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**Cauliflower fractal forms arise from perturbations of floral gene networks**

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**30 One Sentence Summary**

31

32 The molecular making of cauliflowers

33

**34 Abstract**

35

36 Throughout development, plant meristems regularly produce organs in defined spiral, opposite or  
37 whorl patterns, called phyllotaxis. Cauliflowers present an unusual phyllotaxis with a multitude  
38 of spirals nested over a wide range of scales. How such a fractal self-similar organization  
39 emerges from developmental mechanisms has remained elusive. Combining experimental  
40 analyses in *Arabidopsis thaliana* cauliflower-like mutant with modeling, we found that curd self-  
41 similarity arises because the meristems fail to form flowers but keep the “memory” of their  
42 transient passage in a floral state. Additional mutations affecting meristem growth can induce the  
43 production of conical phyllotactic structures reminiscent of the conspicuous fractal Romanesco  
44 shape. This study reveals how fractal-like forms may emerge from the combination of key,  
45 defined perturbations of floral developmental programs and growth dynamics.

46

**47 Main Text**

48

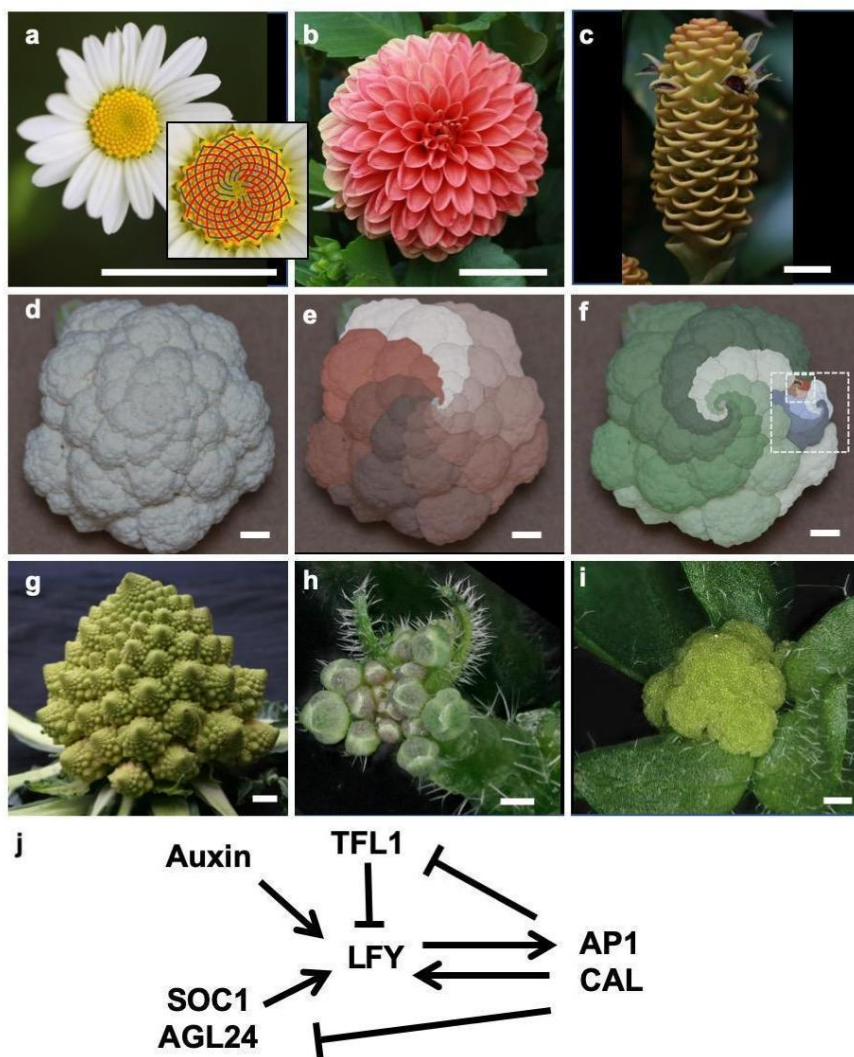
49 Above-ground plant architectures arise from activity of shoot apical meristems (SAM), which  
50 are pools of stem cells that give rise to organs such as leaves, shoots or flowers. The arrangement  
51 of organs on stems is termed phyllotaxis. Plants with a spiral phyllotaxis usually form two  
52 families of organ spirals, visible on compact structures such as flower heads, pine cones or cacti  
53 (Fig. 1a-c). These two families of spirals turn in opposite directions, and come in two  
54 consecutive numbers of the Fibonacci series (Fig. 1a) (1). In cauliflowers, spiral families are  
55 visible not only at one but at several scales (Fig. 1d-f). This self-similar organization culminates  
56 in the Romanesco cultivar where the spirals appear in relief due to their conical shape at all  
57 scales, a geometrical feature conferring the whole curd a marked fractal-like aspect (Fig. 1g).

