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1 **Sugar signaling modulates SHOOT MERISTEMLESS expression and meristem function in**
2 **Arabidopsis**

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5 Filipa L. Lopes^{1,2}, Pau Formosa-Jordan^{2,3}, Alice Malivert^{2,4}, Leonor Margalha¹, Ana Confraria¹,
6 Regina Feil⁵, John E. Lunn⁵, Henrik Jönsson^{2,6,7*}, Benoît Landrein^{2,4*}, and Elena Baena-
7 González^{1§*}

8

9 ¹Instituto Gulbenkian de Ciência, 2780-156 Oeiras, Portugal and GREEN-IT Bioresources for
10 Sustainability, ITQB NOVA, 2780-157 Oeiras, Portugal

11 ²Sainsbury Laboratory University of Cambridge, Bateman St, Cambridge CB2 1LR, United
12 Kingdom

13 ³Max Planck Institute for Plant Breeding Research, D-50829 Cologne, Germany

14 ⁴Laboratoire Reproduction et Développement des Plantes, Université de Lyon, Ecole Normale
15 Supérieure de Lyon, UCB Lyon 1, CNRS, INRA, 69342 Lyon Cedex 07, France

16 ⁵Max Planck Institute of Molecular Plant Physiology, 14476 Potsdam-Golm, Germany

17 ⁶Department of Applied Mathematics and Theoretical Physics, University of Cambridge,
18 Cambridge, UK

19 ⁷Computational Biology and Biological Physics, Lund University, Lund, Sweden

20 [§]Current address: Department of Biology, University of Oxford, South Parks Rd, Oxford
21 OX1 3RB, UK

22

23 *Corresponding authors: henrik.jonsson@slcu.cam.ac.uk, benoit.landrein@ens-lyon.fr and
24 elena.baena-gonzalez@biology.ox.ac.uk

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26

27 **SUMMARY**

28

29 In plants, development of all above-ground tissues is controlled by the shoot apical meristem
30 (SAM) which balances cell proliferation and differentiation to allow life-long growth. To
31 maximize fitness and survival, meristem activity is adjusted to the prevailing conditions through a
32 poorly understood integration of developmental signals with environmental and nutritional
33 information. Here, we show that sugar signals influence SAM function by altering the protein
34 levels of SHOOT MERISTEMLESS (STM), a key regulator of meristem maintenance. STM is
35 less abundant in the inflorescence meristems of plants grown or treated under limiting light
36 conditions, with lower STM levels correlating with lower sugar content in these meristems.
37 Additionally, sucrose but not light is sufficient to sustain STM accumulation in excised
38 inflorescences. Plants overexpressing the α 1-subunit of SUCROSE-NON-FERMENTING1-
39 RELATED KINASE 1 (SnRK1) accumulate less STM protein under optimal light conditions,
40 despite higher sugar accumulation in the meristem. Furthermore, SnRK1 α 1 interacts physically
41 with STM, suggesting a direct local repression. Surprisingly, silencing *SnRK1 α* in the meristem
42 leads to reduced *STM* expression and severe developmental phenotypes previously associated with
43 STM loss-of-function. Altogether, we demonstrate that sugars promote STM accumulation and
44 that the SnRK1 sugar sensor plays a dual role in the SAM, limiting STM abundance under
45 unfavorable conditions but being required for overall meristem organization and integrity. This
46 highlights the importance of sugars and SnRK1 signaling for the proper coordination of meristem
47 activities.

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51 INTRODUCTION

52 Unlike animals, plants generate organs throughout their life cycle. Postembryonic development
53 relies on stem cell reservoirs localized in specialized tissues known as meristems. The shoot apical
54 meristem (SAM) is the site where above-ground organogenesis is initiated, giving rise to leaves,
55 axillary buds, flowers, and the stem. The SAM is organized into functionally distinct subdomains
56 in which cell division and differentiation are tightly coordinated to maintain the integrity and
57 regenerative potential of the meristem. The concerted regulation of the different regions requires
58 the activity of several transcriptional networks and hormone signaling pathways (1).

59 In *Arabidopsis* (*Arabidopsis thaliana*), SAM homeostasis is finely controlled by a negative-
60 feedback loop between WUSCHEL (WUS) and CLAVATA3 (CLV3) (2, 3). WUS is a mobile
61 transcription factor produced in the organizing center that underlies the stem cell layers. WUS
62 moves through plasmodesmata into the overlying stem cells to promote their proliferation and
63 maintain pluripotency (4, 5). In addition, WUS induces stem cells to produce the CLV3 peptide,
64 which in turn diffuses into the organizing center, where it inhibits *WUS* expression. The WUS-
65 CLV3 feedback loop therefore enables a dynamic adjustment of the size of the stem cell pool (6).

66 Meristematic activity is also regulated by members of the THREE-AMINO-ACID-LOOP-
67 EXTENSION (TALE) homeodomain protein superfamily which includes KNOTTED1-like
68 homeobox (KNOX) and BEL-like homeobox (BLH or BELL) transcription factors. One key
69 regulator of the TALE family is SHOOT MERISTEMLESS (STM), which is essential both for
70 SAM establishment and SAM maintenance (7). Unlike WUS, STM is expressed throughout the
71 SAM except at the sites of initiating primordia (7, 8). STM suppresses differentiation
72 independently of WUS and promotes cell division by inducing the expression of *CYCLIN D3*
73 (*CYCD3*) and *ISOPENTENYL TRANSFERASE7* (*IPT7*) (9), which encodes a key enzyme involved
74 in cytokinin (CK) biosynthesis (10). CKs, in turn, are involved in stem cell maintenance,
75 influencing SAM size and organ production through WUS and STM (11–13).

76 Loss-of-function *stm* mutants exhibit defects in SAM formation and maintenance, leading to
77 growth arrest at the seedling stage due to exhaustion of stem cells (7). In addition, the most severely
78 affected mutants like *stm-1* display fusions of cotyledons and other organs, indicating a role for
79 STM also in boundary specification (7, 14, 15). Indeed, incipient organ primordia are formed at
80 sites where *STM* expression is low, coincident with auxin accumulation and the expression of
81 transcription factors (TFs) that promote organ formation and repress *STM*, including

82 ASYMMETRIC LEAVES1 and 2 (AS1 and AS2) and members of the TEOSINTE
83 BRANCHED1/CYCLOIDEA/PCF1 (TCP) family (16–20). In the inflorescence meristem, *STM*
84 expression is also enhanced in boundaries, notably in response to mechanical forces, and is
85 required for correct boundary folding (21).

86 Recent work revealed that *STM* heterodimerizes with *WUS*, enhancing *WUS* binding to the
87 *CLV3* promoter and *CLV3* expression, and repressing stem cell differentiation. Conversely, *WUS*
88 is required for the expression of *STM*, which thereby enhances *WUS*-mediated stem cell activity
89 (22). *STM* is also regulated through an interaction with BELL1-like homeodomain (BLH) proteins
90 (23) and the formation of these heterodimeric complexes is essential for *STM* nuclear localization
91 and, thus, proper function (24). *STM* interacts with the BLH proteins PENNYWISE (PNY),
92 POUNDFOOLISH (PNF), and ARABIDOPSIS THALIANA HOMEODOMAIN GENE1 (ATH1)
93 (24–27) which contribute redundantly with *STM* to meristem initiation and maintenance (28–30).

94 Because of their sessile lifestyle, plants continuously adjust their development to changes in
95 the environment, and this is reflected in the dynamic nature of the SAM. In addition to its
96 maintenance by a network of TFs and hormonal signals, the SAM also responds to environmental
97 signals that influence the relative size of its subdomains and the type and number of organs it
98 produces. One of the external factors that affect meristem activity is light, which can exert a direct
99 effect through photoreceptor-mediated signaling and an indirect effect by driving photosynthesis
100 and sugar production (31–33). Both light and metabolic signals activate the TARGET OF
101 RAPAMYCIN (TOR) protein kinase, which in turn promotes cell proliferation in the SAM *via* an
102 increase in the expression of S-phase genes (34, 35). In addition, TOR induces *WUS* expression
103 partially through an effect on CK degradation (33, 36).

104 TOR activity is often antagonized by SUCROSE NON-FERMENTING1-RELATED
105 KINASE 1 (SnRK1) which, like TOR, translates environmental information into metabolic and
106 developmental adaptations (37–39). SnRK1 is a heterotrimeric protein kinase complex, composed
107 of an α -catalytic subunit and two regulatory β - and γ -subunits. In Arabidopsis, the α -subunit is
108 present in two major isoforms, SnRK1 α 1 and SnRK1 α 2 (also known as KIN10 and KIN11). The
109 SnRK1 complex is activated under low carbon conditions to promote energy saving and nutrient
110 remobilization strategies, whilst TOR is activated in response to nutrient abundance to promote
111 cell proliferation and growth adaptations (37–39).

112 Despite the importance of STM for establishing and maintaining SAM function and our
113 increasing understanding of how STM activity is controlled by other transcriptional regulators, it
114 is unknown if environmental signals modulate STM expression and/or activity. Here, we make use
115 of plants expressing transcriptional and translational STM reporters to investigate this question.
116 We show that STM protein accumulation does not respond to CK but is clearly induced by
117 photosynthesis-derived sugars. We also show that the SnRK1 sugar sensing kinase is active in the
118 SAM and that it is involved in adjusting STM protein levels to the light conditions. Finally, we
119 show that SnRK1 is also required to maintain *STM* expression, meristem organization and
120 integrity.

121

122 **RESULTS**

123 **Light promotes STM protein accumulation**

124 Light is essential for proper plant development and physiology. To investigate a potential
125 regulatory role of light on STM levels, we made use of an Arabidopsis (Col-0) reporter line in
126 which a fluorescently-tagged form of the STM protein (STM-VENUS) is expressed under the
127 control of the *STM* promoter [*pSTM::STM-VENUS* (21, 40)]. We measured STM-VENUS levels
128 in inflorescence meristems from 5-week-old plants grown under, or transiently treated with
129 different light conditions. In one set of experiments, we compared STM-VENUS levels between
130 plants grown under two different irradiances [60 vs. 170 $\mu\text{mol m}^{-2} \text{s}^{-1}$, referred to as low light (LL)
131 and high light (HL), respectively]. Irradiance had a strong impact on STM accumulation, with the
132 mean STM-VENUS levels of plants grown under LL being 76% of those grown under HL (Fig.
133 1A-B). In a second set of experiments, we compared STM-VENUS levels between HL-grown
134 plants transferred to darkness for up to 72 h and their corresponding controls maintained under HL
135 conditions. Incubation under darkness had a very severe impact on STM accumulation, with STM-
136 VENUS levels of plants subjected to a 72 h dark treatment being 39% of those prior to the
137 treatment (Fig. 1A, 1C).

138 To assess whether the impact of light on STM levels was a general effect on protein
139 abundance in meristems, we extracted total proteins from SAMs of plants constantly grown under
140 HL, LL, or treated with 48h of darkness and compared STM levels to those of the housekeeping
141 protein TUBULIN (TUB) by immunoblotting (Fig. 1D). These analyses confirmed the microscopy
142 results regarding STM-VENUS accumulation, showing that, in LL and dark-treated plants, STM

143 levels were 71% and 23%, respectively, of the STM levels in HL. The immunoblots revealed no
144 impact of the light conditions on TUB accumulation, indicating that the lower STM levels were
145 not caused by a general decrease in protein accumulation. Finally, to assess if low STM
146 accumulation could be due to reduced *STM* transcript abundance, we dissected SAMs of plants
147 kept under HL conditions or subjected to 48 h darkness and analyzed *STM* transcript levels by
148 quantitative RT-PCR (qPCR). *STM* levels were not significantly affected by the dark treatment
149 (Fig. 1E), showing that the differences in protein accumulation are not due to changes in *STM*
150 transcription or transcript stability. On the other hand, the levels of *AINTEGUMENTA-LIKE 7*
151 (*AIL7*) and *HOMEODOMAIN PROTEIN 25* (*HB25*), known gene targets of STM (41), were reduced
152 upon the dark treatment (Fig. 1E). This is also consistent with the lower STM-VENUS abundance
153 and indicates decreased STM activity in the SAM in these conditions.

