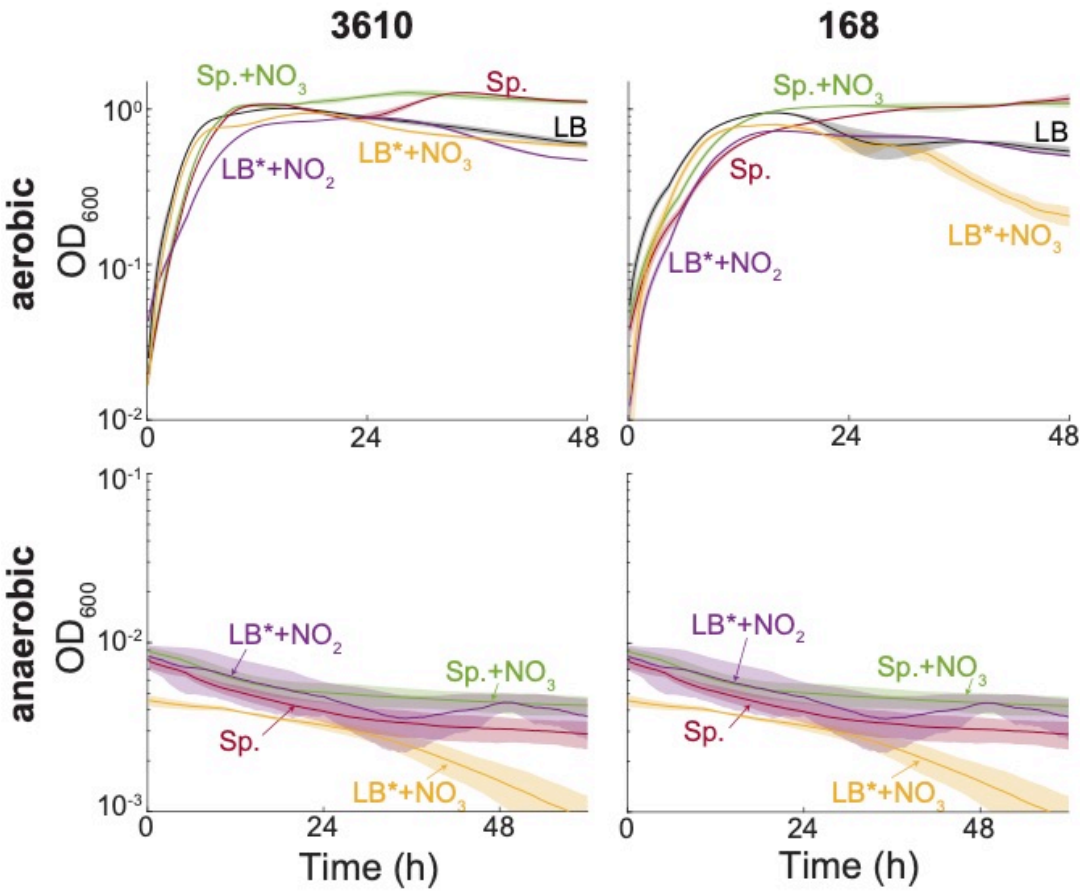

Figure S1: *B. subtilis* does not grow anaerobically on nitrate or nitrite, related to Figure 1.

No growth of *B. subtilis* strain 3610 (bottom left) or strain 168 (bottom right) was observed during anaerobic culturing with nitrate or nitrite. Nitrate may help growth in late exponential and stationary phase during aerobic growth (top panels), as cultures grown in Spizizen medium with nitrate (green) reached their maximum OD_{600} before those grown in Spizizen alone (dark red). *B. subtilis* strains 3610 and 168 were inoculated in aerobic or anaerobic conditions in LB (aerobic only), modified LB+nitrate (LB+NO_3), modified LB+nitrite (LB+NO_2), Spizizen minimal medium (Sp.), and Spizizen minimal medium+nitrate (Sp.+NO_3). Each line represents the mean and shading represents 1 standard deviation (SD), n=3.

