SUPPORTING INFORMATION

Toward the quantitative characterization of arginine phosphorylations in *Staphylococcus aureus*

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ABBREVIATIONS

BE, BioExpress 1000; BIP, 2,2'-bipyridine; TMAD, N,N,N',N'-tetramethylazodicarboxamide

REFERENCES

(1) Bernhardt, J.; Funke, S.; Hecker, M.; Siebourg, J. Visualizing Gene Expression Data via Voronoi Treemaps. *6th Int. Symp. Vor. Diagrams Sci. Eng. ISVD 2009* **2009**, 233–241.

(2) Haft, D. H.; Loftus, B. J.; Richardson, D. L.; Yang, F.; Eisen, J. A.; Paulsen, I. T.; White, O. TIGRFAMs: A Protein Family Resource for the Functional Identification of Proteins. *Nucleic Acids Res.* **2001**, *29* (1), 41–43.

(3) Tanabe, M.; Kanehisa, M. Using the KEGG Database Resource. *Curr. Protoc. Bioinforma.* **2012**, Ch.1, Unit 1.12.

(4) Junker, S.; Maaß, S.; Otto, A.; Michalik, S.; Morgenroth, F.; Gerth, U.; Hecker, M.; Becher, D. Spectral Library Based Analysis of Arginine Phosphorylations in *Staphylococcus Aureus*. *Mol. Cell. Proteomics* **2018**, *17* (2), 335–348.

