SUPPLEMENTARY INFORMATION

Lysine acetylation of the housekeeping sigma factor enhances the activity of the RNA polymerase holoenzyme

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SUPPLEMENTARY METHODS

Translational fusion of GUS reporter and its activity assay

For translational fusion reporter assay for HrdB (AQF52_5901), pGUS (1) was used to construct pGUS-TL. To remove the ribosome binding site (RBS) and the start codon of the glucuronidase (GUS) gene, pGUS was digested with *SpeI*/*ScaI*. The removed region was replaced with an amplified DNA fragment using primer pair (pGTL-F and pGTL-R), resulting in pGUS-TL. It has a multi-cloning site, including *Xba*I, *Kpn*I, *Spe*I, *Nde*I, *Bgl*II, and *Stu*I sites, and the *gusA* gene without the RBS and the start codon. To identify the start codon of the *hrdB* gene, six DNA fragments from -533 to +24 of the *hrdB* gene were cloned into the *Xba*I and *Stu*I sites of pGUS-TL, resulting in pG-

HrdB T1 to T6. The GUS activity was measured as described previously (1). Briefly, cell lysates prepared from the exponentially growing cells were incubated with *p*-nitrophenyl glucuronide, and the optical density at 415 nm (A415) was monitored during incubation at 37°C. The slope of the absorption curve was used to calculate the activity based on the following relationship: Miller Unit = ($A420_{sample}$ – $A420_{blank}$) 1000 / (time (min) × volume (ml) × $A600_{culture}$).

SUPPLEMENTARY FIGURES

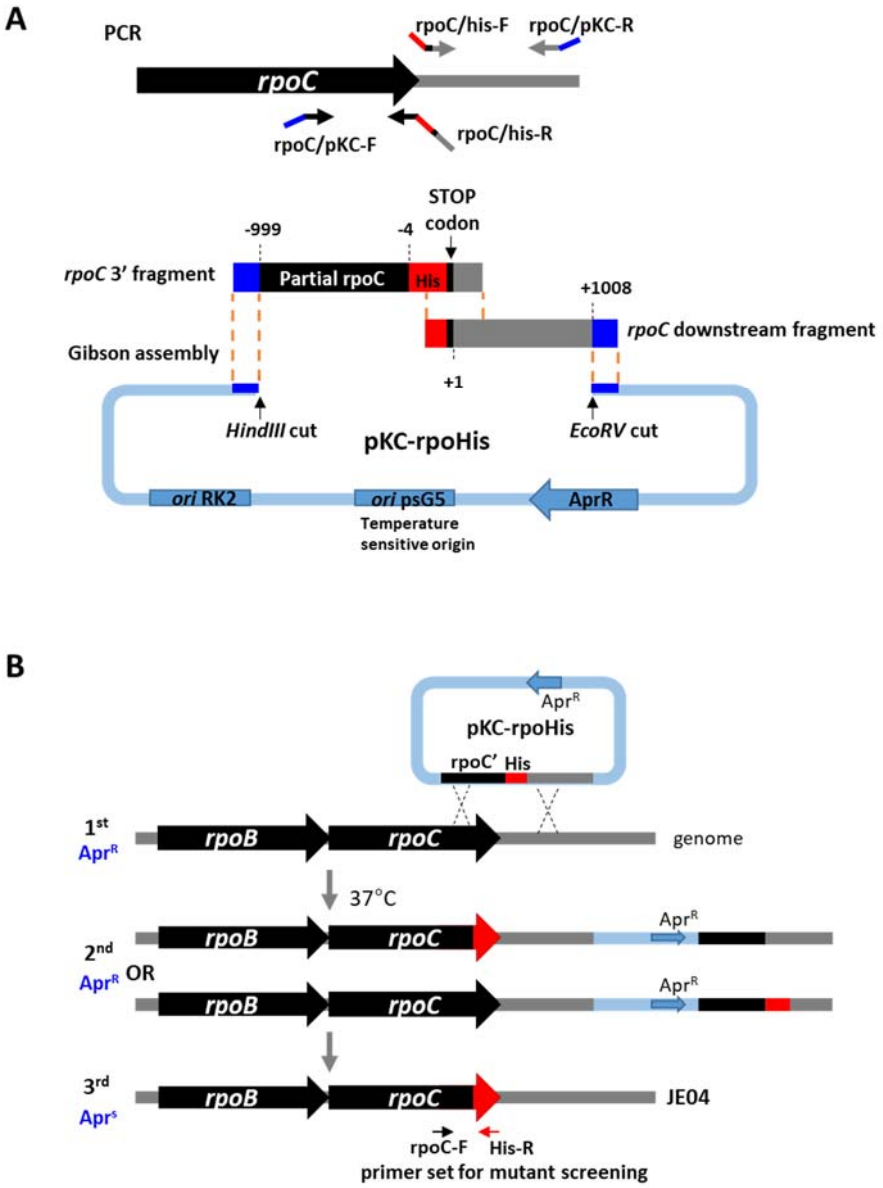


Figure S1. Construction of JE04 expressing His-tagged RpoC

A. Construction of pKC-rpoHis. Each DNA is amplified with primers carrying extensions homologous to the pKC1139 or the tandem His codons. The *rpoC* 3′ and *rpoC* downstream fragments amplified from the Sven15439 genome were cloned into the pKC1139 digested with *Hind*III/*Eco*RV via Gibson assembly.

B. Recombination using pKC-rpoHis and selection of JE04. The pKC-rpoHis plasmid was integrated into the chromosome via homologous recombination. Exconjugants obtained from single crossover were isolated based on apramycin resistance (Apr^R)

and subjected to segregation for Apr-sensitivity (Apr^S) followed by screening using PCR primers (rpoC-F and His-R) to select JE04 from the wild type segregants.

