SUPPLEMENTAL FIGURES

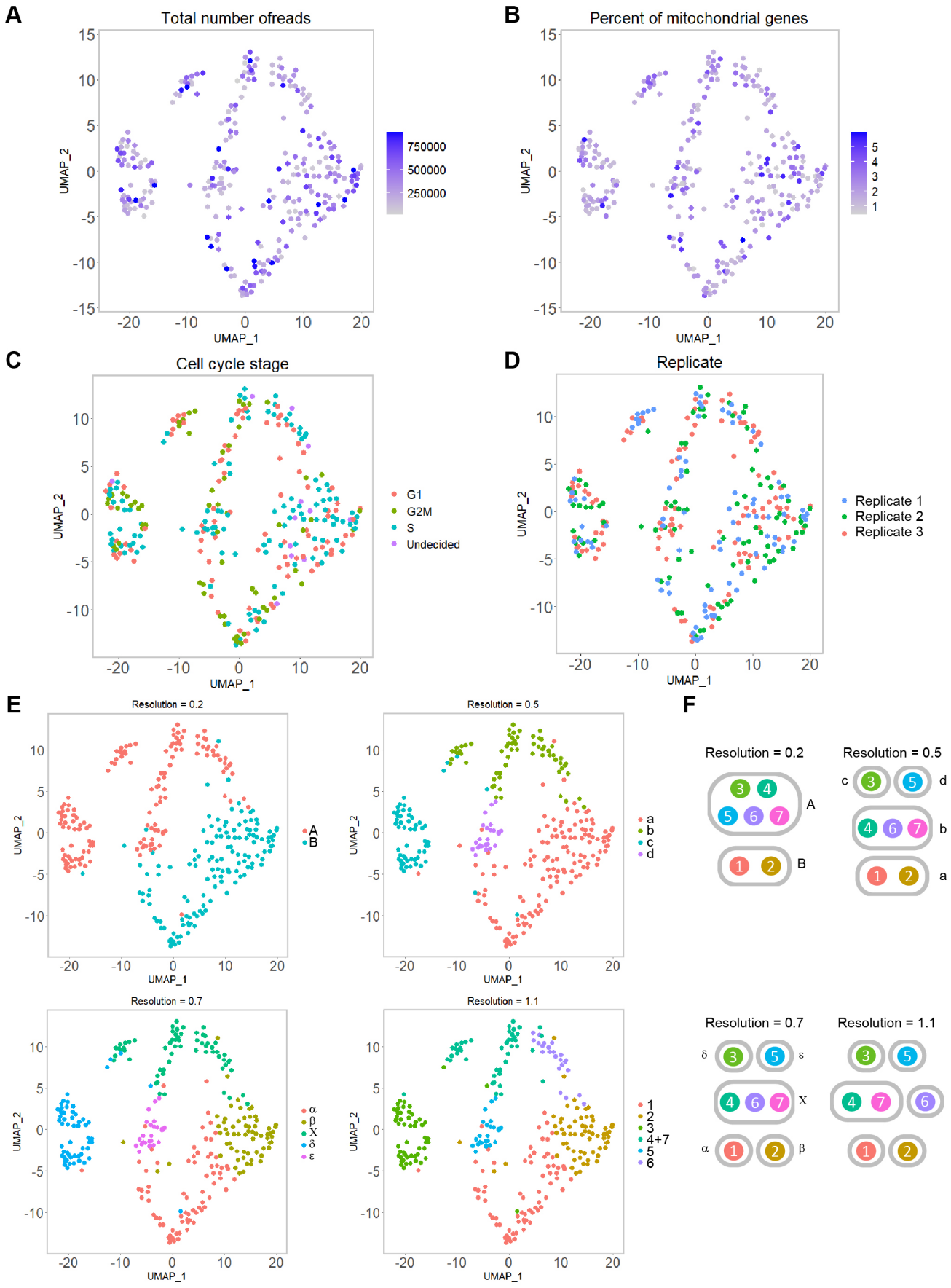


Figure S1. Clustering of cells at different resolutions defines the main LRP cell populations. (A-C) UMAP plots showing (A) the total number of reads in each cell, (B) percent of mitochondrial genes expressed in each cell, and (C) the cell cycle stage of each cell. Note that these features, which represent unwanted sources of variation, do not cluster together in the UMAP representation. **(D)** UMAP plot showing cells collected in each replicate. **(E)** UMAP plots at selected resolutions of 0.2, 0.5, 0.7 and 1.1. **(F)** Schematic representation showing clustering of the final cell populations selected for this study at different resolutions.

