Journal Pre-proof



A critical role for host-derived cystathionine-β-synthase, in *Staphylococcus aureus*-induced udder infection

Shaodong Fu, Bo Yang, Yabin Gao, Yawei Qiu, Naiyan Sun, Zhi Li, Shiyuan Feng, Yuanyuan Xu, Jinqiu Zhang, Zhenhua Luo, Xiangan Han, Jinfeng Miao

PII: S0891-5849(23)01094-8

DOI: https://doi.org/10.1016/j.freeradbiomed.2023.11.001

Reference: FRB 16234

To appear in: *Free Radical Biology and Medicine*

Received Date: 2 September 2023

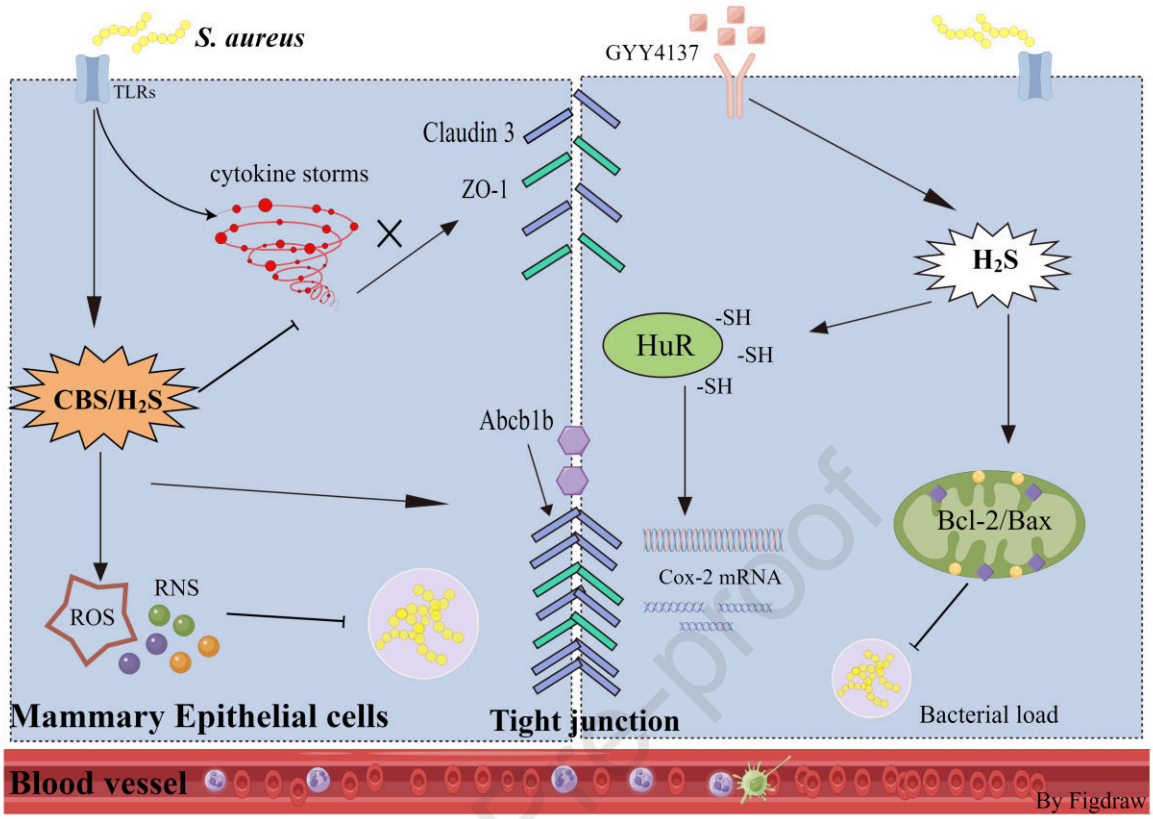
Revised Date: 31 October 2023

Accepted Date: 4 November 2023

Please cite this article as: S. Fu, B. Yang, Y. Gao, Y. Qiu, N. Sun, Z. Li, S. Feng, Y. Xu, J. Zhang, Z. Luo, X. Han, J. Miao, A critical role for host-derived cystathionine-β-synthase, in *Staphylococcus aureus*-induced udder infection, *Free Radical Biology and Medicine* (2023), doi: https://doi.org/10.1016/j.freeradbiomed.2023.11.001.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2023 Published by Elsevier Inc.



Journal Pre-proof

Journal Pre-proof

Free Radical Biology and Medicine, Volume 210, January 2024, pp. 13-24
DOI:10.1016/j.freeradbiomed.2023.11.001

A critical role for host-derived cystathionine-β-synthase in *Staphylococcus aureus*-induced udder infection

Shaodong Fu[1], Bo Yang[1], Yabin Gao[1], Yawei Qiu[1], Naiyan Sun[1], Zhi Li[1], Shiyuan Feng[1], Yuanyuan Xu[1], Jinqiu Zhang[2], Zhenhua Luo[3], Xiangan Han[4], Jinfeng Miao[1*]

[1]MOE Joint International Research Laboratory of Animal Health and Food Safety, Key Laboratory of Animal Physiology & Biochemistry, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China.

[2]Institute of Veterinary Immunology & Engineering, Jiangsu Academy of Agricultural Sciences，Nanjing 210014, China.

[3]School of Water, Energy & Environment, Cranfield University, Cranfield, Bedfordshire, MK43 0AL, United Kingdom.

[4]Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai, 200241, China.

Corresponding author: JF Miao (miaojinfeng@njau.edu.cn)

Journal Pre-proof

ABSTRACT:

Cystathionine-β-synthase (CBS) catalyzes the first step of the transsulfuration pathway. The role of host-derived CBS in *Staphylococcus aureus* (*S. aureus*)-induced udder infection remains elusive. Herein, we report that *S. aureus* infection enhances the expression of CBS in mammary epithelial cells *in vitro* and *in vivo*. A negative correlation is present between the expression of CBS and inflammation after employing a pharmacological inhibitor/agonist of CBS. In addition, CBS achieves a fine balance between eliciting sufficient protective innate immunity and preventing excessive damage to cells and tissues preserving the integrity of the blood-milk barrier (BMB). CBS/H_2S reduces bacterial load by promoting the generation of antibacterial substances (ROS, RNS) and inhibiting apoptosis, as opposed to relying solely on intense inflammatory reactions. Conversely, H_2S donor alleviate inflammation via S-sulfhydrating HuR. Finally, CBS/H_2S promotes the expression of *Abcb1b*, which in turn strengthens the integrity of the BMB. The study described herein demonstrates the importance of CBS in regulating the mammary immune response to *S. aureus*. Increased CBS in udder tissue modulates excessive inflammation, which suggests a novel target for drug development in the battle against *S. aureus* and other infections.

Keywords: *Staphylococcus aureus;* cystathionine-β-synthase; udder infection; blood–milk barrier

Published by Elsevier. This is the Author Accepted Manuscript issued with: Creative Commons Attribution Non-Commercial No Derivatives License (CC:BY:NC:ND 4.0). The final published version (version of record) is available online at DOI:10.1016/j.freeradbiomed.2023.11.001. Please refer to any applicable publisher terms of use.

Journal Pre-proof

Introduction

Mastitis is the most costly infectious disease of of dairy cows. *Staphylococcus aureus* (*S. aureus*) is one of the most important etiologic agents of mastitis [1, 2]. The intracellular survival nature of *S. aureus* is one of the key reasons why mastitis caused by it is so hard to cure. Unfortunately, there are currently no effective vaccines for *S.aureus* infections and antibiotics continue to be the mainstay of treatment. Antimicrobial resistance of *S. aureus* is believed to have a severe negative impact on human health [3, 4]. Therefore, it is essential to identify new molecular targets for drug development against *S. aureus*.

The transsulfuration pathway plays a central role in cellular sulfur metabolism and redox regulation. Cystathionine-β-synthase (CBS) catalyzes the first step of the transsulfuration pathway thereby limiting the rate of this fundamental biological proces [5, 6]. CBS/hydrogen sulfide (H_2S) is an emerging gaseous signaling molecule that regulates many cellular processes. However, the effects of H_2S are often bell-shaped: at lower concentrations, it stimulates cellular bioenergetics, while at toxic concentrations, it suppresses mitochondrial function [7, 8]. Aberrant CBS expression and/or activity contributes to a wide range of disease development, including down syndrome, various cancers and infectious diseases [9-12]. Recently, mounting evidences focus on the importance of host-derived H_2S in microbial pathogenesis, however, research findings are somewhat controversial. For example, Li *et al*. found H_2S significantly impacts cellular responses and viral replication [13], Similarly, Renieris *et al.* reported that H_2S affords protection against *Pseudomonas aeruginosa*

sepsis [14]. Conversely, Rahman *et al.* found that H_2S dysregulates the immune response by suppressing central carbon metabolism thus promoting *tuberculosis* [15]. Previous studies have shown that exogenous H_2S can prevent LPS-induced inflammation by blocking the TLR4/NF-κB pathway and that endogenous H_2S can regulate LPS-induced inflammation through IL-8 signaling both in bovine mammary epithelial cells (MAC-T cells) [16, 17], little attention has been given to the interaction of host derived CBS/H_2S and mastitis pathogens, particularly *S. aureus.*

A previous study pointed out that exogenous H_2S protects the intestinal epithelial barrier function in Caco-2 monolayers from TNF-a and IFN-γ induced injury [18]. Like gut barrier, the blood-milk barrier (BMB) is a natural defense mechanism of mammary tissue that restricts the free exchange of cells and large molecules between blood and milk [19, 20]. The mammary epithelial barrier is formed by tight junctions between the monolayer of secretory mammary epithelial cells. Compromising the integrity of this barrier results in lower milk quality and volume. Additionally, pathogenic bacteria crossing this barrier may cause life-threatening systemic infections [21]. It is crucial to maintain the functional integrity of the BMB for optimal mammary gland health, but there is no data confirming a link between H_2S and the BMB.

And protein S-sulfhydration is a post-translational modification of a hydropersulfide moiety (-SSH) added to specific cysteine residues in targeted proteins [22]. This process is generally facilitated by H_2S and can regulate protein functionn a reversible manner that is critical for precise regulation of homeostasis [23-25]. Recent

studies have proven that H_2S facilitates the S-sulfhydration of Human antigen R (HuR) , leading to a decrease in the stability of COX-2 mRNA in colitis, which confirmed the role of S-sulfhydration in epithelial cells [26]. Thus, we are curious to know if S-sulfhydration by H_2S plays a role in preventing mammary epithelial cell infection induced by *S. aureus.*

Herein, we focused on host-derived CBS/H_2S in the pathogenesis of *S. aureus* infections, and found that CBS is associated with inflammation and the BMB. We then investigated the underlying mechanism of action of CBS/H_2S in *S. aureus*-induced udder infection. These findings shed light on the molecular mechanisms involved in antimicrobial defense and regulation by host sulfur metabolism, providing a promising strategy to tackle the growing issue of mastitis.

