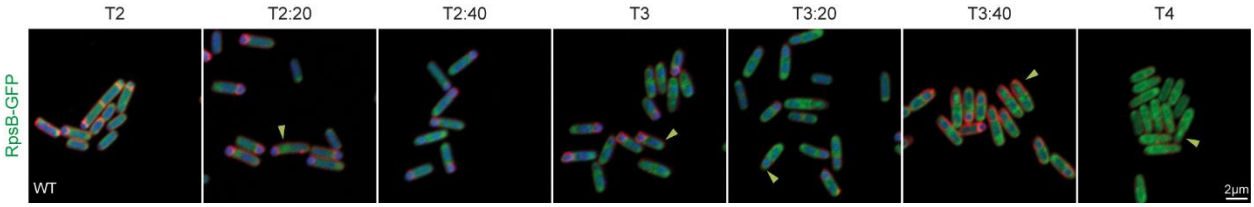
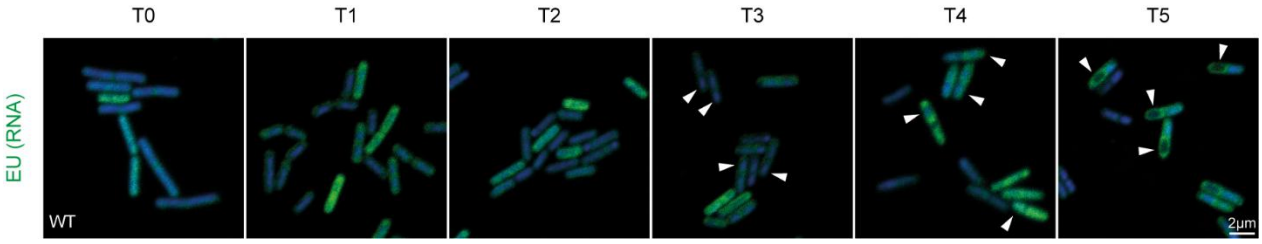


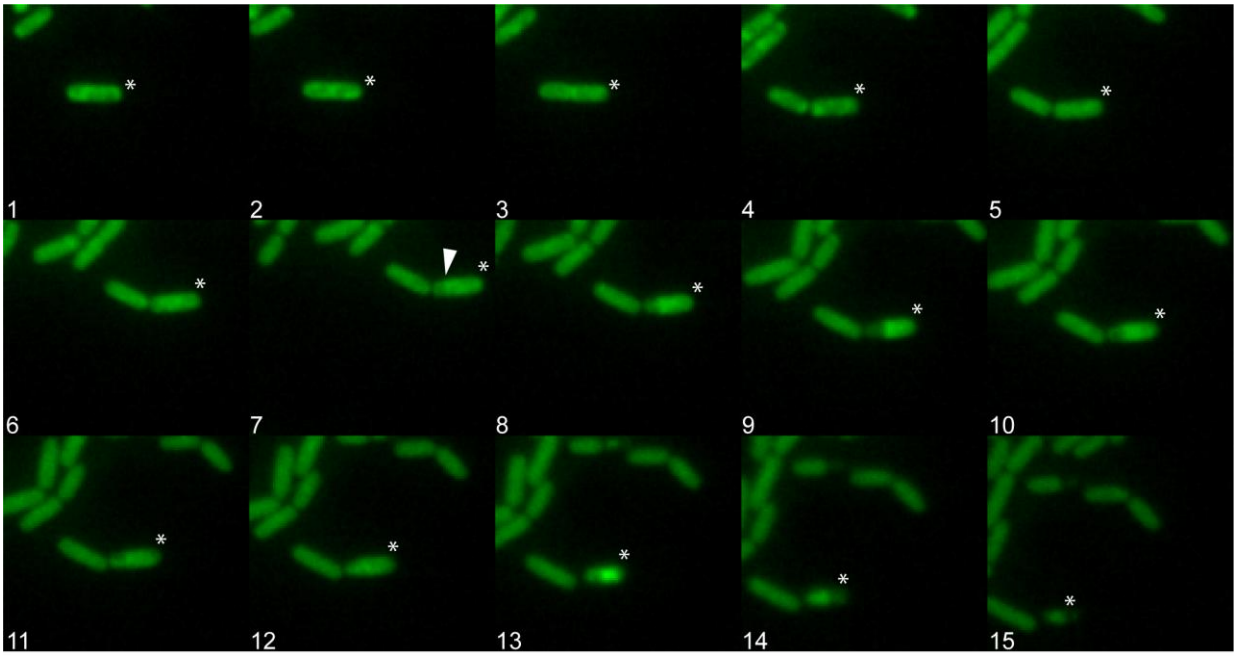
Supplementary Figure 1. Sporulation process of *B. subtilis* expressing GFP-tagged ribosomes (RpsB-GFP).

