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# The root-knot nematode effector MiEFF18 interacts with the plant core spliceosomal protein SmD1 required for giant cell formation

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1 **The Root-Knot Nematode Effector MiEFF18 interacts with the Plant Core Spliceosomal**  
2 **Protein Smd1 Required for Giant Cell Formation**

3  
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50

## 52 Summary

53

54 ● The root-knot nematode *Meloidogyne incognita* secretes specific effectors (MiEFF) and  
55 induces the redifferentiation of plant root cells into enlarged multinucleate feeding "giant  
56 cells" essential for nematode development.

57 ● Immunolocalisations revealed the presence of the MiEFF18 protein in the salivary glands  
58 of *M. incognita* juveniles. *In planta*, MiEFF18 localizes to the nuclei of giant cells  
59 demonstrating its secretion during plant-nematode interactions. A yeast two-hybrid  
60 approach identified the nuclear ribonucleoprotein SmD1 as a MiEFF18 partner in tomato  
61 and *Arabidopsis*. SmD1 is an essential component of the spliceosome, a complex  
62 involved in pre-mRNA splicing and alternative splicing.

63 ● RNA-seq analyses of *Arabidopsis* roots ectopically expressing MiEFF18 or partially  
64 impaired in SmD1 function (*smd1b* mutant) revealed the contribution of the effector and  
65 its target to alternative splicing and proteome diversity. The comparison with  
66 *Arabidopsis* galls data showed that MiEFF18 modifies the expression of genes important  
67 for giant cells ontogenesis, indicating that MiEFF18 modulates SmD1 functions to  
68 facilitate giant cell formation.

69 ● Finally, *Arabidopsis smd1b* mutants exhibited less susceptibility to *M. incognita*  
70 infection, and the giant cells formed on these mutants displayed developmental defects,  
71 suggesting that SmD1 plays an important role in the formation of giant cells and is  
72 required for successful nematode infection.

73

74

75 **Key words:** *Meloidogyne incognita*, Effector, Nucleus, Alternative Splicing, *Arabidopsis*  
76 *thaliana*, *Nicotiana benthamiana*

77

## 79 **Introduction**

80

81 Pathogens have evolved an arsenal of molecules known as effectors, which are secreted *in*  
82 *planta* to manipulate host functions and ensure successful infection. One striking example of  
83 plant cell manipulation is provided by the plant-parasitic root-knot nematodes (RKN) of the  
84 genus *Meloidogyne*. After penetrating the root and migrating to the vascular cylinder, the  
85 microscopic vermiform second-stage juveniles (J2s) induce the transformation of selected  
86 vascular root cells into specialised hypertrophied and multinucleate feeding cells. These ‘giant  
87 cells’ result from successive nuclear divisions without cell division, followed by isotropic cell  
88 growth (Favery *et al.*, 2016). They are several hundred times larger than normal root cells,  
89 contain about 50 to 100 endoreduplicated nuclei and have an expanded endoplasmic reticulum  
90 and numerous organelles (de Almeida Engler & Gheysen, 2013). Giant cells are surrounded  
91 by dividing cells, some of which differentiate into new xylem and phloem cells (Bartlem *et*  
92 *al.*, 2014), leading to the formation of a new organ, the gall. Giant cells act as a strong  
93 metabolic sink and are the sole source of nutrients for the nematode during the sedentary part  
94 of its life cycle in the plant. The pear-shaped RKN females eventually lay their eggs on the  
95 root surface. Interestingly, RKN can induce giant cells in more than 4,000 plant species,  
96 probably by manipulating conserved plant functions (Singh *et al.*, 2013).

97 The major modifications observed in giant cells require extensive transcriptional  
98 reprogramming in root cells. Giant cell formation has been explored at the transcriptomic  
99 level in several plant-RKN interactions, and thousands of differentially expressed genes  
100 (DEG) have been identified in plants (Cabrera *et al.*, 2014; Yamaguchi *et al.*, 2017; Shukla *et*  
101 *al.*, 2018; Postnikova *et al.*, 2015; Favery *et al.*, 2016). Functional analyses of these genes  
102 have highlighted the key roles of microtubule and actin cytoskeleton rearrangements and cell  
103 cycle control in the formation of these multinucleate feeding cells (de Almeida Engler &  
104 Favery, 2011; de Almeida Engler & Gheysen, 2013; Favery *et al.*, 2016; Cabral *et al.*, 2020).  
105 Recent studies have shown that small non-coding RNAs, such as microRNAs (miRNAs) and  
106 small interfering RNAs (siRNAs), play a role in gall development (Jaubert-Possamai *et al.*,  
107 2019).

108 The molecular mechanisms underlying giant cell formation remain poorly understood. It is  
109 assumed that effectors, including, in particular, proteins secreted *in planta* from the three  
110 oesophageal gland cells through a hollow protrusive stylet, are responsible for giant cell  
111 ontogenesis (Mitchum *et al.*, 2013; Truong *et al.*, 2015; Mejias *et al.*, 2019). Various

112 approaches, based on proteomics, transcriptomics and genomics, have been used to  
113 characterise RKN effector repertoires. The sequencing of *M. incognita* mRNAs isolated from  
114 gland cells or from parasitic juveniles *in planta* led to the identification of genes encoding  
115 putative effector proteins expressed specifically in the oesophageal gland cells and more  
116 strongly *in planta*, such as the *Minc18636/Minc15401* genes (Rutter *et al.*, 2014; Nguyen *et*  
117 *al.*, 2018). The expression in the oesophageal glands of about a hundred RKN effectors has  
118 been validated by *in situ* hybridisation (ISH), but secretion *in planta* has been demonstrated  
119 for only a few candidate effectors, by immunolocalisation (Truong *et al.*, 2015). Three RKN  
120 effectors have been shown to accumulate in the nucleus of giant cells (Jaouannet *et al.*, 2012;  
121 Lin *et al.*, 2012; Chen *et al.*, 2017). This targeting event in the host nucleus reflects the need  
122 for various nuclear processes, including transcriptional regulation, to be manipulated, to divert  
123 plant cell fate and disrupt immunity, as reported for other plant pathogens (Deslandes &  
124 Rivas, 2011; Motion *et al.*, 2015). Most RKN candidate effectors are ‘pioneers’ displaying no  
125 significant sequence similarity to any protein in databases and with no known functional  
126 domains; as a result, the functions of only a few RKN effectors have been deciphered (Mejias  
127 *et al.*, 2019). The *M. incognita* 7H08 effector has been shown to have transcriptional activity  
128 *in planta*, but the target genes in the host have yet to be identified (Zhang *et al.*, 2014). The  
129 16D10 effector from *M. incognita* targets Scarecrow-like transcription factors (Huang *et al.*,  
130 2006). In sedentary endoparasitic cyst nematodes, the GLAND4 effector has been shown to  
131 have transcriptional repressor activity against the promoters of two lipid transfer genes  
132 involved in plant defence (Barnes *et al.*, 2018). The 32E03 effector has epigenetic activity,  
133 through the inhibition of *Arabidopsis thaliana* histone deacetylases, thereby modulating host  
134 rDNA gene expression and promoting infection (Vijayapalani *et al.*, 2018).

135 Alternative splicing (AS) is a mechanism by which different forms of mature messenger  
136 RNA (mRNA) are generated from the same gene, from specific transcripts or through the  
137 deletion or retention of an exon/intron sequence (Wilkinson & Charenton, 2020). This  
138 regulatory mechanism results in the production of several related proteins, or isoforms,  
139 thereby increasing proteomic diversity. Plant pathogens have been shown to modulate AS  
140 (Rigo *et al.*, 2019). We show here that the MiEFF18 effector from *M. incognita* accumulates  
141 in the plant cell nucleus and interacts with an essential component of the spliceosome  
142 machinery, the small ribonucleoprotein particle SmD1, in tomato and *Arabidopsis*. Using a  
143 genome-wide transcriptome analysis, we found that MiEFF18 modulated AS, and gene  
144 expression, through a partial impairment of SmD1 activity. We also found that related

145 alternative splicing events occur in *Arabidopsis* upon nematode parasitism. Our findings  
146 further demonstrate that SmD1 is required for RKN infection and giant cell formation. Thus,  
147 MiEFF18 may contribute to giant cell development by modulating the function of a key  
148 component of the spliceosome to promote nematode infection.

149

## 150 **Materials and methods**

151

### 152 **Plant material and growth conditions**

153 All the *A. thaliana* plants used here were of the Columbia 0 ecotype (Col-0). The *smd1a* and  
154 *smd1b* mutants have been described elsewhere (Elvira-Matelot *et al.*, 2016). Seeds of *A.*  
155 *thaliana* Col-0, mutant and transgenic lines were surface-sterilised and sown on Murashige  
156 and Skoog (Duchefa) agar plates (0.5 x MS salts, 1% sucrose, 0.8% agar, pH 6.4) or in a  
157 mixture of soil and sand. Sowings were incubated at 4°C for two days, and then transferred to  
158 a growth chamber with an 8 h photoperiod, at 21°C. For propagation and transformation,  
159 seedlings were transferred to a growth chamber with a 16 h photoperiod, at 21°C. *A. thaliana*  
160 were transformed by the floral dip method (Bent & Clough, 1998). Homozygous transformed  
161 T3 plants were used. *Nicotiana benthamiana* plants were grown on soil, under a 16 h  
162 photoperiod, at 24°C. For the production of plant material for RNA-seq experiments, seeds  
163 were surface-sterilized and sown in liquid MS medium (0.5 x MS salts, 1% sucrose, pH 6.4)  
164 with gentle shaking (70 rpm), under a 12 h photoperiod, at 25°C. Roots were collected after  
165 11 days and immediately frozen in liquid nitrogen until RNA extraction.

