs were analyzed by qPCR normalized to GAPDH, analyzed by Mann Whitney U. Data were pooled from four independent experiments.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
EGR3	Cell Signaling Technology	Cat # 2559; AB_11142006
EGR3	Santa Cruz Biotechnology	Sc-390967
IkappaBzeta	Cell Signaling Technology	Cat # 9244; AB_2151602
IkappaBzeta	Cell Signaling Technology	Cat # 76041; AB_2799879
Rabbit IgG	Cell Signaling Technology	Cat #2729; AB_1031062
IgG Isotype	Cell Signaling Technology	Cat # 3900; AB_1550038
Histone H3	Cell Signaling Technology	Cat # 4620 AB_1904005
IgG-Light chain- HRP	Cell Signaling Technology	Cat # 93702; AB_2800208
Human BD2	Peprotech	500-P161G
Biotinylated Human BD2	Peprotech	500-P161GBt
beta actin	Abcam	Cat# ab49900; AB_867494
rabbit HRP	Thermo Fisher Scientific	Cat# A-11008; AB_143165
mouse HRP	Thermo Fisher Scientific	Cat# 31430; AB_228307
CD45	Thermo Fisher Scientific	Cat# 48-0451-82; AB_1518806
Ly6G	BD Biosciences	Cat# 551461; AB_394208
CD11b	BioLegend	Cat# 101222; AB_493705
Ghost Dye BV510	TONBO Biosciences	Cat # 13-870-T100
Chemicals, Peptides, and Recombinant Proteins		
IL-17A	Peprotech	Cat# 200-17
Human beta defensin 2	Peprotech	Cat # 300-49
DharmaFect Transfection Reagent	Dharmacon	T-2001-03
Opti-MEM I reduced serum medium	Gibco	Cat# 31985-070
Collagenase IV	Gibco	Cat# 17104-019
Streptavidin HRP	R&D systems	DY998
Protease inhibitor cocktail	Thermo Fisher Scientific	Cat# 11697498001
DNase	Qiagen	Cat# 79256
Cell lysis buffer II	Thermo Fisher Scientific	Cat # FNN0021
gentleMACS Dissociator C tubes	Miltenyi Biotec	CAt# 130096334
gentleMACS Dissociator M tubes	Miltenyi Biotec	Cat # 130096335
Critical Commercial Assays		
PerfeCTa SYBR Green FastMix, ROX	Quantabio	Cat# 84071
SsoAdvanced University Syber	Quantabio	Cat # 1725270
SuperScript III First Strand Kits	Thermo Fisher Scientific	Cat# 18080-044
iScript cDNA Synthesis kit	BioRad	Cat# 1708890
West Pico PLUSChemiluminescent Substrate	Thermo Fisher Scientific	Cat# 34580
BCA Protein Assay Kit	Thermo Fisher Scientific	Cat# 23228

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Human oral mucosa single cell atlas	Williams, D.W., Greenwell-Wild, T., Brenchley, L., Dutzan, N., Overmiller, A., Sawaya, A.P., Webb, S., Martin, D., Genomics, N.N., Computational Biology, C., et al. (2021). Human oral mucosa cell atlas reveals a stromal-neutrophil axis regulating tissue immunity. Cell *184*, 4090-4104 e4015. 10.1016/j.cell.2021.05.013.	GSE164241
Nfkbiz^{K13} *2 day OPC RNASeq*		PRJNA955230
Defb3$^{-/-}$ *1 day OPC RNASeq*		SUB13072303
Il17ra$^{-/-}$ *2 day OPC RNASeq*	Conti, H., Bruno, V., Childs, E., Daugherty, S., Hunter, J., Mengesha, B., Saevig, D., Hendricks, M., Coleman, B.M., Brane, L., et al. (2016). IL-17RA signaling in oral epithelium is critical for protection against oropharyngeal candidiasis. Cell Host Microbe *20*, 606-617.	SRP075350
Uncropped original immunoblots	This study	https://data.mendeley.com/datasets/jxpw9krpz2/1
Experimental Models: Cell Lines		
TR146 cells (human oral squamous epithelium, female)	ECACC	Cat # 10032305
Experimental Models: Organisms/Strains		
C57BL/6 mice	The Jackson Laboratory (JAX)	Cat #000664
C57BL/6 mice	Taconic Farms	B6NTac
Nfkbiz$^{fl/fl}$	RIKEN	#RBRC06410 (under MTA)
Act1$^{-/-}$	NIH (Ulrich Siebenlist, deceased)	under MTA
Cebpd$^{-/-}$	NCI (Esta Sterneck)	under MTA
Il17ra$^{-/-}$	Amgen	under MTA
Il22$^{-/-}$	Genentech	under MTA
Defb3$^{-/-}$	MMMRC	under MTA
K13^{Cre}	JAX, Conti, H., Bruno, V., Childs, E., Daugherty, S., Hunter, J., Mengesha, B., Saevig, D., Hendricks, M., Coleman, B.M., Brane, L., et al. (2016). IL-17RA signaling in oral epithelium is critical for protection against oropharyngeal candidiasis. Cell Host Microbe *20*, 606-617.	Cat # 034382
Rosa26^{CreERT2}	JAX	Cat # 008463
K14^{CreERT2}	JAX	Cat # 005107
Candida albicans SC5314	ATCC	MYA-2786
Candida albicans CAF2-1	M. Edgerton, University at Buffalo	N/a
Candida albicans BWP17+CIp30	J. Naglik, King's College London	N/a
Candida albicans als3Δ	S. Filler, Lundquist Inst.	N/a
Candida albicans efgΔ/cph1Δ	A Mitchell, University of Georgia	N/a
Candida albicans ClysΔ	J. Naglik, King's College London	N/a
Oligonucleotides		
	see Table S2	
Software and Algorithms		

REAGENT or RESOURCE	SOURCE	IDENTIFIER
De-Seq2	Bioconductor	doi:10.1186/s13059-014-0550-8
Prism	Graphpad	https://www.graphpad.com/
Biorender	N/a	https://www.biorender.com/