**Staphylococcus aureus** Lpl protein triggers human host cell invasion via activation of Hsp90 receptor

Paula M. Tribelli, Arif Luqman, Minh-Thu Nguyen, Johannes Madlung, Sook-Ha Fan, Boris Macek, Peter Sass, Katharina Bitschar, Birgit Schittek, Dorothee Kretschmer, Friedrich Götz

1 Microbial Genetics, Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), University of Tübingen, Tübingen, Germany
2 Departamento de Química Biológica, FCEyN-UBA, Buenos Aires, Argentina
3 IQUBICEN-CONICET, Buenos Aires, Argentina
4 Institut Teknologi Sepuluh Nopember, Biology Department, Surabaya, Indonesia
5 Division of Microbiology, Paul-Ehrlich Institute, Langen, Germany
6 Proteome Center Tübingen, University of Tübingen, Tübingen, Germany
7 Microbial Bioactive Compounds, Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), University of Tübingen, Tübingen, Germany
8 Department of Dermatology, University of Tübingen, Tübingen, Germany
9 Department of Infection Biology, Interfaculty Institute for Microbiology and Infection Medicine Tübingen (IMIT), University of Tübingen, Tübingen, Germany

**Correspondence**

Friedrich Götz, Department of Microbial Genetics, University of Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany. Email: friedrich.goetz@uni-tuebingen.de

**Abstract**

*Staphylococcus aureus* is a facultative intracellular pathogen. Recently, it has been shown that the protein part of the lipoprotein-like lipoproteins (Lpls), encoded by the *lpl* cluster comprising of 10 *lpls* paralogue genes, increases pathogenicity, delays the G2/M phase transition, and also triggers host cell invasion. Here, we show that a recombinant Lpl1 protein without the lipid moiety binds directly to the isoforms of the human heat shock proteins Hsp90α and Hsp90β. Synthetic peptides covering the Lpl1 sequence caused a twofold to fivefold increase of *S. aureus* invasion in HaCaT cells. Antibodies against Hsp90 decrease *S. aureus* invasion in HaCaT cells and in primary human keratinocytes. Additionally, inhibition of ATPase function of Hsp90 or silencing Hsp90α expression by siRNA also decreased the *S. aureus* invasion in HaCaT cells. Although the Hsp90β is constitutively expressed, the Hsp90α isoform is heat-inducible and appears to play a major role in Lpl1 interaction. Pre-incubation of HaCaT cells at 39°C increased both the Hsp90α expression and *S. aureus* invasion. Lpl1-Hsp90 interaction induces F-actin formation, thus, triggering an endocytosis-like internalisation. Here, we uncovered a new host cell invasion principle on the basis of Lpl-Hsp90 interaction.
INTRODUCTION

Many bacterial pathogens trigger internalisation into non-professional phagocytes, which is crucial to virulence because this shields the pathogen from certain immune defenses, antibiotics and enables the proliferation in relatively protected niches. Although previously considered an exclusively extracellular pathogen, Staphylococcus aureus is now regarded as a facultative intracellular pathogen that triggers proliferation in relatively protected niches. Although previously considered an exclusively extracellular pathogen, S. aureus is now regarded as a facultative intracellular pathogen that triggers internalisation by non-professional phagocytic cells (NPPCs) such as endothelial, epithelial, and mammary cells as well as fibroblasts or osteoclasts and can persist intracellularly for various periods of time (Bayles et al., 1998; Lowy, 1998; Sinha et al., 1999). Clinical studies also indicate a possible role for an intracellular staphylococcal reservoir in recurring diseases, such as rhinosinusitis or osteomyelitis (Clement et al., 2005; Kalinka et al., 2014; Mohamed et al., 2014). Staphylococcus aureus can also survive for long periods inside professional phagocytes, such as macrophages (Kubica et al., 2008) and polymorphonuclear neutrophils (PMN; Voyich et al., 2005). Additionally, infected PMNs can transmit the infection to naive mice (Gresham et al., 2000). Indeed, there is evidence that phagocytes could facilitate S. aureus infection because bacterial cells can survive after phagosomal escape (Koziel et al., 2009; O’Keeffe et al., 2015).

Staphylococcus aureus post-invasion events in non-professional phagocytes are only partially understood. It has been shown that they are able to escape the phagosome, which is associated with the induction of cell death (Bayles et al., 1998; Essen et al., 2001; Menzies & Kourteva, 1998; Wesson et al., 1998) and intracellular persistence (Garzoni & Kelley, 2009). The phagosomal escape is triggered by phenol-soluble modulin alpha (PSMα) (Grosz et al., 2014) and a cyclic dipeptide, phevalin, produced by a non-ribosomal peptide synthetase (NRPS) (Blättner et al., 2016; Zimmermann & Fischbach, 2010).

A prerequisite for any internalisation into NPPCs is host cell adhesion. This step mainly involves fibronectin (Fn), forming a bridge between α5β1 integrin on the cellular side and Fn-binding proteins on the bacteria (Fowler et al., 2000; Grundmeier et al., 2004; Sinha et al., 1999; Tran Van Nhieu & Isberg, 1993). The FnBP-Fn-α5β1 integrin pathway is widely acknowledged to be the main internalisation process. However, there are various so-called secondary mechanisms. These mechanisms mainly involve bacterial serine aspartate repeat-containing protein D (SdrD), clumping factor A (CIFA), serine-rich adhesin for platelets, and the major autolysin, Atl (Josse, Laurent, & Diot, 2017; Zapotoczna, Jevnikar, Majilovic, Kos, & Foster, 2013). These proteins are microbial surface components recognising adhesive matrix molecules (MSCRAMMs) and (except for Atl) have a cell-wall anchoring sequence located in their C-terminal portion (Josse et al., 2017). For example, SdrD binds, for example, directly to Desmoglein 1 in keratinocytes, promoting adhesion (Askarian et al., 2016; Corrigan, Majilovic, & Foster, 2009). Additionally, CIFA can interact through fibrinogen bridges to the alpha-V beta-3 integrin (αV β3) or complex bridge involving von Willebrand factor, the secreted von Willebrand factor binding protein and the αV β3 integrin that promotes adhesion in vascular endothelial cells (Claes et al., 2017; McDonnell et al., 2016). On the other hand, Srp adheres to gp340, a salivary scavenger protein, in A549 cell line (Yang et al., 2014). The major autolysin, Atl, mediates S. aureus internalisation via direct interactions with Hsc70 (Hirschhausen et al., 2010). It has been speculated that the various internalisation mechanisms allow the bacteria to expand their internalisation to changing environmental conditions; for example, in the absence or scarcity of Fn, they can make use of alternative binding partners to trigger invasion (Josse et al., 2017).

Recently, a certain class of lipoproteins, the so-called “lipoprotein-like lipoproteins” (LplS) were found to induce host cell internalisation (Nguyen et al., 2015). The lpl-genes are clustered on a pathogenicity island called vSaα island (non-phage and non-staphylococcal cassette chromosome genomic island), which is present in all S. aureus strains tested so far (Diep et al., 2006; Shahmizrad, Nguyen, & Götz, 2016). The lpl cluster comprises 10 lpl paralogous genes that encode 10 Lpl proteins with high sequence similarity and two accessories genes (Nguyen et al., 2015). When the entire lpl gene cluster is deleted in S. aureus USA300, the mutant showed a marked decrease in invasion of S. aureus into human primary keratinocytes and mouse skin and also showed a decreased pathogenicity in a mouse sepsis model (Nguyen et al., 2015). The Lpl lipoproteins not only trigger host cell invasion, but also delay the G2/M phase transition in HeLa cells (Nguyen et al., 2016). As the number of lpl genes is particularly high in epidemic S. aureus strains, it is assumed that the lpl gene cluster might contribute to increased dissemination and epidemic spreading by shielding the pathogen from the immune defence and antibiotic treatment (Nguyen et al., 2015). However, the mechanism of how Lpl proteins trigger the host cell internalisation was unknown.

