Investigating the apical notch, apical dominance and meristem re-emergence in *Marchantia polymorpha*.

Alan O. Marron[1,2]

[1] Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, United Kingdom

[2] Present Address: Oxford Brookes Centre for BioImaging, Oxford Brookes University, Headington Campus, Oxford OX3 0BP, United Kingdom

Abstract

Meristems are the growth centres of plants. Across all plant groups, the phytohormone auxin controls meristem maintenance and mediates regeneration of new meristems if existing ones are damaged. The Marchantia gemma is an experimentally tractable system featuring two meristematic regions (apical notches). I used laser ablation to precisely manipulate the apical notches, finding that the first apical cell layer is indispensable for meristem maintenance. Within this, a contiguous quorum of cells is required for meristem maintenance, depending on the other apical notch's integrity. Apical notches remodel in response to damage, demonstrating that the apical cell layer acts as a communicating population rather than containing one master-controller Apical Cell and subordinate subapical cells. I show communication between notches and regenerating meristems, with heterogeneity across the gemma tissue for carrying this communication signal. The apical dominance signal represses cell division and requires both sources and sinks, features of auxin-mediated communication. I present a model of Marchantia meristem organization, involving intra-, inter- and extra-meristem communication. This provides a framework for further study of meristem communication, formation and maintenance in Marchantia and a better understanding of plant meristems as a whole.

Significance Statement

Meristems are important as the growth centres of plants. Understanding how meristems function and regenerate, and how existing meristems repress the emergence of new growth centres (apical dominance) is central to understanding how plants develop. Meristems in the model plant Marchantia display regenerative and dominance behaviour typical of plant meristems. Using laser ablation microscopy, I show that meristematic cells act a population and find evidence for communication within and between meristems, including during regeneration. Only certain regions of the plant transmit these signals, and normal meristem dynamics requires tissues acting as signal sources and sinks; features characteristic of the hormone auxin. I present a model for Marchantia meristem organization and regeneration, providing a framework for the study of meristems generally.

Introduction

Meristems are the growth centres of plants, niches where pluripotent stem cells continually divide asymmetrically to produce a daughter and a meristematic cell. The cells at the centre divide slowly, while those at the periphery divide faster. This moves the daughter cells outwards as they mature, where they become differentiated into various tissues and cell types. The meristematic cells remain in the centre to replenish the stem cell pool. This spatial distinction between division and differentiation allows plant meristems to simultaneously sustain both growth and new organ formation (1). In angiosperms the two main meristems are found at either end of the plant: the Shoot Apical Meristem (SAM) and Root Apical Meristem (RAM) (1, 2). The SAM model is thought to be how meristems operated in the early land plants (3). The phytohormone

auxin has a central role in plant meristem function and organization. Auxin is produced in the SAM (4) and flows outwards by polar transport, where it is involved in controlling cell expansion and differentiation (5, 6). Secondary meristem formation is induced by the formation of local auxin maxima and minima arising out of auxin gradients extending from the apical meristems (6). Apically derived auxin promotes shoot growth and inhibits shoot branching by preventing growth of secondary (axillary) meristems in the buds: this is apical dominance. If the SAM is removed or damaged, the flow of auxin is disrupted, apical dominance is removed and the axillary meristems are released from dormancy and begin growing (7). Plants can also regenerate new meristems in response to wounding of existing meristems. This is due to their inherent cellular plasticity, which allows cells from certain tissue types (e.g. embryonic tissue, pericycle) to reprogramme into meristematic tissue in response to signals caused by a wounding-induced changes in auxin and cytokinin ratios (6, 8).

Developing a better insight of the how meristems function is key to understanding plant development. Meristems are vital in agriculture for developing improved crop plant breeds with beneficial apical dominance traits (2). Inducing meristem regeneration is important in vegetative propagation of agricultural crops (9). Controlling the molecular mechanisms for meristem specification and reprogramming therefore offers an avenue for developing new transgenic methods (10). Considerable progress on understanding apical meristems has been made from study of angiosperms, particularly *Arabidopsis* (11), however these systems have limitations, such as difficult access for imaging (particularly live imaging) (12, 13) and the complexity of the gene networks involved (2, 5).

The liverwort *Marchantia polymorpha* has emerged as a new model system in plant biology with numerous advantages that compliment other plant systems. As a bryophyte, Marchantia occupies an important position in the land plant phylogeny (14–17). The Marchantia genome is streamlined, with fewer gene copies for many gene families (18, 19), notably the minimal set of components related to auxin biosynthesis, transport and signalling (18, 20–24). It is highly experimentally tractable, with fast growth rates and well-established transgenic protocols (25, 26). Marchantia produces haploid spores as well as vegetative propagules called gemma (27, 28). Gemmae develop in gemma cups and remain dormant until they are removed from these cups, at which point they germinate and begin growing in a stereotypical pattern of development. Crucially, gemmae have open development where almost all of the cells and tissues are easily accessible for microscopy without the need for dissection, sectioning or clearing (25, 29).

Gemmae have two meristematic growth centres, each located in an apical notch at opposite ends (27) (Fig. 1*A, B*). Cell division is focused around the apical notches with less cell division towards the middle of the gemma. As cells move outwards away from the meristem, division rates reduce, and cell expansion and differentiation increase. The gemma eventually grows into a thallus and branching produces additional meristematic apical notches, however the early stages of gemma development are characterized by growth centred around the two original notches (30). Marchantia shows massive capacity for meristem regeneration, with even single cells capable of reforming full thalli (31–34). In the case of gemma, the basic rules are that if one or other apical notch is removed then the remaining notch maintains apical dominance and prevents regeneration; however, if both apical notches are removed then new cell divisions and eventually meristems emerge in the remaining gemma fragment (31, 34). These new meristems produce apical notches as in normal thallus development from a gemma. Auxin has long been known to be involved in meristem regeneration in Marchantia (35, 36). Auxin is produced in the apical notch and exported to the rest of the thallus, where it inhibits the formation of new meristems (i.e., apical dominance). Removal of the apical notches removes these auxin sources. The levels of auxin initially drop in the remaining thallus, and then rise as new meristems appear (31). Although the Marchantia apical notch has received relatively little attention compared to angiosperm meristems (19) it offers a promising opportunity to gain insights into plant meristems generally.

Previous efforts at studying Marchantia meristem maintenance and regeneration have employed surgical methods such as cutting with scalpel blades (31, 34). Here I use laser ablation

and transgenic marker lines to achieve a more precise disruption of the meristems in gemmae and to get a more sensitive measure of cell division and meristem-related processes (37). My results point towards a model with a dynamic population of apical cells communicating within the apical notch and to the rest of the gemma tissue, and with a balance of auxin sources and sinks maintaining normal meristem activity. Under this model, disruption of this communication and balance induces new meristems to emerge, or existing meristems to lose their identity and become divisionally inert. Given the conserved nature of many developmental pathways in land plants (19), these results are relevant to auxin- and meristem-related processes in land plants as a whole.

Results and Discussion

Laser ablation of transgenic Marchantia lines reveals minimum requirements for meristem regeneration

Marchantia plants expressing an mScarlet membrane marker enabled cell division events in the growing gemma to be observed by highlighting the formation of new cell membranes. Visual inspection of the density of new cell walls and tissue morphology allowed for recognition of developing meristematic regions in the apical notches. Where laser ablation microscopy was used to remove both notches of a 0 days post germination (dpg) gemma (Fig. 1*C*), these membrane marker lines were used to track the regeneration process (Fig. 1*D*). This confirmed previous findings (41), that there are four distinct phases of regeneration. In the first ~24 hours cell division remains largely dormant. Between 24-48 hours after ablation there is rapid, widespread cell division across the gemma. After ~48 hours cell division becomes localized, restricted to specific clusters. Approximately 5 days after ablation (DaA) meristem regeneration has been completed, forming full apical notches with intact apical cells.

I previously generated a range of enhancer trap lines that mark the apical notch/meristem region of the Marchantia gemma (32). Removal of both of the entire apical notch regions of gemmae from these lines resulted in meristem regeneration, which could be observed by emergence of new nuclear mVenus signal in the enhancer trap lines (SI Appendix Fig. S1). The lines used (239-P21, 239-P33, 239-P125, 239-P153) marked the localized clusters of cell division and only persisted in active meristematic regions that went on to produce full apical notches. This meant that the enhancer trap marker signal could be used as an easily observable indicator of where new meristems were regenerating in the gemma. Persistence of localized marker signal also allowed simple verification of which regions were continuing as functioning meristems, whereas loss of marker signal indicates loss of meristem identity and cell division activity.

Previous experiments investigating Marchantia regeneration were limited to relatively crude surgical manipulations using cutting with scalpel blades. Laser ablation microscopy allows for the precise removal of cells in the apical notch. This means that fine scale disruptions can be made in this region, to determine the minimum amount of the apical notch that needs to be removed to induce meristem regeneration. Laser ablation was used to entirely remove one apical notch of a gemma, and to shave off rows of apical notch cells going progressively deeper into the other apical notch. Shaving off just one row of cells was sufficient to induce meristem regeneration, which occurred in a region just behind the ablated notch (Fig. 1*E*, *F* and SI Appendix Fig. S2*A*, *B*). Meristem organisation was disrupted following apical cell ablation, with the notch expanding outwards and cell divisions occurring in the region behind the row of ablated apical cells. This indicates that the apical cell population is indispensable for meristem maintenance. Shaving off the first three rows of cells was required to induce regeneration elsewhere in the gemma, further away from the region immediately surrounding the shaved notch (SI Appendix Fig. S2*C*, *D*).

These results demonstrate that there is a substructure within the apical notch region, with the immediate first row of apical notch cells required to act as meristematic organizers and the second and third rows being in some way “primed” to be induced for meristem regeneration in preference to the rest of the gemma. This is in contrast to regenerations in gemma fragments with completely ablated meristems, where initially cell divisions were widespread across the remaining

gemma tissue (Fig. 1*D*). I infer that because the cells in the second and third rows of the apical notch are still divisionally active, this gives them a “head start” over other parts of the gemma with regards meristem regeneration. Examination of multiple gemmae with both notches completely removed finds that there appears to be no wider predictability or clear bias as to which regions will proceed to form new meristem. This is in contrast to vascular plants, where in the post-embryonic plant only certain somatic cell types are competent for new meristem formation (8). The exception to this is the stalk scar region, which is the part of the gemma that forms first within the gemma cup (27, 28), and which was never observed to initiate cell divisions or to provide the basis for meristem regeneration.

The apical cells function collectively, and this involves intra-notch communication

The current Marchantia literature centres around a model where the key feature of each apical notch meristem is the Apical Cell, a pluripotent cell in the middle of the notch with four division planes (19, 27, 42). Under this hypothesis, the other “subapical” cells in the notch are products of and subordinate to the Apical Cell (19, 42). The Apical Cell has chiefly been defined by morphological characterization from light and electron microscopy images, rather than direct experimental evidence. Experiments on misexpression and knockout lines for the stem-cell promoting peptide factor MpCLE2/CLV3 were found to develop extra or reduced Apical and subapical cells respectively compared to wild-type plants (30), although no clear criteria was given as to how these were classified between the two cell types.

I therefore attempted to use laser ablation to interrogate the standard model of the apical notch by ablating portions of the cells in the crucial first row of apical notch cells (*i.e.*, the Apical Cell or various subapical cells) that appears to act as the meristematic organizer. Removing one side of the apical notch cells did not disrupt meristem function or apical dominance, irrespective of whether or not there was another intact apical notch present in that gemma (SI Appendix Fig. S3*A*, *B*). However, if the notch was ablated so that only a portion of apical notch cells were left intact on one side, then meristem function was disrupted. If the other apical notch in the gemma was intact (SI Appendix Fig. S3*C*) then this demonstrated apical dominance, and the partially ablated notch became inert (*i.e.* non-meristematic). If the other notch was removed (SI Appendix Fig. S3*D*), then new meristematic regions emerged elsewhere in the gemma, and the remaining apical notch cells were insufficient to retain apical dominance over these new meristems. If a few apical notch cells were left intact on each side (SI Appendix Fig. S3*E*, *F*) then the notch became inert irrespective of the status of the other notch- despite the total number of apical notch cells left intact being the same as notches with contiguous portions of apical notch cells that continued to function.

If both sides of the apical notch were removed, but a few cells at the base (including the presumed Apical Cell) were left intact, then the result was dependent on the status of the other notch in the gemma. If the other notch was removed (Fig. 2*A*), then the intact base continued to function as a meristematic apical notch, and retained apical dominance (that is, no new meristematic regions emerged elsewhere in the gemma). If the other notch in the gemma was intact (Fig. 2*B*), then the intact base alone could not sustain meristem functions and became inert, even if the remaining portion was larger than cases where notch function continued with the other notch excised (e.g., Fig. 2*C*). This shows that while the base of the apical notch has a greater functional capacity than the cells on the sides of the notch, this alone is insufficient for full meristematic capabilities and apical dominance if another, fully intact and functional notch is also present. These experiments also confirm that there is long-range communication between apical notches in a gemma.

I then proceeded to ablate portions spanning across the base and sides, including around the centre where, according to the traditional model of Marchantia notch architecture, the Apical Cell is located (Fig. 2*D*, *E*). If only a portion of the apical notch cells were removed, but a sufficient contiguous portion of apical notch cells remained, then not only did the notch continue to function as a meristem, but over the course of the next 48 hours it remodelled itself. The result was that by 3DaA, the centre of the apical notch had shifted to what was the centre of the contiguous portion of remaining apical notch cells on 0DaA. This was irrespective of where the centre of the original notch had been before ablation. The remodelling process did not appear to be influenced by

whether the original location of the Apical Cell was ablated or not. The remodelled apical notches continued to be functional, apically dominant meristems regardless of whether there was another intact apical notch in that gemma. That is to say that during the first 48 hours after ablation, the remodelling process did not permit the initiation of new regions of cell division (if the other apical notch had been removed), nor was it subject to apical dominance-related inhibition if the other notch was intact. This means that the apical notch remodelling is not simply meristem regeneration but is a continuation of the original meristem.

