Genetic variation of staphylococcal LukAB toxin determines receptor tropism

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Staphylococcus aureus has evolved into diverse lineages, known as clonal complexes (CCs), which exhibit differences in the coding sequences of core virulence factors. Whether these alterations affect functionality is poorly understood. Here, we studied the highly polymorphic pore-forming toxin LukAB. We discovered that the LukAB toxin variants produced by S. aureus CC30 and CC45 kill human phagocytes regardless of whether CD11b, the previously established LukAB receptor, is present, and instead target the human hydrogen voltage-gated channel 1 (HVCN1). Biochemical studies identified the domain within human HVCN1 that drives LukAB species specificity, enabling the generation of humanized HVCN1 mice with enhanced susceptibility to CC30 LukAB and to bloodstream infection caused by CC30 S. aureus strains. Together, this work advances our understanding of an important S. aureus toxin and underscores the importance of considering genetic variation in characterizing virulence factors and understanding the tug of war between pathogens and the host.

The Gram-positive bacterium Staphylococcus aureus is responsible for a broad range of invasive diseases. To escape clearance by the immune system, S. aureus employs a wide range of strategies, including the production of potent bicomponent pore-forming toxins known as leukocidins. The leukocidins bind to specific cellular receptors to assemble into oligomeric pores that lead to cell lysis. Among these toxins, LukAB (also known as LukGH4) is the most recently identified leukocidin and is the dominant toxin responsible for S. aureus-mediated phagocyte lysis in ex vivo infection models. The tropism of LukAB towards human phagocytes is mediated by its binding to the integrin component CD11b (encoded by the ITGAM gene). Although the lukAB locus is part of the core S. aureus genome, there is notable nucleotide and amino acid diversity at this locus, up to 18% amino acid diversity in the LukAB toxin compared with up to 5% in other leukocidins. The majority of prior research on LukAB, however, has focused on the prototype toxin produced by the community-acquired methicillin-resistant S. aureus (MRSA) clone USA300, which is a member of clonal complex 8 (CC8)4,8,10–18. In the United States, isolates of the CC8 lineage cause a large proportion of both community-associated and healthcare-associated MRSA infections4,8,10,20. However, other major lineages, such as CC1, CC5, CC15, CC22, CC30 and CC45, contribute substantially to infections both in the United States and globally21.

Here, we set out to characterize the activity of LukAB variants. Phylogenetic analyses indicated that the polymorphisms in lukAB loci are largely CC-specific. Representative LukAB variants from the most diverse lineages exhibited comparable cytotoxic activities against primary human phagocytes. Nevertheless, two closely related variants produced by S. aureus belonging to the CC30 and CC45 lineages killed cells lacking CD11b, the previously established LukAB receptor. By performing a genome-wide CRISPR–Cas9-based screen, we identified hydrogen voltage-gated channel 1 (HVCN1) as a critical cellular receptor for cell killing by CC30 and CC45 LukAB. As with CD11b8,18, LukAB killed mammalian cells containing human but not murine HVCN1. Mapping the LukAB-targeted domain within human HVCN1 enabled the generation of a humanized HVCN1 mouse model (hHVCN1). Phagocytes from hHVCN1 mice were susceptible to CC30 LukAB-mediated cytotoxicity. Compared with wild-type mice, hHVCN1 mice were more susceptible to bacterial burdens following bloodstream infection with both community- and hospital-acquired CC30 S. aureus isolates. Taken together, these findings highlight how lineage-specific changes in the sequence of virulence factors influence the pathogenic potential of bacteria and identify HVCN1 as a human protein targeted by S. aureus.

Results

Sequences of lukA and lukB cluster according to S. aureus CCs. The lukAB locus is a part of the core genome of S. aureus, but it exhibits high allelic variability5,9. To define the extent of lukAB diversity across S. aureus, we analysed the lukA and lukB
sequences from 4,187 publicly available S. aureus genomes as well as the closely related species Staphylococcus argenteus (previously known as CC75) and Staphylococcus schweitzeri. Identical nucleotide sequences were collapsed into a single representative sequence, thereby reducing the number of unique lukA and lukB alleles to 94 and 83, respectively (Supplementary Table 1). Phylogenetic analyses revealed several distinct groups of toxin variants that reflect the overall phylogenetic structure of the Staphylococcus species22 (Fig. 1a,b). Major branches corresponded to the S. aureus CCs CC1/5/8/97, CC10/395, CC398, CC30/45, as well as to S. argenteus (CC75) and S. schweitzeri. Notably, toxins were highly similar within each group, but exhibited long branch lengths between groups (Fig. 1a,b). However, comparisons between groups showed strong levels of purifying selection, which suggests that these

Fig. 1 | CC-specific LukAB variants exhibit different cytotoxic activities towards human leukocytes. a,b, Unrooted maximum likelihood phylogeny for lukA (a) and lukB (b). Branch lengths are in substitutions/site. Key bootstrap values are depicted in green (>90%) or white (>70%) on branches. Major clades are coloured blue (CC1/5/8/97), red (CC30/45), orange (CC10/395), green (CC398), yellow (S. schweitzeri, FSA) and grey (S. argenteus, CC75). c, Recombinant co-purified 6×His-LukA and LukB proteins visualized by total protein staining (1 μg) and immunoblotting (100 ng) with anti-His antibody. One replicate of this experiment was performed. d–g, Intoxication of primary human PMN cells (d), monocytes (e), macrophages (f) and dendritic cells (g) with indicated concentrations of LukAB toxins and cell viability measured using Cell Titer. Data were collected using cells isolated from six independent donors (PMN cells), four donors (monocytes), five donors (monocyte-derived macrophages) and four donors (monocyte-derived dendritic cells), and are represented as the mean ± s.e.m. h, EC\textsubscript{50} values of each toxin calculated from data presented in d–g. See also Extended Data Figs. 1 and 2 and Supplementary Tables 1 and 2.
LukAB variants kill primary human phagocytes. To evaluate the functional significance of the variations observed in LukAB, we cloned and purified LukAB from the following eight CCs that encode the major lukAB alleles: CC1, CC5, CC8, CC30, CC45, S. argenteus (henceforth referred to as CC75), CC398 and S. schweitzeri (strain FSA-084, henceforth referred to as FSA) (Fig. 1c, Extended Data Fig. 2 and Supplementary Table 2). We tested the cytotoxic activity of the LukAB variants towards primary human neutrophils (polymorphonuclear (PMN) cells), monocytes, monocyte-derived macrophages and monocyte-derived dendritic cells. We found that all tested LukAB variants lysed human phagocytes in a dose-dependent manner (Fig. 1d–h). However, while cytotoxicity towards dendritic cells was similar across CCs, we observed much higher variability in toxin activity towards PMN and monocytes (Fig. 1d–h).

Unlike other variants, CC30 and CC45 LukAB are cytotoxic in the absence of CD11b. We sought to determine whether non-CC8 LukAB variants also rely on CD11b for cytotoxicity by evaluating their activity towards CD11b-depleted human monocytic THP1 cells and control cells (Fig. 2a). In cells transduced with scramble short hairpin RNA (shRNA), the LukAB variants exhibited variable but overall comparable cytotoxicity (Fig. 2b). Intoxication with CC30 and CC45 LukAB, two closely related LukAB variants that differ by only four amino acids, resulted in 100% cell death at toxin concentrations as low as 2.5 μg ml⁻¹ (Fig. 2b). As expected, depletion of CD11b by shRNA (Fig. 2a) abolished CC8 LukAB-mediated cytotoxicity. Depletion of CD11b also protected cells from CC1, CC5, CC75, CC398 and FSA LukAB variants (Fig. 2c). Unexpectedly, the CC30 and CC45 LukAB variants killed THP1 cells depleted of CD11b (Fig. 2c). Potencies of both toxins were, however, slightly reduced, as indicated by the increased half-maximum lytic concentration (EC₅₀) values: from 0.1368 μg ml⁻¹ and 0.1835 μg ml⁻¹ in control cells to 0.5425 μg ml⁻¹ and 0.6604 μg ml⁻¹ in CD11b-depleted
To ensure that the findings described above were not specific to THP1 cells, we also used a human promyelocytic leukaemia cell line (HL60)8 differentiated into neutrophil-like cells (PMN-HL60). As with the THP1 cells, CC30 and CC45 LukAB variants killed CD11b-depleted PMN-HL60 cells (Fig. 2d). Furthermore, CD18 (encoded by the ITGB2 gene), which enables surface localization of all β2 integrins24,25, including CD11a, CD11b, CD11c and CD11d, was also dispensable for the cytotoxicity of CC30 and CC45, but not CC8 LukAB (Fig. 2d).

Consistent with the observed reduced dependence on CD11b for cytotoxicity, binding studies using enzyme-linked immunosorbent assays (ELISAs) demonstrated that CC30 LukAB bound more weakly to the CD11b I domain than CC8 LukAB (Fig. 2e). Furthermore, biolayer interferometry revealed that CC30 LukAB...
Fig. 4 | CC30 S. aureus kills leukocytes in a LukAB- and HVCN1-dependent manner. a,b, PCR targeting lukA and hlgA (a) and immunoblot of CC30 LukAB in supernatants of wild-type and ΔlukAB CC30 S. aureus 62300D1 (b). Asterisks indicate nonspecific bands that serve as loading controls. One replicate of this experiment was performed (a). A representative image of two independent experiments is shown (b). c, Viability of human PMN cells following a 2-h infection with nonopsonized wild-type (WT) or isogenic ΔlukAB CC30 S. aureus 62300D1 at the indicated m.o.i. PMN cell lysis was measured by lactate dehydrogenase (LDH) release. Data are from PMN cells isolated from six independent donors represented as the mean ± s.d. Statistical significance was determined by one-way ANOVA; ****P < 0.0001, #P = 0.0119. d,e, Viability of ITGAM shRNA THP1 cells transduced with LentiCRISPRv2 expressing non-targeting sgRNA or HVCN1 sgRNA and infected with nonopsonized (extracellular infection; d) or opsonized (intracellular conditions; e) WT and ΔlukAB CC30 S. aureus 62300D1 for 2 h (m.o.i. = 100). THP1 cell lysis was measured by LDH release. Data from three independent experiments are represented as the mean ± s.d. Statistical significance was determined by t-test (two-tailed); numbers indicate P values.

exhibits lower binding affinity to the CD11b I domain, with an apparent dissociation constant (K_d) of 214 ± 3.28 nM compared with 9.41 ± 0.05 nM for CC8 LukAB (Extended Data Fig. 3). Together, these results indicate that unlike the other CCs, CC30 and CC45 LukAB variants do not require CD11b to kill human phagocytes.

