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Candida Albicans Elicits Protective Allergic Responses Via Platelet Mediated T helper 2 and T helper 17 Cell Polarization

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Summary

Fungal airway infection (airway mycosis) is an important cause of allergic airway diseases such as asthma, but the mechanisms by which fungi trigger asthmatic reactions are poorly understood. Here we leverage wild type and mutant *Candida albicans* to determine how this common fungus elicits characteristic Th2 and Th17 cell-dependent allergic airway disease in mice. We demonstrate that rather than proteinases that are essential virulence factors for molds, *C. albicans* instead promoted allergic airway disease through the peptide toxin candidalysin. Candidalysin activated platelets through the Von Willebrand factor (VWF) receptor GP1bα to release the Wnt antagonist Dickkopf-1 (Dkk-1) to drive Th2 and Th17 cell responses that correlated with reduced lung fungal burdens. Platelets simultaneously precluded lethal pulmonary hemorrhage resulting from fungal lung invasion. Thus, in addition to hemostasis, platelets promoted protection against *C. albicans* airway mycosis through an antifungal pathway involving candidalysin, GP1bα, and Dkk-1 that promotes Th2 and Th17 responses.

Introduction

Severe, treatment refractory asthma and the closely related and often concurrent condition chronic rhinosinusitis (CRS) are causes of serious human morbidity and occasional mortality. Both asthma and CRS are frequently linked to atopy, the predilection to producing IgE antibodies to specific environmental agents. Atopic, or allergic, asthmatics also manifest other signs of systemic inflammation including eosinophilia and local production of predominantly T helper type 2 (Th2) cell cytokines such as interleukin 4 (IL-4), IL-5, and IL-13, but also the Th17 cell cytokine IL-17, all of which are critical for disease expression (Millien et al., 2014; Porter et al., 2011c).

In contrast, an important subset of treatment-refractory asthma is characterized by comparatively reduced expression of Th2, or type 2, cytokines, eosinophils, and atopy and instead affected patients express more prominently type 17 cytokines and neutrophils in addition to the shortness of breath and wheezing that are common to all forms of asthma (Svenningsen and Nair, 2017). Additional asthma "endotypes" have been described, but a coherent mechanism that explains the coordinated development of the Th2 and Th17 cells that largely define them is unknown.

Asthma and CRS are frequently linked to airway mycosis, the non-invasive growth of fungi along the upper or lower airways, which can be caused by molds such as *Aspergillus* spp., *Penicillium* spp., and *Alternaria* spp (Li et al., 2019). Yeasts such as *Candida albicans* are furthermore isolated from up to two-thirds of asthma sputum samples and are as capable as molds of inducing asthma-like type 2 lung inflammation and the characteristic exaggerated

potential for airway constriction, termed airway hyperresponsiveness, that in aggregate protect the host from potentially lethal dissemination of the fungus (Li et al., 2018; Mak et al., 2013; Porter et al., 2011a; Porter et al., 2009; Porter et al., 2014). The type 17 response is also strongly linked to *Candida* and other fungal infections and subjects with inborn errors of immune regulation involving Th17 cell responses such as the hyper-IgE syndromes (e.g., Job's Syndrome; DOCK8 deficiency) are afflicted with severe fungal-related diseases such as mucocutaneous candidiasis, asthma and CRS (Boos et al., 2014; Chu et al., 2012; Engelhardt et al., 2015; Eppinger et al., 1999; Huang and Church, 2018; Milner et al., 2008). Although a long-held perception is that *C. albicans* is not pathogenic when present in the airways of apparently healthy people (Baum, 1960), *Candida spp* are in fact well-known causes of asthma and the related disorder allergic bronchopulmonary mycosis(Knutsen et al., 2012; Masaki et al., 2017; Sandhu et al., 1979).

We have shown previously that proteinases derived from filamentous fungi such as *A. niger* are both necessary and sufficient to induce allergic airway disease in mice, in part by cleaving the clotting factor fibrinogen into fibrinogen cleavage products (FCPs) that signal through Toll like receptor 4 (TLR4) to activate innate immune mechanisms that drive allergic airway inflammation and cell-specific antifungal immunity (Landers et al., 2019b; Millien et al., 2013). Yeasts such as *C. albicans* similarly produce proteinases of the aspartic class that act as key virulence factors in distinct disease contexts (Gropp et al., 2009; Jackson et al., 2007; Meiller et al., 2009; Schaller et al., 1999; Schaller et al., 2000). Genetic, functional, and biochemical

similarities between yeasts and molds suggest that they would elicit allergic disease through similar mechanisms, but this possibility has not been formally assessed.

In this study we demonstrated that in contrast to molds such as *A. niger*, *C. albicans* elicits robust allergic inflammation and allergic airway disease not through its proteinases, but through candidalysin, a non-proteinase peptide toxin (Moyes et al., 2016). We further demonstrated that candidalysin acts on platelets through the von Willebrand factor receptor GP1balpha (GP1bα) to stimulate release of the Wnt pathway antagonist peptide Dickkopf-1 (Dkk-1) that coordinates the development of Th2 and Th17 cell responses during airway mycosis due to *C. albicans*. Platelets simultaneously provided hemostatic defense against fungal invasion of the lung that occurs within hours of airway challenge. Thus, platelets are essential early responding cells to *C. albicans* airway mycosis, providing hemostatic and immune protection through a mechanism involving candidalysin, GP1bα, and Dkk-1.

Results

C. albicans elicits allergic disease through a distinct immunological mechanism. We first determined whether *C. albicans* induces allergic airway disease through the proteinase-FCP-TLR4 signaling pathway that is required for allergic disease induced by molds such as *A. niger* (Millien *et al.*, 2013). Wild type mice challenged intranasally with viable wild type (parental strain) and secreted aspartic proteinase (Sap)-deficient *C. albicans* cells were assessed for key allergic airway disease features 24 h following the final challenge (Fig. S1). In contrast to proteinase-deficient *A. niger* (Porter *et al.*, 2009), *C. albicans* cells lacking broad subsets of *SAP* genes (*SAP1-3* or *SAP4-6*) induced equivalent airway hyperresponsiveness as assessed by increases in respiratory system resistance (R_{RS}) following provocative challenge with escalating doses of acetylcholine comparable to that of isogenic wild type cells (Fig. S1A, B). Although we observed significant decreases in total inflammatory cells and eosinophils in bronchoalveolar lavage fluid (BALF; Fig. S1C), no significant differences were observed regarding Th cell-related cytokines secreted from deaggregated lung (Fig. S1D).

In contrast to studies of *A. niger*-exposed mice (Millien *et al.*, 2013), we further observed no reduction in airway hyperresponsiveness, BALF cell counts, or secreted cytokines from lung homogenates of *Tlr4*$^{-/-}$ mice challenged with wild type *C. albicans* as compared to syngeneic wild type mice as compared to isogenic wild type fungi (Fig. S1, A-D). These observations suggested that the major Saps from *C. albicans* do not generate FCPs that can signal through TLR4 to drive the allergic airway disease phenotype. To confirm this, we compared the proteolytic activities of the highly allergenic proteinases from *A. melleus* (PAM (Landers *et al.*,

2019b)) with Saps with respect to fibrinogen hydrolysis. Whereas PAM yielded fibrinogen cleavage products of the expected size (100-150kDa) (Landers *et al.*, 2019b), purified Saps hydrolyzed fibrinogen without generating detectable FCPs after 2 and 6 h of incubation (Fig. S1E). Recombinant Saps, especially Saps 1, 2 and 3, did generate FCPs, but of smaller molecular size (~37-65 kDa) as compared to PAM (~70-150kDa; Fig. S1F). Thus, unlike molds such as *A. niger*, *C. albicans* induces allergic airway disease through a mechanism operating substantially independently of secreted fungal proteinases and TLR4.

Candidalysin drives allergic airway disease. The inability of *C. albicans* Saps to account for the allergic airway disease induced by this fungus led us to consider the importance of other virulence factors for this phenotype. *C. albicans* expresses several virulence factors in addition to proteinases, including candidalysin, a non-proteinase peptide toxin secreted by hyphal cells that is a potent innate immune activator and mediator of Th17 cell responses (Ho et al., 2020; Kasper et al., 2018; Moyes *et al.*, 2016; Verma et al., 2017; Verma et al., 2018). Therefore, we next determined the importance of candidalysin for *C. albicans*-mediated allergic airway disease comparing wild type mice challenged with isogenic wild type yeast cells (parental strain) or with candidalysin-deficient *ece1Δ/Δ* yeasts. In contrast to proteinase-deficient cells, *ece1Δ/Δ C. albicans* cells induced significantly less airway hyperresponsiveness (Fig. 1, A and B) and substantially reduced BALF cellularity marked especially by fewer macrophages, eosinophils, and neutrophils (Fig. 1C).

Similarly, type 2 cytokine (interleukin (IL)-4, IL-5 and IL-13) and IL-17 secretion from

deaggregated lung was significantly reduced in mice receiving *ece1Δ/Δ C. albicans,* but secretion of gamma interferon (IFN-γ) was not affected (Fig. 1D). *ece1Δ/Δ C. albicans* also failed to induce robust secretion of the innate proinflammatory cytokines IL-1β and IL-6, although TNF secretion was unaffected by the lack of candidalysin (data not shown). These findings were paralleled by reductions in whole lung of major T helper effector subsets that have been linked to human and experimental asthma, especially T helper type 2 cell (Th2; $GATA3^{+}$ (Zheng and Flavell, 1997)) and Th17 cell (RORγt+ (Ivanov et al., 2006)) cells as assessed by flow cytometry (Fig. 1, E and F). We did not detect ($T\text{-}bet^{+}$ (Szabo et al., 2000)) T_H1 cells, suggesting that the secreted IFN-γ (Fig. 1D) derived from non-Th cell sources. These cytokine profiles suggested that, in contrast to *C. albicans* Saps, candidalysin is required not only to promote innate immune responses (Moyes *et al.*, 2016; Verma *et al.*, 2017), but also to induce type 2 and type 17-biased inflammation in the context of *C. albicans*-induced allergic airway disease.

T helper effector cells and their cytokines have all previously been shown to mediate protection against fungal infections, including airway mycosis that is often the underlying cause of asthma and other allergic airway diseases (Ma et al., 2008; Porter *et al.*, 2011a; Porter et al., 2011b; Porter *et al.*, 2009; Porter *et al.*, 2014). The diminished induction of these protective responses by *ece1Δ/Δ C. albicans* suggested that this mutant might be poorly cleared from the lung. Indeed, we found that whereas wild type *C. albicans* was almost completely cleared from lungs 24 h after the final intranasal challenge, approximately 100 CFU/g were recovered from the lungs of each mouse challenged with *ece1Δ/Δ C. albicans* cells (Fig. 1G).

Microscopically, wild type *C. albicans*-challenged lungs were marked by the accumulation of inflammatory cells, including neutrophils and eosinophils, in the alveoli and especially in a peri-bronchovascular distribution. In comparison, *ece1Δ/Δ C. albicans*-challenged lungs showed minimal peri-bronchovascular inflammation (Fig. 1H). As revealed by periodic acid-Schiff staining, goblet cells, a prominent example of airway remodeling that is typical of asthma (Fahy, 2001), were abundant in the airway epithelium of mice challenged with WT *C. albicans*, but scant in the lung epithelium after *ece1Δ/Δ C. albicans* challenge (Fig. 1H). Thus, candidalysin drives both robust allergic airway disease and efficient clearance of *C. albicans* from mouse lung.

The complete spectrum of allergic airway disease elicited by viable filamentous fungi can be comparably reproduced by representative proteinases that they secrete (Kheradmand et al., 2002). Given the minor contribution of *C. albicans* proteinases to allergic responses, we hypothesized that candidalysin from *C. albicans* is alone sufficient to induce allergic airway disease. To test this, we challenged mice intranasally with synthetic, LPS-free candidalysin (CL) or scrambled peptide control (SC) over 17 days using a dose escalating protocol (Fig. S2A) after which the allergic airway disease phenotype was quantified. We found that candidalysin, but not the scrambled peptide control, induced dose-dependent increases in airway hyperresponsiveness as assessed one day after the last challenge as compared to vehicle challenged mice (Fig. S2B). At the highest dose given (16 μmol), candidalysin also provoked a 600% increase in total BALF inflammatory cells consisting primarily of macrophages, but also eosinophils (Fig. S2C). Th2 cell (IL-4, -5, -13) and Th17 cell (IL-17) cytokines (Fig. S2D) as well as innate proinflammatory

cytokines (Fig. S2E) were further elicited from whole lung by candidalysin. Thus, analogous to proteinases from filamentous fungi, candidalysin from *C. albicans* is alone sufficient to induce allergic airway disease. The magnitude of disease induced by exogenously administered candidalysin is reduced compared to that induced by wild type *C. albicans* (Fig. 1), most likely because invading fungal hyphae secrete candidalysin into an invasion pocket in greater concentrations and closer proximity to responsive host cells (e.g., epithelial cells, platelets) than can be achieved with intranasal administration (Moyes *et al.*, 2016).

C. albicans activates platelets to secrete Dkk-1.

Next, we conducted experiments to determine how candidalysin induces the type 2 and type 17 immunity that are typically observed in human asthma (Pene et al., 2008; Zhao et al., 2010) and in response to murine airway mycosis (Porter *et al.*, 2011a). We focused on the potential relationship between candidalysin and the Wnt pathway antagonist peptide Dickkopf-1 (Dkk-1) that coordinates chronic type 2 inflammation in response to *Leishmania major* and dust mite-derived allergens (Chae et al., 2016b). We first quantified Dkk-1 in plasma samples from asthma patients with CRS, a patient group that frequently suffers from *Candida* airway mycosis (Mak *et al.*, 2013; Porter *et al.*, 2014). We found significantly increased Dkk-1 in plasma from asthma and CRS patients compared to control patients with no overt lung or airway disease (Fig. 2A).

Similarly, we found that plasma Dkk-1 was elevated approximately five-fold in mice challenged intranasally with wild type *C. albicans* as compared to sham-challenged control mice, but only two-fold in sera of *ece1Δ/Δ C. albicans*-challenged mice (Fig. 2B). In a dose-

dependent manner, intranasally administered candidalysin alone also induced significant increases in plasma Dkk-1 (Fig. 2C).

Dkk-1 potentially derives from diverse cellular sources, but is thought to be released primarily from platelets in allergic contexts (Chae *et al.*, 2016b). In support of this, challenge of mice with wild type, but not *ece1Δ/Δ, C. albicans* resulted in significantly elevated Dkk-1 in platelets (Fig. 2D) without altering blood platelet counts (Fig. S3A). In contrast, pulmonary $CD41^{+}$ $CXCR4^{+}$ megakaryocytes (Huang and Cantor, 2009) expressed decreased intracellular Dkk-1 after challenge of mice with wild type, but not *ece1Δ/Δ, C. albicans* as assessed by quantifying $Dkk1^{high}$ lung megakaryocytes and Dkk-1 mean fluorescence intensity (MFI) of the same cells (Fig. S3B). Similar to platelets, fungal airway challenge failed to diminish the total number of lung megakaryocytes (Fig. S3C). Thus, Dkk-1 is elevated in both the plasma and platelets of mice with *C. albicans*-induced allergic airway disease, but is diminished in lung megakaryocytes. These observations suggest that upon fungal challenge, lung megakaryocytes more efficiently shed Dkk-1 through enhanced sequestration in platelets.

To further support the possible pulmonary origin of platelets, we challenged wildtype mice with 16 μmol of candidalysin or scrambled control and isolated platelets from the left or right ventricles of mice 2 hours post challenge. We found significantly higher Dkk-1 concentrations in platelets collected from the left ventricle post candidalysin challenge (Fig. S3D). Since blood from the left ventricle represents that draining from the pulmonary circulation, these data indicate that $Dkk\text{-}1^{high}$ platelets originated from pulmonary megakaryocytes.

To determine a potential functional relationship between *C. albicans*, platelets, and Dkk-

1, we incubated human platelets with wild type and *ece1Δ/Δ C. albicans in vitro*. Wild type *C. albicans* cells provoked release of Dkk-1 more than 5-fold above control release amounts, whereas *ece1Δ/Δ C. albicans* induced significantly less Dkk-1 release (Fig. 2E). Furthermore, candidalysin used at two doses (10 or 20 μM) was alone sufficient to provoke release of Dkk-1 from human platelets (Fig. 2F)(Moyes *et al.*, 2016), but not endothelial cells (Fig. S3E). Candidalysin also acutely activated platelets as assessed by its ability to bind to platelets in plasma and enhance expression of surface CD62P (P-selectin) without inducing lysis (Isenberg et al., 1986) (Fig. S4A-D). Together, these results confirm that candidalysin derived from *C. albicans* activates human and mouse platelets to release Dkk-1.