154

155 **The response of STM to light is CK-independent and involves sugars**

156 Several lines of evidence suggest that, like *WUS*, *STM* expression could be directly regulated by
157 CK (11, 42). To investigate whether CK could also regulate STM at the protein level and hence be
158 involved in the response of STM to light, we first tested whether light influenced CK signaling in
159 inflorescence meristems. To this end we used plants expressing the synthetic CK reporter
160 *pTCSn::GFP* (43) in similar experiments as described for the *STM-VENUS* reporter line. In plants
161 grown in LL or subjected to a 48 h dark treatment, *pTCSn::GFP* levels were 74% (Supplementary
162 Fig. S1A-B) and 46% (Supplementary Fig. S1A, S1C) of those in HL plants, respectively. These
163 observations show that CK signaling in inflorescence meristems is, like in vegetative meristems
164 (33) affected by light. We next examined whether CK could impact STM levels in inflorescence
165 meristems. For this, we excised SAMs of HL-grown *STM-VENUS* plants and maintained them
166 under HL *in vitro* (13) for different periods of time in the absence or presence of 500 nM 6-
167 benzylaminopurine (BAP), a synthetic CK. Dissection of the SAMs led to a strong reduction of
168 the STM-VENUS (Supplementary Fig. S1D) and the *pTCSn::GFP* (Supplementary Fig. S1E)
169 reporter signals, as previously described for the *pTCSn::GFP* and *pWUS::GFP* reporters (13).
170 However, in contrast to *pTCSn::GFP* [Supplementary Fig. S1E; (13)], CK could not sustain STM-
171 VENUS levels (Supplementary Fig. S1D), indicating that the effect of light on STM-VENUS is
172 likely CK-independent.

173 Light plays direct signaling functions through various photoreceptors but also signals
174 indirectly through sugars produced by photosynthesis. We therefore wondered whether the effect
175 of light on STM was direct or mediated by sugars. To investigate this, we first measured the levels
176 of sucrose, glucose, and fructose in the rosettes (Supplementary Fig. S2A) and SAMs (Fig. 2A) of
177 HL- and dark-treated plants. We also measured the levels of Tre6P, a regulatory sugar-phosphate
178 that serves as a signal of the plant sucrose status and that is crucial for sucrose homeostasis, growth
179 promotion, and developmental progression (44). In the light, the levels of sucrose, glucose, and
180 Tre6P were, respectively, 2.1-, 2.2-, and 7.9-fold higher in the SAM than in the rosette, whilst
181 fructose accumulated to comparable levels in the two organs (Fig. 2A and Supplementary Fig.
182 S2A). Incubation in the dark led to a marked depletion of sucrose and fructose both in rosettes
183 (15% and 11% of the levels in the light, respectively) and SAMs (8% and 4% of the levels in the
184 light, respectively), with a much milder reduction being observed for glucose, the most abundant
185 sugar in the SAM (44% and 35% of the levels in the light in rosettes and SAMs, respectively).
186 Tre6P levels were also much lower in dark-treated plants (11% and 3% of the levels in the light in
187 rosettes and SAMs, respectively), reflecting the drop in sucrose levels. To further distinguish
188 between a light and a sugar effect, we excised inflorescences at around 3 cm from the apex and
189 placed them for 48 h in liquid medium. Similarly to what was observed in dissected SAMs
190 (Supplementary Fig. S1D), STM-VENUS signal decreased markedly in cut inflorescences as
191 compared to the uncut controls (Fig. 2B). Furthermore, light alone was not sufficient to sustain
192 STM-VENUS expression, as the signal was comparable in cut inflorescences incubated in the light
193 and in the dark (47% and 43% of the levels in the uncut control, respectively). These results,
194 obtained with a double reporter line (*pSTM::STM-VENUS/pSTM::TFP-N7*; Fig. 2B), were similar
195 to those obtained for plants expressing STM-VENUS alone (Supplementary Fig. S2B).

196 To test if the underlying cause was sugar deprivation, we first incubated excised
197 inflorescences of the double marker line for 48 h in darkness in medium supplemented with
198 increasing concentrations of sucrose. Sorbitol, which is not a readily metabolized carbon source,
199 was used as an osmotic control. Sucrose was able to sustain STM-VENUS accumulation, and its
200 effect was dose-dependent, leading to STM-VENUS levels close to those of uncut inflorescences
201 when supplied at a 5% concentration (72% of the uncut control values as compared to 18% in the
202 corresponding 2.5% sorbitol control; Fig. 2C). STM-VENUS levels did not increase in response
203 to sorbitol, indicating that the effects of sucrose were not osmotic. Similar results were obtained

204 for the single STM-VENUS reporter line (Fig. S2C). To test if the observed effects were
205 transcriptional, we monitored the activity of the *pSTM::TFP-N7* transcriptional reporter.
206 Quantification of the *pSTM::TFP-N7* signal revealed no significant repression of *STM* promoter
207 activity upon inflorescence excision and incubation in darkness (Supplementary Fig. S2D),
208 consistent with the results obtained by qRT-PCR in intact plants (Fig. 1E). In addition, incubation
209 in sucrose or sorbitol-containing media had minor effects on TFP levels (Supplementary Fig. S2E)
210 as compared to STM-VENUS (Fig. 2C), with the most severe condition (2.5% sorbitol) leading to
211 65% of the signal in the uncut control as compared to the 18% of the equivalent STM-VENUS
212 samples. This indicates that the effect of sugar deprivation on *STM* levels does not rely on
213 transcriptional regulation of *STM*.

214

215 **The SnRK1 sugar sensor is expressed in the SAM and influences STM levels**

216 One major component of sugar signaling is the SnRK1 protein kinase, that is activated under
217 conditions of low energy and is conversely repressed by sugars (45). Given its well-established
218 role as a sugar sensor and the increasing number of studies implicating SnRK1 in developmental
219 processes (37, 39), we next investigated whether SnRK1 could be involved in the regulation of
220 SAM function through *STM*. To this end, we used a line expressing SnRK1 α 1-GFP under the
221 control of the *SnRK1 α 1* promoter and other gene regulatory regions (46). A clear SnRK1 α 1-GFP
222 signal was detected in the SAM, showing a stronger intensity in the peripheral regions, and
223 developing organs (Fig. 3A). To further confirm this expression and to assess whether SnRK1 α 1
224 might be enriched in the SAM relative to other organs of the plant, we extracted total proteins from
225 rosettes and shoot apices of 6 to 7-week-old plants and compared the relative levels of SnRK1 α 1
226 by immunoblotting (Fig. 3B). For the same amount of total protein, shoot apices contained higher
227 amounts of SnRK1 α 1 suggesting that SnRK1 is relatively more abundant in the SAM than in
228 rosette leaves.

229 To test if SnRK1 is functional in the meristem, we used SAMs dissected from HL- or dark-
230 treated plants (48 h) to measure the activity of the SnRK1 signaling pathway using the expression
231 of downstream target genes (45) as a readout (Fig. 3C). Under control conditions, SnRK1-
232 regulated genes were barely expressed, consistent with the pathway being inactive. However, after
233 48 h of darkness, a marked upregulation of these genes was observed (Fig. 3C), indicating an
234 activation of SnRK1 signaling in the SAM. The induction of SnRK1-regulated genes in darkness

235 was accompanied by a reduction in total SnRK1 α 1 levels (Fig. 3D), consistent with a tight coupling
236 between SnRK1 activity and degradation (46), and by an increase in the relative phosphorylation
237 of the SnRK1 α 1 (T-loop) that is essential for SnRK1 activity (45).

238 To investigate whether SnRK1 is involved in STM regulation, we introgressed the
239 *pSTM::STM-VENUS* reporter construct into a line overexpressing SnRK1 α 1 [*35S::SnRK1 α 1*,
240 hereafter referred to as *SnRK1 α 1-OE*; (47)] and monitored STM-VENUS levels in different light
241 conditions. When plants were grown under HL, the levels of STM-VENUS in the *SnRK1 α 1-OE*
242 were 60% of those in control plants (Fig. 3E), a decrease that could not be explained by differences
243 in *STM* transcript levels (Supplementary Fig. S3). However, the differences between the two
244 genotypes became smaller when plants were grown in LL (STM-VENUS levels in *SnRK1 α 1-OE*
245 were 77% of those in HL plants; Fig. 3E) and negligible when subjected to a 48 h dark treatment
246 (STM-VENUS levels in *SnRK1 α 1-OE* were 97% of those in control plants; Fig. 3F). STM-
247 VENUS levels thus appeared to be constitutively low and largely insensitive to the light conditions
248 in *SnRK1 α 1-OE* plants. This contrasted with control plants which, in response to restrictive light
249 conditions, reduced STM-VENUS accumulation to levels equivalent to those of the *SnRK1 α 1-OE*.
250 Lower STM-VENUS levels in the *SnRK1 α 1-OE* in HL could not be explained by lower sugar
251 accumulation, as these plants had a higher content of sucrose, glucose, and fructose both in the
252 SAM (Fig. 3G) and rosettes (Supplementary Fig. S4), although the differences were not always
253 statistically significant due to the large variation of the *SnRK1 α 1-OE* samples. The levels of Tre6P,
254 known to inhibit SnRK1 activity (48–50), were also markedly higher in the *SnRK1 α 1-OE* SAMs
255 (5.6-fold) and rosettes (5-fold), consistent with previous observations in *SnRK1 α 1-OE* rosettes
256 (51). During the dark treatment, however, all sugars were largely depleted, reaching similarly low
257 levels in control and mutant samples.

258 Altogether these results suggest that SnRK1 is active in the SAM and that it contributes to
259 the adjustment of STM protein levels, inhibiting STM accumulation when sugar levels decline. To
260 further investigate the involvement of SnRK1 on STM regulation, we first used yeast-two-hybrid
261 (Y2H) assays to test if SnRK1 α 1 can interact directly with STM (Fig. 3H). We observed that yeast
262 co-expressing SnRK1 α 1 with STM were able to grow in selective medium but this was not the
263 case when SnRK1 α 1 or STM were expressed individually with the corresponding empty vector
264 controls, suggesting that these two proteins can interact. To determine if the SnRK1 α 1-STM
265 interaction can also occur *in planta*, we next performed co-immunoprecipitation (co-IP)

266 experiments using Col-0 mesophyll cell protoplasts expressing SnRK1 α 1-HA with STM-GFP or
267 with GFP alone as a negative control. Immunoprecipitation with an anti-GFP antibody and
268 subsequent Western blot analyses revealed that SnRK1 α 1 interacts with STM-GFP (Fig. 3I), but
269 not with GFP alone, indicating that the interactions revealed by Y2H may also occur *in planta*.
270 Taken together, our results suggest that the impact of SnRK1 α 1 on STM accumulation may be due
271 to direct action of the SnRK1 kinase on STM in the meristem.

