S1 Methods. Strains and plasmids construction

Strains construction
Deletion mutants from the *Bacillus subtilis* knock-out collection were all confirmed by PCR using an oligonucleotide primer (oKO0) within the erythromycin resistance gene and a gene-specific primer.

**BDR3414** ($\Delta$spoVV $\Delta$gerA::spec) was generated by transforming *B. subtilis* BDR3154 ($\Delta$spoVV) with a PCR product containing the gerA::spec mutation (amplified with oligonucleotide primers oFR5 and oFR8 and template DNA from BDR3371).

**BDR3416** [ycgO::cat] was generated by transforming *B. subtilis* 168 with pKM77. pKM77 (ycgO::cat) is a double-crossover vector for ectopic integration into the nonessential ycgO locus (Rudner Lab stock).

**BDR3430** [ycgO::P$_{hyperspank}$-spoVFAB (erm)] was generated by transforming *B. subtilis* BDR3416 with pFR001.

**BDR3432** [ycgO::P$_{hyperspank}$-spoVFAB (erm) amyE::P$_{xydA}$-spoVV (spec)] was generated by transforming *B. subtilis* BDR342 with pFR002.

**BDR3449** [spoVV-gfp (spec)] was generated by direct transformation of *B. subtilis* 168 with an isothermal assembly product derived from 3 PCR products: 1) a PCR product containing the spoVV gene lacking its stop codon amplified with oligonucleotide primers oFR162 and oFR163 and *B. subtilis* 168 genomic DNA as template; 2) a PCR product containing gfp (mgfpmut3a) and the spec cassette amplified with oligonucleotide primers oFR164 and oFR165 and DNA from pWX429a as template; 3) a PCR product containing the region downstream of spoVV amplified with oligonucleotide primers oFR166 and oFR167 and *B. subtilis* 168 genomic DNA as template.

**BDR3458** ($\Delta$spoIIQ::kan) was generated by two back-crosses into *B. subtilis* 168 with genomic DNA from BCR267 (Rodrigues et al., 2016)

**BDR3465** [ycgO::P$_{yeeK}$-optRBS-spoVV-gfp (erm)] was generated by transforming *B. subtilis* BDR3416 with pFR008.

**BDR3466** [ycgO::P$_{spoVV}$-optRBS-spoVV-gfp (erm)] was generated by transforming *B. subtilis* BDR3416 with pFR009.

**BDR3468** [spoVV ycgO::P$_{yeeK}$-optRBS-spoVV-gfp (erm)] was generated by transforming *B. subtilis* BDR3154 with gDNA from BDR3465.

**BDR3469** [spoVV ycgO::P$_{spoVV}$-optRBS-spoVV-gfp (erm)] was generated by transforming *B. subtilis* BDR3154 with gDNA from BDR3466.

**BDR3471** [gerA::spec spoVV ycgO::P$_{yeeK}$-optRBS-spoVV-gfp (erm)] was generated by transforming *B. subtilis* BDR3414 with gDNA from BDR3465.

**BDR3472** [spoVV spoIIQ::kan ycgO::P$_{spoVV}$-optRBS-spoVV-gfp (erm)] was generated by transforming *B. subtilis* BDR3469 with gDNA from BDR3458.
BDR3474 $\Delta$spollIAH $\Delta$spoVV::spec ycgO::P$\_spoVV$-optRBS-spoVV-gfp (erm)] was generated in two steps: first, B. subtilis BCR1117 was transformed with gDNA from BDR3466, and this intermediate strain was transformed with a PCR product containing $\Delta$spoVV::spec (amplified with oligonucleotide primers oKO260 and oFR3 and gDNA from BDR3312).

BDR3507 $\{ycgO::spoVV-gfp (spec)] was generated by transforming B. subtilis BDR3416 with pFR11.

BDR3507 $\{\Delta$spoVV ycgO::spoVV-gfp (spec)] was generated by transforming B. subtilis BDR3154 with gDNA from B. subtilis BDR3507.

BDR3558 $\{\Delta$spoVV ycgO::cat]] was generated by transforming B. subtilis BDR3154 with pKM77.

BDR3562 $\{\Delta$spoVV ycgO::spoVV(N97A)-gfp (spec)] was generated by transforming B. subtilis BDR3558 with pFR017.

BDR3563 $\{\Delta$spoVV ycgO::spoVV(F302A)-gfp (spec)] was generated by transforming B. subtilis BDR3558 with pFR018.

BDR3564 $\{\Delta$spoVV ycgO::spoVV-gfp (Q310A) (spec)] was generated by transforming B. subtilis BDR3558 with pFR019.

BDR3615 $\{\Delta$spoVV ycgO::spoVV(F141A)-gfp (spec)] was generated by transforming B. subtilis BDR3558 with pFR027.

BDR3632 $\{\Delta$spoVV ycgO::spoVV(G96A)-gfp (spec)] was generated by transforming B. subtilis BDR3558 with pFR024.