SUPPLEMENTAL FIGURES

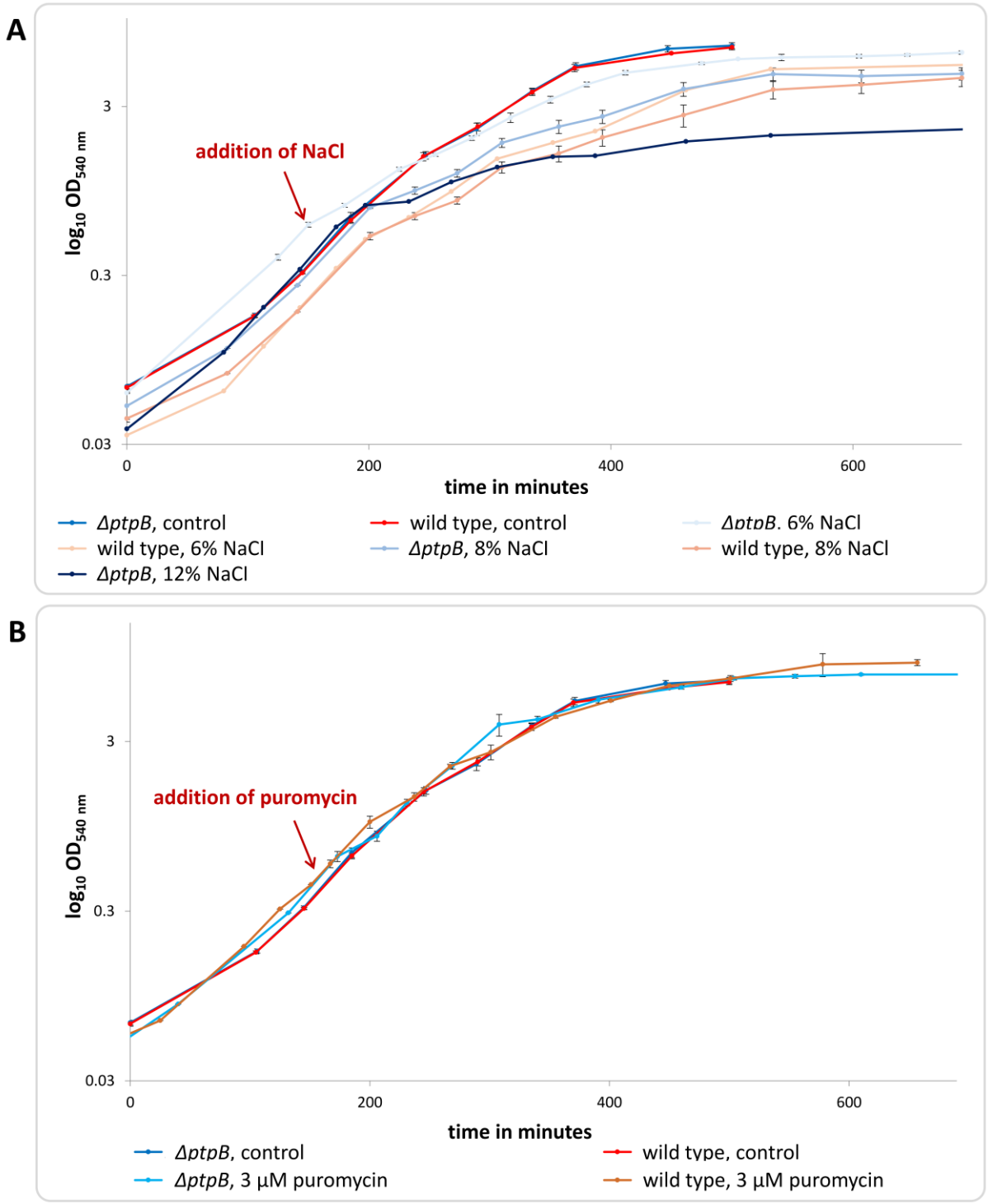


Figure S-1: Growth curves of wild type and *ΔptpB* mutant under stress conditions

The figure displays the comparison of bacterial cell growth between wild type and *ΔptpB* under different stress conditions. **A)** Different concentrations of NaCl. *ΔptpB* growth curves

are colored in shades of blue and wild type curves are depicted in shades of red. **B)** 3 µmol/l puromycin. *ΔptpB* control is shown in dark blue, stressed *ΔptpB* samples are shown in light blue. Wild type control is displayed in red, stressed wild type samples are colored ochraceous.