A flow chart of the outcomes of these ablation experiments is provided in SI Appendix Fig. S4. These experiments demonstrate that the key factor is not the presence or absence of a single, central and essential "Apical Cell" surrounded by dispensable "subapical cells", but instead points towards there being a population of apical cells within the notch that are more-or-less equally competent. What is paramount for meristem integrity is that there is a sufficient number or quorum of these apical cells that are intact and in contiguous contact. The precise limits and requirements for this quorum remain to be determined. The characteristic notch shape of the Marchantia meristem emerges from self-organization of this quorum portion, as an outcome of the biophysics of differential rates of cell division and expansion from the centre of the quorum to the edges (43). The necessity for the portion to be contiguous could mean that intra-notch communication directly between the apical cells is vital to provide a mechanism to regulate faster rates of cell division at the centre of the notch. It may also be that non-contiguous portions compete and cancel each other out if they are in too close proximity. However, the evidence from the ablation experiments demonstrates that the single "Apical Cell" of previous models is not a unique, fixed or critical cell identity, and instead simply emerges from the architecture of the general meristematic apical cell population within the notch.

Meristem regeneration involves communication between regions of cell division

Partial ablation of the apical cells revealed further aspects of Marchantia apical dominance and meristem regeneration. Fig. S5 shows the case of where one apical notch region is removed entirely, and the other is partially ablated. The remaining apical cells of the partially ablated notch do not constitute a quorum initially, and therefore the rest of the gemma is released from apical dominance and proceeds to the early stages of regeneration (SI Appendix Fig. S5*C*). Several patches of cell division can be observed in various regions across the gemma, and by 2DaA regeneration of some of these patches have progressed, as indicated by their having dense patches of fluorescent marker signal (white arrows, SI Appendix Fig. S5*D*, *E*). Simultaneously, the partially ablated original notch has also recommenced cell division and its meristem regeneration is well underway. By 3DaA this region is the most prominent patch of meristem, though emerging patches are visible and have marker signal. Five days after ablation (SI Appendix Fig. S5*F*), the notch that was originally partially ablated has once again exceed the quorum size of apical cells and is now a fully active, mature notch. This notch has established full apical dominance and the other patches have become inert (as indicated by loss of marker signal). However, it can be seen that these aborted meristems did progress far along the regeneration pathway, with significant amounts of cell division that have produced protrusions on the thallus (cyan arrows, SI Appendix Fig. S5*F*).

This observation is especially noteworthy as it demonstrates that loss of meristem function and new meristem emergence is not irreversible. It also reveals that apical dominance in Marchantia is an active process that must be maintained by the meristematic apical notch, can be disrupted and can be re-established. Meristem "maturity" as well as size is a factor in this. This provides a possible checkpoint mechanism for progression through regeneration and prevent multiple meristems emerging in too close proximity. My observations also show that Marchantia apical notches signal to the rest of the plant to inhibit cell division, thus generating the characteristic thalloid growth pattern.

There is a flow of signal from the apical cells

The signal produced at the apical notch must flow outwards in order to influence the rest of the gemma tissue. To investigate this, I used laser ablation to make incisions at a distance from

the apical notch to see if the flow of this signal could be interrupted. Incisions made perpendicular to the notch had no obvious effect (SI Appendix Fig. S6*A*-*E*). Incisions made parallel to the notch (SI Appendix Fig. S6*F*-*O*) had the effect of increasing the rate of cell division proximal to the incision and increasing cell expansion distal to the incision. This effect was not seen if the apical cells proximal to the parallel incision were also ablated. Instead, the cells on both sides of the incision ceased dividing and showed increased cell expansion. This shows that signals from the apical cells are responsible for controlling the balance between cell division and expansion in the region surrounding the apical notch. The cells in this region are the youngest in the gemma and will be acquiring new fates (27, 42, 44), so it is expected that they would be integrating multiple different positional and developmental cues from their surrounding tissue environment. It also reinforces the evidence that the apical cells exist as a population rather than being controlled by a central "Apical Cell", as the effect of removing the apical cells proximal to the incision was not dependent on the status of the cells at the very apex of the notch. Changes in cell division and expansion notwithstanding, it is important to note that there was no evidence for patches of cells distal to the parallel incision entering the early, widespread cell division phase of meristem regeneration. This was the case whether the other apical notch in the gemma was present or not. Therefore, while some signals are dependent on direct flow from the notch, the signals involved in apical dominance and repression of new meristems are capable of being transported around the parallel incisions that were made in the gemma.

Further, more extensive incisions were made to test the limits of the transport of the repressive apical dominance signal (Fig. 3). This found that the location of the tissue forming the connection to the rest of the gemma was crucial. Connections through central zone tissue (Fig. 3*A*-*F*) communicated the repressive signal and did not see any meristem regeneration elsewhere in the gemma. This was the case even when the connections were made from a small amount of tissue only a few cells wide. In contrast, connections formed from the thinner tissue around the gemma periphery (Fig. 3*G*-*L*) appeared not to communicate the repressive signal for apical dominance. Instead, multiple new meristematic regions emerged, even with there being a fully functioning meristematic apical notch present in the gemma. This demonstrates that there is a spatial heterogeneity across the gemma for transport of the repressive signal for apical dominance, suggesting that the signal is carried by a specific mechanism and that not all cell types possess this mechanism.

Signal export to a sink is required to maintain the apical notches as meristems

Making incisions in the central zone tissue from around the apical notch region, so that it is only linked to the rest of the gemma by peripheral connections, effectively isolates the apical notch. If the isolated region is large and includes tissue from outside the notch region, then it continues to grow and develop as normal, even as new meristematic regions emerge elsewhere in the gemma (Fig. 3*K*, *L*). If the isolated region is small and only contains the apical cells and cells immediately surrounding the notch, then the result is different (SI Appendix Fig. S7). As expected, the rest of the gemma is released from apical dominance and proceeds through the early phases of meristem regeneration. The isolated notch sustains meristem identity for the first 24 hours after ablation, as shown by the persistence of mVenus marker signal. 48 hours after ablation the marker signal is fading and cell division has ceased, and by 3DaA the isolated notch has become totally inert. The cells do not divide and instead expand, and no apical notch/meristem marker signal is present whatsoever. This sequence of events is the same as cases where the apical notch and immediately surrounding region is totally disconnected from the rest of the gemma by laser ablation of the intermediary tissue (SI Appendix Fig. S8).

This reinforces the conclusion that the repressive signal that is usually exported from the apical notch cannot be transported through these peripheral connections. It also appears that the meristematic tissue cannot degrade this signal. If it cannot be exported to a sufficient sink of non-meristematic tissue, then it accumulates and has a self-inhibitory effect. This repressive signal

causes loss of meristematic identity: the former apical cells cease dividing, expand, and persist as regular thallus tissue.

The observed self-inhibitory feedback of these isolated apical notches is characteristic of auxin (20, 45). Auxin controls and regulates processes such as apical dominance (7), meristem regulation (5, 6) and meristem regeneration (8) in plants, including Marchantia (35, 36). Auxin is produced in the apical notch and exported to the rest of the thallus. Surgical removal of the apical notch removes this auxin source, causing auxin levels in the remaining thallus fragment to drop initially before rising again as new meristems regenerate (31). A reporter line marking nuclei expressing the auxin biosynthesis gene MpYUC2 (46) shows that in 0dpg plants, MpYUC2 is expressed throughout the gemma with the apical cells having particularly high expression levels, but this becomes more focused around the apical cells in older gemmae (32). A reporter line marking expression of the canonical polar auxin transporter MpPIN1 (17, 21) shows that in 0dpg plants expression is focused around the apical notch regions, as well as being present around the gemma edge and in occasional cells across the central zone. In older gemmae, MpPIN1 expression becomes more focused around the apical cells (32). Collectively, these results show that the apical notch meristem acts as an auxin source in intact gemmae, but that auxin signalling is more widespread.

Canonical PIN distribution does not explain why the repressive signal is carried through central connections but not peripheral connections between the apical notch and the rest of the gemma. As mentioned previously, MpPIN1 expression is high at the gemma edge. The gemma in SI Appendix Fig. S9*A-D* has no MpPIN1 expression in the cells of the central tissue connection, yet the intact notch retains apical dominance over the rest of the gemma and no new meristems emerge. Conversely, SI Appendix Fig. S9*E-H* shows a gemma ablated such that the one remaining notch is only connected to the rest of the gemma by peripheral tissue. The cells of the peripheral connection clearly display MpPIN1 expression (SI Appendix Fig. S9*E-H*), yet meristem regeneration occurs elsewhere in the gemma. It may be the case that other auxin transporters are involved, for example short PIN, ABCB or AUX/LAX transporters (18, 21, 47). The Marchantia non-canonical short PINs are promising candidates as recent work has found that they are localized to the plasma membrane and are capable of auxin export. In particular, MpPINW has an asymmetric distribution in the plasma membrane, and this asymmetry appears to point centrally rather than peripherally in cells around the apical notch that express MpPINW (48).

Relationship between regeneration speed and size of the regenerating gemma fragment

A hypothesis that emerges from these findings is that, in a gemma fragment with both apical notches excised, if there are a greater number of cells that act as auxin sources, combined with a greater number of cells available to act auxin sinks, then regeneration will proceed faster compared to fragments with fewer cells. I tested this by using laser ablation microscopy to remove a certain amount of tissue (expressed as a percentage of the original gemma diameter) from a gemma that had a set amount of the apical notch region removed. The gemmae used were taken from the enhancer trap line 239-P125, a line marking the apical notch/meristem and in which clusters of fluorescent signal re-emerges during regeneration (32). I used the timing reappearance of this signal as a proxy for measuring the speed of the meristem regeneration process. In a 108-hour time course (imaging every 12 hours) it was seen that larger fragments consistently had marker signal reappear faster after ablation compared to smaller fragments (SI Appendix Fig. S10). This supports the hypothesis that having a greater pool of cells to restore the balance between auxin sources and sinks is important for promoting meristem regeneration. An extreme version of this is the case of regeneration from a very small number of isolated thallus cells (32, 33, 49). In contrast to meristem regeneration from full gemmae, which is complete after ~5 days, isolated single cells or small clusters can take at least 5-7 days just for cell divisions to occur, another 4+

days for localized patches of cell division to stabilize, with recognizable notches only appearing 14 days after cell isolation (even longer for protoplasts).

Conclusions

An outline model for the Marchantia apical notch meristem is given in Fig. 4. A population of apical cells are found in the first row of the notch, behind which are 2 rows of cells "primed" to acquire apical cell fate. The centre of the apical cell population forms the apex of the notch by having lower rates of cell division and expansion compared to the edges of the apical cell quorum. The apical cells produce signals controlling cell division and expansion, and signals maintaining apical dominance across the gemma. The apical dominance signal, likely auxin, is carried through the centre of the gemma and not the peripheral regions. If neither meristematic apical notch has a sufficient quorum of apical cells in contiguous contact, or if the apical dominance signal cannot flow to the rest of the gemma, then meristem regeneration occurs elsewhere.

The experimental results we present here refine our understanding of the apical notch substructure, and of the requirements to sustain notch meristematic activity or to induce meristem regeneration. I demonstrate that there is intra- and inter-notch communication in Marchantia, and the role of extra-meristem communication, via auxin biosynthesis and transport, in meristem regeneration. As such developmental processes and pathways show widespread conservation across the land plants, this provides an avenue to provide new insights into the nature of meristem communication, maintenance and regeneration in more complex plant systems.

Materials and Methods

Vector constructs were built according to the Loop Assembly protocol (25, 38) from L0 parts, L1 and L2 plasmids in the OpenPlant Loop Assembly toolkit (25). The lines used experimentally to produce each figure are given in SI Appendix Supporting Text and full plasmid sequences for vector constructs are given in SI Appendix Dataset S1. Plasmid vectors were transformed into Marchantia sporelings (Cam accession) by agrobacterium-mediated transformation according to the methods described previously (25, 26). T0 sporelings and subsequent G1 plants were grown at 21°C under continuous light (intensity= 150 µmol/m^2/s) on 1.2% w/v agar (Melford capsules A20021) plates of Gamborg B5 media with vitamins (Duchefa Biochemie G0210) prepared at half the manufacturer's recommended concentration and adjusted to pH 5.8. The media was supplemented with 100µg/ml Cefotaxime (BIC0111; Apollo Scientific, Bredbury, UK) to reduce bacterial or fungal contamination, and 20µg/ml Hygromycin (10687010; Invitrogen) for selection.

Imaging was carried out using a Leica Sp8 Confocal Microscope, equipped with a hybrid detector and a pulsed white-light laser, with a HC PL APO 10x/0.40 CS2 or a HC PL APO 20x/0.75 CS2 dry objective. Excitation and collection settings for each fluorophore are given in SI Appendix Supplementary Information Text. Images were taken in photon counting mode with sequential scanning. Time gating was active to supress autofluorescence. Maximum-intensity projections of the images were obtained from z-stack series, capturing z-slices at intervals between 1µm-12.5µm. The images were processed using the Leica LasX and Fiji/ImageJ software packages. Figures were produced using the QuickFigures (39) and Stitching (40) Fiji plugins.

