

# 1 **Single-cell transcriptomics of the Arabidopsis floral abscission zone**

2 Isaiah W. Taylor, O. Rahul Patharkar, Che-Wei Hsu, John Baer, Chad E. Niederhuth, Uwe Ohler, Philip N.  
3 Benfey\*, John C. Walker\*

4 \*corresponding authors

5 Philip N. Benfey: [philip.benfey@duke.edu](mailto:philip.benfey@duke.edu)

6 John C. Walker: [walkerj@missouri.edu](mailto:walkerj@missouri.edu)

## 7 **Abstract**

8 Abscission is the programmed separation of plant organs. It is widespread in the plant kingdom with  
9 important functions in development and environmental response. In Arabidopsis, abscission of floral  
10 organs (sepals, petals, and stamen) is controlled by two related receptor-like protein kinases  
11 HAESA/HAE and HAESA LIKE-2/HSL2, which are thought to orchestrate the programmed dissolution of  
12 the pectin-rich middle lamella between cells in the abscission zone, as well as the remodeling of the cell  
13 wall that occurs during abscission. Here, we report transcriptional characterization of the abscission  
14 zones of WT and the abscission deficient *hae hsl2* double mutant at single-cell resolution. We identify  
15 core abscission gene expression programs, as well as perturbations of differentiation dynamics in *hae*  
16 *hsl2*, including detecting expression changes in distinct spatial domains of the abscission zone. We also  
17 report identification of a novel negative regulator of abscission signaling, *MAP KINASE PHOSPHATASE-*  
18 *1/MKP1*. Mutating *MKP1* partially suppresses the abscission defect of *hae hsl2*. We establish the  
19 molecular basis of the suppression as reactivation of the *HAE/HSL2* pathway. Our results provide deep  
20 insight into the biology of abscission and establish it as a model developmental signaling system for  
21 interrogation by single-cell technologies.

## 22 **Introduction**

23 Abscission is a ubiquitous process occurring at multiple stages of the plant life cycle. Shedding of leaves  
24 in the autumn and fruit after ripening are common examples, as is shedding of organs after damage or  
25 infection<sup>1-3</sup>. Historically, selection for grass varieties with defects in seed abscission (also known as  
26 shattering) dramatically increased the efficiency of harvest and is considered one of the critical steps in  
27 domestication<sup>4</sup>. Controlling abscission today is still of agricultural and horticultural relevance. A number  
28 of crops including citrus, cotton, brassica, and some rice varieties suffer yield loss due to abscission<sup>5-8</sup>.

29 The study of abscission dates back to at least the 19<sup>th</sup> century with detailed anatomical analyses<sup>1</sup>. Mid-  
30 20<sup>th</sup> century physiologists used leaf abscission as a model to begin to define molecular regulation of the  
31 process, establishing that interaction of the hormones ethylene and auxin regulate abscission in a  
32 number of species<sup>1</sup>. In the genomics era, abscission of tomato pedicels and Arabidopsis floral organs are  
33 among the best studied systems<sup>9,10</sup>. In particular, Arabidopsis floral abscission has been shown to be  
34 regulated by ethylene, auxin, and their interaction<sup>11-13</sup>, with additional contributions from jasmonic acid  
35 signaling<sup>14</sup>. In Arabidopsis, the site of floral abscission, known as the abscission zone (AZ), is lignified on  
36 the distal margin to create a well-defined separation plane<sup>15</sup>. During abscission, the middle lamella (the  
37 pectin-rich interstitial substance adhering adjacent cells) is degraded enzymatically before the organs  
38 detach<sup>16</sup>.

39 Abscission in Arabidopsis is regulated by two related leucine-rich repeat receptor-like protein kinases  
40 (LRR-RLKs) HAESA/HAE and HAESA LIKE-2/HSL2. The double mutant *hae hsl2* fails to shed its floral  
41 organs [Figure 1A]<sup>17-19</sup>. Several major components of the *HAE/HSL2* signaling pathway regulating  
42 abscission have been discovered [Figure 1B]. In particular, HAE and HSL2 cooperate with members of  
43 the SOMATIC EMBRYOGENESIS RECEPTOR KINASE/SERK family of co-receptor LRR-RLKs to bind the  
44 secreted INFLORESCENCE DEFICIENT IN ABSCISSION/IDA peptide, which activates a MAP kinase cascade  
45 comprised of MAP KINASE KINASE-4 and -5(MKK4/5) and MAP KINASE-3 and -6 (MPK3/6). One validated  
46 target of this MAPK cascade is the transcription factor AGL15, which is phosphorylated in a MAPK-  
47 dependent manner and negatively regulates *HAE* expression, forming a component of a positive  
48 feedback loop<sup>20</sup>. MAPK signaling also triggers expression of downstream abscission genes involved in  
49 pectin degradation, cell wall remodeling, and, ultimately, separation of the abscising organs<sup>18,21-25</sup>, in a  
50 process presumably regulated by activation of additional unidentified transcription factors. In addition,  
51 the spatial pattern of expression of the *IDA* gene has been shown to be controlled by ethylene, providing  
52 a molecular link between IDA-HAE/HSL2 signaling and hormonal regulation of floral abscission<sup>26</sup>.

53 Arabidopsis floral development has been described in stages<sup>27,28</sup>. By stage 15, pollination has occurred  
54 and the silique has elongated just beyond the petals [Figure 1C]. At stage 16, the silique continues to  
55 elongate while floral organs have withered and are nearing abscission. Stage 17 is a long post-abscission  
56 period during which the silique completes elongation. We have previously shown a *HAEpr::HAE-YFP*  
57 transgene can rescue the loss of abscission defect of a *hae hsl2* mutant, and that expression of *HAE*  
58 increases dramatically in the AZ at stage 15 [Fig. 1D]<sup>20,29</sup>. An experimental approach that has proven  
59 useful in identifying genes associated with abscission involves manually dissecting the base of the flower  
60 (called the receptacle) of stage 15 flowers of both WT and *hae hsl2* (at which point major morphological  
61 changes have not yet occurred) and performing transcriptomic comparisons [e.g. <sup>25,30</sup>]. These  
62 experiments indicate that by stage 15, *HAE/HSL2* signaling has been activated to regulate the expression  
63 of a core set of genes primarily involved in pectin degradation and cell wall remodeling.

64 A limitation of these analyses is that the vast majority of cells in the receptacle are not in the AZ, and  
65 only a limited number of genes are known *a priori* to be specific to the AZ<sup>11</sup>. Therefore, it is plausible  
66 that some of the observed gene expression changes are secondary effects of *HAE/HSL2* signaling,  
67 occurring elsewhere in non-AZ cells. In addition, there may be some genuinely differentially expressed  
68 genes in the AZ missed in bulk analyses due to dilution of signal from inclusion of non-AZ cells.  
69 Alternative isolation approaches such as laser capture microdissection of fixed tissue and bulk RNA-Seq  
70 of sorted AZ cells have been employed and are informative<sup>15,31</sup>. However, these techniques fail to  
71 capture global patterns of gene expression both in and outside of the AZ, can be labor intensive, and  
72 offer limited fine-grained expression information.

73 To address these shortcomings, we used single-cell RNA-Sequencing (scRNA-Seq) to compare WT and  
74 *hae hsl2*, performing replicated experiments on floral receptacle derived protoplasts. We supplemented  
75 floral receptacle data with replicated scRNA-Seq on FACS sorted cells expressing either functional HAE-  
76 YFP or kinase dead HAE-YFP K711E (mutated at the conserved catalytic lysine K711). The expression of  
77 both constructs is highly enriched in the AZ, but kinase-dead HAE-YFP K711E does not complement the  
78 mutant phenotype [Figure 1E]. This experimental system is summarized in Figure 1F. The resulting  
79 dataset allowed us to robustly identify AZ cells, which we validated through multiple approaches.  
80 Differential expression analysis between WT and *hae hsl2* identified a large number of genes involved in  
81 pectin degradation, cell wall remodeling, and extracellular barrier formation (cutin/lignin). We provide

82 evidence that single-cell analysis of stage 15 AZs captures differentiating cell populations reflecting the  
83 spatial organization of the AZ, identifying proximal AZ cells (those retained on the plant) and distal AZ  
84 cells (those at the base of the abscising organs). We further show that the *HAE/HSL2* pathway promotes  
85 this differentiation process. Finally, we identify a core set of validated *HAE/HSL2* regulated AZ genes  
86 identifiable by bulk RNA-Seq. We demonstrate the utility of this robust expression signature by  
87 characterizing newly identified suppressors of *hae hsl2* mutated in the *MAP KINASE PHOSPHATASE-*  
88 *1/MKP1* gene. Overall, this study establishes scRNA-Seq as an insightful approach to monitor the  
89 developmental signaling processes occurring during floral abscission.

## 90 **Results**

### 91 **Single-cell RNA-Sequencing identifies floral abscission zone cells**

92 We performed scRNA-Seq (2 replicates each) on stage 15 floral receptacles of WT and *hae hsl2*. We also  
93 profiled replicated samples of FACS sorted cells derived from *hae hsl2* expressing *HAEpr::HAE-YFP*  
94 (complementing) and kinase dead *HAEpr::HAE-K711E-YFP* (non-complementing) lines to enrich for both  
95 WT and mutant AZ cells (Materials and Methods). In total, we obtained data for 4 WT and 4 mutant  
96 replicate samples. After identifying putative multiplets and filtering low quality cells, we integrated all  
97 cells using Seurat<sup>32</sup>, excluding genes whose expression was altered in a separate receptacle protoplast  
98 bulk RNA-Seq experiment (Materials and Methods). We used Louvain clustering to identify  
99 transcriptionally similar groups of cells and embedded them in a 2-dimensional Uniform Manifold  
100 Approximation and Projection (UMAP) [Figure 2A, top panel]. Next, we plotted WT cells on the UMAP  
101 expressing known markers of the AZ: *HAE* and the pectin degrading enzyme-encoding genes  
102 *QUARTET2/QRT2* and *POLYGALACTURONASE ABSCISSION ZONE OF A. THALIANA/PGAZAT* [Figure 2A,  
103 bottom panel]. A compact group of cells (“cluster 11”) was apparent in the UMAP expressing all three AZ  
104 markers. These cells, numbering 869 out of a total of 16169 profiled WT cells (5.4%), grouped together  
105 across a wide range of Louvain clustering resolutions [Figure 2A, pink border, Supplemental Figure 1].  
106 Thus, cluster 11 appears to represent the AZ, which is transcriptionally identifiable and highly distinct  
107 from other cell types in the receptacle.

108 To verify the identity of cluster 11, we took several approaches. First, we sorted *HAE-YFP* expressing  
109 cells and generated and sequenced bulk RNA-Seq libraries (Materials and Methods, Supplemental Table  
110 1). We then calculated cluster-wise Spearman correlation between this dataset and “pseudo-bulk” data  
111 of each of the identified clusters from the single-cell experiment. Pseudo-bulking is a common analytical  
112 technique in single-cell analysis in which expression data for all cells of a particular type are summed  
113 into a single expression vector. Pseudo-bulk correlation analysis therefore provides a genome-wide  
114 measure of similarity between bulk data derived from sorted *HAE* positive cells and each cluster  
115 identified in the single-cell data. As expected, cluster 11 displayed the highest correlation, consistent  
116 with the hypothesis that it represents the AZ [Figure 2B, top panel]. Similarly, we took published bulk  
117 RNA-Seq data from sorted *QRT2*-expressing stage 16 AZ cells and performed an identical correlation  
118 analysis and found that, again, cluster 11 displayed the highest correlation [Figure 2B, bottom panel,  
119 Supplemental Tables 2 and 3]<sup>15</sup>. Next, we identified four cluster 11 enriched genes not previously known  
120 to be specific to the AZ and generated promoter::fluorescent reporter lines [Figure 2C]. All four  
121 reporters displayed highly specific expression in the AZ cells, indicating cluster 11 represents the AZ.