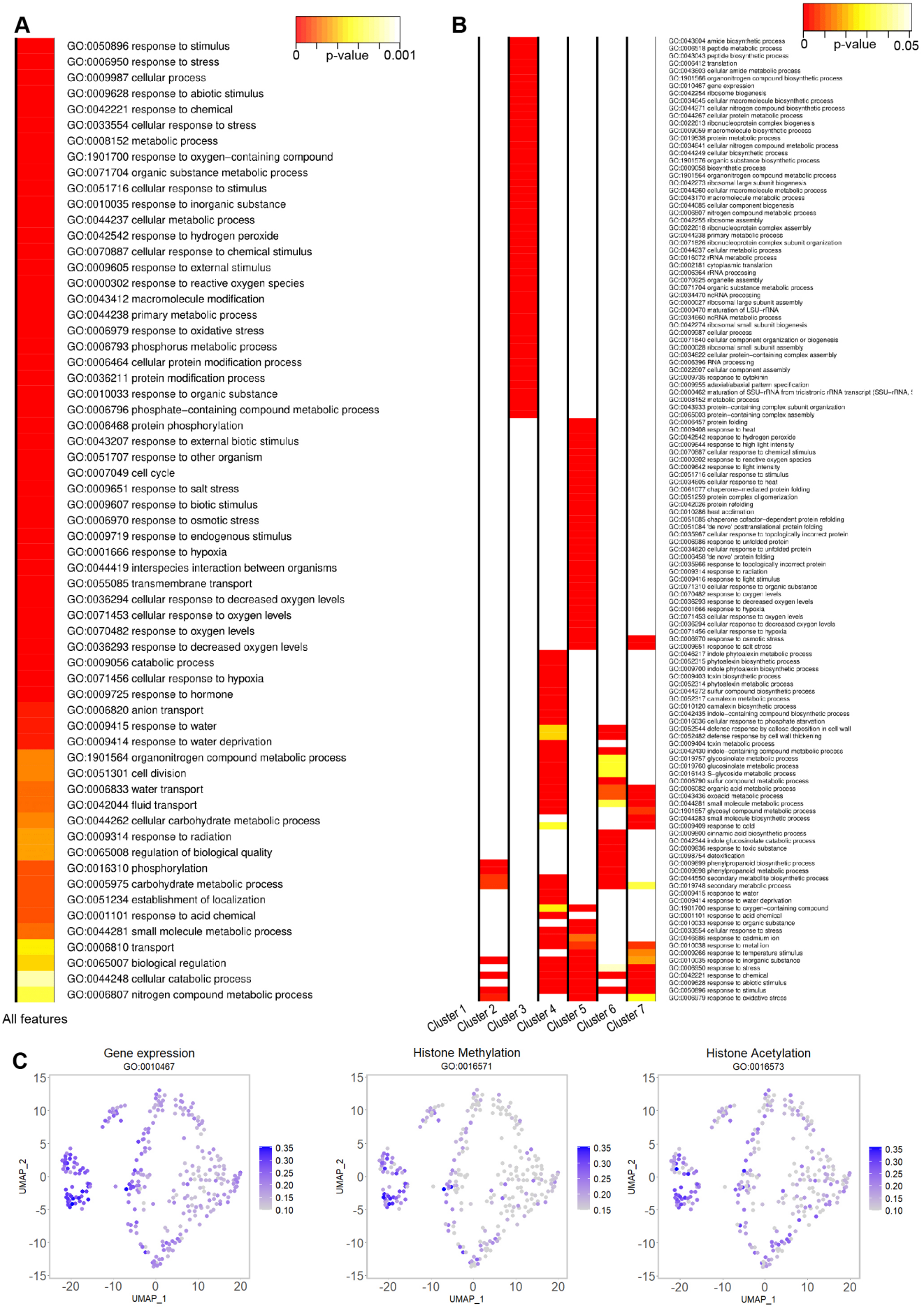


Figure S2. The identified cell populations can be differentiated by predicted functionality. (A) Heatmap representation of the GO terms enriched (p-value <0.001) in the set of 