Figure S1

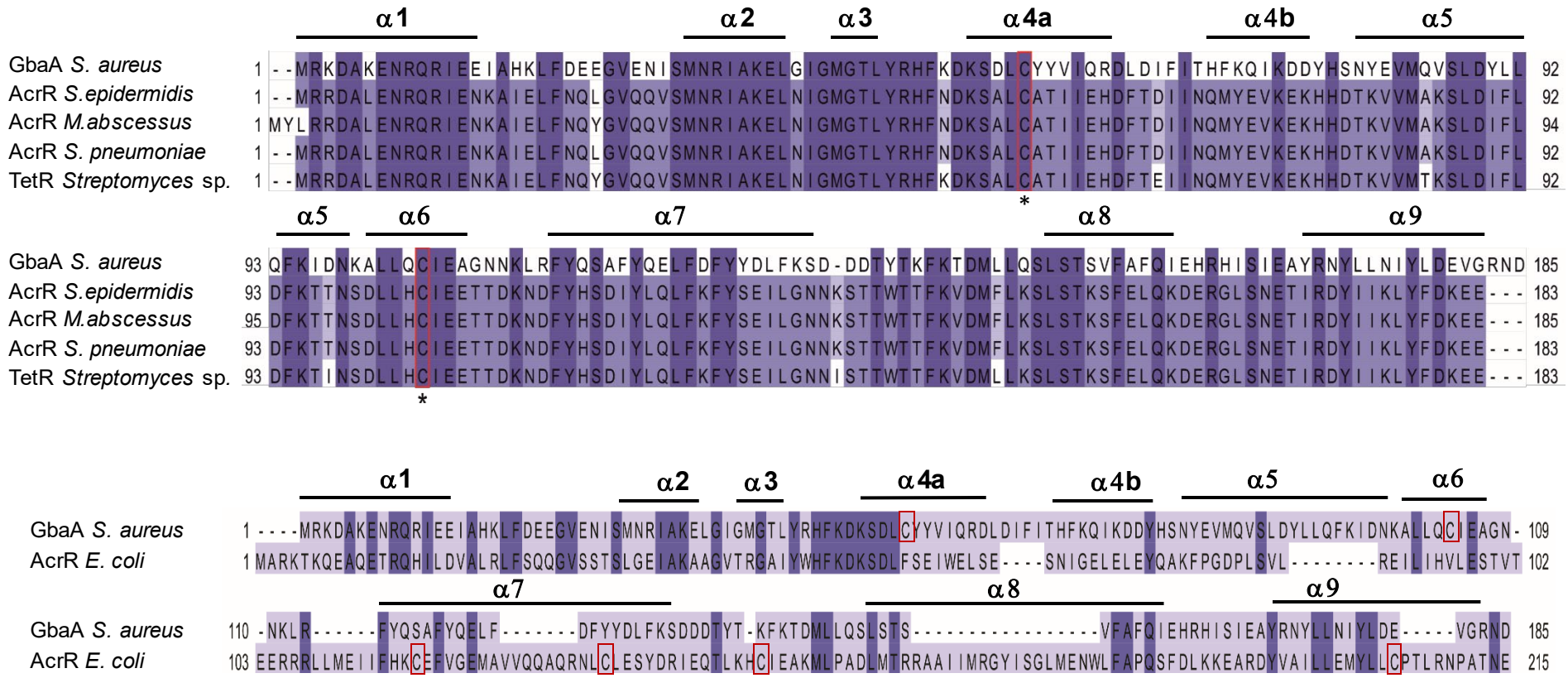


Figure S1. Multiple protein sequence alignments of GbaA homologs. GbaA (SACOL2593) of *S. aureus* COL was aligned with AcrR homologs of *Staphylococcus epidermidis* (A0A3S4K8G5, 48.35%), *Mycobacteroides abscessus* (A0A1U0JWP0, 47.8%), *Streptococcus pneumoniae* (A0A0U0EMJ4, 48.35%), TetR of *Streptomyces* sp. WAC00263 (A0A1V1YYR9, 49.4%) and AcrR of *E. coli* (P0ACS9, 27.17%). The protein sequence comparison was performed with Clustal Omega and is presented in Jalview. Intensity of the blue color gradient is based on 50% sequence identity. The conserved Cys55 and Cys104 of GbaA are marked with asterisks (*).

