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The adherens junctions control susceptibility to *Staphylococcus aureus* α-toxin

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Staphylococcus aureus **is both a transient skin colonizer and a formidable human pathogen, ranking among the leading causes of skin and soft tissue infections as well as severe pneumonia. The secreted bacterial α-toxin is essential for *S. aureus* virulence in these epithelial diseases. To discover host cellular factors required for α-toxin cytotoxicity, we conducted a genetic screen using mutagenized haploid human cells. Our screen identified a cytoplasmic member of the adherens junctions, plekstrin-homology domain containing protein 7 (PLEKHA7), as the second most significantly enriched gene after the known α-toxin receptor, a disintegrin and metalloprotease 10 (ADAM10). Here we report a new, unexpected role for PLEKHA7 and several components of cellular adherens junctions in controlling susceptibility to *S. aureus* α-toxin. We find that despite being injured by α-toxin pore formation, PLEKHA7 knockout cells recover after intoxication. By infecting PLEKHA7$^{-/-}$ mice with methicillin-resistant *S. aureus* USA300 LAC strain, we demonstrate that this junctional protein controls disease severity in both skin infection and lethal *S. aureus* pneumonia. Our results suggest that adherens junctions actively control cellular responses to a potent pore-forming bacterial toxin and identify PLEKHA7 as a potential nonessential host target to reduce *S. aureus* virulence during epithelial infections.**

Staphylococcus aureus | α-toxin | adherens junctions | MRSA | PLEKHA7

The bacterium *Staphylococcus aureus* is not only one of the most important human pathogens resulting in considerable morbidity and mortality (1, 2) but also can be found as a transient skin resident, intermittently colonizing a sizable portion of the healthy population (3). *S. aureus* infections manifest in a diverse array of clinical presentations, but related to its transitory epithelial niche, *S. aureus* predominantly results in skin and soft tissue infections (4, 5). Through local infections bacteria can gain access to deeper tissue and disseminate hematogenously to cause invasive disease such as endocarditis, osteomyelitis, deep tissue abscesses, sepsis, and pneumonia (1). In the face of increasing antibiotic resistance, the widespread prevalence of methicillin-resistant *S. aureus* (MRSA) strains both in hospitals and communities across the globe presents a growing threat to human health worldwide (5, 6). Given the growing difficulty of treating these common and frequently life-threatening infections, understanding host–pathogen interactions that mediate *S. aureus* pathogenesis is imperative.

Chief among the arsenal of *S. aureus* virulence factors, α-toxin (or α-hemolysin) is a critical determinant for pathogenesis in a wide variety of experimental infections, particularly during epithelial infections such as skin abscesses and pneumonia (7–10). After secretion as a soluble monomer, α-toxin oligomerizes on the targeted host cell surface via interactions with its high-affinity metalloprotease receptor, a disintegrin and metalloprotease 10 (ADAM10), forming a 1–3-nm pore that spans the cellular membrane lipid bilayer (11, 12). Originally described solely for its ability to induce lysis of erythrocytes, it is now appreciated that α-toxin exerts pleiotropic effects on a diverse set of host cells (13). In addition to inducing cell death, at sublytic concentrations α-toxin has been described to alter a wide variety of cellular processes, including cell signaling, proliferation, immunomodulation, autophagy, and others (13–17).

Importantly, *S. aureus* uses α-toxin to remodel host epithelia and alter tissue integrity. Engagement of α-toxin with ADAM10 leads to intracellular ion flux across the toxin pore, which enhances the proteolytic activity of ADAM10 through an unknown mechanism (18). ADAM10 is essential for tissue morphogenesis and remodeling and acts on a multitude of extracellular substrates (19), one of which is the adherens junction protein E-cadherin (20). It has been proposed that α-toxin–enhanced ADAM10 cleavage of E-cadherin dismantles the adherens junctions to disrupt the integrity of cell–cell contacts in epithelial tissues during infection to contribute to *S. aureus* pathogenesis (18, 21). However, the molecular components that govern intracellular responses elicited by α-toxin in the targeted host cell remain largely undefined.

To advance understanding of how *S. aureus* α-toxin modulates host cell biology, we conducted a high-throughput genetic screen using human cells (22, 23) to discover novel host factors required

Significance

Staphylococcus aureus **is a major cause of invasive bacterial infection. One prominent virulence factor is α-toxin, a protein that injures the cell by forming a damaging pore across the cell membrane. We conducted a genetic screen to identify host factors that control susceptibility to α-toxin. We discovered that several components of the adherens junction complex modulate α-toxin cytotoxicity. By eliminating expression of the junctional protein plekstrin-homology domain containing protein 7 (PLEKHA7), cells gained the ability to recover from α-toxin injury and mice lacking PLEKHA7 exhibited improved healing from *S. aureus* skin infection and enhanced survival of pneumonia. Our data suggest that targeting nonessential host epithelial junction components can reduce *S. aureus* morbidity by enhancing cellular resilience to α-toxin injury.**

