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Root-knot-nematode-derived mimics of RGF peptides hijack host signalling to orchestrate feeding site formation

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Root-knot nematodes establish long-term parasitic relationships with diverse hosts by inducing specialized feeding cells. However, the molecular mechanisms by which nematodes manipulate this developmental reprogramming process remain largely unknown. Here we identify a class of ROOT MERISTEM GROWTH FACTOR (RGF)-like peptide effectors conserved in root-knot nematodes. *MgRGF* from *Meloidogyne graminicola* and *MiRGF1* from *M. incognita* are expressed in subventral gland cells during early infection and secreted into the host apoplast. Functional analysis reveals that nematode RGFs are critical for feeding site development. Intriguingly, these peptides elicit host-specific outcomes in *Arabidopsis* and rice, involving both cell proliferation and expansion—two processes essential for establishing feeding cell identity. Further genetic and biochemical evidence demonstrates that nematode RGF peptides functionally mimic plant endogenous RGFs by hijacking the host RGI-receptor-mediated signalling pathway to regulate root growth and promote parasitism. Beyond *PLT* transcription factors, *PSY* peptide genes were identified as key downstream components of this RGF signalling cascade in rice. Functional characterization of *OsPSY5* suggests its positive role in promoting cell elongation and facilitating nematode parasitism. Our findings unveil a cross-kingdom mimicry strategy whereby root-knot nematode-secreted RGF peptides co-opt host RGF signalling to orchestrate feeding cell formation, highlighting potential targets for engineering nematode resistance in crops.

Plant-parasitic nematodes are microscopic yet economically devastating pests, causing annual global crop losses exceeding US\$157 billion¹. Among them, root-knot nematodes (RKNs; *Meloidogyne* spp.) and cyst nematodes (*Heterodera* spp. and *Globodera* spp.) are obligate sedentary endoparasites and represent the most damaging and important groups². These nematodes have comparable life cycles, wherein infective second-stage juveniles (J2s) penetrate into host roots and migrate towards the vasculature to induce the establishment of specialized feeding cells³. The hypermetabolic multinucleated

feeding structures—syncytia induced by cyst nematodes and giant cells induced by RKNs—function similarly as nutrient sinks sustaining nematode growth and development; however, the mechanisms of their ontogeny differ substantially^{4–6}. Unlike syncytia, which form through cell wall dissolution and the incorporation of adjacent cells, giant cells develop via cell expansion accompanied by sequential mitosis without cytokinesis^{7,8}. For RKNs, extensive cell division surrounding giant cells culminates in the characteristic root galls.

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Nematodes manipulate host developmental programs to orchestrate intricate root cell modifications, facilitating the initiation and development of feeding cells^{9–12}. Accumulating lines of evidence have pointed to the pivotal roles of phytohormones, particularly auxin and cytokinin, which are central to feeding cell establishment during RKN infection^{13–19}. Genes involved in auxin biosynthesis, transport and signalling are activated in feeding cells, as illustrated by transcriptome and promoter–reporter analysis, while the corresponding mutants exhibit impaired giant cell formation and reduced susceptibility to nematodes^{13,18,19}. Likewise, cytokinin biosynthesis, catabolism and signalling are required for cell cycle activation and giant cell development at infection sites^{14,20,21}.

Recent studies have revealed that multiple developmental pathways are recruited during the formation of RKN-induced galls, therefore conferring meristematic cell identities with transient pluripotency essential for gall organogenesis^{6,22}. Key genes associated with lateral root formation and callus induction, encompassing *LATERAL ORGAN BOUNDARIES-DOMAIN 16* (*LBD16*), *ABERRANT LATERAL ROOT FORMATION 4* (*ALF4*), *AUXIN RESPONSE FACTOR 5* (*ARF5*), *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*AHP6*), *GATA TRANSCRIPTION FACTOR 23* (*GATA23*) and *INDOLE-3-ACETIC ACID* genes, are activated and functionally implicated in gall development, suggesting molecular resemblance between gall formation and these developmental processes^{22–24}. Furthermore, representative markers of root apical meristem, such as *SHORT ROOT* (*SHR*), *SCARECROW* (*SCR*), *SCHIZORIZA* (*SCZ*) and *WUSCHEL-RELATED HOMEBOX 5* (*WOX5*), as well as vascular stem cell markers such as *TDIF RECEPTOR* (*TDR*), *WOX4* and *ARABIDOPSIS THALIANA HOMEBOX 8* (*ATHB8*), exhibit induced expression in proliferating cells of the gall and are required for gall development^{22,25}. A recent study further identified the ETHYLENE RESPONSE FACTOR 115 (*ERF115*) and PHYTOCHROME A TRANSDUCTION 1 (*PAT1*) transcription factor complex, known for wound-induced tissue regeneration, as an important contributor to gall initiation during RKN infection²⁶.

Phytonematodes secrete an arsenal of effector proteins originating from their oesophageal glands to ensure successful parasitism^{27,28}. Remarkably, these nematodes have evolved effector proteins that mimic plant peptide hormones, enabling them to manipulate feeding cell formation or suppress plant defence responses²⁹. Among them, CLAVATA3/EMBRYO SURROUNDING REGION-RELATED (CLE)-like peptide effectors represent one of the best-characterized classes in various cyst nematodes, serving as functional mimics of endogenous plant CLE peptides^{30–32}. The nematode CLE preproteins, expressed in dorsal oesophageal gland cells of cyst nematodes, are delivered into the host cytoplasm as propeptides and processed into mature peptides that function as bioactive ligands in the apoplast³⁰. The plant CLE-receptor-mediated signalling pathways, which play recognized roles in maintaining stem cell pools in the shoot apical meristem, root apical meristem and vascular meristem, are subsequently activated^{33,34}. Both A-type and B-type nematode CLE effectors act synergistically to co-opt these endogenous developmental pathways, ensuring proper syncytium formation^{30,31,35,36}. Intriguingly, CLE-like motifs have also been identified in *Meloidogyne* genomes through sequence mining³⁷. For example, RKN effectors such as *Meloidogyne* Avirulence Protein and 16D10 harbour CLE-like motifs, although their functions as peptide mimics remain elusive^{38,39}. Beyond CLEs, a diverse array of plant peptide mimics, including C-TERMINALLY ENCODED PEPTIDE (CEP), INFLORESCENCE DEFICIENT IN ABSCISSION (IDA), RAPID ALKALINIZATION FACTOR (RALF) and PLANT PEPTIDES CONTAINING SULFATED TYROSINE (PSY), have been discovered in RKNs, suggesting their remarkable capability to reprogram host signalling during adaptation^{11,40–44}. For instance, RKN RALF-like peptides target the host malectin-like receptor kinase FERONIA (FER) to suppress immune responses⁴³. More recently, MigPSYs, functional mimics of plant PSY peptides secreted by RKNs, were shown to promote root growth and enhance nematode infection⁴⁴. Nevertheless, the

biological functions of most nematode-derived peptides during parasitism remain to be elucidated.

In addition to CLE peptides, another group of secreted peptides, ROOT MERISTEM GROWTH FACTORS (RGFs), also known as GOLVEN (GLVs) or CLE-LIKE (CLELs) (referred to as RGFs for simplicity hereafter), play crucial roles in maintaining the root stem cell niche^{45–47}. These peptides have been implicated in regulating diverse biological processes, including lateral root development, root gravitropic responses and innate immunity^{47–50}. The *Arabidopsis* RGF family comprises 11 functionally redundant members, with more than half expressed in the stem cell area and the innermost layer of central columella cells⁵¹. Mature RGF peptides are derived from the carboxy-terminal 13–16 conserved amino acids (aa) of their precursor proteins, following post-translational tyrosine sulfation catalysed by tyrosylprotein sulfotransferase (TPST) and subsequent proteolytic processing^{52,53}. Root growth defects and reduced meristematic activity in the *tpst-1* mutant, owing to a deficiency in tyrosine-sulfated peptides, can be fully rescued by the exogenous application of sulfated RGF1, along with two other sulfated peptides, PHYTOSULFOKINE (PSK) and PSY. Conversely, overexpression of *AtRGF1* or exogenous treatment with synthetic peptide results in an enlarged root apical meristem, while the *rgf1,2,3* triple mutant (with mutations in *RGF1*, *RGF2* and *RGF3*) exhibits reduced meristem size and a short root phenotype, both of which can be restored by exogenous RGF application⁴⁵. A clade of five leucine-rich repeat receptor-like kinases (LRR-RLKs), designated as RGF1-SENSITIVES (RGLs) or RGF RECEPTORS (RGFRs), were independently identified as the receptors of RGF1 by three research groups, and SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE (SERK) family co-receptors were shown to be recruited to initiate downstream signalling^{54–56}. The RGF1–RGL complex activates the Mitogen-Activated Protein Kinase (MAPK) cascade, leading to the upregulation of two AP2-domain transcription factors, *PLETHORA 1* (*PLT1*) and *PLT2*, which modulate proximal meristem activity and root growth^{57,58}. Additionally, the transcription factor *RGF1-INDUCIBLE TRANSCRIPTION FACTOR 1* (*RITF1*) has been demonstrated to mediate RGF1 signalling by regulating reactive oxygen species distribution along root developmental zones, thereby influencing *PLT2* protein stability and meristem size⁵⁹.

Here we identify a class of effectors encoding RGF-like peptides across multiple RKN species. Specifically, we characterized two RGF precursor genes, designated as *MgRGF* (*Meloidogyne graminicola*) and *MiRGF1* (*M. incognita*), and elucidated their critical roles in feeding site establishment in different host plants. Our findings demonstrate that these RGF-like effectors are expressed in the subventral gland cells during early parasitic stages of nematode infection and are secreted into host tissues, where they function as peptide mimics of plant RGF peptides. Intriguingly, we observed distinct effects of these peptides on root growth in different hosts, highlighting host-specific outcomes. These nematode-derived peptides reprogram host developmental pathways via RGF receptors in both *Arabidopsis* and rice, thereby promoting feeding cell development and RKN infection. Furthermore, we revealed that the expression of *PLT* transcription factors, the key downstream regulators of plant RGF signalling, as well as PSY sulfated peptide genes, such as *OsPSY5*, is activated by the nematode-RGF-mediated signalling pathway in favour of nematode parasitism. Collectively, our results unveil a pivotal role of nematode-secreted RGF mimics in hijacking host developmental pathways to facilitate successful infections. These findings reveal important insights into the molecular mechanisms driving RKN feeding site formation and provide potential targets for engineering nematode-resistant crops.

Results

Identification of RGF-like genes in RKNs

Given the established roles of RGF peptides in root development, we wondered whether nematodes might secrete RGF-like peptide mimics. Using the conserved RGF motif (DYxxPxxxPPxHN) of *Arabidopsis* as a

query, we searched the annotated protein database of *M. graminicola* available through Wormbase Parasite (<http://parasite.wormbase.org/index.html>)^{60,61}. We identified a single RGF-like sequence (GenBankID: KAF7636859.1), named *MgRGF*. It consists of 71 aa containing a predicted 21-aa amino-terminal signal peptide followed by two conserved RGF motifs in tandem repeats, with the potential to produce 13-aa (DYFN-PKHHPRRHN) and 14-aa (DYNHFPKGHPPLHN) peptides simultaneously. The sequences of the motifs are not identical, but both contain the canonical residues of the plant RGF family. Notably, the 13-aa RGF motif of *MgRGF* differs from *Arabidopsis* RGF1 by only two residues. We further searched other available sequence databases of nematodes and identified RGF-like orthologues in a diverse range of RKN species, including *M. incognita* (*MiRGF1* and *MiRGF2*), *M. arenaria* (*MaRGF1*, *MaRGF2* and *MaRGF3*), *M. chitwoodi* (*McRGF*), *M. enterolobii* (*MeRGF1* and *MeRGF2*), *M. floridensis* (*MfRGF1* and *MfRGF2*) and *M. javanica* (*MjRGF1*, *MjRGF2* and *MjRGF3*) (Fig. 1a and Supplementary Table 1). However, no RGF-like sequences were found in existing sequence databases of cyst nematodes and other parasitic nematodes, indicating that these genes may play important roles specific to RKN parasitism. Similar to the RGF genes in plants, all nematode RGF genes are encoded by a single exon, except the one in *M. chitwoodi*, which harbours five introns. The identified precursor proteins of nematode RGFs are small (66–72 aa), comprising predicted N-terminal signal peptides and at least one characteristic RGF motif near the C terminus. We observed high sequence similarity shared within Clade I *Meloidogyne* species, but not with distantly related *Meloidogyne* species. In addition, no transmembrane domain was found by TMHMM analysis, suggesting the potential of these proteins as secreted effectors (Supplementary Table 1). We subsequently obtained the coding sequences of *MgRGF* and *MiRGF1* (a 68-aa protein) via reverse transcription (RT)–PCR using RNA extracted from parasitic stages of *M. graminicola* and *M. incognita*, respectively, for further functional analysis.

Secretion of nematode RGF effectors from subventral oesophageal glands during early parasitic stages

We examined the temporal expression patterns of *MgRGF* and *MiRGF1* across different developmental stages of *M. graminicola* and *M. incognita* through RT–qPCR. We found that *MgRGF* is mainly expressed in parasitic J2s (Par-J2s) at 3 days post infection (dpi) and exhibits considerably lower expression in other life stages, including eggs, pre-parasitic J2s and later parasitic stages (third- and fourth-stage juveniles) (Fig. 1b). A similar expression pattern was observed for *MiRGF1*, with peak transcript levels in Par-J2s (Fig. 1c). To determine their spatial expression in nematodes, in situ hybridization was performed to localize the transcripts. Strong signals were detected in the subventral gland cells of Par-J2s in both *M. graminicola* and *M. incognita* when hybridized with the digoxigenin (DIG)-labelled antisense probes specific to *MgRGF* and *MiRGF1*, respectively, whereas no hybridization signals were observed when the corresponding sense probes were used (Fig. 1d,e). Furthermore, a polyclonal antibody was raised using *MgRGF*-derived synthetic peptides for immunolocalization analysis. Consistently, immunofluorescence signals were detected in the subventral glands of Par-J2s (Fig. 1f). Since subventral gland cells are known to be active during early stages of nematode parasitism, our results suggest that RGF effectors may function primarily during early host infection.

To investigate effector secretion, we evaluated the function of *MgRGF* and *MiRGF1* signal peptides using assays previously developed in yeast and *Nicotiana benthamiana*^{62,63}. Using a yeast invertase secretion system, we successfully detected the activity of secreted invertase, as shown by the presence of red products and successful growth on raffinose-containing YPRAA medium, when we expressed SUC2 invertase in fusion with the signal peptides of *MgRGF* and *MiRGF1* in the yeast strain YTK12 (Supplementary Fig. 1a). Alternatively, transient expression assays in *N. benthamiana* leaves demonstrated that

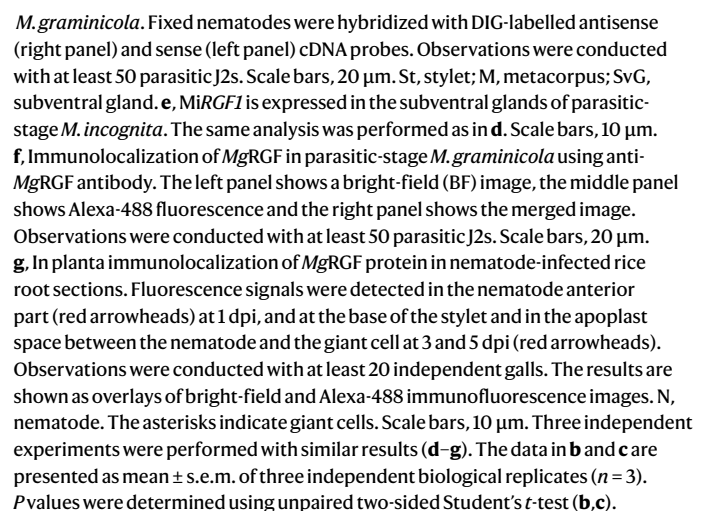
both signal peptides can effectively guide XEG1 out of the plant cell, thus leading to characteristic cell death, confirming that both signal peptides of *MgRGF* and *MiRGF1* act as functional secretion signals (Supplementary Fig. 1b,c). Furthermore, immunohistochemical analysis was conducted on rice gall sections with *M. graminicola* infection. Fluorescence signals were detected in the nematode anterior part and the apoplastic space between the nematode and giant cells after hybridization with anti-*MgRGF* antibodies, whereas no signal was detected when using the secondary antibody alone (Fig. 1g and Supplementary Fig. 2). These results suggest that nematode RGFs secreted from the subventral glands may be delivered directly into the host apoplast via the stylet.

In vitro silencing of nematode RGF effectors impairs nematode parasitism

To investigate the role of nematode RGFs in RKN parasitism, we employed RNA interference experiments to silence the expression of *MgRGF* or *MiRGF1* in *M. graminicola* and *M. incognita*, respectively, and evaluate the resulting effect on nematode infection. Nematodes were in vitro soaked with target-specific double-stranded RNA (dsRNA). Silencing efficiency was confirmed in feeding nematodes by RT–qPCR at 3 dpi with dsRNA-treated nematodes (Fig. 2a). The average numbers of galls and female nematodes were significantly reduced by approximately 31% and 37% in rice roots, respectively, at 7 and 15 dpi with *MgRGF* dsRNA-treated nematodes relative to *GFP* dsRNA or M9 buffer controls (Fig. 2b–d). Additionally, *MgRGF* silencing resulted in a significant reduction (~54%) in the number of feeding sites established by *M. graminicola*, as demonstrated by tissue sectioning and benzyl alcohol/benzyl benzoate (BABB) clearing (Fig. 2e,f), whereas feeding cell size was not significantly altered (Fig. 2g). We also observed enhanced root growth in plants inoculated with *MgRGF*-silenced nematodes compared with those inoculated with control nematodes, most likely due to reduced nematode infection and damage to root tips (Fig. 2d and Supplementary Fig. 3). Similarly, reduced nematode susceptibility was observed in *Arabidopsis* upon silencing of *MiRGF1*, evidenced by the decreased numbers of galls and egg masses per plant (~50% and 48%, respectively), along with the reduced size of single giant cells (~42%) (Fig. 2h–m). For *MiRGF1* silencing, the reduction in feeding cell size was more pronounced, which can be explained by the compromised feeding site establishment. These results demonstrate that both *MgRGF* and *MiRGF1* play crucial roles in the process of feeding site establishment in host roots and are essential for nematode infection.

Exogenous application of nematode RGF peptides regulates root growth, mimicking plant endogenous peptides

To investigate the functional similarity between plant and nematode RGF peptides, we first examined their effects on *Arabidopsis* root growth using synthetic peptides (Supplementary Table 2). Both the 13-aa and 14-aa tyrosine-sulfated peptides derived from *MgRGF*, named *MgRGF1*-Y and *MgRGF2*-Y, along with the 13-aa unmodified peptide (*MgRGF1*), were used for the treatments. In addition, the 13-aa sulfated RGF1 peptide from *Arabidopsis* (*AtRGF1*-Y) was included for comparison. Similar to the *AtRGF1* peptide, nematode peptides can stimulate root growth and meristematic activity, as indicated by increased root length and higher numbers of meristem cortex cells, although the effect of unmodified peptides was less prominent than that of the sulfated forms (Extended Data Fig. 1a–d and Supplementary Fig. 4a,b). Consistent with previous reports, we observed a wavy-root phenotype, especially under treatments with sulfated peptides (Extended Data Fig. 1a). RGF-peptide-treated seedlings exhibited more exaggerated root waving than mock controls when grown on firm agar (1.5%) plates angled at 45° (Extended Data Fig. 1e). Treatment with *MgRGF* peptides also enhanced root growth and restored root meristematic activity of *Arabidopsis tpst-1* (Extended Data Fig. 1f–h) and *rgf1,2,3* mutants to a similar



extent as *At*RGF1 peptide treatment (Extended Data Fig. 1i,j). These results indicate that *Mg*RGF peptides act as ligand mimics of *At*RGF1 in *Arabidopsis* root growth, and sulfation modification is required for full function of the peptides.