2,500 most variable genes among all cell populations. **(B)** Heatmap representation of the GO terms enriched in the top 100 markers for each population and present in at least one of the populations with a p-value <0.001. **(C)** UMAP plots showing the average expression of the genes annotated in three representative GO terms enriched in population 3.

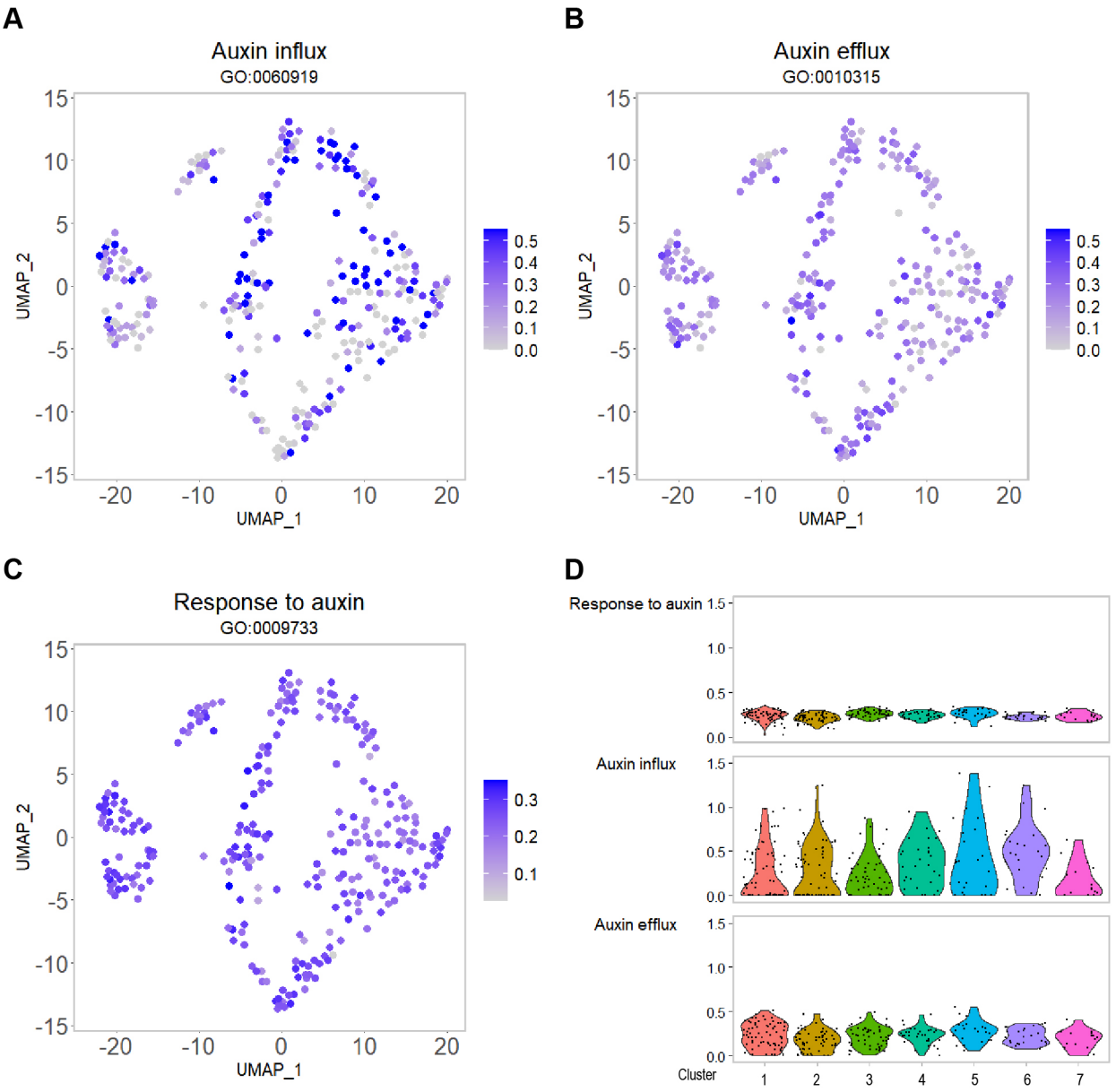
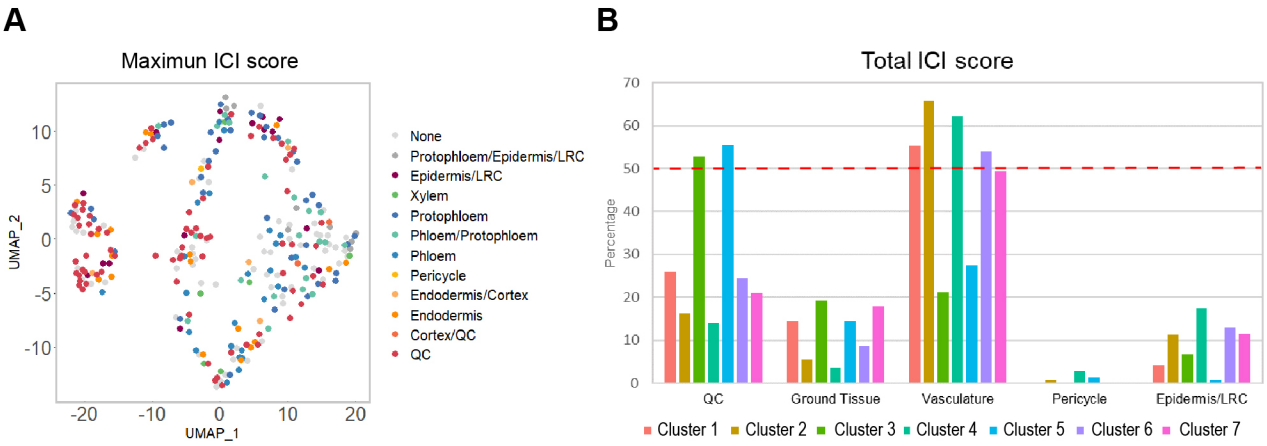