122 While not the focus of this study, we were also curious if we could identify other non-AZ cell types  
123 present in the receptacle. Indeed, many genes associated with specific cell types from Arabidopsis leaf  
124 scRNA-Seq data were present in restricted clusters in our data<sup>33</sup>, allowing us to make tentative  
125 assignments of epidermis, mesophyll, guard cells, etc [Supplemental Figure 2A-C]. This suggests that  
126 floral organs, as modified leaves, retain some of the gene expression patterns of their homologous  
127 organs. There were also clusters that did not express known leaf markers [Supplemental Figure 2B],  
128 likely representing less characterized, flower-specific cell types. For example, we identified a large group  
129 of cells expressing AT3G01420, an alpha dioxygenase encoding-gene previously shown to be highly  
130 enriched in bulk RNA-Seq of developing siliques<sup>34</sup>, suggesting that there are uncharacterized flower-  
131 specific cell types [Supplemental Figure 2D].

### 132 **The abscission zone expresses genes related to pectin degradation, cell wall remodeling, and** 133 **extracellular barrier formation**

134 To explore the biology of the putative AZ, we identified AZ enriched genes using Seurat (Materials and  
135 Methods, Supplemental Tables 4 and 5). Analysis of Gene Ontology (GO) terms found enrichment of  
136 genes associated with middle lamella degradation and cell wall remodeling (pectin catabolic process,  
137 polysaccharide catabolic process, galacturonan metabolic process, etc.) [Figure 2D]. Additionally, there  
138 were terms associated with extracellular barrier formation such as phenylpropanoid biosynthesis, the  
139 major pathway leading to lignin formation, which has been shown to play an important role in the  
140 delineation of the AZ in both Arabidopsis and rice<sup>15,35</sup>. Terms related to Fatty-acyl-CoA metabolism are  
141 associated with genes involved in cutin biosynthesis, a waxy substance deposited on the scar of newly  
142 differentiated epidermal cells after abscission has occurred<sup>15</sup>. Overall, these terms are highly consistent  
143 the AZ being a site of focused cell wall disassembly and barrier formation.

### 144 **The *hae hsl2* mutant exhibits a reduction in expression of genes associated with core abscission zone** 145 **signatures**

146 We next examined the scRNA-Seq data to identify gene expression differences between *hae hsl2* and  
147 WT. Plotting mutant cells by UMAP indicates a similar spectrum of cell types as WT [Figure 3A], with a  
148 similar proportion of putative abscission zone cells numbering 964 out of 24497 profiled mutant cells  
149 (3.9%). To identify differentially expressed genes (DEGs), we performed pseudo-bulk analysis comparing  
150 WT to *hae hsl2* using edgeR<sup>36</sup>. This analysis revealed 302 genes with lower expression and 274 with  
151 higher expression in the double mutant as compared to WT [Supplemental Table 6].

152 GO term enrichment of genes with reduced expression in *hae hsl2* AZ was consistent with known  
153 biology of abscission, including terms related to pectin degradation and cell wall remodeling [Figure 3B,  
154 Materials and Methods]. Terms associated with suberin deposition are likely to reflect biosynthesis of  
155 cutin, a related wax compound with similar biosynthesis, which has been shown to accumulate in  
156 Arabidopsis floral AZs<sup>15</sup>. For visualization, we plotted the expression levels of a number of known and  
157 novel *HAE/HSL2* regulated genes [Figure 3C]. Overall, these results are consistent with the role of  
158 *HAE/HSL2* as central regulators of genes required for breakdown and remodeling of the cell wall during  
159 abscission. Interestingly, GO term analysis of genes with higher expression in *hae hsl2* did not reveal  
160 such clearly biologically interpretable signals and was enriched in terms associated with defense and  
161 hypoxia responses [Supplemental Figure 3]. This suggests there may be novel molecular pathways  
162 repressed by *HAE/HSL2* that can be interrogated in future work.

## 163 **Single-cell RNA-Seq identifies spatial domains of the abscission zone**

164 A recent report provided a detailed examination of the spatial organization of the AZ, identifying a  
165 differentiated group of cells on the distal side at the base of the abscising organ (termed *secession cells*)  
166 and a distinct group on the proximal side of the abscising organ (termed *residuum cells*) [Figure 4A]<sup>15</sup>.  
167 Functionally, secession cells form a lignin “brace,” which is thought to both focus the enzymatic activity  
168 of secreted hydrolases to the middle lamella of the fracture plane at the site of abscission, while also  
169 creating a rigid frame which “chips off” once sufficient weakening of the fracture plane has occurred.  
170 Conversely, the residuum transdifferentiates into epidermal cells exhibiting a protective cuticle layer.

171 This process had previously been detailed in stage 16 flowers, as abscission is occurring<sup>15</sup>. We  
172 hypothesized we may be able to detect evidence of the differentiation process at stage 15 from our  
173 data. Interestingly, when performing low resolution Louvain clustering of only WT AZ cells and  
174 embedding in UMAP space, two groups of cells emerge [Figure 4B, left panel]. We tested the hypothesis  
175 that these cells represent secession and residuum cells by performing cluster-wise Spearman correlation  
176 analysis with previously published bulk RNA-Seq data derived from sorted secession cells and,  
177 separately, sorted residuum cells<sup>15</sup>. Consistent with our hypothesis, we observed a strong association of  
178 one of the two clusters of cells with secession bulk data [figure 4B, middle panel] and strong association  
179 of the second group of cells with residuum bulk data [Figure 4B, right panel]. In addition, we found  
180 enrichment of a number of genes in the putative secession side associated with lignin biosynthesis such  
181 as the tandemly duplicated peroxidase genes *AT4G37520/AT4G37530* [Supplemental Figure 4A]. On the  
182 putative residuum side, we found enrichment of cuticle forming genes such as *ECERIFERUM3/CER3* and  
183 *PERMEABLE CUTICLE1/PEC1* [Supplemental Figure 4B].

## 184 **HAE/HSL2 promote differentiation of residuum and secession cells**

185 We next hypothesized that the *HAE/HSL2* pathway promotes specification of cellular identity of both  
186 secession and residuum cells. To test this hypothesis, we performed low-resolution Louvain clustering,  
187 secession and residuum correlation analysis, and UMAP embedding of mutant cells [Figure 4C, left  
188 panel]. While UMAP is a non-linear dimensionality reduction technique that inexactly represents true  
189 underlying variation, it is interesting that the UMAP embedding of the mutant cells is much less  
190 separated than that of WT. While the two clusters do indeed exhibit complementary correlation with  
191 secession and residuum cells [Figure 4C, middle and right panels], the degree of difference was much  
192 less than in WT. Taken together, these results suggest that in *hae hsl2*, the secession and residuum cells  
193 are not as transcriptionally differentiated as in WT. To test this hypothesis, we performed an analysis  
194 with edgeR to measure enrichment of genes in one group of cells or the other (Materials and Methods).  
195 In WT, we found a total of 846 out of a total of 23974 expressed genes which differed between the two  
196 cell types (Figure 4D, Supplemental Table 7). Using the same criteria in the mutant, we found a  
197 significantly fewer number of only 374 out of 22391 expressed genes differing (Figure 4D, Supplemental  
198 Table 8, p-value < 2.2e-16, Fisher’s Exact Test). This is consistent with the hypothesis that the *HAE/HSL2*  
199 pathway promotes differentiation of both the secession and residuum sides of the AZ.

## 200 **IDA expression is highly enriched in secession cells**

201 Interestingly, while *HAE* is strongly expressed in both secession and residuum cells [Figure 4E], the *IDA*  
202 gene, encoding the secreted ligand of HAE, is the single most strongly enriched gene in secession cells  
203 [Figure 4F, Supplemental Table 7]. This pattern of *IDA* expression is consistent with both published

204 promoter::reporter and sorted bulk RNA-Seq experiments<sup>15</sup>. This suggests an elegant negative  
205 regulatory mechanism by which *IDA-HAE* signaling is activated and attenuated over the course of the  
206 abscission process. In this model, we propose soluble IDA peptide is initially secreted by secession cells  
207 and diffuses across the secession-residuum boundary to activate signaling in both cell types. However,  
208 once cell separation occurs, the source of the activating ligand (the abscising organ) is physically  
209 detached from the plant, leading to signal attenuation.

### 210 **GO term enrichment analysis of secession and residuum cells suggests differing biological function**

211 We were next interested to interrogate the differing biology of the putative secession and residuum  
212 cells. GO term enrichment analysis of secession cell-associated genes in wild type revealed modest  
213 enrichment of pathways related to phenylpropanoid biosynthesis [Figure 4G]. Since one of the main  
214 functions of the phenylpropanoid pathway is lignin formation, this is consistent with the idea that  
215 secession cells are in the early stages of forming a lignin brace. In contrast, genes associated with wild-  
216 type putative residuum cells are enriched in terms related to cellular activity such as protein production  
217 [Figure 4H]. This is consistent with classical observations that AZ cells are sites of high levels of protein  
218 synthesis<sup>37</sup>.

### 219 **Single-cell RNA-Sequencing and bulk RNA-Seq identify a partially overlapping set of DEGs between** 220 **wild type and *hae hsl2***

221 Overall, these results indicate we can recover relevant biological processes and identify and validate  
222 changes in gene expression at the cell-type level in a single analysis. However, a major limiting factor for  
223 single-cell RNA-Seq analysis for routine transcriptional profiling is its expense. Therefore, an optimal  
224 research strategy may be to combine single-cell analysis with bulk RNA-Seq, which is considerably  
225 cheaper, for higher throughput transcriptomics focusing analysis on genes known to change specifically  
226 in a cell type of interest. To explore this possibility, we identified genes expressed at lower levels in *hae*  
227 *hsl2* both by scRNA-Seq and by previously published bulk datasets [Supplemental Table 9]<sup>24,30</sup>. From this  
228 analysis we identified 67 genes [Supplemental Table 10], which were enriched in abscission-associated  
229 gene classes such as those related to pectin modification, fatty acid biosynthesis, and phenylpropanoid  
230 biosynthesis [Supplemental Figure 5]. This confirms bulk RNA-Seq is capable of detecting relevant  
231 changes in gene expression even in a small sub-population of cells such as the AZ in a sample of  
232 receptacle tissue.

### 233 ***hae hsl2* suppressor mutants partially restore the abscission zone gene expression signature**

234 To validate the utility of this core set of *HAE/HSL2* regulated genes, we conducted a series of  
235 experiments on two unpublished *hae hsl2* suppressor mutants isolated from a previously described *hae*  
236 *hsl2* T-DNA suppressor screen<sup>24</sup>. These mutants, which we named *fal-3* and *fal-7* for *facilitated*  
237 *abscission locus*, display weak reduction in adhesion of the floral organs in plants grown in standard  
238 conditions at 22° [Fig 5A, left panels]. However, suppression of the *hae hsl2* phenotype was significantly  
239 increased when plants were grown at 16° [Figure 5A, right panels]. This can be quantified by measuring  
240 the force required to remove petals of stage 16 flowers [Fig 5B]. At 23°, there is a small but significant  
241 reduction in the force required to remove the petals as compared to *hae hsl2*. At 16° there is an  
242 approximately further 40% reduction in breakstrength, which is likely an underestimate of the effect  
243 since at 23° the breakstrength remains somewhat constant after stage 16, but at 16°, by stage 17, the  
244 breakstrength is often unmeasurable because the floral organs have abscised.

245 Based on the phenotypes, we hypothesized that the gene expression changes in *hae hsl2* would be  
246 partially reversed in the *hae hsl2 fal-3* and *hae hsl2 fal-7* suppressors. To test this hypothesis, we  
247 performed stage 15 receptacle bulk RNA-seq of plants grown at 16° comparing WT, *hae hsl2*, *hae hsl2*  
248 *fal-3* and *hae hsl2 fal-7* and examined the expression of the 67 genes identified in the single-cell/bulk  
249 DEG analysis intersection [Supplemental Table 11]. Indeed, in *hae hsl2 fal-3* and *hae hsl2 fal-7*, the  
250 majority of these genes show an intermediate level of expression between WT and *hae hsl2* [Figure 5C].  
251 A simple composite way to measure changes in expression of groups of genes is Parametric Analysis of  
252 Gene Expression/PAGE, which averages expression of a set of genes on a log scale and uses the resulting  
253 approximate normality due to the Central Limit Theorem to perform simple statistical tests<sup>38</sup> (Materials  
254 and Methods). Consistent with the hypothesis that the suppressors have partial reversion of the  
255 abscission gene expression program, the average log<sub>2</sub>(FC) levels for both *hae hsl2 fal-3* and *hae hsl2 fal-*  
256 *7* are intermediate between WT and *hae hsl2* [Figure 5D]. These results confirm that pairing scRNA-Seq  
257 with bulk analysis can produce validated, efficient transcriptomic characterization useful for acquiring  
258 knowledge of molecular pathways.