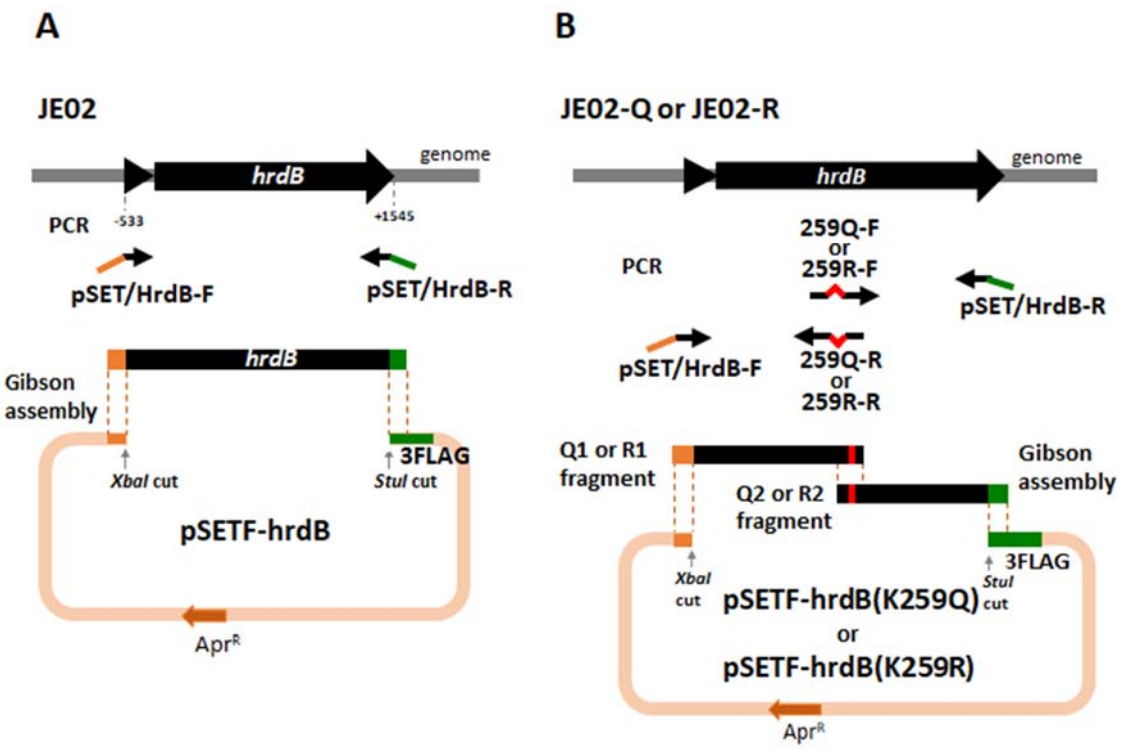


Figure S2. Construction of the integration vectors for FLAG-tagged HrdB

A. Construction of pSETF-hrdB. A DNA fragment of the *hrdB* gene (from -533 to +1545 nt from the start codon) including its promoter region and without stop codon was cloned into the pSET152F digested with *Xba*I/*Stu*I via Gibson assembly.

B. Construction of pSETF-hrdB(K259Q) and pSETF-hrdB(K259R). DNA fragments were amplified using overlapping primer sets designed to incorporate a mutation (K259Q or K259R). Amplicons were assembled with the pSET152F digested with *Xba*I/*Stu*I via Gibson assembly.

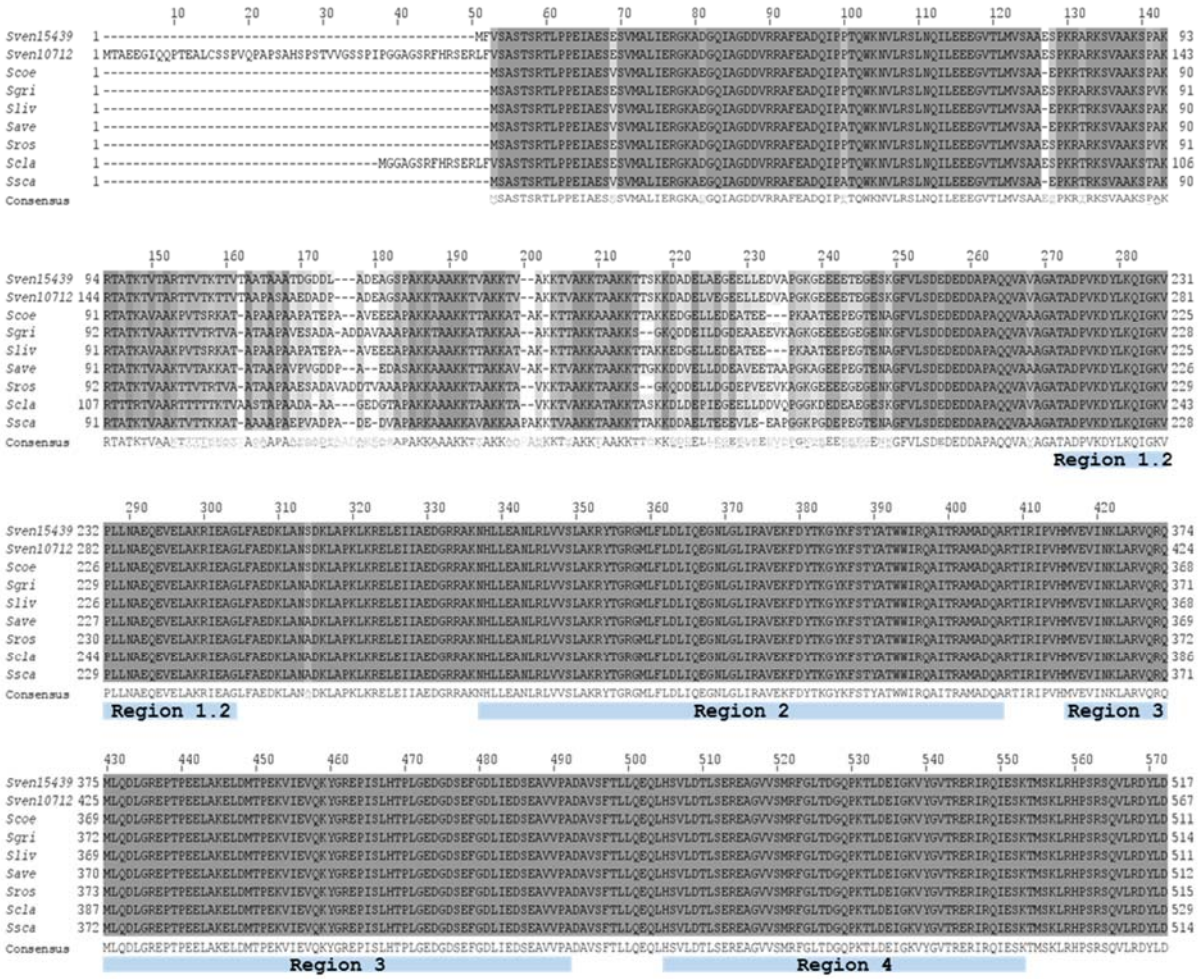


Figure S3. Multiple alignment of the HrdB proteins in *Streptomyces* species. The amino acid conservation (consensus) at each point is indicated below the sequences. Each σ factor domains of HrdB was shown in blue boxes under the sequences. Sven15439, *S. venezuelae* ATCC15439; Sven10712, *S. venezuelae* ATCC10712; Scoe, *S. coelicolor* M145; Sgri, *S. griseus* JCM4626; Sliv, *S. lividans* TK24; Save, *S. avermitilis* ATCC31267; Sros, *S. roseosporus* NRRL11379; Scla, *S. clavuligerus* ATCC27064; Ssca, *S. scabiei* S58.

