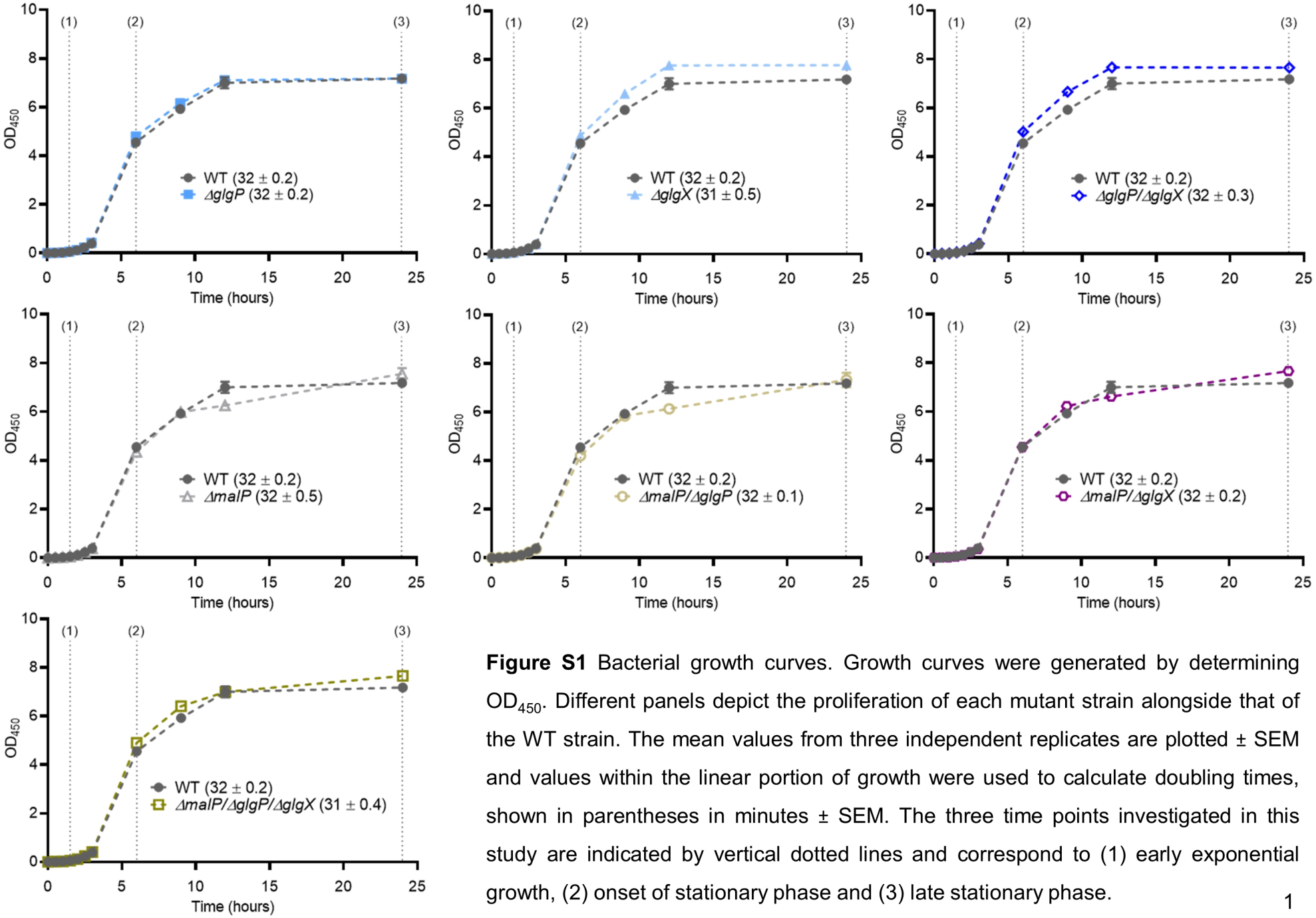


**Figure S1** Bacterial growth curves. Growth curves were generated by determining $OD_{450}$. Different panels depict the proliferation of each mutant strain alongside that of the WT strain. The mean values from three independent replicates are plotted ± SEM and values within the linear portion of growth were used to calculate doubling times, shown in parentheses in minutes ± SEM. The three time points investigated in this study are indicated by vertical dotted lines and correspond to (1) early exponential growth, (2) onset of stationary phase and (3) late stationary phase.