Identification of HVCN1 as the cellular target for the CC30 and CC45 LukAB toxins. To identify the cell surface target of CC30 and CC45 LukAB variants, we employed a genome-wide CRISPR–Cas9 approach26. We introduced two independent genome-wide subpools of single-guide RNAs (sgRNAs) together with Cas9 into CD11b-depleted THP1 cells and subjected the cells to two sequential rounds of CC30 LukAB intoxication. The sgRNAs from the surviving cells were then sequenced, and the frequencies of sgRNAs in the surviving cell population were compared with untreated cells (Fig. 3a). Among the recovered sgRNAs that target genes encoding cell surface proteins, sgRNAs for HVCN1, OGT and ASGR1 were the most highly enriched in the toxin-resistant cells (Fig. 3b and Supplementary Table 3). Studies using individual knockout cell lines revealed that the cytotoxicity of CC30 LukAB was fully dependent on HVCN1, while it remained unaffected or only minimally affected by ASGR1 or OGT depletion (Fig. 3c). HVCN1 encodes hydrogen voltage-gated channel 1, which is primarily found on leukocytes and its major function is to balance charges across the membrane to ensure full NADPH oxidase activity and reactive oxygen species production27–30. CRISPR–Cas9-mediated HVCN1 targeting (Fig. 3d,e) ablated the cytotoxicity of HlgAB, another bicomponent pore-forming toxin that targets CCR2, CXCR1 and CXCR2 (ref. 31), remained unaffected (Fig. 3f). These data demonstrate that HVCN1 is required for CC30 and CC45 LukAB-mediated cell killing.

CC30 S. aureus targets human leukocytes in a LukAB- and HVCN1-dependent manner. To define the role of HVCN1-mediated LukAB cytotoxicity in the virulence of CC30 S. aureus, we generated an isogenic lukAB deletion strain in a community-acquired CC30 S. aureus isolate from our collection (strain 62300D1) (Fig. 4a,b). Potent killing of primary human PMN cells was observed with wild-type CC30 S. aureus, a phenotype that was significantly reduced when lukAB was deleted (Fig. 4c). Similar to what we observed with purified toxins, CC30 S. aureus also killed CD11b-depleted THP1 cells in a LukAB-dependent manner (Fig. 4d). In contrast, cytotoxicity of the wild-type CC30 S. aureus strain in HVCN1-deficient cells was significantly impaired and was indistinguishable from the cytotoxicity observed with the ΔlukAB strain (Fig. 4d).
LukAB mediates cell lysis not only by targeting the extracellular membrane but also when produced by phagocytosed bacteria. Since HVCN1 is transported within phagosomes, we next evaluated whether LukAB produced by S. aureus after phagocytosis targeted HVCN1 for cytotoxicity. CC30 S. aureus cells were opsonized with normal human serum, incubated with THP1 cells to promote bacterial uptake and the viability of the THP1 cells was evaluated. We observed that HVCN1-positive cells were killed by phagocytosed S. aureus in a LukAB- and HVCN1-dependent manner (Fig. 4c). Therefore, CC30 S. aureus targets HVCN1 via LukAB to lyse phagocytes.

HVCN1 sensitizes mammalian cells to CC30 and CC45 LukAB cytotoxicity. To determine whether HVCN1 is sufficient to render mammalian cells susceptible to LukAB, HVCN1 was expressed in Chinese hamster ovary (CHO) cells, which lack endogenous CD11b and HVCN1. As shown in Fig. 5a, control cells expressing firefly luciferase (Fluc) were not affected by CC30 or CC45 LukAB, whereas HVCN1 sensitized CHO cells to both toxins. We hypothesized that HVCN1 facilitates the interaction of LukAB with the plasma membrane of the target cells. Indeed, while only a minimal interaction between CC30 and CC45 LukAB and control cells was observed, a dose-dependent interaction of the toxins with HVCN1-expressing cells was detected (Fig. 5b). Moreover, unlabelled LukAB competed off binding of biotinylated toxin in a dose-dependent manner (Fig. 5c). To further these studies, a pull-down assay was performed to evaluate the direct interaction between LukAB and HVCN1. Analysis of the elution of resin-immobilized HVCN1 revealed that HVCN1 interacts with LukAB, but not LukSF (another bicomponent pore-forming leukocidin used as a negative control) (Fig. 5d). The absence of LukAB in the elution fraction of the empty resin control (TBS buffer) further confirmed that co-elution of LukAB with HVCN1 is not due to a nonspecific LukAB–resin interaction (Fig. 5d). Together, these data support the notion that HVCN1 is a receptor for LukAB.

In addition to phagocytes, HVCN1 is highly expressed in B cells but minimally expressed in T cells (Extended Data Fig. 4). This observation is consistent with the reported higher H+ currents in B cells compared to T cells. To evaluate whether HVCN1 renders lymphocytes susceptible to LukAB, we purified primary B cells and CD4-positive and CD8-positive T cells from human peripheral blood mononuclear cells (PBMCs) and exposed the cells to the toxins. In contrast to the CD4 and CD8 T lymphocytes, which were resistant to both CC30 and CC45 LukAB toxins, human B lymphocytes were highly sensitive to LukAB-mediated membrane damage (Fig. 5e). Therefore, HVCN1 broadens the repertoire of leukocytes that could be targeted by LukAB during infection.

CC30 and CC45 LukAB target human but not murine HVCN1. CC8 LukAB binds with high affinity to the human, but not murine, CD11b I domain. Treatment of murine peritoneal exudate cells with CC8 LukAB binds with high affinity to the human, but not murine, CD11b I domain. Treatment of murine peritoneal exudate cells with CC8 LukAB revealed that murine leukocytes are resistant to LukAB regardless of the toxin lineage (Fig. 6a). In contrast, when the same cells were exposed to LukED, a bicomponent leukocidin that targets both human and murine leukocytes, extensive cell death was observed (Fig. 6a). Since CC30 and CC45 LukAB are closely related and exhibited identical phenotypes with regard to HVCN1, we chose to focus on CC30 LukAB for the rest of the study.

Human HVCN1 and its murine homologue (herein referred to as mHVCN1, also known as mVSOP) share 79.2% amino acid identity. To test whether the observed species specificity of LukAB could be explained by the interspecies differences in HVCN1, CHO cells expressing human HVCN1 or mHVCN1 were exposed to CC30 LukAB. While both HVCN1 proteins were produced, only the cells expressing human HVCN1 were susceptible to LukAB (Fig. 6b).

HVCN1 is composed of 4 transmembrane segments with 2 extracellular loops (one that is 17-amino-acids long and the other 7-amino-acids long) (Fig. 6c). While human HVCN1 and mHVCN1 share close to 80% overall sequence identity, the surface-exposed regions are less than 60% identical between species (Fig. 6c). To test whether these differences affect LukAB targeting, we introduced each of the human extracellular loops into mHVCN1 and produced these chimeric proteins in 293T cells. The chimeric proteins were translationally fused to green fluorescent protein (GFP) via a carboxy-terminal linker to ensure comparable protein levels, and LukAB-mediated cell death was assessed by flow cytometry. These experiments revealed that replacing the first, but not the second, extracellular loop of mHVCN1 with its human orthologue rendered the receptor compatible with CC30 LukAB-mediated killing (Fig. 6d). Collectively, these data demonstrate that CC30 LukAB preferentially targets human HVCN1 and primarily via the first extracellular loop.

Humanizing HVCN1 renders murine leukocytes susceptible to CC30 LukAB. Based on the cytotoxicity data observed in 293T cells (Fig. 6d), we hypothesized that humanizing the first extracellular loop of mHVCN1 in the mouse genome would result in a similar increase in susceptibility to the toxin. Using CRISPR-Cas9 gene editing, we modified murine exon 4 to introduce the corresponding residues of human HVCN1 extracellular loop one (Extended Data Fig. 5). The humanized HVCN1 mice (hHVCN1) were generated in the C57BL/6j background and appeared to be normal in appearance, viability and breeding ability. To evaluate the susceptibility of hHVCN1 mice towards LukAB, we first exposed PECs from wild-type and hHVCN1 mice to CC8 and CC30 LukAB. PECs from wild-type mice were highly resistant to both toxins. In contrast, cells from the hHVCN1 mice were susceptible to CC30 LukAB-mediated membrane damage, while remaining resistant to CC8 LukAB (Fig. 6e). Therefore, facilitating toxin–receptor interaction by humanizing extracellular loop one of mHVCN1 allows for CC30 LukAB-mediated cytotoxicity.

hHVCN1 mice are more susceptible to CC30 S. aureus bloodstream infection. To rule out any unappreciated immunological alterations in the hHVCN1 mice, we infected wild-type and
hHVCN1 mice with $1 \times 10^7$ colony forming units (c.f.u.) of the lukAB-deficient strain LAC$^{13}$ and evaluated bacterial burdens 3 days post-infection. No significant differences in bacterial burdens in the kidneys, liver, heart, spleen or lungs were observed between the wild-type mice and our gene-targeted animals (Extended Data Fig. 5).

