

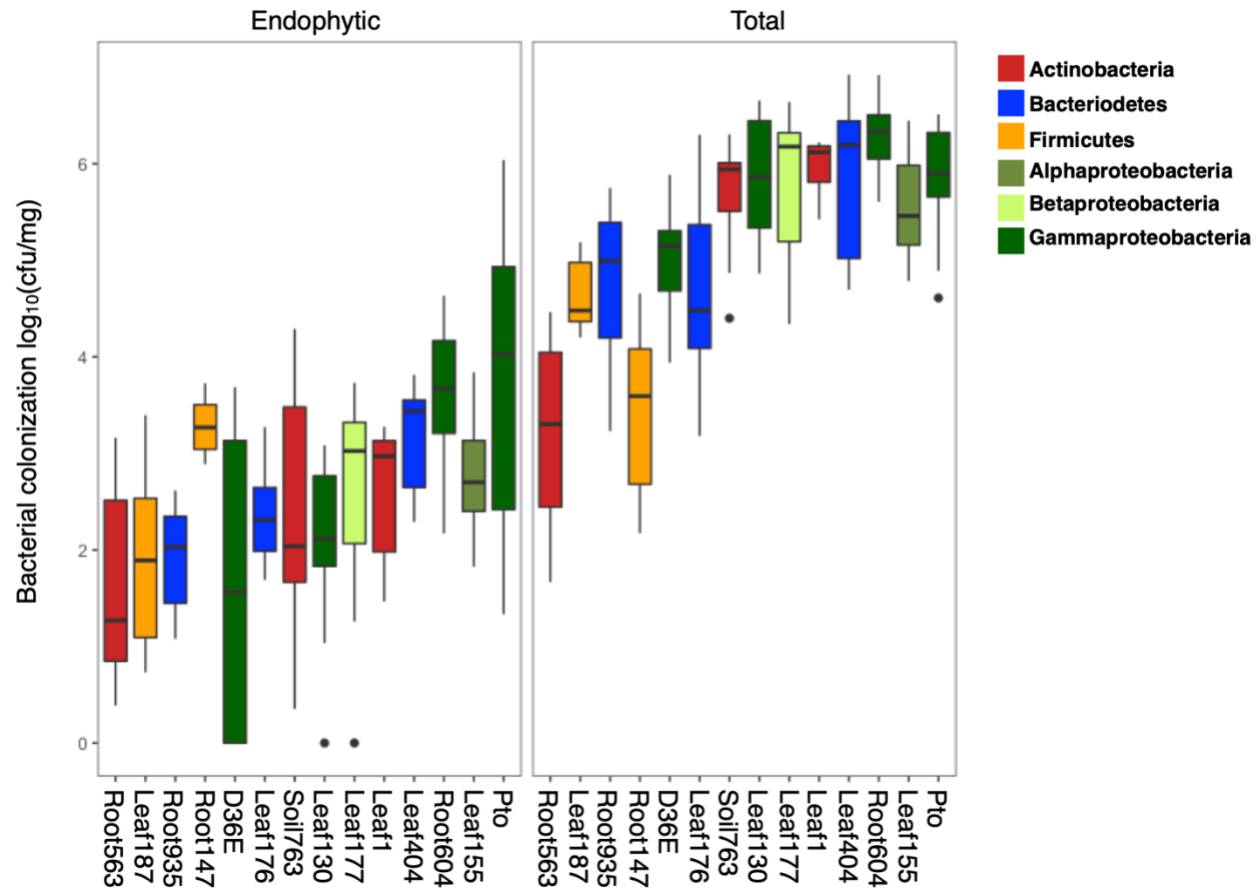
Appendix

Dissecting the co-transcriptome landscape of plants and their microbiota

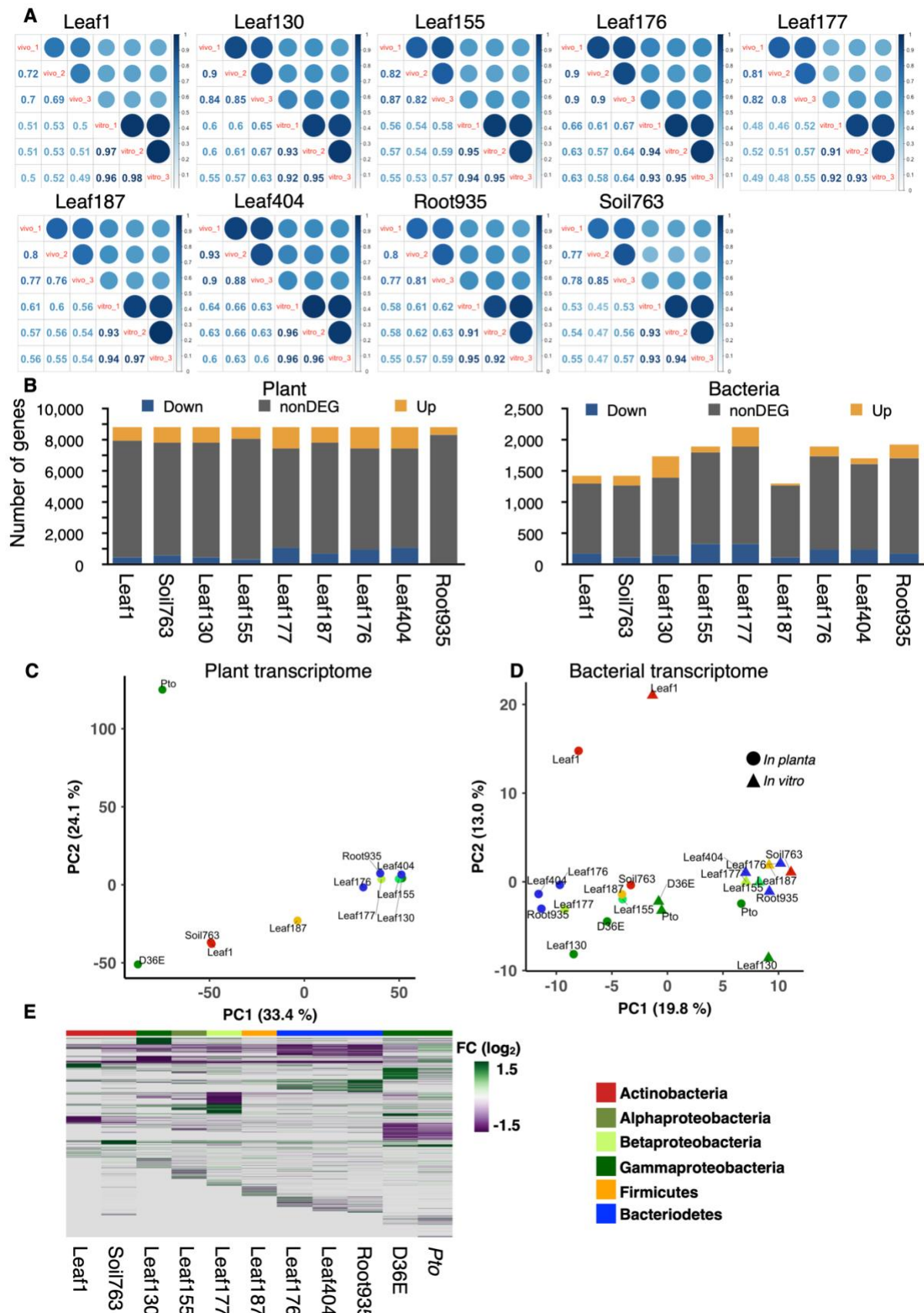
Tatsuya Nobori^{4,5}, Yu Cao⁴, Frederickson Entila⁴, Eik Dahms⁴, Yayoi Tsuda^{1,2,3,4}, Ruben Garrido-Oter^{4,6}, Kenichi Tsuda^{1,2,3,4*}