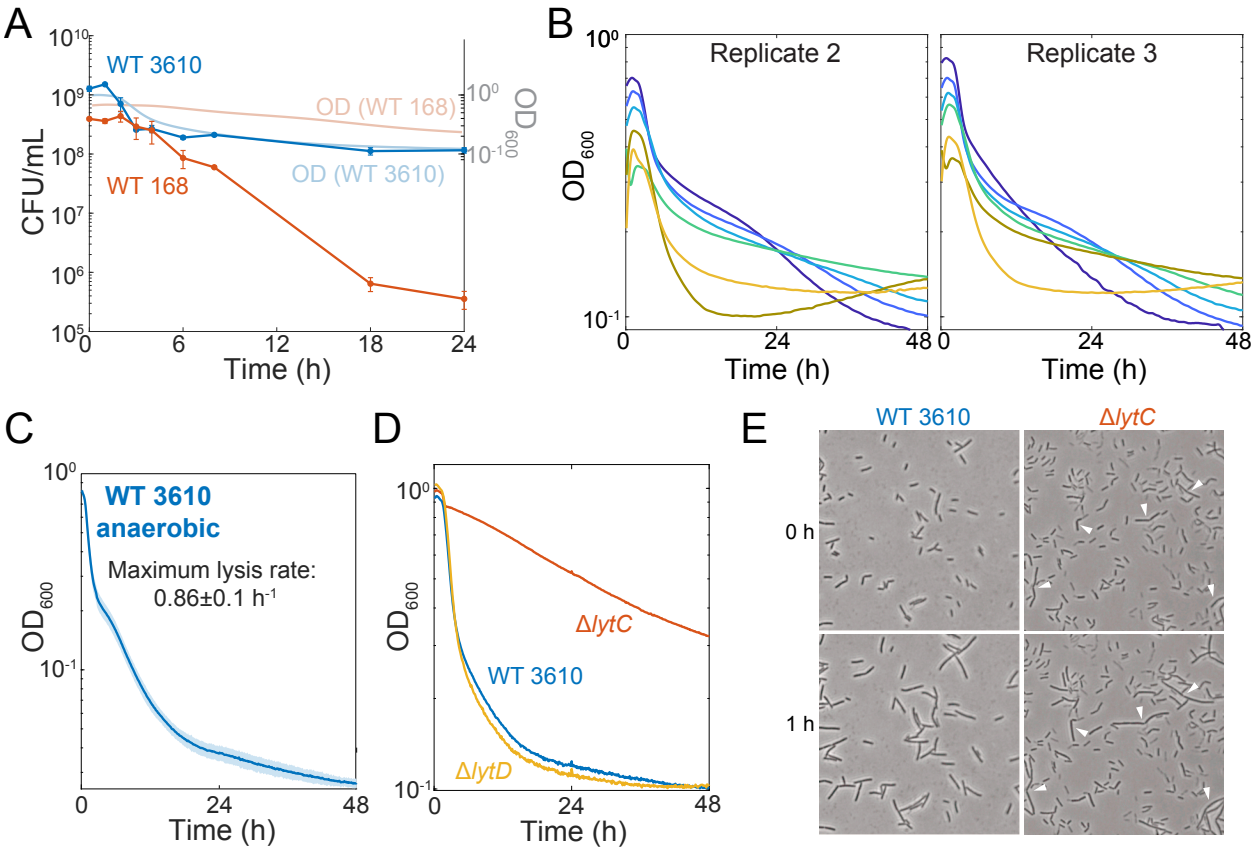

Figure S2: Characterization of oxygen depletion in an anaerobic chamber and of Δ*lytC* and Δ*lytD* mutants, related to Figure 1 and Videos S1 and S2.

(A) The viability of strain 3610 follows OD (faint lines, representing the mean) between 0 and 24 h, while 168 viability drops rapidly around 4 h post-oxygen depletion. Error bars represent 1 SD (n = 3).

(B) Culture lysis depends on cell density at the time of oxygen depletion. Two sets of independent lysis curves that varied in initial OD_{600} are shown; see Figure 1D for the other independent experiment.

(C) Cell lysis is more rapid under anaerobic conditions. Cultures were grown aerobically in 96-well plates to OD_{600}~1 and then transferred to an

anaerobic chamber to rapidly remove oxygen from the culture. Line represents the mean and shading represents 1 SD, *n*=3.

(D) Δ*lytD* cells show similar rates of lysis as wild-type 3610 cells upon oxygen depletion, unlike Δ*lytC* cells that exhibit slower lysis. Strains were grown to OD_{600}~1 and then oxygen was depleted at *t* = 0.

