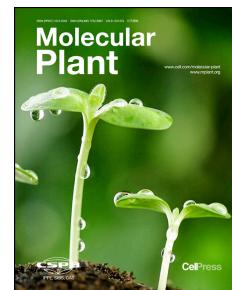


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JASMONATE-ZIM DOMAIN proteins engage Polycomb chromatin modifiers to modulate Jasmonate signaling in *Arabidopsis*

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1 **JASMONATE-ZIM DOMAIN proteins engage Polycomb chromatin**  
2 **modifiers to modulate Jasmonate signaling in *Arabidopsis***  
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21 **Running Title:** Polycomb repression in JA signalling  
22  
23 **Short Summary:** This study reports that the negative regulators of JA signaling pathway  
24 JAZ proteins directly interact and function together with chromatin-associated Polycomb  
25 factors to mediate repressive chromatin modifications and transcriptional repression at  
26 JA-responsive genes in *Arabidopsis*. This reveals a chromatin-based mechanism for  
27 JAZ-mediated transcriptional repression and JA signaling in plants.

**28 ABSTRACT**

29 Jasmonate (JA) regulates various aspects of plant growth and development and stress  
30 responses, with prominent roles in male reproductive development and defenses against  
31 herbivores and necrotrophic pathogens. JASMONATE-ZIM DOMAIN (JAZ) proteins are  
32 key regulators in the JA signaling pathway and function to repress the expression of  
33 JA-responsive genes. Here, we show that JAZ proteins directly interact with several  
34 chromatin-associated Polycomb proteins to mediate repressive chromatin modifications at  
35 part of JA-responsive genes and thus their transcriptional repression in *Arabidopsis*.  
36 Genetic analyses revealed that the developmental defects, including anther and pollen  
37 abnormalities resulting from loss or block of JA signaling, were partially rescued by loss of  
38 Polycomb protein-mediated chromatin silencing (Polycomb repression). We further found  
39 that JAZ-mediated transcriptional repression requires Polycomb proteins at four key  
40 regulatory loci in anther and pollen development. Analysis of genome-wide occupancy of a  
41 Polycomb factor and transcriptome reprogramming in response to JA reveals that Polycomb  
42 repression is involved in the repression of various JA-responsive genes. Taken together, our  
43 study reveals an important chromatin-based mechanism for JAZ-mediated transcriptional  
44 repression and JA signaling in plants.

45 **Key words:** Jasmonate, JA response, Polycomb proteins, Polycomb repression, JAZ  
46 proteins, anther development, H3K27me3, LHP1

47 **INTRODUCTION**

48 The oxylipin hormone Jasmonate regulates various aspects of growth and development and  
 49 stress responses in plants, particularly male reproductive development (stamen  
 50 development) and defenses against insect pests and necrotrophic pathogens (Browse, 2009;  
 51 Chini et al., 2016; Song et al., 2013; Zhai and Li, 2019; Zhang et al., 2017). JA is  
 52 ubiquitous across higher plants and is synthesized in response to developmental cues (e.g.  
 53 anther and pollen development) and environmental stimuli such as wounding, insect  
 54 chewing and pathogen infections (Campos et al., 2014; Wasternack and Hause, 2013).  
 55 Upon its perception, JA triggers a reprogramming of gene expression leading to  
 56 sophisticated responses to developmental and environmental signals (Browse, 2009; Song  
 57 et al., 2013; Zhai and Li, 2019).

58 The active form of JA, Jasmonoyl-isoleucine (JA-Ile), is perceived by a co-receptor  
 59 complex composed of the F-box protein CORONATINE INSENSITIVE1 (COI1) and a  
 60 JAZ protein in Arabidopsis (Sheard et al., 2010; Yan et al., 2009b). The co-receptor  
 61 formation depends on JA, and JA-Ile mediates the binding of a JAZ protein to COI1, a  
 62 subunit of the Skp1/Cullin/F-box SCF<sup>COI1</sup> complex of ubiquitin E3 ligase, and COI1 targets  
 63 JAZs for polyubiquitination and subsequent degradation by 26S proteasomes (Chini et al.,  
 64 2007; Katsir et al., 2008; Sheard et al., 2010; Thines et al., 2007; Yan et al., 2009a; Yan et  
 65 al., 2007). In the absence of JA-Ile (*i.e.* the resting state), JAZs bind to positive  
 66 transcription factors (TFs) of JA signaling, such as basic helix-loop-helix (bHLH) TFs  
 67 including MYC2 to MYC5 and MYB TFs, and inhibit their activities leading to  
 68 transcriptional repression of JA-responsive genes; hence, JAZs are negative regulators of  
 69 JA signaling (Howe et al., 2018; Pauwels and Goossens, 2011). The COI1-dependent  
 70 degradation of JAZ proteins release MYC and MYB TFs. Recent studies reveal that free  
 71 MYC TFs associate with subunits of the Mediator complex, which binds to the RNA  
 72 Polymerase II to promote transcription, to form a transcriptional activation complex and  
 73 thus promote the expression of JA-responsive genes (Chen et al., 2012; Wang et al., 2019;  
 74 Zhang et al., 2015; Zhang et al., 2012).

75 In *Arabidopsis*, there are at least thirteen JAZ proteins, including JAZ1 to JAZ13,  
76 typically composed of three conserved domains, including an amino-terminal domain, ZIM  
77 (ZINC-FINGER EXPRESSED IN INFLORESCENCE MERISTEM) and a  
78 carboxyl-terminus JA-associated (Jas) domain (Chini et al., 2007; Thines et al., 2007;  
79 Thireault et al., 2015). The ZIM domain mediates homo- and hetero-dimerization of JAZs,  
80 whereas the Jas domain, in the presence of JA, mediates the binding of a JAZ protein to  
81 COI1 for degradation (Pauwels and Goossens, 2011). One mechanism for JAZ-mediated  
82 transcriptional repression in the absence of JA is that Jas domains bind to JA-responsive  
83 TFs to interfere with their recruitment of the Mediator complex and thus inhibit their  
84 transcriptional activation activities (Zhang et al., 2015). For instance, the Jas domain of  
85 JAZ9 is interwoven with the N-terminal part of MYC3 (a key bHLH TF to activate  
86 JA-responsive gene expression), which competitively prohibits the association of MYC3  
87 with the Mediator complex and thus inhibits the transcriptional activation mediated by  
88 MYC3 (Zhang et al., 2015). In addition, JAZs associate with the adaptor protein NOVEL  
89 INTERACTOR OF JAZ (NINJA) to repress JA-responsive genes (Pauwels et al., 2010).  
90 NINJA or a transcriptional repression motif at the N termini of JAZ8 and JAZ13 known as  
91 EAR can recruit the transcriptional co-repressors TOPLESS (TPL) and TPL-related  
92 proteins for transcriptional repression (Pauwels et al., 2010; Shyu et al., 2012; Thireault et  
93 al., 2015). TPL has been shown to function together with a histone deacetylase (HDAC) to  
94 repress target gene expression (Long et al., 2006). It is evident that NINJA acts as a  
95 negative regulator of JA signaling with a major role to repress root JA signaling (Acosta et  
96 al., 2013; Pauwels et al., 2010); however, the weak phenotypes of JA response in a *tpl*  
97 mutant suggest that NINJA may engage other, yet to be identified, transcriptional repressors  
98 to repress JA-responsive genes (Pauwels et al., 2010; Wager and Browse, 2012).

99 A defect in JA biosynthesis or perception causes a striking developmental phenotype -  
100 male sterility, because JA signaling is essential for the elongation of stamen filaments,  
101 anther dehiscence and pollen grain maturation and viability (McConn and Browse, 1996;  
102 Song et al., 2013). Stamen development includes anther and pollen development, and is a  
103 complex process that occurs in fourteen stages in *Arabidopsis* (Sanders et al., 1999). Pollen

104 grains are produced within the anther. A single layer of nutritive anther cells, known as  
 105 tapetum, encases developing pollens, provides nourishment and thus promotes pollen grain  
 106 maturation (Gomez et al., 2015; Zhu et al., 2011a). Pollen development includes  
 107 microsporogenesis (from specification of pollen mother cell to haploid microspore) and  
 108 microgametogenesis (from post-meiotic microspore development to mature bi-cellular or  
 109 tri-cellular pollen grains) (Gomez et al., 2015; Sanders et al., 1999). JAZ proteins are  
 110 known to be involved in late anther and pollen development in *Arabidopsis* (Qi et al., 2015;  
 111 Thines et al., 2007). Four JAZ proteins (*e.g.* JAZ1) can interact with two R2R3-MYB TFs  
 112 (MYB21 and MYB24) that function in late anther and pollen development (Song et al.,  
 113 2011). These two MYB TFs, together with the JAZ-interacting bHLH transcription factors  
 114 including MYC2, MYC3, MYC4 to MYC5, form bHLH-MYB complexes; in late stamen  
 115 development, JAZ proteins can inhibit transcriptional activation mediated by these  
 116 bHLH-MYB complexes, through physical association (Qi et al., 2015). JA synthesis is  
 117 initiated upon the transcriptional activation of *DEFECTIVE IN ANTER DEHISCENCE1*  
 118 (encoding an A1 phospholipase) in early stamen development (Ito et al., 2007). JA rise  
 119 leads to COI1-dependent degradation of JAZs in late stamen development, and thus release  
 120 the bHLH-MYB complexes to promote pollen maturation and anther dehiscence (Qi et al.,  
 121 2015; Song et al., 2013).

122 In addition to the JAZ-interacting bHLH-MYB TF complexes, genetic studies in  
 123 *Arabidopsis* have revealed a few key regulatory TFs for anther and pollen development,  
 124 including *SPOROCYTELESS (SPL)/NOZZLE (NZZ)*, *DYSFUNCTIONAL TAPETUM 1*  
 125 (*DYT1*), *ABORTED MICROSPORE (AMS)*, *MALE STERILITY 1 (MS1)*, and several  
 126 additional TFs (Schieffthaler et al., 1999; Xu et al., 2014; Yang et al., 2007; Yang et al., 1999;  
 127 Zhang et al., 2006). *SPL/NZZ* is required for the specification of pollen mother cell,  
 128 whereas *DYT1* acts downstream of *SPL/NZZ* to initiate tapetum development and promote  
 129 meiotic progression (Schieffthaler et al., 1999; Yang et al., 1999; Zhang et al., 2006). *AMS*  
 130 (bHLH TF) and *MS1* (PHD finger TF) act in late stamen development. *AMS1* functions  
 131 downstream of *DYT1* as a master regulator of tapetum development and function, whereas  
 132 *MS1* acts downstream of *AMS1* to promote tapetum degradation and pollen wall formation

133 at late pollen development (Xu et al., 2014; Yang et al., 2007). To date, whether and how JA  
 134 signaling regulates these key regulatory genes of anther development are unclear.

