Supplementary material

Listeria monocytogenes requires the RsbX protein to prevent SigB-activation under non-stressed conditions.

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Supplementary figure legends

Figure S1. (A, B) Expression of SigB regulated genes in different genetic backgrounds. Northern blot analysis showing expression levels of positively (*lmo2230* and *lmo0596*) and negatively (*lmo1699*) SigB regulated genes. The strains (WT, Δ*sigB*, Δ*rsbX* and Δ*rsbX*+*rsbX*) were grown at 37°C (A) or 23°C (B), in BHI medium in light conditions, with constant agitation

(180 r.p.m.). Samples were taken when cultures reached OD_{600} nm~0.8 when RNA was extracted. tmRNA was used as a loading control. n=3. (C, D) Western blot of determining levels of SigB. WT, Δ*sigB*, Δ*rsbX* and Δ*rsbX* + *rsbX* strains were grown in BHI, at 37°C (C) or 23°C (D) in BHI medium in light conditions with constant agitation until OD_{600} nm~0,8 was reached. Samples were taken and protein extracted before Western blot analysis using anti-SigB antibodies. GroEL levels were used as a loading control. n=3.

Figure S2. Relative levels of SigB expression in different genetic backgrounds quantified from Figure 2C and D and Figure S1C and D. The amount of SigB was quantified and normalized to the expression of GroEL in indicated strains. Dunnett's multiple comparisons test was used for statistical analysis. * reflects a *p* value of < 0.05 and ** a *p* value of < 0.01. ns = non significant.

Figure S3. Acid survival assay. WT, Δ*sigB*, Δ*rsbX*, Δ*rsbX* + *rsbX* and Δ*sigB;* Δ*rsbX* strains were grown overnight and resuspended in BHI of pH 2.5, and incubated at 23°C. Samples were taken at indicated time points, spread on agar-plates to determine survival rate (CFU/ml). The graphic shows the average values of 3 biological replicates. A 2-way ANOVA with Multiple comparisons was used for statistical analysis comparing all strains at the different time points with WT. * reflects a *p* value < 0.05.

Figure S4. (A) Biofilm production of WT, Δ*sigB*, Δ*rsbX*, Δ*rsbX* + *rsbX* and Δ*sigB;* Δ*rsbX* strains. Bacteria were statically grown in a 96-well round-bottomed plate, for 24h, 48h and 72h, at 23°C in TSB medium. The graphics show the average values of 3 biological replicates. A 2-way ANOVA with Multiple comparisons was used for statistical analysis comparing all strains at the different time points with WT. * reflects a P value<0.05. (B) Motility assay. WT, Δ*sigB*,

Δ*rsbX* and Δ*sigB;* Δ*rsbX* strains were spotted on a motility agar plate (BHI, 0,3% agar) and grown at bench conditions (~23°C) for 24 hours. n=3. (C) Levels of FlaA in different genetic backgrounds. WT, Δ*sigB*, Δ*rsbX*, Δ*rsbX* + *rsbX and* Δ*sigB;* Δ*rsbX* strains were grown in BHI, at 23°C, with constant agitation (180 r.p.m.) until OD_{600} nm~0.8 was reached. At this time-point, protein was extracted and the levels of FlaA determined by western blot analysis, using anti-FlaA antibodies. A commassie-stained gel was used as a loading control (below). n=3.

Figure S5. *In vivo* RsbT crosslinking experiment. WT, Δ*rsbX* and $RsbT_{N49A}$ (kinase mutant) mutant strains were grown in BHI, at 37°C, until OD_{600} of 0.8 was reached. At this OD, half of the cultures were stressed by 0.5M of NaCl for 5 min. For samples being cross-linked, formaldehyde was added and incubated for 10 mins before protein extraction and western blot using an anti-RsbT antibody. A Δ*rsbR1* mutant strain (also devoid of RsbT expression) was used as a negative control. n=3.

