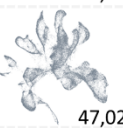
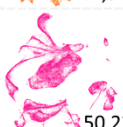
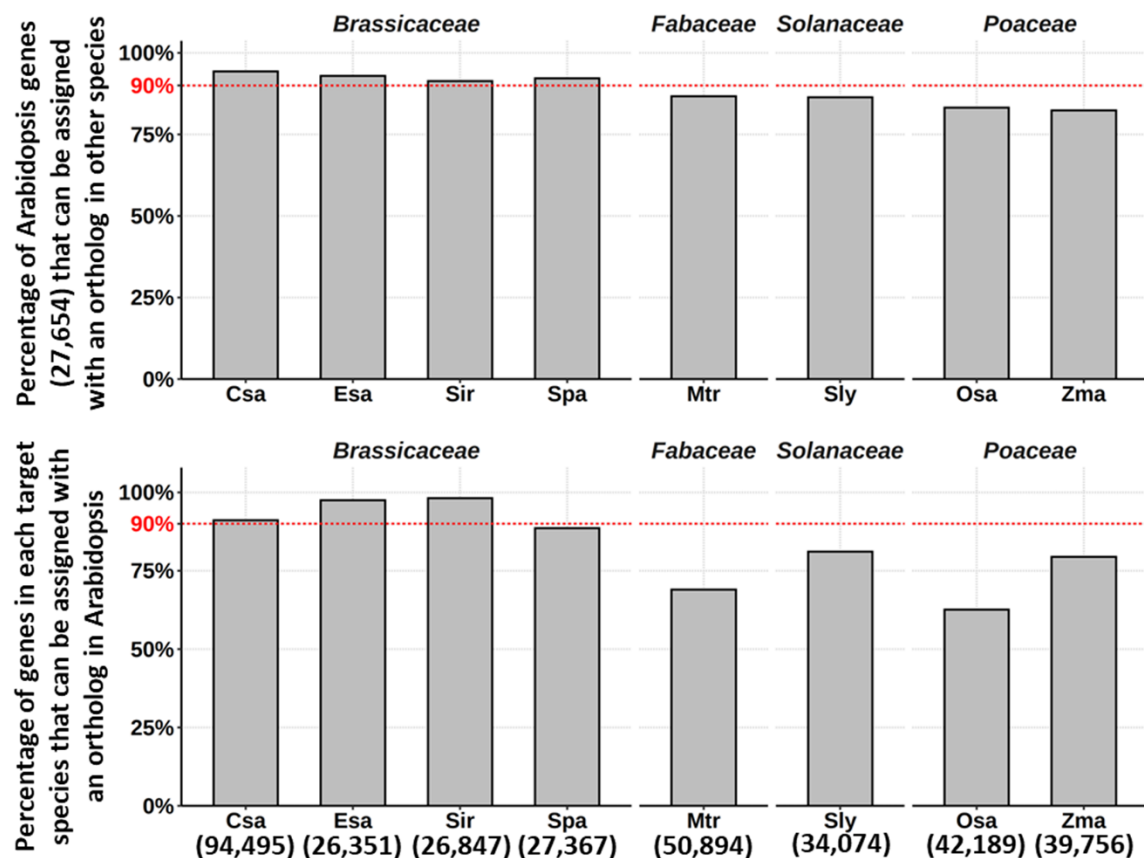
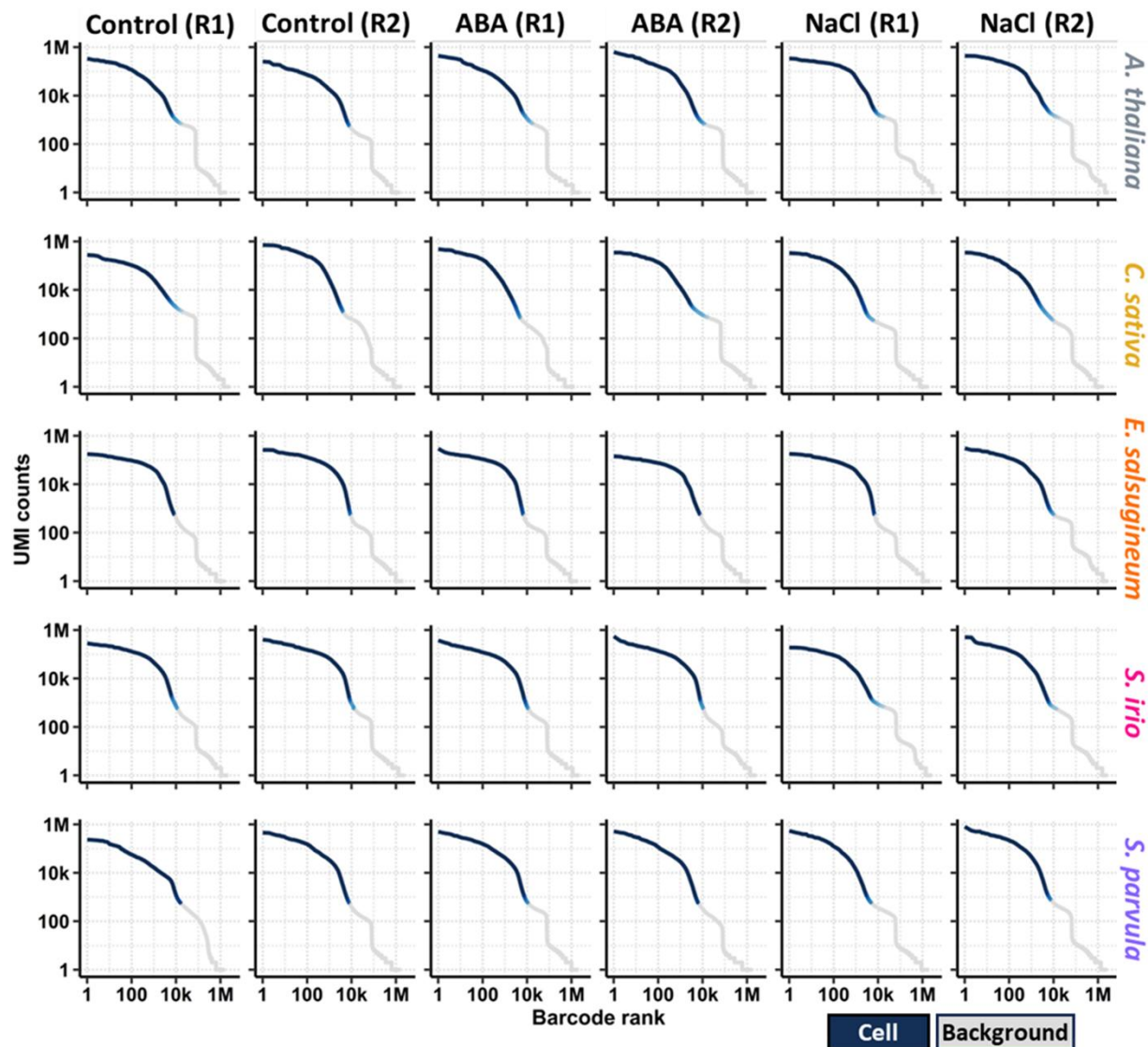


		Input cell counts per conditions (2 replicates)			Preprocessing	Filtered cell counts per condition (2 replicates)			Atlas
		Control	ABA	NaCl		Control	ABA	NaCl	
Lineage I	<i>Camelina sativa</i>	3,848 + 8,871	4,109 + 6,583	3,974 + 4,538	➤ Comparison between pseudo-bulk RNA-seq and whole root RNA-seq.	2,947 + 7,727	3,489 + 5,943	3,365 + 3,971	 27,442
	<i>Arabidopsis thaliana</i>	7,551 + 9,963	9,885 + 8,795	10,615 + 7,627		6,604 + 8,817	8,419 + 7,880	8,953 + 6,355	 47,028
Lineage II	<i>Eutrema salsugineum</i>	8,633 + 8,608	6,498 + 7,869	6,788 + 8,477	➤ Evaluation of the effects of protoplasting.	7,733 + 7,648	5,775 + 6,843	5,975 + 7,566	 41,540
	<i>Sisymbrium irio</i>	10,719 + 8,992	10,039 + 9,316	8,155 + 7,894	➤ Removal of cells with high mitochondrial counts.	9,800 + 8,099	9,077 + 8,455	7,584 + 7,219	 50,234
	<i>Schrenkiella parvula</i>	7,755 + 16,980	10,010 + 6,619	4,435 + 6,986	➤ Removal of doublets. ➤ Check for consistency between replicates.	6,282 + 6,345	8,255 + 5,497	3,649 + 5,990	 36,018

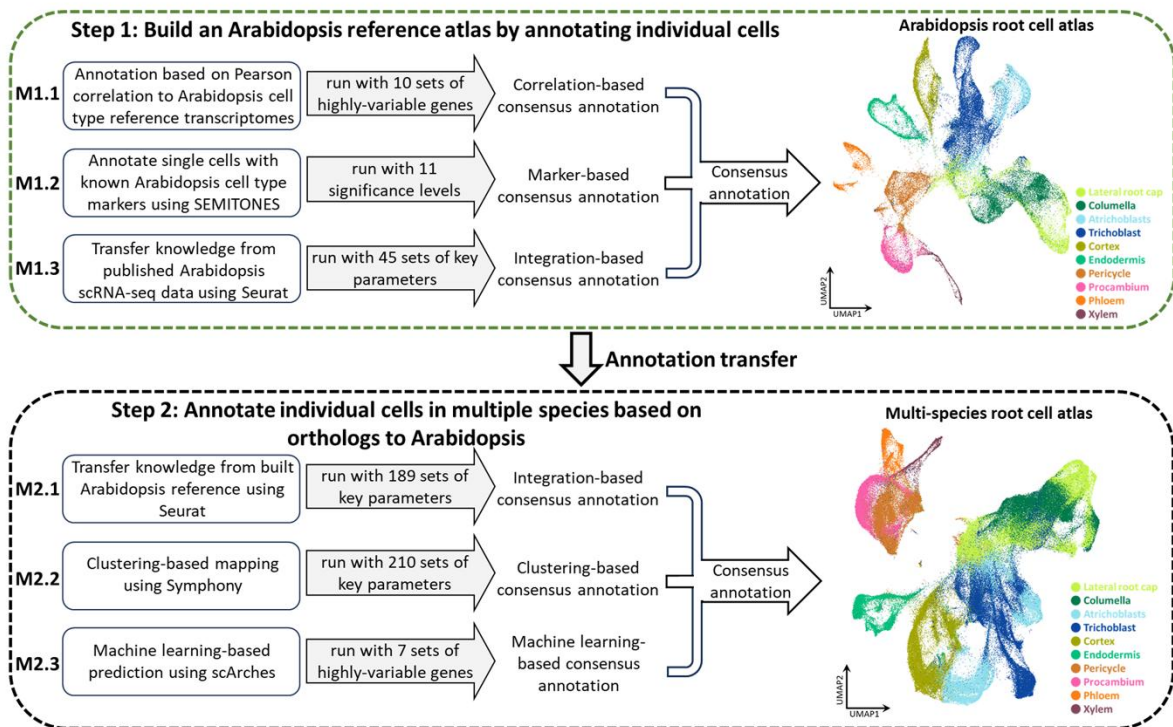
Supplementary Fig. 1. Overview of scRNA-seq dataset for four 5 Brassicaceae species and data preprocessing. The number in each UMAP represents the total number of filtered cells from control, 5 μ M ABA- and 100 mM NaCl-treated samples.



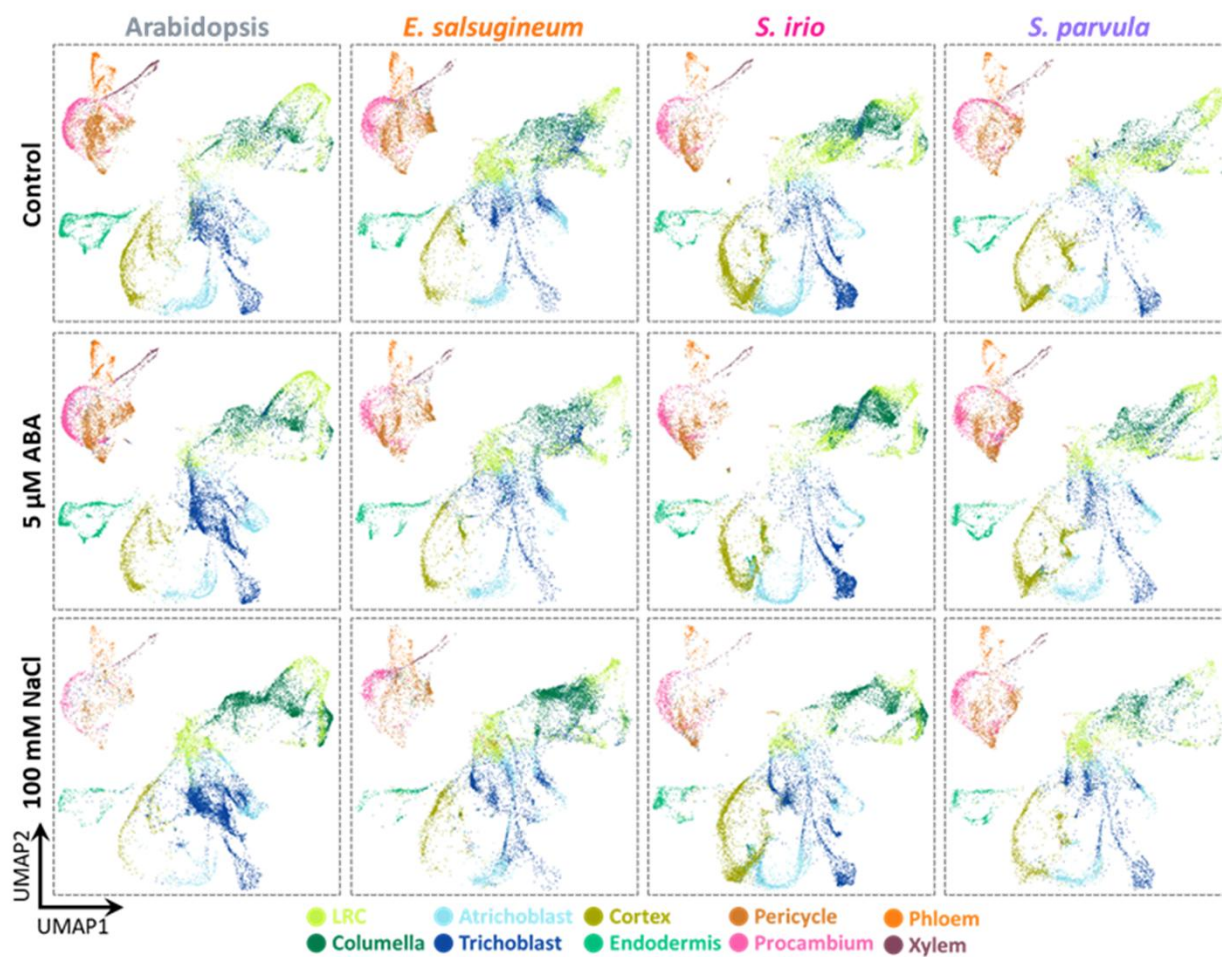
Supplementary Fig. 2. Percentage of genes with orthologs between Arabidopsis and other species. Csa, *Camelina sativa*; Esa, *Eutrema salsugineum*; Spa, *Schrenkiella parvula*; Sir, *Sisymbrium irio*; Mtr, *Medicago truncatula*; Sly, *Solanum lycopersicum*; Osa, *Oryza sativa*; Zma, *Zea mays*. Numbers in parenthesis indicate the numbers of protein-coding genes for each species. Source data are provided as a Source Data file.