Cells were stained with DAPI (blue) to visualise the chromosome and FM4-64 (red), a membrane stain, to track the asymmetric septation and sporulation progress. Samples were taken two hours after sporulation induction (T2) and every twenty minutes of the sporulation process (T2:20-T4). The green arrowheads indicate the movement of the ribosomes in the cell, highlighting the dynamic changes in ribosomal localisation during sporulation. Scale bar is 2 μm.



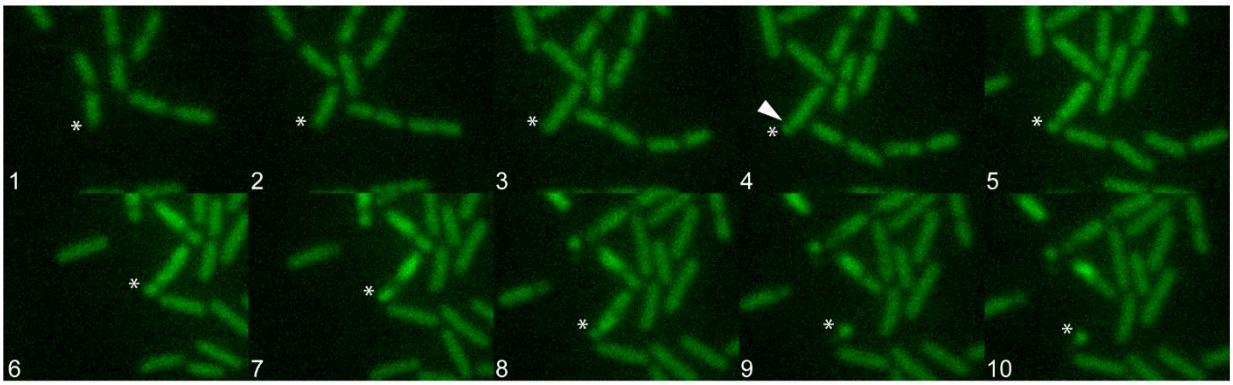
Supplementary Figure 2. Localisation of *de novo* RNA synthesis during sporulation in *B. subtilis*.

Cells were stained with DAPI to visualise the chromosome (blue), and *de novo* RNA synthesis was observed by incorporation and fluorescent tagging of the uridine analog, 5-ethynyluridine (EU, green). Samples were taken before sporulation induction (T0) and every hour of the sporulation process (T1-T5). White arrowheads point to the location of selected forespores. The scale bar is 2 μm.



