eLIFE
elifesciences.org

Figures and figure supplements

A serine sensor for multicellularity in a bacterium

Arvind R Subramaniam, et al.

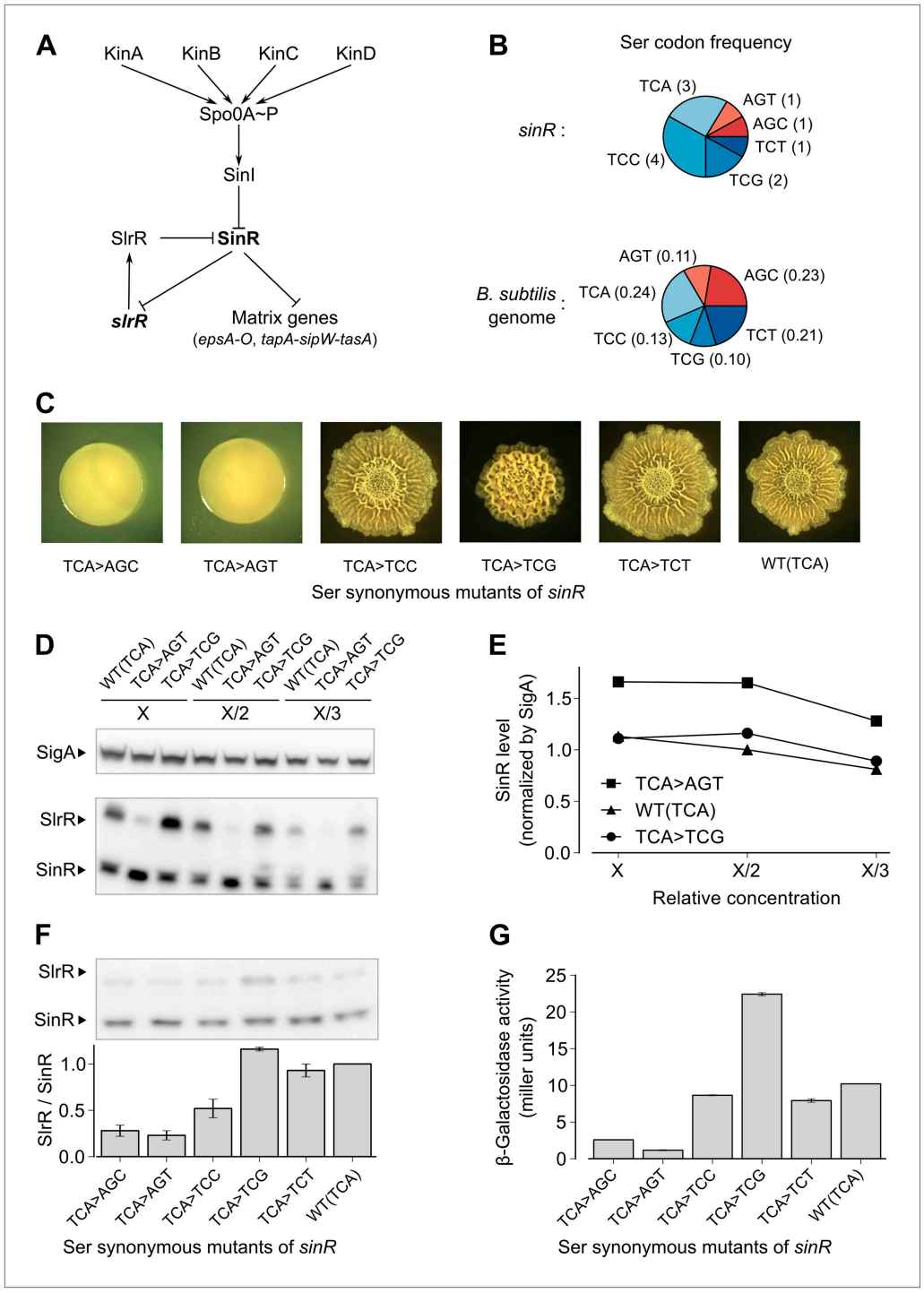

Figure 1. Switching synonymous serine codons in *sinR* affects biofilm formation. (**A**) Regulatory circuit controlling biofilm formation in *B. subtilis*. (**B**) Top: Serine codon usage in the *sinR* coding sequence. Number within parenthesis indicates the frequency of the corresponding codon in *sinR*. Bottom: Average serine codon usage across 4153 protein-coding sequences in the *B. subtilis* genome. Number within parenthesis indicates the relative frequency of each codon in the genome. (**C**) Colony morphology for the wild-type strain and the indicated *sinR* synonymous variants grown on solid biofilm-inducing medium. Three TCA codons in the wild-type sequence of *sinR* were switched to each of the other five serine codons. The wild-type (WT) *sinR* sequence was replaced by the *sinR* synonymous mutant at the native *sinR* locus of the strain 3610. (**D**) SinR protein level during entry into biofilm formation (OD_{600} = 2) measured using an anti-SinR antibody that also cross-reacts with SlrR, a protein that is 85%

Figure 1. Continued on next page

Figure 1. Continued

identical to SinR. Western blot against the RNA polymerase subunit SigA was used as the loading control. Whole cell lysates were loaded at different dilutions (indicated as X, X/2, and X/3). (**E**) Densitometry of SinR bands in (**D**) after normalization by SigA. (**F**) Top panel: Western blot against SinR and SlrR using anti-SinR antibody. Bottom panel: Densitometry ratio of the SlrR and SinR bands in the top panel. Error bars represent standard error