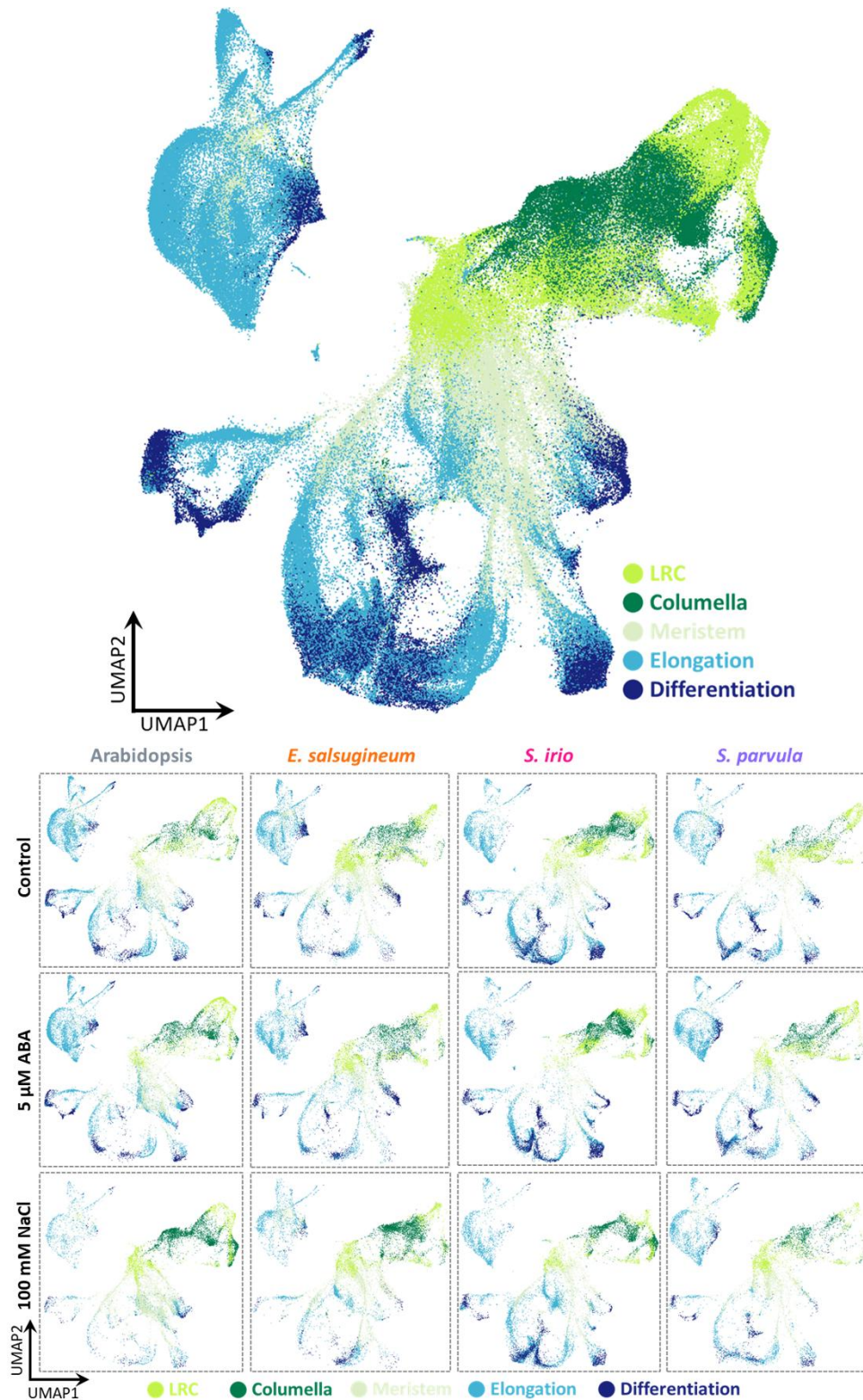
Supplementary Fig. 3. Knee plots for each sample in our study. For each species, samples were harvested from control, 5 μ M ABA-, and 100 mM NaCl-treated conditions. Two independent replicates (R1 and R2) were used for each condition. The high-confidence cell-associated barcodes were clearly separated from the background ones. Source data are provided as a Source Data file.



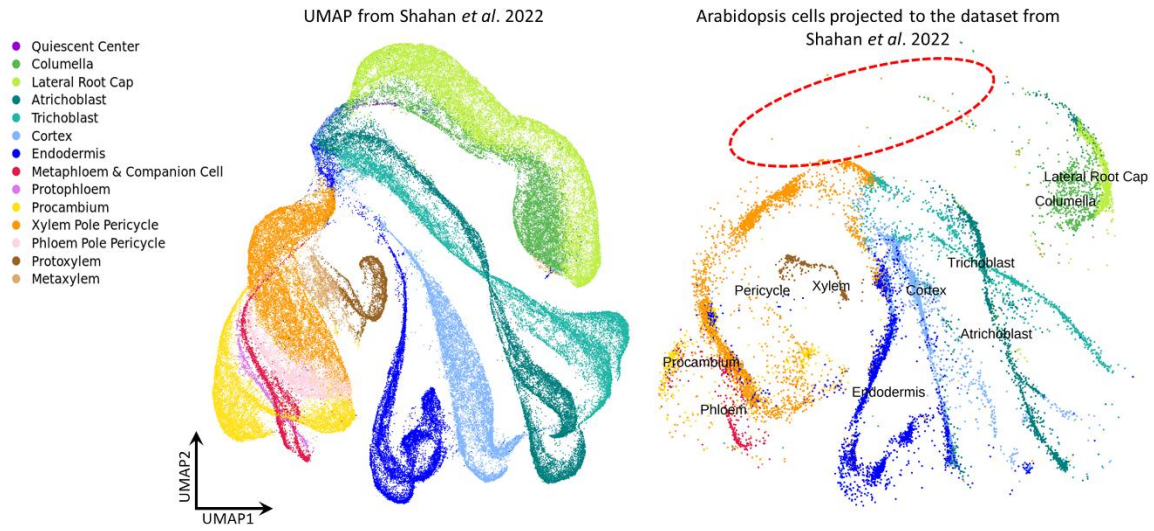
Supplementary Fig. 4. A two-step strategy for cross-species cell type annotations using three independent methods. The Arabidopsis cell type reference transcriptomes were from Li *et al.*, 2016⁶⁵ and Brady *et al.*, 2007⁶⁶. The different sets of highly-variable genes were obtained by categorizing them into different percentile bins. The key parameters for each method were selected based on the tools used, see details in Methods.



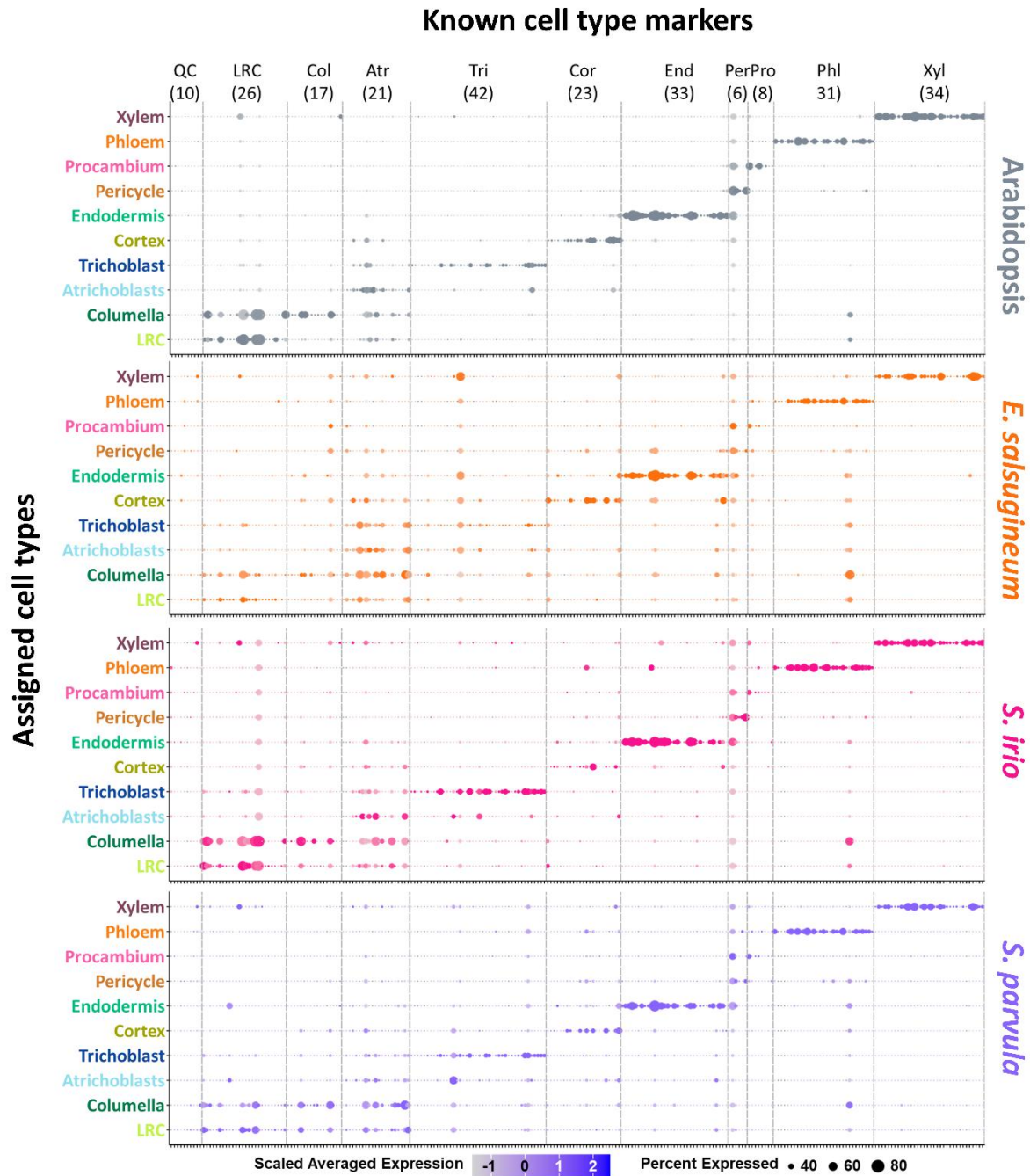
Supplementary Fig. 5. Root cell atlas for each species under different conditions on the integrated UMAP space. All ten root cell types were detected in each species under different conditions. LRC, Lateral Root Cap.



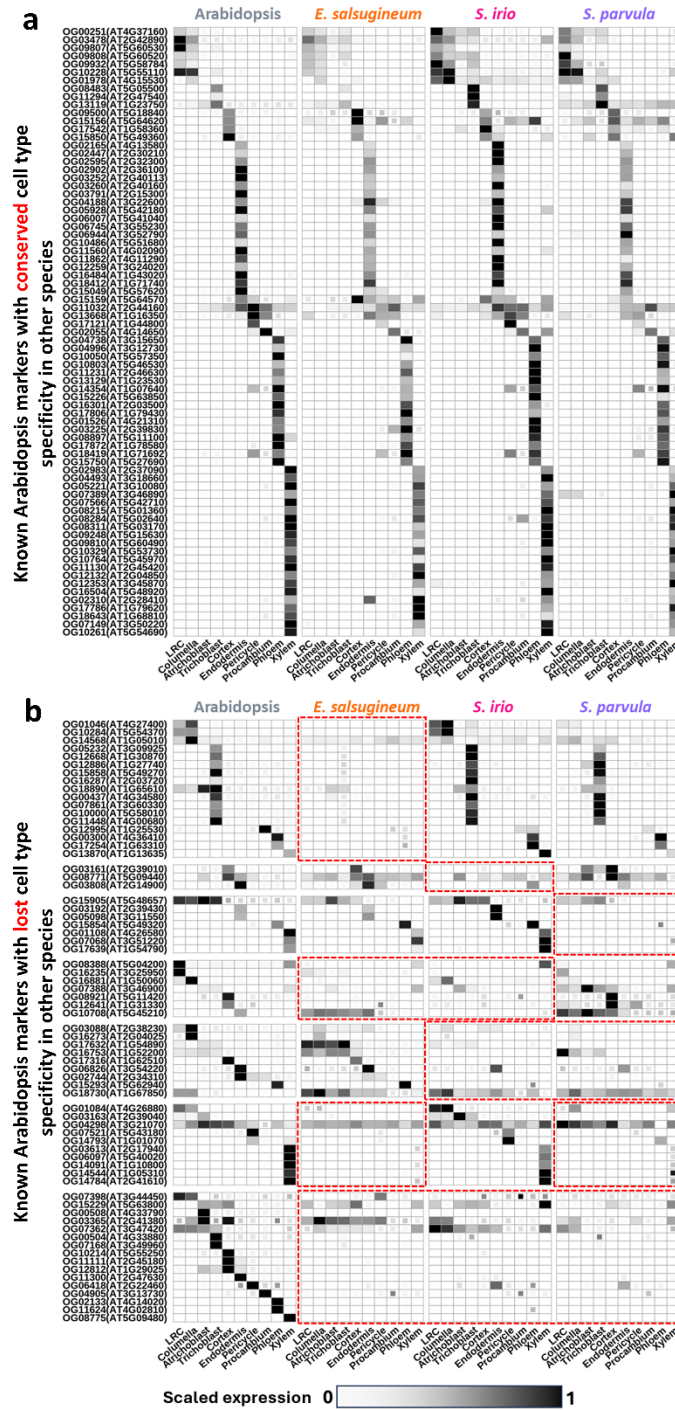
Supplementary Fig. 6. Developmental zone annotation for the integrated root cell atlas from four species (top) and in each species (bottom). All developmental zones were detected in each species under different conditions. LRC, Lateral Root Cap.



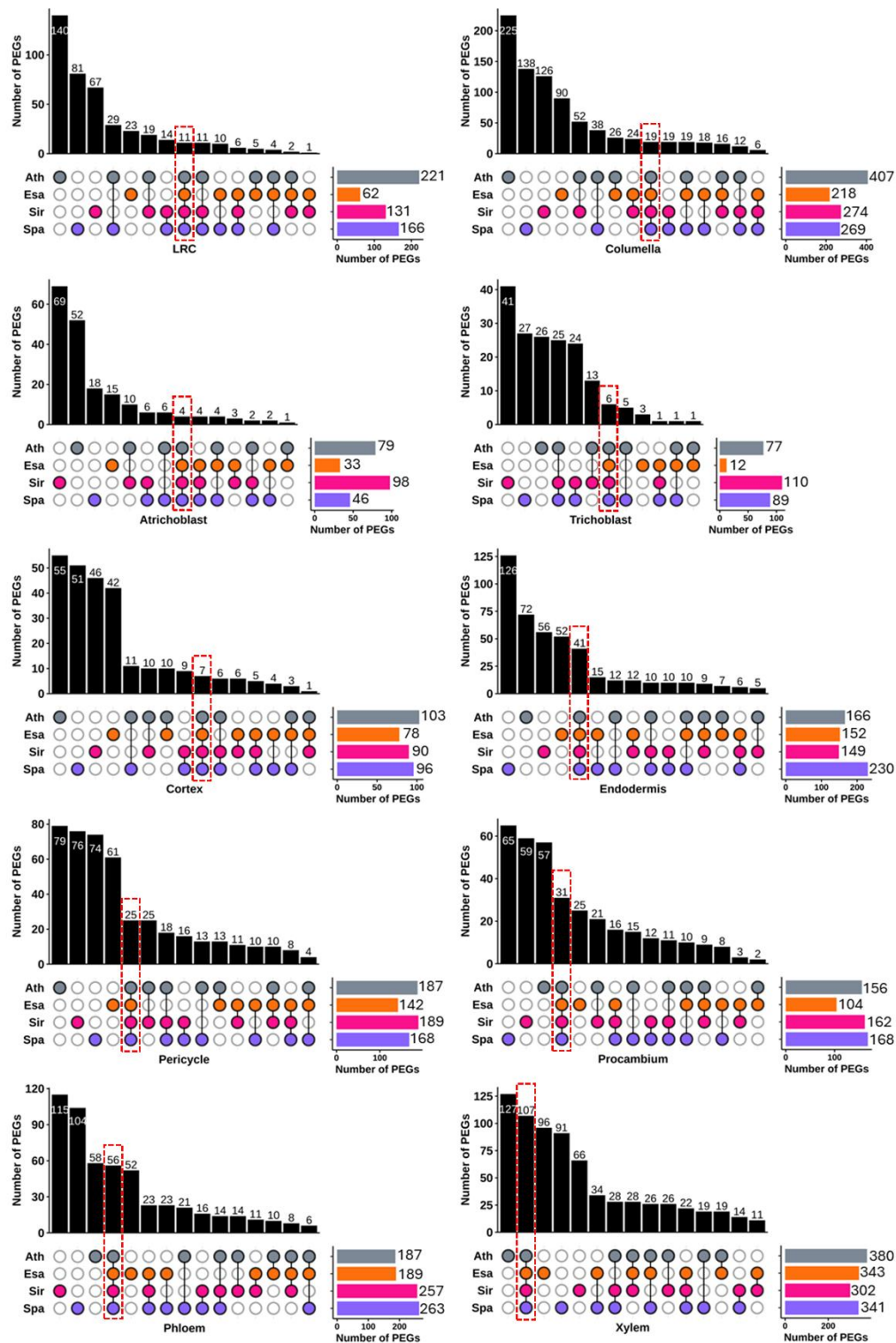
Supplementary Fig. 7. Projection of Arabidopsis control cells from current study to Shahan *et al.*²¹. The red dashed oval marks the expected space for quiescent center cells, currently not detected in the current dataset for Arabidopsis.