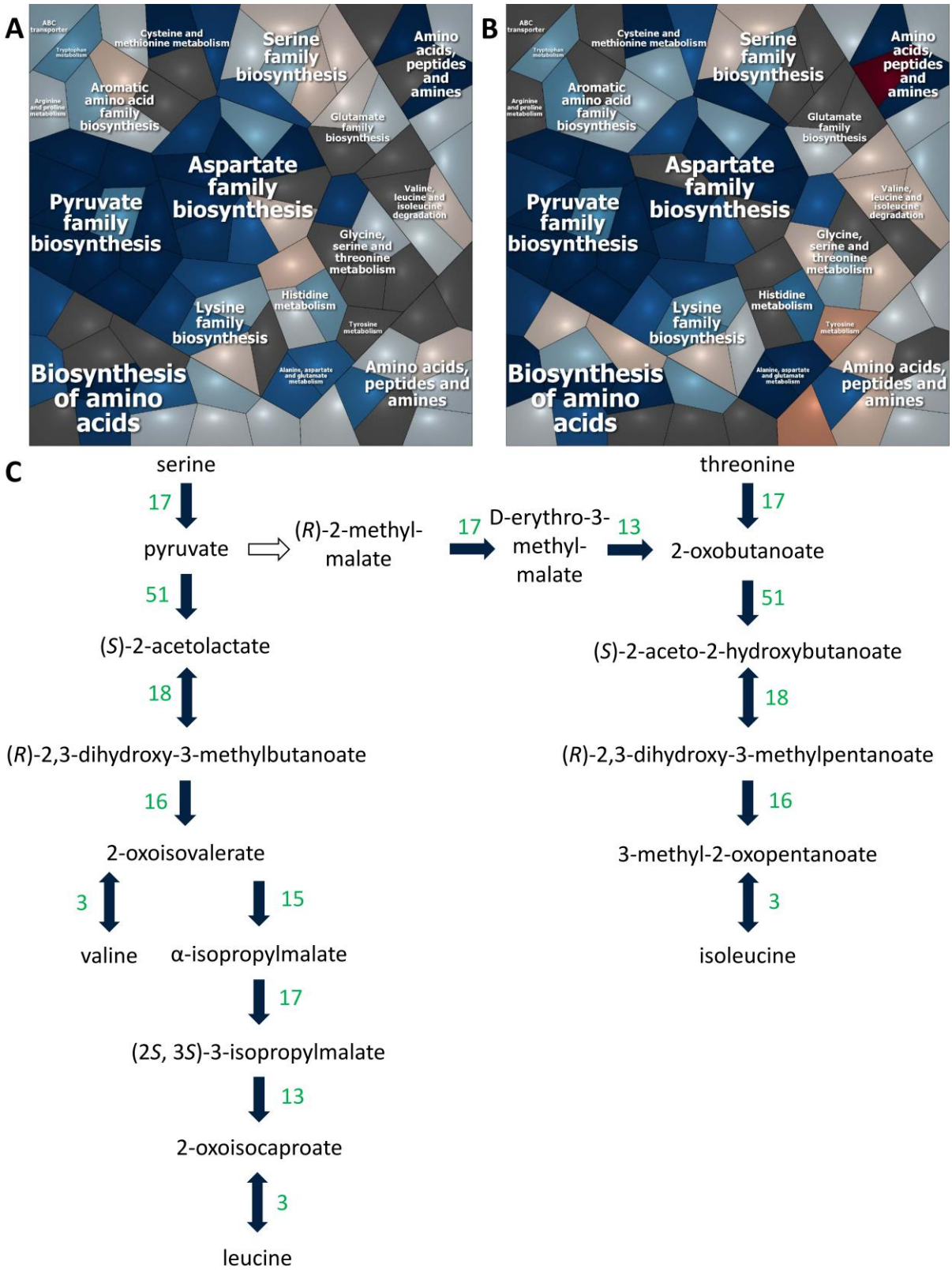
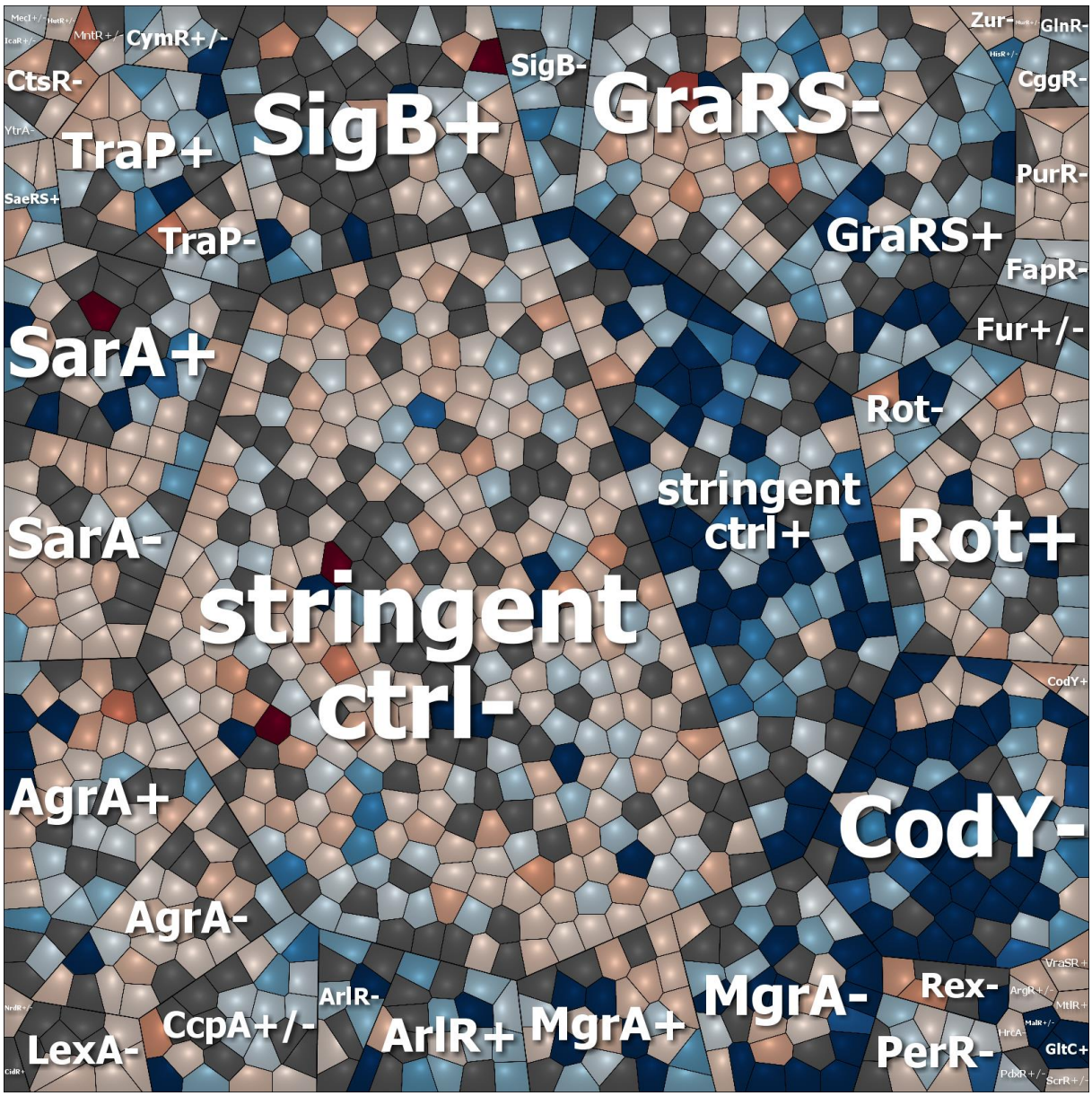


