

Figure S1

YpdA

BrxA

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BrxA_SACOL	1 - MNAYDAYMKE I AQMQRGEL TQNCFTS LETSEAVSEY MNQVNADDTFMV INSTCGCAAGLARPAAVAVATQNEHRPT 77
BrxA_BACSU	1 MSMAYEEYMRQLVVPMMREL TGAGFEELTAEEENFMEKA -- EGGTLVVVNSVCGCAAGLARPAATQAVLQNDKTP 76
BrxA_BACCE	1 - MINFNFFMNDVVRQAREE IVSAGYTELTTPPEADEFK -- RNGTTLVMVNSVCGCAGGIARPAAAHSV - HYDKRPN 73
BrxA_BACAN	1 - MINFNFFMNDVVRQAREE IVSAGYTELTTPPEADEFK -- RNGTTLVMVNSVCGCAGGIARPAAAHSV - HYDKRPN 73
BrxA_STAES	1 - MNNGYEAYMKELAQQMRAELTDNGFTSLETSDDVNQYMQNI DNDDTTFVVIINSTCGCAAGLARPAAVAVAEEQNEVKPD 77
BrxA_STASA	1 - MNAYEAYMNELATQMRSELTGRDFKSLEADE VSNFMTNVGSDDTTFVVIINSTCGCAAGLARPAAVTVVEQNDKKPT 77

BrxB

	*
BrxB_SACOL	1 MDMNFDLYMNGVVEQARNE IESAGYEQLTTAEDVDKVLK-QDGTTLVMI NSVCGCAAGGIARPAAASHAL-HYDVL PDRLVTVFAGO 83
BrxB_BACSU	1 MNMDNFLMNDI VRQARQE ITAAGYTELKTAEEVDEALT-KKGTTLVMVNSVCGCAAGGIARPAAAYHSV-HYDKRPDQLTVFAGO 83
BrxB_BACCE	1 MNMDNFLMNDI VRQARQE ITAAGYTELKTAEEVDAALA-KKGTTLVMVNSVCGCAAGGIARPAAAYHSV-HYDKRPDQLTVFAGO 83
BrxB_BACAN	1 MSNAYEEYMRQMVIPMRQELVRSGFEELTTEANTEFMENTTGTLLVVVNSVCGCAAGLARPSAGQAVVRAEKQPDHLTVFAGO 85
BrxB_STAES	1 MDLNFDLYMNDVVEQARNE IEEAGYQQLTSAEVQDQLQ-QKGTSLVMVNSVCGCAAGGIARPAAAHAL-HYDKLPQLRVTVFAGO 83
BrxB_STASA	1 MDLNFDLYMNDVVEQARNE IEEAGYQQLTSAEVDSVLK-QEGTSLVMVNSVCGCAAGGIARPAAATHAL-HYDKLPQLRVTVFAGO 83
BrxB_SACOL	84 DKEATQRAREYFEGYAPSSPSFALVKDGKITEMIERHQIEGHDMNVINQLQTLFNKMCER 145
BrxB_BACSU	84 DKEATARADYFEGYPPSSPSFAILKDGTIMKMVERHEIEGHEPMAVVAKLOEAEFEEMCEEV 145
BrxB_BACCE	84 DKEATARADYFEGYPPSSPSFAILKDGTIMKMVERHEIEGHEPMAVVAKLOEAEFEEMCEEV 145
BrxB_BACAN	86 DKDATAKMRREYFGEI PPSSPSMALLKGKEVVFIIHRHEIECATMDEIITNLQAFKMCER 144
BrxB_STAES	84 DKEATQQAREYFEGYAPSSPSFALIKDGKITEMIERHQIEGHDMVIVTQLQNLFDNYQEK 145
BrxB_STASA	84 DKEATQRARDYFEGYAPSSPSFALIKDGKITEMIERHQIEGHDMVIVTQLQNLFDNYQEK 145

Fig. S1. ClustalΩ2 protein sequence alignments of BrxA/B and YpdA homologs across firmicutes.

The % identities of the homologs to *S. aureus* BrxA, BrxB and YpdA are given in parenthesis.

BrxA (SACOL1464) of *S. aureus* COL was aligned with BrxA homologs of *Staphylococcus epidermidis* (BrxA_STAES, 79.3%), *Staphylococcus saprophyticus* (BrxA_STASA, 73.1%), *B. subtilis* (BrxA_BACSU, 55.1%), *Bacillus cereus* (BrxA_BACCE, 43.2%) and *Bacillus anthracis* (BrxA_BACAN, 43.2%).

BrxB (SACOL1558) of *S. aureus* COL was aligned with BrxB homologs of *S. epidermidis* (BrxB_STAES, 87.6%), *S. saprophyticus* (BrxB_STASA, 84.1%), *B. subtilis* (BrxB_BACSU, 68.3%), *B. cereus* (BrxB_BACCE, 68.3%) and *B. anthracis* (BrxB_BACAN, 46.3%).

YpdA (SACOL1520) of *S. aureus* COL was aligned with YpdA homologs of *S. saprophyticus* ((YpdA_STASA, 84.1%), *S. epidermidis* (YpdA_STAES, 82.3%), *B. subtilis* (YpdA_BACSU, 62.8%), *B. cereus* (YpdA_BACCE, 61.3%) and *B. anthracis* (YpdA_BACAN, 60.1%). In the lower panel, YpdA of *S. aureus* (SACOL1520) and *B. subtilis* (BSU22950) were aligned with TrxB of *S. aureus* (SACOL0829) and *B. subtilis* (BSU34790). The conserved CGC motif of BrxA/B and the conserved Cys14 residue of YpdA are labeled in red with asterisks (*).

	*
YpdA_SACOL	1 MQKVES I I GGGP CGLSAA IEQKRKG IDTL I I EKGNVVES I I NYPTQHQTFFFSSDKLS I GDVPI VEE 70
YpdA_BACSU	1 M I QEKAI I I GGGP CGLSAA I HLKQIG IDALV I EKGNVVS I I NYPTQHQTFFFSSSEKLE I GDVAF I TENRK 70
YpdA_BACCE	1 MQKETA I I I GGGP CGLAAA I SLQQQG I NPLV I EKGN I VNA I YHYPTQHQTFFFSSSEKLE I GDVAF I TENRK 70
YpdA_BACAN	1 MQKETA I I I GGGP CGLAAA I SLQKVG I NPLV I EKGN I VNA I NYPTQHQTFFFSSSEKLE I GDVAF I TENRK 70
YpdA_STAES	1 M OTIES I I I GGGP CGLSAA I EQKKKG I ETLV I EKGNVVS I I NYPTQHQTFFFSSSDKLS I GDIP I VEDSK 70
YpdA_STASA	1 M QTVES I I I GGGP CGLSAA I EQKKKG I DTLV I EKGNVVS I I NYPTQHQTFFFSSSDKLS I GDIP I VEDSK 70

