Supplemental material

Application of a genetically encoded biosensor for live cell imaging of L-valine production in pyruvate dehydrogenase complex-deficient *Corynebacterium glutamicum* strains

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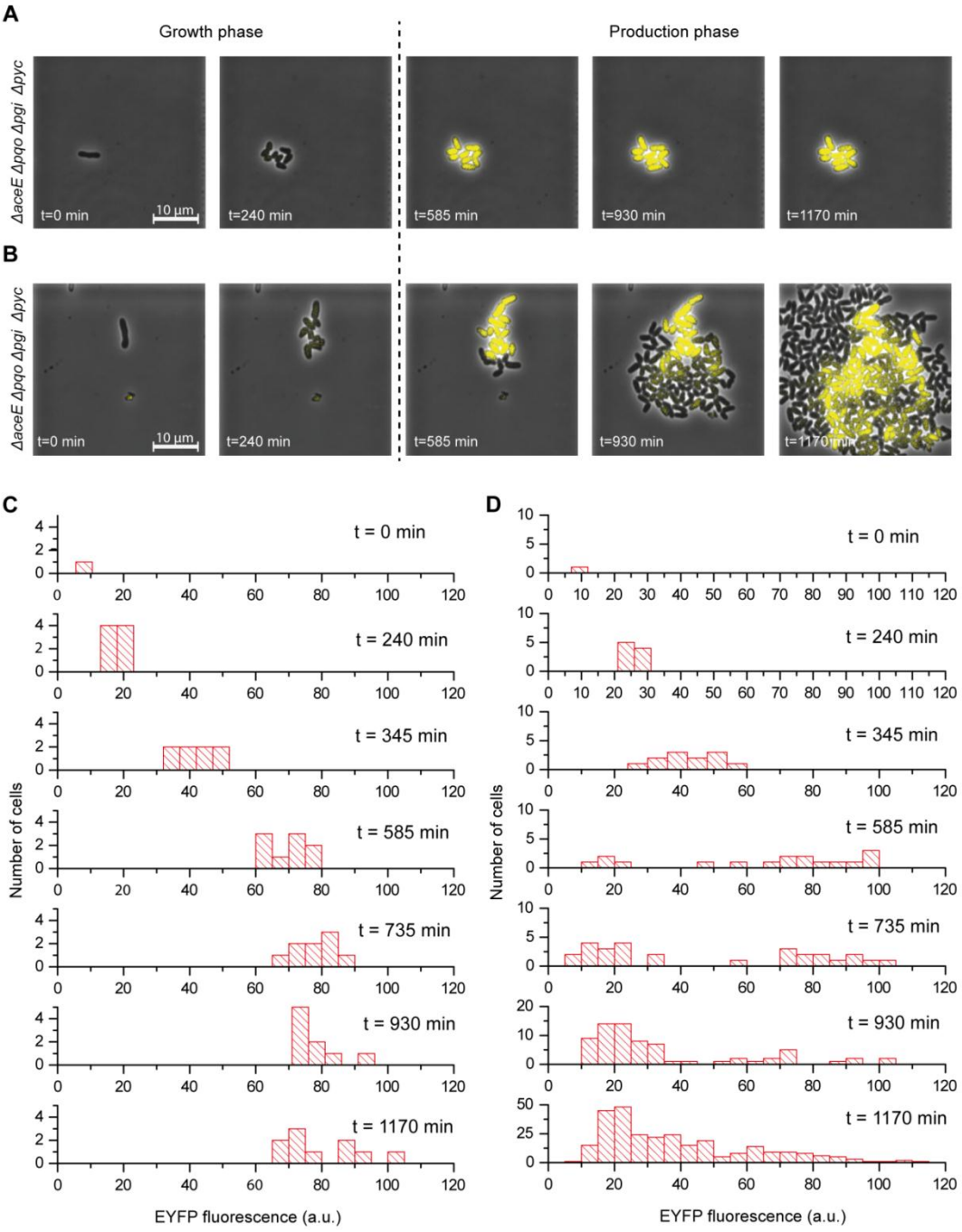

Figure S1. Phenotypic heterogeneity of the Δ*aceE* Δ*pqo* Δ*pgi* Δ*pyc* sensor strain upon switch from growth to production phase. (A) Microcolony showing transition to producing cells or **(B)** a mixture of growing and producing cells after medium switch (initiated after 240 min). In approximately 50% of the recorded colonies one or several single cells continued growth after medium switch. **(C, D)** Fluorescence histograms depicting single cell fluorescence to selected times during growth (0-240 min) and production phase (0-1200 min) of the microcolonies shown in A **(C)** and B **(D)**. Cultivation was performed in CGXII minimal medium containing 154 mM acetate, 222 mM glucose and 0.5% BHI during growth phase or 222 mM glucose and 0.5% BHI during production phase, respectively.