Figure S6. *In vivo* RsbR1 cross-linking and cross-linking reversal. WT, Δ*rsbX* and $RsbT_{N49A}$ strains were grown in BHI, at 37°C, until OD_{600} nm of 0.8. For samples being cross-linked, formaldehyde was added and incubated for 10 minutes. For the reversal of the cross-linking, the same samples were incubated at 95°C, for either 15 minutes (crosslink 15 min) or 30 minutes (crosslink 30 min). At these time-points, protein was extracted and western blot performed using an anti-RsbR1 antibody. The monomeric form of RsbR1 (~30 kD) is indicated by * and the putative dimeric form of RsbR1 (~70kDa) is indicated by **. A Δ*rsbR1* mutant was used as a negative control.

Fig. S1

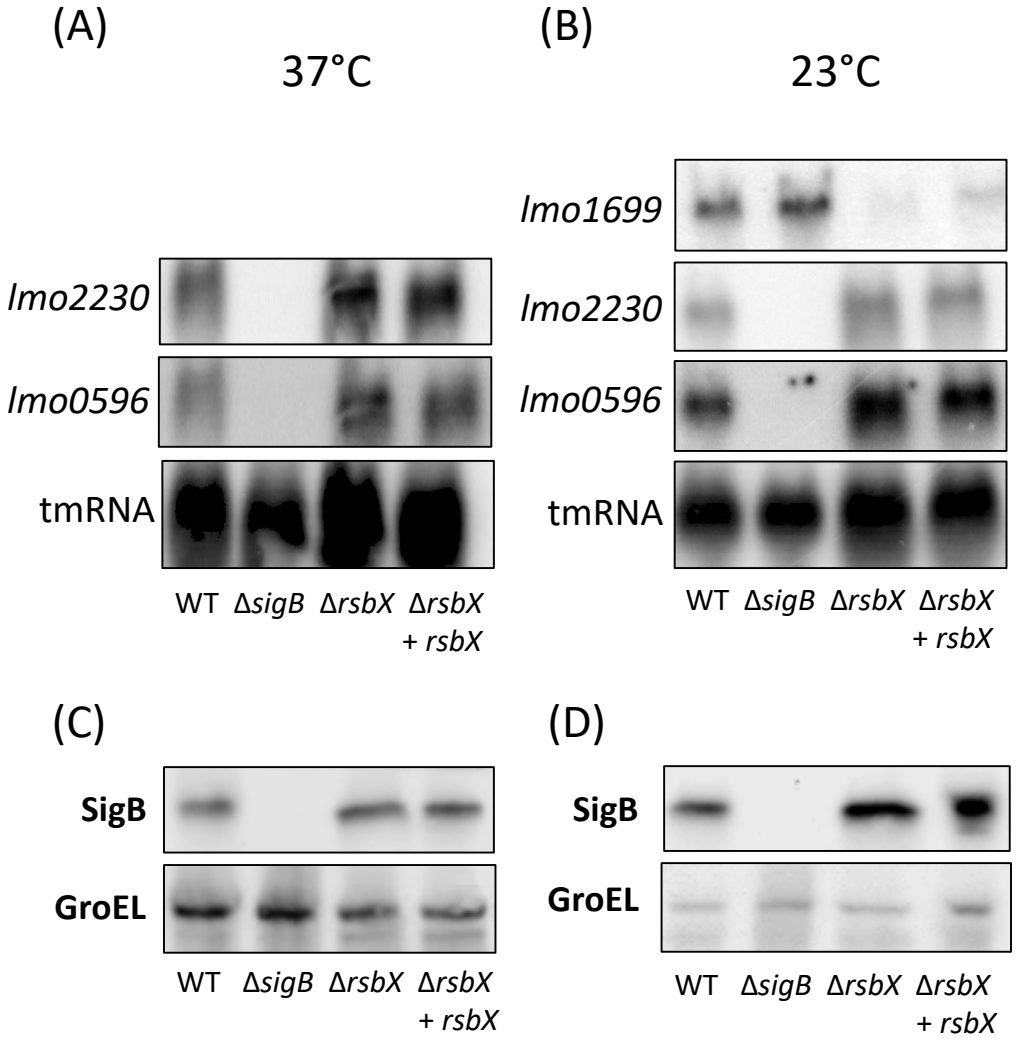

(A)
37°C
lmo2230
lmo0596
tmRNA
WT ΔsigB ΔrsbX ΔrsbX + rsbX
(B)
23°C
lmo1699
lmo2230
lmo0596
tmRNA
WT ΔsigB ΔrsbX ΔrsbX + rsbX
(C)
SigB
GroEL
WT ΔsigB ΔrsbX ΔrsbX + rsbX
(D)
SigB
GroEL
WT ΔsigB ΔrsbX ΔrsbX + rsbX

