Directional transport of the phytohormone auxin is a versatile, plant-specific mechanism regulating many aspects of plant development. The recently identified plant hormones, strigolactones (SLs), are implicated in many plant traits; among others, they modify the phenotypic output of PIN-FORMED (PIN) auxin transporters for fine-tuning of growth and developmental responses. Here, we show in pea and Arabidopsis that SLs target processes dependent on the canalization of auxin flow, which involves auxin feedback on PIN subcellular distribution. D14 receptor- and MAX2 F-box-mediated SL signaling inhibits the formation of auxin-conducting channels after wounding or from artificial auxin sources, during vasculature de novo formation and regeneration. At the cellular level, SLs interfere with auxin effects on PIN polar targeting, constitutive PIN trafficking as well as clathrin-mediated endocytosis. Our results identify a non-transcriptional mechanism of SL action, uncoupling auxin feedback on PIN polarity and trafficking, thereby regulating vascular tissue formation and regeneration.
Plant development is characterized by self-organizing processes, such as the regular patterns of organ initiation at the shoot apical meristem, branching of roots and shoots, the connection of newly formed organs with pre-existing vasculature, or the spontaneous occurrence of vasculature veins in developing leaves. The plant hormone auxin and its directional transport through tissues have been implicated in all these traits. The process that is called auxin canalization establishes narrow auxin transport routes between cells and tissues of relatively high auxin concentration (source), to locations where auxin is being depleted (sink). A self-reinforcing system has been proposed to drive canalization. In this system, auxin feeds back on PIN-FORMED (PIN) auxin transporters by promoting the expression of PIN genes specifically in auxin transport routes and by localizing PINs to plasma membrane (PM) domains facing the auxin sink. Auxin is typically transported basipetally from source to sink and canalization seems to be driven by auxin sink rather than source. For example, developing vasculature tissue is characterized by relatively high auxin contents; therefore, sink strength in such a system primarily depends on PIN-dependent auxin flux rates, depleting auxin from sources. New vein patterns in leaves develop away from a localized sink at the base, opposite to the direction of auxin flow. Vasculature formation and its connection to already existing vascular strands hence is intimately linked to the effects of auxin flux on the subcellular positioning of PINs, which in turn defines auxin flux rates and directionality. 

Related feedback mechanisms controlling PIN polarity have been described for additional developmental processes, such as embryonic axis formation, shoot and root organogenesis, as well as the control of the directional growth of organs.

In spite of the biological significance of PIN proteins function, the mechanisms by which auxin controls polarization of PINs have remained conceptually unclear. Modeling of auxin-mediated polarization has linked auxin feedback on PIN polarity to the auxin effect on PIN subcellular trafficking. PM-associated PINs are internalized in clathrin-coated vesicles in a process called endocytosis that might precede PIN relocation to different (plasma) membrane domains. Numerous pharmacological and genetic determinants that impact on specific cellular events in the control of auxin transport have shaped our current picture of cellular mechanisms and crosstalk therein. For example, treatment with the fungal toxin Brefeldin A (BFA) revealed the constitutive endocytic recycling of PIN proteins. This requires BFA-mediated interference of GNOM ARF-GEF activity, causing PM proteins, including PINs, to aggregate in cells. Notably, auxin itself appears to inhibit the process of endocytosis and antagonize the BFA effect on PIN recycling. In addition to auxin effects on PINs, various other plant hormones can influence PIN-dependent auxin transport, such as cytokinins, brassinosteroids, gibberellins, salicylic acid, abscisic acid, and strigolactones (SLs). However, much remains to be uncovered about the modes of action, by which these plant hormones regulate PINs, thereby ultimately defining plant development.

