1   **Short title: *AtMOB1s* regulate jasmonate and development**

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11   **Title: *AtMOB1* genes regulate jasmonate accumulation and plant development**

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19   **One-sentence summary**

20   A core component of the Hippo pathway plays important roles in regulating jasmonate

21   accumulation and plant development in Arabidopsis.

22

23   **Author contributions**

24   Y.C. conceived and designed research; Z.G., X.Y., X.C., and L.S. performed the

25   experiments; Z.G. and Y.C. analyzed data; Z.G. and Y.C. wrote the paper.

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Abstract

The MOB1 proteins are highly conserved in yeasts, animals, and plants. Previously, we showed that the Arabidopsis *MOB1A* gene (*AtMOB1A/NCP1*) plays critical roles in auxin-mediated plant development. Here, we report that *AtMOB1A* and *AtMOB1B* redundantly and negatively regulate jasmonate (JA) accumulation and function in Arabidopsis development. The two *MOB1* genes exhibited similar expression patterns and the MOB1 proteins displayed similar subcellular localizations and physically interacted in vivo. Furthermore, the *atmob1a atmob1b* (*mob1a/1b*) double mutant displayed severe developmental defects, which were much stronger than those of either single mutant. Interestingly, many jasmonate-related genes were up-regulated in *mob1a/1b*, suggesting that AtMOB1A and AtMOB1B negatively regulate the JA pathways. *mob1a/1b* plants accumulated more JA and were hypersensitive to exogenous JA treatments. Disruption of *MYC2*, a key gene in JA signaling, in the *mob1a/1b* background partially alleviated the root defects and JA hypersensitivity observed in *mob1a/1b*. Moreover, the expression levels of the *MYC2*-repressed genes *PLT1* and *PLT2* were significantly decreased in the *mob1a/1b* double mutant. Our results showed that *MOB1A/1B* genetically interact with *SIK1* and antagonistically modulate JA-related gene expression. Taken together, our findings indicate that AtMOB1A and AtMOB1B play important roles in regulating JA accumulation and Arabidopsis development.

Key words: Hippo pathway, *MOB1A*, *MOB1B*, *SIK1*, jasmonate, Arabidopsis, root, development

Introduction

The Hippo signaling pathway was first described in animals. It plays pivotal roles in controlling cell proliferation, apoptosis, organ growth, and tissue homeostasis (Pan, 2010). Dysregulation of the pathway causes various cancers (Harvey et al., 2013). The

3

67   core components of the pathway, including the Ste20-like kinases MST1/2, the AGC

68   kinase NDR/LATS, and the kinase regulators MOB1 and Sav, form a kinase cascade.

69   Sav interacts with MST1/2 and activates its kinase activity. MST1/2 phosphorylates

70   NDR/LATS kinase and MOB1. Subsequently, MOB1 interacts with NDR/LATS and

71   regulates kinase activity of the latter. The activated NDR/LATS in turn phosphorylates

72   and inactivates the transcriptional co-activator YAP/TAZ (Hansen et al., 2015).

73

74   The MOB1 proteins are highly conserved from yeast (*Saccharomyces cerevisiae*)

75   to plants and animals (Lai et al., 2005; Cui et al., 2016). The yeast *MOB1* is an

76   essential gene required for completion of mitosis and maintenance of ploidy (Luca

77   and Winey, 1998). In Drosophila (*Drosophila melanogaster*), disruption of

78   *MOB1/Mats* results in increased cell proliferation, defective apoptosis, and induction

79   of tissue overgrowth (Lai et al., 2005). In humans (*Homo sapiens*), among the 7

80   homologs of yeast MOB (hMOB1A, 1B, 2A, 2B, 2C, 3, 4), hMOB1A and hMOB1B

81   share more than 95% sequence identity/similarity. A biochemical characterization of

82   hMOBs showed that only hMOB1A and hMOB1B interact with both LATS1 and

83   LATS2 to regulate cell proliferation and apoptosis (Chow et al., 2010). In mouse

84   (*Mus musculus*), the *mob1a/1b* double mutant showed cancer susceptibility and

85   embryonic lethality (Nishio et al., 2012). *Mob1a/1b* double mutation in mouse liver

86   results in the death of more than half of mutant mice within 3 weeks of birth. All

87   survivors eventually develop liver cancers and die by age 60 weeks (Nishio et al.,

88   2016). In addition, tamoxifen-inducible, chondrocyte-specific *Mob1a/b*-deficient mice

89   had chondrodysplasia (Goto et al., 2018).

90

91   The Arabidopsis genome contains four *MOB1* genes: *AtMOB1A*, *AtMOB1B*,

92   *AtMOB1C*, and *AtMOB1D* (Citterio et al., 2006; Vitulo et al., 2007; Cui et al., 2016).

93   *AtMOB1A* is required for tissue patterning of the root tip, and sporophyte and

94   gametophyte development (Galla et al., 2011; Pinosa et al., 2013). Recently, we

95   reported that *AtMOB1A* plays critical roles in auxin-mediated plant development (Cui

96   et al., 2016). The *atmob1a* mutant genetically interacts with mutants in auxin

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97   biosynthesis, signaling, and transport in many developmental processes. Interestingly,

98   the defects of *atmob1a* can be fully rescued by the Drosophila *MOB1* gene *Mats,*

99   suggesting conserved gene functions among different species (Cui et al., 2016). It was

100   shown that AtMOB1A and AtMOB1B interact with SIK1, a Hippo/STE20 homolog,

101   and regulate cell proliferation and expansion in Arabidopsis (Xiong et al., 2016). These

102   findings demonstrate that AtMOB1A plays important roles in plant development.

103   However, the functions of other Arabidopsis *MOB1* genes remain to be elucidated.

104

105   Jasmonates (JAs) are a group of phytohormones including jasmonic acid and its

106   derivatives. They are important in regulating plant growth and development, and plant

107   responses to biotic and abiotic stresses. Jasmonic acid is synthesized from α-linolenic

108   acid via the octadecanoid pathway in plastids and peroxisomes. Following synthesis,

109   jasmonic acid is exported from the peroxisomes into the cytoplasm, where it is

110   conjugated with isoleucine to produce bioactive JA-Ile. In the JA signaling pathway,

111   JA-Ile promotes the interaction between the JA receptor COI1 and JAZ proteins. JAZ

112   can be ubiquitinated and degraded by the 26S proteasome, leading to the release of

113   MYC2, the major transcription factor of jasmonate-mediated gene expression.

114   Consequently, the JA responsive gene expression and JA responses are activated

115   (Huang et al., 2017).

116

117   Here we show that the *mob1a/1b* double mutant displays severe developmental

118   defects at the seedling stage. *AtMOB1B* expression was similar to that of *AtMOB1A*,

119   and the two MOB1 proteins interacted with each other in vivo. Transcriptomic

120   analysis indicated that the expression levels of many genes in the JA pathways were

121   increased in the *mob1a/1b* double mutant. JA contents were also significantly

122   increased in *mob1a/1b*. Consistently, *mob1a/1b* displayed hypersensitivity to

123   exogenous JA treatments and the expression of *MYC2*, which encodes a key

124   transcription factor in JA signaling, was increased. Moreover, *myc2* partially

125   suppressed the developmental defects of *mob1a/1b*. We found that the expression

126   levels of MYC2-repressed *PLT1* and *PLT2* in the *mob1a/1b* double mutant were

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127 significantly decreased. We conclude that AtMOB1A and AtMOB1B regulate JA

128 accumulation, and plant growth and development.

129

130 **Results**

131

132 ***AtMOB1A* and *AtMOB1B* redundantly control plant development**

133   There are four *MOB1* genes in Arabidopsis, namely *AtMOB1A*, *AtMOB1B*,

134 *AtMOB1C*, and *AtMOB1D* (Citterio et al., 2006; Vitulo et al., 2007; Cui et al., 2016).

135 *AtMOB1A* and *AtMOB1B* form a subgroup, whereas *AtMOB1C* and *AtMOB1D* form

136 another clade (Fig. 1A). Previously, we reported that the loss-of-function *MOB1A*

137 mutants, *mob1a-1* and *mob1a-2*, developed short roots and displayed small floral

138 organs and reduced fertility compared to wild type (WT) (Cui et al., 2016).

139   To define functions of the other *AtMOB1* genes, we obtained T-DNA insertion

140 mutants, denoted *mob1b-1*, *mob1c-1*, and *mob1d-1* (Fig. 1B), from the Nottingham

141 Arabidopsis Stock Center. RT-PCR analysis indicated that all of the mutations

142 appeared null (Fig. S1A). However, no obvious developmental defects were observed

143 in the single mutants, suggesting that the *MOB1* genes may have overlapping

144 functions. We then generated the double and triple mutant combinations (Fig. 1C-J).

145 Because *MOB1C* (*At5g20430*) and *MOB1D* (*At5g20440*) are located in tandem on

146 Chromosome V, it is impossible to generate the double mutant or the quadruple

147 mutant by genetic crossing. We thus constructed the *mob1a-2 mob1b-1 mob1c-2*

148 *mob1d-1* quadruple mutant (*mob1a-2/b-1/c-2/d-1*) using CRISPR/Cas9 gene editing

149 technology (Gao et al., 2016) (Zeng et al., 2018) (Fig. 1K, and Fig. S1B) in the

150 *mob1a-2$^{+/-}$/mob1b-1/mob1d-1* (*mob1a-2$^{+/-}$/b-1/d-1*) background.