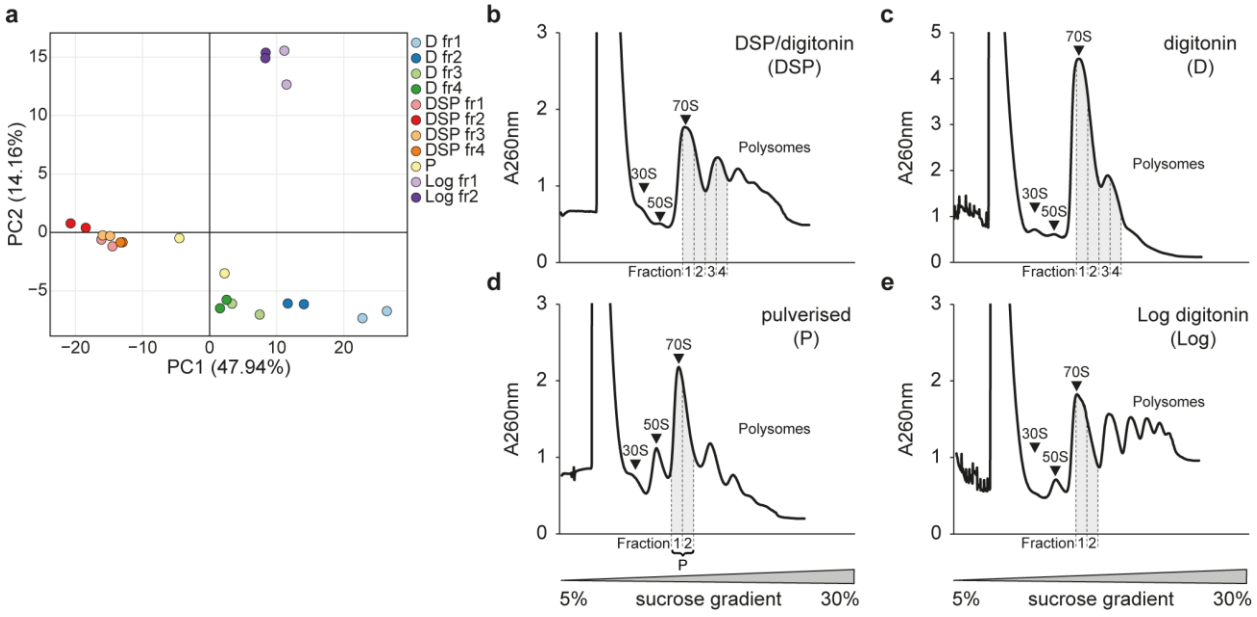
Supplementary Figure 3. Timelapse of sporulating *B. subtilis* WT with GFP-tagged ribosomes (RpsB-GFP). Selected snapshots illustrate the changing morphology of a bacterial cell (marked with an asterisk *) and ribosome localisation during sporulation.

1-4. Dividing cell during vegetative growth – the mother cell divides into two daughter cells; 5-6. Sporulation initiation – a cell decides to stop dividing vegetatively. One of the cells decides to initiate sporulation, while the other becomes dormant. The ribosomes lose their polar location (5) and begin to move away from the pole where the spore will be (6). 7-8. Asymmetric septation – the cell produces an asymmetric septum (indicated with a white arrowhead), the chromosome is translocated into the prespore, and ribosomes are gathered at the asymmetric septum on the mother cell's side; 9. Engulfment of the prespore. The volume of the prespore increases, and the ribosomes are located at the asymmetric septum; 10-12. Translocation of the ribosomes into the spore; spore cortex and core maturation; 13. Mother cell lysis; 14-15. Release of the free mature spore. Full video available in Supplementary Video 1.



