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## Heterogeneous identity, stiffness and growth characterise the shoot apex of Arabidopsis stem cell mutants

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### ► To cite this version:

Léa Rambaud-Lavigne, Aritra Chatterjee, Simone Bovio, Virginie Battu, Quentin Lavigne, et al.. Heterogeneous identity, stiffness and growth characterise the shoot apex of Arabidopsis stem cell mutants. *Development* (Cambridge, England), 2024, 151 (11), pp.dev202810. 10.1242/dev.202810 . hal-04668303

**HAL Id: hal-04668303**

**<https://hal.science/hal-04668303>**

Submitted on 6 Aug 2024

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1 **Heterogeneous identity, stiffness and growth characterise**  
2 **the shoot apex of *Arabidopsis* stem cell mutants**

3  
4 **Running title:** Properties of stem cell mutants

5  
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25  
26 Key words (3-6): shoot apical meristem, *WUSCHEL*, *CLAVATA*, heterogeneities,  
27 morphogenesis, stem cell mechanics

28  
29 Summary statement: Heterogeneities in cell mechanics, growth, function and identity  
30 contribute to buckling in *clavata* mutant shoot apices.

31 **Abstract**

32 Stem cell homeostasis in the shoot apical meristem involves a core regulatory feedback  
33 loop between the signalling peptide CLAVATA3, produced in stem cells, and the  
34 transcription factor WUSCHEL, expressed in the underlying organising centre. *clavata*  
35 mutant meristems display massive overgrowth, which is thought to be caused by stem  
36 cell overproliferation, although it is unknown how uncontrolled stem cell divisions lead to  
37 this altered morphology. Here we reveal local buckling defects in mutant meristems, and  
38 use analytical models to show how mechanical properties and growth rates may  
39 contribute to the phenotype. Indeed, *clavata* meristems are mechanically more  
40 heterogeneous than the wild type, and also display regional growth heterogeneities.  
41 Furthermore, stereotypical wild-type meristem organisation is lost in mutants, in which  
42 cells simultaneously express distinct fate markers. Finally, cells in mutant meristems are  
43 auxin responsive, suggesting that they are functionally distinguishable from wild-type  
44 stem cells. Thus all benchmarks show that *clavata* meristem cells are different from  
45 wild-type stem cells, suggesting that overgrowth is caused by the disruption of a more  
46 complex regulatory framework that maintains distinct genetic and functional domains in  
47 the meristem.

48 **Introduction**

49 The shoot apical meristem (SAM) is a structure at the growing plant tip that gives rise to  
50 all its aboveground tissues. The SAM is sustained by the activity of a small pool of  
51 centrally-located undifferentiated stem cells in the slow-dividing central zone (CZ).  
52 Through successive divisions, their daughter cells exit from the CZ into the surrounding  
53 peripheral zone (PZ), where they may differentiate into lateral organs, such as leaves or  
54 flowers. The CZ is itself maintained via the activity of the underlying organising centre  
55 (OC) (Laux et al., 1996).

56  
57 The regulatory network driving stem cell maintenance in *Arabidopsis* is well studied. At  
58 its core is a feedback loop between WUSCHEL (WUS), a homeobox transcription factor  
59 expressed in the OC, and CLAVATA3 (CLV3), a signalling peptide expressed in the CZ  
60 (Brand et al., 2000; Clark et al., 1995; Laux et al., 1996; Schoof et al., 2000). WUS  
61 moves from the OC to the CZ and induces stem cell identity by directly binding to the  
62 *CLV3* promoter and activating its expression (Daum et al., 2014; Yadav et al., 2011). In  
63 turn, CLV3 binds receptors, such as the leucine-rich-repeat receptor-like kinase CLV1  
64 (Clark et al., 1997) or the receptor-like proteins CLV2 (Kayes and Clark, 1998) and  
65 CORYNE (CRN) (Fletcher et al., 1999; Jeong et al., 1999; Miwa et al., 2008; Müller et  
66 al., 2008). Signalling from these ligand-receptor complexes ultimately leads to the  
67 downregulation of *WUS* in the OC (Miwa et al., 2008; Müller et al., 2006). Additionally,  
68 *CLV3* regulates *WUS* at the transcriptional and post-transcriptional levels, while *WUS*  
69 activates *CLV3* in a concentration-dependent manner (Plong *et al.*, 2021). The absence  
70 of *WUS* activity leads to SAM arrest early in development (Laux et al., 1996), whereas  
71 the loss of the *CLV* genes results in a vast enlargement of the SAM (Clark et al., 1993;  
72 Clark et al., 1995; Kayes and Clark, 1998) through a phenomenon called fasciation.  
73 Several instances of fasciated tissues have been observed in the wild, as well as in crop  
74 plants, including beef tomatoes and the maize *fasciated ear2* mutant (Taguchi-  
75 Shiobara, 2001). It is thought that fasciation is caused by stem cell overproliferation in  
76 the CZ (Brand et al., 2000; Busch et al., 2010; Dao et al., 2022; Kwon et al., 2005;  
77 Lenhard and Laux, 2003; Müller et al., 2008; Nimchuk et al., 2011; Whitewoods et al.,  
78 2018; Wu et al., 2005), or conceivably, by lower rates of cell transit from the CZ to the  
79 PZ (Laufs et al., 1998).

80

81 Over the years, *CLV3* has been the only known genetic marker to study stem cells at  
82 the shoot apex (Müller et al., 2006; Reddy and Meyerowitz, 2005), including for  
83 analysing *c/v* mutants. While microarray analyses have uncovered several other genes  
84 that are enriched in the CZ (Aggarwal et al., 2010; Yadav et al., 2009), none have been  
85 extensively characterised. More recently, atomic force microscopy (AFM) experiments,  
86 which measure cellular mechanical properties, revealed that *CLV3*-expressing cells are  
87 stiffer than surrounding PZ cells, and that the onset of *CLV3* expression in flowers  
88 coincides with an increase in cell wall stiffness (Milani et al., 2014). Thus, although stem  
89 cell identity is both spatially and temporally associated with increased cell stiffness  
90 (Milani et al., 2014), it is unknown how these mechanical patterns are altered when  
91 stem cell regulation is perturbed.

92

93 The core stem cell regulatory network is also influenced by hormone signalling,  
94 including cytokinin and auxin (Gordon et al., 2009; Busch et al., 2010). Exogenous  
95 cytokinin treatment phenocopies the *c/v* mutant by increasing *WUS* expression and  
96 decreasing *CLV1* expression (Lindsay et al., 2006). While exogenous auxin treatment  
97 induces organogenesis markers in the PZ, the CZ itself remains unaffected, displaying  
98 reduced responsivity to auxin (de Reuille et al., 2006). Whereas in the PZ, the auxin  
99 response factor MONOPTEROS (MP) activates the expression of lateral organ identity  
100 genes in an auxin-dependent manner (Berleth and Jürgens, 1993; Bhatia et al., 2016;  
101 Hardtke and Berleth, 1998; Yamaguchi et al., 2013), it also functions to repress CZ  
102 genes that are themselves activators of *CLV3* expression (Luo et al., 2018; Zhao et al.,  
103 2010). Despite these advances, it remains unclear how auxin signalling is perturbed in  
104 *c/v* mutants, and furthermore, whether this plays a direct role in generating the fasciated  
105 *c/v* phenotype.

106

107 In this study, we use quantitative approaches to study the effects of the loss of proper  
108 stem cell regulation that reveal hitherto unobserved cellular and tissular phenotypes,  
109 such as cell size and local surface curvature in *c/v* mutant SAM. Analytical mechanical  
110 models predict that heterogeneities in stiffness and/or growth regimes are sufficient to  
111 account for the changes in surface curvature observed in the mutant. We provide  
112 experimental support for these predictions by showing that both growth and mechanical  
113 properties display regional differences, which are likely caused by the chimeric identities

114 of mutant meristematic cells. Finally, auxin-response quantifications in *c/v* meristems  
115 reveal a behaviour similar to undifferentiated WT PZ cells, rather than to stem cells.

