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Supplemental information

Quantitative analysis of asynchronous transcription-translation and transcription processivity in *Bacillus subtilis* under various growth conditions

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Supplementary Figures

>lacZ

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P297-R: CGACGACAGTATCGGCCTC

P898-F[a]: CGTGACTACCTACGGGTAACAG
P898-R: GCATAACCACCACGCTCATC

P1578-F: GCTGGATCAAATCTGTCGATCC
P1578-R: GGAAGGGCTGGTCTTCATCC

P3105-F: GGCACATGGCTGAATATCGACG
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>araAB

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Ara130-F: ACAGGAAGCCAGCACCTATAC
Ara130-R: TGGAAGAAATCCCGCTGAGC

Ara1477-F: CGGAGCAAATGCTTGATTGGG
Ara1477-R: GGTAAAGCGCCTCGTTCCAT

Ara3141-F: CGGCGAACATGGGAAAACTG
Ara3141-R: TTCAGACGCTTCATGACATGG

>mtlAFD

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Mtl117-F：GCATGAAAGTGAAAGTGCAACG
Mtl117-R: AATGATACCCCACGCGATAAAC

Mtl2926-F: GAATGCACTGGCTGAAGGAATTG
Mtl2926-R: TTCTTCGATCAGCGCTTGCAG

>xynPB

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Xyn162-F: ATGCGTCTGGAGATTTTGCTTG
Xyn162-R: AAACATAGTACCGGCTGCTGC

Xyn2727-F: ACTACAATACAGAGAACTGGACGG
Xyn2727-R: TGTTAATTCAAGAATGCGCCCAAG

Figure S1 qRT-PCR primers used to detect the transcription kinetics of *lacZ*, *araAB*, *mtlAFD* and *xynPB* mRNA. Related to all the transcription kinetics figures in main text (Figure 1 to 5). The *lacZ* sequence started from the transcription site of *lac* operon with the start codon "ATG" being marked in red and bold. The "TGG" (red, bold), coding for the 154th amino acid residue, was mutated to "TAA" for the nonsense mutation study. The number "297", "898", "1578", or "3105" denotes the distance of the reverse primer relative to the transcription start site of *lacZ*. The native *araAB* sequence contains the open reading frame of both *araA* and *araB* as well as the short intergenic region. ara130, ara1477, and ara3141 detect the head, middle, and tail regions of *araAB*, respectively. The number "130", "1477", and "3141" denote the distance of the reverse primer relative to the start codon of *araA*. The native *mtlAFD* sequence contains the open reading frame of all *mtlA*, *mtlF* and *mtlD* as well as the short intergenic region. Mtl117 and Mtl2926 detect the head and tail regions of *mtlAFD* mRNA, respectively. The number "117" and "2926" denote the distance of the reverse primer relative to the start codon of *mtlA*. The native *xynPB* sequence contains the open reading frame of both *xynP* and *xynB* as well as the short intergenic region. Xyn162 and Xyn2727 detect the head and tail regions of *xynPB* mRNA, respectively. The number "162" and "2727" denote the distance of the reverse primer relative to the start codon of *xynP*. Note that all the tail primers are located upstream of (be away from the) the native 3' end intrinsic terminators of the operons.

a. Note that P898 primer was only used in a few cases such as Figure 4C and Figure S9.

