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A 3-component module maintains sepal flatness in *Arabidopsis*

Shouling Xu^{1,8}, Xi He^{1,8}, Duy-Chi Trinh^{2,3}, Xinyu Zhang^{2,4}, Xiaojiang Wu^{1,5}, Dengying Qiu¹, Ming Zhou⁶, Dan Xiang¹, Adrienne H. K. Roeder⁷, Olivier Hamant^{2,*}, Lilan Hong^{1,*}

1. Key Laboratory of Nuclear Agricultural Sciences of Ministry of Agriculture and Zhejiang Province, Institute of Nuclear Agricultural Sciences, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China

2. Laboratoire de Reproduction et Développement des Plantes, Université de Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRAE, Lyon, France

3. University of Science and Technology of Hanoi. Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Vietnam

4. Cell and Developmental Biology Department, John Innes Centre, Norwich NR4 7UH, UK

5. The Advanced Seed Institute, National Key Laboratory of Rice Breeding and Biology, Zhejiang Provincial Key Laboratory of Crop Germplasm, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China

6. State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou, China

7. Weill Institute for Cell and Molecular Biology and Section of Plant Biology, School of Integrative Plant Sciences, Cornell University, Ithaca, NY 14853, USA

8. These authors contributed equally.

* Corresponding authors: olivier.hamant@ens-lyon.fr, lilanhong@zju.edu.cn

Abstract

As in origami, morphogenesis in living systems heavily relies on tissue curving and folding, through the interplay between biochemical and biomechanical cues. In contrast, certain organs maintain their flat posture over several days. Here we identified a pathway, which is required for the maintenance of organ flatness, taking the sepal, the outermost floral organ, in *Arabidopsis* as a model system. Through genetic, cellular and mechanical approaches, our results demonstrate that global gene expression regulator VERNALIZATION INDEPENDENCE 4 (VIP4) fine-tunes the mechanical properties of sepal cell walls and maintains balanced growth on both sides of the sepals, mainly by orchestrating the distribution pattern of AUXIN RESPONSE FACTOR 3 (ARF3). *vip4* mutation results in softer cell walls and faster cell growth on the adaxial sepal side, which eventually cause sepals to bend outward. Downstream of VIP4, ARF3 works through modulating auxin signaling to down-regulate pectin methylesterase VANGUARD1, resulting in

34 decreased cell wall stiffness. Our work unravels a 3-component module, which relates hormonal
35 patterns to organ curvature, and actively maintains sepal flatness during its growth.

36

37 **Introduction**

38 In multicellular organisms, organ morphology exhibits astonishing diversity, especially in three
39 dimensions (Jonsson et al., 2023; Lee et al., 2019; Liang and Mahadevan, 2011; Whitewoods et
40 al., 2020). Understanding the mechanisms controlling three-dimensional morphology of organs is
41 critical for unraveling organ function. The establishment and maintenance of three-dimensional
42 morphology in organs are intricate processes that involve a complex interplay of regulatory
43 mechanisms (Aryal et al., 2020; Huang et al., 2018; Jonsson et al., 2021; LeGoff and Lecuit, 2016).
44 The coordination of cell behaviors such as growth, division and differentiation is crucial to
45 maintain proper development and morphology of organs (Basson, 2012).

46 Within plants, development takes place in mechanically interconnected tissues, where
47 variations in cell expansion at specific locations result in deformations at the organ level, such as
48 buckling or bending (Coen et al., 2004; Echevin et al., 2019; Fendrych et al., 2016). Differential
49 cellular growth between different parts of the tissue is a pivotal factor in the formation of bends in
50 organs such as apical hooks and roots (Aryal et al., 2020; Huang et al., 2018; Jonsson et al., 2021;
51 LeGoff and Lecuit, 2016). During organ buckling or bending, the plant hormones auxin and the
52 cell wall interplay to affect mechanical properties of tissues, leading to mechanical asymmetry and
53 consequently differential growth (Baral et al., 2021; Ikushima et al., 2008; Jonsson et al., 2021).
54 In recent years, only a few modules have been identified to relate hormones, cell wall effectors,
55 tissue mechanics and organ bending (Baral et al., 2021; Jonsson et al., 2021). This is what we
56 undertook here, using the sepal, the outermost floral organ, as a model system.

57 We report that mutation of *VERNALIZATION INDEPENDENCE 4* (*VIP4*), a gene encoding a
58 subunit of the global transcription regulator polymerase-associated factor complex (Paf1C), leads
59 to abnormal sepal curvature, through AUXIN RESPONSE FACTOR 3 (ARF3)-dependent
60 downregulation of pectin methylesterase VANGUARD1, affecting cell wall stiffness.

61

62 **Results**

63 **Mutations in *VIP4* disrupt three-dimensional sepal morphology**

64 To investigate the genes and molecular mechanisms controlling sepal curvature, we screened
65 (Hong et al., 2016) and ultimately isolated two mutants that had altered sepal curvature (Fig. 1).
66 Wild-type (WT) *Arabidopsis thaliana* (henceforth *Arabidopsis*) flowers at maturity have quite flat
67 sepals, while flowers of these two mutants tend to have sepals that bend outward (Fig. 1A). Thus,
68 these two mutants were named *abnormal organ morphology 1 (aom1)* and *abnormal organ*
69 *morphology 2 (aom2)*. Allelic test showed that *aom1* and *aom2* were alleles, although *aom1*
70 flowers showed a stronger sepal outward bending phenotype than *aom2* flowers. In each mutant,
71 the degree of sepal outward bending varied between flowers. To quantify the sepal phenotypes,
72 we recorded the bending angle of the most out-curved sepal in each flower and used it as the sepal
73 outward bending angle of that flower. The bending angle of an out-curved sepal was defined as
74 the angle between the tangent line on the curved part of the sepal and the central axis of the flower
75 (Fig. 1B). We quantified the sepal outward bending angles of mature flowers. 87.4% of *aom1*
76 flowers had a sepal bending angle over 90°, while only 4.5% of *aom2* flowers had a sepal bending
77 angle over 90° (Fig. 1C).

78 Bulked segregant analysis sequencing (BSA-seq) of *aom1* identified a G-to-A point mutation
79 at a splice acceptor site of *VIP4 (AT5G61150)*, which encodes a crucial component protein of the
80 polymerase-associated factor complex (Paf1C) (Fig. 1D). This point mutation disrupted normal
81 mRNA splicing (Supplemental Fig. S1A) and caused a dramatic decrease in *VIP4* transcription
82 level (Supplemental Fig. S1B). Since *aom1* and *aom2* are allelic, we performed Sanger sequencing
83 on *aom2*, and found that it had a G-to-A point mutation at the splice site between the tenth exon
84 and the ninth intron of *VIP4* (Fig. 1D). Given that *vip4-4* exhibits stronger phenotypes than *vip4-*
85 *5*, most work in this study was performed on *vip4-4*. Several transfer-DNA (T-DNA) insertion
86 alleles of *VIP4 (vip4-1, vip4-2, vip4-3)* have been previously reported (Zhang and van Nocker,
87 2002). Thus, we renamed *aom1* and *aom2* as *vip4-4* and *vip4-5*, respectively. To verify that
88 mutations in *VIP4* caused sepals to bend outward, the *VIP4-3*×Myc fusion protein under its
89 endogenous promoter (*pVIP4::VIP4-3*×Myc) was transformed into *vip4-4* and *vip4-5*, and it
90 rescued *vip4-4* and *vip4-5* phenotypes (12/12 and 23/25 rescued in T1, respectively) (Fig. 1E).
91 These results indicate the functionality of the fusion protein and provide additional confirmation
92 of the crucial role of *VIP4* in the regulation of sepal curvature.

93 ***vip4-4* adaxial sepal epidermis has softer cell walls and faster cell growth compared to the**
94 **abaxial epidermis**

95 We determined when during development the abnormality in mutant sepals first occurred. *vip4-4*
96 flowers had a normal sepal morphology at early stages until stage 10 when their sepals began to
97 bend outward, causing the flowers to fail to close (Fig. 1F and 1G). Thus this phenotype is not the
98 result of defective initiation, but instead emerges from sepal growth later on in development.

99 In organs such as roots and apical hooks, asymmetric growth on both sides of the organ drives
100 the bending (Aryal et al., 2020; Jonsson et al., 2021; Jonsson et al., 2023). To check whether a
101 comparable growth pattern occurs in sepals, we live-imaged WT and *vip4-4* flowers from stage 10,
102 i.e. when the sepal outward bending started in *vip4-4* (Fig. 2). Both the adaxial and abaxial sides
103 of the same sepal were imaged and the cellular growth rates of both sides were compared. In WT
104 sepals, adaxial cells and abaxial cells grew at similar rates. In contrast, in *vip4-4* sepals, adaxial
105 cells exhibited slightly, but significantly, faster (1.07 ± 0.018 folds, mean \pm s.d., $n = 3$) growth
106 than abaxial cells (Fig. 2A-2C). These results suggest that, over the duration of sepal growth from
107 stage 10 to stage 14 (i.e. 5 days), this small difference would cumulate and explain the outward
108 bending of *vip4* sepals, while WT sepals would maintain symmetric growth and generate unbent
109 sepals.