Here, we identified the human heat shock protein Hsp90 as the host receptor for Lpl-induced S. aureus USA300 invasion of human keratinocytes using Lpl1 as a model of LplS for in vitro experiments. The Hsp90–Lpl interaction triggers a cascade of reactions including ATPase activity and F-actin formation, indicating that the bacterial internalisation underlies an endocytosis-like process.

RESULTS

2.1 Human Hsp90 interacts with S. aureus Lpl1 protein in pull-down experiments

Previously, it has been shown that Lpl lipoproteins from S. aureus USA300 increase the internalisation into HaCaT cells, a human keratinocyte cell line and also in human primary cells (Nguyen et al.,
2015; Nguyen, Peisl, Barletta, Luqman, & Götz, 2018). As the lipid moiety is anchored in the cytoplasmic membrane, we expect that the protein part interacts with the potential host cell receptor. To capture the host cell receptor, we used Ni-NTA (nikel-nitrilotriacetic acid agarose)-bound Lpl1-his as a bait. Lpl1 from USA300 was used as our model Lpl protein. It was expressed without the lipo signal peptide and with a C-terminal His-tag in S. aureus SA113 (pTX30::ppl1-his) and purified by Ni-NTA affinity chromatography. Purified Lpl1-his was bound to Ni-NTA and loaded with HaCaT cell lysate. After extensive washing, the HaCaT proteins bound to Lpl1-his were eluted with 250 mM imidazole and 500 mM NaCl.

The elution fraction containing the proteins that interacted with the Lpl1-his and the control fraction (in which the cell lysate passed through Ni-NTA without bound Lpl1-his) were separated by SDS-PAGE followed by Coomassie blue staining (Figure S1a).

The most prominent band on the SDS-PAGE was Lpl1-his. There were five lower-sized protein bands that were not present in the control lane and were used for further analysis by Nano-HPLC-MS/MS. The identified HaCaT-specific proteins are listed in Table 1. We only considered HaCaT proteins that were present in the Lpl1-bound Ni-NTA but not in the control column. The most abundant proteins with the highest coverage and posterior error probability (PEP) of 0.01 or lower were the human heat shock Hsp90 alpha (Hsp90α) and beta (Hsp90β) proteins. Both proteins are highly homologous, sharing 94% similarity and 86% identity. Hsp90 proteins are approximately 90 kDa; however, the Lpl1-interacting proteins were about 15 kDa. This indicates that the detected Hsp90 proteins were truncated, most likely due to proteolytic degradation, although a protease inhibitor cocktail was used during the preparation of HaCaT cell lysate.

### 2.2 | Hsp90α and Hsp90β are localised to the cell surface in HaCaT cells

It has been described that both the Hsp90α and Hsp90β isoforms were found on the cellular surface in different cell lines and tissues (Bozza et al., 2014; Eustace et al., 2004; Suzuki & Kulkarni, 2010). Here, we confirmed that both the Hsp90 proteins were localised to the cell surface of HaCaT cells and co-localised with FM 5-95 stained membrane via immunofluorescence analysis (Figure 1).

### 2.3 | Hsp90 antibodies block USA300 adherence and invasion

To further confirm the contribution of Hsp90 in Lpl-triggered invasion into HaCaT cells, we blocked Hsp90 proteins with specific antibodies in invasion assays in the presence of fetal serum to mimic in vivo conditions where soluble fibronectin is present. We used monoclonal antibodies specific against Hsp90α (α-Hsp90α) and Hsp90β (α-Hsp90β) and a polyclonal antibody that recognises both Hsp90α and Hsp90β (α-Hsp90αβ). Pre-incubation of the HaCaT cells with polyclonal α-Hsp90αβ antibodies caused a decrease in the invasion of USA300 from 145.3 ± 40 CFU to 19.1 ± 8.8 CFU per 1 × 10⁵ HaCaT cells, indicating that the antibodies caused a severalfold decrease in invasion (Figure 2a). To further validate our results, we investigated the impact of the polyclonal α-Hsp90αβ antibodies on the internalisation of USA300Δlpl and its complemented mutant USA300Δlpl (pTX-lpl1). In USA300Δlpl, the entire lpl cluster was deleted (Nguyen et al., 2015).

In this strain, the polyclonal α-Hsp90αβ antibodies showed no effect on invasion; however, in the lpl-complemented mutant, the invasion was decreased again (Figure 2a). As fibronectin binding protein (FnBPs)-fibronectin-α5β1 integrin pathway is a key mechanism for S. aureus adherence and invasion into the host cells, we verified that the FnBPs expression was similar in the wild type USA300 and the Δlpl mutant (Figure S1b).

We also investigate whether S. aureus adherence was affected by α-Hsp90α monoclonal antibodies. When HaCaT cells were pre-blocked with α-Hsp90α, S. aureus adherence was almost threefold decreased, from 21,960 ± 3,721 to 7,969 ± 1,951 CFU per 1 × 10⁵ HaCaT cells (p < 0.05, one-way ANOVA).

The invasion frequency was normalised to the unblocked HaCaT cells, which was set at 1.0 and was used as a comparator for further experiments. Pre-incubation with monoclonal α-Hsp90α caused a decrease in invasion from 1.0 to 0.20 ± 0.05, whereas pre-incubation with monoclonal α-Hsp90β caused a decrease in invasion from 1.0 to

| Protein name | Gene name |
|--------------|-----------|
| Heat shock protein HSP 90-beta | HSP90AB1 |
| Heat shock protein HSP 90-alpha | HSP90AA1 |
| Eukaryotic initiation factor 4A-1:Eukaryotic initiation factor 4A-II | EIF4A1:EIF4A2 |
| Tubulin beta-4B chain;Tubulin beta-4A chain | TUBB4B:TUBB4A |
| Tubulin alpha-1B chain;Tubulin alpha-1A chain;Tubulin alpha-3C/D chain;Tubulin alpha-3E chain | TUBA1B:TUBA1A:TUBA3C:TUBA3E:TUBA4A |
| Pre-mRNA-processing factor 19 | PRPF19 |
| Aldose reductase | AKR1B1 |
| Not identified 1 | |
| Not identified 2 | |
| Isocitrate dehydrogenase [NADP] cytoplasmic | IDH1 |
0.27 ± 0.08; in both cases, the invasion was decreased to fivefold and fourfold, respectively. As a control, we used human IgG that has no effect on internalisation (Figure 2b).

We also investigated the influence of Hsp90 proteins in human embryonic kidney 293 cell line which is untransfected by TLR2 (HEK‐0). The result was similar to our earlier observation; α‐Hsp90α decreased the internalisation by fivefold (Figure S2).

Additionally, we investigated the role of Hsp90α in USA300 invasion on primary keratinocytes by using α‐Hsp90α to block Hsp90α. Pre‐incubation with α‐Hsp90α decreased the USA300 invasion by twofold (Figure 2c).