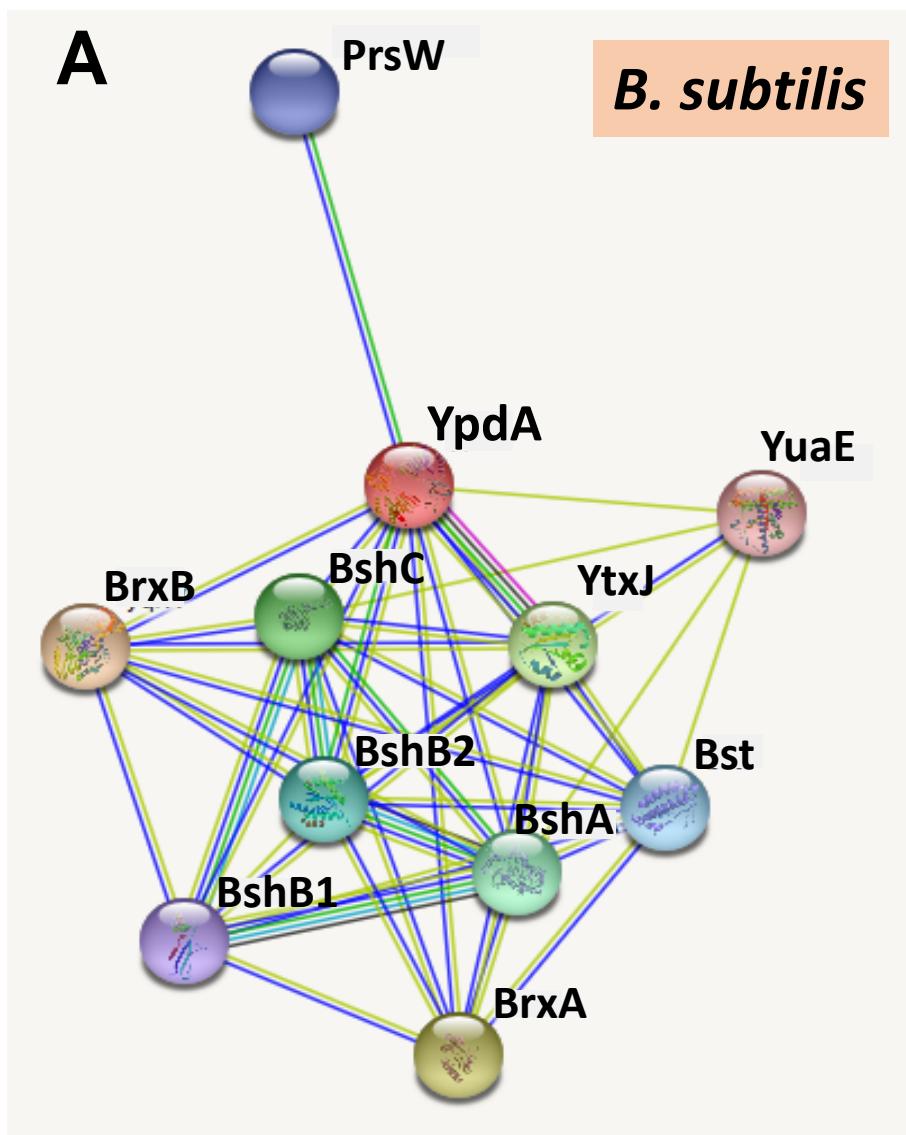
BrxA_SACOL	78 NT VTV FAGQDKEATATMREF I - QQAPSSPSYALFKGQDLVYFMPREF I EGRDIND I AMDLKDADENCK - 145
BrxA_BACSU	77 NT VTV FAGQDKEATAKMREYFTGQEPSSPSMALLKGKEVVFH I PRHE I EGHDMEELMKNLTAFA DAHC - 144
BrxA_BACCE	74 HL VTV FAGQDKEATARAREYFEGYP PSSPSFALLKDGT I TMVERHE I EGHEPMQVIAKLQSYFEENCEEL 144
BrxA_BACAN	74 HL VTV FAGQDKEATARAREYFEGYP PSSPSFALLKDGT I TMVERHE I EGHEPMQVIAKLQSYFEENCEEL 144
BrxA_STAES	78 HK VTV FAGQDKEATQTMRDY I - QQVPSSPSYALFKGQHLVHF I PREH I EGRDIND I AMDLKDADENCK - 145
BrxA_STASA	78 NK VTV FAGQDKEATATMRDY I - QQVPSSPSYALFKGQELKFH I PREH I EGRD I QD I QMD I KDAFDYDQ - 144

YpdA_SACOL	71 PRRNQALVYYREVVKHHQLKVNAFEEVLTVKMNNKFT I T I T T K D V Y E C R F L T I A T G Y Y G Q H N T L E 135
YpdA_BACSU	71 P V R I Q A L S YYREVVKRKRKN I RVNAFEMVRKVTKTQNNT I I F V I E T S K E T Y T P Y C I I A T G Y Y D H P N Y M 136
YpdA_BACCE	71 P V R N Q A L A YYREVVKRKGVRVNAFEEVQVQKGDGF FRVSTKRDGNTET YAAKY I V V A T G Y Y D N P N Y M 140
YpdA_BACAN	71 P V R N Q A L A YYREVVKRKGVRVNAFEEVQVQKGDGEV F Q V E T T K R D G S K E I Y I A K Y I V V A T G Y Y D N P N Y M 140
YpdA_STAES	71 P R R N Q A L V YYREVVKHHQLN I HFEEVLTVKKINNKFA I T I T T K D V Y E C R F L T V A T G Y Y G Q H N T L E 135
YpdA_STASA	71 P H R N Q A L V YYRAVVKHHQLR I N A F E E V L T V K K I N N R F T I T I T T K D V Y E C R F L T V A T G Y Y G Q H N N L E 135

