Mechanics and growth coordination define SOSEKI-based polarity fields.

Marcel Piepers[1], Blanca Jazmin Reyes-Hernández[1], João R D Ramos[2,3] , Neva Bölke[1], Laura Schütz[1], Changzheng Song[1], Anamarija Primc[1], Lotte Bald[1], Roeland MH Merks[2, 3], and Alexis Maizel[1,*]

1: Center for Organismal Studies (COS), University of Heidelberg, Im Neuenheimer Feld 230, 69120 Heidelberg, Germany

2: Mathematical Institute, University Leiden, P.O. Box 9512, 2300 RA, Leiden, The Netherlands

3: Institute of Biology, Leiden University, P.O. Box 9505, 2300 RA, Leiden, The Netherlands

* Corresponding author: Alexis Maizel (alexis.maizel@cos.uni-heidelberg.de)

ORCIDs

MP 0000-0002-0422-7291

BJRH 0000-0001-9748-9751

JRDR 0009-0007-0714-7389

CS 0000-0001-8346-713X

AP 0009-0008-2844-6764

LB 0000-0002-9471-2716

RMHM 0000-0002-6152-687X

AM 0000-0001-6843-1059

Abstract

The formation of organs requires the coordinated growth of cells and tissues relative to the main body axes. Plant growth is typically anisotropic and mechanically coupled through contiguous cell walls, yet how these physical patterns link to cell and tissue polarity remains unclear. Here, we use SOSEKI (SOK) proteins—previously thought to report global polarity fields—as markers to dissect how polarity arises during lateral root (LR) organogenesis. Live imaging revealed that SOK polarisation does not follow a uniform global field but instead responds to local differences in growth and mechanical state between neighbouring tissues. SOKs accumulate at interfaces separating domains of distinct growth behaviour and dissipate under compressive stress. After perturbing tissue interfaces or the mechanical continuity of the tissue, the coherence of the polarity was disrupted, indicating that stable axis establishment requires mechanical coupling across the cell wall network. Our results suggest that SOK polarisation is mechanoresponsive, linking tissue mechanics to polarity and axis establishment.

Main Text

Multicellular development requires the coordinated growth of cells, tissues, and organs relative to the major body axes. In plants, organ growth is characterised by the preservation of contiguous cell wall contacts, is typically anisotropic, and produces specific shapes. While animals rely on canonical polarity proteins, these have not been identified yet in plants, which employ alternative strategies, including auxin transport, cell wall–mediated mechanical continuity, and geometry-based cues. The local orientations of anisotropic expansion, known as the principal directions of growth (PDGs), are inherently linked to organ shape and mechanics. PDGs provide a geometric framework for how growth patterns can inform division orientation and tissue architecture (Hejnowicz & Romberger, 1984). Yet, the molecular mechanisms that connect PDGs to division orientation remain largely unknown.

SOSEKI (SOK) proteins represent a deeply conserved family of polar proteins present throughout land plants. Their polarisation depends on an N-terminal DIX domain that mediates concentration-dependent polymerisation, enabling the formation of discrete polar domains at cell edges. This polymerisation is not merely structural: it provides the high local concentration required to recruit effector proteins such as *ANGUSTIFOLIA*, thereby linking polarity landmarks to cellular functions. Remarkably, DIX-dependent polymerisation is shared with animal polarity proteins such as Dishevelled (Schwarz-Romond *et al*, 2007) and SAR-group

57 “Musketeer” proteins (Kostareli *et al*, 2025), underscoring an ancient and conserved
58 biochemical principle of polarity establishment across kingdoms (van Dop *et al*, 2020). In
59 *Arabidopsis thaliana*, SOKs are expressed in the embryo and proliferating regions of the
60 primary and lateral roots (LRs), and have been proposed to interpret global polarity cues and
61 influence cell division orientation locally (Yoshida *et al*, 2019). However, the nature of the cues
62 that determine SOK localisation is unclear.

63 The post-embryonic formation of lateral organs, such as LRs, requires the
64 respecification of growth and cell division along axes orthogonal to those of the main organ.
65 The growth of these lateral organs is also characterised by unsteady growth fields, in which
66 growth anisotropy varies in direction or between adjacent regions, potentially creating
67 mechanical conflicts (Szymanowska-Pułka et al., 2012). It is unknown when and how the
68 polarity fields are established upon the formation of the new organ axes. In *Arabidopsis*, LRs
69 originate from xylem pole pericycle (XPP) cells that maintain cell proliferation in the
70 differentiation zone and act as founder cells (Dubrovsky *et al*, 2001). LR development follows
71 a stereotyped series of stages (Malamy & Benfey, 1997). Upon initiation (LRI), activated
72 founder cells radially swell and divide asymmetrically to generate a packet of small central
73 cells flanked by larger ones (Fig. 1a, Stage I; (Vermeer *et al*, 2014; Vilches Barro *et al*, 2019;
74 Torres-Martínez *et al*, 2020)). These cells then divide periclinally, forming the two-layered
75 Stage II primordium (Malamy & Benfey, 1997; von Wangenheim *et al*, 2016; Schütz *et al*,
76 2021) (Fig. 1a). Subsequent stages involve continued division and growth, progressively
77 shaping the primordium into an organisation comparable to the primary root. During this
78 process, anisotropic expansion predominates in the central domain (provascular domain),
79 while the surrounding ground tissue expands less, resulting in continuous deformation of the
80 primordium (Malamy & Benfey, 1997; von Wangenheim *et al*, 2016). In the primary root apex,
81 whose geometry remains steady, SOK proteins localise to polar cell edges, faithfully reporting
82 the shoot–root and radial axes that align with the principal directions of growth in a stable
83 growth field (Nakielski, 2008). By contrast, in the emerging LR, SOKs polarise to edges
84 oriented along the new shoot–root and radial axes, which are orthogonal to those of the parent
85 root (Yoshida *et al*, 2019) (Fig. 1a). How SOK polarity is established and reoriented during the
86 formation of new organ axes, and what cues determine this respecification, remains unknown.
87 Here, we show that local mechanical and geometric cues, rather than a global field, define
88 coherent SOK-based polarity fields in developing LRs.