Table of Contents

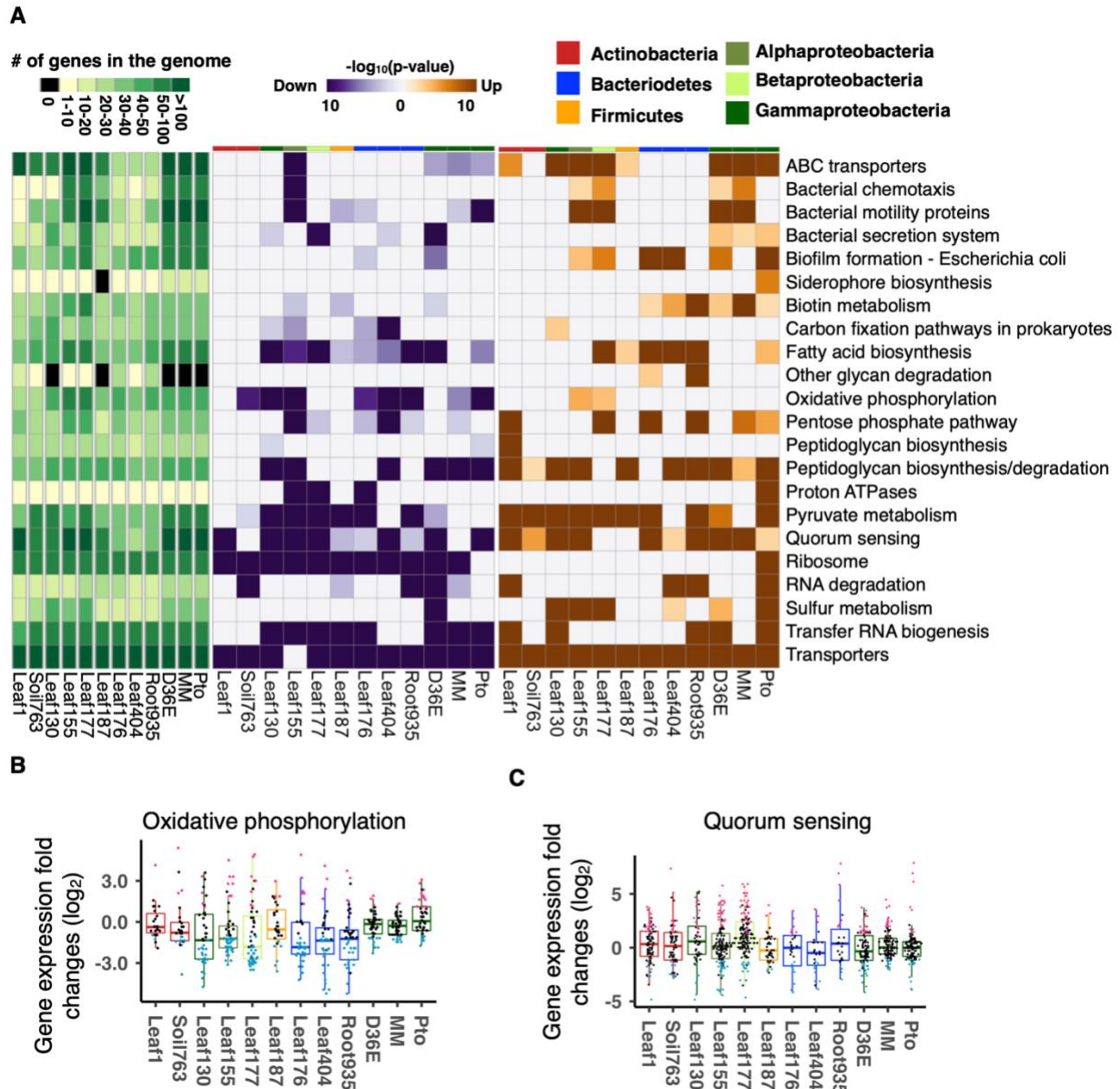
Appendix Figure S1. Commensal strains can colonize in leaf endosphere...	2
Appendix Figure S2. Transcriptome analysis of bacteria...	3
Appendix Figure S3. Conserved and strain-specific regulation of bacterial functions in plants...	5
Appendix Figure S4. Intrapylum comparative transcriptomics of commensals...	6
Appendix Figure S5. Bacterial genes differentially regulated in plants...	8
Appendix Figure S6. Expression of various processes of commensals <i>in planta</i> ...	9
Appendix Figure S7. Expression of genes related to nutrient acquisition processes in commensals...	10
Appendix Figure S8. Integration of plant and bacterial transcriptomes...	12
Appendix Figure S9. Bacterial gene expression <i>in vitro</i> and <i>in planta</i> ...	13
Appendix Figure S10. Expression of commensals genes related to various physiological processes <i>in planta</i> ...	15
Appendix Figure S11. Expression of commensal genes related to various physiological processes <i>in planta</i> ...	17
Appendix Figure S12. Expression of commensal genes related to various physiological processes <i>in planta</i> ...	19
Appendix Figure S13. Expression of commensal genes related to various physiological processes <i>in planta</i> ...	21
Appendix Figure S14. <i>In vitro</i> growth of commensals...	23



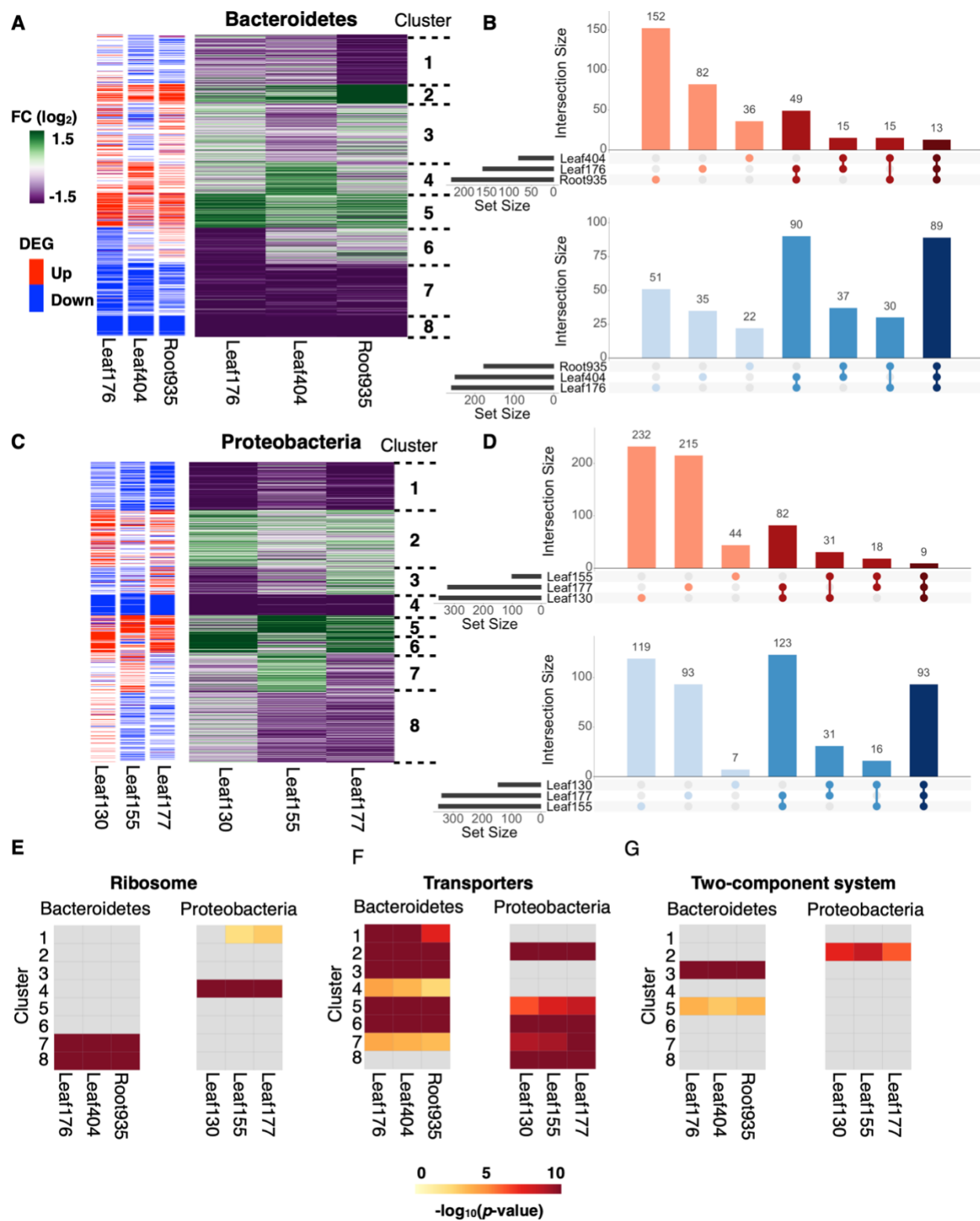
Appendix Figure S1. Commensal strains can colonize in leaf endosphere Bacteria were flood-inoculated to three-week-old *A. thaliana* Col-0 at $\text{OD}_{600} = 0.5$. Bacterial growth was measured three days after inoculation. (Left) Bacteria that entered the leaf endosphere and persisted or grew were counted after washing and sterilizing the leaf surface. (Right) total bacteria (endophytic + epiphytic) were counted without any surface washing and sterilization (see Methods). Three independent replicates were taken for each sample.