SLs represent a recently discovered class of plant growth regulators and their developmental roles and signaling mechanisms are not yet fully characterized. SLs have been shown to influence a range of plant traits including shoot branching, shoot gravitropism, secondary growth, adventitious rooting, as well as lateral rooting and root hair elongation. Many of the processes targeted by SLs also require auxin transport, specifically its canalization as proposed for the classical SL effect on shoot branching. This response has been linked to SL-mediated interference of PIN PM targeting and the modulation of auxin flux, but we still lack insight into the mechanisms by which SLs might impact the sorting of PINs.

Our observations in this study extend the spectrum of physiological SL effects on processes associated with auxin transport canalization, namely leaf venation and vascular tissue regeneration and formation induced by wounding or external auxin sources. At the cellular level, we show that SLs specifically interfere with the feedback of auxin on PIN polarization and clathrin-mediated internalization, providing a mechanistic framework for the molecular action of SLs in many developmental processes.

Results

SLs interfere with auxin canalization in pea. Inhibition of shoot branching is among the best-understood responses of SLs in flowering plants. This is a process that involves auxin canalization, because when buds are released from dormancy, they initiate the formation of PIN1-expressing channels to increase vascular connections with the main vasculature. These channels appear similar to those that form after adding exogenous auxin to the side of the stem. The canalization events can be inhibited by auxin produced in shoot apices, hinting at a possible mechanism, by which dominant shoot tips might control branching. When SLs are applied directly to buds after decapitation, they inhibit bud outgrowth and reduce the transport of auxin (indole-3-acetic acid, [3H]-IAA) from buds into the stem (Supplementary Fig. 1a–d). However, the precise action of SLs in controlling auxin canalization and vascularization is less obvious. Therefore, we explored the effect of synthetic SL, rac-GR24 (hereafter called GR24) using intact or fully decapitated pea (Pisum sativum) plants that had been treated with auxin (indole-3-acetic acid; IAA).

First, we analyzed PIN1 channel and subsequent vasculature formation originating from an artificial lateral auxin source. Lateral, local auxin application in lanolin paste onto pea stems just below a cut (Fig. 1a) was sufficient to induce the formation of PIN1-expressing auxin channels and subsequent vascular connections to the stem vasculature. In our control situation, strong PIN1 expression in the vicinity of the local IAA application was observed with a predominantly lateral PIN1 localization, pointing away from the auxin source (Fig. 1b). An initially large area of PIN1-expressing cells narrowed down about 5 days after auxin application, resulting in the establishment of fully defined and strongly polarized narrow PIN1 channels, often accompanied by differentiated xylem vessels (Fig. 1b). This is in agreement with the classical canalization hypothesis in the absence of competing auxin sources. In contrast, co-application of GR24 interfered with strongly polarized PIN1 expression as well as with the formation of PIN1 channels and continuous de novo vasculature; only occasional fragmented xylem cells appeared instead (Fig. 1b).

Related observations were made when we analyzed PIN1 expression in fully decapitated pea stems. IAA application to the stump (Fig. 1c) led to a massive increase of PIN1 expression in the polarized field below the application site within the first 3 days, while the formation of narrow PIN1-expressing channels accompanied by differentiated xylem strands became visible after 5 days (Fig. 1d). The simultaneous application of GR24 strongly inhibited this process, as we failed to observe a pronounced increase in polarized PIN1 expression and channel formation under these conditions (Fig. 1d).

Together, our findings suggest an inhibitory role of SLs in the formation of new auxin-conducting, PIN-expressing channels induced from auxin sources. This effect of SLs on auxin canalization would offer a plausible explanation for how SLs regulate auxin transport, vascularization and branching.
SLs inhibit vasculature formation and regeneration. To further explore the role of SLs in other processes that have been mechanistically linked to canalization, we examined canalization-dependent vasculature regeneration following wounding, which has recently been established in Arabidopsis (Arabidopsis thaliana) stems (Fig. 2a). This allowed us to use the extensive genetic toolkit in the model species, and also allowed us to test plant-produced, endogenous SLs rather than relying on synthetic SLs.