Furthermore, we examined the effects of *Mg*RGF peptides on rice root growth. Among 13 RGF members in rice, two sulfated RGF peptides, corresponding to the RGF motifs of LOC_Os02g55580 and LOC_Os06g42754, were synthesized for further comparison, owing to their high expression in roots (Supplementary Fig. 5a–e). Interestingly, the effects of peptides on rice root growth, whether nematode- or plant-derived, were unexpected and distinct from what we observed in *Arabidopsis*. Rather than the stimulated root growth in *Arabidopsis*, RGF peptide treatment resulted in dose-dependent root shortening and root tip curling in rice (Fig. 3a,b and Supplementary Figs. 5f–i and 6a,b). Root inhibition was observed at 1 nM peptide concentration and became more severe with increasing peptide concentrations (Supplementary Fig. 6c,d). Consistent with prior observations, sulfated *Mg*RGF peptides caused stronger suppression than unmodified peptides. To elucidate the cellular basis underlying these phenotypic differences, we used 5-ethynyl-20-deoxyuridine (EdU), which labels newly replicated DNA, for root tip analysis. The signals of EdU-labelled cells were significantly increased upon peptide treatment, indicating the elevated cell division in rice root meristem (Fig. 3c,d). Additionally, both *pCYCB1.1::GUS* reporter lines and nitroblue tetrazolium chloride (NBT) staining, commonly used to mark dividing cells and the root meristem zone, corroborated this result (Fig. 3e–h). Longitudinal root sections showed that cell length in the elongation and maturation zones, especially on the inner (concave) side of curled roots, were significantly shorter following peptide treatment, across all radial cell layers, than the untreated controls (Fig. 3i–l). Remarkably, we noticed that the peptide-treated roots were much thicker than the controls. Root cross-sections showed that the root diameters along different developmental zones were significantly increased upon peptide treatment (Fig. 3m,n). Measurements of average cell area revealed that this increased diameter was mainly due to radial cell expansion in different root cell layers (Fig. 3o,p). These findings suggest that the effects of RGF peptides on rice root development are multifaceted, involving alterations in both cell proliferation and expansion. A similar phenotype was observed in tomato, where *Mg*RGF1-Y treatment led to shorter and thicker roots accompanied by enhanced meristematic activity (Extended Data Fig. 2a–d). Nematode infection was also compromised in tomato upon the silencing of the nematode RGF effector (Extended Data Fig. 2e–h). Taken together, all these results support the notion that nematode-derived RGF peptides regulate root growth in a manner resembling that of plant RGFs, albeit with species-specific outcomes. This highlights the functional conservation of these peptides across kingdoms.

Overexpression of nematode RGF genes promotes nematode parasitism

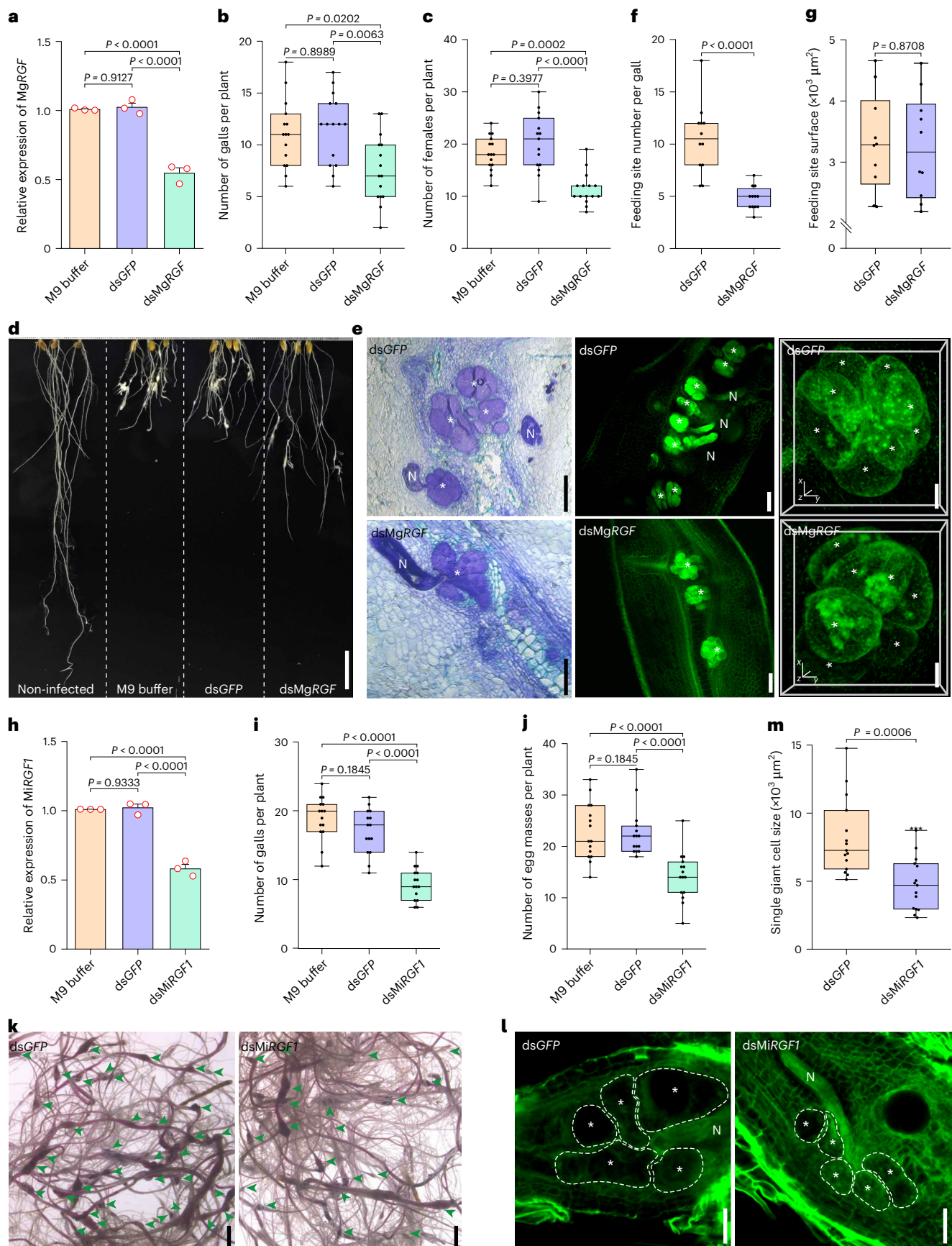
To further elucidate the biological functions of nematode RGFs, we generated transgenic lines of rice and *Arabidopsis* expressing either full-length RGF genes or their signal-peptide-deleted variants. The expression levels of the transgenes were confirmed by RT–qPCR (Extended Data Fig. 3b and Supplementary Fig. 7a,b). Two resulting independent homozygous T₂ lines were used for further analysis. In *Arabidopsis*, expression of both *Mg*RGF and *Mi*RGF1, regardless of signal peptide presence, produced similar root growth phenotypes to those induced by the exogenous application of RGF peptides. The transgenic plants exhibited longer and wavy roots, reduced lateral root formation and increased meristematic cell numbers relative to the controls, mimicking reported hallmark phenotypes triggered by endogenous *Arabidopsis* RGF peptides (Extended Data Fig. 3a–f and Supplementary Fig. 7c–f). Through nano-liquid chromatography coupled to tandem mass spectrometry (nano-LC–MS/MS) analysis, we successfully detected the 13-aa and 14-aa tyrosine-sulfated mature peptides, corresponding to *Mg*RGF1-Y and *Mg*RGF2-Y, in apoplastic peptide extractions from the transgenic lines expressing either full-length *Mg*RGF or its signal-peptide-deleted variant (Extended Data Fig. 3g–j). According to SecretomeP-1.0, the signal-peptide-removed variants were predicted to be secreted through non-classical secretion pathways, thus providing a plausible explanation for the identical phenotypes observed between full-length and truncated proteins (Supplementary Table 3). In contrast, *Mg*RGF expression in rice resulted in shorter and thicker roots, with larger root diameters than the controls, although the changes were less pronounced than those induced by peptide treatment (Fig. 4a–f). The differences in response between the two plant species may reflect the intricate and species-specific nature of RGF-mediated signalling pathways.

To further evaluate the involvement of *Mg*RGF in nematode parasitism, we conducted infection assays with the respective transgenic lines of rice. We observed significantly enhanced susceptibility to nematode infection for all the transgenic lines expressing *Mg*RGF. The average numbers of galls and females increased significantly compared with wild-type controls (Fig. 4g–i). This elevated susceptibility was not due to differences in nematode penetration, as similar numbers of juveniles entered the roots of transgenic and control plants (Supplementary Fig. 8a,b). Histological examination demonstrated that more feeding sites were established by *M. graminicola* in individual galls of the transgenic roots, whereas the feeding cell size was not significantly altered (Fig. 4j–l and Supplementary Fig. 8c,d). In line with this, expression of *Mg*RGF in *Arabidopsis* also resulted in elevated susceptibility, with significantly increased numbers of galls and egg masses, as well as feeding sites per gall (Extended Data Fig. 4a–e). All these findings provide compelling evidence that nematode RGF

Fig. 2 | Silencing of nematode RGF effectors affects nematode parasitism.

a, Evaluation of *Mg*RGF silencing efficiency in feeding nematodes by RT–qPCR. Following in vitro soaking with the indicated dsRNA, 100 J2s per plant were used for inoculation. RT–qPCR analysis was conducted at 3 dpi using *MgACTIN2* and *MgTub* as internal controls. Relative expression levels were calculated relative to the M9 buffer control. **b–d**, Effect of *Mg*RGF silencing on nematode infection. Root galls (**b**) and female nematodes (**c**) were counted at 7 dpi and 15 dpi with dsRNA-treated *M. graminicola* ($n = 15$). The experiments were performed three times with similar results. Representative root phenotypes were photographed at 7 dpi (**d**). Scale bar in **d**, 2 cm. **e–g**, Effect of *Mg*RGF silencing on feeding site establishment. Feeding sites were observed using tissue sectioning (left panels in **e**) and BABB clearing (middle and right panels in **e**) at 7 dpi. Representative images of a single gall and an individual feeding site are shown. The number of feeding sites per gall (**f**) ($n = 12$) and feeding cell size were quantified (**g**) ($n = 10$). Scale bars, 200 μ m (left panels in **e**), 100 μ m (middle panels in **e**) and 50 μ m (right panels in **e**). The asterisks indicate giant cells. **h**, Silencing efficiency of *Mi*RGF1 detected by RT–qPCR. The experiments were performed as in **a**, using *MiGADPH*

and *Mi*18S as internal controls. **i–k**, Silencing of *Mi*RGF1 compromised nematode infection in *Arabidopsis*. Root galls (**i**) were counted at 15 dpi with *M. incognita* ($n = 15$). At 30 dpi, roots were stained with acid fuchsin, and egg masses (**j**) were counted ($n = 15$). The green arrowheads indicate root knots on *Arabidopsis* roots. Representative root phenotypes were photographed at 30 dpi (**k**). Scale bars, 1 mm. **l,m**, Giant cell observation after BABB clearing. Representative images of a nematode feeding site were photographed using confocal microscopy at 30 dpi (**l**). The areas of individual giant cells (**m**) were quantified using ImageJ ($n = 15$). Scale bars, 200 μ m. The asterisks indicate giant cells. The experiments were performed three times with similar results. The data in **a** and **h** are presented as mean $\pm$ s.e.m. of three independent biological replicates ($n = 3$). The data in **b**, **c**, **f**, **g**, **i**, **j** and **m** are presented as box-and-whisker plots with individual data points. The central line indicates the median, the box limits represent the first and third quartiles and the whiskers extend to the minima and maxima. *P* values were determined by Tukey's multiple comparisons test (**a–c**, **h–j**) and unpaired two-tailed Student's *t*-test (**f**, **g**, **m**).



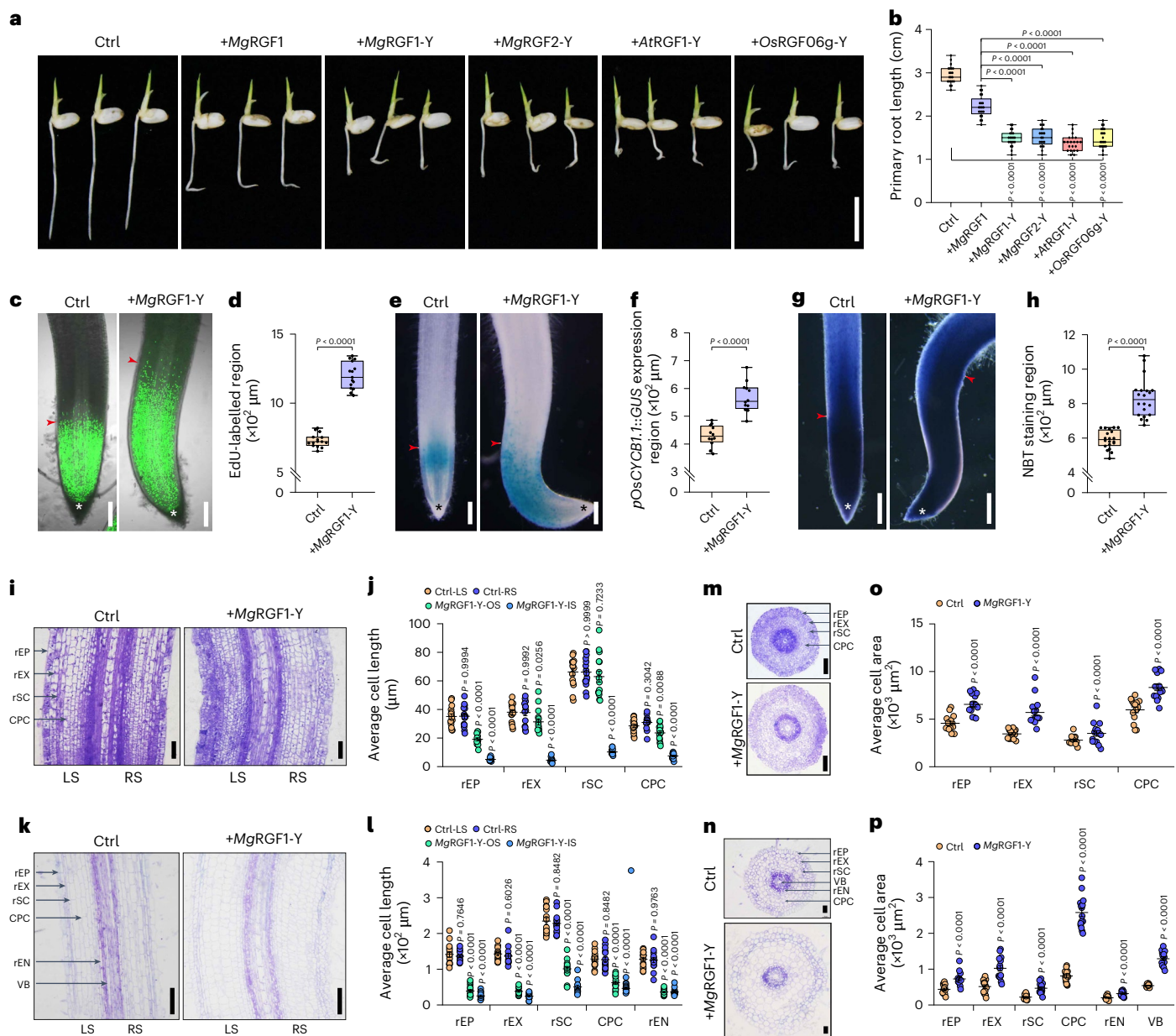


Fig. 3 | Exogenous treatment with nematode RGF peptides regulates root growth and development in rice. **a, b**, Root growth phenotypes under synthetic peptide treatment. Kitaake seedlings were grown vertically on half-strength Murashige and Skoog ($\frac{1}{2}$ MS) plates either alone or supplemented with the indicated peptides (100 nM). Seedlings were photographed (**a**) at 12 h after treatment, and root length (**b**) was measured ($n = 21$). Scale bar, 1 cm. **c, d**, Longitudinal observation of EdU-labelled cells in the root meristem. After 12 h of MgRGF1-Y treatment, roots were imaged with confocal microscopy (**c**), and the sizes of EdU-labelled root meristems (**d**) were measured ($n = 15$). Scale bars, 100 μ m. **e, f**, Expression pattern of *pOsCYCB1.1::GUS* in primary root tips following 12 h of MgRGF1-Y treatment. Roots were photographed using a stereomicroscope (**e**), and the lengths of the stained regions (**f**) were measured ($n = 12$). Scale bars, 200 μ m. **g, h**, Root staining with NBT after 12 h of peptide treatment. Representative root images were photographed using a stereomicroscope (**g**). The stained regions in the meristematic zone (**h**) were measured ($n = 20$). Scale bars, 200 μ m. The red arrowheads mark the boundary region of labelled or stained cells, and the asterisks indicate the quiescent centre (QC) region (**c, e, g**). **i, j**, Median longitudinal sections of the elongation zone after 24 h of 100 nM MgRGF1-Y treatment. Representative root sections were photographed (**i**), and cell length (**j**) across all radial cell layers was measured

($n = 15$). Scale bars, 50 μ m. **k, l**, Observation of longitudinal sections of the maturation zone. The experiments were performed as in **i** and **j**. Scale bars, 50 μ m. **m, n**, Cross-sections of the elongation (**m**) and maturation (**n**) zones after 24 h of 100 nM MgRGF1-Y treatment. Representative root sections were photographed. Scale bars, 50 μ m. **o, p**, Cell area quantification across all radial cell layers in the elongation (**o**) ($n = 15$) and maturation (**p**) ($n = 15$) zones under peptide treatment. LS and RS indicate the left and right sides of the root sections without peptide treatment, respectively; OS and IS indicate the outer and inner sides of the peptide-treated root sections, respectively. VB, vascular bundle; rEP, root epidermis cell; rEX, root exodermis cell; rSC, root sclerenchyma cell; CPC, cortex parenchyma cell; rEN, root endodermis cell. The experiments were performed three times with similar results. 'Ctrl' indicates that no peptide was added. The data in **j, l, o** and **p** are shown as mean $\pm$ s.e.m. with individual data points. The data in **b, d, f** and **h** are presented as box-and-whisker plots with individual data points. The central line indicates the median, the box limits represent the first and third quartiles and the whiskers extend to the minima and maxima. P values were determined by one-way analysis of variance (ANOVA) with Dunnett's multiple comparisons test, comparing the mean of each column with the mean of the control column (**b, j, l**), and unpaired two-tailed Student's t -test (**d, f, h, o, p**).

effectors play pivotal roles in promoting feeding site establishment during nematode parasitism.

Arabidopsis RGI receptors mediate the perception of nematode RGF peptides

The functional similarities between nematode RGF peptides and plant RGFs promoted us to investigate whether these nematode effectors may exploit the host's RGF signalling pathway. In *Arabidopsis*, five LRR-RLKs, RGI1 to RGI5, have been demonstrated as essential receptors for perceiving endogenous RGF peptides during root meristem development. The corresponding quintuple *rgi1,2,3,4,5* mutant displays a short-root phenotype with a decrease in meristem size⁵². To determine whether these RGI receptors are involved in nematode signalling, we first analysed their expression pattern during *M. incognita* infection using promoter–GUS transgenic *Arabidopsis* lines. GUS expression varied along the root, and strong GUS activity was observed predominantly at nematode feeding sites at 3 and 5 dpi with *M. incognita* for all five genes, while the expression was weak or undetectable by 12 dpi, suggesting that RGI receptors primarily function during the early stages of nematode infection (Extended Data Fig. 5a). Cross-sections of galls revealed GUS expression in giant cells and surrounding cells (Extended Data Fig. 5b). We then examined the root growth responses of the *rgi* mutant to the exogenous application of nematode RGF peptide. Relative to the wild-type plants, the quintuple mutant showed complete insensitivity to nematode RGF peptide treatment, demonstrating that these receptors are required for perceiving both plant- and nematode-derived RGF signals (Extended Data Fig. 6a–e).

Furthermore, we performed a nematode infection assay on the *rgi1,2,3,4,5* mutant with *M. incognita* inoculation. Although the roots of the *rgi1,2,3,4,5* mutant are shorter, the number of nematodes penetrating into the roots at 1 dpi was comparable to that in wild-type controls (Extended Data Fig. 6f,g). To minimize potential bias in the interpretation of nematode infection phenotypes arising from significant root growth defects, the root weight was taken into account to standardize infection phenotypes. No significant difference was found in the gall numbers after normalization (Extended Data Fig. 6f). Nevertheless, the quintuple mutant produced fewer egg masses (Extended Data Fig. 6g,h). In line with this, nematode development was delayed in the roots of the *rgi1,2,3,4,5* mutant at 7 and 15 dpi (Extended Data Fig. 6i–n), and giant cell size was significantly smaller than in the controls (Extended Data Fig. 6o,p). These results indicate that the perception of nematode RGFs by *Arabidopsis* RGI receptors is potentially required for successful nematode infection.