Fig. S2

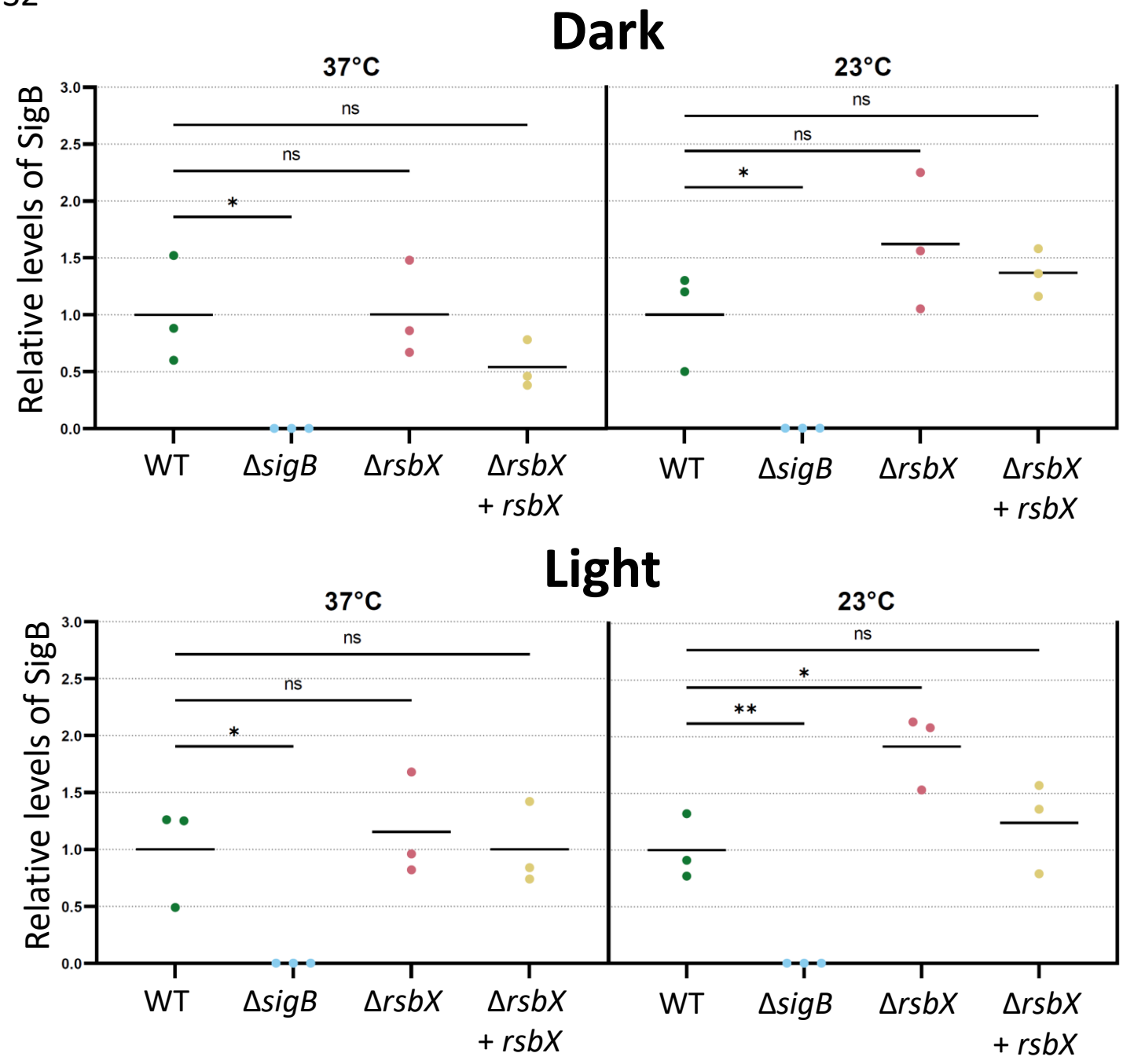

Dark
37°C
23°C
Relative levels of SigB
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ns
ns
*
ns
ns
*
WT
ΔsigB
ΔrsbX
ΔrsbX + rsbX
WT
ΔsigB
ΔrsbX
ΔrsbX + rsbX
Light
37°C
23°C
Relative levels of SigB
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ns
ns
*
ns
*
**
WT
ΔsigB
ΔrsbX
ΔrsbX + rsbX
WT
ΔsigB
ΔrsbX
ΔrsbX + rsbX