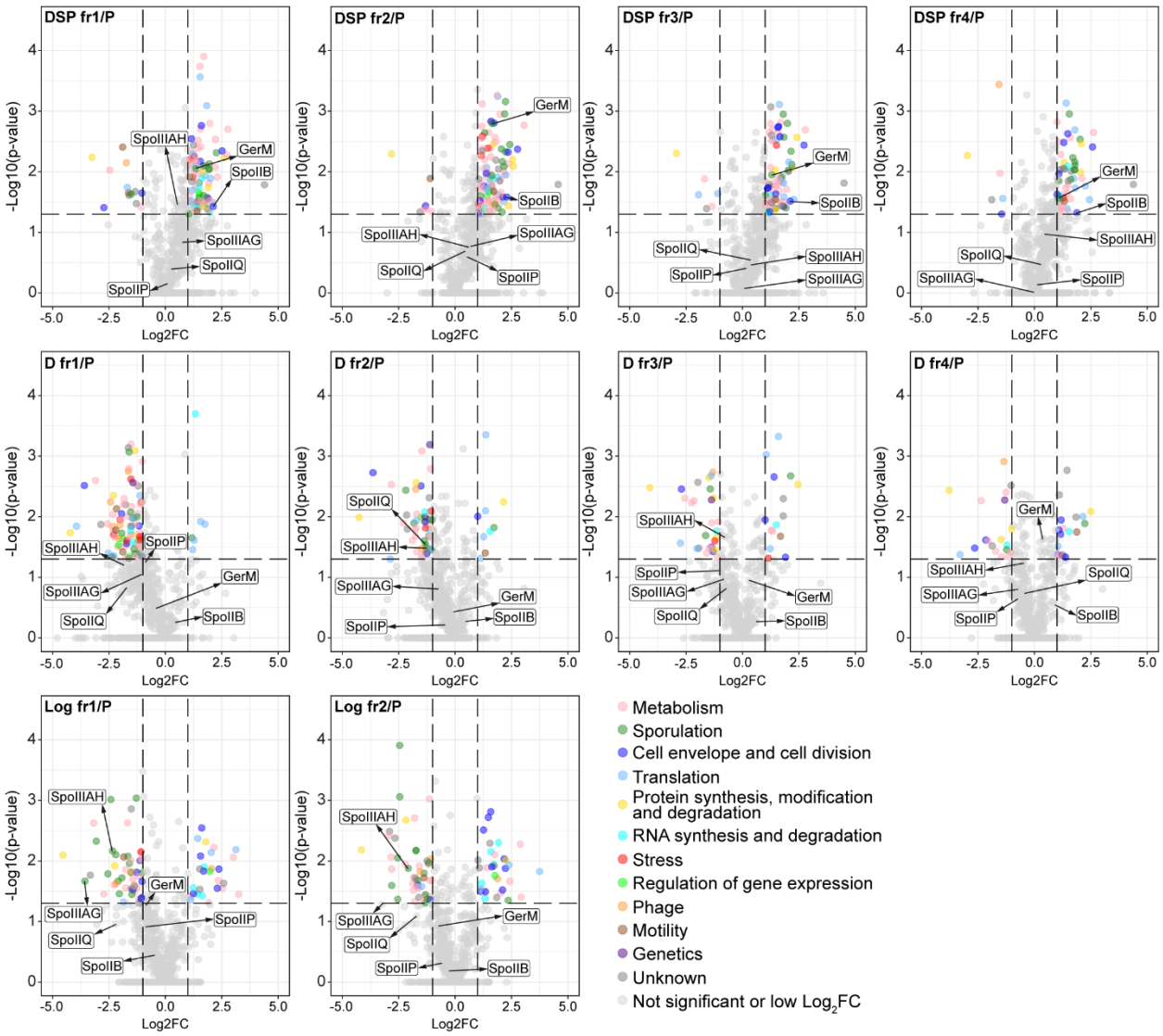
Supplementary Figure 4. Timelapse of sporulating *B. subtilis* WT with GFP-tagged RNA polymerase (RpoC-GFP). Selected snapshots show cell morphology and localisation of transcriptional machinery during sporulation. The observed cell is marked with an asterisk (*).

1-3. A vegetative cell with centrally positioned chromosome co-localised with RNA polymerase. The cell does not divide vegetatively, but instead decides to enter sporulation. 4. Asymmetric division – the cell forms an asymmetric septum (indicated with the white arrowhead) to produce a small prespore. Approximately 25% of the chromosome is localised to the prespore, together with RNA polymerase. 5-6. Chromosome is translocated into the prespore, and colocalization of the transcriptional machinery with DNA is maintained. 7. Spore engulfment. 8. Spore core and cortex maturation – a cell-within-a-cell state, in which the spore moved slightly away from the mother cell pole. 9-10. Mother cell autolysis and mature spore release. Full video available in Supplementary Video 2.



