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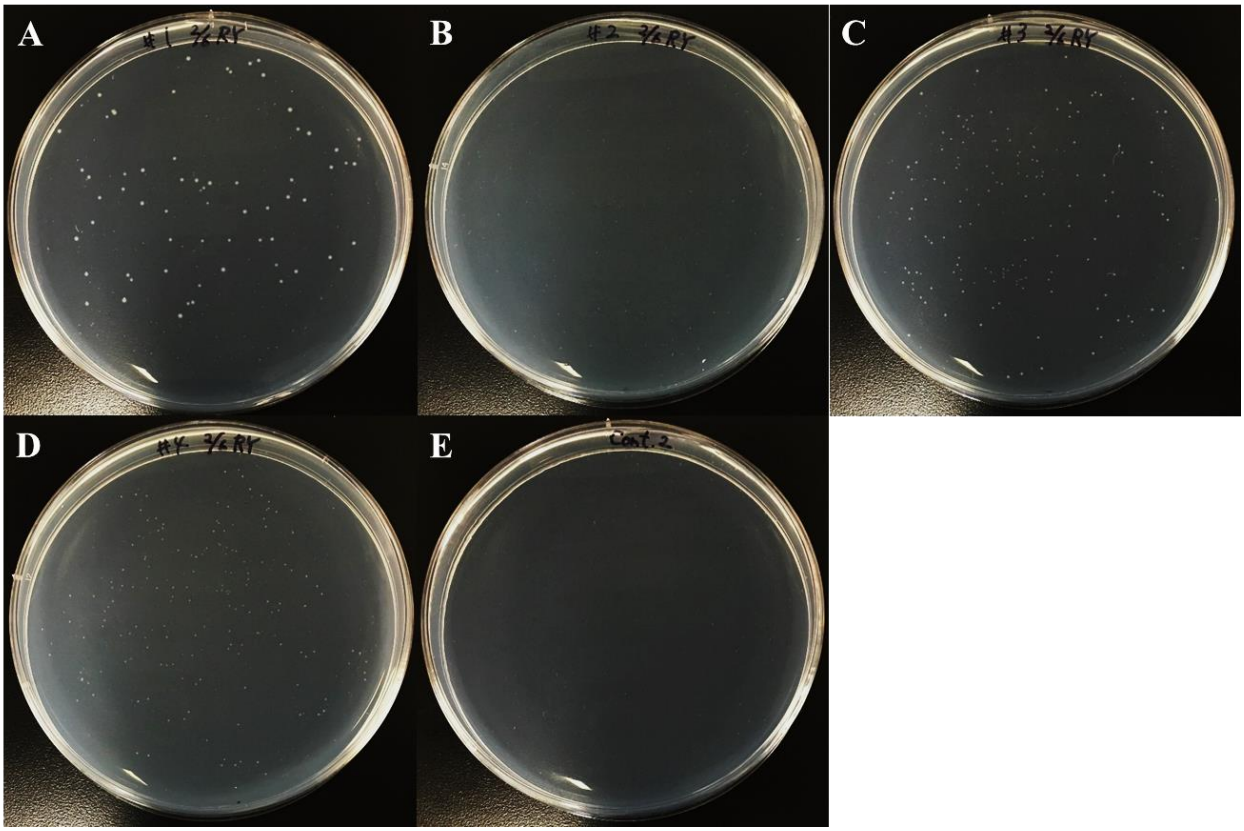
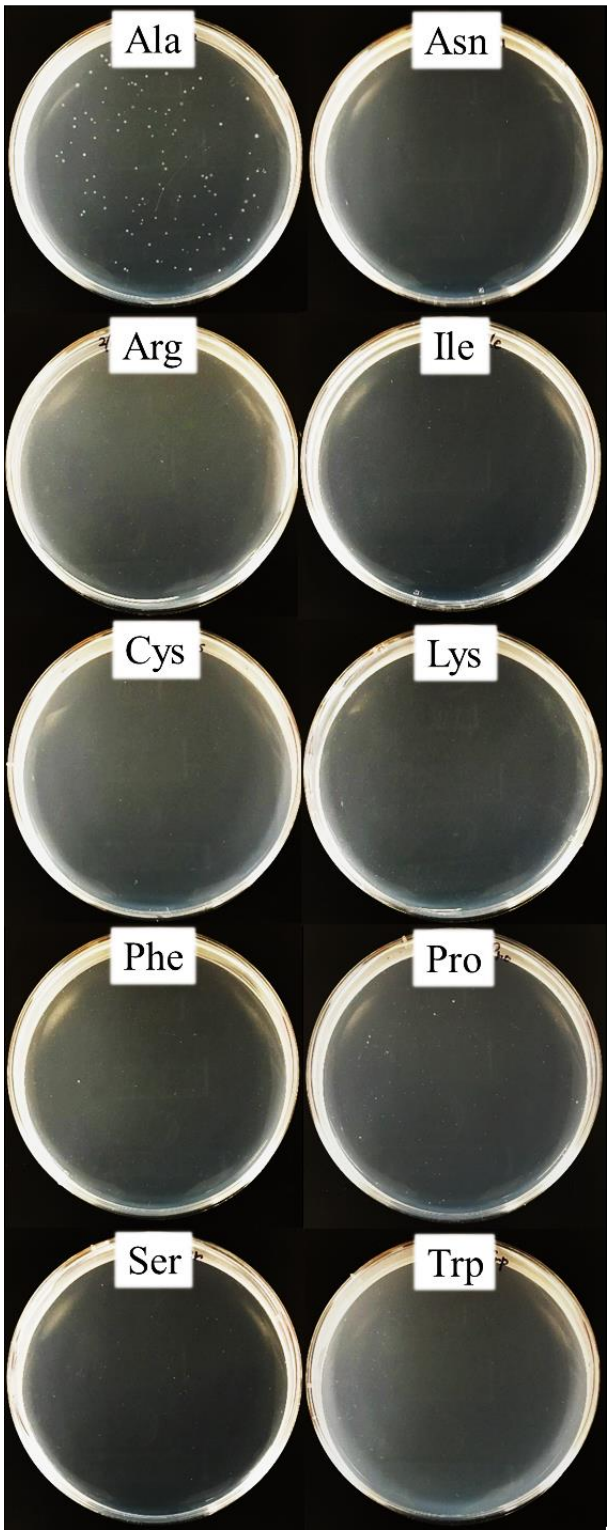
Supplemental Information

Persister Cells Resuscitate Using Membrane Sensors that Activate Chemotaxis, Lower cAMP Levels, and Revive Ribosomes

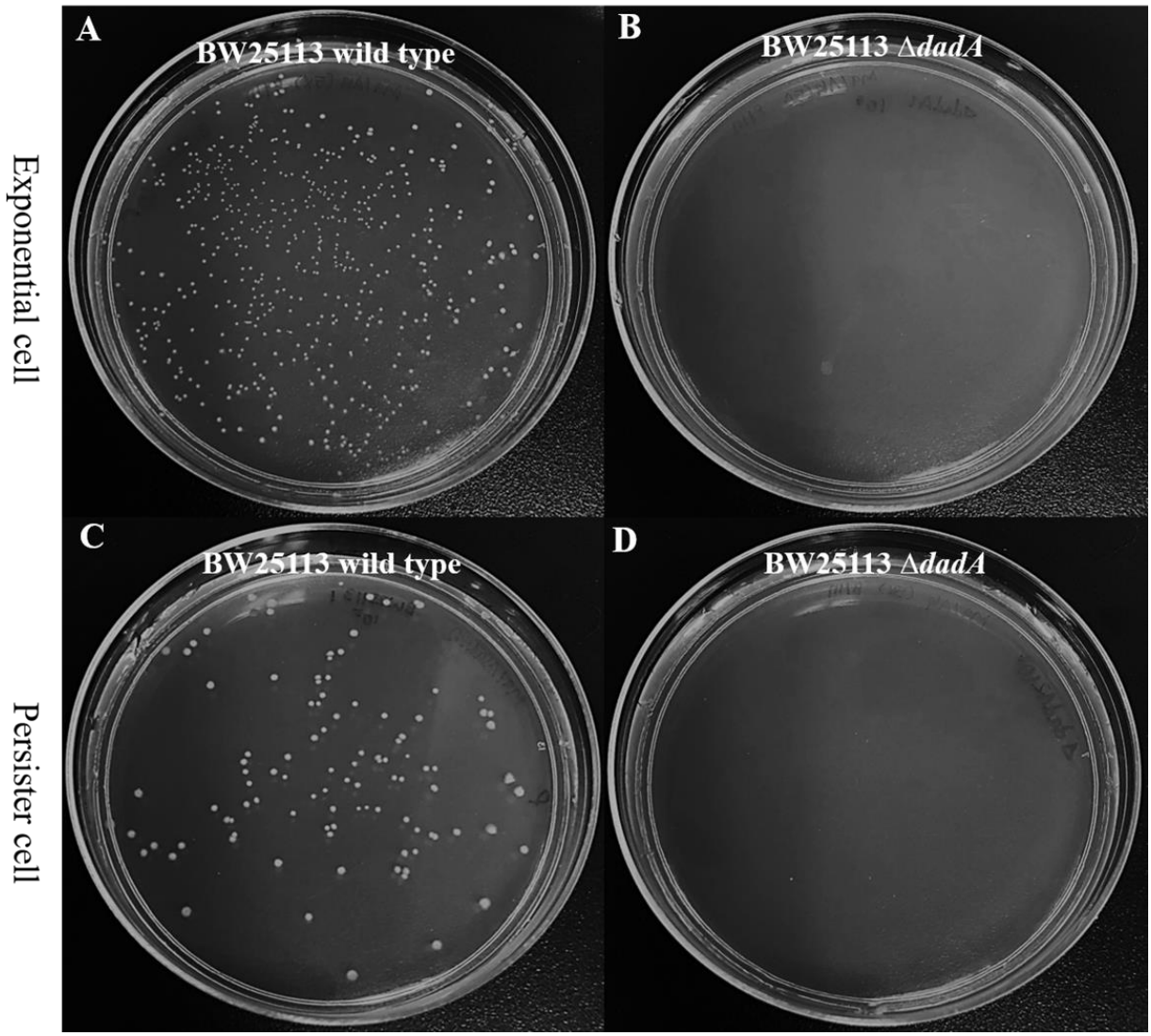
Ryota Yamasaki, Sooyeon Song, Michael J. Benedik, and Thomas K. Wood



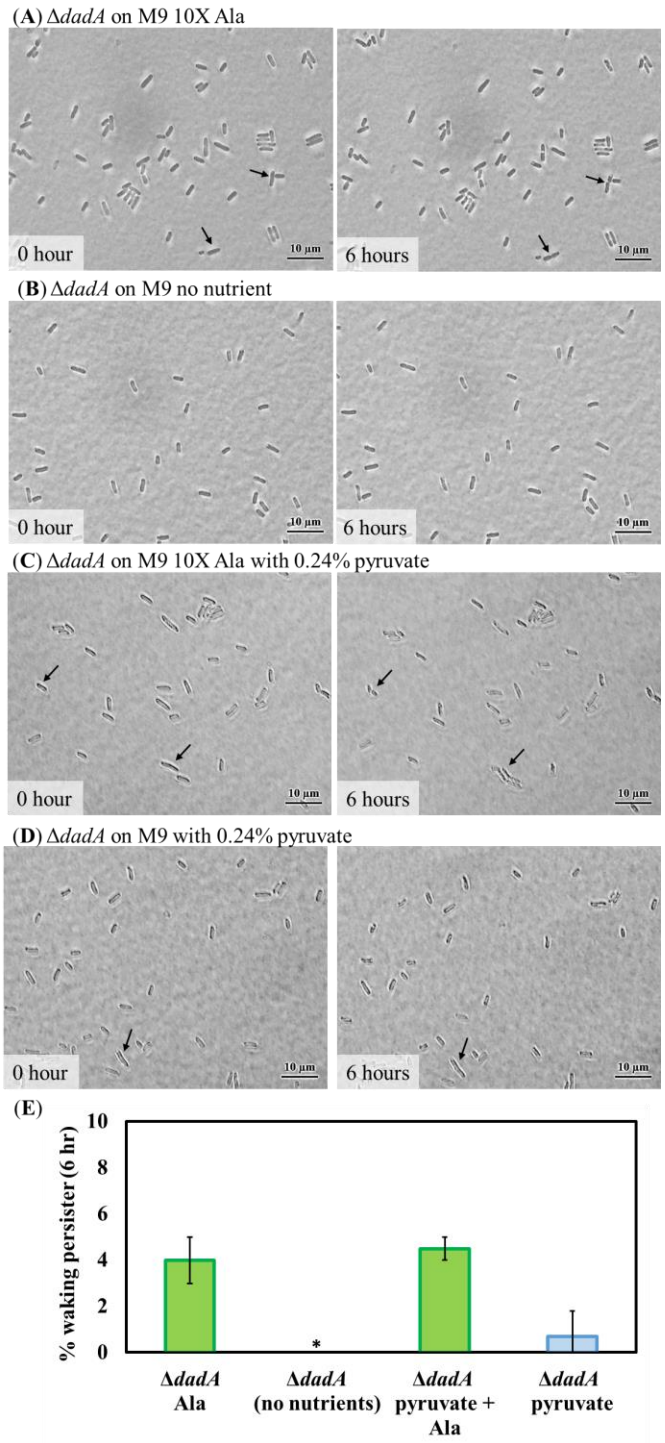
Supplementary Figure 1. Persister cells waking on agar plates with groups of amino acids, Related to Figure 1. *E. coli* BW25113 persister cells were incubated at 37 °C for 4 days on M9 agar plates with groups of five different amino acids: **(A) #1**: Arg, Ser, Cys, Ala, and Phe, **(B) #2**: His, Thr, Gly, Val, and Tyr, **(C) #3**: Lys, Asn, Pro, Ile, and Trp, **(D) #4**: Asp, Glu, Gln, Leu, and Met, and **(E)** no amino acids. These amino acids were used at 1X concentration (**Table S14**). One representative plate of two independent cultures is shown.



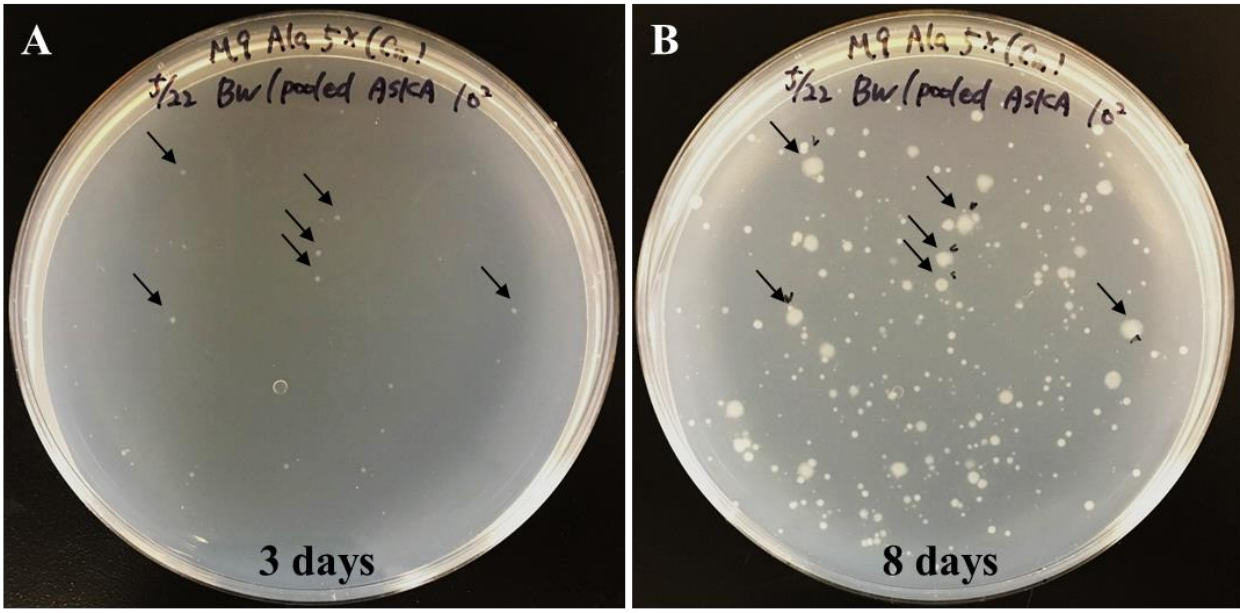
Supplementary Figure 2. Persister cells waking on agar plates with alanine, Related to Figure 1. *E. coli* BW25113 persister cells were incubated at 37 °C for 4 days on M9 agar plates with 10 different amino acids (Arg, Ser, Cys, Ala, Phe, Lys, Asn, Pro, Ile, and Trp). These amino acids were used at 1X concentration (**Table S14**). One representative plate of two independent cultures is shown.