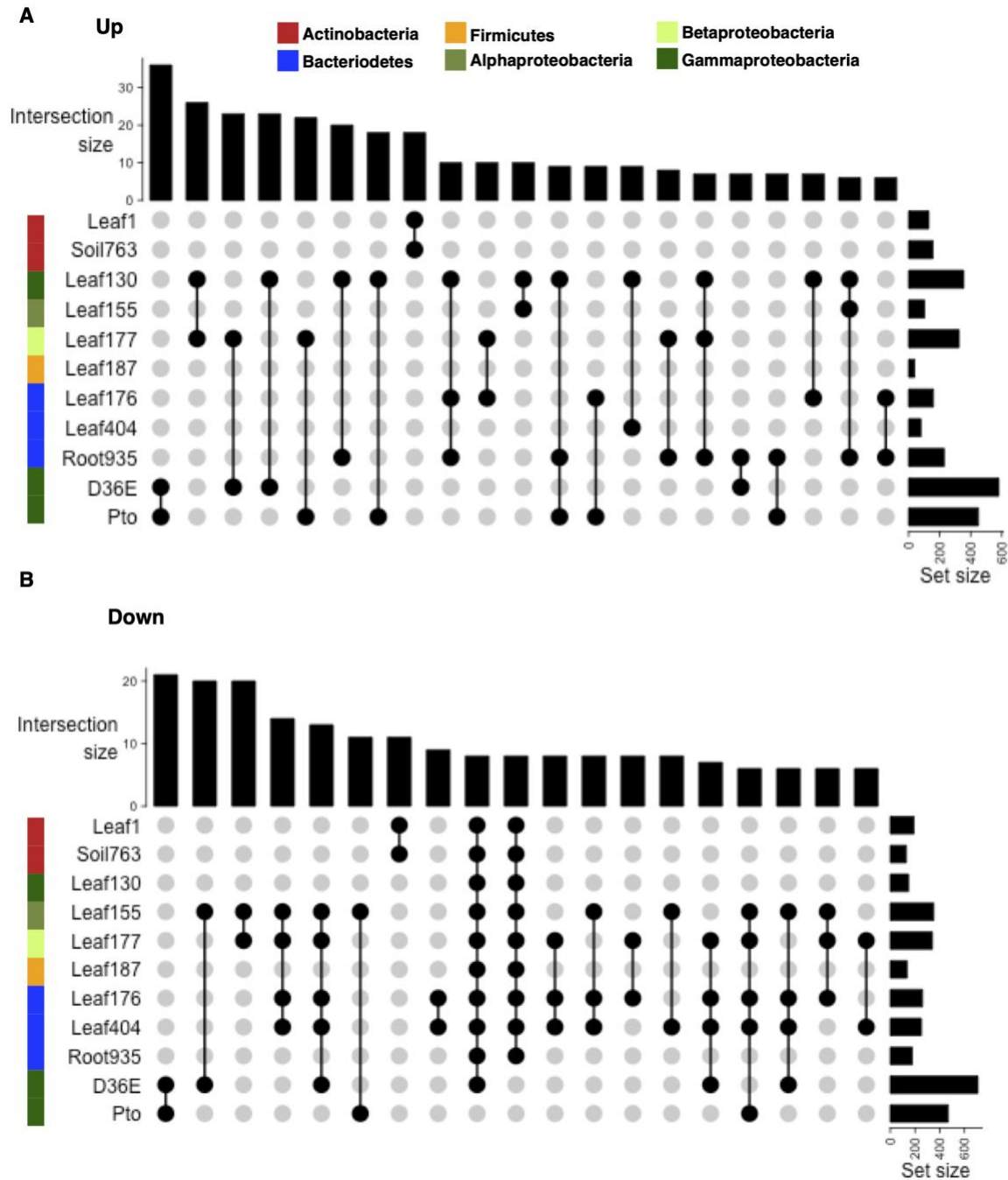
Appendix Figure S2. Transcriptome analysis of bacteria **(A)** The correlation plot of each replicate of bacterial RNA-seq data for individual strains. vivo: bacteria in plants. vitro: bacteria in rich media. **(B)** The number of genes analyzed in plant or bacterial RNA-seq. Differentially regulated genes ($|\log_2FC| > 1$; FDR < 0.01; two-tailed Student's t test followed by Storey's q-value) are colored in blue or yellow. Plant: bacteria-inoculated vs. water-inoculated. Bacteria: *in planta* 6 h vs. *in vitro* (rich media). Bacterial strains used for co-transcriptomics are shown. Plant and bacterial genes with low expression levels across samples were removed. **(C)** Principal component analysis of gene expression fold changes (FCs) of plants inoculated with bacteria used for the co-transcriptome analysis (bacteria-inoculated vs. water-inoculated). **(D)** Principal component analysis of bacterial gene (orthologous group) expression *in planta* and *in vitro*. **(E)** Genes that are present in each strain are colored in red. See Source Data S1 for the gene presence-absence table. The data of *Pto* and D36E are from a previous study (Nobori et al., 2018). Genes not detected or missing in the genome are shown in gray. See Source Data S2 for gene expression data. **(C-E)** The taxonomic affiliation (phylum/class level) of each strain is indicated with different colors.