Dkk-1 drives *C. albicans*-dependent Th2 and Th17 cell responses.

The relevance of Dkk-1 to allergic airway disease induced by airway mycosis is unknown. To address this, we administered to mice challenged with *C. albicans* a Dkk-1 inhibitor (WAY 262611) previously shown to block this peptide in vivo (Chae *et al.*, 2016b) **(**Fig. 2G). In a dose-dependent manner, the Dkk-1 inhibitor progressively reduced airway hyperresponsiveness and BALF inflammatory cell recruitment, most notably reducing BALF eosinophilia **(**Fig. 2 H and I).

Dkk-1 inhibition further resulted in the suppression of secretion of type 2 cytokines (IL-4, IL-5, IL-13) from whole lung, but also inhibited secretion of IL-17 **(**Fig. 2J). Of note, secretion of IL-1β, IL-6 and TNF was not significantly affected by the inhibitor (Fig. 2K). Flow cytometric analysis further revealed the decreased recruitment to whole lung of $GATA3^+$ Th2

and RORγt$^+$ Th17 cells in a manner that correlated with inhibitor dose **(**Fig. 2 L and M). Consequently, *C. albicans*-challenged mice with impaired Th2 and Th17 cell responses due to the Dkk-1 inhibitor had markedly elevated lung fungal burdens (Fig. 2N).

The preceding observations suggested that candidalysin critically influences the induction of Th2 and Th17 cell responses by stimulating the secretion of Dkk-1. To confirm this, we administered synthetic mouse Dkk-1 to mice challenged with *ece1Δ/Δ C. albicans* (Fig. 3A). We found that Dkk-1, but not control peptide, significantly enhanced airway hyperresponsiveness (Fig. 3A and B). Consistent with these observations, exogenously administered Dkk-1 markedly enhanced both the recruitment of inflammatory cells, especially eosinophils, to the airways and the secretion of lung Th2 cytokines, especially IL-4, but also IL-17 (Fig. 3C and D). Although exogenous Dkk-1 did not influence the secretion of innate pro-inflammatory cytokines (Fig. 3E), it did markedly increase recruitment to lung of GATA3$^+$ Th2 and RORγt$^+$ Th17 cells (Fig. 3F and G). Moreover, fungal lung burdens were significantly reduced after exogenous Dkk-1 treatment (Fig. 3H). Dkk-1 alone had no effect on any index of allergic airway disease when administered through the intraperitoneal route (data not shown). Thus, our findings confirm that Dkk-1 is an important mediator of lung Th2 cell responses (Chae *et al.*, 2016b), but critically extend this observation by also revealing that Dkk-1 is equally important for the generation of Th17 cell responses in the context of airway mycosis. These findings further confirm that Dkk-1 is essential for the control of *C. albicans* growth in the lung.

<u>Mechanism of Dkk-1 release from platelets</u>.

We next sought to determine how candidalysin induces release of Dkk-1 from platelets. Although candidalysin acutely induces lysis of host cells at concentrations above 20 μM, suggesting a non-specific mechanism for Dkk-1 release (Moyes *et al.*, 2016), our observation of platelet activation and Dkk-1 release at candidalysin concentrations of 10 and 20 μM suggested that candidalysin potentially activates a specific platelet receptor (Fig. 2F; Fig. S4A-D). To screen for a specific candidalysin receptor, we incubated human platelets with candidalysin after previous addition of blocking antibodies to the receptors P2Y1, P2Y12, $\alpha_2\beta_1$, GPIV, $\alpha_{IIb}\beta_3$, TLR4, CLEC2, GPVI and GP1bα after which we measured Dkk-1 release. We found that only GP1bα blockade inhibited candidalysin-dependent Dkk-1 release. These findings indicated that candidalysin-dependent Dkk-1 release occurs through a specific mechanism and identify GP1bα as a candidate candidalysin receptor (Fig. 4A).

To further establish the potential importance of GP1bα as a mammalian candidalysin receptor, we next attempted to demonstrate direct binding between GP1bα and candidalysin using a modified ELISA in which either candidalysin or GP1bα was used as the capture reagent and His-tagged GP1bα or biotinylated candidalysin were used, respectively, as the second reagent (Fig. 4B, C). Regardless of the configuration, these assays consistently demonstrated concentration-dependent binding between candidalysin and GP1bα (Fig. 4B and C) that was inhibitable by either a blocking anti-GP1bα antibody or VWF A1A2A3 tridomain, the latter representing a fragment of VWF that binds GP1bα through the A1 domain (Fig. 4D) (Azuma et al., 1991). To further demonstrate the interaction between candidalysin and GP1bα, we performed pull-down assays using human platelet lysates. Biotinylated candidalysin, but not its

scrambled control peptide, was found to physically associate with GP1bα from human platelet lysates as determined by western blotting (Fig. 4E). Collectively, these observations confirm that GP1bα on platelets specifically binds to candidalysin.

We conducted additional assays using human platelets to further address the interaction between candidalysin and GP1bα. As assessed by flow cytometry, fluorescently labeled candidalysin readily bound to human platelets in the presence of plasma, but this interaction was blocked approximately 50% by pre-treating platelets with a non-activating antibody against GP1bα (Fig. 5A, S4A-D). We further blocked GP1bα with VWF A1A2A3 tridomain and also observed significantly reduced binding of candidalysin to platelets (Fig. 5B).

The preceding results further confirmed that candidalysin interacts specifically with GP1bα, but in addition suggest that candidalysin can also non-specifically interact with platelets, perhaps by binding to additional receptors or intercalating within the platelet membrane. We therefore carried out further analyses to determine if another platelet receptor, GPIIb/IIIa, which is critical for platelet aggregation and thrombosis (Calvete, 1995; Shattil, 1999), could interact with candidalysin. Using a similar binding assay, we were unable to demonstrate any direct affinity between candidalysin and GPIIb/IIIa (Fig. S4E). Moreover, GPIIb/IIIa expression was unaltered on human platelets stimulated with candidalysin (Fig. S4F). We further demonstrated that candidalysin, in marked contrast to the activating GPIIb/IIIa ligand collagen, failed to induce platelet aggregation in vitro (Fig. S4G).

Despite the non-specific binding of candidalysin to platelets, GP1bα blockade completely inhibited candidalysin-dependent activation of platelets prepared in plasma as assessed by

CD62P upregulation (Fig. 5C). Anti-GP1bα further suppressed CD62P expression lower than that observed in naïve platelets, indicating that GP1bα integrates a variety of inputs to mediate platelet activation. We considered a more complex mechanism whereby candidalysin interacts with plasma VWF to co-activate platelets. However, washed platelets that were prepared free of plasma VWF remained highly susceptible to candidalysin-dependent activation as assessed by Dkk-1 release and autolysis (Fig. 5D, E). Thus, candidalysin interacts with platelets in multiple ways, but only the specific interaction with GP1bα in the absence of VWF is sufficient to mediate platelet activation and Dkk-1 release. Critically, such activation occurs without eliciting platelet aggregation.

Hemostatic role of platelets during airway mycosis.

To confirm the importance of platelets to *C. albicans*-dependent allergic airway disease, we administered 10^5 *C. albicans* cells intranasally to mice depleted >95% of platelets (Fig. 6A). We found that all platelet-depleted mice succumbed to this infectious challenge within 6 h after fungal exposure, whereas *ece1Δ/Δ C. albicans* cells induced no mortality or apparent illness **(**Fig. 6B**)**. Additionally, wild type, platelet-replete mice experienced no mortality or illness after a single challenge with up to 10^7 viable cells of *C. albicans* (data not shown).

In contrast, a single challenge of recombinant candidalysin peptide alone caused no mortality or apparent illness when introduced into the airways of platelet-depleted mice (Fig. 6C), suggesting that the lethal outcomes with viable yeast cells reflected additional requirements such as growing hyphae that are able to extend beyond the airway epithelium and rupture

capillary beds. Examination of deceased animals revealed apparent pulmonary hemorrhage involving most of the lung that was confirmed by histologic analysis (Fig. 6D, E) that also revealed fungal hyphae within terminal airways and alveoli (Fig. S5A). Bronchoalveolar lavage further retrieved gross blood from mouse lungs that contained abundant hemoglobin (Fig. 6F). MicroCT analyses demonstrated that the predominantly normal lung seen in platelet-sufficient, wild type *C. albicans*-challenged mice and platelet-depleted mice receiving *ece1Δ/Δ C. albicans* was largely replaced by an X-ray-dense material, most likely representing blood, that strongly enhanced lung density (measured in Hounsfield units) and attenuated aerated lung volume (Fig. 6G). Of note, platelet depletion or *C. albicans* challenge individually induced no pulmonary hemorrhage (Fig. S5B, C). Thus, in addition to driving type 2 and type 17 immunity through Dkk-1, these findings demonstrate that platelets play an indispensable hemostatic role during acute airway infection with *C. albicans*.

Platelets are necessary for candidalysin-dependent Dkk-1 release and allergic airway disease. While important in revealing the critical hemostatic role of platelets during *C. albicans* airway mycosis, the preceding experiment precluded determining if platelets are also important sources of Dkk-1 that drives type 2 and type 17 responses in this context. We therefore conducted an additional experiment in which normal and platelet-depleted mice were challenged intranasally with synthetic candidalysin, which alone is sufficient to induce allergic airway disease (Fig. S2). As expected, platelet-replete mice developed significant airway hyperresponsiveness, airway eosinophilia, and type 2 and type 17 cytokine responses in comparison to sham-challenged

animals (Fig. S5 D-G). In contrast, mice with sustained depletion of platelets demonstrated no significant inflammatory response to candidalysin and in fact were not significantly different from vehicle-challenged mice for all measured parameters. However, thrombocytopenic mice did manifest epistaxis and one died in response to candidalysin challenge (data not shown). Candidalysin induced more than a 2-fold increase in plasma Dkk-1 in platelet sham-depleted mice but had no effect on plasma Dkk-1 in mice almost entirely deficient in circulating platelets (Fig. S5H). These findings demonstrate that platelets are the primary and probably exclusive source of Dkk-1 in response to candidalysin challenge of the mouse lung and that platelets are essential to both the generation of Th2 and Th17 cell responses and allergic airway disease in this context.

Discussion

Major forms of asthma, including multiple distinct endotypes, are characterized by the presence of Th2 and Th17 cells that are both necessary for disease expression and, in the context of airway mycosis, required for both fungal eradication and prevention of invasion and fungal dissemination. Despite their central importance, the ontogeny of Th2 and Th17 cells in fungal disease has remained largely unexplored, especially in the context of *C. albicans*-mediated allergic disease. We have demonstrated that *C. albicans* elicits Th2 and Th17 responses in experimental allergic airway disease through the secreted peptide toxin candidalysin. We have further demonstrated that platelets respond to candidalysin through the VWF receptor GP1bα to specifically release Dkk-1, a Wnt pathway antagonist peptide that coordinates the generation of Th2 and Th17 cells in this context. Candidalysin and Dkk-1 were required for both expression of allergic airway disease in mice and optimal control of lung fungal burdens, further linking the importance of allergic inflammation to the control of airway mycosis. As only mice with circulating platelets were capable of secreting Dkk-1 into the plasma and responding to candidalysin challenge with Th2 and Th17 cell responses and expressing allergic airway disease, our findings provide substantial evidence that platelets are an important source of Dkk-1 during *C. albicans* airway mycosis. We therefore propose that candidalysin and Dkk-1 may also be important, but clearly not exclusive, determinants of human asthma endotypes.

Secreted proteinases from molds were an early fungal virulence factors described that could elicit robust Th2 cell responses and experimental allergic airway disease. In part, proteinases cleave fibrinogen, immunostimulatory fragments of which signal through TLR4 to elicit allergic

airway disease. While important, this pathway fails to activate Th2 and Th17 cells and instead acts strictly on innate immune cells such as airway epithelial cells, macrophages, and innate lymphoid cells (ILC) (Landers *et al.*, 2019b; Millien *et al.*, 2013). Despite the many proteinases secreted by *C. albicans* (Naglik et al., 2003), they play at most a minor role in inducing robust Th2 and Th17 cell-driven allergic airway disease, in part presumably because these aspartic-class proteinases fail to cleave fibrinogen into immunologically active fragments. Additional studies are required to understand if and how allergenic proteinases from molds also elicit the secretion of Dkk-1 from platelets to drive Th2 and Th17 cell responses.

Rather than being initially detected by a canonical immune cell, our studies demonstrated that megakaryocytes and platelets are indispensable first responders to *C. albicans* airway mycosis. We demonstrated that candidalysin is recognized immunologically through a cognate interaction with GP1bα and not other platelet receptors such as GPIIb/IIIa. The only other known ligand for GP1bα is VWF, an exceptionally large, multimeric protein that is produced by vascular endothelial cells and platelets. Under conditions of shear stress especially in the context of damaged endothelium in which both collagen and VWF become exposed to circulating platelets, the VWF-GP1bα interaction activates platelets to adhere and aggregate, thereby providing an essential hemostatic function. In contrast, ligation of GP1bα with candidalysin failed to elicit thrombosis, but rather activated platelets to express CD62P and secrete Dkk-1 acutely; with more prolonged exposure, candidalysin-activated platelets underwent autolysis rather than aggregation.

During *C. albicans* airway mycosis, but also during invasive aspergillosis (Tischler et al.,

2020), platelets protect the host from the potentially lethal effects of microhemorrhages that likely result from fungal invasion of the airway microvasculature. *C. albicans*-related thrombosis is therefore not the result of candidalysin directly signaling through platelets, but rather most likely the effect of VWF (and collagen) exposed through fungal invasion and tissue disruption. Thrombin (coagulation factor II), which almost certainly is also activated in this context, may also independently induce thrombosis by signaling through proteinase-activated receptors (PARs) (Sambrano et al., 2001). Nonetheless, these observations raise the possibility that GP1bα may signal differentially according to the ligand encountered, eliciting either a predominant secretory or autolytic response through exogenous candidalysin or a thrombotic response through endogenous VWF. Our observations further indicate that these diverse platelet functions operate simultaneously during airway mycosis and raise the possibility that GP1bα ligands may be developed that differentially activate these functions to achieve distinct therapeutic goals.

Important roles for platelets in regulating immunity have long been suspected. By adhering directly to immune and endothelial cells, platelets coordinate leukocyte recruitment to sites of inflammation and tissue injury and therefore play particularly prominent roles in vascular inflammation and sepsis (Rayes et al., 2020), but also inflammatory events that promote tumor growth and metastasis (Stoiber and Assinger, 2020). These effects are largely confined to innate immune cells, but platelets also play a potentially important role in inhibiting Th17 cell differentiation through the release of either soluble factors such as platelet factor 4 or microparticles (Dinkla et al., 2016; Shi et al., 2014).

Platelets are especially strongly linked to asthma pathogenesis. Allergen challenge causes transient reductions in blood platelet counts and platelet-leukocyte aggregates are readily found within the airways and lung tissue of asthma patients and in experimental systems (Pitchford et al., 2008; Shah et al., 2017; Sullivan et al., 2000). The distinct alpha and dense granules of platelets store either peptides or small molecules that powerfully influence eosinophil, neutrophil, dendritic cell (DC), T cell, and endothelial cell recruitment and activation. Platelet depletion or the pharmacologic disruption of platelet activation inhibits asthmatic reactions experimentally (Pitchford et al., 2004; Suh et al., 2016). Nonetheless, prior to this work, little was known of how platelets are activated by allergens to influence Th2 and Th17 cell differentiation and how such differentiation occurs in the context of airway mycosis due to pathogenic fungi such as *C. albicans*.

The Wnt-beta catenin signaling pathway is activated in allergic airway disease and regulates this phenotype in complex ways (Kwak et al., 2015). A primary outcome of Wnt activation appears to be suppression of allergic airway disease (Beckert et al., 2018), although in the context of ultrafine particle challenge, Wnt activation may promote allergic disease (Harb et al., 2020). A suppressive role for Wnt was confirmed by the demonstration that Dkk-1 was required for allergic airway disease due to house dust mite (HDM) allergen (Chae *et al.*, 2016b). However, whereas Dkk-1 influenced only Th2 cell responses in this context, we demonstrated that Dkk-1 plays a much broader immune role, coordinately promoting both Th2 and Th17 cell responses, during airway mycosis due to *C. albicans*.