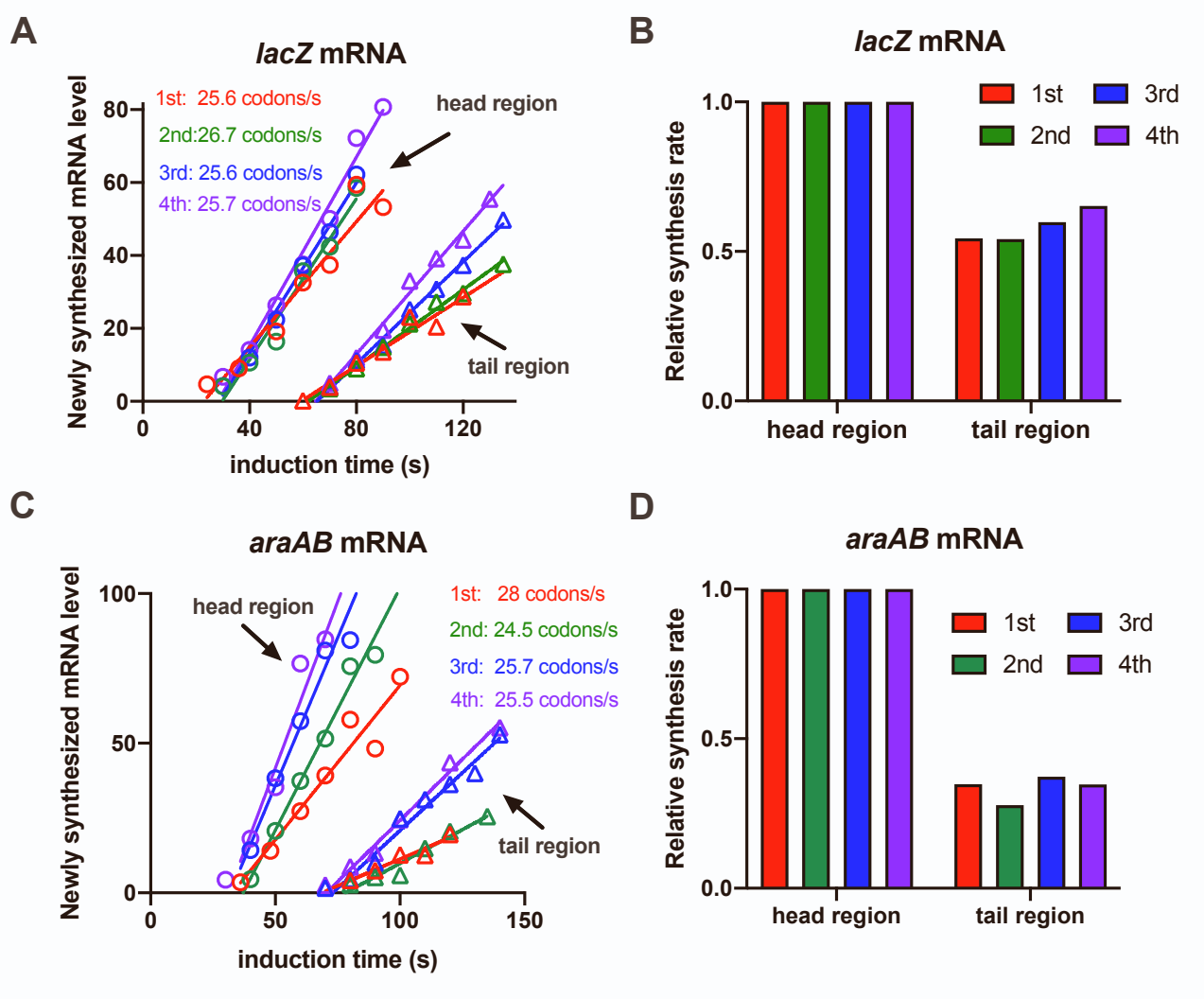

Figure S2 Reproducibility of the transcription elongation rate measurement of *B. subtilis.* Related to Figure 1. Cells were grown in gly+cAA medium. The induction curves of head and tail regions of four replicates for *lacZ* and *araAB* mRNA are shown (panel A and panel C). The transcription time of both head region (T_{head}) and tail regions (T_{tail}) (the X-intercept of the linear line) is very close to each other among different replicates. Therefore, the reproducibility of transcription elongation rate measurement is very high. In addition, we also compare the relative accumulation rate of the head and tail regions of these four replicates. Although the absolute induction fold of mRNA varies mildly among different replicates, we find that the accumulation rate of the tail region is always ~60% and ~30% of that of the head region for *lacZ* mRNA and *araAB* mRNA, respectively (panel B and panel D). Therefore, the reproducibility of transcription processivity measurement is also very high.

Note: the newly synthesized mRNA level equals to M(t)- M(0), where M(t) is the relative mRNA abundance of each time point and M(0) is the basal abundance of time zero, set as “1”.

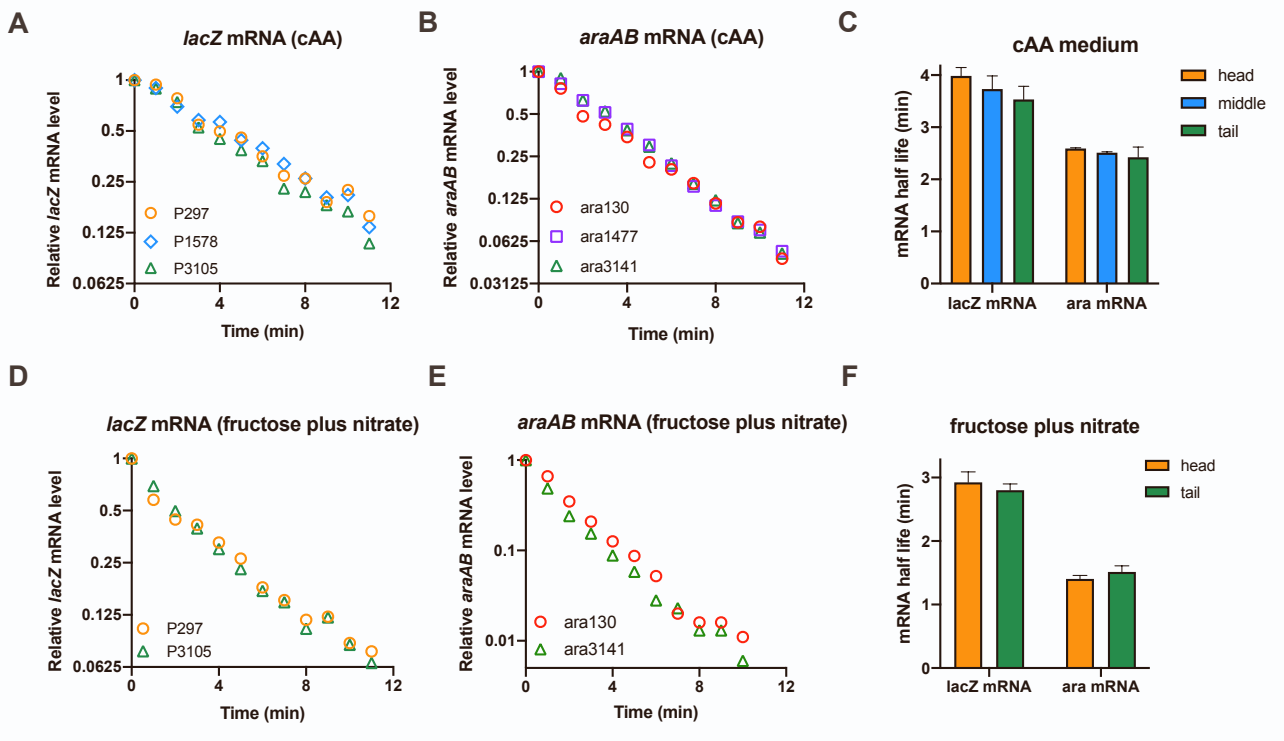

Figure S3 Degradation kinetics of different mRNA sub-regions. Related to Figure 1 and Figure 3. **(A)-(B)** The degradation kinetics of *lacZ* and *araAB* mRNA for cells grown in cAA medium, respectively. **(C)** The mRNA half-lives of *lacZ* and *araAB* mRNA for cells grown in cAA medium. Data are represented as mean +/-SD. **(D)-(E)** The degradation kinetics of *lacZ* and *araAB* mRNA for cells grown in fructose plus nitrate medium, respectively. **(F)** The mRNA half-lives of *lacZ* and *araAB* mRNA for cells grown in fructose plus nitrate medium. Data are represented as mean +/-SD.