(E) After oxygen depletion, the majority of wild-type cells but only a small subset of Δ*lytC* cells resume growth. After 24 h of oxygen depletion, wild-type and Δ*lytC* cultures were spotted onto an LB agarose pad with oxygen and cell growth was monitored. Nearly all wild-type cells grew but only a few Δ*lytC* cells grew (arrowheads). Images shown are frames from Videos S1 and S2.

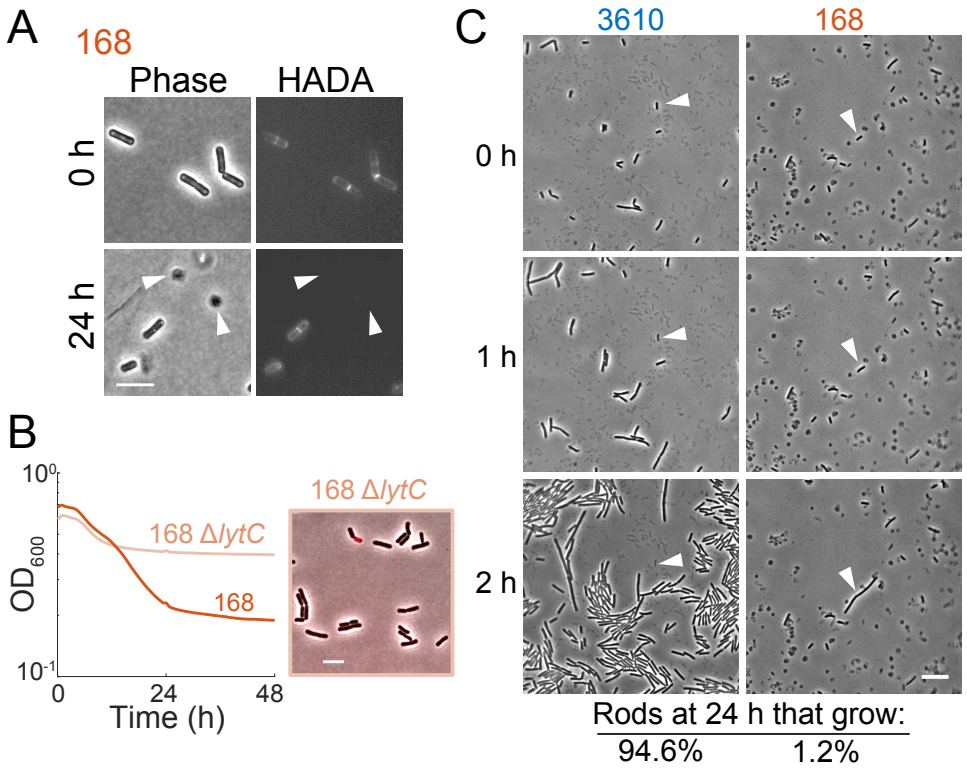

Figure S3: Oxygen depletion causes production of wall-less protoplasts in strain 168 and only a low percentage of cells are able to resume growth, related to Figure 2 and Videos S3 and S4.

(A) Cell-wall staining reveals protoplasts that have lost their cell wall. Phase-contrast and HADA (cell wall) staining of strain 168 cultures at 24 h post-oxygen depletion. Cells were stained with HADA for the last doubling before oxygen was depleted and the dye was present during the duration of depletion. Arrowheads: phase-dark spherical cells that lack cell-wall staining. Scale bar: 5 μm.

(B) Strain 168 Δ*lytC* cells remain rod-shaped following oxygen depletion. Left: OD_{600} of 168 Δ*lytC* exhibited somewhat slower lysis than the parent. Right: overlay of phase-contrast and propidium iodide staining of 168 Δ*lytC* cells.

(C) Almost all strain 3610 cells but only 1% of strain 168 cells grow once oxygen is restored following 24 h of oxygen depletion. Cells from strain 3610 and strain 168 cultures after 24 h of oxygen depletion were spotted onto an LB agarose pad and imaged in time-lapse to determine which cells were capable of growing and dividing. For strain 3610, the arrowhead points to a rare rod-shaped cell that did not grow. For strain 168, the arrowhead points to a rare rod-shaped cell that grew. Scale bar: 10 μm. *n* = 536 and 604 rod-shaped cells for strain 3610 and strain 168, respectively. Images shown are frames from Videos S3 and S4.