Materials and methods

Bacterial strains, cell culture, and treatment

S. aureus ATCC 29213 and EpH4-Ev cells were purchased from American Type Culture Collection (Manassas, USA). Bacteria were inoculated into Luria-Bertani (LB) culture and incubated at 37℃ on a shaker. Following overnight incubation, 30 μL of cultured material was transferred to 3 mL of fresh LB at 37℃ with shaking until the optical density OD_{600} reached 0.6-0.8. EpH4-Ev cells were incubated in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and plated at 80% confluence on 6-well plates. The monolayers were treated with or without 200 μM AOAA and 50 μM SAM for 4 h, 10 μM Vismodegib for 2 h,

Journal Pre-proof

105 or 50 μM GYY4137 for 24 h at 37℃. The treated cells were infected with *S. aureus*
106 at a multiplicity of infection (MOI) of 10 for 3 h at 37 ℃. Supernatants and cells were
107 collected separately and stored at −80 ℃ until use. Other reagents and materials are
108 listed in **Table S1**.

109 **Mice, genotype identification, and establishment of mastitis model**

110 C57BL/6 (WT or $CBS^{+/-}$) mice were purchased from Cyagen Biosciences
111 (Suzhou, China). Animals were maintained under standard conditions of humidity
112 (50%) and temperature (25±2℃) in a 12 h light and 12 h dark cycle in the Laboratory
113 Animal Center of Nanjing Agricultural University. Two females and 1 male were
114 housed together in microisolator cages. Specific pathogen free-grade mice breeding
115 fodder and water were supplied *ad libitum*. Offspring were removed from cages 24 h
116 before bacterial administration. The parturient mice were anesthetized by ether and
117 the teats as well as surrounding areas were disinfected with 75% ethanol. The fourth
118 pair of mammary glands were held lightly with fine forceps and 50 μL *S.aureus*
119 (about 1×10^9 Colony Forming Units [CFU] mL^{-1}) was injected via the mammary
120 ducts using a microsyringe. Twenty four h later, the mice were euthanized. Blood and
121 mammary tissue were collected and stored at −80°C until use. A direct mouse
122 genotyping kit (APExBIO, USA) was used to extract mouse tail DNA, and
123 subsequently amplified using a specific primer pool via Accurate Taq Master mix
124 (AG, Changsha, China). The products of PCR amplification were run on a 1.5%
125 agarose gel to verify their size. All animal experiments were carried out in accordance
126 with the guidelines of the Animal Welfare Council of China and approved by the

Ethical Committee for Animal Experiments of Nanjing Agricultural University (NJAU. No20220509099).

Lentivirus plasmid transfection and RNA sequencing

EpH4-Ev cells were transfected with CBS and mock vector and stably overexpressed cell lines were constructed after selection with puromycin. The expression effect was detected by qPCR. RNA sequencing was performed at Microeco Tech Co. Ltd. (Shenzhen, China). Briefly, RNA was extracted from cells using TRIzol reagent (AG, Changsha, China) and quality analysis was conducted using a Bioanalyzer 2100 (Agilent, Santa Clara, USA). Subsequently, PCR was conducted utilizing Phusion High-Fidelity DNA polymerase, universal PCR primers, and index (X) primer. The amplified PCR products were purified via the AMPure XP system and assessed for quality using a Bioanalyzer 2100. Following cluster generation, library preparation sequencing was done on an Illumina Novaseq platform, thereby generating 150 bp paired-end reads.

Assessment of biochemical parameters and relative enzyme activities

The protein samples from EpH4-Ev cells were extracted using cold lysis buffer and assayed using a BCA Protein Assay Kit. The levels of lactic dehydrogenase (LDH) in cell supernatant were measured using commercial kits according to the manufacturer's instructions. Pro-inflammatory factors (TNF-α and IL-1β) in serum were measured by ELISA kit according to the manufacturer's instructions.

Viable bacterial count assay

The infected cells were washed thrice with PBS containing 100 μg/mL gentamicin for 15 min each at 37℃, followed by a subsequent wash with gentamicin-free PBS. The cells and the supernatant were collected, and an equal number of cells from each group were lysed with sterile water. Then, the samples were serially diluted 10 fold, and 100 μL aliquots of each dilution were inoculated on the LB plate media. CFUs were enumerated via the spread plate method after an 18 h incubation at 37℃.

Histopathologic assay

Tissues were preserved in 10% neutral buffered formalin, then washed with water before dehydration in alcohol solutions ranging from 75 to 100%, with a 5% increase every hour. Next, the tissue was soaked in xylene and embedded in wax for 3 h at 60℃. Three μm sections were stained with hematoxylin and eosin. The resulting histological changes were characterized by light microscopy.

RNA extraction and quantitative real-time polymerase chain reaction

Total RNA was extracted using TRIzol reagent and subjected to reverse transcription using reverse transcriptase (AG, Changsha, China). An aliquot of the resulting cDNA was mixed with 5 μL TB Green PCR Master Mix (AG, Changsha, China) and 0.2 μL of each specific forward and reverse primer. The mixed systems were then analyzed using a Roche LightCycler 96. The reference gene was β-actin and fold changes were calculated as $2^{-\Delta\Delta Ct}$. All primer sequences (listed in **Table S2**) were synthesized by Tsingke Biological Company (Nanjing, China).

Journal Pre-proof

Total protein extraction and Western blotting

The cells and corresponding tissues were washed thrice with ice-cold PBS. They were lysed by incubating with RIPA lysis buffer, containing protease inhibitor phenylmethanesulfonyl fluoride (PMSF, Beyotime, Nantong, China). The lysates were centrifuged at 12,000 × g at 4℃ for 15 min and supernatants collected. Protein concentration was determined using a BCA kit (Beyotime, Nantong, China). SDS loading buffer was added to the protein samples and then boiled at 100℃ for 5 min. Separation of protein samples was done via 12.5% SDS-PAGE, and blotted onto PVDF membranes. To prevent nonspecific binding, the membranes were blocked with 10% non-fat powdered milk for 2 h. The blot was then probed overnight with relevant primary antibody at 4℃. After washing, the blots were incubated with HRP-conjugated secondary antibody at room temperature for 2 h. Signal detection was carried out using an ECL Western Blot Analysis System (Tanon, Shanghai, China). Bands were quantified using ImageJ 1.8 software (NIH, Bethesda, MD, USA).

Immunofluorescent staining

The cell-laden slides were fixed with 4% formaldehyde for 15 min, followed by 3 washes with PBS. Cell membranes were permeated with PBS containing 0.5% Trix-100 for 20 min. Subsequently, the cells were blocked with 5% BSA for 2 h at RT. The slides were incubated overnight at 4℃ with diluted primary antibody. After washing, a secondary antibody was added and the slides were incubated for 2 h at RT. Following another 3 washes with PBS, the cells were exposed to 10 μM DAPI for 10

193 min. The slides went through another 3 washes with PBS, after which an
194 anti-fluorescence quenching sealer was applied and the cells were photographed with
195 an inverted fluorescence microscope (Invitrogen).

196 **Immunohistochemistry**

197 Paraffin-embedded specimens were deparaffinized in xylene. They were then
198 hydrated using a series of graded ethanols and placed in 0.01 mol/L citrate buffer (pH
199 6.0). Subsequently, the samples were heated in a microwave oven over medium-high
200 heat for 10 minutes, followed by incubation with a 3% H_2O_2-methanol solution at RT
201 for 10 min. After primary and HRP-conjugated secondary antibody incubation,
202 diaminobenzidine was used as a chromogen and hematoxylin as a counterstain. The
203 resultant sections were observed with a light microscope and photographed.

204 **Detection of reactive oxygen species (ROS)**

205 The intracellular ROS of EpH4-Ev cells was stained by DCFH-DA according to
206 the instructions (Beyotime, Nantong). Briefly, cells were loaded with 10 μM
207 DCFH-DA for 30 min at 37℃ and washed 3 times with PBS. The entire process was
208 protected from light. The cell samples were immediately analyzed
209 by inverted fluorescence microscopy (Invitrogen).

210 **Biotin switch assay of S-sulfhydration**

211 A biotin switch assay was conducted with standard procedures [27]. The cells were
212 lysed by incubating with RIPA lysis buffer containing PMSF. The lysates were
213 centrifuged at 12,000×g at 4℃ for 15 min and supernatants collected. Protein
214 concentrations were measured via BCA assay. Next, the lysates were added to HEN

Journal Pre-proof

buffer (250 mM HEPES-NaOH supplemented with 1 mM EDTA and 0.1 mM neocuproine) supplemented with 2.5% SDS and 20 mM methyl methanethiosulfonate (MMTS). The samples were shaken at 50℃ for 30 min. Acetone was added to remove MMTS, and proteins precipitated by storing them at -20℃ for 30 min. The pellets were resuspended in HENS buffer containing 4 mM biotin-HPDP. After incubating overnight at RT, the proteins were purified with acetone. Finally, the proteins were dissolved in HENS buffer, purified with streptavidin magpoly beads and subjected to western blotting with an anti-HuR antibody.

Statistical analyses

Data were expressed as the mean±standard error and analyzed by independent samples t-tests or one-way ANOVA followed by the Tukey's multiple comparison test as appropriate. All statistical analyses was performed using GraphPad Prism version 8.0.1 software. A value of $P<0.05$ indicated statistical significance.

Results

CBS expression is elevated in *S. aureus* mastitis

Firstly, we analyzed the relative expression of three crucial enzymes involved in the transsulfuration pathway in mammary gland and EpH4-Ev cells under normal state, including cystathionine c-lyase (CSE), 3-mercaptopyruvate sulphur transferase (3-MPST) and CBS. We found that CSE was primarily expressed in the cells, while CBS dominated in the tissue (**Figure 1A-B**). However, when EpH4-Ev cells were exposed to *S. aureus,* we observed that CBS gene expression was increased by hundreds times, but CSE and 3-MPST presented downward trend and displayed

Journal Pre-proof

small amplitude variations (**Figure 1C-E**). Since CBS plays a role in the conversion of homocysteine to cystathionine, which is a crucial precursor for the production of glutathione (a classic antioxidant), protein synthesis, and H_2S production, it suggests that CBS is a major contributor to the production of H_2S during this period (**Figure 1F**). Further analysis revealed a sharp increase in CBS protein expression in both EpH4-Ev cells and MAC-T cells. (**Figure 1G**). To validate *in vitro* findings, we established a *S. aureus*-induced mastitis model. Western blot analyses and qPCR assays demonstrated higher CBS levels after *S. aureus* stimulation (**Figure 1H-I**). To further corroborate the results described above, we performed immunohistochemistry on mammary tissue sections after *S. aureus* challenge (**Figure 1J**). Overall, these experiments demonstrate that up-regulation of CBS is induced by *S. aureus* both *in vitro* and *in vivo*.