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for α-toxin cytotoxicity. Our screen unexpectedly revealed that multiple components of the cellular adherens junctions modulate susceptibility to α-toxin, suggesting a previously unidentified role for the junctions as critical mediators of α-toxin cytotoxicity—not merely its target. The most significant hit following ADAM10 was plekstrin-homology domain containing protein 7 (PLEKHA7), a cytoplasmic accessory member of the adherens junction complex (24). In PLEKHA7-deficient cells, α-toxin pore formation occurs, but remarkably cells exhibit enhanced recovery from α-toxin injury. Furthermore, we establish the important contribution of PLEKHA7 for *S. aureus* pathogenesis in vivo using MRSA USA300 LAC mouse models of both a self-resolving skin and soft tissue infection and lethal pneumonia.

Results

Human Haploid Genetic Screen Reveals Novel Host Factors Required for α-Toxin Cytotoxicity. To discover host factors required for α-toxin cytotoxicity, we conducted a live/dead genetic screen by intoxicating haploid human cells (HAP1) carrying knockout alleles in essentially all genes through insertional mutagenesis (22, 23). A large-scale library of ∼100 million HAP1 mutagenized cells was treated with recombinant α-toxin, and gene-trap insertion sites were identified in the pool of surviving, toxin-resistant cells. The gene trap insertion sites in the toxin-selected population were compared with a control library that was not exposed to α-toxin (Dataset S1). We identified 46 genes that were significantly enriched in the toxin-treated library ($P < 0.05$) spanning several functional biological categories, suggesting that these genes are essential for α-toxin intoxication (Fig. 1*A* and Dataset S1).

The validity of our screen was confirmed by identification of ADAM10, the α-toxin cell surface metalloprotease receptor (12), which emerged as the most enriched gene in the toxin-selected library (Fig. 1*A*, $P = 7.36 \times 10^{-100}$). Moreover, the screen identified the chaperone protein TSPAN33 (25), which reportedly mediates surface localization of ADAM10 (Fig. 1*A*, $P = 1.09 \times 10^{-25}$). Lipid biogenesis genes, such as sterol response element binding proteins, were also enriched (Fig. 1*A*) and have been attributed roles in cellular innate immune responses against pore-forming toxins (26, 27).

Our screen discovered several genes not previously implicated in α-toxin cytotoxicity. PLEKHA7, the second most enriched gene in our screen (Fig. 1*A*, $P = 1.84 \times 10^{-81}$), is a cytoplasmic, accessory component of adherens junctions (24). PLEKHA7 localizes to the *zonula adhaerens* belt of the apical–junctional complex in epithelial cells, where it directly interacts with p120 catenin, paracingulin, and afadin (24, 28–31). Unlike E-cadherin or p120 catenin, PLEKHA7 is not required for adherens junctions formation, and previous studies suggest a role for PLEKHA7 in regulating the stability of the adherens junctions (24, 28–31). In accord with adherens junctions mediating α-toxin injury, our screen also identified several other junctional components including α-catenin, afadin, and