Thrombopoiesis occurs both in the bone marrow and the lung, with approximately 50% of

megakaryocytes normally found in the lung (Lefrancais et al., 2017). Total lung megakaryocytes did not change after either *C. albicans* challenge or platelet depletion (data not shown), but during fungal airway challenge, total megakaryocyte Dkk-1 decreased while Dkk-1 in platelets, especially platelets isolated from the pulmonary circulation, increased. Megakaryocytes also express GP1bα, which is required for their normal development and function (Kanaji et al., 2004; Meinders et al., 2016), but clearly megakaryocytes *per se* are not a significant source of plasma Dkk-1 during airway mycosis. Our findings therefore indicate that megakaryocytes respond to candidalysin through GP1bα by sequestering Dkk-1 into platelets, thus priming platelets to release enhanced quantities of Dkk-1 upon subsequent encounter with candidalysin. The specific ability of lung megakaryocytes as well as platelets to respond to *C. albicans* in a coordinated manner that critically protects the host suggests that megakaryocytes evolved a partial lung residence to rapidly counter infections with the potential for invasion such as airway mycosis.

In summary, our findings demonstrate that protective lung Th2 and Th17 cell responses against the common mucosa-associated fungus *C. albicans* are coordinated through lung megakaryocytes and platelets. We further demonstrate that *C. albicans* activates megakaryocytes and platelets through recognition of the virulence factor candidalysin by the VWF receptor GP1bα. Rather that eliciting thrombotic responses, candidalysin instead promotes secretion of Dkk-1, which drives the development of both Th2 and Th17 cells. The elaborate and highly specific defensive strategy that has evolved against *C. albicans* differs substantially from the mold proteinase-dependent pathway that also elicits Th2 and Th17 cell

responses, confirming that *C. albicans* is a major independent driver of human allergic diseases through adaptations that favor human infections (Kammer et al., 2020). Further understanding of platelet activation by *C. albicans* and other fungi may yield diagnostic and therapeutic options for asthma and other allergic disorders.

Limitations of Study

We have used primarily mouse studies to determine how the fungus *Candida albicans* elicits protective Th2 and Th17 cells through platelet-dependent Dkk-1 release. Although we included complementary studies with human platelets, additional studies are required to determine if *C. albicans* also elicits Th2 and Th17 cells through this mechanism in human CRS and other disease contexts. Future studies are further required to determine the importance of Dkk-1 for promoting Th2 and Th17 cells in the context of distinct allergenic challenges, including other fungi and common aeroallergens. Additional studies will also be required to understand how candidalysin modifies megakaryocytes to release platelets with an enhanced ability to secrete Dkk-1. Finally, the signaling mechanisms by which candidalysin elicits Dkk-1 release from platelets and Dkk-1 promotes Th2 and Th17 cell development are largely unknown and will require much additional study.

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Author contributions

DBC and FK conceived, designed, and supervised the project. YW and ZZ performed essential experiments, analyzed data, and wrote initial manuscript draft. LS, JEW, XH, YZ, LB, CYC, and JMK performed essential experiments. CV, MAC, BH, AUL, and JRN provided essential reagents and technical advice. All authors edited the manuscript.

Declaration of Interests

DBC is a scientific consultant to Atrapos Therapeutics, LLC and Pulmocide, LLC.

Inclusion and Diversity Statement

We worked to ensure gender balance in the recruitment of human subjects, ethnic or other types of diversity in the recruitment of human subjects, and sex balance in the selection of non-human subjects. One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in science. The author list of this paper includes contributors from the location where the research was conducted who participated in the data collection, design, analysis, and/or interpretation of the work.

Figure Legends

Figure 1. Candidalysin is necessary for the induction of allergic airway disease in mice. (A) C57BL/6 mice were challenged intranasally with 10^5 viable cells of wildtype parental strain or *ece1Δ/Δ C. albicans* as indicated in the timeline. **(B)** Respiratory system resistance (R_{RS}) was assessed after intravenous injection of increasing doses of acetylcholine (Ach). **(C)** Quantitation of cells from bronchoalveolar lavage fluid samples (mac: macrophages; eos: eosinophils; neu: neutrophils; lym: lymphocytes). **(D)** Cytokines quantitated by ELISA from deaggregated lung supernatants. **(E,F)** T cells from lungs analyzed by flow cytometry. **(E)** Representative flow plot of T_H1 (T-bet positive), Th2 (GATA3 positive) and Th17 (RORγt positive) cells from lungs after challenge. **(F)** Aggregate T cell data expressed as percentages and absolute cell numbers. **(G)** *C. albicans* colony forming units (CFU) cultured from lungs. **(H)** Hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining of 5 μm lung sections from mice challenged under the indicated conditions. ($n \geq 4$, mean±S.E.M, n.s.: not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$, using two-tailed Student's t-test (**F**) or one-way ANOVA followed by Tukey's test (**A-D, G**) for multiple comparison. Magnification: 40× and 200×. Bars: 500 or 50μm, respectively). Data are representative of three independent experiments. See also Figure S1 and Figure S2.

Figure 2. **Dkk-1 is secreted by mouse and human platelets in response to candidalysin and is required for robust allergic airway disease and *C. albicans* clearance from lung**. **(A)** Plasma Dkk-1 concentrations from patients with asthma and CRS as compared non-allergic

healthy controls. **(B, C)** Dkk-1 concentrations quantitated from plasma of mice challenged intranasally with **(B)** wild type parental strain or *ece1Δ/Δ C. albicans* or **(C)** candidalysin (CL) or scrambled control (SC). **(D)** Dkk-1 was quantitated from platelets of mice challenged intranasally with wild type or *ece1Δ/Δ C. albicans*. **(E-F)** Human platelets in plasma were incubated with either **(E)** *C. albicans* or **(F)** CL/SC after which secreted Dkk-1 was quantitated. (**G**) C57BL/6 mice were challenged intranasally with *C. albicans* (C.a) and intraperitoneally with Dkk-1 inhibitor (WAY262611) as shown. (**H**) Respiratory system resistance (R_{RS}) was quantitated as in Figure 1. (**I**) Quantitation of cells from the bronchoalveolar lavage fluid (mac: macrophages; eos: eosinophils; neu: neutrophils; lym: lymphocytes). (**J,K**) Cytokines assessed by ELISA from lung homogenate supernatants. (**L,M**) T cell quantitation from lungs as determined by flow cytometry. (**L**) Gating strategy for quantitation of T_H1, Th2 and Th17 cells from lungs. (**M**) Quantitation of T cells assessed as percentages and absolute cell counts. (**N**) Lung fungal burdens. ($n \geqslant 4$, mean ± S.E.M, n.s.: not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$, using one-way ANOVA followed by Tukey's test for multiple comparisons). Data are representative of two independent experiments. See also Figure S3.

Figure 3. Recombinant Dkk-1 enhances allergic airway disease in *ece1Δ/Δ* C. albicans-challenged mice. **(A)** Wildtype mice were challenged intranasally with *ece1Δ/Δ C. albicans* (C.a) and intraperitoneally with recombinant mouse Dkk-1 as shown. **(B)** Respiratory system resistance (R_{RS}) was assessed by increasing intravenous acetylcholine challenge. **(C)**. Quantitation of cells from bronchoalveolar lavage fluid. (**D-E**) Cytokines assessed by ELISA

from lung homogenate supernatants. (**F-G**) T cell quantification from lungs as assessed by flow cytometry. (**F**) Representative flow cytometry plot of T_H1, Th2 and Th17 cells from lungs after challenge. (**G**) Quantitation of T cells as expressed as percentages and absolute cell numbers. (**H**) *C. albicans* CFU retrieved from whole lung ($n \geq 4$, mean ± S.E.M, n.s.: not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$, using two-tailed Student's t-test (**G**) or one-way ANOVA followed by Tukey's test (**A-E**) for multiple comparison). Data are representative of two independent experiments.

Figure 4. Candidalysin primes human platelets to release Dkk-1 via GP1bα. (A) Human platelets were incubated with PBS or candidalysin (CL) at 10μM and with blocking reagents to the indicated platelet receptors as indicated after which secreted Dkk-1 was quantitated. **(B,C)** Schematic diagrams and aggregate data depicting in vitro assays in which the dose- dependent binding of either plate-bound candidalysin or scrambled control peptide (SC) (**B**) or GP1bα (**C**) to the other reagent was determined colorimetrically (OD: optical density). **(D)** Schematic diagram and aggregate data depicting in vitro binding assays with GP1bα blocking reagents (anti-GP1bα antibody; VWF A1A2A3 tridomain) in which the dose-dependent inhibition of binding of candidalysin is colorimetrically quantitated against plates coated with GP1bα. **(E)** Pull down assay of GP1bα from human platelet lysates using biotinylated candidalysin or SC as bait. ($n \geq 4$, mean±S.E.M, *$p<0.05$, **$p<0.01$, ***$p<0.001$ and ****$p<0.0001$ using Kurskal-Wallis test or one-way ANOVA followed by Tukey's test for multiple comparison. Illustrative figures generated at biorenders.com). Data are representative of two independent experiments.

Figure 5. Candidalysin directly binds to human platelets via GP1bα. (A-B) Flow cytometric analysis of human platelets incubated with AF647-conjugated CL (10μM) with or without **(A)** anti-GP1bα antibody or **(B)** VWF A1A2A3 tridomain. **(C)** Flow cytometric analysis of P-selectin (CD62P) on human platelets after incubation with CL (10μM) without or with anti-GP1bα antibody. Representative histograms, percentage quantification, and median fluorescence intensity (MFI) data are shown. **(D-E)** Human platelets were washed and then resuspended in PBS with blocking anti-GP1bα antibody or isotype control antibody prior to addition of candidalysin (CL; 10μM) after which **(D)** platelet counts and **(E)** Dkk-1 concentrations from supernatants were determined. (n=4, mean±S.E.M, ***$p<0.001$ and ****$p<0.0001$ using one-way ANOVA followed by Tukey's test for multiple comparison). Data are representative of two independent experiments. See also Figure S4.

Figure 6. Thrombocytopenic mice rapidly succumb to *C. albicans* airway challenge. (A) Total platelet count in whole blood from mice 2 h after platelet depletion with anti-GP1bα antibody. **(B)** Survival curves (hours) of platelet-depleted mice challenged once intranasally with PBS or 10^5 wild type parental strain or *ece1Δ/Δ C. albicans*. **(C)** Survival curve of platelet-depleted mice challenged intranasally with 16 μmol candidalysin or PBS. (**D-F**) Bronchoalveolar lavage fluid and whole lungs were collected 4 h after platelet-depleted mice were challenged intranasally with wild type or *ece1Δ/Δ C. albicans*. **(D)** Gross appearance of lungs. **(E)** Microscopic appearance of lungs (H&E staining) and **(F)** quantitation of hemoglobin from

BALF. **(G)** Representative microCT-based imaging of platelet sufficient and depleted mice challenged with either wild type or *ece1Δ/Δ C. albicans* as indicated. Bar graphs depict lung density as measured in Hounsfield units and aerated lung volume H: heart; L: lung; #: areas of abnormal alveolar filling ($n \geq 4$, mean±S.E.M, *$p<0.05$, **$p<0.01$, ***$p<0.001$ and ****$p<0.0001$ using one-way ANOVA followed by Tukey's test for multiple comparison, or Log-rank test for survival curves.) Data are representative of two independent experiments. See also Figure S5.

STAR Methods

Detailed methods are provided in the online version of this paper and include the following:

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Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact David B. Corry (dcorry@bcm.edu)

Material availability

This study did not generate new unique reagents

Data and code availability

This paper does not report original codes. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Experimental model and subject details

Animals used in this study

8 week-old C57BL/6J male and female mice (wild type and *Tlr4*$^{-/-}$) were purchased from Jackson Laboratories. All mice were bred and housed at the American Association for Accreditation of Laboratory Animal Care-accredited vivarium at Baylor College of Medicine under specific-pathogen-free conditions. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine and followed federal guidelines.

Method details

Human plasma samples. Deidentified human plasma samples were obtained from healthy control subjects with no allergic airway diseases or those diagnosed with asthma and CRS. Samples were originally obtained after obtaining informed consent under IRBs held at the University of Texas at Houston Health Sciences Center and Baylor College of Medicine.

Fungi and reagents. For experiments in which only wild type fungus was used, wild type *C. albicans* was isolated as described previously (Wu et al., 2019) and validated as *C. albicans* through the San Antonio Center for Medical Mycology. *SAP* gene deletant, *ece1Δ/Δ* (*ECE1* gene encodes the Ece1p protein from which candidalysin is derived), and their parental wildtype strain (BWP17/CIp30) of *C. albicans* were generated as previously described (Hube et al., 1997; Moyes *et al.*, 2016; Sanglard et al., 1997). *C. albicans* was propagated in YPD broth overnight at room temperature and collected in pyrogen-free phosphate buffered saline (PBS; Corning cellgro, Mediatech, Manassus, VA), passed through 40 μm nylon mesh, and washed twice with PBS by centrifugation (10,000 *g*, 5 min, 4°C). Fungal cells were then suspended in PBS and aliquots frozen in liquid nitrogen at 5×10^7/mL. Viability after freezing (> 95%) was confirmed by comparing haemacytometer-derived cell counts to CFU as determined by plating serial dilutions on Sabouraud's agar. Thawed, >95% viable cells were washed once, counted, and suspended in normal saline at indicated concentrations for intranasal challenge.

Candidalysin. Synthetic, biotinylated and Alexafluor 647-labeled candidalysin or scrambled peptide control were obtained commercially and were >98% pure (Peptide Protein Research Ltd, SO32 1QD, UK, or Genscript 08854, NJ, USA). Candidalysin sequence: SIIGIIMGILGNIPQVIQIIMSIVKAFKGNK. Scrambled control (SC) peptide sequence:

IFKIIISKIQIVMGLNGIPIKVAGSQNIGMI. Peptide biotinylation was performed on the N-terminal.

Fibrinogen Cleavage Products (FCPs). FCPs were prepared by suspending human fibrinogen (HCI-0150R; Haematologic Technologies, Essex Junction, Vermont) at 5 mg/mL in PBS. Proteinase from *Aspergillus melleus* (PAM; P4032; Sigma-Aldrich, St. Louis, MO), was added to the fibrinogen solution at a concentration of 6 μg/mL for 2 or 6 hours at 37°C (Landers et al., 2019a). In comparison, human fibrinogen (5 mg/mL) was buffer exchanged to pH 3.5 Tris buffer using Amicon ultra-4 centrifugal filter unit (UFC8010, Millipore Sigma, Burlington, MA), and then incubated with a mixture of secreted aspartic proteinases (Saps) containing predominantly Saps 1-3 isolated as described previously (Schild et al., 2011), or recombinant Saps (Schild *et al.*, 2011), individually or combined at 0.02 mg/mL. Protein lysates were then prepared using NuPAGE 4-12% Bis-Tris Protein Gels, MES SDS Running Buffer, and SimplyBlue SafeStain (Invitrogen, Carlsbad, CA). Samples for protein electrophoresis were diluted at least 1:2 in Tricine sample buffer and Precision Plus Protein Kaleidoscope Prestained Protein Standards were used as the protein electrophoresis molecular weight marker (Bio-Rad, Hercules, CA). See Fig. S6

A1A2A3 von Willebrand factor tridomain protein. Recombinant VWF A1A2A3 tridomain protein was generated using complementary DNA encoding the human VWF A1, A2, and A3 domains (amino acids Q1238-G1874) and inserted via PCR into the pSecTag2B vector (Invitrogen, CA) as described elsewhere (Auton 2007). Recombinant A1A2A3 was expressed in human embryonic kidney (HEK293) cells and purified using affinity chromatography from

conditioned medium. The purified A1A2A3 protein was dialyzed against 1x tris-buffered saline supplemented with 0.1% tween-20 and subjected to gel electrophoresis for verification of purity prior to experimental use (Auton et al., 2007).