Rice RGF peptide receptors are essential for *M. graminicola* parasitism

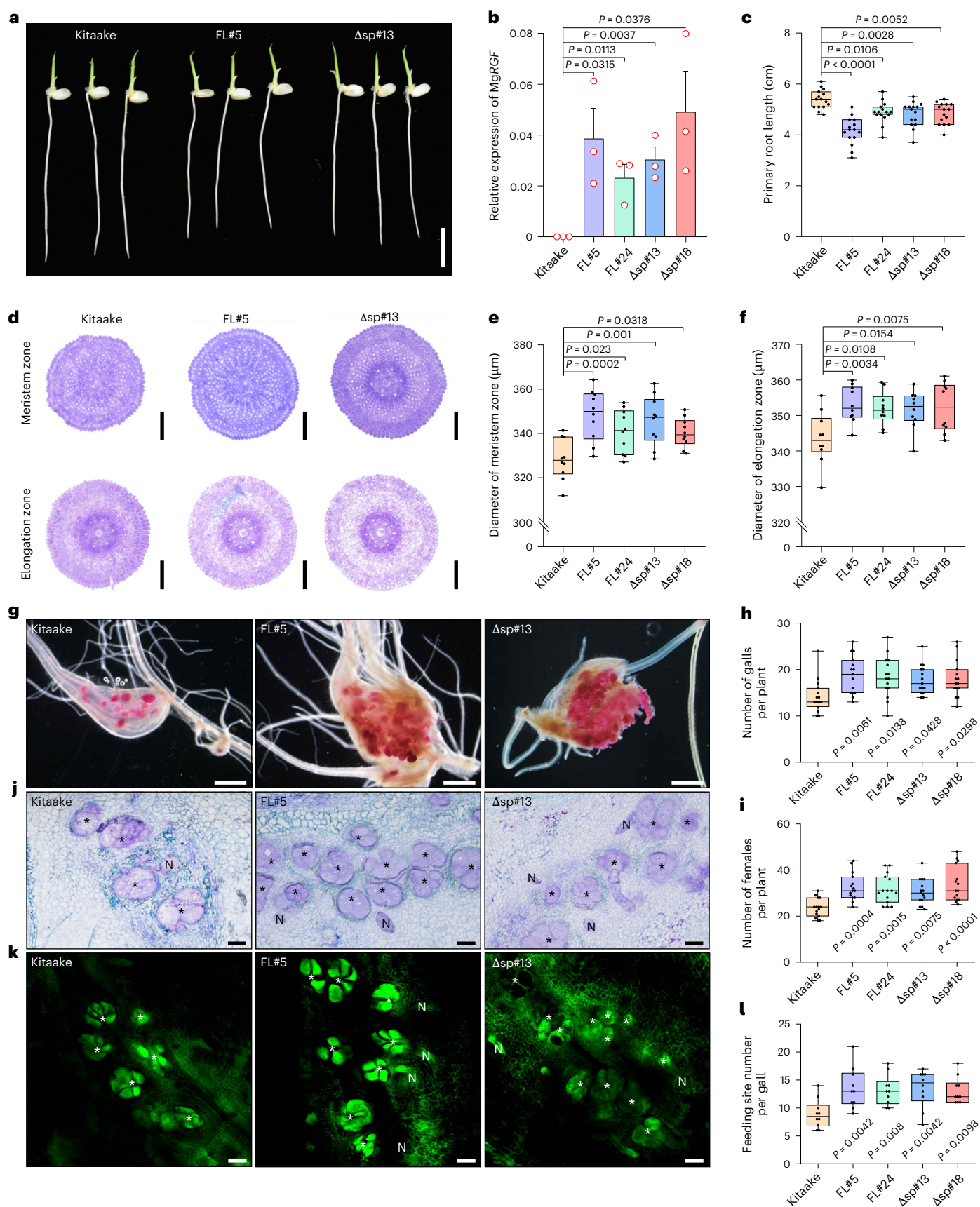
The role of RGF signalling in rice has not been studied before. Through phylogenetic analysis, we identified six rice RGI homologues (designated as OsRGI1 to OsRGI6), which separately clustered with the five

Arabidopsis RGI receptors (Fig. 5a). All these OsRGI proteins contain characteristic domains of LRR receptor kinases and are expressed in root tissues, implicating their potential involvement in root development (Supplementary Fig. 9a–c). Sequence analysis revealed the presence of a conserved Arg-x-Gly-Gly motif in OsRGIs, which was identified as the motif responsible for recognizing the sulfate group of RGF peptides (Supplementary Fig. 10)⁵⁴. Further molecular homology modelling and docking simulation analysis demonstrated interactions between the Arg-x-Gly-Gly motif of OsRGI2 or AtRGI3 and the sulfate group of the MgRGF1-Y and MiRGF1-Y peptides (Supplementary Fig. 11a–d). Subcellular localization confirmed the plasma membrane localization of GFP-tagged OsRGI2 and OsRGI3 in rice protoplasts, in both the presence and absence of RGF peptide (Supplementary Fig. 12a,b). We further expressed the ectodomain of OsRGI2 using an insect-baculovirus expression system and tested the association with biotinylated MgRGF1 peptide through an in vitro pull-down assay. Following immunoprecipitation with an anti-streptavidin antibody, biotinylated MgRGF1 peptide could effectively pull down OsRGI2, whereas an excess of unlabelled RGF peptides could compete for the binding (Fig. 5b). Additionally, high-affinity bindings between the OsRGI2 ectodomain and both nematode and plant RGF peptides were further confirmed through a microscale thermophoresis (MST) assay (Fig. 5c).

To investigate the biological function of OsRGIs, we subsequently generated a series of OsRGI knockout mutants in the Kitaake background using CRISPR–Cas9-mediated gene editing. Considering the high sequence similarity of OsRGIs and the reported functional redundancy among *Arabidopsis* RGI receptors, we performed multiplex genome editing via the polycistronic tRNA–gRNA (PTG)/Cas9 tool⁶⁴. Sequence-specific gRNAs for OsRGI1–5 were tandemly arrayed to target multiple members simultaneously. Homozygous edited lines were confirmed by sequencing (Supplementary Fig. 13a–e). We eventually obtained two independent sets of double (*rgi1,2* and *rgi3,4*), triple (*rgi1,3,4*), quadruple (*rgi1,2,3,4*) and quintuple (*rgi1,2,3,4,5*) mutants. The *rgi1,2* and *rgi3,4* double mutants exhibited root growth comparable to wild-type controls (Supplementary Fig. 14a–d), while the triple mutants displayed a short-root phenotype characterized by fewer meristematic cortex cells and reduced cell division in the meristem zone (Fig. 5d–i). The defects in root growth and development became even more pronounced in quadruple mutants and most severe in the quintuple mutants (Fig. 5d–i), indicating functional redundancy among these receptors. We next examined whether these mutants are defective in RGF responses. While double mutants retained the same sensitivity to MgRGF peptide treatment as wild-type seedlings, the triple and quadruple mutants exhibited reduced sensitivity (Supplementary Fig. 15a,b). The quintuple mutants showed complete insensitivity to peptide treatment, with no detectable changes in root length or meristematic cell division, further supporting the redundant roles of these receptors in peptide perception and meristem growth (Fig. 5j–m). Under field conditions, these mutants displayed dwarfism and reduced yield, with

Fig. 4 | Expression of MgRGF promotes nematode parasitism. **a**, Root growth phenotypes of transgenic rice lines expressing MgRGF with full-length (FL) and signal-peptide-deleted (Δsp) versions. Seedlings from two independent transgenic lines in the Kitaake background were photographed at 5 days after germination. Scale bar, 1.5 cm. **b**, Relative expression levels of MgRGF in the transgenic lines. OsUBQ10 and OsActin were used as internal controls. RT–qPCR was conducted as described previously. **c**, Primary root lengths of overexpression lines. Five-day-old transgenic seedlings were used for the measurements ($n = 15$). **d**, Cross-sections of transgenic lines. The upper panels show sections of the meristem zone, and the lower panels show sections of the elongation zone. Representative images are shown from three independent experiments. Scale bars, 100 μ m. **e, f**, Root diameter measurements in transgenic lines. Cross-sections of the root meristem zone (**e**) and the elongation zone (**f**) were used for quantification ($n = 10$). **g–i**, Nematode infection phenotypes of transgenic lines. Roots were stained with acid fuchsin (**g**) at 15 dpi with

M. graminicola. The numbers of root galls (**h**) and adult females (**i**) were counted at 7 and 15 dpi, respectively ($n = 15$). Scale bars, 1 cm. **j–l**, Feeding site observation in transgenic lines. At 7 dpi, root galls were processed using tissue sectioning (**j**) and BABB clearing (**k**). Observations were conducted with at least 10 independent galls. Representative images of a single gall are shown. The number of feeding sites per gall (**l**) was quantified ($n = 10$). Scale bars, 200 μ m (**j**) and 100 μ m (**k**). The asterisks indicate giant cells. The experiments were performed three times with similar results. The data in **b** are presented as mean $\pm$ s.e.m. with individual data points from three independent biological replicates ($n = 3$). The data in **c**, **e**, **f**, **h**, **i** and **l** are presented as box-and-whisker plots with individual data points. The central line indicates the median, the box limits represent the first and third quartiles and the whiskers extend to the minima and maxima. *P* values were determined by unpaired two-tailed Student's *t*-test (**b**) and one-way ANOVA with Dunnett's multiple comparisons test, comparing the mean of each column with the mean of the control column (**c**, **e**, **f**, **h**, **i**, **l**).



these effects being more prominent for higher-order mutants, though the seed size remained unaffected (Supplementary Fig. 16a–k).

Moreover, we evaluated the function of OsRGI receptors during nematode parasitism. At 1 dpi, the numbers of nematodes that successfully entered the roots were unaffected across all OsRGI mutants compared to wild-type plants (Supplementary Figs. 17a,b and 18a,b). Given the severe root growth phenotype in some mutants, infection phenotypes were normalized to the root weight. At 7 dpi or 15 dpi, double mutants showed no significant difference in nematode infection compared to the wild-type controls (Supplementary Fig. 18c–e). But the higher-order mutants exhibited varying levels of reduction in nematode infection, particularly the quintuple mutants, as shown by lower numbers of galls (Fig. 6a–f). We also observed a substantial decrease in the numbers of females and feeding sites in the root systems of quadruple and quintuple mutants, whereas feeding cell size remained unchanged (Fig. 6a–f and Extended Data Fig. 7a–d). All these findings demonstrate that host RGI-receptor-mediated signalling plays a critical role in facilitating feeding site establishment during nematode parasitism, although we note that root growth defects in *rgi* mutants may complicate nematode infection assessment.

Nematode RGF peptide modulates the expression of *OsPLT2* and *OsPSY5* in rice

Previous studies in *Arabidopsis* have established the MAPK cascade as a key downstream component of RGF signalling that regulates the expression of *PLT1* and *PLT2* transcription factors, which are essential for maintaining the root stem cell niche^{57,59,65}. Consistent with this, treatment with MgRGF1-Y peptide activated MPK3 and MPK6 in both *Arabidopsis* and rice seedlings similar to the effect of AtRGF1-Y peptide, and the activation was abolished in the *rgi1,2,3,4,5* quintuple mutant (Extended Data Fig. 8a,b). In addition, we observed induced promoter activities and protein levels of *PLT1* and *PLT2* upon MgRGF1-Y or AtRGF1-Y peptide treatment in transgenic *Arabidopsis* lines expressing *pPLT1::CFP*, *pPLT2::CFP*, *pPLT1::PLT1-YFP* or *pPLT2::PLT2-YFP*, respectively, whereas the signals were barely detectable in the *rgi1,2,3,4,5* quintuple mutant (Extended Data Fig. 8c).

On the basis of our transcriptome data generated from nematode-infected rice root tips, we found that the expression of *OsPLT2*, but not *OsPLT1*, was significantly upregulated following *M. graminicola* infection⁶⁶. To investigate the expression pattern at nematode infection sites, we generated *pOsPLT2::GUS* stable transgenic lines in the Kitaake background. Unlike *PLT1* and *PLT2* in *Arabidopsis*, which display gradient distributions with maxima in the stem cell area, *OsPLT2* showed low expression in the meristem zone,

with a gradual increase towards the elongation and maturation zones at 3 days post germination. Exogenous application of nematode MgRGF peptide strongly induced the promoter activity of *OsPLT2* (Fig. 7a and Supplementary Fig. 19). Remarkably, its activity was significantly upregulated following nematode inoculation (Fig. 7b). Histological sections of galls revealed strong GUS expression in giant cells and surrounding cells at 3 and 5 dpi (Fig. 7c). RT-qPCR analysis revealed that the gene induction by nematode or peptide treatment was significantly compromised in the quintuple OsRGI receptor mutant (Fig. 7d,e). These observations suggest that nematode RGF peptides activate the expression of *OsPLT2* dependent on OsRGI receptors.

To explore additional components potentially involved in nematode RGF signalling, we analysed the expression of root-growth-related genes using our previously published transcriptome data⁶⁴. We noticed that five out of eight *PSY* family members in rice, encoding another class of tyrosine-sulfated peptides, were significantly upregulated in response to nematode infection (Extended Data Fig. 9a,b). RT-qPCR confirmed the induced expression of *OsPSY2*, *OsPSY4*, *OsPSY5* and *OsPSY6* by both nematode and MgRGF peptide treatments (Extended Data Fig. 9c,d). We chose to focus on *OsPSY5*, which showed the highest level of induction, for further study. *pOsPSY5::GUS* transgenic lines were generated, and the promoter activity was detected in the elongation zone and more evident in the maturation zone (Fig. 7f and Supplementary Fig. 20). Similar to *OsPLT2*, MgRGF peptide treatment led to the upregulation of *OsPSY5* in rice roots. We also observed a substantial increase in the promoter activity of *OsPSY5* following nematode infestation, particularly within giant cells and neighbouring cells (Fig. 7g,h). Importantly, the gene induction patterns of *OsPSY5* were significantly diminished by the removal of OsRGI receptors (Fig. 7i,j). We further compared the expression of *PSY* family genes in *Arabidopsis* following MgRGF peptide treatment. In contrast to rice, no significant changes were detected in *Arabidopsis* under the same treatment condition, although the expression of RGF1-responsive gene *RGF1-INDUCIBLE TRANSCRIPTION FACTOR 1 (RITF1)* was induced as expected. These results point to the regulatory divergence downstream of RGF signalling pathways between *Arabidopsis* and rice (Extended Data Fig. 9e,f). Collectively, these findings indicate that *OsPSY5*, along with *OsPLT2*, could be targeted via RGI-receptor-mediated RGF signalling during nematode parasitism in rice.

OsPSY5 is an important regulator of root growth and nematode parasitism

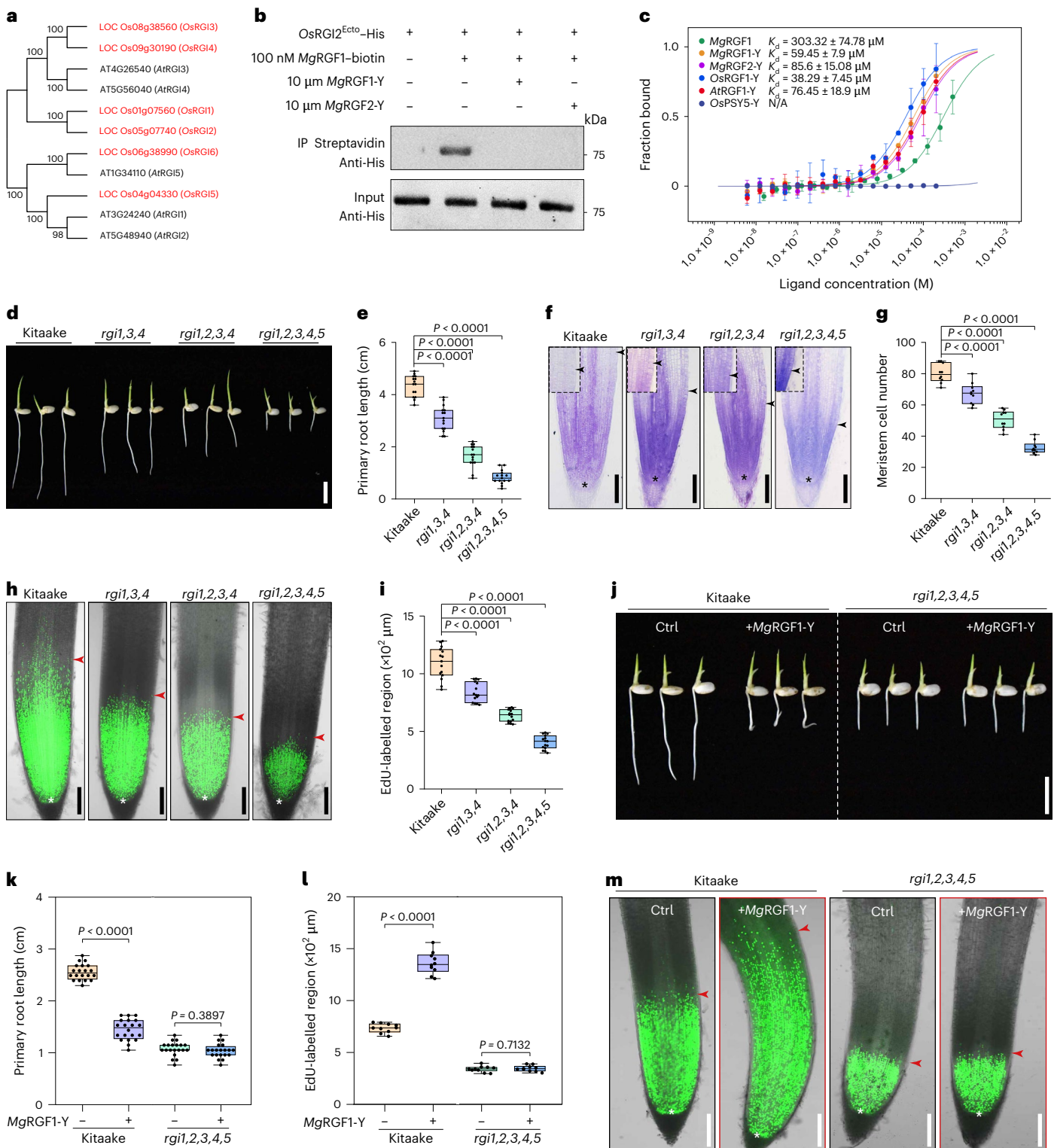
To explore the involvement of *OsPSY5* in root development, we first treated rice seedlings with a synthetic tyrosine-sulfated peptide

Fig. 5 | OsRGIs function as nematode RGF receptors. **a**, Phylogenetic analysis of RGIs from rice and *Arabidopsis*. The phylogenetic tree was generated using the maximum-likelihood method. Rice RGIs are highlighted in red, while *Arabidopsis* RGIs are denoted in black. **b**, Binding of MgRGF1 to the ectodomain of *OsRGI2* (*OsRGI2^{Ecto}*) in a pull-down assay. Purified protein was incubated with 100 nM biotinylated MgRGF peptide for immunoprecipitation. Unlabelled RGF peptide was added as a competitor. Bound and input proteins were detected by immunoblotting with an anti-His antibody. The experiments were repeated twice with similar results. **c**, MST analysis of binding affinity between the ectodomain of *OsRGI2* and peptide ligands. The data points represent the bound fraction (Δ normal/amplitude) measured at different ligand concentrations, and the binding curves indicate the calculated fits. The error bars represent the s.e.m. derived from three independent biological replicates. K_d indicates the dissociation constant. N/A stands for not available. **d,e**, Root growth phenotypes of rice high-order *rgi* mutants. Four-day-old seedlings of triple (*rgi1,3,4*), quadruple (*rgi1,2,3,4*) and quintuple (*rgi1,2,3,4,5*) mutants in the Kitaake background were photographed (**d**), and primary root length (**e**) was measured ($n = 15$). Scale bar, 1 cm. **f,g**, Longitudinal sections of root tips from 4-day-old mutant seedlings. The black arrowheads in the representative root sections (**f**) indicate the proximal end of the root meristem. The insets provide magnified views of the selected region. The asterisks denote QC cells. Scale bars, 200 μ m.

Quantification analysis of meristematic cortex cell number (**g**) was done at the fourth cortical layer in the entire meristem region ($n = 10$). **h,i**, Longitudinal observation of EdU-labelled cells in root tips of the corresponding mutants. The red arrowheads in **h** mark the boundary regions of EdU-labelled cells, and the white asterisks indicate the QC regions. EdU-labelled root apical meristem size (**i**) was measured ($n = 15$). Scale bars, 100 μ m. **j,k**, Root growth of the wild type and the quintuple mutant in response to MgRGF1-Y peptide. Four-day-old seedlings were grown on medium supplemented without or with 100 nM MgRGF1-Y. Representative images (**j**) and primary root length measurements (**k**) are shown ($n = 20$). Scale bar, 1 cm. **l,m**, EdU-labelled cells in the wild type and the quintuple mutant treated with MgRGF1 peptide. Measurements of the EdU-labelled root apical meristem size are shown in **l** ($n = 10$). The arrowheads in **m** indicate the boundary regions of EdU-labelled cells, and the white asterisks indicate the QC regions. Scale bars, 100 μ m. The experiments were performed three times with similar results (**d–m**). The data in **e**, **g**, **i**, **k** and **l** are presented as box-and-whisker plots with individual data points. The central line indicates the median, the box limits represent the first and third quartiles and the whiskers extend to the minima and maxima. *P* values were determined by one-way ANOVA with Dunnett's multiple comparisons test, comparing the mean of each column with the mean of the control column (**e**, **g**, **i**), and unpaired two-tailed Student's *t*-test (**k**, **l**).

(OsPSY5-Y). Our results demonstrated that *OsPSY5-Y* treatment significantly promoted rice root growth, primarily through enhanced cell elongation in the maturation zones (Fig. 7k–n and Supplementary Fig. 21a,b), while meristematic cell proliferation remained unaffected (Supplementary Fig. 21a–d). A consistent effect was found in *Arabidopsis*, where the exogenous application of *OsPSY5-Y* markedly increased cell length (Supplementary Fig. 22a–f). We further generated overexpression lines of *OsPSY5* in Kitaake and evaluated the expression levels using RT–qPCR (Supplementary Fig. 23a).