Figure S-2: Effects on amino acid biosynthesis

The figure highlights the effects of *ptpB* deletion on staphylococcal amino acid biosynthesis. The Voronoi treemaps[1] provide all proteins which were significantly quantified in at least two

biological replicates, sorted according to the respective functional classification scheme according to TIGRFAM[2] or KEGG[3] based annotation. Each cell displays a single protein which is colored in shades of blue if found with higher amounts in wild type cells. It is depicted in shades of red if found in higher amounts in *ΔptpB* cells. **A)** Comparison of wild type and mutant under control conditions. **B)** Comparison of wild type and mutant under stress conditions. **C)** This figure depicts the discussed part of the amino acid biosynthesis pathway of *S. aureus* COL. Blue arrows symbolize enzymes which are encoded in the staphylococcal genome and quantified with significantly higher amounts in wild type cells during comparison with the mutant under stress conditions. If the ratio is > 2, it is additionally written in green numbers. White arrows symbolize reactions *S. aureus* is not capable of.

A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HutR+/-
IcaR+/-
MntR+/-
CymR+/-
CtsR-
SigB-
SigB+
GraRS-
Zur-
HutR+/-
GlnR-
HisR+/-
CggR-
YtrA-
TraP+
PurR-
SaeRS+
TraP-
GraRS+
FapR-
Fur+/-
SarA+
Rot-
stringent ctrl+
Rot+
SarA-
stringent ctrl-
CodY+
AgrA+
CodY-
AgrA-
YraSR+
ArlR-
MgrA-
Rex-
ArgR+/-
MtlR+
NrdR+/-
CcpA+/-
ArlR+
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PerR-
GltC+
CidR+
PdxR+/-
ScrR+/-