Figure S2

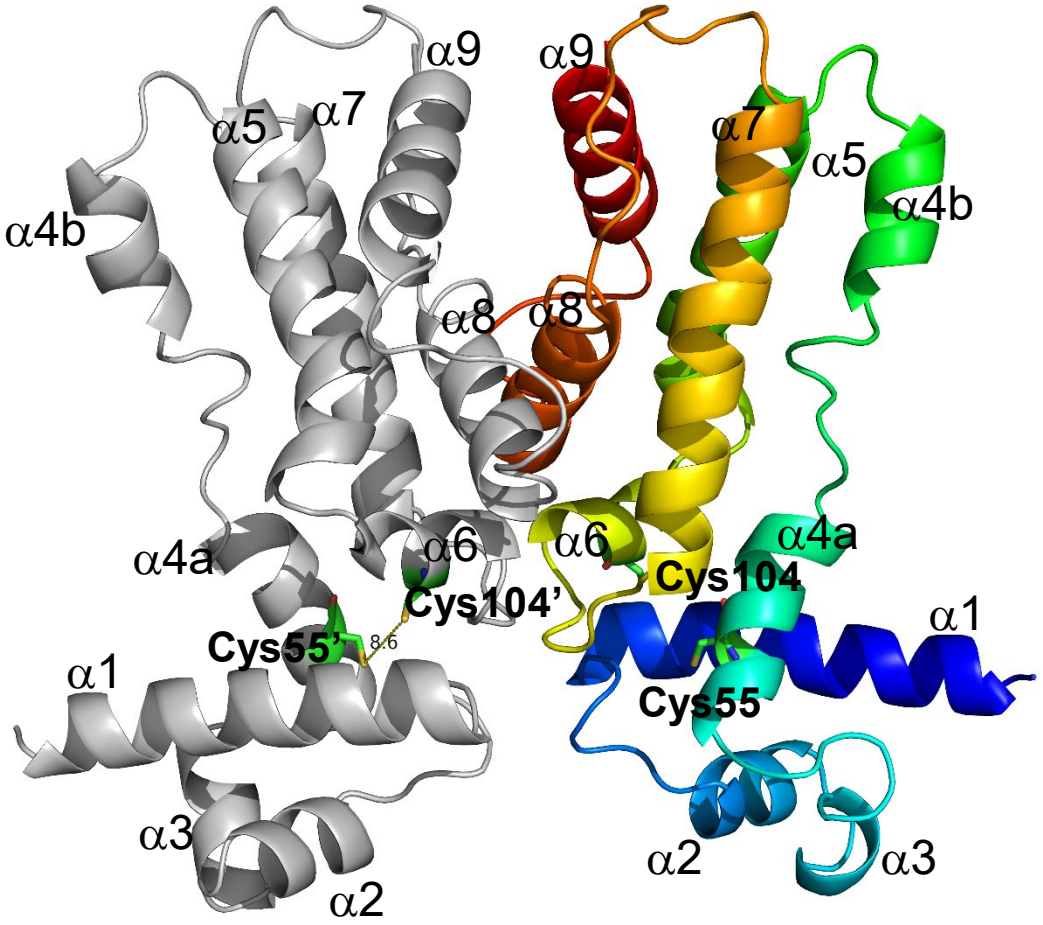


Figure S2. GbaA structural model. The structural model of the TetR repressor GbaA was generated with SWISS-MODEL (https://swissmodel.expasy.org/) and visualized with PyMol based on the template of *E. coli* AcrR (PDB:3GBC), which showed 27.17 % sequence identity to GbaA. The distance of Cys55 and Cys104 was calculated as ~8.6A in each subunit. Cys55 and Cys104 are oxidized to intramolecular disulfides under diamide stress in each subunit. The 9 α-helical domains are numbered based on AcrR template (Li et al., J Mol Biol 2007; 374(3): 591–603 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2254304/)

