

1 **Increased ParB level affects expression of stress response, adaptation and virulence**
2 **operons and potentiates repression of promoters adjacent to the high affinity binding**
3 **sites *parS3* and *parS4* in *Pseudomonas aeruginosa***

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12

13 **Abstract**

14 Similarly to its homologs in other bacteria, *Pseudomonas aeruginosa* partitioning
15 protein ParB facilitates segregation of newly replicated chromosomes. Lack of ParB is not
16 lethal but results in increased frequency of anucleate cells production, longer division time,
17 cell elongation, altered colony morphology and defective swarming and swimming motility.
18 Unlike in other bacteria, inactivation of *parB* leads to major changes of the transcriptome,
19 suggesting that, directly or indirectly, ParB plays a role in regulation of gene expression in
20 this organism.

21 ParB overproduction affects growth rate, cell division and motility in a similar way as
22 ParB deficiency. To identify primary ParB targets, here we analysed the impact of a slight
23 increase in ParB level on *P. aeruginosa* transcriptome. ParB excess, which does not cause
24 changes in growth rate and chromosome segregation, significantly alters the expression of 176
25 loci. Most notably, the mRNA level of genes adjacent to high affinity ParB binding sites
26 *parS1-4* close to *oriC* is reduced. Conversely, in cells lacking either *parB* or functional *parS*
27 sequences the orfs adjacent to *parS3* and *parS4* are upregulated, indicating that direct ParB-
28 *parS3/parS4* interactions repress the transcription in this region. In addition, increased ParB
29 level brings about repression or activation of numerous genes including several transcriptional
30 regulators involved in SOS response, virulence and adaptation. Overall, our data support the
31 role of partitioning protein ParB as a transcriptional regulator in *Pseudomonas aeruginosa*.

32

33 **Introduction**

34 Accurate copying and segregation of genetic material to progeny cells is crucial for
35 survival and maintenance of species identity. The process of bacterial DNA segregation was
36 first deciphered for low-copy-number plasmids [1–4]. Active partitioning of such plasmids
37 during cell division requires three specific plasmid-encoded factors: an NTPase (A-
38 component), a DNA-binding protein (DBP, B-component) and a special site in DNA,
39 designated centromere-like sequence (*parS/parC*). Interactions of DBP with *parS* sequence(s)
40 lead to formation of segrosomes which are then separated by a dynamic NTPase machinery to
41 polar positions assuring their proper segregation during the subsequent cell division [2,3].
42 Plasmidic active partition systems have been classified into three groups based on the type of
43 NTPase and structure of DBP [2,4]. Homologs of plasmidic Type IA partition proteins,
44 Walker-type ATPases (ParAs) and large DBPs with helix-turn-helix motifs (ParBs), which
45 after binding to *parS* spread on DNA and form large nucleoprotein complexes [5], are also
46 encoded on the majority of bacterial chromosomes [3,4,6]. Multiple copies of highly
47 conserved *parS* sequences are mainly clustered in the so-called *ori* domain comprising ca.
48 20% of the chromosome [7].

49 The role of the ParABS systems in accurate bacterial chromosome segregation is
50 widely acknowledged but varies from essential, as exemplified by *Caulobacter crescentus* [8]
51 or *Myxococcus xanthus* [9], to accessory, as in *Bacillus subtilis* [10–13], *Streptomyces*
52 *coelicolor* [14], *Vibrio cholerae* [15,16] or *Pseudomonas aeruginosa* [17–19]. Apart from
53 their well-established role in the segregation of newly replicated *ori* domains through DNA
54 compaction [20–22], proper positioning of *ori* domains in the cell [19,23], Par proteins have
55 also been shown to play a role in the control of DnaA activity and replication initiation
56 [24,25] as well as in coordination of cell cycle and differentiation [26–28]. Chromosomal
57 ParB homologs bind to *parS in vitro*, polymerize on DNA and bridge distant sequences

58 [13,29–32]. Whole-genome analyses using chromatin immunoprecipitation (ChIP) have
59 demonstrated spreading of ParB homologs around *parS* sites for up to 20 kb [5,26–29].
60 Despite the ParB spreading, a transcriptomic analysis in *B. subtilis* did not identify any
61 significant changes in gene expression in a *spo0J* null mutant (Spo0J is a ParB homolog)
62 relative to a WT strain [35]. Limited ParB-dependent transcriptional silencing in the
63 proximity of *parS* sequences has been observed only for several genes in *V. cholerae* [34] and
64 *S. pneumoniae* [36].

65 In *P. aeruginosa* a lack of ParA and/or ParB is not lethal but results in up to 1000-fold
66 increased frequency of production of anucleate cells even during growth in optimal conditions
67 [17,18,37]. Various *par* mutants exhibit longer division time, increase in cell size, altered
68 colony morphology and are impaired in swarming and swimming motility [17,18]. Ten *parS*
69 sites scattered in the chromosome of *P. aeruginosa* have been identified, but only four of
70 them closest to *oriC* seem to be involved in chromosome segregation [33,38]. A
71 transcriptomic analysis of *P. aeruginosa parA* and *parB* mutants has demonstrated changes in
72 expression of hundreds of loci [39], including genes related to stress response but also many
73 known and putative transcriptional regulators, suggesting a direct and/or indirect role of Par
74 proteins in the regulation of gene expression. In test plasmids, ParB of *P. aeruginosa* was
75 found to spread around *parS* and silence nearby promoters [37], but a comparison of the *parB*
76 mutant and WT transcriptomes did not reveal any obvious changes in the expression of genes
77 adjacent to chromosomal *parS* sequences [39]. However, a recent ChIP-seq analysis of ParB
78 distribution on the *P. aeruginosa* chromosome has revealed, in addition to the region around
79 the high affinity *parS1-4* sequences, also secondary ParB binding sites, apparently not related
80 to known *parS* sites [33].

81 Similarly to the lack of ParB, also its excess in *P. aeruginosa* affects the cell cycle, as
82 highlighted by slower growth rate, and causes cell elongation and defects in swarming and

83 swimming. ParB excess manifests in nucleoid condensation and increased frequency of
84 anucleate cells formation [17,37,40,41]. The toxicity of ParB overproduction is significantly
85 diminished when ParB is impaired in its polymerization domain [40] whereas ParB variants
86 defective in interactions with DNA, either due to the inability to form dimers or mutations in
87 the DNA binding domain, demonstrate no toxicity [40,41]. This suggests that DNA binding
88 and spreading of ParB protein is crucial for its biological function.

89 In this project we analysed changes in the transcriptome of *P. aeruginosa* cells with a
90 slightly increased ParB level, not affecting growth rate and chromosome segregation, to
91 define the primary targets of ParB.

92 **Materials and methods**

93 **Bacterial strains and growth conditions**

94 Bacterial strains used in this study are listed in Table 1. Cultures were grown in L
95 broth [42] or on L agar (L broth solidified with 1.5% agar) at 37°C. For selection of plasmids
96 *Escherichia coli* media were supplemented with 150 µg ml⁻¹ (liquid cultures) or 300 µg ml⁻¹
97 (solid media) penicillin (Pn), 10 µg ml⁻¹ chloramphenicol (Cm) or 50 µg ml⁻¹ kanamycin
98 (Km). Chloramphenicol at concentration 75 µg ml⁻¹ (liquid cultures) and 150 µg ml⁻¹ (solid
99 media) was used to maintain plasmids in *P. aeruginosa*. 300 µg ml⁻¹ carbenicillin (Cb) and
100 300 µg ml⁻¹ rifampicin (Rif) was added to the solid medium for the selection of *P. aeruginosa*
101 transconjugants in allele exchange procedure. To induce the expression from the *araBAD*
102 promoter arabinose (Ara) was added to 0.02% to cultures of PAO1161 with pKGB8
103 (*araBADp*) or pKGB9 (*araBADp-parB*) and to 0.1% in PAO1161::*araBADp* or
104 PAO1161::*araBADp-flag-parB* cultures. For MIC analysis of PAO1161 (pKGB8) and
105 PAO1161 (pKGB9) and PAO1161 as a control, strains were grown in Mueller Hinton cation

106 adjusted medium in the range of Ara concentrations (0, 0.02 and 0.2%) and in the presence of
 107 a gradient of piperacillin, ticarcillin, ciprofloxacin, gentamicin, tobramycin or imipenem.

108 **Table 1 Bacterial strains used in this study.**

| Strain | Description | Reference |
|-------------------------------------|---|------------|
| <i>Escherichia coli</i> | | |
| DH5 α | F ⁻ Φ 80lacZ Δ M15 Δ (lacZYA-argF) U169 recA1 endA1 hsdR17 (r _K ⁻ , m _K ⁺) phoA supE44 λ thi-1 gyrA96 relA1 | [43] |
| S17-1 | pro Δ hsdR hsdM ⁺ recA T _p ^R Sm ^R Ω RP4-Tc::Mu Kn::Tn7 | [44] |
| <i>Pseudomonas aeruginosa</i> | | |
| PAO1161 | PAO1161 Rif ^R leu | [17] |
| PAO1161 <i>parB</i> _{null} | PAO1161 Rif ^R leu <i>parB</i> 1-18::tetM, Tc ^R | [17] |
| PAO1161 <i>parS</i> _{null} | PAO1161 Rif ^R with ten <i>parS</i> sequences mutated or deleted to impair ParB binding to these sequences | [38] |
| PAO1161:: <i>araBADp</i> | PAO1161 Rif ^R with <i>araC araBADp</i> T _{rrnB} ¹ cassette integrated in intergenic region between PA5412 and PA5413 (at position 6091513 nt) | this study |
| PAO1161:: <i>araBADp-flag-parB</i> | PAO1161 Rif ^R with <i>araC araBADp-flag-parB</i> T _{rrnB} cassette integrated in intergenic region between PA5412 and PA5413 (at position 6091513 nt) | this study |
| PAO1161:: <i>bexRp-lacZ</i> | PAO1161 Rif ^R Cb ^R with <i>bexRp-lacZ</i> cassette integrated in intergenic region between PA5412 and PA5413 (at position 6091513 nt) | this study |

109 ¹T_{rrnB} transcriptional terminator of *E. coli* *rrnB* gene

110 **Transformation and conjugation procedures**

111 Transformation of *E. coli* and *P. aeruginosa* was performed by standard procedures
 112 [43,45]. Conjugation between transformants of *E. coli* S17-1 and *P. aeruginosa* PAO1161
 113 Rif^R was performed on solid media as described previously [37].

114 Plasmids and DNA manipulations

115 Plasmids used in this study are listed in Table 2. Plasmid manipulations were carried
 116 out by standard procedures [46]. Oligonucleotides used in this work are listed in Table S1. All
 117 new plasmid constructs were verified by sequencing at the Laboratory of DNA Sequencing
 118 and Oligonucleotide Synthesis, IBB PAS.

119 Table 2 Plasmids used in this study.

| Plasmid | Description | Reference |
|----------|---|---------------|
| pABB1.0 | pBBRMCS1 derivative, BHR ¹ , <i>ori</i> _{IncA/C} , Cm ^R | [47] |
| pABB28.2 | pET28a with <i>his</i> -tag replaced by <i>flag</i> -tag, Km ^R | this study |
| pAKE600 | suicide vector, <i>ori</i> V _{MB1} , <i>sacB</i> gene, Pn ^R | [48] |
| pBAD24 | expression vector, <i>ori</i> V _{MB1} , <i>araC</i> <i>araBAD</i> p T _{rmB} cassette, Pn ^R | [49] |
| pCM132 | BHR ¹ , dual replicon promoter-probe vector <i>ori</i> V _{ColE1} , <i>ori</i> V _{RK2} , <i>trfA</i> _{RK2} , <i>oriT</i> _{RK2} , promoter-less <i>lacZ</i> , Km ^R | [50] |
| pET28a | expression vector, <i>ori</i> V _{MB1} , T7p, 6xHis-tag, Km ^R | Novagen, Inc. |
| pKAB132 | pPJB132 with deletion of EcoRI-BglII fragment, control vector, Cm ^R , Km ^R | this study |
| pKAB133 | pPJB132 with PA0011p- <i>lacZ</i> | this study |
| pKAB134 | pPJB132 with PA0011p _{parS3mut} - <i>lacZ</i> | this study |
| pKAB135 | pPJB132 with PA0013p- <i>lacZ</i> | this study |
| pKAB136 | pPJB132 with PA0013p _{parS4mut} - <i>lacZ</i> | this study |
| pKAB240 | pBAD24 with NsiI restriction site between T _{rmB} and <i>blap</i> | this study |
| pKAB241 | pKAB240 with <i>flag</i> tagged <i>P. aeruginosa</i> <i>parB</i> (<i>araBAD</i> p- <i>flag</i> - <i>parB</i>) | this study |
| pKAB242 | pKAB240 with T _{rmB} - <i>mcs</i> - <i>lacZ</i> (partial)-T _{T7} ² cassette inserted as | this study |

| | | |
|---------|--|------------|
| | NsiI fragment | |
| pKAB243 | pKAB242 with insertion of full-length <i>lacZ</i> and <i>mcs</i> , contains complete T_{rmB} - <i>mcs-lacZ</i> - T_{T7} cassette (NsiI fragment) | this study |
| pKAB600 | pAKE600 with <i>P. aeruginosa</i> genomic fragment (coordinates 6090756 - 6092192) with PstI at position 6091513 nt | this study |
| pKAB601 | pKAB600 with <i>araC araBADp T_{rmB}</i> cassette | this study |
| pKAB602 | pKAB600 with <i>araC araBADp-flag-parB T_{rmB}</i> cassette | this study |
| pKAB603 | pKAB600 with deletion of EcoRI restriction site | this study |
| pKAB604 | pKAB603 with T_{rmB} - <i>mcs-lacZ</i> - T_{T7} cassette | this study |
| pKAB605 | pKAB604 with <i>BexRp</i> cloned in the <i>mcs</i> , T_{rmB} - <i>BexRp-lacZ</i> - T_{T7} cassette | this study |
| pKGB8 | pABB1.0 with <i>araC araBADp</i> , expression vector | this study |
| pKGB9 | pKGB8 with <i>araBADp-parB_{P.a.}</i> | this study |
| pKRP10 | <i>ori_{MB1}</i> , contains Cm ^R cassette, Pn ^R | [51] |
| pMKB5.2 | <i>ori_{MB1}</i> , <i>cyaAT18-parB_{P.a.}</i> translational fusion, Pn ^R | [48] |
| pPJB132 | pCM132 with Cm ^R cassette, Cm ^R , Km ^R | this study |

120 ¹ BHR, broad-host-range plasmid

121 ² T_{T7} , T7 phage transcriptional terminator

122

123 Plasmid pKGB8 was constructed by ligation of Eco47III-PstI fragment from pBAD24
 124 containing *araC araBADp* with HincII and PstI digested pABB1.0. Subsequently, EcoRI-SacI
 125 fragment from pMKB5.2 containing *parB* ORF was cloned into pKGB8 to yield plasmid
 126 pKGB9.