151   The seedlings of *mob1a-2/b-1$^{+/-}$* displayed smaller cotyledons, shorter roots,

152 and a longer hypocotyl than single mutants and WT (Fig. 1D-E). The siliques of

153 *mob1a-2/b-1$^{+/-}$* adult plants were very short and completely sterile (Fig. 1F-G). On the

154 other hand, the *mob1a-2$^{+/-}$/b-1* mutant resembled *mob1b-1*. These results indicated

155 that *AtMOB1B* became haploid insufficient in the *mob1a* mutant background. The

156 *mob1a-2/b-1* seedlings were very tiny, and their development was significantly

6

157   retarded. The 21-d-old double-mutant plants had much smaller leaves and shorter

158   roots compared to WT and the single mutants (Fig. S2). To confirm that the

159   phenotypes were caused by the mutations in *AtMOB1A* and *AtMOB1B*, we carried out

160   genetic complementation experiments. Transformation of *mob1a-2/b-1* with the

161   *AtMOB1A* genomic fragment resulted in plants that were similar to *mob1b-1*. We also

162   found that *mob1a-2/b-1* plants harboring the *AtMOB1B* transgene behaved similarly

163   to *mob1a*. Our results demonstrate that the strong developmental defects observed in

164   the *mob1a-2/b-1* mutant are caused by the disruption of both *AtMOB1A* and

165   *AtMOB1B* (Fig. S3).

166   The *mob1a-1/b-1/c-1* and *mob1a-2/b-1/d-1* triple mutants and the

167   *mob1a-2/b-1/c-2/d-1* quadruple mutant displayed phenotypes similar to that of

168   *mob1a-2/b-1* (Fig. 1H-J). Moreover, the *mob1c-2/d-1* double mutant segregated from

169   the *mob1/a-2/b-1/c-2/d-1* quadruple mutant similar to the *mob1c-1* and *mob1d-1*

170   single mutants and WT plants (Fig. 1L). These results suggest that *AtMOB1A* plays a

171   more predominant role than *AtMOB1B*. *AtMOB1A* and *AtMOB1B* appear to be more

172   important in regulating plant development than *AtMOB1C* and *AtMOB1D* under our

173   growth conditions. We therefore focused on *AtMOB1A* and *AtMOB1B* in this work.

174

175   ***AtMOB1A* and *AtMOB1B* show similar expression patterns**

176   To investigate the expression patterns of *AtMOB1A* and *AtMOB1B*, we made

177   constructs containing *AtMOB1A* or *AtMOB1B* genomic DNA sequences fused with

178   the *GUS* gene, which were then expressed in WT plants. *AtMOB1A-GUS* and

179   *AtMOB1B-GUS* were expressed with very similar patterns in cotyledons, true leaves,

180   trichomes, root hairs, primary and lateral roots of seedlings, and floral organs (Fig.

181   S4A-F, and H-M).

182   We also generated transgenic plants containing *AtMOB1B* genomic DNA

183   sequence fused with the *GFP* gene. AtMOB1B-GFP was expressed in the roots, and

184   the fusion protein was localized to the nucleus, cytoplasm, and plasma membrane,

185   similar to AtMOB1A-GFP (Fig. S4G and N). The subcellular localization of

186   AtMOB1B-GFP was similar to that of AtMOB1A-GFP (Cui et al., 2016). These

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187 results are consistent with our hypothesis that *AtMOB1A* and *AtMOB1B* redundantly

188 regulate plant development.

189

AtMOB1A and *AtMOB1B* are necessary for cell proliferation

191   The seedlings of the *mob1a-2/b-1* mutant had very short roots. The lengths of the

192 root elongation zone and the meristem zone were dramatically decreased in the

193 *mob1a-2/b-1* mutant (Fig. 2A-C). The cell numbers of the elongation zone and the

194 meristem zone were also significantly reduced in the double mutant (Fig. 2D-E).

195 Moreover, the root cap columella of *mob1a-2/b-1* was much smaller (Fig. 2F-G).

196 These results suggest that cell division activities are abnormal in *mob1a-2/b-1*. To test

197 this hypothesis, we used *CYCB1;1:GUS,* which is a widely used marker for the G2/M

198 phase of the cell cycle (Colon-Carmona et al., 1999). We introduced this marker into

199 the *mob1a-2/b-1* double and single mutant backgrounds by genetic crossing. The

200 expression of *CYCB1;1:GUS* was decreased significantly in *mob1a-2* and slightly in

201 *mob1b-1* single mutants, and was barely detectable in the *mob1a-2/b-1* double mutant

202 (Fig. 2H-I). These results suggest that both *MOB1A* and *MOB1B* are important for

203 cell proliferation in Arabidopsis roots.

204

AtMOB1A physically interacts with AtMOB1B

206   We tested whether AtMOB1A and AtMOB1B physically interact with each other.

207 We carried out an co-immunoprecipitation (Co-IP) assay in *Nicotiana benthamiana*

208 leaves, which were co-transformed with YFP-tagged AtMOB1B and FLAG-tagged

209 AtMOB1A. The proteins were immuno-precipitated using anti-GFP beads and

210 detected with FLAG antibody. Our results clearly indicated that AtMOB1A and

211 AtMOB1B physically interact in *N. benthamiana* leaves (Fig. 3A).

212   We also performed immuno-precipitation mass spectrometry (IP-MS)

213 experiments using Arabidopsis seedlings transformed with *35S::AtMOB1A-FLAG*.

214 Total protein was extracted from seedlings and immuno-precipitated with anti-FLAG

215 antibody beads. The candidate interacting proteins were then identified by mass

216 spectrometry. A total of 11 AtMOB1A/B peptide sequences were detected as

8

217 AtMOB1A-interacting proteins. Although nine of these were shared by AtMOB1A/B,

218 the other two were AtMOB1B specific (Table S1), indicating that AtMOB1A and

219 AtMOB1B interacted in the IP-MS assay in Arabidopsis.

220   To further confirm the interaction, we carried out the firefly luciferase

221 complementation imaging (LCI) assay (Chen et al., 2008). The results indicated that

222 AtMOB1A directly interacts with AtMOB1B in *N. benthamiana* leaves (Fig. 3B).

223 Combined, these experiments show that AtMOB1A interacts with AtMOB1B in vivo.

224

225 ***AtMOB1A*** **and** ***AtMOB1B*** **negatively regulate the jasmonate pathways**

226   To identify differentially expressed genes (DEGs) between the *mob1a-2/b-1*

227 mutant and WT, we performed RNA-sequencing (RNA-seq) analysis using 10-d-old

228 seedlings of *mob1a-2/b-1* and Col-0, with three biological repeats included of each

229 genotype. A total of 1202 DEGs were identified, including 725 up-regulated and 477

230 down-regulated genes (Supplemental Table S2). Gene Ontology (GO) analysis

231 revealed that many genes in the jasmonic acid (JA) biosynthetic, metabolic, and

232 signaling pathways were significantly enriched (Fig. 4A). In addition, genes involved

233 in several JA-related biological processes were also enriched, including glucosinolate

234 biosynthetic and metabolic processes, and responses to insects and wounding (Huang

235 et al., 2017; Wasternack and Song, 2017) (Fig. 4A and Supplemental Table S3).

236   We identified 89 JA-related DEGs in *mob1a-2/b-1*, which include 83

237 up-regulated and 6 down-regulated genes (Supplemental Table S4). First, the

238 expression of many pivotal genes in JA biosynthesis, including the four *13-LOXs*,

239 *AOS*, *AOC*, *OPR3*, and *OPCL* were dramatically up-regulated (Fig. 4B). Second,

240 *JAR1*, *ILL6*, *IAR3*, *ST2A*, *JOX*, *CYP94B3*, and *CYP94C1* were also up-regulated,

241 which encode enzymes that catalyze a number of key metabolic processes of JA and

242 JA-Ile, including hydrolysis, sulfation, hydroxylation, and carboxylation (Fig. 4C).

243 Third, five *JAZs* and *MYC2* in the JA signaling pathway were markedly up-regulated

244 (Fig. 4D). Moreover, some genes involved in response to JA, such as *VSP2*, *TAT3*,

245 and *JR2*, were also up-regulated (Fig. 4D, Supplement Table S4).

246   To validate the RNA-seq results, we carried out RT-qPCR analysis. The results

9

247 confirmed the increased expression of the up-regulated DEGs in *mob1a-2/b-1*

248 compared to that in the single mutants and WT (Fig. 4E-G). Therefore, AtMOB1A

249 and AtMOB1B likely negatively regulate JA biosynthetic, metabolic, and signaling

250 pathways.

251

252 **JA content is significantly increased in the *mob1a-2/b-1* double mutant**

253   The up-regulation of many genes involved in JA biosynthesis, metabolism, and

254 signaling in the *mob1a-2/b-1* double mutant may lead to an alteration of JA

255 concentrations. To test this hypothesis, we measured endogenous JA contents using

256 the gas chromatography-mass spectrometry (GC-MS) method. Indeed, JA content in

257 the *mob1a-2/b-1* mutant was dramatically increased compared to that in the single

258 mutants and WT plants. JA content was similar in the single mutants and WT (Fig.