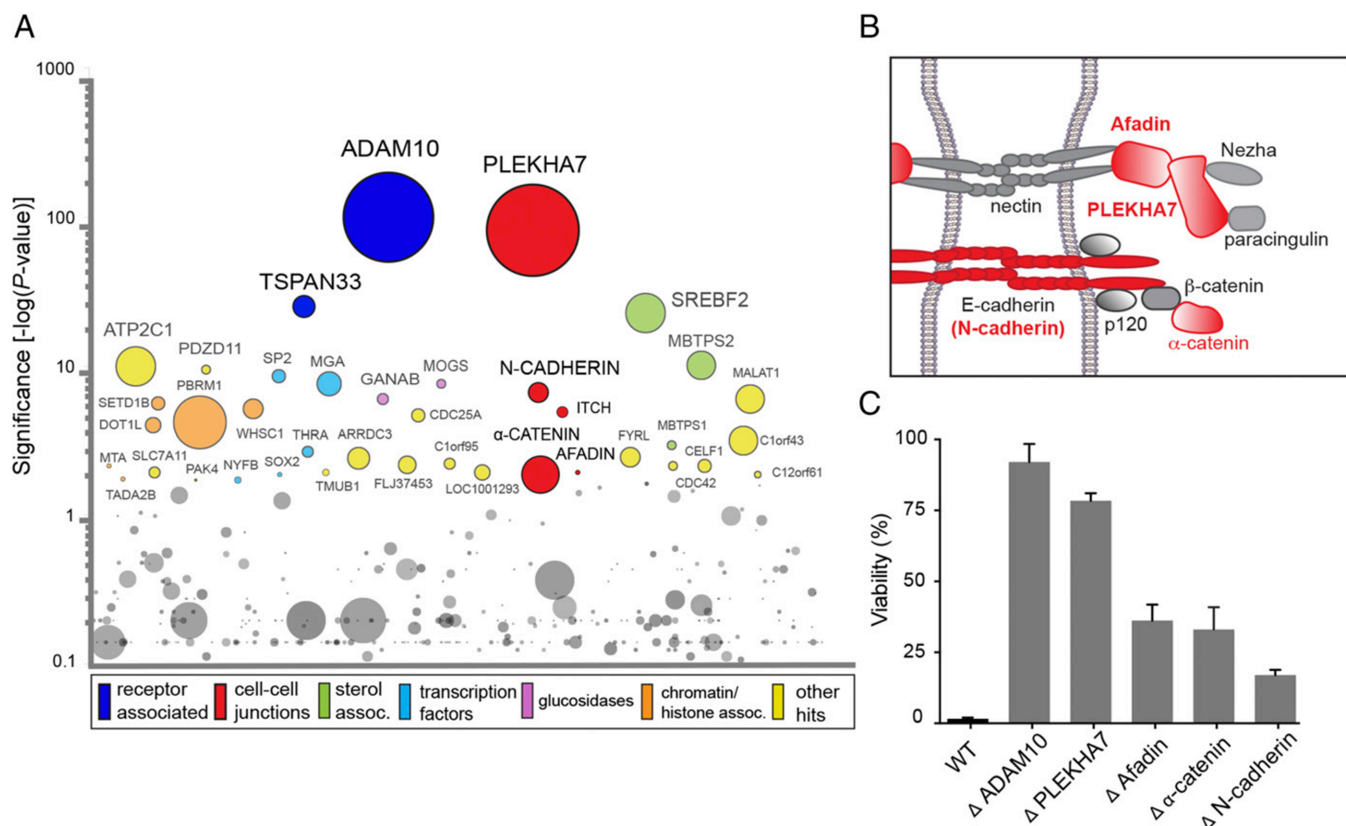

Fig. 1. A haploid genetic screen for cellular host factors required for α-toxin cytotoxicity identifies several components of the adherens junctions. (*A*) A haploid human cell genetic screen identified genes that confer resistance to α-toxin when inactivated by retroviral gene-trap insertion. Each bubble represents a gene, and bubble size corresponds to the number of independent gene-trap insertion events observed in the toxin-selected population. Genes are ranked on the *y* axis according to the significance of enrichment of gene-trap insertions in the α-toxin selected library compared with the nonselected control population (Fisher's exact test, corrected for false discovery rate). Functional groups of significant hits share a common color as indicated and are grouped together horizontally (with other genes in random order along the *x* axis). Gene names are displayed until $P = 0.05$. (*B*) An abridged diagram of the adherens junctional complex. Cell–cell adherens junction proteins found significantly enriched in our α-toxin screen are depicted in red, and associated proteins are shown in gray. (*C*) Viability of WT HAP1 cells or knockout (Δ) HAP1 clones for the indicated genes following treatment with α-toxin (indicated as percentage of nonintoxicated controls; data are mean + SEM; $n = 3$ biological replicates; all differences relative to WT cells are $P = <0.01$, unpaired t test).

N-cadherin (Fig. 1 *A* and *B*). Enrichment of inactivating insertions in multiple adherens junctions complex genes in the toxin-resistant library suggested the novel hypothesis that components of the cellular adherens junctions can control susceptibility to *S. aureus* α-toxin. To test this hypothesis, we used CRISPR/Cas9 gene editing to generate HAP1 knockout (Δ) subclone cell lines for multiple adherens junction genes revealed by our screen (Fig. S1). In accord with our screen results, we find that cells individually lacking the junctional proteins PLEKHA7, afadin, α-catenin, and *N*-cadherin are all significantly less susceptible to α-toxin cytotoxicity than WT controls (Fig. 1*C*). Given that PLEKHA7 was the most important junctional gene modulating susceptibility to α-toxin (Fig. 1*C*), we explored its role further.

The Adherens Junction Component PLEKHA7 Controls Susceptibility to α-Toxin. HAP1 cells lacking PLEKHA7 are markedly more resistant to α-toxin cytotoxicity than WT cells at a wide range of toxin concentrations (Fig. 2*A*). Furthermore, stable expression of a human PLEKHA7-FLAG construct *in trans* fully complements α-toxin cytotoxicity in ΔPLEKHA7 cells (Fig. 2*A*). To determine if PLEKHA7 deletion reduces susceptibility in the context of endogenously expressed levels of α-toxin, we treated WT and ΔPLEKHA7 cells with cell-free supernatants from MRSA strain USA300 LAC and an isogenic mutant MRSA strain lacking the α-toxin gene, USA300 LAC *hla::ermB*. This treatment confirmed that ΔPLEKHA7 cells are also more resistant to endogenous α-toxin (Fig. S2). We find that PLEKHA7 deletion does not confer a generalized injury resistance, as ΔPLEKHA7 cells are not more resistant to cytotoxicity caused by either bacterial pore-forming toxins (streptolysin O, perfringolysin O) or the potassium ionophore nigericin (Fig. S3). PLEKHA7's role in modulating α-toxin susceptibility on well-differentiated epithelial cells was further confirmed by testing the effect of α-toxin on a ΔPLEKHA7 cell line in the Madin–Darby Kidney (MDCK) background. MDCK cells are a widely used in vitro model of a simple epithelium and form a polarized monolayer when grown on transwell filters (32). Consistent with our observations made using HAP1 cells, we find that ΔPLEKHA7 MDCK epithelial monolayers are more resistant to α-toxin cytotoxicity than WT MDCK monolayers (Fig. S4).