BDR3646 $\{\Delta$gerAB::erm $\Delta$spoVV ycgO::spoVV-gfp (wt) (spec)]], BDR3647 $\{\Delta$gerAB::erm $\Delta$spoVV ycgO::spoVV(G96A)-gfp (spec)]], BDR3648 [\Delta$gerAB::erm $\Delta$spoVV ycgO::spoVV(N97A)-gfp (spec)]], BDR3649 [\Delta$gerAB::erm $\Delta$spoVV ycgO::spoVV(F141A)-gfp (spec)]], BDR3650 $\{\Delta$gerAB::erm $\Delta$spoVV ycgO::spoVV(F302A)-gfp (spec)] and BDR3651 [\Delta$gerAB::erm $\Delta$spoVV ycgO::spoVV(Q310A)-gfp (spec)] were generated by transforming B. subtilis BDR3527, BDR3632, BDR3562, BDR3615, BDR3563 and BDR3564, respectively, with a PCR product containing $\Delta$gerAB::erm (amplified with the oligonucleotide primers oFR1 and oFR2 and gDNA from the strain BAM786 as template).

BDR3699 $\{\Delta$spoVF::erm]] and BDR3700 $\{\Delta$gerAB $\Delta$spoVF::erm$)] were generated by direct transformation of B. subtilis 168 and BDR3158, respectively, with a PCR product containing the mutation $\Delta$spoVF::erm [amplified with the oligonucleotide primers oFR60 + oFR61 and gDNA of the strain $\Delta$spoVF::erm (BKE collection) as template].
Plasmids construction

pFR001 [ycgO::P\textsubscript{hyperspank}::spoVFAB (erm)] was constructed in a two-way ligation with a SpeI-SphI PCR product containing the spoVFAB operon (amplified with oligonucleotide primers oDR1247 and oDR1257 and gDNA from \textit{B. subtilis} 168 as template) and pER67 cut with SpeI and SphI. pER67 [ycgO::P\textsubscript{hyperspank} (lacI) (erm)] is a double crossover vector with an IPTG-inducible promoter for ectopic integration at the ycgO locus (Rudner Lab stock).

pFR002 [amyE::P\textsubscript{xylA}::spoVV (spec)] was constructed in a two-way ligation with a Sall-BamHI PCR product containing spoVV (amplified with oligonucleotide primers oDR1250 and oDR1251 and gDNA from \textit{B. subtilis} 168 as template) and pDR150 cut with Sall and BamHI. pDR150 [amyE::P\textsubscript{xylA} (xylR) (spec)] is a double crossover vector with a xylose inducible promoter for ectopic integration at the amyE locus (Rudner Lab stock).

pFR008 [ycgO::P\textsubscript{yeek}::optRBS::spoVV-gfp (erm)] was constructed in a three-way ligation with an EcoRI-BamHI PCR product containing the SigK-responsive yeek promoter (amplified with oligonucleotide primers oFR11 and oFR12 and gDNA from \textit{B. subtilis} 168 as template) and a Nhel-BamHI PCR product containing spoVV with an optimized RBS fused to the \textit{mGFPmut3a} (amplified with oligonucleotide primers oFR13 and oFR15 and gDNA from BDR3449 as template) into pER61 cut with EcoRI and BamHI. pER61 (ycgO::erm) is a double-crossover vector for ectopic integration at the ycgO locus (Rudner Lab stock).

pFR009 [ycgO::P\textsubscript{spoVV}::optRBS::spoVV-gfp (erm)] was constructed in a three-way ligation with a HindIII-Nhel PCR product containing the promoter of spoVV (amplified with oligonucleotide primers oFR16 and oFR17 and gDNA from \textit{B. subtilis} 168 as template) and a Nhel-BamHI PCR product containing spoVV with an optimized RBS fused to the \textit{mGFPmut3a} reporter (amplified with oligonucleotide primers oFR13 and oFR15 and gDNA from BDR3449 as template) into pER61 between HindIII and BamHI sites.

pFR011 [ycgO::spoVV-gfp (spec)] was constructed in a two-way ligation with a SpeI-BamHI PCR product containing spoVV fused to the \textit{mGFPmut3a} reporter (amplified with oligonucleotide primers oFR23 + oFR24 and gDNA from BDR3449 as template) and pKM83 cut with SpeI and BamHI. pKM83 (ycgO::spec) is a double-crossover vector for ectopic integration at the ycgO locus (Rudner Lab stock).

pFR017 [ycgO::spoVV(N97A)-gfp (spec)] was constructed by site-directed mutagenesis using oligonucleotide primers oFR32 + oFR33 and plasmid pFR011.

pFR018 [ycgO::spoVV(F302A)-gfp (spec)] was constructed by site-directed mutagenesis using oligonucleotide primer oFR34 and plasmid pFR011.

pFR019 [ycgO::spoVV(Q310A)-gfp (spec)] was constructed by directed mutagenesis using oligonucleotide primer oFR36 and plasmid pFR011.

pFR024 [ycgO::spoVV(G96A)-gfp (spec)] was constructed by site-directed mutagenesis using oligonucleotide primers oFR40 + oFR41 and plasmid pFR011.

pFR027 [ycgO::spoVV(F141A)-gfp (spec)] was constructed by site-directed mutagenesis using oligonucleotide primers oFR46 + oFR47 and plasmid pFR011.