127 pET28a (Novagen, Inc.) was modified to add *flag*-tag instead of *his*-tag to the coding
 128 sequences. Oligonucleotides #1 and #2 were combined, heated to 100°C for 5 minutes and left
 129 for annealing at room temperature. pET28a vector was digested using NcoI and NdeI and
 130 ligated with obtained synthetic DNA fragment to yield pABB28.2.

131 To facilitate transfer of the *araC-araBADp-T_{rmB}* cassette, an additional NsiI restriction
 132 site was introduced into pBAD24 downstream of T_{rmB} . Two fragments amplified by PCR

133 using pBAD24 as a template and primer pairs #3/#4 and #5/#6, respectively, were used as a
134 template in the second round of PCR with primers #3 and #6 to obtain a 1160 bp product that
135 replaced the HindIII-ScaI fragment in pBAD24 to yield pKAB240.

136 The *flag-parB* fusion was constructed using similar strategy. The *flag* sequence
137 amplified from pABB28.2 using primers #7/#8 and *P. aeruginosa parB* gene amplified from
138 PAO1161 genomic DNA using primers #9/#10 were combined and used as a template in
139 overlap PCR with primers #7/#10. The obtained PCR fragment with *flag-parB* fusion was
140 digested with EcoRI and Sall and ligated into pKAB240 to yield pKAB241.

141 To construct PAO1161::*araBADp* and PAO1161::*araBADp-flag-parB* strains, the
142 suicide plasmids pKAB601 and pKAB602 were constructed. First, the intergenic region
143 between *PA5412* and *PA5413* from PAO1161 chromosome was amplified in two parts with
144 pairs of primers #11/#12 and #13/#14. Primers #12 and #13 introduced a PstI site. The
145 obtained PCR fragments were digested either with MfeI and PstI or PstI and BamHI,
146 respectively, and the mixture was ligated with EcoRI and BamHI digested pAKE600 [48] to
147 yield pKAB600. The *araC-araBADp-T_{rrmB}* cassette from pKAB240 and *araC-araBADp-flag-*
148 *parB-T_{rrmB}* cassette from pKAB241 were excised as NsiI fragments and ligated into PstI site
149 of pKAB600 to yield pKAB601 and pKAB602, respectively. *E. coli* S17-1 strain was
150 transformed with pKAB601 or pKAB602 and the transformants were used as donor strains in
151 conjugation with PAO1161 Rif^R [18]. The cassettes were integrated in the defined region of
152 PAO1161 using the allele exchange procedure [18] to obtain PAO1161::*araBADp* and
153 PAO1161::*araBADp-flag-parB*.

154 pCM132 was modified by insertion of Cm^R cassette (SphI fragment from pKRP10
155 [51]) into SphI site, downstream of *lacZ*, to yield pPJB132. Putative promoter sequences
156 preceding *PA0011* and *PA0013* were amplified on PAO1161 genomic DNA using primer
157 pairs #15/#16 and #17/#18, respectively. The *PA0011p_{parS3mut}* and *PA0013p_{parS4mut}* fragments

158 were amplified on PAO1161 *parS*_{null} genomic DNA [38] as a template using the same primer
159 pairs. PCR products were digested with EcoRI and BamHI and ligated between EcoRI and
160 BglIII sites into pPJB132 to yield plasmids pKAB133 (*PA0011p-lacZ*), pKAB134
161 (*PA0011p_{parS3mut}-lacZ*), pKAB135 (*PA0013p-lacZ*) and pKAB136 (*PA0013p_{parS4mut}-lacZ*).
162 The empty control vector for β -galactosidase measurements (pKAB132) was obtained by
163 digestion of pPJB132 with EcoRI and BglIII, blunting by fill-in with Klenow fragment of
164 DNA PolI and self-ligation.

165 To construct PAO1161 Rif^R strain with *bexRp-lacZ* cassette integrated in intergenic
166 region between *PA5412* and *PA5413*, the suicide plasmid pKAB605 was constructed. First,
167 the *T_{rrmB}* with *mcs*, part of the *lacZ* gene and *T_{T7}* was amplified using overlap PCR strategy.
168 To this end, 2 fragments were amplified on pCM132 plasmid as a template using primer pairs
169 #19/#20 and #21/#22, respectively, and the 3rd fragment was amplified on pET28a using
170 primer pair #23/#24. The three fragments were mixed and used as a template in an overlap
171 PCR with primers #19/#24. The obtained 787 bp product digested with NsiI replaced the
172 *araC-araBADp-T_{rrmB}* in pKAB240 yielding plasmid pKAB242. *mcs-lacZ* was subsequently
173 excised from pCM132 as EcoRI and BlnI fragment and ligated with EcoRI-BlnI digested
174 pKAB242 to yield pKAB243, containing complete *T_{rrmB}-mcs-lacZ-T_{T7}* cassette.

175 The single EcoRI restriction site in pKAB600 plasmid was removed by EcoRI
176 digestion, filling in by Klenow fragment of DNA PolI and plasmid self-ligation to yield
177 pKAB603. The *T_{rrmB}-mcs-lacZ-T_{T7}* cassette excised from pKAB243 by NsiI digestion was
178 ligated into PstI site of pKAB603 to yield pKAB604. The region preceding *bexR* (*PA2432*)
179 gene was amplified on PAO1161 genomic DNA using primers #25/#26, digested with EcoRI
180 and BamHI and ligated with EcoRI and BglIII digested pKAB604 to yield plasmid pKAB605
181 carrying *T_{rrmB}-bexRp-lacZ-T_{T7}* cassette flanked by sequences allowing integration in the
182 intergenic region between *PA5412* and *PA5413*. *E. coli* S17-1 strain transformed with

183 pKAB605 was used as donor in conjugation with PAO1161 Rif^R [18]. The suicide vector was
184 integrated in the defined region of PAO1161 (at position 6091513 nt) using homology
185 recombination [18] and the carbenicillin-resistant integrant (PAO1161::*bexRp-lacZ*) was used
186 as a recipient in a subsequent conjugation with S17-1 cells carrying pKGB8 or pKGB9
187 plasmids. Transconjugants were selected on L agar plates with carbenicillin, chloramphenicol
188 and 40 µg ml⁻¹ X-gal to allow visualization of *bexRp-lacZ* induction immediately after the
189 introduction of plasmids.

190 **RNA isolation**

191 *P. aeruginosa* strains taken from -80°C stocks were grown on L agar plates at 37°C
192 and single colonies were used to inoculate three independent cultures (biological replicates).
193 After overnight growth, the cultures were diluted 1:100 into fresh L broth and grown to the
194 optical density 0.4- 0.6 at 600 nm (OD₆₀₀). RNA was isolated with RNeasy mini kit (Qiagen)
195 according to the manufacturer's protocol for bacterial cells from 2 ml of cultures mixed with 4
196 ml of RNAProtect Bacteria Reagent (Qiagen). Total RNA was digested with DNase (TURBO
197 DNA-free Kit, Ambion) to remove genomic DNA.

198 **Microarray analysis**

199 Microarray analysis was performed essentially as described before [39]. The raw
200 microarray data have been deposited at the NCBI Gene Expression Omnibus database at
201 accession number GSE95647 (release after publication acceptance). Gene expression data
202 were analysed using Partek Genomic Suite v6.6 (Partek Inc., St. Louis, MO). Raw data were
203 processed using GeneChip Robust Multiarray Averaging (GC RMA): background correction,
204 quantile normalization, log₂ transformation and median polish summarization. Analysis of
205 variance (ANOVA) using REML (restricted maximum likelihood) was performed to identify
206 differentially expressed genes. Gene lists were created using a cut-off of $p\text{-value} \leq 0.05$, with a

207 fold change (FC) higher than 2 or lower than -2. Clustering of the genes according to
208 expression pattern changes was performed by K-means clustering [52] using MultiExperiment
209 Viewer v4.9 [53]. The gene expression data for individual replicates were averaged,
210 normalized to zero-mean and unit variance, and subjected to clustering into six clusters, using
211 Pearson correlation as the distance metric and 500 as the maximum number of iterations.

212 **RT-qPCR analysis**

213 cDNA was synthesized with the TranScriba cDNA synthesis kit (A&A Biotechnology,
214 Poland) using 1.5 µg of total RNA per reaction and random hexamer primers. RT-qPCR
215 reactions were performed in a Roche LightCycler 480 using Hot FIREPol EvaGreen qPCR
216 Mix Plus (Solis Biodyne). The reactions were performed with 0.15- 0.3 µl of cDNA in a total
217 volume of 20 µl. Three technical replicates were used for each gene/primer combination. The
218 primers used to amplify target and reference genes are listed in Table S1. Changes in the gene
219 expression were calculated using the Pfaffl method [54] with normalization to the reference
220 gene *nadB* (*PA0761*). RT-qPCR data represent mean for 3 biological replicates and 3
221 technical replicates. All RT-qPCR experiments were repeated twice and representative
222 experiments are shown.

223 **Western blotting**

224 Cultures were grown on L broth to OD₆₀₀ 0.4-0.6. Cells were harvested by
225 centrifugation and stored at -20°C. For each sample a small aliquot was used to estimate the
226 number of c.f.u. ml⁻¹ by plating appropriate dilution of the culture on L agar plates. Pellets
227 were resuspended in 10 mM Tris-HCl (pH 8.0), 1 M NaCl, 0.1 mM EDTA, 5% glycerol. For
228 each sample different volume of the buffer was used to compensate the initial differences in
229 c.f.u. ml⁻¹. Samples were sonicated and aliquots of cleared extracts corresponding to 10⁹ cells
230 were separated by SDS-PAGE on 12% polyacrylamide gels. Additionally, His₆-ParB, purified

231 as described [37], was loaded on each gel. Separated proteins were transferred onto
232 nitrocellulose membranes. The blots were subjected to a two-step immunoreaction including
233 application of rabbit polyclonal anti-ParB antibodies followed by incubation with goat anti-
234 rabbit antibodies conjugated with alkaline phosphatase and were developed by addition of
235 NBT/ BCIP mixture. Band intensity was estimated using ImageJ for at least 3 biological
236 replicates.

237 **Biofilm formation assay**

238 Biofilm formation in static cultures was assayed as described previously with minor
239 modifications [55]. Overnight cultures of *P. aeruginosa* strains in three biological replicates
240 were diluted 1:100 in L broth with or without 0.02% arabinose. 75 $\mu\text{g ml}^{-1}$ chloramphenicol
241 was added to maintain the plasmids. 100 μl aliquots of each diluted culture were transferred
242 into 9 wells of a 96-well flat-bottom polystyrene microplate (Greiner Bio-One). Sterile L
243 broth was used as a negative control. The plate was incubated without shaking at 37°C until
244 OD_{600} reached approximately 0.5. The optical density OD_{600} was measured using a plate
245 reader (BioTek Synergy HT) and bacterial cells, which did not adhere to the surface, were
246 gently removed by aspiration with a pipette. Wells were washed twice with 100 μl of PBS (15
247 mM KCl, 150 mM NaCl, 10 mM NaPi pH 7.4) and biofilm in each well was stained with 100
248 μl of 0.1% crystal violet solution. After 15 min incubation at room temperature, crystal violet
249 solution was aspirated and wells were rinsed three times with distilled water and twice with
250 100 μl of PBS. Subsequently, 100 μl of 96% ethanol was added to each well and left for 10
251 min to dissolve the crystal violet. Well content was mixed by pipetting and OD_{590} was
252 measured using a plate reader and 96% ethanol as a blank. Biofilm formation is presented as
253 proportion $\text{OD}_{590}/\text{OD}_{600}$.