Next, wild-type and hHVCN1 mice were intravenously infected with $5-10 \times 10^7$ c.f.u. of CC30 S. aureus, and bacterial burdens in organs were examined 14 days post-infection. To eliminate potential strain-specific effects, we tested two CC30 clinical isolates: a methicillin-sensitive hospital-acquired bloodstream infection isolate (MUI211) and a community-acquired skin infection isolate (62300D1)$^{40}$. Independent of the isolate, the hHVCN1 mice displayed increased bacterial burdens in the kidneys compared with wild-type mice (Fig. 6f). No significant differences were observed in the other tissues examined (Extended Data Fig. 5). Thus, humanizing mHVCN1 increases the overall susceptibility of mice to renal infections with CC30 S. aureus.
Fig. 6 | LukAB targeting of HVCN1 promotes *S. aureus* pathogenesis. **a**, Intoxication of murine PECs with the indicated concentrations of leukocidins. Membrane damage was detected using the fixable viability dye eFluor 450. Data are presented as the mean ± s.e.m. of three independent experiments. **b**, Inset: immunoblot of HVCN1 in CHO cells expressing Fluc, human HVCN1 (HVCN1) or murine Hvcn1 (mHVCN1). Anti-actin immunoblot is shown above as a loading control. Representative images of four independent samples from one immunoblot are shown (see the corresponding source data for the full gel). Numbers on the left indicate migration of the corresponding molecular weight standards (in kDa). Target protein levels normalized by actin were obtained using ImageJ from four independent protein samples: HVCN1 = 0.310 ± 0.111, mHVCN1 = 0.333 ± 0.066 (mean ± s.d.), *P* = 0.742 as determined by unpaired t-test. Main: CHO cells expressing Fluc, HVCN1 (HVCN1) or Hvcn1 (mHVCN1) were intoxicated with the indicated concentrations of CC30 LukAB. Cell viability was measured using Cell Titer. Data from three independent experiments are presented as the mean ± s.d. Statistical significance was determined by two-way ANOVA; **** *P* ≤ 0.0001. **c**, Schematic architecture of HVCN1 and the amino acid alignments of human and murine extracellular loops generated using Clustal Omega. **d**, Intoxication of Lenti-X 293T cells expressing C-terminal GFP-tagged human, murine and chimeric HVCN1 proteins with the indicated concentrations of CC30 LukAB. Membrane damage was detected using the fixable viability dye eFluor 450. Data from three independent experiments are presented as the mean ± s.d. Statistical significance was determined by two-way ANOVA; **** *P* ≤ 0.0001, ** *P* ≤ 0.01. **e**, Intoxication of PECs from wild-type and hHVCN1 mice with the indicated LukAB concentrations. Membrane damage was detected using propidium iodide (PI) incorporation. Data from five mice per genotype over three independent experiments are presented as the mean ± s.e.m. Statistical significance was determined by two-way ANOVA, and numbers indicate *P* values. **f**, The c.f.u. values in the kidneys of WT and homozygous hHVCN1 mice intravenously infected with MUZZ211 (c.f.u. obtained from 11 WT and 24 hHVCN1 mice) or 62300D1 (c.f.u. obtained from 11 WT and 10 hHVCN1 mice). Data for each isolate are from mice infected over three independent experiments and presented as the mean ± s.e.m. Statistical significance was determined by t-test (two-tailed), and numbers indicate *P* values. See also Extended Data Figs. 5 and 7.
Discussion

Most studies characterizing LukAB and its role in S. aureus immune evasion have focused on the toxin variant produced by CC8 strains, even though multiple other CCs exist, including 11 major ones. Here, we showed that CC30 and CC45 LukAB, which differ only by four amino acids, have diverged substantially from the other LukAB proteins. We established a critical role for HVNC1 in the lytic activity of CC30/45 LukAB variants and determined that the first extracellular loop of HVNC1 is responsible for the human tropism exhibited by these toxins. Finally, we generated a humanized HVNC1 mouse model to study CC30 LukAB, which revealed both increased susceptibility to CC30 LukAB cytotoxic activity in murine leukocytes ex vivo and decreased resistance to bloodstream infection with hospital- and community-acquired CC30 S. aureus in vivo.

Our bioinformatics and functional analyses of the LukAB-encoding alleles showed that while the overall toxin sequence and function are largely conserved, diverse clonal variants exist between CCs but not within them. Along with our selection analyses, this pattern of diversity suggests both strong purifying selection for these toxins and potential inter-CC functional differences such as what we have uncovered herein. CC30 alone accounted for 21% of the methicillin-sensitive S. aureus strains causing disease worldwide between 1961 and 2004 (ref. 42). Moreover, clinical studies have demonstrated that the CC30 lineage is associated with persistent bacteremia and increased risk for haematogenous complications (for example, endocarditis, septic arthritis and vertebral osteomyelitis)42,43. Interestingly, many CC30 isolates harbour attenuating mutations in the global virulence regulator Agr (agrC), a stop codon in the α-toxin-encoding gene (hla), and lack the potent LukED toxin43. These alterations are believed to reduce the cytotoxic potential of contemporary CC30 S. aureus44. It is tempting to speculate that the lack of α-toxin, LukED and reduced function of the Agr master regulatory system could have provided a selective pressure for a LukAB variant that compensates for these toxins.

CC8 LukAB is highly cytotoxic towards human leukocytes, but ~1,000-fold less potent towards murine immune cells45, a phenotype linked to its high binding affinity for human CD11b45. Identification of the human leukocidin receptors provides critical information that can be harnessed to generate transgenic mouse models whereby the human (or humanized) receptors are expressed in the appropriate leukocytes. Recently, the determinants of the human-specific CC8 LukAB–CD11b interaction were defined and used to generate a CD11b-humanized (hCD11b) mouse model45. Interestingly, studies using these mice uncovered that during the initial phase of bloodstream infection, 1–3 days post-infection, LukAB contributes to USA300 bacterial burden only in the liver4. Using the hHVNC1 mice described here, we instead observed increased bacterial burden in the kidneys 14 days post-infection. This disparate tissue tropism could be due to differences in the activity of the LukAB toxins in vivo (for example, CC8 LukAB versus CC30 LukAB) or to altered tropism by the S. aureus isolates (for example, the USA300 strain LAC versus the CC30 strains MUZ211 and 62300D1). Future studies utilizing congenic S. aureus strains are needed to clarify these possibilities. Ultimately, a combination of currently available humanized murine models (for example, hCD11b45, hC5aR1 (ref. 46) and hHVNC1) might be needed to fully define the roles of LukAB and the other leukocidins in vivo.

In conclusion, the findings presented here emphasize the importance of considering the sequence diversity of S. aureus while attempting to characterize the role and function of virulence factors. Moreover, these data highlight that S. aureus lineages associated with human infections have evolved strategies to specifically target the human host. A better understanding of lineage-specific toxin activity and the interaction of S. aureus with the human host will aid in the development of toxin inhibitors to prevent toxin-mediated immune depletion and combat infections caused by diverse S. aureus isolates.

Methods

Ethics statement. Human blood samples were obtained as buffy coats from healthy, anonymous, consenting adult donors (New York Blood Center).

All experiments involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of NYU Langone Health and were performed according to guidelines from the National Institutes of Health (NIH), the Animal Welfare Act, and US Federal Law.

Bacterial strains. The following Escherichia coli strains were used in cloning procedures: DH5α and Endura Competent Cells (Lucigen). E. coli IM308 was plated by J. T. Foster and used to passage plasmid DNA to enable direct transformation of CC30 S. aureus. E. coli T7 LysY/LacQ was used for Flag-tagged CD11b and domain expression. E. coli OverExpress C43(DE3) Chemically Competent Cells (Lucigen) were used for Strep-tagged HVNC1 expression. All E. coli strains were grown in Luria–Bertani broth.

Both CC30 and ST83 S. aureus strains used in this study are listed in the Supplementary Table 4. For recombinant protein expression, S. aureus cultures were prepared in tryptic soy broth (TSB). For PMN and THP1 cell infection studies, S. aureus strains were streaked to single colonies on tryptic soy agar (TSA) plates. Single colonies were inoculated in yeast-casamino acids (YC) broth for overnight culture and then subsequently subcultured 1:100 for 5 h in YC medium with 2% sodium pyruvate (YPC; Fisher Scientific) at 37 °C with shaking46. For in vivo infections, S. aureus strains were streaked to single colonies on TSA plates. Single colonies were inoculated in TSB broth for overnight culture and then subsequently subcultured 1:100 for 3 h in TSB at 37 °C with shaking.

Oligonucleotides. All oligonucleotides used in the study are listed in Supplementary Table 5.

Antibodies. The following antibodies were used: mouse anti-His tag (1:3,000; CSI20563B, Cell Sciences); rabbit anti-HVNC1 (1:1,200; OAPB0114, Aviva Systems Biology); rabbit anti-HVNC1 (1:1,000; PAS-24964, Invitrogen, Thermo Fisher Scientific); mouse anti-HVNC1 (1:1,000; H01D10, Cell Signaling Technology); mouse PE/Cy7 anti-human CD19 (1:100; 302215, BioLegend); mouse FITC anti-human CD3 (1:100; 300406, BioLegend); mouse Alexa Fluor 700 anti-human CD4 (1:100; 325614, BioLegend); mouse PE anti-human CD4 (1:100; 317410, BioLegend); mouse APC anti-human CD8α (1:50; 300912, BioLegend); mouse APC anti-human CD8α (1:100; 301310, BioLegend); mouse Alexa Fluor 700 anti-mouse/human CD11b (1:300; 101222, BioLegend). Additionally, the following isotype control antibodies were used: PE/Cy7 mouse IgG1, k isotype ctrl antibody (1:100; 400126, BioLegend); FITC mouse IgG1, k isotype ctrl antibody (1:100; 400108, BioLegend); Alexa Fluor 700 mouse IgG1, k isotype ctrl antibody (1:100; 400143, BioLegend); PE mouse IgG1, k isotype ctrl antibody (1:100; 400111, BioLegend); APC mouse IgG1, k isotype ctrl antibody (1:100; 400120, BioLegend). The following secondary antibodies were used for immunoblotting: goat anti-mouse IgG (H+L) cross-adsorbed secondary antibody, Alexa Fluor 680 (1:25,000; A-21057, Invitrogen); goat anti-rabbit IgG (H+L) cross-adsorbed secondary antibody, Alexa Fluor 680 (1:25,000; A-21057, Invitrogen). For FACS analysis, the following antibodies were used: PE/Cy7 mouse IgG1, k isotype ctrl antibody (1:100; 400111, BioLegend); APC mouse IgG1, k isotype ctrl antibody (1:100; 400120, BioLegend). The following secondary antibodies were used for immunoblotting: goat anti-mouse IgG (H+L), cross-adsorbed secondary antibody, Alexa Fluor 680 (1:25,000; A-21057, Invitrogen); goat anti-rabbit IgG (H+L), cross-adsorbed secondary antibody, Alexa Fluor 680 (1:25,000; A-21057, Invitrogen).