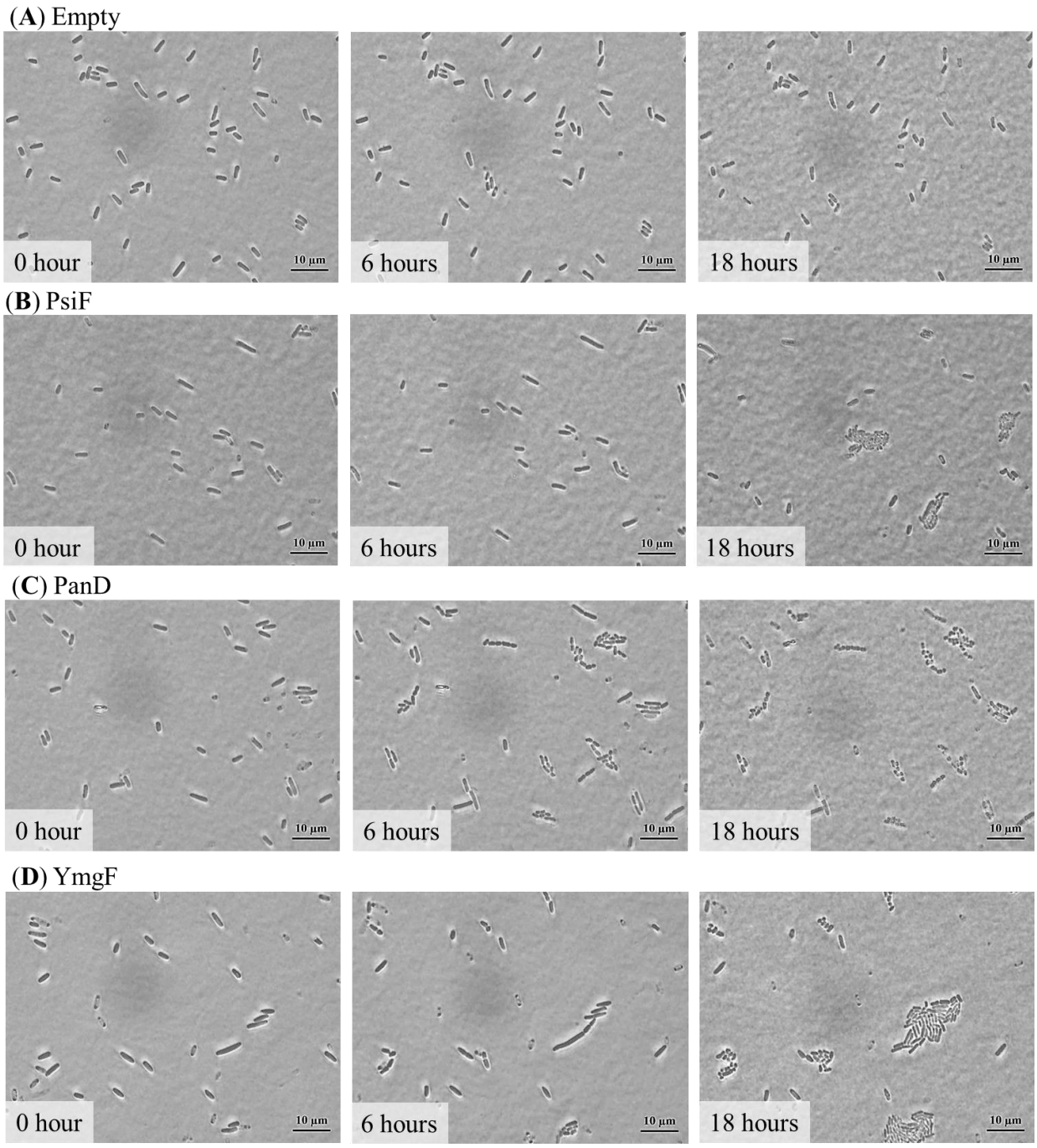
Supplementary Figure 3. Alanine is a waking signal, Related to Figure 2. Exponential wild-type *E. coli* BW25113 (**A**), exponential BW25113 Δ*dadA* (**B**), persister wild-type *E. coli* BW25113 (**C**), and persister BW25113 Δ*dadA* (**D**) incubated at 37 °C on M9 5X Ala agar plates for 3 days.



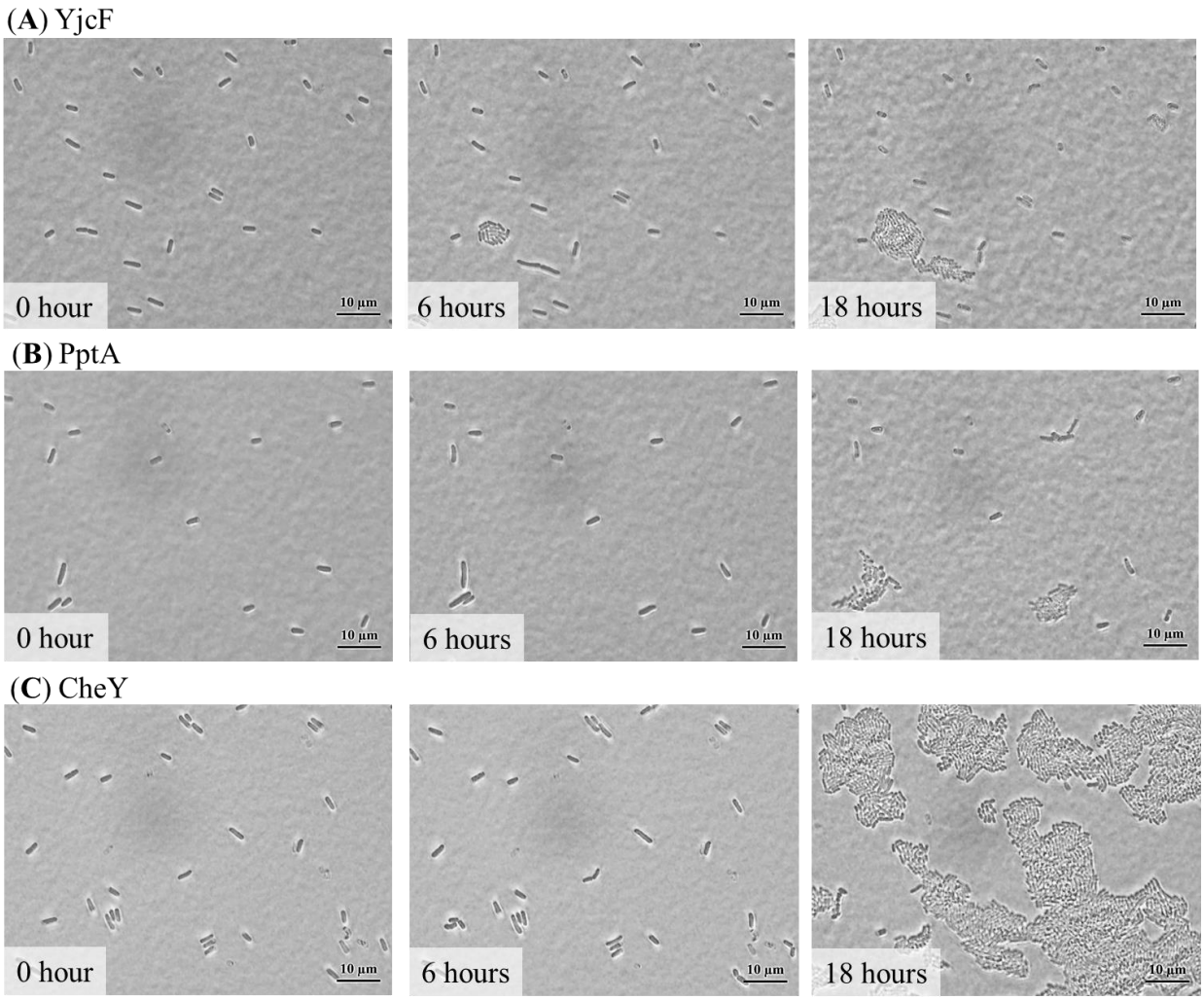
Supplementary Figure 4. Alanine is a waking signal, Related to Figure 2. Persister cells of BW25113 Δ*dadA* on M9 10X Ala gel pads (**A**), on M9 no nutrient gel pads (**B**), on M9 10X Ala with 0.24% pyruvate (**C**), and on M9 with 0.24% pyruvate (**D**) after 6 h at 37°C. Black arrows indicate cells that resuscitate. Scale bar indicates 10 µm. One representative plate of two independent cultures is shown. (**E**) *dadA* persister cell waking (%) after 6 hours. Tabulated cell numbers are shown in **Supplementary Table 3**. Error bars indicate standard deviations. Student's t-test was used to compare Δ*dadA* + Ala and Δ*dadA* (no nutrients) (* indicates a p value < 0.05).



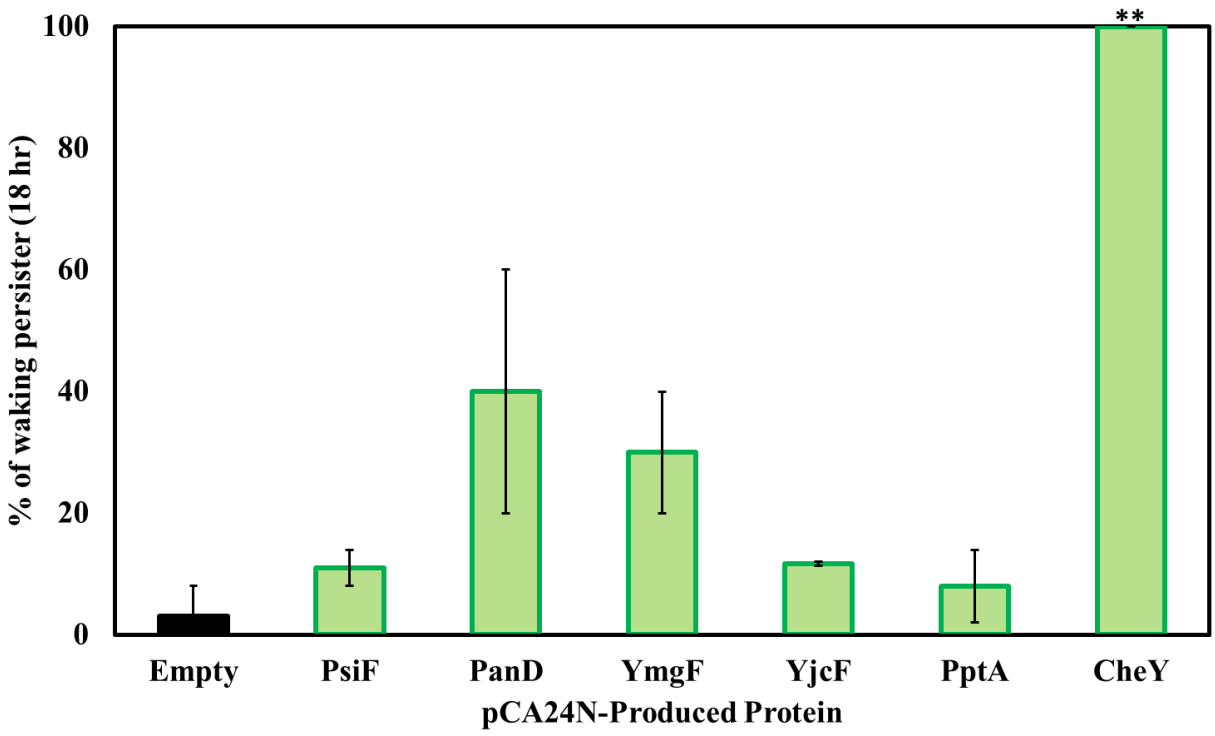
Supplementary Figure 5. Screening persister cell waking with the pooled ASKA plasmids on Ala, Related to Figure 3. *E. coli* BW25113 persisters containing each of the pooled ASKA plasmids were incubated at 37 °C on M9 5X Ala agar plates with 30 μg/mL Cm for (**A**) 3 days and (**B**) 8 days. Faster growing colonies are indicated with black arrows. Prior to forming persister cells, cultures were grown without IPTG with 30 μg/mL Cm. One representative plate of two independent cultures is shown.



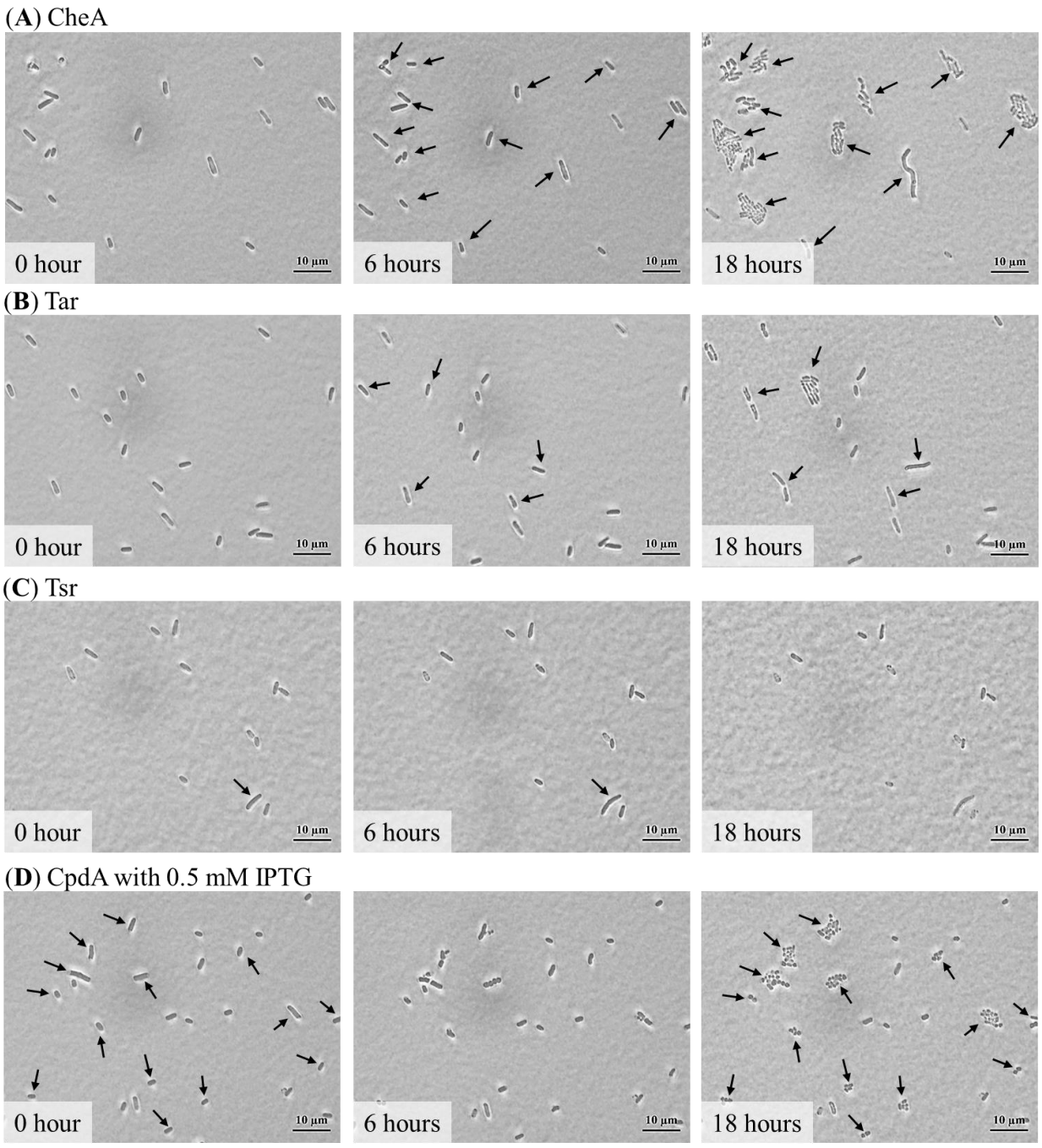
Supplementary Figure 6. Single persister cell waking on Ala related to the ASKA screen, Related to Figure 3. *E. coli* BW25113 persister cells containing (**A**) pCA24N (empty plasmid control), (**B**), pCA24N_*psiF*, (**C**) pCA24N_*panD*, and (**D**) pCA24N_*ymgF* were incubated at 37°C on M9 5X Ala agarose gel pads for 18 h. Scale bar indicates 10 µm. Representative results from two independent cultures are shown. Prior to forming persister cells, cultures were grown without IPTG with 30 µg/mL Cm.