Figure S3

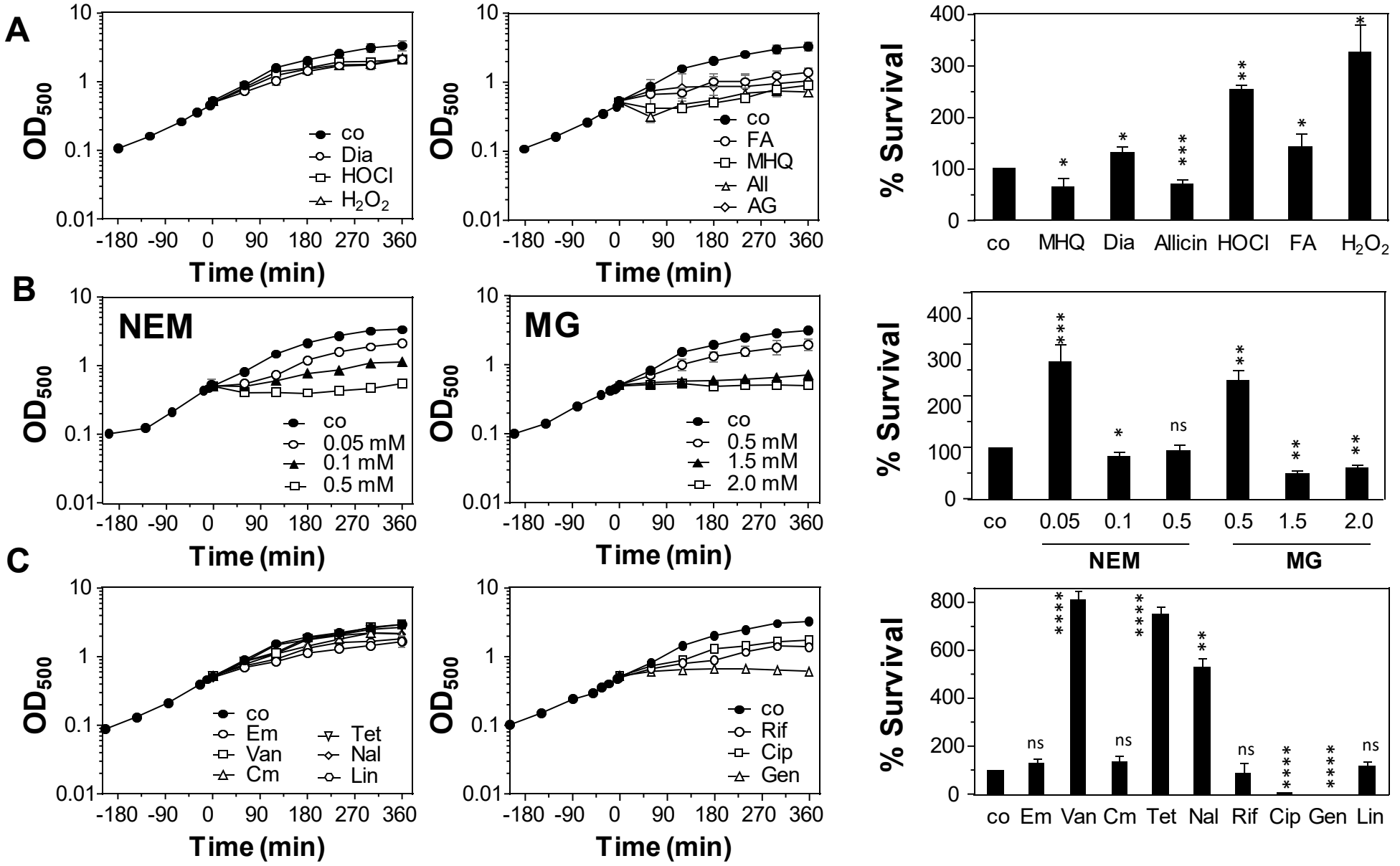


Figure S3. Growth and survival of *S. aureus* COL after treatment with thiol-reactive compounds (A,B) and antibiotics (C) as applied in Fig. 1A-C. To monitor the effect of the compounds on the growth and survival of *S. aureus* COL, cells were cultivated in RPMI medium until an OD_{500} of 0.5 and subjected to 5 µg/ml AGXX®373 (AG), 50 µM methylhydroquinone (MHQ), 2 mM diamide (Dia), 300 µM allicin (All), 1 mM HOCl, 0.75 mM formaldehyde (FA) and 10 mM H_2O_2 **(A)**, to 0.05 - 0.5 mM NEM and 0.5 - 2 mM MG stress **(B)** and to antibiotics, such as 0.25 µg/ml erythromycin (Em), 0.5 µg/ml vancomycin (Van), 4 µg/ml chloramphenicol (Cm), 5 µg/ml tetracycline (Tet), 128 µg/ml nalidixic acid (Nal), 0.1 µg/ml rifampicin (Rif), 32 µg/ml ciprofloxacin (Cip), 2 µg/ml gentamicin (Gen) and 2 µg/ml linezolid (Lin) **(C)**. The percentage of bacterial survival after 2 hours of treatment is calculated versus the untreated control and shown at the right in **A-C**. The results are from 3 biological replicates, error bars represent the standard deviation (SD) and the statistics was calculated using a Student's unpaired two-tailed t-test. The *p*-values were determined at 2h after stress exposure versus the control for growth curves in **A-C** as follows: p=0.0204 Dia, p=0.3874 HOCl, p=0.0570 H2O2, p=0.0327 FA, p=0.0024 MHQ, p≤0.0001 All, p=0.1216 AGXX, p=0.0001-0.0002 NEM, p=0.001-0.0001 MG, p=0.006 Em, p=0.0263 Van, p=0.1111 Cm, p=0.0087 Tet, p=0.8211 Nal, p=0.0019 Lin, p≤0.0001 Rif, p=0.0010 Cip; p=0.0005 Gen. Symbols are defined as: ns p>0.05; *p≤0.05; **p≤0.01; ***p≤ 0.001 and ****p≤0.0001.

Figure S4

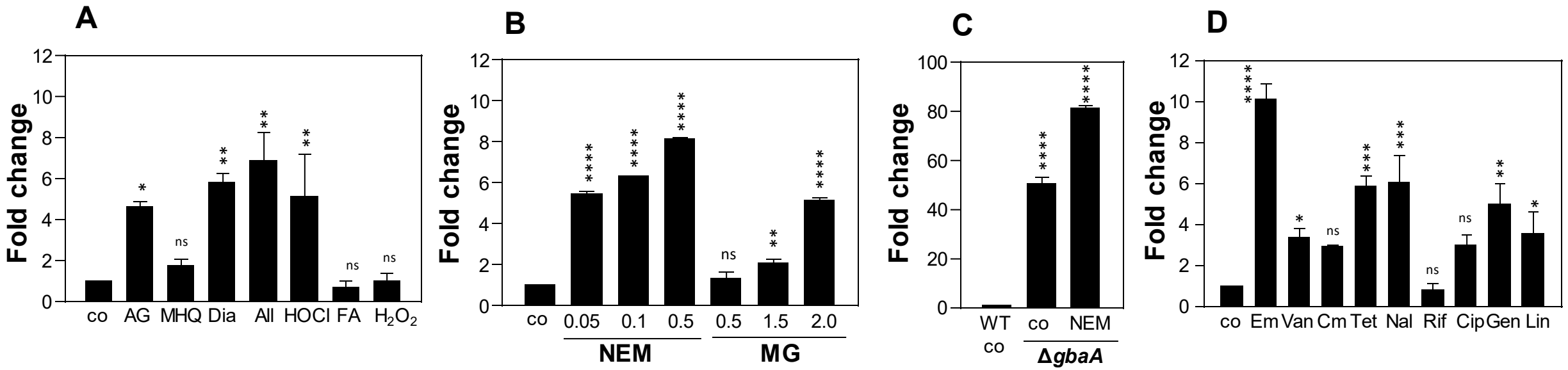


Figure S4. Quantification of the Northern blot transcripts of the *gbaAB* operon in *S. aureus* WT (A,B,D) and the *gbaA* mutant (C) after exposure to thiol-reactive compounds (A-C) and antibiotics (D) based on the band intensities of Fig. 1A-C. The *S. aureus* COL WT was subjected to sub-lethal doses of 5 µg/ml AGXX®373 (AG), 50 µM methylhydroquinone (MHQ), 2 mM diamide (Dia), 300 µM allicin (All), 1 mM HOCl, 0.75 mM formaldehyde (FA) and 10 mM H2O2 **A)** or to increasing doses of 0.05-0.5 mM N-ethylmaleimide (NEM) and 0.5-2 mM methylglyoxal (MG) **B)**. For comparison of the weak transcriptional induction of the *gbaAB* operon after NEM and MG stress in the WT, the *gbaA* mutant was analyzed under control and NEM stress showing full derepression of the *gbaAB* operon in the control **C)**. The Northern blot band intensities were quantified in *S. aureus* COL WT after treatment with sub-lethal doses of antibiotics **D)**, including 0.25 µg/ml erythromycin (Em), 0.5 µg/ml vancomycin (Van), 4 µg/ml chloramphenicol (Cm), 5 µg/ml tetracycline (Tet), 128 µg/ml nalidixic acid (Nal), 0.1 µg/ml rifampicin (Rif), 32 µg/ml ciprofloxacin (Cip), 2 µg/ml gentamicin (Gen) and 2 µg/ml linezolid (Lin) in C). Band intensities of the *gbaAB* operon were quantified from 2 biological replicates using ImageJ and error bars represent the standard deviation (SD). The statistics was calculated in comparison to the WT control (co) using ordinary one-way ANOVA and Dunnet's multiple comparisons test using the Graph prism software. Symbols are defined as: ns $p>0.05$; *$p\leq0.05$; **$p\leq0.01$; ***$p\leq 0.001$ and ****$p\leq0.0001$.

