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Peptidoglycan synthesis drives a single population of septal cell wall synthases during division in *Bacillus subtilis*

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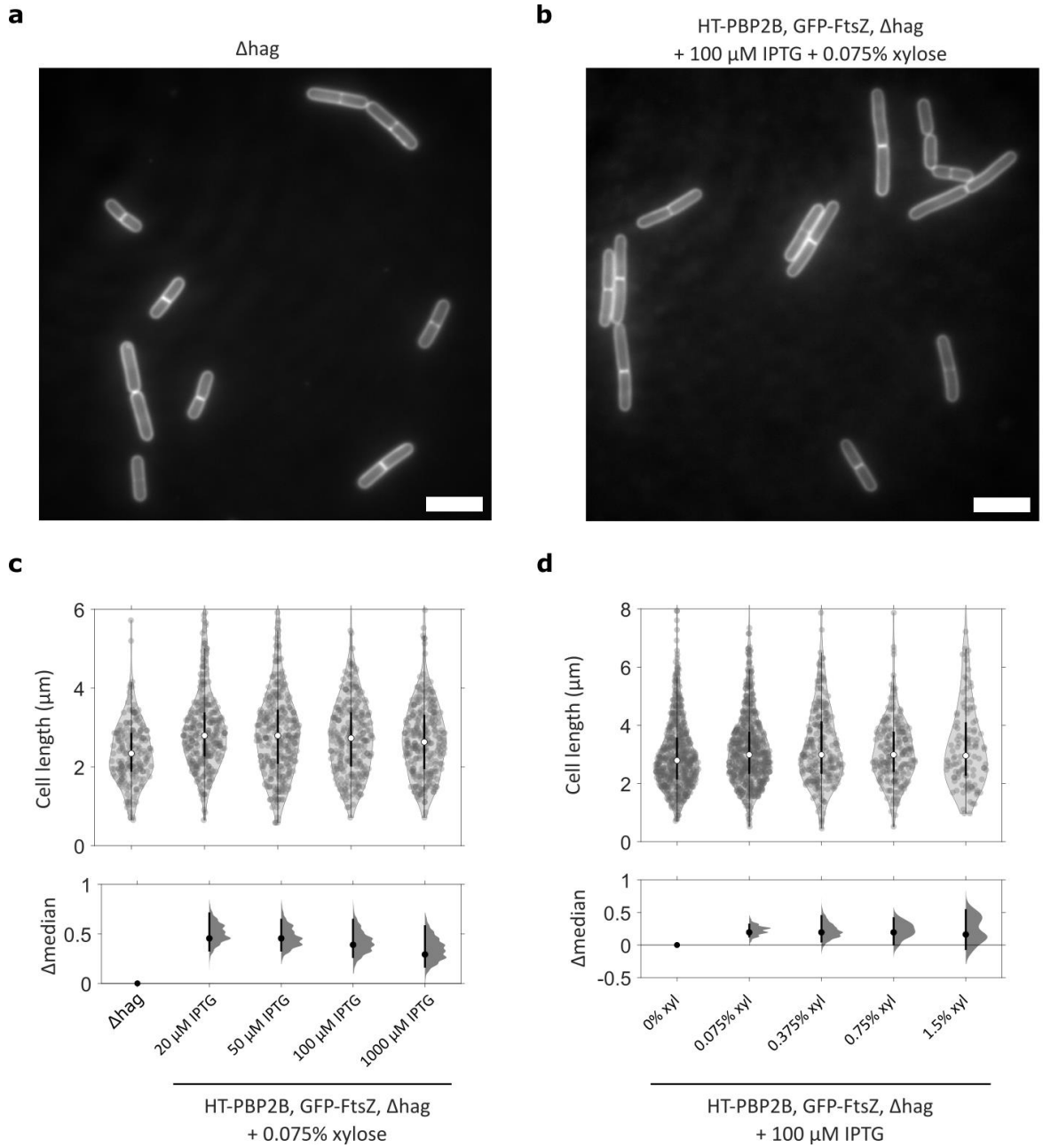
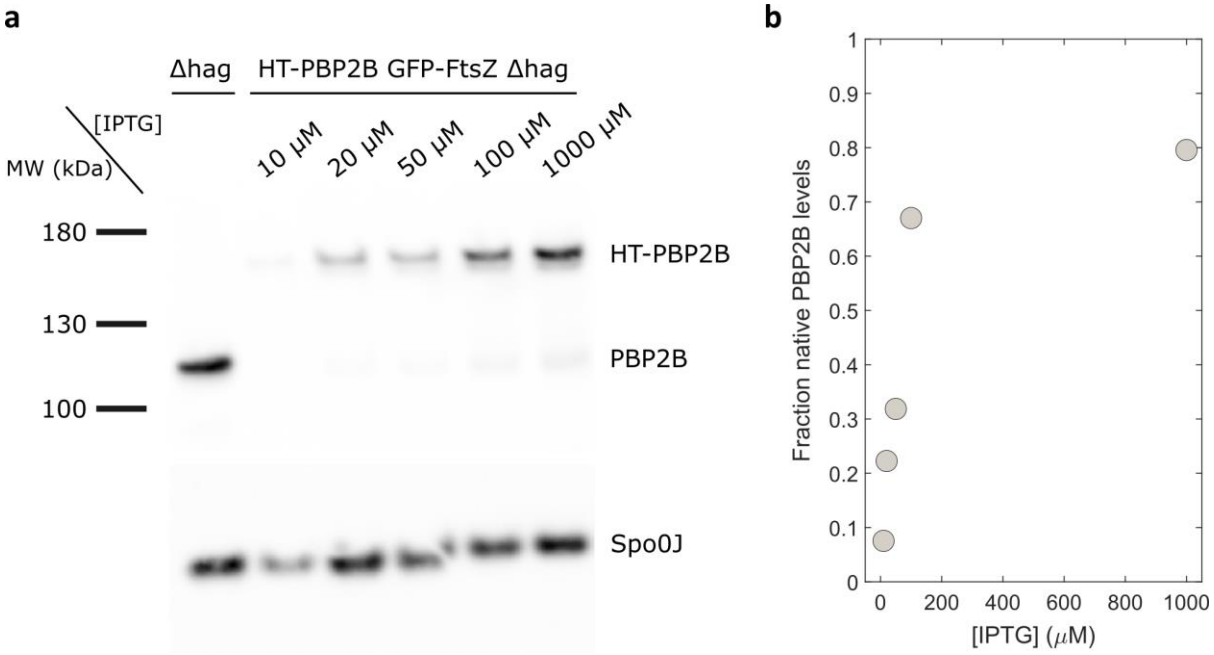
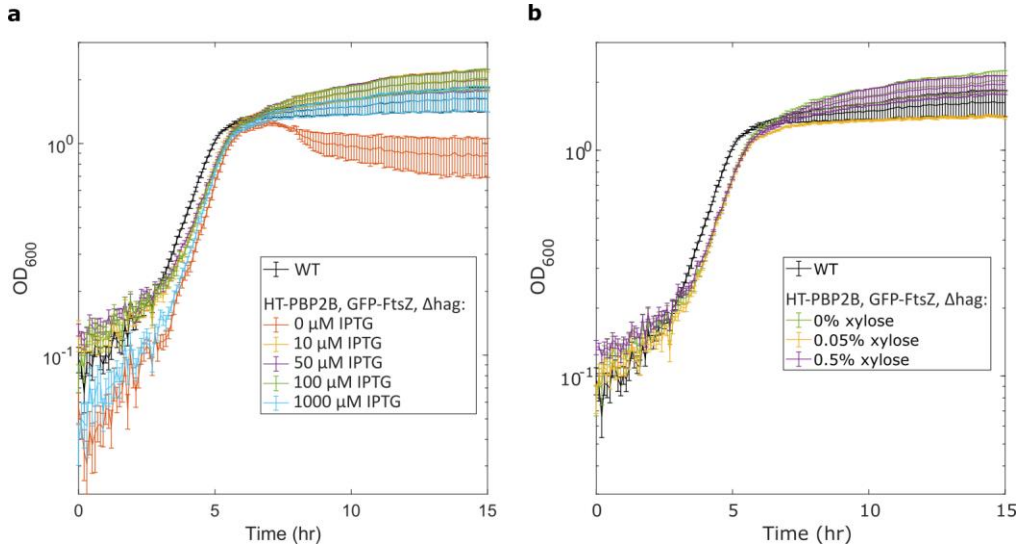
SUPPLEMENTARY FIGURES