110 During the bending process in Arabidopsis apical hooks, the elongation rates of cells exhibit
111 a correlation with the mechanical properties of their walls (Aryal et al., 2020; Jonsson et al., 2021).
112 To determine whether *vip4-4* exhibits differential cell wall mechanical properties on both sides of
113 its sepals, we used atomic force microscopy (AFM) to quantify cell wall stiffness of the abaxial
114 and adaxial sides of WT and *vip4-4* sepals at stage 10. In WT sepals at stage 10, cell walls on the
115 abaxial side were softer than on the adaxial side (Fig. 2D and 2E). At similar stage, *vip4-4* sepals
116 had significantly softer cell walls on both the abaxial and adaxial sides compared with WT
117 counterparts (Fig. 2D and 2E). Strikingly, *vip4-4* sepals exhibited a significant decrease in cell
118 wall stiffness on the adaxial side compared with the abaxial side, in contrast to the WT. The cellular
119 growth rate and cell wall stiffness in WT and *vip4-4* sepals demonstrate that stiffer walls correlate
120 with slower growth in sepal development, in line with what have been reported in other organs
121 (Aryal et al., 2020; Jonsson et al., 2021; Zhu et al., 2020).

122 These measurements on cellular growth rate and cell wall stiffness together support the most
123 parsimonious model that in *vip4-4* sepals, softer cell wall contributes to faster cellular growth on
124 the adaxial side, which leads to growth imbalance between the two sides and eventually organ
125 outward bending.

126

127 **Ectopic *ARF3* accumulation promotes sepal outward bending in *vip4-4* mutant**

128 Having explored the cellular basis leading to outward bending in *vip4-4* sepals, we next
129 investigated genetic mechanisms involved in VIP4's controlling of sepal curvature. Paf1C is
130 known to maintain appropriate gene expression by transcription regulation or epigenetic
131 modification of chromatin (Betz et al., 2002; Ng et al., 2003). Mutations in Paf1C components
132 lead to down-regulation/up-regulation of target genes and affect multiple biological processes (Oh
133 et al., 2008). To identify the biological processes that are regulated by VIP4 during sepal
134 morphogenesis, RNA sequencing (RNA-seq) was performed on WT and *vip4-4* sepals at about
135 stage 10. The RNA-seq analysis identified 3714 differentially expressed genes (DEGs)
136 (Supplemental Dataset S1). Gene ontology (GO) term analysis of DEGs revealed an enrichment
137 of biological processes including "cell wall modification", "response to hormone stimulus" and
138 "pectin metabolic process" (Supplemental Fig. S2A; Supplemental Dataset S2).

139 One of the DEGs up-regulated in *vip4-4* sepals is *ARF3* (Fig. 3), which has been shown to
140 play a crucial role in the abaxial/adaxial tissue patterning of aerial lateral organs (Andres-Robin et
141 al., 2020; Fahlgren et al., 2006; Garcia et al., 2006; Pekker et al., 2005). We hypothesized that
142 *ARF3* might be involved in formation of the differential growth or differential cell wall stiffness
143 on the abaxial/adaxial sides of *vip4-4* sepals. To test this, we disrupted the up-regulation of *ARF3*
144 in *vip4-4* by crossing *vip4-4* with an *ARF3* mutant *arf3-1* and observed that the mutation of *ARF3*
145 partially suppressed sepal outward bending in *vip4-4* (Fig. 3B and 3C).

146 The expression of *ARF3* homolog *ARF4* was also up-regulated in *vip4-4* sepals (Fig. 3A and
147 Supplemental Fig. S3A). Considering the similarities in function between *ARF3* and *ARF4*, we
148 speculated that *ARF3* and *ARF4* might be functionally redundant in regulating sepal curvature.
149 Unfortunately, we could not obtain triple mutants of the *VIP4*, *ARF3* and *ARF4* genes, since *VIP4*
150 and *ARF4* are located very close to each other in the genome. To circumvent this problem, we
151 designed *amiRNA ARF3/4*, an artificial RNA that specifically and simultaneously targeted *ARF3*
152 and *ARF4*. Expressing *amiRNA ARF3/4* in WT produced a very similar phenotype to the *ARF3*
153 and *ARF4* double mutant *arf3-1 arf4-2* (Supplemental Fig. S3B), indicating that *amiRNA ARF3/4*
154 worked. Introducing *amiRNA ARF3/4* into *vip4* mutants could significantly suppress sepal outward
155 bending in *vip4-4* and *vip4-5* (Fig. 3B and 3C and Supplemental Fig. S3C). These results suggest
156 that *ARF3* and *ARF4* function redundantly and are necessary in the outward bending of *vip4* sepals.

157 Since *ARF3* showed higher expression level in WT sepals and more significant up-regulation
158 in *vip4-4* sepals than *ARF4*, we focused on *ARF3* for further study. Having found down-regulation
159 of *ARF3* suppressed the sepal outward bending phenotype in *vip4*, we further investigated whether
160 up-regulation of *ARF3* would promote sepal outward bending. Previous studies showed that *ARF3*
161 expression was regulated by the adaxially expressed mobile *TAS3*-derived trans-acting small
162 interfering RNAs (tasiRNAs) which forms a gradient along the adaxial-abaxial axis and restricts
163 its targets, *ARF3* and *ARF4*, to the abaxial side of the organ (Chitwood et al., 2009; Fahlgren et al.,
164 2006; Garcia et al., 2006; Pekker et al., 2005). We expressed a tasiRNA resistant version of *ARF3*
165 (*pARF3::ARF3m-GFP*) (Liu et al., 2014) to make ARF3 levels less polarized in sepals. Sepal
166 outward bending could be observed in *pARF3::ARF3m-GFP* lines (Fig. 3D), although to a lesser
167 extent compared with *vip4-4*. Since it might not be easy to observe a stronger sepal bending
168 phenotype in *vip4-4*, we introduced *pARF3::ARF3m-GFP* into the weaker allele *vip4-5*, and found
169 the transgene greatly enhanced the *vip4-5* sepal bending phenotype (Fig. 3D). The observations
170 show that ectopic ARF3 accumulation promotes sepal outward bending, and suggest that the
171 asymmetric pattern of ARF3 under the control of VIP4 regulates sepal curvature.

172

173 **VIP4 modulates ARF3 distribution**

174 To investigate this scenario, we first asked whether ARF3 is a direct target of VIP4 (Fig. 4). Since
175 Paf1C is recruited to the active Pol II elongation machinery and regulates transcriptional
176 elongation (Betz et al., 2002), we performed a chromatin immunoprecipitation-qPCR (ChIP-qPCR)
177 assay using *pVIP4::VIP4-3×Myc* inflorescences and found that VIP4 directly binds to *ARF3* gene
178 (Fig. 4A).

179 Second, we checked the localization of ARF3 in WT and *vip4-4* sepals using the
180 *pARF3::ARF3-GFP* (Liu et al., 2014) reporter. We found that at early developmental stages, ARF3
181 was primarily located to the abaxial side of WT sepals, resembling its distribution pattern in leaves
182 (Burian et al., 2022). In *vip4-4* sepals at similar stages, the distribution of ARF3 extended towards
183 the adaxial side (Fig. 4B). The ectopic expression of *ARF3* in *vip4-4* sepals lasted to stage 10,
184 when ARF3 was detected on both sides of *vip4-4* sepals, whereas *ARF3* expression could not be
185 detected on either side of WT sepals at this stage (Fig. 4C). In short, *ARF3* expression in *vip4-4*
186 sepals shows prolonged duration and an expanded domain. We also noticed that WT plants
187 transformed with *pARF3::ARF3-GFP* had normal sepal morphology, while ectopic expression of

188 *ARF3* in WT with *pARF3::ARF3m-GFP* generates sepal outward bending, which is consistent
189 with our scenario in which sepal outward bending in *vip4-4* is mainly caused by ectopic ARF3
190 distribution.

191 As mentioned above, *TAS3* tasiRNAs target *ARF3* and *ARF4*. In our study, we observed a
192 significant decrease in the expression of *TAS3* in *vip4-4* sepals compared to WT (Fig. 4D). The
193 down-regulation of *TAS3* may attenuate *TAS3*'s repression of *ARF3* expression and lead to
194 expanded activity of *ARF3* from the abaxial to the adaxial side of *vip4-4* sepal. ChIP-qPCR showed
195 VIP4 could bind the *TAS3* promoter regions (Fig. 4E). Taken together, these results suggested that
196 VIP4 could regulate ARF3 distribution profile directly by binding to *ARF3* and indirectly by
197 affecting *TAS3* expression.

198

199 **ARF3 acts through auxin signaling in regulating sepal morphology**

200 In the simplest scenario, ARF3 would regulate sepal curvature through auxin signaling. To test
201 this hypothesis, we checked whether auxin signaling is required for the outward bending of *vip4-*
202 *4* sepals (Fig. 5). Auxin signaling is inhibited in the auxin receptor quadruple mutant *tir1-1 afb1-*
203 *1 afb2-1 afb3-1* (Dharmasiri et al., 2005). We generated a *vip4-6 tir1-1 afb1-1 afb2-1 afb3-1*
204 pentuple mutant by knocking out *VIP4* via CRISPR-Cas9 (Supplemental Fig. S4A) in *tir1-1 afb1-*
205 *1 afb2-1 afb3-1* mutant and found that the sepals of the pentuple mutant did not bend outward (Fig.
206 5A), suggesting that functional auxin signaling is necessary in the outward bending process of *vip4*
207 sepals.

