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The tomato *I* gene for Fusarium wilt resistance encodes an atypical leucine-rich repeat receptor-like protein whose function is nevertheless dependent on SOBIR1 and SERK3/BAK1

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SUMMARY

We have identified the tomato *I* gene for resistance to the Fusarium wilt fungus *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) and show that it encodes a membrane-anchored leucine-rich repeat receptor-like protein (LRR-RLP). Unlike most other LRR-RLP genes involved in plant defence, the *I* gene is not a member of a gene cluster and contains introns in its coding sequence. The *I* gene encodes a loopout domain larger than those in most other LRR-RLPs, with a distinct composition rich in serine and threonine residues. The I protein also lacks a basic cytosolic domain. Instead, this domain is rich in aromatic residues that could form a second transmembrane domain. The I protein recognises the *Fol* Avr1 effector protein, but, unlike many other LRR-RLPs, recognition specificity is determined in the C-terminal half of the protein by polymorphic amino acid residues in the LRRs just preceding the loopout domain and in the loopout domain itself. Despite these differences, we show that I/Avr1-dependent necrosis in *Nicotiana benthamiana* depends on the LRR receptor-like kinases (RLKs) SERK3/BAK1 and SOBIR1. Sequence comparisons revealed that the I protein and other LRR-RLPs involved in plant defence all carry residues in their last LRR and C-terminal LRR capping domain that are conserved with SERK3/BAK1-interacting residues in the same relative positions in the LRR-RLKs BRI1 and PSKR1. Tyrosine mutations of two of these conserved residues, Q922 and T925, abolished I/Avr1-dependent necrosis in *N. benthamiana*, consistent with similar mutations in BRI1 and PSKR1 preventing their interaction with SERK3/BAK1.

Keywords: tomato, *Solanum lycopersicum*, *Solanum pimpinellifolium*, Fusarium wilt, *Fusarium oxysporum* f. sp. *lycopersici*, plant disease resistance gene, leucine-rich repeat, receptor-like protein.

INTRODUCTION

The *I* (*Immunity*) gene, introgressed into tomato from *Solanum pimpinellifolium* (accession PI79532), was one of the first plant disease resistance genes deployed against Fusarium wilt disease caused by the soil-borne fungus *Fusarium oxysporum* (Bohn and Tucker, 1939). The effective life of the *I* gene, which conferred resistance against race 1 of *F. oxysporum* f. sp. *lycopersici* (*Fol*), was relatively short because of the emergence of *Fol* race 2 (Alexander and Tucker, 1945). The reason for its short life became apparent with the identification of the corresponding *Fol Avr1* effector gene and the finding that *Avr1* is not required for pathogenicity per se (Houterman *et al.*, 2008). *Avr1* can therefore be readily discarded by the fungus and is absent from *Fol* race 2 (Houterman *et al.*, 2008). The *I-2* gene, also introgressed into tomato from *S. pimpinellifolium* (accession PI126915), was deployed against *Fol* race 2 (Stall and Walter, 1965) but, despite its greater durability, was eventually overcome by the emergence of *Fol* race 3 (Grattidge and O'Brien, 1982; Volin and Jones, 1982). The *I-2* gene encodes a coiled-coil nucleotide-binding leucine-rich repeat (CC-NB-LRR) protein, typical of resistance proteins found in a large number of plants (Simons *et al.*,

1998). The reason for the longer effective life of *I-2* also became apparent with the isolation of the corresponding *Fol Avr2* effector gene and the demonstration that *Avr2* is required for pathogenicity (Houterman *et al.*, 2009). *Avr2* could not therefore be readily discarded by the fungus, but instead required mutations resulting in amino acid substitutions in the Avr2 protein to avoid recognition by I-2 (Houterman *et al.*, 2009). Two genes for *Fol* race 3 resistance have been introgressed into tomato from *Solanum pennellii*. The *I-3* gene from *S. pennellii* accession LA716 (Scott and Jones, 1989) encodes an S-receptor-like kinase (Catanzariti *et al.*, 2015) and the *I-7* gene from *S. pennellii* accession PI414773 (McGrath *et al.*, 1987; Lim *et al.*, 2006) encodes a leucine-rich repeat receptor-like protein (LRR-RLP; Gonzalez-Cendales *et al.*, 2016). The finding that the corresponding *Avr3* effector gene, like *Avr2*, is required for pathogenicity and cannot be readily discarded by the fungus (Rep *et al.*, 2004, 2005) may explain the apparent durability of *I-3*. In contrast, the *I-7* gene has not yet been widely deployed and therefore remains largely untested. Moreover, the corresponding *Avr7* gene has not yet been isolated and its role in pathogenicity remains unknown.

Although the *I* gene is no longer effective against *Fol*, its identification would greatly increase our understanding of *Fol* resistance in tomato, and help inform searches for Fusarium wilt resistance genes in other plant species. More importantly, the discovery that Avr1 suppresses resistance mediated by I-2 and I-3 has given the *I* gene renewed relevance as a gene of practical importance. This discovery led to the proposition that *I-3*-mediated resistance should be safeguarded by deploying *I* alongside *I-3* (Houterman *et al.*, 2008). This circumvents the re-emergence of races carrying both *Avr1* and *Avr3,* which would retain pathogenicity associated with Avr3 while avoiding I-3-mediated resistance. Interestingly, Gonzalez-Cendales *et al.* (2016) found that Avr1 does not suppress resistance mediated by I-7, leading them to postulate that Avr1 is not a general suppressor of resistance but perhaps targets an ENHANCED DISEASE SUSCEPTIBILITY 1 (*EDS1*)-independent signalling pathway. *EDS1* is required for basal defence, resistance mediated by TIR-NB-LRR proteins (where TIR is Toll/interleukin-1 receptor cytosolic-domain homology) and LRR-RLPs, and systemic acquired resistance, but not for resistance mediated by CC-NB-LRR proteins such as I-2 (Parker *et al.*, 1996; Aarts *et al.*, 1998; Hu *et al.*, 2005; Fradin *et al.*, 2009; Breitenbach *et al.*, 2014). Moreover, resistance mediated by the *I* gene, similar to that mediated by *I-7*, is *EDS1* dependent (Hu *et al.*, 2005), suggesting that the *I* gene either encodes a TIR-NB-LRR protein or a LRR-RLP. Here, we confirm this prediction by identifying the *I* gene and showing that it encodes a LRR-RLP. We have also investigated the partner protein requirements for *I*-mediated resistance by virus-induced gene silencing (VIGS) and show that I requires the LRR-receptor-like kinases (RLKs) SOBIR1 (SUPPRESSOR OF BAK1-INTERACTING RLK1 1) and SERK3/*BAK1* (SOMATIC EMBRYOGENESIS RECEPTOR KINASE 3/BRASSINOSTEROID INSENSITIVE 1 ASSOCIATED KINASE 1), like many other LRR-RLPs conferring disease resistance (Liebrand *et al.*, 2013; Du *et al.*, 2015; Larkan *et al.*, 2015; Ma and Borhan, 2015; Postma *et al.*, 2016).