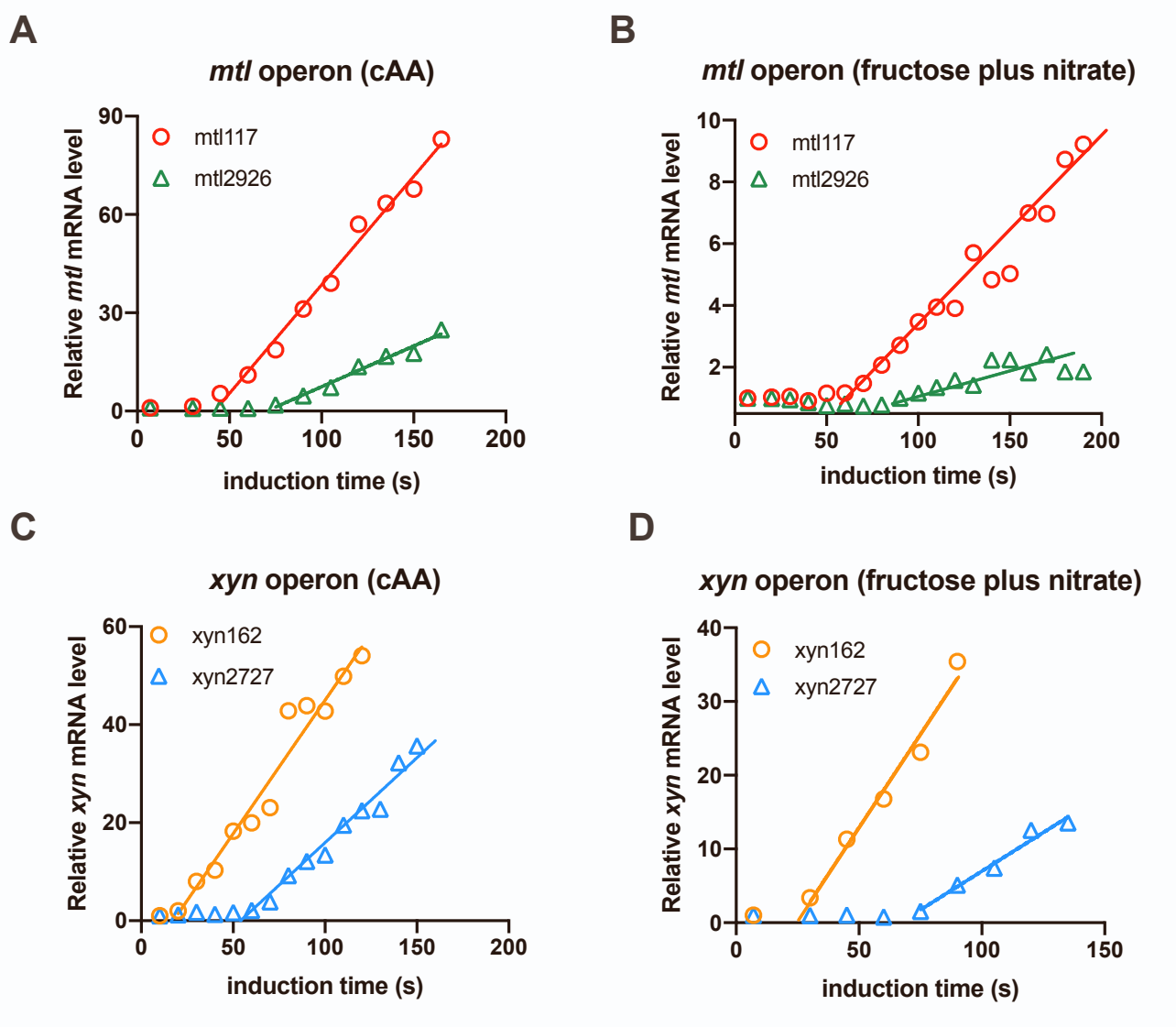

Figure S4 The induction kinetics of the native *mtlAFD* mRNA and *xynPB* mRNA. Related to Figure 1 and Figure 3. **(A)-(B)** The induction kinetics of the native *mtlAFD* mRNA in cAA and fructose plus nitrate medium, respectively. **(C)-(D)** The induction kinetics of the native *xynPB* mRNA in cAA and fructose plus nitrate medium, respectively.

Note: The native *mtlAFD* operon and *xynPB* operon in *B. subtilis* are involved in mannitol utilization and xylan utilization and are induced by mannitol and xylose, respectively. Here we found that, the accumulation rate of the tail region of *mtlAFD* mRNA (detected by mtl2926) is significantly lower than that of the head region (detected by mtl117) in both cAA medium (panel A) and fructose plus nitrate medium (panel B), suggesting the loss of transcription processivity. For the *xynPB* mRNA, the loss of transcription processivity is mild in cAA medium (panel C) but becomes stronger in fructose plus nitrate medium (panel D).