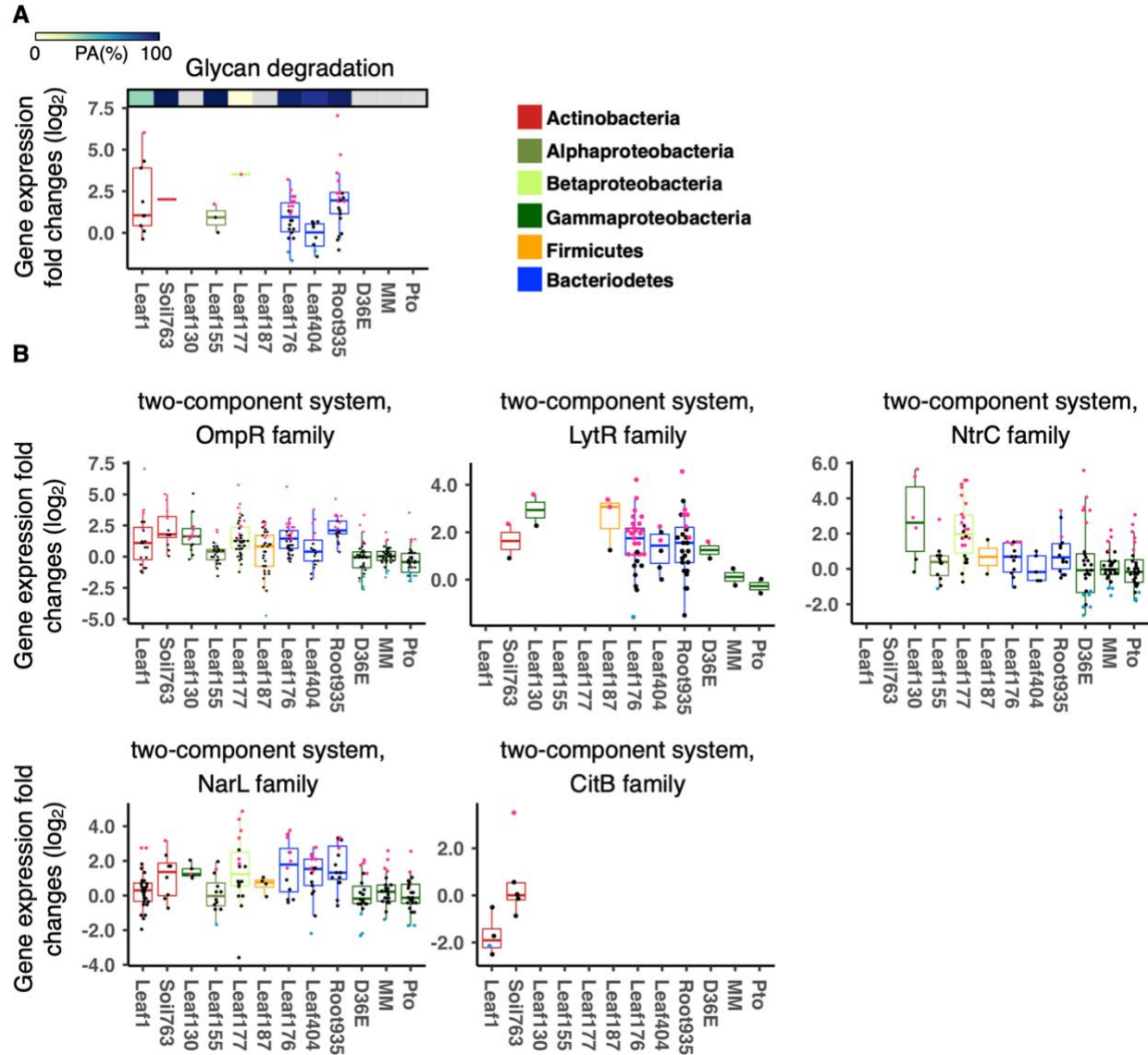
Appendix Figure S3. Conserved and strain-specific regulation of bacterial functions in plants (A) KEGG orthology terms enriched in genes that are significantly up- (orange) or down (purple)-regulated *in planta* compared with *in vitro* (rich media). The heatmaps indicate $-\log_{10}$ p-values (FDR corrected by Benjamini-Hochberg method). The top color bars indicate the taxonomic affiliation (phylum/class level) of each strain. **(B and C)** Expression fold changes (*in planta* vs. *in vitro*) of genes involved in **(B)** Oxidative phosphorylation and **(C)** Quorum sensing. MM, *Pto* grown in a minimal medium. Results are shown as box plots with boxes displaying the 25th–75th percentiles, the centerline indicating the median, whiskers extending to the minimum, and maximum values no further than 1.5 inter-quartile range. Box color indicates the taxonomic affiliation (phylum/class level) of each strain. All individual data points (genes) are overlaid with colors for DEGs (red: upregulated, blue: downregulated, black: non-DEG). **(A-C)** For each condition, mean values of three independent replicates were taken.



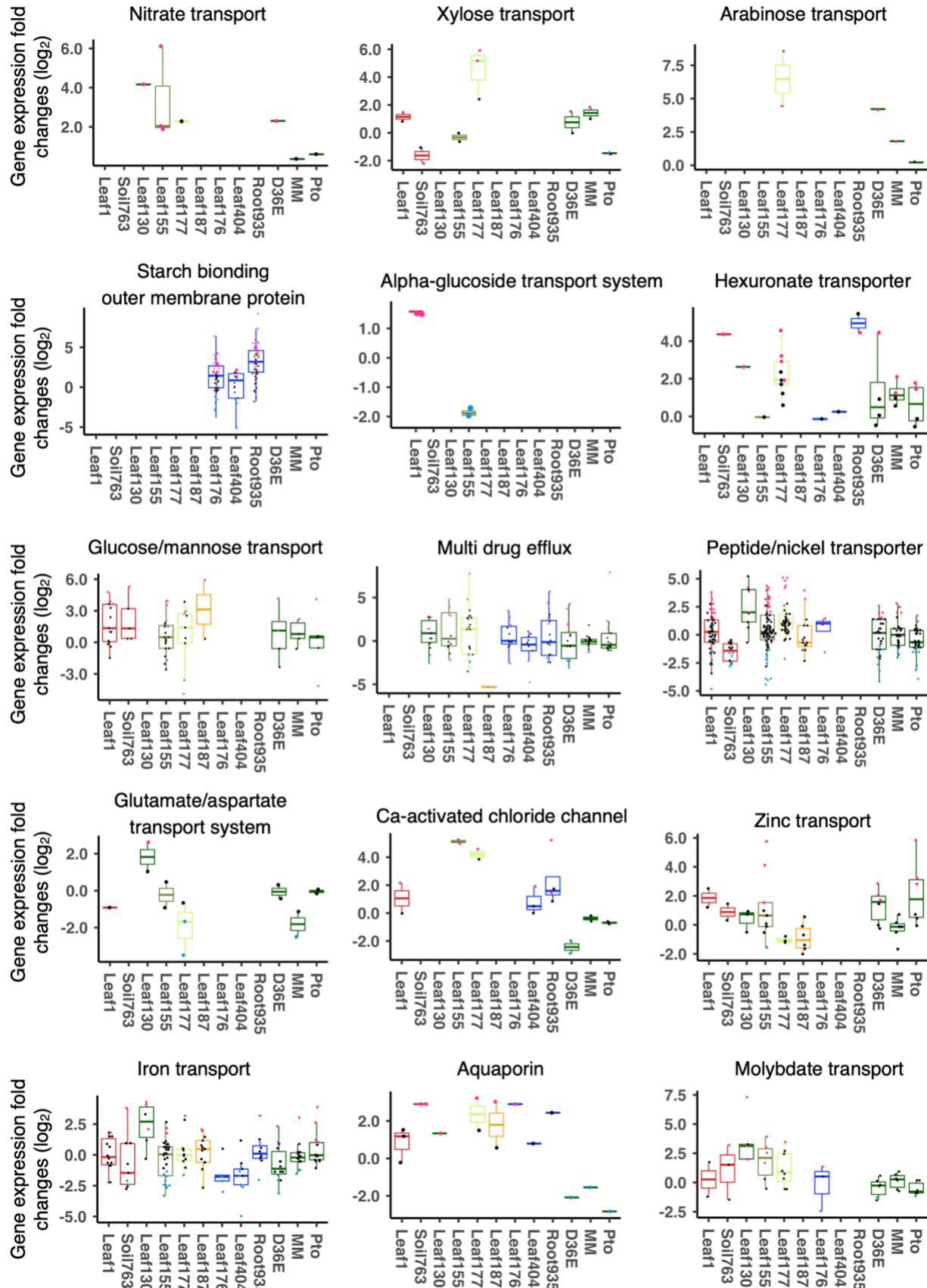
Appendix Figure S4. Intrapylum comparative transcriptomics of commensals (A and C) Gene expression fold changes between *in planta* and *in vitro* (rich media) of **(A)** Bacteroidetes and **(C)** Proteobacteria strains. Orthologous groups shared among three strains were used for the analysis. Differentially expressed genes (DEGs) (*in planta* vs. *in vitro*; $|\log_2FC| > 1$; FDR < 0.01; two-tailed Student's t test followed by Storey's q-value) are indicated in the sidebars. Gene clusters defined by k-means clustering are shown (k = 8). The number of clusters was chosen based on the elbow method. **(B and D)** UpSet intersection plots of DEGs either up- (red) or down (blue)-regulated *in planta* in the **(B)** Bacteroidetes and **(D)** Proteobacteria strains. Intersection size and set size indicate the number of shared DEGs and the number of DEGs in each strain, respectively. **(E-G)** Enrichment analysis of genes with the KEGG orthology terms **(E)** "Ribosome", **(F)** "Transporters", and **(G)** "Two-component system". **(A-G)** For each condition, mean values of three independent replicates were taken.