272

273 **Silencing *SnRK1 α* in the SAM reduces STM expression and disrupts meristem function**

274 To investigate further the possibility that SnRK1 acts locally in the meristem, we designed artificial
275 microRNAs (amiRNAs) targeting both *SnRK1 α 1* and *SnRK1 α 2* in two different regions of the
276 transcripts (*amiRa-1* and *amiRa-2*) and expressed these *amiRNAs* under the 5.7 kb promoter of
277 *STM* in *STM-VENUS* plants (Fig. 4A). Immunoblot analyses confirmed a decrease in the activated
278 form (phosphorylated in the T-loop) of SnRK1 α in all lines, but this was accompanied by a
279 decrease in total SnRK1 α 1 levels only in lines expressing *amiRa-2* (Fig. 4B).

280 To our surprise, depletion of SnRK1 α resulted in decreased STM-VENUS accumulation,
281 both at the protein (Fig. 4C-E) and transcript levels (Supplementary Fig. S5). The decrease in
282 STM-VENUS levels was strongest in the lines with more compromised SnRK1 α activity (*amiRa-*
283 *2* lines) and weaker in the lines where the impact on SnRK1 α activity was moderate or negligible
284 (*amiRa-1* lines; Fig. 4B-E). Lower STM accumulation in the SAM of the *SnRK1 α* amiRNA lines
285 correlated with defects in SAM development, including altered phyllotaxy, reduced bulging and
286 the appearance of bract-like structures in some floral meristems, as well as fusions between
287 adjacent floral meristems (Fig. 4F). Organ fusions were also visible later in development and
288 affected cauline and rosette leaves, petals, siliques, and stems (Fig. 5D,F). Consistent with a
289 previous report (21), STM depletion caused defects in boundary formation, manifested as a
290 decreased curvature at the boundary between the meristem and the incipient organ in the strong
291 *amiRa-2* lines (Supplementary Fig. S6).

292 All *amiRa* lines displayed defects in internode elongation, with an increasing frequency of
293 aberrantly long and aberrantly short internodes (Fig. 5A-B, G-H). Defects in internode elongation
294 resulted in clusters of siliques (Fig. 5A,B,E) and what appeared to be aerial rosettes on the main
295 inflorescence (Fig. 5C,D,F; Supplementary Fig. S7A; Table 1). These phenotypes have been linked
296 to reduced *STM* expression and function (14, 52–54) and, accordingly, they were more severe in

297 the *amiRa-2* plants, where the depletion of STM-VENUS is more pronounced (Fig. 4C-E). Plants
298 expressing *amiRa-2* also exhibited reduced apical dominance with one or two axillary meristems
299 often becoming activated well before flowering (39% and 28% of the *amiRa-2#1*, and *amiRa-2#2*
300 plants, respectively; $n=18$; Supplementary Fig. S7B-C). A less frequent termination of the main
301 meristem was also observed, after which, growth resumed from an axillary meristem (17% of
302 *amiRa-2#1* plants; $n=18$; Supplementary Fig. S7C). Plants expressing *pSTM::amiRa* were also
303 compared to the double reporter line as control (*pSTM::STM-VENUS/pSTM::TFP-N7*) to ensure
304 that the observed phenotypes were not caused by the introgression of an additional *STM* promoter
305 in the genome of the STM-VENUS line (Fig. 4C,D,F; Supplementary Fig. S7D).

306 Collectively, these results indicate that SnRK1 plays critical functions in meristem
307 organization and function and that this involves changes in *STM* expression.

308

309 DISCUSSION

310 The capacity to generate organs throughout development is crucial for plant adaptation to the
311 environment, enabling, amongst others, the replacement of leaves lost due to herbivory, the timing
312 of growth to a specific season, or the switch to flower production when conditions are propitious
313 for reproduction. However, how the SAM perceives environmental information and how this is
314 translated into changes in meristem activity are poorly understood.

315 Here we show that light promotes STM accumulation through sugars. First, a clear
316 correlation between STM-VENUS and SAM sugar levels was observed across different light
317 conditions. STM-VENUS levels were lower in LL-grown or dark-treated plants than in plants
318 grown and maintained under HL (Fig. 1A-C). A similar pattern was observed for sugar
319 accumulation in the inflorescence meristems (Fig. 2A), consistent with a previous report on the
320 impact of limiting photosynthetic rates, and thereby sugar supply to the sinks, on the growth and
321 development of reproductive organs and meristem function (55). Second, STM-VENUS levels
322 declined rapidly when inflorescences were excised from rosettes and this decline was similar in
323 inflorescences maintained in the light or transferred to dark, showing that light is not sufficient to
324 sustain STM levels in this system (Fig. 2B). The reason for this could be that light is sensed in
325 leaves from which a light-related signal travels to the apex and that this remote light sensing and
326 systemic communication is disrupted upon excision of the inflorescence. An alternative
327 explanation is that the signal regulating STM levels is not light itself, but rather photosynthesis-

328 derived sugars. The fact that the decline in STM-VENUS levels triggered by inflorescence
329 excision could be largely suppressed by supplementing sucrose in darkness, argues that sucrose is
330 sufficient to sustain STM-VENUS levels and that the effect of light observed in intact plants is
331 indirect *via* photosynthesis and sugar production. The impact of sucrose on STM is in line with
332 the reported effects of nutrients on *WUS* expression and on meristem function. Sugars contribute
333 to meristem activation by inducing *WUS* in young seedlings (33) and nitrogen promotes *WUS*
334 expression and meristem growth in the inflorescence *via* systemic CK signaling (13). However, in
335 contrast to *WUS*, for which transcriptional regulation plays a major role (13, 33), we could not
336 detect significant changes in *STM* transcript levels under our different growth conditions or
337 treatments (Fig. 1E and Supplementary Fig. S3), supporting that STM is regulated at the protein
338 level. Despite reports linking CK signaling to *STM* expression (11, 42), STM-VENUS levels did
339 not increase in excised meristems treated with CK (Supplementary Fig. S1D). Even though we did
340 not measure *STM* transcript accumulation under these conditions, this could mean that CK signals
341 may influence STM more indirectly, *e.g.* by affecting *WUS* expression and stem cell number (56).

342 The rescue of STM-VENUS levels by sucrose in excised inflorescences suggests that
343 sucrose is sensed locally in the meristem. This is in accordance with the enrichment and activity
344 of the SnRK1 sugar sensor in the SAM (Fig. 3A-D) and with the importance of meristematic
345 SnRK1 for SAM function (Fig. 4 and 5, Supplementary Fig. S6-7). Ubiquitous *SnRK1 α 1*
346 overexpression caused a reduction in STM-VENUS levels under HL conditions (Fig. 3E) despite
347 the high accumulation of soluble sugars and Tre6P in the rosettes and SAMs of the *SnRK1 α -OE*
348 plants (Fig. 3G). In addition, SnRK1 α 1 interacts physically with STM in yeast cells (Fig. 3H) and
349 mesophyll cell protoplasts (Fig. 3I), supporting a close functional connection with STM and a local
350 function for SnRK1 in the meristem.

351 Expressing *amiRs* targeting *SnRK1 α 1* and *SnRK1 α 2* under the control of the *STM* promoter
352 demonstrated that SnRK1 acts locally in the SAM and is crucial for the proper coordination of
353 meristem activities, with reduced SnRK1 activity causing a wide range of developmental defects
354 (Fig. 5). The severity of the phenotypes caused by SnRK1 α depletion contrasts with the apparent
355 lack of developmental defects of the *SnRK1 α -OE* line in our growth conditions. This is consistent
356 with previous studies in which SnRK1 α 1 overexpression causes mostly developmental delays
357 rather than defective organ development and arrangement (37, 39).

358 The finding that sucrose promotes STM-VENUS expression together with the fact that
359 sugars repress SnRK1 activity may at first sight appear to conflict with the decline in STM-
360 VENUS expression and abnormal meristem function caused by *SnRK1 α* silencing. However,
361 despite being generally considered a growth repressor, SnRK1 is also required for cell cycle
362 progression (57) and for normal growth and development (58). Indeed, transient *SnRK1 α 1/ α 2*
363 downregulation *via* virus-induced gene silencing leads to full growth arrest of plants (45) and
364 double *snrk1 α 1 snrk1 α 2* null mutants could thus far not be recovered, suggesting that complete
365 loss of SnRK1 α is embryo lethal (59). A similar duality is observed for the AMP-activated protein
366 kinase (AMPK), the homologue of SnRK1 in animals. Despite serving as a brake for cell
367 proliferation through downregulation of TOR activity (60), AMPK is also essential for normal
368 growth and development. For example, complete loss of the AMPK β 1 subunit leads to cell cycle
369 defects in neural stem and progenitor cells, causing profound abnormalities in brain development
370 in mice (61). Along the same lines, hematopoietic stem cell function in mammals is disrupted both
371 upon inactivation and overactivation of TOR signaling, indicating that a fine balance of this central
372 regulator is required for coordinating proliferation, differentiation, and regeneration (62).

373 The effects of light, sucrose and SnRK1 α 1 overexpression on STM indicate that the
374 underlying mechanisms do not rely on changes in *STM* transcript abundance (Fig. 1-3).
375 Furthermore, the fact that the SnRK1 α 1 and STM proteins interact (Fig. 3H-I) suggests that the
376 impact of SnRK1 on STM may be direct. The consequences of *SnRK1 α* silencing, on the other
377 hand, reveal a more complex scenario, involving reduced accumulation of both the STM transcript
378 and protein, and severe developmental abnormalities. The phenotypes of the *amiRa* lines are
379 highly reminiscent of those described for partial STM loss-of-function (14, 15, 21, 63), including
380 organ fusions, altered phyllotaxy, defective internode elongation with clusters of siliques and
381 leaves and, much less frequently, premature SAM termination (Table 1; Fig. 5; Supplementary
382 Fig. S7). However, whether the impact of SnRK1 α on STM in this case is direct or indirect through
383 other factors requires further investigation. It is plausible that *SnRK1 α* silencing causes hormonal
384 imbalance and/or alterations in the cell cycle that feed back to *STM* expression.

385 Altogether, our work demonstrates that sucrose promotes STM accumulation and that this
386 is counteracted by the SnRK1 sugar sensor, likely to adjust SAM activity to the environment (Fig.
387 6). Nevertheless, SnRK1 is also essential for meristem integrity, adding to the evidence that

388 SnRK1 performs a dual function in the regulation of growth and that its activity needs to be finely
389 balanced.

390

391

392 MATERIALS AND METHODS

393 A list of all primers, antibodies and plant lines used in this study is provided in Supplementary
394 Table 1. Protein extraction and quantification, immunoblotting, RNA extraction, cDNA synthesis,
395 qRT-PCR, sugar measurements, yeast-two-hybrid assays, and protoplast assays were carried out
396 according to protocols described in *SI Materials and Methods*.

397

398 Plant material

399 All *Arabidopsis thaliana* (L.) Heynh. plants used here are in the Columbia (Col-0) background.
400 The *pSTM::STM-VENUS* line (*STM-VENUS*) was generated by transforming Col-0 plants with the
401 plasmid described by Heisler and colleagues (13, 18). The *pTCSn::GFP* line was provided by
402 Bruno Müller (43). The *SnRK1α1-GFP* [*pSnRK1α1::SnRK1α1-GFP::terSnRK1α1/snrk1α1-3*;
403 (46)] and *SnRK1α1-OE* [*35S::SnRK1α1*; (47)] lines were previously described. For expression of
404 *STM-VENUS* in the *SnRK1α1-OE* background, the *SnRK1α1-OE* and *STM-VENUS* lines were
405 crossed, and homozygous progeny was selected on kanamycin and BASTA. For generating the
406 *pSTM::STM-VENUS/pSTM::TFP-N7* line and the *pSTM::amiRa* lines, *STM-VENUS* plants were
407 transformed with a construct to express TFP-N7 or an amiRNA targeting both *SnRK1α1* and
408 *SnRK1α2* (*amiRa-1* or *amiRa-2*) under the *STM* promoter (5.7 kb). Detailed descriptions of the
409 cloning strategy and progeny selection are provided in *SI Materials and Methods*.

