Arabidopsis thaliana root responses to Cd exposure: insights into root tip-specific changes and the role of HY5 in limiting Cd accumulation and promoting tolerance

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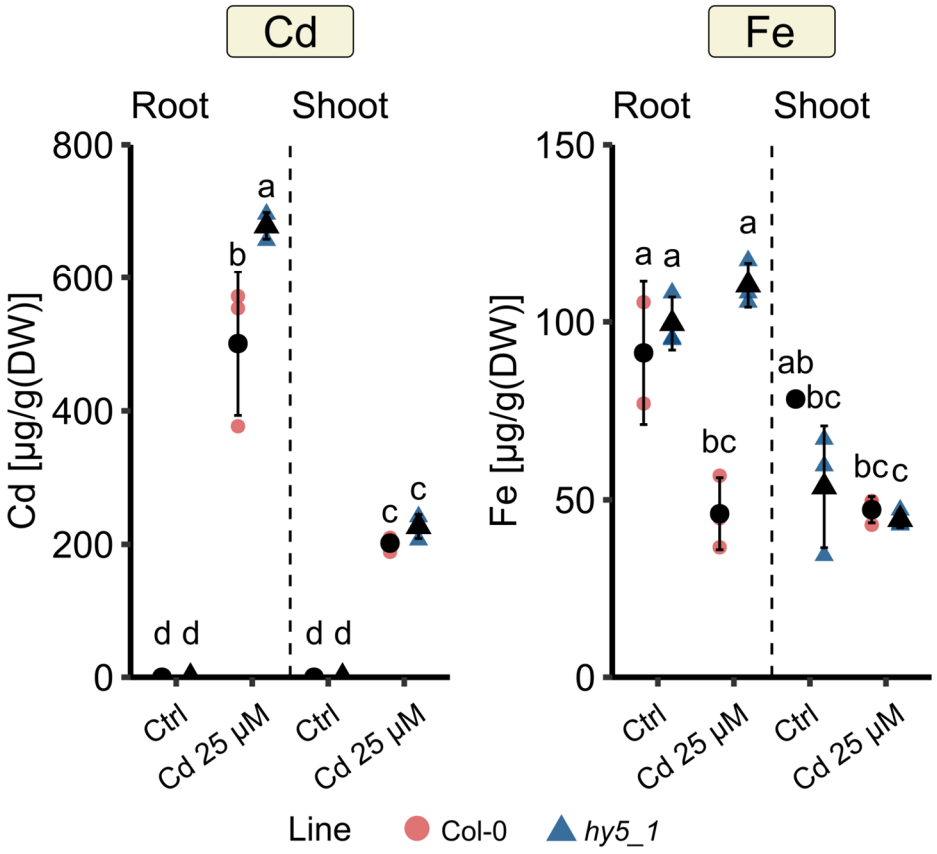
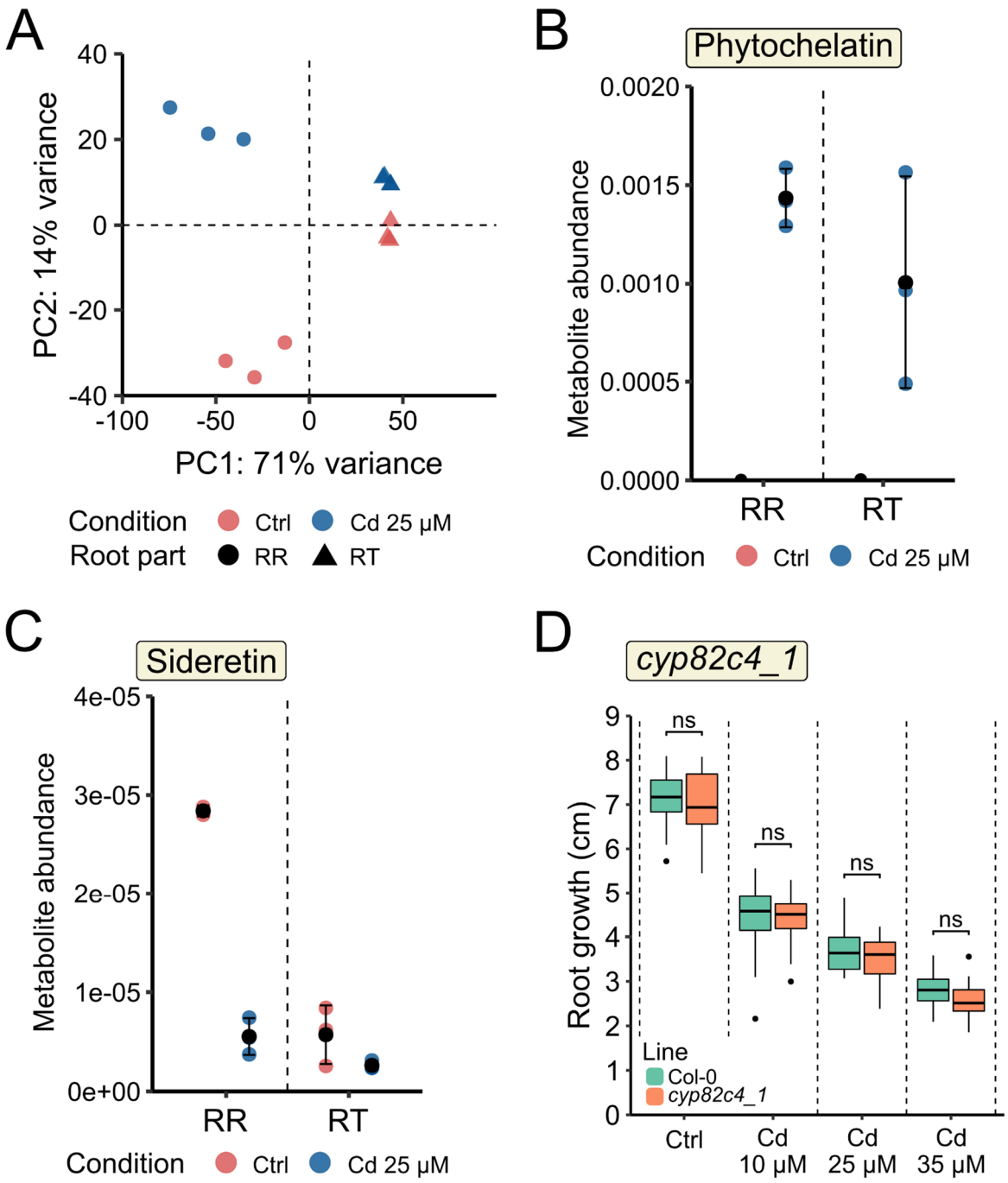
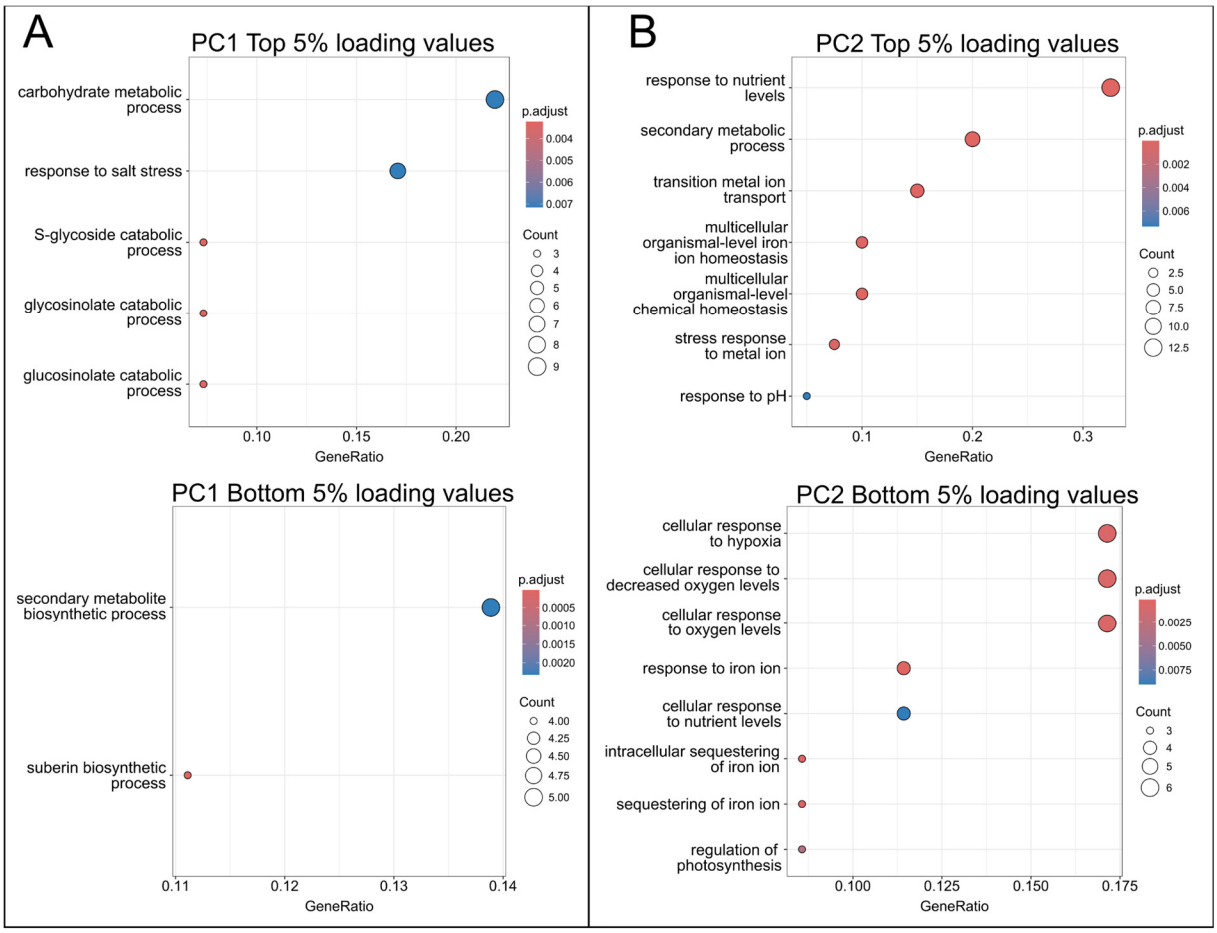
Supplementary Figures