CBS expression is correlated with *S. aureus*-induced inflammatory injury

To gain mechanistic insights into CBS, we used CBS inhibitor aminooxyacetic acid (AOAA) and agonist s-adenosyl methionine (SAM) to treat EpH4-Ev cells. Since the NF-kB pathway plays an important role in oxidative stress and inflammation, we tested marker proteins of this signaling pathway. Western blot was used to determine the protein expression of IkBα, p-IkBα and (p)65. The data indicated markedly increased phospho-p65/p65 and phospho-IkB/IkB ratios (**Figure 2A-C**), followed by a decrease in total IkBα in the AOAA-treated group compared with the control group (**Figure 2D**). This phenomenon was reversed after pretreatment with SAM. We also documented changes in the expression of

inflammation-related molecular markers. The gene expression of pro-inflammatory factors, except IL-6, was up-regulated after AOAA administration compared with control groups. SAM treatment can significantly decrease gene expression of pro-inflammatory factors, particularly TNF-α. To that end, lactate dehydrogenase (LDH) release may serve as a potential marker of cell injury and death. We documented the release of LDH in culture medium in agreement with previous observations. AOAA can exacerbate EpH4-Ev cells damage and SAM largely reverses these changes (**Figure 2E**). Taken together, our study reveals that CBS may enhance the host defense response to *S. aureus,* thus attenuating inflammatory injury.

CBS agonist reduces bacterial burden by an inflammation-independent pathway

The inflammatory response is a double-edged sword since it has the power to cause damage to an organism while also possessing the capability to eliminate harmful intracellular bacteria [28]. Reactive oxygen (ROS) and nitrogen species (RNS) have intracellular bactericidal properties. Our data indicate that CBS inhibition exacerbates inflammatory responses. We therefore studied this effect on *S. aureus* growth and ROS/RNS production in EpH4-Ev cells. In addition, we examined the effect of CBS on inducible nitric oxide synthase (NOS2) expression on both gene and protein levels. Unexpectedly, we found almost complete abolition of NOS2 by AOAA. In contrast, the addition of SAM significantly promotes NOS2 expression (**Figure 3A-C**). Then we evaluated plate counts of intracellular bacteria after gentamicin incubation to kill extracellular bacteria, and

Journal Pre-proof

found that there was a significant decline in mean bacteria loads after treatment with AOAA comparing with control groups. These results are the opposite of what we had expected no significant difference between the AOAA and SAM groups (**Figure 3D-E**). This may be due, in part, to intense inflammation resulting in a decrease of mean bacteria loads post AOAA administration. However, alternative inflammation-independent pathways may exist to compensate for this physiological process in SAM treatment groups. Finally, we quantified intracellular ROS levels. We observed essentially the same data. Cell morphology using bright field microscopy indicated that the AOAA group underwent cell shrinkage compared to the other groups. This surprising outcome suggests that CBS may ameliorate harmful inflammatory responses while promoting otherwise protective innate responses.

CBS contributes to the maintenance of BMB integrity

In order to confirm whether AOAA pretreatment can cause a stronger inflammatory response that exacerbates pathological disorders we measured the expression of ZO-1 and Claudin 3 by western blot analysis. We found a significant downward trend in ZO-1 and Claudin 3 levels with AOAA, whereas SAM displayed the opposite effect (**Figure 4A-C**). Consistent with these results, as presented in **Figure 4D**, ZO-1 immunofluorescent staining also showed decreased expression of ZO-1 with AOAA pretreatment, but effectively recovered ZO-1 expression after SAM pretreatment of *S. aureus* infected EpH4-Ev cells. As a

Journal Pre-proof

result, we propose that CBS may contribute to the maintenance of BMB integrity of inflammation-induced permeability.

CBS induced *Abcb1b* expression is critical for improving BMB integrity

Since CBS can affect the BMB, what could be the underlying mechanism? We created CBS overexpression in EpH4-Ev cells and confirmed that it does (**Figure 5A-B**). Subsequently, we discovered that *S. aureus* infection causes a considerable decrease in the expression of ZO-1 and Claudin 3 tight junction proteins. CBS in EpH4-Ev cells exhibits greater resistance to *S. aureus* infection, particularly an almost complete restoration of Claudin 3 protein expression (**Figure 5C**). Transcriptomic data suggests that normal EpH4-Ev cells display 593 up-regulated and 992 down-regulated genes when exposed to *S. aureus,* while those in CBS EpH4-Ev cells were 558 up-regulated and 992 down-regulated genes (**Figure 5D**). A thorough analysis of differential gene expression identified a significant change in the *Abcb1b* gene as shown in both the volcano plot and the heatmap (**Figure 5E-F**). To determine whether the *Abcb1b* gene plays a direct role in maintaining BMB integrity induced by *S. aureus*, we employed the inhibitor Vismodegib, and found that Claudin 3 protein expression was significantly reduced, even in CBS overexpressed EpH4-Ev cells, indicating that Abcb1b inhibition reversed the protective effect of CBS overexpression on the BMB. Overall, these data provide additional evidence of the crucial role of CBS in the maintenance of the BMB, potentially by modulating the expression of *Abcb1b* gene.

CBS upregulated genes associated with the innate immune response and pathways related to cell receptor interactions.

In order to further define the underlying mechanisms of Abcb1b maintenance of BMB integrity, the Interacting Genes/Proteins database (STRING v11.5) was used to construct the PPI network (**Figure 6A**). After obtaining the differentially expressed genes (DEGs), we conducted GO and KEGG enrichment analyses to construct a bubble diagram that helped identify the functional relevance of these genes (**Figure 6B-C**). The GO enrichment analysis data indicates that the DEGs were mostly associated with the collagen-containing extracellular matrix (ECM), immune system processes and innate immune responses. The KEGG pathway analysis data showed that DEGs were mainly concentrated in cytokine-cytokine receptor interactions, NOD-like receptor signaling pathways, and the coronavirus disease-COVID-19 pathway. Taken together, the CBS genes had increased activity related to innate immune responses, as well as in pathways related to cell receptor interactions.

H_2S donor can significantly reduce inflammation and cell damage induced by *S. aureus*

We found that 100 μM GYY4137 does not have a cytotoxic effect on EpH4-Ev cells (**Figure 7A**). GYY4137 treatment increased the expression of ZO-1 and Claudin 3. There is not a statistically significant difference in p65 when challenged by *S. aureus* (**Figure 7B-E**). While GYY4137 significantly reduces the expression of these proinflammatory factors, it amplifies TNF-α (**Figure 7F**). As expected, western blotting indicates a significant increase in NOS2 protein expression following

GYY4137 treatment compared with the *S. aureus* infection group (**Figure 7G-H**). Due to its direct bactericidal activity, we examined the intracellular bacterial load, and there was a decrease after GYY4137 treatment (**Figure 7I**). Besides the cytotoxic effect mediated by *S. aureus* infection on epithelial cells via inducing apoptosis, the process involves the balanced transcription of anti-apoptotic and pro-apoptotic proteins. We detected the expression of apoptosis-related proteins, Bax and Bcl-2. The ratio of Bcl2/Bax was significantly increased with GYY4137 treatment (**Figure 7J-M**). The studies described above suggest that an H_2S donor significantly reduces inflammation and cell damage induced by *S. aureus* via promoting NO production and inhibiting apoptosis.

CBS-H_2S axis decreases COX-2 expression and the stability of its mRNA by S-sulfhydrating HuR

Given that S-sulfhydration of HuR mediated by endogenous H_2S leads to the amelioration of COX-2 expression in colitis [26]. We found that the total protein expression of HuR increases significantly during *S. aureus* infection, whereas GYY4137 fails to lower it. Conversely, the levels of COX-2 protein (**Figure 8A-D**) and mRNA (**Figure 8E**) decreases markedly, accompanying a significant increase in S-sulfhydrated HuR. Conclusively, HuR S-sulfhydrylation is observed in EpH4-Ev cells, and may potentially play a critical role in the body's response to inflammation.

$CBS^{+/-}$ mice develop severe tissue damage as a result of *S. aureus* infection

Data from experiments in $CBS^{+/-}$ mice examined the effects of CBS on *S. aureus* infection *in vivo*. The mammary glands were congested and swollen following *S.*

Journal Pre-proof

367 *aureus* infection. Hemmorhage was present in the $CBS^{+/-}$ group compared with the
368 WT group (**Figure 9A**). Histologic examination indicated that *S. aureus* infection
369 caused mammary epithelial cells to be disordered and detached accompanied by
370 marked inflammatory cell infiltration. These changes were more pronounced in
371 $CBS^{+/-}$ group (**Figure 9B**). Macrophage infiltration was detected via
372 immunohistochemical staining for CD68 **(Fig 9C).** Serum pro-inflammatory factors
373 TNF-α **(Fig 9D)** and IL-1β **(Fig 9E)** were detected via ELISA. Additionally, serum
374 levels of oxidative stress factor MDA were quantified **(Fig 9F).** The levels of
375 pro-inflammatory factors TNF-α and IL-1β and the oxidative stress marker MDA
376 were remarkably increased in the *S aureus* infection group. The increase in $CBS^{+/-}$
377 mice was more pronounced compared to wild-type mice. Conversely, the bacterial
378 loads in mammary tissue were reduced in the $CBS^{+/-}$ group. To determine BMB
379 integrity, we assayed the expressions of Zo-1 and Claudin 3 tight junction proteins.
380 Consistent with the *in vitro* results, a highly significant reduction in the expression
381 of these tight junction proteins was present in *S. aureus* infected $CBS^{+/-}$ mice.
382 In sum, these results indicate that CBS negatively regulates the magnitude of
383 mammary gland inflammation thereby decreasing tissue damage.
384

385 ## Discussion

386 *S. aureus* is a significant globally-distributed zoonotic pathogen that can infect a
387 variety of epithelial cell types [29,30]. The mammary gland is a vital organ in
388 mammals, responsible for milk production and delivery, and udder infections are a

Journal Pre-proof

major health and economic concern in dairy animals. Identifying molecules involved in host defense against pathogen entry is a promising approach to treatment and prophylaxis.

A major conclusion of the present study is that CBS plays a vital role in host defense against *S. aureus.* Firstly, we observed CBS protein and gene levels increased significantly during *S. aureus*-infection *in vitro* and *in vivo* suggesting a direct link between *S. aureus* infection and CBS/H_2S. We subsequently found the level of expression of CBS was inversely proportional to the degree of inflammatory injury caused by *S. aureus.* The role of CBS in inflammation is controversial so far. Phillips *et al.* noted that upregulation of CBS in colonic epithelium reprogramed metabolism and promoted carcinogenesis [12], Zhu *et al.* found that CBS is essential for AKT-induced senescence and suppresses the development of gastric cancers associated with PI3K/AKT activation [31]. Vila *et al.* reported that CBS gene knockdown promotes inflammation and oxidative stress in immortalized human adipose-derived mesenchymal stem cells [32]. This finding highlighted the strong cell type specificity of the role of CBS in the occurence and development of inflamation. Besides, in intracellular bacterial infections, inflammatory responses aim to clear pathogens yet they often lead to tissue damage. There needs to be a delicate balance between eliciting sufficient protective immunity and causing irreparable tissue damage. Host cells that produce ROS and RNS have bactericidal activity [33]. In this study, we found that CBS inhibitor reduced bacterial burden by exacerbating inflammation leading to a decrease of ROS and RNS.