Next, using PLEKHA7 deletion mutant constructs stably expressed in ΔPLEKHA7 cells (Fig. S5) and subsequently treated with α-toxin, we defined the PLEKHA7 residues necessary and sufficient for restoring α-toxin cytotoxicity to be restricted to the first 538 N-terminal amino acids (Fig. 2 *B* and *C*). This region of

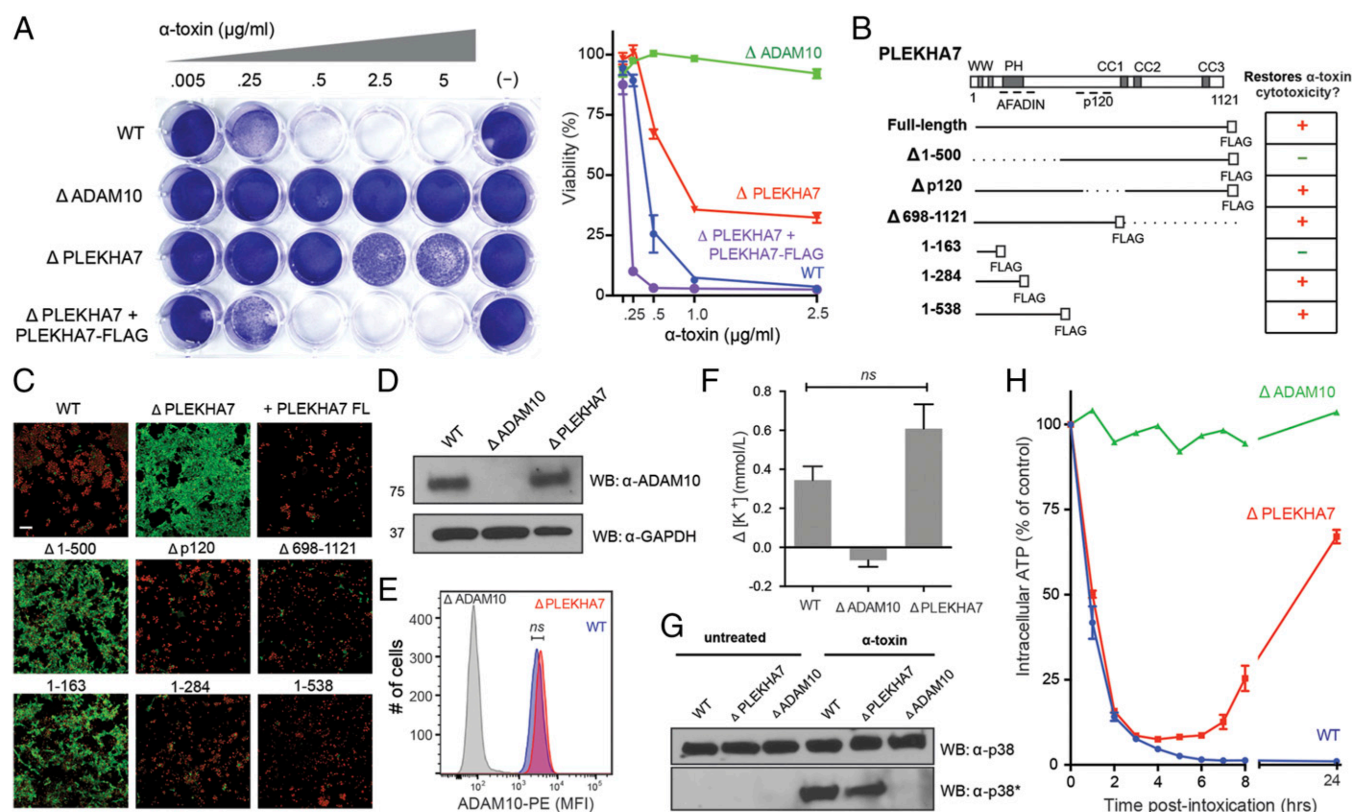

Fig. 2. The adherens junction protein PLEKHA7 controls susceptibility to α-toxin cytotoxicity despite pore formation and cellular injury. (*A*) WT HAP1 cells, knockout (Δ) HAP1 clones, and ΔPLEKHA7 cells stably expressing human PLEKHA7-FLAG were treated with indicated α-toxin concentrations or media only, then subsequently stained with crystal violet (*Left*), or viability was quantified after 24 h of α-toxin treatment (percentage viability shown relative to cell type-specific media controls; data are mean ± SEM; $n = 3$ biological replicates). (*B*) Diagram of deletion human PLEKHA7-FLAG constructs stably expressed by lentiviral transduction in ΔPLEKHA7 HAP1 cells. (*C*) ΔPLEKHA7 HAP1 cells stably expressing the indicated constructs in *B* were treated with α-toxin and 18 h later stained using a fluorescence-based LIVE/DEAD assay, where green fluorescence indicates live cells by esterase activity and red fluorescence demonstrates loss of plasma membrane integrity. (Scale bar, 70 µm.) (*D*) Western blot analysis of ADAM10 and GAPDH expression in whole-cell lysates from WT, ΔADAM10, and ΔPLEKHA7 HAP1 cells. (*E*) Cell surface expression of ADAM10 on WT, ΔADAM10, and ΔPLEKHA7 HAP1 cells as measured by flow cytometric analysis. Data are representative of three independent experiments. (*F*) Change in [K+] in extracellular media following α-toxin treatment of WT, ΔADAM10, and ΔPLEKHA7 HAP1 cells (shown relative to cell type-specific nonintoxicated controls; data are mean ± SEM; $n = 3$ biological replicates; comparison of WT and ΔPLEKHA7, $P = 0.069$, unpaired *t* test). (*G*) Western blot analysis of p38 and phosphorylated p38 in whole-cell lysates from left (nonintoxicated) and right (α-toxin treated) WT, ΔADAM10, and ΔPLEKHA7 HAP1 cells. Data are representative of three independent experiments. (*H*) Time course of intracellular [ATP] in WT, ΔADAM10, and ΔPLEKHA7 HAP1 cells following α-toxin treatment (shown as percentage of cell type-specific nonintoxicated controls; data are mean ± SEM; $n = 3$ biological replicates).

PLEKHA7 encompasses residues that mediate its interaction with afadin (29)—another junctional protein enriched in our screen (Fig. 1*A*) that modulates α-toxin susceptibility (Fig. 1*C*).