Anti-CC30 LukAB monoclonal antibody. The anti-LukAB monoclonal antibody was custom made at Envigo according to their approved standard operating procedures for mouse monoclonal hybridoma generation. Briefly, 5–100 μg of recombinant CC30 LukAB (rLukAB) was emulsified with Freund’s incomplete adjuvant and one to two additional boosts of rLukAB emulsified with Freund’s incomplete adjuvant and one to two additional boosts of rLukAB emulsified with Titermax adjuvant (~1 month apart). The immunized mouse was killed and splenocytes were removed and fused with NS01 myeloma cells to generate hybridomas. The monoclonal hybridoma cell lines were selected using ELISAs. For the ELISAs, Immulon 2HB flat-bottom microtitre plates (Thermo Scientific) were coated with 1 μg ml⁻¹ of antigen. The antigen-coated plates were blocked using 2% milk in saline containing 0.05% Tween-20 for 1 h at room temperature. A total of 50 μl of serially diluted purified antibodies (2 μg ml⁻¹, 0.02 μg ml⁻¹ and 0.002 μg ml⁻¹) or undiluted culture supernatants were added to the ELISA plate and incubated at room temperature for 1 h with rocking, followed by 3 washes of 100 μl per well PBS-T (1X PBS, 0.1% Tween-20, 0.01% NaN₃). Then, 50 μl of goat anti-mouse HRP (1:1,000; Bio-Rad) was added to each well and incubated at room temperature for 1 h with rocking, followed by 3 washes of 100 μl per well PBS-T. Next, 50 μl of 3,3’,5,5’-tetramethylbenzidine (TMB) solution (Thermo Scientific) was added to each well and developed for 3 min, followed by the addition of 50 μl of 1M H₂SO₄. The plates were read at an absorbance of 450 nm on an Envision MultiLabel Plate Reader (PerkinElmer). The anti-CC30 LukAB monoclonal antibody was used for immunoblotting at 1 μg ml⁻¹.

Bioinformatics analysis of LukAB diversity. lukA and lukB diversity screen. Nucleotide sequences of lukA and lukB were used to query a nucleotide database of 4,187 of genomes using blastn. All genomes except five (C0673.fa, C0.98.fa, ST2282a.fa, ST2283a.fa and ST2288a.fa) had a hit to both lukA and lukB sequences (blastn, e-value cut-off = 1×10⁻³). BLAST aligned portions were captured.
and all identical nucleotide sequences were collapsed to a single representative sequence for further analysis using cd-hit. This reduced luka to 94 and lukb to 83 representative sequences (Supplementary Table 1).

Phylogenetic analysis. Gene alignments were done in MAFFT 7.271 (using default settings) and optimized by manually aligning based on codons. Maximum likelihood phylogenies were constructed for luka and lukb with RAxML (v.8.2.4) using the general time-reversal substitution model 16 accounting for among-site rate heterogeneity using the I = 1 category and four rate categories (GTR+Gamma model) with maximum parsimony random-addition starting trees. Node support was evaluated with 100 nonparametric bootstrap pseudoreplicates.15

Selection analysis. Sequences with mid-sequence stop codons (n = 188) were excluded from analysis. Sequences not evenly divisible by three were further trimmed to form a complete codon set. Genes were translated and aligned at the amino acid level using the ClustalW algorithm with default settings as provided in MEGA7 (v.7.0.26)17, and this alignment was imposed on the nucleotide sequence. Whole-gene nonsynonymous (ds) and synonymous (ds) divergence were estimated for all pairs of sequences between the same pair of clades using the between-group method of SNAPGene (v11.0)18. A Jukes–Cantor correction was used with the Nei–Gojobori (1986) method for calculation of pairwise percent identity and divergence tables (Extended Data Fig. 2). Furthermore, MegAlign was used to generate phylogenetic trees and alignment method algorithm with default settings as provided in MegAlign and LukB (listed in Supplementary Table 2) were performed using the ClustalW server.19 Multiple protein alignments of mature (without signal peptide) LukA and LukB were predicted using SignalP 5.0 (https://www.cbs.dtu.dk/services/SignalP/; accessed 2 March 2018). Signal peptides and the location of cleavage sites in full-length LukA and LukB were predicted using SignalP 5.0 (https://www.cbs.dtu.dk/services/SignalP/; accessed 2 March 2018).

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Clade-defining sites. Clade-defining sites were determined using EBf1-aligned fasta_group_diffs.pl (https://github.com/chasewnelson/ebt; accessed 28 March 2018). Specifically, sites were identified at which two or more clades differed in their major (that is, consensus) nucleotides with frequencies of ≥75% in each clade, a criterion chosen to exclude singletons for two members and doubletons for clades with three members. A clade was not considered to have a defining nucleotide at a site if its major nucleotide was present at a frequency <75% or the site contained ≥2 defined (that is, non-gap, non-ambiguous) nucleotides. Among these sites, the nucleotide present in CCl1/5/8 was unique for a nonsynonymous nucleotide difference for 17 sites in luka and 8 sites in lukb. Sites undergoing episodic divergence and phylogeny at internal branches were sought using the MEME approach in HyPhy.20 MEME identified codon 37 in luka (P = 0.0020, likelihood ratio test). No sites were identified in lukb. Comparison of selected mature Luka and Lukb. Signal peptides and the location of cleavage sites in full-length Luka and Lukb were predicted using SignalP 5.0 server.21 Multiple protein alignments of mature (without signal peptide) Luka and Lukb (listed in Supplementary Table 2) were performed using the ClustalW alignment method algorithm with default settings as provided in MegAlign (DNASTar). Furthermore, MegAlign was used to generate phylogenetic trees and pairwise percent identity and divergence tables (Extended Data Fig. 2).

Engineering of mammalian cell lines. Tissue culture. THP1 cells (American Type Culture Collection (ATCC), TIB-202) and HL60 cells (ATCC, CCL-240) were maintained in RPMI 1640 medium (Cellgro) supplemented with 10% fetal bovine serum (FBS; 900-208, Gemini Bio-Products) and 1×/PS (100 U ml−1 penicillin and 1 mg ml−1 streptomycin) at 37 °C with 5% CO2. CHO-K1 cells were maintained in full medium supplemented with 2 mg ml−1 puromycin. LentiCRISPRv2-transduced THP1 cells (American Type Culture Collection (ATCC), CRL-11268) were maintained in DMEM medium with 1-glutamine, 4.5 g per litre glucose and sodium pyruvate (Cellgro) supplemented with 10% FBS and 1×/PS. CHO-K1 CHO-K1 cells were seeded in 24-well tissue-culture plates at a density of 25,000 cells per well and transduced the following day with 100 μl lentiviral pseudoparticles via spinoculation at 1,000 × g for 45 min in medium containing 3% FBS, 20 mg ml−1 HEPES and 4 μg ml−1 polybrene. After 6 h of spinoculation, pseudoparticle-containing medium was removed and replaced with full cell culture medium. To generate stable cell lines, cells were selected with 5 μg ml−1 puromycin for 7 days 48 h after transduction. HVCN1 and mHVCN1 protein expression levels were evaluated by western blotting. Cells were incubated in HEPES lysis buffer (1% Triton X-100, 20 mM HEPES, 137 mM NaCl, 2 mM EDTA, supplemented with 1× Halt Protease Inhibitor Cocktail (Thermo Scientific)) for 15 min on ice, homogenized by passing the sample 10 times through a 21-gauge needle and centrifuged at 14,000 × g for 15 min at 4 °C to separate out cellular debris. The total protein concentration was measured using a Pierce BCA Protein Assay kit (Thermo Scientific). Total protein lysate (20 μg) was loaded per well with 1× Laemmli buffer. Proteins were separated by SDS–PAGE and blotted onto nitrocellulose membranes. After blocking in 5% milk in PBS–T for 1 h at room temperature, membranes were incubated with primary antibodies (anti-HVCN1 (1:1,000; OAPB01154, Aviva Systems Biology) and anti-β-actin (1:1,000; 8H10D10, Cell Signaling Technology)) diluted in 5% milk in PBS–T for 16 h at 4 °C. Membranes were then washed three times with PBS–T for 10 min, then incubated with secondary Alexa-Fluor-680-conjugated antibodies diluted 1:2,500 in 5% milk in PBS–T for 1 h at room temperature. Membranes were imaged using an Odyssey Infrared imaging system (LI-COR). Images were analysed and protein levels quantified using ImageJ.15