116

117

## 118 **Results**

### 119 **Altered cellular and tissular properties characterise fasciated *c/v* SAM**

120 In order to better understand the defects associated with abnormal stem cell regulation,  
121 we first quantified the highly fasciated phenotype of the canonical *c/v3-2* allele (Clark et  
122 al., 1995). WT shoot meristems displayed a stereotypical dome shape, whereas *c/v3-2*  
123 meristems comprised a large centrally-located bulge and two or more arm-like  
124 outgrowths that elongate laterally (Fig. 1A, B, Fig. S1A-C). Further analysis revealed  
125 that the central areas were usually devoid of any discernible cellular organisation,  
126 whereas the lateral arms were often composed of cell files (Fig. S1D, E). These results  
127 were consistent across three *c/v1* and four *c/v3* alleles (Fig. S1A-C), although individual  
128 plants showed varying degrees of fasciation.

129

130 We also detected novel floral phenotypes. First, we frequently observed radially  
131 symmetrical, filament-like organs either growing on the flanks of the meristem or within  
132 the inflorescence itself (Fig. 1C, D). Such filaments were recently noted as aborted  
133 flowers in double mutants of *c/v2* or *c/v3* and with auxin biosynthesis mutants (John et  
134 al., 2023; Jones et al., 2020). Secondly, while the majority (>90%) of flowers showed  
135 previously described phenotypes of supernumerary organs (Clark et al., 1993; Clark et  
136 al., 1995), the rest displayed two types of opposing phenotypes. One group of flowers  
137 bore two or three sepals and petals, a single stamen and no carpels (Fig. 1C, E, F),  
138 reminiscent of the inner-whorl phenotypes of *wus* mutants (Laux et al., 1996), whereas  
139 a second group displayed a tubular phenotype with fused sepals (Fig. 1C). These  
140 flowers were distinctly smaller than those with supernumerary organs. These novel  
141 floral phenotypes were observed in all *c/v1* and *c/v3* alleles in our culture conditions.

142

143 Because it had been suggested that cell size in *c/v* mutants differs from the WT (Laufs  
144 et al., 1998), we wondered whether we could use such changes in cellular morphologies  
145 to further characterise the mutant phenotypes described above. We quantified cell size  
146 in two ways: firstly, we calculated cell areas by acquiring images of meristems from WT  
147 and multiple *c/v1* and *c/v3* SAM, and extracting slices near the midpoints of cells in the

148 L1 and L2 layers, which we then segmented using the MorphoGraphX software (Barbier  
149 de Reuille et al., 2015) (Fig. S1F, G). In WT SAM, the average area was similar in both  
150 cell layers, with L1 cells at  $23.9 \pm 5.8 \mu\text{m}^2$  and L2 cells at  $24.5 \pm 7.2 \mu\text{m}^2$  (Fig. 1G). In  
151 contrast, *clv3-2* mutants exhibited significant differences in the L1 and L2, with L2 cells  
152 on average 69% larger than L1 cells –  $31.2 \pm 9.8 \mu\text{m}^2$  in the L2 versus  $18.4 \pm 5.3 \mu\text{m}^2$   
153 in the L1 (Fig. 1G). Secondly, we measured cell volumes in WT and *clv* SAM, by  
154 generating 3D reconstructions using the MARS pipeline (Fernandez et al., 2010), which  
155 involves imaging samples from multiple angles and fusing those images to generate  
156 high-resolution images that are then segmented in 3D (Fig. S1H). Consistent with our  
157 findings for cell areas, we found that while L1 and L2 cells had comparable volumes in  
158 the WT ( $136.5 \pm 40.5 \mu\text{m}^3$  and  $148.7 \pm 44.6 \mu\text{m}^3$ , respectively), L2 cells in *clv3-2* SAM  
159 were almost twice as large as L1 cells ( $249.1 \pm 130.7 \mu\text{m}^3$  vs  $129.5 \pm 40.9 \mu\text{m}^3$ ) (Fig.  
160 1H). Similar trends were observed for cell size in other *clv1* and *clv3* alleles, suggesting  
161 that altered cell morphology is a general feature of these mutants (Fig. S1I).

162

163 We had noticed that meristems in *clv3* mutants are less smooth than WT meristems.  
164 We quantified this by measuring curvature at the sample surface, using a method that  
165 detects the outer surface in MARS-reconstructed 3D meristems (Kiss et al., 2017). We  
166 used MorphoGraphX to calculate curvature within a 10- $\mu\text{m}$  radius of every pixel on the  
167 surface. In WT plants, the meristem dome and young flowers were regions of uniformly  
168 positive curvature, separated by clearly defined boundary areas with negative curvature  
169 (Fig. 1I). However, the imaged areas of *clv1* and *clv3* SAM, which are distant from the  
170 regions where flowers form, displayed a much more variable surface curvature, with  
171 crests and troughs of varying shapes and depths distributed throughout (Fig. 1J).  
172 Together, our data reveal that *clv* mutants exhibit abnormal cellular traits and buckling  
173 defects that contrast with the stereotypical characteristics of WT meristems.

174

### 175 **Morphoelastic models reveal how growth and stiffness contribute to tissue** 176 **buckling**

177 To elucidate a theoretical basis for the buckling defects of *clv* mutants, we next  
178 generated analytical models of SAM development using a growth and remodelling  
179 framework (Goriely, 2017). Specifically, we adapted a model developed to explore the  
180 biomechanical basis of morphogenesis in unrelated systems that display similar  
181 buckling behaviour to that observed in *clv* mutants (Almet et al., 2019; Moulton et al.,

182 2013). We modelled the SAM as an axially growing planar elastic rod (the outer L1  
183 layer), attached to an elastic foundation (the inner layers), and clamped at the two ends  
184 (Fig. S2). We used the theoretical model to explore how growth stretch, a proxy for  
185 tissue growth, and/or mechanical properties, a proxy for cellular stiffness, might  
186 contribute to the altered morphology of *c/v* mutants (see Supplementary Materials and  
187 Methods for further details).

188

189 First, we used linear stability analysis to plot the output shapes when either the growth  
190 stretch ( $\gamma$ ) alone or the system stiffness ( $\hat{k}$ , the ratio of foundation and rod stiffnesses)  
191 was varied. These analyses showed that when the foundation and rod had similar  
192 mechanical properties ( $\hat{k} = 1$ ) and when growth stretch was at a low, subcritical value  
193 ( $\square^*_{\text{inf}} = 5.82$ ), the output shape resembled the smooth dome of a WT meristem (Fig. 2A).  
194 In contrast, when the growth stretch was raised to a near-critical point ( $\square^*_{\text{inf}} = 8.99$ ), the  
195 size of the dome increased, while remaining smooth. A further increase in growth  
196 stretch generated various modes of buckling (Fig. 2A) that broadly capture the curvature  
197 defects of *c/v* mutants. Next, we investigated the role of system stiffness ( $\hat{k}$ ) on SAM  
198 morphology, by varying stiffness within a background of constant growth stretch ( $\square^* =$   
199 12.23) (Fig. 2B). Whereas no buckling was visible for higher values of stiffness ( $\hat{k} \geq 1$ ),  
200 lowering it ( $\hat{k} = 0.5$ ) led to the appearance of buckling in the rod (Fig. 2B), which was  
201 similar to those obtained using growth values above the critical point (Fig. 2A). Taken  
202 together, these analytical results indicate that in a biological context, cellular growth and  
203 stiffness characteristics could influence tissue architecture of the SAM.

204

205 In contrast to the symmetrical shapes obtained above, *c/v* mutant SAM in fact display  
206 highly variable buckling, such that the positions, numbers and amplitudes of folds differ  
207 within, and between, individual mutant meristems. We reasoned that this variability  
208 might result from local differences in growth, stiffness or both. To investigate the effects  
209 of such heterogeneities on buckling, we replaced constant growth stretch or system  
210 stiffness with one of three distinct functions to generate differential distributions across  
211 the rod (Fig. 2C). Indeed, all three functions generate variable buckling over the length  
212 of the rod when either growth stretch (Fig. 2D, Fig. S3B-B') or system stiffness (Fig. 2E)  
213 is spatially distributed, with the resulting shapes often closely resembling the asymmetry  
214 and variable buckling amplitudes visible in sections through various *c/v* SAM in the  
215 literature as well as in our experiments (Fig. S3F, G). These results suggest that local



216 differences in growth or stiffness within the SAM could explain the phenotypes of *clv*  
217 mutants.