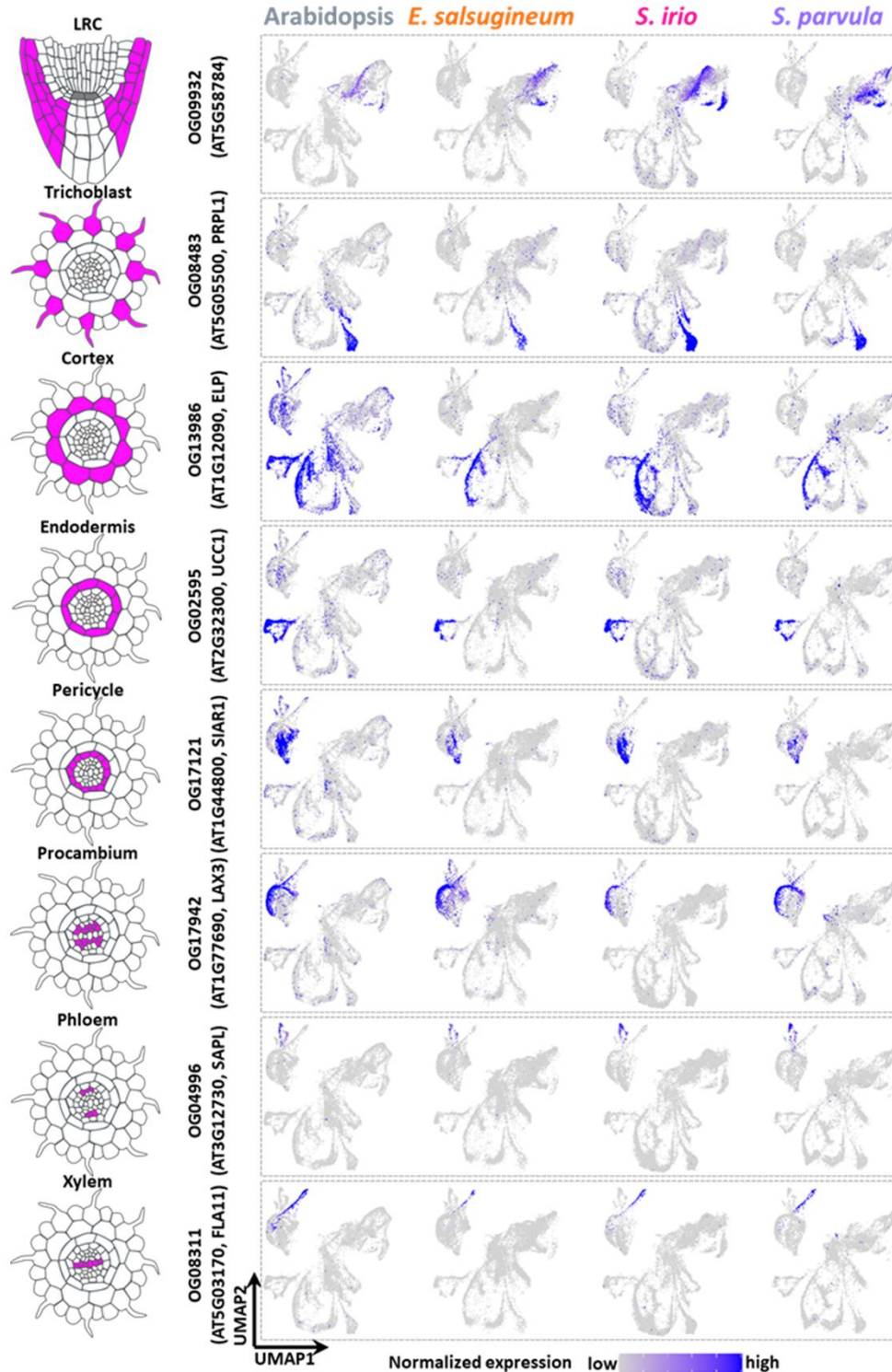
Supplementary Fig. 8. Cell-type expression of known Arabidopsis cell-type markers and their 1-to-1 ortholog in other species under control condition. See Supplementary Data 2 for the list of markers used. Dot size represents the percentage of cells in which each gene is expressed. Dot color intensity indicates the normalized expression of each gene in each cell type. QC, Quiescent center; LRC, Lateral Root Cap; Col, Columella; Atr, Atrichoblast; Tri, Trichoblast; Cor, Cortex; End, Endodermis; Per, Pericycle; Pro, Procambium; Phl, Phloem; Xyl, Xylem.



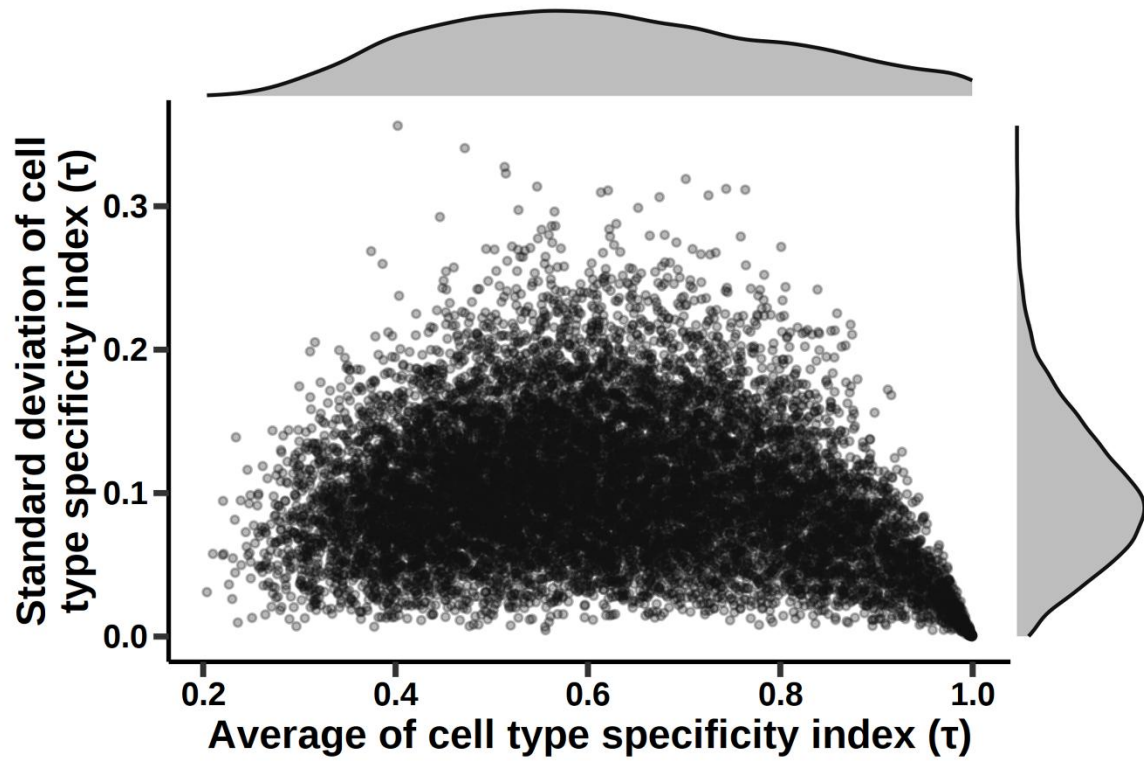
Supplementary Fig. 9. Expression of conserved (a) and diverged (b) Arabidopsis cell type markers across species under control condition. Red boxes mark the species where these cell type markers lost their expression specificity. Tiles with incomplete fill indicate these genes were expressed in less than 10% of cells from the target cell types. They were not considered for marker identification.



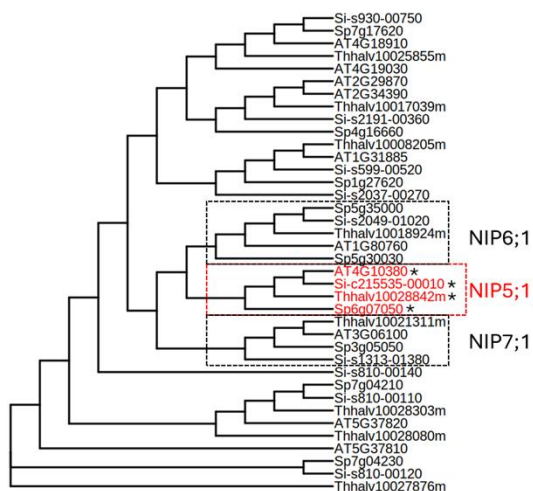
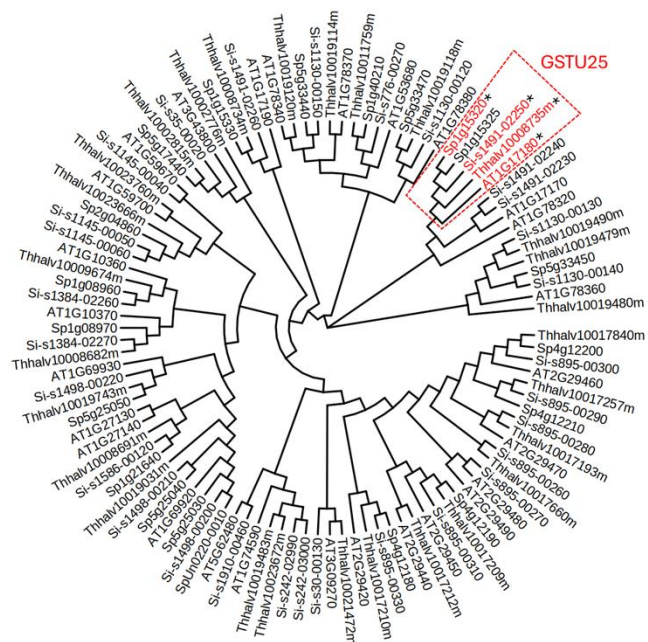
Supplementary Fig. 10. Identification of cell type preferentially enriched genes (PEGs) that were conserved across species for each cell type in the current study. Ath, *Arabidopsis*; Esa, *Eutrema salsugineum*; Spa, *Schrenkiella parvula*; Sir, *Sisymbrium irio*. The conserved PEGs for each cell type are highlighted with red boxes. Source data are provided as a Source Data file.



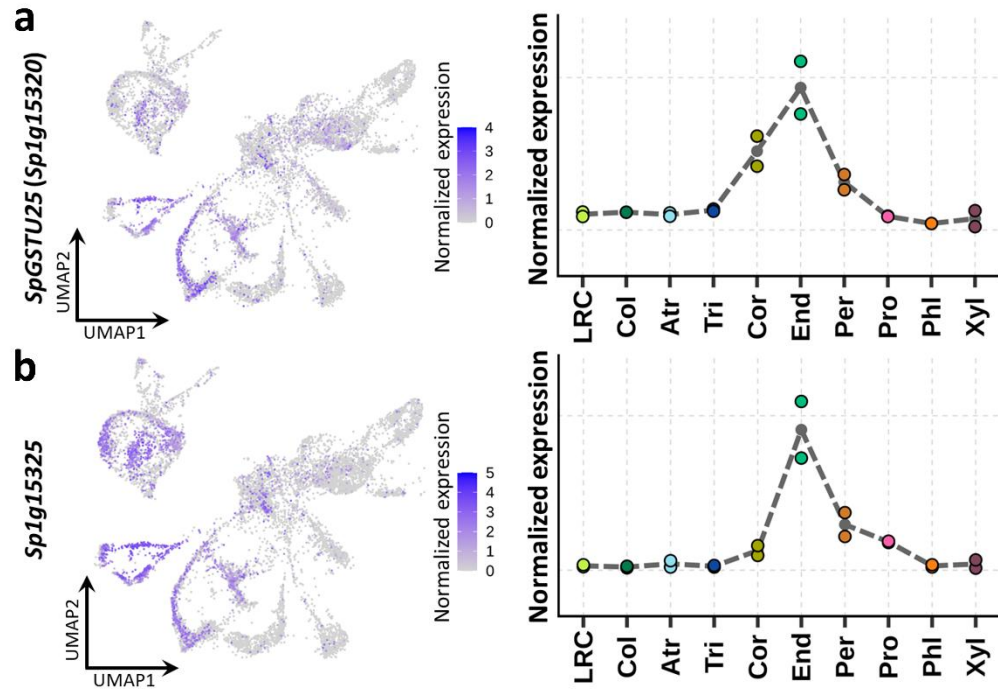
Supplementary Fig. 11. Expression of selected cell type markers in Fig. 2 across species. The expression shown is from the control samples. PRPL1, proline-rich protein-like 1; ELP, extensin-like protein; UCC1, uclacyanin 1; SIAR1, siliques are red 1; LAX3, like aux1 3; SAPL, Sister of APL; FLA11, FASCICLIN-like arabinogalactan-protein 11. Parts of this figure were adapted from images at <https://doi.org/10.6084/m9.figshare.4688761.v2> published under a CC BY 4.0 license (<https://creativecommons.org/licenses/by/4.0/>) (Bouché, Frédéric (2017). *Arabidopsis* - Roots: cross section).



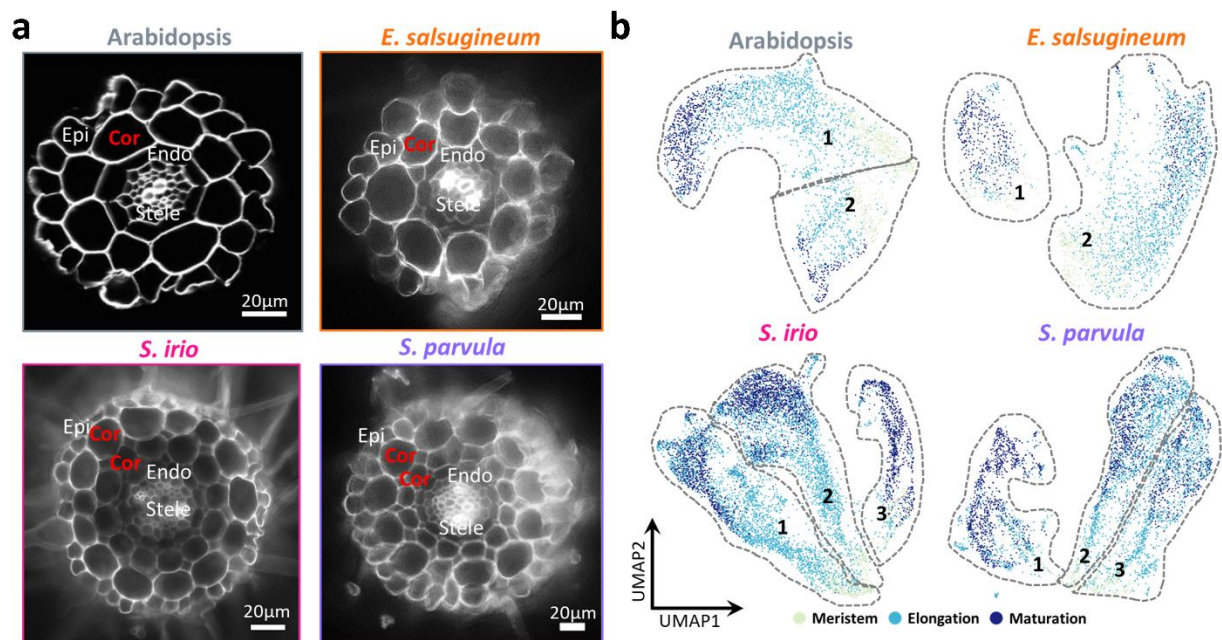
Supplementary Fig. 12. Comparison of the average and the standard deviation of cell type specificity index for the 1-to-1 orthologs. Cell type specificity index (τ) is based on tau metric of tissue specificity in the control samples of each species and calculated as $\sum_{i=1}^N (1 - x_i) / (N - 1)$, where N is the number of cell types and x_i is the expression level in cell type is normalized by the maximal expression of the given gene across all cell types. Cell type specificity index (τ) ranges from 0 to 1, where 0 means broad expression in all cell types and 1 indicates specific expression in one cell type. The cell type specificity indexes for each 1-to-1 orthologs were used to calculate the average and standard deviation for these orthologs.

a**b**

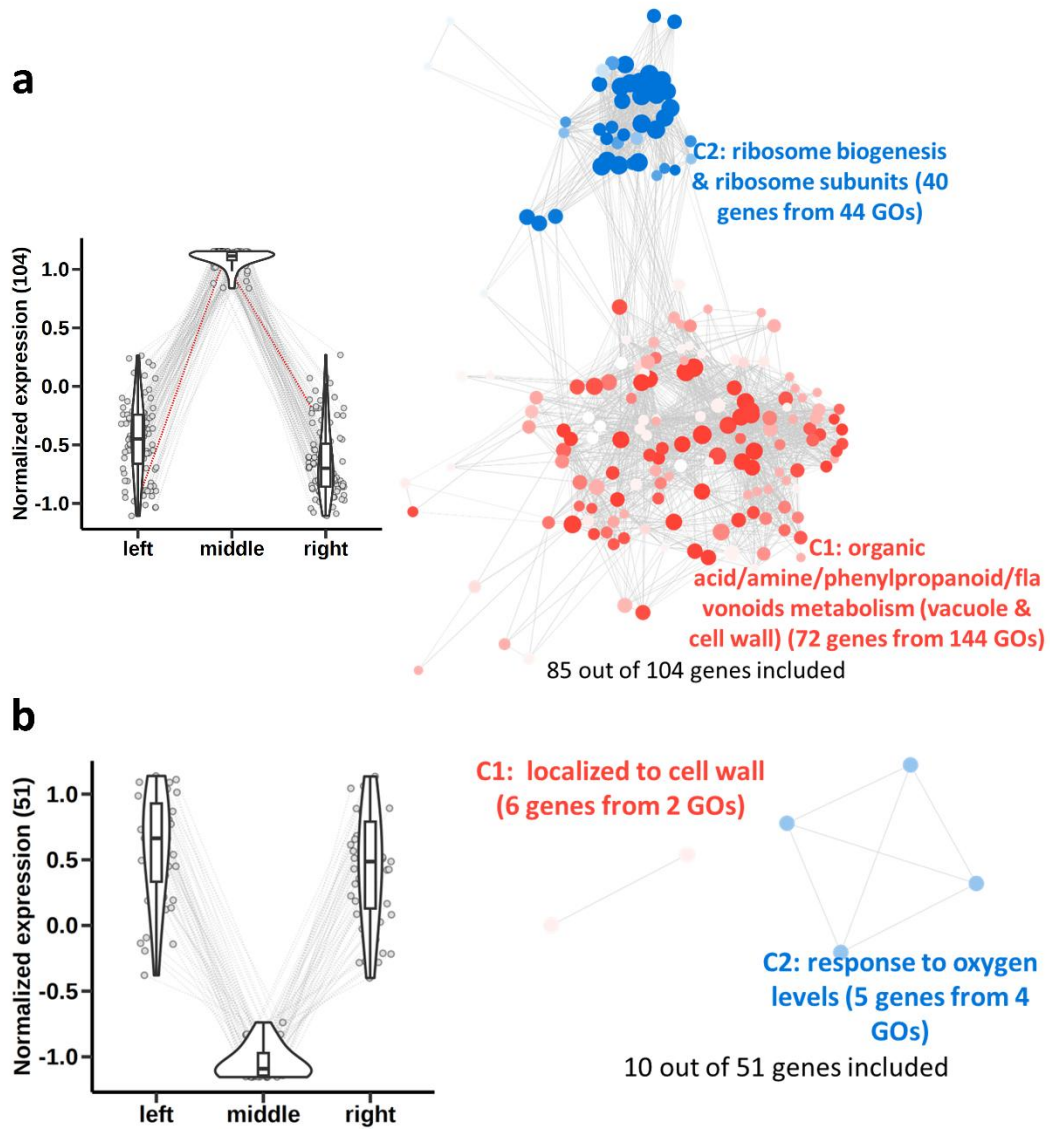
Supplementary Fig. 13. Phylogenetic tree of NIPs (a) and GSTUs (b) from *Arabidopsis*, *S. irio*, *S. parvula*, and *E. salsugineum*. Genes marked with asterisks are the reciprocal best matches of each other.



