Supplemental Figures for

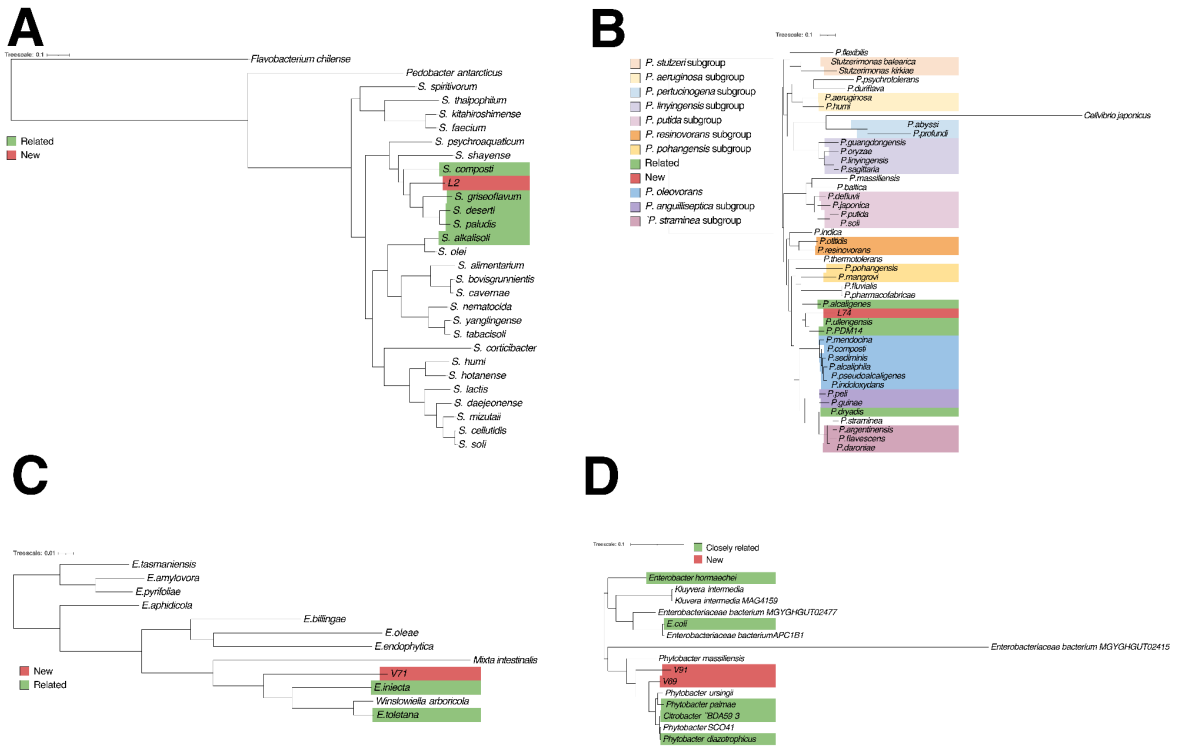
Interspecies systems biology links bacterial metabolic pathways to nematode gene expression, chemotaxis behavior, and survival

Marina Athanasouli[1,2], Tobias Loschko[1], Christian Rödelsperger[1,*]

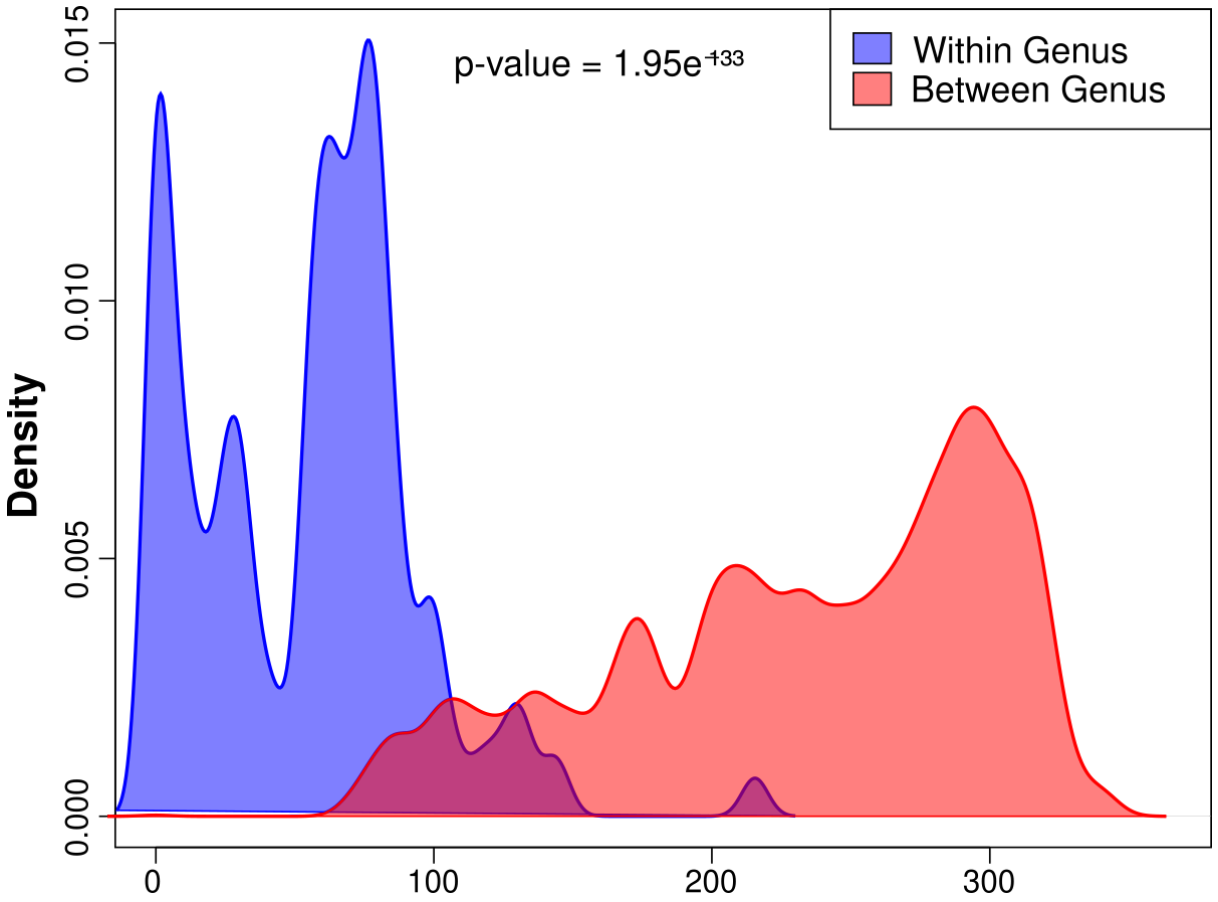