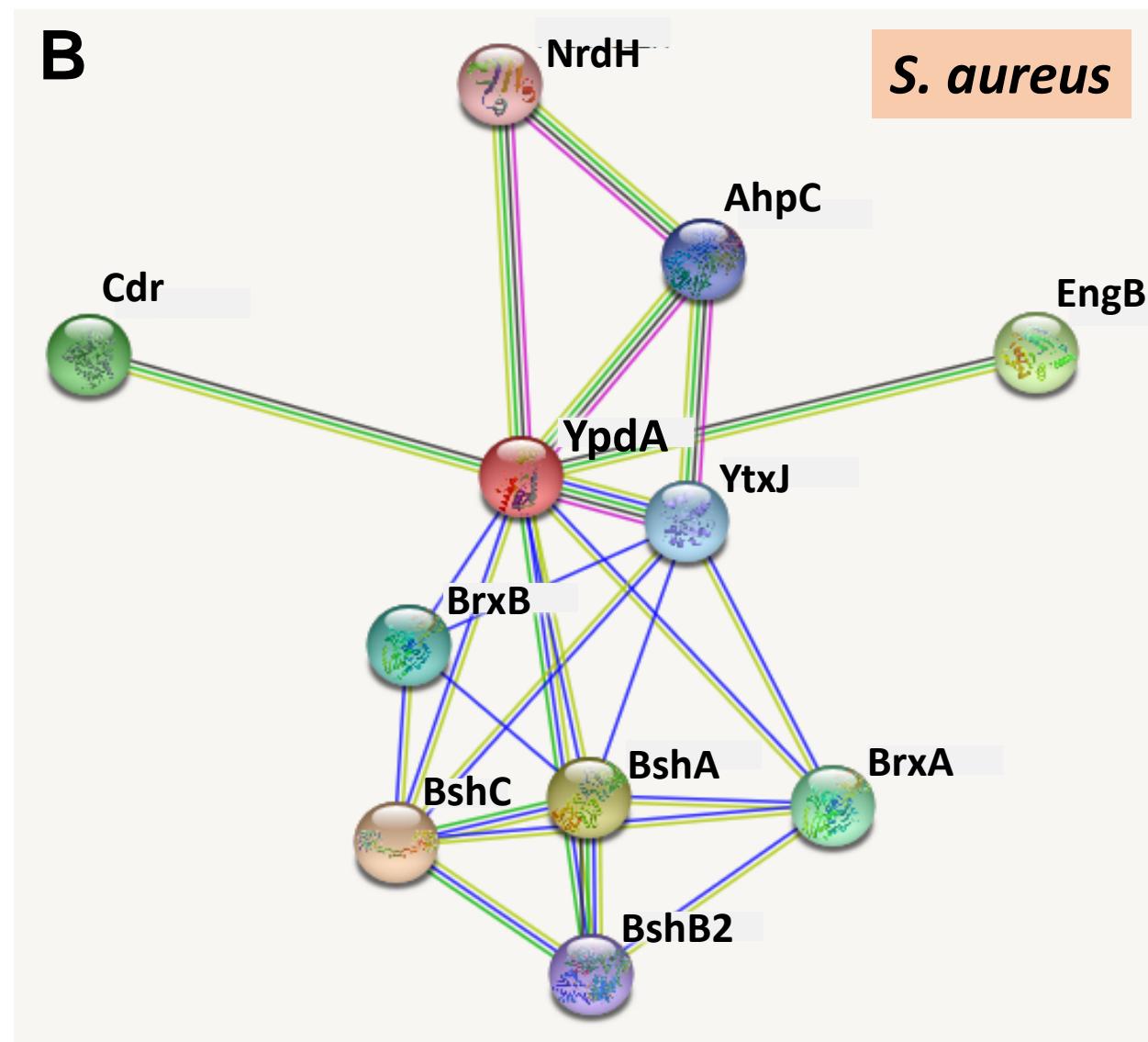
YpdA_SACOL	136 VEGADLPKV FHYFKEAHPYFDQDV V I GGKNSA I DAALELEKAGANVTVL Y R G G D Y S P S I K P W I L P N F T A 205
YpdA_BACSU	137 VPGEDLPKV FHYFKEGHPYFDKDV V I GGKNSV DAALEELVKSGARVTVL Y R G N E Y S P S I K P W I L P E F E A 206
YpdA_BACCE	141 VPGEKLEKVSHYFKEGHPYFDRDV V I GGKNSV DAALEELVKAGARVTVL Y R G G E Y S Q S I K P W I L P E F E A 210
YpdA_BACAN	141 VPGEELPKVVAHYFKEGHPYFDRDV V I GGKNSV DAALEELVKAGARVTVL Y R G S E Y S P S I K P W I L P E F E A 210
YpdA_STAES	136 AEGAELPKV FHYFKEAHPYFNQNVV I GGKNSA DAALELEKAGANVTVL Y R G E Q Y P K A I K P W I L P N F E S 205
YpdA_STASA	136 VEGAELPKV FHYFKEAHPYFDQNI T I I GGKNSA DAALELEKAGANVTVI Y R G S D Y P K A I K P W I L P N F E S 205
YpdA_SACOL	206 L V N H E K I D M E F N A N V T Q I T E D T V T Y E V - N G E S K T I H N D Y V F A M I G Y H P D Y E F L K S V G I Q I N T N E F G T A P V 274
YpdA_BACSU	207 L V R N G T I R M E F G I C V E K I T E N E V F V R S G E K E L I T I K N D F V F A M T G Y H P D H Q F L E K I G V E I D K E - T G R P F 274
YpdA_BACCE	211 L V R N G T I I M Q M F H A V H K E I T E H T L T Y T S - N G E S F T I Q N D F V F A M T G Y H P D H S F L T K M G V R I D K E - T G R P M 277
YpdA_BACAN	211 L V R N G T I I M H M F G A V H K E I T E H T L T Y T V - D G E V N T I Q N D F V F A M T G Y H P D H S F L T K M G V R I D E A - T G R P I 277
YpdA_STAES	206 L V N H E K I T M E F N A T V T K I T D H S V T Y E K - D G Q L I E I D N D Y V F A M I G Y H P D Y D F L K T I G I D I H T N E Y G T A P V 274
YpdA_STASA	206 L V R H E K I I M A F N A N V T K I T E D S V Y Y E Q - N G E T H E I P N D Y V F A M I G Y H P D Y D F L Q S I G I E I N Q N E F G T A P V 274
YpdA_SACOL	275 Y N K E T Y E T N I I E N C Y I A G V I A A G N D A N T I F I E N G K F H G G I I A Q S M L A K K Q T P L E S 328
YpdA_BACSU	275 F N E E T M E T N V E G V F I A G V I A A G N N A N E I F I E N G R F H G G H I A A E I A K R E N H - - - 324
YpdA_BACCE	278 Y T E E T M E T N V E N I F I A G V I A A G N N A N E I F I E N G R F H G G A I T Q T V V S R E Q K - - - 327
YpdA_BACAN	278 Y A E D T M E T N A E N I F I A G V I A A G N N A N E I F I E N G R F H G D A I A Q T I A T R E M - - - 326
YpdA_STAES	275 Y N R E T F E T N V E N I I A G V I A A G N D A N T I F I E N G K Y H G G V I T Q S I L T K K Q T P L E T 328
YpdA_STASA	275 H N K E T Y E T N I I E N C Y I A G V I A A G N D A N T I F I E N G K Y H G G I I T Q N I L S K K Q T P L E S 328