411 CBS agonists not only reduce inflammation, but also decrease bacterial load
412 in part because of their moderate production of ROS and RNS.
413 The above results suggest that up-regulated CBS is able to balance host defense and
414 tissue damage. In general, once bacteria enter a cell, they can trigger host cell
415 apoptosis, restrict the expression of self-toxic factors, halt early cytotoxicity, and
416 adapt to the intracellular environment in order to evade host defenses [34,35]. We
417 suggest that an H_2S donor may prevent apoptosis, thereby limiting bacterial growth.
418 Overall, our data indicate that *S. aureus* infection triggers upregulation of CBS,
419 reducing inflammation and bacterial burden.

420 Based on the observed changes in cell morphology, we focused on the integrity
421 of the BMB. This barrier is a crucial structure for mammary gland function. Tight
422 junctions prevent invasion of microorganisms and regulate the exchange of various
423 substances within the mammary gland [36]. Little is known regarding the correlation
424 between the transsulfuration pathway and tight junction proteins in mammary gland
425 tissue. Most studies have centered around the gastrointestinal system. It has been
426 confirmed that exogenous H_2S can help alleviate damage to the intestinal barrier [37].
427 The data generated in the current study indicates that after exposure to *S. aureus*,
428 upregulation of CBS expression enhances the expression of tight junction proteins,
429 particularly Claudin 3, these experiments employed CBS agonists and inhibitors, H_2S
430 donor and the creation of cell lines that overexpress CBS. RNA-seq
431 transcriptomics show that Abcb1b was the most abundant and was therefore selected
432 for further study. Abcb1b codes P-gp protein. P-gp presence in the blood-brain barrier

Journal Pre-proof

433 (BBB) limits drug accumulation in the brain [38]. It also plays a vital role in
434 preserving intestinal homeostasis by transporting xenobiotics and bacterial toxins [39].
435 And P-gp/Abcb1b is essential for junction reassembly at the blood-testis barrier [40].
436 Vismodegib, a potent inhibitor of P-gp, has undergoing clinical trials for a the
437 treatment of a variety of solid tumors [41]. In the study described herein, the
438 overexpression of CBS on Claudin 3 protein was partially abolished after employing
439 Vismodegib, which indicates that Abcb1b may be the downstream molecule of CBS
440 in the progression of maintaining the BMB. Moreover, the protein-protein interaction
441 networks of Abcb1b were constructed by STRING and detected CD44 protein.
442 Previous studies comfirmed that CD44 could activate macrophages upon *S.aureus*
443 infection triggering release of ROS that mediates activation of acid sphingomyelinase
444 releasing ceramide that causes alterations of the cytoskeleton and internalization of
445 pathogens [42]. As known that maintaining the structure of the cytoskeleton is vital for
446 the proper functioning of the blood-milk barrier. It seems that the regulatory influence
447 of CBS on Abcb1b might be connected to CD44, and is worth further research.
448 Similarly, GO term analysis revealed that many of the DEGs are enriched in the
449 immune process and collagen-containing extracellular matrix suggesting that CBS is
450 important to host immune defense and the extracellular components that play crucial
451 roles in resistance to infection. Enriched KEGG pathways of the DEGs are supported
452 by the three KEGG signaling pathways in which CBS participate, including
453 cytokine-cytokine receptor interactions, coronavirus disease COVID-19 and
454 NOD-like receptor signaling, which in line with previous results in current study that

CBS primarily impacts the structure and functionality of cell membranes, all of these tight junction proteins were predominantly localized to the cell membrane at cell-cell contacts.

Since S-sulfhydration of proteins plays a vital role in the functioning of both endogenous and exogenous H_2S, we investigated whether S-sulfhydration in *S. aureus* infected EpH4-Ev cells occurs, and if so, what is the target protein? Previous studies proved that inhibition of COX-2 mRNA stability by increasing the S-sulfhydration of human antigen R (HuR) may be a mechanisms underlying the anti-inflammatory effect of the CBS-H_2S axis in colitis and other disorders [26], Our data indicates that S-sulfhydration of HuR protein is not cell-type specific in its functionality.

Finally, we further validated our in vitro results in EpH4-Ev cells utilizing an in vivo $CBS^{+/-}$ mouse model. We found $CBS^{+/-}$ mice suffered more significant injuries compared to control mice. This was confirmed via HE stained tissue examination, immunohistochemistry and Elisa. Due to the role of IL-1β in infections caused by *S. aureus* is important only in the early stages[43]. so there is no statistical difference in serum. There is a decrease in the bacterial load of mammary tissue in the $CBS^{+/-}$ group. The reduction of bacteria burden is achieved at the expense of increased inflammatory damage. To this end, the significantly reduced expression of tight junction protein ZO-1 and Claudin 3 after CBS knockdown is consistent with previous in vivo results.

The data from the current study details the importance of host CBS in anti *S. aureus* infection documenting a potential relationship between CBS and BMB for the first time. Mechanistically, CBS maintains a fine balance between eliciting effective

protective immunity and preventing excessive damage to tissues by the generation of direct antibacterial substances, regulating apoptosis related proteins and S-sulfhydrating target proteins (**Fig 10**). With antibiotic resistance and evasion of vaccination on the rise, it is crucial to gain a deeper understanding of the interactions between hosts and pathogens. This will hopefully aid in the identification of new therapeutic targets. Our study helps to elucidate host defense mechanisms against pathogens and suggests that manipulating sulfur metabolism may be a promising tool for drug discovery and vaccine optimization.

Conflicts of interests

The authors declare no competing interests.

Acknowledgments

This project was supported by grants from the National Natural Science Foundation of China (No. 32072867, 32273013 and 32272963), the Jiangsu Province Key Research and Development Program (No. BE2022384) and the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions. The authors declare no competing interests. Dr. Howard Gelberg is thanked for his help in editing this manuscript.

References

[1] Hu X, Guo J, Zhao C, Jiang P, Zhang N, et al.The gut microbiota contributes to the development of *Staphylococcus aureus*-induced mastitis in mice. The ISME Journal. 2020;14(7):1897-1910.

[2] Eckersall PD, Young FJ, Nolan AM, Knight CH, Fitzpatrick JL, et al. Acute phase proteins in bovine milk in an experimental model of *Staphylococcus aureus* subclinical mastitis. J Dairy Sci. 2006;89(5):1488-1501.

Journal Pre-proof

[3] Q R, G L, Z W, J L, W C, et al. Prevalence and characterization of *Staphylococcus aureus* isolates from subclinical bovine mastitis in southern xinjiang, china. J Dairy Sci. 2020;103(4):3368-3380.

[4] Qu Y, Zhao H, Nobrega DB, et al. Molecular epidemiology and distribution of antimicrobial resistance genes of *Staphylococcus species* isolated from chinese dairy cows with clinical mastitis. J Dairy Sci. 2019(2).

[5] Matye D, Gunewardena S, Chen J, et al. TFEB regulates sulfur amino acid and coenzyme a metabolism to support hepatic metabolic adaptation and redox homeostasis. Nat Commun. 2022;1(13):5696.

[6] Sbodio JI, Snyder SH, Paul BD. Regulators of the transsulfuration pathway. Br J Pharmacol. 2019;196(4):583-589.

[7] Jia J, Wang Z, Zhang M, et al. SQR mediates therapeutic effects of H_2S by targeting mitochondrial electron transport to induce mitochondrial uncoupling. Sci Adv. 2020;35(6):z5752.

[8] Dongdong, Wang, Honggang, et al. Hydrogen sulfide and autophagy: a double edged sword. Pharmacol Res. 2018;131:120-127.

[9] T P, Eb R, F A, C S. Overproduction of hs, generated by cbs, inhibits mitochondrial complex IV and suppresses oxidative phosphorylation in down syndrome. Proc Natl Acad Sci U S A. 2019;116(38):18769-18771.

[10] Panagaki T, Lozano-Montes L, Janickova L, et al. Overproduction of hydrogen sulfide, generated by cystathionine β-synthase, disrupts brain wave patterns and contributes to neurobehavioral dysfunction in a rat model of down syndrome. Redox Biol. 2022;51:102233.

[11] V S, Kc C, Vp R, et al. Hydrogen sulfide stimulates *Mycobacterium tuberculosis* respiration, growth and pathogenesis. Nat Commun. 2020;11(1):557.

[12] Phillips CM, Zatarain JR, Nicholls ME, et al. Upregulation of cystathionine-β-synthase in colonic epithelia reprograms metabolism and promotes carcinogenesis. Cancer Res. 2017;77(21):5741-5754.

[13] Li H, Ma Y, Escaffre O, Ivanciuc T, Casola A. Protective role of hydrogen sulfide in paramyxovirus infection. J Virol. 2015;89(10):5557-5568.

[14] G R, De D, K K, et al. Host cystathionine-γ lyase derived hydrogen sulfide protects against *Pseudomonas aeruginosa* sepsis. PLoS Pathog. 2021;17(3):e1009473.

[15] Ma R, Bm C, Kw A, et al. Hydrogen sulfide dysregulates the immune response by suppressing central carbon metabolism to promote *tuberculosis*. Proc Natl Acad Sci U S A. 2020;117(12):6663-6674.

[16] Sun L, Chen L, Wang F, Zheng X, Zhou X, et al. Exogenous hydrogen sulfide prevents lipopolysaccharide-induced inflammation by blocking the TLR4/NF-κB pathway in MAC-T cells. Gene. 2019;710:114-121.

[17] T L, X B, Y G, et al. Cth/hs regulates LPS-induced inflammation through IL-8 signaling in MAC-T cells. Int J Mol Sci. 2022;23(19):11822.

[18] Sw C, J Z, S Z, et al. Protective effect of hydrogen sulfide on TNF-α and IFN-γ-induced injury of intestinal epithelial barrier function in Caco-2 monolayers. Inflamm Res. 2015;64(10):789-797.

[19] O W, Rm B. Invited review: the role of the blood-milk barrier and its manipulation for the efficacy of the mammary immune response and milk production. J Dairy Sci. 2021;104(6):6376-6388.

[20] Li M, Wang Z, Qiu Y, et al. Taurine protects blood-milk barrier integrity via limiting inflammatory response in *Streptococcus uberis* infections. Int Immunopharmacol. 2021;101:108371.