135 Changes in the transcriptional state of a eukaryotic gene (on and off) often involve  
 136 chromatin modifications. Polycomb group (PcG) proteins mediate repressive chromatin  
 137 modifications to silence the expression of developmental genes in plants and animals, *i.e.*  
 138 Polycomb repression (Lanzuolo and Orlando, 2012). PcG proteins are often assembled into  
 139 chromatin-associated complexes. Polycomb repressive complex 2 (PRC2), a histone 3  
 140 lysine-27 (H3K27) methyltransferase complex, is a major PcG complex that is  
 141 evolutionarily-conserved from plants to animals (Forderer et al., 2016; Lanzuolo and  
 142 Orlando, 2012; Merini and Calonje, 2015). The core PRC2 subunits include three structural  
 143 subunits and one H3K27 methyltransferase. PRC2 catalyzes H3K27 trimethylation  
 144 (H3K27me3) that is recognized and bound by a reader protein or protein complex, leading  
 145 to transcriptional repression (Lanzuolo and Orlando, 2012). In *Arabidopsis*, CURLY LEAF  
 146 (CLF) is a major H3K27 methyltransferase constitutively expressed in sporophytic tissues  
 147 (Goodrich et al., 1997; Schatowski et al., 2010). CLF, together with three core structural  
 148 subunits including EMBRYONIC FLOWER 2 [EMF2, a homolog of Su(z)12],  
 149 FERTILIZATION-INDEPENDENT ENDOSPERM (FIE) and *ARABIDOPSIS*  
 150 MULTICOPY SUPPRESSOR OF IRA1 (MSI1), form a PRC2 complex (CLF-PRC2) to  
 151 catalyze genome-wide H3K27me3 in sporophytic tissues (Bemer and Grossniklaus, 2012;  
 152 Mozgova and Hennig, 2015); loss of *CLF* function causes a great reduction of H3K27me3  
 153 in the *Arabidopsis* genome (Jiang et al., 2008; Lafos et al., 2011). H3K27me3 is read by  
 154 LIKE HETEROCHOMATIN PROTEIN 1 (LHP1) and BAH (Bromo adjacent homology)  
 155 domain-containing proteins, which function to maintain and further promote H3K27  
 156 trimethylation at target loci via positive feedbacks (Derkacheva et al., 2013; Li et al., 2018;  
 157 Turck et al., 2007; Zhang et al., 2007). In addition, these H3K27me3 readers interact with  
 158 the plant-specific EMBRYONIC FLOWER 1 (EMF1) that functions to mediate chromatin  
 159 compaction, to execute Polycomb repression at target loci (Calonje et al., 2008; Li et al.,  
 160 2018; Wang et al., 2014).

161 PcG factors are well known to regulate developmental genes and maintain cell

162 type-characteristic gene expression patterns (and thus cell identity) in both plants and  
 163 animals (Lafos et al., 2011; Lanzuolo and Orlando, 2012; Mozgova and Hennig, 2015).  
 164 Although genome-wide H3K27me3 profiling analyses in *Arabidopsis* seedlings have  
 165 revealed that a few genes involved in biosynthesis, transport or signaling of hormones  
 166 including auxin, brassinosteroids and salicylic acid, are marked with H3K27me3 (Lafos et  
 167 al., 2011; Lu et al., 2011; Ramirez-Prado et al., 2019), the roles of Polycomb repression in  
 168 hormone signaling and responses remain elusive.

169 Here, we show that JAZs directly associate with PcG proteins, including LHP1 and  
 170 EMF2 (PRC2), to mediate transcriptional repression of various JA-responsive genes in  
 171 *Arabidopsis*. Genome-wide analysis of LHP1 occupancy reveals that JA rise leads to an  
 172 overall reduction in LHP1 enrichment, and further transcriptome profiling shows that about  
 173 one quarter of JA-induced genes are repressed by *LHP1*. Additional genetic analysis reveals  
 174 that the developmental defects, including anther and pollen abnormalities resulting from  
 175 loss or block of JA signaling, were partly rescued by functional loss of Polycomb  
 176 repression in *lhp1* or a PRC2-defective mutant. We further found that JAZ-mediated  
 177 repression of the master regulatory TFs of stamen development including *DYT1*, *MS1* and  
 178 *AMS1*, requires *LHP1* and PRC2, and that JA rise disrupts PRC2 enrichment to relieve  
 179 transcriptional repression at these loci. Together, our results reveal that JAZs engage PcG  
 180 factors to mediate repressive chromatin modifications and confer transcriptional repression  
 181 at various JA-responsive loci, suggesting that Polycomb repression negatively modulates  
 182 JA signaling and thus JA responses in *Arabidopsis*.

## 183 RESULTS

184 **JAZs directly interact with the H3K27me3 reader LHP1 and the core PRC2 subunit**  
 185 **EMF2**  
 186 Polycomb proteins often function together with transcriptional co-repressors for gene  
 187 repression. In an effort to identify interacting partners of the H3K27me3 reader LHP1  
 188 through a yeast two-hybrid library screening, a fragment of JAZ6 was found to interact

189 with LHP1 (Supplemental Figure 1). We further explored the potential association of LHP1  
 190 with JAZ1 to JAZ13 from *Arabidopsis* using yeast two-hybrid assays, and found that in  
 191 yeast cells LHP1 interacted with full-length JAZ4, JAZ8 and JAZ10, with the ZIM domain  
 192 of JAZ1 or with fragments of JAZ3, JAZ6 or JAZ9, which contains both the ZIM and Jas  
 193 domains (Figure 1A and B). Thus, LHP1 interacts with seven out of the thirteen known JAZ  
 194 proteins. Notably, no interaction was detected between LHP1 and JAZ2, JAZ5, JAZ7,  
 195 JAZ11, JAZ12 or JAZ13 using yeast two-hybrid assays (Supplemental Figure 2).

196 To explore whether other PcG factors could associate with JAZs, we examined the  
 197 association of JAZ proteins with EMF2, a core structural subunit of PRC2 (Mozgova and  
 198 Hennig, 2015; Yoshida et al., 2001). As shown in Figure 1A, EMF2 interacted with the  
 199 full-length JAZ1, JAZ4, JAZ8 and JAZ10, and with a fragment of JAZ3 and JAZ9  
 200 encompassing the ZIM and Jas domains. Further analysis using the ZIM and Jas domains  
 201 from JAZ4 revealed that the ZIM domain mediated the interaction of JAZ4 with EMF2  
 202 (Figure 1C); in addition, we found that the ZIM<sub>JAZ4</sub> domain, like ZIM<sub>JAZ1</sub> interacted with  
 203 LHP1 (Figure 1B-C). These results reveal that the ZIM domains from certain JAZs mediate  
 204 association with PcG proteins.

205 Next, we conducted protein pulldown assays to determine whether JAZ proteins may  
 206 directly interact with Polycomb proteins. Using the *E.coli* extracts containing the LHP1  
 207 tagged by Glutathione S Transferase (GST:LHP1), GST:EMF2, JAZ1:His or JAZ4:His, we  
 208 found that GST:LHP1 and GST:EMF2, but not GST alone, were able to pull down  
 209 JAZ1:His and JAZ4:His, respectively (Supplemental Figure 3A-C), revealing that both  
 210 JAZ1 and JAZ4 can physically associate with Polycomb proteins.

211 Both *JAZ1* and *JAZ4* play an important role in JA-mediated defense, growth and  
 212 development in *Arabidopsis* (Oblessuc et al., 2020; Thines et al., 2007); hence, we further  
 213 verified the association of JAZ1 and JAZ4 with LHP1 and/or PRC2 in *Arabidopsis* using *in*  
 214 *vivo* co-immunoprecipitation (co-IP) assays. First, a transgenic line expressing *JAZ1:HA*  
 215 was crossed to a functional *LHP1:GFP* line (Hou et al., 2010; Sung et al., 2006), and co-IP  
 216 assays were carried out using the resultant F<sub>1</sub> seedlings. As shown in Figure 1D, LHP1:GFP  
 217 was immunoprecipitated by JAZ1:HA, demonstrating that JAZ1 forms a complex with

218 LHP1 in Arabidopsis. Next, we crossed a *JAZ4:Flag* line to the *LHP1:GFP* line and the  
 219 *EMF2:VENUS* line (Sun et al., 2014); subsequent co-IP assays with the resultant F<sub>1</sub>  
 220 seedlings showed that both LHP1:GFP and EMF2:VENUS were immunoprecipitated by  
 221 JAZ4:Flag (Figure 1E and F), confirming that JAZ4 associates with LHP1 and EMF2  
 222 (PRC2) in Arabidopsis. Notably, LHP1 was co-purified with EMF2, CLF and other core  
 223 PRC2 subunits in a previous study (Derkacheva et al., 2013). Taken together, these data  
 224 reveal that certain JAZ proteins can associate with LHP1 and PRC2 to form multi-protein  
 225 complexes.

226

227 **NINJA, a partner of JAZs in the JA signaling pathway, directly interacts with LHP1  
 228 and EMF2**

229 The NINJA adaptor protein binds directly to most JAZs and functions as a transcriptional  
 230 repressor in the JA signaling pathway (Pauwels et al., 2010). We explored whether PcG  
 231 proteins may associate with NINJA, in addition to JAZs, and found that LHP1 interacted  
 232 with NINJA in yeast cells (Supplemental Figure 3D). Next, we performed bimolecular  
 233 fluorescence complementation (BiFC) analysis in which the N-terminal and C-terminal  
 234 EYFP (for ENHANCED YELLOW FLUORESCENT PROTEIN) were fused to the  
 235 full-length LHP1 and NINJA, respectively. The fluorescence was observed in the nuclei of  
 236 onion epidermal cells upon co-expression of nEYFP-LHP1 and NINJA-cEYFP, revealing  
 237 that they directly interacted in the nucleus (Supplemental Figure 3E). In addition, in a  
 238 protein pulldown assay we found that GST:LHP1, but not GST, was able to pull down  
 239 NINJA:His (Supplemental Figure 3F). We further conducted *in vivo* co-IP assays using the  
 240 F<sub>1</sub> progeny from crossing a *NINJA:Flag* line to the *LHP1:GFP* line, and found that  
 241 LHP1:GFP was immunoprecipitated by NINJA:Flag (Figure 1G), demonstrating the  
 242 association of NINJA with LHP1 in Arabidopsis.