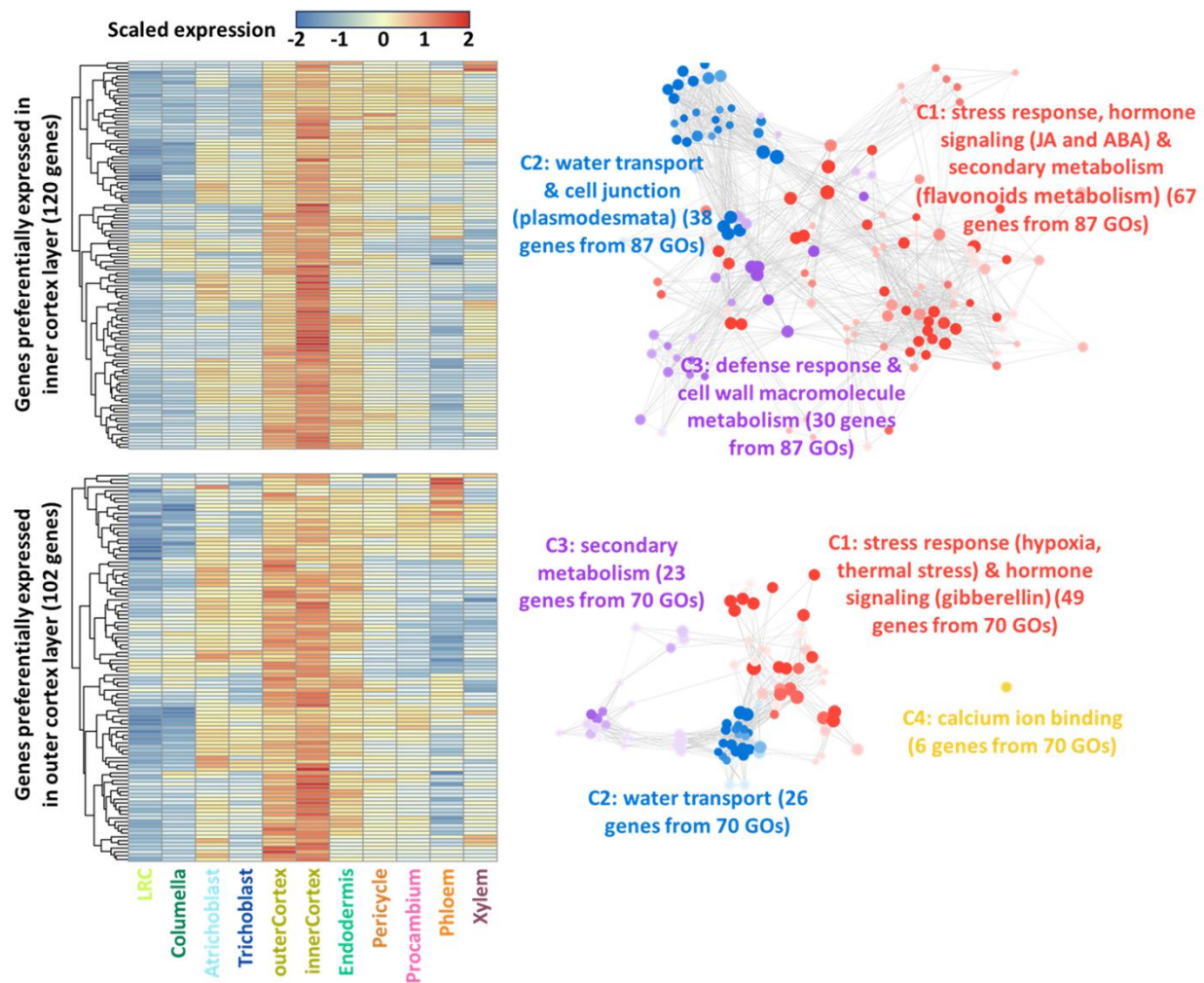
Supplementary Fig. 14. Expression of *SpGSTU25 (Sp1g15320)* (a) and *Sp1g15325* (b) based on scRNA-seq data for *S. parvula* under control condition. Left panels display the expression of the selected genes visualized on UMAP space. Right panels show the pseudo-bulk cell type expression of the selected genes. LRC, Lateral Root Cap; Col, Columella; Atr, Atrichoblast; Tri, Trichoblast; Cor, Cortex; End, Endodermis; Per, Pericycle; Pro, Procambium; Phl, Phloem; Xyl, Xylem. Source data are provided as a Source Data file.



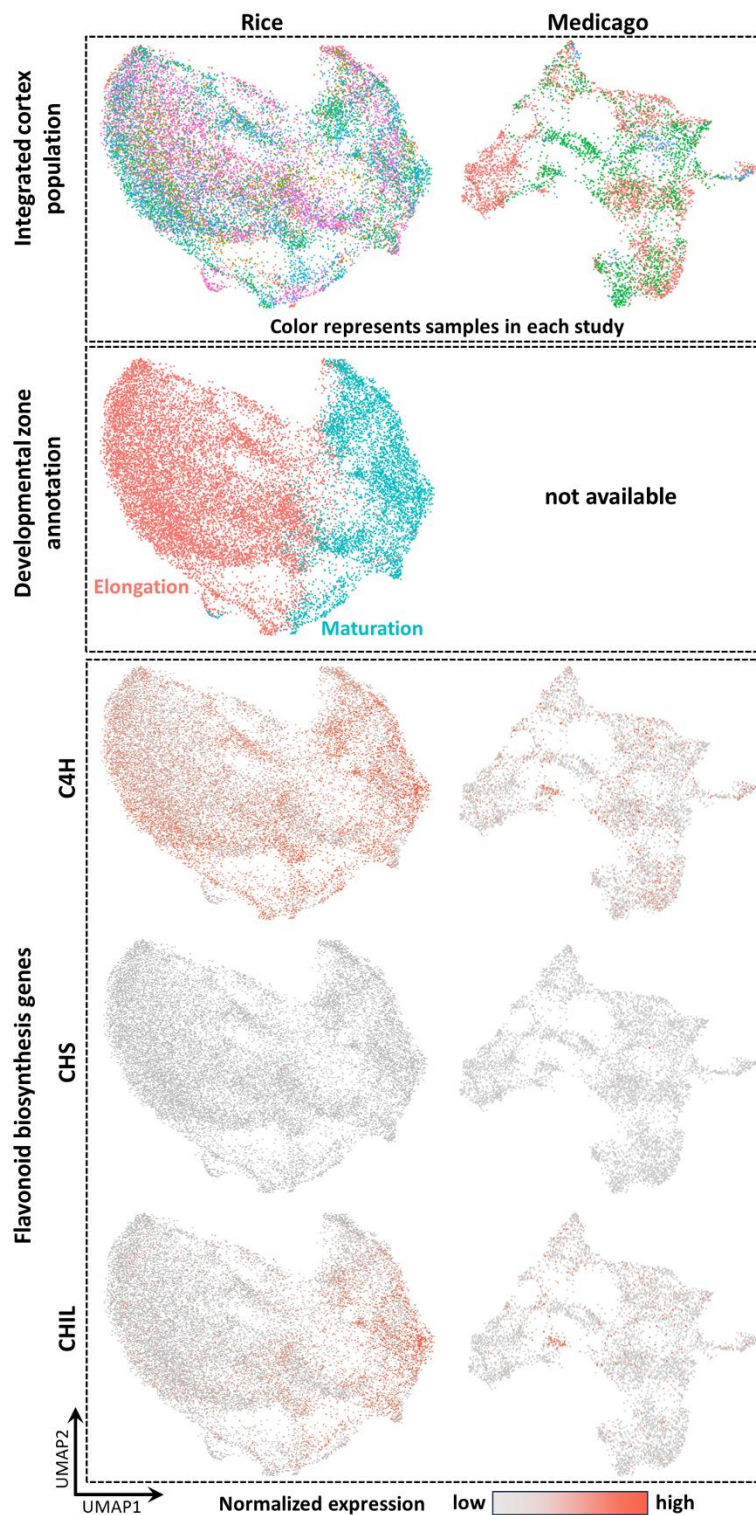
Supplementary Fig. 15. Comparison of cortex cells across species from control samples. a Cross-sections of roots at the maturation zone in each species. Roots were obtained from 5–6-day old seedlings for each species. Scale bars, 20 μm. b UMAP representation of cortex cells in each species. Sub-populations in each species are manually circled based on trajectories identified by Monocle.



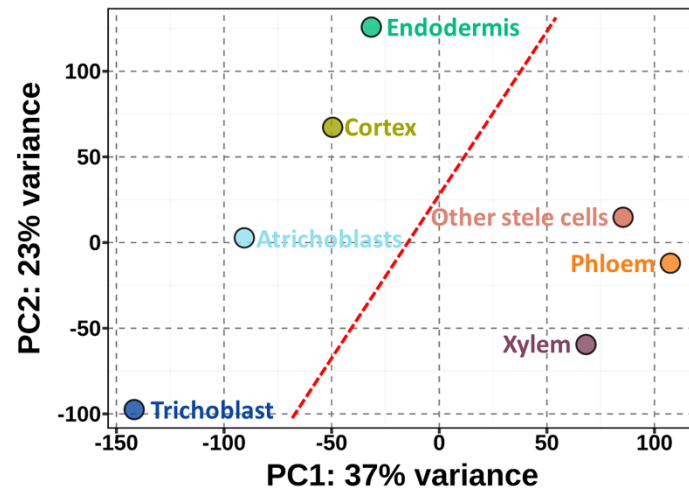
Supplementary Fig. 16. Functional representations of genes that were preferentially expressed (a) and depleted (b) in the middle cortex subpopulation of *S. irio*. Numbers in the parenthesis on the y-axis indicate the number of genes. Box plots represent the median (central value), upper and lower quartile (box limits) with whiskers at $\pm 1.5 \times$ the interquartile range. The red dash line in the line graph in (a) marks the normalized expression of *Si-s1384-01650*, the HCR result of its expression in *S. irio* is shown in Fig. 4d. GO clusters are differently colored and labeled with the representative functional term. Each node represents a gene ontology (GO) term; node size represents genes in the test set assigned to that functional term; GO terms sharing $> 50\%$ of genes are connected with edges; and the shade of each node represents the p -value assigned by the enrichment test at adjusted p values < 0.05 with darker shades indicating smaller p -values.



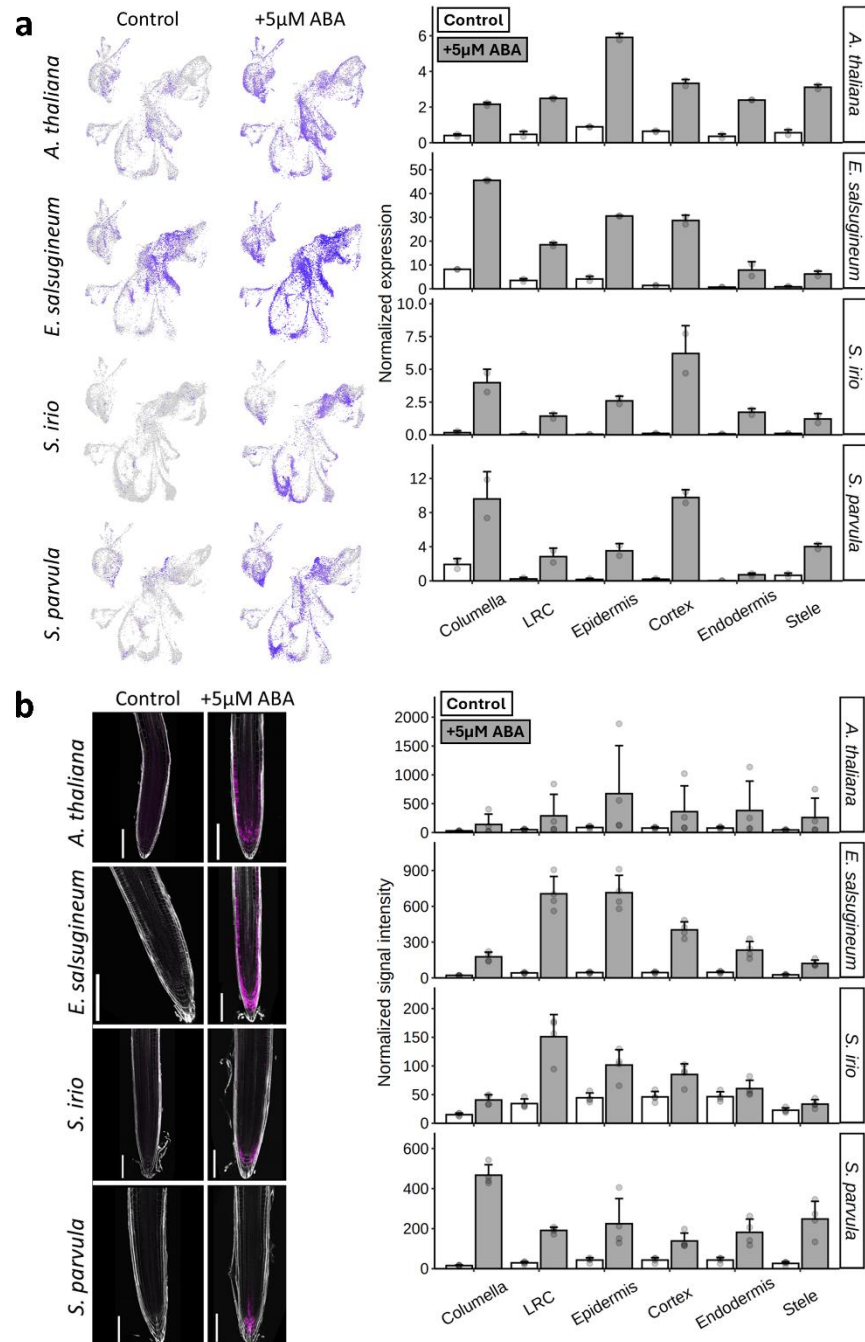
Supplementary Fig. 17. Expression of genes that are preferentially expressed in inner (top) and outer (bottom) cortex layers in *S. irio* and their functional representations. GO clusters are differently colored and labeled with the representative functional term. Each node represents a gene ontology (GO) term; node size represents genes in the test set assigned to that functional term; GO terms sharing > 50% of genes are connected with edges; and the shade of each node represents the p -value assigned by the enrichment test at adjusted p values < 0.05 with darker shades indicating smaller p -values.



Supplementary Fig. 18. The expression of flavonoid biosynthetic genes in rice and Medicago. The spatial separation of the flavonoid pathway in different cortex layers is not conserved in rice and Medicago. C4H, cinnamic acid 4-hydroxylase; CHS, chalcone synthase; CHIL, chalcone isomerase-like.