Two independent homozygous T_2 transgenic lines displayed longer roots than the wild-type controls, due to an increase in cell length (Fig. 8a–d). Meristematic cell proliferation in these overexpression lines remained unchanged (Supplementary Fig. 23c,d). Additionally, *OsPSY5*-knockout lines were constructed using CRISPR–Cas9-mediated gene editing and verified by sequencing (Supplementary Fig. 23b). The *Ospsy5* mutants exhibited a significant reduction in root length, reduced cell elongation in the maturation zone and decreased cell proliferation activity in the meristem zone (Fig. 8e–h and Supplementary Fig. 23e,f).



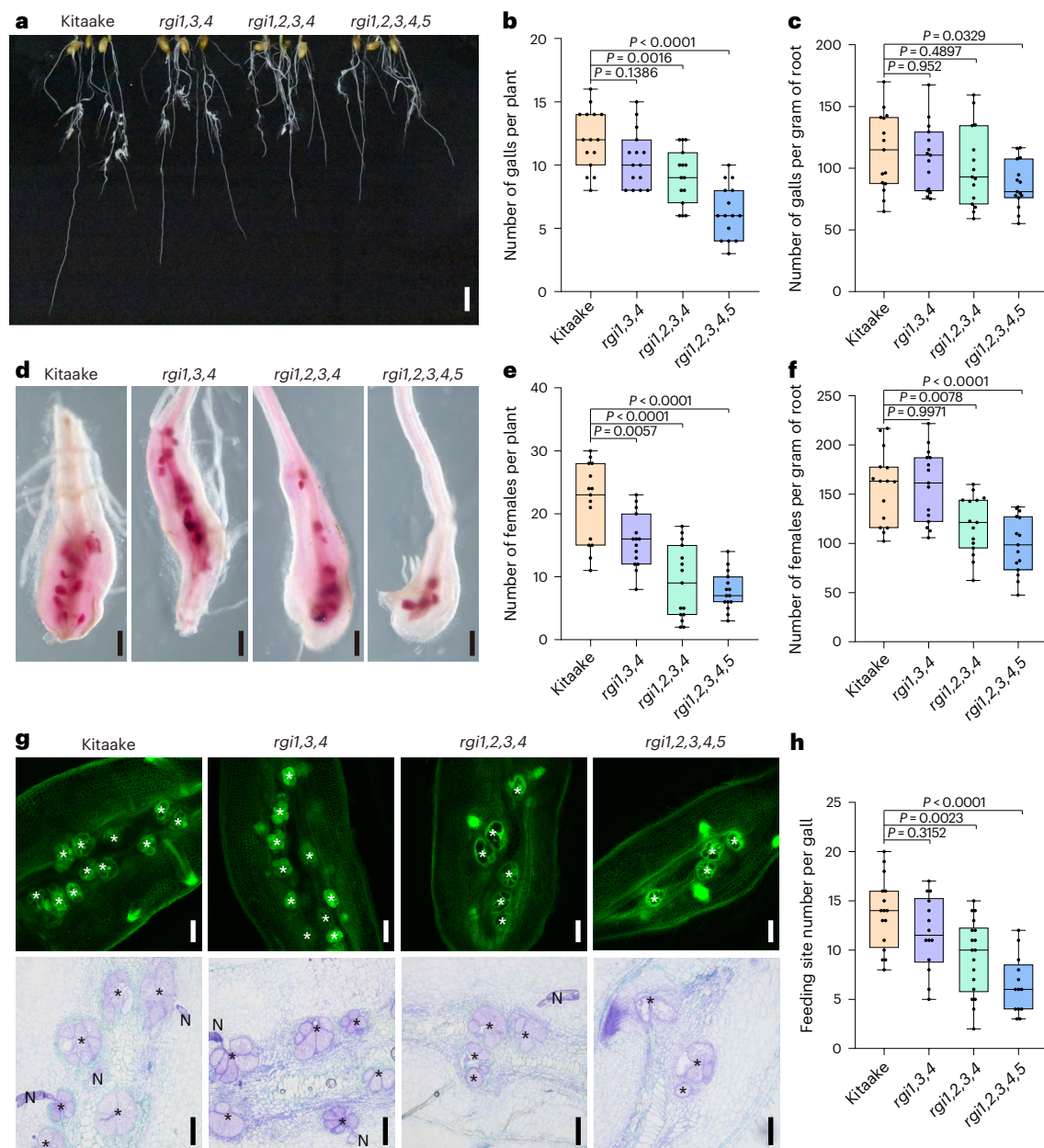


Fig. 6 | OsRGLs are essential for nematode parasitism. **a–c**, Nematode infection phenotype in rice *rgi* mutants. Roots were photographed at 7 dpi with *M. graminicola* (**a**), and the numbers of root galls per root (**b**) and per gram of root (**c**) were counted and quantified ($n = 15$). Scale bar, 1 cm. **d–f**, Acid fuchsin staining of the wild type and *rgi* mutants. Infected roots were stained at 15 dpi (**d**), and the numbers of females per root (**e**) and per gram of root (**f**) were counted and quantified ($n = 15$). Scale bars, 500 μ m. **g, h**, Feeding site observation in *rgi* mutants. The experiments were performed as described previously. Representative images of a single gall per line are shown (**g**). The number of

feeding sites per gall (**h**) was quantified. $n = 16, 14, 18, 13$ from left to right. Scale bars, 200 μ m (upper panels in **g**) and 100 μ m (lower panels in **g**). The asterisks indicate giant cells. Representative data from one of three biological replicates are shown. The data in **b, c, e, f** and **h** are presented as box-and-whisker plots with individual data points. The central line indicates the median, the box limits represent the first and third quartiles and the whiskers extend to the minima and maxima. *P* values were determined by one-way ANOVA with Dunnett's multiple comparisons test, comparing the mean of each column with the mean of the control column.

All these findings indicate that *OsPSY5* promotes root growth primarily through cell elongation, though it may also influence cell proliferation to some extent.

To understand the role of *OsPSY5* in nematode parasitism, we conducted nematode infection assays on *OsPSY5* transgenic lines. Compared with the wild type, *OsPSY5* overexpression lines showed increased numbers of galls and females. More feeding sites were formed within the overexpression roots (Fig. 8i–m). In contrast, knock-out lines showed decreased susceptibility with reduced numbers of

females and fewer feeding sites (Fig. 8i, n–p). The size of nematode feeding cells was not significantly altered in any of the transgenic lines (Supplementary Fig. 23g, h). These results suggest that *OsPSY5*-derived peptide facilitates nematode parasitism and feeding site establishment.

Discussion

Plant parasitic RKNs have a broad host range and pose a major threat to global agriculture⁶⁷. These obligate parasites establish intimate and long-term relationships with their hosts by inducing the formation of

highly specialized feeding sites known as giant cells, a developmental reprogramming process essential for nematode growth and survival^{10,68}. The establishment of giant cells, favoured by secretions released from nematodes, represents a remarkable example of post-embryonic organogenesis; however, the underlying molecular mechanisms remain poorly defined. RGFs, a class of tyrosine-sulfated peptides, have been implicated in the maintenance of the root stem cell niche in *Arabidopsis*⁴⁵. In the present study, we demonstrated that RKNs secrete a class of RGF effector proteins that hijack host RGI-receptor-mediated signalling to promote feeding site establishment during the early stage of nematode infection. We highlight the pivotal roles of nematode RGF peptides in coordinating both cell proliferation and expansion during feeding site development. Furthermore, *PLT* transcription factors and *PSY* peptide genes were identified as key downstream components of the RGF signalling cascade (Extended Data Fig. 10). These findings unveil that RKNs secrete RGF peptide mimics to co-opt plant developmental signalling pathways, priming host cells for their own parasitic advantage.

RGF genes were identified exclusively in all species of RKNs with available high-quality genomes, pointing to a conventional strategy employed by RKNs to parasitize their hosts effectively. The identification of RGF genes outside the plant kingdom is also notable. The corresponding RGF motifs contain the consensus residues of canonical plant RGF peptides; however, sequences outside their conserved domain show low similarity. Although it is conceivable that plant-parasitic nematodes may have acquired RGF genes from their host plants during intimate association, other possibilities such as independent evolution and positive selection cannot be excluded. We also noted high sequence similarity within Clade I *Meloidogyne* species, with identical peptide sequences shared across different species, such as one set shared by *M. arenaria*, *M. javanica* and *M. floridensis*, and another by *M. arenaria*, *M. javanica* and *M. incognita*, suggesting a potential common origin. In line with this, previous comparative genomics of RKNs has revealed that polyploid RKNs, such as *M. arenaria*, *M. javanica* and *M. incognita*, probably shared a common ancestral hybridization origin^{69,70}. Since RGF-like sequences could not be retrieved in the Pratylenchidae family, which was proposed as a close ancestor of RKNs, these genes may have originated from another distant common ancestor. As additional high-quality nematode genomes become available, the underlying evolutionary basis for nematode acquisition of RGF genes will warrant further investigation.

Different nematode groups utilize their unique repertoires of effectors to adapt different hosts and drive distinct feeding cell ontogenies^{27,28}. For cyst nematodes, dorsal-gland-secreted CLE-like peptide effectors have been well characterized as plant peptide mimics to modulate syncytium formation³¹. Here we provide evidence that nematode RGF-like effectors are involved in successful giant

cell establishment during early infection by RKNs. First, RT-qPCR and in situ hybridization analysis demonstrated that nematode RGF effectors are expressed in the subventral gland cells of early parasitic nematodes. Consistently, subventral glands were known to be active during early stages of nematode parasitism, allowing the effectors to fulfil their function in early feeding cell initiation. Immunolocalization confirmed that MgRGF is delivered into host tissues during nematode infection and directly targeted into the apoplast, an interface important for ligand perception by plant receptors on adjacent cells. Second, silencing and transgenic expression analysis of nematode RGF effectors illustrated their importance in feeding site formation. In addition, plant RGI receptors, capable of binding and perceiving nematode RGF ligands, were found to be expressed at nematode feeding sites. Knocking out these receptors resulted in reduced nematode infection and fewer feeding sites, suggesting that multiple receptors may be required for proper feeding site establishment. However, the possibility that root growth defects in *rgi* mutants complicate infection assessment should not be ruled out. Collectively, these findings reveal that nematodes secrete RGF-like peptides to tap into plant RGI-receptor-mediated signalling pathways in favour of giant cell establishment.

Our results illustrate that nematode-derived peptides functionally mimic plant peptide ligands to trigger endogenous developmental cascades essential for root growth via the corresponding receptors. Treatment with nematode RGF peptides compensated root meristem activity in *Arabidopsis tpst-1* and *rgf1,2,3* mutants, resembling the effects conferred by AtRGF1. Ectopic expression of nematode RGFs or exogenous application of these peptides promoted root growth and meristem activity in *Arabidopsis*. In contrast, peptide application to rice or tomato roots produced distinct effects on root growth, resulting in short and thick root phenotypes. Cellular characterization revealed elevated meristem activity despite reduced root length, accompanied by enhanced cell expansion induced by the peptide. To support this, the MAPK cascade and downstream components, such as *PLTs*, were activated by RGF peptide treatment in both *Arabidopsis* and rice. Notably, the gradient distribution of *PLT2* in the stem cell region differs between *Arabidopsis* and rice. In addition, *PSY* genes, which stimulate root growth via cell elongation, were strongly induced in rice, whereas their expression in *Arabidopsis* remained stable under the same duration of RGF peptide treatment, suggesting that downstream responses to RGF signalling are not identical across species. Considering that the anatomy of rice roots differs from that of *Arabidopsis* (for example, by the presence of additional cortex cell layers), this seemingly contradictory root phenotype of rice may reflect a loss of coordination between cell elongation and expansion in the elongation-maturation zone. Two endogenous rice RGF peptides elicited similar phenotypes, but at concentrations differing by an order of magnitude, indicating variations in root sensitivity even to endogenous peptides. These observations

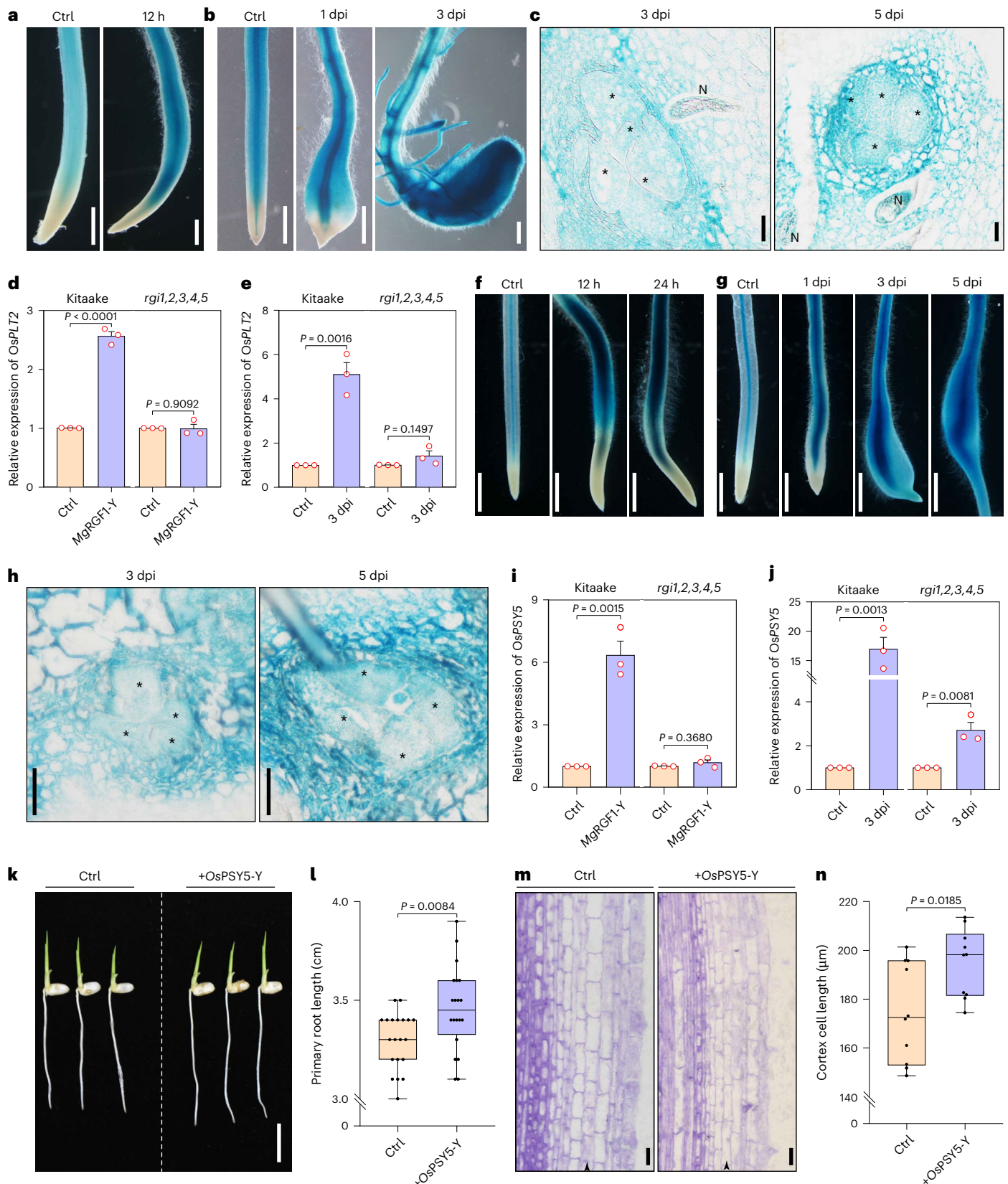
Fig. 7 | OsPLT2 and OsPSY5 are activated by nematode RGF signalling.

a, Expression pattern of *pOsPLT2::GUS* in response to exogenous MgRGF1-Y peptide treatment. Twenty roots were treated with MgRGF1-Y peptide for the indicated time (right). 'Ctrl' indicates that no peptide was added. Scale bars, 500 μ m. **b**, *pOsPLT2::GUS* activity in rice roots following *M. graminicola* infection. Uninfected control as well as infected root tips at 1 dpi and 3 dpi are shown. Twenty roots were used for each treatment. Scale bars, 500 μ m. **c**, Representative histological sections of nematode feeding sites at 3 dpi and 5 dpi. Ten galls were sectioned for observation. The asterisks indicate giant cells. Scale bars, 50 μ m. **d,e**, Induced expression of OsPLT2 is compromised in the *rgi* quintuple mutant. The wild type and *rgi1,2,3,4,5* were subjected to MgRGF1-Y peptide treatment (**d**) or nematode inoculation (**e**). RT-qPCR was quantified relative to controls using *OsUBQ10* and *OsActin* as internal controls. **f**, Expression pattern of *pOsPSY5::GUS* in response to peptide treatment. Twenty roots were observed for each treatment. Scale bars, 1 mm. **g**, *pOsPSY5::GUS* expression pattern at the indicated time points after *M. graminicola* infection. Twenty roots were stained, and representative images are shown. Scale bars, 1 mm. **h**, Representative histological sections of nematode feeding sites at 3 dpi and 5 dpi. Ten galls were sectioned for

observation. The asterisks indicate giant cells. Scale bars, 100 μ m. **i,j**, Induced expression of OsPSY5 is compromised in the *rgi* quintuple mutant. Seedlings were treated with MgRGF1-Y peptide (**i**) or nematodes (**j**). *OsUBQ10* and *OsActin* were used as internal controls. **k,l**, Exogenous treatment with OsPSY5-derived peptide promotes root growth in rice. Kitaake seedlings grown vertically on medium without or with sulfated peptide (100 nM) were photographed (**k**) at 24 h after treatment, and root length (**l**) was measured ($n = 20$). Scale bar, 1 cm. **m,n**, Median longitudinal sections of the root maturation zone under OsPSY5-Y peptide treatment. The black arrowheads in **m** mark the representative layer of CPCs. Scale bars, 200 μ m. The corresponding cell length (**n**) was measured ($n = 10$). Representative images (**a-c**, **f-h**, **k,m**) and data (**l,n**) from one of three biological replicates are shown. The data in **d**, **e**, **i** and **j** are shown as mean $\pm$ s.e.m. with individual data points from three independent biological replicates ($n = 3$). The data in **l** and **n** are presented as box-and-whisker plots with individual data points. The central line indicates the median, the box limits represent the first and third quartiles and the whiskers extend to the minima and maxima. *P* values were determined by unpaired two-tailed Student's *t*-test.

led us to propose that the RGF signalling pathway is subject to delicate regulation, whereas peptide treatment of whole rice roots probably disturbs the homeostasis of cell division and elongation, resulting in growth chaos. The distinct peptide-induced responses between *Arabidopsis* and rice further imply a sophisticated and species-specific nature of peptide signalling networks.

