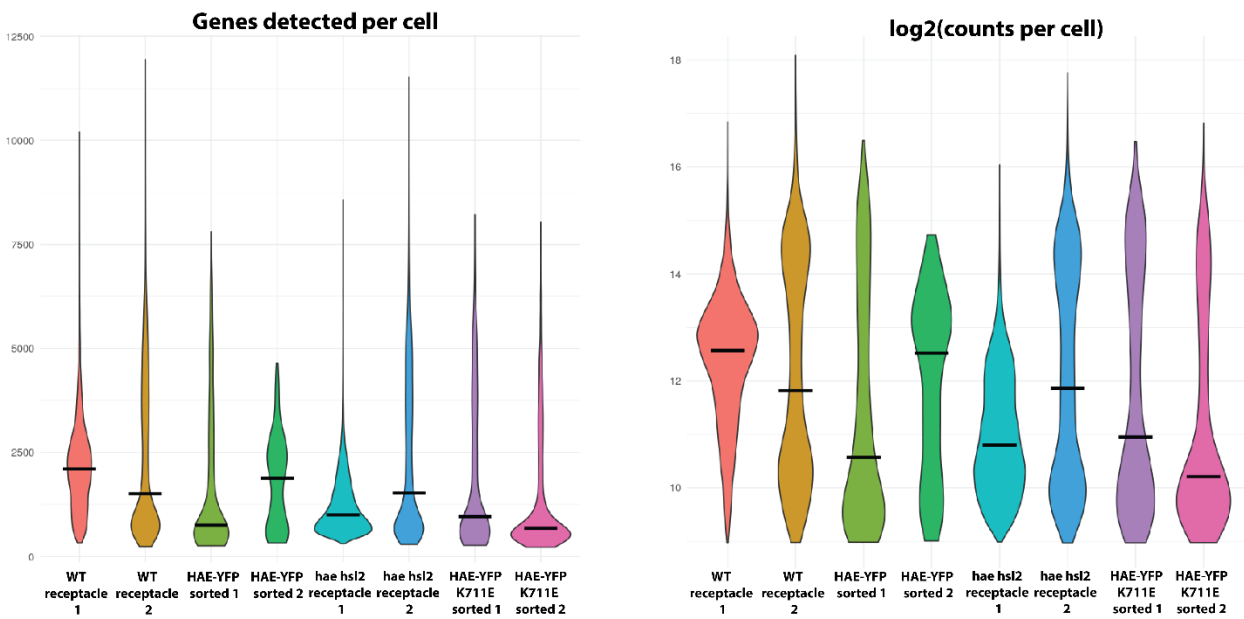
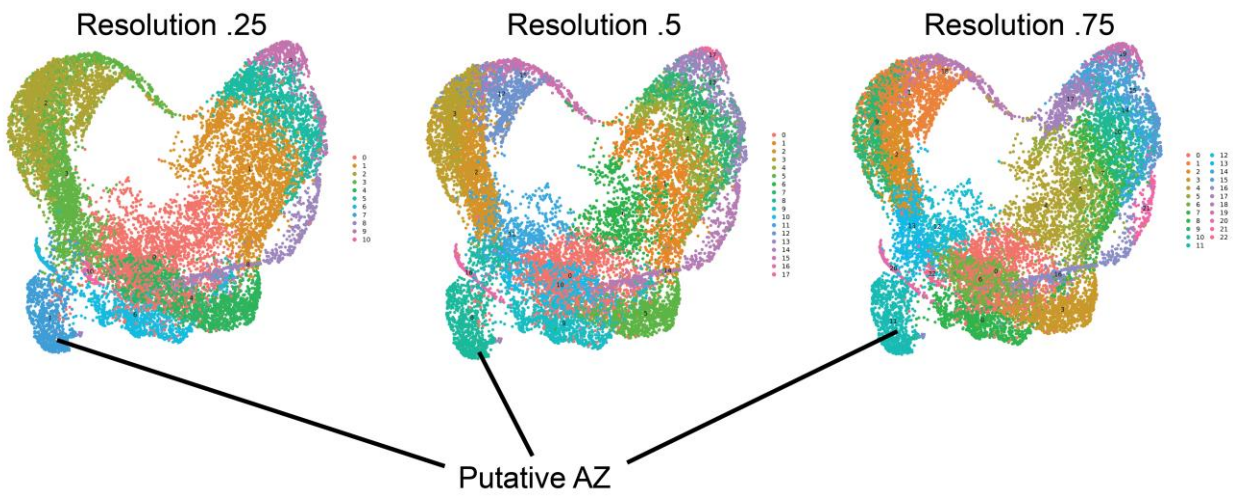