208 Previous studies revealed that ARF3 influences auxin distribution through regulating the
209 expression of auxin transporter genes (Simonini et al., 2017). There were also significant changes
210 observed in the expression of auxin transporter genes in *vip4-4* (Supplemental Fig. S4B). The
211 distribution of auxin in the shoot is generally determined by the polar localization of the auxin
212 efflux carrier PIN-FORMED1 (PIN1) (Heisler et al., 2005). ARF3 has been reported to bind to the
213 *PIN1* genomic region directly and promotes the expression of *PIN1* in previous research (Simonini
214 et al., 2017). *PIN1* was up-regulated in *vip4-4* sepals compared with WT (Supplemental Fig. S4B).
215 It is likely that the altered distribution of ARF3 in *vip4-4* sepals affects PIN1 localization. We
216 checked PIN1 localization pattern in WT and *vip4-4* sepals using *pPIN1::PIN1-GFP* reporter.
217 Cells in WT exhibited PIN1-GFP polarity from the base to the tip of the sepal, whereas cells in
218 *vip4-4* sepals showed no polarized localization of PIN1-GFP (Fig. 5B).

219 To visualize whether the altered PIN1 localization in *vip4-4* sepals disrupts normal auxin
220 distribution, we examined auxin activity pattern in WT and *vip4-4* sepals using the auxin response
221 reporter *pDR5rev::3×VENUS-N7 (DR5)* (Heisler et al., 2005; Zhu et al., 2020). *vip4-4* sepals had
222 broader auxin activity compared to WT at early developmental stages (Fig. 5C and Supplemental
223 Fig. S4C and 4D). In WT, DR5 signal accumulated mainly in the inner tissue of sepals at stage 5.
224 In contrast, in *vip4-4* sepals at similar stages, DR5 signal displayed an expanded distribution
225 domain, with strong signals visible in all cell layers (Fig. 5C). Although neither WT nor *vip4-4*
226 sepals had detectable *DR5* activities at late developmental stages, the expression levels of
227 numerous genes associated with auxin synthesis, signaling, and response were up-regulated in
228 *vip4-4* sepals at stage 10 compared with WT (Supplemental Fig. S4E). Collectively, these data
229 indicate that *vip4-4* sepals have broader and increased auxin levels.

230 A plethora of studies have demonstrated that auxin influences the mechanical properties of
231 cell walls, although the exact influence varies among tissues (Fendrych et al., 2016; Jonsson et al.,
232 2021; Velasquez et al., 2021; Zhu et al., 2020). To investigate the impact of auxin on the
233 mechanical properties of sepal cell walls, we treated WT flowers at stage 10 with indole-3-acetic
234 acid (IAA) and performed osmotic treatment to assess cell wall stiffness in sepals. We found that
235 WT sepals treated with IAA had a greater cell wall shrinkage compared to the mock treated sepals
236 (Supplemental Fig. S5A and 5B), indicating that increased auxin levels softens sepal cell walls.
237 This finding fits with the increased auxin levels and the decreased cell wall stiffness in *vip4-4*
238 sepals. Our results are also consistent with previous research in sepal primordia, which reported
239 that sepal primordia with weaker auxin signaling had stiffer cell walls (Zhu et al., 2020).

240 Altogether, our data indicate that in *vip4-4* sepals, ectopic ARF3 distribution leads to increased
241 and ectopic auxin accumulation which consequently reduce the stiffness of sepal cell walls,
242 disrupting the balanced growth between adaxial and abaxial sides and affecting sepal curvature.

243

244 **ARF3 regulates sepal curvature through regulating the expression of pectin modification** 245 **enzymes.**

246 Previous studies have demonstrated that ARF3 affects the expression of genes encoding pectin
247 methylesterases (Andres-Robin et al., 2018). In our RNA-seq data, we found many genes
248 differentially expressed in *vip4-4* sepals were related to pectin modification (Supplemental Fig.
249 S2B), which prompted us to hypothesize that the ectopic expression of *ARF3* promotes sepal

250 outward bending not only through modulating auxin distribution, but also through regulating the
251 expression of pectin modification enzymes (Fig. 6). Among the *vip4-4* DEGs encoding pectin
252 methylesterases, the *VANGUARD1* (*VGDI*, *AT2G47040*) gene exhibited a very high expression
253 level in WT sepals and a significant down-regulation in *vip4-4* sepals (Fig. 6A and Supplemental
254 Fig. S2B; Supplemental Dataset S3). Studies in pollen tubes showed that *VGDI* potentially
255 contributed to cell wall stiffening (Jiang et al., 2005). In seedlings, *VGDI* was down-regulated
256 shortly after auxin treatment (Supplemental Fig. S6A). In view of these, we investigated whether
257 *VGDI* works downstream of ARF3 and auxin in modulating sepal curvature.

258 First, we checked how the expression of *VGDI* was affected by *ARF3* levels. Since *vip4-5*
259 plants with varied *ARF3/4* levels exhibited a positive correlation between *ARF3/4* levels and the
260 extent of sepal bending, we analyzed *VGDI* expression in these *vip4-5* plants. Flowers of *amiRNA*
261 *ARF3/4 vip4-5* had a significant up-regulation of *VGDI*, while *pARF3::ARF3(m)-GFP vip4-5*
262 flowers had a decreased level of *VGDI* expression, compared with *vip4-5* flowers (Fig. 6B). The
263 expression of *VGDI* was also up-regulated in *arf3-1* inflorescences compared to WT ones
264 (Supplemental Fig. S6B). Together these data reveal a negative correlation between the expression
265 levels of *ARF3* and *VGDI*. To further explore how ARF3 regulated *VGDI*, we generated
266 transgenic plants containing a dexamethasone (DEX) inducible construct *p35S:ARF3m-GR* that
267 expressed the tasiRNA resistant version of ARF3-GR (ARF3m-GR) fusion protein. In
268 *p35S:ARF3m-GR*, the *ARF3m* cDNA was fused translationally to a DNA fragment encoding the
269 hormone binding domain of the glucocorticoid receptor and placed under the constitutive 35S
270 promoter of the cauliflower mosaic virus. After the DEX treatment, *VGDI* expression in
271 *p35S:ARF3m-GR* flowers was down-regulated (Fig. 6C), indicating that ARF3 represses *VGDI*
272 expression.

273 To study how the up-regulated and ectopic expression of *ARF3* in *vip4-4* sepals affect *VGDI*
274 expression in detail, we examined the tissue expression pattern of *VGDI* gene. We introduced a
275 *pVGDI::GFP-GUS* reporter into WT and *vip4-4* plants. However, no visible GFP signal was
276 detected in either WT or *vip4-4* sepals with the *pVGDI::GFP-GUS* transgene. Therefore, we
277 focused on GUS signals. We compared GUS staining in WT and *vip4-4* flowers ranging from stage
278 10 to stage 14 during which stages the sepal outward bending phenotype started and intensified in
279 *vip4-4*. From stage 10 to stage 14, WT had higher *VGDI* expression on both sides of the sepals
280 than *vip4-4* (Fig. 6D and 6E), which together with the fact that *ARF3* was up-regulated and

281 ectopically expressed in *vip4-4* sepals further support the preceding conclusion that *VGD1*
282 expression is negatively regulated by *ARF3*.

283 *VGD1* has been suggested to stiffen the cell wall in pollen tubes (Jiang et al., 2005). To check
284 how the alteration of *VGD1* expression might affect sepal cell wall mechanical properties as well
285 as sepal morphology, we increased the *VGD1* levels in WT by generating a *VGD1* overexpression
286 line *p35S::VGD1*. The *p35S::VGD1* plants had darker leaves, slower vegetative growth, and
287 smaller sepals compared to WT (Supplemental Fig. S7A and 7B). Cell wall stiffness measurement
288 using AFM showed that *p35S::VGD1* sepals had higher cell wall stiffness compared to WT sepals,
289 indicating VGD1 could lead to stiffened cell walls in sepals (Fig. 6F and Supplemental Fig. S6C).
290 Consequently, VGD1 was used to differentially modify cell wall stiffness in sepals. *VGD1*
291 expressed under an adaxial specific *ASYMMETRIC LEAVES2 (AS2)* (Xu et al., 2008) promoter
292 (*pAS2::VGD1*) in *vip4-4* well suppressed the outward bending of *vip4-4* sepals (Fig. 6G),
293 confirming that the softer cell wall on the adaxial epidermis contributes to sepal outward bending
294 in *vip4-4*.

295 Overall, these results provide a complete scenario in which VIP4 directly and indirectly
296 represses ARF3 activity and pattern in sepals, resulting in modified pectin methylesterification and
297 stiffer walls. This ultimately results in a balanced growth rate between adaxial and abaxial sides
298 to maintain flat sepals. Modifications in any of these actors lead to sepal outward bending.

299

300 **Discussion**

301 As previously shown in leaves, maintaining organ flatness does not occur by default. It notably
302 requires controlled growth at the organ margins (Nath et al., 2003). Here we exerted this finding
303 to the entire organ, in the case of the sepal: maintaining unbent sepals for several days requires
304 molecular and mechanical control of cell growth rate.