Fungal cultivation. Sabouraud's agar (BD, Sparks, 21152) was dissolved in water at 50 g/L, and autoclaved for 30 min. Chloramphenicol (Sigma Aldrich, St. Louis) was added to the warm solution at 50 mg/L and the agar was sterilely poured into Petri dishes (VWR, Radnor, 19087) and cooled overnight. Plates were sealed and kept at 4°C until used for fungal growth.

Induction and quantitation of allergic airway disease. C57BL/6 mice were administered 1×10^5 viable cells of *C. albicans* intranasally every other day for 8 challenges as shown in **Figure 1** (Porter *et al.*, 2011a). **Alternatively,** C57BL/6 mice were given 4, 8, and 16 μmol candidalysin or 16 μmol of scrambled peptide control in PBS every other day for 8 challenges as shown in **Figure S2.** Dkk-1 inhibitor (Chae et al., 2016a) (WAY262611, cat: 317700, Millipore Sigma, Burlington, MA. Dose: 10 or 20 μg/kg) and recombinant mouse Dkk-1 (5897-DK-010, R&D systems, Minneapolis, MN. Dose: 5 μg/kg) were given intraperitoneally at the same time and fungal challenge. Mice were analyzed 24 h after the final challenge for allergic airway disease endpoints.

Bronchial constriction in response to increasing dose of acetylcholine (Ach) injected intravenously, bronchoalveolar lavage fluid (BALF) cell differential counting, Dkk-1 quantitation from plasma, ELISA analysis of whole lung, flow cytometry analysis of Th cells and lung histopathology were assessed in response to *C. albicans* challenge as previously described (Porter *et al.*, 2011a). Briefly, mice were anesthetized with etomidate and

intravenously injected with acetylcholine via tail vein to assess AHR as quantified by measuring respiratory system resistance (R_{RS}). Total BALF cells were collected by lavaging whole lung, total cells were enumerated and differential cell counting was performed on modified Giemsa-stained cytospin preparations. Plasma was harvested by retro-orbital bleeding and anti-coagulated by 10% 0.5M EDTA (Thermofisher scientific, Waltham MA). Lungs were harvested and processed as follows. Lungs were cut into small pieces and incubated in digestion buffer (2mg/ml collagenase (#LS004177, Worthington), 0.04mg/ml DNAse (#10104159001, Sigma) 1, 20% FBS in HBSS) for 1 h at 37°C after which they were deaggregated by pressing through a 40 μM nylon mesh and centrifuged at 400 × g for 5 minutes at 4°C. Supernatants were discarded, and 1.5 mL of ACK (Thermofisher scientific, Waltham MA) was added and incubated for 3 min at room temperature for erythrocyte lysis. ACK was then neutralized with 7.5 mL of complete RPMI-1640 (Corning, NY), with 10% FBS and 1% Pen Strep, Gibco, Waltham MA). The resulting leukocyte preparations were centrifuged and prepared for flow cytometry analysis or ELISA.

Cytokine measurements. Cell culture supernatants were analyzed for cytokines by standard ELISA after comparison to recombinant standard. IL-4 (Clone 11B11 and 554390 BD Biosciences, San Jose, CA), IL-5, IL-13 (DY405, DY413, R&D systems, Minneapolis, MN), IL-17 (BMS2017, Thermofisher scientific, Waltham MA), IFN-γ (555142, BD Biosciences, San Jose, CA), IL-1, IL-6, TNF (ab210895, ab213749 and ab212073, Abcam, Cambridge MA) were used as antibodies pairs and capture signals were amplified using Streptavidin-horseradish peroxidase conjugate (51-9002813, BD Biosciences, San Jose, CA). The plate was further

developed using TMB substrate solution (N301, Thermofisher scientific, Waltham MA) and detected at the absorbance wavelength of 450 nm.

Flow cytometry. Total lung cells isolated above were stained with Live/Dead Fix Blue (L34961, Thermofisher scientific, Waltham MA), CD45, CD3, CD4 (103122, 100222, 100412, Biolegend, San Diego, CA). Cells were separated into 3 groups, then permeabilized and fixed using Transcription Factor Buffer Set (562574, BD Biosciences, San Jose, CA), and stained individually for T-bet, GATA3 or RORγt (561265, 560074, 562607, BD Biosciences, San Jose, CA). For megakaryocyte staining, total lung cells were stained with Live/Dead Fix Blue and CD41, CXCR4 (133927, 146507, Biolegend, San Diego, CA), Dkk-1 (BAM1765, R&D systems, Minneapolis, MN) and PE-streptavidin (405203, Biolegend, San Diego, CA). Data were analyzed using FlowJo software (version 10.0.7; Treestar, Ashland, OR). See Figure S6.

Measurement of Dkk-1 from plasma. Whole blood from mice was isolated by retro-orbital puncture and anticoagulated with 10% 0.5 M EDTA and plasma was isolated by centrifugation at 1000 × g for 10 min at 4°C and stored at -80^{o}C until analyzed. Plasma from either mice or humans was diluted 1:10 for Dkk-1 measurement by ELISA (DY1765, R&D systems, Minneapolis, MN) according to the manufacturer's protocol. For human Dkk-1 quantitation, previously banked plasma samples obtained from subjects presenting for evaluation of either chronic obstructive pulmonary disease (COPD) and no prior history of asthma or CRS or CRSwNP were used. Specimens were randomly selected for analysis, excluding those with substantial hemolysis or that demonstrated platelet contamination.

Platelet isolation from mice. Whole blood from mice was isolated by retro-orbital bleeding and

anticoagulated with 10% 0.5 M EDTA. Alternatively, whole blood was collected from the left or the right ventricle of mice using an 18G needle pre-coated with 10% 0.5M EDTA. Platelet rich plasma was isolated by centrifugation at 180 × g for 10 min at room temperature, and platelets were isolated by centrifugation at 1250 × g for 10 min at room temperature. Platelets were then resuspended in Tyrodes buffer (NaCl: 8.19g/L, KCl: 0.2g/L, $NaHCO_3$: 1.01g/L, NaH_2PO_4, 0.055g/L, Glucose: 0.991g/L, $MgCl_2$: 0.49mM, $CaCl_2$: 1.8mM) and counted via flow cytometry. For Dkk-1 quantification, platelets were resuspended in PBS and lysed via sonication.

Preparation of human platelets. Human platelets were isolated from whole blood as platelet rich plasma through the Gulf Coast Blood Center, Houston, TX. For washed, plasma free platelets, PGE1 (sc-201223, Santa Cruz, Dallas, TX) was added to platelet rich plasma to a working concentration of 75 nM. Platelet rich plasma was then centrifuged at 1000 × g for 10 min. The supernatant, platelet poor plasma, was removed using a pipette and platelets were resuspended in CGS buffer (13mM sodium citrate, 30mM glucose, 120mM NaCl, pH: 6.25) containing 75 nM PGE1. Platelets were then centrifuged at 1000 × g for 10 min again. The supernatant was removed and the platelet pellet was resuspended in Tyrodes buffer.

Dkk-1 release assay. Human platelets (1×10^9/mL) were pretreated with/without receptor blocker for 1 h at 37°C (P2Y1: Clopidogrel (Weber et al., 1999), P2Y12: MRS2179 (Baurand et al., 2001), GP2b3a: tirofiban, GPVI: losartan (Taylor et al., 2014) at 100 μM, Millipore Sigma, Burlington, MA; α2β1: 10 μg/mL 910901, Biolegend, San Diego, CA; GPIV: 10 μg/mL ab23680, Abcam, Cambridge MA; GPVI: 10 μg/mL AF3627, CLEC2: 10 μg/mL AF1718(Tsukiji et al., 2018), R&D systems, Minneapolis, MN; TLR4: 10 μg/mL tlrl-prslps,

Invivogen, San Diego, CA; Anti-GP1bα Clone AK2 (Yuan et al., 1999) at 10 μg/mL, Invitrogen, Carlsbad, CA) and then incubated with candidalysin (10 or 20 μM) or *Candida albicans* overnight at 37°C. Cells were centrifuged at 1000 × g, 10 min at 4°C and the supernatants were again centrifuged at 20,000 × g, 10 min, 4°C to remove platelets and yeast cells. The remaining supernatants were diluted 1:10 for Dkk-1 measurement by ELISA (DY1906, R&D systems, Minneapolis, MN).

Alternatively, EOMA cells were seeded in 24 well plates (2×10^6/well) and stimulated with candidalysin (10 or 20 μM) overnight at 37°C. Cells were centrifuged at 400 × g, 5 min at 4°C and the supernatants were used to detect Dkk-1 by ELISA (DY1765, R&D systems, Minneapolis, MN). The lower limit of detection of Dkk-1 in tissue culture media and PBS was 80-100 pg/ml and in plasma, 1.5-2.2 ng/ml.

Platelet activation and binding to candidalysin. Platelets (1×10^9/mL) were pretreated with anti-GP1bα (Clone AK2 at 10 μg/mL, Invitrogen, Carlsbad, CA) or A1A2A3 von Willebrand factor tridomain protein for 1 h at room temperature and then incubated with untagged, Alexafluor-647-tagged candidalysin (10 or 20 μM), or scrambled peptide control for 1 h at room temperature. Activation was assessed by CD62P upregulation (304910, Biolegend, San Diego, CA) and AF-647-candidalysin binding to platelets was assessed by flow cytometry(Nagy Jr et al., 2013).

Alternatively, platelets were treated with biotinylated candidalysin (10 or 20 μM) or scrambled peptide control (20 μM) for 1 hours, washed with CGS buffer, and stained using Alexafluor-647-tagged streptavidin for 30 minutes.

GP1bα-candidalysin binding assay. 96-well plates (9018, Corning, Kennebuck, ME) were coated with 5 μg/mL candidalysin, scrambled peptide control in carbonate buffer (pH = 9.0) overnight at 4°C. Plates were blocked with i-Block (2%) for 2 h at 37°C and incubated with a 2/3 serial dilution of His-tagged GP1bα (4067-GP, R&D systems, Minneapolis, MN) starting from 50 nM for 2 h at 37°C. After washing, plates were incubated with biotinylated anti-His antibody (5 μg/mL, BAM050, R&D systems, Minneapolis, MN) for 2 h at 37°C followed by SAv-HRP (1:250, 51-9002813, BD Biosciences, San Jose, CA). The plate was further developed using TMB substrate solution (N301, Thermofisher scientific, Waltham MA) and detected at the absorbance wavelength of 450 nm. Alternatively, (**Figure 4**) plates were coated with 5 μg/mL GP1bα and a 2/3 serial dilution of biotinylated candidalysin or scrambled peptide control starting from 5 nM was performed followed by addition of SAv-HRP and TMB. As a proper control, binding affinity between candidalysin and an integrin receptor GPIIb/IIIa was also assessed in the same experimental setup (**Fig. S4**).

Inhibition of GP1bα-candidalysin binding. 96 well plates (9018, Corning, Kennebuck, ME) were coated with 0.1 μg/mL GP1bα in carbonate buffer (pH=9.0) overnight at 4°C. Plates were blocked for 2 h at 37°C and incubated with a 1/2 serial dilution of anti-GP1bα antibody (Clone AK2, Invitrogen, Carlsbad, CA) or VWF A1A2A3 tridomain protein for 2 h at 37°C. Without washing, biotinylated candidalysin was added into each well to a final concentration of 0.1 μM and incubated for 1 h at 37°C followed by SAv-HRP and TMB.

GP1bα pulldown. Human platelets were lysed using ChIP lysis buffer (5 mM PIPES, 85 mM KCl, 0.5% NP-40. P7643, P3911, 98379, Sigma Aldrich, St. Louis) and lysates were then buffer

transferred to TBS using Amicon filter unit (UFC801096, Sigma Aldrich, St. Louis) containing protease inhibitor cocktail (78442, Thermofisher scientific, Waltham MA). Immunoprecipitation was carried out using Pierce biotinylated protein interaction pulldown kit (21115 Thermofisher Scientific, Waltham MA). Briefly, 60 μg of biotinylated candidalysin or scrambled peptide control were loaded onto agarose-streptavidin slurry as bait proteins. After biotin blocking, the slurry was incubated with platelet lysate for 1 h at 4°C. The slurry was then washed with acetate buffer containing 0.5 M NaCl, and the eluates were subjected to SDS-PAGE using 5% milk as blocking reagent to detect GP1bα (2 μg/mL, MAB4067, R&D systems, Minneapolis, MN).

Platelet aggregation assay: Blood from healthy individuals was drawn and mixed with sodium citrate. Blood was centrifuged at 189 x g for 15 min at room temperature to obtain platelet-rich plasma (PRP). Platelet aggregation was initiated by addition of collagen (2.5 μg/mL) or increasing concentrations of candidalysin to a 225 μl aliquot of PRP in a four channel Bio/Data PAP-4C aggregometer (Biodata Corporation, Horsham, PA). Platelet aggregation was then assessed after 5 minutes.

Platelet depletion. Wild type mice were give 2 μg/g of platelet depleting antibody intraperitoneally (R300, Emfret Analytics, Würzburg, Germany) as described(Lam et al., 2011). Depletion of platelets from mice in the allergic airway disease model was carried out with intraperitoneal injection of the antibody with each intranasal challenge, 8 times over 2 weeks as described above.

Hemoglobin quantitation. Bronchoalveolar lavage fluid from mice was obtained as above. Free hemoglobin was quantified using a colorimetric kit (ab234046, Abcam, Cambridge MA).

Lung section staining. Lung sections were isolated from mice and stained using H&E, PAS kit or GMS kit (1016460001, 1008200007, Sigma Aldrich, St. Louis).

Measurement of Lung Density and Volumes. Mice were anesthetized, intubated and placed into the CT scanner to generate images in the Baylor College of Medicine Mouse Metabolic and Phenotyping Core. VivoQuantTM image analysis solution software (Invicro, Boston, MA) was used for post-processing of DICOM image data. To determine lung density in Hounsfield unit (HU), the 3D ROI tool was used to generate quantitative analysis. An internal negative and positive control were measured based on the phantom pane located on each image. A standard anatomical landmark for HU measurement was determined for each lung sample. Using the coronal plane, the right and left bronchi were identified, and a 10-point mark was placed lateral to each bronchi. The mean HU per lung was calculated and compared to the negative control HU and positive control HU. Quantitation of lung volume was calculated using segmentation algorithms. OTSU thresholding and ROI connected thresholding functional tools were utilized by measuring the number of voxels assigned to the lung space. Lung volumes, measured in mm^3, represent clear lung space within the lungs.

Quantification and Statistical analysis

Data are presented as means ± standard errors of the means. Significant differences relative to PBS-challenged mice or appropriate controls are expressed by P values of <0.05, as measured two tailed Student's t-test or one-way ANOVA followed by Dunnett's test or Tukey's test for multiple comparison. Survival curves were analyzed using Log-rank test. Human plasma

samples were analyzed using Kruskal-Wallis test. Data normality was confirmed using the Shapiro-Wilk test.

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KEY RESOURCES TABLE

The table highlights the reagents, genetically modified organisms and strains, cell lines, software, instrumentation, and source data **essential** to reproduce results presented in the manuscript. Depending on the nature of the study, this may include standard laboratory materials (i.e., food chow for metabolism studies, support material for catalysis studies), but the table is **not** meant to be a comprehensive list of all materials and resources used (e.g., essential chemicals such as standard solvents, SDS, sucrose, or standard culture media do not need to be listed in the table). **Items in the table must also be reported in the method details section within the context of their use.** To maximize readability, the number of **oligonucleotides and RNA sequences** that may be listed in the table is restricted to no more than 10 each. If there are more than 10 oligonucleotides or RNA sequences to report, please provide this information as a supplementary document and reference the file (e.g., See Table S1 for XX) in the key resources table.

Please note that ALL references cited in the key resources table must be included in the references list. Please report the information as follows:

- **REAGENT or RESOURCE:** Provide full descriptive name of the item so that it can be identified and linked with its description in the manuscript (e.g., provide version number for software, host source for antibody, strain name). In the experimental models section (applicable only to experimental life science studies), please include all models used in the paper and describe each line/strain as: model organism: name used for strain/line in paper: genotype. (i.e., Mouse: $OXTR^{fl/fl}$: B6.129(SJL)-$Oxtr^{tm1.1Wsy/J}$). In the biological samples section (applicable only to experimental life science studies), please list all samples obtained from commercial sources or biological repositories. Please note that software mentioned in the methods details or data and code availability section needs to also be included in the table. See the sample tables at the end of this document for examples of how to report reagents.