Figure S3. Most LRP cells show expression of auxin-transporter and response-to-auxin genes. (A-B) UMAP plots showing average expression of (A) the genes included in the GO term "Auxin influx" and (B) "Auxin efflux". **(C)** UMAP plot showing average expression of the genes included in GO term "Response to auxin". **(D)** Violin plots showing average expression of the genes in the previous GO terms.



FigureS4. Analysis of the Index of Cell Identity (ICI) shows partial similarities of LRP cells to known root identities. (A) UMAP plot showing the maximum ICI score found for each cell. Cells classified as "None" had an ICI score equal to 0 for all cell types. **(B)** Percentage of ICI score found among all cells from each population. %: The ICI scores for each identity were added for every cell within a population and divided by the sum of the ICI scores for all the identities in that population. QC: Quiescent Center. LRC: Lateral Root Cap. Ground tissue includes the ICI scores for endodermis and cortex. Vasculature includes the ICI scores for phloem, protophloem, xylem and protoxylem.

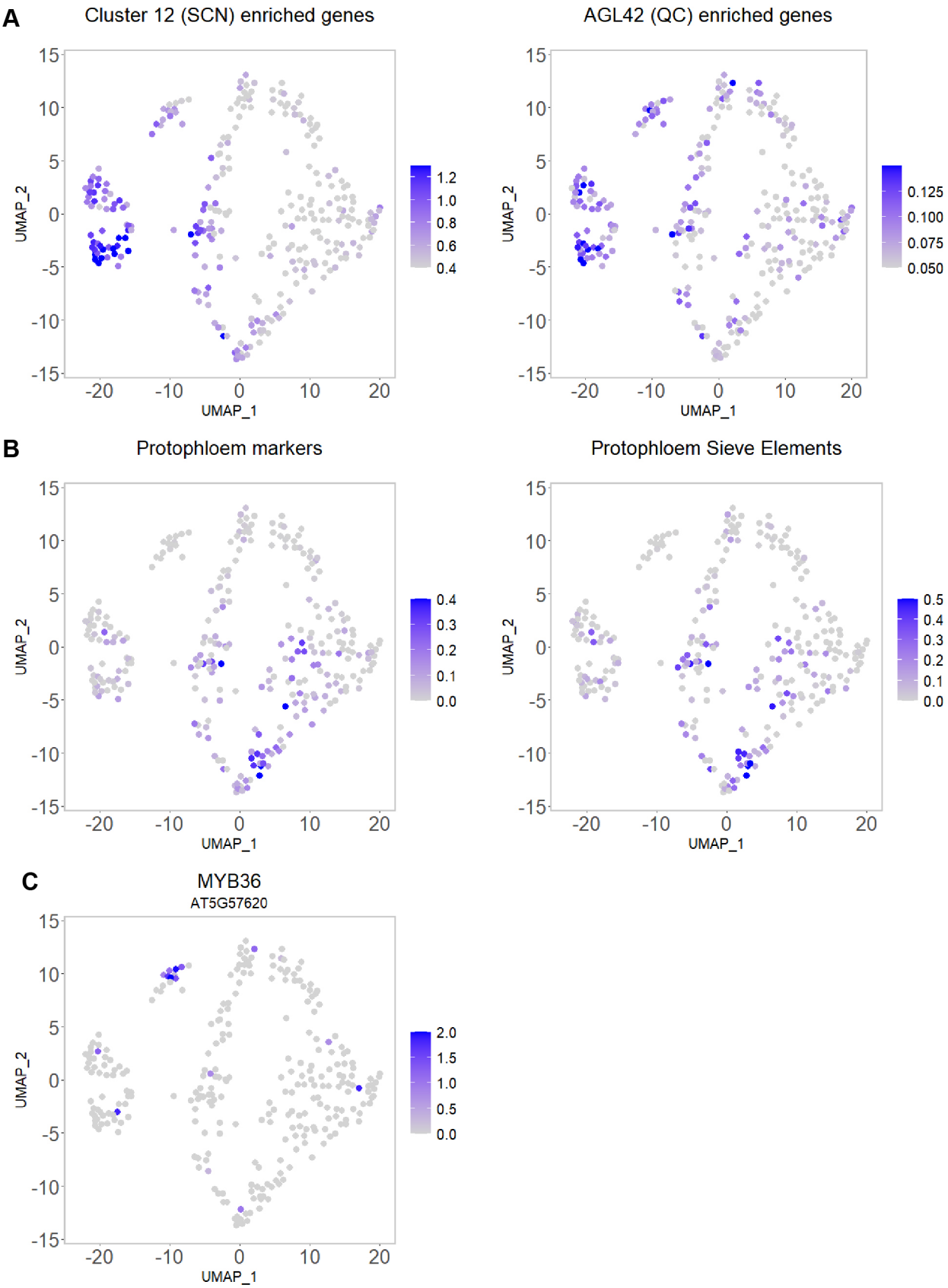


Figure S5. Vasculature markers, stem cell niche (SCN) genes and the LR regulator MYB36 are enriched in some LRP cell populations. (A) UMAP plot showing the average expression of SCN markers as defined in (Zhang et al., 2019) and genes with enriched expression in the QC as profiled in the Root Map using the AGL42 marker (Brady et al., 2007). **(B)** UMAP plot showing the average expression of protophloem markers (Denyer et al., 2019) and protopholem sieve elements (Ryu et al., 2019). **(C)** UMAP plot showing expression of the TF MYB36, which regulates the LRP flanks (Fernández-Marcos et al., 2017).