2.4 | Geldanamycin blocks S. aureus invasion in HaCat cells

Geldanamycin is a well‐known cell permeable anti‐neoplastic compound that competes with ATP binding in Hsp90, thus inhibiting Hsp90 activity (Gorska et al., 2012). Here, we investigated whether this compound affects USA300 internalisation. Indeed, the addition of geldanamycin at a concentration of 5 μM decreased the USA300 invasion in a dose‐dependent manner by approximately threefold (Figure 2d). These results further confirm the role of Hsp90 in USA300 invasion. It should be mentioned that geldanamycin had no growth‐inhibiting effect on S. aureus at all used concentrations.

2.5 | Silencing of Hsp90α expression by siRNA causes a decrease of USA300 invasion

We have shown that the blocking of both Hsp90α and Hsp90β by antibodies caused a fourfold to fivefold decrease in invasion of S. aureus cells. However, for reasons of simplicity, we focus mainly on Hsp90α in the following experiments. Hsp90α is a protein that is inducible by oxidative and heat stress (Prodromou, 2016; Profumo et al., 2018), and is reported to interact with envelope proteins of certain viruses and lipopolysaccharide of Gram‐negative bacteria (Reyes‐Del Valle, Chavez‐Salinas, Medina, & Del Angel, 2005; Triantafilou, Triantafilou, & Dedrick, 2001). Silencing the Hsp90α mRNA expression by an antisense RNA (siRNA) caused a decrease in invasion by approximately twofold (Figure 3a). As a control, random siRNA was used and no effect on invasion was seen (Figure 3a). The silencing of Hsp90α expression was also confirmed by Western blot analysis (Figure S3a).

2.6 | High temperature‐induced expression of Hsp90α causes an increase of USA300 invasion

Hsp90α is an inducible heat shock protein. Its expression increases when the temperature increases, whereas the Hsp90β isoform is constitutively expressed (Prodromou, 2016). An increased body temperature (fever) is frequent during bacterial infection. Therefore, we investigated if an increased temperature upregulates Hsp90α expression, thus leading to an increased USA300 invasion. In order to study this, HaCaT cells were pre‐incubated for 2 hr at 39°C prior to the invasion assay. The higher temperature used resulted in an almost twofold (1.86 ± 0.24) increase in invasion relative to the control at 37°C (Figure 3b). To confirm that the increased invasion was indeed due to the increased Hsp90α expression, we blocked it with α‐Hsp90α; as expected, it caused a decrease in invasion at 39°C (Figure 3b). The increased Hsp90α expression at 39°C was also confirmed by Western blot analysis (Figure S3b).
We investigated if USA300 or Lpl1-his affected Hsp90 expression by using Western blot analysis. Neither USA300 nor purified Lpl1-his affected Hsp90α expression within a period of 6 hr after the addition of USA300 (Figure S4a). In addition, we also tested whether USA300 affects Hsp90α localisation by using flow cytometry analysis in non-permeabilized HaCaT cells. The amount of Hsp90 on HaCaT surface was similar after 1.5 hr exposure of the cells to USA300 (Figure S4b).

**FIGURE 2**  Hsp90 antibodies or inhibitors block USA300 invasion. (a) 1 × 10⁶ HaCaT cells with or without pre-incubation during 1 hr with α-Hsp90αβ or control human IgG (IgG). Cells were further infected with *Staphylococcus aureus* USA300 strain, USA300 Δlpl mutant strain, or USA300 Δlpl + pTXlpl with a MOI of 30 in DMEM medium, supplemented with 10% FBS. Host cells were infected for 1.5 hr followed by lysostaphin treatment for 1.5 hr. Cells were lysed and USA300 CFU was determined by plating on TSA agar plates. The experiments were performed in at least three independent biological replicates. Error bars indicate standard deviation. The statistical was calculated by using Student t test; **p < .01; *p < 0.05, comparing with the control for each strain. (b) Invasion assays using *S. aureus* USA300 wild type were performed as described above but specific isoform antibodies (35 μg ml⁻¹) were used: α-Hsp90α, α-Hsp90β, and human IgG (IgG) as an extra control. Relative invasion was calculated by normalising USA300 invasion in treated HaCaT cells to USA300 invasion in untreated control cells (C). The experiments were performed at least in three independent biological replicates with at least three technical replicates. Error bars indicate standard deviation. The statistical was calculated by using One-way Anova with multiple comparison to invasion in the control (C) sample, **p < 0.01, *p < 0.05. (c) Primary human keratinocytes were cultured in collagen-coated tissue flasks in epidermal keratinocyte base medium. Incubated with or without α-Hsp90α or a control human IgG prior to USA300 invasion using an MOI of 30 were 2.5 × 10⁵ differentiated keratinocytes. Relative invasion was calculated by normalising USA300 invasion in treated cells to USA300 invasion in cells without treatment (C). The experiments were performed using cells from three independent donors with at least three technical replicates. Error bars indicate standard deviation. The statistical was calculated using one-way ANOVA with multiple comparison with the control (C) sample, *p < 0.05. (d) Pretreated with different concentrations of geldanamycin during 1 hr were 1 × 10⁶ cells. Relative invasion was calculated by normalising to invasion in control (C) cells. The experiments were performed in at least three independent biological replicates. Error bars indicate standard deviation. The statistical was calculated by using one-way ANOVA with multiple comparison with the control (C) sample, *p < 0.05, **p < 0.01, ***p < 0.001. USA300, *S. aureus* USA300; MOI, multiplicity of infection; CFU, colony-forming unit; α-Hsp90α, primary mouse antibody to Hsp90α; α-Hsp90β, primary mouse antibody to Hsp90β; α-Hsp90αβ, primary rabbit antibody to Hsp90αβ
3.1 Lpl1-Hsp90α/β interaction boosts F-actin formation

It has been reported that the geldanamycin analogue, 17AAG, an Hsp90 Inhibitor, decreased F-actin formation upon Hsp90 inhibition (Taiyab & Rao Ch, 2011). Therefore, we investigated if actin polymerisation could be enhanced by Lpl1 in an Hsp90-dependent manner. Indeed, F-actin formation could be enhanced by >20%, following treatment of HaCaT cells with Lpl1-his (Figure 4a). This was confirmed by the addition of α-Hsp90α, which caused a decrease of F-actin formation to the control level (Figure 4a). These results showed that Lpl1 triggers F-actin formation in an Hsp90-dependent manner.

3.2 Lpl1-Hsp90α/β interaction has no effect on Hsp90 ATPase activity

Next, we investigated if Lpl1 would directly affect Hsp90 ATPase activity. The addition of Lpl1 to a functional Hsp90α did not significantly affect the ATPase activity, whereas the specific inhibitor
geldanamycin inhibited the ATPase activity by 46% (Figure 4b). These results showed that Lpl1 did not directly affect the ATPase activity of Hsp90.

### 3.3 Lpl1-his interacts directly with purified Hsp90α and Hsp90β

By using far-western blot assay, we could detect interactions between Lpl1-his and purified Hsp90α and Hsp90β, whereas no interaction was seen when bovine serum albumin (BSA) as a control (Figure S5). In order to find out which protein domains of Lpl1 are interacting with the Hsp90 proteins, peptides covering the Lpl1 sequences were synthesised and assayed for invasion and F-actin formation (Table S1). The synthesised peptides covered almost entirely the length of Lpl1 protein. Lpl1 has a conserved “core” region near the N-terminus that increased effect on invasion also exhibited no detectable binding to Hsp90α due to weak binding (Figure S4). All the other peptides that showed no binding proteins bind via fibronectin to α5β1 integrin (Sinha et al., 2000) or human Hsp60 (Dziwanowska et al., 2000), and Atl binds to the heat shock cognate protein Hsc70 (Hirschhausen et al., 2010). However, the host cell receptor for Lpl lipoproteins was still unclear (Nguyen et al., 2015).