Fig. S3

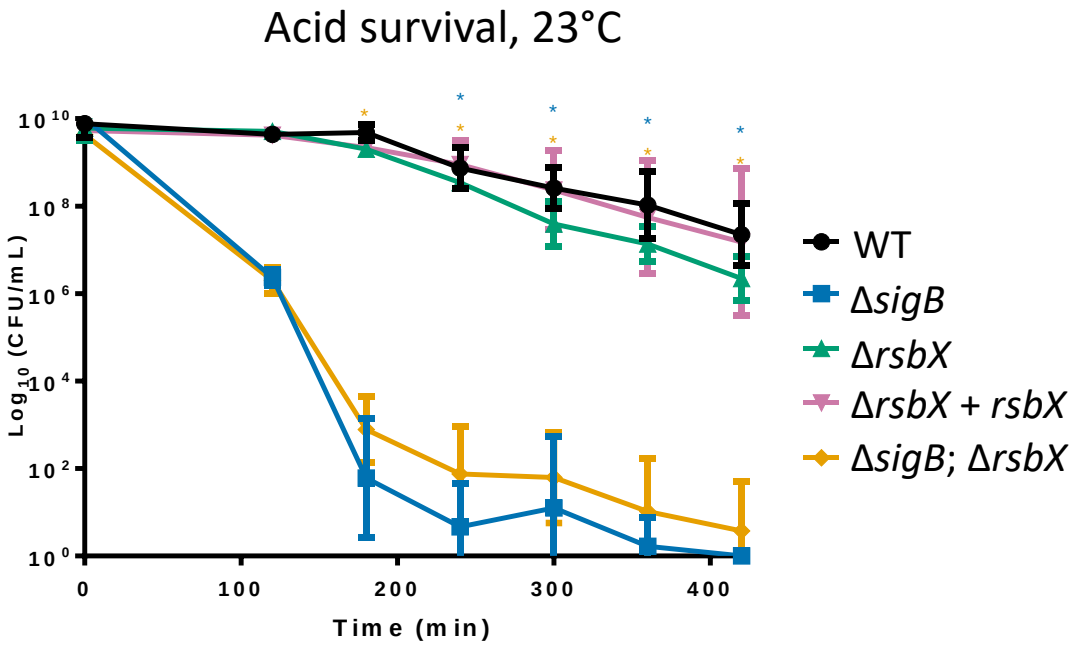
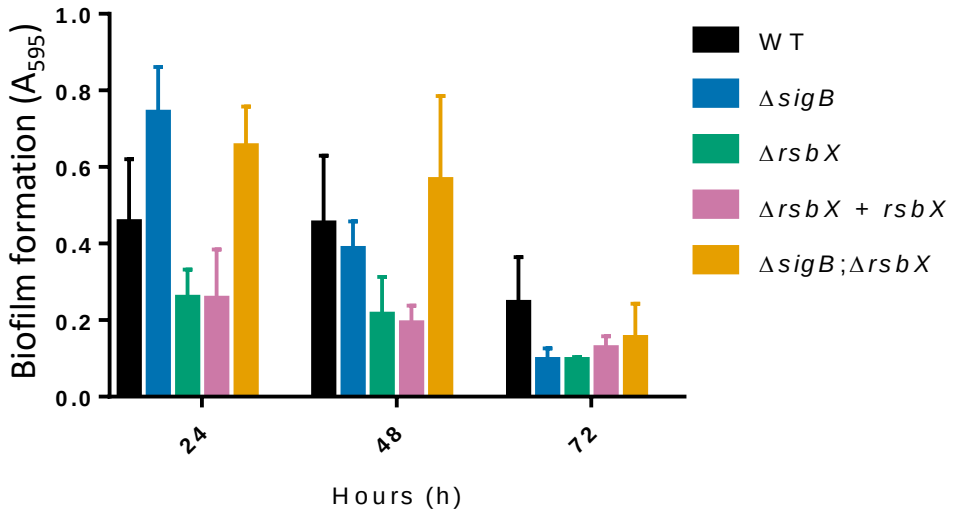
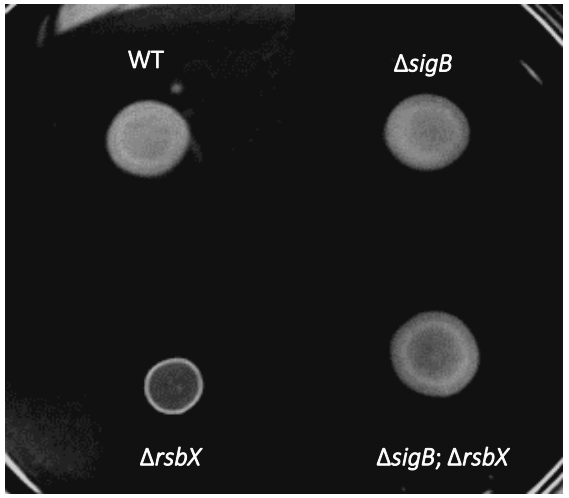