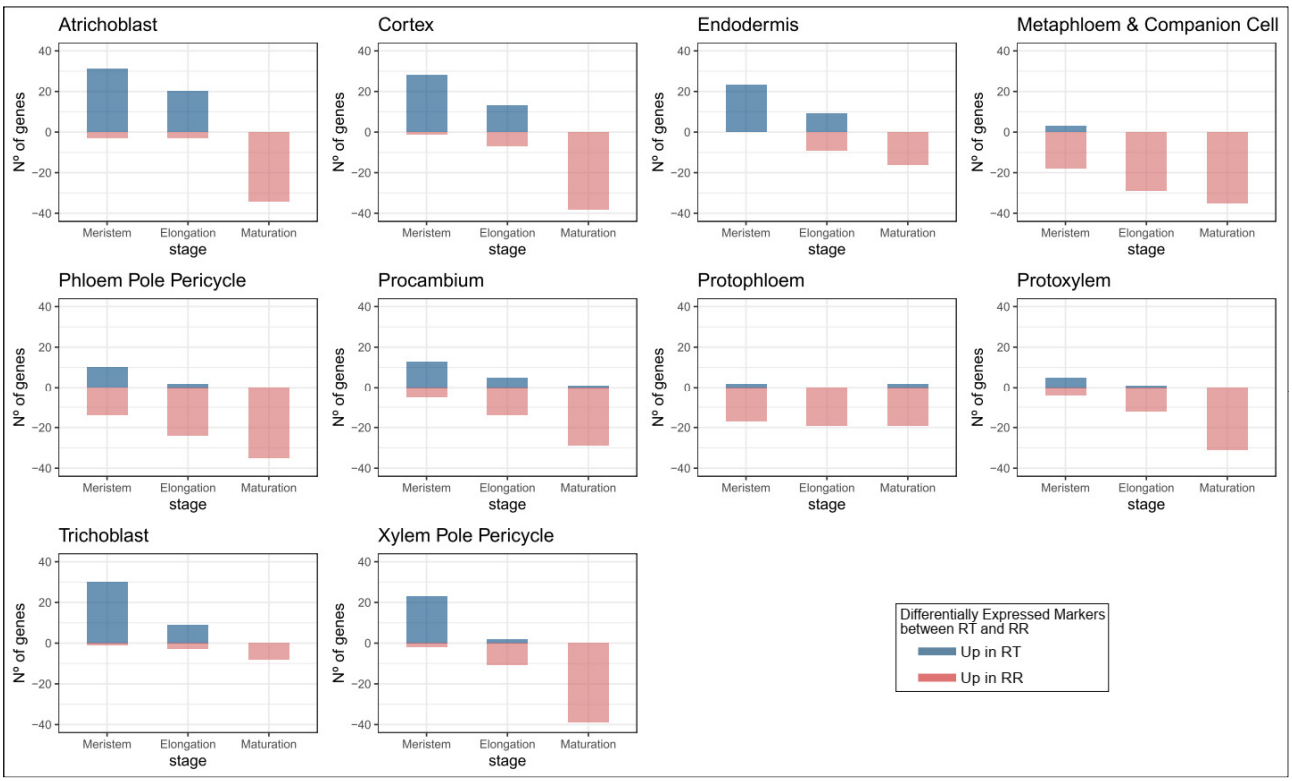
Supplementary Figure S1. ICP-OES measurement of Cd (left) and Fe (right) in Col-0 and *hy5_1* seedlings upon exposure to 25 µM Cd. Seedlings were grown for 7 d on control medium and then transferred to control or Cd medium and exposed for 72 h. Displayed mean values +/- standard deviation of Cd contents as µg per gram dry weight (DW). Letters indicate statistical significance (ANOVA with Tukey test, p≤0.05, data from at least three independent replicates).



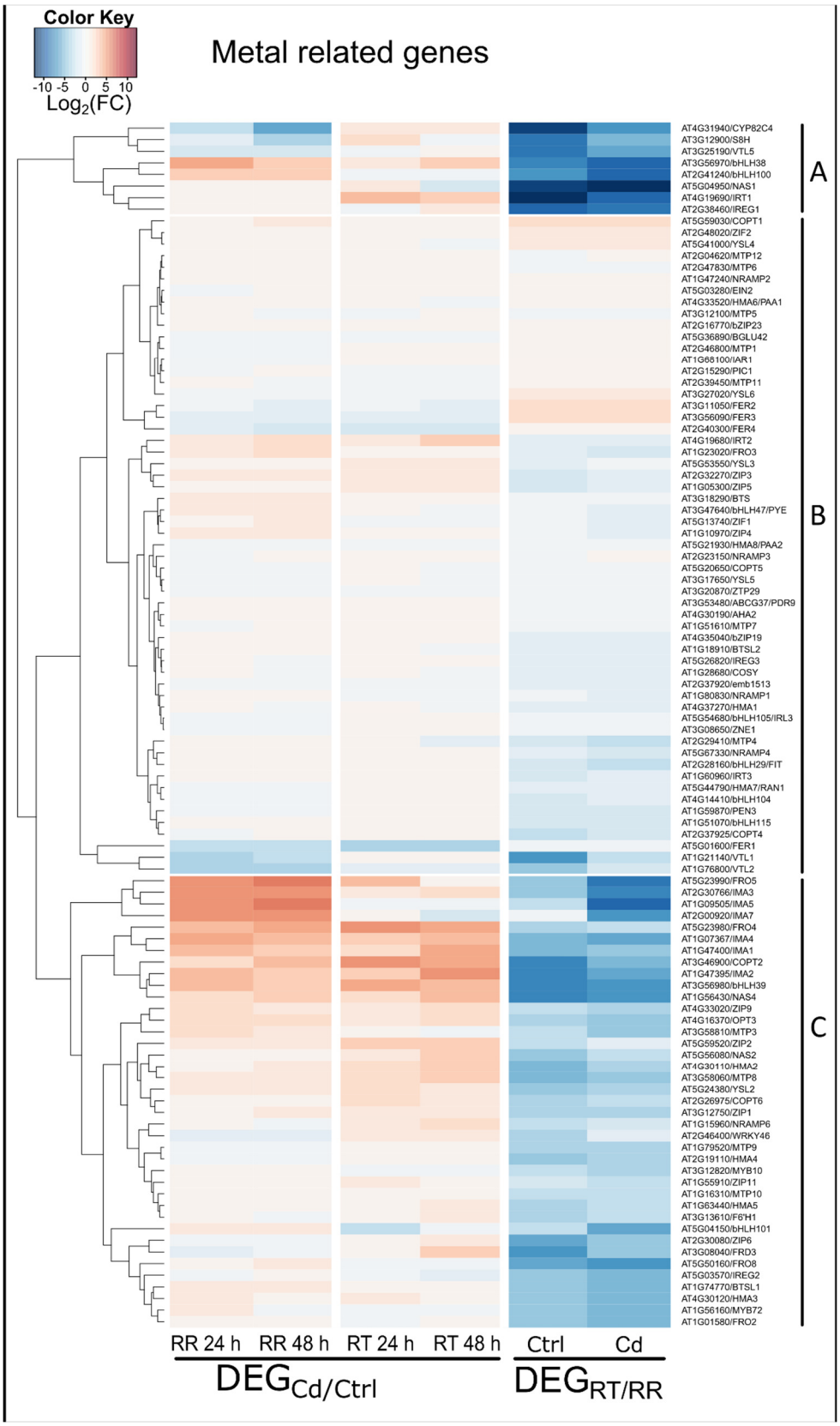
Supplementary Figure S2. Regulation of specialized metabolism in response to 25 µM Cd. A. Principal component analysis of the metabolomic data. Metabolite concentrations were normalized to the amount of collected root pieces per sample. **B**-**C.** Quantification of sideretin and phytochelatin in control and Cd in root tips (RT) and remaining roots (RR). Seedlings were grown for 7 d on control medium and then exposed to 25 µM Cd for 48 h. **D.** Primary root growth of Col-0 and the *cyp82c4* mutant under control and Cd conditions. Statistical significance was tested with Mann Whitney U-Test (n=24-37, 3 independent replicates, p≤0.05)