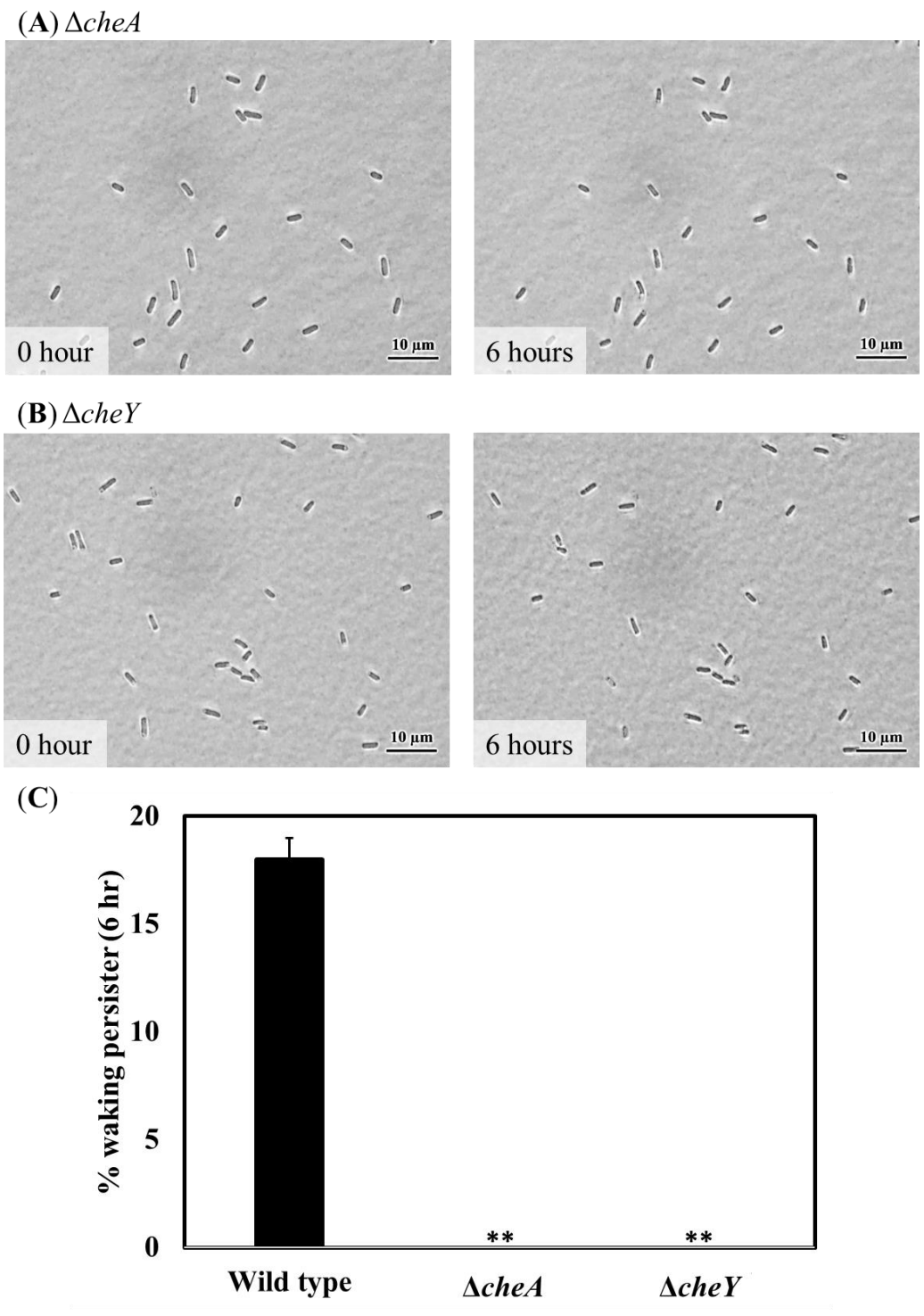
Supplementary Figure 7. Single persister cell waking on Ala related to the ASKA screen, Related to Figure 3. *E. coli* BW25113 persister cells containing (**A**) pCA24N_*yjfF*, (**B**), pCA24N_*pptA*, and (**C**) pCA24N_*cheY* were incubated at 37°C on M9 5X Ala agarose gel pads for 18 h. Scale bar indicates 10 µm. Representative results from two independent cultures are shown. Prior to forming persister cells, cultures were grown without IPTG with 30 µg/mL Cm.



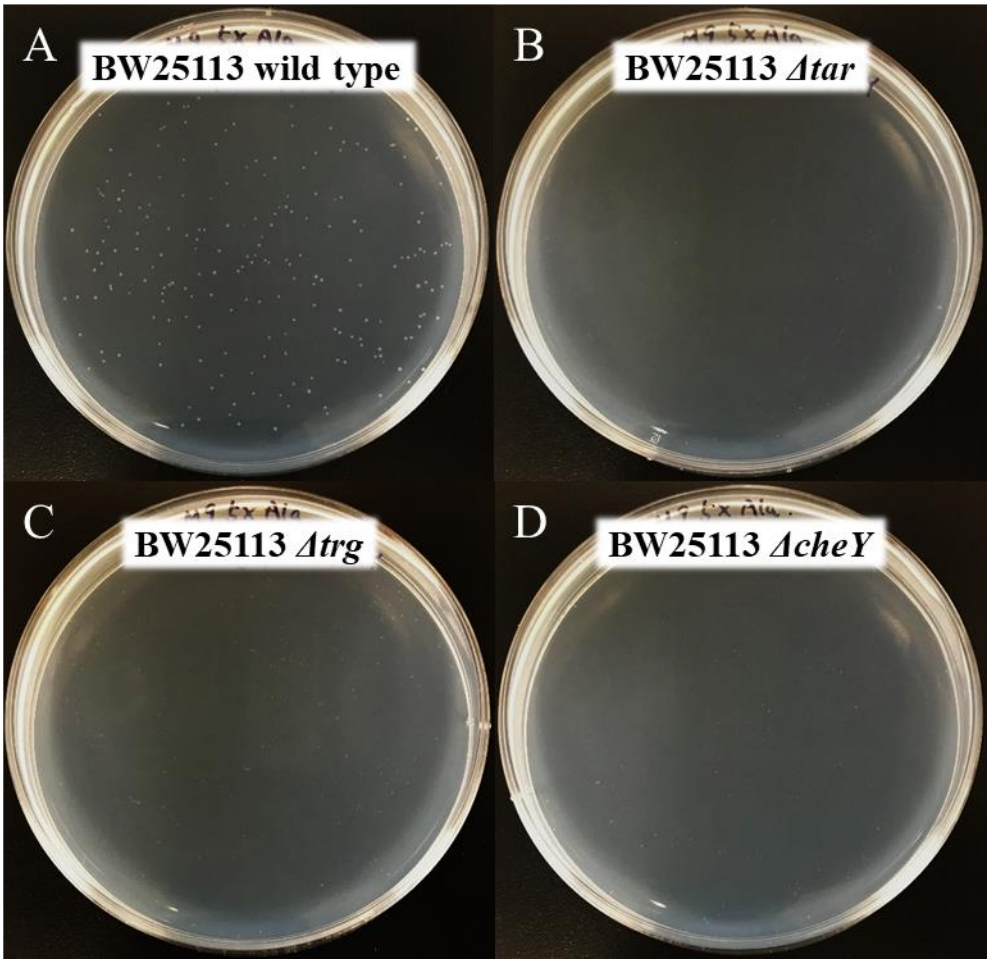
Supplementary Figure 8. Single persister cell waking on Ala related to the ASKA screen, Related to Figure 3. Persister cell waking (%) after 18 hours is shown. Error bars indicate standard deviations. Student's t-tests were used to compare two groups (** indicates a p value < 0.01).



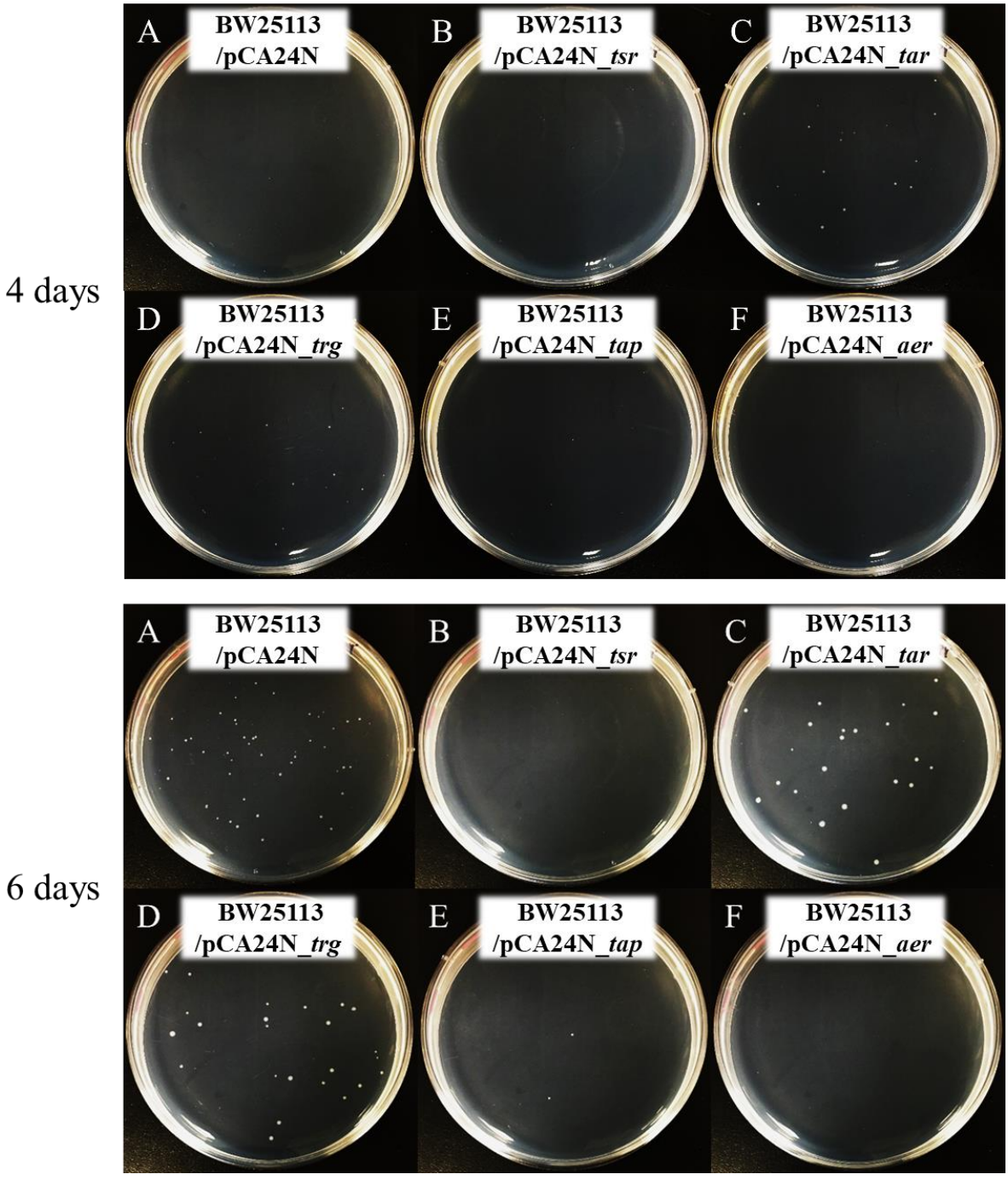
Supplementary Figure 9. Single persister cell waking on Ala after producing chemotaxis-related and cAMP-related proteins, Related to Figure 3. *E. coli* BW25113 persister cells containing (**A**) pCA24N_*cheA*, (**B**) pCA24N_*tar*, (**C**) pCA24N_*tsr*, and (**D**) pCA24N_*cpdA* induced by 0.5 mM IPTG were incubated at 37 °C on M9 5X Ala agarose gel pads for 18 h. Black arrows indicate cells that resuscitate. Scale bar indicates 10 µm. Representative results from two independent cultures are shown. Prior to forming persister cells, cultures were grown without IPTG with 30 µg/mL Cm. Tabulated cell numbers are shown in **Table S5**.



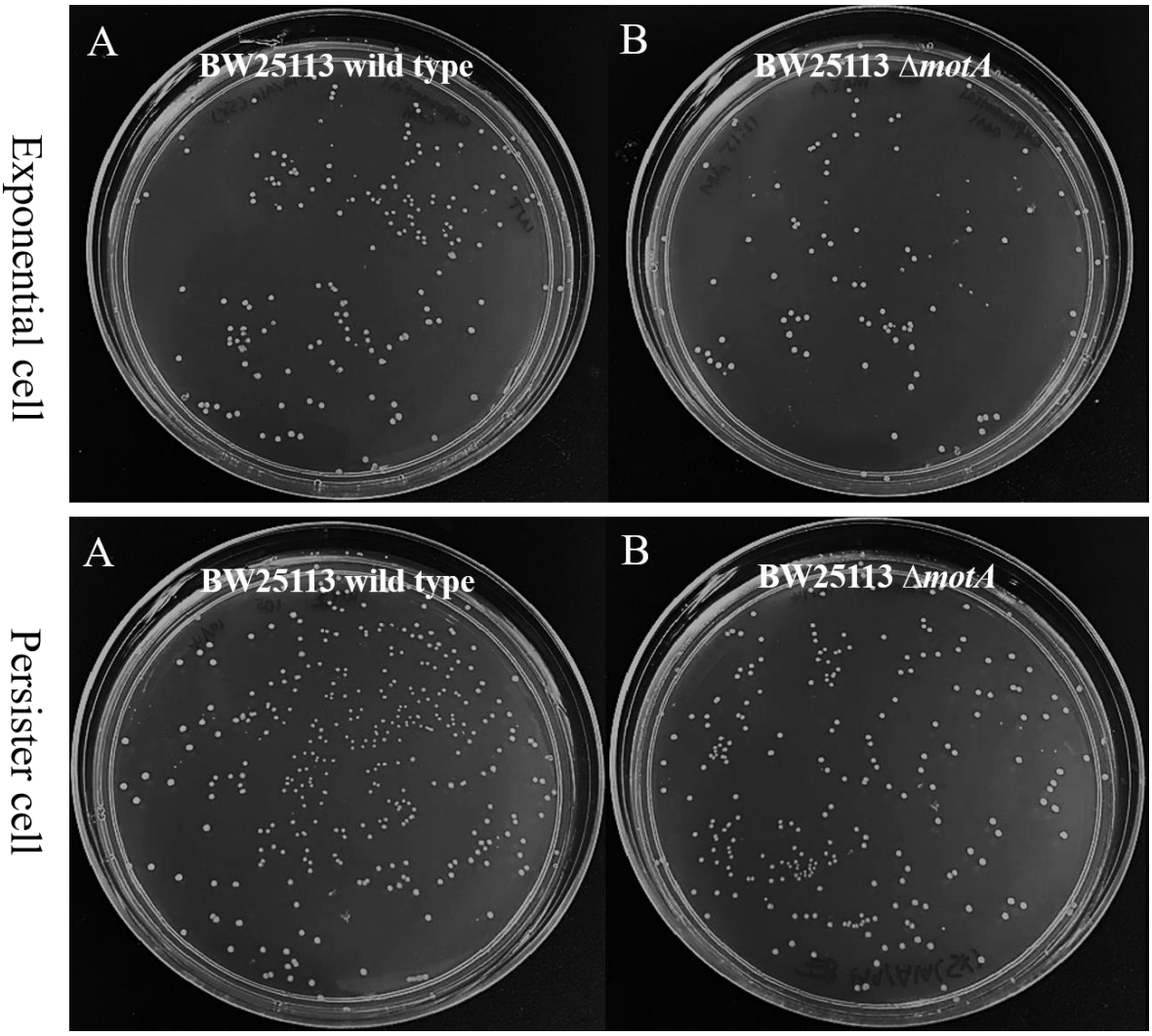
Supplementary Figure 10. Single persister cell waking on Ala after inactivating chemotaxis proteins CheA and CheY, Related to Figure 3. Persister cells of (**A**) BW25113 Δ*cheA* and (**B**) BW25113 Δ*cheY* waking on M9 5X Ala agarose gel pads after 6 h at 37°C. Scale bar indicates 10 µm. Representative results from two independent cultures are shown. (**C**) Persister cell waking (%) after 6 hours is shown. Error bars indicate standard deviations. Student's t-tests were used to compare two groups (** indicates a p value < 0.01).



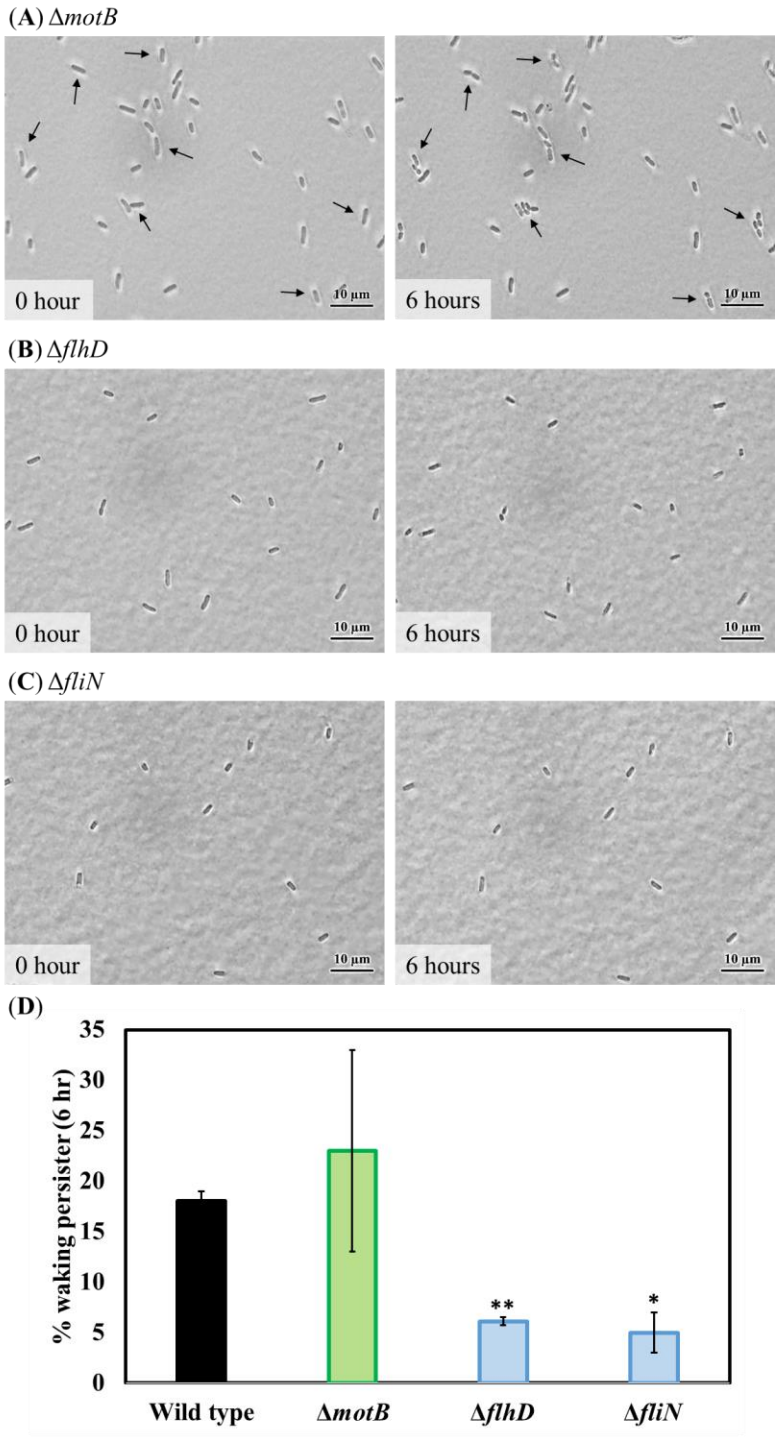
Supplementary Figure 11. Persister cell waking on Ala agar plates after inactivating the chemotaxis-related proteins Tar, Trg, and CheY, Related to Figure 3. Persister cells were incubated at 37 °C on M9 5X Ala agar plates for 2 days. (**A**) wild type BW25113, (**B**) BW25113 Δ*tar*, (**C**) BW25113 Δ*trg*, and (**D**) BW25113 Δ*cheY*. One representative plate of two independent cultures is shown.