89 To investigate the emergence of the SOK polarity fields during LR formation, we
90 mapped the polarisation of SOK1, SOK2, and SOK5 from founder cells activation to LR

91 emergence. Expressed from their native promoters, these three SOKs could all be detected
92 during LR development, albeit with distinct expression strengths and domains (Yoshida *et al*,
93 2019). To ensure reliable tracking of polarity across all cells, we complemented these with
94 RPS5A-driven reporters (Weijers *et al*, 2001) for SOK1, SOK2, and SOK5, as well as an XPP-
95 specific (Vilches Barro *et al*, 2019) SOK1 reporter to assess pre-activation polarisation.
96 Importantly, these misexpression constructs did not alter cell division orientation in the root
97 meristem or LR development, and polarisation remained unperturbed (Fig. S1a, b). These
98 validated reporters enabled us to track the polarisation of SOK1, SOK2, and SOK5 from
99 activation to Stage II (Fig. 1a–c, Fig. S2). In XPP cells, all three proteins polarised as in the
100 primary root meristem, integrating the parent root polarity field (Fig. 1b XPP; schematic in Fig.
101 1c). Upon founder cell activation and radial swelling, however, their polarisation diverged:
102 SOK1 shifted to the edges away from the founder-founder interface and to the periclinal face
103 toward the endodermis, SOK2 concentrated at the first cell wall formed after the founder cell
104 division, and SOK5 decorated edges flanking this interface and the vasculature-facing
105 periclinal edge (Fig. 1b, founder cells). After the first asymmetric division (Stage I), SOK1's
106 divergent polarisation became even more pronounced (Fig. 1b, Stage I, schematic in Fig. 1c).
107 These changes mark the first symmetry-breaking event, where diverging polarity fields replace
108 the parental SOK pattern of XPP centred around the central cell wall. A second symmetry-
109 breaking occurred during the transition to Stage II, when periclinal divisions created a two-
110 layered primordium. Here, SOK1 polarized toward the new periclinal interface between inner
111 and outer cells, SOK2 formed a cross-like pattern marking both the first cell wall formed after
112 the founder cell division and periclinal interfaces, and SOK5 likewise accumulated at the
113 periclinal interface (Fig. 1b, Stage II; schematic in Fig. 1c). Flanking cells that had not yet
114 divided retained their earlier polarisation, highlighting the domain-specific reorganisation of
115 polarity (Fig. S2).

116 Because SOK polarisation in mature roots and embryos defines a polarity "code" for the
117 shoot–root and in–out axes (Yoshida *et al*, 2019), we next asked whether the early symmetry-
118 breaking events already establish these axes (Fig. 1d). In mature roots, endogenous SOK1
119 marks the shoot-out edge, while SOK2 and SOK5 localise to the root-in edge. At Stage II,
120 however, their axial patterns were inconsistent: SOK1 converged toward the new periclinal
121 interface, whereas SOK2 and SOK5 diverged away from it (Fig. 1d). Thus, although both
122 shoot-root and radial information can be detected, the combined SOK patterns cannot be
123 reconciled into a coherent set of shoot–root and radial axes. These observations demonstrate
124 that, early on, SOK proteins do not obey a uniform global polarity field, suggesting that the
125 establishment of the LR axis requires a later maturation phase.

126 To understand how incoherent polarity patterns become progressively organised, we
127 tracked the polarisation of SOK1, SOK2, and SOK5 during stages III–VI of LR development
128 (Movie S1). Strikingly, despite the *pRPS5A* promoter driving uniform expression throughout
129 LR development (Fig. S3), the pRPS5A::SOK1/2/5:YFP signal was not evenly distributed.
130 While polarisation was stable in individual cells, it differed between tissue domains (Fig. 2a).
131 Fluorescence intensity was consistently enriched near the interface separating provascular
132 and ground tissue precursors, corresponding to the prospective pericycle–endodermis (PE)
133 interface (Fig. 2a, Movie S2). To quantify this, we processed light-sheet and confocal datasets
134 spanning stage I to emergence using a custom pipeline that identified the positions of 1,388
135 SOK signal maxima across 462 images, yielding a density map of SOK signal maxima. This
136 analysis confirmed that all three SOK reporters preferentially accumulated near the PE
137 interface throughout LR organogenesis (Fig. 2b). Interestingly, each SOK displayed a slightly
138 different localisation bias. pRPS5A::SOK1:YFP enriched toward ground tissue interfaces,
139 consistent with its known attraction to the endodermis–cortex junction in the root apical
140 meristem (Yoshida *et al*, 2019). pRPS5A::SOK2:YFP accumulation aligned more precisely
141 with the PE interface itself, while pRPS5A::SOK5:YFP was enriched in provascular cells
142 directly adjacent to the PE interface (Movie S1). Together, these patterns suggest that the PE
143 interface constitutes a consistent attractor for SOK polarisation, with each family member
144 responding to slightly differently within this region. Interestingly, the PE interface, which
145 corresponds to the periclinal interface specified upon the Stage I to II transition (Fig. S4, Movie
146 S2), separates the anisotropically growing provascular core from the more isotropic ground
147 tissue. Quantification of cell anisotropy across stages confirmed that growth anisotropy
148 progressively increased in the central provascular domain. In contrast, growth in the ground
149 tissue precursors remained isotropic (Fig. 2c). Importantly, SOK accumulation did not directly
150 correlate with anisotropy at the per-cell level (Fig. S5). Instead, SOKs accumulate near the
151 tissue boundary where domains of differential growth meet (Fig. 2b).