### 259 **Mutation of *MAP KINASE PHOSPHATASE-1/MKP1* underlies the *hae hsl2 fal* suppression phenotype**

260 To identify the causative mutations in *fal-3* and *fal-7* we performed complementation crosses, which  
261 indicated that *fal-3* and *fal-7* are allelic, recessive mutations [Figure 5E]. To identify the underlying  
262 mutations, we performed TAIL-PCR and found *fal-7* harbors a T-DNA insertion in the first exon of *MAP*  
263 *KINASE PHOSPHATASE-1/MKP1*, a gene encoding a phosphatase known to negatively regulate MPK3 and  
264 MPK6 during biotic and abiotic stress signaling in a cool-temperature enhanced manner [Figure 5F]<sup>39,40</sup>.  
265 Because MPK3/6 are also involved in abscission signaling, and because the effect of *fal-3* and *fal-7* is  
266 cold-enhanced, *MKP1* became the leading candidate gene. In *fal-3*, we identified a SNP causing a  
267 premature stop codon in the first exon of *MKP1* leading to truncation of nearly half the protein [Figure  
268 5F]. In addition, segregation analysis of a back-cross population of *hae hsl2* x *hae hsl2 fal-7* indicated the  
269 *hae hsl2* suppression effect is linked to the insertion in *MKP1* [Supplementary Figure 6] confirming that  
270 the suppression effect of *fal-3* and *fal-7* is due to mutation of *MKP1*.

271 These results together indicate *MKP1* is a negative regulator of *HAE/HSL2* signaling, allowing us to revise  
272 our basic model of abscission signaling [Figure 5G]. In the new model, *MKP1* buffers low levels of  
273 aberrant signaling, which could otherwise induce abscission in the absence of activation of the  
274 *HAE/HSL2* pathway. Thus, *MKP1* represents an additional mechanism of regulation that tunes abscission  
275 signal strength.

### 276 **Discussion**

277 This work demonstrates that the abscission zone is transcriptionally distinct as it undergoes rapid  
278 differentiation. The gene expression program of the AZ, a consequence of its highly specialized  
279 physiology involving middle lamella degradation and cell wall remodeling, renders it especially suitable  
280 for single-cell studies due to the ease of cell type identification. Taking advantage of this fact, we have  
281 demonstrated the ability to delineate spatially adjacent cell subtypes, the residuum and secession cells  
282 of the AZ, each with distinct cellular activities. Our work indicates *HAE/HSL2* signaling promotes the  
283 differentiation between these distinct cell types. However, because *HAE* is expressed in both cell types,

284 specificity factors conferring residuum and secession identity are still unknown. Identification of these  
285 factors is a clear target for future research enabled by single-cell technologies.

286 More generally, we have identified a large number of AZ specific genes and molecular pathways,  
287 validating previous results from bulk studies and providing new insights into gene expression and spatial  
288 organization of the AZ, pinpointing downstream genes regulated by *HAE HSL2*. Our identification of a  
289 negative regulator of abscission, *MKP1*, demonstrates that once single-cell information has been  
290 generated and validated, it enables interpretable bulk RNA-Seq analysis as an easier and simpler method  
291 to characterize mutants. Testing the activity and regulation of *MKP1* during floral organ abscission, and  
292 understanding why the suppression effect of *fal-3* and *fal-7* is temperature sensitive, is an exciting  
293 direction for future research.

294 Presumably, the *HAE HSL2* pathway acts by modulating the activity of transcriptional regulators. Future  
295 work combining single-cell RNA-Seq with other techniques such as single-cell ATAC-seq and network  
296 analysis will help identify cis-regulatory motifs and AZ-expressed transcription factors regulating  
297 downstream gene expression programs.

298 It has been reported that there is substantial genetic variation underlying abscission across the plant  
299 kingdom based on differences in the morphology and bulk transcriptome of AZs. Nevertheless, a recent  
300 report showed that *Nicotiana* lines with silenced *HAE* and *IDA* orthologs exhibited reduced perianth  
301 abscission<sup>41</sup>. In addition, expression of both citrus and litchi orthologs of *IDA* can complement the  
302 abscission-deficient *ida* mutant phenotype in Arabidopsis<sup>42,43</sup>. These results suggests that knowledge of  
303 the *HAE/HSL2* pathway will inform regulation of abscission in other dicots. Additionally, we have  
304 recently demonstrated abscission of Arabidopsis cauline leaves upon water stress or pathogen infection  
305 is under similar genetic control as floral abscission<sup>44,45</sup>. We anticipate application of single-cell RNA-Seq  
306 to leaf AZ cells will yield comparably striking information.

307 Abscission is a key plant behavior with critical relevance to agriculture and the study of domestication.  
308 We have uncovered a wealth of biological knowledge about *HAE/HSL2* signaling and expect this work  
309 will propel further investigation to identify regulatory mechanisms controlling AZ function.

## 310 **Methods**

### 311 **Plant growth**

312 The *hae-3 hsl2-3* mutant was used in all experiments<sup>46</sup>. Wild type, *hae-3 hsl2-3*, *hae-3 hsl2-3*  
313 *HAEpr::HAE-YFP*, *hae-3 hsl2-3 HAEpr::HAE-YFP K711E*, *hae-3 hsl2-3 fal-3*, and *hae-3 hsl2-3 fal-7* are all in  
314 the Columbia background. The *fal* suppressor mutants also contain *erecta* and *glabrous* mutations in  
315 order to phenotypically differentiate contaminating wild-type seeds from mutants in the *hae hsl2*  
316 suppressor screen<sup>24</sup>. Plants were grown in a 16-hour light cycle at either 22° or 16°, except for the  
317 breakstrength experiments which were conducted at 23° and 16°. Plants were grown in peat  
318 moss/vermiculite potting soil and fertilized every 3 weeks with ½ strength Miracle-Gro (Scotts Miracle-  
319 Gro Company).

### 320 **Protoplasting and FACS**

321 For each protoplast sample, we isolated approximately 20 1 mm sections of stage 15 floral receptacles  
322 of plants approximately 2 weeks post-bolting, in a similar manner as our previous work<sup>24,30</sup>. For



323 protoplasting, we employed the isolation method of Evrard et al, developed for rice roots, with minor  
324 modifications<sup>47</sup>. In brief, we prepared digest solution as follows: per 30 mls we added 400 mM mannitol  
325 (2.2 g), 20 mM MES hydrate (117 mg), 20 mM KCl (600  $\mu$ L of 1 M KCl), after which the pH was adjusted  
326 to 5.7 with KOH. We would typically make 500 mls of this solution, filter sterilize, and store at 4° for  
327 several months. On the day of protoplasting, per 30 mls, we added 1.25% Cellulase R10 (375 mg), 1.25%  
328 Cellulase RS (375 mg), 0.3% Macerozyme R10 (90 mg), 0.12% Pectolyase-Y23 (36 mg) and heated 10 min  
329 in 60° water bath before cooling to room temperature (all digest enzymes purchased from Duchefa  
330 Biochemie except Pectolyase-Y23, which was purchased from MP Biomedicals). Finally we added 10 mM  
331 CaCl<sub>2</sub> (300  $\mu$ L of 1 M CaCl<sub>2</sub>), 0.1% BSA (30 mg), and 5.38  $\mu$ L of  $\beta$ -mercaptoethanol. We sliced the  
332 receptacles into quarters under a dissecting scope on 4% agarose plates with a fine micro-scalpel #715  
333 (Feather), used forceps to transfer into 5 mls of digest solution in small petri dishes, and vacuum  
334 infiltrated for 7 minutes at -25 inHg in a dome desiccator. We digested the tissue for 2.25 hours at 80  
335 RPM on a rotary platform shaker at 25°, using fine forceps and micro-scalpel to additionally slice the  
336 softened tissue at around 1.5 hours. We next filtered the cells twice through 40  $\mu$ m filters, spun for 5  
337 minutes at 500 x g in a swinging bucket rotor in 5 ml sorting tubes, and rinsed twice with wash buffer  
338 (digest buffer with no enzymes). Between washes we spun cells for 3 minutes at 500 x g. Finally, we  
339 resuspended cells in wash buffer. It should be noted that it is now recognized calcium in the  
340 resuspension buffer reduces the efficiency of reverse transcription<sup>48,49</sup>, so it is recommended that for  
341 follow-up studies, at least in the final resuspension step, calcium be omitted<sup>49</sup>. For receptacle single-cell  
342 samples, cells were counted on a hemacytometer C-chip (SKC, Inc.) and the concentration adjusted to  
343 1000 cells/ $\mu$ L (total yield ranged from approximately 50,000-100,000 cells). For sorted samples, cells  
344 were prepared the same manner and sorted for YFP+ on a BD Diva cell-sorter into either excess wash  
345 buffer (for single-cell samples) or directly into RNA-Later for bulk samples. Single-cell sorted samples  
346 were then spun down and resuspended in 15  $\mu$ L wash buffer. We took 2.5  $\mu$ L of cells for dilution and  
347 counting on a hemacytometer C-chip. Final yield for sorted samples was between 400-3000 cells, all of  
348 which were run on a Chromium chip.

#### 349 **scRNA-Seq library generation**

350 We used the 10x Genomics v3 3' Single-cell RNA-Seq kit for all samples. For the receptacle WT and *hae*  
351 *hsl2* samples we followed the manufacturer's protocol, loading 16,000 cells per sample for a target  
352 capture of 10,000 single cells. For sorted samples, we divided a single reaction into four 25  $\mu$ L reactions  
353 and ran on four lanes of a single chip. For sorted sample library preparation, we reduced the volume of  
354 all reagents only by 1/2, which was enabled by excess reagents accumulated due to prior emulsion failures  
355 (although we expect miniaturization to 1/4-scale library preparations would be feasible). Raw reads have  
356 been deposited at the Sequence Read Archive under BioProject PRJNA857332.

#### 357 **scRNA-Seq library sequencing and preprocessing**

358 Unless otherwise noted, all analyses were performed in R 3.6.3 and are included, along with the output  
359 of sessionInfo(), as Jupyter Notebooks in the Supplemental Code. We used our previously published  
360 scKB procedure to align and produce count matrices for downstream analysis<sup>50</sup>. This pipeline uses  
361 kallisto, bustools, busparse, and BSgenome<sup>51-54</sup> to align and quantify counts to the Arabidopsis TAIR10  
362 genome. The following analyses are recorded in Notebook 1: For samples WT and *hae hsl2* receptacle  
363 samples #1 we had performed species-mixed experiments containing rice and Arabidopsis cells, so we  
364 aligned to a concatenated rice-Arabidopsis MSU7/TAIR10 genome using a combined gff file and retained

365 only reads mapping to Arabidopsis<sup>50,52</sup>. We pooled reads mapping to spliced and unspliced transcripts in  
366 order to make a single matrix of gene expression values. We next ran EmptyDrops<sup>56</sup> in order to identify  
367 putative empty droplets containing no cells with “ignore” parameter = 500 and “lower” parameter =  
368 300. We then constructed Seurat objects with the expression matrices. We finally used doubletFinder<sup>57</sup>  
369 to identify putative doublets using the approximate doublet rate employed by 10X as .004/500 \* #  
370 loaded cells. The resulting Seurat objects were used for downstream analysis. Our estimated number of  
371 recovered cells after all these steps are: WT receptacle #1: 7509 cells, WT receptacle #2: 7571 cells, WT  
372 sorted #1: 878 cells, WT sorted #2: 211 cells, *hae hsl2* receptacle #1: 14492 cells, *hae hsl2* receptacle #2:  
373 7578 cells, *hae hsl2* sorted #1: 1302 cells, *hae hsl2* sorted #2: 1125 cells.