over three replicate Western blots. The SlrR/SinR ratio for each blot was normalized such that the wild-type strain had a ratio of 1. (**G**) Matrix gene expression monitored using a P_{epsA}–*lacZ* transcriptional reporter inserted at the chromosomal *amyE* locus. *β*-galactosidase activity was measured at OD_{600} = 2 in liquid biofilm-inducing medium. Error bars represent standard error of three measurements.

DOI: 10.7554/eLife.01501.003

```
  1 - ttg att ggc cag cgt att aaa caa tac cgt aaa gaa aaa ggc tac tca cta tca gaa cta
      M   I   G   Q   R   I   K   Q   Y   R   K   E   K   G   Y   S   L   S   E   L

 61 - gct gaa aaa gct ggg gta gcg aag tct tat tta agc tca ata gaa cga aat cta caa acg
      A   E   K   A   G   V   A   K   S   Y   L   S   S   I   E   R   N   L   Q   T

121 - aac ccc tcc att caa ttt ctc gaa aaa gtc tcc gct gtt ctg gac gtc tcg gtt cat act
      N   P   S   I   Q   F   L   E   K   V   S   A   V   L   D   V   S   V   H   T

181 - ttg ctc gat gag aaa cat gaa acc gaa tac gat ggt caa tta gat agt gaa tgg gag aaa
      L   L   D   E   K   H   E   T   E   Y   D   G   Q   L   D   S   E   W   E   K

241 - ttg gtt cgc gat gcg atg aca tcc ggg gta tcg aaa aaa caa ttt cgt gaa ttt tta gat
      L   V   R   D   A   M   T   S   G   V   S   K   K   Q   F   R   E   F   L   D

301 - tat caa aaa tgg aga aaa tcc caa aaa gag gag tag
      Y   Q   K   W   R   K   S   Q   K   E   E   *
```

Figure 1—figure supplement 1. *sinR* coding sequence. The three TCA codons (switched in ***Figure 1***) are highlighted in red. The three TCC codons and the two AGC/AGT codons (switched in ***Figure 1—figure supplement 2***) are highlighted in green and blue respectively. The remaining serine codons are shown in yellow.