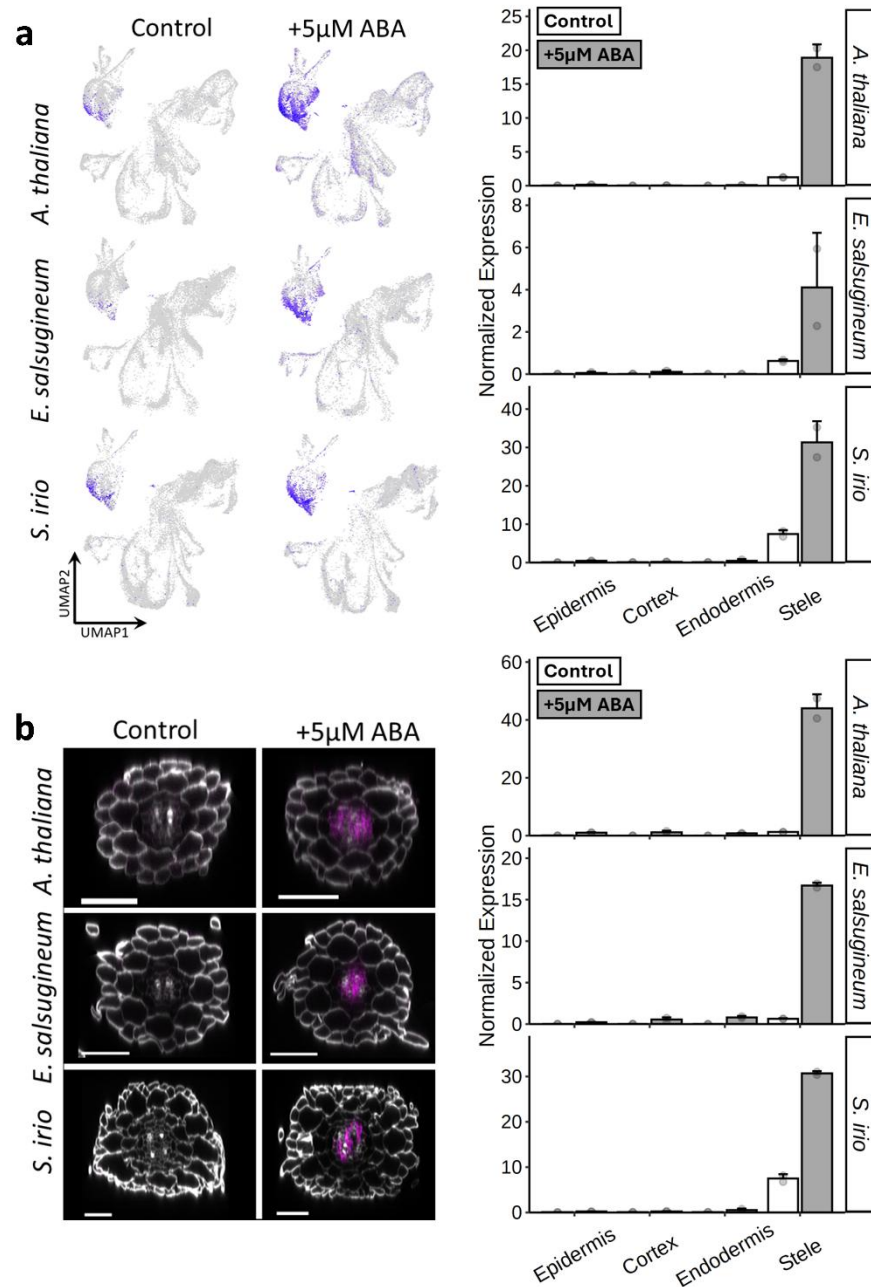


Supplementary Fig. 19. Principal component (PC) analysis of pseudo-bulk cell-type transcriptomes generated from single-nucleus RNA-seq of *Arabidopsis* (Farmer *et al.*, 2021³⁷). Each symbol represents a pseudo-bulk cell-type transcriptome. The red dashed lines separate cell type transcriptome subgroups within each species.



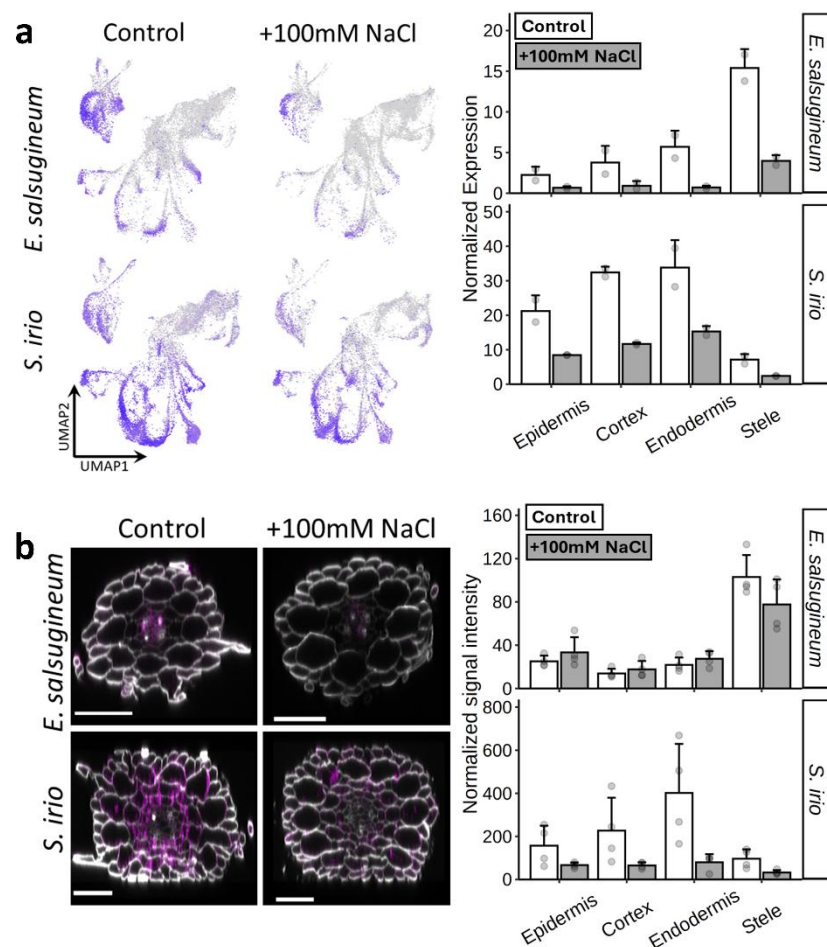
Supplementary Fig. 20. Expression changes of OG04736 orthologs (*AT3G15670*) across species in response to ABA. a Expression of OG04736 orthologs across species from scRNA-seq data under different conditions. Left, UMAP representation of the expression of OG04736 orthologs. Right, pseudo-bulk cell type expression of OG04736 orthologs. b Whole-mount *in situ* HCR detection of expression changes of OG04736 orthologs across species in response to ABA. Left, confocal images of whole-mount HCR showing the detection of OG04736 orthologs across species. Scale bars, 150 μ m. Right, average signal intensity of OG04736 orthologs across defined

cell type groups. Data are presented as mean values $\pm$ SD. Source data are provided as a Source Data file.

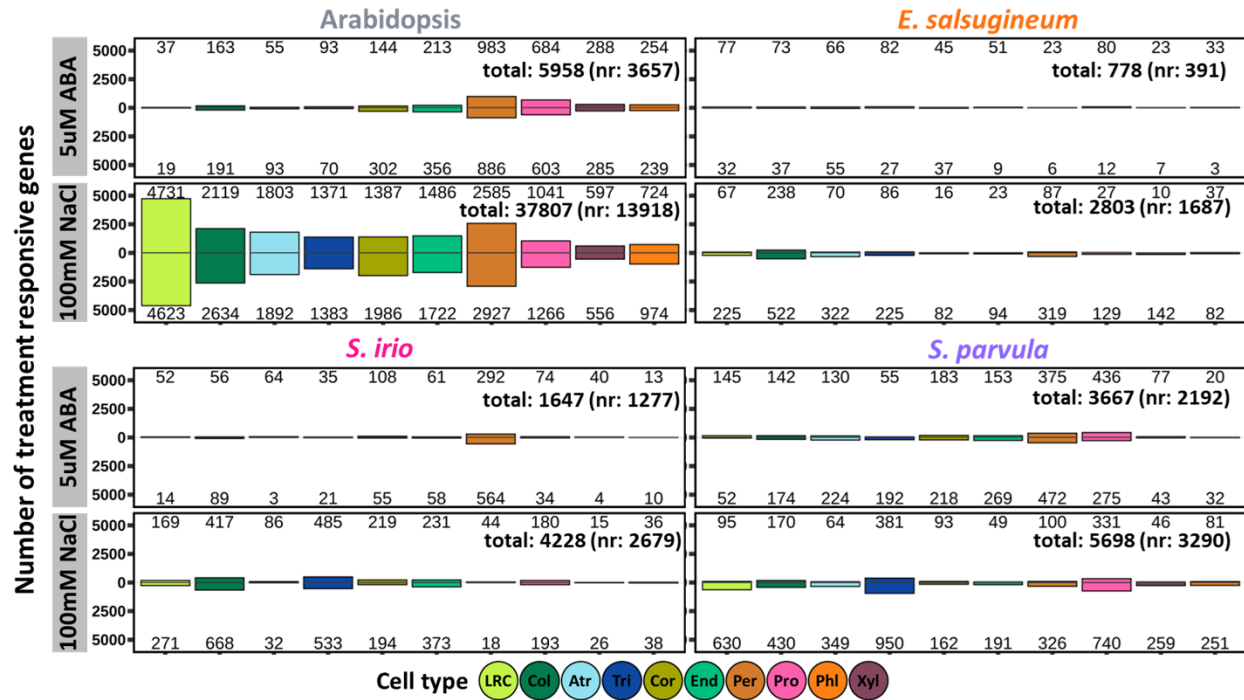


Supplementary Fig. 21. Expression changes of OG03053 orthologs (*AT2G37870*) across species in response to ABA. a Expression of OG03053 orthologs across species from scRNA-seq data under different conditions. Left, UMAP representation of the expression of OG03053 orthologs. Right, pseudo-bulk cell type expression of OG03053 orthologs. b Whole-mount *in situ* HCR detection of expression changes of OG03053 orthologs across species in response to ABA.

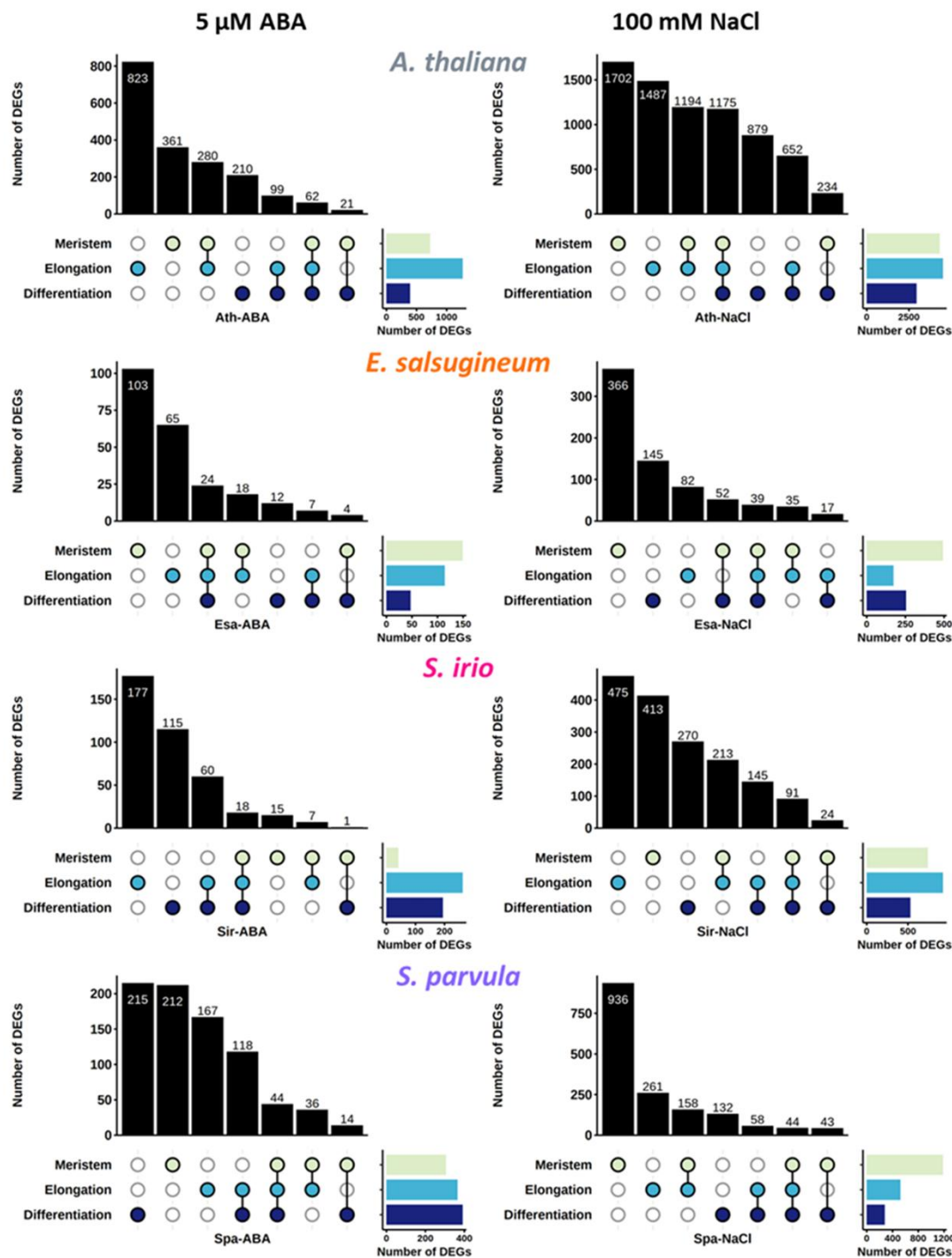
Left, confocal images of whole-mount HCR showing the detection of OG03053 orthologs across species. Scale bars, 50 μ m. Right, average signal intensity of OG03053 orthologs across defined cell type groups. Data are presented as mean values $\pm$ SD. Source data are provided as a Source Data file.



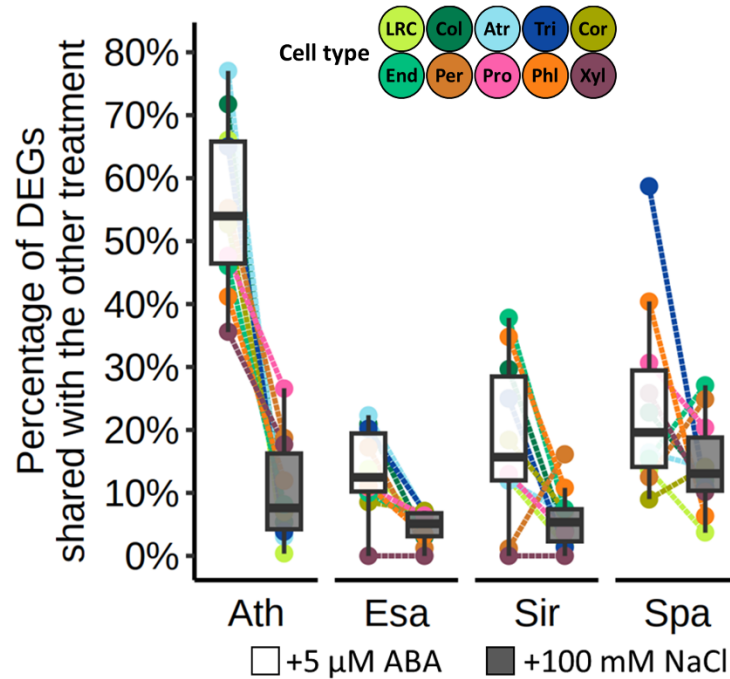
Supplementary Fig. 22. Expression changes of OG10873 orthologs (*AT5G47450*) across species in response to NaCl. a Expression of OG10873 orthologs across species from scRNA-seq data under different conditions. Left, UMAP representation of the expression of OG10873 orthologs. Right, pseudo-bulk cell type expression of OG10873 orthologs. b Whole-mount *in situ* HCR detection of expression changes of OG10873 orthologs across species in response to ABA. Left, confocal images of whole-mount HCR showing the detection of OG10873 orthologs across species. Scale bars, 50 μ m. Right, average signal intensity of OG10873 orthologs across defined cell type groups. Data are presented as mean values $\pm$ SD. Source data are provided as a Source Data file.