305 In this work, through studying Arabidopsis mutants with abnormal sepal outward bending
306 phenotype, we discovered that a generic transcription regulator VIP4 is important for maintaining
307 proper sepal morphology. VIP4 regulates sepal morphogenesis mainly through orchestrating the
308 distribution pattern of ARF3 in sepals, while ARF3 in turn modulates auxin signaling and cell wall
309 modification. In WT sepals, with proper VIP4 and ARF3 function, the mechanical properties of
310 sepal cell walls are fine-tuned to achieve balanced tissue growth, and the sepal three-dimensional
311 morphology is maintained. In *vip4* sepals, *ARF3* is ectopically expressed, resulting in altered auxin

312 distribution and cell wall modification, which leads to differential cell wall mechanical properties
313 and consequently differential growth on two sides of the sepals, hence generating outward bent
314 sepals (Fig. 7). Our work reveals that auxin through cell wall mechanics regulate sepal
315 morphogenesis in Arabidopsis and unravels a 3-component module (VIP4-ARF3-VGD1) that
316 coordinates auxin and cell wall mechanics in sepal development.

317 Similar phenotypes have been reported in other Paf1C mutants (He et al., 2004; Trinh et al.,
318 2023; Zhang and van Nocker, 2002), whereas the precise molecular and cellular mechanisms
319 responsible for their bending remained unidentified. Paf1C mutants also exhibit morphological
320 defects in other organs such as cotyledons, siliques, leaves and shoot apical meristems (Fal et al.,
321 2019; Fal et al., 2017; Trinh et al., 2023), indicating that Paf1C plays important roles in regulating
322 the three-dimensional morphology of organs. Paf1C is involved in transcriptional regulation.
323 Mutations in the Paf1C genes lead to dysregulation of global gene expression and subsequent
324 numerous phenotypic abnormalities (Fal et al., 2019; Fal et al., 2017; He et al., 2004; Oh et al.,
325 2008; Trinh et al., 2023; Zhang and van Nocker, 2002; Zhao et al., 2005). Accordingly, we have
326 identified thousands of genes differentially expressed in *vip4* sepals.

327 Among these genes, *ARF3* exerts a primary role in the outward bending of sepals in *vip4*.
328 *ARF3* expression is regulated at the transcriptional, post-transcriptional and epigenetic levels
329 (Chandler, 2016). In sepals, VIP4 regulates *ARF3* expression at least on two levels: directly by
330 binding to *ARF3* genomic region and indirectly by affecting *TAS3* expression. Via this
331 multifaceted regulation, VIP4 ensures a relatively stable ARF3 distribution pattern, and
332 consequently robust sepal morphogenesis. Paf1C has been implicated in controlling transcriptional
333 noise in Arabidopsis (Ansel et al., 2008; Fal et al., 2019; Trinh et al., 2023). Our data suggests a
334 possible mechanism of Paf1C maintaining the robustness of downstream gene expression.

335 *ARF3* expression can be induced by exogenous auxin (Zhang et al., 2022) and ARF3
336 modulates auxin distribution via regulating the expression of polar auxin transportation genes
337 (Simonini et al., 2017). In addition, ARF3 acts as an auxin signaling component (Kuhn et al., 2020;
338 Simonini et al., 2016; Simonini et al., 2018). In view of VIP4's direct regulation of ARF3
339 distribution and the altered distribution patterns of auxin and auxin polar transporters in *vip4* sepals,
340 we speculate that ARF3 affects sepal morphogenesis through orchestrating auxin distribution, in
341 line with *ARF3*'s role in auxin signaling and pattern specification (Simonini et al., 2017).

342 We cannot rule out the possibility that VIP4 affects auxin distribution through directly
343 regulating the expression of genes pertinent to auxin dynamics, or that auxin signaling exerts
344 impacts on sepal morphology through other ARF3/4 independent pathways. We also cannot rule
345 out the possibility that VGD1, with such a striking effect when overexpressed, triggers
346 compensatory effects on cell wall properties, given the size of the PME family and the complexity
347 of the cell wall (Francoz et al., 2019; Hocq et al., 2017).

348 Although our study focused on the relationship between *ARF3* and genes related to pectin
349 methylesterification, *ARF3* also affects the expression level of genes involved in other cell wall
350 modifications (Supplemental Dataset S3). Numerous studies have demonstrated that auxin activity
351 and cell wall mechanics are highly interrelated in organ morphogenesis (Aryal et al., 2020; Baral
352 et al., 2021; Jonsson et al., 2021; Qi et al., 2017). Our work revealed that auxin and cell wall
353 mechanics also collaborate in shaping three-dimensional sepal morphology, with *ARF3* and its
354 upstream regulator Paf1C acting as overarching coordinators.

355

356 **Methods**

357 **Mutations**

358 In this study, Arabidopsis accession *Col-0* plants were used as wild type (WT). WT
359 seeds were mutagenized with 0.3% (v/v) ethyl methanesulfonate in 10 mL 0.02% (v/v)
360 Tween 20 for 24 hours. M2 plants were examined under a dissecting microscope for the
361 abnormal three-dimensional sepal phenotype. The *vip4-4* and *vip4-5* mutants were
362 back-crossed to *Col-0* three times to segregate unrelated mutations before further
363 characterization. The *vip4-4* mutated gene was identified through BSA-seq following
364 the standard procedure described in ref. (Takagi et al., 2013). The *vip4-4* mutant has a
365 G-to-A mutation at the junction between the fifth intron and sixth exon within the *VIP4*
366 (*AT5G61150*) gene, which disrupts normal mRNA splicing. The *vip4-5* mutant has a
367 G-to-A mutation at the splice junction between the ninth intron and tenth exon. Allelism
368 tests were conducted between *vip4-4* and *vip4-5*. The F1 displayed the *vip4-4* mutant
369 phenotypes, indicating that these two mutants are allelic.

370

371 **Flower staging.**

372 Flowers were staged according to ref. (Smyth et al., 1990).

373

374 **Live imaging of sepal growth.**

375 Live imaging of both sides of sepals and shoot apical meristems was conducted
376 following the sample preparation method outlined in ref. (Stanislas et al., 2017). Briefly,
377 young inflorescences containing different genetic constructs, namely *pUBQ10::Lti6b-*
378 *tdTomato*, *pDR5rev::3×VENUS-N7* (Heisler et al., 2005; Zhu et al., 2020),
379 *pPIN1::PIN1-GFP*, and *pARF3::ARF3-GFP* (Liu et al., 2014) were dissected at the
380 desired developmental stages using tweezers. For imaging the adaxial and abaxial sides
381 of sepals, all flower buds beyond stage 10 were dissected off and one sepal from flower
382 buds at stage 10 was selectively kept for imaging, while smaller flower buds were
383 retained for further observations. The dissected inflorescences were then cultured in the

384 apex culture medium (containing 1% sucrose, 0.25×vitamin mix and 1% agar) as
385 described in ref. (Stanislas et al., 2017). For the analysis of sepal growth, both the
386 adaxial and abaxial sides of the same sepal were imaged on the first and fourth days,
387 respectively. In cases where the size of the sepals exceeded the image acquisition frame,
388 multiple tiles were stitched together using the Stitching plugin in ImageJ (Preibisch et
389 al., 2009).

390 All confocal images were acquired using an SP8 laser-scanning confocal
391 microscope (Leica) equipped with a long-distance 25× (NA 0.95) water-dipping
392 objective and a resonant scanner module with a 1.0 μm z-step, except for the imaging
393 of *pARF3::ARF3-GFP*, which was performed using an upright 980 laser-scanning
394 confocal microscope (Zeiss). Excitation and emission wavelengths for fluorescent
395 proteins are indicated in Supplemental Dataset S4.

396

397 **Transgenic plants.**

398 Otherwise stated, the amplification of PCR fragments was performed using KOD DNA
399 polymerase (Toyobo, CAT KOD-101) and all vectors were generated using T5
400 exonuclease-dependent assembly (Xia et al., 2019).

401 To generate *pVIP4::VIP4-3×Myc*, the *VIP4* sequence from the promoter (2036 bp
402 upstream the start codon) to the sequence before the stop codon was amplified from
403 *Col-0* genomic DNA. The PCR product was cloned into *pRGEcMyc* (He et al., 2018)
404 digested with the restriction enzymes *PstI* and *SpeI*.

405 The 4.1-kb upstream promoter and part of the first exon region of the *AS2* gene
406 were first PCR amplified from WT genomic DNA and cloned into *pENTR/D-TOPO*
407 vectors (Invitrogen) as described in the manual. The resultant vector was LR
408 recombined into the gateway vector *pBGFWG2* (Karimi et al., 2002) to generate the
409 final construct *pAS2::GFP-GUS*.

410 To generate *pVGD1::GFP-GUS*, the *VGD1* promoter (2137 bp upstream of the
411 start codon) was amplified and was inserted into *pAS2::GFP-GUS* digested with *XbaI*
412 and *PmeI*.

413 To generate *p35S::VGD1* and *pAS2::VGD1*, the *VGD1* sequence from the start
414 codon to the stop codon was amplified from WT genomic DNA. The PCR product was
415 cloned into *pCAMBIA1300-35S* (a binary vector derived from *pCAMBIA1300*,
416 containing the 2× CaMV 35S promoter and the CaMV terminator) digested with *KpnI*
417 and *PstI* and *pAS2::GFP-GUS* digested with *NcoI* and *AscI*, respectively.

418 *ARF3/ARF4* knockdown mutants were created through the utilization of an
419 artificial microRNA (*amiRNA*) based knockdown approach (Schwab et al., 2006). To
420 design the *amiRNA* constructs, the Web MicroRNA Designer program available at
421 <http://wmd3.weigelworld.org/> was employed to concurrently target both *ARF3* and
422 *ARF4*. The *amiRNA* construct was constructed with the endogenous *miRNA319a* as the
423 backbone. The final products were cloned into *pCAMBIA1300-35S* digested with *KpnI*
424 and *PstI*.