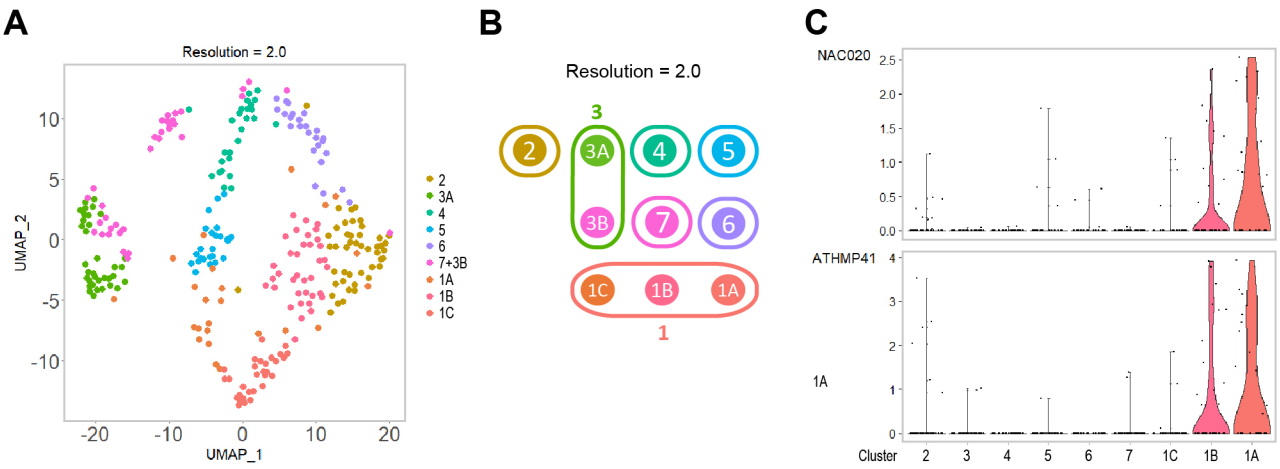


Figure S6. Subclustering reveals heterogeneity of cell identities within cluster 1. (A) UMAP plot at resolution 2.0. **(B)** Schematic representation showing subclustering at resolution 2.0 (filled circles) and how it compares with previous clustering (i.e. with the populations previously defined (outer circles)). A letter after the population number indicates subdivision of the population. **(C)** Violin plots showing the expression of the markers selected for cluster 0 after subclustering at resolution 2.0.