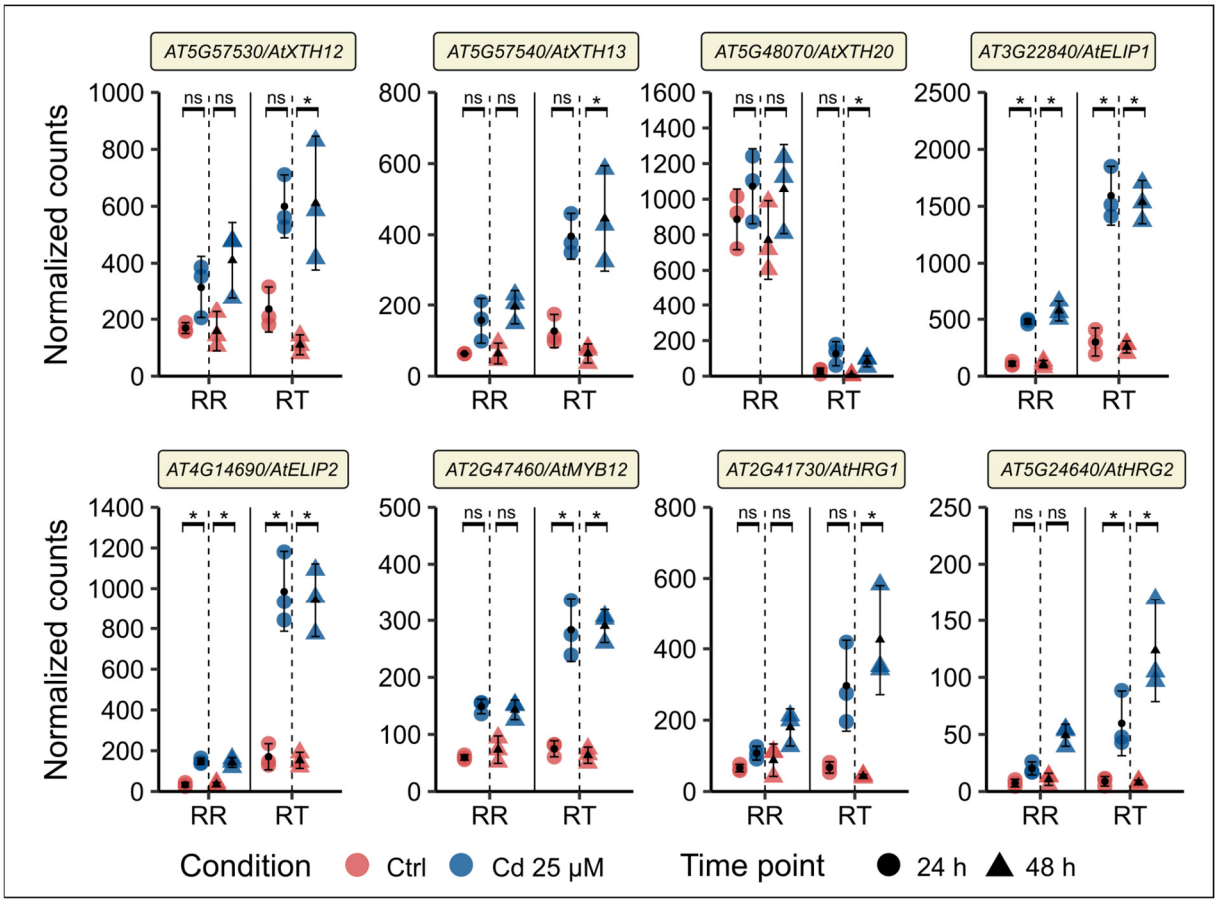
Supplementary Figure S3. GO enrichment analysis on the 5% top and bottom loading values for PC1 (A) and PC2 (B). The analysis was conducted using the ClusterProfiler package in R, which identifies overrepresented GO terms associated with the input gene sets. Enriched GO terms (Biological Process) were selected based on a false discovery rate (FDR) < 0.01. Dot Size represents the number of genes associated with each GO term; dot color, the adjusted p-value (FDR); and GeneRatio the proportion of genes in the input set associated with the GO term.



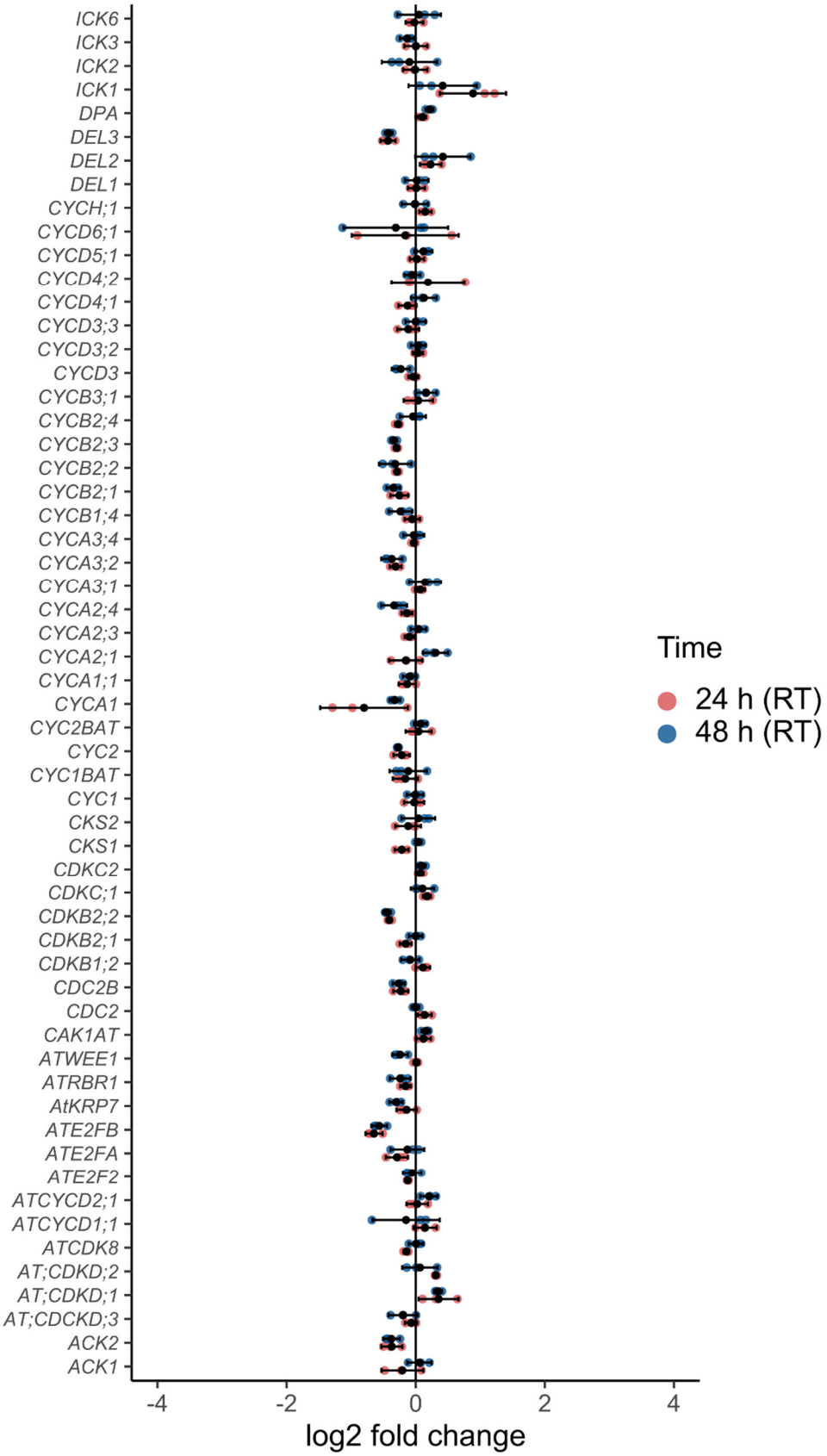
Supplementary Figure S4. Differentially expressed markers between RT and RR. Bulk RNA-Seq data from root tip (RT) and remaining root (RR) samples upon growth in control conditions were cross-referenced with the cell-type specific description of gene expression in roots provided by Shahan *et al.*, (2022). Number of genes differentially expressed ($|\log_2$ fold change$| > 1$ and $p_{adj} \leq 0.05$) between RT and RR samples that are among the 50 genes that are specific markers for each cell line and development stage. Positive and negative values in the Y-axis represent the number of DEGs that are more expressed in RT or RR, respectively.



