

1 **Table S1.** Oligonucleotides used in this study

CC132	GACGTA <sup>ACTCGTTACCGTGAGT</sup>
CC133	TTTGACGCCGTGACAGTCACCGTAT
CC175	atatgatccGCGATTAAAGAAGCGAGCGGAGACCTC
CC546	ATTTATATCATATGTACGAGTTTAAAGATTATTATCAGAATACTG
CC768	GCGCTGATAGACGGAAA <sup>ACTG</sup>
CC770	GACTGCTAGTTCTTTGCCGTTTGC
CC772	CTTGTTACTAGTTAAGCAACAACCAAGTTCATAGC
CC773	CTTGTTGGATCCCTTGAAAATTCCTTGCCGTC
CC1010	GGTTTTAGTCCACTCTCAACTCCTGATC
CC1090	CCACGAAAAGTCTTCCTCAATCTCCGTATACTATTG
CC1091	GGAGGGACTGGCTAATGCTGCGTAAATTAAG
CC1092	ccgaagtatTTTTcactttattaatftgtcgtatgtattcatACCGCTCATAACTCCAATACTTTAAAATTTTCG
CC1093	CGAAAATTTTAAAGTATTGGAGTTATGAGCGGTatgaatacatacgaacaattaataaagtgaaaaaacttcgg
CC1094	ctatttaataacagattaaaaaattataaCATTATGGAGGTTTAAAAAAGAGGCACTCCCTAAG
CC1095	CTTAGGGAGTGCCTCTTTTTTTAAACCTCCATAATGttataatTTTTtaactgttatttaaatag
CC1096	GGATTCAAGAGCGGAGCGATTGCCACAACC
CC1114	CAATTCCGGTGATATTCTCATTTTAGCcatACCGCTCATAACTCCAATACTTTAAAATTTTCG
CC1115	CGAAAATTTTAAAGTATTGGAGTTATGAGCGGTatgGCTAAAATGAGAATATCACCGGAATTG
CC1116	TAAAATATTATATTTTACTGGATGAATIGTTTtagCATTATGGAGGTTTAAAAAAGAGGCACTCCCTAAG
CC1117	CTTAGGGAGTGCCTCTTTTTTTAAACCTCCATAATGctaAAACAATTCATCCAGTAAAATATAATATTTTA
HP279	GCATCGGCATAACCACCACGC

2 Non-hybridising sequences are shown in lower case letters

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1 **Table S2.** Fold-changes in sporulation and competence related transcripts in RNase J1 or RNase  
 2 Y depletion strains compared to wild-type in (Durand et al., 2012a).

Gene	RNase J1↓	RNase Y↓	Profile J1/Y
<b>Sporulation genes</b>			
<i>spo0A</i>	2.9	2.4	UU
<i>spo0B</i>	2.3	1.1	U-
<i>spo0E</i>	6.3	1.4	U-
<i>spoIID</i>	4.5	3.7	UU
<i>spoIIE</i>	5.0	2.3	UU
<i>spoIIIAE</i>	1.0	2.1	-U
<i>spoIIIC</i>	5.1	3.7	UU
<i>spoIIIE</i>	2.4	2.1	UU
<i>spoIIP</i>	2.9	1.9	U-
<i>spoIIR</i>	1.2	2.0	-U
<i>spoIISA</i>	2.0	8.6	UU
<i>spoIISB</i>	5.1	8.5	UU
<i>spoIVFA</i>	2.2	2.5	UU
<i>spoIVFB</i>	3.0	3.2	UU
<i>spoVAEA</i>	1.3	2.4	-U
<i>spoVAF</i>	1.3	2.4	-U
<i>spoVB</i>	1.1	2.2	-U
<i>spoVE</i>	3.1	1.0	U-
<i>spoVFA</i>	1.3	2.1	-U
<i>spoVG</i>	0.2	0.3	DD
<i>spoVID</i>	1.0	2.3	-U
<i>spoVM</i>	2.2	1.0	U-
<i>spoVR</i>	1.7	3.3	-U
<b>Competence genes</b>			
<i>comEB</i>	2.3	2.8	UU
<i>comEC</i>	1.4	2.1	-U
<i>comER</i>	1.2	3.2	-U
<i>comK</i>	2.6	1.0	U-
<i>comN</i>	0.5	0.5	-D
<i>comQ</i>	0.4	0.7	D-
<i>comS</i>	0.4	0.6	D-
<i>comZ</i>	1.5	2.1	-U

3 (U) up-regulated, (D) is down-regulated, (-) unchanged.