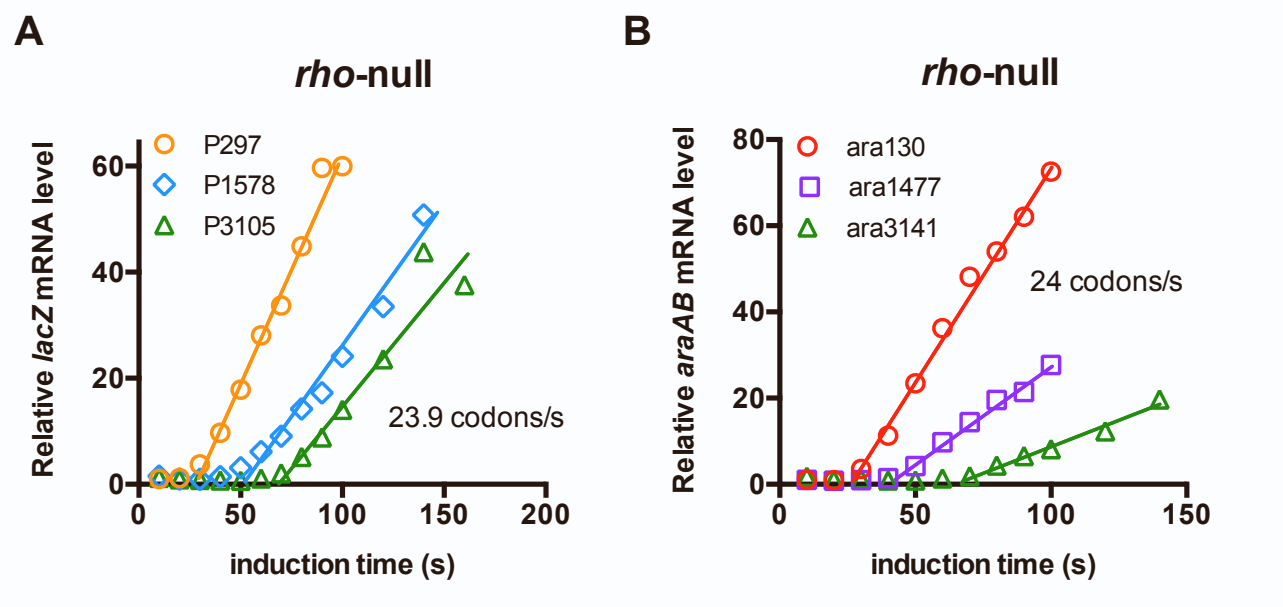

Figure S5 Transcription kinetics of *B. subtilis rho*-null strain grown in gly+cAA medium. Related to Figure 1. **(A)** The induction kinetics of the *lacZ* mRNA detected by three pairs of qRT-PCR primers. **(B)** The induction kinetics of the *araAB* mRNA detected by three pairs of qRT-PCR primers.

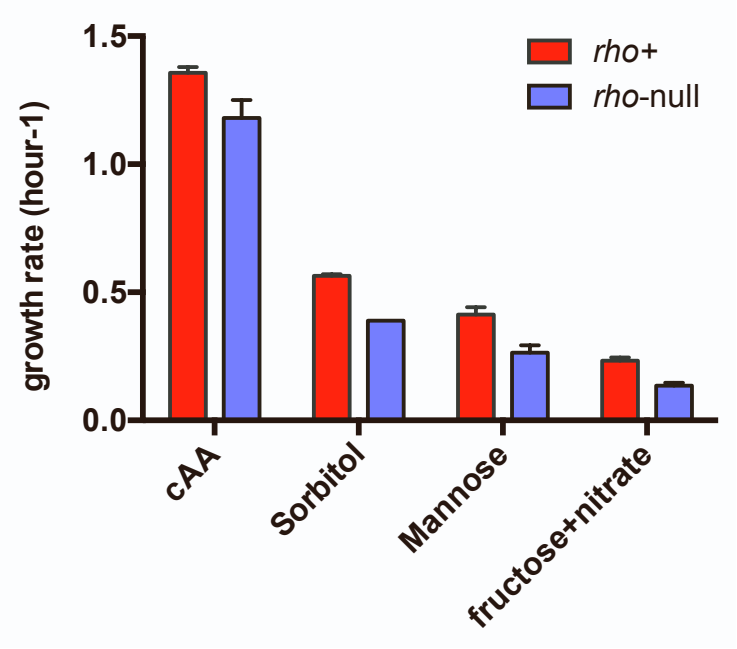

Figure S6 The growth rates of both wild type (*rho*$^+$) strain and *rho*-null strain of *B. subtilis* under different nutrient conditions. Related to Figure 1 and Figure 3. Error denotes standard deviations.

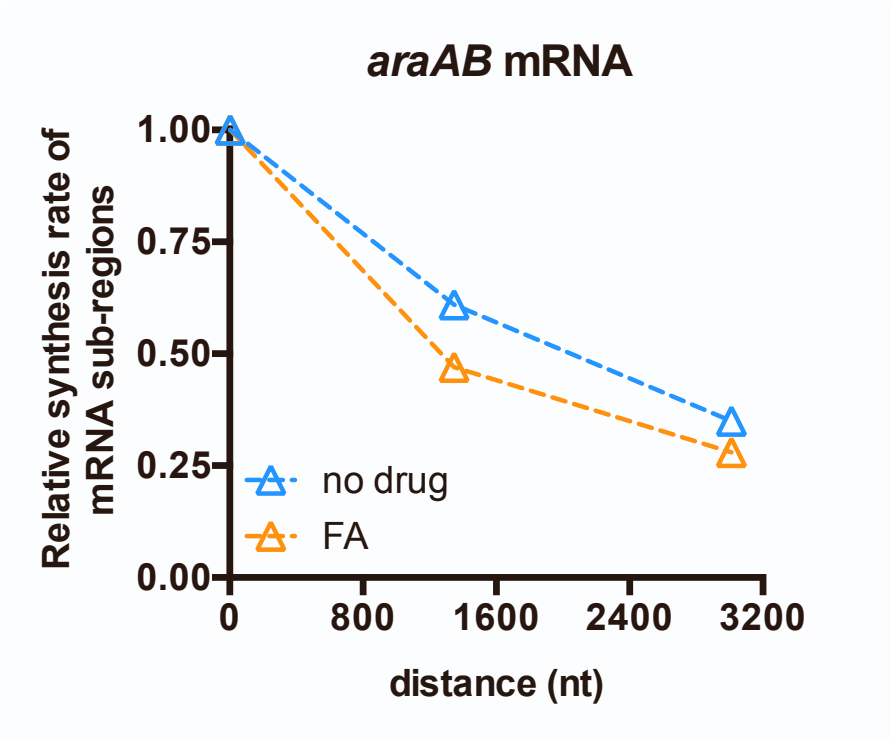

Figure S7 The transcription processivity of *araAB* mRNA in *B. subtilis* (*rho*$^{+}$) under normal condition and fusidic acid treatment. Related to Figure 2. The relative accumulation rates of different mRNA sub-regions are plotted against the hybridization locations of primers for *araAB* mRNA.