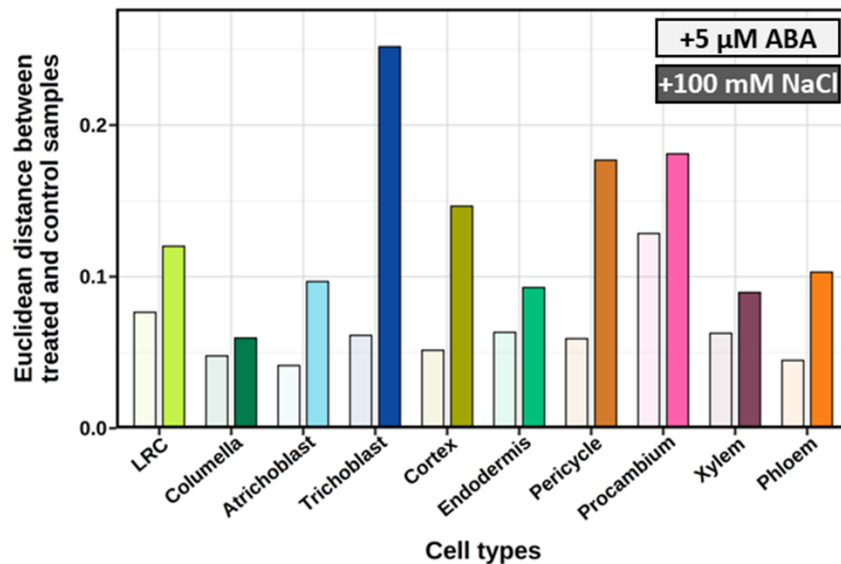
Supplementary Fig. 23. Number of stress responsive genes in each cell type from each species in response to 5 μ M ABA and 100 mM NaCl. Total number of stress responsive genes (nr, non-redundant) from all cell types in each species was shown on the top left corner.



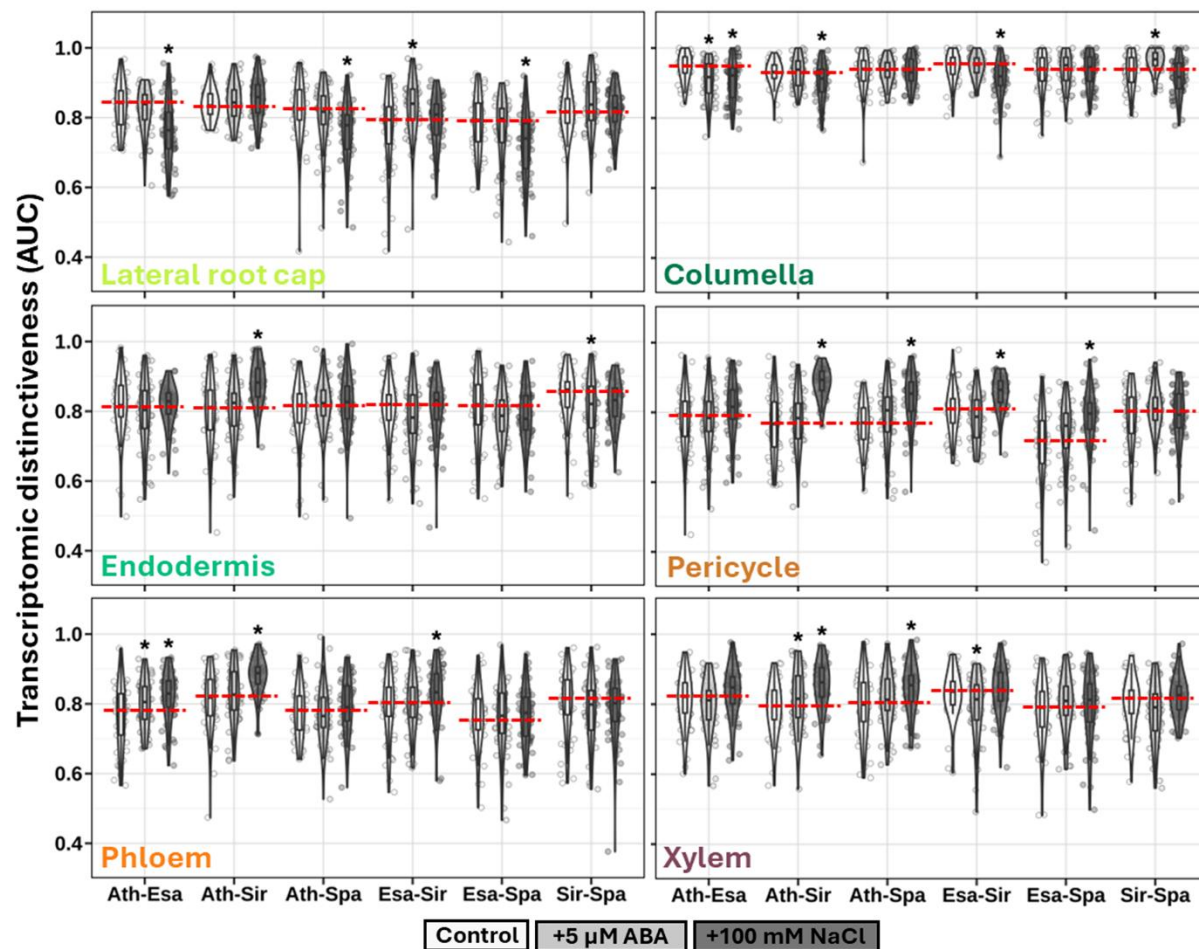
Supplementary Fig. 24. Overlap of stress responsive genes in each developmental zone from each species in response to 5 μ M ABA (left) and 100 mM NaCl (right). Source data are provided as a Source Data file.



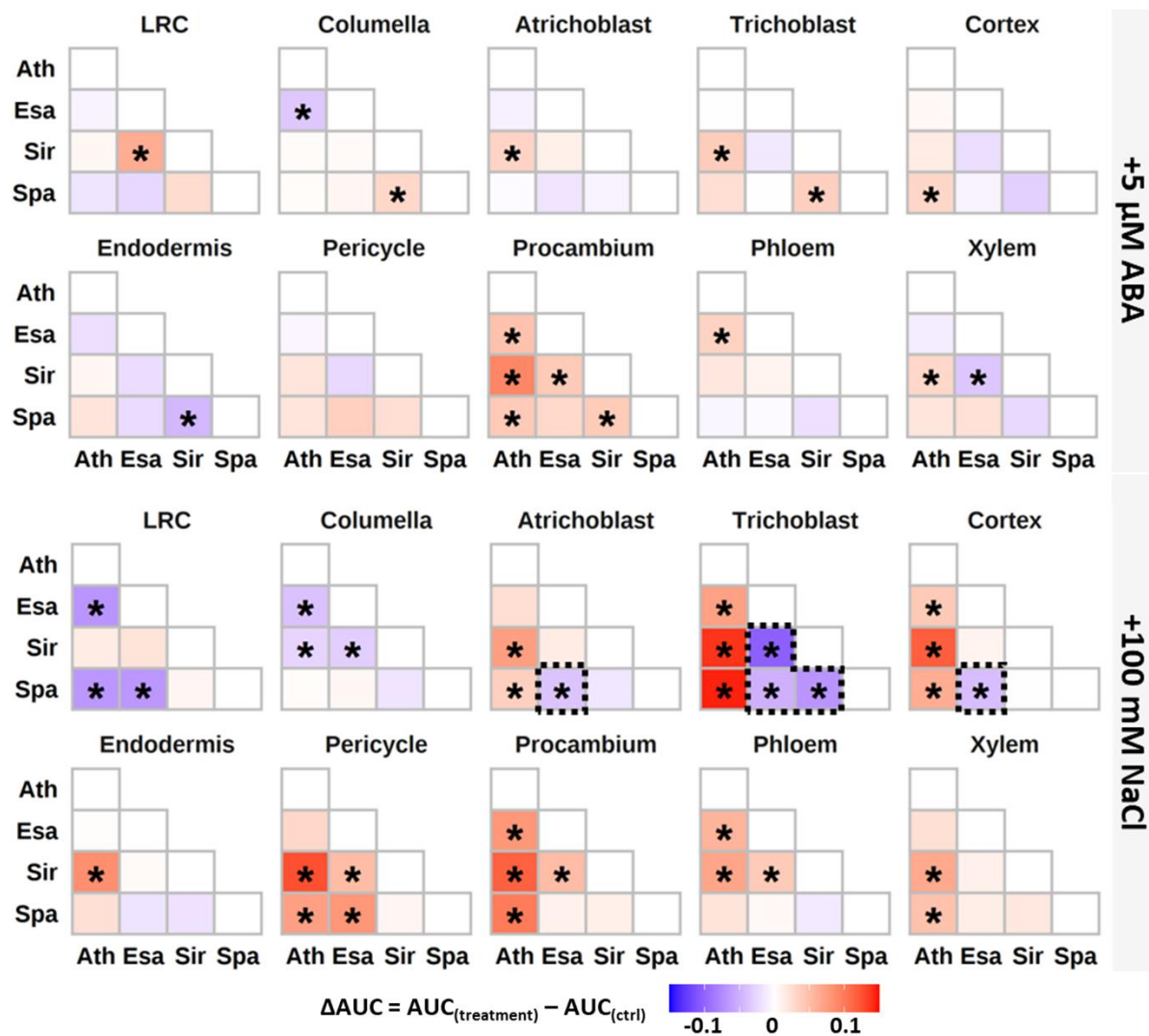
Supplementary Fig. 25. The percentage of DEGs shared between ABA and NaCl treatment. Ath, *Arabidopsis*; Esa, *Eutrema salsugineum*; Sir, *Sisymbrium irio*; Spa, *Schrenkiella parvula*. Source data are provided as a Source Data file.



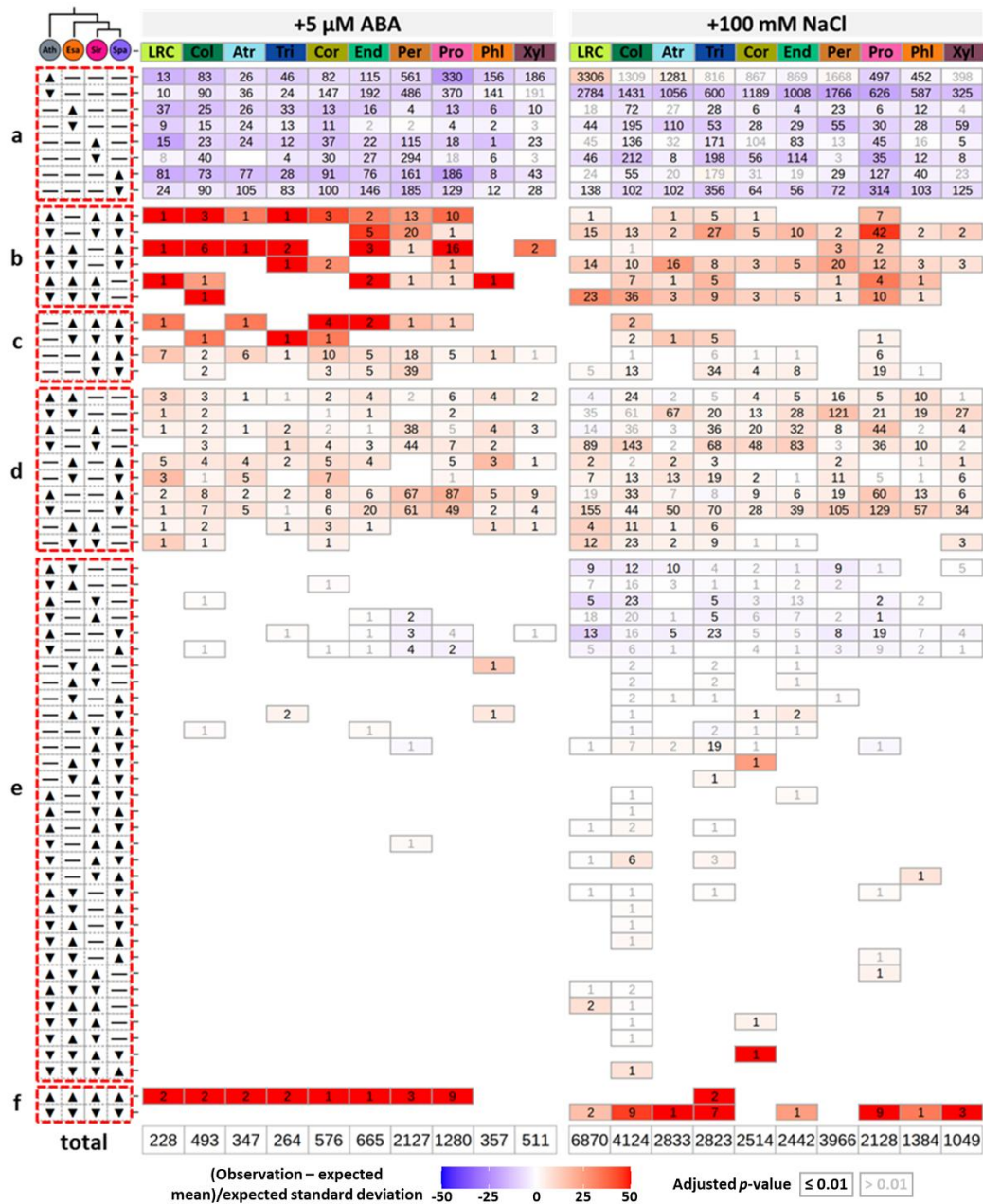
Supplementary Fig. 26. Euclidean distance between treated and control samples in Fig. 5e. The salt-treated samples were generally more distant (greater Euclidean distances) to the control samples than the ABA-treated samples. Source data are provided as a Source Data file.



Supplementary Fig. 27. Transcriptomic distinctiveness between species pairs under different conditions. AUC here quantifies the separability between species for each cell type. AUC = 0.5 indicates random separability; AUC = 1.0 indicates perfect separability. Asterisks indicate significance after comparing to control (n = 50) with Student's t-test at p -value cutoff of 0.05. The red dash lines mark the medians the transcriptomic distinctiveness under control condition. Ath, *Arabidopsis*; Esa, *Eutrema salsugineum*; Sir, *Sisymbrium irio*; Spa, *Schrenkiella parvula*. Source data are provided as a Source Data file.

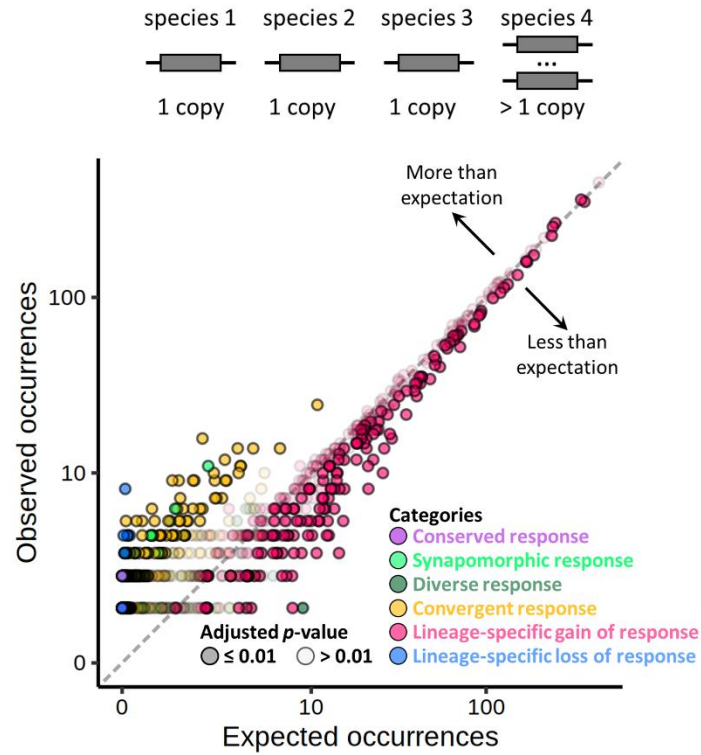


Supplementary Fig. 28. Changes in cell type transcriptome divergence between species in response to ABA (top) and NaCl (bottom). The darker colors indicate larger differences in transcriptome divergence induced by the treatment. Asterisks indicate significance after comparing to control ($n = 50$) with Student's t-test at p-value cutoff of 0.05. The black boxes highlight the comparisons of interest. Ath, *Arabidopsis*; Esa, *Eutrema salsugineum*; Sir, *Sisymbrium irio*; Spa, *Schrenkiella parvula*. Source data are provided as a Source Data file.