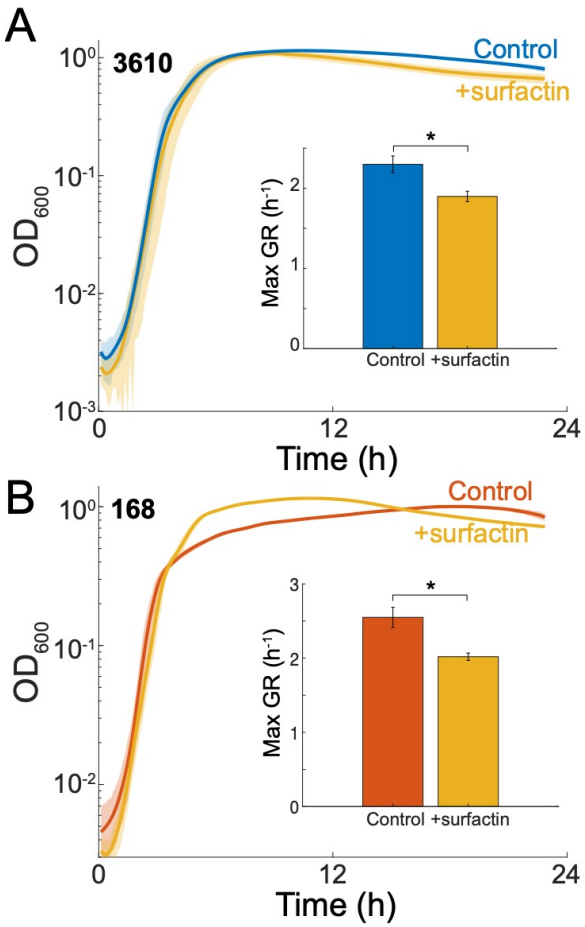

Figure S4: Exogenous surfactin reduces the maximum growth rate of *B. subtilis* when grown aerobically, related to Figure 3.

(A) Surfactin treatment (0.2 mg/mL) significantly reduces the maximum growth rate of strain 3610. Inset: maximum growth rate (the largest positive slope of the $\ln(OD_{600})$ curve) of a control (ethanol-treated) and surfactin-treated (dissolved in ethanol) culture. Shading and error bars represent 1 SD, n=3. *: p<0.05, Student's ttest.

(B) Surfactin treatment (0.05 mg/mL) significantly reduces the maximum growth rate of strain 168. Inset: maximum growth rate (the largest positive slope of the $\ln(OD_{600})$ curve) of a control (ethanol-treated) and surfactin-

treated (dissolved in ethanol) culture. Shading and error bars represent 1 SD, n=3. *: p<0.01, Student's ttest.