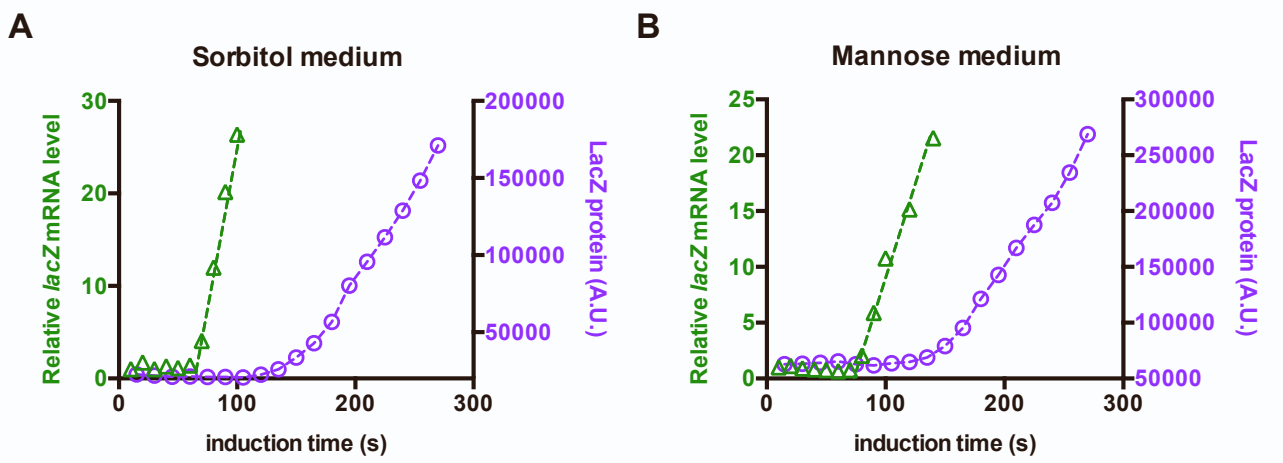

Figure S8 The induction curves of the complete *lacZ* mRNA and LacZ protein for *B. subtilis* (*rho*$^{+}$) under poor nutrients. Related to Figure 3. **(A)** sorbitol medium (doubling time: ~70 min); **(B)** mannose medium (doubling time: ~100 min).

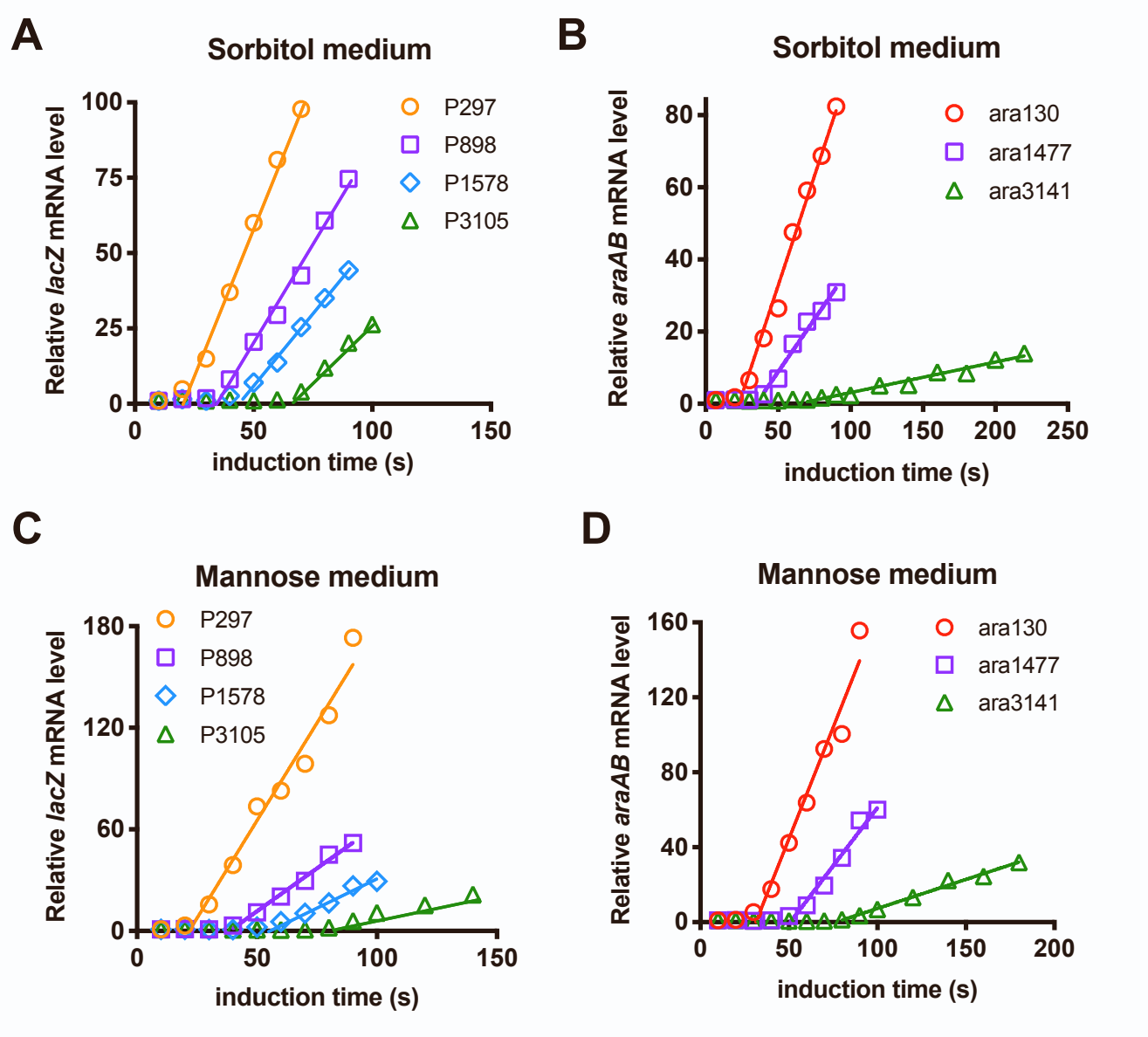

Figure S9 The transcription kinetics of *B. subtilis* (*rho*+) under poor nutrients. Related to Figure 3. **(A)** The induction kinetics of *lacZ* mRNA for *B. subtilis* grown in sorbitol medium. **(B)** The induction kinetics of *araAB* mRNA for *B. subtilis* grown in sorbitol medium. **(C)** The induction kinetics of *lacZ* mRNA for *B. subtilis* grown in mannose medium. **(D)** The induction kinetics of *araAB* mRNA for *B. subtilis* grown in mannose medium.