166

### 167 **RKN infection assay**

168 *M. incognita* strain “Morelos” was multiplied on tomato (*Solanum lycopersicum* cv. “Saint  
169 Pierre”) growing in a growth chamber (25°C, 16 h photoperiod). Freshly hatched J2s were  
170 collected as previously described (Caillaud & Favery, 2016). Three-week-old *Arabidopsis*  
171 seedlings were inoculated with 200 *M. incognita* J2s per plant. Roots were collected six  
172 weeks after infection and stained with 0.5% eosin. The number of females forming egg  
173 masses and root weight were then determined (n=25 to 40 plants per replicates). Three  
174 independent biological replicates were established for each set of conditions. Statistical  
175 analyses were carried out with R software (R Development Core Team, version 3.1.3). The  
176 effect of plant genotype on the number of nematode egg masses was analyzed with  
177 generalised linear models (GLMs) based on a Poisson distribution, for each replicate. We

178 used the Tukey adjustment method ('multcomp' package) for multiple testing. For giant cell  
 179 area measurements, galls were collected 14 days post-infection (dpi), cleared in benzyl  
 180 alcohol/benzyl benzoate (BABB) as previously described (Cabrera *et al.*, 2018) and examined  
 181 under an inverted confocal microscope (model LSM 880; Zeiss). The mean areas of giant  
 182 cells in each gall, for each genotype, and for two biological replicates, were measured with  
 183 Zeiss ZEN software ( $n = 42$  and  $25$  galls for Col-0 and *smd1b*, respectively). The impact of  
 184 the plant genotype on the giant cell surface was analyzed using student t test since the  
 185 dependent variables followed a Normal distribution using a Shapiro-Wilk Test.

### 186 **Plasmid constructs**

187 The *M. incognita* *MiEFF18* and *MiEFF16* coding sequences (CDS) lacking the signal  
 188 peptide, the *S. lycopersicum* *SmD1*, *A. thaliana* *SmD1a* and *SmD1b*, the SV40 Antigen T, and  
 189 the human P53 sequence were amplified by PCR with specific primers (Table S1) and  
 190 inserted into the pDON207 donor vector. They were recombined in pK2GW7  
 191 (P35S:MiEFF18), pK7WGR2 (P35S:mRFP-MiEFF18), pK7FGW2 (P35S:eGFP-SlSmD1,  
 192 P35S:eGFP-MiEFF16) or BiFC (pAM-35SS:GWY-YFPc, pAM-35SS:GWY-YFPn, pAM-  
 193 35SS:YFPc-GWY, pAM-35SS:YFPn-GWY) or, for Y2H, the pB27-GW and pP6-GW  
 194 (Karimi *et al.*, 2007; Caillaud *et al.*, 2009), with Gateway technology (Invitrogen). All the  
 195 constructs were sequenced (GATC Biotech) and transferred into either *Agrobacterium*  
 196 *tumefaciens* strain GV3101 or *Saccharomyces cerevisiae* strain L40ΔGal4 or Y187.

197

### 198 ***N. benthamiana* agroinfiltration**

199 Transient expression was achieved by infiltrating *N. benthamiana* leaves with *A. tumefaciens*  
 200 GV3101 strains harbouring GFP- or mRFP-fusion or BiFC constructs, as previously described  
 201 (Caillaud *et al.*, 2009). Leaves were imaged 48 hours after agroinfiltration, with an inverted  
 202 confocal microscope equipped with an Argon ion and HeNe laser as the excitation source. For  
 203 simultaneous GFP/mRFP imaging, samples were excited at 488 nm for GFP and 543 nm for  
 204 mRFP, in the multi-track scanning mode. GFP or YFP emission was detected selectively with  
 205 a 505-530 nm band-pass emission filter. We detected mRFP fluorescence in a separate  
 206 detection channel, with a 560-615 nm band-pass emission filter.

207

### 208 **Sequence analysis and alignment**



209 *M. incognita* sequences were obtained from *Meloidogyne* genomic resources  
210 ([http://www6.inra.fr/meloidogyne\\_incognita/](http://www6.inra.fr/meloidogyne_incognita/)). We used the MAFFT on the EBI server  
211 (<https://www.ebi.ac.uk/Tools/msa/mafft/>) for sequence alignment. The protein sequences  
212 encoded by the genes were analysed with PHOBIUS (<http://phobius.sbc.su.se/>), PSORT II  
213 (<http://psort.hgc.jp/form2.html>) and NoD (<http://www.compbio.dundee.ac.uk/www-nod/>)  
214 software, for the prediction of signal peptides, non-transmembrane domains, DNA-binding  
215 domains, NLS and NoLS, respectively. BLASTp analyses were carried out with an e-value  
216 threshold of 0.01 and without low complexity against the NCBI non-redundant protein  
217 database, for homologue identification. Interproscan was performed on the proteins to  
218 identify protein signatures referenced in the InterPro database (Mitchell *et al.*, 2015).

219

### 220 **Yeast two hybrid**

221 For the yeast two-hybrid (Y2H) screens, the coding sequences of the MiEFF18 and MiEFF16  
222 effectors without their secretion signals and the *SlSmD1* CDS were inserted into pB27 as C-  
223 terminal fusions with LexA. The constructs were verified by sequencing and used to  
224 transform the L40ΔGal4 (MAT $\alpha$ ) yeast strain. MiEFF18 was used as a bait in a mating  
225 approach, to screen a random-primed cDNA library from tomato roots infected with *M.*  
226 *incognita* and *Ralstonia solanacearum* carried by the Y187 (MAT $\alpha$ ) yeast strain (Hybrigenics  
227 Services, Paris, France). Diploids carrying interactions were selected on a minimal synthetic  
228 defined SD medium lacking tryptophan (W), leucine (L) and histidine (H). The prey  
229 fragments of the positive clones were amplified by PCR and their 5' junctions were  
230 sequenced. The resulting sequences were used to identify the tomato interacting proteins with  
231 the Sol Genomics Network (<https://solgenomics.net/>) blast analysis tools. For pairwise Y2H  
232 assays, full-length controls, baits and candidate targets (MiEFF18 w/o SP, MiEFF16 w/o SP,  
233 SlSmD1, AtSmD1a, AtSmD1b, Antigen T and P53) were inserted into the pB27 or pP6  
234 vector as C-terminal fusions with LexA or Gal4-AD, respectively, verified by sequencing and  
235 used to transform L40ΔGal4 (MAT $\alpha$ ) or Y187 (MAT $\alpha$ ) yeast strain. After mating between  
236 Y187 and L40ΔGal4, diploids were selected on medium lacking tryptophan and leucine, and  
237 interactions were tested on medium lacking tryptophan, leucine and histidine and  
238 supplemented with 0.5 mM 3-amino-1,2,4-triazole (3-AT).

239

### 240 **Western blotting and immunolocalisation**

241 MiEFF18 was inserted into the pET-24a (+) expression vector (Addgene), expressed in  
 242 BL21star (DE3) cells, and purified on HisTrap FF columns (GE Healthcare Life Science).  
 243 The purified protein was used to raise polyclonal antibodies in rabbits (Agro-Bio, La Ferté  
 244 Saint Aubin, France). Western blotting was performed to check the specificity of the antibody  
 245 as previously described (Zhao *et al.*, 2019b). Proteins were transferred onto a nitrocellulose  
 246 membrane with the Trans-Blot Turbo Transfer system (Biorad). The membranes were  
 247 blocked and incubated with  $\alpha$ -MiEFF18 antibody (1:5,000 or 1/10,000) and then with goat  
 248 anti-rabbit secondary antibodies coupled to horseradish peroxidase (HRP; 1:10,000).

249 Immunolocalisation was performed directly on *M. incognita* pre-parasitic J2s with the anti-  
 250 MiEFF18 primary antibody (1:50) and a goat anti-rabbit Alexa Fluor 488-conjugated  
 251 secondary antibody (1:200) (Molecular Probes) as previously described (Jaubert *et al.*, 2005).  
 252 Pre-immune serum was used as a negative control. For *in planta* immunolocalisation, the  
 253 antibodies were affinity-purified (Agro-Bio, La Ferté Saint Aubin, France) and used to  
 254 performed immunolocalisation on *Arabidopsis* gall sections (14 dpi) with the anti-MiEFF18  
 255 purified antibody (1:500) and a goat anti-rabbit Alexa Fluor 488-conjugated secondary  
 256 antibody (1:200) (Molecular Probes) as previously described (Zhao *et al.*, 2019). Images were  
 257 collected with an inverted confocal microscope (model LSM 880; Zeiss).

258

### 259 **Gene expression and alternative splicing analysis**

260 *Arabidopsis* gall and non-infected control root RNA-seq data were generated and described in  
 261 a previous study (Yamaguchi *et al.*, 2017). Total RNA was extracted from the roots of the  
 262 three *Arabidopsis* lines (Col-0, P35S:MiEFF18 and *smd1b*) with TriZol (Invitrogen),  
 263 according to the Invitrogen protocol. The RNA was treated with DNase treatment (Ambion),  
 264 and its quality and integrity were assessed with a Bioanalyzer (Agilent). Libraries were  
 265 constructed with the Tru-Seq Stranded mRNA Sample Prep kit (Illumina®). Paired-end  
 266 sequencing with 75-bp reads was performed on a NextSeq500 perform. A minimum of 30  
 267 million paired-end reads per sample was generated. RNA-seq preprocessing included the  
 268 trimming of library adapters and quality controls with Trimmomatic. Paired-end reads with a  
 269 Phred Quality Score Qscore > 20 and a read length > 30 bases were retained, and ribosomal  
 270 RNA sequences were removed with SortMeRNA. Processed reads were aligned using  
 271 Tophat2 with the following arguments: --max-multihits 1 -i 20 --min-segment-intron 20 --  
 272 min-coverage-intron 20 --library-type fr-firststrand --microexon-search -I 1000 --max-  
 273 segment-intron 1000 --max-coverage-intron 1000 --b2-very-sensitive. Reads overlapping

274 exons were counted per gene with the FeatureCounts function of the Rsubreads package,  
275 using the GTF annotation files from the Araport11 repository  
276 ([https://www.araport.org/downloads/Araport11\\_Release\\_201606/  
277 annotation/Araport11\\_GFF3\\_genes\\_transposons.201606.gff.gz](https://www.araport.org/downloads/Araport11_Release_201606/annotation/Araport11_GFF3_genes_transposons.201606.gff.gz)). The significance of  
278 differential gene expression was estimated with DEseq2, with FDR correction of the  $p$ -value  
279 during pairwise comparisons between genotypes. A gene was considered to be differentially  
280 expressed if its adjusted  $p$ -value (FDR) was  $\leq 0.01$ . Transcripts were quantified on the basis  
281 of pseudo-alignment counts with kallisto on AtRTD2 transcript sequences  
282 ([https://ics.hutton.ac.uk/atRTD/RTD2/AtRTDv2\\_QUASI\\_19April2016.fa](https://ics.hutton.ac.uk/atRTD/RTD2/AtRTDv2_QUASI_19April2016.fa)) with a  $K$ -mer size  
283 of 31 nucleotides. Differential AS events in the AtRTD2 database were detected with  
284 SUPPA2, using default parameters (Trincado *et al.*, 2018). Only events with an adjusted  $p$ -val  
285  $< 0.01$  were retained for further analysis. The dPSI (difference in percent spliced in) values  
286 for each AS were generated by SUPPA2 and plotted in R using ggplot2. Hypergeometric  $p$ -  
287 value was calculated using the phyper function in R taking the total number AS event as the  
288 population size. Gene ontology enrichment analysis was done using the AgriGO server  
289 (<http://bioinfo.cau.edu.cn/agriGO/>) using default parameters. Lists of GO terms were  
290 eventually visualized using REVIGO (<http://revigo.irb.hr/>). Gene family enrichment analysis  
291 was performed using GenFam (<http://mandadilab.webfactional.com>).