254 **β -galactosidase activity assays**

255 β -galactosidase activity was measured as described before [42]. Extracts were
256 prepared from cells grown to OD₆₀₀ 0.4-0.6 in L broth with 75 $\mu\text{g ml}^{-1}$ chloramphenicol, with
257 or without 0.1% arabinose.

258 **Results**

259 **Impact of *parB* overexpression on gene expression in *P. aeruginosa***

260 Our previous microarray analysis revealed altered expression of 1166 genes in ParB-
261 deficient cells of *P. aeruginosa* relative to the wild type PAO1161 (WT) cells [39]. To
262 complement this study, here we analysed the transcriptome of ParB -overproducing cells. To
263 control the level of *parB* expression we constructed a pKGB8 expression vector based on the
264 broad-host-range pBBR1MCS-1 [56], which contains the arabinose (Ara) inducible promoter
265 *araBADp* [49]. Analysis of the growth of PAO1161 (pKGB9 *araBADp-parB*) and PAO1161
266 (pKGB8) cells in the presence of different concentrations of Ara revealed that induction by
267 Ara $\leq 0.02\%$ did not affect the growth (Fig 1A). A slight increase in the ParB level (≤ 2 -fold)
268 was observed even in non-induced cells of PAO1161 (pKGB9) relative to the cells containing
269 the empty vector according to Western blot analysis (Fig 1B). Addition of 0.02% Ara to the
270 medium resulted in a 5-fold increase in the ParB amount per cell in mid-log phase cultures of
271 PAO1161 (pKGB9) in comparison to PAO1161 (pKGB8) (Fig 1B). Such ParB excess did not
272 affect the frequency of anucleate cells formation, cell size, colony morphology or swimming
273 and swarming motility (data not shown) but led to altered biofilm formation under specific
274 conditions (Fig 1C, discussed below).

275 **Fig 1. Effects of ParB excess in *P. aeruginosa*.**

276 (A) Growth of *P. aeruginosa* PAO1161 (pKGB8 *araBADp*) and PAO1161 (pKGB9
277 *araBADp-parB*) strains in L broth with different arabinose concentrations. Data represent
278 mean OD₆₀₀. (B) Western blot analysis of ParB levels in the tested strains. Each lane contains

279 extract from 10^9 cells. Blots were subjected to immunodetection using primary anti-ParB
280 antibodies. Representative blot is shown. Signals on the blots were quantified. Data represent
281 mean ParB level \pm SD relatively to the control strain PAO1161 (pKGB8). Purified His₆-ParB
282 was used to generate standard curves. M – molecular weight marker. (C) Biofilm formation in
283 the static cultures of PAO1161, PAO1161 (pKGB8) and PAO1161 (pKGB9). Strains were
284 grown without or with 0.02% arabinose until OD₆₀₀ 0.5. Biofilm was stained with crystal
285 violet and assessed by measurement of OD₅₉₀. Data represent mean OD₅₉₀/OD₆₀₀ ratio \pm SD
286 from 3 biological replicates. * - *p*-value < 0.05 in two-sided Student's *t*-test assuming equal
287 variance.

288 To determine the impact of an increased ParB level on the transcriptome a microarray
289 analysis was performed on RNA isolated from PAO1161 (pKGB9 *araBADp-parB*) cultures
290 grown under selection in L broth with 0.02% Ara (higher ParB overproduction, hereafter
291 referred to as ParB⁺⁺⁺) or without Ara (mild ParB overproduction, hereafter called ParB⁺)
292 as well as from PAO1161 (pKGB8) cells grown under selection in L broth with 0.02% Ara
293 (empty vector control, hereafter EV) and PAO1161 cells grown in L broth without Ara (wild-
294 type control, hereafter WT). A principal component analysis of the microarray data revealed
295 that the biological replicates for WT and EV form 2 separate groups whereas the biological
296 replicates of ParB⁺ and ParB⁺⁺⁺ form together a separate group, distinct from WT and EV
297 samples (data not shown).

298 Comparative transcriptome analysis of EV vs WT revealed 70 genes, four tRNA
299 genes, and four intergenic regions with an altered expression in response to the presence of
300 the vector [fold change (FC) <-2 or >2, *p*-value <0.05] (Fig 2A, Table S2). 175 genes and 1
301 intergenic region displayed statistically significant difference in expression between
302 ParB⁺⁺⁺ and EV cells using the same criteria. 155 out of 175 genes displayed an altered
303 expression only in the response to the ParB abundance since they were not identified in the

304 EV *vs* WT analysis. The expression of the remaining 20 genes and one intergenic region was
305 affected by the presence of vector as well as ParB overproduction in ParB+++ cells (Fig 2B,
306 Table S2). The comparative analysis of ParB+ and EV transcriptomes revealed altered
307 expression of 157 genes. There was a major overlap between the transcriptomic changes in
308 ParB+ and ParB+++ cells as 122 genes showed similar responses in the ParB+ and ParB+++
309 cells (Fig 2B, Table S2). This is not surprising as the transcription of *parB* (*PA5562*) itself
310 was increased 4- and 12- fold in ParB+ and ParB+++ cells, respectively, in comparison to
311 the EV cells.

312 **Fig. 2. Transcriptome changes in response to ParB overproduction.**

313 (A) Statistics of loci with significant expression change ($FC < -2$ or > 2 , p -value < 0.05). RNA
314 was isolated from PAO1161 cultures grown in L broth without Ara (WT), PAO1161 (pKGB8
315 *araBADp*) cultures grown under selection in L broth with 0.02% Ara (empty vector control,
316 EV), PAO1161 (pKGB9 *araBADp-parB*) cultures grown under selection in L broth without
317 Ara (mild ParB excess, ParB+) or with 0.02% Ara (higher ParB excess, ParB+++). (B) Venn
318 diagram for sets of loci with significant expression change between EV *vs* WT, ParB+ *vs* EV
319 and ParB+++ *vs* EV. (C) Classification of loci with altered expression according to
320 PseudoCAP categories [57]. When a gene was assigned to multiple categories, one category
321 was arbitrarily selected (Table S2). The PseudoCAP categories were grouped into six classes
322 as marked. White and black bars correspond to the numbers of respectively, upregulated and
323 downregulated genes in a particular category.

324 Interestingly, there was also a set of 35 genes with expression changed only in ParB+
325 cells relative to EV cells but not in ParB+++ . Closer inspection of the expression changes of
326 these genes revealed three subgroups (Fig S1A, Table S2): i/ 15 genes were also altered in
327 ParB+++ transcriptome but with a slightly lower fold change ($FC > 1.6$ or $FC < -1.6$, p -value

328 <0.05) and were cut off from the common pool of ParB⁻ affected genes using the criterion of
329 FC, ii/ 6 genes despite their high FC were eliminated in ParB⁺⁺⁺ vs EV analysis because of
330 slightly higher variation of their expression in biological replicates (0.05 < *p*-value < 0.1), iii/
331 remaining 14 genes seemed to be not altered in ParB⁺⁺⁺ transcriptome. Whereas ParB⁺⁺⁺
332 and EV strains were grown under the same conditions (chloramphenicol and 0.02%
333 arabinose), ParB⁺ cells were not exposed to arabinose. To estimate the impact of inducer on
334 gene expression PAO1161 (pKGB8) was grown in medium with or without 0.02% Ara and
335 RT-qPCR analysis of eight loci from the group of 35 genes in question, representing all three
336 subgroups, was performed. The expression of only one gene, *PA2113* (from the bi-cistronic
337 operon *PA2113-2114*) was altered in response to Ara presence in the medium (Fig S1B).
338 *PA2113-2114* belong to the third subgroup in the expression pattern analysis (see above) but
339 also to a distinct set of 4 genes common for EV vs WT and ParB⁺ vs EV lists (Fig 2B, Table
340 S2), hence it is likely that these four genes appeared in our analysis due to the influence of
341 Ara on their expression. Presence of Ara had no influence on the expression of 7 out of 8
342 genes tested, which represent 14 genes due to the operon structures (Fig S1B), confirming that
343 majority of genes with the expression altered in ParB⁺ cells relative to EV cells was identified
344 due to their response to ParB excess.

345 The genes with altered expression were assigned to PseudoCAP categories [57]
346 divided arbitrarily into six classes as described previously [39]. The comparison of the EV
347 and WT transcriptomes revealed that the majority of affected genes belong to three groups:
348 Class II comprising genes encoding membrane proteins, proteins involved in transport of
349 small molecules, and protein secretion systems (18 genes), Class V comprising genes
350 involved in metabolic pathways (21 genes), and Class VI with 22 HUU genes (hypothetical,
351 unclassified, unknown) (Fig. 2C, Table S2). Similarly, most of the genes with altered
352 expression in ParB overproducing cells (ParB⁺⁺⁺ and ParB⁺) relative to EV cells were

353 assigned to the same three classes (II, V and VI). However, almost one third of the genes with
354 expression altered in response to ParB overproduction fall into Class I, mainly to PseudoCAP
355 categories RPTP (related to phage, transposon, plasmids) and SF (secreted factors: toxins,
356 enzymes, alginate). These data suggest that an increased ParB level leads to the activation of
357 stress response.

358 **Different expression patterns of genes affected by ParB excess**

359 To systematize the impact of the vector as well as the increased level of ParB on the
360 PAO1161 transcriptome a K-means clustering analysis was performed [52]. The two lists of
361 altered genes (ParB+ vs EV and ParB+++ vs EV) were combined yielding 211 unique loci
362 (Table S2). Expression data for replicates for each of these loci in the four strains/ growth
363 conditions (WT, EV, ParB+, ParB+++) were averaged, normalized to zero mean and unit
364 variance and grouped into six clusters based on the similarity of their expression patterns (Fig
365 3A and B).

366 **Fig 3. K-means clustering of microarray data.**

367 Expression data for replicates for each of 211 loci displaying altered expression in ParB
368 overproducing cells were averaged, normalized to zero mean and unit variance and grouped
369 into six clusters. **(A)** Expression profiles of individual genes grouped according to the results
370 of K-means clustering. Each horizontal line represents one gene. Red and blue denote that the
371 expression is respectively, above or below the mean expression of a gene across the data set.
372 **(B)** Expression profiles for genes in each cluster. Y-axis represents the difference between
373 expression of a particular gene in tested conditions and the mean expression of this gene in all
374 4 conditions presented as the number of standard deviations that a particular data point differs
375 from the mean. Thick black lines represent the cluster centres. The genes from each cluster
376 and their expression levels in different cells are listed in Table S3.

377 Clusters 1 to 4 contain genes that are upregulated in ParB⁺ and/or ParB⁺⁺⁺ cells
 378 relative to EV cells and clusters 5 and 6 the downregulated ones. A detailed description of the
 379 genes in each cluster as well as their mean expression levels in the four conditions are given
 380 in Table S3.

381 To validate the expression patterns obtained from the microarray analysis the
 382 transcript level for selected genes from each cluster in various samples was analysed using
 383 RT-qPCR. Importantly, for all tested 12 genes their relative levels of expression detected by
 384 RT-qPCR correlated nearly perfectly with the microarray data (Table 3).