152 To test whether the domains of differential growth correspond to differences in
153 mechanical state, we next analysed cell wall properties and cytoskeletal organisation across
154 the developing primordium. Cell wall fluorescence lifetime imaging with the carbohydrate-
155 binding dye CarboTag-BDP (Besten *et al*, 2025), 2025), reporting cell wall network structure,
156 did not reveal evident spatial heterogeneity in wall environments of primordia Stages I to V
157 (Fig. S6). However, in later stages, lifetime quantification revealed significantly longer lifetimes
158 in central domains than in peripheral ones, suggesting differences in cell wall composition or
159 stiffness (Fig. 2d). This result echoes similar conclusions from the primary root (Alonso Baez
160 *et al*, 2025). We then examined cortical microtubule organisation as a proxy for mapping the

161 orientation of mechanical stresses (Verger *et al*, 2018; Vilches Barro, 2018) in developing LR
162 primordia (Fig. 2e). Quantification of the mean anisotropy-weighted microtubule orientation at
163 cell-level regions revealed a supracellular pattern of microtubule orientation (Fig. 2f). In the
164 central domain of stages IV and V, the MT orientation is perpendicular to the PDG, whereas
165 in stage VI, the MT orientation in the provascular domain changed and tended to align with
166 the PDG of the provascular domain, reflecting anisotropic growth in the provascular domain.
167 In contrast, peripheral cells displayed more isotropic microtubule arrays, consistent with their
168 reduced and directionally unconstrained expansion. Together, these data demonstrate that
169 the LR primordium is mechanically heterogeneous: the provascular domain is characterised
170 by denser walls and anisotropic stresses, whereas the surrounding ground tissue has less
171 dense walls and isotropic stresses. Thus, the provascular-ground tissue interface emerges as
172 a boundary between domains of differential growth and distinct mechanical states. This
173 suggests that SOK polarisation may be conditioned by local mechanical heterogeneity rather
174 than a uniform global polarity field.

175 To further explore the relationship between tissue mechanics and SOK polarisation,
176 we developed a mechanical model of the developing LR primordium (Fig. S7a). The model
177 was based on segmented experimental data from *pRPS5A::SOK2* reporter lines at stages IV–
178 VI and was used to calculate local strain distributions across the provascular, ground tissue,
179 and their intervening boundary zone. Importantly, the simulations did not impose any
180 predefined differences in cell-edge properties between regions, allowing the mechanical
181 response to emerge solely from tissue geometry that resulted from growth patterns. The
182 simulated strain distribution revealed lower strain values in the boundary region between the
183 provascular and ground tissue domains (Fig. 2g-i, Fig. S7b, c), demonstrating that tissue
184 geometry alone is sufficient to generate mechanical heterogeneity within the primordium and
185 highlighting this boundary as a mechanically distinct region. We then examined the correlation
186 between SOK2 signal intensity at individual cell edges and the corresponding local strain
187 magnitude (Fig. 2g, h, j, Fig. S7d). We observed a moderate, albeit significant, correlation
188 between the SOK signal and local mechanical strain at cell edges in the boundary and, to a
189 lesser extent, in the ground tissue regions, suggesting a link between polarity and the
190 mechanical landscape of the developing primordium.

191 Building on the observation that SOKs map to domains of differential growth and
192 mechanical state, we next asked how polarity responds when these tissues are subjected to
193 changes in mechanics. LR emergence provides a natural context for this as primordia
194 successively push through overlying tissue layers (Vilches-Barro & Maizel, 2015). The

195 endodermis acts as a mechanical barrier, generating compressive stress at stage II (Stöckle
196 *et al*, 2022). While the cortex and epidermis constrain the shape of the LR primordium (Lucas
197 *et al*, 2013), it remains to be established whether this occurs through compressive stress. To
198 address this, we ablated cortex cells overlying a stage IV primordium and observed an
199 immediate increase in primordium length (7µm in 5 seconds, Fig. S8a). This rapid elongation
200 indicates that cortical cells exert compressive stress. We next asked how SOK polarisation
201 responds to these stresses. Time-lapse imaging of pRPS5A::SOK5:YFP from stage II to
202 emergence revealed a striking correlation between SOK accumulation and growth (Fig. 3a;
203 Movie S3). SOK5 signal intensity increased during periods of elongation but sharply
204 decreased during phases of little or no growth, despite the *RPS5A* promoter being active
205 throughout organogenesis (Fig. S8b). The decline in signal coincided with developmental
206 windows during which the primordium encounters maximum compressive stress beneath the
207 endodermis (stage II) and the cortex (stage IV). A similar pattern was observed for
208 pRPS5A::SOK2:YFP, suggesting that dissipation under stress is a general feature of SOK
209 proteins (Fig. S8c). To confirm this interpretation, we relieved compressive stress by ablating
210 overlying cortex cells and imaged SOK5 signal dynamics. In ablated samples, the SOK5 signal
211 remained consistently high throughout organogenesis, with intensities approximately 3.5-fold
212 greater than in controls (Fig. 3b, Movie S3). To more precisely address the relationship
213 between changes in tissue mechanics induced by cell ablation and SOK polarisation, we
214 extended our mechanical model to simulate strain distribution in lateral root primordia following
215 removal of the overlying cortex and epidermis (Fig. 3c). Using the same mechanical
216 parameters as for intact primordia, the ablation model revealed a significant increase in strain
217 values, and an increased heterogeneity (larger variance) of strain values in periclinal walls
218 compared to non-ablated controls (Fig. 3d). These simulations demonstrate that releasing
219 external compression through ablation shifts mechanical load toward periclinal interfaces.
220 Interestingly, this shift is consistent with experimentally observed enhancement of SOK5
221 polarisation along these surfaces (Fig. 3e). Together, these results suggest that the
222 mechanoresponsive behaviour of SOKs reflects their ability to sense and align with the
223 redistribution of tensile forces within the primordium in response to mechanical perturbation.
224 If correct, a reduction of the tensile forces due to increased compressive forces should lead to
225 a reduction in SOK signal accumulation. To experimentally test this prediction, we perturbed
226 microtubules in the endodermis using the inducible driver–reporter system *pCASP1»PHS1ΔP*
227 to trigger endodermal microtubule depolymerisation, leading to a lack of endodermis thinning
228 and an increase in compressive stresses (Stöckle *et al*, 2022). Dexamethasone (DEX)
229 treatment triggered endodermal microtubule depolymerisation, verified by a marked loss of

230 MAP4:mVenus signal (Fig. 3f, Fig. S8d). In this background, pRPS5A::SOK5:YFP intensity
231 was significantly reduced compared to mock controls, indicating that compressive stress
232 generated by defective endodermal responses dissipates the SOK signal. Together, these
233 results establish that SOK proteins are polarity markers whose accumulation is
234 mechanoresponsive, linking the physical constraints of emergence to the dynamic regulation
235 of polarity fields in the LR primordium.

