APPENDIX

TITLE:

The Torpedo Effect in *Bacillus subtilis*: RNase J1 Resolves Stalled Transcription Complexes

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Figure S1. RT-qPCR validation of RNAseq data.

Expression of selected genes was analyzed by RT-qPCR in wt (LK1371) and *ΔrnjA* (LK1381) strains. New RNA purifications were performed, different from those used in RNAseq experiments. The upper panel for each gene represents RNAseq values (dark colors), the bottom panel represents RT-qPCR values (light colors). Wt – blue and light blue, *ΔrnjA* – red and light red. The RNA level in wt was set as 1. The average value represents four biological replicates, error bars ±SEM.

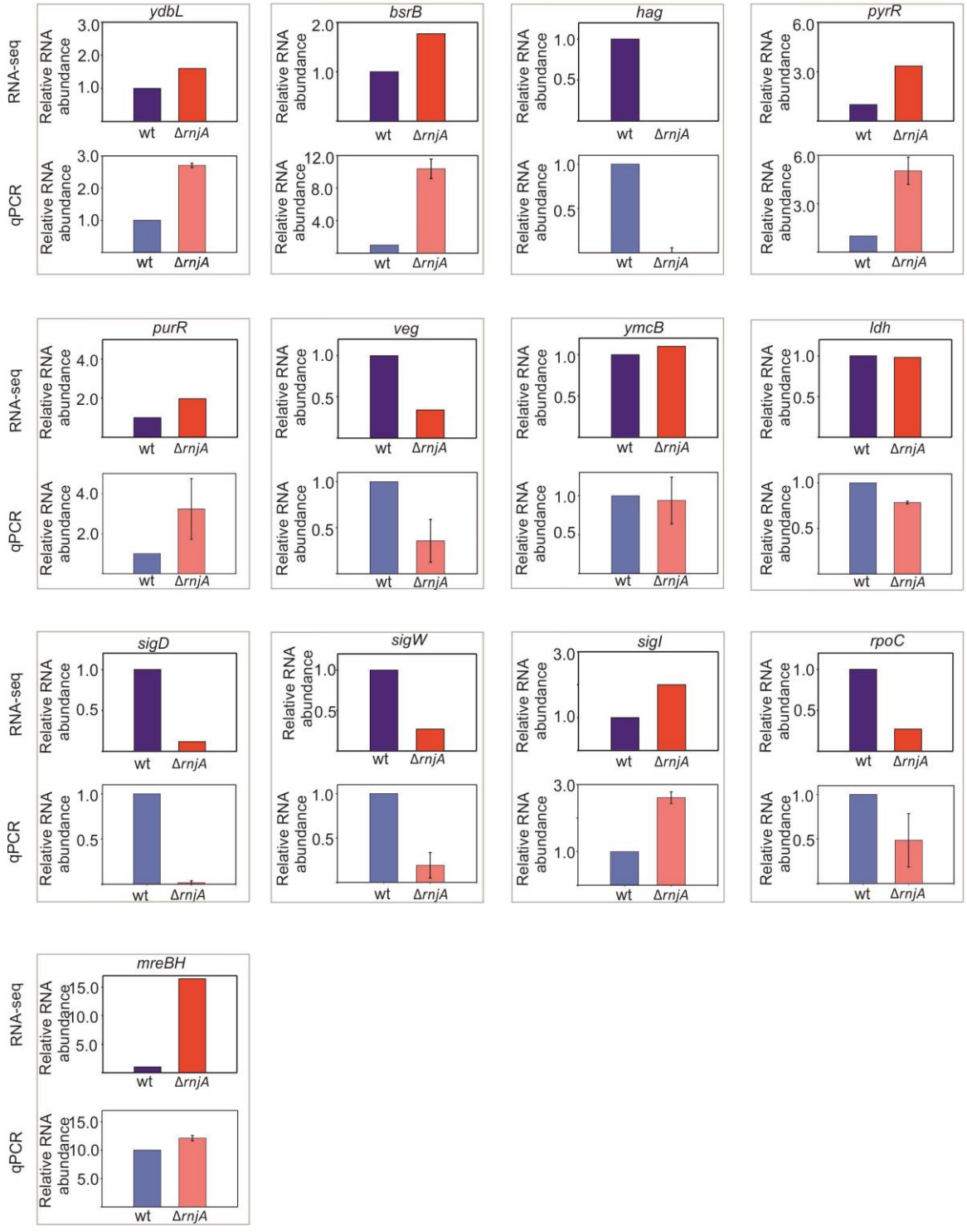


Figure S2. Gene ontology categories of differentially regulated genes in the Δ*rnjA* strain.

