Supplemental Information

Supplemental Experimental Procedures

Materials

S. coelicolor M145 and their derivatives were cultured on MS solid medium or as indicated at 30 ℃. *E. coli* DH5α and *E. coli* BL21 (DE3) were cultured in LB medium at 37 ℃, and they were the host strains for standard plasmid manipulation and protein expression, respectively. The methylation-deficient *E. coli* strain ET12567 was used to propagate DNA into *S. coelicolor* A3(2).

Generation of *S. coelicolor* PL138, PL139 and PL140

First, the DNA sequence of *parB-his* was amplified from *S. coelicolor* genomic DNA with a 12× His sequence embedded in the PCR primer. Then, the resistance gene *aac(3)IV* was integrated into *parB-his* fragment as a selective marker by overlapping PCR. The *parB* gene was finally replaced by *parB-his-aac(3)IV* in the chromosome by homologous recombination (1) generating *S. coelicolor* PL138 [*SCO3887::parB-his-aac(3)IV*]. Based on *S. coelicolor* PL138, another two ParB expression strains were created by further deletion of *Scpat* or *SccobB1*. Briefly, the gene encoding *Scpat* or *Sccobb1* was in-frame replaced by a kanamycin resistance cassette within a cosmid (thiostrepton resistance) first. The resulting cosmids were inserted into *S. coelicolor* PL138 strain to induce double exchanges by homologous recombination (tsr^s, kan^r). The positive strains were verified by PCR and named as PL139 [*Scpat*::*neo*; *SCO3887*::*parB-his-aac(3)IV*] or PL140 [*Sccobb1*::*neo*; *SCO3887::parB-his-aac(3)IV*].

Measurement of growth curve for the wild-type and PL138 *S. coelicolor*

For the determination of growth curves, fresh spores from the wild-type and PL138 *S. coelicolor* were diluted to the same amount (OD600 = 1.5, 100 μl) and pre-germinated in 400μl LB liquid medium at 50 ℃ for 10 min. Then, a total of 500 μl spores of each strain were grown on MS agar plates (diameter 150 mm) covered with sterile plastic cellophane at 30 ℃ and harvested at different time points (24, 30,36, 48, 60, 72 and 84 h). Cells were dried

31 at 65 ℃ for 2 days to measure the dry weights of samples.

32

33 **Expression and purification of *Sc*Pat, *Sc*CobB1, *Sc*CobB2, ParB, and ParB derivatives**

34 The gene coding sequences for *Sc*Pat, *Sc*CobB1, *Sc*CobB2, and ParB were amplified
35 from *S. coelicolor* genomic DNA with corresponding primers. DNA fragments of *Scpat*,
36 *SccobB1*, and *SccobB2* were digested by *Eco*R I and *Xho* I, and ligated to pET28b to construct
37 the expression vectors. For heterologous expression of ParB, the *parB* DNA fragment was
38 digested with *Eco*R I and *Xba* I, and then ligated to pET28b to construct pET28b-*parB*. The
39 expression vectors for $ParB^{K183Q}$, $ParB^{K183R}$, $ParB^{K183A}$, $ParB^{K187Q}$ $ParB^{K187R}$, and $ParB^{K187A}$
40 were built by site-directed mutagenesis Kit (Thermo Fisher, USA), using pET28b-*parB* as the
41 template. Primer sequences are listed in Table S3.

42 All these plasmids were transformed into the *E. coli* BL21 (DE3). The correct
43 monoclones were grown overnight in 5 ml LB medium containing kanamycin (50 μg mL^{-1}),
44 and then subcultured into 500 ml LB liquid medium. When the OD_{600} reached to 0.6, cells were
45 translocated to 16 ℃ and the corresponding proteins were induced with 0.5 mM IPTG for 12
46 hours. The purification process for these proteins was the same as the endogenous ParB
47 purification.

48

49 **Western blotting detection**

50 Standard western blotting procedures were followed for the detection of acetylation
51 level of ParB and ParB variants. Briefly, the proteins were separated by SDS-PAGE (10% or
52 12% acrylamide) and then transferred to a nitrocellulose membrane. For western blotting, 100
53 mM Tris-HCl (pH 7.5) with 0.5% (v/v) Tween-20 and 1% peptone (AMRESCO) was used for
54 blocking buffer and 100 mM Tris-HCl (pH 7.5) with 0.05% (v/v) Tween-20 and 0.1% peptone
55 was used for primary and secondary antibody buffers. The nitrocellulose membrane was treated
56 with an ECL western blotting Kit (GE Healthcare) and visualized using an ImageQuant LAS
57 4000 mini (GE Healthcare) equipped with the ImageQuant TL software (GE Healthcare).
58 Unless otherwise indicated, coomassie blue staining was used for the loading control.

59

60 ***In vitro* acetylation and deacetylation of ParB peptides**

61 The *in vitro* peptide acetylation system contains 50 mM Tris-HCl (pH 8.0), 1.55 μM
62 *Sc*Pat and 295 μM peptide ParB^{K183}, in the presence or absence of 0.2 mM acetyl-CoA. The *in*
63 *vitro* peptide deacetylation system contains 50 mM Tris-HCl (pH 8.0), 6.0 mM $MgCl_2$, 6.0 μM
64 *Sc*CobB1 or 7.5 μM *Sc*CobB2 and 1.43 mM peptide ParB^{K183Ac}, in the presence or absence of
65 1.0 mM NAD^+. The reactions were performed in a 50 μl volume at 30°C for 2 hours, and
66 terminated by adding 1% (v/v) trifluoroacetic acid (TFA). After treatments, samples were
67 centrifuged at 15,000 $\times g$ for 10 mins, and the supernatants were analyzed *via* HPLC using the
68 Aeris peptide XB-C18 column (150 × 4.6 mm, 3.6 μm; Phenomenex).
69
70 **Construction of fluorescent ParB and ParB variants in *S. coelicolor***
71 To monitor the formation of ParB segregation complex in *S. coelicolor*, an integration
72 plasmid encoding the in-fusion ParB-eGFP protein was generated and propagated into *S.*
73 *coelicolor* chromosome. Specifically, the *parB-egfp* fragment including the native *parAB*
74 promotor (containing both *parABp$_1$* and *parABp$_2$*) was fused by overlapping PCR. The stop
75 codon of *parB* gene was replaced by the linker sequence 'tctagaggtcgccac'. The fused *parABp-*
76 *parB-egfp* fragment was digested by *Bgl* II and *Not* I and then ligated into pSET152a plasmid.
77 The resulting plasmid was propagated into the *S. coelicolor* cells (WT, Δ*parb*, Δ*Scpat*,
78 Δ*SccobB1*, or Δ*SccobB2*) and integrated at the *attB* sites of their chromosomes, respectively.
79 Using the plasmid of pSET152a-parB-eGFP as the template, ParB^{K183Q}-eGFP and ParB^{K183R}-
80 eGFP were built by site-directed mutagenesis Kit (Thermo Fisher, USA). These three plasmids
81 were propagated into *S. coelicolor* Δ*parb* strain and integrated into the chromosome, generating
82 *ΔparB*(*parB-egfp*), *ΔparB*(*parB^{K183Q}-egfp*) and *ΔparB*(*parB^{K183R}-egfp*) strains, respectively. We
83 further built *ΔSccobB1ΔparB* (*parB^{K183R}-egfp*) strain by deletion of *SccobB1* gene based on
84 *ΔparB*(*parB^{K183R}-egfp*) cell using the PCR-targeted method (1).
85 The correct clones were selected by flooding the MS solid plates with thiostrepton (10
86 μg mL^{-1}) after 16 hours' culture, and confirmed by cloning PCR. To determine the expression
87 level of the fluorescent ParB or ParB variants, cells were harvested by centrifuging at 6000 $\times g$
88 for 10 mins and resuspended in cold Tris-HCl buffer (50 mM, pH 8.0) containing NaCl (500
89 mM), imidazole (10 mM), EDTA (1 mM) and phenylmethanesulfonyl fluoride (1 mM). Then,
90 cells were disrupted by an EmulsiFlex-C5 cell disruptor and the collected supernatants were

91 performed western blotting with an anti-eGFP antibody.