236 To test whether the mechanoresponsive behaviour of SOKs extends beyond the root,
237 we examined their localisation in the shoot apical meristem (SAM), an organ where tissue
238 dynamics generate heterogeneous stress patterns (Alonso-Serra *et al*, 2024; Shi *et al*, 2018;
239 Hamant *et al*, 2008; Uyttewaal *et al*, 2012). Despite uniform activity of the *pRPS5A* promoter
240 (Fig. S9a), pRPS5A::SOK1/2/5:YFP signals were specifically absent from creases near the
241 peripheral zone and at sites of incipient primordia (Fig. S9b). These regions are characterised
242 by minimal growth and elevated mechanical stress arising from organ outgrowth and boundary
243 formation. Thus, as in LR primordia, SOKs dissipate from mechanically stressed zones in the
244 SAM, suggesting that their stress response is a general feature of plant organogenesis rather
245 than an organ-specific phenomenon.

246 Our previous results showed that SOK polarity reflects mechanical heterogeneity and
247 is mechanoresponsive. We next asked how cell and tissue interfaces, as well as mechanical
248 continuity, contribute to the establishment and maintenance of SOK polarity patterns. We first
249 live-imaged gSOK1::YFP in response to the targeted ablation of the overlying endodermis at
250 Stage II. Removal of the endodermis resulted in a loss of edge polarity in the adjacent outer
251 central cells (Fig. 4a), a phenotype reminiscent of natural endodermal thinning during stage II,
252 which also coincides with reduced SOK1 polarity (Fig. 4b). These results indicate that the
253 endodermis is required not only as a structural contact but may also serve as a conduit for
254 transmitting mechanical cues that stabilise SOK1 polarity at adjacent cell edges. We then
255 asked whether the cell wall network within the primordium is also playing a role in SOK1
256 polarity. For this, we performed single-cell ablations in stage I and II primordia (Fig. 4c). Of 24
257 ablation events, half led to an immediate abortion of the primordium development. In contrast,
258 the others continued for some time but eventually arrested. In this group, the SOK1 signal was
259 frequently lost, although transient polarity reorientations were observed near the ablation site
260 (Fig. 4c, Fig. S10a). These results indicate that targeted alteration of the cell wall network is
261 sufficient to reconfigure polarity fields and alter organ-level behaviour. Because SOK
262 polarisation maps to the provascular–ground tissue boundaries, where domains of differential
263 growth and distinct mechanical states meet, we next asked whether altering root radial

264 patterning would affect SOK polarisation. To this end, we examined SOK1 polarity in the
265 *scarecrow* (*scr)* mutant, which is defective in proper ground tissue patterning and fails to
266 establish the outer layer during the stage II–III transition (Goh *et al*, 2016). In wild type, SOK1
267 polarity gradually shifts from the PE interface toward the ground tissue interface during stages
268 II–IV. In *scr* mutants, however, polarity was disrupted throughout the primordium, including
269 provascular cells normally unaffected by *SCR* function (Fig. 4d). This indicates that proper
270 patterning and differentiation of the ground tissue are required for long-range polarity
271 coherence. We can speculate that in the *scr* mutant, changes in the mechanical properties
272 and/or the topology of the tissue cell wall network alter the transmission of mechanical cues,
273 stabilising SOK polarity across the primordium.

274 We next focused on the role of the PE interface, which depends on the
275 PLETHORA3/5/7 (PLT3/5/7) transcription factors (Du & Scheres, 2017). In *plt3/5/7* triple
276 mutants, the PE interface is rarely formed, yet SOK1 maintained its divergent edge polarity in
277 stage I primordia unless the overlying endodermis was thinned, reiterating that the
278 endodermis-primordium interface affects SOK1 polarity (Fig. 4e). Remarkably, in rare *plt3/5/7*
279 primordia that underwent a periclinal division in some cells, SOK1, SOK2, and SOK5
280 repolarised to this new interface, resembling the wild type pattern (Fig. 4f early, Fig. S10b).
281 This wild-type-like pattern was absent in older primordia (Fig. 4f early, Fig. S10b), which were
282 characterised by more isotropically oriented cortical microtubules than in wild type and by
283 deformed cells growing isotropically (Fig. S10d). Together, these data suggest that, while the
284 transient establishment of a correct cellular layout can account for proper SOK polarisation
285 independent of specific cell identity, changes in growth behaviour lead to SOK repolarisation.
286 To investigate when mispolarisation occurred, we recorded pRPS5A::SOK2:YFP in *plt3/5/7*
287 and found that mispolarisation coincided with growth arrest, confirming the
288 mechanosresponsiveness of SOK accumulation (Fig. S10d). Together, these data show that
289 SOK polarity depends on the structural presence of cell wall networks and their mechanical
290 continuity. Disruption of interfaces, whether by ablation or genetic perturbation, redistributes
291 mechanical stresses and alters polarity at the organ level. In contrast, perturbations in growth
292 or geometry that preserve cell–cell contacts typically retain wild-type-like polarity. Thus, the
293 mechanical continuity of the cell wall network emerges as a key determinant of SOK polarity
294 during organogenesis.

295 Together, our results reveal that SOK proteins provide a molecular link between growth
296 fields and axis formation in plants. Rather than reflecting a uniform global field, SOK polarity
297 emerges from the physical architecture of tissues—interfaces, boundaries, and the continuity

of the cell wall network. These findings establish SOKs as sensitive reporters of mechanical organisation in living tissues and highlight how the coupling between mechanics and polarity underpins the self-organisation of plant organs. Future work should aim at clarifying SOSEKI proteins are direct mechanosensors or act downstream of mechanical signal transduction as well as elucidating the developmental functions of SOKs. This will be key to understanding how plants integrate mechanical information to coordinate growth, patterning, and morphogenesis.

305 **Methods**

306 **Plant material and growth conditions**

307 All experiments were performed in *Arabidopsis thaliana* (Col-0 background). An overview of
308 plant lines used is provided in Supplementary Table S1. SOSEKI marker lines (*gSOK1–*
309 *5::YFP*) were described previously (Yoshida *et al*, 2019) and were crossed with plasma
310 membrane marker lines (pUBQ10::LTI6B:mCherry; (Elsayad *et al*, 2016)). SOK reporters
311 were introgressed into the *plt3/5/7* and *scr* mutants backgrounds (Hofhuis *et al*, 2013; Goh *et*
312 *al*, 2016), by transformation or crossing. Seeds were surface-sterilised (70% ethanol, 0.05%
313 SDS), stratified for 24 h at 4 °C in darkness, and sown on half-strength Murashige and Skoog
314 (½MS) medium (pH 5.7) supplemented with 1% phytoagar. Plants were grown under long-day
315 conditions (16 h light/8 h dark, 22 °C). For some experiments, plates were supplemented with
316 1% sucrose. Soil-grown plants were cultivated under the same photoperiod and temperature.