385 **Table 3 Validation of the microarray data by RT-qPCR.**

| Cluster | Gene ID | WT | | EV | ParB ⁺ | | ParB ⁺⁺⁺ | |
|---------|---------------|------|-------------|----------|-------------------|-------------|---------------------|-------------|
| | | MA | qPCR | qPCR | MA | qPCR | MA | qPCR |
| 1 | <i>PA0612</i> | 0.66 | 0.76 ± 0.22 | 1 ± 0.06 | 6.54 | 5.62 ± 0.97 | 10.57 | 9.69 ± 3.3 |
| 1 | <i>PA0985</i> | 0.83 | 0.87 ± 0.16 | 1 ± 0.32 | 3.57 | 5.7 ± 1.67 | 5.02 | 7.1 ± 2.14 |
| 1 | <i>PA4635</i> | 0.60 | 0.48 ± 0.08 | 1 ± 0.12 | 2.95 | 1.42 ± 0.47 | 4.63 | 2.39 ± 1.12 |
| 1 | <i>PA5562</i> | 0.93 | 0.94 ± 0.21 | 1 ± 0.08 | 4.31 | 2.49 ± 0.43 | 12.06 | 17.3 ± 3.21 |
| 2 | <i>PA2432</i> | 0.62 | 0.57 ± 0.18 | 1 ± 0.29 | 8.36 | 5.13 ± 1.13 | 7.90 | 6.74 ± 0.1 |
| 2 | <i>PA3661</i> | 1.16 | 1.15 ± 0.45 | 1 ± 0.18 | 17.50 | 5.78 ± 2.23 | 23.34 | 8.39 ± 4.41 |
| 2 | <i>PA5471</i> | 0.24 | 0.28 ± 0.08 | 1 ± 0.25 | 2.24 | 2.01 ± 0.66 | 2.50 | 2.34 ± 0.34 |
| 3 | <i>PA2008</i> | 2.44 | 3.03 ± 1.06 | 1 ± 0.27 | 6.26 | 7.91 ± 1.36 | 3.81 | 3.91 ± 0.09 |
| 4 | <i>PA4659</i> | 1.73 | 0.97 ± 0.13 | 1 ± 0.13 | 1.83 | 2.23 ± 0.22 | 2.13 | 2.06 ± 0.1 |
| 5 | <i>PA0996</i> | 0.75 | 0.57 ± 0.01 | 1 ± 0.15 | 0.58 | 0.63 ± 0.22 | 0.31 | 0.33 ± 0.04 |
| 5 | <i>PA1003</i> | 0.87 | 0.73 ± 0.13 | 1 ± 0.24 | 0.72 | 0.58 ± 0.05 | 0.45 | 0.44 ± 0.04 |
| 6 | <i>PA0011</i> | 0.96 | 1.01 ± 0.2 | 1 ± 0.24 | 0.37 | 0.41 ± 0.03 | 0.20 | 0.24 ± 0.00 |

386 Relative transcript level of selected genes in replicates of *P. aeruginosa* cultures assessed by microarrays and
 387 RT-qPCR. WT - PAO1161 grown in L broth, EV - PAO1161 (pKGB8) grown in L broth with 0.02% Ara,

388 ParB+ - PAO1161 (pKGB9) grown in L broth and ParB+++ - PAO1161 (pKGB9) grown in L broth with 0.02%
389 Ara. Microarray (MA) data represent mean expression and RT-qPCR (qPCR) data represent mean \pm SD from
390 three biological replicates.

391 **Genes upregulated in response to *parB* overexpression**

392 Clusters 1 (64 genes and one intergenic region), 2 (48 genes), 3 (21 genes) and 4 (12
393 genes) contain loci upregulated in response to ParB overproduction. For eight of these loci an
394 increased transcript level is also observed in EV cells relative to WT cells (Table S3, Table 4).
395 Among these loci are *PA5471* gene encoding ArmZ, an inhibitor of MexZ which negatively
396 regulates the *mexXY* operon encoding the multidrug efflux pump MexXY [58,59] and *pyeR*
397 (*PA4354*) encoding a transcriptional regulator of biofilm formation [60]. Additionally, Cluster
398 1 contains the *arr* (*PA2818*) gene predicted to encode a phosphodiesterase whose substrate is
399 cyclic di-guanosine monophosphate (c-di-GMP), a bacterial secondary messenger that
400 regulates cell surface adhesiveness, virulence and biofilm formation [61].

401 The biofilm formation was checked in the cultures of PAO1161 (pKGB9 *araBADp-*
402 *parB*) in comparison to PAO1161 (pKGB8) during growth under static conditions. No
403 difference was observed between the strains in the overnight cultures (data not shown).
404 Notably, a significant increase in biofilm formation was observed in dividing cultures of
405 PAO1161 (pKGB9 *araBADp-parB*) relative to PAO1161 (pKGB8), which correlates with the
406 altered expression of genes involved in regulation of biofilm formation and identified in our
407 study. No effect of arabinose presence on biofilm formation was detected (Fig 1C).
408 Transcriptomic analysis also implicated that ParB excess could alter the response to antibiotic
409 presence. The MICs for six antibiotics from β -lactam, aminoglycoside and fluoroquinolone
410 groups (piperacillin, ticarcillin, imipenem, gentamicin, tobramycin and ciprofloxacin) were
411 tested but no significant differences between PAO1161 (pKGB9) and control
412 PAO1161(pKGB8) strains grown with and without arabinose were detected (data not shown).

Table 4 Selected genes whose expression was identified as ParB regulated.

| ID | Gene | Fold change | | | Cluster | Description |
|---|---------------|-------------|-------------|---------------|---------|---|
| | | EV vs WT | ParB+ vs EV | ParB+++ vs EV | | |
| Genes induced by the presence of vector as well as ParB excess | | | | | | |
| PA0805 | | 3,3 | | 2,2 | 1 | hypothetical protein |
| PA1394 | | 2,1 | 2,0 | 2,7 | 1 | hypothetical protein |
| PA2288 | | 2,2 | 2,3 | 4,2 | 1 | hypothetical protein |
| PA4354 | <i>pyeR</i> | 3,2 | | 2,3 | 1 | ArsR-family transcriptional repressor |
| PA4826 | | 2,6 | | 2,5 | 1 | hypothetical protein |
| PA5470 | | 7,1 | 2,3 | 3,0 | 1 | probable peptide chain release factor |
| PA5471 | <i>armZ</i> | 4,1 | 2,2 | 2,5 | 2 | MexZ anti-repressor |
| Selected phage-related genes and pyocins | | | | | | |
| PA0610 | <i>prtN</i> | | 2,8 | 5,4 | 1 | transcriptional regulator PrtN |
| PA0612 | <i>ptrB</i> | | 6,5 | 10,6 | 1 | repressor PtrB |
| PA0907 | <i>alpA</i> | | 3,4 | 4,3 | | transcriptional regulator |
| PA0985 | <i>pyoS5</i> | | 3,6 | 5,0 | 1 | pyocin S5 |
| PA1150 | <i>pys2</i> | | 2,9 | 4,1 | 1 | pyocin S2 |
| PA3866 | | | 2,6 | 4,1 | 1 | Pyocin S4 |
| Genes related to type VI export | | | | | | |
| PA0083 | <i>hsiB1</i> | | 2,0 | 2,3 | 2 | sheath protein, Hcp secretion island I |
| PA0084 | <i>tssC1</i> | | 2,2 | 2,1 | 2 | TssC1, Hcp secretion island I |
| PA0085 | <i>hcp1</i> | | 2,3 | 2,2 | 2 | Hcp1, Type VI secretion system effector |
| PA0087 | <i>tssE1</i> | | | 2,2 | 2 | component of Hcp secretion island I |
| PA0089 | <i>tssG1</i> | | 2,2 | 2,5 | 2 | component of Hcp secretion island I |
| PA0090 | <i>clpV1</i> | | 2,7 | 2,7 | 2 | putative ATPase, Hcp secretion island I |
| PA0091 | <i>vgrG1a</i> | | 2,6 | 2,8 | 2 | component of Hcp secretion island I |
| PA0095 | <i>vgrG1b</i> | | 2,1 | | 4 | secreted protein of unknown function |
| PA1844 | <i>tse1</i> | | | 2,0 | 1 | toxin |
| PA1845 | <i>tsi1</i> | | 2,2 | 2,6 | 2 | immune protein |
| Genes regulated by BexR | | | | | | |
| PA2432 | <i>bexR</i> | | 8,4 | 7,9 | 2 | bistable expression regulator |
| PA2433 | | | 3,4 | 4,5 | 1 | hypothetical protein |
| PA1202 | | | 6,9 | 5,8 | 2 | probable hydrolase |
| PA1203 | | | 3,3 | 3,2 | 2 | hypothetical protein |
| PA1204 | | | 2,3 | 2,2 | 2 | NAD(P)H quinone oxidoreductase |
| PA1205 | | | 2,5 | 2,3 | 2 | conserved hypothetical protein |
| PA2698 | | | 2,1 | 2,1 | 2 | probable hydrolase |
| PA1337 | <i>ansB</i> | | | -2,0 | 6 | glutaminase-asparaginase |
| PA1338 | <i>ggt</i> | | | -2,5 | 6 | gamma-glutamyltranspeptidase precursor |
| PA1340 | <i>aatM</i> | | | -2,8 | 6 | putative amino acid transporter |
| PA1341 | <i>aatQ</i> | | | -3,1 | 6 | putative amino acid transporter |
| Genes involved in PQS synthesis | | | | | | |
| PA0996 | <i>pqsA</i> | | | -3,2 | 5 | PqsA, probable coenzyme A ligase |
| PA0997 | <i>pqsB</i> | | | -3,5 | 5 | PqsB, probable beta-keto-acyl-acyl-carrier protein synthase |
| PA0998 | <i>pqsC</i> | | -2,0 | -3,5 | 5 | PqsC, probable beta-keto-acyl-acyl-carrier protein synthase |
| PA0999 | <i>pqsD</i> | | -2,1 | -3,8 | 5 | 3-oxoacyl-[acyl-carrier-protein] synthase III |

| | | | | | |
|--|--------------|------|------|---|---|
| <i>PA1000</i> | <i>pqsE</i> | -2,4 | -3,7 | 5 | quinolone signal response protein |
| <i>PA1001</i> | <i>phnA</i> | -2,5 | -4,6 | 5 | anthranilate synthase component I |
| <i>PA1002</i> | <i>phnB</i> | | -3,1 | 5 | anthranilate synthase component II |
| <i>PA1003</i> | <i>mvfR</i> | | -2,2 | 5 | transcriptional regulator MvfR |
| Pyochelin and pyoverdine biosynthesis genes | | | | | |
| <i>PA4220</i> | | -2,0 | | 5 | hypothetical protein |
| <i>PA4221</i> | <i>fptA</i> | -2,6 | | 5 | Fe(III)-pyochelin outer membrane receptor precursor |
| <i>PA4224</i> | <i>pchG</i> | -2,6 | -2,5 | 6 | pyochelin biosynthetic protein PchG |
| <i>PA4225</i> | <i>pchF</i> | -2,4 | -2,9 | 6 | pyochelin synthetase |
| <i>PA4228</i> | <i>pchD</i> | -2,9 | -2,3 | 5 | pyochelin biosynthesis protein PchD |
| <i>PA4229</i> | <i>pchC</i> | -2,2 | | 5 | pyochelin biosynthetic protein PchC |
| <i>PA4230</i> | <i>pchB</i> | -2,4 | -2,1 | 6 | salicylate biosynthesis protein PchB |
| <i>PA4231</i> | <i>pchA</i> | -2,5 | -2,3 | 5 | salicylate biosynthesis isochorismate synthase |
| <i>PA2384</i> | | -3,0 | | 6 | transcriptional regulator |
| <i>PA2403</i> | | -3,1 | | 6 | hypothetical protein |
| <i>PA2406</i> | | -2,7 | -3,2 | 6 | hypothetical protein |
| <i>PA2409</i> | | -3,0 | -2,8 | 6 | probable permease of ABC transporter |
| <i>PA2410</i> | | -3,0 | -3,2 | 6 | hypothetical protein |
| <i>PA2411</i> | | -2,2 | | 5 | probable thioesterase |
| Transcriptional regulators | | | | | |
| <i>PA0515</i> | | 3,1 | 3,0 | 4 | probable transcriptional regulator |
| <i>PA1196</i> | | 3,7 | 3,5 | 2 | probable transcriptional regulator |
| <i>PA2583</i> | | | 2,3 | 1 | sensor/response regulator hybrid |
| <i>PA4659</i> | | | 2,1 | 4 | probable transcriptional regulator |
| <i>PA4896</i> | | -2,1 | | 5 | probable sigma-70 factor, ECF subfamily |
| Genes adjacent to <i>parS1-4</i> | | | | | |
| <i>PA0004</i> | <i>gyrB</i> | | -2,0 | 6 | DNA gyrase subunit B |
| <i>PA0005</i> | <i>lptA</i> | | -2,1 | 6 | lysophosphatidic acid acyltransferase, LptA |
| <i>PA0006</i> | | | -2,1 | 6 | conserved hypothetical protein |
| <i>PA0008</i> | <i>glyS</i> | | -3,1 | 6 | glycyl-tRNA synthetase beta chain |
| <i>PA0009</i> | <i>glyQ</i> | | -2,5 | 6 | glycyl-tRNA synthetase alpha chain |
| <i>PA0011</i> | <i>htrBI</i> | -2,7 | -4,9 | 6 | 2-OH-lauroyltransferase |
| <i>PA0012</i> | | -2,6 | -3,7 | 6 | hypothetical protein |
| <i>PA0013</i> | | -3,4 | -3,5 | 6 | conserved hypothetical protein |
| Genes adjacent to <i>parS6</i> | | | | | |
| <i>PA0492</i> | | -6,7 | -2,8 | 6 | conserved hypothetical protein |
| <i>PA0493</i> | | -4,3 | -2,4 | 6 | probable biotin-requiring enzyme |
| <i>PA0494</i> | | -3,0 | -2,2 | 6 | probable acyl-CoA carboxylase subunit |
| <i>PA0495</i> | | -3,6 | -2,1 | 6 | hypothetical protein |

414

415 A remarkable feature of clusters 1 and 2, comprising the majority of ParB -upregulated
 416 genes (Table S3), is a high proportion of phage-related genes (*PA0610*, *PA0612-0641*,
 417 *PA0643-0648*, *PA0717*, *PA0718*, *PA0907*, *PA0909-0911*) as well as pyocin-encoding genes
 418 (*PA0985*, *PA1150*, *PA3866*). Induction of bacteriophage genes and pyocin production in *P.*

419 *aeruginosa* have been shown to be a hallmark of the SOS response [62], suggesting that *parB*
420 overexpression induces a DNA damage signal. In this bacterium SOS response is coordinated
421 not only by LexA (*PA3007*), but also by two structurally related repressors, PrtR (*PA0611*)
422 and AlpR (*PA0906*) [62,63]. Significantly, expression of none of these genes is affected by
423 ParB excess. PrtR is an inhibitor of *prtN* (*PA0610*), a transcriptional activator of pyocin
424 synthesis genes [64]. *prtN* is induced 5.4-fold in ParB+++ cells suggesting a relief of the
425 PrtR-mediated repression in these cells. Similarly, genes controlled by AlpR (*PA0907*,
426 *PA0909-PA0911*) are induced in response to ParB overproduction. Further studies are
427 required to determine the effect on the expression of the PrtR- and AlpR- regulated genes
428 apparently without a change in the amounts of transcript level of these two repressors.