Comparison of up- and down-regulated genes of the *ΔrnjA* (LK1381) strain categorized into six main ontology categories according to (Zhu and Stulke, 2018). Green bars: upregulated genes, orange bars: down-regulated genes; grey bars: no differences between *wt* (LK1371) and *ΔrnjA* (LK1381) strain.

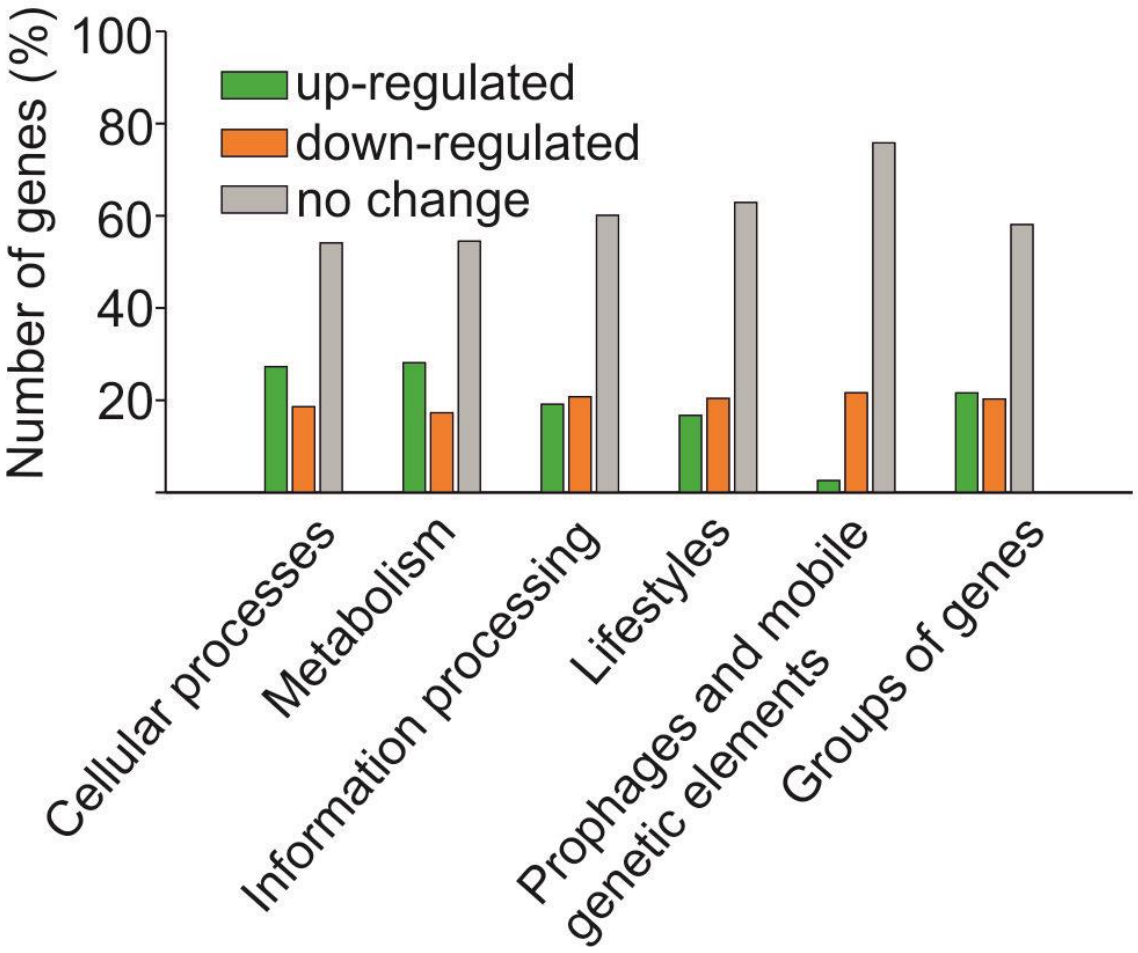


Figure S3. qPCR validation of ChIPseq data.

RNAP occupancy on selected genes was analyzed by qPCR of immunoprecipitated DNA (for details on immunoprecipitation see the Materials and Methods section). Primers used in the validation are in the Key Resources Table. PCR conditions are described in the Materials and Methods section. Wt was set as 1. The average value (ratio of Δ*rnjA* to wt) represents three biological replicates (new immunoprecipitations of DNA, different from those used for ChIPseq, were performed), error bars indicate ±SD.