425 For the overexpression of *ARF3m-GR*, *ARF3m-GR* was amplified using
426 *pARF3::ARF3m-GR* (Liu et al., 2014) as a template and then cloned into
427 *pCAMBIA1300-35S* digested with *KpnI* and *PstI*.

428 To generate *VIP4* mutation via CRISPR-Cas9, two guide sequences targeting *VIP4*
429 were designed and then inserted into *pHEE401* vector, according to the guide of ref.
430 (Xing et al., 2014).

431 All primers used to construct the vectors are listed in Supplemental Dataset S5.
432 All of the final constructs including *pARF3::ARF3-GFP* and *pARF3::ARF3m-GFP*
433 (Liu et al., 2014) were verified by sequencing and transformed into the corresponding
434 plants by *Agrobacterium*-mediated floral dipping. Note that, *pVGD1::GFP-GUS* and
435 *pARF3::ARF3-GFP* were transformed into F1 plants (the hybrid of *vip4-4* and WT) .
436 All T1 plants were grown in plates with corresponding antibiotic resistance

437 (Kanamycin, Hygromycin and Basta). The surviving plants or plants with elongated
438 hypocotyls were then checked for sepal phenotypes or fluorescence at the microscope.
439

440 **RNA-seq**

441 For sample collection, the WT and *vip4-4* sepals at stage 10 were dissected under a
442 stereomicroscope and collected into Eppendorf tubes with liquid nitrogen. Three
443 biological replicates were collected, each replicate combining sepals from different
444 individual plants. RNA extraction was done using a commercial kit (Easy Plant RNA
445 Kit, Easy-Do, CAT DR0406050) following the manual. Library preparation was carried
446 out, and their quality was assessed with an Agilent 2100 Bioanalyzer (Agilent
447 Technologies). Subsequently, sequencing was performed on a HiSeq 2500 (Illumina)
448 in accordance with the manufacturer's guidelines. Raw reads were subjected to cleaning
449 and alignment to the TAIR10 Arabidopsis reference genome using Bowtie2, as
450 described in ref. (Langmead and Salzberg, 2012). Genes with more than 2.0-fold
451 change in expression and P value < 0.01 were considered to be differentially expressed
452 genes. GO enrichment was determined using agriGO as described in ref. (Du et al.,
453 2010) and Heatmap was made on <https://hiplot.com.cn/>.

454

455 **Quantitative RT-PCR**

456 Total RNA was isolated from inflorescences (with mature flowers removed) using the
457 Easy Plant RNA Kit (Easy-Do, CAT DR0406050). For DEX treatment experiments,
458 transgenic inflorescences were treated by 10 μ M DEX for 3 d. First-strand cDNA was
459 synthesized using oligo d(T) and reverse transcriptase (Vazyme). qPCR was performed
460 using Hieff qPCR SYBR Green master mix (Yeasen, CAT 11201ES08) in a
461 LightCycler 480 (Roche) according to the manufacturers' instructions. Three technical
462 replicates were carried out. All primers used for qRT-PCR were listed in Supplemental
463 Dataset S5. Quantification of *Actin* gene served as a control.

464

465 **ChIP assay**

466 ChIP assays were performed as previously described in ref. (Bowler et al., 2004). The
467 inflorescences were treated with 1% (v/v) formaldehyde to cross-link the protein-DNA
468 complexes on ice. After isolation and sonication, samples were centrifuged at 12,000 g
469 for 10 min at 4°C. The supernatants were precleared with 20 uL protein-A agarose
470 beads for at least 1 hour, then incubated with 20 uL Myc-Nanoab-agarose beads
471 (Lablead, CAT MNA-25-500) overnight. The precipitated DNA samples were
472 quantified by qPCR using primers listed in Supplemental Dataset S5. Quantification of
473 the *Actin* gene served as a control.

474

475 ***VGDI* expression level and pattern analysis by GUS staining**

476 *pVGDI::GFP-GUS* was utilized to detect the expression pattern of *VGDI* in WT and
477 *vip4-4* sepals during stage 10 to stage 14. *pVGDI::GFP-GUS* in WT and
478 *pVGDI::GFP-GUS* in *vip4-4* were derived from the same genetic line. GUS staining
479 was conducted following the procedure described in ref. (Sessions et al., 1999). Briefly,
480 flowers were subjected to overnight staining with a solution containing 50 mM sodium
481 phosphate buffer (pH 7.0), 0.2% (v/v) Triton-X-100, 10 mM potassium ferrocyanide,
482 10 mM potassium ferricyanide, and 1 mM X-gluc at 37 °C. Subsequently, the stained
483 tissue was dehydrated and cleared using a series of ethanol solutions. GUS-stained
484 sepals were then photographed using a Leica binocular.

485

486 **Image processing for growth quantification.**

487 The procedures for image processing and growth quantification were carried out
488 following the methodology described in ref. (Hong et al., 2016; Zhu et al., 2020). In
489 brief, the confocal stacks obtained from live imaging of sepal growth were converted
490 from LSM to TIFF format using FIJI. The MorphoGraphX (MGX) 3D image analysis
491 software (de Reuille et al., 2015) was utilized for growth analyses. The surfaces of the
492 samples were detected and meshes representing the surfaces were generated.

493 Fluorescent signals were projected onto the meshes, and the cells were segmented
494 within the meshes.

495

496 **AFM**

497 AFM was conducted to examine the cell wall stiffness of sepals at stage 10. The sepals
498 were carefully removed from the flowers and placed in a solid growth medium in a Petri
499 dish, following the protocol described in ref. (Bovio et al., 2019), with minor
500 modifications. An R=400 nm spherical-end tip with a nominal force constant of 42 N/m
501 was used. Prior to the measurements, each cantilever was calibrated using indentation
502 on sapphire and thermal tune, both conducted in water.

503

504 **Osmotic treatments measuring sepal stiffness**

505 WT and *vip4-4* sepals at stage 10 were subjected to 0.4 M NaCl treatment for 30 min.
506 The areas of the sepals before and after the treatment were compared using MGX,
507 following the methodology described in ref. (Hong et al., 2016; Kierzkowski et al.,
508 2012). For IAA treatment experiments, WT inflorescences were treated by 1 mM IAA
509 for 1 d. The flowers at stage 10 were then processed according to the above-mentioned
510 methods.

511

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528

529 **Author contributions**

530 Conception and design of experiments: SX, AHKR, OH, and LH. Isolation and
531 characterization of *vip4-4* and *vip4-5* mutants: SX, and LH. BSA-seq analysis: LH and
532 ZM. Live imaging and analysis: SX, D-CT, XH, and LH. AFM analysis: SX. RNA-seq
533 analysis: XW, DQ and MZ, qRT-PCR analysis: SX, XH and XZ. Sepal phenotypes
534 quantification: SX, DX and DQ. CHIP-qPCR analysis: SX and XH. Manuscript writing:
535 SX and LH. Manuscript revising and editing: SX, XH, D-CT, XZ, XW, DQ, MZ, DX,
536 AHKR, OH, and LH.

537

538 **Competing interests**

539 The authors declare no competing interests.

540

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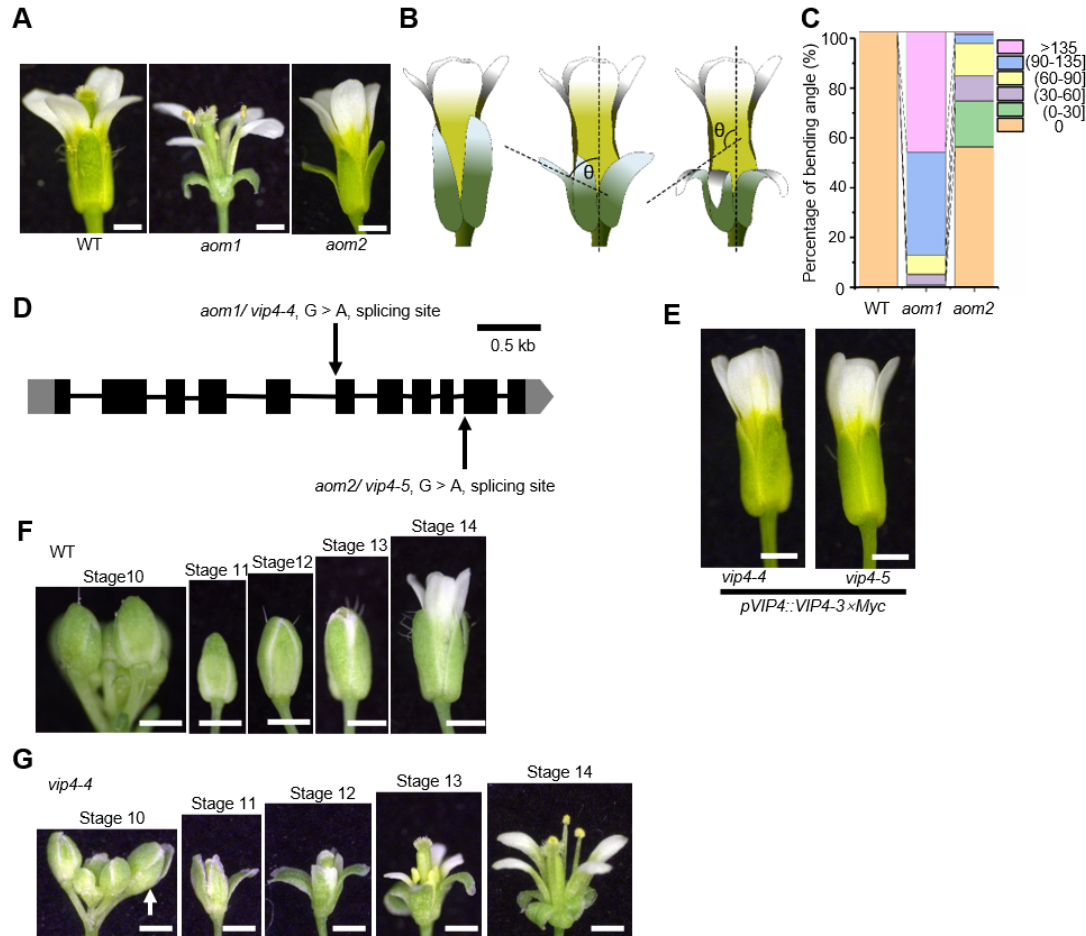
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755

756

Figure 1. Mutations in the *VIP4* gene lead to sepal outward bending.