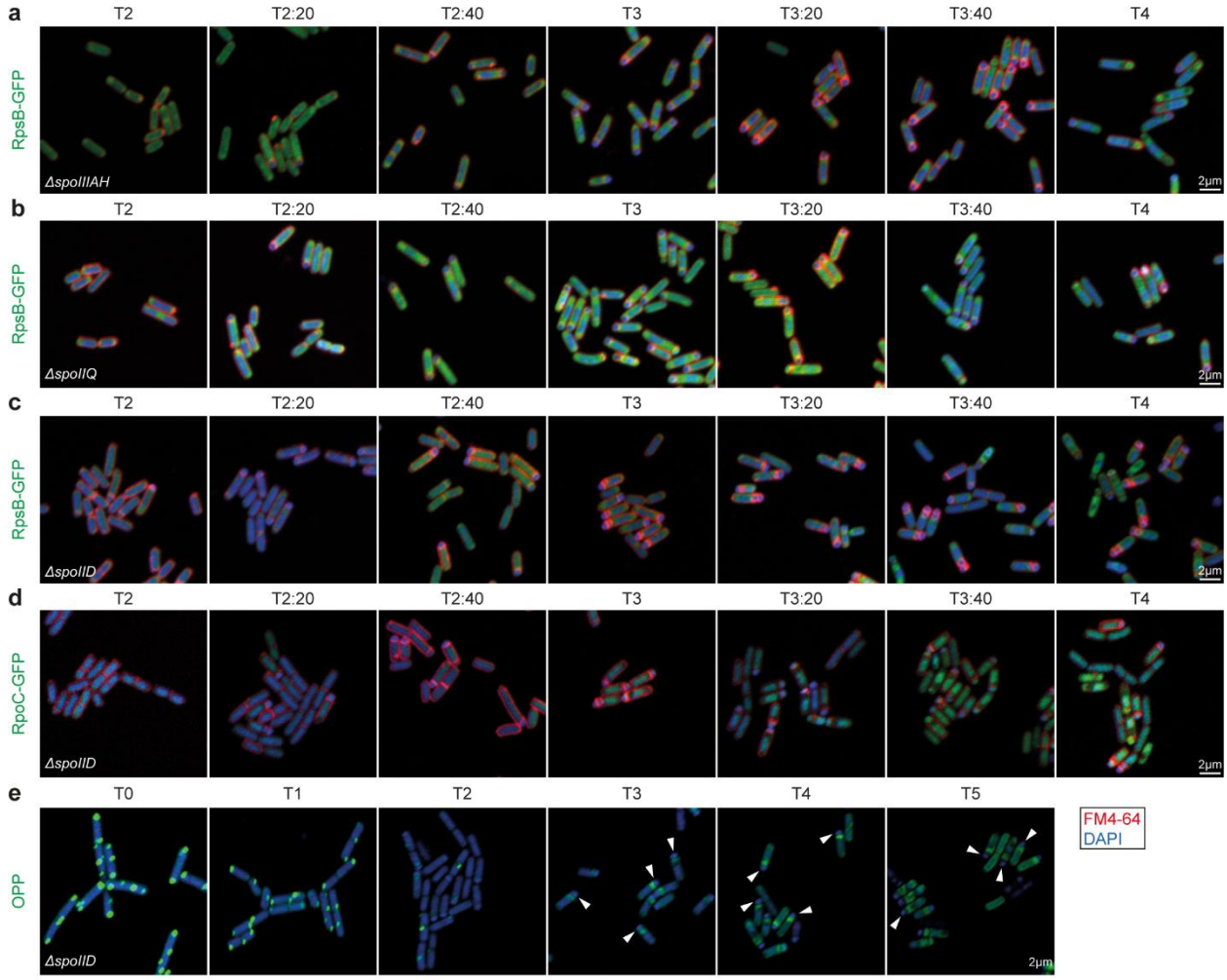
Supplementary Figure 5. Principal Component Analysis of mass spectrometry data and polysome profiles of *B. subtilis* cells.

(**a**) Principal Component Analysis (PCA) plot of the log2 normalised reporter intensities for all samples. Each point represents a biological replicate from the indicated fraction (see legend). The percentage of variance explained by each principal component is shown in parentheses on the axis labels. PC1 describes data variance based mostly on preservation of ribosomes in their native state (lysis-dependent) and PC2 based on growth stage (sporulation vs logarithmic). The plots model 62.1% of the total data variance. Polysome profiles of lysates prepared by (**b**) DSP cross-linking and digitonin-based lysis (DSP), (**c**) digitonin-based lysis (D), (**d**) flash-freezing and pulverised with mortar and pestle, of the sporulating *B. subtilis* (P), (**e**) digitonin-based lysis of culture harvested in the logarithmic phase (Log). Black arrows indicate peaks corresponding to 30S and 50S ribosomal subunits and 70S ribosomes. Dotted vertical lines indicate fractions selected for mass spectrometry analysis.