#### 374 **Exploratory analysis of scRNA-Seq data and identification of the AZ**

375 Sample integration (Notebook 2) was performed by running SCTransform on each sample before  
376 integrating, excluding mitochondrial genes, plastid genes, and genes altered by protoplasting (defined as  
377 those with absolute value of  $\log_2(\text{FC}) > 1$ ) [Supplemental Table 12]. We performed PCA, constructed a  
378 shared nearest neighbor graph, and identified clusters using the SLM algorithm. Visualization was  
379 performed by UMAP embedding.

380 Identification of the AZ (Notebook 3) was performed by first plotting *HAE*, *QRT2*, and *PGAZAT* in WT cells  
381 of the UMAP embedding with the “min” parameter set to .5. We next calculated the pseudobulk  
382 expression profile for all clusters identified at resolution .75 by summing all counts for each gene. We  
383 next calculated Spearman correlation with bulk sorted *HAE+* and *QRT2+* RNA-Seq data. The *HAE* data  
384 (Supplemental Table 1) was Lexogen Quant-seq 3' and consequently is directly convertible to Transcripts  
385 per Million (TPM) making it comparable to 3' RNA-Seq data generated by Chromium 10X. Because the  
386 *QRT2* data (Supplemental Table 2) include separate secession and residuum derived cells, we first  
387 created an approximate composite AZ transcriptome by summing the counts per million for both  
388 secession and residuum datasets with equal weighting. This dataset was full-transcript RNA-Seq, so we  
389 estimated TPM using the formula below (Supplemental Table 3):

$$TPM_{geneX} = \frac{\frac{count_{geneX}}{length_{geneX}}}{\sum \left( \frac{count_{each\ gene}}{length_{each\ gene}} \right)} * 1,000,000$$

390 We then plotted the correlation value for each cluster on the previously generated UMAP embedding.

#### 391 **Promoter cloning**

392 Fragments ranging in size from 1 to 2.5 kilobases upstream of *MYB62*, *PECTIN LYASE-LIKE*, and *GDSL-*  
393 *TYPE ESTERASE* were PCR amplified with PfuUltrall polymerase and cloned into pENTR (ThermoFisher),  
394 then recombined into pMDC111<sup>58</sup> to create promoter::GFP fusions (primers are listed in Supplemental  
395 Table 13). The *FAR5* upstream region was PCR amplified by KAPA Hifi polymerase (Roche) and cloned  
396 into an *Oryza sativa* H2B-VENUS fusion construct created by gene synthesis (Twist Bioscience). The *H2B-*  
397 *VENUS* construct was first cloned into pENTR and recombined into pGWB501<sup>59</sup>. This construct was  
398 created to include two AarI sites for Golden Gate cloning of promoter fragments in one step. Primers are  
399 included in Supplemental Table 13. Siliques were imaged on a Zeiss Axiozoom stereomicroscope with UV  
400 illumination.

## 401 **Non-Abscission zone Cell Annotation**

402 Clusters were defined by twice running the Seurat FindClusters function with both a low modularity  
403 parameter ( $res = 2$ ) and a high modularity parameter ( $res = 200$ ), which results in clusters containing  
404 hundreds/thousands of cells (broadly-resolved) and those having only tens of cells each (finely-  
405 resolved), respectively. We then calculated expression z-scores of known marker genes [Supplemental  
406 Table 14] in each cluster (both broadly and finely-resolved). These clusters were then annotated by  
407 comparing the average marker gene z-scores. Cells that were annotated with the same cell identity in  
408 broadly-resolved and finely-resolved clusters were considered confidently annotated. While those that  
409 were not were labeled as “Unknown.” This annotation approach combining results of complementary  
410 modularity resolution was particularly useful for annotating rare cell-types while maintaining low noise  
411 levels (Notebook 4).

## 412 **AZ pseudo-bulk DEG analysis comparing WT and *hae hsl2***

413 We performed pseudo-bulking for the AZ (Seurat cluster 11) for each WT and *hae hsl2* sample, yielding  
414 four samples for each genotype (Notebook 5). We then used edgeR to normalize and calculate CPM and  
415 LCPM matrices. We modeled whether the cells were sorted as a nuisance factor (to account for variation  
416 due to FACS), and otherwise only included genotype as a factor (WT or mutant). We then constructed a  
417 contrast testing the hypothesis that the genotype factor for the difference in expression between WT  
418 and *hae hsl2* for each gene was equal to 0. The output of edgeR has been included as Supplemental  
419 Table 6. A file with our edgeR functions has been included in Supplemental Code. This code requires an  
420 Arabidopsis annotation file derived from TAIR10 included as Supplemental Table 15.

## 421 **GO term analysis**

422 For GO analysis we used shinyGO v 0.76<sup>60</sup>. For all analyses we displayed Biological Process terms at FDR  
423  $< .01$ . For GO analysis of WT AZs we used the output of the Seurat FindAllMarkers function to select  
424 genes  $\log_2(\text{Fold Enrichment}) > 1$  with  $\text{FDR} < .05$  in the AZ. We used all genes from the FindAllMarkers  
425 analysis as a universe (IE, genes expressed in at least one cluster). For the WT-mutant DE GO analysis,  
426 we took genes defined as DE  $\log_2(\text{FC}) > 1$  with  $\text{FDR} < .05$  with all genes expressed in at least 3 samples as  
427 universe. GO analysis of the bulk DE/single-cell DE intersection was performed with a universe as the  
428 genes defined to be expressed in the WT/*hae hsl2* pseudo-bulk DEG analysis.

## 429 **AZ subclustering**

430 AZ cells (those from cluster 11) were sub-divided into those of WT and *hae hsl2* origin, and each dataset  
431 was reintegrated using a similar process as above (Notebook 6). We then performed coarse clustering  
432 with  $resolution = .1$  in Seurat which identified two transcriptionally distinct groups for both genotypes.  
433 We performed pseudo-bulk Spearman correlation analysis comparing expression of each of the two  
434 clusters in each genotype to previously published FACS secession and residuum datasets<sup>15</sup>. For  
435 enrichment analysis we took the pseudo-bulked putative residuum cells and secession cells and  
436 performed edgeR analysis as above, except we pooled the cells derived from the two FACS samples for  
437 both mutant and WT due to a particularly low number of cells in WT sorted sample #1. The secession  
438 and residuum associated genes are those with  $\text{FDR} < .05$  and  $\log_2(\text{fold enrichment}) > 1$  in the respective  
439 cell types. We will note because the cells were clustered first before running the edgeR analysis, the  
440 resulting gene lists were not unbiased estimates of differential expression. However, because we

441 performed identical procedures for WT and mutant and our Fisher's exact test is testing the hypothesis  
442 that the proportion of genes in this "enriched" set is different between the two genotypes.

#### 443 **Bulk RNA-Seq library generation and sequencing**

444 For the sorted *HAE-YFP* bulk samples, we sorted into 20  $\mu$ l of RNA-Later then used the magnetic bead  
445 based Direct-zol-96 MagBead RNA-Isolation kit (Zymo) to isolate RNA. For the protoplast test, we cut  
446 three receptacles per replicate and digested as above. We harvested the tissue by spinning at 500 x g  
447 and removing supernatant, leaving digested cells and undigested tissue in place, before freezing in liquid  
448 N<sub>2</sub>. We simultaneously collected three receptacles per replicate in our unprotoplasted control where  
449 tissue was placed in 10  $\mu$ l of RNA-Later in the cap of a 1.7 ml microcentrifuge tube, tapped to the  
450 bottom of the tube, immediately frozen in liquid N<sub>2</sub>, homogenized with a blue pestle, and performed  
451 RNA isolation as above. We performed three replicates in each condition.

452 For the *fal-3/fal-7* bulk RNA-Seq experiment, we isolated receptacles from three stage 15 flowers for  
453 each replicate of *er gl* (WT grandparent), *er gl hae-3 hsl2-3* (mutant parent), and *er gl hae-3 hsl2-3 fal-*  
454 *3/fal-7* (suppressors). Tissue was placed into the cap of a 1.7 ml tube containing 10  $\mu$ l RNA-Later. After  
455 tapping the tissue to the bottom of the tube, we froze in liquid nitrogen. We then ground the tissue in  
456 liquid N<sub>2</sub> using blue pestles, and used the Zymo RNA-isolation kit as before. For all samples, RNA  
457 integrity was checked with Bioanalyzer RNA Nano kit and quantified by Qubit.

458 For library generation, we used Lexogen Quantseq RNA-Seq using the manufacturer's protocol, with  
459 instructions for "Low input" for the sorted samples due to input of only 5-10 ng total RNA input per  
460 sample. We used the Unique Molecular Identifier (UMI) PCR add-on kit (Lexogen). Libraries were  
461 indexed and sequenced on an Illumina NextSeq, High Output setting. Reads were aligned to the TAIR10  
462 genome using the STAR aligner, deduplicated using UMI-Tools, and counted with HTSeq-Count. Counts  
463 were analyzed with edgeR. We defined "expressed genes" to be those with observed reads in three or  
464 more libraries. Raw reads have been deposited at the Sequence Read Archive under BioProject  
465 PRJNA857332.

#### 466 **PAGE analysis of abscission-associated gene expression in *fal* mutants**

467 We constructed an expression matrix for the 67 bulk/single-cell intersection gene set for WT, *hae hsl2*,  
468 *hae hsl2 fal-3*, and *hae hsl2 fal-3* using the log<sub>2</sub>(CPM) values generated by edgeR (Notebooks 7 and 8).  
469 We then summed the log<sub>2</sub>(CPM) values of each gene for each sample and normalized to the average  
470 summed values of the *hae hsl2* samples before dividing by the number of genes in the analysis. The  
471 resulting quantity represents the average log<sub>2</sub>(FC) for each sample compared to the *hae hsl2* average.  
472 Last, we performed pairwise T-tests with Bonferroni Correction assuming equal variance.

#### 473 **Breakstrength measurements**

474 The breakstrength of petals of stage 16 flowers (ie, silique 1-2 mm above petals) in *er gl hae-3 hsl2-3*  
475 and *er gl hae-3 hsl2-3 fal-3/7* were measured using our previously described petal break strength meter  
476 and analysis script<sup>61</sup>. In brief, the petals were clamped to the meter and the flower pulled down with  
477 forceps until the petal detached. The maximum voltage was extracted from the output file of the meter.  
478 This voltage reading was converted to an equivalent force after calculation of a standard curve based on  
479 voltage readings of the meter attached to a varying number of objects of known weight (ie, paper clips).

480 For measurements taken at each temperature, we performed pairwise T-tests with Bonferroni  
481 Correction assuming equal variance.

#### 482 ***fal* mutant identification**

483 The *hae-3 hsl2-3* suppressor screen was performed as previously described<sup>24</sup>. For identification, we used  
484 TAIL-PCR<sup>62</sup> to amplify a PCR fragment in *fal-7* which was analyzed by Sanger sequencing. For *fal-3*, we  
485 used TAIL-PCR to identify an insertion in an exon of AT2G07690, which is a member of the  
486 Minichromosome Maintenance gene family involved in initiation of DNA replication. However, given the  
487 similar phenotype of *fal-7* we hypothesized there may exist an additional mutation in *MKP1*. We  
488 designed Sanger sequencing primers and tiled the coding sequence, detecting a G-> A SNP at bp 1357 in  
489 the *MKP1* coding sequence using primers listed in Supplemental Table 13. We performed linkage  
490 analysis in the *hae hsl2 x hae hsl2 fal-7* F2 population using genotyping primers listed in Supplemental  
491 Table 13.

#### 492 **Conflict of Interest**

493 The authors declare they have no conflict of interest.

#### 494 **Funding**

495 This work was supported by NSF MCB0743955 to JCW, NIH 1R35GM131725 to PNB, who is also  
496 supported by the Howard Hughes Medical Institute as an investigator, USDA 2021-67034-35139 to IWT,  
497 and Deutsche Forschungsgemeinschaft (DFG) 2403 to UO and CWH.

498 1. Addicott, F. T. & Lynch, R. S. Physiology of abscission. *Annual Review of Plant Physiology* **6**, 211–238  
499 (1955).