292

### 293 **Reverse transcription-quantitative PCR**

294 Total RNA was extracted from plantlets or roots extracted with TriZol (Invitrogen) and  
295 subjected (1  $\mu\text{g}$  of total RNA) to reverse transcription with the Superscript IV reverse  
296 transcriptase (Invitrogen). qPCR analyses were performed as described by Nguyen *et al.*  
297 (2018). We performed qPCR on triplicate samples of each cDNA from three independent  
298 biological replicates. *OXA1* (*At5g62050*) and *UBQ10* (*At4g05320*) were used for the  
299 normalization of RT-qPCR data. Quantifications and statistical analyses were performed with  
300 SATqPCR (Rancurel *et al.*, 2019), and the results are expressed as normalised relative  
301 quantities. For the validation of alternatively spliced genes, two pairs of primers, specifically  
302 amplifying one or the two isoforms of the gene concerned, were designed (Table S1) and used  
303 for RT-qPCR assays with the parameters described above for the DEG. The *UBQ10* reference  
304 gene was used for normalization of the alternatively spliced genes.

305

## 306 Accession numbers

307 Sequence data from this article can be found in the *Arabidopsis* Information Resource  
 308 (<https://www.arabidopsis.org/>), Solgenomics (<https://solgenomics.net/>) and GenBank/EMBL  
 309 databases under the following accession numbers: *Minc18636* (KX907770), *Minc15401*  
 310 (MT591034), *Minc16401* (MT591035), *AtSmD1a* (*At3g07590*), *AtSmD1b* (*At4g02840*),  
 311 *OXA1* (*At5g62050*), *UBQ10* (*At4g05320*), *FAD/NAD(P)-binding oxidoreductase*  
 312 (*At5g11330*), *U1 snRNP 70K* (*At3g50670*), *ribosomal protein S21 family protein*  
 313 (*At3g26360*), *RNA-binding (RRM/RBD/RNP motifs)* (*At3g04500*), *MCM10* (*At2g20980*),  
 314 *prenylated RAB acceptor 1.E* (*At1g08770*), *defensin* (*At5g33355*), *Solanum lycopersicum*  
 315 *SlSmD1a* (*Solyc06g084310.2.1*; MT598822) and *SlSmD1b* (*Solyc09g064660.2.1*;  
 316 MT598823). The transcriptome data are available at the Sequence Read Archive (SRA) via  
 317 accession numbers PRJDB5797 (*A. thaliana* galls at 5 and 7 dpi with *M. incognita* and non-  
 318 inoculated roots; Yamaguchi *et al.*, 2017) and GSE153171 (*A. thaliana* Col-0/  
 319 P35S:MiEFF18/*smd1b* roots).

320

321

## 322 Results

323

### 324 **MiEFF18 is a secreted effector that localises to the nucleoplasm and nucleolus of plant** 325 **cells**

326 MiEFF18 is a putative *M. incognita* secreted effector encoded by the *Minc18636* gene.  
 327 *Minc18636* and its paralog, *Minc15401*, are more strongly expressed at the juvenile parasitic  
 328 stages than at the J2 pre-parasitic stage, and are specifically expressed in the subventral  
 329 oesophageal glands (SvG) of both pre-parasitic and parasitic juveniles (Rutter *et al.*, 2014;  
 330 Nguyen *et al.*, 2018). MiEFF18 displays no similarity to any sequences out of the genus  
 331 *Meloidogyne* or motifs included in public databases. MiEFF18 is a 312-amino acid (aa)  
 332 protein with a signal peptide for secretion (aa 1 to 21, according to the Phobius prediction tool  
 333 (Käll *et al.*, 2007), an N-terminal region rich in aspartic acid and glutamic acid (D-E; 55%)  
 334 and a C-terminal region enriched in lysine (K; 40%) (Fig. 1a; Fig. S1). *In silico* assays  
 335 predicted the presence of several nuclear localisation signals (NLS) and one nucleolar  
 336 localisation signal (NoLS) in MiEFF18 (Fig. 1a), suggesting that this protein would be  
 337 imported into the nuclei of host plant cells. We produced specific antibodies against the  
 338 complete MiEFF18 protein in *E. coli*, to check that this protein was, indeed, secreted *in planta*

339 (Fig. S2a-c). As expected, immunolocalisation experiments on pre-parasitic J2s showed the  
340 MiEFF18 to be present in the two SvGs (Fig. 1b-e), consistent with published *in situ*  
341 hybridisation results (Rutter *et al.*, 2014; Nguyen *et al.*, 2018). Within the SvGs and their  
342 secretory tracks, MiEFF18 localised with punctate structures corresponding to secretory  
343 granules (Fig. 1c-e), consistent with its secretion during plant-nematode interactions. No  
344 signal was observed, with the exception of a non-specific signal, with the pre-immune serum  
345 in pre-parasitic J2s (Fig. S2b-c). To demonstrate secretion of MiEFF18 *in planta*, we used  
346 affinity-purified antibodies to immunolocalise MiEFF18 on gall sections (Fig. S2d). MiEFF18  
347 production occurs in the SvGs of parasitic juveniles (Fig. 1f). In galls, we detected MiEFF18  
348 in giant cells where it accumulated in the nuclei (Fig. 1g-h'), confirming its injection within  
349 the host cells during *M. incognita* parasitism. No signal was detected within the giant cells  
350 when using only the Alexa Fluor 488-conjugated secondary antibody (Fig. S2e-f). These  
351 results provide evidence for the secretion of MiEFF18 *in planta* during parasitism and its  
352 targeting to the plant cell nucleus.

353

#### 354 **MiEFF18 interacts with the spliceosomal ribonucleoprotein Smd1**

355 We investigated the effector function of MiEFF18, by performing a yeast two-hybrid (Y2H)  
356 screen to search for interactors in tomato. In this system, we used MiEFF18 without its signal  
357 peptide as a bait, and a tomato root cDNA library from healthy and *M. incognita*-infected  
358 roots (Hybrigenics) as the prey. We screened 48.5 million interactions between MiEFF18 and  
359 proteins encoded by the cDNA library. We identified one major target, a ribonucleoprotein,  
360 Smd1, which was captured 26 times, whereas other candidates were captured only one to four  
361 times (Fig. S3). There are two genes encoding Smd1 proteins with 100% aa identity in  
362 *Solanum lycopersicum* (*SlSmd1a\_Solyc06g084310* and *SlSmd1b\_Solyc09g064660*). Using a  
363 pairwise Y2H approach, we independently validated the interaction between the full-length  
364 sequences of MiEFF18 and SlSmd1 (*Solyc06g084310*) (Fig. 2a). As a control, we  
365 investigated the interaction between SlSmd1 and another *M. incognita* effector, MiEFF16,  
366 encoded by the *Minc16401* gene, expressed in the subventral glands, and also localising to the  
367 nucleoplasm and the nucleolus of plant cells following transient expression in *Nicotiana*  
368 *benthamiana* leaves (Fig. S4). No interaction was observed between MiEFF16 and SlSmd1 in  
369 yeast (Fig. 2a).

370 We investigated the colocalisation of MiEFF18 and its target, Smd1, in plant cells, by  
371 transiently expressing constructs encoding RFP-MiEFF18 and the GFP-SlSmd1 fusion

372 proteins in *N. benthamiana*. We confirmed the colocalisation of MiEFF18 and SlSmD1 in the  
 373 nucleoplasm and nucleolus (Fig. 2b). SlSmD1 was also localised in nucleoplasmic speckles,  
 374 whereas MiEFF18 was not detected in these structures (Fig. 2b). We used a bimolecular  
 375 fluorescent complementation (BiFC) assay for the validation and localisation *in planta* of the  
 376 interaction between MiEFF18 and SlSmD1. Using three combinations of BiFC vectors, we  
 377 showed that MiEFF18 and SlSmD1 interacted strongly in the nucleolus, with a weaker signal  
 378 observed in the cytoplasm and the nucleoplasm (Fig. 2c and Fig. S5). No interaction was  
 379 observed between MiEFF16 and SlSmD1, with the various BiFC constructs used (Fig. S5).

380 Two genes, *AtSmD1a* (*At3g07590*) and *AtSmD1b* (*At4g02840*), encode SmD1 proteins in  
 381 *A. thaliana*. Using knock-out (KO) mutant lines, Elvira-Matelot et al., (2016) demonstrated  
 382 that these two genes encode proteins with redundant activities, and that the *smd1a smd1b*  
 383 double mutant is lethal, as expected for a core component of the spliceosome. The *smd1b*  
 384 single mutant displays developmental and splicing defects, whereas the *smd1a* single mutant  
 385 develops normally. *AtSmD1b* would account for a larger proportion of the total activity,  
 386 probably due to its stronger expression in all tissues compared to *AtSmD1a* (Elvira-Matelot et  
 387 al., 2016). Using a pairwise Y2H approach, we validated the interaction of the MiEFF18  
 388 effector with *AtSmD1a* and *AtSmD1b* (Fig. 2a). Overall, these results demonstrate that the  
 389 MiEFF18 effector specifically interacts, in yeast and *in planta*, with the tomato and  
 390 *Arabidopsis* spliceosomal SmD1 core proteins.