RESULTS

Identification of the *S. pimpinellifolium* introgression carrying the *I* gene

Sela-Buurlage *et al.* (2001) showed that the *I* gene is located close to the restriction fragment length polymorphism (RFLP) marker TG523. The TG523 sequence corresponds to nucleotides 37053013707614 on the sequence-based physical map of tomato chromosome 11 (SL2.50ch11; https://solgenomics.net/) and overlaps the single nucelotide polymorphisms (SNPs) solcap_snp_sl_100072 and solcap_snp_sl_20977 (Table S1). Both of these SNPs were included among 7720 SNPs used by Sim *et al.* (2012) to genotype a collection of 426 tomato accessions. These accessions included the cultivars Heinz 1706 and M82, which carry the *I* gene, and Moneymaker, which does not (Sela-Buurlage *et al.*, 2001; Ozminkowski, 2004; Gonzalez-Cendales *et al.*, 2016). By analysing the SNPs surrounding solcap_snp_sl_100072 and solcap_snp_sl_20977 we were able to identify introgressions of *S. pimpinellifolium* DNA likely to carry the *I* gene in Heinz 1706 and M82 (Table S1 in the Supporting Information). The Heinz 1706 introgression is approximately 1.1 Mb and carries 107 genes, whereas the M82 introgression is approximately 1 Mb with 98 genes. The M82 introgression is shorter at one end compared to that of Heinz 1706, suggesting a reduction by recombination with *S. lycopersicum* DNA during the breeding of M82 (Table S1). Cleaved amplified polymorphic sequence (CAPS) markers developed from SNPs corresponding to restriction site polymorphisms located near the ends of the M82 introgression and one near the middle were used to confirm the polymorphisms between M82 and Moneymaker (Gonzalez-Cendales *et al.*, 2014; see Table S1).

The annotations (available at https://solgenomics.net/) of the 98 genes in the M82 introgression were examined for genes corresponding to one of the three major resistance gene classes, i.e. encoding CC-NB-LRRs, TIR-NB-LRRs or LRR-RLPs. In this way, four *I* gene candidates were identified in the M82 introgression, three TIR-NB-LRR genes, *Solyc11g011080*, *Solyc11g011090* and *Solyc11g011350*, and one LRR-RLP gene, *Solyc11g011180*. The TIR-NB-LRR gene *Solyc11g011080* is an orthologue of the potato *Y-1* gene associated with resistance to potato virus Y (PVY) (Vidal *et al.*, 2002) and, along with its paralogues *Solyc11g011090* and *Solyc11g011350*, is also a close homologue

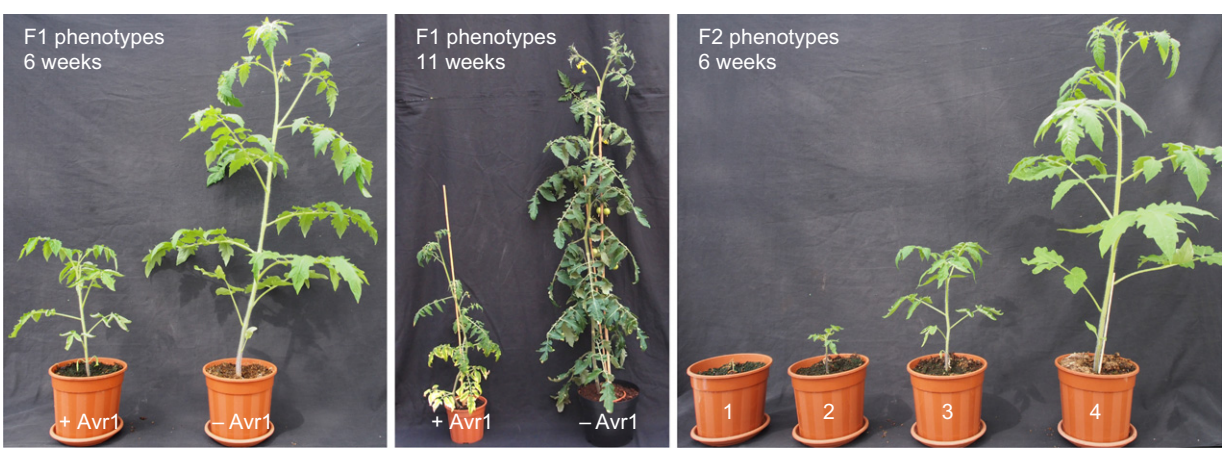
of the tobacco *N* gene for resistance to tobacco mosaic virus (TMV) (Whitham *et al.*, 1994). Owing to this association with viral resistance, we considered these genes unlikely to be involved in resistance to *Fol* and therefore focused on *Solyc11g011180* as the most likely candidate for *I*.