B

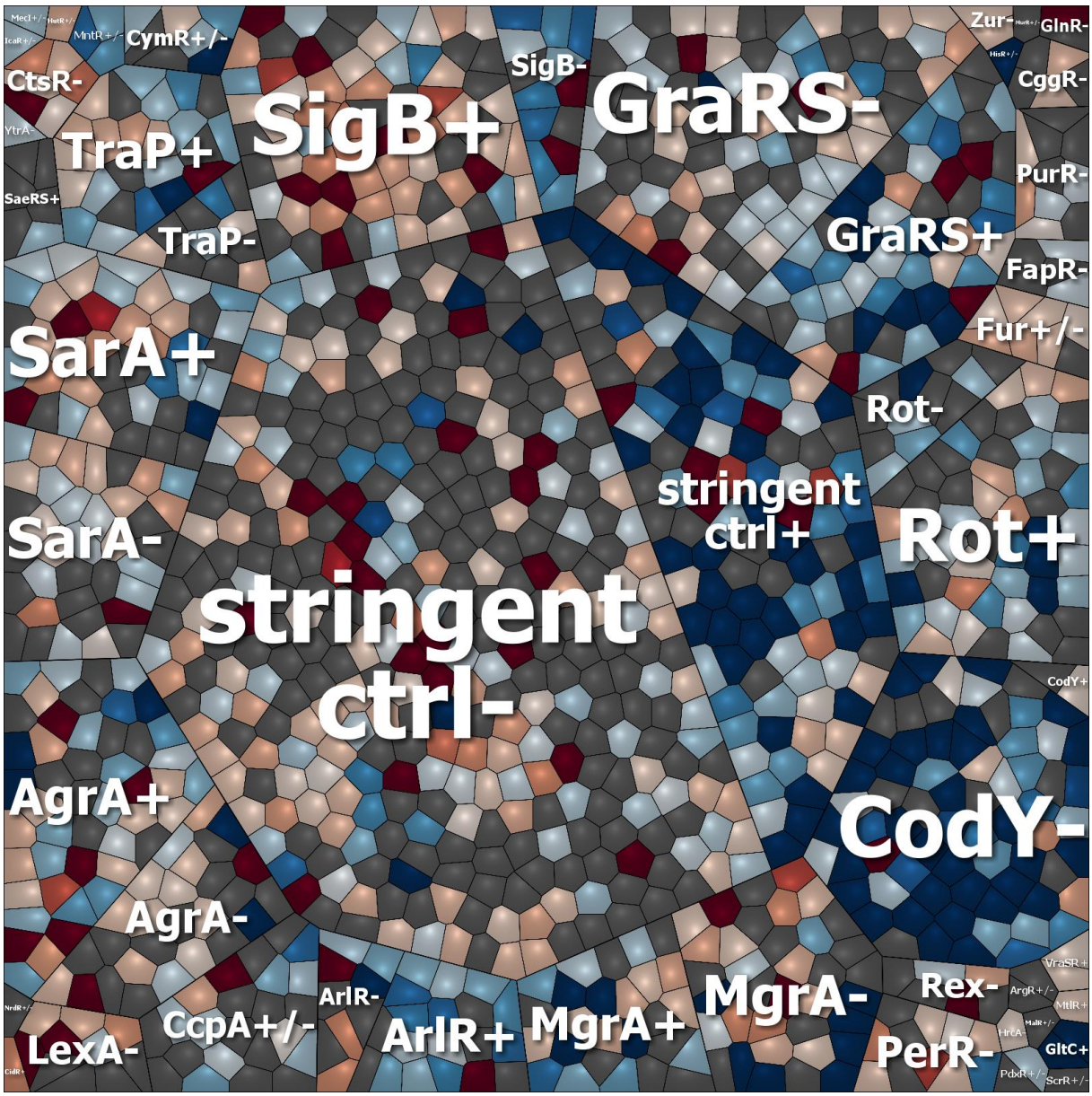

Figure S-3: Voronoi treemaps of proteome quantification

Comparison of quantified proteins between *ΔptpB* and wild type under control conditions (A) or under stress conditions (B). The Voronoi treemaps[1] provide all proteins which where significantly quantified in at least two biological replicates, sorted according to the respective regulons. Each cell displays a single protein which is colored in shades of red if found with higher amounts in *ΔptpB* cells. It is depicted in shades of blue if found in higher amounts in wild type cells.