391

### 392 **MiEFF18 and SmD1 modulate the alternative splicing of plant genes**

393 As MiEFF18 interacts with SmD1, a core component of the spliceosome, we investigated the  
 394 possible accumulation of similar mis-spliced transcripts in the homozygous *smd1b* mutant and  
 395 an *Arabidopsis* *MiEFF18*-expressing line, relative to the wild type. We generated transgenic  
 396 plants expressing the MiEFF18 effector under the control of a 35S promoter (Fig. S6a).  
 397 Noteworthy, the *MiEFF18*-expressing lines #8.6 and #13.6 exhibited a decreased  
 398 susceptibility to *M. incognita*, indicating that the continued and excessive presence of  
 399 MiEFF18 may be detrimental to feeding site formation (Fig. S6b). We performed RNA-  
 400 sequencing (RNA-seq) on total RNA isolated from the roots of 11-day-old *Arabidopsis*  
 401 seedlings, Col-0, *smd1b* and *MiEFF18*-expressing line #13.6. Biological triplicates were run  
 402 for all samples. We then performed transcript quantification with SUPPA2, which is a  
 403 computational tool that calculate relative inclusion values of alternative splicing events, based  
 404 of transcript level quantification in RNA-seq data (Trincado et al., 2018; Table S2 and S3).

405 The five main categories of AS events were detected: intron retention (IR), exon skipping  
 406 (ES), alternative 5' splice site (A5), alternative 3' splice site (A3) and mutually exclusive  
 407 exons (MX) (Fig. 3a; Fig. S7). In total, we identified 249 and 593 differential splicing events,  
 408 affecting 222 and 463 genes, in the *MiEFF18*-expressing line and the *smd1b* mutant,  
 409 respectively (Fig. 3a-b; Fig. S7). A high degree of overlap was observed between the two  
 410 lines, with 113 AS events and 107 alternatively spliced genes common to the two lines  
 411 (hypergeometric  $p$  value  $< 4.666e-117$ ; Fig. 3b and Fig. S7). We also compared the dPSI  
 412 corresponding to the change in each AS event in both *MiEFF18*-expressing lines and the  
 413 *smd1b* mutants (Fig. S8). We found that the global change in AS relative to the wild-type root  
 414 was significantly positively correlated in the two lines ( $p < 2e-16$ ,  $R^2 = 0.2406$ ). We observed an  
 415 almost perfect positive correlation if the analysis was restricted to significant differential  
 416 splicing events in both lines ( $p < 2e-16$ ,  $R^2 = 0.7613$ ). The genes concerned belonged to various  
 417 families, e.g. the UDP-glucuronate decarboxylase (UXS), the heat shock protein 90 (HSP)  
 418 and auxin-responsive (AUX/IAA) gene families (Table S4). The GO analysis however  
 419 showed no significant enrichment in any term among the genes displaying AS in the  
 420 *MiEFF18*-expressing line or the *smd1b* mutant. Using RT-qPCR, we validated an IR  
 421 occurring in the *MiEFF18*-expressing line and the *smd1b* mutant in a the *FAD/NAD(P)-*  
 422 *binding oxidoreductase (At5g11330)* gene, and an A3 event in *RNA-binding protein*  
 423 *(At3g04500)*, an IR in the *ribosomal protein S21 family protein (At3g26360)* and an A5 event  
 424 in *U1 snRNP 70K (At3g50670)* genes occurring in the *smd1b* mutant (Fig. 3e; Fig.S9). Thus,  
 425 MiEFF18 can modulate AS through SmD1, as the ectopic expression of *MiEFF18* partially  
 426 mimics the global change in AS pattern observed in the *smd1b* mutant line.

427

#### 428 ***M. incognita* triggers alternative splicing during giant cell formation**

429 We used available RNA-seq data from *Arabidopsis* galls at 5 and 7 dpi and from non-  
 430 inoculated Col-0 roots (Yamaguchi et al., 2017) to investigate AS events during giant cell  
 431 formation in *Arabidopsis*. SUPPA analysis identified 411 and 443 genes that underwent AS in  
 432 response to *M. incognita* infection at 5 and 7 dpi, respectively (Fig.3a; Table S5 and S6). In  
 433 total, 701 genes were alternatively spliced at either 5 or 7 dpi (Fig. 3c), representing 840  
 434 different AS events (Fig. S7d). GO analysis on these 701 AS genes revealed highly significant  
 435 enrichment in the term “post embryonic development” ( $p$ -value= $5.8e-07$ ), including 10  
 436 *EMBRYO DEFECTIVE (EMB)* genes (*EMB 1353*, *EMB1995/ATS2*, *EMB1629/APO2*, *EMB*  
 437 *2728/RPE*, *EMB76/DCL1*, *EMB1006*, *EMB1379*, *EMB2768*, *EMB1401/EIF2 BETA* and

438 *EMB1796/NUWA*) and genes involved in hormone signalling (e.g. the gibberellin receptor GA  
 439 INSENSITIVE DWARF1C, the cytokinin receptor WOODEN LEG (*WOL/CRE1*) and the  
 440 auxin-responsive IAA28). In addition we noticed an enrichment in GO terms “nucleotide  
 441 binding” ( $p$ -value=2.6e-05), “single-stranded DNA binding” ( $p$ -value=5.6e-05) and  
 442 “ribonucleotide binding” ( $p$ -value=5.1e-04) (Table S7). These results provide a first insight  
 443 into the importance of AS as a regulatory mechanism involved in giant cell formation.

444 We then investigated whether the modulation of SmD1 function by the MiEFF18 effector  
 445 could account for the AS observed upon RKN infection. Interestingly, 34.2% (76 genes) and  
 446 24.8% (115 genes) of the genes displaying AS changes in the *MiEFF18*-expressing line and in  
 447 the *smd1b* mutant, respectively, were also affected at 5 or 7 dpi with *M. incognita*; this  
 448 corresponds to significant enrichment (hypergeometric  $p$ -value < 2.0e-61) (Fig. 3d). In total,  
 449 39 of the genes displaying AS were common to the three sets of conditions, suggesting that  
 450 the MiEFF18 effector and SmD1 may be at least partly responsible for the AS occurring in  
 451 roots in response to RKN infection. These genes included those involved in hormone  
 452 signalling, such as the auxin-responsive *IAA27*, the *CALCIUM-DEPENDENT PROTEIN*  
 453 *KINASE 4 (CPK4)* involved in ABA signalling, and genes encoding RNA-binding proteins,  
 454 such as *GLYCINE-RICH RNA-BINDING PROTEIN 2 (ATGRP2)* or *NUCLEAR TRANSPORT*  
 455 *FACTOR 2 (NTF2)*. Thus MiEFF18 could account for AS triggered in *Arabidopsis* following  
 456 infection with *M. incognita* to modulate giant cell proteome.

457

#### 458 **MiEFF18 and SmD1 modulate expression of plant genes involved in giant cell formation**

459 Using RNA-seq data, we also identified 511 and 1,160 differentially expressed genes (DEGs)  
 460 in the *Arabidopsis MiEFF18*-expressing line and the homozygous *smd1b* mutant,  
 461 respectively, relative to wild-type Col-0 plants (Fig. 4a-c; Table S8 and S9). We found that  
 462 187 DEGs (130 upregulated and 57 downregulated genes) were common to *MiEFF18*-  
 463 expressing and *smd1b* plants. Interestingly, 38.0% of the DEGs in the *MiEFF18*-expressing  
 464 line and the *smd1b* mutant were also differentially expressed at 5 and/or 7 dpi with *M.*  
 465 *incognita* (Fig. 4b-c; Fig. S10; Table S10 and S11). RT-qPCR was used to confirm the RNA-  
 466 seq data (Fig. 4d). We validated the upregulation of the DNA replication-related *MCM10*  
 467 (*At2g20980*) gene and the downregulation of the *Prenylated RAB acceptor 1.E (At1g08770)*  
 468 and a defensin (*At5g33355*) genes in the *MiEFF18*-expressing line and/or the *smd1b* mutant,  
 469 relative to Col-0. These results are consistent with the modulation of plant gene expression by  
 470 MiEFF18, through interaction with the SmD1 protein. A GO term analysis highlighted an



471 overrepresentation of genes involved in “microtubule-based movement” (p-value=9.1e-25)  
472 and “cell cycle process” (p-value=4.9e-8) in the *MiEFF18*-expressing line, whereas GO terms  
473 associated with “plant-type cell wall organization” (p-value=1.1e-05), “response to stimulus”  
474 (p-value=4.1e-05) and “response to oxidative stress” (p-value=4.1e-05) were overrepresented  
475 in the *smd1b* mutant (Fig. 4e, Fig. S11, Table S7). Interestingly, four GO terms were  
476 overrepresented in all three sets of conditions: “cytoskeleton organization”, “cytoskeletal  
477 protein binding”, “microtubule binding” and “tubulin binding” (Fig. 4e, Table S7).

478

### 479 ***AtSmD1b* is instrumental to root knot nematode parasitism**

480 We investigated the possible role of Sm proteins, and SmD1 in particular, in RKN parasitism.  
481 We began by browsing transcriptomic data to determine whether the expression of *Sm* genes  
482 in galls was induced by *M. incognita* infection. Genes encoding the core Sm protein  
483 components of the spliceosome, including *AtSmD1a*, are generally induced upon infection  
484 (Table S12), suggesting a possible role in the plant-nematode interaction. We investigated the  
485 function of *Arabidopsis AtSmD1* genes during parasitism further, by inoculating the *smd1a*  
486 and *smd1b Arabidopsis* knockout mutants (Elvira-Matelot et al., 2016) with *M. incognita* J2s.  
487 Inoculation resulted in a mean decrease of 30% in the number of females producing egg  
488 masses in *smd1b* plants relative to wild-type Col-0 (Fig. 5a). Inoculation had no significant  
489 effect on the number of females producing egg masses in *smd1a* plants. This result is  
490 consistent with *AtSmD1b* being strongly expressed in *Arabidopsis*, whereas *AtSmD1a* is not  
491 (Elvira-Matelot et al., 2016). We investigated whether the giant cells formed on the *smd1b*  
492 plants displayed developmental defects. We observed these giant cells directly, under a  
493 confocal microscope, after BABB clearing. A comparison of the mean surface areas of the  
494 giant cells in each gall showed that giant cells from *smd1b* plants were 37% smaller than  
495 those from control plants (Fig. 5b and 5c). Thus, the *AtSmD1b* protein plays an important role  
496 in the formation of giant cells and is required for successful nematode infection.