Co-segregation of the *Solyc11g011180* introgression with phenotypic response to an *Avr1* transgene in tomato

As additional confirmation that the *Solyc11g011180* introgression contains the *I* gene, tomato cultivar GCR161 (carrying the *I* gene) was crossed to a transgenic line of tomato cultivar C32 (lacking *I*) carrying an intronless *Avr1* transgene expressed constitutively by the CaMV 35S promoter. The parental line expressing Avr1 showed an unaltered phenotype compared with untransformed C32, but the F_1 of the cross to GCR161 showed a stunted phenotype with delayed acropetal chlorosis followed by necrosis (Figure 1a), and the F_2 progeny segregated for this phenotype (Figure 1). The F_1 phenotype was very similar to that described for the autoactive M205 mutant of the tomato *Cf-9* resistance gene described by Barker *et al.* (2006), except that the phenotype was more severe at 33°C rather than suppressed (Figure S1).

Segregation of the *Avr1* transgene and the *Solyc-11g011180* introgression among the F_2 progeny was assessed by PCR analysis of genomic DNA extracted from 90 F_2 seedlings using *Avr1*-specific primers and CAPS marker 21040 (Gonzalez-Cendales *et al.*, 2014) located in the nearby *Solyc11g011090* gene (Tables S1 and S2, Figure S2). All but one of the F_2 plants showing a stunted phenotype carried both the introgression and the *Avr1* transgene, and vice versa (Figure 1b), consistent with a resistance response mediated through Avr1 recognition, and supporting the conclusion that this introgression carries the *I* gene. One stunted plant was genotyped as carrying *Avr1,* but lacking the introgression based on CAPS 21040 analysis (Figures 1b and S2). PCR amplification and sequencing of

(a)



(b)

F2 genotypes	F2 phenotypes				Total
	1	2	3	4	
A/- I/I	5	7	0	0	12
A/- I/i	0	12	15	0	27
A/- i/i	0	0	1*	23	24
a/a I/I	0	0	0	8	8
a/a I/i	0	0	0	13	13
a/a i/i	0	0	0	5	5
Total	**5**	**19**	**16**	**49**	**89**

Figure 1. Genotypes and phenotypes of tomato plants containing the *I* introgression and a 35S:*Avr1* transgene.
(a) The left and middle photographs show 6- and 11-week-old F_1 plants, respectively, from crosses between tomato cultivars GCR161, containing the *I* gene, and C32, with or without the 35S:*Avr1* transgene. The right photograph shows the range of phenotypes represented among 6-week-old F_2 progeny from the cross between GCR161 and C32 containing the *35S:Avr1* transgene. Key: 1, dying; 2, severely stunted, chlorotic and necrotic; 3, stunted with curling basal leaves (similar to the F_1 phenotype); 4, healthy.
(b) F_2 genotypes were scored by PCR as either the *35S:Avr1* transgene present (*A*) or absent (*a*) and either the *I* introgression present (*I*) or absent (*i*) as determined by CAPS 21040 (*Solyc11g011090*) marker analysis. Phenotypes were scored as described in (a). The asterisk indicates a recombinant plant homozygous for $Solyc11g011090^{C32}$ as determined by CAPS 21040 marker analysis but heterozygous for $Solyc11g011180^{GCR161}$ as determined by sequence analysis of the *Solyc11g011180* gene (see Figure S3).

the 3′ end of the *Solyc11g011180* gene from this plant revealed it to be heterozygous for the introgressed allele of *Solyc11g011180* (Figure S3). This plant was therefore a recombinant between *Solyc11g011090* and *Solyc-11g011180*, localising *I* to a region of no more than 329 kb carrying 33 genes including *Solyc11g011180* but excluding *Solyc11g011080* and *Solyc11g011090*. This result added further support to our view that *Solyc11g011180* was the most likely candidate for the *I* gene.

Some F_2 plants showed a more severe phenotype than the F_1 (Figure 1a), and many of these were homozygous for the introgressed allele of CAPS 21040 (Figure 1b, Table S3), suggesting that in this context the *I*-mediated response to *Avr1* is a semi-dominant phenotype. As observed in the autoactive M205 mutant of *Cf-9*, and in progeny of a cross between tomato lines carrying *Cf-9* on the one hand and an *Avr9* transgene on the other (Hammond-Kosack *et al.*, 1994; Barker *et al.*, 2006), roots of the stunted F_2 plants did not show macroscopic necrosis even in the most severely affected seedlings.

Identification of the *I* gene by co-expression with *Avr1* in *Nicotiana benthamiana*

To test whether *Solyc11g011180* was the *I* gene, we used *Agrobacterium tumefaciens*-mediated expression in *N. benthamiana* leaves to test whether *Solyc11g011180* could trigger Avr1-dependent necrosis. The coding region of the *Solyc11g011180* gene was PCR amplified from M82 and Moneymaker genomic DNA and cloned into the binary vector pL2 containing the CaMV 35S promoter (Gonzalez-Cendales *et al.*, 2016). The *Avr1* gene was amplified from *Fol* race 1 genomic DNA, and also cloned into the pL2 vector. *Avr1* contains a single intron (Houterman *et al.*, 2007) that may not be spliced correctly when expressed *in planta*. We therefore generated a second pL2-*Avr1* construct that lacked the intron by overlap extension PCR, as described by Heckman and Pease (2007). Co-expression of the M82 allele of *Solyc11g011180* (designated *Solyc11g011180^{M82}*) with intronless *Avr1* was found to induce necrosis (Figure 2a) whereas co-expression with *Avr1* containing the intron did not, indicating, firstly, that *Solyc11g011180^{M82}* is able to recognise *Avr1* and therefore corresponds to the *I* gene and, secondly, that the intron in *Avr1* is not correctly spliced when expressed in *N. benthamiana*. All subsequent experiments were conducted using intronless *Avr1*. Co-expression of the Moneymaker allele of *Solyc11g011180* (designated *Solyc11g011180^{MM}*) and intronless *Avr1* produced only a weak chlorotic response (Figure 2b, c), confirming the recognition specificity of the *I* gene.