In control experiments, we observed vascular regeneration initiated with a broad PIN1 expression field and auxin accumulation above the wound about 2 days after wounding. This was subsequently followed by the establishment of narrow, polarized PIN1-expressing, auxin-conducting channels circumventing the wounded site during the next days (Supplementary Fig. 2a). Strikingly, vasculature regeneration in the SL biosynthesis mutants more axillary growth (max)1-I and max4-1 occurred as fast as in control (Supplementary Fig. 2b), but the regenerated vasculature in the mutants was more abundant than that in the wild-type control (Fig. 2b). To test the effect of increased endogenous SLs, we employed conditional overexpression of the cytochrome P450 monoxygenase MAX1 in the max1 mutant background (DEX>MAX1 max1-1; hereafter named DEX>MAX1). In un-induced control plants, the first vessels along the wound appeared after 4 days and fully regenerated vasculature was observed after 6 days (Fig. 2c, Supplementary Fig. 2c). In contrast, although we detected clusters of isolated vessel-like cells that developed from callus in both untreated and dexamethasone (Dex)-treated plants (Fig. 2c), there was no regeneration of vasculature around the wound observed after Dex induction (Fig. 2c, Supplementary Fig. 2c). These results are in line with the observations that we made in pea, substantiating an inhibitory role for SLs in the regulation of canalization-mediated vasculature regeneration.

We also determined the efficiency of vasculature regeneration in mutants affected in SL/karrikin-related signaling, including a mutant allele affected in the MAX2 F-box protein (max2-3) and a double mutant affected in SL/karrikin receptors dwarf14-1 hyposensitive to light-3 (dl4-1 htl-3)42. In both genotypes, regeneration occurred faster (Fig. 2d) and the regenerated strands were more abundant compared with wild-type control (Supplementary Fig. 2d), suggesting that SL/karrikin signaling normally restricts vasculature regeneration.

To directly assess whether SL/karrikin signaling is involved in auxin channel formation, we analyzed the expression of the DR5 auxin response reporter (DR5rev::GFP) during regeneration. Comparison between wild type and max2-3 revealed that the DR5-positive channels formed faster and more abundantly when SL/karrikin signaling was compromised (Fig. 2e, f). Consistently, the layer of regenerated vasculature was also formed earlier and thicker in the max2-3 mutant (Fig. 2e, f).

Together, these results identify SLs as crucial regulators of vasculature regeneration after wounding, and that increased SL levels inhibit, whereas decreased SL biosynthesis or compromised SL signaling promotes, canalization-mediated vasculature regeneration. Another presumably auxin canalization-dependent process that involves vasculature patterning along auxin channels is de novo leaf venation formation2,6,8,43. We questioned whether SLs might participate in this process as well, and thus examined leaf vascular development in presence of GR24 or upon induction of endogenous SL biosynthesis. After growth on GR24, simplified leaf vascular network patterns with occasional discontinuities were detected (Supplementary Fig. 3a, b). Dex-treated DEX>MAX1 plants also caused more simplified leaf veins with more free ends (Supplementary Fig. 3c, d). This two-component glucocorticoid system can occasionally cause non-specific growth defects44. However, our Dex-treated transgenic plants expressing only the chimeric GAL4-VP16-GR (GVG) transcription factor grew normally and leaf vasculature was unaffected (Dex: 11.1 free-ending veins per leaf, n = 20 leaves; Control: 10.7 free-ending veins per leaf, n = 20 leaves). Therefore, these data support the
Fig. 2 SL regulation of vasculature regeneration after wounding in Arabidopsis stems. a Scheme representing spatial changes around a wound during vascular tissue regeneration in incised stems of Arabidopsis. Wounding is made in the basal part of inflorescence stem just above the rosette leaves to disturb the longitudinal continuum of the vascular cambium. Green line represents development of regenerated vessel strands around a wound. Red line represents auxin-mediated channels formation. Green circles represent the groups of vessel-like cells developed from outer cortex or callus in the neighborhood of the wound. b Vascular tissue regeneration in SL biosynthesis defective mutants max1-1 and max4-1. Line segments indicate the thickness of regenerated vasculature; above the wound (1), close to the wound (2), below the wound (3). Scale bars: 100 µm. c Vascular tissue regeneration in wounded DEX–MAX1 plants. Data are expressed as mean ± s.e.m. (n ≥ 22 inflorescence stems). Means with different letters are significantly different at P < 0.05 (one-way ANOVA with Fisher LSD test). d Vascular tissue regeneration in SL/karrikin signaling-defective mutants max2–3 and d14–1 htl–3. Data are expressed as mean ± s.e.m. (n ≥ 15 inflorescence stems). Means with different letters are significantly different at P < 0.05 (one-way ANOVA with Fisher LSD test). e, f The formation of auxin channels around a wound as inferred from DR5rev::GFP expression during vascular tissue regeneration. Data are expressed as mean ± s.e.m. (n ≥ 24 inflorescence stems). Means with different letters are significantly different at P < 0.05 (one-way ANOVA with Fisher LSD test). Right panels in f are merged images of fluorescence and light transmitted signals. Arrowheads indicate abundant channels. Asterisks indicate regenerated vasculature. Scale bars: 100 µm. The above experiments were repeated twice with similar results. Images shown are representative of each treatment. Source data of c–e are provided in the Source data file.