Laser ablation experiments were performed using a Leica LMD6000 laser dissection microscope system equipped with a solid state 355nm cutting laser and controlled with the Leica LMD6 software. Ablation performed under the 10x/0.3 HCX PL FL objective lens used settings 60 power, 45 aperture, 25 speed; ablation performed under the 40x/0.6 HCX PL FL objective lens used 60 power, 35 aperture, 20 speed. All gemmae used for ablation were taken directly from the gemma cup of the parent thallus (i.e., 0dpg). Intact gemmae were also taken from the same cups and used as controls to verify regular growth and marker expression in that plant line. Gemmae were planted on 50mm agar plates and all ablation and imaging was performed directly on the plates to minimize mechanical disruption to the plants. For wholescale notch removal a 90µm diameter circle was drawn with its centre point at the apical notch, and all tissue within this circle

was excised. All other cells or regions ablated are given in a schematic accompanying the relevant figures. Gemma growth responses and marker gene expression were observed and consistent in at least three replicates, unless otherwise noted.

Propidium iodide (PI) staining was carried out to verify that the laser ablation settings did not cause widespread stress or uncontrolled damage to the gemma. WT and enhancer trap line gemmae were incubated in 10µl of 100µg/ml PI (Sigma) on a glass coverslip for 10 minutes at room temperature, ensuring that the gemma was not otherwise mechanically damaged or desiccated during incubation. Gemma staining and imaging was carried out before and/or after ablation in various combinations to ensure that any PI signal relating to cellular damage was solely connected to the laser ablation. All experiments showed that the laser ablation settings used did kill the targeted cells, that cellular damage was localized to the visible ablation zone and that the ablation process did not cause more widespread cell death or stress to the gemma (SI Appendix Fig. S11). The results validated laser ablation microscopy as an approach to study the microarchitecture of the apical notch region.

Acknowledgments

I would like to thank members of the lab working with Marchantia, particularly Susanna Sauret-Güeto, Jenna Rever, Harriet Kempson, Sze Wai Tse and Eftychios Frangedakis, for their advice and support. I would especially thank Marius Rebmann for providing plant lines and his valuable intellectual input into this research. Mihails Delmans developed some of the concepts related to these experiments. Jim Haseloff provided funding and influenced the development of this project. I thank Alexander Jones (Sainsbury Laboratory, Cambridge, UK) for constructive comments on this manuscript, Satoshi Naramoto (Hokkaido University, Japan) for kindly providing the 5' promoter sequence for MpYUC2 to create the vector construct, and Kuan-Ju Lu (National Chung Hsing University, Taiwan) for kindly providing image data on Marchantia non-canonical short PIN expression. This work was funded as part of the Biotechnology and Biological Sciences Research Council/ Engineering and Physical Sciences Research Council OpenPlant Synthetic Biology Research Centre Grant BB/L014130/1 to Prof. Jim Haseloff and Biotechnology and Biological Sciences Research Council grant BB/F011458/1 for confocal microscopy to Prof. Jim Haseloff.

References

1. M. F. Schwartz, *et al.*, Divide and Conquer: The Initiation and Proliferation of Meristems. *Critical Reviews in Plant Sciences* **40**, 147–156 (2021).
2. M. Kitagawa, D. Jackson, Control of Meristem Size. *Annual Review of Plant Biology* **70**, 269–291 (2019).
3. J. de Vries, *et al.*, Cytokinin-induced promotion of root meristem size in the fern *Azolla* supports a shoot-like origin of euphyllophyte roots. *New Phytologist* **209**, 705–720 (2016).
4. T. Vernoux, F. Besnard, J. Traas, Auxin at the Shoot Apical Meristem. *Cold Spring Harbor Perspectives in Biology* **2**, a001487 (2010).
5. M. Pernisová, T. Vernoux, Auxin Does the SAMba: Auxin Signaling in the Shoot Apical Meristem. *Cold Spring Harbor Perspectives in Biology* a039925 (2021).
6. Y. Wang, Y. Jiao, Auxin and above-ground meristems. *Journal of Experimental Botany* **69**, 147–154 (2018).
7. M. A. Domagalska, O. Leyser, Signal integration in the control of shoot branching. *Nature Reviews Molecular Cell Biology* **12**, 211–221 (2011).
8. M. Ikeuchi, Y. Ogawa, A. Iwase, K. Sugimoto, Plant regeneration: cellular origins and molecular mechanisms. *Development* **143**, 1442–1451 (2016).

9. L. Lassois, P. Lepoivre, R. Swennen, I. van den Houwe, B. Panis, Thermotherapy, chemotherapy, and meristem culture in banana. *Methods in Molecular Biology* **11013**, 419–433 (2013).
10. M. F. Maher, *et al.*, Plant gene editing through de novo induction of meristems. *Nature Biotechnology* **38**, 84–89 (2020).
11. M. K. Barton, Twenty years on: The inner workings of the shoot apical meristem, a developmental dynamo. *Developmental Biology* **341**, 95–113 (2010).
12. Y. Geng, Y. Zhou, Confocal Live Imaging of Shoot Apical Meristems from Different Plant Species. *Journal of Visualized Experiments* e59369 (2019).
13. N. Prunet, T. P. Jack, E. M. Meyerowitz, Live confocal imaging of Arabidopsis flower buds. *Developmental Biology* **419**, 114–120 (2016).
14. D. Su, *et al.*, Large-Scale Phylogenomic Analyses Reveal the Monophyly of Bryophytes and Neoproterozoic Origin of Land Plants. *Molecular Biology and Evolution* **38**, 3332–3344 (2021).
15. S. Naramoto, Y. Hata, T. Fujita, J. Kyozuka, The bryophytes *Physcomitrium patens* and *Marchantia polymorpha* as model systems for studying evolutionary cell and developmental biology in plants. *The Plant Cell* **34**, 228–246 (2022).
16. H. Kato, R. Nishihama, D. Weijers, T. Kohchi, Evolution of nuclear auxin signaling: Lessons from genetic studies with basal land plants. *Journal of Experimental Botany* **69**, 291–301 (2018).
17. T. Viaene, C. F. Delwiche, S. A. Rensing, J. Friml, Origin and evolution of PIN auxin transporters in the green lineage. *Trends in Plant Science* **18**, 5–10 (2013).
18. J. L. Bowman, *et al.*, Insights into Land Plant Evolution Garnered from the Marchantia polymorpha Genome. *Cell* **171**, 287-304.e15 (2017).
19. T. Kohchi, K. T. Yamato, K. Ishizaki, S. Yamaoka, R. Nishihama, Development and Molecular Genetics of Marchantia polymorpha. *Annu Rev Plant Biol* **72**, 677–702 (2021).
20. S. K. Mutte, *et al.*, Origin and evolution of the nuclear auxin response system. *eLife* **7**, 1–25 (2018).
21. T. Bennett, PIN proteins and the evolution of plant development. *Trends in Plant Science* **20**, 498–507 (2015).
22. H. Kato, *et al.*, Design principles of a minimal auxin response system. *Nature Plants* **6**, 473–482 (2020).
23. H. Kato, *et al.*, Auxin-Mediated Transcriptional System with a Minimal Set of Components Is Critical for Morphogenesis through the Life Cycle in *Marchantia polymorpha*. *PLoS Genetics* **11**, 1–26 (2015).
24. E. Flores-Sandoval, D. M. Eklund, J. L. Bowman, A Simple Auxin Transcriptional Response System Regulates Multiple Morphogenetic Processes in the Liverwort *Marchantia polymorpha*. *PLOS Genetics* **11**, e1005207 (2015).
25. S. Sauret-Güeto, *et al.*, Systematic Tools for Reprogramming Plant Gene Expression in a Simple Model, *Marchantia polymorpha*. *ACS Synthetic Biology* **9**, 864–882 (2020).
26. K. Ishizaki, R. Nishihama, K. T. Yamato, T. Kohchi, Molecular genetic tools and techniques for *Marchantia polymorpha* research. *Plant and Cell Physiology* **57**, 262–270 (2016).
27. M. Shimamura, *Marchantia polymorpha*: Taxonomy, phylogeny and morphology of a model system. *Plant and Cell Physiology* **57**, 230–256 (2016).
28. H. Kato, Y. Yasui, K. Ishizaki, Gemma cup and gemma development in *Marchantia polymorpha*. *New Phytologist* **228**, 459–465 (2020).
29. J. Westermann, E. Koebke, R. Lentz, M. Hülskamp, A. Boisson-Dernier, A Comprehensive Toolkit for Quick and Easy Visualization of Marker Proteins, Protein–Protein Interactions and Cell Morphology in *Marchantia polymorpha*. *Frontiers in Plant Science* **11**, 569194 (2020).

30. Y. Hirakawa, *et al.*, Induction of Multichotomous Branching by CLAVATA Peptide in *Marchantia polymorpha*. *Current Biology* **30**, 3833-3840.e4 (2020).
31. S. Ishida, *et al.*, Diminished Auxin Signaling Triggers Cellular Reprogramming by Inducing a Regeneration Factor in the Liverwort *Marchantia polymorpha*. *Plant and Cell Physiology* **63**, 384–400 (2022).
32. A. O. Marron, *et al.*, An Enhancer Trap System to study tissue development in *Marchantia polymorpha*. *The Plant Journal* (2023).
33. K. Ono, K. Ohyama, O. L. Gamborg, Regeneration of the liverwort *Marchantia polymorpha* L. from protoplasts isolated from cell suspension culture. *Plant Science Letters* **14**, 225–229 (1979).
34. R. Nishihama, *et al.*, Phytochrome-mediated regulation of cell division and growth during regeneration and sporeling development in the liverwort *Marchantia polymorpha*. *Journal of Plant Research* **128**, 407–421 (2015).
35. N. C. Maravolo, P. D. Voth, Morphogenic Effects of Three Growth Substances on Marchantia gemmalings. *Botanical Gazette* **127**, 79–86 (1966).
36. J. A. Rota, N. C. Maravolo, Transport and Mobilization of 14C-Sucrose During Regeneration in the Hepatic, *Marchantia polymorpha*. *Botanical Gazette* **136**, 184–188 (1975).
37. D. Reinhardt, M. Frenz, T. Mandel, C. Kuhlemeier, Microsurgical and laser ablation analysis of interactions between the zones and layers of the tomato shoot apical meristem. *Development* **130**, 4073–4083 (2003).
38. B. Pollak, *et al.*, Loop assembly: a simple and open system for recursive fabrication of DNA circuits. *New Phytologist* **222**, 628–640 (2019).
39. G. Mazo, QuickFigures: A toolkit and ImageJ PlugIn to quickly transform microscope images into scientific figures. *PLoS One* **16**, e0240280 (2021).
40. S. Preibisch, S. Saalfeld, P. Tomancak, Globally optimal stitching of tiled 3D microscopic image acquisitions. *Bioinformatics* **25**, 1463–1465 (2009).
41. H. Dickson, Polarity and the Production of Adventitious Growing Points in *Marchantia polymorpha*. *Annals of Botany* **46**, 683–684 (1932).
42. H. Suzuki, C. J. Harrison, M. Shimamura, T. Kohchi, R. Nishihama, Positional cues regulate dorsal organ formation in the liverwort *Marchantia polymorpha*. *Journal of Plant Research* **133**, 311–321 (2020).
43. J. E. Solly, N. J. Cunniffe, C. J. Harrison, Regional Growth Rate Differences Specified by Apical Notch Activities Regulate Liverwort Thallus Shape. *Current Biology* **27**, 16–26 (2017).
44. P. Apostolakos, B. Galatis, Studies on the development of the air pores and air chambers of *Marchantia paleacea* - IV. Cell plate arrangement in initial aperture cells. *Protoplasma* **128**, 136–146 (1985).
45. R. S. Smith, E. M. Bayer, Auxin transport-feedback models of patterning in plants. *Plant, Cell and Environment* **32**, 1258–1271 (2009).
46. X. Cao, *et al.*, The roles of auxin biosynthesis YUCCA gene family in plants. *International Journal of Molecular Sciences* **20**, 8–10 (2019).
47. W. A. Peer, J. J. Blakeslee, H. Yang, A. S. Murphy, Seven things we think we know about auxin transport. *Molecular Plant* **4**, 487–504 (2011).
48. H. Tang, *et al.*, The Unexpected Membrane Targeting of Marchantia Short PIN Auxin Exporters Illuminates Sequence Determinants and Evolutionary Significance.
49. M. Bopp, R. Vicktor, Protoplasts of *Marchantia polymorpha* and its Development. *Plant and Cell Physiology* **29**, 497–501 (1988).