DOI: 10.7554/eLife.01501.004

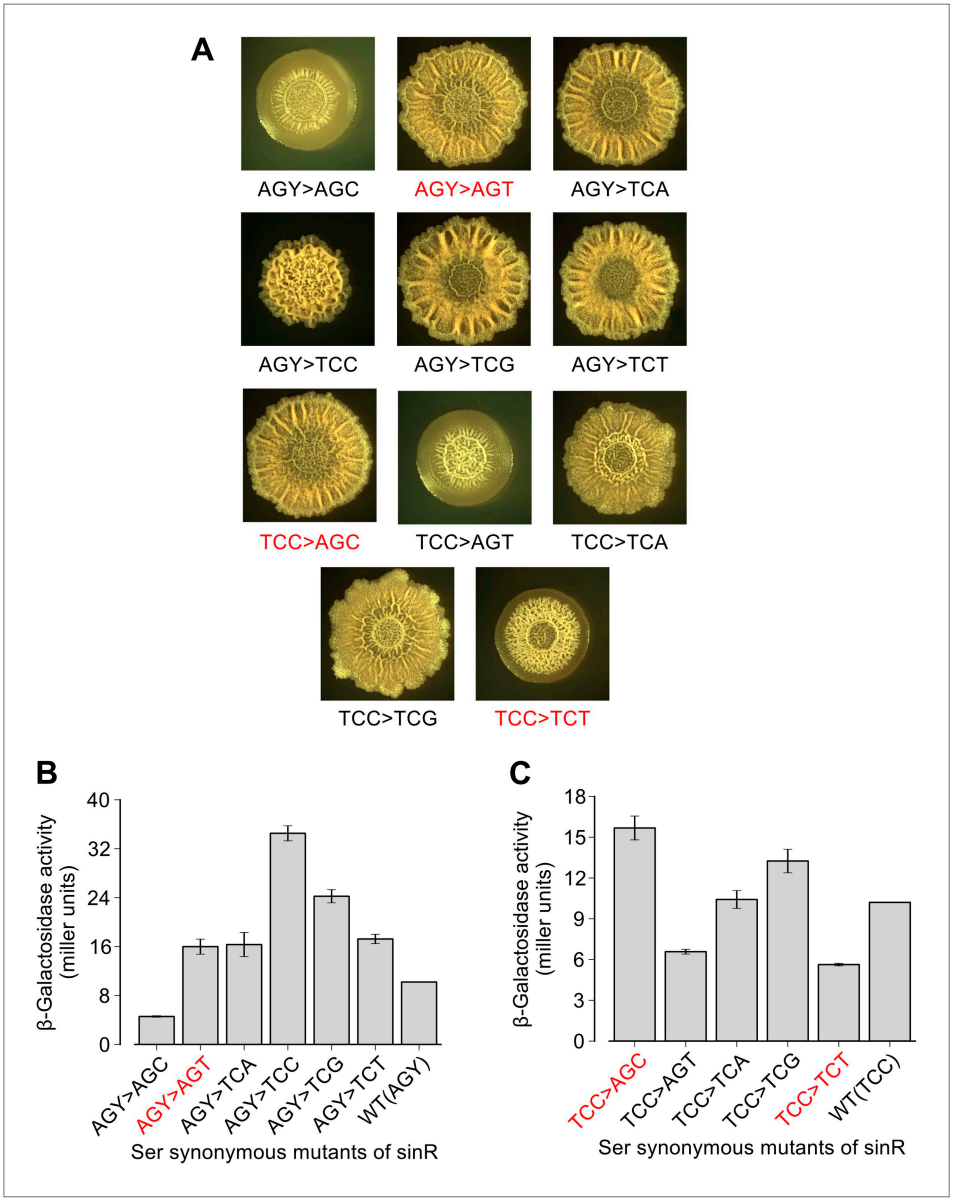

Figure 1—figure supplement 2. Effect of TCC and AGC/AGT synonymous substitutions in the *sinR* gene on colony morphology and biofilm reporter activity. (**A**) Colony morphology for the indicated *sinR* synonymous variants grown on solid biofilm-inducing medium. Either three TCC codons or two AGC/AGT (AGY) codons in the wild-type sequence of *sinR* were switched to remaining serine synonymous codons. The wild-type (WT) *sinR* sequence was replaced by the *sinR* synonymous variant at the native *sinR* locus of the strain 3610. Colony morphology of the wild-type strain is shown in ***Figure 1***. (**B** and **C**) Matrix gene expression monitored using a P_{epsA}–*lacZ* transcriptional reporter inserted at the chromosomal *amyE* locus. Strains were grown in liquid biofilm-inducing medium and *β*-galactosidase activity was measured at an OD_{600} = 2. Error bars represent standard error of three measurements. The synonymous variants highlighted in red do not follow the hierarchy between TCN and AGC/AGT codons seen for the six TCA synonymous variants in ***Figure 1***.