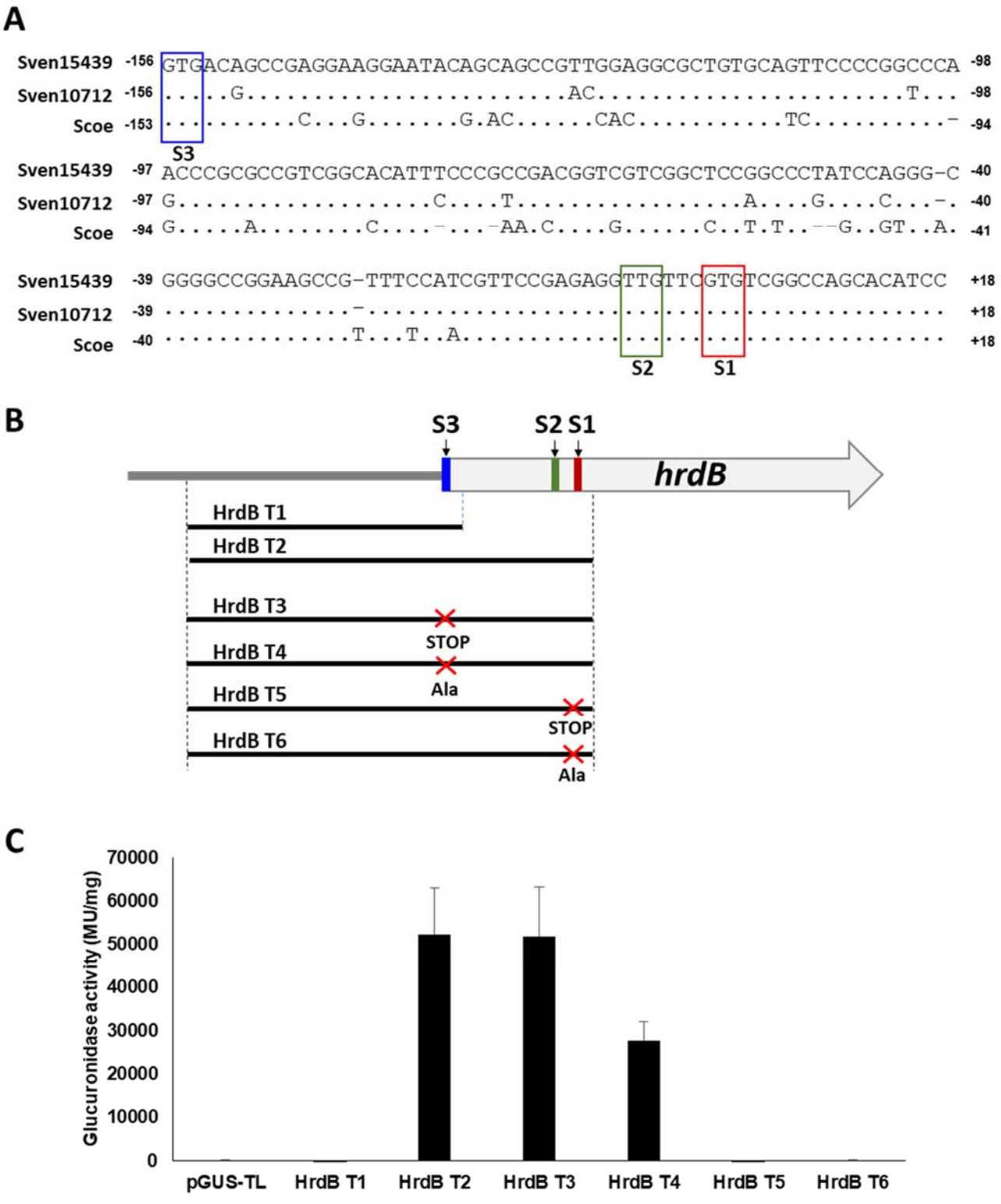


Figure S4. Translation start site of HrdB.

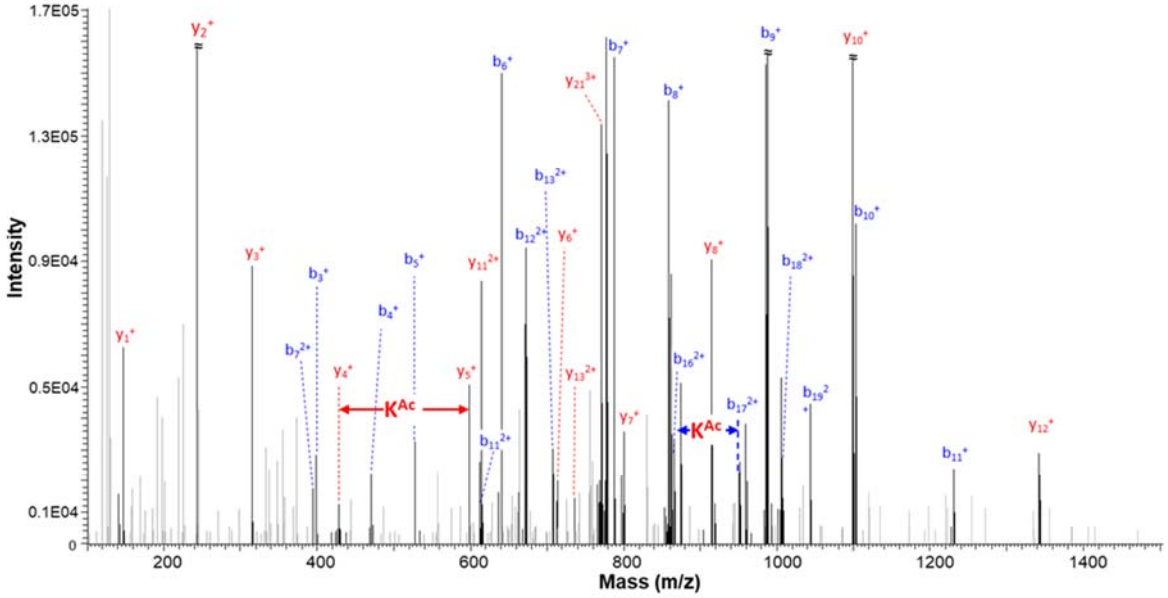
A. Nucleotide sequence alignment of the upstream regions of the *hrdB* genes from *Streptomyces* species. S1-3 indicate the annotated start codons of HrdB in Scoe (red),

Sven15429 (green), and Sven10712 (blue), respectively. The numbers at the right indicate the nucleotide positions relative to the first G of S1 as the +1.