[1] Department for Integrative Evolutionary Biology, Max Planck Institute for Biology, Max-Planck-Ring 9, 72076 Tübingen, Germany

[2] Department of Internal Medicine I and M3 Research Institute, University Clinic Tübingen, Otfried-Müller-Strasse 37, Tübingen, 72076, Germany

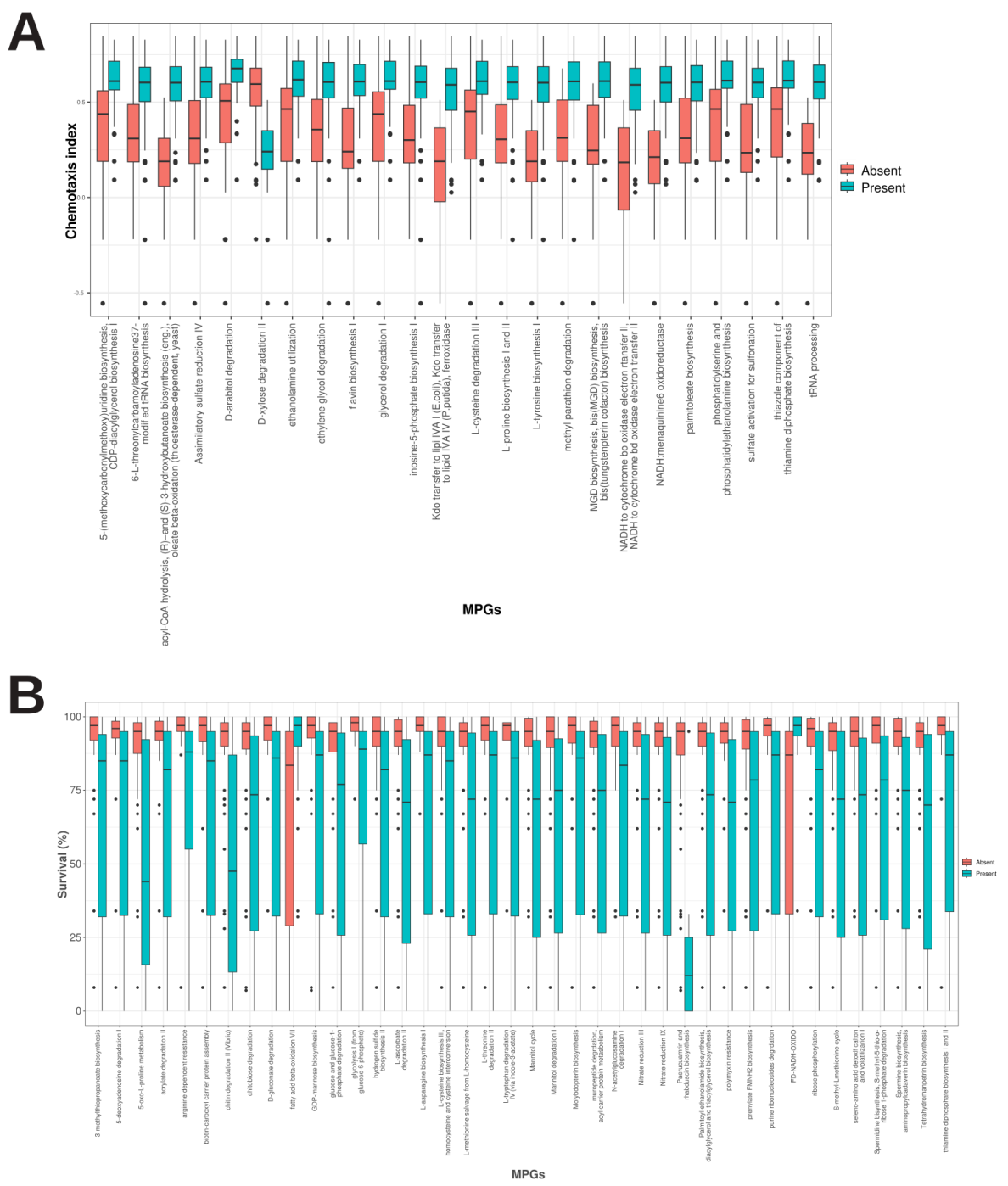