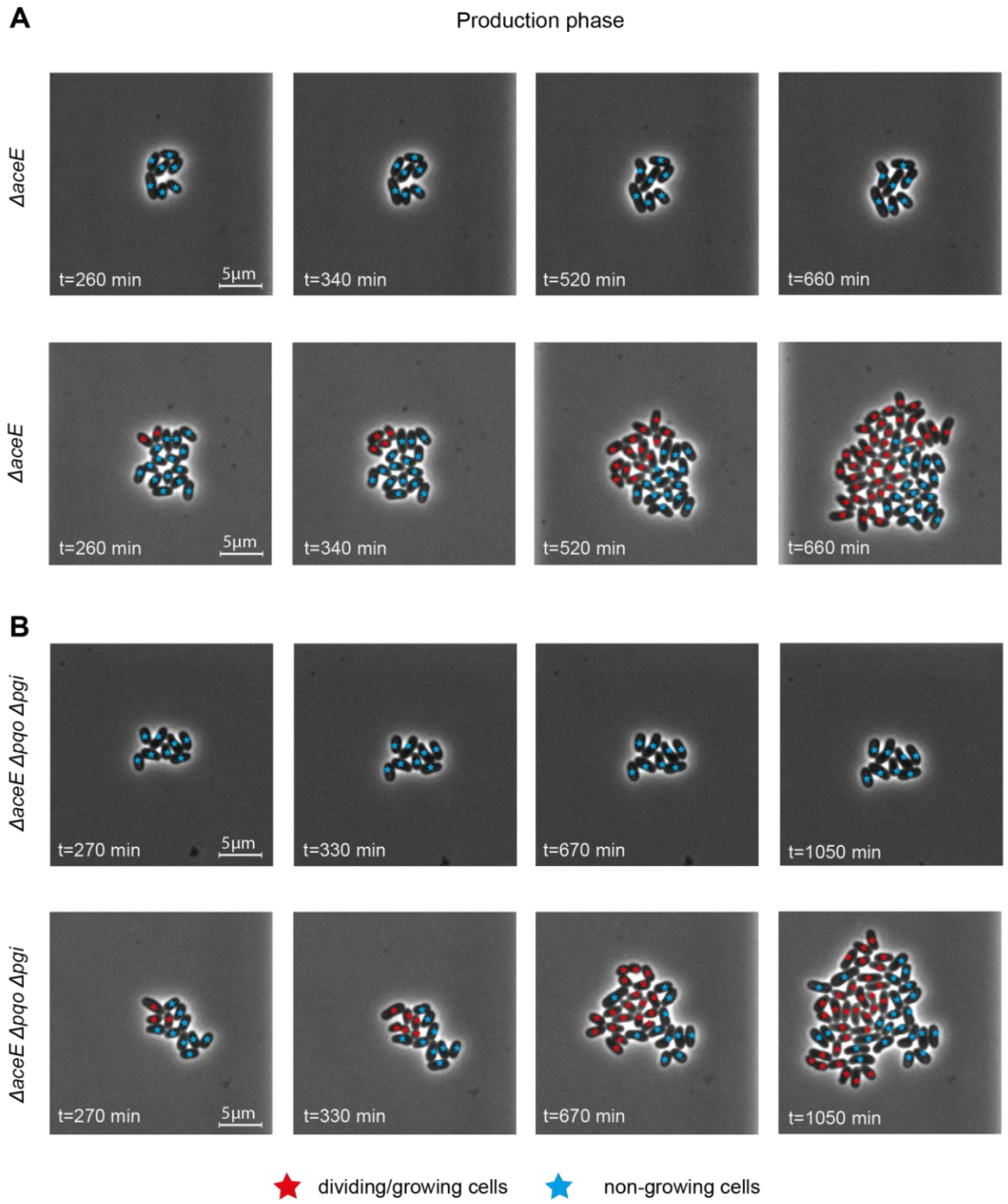

Figure S2. Phenotypic heterogeneity of Δ*aceE* and Δ*aceE* Δ*pqo* Δ*pgi* upon switch from growth to production phase. (A) Δ*aceE* microcolonies where all cells stopped growth (blue stars) upon transition to the production phase (upper row) or a mixture of growing (red stars) and non-growing cells (lower row) after initiation of the production phase. In approximately 50% of the recorded colonies one or several single cells continued growth after medium switch (initiated after 250 min). **(C)** Δ*aceE* Δ*pqo* Δ*pgi* microcolonies. In the upper row, all cells stopped growth whereas in the lower row a microcolony is shown were some cells continued growth after initiation of the production phase. In approximately 50% of the recorded colonies one or several single cells continued growth after medium switch (initiated after 250 min). These findings confirm that the phenotypic split shown in Figure 5 is not due to the presence of the Lrp-sensor. Cultivation was performed in CGXII minimal medium containing 154 mM acetate, 222 mM glucose and 0.5% BHI during growth phase or 222 mM glucose and 0.5% BHI during production phase, respectively.