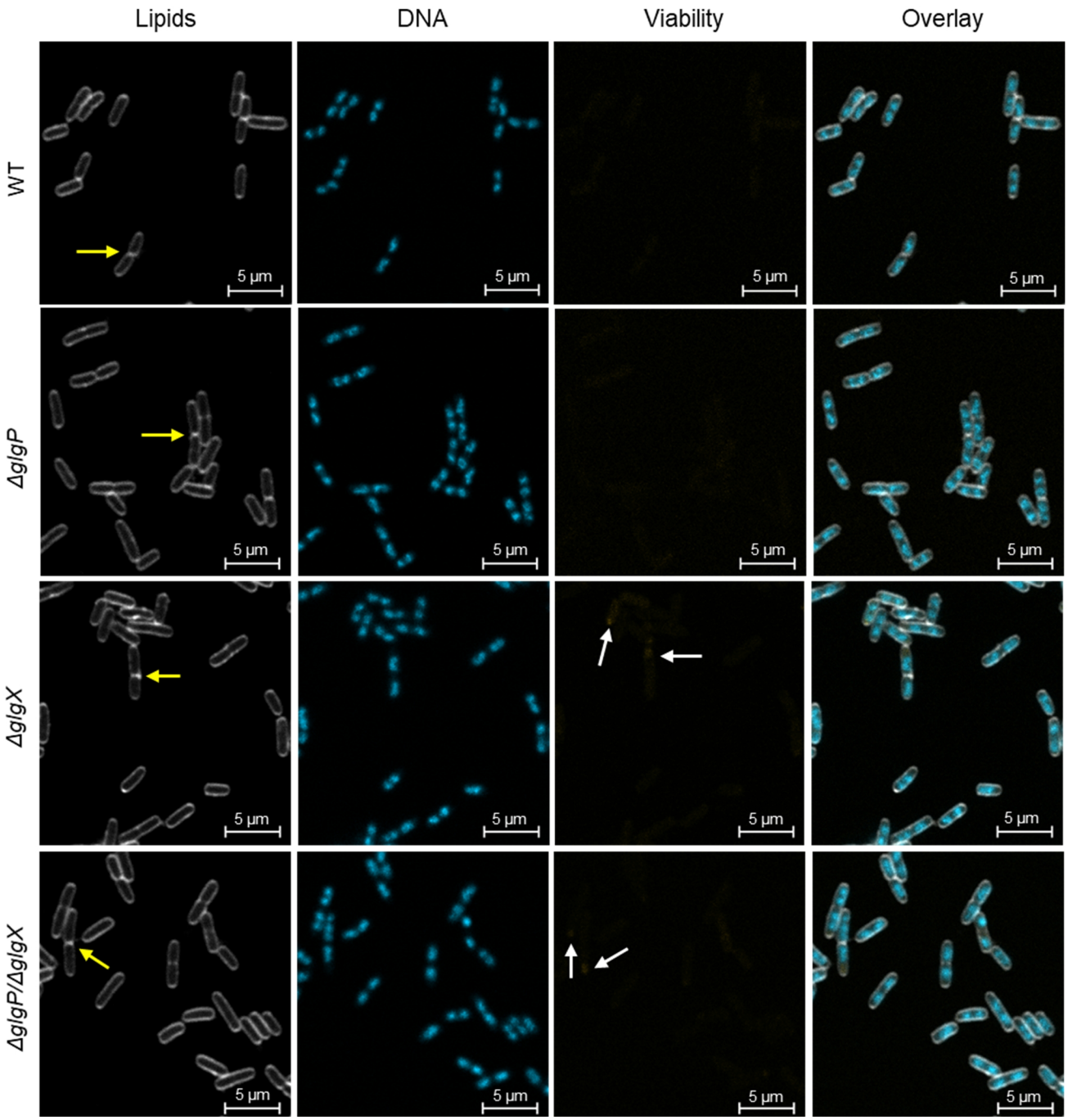


**Figure S2a** Representative confocal images of *E. coli* cells in early exponential phase. Cell membranes, DNA and viability were assessed simultaneously using multi-color fluorescent staining. The cell periphery is shown in white, DNA in light blue and proteins tagged by the viability dye are shown in yellow. Yellow arrows demarcate invaginating septa, with lipids in the new cell wall tagged by the membrane dye. White arrows indicate putative inclusion bodies, highlighted by the amine-reactive viability stain.

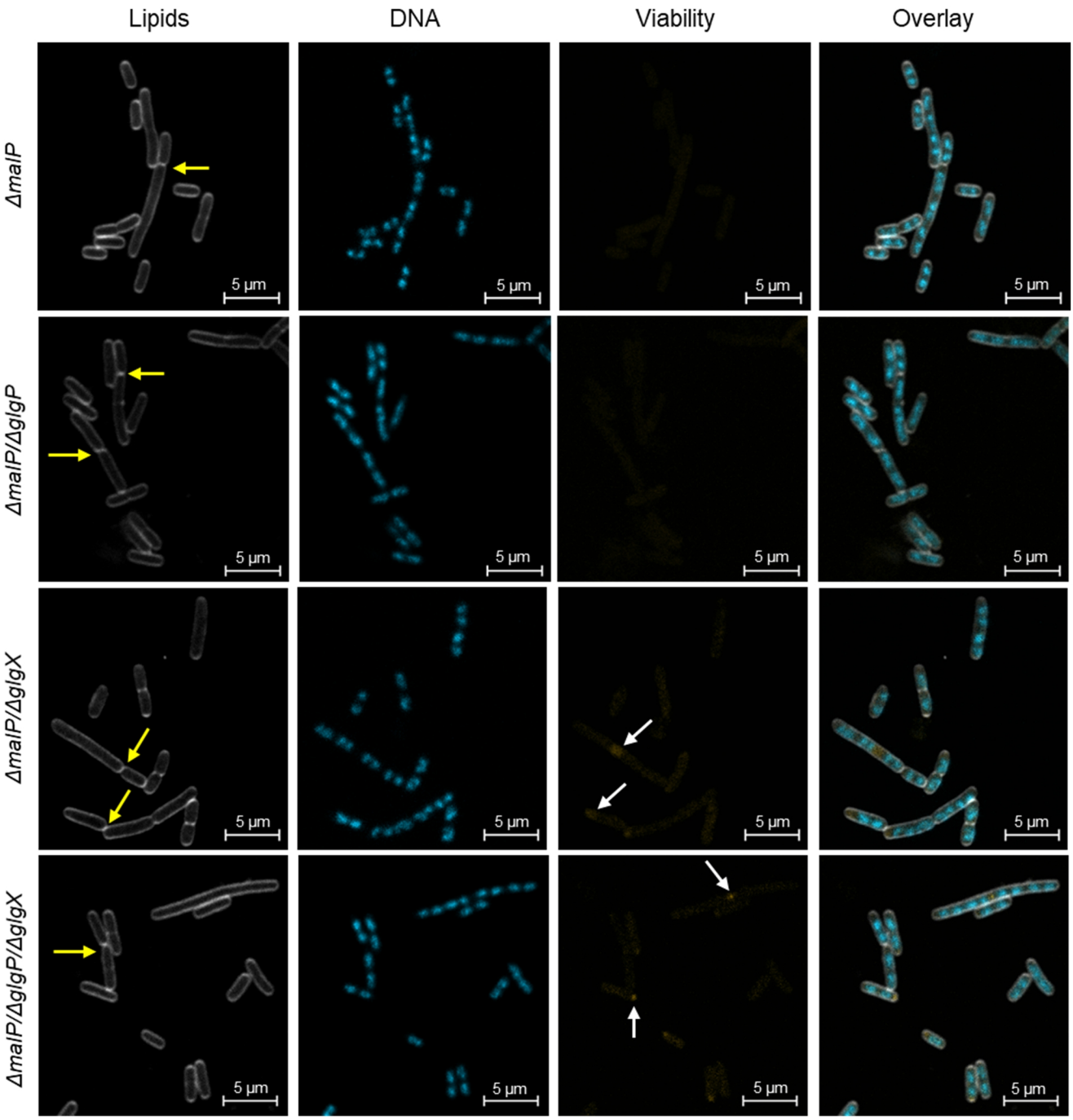


**Figure S2b** Representative confocal images of *E. coli* cells in early exponential phase. Cell membranes, DNA and viability were assessed simultaneously using multi-color fluorescent staining. The cell periphery is shown in white, DNA in light blue and proteins tagged by the viability dye are shown in yellow. Yellow arrows demarcate invaginating septa, with lipids in the new cell wall tagged by the membrane dye. White arrows indicate putative inclusion bodies, highlighted by the amine-reactive viability stain.