Confirmation that *Solyc11g011180^{M82}* is the *I* gene by transgenic complementation

To test whether *Solyc11g011180^{M82}* is able to confer *Fol* resistance in tomato, *A. tumefaciens* containing pL2-*Solyc-11g011180^{M82}* was used to transform the *Fol* susceptible tomato cultivar Moneymaker. Three transgenic lines were generated but only one produced seed. Seeds from this line were germinated and 30 of the resulting seedlings were screened for presence of the *Solyc11g011180^{M82}* transgene (Figure S4). These plants segregated 27:3 for presence versus absence of the transgene, consistent with either one ($\chi^2 = 3.60$, $P > 0.05$) or two ($\chi^2 = 0.44$, $P > 0.50$) transgene loci. These same 30 plants were tested for resistance to *Fol* race 1, which carries the *Avr1* gene, and were found to segregate 27:3 for resistance versus susceptibility (Figure 3b, d) in complete concordance with segregation of the transgene (Figure S4). An additional 15 seedlings were screened with *Fol* race 2, which lacks *Avr1*, and the majority were susceptible (Figure 3c, d). A few plants appeared to be resistant, perhaps owing to over-expression of the *I* gene or poor infection. These results confirm that *Solyc-11g011180^{M82}* is the *I* gene.

The I protein recognises the Avr1 homologue from *F. oxysporum* isolate Fo5176 but the *I* gene does not confer resistance to Fo5176 in Arabidopsis

Isolate Fo5176 of *F. oxysporum*, able to infect *Arabidopsis thaliana* (Arabidopsis), has an Avr1 homologue that plays

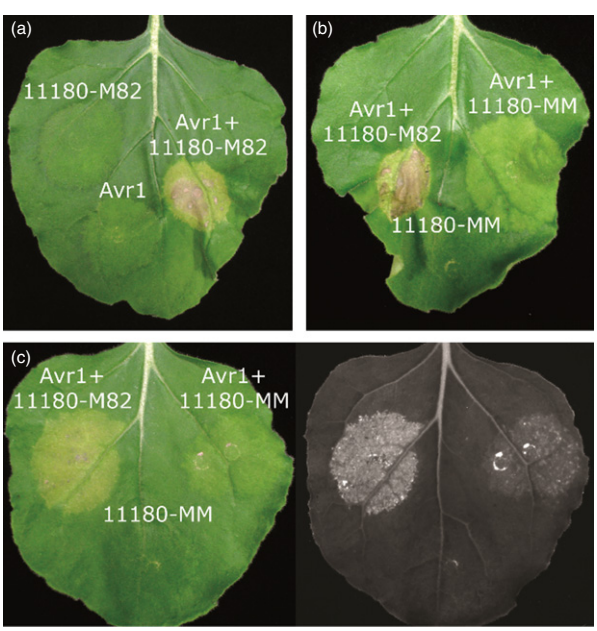


Figure 2. Identification of the *I* gene by *Agrobacterium tumefaciens*-mediated co-expression with intronless *Avr1* in *Nicotiana benthamiana*.
(a) Co-expression of *Avr1* (Avr1) and *Solyc11g011180^{M82}* (11180-M82) caused necrosis, while no response was seen when either gene was expressed on its own.
(b) Co-expression of *Avr1* and *Solyc11g011180^{MM}* (11180-MM) only gave a weak chlorotic response.
(c) The image on the left shows a weak necrotic response following co-expression of *Avr1* and *Solyc11g011180^{M82}*, illustrating the variability of the cell death phenotype induced by this interaction. The image on the right shows autofluorescence associated with cell death in the same leaf visualised under ultraviolet light. Leaves were photographed 8 days after infiltration with *A. tumefaciens*.

an important role in pathogenicity on Arabidopsis and differs from *Fol* Avr1 by only two residues (Thatcher *et al.*, 2012). To test whether the tomato I protein is able to recognise Fo5176 Avr1 (designated $Avr1^{Fo5176}$), an intronless version of *Avr1*, was amplified from Fo5176 genomic DNA using overlap extension PCR (as described for *Fol Avr1*), and cloned into the pL2 binary vector. Co-expression of $Avr1^{Fo5176}$ with *I* was found to induce necrosis in *N. benthamiana* (Figure S5), indicating that the I protein is able to recognise $Avr1^{Fo5176}$.

To test whether the *I* gene enables recognition of Fo5176 in Arabidopsis, *A. tumefaciens* carrying pL2-*I* was used to transform Fo5176-susceptible Arabidopsis ecotype Col-0 (Figure S6). Pathogenicity tests were carried out on kanamycin-resistant T_2 seedlings from three single-locus transformants carrying the 35S:*I* transgene and expressing *I* transcripts in roots (Figure S6a). All three lines showed unaltered susceptibility to Fo5176 (Figure S6b), indicating that the *I* gene does not confer resistance to Fo5176 in Arabidopsis. However, subsequent investigation of the *I* transcripts from these lines revealed incorrect splicing resulting in frame-shifted transcripts encoding a truncated protein (see below).