259 5A). These results suggest that AtMOB1A and AtMOB1B negatively regulate JA

260 accumulation.

261

262 **The *mob1a-2/b-1* double mutant is hypersensitive to exogenous Me-JA treatment**

263   It is known that JA can promote leaf senescence (He et al., 2002; Xiao et al., 2004;

264 Shan et al., 2011). We tested whether the *mob1* single and double mutants responded

265 to exogenous JA treatments differently than WT. Five-d-old seedlings of WT,

266 *mob1a-2* and *mob1b-1* single mutants, and the *mob1a-2/b-1* double mutant were

267 transferred onto 1/2 MS plates containing different concentration (0, 10, 25, 50, 100,

268 and 200 μM) of Me-JA and grown for a further 14 d. The leaves of the double mutant

269 became senescent and yellow when treated with Me-JA at 100 μM and 200 μM,

270 whereas the WT and the single mutants remained green (Fig. S4). In addition,

271 RNA-seq results indicated that several senescence-associated genes displayed altered

272 expression in the double mutant (Table S5), including up-regulated

273 *Senescence-associated gene 21* (*SAG21/*AT4G02380),

274 *Senescence/dehydration-associated protein-related A*/(AT4G15450), and

275 *ERD7/Senescence/dehydration-associated protein-related* (AT2G17840), and

276 down-regulated *Rubisco Activase* (*RCA*), and *Senescence-associated family protein*

10

277   (AT1G66330). These results are consistent with the observed increase in JA content in

278   the double mutant.

279

Disruption of *MYC2* partially rescues the developmental defects and JA

281   **hypersensitivity of *mob1a-2/b-1***

282   The increased expression of JA-related genes and JA content in the *mob1a-2/b-1*

283   double mutant suggested that *AtMOB1A* and *AtMOB1B* negatively regulate JA

284   accumulation. To test the biological relevance of these observations, we analyzed the

285   genetic interaction between *myc2-2* and the *mob1a-2/b-1* double mutant. *MYC2*

286   encodes a key downstream transcription factor that regulates diverse aspects of JA

287   responses, and its expression was also found to be up-regulated in *mob1a-2/b-1* (Table

288   S4, Fig. 4G). We introduced the *myc2-2* mutation into the *mob1a-2/b-1* background

289   by genetic crossing. The *myc2-2 mob1a-2/b-1* triple mutant developed longer roots

290   than the *mob1a-2/b-1* double mutant (Fig. 5B-D), indicating that the *myc2-2* mutation

291   can partially suppress the root defects of *mob1a-2/b-1*.

292   Because *MYC2* plays critical roles in activating JA-induced leaf senescence (Qi

293   et al., 2015), we examined JA-induced senescence in the *myc2-2 mob1a-2/b-1* triple

294   mutant. It was clear that the *myc2-2* mutation repressed the JA hypersensitivity of the

295   *mob1a-2/b-1* double mutant. Both the morphological defects and decreased

296   chlorophyll content of *mob1a-2/b-1* were suppressed by *myc2-2*. (Fig. 5E-F). These

297   results suggest that the root defects and JA hypersensitivity of the *mob1a-2/b-1* double

298   mutant are partially caused by over-accumulation of JA, and are dependent on

299   *MYC2*-mediated JA signaling.

300

Expression levels of *PLT1* and *PLT2* are decreased in the *mob1a-2/b-1* double

302   **mutant**

303   *PLETHORA (PLT) 1* and *PLT2* encode proteins belonging to the AP2 class of

304   transcription factors, and are essential for root stem cell niche patterning (Aida et al.,

305   2004; Galinha et al., 2007). It is known that MYC2 represses the expression of *PLT1*

306   and *PLT2*, which restricts root meristem activity and inhibits primary root growth

11

307   (Chen et al., 2011). To investigate the expression of *PLT1* and *PLT2* in *mob1a-2/b-1*,

308   we introduced markers *pPLT1:CFP* and *pPLT2:CFP* (Galinha et al., 2007) into the

309   mutant backgrounds by genetic crossing. The expression levels of *pPLT1:CFP* and

310   *pPLT2:CFP* were significantly decreased in the *mob1a-2/b-1* double mutant

311   compared to that in the single mutants and WT (Fig. 6A-D). We further introduced the

312   *pPLT1:PLT-YFP* and *pPLT2:PLT-YFP* fusions (Galinha et al., 2007) into the mutant

313   backgrounds by genetic crossing, and found that, in agreement, the resulting protein

314   levels were also significantly decreased in *mob1a-2/b-1* compared to that in the single

315   mutants and WT (Fig. 6E-H). To examine the root stem cell identity, we introduced

316   the quiescent center (QC) marker *pWOX5::GFP* into the mutant backgrounds by

317   genetic crossing. The GFP signals were detected in the QC cells (Fig. S5), indicating

318   that the root stem cell identity was still maintained. These results suggest that

319   up-regulated *MYC2* expression represses *PLT1/2* expression in the *mob1a-2/b-1*

320   double mutant, which at least partially accounts for the root developmental defects.

321

Genetic interaction between *mob1a-2/b-1* and *sik1* mutants

323   It was previously reported that the Hippo/STE20 homolog SIK1 interacts with

324   MOB1 to regulate cell proliferation and cell expansion in Arabidopsis (Xiong et al.,

325   2016). *sik1* mutant plants are smaller than WT (Xiong et al., 2016) and the JA levels

326   in the *sik1* mutant are decreased compared to WT (Zhang et al., 2018). We generated a

327   *sik1 mob1a-2/b-1* triple mutant by genetic crossing, and found that seedlings of the

328   resulting triple mutant were smaller than *mob1a-2/b-1* double-mutant seedlings (Fig.

329   7A-C). Because JA levels are increased in *mob1a-2/b-1* but decreased in *sik1* mutant,

330   we examined the expression of several JA-responsive genes in these mutant

331   backgrounds. The expression levels of *JAZ1*, *JAZ2*, *JAZ5*, *JAZ9*, *JAZ19*, and *MYC2*

332   were increased in *mob1a-2/b-1* but significantly decreased in the *sik1* mutant. The

333   expression levels of these genes were increased in the *sik1 mob1a-2/b-1* triple mutant

334   compared to in the *sik1* mutant (Fig. 7D). These results suggest that changes to

335   JA-responsive gene expression caused by the *mob1a-2/b-1* mutations were partially

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336 alleviated by the *sik1* mutation in the *sik1 mob1a-2/b-1* triple mutant, consistent with

337 the observed differences in JA levels in *mob1a-2/b-1* and *sik1*.

338

339 **Discussion**

340   In this paper, we report that *AtMOB1A* and *AtMOB1B* genetically and physically

341 interact to control Arabidopsis development. The *mob1a-2/b-1* double mutant was

342 reduced in size with severe developmental defects, and the expression levels of many

343 genes in the JA biosynthetic, metabolic, signaling, and responses pathways were

344 up-regulated. Consistent with these observations, *mob1a-2/b-1* accumulated much

345 more JA than the single mutants and WT, and the double mutant was hypersensitive to

346 JA treatment. Disruption of the key JA signaling gene *MYC2* partially alleviated

347 *mob1a-2/b-1* root defects and JA hypersensitivity. *mob1a-2/b-1* was associated with

348 decreased expression of *PLT1/2*, suggesting that altered expression of these critical

349 root development genes partially accounts for the observed root defects.

350

351 **AtMOB1A and AtMOB1B interact to regulate growth and development**

352   Previously, it has been shown that AtMOB1A is important for many plant

353 development processes (Citterio et al., 2006; Pinosa et al., 2013; Cui et al., 2016), and

354 that AtMOB1A plays critical roles in auxin-mediated development (Cui et al., 2016).

355 Although the *mob1a* single mutant plants displayed strong developmental defects and

356 the *mob1b* phenotype was similar to WT, *mob1a-2/b-1* showed an enhanced mutant

357 phenotype compared to *mob1a-2* and *mob1b-1*, indicating that *AtMOB1A* and

358 *AtMOB1B* have unique and overlapping functions. Interestingly, such genetic

359 interaction and redundant or overlapping function of MOB1A/B were also observed

360 in mouse. The mouse *mob1a/b* double mutant exhibited embryonic lethality or severe

361 cancer susceptibility (Nishio et al., 2012). *Mob1a/1b* double mutation in mouse liver

362 resulted in death within 3 weeks of birth or liver cancers and death by age 60 weeks

363 (Nishio et al., 2016).