	*
YpdA_SACOL	1 - - M Q K V E S I I I G G G P C G L S A A I E Q K R K G I D T L I I E K G N V V E S - - - - I N Y P T H Q T F F S S D K L S I G D V P F I V E E 68
YpdA_BACSU	1 - - M I Q E K A I I I G G G P C G L S A A I H L K Q I G I D A L V I E K G N V V N S - - - - I N Y P T H Q T F F S S E K L E I G D V A F I T E N 68
TrxB_SACOL	1 - M T E I D F D I A I I I G A G P A G M T A A V Y A S R A N L K T V M I E R G I P G Q M A N T E E V E N F P G F E M I T G P D L - - - - S T K M F - - E H 70
TrxB_BACSU	1 M S E E K I Y D V I I I I G A G P A G M T A A V Y T S R A N L S T L M I E R G I P G Q M A N T E D V E N Y P G F E S I L G P E L - - - - S N K M F - - E H 71
YpdA_SACOL	69 S K P R R N Q A L V Y Y R E V V K H H Q L K V N A F E E V L T V K K - M N N K F T I T T T K D V Y E C R F L T I A T G Y Y G Q H N T L E V E G A D L P - - 142
YpdA_BACSU	69 R K P V R I Q A L S Y Y R E V V K R K N I R V N A F E M V R K V T K T Q N N T F V I E T S K E T Y T P Y C I I A T G Y Y D H P N Y M C V P G E D L P - - 143
TrxB_SACOL	71 A - - K K F G A V V Q Y G - - - - - - D I K S V E D K G - E Y K V I F G N K L E T A K V I I A T G A E - - Y K K I G V P G E Q E L G G 128
TrxB_BACSU	72 A - - K K F G A E Y A Y G - - - - - - D I K E V I D G K - E Y K V V K V K A G S K E Y K A R A V I I A A G E - - Y K K I G V P G E K E L G G 129
YpdA_SACOL	143 - - K V F H Y F K E A H P Y F D Q D V V I I G G K N S A I D A A L E L E K A G A N V T V L Y R G G D Y S P S I K P W I L P N F T A L V N H E K I D M E F N 217
YpdA_BACSU	144 - - K V F H Y F K E G H P Y F D K D V V V I I G G K N S V D A A L E L V K S G A R V T V L Y R G N E Y S P S I K P W I L P E F E A L V R N G T I R M E F G 218
TrxB_SACOL	129 R G V S Y C A V C D G A F F K N K R L F V I I G G D S V A V E E G T F L T K F A D K V T I V H R R D E L R A Q R - - I L Q - - D R A F K N D K I D F I W S 200
TrxB_BACSU	130 R G V S Y C A V C D G A F F K G K E L V V V G G D S V A V E E G V Y L T R F A S K V T I V H R R D K L R A Q S - - I L Q - - A R A F D N E K V D F L W N 201
YpdA_SACOL	218 A N V T C I T E D T V - - - - - T Y E V N - - G E S K T I H N D Y V F A M I G Y H P D Y E F L K S V G I Q I N T N E F G T A P M Y N K E T Y E T N I E N C 287
YpdA_BACSU	219 A O V E K I T E N E V - - - - - V F R S G E K E L I T I K N D F V F A M T G Y H P D H Q F L E K I G V E I D K E - - T G R P F F N E E T M E T N V E G V 287
TrxB_SACOL	201 H T L K S I N E K D G K V G S V T L T S T K D G S E E T H E A D G V F I Y I G M K P L T A P F K D L G I T N D V G - - - Y I V T K D D M T T S V P G I 272
TrxB_BACSU	202 K T V K E I H E E N G K V G V N T L V D T V G E E S E F K T D G V F I Y I G M L P L S K P F E N L G I T N E E G - - - Y I E T N D R M E T K V E G I 273
YpdA_SACOL	288 Y I A G V I A A G N D A N T I F I E N G K F H G G I I A Q S M L A K K Q T P L E S - - - - - 328
YpdA_BACSU	288 F I A G V I A A G N N A N E I F I E N G R F H G G H I A A E I A K R E N H - - - - - 324
TrxB_SACOL	273 F A A G D V R D K G L R Q - I V T A - - T G D G S I I A Q S S A E Y I E H L N D Q A - - - - - 311
TrxB_BACSU	274 F A A G D I R E K S L R Q - I V T A - - T G D G S I I A Q S V Q H Y V E E L Q E T L K T L K 316

Figure S2



<https://string-db.org/cgi/network.pl?taskId=nLwfWwHpDe6G>



<https://string-db.org/cgi/network.pl?taskId=EBilN4U7gOB>

Fig. S2. Phylogenomic profiling of YpdA interaction networks with the BSH biosynthesis enzymes (BshA, BshB1/2, BshC) and bacilliiredoxins BrxA/B (YphP/YqiW) in *Bacillus subtilis* (A) and *Staphylococcus aureus* NCTC 8325 as revealed by EMBL STRING search (<https://string-db.org>). The green and blue lines denote co-localization and co-occurrence of genes in genomes, respectively.

Figure S3

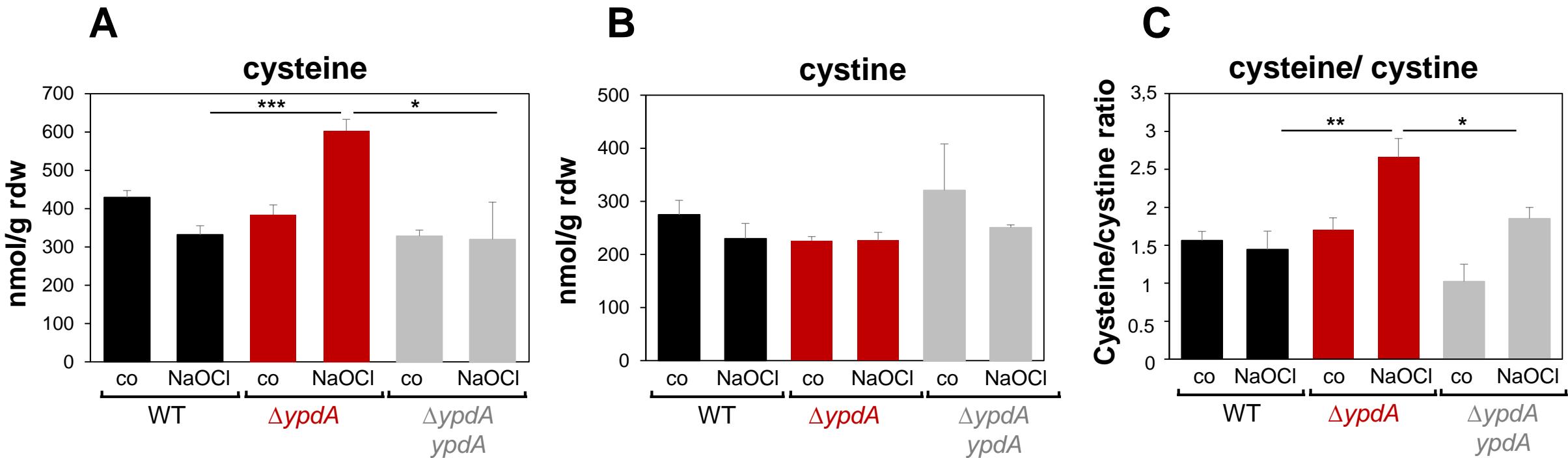


Fig. S3. Levels of cysteine (A) and cystine (B) and the cysteine/cysteine ratio (C) under control and NaOCl stress in *S. aureus* COL WT, the $\Delta ypdA$ mutant and $ypdA$ complemented strain. *S. aureus* strains were grown in RPMI and exposed to 2 mM NaOCl stress for 30 min at an OD_{500} of 0.9. mBBr-labeled LMW thiols and disulfides were measured by HPLC thiol metabolomics. Mean values and SD of 3 biological replicates are shown. ns p > 0.05; *p ≤ 0.05 **p ≤ 0.01 and ***p ≤ 0.001.