497

## 498 **Discussion**

499

### 500 **MiEFF18 interacts with a nuclear spliceosomal protein**

501 *Meloidogyne* spp. are among the most devastating plant pathogens, but our understanding of  
502 the molecular basis of RKN pathogenicity remains limited. RKN secrete hundreds of  
503 effectors, enabling them to overcome host defences and to induce the redifferentiation of root

504 cells into permanent feeding cells. However, the functions of most of these effectors remain to  
505 be determined (Mitchum *et al.*, 2013; Truong *et al.*, 2015; Vieira & Gleason, 2019; Mejias *et*  
506 *al.*, 2019). One of the predicted secreted effectors, MiEFF18, has been shown to be  
507 specifically overexpressed within the nematode subventral oesophageal glands at an early  
508 stage of parasitism (Rutter *et al.*, 2014; Nguyen *et al.*, 2018).

509 We showed, by immunolocalisation studies on J2s, that MiEFF18 was present in secretory  
510 granules in the subventral gland cells. In plant-parasitic nematodes, these structures are  
511 thought to be involved in the delivery of secretions from the oesophageal glands to the stylet,  
512 through which they are secreted into the host tissues (Sundermann & Hussey, 1988; Hussey &  
513 Mims, 1990; Wang *et al.*, 2010). Immunolocalisation on gall sections further demonstrated  
514 MiEFF18 secretion within the giant cells, where it accumulated in the nuclei, validating the *in*  
515 *silico*-predicted nuclear localisation of this effector *in planta*. Secretion has been  
516 demonstrated experimentally for very few effectors, and even fewer have been shown to be  
517 delivered to the giant cells. *M. incognita* MiMIF-2 (Zhao *et al.*, 2019a) was localised in the  
518 cytoplasm, whereas the other effectors (Mi-EFF1, MjNULG1a, MgGPP and Mg16820) were  
519 immunolocalised in giant cell nuclei (Jaouannet *et al.*, 2012; Lin *et al.*, 2012; Chen *et al.*,  
520 2017b; Naalden *et al.*, 2018). Our findings support the notion that the nucleus is a key cellular  
521 compartment that must be targeted by the parasite, for the regulation of nuclear processes  
522 essential for giant cell development, such as cell cycle regulation and transcription (Hewezi &  
523 Baum, 2013; Quentin *et al.*, 2013).

524 Using a Y2H screen, we identified the nuclear spliceosomal SmD1 protein as a potential  
525 target of MiEFF18. SmD1, together with six other small ribonucleoprotein particle (Sm)  
526 proteins (SmB, SmD2, SmD3, SmE, SmF and SmG), forms a heptameric ring structure  
527 surrounding the U-rich small nuclear RNAs (snRNAs) (Matera & Wang, 2014). These snRNP  
528 complexes are core components of the spliceosome and play a key role in pre-mRNA splicing  
529 (i.e. the correct removal of introns from pre-RNA). When the Sm ring is assembled on the  
530 different snRNA molecules in the cytoplasm, it can enter the nucleus, where it initially  
531 accumulates in Cajal bodies, and finally, the fully assembled spliceosome executes splicing in  
532 the nucleoplasm and, more specifically, in nuclear speckles. Thus, in plants, SmD1 may  
533 localise to the nucleoplasm, nucleolus, nuclear speckles, Cajal bodies and cytoplasm,  
534 consistent with previous reports (Pendle *et al.*, 2005; Fujioka *et al.*, 2007; Elvira-Matelot *et*  
535 *al.*, 2016; Huertas *et al.*, 2019). We validated the localisation of SmD1 in the cytoplasm and  
536 the nucleus, where it could interact with MiEFF18.

537

**538 Ectopic MiEFF18 expression mimics the effect of SmD1 impairment on AS**

539 The finding that the ectopic expression of MiEFF18 *in planta* mimics characteristics of the  
540 *smd1b* mutation provides further evidence in favour of SmD1 being the target of MiEFF18.  
541 AtSmD1b has recently been shown to modulate the AS of specific transcripts (Elvira-Matelot  
542 et al., 2016). In *Arabidopsis*, 70% of the genes may be alternatively spliced, and AS has been  
543 shown to play a significant role in plant development and responses to abiotic stresses (Reddy  
544 et al., 2013; Staiger and Brown, 2013). AS provides a layer of genetic regulation mediating  
545 rapid responses to different stimuli by increasing proteomic diversity. It can affect the  
546 stability of a transcript, particularly if the 5'UTR or 3'UTR is concerned. It can also lead to a  
547 loss/gain of protein function if the open reading frame is modified, by a frameshift or the  
548 creation of a new premature stop codon (Chaudhary *et al.*, 2019). Only a few studies to date  
549 have focused on plant Sm proteins. They investigated the *Arabidopsis* SmD3 (Swaraz *et al.*,  
550 2011) and SmE (Huertas *et al.*, 2019) proteins, and data are also available for the Sm-Like  
551 protein LSm8, another core component of the spliceosome (Carrasco-López *et al.*, 2017).  
552 Genome-wide AS analysis has confirmed the role of SmE and LSm8 in regulating AS in  
553 *Arabidopsis*, enabling plants to adapt to unfavourable abiotic environments. We expand here,  
554 by a transcriptomic approach, the role of AtSmD1b in regulating AS, and we reveal its crucial  
555 function in a biotic interaction. Our RNAseq data showed that MiEFF18 could coordinate this  
556 AtSmD1b function during RKN parasitism. Indeed, half of the splicing events, in 107 genes,  
557 induced by the ectopic expression of *MiEFF18* in *Arabidopsis*, were also induced by  
558 *AtSmD1b* mutation, suggesting that MiEFF18 controls susceptibility to RKN by directly  
559 modulating the host cell transcriptome.

560

**561 Alternative splicing occurs upon RKN parasitism in *Arabidopsis***

562 AS may play an important role in plant responses to pathogens (Rigo *et al.*, 2019). Very few  
563 studies have reported the AS events occurring in plants in response to infection with bacterial,  
564 viral or fungal pathogens (Howard *et al.*, 2013; Mandadi & Scholthof, 2015; Rubio *et al.*,  
565 2015; Song *et al.*, 2017; Zheng *et al.*, 2017; Bedre *et al.*, 2019; Ma *et al.*, 2019; Zhang *et al.*,  
566 2019; Wang *et al.*, 2020). Specific AS events occur in plants in response to a pathogen. A  
567 number of different, specific splice variants have, for example, been shown to accumulate in  
568 wheat in response to infection with two fungal pathogens, *Blumeria graminis* f. sp. *tritici* and  
569 *Puccinia striiformis* f. sp. *tritici* (Zhang et al., 2019). However, the mechanisms regulating the

570 specificity of the AS of pre-mRNA and controlling stress responses remain poorly understood  
 571 (Catalá *et al.*, 2019). We provide here a transcriptome-wide description of the AS events  
 572 occurring in galls 5 and 7 dpi with *M. incognita*. We show that, in galls, AS genes exhibited  
 573 significant alternative 3' splice site selection rather than intron retention, which is usually  
 574 predominant in plant response to stress (Laloum *et al.*, 2018). In addition, in galls AS occurs  
 575 in genes specifically related to giant cell ontogenesis. Indeed, we show enrichment in genes  
 576 related to post-embryonic organogenesis among the genes displaying AS in galls. The  
 577 developmental reprogramming required for giant cell formation involves modulation of the  
 578 expression of genes involved in root cell identity and root development (Yamaguchi *et al.*,  
 579 2017; Olmo *et al.*, 2020). The Mi16D10 effector has been shown to manipulate two of these  
 580 proteins, both of which are SCARECROW-like transcription factors regulating gene  
 581 expression during root organogenesis (Huang *et al.*, 2006). Our results suggest that MiEFF18,  
 582 by interfering with AtSmD1b function, may affect these processes in a broader manner,  
 583 providing transcriptional control over several of these genes.

584 Recently, effectors have been shown to interfere with the plant spliceosome machinery.  
 585 The PsAvr3c effector, secreted by the plant pathogenic oomycete *Phytophthora sojae*, has  
 586 been shown to interfere with the soybean serine/lysine/arginine-rich protein GmSKRP1,  
 587 modifying the pattern of AS in the host plant to subvert immunity (Huang *et al.*, 2017).  
 588 Similarly, the *H. schachtii* 30D08 effector has been shown to interact with the *Arabidopsis*  
 589 SMU2 auxiliary spliceosomal protein. The 30D08 protein allows the cyst nematode to alter  
 590 pre-mRNA splicing and the expression of genes involved in feeding site development (Verma  
 591 *et al.*, 2018). We can, thus, hypothesize that, acting through its interaction with a core  
 592 spliceosomal protein, MiEFF18 modulates the AS occurring in giant cells upon plant-RKN  
 593 interaction.