DOI: 10.7554/eLife.01501.005

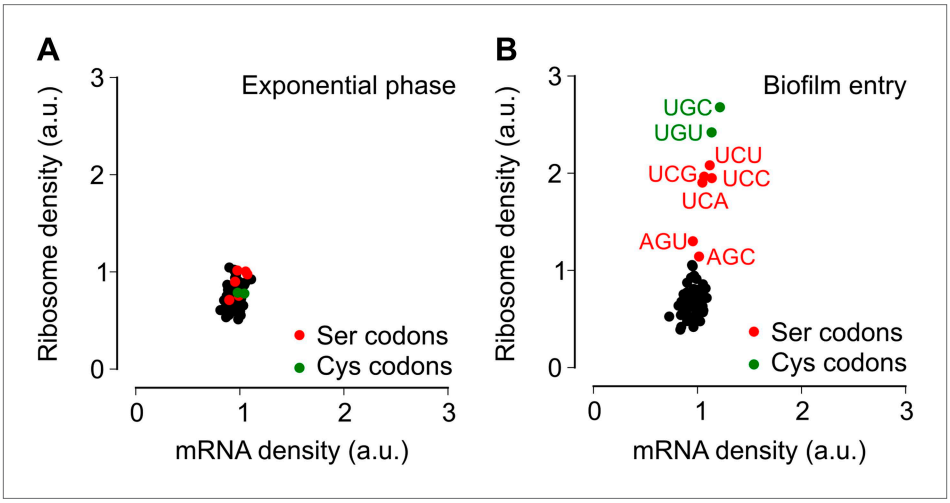

Figure 2. Entry into biofilm formation is accompanied by codon-specific increase in ribosome density. Genome-wide median ribosome density and total mRNA density at 61 sense codons (excluding start and stop codons) (**A**) during exponential phase growth (OD_{600} = 0.6), and (**B**) during stationary phase when biofilm formation is induced (OD_{600} = 1.4). The six serine (red) and two cysteine (green) codons are highlighted. Genome-wide ribosome density and total mRNA density were measured by deep-sequencing of ribosome-protected mRNA fragments and total mRNA fragments respectively, of a *B. subtilis* 3610 derivative (Δ*epsH*) grown in liquid biofilm-inducing medium.
DOI: 10.7554/eLife.01501.006