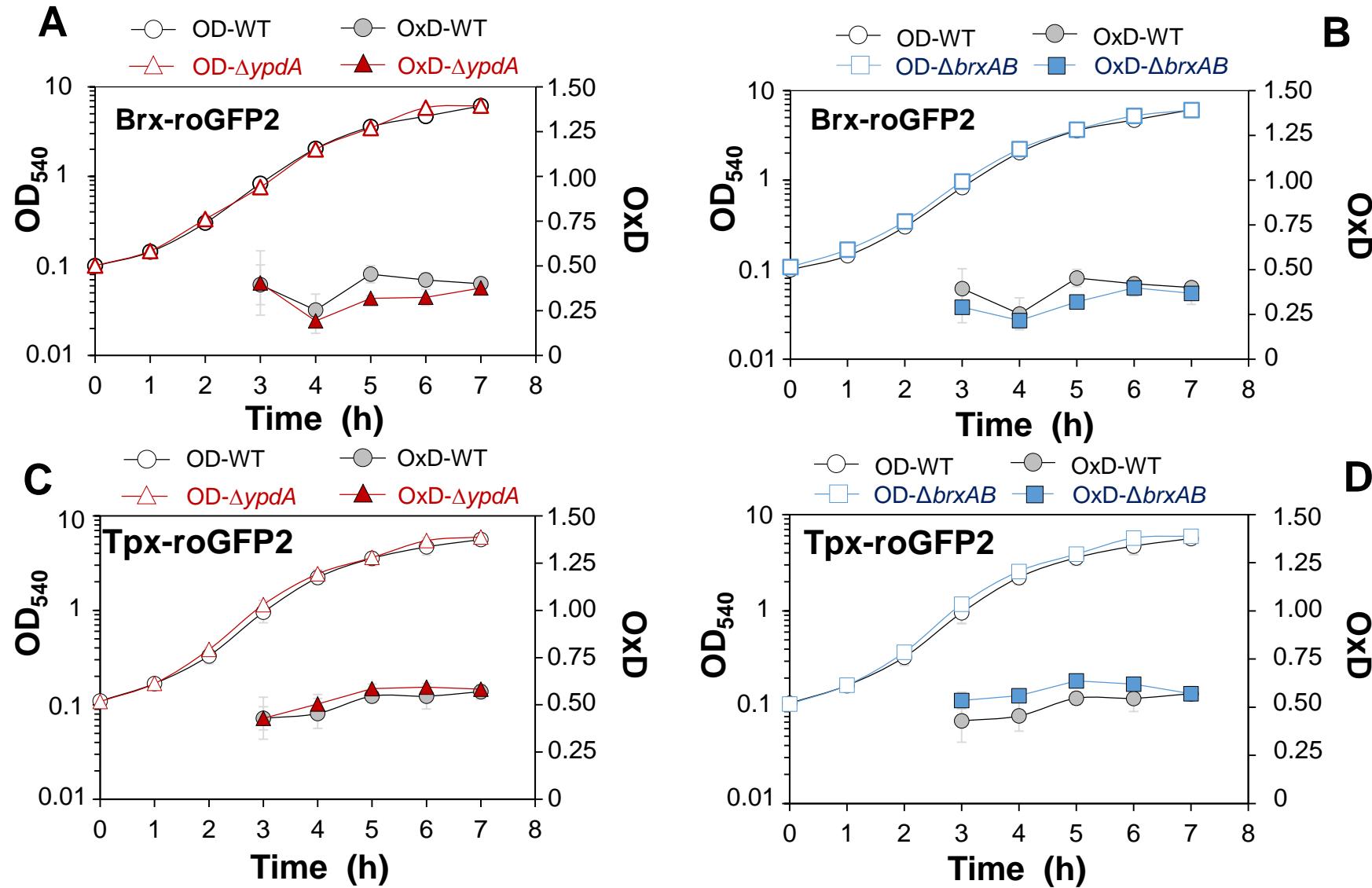
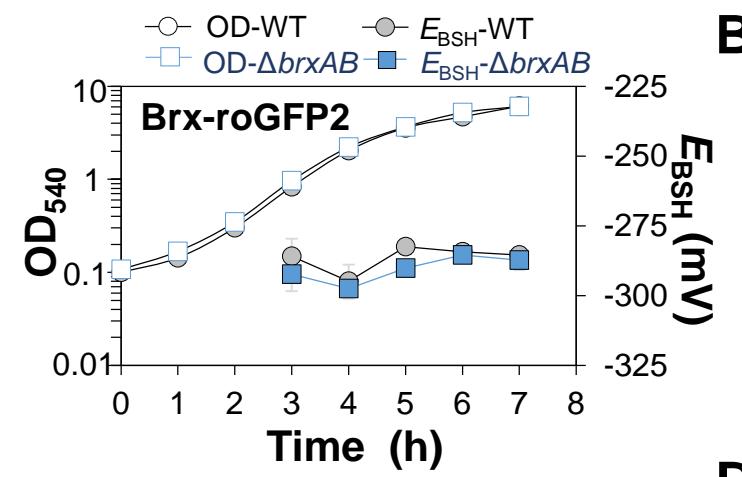
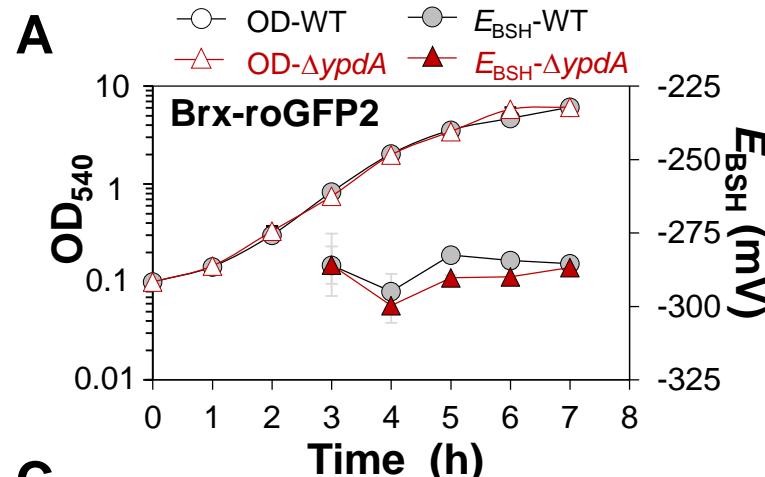
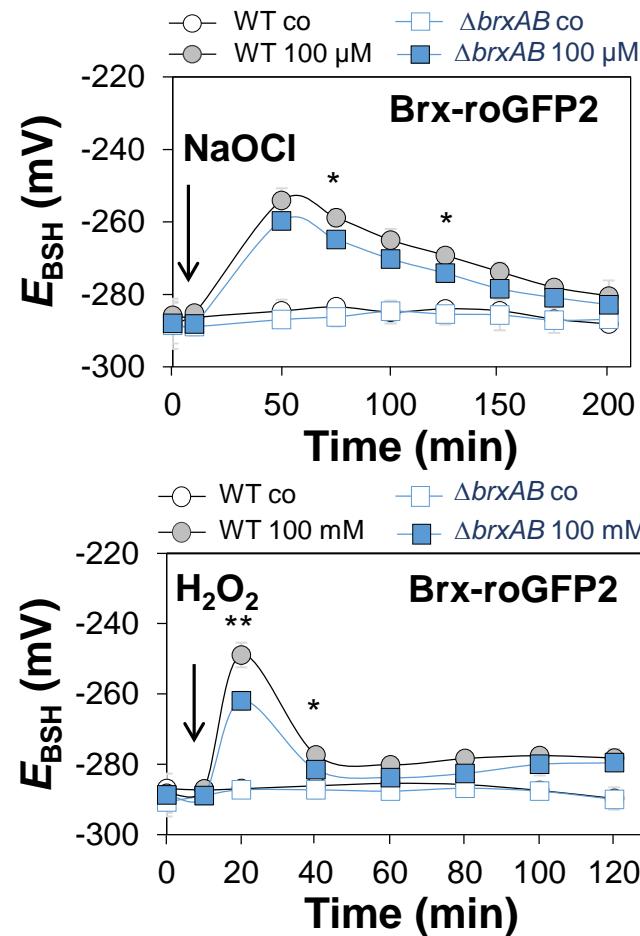
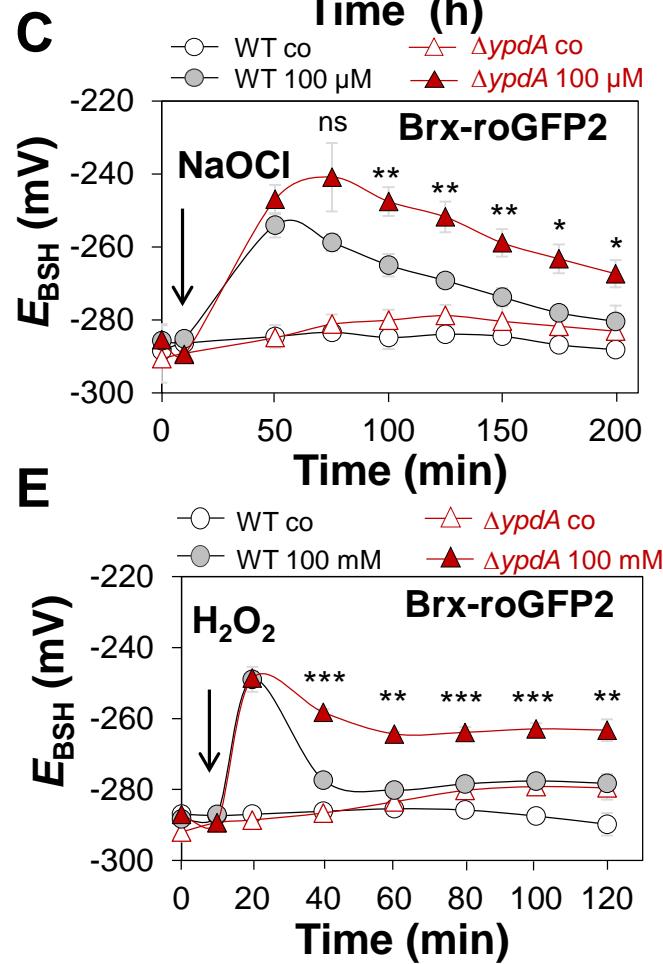
Figure S4