500 2. Taylor, J. E. & Whitelaw, C. A. Signals in abscission. *New Phytologist* **151**, 323–340 (2001).

501 3. Patharkar, O. R. & Walker, J. C. Connections between abscission, dehiscence, pathogen defense,  
502 drought tolerance, and senescence. *Plant Science* **284**, 25–29 (2019).

503 4. Yu, Y. & Kellogg, E. A. Inflorescence abscission zones in grasses: diversity and genetic regulation.  
504 *Annual Plant Reviews* **1**, 1–35 (2018).

505 5. Dutta, S. K., Gurung, G., Yadav, A., Laha, R. & Mishra, V. K. Factors associated with citrus fruit  
506 abscission and management strategies developed so far: A review. *New Zealand Journal of Crop and*  
507 *Horticultural Science* 1–22 (2022).

508 6. Kaur, R., Deol, J. & Dass, A. Physiology of abscission and crop regulation in cotton-a review. (2016).

- 509 7. Hossain, S., Kadkol, G., Raman, R., Salisbury, P. & Raman, H. Breeding Brassica napus for shatter  
510 resistance. *In Plant Breeding* (2011).
- 511 8. Li, L.-F. & Olsen, K. To have and to hold: selection for seed and fruit retention during crop  
512 domestication. *Current topics in developmental biology* **119**, 63–109 (2016).
- 513 9. Tranbarger, T. J. & Tadeo, F. R. Diversity and functional dynamics of fleshy fruit abscission zones.  
514 *Annual Plant Reviews online* 1–64 (2018).
- 515 10. Patharkar, O. R. & Walker, J. C. Advances in abscission signaling. *J Exp Bot* **69**, 733–740 (2018).
- 516 11. Meir, S. *et al.* Re-evaluation of the ethylene-dependent and-independent pathways in the  
517 regulation of floral and organ abscission. *Journal of experimental botany* **70**, 1461–1467 (2019).
- 518 12. Ellis, C. M. *et al.* AUXIN RESPONSE FACTOR1 and AUXIN RESPONSE FACTOR2 regulate  
519 senescence and floral organ abscission in Arabidopsis thaliana. *Development* **132**, 4563–4574 (2005).
- 520 13. Basu, M. M. *et al.* The manipulation of auxin in the abscission zone cells of Arabidopsis flowers  
521 reveals that indoleacetic acid signaling is a prerequisite for organ shedding. *Plant physiology* **162**, 96–  
522 106 (2013).
- 523 14. Kim, J. *et al.* New clothes for the jasmonic acid receptor COI1: delayed abscission, meristem  
524 arrest and apical dominance. *PloS one* **8**, e60505 (2013).
- 525 15. Lee, Y. *et al.* A lignin molecular brace controls precision processing of cell walls critical for  
526 surface integrity in Arabidopsis. *Cell* **173**, 1468–1480 (2018).
- 527 16. Patterson, S. E. Cutting Loose. Abscission and Dehiscence in Arabidopsis. *Plant Physiology* **126**,  
528 494–500 (2001).
- 529 17. Jinn, T. L., Stone, J. M. & Walker, J. C. HAESA, an Arabidopsis leucine-rich repeat receptor kinase,  
530 controls floral organ abscission. *Genes Dev.* **14**, 108–117 (2000).
- 531 18. Cho, S. K. *et al.* Regulation of floral organ abscission in Arabidopsis thaliana. *Proc. Natl. Acad. Sci.*  
532 *U.S.A.* **105**, 15629–15634 (2008).

- 533 19. Stenvik, G.-E. *et al.* The EPIP peptide of INFLORESCENCE DEFICIENT IN ABSCISSION is sufficient to  
534 induce abscission in arabidopsis through the receptor-like kinases HAESA and HAESA-LIKE2. *Plant Cell*  
535 **20**, 1805–1817 (2008).
- 536 20. Patharkar, O. R. & Walker, J. C. Floral organ abscission is regulated by a positive feedback loop.  
537 *Proceedings of the National Academy of Sciences* 201423595 (2015).
- 538 21. Butenko, M. A. *et al.* INFLORESCENCE DEFICIENT IN ABSCISSION controls floral organ abscission  
539 in Arabidopsis and identifies a novel family of putative ligands in plants. *The Plant Cell Online* **15**,  
540 2296–2307 (2003).
- 541 22. Santiago, J. *et al.* Mechanistic insight into a peptide hormone signaling complex mediating floral  
542 organ abscission. *Elife* **5**, e15075 (2016).
- 543 23. Meng, X. *et al.* Ligand-induced receptor-like kinase complex regulates floral organ abscission in  
544 Arabidopsis. *Cell reports* **14**, 1330–1338 (2016).
- 545 24. Taylor, I., Baer, J., Calcutt, R. & Walker, J. C. Hypermorphic SERK1 mutations function via a  
546 SOBIR1 pathway to activate floral abscission signaling. *Plant physiology* **180**, 1219–1229 (2019).
- 547 25. Niederhuth, C. E., Patharkar, O. R. & Walker, J. C. Transcriptional profiling of the Arabidopsis  
548 abscission mutant *hae hsl2* by RNA-Seq. *BMC Genomics* **14**, 37 (2013).
- 549 26. Butenko, M. A. *et al.* Ethylene-dependent and -independent pathways controlling floral  
550 abscission are revealed to converge using promoter::reporter gene constructs in the *ida* abscission  
551 mutant. *J. Exp. Bot.* **57**, 3627–3637 (2006).
- 552 27. Müller, A. Zur Charakterisierung der Blüten und Infloreszenzen von *Arabidopsis thaliana* (L.)  
553 Heynh. *Die Kulturpflanze* **9**, 364–393 (1961).
- 554 28. Smyth, D. R., Bowman, J. L. & Meyerowitz, E. M. Early flower development in Arabidopsis. *The*  
555 *Plant Cell* **2**, 755–767 (1990).

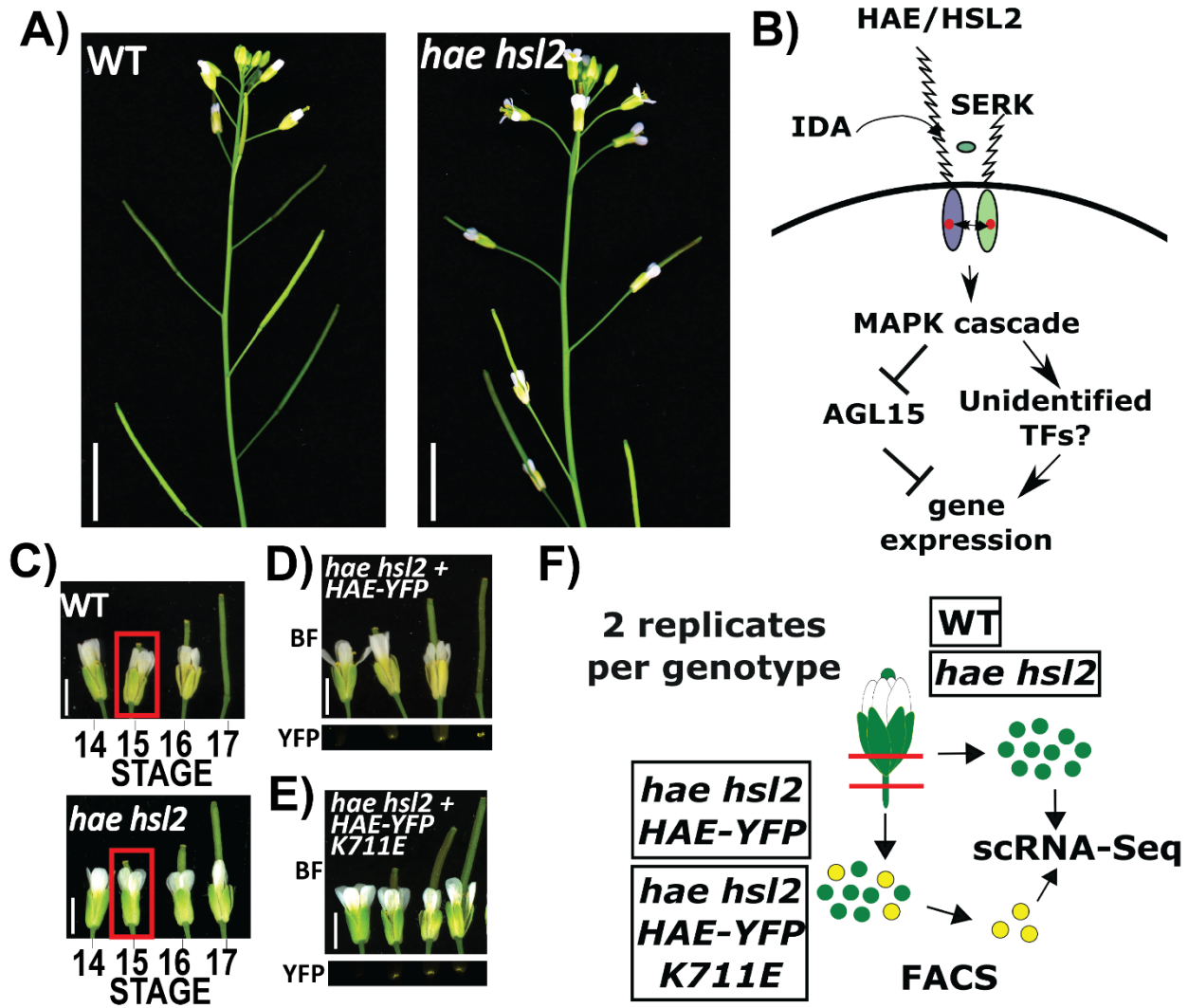
- 556 29. Taylor, I. *et al.* Analysis of Phosphorylation of the Receptor-Like Protein Kinase HAESA during  
557 Arabidopsis Floral Abscission. *PLoS ONE* **11**, e0147203 (2016).
- 558 30. Taylor, I. & Walker, J. C. Transcriptomic evidence for distinct mechanisms underlying abscission  
559 deficiency in the Arabidopsis mutants haesa/haesa-like 2 and nevershed. *BMC Research Notes* **11**,  
560 754 (2018).
- 561 31. Cai, S. & Lashbrook, C. C. Stamen abscission zone transcriptome profiling reveals new candidates  
562 for abscission control: enhanced retention of floral organs in transgenic plants overexpressing  
563 Arabidopsis ZINC FINGER PROTEIN2. *Plant physiology* **146**, 1305–1321 (2008).
- 564 32. Stuart, T. *et al.* Comprehensive integration of single-cell data. *Cell* **177**, 1888–1902 (2019).
- 565 33. Zhang, T.-Q., Chen, Y. & Wang, J.-W. A single-cell analysis of the Arabidopsis vegetative shoot  
566 apex. *Developmental Cell* **56**, 1056–1074 (2021).
- 567 34. Klepikova, A. V., Kasianov, A. S., Gerasimov, E. S., Logacheva, M. D. & Penin, A. A. A high  
568 resolution map of the Arabidopsis thaliana developmental transcriptome based on RNA-seq profiling.  
569 *The Plant Journal* **88**, 1058–1070 (2016).
- 570 35. Zhou, Y. *et al.* Genetic control of seed shattering in rice by the APETALA2 transcription factor  
571 SHATTERING ABORTION1. *The Plant Cell* **24**, 1034–1048 (2012).
- 572 36. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential  
573 expression analysis of digital gene expression data. *Bioinformatics* **26**, 139–140 (2010).
- 574 37. Sexton, R. & Roberts, J. A. Cell biology of abscission. *Annual Review of Plant Physiology* **33**, 133–  
575 162 (1982).
- 576 38. Kim, S.-Y. & Volsky, D. J. PAGE: Parametric Analysis of Gene Set Enrichment. *BMC Bioinformatics*  
577 **6**, 144 (2005).
- 578 39. Ulm, R. *et al.* Distinct regulation of salinity and genotoxic stress responses by Arabidopsis MAP  
579 kinase phosphatase 1. *The EMBO Journal* **21**, 6483–6493 (2002).