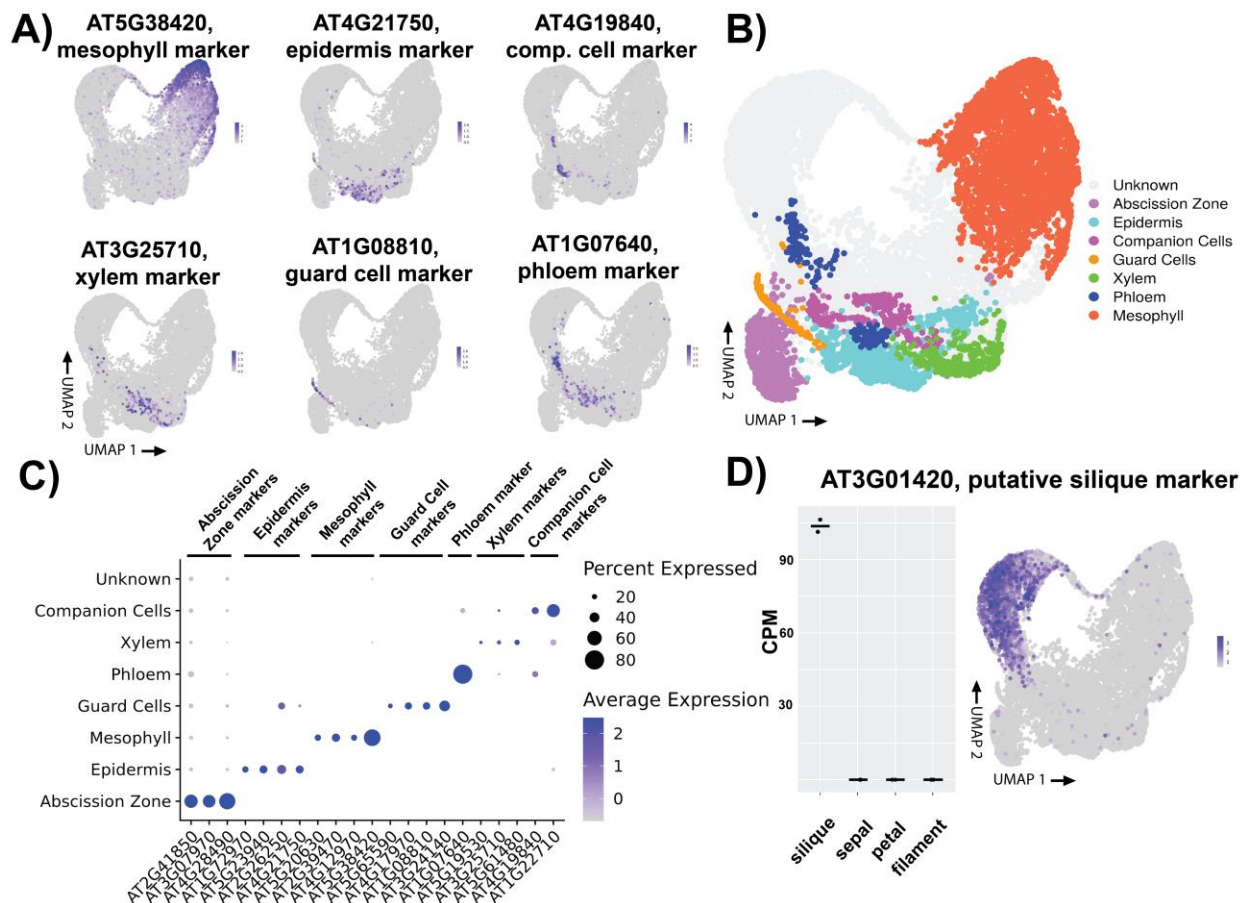
Supplemental Figures



Supplemental Figure 1: Plots of the number of genes detected per cell and log2(counts per cell)