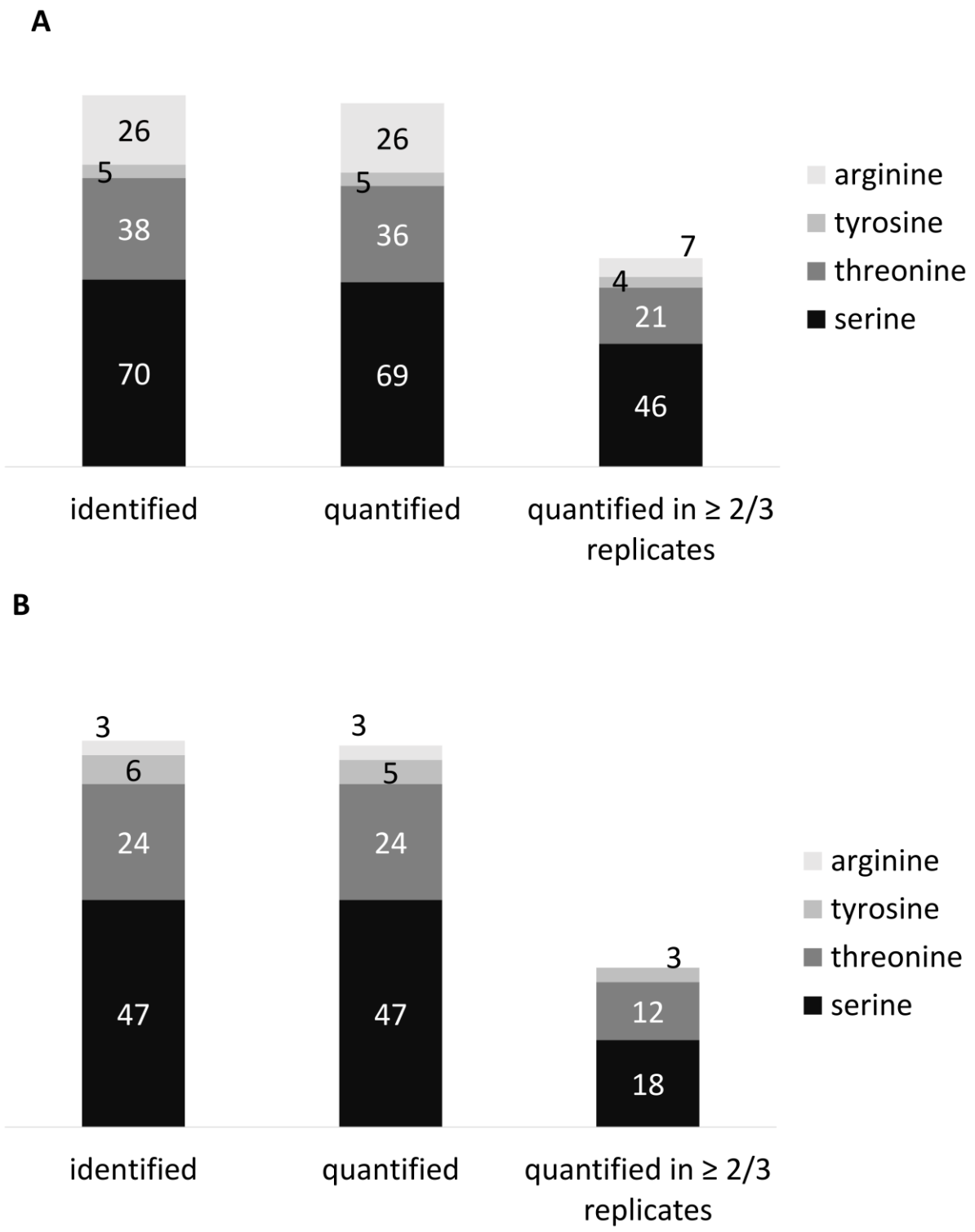

Figure S-4: Number of phosphopeptides

The figure shows the number of phosphopeptides identified, quantified and quantified in at least two out of three biological replicates for the SILAC-labeled samples enriched by TiO_2.

Results are differentiated according to the respective amino acid. **A)** Phosphopeptides in *ΔptpB* samples. **B)** Phosphopeptides in wild type samples.