PLEKHA7-Deficient Cells Exhibit Enhanced Resilience to α-Toxin Injury. To interrogate the mechanism underlying PLEKHA7 modulation of α-toxin cytotoxicity, we first assessed whether PLEKHA7 deletion alters expression of the toxin receptor ADAM10. Western blot of whole-cell lysates demonstrates that ΔPLEKHA7 cells express comparable levels of ADAM10 as WT controls (Fig. 2*D*). To determine whether ADAM10 is localized at the plasma membrane in the absence of PLEKHA7, we quantified surface-available ADAM10 by flow cytometry and found no significant decrease in cell surface ADAM10 expression between WT and ΔPLEKHA7 cells (Fig. 2*E*). Consistent with these findings, time-lapse video microscopy reveals that WT and ΔPLEKHA7 cells, but not ΔADAM10 cells, develop cytopathic effects following intoxication. However, we observe that individual ΔPLEKHA7 cells recover from this initial injury and survive intoxication, in contrast to WT cells (Movie S1).

We next sought to determine whether ΔPLEKHA7 cells are more resistant to α-toxin cytotoxicity because of a defect in α-toxin pore formation spanning the targeted host cell surface. To do so, we assessed functional outcomes of pore formation and subsequent cellular injury in α-toxin–treated ΔPLEKHA7 cells using several distinct assays. Rapid efflux of intracellular potassium is an early effect of α-toxin pore formation (33). We observe an increase in extracellular potassium relative to nonintoxicated controls following intoxication of WT and ΔPLEKHA7 cells, but not ΔADAM10 cells (Fig. 2*F*). Another functional consequence of α-toxin injury known to be dependent on pore formation is the activating phosphorylation of the cellular stress response kinase p38 (34). Consistent with cellular injury occurring in the absence of PLEKHA7, we observe α-toxin–dependent p38 activation in WT and ΔPLEKHA7 cells but not ΔADAM10 cells (Fig. 2*G*). Because intracellular ATP depletion is a hallmark of α-toxin damage and ATP repletion is associated with enhanced recovery from α-toxin injury (16, 35), we quantified changes in intracellular ATP levels following intoxication. Shortly after α-toxin treatment, both WT and ΔPLEKHA7 cells quickly deplete intracellular ATP. At later time points, however, only ΔPLEKHA7 cells restore intracellular ATP (Fig. 2*H*). From these studies, we conclude that PLEKHA7 controls susceptibility to α-toxin in a step downstream of ADAM10 recognition and α-toxin pore formation. PLEKHA7 deletion does not strictly prevent damage caused by α-toxin, but rather cells lacking PLEKHA7 exhibit enhanced resilience to and recovery from α-toxin injury.

