

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

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1	Heterogeneous identity, stiffness and growth characterise
2	the shoot apex of Arabidopsis stem cell mutants
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4	Running title: Properties of stem cell mutants
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28	
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30	contribute to buckling in <i>clavata</i> 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

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

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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 turn, CLV3 binds receptors, such as the leucine-rich-repeat receptor-like kinase CLV1 63 64 (Clark et al., 1997) or the receptor-like proteins CLV2 (Kayes and Clark, 1998) and CORYNE (CRN) (Fletcher et al., 1999; Jeong et al., 1999; Miwa et al., 2008; Müller et 65 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 *clv* 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 clv 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 clv mutants, and furthermore, whether this plays a direct role in generating the fasciated 105 *clv* 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 *clv* 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

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114 115 of mutant meristematic cells. Finally, auxin-response quantifications in *clv* meristems 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 *clv* 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 clv3-2 allele (Clark et 122 al., 1995). WT shoot meristems displayed a stereotypical dome shape, whereas clv3-2 123 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 *clv1* and four *clv3* 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 clv2 or clv3 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 al., 1995), the rest displayed two types of opposing phenotypes. One group of flowers 136 137 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 *clv1* and *clv3* alleles in our culture conditions.

142

Because it had been suggested that cell size in *clv* mutants differs from the WT (Laufs et al., 1998), we wondered whether we could use such changes in cellular morphologies to further characterise the mutant phenotypes described above. We quantified cell size in two ways: firstly, we calculated cell areas by acquiring images of meristems from WT and multiple *clv1* and *clv3* 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 cell layers, with L1 cells at 23.9 \pm 5.8 μ m² and L2 cells at 24.5 \pm 7.2 μ m² (Fig. 1G). In 150 151 contrast, clv3-2 mutants exhibited significant differences in the L1 and L2, with L2 cells on average 69% larger than L1 cells $-31.2 + 9.8 \text{ }\mu\text{m}^2$ in the L2 versus $18.4 + 5.3 \text{ }\mu\text{m}^2$ 152 153 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 the WT (136.5 \pm 40.5 μ m³ and 148.7 \pm 44.6 μ m³, respectively), L2 cells in *clv*3-2 SAM 158 159 were almost twice as large as L1 cells (249.1 \pm 130.7 μ m³ vs 129.5 \pm 40.9 μ m³) (Fig. 1H). Similar trends were observed for cell size in other *clv1* and *clv3* alleles, suggesting 160 161 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-µm 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. 1). 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

To elucidate a theoretical basis for the buckling defects of *clv* mutants, we next generated analytical models of SAM development using a growth and remodelling framework (Goriely, 2017). Specifically, we adapted a model developed to explore the biomechanical basis of morphogenesis in unrelated systems that display similar 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 *clv* 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 (y) 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 $(\Box_{inf}^*=5.82)$, the output shape resembled the smooth dome of a WT meristem (Fig. 2A). 193 194 In contrast, when the growth stretch was raised to a near-critical point (\Box^*_{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 *clv* 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 ($\Box^* =$ 199 12.23) (Fig. 2B). Whereas no buckling was visible for higher values of stiffness ($\hat{k} \ge 1$), lowering it ($\hat{k} = 0.5$) led to the appearance of buckling in the rod (Fig. 2B), which was 200 201 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, *clv* 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 clv 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.

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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. S4A-C). In the WT, cells had a mean size of 22.8 \pm 5.8 μ m² at the start of the 239 240 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 contrast, cells at the very top of the c/v3 meristem had an initial size of 17 ± 6.2 μ m² 242 243 and had grown 1.5 ± 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; growth and size, r = -0.23, p = 0.07) (Fig. 3I-J, Fig. S4D). *clv3* mutants, however, 255 256 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

Loss of *CLV* activity is associated with reduced and variable epidermal cell 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 cells in WT, clv1 and clv3 SAM using atomic force microscopy (AFM), as published 272 273 (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 *clv* 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 *clv* 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 *clv* mutants.

294

295 Genetic domain separation is lost in *clv* 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 *clv* 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

One explanation for why some patches of cells in *clv3* mutants expressed neither CZ nor OC identity was that they might instead bear other identities. To test this, we localised mRNA for the *MP* gene (Hardtke and Berleth, 1998), which is expressed mainly in the WT PZ (Zhao et al., 2010) (Fig. 5E). In *clv* SAM, the PZ is thought to be restricted to areas at the base of the enlarged dome, in the region where flowers form. However, we found that in *clv3* mutants, *MP* is expressed in a broad and patchy manner (Fig. 5F-F'), similarly to the *APUM10* and *WUS* patterns. Taken together, our results suggest that identities of the three meristematic zones are present in overlapping 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

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

342

343 **Exogenous auxin elicits a strong response in** *clv***3 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 indicator of stem cell identity. Thirdly, MP, a polarity regulator of the auxin efflux
transporter PIN1, which itself promotes auxin accumulation and drives organogenesis
(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::GFPer*, 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

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

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

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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 recent study shows that cell expansion in parts of the meristem can be accounted for by 463 464 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 auxin source (Shi et al., 2018). The presence of fewer primordia during early 495 496 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