410

411 Plant growth conditions

412 For most experiments, seedlings were initially grown in short-day conditions (8 h/16 h light/dark
413 period) for 3-4 weeks and then transferred to continuous light (24 h light, temperature: 22°C) and
414 kept under an irradiance of 60 (LL) or 170 (HL) $\mu\text{mol m}^{-2} \text{s}^{-1}$, provided by white fluorescent
415 lamps. Unless otherwise indicated, plants were grown in HL conditions. For dark treatment, plants
416 grown under HL were put into the dark for 24, 48 or 72 h. For the assays with excised
417 inflorescences and *STM-VENUS* imaging of *amiRa* lines, seedlings were grown in short-day

418 conditions (8 h/16 h light/dark period) for 3-4 weeks and then transferred to long-day conditions
419 (16 h/8 h light/dark period; 22°C/18°C).

420 For experiments involving SAM imaging, gene expression or protein analyses, plants were
421 grown in the indicated conditions until bolting, after which SAMs were dissected at the beginning
422 of the flowering stage when the main inflorescence reached 3-5 cm in height.

423 For phenotyping the *amiRa* lines, seeds were germinated, and plants grown under equinoctial
424 conditions (12 h/12 h light/dark period; 100-110 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 22°C/18°C). Phenotypes were
425 scored when flowering was completed [stage 6.90; (64)].

426

427 **SAM imaging and quantification**

428 For meristem imaging, the main inflorescence meristem of plants at the beginning of the flowering
429 stage was cut 1-2 cm from the tip, dissected under a binocular stereoscopic microscope to remove
430 all the flowers down to stage 3 [as defined in (65)] and transferred to a box containing Arabidopsis
431 apex culture medium without sucrose (ACM: 2.2 g/l Duchefa Biochemie ([www.duchefa-](http://www.duchefa-biochemie.com)
432 [biochemie.com](http://www.duchefa-biochemie.com))-MS basal salt mixture with vitamins, pH adjusted to 5.8 with KOH, and 1.6%
433 (w/v) agarose added).

434 For the time-lapse experiments examining the effect of exogenous CK, meristems were
435 dissected from HL-grown plants and placed in a box of ACM with 1% (w/v) sucrose and 500 nM
436 BAP or the equivalent volume of the BAP solvent (DMSO) as control. Meristems were thereafter
437 returned to the constant HL cabinet for the indicated times and covered with water for imaging.

438 For the experiments examining the effect of exogenous sugar, inflorescences were
439 dissected at about 3 cm from the apex from HL-grown plants at the beginning of the flowering
440 stage and placed in a 2 mL Eppendorf tube containing 2 mL of liquid ACM (no sucrose) covered
441 with parafilm pierced with a needle so that the inflorescence could be held in air while the base of
442 the stem was submerged in the solution, supplemented with the indicated concentrations of sucrose
443 or sorbitol as osmotic control. Sorbitol and sucrose were used at roughly equivalent concentrations,
444 with 1% sorbitol and 2% sucrose corresponding to 54 mM and 58 mM, respectively. Inflorescences
445 were thereafter kept in darkness inside the growth cabinet for the indicated times. Meristems were
446 then dissected from the excised inflorescences, transferred to a box containing the same sucrose-
447 or sorbitol-supplemented solid (1.6% agarose) medium and covered with water for imaging.

448 Meristems were imaged in water using a 20X long-distance water-dipping objective
449 mounted either on a LSM880 (Zeiss; www.zeiss.com) or a SP8 (Leica; www.zeiss.com) confocal
450 microscope. Z-stacks of 1-2 μm spacing were taken and the spacing was kept constant within a
451 single experiment.

452 Confocal Z stacks were analyzed using the ImageJ software (<https://fiji.sc/>) and a custom-
453 made code written in Matlab (Mathworks Inc., Natick, MA). ImageJ was used for generating the
454 panel figures showing the fluorescence reporters in the SAMs. To generate the panels, z-
455 projections (sum slices) of meristems expressing TFP, GFP or VENUS were performed, and the
456 Fire color code was used to represent the signal. The expression levels of the different fluorescence
457 reporters were analyzed by using the Matlab code (see
458 <https://gitlab.com/sluc/teamHJ/pau/RegionsAnalysis>). *pTCSn::GFP* reporter was analyzed by
459 using a previously described pipeline (13), which measures total fluorescence intensity of circular
460 expression domains. STM-VENUS signal and TFP signal were analyzed by using a new pipeline
461 that consists on the following. Firstly, a z-sum intensity projection is performed followed by a
462 gaussian blur with a smoothing kernel with standard deviation $\sigma = 5 \mu\text{m}$. Second, a region of
463 interest (SAM core excluding the emerging floral organs) is drawn on the image projection and
464 the mean intensity of the region is extracted. The mean intensity was chosen as a measure of the
465 STM-VENUS and TFP levels instead of the total intensity to minimize the influence of manually
466 drawing the region of interest and the new buds on the signal.

467 To measure the correlation between the folding of the boundary and the size of the primordia
468 in *pSTM::amiRa-1* and *amiRa-2* lines, maximal projections were performed to outline the stage-2
469 primordia (65) and measure their projected area. Longitudinal sections passing through the middle
470 of the primordia were also performed to measure the folding of the boundary using the angle tool
471 of ImageJ as previously described (21). The relationship between primordia size and the folding
472 of the boundary appeared to be more linear for smaller primordia. Therefore, only stage-2
473 primordia up to $4000 \mu\text{m}^2$ in size were considered for these measurements.

474

475

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488

489 **FIGURE LEGENDS**

490 **Figure 1. Effect of light on STM expression.** A-C, STM-VENUS expression in SAMs of
491 *pSTM::STM-VENUS* plants grown under high light (HL) or low light (LL) conditions or
492 transferred from HL to darkness (D) or kept under HL for the indicated times. **A**, Representative
493 STM-VENUS images of SAMs from HL and LL-grown plants and of plants transferred to D for
494 48 h. Scale bar, 50 μ m. **B**, **C**. Quantification of STM-VENUS signal. **B**, Plots show SAM
495 measurements of plants grown as 3 independent batches normalized by the mean of the HL
496 condition of each batch (HL, $n=44$; LL, $n=45$). Student's *t*-test (*p*-value shown). **C**, Plots show
497 SAM measurements of plants grown as 2-3 independent batches normalized by the mean of the
498 HL condition of each batch (0h, $n=18$; 24 h L, $n=19$; 24 h D, $n=18$; 48 h L, $n=19$; 48 h D, $n=18$;
499 72 h L, $n=9$; 72 h D, $n=12$). Different letters indicate statistically significant differences (Kruskal-
500 Wallis with Dunn's test; $p<0.05$). **D**, Immunoblot analyses of STM protein levels in SAMs of
501 *pSTM::STM-VENUS* plants grown under HL or LL conditions or grown in HL and transferred to
502 D for 48 h. TUBULIN (TUB) and Ponceau staining serve as loading controls. Numbers refer to
503 mean STM-VENUS amounts in LL and D as compared to HL ($n=2$; each a pool of 5 SAMs; in
504 parenthesis, SEM). **E**, RT-qPCR analyses of *STM* and *STM* target genes *AIL7* and *HB25* in SAMs
505 of *pSTM::STM-VENUS* plants grown in HL and transferred to D or kept in HL for 48 h. Graphs
506 show the average of 3 independent samples, each consisting of a pool of 5 SAMs. Paired ratio *t*-
507 test (*p*-values shown).

508

509 **Figure 2. Effect of sugars on STM levels.** **A**, Effect of light on the levels of soluble sugars in
510 SAMs of *pSTM::STM-VENUS* plants grown in high light (HL) and transferred to darkness (D) or
511 kept in HL for 48 h. Suc, sucrose; Tre6P, trehalose 6-phosphate; Glc, glucose; Fru, fructose. Plots
512 show measurements of 5-6 samples, each consisting of a pool of 5 SAMs from plants grown as 2
513 independent batches. Welch's *t*-test (*p*-value shown). **B**, Effect of light on STM-VENUS levels in
514 cut inflorescences. Inflorescences of *pSTM::STM-VENUS/pSTM::TFP-N7* plants grown under HL
515 were cut and placed in medium without sugar for 48 h under HL (L) or dark (D) conditions, after
516 which the SAMs were dissected and imaged (VENUS). Upper panel, representative STM-VENUS
517 images of SAMs. Scale bar, 50 μ m. Lower panel, plots showing SAM measurements of plants
518 grown as 1-2 independent batches normalized by the mean of the uncut condition of each batch
519 (uncut, *n*=31, 2 batches; 48 h L, *n*=14, 1 batch; 48 h D, *n*=21, 2 batches). Different letters indicate
520 statistically significant differences (Kruskal-Wallis with Dunn's test; *p*<0.05). **C**, Effect of sugar
521 on STM-VENUS levels in cut inflorescences. Inflorescences of *pSTM::STM-VENUS/pSTM::TFP-*
522 *N7* plants grown under HL condition were cut and placed under darkness for 48 h in medium with
523 sucrose (Suc; 2% and 5%) or sorbitol (Sor; 1% and 2.5%) as osmotic control. SAMs were
524 thereafter dissected and imaged (VENUS). Upper panel, representative STM-VENUS images of
525 SAMs. Scale bar, 50 μ m. Lower panel, plots showing SAM measurements of plants grown as 1-3
526 independent batches normalized by the mean of the uncut condition of each batch (uncut, *n*=31, 3
527 batches; 1% Sor, *n*=21, 2 batches; 2% Suc, *n*=28, 3 batches; 2.5% Sor, *n*=7, 1 batch; 5% Suc, *n*=6,
528 1 batch). Different letters indicate statistically significant differences (Kruskal-Wallis with Dunn's
529 test; *p*<0.05). The same batches of HL-grown uncut plants served as controls for the experiments
530 shown in (B) and (C).

531
532 **Figure 3. SnRK1 is expressed in the SAM and affects STM response to light.** **A**, SnRK1 α 1-
533 GFP imaging in the SAM. Right panel, SAM longitudinal section. Scale bars, 50 μ m. **B**,
534 Immunoblot analyses of SnRK1 α 1 in SAMs and rosette leaves of *pSTM::STM-VENUS* plants
535 grown under high light (HL). 35 μ g of total protein was loaded from SAM and leaf extracts.
536 Ponceau staining serves as loading control. Similar results were obtained from two independent
537 experiments. **C**, RT-qPCR analyses of SnRK1 signaling marker genes (*DIN10*, *SEN5*, *DRM2*) in
538 SAMs of *pSTM::STM-VENUS* plants grown in HL and transferred to darkness (D) or kept in HL
539 for 48 h. Graphs show the average of 3 independent samples, each consisting of a pool of 5 SAMs.