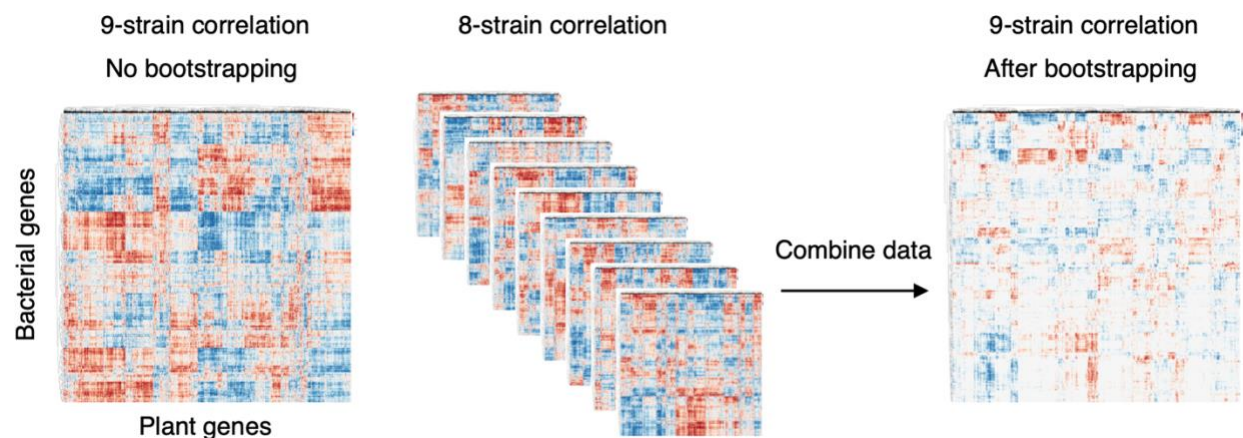
Appendix Figure S5. Bacterial genes differentially regulated in plants UpSet intersection plots of differentially expressed genes (DEGs; $|\log_2FC| > 1$; FDR < 0.01; two-tailed Student's t test followed by Storey's q-value) either **(A)** up- or **(B)** downregulated *in planta*. Intersection size and set size indicate the number of shared DEGs and the number of DEGs in each strain, respectively. Combinations of more than one strain with intersection size > 5 are shown. The color sidebars indicate the taxonomic affiliation.



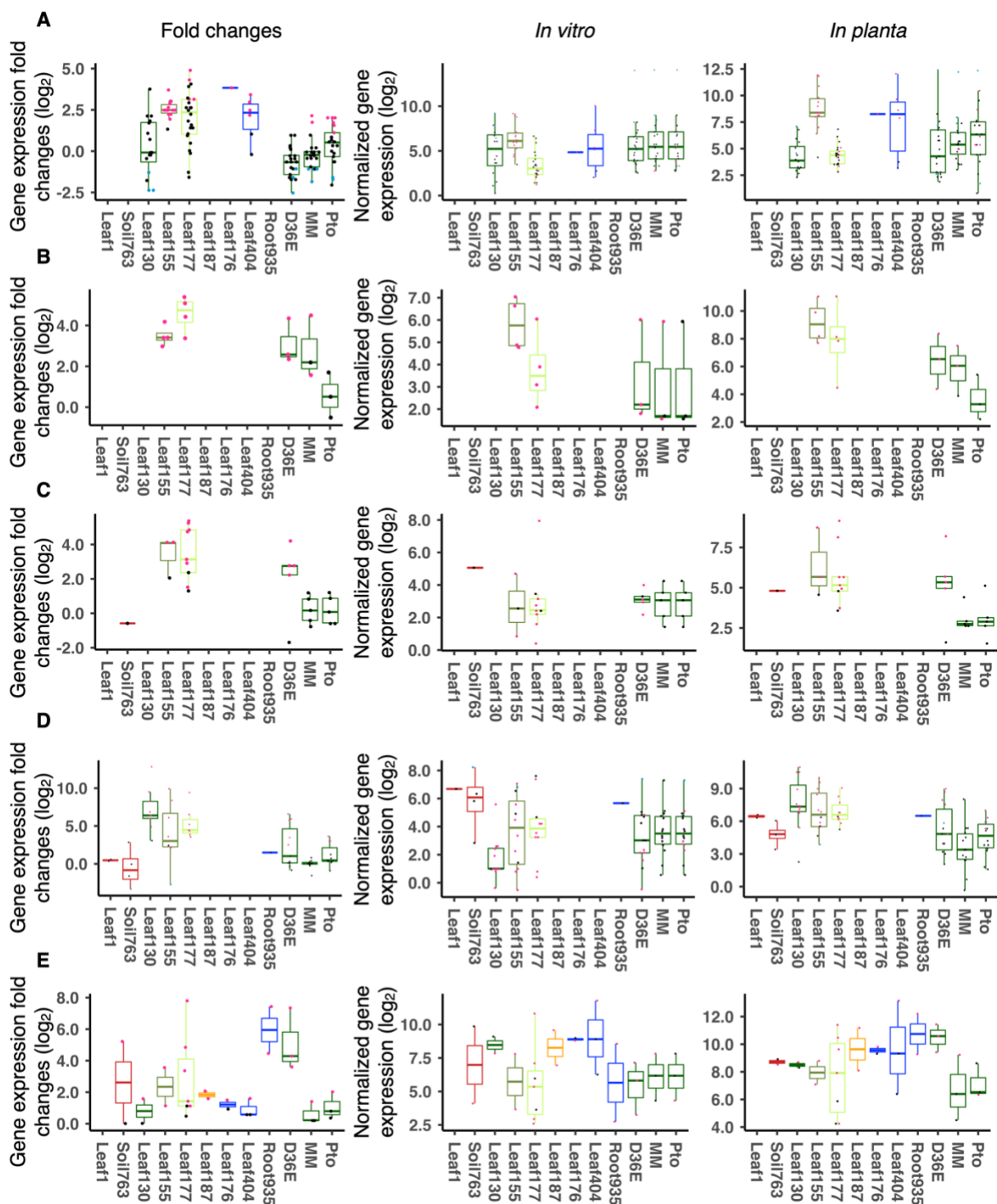
Appendix Figure S6. Expression of various processes of commensals *in planta*
 Expression fold changes (*in planta* vs. *in vitro*) of genes related to **(A)** glycan degradation and **(B)** two-component system. Results are shown as box plots with boxes displaying the 25th–75th percentiles, the centerline indicating the median, whiskers extending to the minimum, and maximum values no further than 1.5 inter-quartile range. Box color indicates the taxonomic affiliation (phylum/class level) of each strain. All individual data points (genes) are overlaid with colors for DEGs (red: upregulated, blue: downregulated, black: non-DEG). **(A)** The top bar indicate the ratio of PA genes in each strain. All individual data points (genes) are overlaid to the box plots with colors for DEGs (red: upregulated, blue: downregulated, black: non-DEG). **(A-B)** For each condition, mean values of three independent replicates were taken.