- **SOURCE:** Report the company, manufacturer, or individual that provided the item or where the item can be obtained (e.g., stock center or repository). For materials distributed by Addgene, please cite the article describing the plasmid and include “Addgene” as part of the identifier. If an item is from another lab, please include the name of the principal investigator and a citation if it has been previously published. If the material is being reported for the first time in the current paper, please indicate as “this paper.” For software, please provide the company name if it is commercially available or cite the paper in which it has been initially described.

- **IDENTIFIER:** Include catalog numbers (entered in the column as “Cat#” followed by the number, e.g., Cat#3879S). Where available, please include unique entities such as RRIDs, Model Organism

Database numbers, accession numbers, and PDB, CAS, or CCDC IDs. For antibodies, if applicable and available, please also include the lot number or clone identity. For software or data resources, please include the URL where the resource can be downloaded. Please ensure accuracy of the identifiers, as they are essential for generation of hyperlinks to external sources when available. Please see the Elsevier list of data repositories with automated bidirectional linking for details. When listing more than one identifier for the same item, use semicolons to separate them (e.g., Cat#3879S; RRID: AB_2255011). If an identifier is not available, please enter "N/A" in the column.

- ***A NOTE ABOUT RRIDs:*** We highly recommend using RRIDs as the identifier (in particular for antibodies and organisms but also for software tools and databases). For more details on how to obtain or generate an RRID for existing or newly generated resources, please visit the RII or search for RRIDs.

Please use the empty table that follows to organize the information in the sections defined by the subheading, skipping sections not relevant to your study. Please do not add subheadings. To add a row, place the cursor at the end of the row above where you would like to add the row, just outside the right border of the table. Then press the ENTER key to add the row. Please delete empty rows. Each entry must be on a separate row; do not list multiple items in a single table cell. Please see the sample tables at the end of this document for relevant examples in the life and physical sciences of how reagents and instrumentation should be cited.

TABLE FOR AUTHOR TO COMPLETE

Please upload the completed table as a separate document. ***Please do not add subheadings to the key resources table.*** *If you wish to make an entry that does not fall into one of the subheadings below, please contact your handling editor.* ***Any subheadings not relevant to your study can be skipped.*** *(**NOTE:** For authors publishing in Cell Genomics, Cell Reports Medicine, Current Biology, and Med, please note that references within the KRT should be in numbered style rather than Harvard.)*

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-mouse CD45 (Alexafluor 488)	Biolegend	Cat# 103122, RRID:AB_493531
Anti-mouse CD3 (APC-Cy7)	Biolegend	Cat# 100222 RRID:AB_2242784

Anti-mouse CD4 (APC)	Biolegend	Cat# 100412 RRID:AB_312697
Anti-mouse T-bet (PE)	BD Biosciences	Cat# 561265 RRID:AB_10565980
Anti-mouse GATA3 (PE)	BD Biosciences	Cat# 560074 RRID:AB_1645330
Anti-mouse RORγt (PE)	BD Biosciences	Cat# 562607 RRID:AB_11153137
Anti-mouse CD41 (APC-Cy7)	Biolegend	Cat# 133927 RRID:AB_2572131
Anti-mouse CXCR4 (APC)	Biolegend	Cat# 146507 RRID:AB_2562784
Anti-human α2β1	Biolegend	Cat# 910901 RRID:AB_2565103
Anti-human GPIV	Abcam	Cat# ab23680 RRID:AB_447608
Anti-human GPVI	R&D systems	Cat# AF3627 RRID:AB_2114072
Anti-human CLEC2	R&D systems	Cat# AF1718 RRID:AB_2083455
Anti-mouse GP1bα	Invitrogen	Cat# MA5-16564 RRID:AB_2538068
Anti-human CD62P (APC)	Biolegend	Cat# 304910 RRID:AB_314482
biotinylated anti-His antibody	R&D systems	Cat# BAM050 RRID:AB_356845
Anti-mouse GP1bα for western	R&D systems	Cat# MAB4067 RRID:AB_2113776
Platelet depleting antibody	Emfret	Cat# R300 RRID:AB_2721041
Bacterial and virus strains		

Biological samples		
Human plasma samples	University of Texas at Houston Health Sciences Center and Baylor College of Medicine	https://www.uth.edu/ https://www.bcm.edu/
Human platelets rich plasma	Gulf Coast Blood Center	https://www.giveblood.org/
Chemicals, peptides, and recombinant proteins		
YPD Broth	BD	DF0428-17-5
Sabouraud's agar	BD	DF0109-08-2
Chloramphenicol	Sigma Aldrich	C0378-100G
Recombinant Candidalysin (>98% purity): SIIGIIMGILGNIPQVIQIIMSIVKAFKGNK	Peptide Protein Research Ltd Genscript	N/A
Recombinant Scrambled control (SC) peptide (>98% purity): IFKIIISKIQIVMGLNGIPIKVAGSQNIGMI	Peptide Protein Research Ltd Genscript	N/A
Recombinant Biotinylated Candidalysin (>98% purity): (Bio)-SIIGIIMGILGNIPQVIQIIMSIVKAFKGNK	Genscript	N/A
Recombinant Biotinylated Scrambled control (SC) peptide (>98% purity): (Bio)-IFKIIISKIQIVMGLNGIPIKVAGSQNIGMI	Genscript	N/A
Recombinant AF647 tagged Candidalysin (>98% purity): (AF647)-SIIGIIMGILGNIPQVIQIIMSIVKAFKGNK	Peptide Protein	N/A
Fibrinogen Cleavage Products	Haematologic Technologies, Essex Junction	HCI-0150R
Proteinase from *Aspergillus melleus*	Sigma-Aldrich	P4032
Secreted aspartic proteinases (Saps)	Dr. Bernhard Hube Schild et al., 2011	N/A
Recombinant VWF A1A2A3 tridomain protein	Dr. Miguel Cruz Auton et al., 2009	N/A
Dkk-1 inhibitor: WAY262611	Millipore Sigma	317700
recombinant mouse Dkk-1	R&D systems	5897-DK-010
acetylcholine	Sigma-Aldrich	A6625-25G
EDTA	Thermofisher scientific	AM9260G
collagenase	Worthington	#LS004177
DNAse	Sigma-Aldrich	10104159001

HBSS	Thermofisher scientific	14175095
ACK	Thermofisher scientific	A1049201
RPMI-1640	Corning	10-040
FBS	Gibco	26140079
Penicillin-Streptomycin	Gibco	15140122
Streptavidin-horseradish peroxidase conjugate	BD Biosciences	51-9002813
TMB substrate solution	Thermofisher scientific	N301
Live/Dead Fix Blue	Thermofisher scientific	L34961
Transcription Factor Buffer Set	BD Biosciences	562574
PE-streptavidin	Biolegend	405203
PGE1	Santa Cruz	sc-201223
Clopidogrel	Millipore Sigma	120202-66-6
MRS2179	Millipore Sigma	101204-49-3
tirofiban	Millipore Sigma	144494-65-5
losartan	Millipore Sigma	124750-99-8
LPS-RS	invivogen	tlrl-prslps
i-Block	Invitrogen	T2015
His-tagged GP1bα	R&D systems	4067-GP
SAv-HRP	BD Biosciences	51-9002813
His-tagged GPIIb/IIIa	R&D systems	7148-A2
ChIP lysis buffer	Santa Cruz	sc-45000
protease inhibitor cocktail	Thermofisher scientific	78442
sodium citrate	Millipore Sigma	6132-04-3
collagen	Millipore Sigma	232-697-4
Critical commercial assays		
ELISA Antibody Pair: mouse IL-4	BD Bioscience	Cat# 559062, 554390 RRID:AB_397187, AB_395362
ELISA Antibody Pair: mouse IL-5	R&D systems	Cat# DY405
ELISA Antibody Pair: mouse IL-13	R&D systems	Cat# DY413
ELISA Antibody Pair: mouse IL-17	Thermofisher scientific	Cat# BMS2017
ELISA Antibody Pair: mouse IFN-γ	BD Biosciences	Cat# 555142
ELISA Antibody Pair: mouse IL-1	Abcam	Cat# ab210895
ELISA Antibody Pair: mouse IL-6	Abcam	Cat# ab213749
ELISA Antibody Pair: mouse TNF	Abcam	Cat# ab212073

ELISA Antibody Pair: mouse Dkk-1	R&D systems	Cat# DY1765
ELISA Antibody Pair: human Dkk-1	R&D systems	Cat# DY1906
Pierce biotinylated protein interaction pulldown kit	Thermofisher scientific	Cat# 21115
Hemoglobin quantification kit	Abcam	Cat# ab234046
PAS staining kit	Millipore Sigma	Cat# 1016460001
GMS kit	Millipore Sigma	Cat# 1008200007
Deposited data		
Experimental models: Cell lines		
HEK293	ATCC	**CRL-1573**
EOMA	ATCC	**CRL-2586**
Experimental models: Organisms/strains		
Mice: C57BL/6J	The Jackson Laboratory	Stock number: 000664
Mice: TLR4$^{-/-}$	The Jackson Laboratory	Stock number: 007227
C. albicans: WT (clinical isolate)	Wu et al., 2019	N/A
C. albicans: *SAP1-3 Δ / Δ*. Sap1, Sap2, Sap3 triple gene deletant sap1 Δ ::hisG/sap1 Δ ::hisG sap2 Δ ::hisG/sap2 Δ ::hisG sap3 Δ ::hisG/sap3 Δ ::hisG rps1::URA3	Dr. Bernhard Hube Hube et al., 1997; Moyes *et al.*, 2016;	N/A
C. albicans: *SAP4-6 Δ / Δ* Sap4, Sap5, Sap6 triple gene deletant sap1 Δ ::hisG/sap1 Δ ::hisG sap2 Δ ::hisG/sap2 Δ ::hisG sap3 Δ ::hisG/sap3 Δ ::hisG rps1::URA3	Dr. Bernhard Hube Hube et al., 1997;	N/A
C. albicans: Candidalysin gene deletant ece1::HIS1/ece1::ARG4; RPS1/rps1::URA3	Dr. Bernhard Hube Dr. Julian Naglik Moyes *et al.*, 2016;	N/A
C. albicans: BWP17/CIp30 Parental wildtype isogenic strain Rps1::(HIS1 ARG4 URA3)	Dr. Bernhard Hube Dr. Julian Naglik Hube et al., 1997; Moyes *et al.*, 2016;	N/A
Oligonucleotides		

Recombinant DNA		
complementary DNA encoding the human VWF A1, A2, and A3 domains (amino acids Q1238-G1874)	Auton et al., 2009	N/A
Software and algorithms		
FlowJo software (version 10.0.7)	LLC	https://www.flowjo.com
GraphPad PRISM v.6.0.1	GraphPad software	https://www.graphpad.com
Other		
Amicon ultra-4 centrifugal filter unit	Millipore Sigma	UFC8010
NuPAGE 4-12% Bis-Tris Protein Gels	Invitrogen	NP0335BOX
MES SDS Running Buffer	Invitrogen	NP0002
SimplyBlue SafeStain	Invitrogen	LC6060
Precision Plus Protein Kaleidoscope Prestained Protein Standards	Bio-rad	#1610375
pSecTag2B vector	Invitrogen	V90020
96-well plates	Corning	9018
Amicon filter unit	Sigma Aldrich	UFC801096