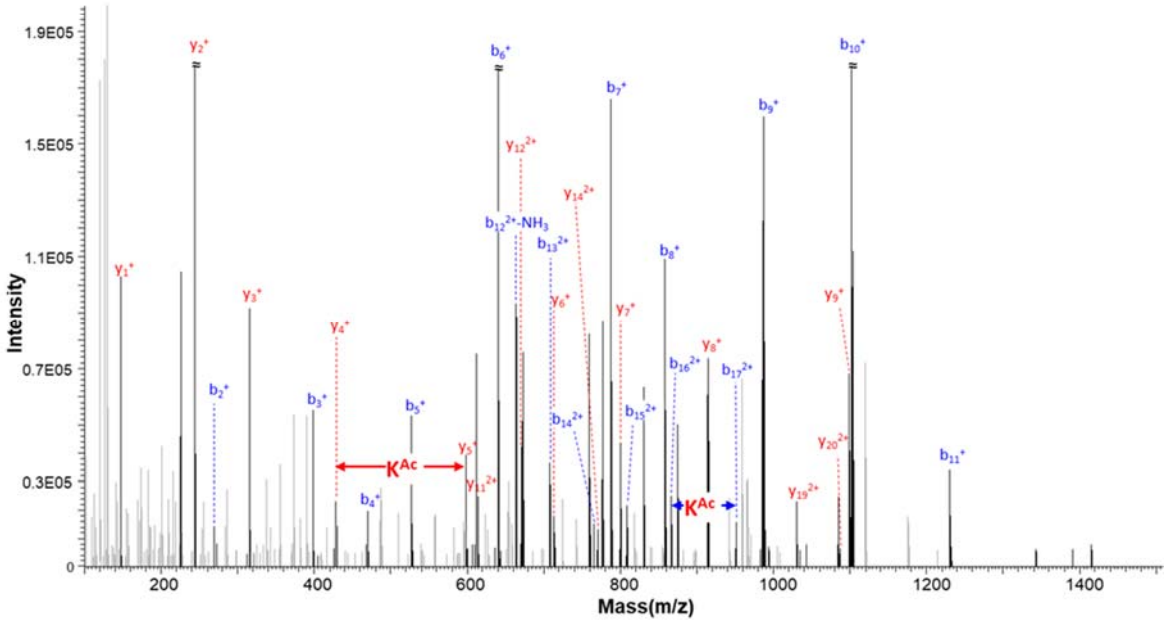
B. Translational fusions of the *hrdB* upstream regions (HrdB T1-6) to β-glucuronidase (GUS). The upstream DNA fragments from -533 to -133 (HrdB T1) or to +24 (HrdB T2~6) with or without the designated mutations: STOP represents a GTG-to-TGA mutation; Ala represents a GTG-to-GCC mutation.

C. GUS activity of the translational fusions in B. The GUS activity is expressed in Miller units (MU) per mg of total protein in the cell lysate. The data represent the average values with the standard errors from three independent experiments.