Figure S5

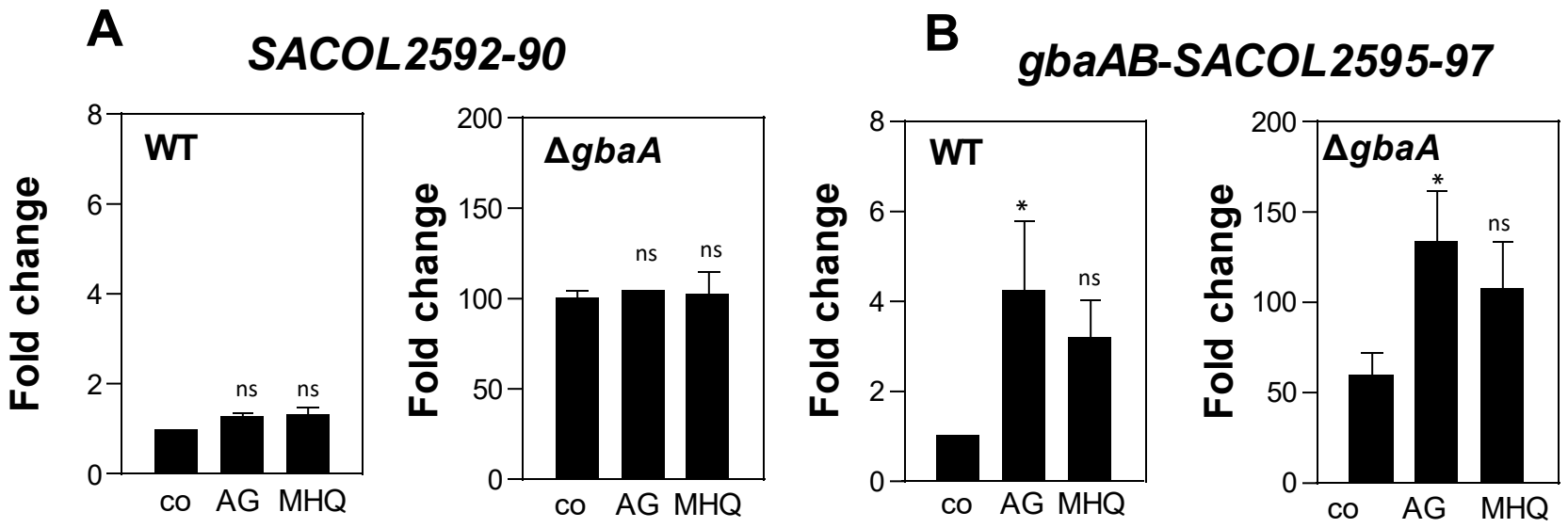


Figure S5. Quantification of the Northern blot transcripts of the *SACOL2592-90* (A) and *gbaAB* operons (B) in *S. aureus* WT and the *gbaA* mutant after AGXX® and MHQ stress based on the band intensities of Fig. 2C,D. Band intensities of the Northern blot transcripts were quantified from 2 biological replicates using ImageJ and error bars represent the standard deviation (SD). The statistics of the stress sample was calculated in comparison to the WT and *gbaA* mutant controls (co) using ordinary one-way ANOVA and Dunnet's multiple comparisons test using the Graph prism software. Symbols are defined as: ns $p>0.05$; ***$p\leq 0.001$ and ****$p\leq 0.0001$.