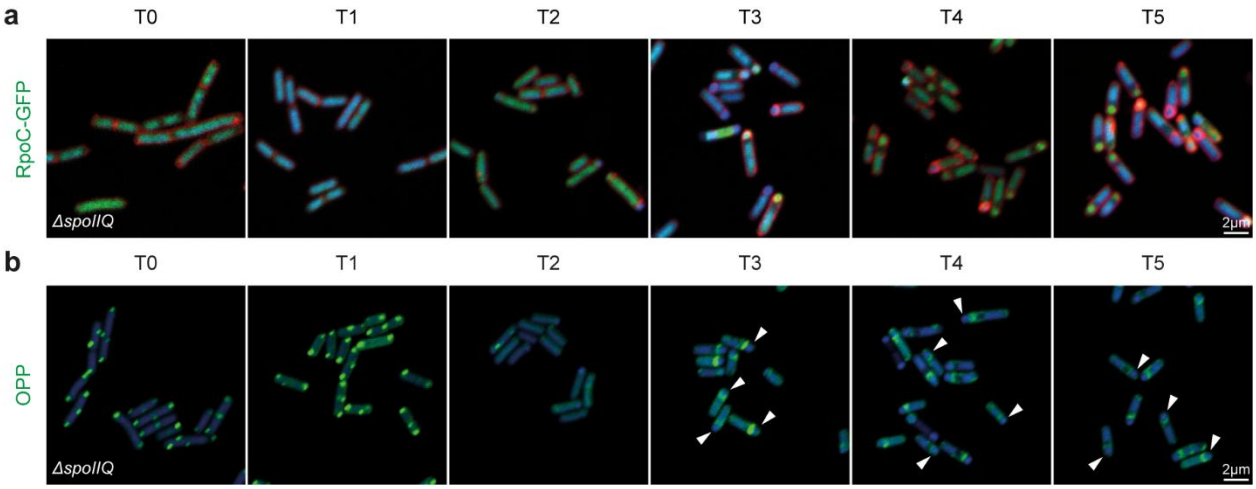
Supplementary Figure 6. Volcano plots illustrating the mass spectrometry proteomics data for *B. subtilis* cells compared based on growth stage and lysis method.

Cell lysates were prepared using three different methods: (1) DSP cross-linking followed by digitonin-based lysis (DSP); (2) digitonin-based lysis alone (D); and (3) pulverisation of flash-frozen cells (P), serving as a control of efficient and biologically accurate lysis. For the control of the growth stage we used the culture harvested during logarithmic growth phase followed by digitonin lysis (Log). The x-axis shows the calculated Log2 fold change (Log2FC) compared to the P sample, and p-values are represented on the y-axis as -Log10(p-value) (Student's T-test, two-sided; no adjustments for multiple comparisons). The vertical and horizontal dotted lines indicate a Log2(Fold Change) of ±1 (Fold Change = ±2) and a p-value of 0.05, respectively. Enriched proteins are categorized into functional groups (see legend) and color-coded accordingly. Protein components of the feeding tube channel (SpoIIIAH-AG, SpoIIQ, and GerM) and the peptidoglycan degradation module (SpoIIP and SpoIIB) are labeled. Source data are provided as a Source Data file.



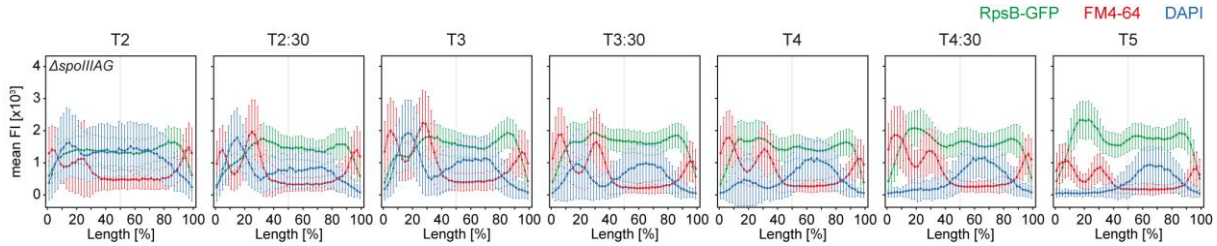
Supplementary Figure 7. Localisation of translational and transcriptional machineries in B. subtilis mutant strains.

(**a**) *ΔspoIIIAH*, (**b**) *ΔspoIIQ* and (**c**) *ΔspoIID* strains with fluorescently tagged RpsB-GFP protein; (**d**) *ΔspoIID* strain with fluorescently labelled RNA polymerase subunit β' (RpoC-GFP). Cells were stained with DAPI to visualise the chromosome and FM4-64, a membrane stain, to track the asymmetric septation and sporulation progress. Samples were taken every 20 minutes starting two hours post sporulation induction (T2) up to four hours post sporulation induction (T4). Scale bar is 2 μm. (**e**) Microscopic images of *ΔspoIID* strain treated with OPP and stained with Alexa 488 and DAPI, illustrating active translation during sporulation (T0 – T5). White arrowheads point to the location of selected forespores. Scale bar is 2 μm.