The *I* gene is alternatively spliced and encodes an atypical LRR-RLP protein

According to the current tomato genome sequence annotation (http://solgenomics.net/organism/Solanum_lycopersicum/genome), *Solyc11g011180* is predicted to contain five introns and encode a 994-amino-acid protein. However, the RNA sequencing (RNAseq) data reported by Gonzalez-Cendales *et al.* (2016) for root transcripts from mock- and *Fol*-inoculated M82 and Tristar plants (both containing the *I* gene) showed that in the majority of transcripts intron 5 is not spliced out but instead encodes a LRR sequence contiguous with flanking LRRs (Figure S7). Splicing was evident in a minority of transcripts, but did not occur at the splice donor and acceptor sites predicted for intron 5. The actual splice donor site for intron 5 was 21 nucleotides upstream of that predicted, and the actual splice acceptor site was eight nucleotides upstream of that predicted. The resulting frame-shifted splice product is predicted to encode a truncated protein of only 614 amino acids. Interestingly, RT-PCR analysis showed the presence of both intron 5-spliced and -unspliced transcripts in the roots of uninfected M82 plants, 35S:*I* Arabidopsis plants and in *N. benthamiana* leaves agroinfiltrated with *35S:I* (Figure S8). In each case, direct sequencing of the spliced products confirmed use of the same splice donor and acceptor sites as determined from the RNAseq data.

Surprisingly, the RNAseq data reported by Gonzalez-Cendales *et al.* (2016) also showed that a second splice acceptor site 4 bp upstream of the predicted splice site is used for intron 2 in approximately half of the *I* gene transcripts. Whilst the functional *I* gene has four introns and encodes a LRR-RLP of 1022 amino acids containing 31 LRRs when spliced correctly (Figure 4), the alternative splice product contains a frameshift generating a transcript encoding a severely truncated protein of only 150 amino acids. Direct sequencing of RT-PCR products derived from roots of 35S:*I* Arabidopsis plants showed that intron 2 of the *I* gene is not spliced correctly in Arabidopsis, thereby providing an explanation for the lack of *I* gene function in this plant.

The predicted I protein shows only limited sequence similarity to other LRR-RLPs involved in plant defence, including the Arabidopsis *RFO2* and tomato *I-7* genes for Fusarium wilt resistance (Shen and Diener, 2013; Gonzalez-Cendales *et al.*, 2016). The 31 LRRs of I are separated into two domains of 27 and 4 LRRs by a loopout or island domain (Figure 4), as found in a number of other LRR-RLPs involved in plant defence (Figures S8). However, the I protein has a much longer loopout domain than other LRR-RLPs (55 residues compared with an average of about 30) and it is rich in serine and threonines (15 out of 55 residues; Figures 4 and S8). Similarly, whilst the I protein has an acidic extra-cytosolic juxtamembrane domain like other LRR-RLPs involved in plant defence, it lacks a basic cytosolic domain (Figures 4 and S8). Instead, the cytosolic domain is relatively hydrophobic and rich in aromatic residues (eight tyrosines, three phenylalanines and two tryptophans; a total of 13 out of 27 residues), leading to a prediction of a possible second transmembrane domain or, if cytosolic, multiple potential YxxØ endocytosis signals (Bonifacino and Traub, 2003; Figures 4 and S8). A second transmembrane domain would leave a loop of only five residues exposed to the cytosol and only three residues exposed to the apoplast at the C-terminus. Neither transmembrane domain carries the GxxxG motif typical of the transmembrane domains of most other LRR-RLPs involved in plant defence (Figure S9; Bi *et al.*, 2016).

To determine the residues involved in recognition specificity, we sequenced the Moneymaker allele of *I*, here designated *i*, and found that it encodes a protein differing by only 13 amino acid substitutions from that encoded by *I* (Figures 4 and S10). In contrast to other LRR-RLPs involved in plant defence, most of these substitutions were found in the C-terminal half of the protein and were localised to a small region comprising LRRs 25–27 (six substitutions) and the adjacent loopout domain (five substitutions). There were only two substitutions in the N-terminal half of the protein i.e. in LRRs 5 and 18. To test whether Avr1 recognition was determined by these two residues, reciprocal domain swaps were constructed between *I* and *i* at LRR19 and *A. tumefaciens*