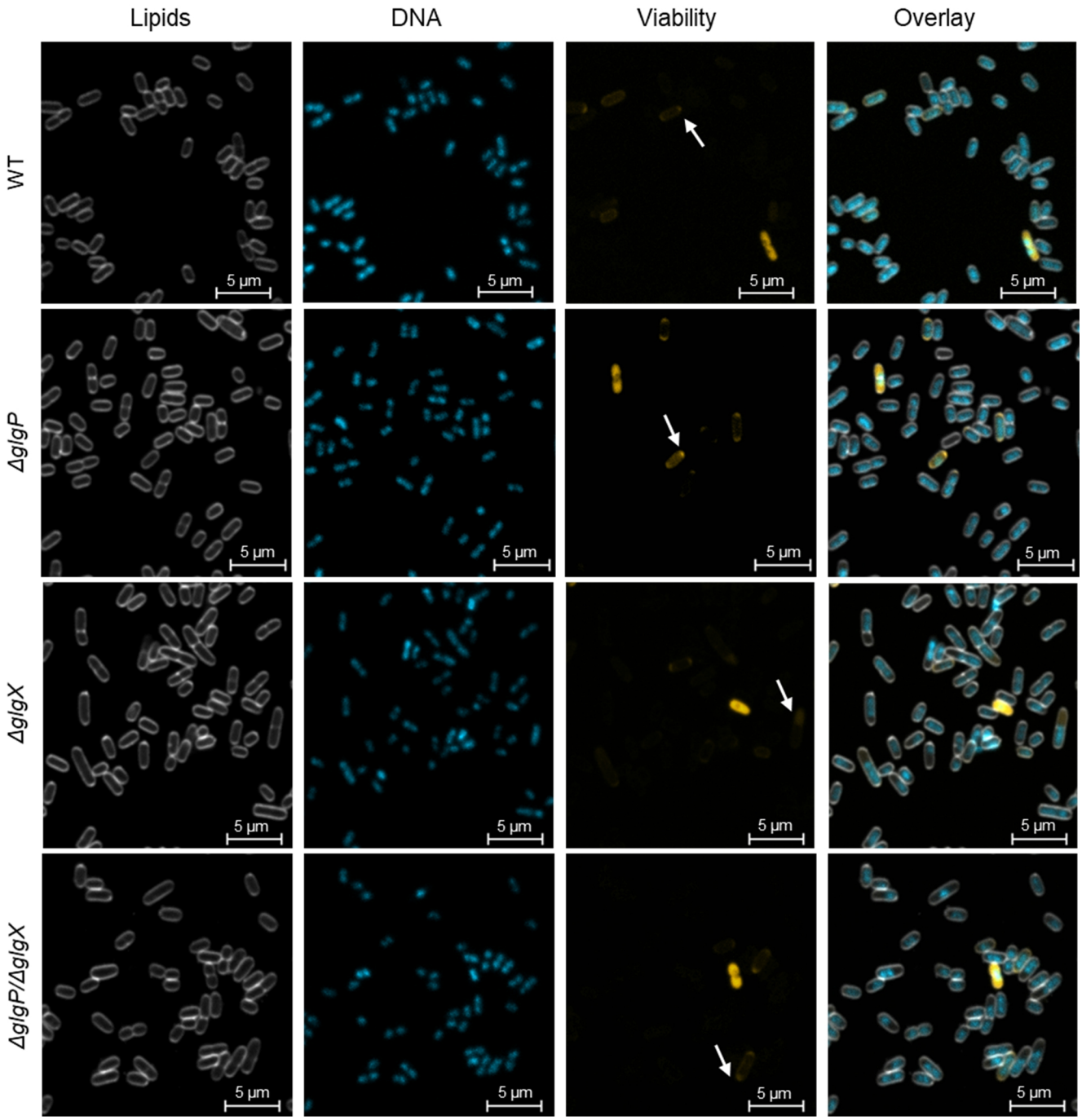


**Figure S3a** Representative confocal images of *E. coli* cells entering stationary phase. The cell periphery is shown in white, DNA in light blue and polypeptides in yellow. Arrows indicate foci which appear to be protein masses, illuminated upon excitation of the viability stain.

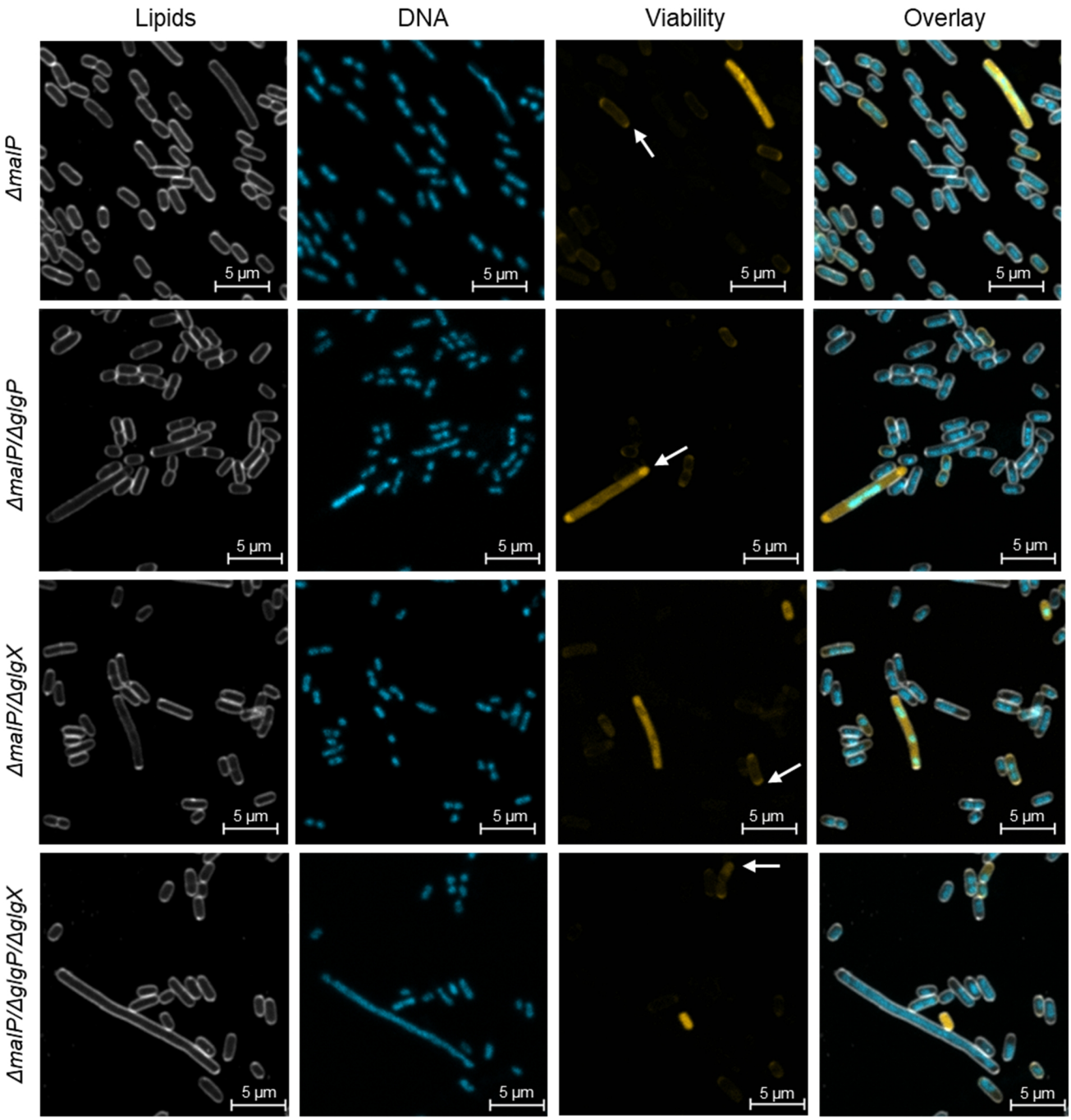


**Figure S3b** Representative confocal images of *E. coli* cells entering stationary phase. The cell periphery is shown in white, DNA in light blue and polypeptides in yellow. Arrows indicate foci which appear to be protein masses, illuminated upon excitation of the viability stain.

