

**Fig. S1:** *cdsA* mutants are recessive to wild-type. EOPs of strains supplemented with 200 mg/l vancomycin. IPTG was not supplemented in media to avoid overexpression of *cdsA*.

## **Cell volume**



**Fig. S2:** *cdsA* mutant cell size does not differ from wild-type. Cell dimensions were quantified with MicrobeTracker version 0.936 (21), using MATLAB version R2013a. Error bars represent the standard error of the mean (n = 3 replicates of approximately 100 cells each). *P*-values were calculated using a Student's *t*-test. All *p*-values were > 0.05.



**Fig. S3:** All PA species detected accumulate in the *cdsA* strains, the *pgsA444* strain, and the *plsC* overexpression strain. PA and PS were quantified by Q-TOF LC/MS. Each PA species (PA (36:2) m/z 699, PA (34:1) m/z 673, PA (34:2) m/z 671, PA (32:1) m/z 645) was normalized to sum of all PS species. The change of PA/PS is reported  $\pm$  standard error from three experiments.

## Reverse-Phase LC-MS EIC plot



Retention time (min)

## ESI-MS [M-H]<sup>-</sup>



**Figure S4:** Retention time and ESI-MS of each PA species. PA (32:1) m/z 645, PA (34:2) m/z 671, PA (34:1) m/z 673, PA (36:2) m/z 699 accumulated in *cdsA9* strain, which was used as a representative example.



**Fig. S5:** ESI (Electrospray ionization)-TIC (total ion current) chromatogram from reverse-phase LC-MS analysis of phosphatidic acid (PA) and cardiolipin (CL). This chromatogram is from a *cdsA9* strain, which was used as a representative example. Monoglyceride (MG), Phosphatidylserine (PS), Phosphatidylglycerol (PG), Phosphatidylethanolamine (PE), Diglyceride (DG) are also shown on the chromatogram.

Strain	growth rate ( $\mu$ ) ± SEM
wild-type	$3.1 \pm 0.2$
cdsA7	$3.3 \pm 0.1$
cdsA9	$3.0 \pm 0.2$

**Table S1:** *cdsA* mutant growth rate does not differ from wild-type. Strains were subcultured and  $OD_{600nm}$  readings were taken every 20 minutes.  $OD_{600nm}$  was plotted against time on a logarithmic scale. Linear regression analysis was used to determine a growth rate ( $\mu$ ) for each strain. The growth rate is reported  $\pm$  standard error of the mean from three technical replicates for three biological replicates per strain.