Figure S6

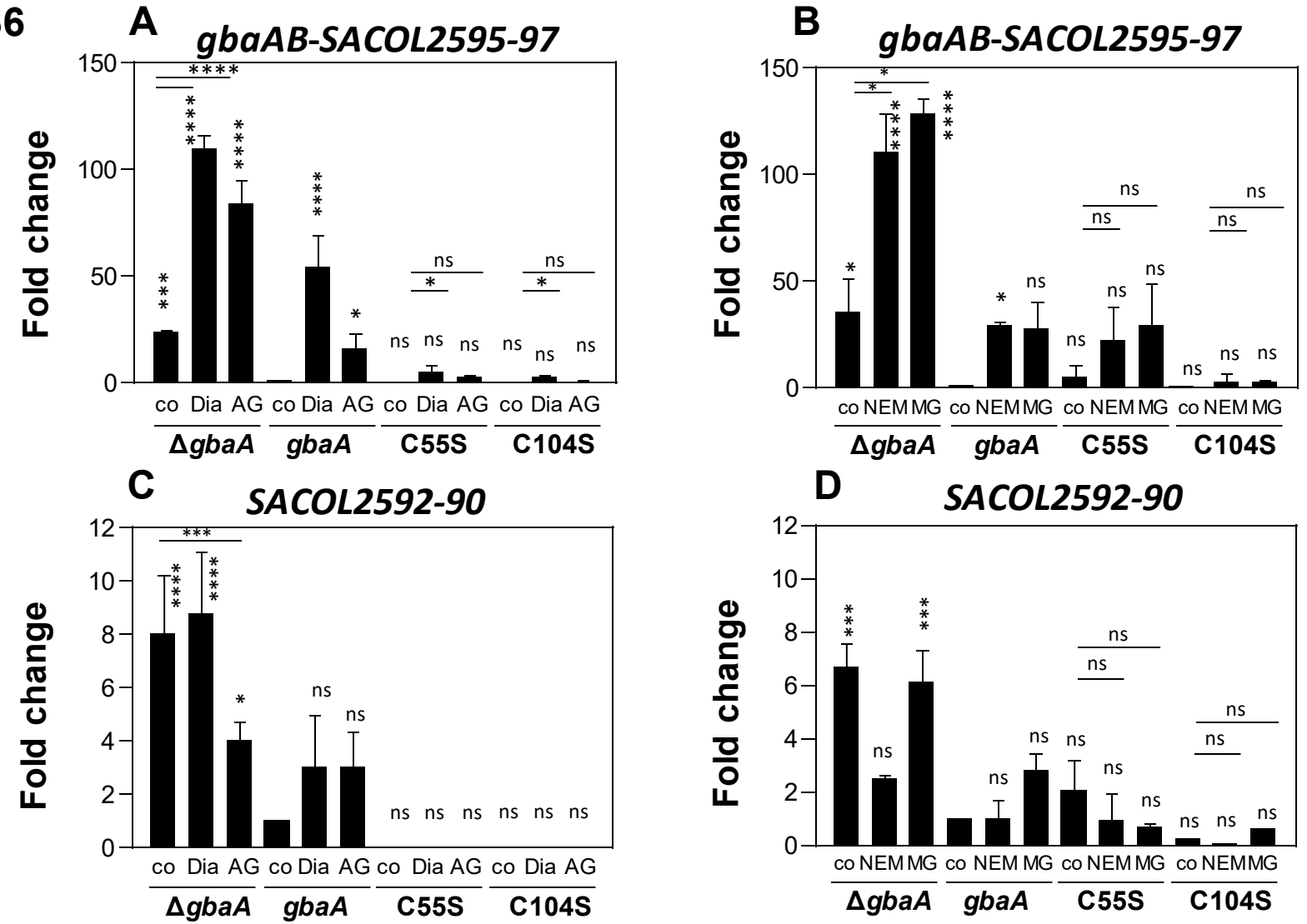


Figure S6. Quantification of the Northern blot transcripts of the *gbaAB* operon (A, B) and *SACOL2592-90* operon (C, D) in the *S. aureus* COL Δ*gbaA* mutant and the *gbaA, gbaAC55S* and *gbaAC104S* complemented strains after exposure to 2 mM diamide (Dia) and 5 µg/ml AGXX® (AG) (A,C) as well as 0.3 mM NEM and 2 mM methylglyoxal (MG) stress (B, D) based on the band intensities of Fig. 4A-D. Band intensities of the *gbaAB* and *SACOL2592-90* operons were quantified from 2 biological replicates using ImageJ and error bars represent the standard deviation (SD). The statistics was calculated in comparison to the untreated *gbaA* complemented strain (*gbaA* co) and in stressed samples versus controls using ordinary one-way ANOVA and Dunnet's multiple comparisons test using the Graph prism software. Symbols are defined as: ns $p>0.05$; *$p\leq0.05$; **$p\leq0.01$; ***$p\leq 0.001$ and ****$p\leq0.0001$.