317 **Plant transformation and selection**

318 Stable transformation was performed using the floral dip method (Clough & Bent, 1998) with
319 *Agrobacterium tumefaciens* strain GV3101. Transformants were selected either by antibiotic
320 resistance (BASTA, hygromycin, kanamycin) or by fluorescence-accumulating seed
321 technology (FAST; (Shimada *et al*, 2010)).

322 **Inducible expression**

323 For endodermis-specific microtubule depolymerisation, we used the *pCASP1>>PHS1ΔP*
324 system (Stöckle *et al*, 2022). Seedlings were transferred at 7 d after germination (DAG) to
325 ½MS medium containing 15 µM dexamethasone (DEX) and imaged after ~24 h.

326 **Sample preparation and staining**

327 Seedlings were fixed in 4% paraformaldehyde in PBS for 2 h at room temperature or overnight
328 at 4 °C, then washed and cleared using ClearSee (Kurihara *et al*, 2015). Cell walls were
329 counterstained with 1 mg/mL calcofluor white. For live imaging, seedlings were mounted in ½
330 MS medium on agar slices, in chambered coverglass systems, or in glass/FEP capillaries for
331 light-sheet microscopy.Confocal and light-sheet microscopy

332 Confocal imaging was performed on Leica SP5II and SP8 systems equipped with water-
333 immersion objectives and white-light or multi-photon lasers. Excitation/emission settings are

provided in Supplementary methods. For live imaging, z-stacks were acquired every 30 min with step sizes of 0.5–1 µm. Light-sheet imaging was performed using a Luxendo MuViSPIM (Bruker) with dual-sided illumination and 40× or 60× water-immersion objectives. Time-lapse recordings spanned up to three days, with z-stacks collected at 30-minute intervals. Additional details are provided in the Supplemental Materials.

Laser ablation

Targeted ablations were performed either with two-photon excitation on a Leica SP8 system (960 nm, 20–30% power) or with an integrated UV ablation laser on the MuViSPIM. Exposure times were adjusted until visible damage was achieved. Additional details are provided in the Supplemental Materials.

Image processing and quantification

An extensive description is provided in the Supplemental Materials. Images were processed in Fiji. Light-sheet data were corrected and cropped using BigDataProcessor2 (Tischer *et al*, 2019). Drift correction of confocal timelapses was done with the Fiji Registration plugin. For weak signals, maximum intensity projections of 2–10 slices were generated. SOK signal maxima were quantified using a custom Python pipeline on 462 images (1388 maxima) and plotted relative to developmental stage. CarboTag-BDP fluorescence lifetimes were fitted with a two-component exponential decay model in LAS X software (Leica) and analysed in R. Microtubule orientation was quantified from 2D cortical images using FibrilJ (Boudaoud *et al*, 2014), with anisotropy-weighted mean orientations plotted per cell group. Growth anisotropy and principal growth directions were determined with MorphoGraphX (Strauss *et al*, 2022). Additional details are provided in the Supplemental Materials.

Molecular cloning

All constructs (listed in Table S1) were generated by GreenGate cloning (Lampropoulos *et al*, 2013; Piepers *et al*, 2023).

360 **Data availability**

361 All raw images, processed data, and biological resources are available from the corresponding
362 author upon reasonable request. Custom scripts for image analysis are available from
363 https://doi.org/10.5281/zenodo.17598920.

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371 **Ethics declarations**

372 The authors declare no competing interests.

373 **Contributions**

374 A.M. and M.P. conceived the study. M.P. performed wet bench experiments under the
375 supervision of A.M. and, with help from or material generated by B.J.R.H, N.B., L.S. C.S., A.P.
376 and L.B.. J.R.D.R. performed the computational modelling with input from A.M. and R.M.H.M,
377 who provided feedback on interpretation. M.P. and A.M. wrote the manuscript with input from
378 all authors.
379

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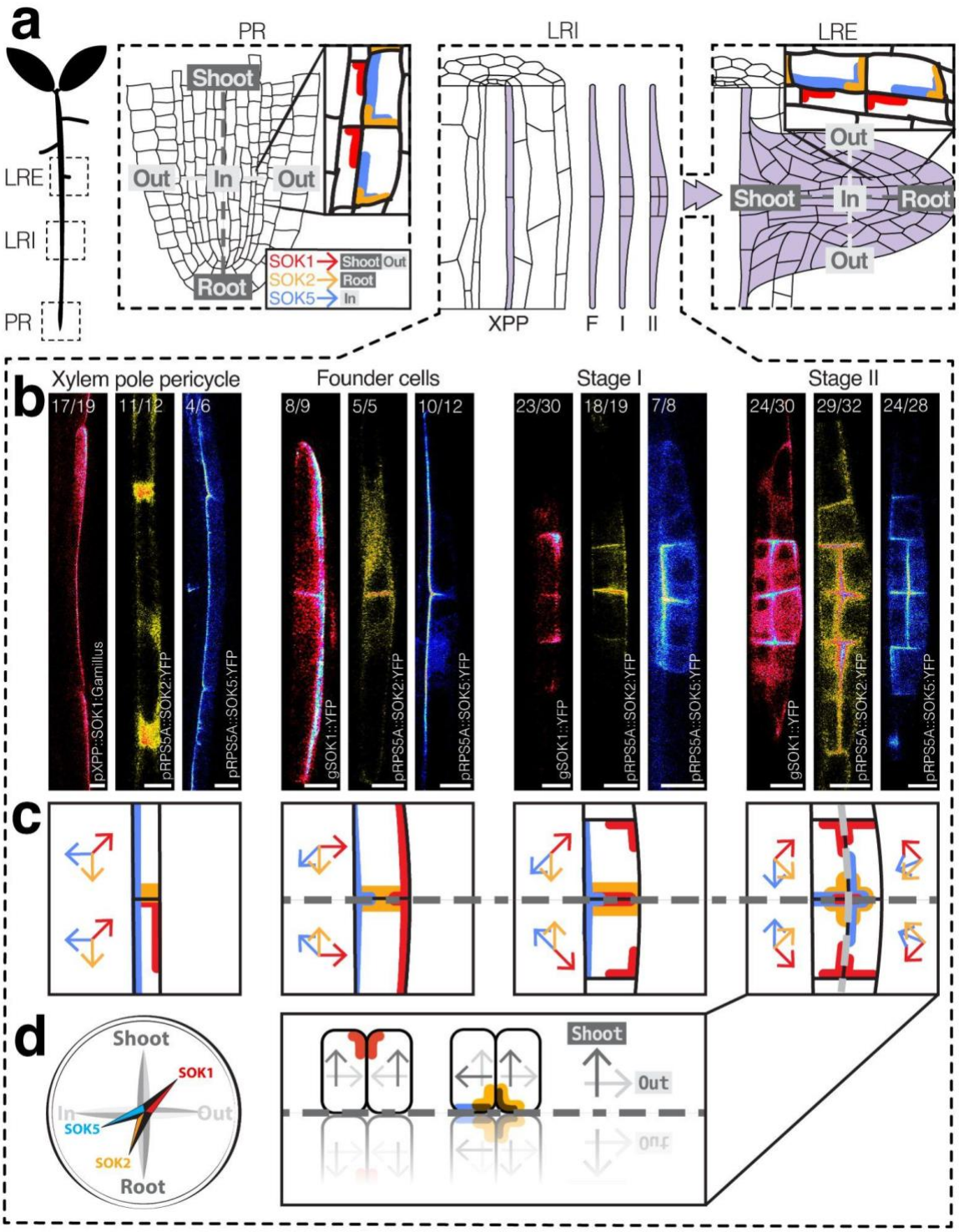
491 **Figures and legends**