- 580 40. Bartels, S. *et al.* MAP kinase phosphatase1 and protein tyrosine phosphatase1 are repressors of  
581 salicylic acid synthesis and SNC1-mediated responses in Arabidopsis. *The Plant Cell* **21**, 2884–2897  
582 (2009).
- 583 41. Ventimilla, D. *et al.* IDA (INFLORESCENCE DEFICIENT IN ABSCISSION)-like peptides and HAE  
584 (HAESA)-like receptors regulate corolla abscission in *Nicotiana benthamiana* flowers. *BMC plant*  
585 *biology* **21**, 1–14 (2021).
- 586 42. Estornell, L. H. *et al.* The IDA peptide controls abscission in Arabidopsis and Citrus. *Frontiers in*  
587 *plant science* **6**, 1003 (2015).
- 588 43. Ying, P. *et al.* Identification and molecular characterization of an IDA-like gene from litchi,  
589 LcIDL1, whose ectopic expression promotes floral organ abscission in Arabidopsis. *Scientific Reports*  
590 **6**, 1–11 (2016).
- 591 44. Patharkar, O. R. & Walker, J. C. Core Mechanisms Regulating Developmentally Timed and  
592 Environmentally Triggered Abscission. *Plant Physiol.* **172**, 510–520 (2016).
- 593 45. Patharkar, O. R., Gassmann, W. & Walker, J. C. Leaf shedding as an anti-bacterial defense in  
594 Arabidopsis cauline leaves. *PLoS genetics* **13**, e1007132 (2017).
- 595 46. Niederhuth, C. E., Cho, S. K., Seitz, K. & Walker, J. C. Letting Go is Never Easy: Abscission and  
596 Receptor-Like Protein Kinases. *Journal of integrative plant biology* **55**, 1251–1263 (2013).
- 597 47. Evrard, A. *et al.* Fluorescence-activated cell sorting for analysis of cell type-specific responses to  
598 salinity stress in Arabidopsis and rice. in *Plant Salt Tolerance* 265–276 (Springer, 2012).
- 599 48. Tan, C. K. *et al.* Functional characterization of RNA-dependent DNA polymerase and RNase H  
600 activities of a recombinant HIV reverse transcriptase. *Biochemistry* **30**, 2651–2655 (1991).
- 601 49. Denyer, T. & Timmermans, M. C. Crafting a blueprint for single-cell RNA sequencing. *Trends in*  
602 *Plant Science* (2021).

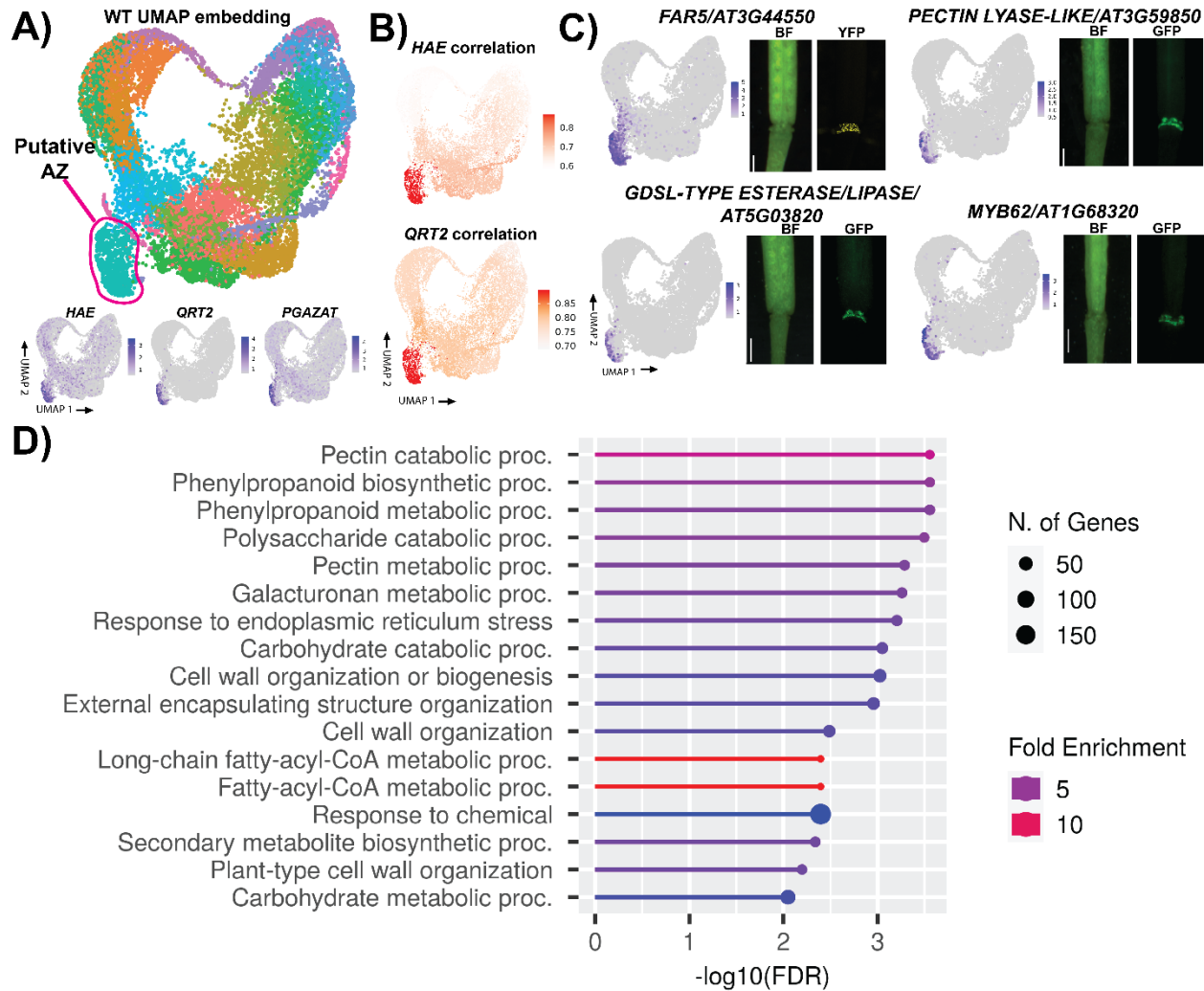
- 603 50. Shahan, R. *et al.* A single-cell Arabidopsis root atlas reveals developmental trajectories in wild-  
604 type and cell identity mutants. *Developmental cell* **57**, 543–560 (2022).
- 605 51. Bray, N. L., Pimentel, H., Melsted, P. & Pachter, L. Near-optimal probabilistic RNA-seq  
606 quantification. *Nature biotechnology* **34**, 525–527 (2016).
- 607 52. Melsted, P., Ntranos, V. & Pachter, L. The barcode, UMI, set format and BUSTools. *Bioinformatics*  
608 **35**, 4472–4473 (2019).
- 609 53. Moses, L. & Pachter, L. BUSpaRse: kallisto| bustools R utilities. *R package version 1*, (2019).
- 610 54. Pagès, H. BSgenome: Software infrastructure for efficient representation of full genomes and  
611 their SNPs. *R package version 1.64.0*, <https://bioconductor.org/packages/BSgenome> (2020).
- 612 55. Kawahara, Y. *et al.* Improvement of the *Oryza sativa* Nipponbare reference genome using next  
613 generation sequence and optical map data. *Rice* **6**, 1–10 (2013).
- 614 56. Lun, A. T., Riesenfeld, S., Andrews, T., Gomes, T. & Marioni, J. C. EmptyDrops: distinguishing cells  
615 from empty droplets in droplet-based single-cell RNA sequencing data. *Genome biology* **20**, 1–9  
616 (2019).
- 617 57. McGinnis, C. S., Murrow, L. M. & Gartner, Z. J. DoubletFinder: doublet detection in single-cell  
618 RNA sequencing data using artificial nearest neighbors. *Cell systems* **8**, 329–337 (2019).
- 619 58. Curtis, M. D. & Grossniklaus, U. A gateway cloning vector set for high-throughput functional  
620 analysis of genes in planta. *Plant physiology* **133**, 462–469 (2003).
- 621 59. Nakagawa, T. *et al.* Development of series of gateway binary vectors, pGWBs, for realizing  
622 efficient construction of fusion genes for plant transformation. *Journal of bioscience and*  
623 *bioengineering* **104**, 34–41 (2007).
- 624 60. Ge, S. X., Jung, D. & Yao, R. ShinyGO: a graphical gene-set enrichment tool for animals and  
625 plants. *Bioinformatics* **36**, 2628–2629 (2020).

- 626 61. Lease, K. A., Cho, S. K. & Walker, J. C. A petal breakstrength meter for Arabidopsis abscission  
627 studies. *Plant Methods* **2**, 2 (2006).
- 628 62. Liu, Y.-G., Mitsukawa, N., Oosumi, T. & Whittier, R. F. Efficient isolation and mapping of  
629 Arabidopsis thaliana T-DNA insert junctions by thermal asymmetric interlaced PCR. *The Plant Journal*  
630 **8**, 457–463 (1995).
- 631



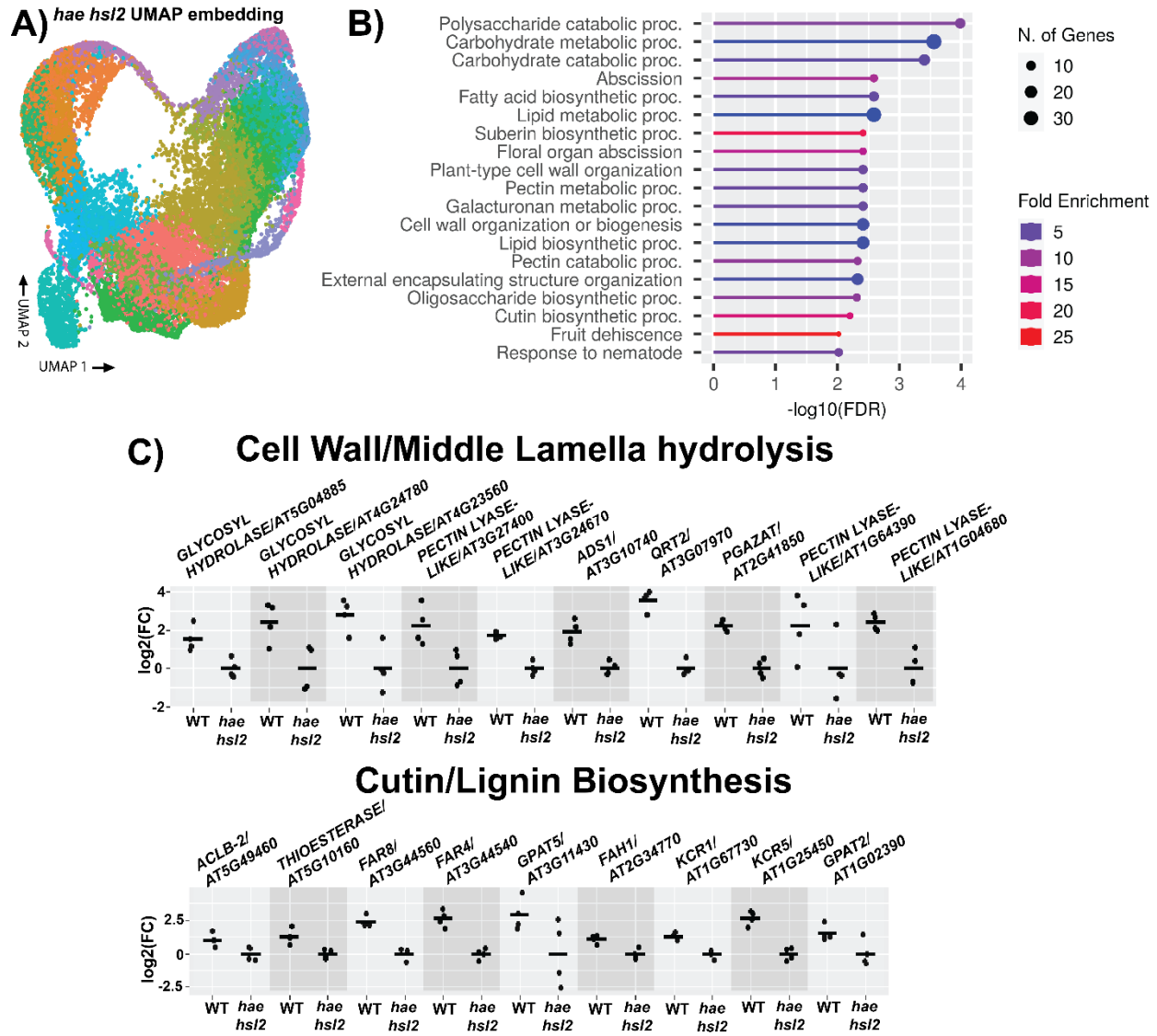
**Figure 1: Background and experimental system**

- A) Floral abscission phenotype of WT and *hae hsl2* mutant. Scale bar = 10 mm.
- B) Schematic diagram of HAE/HSL2 signaling pathway.
- C) Flower stages in WT and *hae hsl2* mutant. Scale bar = 2 mm.
- D) Phenotype of transgenic *hae hsl2* expressing wild-type *HAEpr::HAE-YFP* (top: bright-field/BF, lower: YFP). Scale bar = 2 mm.
- E) Phenotype of transgenic *hae hsl2* expressing mutant *HAEpr::HAE-YFP K711E* (top: bright-field/BF, lower: YFP). Scale bar = 2 mm.
- F) Diagram of AZ single-cell isolation and experimental procedure.