594

#### 595 **MiEFF18 and SmD1 regulate the expression of genes involved in giant cell ontogenesis**

596 A broad reprogramming of transcription occurs upon RKN infection, as already demonstrated  
 597 in many plants, including *Arabidopsis* (Escobar *et al.*, 2011; Favery *et al.*, 2016; Yamaguchi  
 598 *et al.*, 2017). Thousands of plant genes involved in diverse processes, including cell cycle  
 599 activation, cell wall modification, and hormone and defence responses, are differentially  
 600 expressed during RKN parasitism (Favery *et al.*, 2016). Ectopic expression of *MiEFF18* and  
 601 partial impairment of SmD1 activity (using the *smd1b* mutant) had similar effects on the  
 602 expression of various genes differentially expressed upon *M. incognita* infection and giant cell

603 formation in *Arabidopsis*. In particular, genes involved in DNA replication (e.g. the MCM  
604 gene family), in DNA repair and in microtubule network regulation (e.g. encoding kinesins or  
605 the MAP65 proteins), or encoding proteins involved in spindle assembly (MAP70-1; IQ  
606 DOMAIN 31; TPX2) were upregulated in the *Arabidopsis* lines studied here. This finding is  
607 consistent with the synchronised activation of cell cycle processes, such as acytokinetic  
608 mitoses and DNA amplification, that occurs during giant cell formation (De Almeida Engler  
609 & Gheysen, 2013; Favery *et al.*, 2016). Deregulation of the expression of key regulators of the  
610 cell cycle and of cytoskeleton regulators through mutations (e.g. *map65-3* or *wee1.1*), or  
611 ectopic expression (e.g. *Kip-Related Protein (KRP)*-expressing lines), leads to defective giant  
612 cell development (Caillaud *et al.*, 2008; Coelho *et al.*, 2017; Vieira & de Almeida Engler,  
613 2017; Cabral *et al.*, 2020).

614 We show here that constitutive expression of the MiEFF18 effector decreases the  
615 susceptibility of *Arabidopsis* to *M. incognita*. However, the ectopic expression of MiEFF18  
616 may not reflect what happens under physiological conditions in a giant cell, where the effector  
617 must be timely delivered in a precise amount. The excess of some effectors in plants may  
618 modify plant physiology and cell function, and confer plant resistance to biotic and/or abiotic  
619 stresses. Such observations could be made when expressing *in planta* oomycete effectors (e.g.  
620 PsCRN161 or PsCRN115; Rajput *et al.* 2015) or cyst nematode effectors (e.g. Hs32E03 and  
621 Hs30D08; Vijayapalani *et al.* 2018 and Verma *et al.* 2018). In addition, the partial  
622 impairment of SmD1 function affects the susceptibility of *Arabidopsis* to RKN, impacting  
623 giant cell development. Altogether our results demonstrate that MiEFF18 effector interacts  
624 with AtSmD1b and may perturbate its homeostasis to facilitate the *de novo* formation of the  
625 giant feeding cells unique to RKN parasitism, by regulating key developmental processes.

626 The answer on how the EFF18 effector manipulates the SmD1 function may come from an  
627 analysis of the structure of the MiEFF18. The K-rich C-terminal part of the effector, carrying  
628 NLS and NoLS, undoubtedly mediates import into the nucleus, and the N-terminal part of the  
629 molecule carries D/E repeats, which are often found in DNA/RNA mimic proteins (Chou &  
630 Wang, 2015). These proteins regulate the activity of various DNA/RNA-binding proteins  
631 involved in diverse nuclear processes, such as chromatin assembly, DNA repair or  
632 transcriptional regulation (Chou & Wang, 2015; Wang *et al.*, 2019). Further studies of this  
633 effector-target pair and associated RNAs would improve our understanding of the role and  
634 regulation of the spliceosome machinery in plants and might lead to the development of

635 applications in new control strategies based on the loss of a susceptibility gene essential for  
636 development of the disease.

637

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658

### 659 **Author contributions**

660 J.M. designed and performed experiments, and interpreted results; J.M., Y.P.C. and N.M.T.  
661 performed yeast two-hybrid assays and generated constructs; SW, J.B. and M.D.C. performed  
662 the transcriptome analysis and analysed AS data; H.V. and N.B. contributed material and  
663 analysed the data; N.M. produced the nematodes and tomato plants; J.M., J.B., H.V., P.A.,  
664 B.F. and M.Q. wrote the article; P.A., B.F. and M.Q. obtained funding, designed the work and  
665 supervised the experiments and data analyses; all the authors read and edited the article.

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667

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- 887

889 **Figure legends**

890

891 **Fig. 1** MiEFF18 is a secreted effector that localises to the nucleus and nucleolus of plant cells.  
 892 (a) Schematic diagram of the MiEFF18 protein. The predicted secretion signal peptide (SP;  
 893 red box), the aspartic acid and glutamic acid (D-E)-rich region (purple box), the lysine (K)-  
 894 rich C-terminal region (yellow box), nuclear localisation signals (NLS) and the nucleolar  
 895 localisation signal (NoLS) are shown. The NLS pat4 (KKPK, aa 235-238) and pat 7  
 896 (PAKKGKK, aa 292-298) are indicated in grey and the bipartite region  
 897 (KGAAKVAKKDTKKPKD, aa 223-239) is shown in black. (b) Schematic diagram of a  
 898 section through a J2. (c-e) Immunolocalisation of MiEFF18 in the subventral glands (SvGs)  
 899 of *M. incognita* pre-parasitic J2s. (f) Immunolocalisation of MiEFF18 in the subventral glands  
 900 (SvGs) of parasitic *M. incognita*. Confocal images of J2s treated with rabbit anti-MiEFF18  
 901 serum and goat anti-rabbit Alexa Fluor 488 secondary antibodies are shown. Fluorescence  
 902 signals are visible in the secretory granules of the subventral glands (magnification in the  
 903 insets) and in the secretory tracts (arrow). Corresponding bright-field images of the juveniles  
 904 are shown in the left. Bars = 10  $\mu$ m. m, metacarpus, n, nucleus, SvGs, subventral glands. (g-  
 905 h') Localization of the secreted MiEFF18 protein in plant tissues. MiEFF18 accumulated in  
 906 the giant cell nuclei. Images of Alexa Fluor 488 fluorescence, DAPI-stained nuclei and  
 907 overlays are shown. (h') is an enlargement of the area framed in (h). \*, giant cell. Bars = 10  
 908  $\mu$ m.

909

910 **Fig. 2** The MiEFF18 effector interacts with SmD1 proteins in the nucleus and nucleolus of  
 911 plant cells. (a) Diploid yeasts containing the bait and prey plasmids carrying controls,  
 912 effectors or SmD1 proteins (*Solanum lycopersicum* SlSmD1 and *Arabidopsis thaliana*,  
 913 AtSmD1a and AtSmD1b) were spotted on plates. SD-WL corresponds to the non-selective  
 914 medium without tryptophan (W) and leucine (L). Only yeasts carrying a protein-protein  
 915 interaction can survive on the SD-WLH (H, histidine) + 0.5 mM 3-AT selective medium.  
 916 Murine p53 and SV40 T-antigen T (anti T) were used as a positive control, and MiEFF16 was  
 917 used as a negative control. (b) Colocalisation of RFP-MiEFF18 and GFP-SlSmD1 in *N.*  
 918 *benthamiana* epidermal leaf cells. RFP and GFP were used as a nucleocytoplasmic control.  
 919 Bars = 5 $\mu$ m. (c) MiEFF18 and SlSmD1 interact together in the nucleolus, nucleoplasm and  
 920 cytoplasm in *N. benthamiana* cells. YFP fluorescence confocal images of bimolecular  
 921 fluorescence complementation (BiFC) experiments with different combinations of YFPc or

922 YFNn fused, at the C- or N-terminus, to SLSmD1 and MiEFF18, expressed in *N. benthamiana*  
 923 epidermal cells. The MiEFF16 effector was used as a negative control. Bars = 10  $\mu$ m.

924

925 **Fig. 3** Smd1b modulates alternative splicing in *Arabidopsis* roots. (a) *Arabidopsis* genes with  
 926 alternative splicing (AS) events (intron retention, exon skipping, alternative 5' splice site,  
 927 alternative 3' splice site, mutually exclusive exons) in the *MiEFF18*-expressing line and the  
 928 *smd1b* mutant, relative to Col-0 roots, and in galls five and seven days post inoculation (dpi)  
 929 with *M. incognita*, relative to uninfected *Arabidopsis* Col-0 roots. (b) Venn diagram showing  
 930 the overlap between alternatively spliced genes in the *MiEFF18*-expressing line and *smd1b*  
 931 mutant plants. (c) Venn diagram showing the overlap between alternatively spliced genes in  
 932 *M. incognita*-induced galls at 5 and 7 dpi. (d) Venn diagram showing the overlap between  
 933 genes affected in the *MiEFF18*-expressing line, *smd1b* mutant and in *M. incognita*-induced  
 934 galls at 5 or 7 dpi. (e) Validation of the changes in AS pattern detected in the roots of  
 935 *Arabidopsis* *MiEFF18*-expressing line, *smd1b* mutant and wild-type Col-0 by RT-qPCR. Data  
 936 were normalised using *UBQ10* as a reference gene. Asterisks indicate significant differences  
 937 (\*\*P < 0.001, \*\*\*P < 0.0001) compared to wild-type plants, as determined by t-student test  
 938 (SatqPCR software). Error bars indicate the SE. Left panels show the part of the alternately  
 939 spliced genes (the black boxes represent the exons, the lines represent the introns) and the  
 940 read mapping of the RNAseq (y-axis).

941

942 **Fig. 4** MiEFF18 and SMD1b regulate transcript accumulation in *Arabidopsis* root. (a)  
 943 Quantification of differentially expressed genes (DEG) in the roots of the *MiEFF18*-  
 944 expressing *Arabidopsis* line (EFF18) and the *smd1b* mutant, relative to Col-0 roots. The  
 945 overlap between genes differentially expressed (up: induced; down: repressed) in the EFF18  
 946 line and the *smd1b* mutant is shown. (b) Venn diagram showing the overlaps between genes  
 947 induced (up) in the *MiEFF18*-expressing line, the *smd1b* mutant and in *M. incognita*-induced  
 948 galls at 5 or 7 dpi. (c) Venn diagram showing the overlaps between genes repressed (down) in  
 949 the *MiEFF18*-expressing line, the *smd1b* mutant and in *M. incognita*-induced galls at 5 or  
 950 7dpi. (d) Validation of the expression of DEG identified in the *smd1b* mutant and/or the  
 951 *MiEFF18*-expressing line, by RT-qPCR. Data were normalized against *UBQ10* and *OXA1* as  
 952 constitutive genes. Asterisks indicate significant differences (\*P < 0.01) between *MiEFF18*-  
 953 expressing line or the *smd1b* mutants compared to wild-type (Col-0) plants, as determined by  
 954 t-student test (SatqPCR software). Error bars indicate the SE. (e) Enrichment in GO terms for

955 biological processes among DEGs in the *MiEFF18*-expressing line, *smd1b* mutant and in galls  
 956 five and seven days after inoculation with *M. incognita*. Only GO terms displaying  
 957 statistically significant enrichment (FDR<0.05) in at least two sets of conditions are  
 958 presented.