PLEKHA7 Contributes to the Severity of MRSA Skin and Pneumonia Infections in Vivo. Given α-toxin's critical role in *S. aureus* pathogenesis during skin and lung infections and the expression of PLEKHA7 in epithelial tissues at the adherens junctions, we hypothesized that PLEKHA7 may contribute to *S. aureus* pathogenesis during an in vivo infection. To test the role of PLEKHA7 during MRSA infection, we made use of previously unpublished PLEKHA7$^{-/-}$ whole-body transgenic mice (Fig. S6). Consistent with a recently described PLEKHA7-deficient rat (36), PLEKHA7$^{-/-}$ mice are viable and fecund, exhibiting no gross developmental defects.

We first examined the contribution of PLEKHA7 during a self-limiting MRSA skin and soft tissue infection. In this model, *S. aureus* is superficially introduced into the ear pinnae using a shallow needle, resulting in a necrotic, inflammatory lesion that resolves with tissue loss (37). Confirming the importance of α-toxin for this infection, USA300 LAC *hla::ermB* isogenic mutant infections result in significantly decreased lesion size compared with WT USA300 LAC (Fig. S7). To assess the contribution of PLEKHA7 to disease during a skin and soft tissue infection, we infected WT and PLEKHA7$^{-/-}$ mice with WT USA300 LAC and followed disease development in individual animals over time. We observe that both WT and PLEKHA7$^{-/-}$ mice developed similar-sized necrotic lesions at early time points (Fig. 3 *A* and *B*) indistinguishable by histopathology and bacterial burden (Figs. S8 and S9). However, despite an initial similarity between lesions in WT and PLEKHA7$^{-/-}$ mice, at day 14 postinfection, we found that PLEKHA7$^{-/-}$ mice had resolved the lesions with significantly less tissue loss than WT controls (Fig. 3*B* and Movie S2). Paralleling our in vitro observations at the cellular level, these data support an enhanced recovery phenotype for PLEKHA7$^{-/-}$ mice during a superficial MRSA skin infection.

Upon observing that a self-limiting MRSA skin infection in PLEKHA7$^{-/-}$ mice results in significantly reduced pathology, we sought to assess the importance of PLEKHA7 during an acute, lethal MRSA pneumonia (7, 8). Although both groups developed comparable hypothermia following infection, only PLEKHA7$^{-/-}$ animals showed an enhanced, significant recovery of core temperature by 24 h postinfection, and more animals fully recovered without antibiotic treatment (Fig. 3 *C* and *D*). We find that PLEKHA7$^{-/-}$ mice survived USA300 LAC pneumonia significantly better than WT controls (Fig. 3*D*). To explore the strength of this phenotype, we challenged mice with a threefold higher inoculum and measured similar bacterial loads in lung tissue homogenates at 6 h and 24 h postinfection in both groups (Fig. 3*E*). Despite this higher inoculum, PLEKHA7$^{-/-}$ mice exhibit an increased mean time to death relative to WT control animals (Fig. 3*F*), highlighting the contribution of PLEKHA7 to MRSA pneumonia virulence.

Discussion

S. aureus can transiently and asymptomatically colonize the human skin epithelium and also cause significant morbidity and mortality, predominantly through skin and soft tissue infections that can progress to dangerous systemic disease. Due to the critical importance of *S. aureus* α-toxin for both pathogenesis and its interface with host epithelia, we conducted a genetic screen to identify novel host mediators of α-toxin cytotoxicity. In addition to the toxin receptor ADAM10, our screen identified the intracellular junctional protein PLEKHA7 and several other members of the epithelial adherens junction complex as host factors that modulate α-toxin injury.

Our data suggest a previously unidentified biological role for the adherens junctions in controlling cellular injury caused by a potent bacterial cytotoxin. It is well established that many diverse pathogens and their virulence factors have evolved to target the cellular junctions to facilitate attachment, entry, and invasion across tissue barriers (38–40). Indeed, α-toxin has been shown to alter epithelial barrier integrity by enhancing ADAM10 cleavage of the ectodomain of E-cadherin (18, 21). It is increasingly appreciated, however, that microbial interactions with the host epithelium and cell–cell junctions are not restricted to mechanical disruption of barrier function (39, 41). Mounting evidence illustrates that bacteria can actively modulate other important aspects of junction function, such as altering cell polarity (42–44) and intracellular signals emanating from the junctions (45–47). Our findings build importantly on these observations, revealing not only that *S. aureus* α-toxin does target the junctions but also that α-toxin acts through adherens junctions components to mediate its cytotoxicity. Considering that *S. aureus* is an epithelial colonizer, our data suggest the likely existence of more subtle biological interplay between *S. aureus* and the host adherens junctions at sublytic concentrations of α-toxin to facilitate bacterial modification of its replicative niche during colonization.