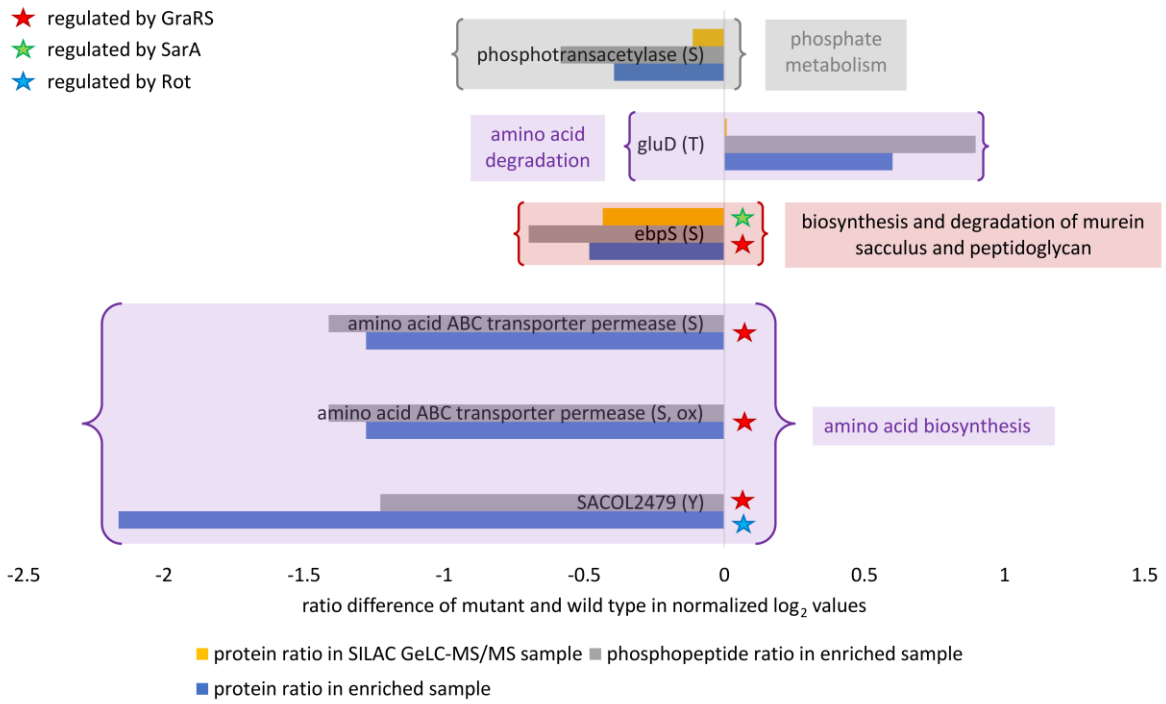

Figure S-5: Quantified phosphopeptides

The figure compares average protein ratios of classical proteome quantification (GeLC-MS/MS, yellow bar), protein ratio of enriched proteins (blue bar) and ratio of enriched phosphopeptides (grey bar) of at least two biological replicates of wild type and mutant. It shows the difference of *ΔptpB* and wild type ratio which means that peptides with positive ratios were found with higher amounts in *ΔptpB* cells. The figure focuses on phosphopeptides where both phosphopeptide and phosphoprotein amounts are significantly different. The brackets assort the peptides to proteins belonging to distinct functional groups. The respective metabolic pathway is written next to the brackets. The stars contain information about involved regulatons. Proteins with a red star are known to be regulated by the GraRS system, proteins with a green star by SarA and proteins with a blue star by Rot.

SUPPLEMENTAL TABLES

Table S-1: Stress conditions for phenotypic characterization

The following stress conditions were applied for the growth curves of the phenotypic characterization of *S. aureus* COL *ΔptpB*.

condition	stress inducer
control	no stress
heat stress	40 °C, 42 °C, 43 °C, 48 °C
oxidative stress (H_2O_2)	10 mmol/l, 50 mmol/l, 100 mmol/l
oxidative stress (TMAD)	500 µmol/l, 1 mmol/l, 2 mmol/l, 4 mmol/l
oxidative stress (methyl viologen)	10 nmol/l, 20 nmol/l, 50 nmol/l, 100 nmol/l, 10 µmol/l, 100 µmol/l, 1 mmol/l
iron limitation (cultivated in BE)	0.01 mmol/l BIP, 0.05 mmol/l BIP, 0.1 mmol/l BIP, 0.2 mmol/l BIP, 0.3 mmol/l BIP, 0.4 mmol/l BIP, 0.5 mmol/l BIP, 0.6 mmol/l BIP, 0.75 mmol/l BIP, 1 mmol/l BIP
salt stress	6% NaCl, 8% NaCl, 12% NaCl
antibiotic stress	3 µmol/l puromycin

TMAD = *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide; methyl viologen = 1,1'-dimethyl-4,4'-bipyridinium dichloride; BE = BioExpress 1000 (Cambridge Isotope Laboratories, Inc., Tewksbury, MA, USA); BIP = 2,2'-bipyridine

Table S-2: Phosphorylated peptides identified within at least two replicates of *ΔptpB* after oxidative stress

Table S-2 lists all phosphorylated peptides identified within at least two out of four biological replicates of *ΔptpB* after oxidative stress. It is provided as separate spreadsheet file.

Table S-3: Phosphoproteome comparison under control and oxidative stress conditions

This table compares all phosphosite and phosphoprotein identifications under control and stress conditions of *ΔptpB*. Identifications under control conditions were extracted from Junker *et al*.[4]. It is provided as separate spreadsheet file.

Table S-4: Results of proteome quantification

This table provides normalized $\log_2$ ratios for all proteins which were quantified in at least two biological replicates. All significant results ($p < 0.01$ according to the ANOVA if the protein was quantified in all conditions and $p < 0.01$ according to the *t*-test if the protein was not quantified in all four conditions) are colored in green. It is provided as separate spreadsheet file.

Table S-5: Results of phosphopeptide quantification

This table provides normalized $\log_2$ ratios for all quantified phosphopeptides. It is provided as separate spreadsheet file.