959

960 **Fig. 5** AtSmD1b is instrumental to root-knot nematode parasitism. (a) Box-and-whisker plots  
 961 of females producing egg masses per plant in Col-0 control line, *smd1a*, *smd1b* lines six  
 962 weeks post infection with 200 *M. incognita* J2s. The three independent experiments are  
 963 presented. The effect of plant genotype on the number of nematode egg masses was analyzed  
 964 with generalized linear models (GLMs) based on a Poisson distribution, for each replicate.  
 965 We used the Tukey adjustment method ('multcomp' package) for multiple testing. Different  
 966 letters indicate statistically significant difference between each column. (b) Galls of Col-0 and  
 967 *smd1b* plants collected two weeks post infection to measure the surface of the giant cells  
 968 (dotted line) using BABB clearing method (Cabrera et al., 2018). (c) Box-and-whisker plot of  
 969 giant cell size ( $\mu\text{m}^2$ ) measures on Col-0 and *smd1b* plants. The impact of the plant genotype  
 970 on the surface of giant cells was analysed using student t test. Combined data from two  
 971 independent biological replicates are shown (n=42 and n=25). Significance of terms: \*\*\*P <  
 972 0.001.

### Supporting information

973 **Table S1** Primers used in this study.

974 **Table S2** Altered splicing events identified in the *Arabidopsis* MiEFF18-expressing line

975 **Table S3** Altered splicing events identified in the *Arabidopsis smd1b* mutant line.

976 **Table S4** Gene family (GenFam) enrichment analyses.

977 **Table S5** Altered splicing events identified in *Arabidopsis thaliana* at 5 dpi with *Meloidogyne*  
 978 *incognita*.

979 **Table S6** Altered splicing events identified in *Arabidopsis thaliana* at 7 dpi with *Meloidogyne*  
 980 *incognita*.

981 **Table S7** Gene Ontology (GO) analyses.

982 **Table S8** Differentially expressed genes identified in the *Arabidopsis* MiEFF18-expressing  
 983 line

984 **Table S9** Differentially expressed genes identified in the *Arabidopsis smd1b* mutant line.



985 **Table S10** Differentially expressed genes identified in *Arabidopsis thaliana* at 5 dpi with  
986 *Meloidogyne incognita*.

987 **Table S11** Differentially expressed genes identified in *Arabidopsis thaliana* at 7 dpi with  
988 *Meloidogyne incognita*.

989 **Table S12** Expression of the different *Arabidopsis* small nuclear ribonucleoprotein Sm core  
990 genes during *M. incognita* infection.

991

992 **Fig. S1** Alignment of the Minc18636 and Minc15401 proteins.

993 **Fig. S2** Specificity of the  $\alpha$ -MiEFF18 and pre-immune serums.

994 **Fig. S3** Results of the yeast two-hybrid screen using MiEFF18 as a bait against the tomato  
995 root cDNA library.

996 **Fig. S4** Minc16401 encodes a putative effector targeting the plant cell nucleus and nucleoli.

997 **Fig. S5** Bimolecular fluorescence complementation (BiFC) experiments in *N. benthamiana*  
998 cells showed that SISmD1 interact with MiEFF18, but not with MiEFF16.

999 **Fig. S6** *MiEFF18*-expression in *Arabidopsis* transgenic lines altered *M. incognita*  
1000 reproduction.

1001 **Fig. S7** MiEFF18 and SmD1b modulate alternative splicing in *Arabidopsis* roots.

1002 **Fig. S8** Effect of *MiEFF18* expression and *smd1b* mutation on AS are positively correlated.

1003 **Fig. S9** Example of alternative splicing qPCR validation for the U1-70K mRNA isoform.

1004 **Fig. S10** Venn diagrams of differentially expressed genes (DEG) in roots of the MiEFF18-  
1005 expressing line, the *smd1b* mutant and in *M. incognita*-induced galls at 5 or 7dpi.

1006 **Fig. S11** Gene ontology (GO)-term enrichment of differentially expressed genes (DEG) in the  
1007 *MiEFF18*-expressing line and the *smd1b* mutant.