243 Next, we found that EMF2 was able to interact directly with NINJA:His in a protein  
 244 pulldown assay (Supplemental Figure 3G). Furthermore, we carried out co-IP with the F<sub>1</sub>  
 245 progeny from a cross of the *NINJA:Flag* line to the *EMF2:VENUS* line, and found that  
 246 EMF2:VENUS was co-immunoprecipitated by NINJA:Flag (Figure 1H). Thus, NINJA

247 associates with LHP1 and EMF2 (PRC2) *in vivo*. Interestingly, we observed that the  
 248 NINJA-interacting protein TPL did not interact with neither LHP1 nor EMF2 in yeast cells  
 249 (Supplemental Figure 4). Taken together, these results suggest that the NINJA adaptor,  
 250 together with JAZs, engages PcG-mediated chromatin silencing (i.e. JAZ-NINJA-PcG) to  
 251 negatively modulate JA responses.

252

253 **Loss of *LHP1* or PRC2 function partially rescues anther and pollen abnormalities  
 254 resulting from loss or block of JA signaling**

255 JA signaling is essential for stamen development and the obvious developmental  
 256 phenotypes of a mutant with a defect in JA synthesis or perception are anther dehiscence  
 257 failure and pollen inviability (Browse, 2009; Song et al., 2013). Given the association of  
 258 NINJA and the seven JAZ proteins with several PcG factors, it was of interest to explore  
 259 the genetic roles of Polycomb repression for JA-mediated stamen development. First, we  
 260 examined anther dehiscence upon functional loss of *LHP1* or PRC2. As *emf2* skips  
 261 vegetative growth and is inviable (Yoshida et al., 2001), we examined a loss-of-function  
 262 mutant in *CLF*, which encodes the major H3K27 methyltransferase and forms a PRC2  
 263 complex with EMF2 (Kim et al., 2010; Mozgova and Hennig, 2015). Both mature *clf* and  
 264 *lhp1* anthers were able to dehisce (Figure 2A and B). These two mutations were introduced  
 265 into a loss of function *coi1* mutant that is unable to perceive JA signals (Devoto et al., 2002;  
 266 Xie et al., 1998). All *coi1* anthers failed to dehisce and release mature pollen grains, but in  
 267 double mutants of *coi1* with *clf* or *lhp1*, mature anthers were partially dehisced (Figure 2A).  
 268 We observed that 33 out of 337 *coi1 lhp1* anthers and 63 out of 348 *coi1 clf* anthers were  
 269 partially dehisced. In addition, we further examined pollen viability in these mutants. *lhp1*  
 270 and *clf* pollen grains exhibited a moderate reduction in viability, indicating a role of  
 271 Polycomb repression for pollen development. All examined *coi1* pollen grains were  
 272 unviable and failed to produce a pollen tube on a pollen grain-germination medium, but  
 273 about 10% pollen grains of *coi1 lhp1* and *coi1 clf* were able to produce pollen tubes (Figure  
 274 2C). These results show that loss of *LHP1* or *CLF* function can partially rescue the *coi1*  
 275 mutant, suggesting that Polycomb repression plays a negative role in COI1-mediated JA

276 signaling.

277 We further explored the role of Polycomb repression in JAZ-mediated repression of JA  
 278 response in stamen development. As there is no JA-related phenotype in single  
 279 loss-of-function *JAZ* mutants, a mutant constitutively expressing a gain-of-function *JAZ1*  
 280 gene without the *Jas* domain (*JAZ1ΔJas*) (Thines et al., 2007), was pollinated by *clf* or *lhp1*.  
 281 The *JAZ1ΔJas* protein cannot be bound by COI1 and is stable in the presence of JA, and  
 282 thus constitutively blocks JA signaling transduction (Melotto et al., 2008; Thines et al.,  
 283 2007). The *JAZ1ΔJas* mutant displays a failure of anther dehiscence, suggesting that *JAZ1*  
 284 plays a role in anther development (Thines et al., 2007). We found that about 95% mature  
 285 *JAZ1ΔJas* anthers failed to dehisce, but in double mutants of *JAZ1ΔJas* with *clf* or *lhp1*, the  
 286 anthers were partially dehisced: 32 out of 120 *JAZ1ΔJas lhp1* anthers and 36 out of 120  
 287 *JAZ1ΔJas clf* anthers were partially dehisced (Figure 2B). Further analysis of pollen  
 288 viability revealed that 90% mature *JAZ1ΔJas* pollen grains were unviable and failed to  
 289 produce a pollen tube, but about 30% *JAZ1ΔJas lhp1* and *JAZ1ΔJas clf* pollen grains were  
 290 able to produce pollen tubes (Figure 2D). Hence, loss of *LHP1* or *CLF* function partially  
 291 released the constitutive repression of JA signaling in *JAZ1ΔJas*. Together, these genetic  
 292 analysis results suggest that Polycomb repression negatively regulates JA signaling and  
 293 responses in stamen development.

294

295 **Both *LHP1* and PRC2 are required for *JAZ1*-mediated repression of master  
 296 transcription factors in anther and pollen development**

297 Anther and pollen development consists of two phases: microsporogenesis (early  
 298 development) and microgametogenesis (late development), and is further divided into  
 299 fourteen stages in *Arabidopsis* (Sanders et al., 1999). This development is regulated by a  
 300 genetic pathway consisting of a few master TFs, among which are *DYT1*, *AMS* and *MS1*  
 301 (Supplemental Figure 5; (Gomez et al., 2015; Zhu et al., 2011a); in addition, the  
 302 JAZ-interacting bHLH-MYB complexes act to promote pollen maturation and anther  
 303 dehiscence (Qi et al., 2015). We noticed that all of the seven LHP1/EMF2-interacting JAZ  
 304 proteins have been previously shown to physically interact with bHLH-MYB complex

305 members (Pauwels and Goossens, 2011; Qi et al., 2015; Song et al., 2011), and observed  
 306 that these *JAZ*s were expressed in floral buds (Supplemental Figure 6); hence, we explored  
 307 the role of *JAZ*s and Polycomb proteins in the regulation of *DYT1*, *AMS* and *MS1* during  
 308 anther and pollen development.

309 *DYT1*, *AMS* and *MS1* are expressed specifically in early (*DYT1*) and late (*AMS* and  
 310 *MS1*) anther development during flower development (Xu et al., 2014; Yang et al., 2007;  
 311 Zhang et al., 2006; Zhu et al., 2011a). We extracted total RNAs from floral buds with early  
 312 or late development anthers, and the expression of these three master TFs was measured in  
 313 wild type (WT) and *JAZ1ΔJas*. Consistent with the male-sterile phenotype in *JAZ1ΔJas*, the  
 314 constitutive blocking of JA signaling greatly suppressed *DYT1* expression in early anther  
 315 development, and *AMS* and *MS1* expression at late development (Figure 3A); in addition,  
 316 *JAZ1* expression itself was suppressed throughout anther development by the constitutive  
 317 blocking of JA signaling in *JAZ1ΔJas* (Figure 3A). Thus, all of these three master TFs as  
 318 well as *JAZ1* are negatively regulated by *JAZ*-mediated repression in anther development,  
 319 suggesting a role of JA signaling from early to late anther development.

320 As *JAZ* proteins interact with LHP1 and PRC2, next, we determined whether  
 321 Polycomb repression, like *JAZ*s, mediates transcriptional repression of *DYT1*, *AMS* and  
 322 *MS1* during anther and pollen development. We found that loss of *CLF* or *LHP1* function  
 323 led to a great de-repression of *DYT1* specifically in early anther development, and of *AMS*  
 324 and *MS1* in late anther development (Figure 3A); hence, Polycomb repression indeed  
 325 mediates repression of these three master TFs of anther and pollen development. We further  
 326 found that introduction of *clf* or *lhp1* into *JAZ1ΔJas* partially relieved the  
 327 *JAZ1ΔJas*-mediated suppression of *DYT1*, *AMS*, *MS1* and *JAZ1* during anther development  
 328 (Figure 3A), consistent with the partial rescue of the anther and pollen abnormalities in  
 329 *JAZ1ΔJas* by *clf* and *lhp1* (Figure 3A). Thus, Polycomb repression is required for  
 330 *JAZ*-mediated repression of these JA responsive master TFs in anther development. Notably,  
 331 loss of *CLF* or *LHP1* function did not cause a de-repression of *AMS* and *MS1* in early  
 332 anther development, or *DYT1* in late anther development (Figure 3A), suggesting that  
 333 regulation of these genes involves not only Polycomb repressors, but also development

334 stage-specific factors such as JAZs-interacting transcriptional activators.

335

336 **JA regulates CLF (PRC2) enrichment and H3K27 trimethylation at *DYT1, AMS, MS1***  
 337 **and *JAZ1* in anther development**

338 To determine whether Polycomb proteins directly function at the master TF loci of anther  
 339 and pollen development, we carried out chromatin immunoprecipitation (ChIP) using a  
 340 fully-functional *CLF:GFP* line (Gu et al., 2014b). We found that CLF:GFP bound to the  
 341 region around the transcription start site (TSS) of *DYT1, AMS, MS1* and *JAZ1* in both early  
 342 and late anther development stages; hence, these four genes are regulated directly by  
 343 Polycomb repression. We further applied 5-μm JA on developing anthers for three hour (h),  
 344 and all four genes were induced by JA (Supplemental Table 1), confirming that these genes  
 345 are JA responsive. Subsequently, we conducted ChIP assays using the JA-treated floral buds  
 346 with early- or late-development anthers, and found that CLF enrichment was apparently  
 347 reduced at *DYT1* in early development (but not in late development), at *AMS* and *MS1* in  
 348 late development, and at *JAZ1* in both early and late development (Figure 3B). These  
 349 results are consistent with JAZ-mediated repression of *DYT1* specifically in early  
 350 development, *AMS* and *MS1* in late development, and *JAZ1* through anther development  
 351 (Figure 3A), and reveal that CLF (PRC2) binding to these gene chromatin is partly  
 352 dependent on JAZ proteins. Notably, upon JA application CLF is still enriched at *DYT1* in  
 353 late anther development and at *AMS* and *MS1* in early anther development (Figure 3B),  
 354 suggesting that additional factors other than JAZs contribute to CLF (PRC2) recruitment  
 355 /enrichment at these loci.