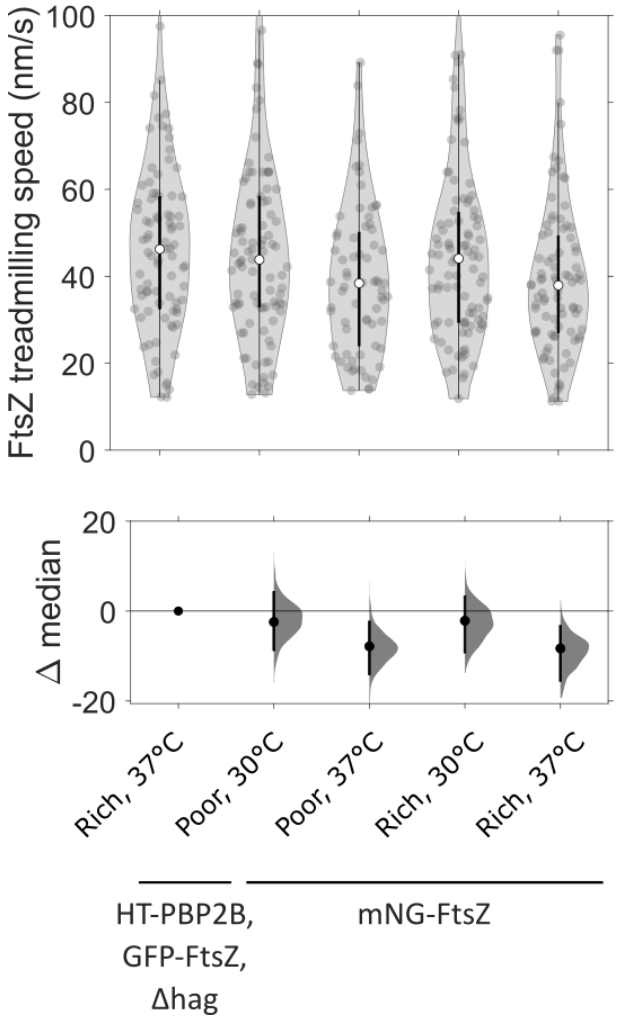
Supplementary Figure 1. Effect of HT-PBP2B and GFP-FtsZ induction levels on cell morphology. Lengths of HT-PBP2B GFP-FtsZ Δhag cells (strain SH147) were measured by microscopy of Nile Red-stained cells and compared to those of Δhag cells (strain SH211) (Supplementary Table 1). Both strains were grown in PHMM at 30°C. **(a)** Micrograph of *Δ*hag cells. **(b)** Micrograph of HT-PBP2B, GFP-FtsZ, Δhag cells with 100 µM IPTG and 0.075% xylose (standard experimental conditions). **(c)** HT-PBP2B expression was induced at the level specified by the [IPTG] shown, while GFP-FtsZ levels were held constant (0.075% xylose induction). **(d)** GFP-FtsZ expression was induced at the level specified by the [xyl] concentration shown, while the HT-PBP2B levels were held constant (100 µM IPTG induction). Violin plots: white circles, median; thick black lines, interquartile range; thin black lines, 1.5x interquartile range. DABEST plots: black circle, median difference between indicated conditions; black lines, 95% confidence interval of median difference. Scale bars: 5 µm. Sample sizes are listed in Supplementary Table 6.



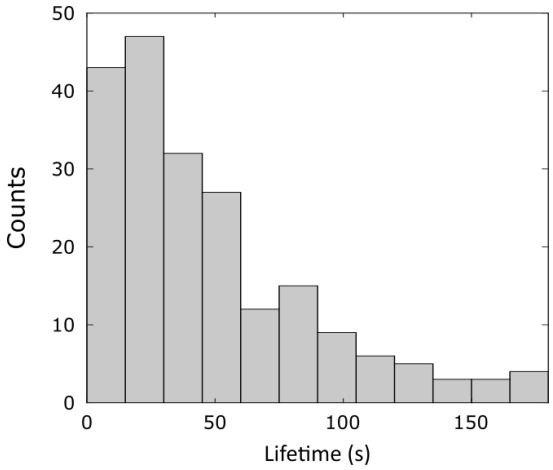
Supplementary Figure 2. Quantification of PBP2B levels across [IPTG]. (a) Western blot of HT-PBP2B GFP-FtsZ Δhag (strain SH147; Supplementary Table 1) and Δhag (strain SH211). Cultures were grown in rich media at 37°C with varying [IPTG] to induce HT-PBP2B expression. 5 µg of total protein from lysate was blotted. Spo0J was used as a loading control on a separate blot. The membranes were incubated with polyclonal antibodies specific for PBP2B or Spo0J. Bands were visualised by a chemiluminescence system. **(b)** Protein expression levels of HT-PBP2B across [IPTG] based on the normalized integrated intensities of bands in the Western blot. Sample sizes are listed in Supplementary Table 6. Uncropped images are provided as Supplementary Figure 12.