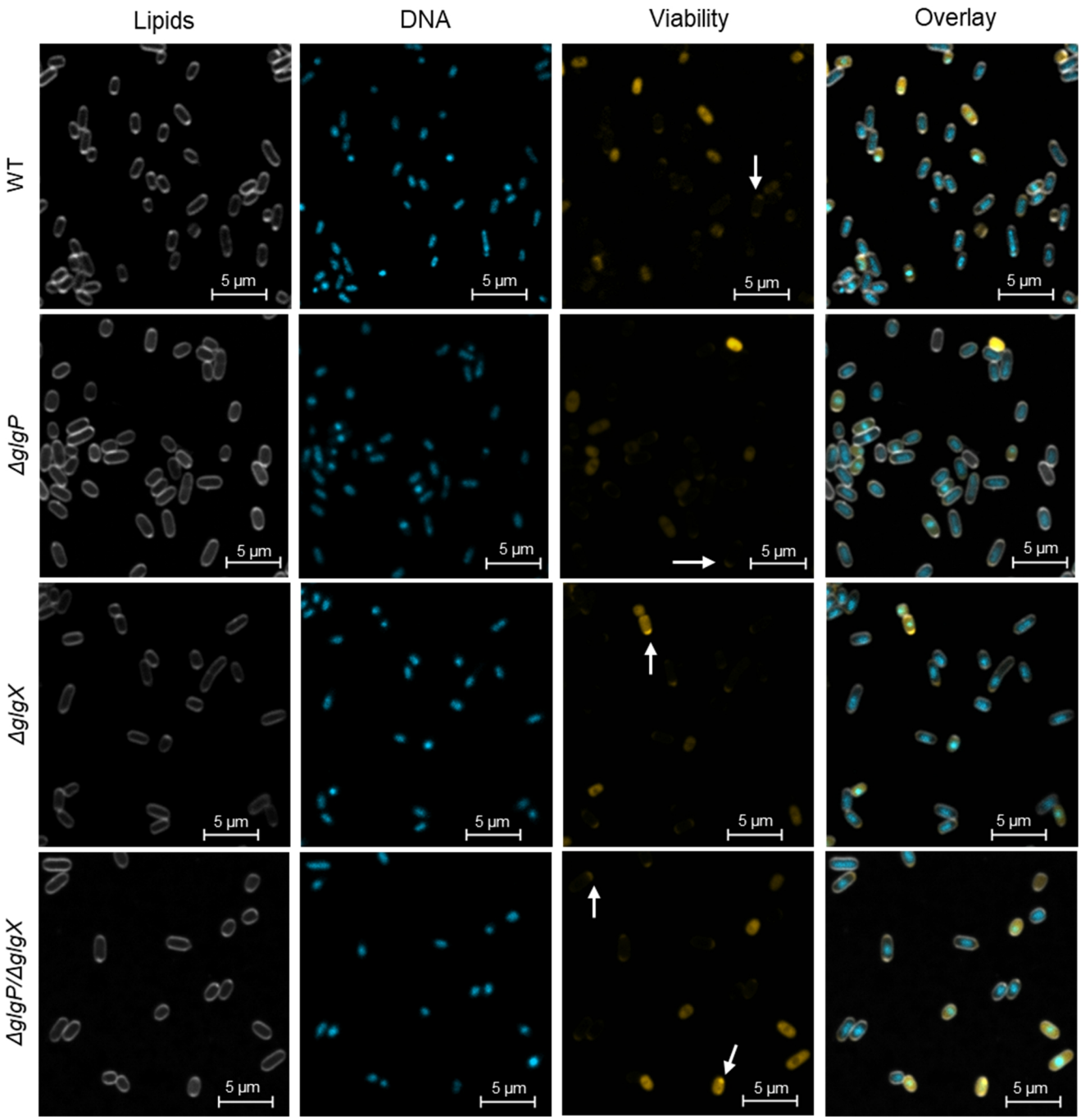


**Figure S4a** Representative confocal images of *E. coli* strain in late stationary phase. The cell periphery was assessed alongside DNA and proteins, shown here in white, light blue, and yellow, respectively. Arrows point to protein aggregates, highlighted by the amine-reactive dye used to assay viability.