757

(A) Wild-type (WT), *aom1* and *aom2* flowers at maturity (stage 14). Sepals in *aom1* and *aom2* mutants exhibit outward bending compared to the WT. Scale bars, 0.5 mm.

758

759

(B) Schematic diagram illustrating how to define the bending angle (θ) of a sepal.

760

(C) *aom1* displays a stronger sepal bending phenotype compared to *aom2*. The sepal bending angle was quantified for WT and *aom1*, *aom2* based on the criteria shown in panel (B). The bending angle of WT sepal was set to 0.

761

762

(D) Mutation sites of *vip4* alleles isolated in our study.

763

(E) T1 plants of *vip4-4* and *vip4-5* transformed with *pVIP4::VIP4-3xMyc*. Scale bars, 0.5 mm.

764

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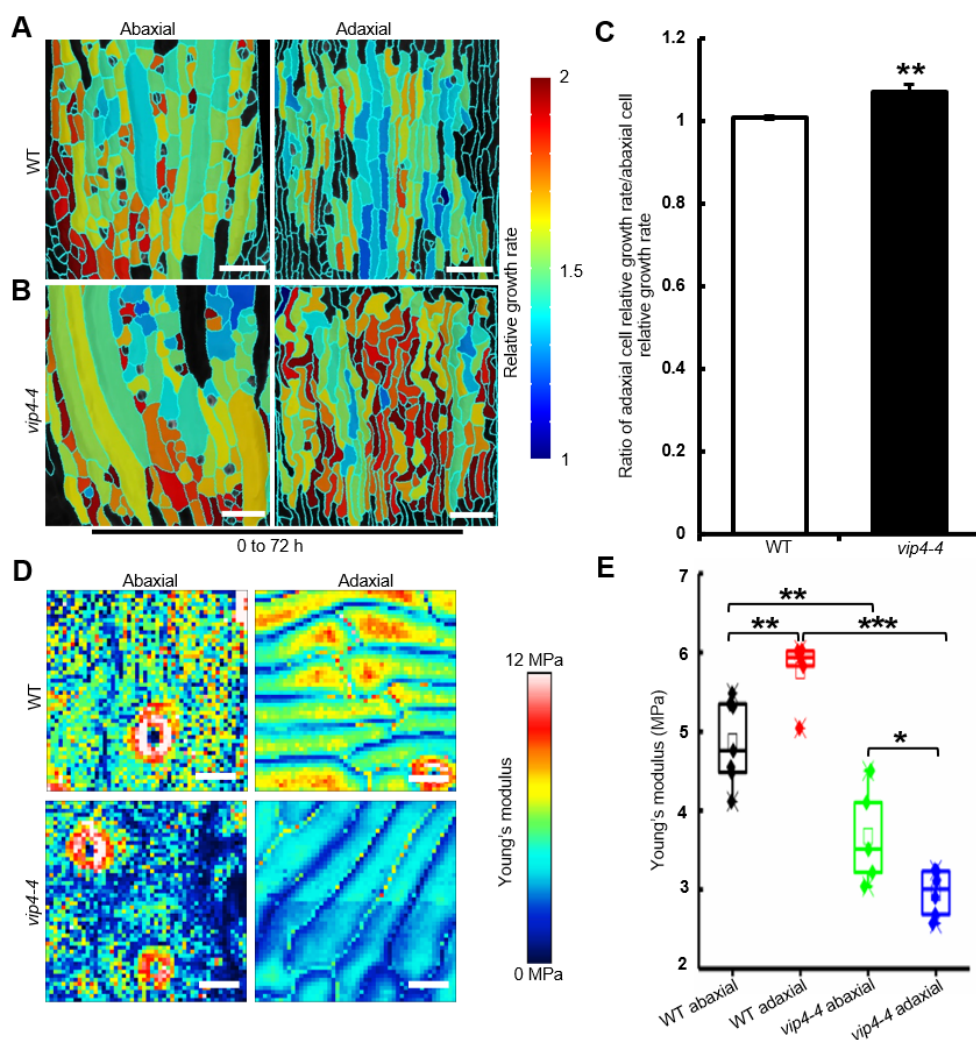
766

(F and G) Developmental progression of sepals in WT and *vip4-4*. The flowers of *vip4-4* (G) exhibit normal sepal shape in the early stage of development similar to WT (F).

767

768

vip4-4 sepals start to bend outward from stage 10. Scale bars, 0.5 mm.



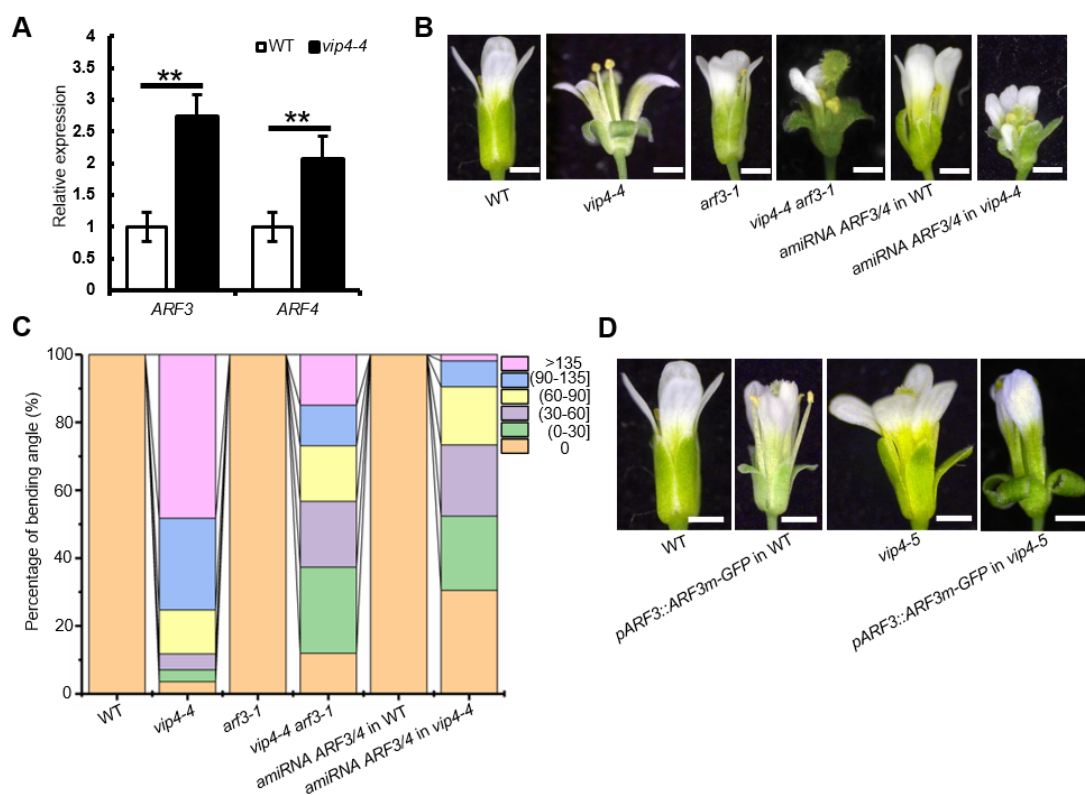
769

770 **Figure 2. *vip4-4* sepals show differential cell growth and cell wall stiffness**

771 **(A and B)** Heatmaps show 72-hour cellular growth rates on the abaxial (left) side and
772 adaxial (right) side of WT **(A)** and *vip4-4* **(B)** sepals at stage 10. The sepal cellular
773 growth rate was quantified through live imaging. Red and blue colors indicate high and
774 low relative growth rate, respectively. The growth rate is calculated as the ratio of cell
775 area at 72 h to cell area at 0 h. $n = 3$ biological flowers, all showing similar trends. Scale
776 bars, 50 μm .

777 **(C)** The ratios of adaxial cell relative growth rate to abaxial cell relative growth rate in
778 WT and *vip4-4* sepals. In *vip4-4* sepals, adaxial cells grow faster than abaxial cells,
779 while in WT sepals, adaxial cells and abaxial cells have similar growth rates. Data are
780 mean \pm s.d., $n = 3$ flowers. Two-tailed Student's t test, $**P < 0.01$. Exact P value is
781 listed in Supplemental Dataset S6.