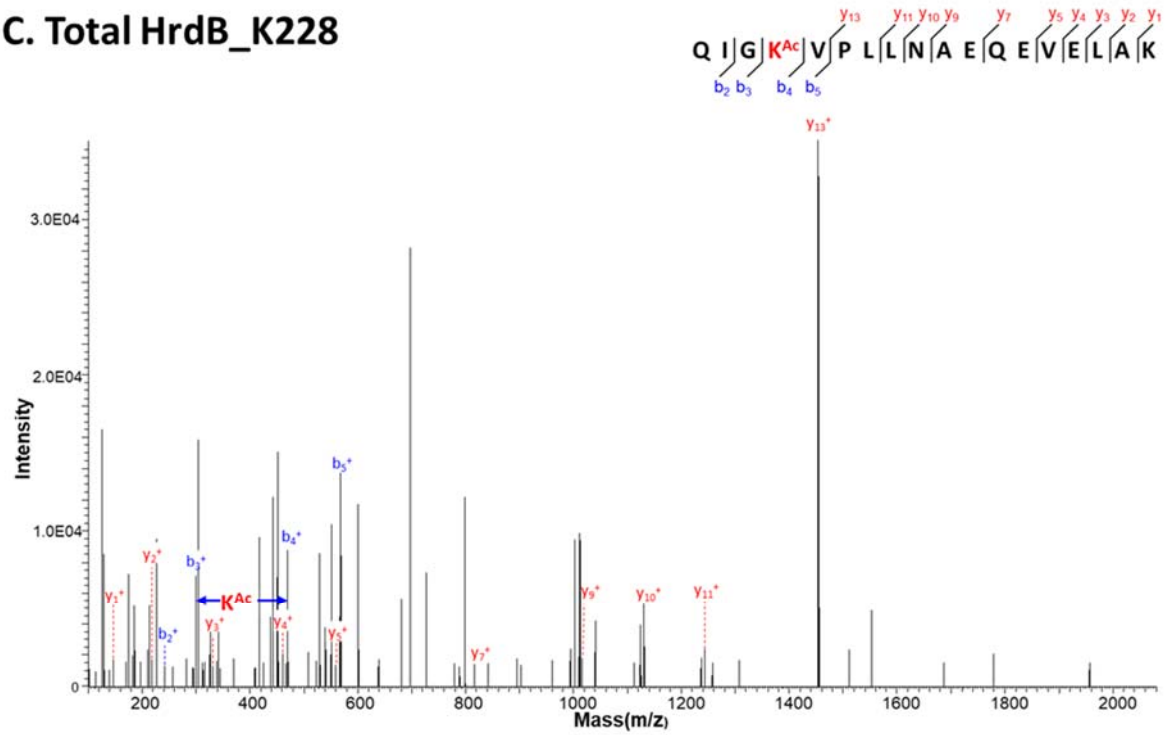
A. Total HrdB_K259



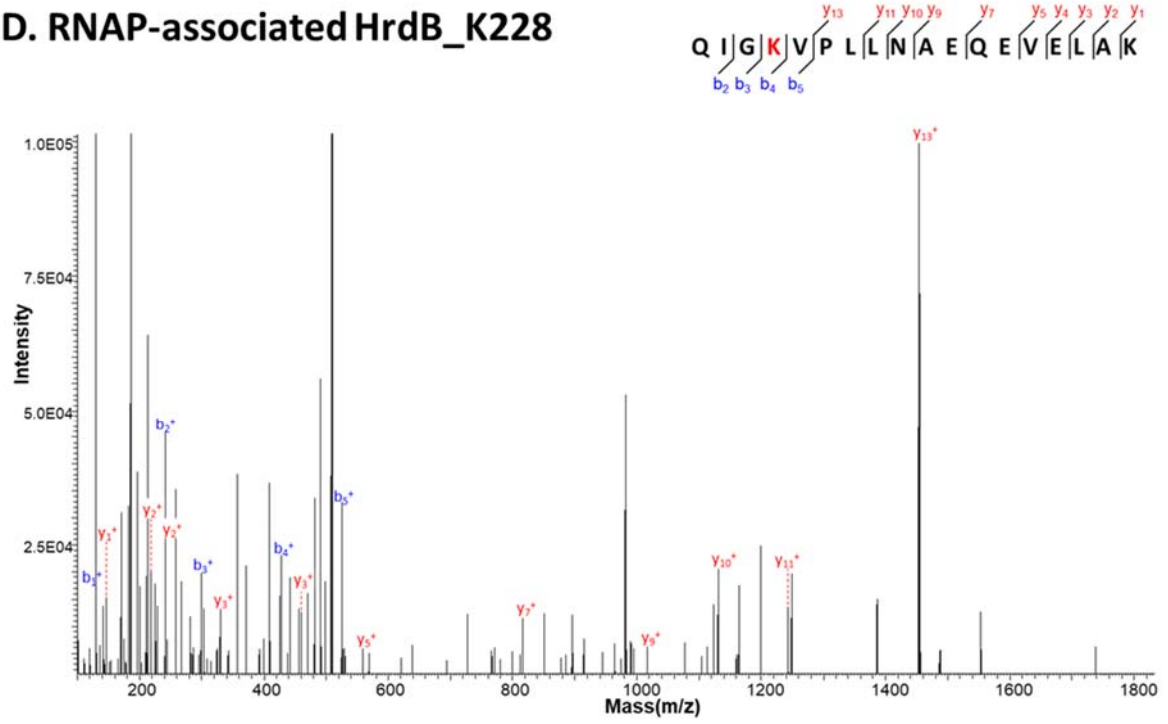
B. RNAP-associated HrdB_K259



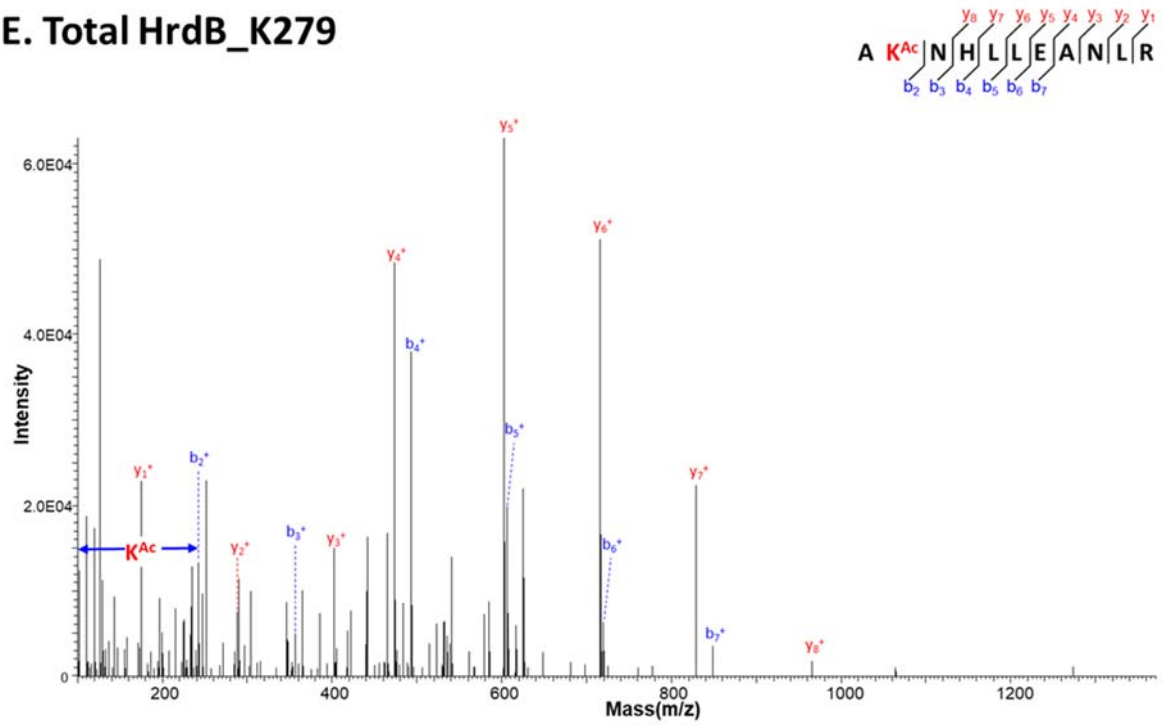
C. Total HrdB_K228



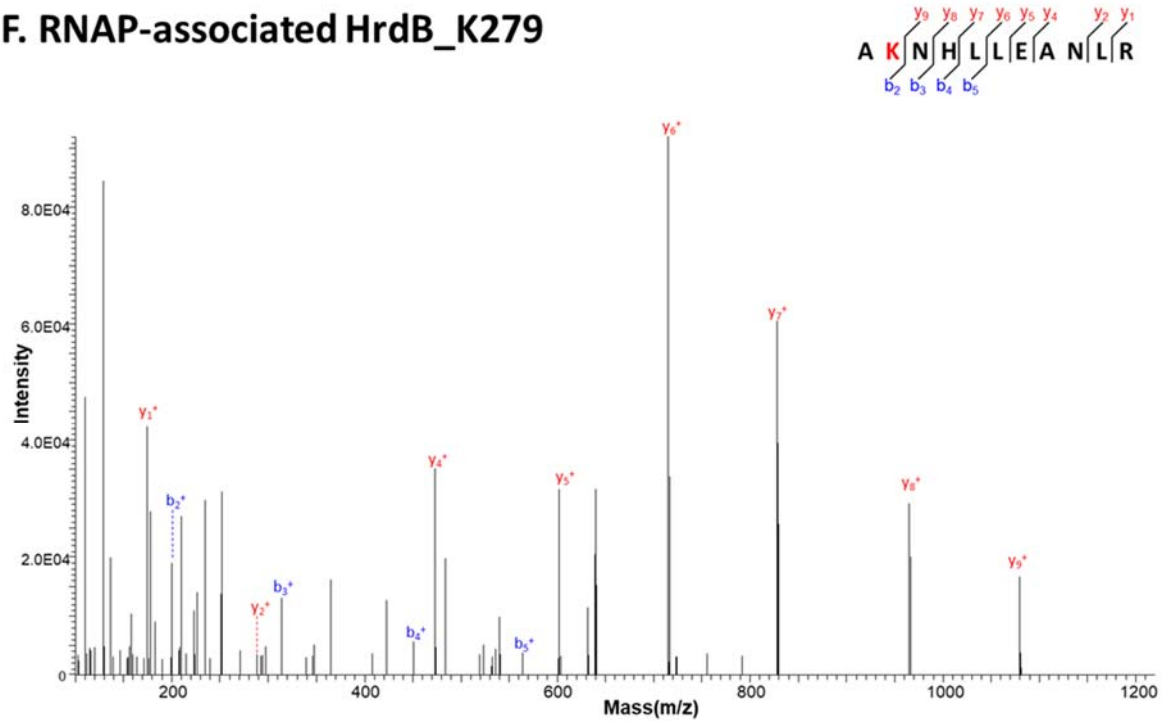
D. RNAP-associated HrdB_K228



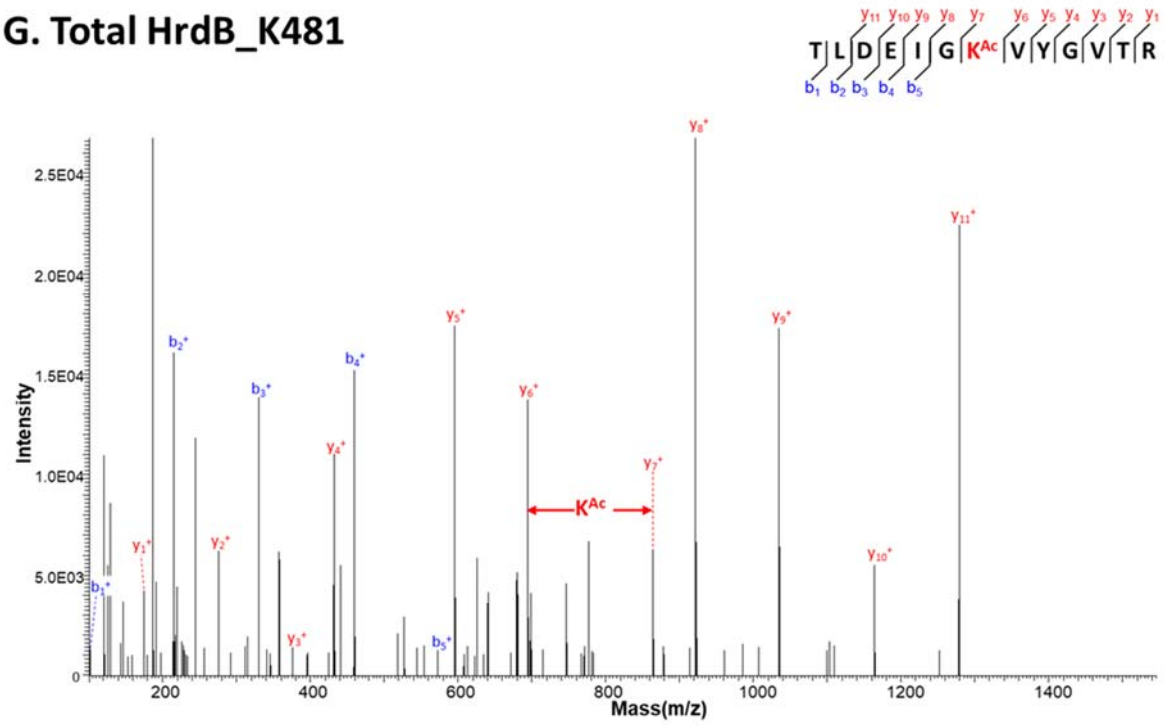
E. Total HrdB_K279



F. RNAP-associated HrdB_K279



G. Total HrdB_K481



H. RNAP-associated HrdB_K481