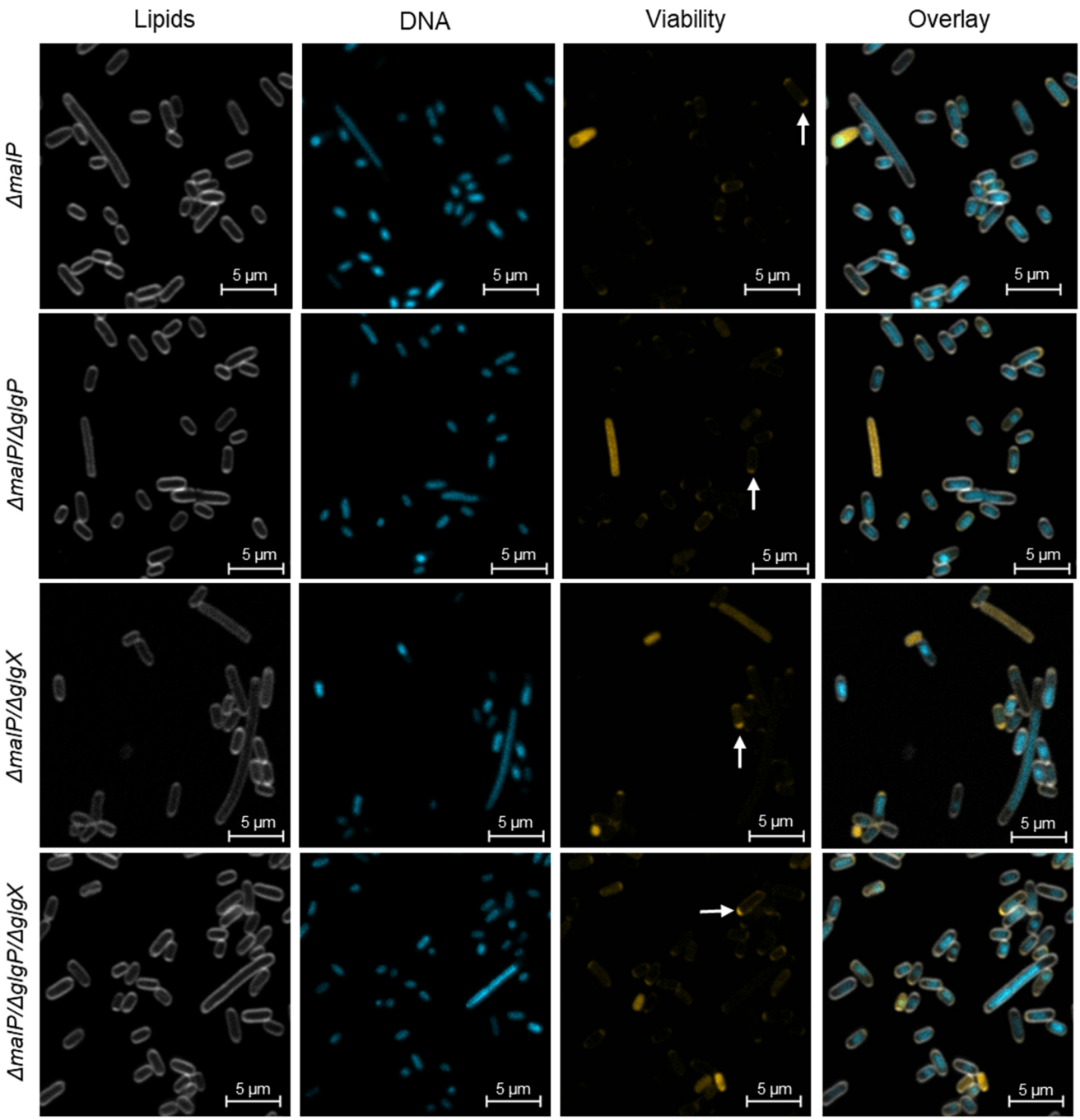


**Figure S4b** Representative confocal images of *E. coli* strain in late stationary phase. The cell periphery was assessed alongside DNA and proteins, shown here in white, light blue, and yellow, respectively. Arrows point to protein aggregates, highlighted by the amine-reactive dye used to assay viability.

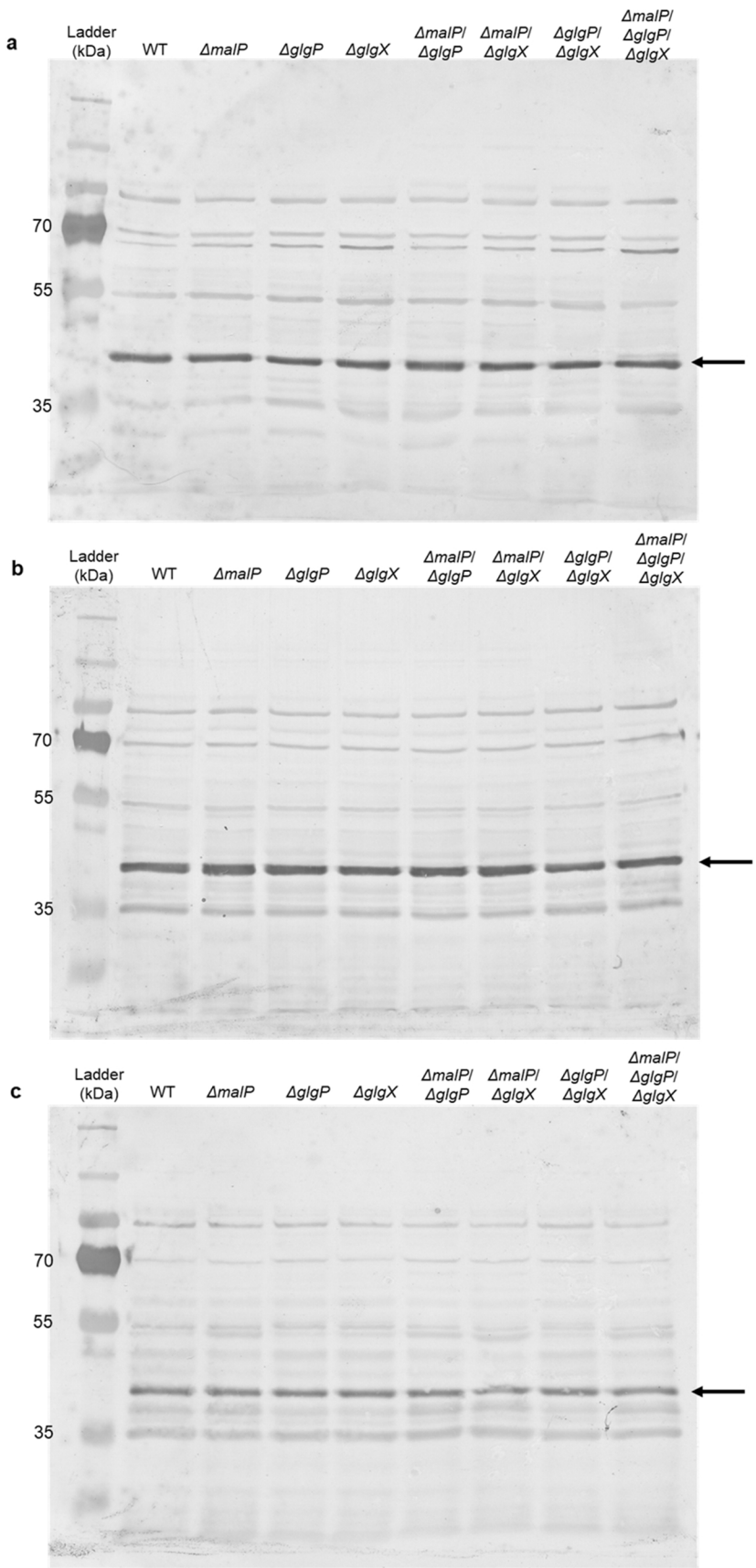


**Figure S5** Immunoblot analysis of relative FtsZ levels. Protein extracts were prepared from cells (**a**) growing exponentially, (**b**) approaching stationary phase or (**c**) cultured to late stationary phase. Ten µg total protein was loaded in each lane, separated by 10% (w/v) SDS-PAGE and blotted onto nitrocellulose membranes, before being probed with an α-FtsZ antibody. The arrow indicates the position of FtsZ (~40 kDa) as estimated from the protein molecular size marker (PageRuler Plus, Thermo Fisher Scientific).