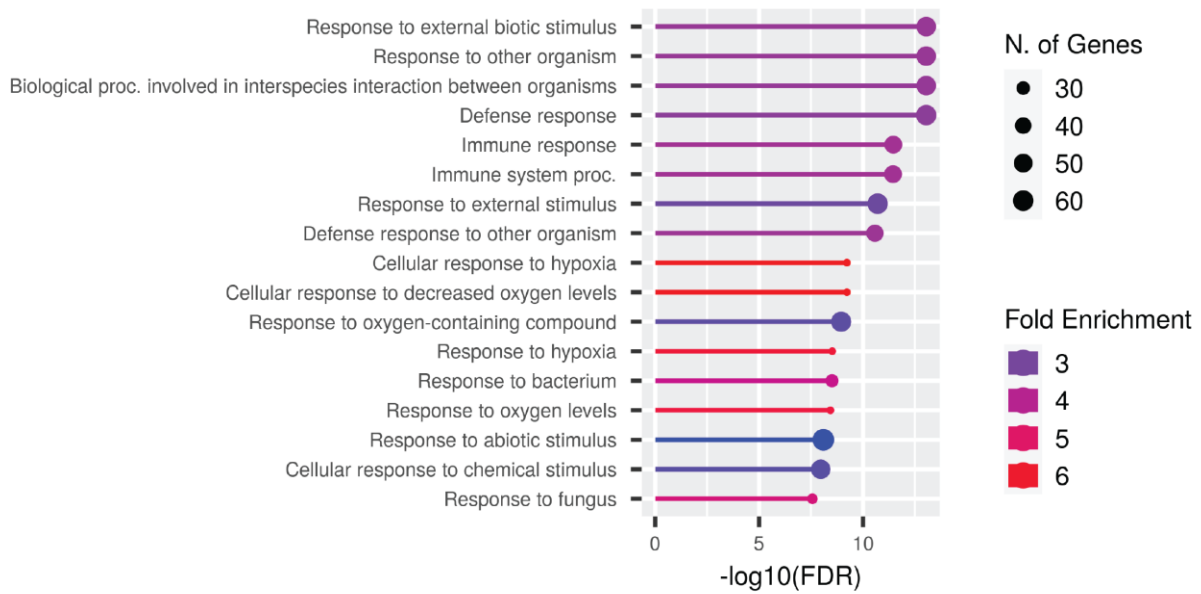


Supplemental Figure 2: The putative AZ cluster is similar across a range of clustering resolutions