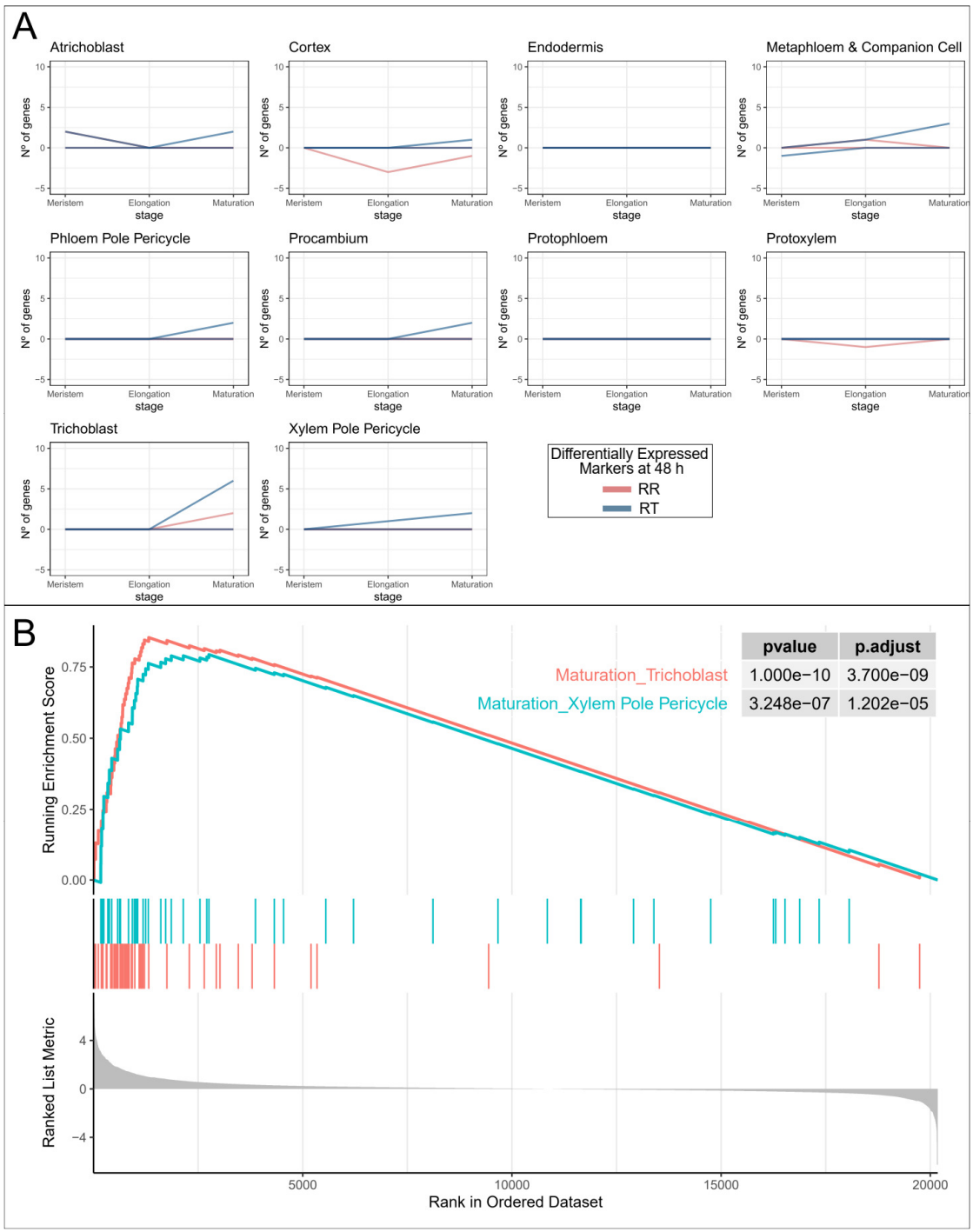
Supplementary Figure S5. Expression of metal-related genes after 24 h and 48 h of Cd treatment in *A. thaliana* roots. Using a manually curated list of metal related genes, the heatmap shows the log2(fold change) of gene expression between conditions. In the $DEG_{Cd/Ctrl}$ columns, positive values indicate upregulation of genes after Cd treatment, while negative values indicate downregulation, in both the remaining root (RR) and root tip (RT) after 24 h or 48 h of treatment. In the $DEG_{RT/RR}$ columns, positive values indicate higher expression in RT compared to RR, while negative values indicate higher expression in RR compared to RT.



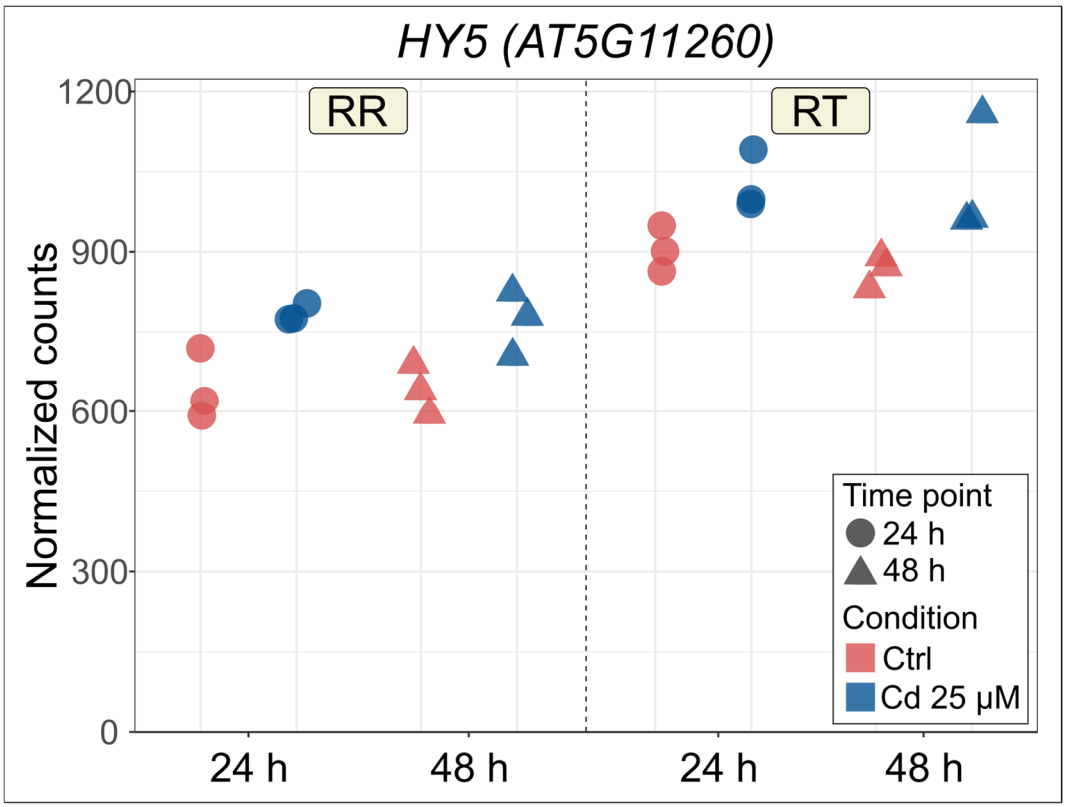
Supplementary Figure S6. Gene expression and phenotypic analysis of root tip responsive genes in *A. thaliana*. A. Expression of genes that display a specific or more pronounced response to Cd in RT compared to RR. Displayed are mean values ± standard deviation. Asterisks indicate statistical significance of DEG (DESeq2, $|\log_2$ fold change$| > 1$ and $p_{adj} \leq 0.05$, data from three independent replicates).