[21] X H, S L, R M, et al. The rumen microbiota contributes to the development of mastitis in dairy

cows. Microbiol Spectr. 2022;10(1):e251221.
[22] Zhao S, Song T, Gu Y, et al. Hydrogen sulfide alleviates liver injury via s-sulfhydrated-Keap1/Nrf2/LRP1 pathway. Hepatology. 2021;73(1):282-302.
[23] Meng G, Zhao S, Xie L, Han Y, Ji Y. Protein S-sulfhydration by hydrogen sulfide in cardiovascular system. Br J Pharmacol. 2018;175(8):1146-1156.
[24] Módis K, Ju YJ, Ahmad A, et al. S-sulfhydration of atp synthase by hydrogen sulfide stimulates mitochondrial bioenergetics. Pharmacol Res. 2016:116-124.
[25] Gupta R, Sahu M, Tripathi R, Ambasta RK, Kumar P. Protein s-sulfhydration: unraveling the prospective of hydrogen sulfide in the brain, vasculature and neurological manifestations. Ageing Res Rev. 2022;76:101579.
[26] S G, Z H, J Z, et al. CBS-HS axis preserves the intestinal barrier function by inhibiting COX-2 through Sulfhydrating human antigen R in colitis. J Adv Res. 2023;44:201-212.
[27] Sen N, Paul B, Gadalla M, et al. Hydrogen sulfide-linked sulfhydration of NF-κB mediates its antiapoptotic actions. Mol Cell. 2012;45(1):13-24.
[28] T X, Y G, X Q. Ubiquitination-mediated inflammasome activation during bacterial infection. Int J Mol Sci. 2019;20(9):2110.
[29] Seitaro N, Masanori M, Yuki K, et al. *Staphylococcus* aureus virulent psmα peptides induce keratinocyte alarmin release to orchestrate IL-17-dependent skin inflammation. Cell Host Microbe. 2017;22(5):667-677.
[30] Huvenne W, Callebaut I, Reekmans K, et al. *Staphylococcus aureus* enterotoxin b augments granulocyte migration and survival via airway epithelial cell activation. Allergy. 2010;65(8):1013-1020.
[31] Zhu H, Kt C, Huang X, et al. Cystathionine-β-synthase is essential for AKT-induced senescence and suppresses the development of gastric cancers with PI3k/AKT activation. elife. 2021;11:e71929.
[32] Vila F, Latorre J, Ortega F, Navarrete JM, et al. Permanent cystathionine-β-synthase gene knockdown promotes inflammation and oxidative stress in immortalized human adipose-derived mesenchymal stem cells, enhancing their adipogenic capacity. Redox Biol. 2020;42:101668.
[33] B LVA, C RIJ, A ZR, D LBC, D PPC. Self-defense of macrophages against oxidative injury: fighting for their own survival. Redox Biol. 2019;26:101261.
[34] Jia F, Ma W, Zhang X, Wang D, Zhou X, et al. Matrine and baicalin inhibit apoptosis induced by panton-valentine leukocidin of *Staphylococcus aureus* in bovine mammary epithelial cells. J Dairy Sci. 2020;103(3):2731-2742.
[35] X Z, X H, X R. Apoptosis induced by *Staphylococcus aureus* toxins. Microbiol Res. 2017;205:19-24.
[36] Frster C. Tight junctions and the modulation of barrier function in disease. Histochem Cell Biol. 2008;130(1):55-70.
[37] Chen S, Bu D, Ma Y, et al. GYY4137 ameliorates intestinal barrier injury in a mouse model of endotoxemia. Biochem Pharmacol. 2016;118:59-67.
[38] Watchko, Jon F. P-glycoprotein in the developing human blood–brain barrier. Pediatr Res. 2016;79(5):806.
[39] Foley SE, Tuohy C, Dunford M, et al. Gut microbiota regulation of p-glycoprotein in the intestinal epithelium in maintenance of homeostasis. Microbiome. 2021;9(1):183.
[40] Su L, Cheng YC, Lee WM, et al. Abcb1a and abcb1b genes function differentially in blood–testis

barrier dynamics in the rat. Cell Death Dis. 2017;8(9):e3038.

[41] Y Z, J L, Mg P, et al. Hedgehog pathway inhibitor hhantag691 is a potent inhibitor of ABCG2/BCRP and ABCB1/PGP. Neoplasia. 2009;11(1):96-101.

[42] Li C, Wu Y, Orian-Rousseau V, Zhang Y, Grassme H, et al. Regulation of *Staphylococcus aureus* infection of macrophages by cd44, reactive oxygen species, and acid sphingomyelinase. Antioxid Redox Signal. 2018;28(10):916-934.

[43] Oviedo-Boyso J, Valdez-Alarcón JJ, Cajero-Juárez M, et al. Innate immune response of bovine mammary gland to pathogenic bacteria responsible for mastitis. J Infect. 2007;54(4):399-409.

Journal Pre-proof

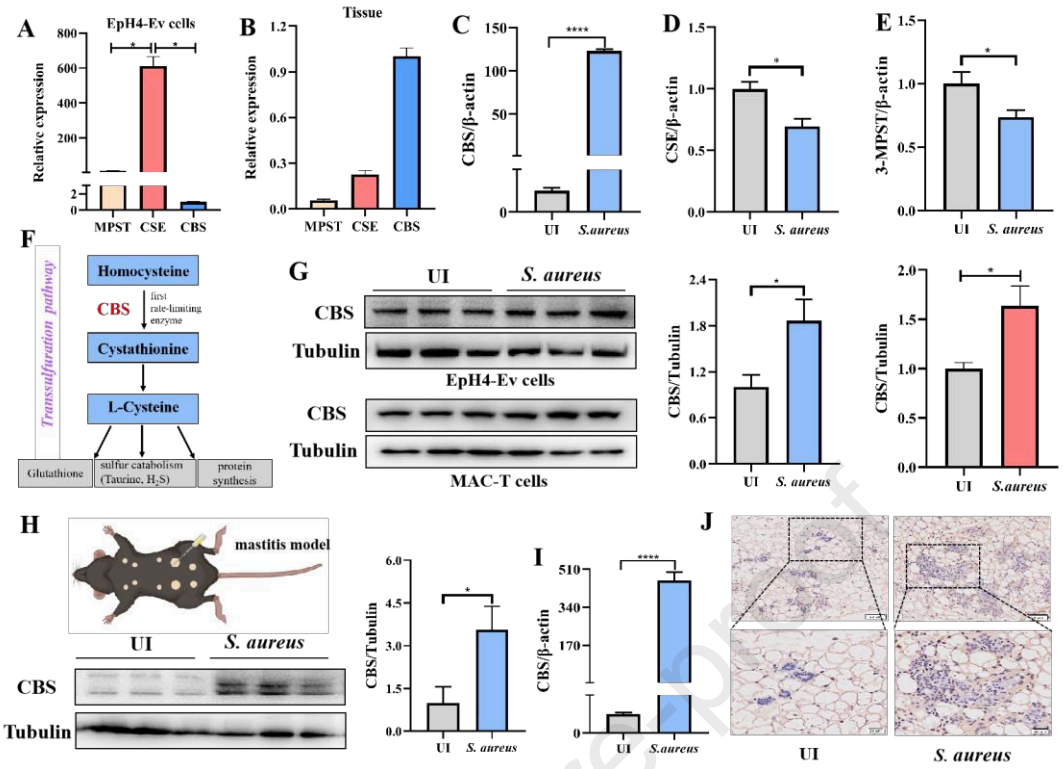


Figure 1. The expression of CBS is increased in mastitis caused by *S. aureus*. Relative expression levels of three crucial enzymes involved in transsulfuration pathway in mammary gland tissue (A) and EpH4-Ev cells (B). Gene expression of CBS (C), CSE (D) and 3-MPST (E) in uninfected and *S. aureus*-infected EpH4-Ev cells. (F) Endogenous transsulfuration pathway that generates glautathione, taurine and H_2S. (G) Western blot of CBS expression in uninfected (UI) and *S. aureus*-infected EpH4-Ev cells and MAC-T cells. (H) Mice mastitis model establishment, and protein expression of CBS in uninfected mice (UI) and those infected with *S. aureus* confirmed by qPCR (I) and immunohistochemistry (J) (n=6). Data are presented as means±SEMs (n=3, unless otherwise indicated). *(P<0.05)=significantly different.

Journal Pre-proof

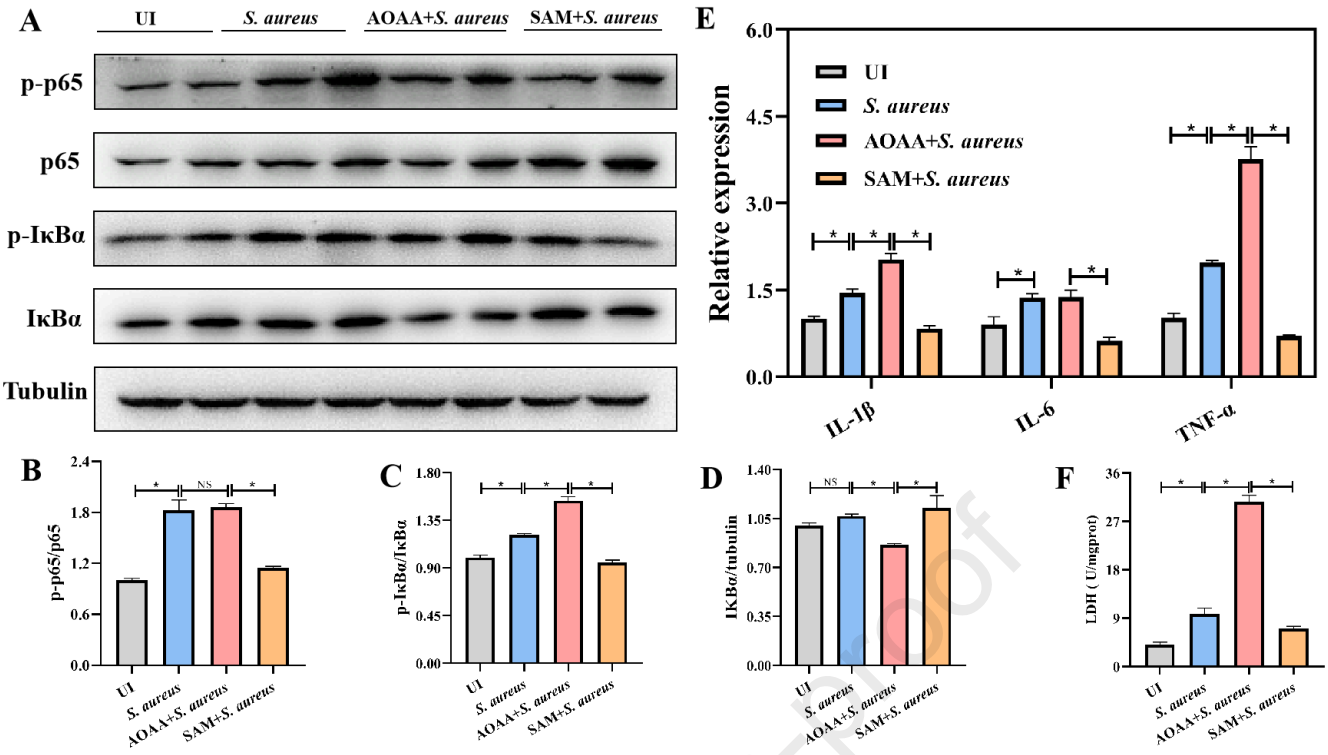


Figure 2. The degree of *S. aureus*-induced inflammatory injury is related to CBS expression. (A-D) Western blot showing expression of (p)IκBα, (p)65 in uninfected and *S. aureus*-infected EpH4-Ev cells, with or without AOAA and SAM. (E) Gene expression of pro-inflammatory factors. (F) The release of lactate dehydrogenase (LDH) in the culture supernatant. Data are presented as means ± SEMs (n=3). *(P<0.05)=significantly different.