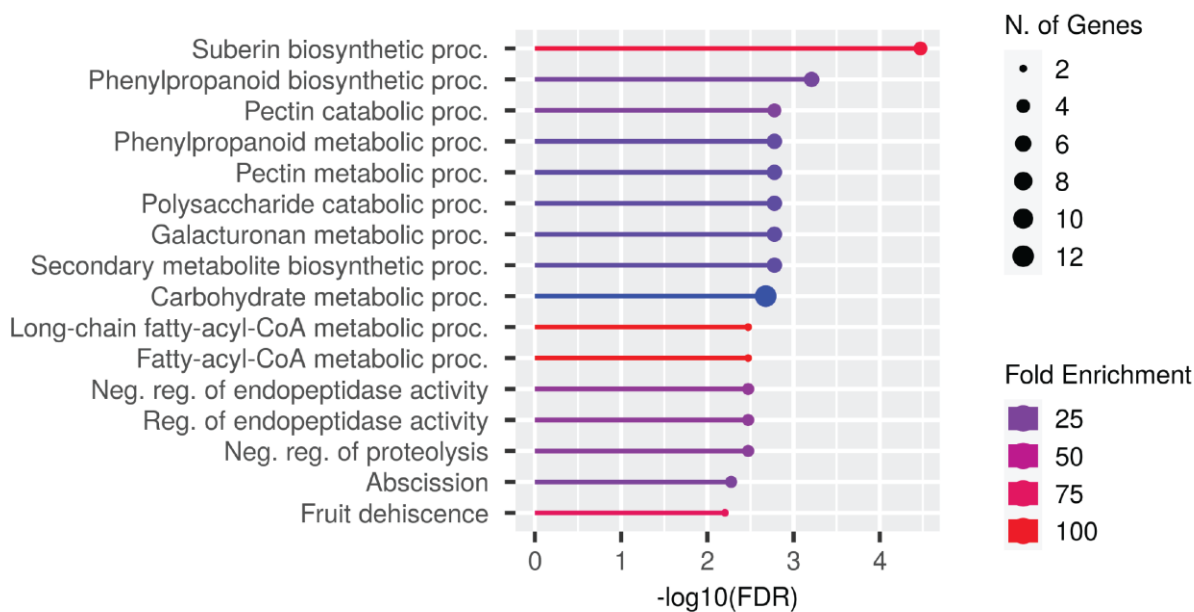


Supplemental Figure 3: Identification of additional cell types

- A) Plotting cell type markers identified from a prior single-cell study of Arabidopsis leaves.
- B) Tentative cell-type identification based on expression of known marker cell-type marker genes.
- C) Distribution of marker gene expression across putative cell types.
- D) Expression of putative silique marker gene in previously published bulk data (left panel), and expression of the same putative silique marker gene from our single-cell data (right panel).



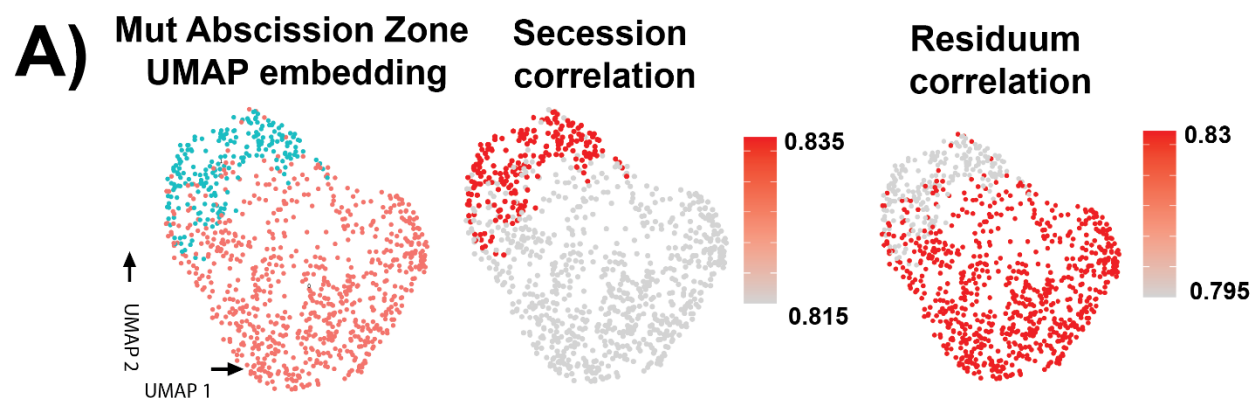
Supplemental Figure 4: GO term enrichment analysis of genes higher in *hae hsl2* compared to WT AZs.