92

93 **Scanning electron microscopy**

94 The wild-type, Δ*Scpat*, Δ*SccobB1*, Δ*SccobB2*, and Δ*parB S. coelicolor* strains were

95 inoculated on MS agar with inserted coverslips and then incubated at 30 ℃ for 4 days. After

96 rapid dehydration with a graded ethanol series (70, 80, 90, 95, and 100%) and concentrated

97 amyl acetate, the samples were dried with the HCP-2 critical point dryer (Hitachi, Tokyo,

98 Japan) according to the supercritical drying method. The cells were subsequently coated with

99 gold using the JFC-1600 Auto Fine Coater (Jeol, Tokyo, Japan) and then examined using a

100 JSM-6360LV scanning electron microscope (Jeol, Tokyo, Japan).

101

102 **Real-Time PCR**

103 The *S. coelicolor* wild-type strain was cultured under the same condition as described

104 in growth curve measurement. Cells were collected by centrifugation at the time points of 24,

105 30, 36, 48, 60, 72 and 84 h, respectively, and were stored in liquid nitrogen immediately. The

106 total RNA was extracted by using TRIzol (Thermo Fisher Scientific) and the SV Total RNA

107 Isolation System (Promega). DNA was removed by digestion with recombinant DNase I

108 (TaKaRa). The cDNA synthesis was executed by using the PrimeScriptTM 1st Strand cDNA

109 Synthesis Kit (TaKaRa) in a 20 μl reaction system. Real-time PCR assay was carried out with

110 SYBRR Premix Ex TaqTM II (TaKaRa) according to the manufacturer's instructions. At least

111 three independent samples were tested, the transcription of *hrdB* was used as an internal

112 control. The relative transcript levels of tested genes were normalized to *hrdB* gene

113 transcription and determined using the 2-ΔΔCT method.

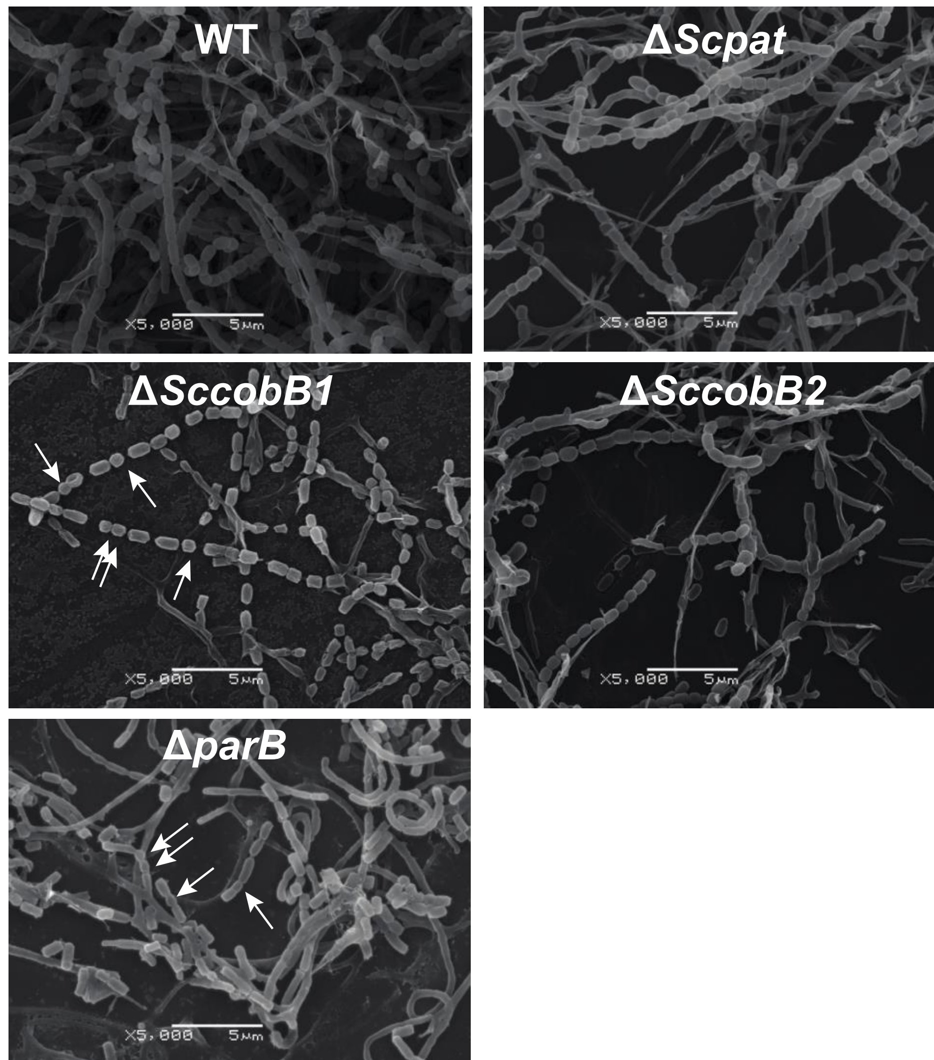
114

115

116 **Supplemental Figures**

117

118 **Figure S1**



119

120 **Figure S1. Scanning electron microscopy analysis reveals that Δ*ScobB1* produces**

121 **abnormal spores.** The wild type, Δ*Scpat*, Δ*SccobB1*, Δ*SccobB2*, and Δ*parB* of *S. coelicolor*

122 cells were inoculated onto the acute-angled junction of coverslips that inserted on MS solid

123 medium for 3 days and performed for SEM imaging. Arrows point out some abnormal spores

124 with relatively smaller sizes were found in Δ*SccobB1* and Δ*parB*. (Scale bars, 5 μm.)

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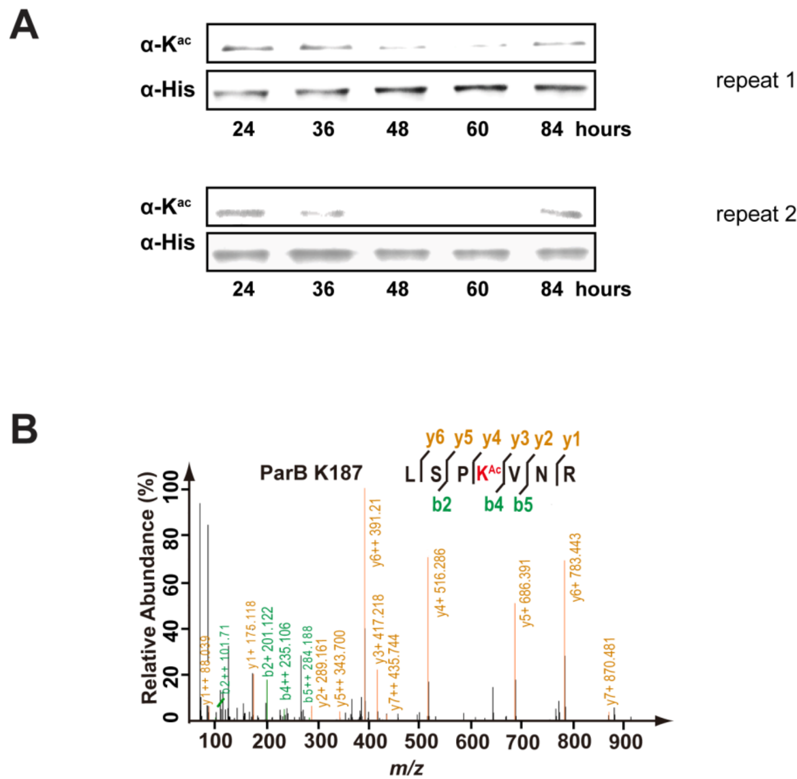
126

127 **Figure S2**



128

129 **Figure S2.** (A) The acetylation level of *S. coelicolor* ParB decreases after a 48-h cultivation

130 (corresponding to Figure 1B). Two independent results of triplicates were shown here. Cells

131 from the *S. coelicolor* PL138 [*SCO3887::parB-his-aac(3)IV*] were grown on MS solid

132 medium and their ParB acetylation levels were analyzed at different time points (1.0, 1.5, 2.0,

133 2.5, and 3.5 days). The acetylation level was determined by western blot using the anti-

134 acetyllysine antibody and visualization the proteins using the anti-His antibody.