Journal Pre-proof

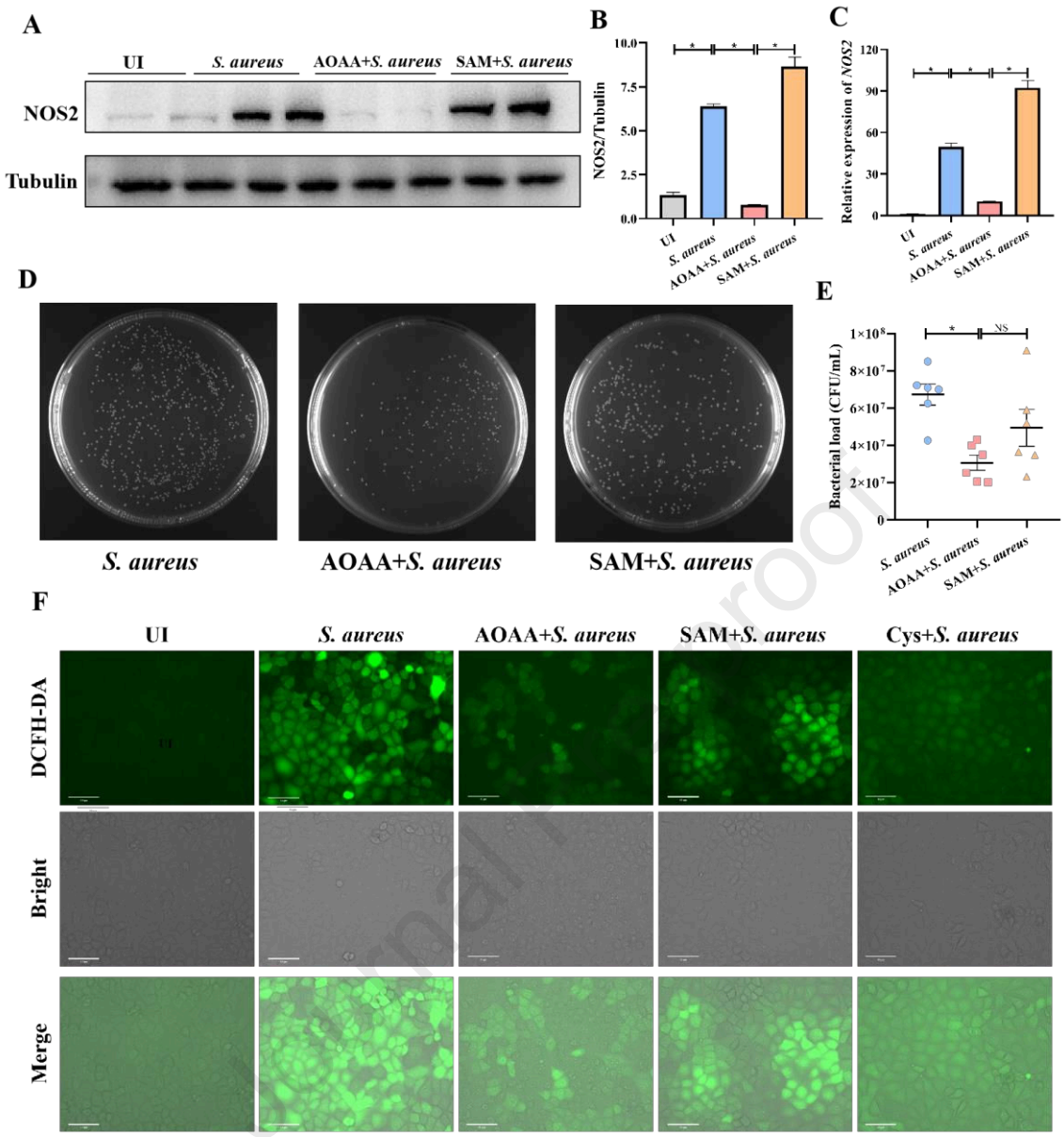


Figure 3. CBS agonists lower bacteria by utilizing a noninflammatory pathway. (A-B) Western blot showing expression of NOS2 in uninfected and *S. aureus*-infected EpH4-Ev cells, with or without AOAA and SAM, and validated by qPCR (C). (D-E) Intracellular growth profile of *S. aureus.* (D) ROS content of EpH4-Ev cells following the use of AOAA and SAM, with cysteine positive control. Data are presented as the means±SEMs (n=3). *(P<0.05)=significantly different.

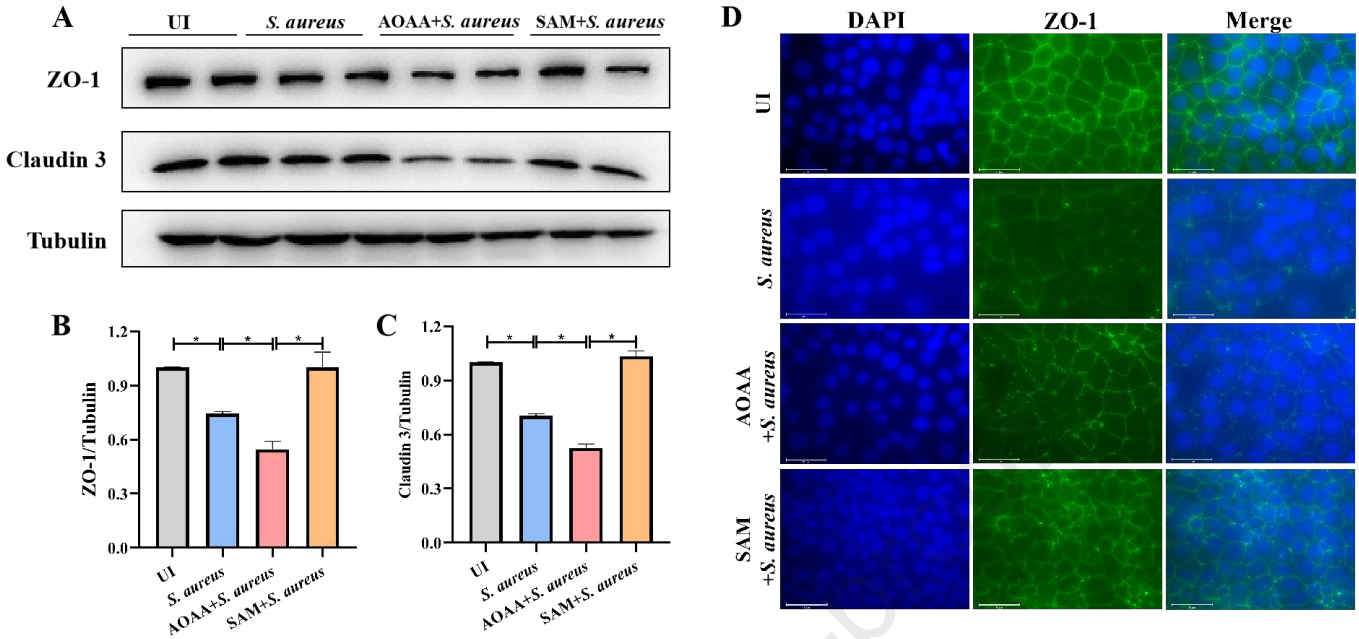


Figure 4. CBS plays a role in preserving the integrity of the BMB. (A-C) The expression of ZO-1 and Claudin 3 in EpH4-Ev cells, both uninfected and infected with *S. aureus*, was analyzed using western blot. Additionally, the effect of AOAA and SAM were also studied. The results were confirmed by immunofluorescence (D). Data are presented as the means±SEMs (n=3). *(P<0.05)=significantly different.