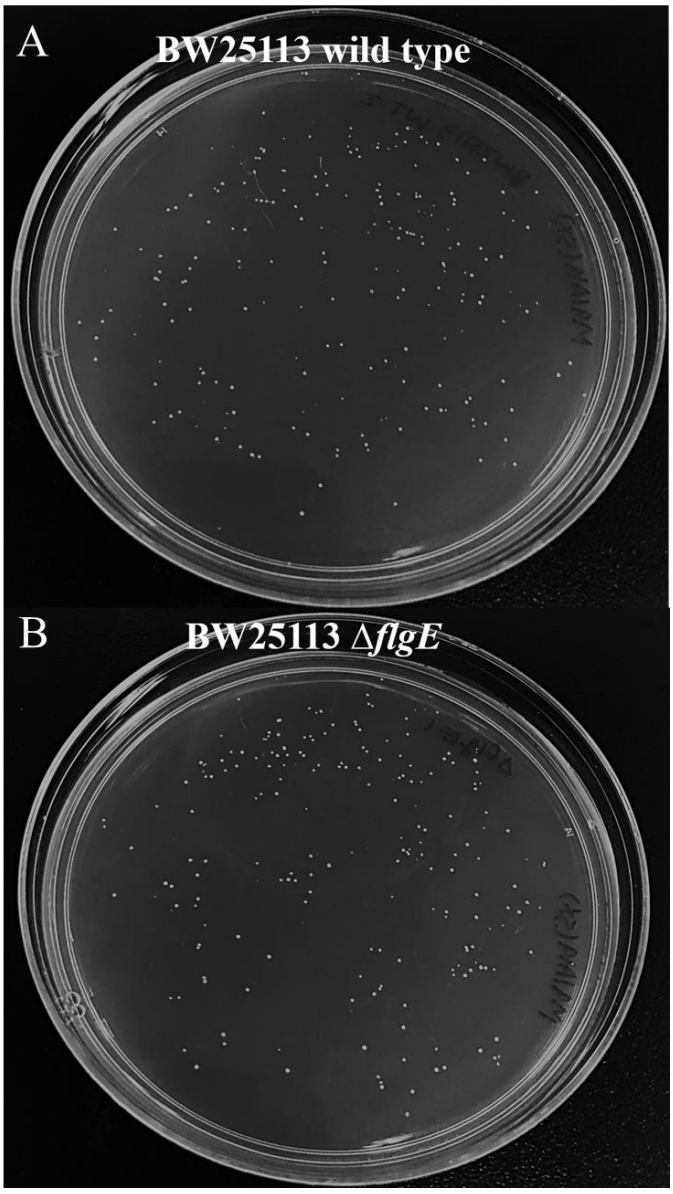
Supplementary Figure 12. Persister cell waking on Ala agar plates after producing chemotaxis proteins, Related to Figure 3. Persister waking after 4 days (upper panel) and after 6 days (lower panel) incubation at 37 °C on M9 5X Ala agar plates. (**A**) BW25113/pCA24N, (**B**) BW25113/pCA24N_*tsr*, (**C**) BW25113/pCA24N_*tar*, (**D**) BW25113/pCA24N_*trg*, (**E**) BW25113/pCA24N_*tap*, and (**F**) BW25113/pCA24N_*aer*. Prior to forming persister cells, cultures were grown without IPTG with 30 μg/mL Cm. One representative plate of two independent cultures is shown.



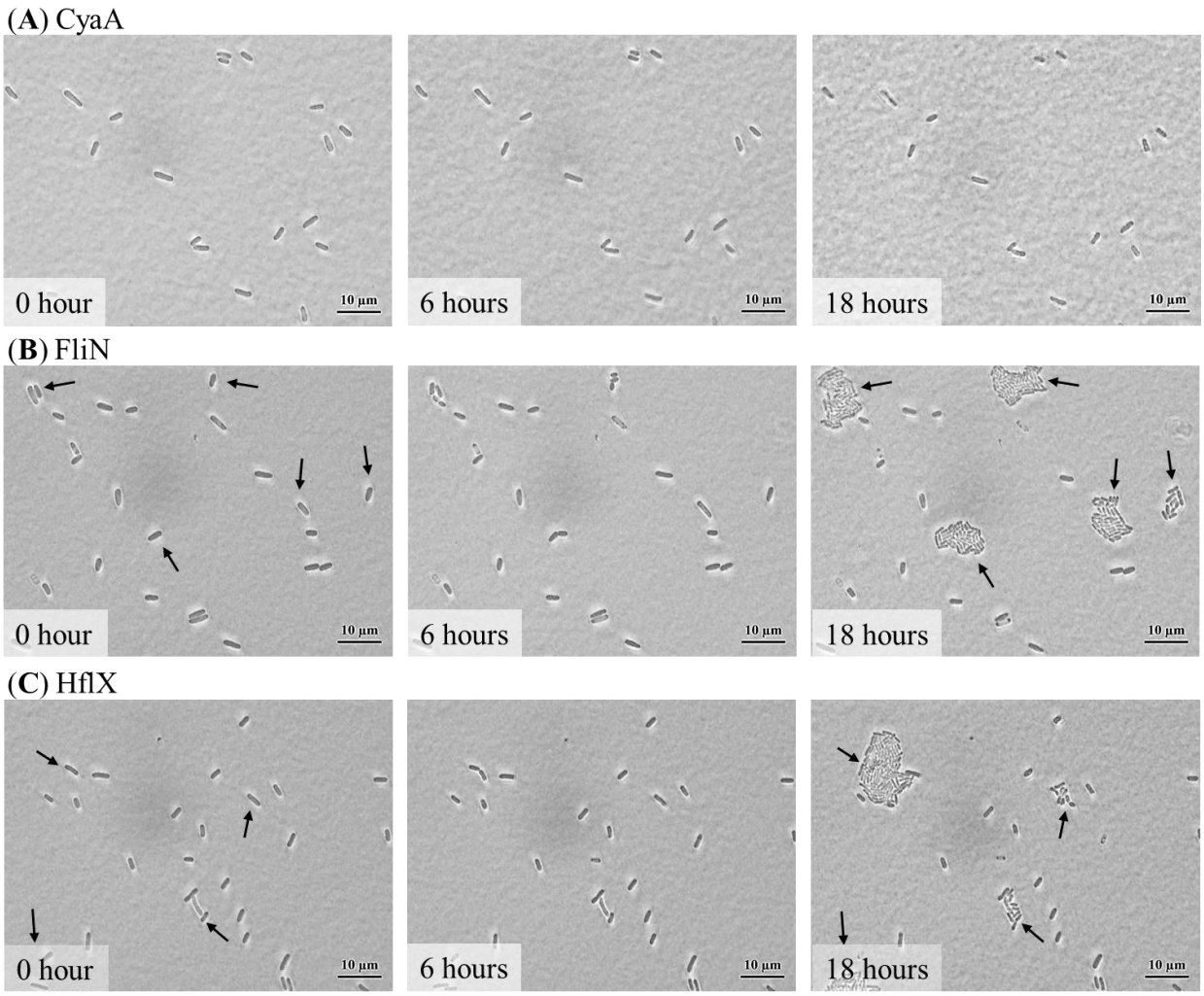
Supplementary Figure 13. Persister cell waking on Ala agar plates after inactivating flagellar motor complex proteins MotA, Related to Figure 3. Cells were incubated at 37 °C on M9 5X Ala agar plates for 3 days. **Upper panel**: Exponential cells (**A**) wild type BW25113 and (**B**) BW25113 Δ*motA*. **Lower panel**: Persister cells of (**A**) wild type BW25113 and (**B**) BW25113 Δ*motA*. One representative plate of two independent cultures is shown.



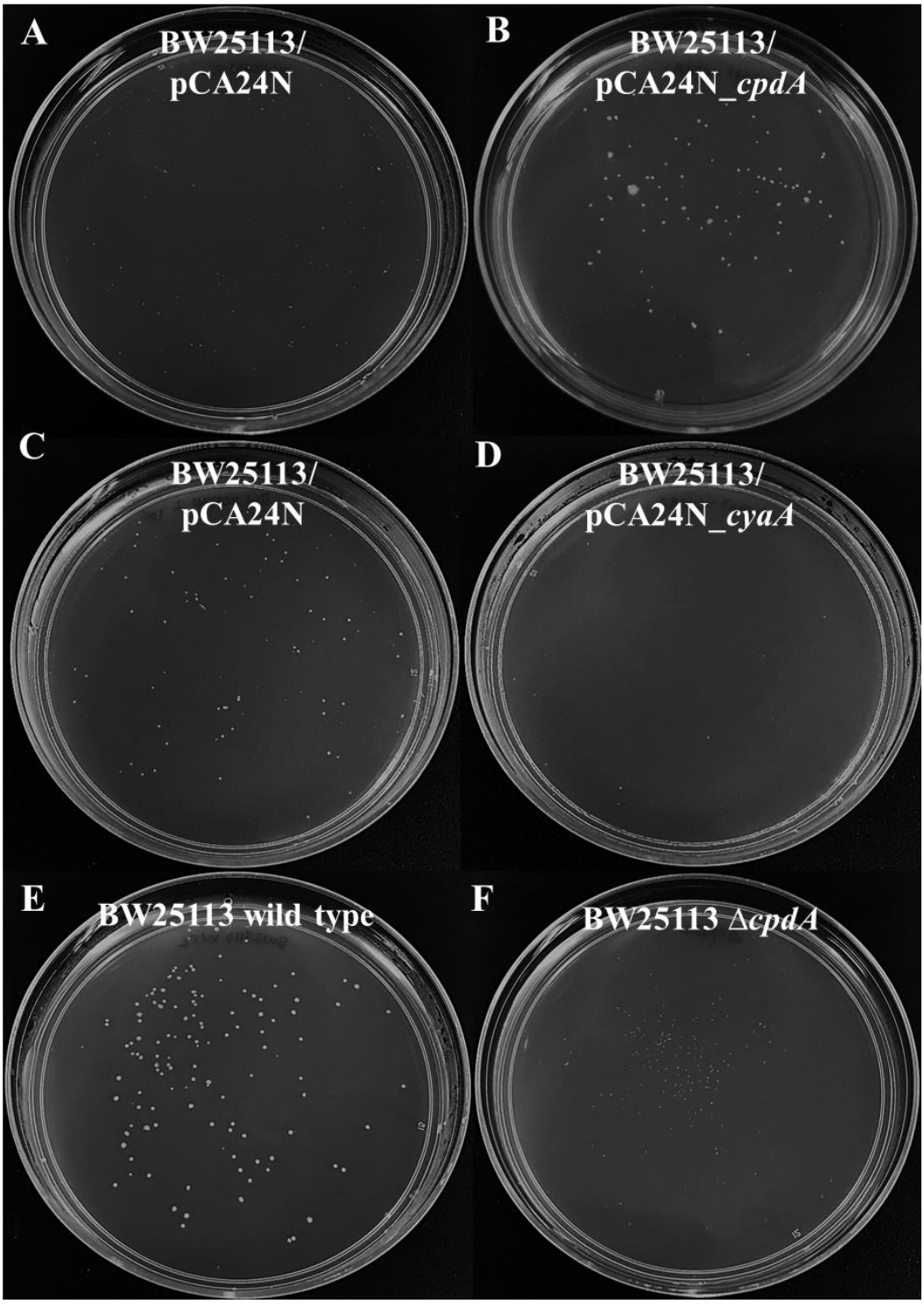
Supplementary Figure 14. Single persister cell waking on Ala after inactivating flagellar proteins MotB, FhlD, and FliN, Related to Figure 3. Persister cells of (**A**) BW25113 Δ*motB*, (**B**) BW25113 Δ*flhD*, and (**C**) BW25113 Δ*fliN* waking on M9 5X Ala agarose gel pads after 6 h at 37°C. Black arrows indicate cells that resuscitate. Scale bar indicates 10 µm. Representative results from two independent cultures are shown. (**D**) Persister cell waking (%) after 6 hours is shown. Error bars indicate standard deviations. Student's t-tests were used to compare two groups (* indicates a p value < 0.05 and ** indicates a p value < 0.01). Tabulated cell numbers are shown in **Table S7**.



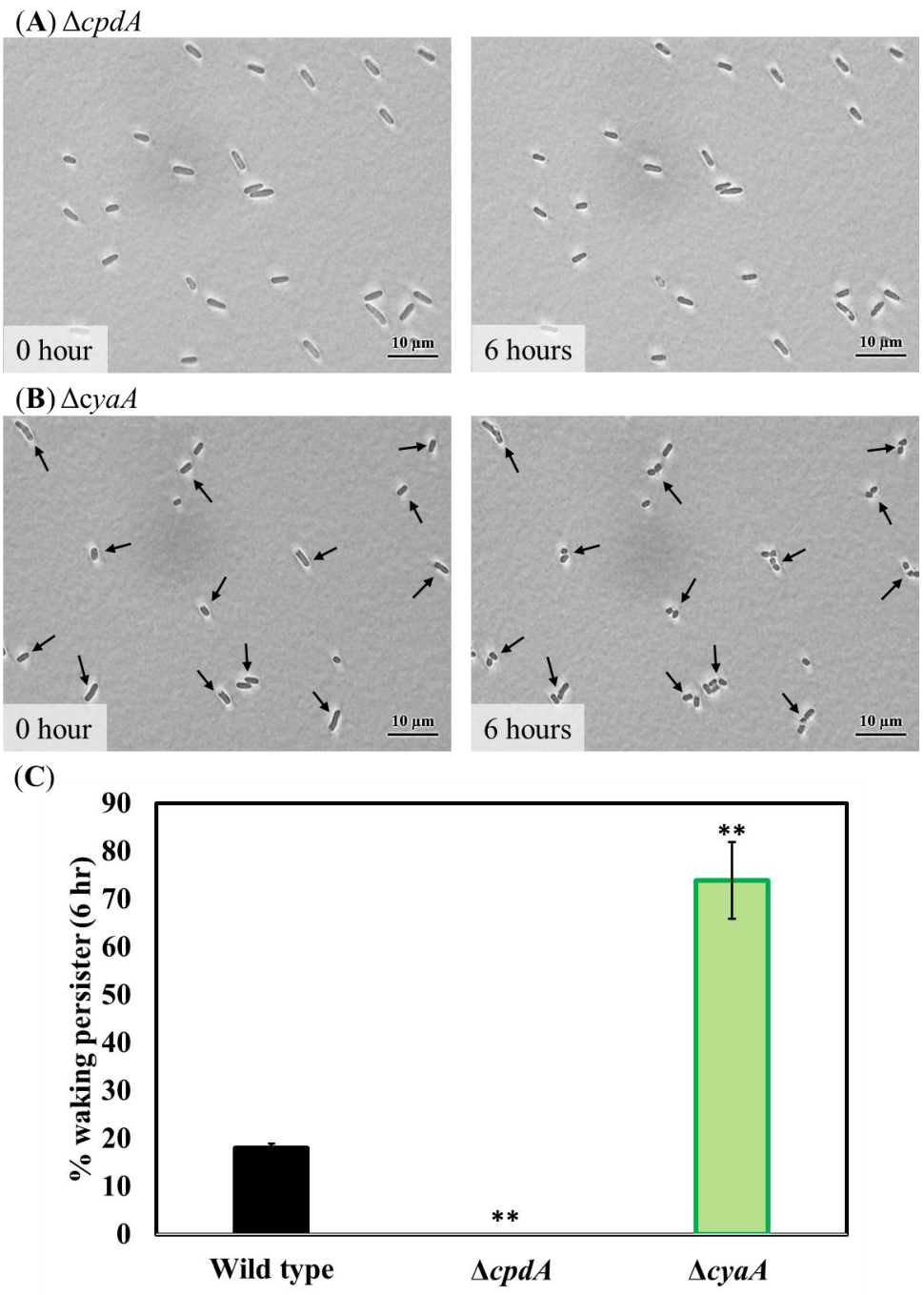
Supplementary Figure 15. Persister cell waking on Ala agar plates after inactivating the FlgE flagellum hook protein, Related to Figure 3. *E. coli* BW25113 persister cells of (**A**) wild type BW25113 and (**B**) BW25113 Δ*flgE* were incubated at 37 °C on M9 5X Ala agar plates for 2 days. One representative plate of two independent cultures is shown.