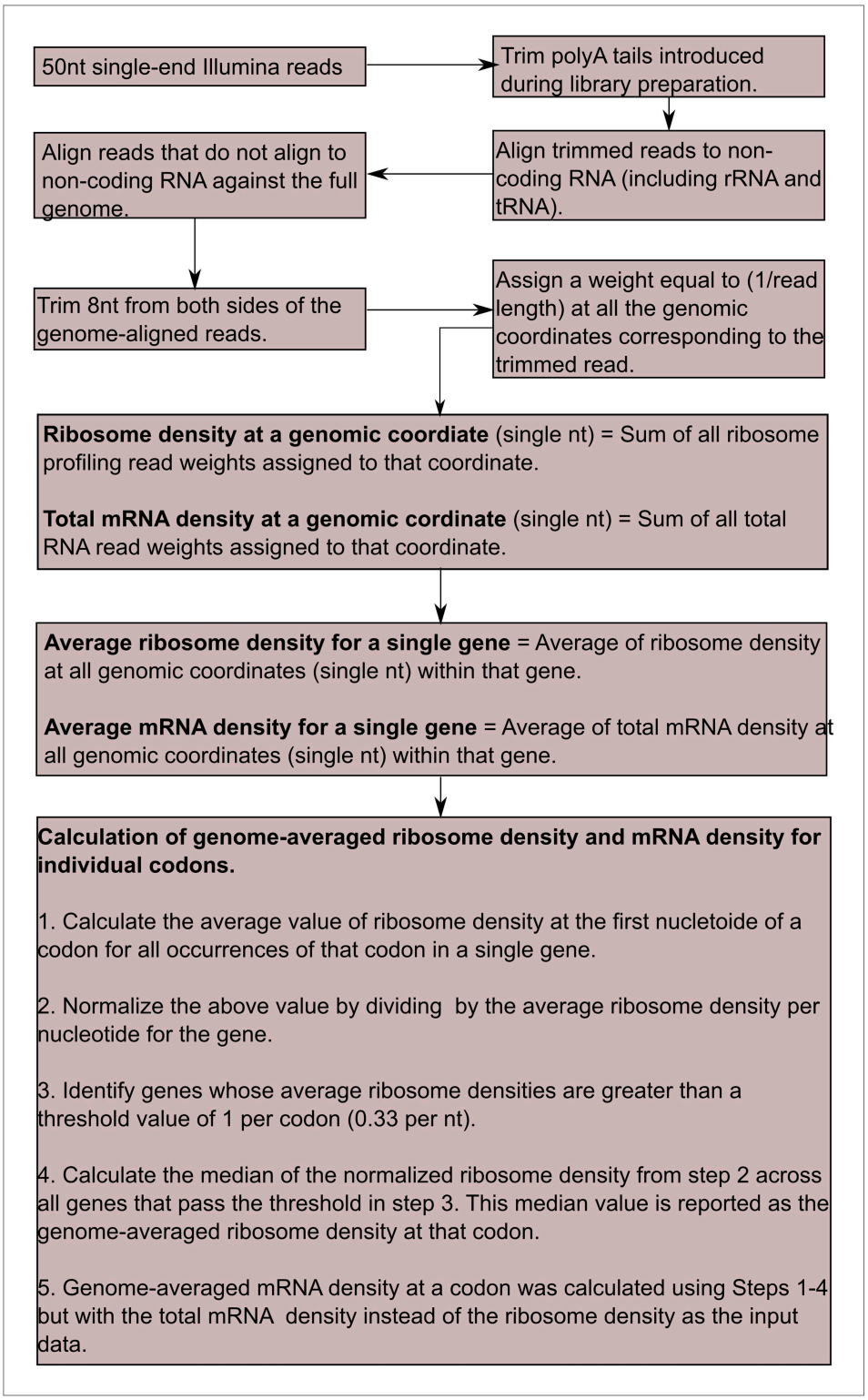

Figure 2—figure supplement 1. Computational workflow for deep-sequencing data analysis. All steps outlined here were performed in Bash and Python programming languages. For further details on individual steps, see 'Materials and methods'.

DOI: 10.7554/eLife.01501.007

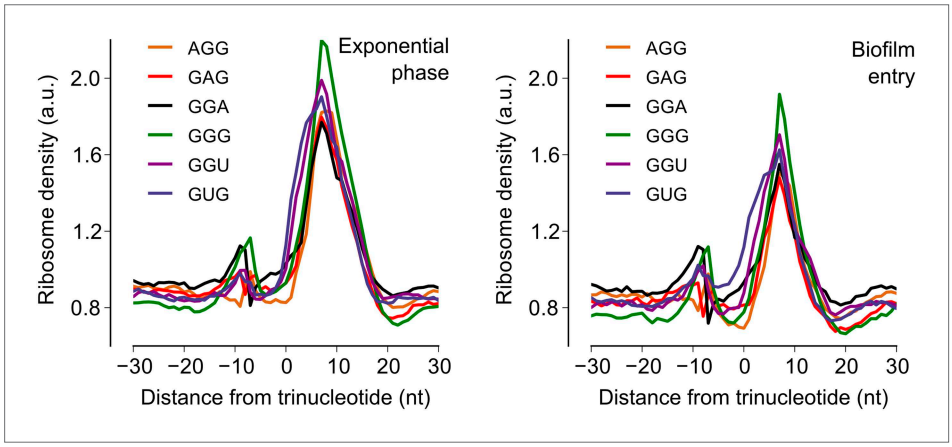

Figure 2—figure supplement 2. Increase in ribosome density downstream of Shine-Dalgarno-like trinucleotide sequences. Median ribosome density across all protein coding sequences was computed for the 60 nt region around each of six Shine-Dalgarno-like trinucleotide sequences (***Li et al., 2012***) for the exponential phase sample (left-hand panel) and the biofilm entry sample (right-hand panel).