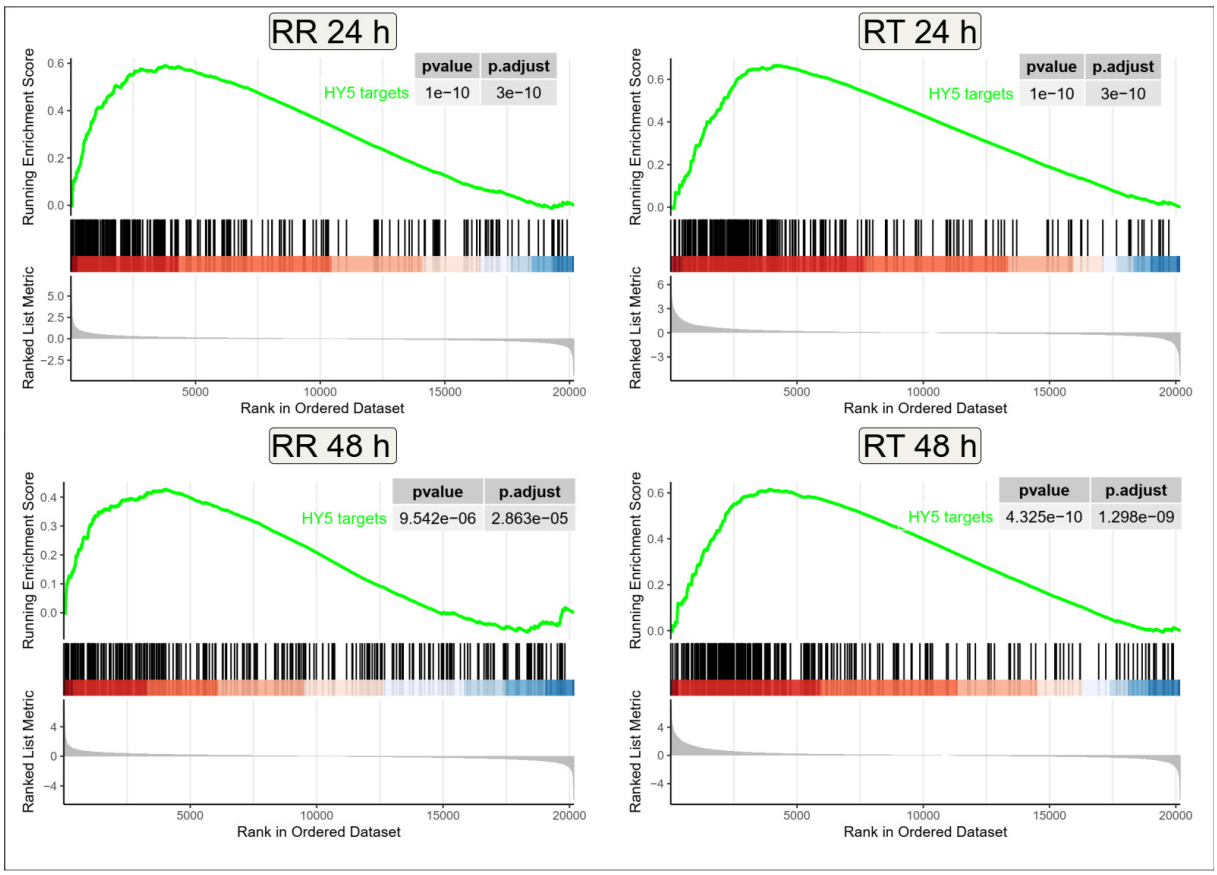
Supplementary Figure S7. Effect of 25 µM Cd on expression of cell cycle-related genes from Vandepoele *et al.*, (2002) in root tips (RT) after 24 h and 48 h. Values were obtained by dividing the DESeq2 normalized counts for each gene in the Cd-exposed sample by the mean of the respective control sample. Values are depicted as log2-fold changes.



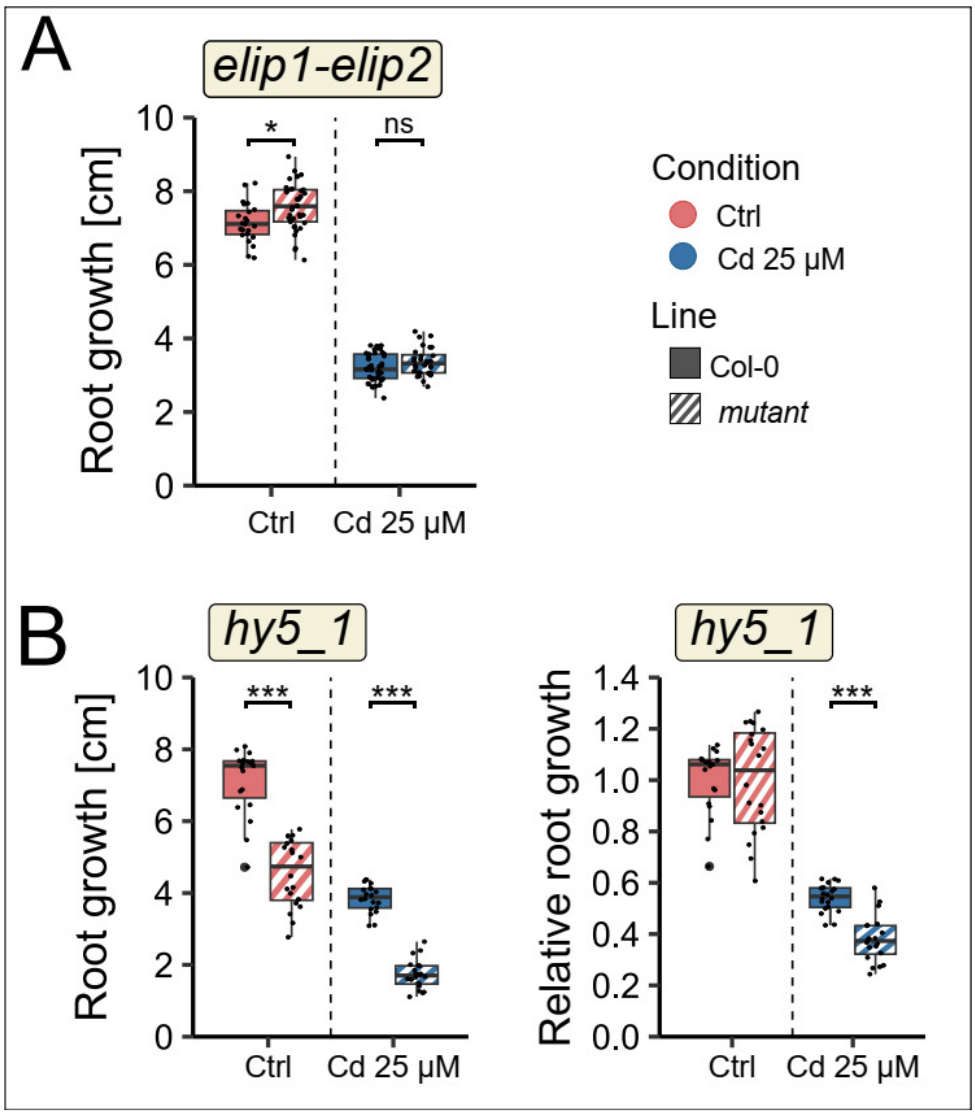
Supplementary Figure S8. Impact of Cd excess on differentiation in *A. thaliana* roots. Progression of the differentiation in the root tips upon Cd excess at 48 h. Bulk RNA-Seq data from root tip (RT) and remaining root (RR) samples upon growth in control or Cd treatments conditions were cross-referenced with the cell-type specific description of gene expression in roots provided by Shahan *et al.*, (2022). (**A**) Number of genes differentially expressed ($|\log_2$ fold change$| > 1$ and $p_{adj} \leq 0.05$) between Cd-treated and control samples that are among the 50 genes that are specific markers for each cell line and development stage. Positive and negative values in the Y-axis represent the number of DEGs that are upregulated or downregulated after Cd treatment, respectively. (**B**) Gene Set Enrichment Analysis of Developmental Markers. The plot shows the enrichment profiles of gene sets derived from Shahan *et al.*, (2022) between Cd-treated and control samples that were statistically significant (adjusted p-value < 0.05, Bonferroni correction).



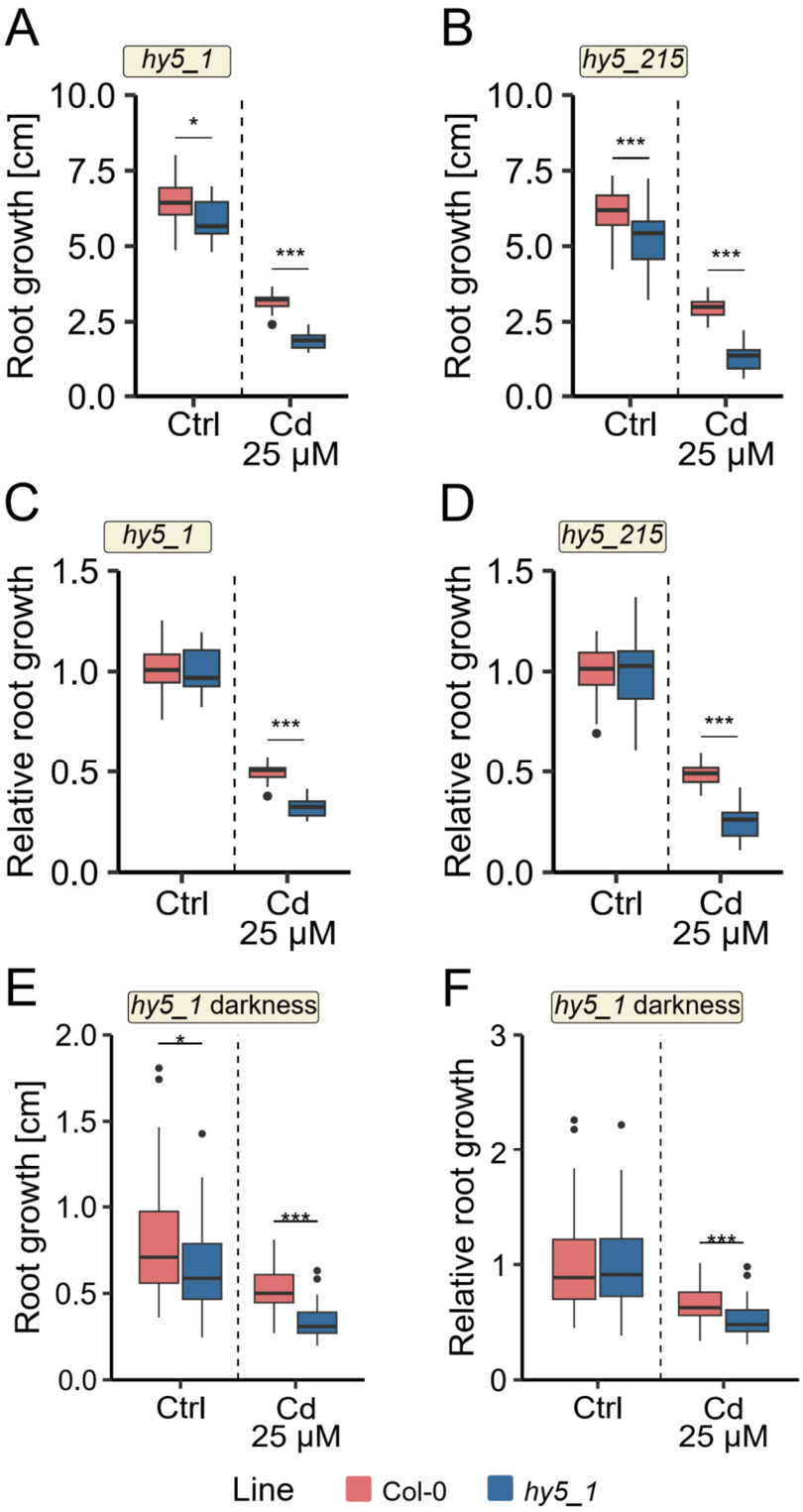
Supplementary Figure S9. Expression pattern of *HY5*. Normalized counts (DESeq2-processed) of HY5 transcripts in root tips (RT) and root rest (RR) under control conditions and after 24 h and 48 h Cd treatment. Individual data points represent biological replicates (n = 3).