notion that SLs regulate vasculature regeneration as well as de novo formation during venation patterning in leaves.

SLs interfere with auxin-mediated PIN polarization. The mechanism by which a local auxin source promotes the formation of auxin channels and vascularization is largely unknown. The classical canalization hypothesis proposes positive auxin feedback on auxin transport directionality, which can be realized at the cellular level by the effect of auxin on PIN polar distribution. This can be visualized by auxin-mediated PIN polarity rearrangements in Arabidopsis roots. In primary roots, PIN2 localizes to the apical side of epidermal cells, and preferentially to the basal cell side in the young cortex cells. Auxin (synthetic 1-naphthaleneacetic acid; NAA or natural; IAA) treatments led to rearrangement in PIN2 distribution to the outer lateral sides of cortex cells. This PIN lateralization effect of auxin can regulate PIN polarity and, in particular, how a localized auxin source can lead to the coordinated polarity changes in a whole field of cells, is conceptually unclear. Modeling of canalization processes at the organ and tissue levels, but also auxin-mediated polarization of PIN transporters at the level of individual cells.

SLs interfere with auxin effect on PIN endocytic recycling. How auxin can regulate PIN polarity and, in particular, how a localized auxin source can lead to the coordinated polarity changes in a whole field of cells, is conceptually unclear. Modeling of canalization and PIN polarization suggests that auxin feedback on PIN polarity can be related to the known inhibitory auxin effect on PIN internalization in individual cells. PIN proteins are known to constitutively cycle between the PM and the endosomes.
This cycling is sensitive to BFA\textsuperscript{18}, which preferentially inhibits PIN trafficking to the PM\textsuperscript{22,47}, leading to the intracellular accumulation of constitutively cycling PIN proteins\textsuperscript{18}. Previous studies have shown that PIN endocytosis and constitutive recycling are important in determining PIN polarity\textsuperscript{48–50}, and intracellular PIN accumulation is rapidly and transiently inhibited by auxin itself\textsuperscript{15}.

We investigated the SL effect on auxin-mediated inhibition of PIN endocytic recycling. As shown previously\textsuperscript{15}, PIN proteins accumulated intracellularly after BFA treatment and such internalization was inhibited by NAA (Fig. 3e). GR24 treatment showed no effect on BFA-induced PIN intracellular accumulation (Supplementary Fig. 4a, b), but it clearly interfered with NAA-mediated inhibition of PIN internalization. This was reflected by
increased accumulation of PIN1 and PIN2 in BFA-induced compartments, upon co-treatment with NAA/GR24 (Fig. 3e, f, Supplementary Fig. 4c–e). Similarly, 5DS also interfered with the auxin effect on the BFA-induced PIN intracellular accumulation (Fig. 3h, Supplementary Fig. 4f).