DOI: 10.7554/eLife.01501.008

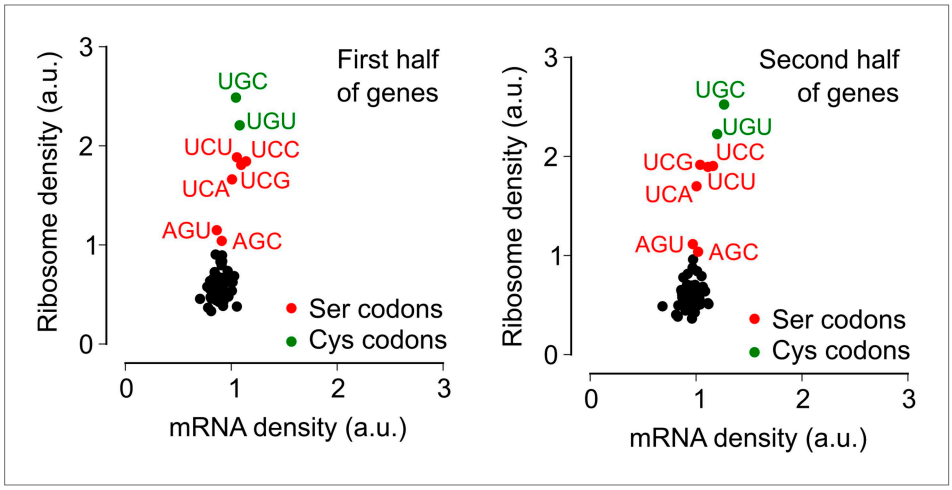

Figure 2—figure supplement 3. Context independence of ribosome and mRNA densities during biofilm formation. Each gene was conceptually divided into two equal halves and the ribosome density and mRNA density was computed separately for codons located either in the first half (left-hand panel) or in the second half (right-hand panel) of each gene. All other analysis steps were identical to those in ***Figure 2***.