58

59 Cauliflowers (*Brassica oleracea* var. *botrytis*) were domesticated from cabbages (2). The  
60 cauliflower inflorescence (the shoot bearing flowers) takes a curd shape because each emerging

61 flower primordia never matures to the floral stage but instead generates more curd-shaped  
62 inflorescences (2, 3). In *B. oleracea*, the genetic modifications causing curd development are still  
63 debated and likely affect multiple genes (2–5). However, cauliflower-like structures also exist in  
64 the model brassicaceae *Arabidopsis thaliana* and are caused by a double mutation in *APETALA1*  
65 (*API*) and *CAULIFLOWER* (*CAL*) (Fig. 1h-i), two paralogous genes encoding MADS-box  
66 transcription factors (TF) promoting floral development (6, 7). The *Arabidopsis* molecular  
67 regulators governing the development of shoots and flowers have been largely identified (8–  
68 10)(Table S1). Network models based on these regulators have been proposed to explain wild-  
69 type flower development (11–14). However, whether variants of these networks are able to  
70 account for development of *Arabidopsis ap1 cal* curds is unknown.

71  
72 To address this question, we first built a network of the main regulators involved in both flower  
73 and curd development. Then, we embedded this network within a 3D computational model of  
74 plant development to understand how mutations could transform wild-type (WT) inflorescences  
75 into curds.



76

77 **Figure 1: Illustrations of phyllotactic spirals on plant inflorescences**

78 (a) Daisy capitulum: the two families of spirals are indicated in the close-up (13 blue spirals and  
 79 21 red). (b) Dahlia composite flower (c) Zingiber inflorescence. (d-f) *Brassica oleracea* var.  
 80 *botrytis* cauliflower with (e) 8 counterclockwise (brown family) and (f) 5 clockwise (green  
 81 family) main spirals. Dashed rectangles show families of spirals nested over several scales (g)  
 82 Romanesco curd, (h) *Arabidopsis* wild-type inflorescence (h) and *ap1 cal* curd (i), Bar = 2 cm (a-  
 83 g), 500  $\mu$ m (h-i). (j) Interactions between major floral regulators; arrows depict activation  
 84 whereas barred lines indicate repression.

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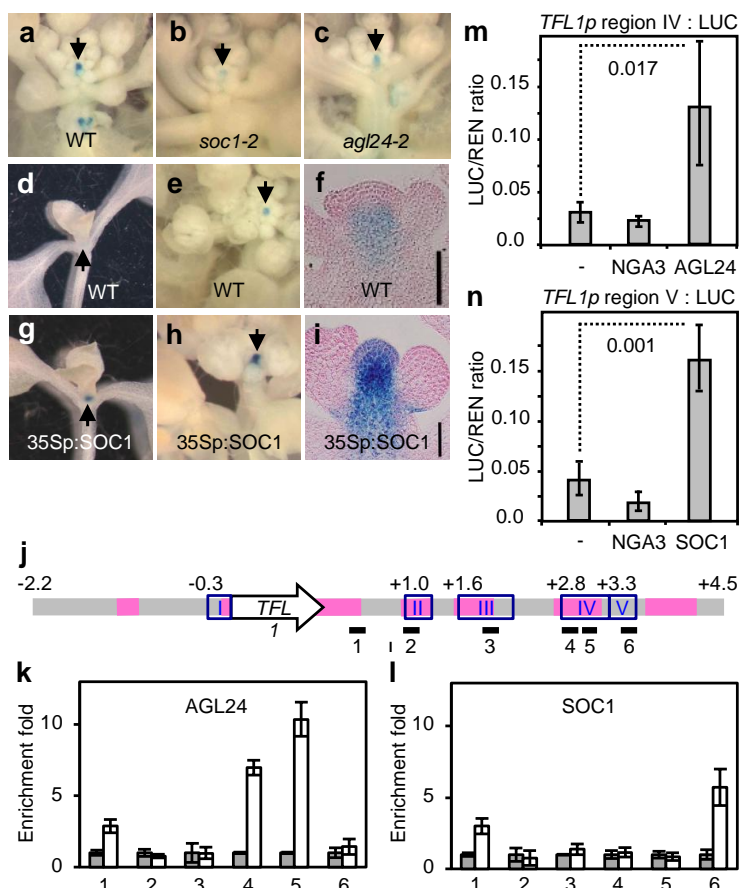
**88 The genetic basis of cauliflower curds**