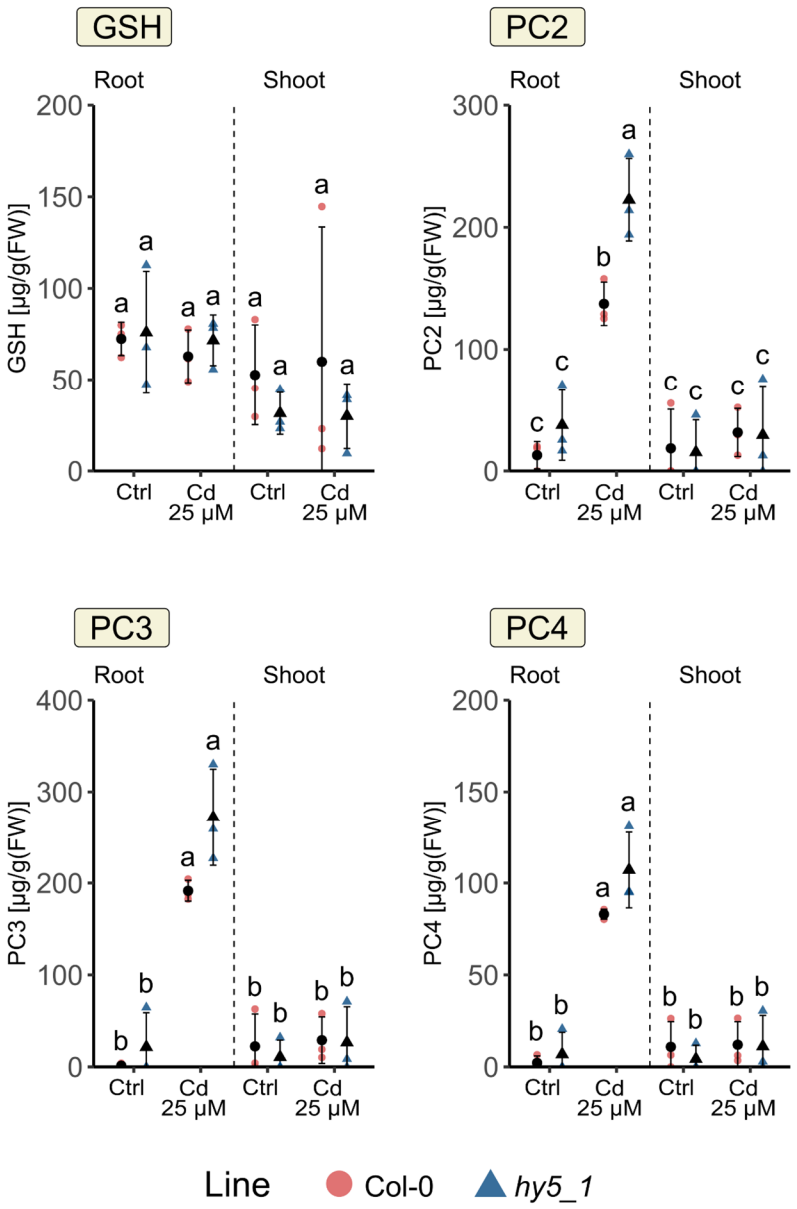
Supplementary Figure S10. Gene Set Enrichment Analysis of HY5 targets genes. The plot shows the enrichment profiles of HY5 target genes from Burko *et al.*, (2020) between control and Cd-treated samples (Adjusted p-value < 0.05, Bonferroni correction).



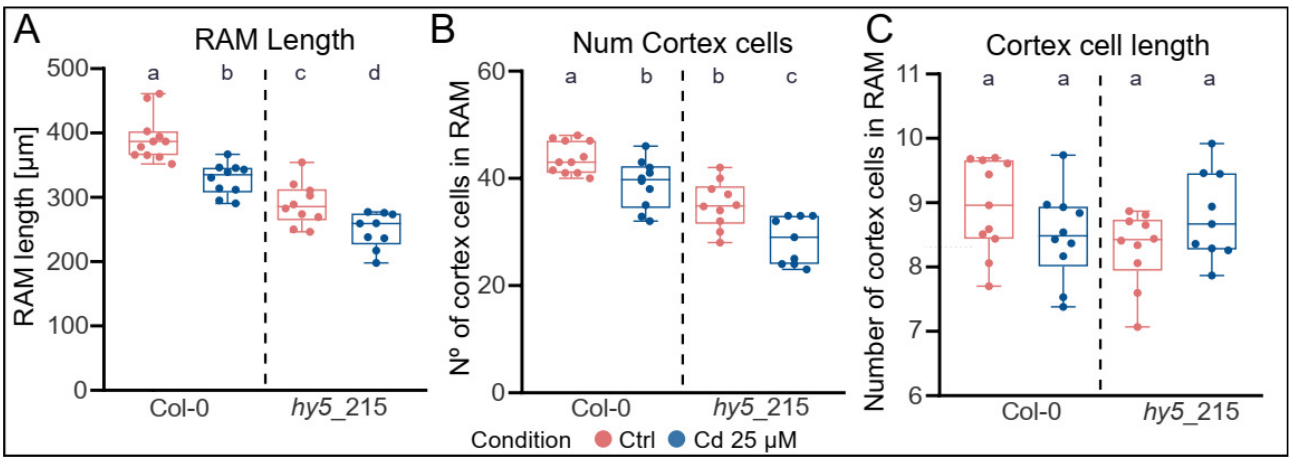
Supplementary Figure S11. Phenotypic analysis of root tip-responsive gene mutants under Cd stress. Primary root growth of *A. thaliana* mutants for candidate genes involved in the Cd response. Seedlings were grown for 7 days on control plates and then transferred to either control or Cd-containing plates for an additional 7 days. Asterisks denote statistically significant differences ($p \leq 0.05$, Mann-Whitney U-test; data from three independent biological replicates, n = 15–30 seedlings per replicate).