356 PRC2 deposits H3K27me3 for transcriptional repression (Mozgova and Hennig, 2015);  
 357 hence, it was of interest to examine H3K27me3 changes at *DYT1, AMS, MS1* and *JAZ1*  
 358 upon JA induction. We found that upon a three-hour induction the levels of H3K27me3  
 359 were reduced at *DYT1* in early anther development, at *AMS* and *MS1* in late development,  
 360 and at *JAZ1* in both early and late anther development (Figure 3C), consistent with the  
 361 apparent reductions of CLF enrichment at each locus in early and/or late anther  
 362 development (Figure 3B). Taken together, these results reveal that upon JA rise and

363 consequent degradation of JAZs, PRC2 enrichment is reduced and that Polycomb  
 364 repression is disrupted at the master TFs of anther development.

365 **The gain-of-function *JAZ1ΔJas* gives rise to elevated CLF (PRC2) enrichment at  
 366 master TFs in anther development**

367 To further determine whether JAZs function to promote CLF (PRC2) binding to *DYT1*,  
 368 *AMS*, *MS1* and *JAZ1* chromatin, we carried out ChIP with anti-CLF using the *JAZ1ΔJas*  
 369 line. The stable or gain-of-function *JAZ1ΔJas* resulted in apparent increases of CLF  
 370 enrichment at *DYT1* in early anther development, at *AMS* and *MS1* in late anther  
 371 development, and at *JAZ1* from early to late anther development (Figure 4), in opposite to  
 372 the reductions of CLF enrichment upon JA application (Figure 3B). Taken together, these  
 373 results reveal that JAZs engage Polycomb proteins to mediate repression of the master TFs  
 374 during anther development.

375

376 **Polycomb silencing mediates repression of various JA-responsive genes**

377 Given the interactions of JAZ proteins with LHP1 and PRC2, it was of great interest to  
 378 explore the potential role of LHP1 and/or CLF for genome-wide regulation of  
 379 JA-responsive genes. Using ChIP coupled with high-throughput sequencing (ChIP-seq), we  
 380 examined genome-wide occupancy of LHP1 in response to JA rise at a seedling stage as JA  
 381 responses in multiple organs or tissues can be captured in the seedling. Notably, we chose  
 382 to examine the genome-wide occupancy of LHP1, instead of CLF, due to technical  
 383 challenges in ChIP-seq assays on CLF occupancy (Shu et al., 2019). We grew the seedlings  
 384 expressing a fully-functional LHP1:Flag (Supplemental Figure 5A) in liquid media,  
 385 followed by a three-hour treatment with 5-μM JA or mock (5,000x diluted ethanol). It was  
 386 found that LHP1:Flag was enriched in a total of 3,675 loci in mock and/or JA-treated  
 387 seedlings (Supplemental Table 2 and Supplemental Figure 7D). LHP1 occupancy was  
 388 mainly in gene-body regions (from transcription start site /TSS to transcription end site  
 389 /TES), because H3K27 trimethylation occurs largely in these regions (Lafos et al., 2011;  
 390 Turck et al., 2007; Zhang et al., 2007). We found that JA rise led to an apparent overall

391 reduction in LHP1 enrichment in these 3,675 loci (Figure 5A), indicating that degradation  
 392 of JAZs upon JA rise gives rise to an overall disruption of LHP1 association with  
 393 H3K27me3-marked regions.

394 Next, we conducted transcriptome profiling through high-throughput sequencing  
 395 (RNA-seq) to examine overlapping of JA-inducible genes with *LHP1*-repressed genes in  
 396 liquid-cultured seedlings. LHP1 functions in partial redundancy with several BAH-domain  
 397 readers of H3K27me3 to mediate Polycomb silencing (Li et al., 2018), and loss of *LHP1*  
 398 function results in only a moderate transcriptome change (Zhou et al., 2016); hence, we  
 399 used a fold-change cutoff value of 1.4 (FDR< 0.05) to score *LHP1*-regulated genes, and  
 400 found that 1,469 genes were upregulated in *lhp1* (Supplemental Table 3 and Supplemental  
 401 Figure 8). In addition, we found that 1,222 genes were upregulated following a three-hour  
 402 JA treatment (cut off: 1.4 fold; FDR< 0.05) (Supplemental Table 3), among which about  
 403 one quarter (289 genes) were de-repressed in *lhp1* (Figure 5B and Supplemental Tables 3  
 404 and 4), indicating that *LHP1* plays an important role in the repression of various  
 405 JA-responsive genes.

406 To further explore the role of LHP1 in JAZ-mediated repression of JA-inducible genes,  
 407 we overlapped the list of LHP1-enriched loci with the list of JA-induced genes, and found  
 408 that 177 LHP1-bound loci were induced by JA (Figure 5B). Moreover, we observed that JA  
 409 rise resulted in a reduction in LHP1 enrichment in a gene-body region or across gene  
 410 bodies in a majority of these 177 loci (Figure 5C). We further selected and examined six  
 411 loci (out of the 177 loci) with a varying degree of reduction in LHP1 enrichment upon JA  
 412 application (five loci with a moderate or slight reduction), based on the ChIP-seq data of  
 413 genome-wide LHP1 occupancy (Figure 6A-B). Using ChIP-qPCR, we found that all six  
 414 loci exhibited an apparent reduction in LHP1 enrichment in the examined gene-body  
 415 regions following a three-hour JA application (Figure 6A-B); in addition, these genes  
 416 indeed were upregulated by JA (Figure 6C). We further found that these six genes were all  
 417 repressed by *LHP1* (Figure 6C), and that four (*AT4G37400*, *AT4G09030*, *AT4G25020* and  
 418 *AT5G67300*) displayed a lesser response to JA treatment in *lhp1*, compared to wild-type  
 419 seedlings (Figure 6C), indicating that JA induction of these genes is partly through a

420 disruption of *LHP1* function. Taken together, these results suggest that at part of  
 421 JA-responsive loci JA rise-triggered degradation of JAZ proteins results in a disruption of  
 422 LHP1 binding, leading to a relieving of Polycomb repression and consequent de-repression  
 423 (upregulation).

## 424 DISCUSSION

425 We have uncovered that in JA signaling JAZ proteins and Polycomb factors including  
 426 LHP1 and PRC2 interact and function in concert to mediate transcriptional repression at  
 427 various JA-responsive genes in *Arabidopsis*. We further explored the role of JAZ-PcG in  
 428 stamen development, and found that loss of Polycomb repression partially rescues the  
 429 anther and pollen development abnormalities resulting from loss or block of JA signaling,  
 430 and that JAZ-mediated transcriptional repression engages and requires Polycomb proteins  
 431 at four key regulatory loci in anther and pollen development. Our study reveals a novel  
 432 chromatin-based mechanism for JAZ-mediated transcriptional repression and JA signaling  
 433 in plants.

434 In JA signaling JAZ proteins are known to be able to physically associate with  
 435 transcriptional activators (*e.g.* bHLH and MYB TFs) for JA-responsive genes to inhibit  
 436 their activities (Chini et al., 2009; Fernandez-Calvo et al., 2011; Niu et al., 2011; Qi et al.,  
 437 2015; Qi et al., 2011; Zhang et al., 2015; Zhu et al., 2011b). In addition, it has been shown  
 438 that most JAZs interact with the NINJA adaptor, which engages the TPL co-repressor and  
 439 its associated histone deacetylases (HDACs) to repress JA-responsive genes (Long et al.,  
 440 2006; Pauwels et al., 2010). Furthermore, it has been reported that JAZ1 directly interacts  
 441 with the HISTONE DEACETYLASE 6 (HDA6) to repress the expression of several genes  
 442 that are co-regulated by JA and the plant hormone ethylene, and thus integrate the JA and  
 443 ethylene signaling pathways (Zhu et al., 2011b). These findings reveal that histone  
 444 deacetylation plays a role in JAZ-mediated transcriptional repression; however, both *tpl* and  
 445 *hda6* exhibit a weak JA-response phenotype (Pauwels et al., 2010; Zhu et al., 2011b),  
 446 suggesting that JAZs and NINJA may engage additional repressive chromatin modifiers for

447 transcriptional repression. In this study, we found that NINJA and a few JAZ proteins  
 448 associate with the PcG factors including LHP1 and EMF2 /PRC2; notably, the H3K27me3  
 449 reader LHP1 physically associates and functions together with PRC2 for H3K27  
 450 trimethylation in the Arabidopsis genome (Derkacheva et al., 2013). These findings reveal  
 451 that a JAZ protein, NINJA, LHP1 and PRC2 can associate together, and such an assembly  
 452 may interact with TPL-HDACs and conduct multiple repressive chromatin modifications  
 453 such as H3K27 trimethylation and histone deacetylation to establish a repressive chromatin  
 454 environment at part of JA-responsive loci for transcriptional repression (Figure 7).

455 We have observed that seven of out of the 13 Arabidopsis JAZ proteins, including  
 456 JAZ1, JAZ3, JAZ4, JAZ6, JAZ8, JAZ9 and JAZ10, interact with LHP1, among which six  
 457 JAZs interact with EMF2. In our yeast two-hybrid (YTH) assays, we noticed that several  
 458 JAZ fragments, but not full-length proteins, interacted with LHP1 and/or EMF2 in the  
 459 GAL4-based YTH (Figure 1 and Supplemental Figure 2). One possibility is that in yeast  
 460 cells the LHP1/EMF2-interacting interfaces (e.g. the ZIM domains) in these full-length  
 461 JAZs may not be properly configured /exposed to mediate protein interactions. Notably, in  
 462 our GAL4-based YTH no interaction was observed between the remaining five JAZs and  
 463 LHP1 or EMF2; it is likely that other protein-protein interaction assays such as LexA-based  
 464 YTH, BiFC and co-IP may uncover additional LHP1/PRC2-interacting JAZs.

465 Our analysis of genome-wide LHP1 occupancy in response to JA and *LHP1* function in  
 466 repression of individual JA-responsive genes, characterization of JAZ- and PcG-mediated  
 467 transcriptional repression of the regulatory TFs in anther development, and genetic rescues  
 468 of loss or block of JA signaling by PcG mutants together reveal that Polycomb repression  
 469 plays an important role to regulate part of JA-responsive genes. These findings, thus far,  
 470 show that there are three mechanisms for JAZ-mediated transcriptional repression: direct  
 471 inhibition of a transcription activator by JAZs, histone deacetylation primarily by  
 472 JAZ-NINJA-TPL-HDAC (at certain loci by JAZ1-HDAC or JAZ8-TPL-HDAC) and  
 473 Polycomb repression by JAZ-NINJA-PcG. Individual JAZs at discrete target loci may  
 474 engage one or a combination (two or three) of these mechanisms for transcriptional  
 475 repression. It is noteworthy that Polycomb repression or PcG factors are involved in

476 transcriptional repression of part of the JA-responsive loci.