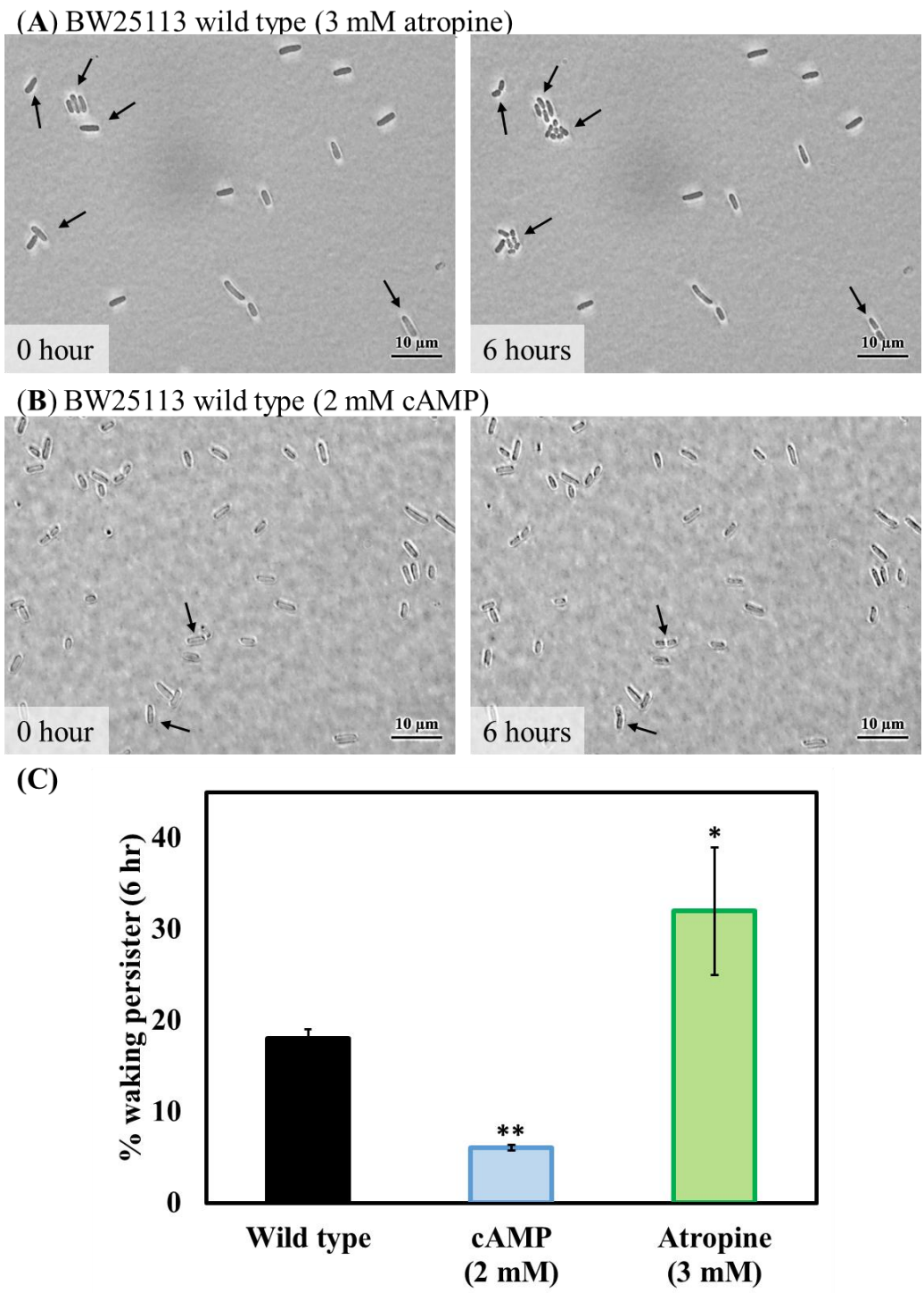
Supplementary Figure 16. Single persister cell waking on Ala after producing cAMP-related, flagella-related, and ribosome-resuscitation-related proteins, Related to Figure 3. *E. coli* BW25113 persister cells containing (**A**) pCA24N_*cyaA*, (**B**) pCA24N_*fliN*, and (**C**) pCA24N_*hflX* were incubated at 37 °C on M9 5X Ala agarose gel pads for 18 h. Black arrows indicate cells that resuscitate. Scale bar indicates 10 µm. Representative results from two independent cultures are shown. Prior to forming persister cells, cultures were grown without IPTG with 30 µg/mL Cm. Tabulated cell numbers are shown in **Table S5**.



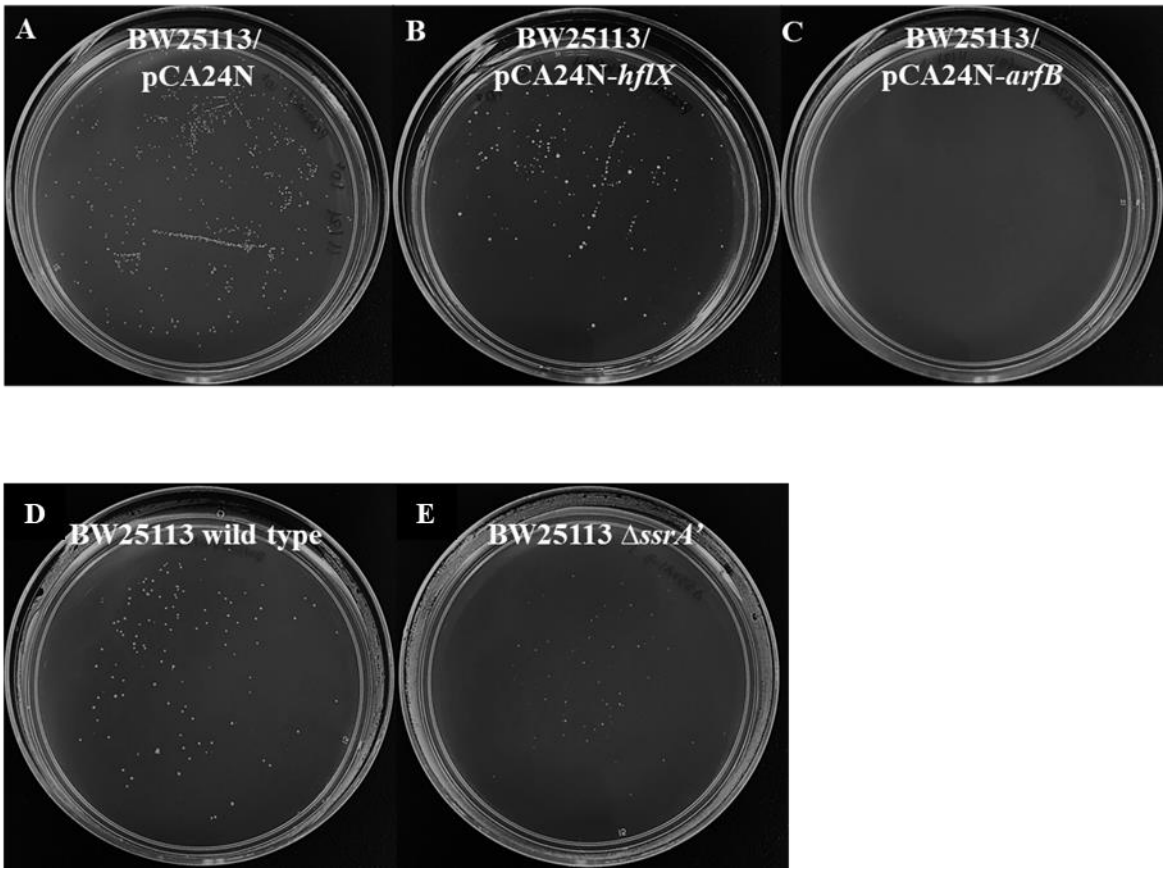
Supplementary Figure 17. Persister cell waking on Ala agar plates after inactivating or producing proteins related cAMP, Related to Figure 3. *E. coli* BW25113 persister cells with (**A**) pCA24N after 4 days, (**B**) pCA24N_*cpdA* after 4 days, **(C)** pCA24N after 5 days, **(D)** pCA24N_*cyaA* after 5 days, (**E**) wild type BW25113 after 3 days, and **(F)** BW25113 Δ*cpdA* after 3days. Plates were incubated at 37 °C and contain M9 5X Ala. One representative plate of two independent cultures is shown.



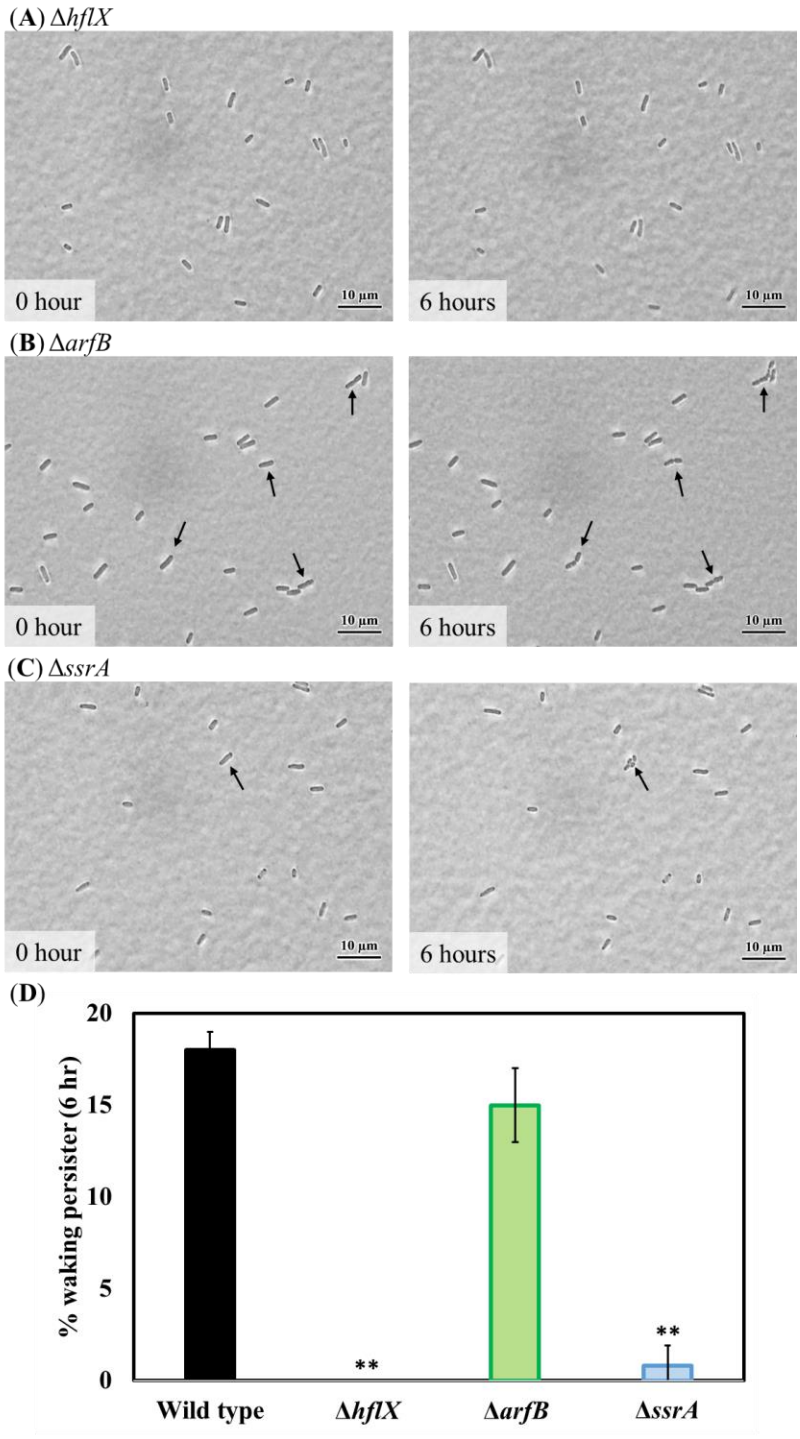
Supplementary Figure 18. Single persister cell waking on Ala after inactivating cAMP-related proteins CpdA and CyaA, Related to Figure 3. Persister cells of (**A**) BW25113 Δ*cpdA* and (**B**) BW25113 Δ*cyaA* waking on M9 5X Ala agarose gel pads after 6 h at 37°C. Scale bar indicates 10 µm. Representative results from two independent cultures are shown. (**C**) Persister cell waking (%) after 6 hours is shown. Error bars indicate standard deviations. Student's t-tests were used to compare two groups (** indicates a p value < 0.01). Tabulated cell numbers are shown in **Table S8**.



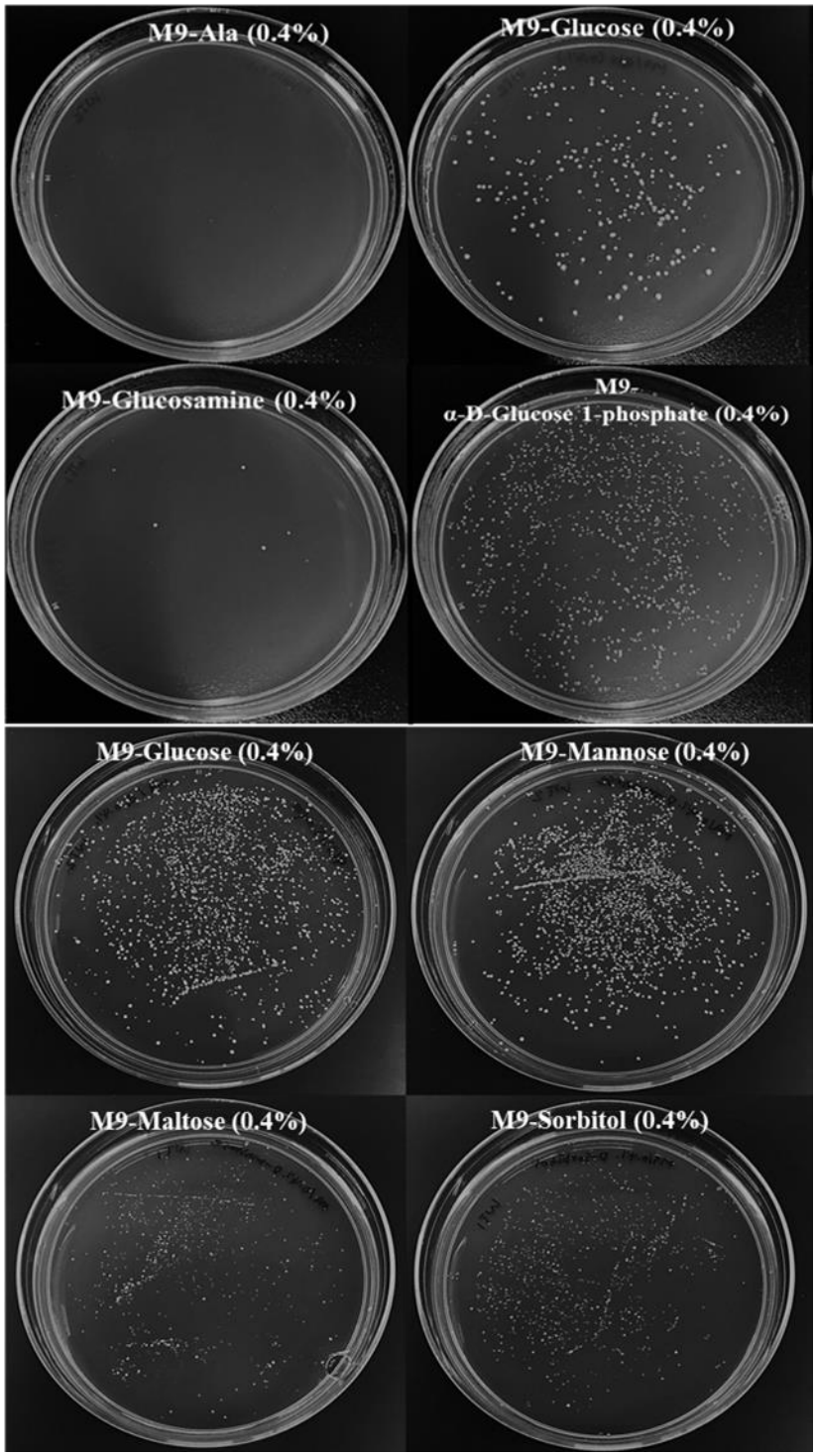
Supplementary Figure 19. Single persister cell waking on Ala with exogenous atropine and cAMP, Related to Figure 3. Persister cells of wild-type BW25113 waking on M9 5X Ala on agarose gel pads with (**A**) 3 mM atropine or (**B**) 2 mM cAMP after 6 h at 37°C. Black arrows indicate cells that resuscitate. Scale bar indicates 10 µm. Representative results from two independent cultures are shown. (**C**) Persister cell waking (%) after 6 hours is shown. Error bars indicate standard deviations. Student's t-tests were used to compare two groups (* indicates a p value < 0.05 and ** indicates a p value < 0.01).