429 The ParB excess also induces genes encoding components of the Hcp secretion island
430 HSI-1 [65] (*PA0083-0085*, *PA0087*, *PA0089-0091*) and the toxin/immunity proteins TseI
431 (*PA1844*) and TsiI (*PA1845*). The HSI-1 together with two other secretion islands participate
432 in *P. aeruginosa* virulence, inter- and intraspecies antagonism, biofilm formation, and stress
433 sensing [66].

434 Overexpression of *parB* also results in an 8-fold increase in the expression of the
435 transcriptional regulatory gene *PA2432* encoding a bistable response regulator BexR [67]. Six
436 genes, *PA1202*, *PA1203*, *PA1204*, *PA1205*, *PA2433* and *PA2698*, previously identified as
437 upregulated in response to BexR overproduction [67], are also upregulated in ParB-
438 overproducing strains. Similarly, five genes downregulated in response to BexR
439 overproduction, *PA0998*, *PA1337*, *PA1338*, *PA1340* and *PA1341*, are also downregulated in
440 the analysed ParB -overproducing cells (Table 4).

441 The expression of *bexR* gene is known to be bistable, meaning that this gene switches
442 between OFF and ON states in cells of a genetically identical bacterial population [67]. The

443 microarray analysis was performed on three independent biological isolates of PAO1161
444 (pKGB9 *araBADp-parB*) and all demonstrated a strong induction of *bexR* (PA2432)
445 expression (*p*-value 3E-06) strongly suggesting that ParB excess modulates the BexR regulon
446 through induction of *bexR* expression. To monitor the effect of ParB excess on promoter of
447 *bexR* the strain PAO1161::*bexRp-lacZ* was constructed with the promoter-reporter cassette
448 inserted in a non-coding region of the genome. A single colony (white on L agar with X-gal)
449 was inoculated and the cells were used as the recipients in conjugation with either S17-1
450 (pKGB8) or S17-1 (pKGB9 *araBADp-parB*). Conjugants were plated on selective medium
451 containing X-gal to visualize the expression of *bexRp-lacZ* transcriptional fusion. Conjugants
452 PAO1161::*bexRp-lacZ* (pKGB8) formed typical white/transparent colonies with a very low
453 frequency of blue ones (less than 0.1%) whereas majority of PAO1161::*bexRp-lacZ* (pKGB9)
454 conjugants formed blue colonies (Fig 4), confirming that ParB induces *bexRp* expression.

455 **Fig 4. ParB excess induces the expression of chromosomal *bexRp-lacZ* transcriptional**
456 **fusion.**

457 PAO1161::*bexRp-lacZ* strain contains *bexRp-lacZ* transcriptional fusion inserted in the
458 intergenic region of PAO1161 genome. White colony from L agar with X-gal was inoculated
459 and used as a recipient in conjugation with either S17-1 (pKGB8) or S17-1 (pKGB9
460 *araBADp-parB*) donor cells. Conjugants were grown on selective L agar plates supplemented
461 with X-gal. The photographs show representative plates of PAO1161::*bexRp-lacZ* with both
462 plasmids.

463 Clusters 3 and 4 group 33 genes with mRNA level reduced in response to plasmid/
464 chloramphenicol/ arabinose (EV) but then increased by ParB excess. Twelve of the 33 genes
465 in this group belong to the PseudoCAP category carbon compounds catabolism (CCC) and
466 eight genes to the amino acid biosynthesis and metabolism (AABM) category (Table S3). The

467 altered expression of genes from these categories suggests that ParB overproduction may
468 interfere with primary metabolism as an adaptive measure. Interestingly, three out of four
469 putative arabinose-dependent genes have also been classified into cluster 4. Additionally,
470 cluster 4 contains two putative transcriptional regulators: *PA0515*, which is a part of the *nir*
471 operon (denitrification operon), and *PA4659* with unknown function.

472 **Genes downregulated in response to *parB* overexpression**

473 Clusters 5 (31 genes) and 6 (34 genes) contain genes downregulated in response to
474 ParB overproduction but differ from each other by a moderate induction in EV cells seen in
475 Cluster 5. This cluster contains all genes from two operons, *pqsABCDE* (*PA0996-1000*) and
476 *phnAB* (*PA1001* and *PA1002*), involved in the production of *Pseudomonas* quinolone quorum
477 sensing signal (PQS) [68,69]. Interestingly, the same cluster contains *PA1003* (*mvfR/pqsR*)
478 which encodes a positive regulator of the both operons [70,71], suggesting that the
479 downregulation of the *pqs* and *phn* operons is a direct result of lower *mvfR* expression.
480 Interestingly, MvfR also acts as a negative regulator of type VI secretion system HSI-1 [66],
481 components of which are upregulated in response to ParB excess (Table 4 and see above).

482 Five genes (*PA4220*, *PA4221*, *PA4228*, *PA4229*, *PA4231*) from Cluster 5 and three
483 genes (*PA4224*, *PA4225*, *PA4230*) from Cluster 6 are involved in the biosynthesis and
484 transport of a siderophore, pyochelin. Pyochelin synthesis in *P. aeruginosa* cells is positively
485 regulated by the transcriptional regulator PA2384 [72], which is downregulated in ParB+
486 cells. Interestingly, biosynthesis of another siderophore, pyoverdine, also seems to be
487 negatively affected by ParB excess as the genes *PA2403*, *PA2406*, *PA2409* and *PA2410*
488 (Cluster 6) from the pyoverdine biosynthesis operon are significantly downregulated in ParB-
489 overproducing cells (Table 4).

490 **Analysis of ParB-related transcriptional silencing around *parS* sites**

491 Analysis of the microarray data revealed that ParB overproduction reduces the
492 expression of a number of genes in close proximity of *parS1-4* (Table 4). Closer inspection of
493 the expression changes in this region revealed that genes *PA0003-PA0015*, with the exception
494 of *PA0007*, *tag* (*PA0010*) and *PA0014*, show a significant (30%-80%, p -value <0.05), ParB
495 dose-dependent downregulation (Fig 5A). RT-qPCR analysis revealed that in fact all genes
496 from *PA0004* to *PA0014* are subject to significant downregulation (Fig 5B), suggesting that
497 ParB bound to *parS1-4* negatively influences the expression of adjacent genes. To verify this
498 hypothesis we analysed the expression of these genes in the *parB_{null}* mutant, which does not
499 produce ParB [18], and in the *parS_{null}* mutant in which the *parS1-4* sequences had been
500 mutated, so the ParB binding to these sequences was impaired [38]. In the both mutants only
501 genes from *PA0010* to *PA0015* displayed significantly increased expression (Fig 5C)
502 indicating that at its native level ParB acts as a major negative regulator of genes adjacent to
503 intergenic *parS3* and *parS4* but not for the genes adjacent to intragenic *parS1* and *parS2*.
504 Regulation of the expression of genes in *parS1-parS4* region seems to be a direct consequence
505 of ParB binding to *parS1-parS4* as ParB overproduction in *parS_{null}* cells did not lower the
506 expression of *dnaA-trkA* (*PA0001-PA0016*) genes relatively to the control *parS_{null}* cells
507 carrying empty vector (Fig 5D, also see below).

508 **Fig 5. Influence of ParB on gene expression in the *parS1-4* region.**

509 (A) Mean level of expression of *dnaA-def* (*PA0001-PA0019*) genes in ParB⁺ and ParB⁺⁺⁺
510 cells relative to EV as revealed by microarray analysis. Filled markers indicate statistically
511 different expression relative to EV (p -value < 0.05 in ANOVA test). Arrangement of the
512 genes in the chromosome is shown below. Operons (according to the DOOR 2.0 database
513 [73]) are marked in grey. (B) Expression of *PA0001-PA0016* (*dnaA-trkA*) genes in ParB⁺ and
514 ParB⁺⁺⁺ cells relative to EV cells. (C) RT-qPCR analysis of expression of *dnaA-trkA* genes
515 in *parB_{null}* and *parS_{null}* strains relative to WT cells. Cells were grown in L broth. (D) RT-

516 qPCR analysis of expression of *dnaA-trkA* genes in *parS*_{null} (pKGB9 *araBADp-parB*) and
517 *parS*_{null} (pKGB8 *araBADp*) relative to EV [PAO1161 (pKGB8 *araBADp*)]. Cells were grown
518 in L broth supplemented with chloramphenicol and 0.02% arabinose. RT-qPCR data represent
519 mean \pm SD from three biological replicates. Filled symbols indicate significantly different
520 expression (p -value < 0.05 in two-sided Student's t -test assuming equal variance) relative to
521 the control cells labelled as blue squares. The differences in expression of genes in *parS*_{null}
522 (pKGB9) strain relative to *parS*_{null} (pKGB8) strain are not statistically significant.

523 A similar inspection of the expression changes in proximity (± 10 adjacent genes) of
524 the remaining six *parS* sequences [38] revealed that overproduction of ParB lowers the
525 expression of genes adjacent to *parS6* (Fig 6A, *PA0492-PA0496*) but not to the other *parS*
526 sequences (data not shown). These results were confirmed by RT-qPCR (data not shown).
527 However, the expression of *PA0492*, *PA0493* and *PA0494* as assayed by RT-qPCR was not
528 significantly changed in *parB*_{null} or *parS*_{null} cells in comparison with the WT strain (Fig 6B),
529 indicating that at its native abundance ParB is not a major effector of genes in this region.

530 **Fig 6. Influence of ParB level on expression of genes adjacent to *parS6*.**

531 (A) Mean level of expression of *PA0488-PA0498* genes in ParB⁺ and ParB⁺⁺⁺ cells relative
532 to EV cells (microarray data). Filled markers indicate statistically different expression relative
533 to EV (p -value < 0.05 in ANOVA test). Operons (according to the DOOR 2.0 database [73])
534 are labelled with different shades of grey. (B) RT-qPCR analysis of expression of *PA0492*,
535 *PA0493* and *PA0494* genes in *parB*_{null} and *parS*_{null} strains relative to WT cells. Data represent
536 mean \pm SD from three biological replicates. The differences between strains / conditions are
537 not statistically significant (p -value > 0.05 in two-sided Student's t -test assuming equal
538 variance).

539 **Regulation of *PA0011* and *PA0013* promoters by ParB interactions with *parS3* and**
540 ***parS4* sequences**

541 To further confirm that ParB binding to *parS3* and *parS4* affects the expression of
542 adjacent genes the intergenic regions preceding *PA0011* and *PA0013* and their respective
543 variants carrying mutated *parS3* and *parS4* (Fig 7A) [38] were cloned upstream of a
544 promoter-less *lacZ* cassette in pPJB132, a derivative of pCM132 [50]. To produce an excess
545 of ParB from the chromosome strain PAO1161::*araBADp-flag-parB* was constructed with the
546 expression cassette inserted in a non-coding region of the genome (Table 1). Growth of the
547 PAO1161::*araBADp-flag-parB* cells in a medium containing 0.1% arabinose results in the
548 overproduction of Flag-ParB to a level that is not toxic for the cells (data not shown). All four
549 pPJB132 derivatives were introduced into PAO1161::*araBADp-flag-parB* as well as into
550 control strain PAO1161::*araBADp* with the empty expression cassette inserted in the same
551 genomic position.

552 Analysis of the β -galactosidase activity in the transformants of the control strain
553 revealed no effect of 0.1% arabinose on the promoters tested (Fig 7B). The nucleotide
554 substitutions in *parS3* modifications (Fig 7A) did not affect the *PA0011p* activity (Fig 7B).
555 Surprisingly, replacing *parS4* by a NcoI restriction site (deletion of 10 bp) resulted in a minor
556 increase of the promoter strength in the *PA0013p_{parS4mut}-lacZ* fusion (Fig 7B). DNA sequence
557 analysis of promoters' regions does not give a clear explanation of such effect (Fig 7A). The
558 β -galactosidase activities detected in transformants of PAO1161::*araBADp-flag-parB* were
559 similar to those in the corresponding control strain and when the strains were grown without
560 arabinose. However, upon induction of *flag-parB* expression with 0.1% arabinose a reduction
561 of β -galactosidase activity was observed in PAO1161::*araBADp-flag-parB* transformants
562 carrying plasmids with the *PA0011p-lacZ* and *PA0013p-lacZ* transcriptional fusions but not
563 plasmids with the *PA0011p_{parS3mut}-lacZ* or *PA0013p_{parS4mut}-lacZ* transcriptional fusions (Fig

564 7C). These data confirmed that ParB negatively regulates expression of these two promoters
565 *in vivo* through interactions with *parS* sites.