Note that some of these short-term pharmacological experiments required high concentrations of rac-GR24. High GR24 concentrations may impact on photoreceptor pathways31 and the use of rac-GR24 may lead to non-SL responses due to stereoisomer specificity52. However, 5 μM GR24 or higher can be required to trigger responses, particularly in roots37,53–55. We aimed to resolve these issues by testing the transgenic line DEX×>MAX1 that stimulates endogenous SL biosynthesis29, and also comparing GR24 treatment responses with SL mutants. The same antagonistic SL effect on auxin-mediated inhibition of PIN internalization was observed in Dex-treated DEX×>MAX1 line (Supplementary Fig. 4g–k). Furthermore, we tested whether the effect of SLs on PIN trafficking depends on SL signaling components. In the absence of GR24, BFA-induced PIN internalization or NAA-mediated inhibition was similar in the max2 SL/karrakin signaling mutant or the d14 SL-specific signaling mutant as that of the wild type (Fig. 3e–g, Supplementary Fig. 4d, e). Importantly, these mutants showed less sensitivity to GR24 in counteracting the NAA action on PIN endocytic trafficking (Fig. 3e–g, Supplementary Fig. 4d, e), which appeared consistent with the results from other pharmacological studies.

In summary, our findings imply that synthetic or endogenous SLs interfere with the antagonistic auxin effect on BFA-induced intracellular accumulation of PINs, by acting via D14- and MAX2-dependent SL signaling.

SLs interfere with auxin effect on endocytosis. PIN proteins are internalized by clathrin-mediated endocytosis (CME)19 and this endocytic pathway is inhibited by auxin through a TIR1-independent mechanism16. Notably, in shoots, SL action has been linked to clathrin-mediated PIN internalization, acting independently of de novo protein synthesis37. To gain further insights into the mode of SL action in uncoupling auxin feedback (Supplementary Fig. 4g–k). Furthermore, we tested whether the effect of SLs on PIN trafficking depends on SL signaling components. In the absence of GR24, BFA-induced PIN internalization or NAA-mediated inhibition was similar in the max2 SL/karrakin signaling mutant or the d14 SL-specific signaling mutant as that of the wild type (Fig. 3e–g, Supplementary Fig. 4d, e). Importantly, these mutants showed less sensitivity to GR24 in counteracting the NAA action on PIN endocytic trafficking (Fig. 3e–g, Supplementary Fig. 4d, e), which appeared consistent with the results from other pharmacological studies.

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SL/karrikin signaling interferes with auxin feedback. SLs and karrikins act via MAX2-dependent signaling, and we therefore questioned whether both signals exert long-term effects on vasculature patterning in response to external auxin sources (droplets of auxin in lanolin). IAA application promoted the formation of PIN1-mediated auxin channels and regeneration of vascular strands in Arabidopsis, whereas GR24 treatment alone had no influence neither on auxin channel nor on vascular tissue formation (Supplementary Fig. 6a, b). Although SL signaling is essential for lateral bud development, in this experimental set-up, regardless of the presence or absence of lateral buds, GR24 effectively inhibited IAA-induced formation of PIN1-GFP channels and vascular strands from the position where IAA and GR24 were co-applied (Supplementary Fig. 6a, b). Notably, antagonistic effects on auxin channel and vasculature formation were also observed in response to karrikinolide (KAR1; Supplementary Fig. 6a, b).