364   On the other hand, AtMOB1A and AtMOB1B proteins physically interact in vivo.

365 The interaction between hMOB1A and hMOB1B was also reported in humans (Wang

13

366 et al., 2014), suggesting that the interaction between these two proteins is also

367 conserved. Previously, it was reported that Arabidopsis serine/threonine kinase 1

368 (SIK1) is a Hippo homolog, and that AtMOB1A and AtMOB1B interact with SIK1

369 (Xiong et al., 2016). These genetic and physical interactions suggest that SIK1,

370 AtMOB1A, and AtMOB1B form a large protein complex. Interestingly, the *sik1*

371 *mob1a-2/b-1* triple mutant displayed stronger developmental defects compared to the

372 *mob1a-2/b-1* double mutant, suggesting that there might be additional components

373 involved in regulating the affected developmental processes. There are 10 *SIK1*-like

374 genes in the MAP4 Kinase family (Zhang et al., 2018). It is possible that other

375 members of the MAP4 family play redundant roles with SIK1. Recently, it was shown

376 that SIK1 associates with, phosphorylates, and stabilizes the central immune regulator

377 BIK1 (Zhang et al., 2018). On the other hand, MOB1s are adaptor/scaffolding

378 proteins. It is not clear whether SIK1 phosphorylates MOB1 and/or MOB1 activates

379 SIK1 kinase activity.

380   Because the phenotypes of the *mob1a-2/b-1* double mutant are stronger than those

381 of the *mob1a-2* and *mob1b-1* single mutants, it is likely that AtMOB1B and

382 AtMOB1A proteins also form homodimer/oligomers. Also, because the *mob1a-2*

383 mutant displayed strong developmental defects whereas *mob1b-1* was largely normal,

384 it is likely that AtMOB1A plays the dominant role. Indeed, a total of 11 AtMOB1A/B

385 peptide sequences were detected as AtMOB1A-interacting proteins. Nine of these

386 were shared by AtMOB1A/B, and the remaining two were AtMOB1B specific (Table

387 S1). These results indicate AtMOB1A and AtMOB1B interacted in the IP-MS assay in

388 Arabidopsis, and further suggest that AtMOB1A and AtMOB1B form

389 homodimer/oligomers.

390

AtMOB1A and AtMOB1B modulate JA accumulation

392   JAs are important in regulating plant growth and development as well as plant

393 responses to biotic and abiotic stresses. JA inhibits primary root growth and promotes

394 leaf senescence (Huang et al., 2017). We found that *mob1a-2/b-1* mutant plants had a

395 very small growth habit, with small cotyledons and short roots. It was reported that JA

14

treatment markedly reduces the expression of *CYCB1;1:GUS* and represses cell

division activity in Arabidopsis root meristems (Chen et al., 2011). We showed that

the expression of *CYCB1;1:GUS* was dramatically decreased in *mob1a-2/b-1* mutant

plants (Fig. 2), which is consistent with their higher JA level (Fig. 5). These results

suggest that the short root phenotype of the *mob1a-2/b-1* double mutant could be

partially caused by JA-induced repression of root cell division.

The expression levels of many genes involved in JA biosynthetic, metabolic, and

signaling pathways were increased, and JA content was elevated in *mob1a-2/b-1*.

Moreover, the expression of *MYC2* was increased and the expression of *PLT1/2* was

decreased in the double-mutant plants. These observations are consistent with the

findings that JA reduces the expression levels of *PLT1* and *PLT2*, which is mediated

by the direct binding of MYC2 to the promoters of *PLT1* and *PLT2* to repress their

expression (Chen et al., 2011). Moreover, disruption of *MYC2* in the *mob1a-2/b-1*

background partially alleviated the short-root phenotype, suggesting that the root

defects were at least partially caused by increased endogenous JA. The *mob1a-2/b-1*

double mutant was hypersensitive to exogenous Me-JA treatment in terms of leaf

senescence. JA-repressed *RCA*, which plays an important role in JA-induced leaf

senescence (Shan et al., 2011), was among the few down-regulated DEGs in

mob1a-2/b-1 plants. Our RNA-seq analysis also revealed that expression of some JA

responsive genes was altered in the *mob1a-2/b-1* double mutant, such as the

upregulation of *VPS2*, *TAT,* and *LOX3,* the most prominent marker genes responding

to wounding activated by *MYC2* in the JA signaling pathway (Titarenko et al., 1997;

Lorenzo et al., 2004). More importantly, the JA hypersensitivity of the *mob1a-2/b-1*

double mutant was markedly alleviated by the *myc2* mutation in *myc2-2 mob1a-2/b-1*

plants. These results suggest that the elevated JA content in the *mob1a-2/b-1* double

mutant caused the JA-related phenotypes. Intriguingly, disruption of *MYC2* only

mildly suppressed the extreme short-root phenotype observed in *mob1a-2/b-1*,

suggesting that other factors besides JA pathways, such as auxin signaling, are also

involved in regulating root growth in the *mob1a-2/b-1* double mutant. This hypothesis

is consistent with our previous report that *AtMOB1A* plays a role in auxin-mediated

15

426   development (Cui et al., 2016). It is likely that *AtMOB1B* plays similar roles in

427   modulating auxin signaling.

428   Interestingly, it was reported that the expression of *JAZ* genes (*JAZ1*, *JAZ2*, *JAZ5*,

429   *JAZ6*, *JAZ9*, and *JAZ12*)*,* and *MYC* genes (*MYC2*, *MYC3*, and *MYC4*) was repressed

430   in the *sik1* mutant (Xiong et al., 2016), suggesting that *SIK1* also plays roles in

431   JA-related development. However, because the expression of some of the *JAZs* and

432   *MYCs* in the *mob1a-2/b-1* double mutant was up-regulated, it seems that *AtMOB1A/B*

433   and *SIK1* play different roles in modulating JA pathways. Indeed, JA levels were

434   decreased in the *sik1* mutant compared to WT (Zhang et al., 2018), whereas they were

435   increased in *mob1a-2/b-1* (Fig. 5A). These findings suggest that *SIK1* promotes and

436   *MOB1A/B* represses JA levels. Consistently, the expression levels of *JAZs* and *MYC2*

437   were increased in the *sik1 mob1a-2/b-1* triple mutant compared to in the *sik1*mutant

438   (Fig. 7). It is intriguing how components in the same protein complex would

439   antagonistically modulate JA levels. The *sik1 mob1a-2/b-1* triple-mutant plants

440   showed compromised regulation of JA-related genes, but displayed more severe

441   developmental defects than *sik1* or the *mob1a-2/b-1* double mutant. These results

442   suggest that *MOB1* and *SIK1* may function similarly in controlling cell proliferation,

443   but may have opposite roles in regulating JA levels and JA-related gene expression.

444   The exact molecular mechanisms for such complex regulation are not currently

445   understood. It was reported that the *sik1* mutant exhibited significantly higher levels

446   of basal salicylic acid (SA) and decreased levels of JA (Zhang et al., 2018). Since

447   SIK1 plays a role in antibacterial immunity response (Zhang et al., 2018) and MOB1

448   and SIK1 physically interact, it is possible that MOB1 plays a role in biotic stress

449   responses, perhaps through JA signaling.

450

451   **AtMOB1A and AtMOB1B act as key regulators in auxin- and JA-mediated plant**

452   **growth**

453   Previously, we reported that AtMOB1A plays critical roles in auxin-mediated plant

454   development. We isolated the *mob1a/ncp1* mutant as an enhancer of *pid*, and showed

455   that it had strong genetic interactions with mutants in auxin biosynthesis, polar

16

transport, and signaling (Cui et al., 2016). Disruption of *AtMOB1A* led to a reduced

sensitivity to exogenous auxin. Our previous results demonstrated that AtMOB1A

plays an important role in Arabidopsis development by promoting auxin signaling

(Cui et al., 2016). Our results presented in this paper clearly indicate that AtMOB1A

and AtMOB1B also act as key regulators of JA-mediated plant growth. It is likely that

AtMOB1A and AtMOB1B physically interact with each other and are components of

a large protein complex which includes SIK1 (Xiong et al., 2016). AtMOB1A and

AtMOB1B promote auxin signaling and cell proliferation; however, the two proteins

repress JA accumulation. Thus, the AtMOB1-containing protein complex likely

regulates the crosstalk between auxin- and JA-mediated plant growth and

development. A molecular framework for JA-induced inhibition of root growth

through interaction with auxin pathways is well established, in which

MYC2-mediated repression of PLT expression integrates JA action into the auxin

pathway in regulating root meristem activity and stem cell niche maintenance (Chen

et al., 2011). Auxin upregulates *PLT1* and *PLT2* transcripts and positively regulates

stem cell niche maintenance and meristem activity (Aida et al., 2004), and JA

downregulates *PLT1* and *PLT2* expression and negatively regulates stem cell niche

maintenance and meristem activity (Chen et al., 2011). We showed that *PLT1/2*

expression is repressed in the *mob1a-2/b-1* double mutant, which could be an

integrative outcome of elevated JA content and reduced auxin signaling. It would be

interesting to further explore the mechanisms by which AtMOB1A/B control JA

accumulation and the crosstalk with auxin signaling.

Materials and Methods

Plant materials and growth condition

    All *Arabidopsis thaliana* materials used in this work were in the Col-0 ecotype

background. Seeds were surface sterilized for 15 min in 70% (v/v) and then 100%

(v/v) ethyl alcohol. The seeds were then sowed on half-strength Murashige and Skoog

(1/2 MS) medium containing 0.8% (w/v) agar. The plates were transferred to 4°C for

17

486   3 d in darkness for vernalization. Plants were grown at 22°C under a 16-h light/8-h

487   dark cycle. The T-DNA insertion lines, including *mob1 a-2* (GK_719G04), *mob1 b-1*

488   (SALK_062070), and *mob1 d-1* (SALK_053800) were purchased from the NASC.