477 We have found that NINJA-JAZs and PcG factors function together to mediate  
 478 transcriptional repression of various JA-responsive genes, but the extent of how  
 479 NINJA-JAZs and PcG function collaboratively may vary at individual loci. At the six  
 480 examined loci that are directly repressed by LHP1, JA rise causes a great reduction in LHP1  
 481 enrichment and induces these gene expression (Figure 6). We observed that LHP1 was not  
 482 fully eliminated at these six loci following the three-hour JA application, indicating that the  
 483 remaining JAZs at these loci may partly stabilize LHP1 binding or that LHP1 binding is not  
 484 totally dependent on JAZs. Moreover, in addition to LHP1, other H3K27me3 readers may  
 485 bind to these JA-responsive loci, and a possible reduction in their occupancy in response to  
 486 JA may contribute to these gene induction by JA in *lhp1* (Figure 6C). At the four key  
 487 regulatory genes of anther development (*DYT1*, *AMS*, *MS1* and *JAZ1*), their repression by  
 488 JAZs, as reflected by *JAZ1ΔJas*, essentially requires Polycomb factors (LHP1 and PRC2)  
 489 (Figures 3A and 4). Interestingly, the Polycomb repression at these four loci, as reflected by  
 490 CLF/PRC2 enrichment, appears to be partly dependent on JAZ proteins, because CLF  
 491 enrichment is reduced (but not eliminated) at these loci upon JA rise (Figure 3B). In short,  
 492 our findings reveal that JAZs and Polycomb factors directly interact and function together  
 493 to mediate repression of JA-responsive genes.

494 Many JA-responsive genes respond to JA rise only in particular tissues and/or at certain  
 495 developmental stages (Browse, 2009; Pauwels and Goossens, 2011); hence, JAZs are  
 496 involved in these gene regulation only in certain tissues and/or stages. For instance, JAZs  
 497 are involved in *AMS* and *MS1* repression only at late anther development, but not at early  
 498 development; therefore, JAZ proteins are required for PRC2 enrichment at *AMS* and *MS1*  
 499 only at late anther development (Figures 3B and 4), whereas the binding of PRC2 to both  
 500 loci at early anther development is independent of JAZs.

501 In response to JA, levels of the repressive chromatin mark H3K27me3 (deposited by  
 502 CLF/PRC2) at certain JA loci are reduced over a time scale of hours, as exemplified by the  
 503 regulatory loci of anther development (*DYT1*, *AMS*, *MS1* and *JAZ1*). The levels of  
 504 H3K27me3 can be dynamically modulated by H3K27 methyltransferases (PRC2) and

505 de-methylases (Mozgova and Hennig, 2015; Xiao et al., 2016). The prompt loss of  
 506 H3K27me3 at a JA-responsive gene following loss of CLF binding suggests that there is  
 507 active H3K27 demethylation at JA-responsive loci. Indeed, a previous study in rice has  
 508 revealed that a JmjC domain-containing H3K27 demethylase mediates the removal of  
 509 H3K27me3 at several JA-responsive genes (Li et al., 2013). Hence, JAZ-NINJA-PcG  
 510 (PRC2) and H3K27 demethylases may act antagonistically to dynamically control the  
 511 levels of H3K27me3 at certain JA-regulated loci.

512 The readily noticeable developmental phenotype resulting from a defect in JA  
 513 biosynthesis or signaling under normal growth conditions, is male sterility. Our study  
 514 reveals that JAZs function together with Polycomb factors to regulate master TFs in the  
 515 genetic pathway for anther and pollen development and thus this developmental process, in  
 516 line with the essential role of JA for male sterility including pollen maturation and anther  
 517 dehiscence (Browse, 2009; Song et al., 2013). Developing pollen grains are encased in the  
 518 nutritive tapetum that provides nourishment and plays an important role in male meiosis  
 519 progression and pollen grain maturation (Gomez et al., 2015; Lei and Liu, 2019; Zhu et al.,  
 520 2011a). The master TFs, *DYT1*, *AMS* and *MS1*, act in tapetum initiation, function and  
 521 degradation, respectively, and loss-of-function *dyt1*, *ams* and *ms1* mutants are male sterile  
 522 due to tapetum dysfunction and premature microspore or pollen degeneration (Gomez et al.,  
 523 2015; Xu et al., 2014; Yang et al., 2007; Zhang et al., 2006; Zhu et al., 2011a). We found  
 524 that JAZ-PcG mediates transcriptional repression of *DYT1* in early anther development and  
 525 *AMS* and *MS1* in late development. JA synthesis is initiated in early anther development at  
 526 around stage 6 (Ito et al., 2007), at which *DYT1* is expressed (Zhang et al., 2006; Zhu et al.,  
 527 2011a). JA rise in late anther development conceivably causes JAZ degradation, relieving  
 528 of Polycomb repression at and transcriptional activation of both *AMS* and *MS1*. Defects in  
 529 pollen grain maturation and anther dehiscence in loss /block of JA signaling in *coi1* and the  
 530 *JAZ1ΔJas* line may be attributed to a dysfunction of tapetum development, function and/or  
 531 degradation resulting from the constitutive JAZ-PcG-mediated transcriptional repression of  
 532 *DYT1*, *AMS* and *MS1*. In short, our study has revealed a previously undescribed role for  
 533 JAZs in anther development: engaging Polycomb factors to regulate master TFs for tapetum

534 development, function and degradation. Notably, in addition to JA-mediated anther  
 535 development, we observed that Polycomb repression is also involved in root growth  
 536 inhibition by JA, as both *clf* and *lhp1* primary roots display a hypersensitive response to JA  
 537 treatment (Supplemental Figure 9).

538       Turning on the expression of the master regulators *DYT1*, *AMS* and *MS1* in anther  
 539 development requires not only to remove JAZs and PcG factors but also a transcriptional  
 540 activator, as *DYT1*, and *AMS* and *MS1* are not expressed in late and early anther  
 541 development upon loss of Polycomb repression, respectively. *AMS* and *MS1* expression is  
 542 activated by *MYB35* and *MYB80*, respectively (Gomez et al., 2015). It has been shown that  
 543 MYB TFs including MYB21 and MYB26 associate with MYC2 to MYC5 to mediate  
 544 transcriptional activation of JA-responsive genes in anther development (Qi et al., 2015;  
 545 Song et al., 2011); whether *MYB35* and *MYB80* function together with these TFs to  
 546 activate *AMS* and *MS1* expression in response to JA, remains to be determined in future  
 547 study.

548       Polycomb silencing, first discovered in *Drosophila*, is well known to regulate  
 549 developmental genes and maintain cell type-characteristic gene expression patterns and  
 550 thus cell identity in plants and animals (Lanzuolo and Orlando, 2012; Mozgova and Hennig,  
 551 2015). We have uncovered a previously unrecognized role of Polycomb repression for  
 552 hormone signaling. In response to JA, the binding of CLF (PRC2) to the examined  
 553 JA-regulated master TFs of anther and pollen development is dynamically regulated, and a  
 554 change can occur as soon as within three hours, leading to swift changes in H3K27  
 555 trimethylation and thus transcriptional activation of JA-responsive genes. JA is synthesized  
 556 in response to developmental cues (*e.g.* anther and pollen development) and environmental  
 557 stimuli, such as wounding, insect chewing and pathogen infections (Campos et al., 2014;  
 558 Wasternack and Hause, 2013). The levels of JA in plant tissues are dynamically controlled  
 559 to ensure proper growth and development as well as prompt and adequate response to biotic  
 560 and abiotic stresses. Our findings show that the evolutionarily-conserved PcG-mediated  
 561 Polycomb silencing mechanism is utilized to dynamically modulate gene expression in  
 562 response to a hormone in plants.

563 **METHODS**564 **Genetic Materials and Plant Growth Condition**

565 The Arabidopsis mutants including *clf-81*, *coi1-1*, *lhp1-3*, the *LHP1:GFP* line, the  
 566 *CLF:GFP* line, the *EMF2:VENUS* line and the *JAZ1ΔJas* line were described previously  
 567 (Gu et al., 2014b; Schubert et al., 2006; Sun et al., 2014; Sung et al., 2006; Thines et al.,  
 568 2007; Xie et al., 1998). Plants were grown in long days (16-h light/8-h dark) under cool  
 569 white fluorescence light at about 22°C. All the transgenic plants created were in the  
 570 Columbia (Col) background. The *JAZ1ΔJas* line was maintained as a heterozygote via  
 571 pollination by Col-0 (WT).

572 **Yeast Two-Hybrid (Y2H) Analysis**

573 The Y2H library screening was conducted by Hybrigenics Services (Paris, France) using  
 574 *LHP1* as a bait to screen an Arabidopsis cDNA library prepared from 1-week-old seedlings.  
 575 Y2H assays were conducted using *Matchmaker GAL4 Two-Hybrid System* (Clontech)  
 576 following the manufacturer's instructions. Synthetic media without tryptophan, leucine,  
 577 histidine, and adenine were used for yeast growth.

578 **Plasmid Construction**

579 For *JAZ4:Flag* construction, the full-length *JAZ4* coding sequence except the stop codon  
 580 (0.9 kb) was fused in frame with a *Flag* tag (three copies) in *pPZPY112* vector  
 581 (Hajdukiewicz et al., 1994). To create *pNINJA-NINJA:Flag* construction, a 2.8-kb genomic  
 582 fragment of *NINJA* (including a 1.5-kb promoter region) without the stop codon was fused  
 583 in frame with a *Flag* tag (three copies) and then inserted into the *pHGW* vector (Karimi et  
 584 al., 2005) via *Gateway technology*.

585 To generate *GST:LHP1* and *GST:EMF2*, the full length coding sequence for *LHP1* (1.3  
 586 kb) or *EMF2* (1.9 kb) was cloned into *pGEX-4T-1* vector in-frame with the glutathione  
 587 S-transferase coding sequence. To create *JAZ1:His*, *JAZ4:His* and *NINJA:His*, the coding

588 sequence of *JAZ1* (0.8 kb), *JAZ4* (0.9 kb) or *NINJA* (1.3 kb) was cloned into *pET-28* in  
 589 frame with six copies of *His*.