1008

1009

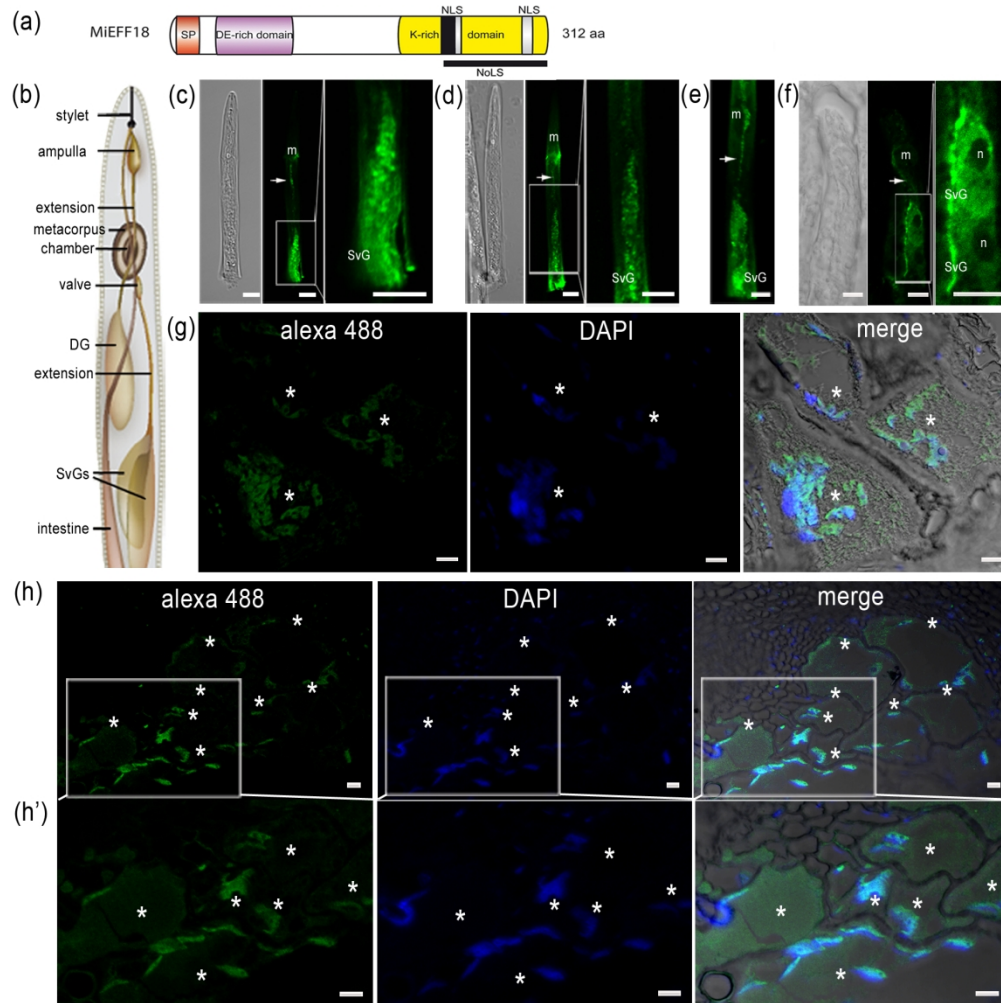


Figure 1

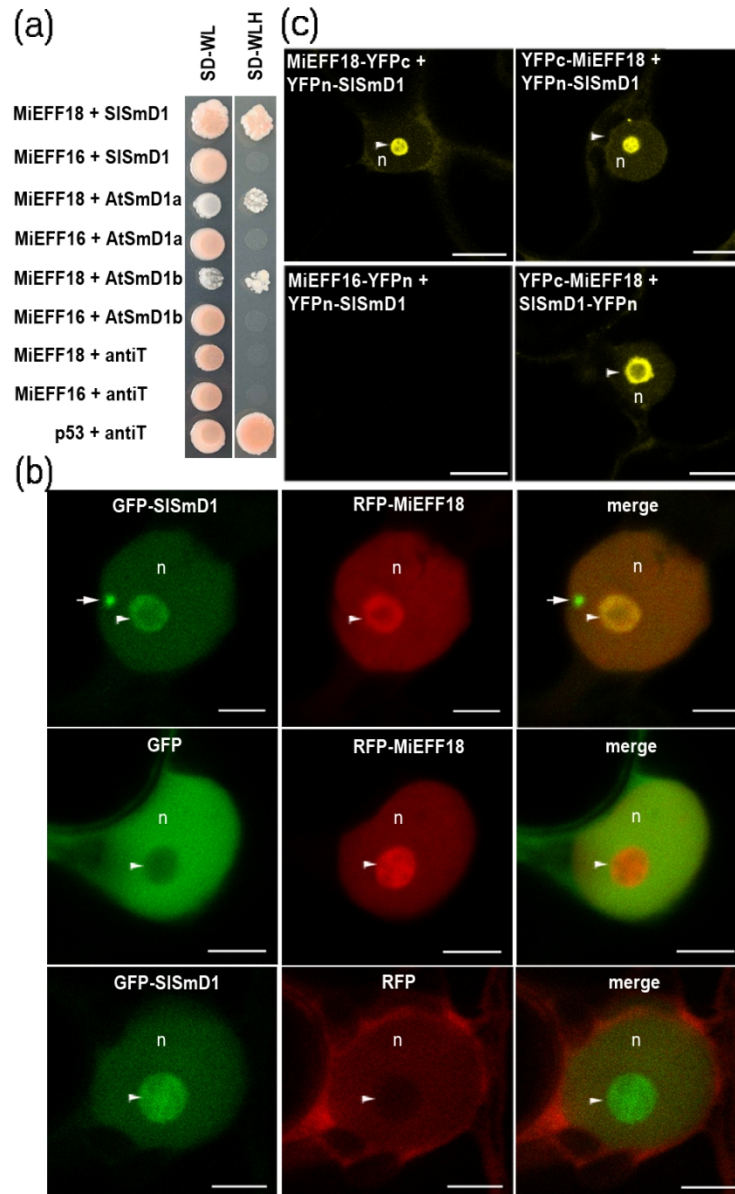


Figure 2

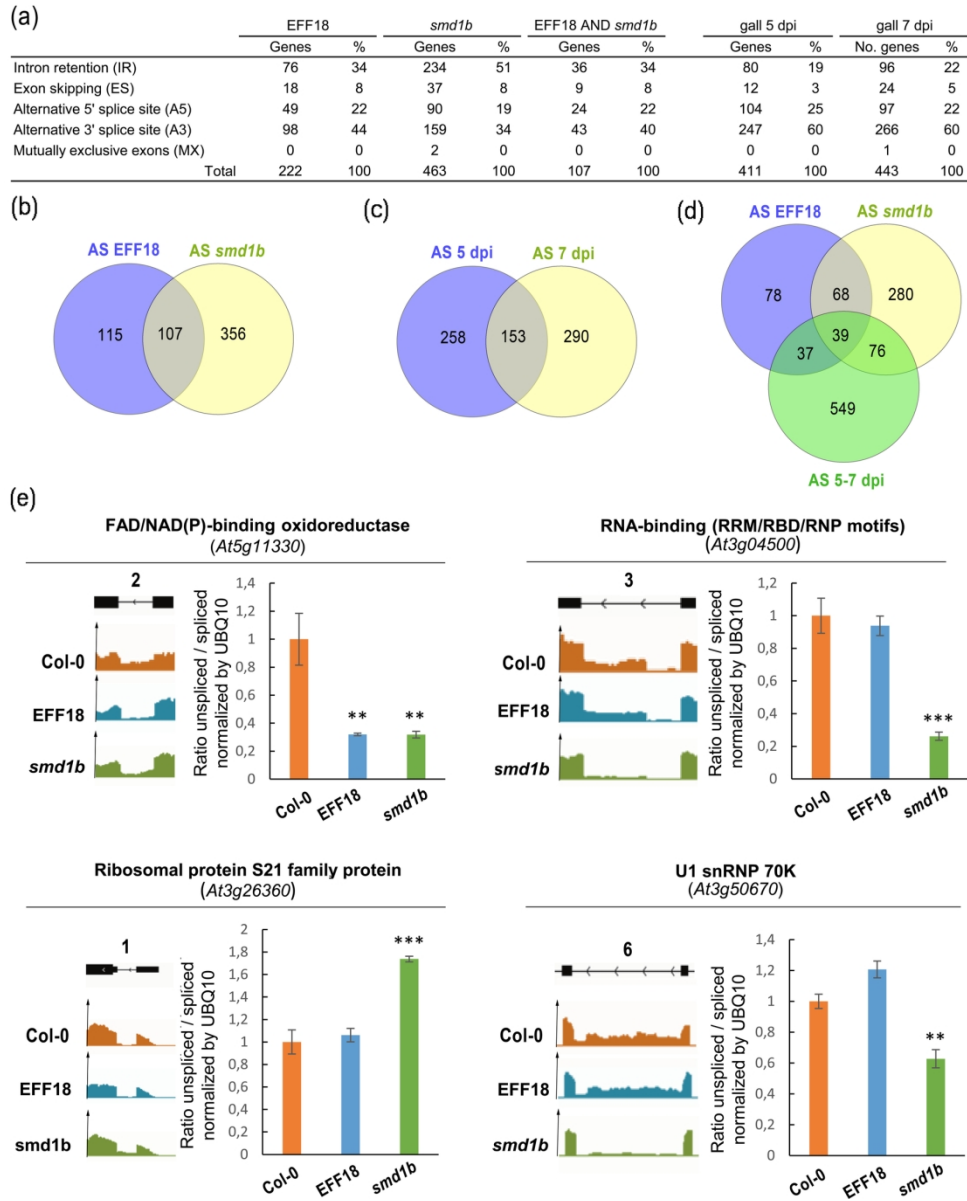


Figure 3

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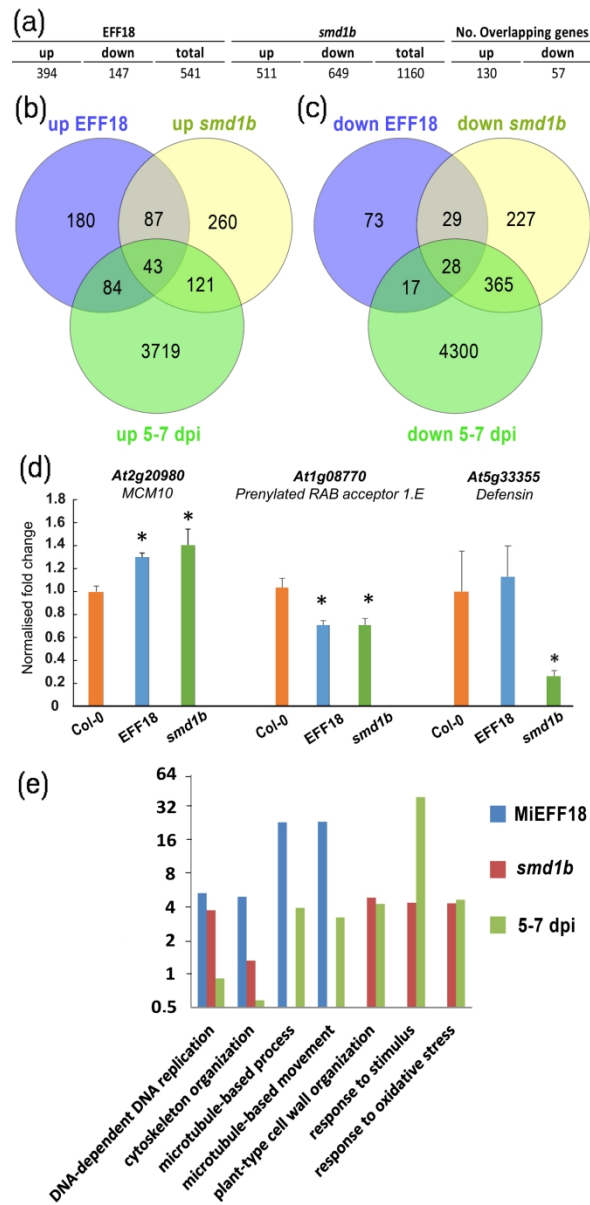


Figure 4

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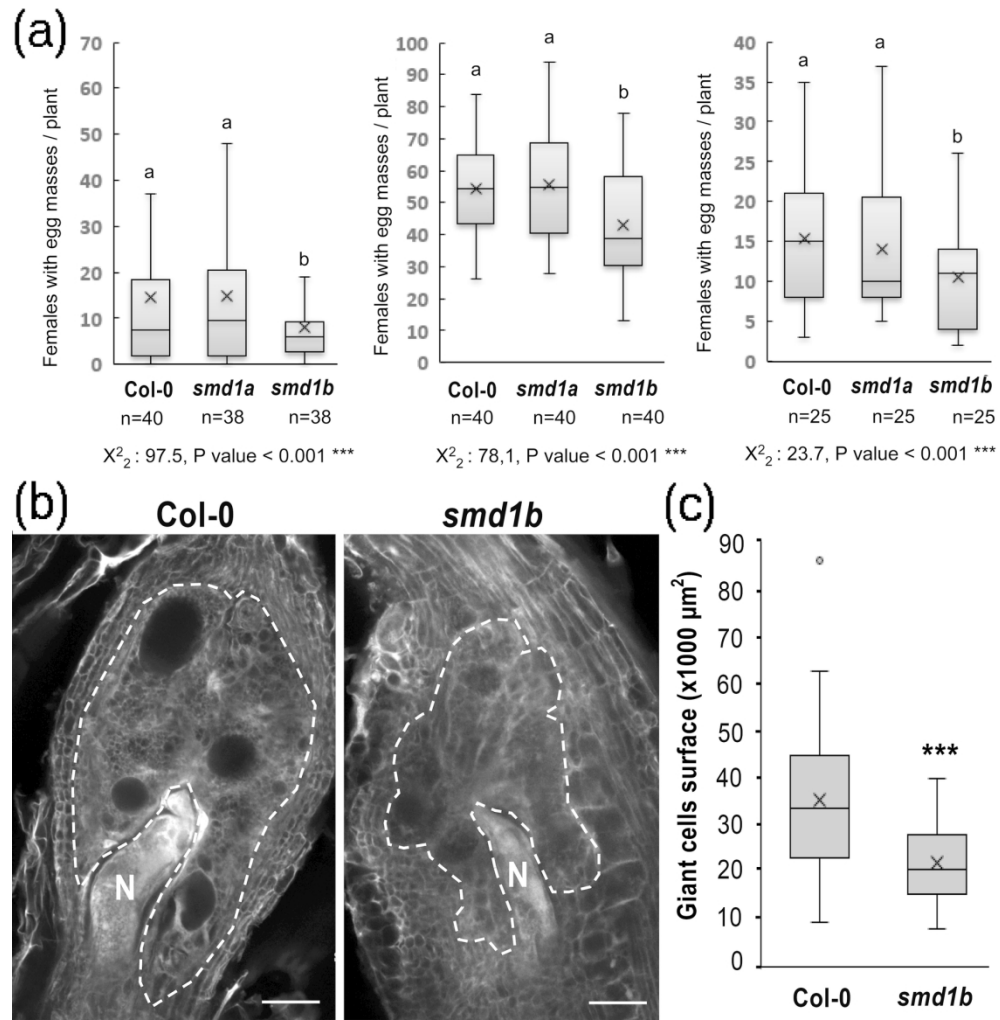


Figure 5

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