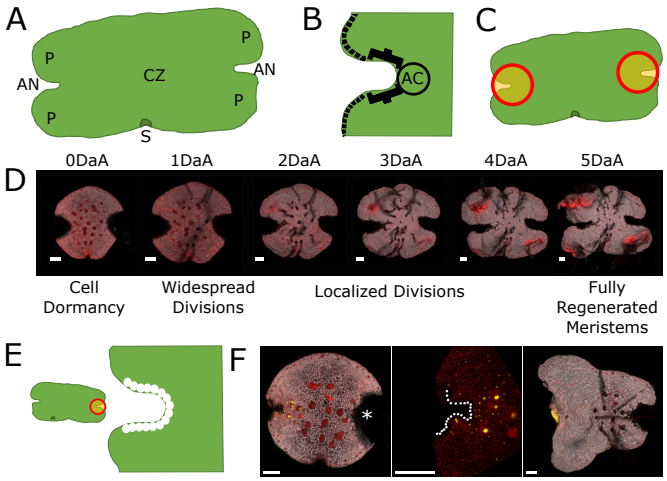

Figure 1. Fine scale ablation shows that apical cells are indespensible for meristem function. (A) Schematic outline of the Marchantia gemma showing the full gemma, with two apical notches (AN) at either end. To the sides of the notches are the peripheral regions (P) and behind the notches is the central zone (CZ). The stalk scar (S) is where the base of the gemma was attached to the parent plant in the gemma cup. (B) shows a close-up of an apical notch. AC marks the "Apical Cell" at the apex of the notch, flanked by subapical cells (brackets) extending out of the "sides" of the notch. These cells are all in the first row of cells. Further out of the notch the first row of cells are comprised of margin tissue (dashed line) that forms from old notch cells and extends around the edge of the gemma and thallus (32). (C) is an outline schematic of the ablation pattern for complete excision of all notches (marked in orange with a red outline). (D) shows a time course of a fragment of an enhancer trap line expressing an mScarlet membrane marker following notch excision by laser ablation, with the four main stages of meristem regeneration marked to the right. The overlap and fading between the marked stages indicates the uncertainty and variability of the timing of the stage transitions. Images were acquired at the indicated time-points after meristem removal (days after ablation, DaA). (E) shows a fine scale gemma ablation experiment, with a schematic outline of the ablation patterns used (orange bounded by red denotes excised tissue, white circles indicate ablated cells). (F) shows, from left to right, an image of the 0DaA whole gemma (asterisk marking excised notch), a close up of the 0DaA notch (ablated cells marked by white circles) and an image of the 4DaA gemma. This demonstrates that removal of only a small region of the first row of cells around the apical cells is sufficient to induce meristem regeneration, just behind the precisely ablated notch. The gemma shown is from the enhancer trap notch/meristem marker line ET239-P153. See SI Appendix Fig. S2*B* for the full time series. Confocal images show membrane marker expression in red, nuclear mVenus enhancer trap signal in yellow and chlorophyll autofluorescence in grey. All scale bars= 100µm.

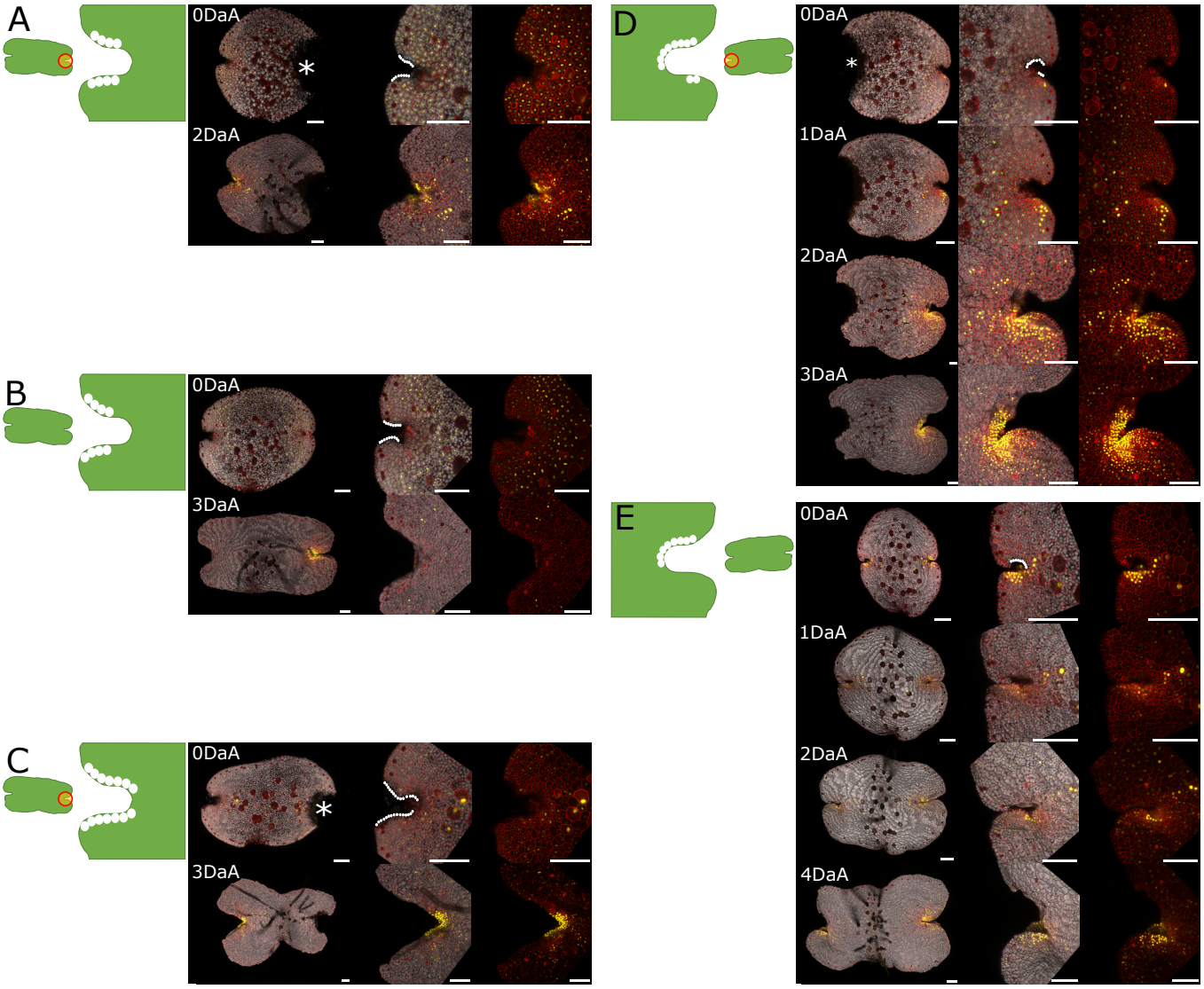

Figure 2. The apical notch can remodel even after partial ablation across the notch side and base, independent of the status of the other gemma apical notch. (A) Ablation of the notch sides, leaving the notch base (including the "Apical Cell") intact, does not disrupt meristem status if the other apical notch in the gemma is excised. (B) if the other apical notch is intact then meristem function ceases because of apical dominance. (C) Meristematic activity continues even if more of the apical notch is ablated and fewer cells are left intact at the base, as long as the other notch is excised. (D) and (E) demonstrate that partially ablating the notch such that the intact portion spans across the base and one side of the notch, but excludes the "Apical Cell" location, results in remodelling of the apical notch and maintenance of meristematic function, as indicated by continuing mVenus expression. The apex of the notch changes so that it is found where the centre of the intact portion was. This is irrespective of whether the other notch in the gemma has been excised (D) or is intact (E). In each figure a schematic outline of the ablation pattern is shown with white circles marking the location of ablated cells and orange bounded by red denoting excised tissue. A time course is given in the format whole gemma, notch close up, notch close-up without the chlorophyll autofluorescence channel. Gemmae were imaged at the time points indicated. In the 0DaA images, white circles mark ablated cells, asterisks denote excised notch. The enhancer trap notch/meristem marker lines used were ET239-P21 (A, B, D, E) and ET239-P153 (C). Scale bars=100µm.

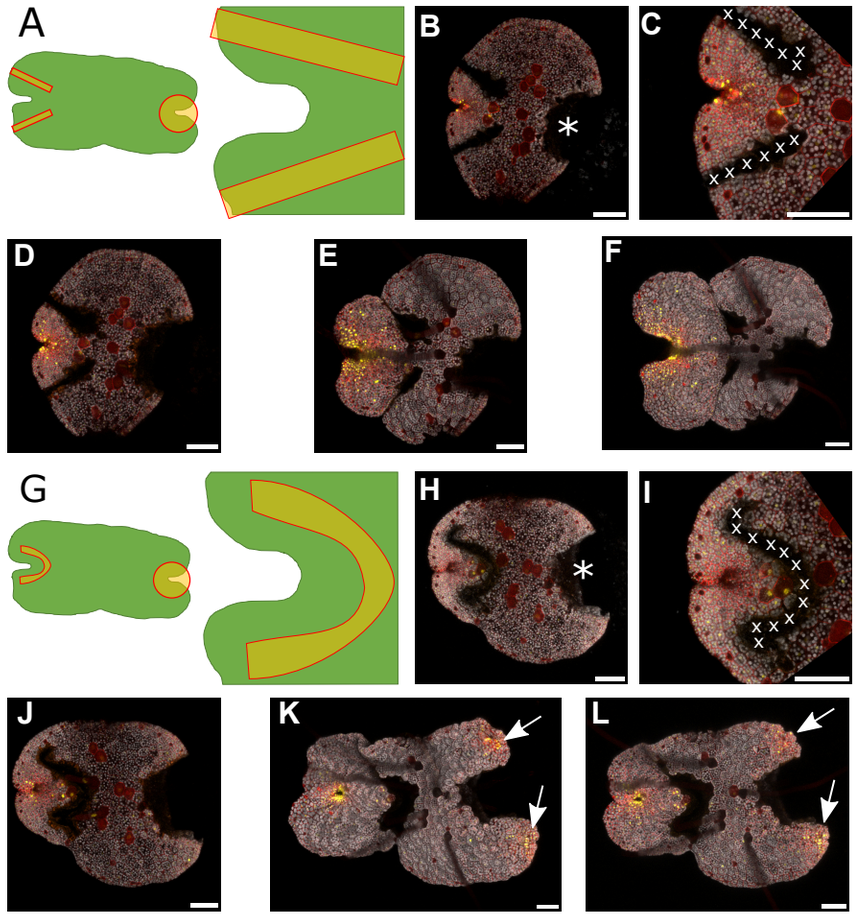

Figure 3. Apical notch isolation demonstrates that there is heterogeneity across the gemma for carrying signals from the apical notch. (A) shows an outline schematic of the ablation pattern used on the 0dpg gemma shown in (B) (whole gemma) and (C) (close-up of notch). (D)-(F) shows a time course from 1DaA (D), through 2DaA (E) until 3DaA (F). The isolated notch continues to be meristematically active. The gemma fragment attached to this notch by a central connection does not show any evidence for the emergence of new meristems. (G) shows an outline schematic of the ablation pattern used on the 0dpg gemma shown in (H) (whole gemma) and (I) (close-up of notch). (J)-(L) shows a time course from 1DaA (J), through 2DaA (K) until 3DaA (L). The isolated notch continues to be meristematically active. However, the gemma fragment attached to this notch by the peripheral connections does have new meristems emerging (white arrows). Asterisk marks excised notch, X marks removed tissue, orange bounded in red denotes excised tissue in the outline schematics. Gemmae were from the enhancer trap notch/meristem marker line ET239-P153. Scale bars= 100µm.

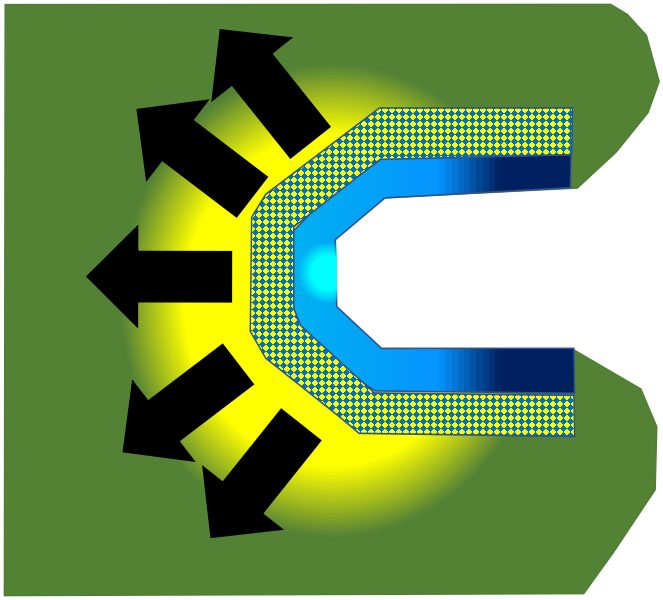

Figure 4. An outline schematic model of meristem organization and regeneration in Marchantia. The first row of cells contains the critical meristem (in blue). The cells at the centre of this population (wherever that centre is) become distinct, from the cells towards the edge of the apical notch (blue gradient). The cells in the two rows behind this are primed to form new meristem (blue hatching). The apical cells emit a signal controlling the balance of cell division and expansion in the region surround the notch (yellow gradient). The meristem emits a repressive signal (auxin) that maintains apical dominance, transmitted through the central region (black arrows) to sink tissues. If this transmission between sources and sinks is disrupted, then the signal accumulates, causing self-repression in the apical region and the loss of meristem identity, while new meristems emerge elsewhere in the gemma.