218

219 Finally, we asked whether variations in system stiffness and growth stretch could act  
220 together to generate even greater differences in buckling characteristics. To address  
221 this question, we used a cosine spatial growth stretch distribution and examined how  
222 buckling was affected when system stiffness ( $\hat{k}$ ) was varied between 0.5 and 1.5.  
223 Indeed, for identical distributions of growth, rod buckling varied locally as a function of  
224 stiffness (Fig. 2F); these analyses suggest that small changes at the cellular level could  
225 alter shape locally.

226

227 Taken together, our results indicate that growth and mechanical traits are sufficient to  
228 describe the differences in meristem shape between WT and *clv* SAM. Furthermore,  
229 local variations of these two cellular parameters could lead to the local curvature defects  
230 observed in *clv* meristems.

231

### 232 **Heterogeneous growth correlates with buckling in *clv* mutants**

233 We tested predictions from the analytical model by first examining whether *clv* mutants  
234 display local heterogeneities in growth and, more specifically, whether these differences  
235 correlate with areas of differential curvature. We did this by dissecting WT and *clv3-7*  
236 meristems, placing them on culture medium and imaging them once a day over 72  
237 hours by confocal microscopy. We then segmented the image stacks, tracked cell  
238 lineages, and analysed cell size, growth and curvature over the tissue (Fig. 3A-H, Fig.  
239 S4A-C). In the WT, cells had a mean size of  $22.8 \pm 5.8 \mu\text{m}^2$  at the start of the  
240 experiment and over 72 hours their daughters had grown  $5.5 \pm 1.3$  fold (Fig. 3A-C). Of  
241 these, lineages that remained entirely within the meristem dome grew  $4.9 \pm 1.1$  fold. In  
242 contrast, cells at the very top of the *clv3* meristem had an initial size of  $17 \pm 6.2 \mu\text{m}^2$   
243 and had grown  $1.5 \pm 0.3$  fold over 72 hours (Fig. 3E-G). This is consistent with previous  
244 studies showing reduced proliferation in *clv3-1* mutants (Laufs et al., 1998).

245

246 In the WT, a gradient of differential growth is observed from the centre to the periphery  
247 of the meristem, whereas in the mutant, groups of cells with varying growth rates are  
248 seemingly scattered across the enlarged meristem (Fig. 3C, G). To determine whether  
249 this observation is relevant in the context of buckling in *clv* meristems, we then

250 measured curvature at the surface of WT and mutant samples, determined the mean  
251 value per cell (Fig. 3D, H, Fig. S4C), and tested correlations between cell size, growth  
252 rate and local curvature (Fig. 3I-L, Fig. S4D-E). WT meristems showed no significant  
253 correlations between any parameters (Pearson's correlation coefficients ( $r$ ) between:  
254 growth and curvature,  $r = -0.17$ ,  $p = 0.18$ ; size and curvature,  $r = -0.035$ ,  $p = 0.79$ ;  
255 growth and size,  $r = -0.23$ ,  $p = 0.07$ ) (Fig. 3I-J, Fig. S4D). *clv3* mutants, however,  
256 displayed significant moderate or strong positive correlations between all parameters  
257 (Pearson's correlation coefficients between: growth and curvature,  $r = 0.59$ ,  $p < 0.001$ ;  
258 size and curvature,  $r = 0.41$ ,  $p < 0.001$ ; growth and size,  $r = 0.36$ ,  $p < 0.001$ ) (Fig. 3K-L,  
259 Fig. S4E). To account for differences in numbers of cell lineages between genotypes,  
260 we confirmed that significant correlations also exist in randomly selected subsets of  
261 mutant lineages equal in size to the WT (Fig. S4F). These analyses show that in *clv3*  
262 mutants, both cell size and growth rate are variable across the tissue and are linked to  
263 local buckling characteristics.

264

#### 265 **Loss of *CLV* activity is associated with reduced and variable epidermal cell** 266 **stiffness**

267 In addition to growth heterogeneity, our analytical models predicted that buckling is also  
268 dependent on stiffness changes in the SAM. We had previously shown that *CLV3*-  
269 expressing CZ cells are stiffer than PZ cells (Milani et al., 2014). Because the *clv*  
270 phenotype is thought to be caused by stem cell overproliferation, we predicted that cells  
271 in *clv* meristems might bear higher values of stiffness. To test this, we probed epidermal  
272 cells in WT, *clv1* and *clv3* SAM using atomic force microscopy (AFM), as published  
273 (Milani et al., 2014; Beauzamy et al., 2015) (Fig. S5A-D). The resultant force-  
274 displacement curves at each measured point from individual AFM scans were analysed  
275 to determine the apparent Young's modulus up to a depth of 100 nm, and these values  
276 were subsequently corrected to remove artefacts caused by local slope (Fig. 4A-D). The  
277 data were either pooled and plotted as density curves (Fig. 4E, F) or analysed by  
278 individual scans as box plots (Fig. 4G, H). When compared to the appropriate WT  
279 accession, we found that both *clv3-2* (Fig. 4E, G) and *clv1-8* cells (Fig. 4F, G) were  
280 significantly softer, with apparent Young's moduli that were 15-20 % lower than WT  
281 cells (Fig. 4G, Tables S1, S2). Similar trends were observed in other *clv* alleles (Fig.  
282 S5E, Table S1).

283

284 While these results show that *c/v* mutant SAM bear different physical characteristics to  
285 WT cells, they do not reveal whether they also display greater heterogeneity. To test  
286 this, we plotted standard deviation values for each scanned region by genotype (Fig.  
287 4H) and found that *c/v* mutant samples indeed display a significantly greater variability  
288 in values of apparent Young's moduli when compared to the WT. This altered regime is  
289 also visible in the density plots (Fig. 4E, F), where mutant distributions are notably  
290 broader than in the WT. Furthermore, scatter plots of the dataset show that mean and  
291 standard deviation are not correlated (Fig. S5F-I). Consistent with our analytical models,  
292 these results suggest that altered local stiffness and increased heterogeneity could  
293 indeed contribute to tissue buckling in *c/v* mutants.

294

### 295 **Genetic domain separation is lost in *c/v* SAM**

296 We reasoned that the local heterogeneities in growth and stiffness described above  
297 might be caused by underlying variations in genetic identity. To examine this, we first  
298 retested the expression patterns of OC and CZ markers using mRNA *in situ*  
299 hybridisation assays in WT and different *clv3* alleles. We used *WUS* to examine OC  
300 identity (Laux et al., 1996; Mayer et al., 1998), whereas for the CZ, we tested several  
301 genes previously identified as enriched in *CLV3*-expressing cells (Yadav et al., 2009)  
302 and retained the *APUM10* gene, which encodes a member of the Puf family, with a  
303 conserved Pumillio homology domain (Tam et al., 2010). *APUM10* was observed in a  
304 few centrally-located cells of the L1 and L2 in WT SAM (Fig. 5A), and *WUS* in an  
305 underlying group of cells (Fig. 5B). In *clv3* mutants, we detected both *APUM10* and  
306 *WUS* in broad domains occupying almost the entire meristem, with *APUM10* expressed  
307 principally in the L2 layer (Fig. 5C, C') and *WUS* in deeper layers (Fig. 5D-D'). However,  
308 both genes were expressed in a discontinuous manner, with no detectable expression  
309 in scattered groups of cells. An inspection of published expression data in *c/v* mutants  
310 revealed that such patchy expression was frequently visible, but had gone unremarked  
311 (Brand et al., 2000; Kinoshita et al., 2015; Schuster et al., 2014).