782 **(D and E)** *vip4-4* sepals have softer cell wall. **(D)** AFM measurement of cell wall
783 stiffness (Young's modulus) of both sides for WT and *vip4-4* sepals at stage 10. In the
784 heatmap, stiff points are shown in red and soft points are shown in blue. Scale bars, 10
785 μm . **(E)** The average apparent elastic modulus calculated from AFM measurements. n
786 = 7 for WT abaxial, $n = 5$ for WT adaxial, $n = 5$ for *vip4-4* abaxial, $n = 6$ for *vip4-4*
787 adaxial. Two-tailed Student's t test, $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. Exact P
788 values are listed in Supplemental Dataset S6. For the boxplots, the box extends from
789 the lower to upper quartile values of the data, with a line at the median.



790

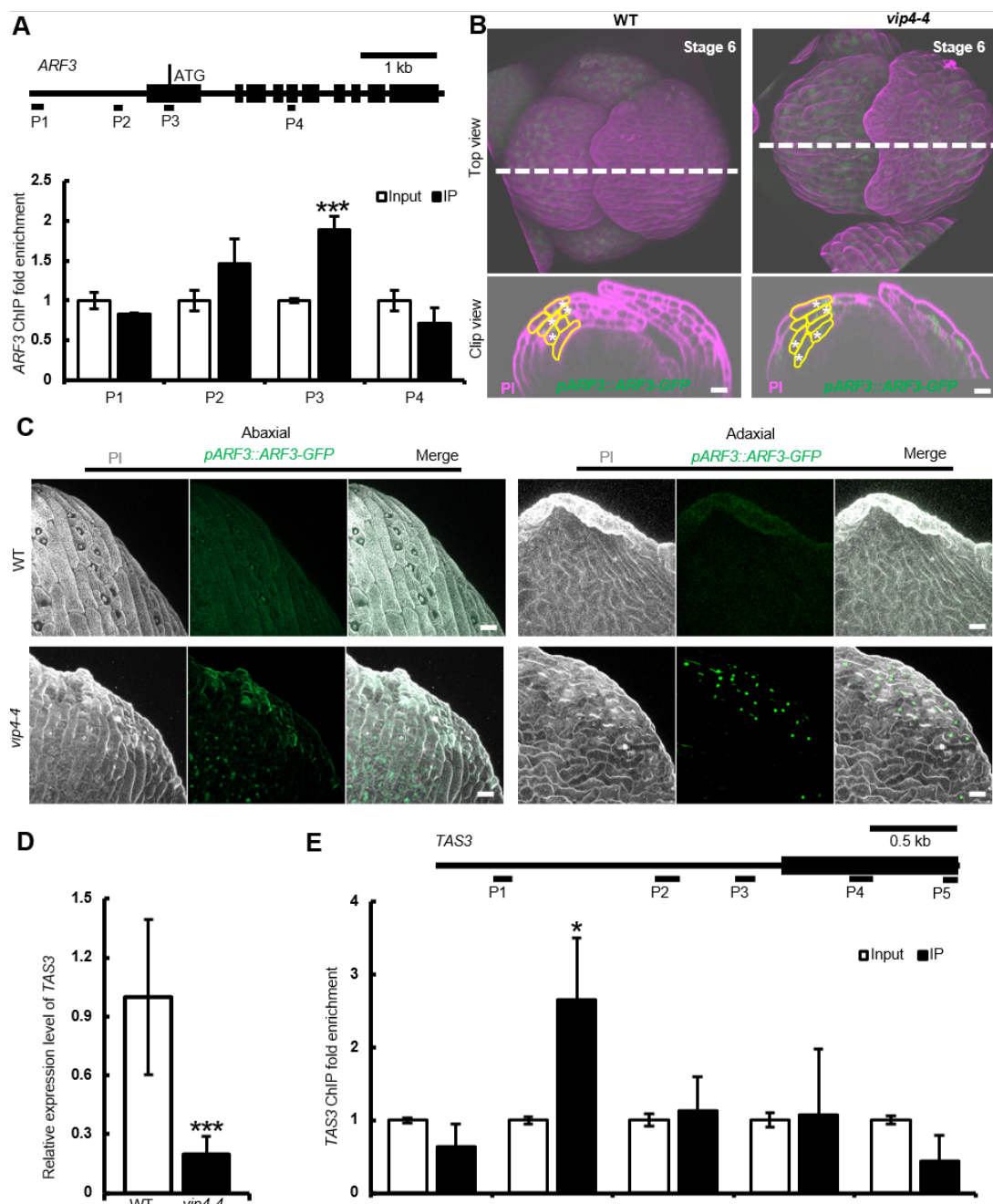
791 **Figure 3. *ARF3* expression levels affect sepal curvature.**

792 **(A)** RNA-seq analysis revealing up-regulation of *ARF3* and *ARF4* in *vip4-4* sepals
 793 compared to WT. Data are mean \pm s.d., $n = 3$ replicates. Adjusted P value (P -adj) is
 794 obtained via Benjamini-Hochberg correction. ** P -adj < 0.01 . Exact P -adj are listed in
 795 Supplemental Dataset S6.

796 **(B)** Flowers of WT, *vip4-4*, *arf3-1*, *vip4-4 arf3-1*, *amiRNA ARF3/4* in WT and *amiRNA*
 797 *ARF3/4* in *vip4-4*. Scale bars: 0.5 mm.

798 **(C)** Quantification of sepal bending angle of the indicated lines, showing reduced sepal
 799 bending angles in the *vip4-4* background when *ARF3* was mutated or knocked down.
 800 The bending angle of WT sepal was set to 0.

801 **(D)** Flowers of WT, *pARF3::ARF3m-GFP* in WT, *vip4-5*, *pARF3::ARF3m-GFP* in
 802 *vip4-5*, showing increased *ARF3* expression level could lead to or enhance sepal
 803 outward bending. WT is reproduced from Fig. 2B. Scale bars: 0.5 mm.



804

805 **Figure 4. VIP4 regulates *ARF3* expression profile through binding to *TAS3* and**
 806 ***ARF3*.**

807 (A) Diagrams illustrating the gene structure of *ARF3* with ChIP-qPCR target positions
 808 shown and ChIP-qPCR results showing the enrichment of VIP4 in *ARF3* genomic
 809 region using anti-Myc antibody. Data are mean \pm s.d., $n = 3$ replicates. Two-tailed
 810 Student's *t* test, *** $P < 0.001$. Exact *P* value is listed in Supplemental Dataset S6.

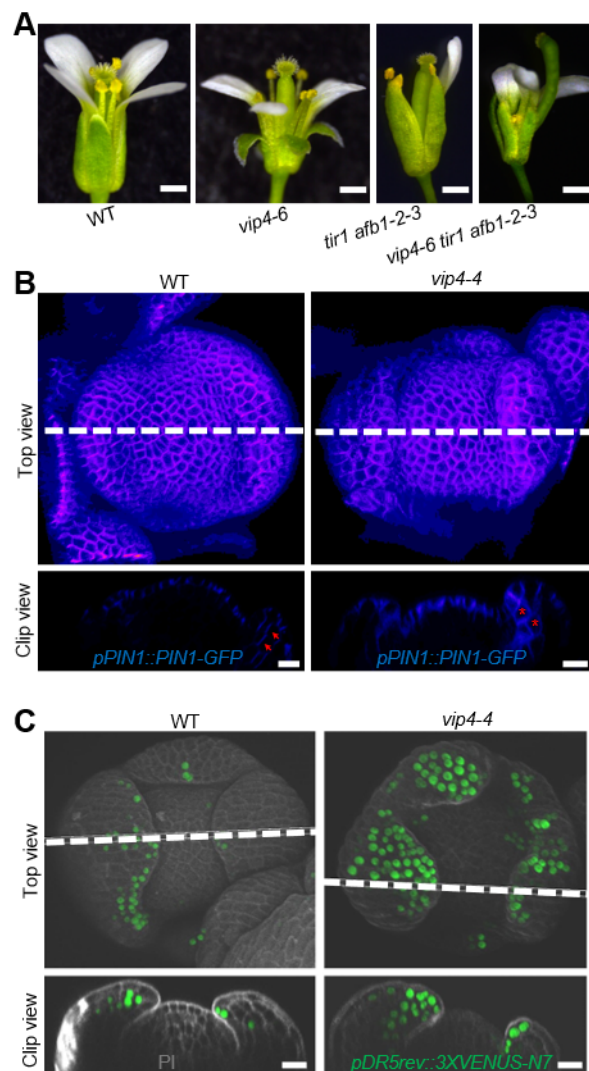
811 (B) *ARF3* distribution pattern in WT and *vip4-4* sepals at stage 6. Confocal images
 812 showing the distribution of *ARF3* (*pARF3::ARF3-GFP*, green) in WT and *vip4-4*
 813 flowers at stage 6 stained with PI (magenta). Upper panels show the front view of the

814 flowers. Lower panels show the side view of clips at the white lines in upper panels.
815 *ARF3* is expressed in the abaxial three layers of cells in WT sepals, while in *vip4-4*
816 sepals *ARF3* is distributed in all the four layers of cells. Some cells in the transverse
817 sections are outlined in yellow to show the four layers of sepal cells. Asterisks mark
818 *ARF3*-GFP signals. Scale bars, 20 μm .

819 **(C)** *ARF3* distribution pattern in WT and *vip4-4* sepals at stage 10. *ARF3*-GFP signals
820 could not be detected on either sides of sepals at stage10 in WT, while remain visible
821 in *vip4-4* sepals at the same stage (some *ARF3*-GFP signals highlighted with red
822 circles). Gray, propidium iodide (PI) staining the cell wall; green, *pARF3::ARF3*-GFP.
823 Scale bars, 20 μm . n= 10 flowers for WT. n=8 flowers for *vip4-4*.