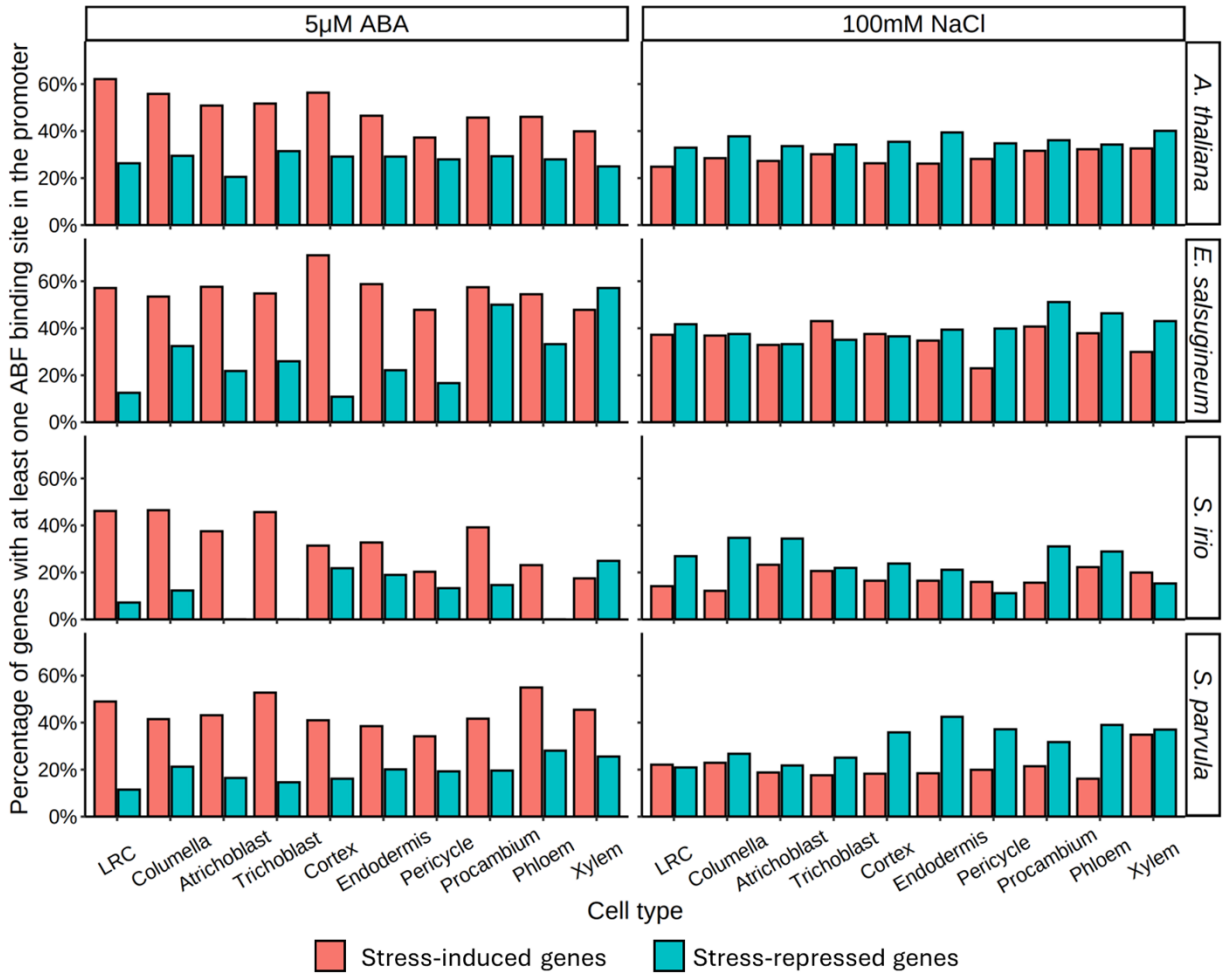


Supplementary Fig. 29. Differences between observed and bootstrapped (10,000 times) occurrences of different ortholog response patterns. The patterns were grouped according to orthologs showing a, lineage-specific gain of response; b, lineage-specific loss of response; c, synapomorphic response; d, convergent response; e, diverse response; f, conserved response. Number of orthologs for each pattern in each cell type is indicated in the corresponding tile. The differences were measured as the number of standard deviations between the overserved occurrences and the corresponding bootstrapped means and indicated with the shade of the color with darker colors corresponding to larger differences. Black and grey text represent significant and insignificant deviation of the observed occurrences from the expected distribution,

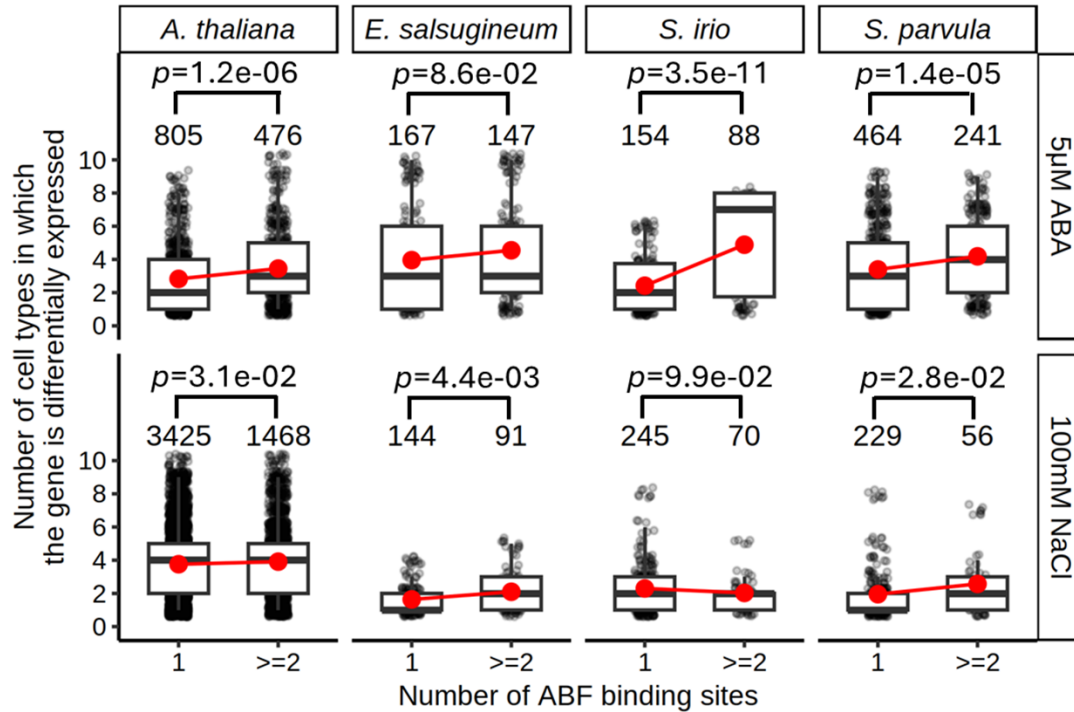
respectively, at Benjamini-Hochberg-adjusted p -value ≤ 0.01 . Source data are provided as a Source Data file.



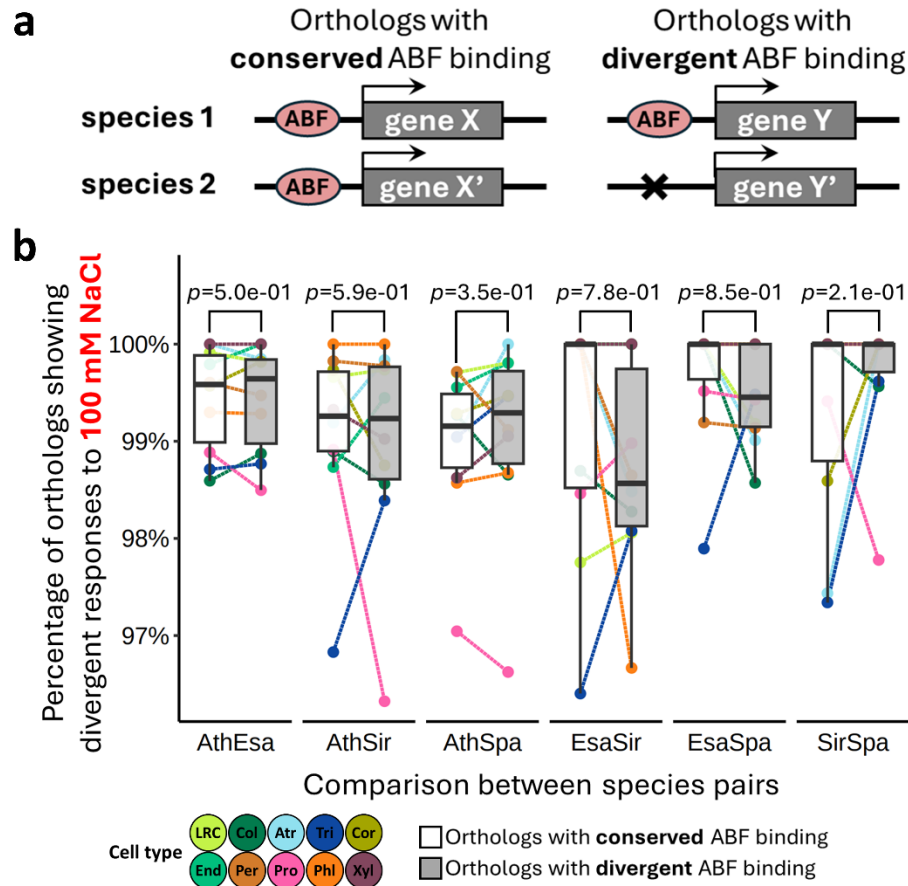
Supplementary Fig. 30. Differences between observed and bootstrapped (10,000 times) occurrences of response patterns among all ortholog groups that have been duplicated only in one species. The darker and lighter color intensities represent significant and insignificant deviation of the observed occurrences from the expected distribution, respectively, at Benjamini-Hochberg-adjusted p -value ≤ 0.01 . Source data are provided as a Source Data file.



Supplementary Fig. 31. Percentage of genes with ABF binding among genes induced by 5μM ABA (left) or 100mM NaCl (right). ABF binding was defined as the presence of ABF binding events in the 2-kilobase upstream of the transcription start site using the DAP-seq data from Sun *et al.*, 2022⁹. Source data are provided as a Source Data file.

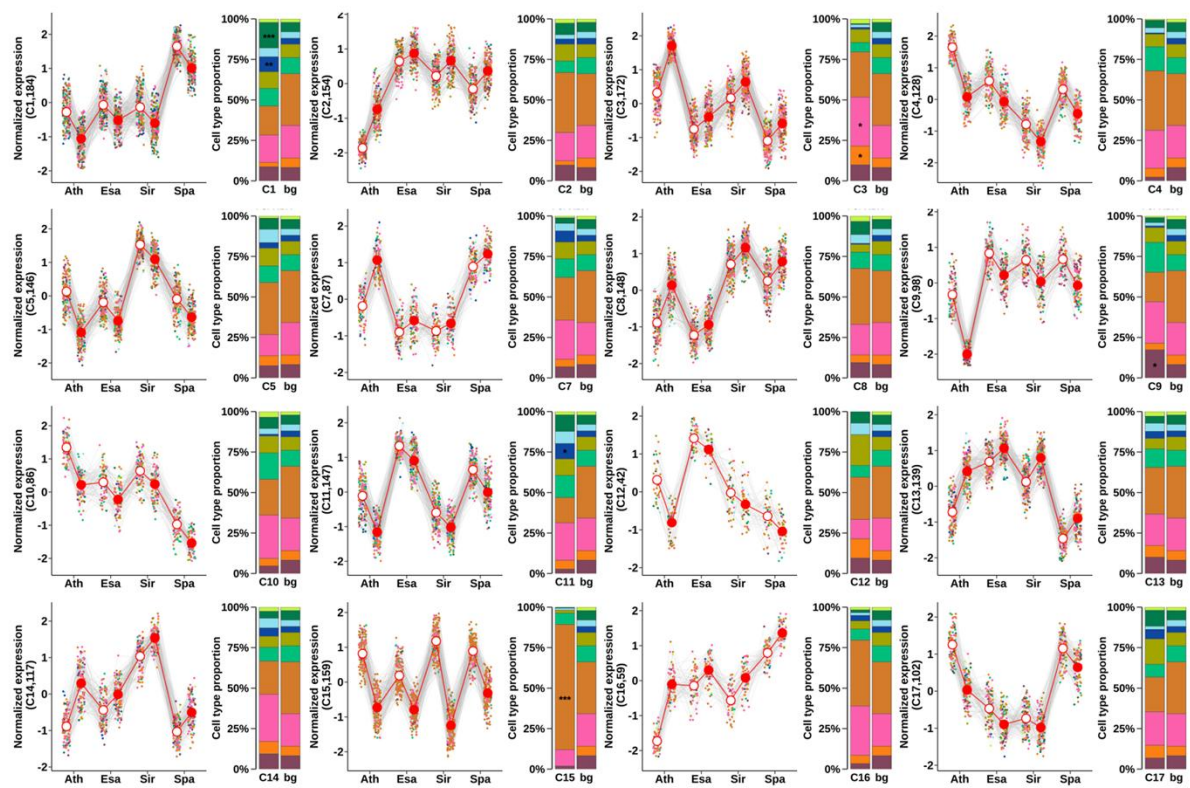


Supplementary Fig. 32. Number of responsive cell types for genes with different numbers of ABF binding sites. ABF binding was defined as the presence of ABF binding events in the 2-kilobase upstream of the transcription start site using the DAP-seq data from Sun *et al.*, 2022⁹. Significance was represented by *p*-values determined by Student's t-test and shown on the top of each comparison. Numbers above each box indicate the number of genes included in each group. Source data are provided as a Source Data file.

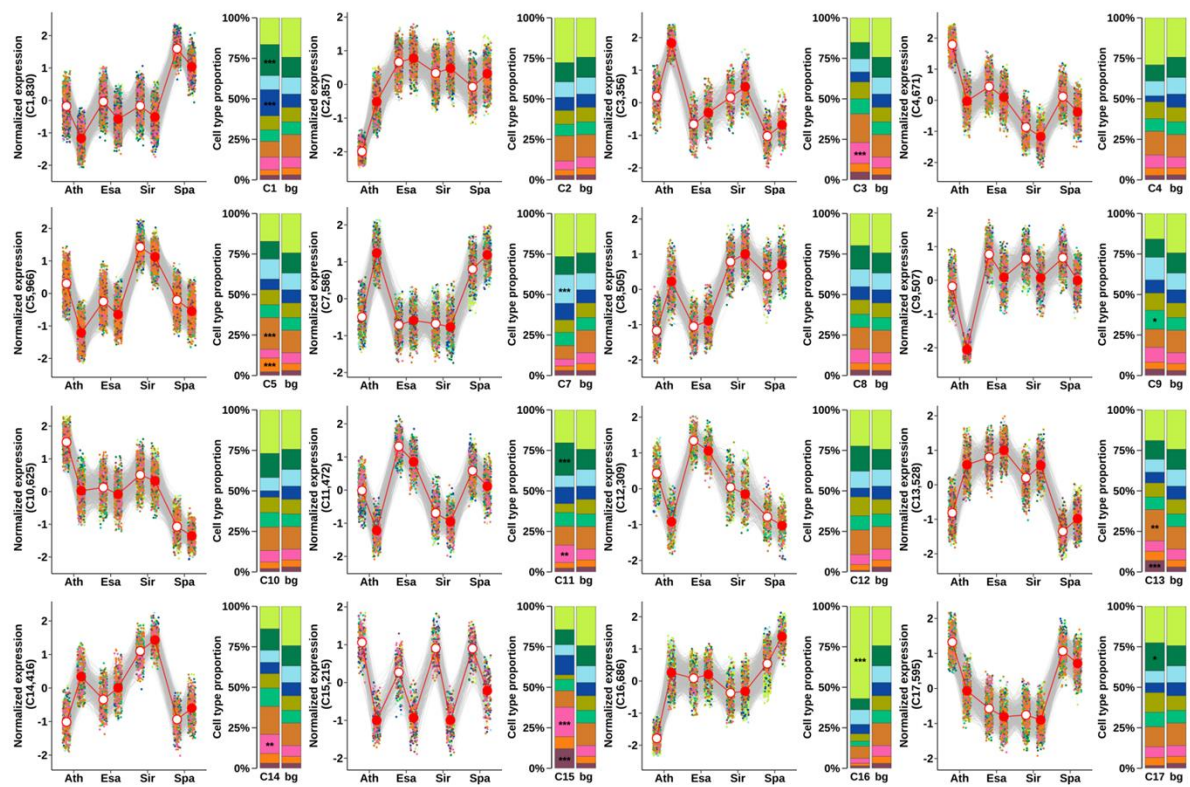


Supplementary Fig. 33. The relationship between ABF binding and stress responses of orthologs between species. a Categorization of orthologs with different ABF binding events. ABF binding was defined as the presence of ABF binding events in the 2-kilobase upstream of the transcription start site using the DAP-seq data from Sun *et al.*, 2022⁹. b Percentage of divergently regulated orthologs (in response to 100 mM NaCl) with conserved or divergent ABF binding. Significance was represented by p -values determined by Wilcoxon test and shown on the top of each comparison.