540 Paired ratio *t*-test (*p*-values shown). **D**, Left, representative immunoblot of SnRK1 α T-loop
541 phosphorylation in SAMs of the plants described in **(C)**, using antibodies recognizing the T175
542 phosphorylation (phospho-SnRK1 α) or the total SnRK1 α protein. Right, quantification of the
543 mean SnRK1 α phosphorylation (phospho-SnRK1 α /total SnRK1 α) in D as compared to the ratio
544 in HL (*n*=3; each a pool of 5 SAMs). Paired ratio *t*-test (*p*-value shown). **E-F**, STM-VENUS
545 expression in SAMs of control and *SnRK1 α 1-OE* plants grown under HL or low light (LL)
546 conditions (**E**) or grown under HL and transferred to darkness (D) or kept under HL for 48 h (**F**).
547 The same batches of HL-grown plants served as controls for the experiments shown in (**E**) and
548 (**F**). HL and LL-grown STM-VENUS samples are replotted from Fig. 1B as a reference. Plots
549 show SAM measurements of plants grown as 3 independent batches normalized by the mean of
550 the HL condition of each batch (control, HL: *n*=44, LL: *n*=45, D: *n*=45; *SnRK1 α 1-OE*, HL: *n*=45,
551 LL: *n*=45; D: *n*=45). Different letters indicate statistically significant differences (Kruskal-Wallis
552 with Dunn's test; *p*<0.05). **G**, Effect of light on the levels of sugars in SAMs of *SnRK1 α 1-OE*
553 plants as compared to the control. Suc, sucrose; Tre6P, trehalose 6-phosphate; Glc, glucose; Fru,
554 fructose. Plots show measurements of 5-6 samples, each consisting of a pool of 5 SAMs from
555 plants grown as 2 independent batches. Welch's *t*-test (mutant vs. control for each condition; *p*-
556 values shown). **H**, Yeast-two hybrid assays examining the interaction of SnRK1 α 1 with STM.
557 Protein interaction was determined by monitoring yeast growth in medium lacking Leu, Trp and
558 His (-L-W-H) compared with control medium only lacking Leu and Trp (-L-W). Upper panel,
559 yeast growth in cells co-expressing AD-STM, with BD-SnRK1 α 1. Lower panel, negative controls
560 of yeast transfected with the indicated AD/BD-constructs and the complementary BD/AD-empty
561 vectors. BD and AD, DNA binding and activation domains of the GAL4 transcription factor,
562 respectively. Increasing dilutions of transformed yeast cells are shown (10^{-1} , 10^{-2} , 10^{-3}).
563 Experiments were performed three times with similar results. **I**, Co-immunoprecipitation (co-IP)
564 experiments using Arabidopsis Col-0 mesophyll cell protoplasts co-expressing SnRK1 α 1-HA with
565 STM-GFP or GFP alone. GFP-tagged proteins were immunoprecipitated and co-
566 immunoprecipitation of SnRK1 α 1 was assessed by immunoblotting with an HA antibody.
567 Arrowheads, STM-GFP (upper) and GFP (lower). Experiments were performed three times with
568 similar results.
569

570 **Figure 4. Silencing *SnRK1α* in the SAM compromises STM expression.** **A.** Schematic
571 localization of *amiRa-1* and *amiRa-2* target sites (grey triangles) in the *SnRK1α1* and *SnRK1α2*
572 transcripts. Yellow blocks correspond to exons. **B.** Immunoblot analyses of SnRK1α T-loop
573 phosphorylation in SAMs of plants expressing *pSTM::amiRa-1* (*amiRa-1*) or *pSTM::amiRa-2*
574 (*amiRa-2*), using antibodies recognizing total SnRK1α1 or SnRK1α phosphorylated on T175
575 (phospho-SnRK1α). Ponceau staining serves as loading control. Numbers refer to mean SnRK1α1
576 amounts or mean SnRK1α phosphorylation (phospho-SnRK1α/total SnRK1α1) in the *amiRa* lines
577 relative to the control *STM-VENUS* line ($n=2$; each a pool of 5 SAMs; in parenthesis, SEM). **C.**
578 Representative meristems expressing *STM-VENUS* together with *pSTM::amiRa-1*,
579 *pSTM::amiRa-2* or *pSTM::TFP-N7* as a control, and whose membranes were labelled with FM4-
580 64. Left panels show the sum-slice projections of the *STM-VENUS* signal (color-coded using the
581 Fire representation in ImageJ), and the right panels, the sum-slice projection of the FM4-64
582 signal. Scale bars, 50 μm. *P1* and *P2*, youngest visible and older flower primordia, respectively. **D.**
583 Quantification of the *STM-VENUS* signal in the SAMs shown in (C). Plots show SAM
584 measurements of plants grown as 2 independent batches (except *amiRa-1#2*, which grown as a
585 single batch) normalized by the mean of the control line of each batch (control, $n=34$; *amiRa-2#1*,
586 $n=16$; *amiRa-2#2*, $n=22$; *amiRa-1#1*, $n=25$; *amiRa-1#2* $n=10$). Different letters indicate
587 statistically significant differences (Kruskal-Wallis with Dunn's test; $p<0.05$). **E.** Immunoblot
588 analyses of *STM-VENUS* protein levels in SAMs of the *amiRa* lines as compared to the *STM-*
589 *VENUS* control using antibodies recognizing STM. TUBULIN (TUB) and Ponceau staining serve
590 as loading controls. Numbers refer to mean *STM-VENUS* amounts in the *amiRa* lines as compared
591 to the control ($n=2$; each a pool of 5 SAMs; in parenthesis, SEM). **F.** Sum-slice projection of
592 control line and *amiRa-2* showing additional defects in meristem organization. Red arrows point
593 at bract-like structures while yellow arrows point at fusions between adjacent floral primordia.
594 Scale bars: 50 μm.

595
596 **Figure 5. Silencing of *SnRK1α* in the SAM affects meristem function and plant architecture.**
597 **A-F,** Representative images of control (*STM-VENUS*, **A,C**) and *amiRa* (**B,D,E,F**) plants showing
598 irregular internode length (**A,B**), clusters of leaves (**C,D,F**) and siliques (**A,B,E**), and termination
599 of the main inflorescence (**F**) in the *amiRa* lines. Insets show organ fusion between leaves of an
600 aerial rosette (**D**) and between pedicels and the stem (**F**). **G-H,** Quantification of the internode

601 length defects in control and two independent lines of *amiRa-1* and *amiRa-2* mutants. Internode
602 length was determined by measuring the length of the internodes between paraclades (**G**) and
603 between the first 12 siliques (**H**), all counted acropetally. Internode length was scored in the
604 indicated size ranges from the main inflorescence of 18 plants of each genotype. Graphs show the
605 relative frequencies of each size class in the total number of internodes scored. All phenotypes
606 were scored from plants grown under equinoctial conditions until the completion of flowering.

607

608 **Figure 6. Model for the role of sugars and SnRK1 signaling in the SAM.** Under favorable
609 conditions, basal SnRK1 activity is required for meristem organization, with local *SnRK1α*
610 silencing causing reduced *STM* expression and severe phenotypes related to SAM dysfunction.
611 The mechanisms underlying these SnRK1 effects remain unknown (question mark). Under
612 limiting light conditions or other unfavorable situations, sugar levels decrease, leading to a strong
613 activation of SnRK1 signaling. This results in decreased STM protein accumulation, potentially
614 through direct action of SnRK1 α 1 on STM to reduce SAM activity and growth.

615

616 **Table 1.** Quantitative analyses of *amiRa* phenotypes. Measurements were taken from the main
617 inflorescence of 18 plants of the *amiRa* lines and the *STM-VENUS* control line after flowering was
618 completed. Numbers are averages and SD. *p*-values refer to differences between each mutant and
619 the control (Kruskal-Wallis with Dunn's test).

620

621 SUPPLEMENTARY FIGURE LEGENDS

622 **Supplementary Figure S1. Effect of cytokinin on STM levels.** **A**, Representative GFP images
623 from *pTCSn::GFP* SAMs from plants grown under high light (HL; 170 $\mu\text{mol m}^{-2} \text{s}^{-1}$) or low light
624 (LL; 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$) conditions and of plants transferred from HL to darkness (D) for 48 h. Scale
625 bar, 50 μm . **B**, GFP quantification from SAMs of *pTCSn::GFP* plants grown under HL or LL
626 conditions. Plots show SAM measurements of plants grown as 3 independent batches normalized
627 by the mean of the HL condition of each batch (HL: $n=44$, LL: $n=41$). Student's *t*-test (*p*-values
628 shown). **C**, GFP quantification from SAMs of *pTCSn::GFP* plants grown in HL and transferred to
629 D or kept under HL for 48 h. Plots show SAM measurements of plants grown as 3 independent
630 batches normalized by the mean of the HL condition of each batch (HL, $n=44$; 48 h D, $n=45$).
631 Student's *t*-test (*p*-values shown). **D**, Effect of cytokinin (BAP) application on the expression of

632 the STM-VENUS reporter. SAMs of plants grown under HL conditions were excised and placed
633 in medium supplemented or not with 500 nM BAP for the indicated times. Plots show SAM
634 measurements of plants grown as 2 independent batches normalized by the mean of the uncut (0h)
635 condition of each batch ($n=14$ for each of the indicated conditions). Different letters indicate
636 statistically significant differences (Kruskal-Wallis with Dunn's test; $p<0.05$). **E**, Effect of
637 cytokinin (BAP) application on the activity of the *pTCSn::GFP* reporter. SAMs of plants grown
638 under HL conditions were excised and placed in medium supplemented or not with 500 nM BAP
639 for the indicated times. Plots show SAM measurements of plants grown as 2 independent batches
640 normalized by the mean of the uncut (0h) condition of each batch (all 0 nM BAP conditions, $n=12$;
641 0h 500 nM BAP, $n=12$; 24 h 500 nM BAP, $n=10$; 48 h 500 nM BAP, $n=10$). Different letters
642 indicate statistically significant differences (Kruskal-Wallis with Dunn's test; $p<0.05$).