590 To create *pLHP1-LHP1:Flag*, a 3.5-kb region encompassing the *LHP1* gene including  
 591 1.4 kb upstream of the start codon and the entire genomic coding sequence (minus the stop  
 592 codon), was fused in frame with a *Flag* tag (nine copies) and subsequently inserted into  
 593 *pBGW* (Karimi et al., 2005) via *Gateway technology*.

#### 594 **BiFC Assay**

595 Full-length coding sequences for *LHP1* (with the stop codon) and *NINJA* (minus the stop  
 596 codon) were cloned in frame with the coding sequence for an N-terminal EYFP fragment in  
 597 the *pSAT1-nEYFP-C1* vector and a C-terminal EYFP fragment in the *pSAT1A-cEYFP-N1*  
 598 vector (Lee et al., 2008). Two constructs were co-infected into onion epidermal cells by  
 599 bombardment using *Helium Biolistic Gene Transformation System* (Bio-Rad). Transfected  
 600 onion epidermal cells were cultured under consistent light for 24 h and EYFP fluorescence  
 601 was observed under a Zeiss *LSM 5 EXCITER* upright laser scanning confocal microscopy  
 602 (Zeiss).

#### 603 **Co-immunoprecipitation**

604 Co-IP assays were conducted as described previously with minor modifications (Jiang et al.,  
 605 2011). Briefly, 10-day-old seedlings expressing respective tagged proteins were treated with  
 606 10 µM MG132 (Calbiochem) for 6 h before harvest. Total proteins were extracted and  
 607 immunoprecipitated with anti-Flag M2 affinity gel (Sigma) and anti-HA affinity gel  
 608 (Sigma), followed by western blotting using anti-Flag (Sigma), anti-HA (Roche) or  
 609 anti-GFP (Invitrogen).

#### 610 **Protein Pulldown Assay**

611 Tagged *LHP1*, *EMF2*, *JAZ1*, *JAZ4* and *NINJA* were introduced into the *E.coli* BL21 (DH3)  
 612 strain, and the expression of fusion proteins were induced by IPTG at 30°C for 4 h  
 613 (GST:LHP1 and NINJA:His) or at 16°C overnight (GST:EMF2, JAZ1:His and JAZ4:His).

614 Cell cultures were harvested by centrifugation and resuspended in 2.0-ml PBS (Phosphate  
615 Buffered Saline) (pH 7.4) with 1x Roche protease inhibitor, followed by sonication and  
616 centrifugation. To examine physical association of paired proteins, equal volume of  
617 supernatants with mixed and further incubated at 4°C for 2 hr, followed by pulldown of  
618 Glutathione-linked resins (20 µl per mixture; Sigma). The resins were washed three times  
619 with PBS; subsequently, immunoblotting was conducted with anti-His (Sigma-Aldrich) to  
620 detect JAZ1:His, JAZ4:His or NINJA:His in the pulldowns.

621 **Pollen Germination Assay**

622 Mature pollen grains were harvested and cultured in the liquid medium containing 2 mM  
623 CaCl<sub>2</sub>, 1.65 mM boric acid and 17% sucrose (pH 7.0) (Yuan et al., 2009). Pollen grains  
624 were cultured under consistent light for 24 h and checked under light microscope. More  
625 than one hundred pollen grains were counted from individual plants, and three biological  
626 replicates were carried out.

627 **JA Application**

628 JA (Sigma) was dissolved in ethanol at 25 mM (stock) and diluted in water before use. For  
629 JA-responsive gene expression analyses in seedlings (RNA-seq), six day (d)-old seedlings  
630 grown in liquid half-strength MS media supplemented with 1% sucrose was treated with  
631 5-µm JA (JA stock was added into the liquid media; diluted 5,000x). To analyze  
632 JA-responsive gene expression in anther development, inflorescences were sprayed with  
633 5-µM JA, 3 h before harvest (mock: 5,000x diluted ethanol). For JA-mediated root growth  
634 inhibition assay, seedlings (3 d after germination) were transferred to half-strength MS  
635 plates supplemented with 10 µM JA (mock: 2,500x diluted ethanol), and root length was  
636 scored 7 d later.

637 **Gene Expression Analysis**

638 For anther development gene expression analysis, inflorescence with floral buds were  
639 treated with 5 µM Jasmonic acid (Sigma) for 3 h before harvest. Closed floral buds were

640 collected and separated into two groups: buds with emerging petal primordia (harvested as  
 641 a mixture of approximate flower development stages 1 to 9, bearing early-developing  
 642 anthers approximately on stages 1 to 7), and a mixture of buds from the ones with short  
 643 stamens to the ones about to open (approximate flower development stages 10-12 with  
 644 late-development anthers approximately on stages 8 to 12) (Sanders et al., 1999; Smyth et  
 645 al., 1990). Total RNAs were extracted using the *RNeasy mini* kit (Qiagen) according to the  
 646 manufacturer's instructions, and subsequently were reverse-transcribed into cDNAs,  
 647 followed by quantitative PCR in *QuantStudio 6 Flex Real-Time PCR System* with *SYBR*  
 648 *Green PCR master mix* (ABI). All expression analyses were conducted with three  
 649 biological repeats. Melting curve was analyzed to check the specificity of amplified  
 650 products. The relative RNA expression levels were normalized to *TUB2* following the  $2^{-\Delta\Delta Ct}$   
 651 method (Livak and Schmittgen, 2001). Primers for RNA expression analysis are listed in  
 652 Supplemental Table 5.

653 For RNA-seq analysis, total RNAs were extracted from 6 day-old seedlings treated  
 654 with 5- $\mu$ m JA or mock; subsequently, mRNAs were purified using the *NEBNext Poly(A)*  
 655 *mRNA Magneic Isolation Module* kit (New England Biolabs /NEB), and cDNA libraries  
 656 were constructed using the *NEBNext Library Prep* kit (NEB) and further sequenced with  
 657 NovaSeq 6000 (Illumina). Paired-end reads of RNA-seq libraries were mapped to Col-0  
 658 genome (TAIR10) using TopHat with default parameters (Trapnell et al., 2009). The read  
 659 counts of the genes were calculated by htseq-count tool of HTSeq (Anders et al., 2015), and  
 660 CPM (counts per million mapped) or RPKM (reads per kilo base per million mapped) was  
 661 used to measure the expression level of a gene. edgeR was applied to identify the  
 662 differentially expressed genes (DEGs) with FDR<0.05 and more than 1.4 fold-change  
 663 (Robinson et al., 2010). Three biological replicates were conducted for each sample. The  
 664 statistical test of the overlap between two gene lists was performed at  
 665 <http://nemates.org/MA/progs/overlap stats.html>.

## 666 Chromatin Immunoprecipitation (ChIP)

667 ChIP assays were carried out as previously described with minor modifications (Wang et al.,

668 2014). CLF-, CLF:GFP- or LHP1:Flag-bound or H3K27me3-marked chromatin was  
 669 immunoprecipitated with anti-CLF (Tao et al., 2019), anti-GFP (Abcam), anti-Flag M2  
 670 magnetic beads (Sigma) or anti-H3K27me3 (Millipore), respectively, and the amount of  
 671 each precipitated DNA fragment was measured by qPCR using *QuantStudio 6 Flex*  
 672 *Real-Time PCR System* (ABI), and subsequently normalized to an internal control (*TUB2* or  
 673 *TUB8*) or input DNA. Primers are listed in Supplemental Table 6. Three biological repeats  
 674 were conducted.

675 **ChIP-seq**

676 Nuclei were extracted from liquid-cultured *LHP1:Flag* seedlings as previously described  
 677 (Louwers et al., 2009), followed by chromatin immunoprecipitation with anti-Flag M2  
 678 magnetic beads (Sigma). DNA libraries were constructed using *NEBNext Ultra II DNA*  
 679 *Library Prep Kit for Illumina* (NEB), and subsequently were sequenced on NovaSeq 6000  
 680 (Illumina). Three biologically independent samples treated by 5 $\mu$ M JA or mock (5000x  
 681 diluted ethanol) were analyzed.

682 Raw paired-end reads (21 to 26 million per sample) were cleaned by Trimomatic  
 683 (Bolger et al., 2014), to trim the adapter sequences, remove low-quality bases and filter  
 684 short reads (< 36 nt); subsequently, the clean reads were mapped to the *Arabidopsis*  
 685 *thaliana* genome (TAIR10) by Bowtie2 (Langmead and Salzberg, 2012) using default  
 686 parameters. Mapped reads with low mapping quality (MAPQ < 30) or with multiple  
 687 positions in the genome were eliminated by SAMtools (version 1.3, Li et al., 2009).  
 688 Enriched peaks were identified by MACS2 (version 2.2.7.1, (Zhang et al., 2008) with a  
 689 cut-off *p* value 0.01. Target-gene regions were set as the range from 1.0 kb upstream of  
 690 transcription start site (TSS) to 1.0 kb downstream of transcription end site (TES). Target  
 691 genes were defined by overlapping of the peaks from at least two of the three  
 692 biologically-independent samples, and were annotated by annotatePeak function in  
 693 ChIPseeker package (Yu et al., 2015).

694 The read coverage (reads per million/RPM, at single nucleotide resolution) was

695 generated by genomecov in BEDTools (version 2.25.0, Quinlan and Hall, 2010), and RPM  
 696 values were converted to bigwig files by *wigToBigwig* from the *UCSC Kent* software  
 697 package. Scores of read coverage of LHP1 occupancy in the JA- and mock-treated  
 698 seedlings were calculated by computeMatrix in deepTools (version 2.4.1, Ramirez et al.,  
 699 2016) with bigwig files, and visualization of the scores was performed by plotProfile in  
 700 deepTools (version 2.4.1, Ramirez et al., 2016). Read coverage of individual genes was  
 701 visualized by the Gviz package (Hahne and Ivanek, 2016).

702 To examine the differences of LHP1 occupancy in JA-treated vs mock, at the 177  
 703 JA-responsive loci that are bound by LHP1, RPM score differences (JA minus mock) over  
 704 these loci (gene body plus 0.5 kb upstream TSS and 0.5 kb downstream TES) were  
 705 calculated by computeMatrix in deepTools (version 2.4.1, Ramirez et al., 2016) with bigwig  
 706 files, and visualized by plotProfile and plotHeatmap in deepTools (version 2.4.1, Ramirez  
 707 et al., 2016).