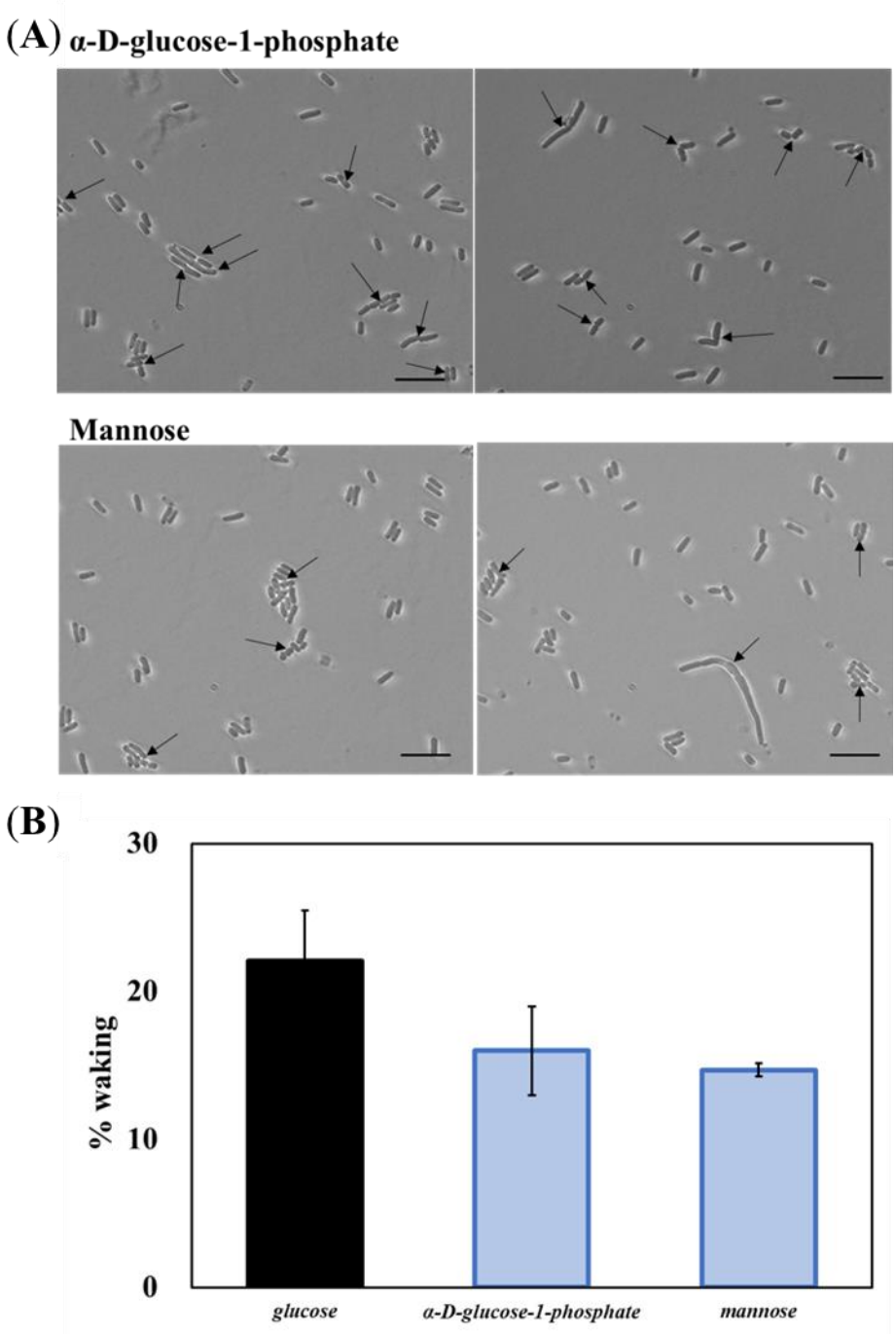
Supplementary Figure 20. Persister cells waking on Ala agar plates after producing HflX and ArfB and inactivating SsrA, Related to Figure 3. *E. coli* BW25113 persister cells with (**A**) empty plasmid, (**B**) pCA24N_*hflX*, and (**C**) pCA24N_*arfB* were incubated at 37 °C on M9 5X Ala agar plates for 6 days. *E. coli* BW25113 persister cells with (**D**) wild type and (**E**) BW25113 Δ*ssrA'*. One representative plate of two independent cultures is shown.



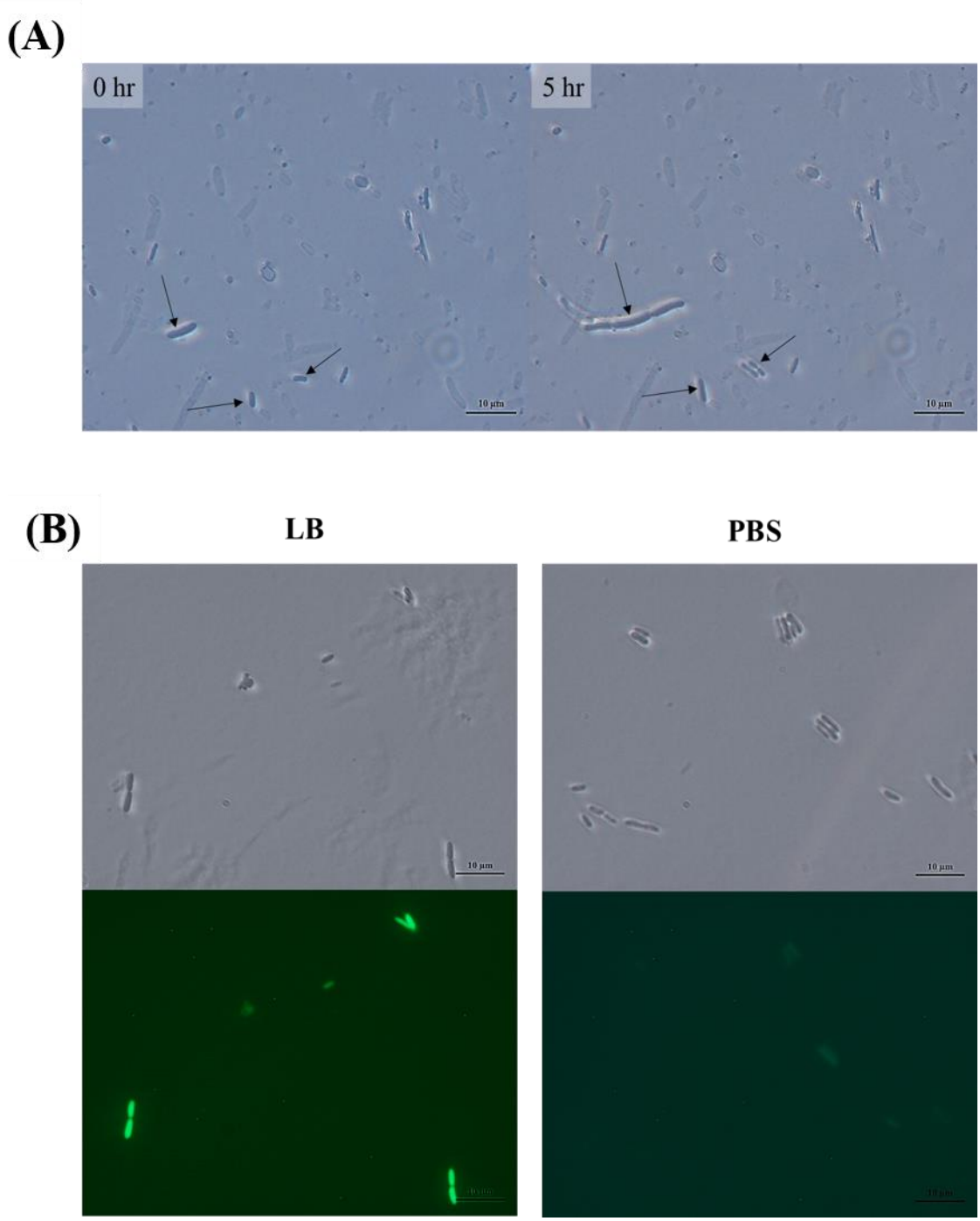
Supplementary Figure 21. Single persister cell waking on Ala after inactivating ribosome rescue proteins, Related to Figure 3. Persister cells of (**A**) BW25113 Δ*hflX*, (**B**) BW25113 Δ*arfB*, and (**C**) BW25113 Δ*ssrA*, waking on M9 5X Ala agarose gel pads after 6 h at 37°C. Black arrows indicate cells that resuscitate. Scale bar indicates 10 µm. Representative results from two independent cultures are shown. (**D**) Persister cell waking (%) after 6 hours is shown. Error bars indicate standard deviations. Student's t-tests were used to compare two groups (** indicates a p value < 0.01). Tabulated cell numbers are shown in **Table S9**.



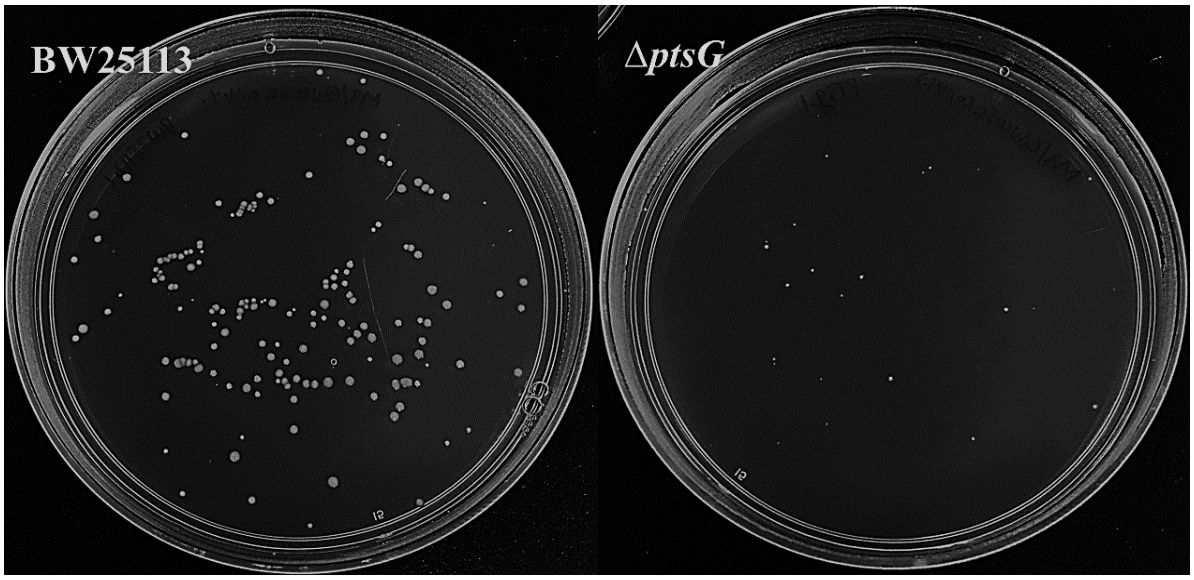
Supplementary Figure 22. Persister cell waking on agar plates with polysaccharides as the sole carbon source, Related to Figure 4. Persister cell waking of the wild type strain on M9 agar plates with alanine (0.4%), glucose (0.4%), glucosamine (0.4%) (*D*-glucosamine + *N*-acetyl-*D*-glucosamine), α-*D*-glucose 1-phosphate (0.4%), mannose (0.4%), maltose (0.4%), and sorbitol (0.4%). Plates were incubated at 37°C for 3 days. One representative plate of two independent cultures is shown.



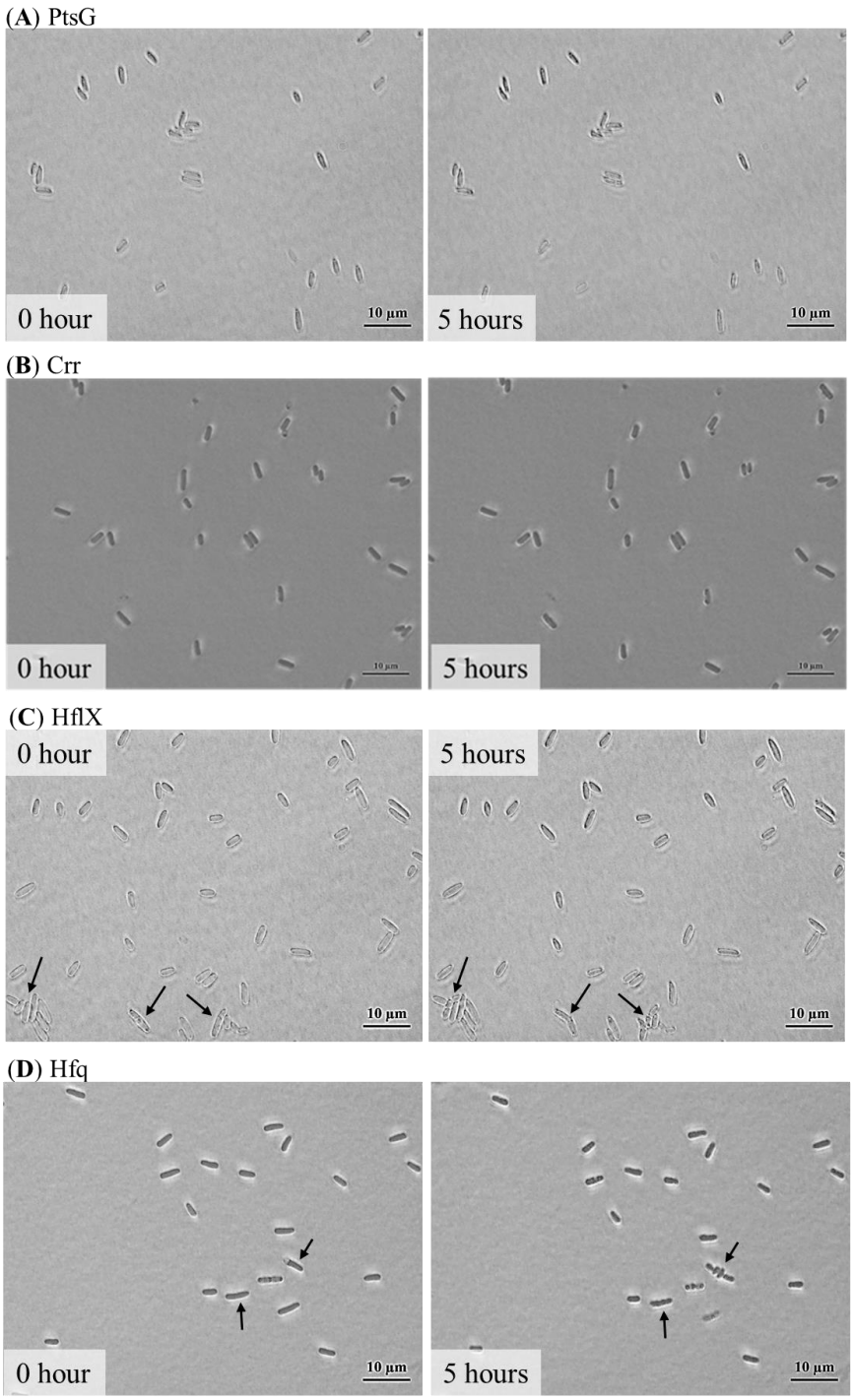
Supplementary Figure 23. Persister cell waking on agar plates with polysaccharides as the sole carbon source, Related to Figure 4. (**A**) Persister cell waking of the wild type strain on M9 0.4% α-*D*-glucose 1-phosphate and 0.4% mannose agarose gel pads after 3 h at 37°C. Black arrows indicate cells that resuscitate. Scale bar indicates 10 µm. Representative results from two independent cultures are shown. (**B**) Persister cell waking (%) on the three best sugars (glucose, α-*D*-glucose 1-phosphate, mannose) after 3 h. Error bars indicate standard deviations.



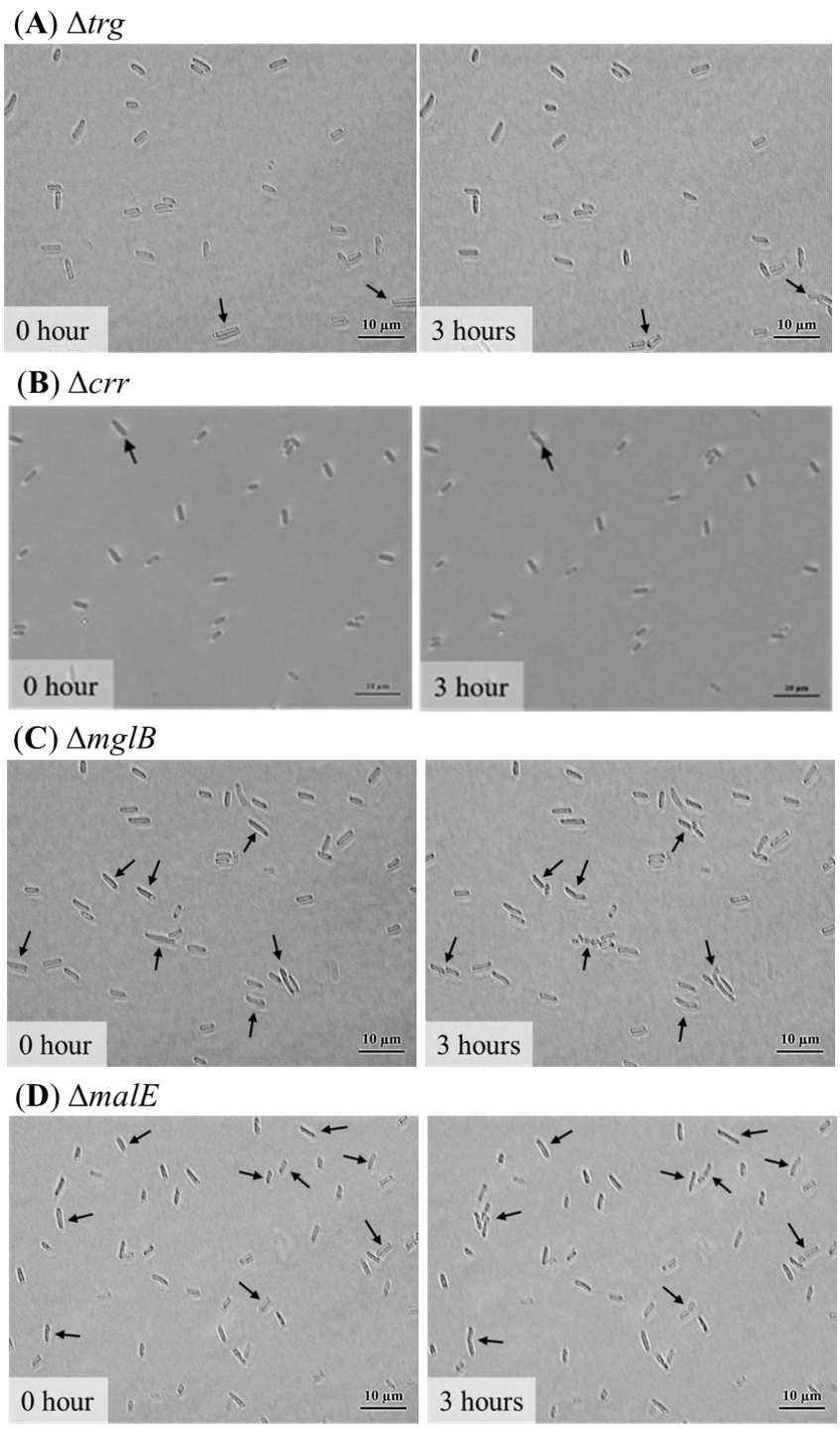
Supplementary Figure 24. Biofilm persister cells waking via ribosome activation induced by nutrients, Related to Figure 4. (**A**) Persister cells harvested from biofilms of *E. coli* BW25113/pCA24N waking on a M9 0.4% glucose agar gel pad after 5 h at 37°C. Black arrows indicate cells that resuscitate. (**B**) Active 70S ribosomes in single persister cells for MG1655-ASV on LB (left) and PBS (light). Cells are shown on LB agarose pads, and PBS gel pads after 1 h at 37°C. The **upper panel** indicates dark field and the **lower panel** indicates shows GFP fluorescence. Scale bars indicate 10 µm. Representative results from two independent cultures are shown