Our in vitro studies demonstrate that PLEKHA7 modulates susceptibility to α-toxin in both a human cell line and an in vitro model of a simple, polarized epithelium. Surprisingly, further mechanistic investigations revealed that PLEKHA7 controls susceptibility to α-toxin downstream of functional pore formation, suggesting that pore formation by itself is not sufficient to cause cell death. Rather,

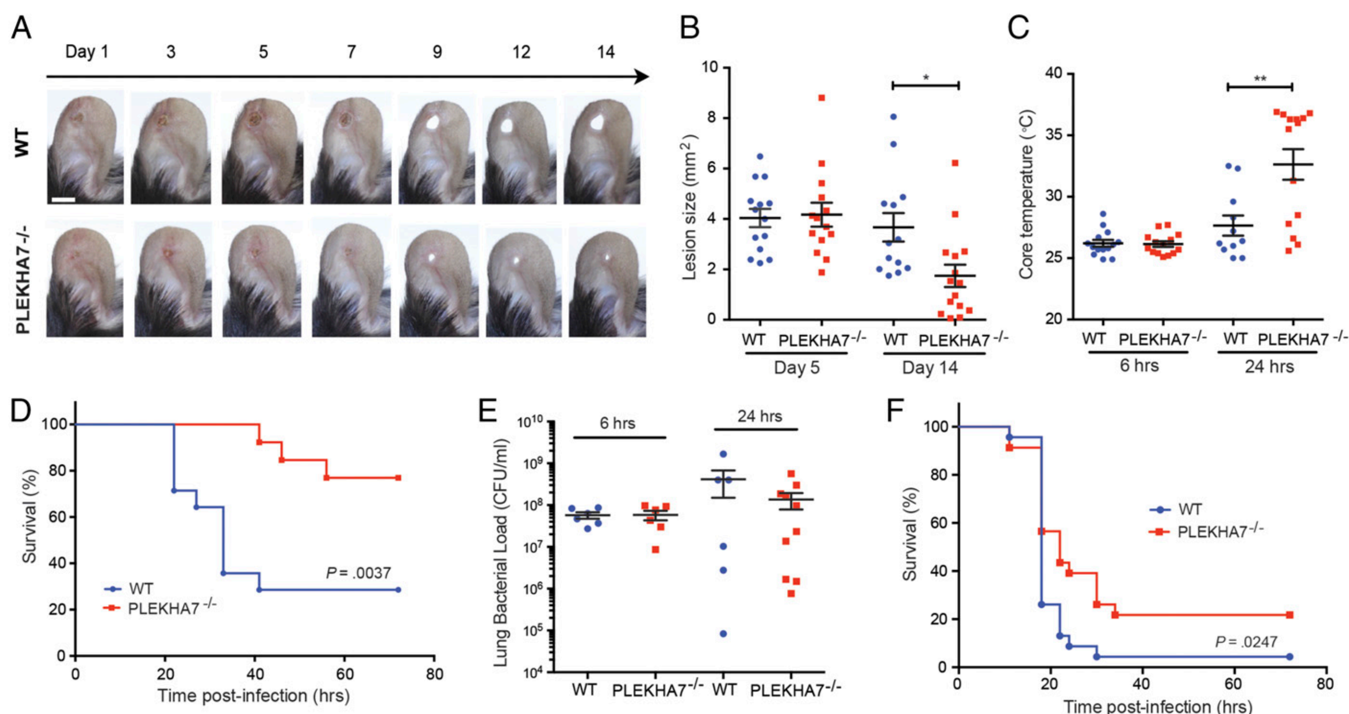

Fig. 3. PLEKHA7 contributes to the severity of MRSA skin and pneumonia infections in vivo. (*A*) Images of ear skin and soft tissue infection progression within individual animals over time from representative WT and PLEKHA7$^{-/-}$ mice following infection with MRSA strain USA300 LAC. (Scale bar, 5 mm for all panels.) (*B*) Lesion size (mm^2) in WT and PLEKHA7$^{-/-}$ mice at day 5 and day 14 in superficial ear infection with USA300 LAC. Data are mean ± SEM from two independent experiments, representing $n = 15$ animals in each group. $P = 0.0116$, unpaired *t* test. (*C*) Core body temperature of USA300 LAC-infected WT and PLEKHA7$^{-/-}$ mice as shown in *D* at 6 h and 24 h postinfection ($P = 0.0047$, unpaired *t* test). At 6 h, $n = 14$ animals in each group; at 24 h, $n = 11$ WT and $n = 14$ PLEKHA7$^{-/-}$ animals. (*D*) Survival analysis of PLEKHA7$^{-/-}$ mice and WT controls after infection with *S. aureus* USA300 LAC. $n = 14$ animals in each group; $P = 0.0037$, log-rank test. (*E*) USA300 LAC bacterial density measurements from infected lungs of WT and PLEKHA7$^{-/-}$ mice at 6 h ($n = 6$ animals per group) and 24 h postinfection ($n = 6$ WT animals and 10 PLEKHA7$^{-/-}$; data are mean ± SEM). (*F*) Survival analysis of PLEKHA7$^{-/-}$ mice and WT controls after infection with a threefold higher inoculum of *S. aureus* USA300 LAC than in *D*. $n = 23$ animals in each group; $P = 0.0247$, log-rank test.