Acid survival, 23°C
10^{10}
10^{8}
10^{6}
10^{4}
10^{2}
10^{0}
Log_{10} (CFU/mL)
0
100
200
300
400
Time (min)
WT
Δ*sigB*
Δ*rsbX*
Δ*rsbX* + *rsbX*
Δ*sigB*; Δ*rsbX*

Fig. S4

(A)


Biofilm formation, 37°C
Biofilm formation (A_{595})
1.0
0.8
0.6
0.4
0.2
0.0
24
48
72
Hours (h)
WT
ΔsigB
ΔrsbX
ΔrsbX + rsbX
ΔsigB;ΔrsbX

(B)


Motility
WT
ΔsigB
ΔrsbX
ΔsigB; ΔrsbX

(C)

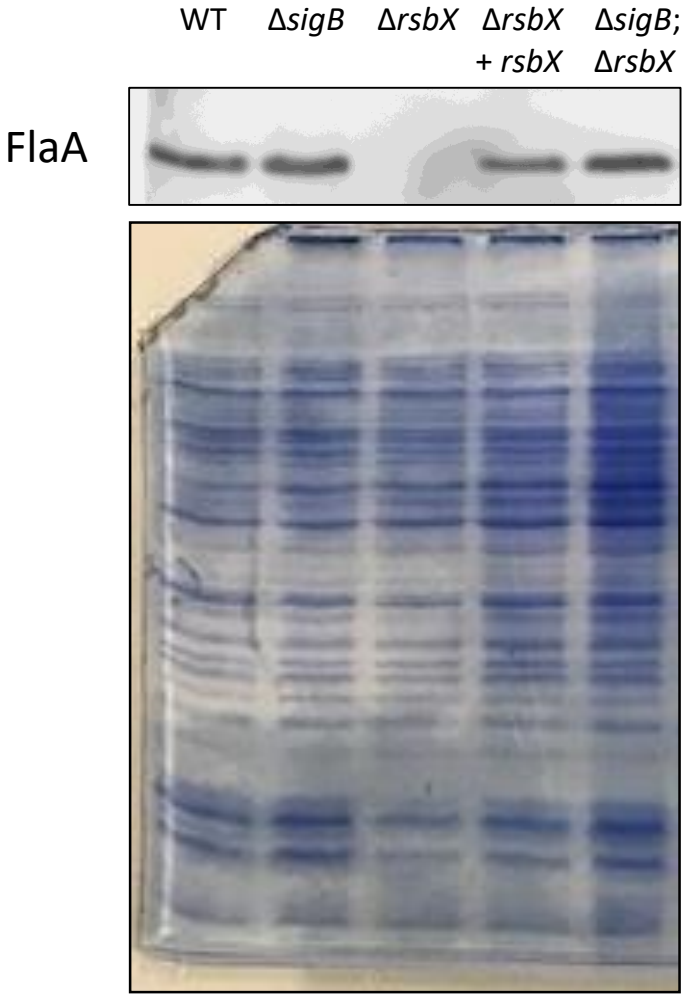