312

313 One explanation for why some patches of cells in *clv3* mutants expressed neither CZ  
314 nor OC identity was that they might instead bear other identities. To test this, we  
315 localised mRNA for the *MP* gene (Hardtke and Berleth, 1998), which is expressed  
316 mainly in the WT PZ (Zhao et al., 2010) (Fig. 5E). In *c/v* SAM, the PZ is thought to be  
317 restricted to areas at the base of the enlarged dome, in the region where flowers form.

318 However, we found that in *clv3* mutants, *MP* is expressed in a broad and patchy manner  
319 (Fig. 5F-F'), similarly to the *APUM10* and *WUS* patterns. Taken together, our results  
320 suggest that identities of the three meristematic zones are present in overlapping  
321 groups of cells in *clv* apices.

322

323 We thought it possible that normal domain organisation might exist at a more local level.  
324 In order to investigate the positions of the OC and PZ markers relative to the CZ marker  
325 within *clv* SAM, we performed pairwise *in situ* hybridisations using alternating sections  
326 from individual fixed meristems, with one probe used on even-numbered sections, and a  
327 second on odd-numbered sections. In WT meristems, the *APUM10*, *WUS* and *MP*  
328 expression domains were never detected in similar regions within adjacent sections  
329 (Fig. 5G, G', J, J'). However in *clv3* mutants, *APUM10* and *WUS* expression overlapped  
330 across the entire SAM (Fig. 5H-I', Fig. S6), except for a few areas where *APUM10* was  
331 observed but *WUS* was not (Fig. 5H', I') and rare areas where *WUS* was expressed  
332 without *APUM10*. Similarly in *clv3* mutants, *APUM10* and *MP* were expressed in  
333 overlapping patterns, except for a few areas where *MP* was expressed without *APUM10*  
334 (Fig. 5K-L', Fig. S7) and no instances where *APUM10* was expressed without *MP*. We  
335 also observed a few cell patches devoid of any signal.

336

337 Together, these results show that *clv3* meristems comprise a heterogeneous group of  
338 cells with mixed genetic identities, rather than a homogeneous population of stem cells.  
339 Furthermore, we observed similar expression patterns in SAM from other alleles of *clv1*  
340 and *clv3* (Fig. S8), suggesting that chimeric cell identities are a general feature of  
341 fasciated *clv* meristems.

342

### 343 **Exogenous auxin elicits a strong response in *clv3* meristems**

344 Because cells in fasciated *clv3* SAM displayed traits not usually associated with stem  
345 cells, such as chimeric cell identities and reduced stiffness, we wished to better  
346 understand if they were also functionally different from stem cells. We chose auxin  
347 response as a functional readout for the following reasons: firstly, auxin-associated  
348 genes are differentially expressed in WT and *clv3* mutants (John et al., 2023). Secondly,  
349 a key aspect of organogenesis at the shoot apex is the presence of a CZ that is  
350 refractive to auxin signalling (de Reuille et al., 2006; Douady and Couder, 1996; Galvan-  
351 Ampudia et al., 2020; Vernoux et al., 2011), suggesting that auxin insensitivity is an

352 indicator of stem cell identity. Thirdly, MP, a polarity regulator of the auxin efflux  
353 transporter PIN1, which itself promotes auxin accumulation and drives organogenesis  
354 (Bhatia et al., 2016), is misregulated in *clv* mutants (Fig. 5E, F).

355

356 In order to test how *clv* mutants respond to auxin, we dissected shoot apices from WT,  
357 *clv1* and *clv3* plants carrying the auxin signalling reporter, *pDR5rev::GFP*, and treated  
358 them with exogenous auxin as described (Galvan-Ampudia et al., 2020), while  
359 monitoring expression every 24 hours. As is well understood, in 100 % of WT samples  
360 prior to treatment, GFP was localised in small groups of cells corresponding to the  
361 positions of floral anlagen, accompanied by low expression in the geometric centre of  
362 the SAM (Fig. 6A). 24 hours later, we observed high levels of GFP throughout the  
363 periphery of the SAM and in internal layers, whereas expression levels at the centre  
364 remained low (Fig. 6B). *clv* meristems differed from the WT in that prior to auxin  
365 treatment, we detected only very basal levels – several thousand-fold lower than in the  
366 WT – of GFP (Fig. 6C), whereas 24 hours after treatment, a clear response was visible  
367 in all samples (Fig. 6D). More specifically, a minority of meristems (1 out of 11 *clv3*  
368 SAM) showed high levels of GFP in both outer and inner layers (Fig. S9A, B), whereas  
369 in all other samples (10 out of 11 *clv3*, and 5 out of 5 *clv1*) expression levels were  
370 variable and patchy in outer layers, and high in inner layers (Fig. 6D).

371

372 We next quantified this response by segmenting image stacks of WT (Fig. 6E, F) or  
373 mutant (Fig. 6I, J) meristems before and after auxin treatment, and extracted average  
374 fluorescence values per cell (Fig. 6G, H, K, L). For each cell, we then calculated the  
375 ratio of expression between the two time points, normalised this across the sample, and  
376 compared values in the mutant to values for cells in either the geometric centre or the  
377 periphery of WT meristems. A histogram of cell signal intensities shows that before  
378 auxin treatment, 22 % of WT cells and 100 % of mutant cells were in a low-expressing  
379 category of signal intensities under an arbitrary threshold set to 1/10th of the highest  
380 value (Fig. S9C). After treatment with auxin, only 6.4 % of WT and 5.9 % of mutant cells  
381 remained in this category, suggesting that the *clv* meristem responded to exogenous  
382 auxin in a similar manner to the WT. Furthermore, plots of normalised intensity change  
383 per lineage show that cells from the putative WT CZ and WT PZ, as well as from the  
384 *clv3* mutant had significantly different distributions from each other, although  
385 qualitatively the mutant resembled the PZ more than the CZ (Fig. 6M). Similar results

386 are obtained for *clv1* mutants carrying the *DR5* reporter (Fig. S9D, E). Taken together,  
387 these results show that cells in *clv* mutant SAM are capable of responding to auxin  
388 signalling, an output more associated with the WT PZ, where organ specification  
389 occurs.

390

391

## 392 **Discussion**

393 The development of fasciated meristems due to the loss of *CLV* activity in *Arabidopsis*  
394 shoot apical stem cells has generally been interpreted as being caused by a massive  
395 increase in stem cell numbers (Brand et al., 2002; Busch et al., 2010; Dao et al., 2022;  
396 Kwon et al., 2005; Lenhard and Laux, 2003; Müller et al., 2006; Nimchuk et al., 2011;  
397 Whitewoods et al., 2018; Wu et al., 2005). Direct experimental evidence for this comes  
398 principally from a *CLV3* transcriptional reporter, which is expressed throughout the  
399 enlarged meristem of *clv* mutants (Brand et al., 2002; Reddy and Meyerowitz, 2005).  
400 However, because *WUS* protein is known to directly activate *CLV3* transcription (Yadav  
401 et al., 2011), and because *WUS* is expressed throughout *clv* meristems (Schoof et al.,  
402 2000), it is not surprising that *CLV3* reporter expression is broad in *clv* mutant. We  
403 suggest that the *CLV3* reporter might thus not be an appropriate marker to study cell  
404 identity in *clv* meristems. Furthermore, the use of the *CLV3* promoter for various  
405 misexpression studies of the role of stem cells in meristem maintenance might be  
406 inappropriate.

407

408 An alternate way of deciphering regional identities is by characterising cellular  
409 properties, which we have done using recently-developed approaches to precisely  
410 quantify cell volumes, areas and surface curvatures. Although we show that epidermal  
411 and subepidermal cell sizes in *clv* meristems are different from the WT, the effect of  
412 such changes on meristem structure is difficult to estimate. One possibility is that they  
413 generate a higher frequency of cell boundary overlaps between the L1 and L2 layers,  
414 which could generate a mechanically fragile structure, akin to a wall built with smaller  
415 cells in one layer and much larger bricks in the next.