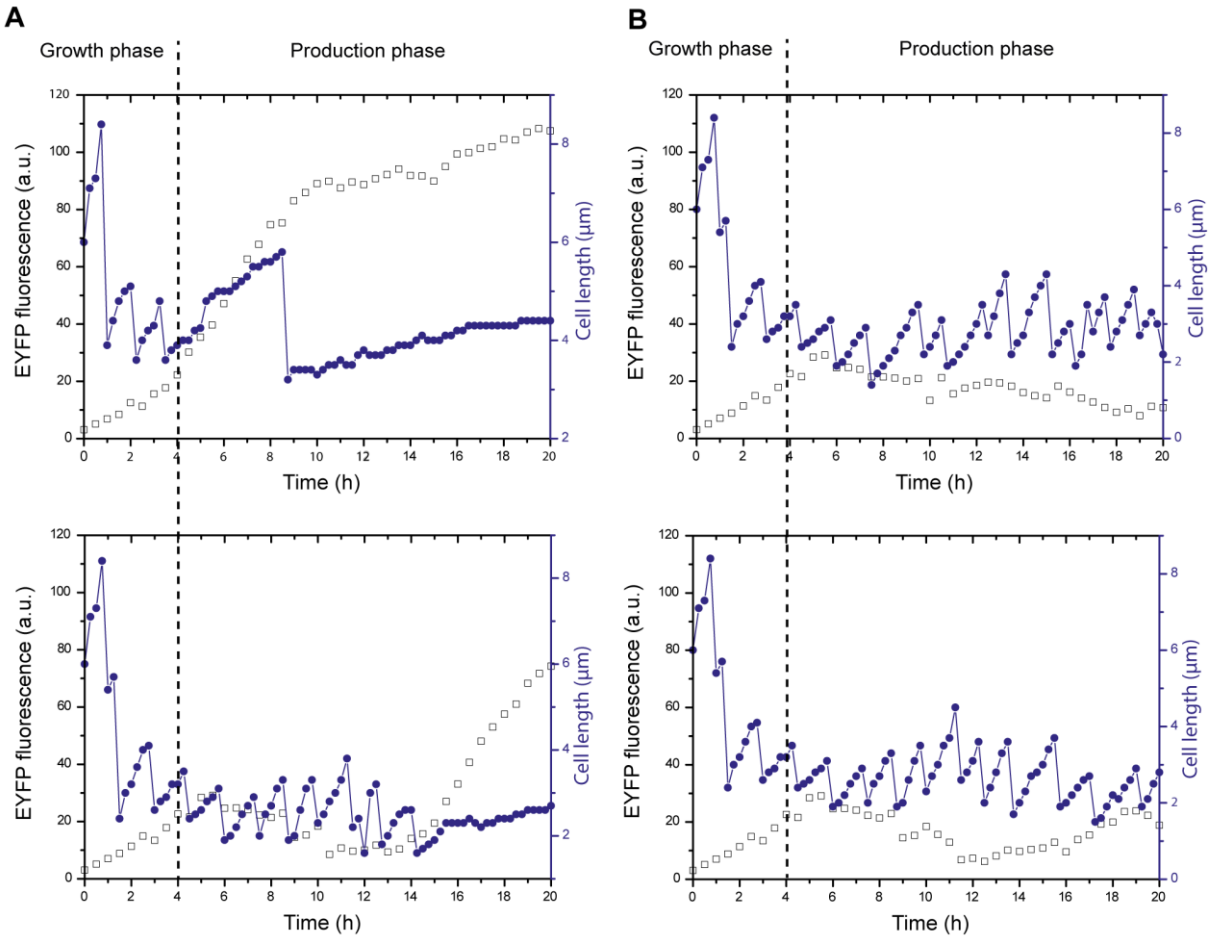

Figure S3. Single cell traces of the Δ*aceE* Δ*pqo* Δ*pgi* Δ*pyc* sensor strain upon switch from growth to production phase. (A) Single cell traces showing the switch from growth (cell length=blue line) to production (fluorescence=squares) after several cell divisions during production phase (t=8.5 h, t=15.0 h). **(B)** Single cell traces showing no switch from growth to production. Single cell traces are taken from the cultivation of Δ*aceE* Δ*pqo* Δ*pgi* Δ*pyc* sensor strain shown in Figure S1.