89 In *Arabidopsis*, flowers are initiated by the TF LEAFY (*LFY*) (Fig. 1j) (Table S1). *LFY* is  
90 upregulated by the SUPPRESSOR-OF-OVEREXPRESSION-OF-CO 1 (*SOC1*) and  
91 AGAMOUS-LIKE 24 (*AGL24*) MADS-box proteins (induced throughout the inflorescence  
92 meristem by environmental and endogenous cues) and by auxin phytohormone maxima that  
93 mark floral meristem initiation sites. *LFY* is expressed specifically in floral primordia because its  
94 induction in the SAM is repressed by the *TFL1* inflorescence identity protein. In the floral  
95 primordium, *LFY* induces *API* and *CAL* (*API/CAL*) that positively feedback on *LFY* and repress  
96 both *SOC1/AGL24* and *TFL1*, thereby stabilizing the floral fate of the new meristem. In the *apl*  
97 *cal* cauliflower mutant, the *API/LFY* positive feedback is absent and *TFL1* is not repressed by  
98 *API/CAL* in the nascent floral meristem. Consequently, young flower primordia cannot maintain  
99 *LFY* expression and start themselves expressing *TFL1*. As a result, they lose their floral identity  
100 and become inflorescence meristems (6). Whereas *TFL1* repression in nascent flower primordia  
101 is well understood, the factors directly responsible for its upregulation in *apl cal* and  
102 inflorescence meristems are unknown.

103  
104 To complete our network, we thus searched for direct positive regulators of *TFL1*, other than  
105 *LFY* (that induces *TFL1* (15) but is not active in inflorescence meristems). *TFL1* is indirectly  
106 regulated by day length (16): in long days (LD) *TFL1* is up-regulated by *CONSTANS* (*CO*) and  
107 *FT*, two key upstream effectors of the LD pathway (11, 17–19) (Fig. S1). To search for direct  
108 regulators, we examined *SOC1* and *AGL24* that act downstream of *CO* and *FT* in the LD  
109 pathway (9). Loss- and gain-of-function experiments demonstrated that both *SOC1* and *AGL24*  
110 induce *TFL1* (Fig. 2a-i) and Chromatin Immuno-Precipitation showed that these two TFs bind to  
111 the *TFL1* regions that regulate its expression in the SAM (20) (Fig. 2j-l). These regions were  
112 sufficient to activate a *TFL1* reporter construct by *SOC1* and *AGL24* in a transient assay (Fig.  
113 2m-n) confirming that both MADS-box TFs are direct regulators of *TFL1*. Since *XAANTAL2*  
114 (*XAL2*), a homolog of *SOC1* and *AGL24* also bound to and induced *TFL1* (21), we aggregated  
115 the activities of *SOC1*, *AGL24* and *XAL2* into a SAX proxy acting as *TFL1* positive regulator  
116 (Fig. 3a).

117

118 We thus created the SALT network (for SAX, AP1/CAL, LFY, and TFL1; Fig. 3a) made of  
 119 these 4 regulator sets, auxin (22), and F, a flower inducing signal (a proxy for the FT florigen)  
 120 that increases when the plant ages or is exposed to flower-inducing environmental conditions  
 121 (23, 24). We also added a short-lived transient early Repressor of *TFL1* (eREP), as a proxy for  
 122 *TFL1* early repression in the young flower bud performed by the redundant activities of SOC1,  
 123 AGL24, SHORT VEGETATIVE PHASE, and SEPALLATA4 (25).



124  
 125 **Fig. 2: AGL24 and SOC1 are direct positive regulators of *TFL1*.**  
 126 (a-c), *TFL1p*:GUS activity in WT (a), *soc1-2* (b) and *agl24-2* (c) inflorescence apices. (d-i),  
 127 *TFL1p*:GUS activity (blue signal) in WT (d-f) and *35Sp:SOC1* (g-i) apices at vegetative (d,g)  
 128 and flowering (e,f,h,i) stages. (f-i), longitudinal sections through flowering shoots. Arrows mark  
 129 the SAM. Scale bars in (f) and (i), 40  $\mu$ m. (j-l) Structure of *TFL1* locus, with regions conserved  
 130 in Brassicaceae (pink lines), regulatory regions (20) (blue boxes I-V), and fragments used in  
 131 ChIP (black lines 1-6). ChIP experiments on plants expressing a tagged version of AGL24 (k,

132 white bars) or the WT SOC1 protein (l, white bars) or on control plants (grey bars, see Material  
133 and Methods), show that AGL24 binds region IV (k, fragments 4-5) and SOC1 region V (l,  
134 fragment 6). A representative biological replicate is shown with the mean  $\pm$  SE for three  
135 technical replicates. (m,n) Transient assays showing transactivation of the LUCIFERASE (LUC)  
136 reporter driven by region IV (activation by 35Sp:AGL24) and region V (activation by  
137 35Sp:SOC1). NGA3 is an unrelated TF used as negative control. Bars denote the mean and  
138 standard deviation of three independent biological replicates. *P* values are for the equality of  
139 means (Student's t-test).