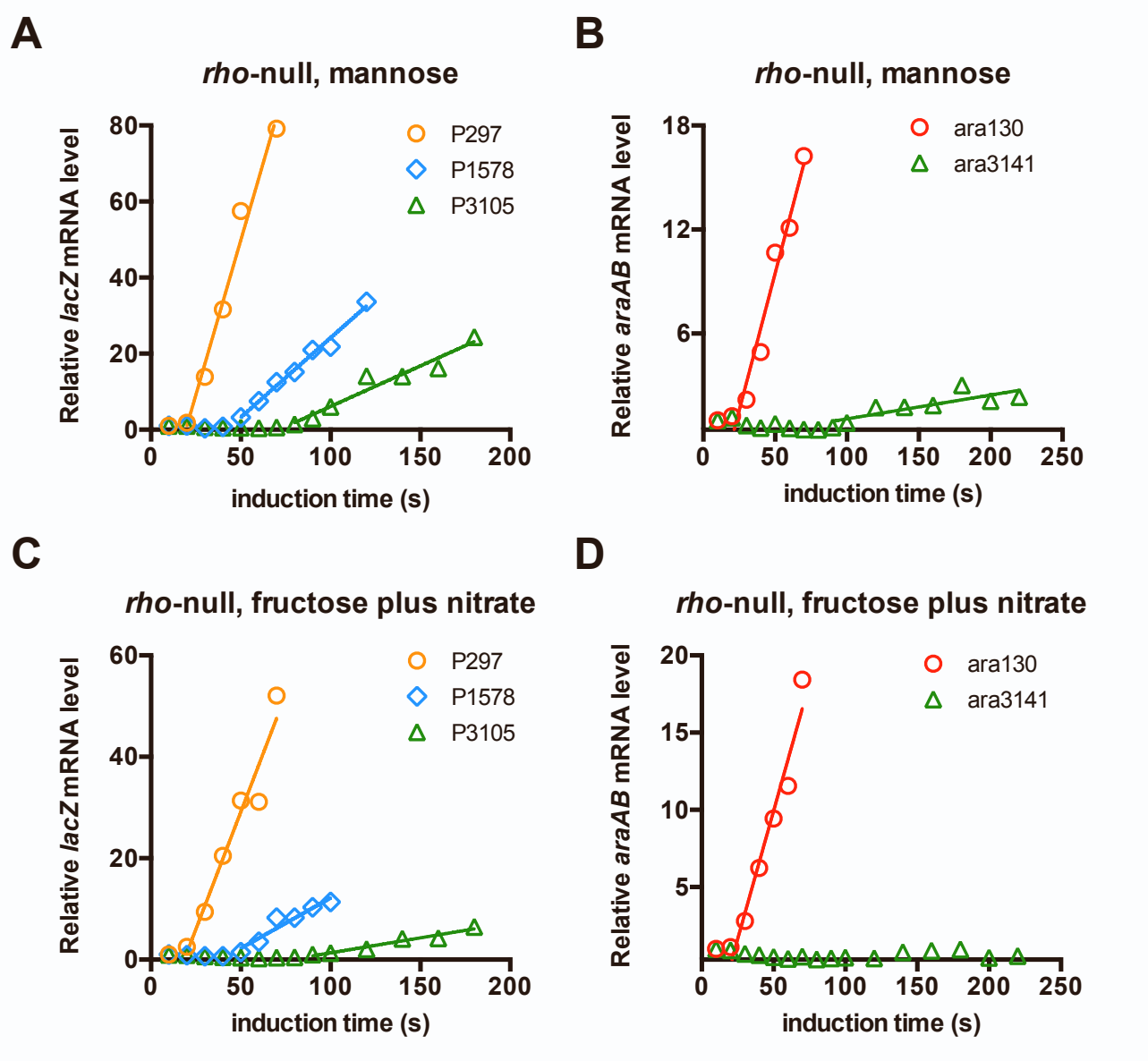

Figure S10 The transcription kinetics of *B. subtilis rho*-null mutant under poor nutrients. Related to Figure 3. **(A)** The induction kinetics of *lacZ* mRNA in *rho*-null mutant grown in mannose medium. **(B)** The induction kinetics of *araAB* mRNA in *rho*-null mutant grown in mannose medium. **(C)** The induction kinetics of *lacZ* mRNA in *rho*-null mutant grown in fructose plus nitrate medium. **(D)** The induction kinetics of *araAB* mRNA in *rho*-null mutant grown in fructose plus nitrate medium. From panel B and panel D, the induction-fold of *araAB* mRNA in *rho*-null mutant becomes somehow much lower than that in wild type cells (see Figure S9 and 3F). However, the loss of transcription processivity still occurs strongly in the *rho*-null mutant.