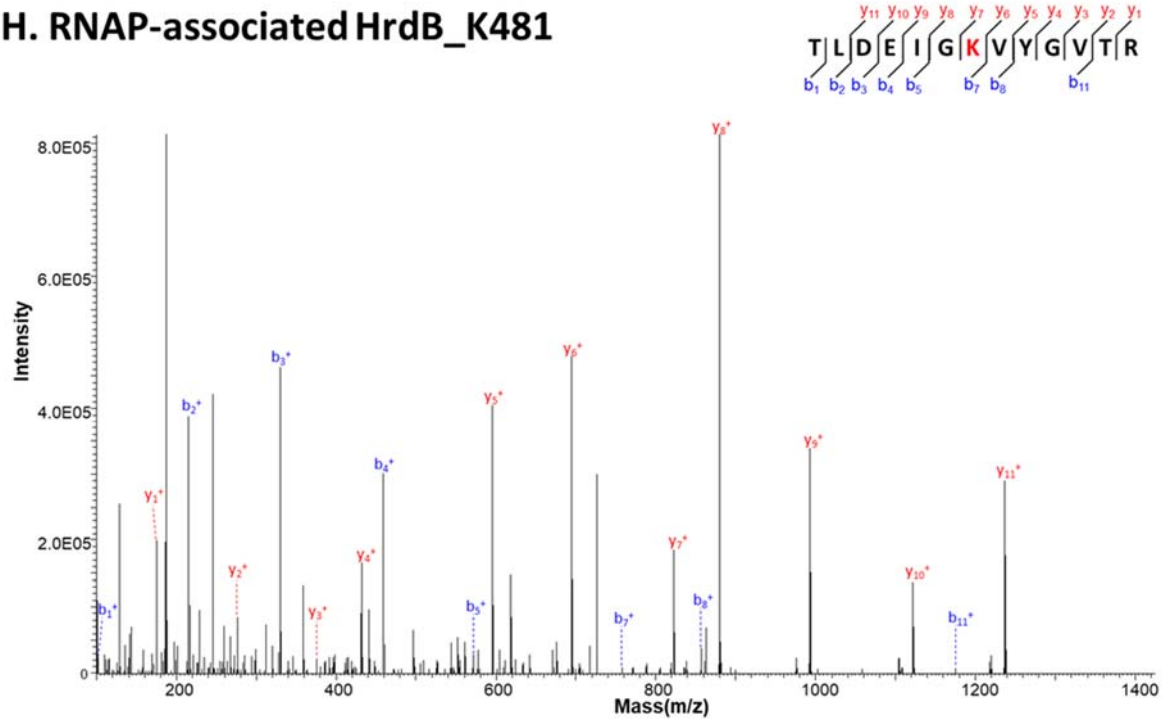


Figure S5. Determination of the AcK residues in HrdB.

MS/MS spectra are shown for the lysine acetylated and non-acetylated HrdB peptides in the total HrdB (A, C, E, and G) and in the RNAP-associated HrdB (B, D, F, and H) at the early exponential phase, which contain one of the 4 lysine residues of HrdB: K259 (A and B), K228 (C and D), K279 (E and F), and K481 (G and H).