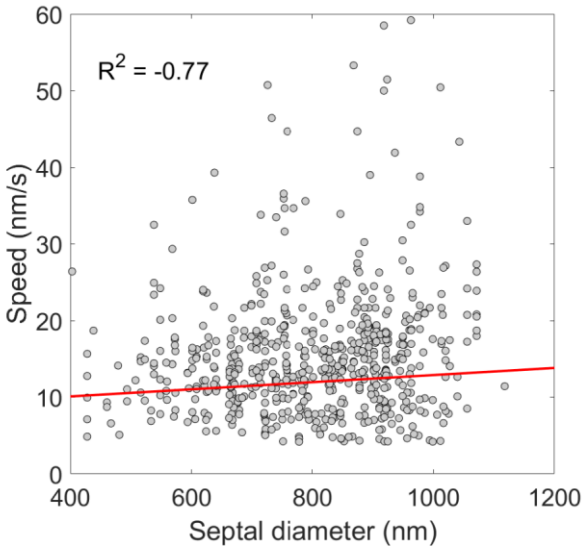
Supplementary Figure 3. Effect of HT-PBP2B and GFP-FtsZ induction on growth in liquid culture. Growth of HT-PBP2B GFP-FtsZ Δhag cultures (strain SH147) was monitored for 15 hours using a FLUOStar OPTIMA plate reader (BMG Labtech) and compared to that of wild-type (PY79) (Supplementary Table 1). Both strains were grown in LB at 30°C. Mean values ± SD of triplicate repeats are plotted. **(a)** HT-PBP2B expression was induced at the level specified by the [IPTG] shown, while GFP-FtsZ was not induced (0% xylose). **(b)** GFP-FtsZ expression was induced at the level specified by the [xylose] shown, while HT-PBP2B expression was induced with a constant 100 µM IPTG.



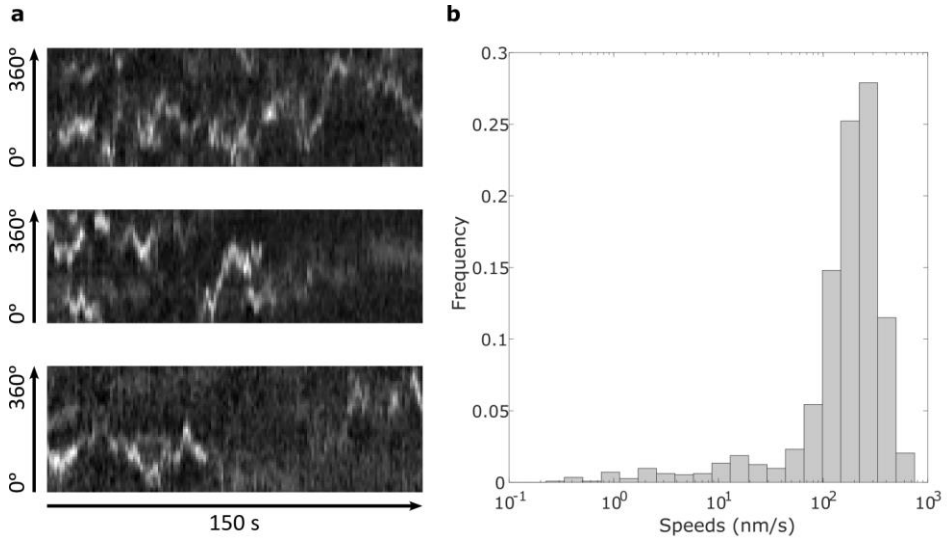
Supplementary Figure 4. Speed of FtsZ treadmilling in HT-PBP2B, GFP-FtsZ, Δhag strain compared to previous measurements. FtsZ treadmilling speeds were measured by TIRF microscopy using a HT-PBP2B, GFP-FtsZ, Δhag strain (strain SH147; Supplementary Table 1) and compared to treadmilling speeds in a strain expressing mNeonGreen-FtsZ (mNG-FtsZ) from an IPTG-inducible promoter (strain bWM4; Supplementary Table 1) measured across different growth conditions (poor vs. rich media, 30°C vs. 37 °C). HT-PBP2B, GFP-FtsZ, Δhag cultures were grown in rich media (PHMM) at 37°C with 0.075% xylose to induce a low level of GFP-FtsZ expression. Treadmilling speeds were determined by manually tracing filament trajectories on kymographs, as done previously[1]. Data for all mNG-FtsZ conditions are taken from Whitley et al[1]. Violin plots: white circles, median; thick black lines, interquartile range; thin black lines, 1.5x interquartile range. DABEST plots: black circle, median difference between indicated conditions; black lines, 95% confidence interval of median difference. Sample sizes are listed in Supplementary Table 6.