643

644 **Supplementary Figure S2. Effect of sugar on STM promoter activity. A**, Effect of light on the
645 levels of soluble sugars in rosettes of *pSTM::STM-VENUS* plants grown in high light (HL) and
646 transferred to darkness (D) or kept in HL for 48 h. Suc, sucrose; Tre6P, trehalose 6-phosphate;
647 Glc, glucose; Fru, fructose. Plots show measurements of 6 whole rosettes from plants grown as
648 one batch. Welch's *t*-test (p -value shown). **B**, Effect of light on STM-VENUS levels in cut
649 inflorescences. Inflorescences of *pSTM::STM-VENUS* plants grown under HL were cut and placed
650 in medium without sugar for 48 h under HL (L) or dark (D) conditions, after which the SAMs were
651 dissected and imaged (VENUS). Upper panel, representative STM-VENUS images of SAMs.
652 Scale bar, 50 μ m. Lower panel, plots showing SAM measurements of plants grown as one batch
653 normalized by the mean of the uncut condition (uncut, $n=7$; 48 h L, $n=7$; 48 h D, $n=7$). Different
654 letters indicate statistically significant differences (Kruskal-Wallis with Dunn's test; $p<0.05$). **C**,
655 Effect of sugar on STM-VENUS levels in cut inflorescences. Inflorescences of *pSTM::STM-*
656 *VENUS* plants grown under HL condition were cut and placed under darkness for 48 h in medium
657 with 2% sucrose or 1% sorbitol as osmotic control. SAMs were thereafter dissected and imaged
658 (VENUS). Upper panel, representative STM-VENUS images of SAMs. Scale bar, 50 μ m. Lower
659 panel, plots showing SAM measurements of plants grown as one batch normalized by the mean of
660 the uncut condition (uncut, $n=7$; 1% Sor, $n=7$; 2% Suc, $n=7$). Different letters indicate statistically
661 significant differences (Kruskal-Wallis with Dunn's test; $p<0.05$). The same batches of HL-grown
662 uncut plants served as controls for the experiments shown in **(B)** and **(C)**. **D**, Effect of light on

663 *STM* promoter activity in cut inflorescences. Inflorescences of *pSTM::STM-VENUS/pSTM::TFP-*
664 *N7* plants grown under HL were treated as in (B) and dissected SAMs were imaged (TFP). Upper
665 panel, representative *pSTM::TFP-N7* images of SAMs. Scale bar, 50 μm . Lower panel, plots
666 showing SAM measurements of plants grown as 2 independent batches (except 48h L samples,
667 which generated from a single batch) normalized by the mean of the *uncut* condition of each batch
668 (*uncut*, $n=17$; 48 h L, $n=7$; 48 h D, $n=14$). Different letters indicate statistically significant
669 differences (Kruskal-Wallis with Dunn's test; $p<0.05$). **E**, Effect of sugar on *STM* promoter
670 activity in cut inflorescences. Inflorescences of *pSTM::STM-VENUS/pSTM::TFP-N7* plants
671 grown under HL were cut and placed under darkness for 48 h in medium with sucrose (Suc; 2%
672 and 5%) or sorbitol (Sor; 1% and 2.5%) as osmotic control. SAMs were thereafter dissected and
673 imaged (TFP). Upper panel, representative *pSTM::TFP-N7* images of SAMs. Scale bar, 50 μm .
674 Lower panel, plots showing SAM measurements of plants grown as 2-3 independent batches
675 (except 2.5% Sor and 5% Suc, which were grown as a single batch) normalized by the mean of the
676 *uncut* condition of each batch (*uncut*, $n=24$; 1% Sor, $n=14$; 2% Suc, $n=21$; 2.5% Sor, $n=7$; 5% Suc,
677 $n=6$). Different letters indicate statistically significant differences (Kruskal-Wallis with Dunn's
678 test; $p<0.05$).

679

680 **Supplementary Figure S3. Effect of ubiquitous SnRK1 α 1 overexpression on *STM* levels.** RT-
681 qPCR analyses of *STM* in SAMs of control and *SnRK1 α 1-OE* plants grown under high light
682 conditions ($170 \mu\text{mol m}^{-2} \text{s}^{-1}$). Graphs correspond to the average of 3 independent samples, each
683 consisting of a pool of 5 SAMs. Paired ratio *t*-test (*p*-value shown).

684

685 **Supplementary Figure S4. Effect of ubiquitous SnRK1 α 1 overexpression on the**
686 **accumulation of soluble sugars.** Control and *SnRK1 α 1-OE* plants were grown under HL
687 conditions and transferred to darkness (D) or kept under HL for 48 h. Plots show measurements of
688 6 whole rosettes from plants grown as one batch, with *SnRK1 α 1-OE* values expressed in
689 comparison to the control. Suc, sucrose; Tre6P, trehalose 6-phosphate. Glc, glucose; Fru, fructose.
690 Welch's *t*-test (mutant vs. control for each condition; *p*-value shown).

691

692 **Supplementary Figure S5. Effect of SnRK1 α depletion in the SAM on *STM* levels.** RT-qPCR
693 analyses of *STM* in SAMs of control and *pSTM::amiRa* plants grown under high light conditions

694 (170 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Two independent lines of *pSTM::amiRa-2* (*amiRa-2#1* and *amiRa-2#2*) and
695 *pSTM::amiRa-1* (*amiRa-1#1* and *amiRa-1#2*) were used. Graph shows measurements from 2
696 independent samples, each consisting of a pool of 5 SAMs.

697

698 **Supplementary Figure S6. Effect of SnRK1 α depletion in the SAM on organ boundary**

699 **formation. A,** Representative meristems expressing *STM-VENUS* together with *pSTM::amiRa-1*,

700 *pSTM::amiRa-2* or *pSTM::TFP-N7* as a control, and whose membranes were labelled with FM4-

701 64. Left panels show the sum-slice projection of the FM4-64 signal and the right panels, two

702 longitudinal sections showing the SAM-organ boundary of two stage-2 primordia. Note the

703 reduced folding of the boundary and reduced bulging of the primordia in the *pSTM::amiRa-2* line.

704 Scale bars, projections: 50 μm , sections: 20 μm . **B,** Folding angle of the boundary as a function of

705 the size of the primordium in control plants (expressing *pSTM::TFP-N7*) as compared to *amiRa-2*

706 (upper graph) or *amiRa-1* plants (lower graph). Plants in the *amiRa-2* experiment were grown as

707 3 independent batches. Control, $n=126$ primordia from 29 SAMs; *amiRa-2#1*, $n=121$ primordia

708 from 25 SAMs; *amiRa-2#2*, $n=120$ primordia from 26 SAMs). Plants in the *amiRa-1* experiment

709 were grown as 2 independent batches. Control, $n=95$ primordia from 22 SAMs; *amiRa-1#1*, $n=84$

710 primordia from 22 SAMs; *amiRa-1#2*, $n=87$ primordia from 21 SAMs. Data were fitted using a

711 linear regression (see Methods). #1 and #2 denote two independent lines for the indicated *amiRs*.

712

713 **Supplementary Figure S7. Silencing of SnRK1 α in the SAM compromises meristem function**

714 **and plant architecture. A,** Representative images of plants expressing *STM-VENUS* (control)

715 together with *pSTM::amiRa-1* or *pSTM::amiRa-2* and grown under equinoctial conditions until

716 the completion of flowering. **B-C,** Representative images of 22d-old plants of the most severely

717 affected *amiRa* line (*amiRa-2#1*) showing activation of axillary meristems before flowering (red

718 arrows in **B-C**) and termination of the main meristem (white arrow in **C**). **D,** Representative images

719 of plants expressing *STM-VENUS* together with *pSTM::TFP-N7* (control), *pSTM::amiRa-1*, or

720 *pSTM::amiRa-2*, and grown under short-day conditions for 3 weeks and then transferred to long-

721 day conditions until the completion of flowering. #1 and #2 denote two independent lines for the

722 indicated *amiRs*.

723

724

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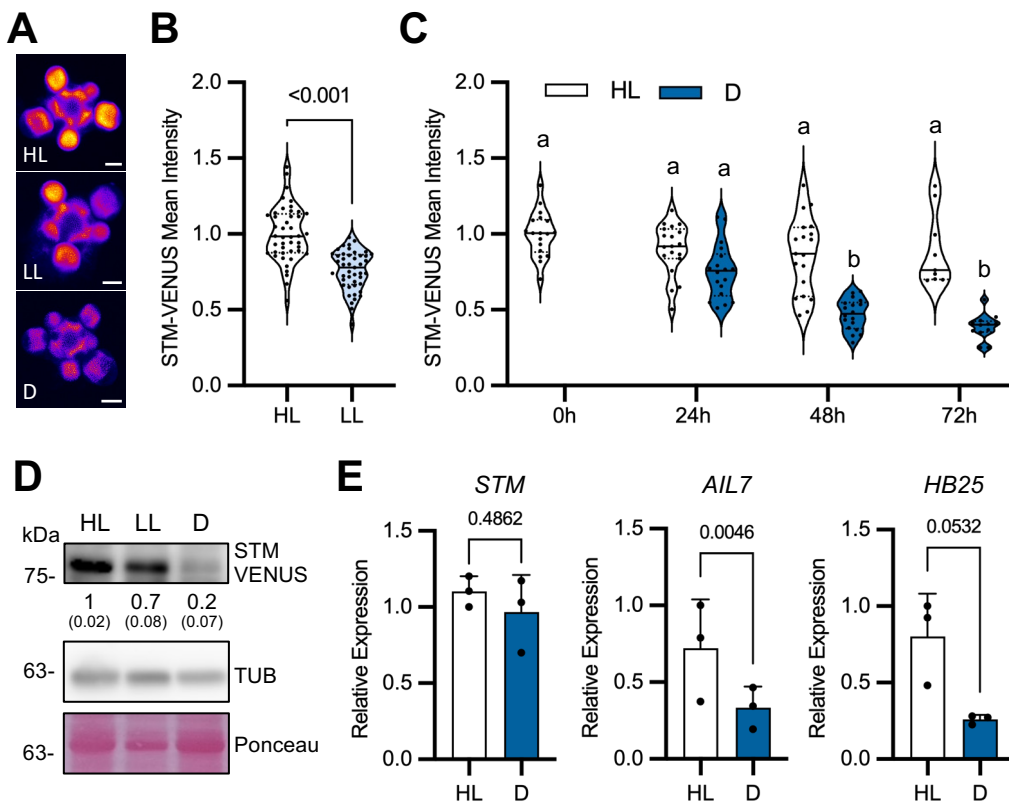


Figure 1. Effect of light on STM expression. A-C, STM-VENUS expression in SAMs of *pSTM::STM-VENUS* plants grown under high light (HL) or low light (LL) conditions or transferred from HL to darkness (D) or kept under HL for the indicated times. **A**, Representative STM-VENUS images of SAMs from HL and LL-grown plants and of plants transferred to D for 48 h. Scale bar, 50 μ m. **B**, **C**. Quantification of STM-VENUS signal. **B**, Plots show SAM measurements of plants grown as 2-3 independent batches normalized by the mean of the HL condition of each batch (HL, $n=44$; LL, $n=45$). Student's *t*-test (*p*-value shown). **C**, Plots show SAM measurements of plants grown as 3 independent batches normalized by the mean of the HL condition of each batch (0h, $n=18$; 24 h L, $n=19$; 24 h D, $n=18$; 48 h L, $n=19$; 48 h D, $n=18$; 72 h L, $n=9$; 72 h D, $n=12$). Different letters indicate statistically significant differences (Kruskal-Wallis with Dunn's test; $p < 0.05$). **D**, Immunoblot analyses of STM protein levels in SAMs of plants grown under HL or LL conditions or grown in HL and transferred to D for 48 h. TUBULIN (TUB) and Ponceau staining serve as loading controls. Numbers refer to mean STM-VENUS amounts in LL and D as compared to HL ($n=2$; each a pool of 5 SAMs; in parenthesis, SEM). **E**, RT-qPCR analyses of *STM* and *STM* target genes *AIL7* and *HB25* in SAMs of *pSTM::STM-VENUS* plants grown in HL and transferred to D or kept in HL for 48 h. Graphs show the average of 3 independent samples, each consisting of a pool of 5 SAMs. Paired ratio *t*-test (*p*-values shown).