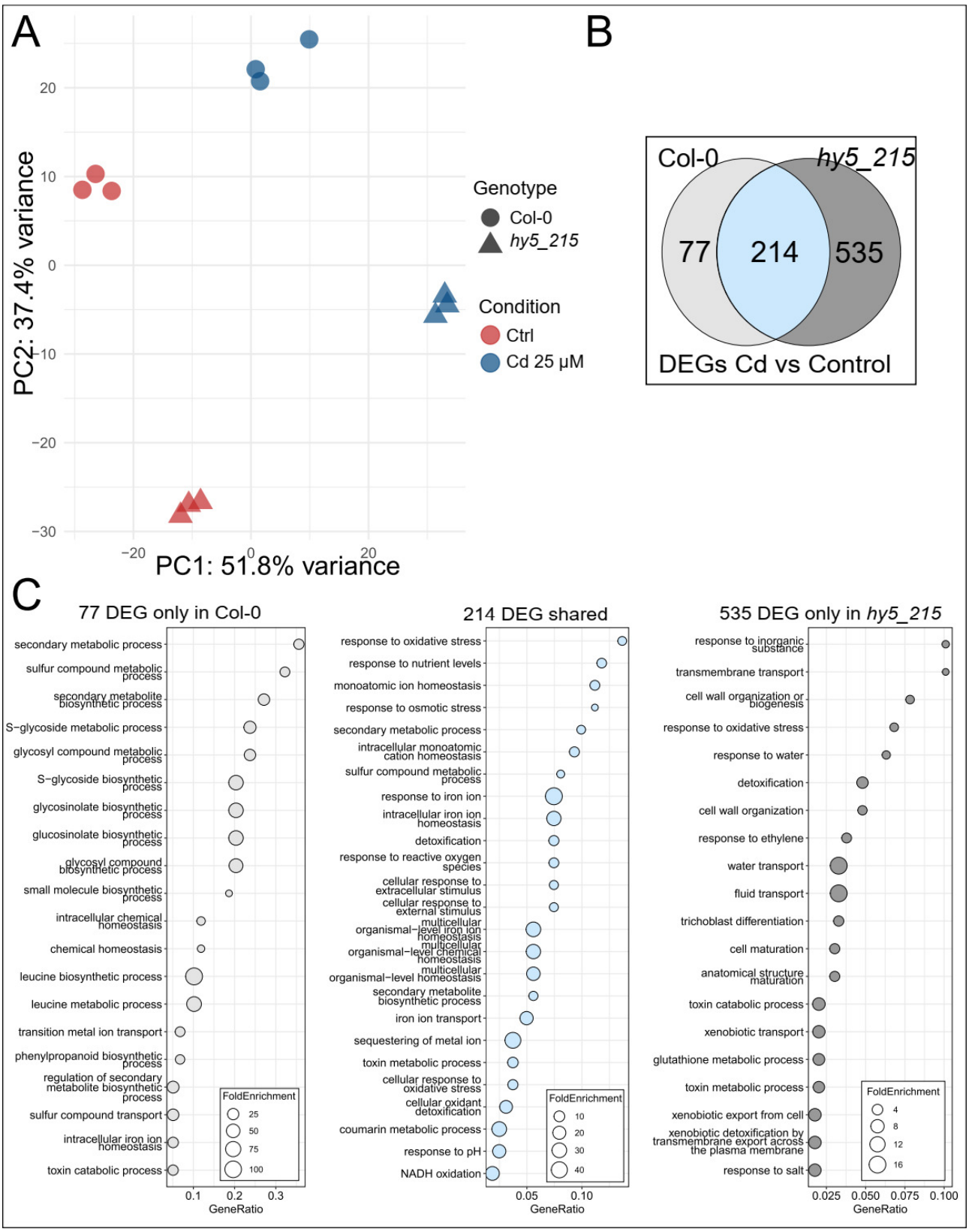
Supplementary Figure S12. Primary root growth of *hy5_1* and *hy5_215* under exposure to 25 µM Cd in a root covered system (A-D) and in darkness (E-F). Seedlings were grown on control medium for 7 d and then transferred to control or Cd medium and grown for additional 7 d. Values are depicted as absolute growth (**A-B**) and growth relative to the respective control (**C-D**). Asterisks indicate statistical significance (Mann Whitney U-Test, n=40-50, 3 independent replications, p≤0.05). (**E-F**) Primary root growth of *hy5_1* under exposure to 25 µM Cd in darkness. Seedlings were grown on control medium for 7 d and then transferred to control or Cd medium and grown for additional 7 d. Values are depicted as absolute growth (left) and growth relative to the respective control (right). Asterisks indicate statistical significance (Mann Whitney U-Test, n=37-43, 3 independent replicates, p≤0.05).



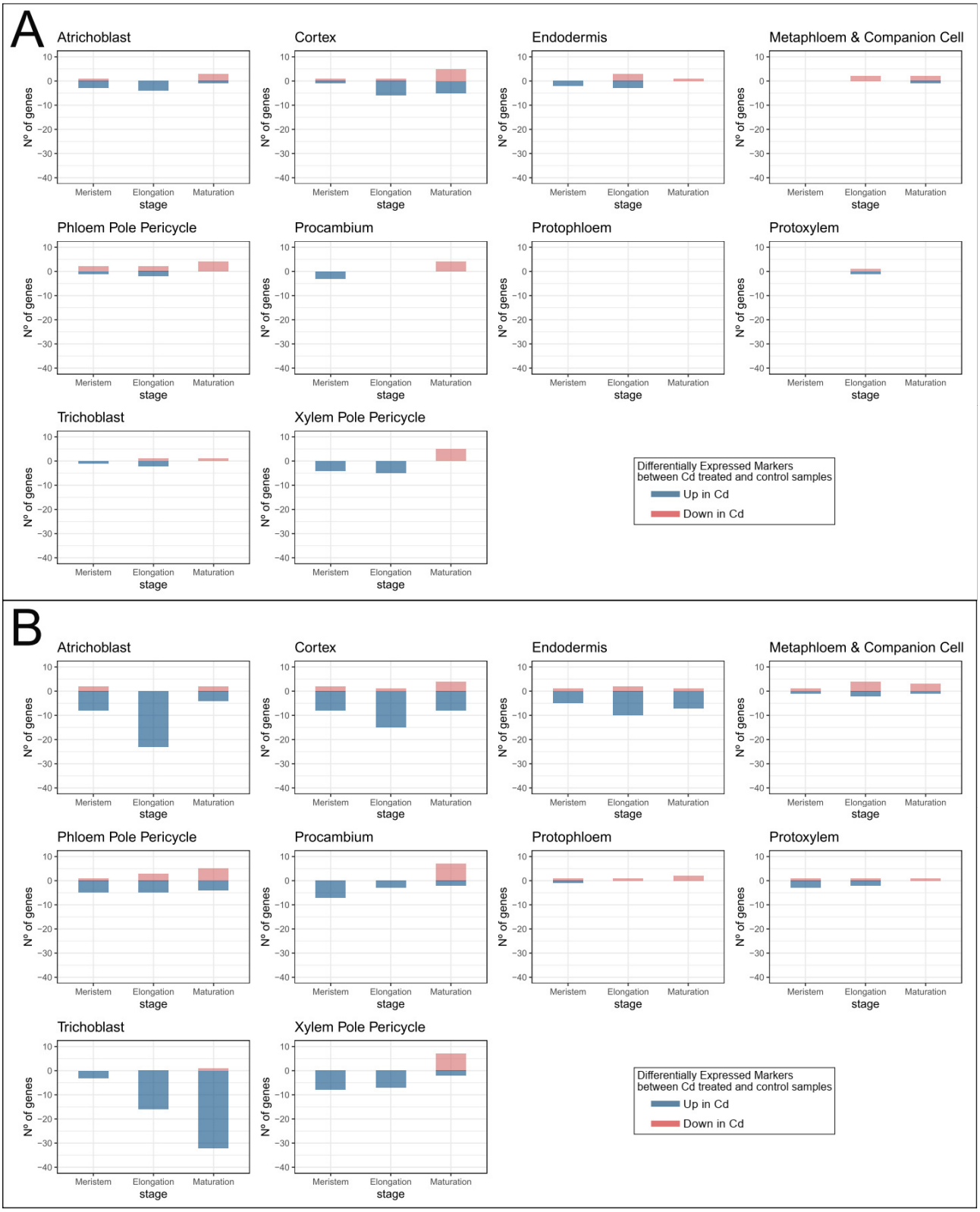
Supplementary Figure S13. Phytochelatin (PC) and glutathione contents in Col-0 and *hy5_1* upon exposure to 25 µM Cd. Seedlings were grown for 9 d on control medium and then transferred to control or Cd medium and exposed for 72 h. Displayed are mean values +/- standard deviations of contents in µg per gram fresh weight (FW). Letters indicate statistical significance (ANOVA with Tukey HSD, $p \leq 0.05$, data from three independent replications).