We also observed that sulfated peptides exhibit higher biological activity and binding affinity to receptors than their unsulfated forms. Currently, it is technically challenging to directly determine the mature form and modification status of nematode-secreted peptides in planta. Given that the *TPST* homologues (the genes responsible for tyrosine sulfation) are present in the genomes of RKNs, it is tempting to



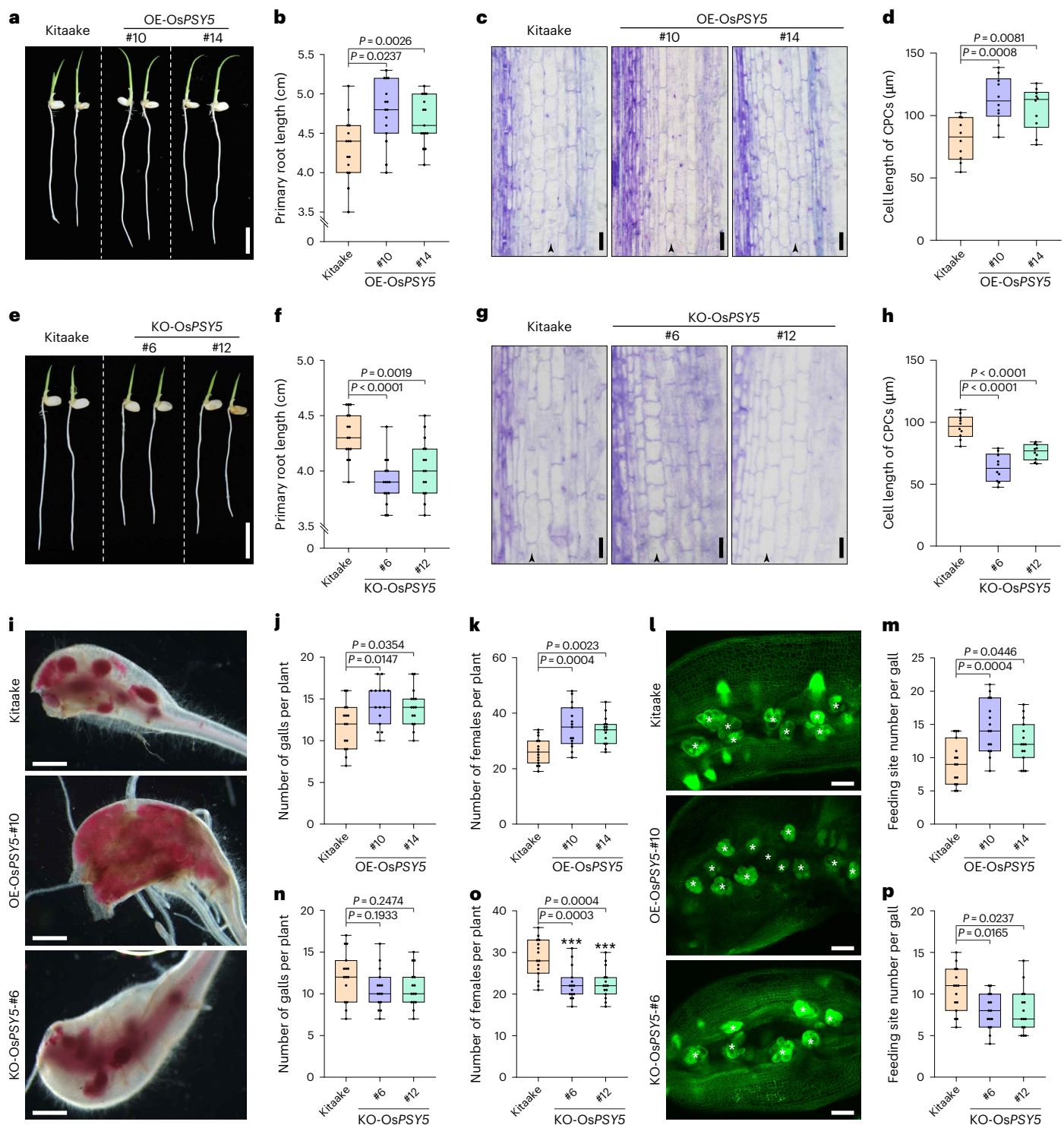


Fig. 8 | *OsPSY5* plays an important role in nematode parasitism. a, b, Effect of *OsPSY5* overexpression (OE) on root growth. Four-day-old seedlings were photographed (a), and primary root length (b) was measured ($n = 15$). Scale bar, 1 cm. **c, d**, Longitudinal sections of wild-type and *OsPSY5* overexpression lines. Four-day-old seedlings were sectioned across the differentiation zone (c). The black arrowheads mark the representative layer of CPCs. The corresponding cell length (d) was measured ($n = 10$). Scale bars, 200 μm . **e, f**, Effect of *OsPSY5* knockout (KO) on root growth. Four-day-old seedlings were photographed (e), and primary root length measurements are shown in f ($n = 15$). Scale bar, 1 cm. **g, h**, Longitudinal sections of wild-type and *OsPSY5* knockout lines as described previously. Representative images (g) and cell length measurements ($n = 10$) (h) are shown. Scale bars, 200 μm . **i–p**, Nematode infection phenotypes in transgenic lines. Two independent homozygous lines were generated for

OsPSY5 overexpression and knockout. Representative images of root galls are shown by acid fuchsin staining (i). Root galls (j, n) and female nematodes (k, o) were counted at 7 dpi and 15 dpi with *M. graminicola*, respectively ($n = 15$). Scale bars, 1 mm. Feeding site observation (l) was performed by BABB clearing. The experiments were performed as described previously. Representative images of a single gall per line are shown. The number of feeding sites per gall (m, p) was quantified ($n = 15$). The white asterisks represent feeding sites. Scale bars, 200 μm . The experiments were performed three times with similar results. The data are presented as box-and-whisker plots with individual data points (b, d, f, h, j, k, m–p). The central line indicates the median, the box limits represent the first and third quartiles and the whiskers extend to the minima and maxima. *P* values were determined by one-way ANOVA with Dunnett's multiple comparisons test, comparing the mean of each column with the mean of the control column.

speculate that nematode peptides may be sulfated within the nematode prior to secretion^{44,71}. With respect to peptide processing, subtilisin-like proteinases (such as SBT6.1 and SBT3.8) have been reported to cleave endogenous plant RGF precursors^{52,53}. We successfully detected mature tyrosine-sulfated peptides in apoplastic peptide extractions of *MgRGF* transgenic lines, indicating that nematode RGF precursors can be properly processed once secreted into the plant. We proposed that nematode RGF preproteins may be processed into proproteins via the endoplasmic reticulum–Golgi secretory pathway in the subventral glands, secreted into the host apoplast and subsequently cleaved into bioactive peptides by apoplastic proteinases. It is unclear whether the short sequence between the signal peptide and the conserved RGF domain contributes to this process. The mechanistic details regarding nematode peptide modification and processing remain to be elucidated in future.

Feeding cell formation is a complex process, accompanied by dramatic cellular changes and massive transcriptional reprogramming⁴. RKNs target vascular parenchyma cells and transform them into hypertrophied multinucleate giant cells through repeated acytokinetic mitosis and endoreduplication, increasing cell volume by up to 100 times^{3,4}. Concurrently, the extensive division of neighbouring pericycle and cortical cells leads to localized root swelling, ultimately giving rise to characteristic gall symptoms⁷². Limited hyperplasia and hypertrophy are often associated with suboptimal giant cell development. Emerging evidence has illustrated that giant cells appear to share developmental characteristics with meristematic cells. Within galls, different developmental programs are recruited to drive the induction of pluripotent cells that subsequently differentiate for gall regeneration^{4,6,7,23}. Our studies show that sulfated peptides, including RGFs and PSYs derived from both RKNs and host cells, act cooperatively as essential signals to trigger cell reprogramming, potentially dedifferentiating the selected plant cells to generate a novel cell type. Interestingly, PSY peptides have recently been reported to repress stress signalling activated by PSY RECEPTORS to enable root growth⁷³. This PSY-peptide-mediated trade-off between growth and stress responses raises the possibility that the induction of PSYs in nematode feeding sites may facilitate nematode invasion, not only by promoting cell growth but also by suppressing PSY-RECEPTOR-mediated defence responses (Extended Data Fig. 10).

A recent study identified sulfated PSY peptides only in Clade I *Meloidogyne* species, but not in other nematode species, such as *M. graminicola*⁴⁴. Although *M. graminicola* lacks nematode-derived PSYs, it instead activates rice endogenous PSY genes. The interplay of multiple sulfated peptides from both nematode and host may act synergistically to magnify plant responses during the parasitic process. As previously hypothesized, an advantage of nematode-secreted mimics is the local increase of the peptide concentrations, presumably bypassing plant endogenous feedback regulation and constitutively activating downstream pathways required for feeding cell formation²⁹. Considering the established roles of phytohormones in regulating nematode-induced feeding cells, an incredibly complex network is probably active, involving interactions or crosstalk between peptide and hormone signalling pathways during feeding site formation. How *OsPLT2* and *OsPSYs* are transcriptionally activated remains to be elucidated. How multiple pathways are coordinated in time and space during nematode parasitism presents a challenging yet exciting topic for future exploration.

Deciphering the developmental reprogramming pathways manipulated by plant-parasitic nematodes is imperative, given that nematode survival is entirely dependent on successful feeding site establishment. Our study highlights the critical role of nematode RGF signalling in RKN feeding cell formation. These findings not only enrich our understanding of RGF peptide signalling in fundamental plant development but also provide potential targets for engineering nematode resistance in crops through the disruption of nematode RGF signalling.

Methods

Nematode culture, plant materials and growth conditions

Meloidogyne graminicola and *M. incognita* cultures, initially propagated from a single egg mass, were maintained on rice (*Oryza sativa* L. Nipponbare) and tomato (*Solanum lycopersicum*), respectively^{66,74}. The infested plants were grown in a sand-and-potting-soil mixture (3:1) under controlled conditions at 28 °C with a 16-h light/8-h dark photoperiod, 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity and 70–75% relative humidity. Eggs were purified from infected roots and hatched in sterile water as described previously⁷⁵. J2s were used for inoculation experiments. Different parasitic life stages of the nematodes were collected from roots as previously described⁷⁶.

Rice cultivars Kitaake and Nipponbare were used in this study. For agronomic trait measurements, rice plants were planted in a standard paddy field in Wuhan, China. *Arabidopsis* mutant and transgenic lines are in the *A. thaliana* ecotype Columbia (Col-0) background. The *tpst-1*, *rgf1.2,3*, *rgi1.2,3,4,5*, *pRG11::GUS*, *pRG12::GUS*, *pRG13::GUS*, *pRG14::GUS*, *pRG15::GUS*, *pPLT1::CFP*, *pPLT2::CFP*, *pPLT1::PLT1-YFP*, *pPLT2::PLT2-YFP* and *pOsCYCB1.1::GUS* lines have been reported in previous studies^{54,77}.

Both rice and *Arabidopsis* seeds were surface-sterilized in 75% (v/v) ethanol for 2 min, followed by treatment with 1% (w/v) sodium hypochlorite for 10 min (*Arabidopsis*) or 15 min (rice), and then rinsed thoroughly at least five times with sterile deionized water. The seedlings were then grown on $\frac{1}{2}$ MS medium containing 1% (w/v) sucrose and 0.7% (w/v) agar at pH 5.8. *Arabidopsis* and rice seedlings were cultured under a 16-h light/8-h dark photoperiod at 22 °C and 28 °C, respectively. For peptide treatment, synthetic peptides were added to the medium at the indicated concentrations.

Sequence analysis

Amino acid sequences of nematode RGFs were retrieved from different *Meloidogyne* species using the annotated genome databases in Wormbase Parasite (<http://parasite.wormbase.org/index.html>). Sequences of the rice *RGF* and *PSY* gene families were obtained from Phytozome v.13 (<https://phytozome-next.jgi.doe.gov/>). Conserved domains were predicted using the online tools SignalP v.6.0 (<https://services.healthtech.dtu.dk/services/SignalP-6.0/>), SMART v.10 (<http://smart.embl-heidelberg.de/>) and TMHMM v.2.0 (<https://services.healthtech.dtu.dk/services/TMHMM-2.0/>). Multiple sequences were aligned via MAFFT v.7 (<https://mafft.cbrc.jp/alignment/software/>). Phylogenetic analysis was performed using the MAFFT algorithm of MEGA11 (ref. 78). A bootstrap consensus tree was constructed inferred from 1,000 replicates using the maximum-likelihood method. Non-classical protein secretion was predicted using SecretomeP-1.0 (<https://services.healthtech.dtu.dk/services/SecretomeP-1.0/>).

Vector construction

For overexpression analysis, the full-length coding sequences and the signal-peptide-deleted versions of *MgRGF* and *MiRGF1* were amplified from parasitic J2s of *M. graminicola* and *M. incognita* using gene-specific primers. The PCR products were digested with *Sall* and *XbaI* and ligated into the pCambia1300 binary vector under the control of the CaMV35S promoter. The constructs were introduced into rice variety Kitaake and *Arabidopsis* Col-0 via *Agrobacterium tumefaciens*-mediated transformation to generate independent lines for subsequent functional analysis. RT-qPCR was performed to evaluate gene expression levels.

For yeast secretion assays, the signal peptide sequences of *MgRGF* and *MiRGF1* were cloned into the pSUC2 vector to generate in-frame fusions with invertase. The resulting vectors and control vectors were transformed into the yeast strain YTK12 for further analysis (courtesy of W. Yin at Huazhong Agricultural University). For secretion analysis in *N. benthamiana*, the signal peptide fragments were fused to the C terminus of XEG1 in the vector pGR107 (courtesy of W. Yin at HZAU).

Targeted mutagenesis of candidate genes was performed using PTG CRISPR–Cas9 technology⁶⁴. Briefly, gene-specific gRNAs were

designed for each target, and the assembled PTG fragments were inserted into the pRGE32 binary vector (courtesy of K. Xie at HZAU). The resulting constructs were transformed into *Kitaake*. The independent transgenic plants were genotyped by PCR using primers flanking the target sites, and mutations were confirmed by sequencing. T₁ or T₂ homozygous transgenic lines were used for phenotypic analysis.

For subcellular localization, the coding sequences of *OsRGI2* (LOC_Os05g07740) and *OsRGI3* (LOC_Os08g38560) were subcloned into the pRTV-cGFP binary vector via BamHI and HindIII digestion to generate GFP fusion constructs.

To generate promoter–GUS reporter constructs, the 2-kb genome fragments upstream of the ATG start codon of *OsPLT2* and *OsPSY5* were amplified and subcloned into the pCAMBIA2391Z vector with Sall and EcoRI. T₂ homozygous transgenic lines in the *Kitaake* background were used for subsequent analysis.

For recombinant protein expression, the gene sequence encoding the extracellular LRR domain of *OsRGI2* was subcloned into the pFastbac I plasmid in-frame with an N-terminal signal peptide and a C-terminal His-tag via BamHI and XhoI digestion.

All primers used to construct vectors are listed in Supplementary Table 4. All constructs were verified by sequencing.

Nematode infection assay

Four-day-old rice seedlings (approximately 2 cm in root length) were inoculated with 100 *M. graminicola* J2s per plant or with 0.1% (w/v) agarose as controls. The infection levels were evaluated at 7 dpi by counting the number of galls per plant and at 15 dpi by counting the number of female nematodes per plant. For rice RKNs, hook-shaped galls are primarily established in rice root tips, with numerous feeding sites within a single gall. The females and eggs remain embedded in the root tissue. Thus, the egg sacs are difficult to quantify, and females were counted after gall dissection. Acid fuchsin staining was performed to visualize galls and nematode development under a stereomicroscope (Olympus SZX16). Galls were boiled in 0.8% (v/v) acetic acid and 0.013% (w/v) acid fuchsin for 3 min and then destained in acidified glycerol. Feeding sites were observed by tissue sectioning and BABB clearing. To facilitate the measurement of feeding cell size in rice, 35 J2s were used for inoculation.

Nematode infection assays on *Arabidopsis* or tomato plants were performed as described previously⁷⁹. Fourteen-day-old seedlings were inoculated with 300 *M. incognita* J2s per plant in a sand:soil (3:1) mixture. Roots were collected at 35 dpi and stained with acid fuchsin. Egg masses are visible on the root surface. Galls and egg masses were quantified under a stereomicroscope (Olympus SZX16). Giant cell size was measured using Fiji ImageJ v.1.52.

RNA extraction and RT–qPCR analysis

Total RNA was extracted from various developmental stages of *M. graminicola* and *M. incognita*, as well as from rice and *Arabidopsis* roots, using the TransZol Up Plus RNA Kit (TransGen Biotech). Parasitic stages of nematodes were collected from infected roots as previously described⁸⁰. RNA concentration was determined using a NanoDrop-1000 spectrophotometer (Thermo Scientific). Subsequently, first-strand cDNA was synthesized with the ABScript II cDNA First-Strand Synthesis Kit (ABclonal), and RT–qPCR was conducted with 2X Universal SYBR Green Fast qPCR Mix (ABclonal) on a Bio-Rad iCycler iQ5 real-time PCR system. Data were analysed using the $2^{-\Delta\Delta CT}$ or $2^{-\Delta CT}$ method⁸¹. As previously reported^{57,66,82–87}, the following genes were used as internal controls: *MgACTIN2* (KAF7632952) and *MgTub* (NXFT01002159.1.5702_g) for *M. graminicola*, *MiGAPDH* (Minc3s07075g40689) and *Mi18S* (U81578) for *M. incognita*, *OsUBQ10* (LOC_Os02g06640) and *OsActin* (LOC_Os03g50885) for rice, and *AtActin2* (AT3G18780) and *AtTUB2* (At5g62690) for *Arabidopsis*. The experiments were repeated three times, with three technical replicates performed for each reaction.

In situ hybridization and immunolocalization analysis

In situ hybridization was performed with approximately 5,000 parasitic J2s of *M. graminicola* and *M. incognita*. A 153-bp fragment of *MgRGF* and a 126-bp fragment of *MiRGF1* were amplified to synthesize DIG-labelled sense and antisense cDNA probes using a DIG DNA labelling kit (Roche). Nematodes were fixed in 2% paraformaldehyde in M9 buffer at 4 °C for 18 h followed by 4 h of incubation at room temperature. Fixed nematodes were cut with a razor blade to facilitate probe penetration, then permeabilized with proteinase K (0.5 mg ml⁻¹) for 30 min at room temperature and subsequently incubated sequentially in cold methanol and acetone. Hybridization was carried out overnight at 42 °C in hybridization buffer (50% formamide, 4× SSC, 1% blocking reagent, 2% SDS, 1× Denhardt's solution, 0.2 mg ml⁻¹ fish sperm DNA and 0.166 mg ml⁻¹ yeast tRNA) containing 50 ng of DIG-labelled probe⁸⁸. After hybridization, the signals were detected using an anti-DIG Fab antibody conjugated to alkaline phosphatase (Roche, 1:1,000). The stained nematodes were observed with a light microscope (Nikon Eclipse 80i) and photographed using a digital camera (Nikon DS-Ri1).

A polyclonal antibody against *MgRGF* was produced by ABclonal using two synthetic peptides (DYNHFKGHPPLHNK and KDSDY-FNPKHHPPRHNIHK) for rabbit immunization. Immunolocalization was performed with parasitic J2s of *M. graminicola* using the anti-*MgRGF* primary antibody at a 1:100 dilution, followed by an AlexaFluor-488-conjugated goat anti-rabbit secondary antibody at a 1:200 dilution⁸⁹. For in planta immunolocalization, sampled rice galls were fixed in 8% paraformaldehyde in PIPES buffer (50 mM, pH 6.9) overnight at 4 °C, dehydrated through an ethanol series, embedded in butyl-methylmethacrylate and then processed into 5-μm-thick sections⁹⁰. The sections were rehydrated and blocked in 1% bovine serum albumin in PIPES buffer for 30 min. The anti-*MgRGF* antibody (1:100 dilution) and an AlexaFluor-488-conjugated goat anti-rabbit secondary antibody (1:500 dilution) were used for immunolocalization. Negative controls were hybridized by using only the secondary antibody. Images were captured using a Nikon A1 HD25 confocal microscope with 488 nm excitation and 505–530 nm emission for GFP (Nikon).

In vitro silencing of RGF genes in nematodes

The 216-bp and 207-bp cDNA fragments of *MgRGF* and *MiRGF1* were amplified, and the corresponding dsRNAs were synthesized using the T7 RNAi Transcription Kit (Vazyme) according to the manufacturer's instructions. dsRNA of *GFP* was prepared and used as a control. Approximately 10,000 freshly hatched J2s were soaked in M9 buffer containing 2–5 mg ml⁻¹ dsRNA and 1% resorcinol for 6 h at room temperature on a rotator⁹¹. Following incubation, nematodes were washed five times with RNase-free water, and 100 J2s per plant were used to inoculate rice and *Arabidopsis* seedlings. After 3 dpi, total RNA was extracted from infected roots, and RT–qPCR was conducted to evaluate the gene silencing efficiency.

Signal peptide secretion analysis

The signal peptide secretion analysis in yeast was performed as described previously⁶³. The yeast transformants were streaked onto CMD-W (0.67% yeast nitrogen base without amino acids, tryptophan dropout supplement, 2% sucrose, 0.1% glucose and 2% agar) and YPRAA (1% yeast extract, 2% peptone, 2% raffinose, 2 μg l⁻¹ antimycin A and 2% agar) media plates for invertase secretion assays. Invertase enzymatic activity was detected by the reduction of 2,3,5-triphenyltetrazolium chloride to a red-coloured insoluble triphenylformazan.

To verify the function of signal peptide in *N. benthamiana*, *Agrobacterium tumefaciens*-mediated transient expression was carried out to evaluate cell death symptoms triggered by XEG1 secretion⁶². *Agrobacterium* strain EHA105 carrying the indicated vectors was resuspended to a final concentration of OD₆₀₀ = 0.4 in infiltration buffer (10 mM MES, pH 5.6, 10 mM MgCl₂ and 0.2 mM acetosyringone). After syringe

infiltration, plants were cultured at 22 °C for 48 h under a 16-h light/8-h dark photoperiod with a light intensity around 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Root growth analysis

The peptides, with or without tyrosine sulfation, were chemically synthesized with >95% purity (SciLight Biotechnology). Synthetic peptides were dissolved in double-distilled water to a final stock concentration of 1 mM and diluted to the indicated concentrations for subsequent treatments. Seedling growth and root length were monitored at the specified time points. The corresponding peptide sequences are listed in Supplementary Table 2.

Histological observation of rice roots

Rice roots or root galls were embedded in Technovit 7100 resin (Heraeus Kulzer) according to the manufacturer's protocol. Longitudinal or transverse sections of 5- μm thickness were cut using a Leica RM2265 microtome (Leica). The sections were stained with 0.05% toluidine blue for 3–5 min, rinsed three times with double-distilled water and examined under a light microscope (Nikon Eclipse 80i). Cell number in the root meristem region was counted with all cells in the fourth cortical layer of the root. The average cell length and cell area in the root elongation and maturation zones were quantified across all radial cell layers using ImageJ.

EdU staining

Rice EdU staining was conducted using a BeyoClick EdU-488 kit (Beyotime) according to the manufacturer's protocol. Briefly, roots of 4-day-old rice seedlings were treated in 20 μM EdU solution for 4 h. Following 30 min of fixation in 4% paraformaldehyde, the samples were incubated with Azide 488 for an additional 30 min. Then images were taken using a confocal microscope with an excitation laser wavelength at 488 nm and an emission filter of 505–530 nm (Nikon A1 HD25).

NBT staining

Rice roots were vacuum-infiltrated with 0.1% NBT solution for 15 min in the dark at room temperature, followed by two rinses with distilled water. The stained roots were photographed using a stereomicroscope (Olympus SZX16). The stained region within the meristematic zone was measured using ImageJ.