Figure 1

Fig 1

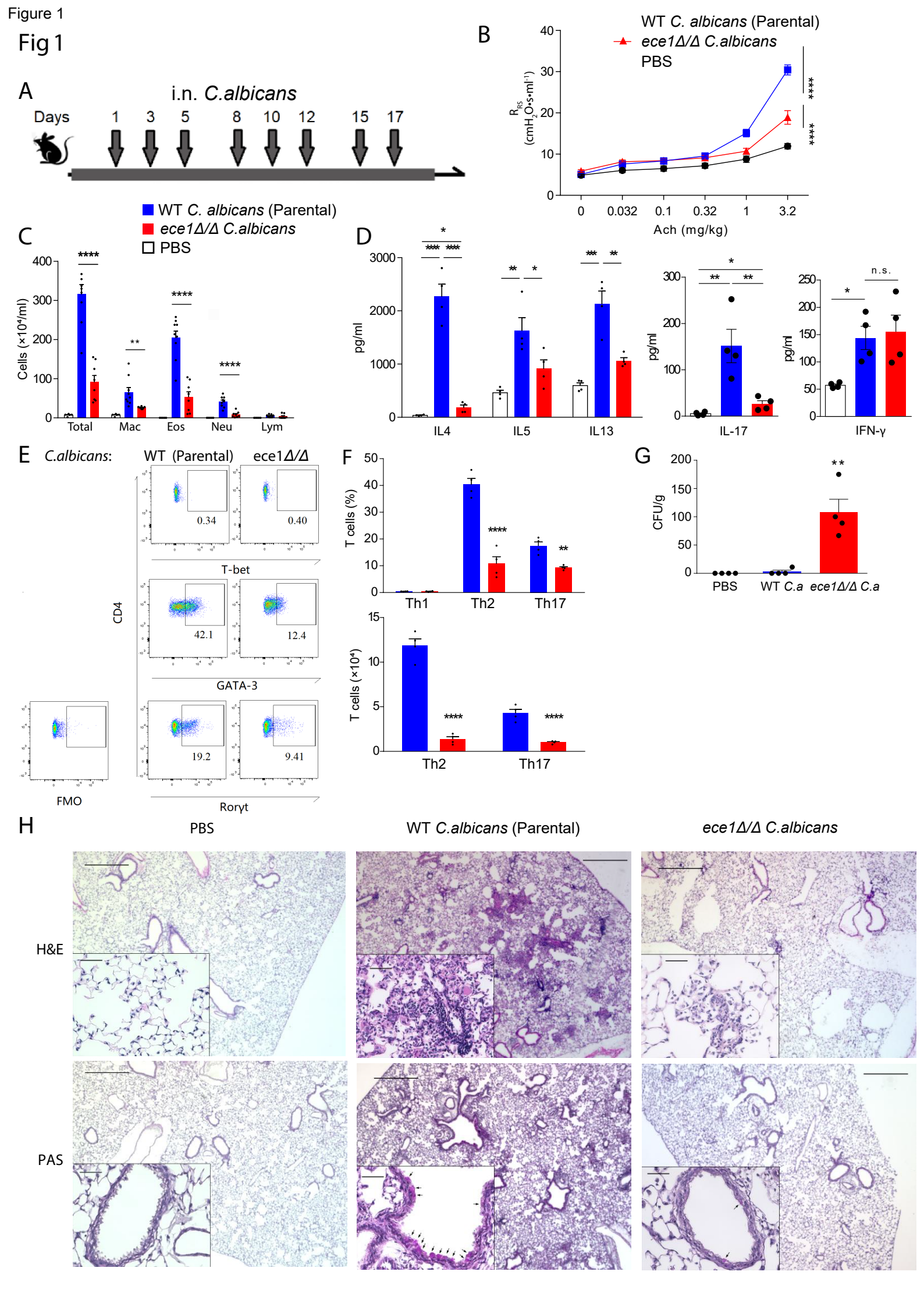


Figure 2
Fig 2

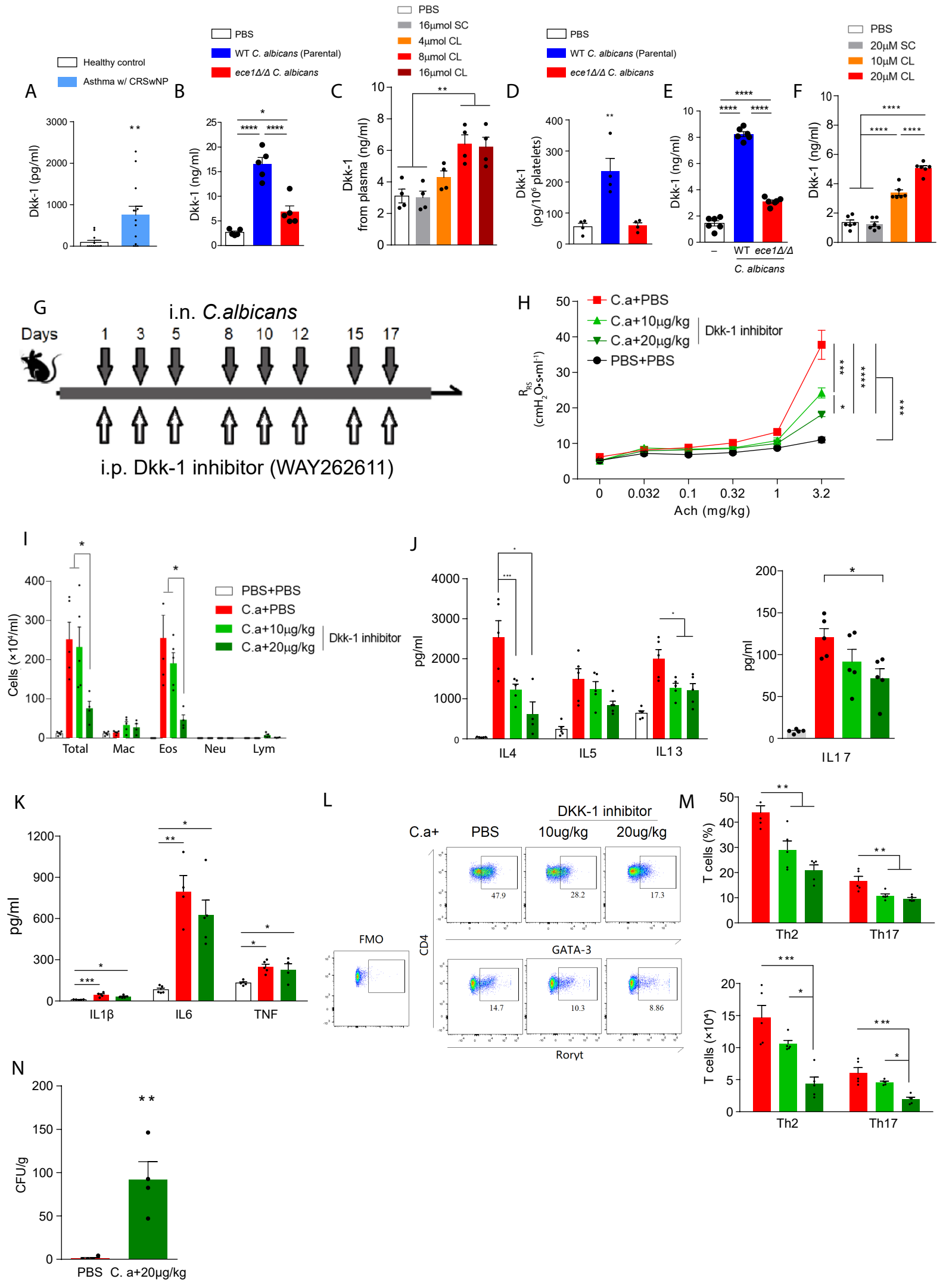


Figure 3

Fig 3

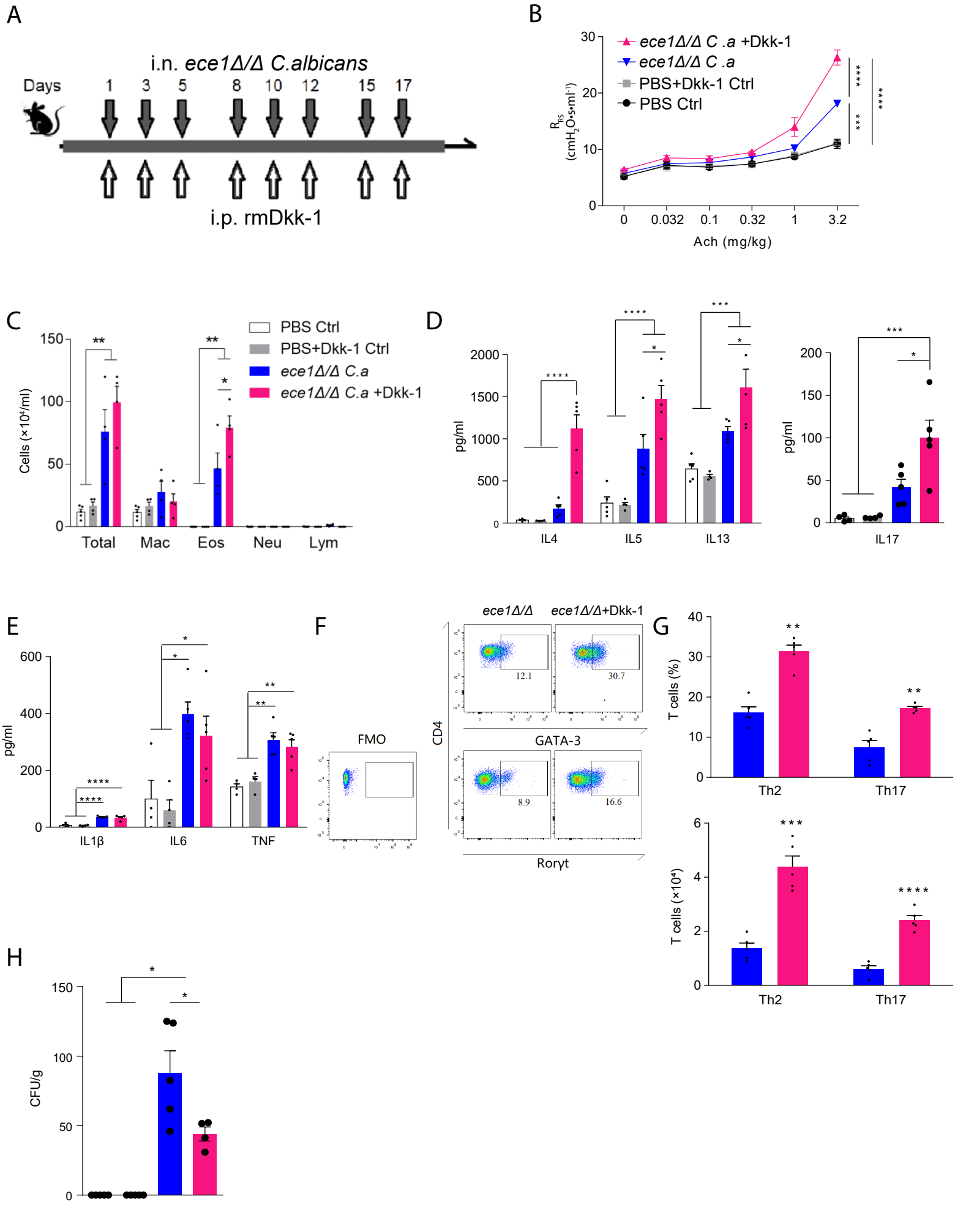


Figure 4
Fig 4

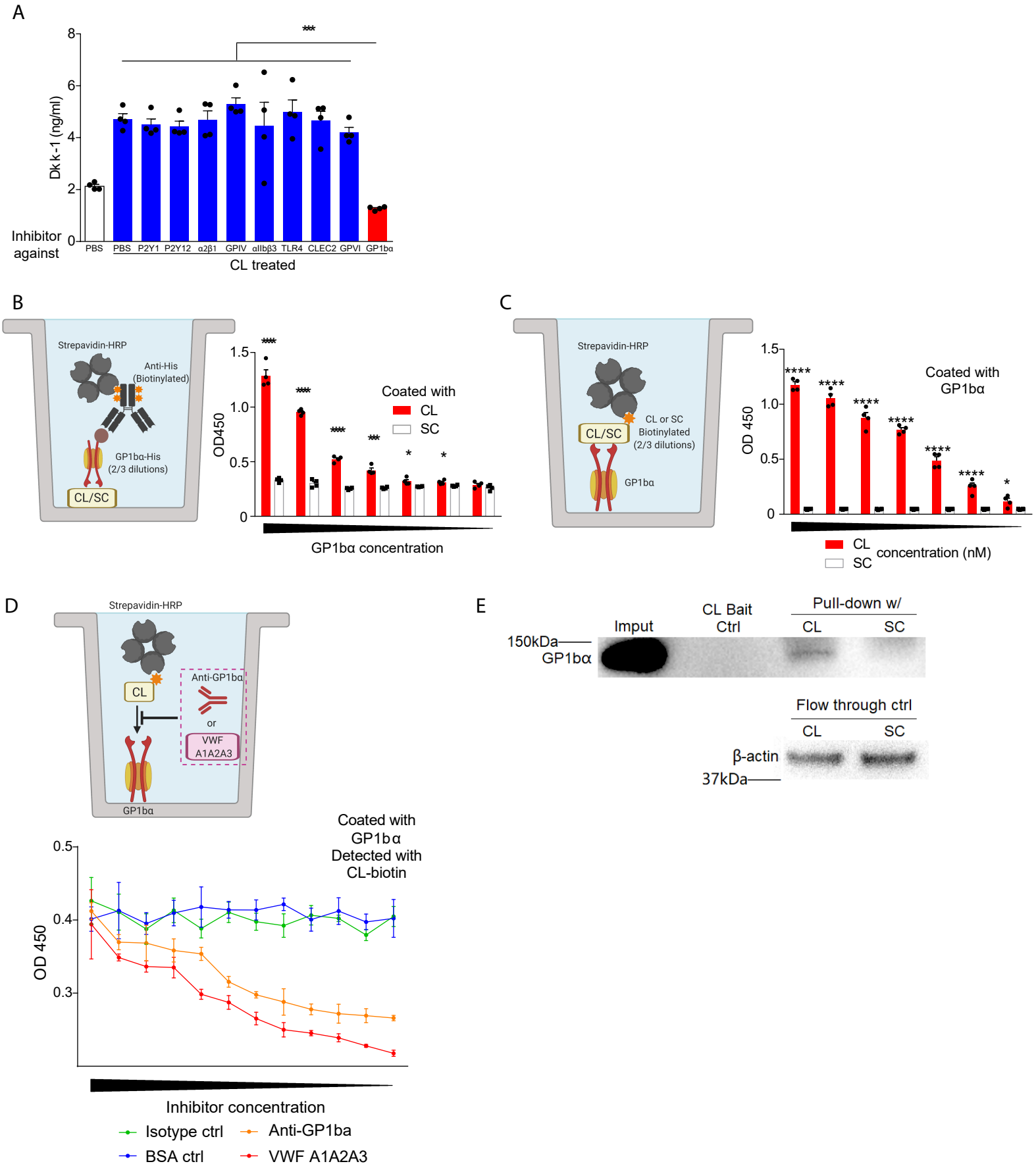


Figure 5

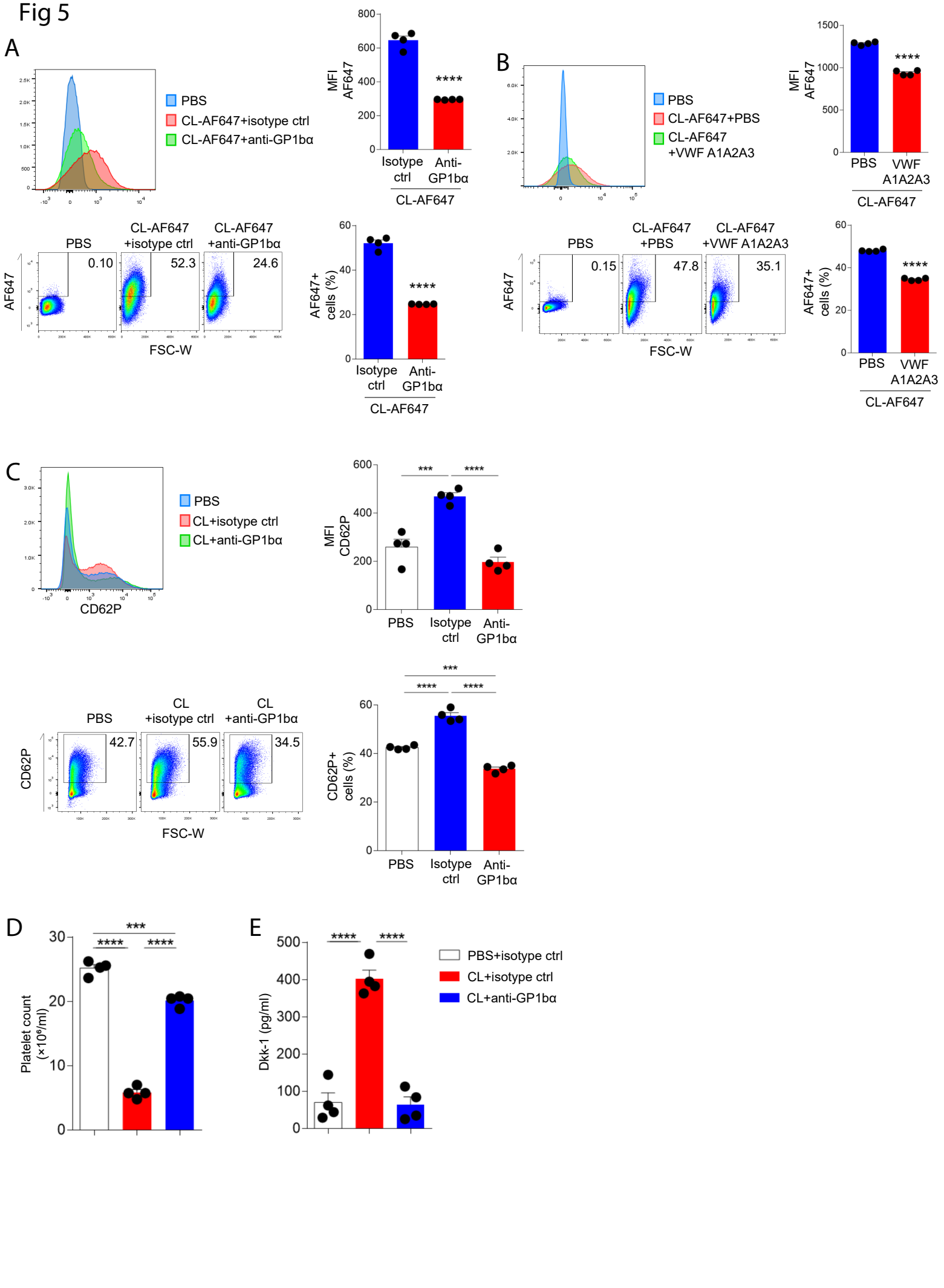
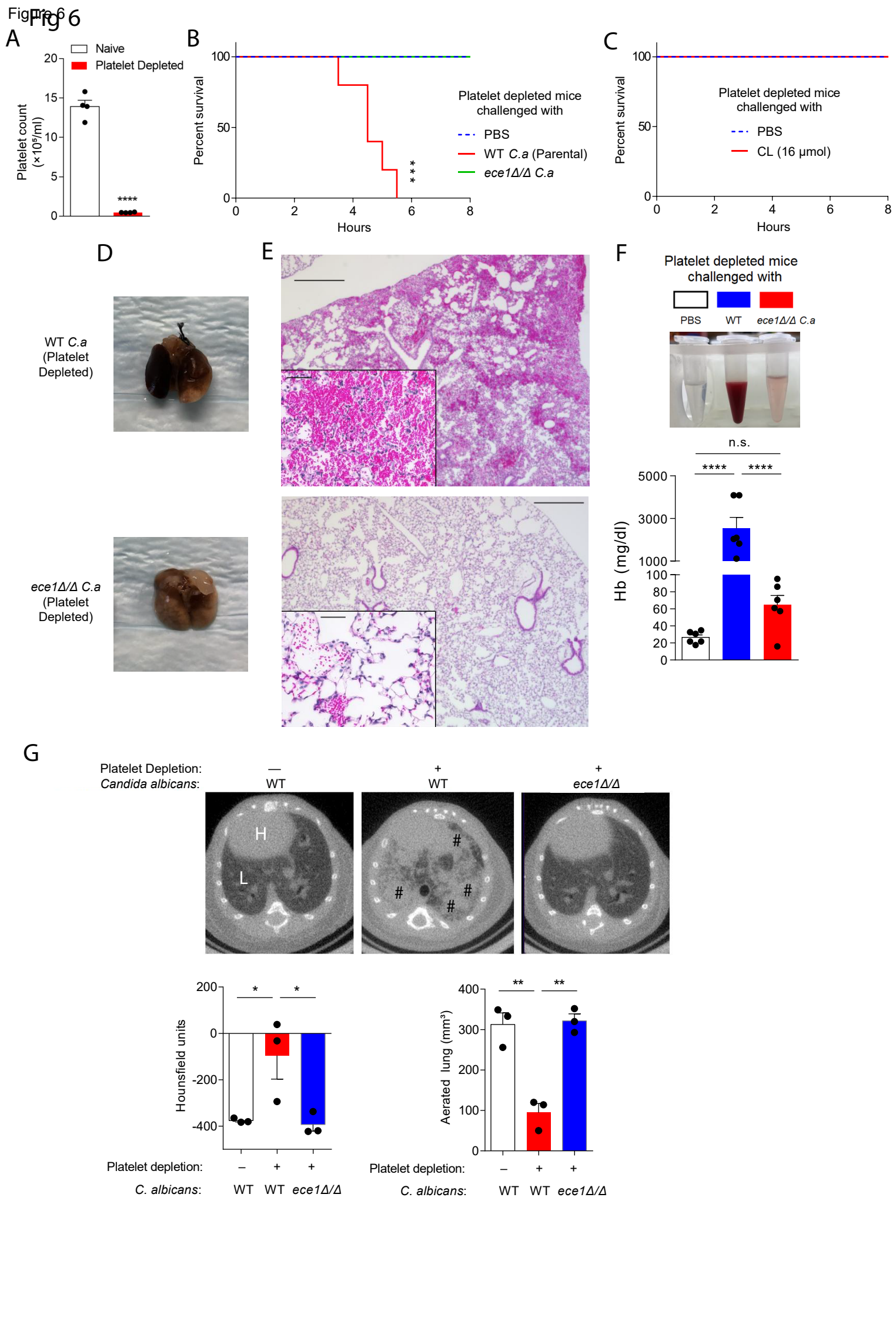
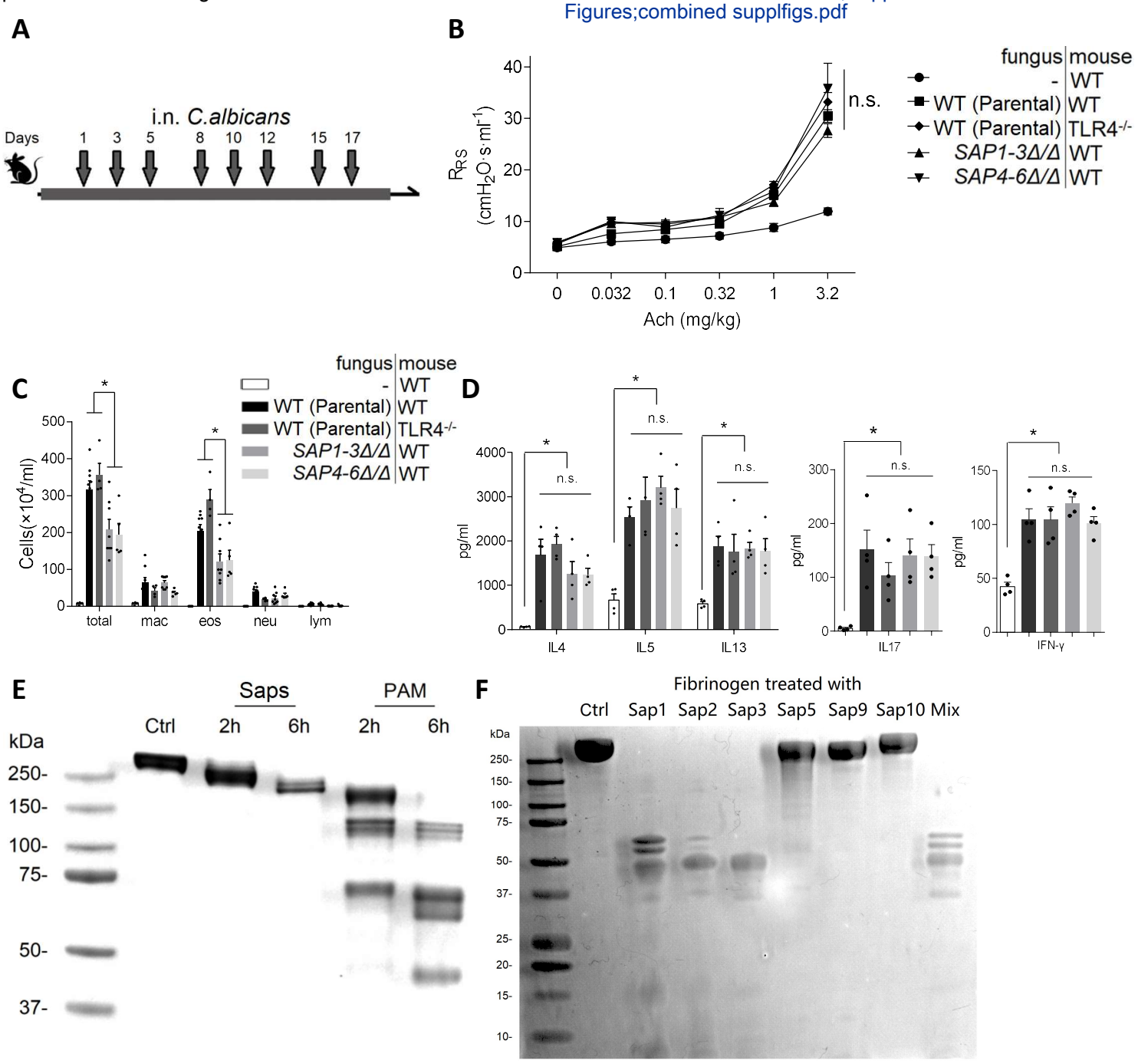