416

417 Our analytical model attempts to explain meristem morphology through a mechanical  
418 framework, in contrast to various other modelling studies, which have shed light on  
419 different aspects of meristem shape and function, such as the role of auxin in

420 phyllotactic patterning or homeostasis of the CLV-WUS feedback loop (Banwarth-Kuhn  
421 et al., 2022; Battogtokh and Tyson, 2022; Chickarmane et al., 2012; Fujita et al., 2011;  
422 Galvan-Ampudia et al., 2020; Gruel et al., 2016; Heisler et al., 2005; Jönsson et al.,  
423 2005; Jönsson et al., 2006; Klawe et al., 2020; Michael et al., 2023; Plong et al., 2021).  
424 Our results suggest that the specific buckling-like patterns displayed by *clv* mutants can  
425 be explained by a combination of local heterogeneities in growth rates and in  
426 mechanical properties. Indeed, we show that mutant tissues are 15-20% less stiff than  
427 the WT and display a wider range of stiffnesses. We had expected *clv* cells to be stiffer  
428 than WT cells, because *CLV3*-expressing WT CZ cells are stiffer than PZ cells (Milani et  
429 al., 2014), and because the prevailing view in the literature is that cells in *clv* SAM bear  
430 stem cell identity. Our findings indicate that this view might be incorrect.

431  
432 A wavy surface can arise from buckling with the epidermis growing more than  
433 subepidermal layers, or from differences along the epidermis, with accelerated growth in  
434 bumps or growth constriction in the valleys. The results presented here cannot fully  
435 distinguish between these scenarios. However, a recent study revealed that cellular  
436 turgor pressure is anticorrelated to the size and number of neighbours of a given cell,  
437 but is correlated to its growth rate, such that smaller cells with fewer neighbours display  
438 higher pressure and higher growth (Long et al., 2020). In this context, our results  
439 showing a large epidermal-to-subepidermal cell size differential suggest that  
440 subepidermal cells in *clv* mutants are less stiff and grow less than the significantly  
441 smaller epidermal cells, which is consistent with predictions from our analytical model,  
442 that buckling occurs when inner layers are less stiff than the L1. A further experimental  
443 validation of this would require techniques to measure stiffness or pressure in internal  
444 layers. In the WT, although a growth rate differential is observed between the centre  
445 and the periphery, buckling would not necessarily occur if the intrinsic growth rate of the  
446 epidermis falls below the threshold predicted by the model and determined by growth  
447 and stiffness of the different layers.

448  
449 That tissue-level shape robustness emerges from cell growth and cell size variability  
450 averaged through space and time has been shown in multiple contexts (Hervieux et al.,  
451 2016; Hong et al., 2016; Kamimoto et al., 2016; Tsugawa et al., 2017). Consistent with  
452 previous studies (Laufs et al., 1998), we find that *clv* mutants grow at a much lower rate  
453 than the WT, indicating that the enlarged meristem of mature plants is likely to be a

454 result of the amplification of early changes through time, rather than due to increased  
455 proliferation later in development. Evidence for such a scenario is provided by Reddy &  
456 Meyerowitz, who showed that the immediate effect of the loss of CLV3 is an increase in  
457 proliferation in the meristem. A recent study has shown that impairing cellulose  
458 synthesis decreases both cell proliferation and cell stiffness, leading to smaller SAM,  
459 and that in the context of a *clv1* mutant, it results in a dramatic reduction of the fasciated  
460 phenotype (Sampathkumar et al., 2019). Since we now show that *clv* mutants  
461 themselves have slower growth and reduced stiffness, it is not evident how a further  
462 reduction of both parameters could result in an amelioration of the *clv* phenotype. A  
463 recent study shows that cell expansion in parts of the meristem can be accounted for by  
464 stress-dependent orientation of cell division planes (Banwarth-Kuhn et al., 2022). Thus  
465 the early effects of lowered cellulose synthase activity in reducing tensile stress may  
466 prevent the *clv* phenotype from developing in plants with impaired cellulose synthase  
467 activity. Furthermore, an important distinction is that our study focuses on local buckling,  
468 rather than on global fasciation.

469

470 Mechanical and growth heterogeneities in *clv* mutants may be caused by underlying  
471 perturbations in genetic patterns. Variable and patchy expression of CZ and OC  
472 markers are in fact visible in the literature (Brand et al., 2000; Reddy and Meyerowitz,  
473 2005; Schlegel et al., 2021; Schoof et al., 2000), although few studies have remarked  
474 upon, or interpreted, these data. It is unclear precisely how this pattern arises in the  
475 mutant and how it behaves over time. Furthermore, chimeric identities might exist in  
476 regions of fate transit within WT meristems, for instance at the CZ-PZ or the PZ-flower  
477 boundaries. In this context, it would be informative to look at the expression of a related  
478 negative feedback loop that functions specifically in the PZ (Schlegel et al., 2021).

479

480 In the absence of external stimuli, cells in *clv* meristems clearly do not undergo  
481 differentiation, like WT CZ cells. However, we show that cells in *clv* meristems are more  
482 akin to WT PZ cells in their capacity to respond to auxin signalling, perhaps due, at least  
483 in part, to their heterogeneous identities. Bhatia et al. (Bhatia et al., 2016) show that for  
484 organogenesis to occur, spatial differences in MP are a necessary condition. This is  
485 indeed the case in *clv* mutants, where *MP* expression is both broad and patchy,  
486 suggesting that improper auxin distributions or signalling contribute to the *clv*  
487 phenotype. It is thus unclear why organogenesis is affected in *clv* plants. One possibility



488 is that CLV signalling might regulate auxin activity directly, such as in the moss (Nemec-  
489 Venza et al., 2022). Alternatively, because auxin accumulation in space and time is  
490 crucial for organogenesis (Galvan-Ampudia et al., 2020; Reddy and Meyerowitz, 2005;  
491 Reinhardt et al., 2000; Vernoux et al., 2000), it is possible that the abnormal shapes of  
492 *clv* SAM do not allow proper auxin patterns to be generated over most of the meristem.  
493 In this model, an initial change in the geometry of *clv* SAM would lead to perturbations  
494 in auxin fluxes, thereby inhibiting the production of lateral organs, which are a major  
495 auxin source (Shi et al., 2018). The presence of fewer primordia during early  
496 development (e.g. embryogenesis or the vegetative phase) could generate even lower  
497 auxin levels in the SAM and lead to an increase in size (Shi et al., 2018). In support of  
498 this, the novel floral phenotypes we reveal are reminiscent of certain auxin pathway  
499 components (Cheng et al., 2006). Furthermore, it was recently shown that *clv* SAM are  
500 sensitised to auxin levels (John et al., 2023; Jones et al., 2020). A detailed analysis of  
501 auxin fluxes and auxin signalling in *clv* meristems will be necessary to understand the  
502 role of auxin in fasciation.

503

504 Taken together, all examined parameters in this study show that cells in *clv* SAM are  
505 different from WT CZ cells, and that they do not fit the current idea of typical stem cells.  
506 We suggest that stem cell identity needs to account for genetic, mechanical and  
507 functional parameters. Reporters for diverse cellular properties, as well as detailed real-  
508 time analyses, will help elucidate how chimeric identities arise and what their effects are  
509 on plant architecture.

510

511

## 512 **Materials and methods**

### 513 *Plant material and culture conditions*

514 The following plant lines were used in this study: *Ler* and *Col-0* as wild types, *clv3-2*  
515 (Clark et al., 1995), *clv3-7* (Fletcher et al., 1999), *clv3-9* (Nimchuk et al., 2015), *clv1-8*  
516 (Medford et al., 1992), *clv1-11* (Dievart, 2003), *pCLV3::GFP<sub>er</sub>* (Reddy and Meyerowitz,  
517 2005), *pDR5rev::GFP<sub>er</sub>* (Friml et al., 2003), *pDR5::3xVENUS-N7* (Vernoux et al., 2011),  
518 and *pUBQ10::LTI6b-TdTomato* (Shapiro et al., 2015).