[*] Corresponding author: christian.roedelsperger@tuebingen.mpg.de



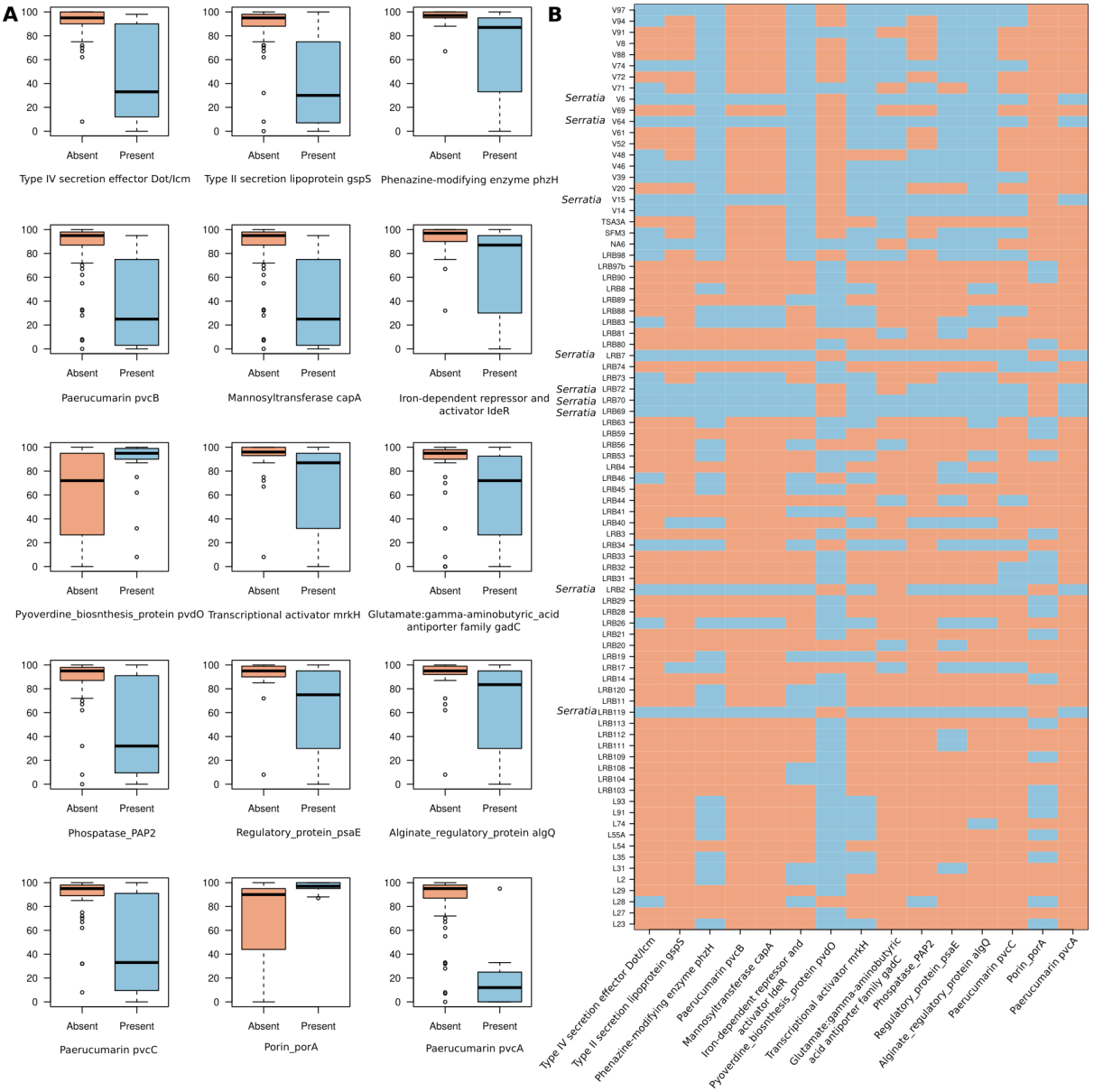
Supplemental Figure S1. Reconstruction of bacterial phylogenies to characterize novel strains. Potentially novel bacterial strains in our collection were integrated into previously published phylogenies by reconstructing species trees that include the candidate genome together with their best hits in the non-redundant version of NCBI. (A) The phylogeny for *Sphingobacterium* L2 (B) The phylogeny for *Pseudomonas* L74. (C) The phylogeny for *Erwinia* V71. (D) The phylogeny for *Phytobacter* V69 and V91.