492

493 **Figure 1 | Two symmetry-breaking events pattern early lateral root primordia.**

494 **a**, Schematic of *Arabidopsis* root zones showing the primary root (PR), lateral root initiation

495 (LRI), and lateral root emergence (LRE). Insets illustrate SOSEKI (SOK1 in red, SOK2 in

496 orange, SOK5 in blue) localisation in the PR and emerged LR, reporting the shoot–root and
497 in–out axes.
498 **b**, Representative images of fluorescently tagged SOK1, SOK2, and SOK5 reporters in XPP
499 cells, founder cells, Stage I, and Stage II primordia. Numbers indicate the frequency of
500 observed patterns across independent samples. Scale bars, 10 μm. See also Movie S1.
501 **c**, Consensus schematics of SOK polarisation at each stage. Arrows denote the orientation of
502 SOK polarisation in each cell. The grey dashed lines indicate axes of symmetry. **d**, At Stage
503 II, the shoot-root and in-out axes inferred from SOK polarisation are incoherent between the
504 inner and outer cells.

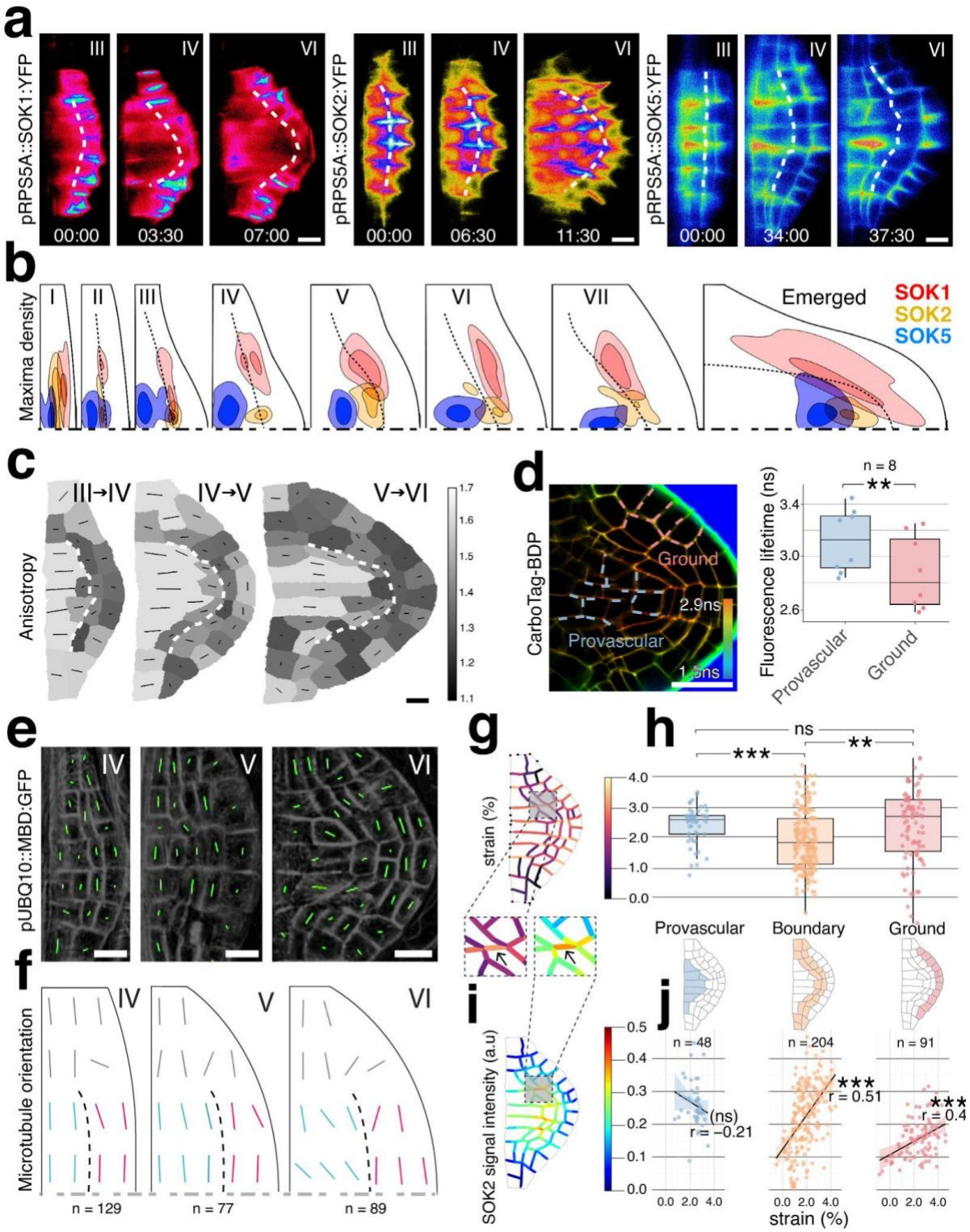


Figure 2 | SOK polarisation highlights domains of differential tissue growth.