We also tested the interaction of Hsp90α with the mentioned peptides that increased *S. aureus* invasion and F-actin formation using far-western blot assay. P10 and P11 were found to interact with Hsp90α, as well as with Hsp90β. No interaction with P2 was seen, presumably due to weak binding (Figure S4). All the other peptides that showed no effect on invasion also exhibited no detectable binding to Hsp90α.

### 4 DISCUSSION

Almost all pathogenic bacteria have developed the ability to directly invade or to trigger invasion into non-phagocytic host cells. Triggering the internalisation of pathogenic bacteria into the host cells is a survival strategy because the human body is constantly patrolled by immune cells and contains antibodies and other molecules that can target and kill the bacteria. Therefore, the environment inside the host cells provides a safe place to avoid detection by the immune system. Internalised staphylococci may induce apoptosis, autophagy, or exist freely within the cytoplasm (Mauthe et al., 2012; Menzies & Kourteva, 1998).

There are several reports describing *S. aureus* surface proteins that interact with specific receptors of the host cell. For example, fibronectin-binding proteins bind via fibronectin to α5β1 integrin (Sinha et al., 2000) or human Hsp60 (Dziwanowska et al., 2000), and Atl binds to the heat shock cognate protein Hsc70 (Hirschhausen et al., 2010). However, the host cell receptor for Lpl lipoproteins was still unclear (Nguyen et al., 2015).

Here, we show that Lpl1, which served as a model Lpl-lipoprotein, interacts with the human heat shock protein Hsp90 to trigger host cell invasion in HaCaT cells and primary keratinocytes. Hsp90 (90 kDa) proteins are expressed abundantly in a variety of cell types amounting to 2–3% of total proteins. They represent molecular chaperones and have a central role in protein homeostasis under stress conditions (Albrecht, Raue, Rosenstein, Nieselt, & Götz, 2012). At least two isoforms can be found in humans, the inducibly expressed Hsp90α and...
the constitutively expressed Hsp90β (Zuehike, Beebe, Neckers, & Prince, 2015).

The activities of Hsp90 proteins are diverse and not completely analysed. They are found to be membrane-bound, intracellularly, and are also as secreted form. Hsp90α for example, is membrane-bound but can be secreted in response to tissue injury. A well-characterised function of secreted Hsp90α is to promote cell motility, a crucial event for both wound healing and cancer (Li, Sahu, & Tseng, 2012). The anchoring of Hsp90α and Hsp90β to the plasma membrane involves co-localisation with heparan sulfate proteoglycans (HSPGs) on the cell surface (Snigireva, Vrublevskaya, Afanasyev, & Morenkov, 2015). Hsp90α secretion is proposed to occur through the exosome pathway (Snigireva, Vrublevskaya, Skarga, & Morenkov, 2016). The presence of extracellular Hsp90 protein was found in normal tissues like dermal, epidermal, endothelial cells and in the nervous system (Cheng et al., 2008; Cheng et al., 2011; Miyakoshi et al., 2017; Profumo et al., 2018; Sidera, Samiotaki, Yfant, Panayotou, & Patsavoudi, 2004), and also during pathological processes like cancer or autoimmune diseases (Albrecht et al., 2012; Eustace et al., 2004; Tsutsuji & Neckers, 2007; Weidle, Maisel, Klostermann, Schiller, & Weiss, 2011).

Using specific antibodies against both α and β forms, we could block the invasion of S. aureus, suggesting that both forms can trigger invasion. However, we speculate that Hsp90α primarily functions to facilitate Lpl-triggered invasion for the following reasons: (a) We tried to silence Hsp90α and Hsp90β expression by siRNA. This worked perfectly with Hsp90α, whereas silencing of Hsp90β expression was unsuccessful, which is in agreement with other reports (Didelot et al., 2008; Lee, Jang, & Chung, 2010). Silencing of Hsp90α caused a significant decrease in Lpl-triggered invasion. (b) In particular, the Hsp90α has been reported to interact with pathogens or defined compounds. For example, Hsp90α serves as a receptor for dengue virus (Reyes-Del Valle et al., 2005), adhesin A of Neisseria meningitidis (Bozza et al., 2014), and is involved in the uptake of diphtheria toxin in host cells (Schuster et al., 2017).

Expression of Hsp90α is inducible by oxidative stress and increased temperature. Therefore, we investigated whether increased temperature such as 39°C affects invasion. Interestingly, pretreatment of HaCaT cells at 39°C prior to the invasion assay led to an almost twofold increase in invasion relative to the control at 37°C. This suggests that fever favours S. aureus host cell invasion by upregulating Hsp90α (Figure S2c). Fever is an evolutionarily conserved response that promotes T-lymphocyte trafficking through Hsp90-induced alpha4 integrin activation and signalling in T cells, thus enhancing immune surveillance during infection (Lin et al., 2019). However, fever induces many factors, among them are the pyrogenic cytokine interleukin-6 (IL-6) which is involved in the mobilisation of lymphocytes to the lymphoid organs that are the staging ground for immune defence (Evans, Repasky, & Fisher, 2015). Although fever increases the migration of T-lymphocytes, some pathogens like S. aureus try to escape the lymphocyte killing by hiding in nonprofessional cells.

At present, we do not know which partners are involved in the Lpl-Hsp90-triggered signalling cascade. Proteins and complexes described as potential clients of Hsp90 are growing constantly and include kinases and receptors (Miya, Nakamoto, & Neckers, 2013). For example, it is assumed that Hsp90 interacts with the extracellular domain of the Her-2 receptor in the membrane and this interaction triggers signalling in which the cytoplasmic Hsp90 also participates in the actin polymerisation (Sidera & Patsavoudi, 2008; Taiyab & Rao Ch, 2011). The Lpl-Hsp90-induced internalisation of S. aureus is probably on the basis of a zipper mechanism in which an intracellular signalling cascade involving the activation of adapter proteins and kinases and the formation of F-actin leading to endocytosis (Colonne, Winchell, & Voith, 2016). Thus, rapid actin polymerisation causes internalisation of the pathogen into non-phagocytic cells.

In addition, we attempted to identify the Lpl1 epitopes that trigger S. aureus internalisation, increased F-actin formation and displayed direct interaction with Hsp90α. We found two overlapping peptides, which consist of 64 and 38 amino acids and both are located at the C-terminal part of Lpl1 (Figure S5). This finding is reasonable, as this part is the tip of the Lpl proteins and most likely protrudes out of the cell wall. The similarity of the two epitopes to other Lpl-proteins is around 65%.

The lpl gene cluster is localised on a pathogenicity island, termed vSaC. This island is predominant in S. aureus clonal complexes that are spreading worldwide. The key for any additional invasion mechanism lies in the increased ability of a pathogen to hide (and multiply) intracellularly in a safe environment. The internalised cells are protected not only from the host’s own innate and adaptive immune response, but also from antibiotics. Interestingly, the number of tandem lpl's is particularly high in S. aureus clones that are known to spread worldwide. For example, the USA300 lineage is spreading rapidly and this lineage is distinguished by a high number of tandem lpl repeats (Carrel, Perencevich, & David, 2015; Tickler et al., 2017). We posit that the Lpls play a role in the rapid spreading of such lineages, as intracellular pathogens are better protected. With the identification of Hsp90 as a receptor for Lpls, we laid the basis for the development of drugs that block Hsp90 mediated invasion.