824 **(D)** RNA-seq data showing downregulation of *TAS3* in *vip4-4* sepals compared to WT.
825 Data are mean \pm s.d., n = 3 replicates. *P*-adj is obtained via Benjamini-Hochberg
826 correction. ****P*-adj < 0.001. Exact *P*-adj is listed in Supplemental Dataset S6.

827 **(E)** Diagrams illustrating the gene structure of *TAS3* with ChIP-qPCR target positions
828 shown and ChIP-qPCR results showing the enrichment of VIP4 in *TAS3* promoter using
829 anti-Myc antibody. Data are mean \pm s.d., n = 3 replicates. Two-tailed Student's t test,
830 **P* < 0.05. Exact *P* value is listed in Supplemental Dataset S6.



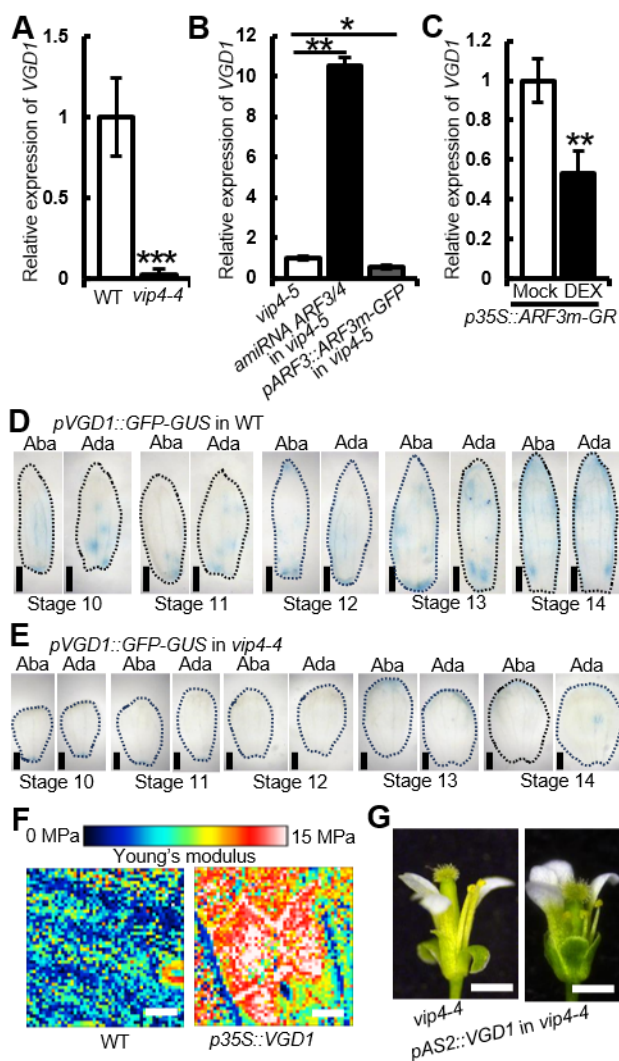
831

832 **Figure 5. Auxin signaling is involved in the outward bending of *vip4-4* sepals.**

833 (A) Flowers of WT, *vip4-6*, *tir1 afb1-2-3*, and *vip4-6 tir1 afb1-2-3*. Inhibiting auxin
834 signaling in *vip4* suppresses sepal outward bending. Scale bars: 0.5 mm.

835 (B) Confocal images showing PIN1 (*pPIN1::PIN1-GFP*, blue) distribution in WT and
836 *vip4-4* flowers at stage 4. Upper panels show the front view of the flowers. Lower
837 panels show the side view of clips at the white lines in upper panels. Cells in WT exhibit
838 PIN1-GFP polarity from the base to the tip of the sepal (as the red arrows point), while
839 cells in *vip4-4* sepals show no polarized localization of PIN1-GFP (marked with
840 asterisks). Scale bars, 20 μm, n = 5 flowers. LUT is gem in ImageJ.

841 (C) Confocal images showing auxin distribution (*pDR5rev::3xVENUS-N7*, green) in
842 WT and *vip4-4* flowers at stage 5 stained with PI (gray). Upper panels show the front
843 view of the flowers. Lower panels show the side view of clips at the white lines in upper
844 panels. DR5 signals accumulate at the tips of four sepals in WT, while have a broader
845 distribution in *vip4-4* sepals. Scale bars, 20 μm. n = 5 flowers.



846

847 **Figure 6. ARF3 may affect cell wall stiffness by regulating *VGD1* expression.**

848 **(A)** RNA-seq analysis revealing down-regulation of *VGD1* in *vip4-4* sepals compared
 849 to WT. Data are mean \pm s.d., $n = 3$ biological replicates. P -adj is obtained via
 850 Benjamini-Hochberg correction. *** P -adj < 0.001 . Exact P -adj is listed in
 851 Supplemental Dataset S6.

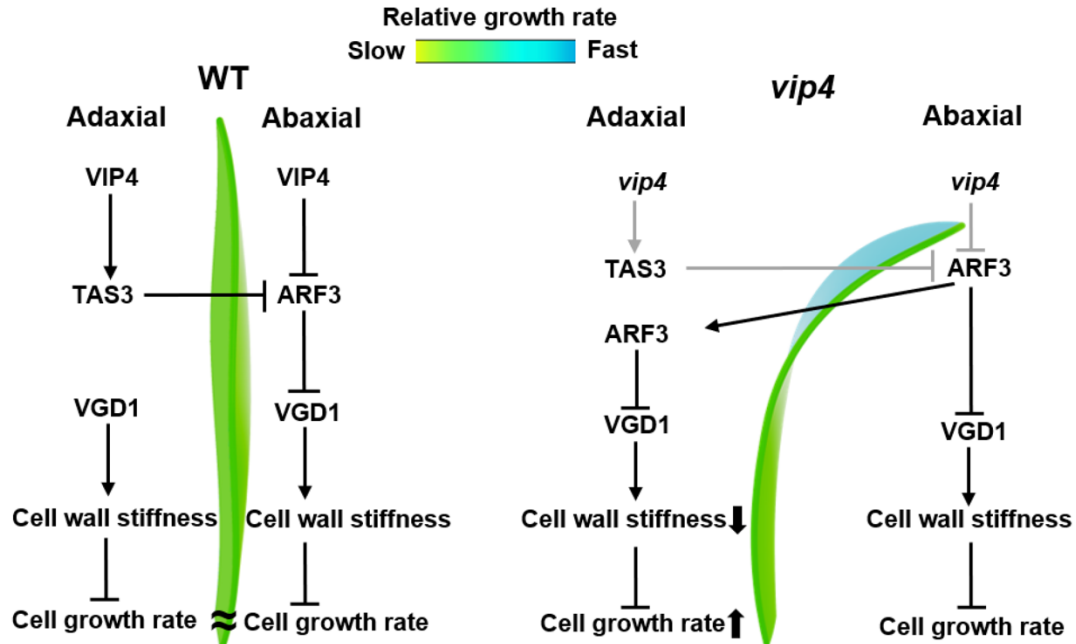
852 **(B and C)** *ARF3* negatively regulates *VGD1* expression. **(B)** *VGD1* expression is up-
 853 regulated by *amiRNA ARF3/4* while down-regulated by *ARF3* overexpression
 854 (*pARF3::ARF3m-GFP*) in *vip4-5* background. **(C)** DEX-treated *p35S:ARF3m-*
 855 *GR* flowers showing down-regulation of *VGD1* in contrast to solvent DMSO-treated
 856 flowers. Data are mean \pm s.d., $n = 3$ biological replicates. Two-tailed Student's t test,
 857 * $P < 0.05$, ** $P < 0.01$. Exact P values are listed in Supplemental Dataset S6.

858 **(D and E)** *VGD1* expression profiles during late development stage in WT **(D)** and
 859 *vip4-4* **(E)** sepals. From stage 10 to stage 14, WT shows higher *VGD1* expression level

860 on both sides of the sepals compared to *vip4-4* (sepals outlined with dashed lines). Scale
861 bars, 200 μm .

862 **(F)** Overexpression of *VGDI* stiffens cell walls in sepals. AFM measurements show
863 increased cell wall stiffness in *p35S:VGDI* lines. Scale bars, 10 μm .

864 **(G)** *pAS2::VGDI* suppresses the *vip4-4* sepal outward bending by hardening the adaxial
865 cell wall. Scale bars, 0.5 mm.



866

867 **Figure 7. Summary model: a 3-component module controls sepal curvature.**

868 In WT sepals, ARF3 expression is restricted to the adaxial side of the sepals due to the
869 presence of functional VIP4 and appropriate expression level of *TAS3*. The proper
870 distribution of ARF3 plays a crucial role in regulating cell wall stiffness by influencing
871 the expression level of *VGD1*. This regulation, in turn, contributes to maintaining
872 balanced tissue growth on both sides of the WT sepal, resulting in a flat sepal structure.
873 However, in *vip4-4* sepals with a mutated *VIP4* gene, there is an up-regulation of *ARF3*
874 expression and a down-regulation of *TAS3* expression. This leads to the ectopic
875 expression of *ARF3*, disrupting its normal adaxial confinement. The abnormal
876 distribution of ARF3 results in an accelerated cell growth rate with softer cell walls by
877 mediating *VGD1* expression. Consequently, this abnormal growth pattern leads to the
878 outward bending of the sepal.

879