489   The *mob1 a-2* (GK_719G04) mutant was genotyped as described previously (Cui et

490   al., 2016). For genotyping *mob1 b-1* (SALK_062070), the primers

491   5'-GGATGAAGTGTTTGAAGC-3' and 5'-GCTGAGTAATGG TTGTGA-3'

492   combined with JMLB1 were used. For genotyping *mob1 d-1* (SALK_053800), the

493   primers 5'-GGGCAAAGTCCAAATCCT-3' and 5'-CCGCTTCACGAAATCCTC-3'

494   combined with JMLB1 were used.

495

496   **DNA constructs and plant transformation**

497   For the expression patterns of *ATMOB1B*, the plasmid was constructed with its

498   genomic DNA fragment containing the coding region alongside up- and down-stream

499   regulatory sequences with the GFP or GUS gene inserted before the stop code. The

500   *AtMOB1B* gene was divided into parts A and B. The part A was amplified using

501   primers 5'-CGGGGTACCGCAGGAATACTATTGGGCCTG-3' and

502   5'-ACTGGGCCCGTAAGGTGCAATGATAGATTC-3'. The part B was amplified

503   using primers 5'-ACTGGGCCCACCAAACAAAACCCAAATCCTC-3' and

504   5'-ACTGTCGACGGCTCCAACACAAAATACCTC-3'. The two PCR products

505   were double digested with *KpnI* and *Apa I*, or *ApaI* and *SalI*, respectively, and cloned

506   into the *KpnI-SalI* sites of vector *pPZP211* to generate *pPZP211-gAtMOB1B*

507   construct. The GUS or GFP gene was inserted immediately before the stop code using

508   restriction site of *ApaI*. The *pPZP211-gAtMOB1B-GUS* or

509   *pPZP211-gAtMOB1B-GFP* construct was transformed into *Agrobacterium*

510   *tumefaciens* strain GV3101, and then Arabidopsis plants were transformed by floral

511   dipping. The transgenic seedlings were selected on 1/2 MS plates with 50 µg/mL

512   kanamycin. The *pPZP211-gAtMOB1A* (Cui et al., 2016) and

513   *pPZP211-gAtMOB1B-GFP* constructs were used in the complementation experiments

514   of the *mob1a-2/b-1* mutants (Fig. S3).

515

18

Phenotypic analysis, statistical analysis, and microscopy

Seedlings and roots were photographed and their lengths were measured using NIH

ImageJ software (https://imagej.nih.gov/ij/). Seedlings were mounted in HCG

(H_2O-Chloral hydrate-Glycerine) solution (Chloral hydrate:water:glycerol = 8:3:1)

and the root meristem cells analyzed on a Leica microsystems DM4500B microscope.

Statistical significance was evaluated by Student's *t*-test analysis or one-way ANOVA

analysis followed by LSD test (SPSS). Histochemical staining for GUS activity in

plants was performed as described previously (Cui et al., 2016). For the Lugol

staining, roots were incubated in the Lugol solution for 3–5 min, and then washed in

water once, and mounted in HCG solution for microscopy analysis. GFP, YFP, CFP,

and FM4-64 fluorescence was imaged under a confocal laser scanning microscope

Olympus FV1000MPE following the manufacturer's instructions. The fluorescence

intensities were measured using ImageJ for quantification analysis.

Co-IP assays and Mass Spectrometry

For the Co-IP assay of AtMOB1A and AtMOB1B, *pSuper1300:AtMOB1A-Flag*

and *pEarleyGate 104-35S:YFP-AtMOB1B* plasmids were constructed and introduced

into *Agrobacterium tumefaciens* strain GV3101, then *Nicotiana benthamiana* leaves

were transformed by injection. Leaves were ground in liquid nitrogen and proteins

were extracted with same volume of extraction buffer [100 mM HEPES (pH 7.5), 5

mM EDTA, 5 mM EGTA, 10 mM NaF, 5% (v/v) Glycerol, 10 mM Na_3VO_4, 10 mM

DTT, 1 mM PMSF, 0.1% (v/v) Triton X-100, protease inhibitor cocktail (Sigma)].

Samples were mixed twice quickly following incubating on ice for 30 min and then

centrifuged at 14000 *g* at 4°C for 30 min. The supernatant was incubated with

anti-Flag agarose (Sigma) or Anti-GFP-mAb agarose (MBL) in IP buffer [20 mM

Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4, 1 mM

NaF, 10 mM β-glycerophosphate, 0.1% (v/v) Triton X-100, protease inhibitor cocktail

(Sigma)] at 4°C for 2 h with gentle shaking. The agarose was collected and washed

three times with PBS, boiled in 2×SDS loading buffer for 5 min and examined by

19

546 immunoblot analysis with anti-GFP (CWBIO) or anti-Flag (Abmart) antibodies.

547 For Mass Spectrometry, *pSuper1300:AtMOB1A-Flag* vector was introduced into

548 Agrobacterium. Arabidopsis plants were transformed by the floral dipping method. T4

549 generation transgenic plants were used for extracting total protein and IP using the

550 method described above. The AtMOB1A interacting proteins were examined by Mass

551 Spectrometry.

552

553 **LCI assay**

554 The LCI assay was performed as previously described (Chen et al., 2008). *A.*

555 *tumefaciens* bacteria strain GV3101 containing *pCAMBIA1300-AtMOB1A-nLUC*,

556 *pCAMBIA1300-cLUC-AtMOB1B*, *pCAMBIA1300-nLUC*, and *pCAMBIA1300-cLUC*

557 were injected into *N. benthamiana* leaves. The empty cLUC and nLUC vectors were

558 used as negative controls. The plants were incubated in darkness overnight, and the

559 leaves were harvested after 2–3 d. Leaves were incubated in D-Luciferin, Potassium

560 Salt (Goldbio) solution in darkness for 3 min and the luciferase signals were analyzed

561 with a Tanon 5200 Chemiluminescent Imaging System (Tanon, Shanghai, China). The

562 imaging exposure time was 3 min. Eight leaves were analyzed for each experiment,

563 with three biological replicates in total.

564

565 **RNA Extraction, RT-qPCR, and RNA-Seq**

566 The samples were collected from 10-d-old whole seedlings of Col-0, *mob1a-2*,

567 *mob1b-1*, and *mob1a-2 b-1*. Total RNA was extracted using a Biozol kit (Biomiga)

568 according to the manufacturer's instructions. The first-strand cDNA synthesis was

569 performed using M-MLV Reverse Transcriptase (Promega). The qPCR analysis was

570 performed using a light cycle 96 (Roche) and SYBR Green I (Takara). *ACTIN2*

571 (*AT3G18780*) was used as an internal control. All experiments were performed with

572 three independent biological replicates. Statistical significance was evaluated by

573 one-way ANOVA analysis (multiple comparison by Tukey post test). The primers

574 used for RT-qPCR analysis are listed in Table S6.

575 For RNA-seq, mRNA enrichment, cDNA library construction, and single-end

20

576 sequencing were performed by BGI (www.genomics.org.cn). Filtered Clean-reads

577 were mapped to the reference genome (TAIR10) available at TAIR

578 (http://www.arabidopsis.org). The gene expression quantitation was calculated using

579 the RSEM tool (Li and Dewey, 2011). The DEGs were selected according the

580 threshold value fold change ≥ 2 ($\log_2$ ratio ≥ 1.0) and deviation probability ≥ 0.7

581 through comparing the gene expression quantitation of mutants and wild-type. GO

582 enrichment analysis was carried out using AmiGO 2 program

583 (http://amigo.geneontology.org/amigo).

584

585 **Measurement of endogenous JA by GC-MS**

586   Fourteen-d-old whole plants of every genotype were collected in triplicate

587 replicates. The samples were homogenized quickly in liquid N_2, then powdered

588 samples were weighed dry-frozen. Samples were extracted with 80% (v/v) methanol

589 containing 0.2 ng [$^2H_6$]JA as an internal standard and incubated overnight at 4°C.

590 After centrifugation at 5,976 *g* for 5 min, the supernatant layer was collected in new

591 glass tubes and dried with nitrogen gas. The samples were dissolved in 2.5%

592 (v/v)ethyl acetate and the supernatant were dried with nitrogen gas. Following the

593 addition of 23% (v/v) methanol and incubation for 2 h at -20°C, samples were

594 centrifuged at 10,625 *g* for 7 min and the supernatant was dried with nitrogen gas.

595 Samples were dissolved in 30 µl BSTFA with 3 µl pyridine and incubated for 30 min

596 at 80°C, then analyzed using GC-MS (7890A-7000B, Agilent, USA). The amount of

597 JA present in plant samples was calculated based on the internal standards and weight

598 of the tissues and retention time.

599

600 **Measurement of chlorophyll content**

601 Measurement of chlorophyll content was performed as previously described (Qi et al.,

602 2015). The leaves was detached and weight. For chlorophyll extraction, the leaves

603 were incubated in 80% (v/v) acetone in the dark. Absorbances were measured at 645

604 and 663 nm using a spectrophotometer (Beckman Coulter DU-800). Chlorophyll

605 contents were calculated and expressed as a ratio of the chlorophyll content of Col-0

21

606   treated with mock.