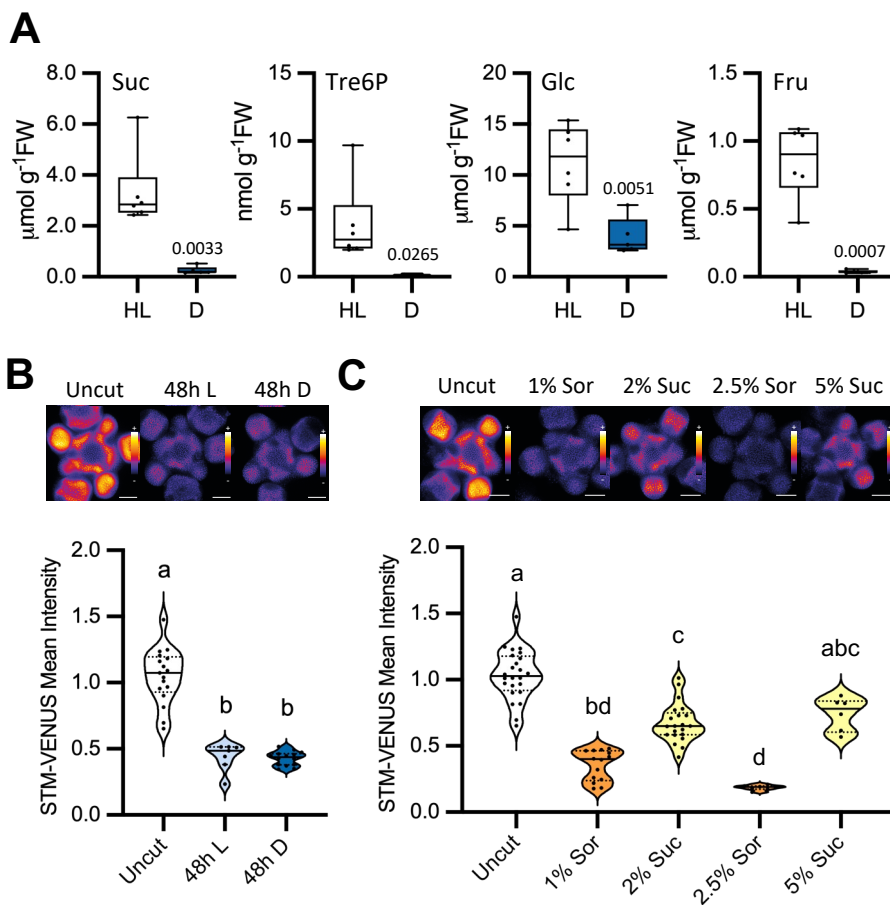


Figure 2. Effect of sugars on STM levels. **A**, Effect of light on the levels of soluble sugars in SAMs of *pSTM::STM-VENUS* plants grown in high light (HL) and transferred to darkness (D) or kept in HL for 48 h. Suc, sucrose; Tre6P, trehalose 6-phosphate; Glc, glucose; Fru, fructose. Plots show measurements of 5-6 samples, each consisting of a pool of 5 SAMs from plants grown as 2 independent batches. Welch's *t*-test (*p*-value shown). **B**, Effect of light on STM-VENUS levels in cut inflorescences. Inflorescences of *pSTM::STM-VENUS/pSTM::TFP-N7* plants grown under HL were cut and placed in medium without sugar for 48 h under HL (L) or dark (D) conditions, after which the SAMs were dissected and imaged (VENUS). Upper panel, representative STM-VENUS images of SAMs. Scale bar, 50 μm . Lower panel, plots showing SAM measurements of plants grown as 1-2 independent batches normalized by the mean of the uncut condition of each batch (uncut, $n=31$, 2 batches; 48 h L, $n=14$, 1 batch; 48 h D, $n=21$, 2 batches). Different letters indicate statistically significant differences (Kruskal-Wallis with Dunn's test; $p<0.05$). **C**, Effect of sugar on STM-VENUS levels in cut inflorescences. Inflorescences of *pSTM::STM-VENUS/pSTM::TFP-N7* plants grown under HL condition were cut and placed under darkness for 48 h in medium with sucrose (Suc; 2% and 5%) or sorbitol (Sor; 1% and 2.5%) as osmotic control. SAMs were thereafter dissected and imaged (VENUS). Upper panel, representative STM-VENUS images of SAMs. Scale bar, 50 μm . Lower panel, plots showing SAM measurements of plants grown as 1-3 independent batches normalized by the mean of the uncut condition of each batch (uncut, $n=31$, 3 batches; 1% Sor, $n=21$, 2 batches; 2% Suc, $n=28$, 3 batches; 2.5% Sor, $n=7$, 1 batch; 5% Suc, $n=6$, 1 batch). Different letters indicate statistically significant differences (Kruskal-Wallis with Dunn's test; $p<0.05$). The same batches of HL-grown uncut plants served as controls for the experiments shown in (B) and (C).

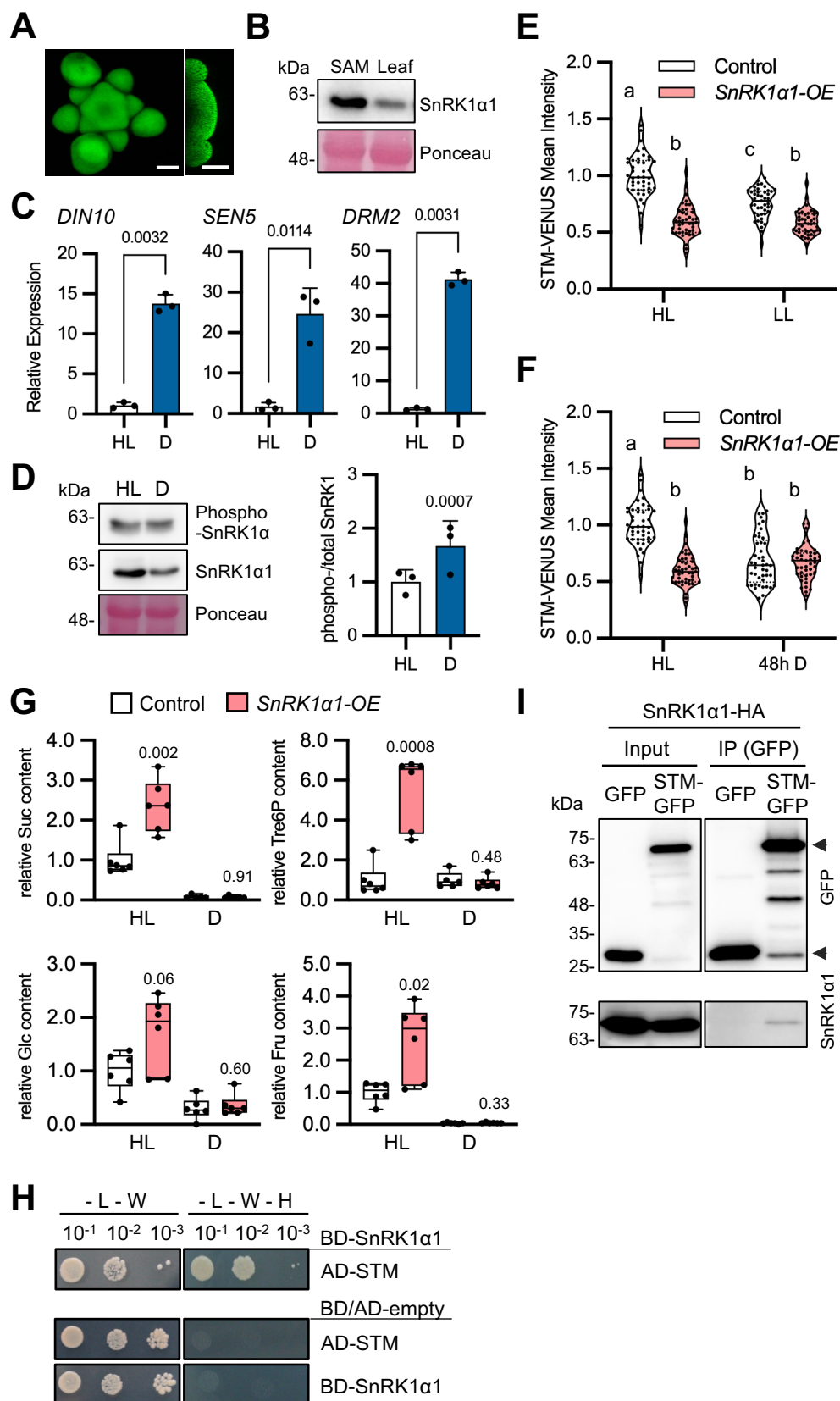


Figure 3. SnRK1 is expressed in the SAM and affects STM response to light. **A**, SnRK1 α 1-GFP imaging in the SAM. Right panel, SAM longitudinal section. Scale bars, 50 μ m. **B**, Immunoblot analyses of SnRK1 α 1 in SAMs and rosette leaves of *pSTM::STM-VENUS* plants grown under high light (HL). 35 μ g of total protein was loaded from SAM and leaf extracts. Ponceau staining serves as loading control. Similar results were obtained from two independent experiments. **C**, RT-qPCR analyses of SnRK1 signaling marker genes (*DIN10*, *SEN5*, *DRM2*) in SAMs of *pSTM::STM-VENUS* plants grown in HL and transferred to darkness (D) or kept in HL for 48 h. Graphs show the average of 3 independent samples, each consisting of a pool of 5 SAMs. Paired ratio *t*-test (*p*-values shown). **D**, Left, representative immunoblot of SnRK1 α 1 T-loop phosphorylation in SAMs of the plants described in (C), using antibodies recognizing the T175 phosphorylation (phospho-SnRK1 α) or the total SnRK1 α 1 protein. Right, quantification of the mean SnRK1 α phosphorylation (phospho-SnRK1 α /total SnRK1 α) in D as compared to the ratio in HL (*n*=3; each a pool of 5 SAMs). Paired ratio *t*-test (*p*-value shown). **E-F**, STM-VENUS expression in SAMs of control and *SnRK1 α 1-OE* plants grown under HL or low light (LL) conditions (E) or grown under HL and transferred to darkness (D) or kept under HL for 48 h (F). The same batches of HL-grown plants served as controls for the experiments shown in (E) and (F). Control HL and LL-grown STM-VENUS samples are replotted from Fig. 1B as a reference. Plots show SAM measurements of plants grown as 3 independent batches normalized by the mean of the control HL condition of each batch (control, HL: *n*=44, LL: *n*=45, D: *n*=45; *SnRK1 α 1-OE*, HL: *n*=45, LL: *n*=45; D: *n*=45). Different letters indicate statistically significant differences (Kruskal-Wallis with Dunn's test; *p*<0.05). **G**, Effect of light on the levels of sugars in SAMs of *SnRK1 α 1-OE* plants as compared to the control. Suc, sucrose; Tre6P, trehalose 6-phosphate; Glc, glucose; Fru, fructose. Plots show measurements of 5-6 samples, each consisting of a pool of 5 SAMs from plants grown as 2 independent batches. Welch's *t*-test (mutant vs. control for each condition; *p*-values shown). **H**, Yeast-two hybrid assays examining the interaction of SnRK1 α 1 with STM. Protein interaction was determined by monitoring yeast growth in medium lacking Leu, Trp and His (-L-W-H) compared with control medium only lacking Leu and Trp (-L-W). Upper panel, yeast growth in cells co-expressing AD-STM, with BD-SnRK1 α 1. Lower panel, negative controls of yeast transfected with the indicated AD/BD-constructs and the complementary BD/AD-empty vectors. BD and AD, DNA binding and activation domains of the GAL4 transcription factor, respectively. Increasing dilutions of transformed yeast cells are shown (10^{-1} , 10^{-2} , 10^{-3}). Experiments were performed three times with similar results. **I**, Co-immunoprecipitation (co-IP) experiments using Arabidopsis Col-0 mesophyll cell protoplasts co-expressing SnRK1 α 1-HA with STM-GFP or GFP alone. GFP-tagged proteins were immunoprecipitated and co-immunoprecipitation of SnRK1 α 1 was assessed by immunoblotting with an HA antibody. Arrowheads, STM-GFP (upper) and GFP (lower). Experiments were performed three times with similar results.