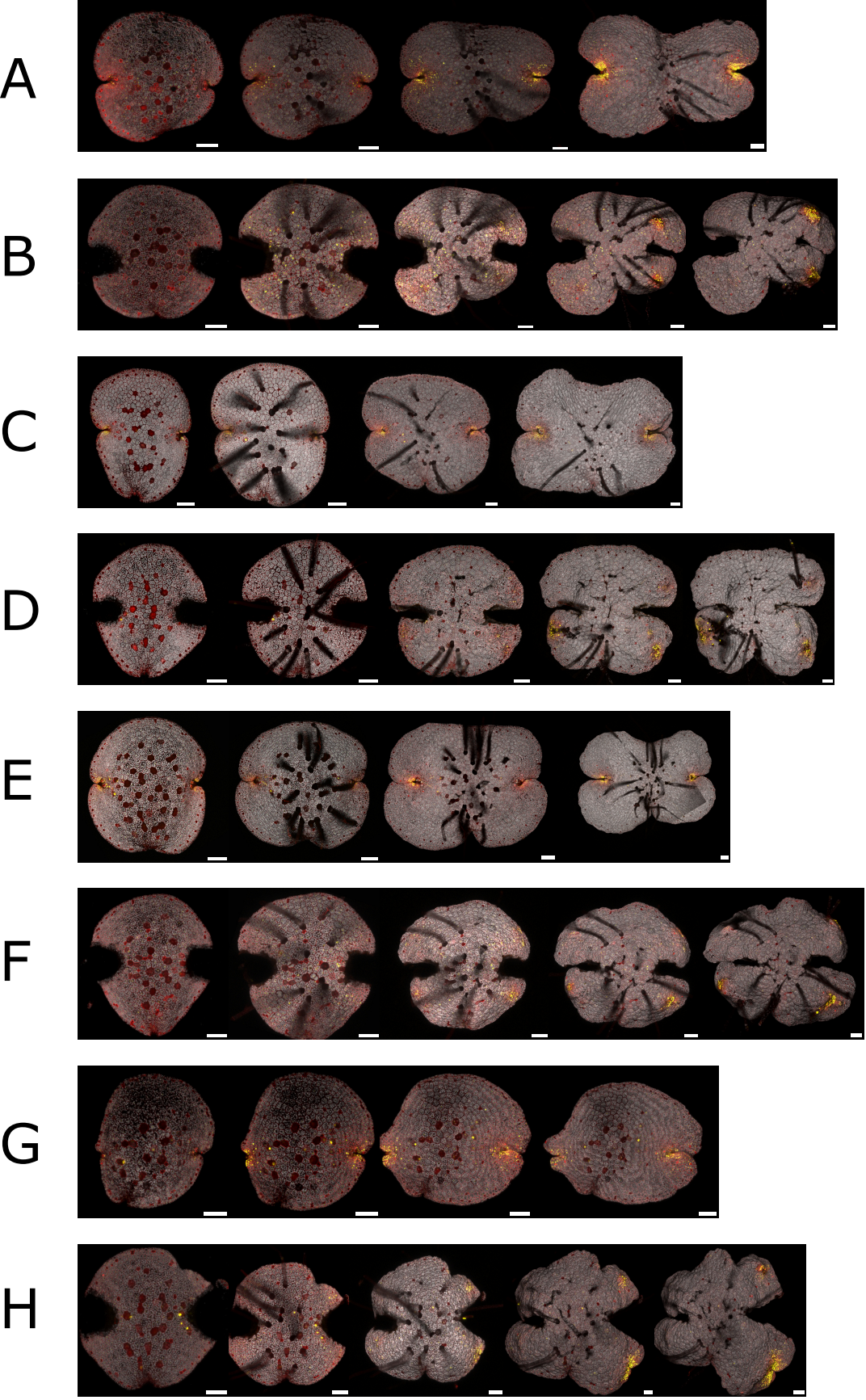

Fig. S1. Enhancer trap Apical Notch/Meristem marker lines used in this study. (A) is an intact gemma from line ET239-P21 imaged daily from 0dpg until 3dpg. (B) is a gemma from line ET239-P21 with notches removed by laser ablation, imaged daily from 0DaA until 4DaA. (C) is an intact gemma from line ET239-P33 imaged daily from 0dpg until 3dpg. (D) is a gemma from line ET239-P33 with notches removed by laser ablation, imaged daily from 0DaA until 4DaA. (E) is an intact gemma from line ET239-P125 imaged daily from 0dpg until 3dpg. (F) is a gemma from line ET239-P125 with notches removed by laser ablation, imaged daily from 0DaA until 4DaA. (G) is an intact gemma from line ET239-P153 imaged daily from 0dpg until 3dpg. (H) is a gemma from line ET239-P153 with notches removed by laser ablation, imaged daily from 0DaA until 4DaA. In all lines, mVenus (in yellow) is strongly expressed in the dense cluster of cells in and around the meristematic apical notch. Reappearance of dense clustered mVenus expression following apical notch removal occurs in all lines and indicates the meristem regeneration. All lin es express a constitutive mScarlet membrane marker (in red) and the chlorophyll autofluorescence in grey. The ablation pattern used is as in Fig. 1C. Scale bars= 100µm.

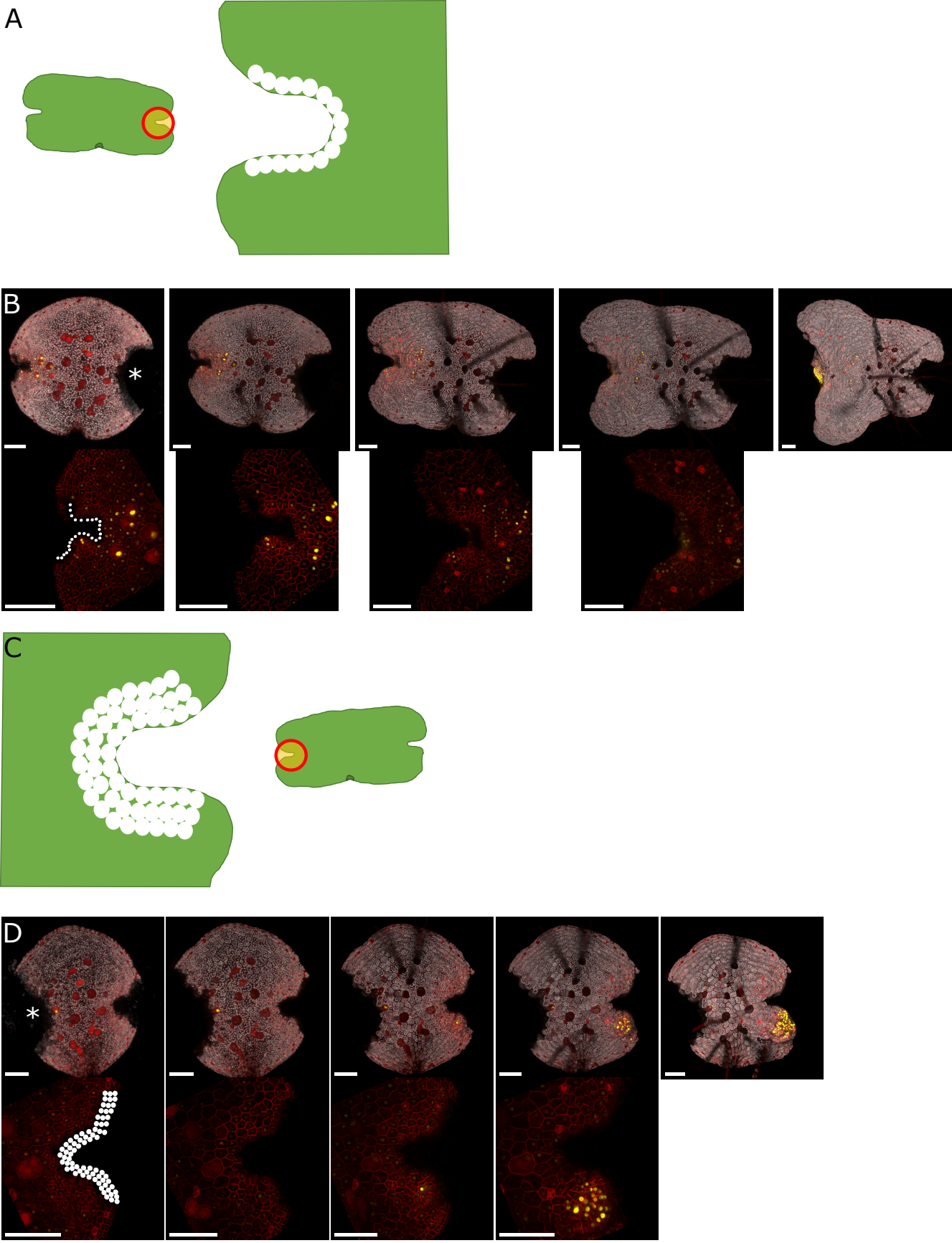

Fig. S2. Shaving cells off from the apical notch shows that the first three rows of cells are critical in determining meristem regeneration and its location. (A) and (C) show schematic outlines for the ablation of the first row and first three rows of cells in the apical notch, respectively. White circles indicate ablated cells, orange bounded by red shows tissue removal. (B) and (D) are gemma from the enhancer trap notch/meristem marker line ET239-P153 imaged daily during a time course from 0DaA until 3DaA (notch close-up) or 4DaA (whole gemma). White circles mark the position of cells removed by laser ablation in the notch close-up images of the 0DaA gemma. One notch was excised entirely (asterisk in the 0DaA whole gemma image). (B) shows a gemma where only the first row of cells in from the edge around the notch was removed. This is sufficient to induce meristem regeneration by 4 DaA, but this occurs at the shaved notch, as seen by the region of mVenus signal. (D) shows a gemma where the first three rows of cells in from the edge around the notch were removed. This was sufficient to allow meristem regeneration to occur elsewhere in the gemma, away from the site of the shaved notch. Scale bars= 100μm.

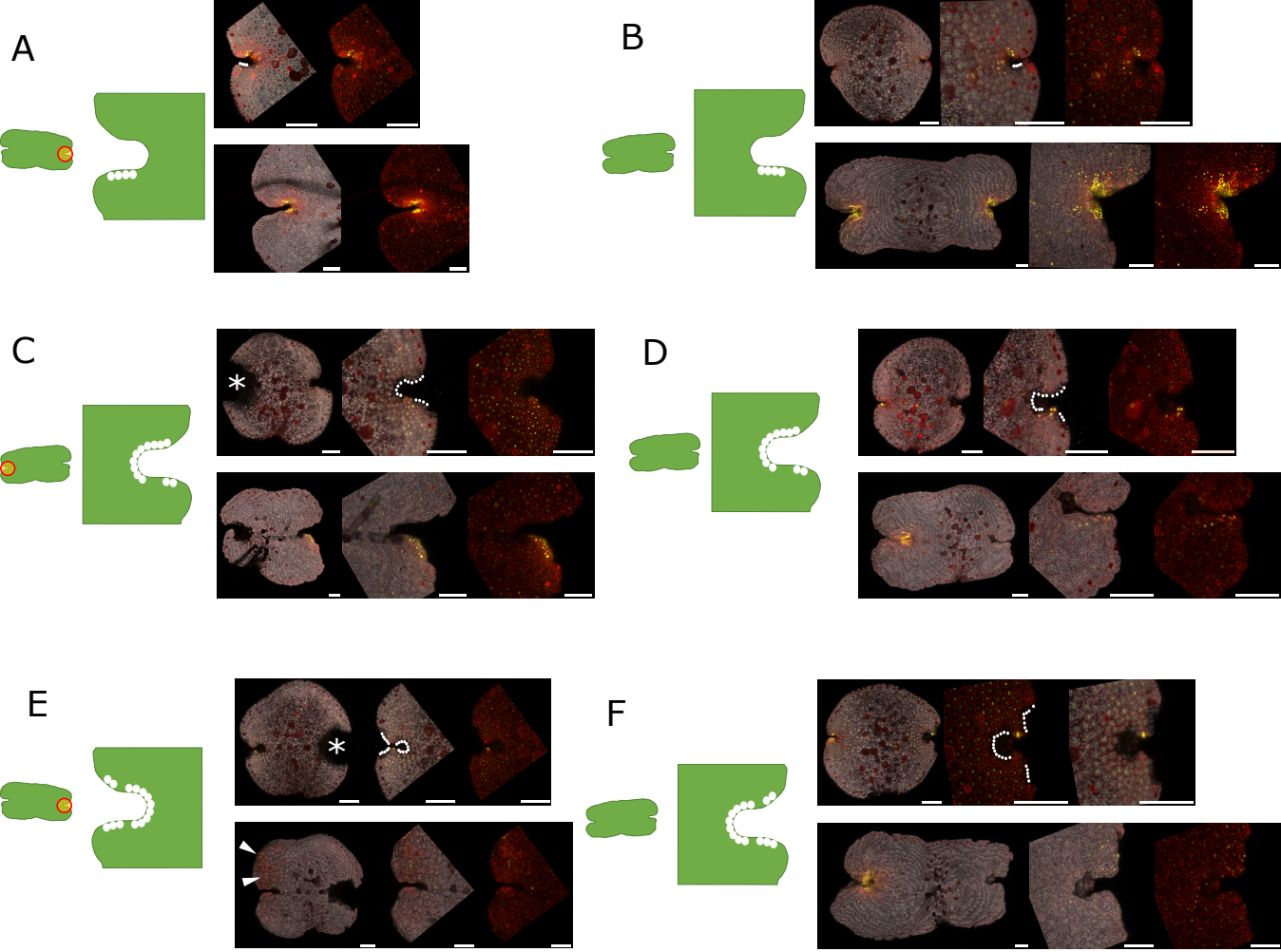

Fig. S3. Precise partial albations of the apical notch. (A) and (B) demonstrate that ablating one side of the apical notch does not disrupt meristematic function irrespective of whether the other notch in the gemma is intact or not. Ablation of the notch (including the "Apical Cell"), leaving only a portion of one side intact does not disrupt meristem function if the other notch is excised (C) but if the other notch is intact (D) then meristematic function ceases. Leaving only a few cells intact on both sides of the notch does not sustain meristematic function, irrespective of whether the other notch is excised (E) or intact (F). If the other notch is excised then meristem regeneration occurs elsewhere in the gemma fragment (arrows, E). This is despite the total number of intact apical cells being greater than in the ablation experiment shown in (C). In each figure a schematic outline of the ablation pattern is given (whole gemma and notch close-up), with images of the 0DaA gemma on the top row (whole gemma, notch close-up, notch close-up without the chlorophyll autofluorescence channel). The bottom row gemma shown are (A) 2DaA, (B) 3DaA, (C) 4DaA, (D) 4DaA, (E) 3DaA, (F) 3DaA. The enhancer trap lines used were ET239-P125 (A), ET239-P21 (B, C, E, F) and ET239-P153 (D). Asterisks denotes removed notch, white circles denotes the location of ablated cells, orange bounded by red marks excised tissue. Scale bars= 100μm.