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1 **Table S3.** Fold-changes in cell envelope biosynthesis related transcripts in RNase J1 or RNase Y  
 2 depletion strains compared to wild-type in (Durand et al., 2012a).

Name	RNase J1↓	RNase Y↓	Profile J1/Y
<b>Biosynthesis of peptidoglycan</b>			
<i>gcaD</i>	1.1	0.4	-D
<i>glmS</i>	2.2	0.7	U-
<i>murAA</i>	2.4	3.0	UU
<i>murB</i>	2.5	4.9	UU
<i>murG</i>	2.5	5.8	UU
<i>racE</i>	2.3	1.3	U-
<i>spoVB</i>	1.1	2.2	-U
<i>spoVE</i>	3.1	1.0	U-
<i>yabM</i>	2.7	3.9	UU
<i>ykuD (ldt)</i>	4.2	8.6	UU
<i>ytgP</i>	2.1	3.7	UU
<i>yycJ (walJ)</i>	3.4	2.4	UU
<b>Biosynthesis of lipoteichoic acid</b>			
<i>dgkA</i>	1.9	2.1	-U
<i>ltaSA</i>	1.0	3.6	-U
<i>yqgS</i>	3.0	1.7	U-
<i>yvgJ</i>	1.5	2.1	-U
<b>Biosynthesis of teichoic acid</b>			
<i>dltA</i>	2.3	3.1	UU
<i>dltB</i>	2.3	2.7	UU
<i>dltC</i>	2.4	2.6	UU
<i>dltD</i>	2.4	2.8	UU
<i>dltE</i>	3.1	2.8	UU
<i>ggaB</i>	1.1	0.4	-D
<i>mnaA</i>	2.0	0.9	U-
<i>pgcA (gtaC)</i>	2.1	1.7	U-
<i>tagA</i>	1.9	2.7	-U
<i>tagB</i>	1.2	2.1	-U
<i>tagO</i>	1.4	2.9	-U
<b>Biosynthesis of teichuronic acid</b>			
<i>tuaA</i>	1.0	4.5	-U
<i>tuaA</i>	1.1	6.0	-U
<i>tuaB</i>	1.6	5.5	-U
<i>tuaC</i>	1.4	4.3	-U
<i>tuaD</i>	1.4	3.9	-U
<i>tuaE</i>	1.2	3.1	-U
<i>tuaF</i>	1.2	2.6	-U
<i>tuaG</i>	1.3	2.6	-U
<i>tuaH</i>	1.2	2.5	-U

**Penicillin binding proteins**

<i>dacB</i>	2.4	2.2	UU
<i>dacC</i>	0.5	0.6	D-
<i>pbpA</i>	1.0	2.1	-U
<i>pbpC</i>	0.7	0.2	-D
<i>pbpE</i>	0.4	0.5	D-
<i>pbpG</i>	1.6	3.8	-U
<i>pbpH</i>	3.0	1.0	U-
<i>pbpI</i>	1.4	4.5	-U
<i>pbpX</i>	1.4	2.4	-U
<i>ponA</i>	1.5	2.4	-U

**Export of anionic polymers and attachment to peptidoglycan**

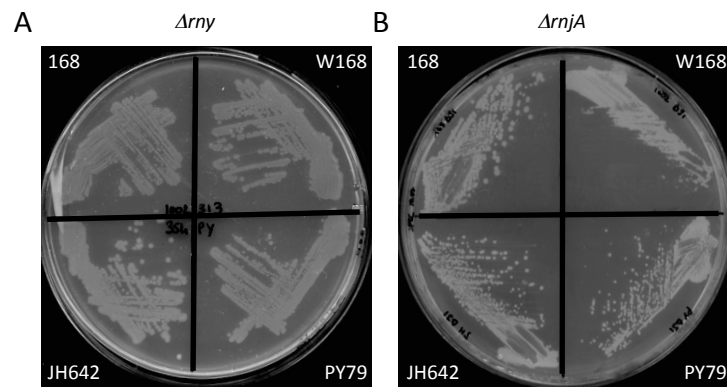
<i>lytR (tagU)</i>	3.1	0.5	U-
<i>tagG</i>	0.8	4.0	-U
<i>tagH</i>	1.1	3.7	-U
<i>yvhJ (tagV)</i>	3.0	1.3	U-
<i>ywtF (tag T)</i>	2.4	4.7	UU