607

Accession numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries

under accession numbers JAZ1 (AT1G19180), JAZ2 (AT1G74950), JAZ3

(AT3G17860), JAZ4 (AT1G48500), JAZ5 (AT1G17380), JAZ6 (AT1G72450), JAZ7

(AT2G34600), JAZ8 (AT1G30135), JAZ9 (AT1G70700), JAZ10 (AT5G13220),

JAZ11 (AT3G43440), and JAZ12 (AT5G20900).

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Supplemental Data

Supplemental Figure S1. Analysis of T-DNA insertion lines of AtMOB1 genes and

generation of CRISPR line of AtMOB1C.

Supplemental Figure S2. Representative images of 21-d-old plants of Col-0,

mob1a-2, *mob1b-1*, and *mob1a-2/b-1* mutants.

Supplemental Figure S3. Complementation of *mob1a-2/b-1* with a genomic DNA

fragment of *AtMOB1A* or *AtMOB1B*.

Supplemental Figure S4. Expression patterns of *AtMOB1A* and *AtMOB1B* and

subcellular localization of the proteins.

Supplemental Figure S5. JA treatment of seedlings at different concentrations.

Supplemental Figure S6. *pWOX5:GFP* expression in the root of 6-d-old seedlings.

Supplemental Table S1. MOB1B was identified by IP-MS assays in MOB1A-Flag

transgenic plants.

Supplemental Table S2. List of differentially expressed genes (DEGs).

Supplemental Table S3. Enriched GO categories in biological process of the DEGs.

Supplemental Table S4. DEGs involved in JA related pathway.

22

Acknowledgements

Figure legends

Figure 1.   Analysis of loss-of-function *atmob1*mutants.

(A) A phylogenetic tree of AtMOB1 family proteins. (B) Schematic representation of *AtMOB1* gene structures and positions of the T-DNA insertion. The 5-bp deletion in *mob1c-2* mutant was designated as "Del-agGAG" in *AtMOB1C*. (C-D) 6-d-old seedlings of Col-0, *mob1a-2*, *mob1b-1*, *mob1a-2/b-1*$^{+/-}$, and *mob1a-2/b-1* mutants. (E) Electron micrograph of a *mob1a-2/b-1* double-mutant seedling. (F-G) Adult plants (F) and siliques (G) of Col-0, *mob1a-2*, *mob1b-1*, and *mob1a-2/b-1*$^{+/-}$. (H-K) 6-d-old seedlings of *mob1a-2/b-1* (H), *mob1a-2/b-1/c-1* (I), *mob1a-2/b-1/d-1* (J), and *mob1a-2/b-1/c-2/d-1* (K). (L) 6-d-old seedlings of Col-0, *mob1c-1*, *mob1d-1*, and *mob1c-2/d-1*. Scale bars: 5 mm (C, D), 100 μm (E, H-K).

Figure 2.   The *mob1a-2 b-1* mutant displays strong defects in root tips.

656 (A) The root tips of 6-d-old seedlings of Col-0, *mob1a-2*, *mob1b-1*, and *mob1a-2/b-1*

657 mutants are shown. The meristem zone is marked with two arrowheads. (B-E)

658 Measurements of the lengths of root elongation zone (B) and meristem (C), and cell

659 numbers in elongation zone (D) and meristem (E). (F) Root tips of 6-d-old seedlings

660 stained with FM4-64. (G) The columella root cap cell of 6-d-old seedlings revealed by

661 Lugol staining. (H) *CYCB1;1:GUS* expression in the root of 6-d-old seedlings. (I)

662 Quantification of cells with *CYCB1;1:GUS* signal in (H). Data represent means ± SD.

663 Different letters represent the significance at the $P < 0.001$ level (one-way ANOVA,

664 LSD test); n > 20. Scale bars: 200 μm (A), 50 μm (F-H).

665 **Figure 3. Physical interaction between AtMOB1A and AtMOB1B proteins in**

666 **vivo.**

667 (A) Co-IP assay of AtMOB1A and AtMOB1B. AtMOB1B-GFP was

668 immuno-precipitated by using anti-GFP agarose beads, followed by immunoblot with

669 anti-Flag antibody, and AtMOB1A-Flag was detected. (B) LCI assays showing the

670 interaction between MOB1A and MOB1B in *N. benthamiana* leaf cells. The left panel

671 shows the combinations of agrobacteria containing the indicated plasmids used to

672 co-infiltrate into different leaf regions of the right panel. Empty cLUC and nLUC

673 vectors were used as negative controls. The experiments were carried out with three

674 independent biological repeats.

675 **Figure 4. Expression of genes involved in JA biosynthesis, metabolism, and**

676 **signaling are altered in the *mob1a-2/b-1* double mutant.**

677 (A) Functional assignment of the DEGs by GO enrichment analysis. Bars represent –

678 lg (P value) and the P values were adjusted by the Bonferroni approach. (B-D) The

679 DEGs in jasmonic acid biosynthesis (B), metabolic processes of JA and JA-Ile (C),

680 and JA signaling pathway and response to JA (D). The Heatmap indicates the ratio

681 being up-regulated. Scale colors represent log2(ratio). α-LeA, α-linolenic acid;

682 13-HPOT, 13-hydroperoxylinoleic acid; 12,13-EOT, 12,13-epoxyoctadecatrienoic

24

683   acid; OPDA, 12-oxophytodienoic acid; OPC-8,

684   3-oxo-2-(2-pentenyl)-cyclopentane-1-octanoic acid; PLA1, phospholipase A1;

685   13-LOX, 13-lipoxygenase; AOS, allene oxide synthase; AOC, allene oxide cyclase;

686   OPR, OPDA reductase; OPCL, OPC-8-CoA ligase; ST2A, 12-OH-JA sulfotransferase;

687   JOX, jasmonic acid oxidase; JAR1, jasmonoyl isoleucine syntase; ILL6 and IAR3,

688   IAA-amino acid hydrolase; CYP94C1; 12-OH-JA-Ile carboxylase; JAZ,

689   jasmonate-zim-domain protein; MYC2, bHLH zip transcription factor; VSP2,

690   vegetative storage protein 2; TAT3, tyrosine aminotransferase 3; JR2, jasmonic acid

691   responsive 2; RAP2.6L, ERF/AP2 transcription factor; ANAC081, Arabidopsis NAC

692   domain containing protein 81. (E-G) Relative expression of the DEGs in JA

693   biosynthesis (E), metabolism (F), and signaling (G) in Col-0, *mob1a-2, mob1b-1*, and

694   *mob1a-2/b-1*. 10-d-old seedlings were collected for RNA extraction and RT-qPCR

695   analysis. *ACTIN2* was used as an internal control. The expression levels of the

696   indicated genes in Col-0 were set to 1. Error bars represent the SD of three biological

697   repeats. Different letters indicate significant difference at $P < 0.001$ (one-way ANOVA,

698   Tukey post test).

699   **Figure 5. AtMOB1A and AtMOB1B negatively regulate JA accumulation.**

700   (A) Measurement of the JA contents in seedlings of Col-0, *mob1a-2*, *mob1b-1*, and

701   *mob1-2/b-1* mutants by using GC-MS. Seedlings were grown on 1/2 MS medium for

702   14 d after germination. Data represent means ± SD of three independent biological

703   repeats. Different letters represent statistical significance at the $P < 0.001$ level

704   (one-way ANOVA, LSD test). (B-C) 6-d-old seedlings of Col-0, *myc2-2*,

705   *mob1a-2/b-1*, and *myc2-2 mob1a-2/b-1* mutants. Seedlings were grown on 1/2 MS

706   medium for 6 d after germination. Scale bars: 2 mm (B), 1 mm (C). (D) Measurement

707   of root length of Col-0, *myc2-2*, *mob1a-2/b-1*, and *myc2-2 mob1a-2/b-1* mutants. Data

708   represent means ± SD (n ≥ 20) with significant differences determined by Student's

709   *t*-test. [***]$P < 0.001$ compared to *mob1-2 b-1*. (E) *mob1a-2/b-1* mutants were

710   hypersensitive to exogenous JA treatment. 5-d-old seedlings grown on 1/2 MS plates

25

were transferred to the 1/2 MS plates without (Control, upper panels) or with (JA,

lower panels) 100 µM Me-JA and grown for 2 weeks. Scale bars: 2 mm. (F)

Measurement of chlorophyll contents. The chlorophyll contents of indicated mutants

are relative to that in Col-0, which was set to 1.0. Data represent means ± SD (n = 15).

Student's *t*-test. ***P < 0.001 compared to control.

Figure 6. Expression levels of *PLT1* and *PLT2* are reduced in *mob1a-2/b-1*.

(A, C, E, G) Representative expression of *PLT1:CFP* (A), *PLT2:CFP* (C),

PLT1:PLT1-YFP (E), and *PLT2:PLT2-YFP* (G) in the root tips of 6-d-old Col-0,

mob1a -2, *mob1b-1*, and *mob1a-2/b-1* mutant seedlings. Scale bars: 50 µm. (B, D, F,

H) Quantification of CFP (A, C) and YFP (E, G) fluorescence, respectively. The

fluorescence strength of Col-0 was set to 1. Data represent means ± SD (n = 15).