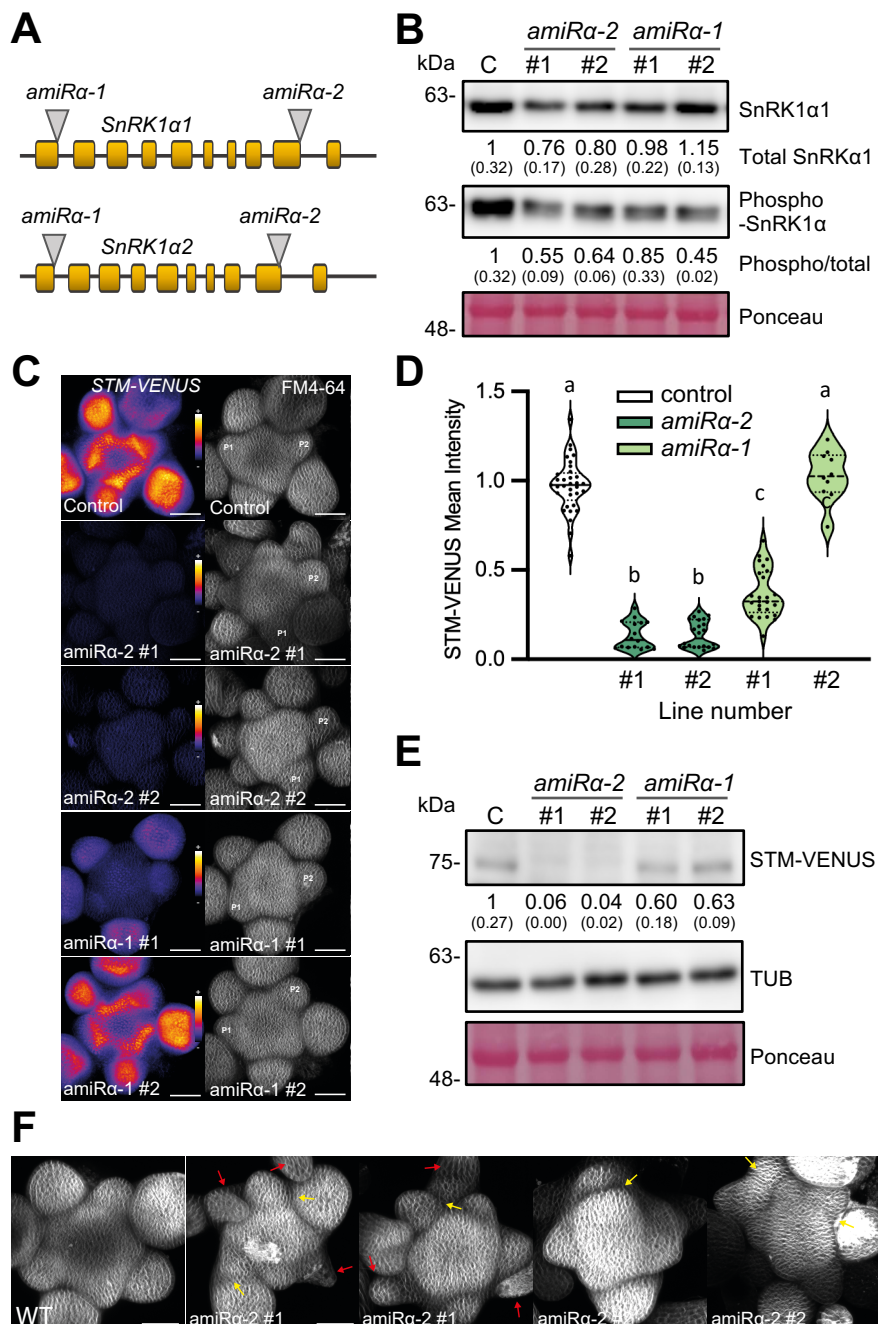


Figure 4. Silencing *SnRK1α* in the SAM compromises STM expression. **A**, Schematic localization of *amiRa-1* and *amiRa-2* target sites (grey triangles) in the *SnRK1α1* and *SnRK1α2* transcripts. Yellow blocks correspond to exons. **B**, Immunoblot analyses of SnRK1α T-loop phosphorylation in SAMs of plants expressing *pSTM::amiRa-1* (*amiRa-1*) or *pSTM::amiRa-2* (*amiRa-2*), using antibodies recognizing total SnRK1α1 or SnRK1α phosphorylated on T175 (phospho-SnRK1α). Ponceau staining serves as loading control. Numbers refer to mean SnRK1α1 amounts or mean SnRK1α phosphorylation (phospho-SnRK1α/total SnRK1α1) in the *amiRa* lines relative to the control *STM-VENUS* line ($n=2$; each a pool of 5 SAMs; in parenthesis, SEM). **C**, Representative meristems expressing *STM-VENUS* together with *pSTM::amiRa-1*, *pSTM::amiRa-2* or *pSTM::TFP-N7* as a control, and whose membranes were labelled with FM4-64. Left panels show the sum-slice projections of the *STM-VENUS* signal (color-coded using the Fire representation in ImageJ), and the right panels, the sum-slice projection of the FM4-64 signal. Scale bars, 50 μm . *P1* and *P2*, youngest visible and older flower primordia, respectively. **D**, Quantification of the *STM-VENUS* signal in the SAMs shown in (C). Plots show SAM measurements of plants grown as 2 independent batches (except *amiRa-1#2*, which grown as a single batch) normalized by the mean of the control line of each batch (control, $n=34$; *amiRa-2#1*, $n=16$; *amiRa-2#2*, $n=22$; *amiRa-1#1*, $n=25$; *amiRa-1#2* $n=10$). Different letters indicate statistically significant differences (Kruskal-Wallis with Dunn's test; $p<0.05$). **E**, Immunoblot analyses of *STM-VENUS* protein levels in SAMs of the *amiRa* lines as compared to the *STM-VENUS* control using antibodies recognizing STM. TUBULIN (TUB) and Ponceau staining serve as loading controls. Numbers refer to mean *STM-VENUS* amounts in the *amiRa* lines as compared to the control ($n=2$; each a pool of 5 SAMs; in parenthesis, SEM). **F**, Sum-slice projection of control line and *amiRa-2* showing additional defects in meristem organization. Red arrows point at bract-like structures while yellow arrows point at fusions between adjacent floral primordia. Scale bars: 50 μm .

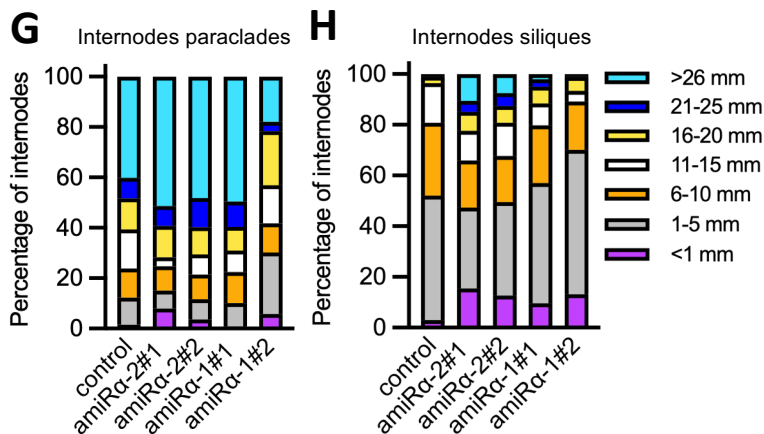


Table I. Number of organs per stem node in *amiRa* plants

| Line | Siliques per node | p-value | Leaves per node | p-value |
|------------------|-------------------|---------|-----------------|---------|
| control | 1.4 ± 0.5 | | 1.9 ± 0.8 | |
| <i>amiRa-2#1</i> | 2.3 ± 0.8 | <0.0001 | 3.8 ± 1.7 | <0.0001 |
| <i>amiRa-2#2</i> | 2.2 ± 0.5 | <0.0001 | 3.6 ± 1.5 | <0.0001 |
| <i>amiRa-1#1</i> | 2.0 ± 0.5 | <0.0001 | 2.8 ± 1.3 | <0.0001 |
| <i>amiRa-1#2</i> | 1.8 ± 0.7 | 0.0004 | 2.1 ± 0.9 | >0.9999 |

Figure 5. Silencing of *SnRK1α* in the SAM affects meristem function and plant architecture. A-F, Representative images of control (*STM-VENUS*, A,C) and *amiRa* (B,D,E,F) plants showing irregular internode length (A,B), clusters of leaves (C,D,F) and siliques (A,B,E), and termination of the main inflorescence (F) in the *amiRa* lines. Insets show organ fusion between leaves of an aerial rosette (D) and between pedicels and the stem (F). G-H, Quantification of the internode length defects in control and two independent lines of *amiRa-1* and *amiRa-2* mutants. Internode length was determined by measuring the length of the internodes between paraclades (G) and between the first 12 siliques (H), all counted acropetally. Internode length was scored in the indicated size ranges from the main inflorescence of 18 plants of each genotype. Graphs show the relative frequencies of each size class in the total number of internodes scored. All phenotypes were scored from plants grown under equinoctial conditions until the completion of flowering.

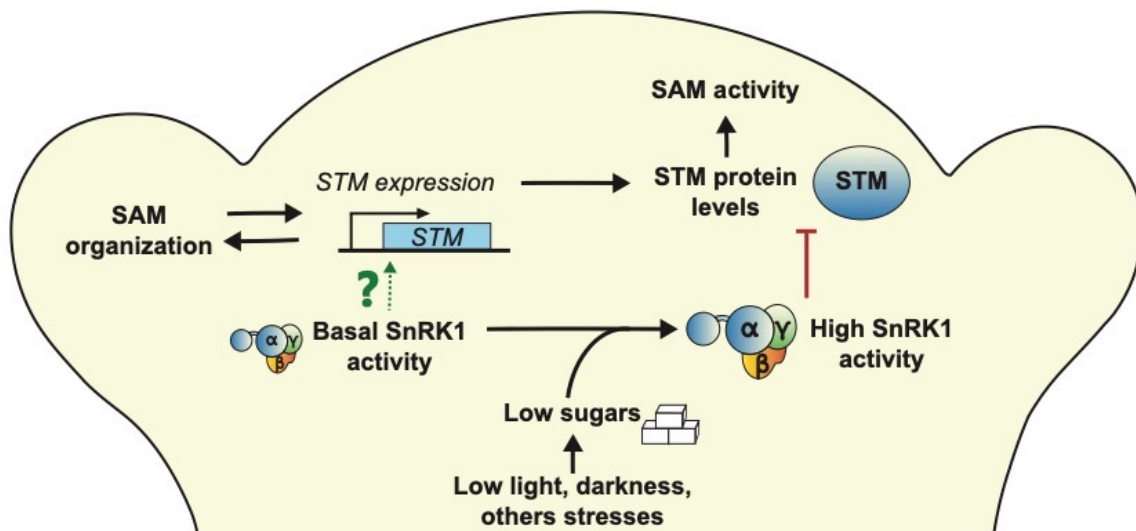


Figure 6. Model for the role of sugars and SnRK1 signaling in the SAM. Under favorable conditions, basal SnRK1 activity is required for meristem organization, with local *SnRK1a* silencing causing reduced *STM* expression and severe phenotypes related to SAM dysfunction. The mechanisms underlying these SnRK1 effects remain unknown (question mark). Under limiting light conditions or other unfavorable situations, sugar levels decrease, leading to a strong activation of SnRK1 signaling. This results in decreased STM protein accumulation, potentially through direct action of SnRK1 α 1 on STM to reduce SAM activity and growth.