a



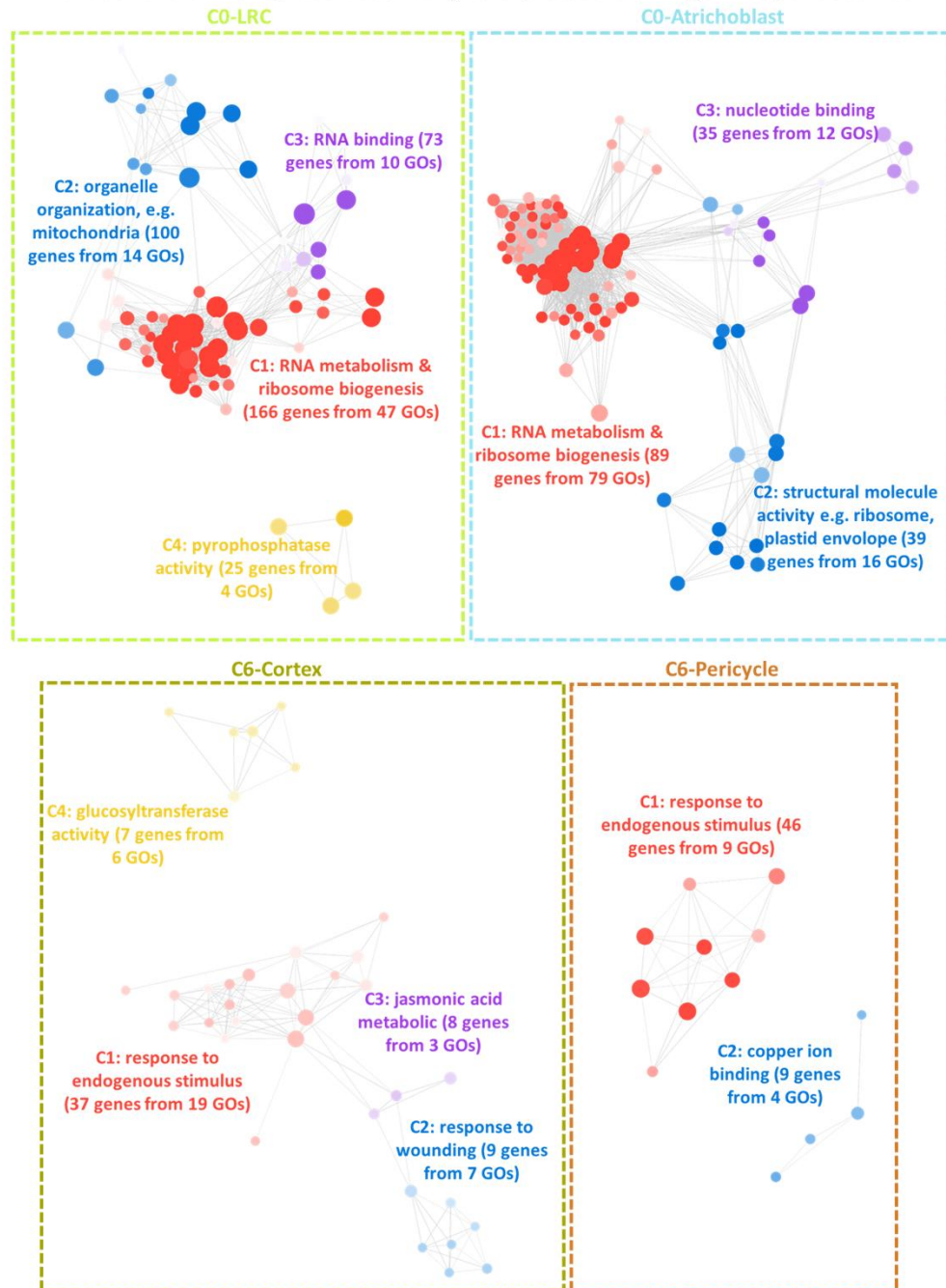
b



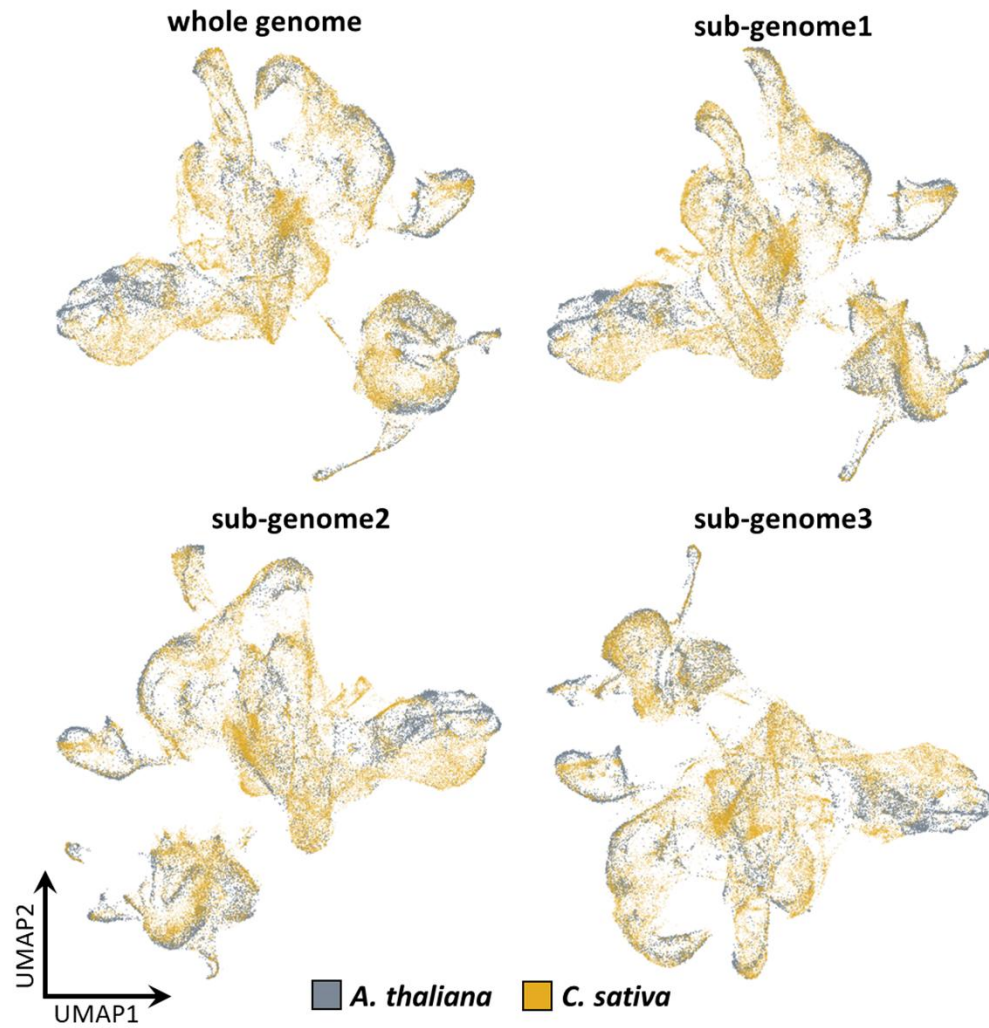
Cell type ● LRC ● Col ● Atr ● Tri ● Cor ● End ● Per ● Pro ● Phl ● Xyl Condition ○ Control ● Treatment

Supplementary Fig. 34. Contributions of each cell type to co-expression networks identified from stress-responsive orthologs under 5 μ M ABA (a) and 100 mM NaCl (b). Cluster ID and the number of orthologs in the cluster are shown in parentheses in the Y-axis labels. Cell type proportions of the cluster were compared to background (bg) where orthologs from all clusters were averaged (see Methods for details). Asterisks represent significance determined by hypergeometric test, * p -value < 0.01, ** p -value < 0.001, *** p -value < 0.0001. LRC, Lateral Root Cap; Col, Columella; Atr, Atrichoblast; Tri, Trichoblast; Cor, Cortex; End, Endodermis; Per, Pericycle; Pro, Procambium; Phl, Phloem; Xyl, Xylem. Ath, Arabidopsis; Esa, *Eutrema salsugineum*; Sir, *Sisymbrium irio*; Spa, *Schrenkiella parvula*. Source data are provided as a Source Data file.

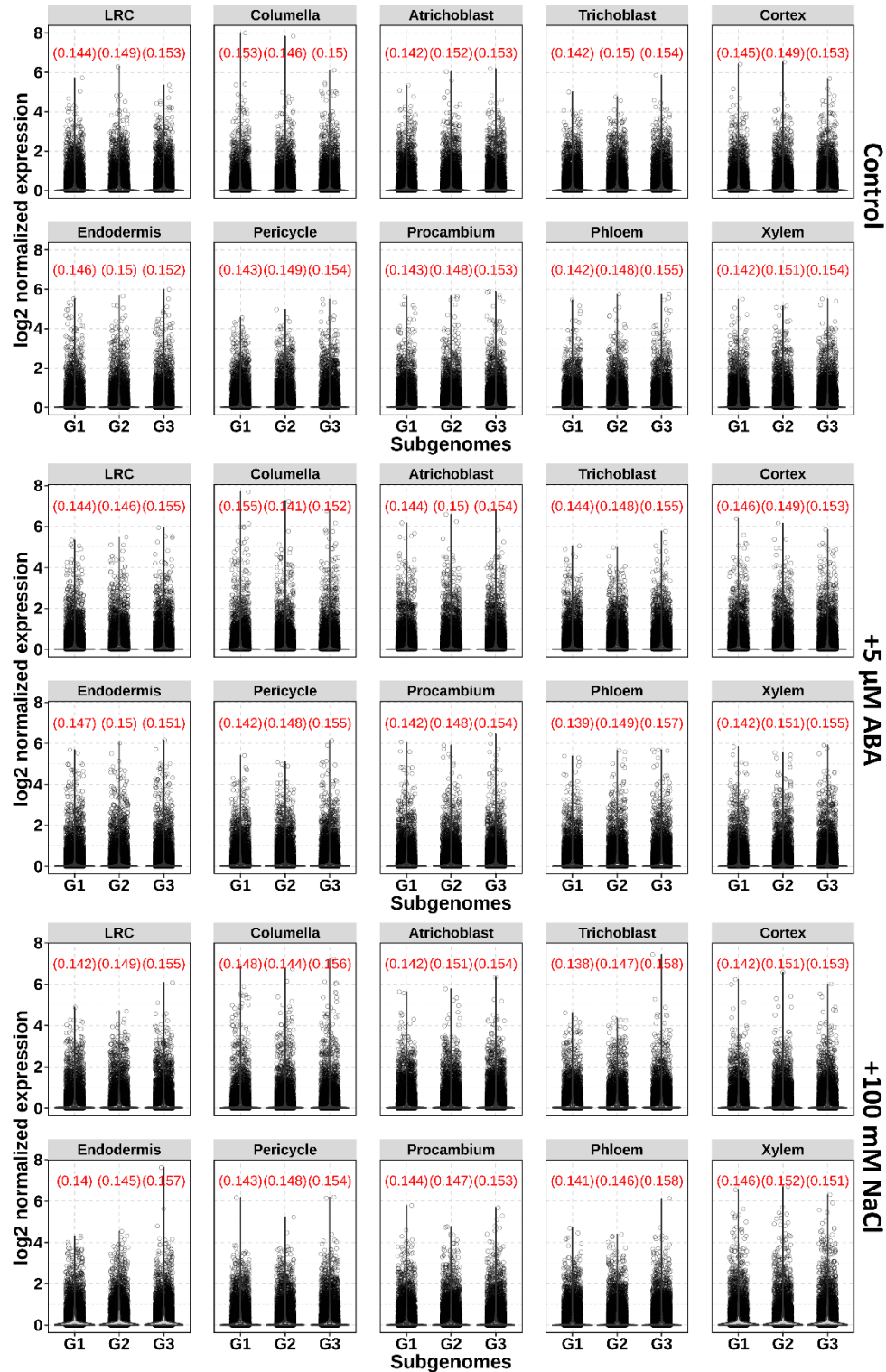
Functional themes represented among co-expressed orthologs in response to NaCl



Supplementary Fig. 35. Functional representations of co-expressed orthologs in response to NaCl. GO clusters are differently colored and labelled with the representative functional term. Each node represents a gene ontology (GO) term; node size represents genes in the test set assigned to that functional term; GO terms sharing $> 50\%$ of genes are connected with edges; and the shade of each node represents the p -value assigned by the enrichment test at adjusted p values < 0.05 with darker shades indicating smaller p -values.



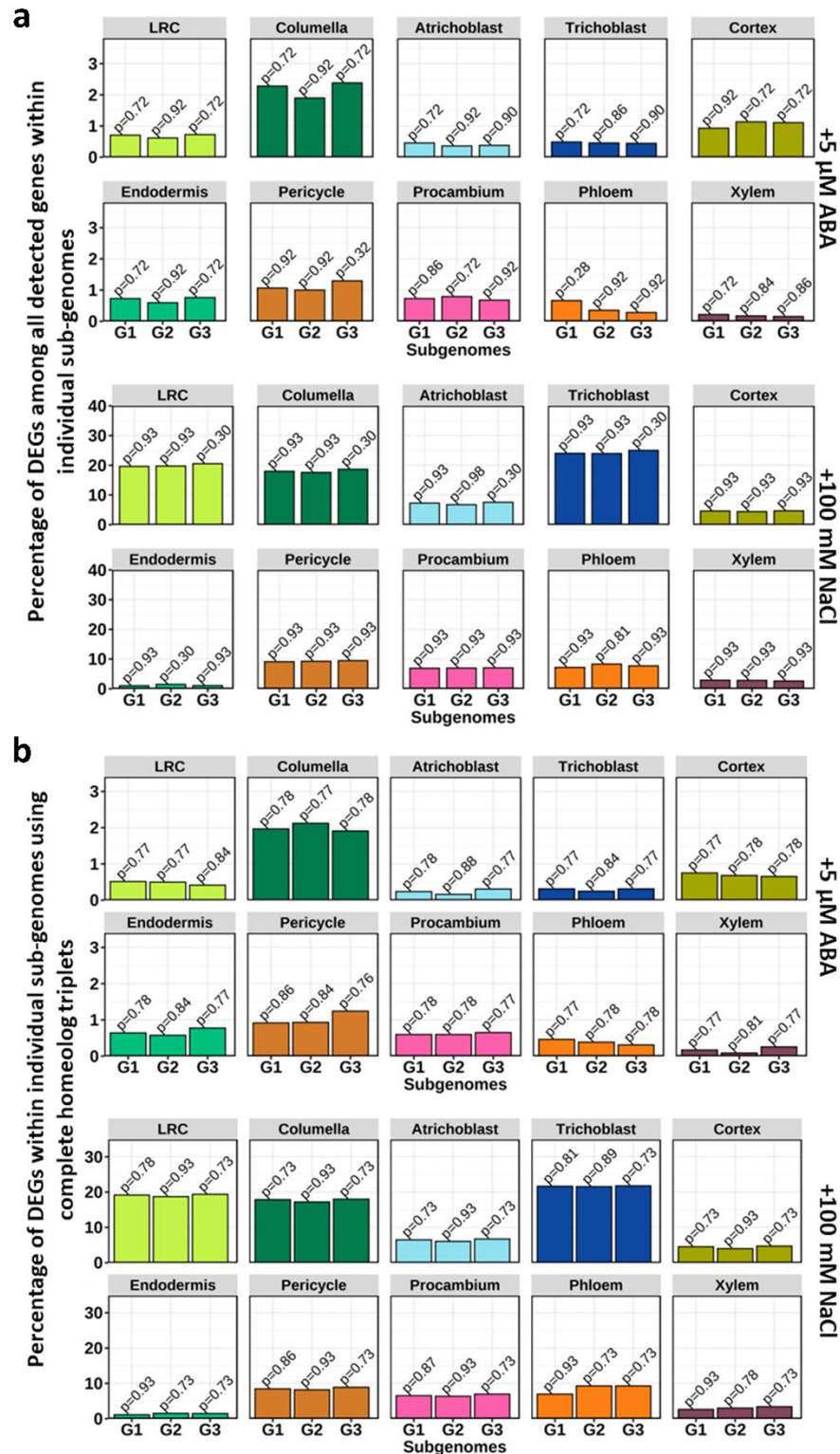
Supplementary Fig. 36. Integration between Arabidopsis and *C. sativa* using different sub-genomes in *C. sativa*.



Supplementary Fig. 37. Overall transcriptome expression levels separated by the three sub-genomes for each cell type under different conditions. The number of genes considered for sub-genome G1, G2, and G3 is 21,848, 21,682, and 23,066, respectively. Numbers in red indicate the averages of transcript levels.



Supplementary Fig. 38. Comparison of GO functional enrichment among DEGs in response to ABA (top) and NaCl (bottom) for each cell type across species. The enrichment in *C. sativa* was performed on DEGs combined from all sub-genomes. Endodermis, phloem and xylem cells in *C. sativa* were removed from the comparison for NaCl treatment due to insufficient number of cells (< 50 cells). The detailed list of GO terms is present in Supplementary Data 6. The shade represents the p -value assigned by the enrichment test at adjusted p -value < 0.05 with darker shades indicating smaller p -values.



Supplementary Fig. 39. Percentage of DEGs in each sub-genome of *C. sativa*. The percentage for each genome was calculated considering all detected genes within individual sub-genomes (a) and only the complete homeolog triplets (b). Numbers on top of the bars represent adjusted p -values from hypergeometric test. Source data are provided as a Source Data file.