Different letters represent statistical significance at the P < 0.001 level (one-way

ANOVA, LSD test).

Figure 7. Genetic interaction between *MOB1A/B* and *SIK1*.

(A-B) 12-d-old seedlings of Col-0, *sik1-1*, *mob1a-2/b-1*, and *mob1a-2/b-1 sik1-1*

mutants. (C) Close-up of seedlings in (B). Note the triple mutant was smaller than the

double mutant. Scale bars: 2 mm (A-C). (D) Relative expression of JA

signaling-related genes in 12-d-old seedlings of Col-0, *mob1a-2/b-1*, *sik1-1*, and

mob1a-2/b-1 sik1-1. *ACTIN2* was used as an internal control. The expression levels of

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Figure 1. Analysis of loss-of-function *atmob1* mutants.

(A) A phylogenetic tree of AtMOB1 family proteins. (B) Schematic representation of *AtMOB1* gene structures and positions of the T-DNA insertion. The 5-bp deletion in *mob1c-2* mutant was designated as "Del-agGAG" in *AtMOB1C*. (C-D) 6-d-old seedlings of Col-0, *mob1a-2*, *mob1b-1*, *mob1a-2/b-1*^{+/-}, and *mob1a-2/b-1* mutants. (E) Electron micrograph of a *mob1a-2/b-1* double-mutant seedling. (F-G) Adult plants (F) and siliques (G) of Col-0, *mob1a-2*, *mob1b-1*, and *mob1a-2/b-1*^{+/-}. (H-K) 6-d-old seedlings of *mob1a-2/b-1* (H), *mob1a-2/b-1/c-1* (I), *mob1a-2/b-1/d-1* (J), and *mob1a-2/b-1/c-2/d-1* (K). (L) 6-d-old seedlings of Col-0, *mob1c-1*, *mob1d-1*, and *mob1c-2/d-1*. Scale bars: 5 mm (C, D), 100 μm (E, H-K).

Figure 2. The *mob1a-2 b-1* mutant displays strong defects in root tips.
(A) The root tips of 6-d-old seedlings of Col-0, *mob1a-2*, *mob1b-1*, and *mob1a-2/b-1* mutants are shown. The meristem zone is marked with two arrowheads. (B-E) Measurements of the lengths of root elongation zone (B) and meristem (C), and cell numbers in elongation zone (D) and meristem (E). (F) Root tips of 6-d-old seedlings stained with FM4-64. (G) The columella root cap cell of 6-d-old seedlings revealed by Lugol staining. (H) *CYCB1;1:GUS* expression in the root of 6-d-old seedlings. (I) Quantification of cells with *CYCB1;1:GUS* signal in (H). Data represent means ± SD. Different letters represent the significance at the $P < 0.001$ level (one-way ANOVA, LSD test); n > 20. Scale bars: 200 μm (A), 50 μm (F-H).