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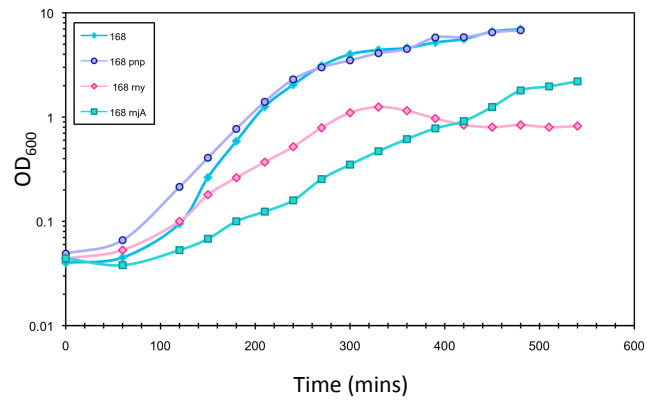
1 (U) up-regulated, (D) is down-regulated, (-) unchanged.

2

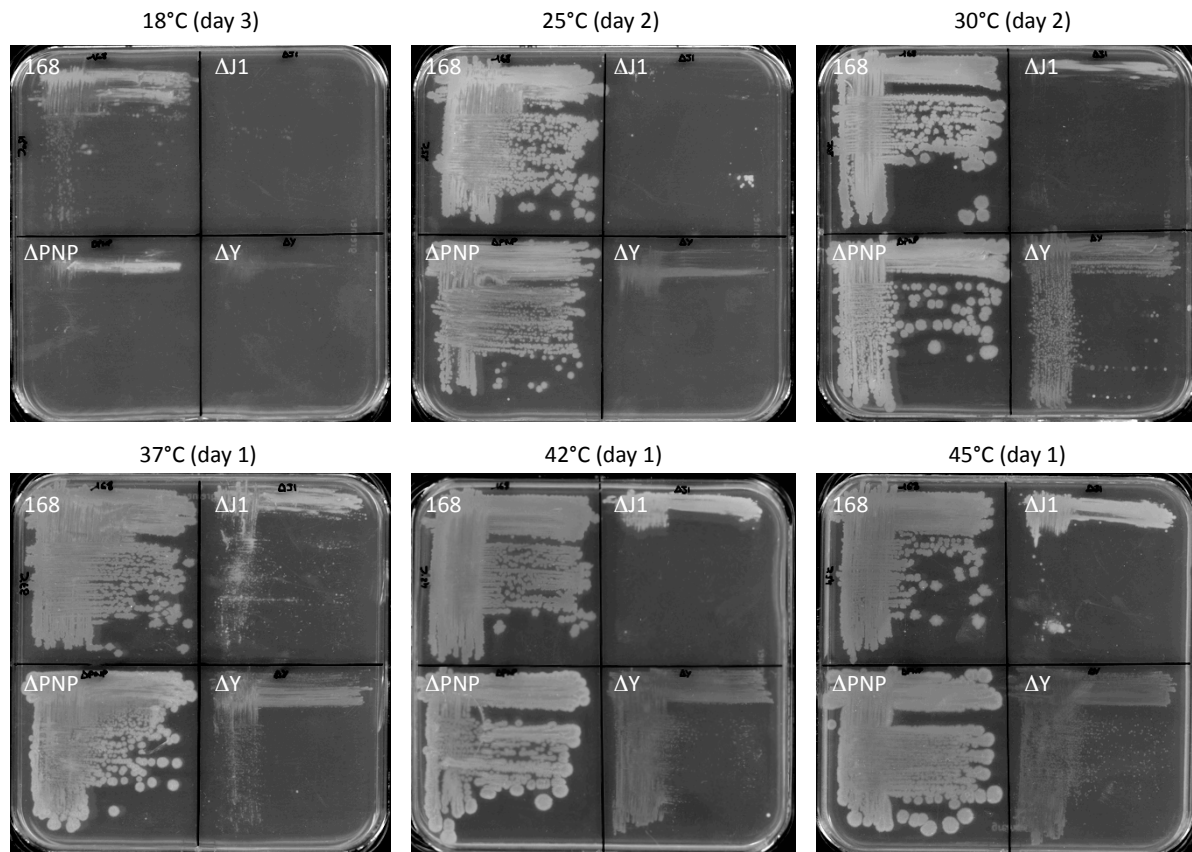
3



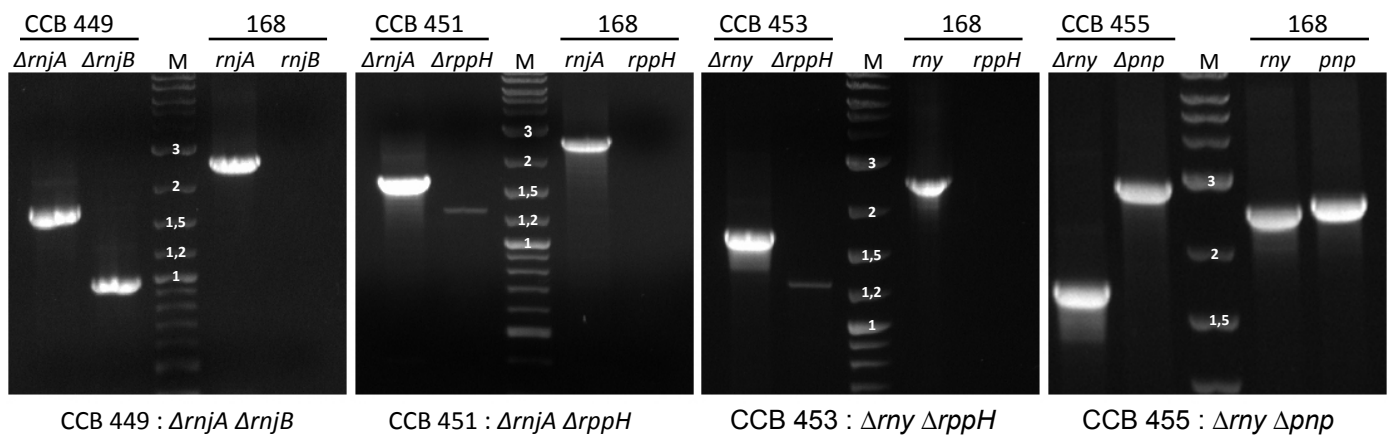
**Figure S1.** Deletion mutants of RNase Y and J1 are viable in four commonly used *B. subtilis* genetic backgrounds. Luria Bertani (LB) plates showing growth of (A)  $\Delta rny$  and (B)  $\Delta rnjA$  mutants in strains 168 *trpC2*, W168 *trp<sup>+</sup>*, JH642 *trpC2 pheA1* and PY79  $\Delta SP\beta$ .



**Figure S2.** Growth curves of RNase mutant strains in liquid medium. Cells were grown in 2xYT medium in Erlenmeyer flasks with orbital shaking at 200 rpm and an air/liquid ratio of 10.



**Figure S3.** Effect of temperature on RNase mutants. Cells were streaked out on LB plates and incubated at the temperatures indicated for the times shown.



**Figure S4.** Confirmation of double mutants by PCR. Deletions were verified by colony PCR using the following oligonucleotide pairs: *rnjA* (CC1090/1096); *rny* (CC768/770); *pnp* (CC132/133); *rnjB* (CC175/1010); *rppH* (HP279/CC546). For *rppH* and *rnjB*, the lower oligonucleotide hybridizes to the antibiotic resistance cassette and therefore only yields a product in the mutant strain. A DNA marker (M) is shown in the middle of the gel. Expected sizes were as follows: *rnjA* (WT/mutant: 2.57 vs 1.66 bp), *rny* (WT/mutant: 2.54 vs 1.73 bp), *pnp* (WT/mutant: 2.59 vs 2.8 kb), *rnjB* (WT/mutant: no product vs 996 bp); *rppH* (WT/mutant: no product vs 1.30 kb).