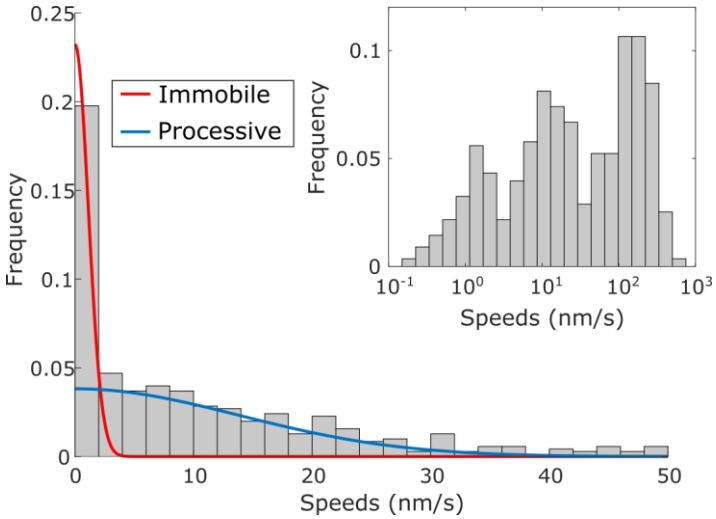
Supplementary Figure 5. Lifetime of immobile HT-PBP2B tracks. Histogram of lifetimes for immobile track segments from HT-PBP2B GFP-FtsZ Δhag cells (strain SH147) grown in PHMM at 30°C. Here, immobile is defined as a track segment with speed less than 4 nm/s. Sample sizes are listed in Supplementary Table 6.



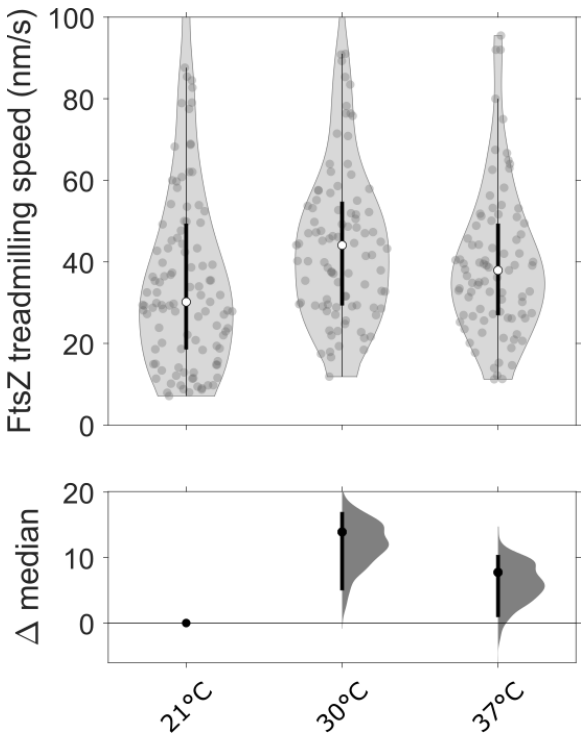
Supplementary Figure 6. Correlation between HT-PBP2B speed and septal diameter. Scatter plots showing the speeds of processive HT-PBP2B track segments (speeds between 4 and 60 nm/s) and the associated diameters of the septa around which they were moving under our standard conditions (rich media, 30°C).