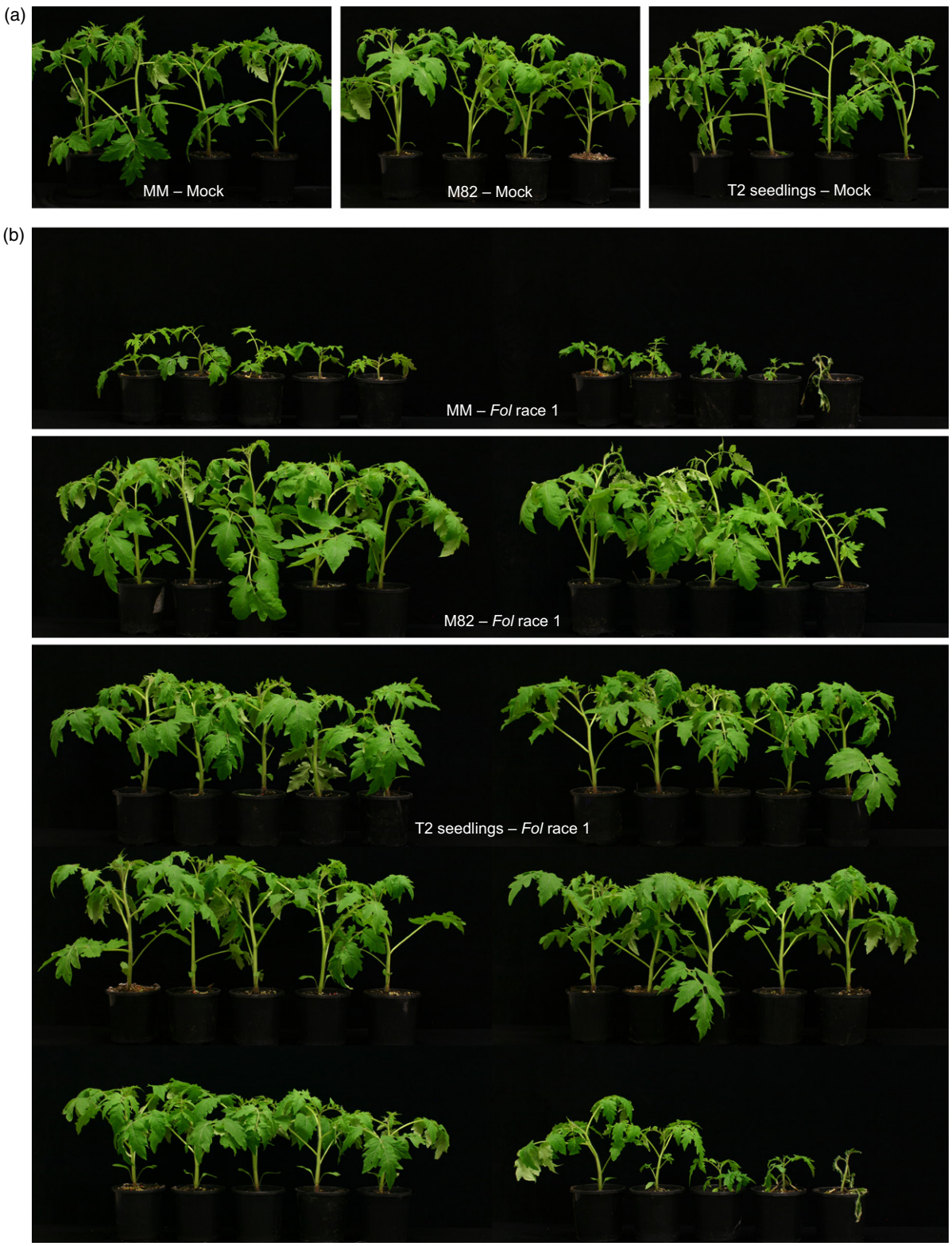
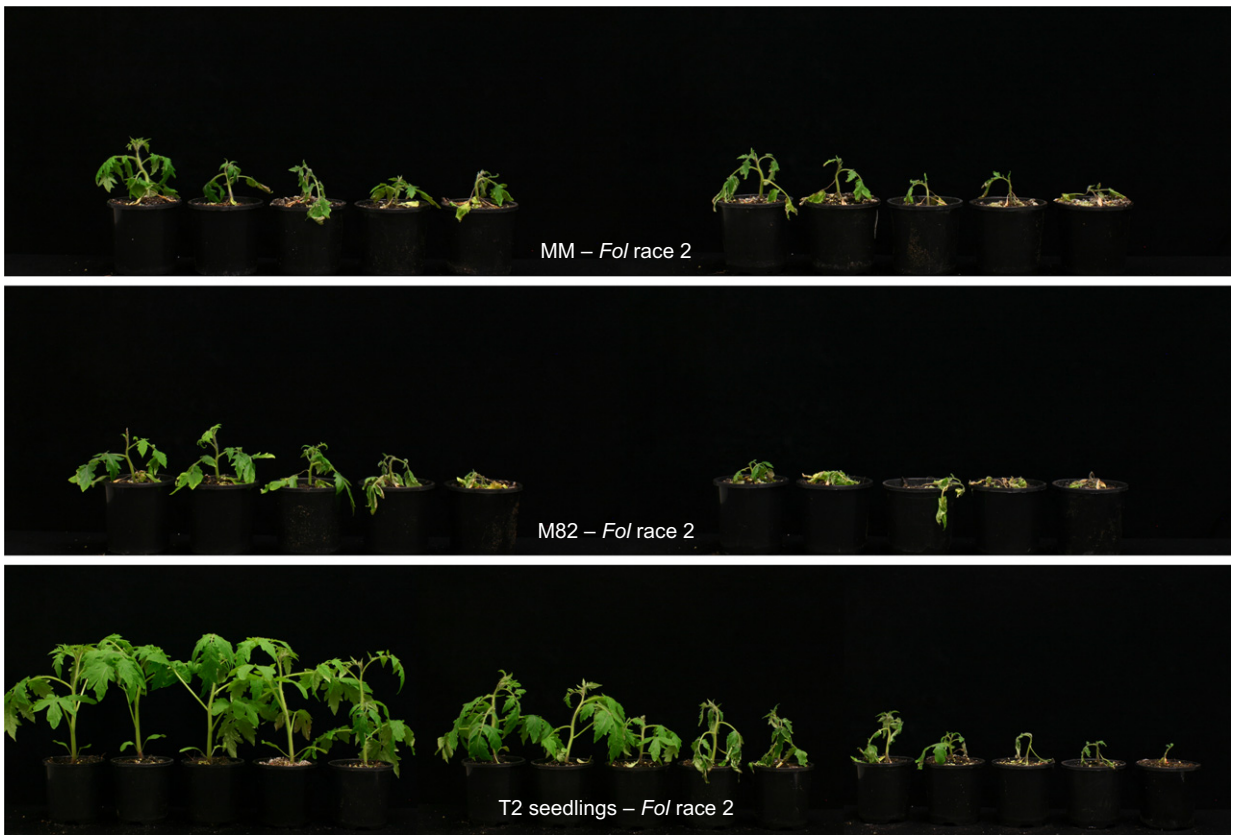


Figure 3. Disease assays on Moneymaker T_2 plants carrying the 35S:*Solyc11g011180*^{M82} transgene.
(a) Mock-inoculated M82 (carrying the *I* gene), MM (Moneymaker; lacking the *I* gene) and T_2 plants (segregating for the 35S:*Solyc11g011180*^{M82} transgene).
(b) M82, MM and T_2 plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) race 1.
(c) M82, MM and T_2 plants inoculated with *Fol* race 2.
(d) Disease scores for plants shown in (a)–(c).