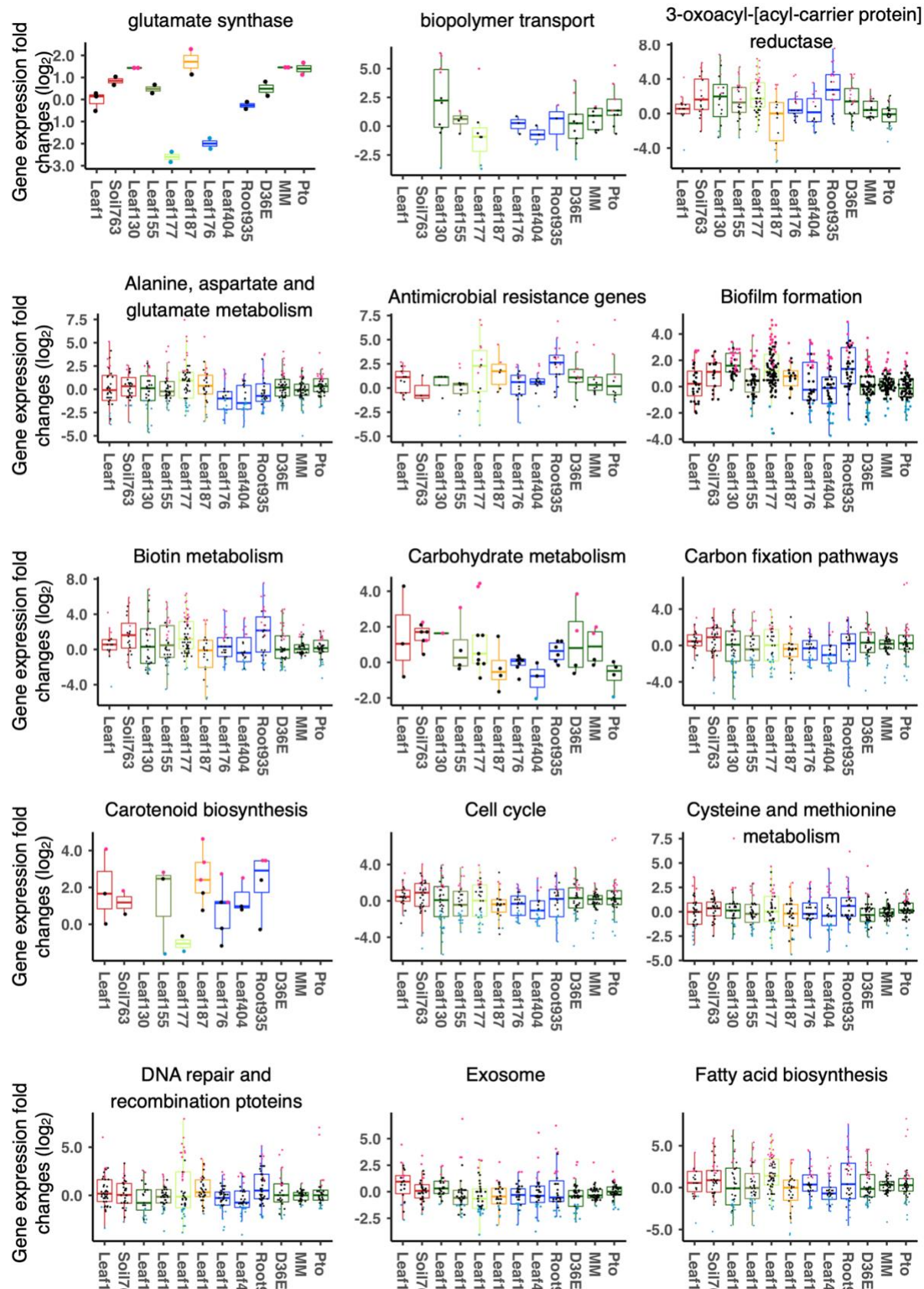
Appendix Figure S7. Expression of genes related to nutrient acquisition processes in commensals Expression fold changes (*in planta* vs. *in vitro*) of genes related to nutrient transporters. Results are shown as box plots with boxes displaying the 25th–75th percentiles, the centerline indicating the median, whiskers extending to the minimum, and maximum values no further than 1.5 inter-quartile range. Box color indicates the taxonomic affiliation (phylum/class level) of each strain (see Fig. S6 for the color code). All individual data points (genes) are overlaid with colors for DEGs (red: upregulated, blue: downregulated, black: non-DEG). For each condition, mean values of three independent replicates were taken.



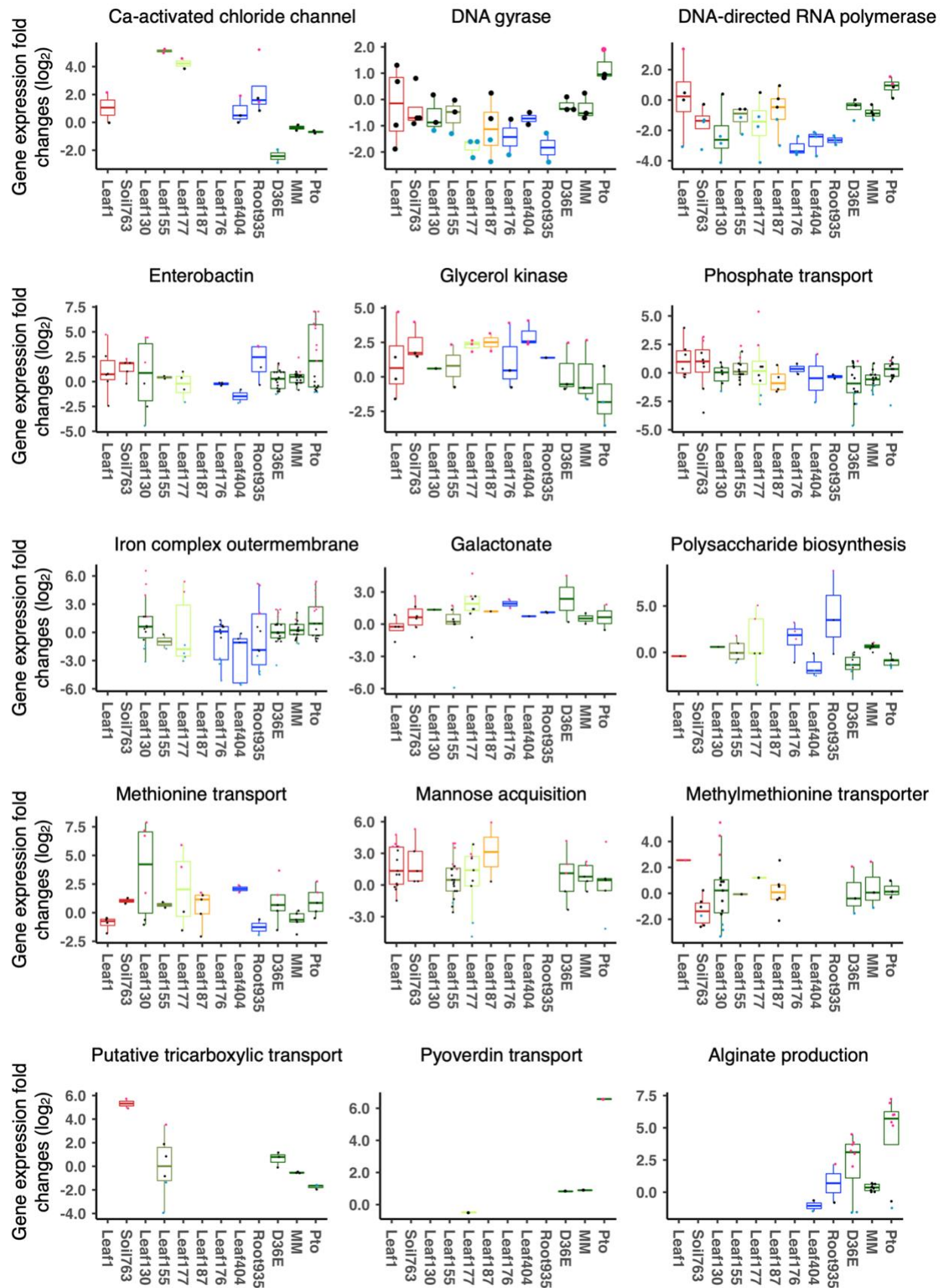
Appendix Figure S8. Integration of plant and bacterial transcriptomes Schematic diagram showing a bootstrapping approach to evaluate correlations between individual plant and bacterial genes. To obtain robust correlation scores, Pearson's correlation coefficients were calculated using all the combinations of eight strains as well as using all the nine strains. Among these 8-strain and 9-strain datasets, the weakest correlation coefficient value was used for each combination of a bacterial OG and a plant gene ("Combining data").