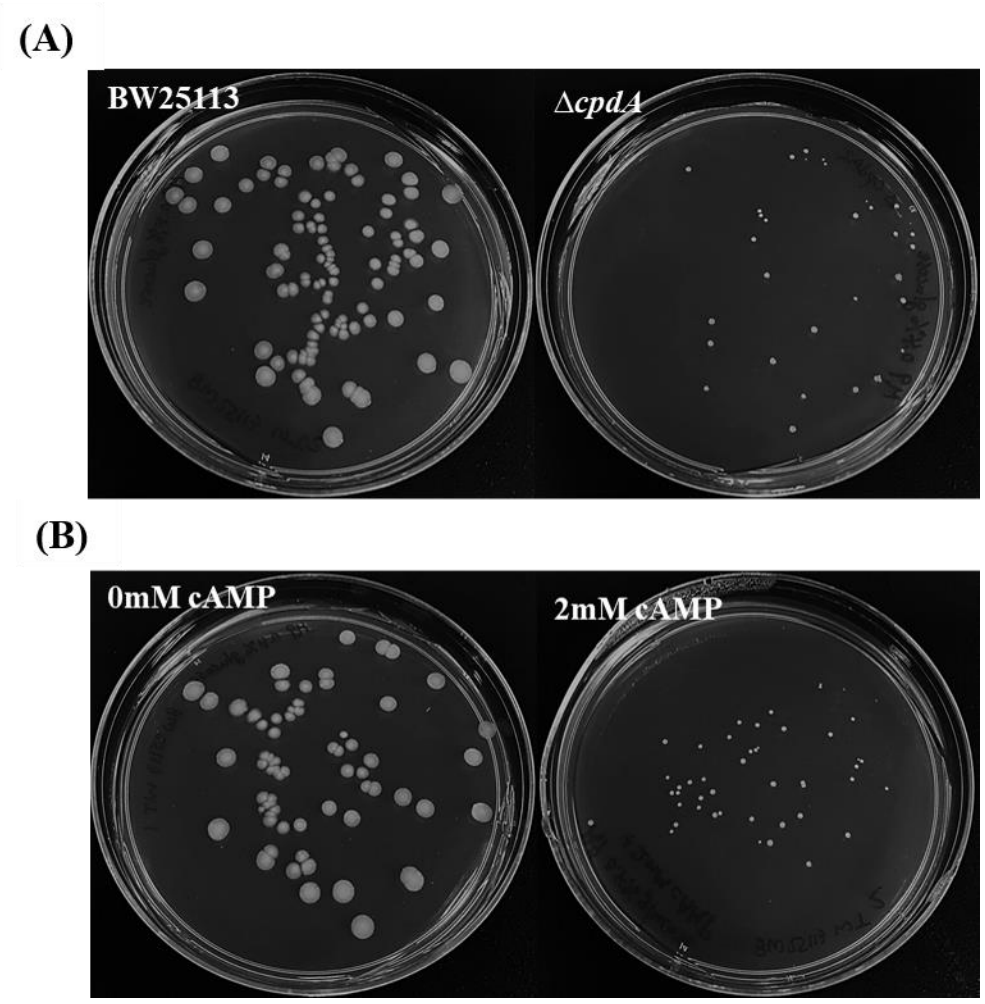
Supplementary Figure 25. Persister cell waking on glucose agar plates after inactivating PtsG, Related to Figure 5. Persister cell waking of the wild type (left panel) and the Δ*ptsG* mutant (right panel) on M9 0.4% glucose agar plates incubated at 37 °C for 2 days. One representative plate of two independent cultures is shown.



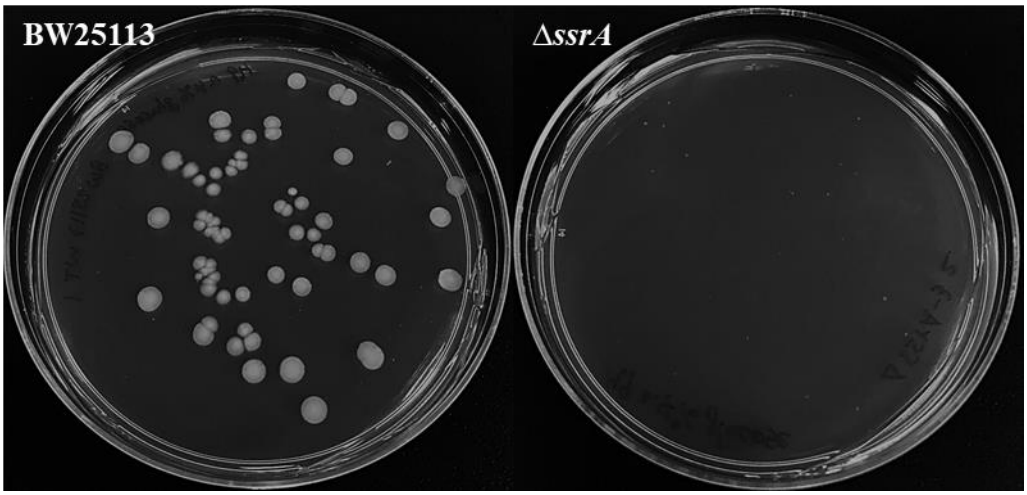
Supplementary Figure 26. Single persister cell waking on glucose after producing proteins related to transport, ribosome resuscitation, and sRNA, Related to Figure 6. *E. coli* BW25113 persister cells containing (**A**) pCA24N_*ptsG*, (**B**) pCA24N_*crr*, (**C**) pCA24N_*hflX*, and (**D**) pCA24N_*hfq* were incubated at 37 °C on M9 0.4% glucose agarose gel pads for 5 h. Black arrows indicate cells that resuscitate. Scale bar indicates 10 µm. Representative results from two independent cultures are shown. Prior to forming persister cells, cultures were grown without IPTG with 30 µg/mL Cm. Tabulated cell numbers are shown in **Table S12**.



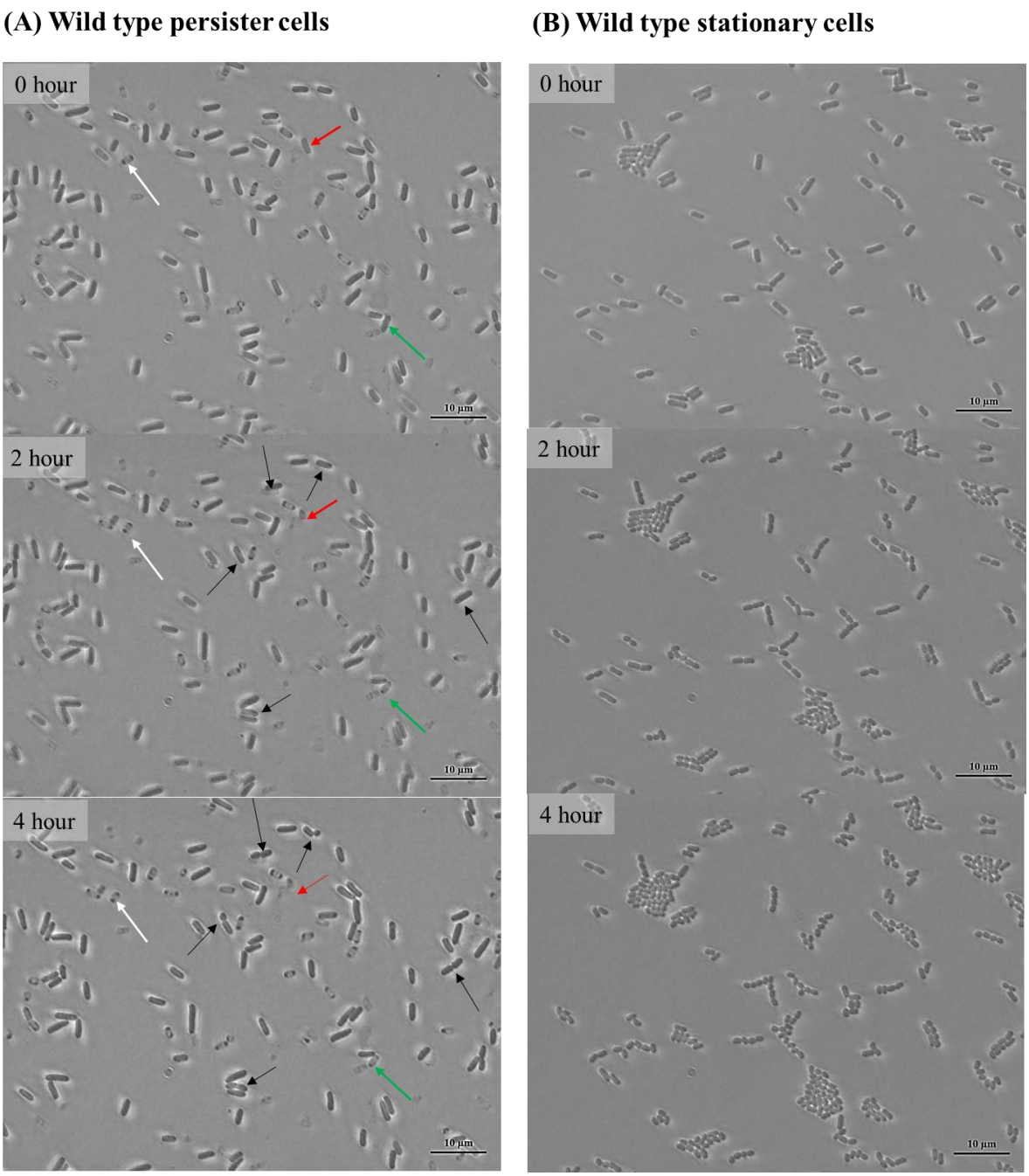
Supplementary Figure 27. Single persister cell waking on glucose after inactivating proteins related to transport, Related to Figure 5. *E. coli* BW25113 persister cells isogenic deletion mutants (**A**) Δ*trg*, (**B**) Δ*crr*, (**C**) Δ*mglB*, and (**D**) Δ*malE* were incubated at 37 °C on 0.4% glucose agarose gel pads for 3 h. Black arrows indicate cells that resuscitate. Scale bar indicates 10 µm. Representative results from two independent cultures are shown. Tabulated cell numbers are shown in **Table S11**.



Supplementary Figure 28. Persister cell waking on glucose agar plates after increasing cAMP, Related to Figure 5. Persister cell waking of (**A**) wild type and the Δ*cpdA* mutant and (**B**) wild type with 0 mM and 2 mM cAMP on M9 0.4% glucose agar plates incubated at 37 °C on for 2 days. One representative plate of two independent cultures is shown.



Supplementary Figure 29. Persister cell waking on glucose agar plates for Δ*ssrA*, Related to Figure 5. Persister cell waking for the wild type (left panel) and the Δ*ssrA* mutant (right panel) on M9 0.4% glucose agar plates incubated at 37 °C for 2 days. One representative plate of two independent cultures is shown.



Supplementary Figure 30. Stationary-phase cells revive differently than persister cells, Related to Figure 5 and Figure 6. Persister cell waking (**A**) and revival of stationary-phase cells (turbidity 2.0 at 600 nm) (**B**) for the wild type strain on M9 0.4% glucose agar plates incubated at 37°C for 4 hours. For the stationary-phase cells, 98 ± 2% of the cells begin growing immediately. For the persister cells, black arrows indicate all waking cells (7 ± 2%), white arrows indicate representative ghost cells (cells with cytosolic components condensed at the poles), green arrows indicate representative cells that become ghost cells, and red arrows indicate representative cells that lyse (A). Scale bars indicate 10 µm. Representative results from two independent cultures are shown.

Table S1. Single *E. coli* persister cell waking by Ala, Related to Figure 1. Single persister cells were observed using light microscopy (Zeiss Axio Scope.A1). The total number of *E. coli* BW25113 persister cells that wake on M9 5X Ala, M9 5X Asn, and no amino acid (aa) gel pads is shown after 6 h at 37 °C. Also, growth of exponential cells on gel pads that lack amino acids is shown after 6 h at 37 °C. 5X Ala and 5X Asn is 375 μg/mL. Total waking cells indicates the number of dividing or elongating cells. These results are the combined observations from two independent experiments, and standard deviations are shown. The microscope images are shown in **Fig. 1**. A Student's t-test was used to compare Ala vs. Asn (* indicates a p value < 0.05).

	Total cells	**Dividing cells**	**Elongating cells**	**Total waking cells**	**% waking**	**Fold-change**
Ala	51	7	2	9	18 ± 1	9*
Asn	46	1	0	1	2 ± 2	1
no aa	56	0	0	0	0 ± 0	-∞
no aa (expon. cells)	104	12	30	12	12 ± 4	-

Table S2. Single persister cells wake primarily by sensing nutrients rather than spontaneously, Related to Figure 2. Single persister cells were observed using light microscopy (Zeiss Axio Scope.A1). The total number of *E. coli* BW25113 wild type persister cells on the NaCl, NaCl + Amp, and NaCl + Amp + Ala agarose gel pads is shown after 6 h at 37 °C. Dead cells were identified by their disappearance. The concentration of NaCl was 0.85 %, Amp was 100 μg/mL, and Ala was 375 μg/mL. Fold-changes was based on NaCl + Amp. These results are the combined observations from two independent experiments, and standard deviations are shown. The microscope images are shown in **Fig. 2**. A Student's t-test was used to compare NaCl + Amp vs. NaCl + Amp + Ala (** indicates a p value < 0.0003).

	Total cells	**Dead cells**	**% of dead cells**	**Fold-change**
NaCl	144	0	0 ± 0	-
NaCl + Amp	164	13	7.5 ± 0.7	1
NaCl + Amp + Ala	135	47	33 ± 6	4.4**

Table S3. Single persister cells wake with alanine as a signal, Related to Figure 2. Single persister cells were observed using light microscopy (Zeiss Axio Scope.A1). The total number and waking number of persister cells of BW25113 Δ*dadA* on M9 10X Ala, no nutrients, pyruvate (final conc. 0.24%) + 10 X Ala, and pyruvate (final conc. 0.24%) agarose gel pads are shown after 6 h at 37°C. These results are the combined observations from two independent experiments. The microscope images are shown in **Supplementary Figure 4**. Student's t-test was used to compare Δ*dadA* + Ala and Δ*dadA* (no nutrients) (* indicates a p value < 0.05).

	Total cells	**Total waking cells**	**% waking**	**Fold-change**
Δ*dadA* + Ala	345	16	4 ± 1	1
Δ*dadA* (no nutrients)	223	0	0 ± 0	-∞ *
Δ*dadA* + pyruvate + Ala	303	14	4.5 ± 0.5	1.1
Δ*dadA* + pyruvate	327	3	0.7 ± 1.1	-5.7

Table S4. Enhanced single persister cell waking on Ala via proteins identified by the ASKA library search, Related to Figure 3. Single persister cells were observed using light microscopy (Zeiss Axio Scope.A1). The total number and waking number of persister cells of BW25113 producing the indicated proteins (PsiF, PanD, YmgF, YjcF, PptA, and CheY) are shown. Fold-change in waking is relative to BW25113 with the empty plasmid pCA24N. These results are the combined observations from two independent experiments after 18 hours, and standard deviations are shown. The microscope images are shown in **Supplementary Figure 6-8**. Student's t-tests were used to compare Empty and the other groups (** indicates a p value < 0.01).

	Total cells	**Waking cells**	**% waking**	**Fold-change**
Empty	103	2	3 ± 5	1
PsiF	91	10	11 ± 3	6.1
PanD	105	43	40 ± 30	22.8
YmgF	87	28	30 ± 10	17.9
YjcF	120	14	11.7 ± 0.3	6.5
PptA	118	15	8 ± 6	7.1
CheY	93	93	100 ± 0	33**

Table S5. Single persister cell waking on Ala after producing chemotaxis-related, cAMP-related, and ribosome-resuscitation-related proteins, Related to Figure 3. Single persister cells were observed using light microscopy (Zeiss Axio Scope.A1). The total number and waking number of persister cells are shown. Fold-change in waking is relative to BW25113 with the empty plasmid pCA24N (empty vector data from **Table S4**). IPTG was used only for producing CpdA as indicted, otherwise, protein production was from the leaky promoter. M9 5X Ala with 3 mM atropine in the agarose gel pad was used with cells producing CpdA. These results are the combined observations from two independent experiments after 18 hours, and standard deviations are shown. The microscope images are shown in **Fig. 3, Supplemental Fig. 9, 16**. Student's t-tests were used to compare Empty and the other groups (* indicates a p value < 0.05 and ** indicates a p value < 0.01).