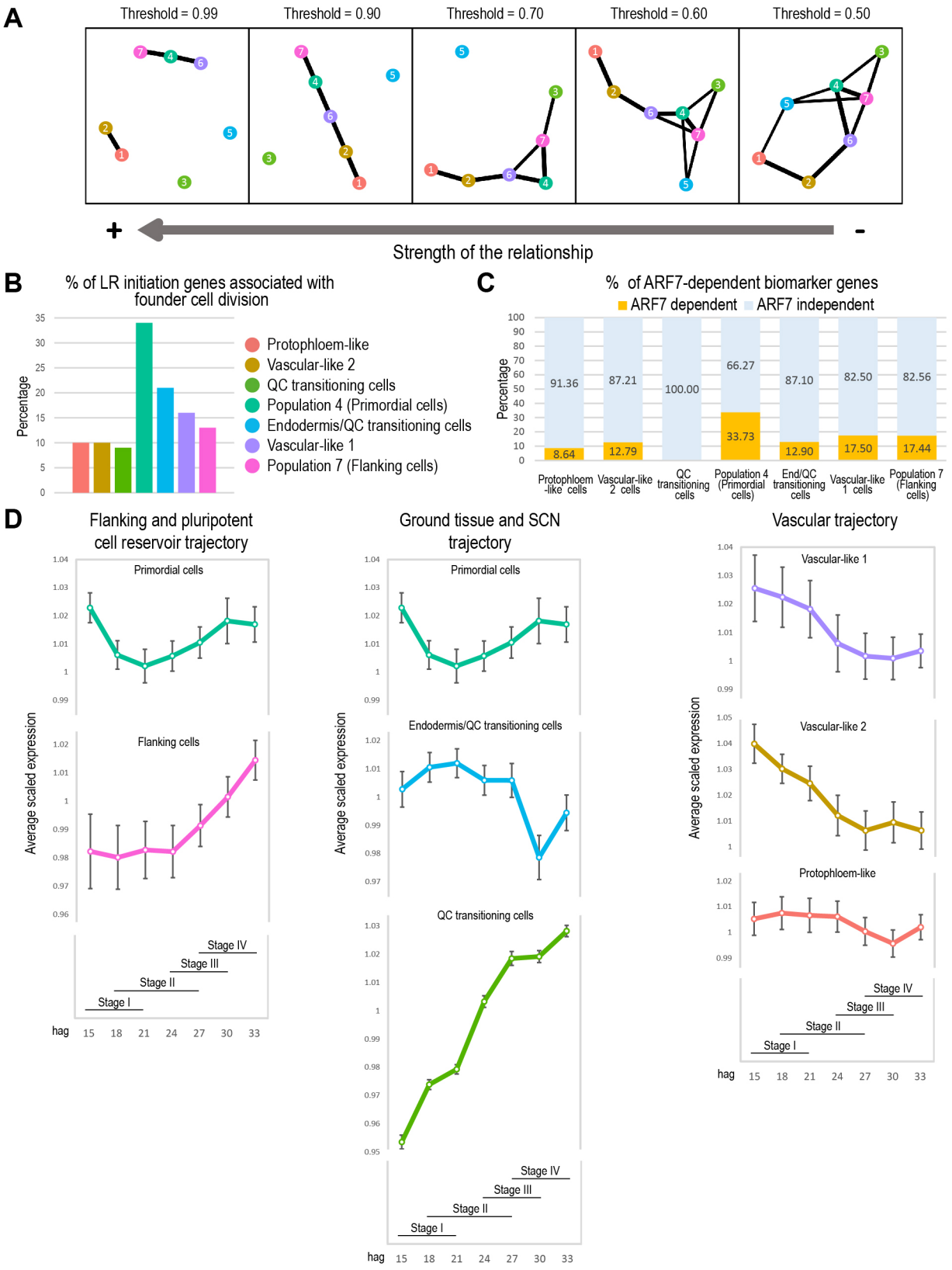


Figure S7. Partition-based Graph Abstraction analysis (PAGA) and comparison with previous LR initiation datasets support spatial temporal relationships among the identified cell populations. (A) Plots showing the relationships that the Partition-based Graph Abstraction analysis (PAGA) predicts among cell populations. Colored dots represent the cell populations. Black lines represent the relationships among cell populations. The thickness of the line is directly proportional to the strength of the relationship at that corresponding threshold. Note that each plot shows a different threshold. **(B)** Percentage of differentially regulated genes (DEG) in pericycle cells stimulated by hormone supplementation to undergo LR initiation and defined as LR initiation genes associated with founder cell division by (De Smet et al., 2008; Parizot et al., 2010) for the top 100 biomarkers in the seven cell populations identified in this study. **(C)** Percentage of ARF7-dependent biomarker genes for the seven cell population in this study. ARF7-dependency for the top 100 biomarkers in each cell population was calculated according to ARF7 gene dependencies established by (Lavenus et al., 2015). **(D)** Centroid expression in gravistimulated whole roots (LR initiation dataset defined by (Voß et al., 2015)) for the seven cell populations identified in this study. Stages and hours after gravistimulation (hag) are those in (Voß et al., 2015). Centroids were calculated as the average expression of the scaled data of the top 100 biomarkers for each cell population.