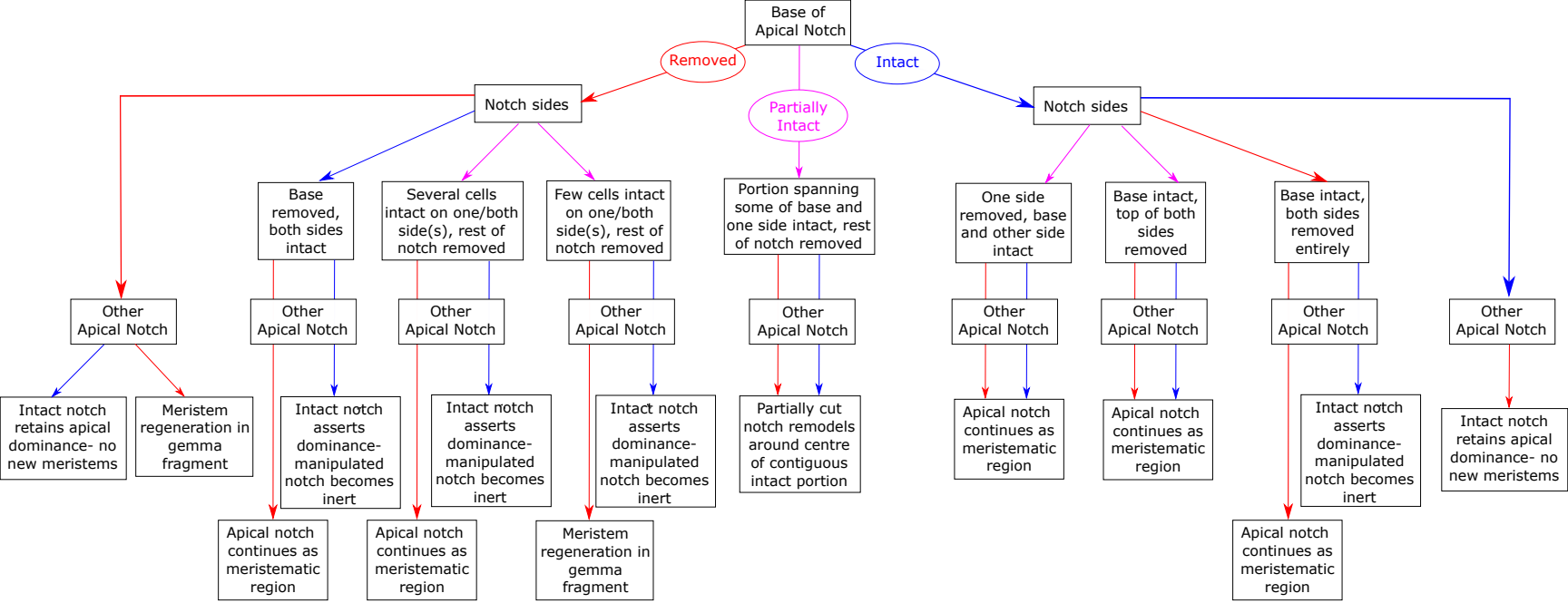

Fig. S4. Flow chart of outcomes of cutting experiments. The chain goes from the status of the base of the notch, through the status of notch sides and to the status of the other notch in the gemma. Red arrows indicate complete removal by laser ablation, magenta arrows indicate partial removal by laser ablation and blue arrows indicate where a structure is left fully intact.

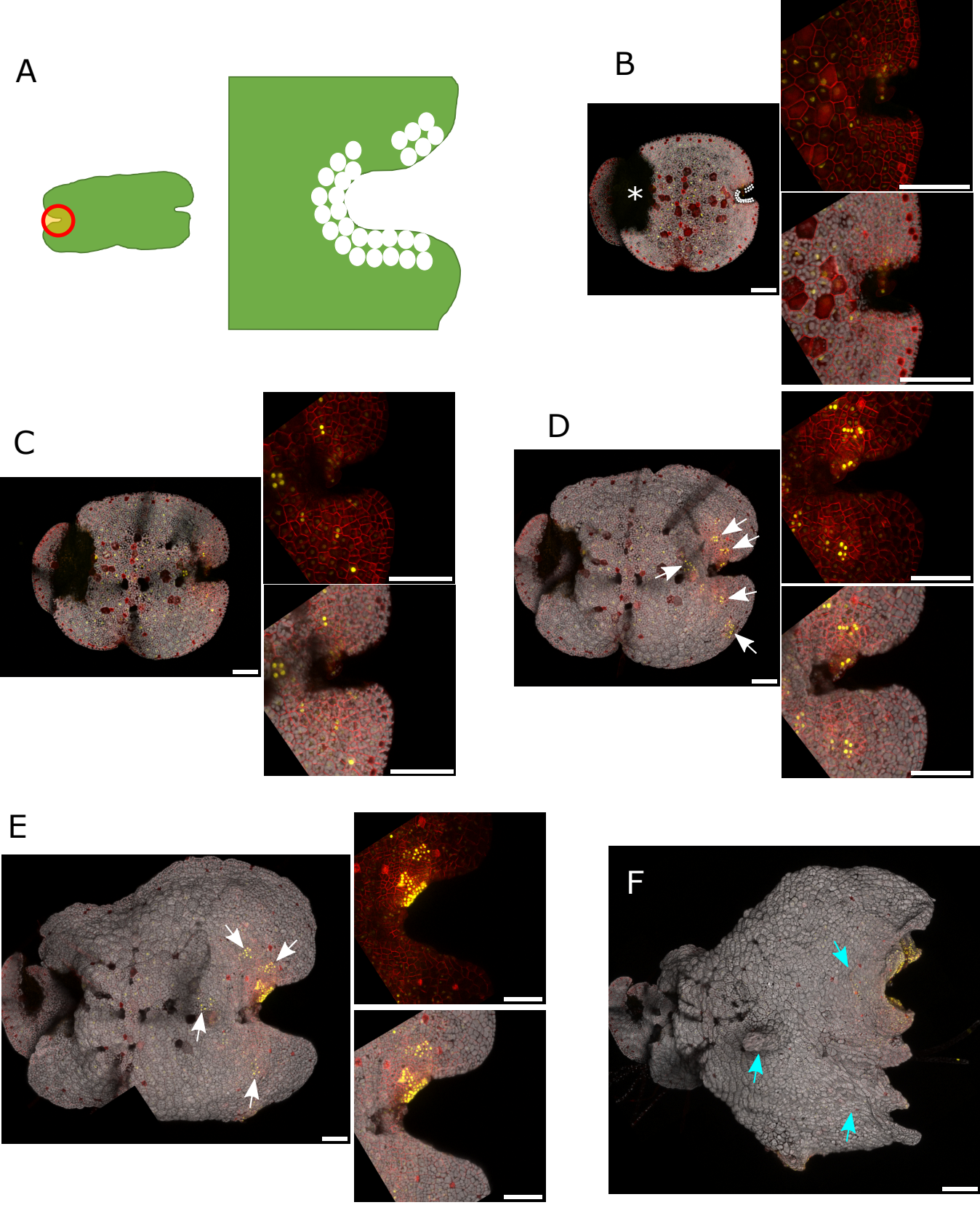

Fig. S5. Re-establishment of apical dominance by a partially ablated apical notch. (A) shows an outline schematic of the ablation pattern used. White circles mark ablated cells, orange bounded by red marks excised tissue. (B)-(F) shows a time course of an enhancer trap notch/meristem marker line ET239-P125 gemma. The images shown are whole gemma, ablated notch close-up and apical notch close-up without the chlorophyll autofluroescence channel. (B) is the 0DaA gemma, with an asterisk marking the excised notch and white circles denoting ablated apical cells. (C) is the same gemma imaged at 1DaA, (D) at 2DaA, (E) at 3DaA. (F) is the whole gemma imaged at 5DaA. Although the original, partially ablated notch remains active (as indicated by the continuation of mVenus marker signal), new patches of cell division emerge at 2DaA (D, white arrows). Some of these persist at 3DaA (E, white arrows). However, by 5DaA the original notch has reformed and re-established apical dominance, as demonstrated by this notch being the only region of the gemma with mVenus marker signal. The areas that were patches of cell division are now inert (no mVenus signal) and are left as protrusions on the gemma surface (cyan arrows). These observations were only recorded in one set of gemma ablations. Scale bars= 100µm.

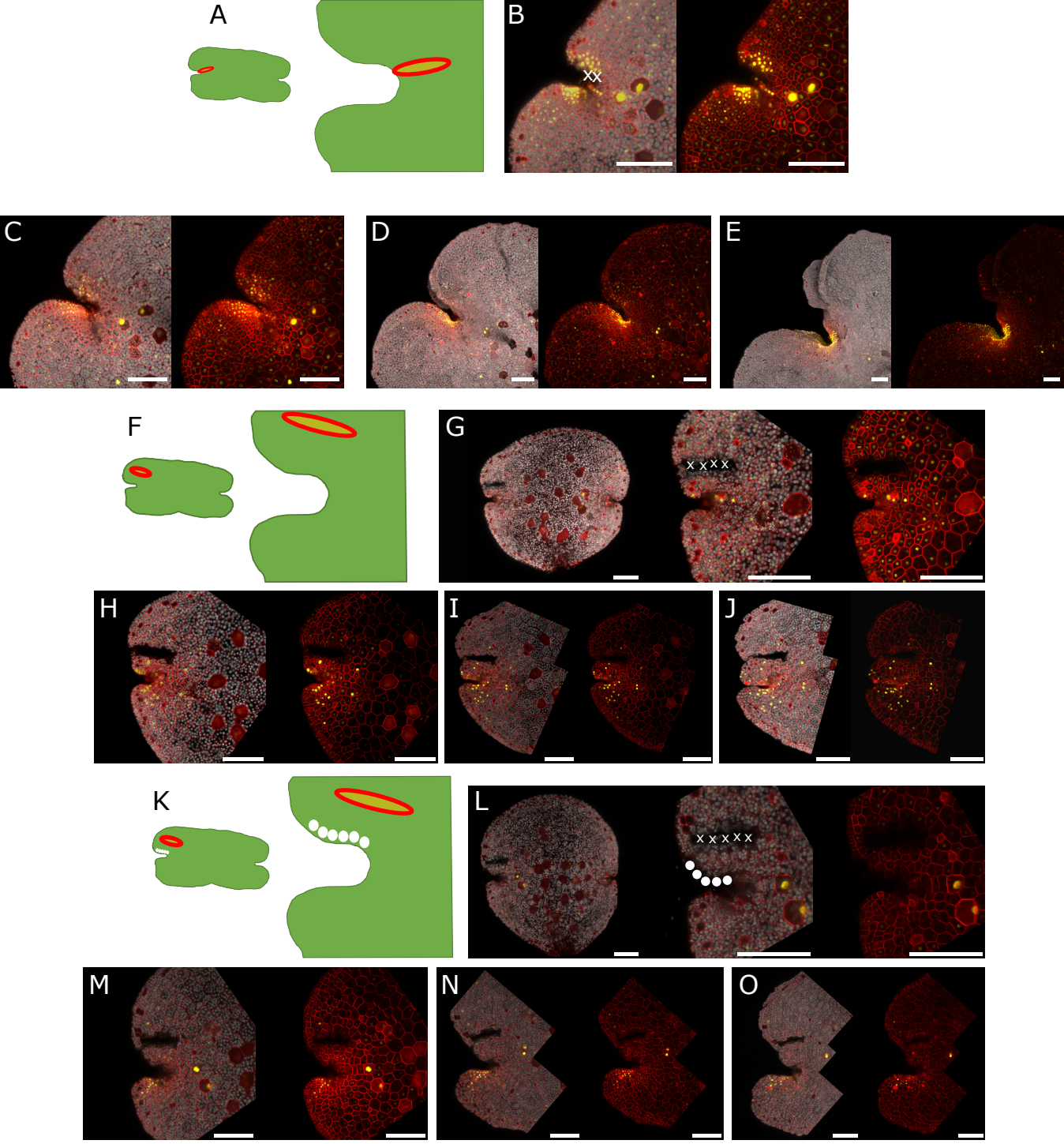

Fig. S6. Ablation perpendicular to the apical notch has little effect, whereas ablation parallel to the apical notch alters the balance between cell division and expansion either side of the ablation site. (A) is an outline schematic of the perpendicular ablation pattern, with orange bounded by red denoting excised tissue. (B) shows a close-up of the ablated notch, with white X marking excised tissue. (C) is the same gemma imaged at 3DaA, (D) at 2DaA, (E) at 3 DaA. Ablation perpendicular to the apical cells has no obvious effect on notch function, other than the z-axis thallus split in the gemma appearing earlier on the ablated side of the notch, at 3DaA. (F) and (K) are schematic outlines of the parallel ablation patterns, with orange bounded by red marking excised tissue and white circles indicating ablated cells. (G)-(J) is a daily time course from 0DaA until 3DaA of a gemma ablated in the patterns shown in (F), denoted by white X in (G) and (L). Proximal to the cut site there is increased cell division, whereas distal to the cut site there is less cell division and more cell expansion, compared to the cells at an equivalent position on the uncut side of the notch. (L)- (O) is a daily time course from 0DaA until 3DaA of a gemma ablated as in (K), where in addition to the ablation parallel to the apical notch (white X in L) the apical cells proximal to the cut site have also been ablated (white circles in L). In this case the cells on both sides of the cut show reduced division and increased cell expansion compared to the cells at an equivalent position on the uncut side of the apical notch. Gemma images are given in the format whole gemma (G and L only), notch close-up, notch close-up without the chlorophyll autofluorescence channel. Gemma used are from the enhancer trap notch/meristem marker lines ET239-P125 for the perpendicular ablation experiment (B-E) and ET239-P153 for the parallel ablation experiments (G-J and L-O). Scale bars= 100µm.