519

520

521

522 Seeds were germinated on soil and placed in short-day conditions (8 hrs light, 21°C, 50-  
523 60% humidity and 16 hrs dark, 19°C, 50-60% humidity) for ten days. Seedlings were  
524 then transplanted into individual pots and placed back in the short-day conditions. Two  
525 weeks after transplantation, plants were transferred from short-day to long-day  
526 conditions (16 hrs light period, 21°C, 60% humidity and 8 hrs dark period, 19°C, 60%  
527 humidity) until flowering, when they were used for further experiments, as described  
528 below. Light sources were LED fixtures (Valoya, C75, spectrum NS12), with an intensity  
529 of 150  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ .

530

### 531 *CRISPR mutagenesis of CLV3*

532 Generation of *CLV* mutants was carried out using a multiplexing CRISPR system  
533 (Stuttman et al., 2021) to create the *clv3-21* allele in Col-0 plants carrying a  
534 *pUBQ10::LTI6b-tdTomato* reporter construct. Two guide RNAs targeting *CLV3* (5'-  
535 gATCTCACTCAAGCTCATGC-3' and 5'-TCAAGGACTTTCCAACCGCA-3') were  
536 designed using the Benchling software ([www.benchling.com](http://www.benchling.com)) and placed under the  
537 control of the *U6* promoter in the pDGE333 and pDGE334 'level 0' shuttle vectors as  
538 described (Stuttman et al., 2021). These sgRNA constructs were subsequently  
539 assembled in the CAS9-carrying pDGE347 binary vector, along with the FAST-red  
540 marker (fluorescence-accumulating seed technology) (Shimada et al., 2010) and the  
541 *bialophos resistance* gene (*BAR*) that confers resistance to glufosinate ammonium  
542 (Basta) for positive selection of transformants. T1 generation seeds that were positive  
543 for FAST-red were germinated on plates containing Basta and target genes from  
544 putative transformants were sequenced by Sanger sequencing, screened for *clv*-like  
545 phenotypes in the T2 and T3 generations and resequenced to generate independent  
546 homozygous lines. The *clv3-21* allele bears an insertion of an Adenine after the second  
547 base pair of exon 2, thus leading to a frameshift at residue 24 of the *CLV3* protein and  
548 the creation of a stop codon at residue 46, 23 amino acids prior to the start of the CLE  
549 domain.

550

### 551 *Plant dissection and preparation for confocal microscopy*

552 Plants were grown as described and dissected 2-5 days after bolting. An approximately  
553 1 cm region of primary or secondary meristems was placed in apex culture medium  
554 (ACM): ½ MS medium, 1% [w/v] sucrose, pH adjusted to 5.8 with 1M KOH, 0.8% [w/v]  
555 agarose, supplemented with 1X vitamins (1000X filtered stock: 5 g myo-inositol, 0.05 g

556 nicotinic acid, 640.05 g pyridoxine hydrochloride (B6), 0.5 g thiamine hydrochloride  
557 (B1), 0.1 g glycine, H<sub>2</sub>O to 50 mL) and cytokinins (BAP; 125-175 nM final). Older flower  
558 buds were removed until the shoot meristem was sufficiently exposed for  
559 experimentation. Where necessary, dissected samples were placed in a growth cabinet  
560 under long-day conditions until treated.

561

#### 562 *Bright field photos*

563 Bright field photographs of plants were taken with a binocular Nikon SMZ18 equipped  
564 with an objective Nikon SHR Plan Apo 1X WD:60, and an ORCA-Flash4.OLT camera.

565

#### 566 *Confocal imaging*

567 Imaging was carried out on a Leica TCS SP8 (DM6000 CS) upright confocal laser  
568 scanning microscope, equipped with a 25x water dipping lens (Leica HC FLUOTAR L  
569 25x/0.95 W VISIR) and a Leica HyD hybrid detector. Either plants expressed the  
570 *pUBQ10::LTI6b-TdTomato* fluorescent plasma membrane marker, or FM4-64  
571 (ThermoFisher Scientific, T13320) was used to mark the plasma membrane as  
572 previously described (Fernandez et al., 2010). FM4-64 was excited with a 488 nm laser  
573 diode and detected at 600-640 nm. GFP was excited at 488 nm and detected at 500-  
574 520 nm. VENUS was excited at 514 nm and detected at 520-535 nm. tdTomato was  
575 excited at either 514 or 552 nm and detected at 560-600 nm.

576

#### 577 *Image analysis*

578 Fiji (Schindelin et al., 2012) was used to generate 2D projections from 3D confocal  
579 stacks (3D viewer plugin) and to generate orthogonal slices.

580

581 The MultiAngle Reconstruction and Segmentation (MARS) pipeline (Fernandez et al.,  
582 2010) was used to generate 3D reconstructions of confocal stacks. For this, shoot  
583 meristems were imaged from multiple angles by means of a custom-made device to tilt  
584 and/or rotate the sample by 15-20 degrees between acquisitions. After fusion using  
585 MARS, the external contours of the sample were detected using the Level Set Method  
586 (Kiss et al., 2017) and this contour was then used to segment the 3D reconstructed  
587 sample using a watershed segmentation algorithm. Cells were then extracted from the  
588 segmented image for the L1 and L2 tissue layers and the volume of each cell was  
589 calculated.

590

591 MorphoGraphX (Barbier de Reuille et al., 2015; Kiss et al., 2017) was used to measure  
592 cell size, growth and curvature for individual image stacks. For cell size, we determined  
593 the image slice within the L1 and L2 layers that was orthogonal to the axis of  
594 acquisition, and which contained the midline of the group of cells near the centre of the  
595 image (in order to avoid cells that had been imaged from the side). We then segmented  
596 and extracted cell areas. For growth, we first identified the tissue surface, generated the  
597 mesh, projected signal from the first cell layer (usually 5.5-6  $\mu\text{m}$ ), watershed-segmented  
598 the mesh, and determined cell lineages over two or more time points. Minimal curvature  
599 was calculated within a 10- $\mu\text{m}$  radius for each point in the image, and for segmented  
600 tissues, was then normalised to the size of each cell. R was used to plot the data and  
601 run statistical tests.

602

### 603 *Statistical analysis*

604 Plots and statistical analyses were done with R studio version 2023.03.0 (Posit team,  
605 2023) using the *ggplot2* package (Wickham, H., 2016). Welch's t-tests were used to  
606 compare the mean of two independent groups: cell areas and cell volumes in different  
607 cell layers, and the apparent Young's modulus in WT and mutant SAM. When we use  
608 boxplots, the boxes extend from the first to the third quartile and the whiskers from 10%  
609 to 90% of the values, the solid black line represents the median of the distribution.  
610 Throughout the manuscript, average values are means  $\pm$  s.d. On scatter plots, the  
611 regression line represents the best linear fit of the data, along with its 95% confidence  
612 interval. Pearson's correlation coefficients and the related p-values were calculated with  
613 *ggplot2*.

614

### 615 *Analytical models*

616 We used a morphoelastic framework, which involves a multiplicative decomposition of  
617 the deformation gradient into growth tensor, describing changes in mass or density, and  
618 an elastic tensor, accounting for the elastic material response, commonly used in the  
619 literature (Goriely and Ben Amar, 2005; Moulton et al., 2013). The outer L1 layer of cells  
620 in the SAM is modelled as a planar elastic rod attached to an elastic foundation that  
621 represents the inner layers (Almet et al., 2019; Moulton et al., 2013) (Fig. S2). The  
622 elastic rod grows axially in the x-direction, which generates compressive stresses due to  
623 clamped boundary conditions at the two rod ends. These stresses lead to instability,

624 and buckling that result in the formation of folded structures, which closely resemble the  
625 shapes obtained in our experimental data. We used the model to explore differences in  
626 the growth rates and stiffness in the L1 and L2 layers of the various groups in our study  
627 (see supplementary Materials and Methods for further details).