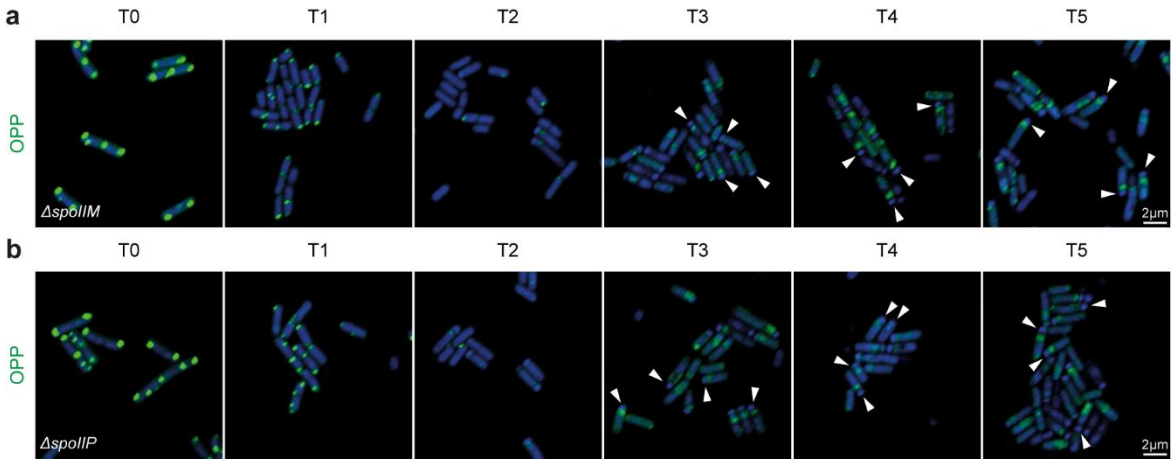
Supplementary Figure 8. Spatial coordination of transcription factors and translation in *ΔspoIIQ* strain during sporulation.

(**a**) Microscopic images show the localization of GFP-tagged β' subunit of RNA polymerase (RpoC, green) in *ΔspoIIQ* strain during five hours into sporulation. The cells were stained with DAPI (blue) to highlight the chromosome and FM4-64 (red) to indicate cell membrane. (**b**) Microscopic images of *B. subtilis ΔspoIIQ* cells treated with OPP and stained with Alexa 488 (green) and DAPI (blue). Samples were collected before sporulation induction (T0) and at hourly intervals throughout the first five hours of the sporulation process (T1-T5). White arrowheads point to the location of selected forespores. The scale bar is 2 μm.



Supplementary Figure 9. Mean fluorescence intensity plots in Δ*spoIIIAG* mutant.

The localisation of ribosomes is shown in green (RpsB-GFP), chromosome in blue (DAPI) and cell membrane in red (FM4-64). Cell lengths were normalised and fluorescence intensity was measured along the cell long axis. n=143, n=101, n=147, n=190, n=162, n=122, and n=119 cells were analysed at T2, T2:30, T3, T3:30, T4, T4:30, and T5, respectively. Error bars represent ±SD (standard deviation) of the fluorescence intensities. Source data are provided as a Source Data file.



Supplementary Figure 10. Translation in strains impaired in spore peptidoglycan rearrangement is lessened in forespores.

Microscopic images of (**a**) *ΔspoIIM* and (**b**) *ΔspoIIP* strains treated with OPP and stained with Alexa 488 and DAPI, illustrating active translation during sporulation (T0 – T5). White arrowheads point to the location of selected forespores. Scale bar is 2 μm.