135 (B) HPLC/MS-MS analysis identified Lys-187 as one of the other ParB acetylation site

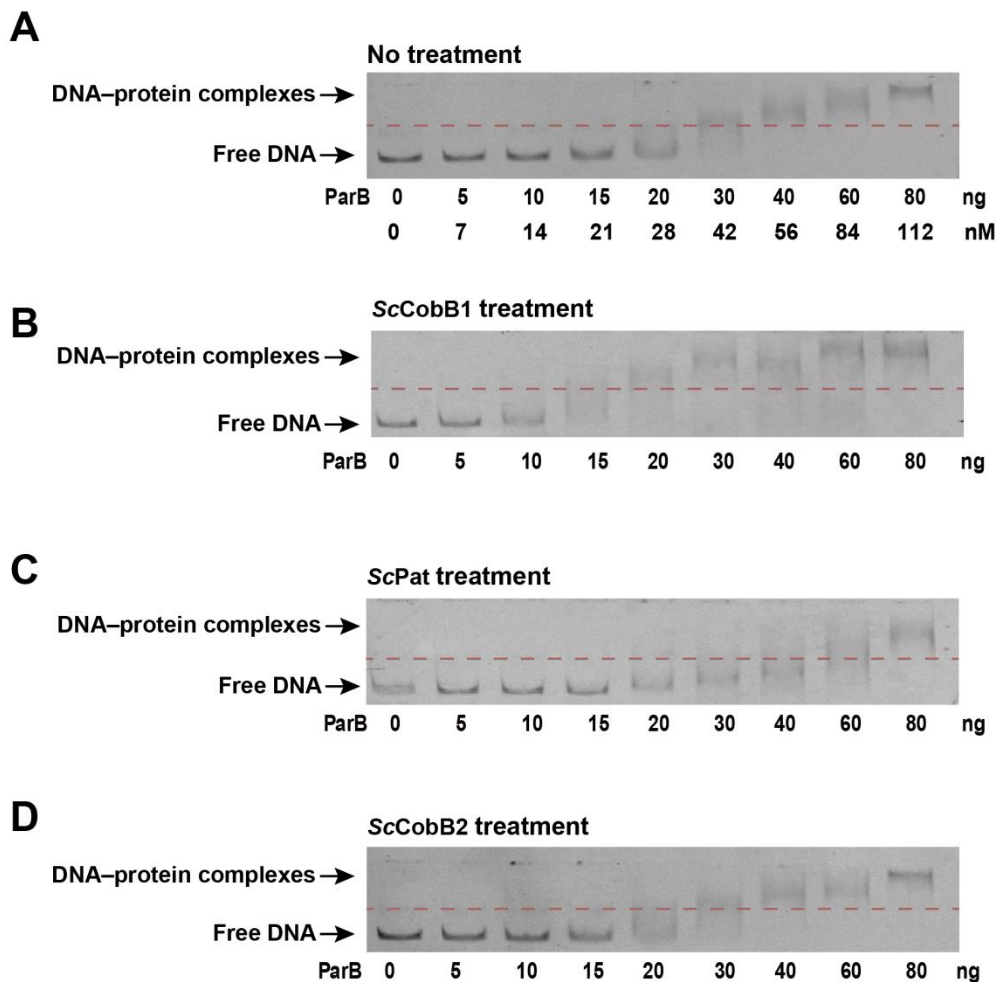
136 (corresponding to Figure 1D). The ParB protein isolated from *S. coelicolor* after a 36-h

137 cultivation was digested and subjected to HPLC/MS-MS analyses.

138

139

140 **Figure S3**



141

142 **Figure S3. Deacetylation increases the DNA-binding affinity of ParB to *parS* while**
143 **acetylation decreases its DNA-binding affinity to *parS* (corresponding to Figure 3D).** A
144 series of EMSAs were completed by incubating *parS* with varying concentrations of ParB
145 proteins (0–112 nM), which were pre-treated with *Sc*Pat, *Sc*CobB1, or *Sc*CobB2. Free DNA
146 and DNA bound (indicated by arrows) were separated by 5% polyacrylamide gel
147 electrophoresis. The ParB protein without any treatment was used as the control. The
148 background-subtracted signal intensity was measured, and the binding constant K_D was
149 calculated based on these results.

150

151

152 **Figure S4**

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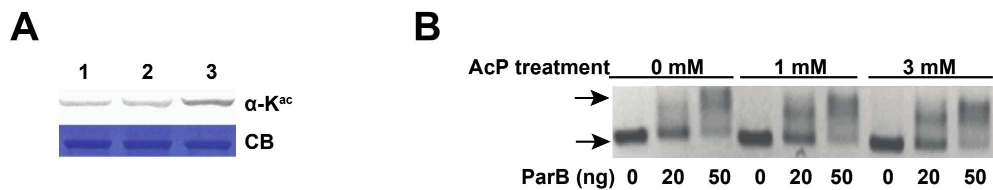
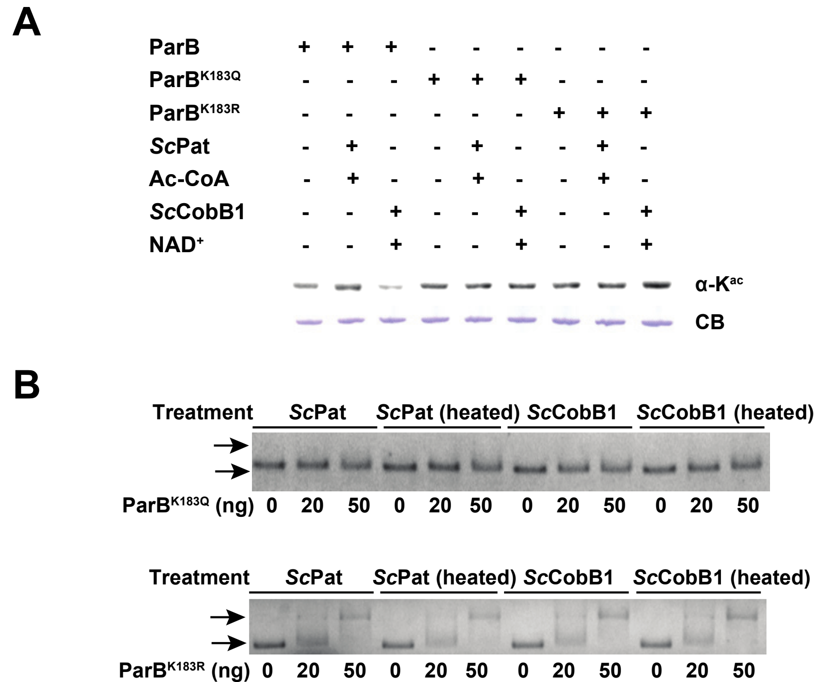


154

155 **Figure S4. The acetylation by AcP is not specific to the lysine site(s) pivotal for ParB**
156 **binding activity.** (A) ParB proteins were incubated with 0 mM (line 1), 1 mM (line 2), 3 mM
157 (line 3) acetyl-phosphate for 2 hours, respectively, and then performed western blotting using
158 pan-anti-acetyllysine antibody. The coomassie blue staining (CB) of ParB was set as the
159 loading control. (B) EMSA assay was carried out using a FAM-labelled fragment containing
160 two consecutive *parS* sequences. DNA fragments were incubated with varying concentrations
161 of ParB proteins (0-50 ng), which were pre-treated by indicated acetyl-phosphate. Free DNA
162 and DNA-protein complexes (shown by arrow, respectively) were separated in 2% agarose
163 gel (0.25 × TBE, at 20 V cm^{-1}, 4 ℃).