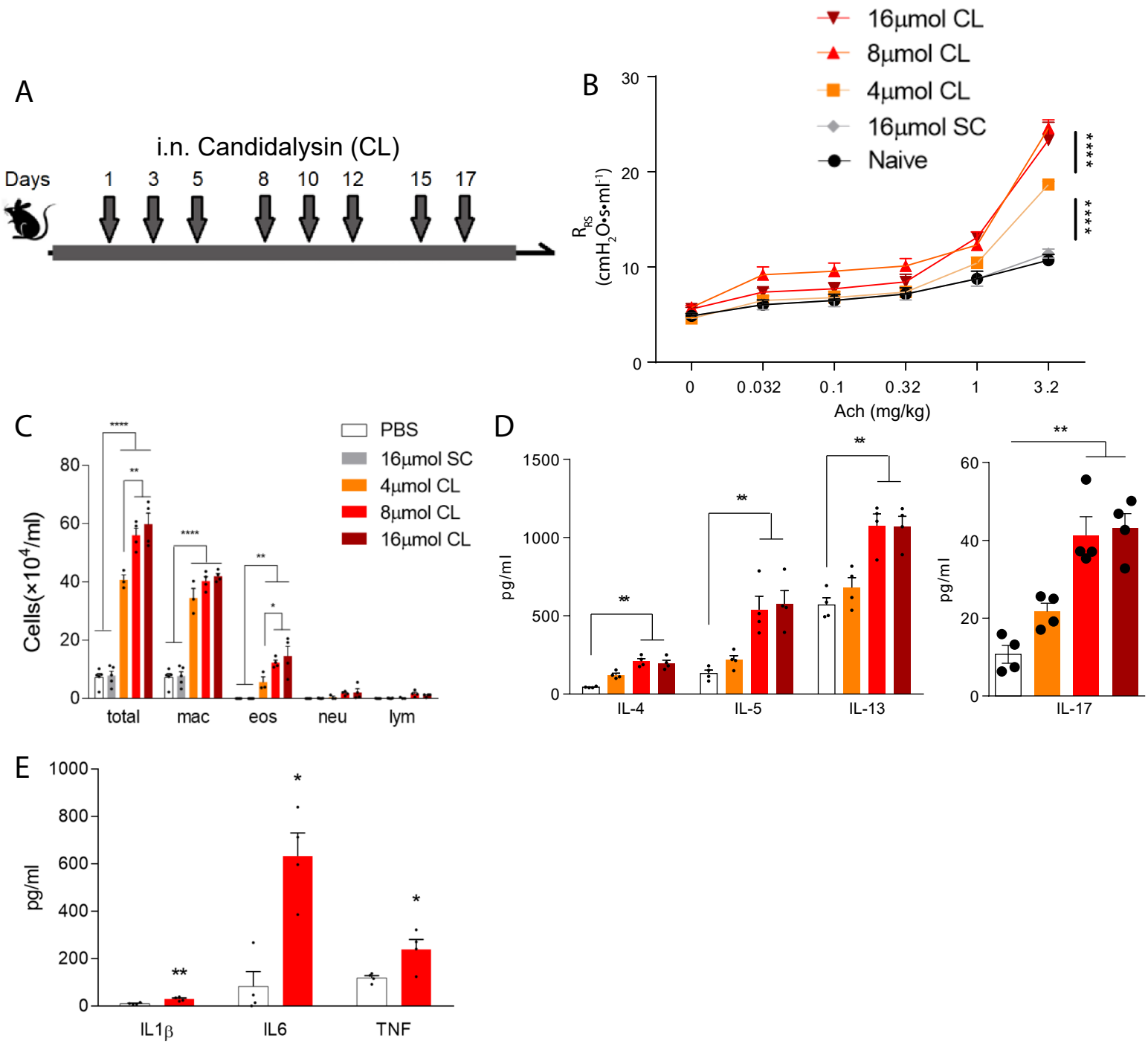
Fig 5
A
PBS
CL-AF647+isotype ctrl
CL-AF647+anti-GP1bα
MFI AF647
Isotype ctrl
Anti-GP1bα
CL-AF647
PBS
CL-AF647 +isotype ctrl
CL-AF647 +anti-GP1bα
0.10
52.3
24.6
AF647
FSC-W
AF647+ cells (%)
B
PBS
CL-AF647+PBS
CL-AF647 +VWF A1A2A3
PBS
CL-AF647 +PBS
CL-AF647 +VWF A1A2A3
0.15
47.8
35.1
VWF A1A2A3
C
PBS
CL+isotype ctrl
CL+anti-GP1bα
CD62P
MFI CD62P
PBS
CL +isotype ctrl
CL +anti-GP1bα
42.7
55.9
34.5
CD62P+ cells (%)
D
Platelet count (×10⁶/ml)
E
Dkk-1 (pg/ml)
PBS+isotype ctrl
CL+isotype ctrl
CL+anti-GP1bα

Figure 6

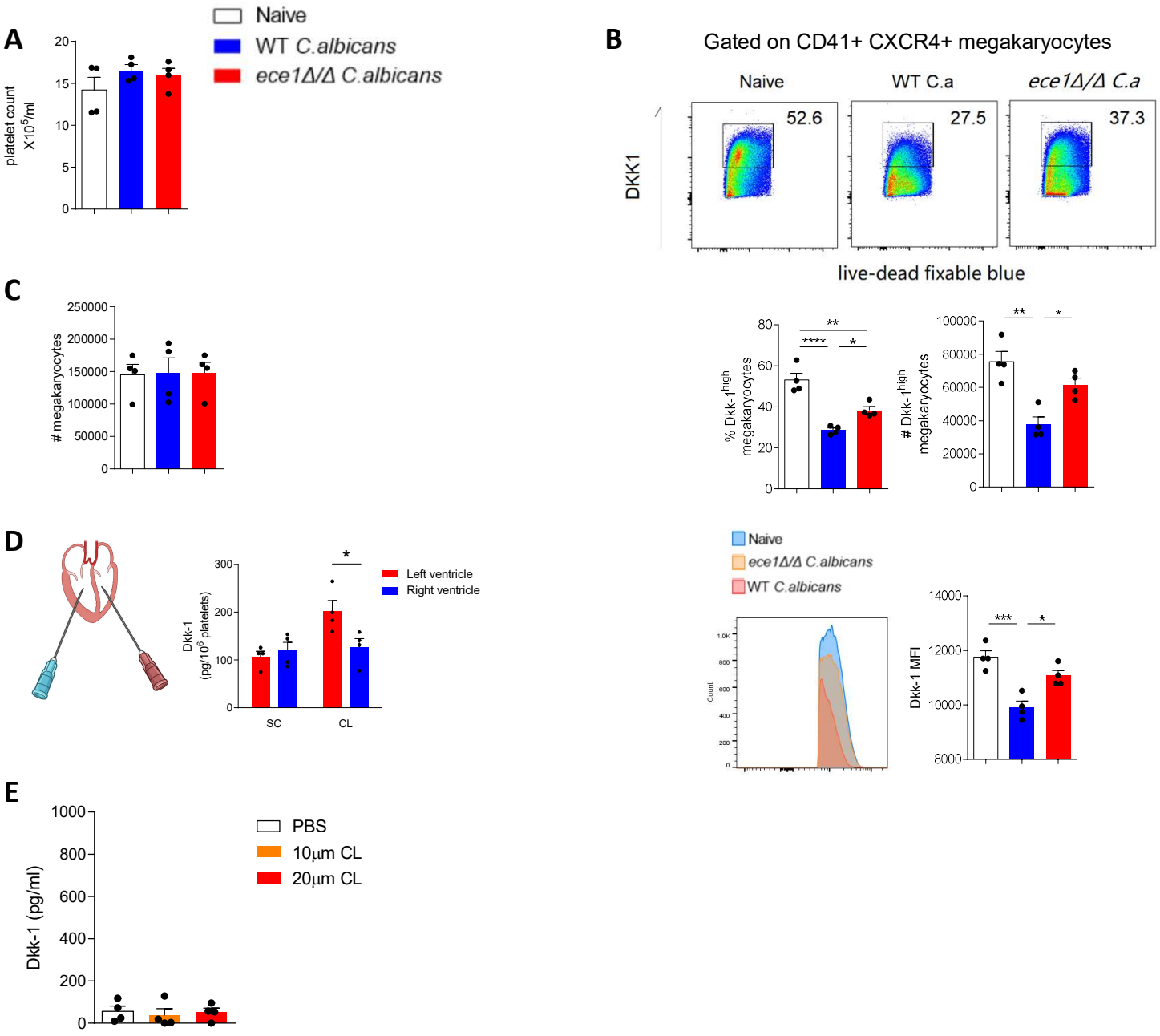

A
Naive
Platelet Depleted
Platelet count (×10⁵/ml)
20
15
10
5
0
****
B
Percent survival
100
50
0
0
2
4
6
8
Hours
***
Platelet depleted mice challenged with
PBS
WT C.a (Parental)
ece1Δ/Δ C.a
C
Percent survival
100
50
0
0
2
4
6
8
Hours
Platelet depleted mice challenged with
PBS
CL (16 µmol)
D
WT C.a (Platelet Depleted)
ece1Δ/Δ C.a (Platelet Depleted)
E
F
Platelet depleted mice challenged with
PBS
WT
ece1Δ/Δ C.a
n.s.
****
****
Hb (mg/dl)
5000
3000
1000
100
80
60
40
20
0
G
Platelet Depletion:
Candida albicans:
—
WT
+
WT
+
ece1Δ/Δ
H
L
#
#
#
#
Hounsfield units
200
0
-200
-400
*
*
Platelet depletion:
C. albicans:
– + +
WT WT ece1Δ/Δ
Aerated lung (mm³)
400
300
200
100
0
**
**
Platelet depletion:
C. albicans:
– + +
WT WT ece1Δ/Δ