Supplemental Figure 5: GO term enrichment analysis of genes lower in *hae hsl2* compared to WT from both bulk and single-cell RNA-Seq.

genotype	<i>+/+</i>	<i>+/fal-7</i>	<i>fal-7/fal-7</i>
suppression phenotype	0/8	0/34	18/18

Supplemental Figure 6: Association of phenotype and genotype in an F2 *hae hsl2* x *hae hsl2 fal-7* backcross population.

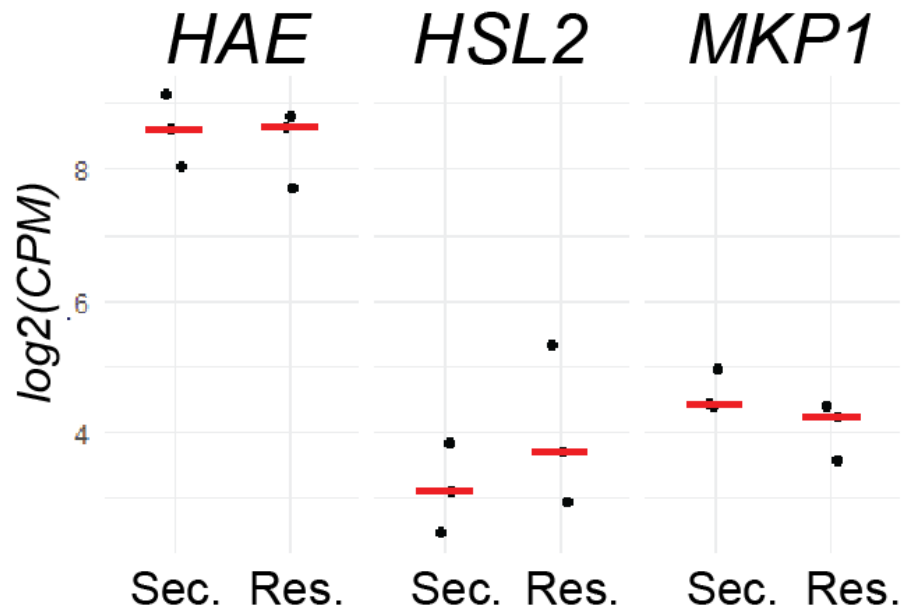


B)

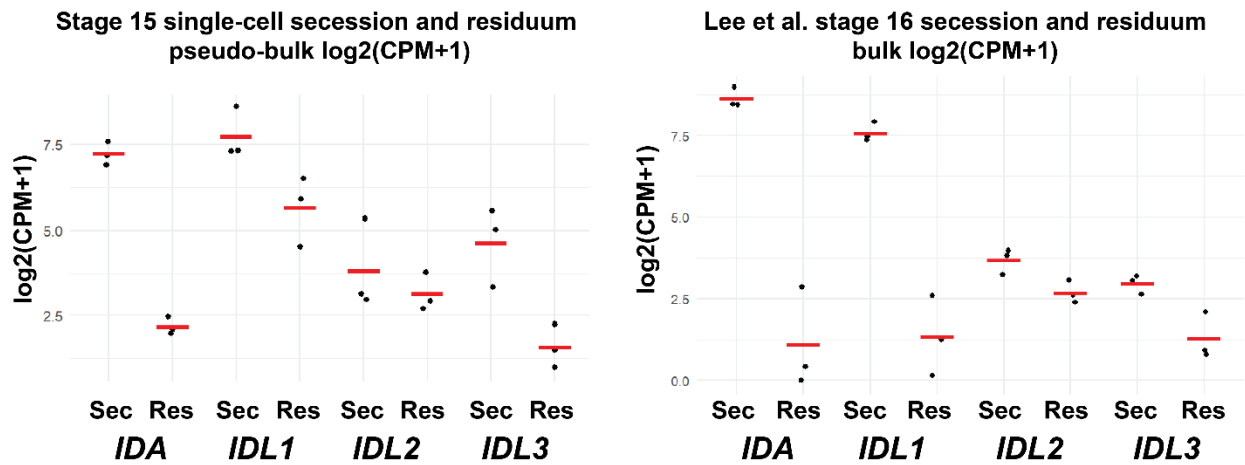
	Secession-associated genes	Residuum-associated genes	Total genes
WT	306	540	846
<i>hae hsl2</i>	178	196	374