Figure S7

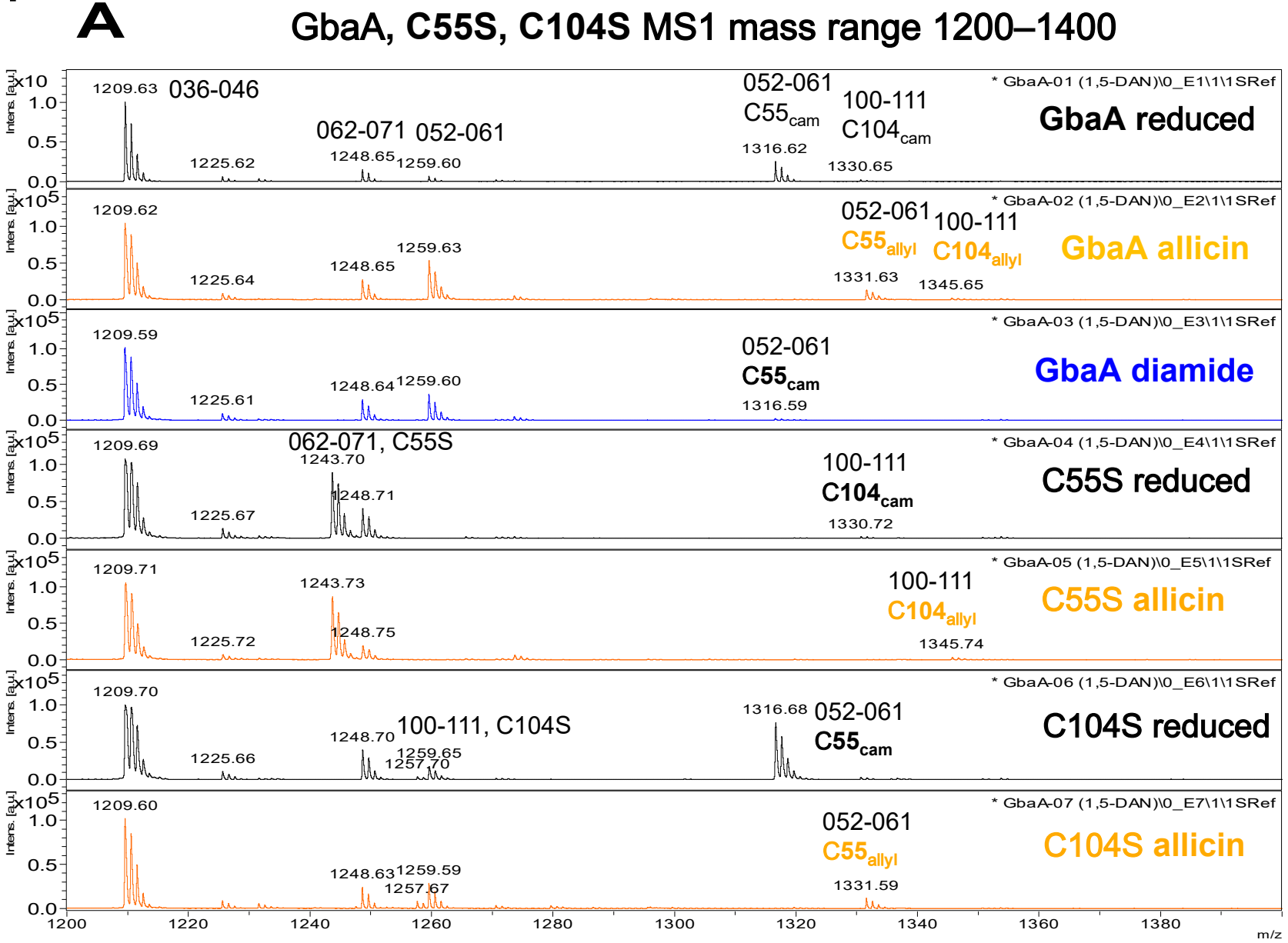
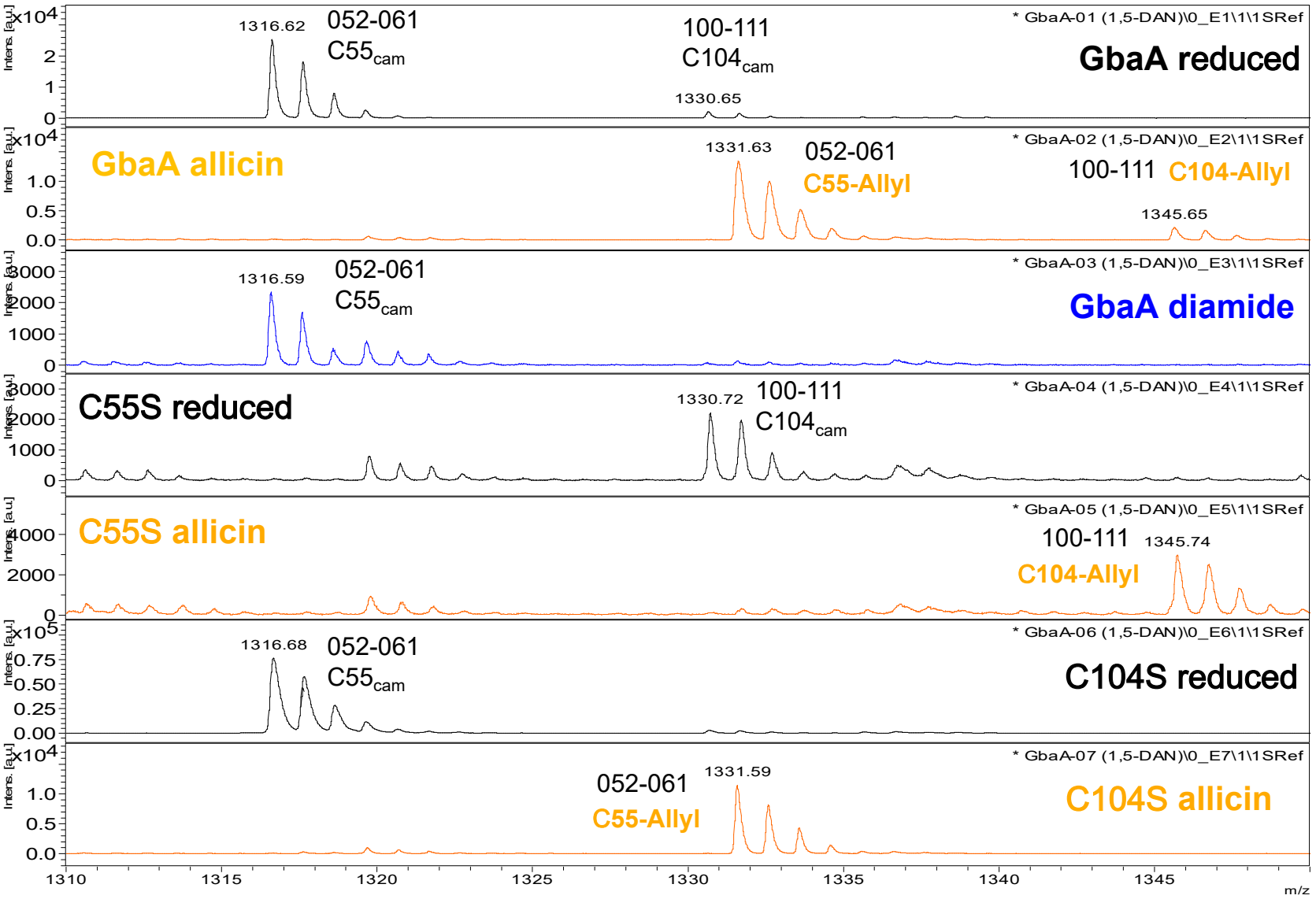


Figure S7. Identification of disulfides in GbaA, the GbaAC55S and GbaAC104S mutants after diamide and allicin using MALDI-TOF MS *in vitro*. GbaA proteins were reduced with 10 mM DTT, treated with 1 mM diamide or 1 mM allicin, separated by non-reducing SDS-PAGE, tryptic digested and analyzed using MALDI-TOF-MS. The overview MS1 scan is displayed in the mass ranges m/z= 1200-1400 **(A)**, 1310-1350 **(B)** and 2300-2650 **(C)**.