Moreover, we also examined short-term effects of KAR1 on auxin feedback at the subcellular level. As in case with SLs, KAR1 treatment antagonized NAA-mediated PIN2 lateralization (Supplementary Fig. 6c), and attenuated the NAA effect on BFA-induced PIN2 intracellular accumulation as well (Supplementary Fig. 6d). Taken together, these data suggest that MAX2-dependent SL and karrikin signaling interfere with auxin feedback control of canalization at the tissue level as well as PIN polarity and trafficking at the cellular level.

Discussion
Our observations extend the current knowledge about developmental processes regulated by SLs and provide insights into the
cellular mechanism of SL action. We show that SLs negatively regulate vascularization of leaves, vasculature regeneration after wounding as well as de novo formation of vasculature from artificial exogenous auxin sources. These processes, together with well-documented effects of SLs on shoot branching, are thought to at least partially depend on the canalization of auxin flow through narrowed auxin-conducting channels that demarcate future vasculature.

Prerequisites for canalization involve the feedback regulation of directional auxin transport, as manifested at the cellular level by the auxin effect on polar, subcellular localization of PIN auxin transporters.44-46. Our results show that endogenous as well as exogenous SLs interfere with canalization-dependent developmental processes, and specifically interfere with auxin feedback on PIN polarity and clathrin-mediated endocytosis of PIN proteins. This SL action does not require the regulation of transcription and occurs through the known D14- and MAX2-mediated signaling pathways. Thus, SLs may repress a mechanism that enables auxin to inhibit PIN internalization and polar transport, or SLs may modulate auxin bioavailability in the cellular context. This is indicated by a proposed role for SLs in the regulation of auxin biosynthesis in context of shoot gravitropism.47 However, auxin biosynthesis and auxin levels could also be repressed as a consequence of inhibition of auxin transport.60. Moreover, in our tests, SLs also inhibit the action of exogenously applied auxin, suggesting SLs act downstream of auxin biosynthesis.

It was suggested previously that, in context of shoot branching, SLs destabilize PINs via promoting their internalization from the PM.29,37,61 However, our observations in roots suggest that SLs do not affect endocytosis or PIN internalization per se, but specifically uncouple the effect of auxin on endocytosis and trafficking processes. Alternatively, SLs may divert endocytic PIN trafficking into an auxin-insensitive pathway, thus making PIN retrieval from membranes more efficient and possibly auxin-insensitive. In any case, given that SLs also interfere with canalization-mediated processes in context of branching and vascular tissue formation and regeneration in shoots, it is likely that the above-mentioned PIN1-GFP-based observations in shoots29,37,61 are in fact results of the here-identified SL effects on auxin feedback on PIN internalization.

Our findings identify a cellular mechanism, acting downstream of D14 and MAX2-dependent SL and karrikin signaling, and provide a mechanistic framework for the important part of developmental roles of the pathways, including vascularization and the regulation of root and shoot architecture. Further work should identify the precise molecular links between the SL/karrikin-related pathways and auxin feedback on PIN polarity.

Methods

Plant materials. The following transgenic plants and mutants have been described previously: DR5rev::GFP:42; PIN1::PIN1-GFP:41; CLE-CLE-GFP:97; max1-1:43; DEX::MAX1, max1-294; max5-394; max1-1:46; d14-1:46; d14-1 hsl6.45. Arabidopsis was stably transformed with pTA700267 to only express the empty Dex-inducible GVG cassette. This cassette can occasionally cause unspecific expression of de novo protein synthesis or transcription in both experiments, 50 µM CHX or actinomycin D was used to repress as a consequence of inhibition of auxin transport.60.

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Gene expression analyses. For gene expression of PsDM1, GR24 (0.03 mM) in water lanolin paste was applied on the upper axillary bud of decapitated plants as a ring. PsDM1 expression was then followed in the untreated lower and treated upper axillary buds. Total RNA was extracted from buds of pea plants using the RNeasy Plant Mini Kit (Qiagen). RNase-free DNase I (Qiagen) was used to remove genomic DNA. RNA was then reverse transcribed using the SuperScript III cDNA kit (Invitrogen). CDNA were used to detect PsDM1 gene expression by quantitative Real-Time PCR (qRT-PCR) using LC 480 SYBR Green I Master Mix (Roche Diagnostics) with the specific primers (Supplementary Table 1). The gene expression normalization was performed by using the combination of three reference genes (Dof-tubulin, PsActin, and PsPEF1-a).