In conclusion, we propose a model (Figure 6) in which the membrane-bound S. aureus Lpls interact with the cell surface localised Hsp90. This interaction triggers a cascade of reactions involving ATPase in an indirect manner, given that geldanamycin blocked S. aureus invasion and the ATPase activity and F-actin formation are not affected by Lpl1, resulting in an endocytosis-like engulfment of bacteria.

5 | EXPERIMENTAL PROCEDURES

5.1 | Bacterial strains, antibodies, and cell lines

Bacterial strains used in this study are listed in Table S2. Bacteria were grown aerobically at 37°C in either basic medium, BM (1% soy peptone, 0.5% yeast extract, 0.5% NaCl, 0.1% glucose and 0.1% K2HPO4, pH 7.4) or tryptic soy broth (TSB, Sigma). When appropriate, the media was supplemented with tetracycline (25 μg/ml, Carl Roth,
The supernatant was incubated with Ni-NTA super flow beads (Qiagen, Germany). After overnight incubation, the Ni-NTA beads were extensively washed and eluted with buffer containing 400 mM imidazole. Lpl1 was concentrated via centrifugal ultra-filter unit with a molecular mass cut-off of 10 kDa (Sartorius AG, Göttingen, Germany). The obtained protein was dialysed using D-Tube Dialyzer Maxi MWCO 6-8 kDa. (Novagen Cod) with DPBS (phosphate buffer saline without calcium or magnesium, Gibco). Finally, the Lpl1-his purification was verified by SDS-PAGE and the total protein amount was determined using a Bradford assay kit.

### 5.3 Synthesis of Lpl1 derivative peptides

Lpl1 derivative peptides were designed on the basis of the sequence (Nguyen et al., 2015) and structural analysis performed with Phyre2 (Kelley, Mezulis, Yates, Wass, & Sternberg, 2015) and listed in Table S1. The peptides were synthesised by Apeptide (Shanghai, China) with a purity of >95%. The peptides solution at 1 mg/ml was prepared in water and stored at −20°C.

### 5.4 Pull-down experiments

Pull-down experiments were performed as described before (Hirschhausen et al., 2010). Briefly, confluent HaCaT cells from one cell culture flask (75 cm², Greiner Bio-One) were detached, washed twice with DPBS, and then resuspended in lysis buffer (containing 30 mM HEPES, 1 mM EDTA, 14 mM NaCl, 2 mM MgCl₂, 1% Triton X-100, 1 mM DTT, and protease inhibitors). The complete cell lysis was assayed by additional mechanical disruption using a 21-G needle (BD Bioscience). Next, the cell lysate was centrifuged. A 10 μl disposable column (Pierce) containing 500 μl of the Ni-NTA agarose (Qiagen) equilibrated with washing buffer (30 mM HEPES, 10 mM imidazole, 500 mM NaCl, 10 mM b-mercaptoethanol) was used. Then, 25–50 μg of Lpl1-his protein was bound to the Ni-NTA column and washed three times with three-column volumes with washing buffer. Next, 500 μl of the HaCaT cell lysate was added and the column was intensively washed with washing buffer (five washes with three-column volumes). Bound proteins were eluted with elution buffer (30 mM HEPES, 250 mM imidazole, 500 mM NaCl, 10 mM b-mercaptoethanol). The elution fraction was precipitated with Strata Clean Resin and consequently analysed by Nano-HPLC-MS/MS analysis.

### 5.5 Nano-HPLC-MS/MS analysis

Nano-HPLC-MS/MS was used to analyse the protein bands from the Lpl1-HaCat cells pull-down experiment. Excised protein bands were digested in-gel using trypsin. Nano-HPLC-MS/MS analysis was done on an LC-MS/MS analysis on a Proxeon Easy-nLC 1200, coupled to a LTQ Orbitrap Elite mass spectrometer with a setting of 60 min gradient and Top15 CIDMS. Data was processed using MaxQuant software suite v.1.5.2.8 (Cox and Mann, 2008). Employing the Andromeda search engine (Cox et al., 2011), the spectra were searched against Homo sapiens and S. aureus and a database comprising the sequence of Lpl1-his. False discovery rate (FDR) was set to 1% at both the protein and peptide level and posterior error probability (PEP) was set to 0.01, or smaller, for each peptide to consider a valid identification. The most abundant proteins with high coverage and...
low FDR and PEP below 0.01 were Hsp90 alpha (Hsp90α) and beta (Hsp90β) proteins. Both of the isoforms of Hsp90 were found in the analysed samples: alpha Hsp90 (Accession No: P_005339) and beta Hsp90 (Accession No: P_001258899) encoded by HSP90AA1 and HSP90AB1, respectively.

5.6 Infection and adherence assays of HaCaT cells

Invasion assay was performed as described previously (Nguyen, Peisl, et al., 2018). For bacterial infection, 2.5 × 10⁵ HaCaT cells were seeded into 24-well plates and incubated at 37°C under 5% CO₂ for 48 hr. After 48 hr, cells were washed two times with DPBS and then 1 ml DMEM supplemented with 10% FBS, but without the addition of antibiotics. Afterwards, the cells, in the presence of DMEM supplemented with 10% FBS, were incubated with different antibodies or peptides (Table S1 and Table S2) for 1 hr prior to the invasion of bacteria. Before stimulation, the strains were cultured for 16 hr in TSB and washed twice with DPBS. Bacterial pellets were resuspended in DMEM/F-12 without any supplements and incubated with host cells for 1.5 hr. For detection of invaded bacteria, cells were treated with 2.5 μg ml⁻¹ lysostaphin (Sigma-Aldrich, Germany) for an additional 1.5 hr to remove extracellular bacteria. Keratinocytes were lysed with 0.1% Triton X-100, 0.5% Trypsin, and 0.3 mgml⁻¹ DNase in DPBS. Serial dilutions were performed and 10 μl of several dilutions were seeded on agar plates. Internalised bacteria were determined on the basis of the calculation of bacterial colonies grown on the agar plates. In some experiments, cells were pre-incubated for 1 hr with α-Hsp90α, α-Hsp90β, or α-Hsp90αβ or human IgG as a control (Table S2) in different concentrations (17.5 or 35 μg ml⁻¹).

Adherence experiments were performed in the presence of 10% FBS similar to the invasion assays but without the addition of lysostaphin. HaCaT cells were lysed and serial dilutions of the lysates were performed. Ten microliters of the dilutions were seeded on agar plates. Internalised bacteria were not considered because their contribution to adherence is very low. Adhered bacteria were determined as CFU per 10⁶ HaCaT cells.

5.7 Hsp90α induction and siRNA experiments

For siRNA-mediated gene silencing, predesigned RNA oligonucleotide targeted against human Hsp90α (siRNAHsp90α) or a random siRNA negative control (Silencer™ Negative Control No. 1 siRNA, Thermo Scientific) were used. HaCat cells of 2.5 × 10⁶ were seeded in 24-well plates and after 24 hr, cells were transfected with 6 μl of 10 μM dsRNA oligonucleotide or without dsRNA using Lipofectamin RNAiMAX reagent according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA). After 24 hr, invasion assay was performed as described above. For heat shock Hsp90α induction, 24-wells plate with 1 × 10⁶ HaCaT cells per well was pre-incubated for 2 hr at 39°C prior to the invasion assay.