B


GbaA, C55S, C104S MS1 mass range 1310–1350
* GbaA-01 (1,5-DAN)\0_E1\1\1SRef
GbaA reduced
1316.62
052-061
C55cam
100-111
C104cam
1330.65
* GbaA-02 (1,5-DAN)\0_E2\1\1SRef
GbaA allicin
1331.63
052-061
C55-Allyl
100-111 C104-Allyl
1345.65
* GbaA-03 (1,5-DAN)\0_E3\1\1SRef
GbaA diamide
1316.59
052-061
C55cam
* GbaA-04 (1,5-DAN)\0_E4\1\1SRef
C55S reduced
1330.72
100-111
C104cam
* GbaA-05 (1,5-DAN)\0_E5\1\1SRef
C55S allicin
100-111 1345.74
C104-Allyl
* GbaA-06 (1,5-DAN)\0_E6\1\1SRef
C104S reduced
1316.68
052-061
C55cam
* GbaA-07 (1,5-DAN)\0_E7\1\1SRef
C104S allicin
052-061
1331.59
C55-Allyl
Intens. [a.u.]
m/z
1310
1315
1320
1325
1330
1335
1340
1345

C

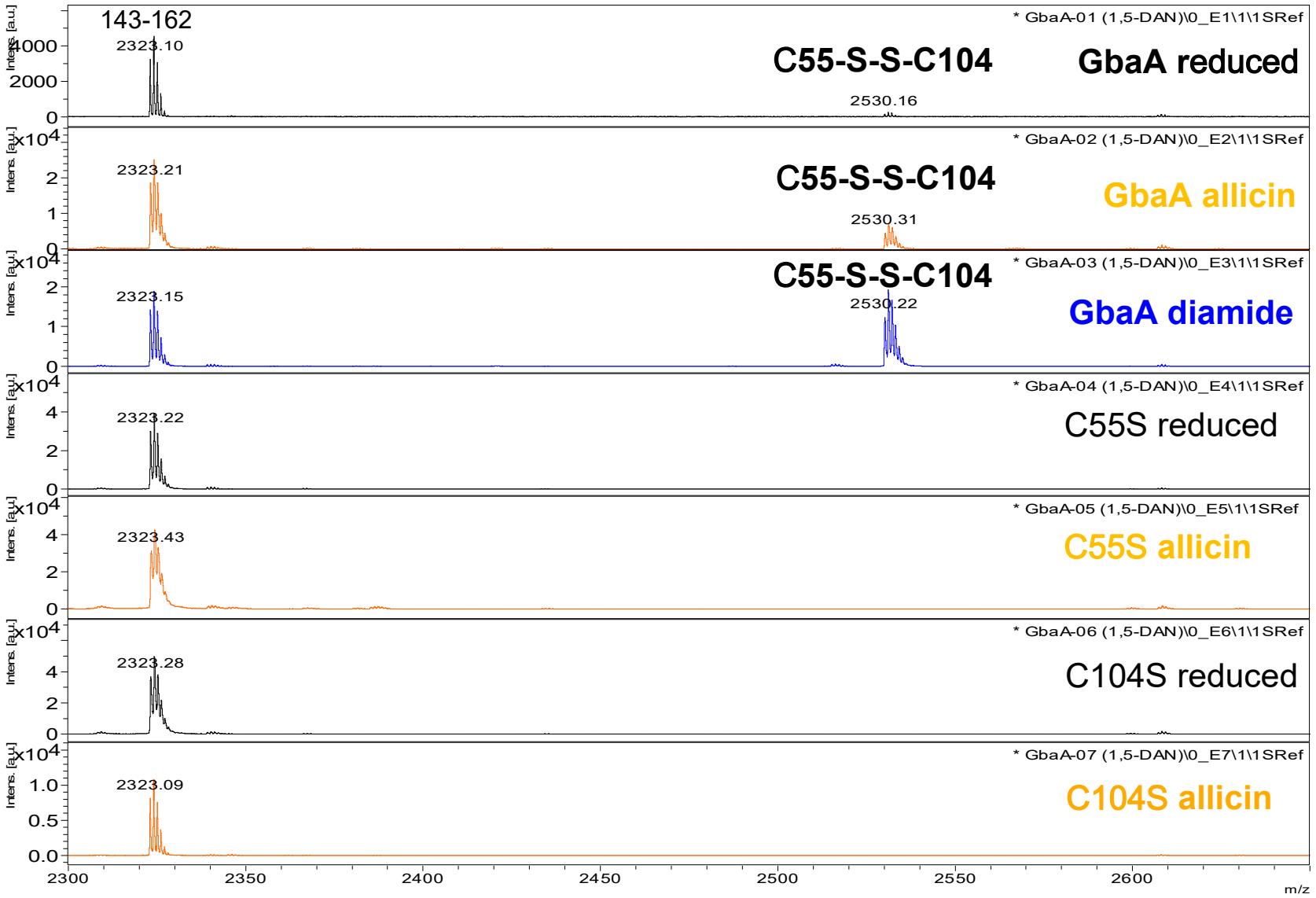

GbaA, C55S, C104S MS1 mass range 2300–2650
(GbaA intramolecular C55-C104 disulfide)
143-162
2323.10
* GbaA-01 (1,5-DAN)\0_E1\1\1SRef
C55-S-S-C104
GbaA reduced
2530.16
2323.21
* GbaA-02 (1,5-DAN)\0_E2\1\1SRef
C55-S-S-C104
GbaA allicin
2530.31
2323.15
* GbaA-03 (1,5-DAN)\0_E3\1\1SRef
C55-S-S-C104
GbaA diamide
2530.22
2323.22
* GbaA-04 (1,5-DAN)\0_E4\1\1SRef
C55S reduced
2323.43
* GbaA-05 (1,5-DAN)\0_E5\1\1SRef
C55S allicin
2323.28
* GbaA-06 (1,5-DAN)\0_E6\1\1SRef
C104S reduced
2323.09
* GbaA-07 (1,5-DAN)\0_E7\1\1SRef
C104S allicin
Intens. [a.u.]
m/z
2300
2350
2400
2450
2500
2550
2600