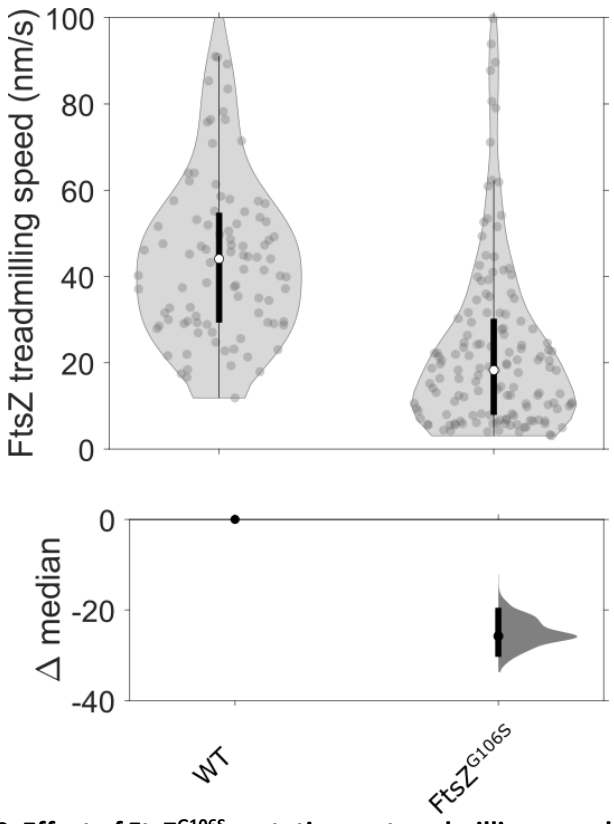
Supplementary Figure 7. Motion of HT-PBP2B molecules outside the septal ring area. (a) Example radial kymographs of HT-PBP2B (rich media, 30°C) showing motion outside the septal ring area (*i.e.* at a random section of the cell sidewall). **(b)** Histogram of HT-PBP2B speeds for molecules observed outside the septal ring area plotted on logarithmic x axis, measured from linear segments on kymographs.



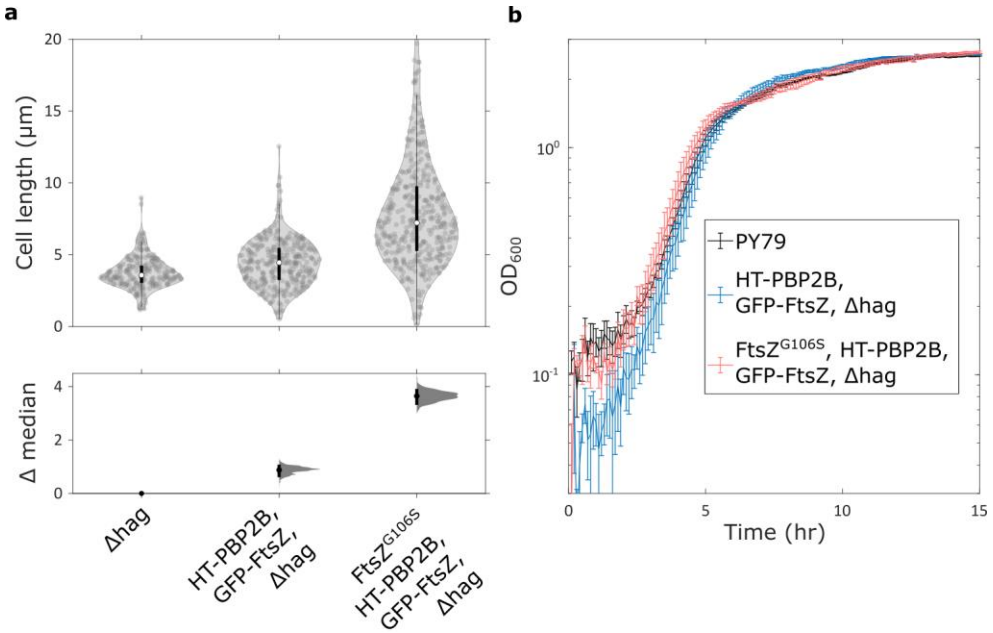
Supplementary Figure 8. Speeds of HT-PBP2B molecules with high inducer concentration. Histogram of HT-PBP2B speeds in HT-PBP2B GFP-FtsZ Δhag cells (strain SH147; Supplementary Table 1) grown in rich media with 1 mM IPTG and 0.075% xylose at 30°C with 250-500 pM JFX554 HaloTag ligand and imaged using VerCINI. Red and blue lines show fits to the data. *Inset*: Histogram of speeds plotted on logarithmic x axis, showing three populations.