we determined that PLEKHA7-deficient cells are more resilient than WT cells and better recover from injury caused by α-toxin, ultimately exhibiting enhanced survival from intoxication. This conclusion is supported by time-lapse video microscopy revealing individual cells recovering from intoxication, as well as population-level assays quantifying cellular viability and the kinetics of intracellular ATP repletion in α-toxin–treated PLEKHA7-deficient cells.

We speculate that adherens junctions may regulate cytotoxicity through controlling resolution of pores and cellular membrane repair, or alternatively may act to transmit prodeath intracellular signals or localize injury caused by pore-forming toxins. PLEKHA7 is known to link microtubules to the adherens junctions and regulate stability of the junctions (24, 30), which may serve to coordinate these hypothesized functions. Our data support a new biological role for intracellular components of the adherens junctions in regulating cellular injury in response to α-toxin, a paradigm that warrants future investigation.

The relevance of PLEKHA7 for determining the outcome of in vivo MRSA bacterial infections was demonstrated in two relevant infection models, a self-resolving skin infection (37) and a lethal pneumonia (7, 8). Some canonical adherens junctions proteins such as E-cadherin and p120 catenin are essential for junction formation, and systemic knockouts are embryonic lethal (48, 49). In contrast, our previously unpublished PLEKHA7$^{-/-}$ mice and a recently described PLEKHA7$^{-/-}$ rat model (36) are healthy and fecund, exhibiting no gross developmental or epithelial pathology. From this we infer that PLEKHA7 is not an essential junctional protein in vivo but rather may serve to regulate some previously unidentified aspect of junction function under specific conditions. We find that systemic PLEKHA7 deletion in vivo attenuates the pathogenicity of the clinically relevant MRSA USA300 LAC strain in mouse models of both a self-resolving skin infection as well as a lethal pneumonia. In both epithelial infection models, we observe an initial similarity in pathology between the WT- and PLEKHA7$^{-/-}$-infected animals; however, at later time points, PLEKHA7$^{-/-}$-infected animals recover better than WT-infected controls. These results suggest that targeting nonessential components of the host adherens junctions could potentially reduce MRSA morbidity by enhancing resilience to and recovery from α-toxin injury. The increasing prevalence of drug-resistant MRSA strains underscores the urgent need to develop host cellular targets of *S. aureus* virulence, which may have future utility as adjunctive therapy.

Materials and Methods

Haploid Human Cell Genetic Screen. HAP1 cells were mutagenized with a retroviral gene trap to cause inactivating mutations throughout the genome, and a haploid genetic screen was performed as previously described (22, 23). For a complete description of the haploid genetic screen, see *SI Materials and Methods*.

Genome Engineering and PLEKHA7 Cloning. Clustered regularly interspaced short palindromic repeats (CRISPR) sequence-targeting sequences were designed using the Zhang Lab CRISPR design tool (crispr.mit.edu), and oligos corresponding to the guide RNA sequences were directly cloned into the Zhang laboratory-generated Cas9-expressing plasmid px458 using the Gibson Assembly Reaction (NEB). A complete description of HAP1 and MDCK genome engineering, guide target sequence oligos, and PLEKHA7 construct cloning is presented in *SI Materials and Methods*.

Bacterial Strains and Culture. The MRSA strain USA300 LAC was kindly provided by Fabio Bagnoli. A detailed description of the generation of the α-toxin isogenic mutant strain *hla::ermB* and the complemented *hla::ermB-phla*

is provided in *SI Materials and Methods*. Bacteria were grown in tryptic soy broth at 37° and prepared as indicated for animal infections.

Generation and Validation of PLEKHA7$^{-/-}$ Transgenic Mice. A detailed description of the generation and validation of *Plekha7(LacZ)* mutant mice is provided in *SI Materials and Methods*.

Murine *S. aureus* Superficial Skin and Pneumonia Infection Models. Murine models of MRSA superficial skin and pneumonia infections were carried out as previously described (7, 8, 37) with minor modifications. Inoculum preparation, infection conditions, and postinfection procedures are fully presented in *SI Materials and Methods*.

Other Procedures. Detailed descriptions of all other procedures are available in *SI Materials and Methods*. Animal experiments were carried out with the approval of the Institutional Animal Care and Use Committees of Stanford University School of Medicine and the RIKEN Center for Developmental Biology.

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