708 To validate ChIP-seq results, ChIP assays with anti-Flag M2 magnetic beads (Sigma)  
 709 were performed in three biologic replicates. qPCR was conducted to examine LHP1:Flag  
 710 enrichment at selected genes. Level of each amplified DNA fragment was normalized to  
 711 *TUB2*. Primers were listed in Supplemental Table 6.

## 712 Data Availability

713 The raw RNA-seq and ChIP-seq data are available at the NCBI GEO repository under  
 714 accession number GSE167639.

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978 **Figure Legends**

979 **Figure 1. NINJA and JAZ proteins interact with LHP1 and EMF2.** (A) JAZs interacted  
 980 with LHP1 and EMF2 in yeast cells. Full-length LHP1 and EMF2 were fused with the  
 981 GAL4-BD domain and full-length JAZ1, JAZ4, JAZ8 and JAZ10 and a fragment of JAZ3,  
 982 JAZ6 or JAZ9, were fused with GAL4-AD. Yeast cells were spotted on the stringent  
 983 selective medium without Ade (A), His (H), Leu (L) and Trp (W). (B) JAZ1, through its  
 984 ZIM domain containing fragment (aa 101-180), but not Jas (aa 181-253), interacted with  
 985 LHP1 in yeast cells. (C) JAZ4, through its ZIM domain (aa143-178), interacted with LHP1  
 986 and EMF2 in yeast cells. (D) Co-immunoprecipitation assay of JAZ1:HA with LHP1:GFP.  
 987 Anti-HA affinity gel was used to immunoprecipitate LHP1:GFP from the F<sub>1</sub> seedlings of  
 988 doubly hemizygous *JAZ1:HA* and *LHP1:GFP*, followed by western blotting using anti-HA  
 989 and anti-GFP antibodies. Seedlings were pre-treated with the protease inhibitor MG132  
 990 before harvest. (E-F) Co-immunoprecipitation assays of JAZ4:Flag with LHP1:GFP (E)  
 991 and EMF2:VENUS (F). Anti-Flag M2 affinity gels were used to immunoprecipitate  
 992 LHP1:GFP from the seedlings expressing both *JAZ4:Flag* and *LHP1:GFP*, or  
 993 EMF2:VENUS from the seedlings expressing both *JAZ4:Flag* and *EMF2:VENUS*,  
 994 followed by western blotting using anti-Flag and anti-GFP. Seedlings were pre-treated with  
 995 MG132 before harvest. (G-H) Co-IP assays of NINJA:Flag with LHP1 (G) and EMF2 (H).  
 996 Total protein extracted from the F<sub>1</sub> seedlings of doubly hemizygous *NINJA:Flag* and  
 997 *LHP1:GFP* or *NINJA:Flag* and *EMF2:VENUS*, were subjected to immunoprecipitations  
 998 with anti-Flag M2 affinity gels, followed by western blotting with anti-Flag and anti-GFP.  
 999 Seedlings were pre-treated with MG132 before harvest.

1000 **Figure 2. *lhp1* and *clf* partly rescue anther and pollen abnormalities in *coi1* and  
 1001 *JAZ1ΔJas*.** (A-B) Rescue of the anther dehiscence defects in *coi1* (A) and *JAZ1ΔJas* (B) by  
 1002 *clf* and *lhp1*. Shown are representative scanning electron micrographs of anthers; scale bars  
 1003 for 30 μm. (C-D) *lhp1* and *clf* rescued inviable pollen grains of *coi1* (C) and *JAZ1ΔJas* (D).  
 1004 Pollen grains were cultured in a germination medium under constant light for 24 hour, and

1005 over 300 pollen grains were examined for each line, error bars for S.D. of three biological  
 1006 repeats. One-way ANOVA was conducted, and letters indicate statistically significant  
 1007 differences.

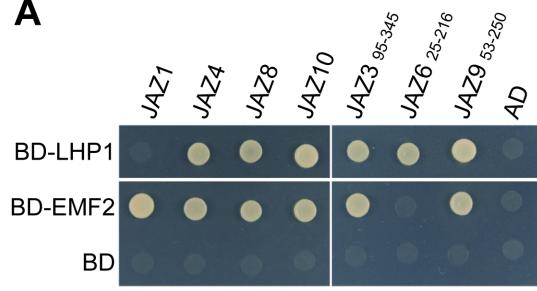
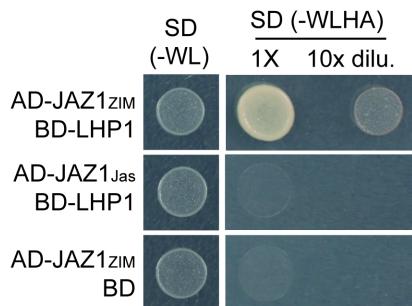
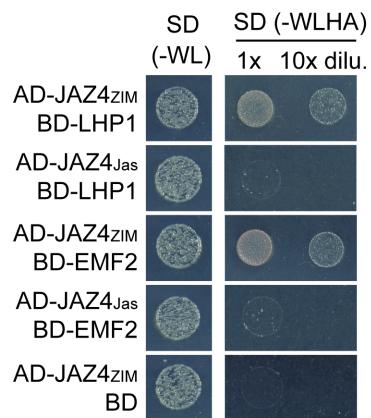
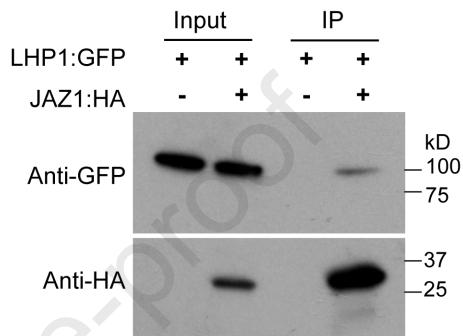
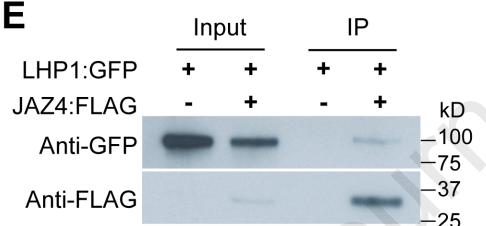
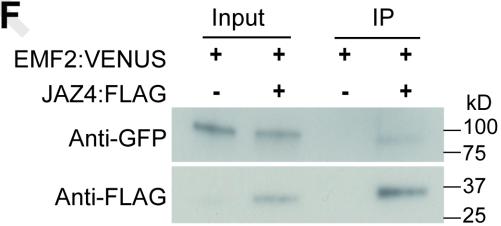
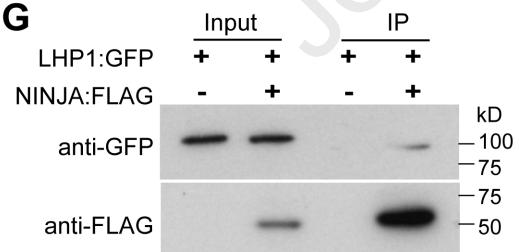
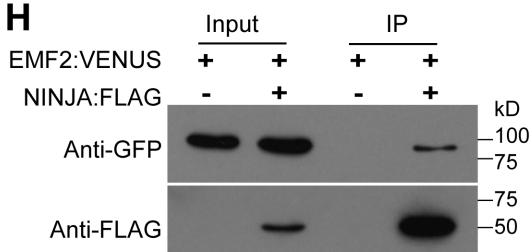
1008 **Figure 3. Functional analysis of *LHP1* and *CLF* in JAZ-mediated transcriptional  
 1009 repression of four key regulatory transcription factors in anther and pollen  
 1010 development. (A)** Expression analysis of *DYT1*, *AMS*, *MS1* and *JAZ1* in anther  
 1011 development. Floral buds were separated into two groups: buds bearing anthers  
 1012 approximately at development stages 1 to 7 (early development /microsporogenesis), and  
 1013 buds with anthers approximately at stages 8-12 (late development /microgametogenesis).  
 1014 Transcript levels were quantified by qPCR and normalized to the endogenous control  
 1015 *TUBULIN2* (*TUB2*). Error bars for S.D. of three biological repeats. One-way ANOVA was  
 1016 conducted, and letters (in the same color) denote statistically distinct groups ( $p < 0.01$ ).  
 1017 Note that *JAZ1ΔJas* is heterozygous. **(B)** ChIP analysis of CLF:GFP enrichment at *DYT1*,  
 1018 *AMS*, *MS1* and *JAZ1*. At panel bottom are schematic drawings of gene structures; the  
 1019 regions (a or b) examined by ChIP-qPCR are indicated. Floral buds of the *CLF:GFP* line  
 1020 (in *clf*) were treated with 5  $\mu$ M JA for 3 h. Relative enrichments of CLF:GFP at each  
 1021 examined region were first normalized to the endogenous control *TUBULIN8* (*TUB8*), and  
 1022 values are relative enrichments in *CLF:GFP mock* or *CLF:GFP JA* over CK (untreated  
 1023 WT). **C.** ChIP analysis of H3K27me3 in response to JA. WT floral buds were treated with 5  
 1024  $\mu$ M JA for 3 h. Levels of the immunoprecipitated DNA fragments were quantified by qPCR  
 1025 and normalized to input DNA. **(B-C)** Values are mean  $\pm$  S.D. of three biological repeats.  
 1026 Two-tailed *t* tests were conducted (\*\*  $p < 0.01$ ).