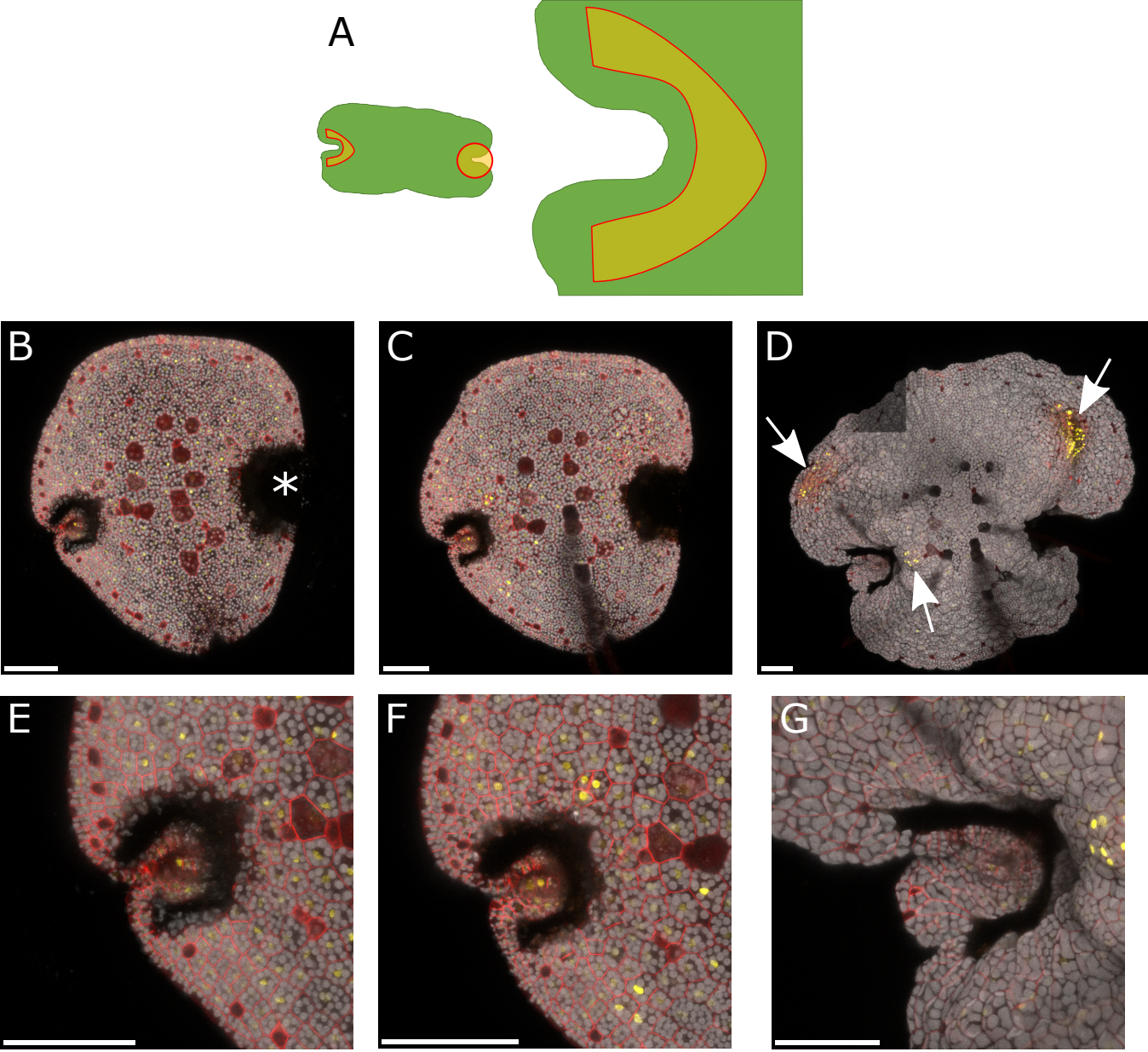

Fig. S7. Self-inhibition of an isolated meristematic notch region. (A) shows an outline schematic where the gemma was ablated so that one notch was excised entirely and an incision was made behind the other notch, such that the notch and only a small amount of surrounding tissue was connected to the rest of the gemma just by the peripheral regions to either side of the notch. Excised tissue is marked by orange bounded by red. (B)-(D) shows the whole gemma (0DaA, 1DaA and 3DaA), asterisk marks excised notch; (E)-(G) shows a close up of the isolated notch region (0DaA, 1DaA and 3DaA). By 3DaA, new meristematic regions were emerging, as shown by the newly formed patches of mVenus expression (white arrows). The isolated notch initially had mVenus expression (i.e., was meristematically active) but by 3DaA this signal had disappeared, with the cells losing meristematic identity, ceasing division and instead expanding. This suggests that the peripheral connections are not capable of exporting the repressive apical dominance signal, and that without a sufficient number of cells in the isolated portion to act as a sink for this signal it causes the isolated notch to self-inhibit. Gemma is from the enhancer trap notch/meristem marker line ET239-P125. Scale bars= 100µm.

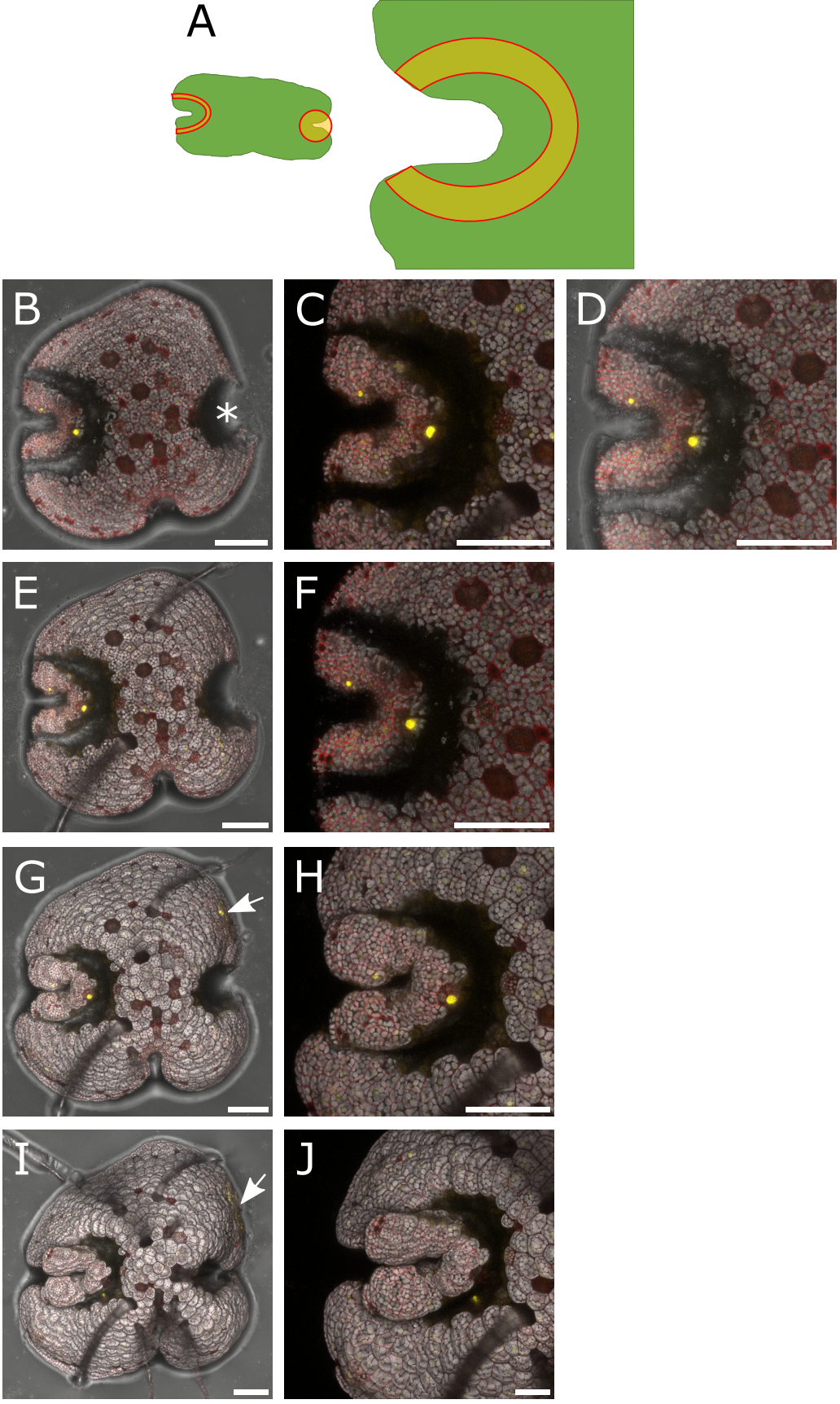

Fig. S8. Entirely ablating the tissue surrounding the apical notch results in the isolated notch to lose meristematic identity and become divisionally inert. (A) shows an outline schematic of the ablation pattern, where one notch was entirely excised and laser ablation used to excise all tissue surrounding the other notch (excised tissue shown in orange bounded by red), which consisted of an intact apical notch and a small number of surrounding cells. The confocal images show a gemma from the enhancer trap notch/meristem marker line ET239-P21 imaged daily during a time course from 0DaA (B,C,D) through 1DaA (E,F), 2DaA (G,H) until 3DaA (I,J). Appearance of new mVenus signal by 2DaA (G) shows that this isolation removed the effects of apical dominance on the rest of the gemma and allowed a new patch of meristem to emerge (white arrow in G and I). The isolated notch itself shows loss of mVenus signal, indicating that this region has lost its meristematic identity. By 3DaA cell division has ceased and the remaining cells expand (J). This indicates that without a sufficient number of cells in the isolated portion to act as a sink for the repressive apical dominance signal it causes the isolated notch to self-inhibit. (B), (D), (E), (G) and (I) include the transmitted light channel in addition to the mVenus, mScarlet and chlorophyll autofluorescence emission channels. Scale bars= 100µm.

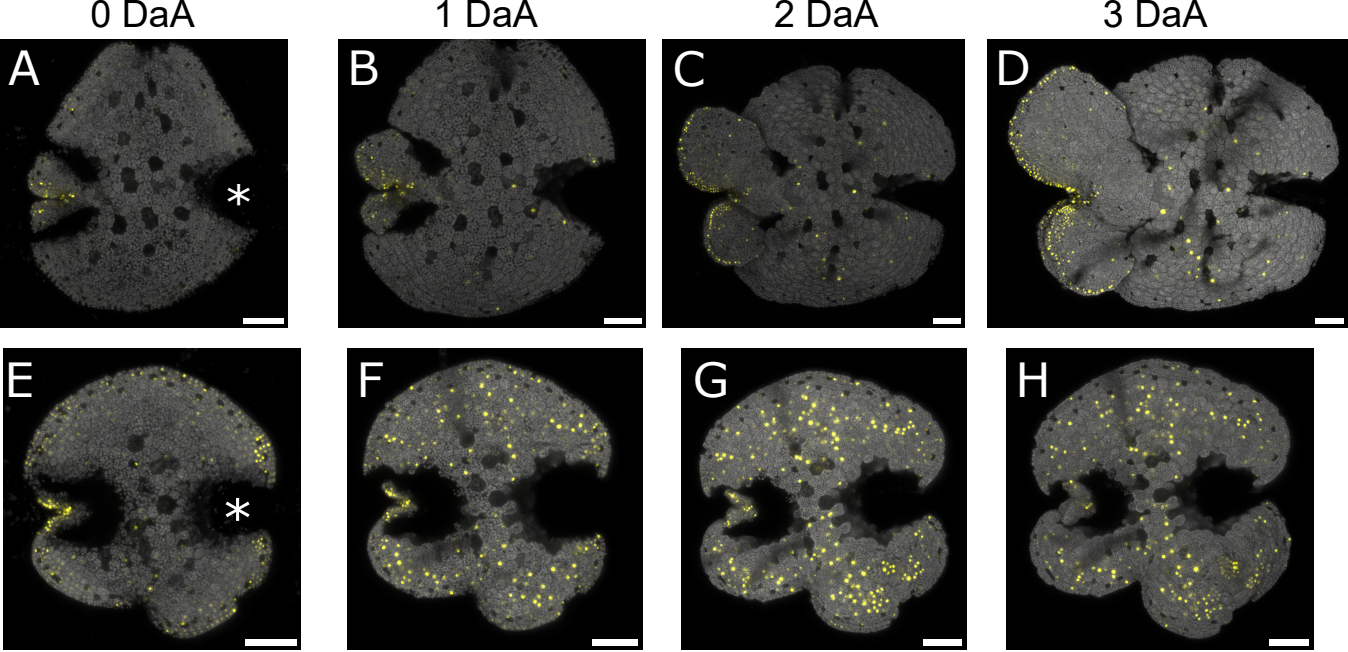

Fig. S9. Notch isolation in an MpPIN1 gemma. Laser ablation was used to excise one notch entirely (marked by asterisk) and to make incisions in the gemma tissue such that the other remaining notch was isolated, save for a connection formed from central (A-D) or peripheral (E-H) tissue. Gemma were followed over a daily time course from 0DaA (A, E), through 1DaA (B, F), 2DaA (C, G)until 3DaA (D, H) as indica ted. In the gemma with central connections, MpPIN1 expression is clearly localized at the notch and, as new tissue is formed, at the gemma edge with only occasional expression in the central zone. MpPIN1 expression appears to be absent in the central connection itself. No new meristems emerge in these plants (see Fig. 3A-F). In the gemma with peripheral connections, new regions of cell division appear throughout the gemma, as indicated by proliferation on MpPIN1 expression throughout the gemma, and the original notch shows reduction in MpPIN1 expression. By 3DaA (H) the original notch is not dividing as a meristem and new meristematic regions are appearing, as shown by the clusters of MpPIN1 expression (also see Fig. 3G-K). Though MpPIN1 is strongly localized to the region forming the peripheral connection early on (E, F), this connection apparently does not conduct sufficient signal from the intact notch to maintain apical dominance. This suggests that PIN-type auxin transporters are not responsible for conducting the apical dominance signal. Scale bars= 100µm.