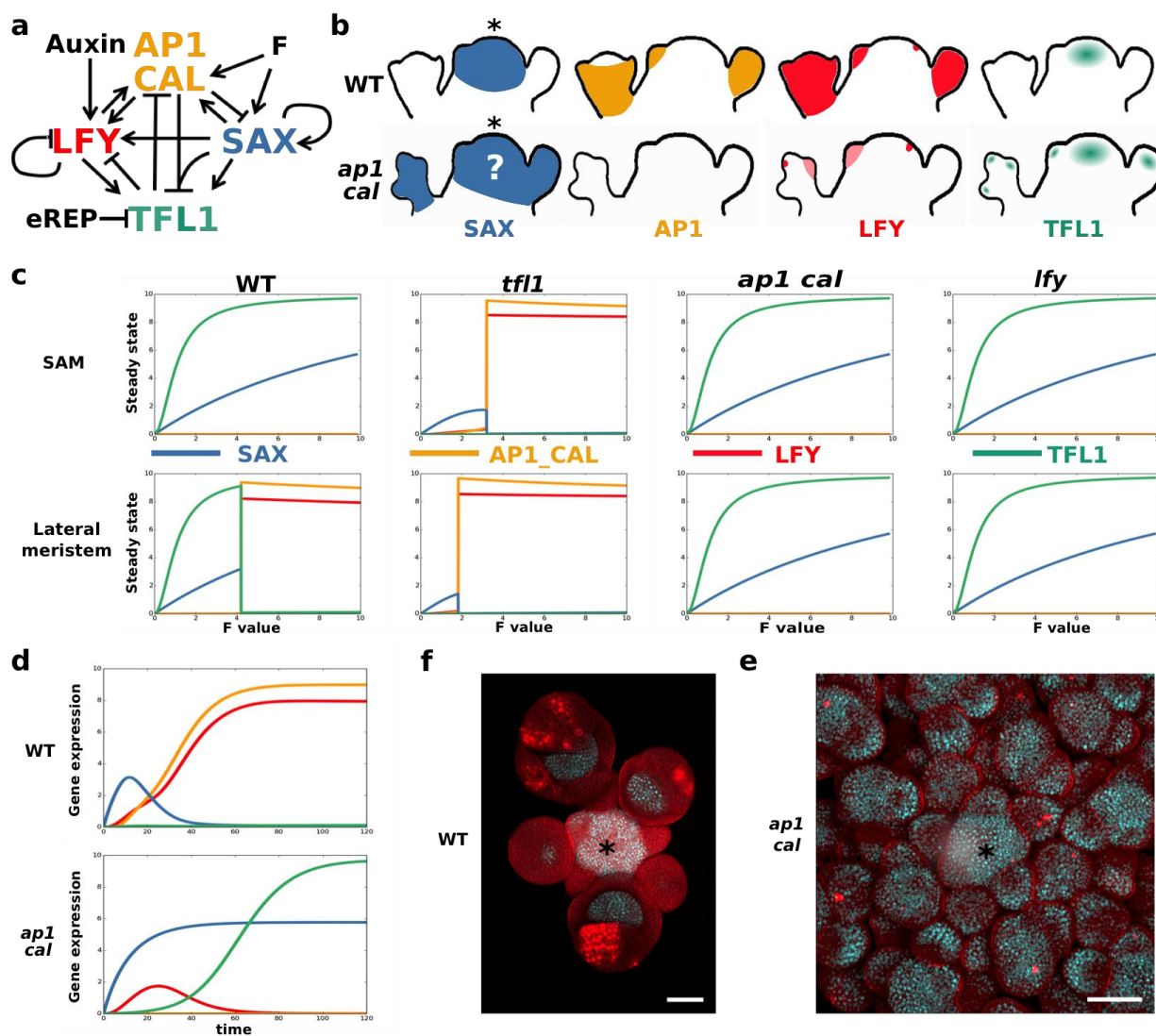
140  
141 The steady states of the SALT network correspond to the gene expression patterns observed in  
142 wild-type vegetative (low SALT values), inflorescence (high TFL1/SAX, low AP1/CAL/LFY)  
143 and flower (low TFL1/SAX, high AP1/CAL/LFY) meristems (Fig. 3b,c, Fig. S2). Above an *F*  
144 threshold value, the network generates a flower or an inflorescence state depending on *F* and  
145 auxin values. Simulations of *tfl1*, *lfy*, *ap1 cal* mutants produce expected outputs consistent with  
146 experimentally reported gene expressions (6, 16, 26, 27) (Fig. 3b, c). The simulated *sax* mutant  
147 did not reach a floral state, consistent with the late flowering behavior of the *soc1 agl24* double  
148 mutant (28).