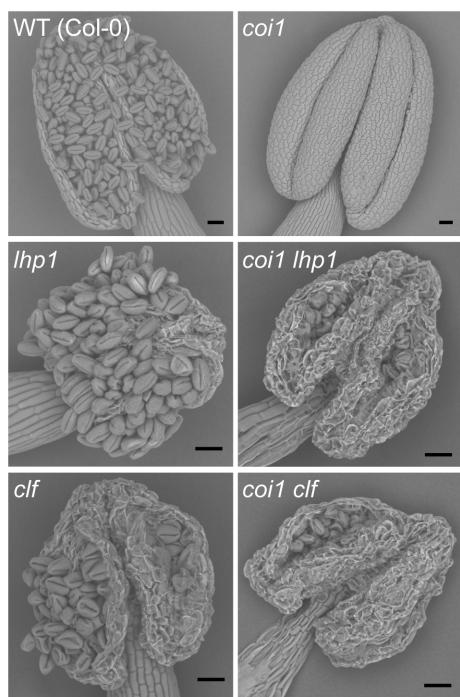
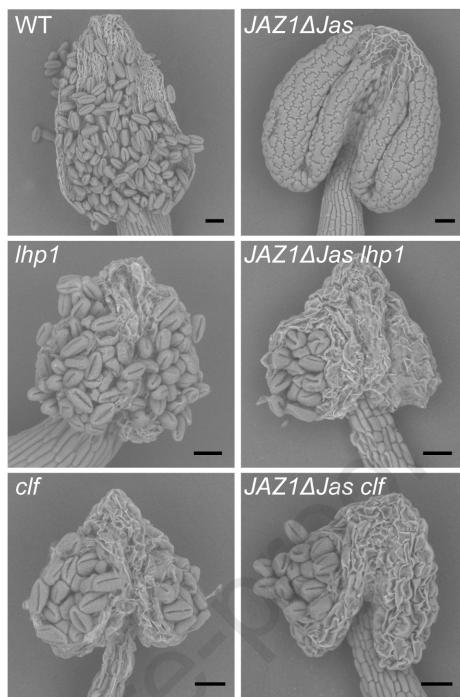
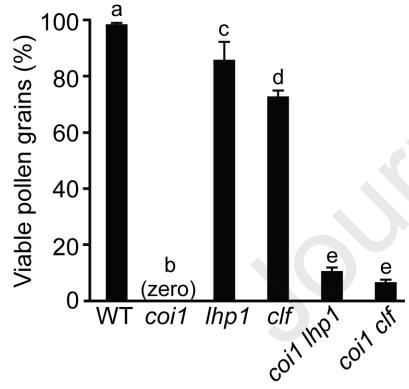
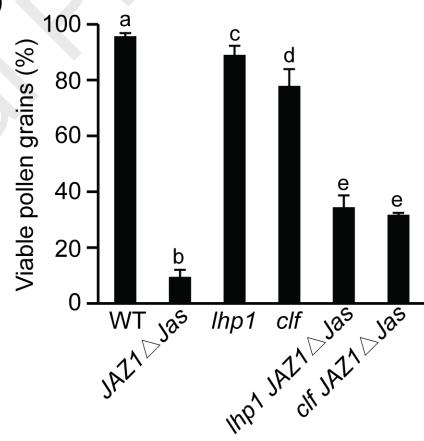
1027 **Figure 4. ChIP analysis of CLF enrichment at *DYT1*, *AMS*, *MS1* and *JAZ1* in anther  
 1028 development in the gain-of-function *JAZ1ΔJas* line.** Immunoprecipitated DNA fragments  
 1029 by rabbit polyclonal anti-CLF were quantified by qPCR. Values are relative CLF  
 1030 enrichments in WT and *JAZ1ΔJas* over CK (WT immunoprecipitated by rabbit serum), and  
 1031 mean  $\pm$  S.D. of three biological repeats. Two-tailed *t* tests were conducted (\*\*  $p < 0.01$ ).

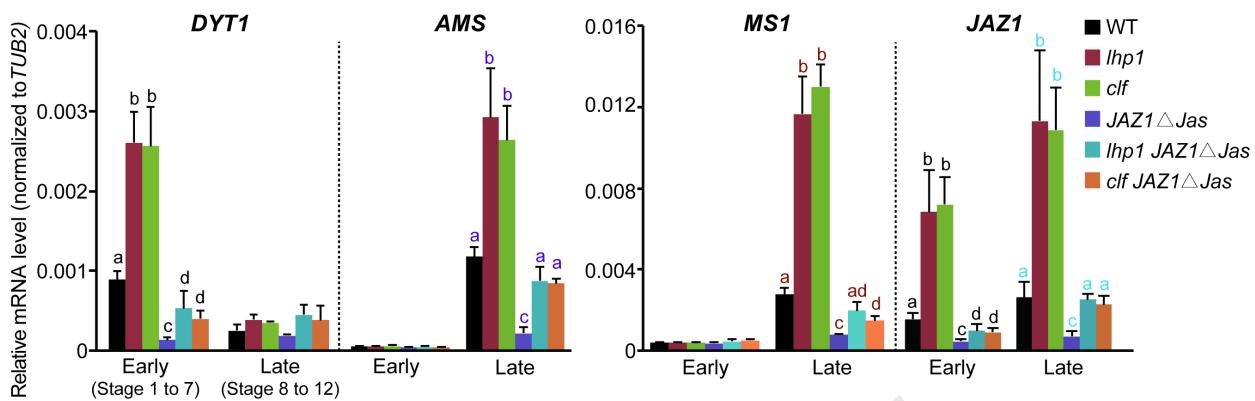
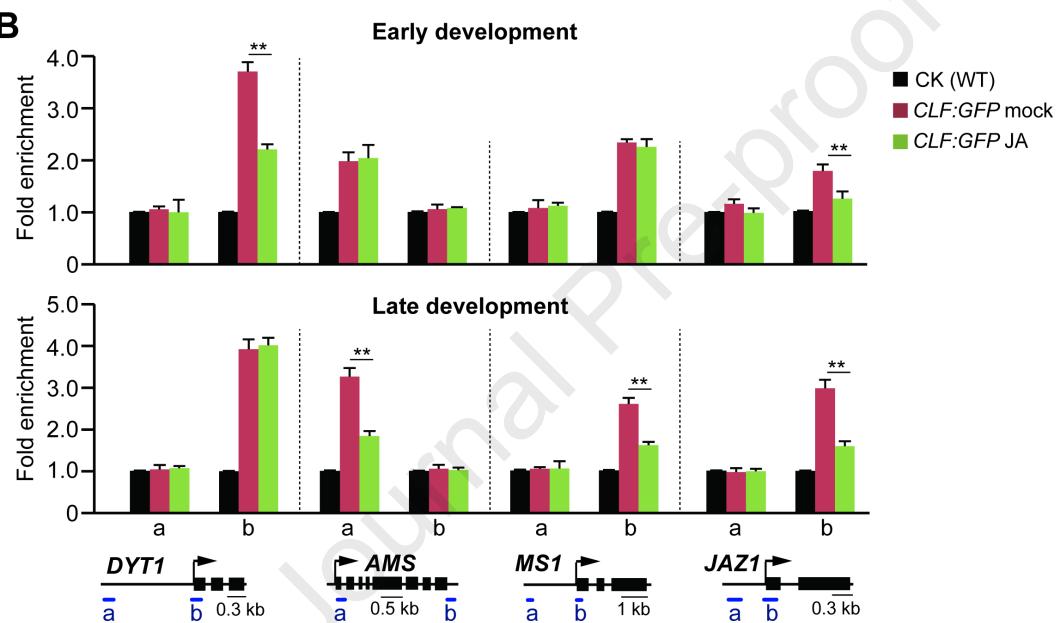
1032 **Figure 5. LHP1 mediates repression of various JA-responsive genes in the *Arabidopsis***  
 1033 **genome.** **(A)** Metagene plots of LHP1:Flag occupancy at target loci in response to JA  
 1034 application. The y-axis scales represent read coverage (RPM /reads per million, average of  
 1035 three biological replicates). TSS, transcription start site; TES, transcription end site. Plotted  
 1036 gene-body regions are divided into bins and scaled to 2 kb. **(B)** Overlapping of the  
 1037 LHP1-bound loci (in mock) with JA-induced genes or the genes de-repressed in the *lhp1*  
 1038 mutant. RNA-seq analyses were conducted in three biological repeats. *P* values of the  
 1039 indicated overlappings are denoted (Fisher's exact test). **(C)** Heatmap showing the read  
 1040 coverage difference in LHP1:Flag occupancy at the 177 JA-inducible loci in response to JA  
 1041 rise. Blue /light blue color indicates a reduction of LHP1:Flag occupancy following a  
 1042 three-hour JA application.

1043 **Figure 6. Analysis of LHP1-mediated repression in six selected JA-responsive genes in**  
 1044 **seedlings.** **(A-B)** ChIP analysis of LHP1:Flag binding to JA-responsive genes in response  
 1045 to JA application. On the top of each panel are genome browser views of LHP1:Flag  
 1046 occupancy at the indicated genes, as revealed by ChIP-seq. RPM for read coverage.  
 1047 Schematic drawings represent gene structures (black boxes for exons, and white arrows for  
 1048 transcription direction); the regions examined by ChIP-qPCR are indicated by a, b and c. At  
 1049 bottom of each panel are relative enrichments of LHP1:Flag at the regions examined by  
 1050 ChIP-qPCR. The fold enrichments were first normalized to the endogenous control *TUB2*,  
 1051 followed by normalization to CK (wild type). Error bars for S.D. of three biological repeats.  
 1052 Asterisks denote statistically-significant difference revealed by two-tailed *t* tests (\* *p*<0.05,  
 1053 \*\* *p*<0.01, and \*\*\**p*<0.001). **(C)** Analysis of JA induction of LHP1-bound loci. Transcript  
 1054 levels in 5 μM JA- or mock-treated (3 hr) samples were quantified by qPCR and  
 1055 normalized to *TUB2*. Folds of JA induction in WT and *lhp1* are denoted by values above  
 1056 graph bars. Error bars for S.D. of three biological replicates. The letters in the same color  
 1057 indicate statistically significant difference (*p* <0.05, one-way ANOVA).

1058 **Figure 7. A working model for Polycomb repression to negatively modulate JA**  
1059 **responses.** At the resting state (without bioactive JA), on one hand, JAZ proteins physically  
1060 interact with a transcription factor (e.g. MYC2) to inhibit its activity for transcriptional  
1061 activation; on another hand, JAZs and the co-adaptor NINJA directly associate with PcG  
1062 factors (LHP1 and CLF-PRC2), and such an assembly may contain a TPL-HDAC module  
1063 and conduct multiple repressive chromatin modifications such as H3K27 trimetylation and  
1064 histone deacetylation to establish a repressive chromatin environment at part of  
1065 JA-responsive loci for transcriptional repression. Upon JA rise, JAZs are targeted for  
1066 degradation by 26S proteasomes; subsequently, the repressive chromatin modifiers are  
1067 partly lost at JA loci. Furthermore, a JmjC domain-containing demethylase may remove  
1068 H3K27me3 at certain JA loci, as indicated by a previous study in rice (Li et al., 2013).  
1069 These actions, together with a functional release of the JAZ-inhibited transcription factors,  
1070 result in transcriptional activation. Soon after the transmission of JA signal (JA decline),  
1071 newly-synthesized JAZ proteins re-load the repressive chromatin modifiers, leading to  
1072 de-sensitization of the cell to JA.

**A****B****C****D****E****F****G****H**

**A****B****C****D**

**A****B****C**