164

165 **Figure S5**



166

167 **Figure S5. Neither the acetylation level nor the DNA-binding affinity of ParB variants at**
168 **Lys-183 is altered in response to *Sc*Pat or *Sc*CobB1 treatment.** (A) ParB and ParB variants
169 (ParB^{K183Q} and ParB^{K183R}) were incubated with *Sc*Pat in presence of acetyl-CoA, or *Sc*CobB1
170 in presence of NAD$^+$. The acetylation level of ParB was determined by western blotting using
171 a pan-anti-acetyllysine antibody. The coomassie blue staining (CB) of ParB was set as the
172 loading control. (B) ParB^{K183Q} and ParB^{K183R} were incubated with active *Sc*Pat (or *Sc*CobB1)
173 or heat-inactivated *Sc*Pat (or *Sc*CobB1). EMSA assays were carried out using a FAM-labelled
174 fragment containing two consecutive *parS* sequences. DNA fragments were incubated with
175 varying concentrations of pre-treated ParB variants (0-50 ng). Free DNA and DNA-protein
176 complexes (shown by arrow, respectively) were separated in 2% agarose gel (0.25×TBE, at
177 20 V cm^{-1}, 4 ℃).

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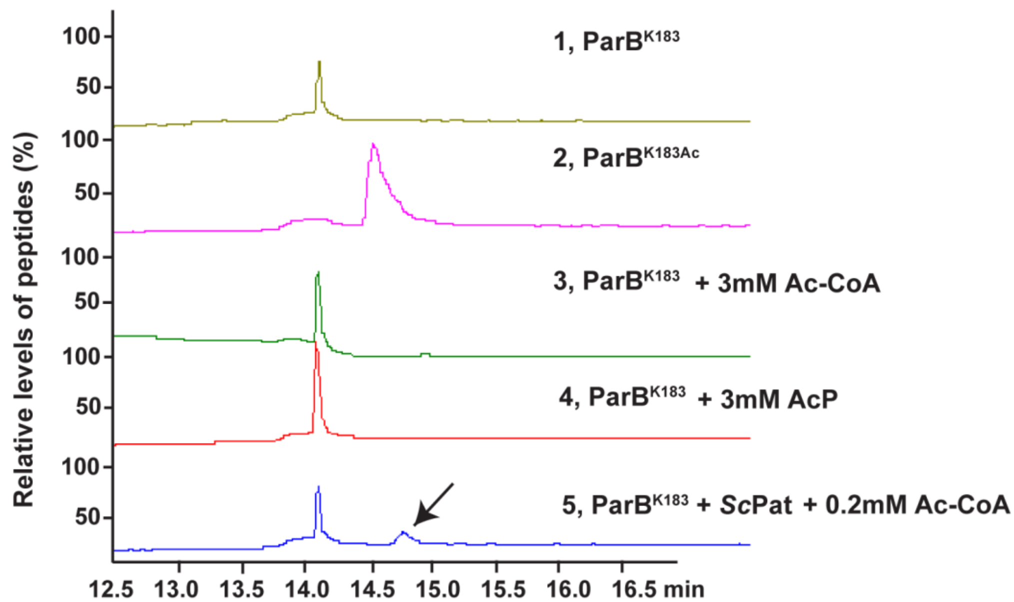
184 **Figure S6**

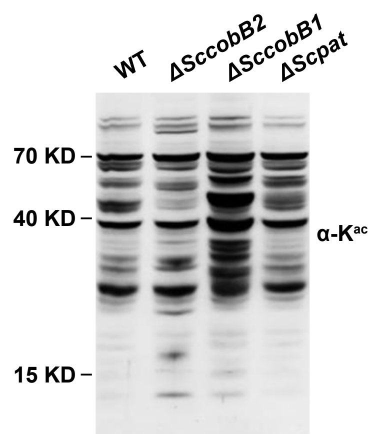


185

186 **Figure S6. Treatments with high concentration of Acetyl phosphate (AcP) or acetyl-CoA**
187 **(Ac-CoA) failed to acetylate ParB^{K183} peptide.** The ParB peptide with an acetylated Lys-183
188 residue were synthesized and incubated with 3 mM Ac-CoA, 3 mM AcP or 0.2 mM Ac-CoA
189 in present of *Sc*Pat at 30 ℃ for 2 h. Reactions were terminated by adding 1% TFA, after which
190 the samples were analyzed by HPLC. The arrow indicates the retention time of the acetylated
191 ParB peptide (ParB^{K183Ac}).

192

193 **Figure S7**



194

195 **Figure S7. *Sc*CobB1 has a global regulatory role in *S. coelicolor* acetylome.** Western blot

196 was used to examine the whole protein extracts from the wild-type, Δ*Scpat,* Δ*SccobB1*, and

197 Δ*SccobB2* cells after a 48-h cultivation in TSB liquid medium.

198

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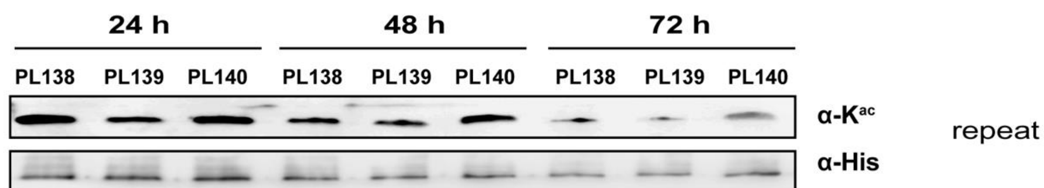
200