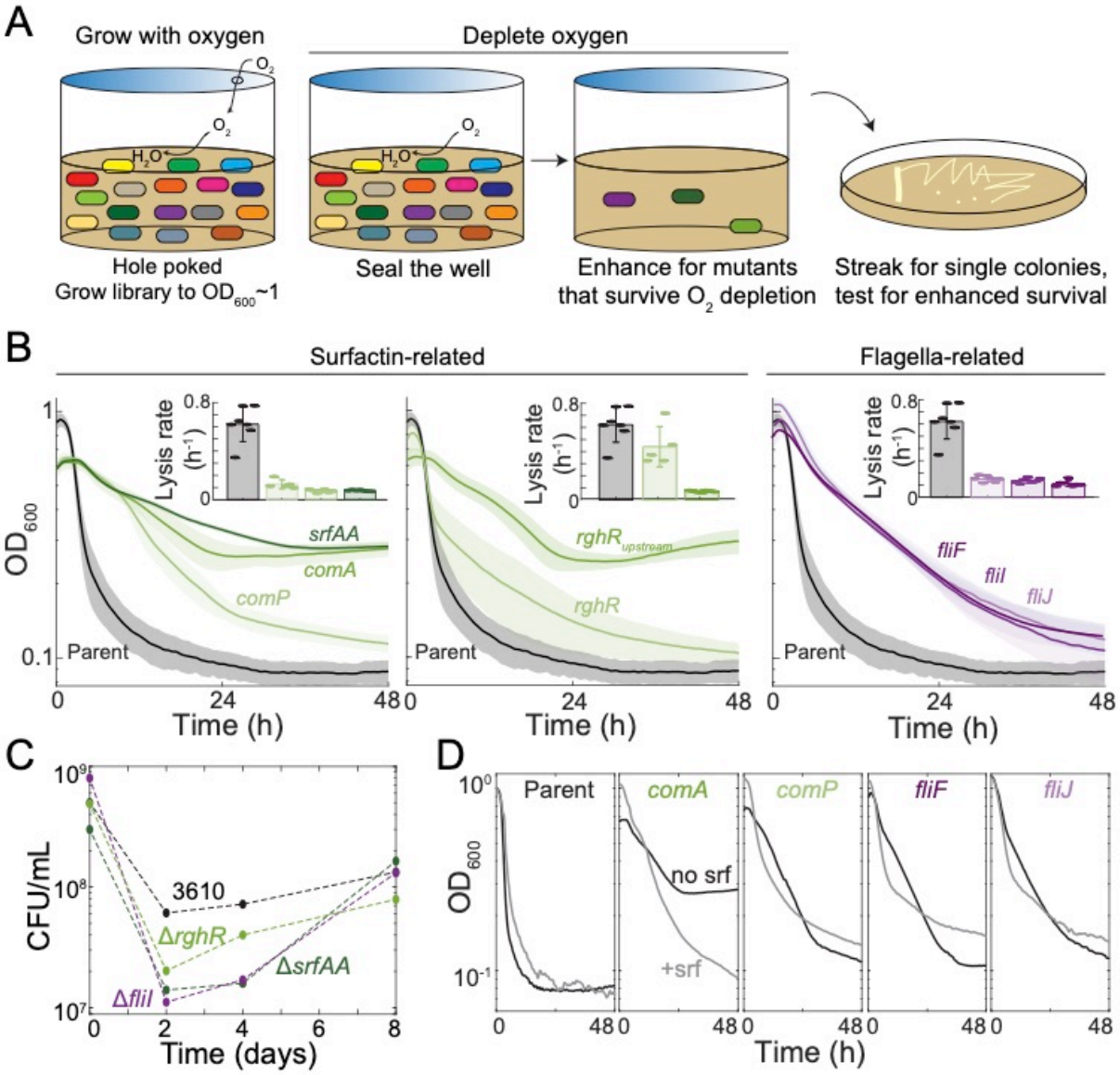

Figure S5: Characterization of hits from transposon screen, related to Figure 4.

(A) Schematic of transposon screen design. A transposon mutant library was grown to OD_{600}~1, and then oxygen was depleted. We screened cells that were able to recover after oxygen depletion for oxygen-depletion phenotypes.

(B) Transposon mutants have a reduced lysis rate. OD_{600} curves during oxygen depletion of mutants related to surfactin regulation (green) or flagella

(purple). Lines represent the mean and shading represents 1 SD, *n*=5. Inset: maximum lysis rates. Error bars represent 1 SD, *n*=5.

(C) Viability of clean deletions of *srfAA*, *fliI*, and *rghR* is lower than that of 3610 2 and 4 days following oxygen depletion.

(D) Addition of 48 μM surfactin increases the lysis rate of hits from transposon mutant screen.