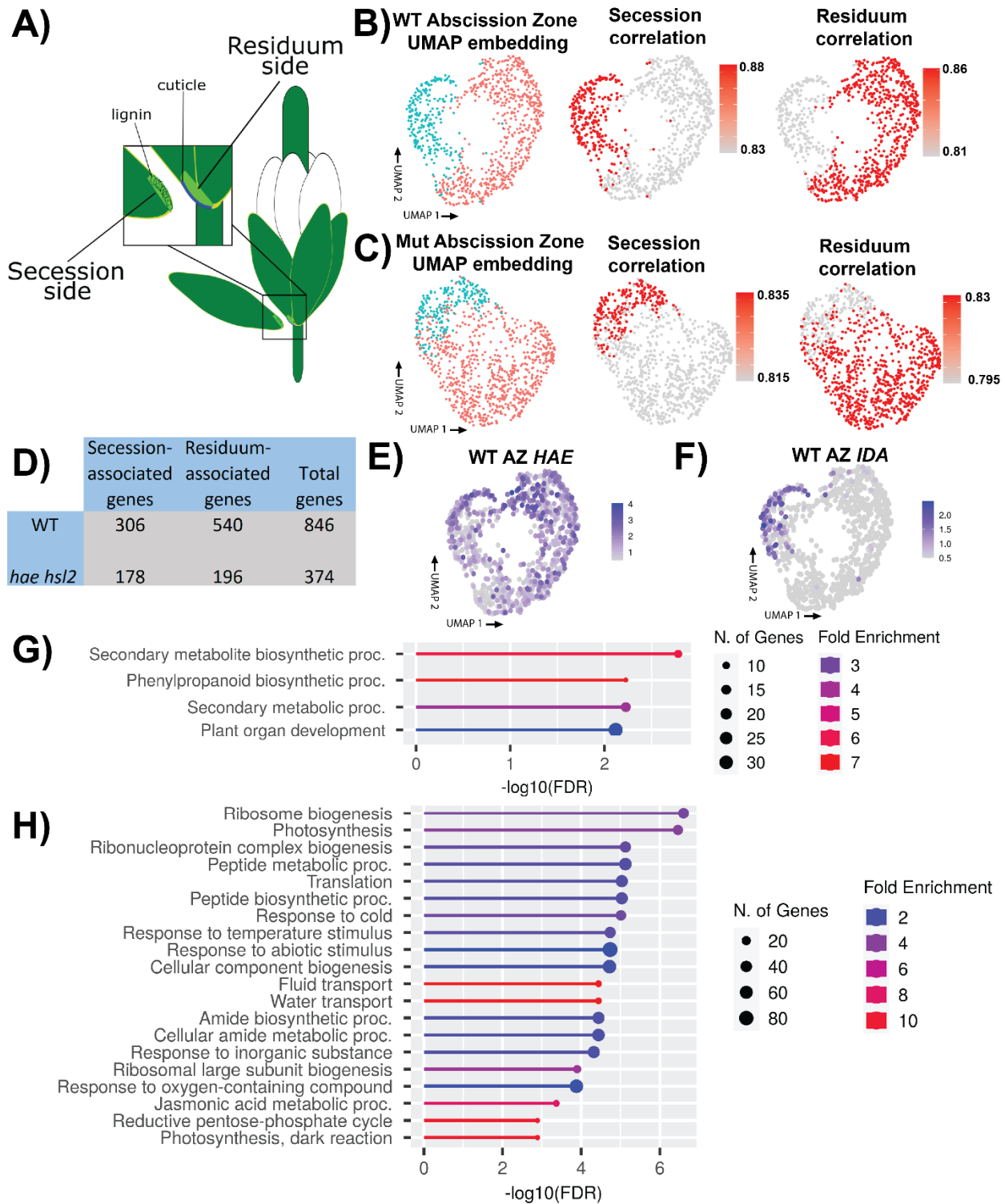
**Figure 2: Identification and characterization of AZ cells by single-cell RNA-Sequencing**

- UMAP embedding of WT cells with putative AZ cluster circled in pink (top) and expression of AZ marker genes on UMAP (bottom). Expression is on Seurat SCT scale.
- Cluster-wise pseudo-bulk Spearman correlation with sorted bulk data of *HAEpr::HAE-YFP* (top) or *QRT2pr::GFP* (bottom).
- Expression of 4 putative AZ marker genes in single-cell UMAP embedding and in the young siliques of *promoter::fluorescent protein* expressing transgenic plants (bright-field/BF and YFP or GFP). Expression is on Seurat SCT scale. Scale bar = .7 mm.
- Gene Ontology Biological Process term enrichment of AZ specific genes.



**Figure 3: Analysis of differentially expressed genes in the *hae hsl2* mutant AZ**

- UMAP embedding of *hae hsl2* cells.
- Gene Ontology Biological Process term enrichment of DE genes higher in WT (FDR < .05, log<sub>2</sub>(FC) > 1).
- Expression of cell wall/middle lamella hydrolysis genes (top) and cutin/lignin biosynthesis genes (bottom). Scale is log<sub>2</sub>(FC) for each sample relative to the average of *hae hsl2*.

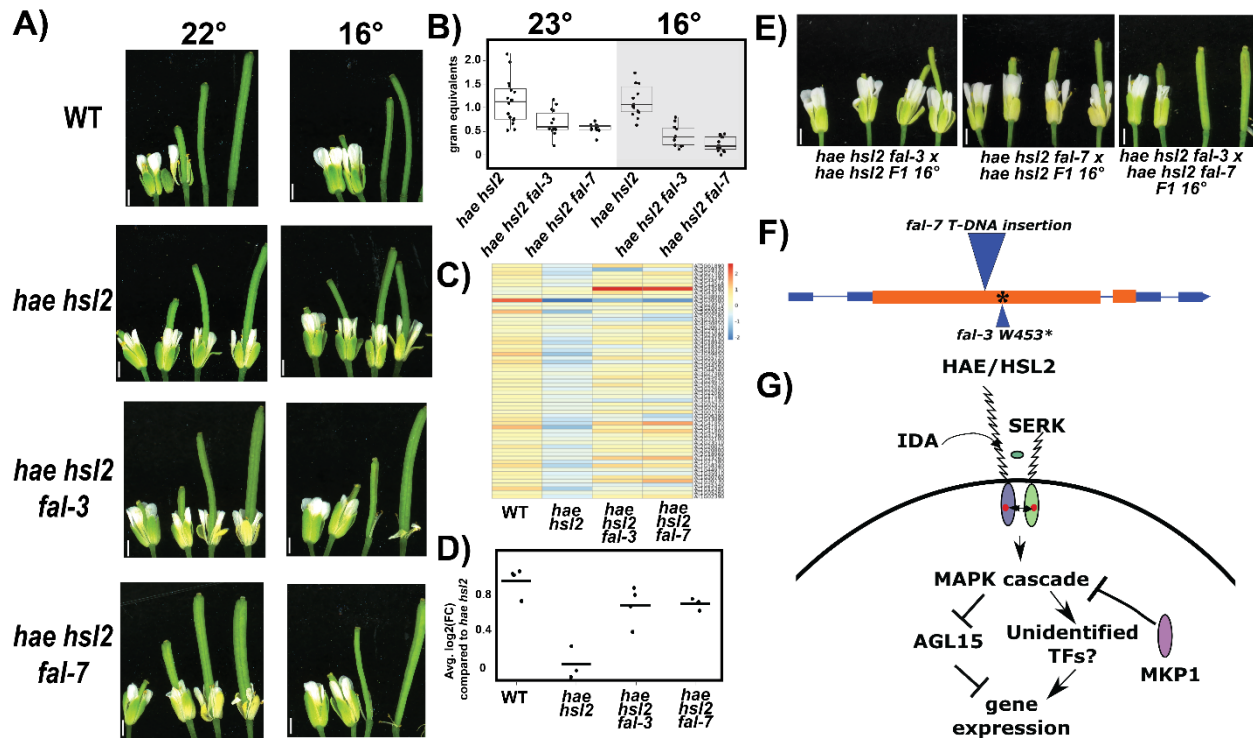


**Figure 4: Spatial analysis of the AZ**

- A) Schematic view of the spatial organization of the AZ.  
 B) Low resolution Louvain clustering and UMAP embedding of WT AZ cells (left) and Spearman correlation with sorted bulk seccion data (middle) or residuum data (right).

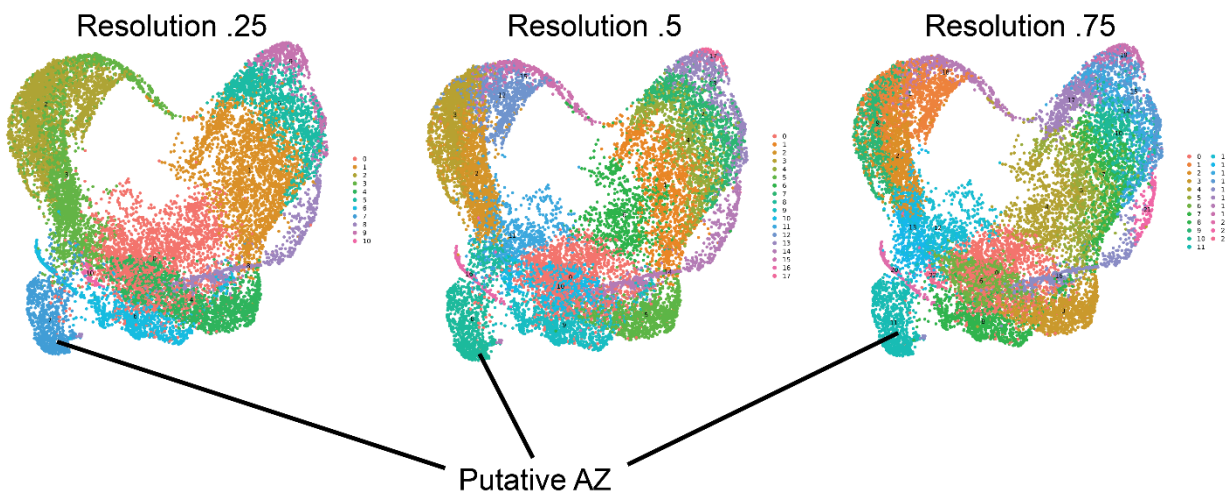
- C) Low resolution Louvain clustering and UMAP embedding of *hae hsl2* AZ cells (left) and Spearman correlation with sorted bulk secession data (middle) or residuum data (right).
- D) Tabulation of differences in the number of secession and residuum-associated genes between WT and *hae hsl2*.
- E) Expression of *HAE* on SCT scale across the AZ.
- F) Expression of *IDA* on SCT scale across the AZ.
- G) Gene Ontology Biological Process term enrichment of genes enriched in putative secession cells.
- H) Gene Ontology Biological Process term enrichment of genes enriched in putative residuum cells.