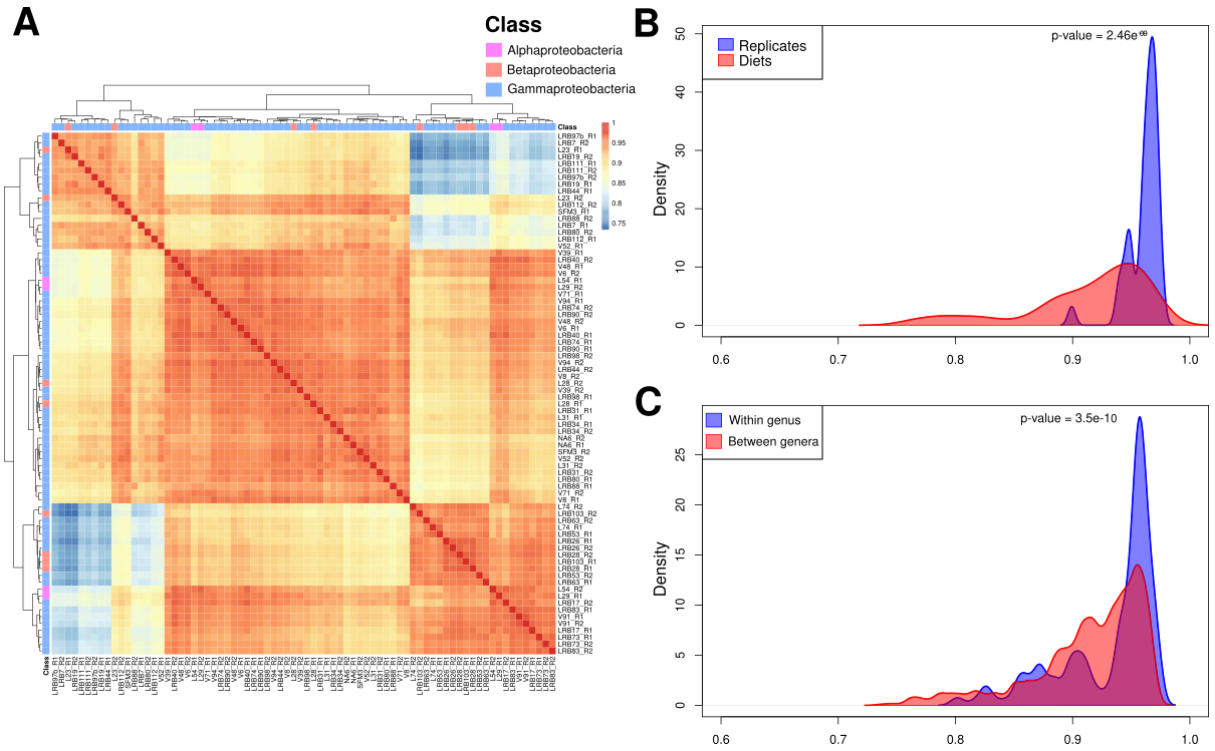
Supplemental Figure S2. Phylogenetic signature of metabolic potential. Pairwise comparisons of the MPGs present in strains of the same genus versus strains of different genera indicated that closely related bacteria share a higher number of MPGs and thus, might exhibit a genus-specific metabolic signature.



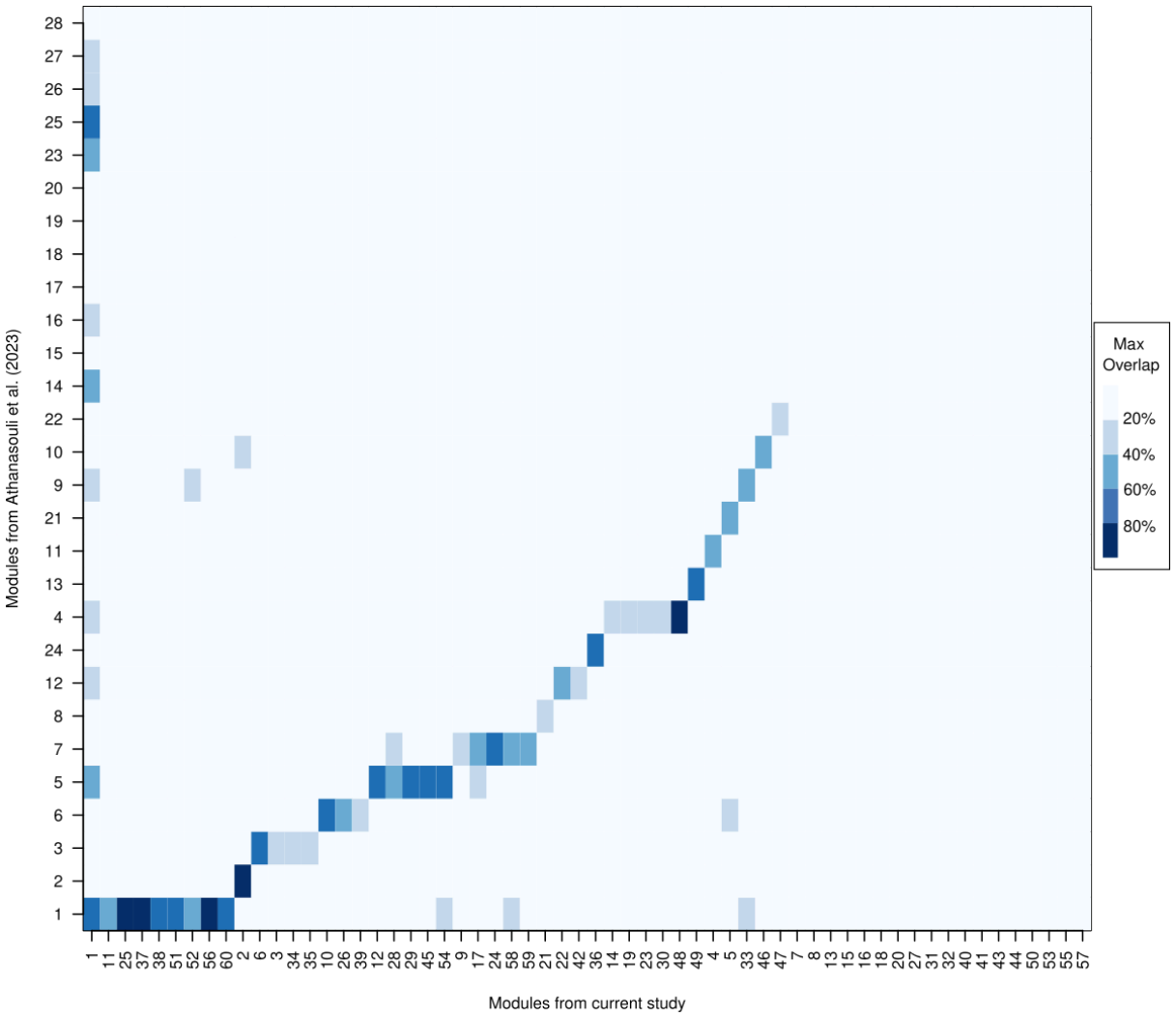
Supplemental Figure S3. Association of MPGs with chemoattraction and survival. (A) Chemotaxis indices were obtained by transferring 50-200 J4/adult worms grown on *E. coli* OP50 to a fresh plate with equal amounts of test bacteria and control bacteria (*E. coli* OP50) and quantifying the amount of worms on spots with the test bacteria relative to the control after three hours (Akduman et al. 2018). Association analysis with the MPGs predicted 24 MPGs with a significant impact on chemotaxis behavior. **(B)** The boxplots show the survival data (survival after five days exposure) for all significantly associated MPGs.