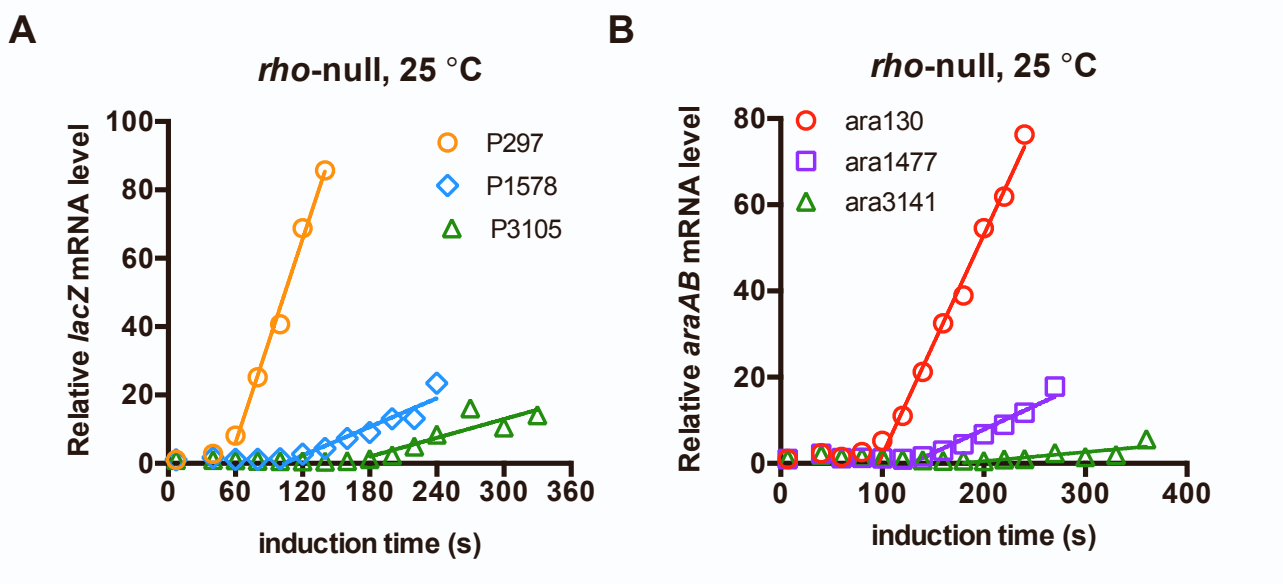

Figure S11 The transcription kinetics of *B. subtilis rho*-null strain under 25°C in gly+cAA medium. Related to Figure 4. (A) The induction kinetics of the *lacZ* mRNA detected by three pairs of qRT-PCR primers. (B) The induction kinetics of the *araAB* mRNA detected by three pairs of qRT-PCR primers.

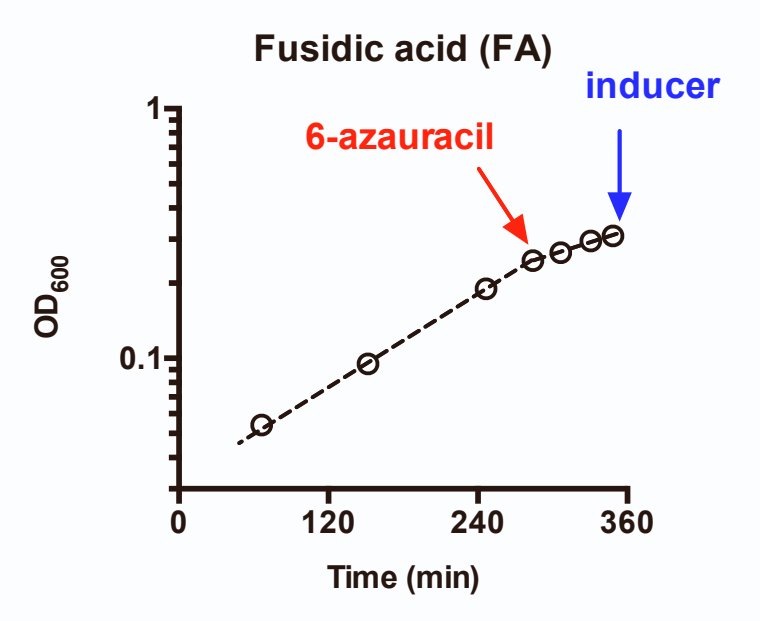

Figure S12 Cell growth with fusidic acid (FA) plus 6-azauracil (6-aza). Related to Figure 5. *B. subtilis* cells were first exponentially grown to OD_{600}~0.25 in gly+cAA medium supplemented with 0.2 μg/mL FA. 500 μg/mL 6-aza was then added to deplete the cellular nucleotides pools. Cell culture was further incubated for 1 hour before measuring the transcription kinetics.