Propidium iodide staining

Roots of *Arabidopsis* seedlings were stained with 0.02 mg ml^{-1} propidium iodide for 1–2 min and then imaged using a Nikon A1 HD25 confocal microscope with an excitation laser wavelength at 561 nm and an emission filter of 587–610 nm (Nikon).

Histochemical GUS assay

At the indicated time points, root fragments of rice and *Arabidopsis* with or without nematode infection were vacuum-infiltrated twice for 10 min each with GUS staining solution (100 mM Tris-HCl, pH 7.0, 50 mM NaCl, 1 mM 5-bromo-4-chloro-3-indolyl- β -glucuronic acid, 1 mM potassium ferricyanide, pH 7.0 and 0.06% (v/v) Triton X-100) and then incubated overnight at 37 °C. The reaction was stopped with 70% (v/v) ethanol. Stained roots were visualized with a stereomicroscope (Olympus SZX16) or embedded in Technovit 7100 resin for cross-sectioning.

Gall imaging

Fixation and clearing of gall samples were conducted following the protocol described previously⁹². Briefly, galls were vacuum-fixed with 2.5% (v/v) glutaraldehyde in 50 mM sodium phosphate buffer for 15 min at room temperature and then left overnight at 4 °C. Fixed galls were subsequently rinsed twice for 5 min with sodium phosphate buffer and dehydrated stepwise in 30%, 50%, 70% and 90% (v/v) ethanol under vacuum twice for 20 min each step. The dehydrated galls were rinsed twice for 20 min with pure ethanol and stored overnight in pure ethanol

at 4 °C for the subsequent clearing process. The samples were transferred to 100% BABB (benzyl alcohol and benzyl benzoate in a 1:2 ratio, v/v) for 2 days at 4 °C. Glutaraldehyde autofluorescence was observed under a Nikon A1 HD25 confocal microscope with an excitation laser wavelength at 488 nm and an emission filter of 505–530 nm (Nikon).

Protein structure prediction and molecular docking

The protein crystal structure of AtRGFR1 (PDB: 5z33) was downloaded from the PDB database (<https://www1.rcsb.org/>). The protein structures of OsRGI2 were predicted with AlphaFold2 (ref. 93). The structures of nematode RGF peptides were generated on the basis of the crystal structure of the AtRGF1–AtRGFR1^{ECD} complex by altering the corresponding residues of AtRGF1 (ref. 56). Molecular docking between RGF peptides and receptors was performed with Autodock Vina⁹⁴, and the conformation with the highest affinity was selected as the final docking conformation. Structural images were displayed using PyMOL v.3.1.6.1 (Schrödinger, LLC).

Subcellular localization in rice protoplasts

Rice protoplasts were isolated from 14-day-old *rgi1,2* and *rgi3,4* mutant seedlings using enzymatic digestion in solution containing 1.5% cellulase R10 and 0.75% Macerozyme R10 (ref. 95). The protoplasts were then transfected with 10 μl of plasmids (5–10 μg), incubated in W5 solution for 16 h and visualized under a confocal microscope excited at 488 nm with an emission filter of 500–550 nm. The protoplasts were treated with 100 nM RGF peptide for 3 h to evaluate the impact on subcellular localization.

In vitro peptide binding assay

The extracellular domain of OsRGI2 was expressed using the Bac-to-Bac Baculovirus Expression System (Thermo Fisher Scientific). Two hundred millilitres of High Five cells (2×10^6 cells per ml) were infected with 30 ml of recombinant baculovirus and cultured at 22 °C for 60 h with gentle shaking. The secreted protein was affinity-purified with Ni-NTA resin (GenScript) for subsequent pull-down experiments and binding affinity assays. For immunoprecipitation, 500 ng of purified protein was incubated with 100 nM biotinylated MgRGF1 peptide in reaction buffer (50 mM Tris-HCl, pH 7.5, 1% Nonidet P40) at 4 °C for 2 h with gentle agitation. Unlabelled RGF peptides were added as competitors. The mixture was immunoprecipitated with BeyoMag Streptavidin Magnetic Beads (Beyotime) for 4 h at 4 °C, followed by five washes with reaction buffer. The eluted and input proteins were detected by immunoblotting with an anti-His antibody at a 1:5,000 dilution.

To evaluate the binding affinities, MST assays were performed using a Monolith NT.115 instrument (NanoTemper). The purified protein was labelled with fluorescence using a RED-tris-NTA His-Tag Labeling Kit (MO-L018; NanoTemper) according to the manufacturer's instructions. The RGF peptides were diluted in a serial concentration gradient ranging from 0.0061 to 200 μM . After incubation at room temperature for 5–10 min, approximately 5 μl of the labelled protein and peptide mixture was loaded into capillaries (MO-K022; NanoTemper). Measurements were conducted in sodium phosphate buffer supplemented with 0.05% Tween 20, using constant instrument settings (20% LED power, medium MST power). Signal-to-noise ratios above 10 were considered significant. The assays were repeated three times for each affinity measurement. Data analyses were performed using MO.Affinity Analysis v.2.3 (NanoTemper).

MAPK activation assay

Five-day-old rice and *Arabidopsis* seedlings grown on solid $\frac{1}{2}$ MS medium were carefully transferred to liquid medium, acclimated for at least 3 h and then treated with 10 μM of sulfated MgRGF1 or AtRGF1 peptides for the indicated times. Following treatment, seedling roots were ground, and total proteins were extracted in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 10% glycerol

(v/v), 1 mM PMSF and 1× complete protease inhibitor cocktail). Equal amounts of protein samples were separated by 10% SDS–PAGE, and phosphorylated MPK3 and MPK6 were detected by immunoblotting using an anti-phospho-p44/p42 MAPK monoclonal antibody (α-pERK, Cell Signaling Technology) at a 1:3,000 dilution. Equal loading was assessed using an anti-actin immunoblot.

Apoplastic peptide analysis

Extraction of apoplastic peptides and nano-LC–MS/MS analysis were conducted as previously described⁹⁶. Seven-day-old transgenic *Arabidopsis* plants expressing full-length MgRGF or its signal-peptide-deleted variant were cultured in liquid ½MS medium for 12 days. The submerged culture medium containing secreted peptides was subjected to *o*-chlorophenol extraction⁴⁵. Precipitated peptides were dissolved in 500 µl of 0.1% TFA. Separation was carried out on an EASY-nLC 1200 system (Thermo Scientific) with a PepMap C18 trap column (3 µm, 75 µm × 2 cm, Thermo Scientific) and an analytical column (2 µm, 75 µm × 25 mm, Thermo Scientific) at a flow rate of 500 nL min^{−1}, using a gradient of 2–90% acetonitrile containing 0.1% formic acid. A 30-min blank gradient was run between samples to minimize carryover. Mass spectra analysis was performed on an Orbitrap Fusion Lumos mass spectrometer in data-dependent acquisition mode, using full MS scans (*m/z* 350–1,250) at 60,000 resolution with HCD fragmentation at 35% collision energy. Dynamic exclusion was set to 25 s. The data were analysed with PEAKS Studio v.13 (Bioinformatics Solutions Inc.) by searching against the MgRGF peptide sequences.

Statistical analysis

Significant differences were determined by unpaired two-tailed Student's *t*-test or one-way ANOVA using GraphPad Prism v.8.01 (GraphPad Software, Inc.).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Source data are provided with this paper. All other data supporting the findings of the study are available in the article and Supplementary Information.

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Author contributions

Xiaoli Guo conceived and supervised the project. Xiaoli Guo, W.L. and J.M. designed the experiments. Xiaoli Guo and W.L. wrote the paper with input from all coauthors. W.L. performed most of the experiments and data analysis. J.M. constructed most of the transgenic materials and contributed to the root growth and disease assay. X.S., Y.W. and Xiaolin Guo built the MgRGF transgenic materials, assisted with nematode isolation and contributed to the peptide treatment experiments. D.D. contributed to nematode sequencing analysis. J.L., C.C. and K.X. provided some reporter lines and *Arabidopsis* mutants. D.P. and G.W. provided nematode materials and critical feedback. All authors approved the submitted version of the paper.

Competing interests

The authors declare no competing interests.

Additional information

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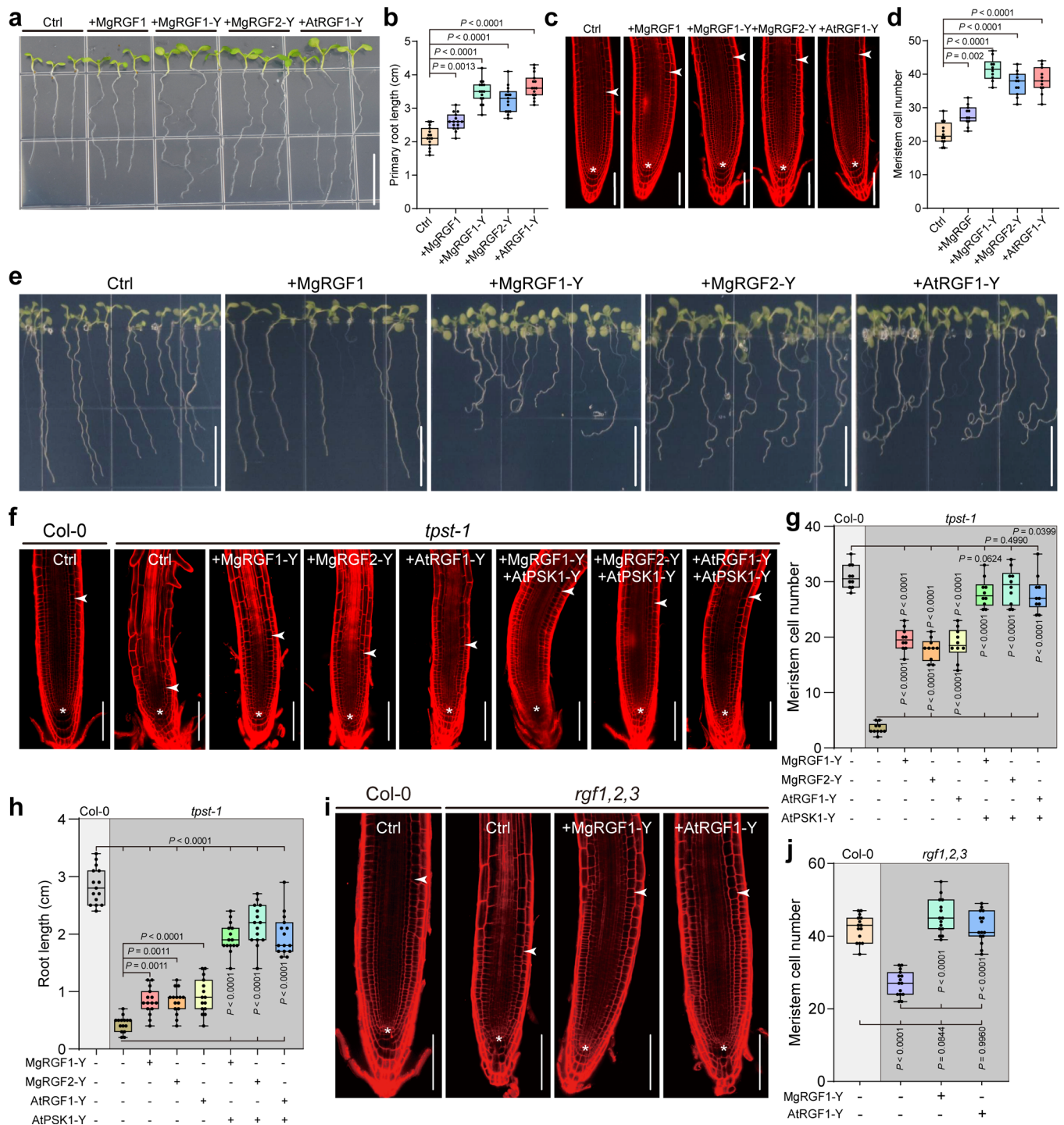
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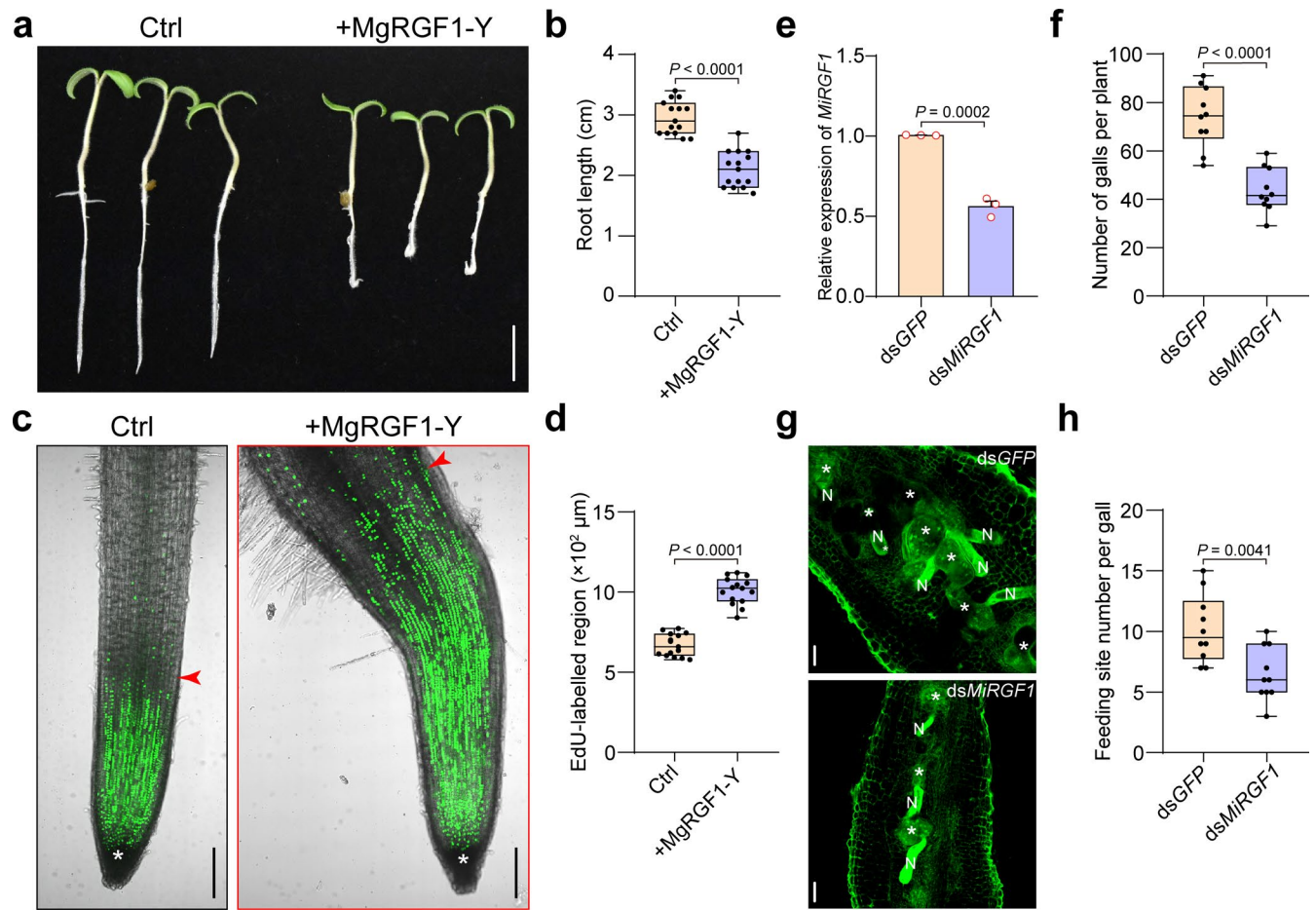
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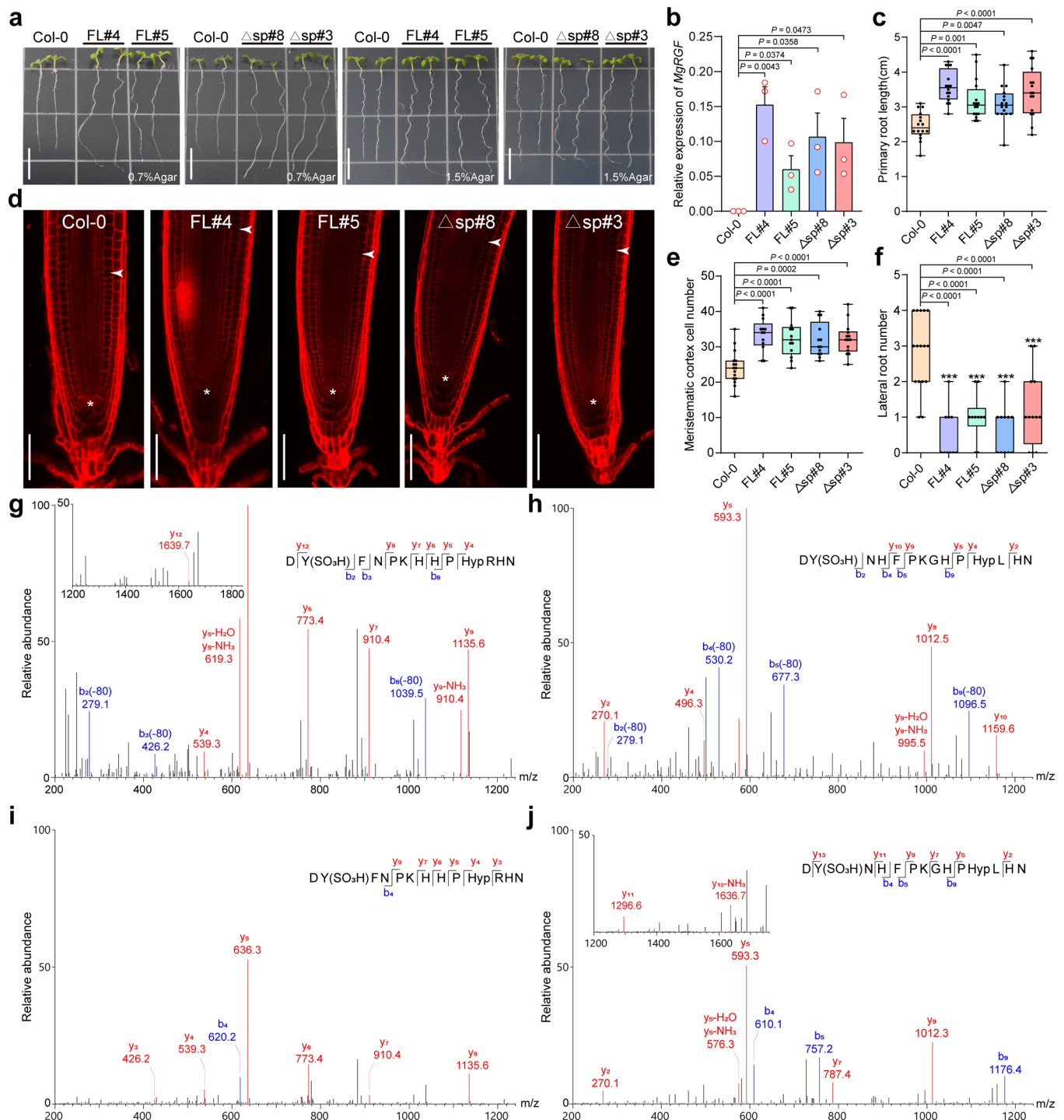
Extended Data Fig. 1 | MgRGF peptides promotes root development in *Arabidopsis*. **a, b**, Root growth phenotype of Col-0 seedlings on vertical plates supplemented with or without 100 nM of the indicated peptides. Seedlings were photographed (**a**) and measured (**b**) at 7 days after germination ($n = 15$). Ctrl indicate no peptide added. Scale bars, 1 cm. **c, d**, Confocal images of Col-0 root tips stained with PI. Four-day-old seedlings were used for the staining (**c**). Number of meristematic cortex cells (**d**) was measured. $n = 12, 11, 10, 11, 11$ in **d** from left to right. Scale bars, 100 μ m. **e**, Seven-day-old seedlings of Col-0 grown on 1.5% agar plates angled at 45°. Seedlings were grown vertically for 2 d on medium supplemented with the indicated peptides, then inclined for an additional 5 d. Representative images are shown, with at least 20 plants observed per treatment in each replicate. Scale bars, 1 cm. **f-h**, Root meristem phenotypes of *tpst-1* grown in the absence or presence of the indicated peptides. Four-day-old seedlings were used for the PI staining and confocal imaging (**f**). Scale bars,

100 μ m. Number of meristematic cortex cells (**g**) was measured ($n = 10$). Root length (**h**) of *tpst-1* seedlings was measured at 7 days after germination ($n = 15$). **i, j**, Root meristem phenotypes of *rgf1,2,3* grown in the absence or presence of indicated peptides. Confocal images showed root meristem of *rgf1,2,3* stained with PI (**i**). Scale bars, 100 μ m. Number of meristematic cortex cells (**j**) was measured ($n = 15$). Experiments were performed three times with similar results. White arrowheads mark the boundary between meristem and elongation zones and white asterisks indicate the QCs (**c, f, i**). Data in **b, d, g, h** and **j** are presented as box-whisker plots with individual data points. The central line indicates the median; box limits represent the first and third quartiles; whiskers extend to the minima and maxima. P values were determined by one-way ANOVA with Dunnett's multiple comparisons test, comparing the mean of each column with the mean of the control column.