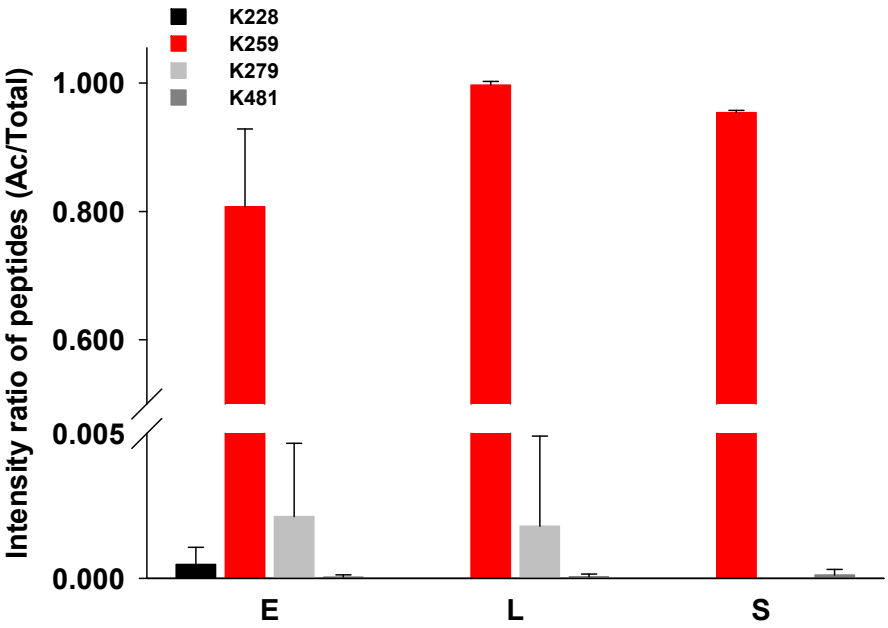


Figure S6. Acetylation of lysine residues in the total HrdB.

Acetylation ratio (acetylated vs total) of HrdB in the immunoprecipitates (i.e. total HrdB) at early- (E) and late- (L) exponential and stationary (S) phases was determined from the LC-MS/MS data such as shown in Fig. S5. The data represent the average values with standard errors from four independent experiments.

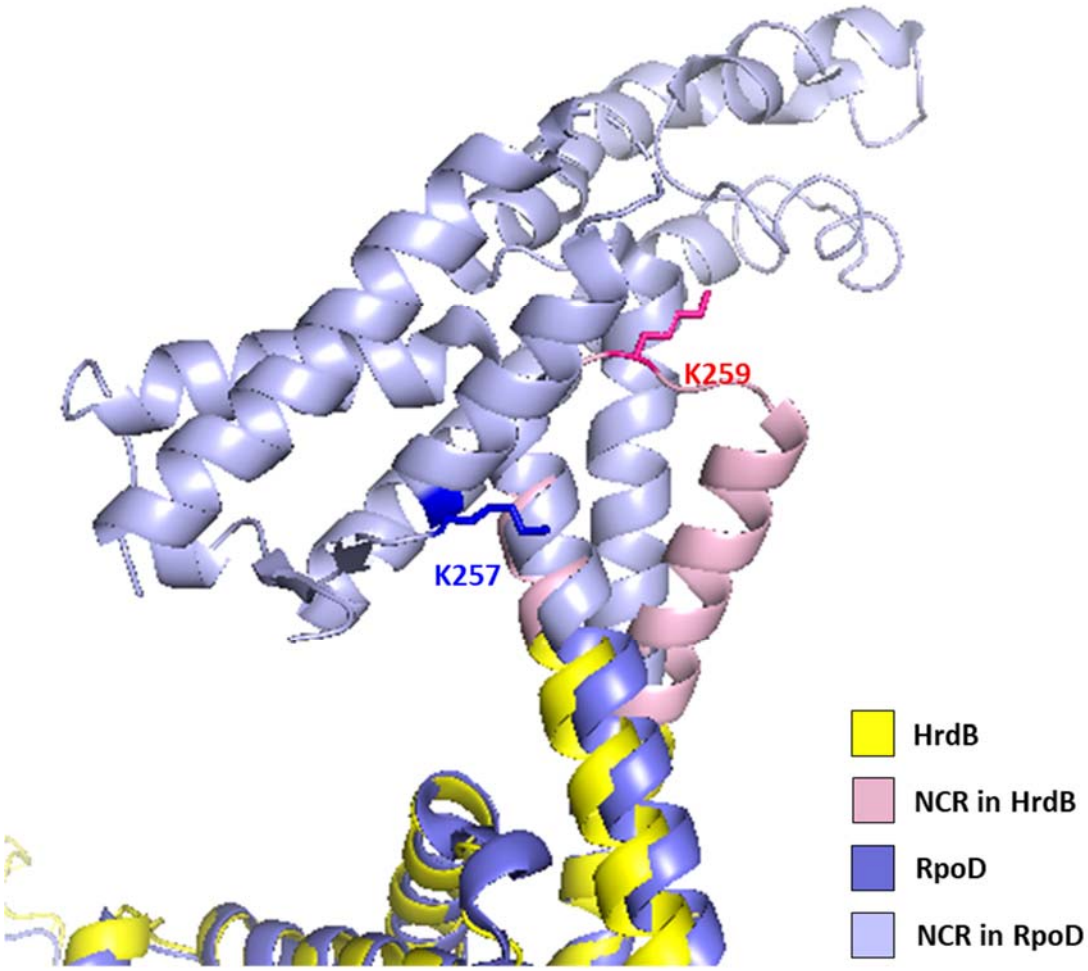


Figure S7. Location of an acetylated lysine residue in *E. coli*.

The structure of the *E. coli* RpoD is shown in the *E. coli* RNAP holoenzyme structure from RSC Protein Data Bank (4XSX). The RpoD (purple) is aligned with the modeled HrdB (yellow) using PyMol. The non-conserved region (NCR) in HrdB is shown in pink, and the NCR in RpoD is shown in light-purple. The acetylated lysine residue in RpoD (K257) and HrdB (K259) are shown in blue and red, respectively.