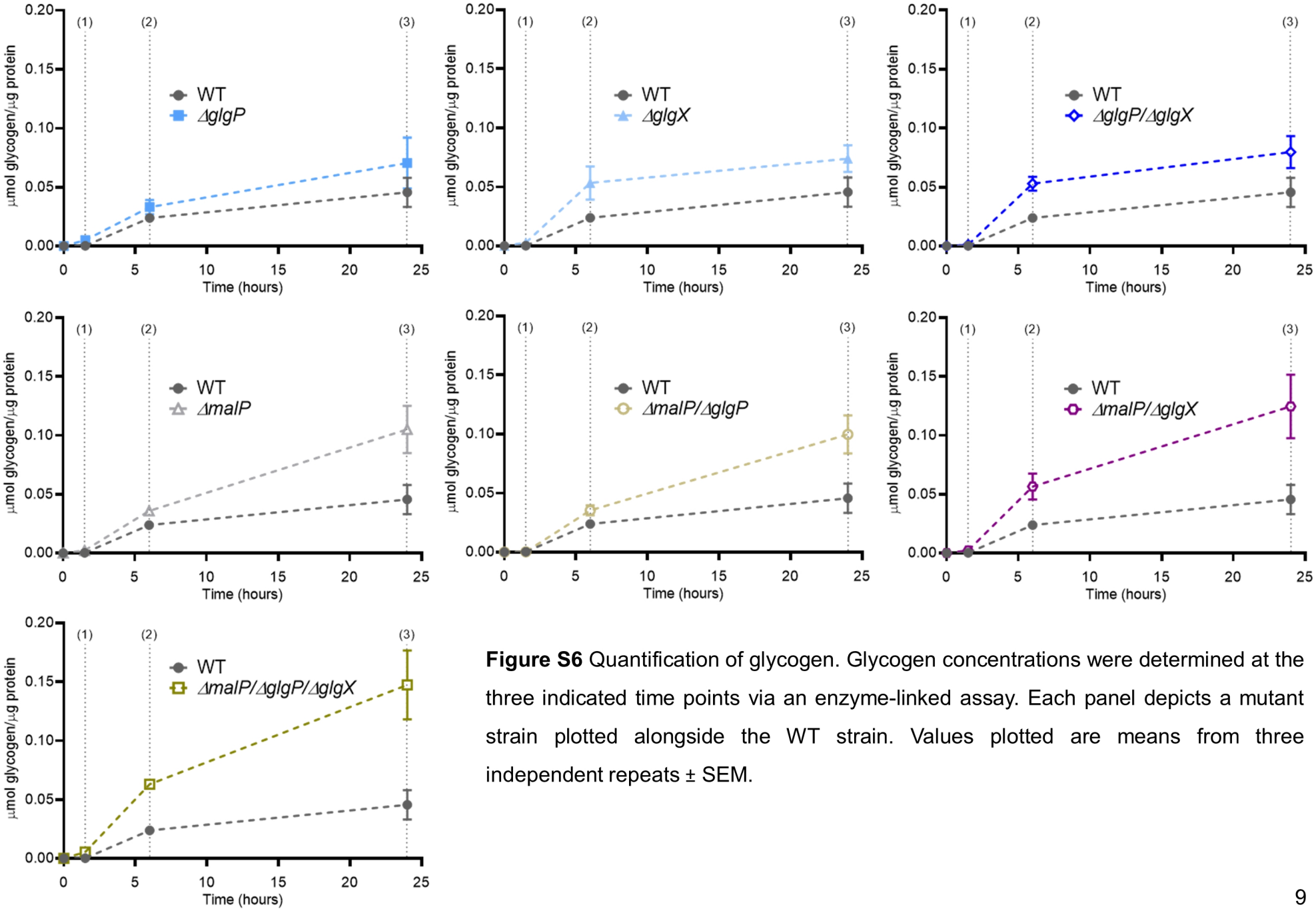


**Figure S6** Quantification of glycogen. Glycogen concentrations were determined at the three indicated time points via an enzyme-linked assay. Each panel depicts a mutant strain plotted alongside the WT strain. Values plotted are means from three independent repeats ± SEM.

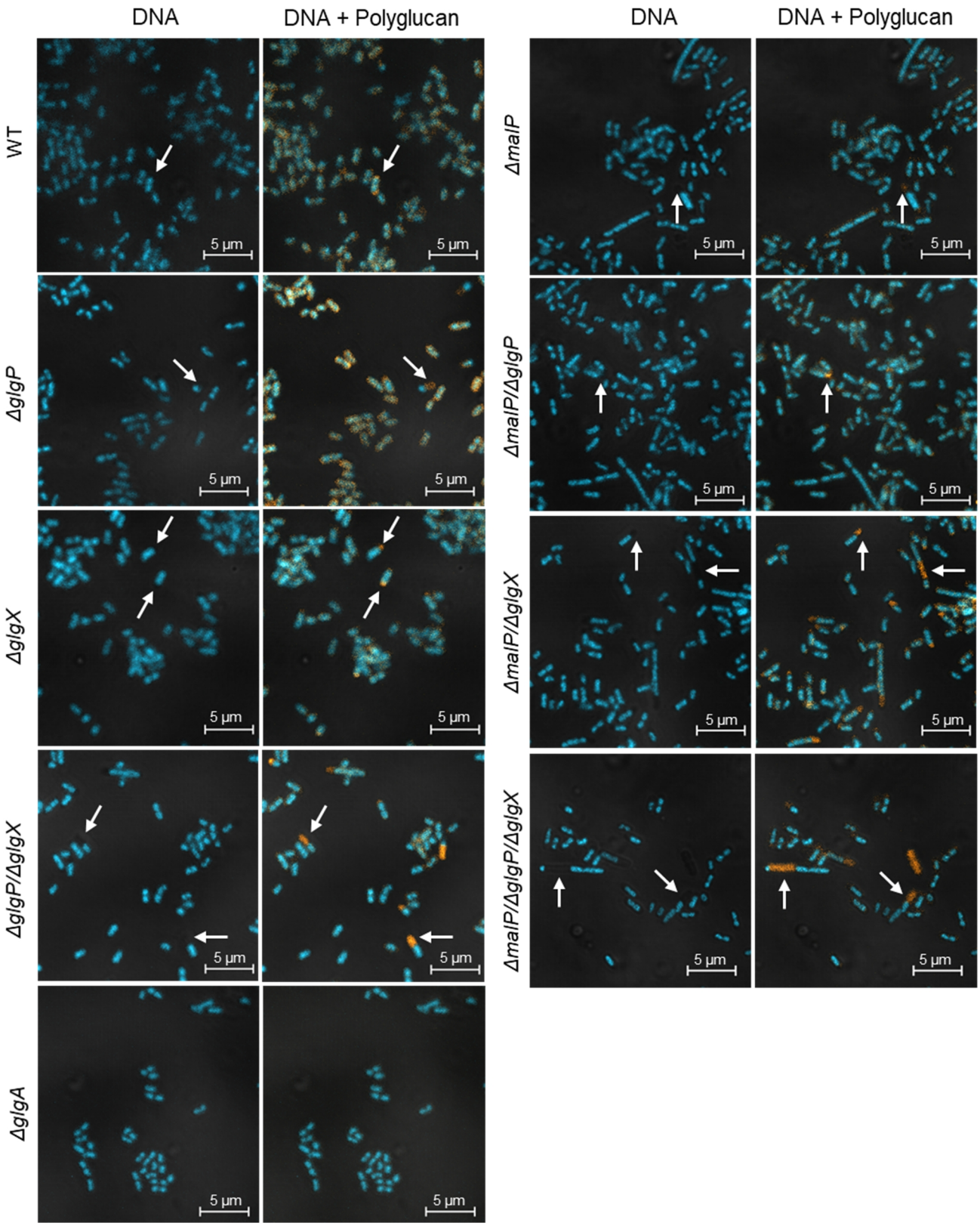


**Figure S7** Confocal and phase contrast imagery of PAS-stained *E. coli* cells entering stationary phase. PAS staining procedures were performed as described and polyglucan displayed in orange, while DAPI-stained DNA is shown in light blue. White arrows point to glycogen aggregates.