Generation of THP1 shRNA cell lines. THP1 cell lines expressing scramble shRNA or ITGAM (CD11b)-targeting shRNA (CS) were generated as previously described.22 Knockdown of CD11b by shRNA in THP1 cells was verified as previously described: Briefly, cells were stained with anti-CD11b–APC (clone ICRF44, 301310, BioLegend) or APC-conjugated isotype control (clone MOPC-21, 400120, BioLegend) antibodies for 30 min on ice, then washed twice with 1× PBS + 2% FBS + 0.05% sodium azide (FACS buffer) and resuspended in FACS buffer. Flow cytometry data were acquired using a CytoFLEX Flow cytometer (Beckman Coulter). Data were analysed using FlowJo (v10.7.1) software (TreeStar). LentiCRISPRv2 GeCKO transduction of THP1 cells. ITGAM and CD11b targeting shRNAs (CS) were generated in pLV-GeCKO v2.23 LentiCRISPRv2 GeCKO transduction of THP1 cells. ITGAM and CD11b targeting shRNAs (CS) were generated in pLV-GeCKO v2.23 LentiCRISPRv2 GeCKO transduction of THP1 cells. ITGAM and CD11b targeting shRNAs (CS) were generated in pLV-GeCKO v2.23 LentiCRISPRv2 GeCKO transduction of THP1 cells. ITGAM and CD11b targeting shRNAs (CS) were generated in pLV-GeCKO v2.23 LentiCRISPRv2 GeCKO transduction of THP1 cells. ITGAM and CD11b targeting shRNAs (CS) were generated in pLV-GeCKO v2.23
Isolation of human monocytes and generation of monocye-derived macrophages and dendritic cells. Fresh human PBMCs were isolated as described above. Cells were washed twice in 1× PBS, counted and resuspended in 950 ml ice-cold MACS buffer (1% 2 mM EDTA and 0.5% BSA) per 100 × 10^6 PBMCs. 50 μl Microbeads (Miltenyi Biotec) per 100 × 10^6 PBMCs were added, and cells were incubated for 20 min at 4°C. Following incubation, cells were washed in 25 ml MACS buffer, resuspended in 10 ml MACS buffer, passed through a 0.4-μm cell strainer and divided into two 15-ml tubes per donor. Magnetic separation was then performed using an autoMACS Pro Separator following the manufacturer’s instructions (Posseladt program). CD14-positive-selected cells (monocytes) were kept on ice, centrifuged at 1,200 × g for 5 min at 4°C to remove MACS buffer, resuspended in MDM buffer (RPMI, 10% FBS, 10 mM HEPES and 1% P/S) at a concentration of ~1 × 10^6 cells per ml, and divided into 10-ml dishes (10–20 ml per dish). CD14-negative cells were kept on ice, centrifuged at 1,200 × g for 5 min at 4°C to remove MACS buffer, resuspended in PBMC freezing medium (RPNI, 40% FBS, 10% DMSO) at about 50 × 10^5 cells per ml, frozen overnight at −80°C and stored in the gas phase in of a liquid nitrogen tank until further use. To differentiate monocytes into macrophages, 0.05 μg/ml granulocyte–macrophage colony-stimulating factor was added. To obtain monocye-derived dendritic cells, interleukin-4 (2.82 × 10^−10 M) and granulocyte–macrophage colony-stimulating factor (1.08 × 10^−10 M) per ml) were added. Plates were kept in the tissue culture incubator at 37°C with 5% CO2 and treatment was repeated 48 h later. At 48 h after the second treatment, medium containing loosely attached cells was collected. The remaining cells were detached by gently washing with cold 1× PBS twice, incubating for 5 min at 4°C and washing twice with MDM medium.

Toxin exposure of human PMN cells, monocytes, monocye-derived macrophages, dendritic cells, THP1 and PMN-HL60 cells. Cells of interest were counted and resuspended at 1.11 × 10^6 cells per ml. A total of 1 × 10^6 cells per well in a total volume of 100 μl were incubated with the indicated concentrations of purified toxins for 1 h. Unbound toxins were removed and cells resuspended in 100 μl Complete RPMI (Corning, Fisher Scientific) and plated in 96-well plates. PMN cells, monocytes, monocye-derived macrophages, dendritic cells and PMN-HL60 cells were incubated with toxin for 1 h at 37°C, 5% CO2. THP1 cells were incubated for 2 h at 37°C, 5% CO2. Cell viability was measured by adding 10 μl of the CellTiter 96 AQueous One Solution from the Cell Proliferation Assay kit (MTS, Promega) to the wells, and incubating the cells for an additional 1 h at 37°C, 5% CO2, (1.5 h for THP1 cells). Absorbance at 492 nm was measured using an EnVision Multimode plate reader (PerkinElmer).

Selection of CC398 lukAB-resistant LentiCRISPRv2 THP1 cells. LentiCRISPRv2 -GeCKO-transduced THP1 cells were exposed to 0.67 μg/ml CC398 lukAB in two sequential rounds. After the first round of toxin exposure, cells were allowed to recover for 10 days. After the second round, surviving cells were expanded, genomic DNA was extracted using a DNA Isolation Kit for Cells and Tissues (Roche) and sgRNA was amplified by nested PCR and sequenced as previously described[18]. As a control, LentiCRISPRv2-GeCKO-transduced THP1 cells that were not exposed to LukA/B were also kept in culture before isolating the DNA. Briefly, in the first round of PCR, primers VJT1627 and VJT1627 were used to anneal upstream and downstream of the sgRNA sequence. The PCR product was then subjected to the second round of amplification with the universal reverse primer VJT1667 in combination with the forward primers VJT1663, VJT1664, VJT1665 and staggered 5′-primer VJT1666 in combination with staggered 5′-primer VJT1666. Eight independent reactions were performed per sample to maintain diversity and prevent PCR bias. The PCR products containing sgRNAs were pooled and sequenced by NextSeq HighOutput (150 cycles) (Illumina). Reads for each sgRNA were normalized to counts per million total reads. Enrichment of each individual sgRNA was calculated and compared with the unselected THP1 cells and expressed as the fold-enrichment. Data for sgRNAs enriched >100-fold are plotted as the number of enriched sgRNAs per gene versus the mean fold-enrichment per gene.

Toxin exposure of CHO-K1 cells. For toxin exposure, CHO-K1 cells generated as described above were detached from tissue-culture-treated plates and resuspended in 2 μl of the stock solution. 50 μl Heps, 0.53 mM EDTA in HBSS (Corning, Fisher Scientific), resuspended in full medium, counted and resuspended at 1.11 × 10^6 cells per ml. Cells (1 × 10^6 per well) in a total volume of 100 μl were incubated with the indicated concentrations of purified recombinant LukA/B in flat-bottom tissue-culture-treated 96-well plates for 1 h at 37°C, 5% CO2. Cell viability was measured by adding 10 μl of the CellTiter 96 AQueous One Solution Cell Proliferation Assay Kit (MTS, Promega) to the wells, and incubating the cells for an additional 1 h at 37°C, 5% CO2. Absorbance at 492 nm was measured using an EnVision Multimode plate reader (PerkinElmer).

Isolation and toxin exposure of human B cells. Cryopreserved human CD14-monocyte-depleted PBMCs were thawed at 37°C and resuspended in 10 ml of RPMI, 10% FBS. Cells were then treated with Benzonase nuclease (Millipore), incubated at room temperature for 10 min and passed through a 0.4-μm cell strainer into a fresh tube. Cells were centrifuged at 1,500 × g for 5 min, and B lymphocytes were isolated using an EasySep Human B Cell Isolation kit (StemCell).

Isolation of primary human PMN cells and PBMCs. Primary human PMN cells were isolated using a Ficol–Paque method as previously described[7]. To isolate PBMCs, the fucose-labeled layer on the Ficol layer was collected and transferred into a new tube. RPMI medium was then added to bring the volume to 50 ml and the tubes were centrifuged at 1,500 × g for 15 min. Then, the supernatant was removed and cells resuspended in 20 ml RPMI, 0.1% human serum albumin and 10 mM HEPES.
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Isolation of murine PECs. Eight- to 10-week-old wild-type or hHVCN1 C57BL/6 mice were intraperitoneally injected with 1 × 10^6 c.f.u. heat-killed S. aureus strain Newman at 24 and 48 h before collection. Mice were euthanized with CO_2, sprayed with 70% ethanol and mounted on a Styrofoam block on its back. Murine PECs were collected as follows: 5 ml of RPMI, 10% FBS was injected into the peritoneal cavity using a BD Angiocath i.v. catheter (18G 1.88IN, 381147) and the peritoneum was washed twice in 100 μl 1× PBS and resuspended in 50 μl FACS/Fix buffer (1× PBS, 2% FBS, 2% paraformaldehyde (PFA) and 0.05% (w/v) sodium azide). Flow cytometry data were acquired using a CytoFLEX flow cytometer (Beckman Coulter). Data were analysed using FlowJo (v.10.7.1) software (TreeStar). A purity of 75–95% CD11b-positive cells was achieved. B cell membrane damage was determined as the percentage of PE/Cy7 (CD19)-positive cells stained with the eFluor 450 viability dye.

Ex vivo S. aureus infection. Generation of LukAB CC30 S. aureus strain. An isogenic mutant lacking LukAB was constructed using a plMAY plasmid as previously described. Briefly, 500-bp sequences flanking the lukAB locus were PCR amplified using the primers VT1878 and VT1879 for the upstream fragment and the primers VT1880 and VT1881 for the downstream fragment from MRS252 genomic DNA. The gene encoding the kanamycin resistance marker aph-a3 was PCR amplified from the pBT plasmid using the primers VT1882 and VT1883 containing sequence overlapping with both up- and downstream of the CC30 lukAB locus. The PCR amplicons were then assembled by splicing over a PCR extension PCR, digested with KpnI and SacI, ligated into a previously digested and dephosphorylated plMAY vector and then transformed into DH5α E. coli cells following standard cloning procedures. Correct insertion was verified by sequencing, and plasmids were transformed into CC30 S. aureus hlgA-comparative IM0B cells. Deletion of the lucAB locus was achieved by allelic replacement as previously described. Mutagenesis was confirmed by PCR using the primers VT1878 and VT353 for lukAB (expected product size 1.5 kb) and the primers VT599 and VT600 for hlgA as a positive control (expected product size 1 kb). Additionally, proteins secreted by wild-type cells and lucAB mutant cells infected with lucAB following infection in HEPES buffer 1× PBS or 2% FBS and 0.05% (w/v) sodium azide. To equalize levels of protein expression between samples, during flow cytometry analysis, cells were first gated on populations with equal forward and side scatter and CD14+CD11b+ cells were then selected. The lucAB locus was determined as a percentage of these cells stained with the eFluor 450 viability dye.