Supplemental Figure 7: Sub-clustering and differential enrichment analysis of mutant AZ cells

- A) UMAP and coloration of low-resolution mutant AZ cell clusters (left), along with correlation to bulk secession and residuum RNA-Seq data (right)
- B) Table of differentially enriched genes in secession and residuum cells across both WT and mutant samples



Supplemental Figure 8: Pseudobulk $\log_2(\text{CPM})$ of *HAE*, *HSL2*, and *MKP1* in putative secession and residuum cells.



Supplemental Figure 9: Transcript abundance for *IDA* family genes in secession and residuum cells from stage 15 flower single-cell data (this paper) and reanalyzed data from Lee et al. stage 16 flower bulk data. IDA: At1g68765, IDL1: AT3G25655, IDL2: AT5G64667, IDL3: At5g09805

Supplemental Dataset Legends

Supplemental Dataset 1- Median UMIs, Median genes detected, and number of cells called per sample.

Supplemental Dataset 2- Average and replicate-specific transcripts per million (TPMs) for 3 replicate samples of FACS sorted *HAE-YFP* YFP+ cells.

Supplemental Dataset 3- Lee et al data. Average and replicate-specific counts per million (CPMs) for 3 replicate samples of FACS sorted *QRT2-YFP* YFP+ cells for both residuum and secession cells.

Supplemental Dataset 4- Lee et al data. pseudoTPM for the average *QRT2-YFP* YFP+ cells for both residuum and secession cells. All other data in this study is 3' sequencing and intrinsically TPM. We transformed the full-length transcript measurements of Lee et al to pseudo-TPM using the formula in Materials and Methods. This puts the data on a similar scale for purposes of calculating correlation, etc.

Supplemental Dataset 5- Output of Seurat FindMarkers function reporting differentially enriched genes from the WT putative abscission zone cluster ("cluster 11") compared to all other cells in the WT samples. "pct.1" represents the percentage of cells in cluster 11 where each gene was detected, and "pct.2" is the percentage of cells outside cluster 11 where each gene was detected.

Supplemental Dataset 6- All genes detected in at least one cluster in the single-cell data, used for Gene Ontology analysis.

Supplemental Dataset 7- edgeR analysis of pseudo-bulk data comparing WT and mutant samples of abscission zone cells. "WT_1", "WT_2", "mut_1", and "mut_2" refer to the unsorted cells isolated from receptacles and identified as putative abscission zone cells. The other samples are sorted HAE-YFP or HAE-K711E-YFP samples referred as "WT_#" and "mut_#" respectively.

Supplemental Dataset 8- Intersection of genes determined to be statistically significantly higher in WT compared to mutant in the pseudo-bulk single-cell analysis and prior receptacle bulk analysis.

Supplemental Dataset 9- TPMs of Columbia, *hae-3 hsl2-3*, *hae-3 hsl2-3 fal-3*, *hae-3 hsl2-3 fal-7* bulk RNA-Seq.

Supplemental Dataset 10- edgeR output of pseudo-bulk differential enrichment analysis comparing putative secession and residuum cells in WT.

Supplemental Dataset 11- edgeR output of pseudo-bulk differential enrichment analysis comparing putative secession and residuum cells in mutant.

Supplemental Dataset 12- edgeR output of receptacle protoplasting experiment. Receptacles were either dissected and directly frozen in N2 or subjected to the protoplasting procedure before harvesting all cells and tissue before RNA preparation.

Supplemental Dataset 13- Primers for cloning, genotyping, and sequencing.

Supplemental Dataset 14- Marker genes for known shoot-tissue cell types.

Supplemental Dataset 15- Gene annotations used in all gene lists.