Journal Pre-proof

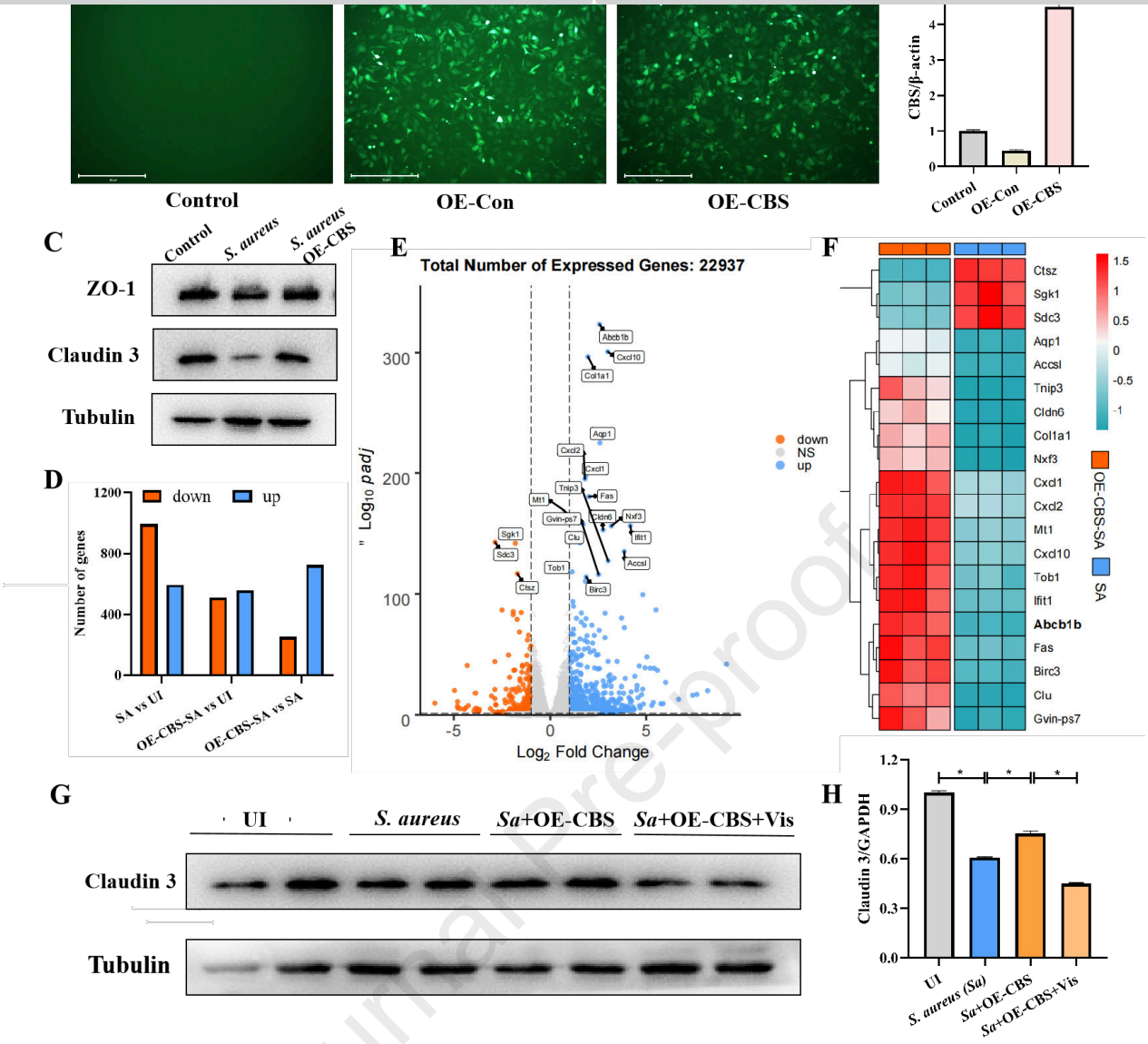


Figure 5. The induction of *Abcb1b* expression by CBS enhances the integrity of the BMB. (A-B) Fluorescence indicates that the plasmid was successfully transfected into EpH4-Ev cells. qPCR verifies the results. (C) The expression of ZO-1 and Claudin 3 in EpH4-Ev cells and OE-CBS EpH4-Ev cells during *S. aureus* infection. (D) Up- and down-regulation of differential genes. (E-F) Volcano plot and heatmap of differential genes. (G-F) The expression of Claudin 3 in EpH4-Ev cells and OE-CBS EpH4-Ev cells with or without Vismodegib during *S. aureus* infection. Data are presented as the means±SEMs (n=3). *(P<0.05)=significantly different.

Journal Pre-proof

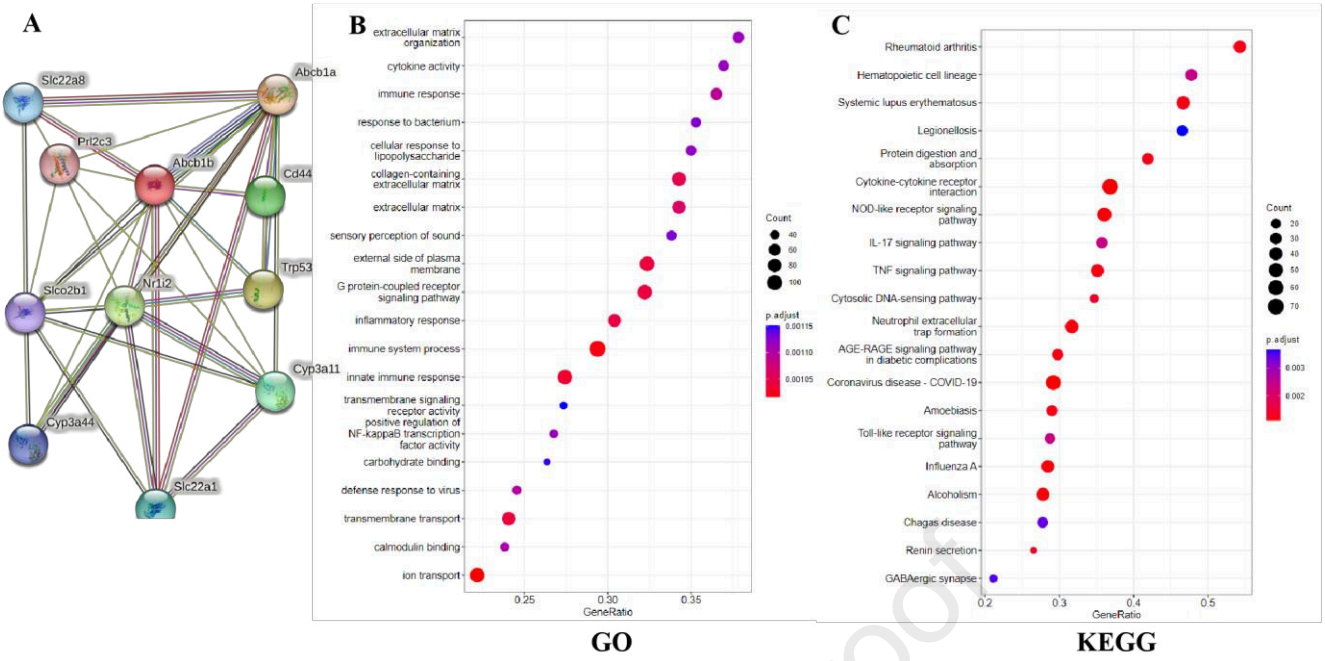


Figure 6 CBS increases gene expression linked to the natural defense system of the body, and pathways connected to communication between cells. (A) Protein-to-protein interaction network analyzed using STRING. GO enrichment analysis (B) and KEGG enrichment analysis (C). Size of the bubble represents the number of genes enriched in the GO terms and KEGG pathways. The color of the bubble corresponds to the FDR p value.

Journal Pre-proof

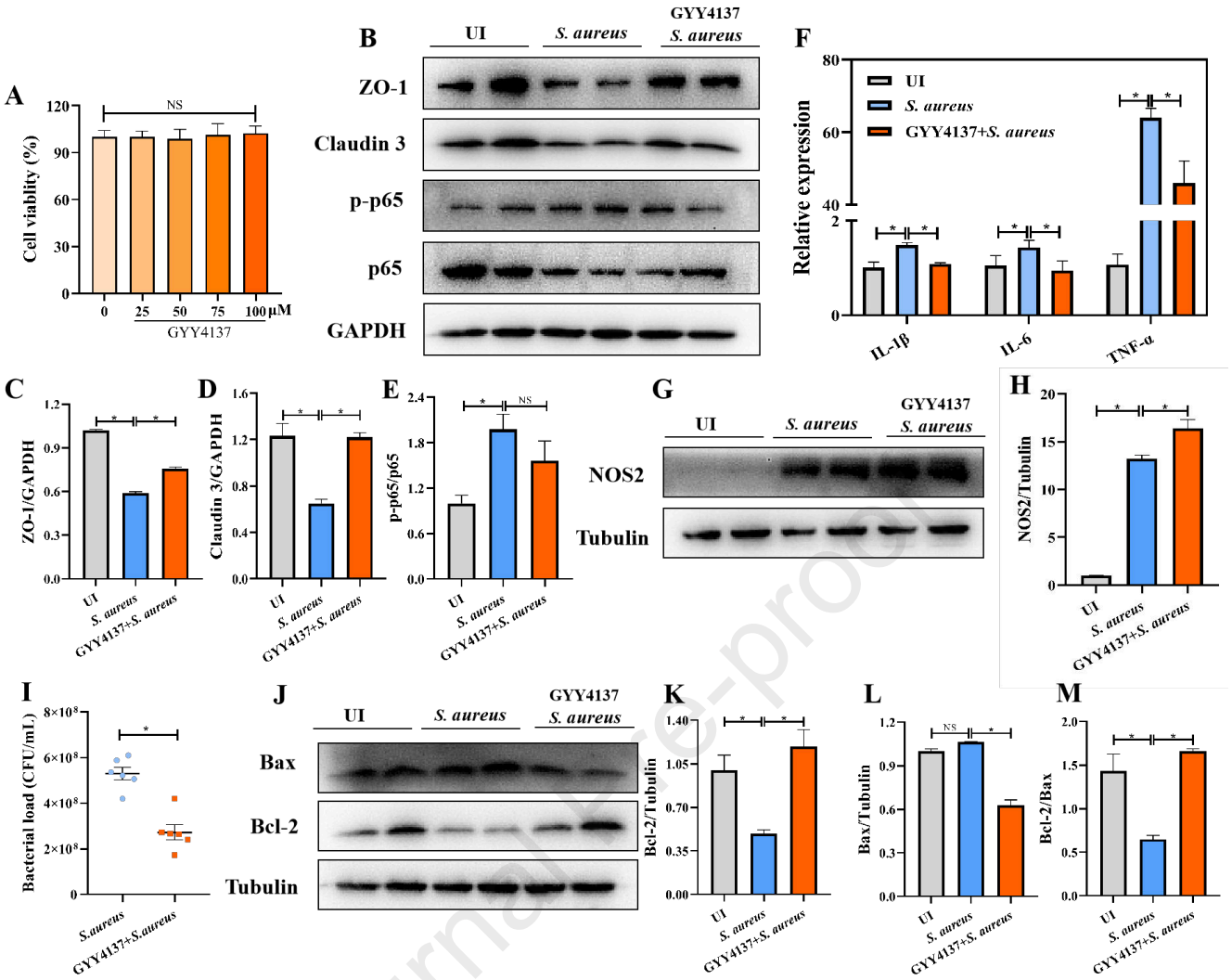


Figure 7. GYY4137 shows potential in reducing inflammation and cell damage caused by *S. aureus*. (A) Cytotoxicity of GYY4137 on EpH4-Ev cells was determined using a CCK8 kit. (B-E) Western blot showing expression of ZO-1, Claudin 3 and (p)65 in uninfected and *S. aureus*-infected EpH4-Ev cells after being preprocessed with GYY4137. (F) Gene expression of pro-inflammatory factors. (G-H) NOS2 protein level. (I) Intracellular growth of *S. aureus*. (J-M) Bax and Bcl-2 protein levels. Data are presented as the means±SEMs (n=3). *(P<0.05)=significantly different.