	Total cells	**Waking cells**	**% waking**	**Fold-change**
Empty	103	2	3 ± 5	1
CheA	93	77	82 ± 2	27**
CheY	93	93	100 ± 0	33**
Tar	123	33	28 ± 7	9
Trg	97	23	24 ± 7	8
Tsr	144	4	2.78 ± 0.05	-1.1
CpdA	128	21	25 ± 24	8
CpdA (0.5 mM IPTG)	115	52	45 ± 1	15**
CpdA + 3 mM atropine	77	71	92 ± 3	31**
CyaA	137	3	2.1 ± 0.5	-1.4
FliN	142	18	13 ± 2	4
HflX	126	25	20.0 ± 0.7	7*

Table S6. Persister cell formation on LB, Related to Figure 3. The percentage of persistence for single-gene knockouts (relative to BW25113 wild-type cells) and for BW25113 cells harboring pCA24N (relative to BW25113/pCA24N) was quantified for each strain by growing cells to the exponential phase in LB (turbidity of 0.8 at 600 nm), by treating with rifampicin and ampicillin to form persisters and to remove non-persisters, and by counting the number of colonies on LB plates after 1 day (the number of colonies indicates the number of cells that survive antibiotic treatment at 10X MIC). The specific growth rates (1/h) in LB are relative to BW25113 wild-type cells (0.81 ± 0.01) for the single-gene knockouts and relative to BW25113/pCA24N (0.92 ± 0.06) for cells producing the indicated protein; these specific growth rates were calculated by measuring the change in turbidity during exponential growth for two replicates for each independent culture in 96-well plates at 37°C. The results are the combined observations from two independent experiments, and standard deviations are shown.

	% persister formation	% growth rate
Wild type	100.0 ± 0.1	100 ± 1
Δ*arfB*	98.9 ± 0.9	98 ± 43
Δ*cheA*	99 ± 1	110 ± 31
Δ*cheY*	101.6 ± 0.1	120 ± 10
Δ*cpdA*	100.8 ± 0.3	98 ± 4
Δ*crp*	102 ± 1	96 ± 6
Δ*cyaA*	93.9 ± 2.4	109 ± 4
Δ*dadA*	97 ± 4	104 ± 8
Δ*flgE*	101.2 ± 0.6	129 ± 20
Δ*flhD*	101.1 ± 0.2	112 ± 1
Δ*fliN*	98.1 ± 0.8	108 ± 11
Δ*hflX*	100.4 ± 0.2	118 ± 4
Δ*motB*	100.7 ± 0.4	104 ± 6
Δ*motA*	99.1 ± 0.2	110 ± 17
Δ*ssrA'*	101 ± 2	99 ± 8
Δ*tar*	98.3 ± 0.1	97 ± 6
Δ*trg*	98.7 ± 0.4	86 ± 4
Δ*ptsG*	97 ± 2	86 ± 4
Δ*crr*	97 ± 1	113 ± 4
Δ*mglB*	98± 2	111 ± 2
Δ*malE*	100.8± 0.4	111 ± 4
Δ*smpB*	96± 2	121 ± 63
pCA24N	100.0 ± 0.5	100 ± 6
ArfB	97.4 ± 0.7	86 ± 7
CheA	95.6 ± 0.6	73 ± 6
CheY	99.6 ± 0.5	80 ± 8
CpdA	99.7 ± 0.5	114 ± 8
Crp	98.2 ± 0.7	82.0 ± 6
CyaA	99.7 ± 0.7	103 ± 11
FlhD	98.0 ± 0.7	107 ± 8
FliN	97.6 ± 0.6	94 ± 13

HflX	97.7 ± 0.9	79 ± 6
Tar	98.8 ± 0.8	77 ± 7
Trg	97.9 ± 0.7	77 ± 6
PtsG	97 ± 1	81 ± 7
Crr	93.6 ± 0.2	81 ± 6

Table S7. Single persister cell waking on Ala after inactivating flagellar proteins MotB, FhlD, and FliN, Related to Figure 3. Single persister cells were observed using light microscopy (Zeiss Axio Scope.A1). The total number and waking number of persister cells are shown. Fold-change in waking is relative to wild-type BW25113 (wild-type data from **Table S1**). These results are the combined observations from two independent experiments after 6 hours, and standard deviations are shown. The microscope images are shown in **Supplementary Figure 14**. Student's t-tests were used to compare wild type and the other groups (* indicates a p value < 0.05 and ** indicates a p value < 0.01).

	Total cells	**Waking cells**	**% waking**	**Fold-change**
Wild type	51	9	18 ± 1	1
Δ*motB*	57	17	30 ± 10	1.7
Δ*flhD*	81	5	6.1 ± 0.4	-3**
Δ*fliN*	96	5	5 ± 2	-3.6*

Table S8. Single persister cell waking on Ala after inactivating CpdA and CyaA, Related to Figure 3. Single persister cells were observed using light microscopy (Zeiss Axio Scope.A1). The total number and waking number of persister cells are shown. Fold-change in waking is relative to wild-type BW25113 (wild-type data from **Table S1**). These results are the combined observations from two independent experiments after 6 hours, and standard deviations are shown. The microscope images are shown in **Supplementary Figure 18**. Student's t-tests were used to compare wild type and the other groups (** indicates a p value < 0.01).

	Total cells	**Waking cells**	**% waking**	**Fold-change**
Wild type	51	9	18 ± 1	1
Δ*cpdA*	79	0	0 ± 0	-∞**
Δ*cyaA*	93	68	74 ± 8	4.1**

Table S9. Single persister cell waking on Ala after inactivating ribosome rescue proteins HflX and ArfB and inactivating trans-translation (SsrA), Related to Figure 3. Single persister cells were observed using light microscopy (Zeiss Axio Scope.A1). The total number and waking number of persister cells are shown. Fold-change in waking is relative to wild-type BW25113 (wild-type data from **Table S1**). These results are the combined observations from two independent experiments after 6 hours, and standard deviations are shown. The microscope images are shown in **Supplementary Figure 21**. Student's t-tests were used to compare wild type and the other groups (** indicates a p value < 0.01).

	Total cells	**Waking cells**	**% waking**	**Fold-change**
Wild type	51	9	18 ± 1	1
Δ*hflX*	96	0	0 ± 0	-∞**
Δ*arfB*	101	15	15 ± 2	-1.2
Δ*ssrA*	123	1	0.8 ± 1.1	-22.5**

Table S10. Single persister cell waking on glucose or alanine gel pads, Related to Figure 4. Single wild-type persister cells were observed using light microscopy (Zeiss Axio Scope.A1). The total number and waking number of persister cells on minimal medium with 0.4% glucose and 0.04% alanine are shown (0.4% glucose inhibited resuscitation with Ala). Fold-change in waking is relative to alanine (wild-type Ala data from **Table S1**). These results are the combined observations from two independent experiments after 6 hours, and standard deviations are shown. The microscope images are shown in **Figure 4**. Student's t-test was used to compare glucose vs. alanine (** indicates a p value < 0.01).

	Glucose	**Alanine**
Total cells	354	51
Waking cells	326	9
Waking %	92 ± 2	18 ± 1
Fold-change	5.1**	1

Table S11. Single persister cell waking on glucose gel pads, Related to Figure 5. Single persister cells were observed using light microscopy (Zeiss Axio Scope.A1). The total number and waking number of persister cells are shown. Fold-change in waking is relative to wild-type BW25113 (same field of view with 354 cells as in **Table S10** but cells wake here for only 3 h vs. 6 h in **Table S10**). These results are the combined observations from two independent experiments after 3 hours, and standard deviations are shown. The microscope images are shown in **Figure 5, Supplementary Fig. 27**. Student's t-tests were used to compare wild type and the other groups (* indicates a p value < 0.05 and ** indicates a p value < 0.01).

	Total cells	**Waking cells**	**% waking**	**Fold-change**
wild type	354	74	22 ± 3	1
Δ*trg*	283	12	5 ± 1	-4.6*
Δ*ptsG*	212	1	0.3 ± 0.5	-69.2*
Δ*crr*	369	44	11±3	-2
Δ*mglB*	311	55	17.8 ± 0.5	-1.2
Δ*malE*	329	77	23.3 ± 0.5	1.1
Δ*cpdA*	305	20	6 ± 2	-3.7*
Δ*cyaA*	279	139	50 ± 2	2.3**
Δ*hfq*	201	23	12 ± 1	-1.9
Δ*smpB*	352	132	35 ± 5	1.6
Δ*ssrA*	316	22	6.9 ± 0.1	-3.2*

Table S12. Single persister cell waking on glucose gel pads, Related to Figure 6. Single persister cells were observed using light microscopy (Zeiss Axio Scope.A1). The total number and waking number of persister cells are shown. Fold-change in waking is relative to BW25113 with the empty plasmid pCA24N. These results are the combined observations from two independent experiments after 5 hours, and standard deviations are shown. The microscope images are shown in **Figure 6, Supplementary Fig. 26**. Student's t-tests were used to compare Empty and the other groups (* indicates a p value < 0.05 and ** indicates a p value < 0.01).

	Total cells	Waking cells	% waking	Fold-change
Empty	284	7	2.5 ± 0.3	1
PtsG	195	0	0 ± 0	-∞*
Crr	356	5	1.4 ± 0.2	-1.8
CheY	339	129	39 ± 4	15.9**
CpdA	269	104	39 ± 1	15.9**
HflX	321	11	3.41 ± 0.07	1.4
Hfq	207	11	5.0 ± 0.9	2

Table S13. Bacterial strains and plasmids used in this study**, Related to Figures 1, 2, 3, 4, 5, and 6**.

Strains and Plasmids	Features	Source
E. coli BW25113	*rrnB3 ΔlacZ4787 hsdR514* Δ(*araBAD*)*567* Δ(*rhaBAD*)*568 rph-1*	(Baba et al., 2006)
E. coli BW25113 Δ*arfB*	Δ*arfB*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*cheA*	Δ*cheA*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*cheY*	Δ*cheY*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*cpdA*	Δ*cpdA*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*crp*	Δ*crp*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*cyaA*	Δ*cyaA*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*dadA*	Δ*dadA*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*flgE*	Δ*flgE*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*flhD*	Δ*flhD*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*fliN*	Δ*fliN*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*hflX*	Δ*hflX*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*motA*	Δ*motA*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*motB*	Δ*motB*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*ssrA'*	Δ*ssrA*, Km^R (161 of 363 nt deleted with attL intact)	(Wang et al., 2009)
E. coli BW25113 Δ*tar*	Δ*tar*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*trg*	Δ*trg*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*arfB*	Δ*arfB*, Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*ptsG*	*ΔptsG,* Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*mglB*	*ΔmglB,* Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*malE*	*ΔmalE,* Km^R	(Baba et al., 2006)
E. coli BW25113 Δ*smpB*	*ΔsmpB,* Km^R	(Baba et al., 2006)
E. coli MG1655-ASV	*rrnbP1::*GFP[ASV]	(Shah et al., 2006)
P. aeruginosa PA14	Wild type	(Liberati et al., 2006)
Plasmids		
pCA24N	Cm^R; *lacI*^{q}	(Kitagawa et al., 2005)
pCA24N_*aer*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*aer*$^+$	(Kitagawa et al., 2005)
pCA24N_*arfB*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*arfB*$^+$	(Kitagawa et al., 2005)
pCA24N_*cheA*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*cheA*$^+$	(Kitagawa et al., 2005)
pCA24N_*cheY*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*cheY*$^+$	(Kitagawa et al., 2005)
pCA24N_*cpdA*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*cpdA*$^+$	(Kitagawa et al., 2005)
pCA24N_*crp*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*crp*$^+$	(Kitagawa et al., 2005)
pCA24N_*cyaA*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*cyaA*$^+$	(Kitagawa et al., 2005)
pCA24N_*flhD*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*flhD*$^+$	(Kitagawa et al., 2005)
pCA24N_*fliN*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*fliN*$^+$	(Kitagawa et al., 2005)
pCA24N_*hflX*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*hflX*$^+$	(Kitagawa et al., 2005)
pCA24N_*panD*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*panD*$^+$	(Kitagawa et al., 2005)
pCA24N_*pptA*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*pptA*$^+$	(Kitagawa et al., 2005)
pCA24N_*psiF*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*psiF*$^+$	(Kitagawa et al., 2005)
pCA24N_*tap*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*tap*$^+$	(Kitagawa et al., 2005)
pCA24N_*tar*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*tar*$^+$	(Kitagawa et al., 2005)
pCA24N_*trg*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*trg*$^+$	(Kitagawa et al., 2005)
pCA24N_*tsr*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*tsr*$^+$	(Kitagawa et al., 2005)
pCA24N_*yjcF*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*yjcF*$^+$	(Kitagawa et al., 2005)

pCA24N_*ymgF*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*ymgF*$^+$	(Kitagawa et al., 2005)
pCA24N_*ptsG*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*ptsG*$^+$	(Kitagawa et al., 2005)
pCA24N_*crr*	Cm^R; *lacI*^{q}, $P_{T5\text{-}lac}$::*crr*$^+$	(Kitagawa et al., 2005)

Km^R and Cm^R indicate kanamycin and chloramphenicol resistance, respectively.

Table S14. Supplementation levels (1X) for amino acids in M9 minimal medium**, Related to Figure 1**.

No.	Amino acid	Final concentration (µg/mL)
1	1 % L-alanine	75
2	2 % L-arginine	145
3	1 % L-asparagine	75
4	1 % L aspartic acid	75
5	2 % L-cysteine	50
6	2 % L-histidine	42
7	1 % L-glutamic acid	75
8	1 % L-glutamine	75
9	2 % glycine	110
10	1 % L-isoleucine	42
11	1 % L-leucine	41
12	1 % L-lysine	75
13	2 % L-methionine	25
14	1 % L-phenylalanine	75
15	4 % L-proline	164
16	2 % L-serine	42
17	2 % L-threonine	82
18	0.25 % L-tryptophan	18
19	1 % L-tyrosine	75
20	1 % L-valine	42

TRANSPARENT METHODS

Bacterial strain and growth conditions. The bacterial strains and plasmids used in this study are listed in **Table S13**. *E. coli* BW25113 and its isogenic mutants (Baba et al., 2006), and the pCA24N-based plasmids from the *E. coli* ASKA collection (Kitagawa et al., 2005) were used. Lysogeny broth (Sambrook et al., 1989) was used for routine cell growth, and M9 medium supplemented with amino acids (Rodriguez and Tait, 1983) was used for waking studies; strains were grown at 37 °C. Each of the 20 amino acids stock solutions were prepared as indicated in **Table S14** (Rodriguez and Tait, 1983).

Observation of persister cells on agarose gel pads. We utilized our previous method for converting up to 70% of the cell population into persister cells (Kim et al., 2018a; Kim et al., 2018b; Kwan et al., 2013): exponentially-growing cells (turbidity of 0.8 at 600 nm) were treated with rifampicin (100 μg/mL for 30 min) to form persister cells by stopping transcription and non-persister cells were removed by lysis via ampicillin treatment (100 μg/mL for 3 h, 10X minimum inhibitory concentration (Kwan et al., 2013)). Cells were harvested at 17,000 g for 1 min and washed with 1x phosphate buffered saline buffer (Dulbecco and Vogt, 1954) (PBS) buffer twice to remove all possible carbon sources, then re-suspended with 1 mL of 1x PBS. To obtain biofilm persister cells, *E. coli* BW25113/pCA24N and *E. coli* MG1655-ASVGFP were cultured for 48 h at 37°C in the 96 well plates with LB; the biofilm formation was confirmed by crystal violet staining (Fletcher, 1977). The biofilm was washed two times with PBS to remove the planktonic cells, and the biofilm cells were removed by sonicating it for 5 sec at 30% power (Sonic Dismembrator 60, Fisher Scientific). The biofilm cells were resuspended in LB containing ampicillin (100 μg/mL) for 3 h to remove any non-persister cells. Gel pads of 1.5% agarose were prepared (Kim et al., 2018b) and 5 μL of persister cells were added, kept at 37°C, and observed using a light microscope (Zeiss Axio Scope.A1, bl_ph channel at 1000 ms exposure) every hour. The fluorescence intensity of the ribosome GFP reporter strain, MG1655-ASVGFP, was monitored using a fluorescence microscope (Zeiss Axioscope.A1, GFP channel at 10,000 ms exposure) (Kim et al., 2018b).

Observation of persister cells on motility gel pads. An M9-gradient glucose motility gel pad (0.3% agar) was prepared by solidifying for 1.5 hours and adding 10 μL of 5 wt% glucose and 10 μL of persister cells.

The movement of the persister cell was observed using light microscopy (Zeiss Axio Scope.A1, bl_ph channel at 1000 ms exposure).

Persister resuscitation screen. All 4,267 ASKA clones (GFP-) (Kitagawa et al., 2005) were combined, grown to a turbidity of 2 at 600 nm in LB medium, and their plasmids isolated using a plasmid DNA Mini Kit I (OMEGA Bio-tek, Norcross, GA, USA). The pooled ASKA plasmids (1 µL containing 30 ng of DNA) was electroporated into 50 µL of *E. coli* BW25113 competent cells, 1 mL LB medium was added, and the cells were grown to a turbidity of 0.5 in LB medium. Chloramphenicol was added (final conc. 30 µg/mL) to the culture, and the cells were incubated at 250 rpm to a turbidity of 0.8. Rifampicin followed by ampicillin was added to make persister cells, the persister cells were washed twice with 1x PBS buffer, diluted 100-fold 1x PBS buffer, and 100 µL was plated on M9 5X Ala (Cm) agar plates and incubated at 37°C; faster colony appearance indicated faster persister resuscitation.

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