566 **Fig 7. Influence of ParB on the activities of PA0011 and PA0013 promoters.**

567 (A) DNA sequences preceding PA0011 (PA0011p) and PA0013 (PA0013p) and their mutated
568 versions, PA0011p_{parS3mut} and PA0013p_{parS4mut}, cloned upstream of promoter-less *lacZ* cassette
569 in pPJB132 are presented. Putative promoters' motifs, RBS sequences and start codons are
570 indicated. Promoter -35 and -10 boxes were predicted using BPPROM [74]. β -galactosidase
571 activity was measured in extracts from PAO1161::*araBADp* (control strain) (B) and
572 PAO1161::*araBADp-flag-parB* (ParB-overproducing strain) (C) cells carrying pPJB132
573 derivatives as indicated. Data represent mean activity from at least three cultures \pm SD. * - *p*-
574 value < 0.05 in two-sided Student's *t*-test assuming equal variance.

575 **Discussion**

576 In *P. aeruginosa*, partitioning protein ParB plays a non-essential but important role in
577 segregation of chromosomes. Its binding to at least one of the four *parS* sequences in the
578 *parS1-4* cluster, closest to *oriC*, is necessary and sufficient for accurate segregation [33,38].
579 The role of the remaining six *parS* sequences (four in the *ori* domain and two, *parS7* and
580 *parS8*, close to the terminus domain [38]) is not fully understood. Ten similarly distributed
581 *parS* sequences in *B. subtilis* have been shown to participate in SMC recruitment,
582 condensation and juxtapositioning of chromosome arms [75]. Several reports have indicated
583 that partitioning proteins may also modulate transcription of certain genes [34,36] but *P.*
584 *aeruginosa* seems to be unique, as inactivation of *parA* or *parB* leads to large-scale changes
585 of its transcriptome [39]. A lack or an excess of ParB protein is manifested by similar
586 phenotypic changes, including erratic chromosome segregation, increased rate of formation of
587 anucleate cells, cell elongation, disturbed division and defects in swarming and swimming

588 [17,18,37]. To find out how such multiple effects arise, a microarray-based transcriptomic
589 analysis was performed of PAO1161 derivatives in which ParB level was increased either 2-
590 or 5- fold in comparison with the strain carrying empty vector. Since after binding to a
591 specific *parS* sequence ParB has the ability to spread on the adjacent DNA, we mainly
592 focused on expression of genes in the vicinity of *parS* sequences.

593 At tested ParB concentrations its binding to *parS* sites has no pronounced effect on
594 expression of genes around them with the exception of genes located in proximity of *parS1-4*
595 and *parS6*. Microarray and RT-qPCR data show convincingly that all the genes adjacent to
596 *parS1-4* (*PA0003* to *PA0015*) are downregulated in the presence of ParB excess (Fig 3A and
597 B). The interference of ParB with the expression of a vital replication operon (*dnaA-dnaN-
598 recF-gyrB*), likely caused by ParB binding to *parS1/parS2* sites within the *recF* gene, may
599 underlie the defects in cell division caused by a ParB excess larger than studied here. In
600 contrast, in cells lacking a functional ParB protein or carrying mutated *parS1-4* sequences
601 unable to bind ParB, RT-qPCR analysis revealed only upregulation of *PA0010-PA0015* genes,
602 located in proximity of the intergenic *parS3* and *parS4*. Linking the regions preceding
603 *PA0011* and *PA0013* to the promoter-less *lacZ* cassette confirmed the presence of functional
604 promoters in the cloned fragments. Both promoters were repressed by ParB excess provided
605 functional *parS3* and *parS4* were present, which confirmed that ParB binding directly affects
606 transcription in this region.

607 The product of *PA0011*, the 2-OH-lauroyltransferase HtrB1 is involved in lipid A
608 biosynthesis important for integrity of the outer membrane and cell envelope [76–78]. *htrB1*
609 expression is induced by sub-inhibitory concentrations of different antibiotics [78] or growth
610 at low temperature [77]. Inactivation of *htrB1* leads to pleiotropic effects, manifested by
611 increased susceptibility to surfactants and antibiotics, impaired swarming motility, growth
612 defects and induction of type III secretion system. Some of these effects are also observed

613 under ParB excess. Conversely, expression of another HtrB homolog (*PA3242*) is
614 significantly reduced in *parB*_{null} mutant [39]. Since inactivation of either of the *htrB* genes in
615 *P. aeruginosa* PAO1 leads to motility defects [77,79], this could explain the impaired motility
616 seen both when ParB is missing and when it is present in excess. Notably, also other genes
617 adjacent to *parS3* and *parS4* encode proteins with putative roles in response to cellular stress.
618 *tag* (*PA0010*) encodes a putative 3-methyladenine glycosidase I [80]. *PA0012* encodes a
619 protein with a predicted PilZ domain, which could bind cyclic di-GMP, an important
620 signalling molecule in *P. aeruginosa* [81,82]. Further studies are needed to characterize the
621 biological functions of all the genes adjacent to *parS3* and *parS4* and the role of ParB in their
622 regulation.

623 Apart from the genes in proximity to *parS3*/*-parS4*, also those close to *parS6* showed a
624 reduced expression in response to ParB excess, however, their expression was not altered in
625 *parB*_{null} and *parS*_{null} strains, suggesting that here the ParB involvement is a part of a more
626 complex regulatory circuit. No changes were observed in the transcription of genes adjacent
627 to *parS5* and *parS7*-*parS10* upon ParB excess. Indeed, a recent ChIP-seq analysis did not
628 indicate binding of ParB to these sequences *in vivo* in cells grown in minimal medium [33],
629 but instead showed the presence of nine additional ParB-bound sites. However, none of the
630 genes adjacent to those regions changed expression in response to ParB excess in cells grown
631 in conditions tested here (data not shown). It is likely that the interaction of ParB with such
632 secondary ParB binding sites, which is not required for chromosome segregation, may
633 strongly depend on experimental conditions. It would be therefore interesting to compare
634 ParB ChIP data obtained for cells grown in minimal vs. rich medium.

635 A comparison of the transcriptional changes in *parB* -deficient cells and cells
636 expressing ParB excess identified 87 genes found in both studies (Table S4) [39]. Expression
637 of 83 genes similarly responded to either lack or abundance of ParB. Majority of them seem

638 to be involved in the stress adaptation. Only *PA0293*, *PA1894*, *PA2113* and *PA2792* showed
639 opposite trends of the response. These four genes are downregulated in *parB* -deficient cells
640 and upregulated in response to ParB excess, hence it is unlikely that, akin to genes in the
641 proximity of *parS3* and *parS4*, ParB directly affects their expression.

642 Our data indicate that ParB excess downregulates two operons *pqsABCDE* and *phnAB*
643 involved in quinolone quorum sensing signalling [68,69], probably indirectly by repressing
644 *PA1003* (*mvfR/pqsR*) coding for a transcriptional activator of the both operons [70,71].
645 Additionally, the ParB excess interferes with expression of key virulence determinants, two
646 pathways involved in the production of the siderophores pyochelin and pyoverdine.

647 The extreme adaptability and excellent survival of *Pseudomonas* species have been
648 claimed to originate from the plasticity of gene expression facilitated by the high number of
649 transcriptional regulators encoded in their genomes [83,84]. ParB excess leads to the
650 induction of several genes encoding known and putative transcriptional regulators (Table 4).
651 Among them is *PA2432* encoding the bistable response regulator BexR [67]. Bistability refers
652 to a phenotypic heterogeneity within an isogenic population which allows a fraction of cells to
653 survive in otherwise lethal conditions [85]. The BexR regulon comprises a diverse set of up-
654 and down- regulated genes related to virulence and quorum sensing [67]. Importantly, the
655 *bexR* induction in response to ParB excess is highly reproducible and approaches the ~10-fold
656 induction maximum found in the subset of cells which have switched on the BexR regulon
657 [67]. The mobilization of pKGB9 (*araBADp-parB*) and pKGB8 (*araBADp*) plasmids into the
658 test strain PAO1161::*bexRp-lacZ* with “OFF” phenotype demonstrated “ON” state of *bexRp*
659 in nearly all conjugants with pKGB9 and in a tiny fraction of conjugants with pKGB8
660 (<0.1%). This confirms that the ParB excess induces the *bexR* gene in all cells rather than in a
661 specific subpopulation only. So far the mechanism of *bexR* induction in response to ParB
662 excess remains unknown.

663 Our study also revealed that ParB excess leads to the induction of bacteriophage and
664 pyocin encoding genes, which in *P. aeruginosa* are hallmarks of the SOS response [62,86].
665 Whereas the SOS response had initially been linked exclusively to DNA damage signals, it
666 was later established to be part of a broad stress reaction [87,88]. SOS response is tightly
667 connected to cell growth inhibition, cell-cycle checkpoints and even programmed cell death
668 [89–91]. The phage- and pyocins-related genes are among the genes responding similarly to
669 the *parB* deficiency and ParB excess (Table S4), suggesting that any imbalance in the
670 ParA/ParB/*parS* system may trigger SOS response.

671 Altogether, our data indicate that the level of partitioning protein ParB in *P.*
672 *aeruginosa* influences the expression of numerous genes including those involved in
673 virulence, stress response and quorum sensing. Further studies are needed to decipher whether
674 these effects are a direct result of ParB binding to DNA (specific or non-specific), interactions
675 with other proteins or are caused indirectly, for example by topological changes.

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949

950 **Supporting information captions**

951 **S1 Figure.** Analysis of genes identified as altered in ParB⁺ vs EV but not ParB⁺⁺⁺ vs EV.
952 Abbreviation ParB⁺ corresponds to PAO1161 (pKGB9 *araBADp-parB*) strain grown without
953 arabinose, EV corresponds to PAO1161 (pKGB8 *araBADp*) grown with arabinose and
954 ParB⁺⁺⁺ for PAO1161 (pKGB9 *araBADp-parB*) cells grown with arabinose. (A) Fold
955 change- and *p*- values for the 35 genes in ParB⁺ vs EV and ParB⁺⁺⁺ vs EV analysis. First
956 subgroup of genes with $-1.6 > FC > -2$ or $1.6 < FC < 2$ and *p*-value < 0.05 in ParB⁺⁺⁺ vs EV
957 comparison is indicated in green. The second subgroup of genes with $0.05 < p\text{-value} < 0.1$ in
958 ParB⁺⁺⁺ vs EV comparison is indicated in blue. The third subgroup of genes with *p*-value
959 > 0.1 and $-1.5 < FC < 1.5$ is left uncoloured. Genes in operons are marked with arrows. (B)
960 Impact of arabinose on the expression of selected genes. PAO1161 (pKGB8 *araBADp*) cells
961 were grown in L broth containing chloramphenicol with or without 0.02% arabinose. RT-
962 qPCR was performed cDNA synthesized on RNA isolated from cells harvested at OD₆₀₀ 0.5.
963 Data represent mean \pm SD from three biological replicates. Expression values for all genes are
964 shown relative to the cells from cultures without arabinose. * - *p*-value < 0.05 in two-sided
965 Student's *t*-test assuming equal variance.

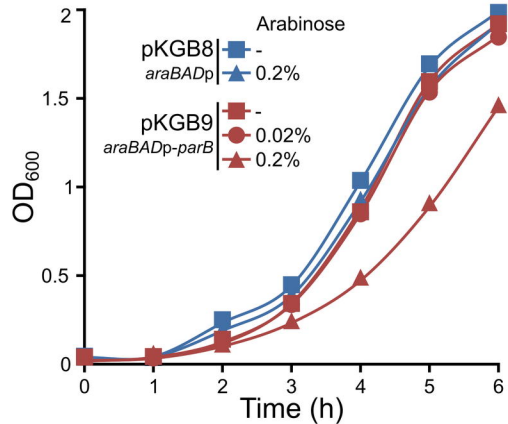
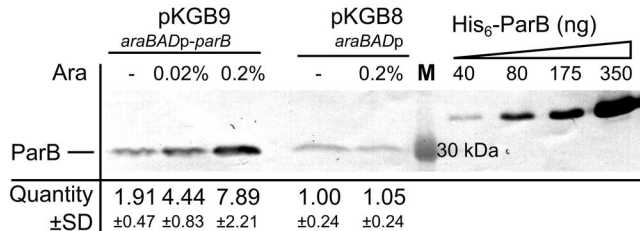
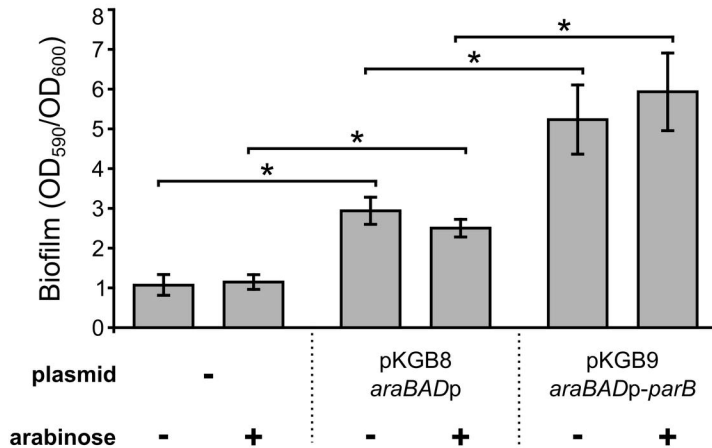
966 **S1 Table.** Oligonucleotides used in this work.