Fig. S4. The basal level OxD of the Brx-roGFP2 and Tpx-roGFP2 biosensors is not affected in *ΔypdA* and *ΔbrxAB* mutants during the growth. *S. aureus* COL WT, *ΔypdA* and *ΔbrxAB* mutants expressing Brx-roGFP2 (A,B) and Tpx-roGFP2 (C,D) were grown in LB medium and the OxD values were determined along the growth curve. Mean values and SD of 3 biological replicates are shown. The corresponding E_{BSH} changes for S4AB were calculated using the Nernst equation in Fig. S5AB.



B



D

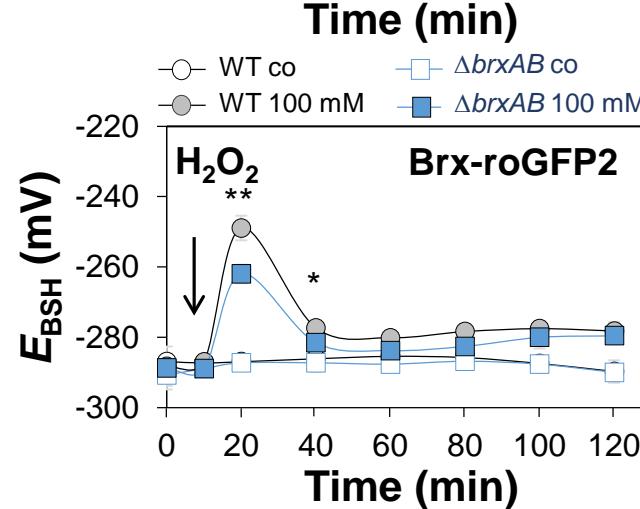
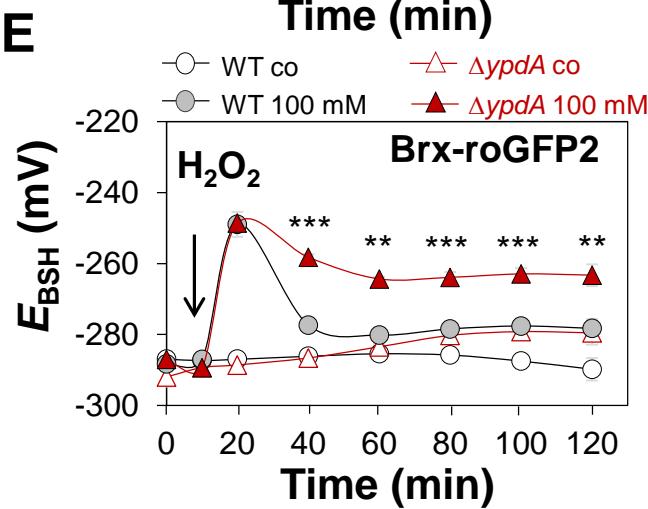


Fig. S5. YpdA and BrxAB do not affect the basal E_{BSH} level during the growth (A, B), but the Δ ypdA mutant is impaired to regenerate the reduced E_{BSH} during recovery from oxidative stress (C, E).