DOI: 10.7554/eLife.01501.009

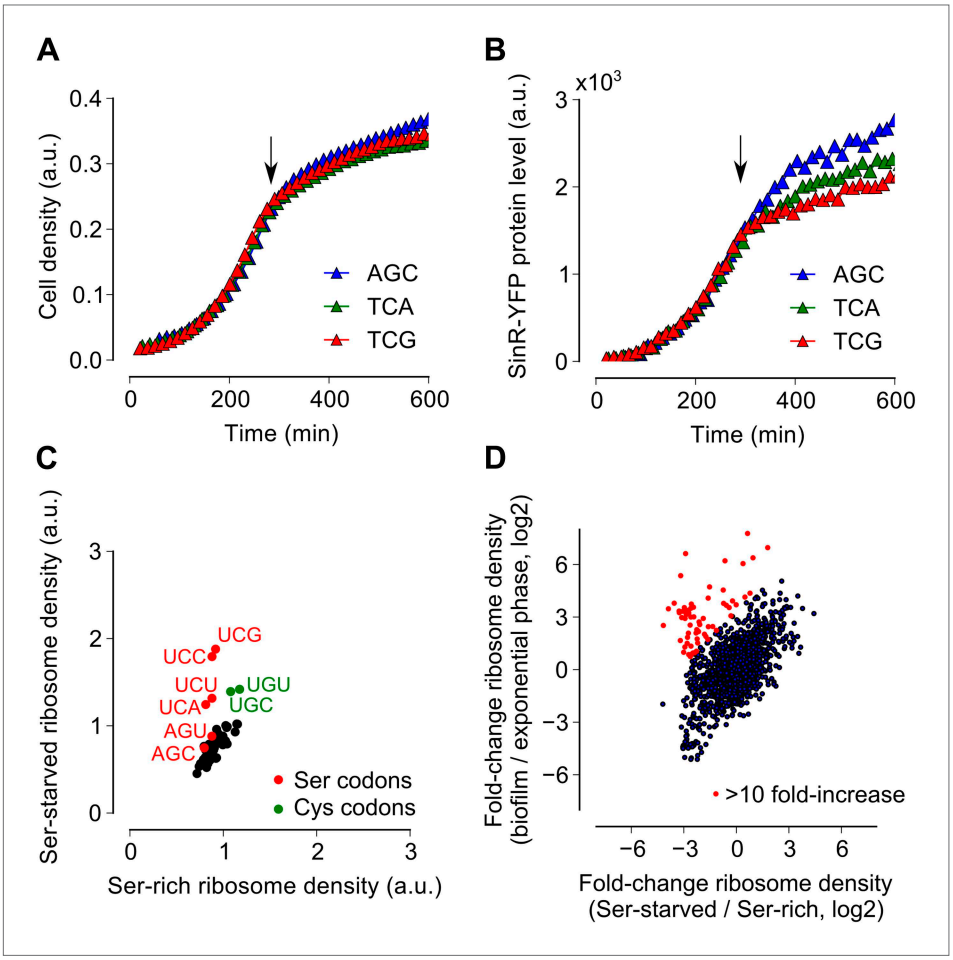

Figure 3. Serine starvation reduces translation speed and inhibits SinR synthesis in a codon-specific manner. (**A** and **B**) Three *sinR* synonymous variants were synthesized with 10 serine codons switched to AGC, TCA or TCG. The variants were expressed as SinR-YFP fusions from the *amyE* locus under the control of a *lac* promoter in a 3610-Δ*serA* serine auxotroph strain growing in serine-limited medium. Black arrow around 300 min indicates the onset of serine starvation caused by depletion of exogenously-added serine in the growth medium. Cell density (**A**) and the corresponding SinR-YFP protein level (**B**) were monitored using a 96-well spectrophotometer. (**C**) Genome-wide median ribosome density for 61 sense codons (excluding start and stop codons) during serine starvation (vertical axis) and serine-rich growth (horizontal axis) of a serine auxotrophic strain. (**D**) Fold-change in average ribosome density for individual genes upon biofilm entry (vertical axis) or serine starvation (horizontal axis). Genes that were preferentially up-regulated at least 10-fold upon biofilm entry in comparison to serine starvation are highlighted in red (68 genes, ***Table 1***). Only genes with a minimum of 100 ribosome profiling reads in at least one of the samples were included in this analysis (1724 genes) and the reported log2 fold-changes are median-subtracted values across this gene set.

DOI: 10.7554/eLife.01501.010

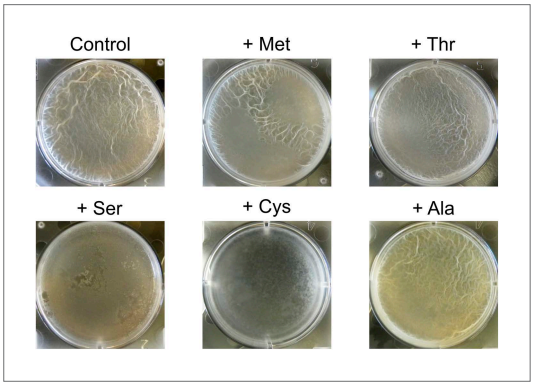

Figure 3—figure supplement 1. Addition of excess serine or cysteine blocks pellicle formation by *B. subtilis*. Single amino acids were added at 300 μg ml^{-1} to liquid MSgg medium. Biofilm formation of 3610 was assayed visually by pellicle formation at the air-liquid interface 48 hr after inoculation. Serine and cysteine were found to block pellicle formation out of all 20 amino acids tested.