Figure S8

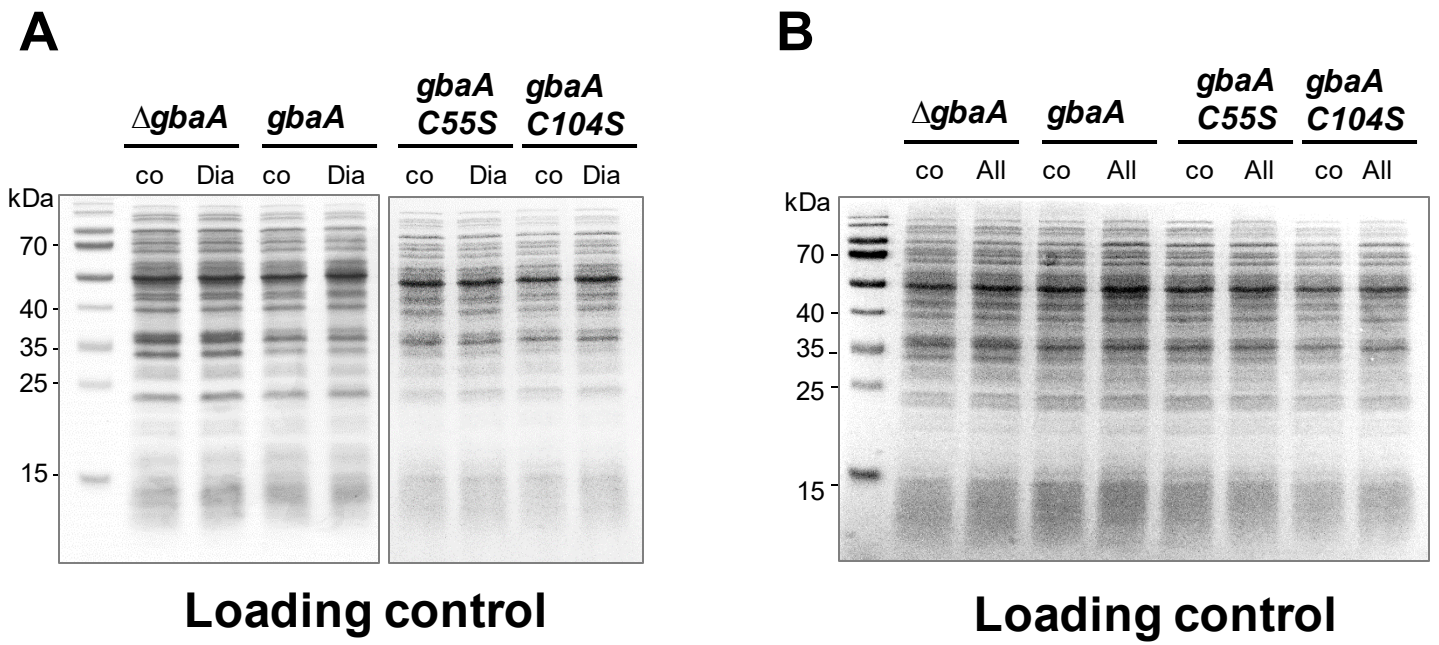


Figure S8. Loading control of non-reducing SDS-PAGE of protein extracts of the *S. aureus* COL *gbaA* mutant and *gbaA, gbaAC55S and gbaAC104S* complemented strains after diamide (A) and allicin (B) stress. The same protein extracts used for Western blot analyses in Fig. 6D,E and Fig. 7D,E were separated by non-reducing SDS-PAGE analysis and stained with Coomassie Blue.

Figure S9

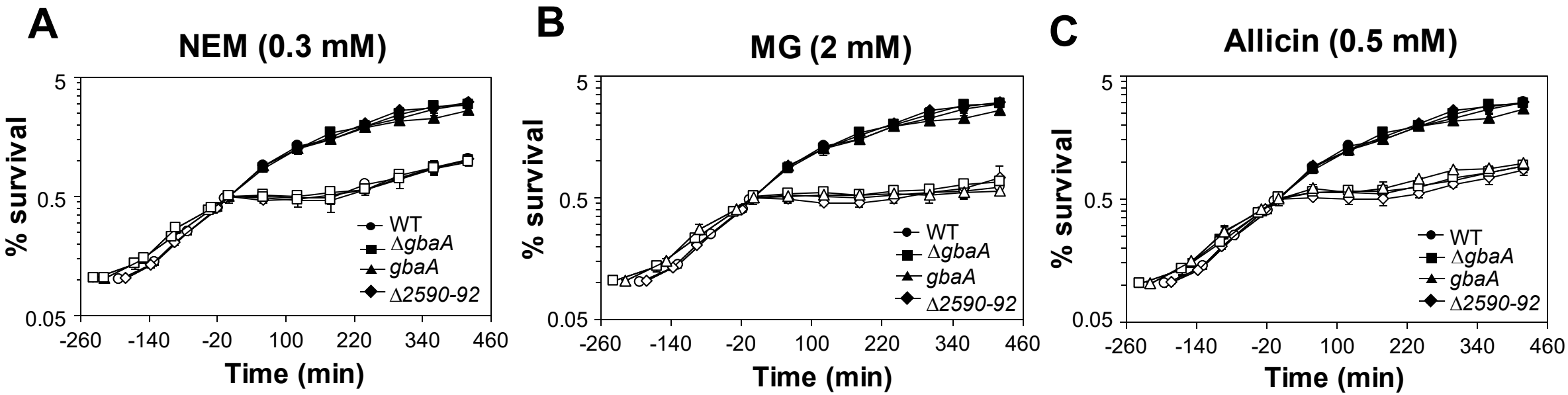


Figure S9. Growth curves of *S. aureus* COL WT, the *gbaA* and *SACOL2590-92* mutants and the *gbaA* complemented strains after exposure to NEM (A), MG (B) and allicin (C). For growth curves, *S. aureus* strains were cultivated in RPMI medium until an OD_{500} of 0.5 and subjected to 0.3 mM NEM **(A)**, 2 mM MG **(B)** and 0.5 mM allicin **(C)**. The results are from 4 biological replicates and error bars represent the standard deviation (SD). No significant growth difference was observed for the *gbaA* and *SACOL2590-92* mutants and the *gbaA* complemented strains in comparison to the WT strain.