SUPPLEMENTARY TABLES

Table S1. Strains and plasmids used in this study

Strains or plasmids	Relevant properties or sequences	Sources or references
Strains		
E. coli DH5α	*supeE44*, *ΔlacU169* (φ*80lacZΔM15*), *hsdr*17 (r_K-, m_K-), *recA1*, *endA1*, *gyrA96, thi-1*, *relA1*	Laboratory Stock
E. coli BL21(DE3)	F-*ompT* r_B-m_B-(DE3)/pLys	Laboratory Stock
Streptomyces venezuelae ATCC15439	Wild-type	Laboratory Stock
JE01	*S. venezuelae* with pSET152F	This study
JE02	*S. venezuelae* with pSETF-hrdB	This study
JE02-Q	*S. venezuelae* with pSETF-hrdB(K259Q)	This study
JE02-R	*S. venezuelae* with pSETF-hrdB(K259R)	This study
JE03	*S. venezuelae* with pGUS-TL	This study
JE03-1	*S. venezuelae* with pG-HrdB T1	This study
JE03-2	*S. venezuelae* with pG-HrdB T2	This study
JE03-3	*S. venezuelae* with pG-HrdB T3	This study
JE03-4	*S. venezuelae* with pG-HrdB T4	This study
JE03-5	*S. venezuelae* with pG-HrdB T5	This study
JE03-6	*S. venezuelae* with pG-HrdB T6	This study

JE04	RpoC-6×His tagged in *S. venezuelae*	This study
Plasmids		
pSET152F	pSET152 derivative used for C-terminus 3×FLAG tagging with apramycin resistance gene	(2)
pSETF-hrdB	pSET152F derivative carrying a DNA fragment (2,078 bp) for expression of the HrdB-FLAG protein in *S. venezuelae*	This study
pSETF-hrdB(K259Q)	pSET152F derivative carrying a DNA fragment (2,078 bp) for expression of the HrdB-FLAG mutein (K259Q)	This study
pSETF-hrdB(K259R)	pSET152F derivative carrying a DNA fragment (2,078 bp) for expression of the HrdB-FLAG mutein (K259R)	This study
pKC1139	*E. coli-Streptomyces* shuttle vector for gene deletion in *S. venezuelae* with apramycin resistance gene	(3)
pKC-rpoHis	pKC1129 derivative for construction of RpoC-6×His tagging strains	This study
pGUS	Promoter probe vector containing the *gusA* gene	(1)
pGUS-TL	pGUS derivative having multi cloning site (*XbaI*, *KpnI*, *SpeI*, *NdeI*, *BglII*, and *StuI*) and with neither RBS nor start codon of the *gusA* gene	This study
pG-HrdB T1	pGUS-TL derivative carrying the DNA fragment (401 bp) for the HrdB upstream region from -533 to -133	This study
pG-HrdB T2	pGUS-TL derivative carrying DNA fragment (557 bp) for the HrdB upstream region from -533 to +24	This study
pG-HrdB T3	pG-HrdB T2 derivative with a codon change from GTG to TGA at S3 (-156 to -154)	This study
pG-HrdB T4	pG-HrdB T2 derivative with a codon change from GTG to GCC at S3 (-156 to -154)	This study

pG-HrdB T5	pG-HrdB T2 derivative with a codon change from GTG to TGA at S1 (+1 to +3)	This study
pG-HrdB T6	pG-HrdB T2 derivative with a codon change from GTG to GCC at S1 (+1 to +3)	This study

Table S2. Oligonucleotides used in this study

Purpose	Primer name	Sequences (5′ to 3′)
Plasmid construction		
pGUS-TL	pGTL-F[a]	ACT AGT CAT ATG TCG CAG ATC TTA AGG CCT C*TG CGG CCC GTC GAA ACC CCG ACC C*
	pGTL-R[b]	GCC AGC GGG GCG AAG TAC TCC C
pKC-rpoHis	rpo/pKC-F[c]	CGA CGG CCA GTG CCA GGT ACG TCT GCG TTC TGC
	rpo/his-R[c,d]	CGC ACT AC*T TAG TGG TGG TGG TGG TGG TG*C TGG TTG TAC GGA CCG TA
	rpo/his-F[d,e]	*CAC CAC TAA* GTA GTG CGA ACC GAA GGG
	rpo/pKC-R[c]	CAT GAT TAC GAA TTC GAT GTC CGT CAT CGG CCA CCT
	rpoC-F	CCT GGT CGG CCT CAA GGA GA
	His-R	GTG GTG GTG GTG GTG GTG CTG GTT G
pSETF-HrdB	pSET/HrdB-F[f]	GCT TGG GCT GCA GGT CGA CTT GCG ACC GCC CGA GCG AG
	pSET/HrdB-R[f]	TCC TTG TAG TCC TCG AGA GGG TCG AGG TAG TCG CGC AG
HrdB Q	259Q-F[g]	CGG ACC AGC TCG CGC CGA AGC TCA A
	259Q-R[g]	CGC GAG CTG GTC CGA GTT CGC CAG C
HrdB R	259R-F[g]	CGG ACC GCC TCG CGC CGA AGC TCA A
	259R-R[g]	GCG AGG CGG TCC GAG TTC GCC AGC T
RT-qPCR		
16s rRNA	16s-F	GGG AGC GAA CAG GAT TAG ATA C

	16s-R	CTT TGA GTT TTA GCC TTG CGG
hrdB	hrdB-F	CGA GTC CGA GTC TGT GAT G
	hrdB-R	CGA GGA TCT GAT TGA GGC TG
ChIP-qPCR		
rpoB	rpoB-F	GTC ACC AGG GGC ATA CCT TC
	rpoB-R	TGC TTT GCG GCA GAC TTT TC
atpL	atpL-F	GTA GTG CGA CGG TCT GTT G
	atpL-R	ATG ACG GTG TGC AGA AGG

a: *SpeI*, *NdeI*, *BglII*, and *StuI* sites are underlined, with the sequence from +4 to +28 of the *gusA* gene italicized.

b: The innate *ScaI* site within the *gus* gene is underlined.

c: The sequences from pKC1139 are underlined.

d: The 6xHis-tag and the stop codon are italicized.

e: The *rpoC* region is underlined.

f: The sequences from pSET152F are underlined.

g: The changed codons are underlined.

Table S3. LC-MS/MS data for the most abundant proteins in the HrdB-containing gel slice based on spectral counts

Protein Name	GenBank	Peptide spectrum No.	Unique peptides	Predicted Protein size
60kDa chaperonin	ALO10479	276	65	56.9 kDa
Ribonuclease J	ALO11404	146	49	61.1 kDa
60kDa chaperonin	ALO10061	119	54	57.0 kDa
HrdB	ALO11494	106	43	56.5 kDa

REFERENCES

1. Myronovskyi, M., Welle, E., Fedorenko, V. and Luzhetskyy, A. (2011) β-Glucuronidase as a sensitive and versatile reporter in actinomycetes. *Applied and environmental microbiology*, **77**, 5370-5383.
2. Kim, J.-E. (2019) Expression and regulation of RNA polymerase sigma factors in *Steptomyces venezuelae*. Doctor of Philosophy, Seoul National University.
3. Bierman, M., Logan, R., O'brien, K., Seno, E., Rao, R.N. and Schoner, B. (1992) Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. *Gene*, **116**, 43-49.