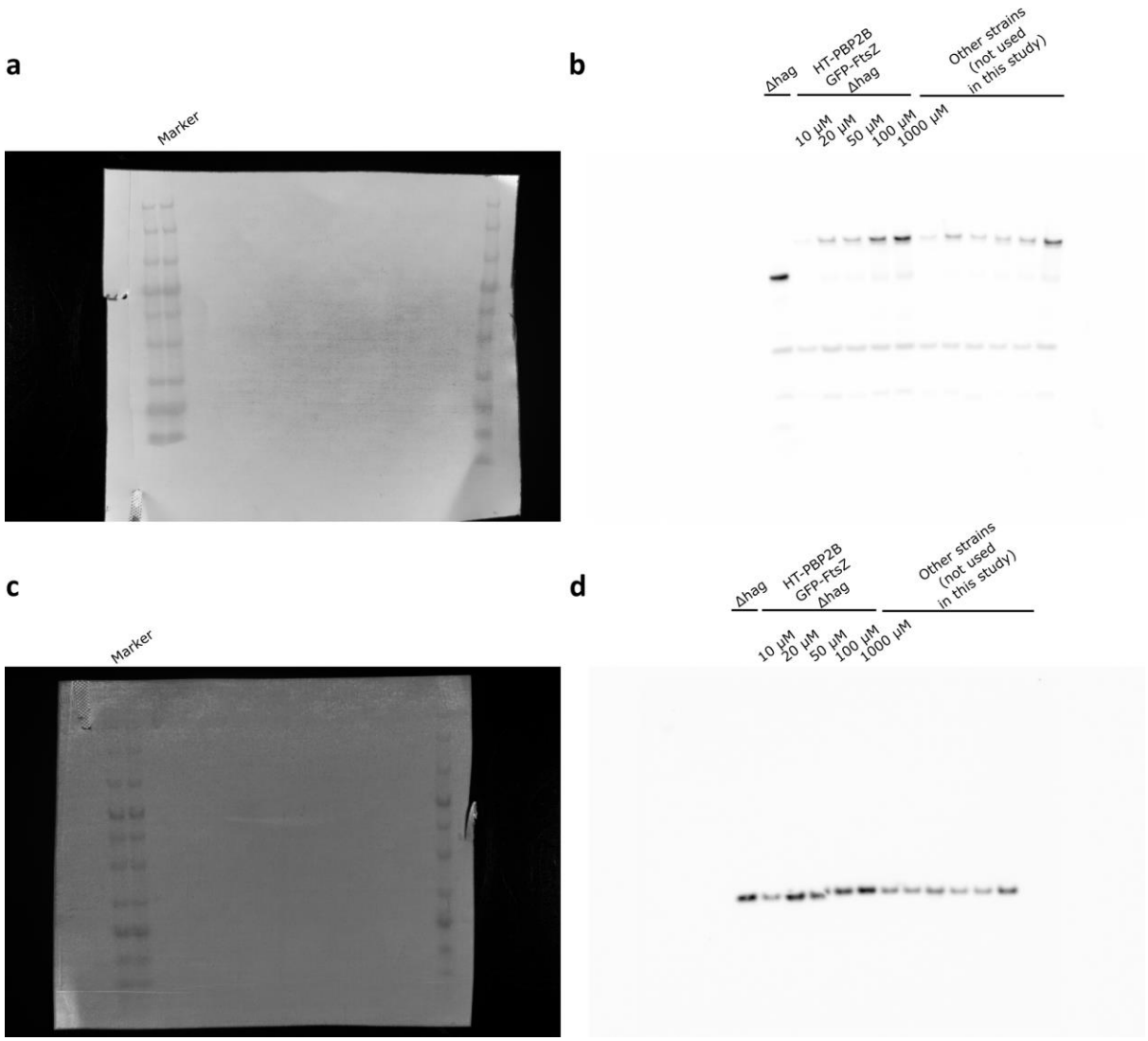
Supplementary Figure 9. Effect of temperature on FtsZ treadmilling speed. FtsZ treadmilling speeds were measured by TIRF microscopy using a strain expressing mNeonGreen-FtsZ from an IPTG-inducible promoter (strain bWM4; Supplementary Table 1) grown in rich media at 21°C and compared to previous measurements of the same strain in the same media under varying temperatures. Cultures were incubated with 25 µM IPTG 1 hr prior to imaging to induce mNeonGreen-FtsZ expression. Treadmilling speeds were determined by manually tracing filament trajectories on kymographs, as done previously[1]. Data for 30°C and 37°C are taken from Whitley et al. 2021[1]. Violin plots: white circles, median; thick black lines, interquartile range; thin black lines, 1.5x interquartile range. DABEST plots: black circle, median difference between indicated conditions; black lines, 95% confidence interval of median difference. Sample sizes are listed in Supplementary Table 6.