a, Time-lapse light-sheet imaging of SOK1, SOK2, and SOK5 reporters from LR primordium stages III to VI. SOK polarisation is stable in individual cells and is consistently enriched near the pericycle–endodermis interface (dashed line). Scale bars, 10 µm. Time in hh:mm format.

b, Consensus density maps highlighting the regions with the highest SOK1 (red), SOK2 (orange), and SOK5 (blue) expression across developmental stages. Developmental stages and number of cells analyzed: I (n = 126), II (n = 216), III (n = 294), IV (n = 234), V (n = 122), VI (n = 69), VII (n = 114), emerged (n = 213). Range of LR height at each stage: I: 8-15µm, II: 15-23µm, III: 23-30µm, IV: 30-38µm, V: 38-45µm, VI: 45-50µm, VII: 50-60µm, emerged: 60-130µm. Only half of the LR primordium is represented. The black dashed line is the pericycle-endodermis interface.

c, Quantification of cell growth anisotropy between successive stages (III–IV, IV–V, V–VI). Higher anisotropy is detected in the central domain. Scale bar, 10 µm.

d, Left, confocal FLIM imaging of CarboTag-BDP in stages III–V of LR primordia. Color scale denotes fluorescence lifetime (ns). Right, quantification of CarboTag-BDP fluorescence lifetimes in epidermal, ground tissue, pericycle-endodermis junction, and provascular domains. Provascular and pericycle show significantly higher values (**$p<0.01$).

e, Confocal imaging of cortical microtubule organisation (pUBQ10::MBD:GFP, grey) at stages IV–VI. Microtubule orientation and anisotropy in each cell are indicated by green overlays. Scale bars, 10 µm.

f, Schematics of average microtubule orientation. Central tissues show alignment along anisotropic growth directions, while peripheral tissues remain isotropic. The black dashed line is the pericycle-endodermis interface.

g, Predicted strain pattern obtained after pressurising the cellular template of a Stage V primordium.

h, Boxplots showing the distribution of strain across the provascular, boundary, and ground tissue domains.

i, SOK2 cortical signal intensity mapped on the same pressurised template as in g.

j, Correlation between strain magnitude and SOK2-YFP signal intensity for each tissue domain.