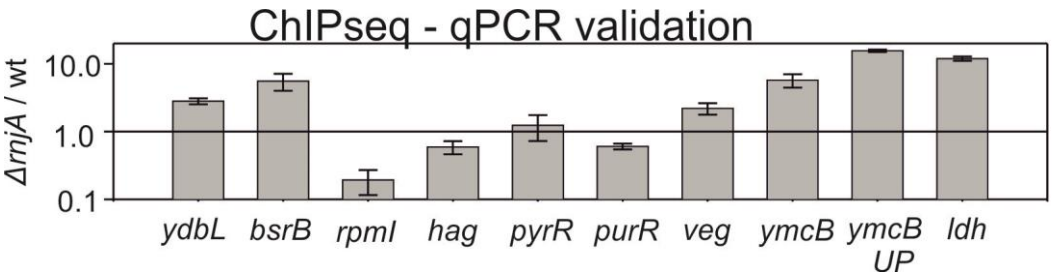


Figure S4. Super-resolution microscopy of LK2328.

Structured illumination microscopy (SIM) of exponentially growing cells of *Bacillus subtilis*, containing RNase J1 fused to GFP (green fluorescent protein) and RNAP to mCherry (red) [strain LK2328]. The scale bar below the Figure represents 3 μM.

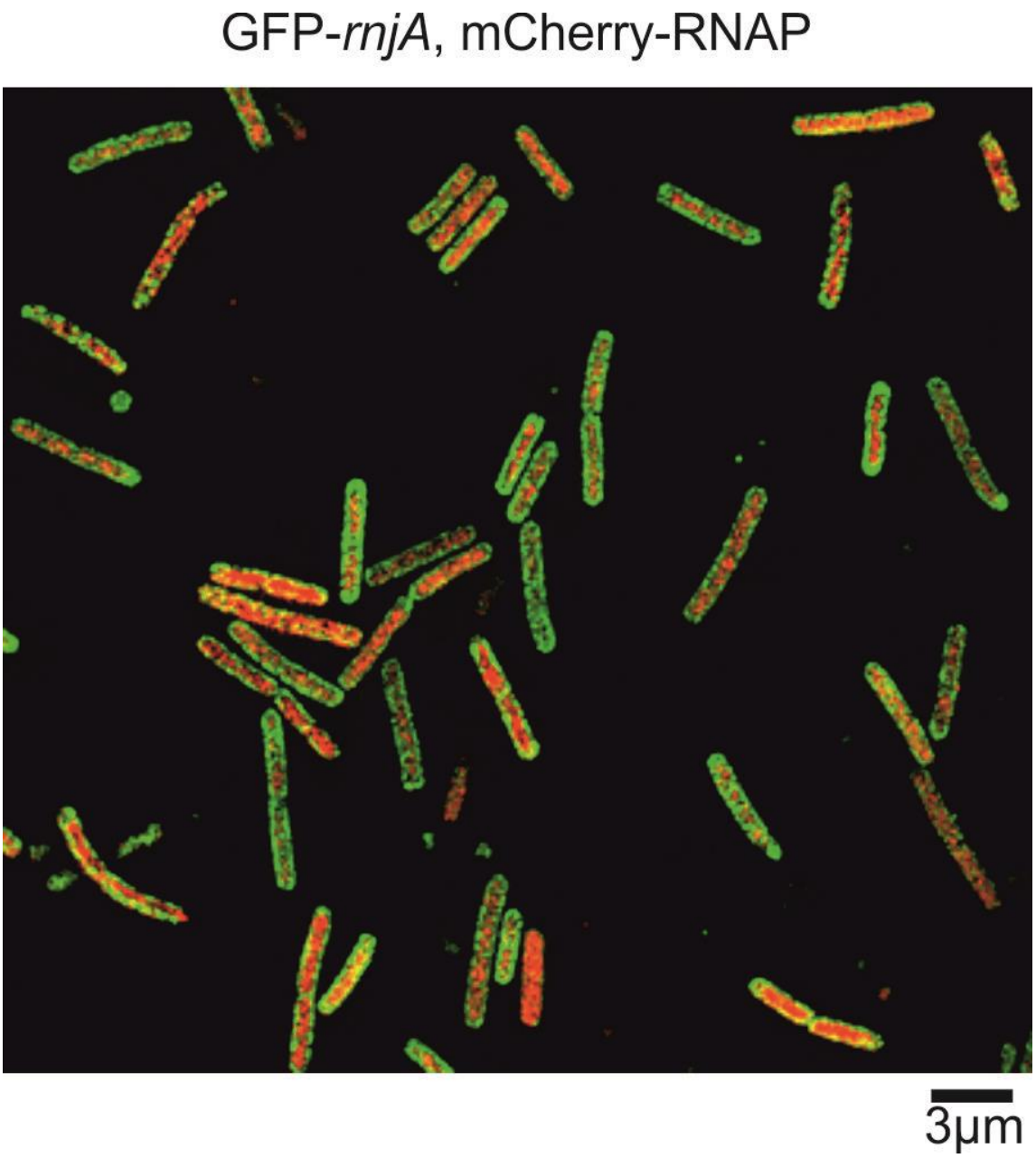


Figure S5. Effects of the absence of Rho or RNase J1 on RNA accumulation: a correlation.