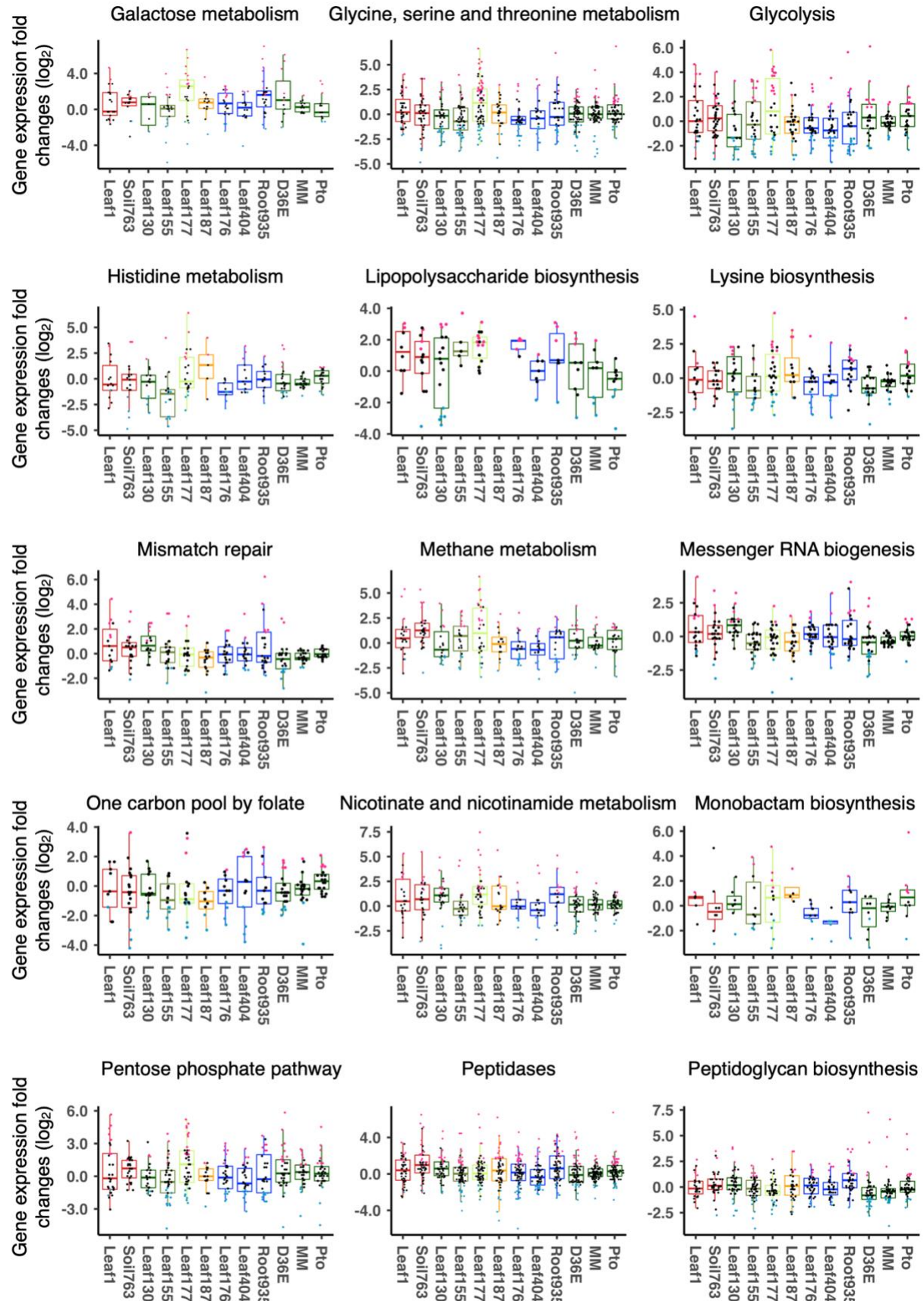
Appendix Figure S9. Bacterial gene expression *in vitro* and *in planta* Expression of bacterial genes related to (A) Type 6 secretion system (B) Glycerol transport (C) Urea transport (D) Sulfur transport (E) Catalase. Results are shown as box plots with boxes displaying the 25th–75th percentiles, the centerline indicating the median, whiskers extending to the minimum, and maximum values no further than 1.5 inter-quartile range. Box color indicates the taxonomic affiliation (phylum/class level) of each strain. All individual data points (genes) are overlaid with colors for DEGs (red: upregulated, blue: downregulated, black: non-DEG). For each condition, mean values of three independent replicates were taken.



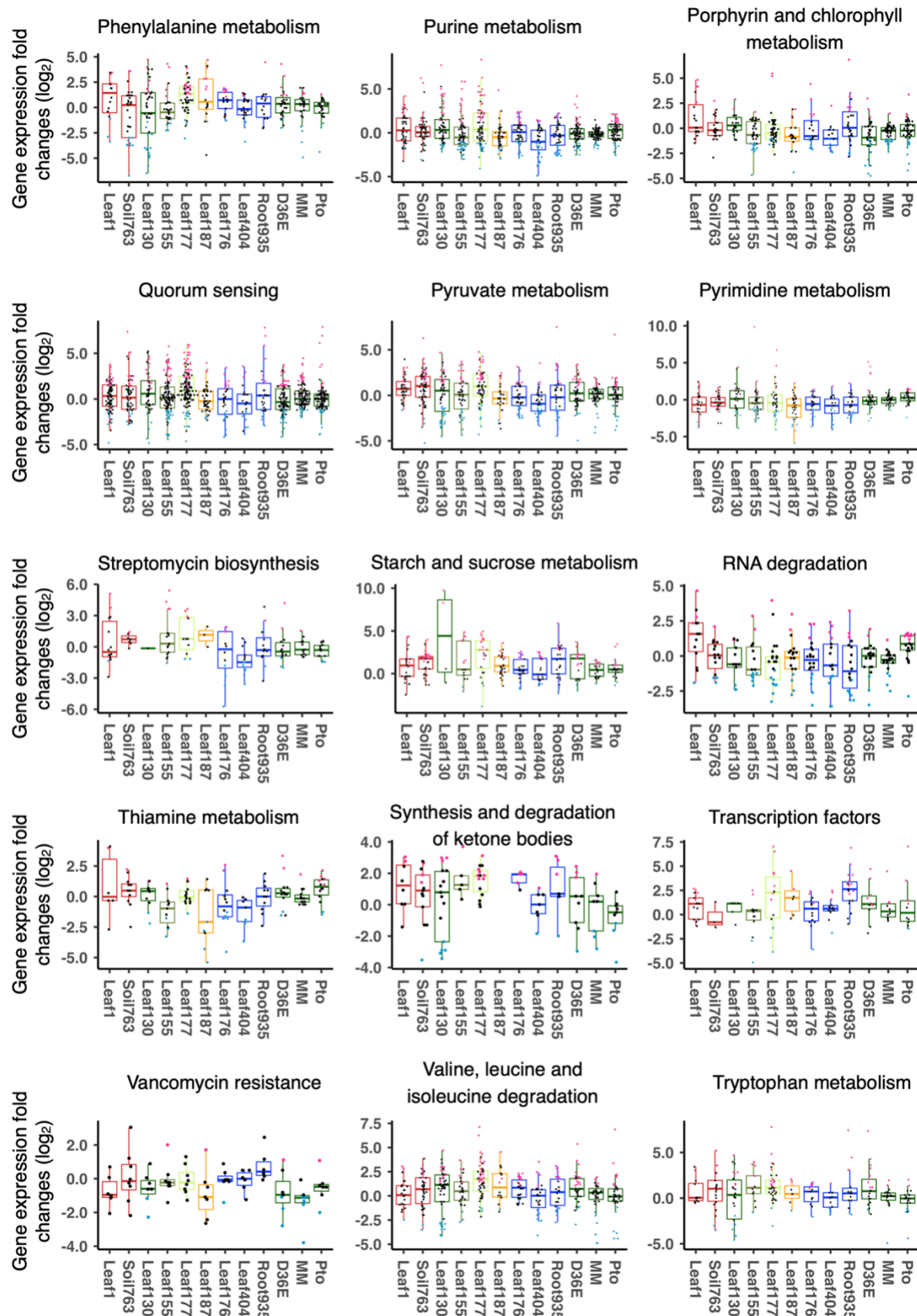
Appendix Figure S10. Expression of commensals genes related to various physiological processes *in planta* Expression fold changes (*in planta* vs. *in vitro*) of genes related to various functions. Results are shown as box plots with boxes displaying the 25th–75th percentiles, the centerline indicating the median, whiskers extending to the minimum, and maximum values no further than 1.5 inter-quartile range. Box color indicates the taxonomic affiliation (phylum/class level) of each strain. All individual data points (genes) are overlaid with colors for DEGs (red: upregulated, blue: downregulated, black: non-DEG). For each condition, mean values of three independent replicates were taken.