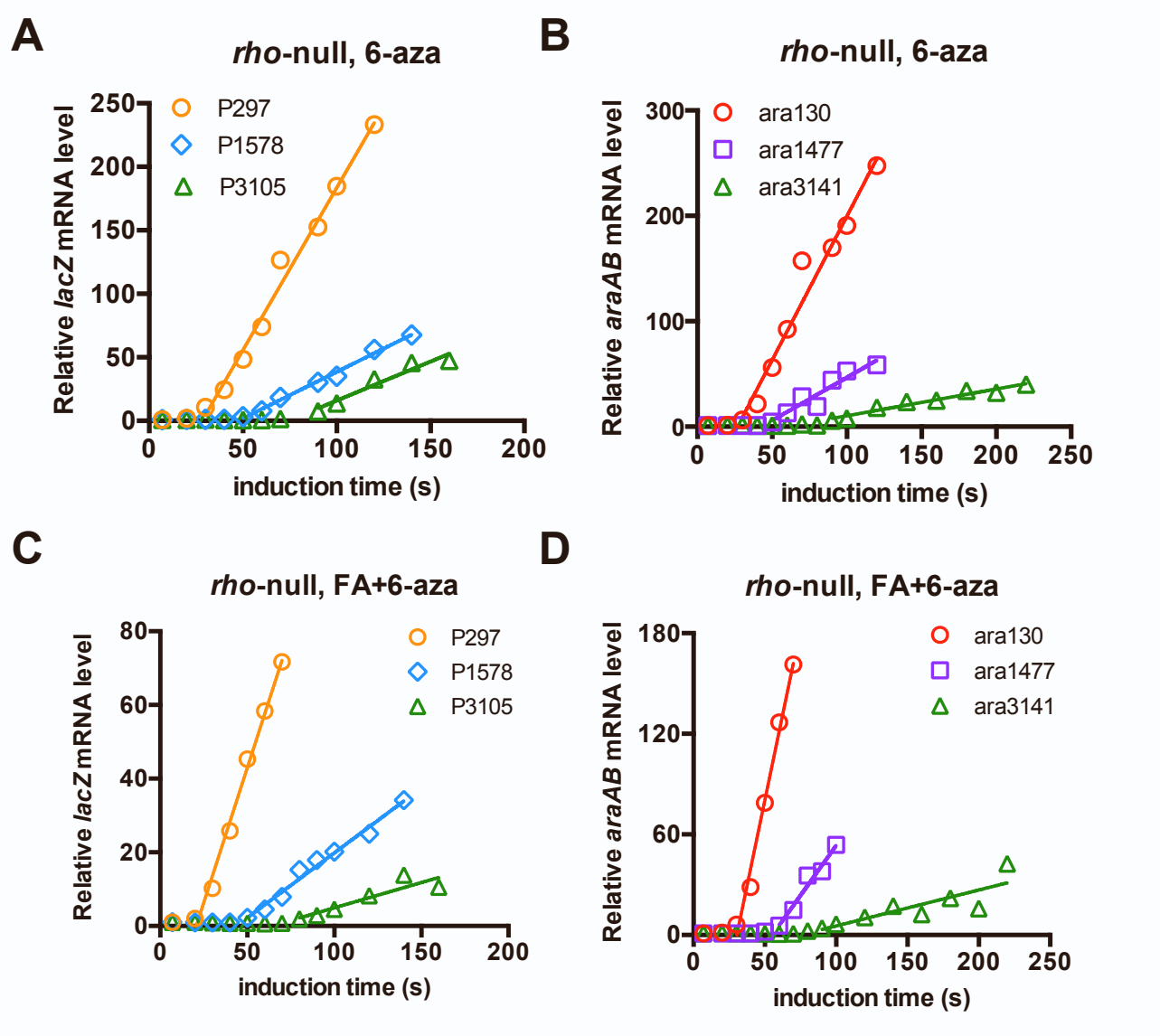

Figure S13 The transcription kinetics of *B. subtilis rho*-null mutant treated with 6-azauracil (6-aza). Related to Figure 5. (A)-(B) The induction kinetics of *lacZ* mRNA and *araAB* mRNA in *B. subtilis rho*-null mutant treated with 6-aza. (C)-(D) The induction kinetics of *lacZ* mRNA and *araAB* mRNA in *B. subtilis rho*-null mutant treated with 6-aza. Being different from Panel A and B, Cells were grown with 0.2 μg/mL fusidic acid.

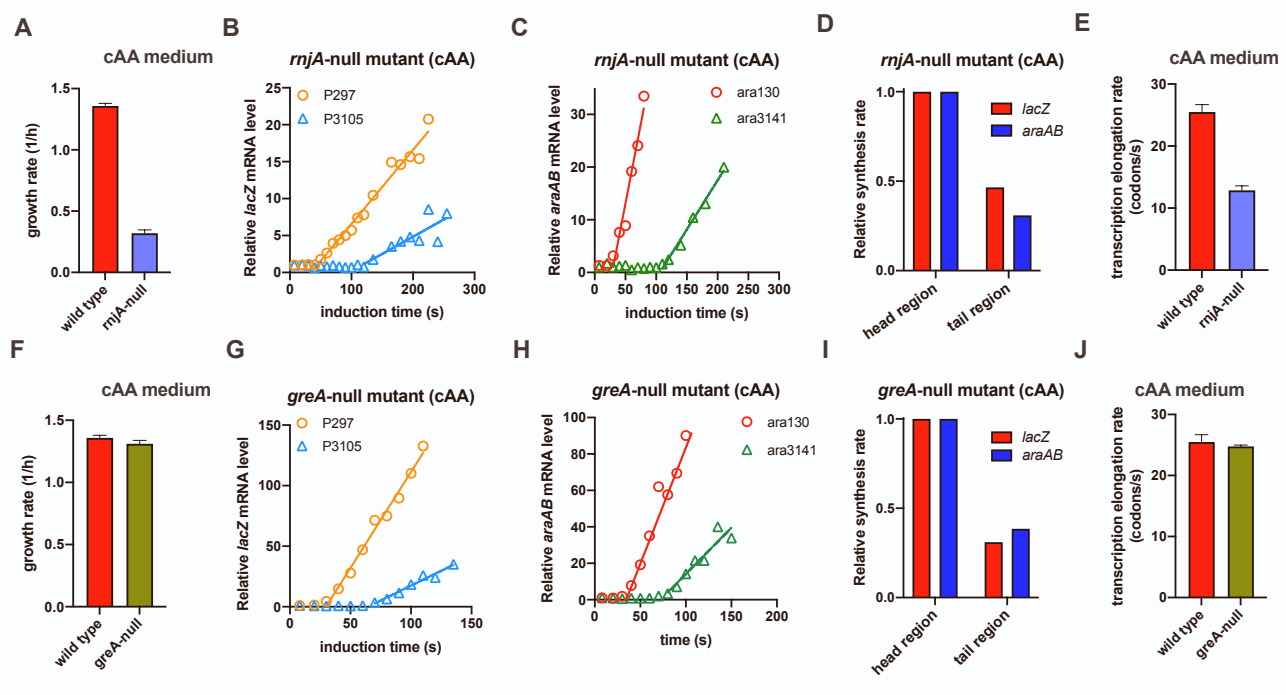

Figure S14 The transcription kinetics of *B. subtilis rnjA*-null mutant *and greA*-null mutant. Related to Figure 1. **(A)** The growth rates of wild type strain and *rnjA*-null strain in cAA medium. The *rnjA*-null strain grows much slower than wild type, being consistent with previous reports such as Sikova *et al* 2020. Data are represented as mean +/-SD. **(B)-(C)** The induction kinetics of the *lacZ* mRNA and *araAB* mRNA in *rnjA*-null strain grown in cAA medium. Here, the mRNA accumulation rate of the tail region is lower than that of the head region, suggesting the loss of transcription processivity. **(D)** The relative accumulation rate of mRNA head and tail regions in *rnjA*-null strain. The accumulation rate of the head region is set as "1". **(E)** The transcription elongation rates of wild type strain and *rnjA*-null strain in cAA medium. Data are represented as mean +/-SD. **(F)** The growth rates of wild type strain and *greA*-null strain in cAA medium. Data are represented as mean +/-SD. **(G)-(H)** The induction kinetics of the *lacZ* mRNA and *araAB* mRNA in *greA*-null strain grown in cAA medium. We found again the mRNA accumulation rate of the tail region is lower than that of the head region, suggesting the loss of transcription processivity. **(I)** The relative accumulation rate of mRNA head and tail regions in *greA*-null strain. The accumulation rate of the head region is set as "1". **(J)** The transcription elongation rates of wild type strain and *greA*-null strain in cAA medium. Data are represented as mean +/-SD.