628

#### 629 *Atomic force microscopy (AFM) and data analysis*

630 Meristems were dissected to remove all flower buds older than stage 3 the day before  
631 AFM observations and placed in ACM in 60 mm plastic Petri dishes. Acquisitions were  
632 carried out on either the dome of WT plants or on lateral outgrowths of *clv* meristems.  
633 Samples were probed in distilled water at room temperature on a stand-alone JPK  
634 Nanowizard III microscope equipped with a CellHesion module (100  $\mu\text{m}$ -range Z-piezo)  
635 using the Quantitative Imaging mode (QI). We used a silica spherical tip with a radius of  
636 400 nm (Special Development SD-sphere-NCH, Nanosensors) mounted on a silicon  
637 cantilever with a nominal force constant of 42  $\text{N}\cdot\text{m}^{-1}$ . Scan size was generally a square  
638 field of 45 x 45  $\mu\text{m}$  with pixel size of approximately 500 nm. An applied force trigger of 1  
639  $\mu\text{N}$  was used in order to indent only the cell wall (Milani et al., 2011; Tvergaard and  
640 Needleman, 2018), with a ramp size of 2  $\mu\text{m}$ , an approach speed of 10  $\mu\text{m}\cdot\text{s}^{-1}$  and a  
641 retraction speed of 100  $\mu\text{m}\cdot\text{s}^{-1}$ . Apparent Young's modulus was obtained by fitting the  
642 first 100-150 nm of the force displacement curve with a Hertz model and subsequently  
643 corrected for the effect of slope (see supplementary Materials and Methods for further  
644 details).

645

#### 646 *RNA in situ hybridisations*

647 RNA *in situ* hybridisations on sections were performed according to published protocols  
648 (Long et al., 1996). Meristems were dissected just after bolting, fixed in FAA  
649 (formaldehyde 3.7% [v/v], ethanol 50% [v/v], acetic acid 5% [v/v], H<sub>2</sub>O to final volume),  
650 washed, dehydrated and embedded in paraplast. Embedded samples were cut (10  $\mu\text{m}$ -  
651 thick) and attached to pre-coated glass slides (Superfrost Plus Gold, Fisher Scientific).  
652 Antisense probes were made from PCR products using cDNA from inflorescences as a  
653 template, except for *CLV3*, which was amplified using genomic DNA (Table S3). Those  
654 PCR products were transcribed into RNA and then labelled using digoxigenin (DIG)-  
655 UTP. All probes were filtered on columns (CHROMA SPIN-30 columns, Clontech) to  
656 remove remaining nucleotides. Immunodetection was performed using an anti-DIG  
657 antibody coupled to alkaline phosphatase (Anti-Digoxigenin-AP, Fab fragments,

658 Roche), whose activity was detected by the chromogenic method using NBT/BCIP  
659 (Roche). Sections were finally washed with water and observed under a Zeiss Imager  
660 M2 microscope equipped with an AxioCam Mrc camera, and 10 X or 20 X objectives in  
661 DIC (differential interference contrast) mode.

662

### 663 *Hormone treatments*

664 Auxin treatments were performed by immersing dissected SAM in 1 or 2 mM Indole-3-  
665 acetic acid (IAA) solution (Sigma) for five hours as described (Galvan-Ampudia et al.,  
666 2020). *DR5* reporter expression was monitored in the samples by confocal microscopy  
667 approximately 24 hours post-treatment. Treatments were carried out on *clv1-8* (in the  
668 Col-0 accession, as in Fig. S9D, E), *clv3-2* plants (in the Ler accession, as in Fig. S9A,  
669 B), or in F1 plants of a cross between *clv3-2* and *clv3-21* (mixed Col-0 and Ler  
670 background, as in Fig. 6C-D, I-L).

671

### 672 **Acknowledgements**

673 We thank V. Mirabet and N. Dubrulle for help with preliminary AFM measurements, A.  
674 Kiss for help with 3D segmentations and surface curvature detection, Zofia Haftek-  
675 Terreau and G. Gilbert for help with *in situ* hybridisations, and Aurélie Chauvet for help  
676 with molecular biology. We thank the PLATIM/LyMIC imaging facility of the SFR  
677 Biosciences (UMS3444/CNRS, US8/Inserm, ENS de Lyon, UCBL1). We thank A.  
678 Lacroix, J. Berger, P. Bolland, H. Leyral, and I. Desbouchages for assistance with plant  
679 growth and logistics. This article is based on LRL's PhD thesis: Rambaud-Lavigne, L. E.  
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682

683

### 684 **Author contributions**

685 Conceptualization: LRL, AB, PD; Methodology: AC, NG, SB, AB; Software & Modeling:  
686 AC, NG; Validation: LRL, VB, PD; Formal Analysis: QL, AC, LRL, PD; Investigation:  
687 LRL, SB, VB, PD; Data Curation: LRL, PD; Writing - Original Draft Preparation: LRL,  
688 AC, NG, PD; Writing - Review & Editing: LRL, AC, SB, NG, AB, PD; Visualisation: LRL,  
689 AC, QL, NG, PD; Supervision: NG, AB, PD; Funding Acquisition: LRL, NG, PD.

690

691 **Competing interests**

692 No competing interests declared.

693

694 **Funding**

695 This work was supported by a PhD fellowship from the Ecole Normale Supérieure de  
696 Lyon to LRL., and a Science Engineering Research Board grant (SERB-POWER) to  
697 NG.

698

699 **Data availability**

700 All datasets and scripts are available at <https://doi.org/10.57745/N6QM41>.

701

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982 **Figure Legends**

983 **Figure 1. Altered cell and tissue properties characterise fasciated SAM**

984 **(A, B)** Bright field images showing dissected WT *Ler* (A) and *clv3-2* (B) SAM. The  
985 asterisk in (B) shows the central outgrowth and arrows point out two linear elongations.  
986 **(C-F)** Bright field images showing a dissected *clv3-21* SAM with filaments (arrows) on  
987 the side of the stem and abnormally shaped flowers (arrowheads) (C), a close-up of the  
988 filaments (D), and *clv3-21* abnormally-shaped flowers (E, F). **(G, H)** Boxplots of cell  
989 areas (G) or cell volumes (H) in the L1 (black) and L2 (grey) cell layers of WT and *clv3-*  
990 *2* SAM. Mean  $\pm$  s. d. areas (G) are  $23.9 \pm 5.8 \mu\text{m}^2$  (WT L1) and  $24.5 \pm 7.2 \mu\text{m}^2$  (WT  
991 L2); and  $18.4 \pm 5.3 \mu\text{m}^2$  (*clv3* L1) and  $31.2 \pm 9.8 \mu\text{m}^2$  (*clv3* L2). *P*-values (Welch's t-  
992 test) = 0.545 (WT) and  $4.65\text{e-}54$  (*clv3-2*). *n* = 103 (L1) and 115 (L2) cells from 3 WT  
993 SAM; *n* = 400 (L1) and 244 (L2) cells from 3 *clv3-2* SAM. Mean  $\pm$  s. d. volumes (H) are  
994  $136.5 \pm 40.5 \mu\text{m}^3$  (WT L1) and  $148.7 \pm 44.5 \mu\text{m}^3$  (WT L2); and  $129.5 \pm 40.9 \mu\text{m}^3$  (*clv3*  
995 L1) and  $249.1 \pm 130.7 \mu\text{m}^3$  (*clv3* L2). *P*-values (Welch's t-test) =  $2.32\text{e-}9$  (WT) and  
996  $1.53\text{e-}204$  (*clv3-2*). *n* = 1468 (L1) and 529 (L2) cells from 4 WT SAM; 6426 (L1) and  
997 3428 (L2) cells from 3 *clv3-2* SAM. **(I, J)** Colour maps quantifying minimal curvature in  
998 representative WT *Col-0* (I) and in *clv3-2* (J) SAM. The *clv3-2* SAM (J) is only a small  
999 region of the entire meristem. Colourmap: warm helix (I, J). Scale bars: 30  $\mu\text{m}$  (A, B, I,  
1000 J), 1 mm (C), 200  $\mu\text{m}$  (D), 100  $\mu\text{m}$  (E, F).