967 **S2 Table.** Transcriptomic changes between analyzed PAO1161 strains. (A) Comparison of
968 PAO1161 (pKGB8 *araBADp*) (EV) vs PAO1161 (WT). (B) Comparison of PAO1161
969 (pKGB9 *araBADp-parB*) grown without arabinose (ParB⁺) vs EV. (C) Comparison of

970 PAO1161(pKGB9 *araBADp-parB*) grown with 0.02% arabinose (ParB⁺⁺⁺) vs EV. **(D)** List
971 of 211 loci displaying altered expression in ParB overproducing cells.

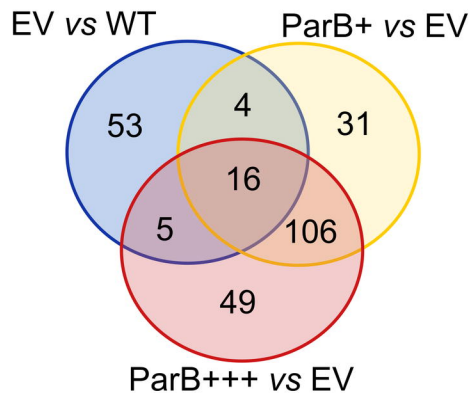
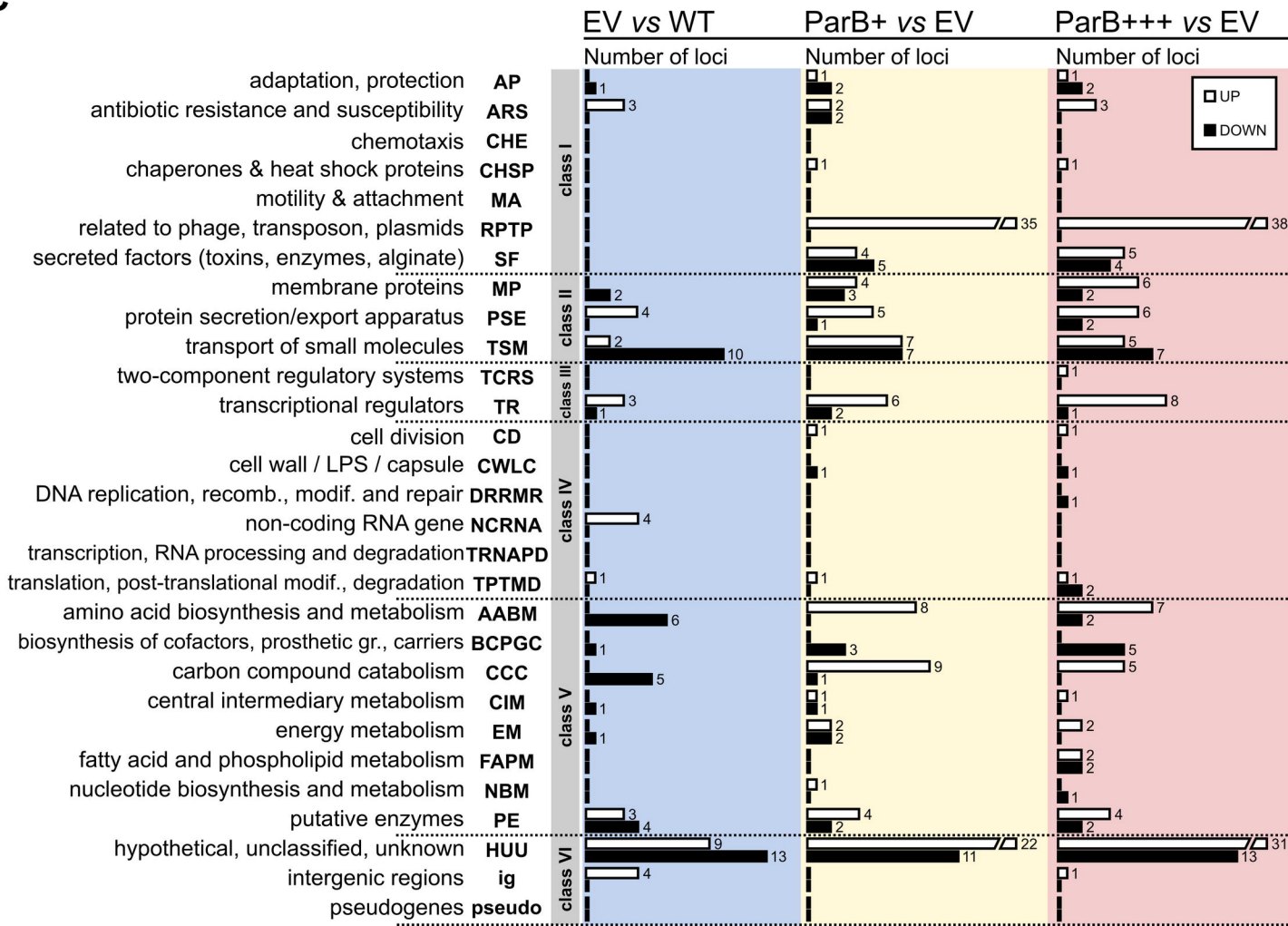
972 **S3 Table.** K-means clustering of ParB dependent loci. Mean levels of transcripts for all loci
973 are shown relative to PAO1161 (pKGB8 *araBADp*).

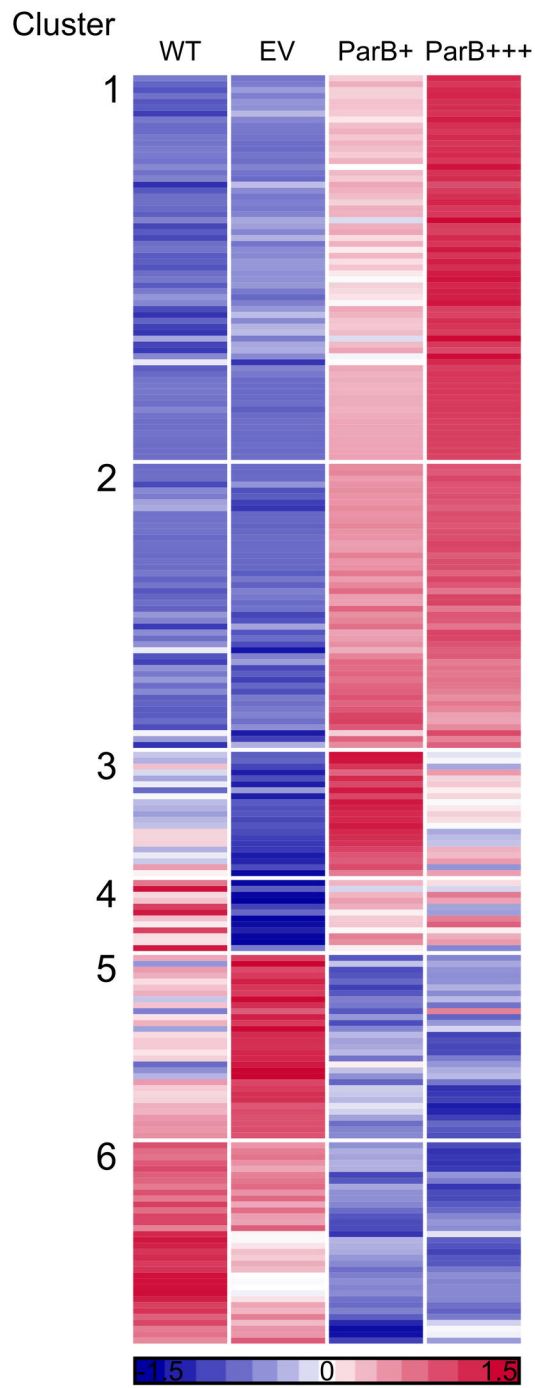
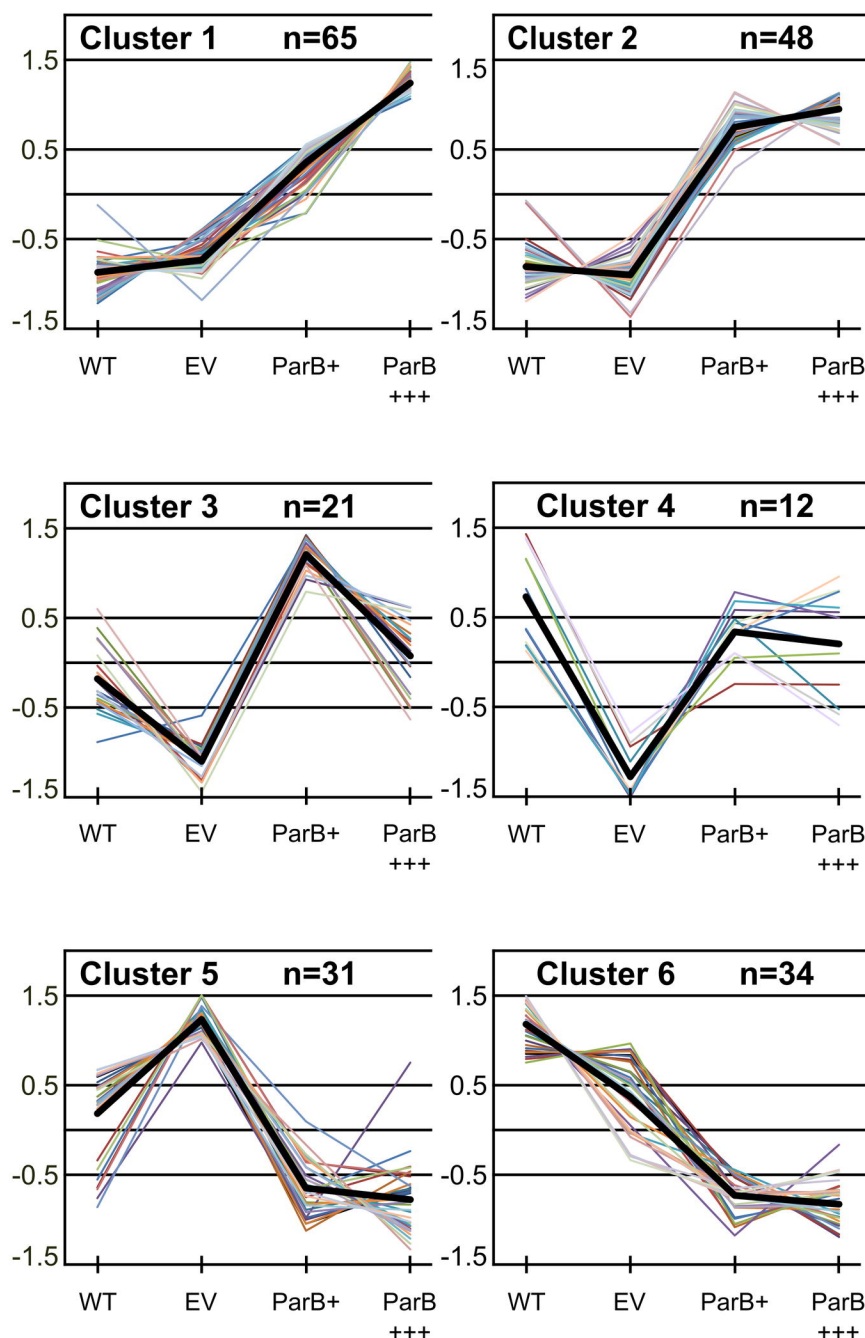
974 **S4 Table.** Genes with altered expression in response to ParB deficiency (PAO1161 *parB_{null}*)
975 and ParB overproduction [PAO1161 (pKGB9 *araBADp-parB*) grown with or without 0.02%
976 arabinose vs PAO1161 (pKGB8 *araBADp*)].