5.8 Primary human keratinocytes invasion

Primary human keratinocytes were isolated from human foreskin after routine circumcision from the Loretto Clinic in Tübingen as previously described (Nguyen et al., 2018). Primary human keratinocytes were cultured in collagen-coated tissue flasks (Corning, BioCoat™) in epidermal keratinocyte medium (CELLnTEC) at 37°C under 5% CO₂. At 24 hr prior to experiments, keratinocytes were differentiated with 1.7 mM CaCl₂ in epidermal keratinocyte base medium (CELLnTEC). Confluent cells, approximately 2.5 × 10⁶ cells per well, were used for USA300 invasion experiments with an MOI of 30. For blocking, cells were incubated with α-Hsp90α or human IgG (17.5 μg ml⁻¹) for 1 hr prior to the invasion assay.

5.9 F-Actin measurement

Seeded into black cell culture microplate (Greiner, Germany) were 2.5 × 10⁶ HaCat cells in 200 μl for 48 hr prior to incubation with 35 μg ml⁻¹ of Lpl1 or the derivative peptides for 1 hr. In some cases, HaCaT cells were pre-incubated with 35 μg ml⁻¹ of α-Hsp90α antibody for 1 hr prior to the addition of Lpl1 or peptide. F-actin levels were measured as described before (Nguyen, Peisl, et al., 2018), using ActinGreen™ 488 ReadyProbes® (Thermo Fischer). The treated cells were washed with DPBS, permeabilized with 0.25% (v/v) Triton X-100, stained with the dye for 30 min and washed again with DPBS. Then, the fluorescence was measured at 495 nm for the excitation and 518 nm for the emission using Tecan Reader.

5.10 Immunofluorescence of surface Hsp90

To detect Hsp90α on the cellular surface, an immunofluorescence assay was carried out. Briefly, 2.5 × 10⁶ of HaCaT cells were seeded in CellView glass bottom culture dish (Greiner, Germany) for 36 hr. Afterwards, the cells were washed three times with DPBS and fixed with paraformaldehyde 4% in DPBS at pH 7 before incubating it for 10 min at room temperature. The cells were washed again three times with DPBS and blocked with 3% of BSA (Sigma) in DPBS for 30 min at room temperature. The cells were washed and incubated overnight at 4°C with the corresponding antibodies. α-Hsp90α or α-Hsp90β mouse monoclonal antibodies (Table S2) used were diluted at 1:50. Cells were intensely washed and incubated with the secondary antibody donkey α-mouse IgG H&L pre-absorbed Alexa Fluor® 488 (Abcam) in a 1:450 dilution for 1 hr at room temperature. Cell membranes were stained with FM5-95 in a 1:1000 dilution for 5 min. Controls with incubation of the cells with only the secondary antibody was also performed. Cells were imaged using the Zeiss Axio Observer Z1 fluorescence microscope equipped with a C Plan-Apo 63x/1.4 Oil DIC objective (Zeiss) and an ORCA-Flash 4.0 LT camera (Hamamatsu). Images were acquired and analyzed via ZEN 2.6 imaging software package (Zeiss) and Fiji software (Schindelin et al., 2012).
5.11 | Far-western blot experiments

The study of Hsp90α or Hsp90β binding with Lpl1-his proteins was performed by far-western blot assay according to previous study (Nguyen, Peis, et al., 2018). Briefly, 10 μg of Lpl1-his or BSA (as a negative control) was loaded in a polyacrylamide gel in native conditions. Proteins were transferred to a PVDF nitrocellulose membrane (Bio-Rad, USA) and was blocked with 3% BSA for 1 hr. The blocked membrane was incubated with 20 μg of recombinant Hsp90α or Hsp90β recombinant protein (Abcam) overnight at 4°C. For immunoblotting, monoclonal specific α-Hsp90α or α-Hsp90β antibodies (Abcam) were used as first antibody and goat-α-mouse IgG (Sigma, Germany) as secondary antibody. The detection of the reaction was performed with BCIP®/NBT solution (Sigma, Germany) according to the manufacturer's instructions. In the case of synthetic peptides, 2 μg of each peptide was blotted directly to the PVDF nitrocellulose membrane following the same steps described above but with 6 μg of recombinant Hsp90α.

For fibronectin binding proteins (FnBPs) detection, we followed the protocol described previously with modifications (Mongodin et al., 2002). Briefly, cells from S. aureus USA300 wild type and Δipl strain were lysed with lysostaphin and treated with DNase. Protein concentration was determined using Bradford assay and 10 μg of proteins were loaded into a polyacrylamide gel in native conditions. For immunoblotting, the steps described above were followed but the membrane was incubated with 30 μg of fibronectin (Sigma Aldrich, Germany) overnight at 4°C. After extensive washes, the membrane was incubated with monoclonal primary antibody to human fibronectin (clone FN3, eBioscience) overnight at 4°C and with GapA polyclonal primary antibody as a loading control (Nega et al., 2015). Goat-α-mouse IgG (Sigma, Germany) as secondary antibody. The detection of the reaction was performed with BCIP®/NBT solution (Sigma, Germany) according to the manufacturer's instructions.

5.12 | Western blot experiments

Western blot experiments were performed using standard techniques. Briefly, protein concentration in the samples was determined by Bradford assay. Proteins were run on an SDS-page and then transferred to a PVDF nitrocellulose membrane and blocked with 3% BSA. The blocked membrane was incubated with α-Hsp90α (Thermo Fisher, Table S2) or α-GDPH (Thermo Fisher, Table S2) as a loading control (Thermo Fisher) and goat-α-rabbit IgG or goat-α-mouse IgG was used as secondary antibody. Pre-stained protein ladder (Fermentas) was used as molecular weight marker.

5.13 | Flow cytometry assay (FACS)

In a MOI of 30 as described previously, 1 × 10⁵ HaCaT cells were exposed to USA300. Cells were washed with DPBS and further incubated for 1 hr with DMEM/F-12, supplemented with lysostaphin and 2 mM of EDTA. Cells were desegregated, centrifuged, and incubated for 30 min with α-Hsp90α in PBS and 1% fetal calf serum (FCS) in 1:100 dilution or with an isotype control (Sigma) or the secondary antibody alone as control. The cells were washed in PBS and 1% FCS and further incubated for 30 min with donkey α-mouse IgG H&L pre-absorbed Alexa Fluor® 488 (Abcam) in 1:1000 dilution. Cells were resuspended in 100 μl of PBS, and 4% paraformaldehyde was added to a final volume of 300 μl. Fluorescence intensity was determined with a BD FACScan. We analysed 10 000 events and the data analysis was performed with FlowJo 10.

5.14 | Ethic statements

Keratinocyte isolation from human foreskin was approved by the ethics committee of the medical faculty of the University Tübingen (654/2014BO2) and performed according to the principles of the Declaration of Helsinki.

5.15 | Statistical analysis

Student’s t tests or one-way analysis of variance (ANOVA) were employed whenever appropriate to compare the difference of means. Statistical analysis was performed by GraphPad Prism. The significance level was set as follows: p value > 0.05 was considered not significant (ns). In figures, significant differences are depicted as follows: *p < 0.05; **p < 0.01; ***p < 0.001; and ****p < 0.0001.