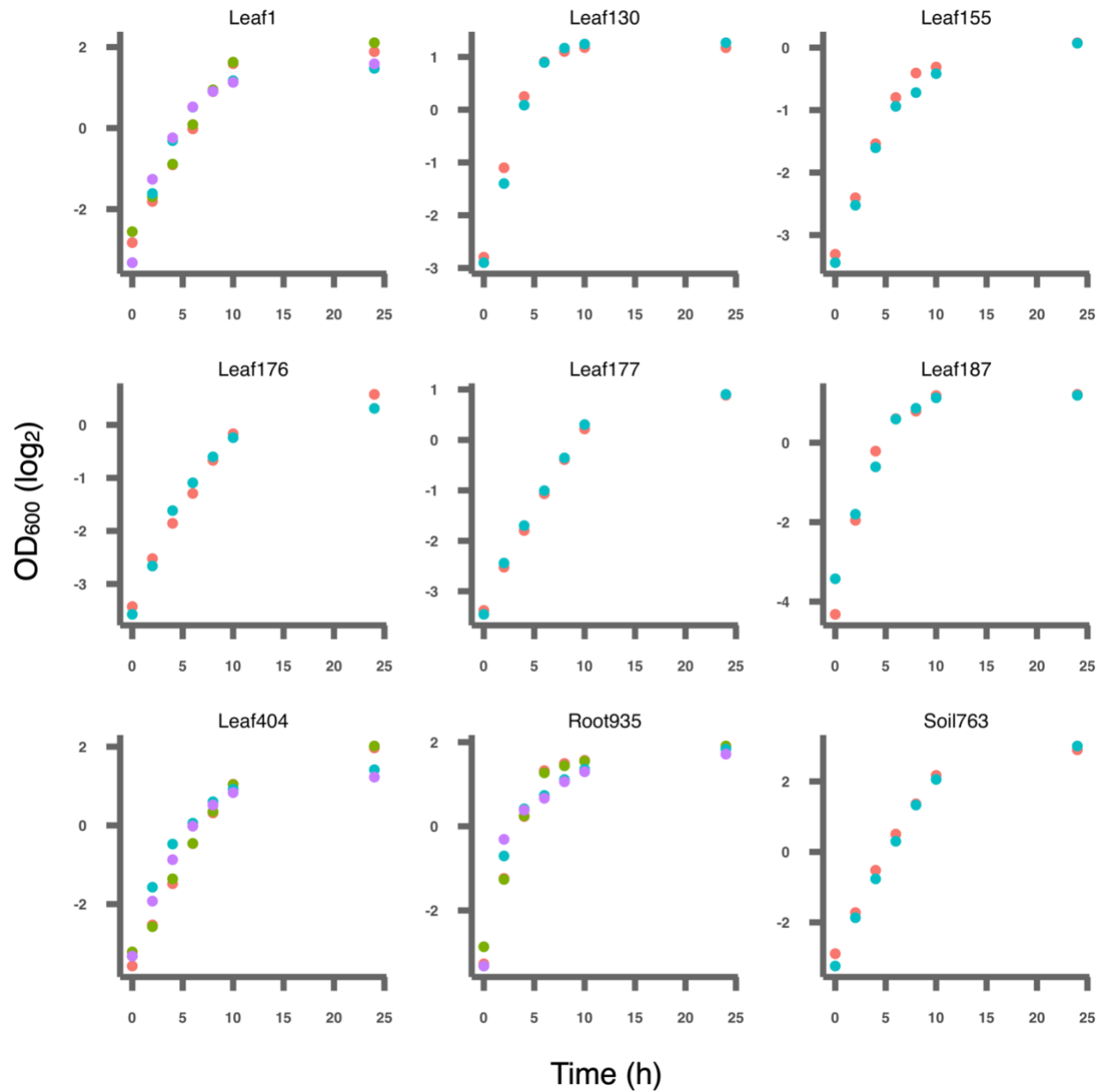
Appendix Figure S11. Expression of commensal genes related to various physiological processes *in planta* Expression fold changes (*in planta* vs. *in vitro*) of genes related to various functions. Results are shown as box plots with boxes displaying the 25th–75th percentiles, the centerline indicating the median, whiskers extending to the minimum, and maximum values no further than 1.5 inter-quartile range. Box color indicates the taxonomic affiliation (phylum/class level) of each strain. All individual data points (genes) are overlaid with colors for DEGs (red: upregulated, blue: downregulated, black: non-DEG). For each condition, mean values of three independent replicates were taken.



Appendix Figure S12. Expression of commensal genes related to various physiological processes *in planta* Expression fold changes (*in planta* vs. *in vitro*) of genes related to various functions. Results are shown as box plots with boxes displaying the 25th–75th percentiles, the centerline indicating the median, whiskers extending to the minimum, and maximum values no further than 1.5 inter-quartile range. Box color indicates the taxonomic affiliation (phylum/class level) of each strain. All individual data points (genes) are overlaid with colors for DEGs (red: upregulated, blue: downregulated, black: non-DEG). For each condition, mean values of three independent replicates were taken.



Appendix Figure S13. Expression of commensal genes related to various physiological processes *in planta* Expression fold changes (*in planta* vs. *in vitro*) of genes related to various functions. Results are shown as box plots with boxes displaying the 25th–75th percentiles, the centerline indicating the median, whiskers extending to the minimum, and maximum values no further than 1.5 inter-quartile range. Box color indicates the taxonomic affiliation (phylum/class level) of each strain. All individual data points (genes) are overlaid with colors for DEGs (red: upregulated, blue: downregulated, black: non-DEG). For each condition, mean values of three independent replicates were taken.



Appendix Figure S14. *In vitro* growth of commensals Commensal bacteria were cultured in rich media. Replicates are shown in different colors.