Correlations are shown for relative gene expression values between the Δ*rho* mutant [expressed as log2 fold change vs wt; data taken from (Nicolas et al., 2012)] and relative gene expression values for the Δ*rnjA* mutant [expressed as log2 fold change vs wt]. Only class IV genes for which expression data were available from both Δ*rnjA* and Δ*rho* mutants were used in this comparison (1609 out of 1654 class IV genes).

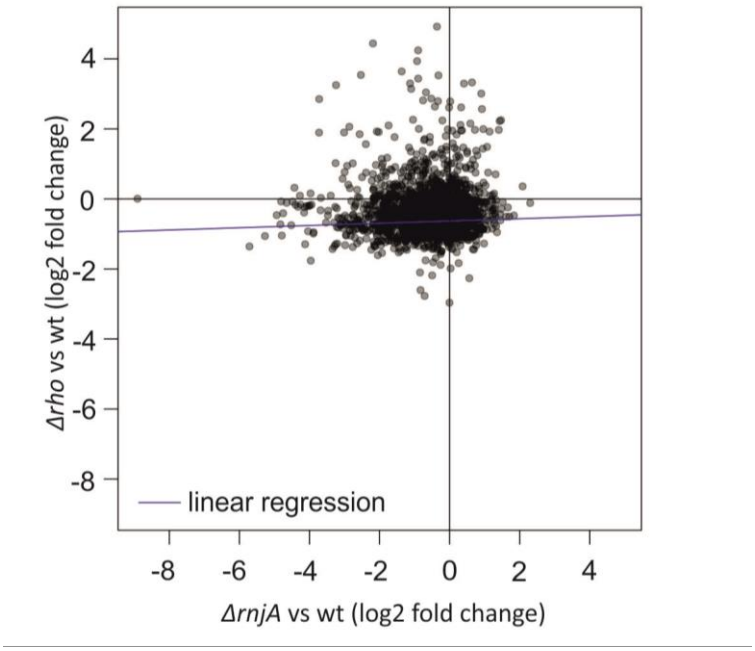
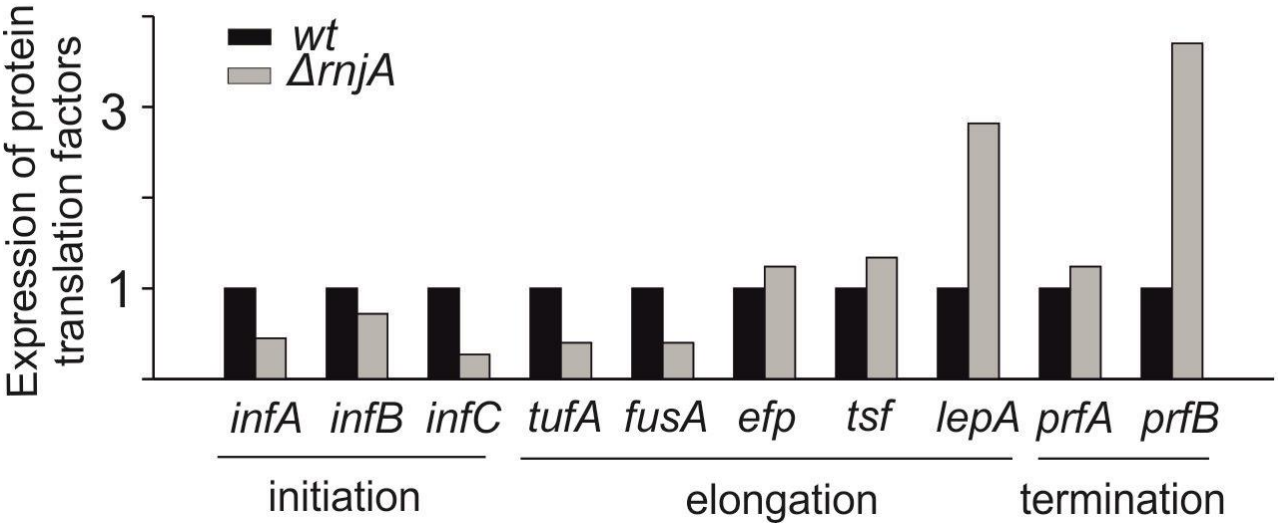


Figure S6. mRNA levels of protein translation factors in Δ*rnjA* compared to wt.

Relative expression of protein translation genes (mRNA levels) in the Δ*rnjA* strain (normalized to wt [set as 1]).



Supplemental References

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