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LukAB cell binding and competition assays. For the binding assays, 1 × 10^6 CHO-K1 cells expressing Fluc, HVCN1 or mHVCN1 were incubated on ice for 10 min with a titration of biotinylated CC30 and CC45 LukAB in a total volume of 10 μl of ice-cold full media. Following incubation, cells were washed twice with cold 1× PBS and stained with PerCP/Cy5.5 streptavidin (405214, BioLegend) diluted 1/200 in 1× PBS for 20 min. Cells were then washed twice with cold 1× PBS and resuspended in 50 μl FACS/Fix buffer (1× PBS, 2% FBS, 2% PFA and 0.05% (w/v) sodium azide). Cell-bound toxins were detected by flow cytometry (CytoFLEX flow cytometer (Beckman Coulter)), and data shown as the percentage of cells stained with PerCP/Cy5.5 (streptavidin).

For the competition assays, 1 × 10^6 CHO-K1 cells expressing Fluc, HVCN1 or mHVCN1 were incubated on ice for 10 min with 3 μg ml−1 biotinylated CC30 or CC45 LukAB and a titration of unlabelled CC30 or CC45 LukAB in a total volume of 10 μl of ice-cold full media. LukAB binding was measured as described above.

Transient transfection and intoxication of Lenti-X 293T cells. Lenti-X 293T cells were seeded in 6-well tissue-culture plates at a density of 4.8 × 10^5 cells per well and transfected the following day with 400 ng pCMV6-AC-GFP plasmids encoding Fluc, HVCN1 (human, HVCN1), Hven1 (murine, mHVCN1) or cimerecs. For each transfection, 2 μl XtremeGene 9 (Roche) was combined with 400 ng total DNA in 50 μl OptiMEM (Gibco) and added to cells in 1 ml DMEM, 5% FBS. Transfections were carried out for 6 h followed by a change in medium to 2 ml DMEM, 10% FBS per well. Cells were collected at 48 h post-transfection with Accumax (Innovative Cell Technologies) and counted using a Countess II automated cell counter (Thermo Fisher). Cells were intoxicated for 30 min with the indicated concentration of purified recombinant LukAB toxins in tissue-culture-treated 96-well plates. Cells were then washed twice in 100 μl 1× PBS and stained for 20 min with the eFluor 450 viability dye eFluor 450 at 1:5000 in 1× PBS on ice. Finally, cells were washed twice with 100 μl 1× PBS and resuspended in 60 μl FACS/Fix buffer (1× PBS, 2% FBS, 2% PFA and 0.05% (w/v) sodium azide). To equalize levels of protein expression between samples, during flow cytometry analysis, cells were first gated on populations with equal forward and side scatter and CD14+CD11b+ cells were then selected. The lucAB locus was determined as a percentage of these cells stained with the eFluor 450 viability dye.

Isolation and toxin exposure of human T cells. Cryopreserved human PBMCs were thawed at 37 °C and resuspended in 10 ml of RPMI, 10% FBS, CD4− or CD8− positive T lymphocytes were isolated using an EasySep Human CD4+ T Cell Isolation kit (StemCell Technologies) or an EasySep Human CD8+ T Cell Isolation kit (StemCell Technologies), respectively, following the manufacturer’s instructions. A total of 2 × 10^6 cells per well were intoxicated for 3 h at 37 °C, 5% CO_2, with the indicated concentration of purified recombinant LukAB treated-96 well V-bottom plates. Cells were then washed twice in 100 μl 1× PBS and stained with ethidium fluochrome viability dye eFluor 450 (Invitrogen) at 1:1,500 in 1× PBS. Finally, cells were washed twice in 100 μl 1× PBS and resuspended in 50 μl FACS/Fix buffer (1× PBS, 2% FBS, 2% PFA and 0.05% (w/v) sodium azide). Flow cytometry data were acquired using a CytoFLEX flow cytometer (Beckman Coulter). Data were analysed using FlowJo (v.10.7.1) software (TreeStar). A purity of 75–95% CD11b-positive cells was achieved. B cell membrane damage was determined as the percentage of PE/Cy7 (CD19)-positive cells stained with the eFluor 450 viability dye.

Ex vivo toxin exposure of freshly isolated murine cells analysed by flow cytometry. Approximately 2 × 10^6 cells isolated as described above were added per well to tissue-culture-treated 96-well V-bottom plates and incubated with the indicated concentration of purified recombinant CC8, CC30 or CC45 LukAB or LukED. Cells were intoxicated for 2 h at 37 °C, 5% CO_2. To measure toxin-induced membrane damage, cells were washed twice in 100 μl 1× PBS, and stained with the eBioscience fixable viability dye eFluor 450 (Invitrogen) at 1:3,500 in 1× PBS for 20 min on ice. Finally, cells were washed twice in 100 μl 1× PBS and resuspended in 50 μl FACS/Fix buffer (1× PBS, 2% FBS, 2% PFA and 0.05% (w/v) sodium azide). Flow cytometry data were acquired using a CytoFLEX flow cytometer (Beckman Coulter). Data were analysed using FlowJo (v.10.7.1) software (TreeStar) and presented as the percentage of cells stained with the eFluor 450 viability dye.

Ex vivo toxin exposure of freshly isolated murine cells analysed by microscopy. Approximately 1.5 × 10^6 cells isolated as described above were added per well to tissue-culture-treated 96-well black wall-clear bottom-plates and incubated with the indicated concentration of purified recombinant CC8, CC30 or CC45 LukAB or LukED. Cells were intoxicated for 3 h at 37 °C, 5% CO_2, followed by staining with propidium iodide solution (1:500; G Biosciences, 786-1272) and NucBlue Live ReadPrapse reagent ( Hoechst 33342) (1:8; Thermo Fisher Scientific, R37605) for 20 min at room temperature. Stained cells were imaged using a CellSight CX7 High-Content Screening (HCS) platform (Thermo Fisher) with the ×4 objective acquiring 9 fields per well to cover the entire 96-well plate. Images were analysed using HCS Studio software, and toxin-induced membrane damage was quantified as a percentage of propidium-iodide-positive cells.
before incubating for 2 h at 37 °C, 5% CO₂. Cell lysis was evaluated using a CytoTox-ONE Homogeneous Membrane Integrity assay (Promega). The m.o.i. values were confirmed by serially diluting the input cultures and counting c.f.u. on TSA plates.

**LukAB interaction with the CD11b I domain in vitro.** Human Flag and 6xHis-tagged CD11b I domain was purified as previously described. Protein biotinylation. Proteins were biotinylated using EZ-Link NHS-PEG4-biotin, No-Weigh Format (Thermo Scientific) according to the manufacturer's instructions. Briefly, proteins were purified as described above and dialysed overnight in 1x PBS. EZ-Link NHS-PEG4-biotin reagent was added to the protein at a 1:1 molar ratio and incubated for 30 min at room temperature. Unreacted biotin reagent was removed by dialysing overnight in 1x PBS. Labled protein was then filter-sterilized, aliquoted and stored at −80°C.

**ELISA.** ELISAs used to measure the CD11b I domain–LukAB interaction were performed as recently described. As a loading control, plates were coated with a series of 1:2 dilutions of CC8 and CC30 LukAB starting at 1 mg·ml⁻¹.

**Biolayer interferometry.** Kᵣ values for CC8 and CC30 LukAB binding to the CD11b I domain were determined by biolayer interferometry using an Octet RED96 system (Pall ForteBio). Biotinylated CD11b I domain (10 μg·ml⁻¹) was loaded onto streptavidin-coated biosensor chips and kinetic data were obtained using the BioLayer Interferometer system (Pall ForteBio). The gene was then cloned into a pET24a (+) vector using NdeI and restriction enzymes (New England BioLabs). The gene was then confirmed by DNA sequence analysis. Following lysis, cell debris was pelleted by centrifugation (15,000 g, 30 min, 4 °C) and the soluble fraction was eluted with 1.25 ml wash buffer supplemented with 50 mM biotin. Next, 250 ng of LukAB or LukSF was added to the resin and allowed to bind for 5 min. Excess toxin was washed with 20 ml wash buffer. HVCN1–Strep was eluted with 1.5 ml wash buffer supplemented with 50 mM biotin. Two independent founder mice with the modified Hvcn1 exon 4 were backcrossed to wild-type C57BL/6J mice (The Jackson Laboratory), and the progeny were intercrossed to produce homozygous breeder pairs. Homozygous G1, G2 and G4 animals from both lines were used for downstream studies. Mice were overall mortality was ≥30% weight loss, at which time the animals were euthanized. At 14 days post-infection, mice were euthanized with CO₂. The live weight and body fat mass were determined. For dose–response experiments, statistical significance was determined by two-way ANOVA. Data analysis was performed using GraphPad Prism 8 software.

**Pull-down assay.** Streptacitin XT Superflow High Capacity resin (IBA Lifesciences) was incubated with 50 μg of purified HVCN1–Strep (or TBS as a control) for 1 h at 4°C. All assay steps were performed at 4°C. Resin was loaded onto a column and washed with 20 ml wash buffer (150 mM Tris pH 8, 150 mM NaCl and 1 mM EDTA). A total of 30 μg of LukAB or LukSF was added to the resin and allowed to bind for 5 min. Excess toxin was washed with 20 ml wash buffer. HVCN1–Strep was eluted with 1.5 ml wash buffer supplemented with 50 mM biotin. Two independent founder mice with the modified Hvcn1 exon 4 were backcrossed to wild-type C57BL/6J mice (The Jackson Laboratory), and the progeny were intercrossed to produce homozygous breeder pairs. Homozygous G1, G2 and G4 animals from both lines were used for downstream studies. Mice were euthanized with carbon dioxide at 14 days post-infection, mice were euthanized with CO₂. The live weight and body fat mass were determined. For dose–response experiments, statistical significance was determined by two-way ANOVA. Data analysis was performed using GraphPad Prism 8 software.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.
Data availability
The data generated during this study are available upon request with no restrictions. Source data are provided with this paper.