149  
150 The modelled gene expression dynamics (Fig. 3d) illuminate the fundamental differences  
151 between WT and cauliflower meristems: in a WT flower primordium, *F* induces *SAX*. *SAX* and  
152 auxin induce *LFY*, that, together with *F*, induce *API/CAL*. *API* positively feeds back on *LFY* and  
153 represses *SAX* (Fig. 3d). *TFL1* expression, that could be induced by *SAX* and *LFY* in early floral  
154 stages, is constantly repressed, first by eREP and later by *SAX* plus *API/CAL*. High *API/CAL*  
155 and *LFY* with low *TFL1* and *SAX* expression stabilize the floral fate. In contrast, in the *ap1 cal*  
156 flower primordia, the absence of *API/CAL* activity has two consequences: i) *LFY* expression is  
157 upregulated only transiently since *API/CAL* positive feedback is missing (Fig. 3d) and ii) *SAX*  
158 genes are not repressed by *API* and thus induce *TFL1* in nascent flower meristems. *TFL1*  
159 represses *LFY* even further and the meristem returns to a shoot meristem state (Fig. 3d). Note  
160 that, the early *LFY* induction would likely be reinforced (while remaining transient) by  
161 incorporating the recently discovered direct induction of *LFY* by the *F* partner protein *FD* (29).  
162 The SALT model predicts that *SAX* expression should extend over the entire cauliflower. We



163 analyzed a SOC1-GFP reporter line and indeed observed expansion of its expression domain in  
164 *ap1 cal* as compared to WT (Fig. 3e, f).

165



166

167 **Fig. 3: SALT Gene Regulatory Network model and experimental validation.**

168 (a) SALT GRN network structure (b) Known expression patterns of *SAX*, *API/CAL*, *LFY*, and  
169 *TFL1* in the SAM and lateral primordia of WT and *ap1 cal* mutant. The question mark indicates  
170 a predicted expression pattern of the model. (c) WT, *tf11*, *ap1 cal* and *lfy* steady states of the  
171 model at different F values in the SAM (low auxin) and in lateral meristems (high auxin). The  
172 genetic identity predicted for WT and all mutant meristems correspond to the experimentally  
173 observed phenotypes. (d) Temporal simulation of gene expression in lateral primordia with high

174 F value. (e, f) Expression of the SOC1:GFP (white/light blue signal) reporter construct in WT (e)  
175 and in the *ap1-7 cal-1* mutant (f) inflorescences. Asterisks mark the SAM. Bar = 50  $\mu$ m.

176

177 The SALT network thus recapitulates realistic gene expressions driving meristem fates.  
178 However, a plant architecture does not only depend on meristem fates but also on  
179 morphodynamic parameters including molecular thresholds for fate decisions, organ growth rate,  
180 delay for meristems to start organ production and organ production rate which are independently  
181 regulated. Plant inflorescence architecture thus emerges from the complex interaction between  
182 the floral GRN and morphodynamic parameters. This is illustrated here by the *lfy* and *ap1 cal*  
183 mutants that have the same GRN outputs (Fig. 3c) but markedly different architectures (6, 27).  
184 To study how this interaction operates in Arabidopsis, we integrated the SALT GRN in a 3D  
185 plant computational model implemented as an L-system (see Supplementary materials Modeling  
186 Methods).