DOI: 10.7554/eLife.01501.011

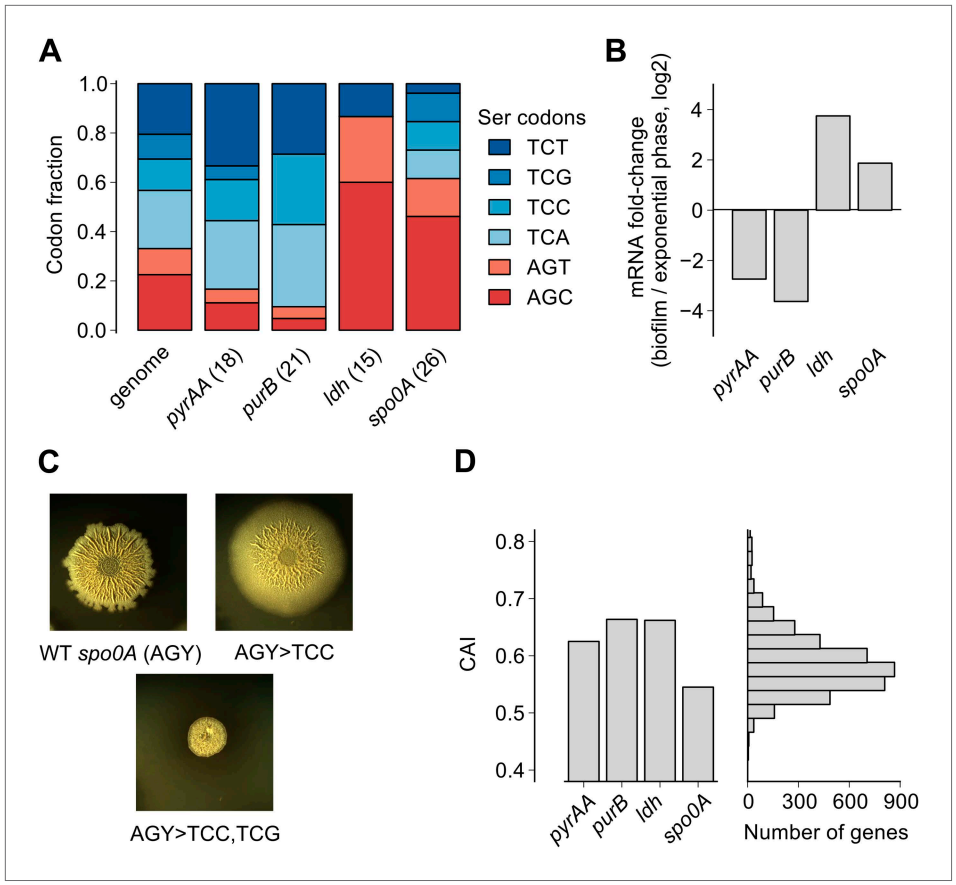

Figure 4. Serine codon bias of biofilm-regulated genes reflects their expression under serine starvation. (**A**) Relative serine codon fraction in genes for nucleotide biosynthesis (*pyrAA*, *purB*), lactate dehydrogenase (*ldh*) and a sporulation regulator (*spo0A*). Numbers in parentheses indicate the number of serine codons in each gene. Relative fraction of serine codons across the *B. subtilis* genome is shown for comparison. (**B**) Fold-change (expressed in log2 units) in average ribosome density upon biofilm entry for the four genes shown in **A**. (**C**) Colony morphology of a wild-type strain and two *spo0A* synonymous variants grown on solid biofilm-inducing medium. Seven AGC/AGT codons in wild-type *spo0A* were replaced by either 7 TCC codons or 3 TCC and 4 TCG codons and inserted at the chromosomal *spo0A* locus. Both the wild-type *spo0A* and the synonymous *spo0A* variants were inserted with a downstream selection marker. (**D**) Left: Codon Adaptation Index (CAI) for the four genes shown in **A**. Right: Distribution of CAI values for 4153 protein-coding sequences of *B. subtilis*.

DOI: 10.7554/eLife.01501.013