1001 **Figure 2. Growth and stiffness influence SAM shapes via growth-induced**  
1002 **buckling**  
1003 **(A)** Deformed shapes due to growth-induced buckling obtained for different values of  
1004 critical growth rates ( $\gamma^*$ ) causing various buckling modes, with the stiffness of the  
1005 underlying foundation fixed ( $\hat{k} = 1$ ). **(B)** Deformed shapes due to stiffness-induced  
1006 buckling obtained for different values of foundation stiffness ( $\hat{k} = 0.5, \hat{k} = 1, \hat{k} = 1.5$ ),  
1007 with the growth rate fixed ( $\gamma^* = 12.23$ ). **(C)** The choice of growth law affects the  
1008 deformed shapes. We select three different forms of growth laws for the same value of  
1009 growth rate  $\gamma_0$ : cosine, linear and exponential. **(D)** Deformed shape obtained as a result  
1010 of growth-induced buckling in the case of cosine growth distribution. **(E)** Deformed  
1011 shape obtained as a result of stiffness-induced buckling in the case of cosine stiffness  
1012 distribution. **(F)** Effect of foundation stiffness on the asymmetric rod shapes obtained  
1013 using a cosine growth distribution. The colour bar in (D, E) represents the magnitude of  
1014 growth and stiffness, respectively, that was varied along the length of the rod.

1015 **Figure 3. Regional growth heterogeneities correlate with surface buckling in *clv***  
1016 **mutants**  
1017 **(A-H)** 3D surface reconstruction of a WT Col-0 (A-D) and a *clv3-7* (E-H) SAM,  
1018 expressing the *pUBQ10::Lti6b-tdTomato* membrane marker, at the start of the  
1019 experiment (A, E) and 72 h later (B, F). Solid white line in (A) and (B) delineates the  
1020 same group of cells. Segmented cells are coloured by lineage and outlines of  
1021 unsegmented cells are shown. For each lineage, heatmaps for either growth fold-  
1022 change over 72 h (C, G) or minimal curvature (D, H) are represented on the second  
1023 time point. **(I-L)** Scatter plots of minimal curvature per lineage as a function of fold-  
1024 growth per lineage (I, K), or of minimal curvature per lineage as a function of initial  
1025 lineage surface area (J, L) in WT (I, J) and *clv3-7* (K, L) SAM. Regression lines  
1026 representing the best fit are shown in blue, and confidence intervals as shaded regions  
1027 around them.  $n = 64$  WT lineages (I, J);  $n = 1064$  *clv3* lineages (K, L). Pearson's  
1028 correlation coefficients ( $r$ ) are shown on the plots.  $P$ -values:  $p = 0.18$  (I);  $p = 0.79$  (J);  $p$   
1029  $< 0.001$  (K);  $p < 0.001$  (L). Colourmap: Turbo (C, G); warm helix (D, H). Scale bars: 20  
1030  $\mu\text{m}$  (A-H).

1031 **Figure 4. Loss of *clv* is associated with reduced and heterogenous stiffness**  
1032 **(A-D)** Maps showing apparent Young's moduli derived from atomic force microscope  
1033 scans of representative *Ler* (A), *Col-0* (B), *clv3-2* (C), and *clv1-8* (D) SAM. **(E, F)**  
1034 Density plots of apparent Young's moduli from all probed WT (*Ler*) and *clv3-2* (E), and  
1035 WT (*Col-0*) and *clv1-8* (F) samples. **(G-H)** Box plots of either mean values (G) or  
1036 standard deviation values (H) of apparent Young's moduli measurements of scans from  
1037 all probed samples. Black dots show values of different samples probed by a single  
1038 scan, whereas coloured dots represent values from samples bearing multiple non-  
1039 overlapping scans, with each colour identifying an individual sample. Overall mean  
1040 values derived from individual mean apparent Young's moduli (G): *Ler*,  $12.2 \pm 1.2$  MPa;  
1041 *clv3-2*,  $9.7 \pm 0.7$ ; *Col-0*,  $10.58 \pm 0.4$ ; and *clv1-8*,  $8.94 \pm 0.5$ . Overall mean values  
1042 derived from individual standard deviations (H): *Ler*,  $1.7 \pm 0.3$  MPa; *clv3-2*,  $1.9 \pm 0.4$ ;  
1043 *Col-0*,  $1.2 \pm 0.2$ ; *clv1-8*,  $1.9 \pm 0.3$ . *P*-values (Welch's t-test): WT-*clv3*,  $6.65e-8$  and  
1044 WT-*clv1*,  $1.85e-7$  (G); WT-*clv3*,  $3.4e-2$  and WT-*clv1*,  $7.1e-5$  (H). n (non-overlapping  
1045 scans/independent meristems): *Ler*, 18/11; *Col-0*, 8/2; *clv3-2*, 34/8; and *clv1-8*, 16/1.

1046 **Figure 5. Gene expression is heterogenous in *clv3* SAM**  
1047 **(A-F')** Pattern of mRNA localisation for *APUM10* (A, C, C'), *WUS* (B, D, D'), and *MP* (E-  
1048 F') in WT *Ler* (A, B, E) or *clv3-2* (C, C', D, D', F, F') SAM. (C', D', and F') are magnified  
1049 views of the boxed regions in (C, D, and F), respectively. **(G-L')** Pairwise localisation of  
1050 *APUM10* (G, J, H, H', K, K'), *WUS* (G', I, I'), and *MP* (J', L, L') transcripts on alternating  
1051 sections of meristems from individual WT *Ler* (G, G', J, J') or *clv3-2* (H-I', K-L') plants.  
1052 Arrowheads (H',I', K', L') indicate zones where only one of the two transcripts is  
1053 detected, asterisks (K', L') indicate regions where neither is detected, and arrows  
1054 indicate areas where both are detected. (H', I', K', and L') are magnified views of the  
1055 boxed regions shown in (H, I, K, and L), respectively. Section thickness: 10  $\mu\text{m}$ . Scale  
1056 bars: 50  $\mu\text{m}$  (A-C, E, F, G, G', H', I', J, J', K', L'); 100  $\mu\text{m}$  (D, H, I, K, L); 25  $\mu\text{m}$  (C', D',  
1057 F').

1058 **Figure 6. *clv3* meristematic cells respond strongly to exogenous auxin treatment**  
1059 **(A-D)** Projections of confocal stacks showing the effect of IAA on WT Col-0 (A, B) and  
1060 *clv3-2/clv3-21* (C, D) SAM expressing the *pUBQ10::Lti6b-tdTomato* and *pDR5::GFP<sub>er</sub>*  
1061 reporters. Samples are shown either before (A, C) or 25 h after (B, D) a 5-hour IAA  
1062 treatment. Orthogonal views of the SAM are shown alongside each panel. n = 12 WT  
1063 SAM (5 independent experiments), n = 11 *clv3* SAM (4 independent experiments). **(E-L)**  
1064 Quantification of response to IAA treatment observed in representative SAM from WT  
1065 Col-0 (E-H) or *clv3-2/clv3-21* heterozygotes (I-L) carrying the *pUBQ10::Lti6b-tdTomato*  
1066 and *pDR5::GFP<sub>er</sub>* reporters. Views of the SAM surface before (E, I) and after (F, J)  
1067 treatment showing segmented cells coloured by lineage and unsegmented cells as grey  
1068 outlines. Numbers in (E) represent the phyllotactic order of flowers from oldest to  
1069 youngest. Heatmaps show DR5 signal per cell presented as a concentration (G, H, K, L)  
1070 with a common colour scale (G). **(M)** Violin plot reflecting distributions of DR5 fold-  
1071 change per cell lineage between *clv3* SAM and putative WT CZ and PZ (shown in E-L).  
1072 *P*-values < 0.001 (Welch's t-test). n = 12 cells for WT CZ; n = 300 cells for WT PZ; n =  
1073 713 cells for *clv3*. Colourmap: Turbo (G, H, K, L). Scale bars: 50  $\mu$ m (top views in A-D);  
1074 20  $\mu$ m (orthogonal sections in A-D, F-H, J, L); 10  $\mu$ m (E, I, K).