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

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512 Materials and methods

513 Plant material and culture conditions

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

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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 of 150 µmol.m⁻².s⁻¹. 529

530

531 CRISPR mutagenesis of CLV3

532 Generation of CLV mutants was carried out using a multiplexing CRISPR system 533 (Stuttmann 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 and gATCTCACTCAAGCTCATGC-3' 5'-TCAAGGACTTTCCAACCGCA-3') were 536 designed using the Benchling software (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 (Stuttmann 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): ¹/₂ 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

nicotinic acid, 640.05 g pyridoxine hydrochloride (B6), 0.5 g thiamine hydrochloride
(B1), 0.1 g glycine, H2O to 50 mL) and cytokinins (BAP; 125-175 nM final). Older flower
buds were removed until the shoot meristem was sufficiently exposed for
experimentation. Where necessary, dissected samples were placed in a growth cabinet
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.

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 µm), watershed-segmented 598 the mesh, and determined cell lineages over two or more time points. Minimal curvature 599 was calculated within a 10-µm 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

590

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,

and buckling that result in the formation of folded structures, which closely resemble the
shapes obtained in our experimental data. We used the model to explore differences in
the growth rates and stiffness in the L1 and L2 layers of the various groups in our study
(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 µm-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 N.m⁻¹. Scan size was generally a square 638 field of 45 x 45 µm with pixel size of approximately 500 nm. An applied force trigger of 1 639 µN was used in order to indent only the cell wall (Milani et al., 2011; Tvergaard and Needleman, 2018), with a ramp size of 2 µm, an approach speed of 10 µm.s⁻¹ and a 640 641 retraction speed of 100 µm.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], H₂O to final volume), 650 washed, dehydrated and embedded in paraplast. Embedded samples were cut (10 µm-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-Digoxygenin-AP, Fab fragments,

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

662

663 Hormone treatments

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

671

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684 Author contributions

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

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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 c/v3-21 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 clv3-2 SAM. Mean + s. d. areas (G) are 23.9 + 5.8 μ m² (WT L1) and 24.5 + 7.2 μ m² (WT 990 L2); and 18.4 \pm 5.3 μ m² (*clv*3 L1) and 31.2 \pm 9.8 μ m² (*clv*3 L2). *P*-values (Welch's t-991 992 test) = 0.545 (WT) and 4.65e-54 (c/v3-2). n = 103 (L1) and 115 (L2) cells from 3 WT SAM; n = 400 (L1) and 244 (L2) cells from 3 clv3-2 SAM. Mean \pm s. d. volumes (H) are 993 136.5 ± 40.5 μ m³ (WT L1) and 148.7 ± 44.5 μ m³ (WT L2); and 129.5 ± 40.9 μ m³ (*clv3*) 994 L1) and 249.1 \pm 130.7 μ m³ (*clv*3 L2). *P*-values (Welch's t-test) = 2.32e-9 (WT) and 995 996 1.53e-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 µm (A, B, I, J), 1 mm (C), 200 µm (D), 100 µm (E, F). 1000

Figure 2. Growth and stiffness influence SAM shapes via growth-induced buckling

1003 (A) Deformed shapes due to growth-induced buckling obtained for different values of 1004 critical growth rates (γ^*) causing various buckling modes, with the stiffness of the underlying foundation fixed ($\hat{k} = 1$). (B) Deformed shapes due to stiffness-induced 1005 buckling obtained for different values of foundation stiffness ($\hat{k} = 0.5$, $\hat{k} = 1$, $\hat{k} = 1.5$), 1006 with the growth rate fixed ($\gamma^* = 12.23$). (C) The choice of growth law affects the 1007 1008 deformed shapes. We select three different forms of growth laws for the same value of 1009 growth rate γ_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); p1029 < 0.001 (K); p < 0.001 (L). Colourmap: Turbo (C, G); warm helix (D, H). Scale bars: 20 1030 µm (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; c/v_{3-2} , 9.7 \pm 0.7; Col-0, 10.58 \pm 0.4; and c/v_{1-8} , 8.94 \pm 0.5. Overall mean values 1041 1042 derived from individual standard deviations (H): Ler, 1.7 ± 0.3 MPa; clv3-2, 1.9 ± 0.4 ; Col-0, 1.2 ± 0.2; *clv1-8*, 1.9 ± 0.3. *P*-values (Welch's t-test): WT-*clv3*, 6.65e-8 and 1043 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-F') in WT Ler (A, B, E) or clv3-2 (C, C', D, D', F, F') SAM. (C', D', and F') are magnified 1048 1049 views of the boxed regions in (C, D, and F), respectively. (G-L') Pairwise localisation of APUM10 (G, J, H, H', K, K'), WUS (G', I, I'), and MP (J', L, L') transcripts on alternating 1050 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 µm. Scale 1056 bars: 50 µm (A-C, E, F, G, G', H', I', J, J', K', L'); 100 µm (D, H, I, K, L); 25 µm (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::GFPer 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::GFPer 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 *clv*3. Colourmap: Turbo (G, H, K, L). Scale bars: 50 µm (top views in A-D); 1074 20 µm (orthogonal sections in A-D, F-H, J, L); 10 µm (E, I, K).