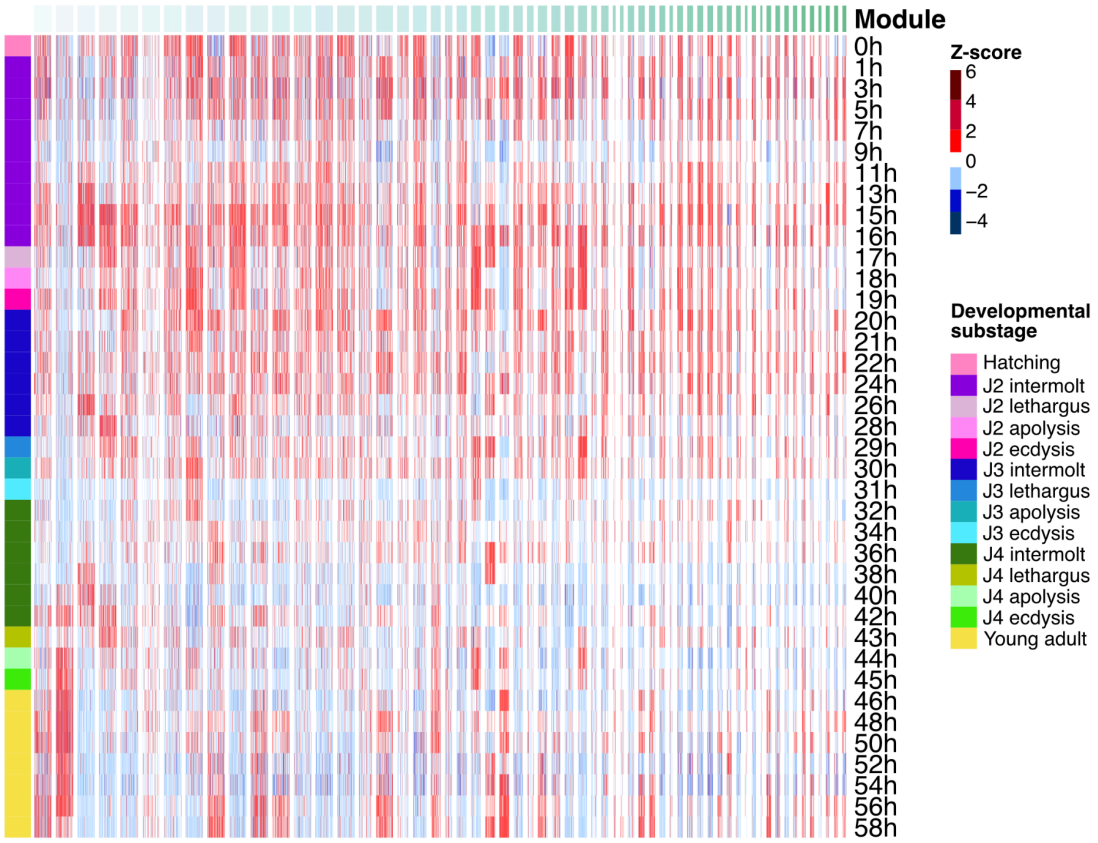
Supplemental Figure S4. Association of bacterial virulence factors with nematode survival. (A) The core protein set of the Virulence Factor DataBase (VFDB) analysis was clustered together with the bacterial protein sets into orthogroups which were then used to test for associations with survival data. The boxplots show the most significantly associated orthogroups, labeled with a representative VFDB member. While most cases point towards reduced survival in the presence of a candidate virulence factor, pyoverdine and porins show opposite patterns. This could potentially be explained by co-occurrence or mutual exclusion with other factors. **(B)** The heatmap shows the presence/absence pattern of the candidate orthogroups across the bacterial genomes.



Supplemental Figure S5. Variation in the transcriptomic response of the nematode. (A) Correlation analysis and clustering of the transcriptomes from *P. pacificus* nematodes in response to 38 different bacteria. The overall transcriptomes are highly similar with most pairwise comparisons showing correlation coefficients >0.9. **(B)** Biological replicates exhibited significantly higher correlation coefficients compared to transcriptomes from different bacterial diets. **(C)** Transcriptomes of bacteria from the same genus showed significantly higher correlation coefficients compared to dietary samples from different genera.



Supplemental Figure S6. Comparison of coexpression networks.
We calculated the gene set overlap between modules from the current and our previous study (Athanasouli et al. 2023). The color code indicates the percentage of genes in the smaller module that is shared with a given larger module from the other study. The majority the modules from the current study show substantial overlap with only a single module from the previous study indicating robust coexpression signals across studies.



Supplemental Figure S7. Visualization of the co-expression modules with developmental time course data from Sun et al (2021). The top modules of the co-expression network were visualized with developmental time-course data. This revealed developmental signatures in various modules.