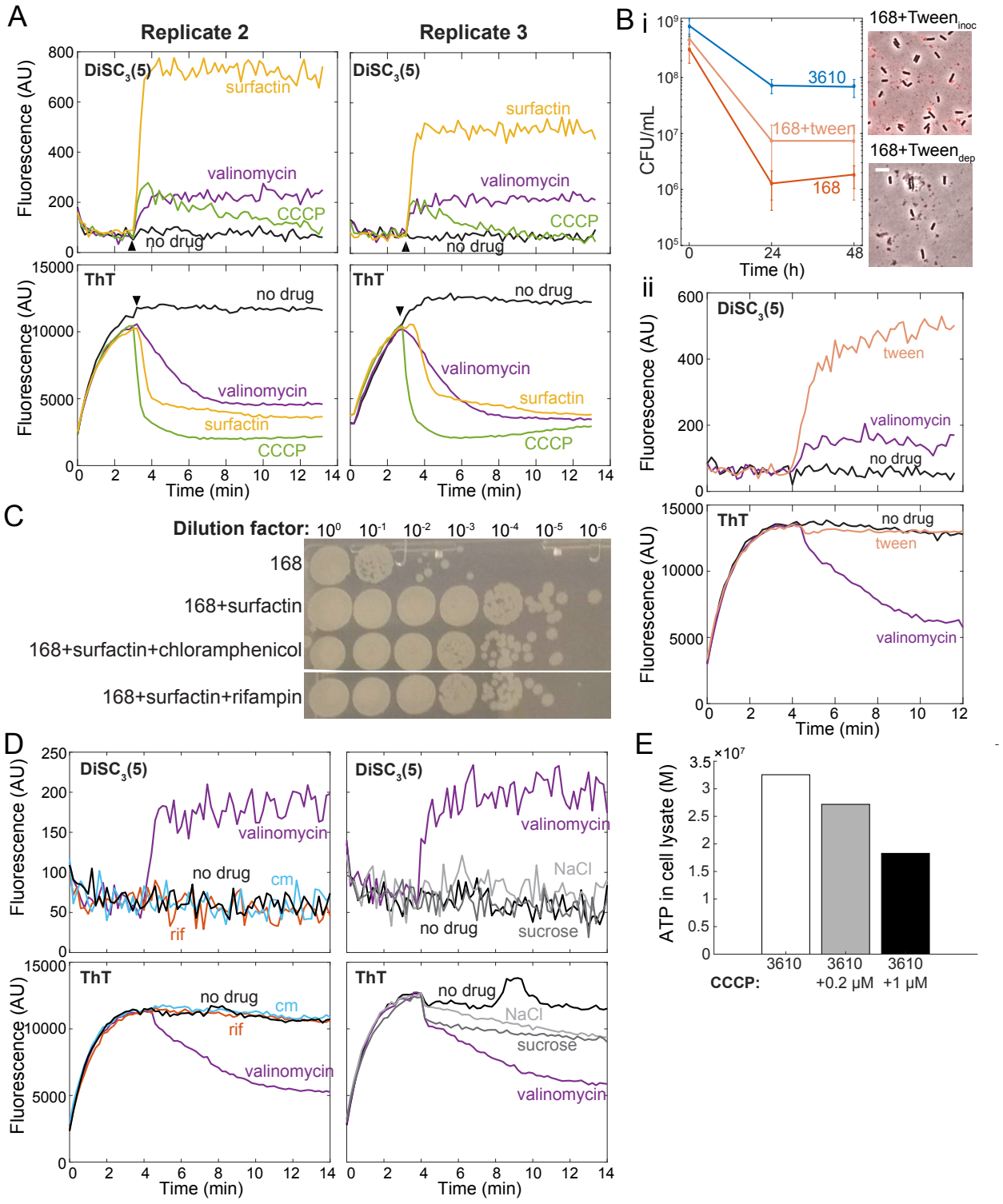

Figure S6: Membrane potential assays of strain 168 cells undergoing various treatments, antibiotic+surfactin viability data, and ATP assay control, related to Figures 5 and 6.

(A) $DiSC_3(5)$ (top) and ThT (bottom) both show that surfactin (48 μM), valinomycin (50 μM), and CCCP (5 μM) cause membrane depolarization. The time of drug addition is marked by the black arrow. Two experimental replicates are shown (replicate 1 is shown in Figure 5).

(B) (i) Tween 80 treatment of strain 168 cells removes protoplasts (right, scale bar: 5 μm) but does not significantly improve viability in plating efficiency measurements (left, n=3; p>0.05). (ii) Tween 80 alters $DiSC_3(5)$ fluorescence (top) but not ThT (bottom) as compared to untreated strain 168 cells.

(C) Adding chloramphenicol (5 μg/mL) or rifampin (1 μg/mL) to surfactin-treated cells does not reduce viability. A representative plating efficiency image of 4 independent experiments is shown.

(D) Left: chloramphenicol (5 μg/mL) and rifampin (1 μg/mL) do not depolarize strain 168 cells in either $DiSC_3(5)$ (top) or ThT (bottom) assays. Right: 300 mM NaCl and 300 mM sucrose do not depolarize cells when measured by $DiSC_3(5)$ (top), and only slightly affect ThT measurements (bottom).

(E) Sub-inhibitory concentrations of CCCP reduce ATP levels of strain 3610 cells when grown aerobically. ATP levels were normalized to OD at the time of the assay (OD_{600} values were 1.04, 0.65, and 0.57 for untreated, 0.2 μg/mL, and 1 μg/mL, respectively).