187

### 188 **A multi-scale model generates Arabidopsis cauliflower structures**

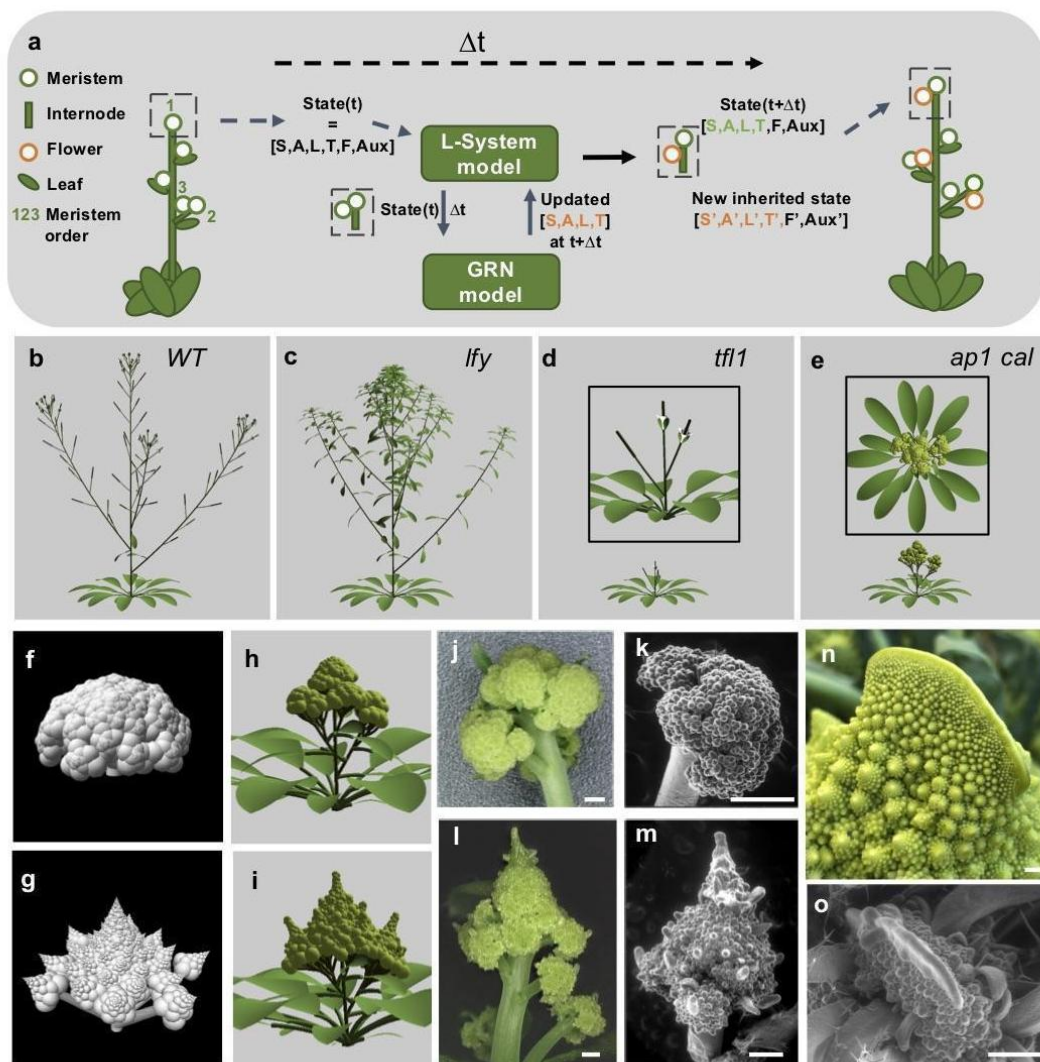
189 The 3D model is made of the 4 types of organs that shape plant above-ground architecture:  
190 meristems, internodes, leaves and flowers (Fig. 4a, Supplementary materials). Each meristem's  
191 identity (vegetative, inflorescence and floral) is determined by the GRN steady state, computed  
192 at each time step as a function of the meristem's previous state and external factors (auxin and  
193 F). The GRN model is implemented as single compartment ordinary differential equations  
194 (Supplementary materials Modeling Methods). We assume that the GRN dynamics is faster than  
195 growth and reaches its steady state within a time step. A set of growth rules defines meristem  
196 production: a vegetative meristem produces a compressed stem (non-elongated internodes) with  
197 rosette leaves; an inflorescence meristem produces an elongating internode, a cauline leaf and a  
198 new shoot meristem in the leaf axil; a floral meristem produces an internode terminating with a  
199 flower meristem, devoid of bracts (leaf-like organs subtending flowers) since they are repressed  
200 by LFY (6)). Each newly generated axillary meristem begins with maximal auxin level (22),  
201 SAX/LFY/AP1/CAL values inherited from the parent meristem, together with a fraction of the  
202 parent TFL1 value as, in the real plant, this non-cell autonomous protein is present in the  
203 primordia region (30). To match the wild-type plant architecture, indeterminate meristems at  
204 orders  $>2$  (Fig. 4a) were kept quiescent, a likely effect of apical dominance (the inhibition of

205 lateral meristem outgrowth) (Fig. S3a). The model also contains rules describing organ growth  
206 dynamics (internode and leaf elongation, flower growth, organ production rate, growth initiation  
207 delay). Simulated plants start with a single vegetative SAM and repeatedly produce new organs  
208 according to the GRN, the morphodynamic rules and an input value of F.