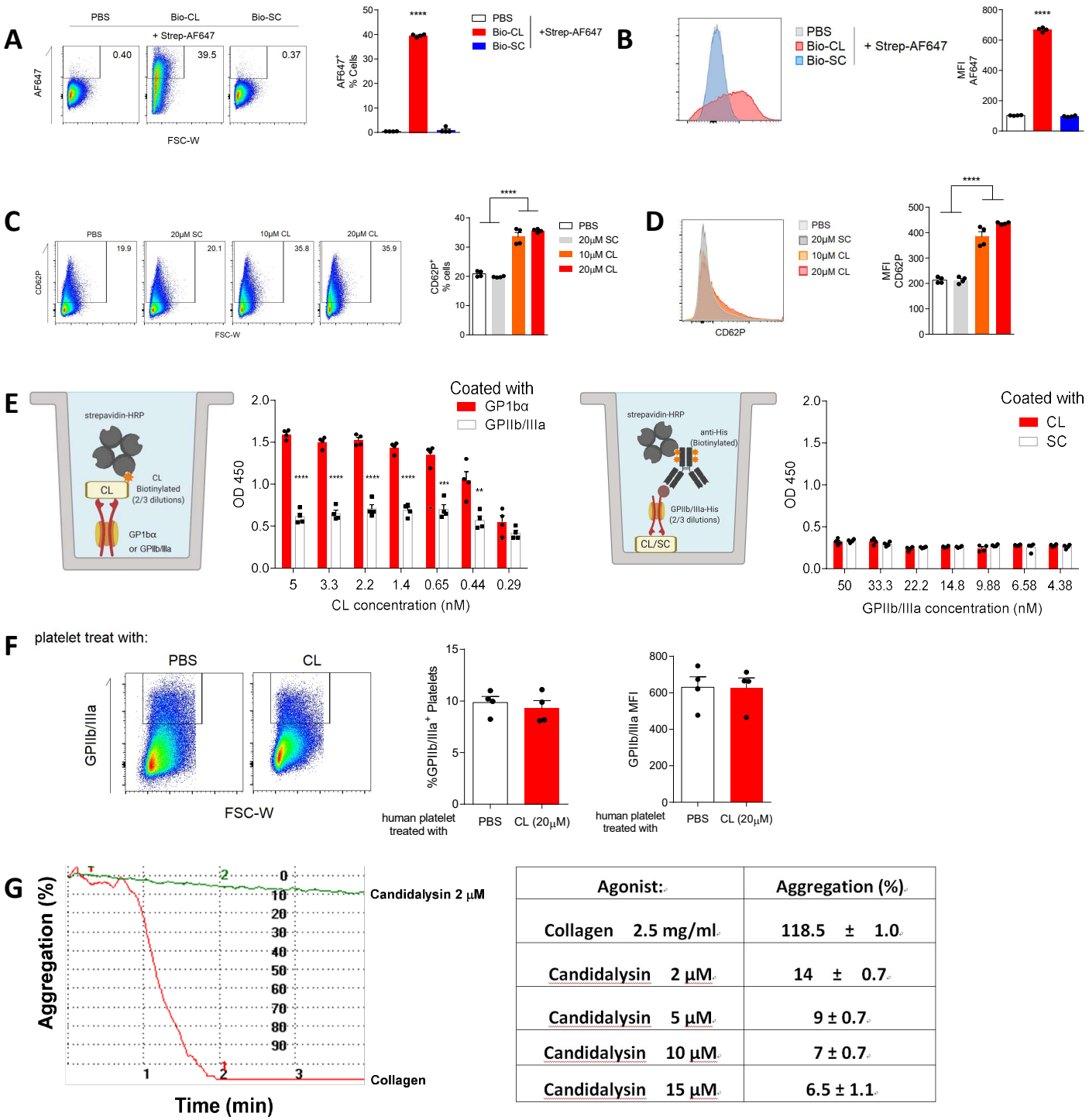
Extended Data Figure 1, related to Figure 1. Proteinase from *C. albicans* is not required for allergic airway disease. (A) Wild type (WT) or TLR4$^{-/-}$ C57BL/6 mice were challenged intranasally with 10^5 viable cells of WT (Parental control), secreted aspartic proteinase 1,2,3 triple deficient (*SAP1-3Δ/Δ* or *SAP4-6Δ/Δ*) *C. albicans* every two days over 17 days. **(B)** Respiratory system resistance (R_{RS}) was assessed in response to increasing intravenous acetylcholine (Ach) challenges. **(C)** Quantitation of cells from bronchoalveolar lavage fluid (mac: macrophages; eos: eosinophils; neu: neutrophils; lym: lymphocytes). **(D)** Cytokines quantitated by ELISA from deaggregated lung. **(E-F)** SDS-PAGE gel electrophoresis assay showing degradation of fibrinogen by purified secreted aspartic proteinases (Saps) from *C. albicans* or the proteinase from *Aspergillus melleus* (PAM) over the indicated times **(E)**, or by recombinant Saps individually or combined for 6 hours **(F)**. (n≥4, mean±S.E.M, n.s.: not significant, *p<0.05, **p<0.01, using one-way ANOVA followed by Tukey's test for multiple comparisons). Data are representative of three independent experiments



Extended Data Figure 2, related to Figure 1. Candidalysin induces allergic airway disease. **(A)** Protocol for administering synthetic candidalysin intranasally to anesthetized wildtype mice. **(B)** Respiratory system resistance (RRS), **(C)** BALF cells, and **(D-E)** lung cytokines were quantitated as in Figure 1. (n≥4, mean±S.E.M, *p<0.05, **p<0.01 and ****p<0.0001 using one-way ANOVA followed by Tukey's test for multiple comparison.) Data are representative of three independent experiments.

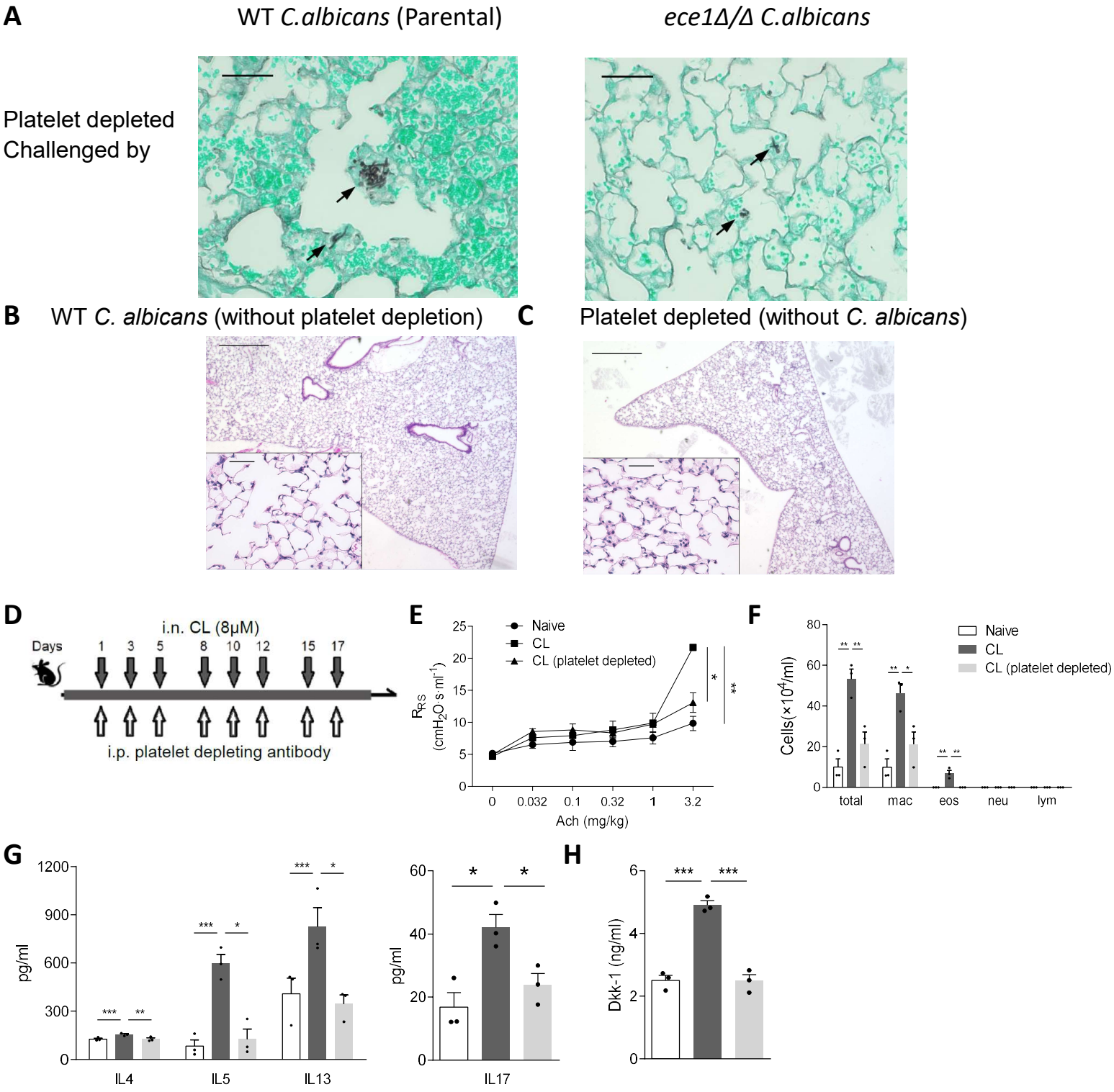


Extended Data Figure 3, related to Figure 2. Platelet and pulmonary megakaryocytes reacts to Candidalysin. (A-C) Anesthetized wildtype C57BL/6 mice were challenged intranasally 8 times over 17 days with WT (parenta) or *ece1Δ/Δ C. albicans*. **(A)** Platelet count from mice post challenge. Lungs were removed 24 hours after the final challenge and **(B)** quantified for Dkk-1^{high} megakaryocytes (as indicated by the gating box) and Dkk-1 MFI. **(C)** Total megakaryocytes were quantified by flow cytometry. **(D)** Mice are challenge intranasally with 16µmol of candidalysin (CL) or scrambled control (SC) and platelets were isolated from left and right ventricle 2 hours post challenge. Dkk-1 quantified from platelets from the left and right ventricle. **(E)** EOMA cells were treated with candidalysin at 10 and 20µM. Dkk-1 was quantified in the supernatant Illustrative figures generated at biorenders.com (n≥4, mean±S.E.M, n.s.: not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 using one-way ANOVA followed by Tukey's test for multiple comparisons)

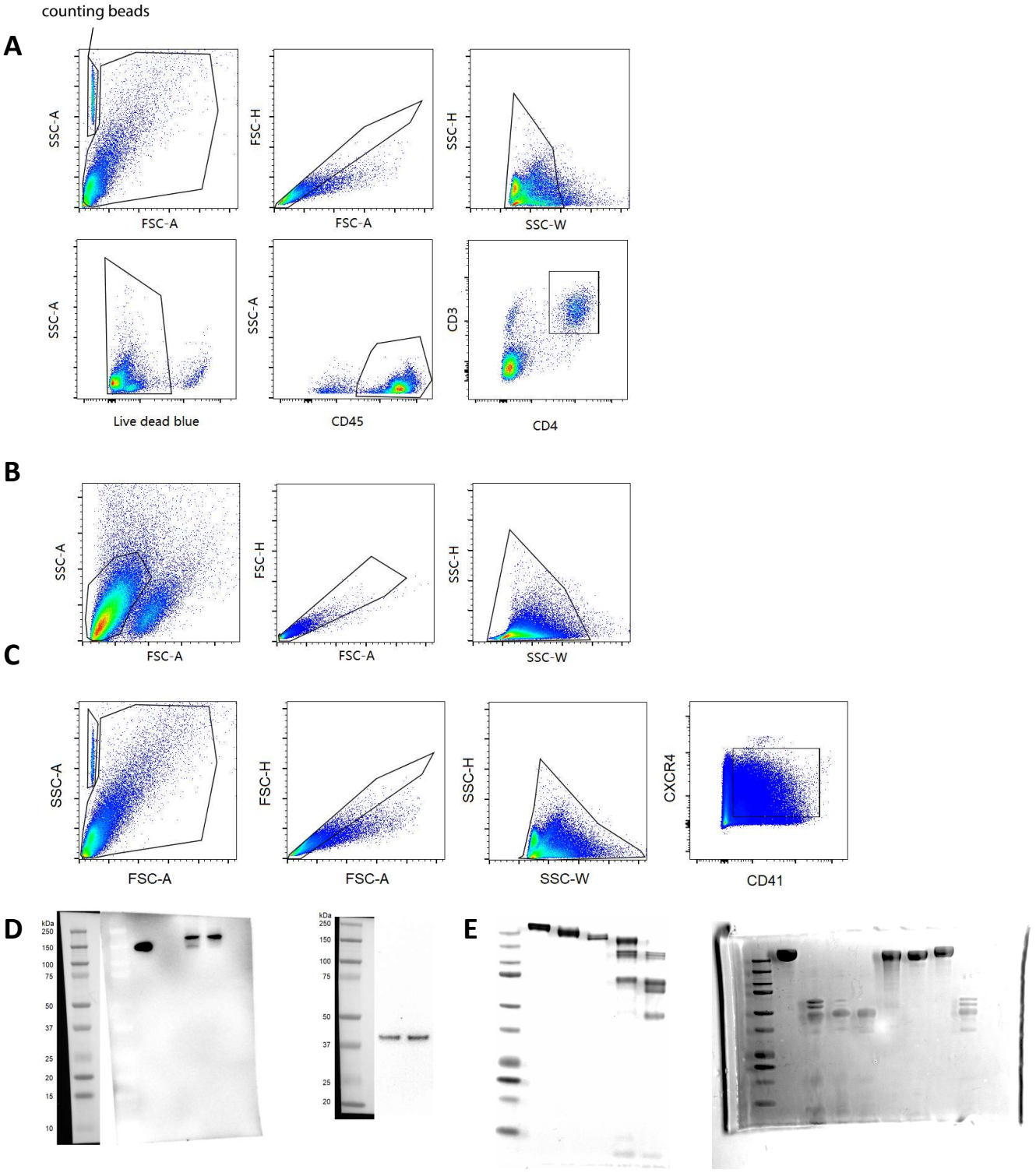


Agonist:	Aggregation (%)
Collagen 2.5 mg/ml	118.5 ± 1.0
Candidalysin 2 μM	14 ± 0.7
Candidalysin 5 μM	9 ± 0.7
Candidalysin 10 μM	7 ± 0.7
Candidalysin 15 μM	6.5 ± 1.1

Extended Data Figure 4, related to Figures 2,4,5. **Candidalysin binds to and activates human platelets. (A-B)** Human platelets were treated with 10μM biotinylated candidalysin (Bio-CL) or scrambled control (Bio-SC), followed by Streptavidin-Alexafluor 647 after which flow cytometry was used to determine binding as (**A**) AF647 % positive cells and (**B**) median fluorescence intensity (MFI). **(C-D)** Human platelets were prepared with candidalysin (CL) (10 or 20μM), scrambled control (SC) or PBS after which flow cytometry was used to determine the change in expression of the activation marker P-selectin (CD62P) expressed as (**C**) % positive cells and (**D**) mean fluorescence intensity (MFI). **(E)** Schematic diagrams and aggregate data depicting in vitro assays in which the dose-dependent binding of plate-bound GP1bα or GPIIb/IIIa to candidalysin was determined colorimetrically. **(F)** Human platelets were prepared with 20μM candidalysin (CL) or PBS after which flow cytometry was used to determine the change in expression of the activation marker GPIIb/IIIa expressed as % positive cells and mean fluorescence intensity (MFI). **(G)** Percentage aggregation for platelets in response to the indicated doses of collagen or candidalysin Data are representative of three independent experiments. (n≥4, mean±S.E.M, n.s.: not significant, **p<0.01,



Extended Data Figure 5, related to Figure 6. Lung histology of mice after platelet depletion or *C.albicans* challenge, and candidalysin failed to induce airway hyperreactivity to platelet depleted mice. (A) GMS staining on lung sections from wildtype plateled depleted mice after *C.albicans* challenge.(**B-C**) Wildtype, platelet-sufficient mice were challenged once with *C. albicans* intranasally (**B**) or platelet depleted without *C. albicans* challenge (**C**). Lungs were removed 4 hours after either challenge and H&E staining on 5 μm lung sections was performed. Reference bar: 500 and 50μm, respectively. Magnification: **A**: 200x, **B-C**: 40× (Inset: 200×). **(D)** Wild type mice were challenged intranasally with 8μM of recombinant candidalysin intranasally, with or without platelet depleting antibody intraperitoneally every two days over 17 days. **(E)** Respiratory system resistance (R_{RS}) was assessed in response to increasing intravenous acetylcholine (Ach) challenges. **(F)** Quantitation of cells from bronchoalveolar lavage fluid (mac: macrophages; eos: eosinophils; neu: neutrophils; lym: lymphocytes). **(G)** Cytokines quantitated by ELISA from deaggregated lung. **(H)** Dkk-1 quantitated by ELISA from mouse plasma. (n=3, mean±S.E.M, n.s.: not significant, *p<0.05, **p<0.01, ***p<0.001, using one-way ANOVA followed by Tukey's test for multiple comparisons). Data are representative of two independent experiments



Extended Data Figure 6, related to STARR Methods. Gating strategy for **(A)** TH cells, (**B**) Platelets and (**C**) megakaryocytes. Uncropped membranes from western blot of GP1Bα pulldown and **(D)** cleavage product from Saps or PAM