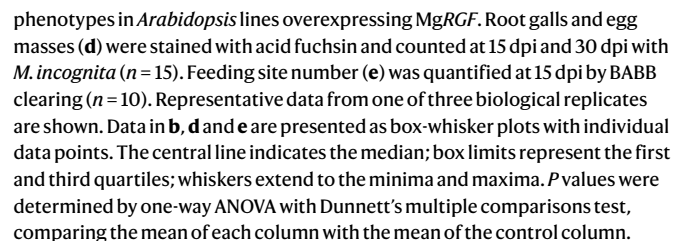
Extended Data Fig. 2 | Nematode RGF effector regulates root growth and promotes nematode parasitism in tomato. **a, b**, Root growth phenotypes under 100 nM synthetic peptide treatment. Tomato seedlings were photographed (**a**) at 12 h after treatment, and root length (**b**) was measured ($n = 15$). Ctrl indicate no peptide added. Scale bars, 1 cm. **c, d**, Longitudinal observation of EdU-labelled cells in the root meristem. After 12 h of MgRGF1-Y treatment, roots were imaged with confocal microscopy (**c**), and the size of EdU-labelled root meristems (**d**) was measured ($n = 15$). Scale bars, 100 μm . Red arrowheads in **c** mark the boundary region of EdU-labelled cells and white asterisks indicate the QC region. **e**, Silencing efficiency of *MiRGF1* detected by RT-qPCR. The experiments were performed using *MiGAPDH* and *Mi18S* as internal controls. **f**, Effect of *MiRGF1* silencing on nematode infection. Root galls were counted at 30 dpi with

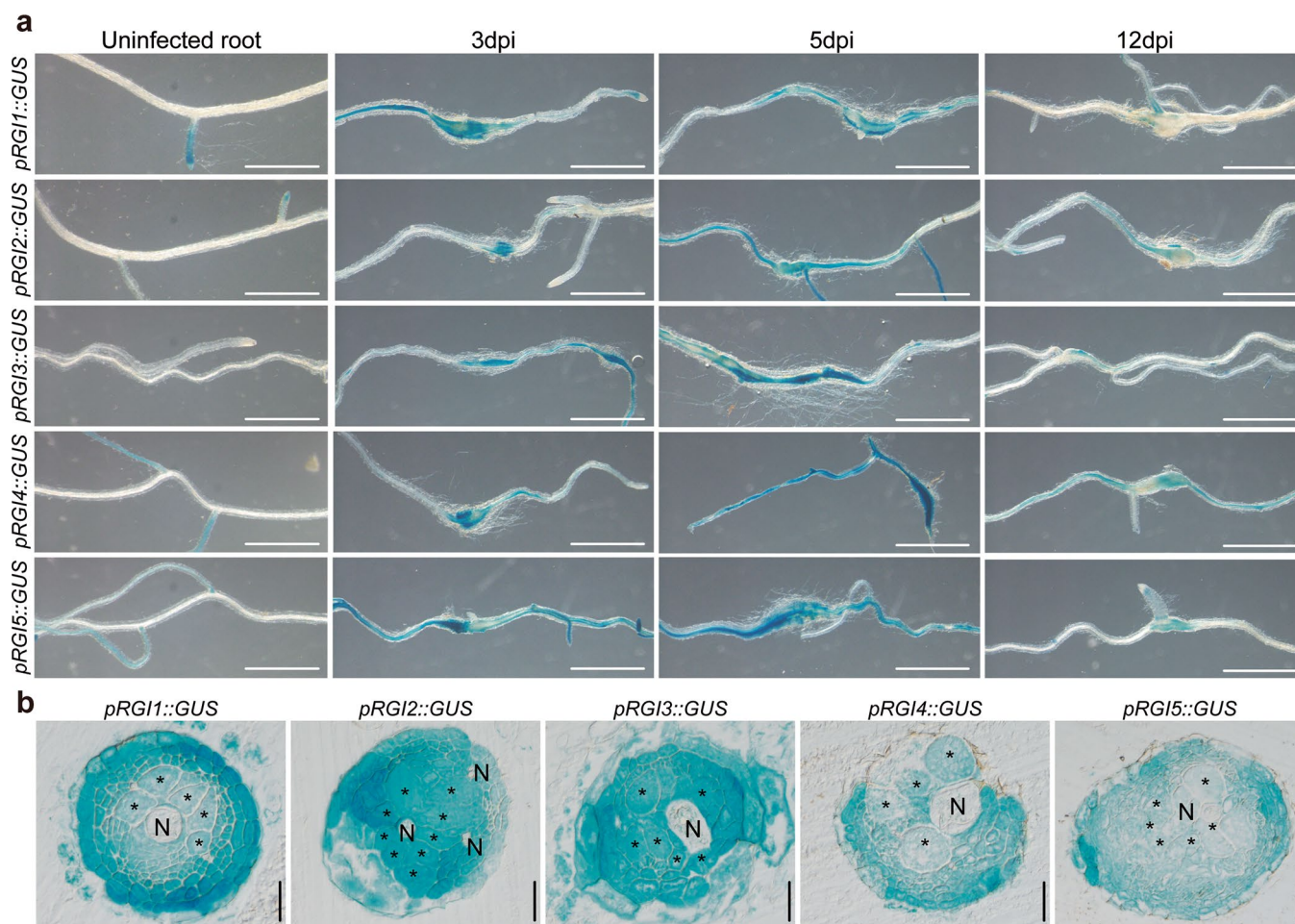
dsRNA-treated *M. incognita* ($n = 10$). **g, h**, Effect of *MiRGF1* silencing on feeding site establishment. Feeding sites (**g**) were observed using BABB clearing at 10 dpi ($n = 10$). Representative images of a single gall are shown. The number of feeding sites per gall (**h**) was counted. Ten galls were analyzed per treatment. Scale bar in **g**, 100 μm ; N: nematode; Asterisks: feeding site. Experiments were performed three times with similar results. Data in **e** are presented as mean $\pm$ s.e.m. with individual data point of three independent biological replicates ($n = 3$). Data in **b, f, d** and **h** are presented as box-whisker plots with individual data points. The central line indicates the median; box limits represent the first and third quartiles; whiskers extend to the minima and maxima. P values were determined by unpaired two-tailed Student's t -test.



Extended Data Fig. 3 | Overexpression of *MgRGF* promotes root development in *Arabidopsis*. **a**, Root phenotypes of Col-0 and two independent transgenic lines overexpressing full-length or signal peptide-deleted version of *MgRGF*. Seedlings grown on 0.7% agar or 1.5% agar were photographed at 7 days after germination. Representative images are shown from three independent experiments, with at least 15 plants examined per treatment in each replicate. Scale bar, 1 cm. **b**, Relative expression levels of *MgRGF* by RT-qPCR using *AtActin2* and *AtTUB2* as internal controls. **c**, Measurements of primary root length ($n = 16$). **d**, Confocal images of root meristem of Col-0 and transgenic lines. Seven-day-old seedlings were stained with PI for imaging. White arrows indicate the boundary between root meristem and elongation zones. White asterisks indicate the QCs. Representative images are shown from three independent experiments, with at least 10 roots examined per treatment in each replicate. Scale bars, 100 μ m. **e**, Measurements of the number of meristematic cortex cells in Col-0 and

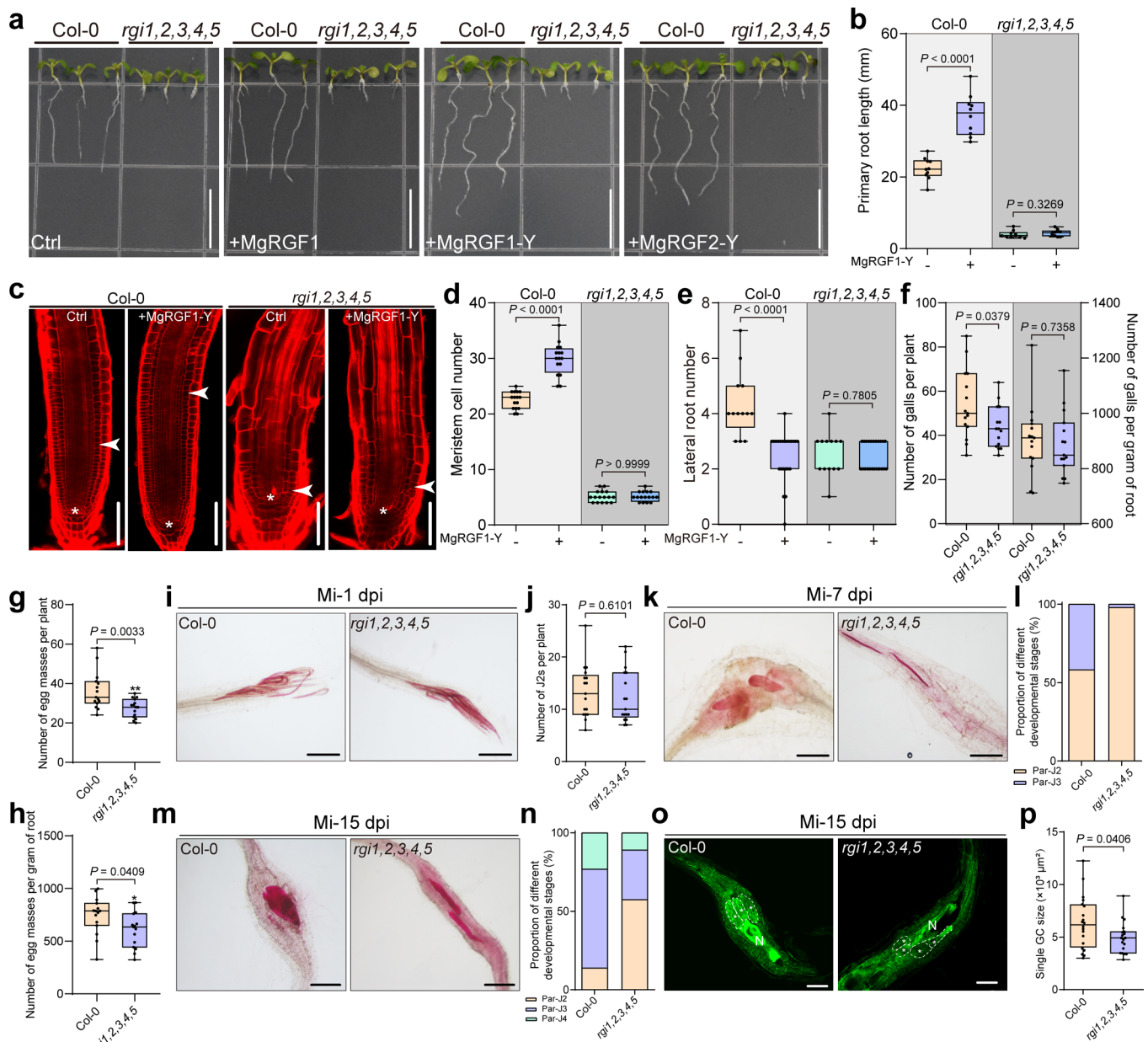
transgenic lines. $n = 16, 13, 13, 14$ from left to right. **f**, Measurements of lateral root number. $n = 16, 10, 10, 11, 12$ from left to right. **g–j**, Nano-LC-MS/MS spectra of tyrosine-sulfated mature peptides. *MgRGF1-Y* (**g, i**) and *MgRGF2-Y* (**h, j**) were identified in apoplastic extraction of the transgenic lines expressing full-length *MgRGF* (**g, h**) or its signal peptide-deleted variant (**i, j**). Red and blue lines indicate matched y-ions and b-ions, respectively. Experiments were performed three times with similar results. Data in **b** are presented as mean $\pm$ s.e.m. with individual data point of three independent biological replicates ($n = 3$). Data in **c**, **e** and **f** are presented as box-whisker plots with individual data points. The central line indicates the median; box limits represent the first and third quartiles; whiskers extend to the minima and maxima. *P* values were determined by one-way ANOVA with Dunnett's multiple comparisons test, comparing the mean of each column with the mean of the control column.





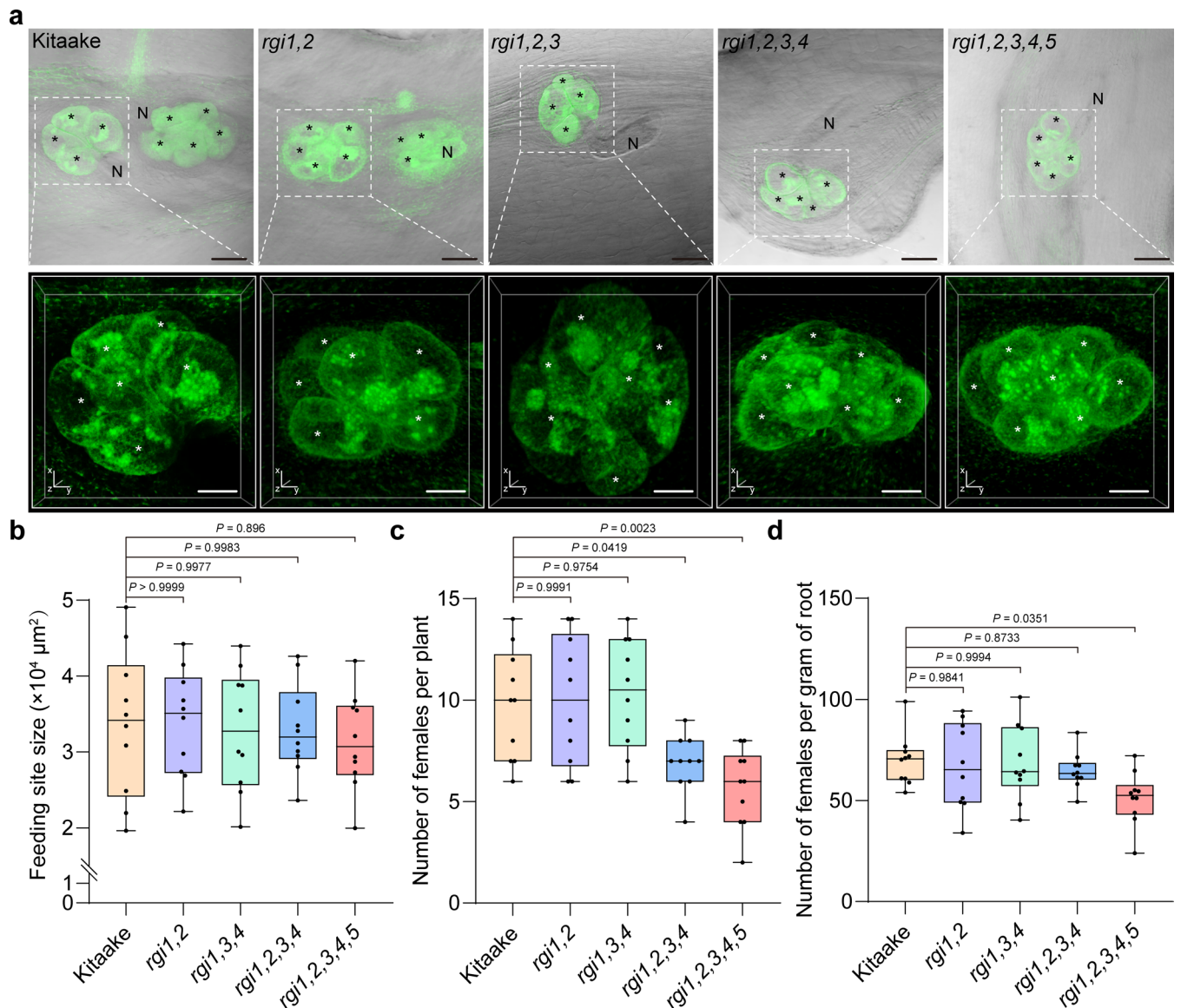
Extended Data Fig. 5 | Expression of *AtRGII-5* is activated in galls induced by *M. incognita* in *Arabidopsis*. **a**, GUS staining of *pRGII1::GUS*, *pRGII2::GUS*, *pRGII3::GUS*, *pRGII4::GUS*, and *pRGII5::GUS* lines infected with *M. incognita*. Twelve-day-old seedlings were used for inoculation. Twenty roots were stained and representative images are shown. Strong GUS activity was observed in galls

at 3 and 5 dpi, which diminished by 12 dpi. The experiments were performed three times with similar results. Scale bars, 500 μ m. **b**, Histological sections of galls at 5 dpi. Ten galls were observed with similar results. Scale bars, 50 μ m. N: nematode; Asterisks: giant cells.



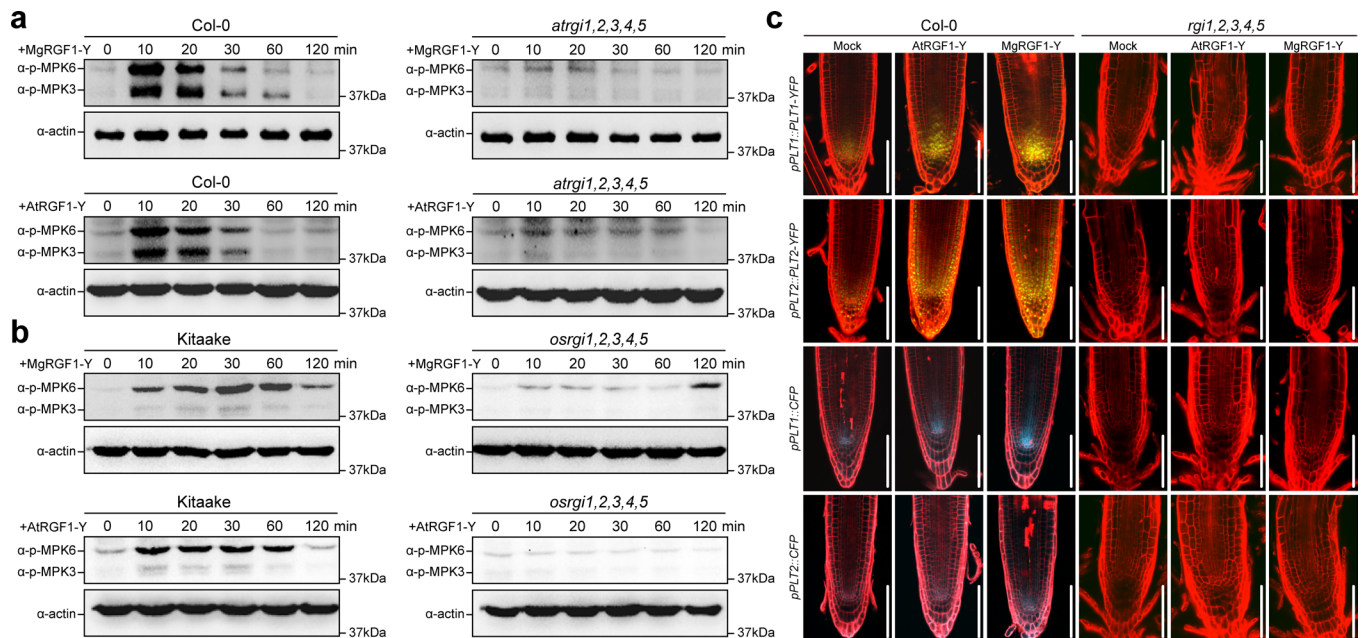
Extended Data Fig. 6 | Arabidopsis *rgi1,2,3,4,5* mutant shows insensitivity to nematode RGF and delayed development of *M. incognita*. **a**, Phenotypes of Col-0 and the *rgi1,2,3,4,5* mutant grown on medium for 7 days without or with 100 nM indicated peptides. Scale bars, 1 cm. **b**, Measurements of primary root length under 100 nM MgRGF1-Y treatment ($n = 10$). **c**, Confocal images of root tips from Col-0 and *rgi1,2,3,4,5* mutant. Seven-day-old seedlings grown with 100 nM MgRGF1-Y were stained and imaged ($n = 10$). White arrows indicate the boundary between root meristem and transition zones. White asterisks indicate the QC. Scale bars, 100 μm . **d**, Measurements of the number of meristematic cortex cells in Col-0 and mutant plants ($n = 16$). **e**, Measurements of lateral root number. $n = 13, 23, 13, 23$ from left to right. **f**, Nematode infection phenotypes in Col-0 and the *rgi1,2,3,4,5* mutant. The numbers of root galls per root and per gram of root were counted and quantified ($n = 15$). **g**, **h**, The numbers of egg masses per root (**g**) and per gram of root (**h**) were counted and quantified ($n = 15$). **i**, Nematode penetration phenotype of wild-type (Col-0) and *rgi1,2,3,4,5* mutant by acid fuchsin staining at 1 dpi with *M. incognita*. Scale bars, 200 μm . **j**, Average

number of *M. incognita* juveniles per plant at 1 dpi ($n = 20$). **k**, Nematode infection phenotypes of wild-type (Col-0) and *rgi1,2,3,4,5* mutant by acid fuchsin staining at 7 dpi. Scale bars, 200 μm . **l**, Proportion of different nematode developmental stages at 7 dpi. $n = 10, 9$ for Col-0 and *rgi1,2,3,4,5* mutant. **m**, Nematode infection phenotypes of wild-type (Col-0) and *rgi1,2,3,4,5* plants by acid fuchsin staining at 15 dpi. Scale bars, 200 μm . **n**, Proportion of different nematode developmental stages at 15 dpi ($n = 8$). **o**, Gall imaging by BABB clearing in wild-type (Col-0) and *rgi1,2,3,4,5* plants at 15 dpi. Scale bars, 200 μm . **p**, Single giant cell size quantification. $n = 20, 19$ for Col-0 and *rgi1,2,3,4,5* mutant. Scale bar, 200 μm . N: nematode; Asterisks: giant cells. Representative images (**a**, **c**, **i**, **k**, **m**, **o**) and data (**b**, **d**, **h**, **j**, **l**, **n**, **p**) from one of three biological replicates are shown. Data in **b**, **d**, **e**, **f**, **g**, **h**, **j** and **p** are presented as box-whisker plots with individual data points. The central line indicates the median; box limits represent the first and third quartiles; whiskers extend to the minima and maxima. P values were determined by unpaired two-tailed Student's t -test.