(c)



(d)

Inoculation	Line	Disease scores					
		0	**1**	**3**	**5**	**7**	**9**
Mock	MM	4	0	0	0	0	0
	M82	4	0	0	0	0	0
	T2	4	0	0	0	0	0
Fol race 1	MM	0	0	0	0	9	1
	M82	10	0	0	0	0	0
	T2	27	0	0	0	2	1
Fol race 2	MM	0	0	0	0	2	8
	M82	0	0	0	0	2	8
	T2	4	0	1	0	3	7

0 = no reaction, healthy plant
1 = slight browning of vascular tissue, no wilting of leaves
3 = browning of vascular tissue, slight wilting of leaves
5 = browning of vascular tissue, wilting of leaves, slight chlorosis in leaves
7 = strong browning of vascular tissue, severe wilting, strong chlorosis leaves
9 = leaf necrosis, plant death

Figure 3. Continued.

carrying these domain swaps were co-infiltrated into *N. benthamiana* leaves together with *A. tumefaciens* carrying *Avr1*. Only the domain swap containing the C-terminal portion of I responded to Avr1 (Figure 5), indicating that the polymorphic residues in LRRs 5 and 18 do not provide I specificity.

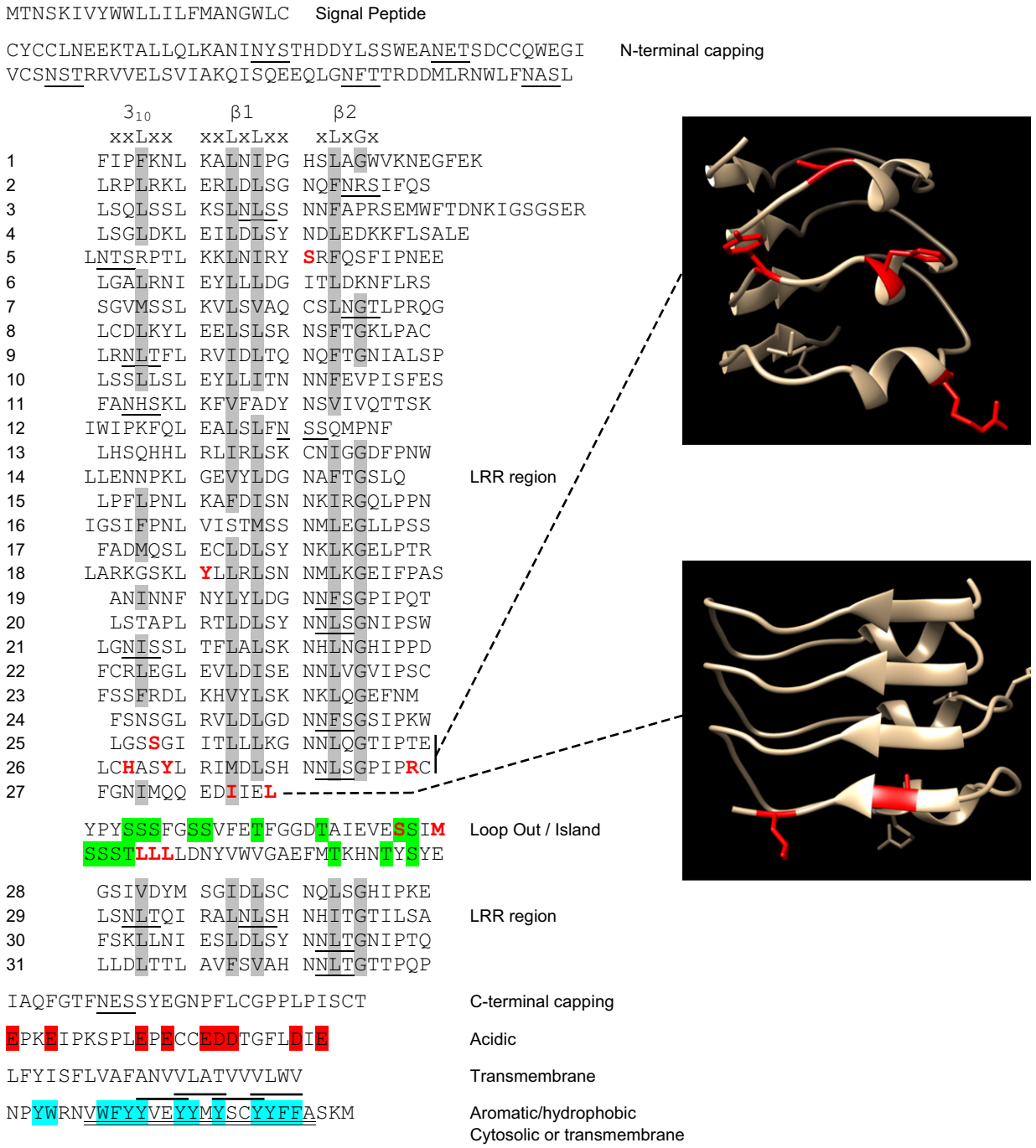


Figure 4. Annotated sequence of the I protein

The predicted sequence of the I protein is shown divided into eight domains: signal peptide, N-terminal leucine-rich repeat (LRR)-capping region, extracellular LRR region (with LRRs numbered 1–31), divided into two blocks by a loopout region or island domain, C-terminal LRR-capping region, acidic domain, transmembrane domain, and hydrophobic cytosolic or second transmembrane domain. The locations of predicted 3_{10} (xxLxx), β1 (xxLxLxx) and β2 (xLxGx) motifs are shown above the LRR domain. The positions of amino acid differences between I and i are shown using red letters. Serine and threonine residues in the loopout domain are highlighted in green, acidic residues in the acidic domain are highlighted in red and aromatic residues in the C-terminal cytosolic or transmembrane domain are highlighted in blue. Putative *N*-glycosylation sites are underlined and putative endocytosis motifs are overlined. A possible second transmembrane domain is double underlined. A structural model of LRRs 24–27 was generated with 99% confidence from PDB accessions 3RGX (BRI1; She *et al.*, 2011), 4LSX (BRI1 interacting with SERK1; Santiago *et al.*, 2013), 4J0M (BRL1; She *et al.*, 2013), 4Z62 (PSKR1; Wang *et al.*, 2015), 4MNA (FLS2; Sun *et al.*, 2013b) and 4MN8 (FLS2 interacting with flg22 and BAK1; Sun *et al.*, 2013b) using Phyre2 (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index; Kelley *et al.*, 2015). The ribbon representations of the model showing polymorphic residues in LRRs 24 and 25 (upper panel) and LRR 27 (lower panel) were generated using UCSF Chimera (available from https://www.cgl.ucsf.edu/chimera/; Pettersen *et al.*, 2004). Note that the model has been rotated in the lower panel compared with the upper panel.