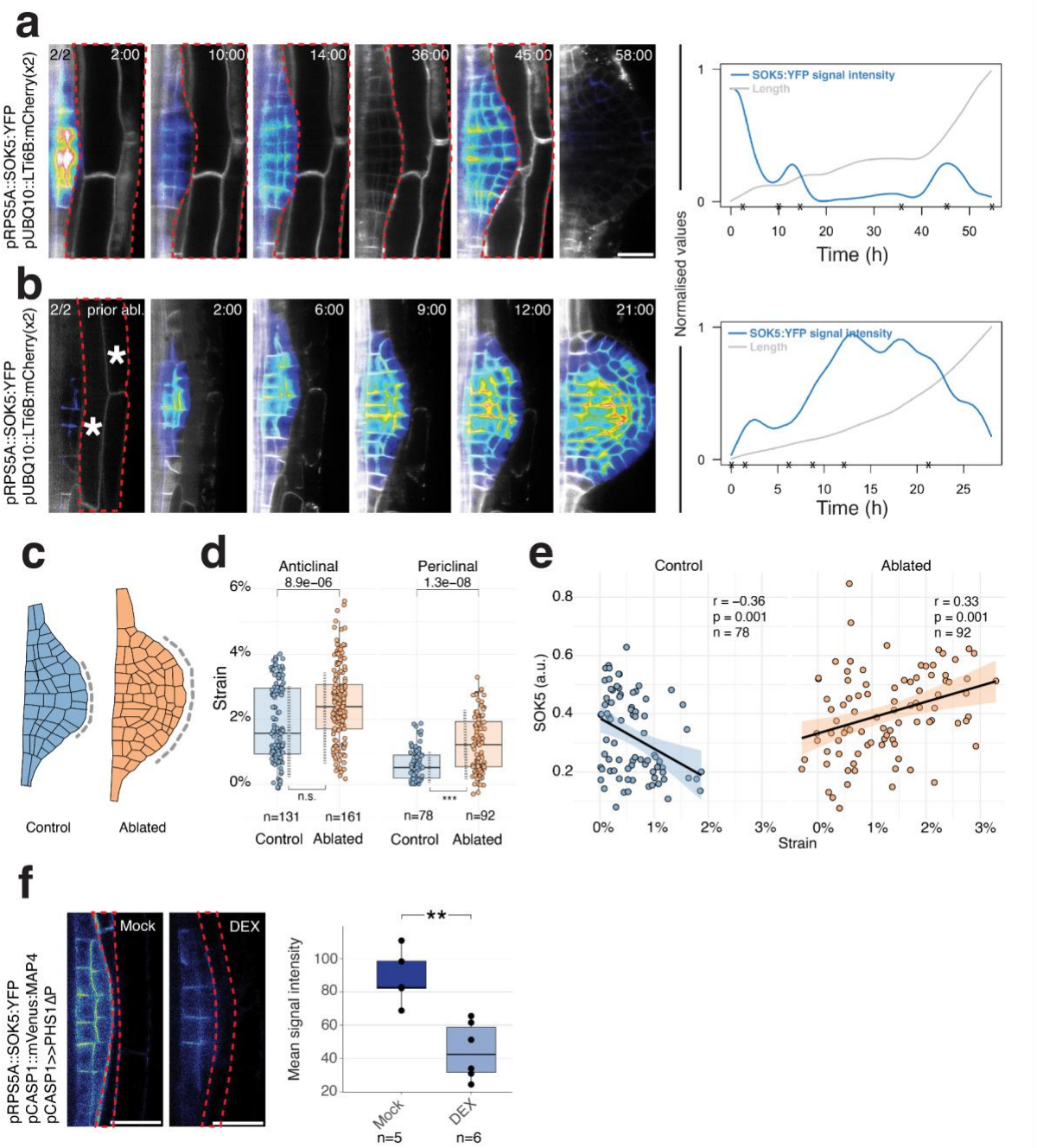
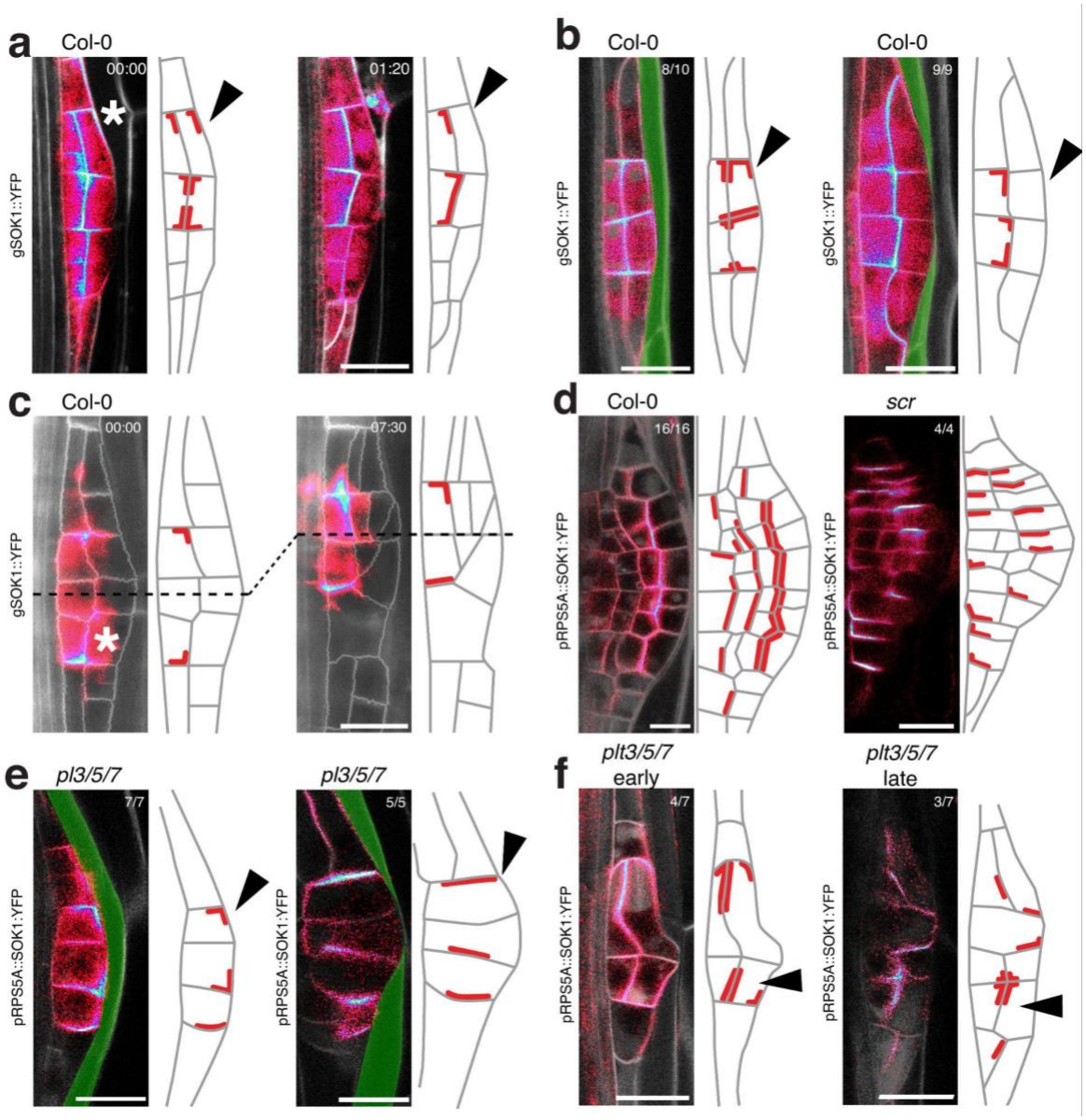


Figure 3 | SOSEKI accumulation is mechanoresponsive during lateral root emergence. **a**, **b** Time-lapse light-sheet imaging of pRPS5A::SOK5:YFP (blue) with plasma membrane marker (grey) from stage II to emergence in wild type (a) and after targeted ablation of overlying cortex cells (asterisks) to relieve compressive stress. Right: quantification of primordium length (grey) and SOK5 signal intensity (blue) over time.

c, schematic of the meshes used for turgor-induced strain simulation in control and ablated conditions. The dashed lines indicate the range of external vertices set free to move. In the ablated case, the fraction of free-to-move vertices is increased compared to the control condition to account for the absence of the overlying tissue.

546 **d**, boxplot showing the distribution of strain in anticlinal and periclinal walls after pressurising
547 the cellular template in control and ablated conditions. P-values for the comparisons of the
548 mean are indicated above. The vertical dashed lines indicate the distributions' variances and
549 the symbols below indicate the result of a Levene's test for equality of variance (***, p<0.001).
550 Anticlinal walls are perpendicular to the primordium surface, periclinal ones parallel.
551 **e**, Correlation between strain magnitude and SOK5-YFP signal intensity in periclinal walls in
552 control and ablated conditions.
553 **f**, Confocal imaging of pRPS5A::SOK5:YFP upon dexamethasone (DEX)-inducible
554 depolymerisation of endodermal microtubules using pCASP1»PHS1ΔP. Scale bars, 20 µm.



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556 **Figure 4 | SOK polarity depends on tissue interfaces and mechanical continuity.**

557 **a-f**, Confocal or light-sheet (c) sections through LR primordia expressing the indicated SOK
558 reporter. In each case, the images and matching schematic depict the consequence of the
559 following perturbations on SOK polarisation: (a) ablation of endodermis, (b) crossing of
560 endodermis, (c) ablation of a cell in the LR primordium, (d) *scr* mutant, (e) crossing of the
561 endodermis or (f) formation of a periclinal interface in *plt3/5/7* mutant. Asterisks mark ablated
562 cells. Black arrowheads highlight changes in polarisation. Scale bars: 20 µm.