Code availability
Any code generated during this study is available upon request with no restrictions.

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Author contributions

S.S.P, D.B.A.J., K.M.B. and V.J.T. designed the study. S.S.P, D.B.A.J., K.M.B. and J.K.I. performed experiments. C.W.N., A.N., A.M.M. and P.J.P. performed the bioinformatics analyses, K.T. generated the monoclonal antibody. E.E.Z. performed isolation and sorting of the primary human leukocytes, and R.C. purified recombinant toxins. B.S. provided bacterial strains and insight into the clinical relevance of CC30 strains. S.S.P, K.M.B., S.Y.K. and S.B.K. generated the HVCN1 mice. N.V. and M.B.P bred the mice. R.A.P., A.M. and M.D. provided guidance and assisted with microscopy and image analyses. D.C.E. and G.B. provided expertise with purification of recombinant HVCN1–Strep as well as with Octet measurements. S.S.P and V.J.T. wrote the manuscript, and all authors commented on the manuscript.

Competing interests

V.J.T is an inventor on patents and patent applications filed by New York University, which are currently under commercial license to Janssen Biotech Inc. (patent nos. US10669932; US10202440; US10087243; MY-175062-2; BR112013032774-0; 60 2014 068 192.1; 40000719B;119064089; 10-2650267; 2012273125; 20122731132; 10,781,246; 10,301,378; 9,783,597; 9,657,103; 9,481,723; 9,480,726; 9,091,689; 8,846,609; 6758363; 6452765; 6,170,913; 6,993,760; 3441474; 3403669; 3011812; 2,726,754; 272074; 2,635,462; 2,613,135; 2,609,650; 730359; 710,439; 619,942; 619,938; 357,938; 343,589; 340,446; and 229922). Janssen Biotech Inc. provides research funding and other payments associated with the licensing agreement. All other authors declare no conflicts of interest.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41564-021-00890-3. Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41564-021-00890-3. Correspondence and requests for materials should be addressed to V.J.T. Peer review information Nature Microbiology thanks the anonymous reviewers for their contribution to the peer review of this work. Peer reviewer reports are available. Reprints and permissions information is available at www.nature.com/reprints. Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations. © The Author(s), under exclusive licence to Springer Nature Limited 2021.
Extended Data Fig. 1 | Nonsynonymous (\(d_N\)) and synonymous divergence (\(d_S\)) values and ratios for lukA and lukB between clades. a–b, Mean between-clade \(d_N\) and \(d_S\) values are shown below the diagonal, after applying a Jukes-Cantor correction for multiple substitutions at the same sites. Error bars show the standard error of the mean (SEM), estimated using 10,000 bootstrap replicates (codon unit). Corresponding mean between-clade \(d_N/d_S\) ratios are shown above the diagonal, with significance evaluated using a Z-test of the null hypothesis that \(d_N = d_S\) (10,000 bootstrap replicates, codon unit). All P-values are <1.82x10^-6 (see corresponding Figure Source Data) such that asterisks *** indicate a significance level of 0.0001 after Bonferroni correction. Mean between-clade estimates were derived by comparing each member of one clade to each member of a second clade for all pairs of clades. For lukA, the numbers of taxa (\(n\)) and independent aligned codon positions (\(L\)) for each clade are \(n = 49\) and \(L = 1013\) (clade 1/5/8); 6 and 1013 (30/45); 2 and 1008 (10/395); 8 and 1012 (398); 3 and 860 (S. schweitzeri, labeled as S. sch); and 6 and 1010 (S. argenteus, labeled as S. arg). For lukB, the numbers of taxa (\(n\)) and independent aligned codon positions (\(L\)) for each clade are \(n = 51\) and \(L = 1004\) (clade 1/5/8); 8 and 1004 (30/45); 3 and 1004 (10/395); 3 and 1004 (398); 5 and 1004 (S. schweitzeri); and 6 and 966 (S. argenteus). The number of aligned codon positions (\(L\)) are given after eliminating positions with \(\leq 2\) defined (non-gap, non-ambiguous) codons in either clade, separately for each between-clade comparison.
Extended Data Fig. 2 | Comparison between amino acid sequences of selected mature LukA and LukB. a,c, Phylogenetic tree based on amino acid sequences of mature LukA (a) and LukB (c) produced by *S. aureus* belonging to the indicated clonal complexes (CCs). The branch length in the tree is proportional to the number of amino acid substitutions per 100 residues. b,d, Percent identity and divergence of mature LukA (b) and LukB (d) proteins produced by *S. aureus* belonging to the indicated CCs.
Extended Data Fig. 3 | Biolayer interferometry binding curves of CC30 and CC8 LukAB variants binding to CD11b I-domain. a,b, Binding curves of CC30 (a) and CC8 (b) LukAB toxins to CD11b I-domain. The association and dissociation kinetics of LukAB with the I-domain coated sensor are represented in blue. Toxin concentrations are 400 nM, 200 nM, and 100 nM for CC30 LukAB, or 125 nM, 62.5 nM, and 31.3 nM for CC8 LukAB. Red curves show the best global fit using a 1:1 binding model.
Extended Data Fig. 4 | HVCN1 expression in human white blood cells. Consensus human blood cell type expression of HVCN1 derived from RNA-seq data from internally generated Human Protein Atlas (HPA) data. Transcript expression values are presented as Normalized eXpression (NX), resulting from the internal normalization pipeline for 18 blood cell types and total peripheral blood mononuclear cells (PBMC). Data is available at v20.proteinatlas.org/ENSG00000122986-HVCN1/blood, Human Protein Atlas available from www.proteinatlas.org34.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Generation and evaluation of the HVCN1 humanized mouse model. a, Schematic representation of murine Hvcn1 locus and DNA template used to humanize exon 4. b, Genotyping strategy using genomic DNA isolated from wild type (WT), heterozygous (het), and homozygous (homo) hHVCN1 mice using primers VJT2065 and VJT2069. Images are representative of multiple independent experiments as routinely performed for hHVCN1 mouse genotyping. c–g, CFUs in the kidneys (c), livers (d), hearts (e), spleens (f), and lungs (g) collected from WT and hHVCN1 mice infected intravenously with $1 \times 10^7$ CFU of lukAB-deficient USA300 strain LAC. Data from 11 WT and 10 hHVCN1 mice are represented as mean values ±SEM. Statistical significance was determined by t-test (two-tailed), numbers above bars indicate $P$ values. h–k, CFUs in the livers (h), hearts (i), spleens (j), and lungs (k) collected from WT and hHVCN1 mice infected intravenously with 5-10x$10^7$ CFU CC30 S. aureus MUZ211 (CFU obtained from 11 WT and 24 hHVCN1 mice) and 62300D1 (CFU obtained from 11 WT and 10 hHVCN1 mice). Data for each isolate are from mice infected over three independent experiments and is represented as mean values ±SEM. Statistical significance was determined by t-test (two-tailed), numbers above bars indicate $P$ values.
Extended Data Fig. 6 | Flow cytometry gating (part 1). a, Flow cytometry gating scheme utilized to measure surface CD11b levels in scramble shRNA (top) and ITGAM shRNA (bottom) expressing THP1 cell (Fig. 2a) using APC-conjugated anti-CD11b antibody. b, Flow cytometry gating scheme utilized to measure binding of biotinylated LukAB (CC30 LukAB is shown as an example) to CHO cells expressing Fluc (top) or HVCNI (bottom) using PerCP/Cy5.5-conjugated streptavidin staining (Fig. 5b,c). c, Flow cytometry gating scheme utilized to measure membrane damage in B cells following treatment with PBS control (top) and LukAB (CC30 LukAB is shown as an example, bottom) using Fixable Viability Dye eFluor™ 450 (Fig. 5e). d–e, Flow cytometry gating scheme utilized to measure membrane damage in CD4-positive (d) and CD8-positive (e) T cells following treatment with PBS control (top) and LukAB (CC30 LukAB is shown as an example, bottom) using Fixable Viability Dye eFluor™ 450 (Fig. 5e).
Extended Data Fig. 7 | Flow cytometry gating (part 2). a, Flow cytometry gating scheme utilized to measure membrane damage in PECs after treatment with PBS control (top) and leukocidins (LukED is shown as an example, bottom) using Fixable Viability Dye eFluor™ 450 (Fig. 6a). b, Flow cytometry gating scheme utilized to measure membrane damage in Lenti-X 293 T cells expressing C-terminal GFP-tagged wildtype HVCN1 and chimeric proteins (human HVCN1 is shown as an example) following treatment with PBS control (top) and CC30 LukAB (bottom) using Fixable Viability Dye eFluor™ 450 (Fig. 6d).
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Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Software and code
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Bioinformatic analysis of LukAB diversity:
LukA and LukB diversity screen: Nucleotide sequences of lukA and lukB were used to query a nucleotide database of 4,187 of genomes using blastn. All genomes except 5 (C0673.fa, CO_98.fa, ST2282.fa, ST2283.fa, ST228.fa) had a hit to both lukA and lukB sequences (blastn, evalue cutoff=1x10^-5). BLAST aligned portions were captured, and all identical nucleotide sequences were collapsed to a single representative sequence for further analysis using cd-hit. This reduced LukA to 94 and LukB to 83 representative sequences (see Supplementary Table 1).

Phylogenetic Analysis: Gene alignments were done in MAFFT 7.271 (default settings) and optimized by aligning manually based on codons. Maximum likelihood phylogenies were constructed for lukA and lukB with RAxML v8.2.4 using the general time-reversible (GTR) substitution model accounting for among-site rate heterogeneity using the \( \Gamma \) distribution and four rate categories (GTRGAMMA model) with maximum parsimony random-addition starting trees. Node support was evaluated with 100 nonparametric bootstrap pseudoreplicates.