ACKNOWLEDGEMENT

We would like thank to Mulugueta Nega for provide the Lpl1 non-related peptide. This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG: SFB766 and TR156). PMT is supported by a postdoctoral Alexander von Humboldt fellowship and by CONICET, Argentina. SHF is supported by a PhD fellowship from the German Academic Exchange Service (DAAD) and by the Graduate College GRK1708 (DFG). The funders had no role in study design, data collection and analysis or decision to publish.

COMPETING INTERESTS

The authors declare no competing financial, personal, or professional interests.

ORCID

Paula M. Tribelli 🌐 https://orcid.org/0000-0001-9558-6275

Friedrich Götz 🌐 https://orcid.org/0000-0002-2042-0518

REFERENCES

Albrecht, T., Raue, S., Rosenstein, R., Nieselt, K., & Götz, F. (2012). Phylogeny of the staphylococcal major autolysin and its use in genus and species typing. Journal of Bacteriology, 194(10), 2630–2636. https://doi.org/10.1128/JB.06609-11

Askarian, F., Ajayi, C., Hanssen, A. M., van Sorge, N. M., Pettersen, I., Diep, D. B., ... Johannessen, M. (2016). The interaction between
Staphylococcus aureus SdrD and desmosigel 1 is important for adhesion to host cells. Scientific Reports, 6, 22134. https://doi.org/10.1038/srep22134

Bayles, K. W., Wesson, C. A., Liou, L. E., Fox, L. K., Bohach, G. A., & Trumble, W. R. (1998). Intracellular Staphylococcus aureus escapes the endosome and induces apoptosis in epithelial cells. Infection and Immunity, 66(1), 336–342.

Blättner, S., Das, S., Paprotka, K., Eilers, U., Kirschke, M., Kretschmer, D., ... Fraunholz, M. J. (2016). Staphylococcus aureus exploits a non-ribosomal cyclic dipetide to modulate survival within epithelial cells and phagocytes. PLoS Pathogens, 12(9), e1005857. https://doi.org/10.1371/journal.ppat.1005857

Bozza, G., Capitani, M., Montanari, P., Benucci, B., Biancucci, M., Nardi, V., ... Merola, M. (2014). Role of ARF6, Rab11 and external Hsp90 in the trafficking and recycling of recombinant-soluble Neisseria meningitidis adhesin A (rNadA) in human epithelial cells. PLoS ONE, 9(10), e110047. https://doi.org/10.1371/journal.pone.0110047

Carrel, M., Perencevich, E. N., & David, M. Z. (2015). USA300 Methicillin-resistant Staphylococcus aureus, United States, 2000-2013. Emerging Infectious Diseases, 21(11), 1973–1980. https://doi.org/10.3202/eid2111.150452

Cheng, C. F., Fan, J., Fedesco, M., Guan, S., Li, Y., Bandyopadhyay, B., ... Li, W. (2008). Transforming growth factor alpha (TGFalpha)-stimulated secretion of HSP90alpha: using the receptor LRP-1/CD91 to promote human skin cell migration against a TGFbeta-rich environment during wound healing. Molecular and Cellular Biology, 28(10), 3344–3358. https://doi.org/10.1128/MCB.01287–07

Claes, J., Liesenberghs, L., Peertmans, M., Veloso, T. R., Missiakas, D., Schneewind, O., ... Vanasse, T. (2017). Clumping factor A, von Willebrand factor-binding protein and von Willebrand factor anchor Staphylococcus aureus to the vesSEL wall. Journal of Thrombosis and Haemostasis, 15(5), 1099–1109. https://doi.org/10.1111/jth.13653

Clement, S., Vaudaux, P., Francois, P., Schrenzel, J., Hugger, E., Kampf, S., ... Lacroix, J. S. (2005). Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent Staphylococcus aureus rhinosinusitis. The Journal of Infectious Diseases, 192(6), 1023–1028. https://doi.org/10.1086/432735

Colonel, P. M., Winchell, C. G., & Voth, D. E. (2016). Hijacking host cell highways: manipulation of the host actin cytoskeleton by obligate intracellular bacterial pathogens. Frontiers in Cellular and Infection Microbiology, 6, 107. https://doi.org/10.3389/fcimb.2016.00107

Corrigan, R. M., Majilovic, H., & Foster, T. J. (2009). Surface proteins that promote adherence of Staphylococcus aureus to human desquamated nasal epithelial cells. BMC Microbiology, 9, 22. https://doi.org/10.1186/1471-2180-9-22

Didelot, C., Lanneau, D., Brunet, M., Bouchot, A., Cartier, J., Jacquel, A., ... Garrido, C. (2008). Interaction of heat-shock protein 90 beta isoform (HSP90 beta) with cellular inhibitor of apoptosis 1 (c-IAP1) is required for cell differentiation. Cell Death and Differentiation, 15(5), 859–866. https://doi.org/10.1038/cdd.2008.5

Diep, B. A., Gill, S. R., Chang, R. F., Phan, T. H., Chen, J. H., Davidson, M. G., ... Perdreau-Remington, F. (2006). Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant Staphylococcus aureus. Lancet, 367(9512), 731–739. https://doi.org/10.1016/S0140-6736(06)68231-7

Dziewanowska, K., Carson, A. R., Patti, J. M., Deobald, C. F., Bayles, K. W., & Bohach, G. A. (2000). Staphylococcal fibronectin binding protein interacts with heat shock protein 60 and integrins: role in internalisation by epithelial cells. Infection and Immunity, 68(11), 6321–6328. https://doi.org/10.1128/IAI.68.11.6321-6328.2000

Esen, M., Schreiner, B., Jendrossek, V., Lang, F., Fassbender, K., Grassme, H., & Gultbins, E. (2001). Mechanisms of Staphylococcus aureus induced apoptosis of human endothelial cells. Apoptosis, 6(6), 431–439. https://doi.org/10.1023/A:1012445925628

Eustace, B. K., Sakurai, T., Stewart, J. K., Yimlamai, D., Unger, C., Zehetmeier, C., ... Jay, D. G. (2004). Functional proteomic screens reveal an essential extracellular role for hsp90 alpha in cancer cell invasiveness. Nature Cell Biology, 6(6), 507–514. https://doi.org/10.1038/ncb1131

Evans, S. S., Repasky, E. A., & Fisher, D. T. (2015). Fever and the thermal regulation of immunity: The immune system feels the heat. Nature Reviews. Immunology, 15(6), 335–349. https://doi.org/10.1038/nri3843

Fowler, T., Wann, E. R., Joh, D., Johansson, S., Foster, T. J., & Hook, M. (2000). Cellular invasion by Staphylococcus aureus involves a fibronectin bridge between the bacterial fibronectin-binding MSCRAMMs and host cell beta1 integrins. European Journal of Cell Biology, 79(10), 672–679. https://doi.org/10.1078/0171-9335-00104

Garzoni, C., & Kelley, W. L. (2009). Staphylococcus aureus: new evidence for intracellular persistence. Trends in Microbiology, 17(2), 59–65. https://doi.org/10.1016/j.tim.2008.11.005

Gorska, M., Popowska, U., Sielicka-Dudzin, A., Kuban-Jankowska, A., Sawczuk, W., Knap, N., ... Wozniak, F. (2012). Geldanamycin and its derivatives as Hsp90 inhibitors. Frontiers in Biosci (Landmark Ed), 17, 2269–2277. https://doi.org/10.2741/4050

Gresham, H. D., Lowrance, J. H., Caver, T. E., Wilson, B. S., Cheung, A. L., & Lindberg, F. P. (2000). Survival of Staphylococcus aureus inside neutrophils contributes to infection. Journal of Immunology, 164(7), 3713–3722. https://doi.org/10.4049/jimmunol.164.7.3713