201 **Figure S8**



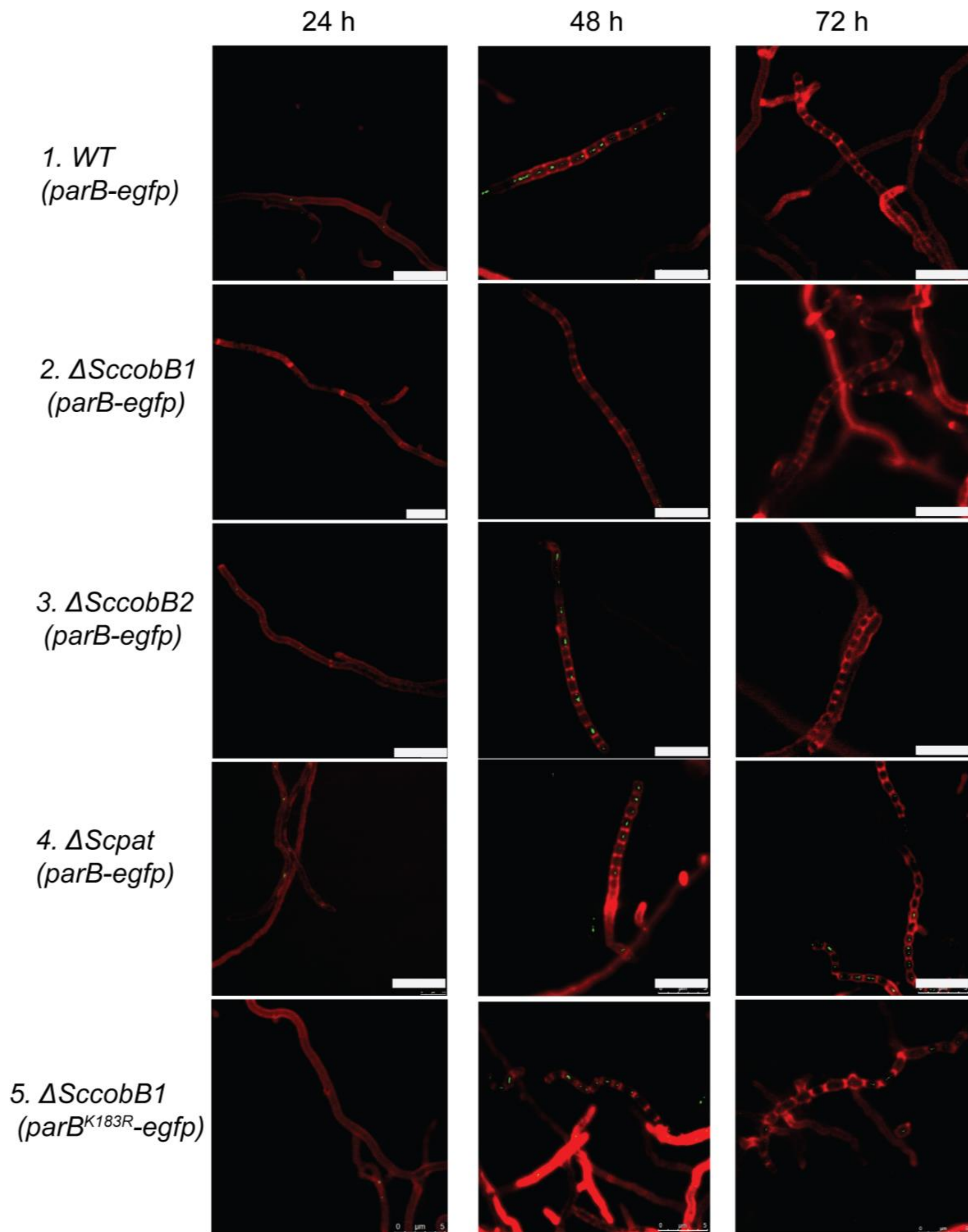
202

203 **Figure S8. The acetylation level of ParB-His proteins from PL138 (WT), PL139 (Δ*Scpat*),**
204 **and PL140 (Δ*SccobB1*) at different time points grown in TSB liquid medium**
205 **(corresponding to Figure 5).** One independent results of two replicates was shown here. The
206 acetylation level was determined by western blot using the anti-acetyllysine antibody and
207 visualization the proteins using the anti-His antibody.

208

209

210 **Figure S9**



211

212 **Figure S9.** The formation of ParB segregation complexes at different growth time in *S.*

213 *coelicolor* strains. Fluorescent microscopy was performed for wild-type, Δ*SccobB1*,

214 *ΔSccobB2*, Δ*Scpat* and Δ*SccobB1*(*parB*^{K183R}) strains. Representative images were displayed

215 for the cells collected at indicated time (24, 48, and 72 h) cultured on MS solid medium. Cell

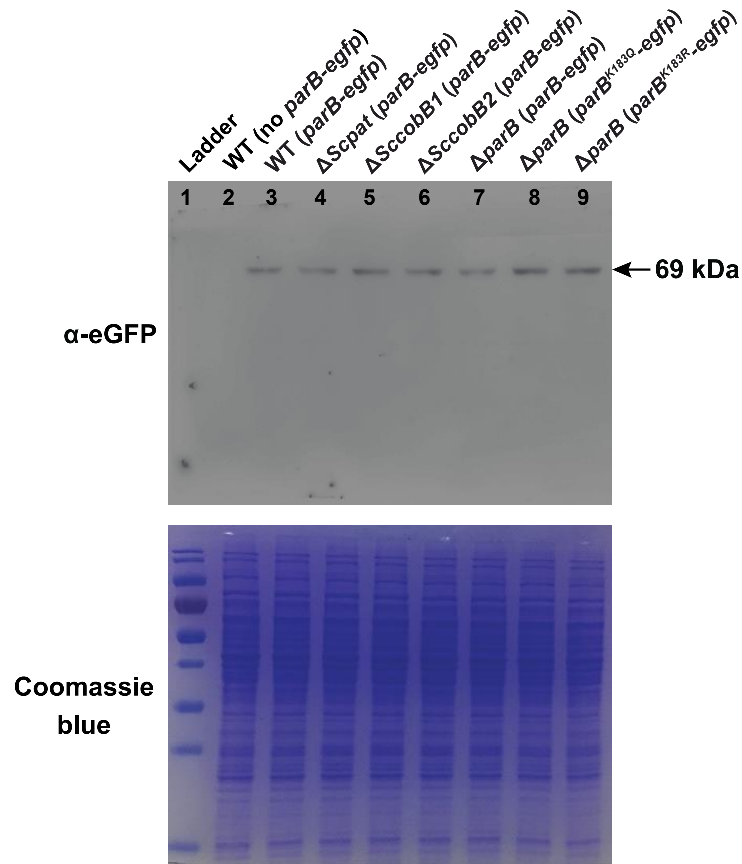
216 wall (red) and ParB segregation complex (green) were showed. All scale bars, 5 μm.

217

218

219 **Figure S10**

220



221

222 **Figure S10. Western blotting analysis indicates the expression levels of ParB and ParB**

223 **variants are similar among different *S. coelicolor* cells.** The expression levels of ParB

224 proteins were determined by detecting the abundance of ParB-eGFP with an anti-eGFP

225 antibody in *S. coelicolor* strains after a 48-hour cultivation. The expression levels of ParB

226 variants, *i.e.*, $ParB^{K183Q}$-eGFP and $ParB^{K183R}$-eGFP, were also determined. Their coomassie

227 blue staining of the whole protein extracts were set as the loading control.

228

229

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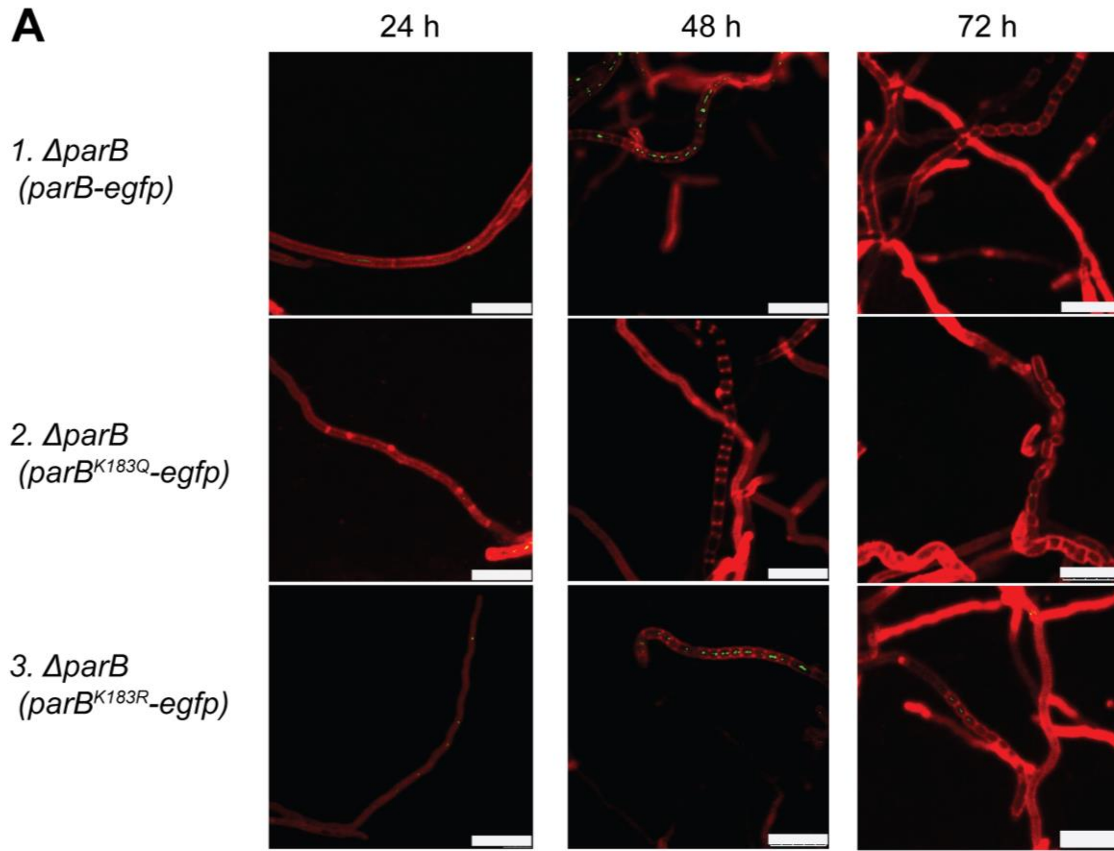
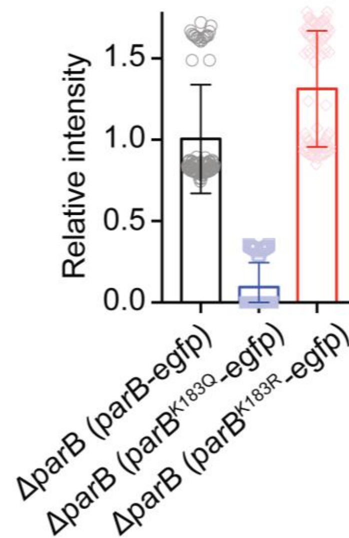
231