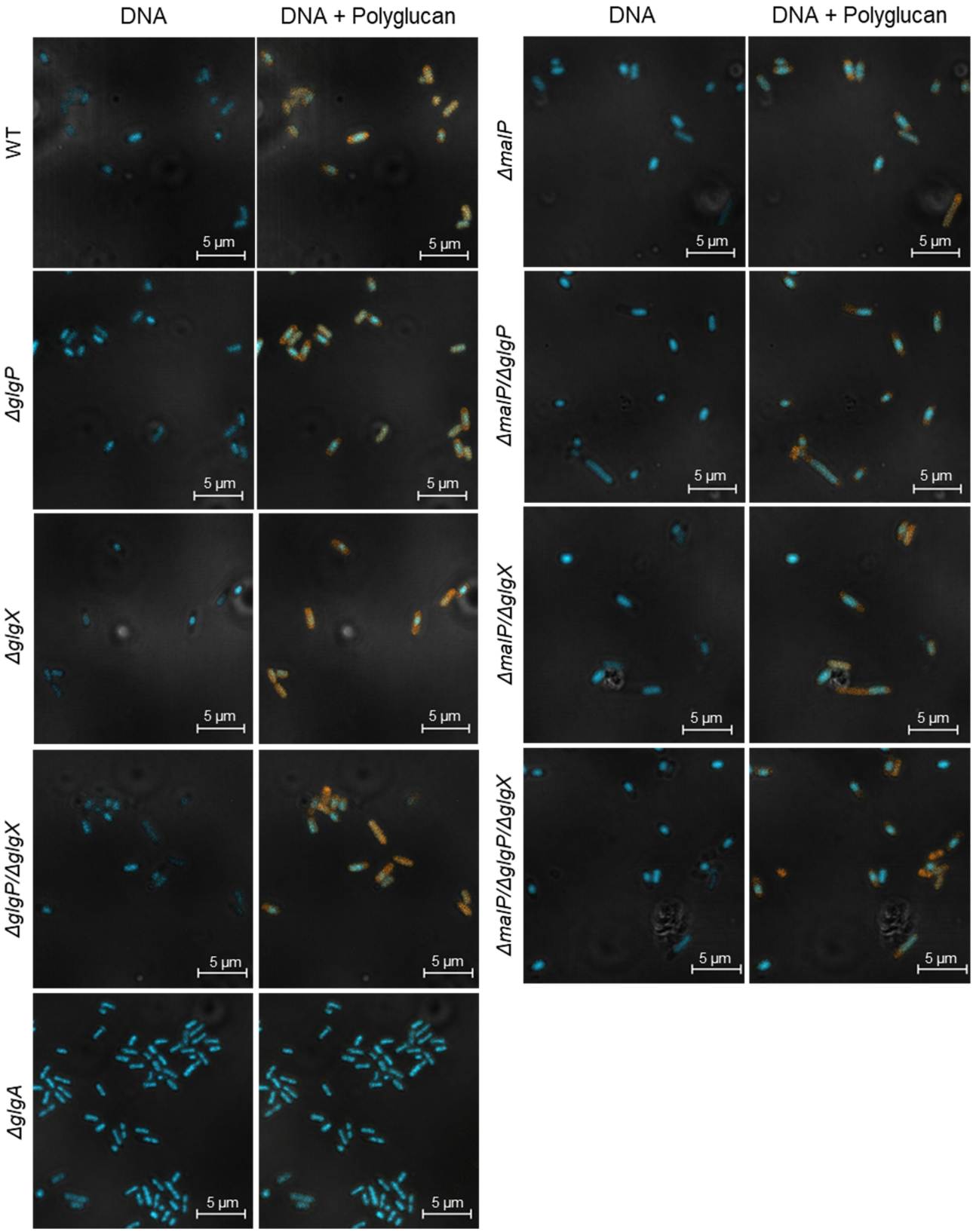


**Figure S8** Confocal and phase contrast imagery of PAS-stained *E. coli* cells in late stationary phase. PAS staining procedures were performed as described and polyglucan displayed in orange, while DAPI-stained DNA is shown in light blue. White arrows point to glycogen aggregates.