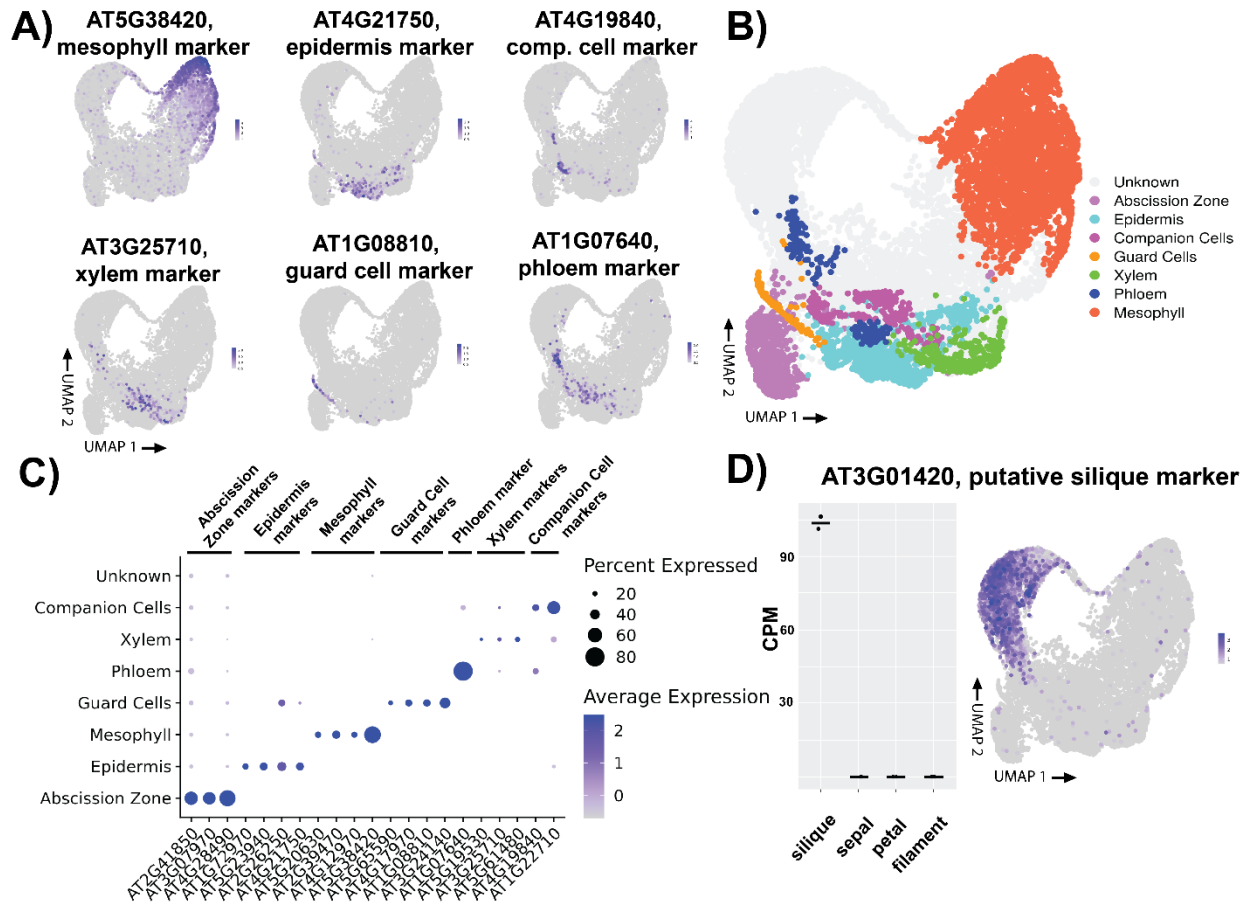


**Figure 5: Analysis and identification of *hae hsl2* suppressors**

- Phenotypes of WT, *hae hsl2*, *hae hsl2 fal-3*, and *hae hsl2 fal-7* at 22° (left) or 16° (right). Siliques were gently tapped to remove remnant floral organs. Scale bar = 1 mm.
- Breakstrength phenotypes of *hae hsl2*, *hae hsl2 fal-3*, and *hae hsl2 fal-7* at 23° (left) or 16° (right).
- Heatmap of expression values for 67 genes identified as higher in WT in both bulk and single-cell DE analysis comparing WT to *hae hsl2*. Values are log<sub>2</sub>(FC) compared the overall average expression across all genotypes.
- Average log<sub>2</sub>(FC) comparing each sample to the average of *hae hsl2* for the genes in part C.
- Complementation crosses of *fal* mutants. Scale bar = 1 mm.
- Gene model of *MKP1* depicting mutations in *fal-3* and *fal-7*. Orange colors represent exons, blue represent UTRs, and thin lines represent introns.
- Revised schematic diagram of HAE/HSL2 signaling pathway

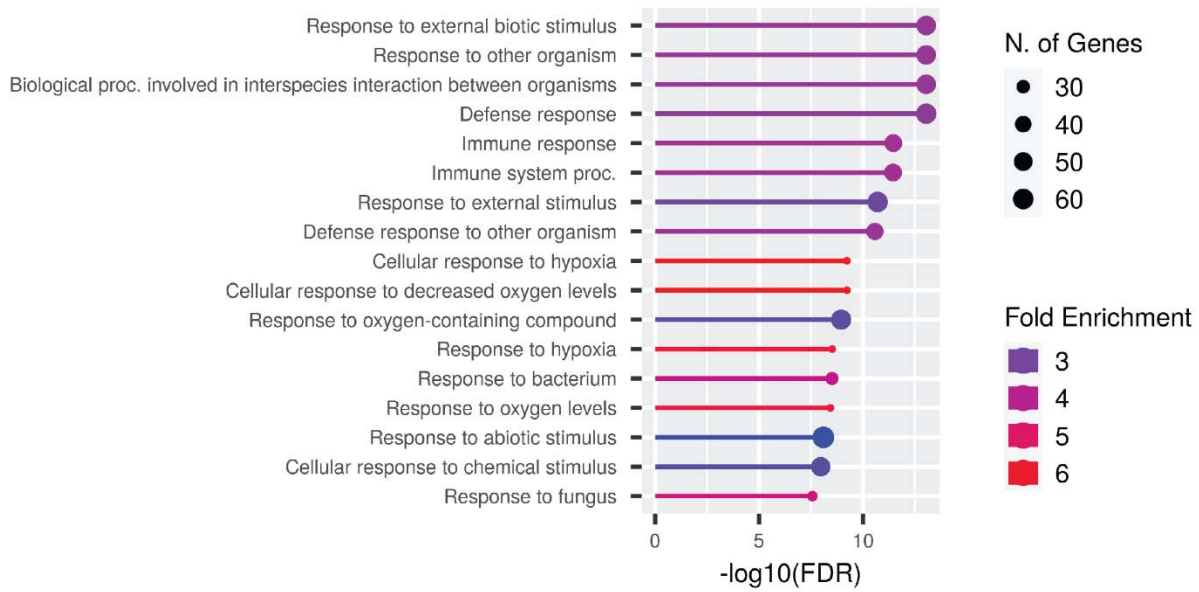


**Supplemental Figure 1:** The putative AZ cluster is similar across a range of clustering resolutions

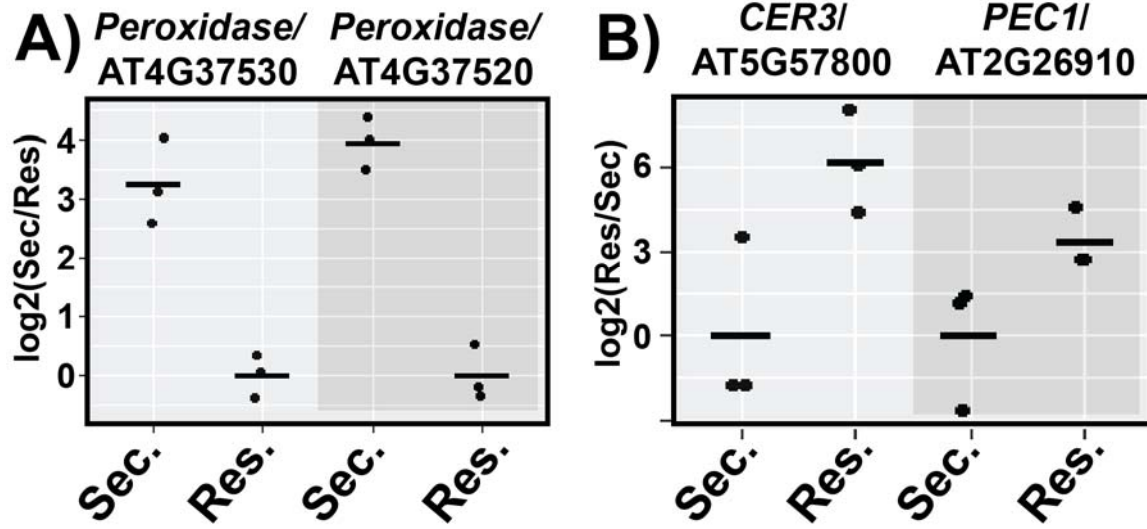


**Supplemental Figure 2: Identification of additional cell types**

- Plotting cell type markers identified from a prior single-cell study of Arabidopsis leaves.
- Tentative cell-type identification based on expression of known marker cell-type marker genes.
- Distribution of marker gene expression across putative cell types.
- 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).

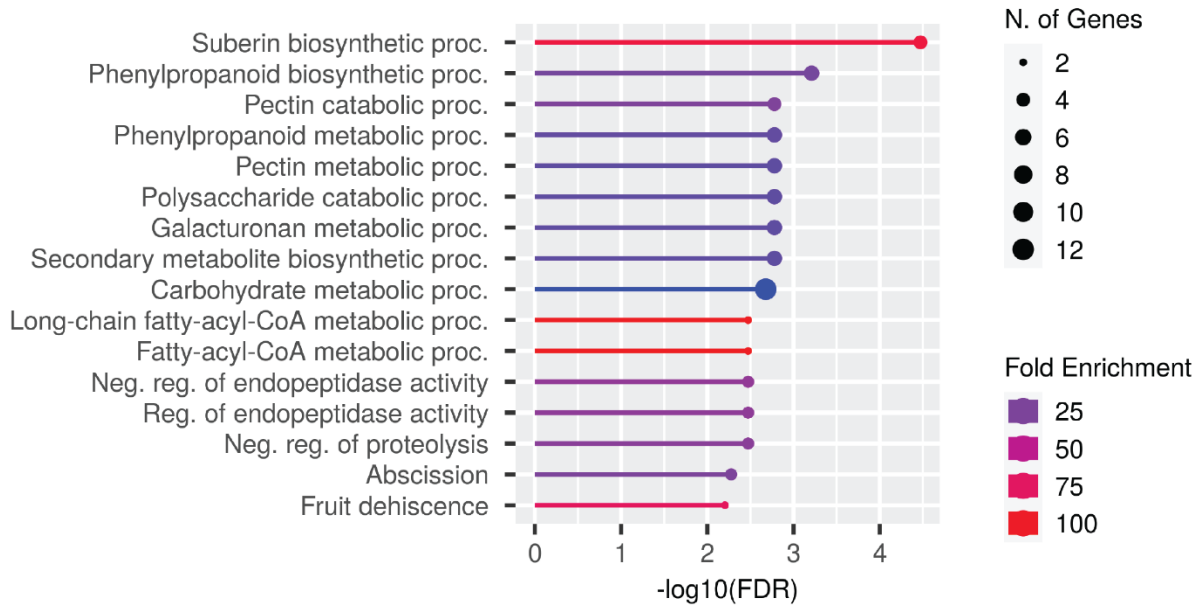


**Supplemental Figure 3: GO term enrichment analysis of genes higher in *hae hsl2* compared to WT AZs.**



**Supplemental Figure 4:** Expression of putative secession and residuum cell markers

- A) Relative log<sub>2</sub>(fold enrichment) of two peroxidase genes in secession cells.
- B) Relative log<sub>2</sub>(fold enrichment) of two cutin biosynthesis genes in residuum cells.



**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.