232 **Figure S11**





233

234 **Figure S11. ParB^{K183R}-eGFP forms ParB segregation complex while ParB^{K183Q}-eGFP**

235 **fails to form bright segregation foci.** Fluorescent microscopy was performed for ParB-

236 eGFP, ParB^{K183Q}-eGFP and ParB^{K183R}-eGFP in *S. coelicolor* Δ*parB* strains. (A) Images of the

237 cells cultured on MS solid medium at indicated hours (24, 48, and 72 h). Cell wall (red) and

238 ParB segregation complex (green) were showed. All scale bars, 5 μm. (B) The focus
239 intensities were quantified by measurement of the average fluorescence signal for the 48-h
240 cultivated aerial hyphae. The value of background-subtracted signal intensity was calculated
241 by ImageJ and normalized against the cell number (150 cells from each sample). The value of
242 the Δ*parB* (*parB-egfp*) sample at 48 h growth stage was set as 100%.
243
244
245
246

Figure S12

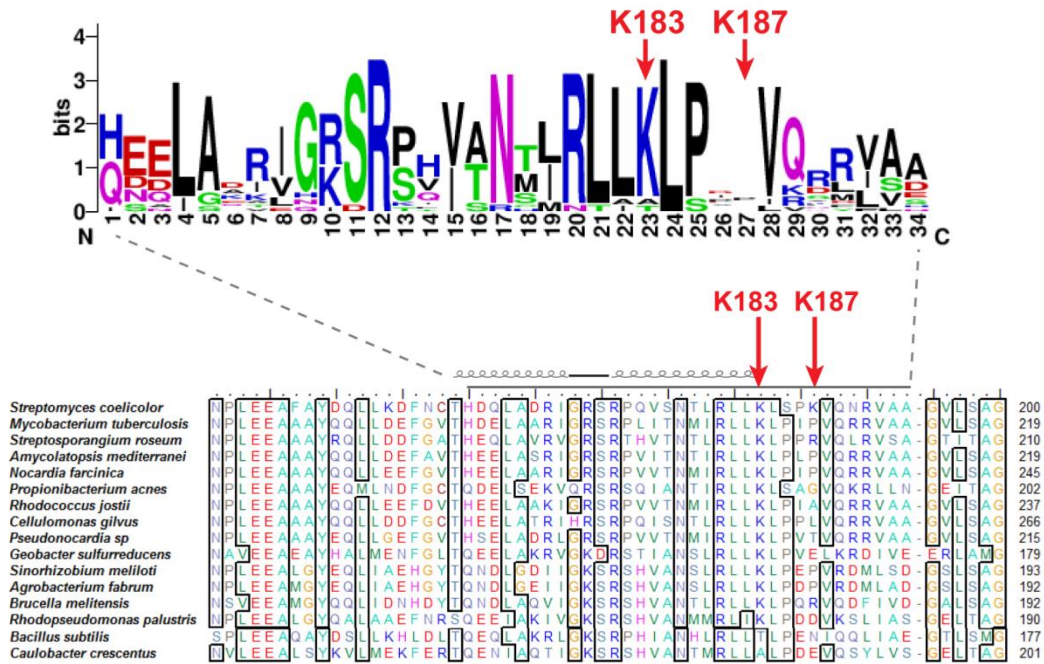


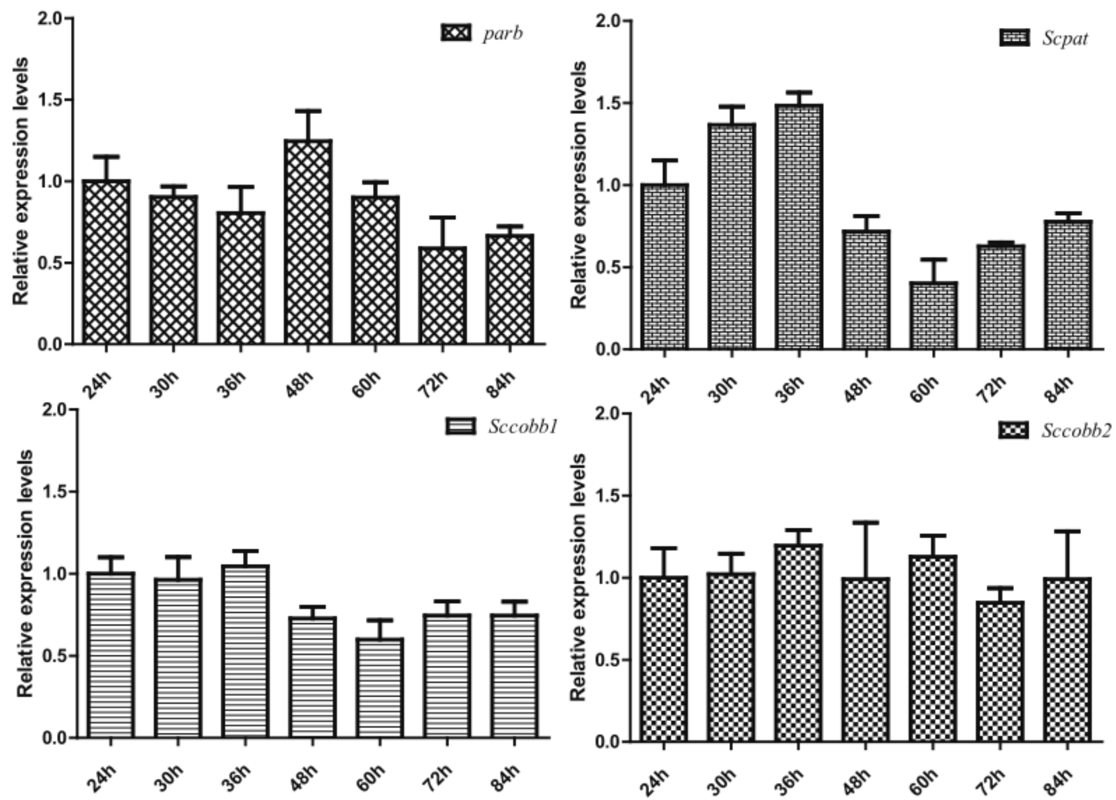
Figure S12. The ParB Lys-183 is conserved among most actinobacteria and some representative proteobacteria. Sequences including the HTH DNA-binding motifs (amino acids 161–194 in *S. coelicolor* ParB) were compared using WebLogo (http://weblogo.berkeley.edu/logo.cgi) and are graphically represented in the top panel. Sequence alignments of the ParB HTH DNA-binding motif from different bacterial species are provided in the lower panel. The numbers to the right of the panel represent the corresponding amino acid positions in the ParB proteins from different organisms. Red arrows indicate K183 and K187 of *S. coelicolor* ParB. The following sequences were acquired from the KEGG database: *Streptomyces coelicolor* SCO3887; *Mycobacterium tuberculosis* HKBT2_4143; *Streptosporangium roseum* Sros_9383; *Amycolatopsis mediterranei* AMED_9359; *Nocardia farcinica* nfa56660; *Propionibacterium acnes* TIA1EST1_11540; *Rhodococcus jostii* RHA1_ro03654; *Cellulomonas gilvus* Celgi_3200; *Pseudonocardia* sp. AD017_13410; *Geobacter sulfurreducens* GSU0107; *Sinorhizobium meliloti* SMc02801; *Agrobacterium fabrum* Atu2828; *Brucella melitensis* BMEI0010;

263 *Rhodopseudomonas palustris* RPA0291; *Bacillus subtilis* BSU40960 (Spo0J); and

264 *Caulobacter crescentus* CCNA_03868.