209 By adjusting the GRN and morphodynamic parameters within a range of plausible values  
210 (Supplementary materials), we successfully calibrated the model to produce realistic  
211 architectures for wild-type and *lfy* plants (Supplementary Movies 1-2), as well as for the *tfl1*  
212 mutant (Fig. 4b-d) and a non-flowering phenotype for the *sax* mutant. However, our simulations  
213 could not generate a realistic *apl cal* mutant growing without bract/cauline leaves and displaying  
214 high order meristems (Fig. S3a-b) suggesting that the cauliflower phenotype involves additional  
215 regulations. We reasoned that laterally produced *apl cal* inflorescence meristems are different  
216 from those produced in other genotypes as, according to our GRN, they have been transiently  
217 exposed to LFY expression (Fig. 3d). Several pieces of evidence suggest that this transient LFY  
218 expression, already known to repress bracts (6), could also contribute to high-order meristem  
219 release. First, the outgrowth of otherwise inhibited axillary meristems in the rosette is stimulated  
220 by ectopic expression of LFY (or a LFY allele) (31, 32). Second, it was established that the *lfy*  
221 *apl cal* triple mutant does not form cauliflowers (6) and we found that, in this mutant, the  
222 number of high-order meristems is significantly reduced as compared to *apl cal* (Fig. S3d-h),  
223 thus supporting our hypothesis.

224 We abstracted this critical molecular pathway, by introducing in the model a factor X  
225 upregulated when LFY exceeds a minimal threshold level. Upregulated factor X releases high-  
226 order meristem growth and suppresses the bract. This was sufficient to unlock the recursive  
227 growth of lateral meristems and to generate the *apl cal* curd structure that arises from the  
228 transient but irreversible exposure of meristems to the floral signal without any alteration of wild  
229 type growth dynamics (Fig. 4e,h, Supplementary Movie 3). Overall, our work shows that the *apl*  
230 *cal* and *lfy* architectures are different (Fig. 3c) because the molecular histories of their  
231 inflorescence meristems are different, thereby revealing the existence of a developmental  
232 hysteresis.

233



234

235 **Fig. 4: Simulation and assessment of a GRN-based plant development model.**

236 (a) Schematic representation of the multi-scale model of Arabidopsis development. Each  
 237 meristem state is composed of signal levels (auxin, F) and a GRN steady state. At time  $t$ , the  
 238 plant is made up of a collection of organs (left). At time  $t+\Delta t$  (right) the model updates the signal  
 239 levels and GRN state in each meristem. The steady state defines the identity of the meristems  
 240 (vegetative, inflorescence or flower) used to compute meristem lateral productions. Green  
 241 numbers indicate meristem order (b-e). Plant morphologies obtained in the WT (b), *lfy* (c), *tfl1*  
 242 (d) and *ap1 cal* (e) simulations. Simulated morphologies with constant (f,h) or increased  
 243 meristem size (g,i) in a simplified (f,g) and the Arabidopsis model (h,i). Light micrographs (j,l,n)  
 244 and s.e.m (k,m,o) of cauliflower structures in Arabidopsis *ap1 cal* (j, k), Arabidopsis *ap1 cal*

245 *clv3* (l, m, o) and Romanesco (n). Uninduced *API:GR* transgene is present in plants j-m. Scale  
246 bars = 500  $\mu$ m.

247

## 248 **Growth dynamics define cauliflower and Romanesco curd structures**

249 Our work in *Arabidopsis* offers a conceptual framework to explain how inflorescence  
250 architecture emerges from coupling a floral GRN to morphodynamic parameters. We wondered  
251 whether modifications affecting components of this framework could also explain the  
252 architecture of the cauliflowers that arose during domestication, namely the edible *Brassica*  
253 *oleracea* (*Bo*) var. *botrytis* (*Bob*) and its Romanesco variant. Whether similar genetic defects as  
254 in *Arabidopsis* are responsible for curd development in *B. oleracea* is still debated (4, 5). To  
255 further investigate this point, we analysed RNA-seq data of *Bob* curds: we confirmed the  
256 previously identified mutation in the *BobCAL* gene (Fig. S4a)(4, 5, 7) and observed that the two  
257 *API* paralogs (*BobAPI-a* and *BobAPI-c*) are expressed at much lower levels than in cabbage (*Bo*  
258 var. *capitata*) inflorescences (Fig. S4b). These functional proteins are induced with a delay only  
259 when the cauliflower elongates and start forming normal flowers (3, 33). Comparing cauliflower  
260 and cabbage sequences, we identified differences in binding sites for candidate regulators of  
261 *BoAPI* that could account for their delayed activation (Fig. S4d). The combination of *BoCAL*  
262 inactivation and *BobAPI-a/c* expression delay (heterochrony due to *cis* or *trans* mutations) thus  
263 likely participates to *Bob* curd development. Similar to *Arabidopsis ap1 cal*, cauliflowers have  
264 meristems of higher maximal order ( $n \geq 7$ ) than cabbages ( $n = 3-4$ ) (Fig. S5). Nevertheless, the  
265 development of single massive cauliflower curds is not the exact equivalent of the *Arabidopsis*  
266 mutant (3, 5) and involves additional multifactorial alterations of morphodynamics parameters  
267 (such as reduction of internode elongation and branches diameter increase).