Selection Analysis: Sequences with mid-sequence STOP codons (n=188) were excluded from analysis. Sequences not evenly divisible by 3 were end-trimmed to form a complete codon set. Genes were translated and aligned at the amino acid level using the ClustalW algorithm with default settings as provided in MEGA7 version 7.0.26, and this alignment was imposed on the nucleotide sequence. Whole-gene nonsynonymous (dN) and synonymous (dS) divergence were estimated for all pairs of sequences between each pair of clades using the between-group method of SNPGenie version 1.0 (https://github.com/chasewnelson/spngenie). A Jukes-Cantor correction was used with the Nei-Gojobori (1986) method because synonymous p distances approached 0.555. Standard errors were determined using a bootstrap procedure with 10,000 replicates. Individual codons exhibiting potential positive selection were identified using the FUBAR method of HyPhy version 2.3.420171008beta(MP) (https://veg.github.io/hyphy-site/). All genes exhibited similar, significant levels of purifying selection (dN<dS; P<0.0001; Bonferroni correction). Two codons in lukA were identified as exhibiting positive selection with a posterior probability of >90%: lukA codons 50 and 148. All statistical operations were performed in R version 3.2.0.

Clade-defining sites: Clade-defining sites were determined using EBT::aligned_fasta_group_diffs.pl (https://github.com/chasewnelson/ebt; accessed March 28, 2018). Specifically, sites were identified at which two or more clades differed in their major (i.e., consensus) nucleotides.

Data collection

| Bioinformatic analysis of LukAB diversity: LukA and LukB diversity screen: Nucleotide sequences of lukA and lukB were used to query a nucleotide database of 4,187 of genomes using blastn. All genomes except 5 (C0673.fa, CO_98.fa, ST2282.fa, ST2283.fa, ST228.fa) had a hit to both lukA and lukB sequences (blastn, evalue cutoff=1x10^-5). BLAST aligned portions were captured, and all identical nucleotide sequences were collapsed to a single representative sequence for further analysis using cd-hit. This reduced LukA to 94 and LukB to 83 representative sequences (see Supplementary Table 1). |
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with frequencies of ≥75% in each clade, a criterion chosen to exclude singletons for clades with 2 members and doubletons for clades with 3 members. A clade was not considered to have a defining nucleotide at a site if its major nucleotide was present at a frequency <75% or the site contained ≤2 defined (i.e., non-gap, non-ambiguous) nucleotides. Among these sites, the nucleotide present in CC1/5/8 was unique for a nonsynonymous nucleotide difference for 17 sites in lukA and 8 sites in lukB. Sites undergoing episodic diversifying selection at internal branches were sought using the MEME approach in HyPhy. MEME identified codon 37 in lukB (P=0.0020, LRT). No sites were identified in lukA.

Comparison of selected mature LukA and LukB: Signal peptides and the location of cleavage sites in full-length LukA and LukB were predicted using SignalP 5.0 server. Multiple protein alignments of mature (without signal peptide) LukA and LukB (listed in Supplementary Table 2) were performed using ClustalW alignment method algorithm with default settings as provided in MegAlign (DNASTar, Madison, WI). Further, MegAlign was used to generate phylogenetic trees and pairwise percent identity and divergence tables (presented in Extended Data Supplementary Figure 2).

Human HVCN1 gene (NM_032369) with a short linker and C-terminal Strep-tag (SA-WSHPQFEK) was codon optimized for expression in E. coli using publicly available gene optimization algorithm GeneArt (Thermo Fisher).

Data analysis
GraphPad Prism (v. 8) was used to plot data and carry out statistical analyses. Flowjo (version 10.7.1) was used to analyze flow cytometry data. HCS Studio software was used to analyze microscopy images obtained using CellInsight CX7 High-Content Screening (HCS) Platform (Thermo Fisher Scientific). MegAlign (DNASTar) was used to generate phylogenetic trees and pairwise percent identity/divergence tables. Immunoblot mages were analyzed and protein levels quantified using ImageJ 1.51s as described in the manuscript.

Data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
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Life sciences study design
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| Sample size | No statistical methods were used to determine sample size. The sample size was chosen to obtain significant results as per years of experience using these models. |
| Data exclusions | No data points were excluded from analysis. |
| Replication | Multiple independent experiments were carried out and all attempts to reproduce data were successful. The number of independent experiments are listed in the figure legends. |
| Randomization | Allocation was random. |
| Blinding | Investigators were not blinded to mouse genotype groups, since determination of organ CFU burden is a quantitative and not subjective assay. |

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.
### Antibodies

**Antibodies used**

1. Mouse anti-His tag (CSI20563B, Cell Sciences)
2. Rabbit anti-HVCN1 (OAPB01154, Aviva Systems Biology)
3. Mouse anti-β-Actin (8H10D10, Cell Signaling Technology)
4. Mouse PE/Cy7 anti-human CD19 (302215, BioLegend)
5. Mouse FITC anti-human CD3 (300406, BioLegend)
6. Mouse Alexa Fluor® 700 anti-human CD14 (317410, BioLegend)
7. Mouse APC anti-human CD4 (300912, BioLegend)
8. Mouse APC anti-human CD8α (301310, BioLegend)
9. Mouse Alexa Fluor® 700 anti-mouse/human CD11b (101222, BioLegend)
10. Mouse Alexa Fluor® 700 anti-mouse/human CD11b (101222, BioLegend)
11. PE/Cy7 Mouse IgG1, κ Isotype Ctrl Antibody (400126, BioLegend)
12. FITC Mouse IgG1, κ Isotype Ctrl Antibody (400108, BioLegend)
13. Alexa Fluor® 700 Mouse IgG1, κ Isotype Ctrl Antibody (400143, BioLegend)
14. PE Mouse IgG1, κ Isotype Ctrl Antibody (400111, BioLegend)
15. APC Mouse IgG1, κ Isotype Ctrl Antibody (400120, BioLegend)
16. Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 680 (A-21057, Invitrogen)
17. Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 680 (A-21076, Invitrogen)
18. Mouse anti-CC30 LukAB monoclonal antibody (generated in this study, used at 1 μg/ml)
19. Rabbit anti-HVCN1 [AHC-001, Alomone Labs]
20. Rabbit anti-HVCN1 (PA5-24964, Invitrogen, Thermo Fisher Scientific)

**Validation**

Antibodies were validated by the manufacturer, via western blot or flow cytometry, found on the respective product pages. We also validated the specificity of the antibodies (anti-HVCN1, anti-LukAB, anti-CD11b) by using knockout samples to distinguish specific signals.

### Eukaryotic cell lines

**Policy information about cell lines**

**Cell line source(s)**

1. THP1 (ATCC, TIB-202)
2. HL60 (ATCC, CCL-240)
3. CD18 CHO-K1 (provided by Dr. Radim Osicka, Institute of Microbiology of the Czech Academy of Sciences)
4. Lenti-X 293T (Takara Bio USA, 632180)

**Authentication**

THP1 and HL-60 were authenticated by ATCC. Lenti-X 293T were authenticated by Takara Bio USA. All cell lines were identified by morphology.

**Mycoplasma contamination**

All cell lines tested negative for contamination.

**Commonly misidentified lines**

No commonly misidentified cell lines were used.

### Animals and other organisms

**Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research**

**Laboratory animals**

Female and male WT C57Bl/6 (The Jackson Laboratory), female and male hHVCN1 (this study) mice were used at 8-10 weeks of age. No sex difference was observed. Pseudopregnant CD-1 females (Charler River Laboratory) 8-9 weeks of age were used to implant zygotes.

**Wild animals**

No wild animals were used in this study.

**Field-collected samples**

No field-collected samples were used in this study.

**Ethics oversight**

All experiments involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of NYU Langone.
Ethics oversight

Health and were performed according to guidelines from the National Institutes of Health (NIH), the Animal Welfare Act, and US Federal Law.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

1. For isolated human T and B cells as well as freshly isolated murine cells: cells were washed twice in 100 μl 1xPBS and resuspended in 50 μl FACS/Fix buffer (1x PBS/2% FBS/2% PFA/0.05% (w/v) sodium azide).
2. For Lenti-X 293T cells: Cells were washed twice with 100 μl 1xPBS and resuspended in 60 μl FACS/Fix buffer (1x PBS/2% FBS/2% PFA/0.05% (w/v) sodium azide).
3. For CHO cells in toxin-binding studies: cells were then washed twice with cold 1xPBS and resuspended in 50 μl FACS/Fix buffer (1x PBS/2% FBS/2% PFA/0.05% (w/v) sodium azide).
4. For THP1 cells: cells were washed twice with 1× PBS+2% FBS + 0.05% sodium azide (FACS buffer) and resuspended in FACS buffer.

Instrument

CytoFLEX Flow Cytometer (Beckman Coulter Life Sciences)

Software

FlowJo (version 10.7.1)

Cell population abundance

Typically 10,000 singlets from the live gate were analyzed.

Gating strategy

1. Live cells were gated first using FSC/SSC. Then singlet population was gated. For T and B cells: the CD19 (B cell), CD4 (CD4 T cell), or CD8a (CD8 T cell) positive population was gated. The percentage of these cells stained with eFluor™ 450 viability dye was determined.
2. For CHO cells in toxin-binding studies: percentage of PerCP/Cy5.5 (streptavidin)-positive cells was determined.
3. For Lenti-X 293T cells: To ensure equal levels of protein expression between samples, during flow cytometry analysis cells were first gated on populations with equal GFP fluorescence intensity. Cell death was then determined as a percentage of these cells stained with the eFluor™ 450 viability dye.
4. For freshly isolated murine cells: percentage of cells stained with the eFluor™ 450 viability dye was determined.
5. For THP1 cells: Live cells were gated first using FSC/SSC. Then singlet population was gated. Percentage of APC (CD11b)-positive cells was determined.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.