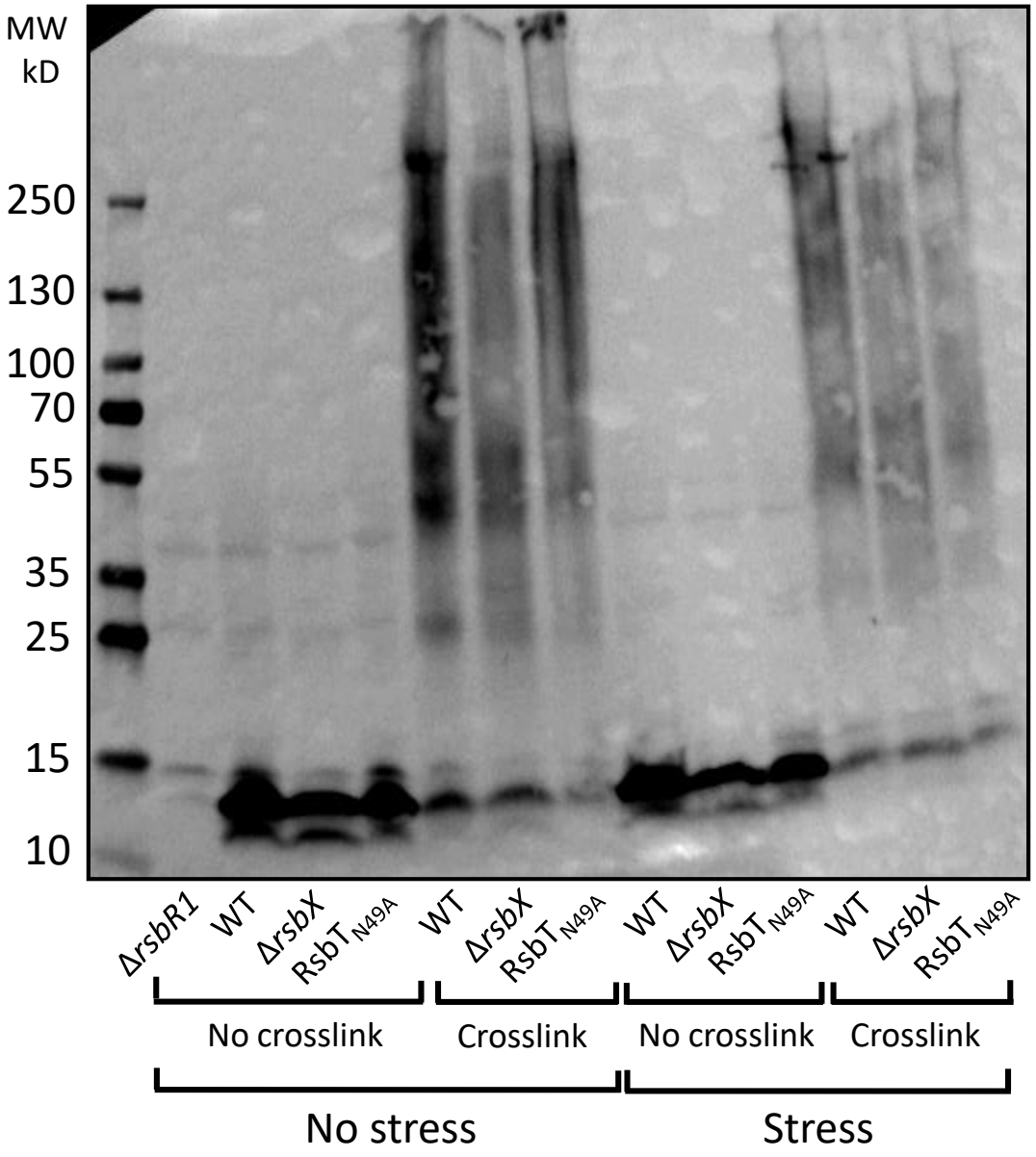
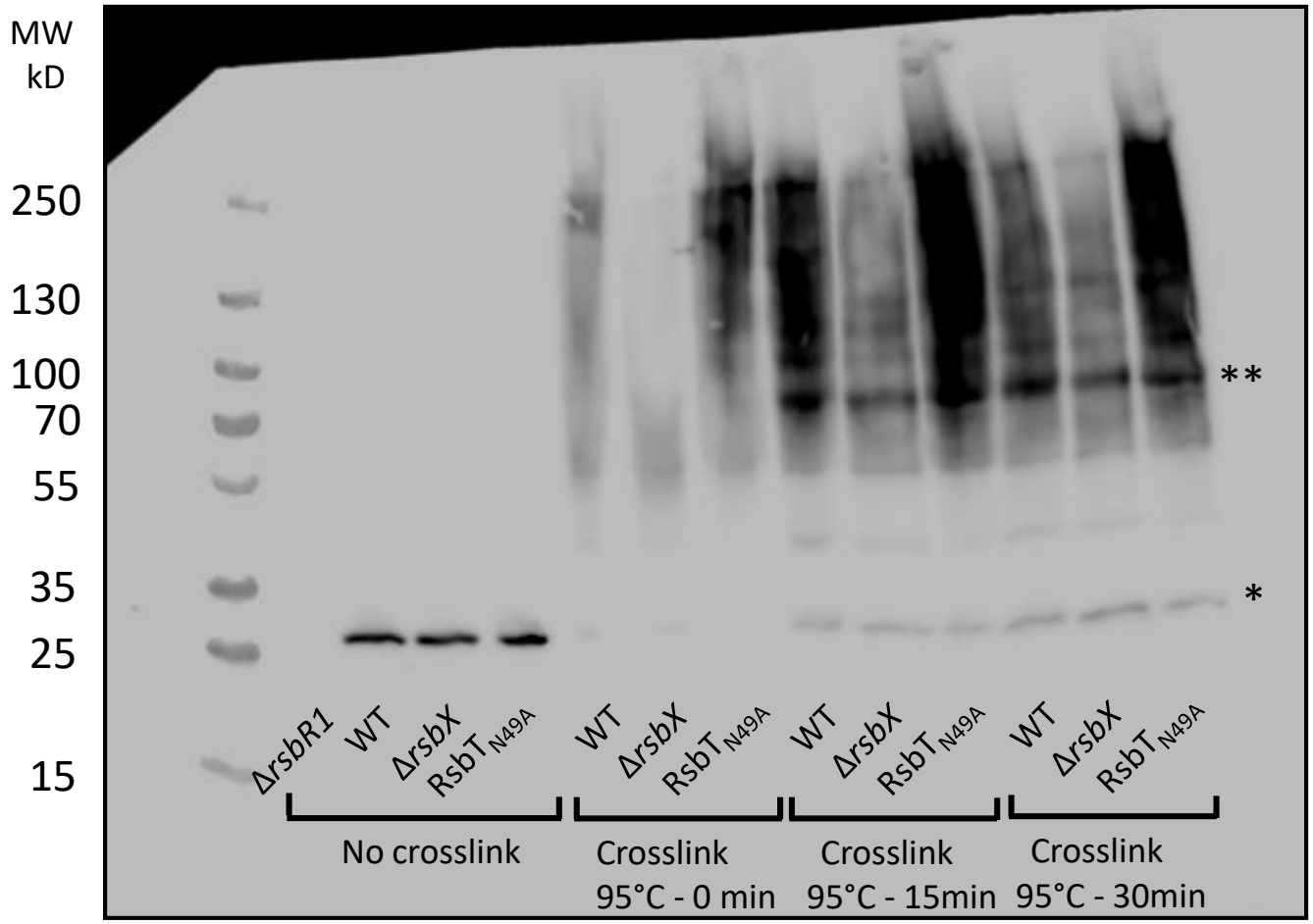

WT
ΔsigB
ΔrsbX
ΔrsbX + rsbX
ΔsigB; ΔrsbX
FlaA

Fig. S5



Monomeric RsbT

Fig. S6



Monomeric RsbR