A

	Input			IP (anti-GFP)		
AtMOB1A-Flag	+	-	+	-	+	+
AtMOB1B-YFP	-	+	+	+	-	+

α-GFP

α-Flag

B
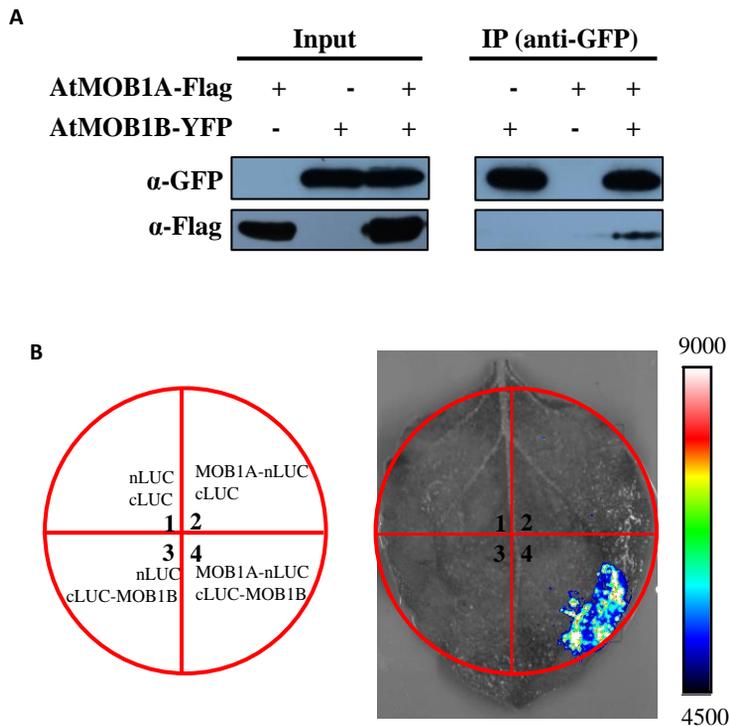
Figure 3. Physical interaction between AtMOB1A and AtMOB1B proteins in vivo.
(A) Co-IP assay of AtMOB1A and AtMOB1B. AtMOB1B-GFP was immuno-precipitated
by using anti-GFP agarose beads, followed by immunoblot with anti-Flag antibody, and
AtMOB1A-Flag was detected. (B) LCI assays showing the interaction between MOB1A
and MOB1B in *N. benthamiana* leaf cells. The left panel shows the combinations of
agrobacteria containing the indicated plasmids used to co-infiltrate into different leaf
regions of the right panel. Empty cLUC and nLUC vectors were used as negative
controls. The experiments were carried out with three independent biological repeats.
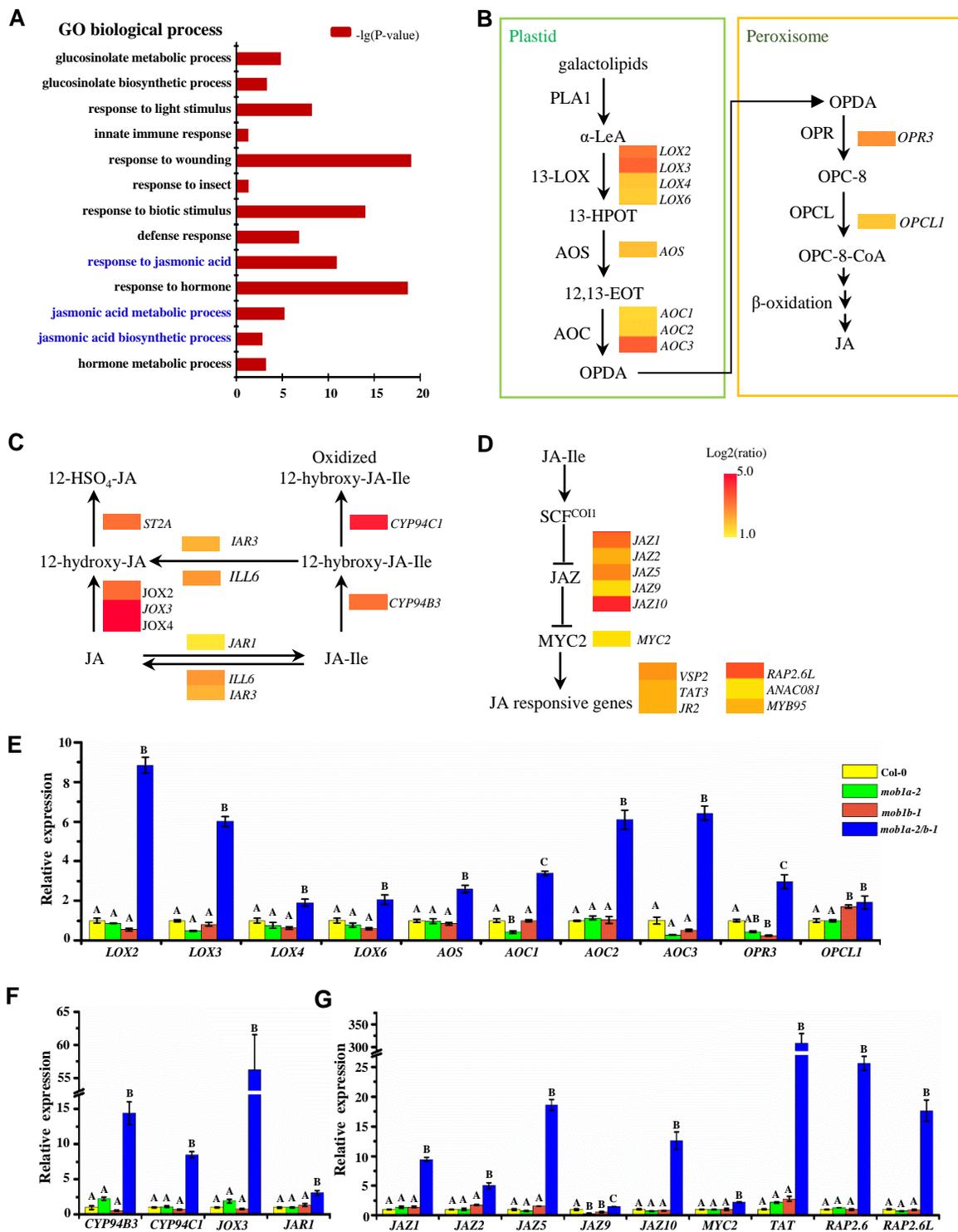
Figure 4. Expression of genes involved in JA biosynthesis, metabolism, and signaling are altered in the *mob1a-2/b-1* double mutant.
(A) Functional assignment of the DEGs by GO enrichment analysis. Bars represent –lg (P value) and the P values were adjusted by the Bonferroni approach. (B-D) The DEGs in jasmonic acid biosynthesis (B), metabolic processes of JA and JA-Ile (C), and JA signaling pathway and response to JA (D). The Heatmap indicates the ratio being up-regulated. Scale colors represent log2(ratio). α-LeA, α-linolenic acid; 13-HPOT, 13-hydroperoxylinoleic acid; 12,13-EOT, 12,13-epoxyoctadecatrienoic acid; OPDA, 12-oxophytodienoic acid; OPC-8, 3-oxo-2-(2-pentenyl)-cyclopentane-1-octanoic acid; PLA1, phospholipase A1; 13-LOX, 13-lipoxygenase; AOS, allene oxide synthase; AOC, allene oxide cyclase; OPR, OPDA reductase; OPCL, OPC-8-CoA ligase; ST2A, 12-OH-JA sulfotransferase; JOX, jasmonic acid oxidase; JAR1, jasmonoyl isoleucine syntase; ILL6 and IAR3, IAA-amino acid hydrolase; CYP94C1; 12-OH-JA-Ile carboxylase; JAZ, jasmonate-zim-domain protein; MYC2, bHLH zip transcription factor; VSP2, vegetative storage protein 2; TAT3, tyrosine aminotransferase 3; JR2, jasmonic acid responsive 2; RAP2.6L, ERF/AP2 transcription factor; ANAC081, Arabidopsis NAC domain containing protein 81. (E-G) Relative expression of the DEGs in JA biosynthesis (E), metabolism (F), and signaling (G) in Col-0, *mob1a-2, mob1b-1*, and *mob1a-2/b-1*. 10-d-old seedlings were collected for RNA extraction and RT-qPCR analysis. The expression levels of the indicated genes in Col-0 were set to 1. Error bars represent the SD of three biological repeats. Different letters indicate significant difference at P < 0.001 (one-way ANOVA, Tukey post test).
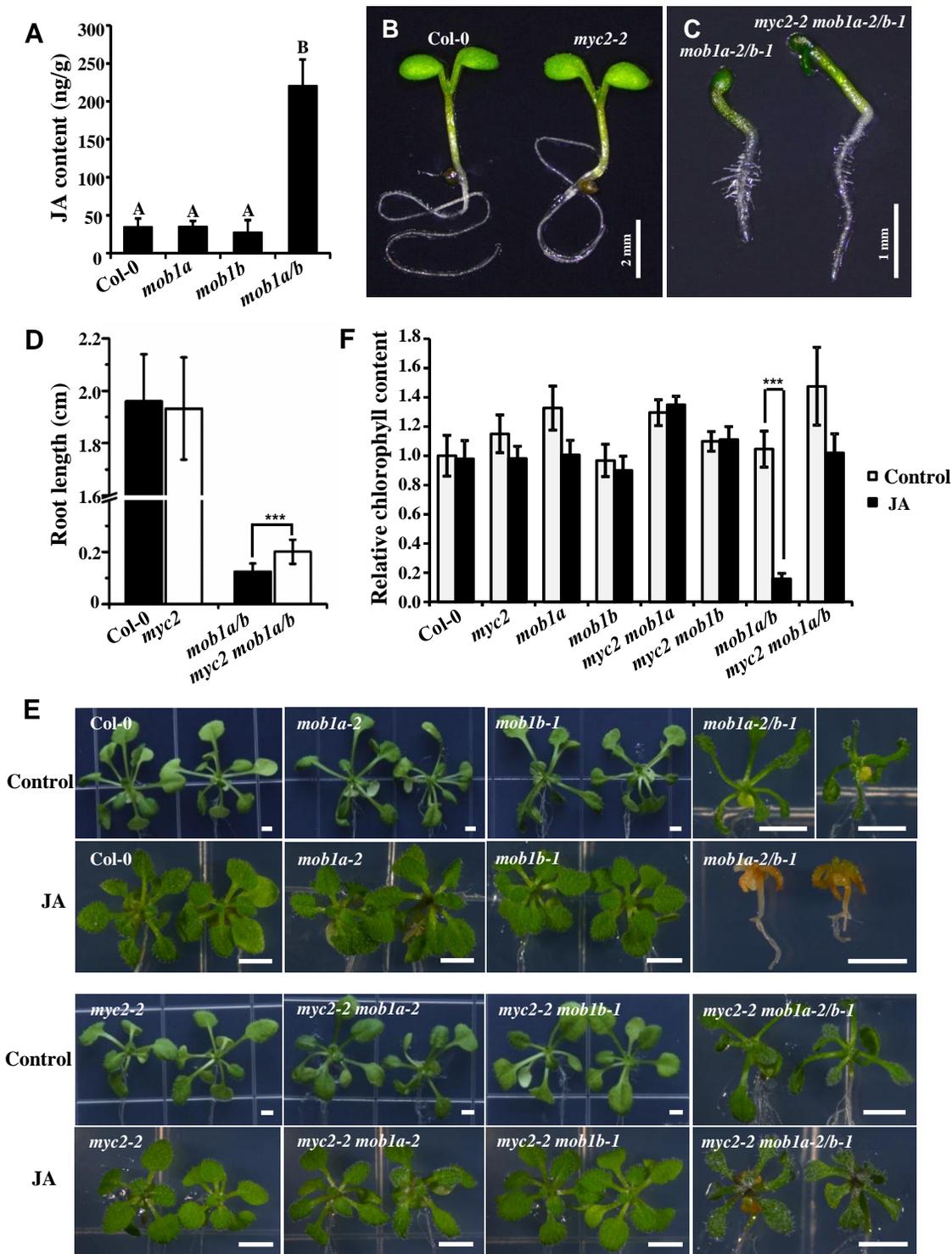
Figure 5. AtMOB1A and AtMOB1B negatively regulate JA accumulation. (A) Measurement of the JA contents in seedlings of Col-0, *mob1a-2*, *mob1b-1*, and *mob1-2/b-1* mutants by using GC-MS. Seedlings were grown on 1/2 MS medium for 14 d after germination. Data represent means ± SD of three independent biological repeats. Different letters represent statistical significance at the P < 0.001 level (one-way ANOVA, LSD test). (B-C) 6-d-old seedlings of Col-0, *myc2-2*, *mob1a-2/b-1*, and *myc2-2 mob1a-2/b-1* mutants. Seedlings were grown on 1/2 MS medium for 6 d after germination. Scale bars: 2 mm (B), 1 mm (C). (D) Measurement of root length of Col-0, *myc2-2*, *mob1a-2/b-1*, and *myc2-2 mob1a-2/b-1* mutants. Data represent means ± SD (n ≥ 20) with significant differences determined by Student's *t*-test. ***P < 0.001 compared to *mob1-2 b-1*. (E) *mob1a-2/b-1* mutants were hypersensitive to exogenous JA treatment. 5-d-old seedlings grown on 1/2 MS plates were transferred to the 1/2 MS plates without (Control, upper panels) or with (JA, lower panels) 100 μM Me-JA and grown for 2 weeks. Scale bars: 2 mm. (F) Measurement of chlorophyll contents. The chlorophyll contents of indicated mutants are relative to that in Col-0, which was set to 1.0. Data represent means ± SD (n = 15). Student's *t*-test. ***P < 0.001 compared to control.

Figure 6. Expression levels of *PLT1* and *PLT2* are reduced in *mob1a-2/b-1*.
(A, C, E, G) Representative expression of *PLT1:CFP* (A), *PLT2:CFP* (C), *PLT1:PLT1-YFP* (E), and *PLT2:PLT2-YFP* (G) in the root tips of 6-d-old Col-0, *mob1a -2*, *mob1b-1*, and *mob1a-2/b-1* mutant seedlings. Scale bars: 50 µm. (B, D, F, H) Quantification of CFP (A, C) and YFP (E, G) fluorescence, respectively. The fluorescence strength of Col-0 was set to 1. Data represent means ± SD (n = 15). Different letters represent statistical significance at the P < 0.001 level (one-way ANOVA, LSD test).

Figure 7. Genetic interaction between *MOB1A/B* and *SIK1*.
(A-B) 12-d-old seedlings of Col-0, *sik1-1*, *mob1a-2/b-1* and *mob1a-2/b-1 sik1-1* mutants. (C)
Close-up of seedlings in (B). Note the triple mutant was smaller than the double mutant. Scale
bars: 2 mm (A-C). (D) Relative expression of JA signaling-related genes in 12-d-old seedlings
of Col-0, *mob1a-2/b-1*, *sik1-1* and *mob1a-2/b-1 sik1-1*. *ACTIN2* was used as an internal
control. The expression levels of the indicated genes in Col-0 were set to 1. Different letters
indicate significant difference at P < 0.001 (n = 3, one-way ANOVA, Tukey post test).

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