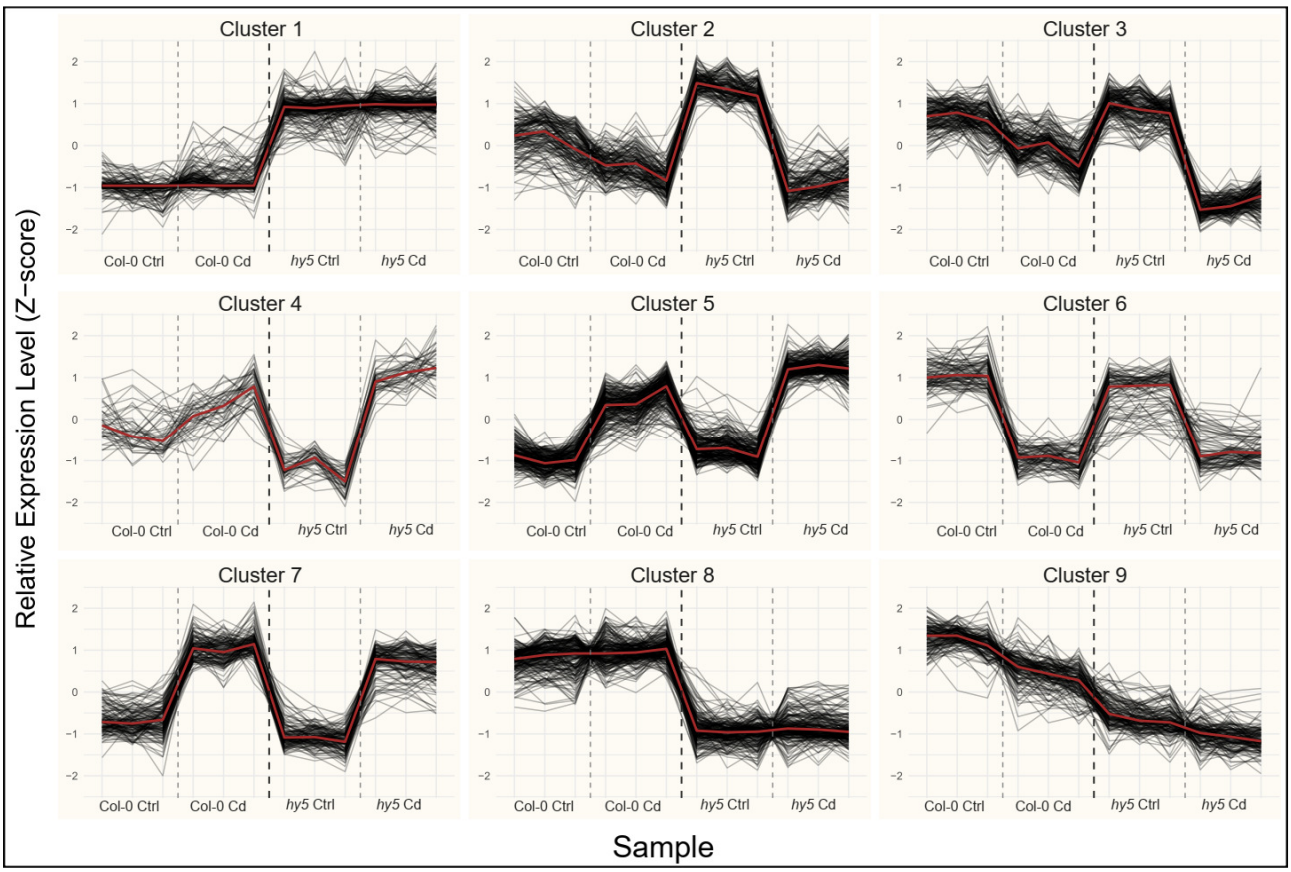
Supplementary Figure S14. **A** RAM size in Col-0 and *hy5* mutant lines. RAM size was defined as the distance from the quiescent center to the first elongated cortex cell. **B.** Number of cortex cells in the RAM. **C.** Cortex cell length. Cortex cell length was determined by dividing the length of RAM by the number of cortex cells in each meristem.Measurements originate from 9-11 seedlings per condition and time point (n=9-11) and one replication. Letters indicate statistically different groups (ANOVA with Tukey test, $p \leq 0.05$**)**.



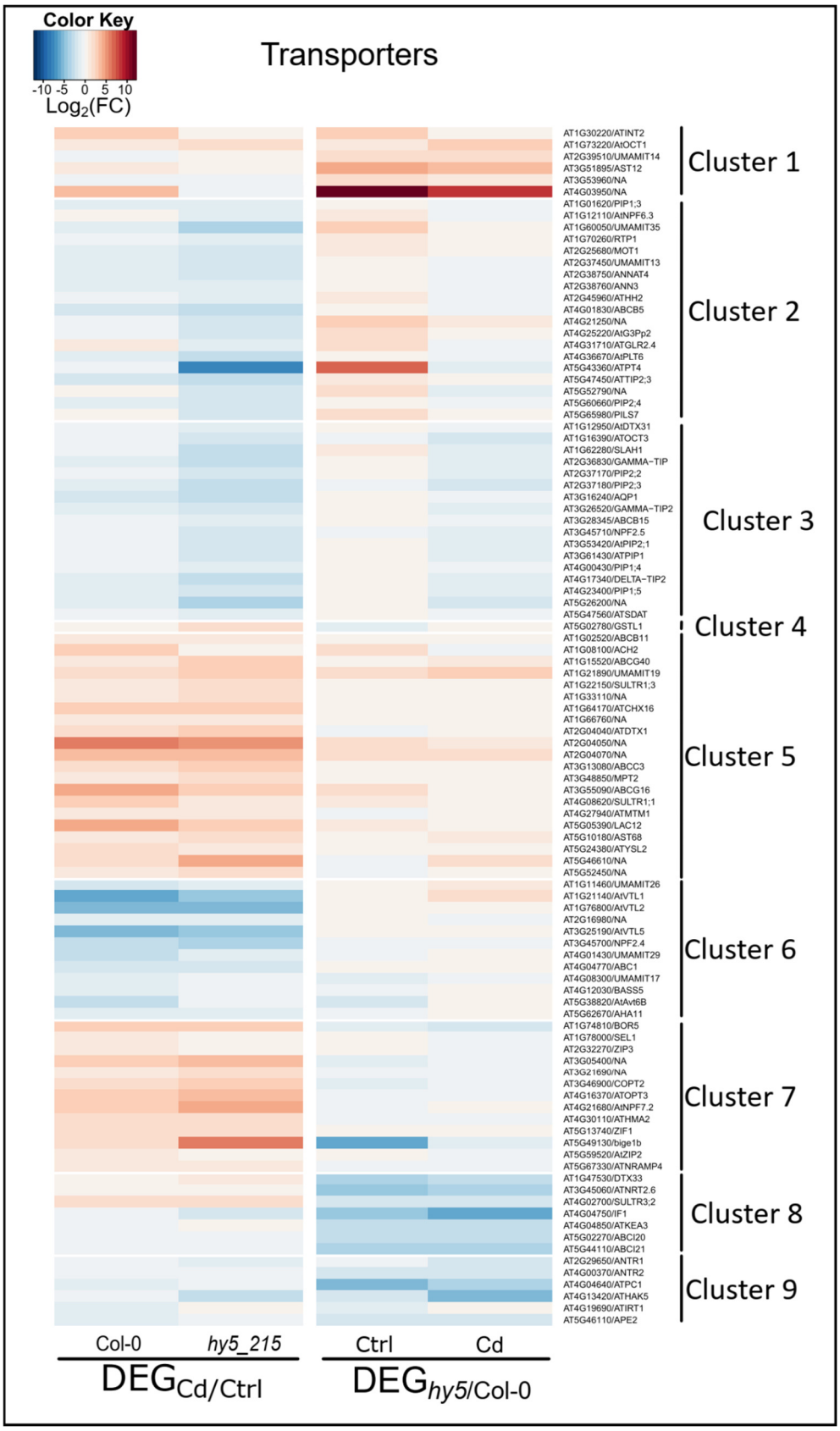
Supplementary Figure S15.Differential Expression Analysis in Col-0 and the *hy5_215* mutant using whole roots. **A.** Principal component analysis showing the distribution of samples according to PC1 and PC2. The percentage of variance explained by each PC is indicated in the corresponding axis. **B**. Venn diagram showing the number of DEG between Cd-treated samples and control**. C**. GO enrichment analysis for genes in B. The plots show the top GO terms with the lowest adjusted p-values (FDR), ordered by Gene Ratio (ratio of input genes that are annotated in a term). Circle size corresponds to fold enrichment.



Supplementary Figure S16. Differentially expressed markers between Cd treated and control samples in the roots of *A. thaliana* Col-0 (A) and *hy5_215* (B) mutant. Bulk RNA-Seq data from whole roots samples upon growth in control and Cd conditions were cross-referenced with the cell-type specific description of gene expression in roots provided by Shahan *et al.*, (2022). Number of genes differentially expressed ($|\log_2$ fold change$|$ > 1 and $p_{adj} \leq 0.05$) that are among the 50 genes that are specific markers for each cell line and development stage. Positive and negative values in the Y-axis represent the number of DEGs that are upregulated or downregulated in Cd treated samples, respectively.



Supplementary Figure S17. Clustering and expression patterns of 1377 genes under Cd exposure in *Arabidopsis thaliana* Col-0 and *hy5_215* mutant roots. Self-Organizing Map analysis identified nine distinct gene expression clusters across four experimental conditions: (1) Col-0 control, (2) Col-0 Cd-treated, (3) *hy5* control, and (4) *hy5* Cd-treated samples, with three biological replicates per condition. Normalized expression data were obtained using DESeq2's variance stabilization transformation (VST), followed by Z-score normalization for each gene to minimize the influence of absolute expression levels on clustering. Line plots display individual gene expression patterns (black lines) and the median profile of each cluster (red line).



Supplementary Figure S18. Expression of transporter genes under Cd exposure in *Arabidopsis thaliana* Col-0 and *hy5_215* mutant roots. Using a list of predicted transporter genes from TransportDB, the heatmap shows the log2(fold change) of gene expression between conditions. In the $DEG_{Cd/Ctrl}$ columns, positive values indicate upregulation of genes after Cd treatment, while negative values indicate downregulation, in both Col-0 and *hy5_215* mutant roots after 48 h of treatment. In the $DEG_{hy5\_215/Col\text{-}0}$ columns, positive values indicate higher expression in *hy5_215* compared to Col-0, while negative values indicate higher expression in Col-0 compared to *hy5_215*.