Supplementary Table 1. List of (**a**) strains, (**b**) primers and (**c**) plasmids used in this study.

a. Strains		
Strain	**Description**	**Reference**
WT	*Bacillus subtilis subsp. subtilis trpC2*	Koo *et al.*[1]
WT RpsB-GFP	*Bacillus subtilis subsp. subtilis trpC2 rpsB:gfp SpR*	Iwanska *et al*. 2024[2]
WT RpoC-GFP	*Bacillus subtilis subsp. subtilis trpC2 rpoC:gfp SpR*	This study
ΔspoIIIAH	*Bacillus subtilis subsp. subtilis trpC2 ΔspoIIIAH::erm*	Koo *et al.*[1]
ΔspoIIQ	*Bacillus subtilis subsp. subtilis trpC2 ΔspoIIQ::erm*	Koo *et al.*[1]
ΔspoIID	*Bacillus subtilis subsp. subtilis trpC2 ΔspoIID::erm*	Koo *et al.*[1]
ΔspoIIIAH RpsB-GFP	*Bacillus subtilis subsp. subtilis trpC2 ΔspoIIIAH::erm rpsB:gfp SpR*	This study
ΔspoIIQ RpsB-GFP	*Bacillus subtilis subsp. subtilis trpC2 ΔspoIIQ::erm rpsB:gfp SpR*	This study
ΔspoIIQ RpoC-GFP	*Bacillus subtilis subsp. subtilis trpC2 ΔspoIIQ::erm rpoC:gfp SpR*	This study
ΔspoIID RpsB-GFP	*Bacillus subtilis subsp. subtilis trpC2 ΔspoIID::erm rpsB:gfp SpR*	This study
ΔspoIID RpoC-GFP	*Bacillus subtilis subsp. subtilis trpC2 ΔspoIID::erm rpoC:gfp SpR*	This study
b. Primers		
Primer	**Sequence**	
rpsB		
UPFOR	GATACCTACGCCTCGTTTAGAATTCGCGGCGCAATC	
UPREV	CATTGATCCGCTGCCTGATCCGGACGCAGTTGTTGTTTCTGTTTC	
MID1FOR	GAAACAGAAACAACAACTGCGTCCGGATCAGGCAGCGGATCAATG	
MID1REV	GTATTTTTCCGTTAATCAAATTGCTCATTCACTTATAGAGTTCATCCATACC	
MID2FOR	GGTATGGATGAACTCTATAAGTGAATGAGCAATTTGATTAACGGAAAAATAC	
MID2REV	GTCCCTCTTATCACCTTTTGAATAGGTAATTGAGAGAAGTTTCTATAGAATTTTTC	
DOWNFOR	GAAAAATTCTATAGAAACTTCTCTCAATTACCTATTCAAAAGGTGATAAGAGGGAC	
DOWNREV	GTGTCTGCGCTCCGTAATATTCAACCGTTACTTTATCTAATAATG	
rpoC		
UPFOR	CTGATGTTAAAGCTGTTATCGCACAGCAGC	
UPREV	CATTGATCCGCTGCCTGATCCGGATTCAACCGGGACCATATCGTCAG	
MID1FOR	CTGACGATATGGTCCCGGTTGAATCCGGATCAGGCAGCGGATCAATG	
MID1REV	GTATTTTTCCGTTAATCAAATTGCTCATTCACTTATAGAGTTCATCCATACC	
MID2FOR	GGTATGGATGAACTCTATAAGTGAATGAGCAATTTGATTAACGGAAAAATAC	
MID2REV	CAGTCTTTCAGCAGAGTTAAATCAGTAATTGAGAGAAGTTTCTATAGAATTTTTC	
DOWNFOR	GAAAAATTCTATAGAAACTTCTCTCAATTACTGATTTAACTCTGCTGAAAGACTG	
DOWNREV	ATGAGCGTTTGCTTGAAGACGGTCTCTTAAAG	
c. Plasmids		
Plasmid	**Description**	
pSHP2[3]	Used as a template for GFP and spectinomycin resistance cassette	

References

1. Koo, B. M. *et al.* Construction and Analysis of Two Genome-Scale Deletion Libraries for Bacillus subtilis. *Cell Syst.* **4**, 291-305.e7 (2017).

2. Iwańska, O. *et al.* Translation in Bacillus subtilis is spatially and temporally coordinated during sporulation. *Nat. Commun. 2024 151* **15**, 1–13 (2024).

3. Murina, V. *et al.* ABCF ATPases Involved in Protein Synthesis, Ribosome Assembly and Antibiotic Resistance: Structural and Functional Diversification across the Tree of Life. *J. Mol. Biol.* **431**, 3568–3590 (2019).