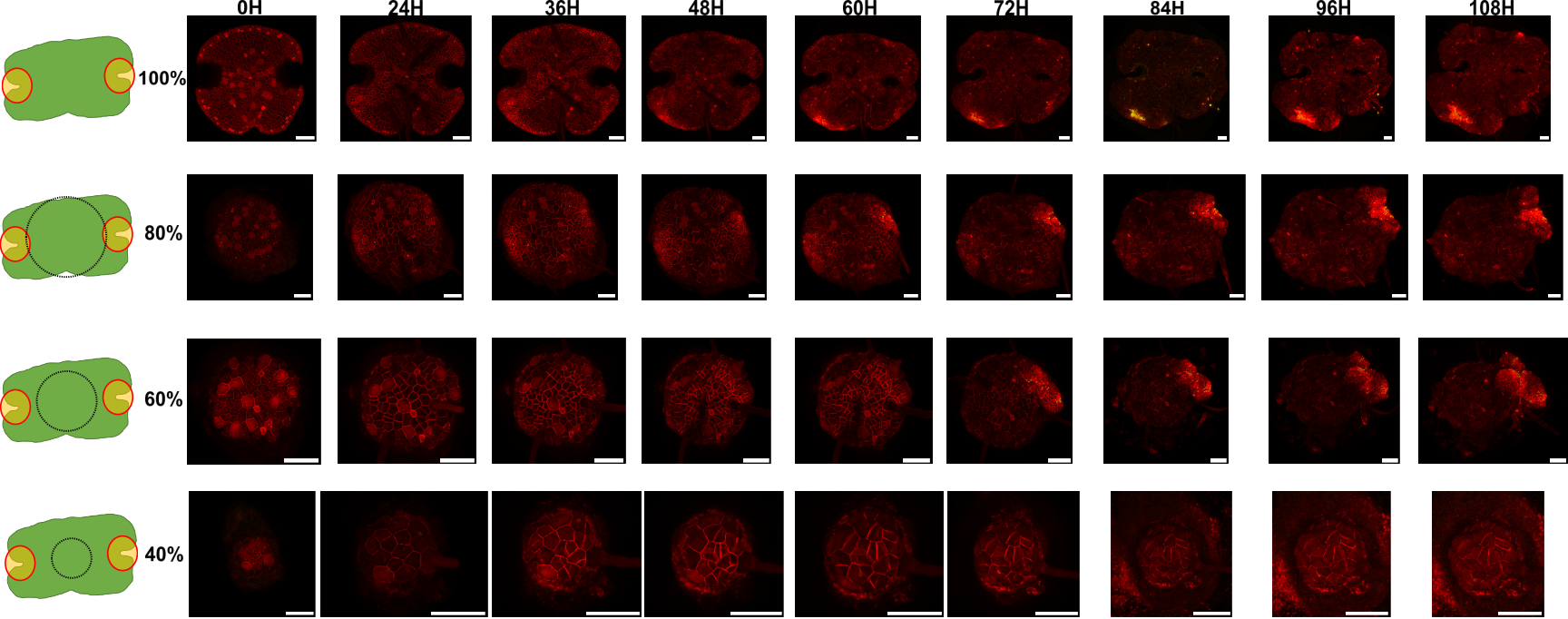

Fig. S10. Size versus Regeneration Speed in the Enhancer Trap Notch/Meristem Marker Line ET239-P125. On the far left of each row is an outline schematic of the ablation pattern used, with orange bounded by red indicating excised tissue. The dotted line shows the laser ablation circle trace used, with a diameter calculated as a percentage of the notch-notch distance. Only the gemma portion within the circle trace was retained, with all tissue outside of this destroyed. Meristem regeneration, as indicated by the appearance of mVenus marker signal, occurs faster in larger fragments versus smaller fragments. This suggests that having a larger pool of cells to act as auxin sinks for the emerging auxin sources (meristems) allows for faster progression through Stages 2 and 3 of regeneration. Time indicated is in hours after ablation. Scale bars= 100μm.

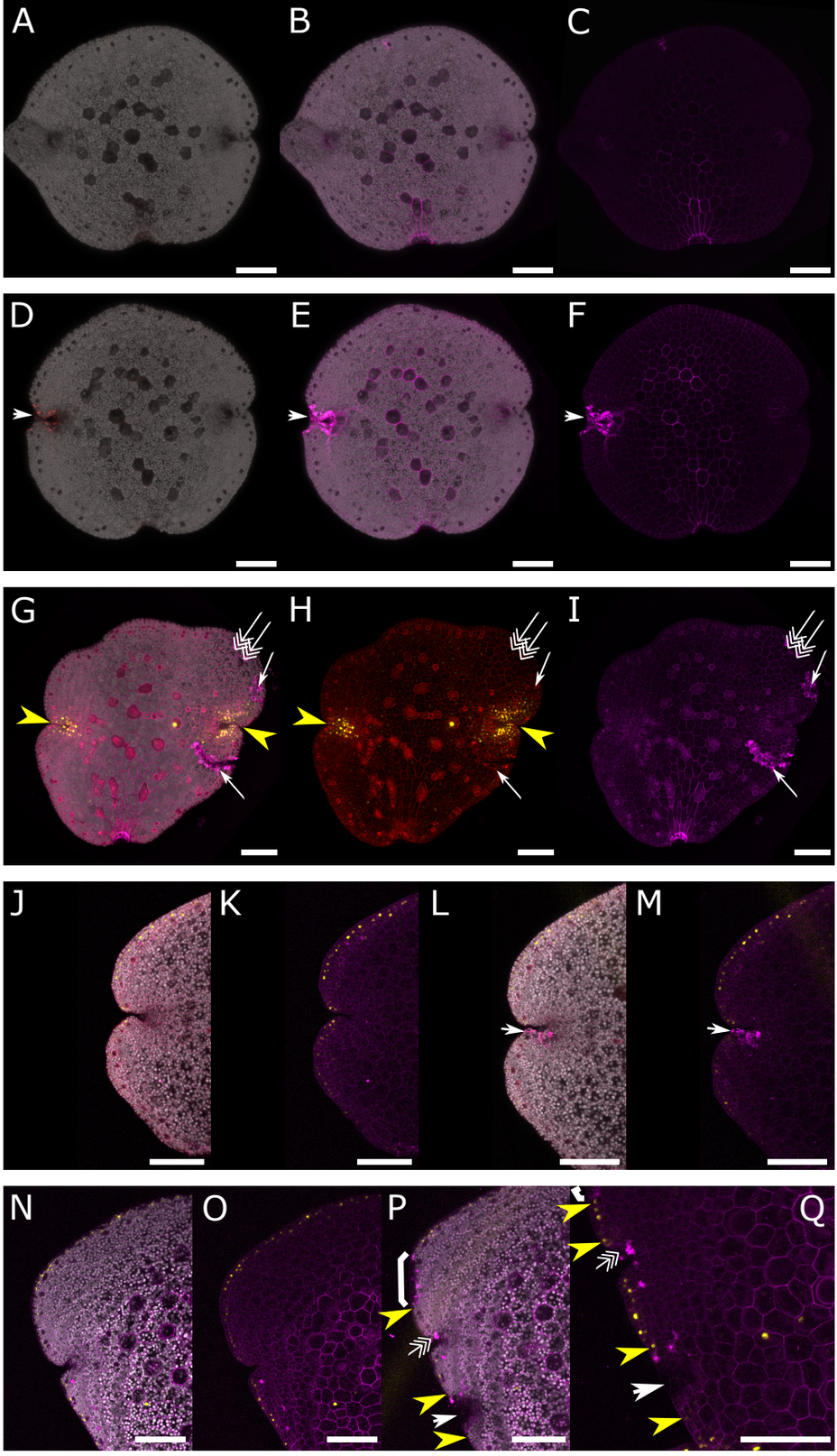

Fig. S11. Propidium iodide staining shows that laser ablation effectively kills cells without causing widespread cell death or stress to the wider gemma. Propidium iodide (PI) staining is shown in magenta. PI stains cell walls but does not penetrate the cell membranes of living cells, however upon cell death it can penetrate into the remains of the cell, upon which it stains the nucleus (with emission into the mScarlet channel wavelengths). (A)-(C) shows an intact WT gemma, with no nuclear PI staining. (D)-(F) shows that an apical notch subjected to laser ablation has nuclear and intracellular PI staining, demonstrating that this treatment effectively kills cells in the Marchantia gemma. The damage (also indicated by loss of chlorophyll autofluorescence and mechanical disruption) is restricted to the region targeted by the laser, with neighbouring cells and the wider gemma left intact and alive. (G)-(I) shows a gemma from an enhancer trap line (ET239-P14) following various laser ablation targeting treatments. Excision of tissues (white arrows) only kills cells that feature multiple other obvious signs of cell damage (loss of chlorophyll autofluorescence, membrane destruction and mechanical disruption) and does not cause widespread cell death or stress across the wider gemma. Single cell targeting ablation (double white arrows) only kills the cells targeted. Laser ablation treatment does not have off-target effects on mVenus marker expression, for example around the notches (yellow arrows). (J) and (K) show a gemma region before ablation; (L) and (M) show the same region afterwards. The lower side of the apical notch has been ablated (white arrows in L and M) with no damage caused to the other side of the apical notch. (N) and (O) show a gemma region before ablation, (P) shows the same region after ablation and (Q) shows the same gemma in a higher magnification post-ablation image. The ablation patterns used were edge ablation (white brackets), single cell targeting ablation (double white arrows) and tissue excision (white arrows). Only the areas directly targeted show cell death, with agreement between PI staining, loss of chlorophyll autofluorescence and mechanical disruption. Neighbouring cells are unaffected, with enhancer trap marker signal continuing unaffected (yellow arrows). (J)-(Q) show gemma from the enhancer trap line ET239-P64. (A) and (D) show the chlorophyll autofluorescence and mScarlet emssion channels overlaid; (B) and (E) show chlorophyll autofluorescence and PI emission channels overlaid; (C), (F) and (I) show PI emission channels only. (G), (J) and (L) show chlorphyll autofluorescence, mScarlet, PI emission and mVenus channels overlaid. (H) shows mScarlet and mVenus channels overlaid. (K), (M), (O) and (Q) shows PI emssion and mVenus channels overlaid. (N) and (P) show chlorophyll autofluorescence, PI emission and mVenus channels overlaid. Scale bars= 100µm.

Supporting Information

Transgenic plant lines used for each figure. ET numbers (ET239-PXXX) refer to the Marchantia enhancer trap screening project (ref. 32)

Fig. 1 D: L2_239-CsA (ET239-P64) F: L2_239-CsA (ET239-P153)
Fig. 2 L2_239-CsA (A: ET239-P21 B: ET239-P21 C: ET239-P153 D: ET239-P21 E: ET239-P21)
Fig. 3 L2_239-CsA (ET239-P153)

Fig. S1 L2_239-CsA (A, B: ET239-P21 C,D: ET239-P33 E,F: ET239-P125 G,H: ET239-P153)
Fig. S2 L2_239-CsA (ET239-P153)
Fig. S3 L2_239-CsA (A:ET239-P125 B: ET239-P21 C: ET239-P21 D: ET239-P153 E: ET239-P21 F: ET239-P21)
Fig. S5 L2_239-CsA (ET239-P125)
Fig. S6 L2_239-CsA (B-E: ET239-P125 G-O: ET239-P153)
Fig. S7 L2_239-CsA (ET239-P125)
Fig. S8 L2_239-CsA (ET239-P21)
Fig. S9 L2_268-CsA
Fig. S10 L2_239-CsA (ET239-P125)
Fig. S11 A-F: Wild Type (Cam accession) G-I: L2_239-CsA (ET239-P14) J-Q: L2_239-CsA (ET239-P64)

Table S1. Excitation and collection wavelengths used in confocal microscope imaging

Flurophore	Excitation Wavelengths (nm)	Collection Wavelengths (nm)
mVenus	515 (White Light Laser)	522-541
mScarlet	569 (White Light Laser)	589-625
Chlorophyll	442 (Diode)	670-701
Propidium Iodide	488, 535 (White Light Laser)	592 - 650

Dataset S1 (separate file). Annotated sequence description of the plasmids used to generate the transgenic lines used in the experiments: L2_239-CsA (16,019 bp) and L2_268-CsA (19,174 bp). The sequences and annotations are provided in Genbank format.