Gene hit	# independent hits	Gene function
srfAA	1	surfactin synthetase
comA	3	*comP*/*comA* quorum sensing response regulator
comP	4	*comP*/*comA* quorum sensing sensor histidine kinase
rghRA	1	transcriptional repressor
rghRA-upstream	1	transcriptional repressor
fliI	1	flagella chaperone
fliF	1	flagella chaperone
fliJ	1	flagella basal ring

Table S1: Information about hits from transposon screen. Related to Figure 4.

Strain name	Nickname	Genotype	Reference or source
HA10	3610	*trpC+, rapP+, sfp+, epsC+, swrA+, degQ+, pBS32*	Kearns lab
DK5073	3610 *ΔlytC*	3610, Δ*lytC*	This work
DK5075	3610 *ΔlytD*	3610, Δ*lytD*	This work
HA1	168	*trpC2*	Carol Gross lab
BKE35620	168 *ΔlytC*	168, Δ*lytC::MLS*	[S1]
HA1417	168*sfp+*	168, *swrAfs, sfp^0, amyE::Psfp-sfp cmR*	This work
HA1161	parent	3610, Δ*SPB,* Δ*PBSX,* Δ*pBS32*	Kearns lab
HA1235	N/A	3610, Δ*SPB,* Δ*PBSX,* Δ*pBS32, pMarA-kan*	This work
HA1414	N/A	168, *pMarA-kan*	This work
Back-crossed transposon mutagenesis strains			
HA1225	*comA*	3610, Δ*SPB,* Δ*PBSX,* Δ*pBS32, comA::pMarA-kan*	This work
HA1227	*comP*	3610, Δ*SPB,* Δ*PBSX,* Δ*pBS32, comP::pMarA-kan*	This work
HA1228	*fliJ*	3610, Δ*SPB,* Δ*PBSX,* Δ*pBS32, fliJ::pMarA-kan*	This work
HA1229	*fliI*	3610, Δ*SPB,* Δ*PBSX,* Δ*pBS32, fliI::pMarA-kan*	This work
HA1233	*fliF*	3610, Δ*SPB,* Δ*PBSX,* Δ*pBS32, fliF::pMarA-kan*	This work
HA1234	*srfAA*	3610, Δ*SPB,* Δ*PBSX,* Δ*pBS32, srfAA::pMarA-kan*	This work
HA1281	*yvaN*	3610, Δ*SPB,* Δ*PBSX,* Δ*pBS32, yvaN::pMarA-kan*	This work
HA1282	*yvaN-us (upstream)*	3610, Δ*SPB,* Δ*PBSX,* Δ*pBS32, yvaN-us::pMarA-kan*	This work
Clean deletions of transposon mutagenesis strains			
HA1369	Δ*srfAA*	3610, *srfAA*	This work
HA1374	Δ*fliI*	3610, *fliI*	This work
HA1377	Δ*rghRA*	3610, *rghRA*	This work
Deletion and depletion strains			
BKE03480	N/A	168, *srfAA::MLS*	[S1]
BKE16240	N/A	168, *fliI::MLS*	[S1]

BKE33660	N/A	168*, rghRA::MLS*	[S1]
CAG74772	168 *trxA* ↓	168, *lacA*::*Pxyl-dcas9*(ErmR), *amyE::Pveg-sgRNA-trxA*(cmR)	[S2]
CAG74814	168 *trxB* ↓	168, *lacA*::*Pxyl-dcas9*(ErmR), *amyE::Pveg-sgRNA-trxB*(cmR)	[S2]

Table S2: List of strains used in this study, related to STAR Methods.

Supplemental References

S1. Koo, B.M., Kritikos, G., Farelli, J.D., Todor, H., Tong, K., Kimsey, H., Wapinski, I., Galardini, M., Cabal, A., Peters, J.M., et al. (2017). Construction and Analysis of Two Genome-Scale Deletion Libraries for *Bacillus subtilis*. Cell Syst *4*, 291-305 e297.

S2. Peters, J.M., Colavin, A., Shi, H., Czarny, T.L., Larson, M.H., Wong, S., Hawkins, J.S., Lu, C.H.S., Koo, B.M., Marta, E., et al. (2016). A Comprehensive, CRISPR-based Functional Analysis of Essential Genes in Bacteria. Cell *165*, 1493-1506.