265

266 **Figure S13**

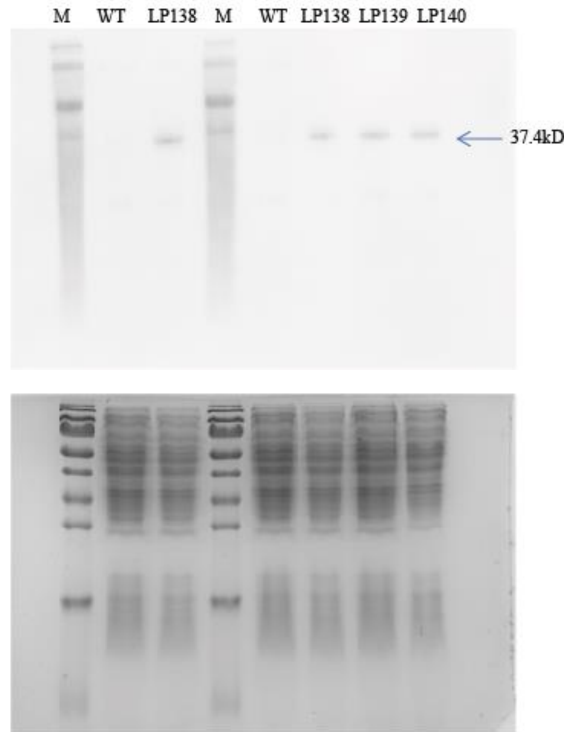


267

268 **Figure S13. Transcriptional profiles of selected genes throughout *S. coelicolor* life cycle.**
269 qPCR analysis of the transcriptional expression profiles of *parb*, *Scpat*, *SccobB1* and *SccobB2*
270 genes in wild type *S. coelicolor* on MS solid medium. The relative transcript levels of these
271 genes were obtained individually after normalization to the internal reference *hrdB* at the
272 indicated time points. The data show that the transcriptional levels of these genes were barely
273 changed (≤ 3 folds) during the cell cycle. The relative value for the expression of each gene at
274 24 h was arbitrarily assigned as value 100%. Error bars indicate the standard deviation from
275 three independent biological replicates.
276

277 **Figure S14**

278



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280

281 **Figure S14. Western blotting analysis indicates ParB-His is specifically detected by the**
282 **anti-His antibody in cells (corresponding to Figure 1 and Figure 5).** The expression of ParB-
283 His fusion proteins in LP138, LP139 and LP140, and their cell extracts were compared with
284 that of wild-type cells (no tagged ParB). Their cell extracts were isolated and detected with
285 anti-His antibody by western blotting (upper panel). The coomassie blue staining of the cell
286 extracts were set as the loading control (lower panel).

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289

290 **Table S1. Bacterial strains used in this study.**

Strains	Relevant Genotype	Reference
E. coli DH5α	FØ80d *lacZ* Δ*M*(*lacZYA-argF*) U169 *deoR recA1 endA1 hsdR17*(r^-km^-k) *supE44* λ^- *thi*$^-$ *gyr96 relA1*	GIBCO-BRL
E. coli BL21(DE3)	F'*ompT* rB^-mB^- (λDE3)	Novagen
E. coli BW25113/*pIJ790*	Δ(*araD-araB*)567, Δ*lacZ4787*(::*rrnB-4*), *lacIp-4000* (*lacIQ*), λ^-, *rpoS369*(Am), *rph-1*, Δ(*rhaD-rhaB*)*568*, *hsdR514*; Cml^R	Datsenko and Wanner, 2000 (2)
E. coli ET12567/*pUZ8002*	Non-methylating, Cml^R, Kan^R	Life&Technologies Inc
S. enterica G2466	Recombinant *galE* mutant from the wild-type LT2	Hone, D. *et al.*, 1987 (3)
S. enterica G2466 Δ*pat*	G2466 *pat::cat*	our lab
S. enterica G2466 Δ*cobB*	G2466 *cobB::cat*	our lab
S. coelicolor M145	Wild type, $SCP1^-$, $SCP2^-$	Bentley, 2002 (4)
S. coelicolor M145 Δ*Scpat*	*SCO5842::neo*	This study
S. coelicolor M145 Δ*SccobB1*	*SCO0452:: aac(3)IV*	This study
S. coelicolor M145 Δ*SccobB2*	*SCO6464:: aac(3)IV*	This study
S. coelicolor M145 Δ*parB*	*SCO3887::neo*	This study
S. coelicolor M145 Δ*parB* (*parB-egfp*)	*SCO3887::neo*; *attB::parB-egfp-aac(3)IV*	This study
S. coelicolor M145 Δ*parB* (*parB*^{K183Q}*-egfp*)	*SCO3887::neo*; *attB::parB*^{K183Q}*-egfp-aac(3)IV*	This study
S. coelicolor M145 Δ*parB* (*parB*^{K183R}*-egfp*)	*SCO3887::neo*; *attB::parB*^{K183R}*-egfp-aac(3)IV*	This study
S. coelicolor M145 (*parB-egfp*)	*attB::parB-egfp-aac(3)IV*	This study
S. coelicolor M145 Δ*Spat* (*parB-egfp*)	*SCO5842::neo*; *attB::parB-egfp-aac(3)IV*	This study
S. coelicolor M145 Δ*ScobB1* (*parB-egfp*)	*SCO0452:: aac(3)IV*; *attB::parB-egfp-aac(3)IV*	This study
S. coelicolor M145 Δ*ScobB2* (*parB-egfp*)	*SCO6464:: aac(3)IV*; *attB::parB-egfp-aac(3)IV*	This study
S. coelicolor M145 Δ*ScobB1* (*parB*^{K183R}*-egfp*)	*SCO0452:: aac(3)IV*; *attB::parB*^{K183R}*-egfp-aac(3)IV*	This study
S. coelicolor M145 Δ*Spat* (*parB*^{K183Q}*-egfp*)	*SCO5842::neo*; *attB::parB*^{K183Q}*-egfp-aac(3)IV*	This study
PL138	*SCO3887::parb-his-aac(3)IV*	This study
PL139	*SCO5842::neo*; *SCO3887::parb-his-aac(3)IV*	This study

PL140	*SCO0452:: ::neo*; *SCO3887::parb-his-aac(3)IV*	This study

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293 **Table S2. Plasmids and Cosmids used in this study.**