Vascular tissue formation analyses. Young Arabidopsis plants with inflorescence stems having primary tissue architecture (vascular bundles separated by interfascicular parenchyma sectors) were used for analyzing vasculature regeneration and formation after wounding or from local application of compounds according
to method described in previous study. Briefly, first step was aimed to obtain a closed ring of active vascular cambium and secondary tissue architecture in immature inflorescences. Second step was to analyze regeneration of incised vascular cambium and formation of new vessels in wounded stems.

**In situ expression and localization analyses.** In Arabidopsis, whole-mount immunolocalization was performed following the published protocol. Antibodies were diluted as follows: 1:1000 for rabbit anti-PIN (produced and processed in lab); 1:1000 for rabbit anti-PIN (produced and processed in lab); and 1:500 for CY3-conjugated anti-rabbit secondary antibody (Sigma, C2306). In pea, water lanolin pastes containing IAA (0.16 µM), or IAA/GR24 (0.16 µM/0.09 µM) were applied on the stem stump or on the stem 2 mm below lateral incision. Immunolocalization was performed on longitudinal pea stem segments as described for Arabidopsis stems. The Arabidopsis anti-PIN1 antibody can also recognize the homologous PIN protein in pea. Antibodies were diluted as follows: 1:1000 for rabbit anti-PIN (produced and processed in lab); and 1:500 for CY3-conjugated anti-rabbit secondary antibody (Sigma, C2306). All the fluorescence signals were evaluated on Zeiss LSM 700, Zeiss LSM 710, Zeiss Observer. Z1, Leica TCS SP2, Olympus Fluoview FV1000, or Olympus Fluoview 200 confocal scanning microscopes. Unless otherwise noted, the same microscope settings were usually used for each independent experiment and pixel intensities were taken into account when comparing the images between different samples. Images were finally assembled in Adobe Photoshop CC 2015 and Adobe Illustrator CS6.

**Data availability**

The data supporting the findings of this study are available within the paper and its supplementary information files, or from the corresponding authors upon reasonable request. The source data underlying Figs. 2c–e, 3b, 6b, 7b, 7c, 11b, 12b, 13b, 17–20, 22–25, 27–29, 31, 32, 34–36, 38–40, and Supplementary Figs. 1b–d, 2b, 3b, 4a, b, e, f, i–l, 5c–h, 6d–b are provided as a Source data file.

**Received:** 13 October 2018; **Accepted:** 15 June 2020; **Published online:** 14 July 2020

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Acknowledgements

We are grateful to David Nelson for providing published materials and extremely helpful comments, and Elizabeth Dun and Christine Beveridge for helpful discussions. The research leading to these results has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (742985). This work was also supported by the Beijing Municipal Natural Science Foundation (5192011), Beijing Outstanding University Discipline Program, the National Natural Science Foundation of China (31570309), CEITEC 2020 (LQ1401) project with financial contribution made by the Ministry of Education, Youth and Sports of the Czech Republic within special support paid from the National Program of Sustainability II funds, Australian Research Council (FT180100881), and China Postdoctoral Science Foundation (2019M660864).

Author contributions

J.Z. and J.F. designed and supervised the study. J.Z., E.M., J.B., M.G., P.K., Z.M., Y.L., Y.W., T.P., M.V., V.R., S.P., R.H. and P.T. performed all the experiments. J.Z., C.L., P.B.R. and J.F. wrote the paper, which was discussed and approved by all authors.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41467-020-17252-y.

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Peer review information Nature Communications thanks Yohann Boutte for his contribution to the peer review of this work.

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