A**B****C**

A

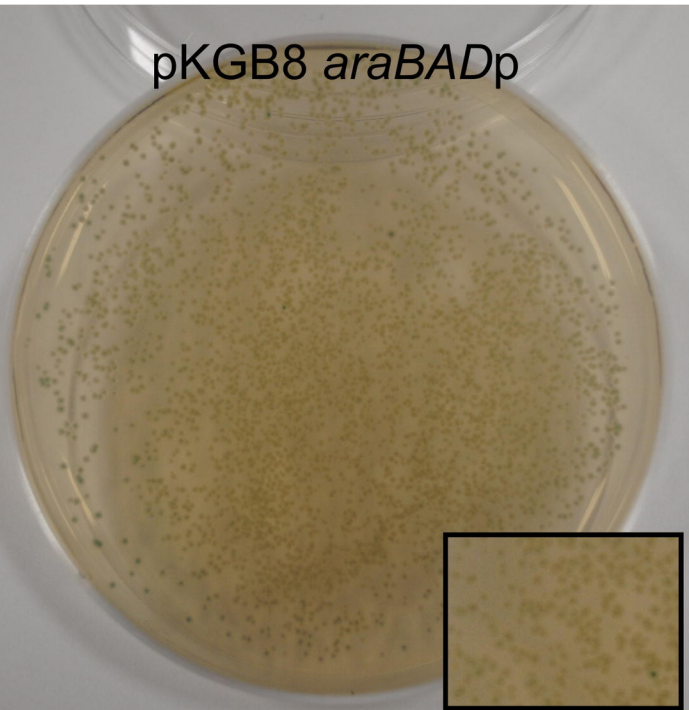
| | Total | UP | DOWN |
|---------------|-------|-----|------|
| EV vs WT | 78 | 33 | 45 |
| ParB+ vs EV | 157 | 114 | 43 |
| ParB+++ vs EV | 176 | 129 | 47 |

B**C**

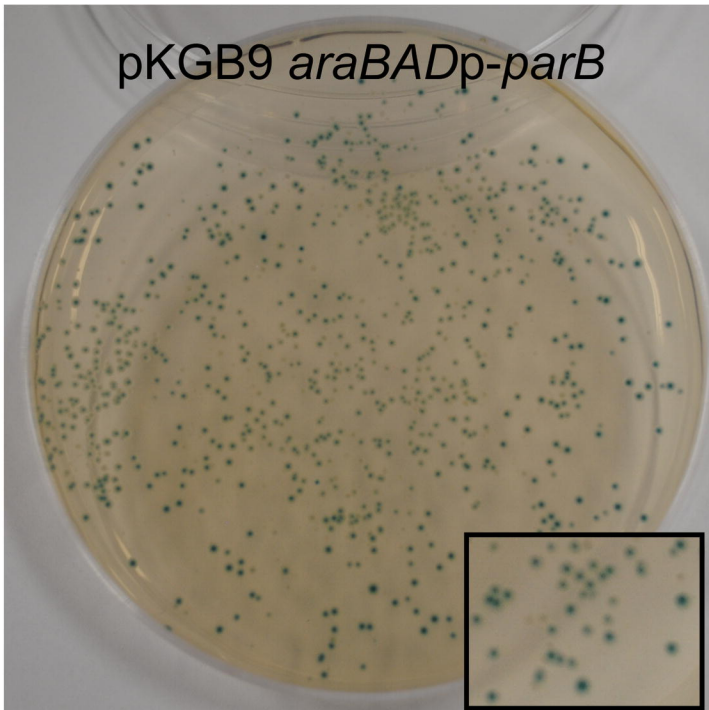
A**B**

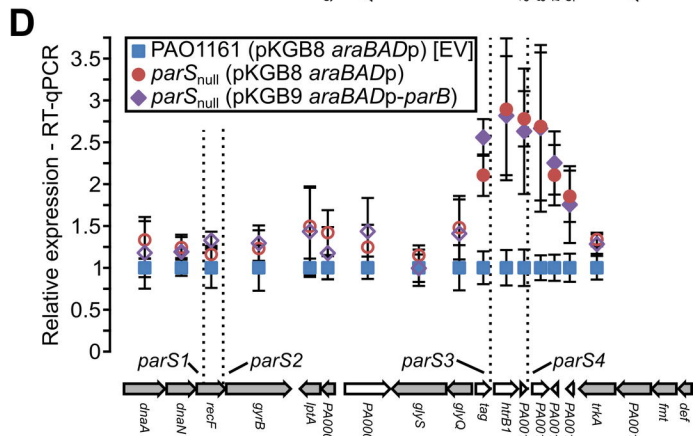
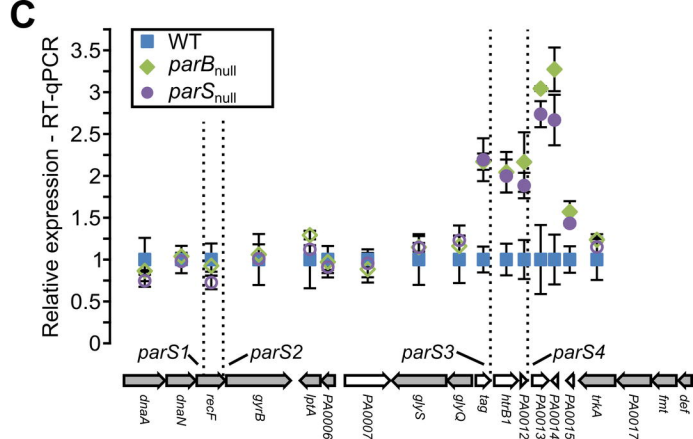
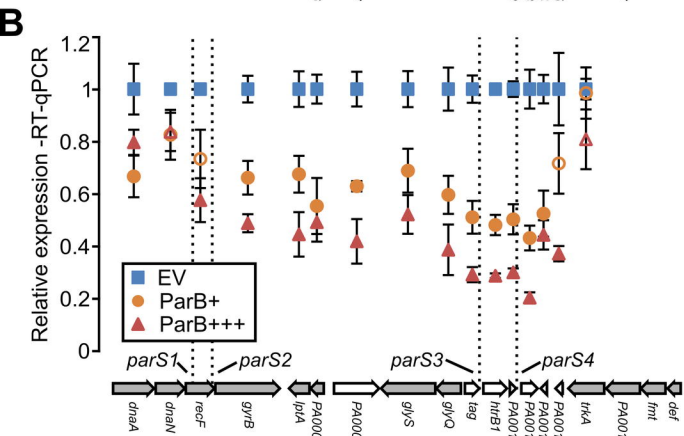
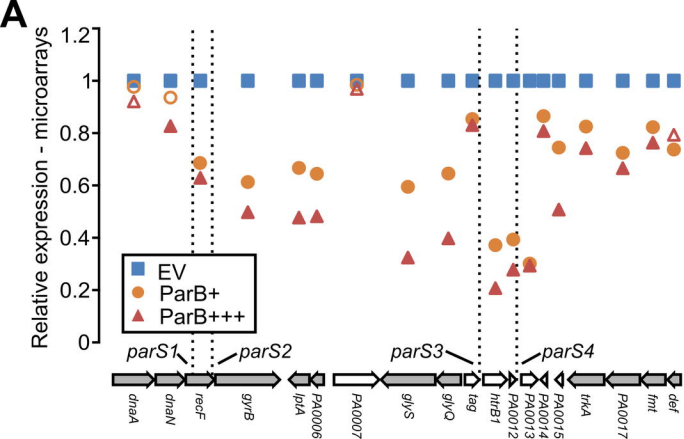
PAO1161::*bexRp-lacZ*

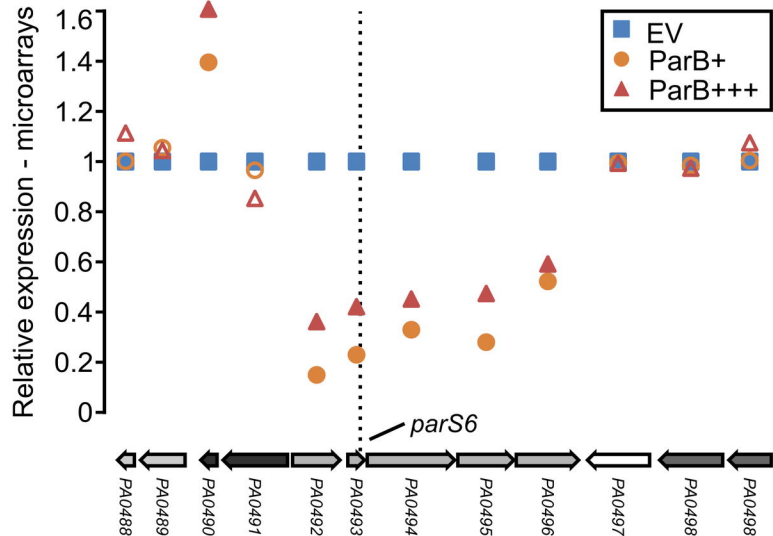
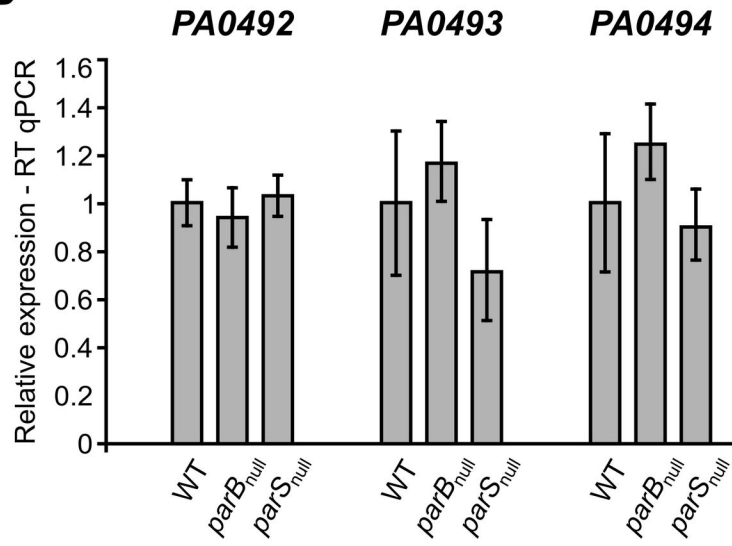
pKGB8 *araBADp*

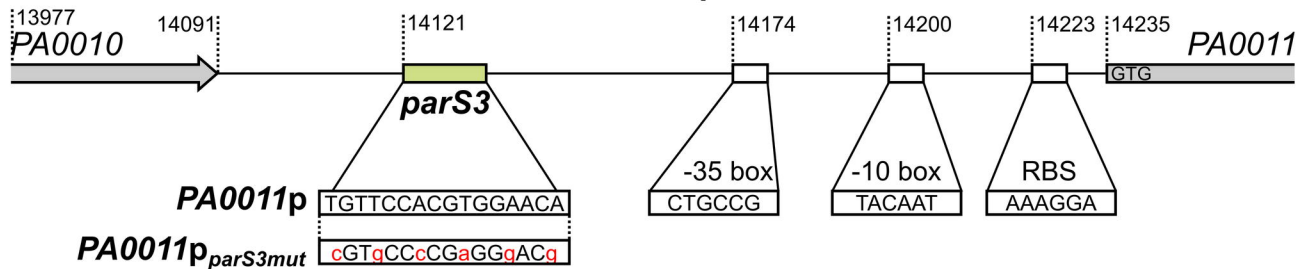
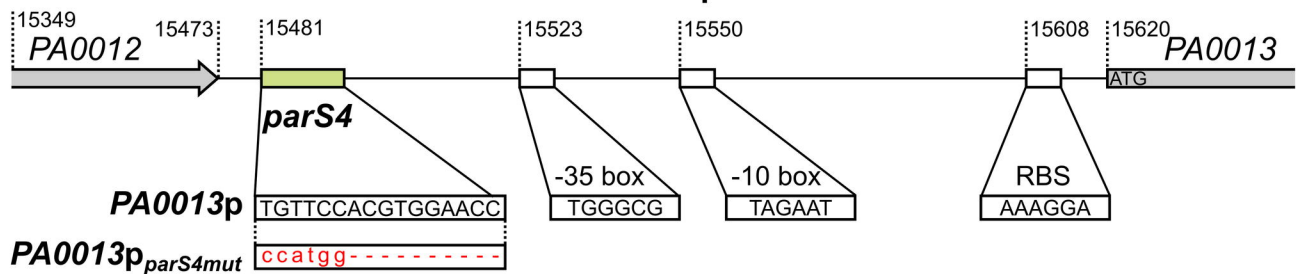
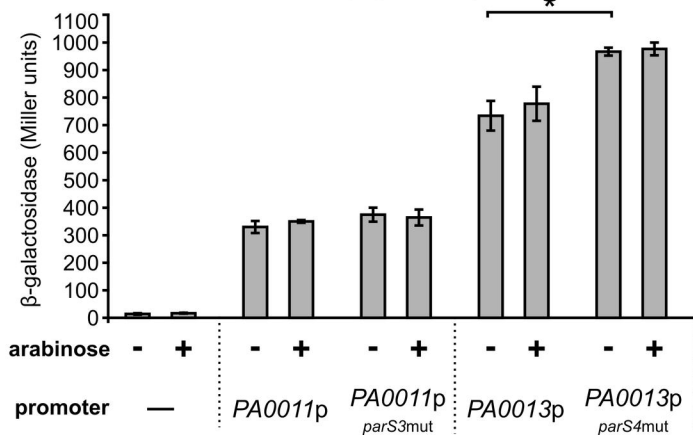


pKGB9 *araBADp-parB*





A**B**

A**PA011p****PA013p****B****PA01161::araBADp (control)****C****PA01161::araBADp-flag-parB**