Supplementary Figure 10. Effect of FtsZ^{G106S} mutation on treadmilling speed. FtsZ treadmilling speeds were measured for cells expressing FtsZ^{G106S} (strain SH203; Supplementary Table 1) in rich media at 30°C and compared to treadmilling speeds in a strain expressing mNeonGreen-FtsZ from an IPTG-inducible promoter (strain bWM4) measured under the same conditions. FtsZ^{G106S} cells were incubated with 0.075% xylose to induce GFP-FtsZ expression. Treadmilling speeds were determined by manually tracing filament trajectories on kymographs, as done previously[1]. Data for WT are taken from Whitley et al. 2021[1]. Violin plots: white circles, median; thick black lines, interquartile range; thin black lines, 1.5x interquartile range. DABEST plots: black circle, median difference between indicated conditions; black lines, 95% confidence interval of median difference. Sample sizes are listed in Supplementary Table 6.



Supplementary Figure 11. Effect of FtsZ^{G106S} mutation on cell lengths and growth in liquid culture. **(a)** Lengths of FtsZ^{G106S} HT-PBP2B GFP-FtsZ Δhag cells (strain SH203) were measured by microscopy of Nile Red-stained cells and compared to those of HT-PBP2B GFP-FtsZ Δhag and Δhag cells (strains SH147 and SH211, respectively). All strains were grown in PHMM at 30°C. HT-PBP2B expression was induced with 100 µM IPTG in both strains containing HT-PBP2B, while GFP-FtsZ was not induced in either case (0% xylose). Violin plots: white circles, median; thick black lines, interquartile range; thin black lines, 1.5x interquartile range. DABEST plots: black circle, median difference between indicated conditions; black lines, 95% confidence interval of median difference. Sample sizes are listed in Supplementary Table 6. **(b)** Growth was monitored for 15 hours using a FLUOStar OPTIMA plate reader (BMG Labtech) at 30°C. Mean values ± SD of triplicate repeats are plotted. All strains were grown in LB at 30°C. HT-PBP2B expression was induced with 100 µM IPTG in both strains containing HT-PBP2B, while GFP-FtsZ was not induced in either case (0% xylose).



Supplementary Figure 12. Uncropped images of anti-PBP2B and anti-Spo0J Western blots. Uncropped Western blot images of HT-PBP2B GFP-FtsZ Δhag (strain SH147; Supplementary Table 1) and Δhag (strain SH211), corresponding to cropped images in Supplementary Figure 2. **(a)** White light illumination of anti-PBP2B blot. **(b)** Chemiluminescence of anti-PBP2B blot. **(c)** White light illumination of anti-Spo0J blot. **(d)** Chemiluminescence of anti-Spo0J blot.

REFERENCES

1. Whitley, K. D. *et al.* FtsZ treadmilling is essential for Z-ring condensation and septal constriction initiation in Bacillus subtilis cell division. *Nat. Commun.* **12**, 2448 (2021).