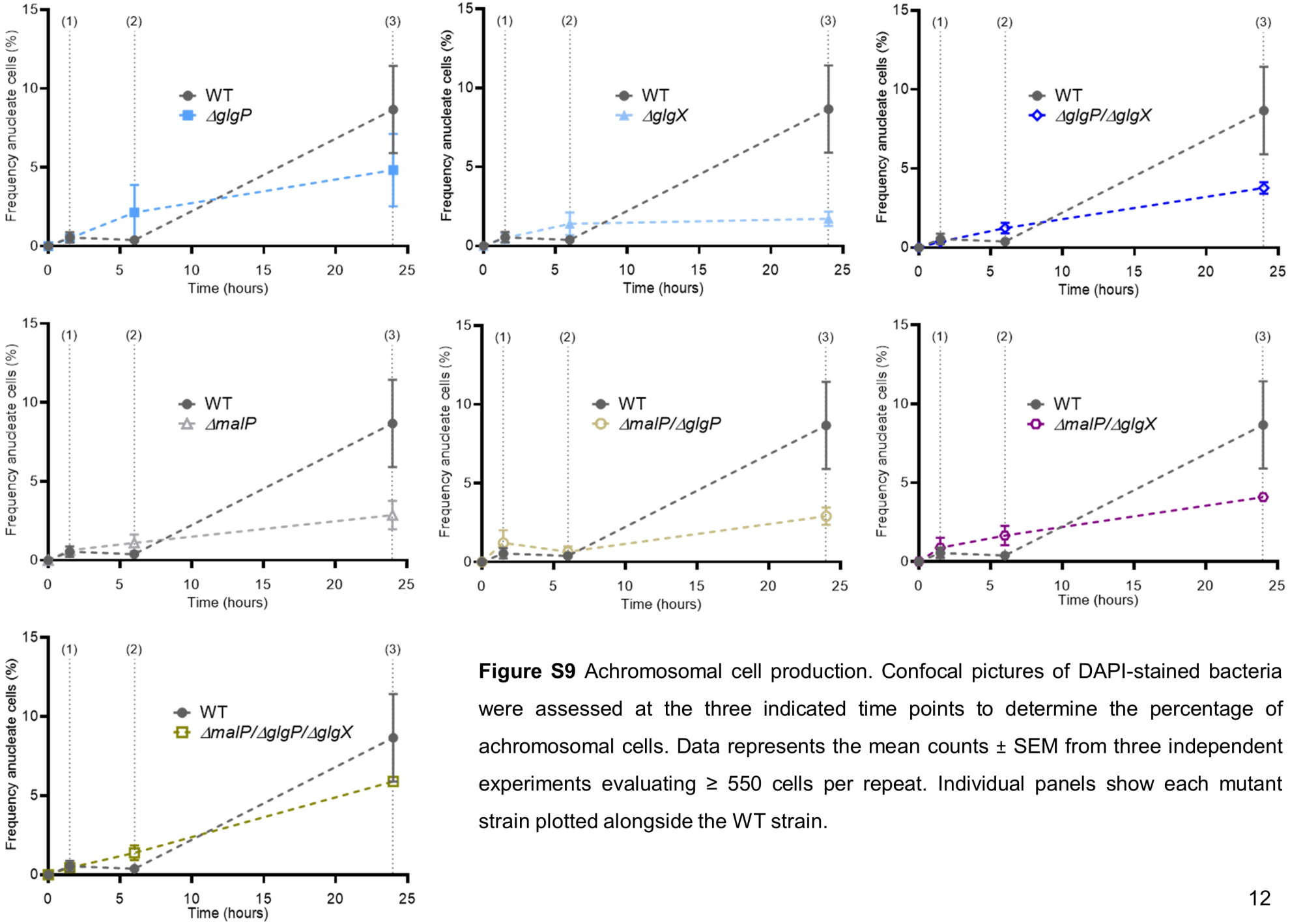


**Figure S9** Achromosomal cell production. Confocal pictures of DAPI-stained bacteria were assessed at the three indicated time points to determine the percentage of achromosomal cells. Data represents the mean counts ± SEM from three independent experiments evaluating ≥ 550 cells per repeat. Individual panels show each mutant strain plotted alongside the WT strain.

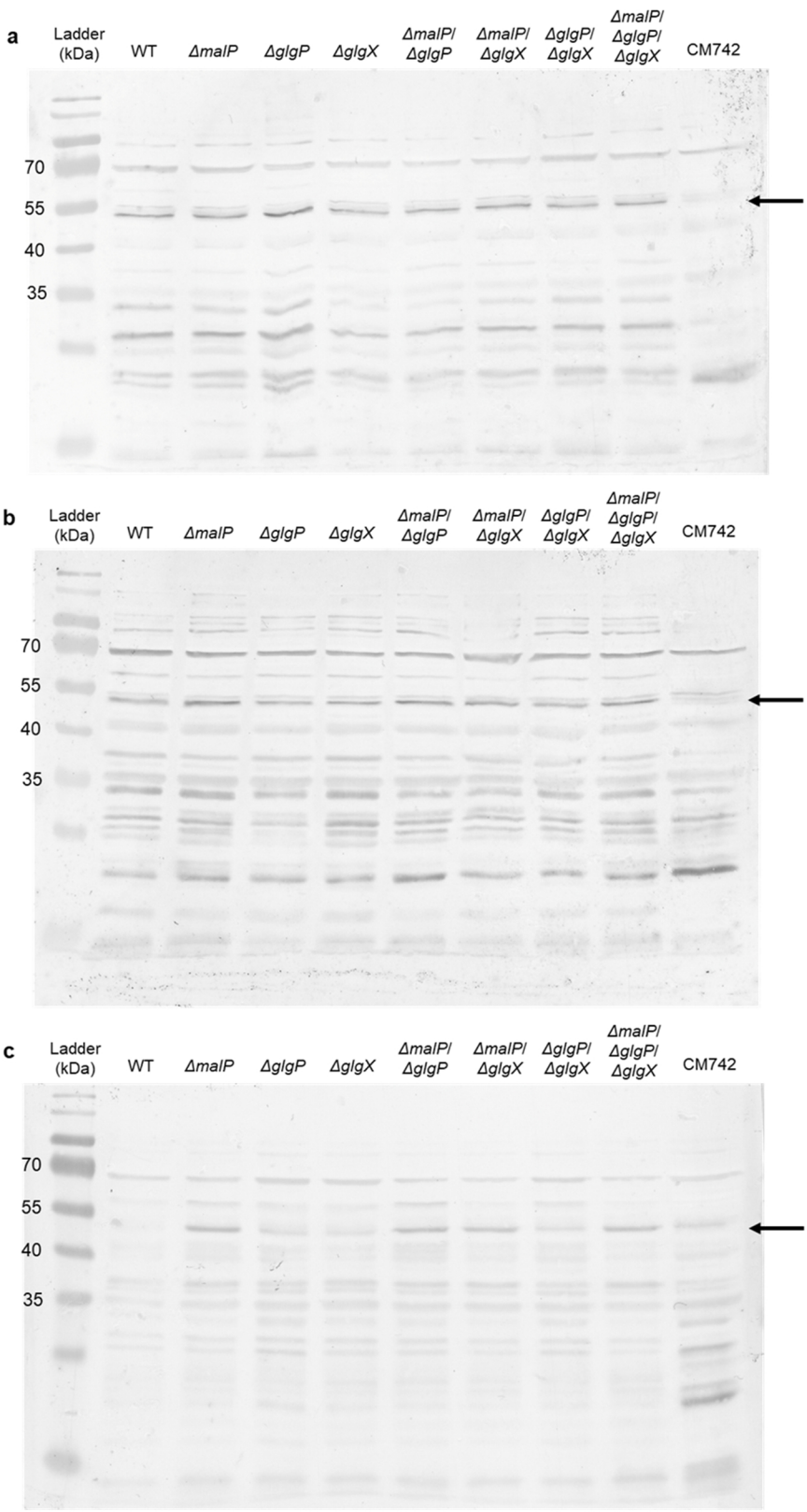


**Figure S10** Immunoblot examination of relative DnaA amounts. Soluble protein was extracted from cells (**a**) growing exponentially, (**b**) entering stationary phase or (**c**) grown late into stationary phase. Ten µg total protein was separated by 10% (w/v) SDS-PAGE and blotted onto nitrocellulose membranes, before being probed with an α-DnaA antibody. The arrow points to the position of DnaA (~53 kDa) as estimated from the protein molecular size marker (PageRuler, Thermo Fisher Scientific). An extract containing 10 µg of soluble protein from a mutant *dnaA46* strain (CM742), cultured exponentially under non-permissive conditions, was included as a negative control.