The E_{BSH} changes were measured in *S. aureus* COL WT, Δ ypdA and Δ brixAB mutants expressing Brx-roGFP2 along the growth curve in LB (A, B) and after exposure to 100 μ M NaOCl (C, D) or 100 mM H_2O_2 stress (E, F) in Belitsky minimal medium (BMM). The E_{BSH} values were calculated using the Nernst equation based on the OxD values of Fig. S4AB and Fig. 4A-D.

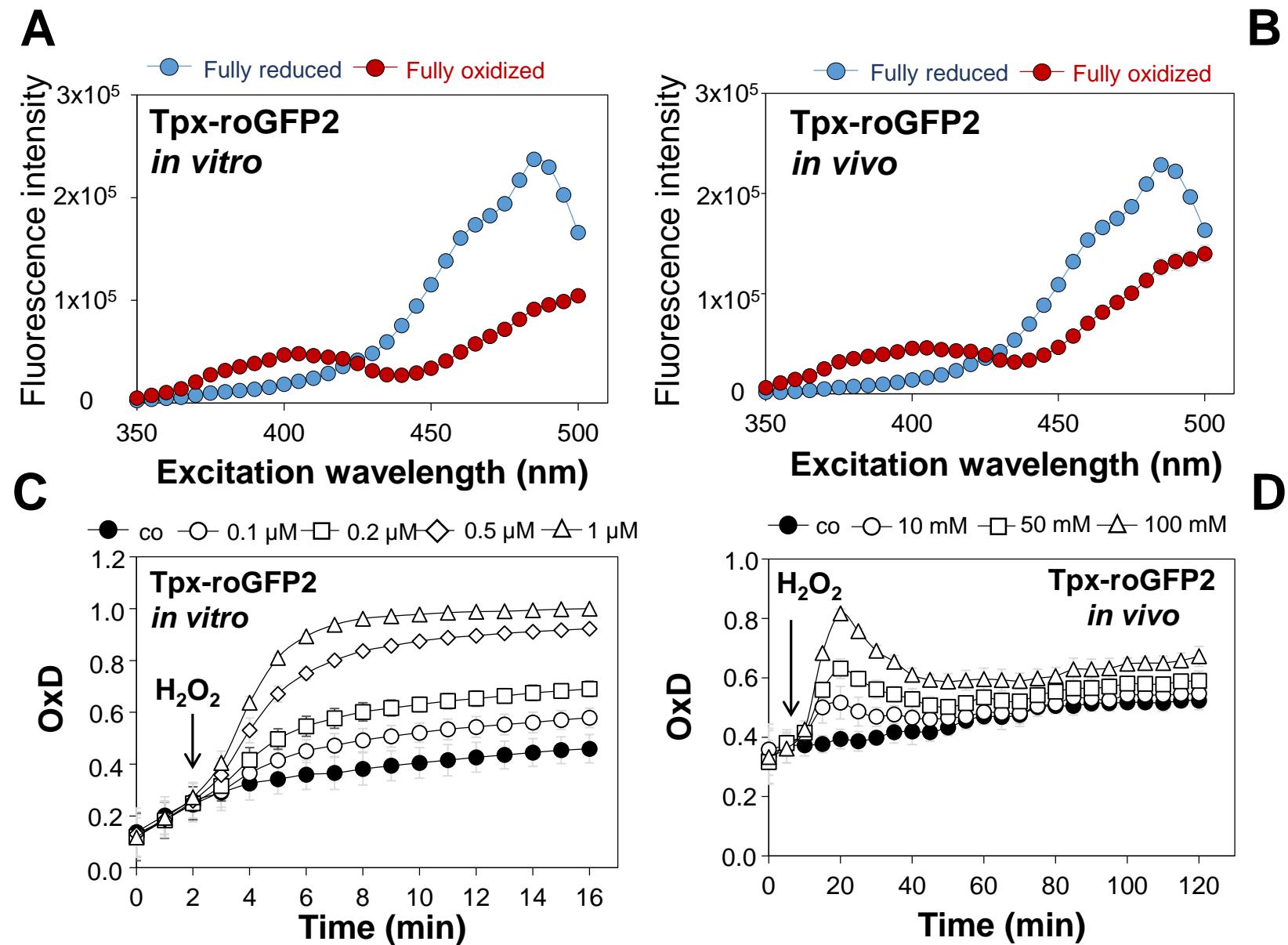
Figure S6

Fig. S6. Responses of Tpx-roGFP2 *in vitro* (A,C) and *in vivo* inside *S. aureus* COL after exposure to H_2O_2 (B,D). (A, B) The ratiometric Tpx-roGFP2 response in the DTT-treated fully reduced and diamide-treated fully oxidized state *in vitro* (A) and inside *S. aureus* COL *in vivo* (B). (C) Purified Tpx-roGFP2 (1 μM) responds specifically to low levels H_2O_2 (0.1-1 μM H_2O_2) *in vitro*. (D) Tpx-roGFP2 inside *S. aureus* COL is rapidly and reversible oxidized by sub-lethal 1-100 mM H_2O_2 *in vivo*. Mean values and SD of 3-5 replicates are shown.

Figure S7

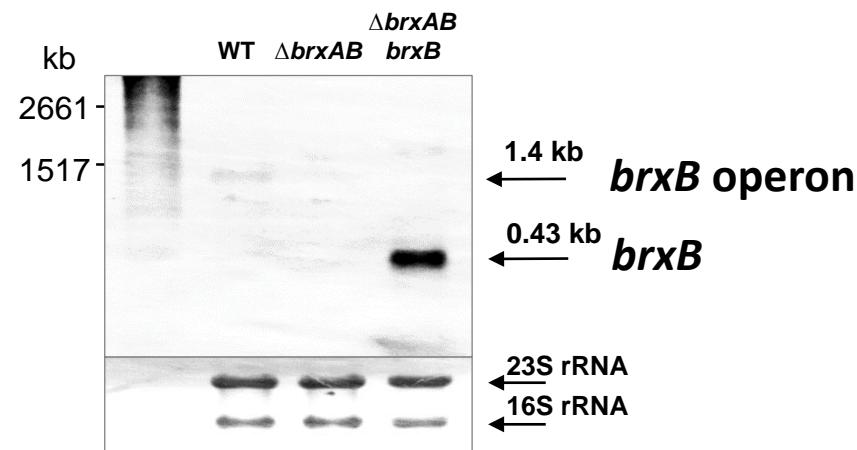


Fig. S7. Northern blot analysis of *brxB* transcription in the *S. aureus* COL WT, $\Delta brxA B$ mutant and in the *brxB* complemented strain using a *brxB*-specific RNA probe. RNA was isolated of *S. aureus* cells grown in LB to an OD_{540} of 2.0 with 1% xylose to induce *brxB* expression in the pRB473-*brxB* complemented $\Delta brxA B$ mutant strain. *brxB* transcription in the *brxB* complemented strain is shown by the 0.43 kb band on the Northern blot. Methylene blue stained bands for 16S and 23S rRNAs indicate the RNA loading controls below the Northern blot image.