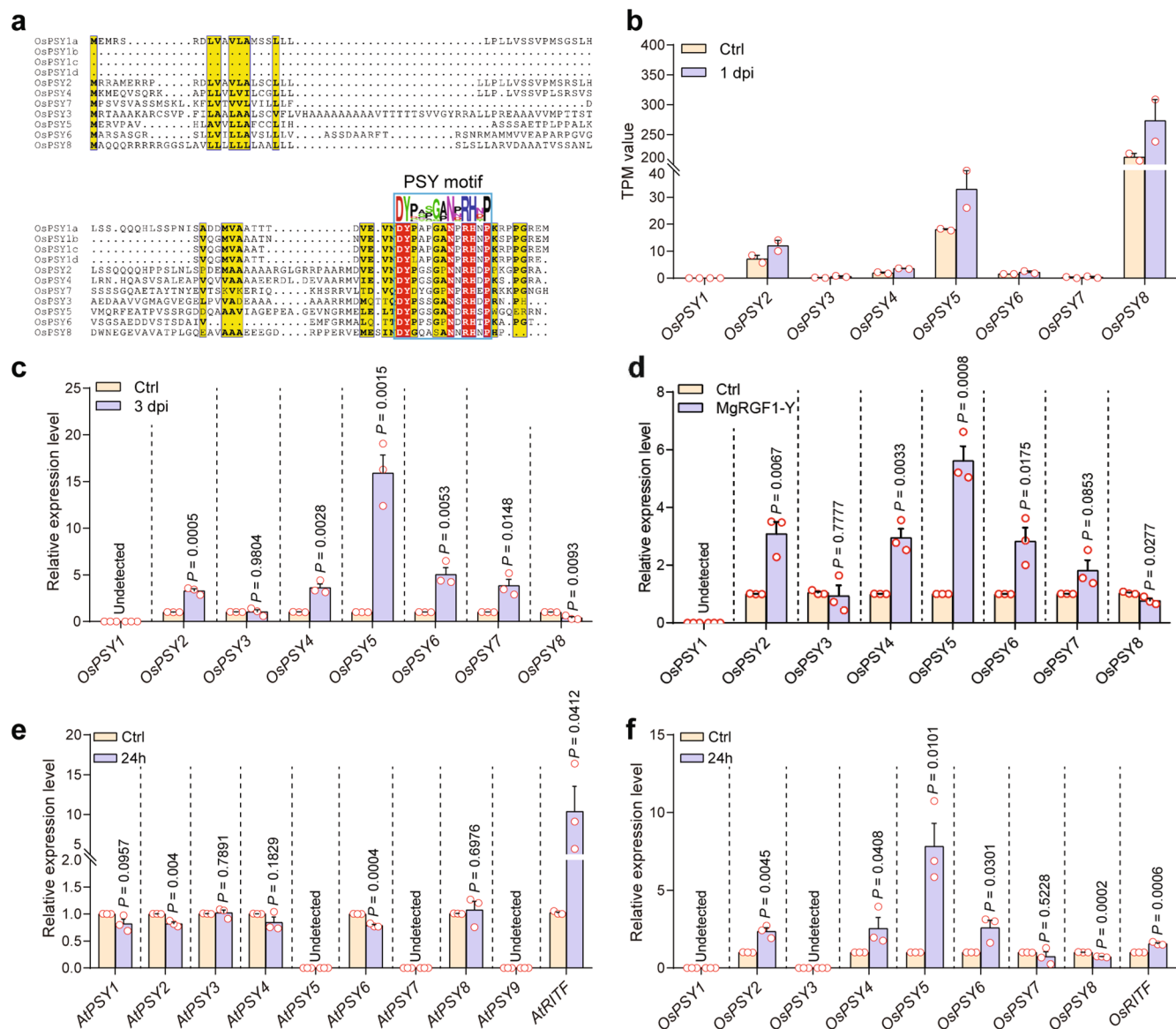
Extended Data Fig. 7 | The size of feeding sites is not significantly affected in rice *rgi* mutants. a, Feeding site observation in rice *rgi* mutants at 7 dpi with 35 ppj2s. Representative images of a single gall are shown after BABB clearing. Scale bars, 100 μm (upper panel) and 50 μm (lower panel). N: nematode; Asterisks: giant cells. **b**, Quantification of feeding cell size ($n = 10$). **c**, **d**, The number of females per root (**c**) and per gram of root (**d**) was counted and quantified in *rgi*

mutant ($n = 10$). Experiments were performed three times with similar results. Data in **b**, **c** and **d** is presented as box-whisker plots with individual data points. The central line indicates the median; box limits represent the first and third quartiles; whiskers extend to the minima and maxima. *P* values were determined by one-way ANOVA with Dunnett's multiple comparisons test, comparing the mean of each column with the mean of the control column.



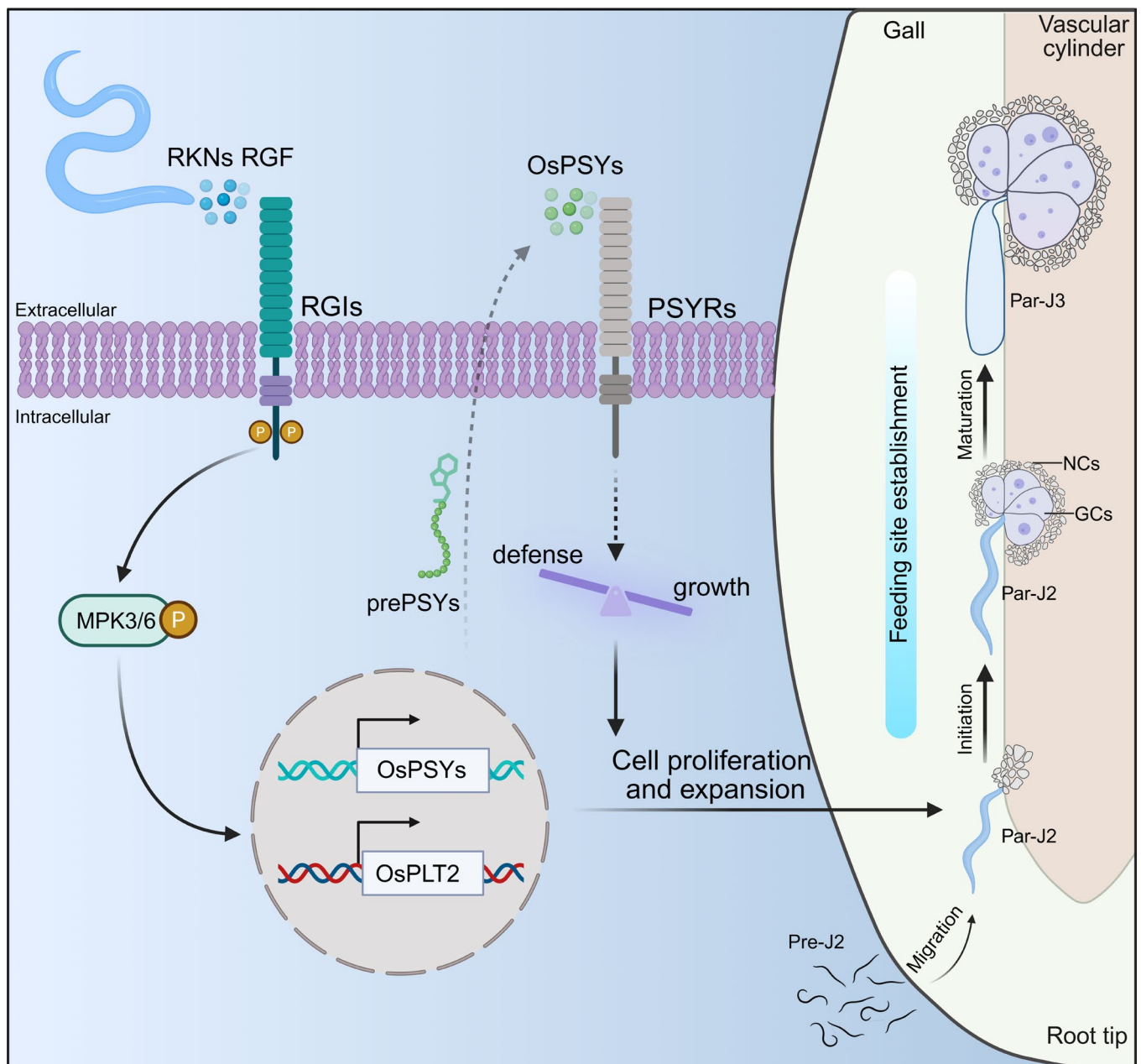
Extended Data Fig. 8 | Treatment with MgRGF1 or AtRGF1 peptide activates MAPK cascade and PLT1/2 expression through RGI receptors. a, b, MPK3 and MPK6 activation upon MgRGF1 or AtRGF1 peptide treatment. Wild-type seedlings and the corresponding *rgi* mutants were tested in *Arabidopsis* (a) and rice (b). Five-day-old seedlings were treated with 10 μM sulfated peptides for the indicated times. Phosphorylated MPK3/6 was detected using an anti-pERK antibody. Equal loading was shown by anti-actin immunoblot. The experiments

were performed three times with similar results. **c,** Peptide-induced promoter activities and protein levels of PLT1/2 were blocked in the *Arabidopsis rgi* mutant. Representative confocal images show root meristems of the transgenic seedlings expressing pPLT1::CFP, pPLT2::CFP, pPLT1::PLT1-YFP, or pPLT2::PLT2-YFP in wild-type and *rgi* mutant backgrounds. The experiments were performed three times with similar results. Scale bars, 100 μm.



Extended Data Fig. 9 | Sequence and expression analysis of *PSY* family genes in rice. **a**, Multiple sequence alignment and Web-Logo analysis of rice *PSY*s. Conserved residues are highlighted in blue box. **b**, Gene expression levels of *OsPSY* genes at 1 dpi with *M. graminicola*. TPM values were retrieved from previously published RNA-seq data⁶⁶. **c**, **d**, Relative expression analysis of *OsPSY* genes by RT-qPCR in Kitaake root tips after 3 days post *M. graminicola* inoculation (**c**) and 12 h *MgRGF1-Y* treatment (**d**). **e**, **f**, Relative expression levels of *PSY* family

genes in root tips of *Arabidopsis* (**e**) and rice (**f**) after 24 h *MgRGF1-Y* treatment. Expression levels were quantified using the $2^{-\Delta\Delta Ct}$ method. *AtActin2* and *AtTUB2* served as internal controls for *Arabidopsis*, while *OsUBQ10* and *OsActin* were used for rice. Data in **c**, **d**, **e** and **f** were presented as mean $\pm$ s.e.m. with individual data point of three independent biological replicates ($n = 3$). *P* values were determined by unpaired two-tailed Student's *t*-test.



Extended Data Fig. 10 | A proposed model for nematode RGF signaling during feeding site formation. RKNs secrete RGF-like peptide mimics that specifically bind to the host RGI receptors and activate RGI receptor-mediated signaling pathway to regulate cell proliferation and expansion—two processes critical for establishing feeding cell identity. Downstream components, including PSY peptide genes and PLT transcription factors, are activated to coordinate giant cell development and promote parasitism. Solid lines indicate regulation tested in this study, whereas dashed lines represent proposed regulation. Based on

recent findings regarding the PSY peptide-mediated trade-off between growth and stress responses, the induction of PSYs at nematode feeding sites may further facilitate nematode infection by suppressing PSYR-mediated defense responses⁷³. RKNs: root-knot nematodes; RGFs: ROOT MERISTEM GROWTH FACTORS; MPK3/6: Mitogen-Activated Protein Kinase 3/6; PSY: PLANT PEPTIDES CONTAINING SULFATED TYROSINE; PLT2: PLETHORA 2; NCs: neighboring cells; GCs: giant cells. Figure created in BioRender; Li, W. <https://biorender.com/tsfuzix> (2026).

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| ☐ | ☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ A description of all covariates tested |
| ☒ | ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	For RT-qPCR: Bio-Rad iCycler iQ5 real-time PCR system. For images and analysis: Nikon D3400 Camera, Nikon Eclipse 80i with Nikon DS-Ri1 camera, Nikon A1 HD25 confocal microscope, Olympus SZX16 stereomicroscope, Fiji ImageJ v.1.52. For sections: Leica RM2265 Fully Motorized Rotary Microtome, Leica VT1200 S Fully automated vibrating blade microtome. For microscale thermophoresis: Monolith NT.115 instrument.
Data analysis	For sequences aligned and phylogenetic analysis: MAFFT version 7 and MEGA 11. For plotting and statistical analysis of biological data: Microsoft Excel 2019 and GraphPad Prism 8.0.1. For protein prediction and presentation: AlphaFold2, Autoduck Vina, PyMOL v2.5.2 For microscale thermophoresis: MO.Affinity Analysis v2.3

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Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Amino acid sequences of nematode RGFs were retrieved from different Meloidogyne species using the annotated genome databases in Wormbase Parasite (<http://parasite.wormbase.org/index.html>). Sequences of the rice RGI and PSY gene families were obtained from Phytozome v13 (<https://phytozome-next.jgi.doe.gov/>). The protein crystal structure of plant peptide hormone receptor RGFR1 in complex with RGF1 (PDB: 5HYX) was downloaded from the PDB database (<https://www1.rcsb.org/>). Source data are provided with this paper. All data supporting the findings of the study are available in the article and Supplementary Information.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

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All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample sizes were mainly determined based on the prior experiences or established protocol in the field.
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Replication	All experiments were performed three times independently, and the data are reproducible. For images and photographs, representative results are shown. The information is provided in the figure legends.
Randomization	All samples were arranged or collected randomly.
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Behavioural & social sciences study design

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Study description	Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).
Research sample	State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.

Sampling strategy	Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.
Data collection	Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.
Timing	Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Non-participation	State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.
Randomization	If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.
Research sample	Describe the research sample (e.g. a group of tagged <i>Passer domesticus</i> , all <i>Stenocereus thurberi</i> within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.
Sampling strategy	Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.
Data collection	Describe the data collection procedure, including who recorded the data and how.
Timing and spatial scale	Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Reproducibility	Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.
Randomization	Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.
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Field work, collection and transport

Field conditions	Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).
Location	State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).
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Disturbance	Describe any disturbance caused by the study and how it was minimized.

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

- n/a ☐ Involved in the study
- ☐ ☒ Antibodies
- ☐ ☒ Eukaryotic cell lines
- ☒ ☐ Palaeontology and archaeology
- ☒ ☐ Animals and other organisms
- ☒ ☐ Clinical data
- ☒ ☐ Dual use research of concern
- ☐ ☒ Plants

Methods

- n/a ☐ Involved in the study
- ☒ ☐ ChIP-seq
- ☒ ☐ Flow cytometry
- ☒ ☐ MRI-based neuroimaging

Antibodies

Antibodies used

anti-DIG-AP: Fab antibody conjugated to alkaline phosphatase, Roche, Cat. No.11093274910, 1:500 dilution.
 anti-MgRFG: Rabbit polyclonal antibody, Custom-produced by Abclonal, 1:100 dilution.
 AlexaFluor 488-conjugated goat anti-rabbit IgG: Goat polyclonal antibody, Invitrogen, Cat. No.A-11008, 1:200 dilution for nematode and 1:500 dilution for gall sections.
 anti-His: Mouse monoclonal antibody, GenScript, Cat. No. A00186, 1:5000 dilution.
 HRP-conjugated goat anti-mouse IgG: Goat polyclonal antibody, Bio-Rad, Cat. No. 1706516, 1:10000 dilution.
 α-phospho-p44/p42 MAPK monoclonal antibody, α-pERK, Cell Signaling Technology, Cat. 9101S, 1:2000 dilution.
 α-Actin (plant specific) Mouse mAb, Abclonal, AC009, 1:5000 dilution.
 HRP-conjugated Goat anti-Rabbit IgG (H+L), Abclonal, AS014, 1:10000 dilution.

Validation

The validation statements of commercial antibodies are available from manufacturers:
<https://www.sigmaaldrich.cn/CN/zh/product/roche/11093274910>
<https://www.thermofisher.cn/cn/zh/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11008>
https://www.genscript.com/antibody/A00186-THE_His_Tag_Antibody_mAb_Mouse.html
https://www.bio-rad.com/SearchResults?search_api_fulltext=1706516
<https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-antibody/9101>
<https://abclonal.com.cn/catalog/AC009>
<https://abclonal.com.cn/catalog/AS014>

Eukaryotic cell lines

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Cell line source(s)

High Five cell lines from Thermo Fisher Scientific. <https://www.thermofisher.cn/order/catalog/product/B85502>

Authentication

High Five cell lines were verified by manufacturer's website and identity of these cell lines were frequently checked by their morphological features. <https://www.thermofisher.cn/order/catalog/product/B85502>

Mycoplasma contamination

High Five cell lines were not tested for mycoplasma contamination.

Commonly misidentified lines (See [ICLAC](#) register)

No commonly misidentified cell lines are used in this study.

Palaeontology and Archaeology

Specimen provenance

Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.

Specimen deposition

Indicate where the specimens have been deposited to permit free access by other researchers.

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If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

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Reporting on sex

Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.

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Study protocol

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Data collection

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Outcomes

Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

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Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes
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☒	☐ National security
☒	☐ Crops and/or livestock
☒	☐ Ecosystems
☒	☐ Any other significant area

Does the work involve any of these experiments of concern:

No	Yes
☒	☐ Demonstrate how to render a vaccine ineffective
☒	☐ Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐ Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐ Increase transmissibility of a pathogen
☒	☐ Alter the host range of a pathogen
☒	☐ Enable evasion of diagnostic/detection modalities
☒	☐ Enable the weaponization of a biological agent or toxin
☒	☐ Any other potentially harmful combination of experiments and agents

Seed stocks

Novel plant genotypes

Authentication

Wild type cultivar: Kitaake and Nipponbare (rice), Columbia-0 (*Arabidopsis thaliana*). Transgenic lines and mutants: pOsCYCBB1.1::GUS (rice). rg1/2/3/4/5 (quintuple mutant, *Arabidopsis thaliana*), pRG1::GUS, pRG2::GUS, pRG3::GUS, pRG4::GUS, pRG5::GUS (promoter-reporter lines, *Arabidopsis thaliana*), pLPT1::CFP, pLPT2::CFP, pLPT1::LPT1-YFP, pLPT2::LPT2-YFP (promoter-reporter lines, *Arabidopsis thaliana*), pCRISPR/Cas9-Generated OsB1 mutants: double mutants: rg1,2 and rg3,4; Higher-order mutants: rg1,3,4 (triple mutant), rg1,2,3,4 (quadruple mutant), rg1,2,3,4,5 (quintuple mutant). CRISPR/Cas9-Generated OsPSY5 Mutants: ospsy5. Overexpression Lines: 35S::MgRGF (full-length), 35S::MgRGFΔSP (signal peptide-deleted), 35S::OsPSY5; Transgenic reporter lines: pOsPLT2::GUS (PLETHORA2 promoter-driven GUS), pOsPSY5::GUS (PSY5 promoter-driven GUS). *Arabidopsis thaliana* transgenic lines: 35S::MgRGF (full length), 35S::MgRGFΔSP (signal peptide-deleted), 35S::MiRGF (full length), 35S::MiRGFΔSP (signal peptide-deleted) — pOsCYCBB1.1::GUS was previously reported (Zhou et al., 2020, Front. Plant Sci) rg1/2/3/4/5, pRG1::GUS, pRG2::GUS, pRG3::GUS, pRG4::GUS, pRG5::GUS, pLPT1::CFP, pLPT2::CFP, pLPT1::LPT1-YFP, pLPT2::LPT2-YFP were previously reported (Ou et al., 2016, Cell Res). tpst-1, rgf1,2,3 were previously reported (Matsuzaki et al., 2010, Science). Novel plant genotypes were assessed by PCR-based gene amplification and Sanger sequencing.

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission

Provide a list of all files available in the database submission.

Genome browser session
(e.g. [UCSC](#))

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.

Data quality

Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

Plots

Confirm that:

- ☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☐ All plots are contour plots with outliers or pseudocolor plots.
- ☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.

Instrument

Identify the instrument used for data collection, specifying make and model number.

Software

Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.

Cell population abundance

Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Gating strategy

Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.

- ☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI

☐ Used

☐ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

(See [Eklund et al. 2016](#))

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a | Involved in the study

☐ ☐ Functional and/or effective connectivity

☐ ☐ Graph analysis

☐ ☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.