Plasmids	Description	Reference
pBluescriptII KS+	Cloning vector for *E. coli,* Amp^R	Merck
pSET152a	Chromosome integration vector for *S. coelicolor, aac(3)IV, tsr*	Kuhstoss, 1991 (5)
pET-28b (+)	Expression vector for *E. coli,* Kan^R	Novagen
pIJ8660	Chromosome integration vector for *S. coelicolor*, aac*(3)IV*	Sun, 1999 (6)
pHY773	Template plasmid for *aac(3)IV*	Our lab
pHY779	Template plasmid for *neo*	Our lab
Cosmid-*ParAB*	cosmid containing *parA parB*	Qin lab
Cosmid-*Pat*	cosmid containing *Scpat*	Qin lab
Cosmid-*CobB1*	cosmid containing *Sccobb1*	Qin lab
Cosmid-*CobB2*	cosmid containing *Sccobb2*	Qin lab
Cosmid-*ParB-His*	cosmid containing *parB-his-aac(3)IV*	This study
Cosmid-Δ*Pat1*	cosmid containing *Scpat::neo*	This study
Cosmid-Δ*CobB1*	cosmid containing *Sccobb1::aac(3)IV*	This study
Cosmid-Δ*CobB2*	cosmid containing *Sccobb2::aac(3)IV*	This study
Cosmid-Δ*ParB*	cosmid containing *parB::neo*	This study
pSET152a-*parB-eGFP*	Chromosome integration plasmid for *parB-egfp*	This study
pSET152a-$parB^{K183Q}$-*eGFP*	Chromosome integration plasmid for $parB^{K183Q}$-*egfp*	This study
pSET152a-$parB^{K183R}$-*eGFP*	Chromosome integration plasmid for $parB^{K183R}$-*egfp*	This study
pET-28b-*Scpat*	*Sc*Pat expression clone	This work
pET-28b-*SccobB1*	*Sc*CobB1 expression clone	This work
pET-28b-*SccobB2*	*Sc*CobB2 expression clone	This work
pET-28b-*parB*	ParB expression clone	This work
pET-28b-$parB^{K183Q}$	$ParB^{K183Q}$ expression clone	This study
pET-28b-$parB^{K183R}$	$ParB^{K183R}$ expression clone	This study
pET-28b-$parB^{K187Q}$	$ParB^{K187Q}$ expression clone	This study
pET-28b-$parB^{K187R}$	$ParB^{K187R}$ expression clone	This study
pET-28b-$parB^{K183A}$	$ParB^{K183A}$ expression clone	This study
pET-28b-$parB^{K187A}$	$ParB^{K187A}$ expression clone	This study

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298 **Table S3. Primers used in this study.**

Primers name	Sequences（5'-3'）
parB-3887-M-F	TTGCCTCGGCGGCCTTGGGGAGCGCGGCTTCTGCGGCCCC TGGGGCGATG ATTCCGGGGATCCGTCGACC
parB-3887-M-R	CGCTCCTACGGCAGTCGGAGCGCCCCTGGACGCTCCGCCT ACCGGAGTCA TGTAGGCTGGAGCTGCTTC
parB-3887-C-F	AGGGGACCCAGTGAGTGAG
parB-3887-C-R	GGTATGCACCGGACACAGTC
pat-5842-M-F:	gcgccgttgtcgtacgcgagccgtactctggggcccatgATTCCGGGGATCCGT CGACC
pat-5842-M-R:	ggcacgggcaccgggccgggtacggggcgtggcgggtcaTGTAGGCTGGAGC TGCTTC
pat-5842-C-F:	tcagaggcgttgaagttgtc
pat-5842-C-R:	gttctggtcgaacgtggtct
cobB-0452-M-F:	cgccggggaccgcggcgcgttccgtacccttgccgcatgATTCCGGGGATCCGT CGACC
cobB-0452-M-R:	agatttccggtcccgtgcgcgacgcgggctgcctggtcaTGTAGGCTGGAGCTG CTTC
cobB-0452-C-F:	tggacagtgcaagtctgtca
cobB-0452-C-R:	ccgttgacgaggttcttgat
cobB-6464-M-F:	cgataaccgttcgccggccgcaccccggcgcccctaccctcgaccgcatgATTCCGGG GATCCGTCGACC
cobB-6464-M-R:	cgacgccgtgttctgacgggcctgcggctttcggttccagccgcggctcaTGTAGGCTG GAGCTGCTTC
cobB-6464-C-F:	tgactacgccacctacatcg
cobB-6464-C-R:	gcgactactacgccttcacc
ParB-his-M-F	TGCAGAAGGGCCTTCTGGAGGGCGAGGACGAGGACGGGG ACGCCGAGTCCCACCACCACCACCACCACCA
ParB-his-M-R	CGTTCGCATGGCCCCTGCCTTCCTCCCAGAGGTACCGCCA GGTGACAACGATTCCGGGGATCCGTCGACC
ParB-his-C-F	gtccacagctccttccgtag
ParB-his-C-R	accaacttgccatcctgaag
His-apr-F	CACCACCACCACCACCACCACCACCACCACCACCACTGAT GTAGGCTGGAGCTGCTTC
His-apr-R	ATTCCGGGGATCCGTCGACC
parB-E-F	GCTCTAGACATCATCATCATCATCACAGCAGCGGCatgccgct gctaccgaacga
parB-E-R	CGGAATTCtcaggactcggcgtccccgtcct
cobB-6464-E-F:	cccAAGCTTatgaccggcaagcctctcg
cobB-6464-E-R:	gcTCTAGAtcagcccagcccgcgcagca
cobB-0452-E-F:	cccAAGCTTatgcgcatgcgccccactc
cobB-0452-E-R:	gcTCTAGAtcaggccgtcgccgcgtccC
pat-5842-E-F:	ggGATATCatgcagacctcgtcggaccg
pat-5842-E-R:	gcTCTAGAtcagtaggccggcagggtcc

parS-H-F	AGGCATACAAGCACCTGCTGGAGCTTCGCC
parS-H-R	FAM-CGAGACGGAGTGCCGGAAGGTCGACGGAGC
$ParB^{K187Q}$-F	CAAGTGCAGAACCGGGTGGC
$ParB^{K187R}$-F	CGTGTGCAGAACCGGGTGGC
$ParB^{K183Q}$-F	CAACTCTCCCCGAAGGTGCAGAACC
$ParB^{K183R}$-F	CGTCTCTCCCCGAAGGTGCAGAACC
$ParB^{K187A}$-F	GCGGTGCAGAACCGGGTGGC
$ParB^{K187A}$-F	GCGCTCTCCCCGAAGGTGCA
$ParB^{K183-R}$	CAGCAGACGCAGGGTGTTGG
$ParB^{K187-R}$	CGGGGAGAGCTTCAGCAGAC
ParB-promotor-F	GAAGATCTTACTCCACACAAGCTGCCCT
ParB-promotor-R	TCGTTCGGTAGCAGCGGCATGACCCGGGTCTGCTCGGG
EGFP-ParB-F	ATGCCGCTGCTACCGAACGA
EGFP-ParB-R	gtggcgaccTCTAGActtgtacagctcgtccatgc
EGFP-F	TCTAGAggtcgccacCCGCTGCTACCGAACGAGCG
EGFP-R	cgGAATTCtcaGGACTCGGCGTCCCCGTCCT
ParB-C-F	GACTTCAACTGCACGCATGA
ParB-C-R	ttacttgtacagctcgtccatgc
ParB-tran-F	cttcttctggacgcgctc
ParB-tran-R	tcatgcgtgcagttgaagtc
Cobb1-tran-F	gtgatcacccagaacgtcga
Cobb1-tran-R	cgcaggacagacagacga
Pat-tran-F	gtgtccaccttcgtctcctc
Pat-tran-R	ggactccaggtacatcagcg
HrdB-tran-F	tggtcgaggtcatcaacaag
HrdB-tran-R	tggacctcgatgaccttctc

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302 ***parS* probe used in this study**
303 AGGCATACAAGCACCTGCTGGAGCTTCGCCTGGAGAACGGGCCGATGGAGCACG
304 ACGCGGCGGTGGCAGCACTGAAGGAATGGTGGGCTCAGCACCCGGACAACGCAT
305 GAGAACGGGCGGCACGTTTCACGTGAAACGTGCCGCCCGCGGGTCATGTTTCACG
306 TGAAACATGACCGTGAAGCAGAACACGGAGAGTGGTCTGTGCCTCAGGCCTCGC
307 CGAGGCAGAGCAGGAACTCTTCACCGTCGCCGGTTTCGGCGTAGAAGCCGGTGG
308 CTCCGTCGACCTTCCGGCACTCCGTCTCG
309
310 About 200k Dalton. The two copies of reverse complemental sequences in probe are
311 highlighted in green.
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317 **Supplemental References**

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