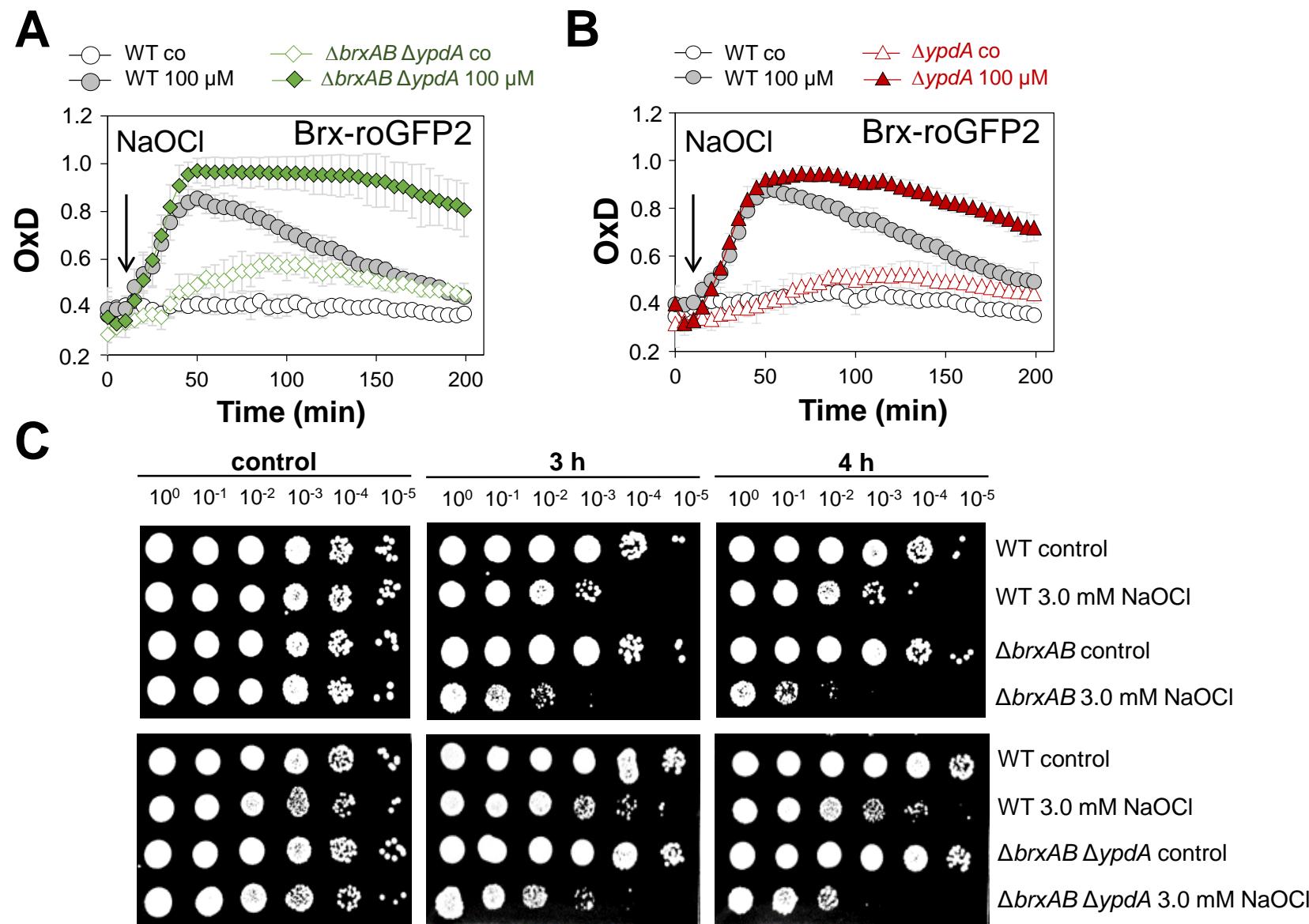
Figure S8

Fig. S8. Brx-roGFP2 measurements and survival assays indicate that the *S. aureus* $\Delta brxAB \Delta ypdA$ triple mutant is similar defective to rescue E_{BSH} as the $\Delta ypdA$ mutant and similar sensitive to NaOCl stress as the $\Delta brxAB$ mutant. (A, B) Brx-roGFP2 response in the *S. aureus* COL WT, $\Delta ypdA$ and $\Delta brxAB \Delta ypdA$ mutants under 100 μM NaOCl stress. (C) Survival assays of the *S. aureus* COL WT, $\Delta brxAB$ and $\Delta brxAB \Delta ypdA$ mutants at 3-4 hours after exposure to 3.0 mM NaOCl.

Figure S9

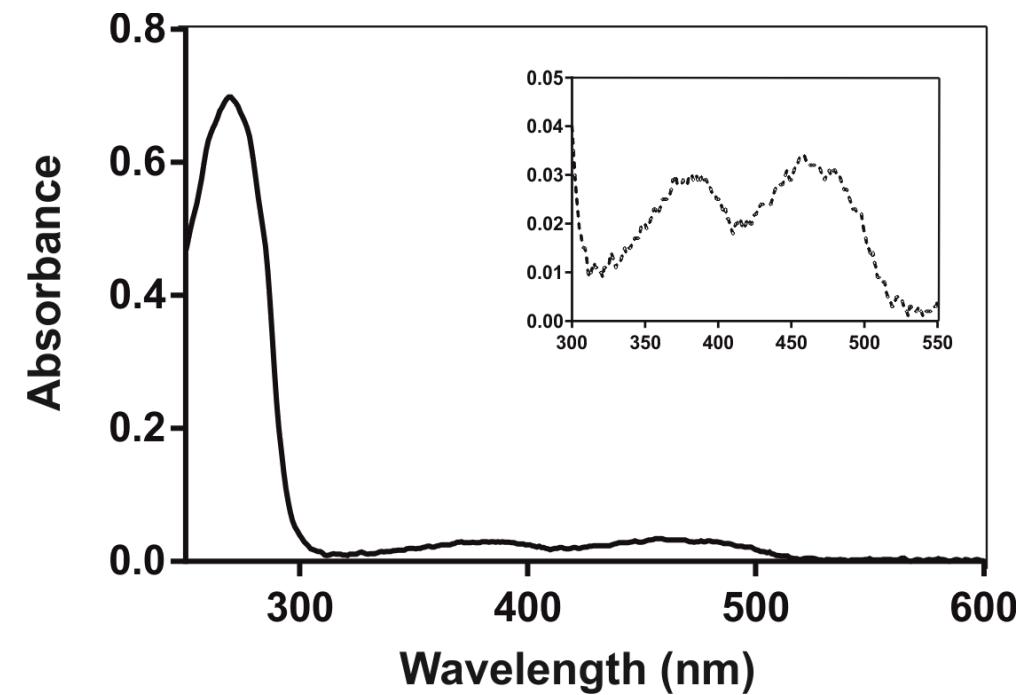


Fig. S9. The UV-visible absorption spectrum of purified yellow coloured YpdA protein indicates that YpdA is a flavoprotein containing the FAD co-factor with absorbance peaks at 375 and 450 nm (insert).