Journal Pre-proof

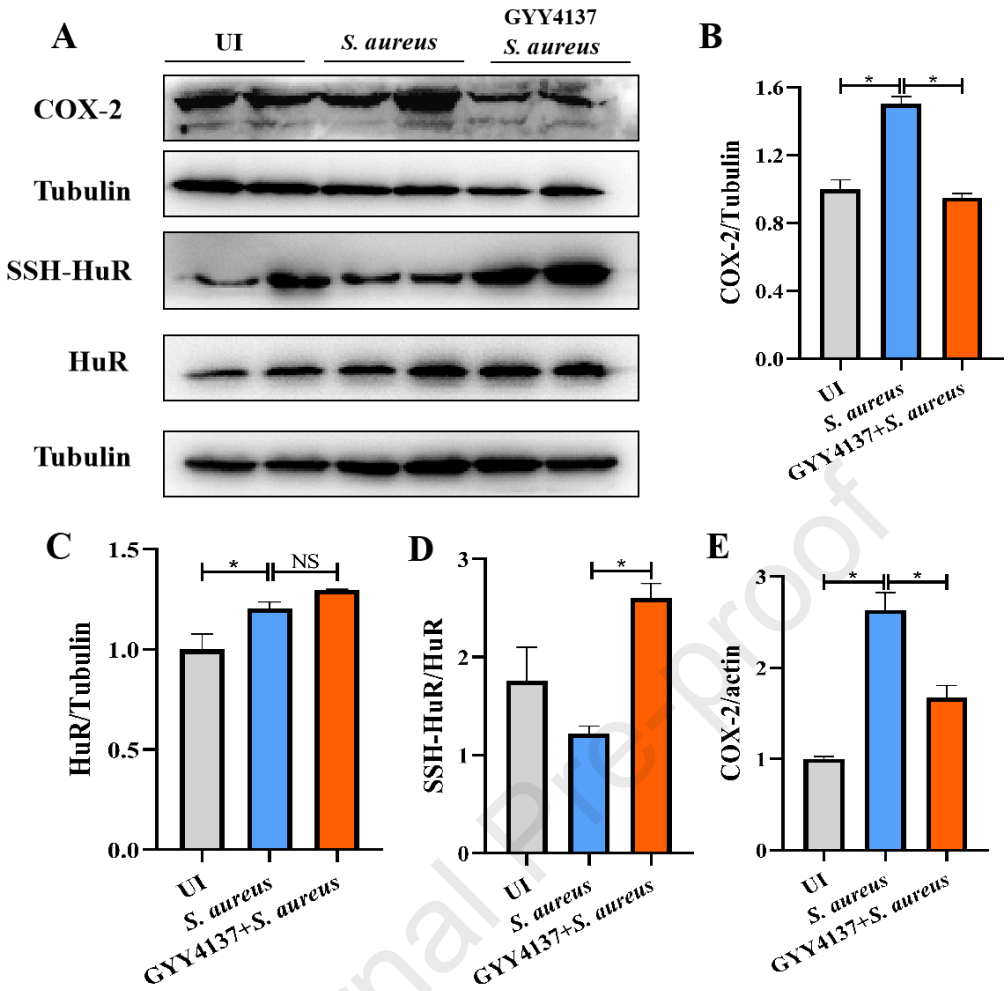


Figure 8 The CBS-H_2S axis decreases expression of COX-2 and stabilizes mRNA through S-sulfhydration of HuR. (A) Representative western blot images of COX-2, HuR and SSH-HuR proteins in EpH4-Ev cells with or without GYY4137. (B-D) Relative band intensity. (E) Gene expression of COX-2. Data are presented as the means±SEMs (n=3). *(P<0.05)=significantly different.

Journal Pre-proof

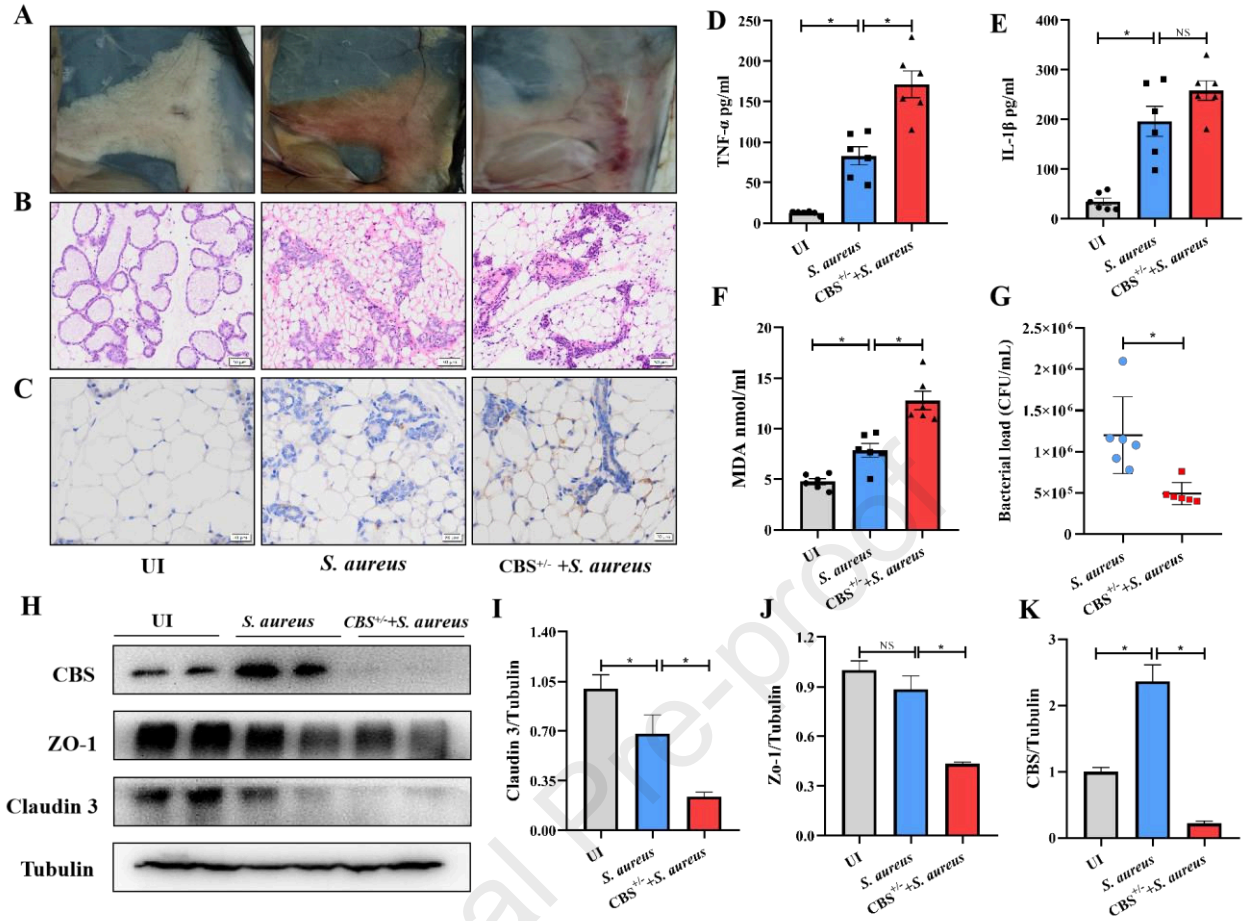


Figure 9 CBS$^{+/-}$ mice suffer significant tissue damage in response to *S. aureus* infection. Representative images of visual observation (A) and HE staining (B) of mammary gland of WT and CBS$^{+/-}$ mice after *S. aureus* infection. (C) Positive staining for macrophage marker CD68 during *S. aureus* infection. Comparison of serum pro-inflammatory factors including TNF-α (D) and IL-1β (E) oxidative stress marker MDA (F) as well as bacterial load (G) between WT and CBS$^{+/-}$ mice with *S. aureus* infection. (H) Representative western blot images of CBS, ZO-1 and Claudin 3 proteins in WT and CBS$^{+/-}$ mice with *S. aureus*. (I-K) Relative band intensity. Data are presented as the means±SEMs (n=5/6). *(P<0.05)=significantly different.

Journal Pre-proof

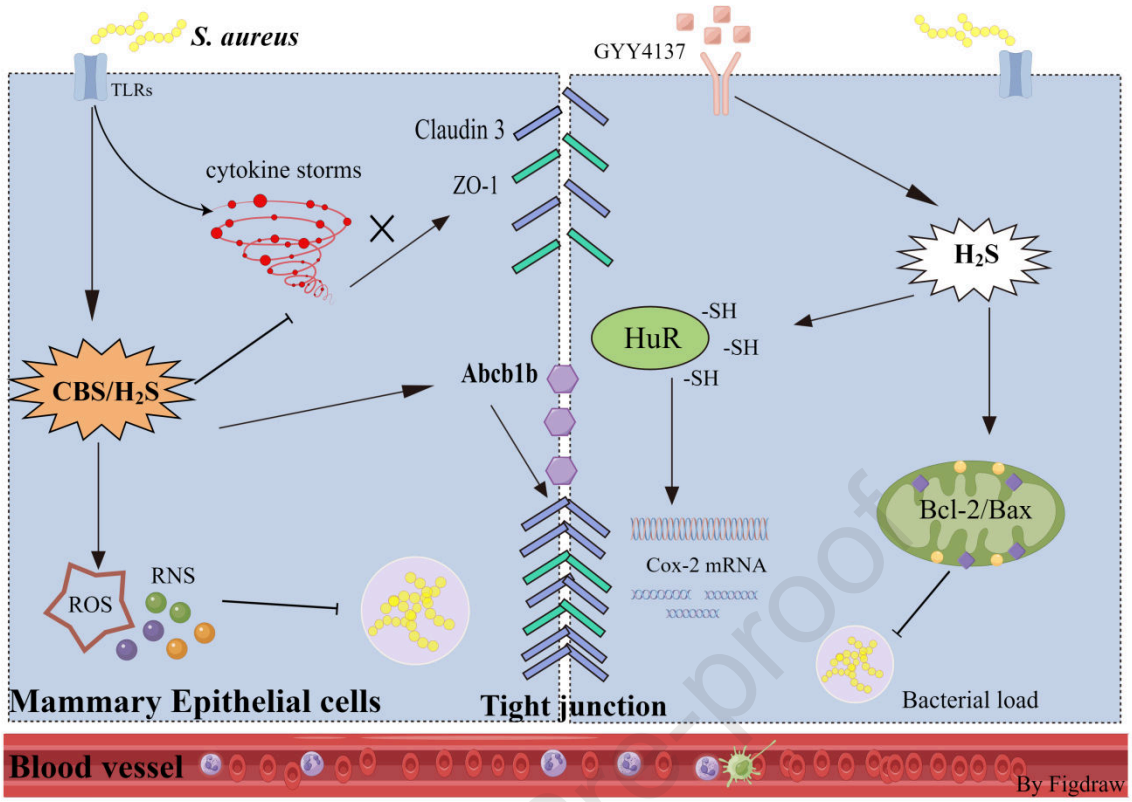


Figure 10. Schematic model showing how CBS attenuates inflammation and maintains the integrity of the BMB during *S. aureus* infection.

Journal Pre-proof

Journal Pre-proof

Highlights

1. *S. aureus* infection in the udder results in an increase in CBS.

2. CBS plays a role in the negative regulation of inflammation.

3. CBS contribute to maintain BMB integrity via promoting Abcb1b expression

Journal Pre-proof

Journal Pre-proof

Conflicts of interests

The authors declare no competing interests.

Journal Pre-proof



https://dspace.org/

A critical role for host-derived cystathionine--synthase, in Staphylococcus aureus-induced udder infection

Fu, Shaodong; Yang, Bo; Gao, Yabin; Qiu, Yawei; Sun, Naiyan ...

2024-01-01
Elsevier
https://doi.org/10.1016/j.freeradbiomed.2023.11.001
Fu S, Yang B, Gao Y, et al., (2024) A critical role for host-derived cystathionine--synthase, in Staphylococcus aureus-induced udder infection. Free Radical Biology and Medicine, Volume 210, January 2024, pp. 13-24

Downloaded from a DSpace repository. https://dspace.org/