Grosz, M., Kolter, J., Paprotka, K., Winkler, A. C., Schafer, D., Chatterjee, S. S., ... Fraunholz, M. (2014). Cytoplasmic replication of Staphylococcus aureus upon phagosomal escape triggered by phenol-soluble modulin alpha. Cellular Microbiology, 16(4), 451–465. https://doi.org/10.1111/cmi.12233

Grundmeier, M., Hussain, M., Becker, P., Heilmann, C., Peters, G., & Sinha, B. (2004). Truncation of fibronectin-binding proteins in Staphylococcus aureus strain Newman leads to deficient adherence and host cell invasion due to loss of the cell wall anchor function. Infection and Immunity, 72(12), 7155–7163. https://doi.org/10.1128/IAI.72.12.7155-7163.2004

Hirschhausen, N., Schlesier, T., Schmidt, M. A., Götz, F., Peters, G., & Heilmann, C. (2010). A novel staphylococcal internalization mechanism involves the major autoysin Atl and heat shock cognate protein Hsc70 as host cell receptor. Cellular Microbiology, 12(12), 1746–1764. https://doi.org/10.1111/j.1462-2503.2010.01506.x

Josse, J., Laurent, F., & Diot, A. (2017). Staphylococcal adhesion and host cell invasion: Fibronectin-binding and other mechanisms. Frontiers in Microbiology, 8, 2433. https://doi.org/10.3389/fmicb.2017.02433

Kalinka, J., Hachmeister, M., Geraci, J., Sordelli, D., Hansen, U., Niemann, S., ... Tuchscherer, L. (2014). Staphylococcus aureus isolates from chronic osteomyelitis are characterized by high host cell invasion and intracellular adaptation, but still induce inflammation. International Journal of Medical Microbiology, 304(8), 1038–1049. https://doi.org/10.1016/j.ijmm.2014.07.013

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., & Sternberg, M. J. (2015). The Phyre2 web portal for protein modeling, prediction and
Sinha, B., Francois, P. P., Nusse, O., Foti, M., Hartford, O. M., Vaudaux, P., ... Krause, K. H. (1999). Fibronectin-binding protein acts as Staphylococcus aureus invasin via fibronectin bridging to integrin alpha5beta1. *Cellular Microbiology*, 1(2), 101–117. https://doi.org/10.1046/j.1462-5822.1999.00011.x

Snigireva, A. V., Vrublevskaya, V. V., Afanasyev, V. N., & Morenkov, O. S. (2015). Cell surface heparan sulfate proteoglycans are involved in the binding of Hsp90alpha and Hsp90beta to the cell plasma membrane. *Cell Adhesion & Migration*, 9(6), 460–468. https://doi.org/10.1080/19336918.2015.1103421

Snigireva, A. V., Vrublevskaya, V. V., Skarga, Y. Y., & Morenkov, O. S. (2016). The role of membrane-bound heat shock proteins Hsp90 in migration of tumor cells in vitro and involvement of cell surface heparan sulfate proteoglycans in protein binding to plasma membrane. *Biofizika*, 61(2), 328–336.

Suzuki, S., & Kulkami, A. B. (2010). Extracellular heat shock protein HSP90beta secreted by MG63 osteosarcoma cells induces activation of latent TGF-beta1. *Biochemical and Biophysical Research Communications*, 398(3), 525–531. https://doi.org/10.1016/j.bbrc.2010.06.112

Taiyab, A., & Rao Ch, M. (2011). HSP90 modulates actin dynamics: Inhibition of HSP90 leads to decreased cell motility and impairs invasion. *Biochimica et Biophysica Acta*, 1813(1), 213–221. https://doi.org/10.1016/j.bbbaproc.2010.09.012

Tickler, I. A., Goering, R. V., Mediavilla, J. R., Kreiswirth, B. N., Tenover, F. C., & Consortium, H. A. I (2017). Continued expansion of USA300 Staphylococcus aureus resistant MRSA among hospitalized patients in the United States. *Diagnostic Microbiology and Infectious Disease*, 88(4), 342–347. https://doi.org/10.1016/j.diagmicrobio.2017.04.016

Tran Van Nhieu, G., & Isberg, R. R. (1993). Bacterial internalization mediated by beta 1 chain integrins is determined by ligand affinity and receptor density. *The EMBO Journal*, 12(5), 1887–1895. https://doi.org/10.1002/j.1460-2075.1993.tb05837.x

Triantafilou, K., Triantafilou, M., & Dedrick, R. L. (2001). A CD14-independent LPS receptor cluster. *Nature Immunology*, 2(4), 338–345. https://doi.org/10.1038/86342

Tsutsumi, S., & Neckers, L. (2007). Extracellular heat shock protein 90: A role for a molecular chaperone in cell motility and cancer metastasis. *Cancer Science*, 98(10), 1536–1539. https://doi.org/10.1111/j.1349-7006.2007.00661.x

Voyich, J. M., Braughton, K. R., Sturdevant, D. E., Whitney, A. R., Said-Salim, B., Porcella, S. F., ... DeLeo, F. R. (2005). Insights into mechanisms used by Staphylococcus aureus to avoid destruction by human neutrophils. *Journal of Immunology*, 175(4), 3907–3919. https://doi.org/10.4049/jimmunol.175.6.3907

Weidle, U. H., Maisel, D., Klostermann, S., Schiller, C., & Weiss, E. H. (2011). Intracellular proteins displayed on the surface of tumor cells as targets for therapeutic intervention with antibody-related agents. *Cancer Genomics Proteomics*, 8(2), 49–63.

Wesson, C. A., Liou, L. E., Todd, K. M., Bohach, G. A., Trumble, W. R., & Bayles, K. W. (1998). *Staphylococcus aureus* Agr and Sar global regulators influence internalization and induction of apoptosis. *Infection and Immunity*, 66(11), 5238–5243.

Yang, Y. H., Jiang, Y. L., Zhang, J., Wang, L., Bai, X. H., Zhang, S. J., ... Zhou, C. Z. (2014). Structural insights into SraP-mediated *Staphylococcus aureus* adhesion to host cells. *PLoS Pathogens*, 10(6), e1004169. https://doi.org/10.1371/journal.ppat.1004169

Zapotoczna, M., Jevnikar, Z., Mijailovic, H., Kos, J., & Foster, T. J. (2013). Iron-regulated surface determinant B (IsdB) promotes *Staphylococcus aureus* adherence to and internalization by non-phagocytic human cells. *Cellular Microbiology*, 15(6), 1026–1041. https://doi.org/10.1111/cmi.12097

Zimmermann, M., & Fischbach, M. A. (2010). A family of pyrazinone natural products from a conserved nonribosomal peptide synthetase in *Staphylococcus aureus*. *Chemistry & Biology*, 17(9), 925–930. https://doi.org/10.1016/j.chembiol.2010.08.006

Zuehlke, A. D., Beeke, K., Neckers, L., & Prince, T. (2015). Regulation and function of the human HSP90AA1 gene. *Gene*, 570(1), 8–16. https://doi.org/10.1016/j.gene.2015.06.018

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Tribelli PM, Luqman A, Nguyen M-T, et al. *Staphylococcus aureus* Lpl protein triggers human host cell invasion via activation of Hsp90 receptor. *Cellular Microbiology*. 2020;22:e13111. https://doi.org/10.1111/cmi.13111