268

269 The conical shapes appearing in Romanesco spirals at all scales (Fig. 1f) represent an additional  
270 geometric variation obtained through domestication that seems to be associated with a change in  
271 morphodynamic parameters. Indeed, several such parameters remain constant during cauliflower  
272 development but vary in Romanesco (34): i) the plastochron, the time between two successive  
273 meristem productions, ii) the number of visual spirals originating from a given meristem, iii) the  
274 time (measured in number of plastochrons) needed before a lateral primordium starts producing  
275 its own primordia (or lateral production onset delay), and iv) the size of the meristems. Whether

276 some of these parameters are causal to the Romanesco phenotype remains unclear but  
277 phyllotaxis studies (1, 35, 36) indicate that the first three parameters are linked to the meristem  
278 size: an augmentation of the size of the meristem central zone should decrease the plastochron,  
279 which in turn increases the number of spirals, and the lateral production onset delay. We thus  
280 hypothesized that passing from a constant to a decreasing plastochron in meristems could change  
281 cauliflower into Romanesco morphologies. We first tested this *in silico* using a simplified, purely  
282 geometric model of curd growth, independent from the Arabidopsis GRN and specific growth  
283 dynamics (Supplementary materials). A decreasing plastochron was sufficient to produce  
284 Romanesco shapes (Fig. 4g) whereas constant values of this parameter produce cauliflower  
285 morphologies (Fig. 4f).

286  
287 We then introduced the same change in the more complex GRN-based, Arabidopsis cauliflower  
288 architectural model, while keeping its organ growth dynamics as calibrated on the WT. Although  
289 not as complete as in the purely geometric model, the curd changed towards a “Romanesco-like”  
290 morphology with typical conical curd shapes (Fig. 4h, i). We then tested this hypothesis  
291 experimentally in Arabidopsis by altering the size of the meristem directly. We achieved this by  
292 introducing a mutation in the *CLAVATA3 (CLV3)* gene that controls meristem homeostasis and  
293 induces an increase of the meristem central zone during growth (37, 38). As predicted by our  
294 analysis, introduction of a *clv3* mutation in *ap1 cal* Arabidopsis mutant modified the curd shape,  
295 which lost its round morphology and acquired a more conical shape, with similar structures at  
296 different scales, features recognized as hallmarks of Romanesco curds (39) (Fig. 4l-m). Two  
297 additional pieces of evidence support the hypothesis that meristem homeostasis is perturbed in  
298 Romanesco curds: they occasionally show fasciation, a feature typical of meristem enlargement  
299 also observed in *clv3* or *ap1 cal clv3* mutants (Fig. 4n,o)(37). Moreover, the expression of *CLV3*  
300 (and possibly two other genes acting in the same pathway)(38) are lower in Romanesco curds  
301 than in cauliflowers (Fig. S6). Altogether, these observations establish that meristem size  
302 regulates the final curd morphology through control of plastochron value.

303  
304 These results reveal how fractal patterns can be generated through growth and developmental  
305 networks that alter identities and meristem dynamics. Our data, GRN and growth models now  
306 clarify the molecular and morphological changes over time by which meristems gain different

307 identities to form the highly diverse and fascinating array of plant architectures found throughout  
308 nature and crops.

309

310 **References and Notes:**

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455

456 **Author contributions**

457 ChG and FP conceived the study

458 ChG, EA, EF performed the modelling

459 ASM, CaG, DB, FM, FP, GT, MK, MLM, VG designed and performed the plant experiments

460 NP performed the confocal imaging experiment

461 JL analysed the RNA-seq and genomic data

462 ChG, FP and EA wrote the paper with the help of all authors

463

464 **Competing interests**

465 The authors declare no competing interests.

466

467 **Data and Materials Availability**

468

469 All data are in the main paper or the supplement.

470

471 All plant materials are available upon request.

472 The following secure token has been created to allow review of record GSE150627 while it

473 remains in private status: khkjgckmdtkhpgb.

474 All source codes to run the simulations are available as supplementary archive file (description

475 of installation and execution available as README.txt.

476

477

478 **Supplementary Materials:**

479 Materials and Methods

480 Figures S1 to S6

481 Tables S1 to S3

482 Movies S1 to S3

483 Code archive file: Architecture-model.zip

484 References (41-108)

485 MDAR Reproducibility Checklist

486

487

488