Virus-induced gene silencing of *SOBIR1* and *SERK3*/*BAK1* reduces the necrotic response induced by co-expression of *I* and *Avr1* in *N. benthamiana* leaves

Given these differences in the C-terminus of the I protein compared with other LRR-RLPs involved in plant defence, it was possible that the I protein might also activate plant defences differently. We therefore investigated whether the I protein requires the LRR-RLKs SOBIR1 and SERK3/BAK1, which are required for normal function of many, if not all, LRR-RLPs involved in plant defence (Liebrand *et al.*, 2013; Albert *et al.*, 2015; Ma and Borhan, 2015; Postma *et al.*, 2016). We used VIGS to silence SOBIR1 and SERK3/BAK1 in *N. benthamiana* seedlings, then tested these plants for induction of necrosis following co-expression of I and Avr1. In this experiment, tomato Cf-9, which requires SOBIR1 and SERK3/BAK1 for normal function (Liebrand *et al.*, 2013; Postma *et al.*, 2016), and barley MLA10, which does not, were used as controls. Both I/Avr1 and Cf-9/Avr9 triggered necrosis in control GFP-silenced plants but only chlorosis in SOBIR1- and SERK3/BAK1-silenced plants, whereas the autoactive coiled-coil domain of MLA10 (Bai *et al.*, 2012) still triggered necrosis in SOBIR1- and SERK3/BAK1-silenced plants (Figure 6). These results suggest that the I protein depends on SOBIR1 and SERK3/BAK1 for normal function.

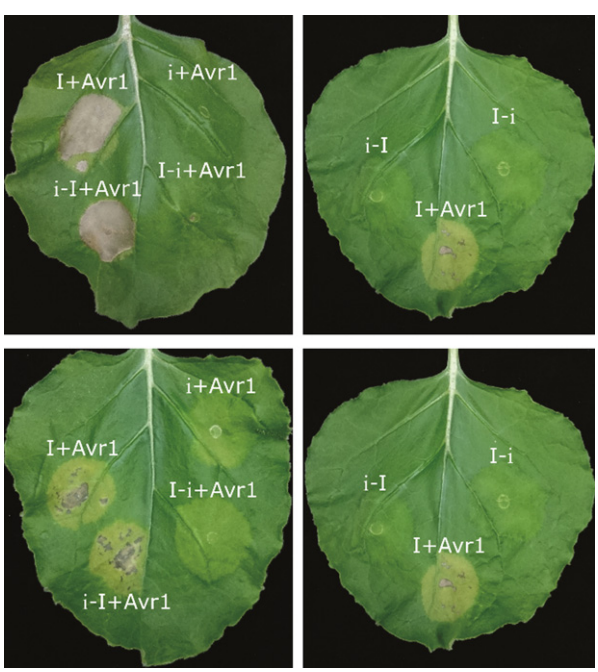


Figure 5. Testing of domain swaps to determine whether polymorphisms in the N- or C-terminal halves of the I protein control Avr1 recognition specificity.
Avr1 (Avr1) was co-expressed in *Nicotiana benthamiana* with either the *I* gene (I), or its Moneymaker allele (i), or a domain swap encoding the N-terminus of I and the C-terminus of i (I–i), or the reciprocal swap (i–I). The domain swaps were also expressed individually as controls for possible autoactivation. Leaves were photographed 10 days after infiltration with *Agrobacterium tumefaciens*.

Mutations of putative SERK3/BAK1 interaction sites in the I protein abolish Avr1-dependent necrosis in *N. benthamiana*

Structural analysis of the brassinosteroid-dependent interaction between BRI1 and SERK3/BAK1 has shown that SERK3/BAK1 interacts with BRI1 via the last LRR and the C-terminal LRR-capping domain and via brassinosteroid bound to the loopout domain (Sun *et al.*, 2013a). A similar interaction has been shown for the brassinosteroid-dependent interaction between BRI1 and SERK1 (SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1) and the phytosulphokine-dependent interaction between PSKR1 and SERK1 (Santiago *et al.*, 2013; Wang *et al.*, 2015). Given the similar structure of loopout followed by four LRRs, we postulated that the LRR-RLPs also interact with SERK3/BAK1 and perhaps SERK1 in a similar manner.

Several BRI1 and PSKR1 residues important for interaction with SERK3/BAK1 and SERK1 are conserved in LRR-RLPs involved in plant defence responses (Figure 7a). These include T726 in the last LRR of BRI1, and Q747 and T750 in the C-terminal LRR-capping domain of BRI1 (Santiago *et al.*, 2013; Sun *et al.*, 2013a), and similarly Q616 (equivalent to BRI1 Q747) and T619 (equivalent to BRI1 T750) in the C-terminal LRR-capping domain of PSKR1 (Wang *et al.*, 2015). Sequence and positional conservation of these residues in the LRR-RLPs (Figure 7a, b) supports the hypothesis that the last LRR and the C-terminal LRR-capping domain of these LRR-RLPs are involved in the interaction with SERK3/BAK1 and SERK1. To test this hypothesis, we mutated the corresponding residues in the C-terminal capping domain of the I protein (Q922 and T925) to tyrosine so as to disrupt interaction with SERK3/BAK1 as described for BRI1 T750 and PSKR1 T619 (Sun *et al.*, 2013a; Wang *et al.*, 2015), which correspond to T925 of I. Tyrosine mutations of Q922 and T925 were found to abolish necrosis following co-expression of I and Avr1 in *N. benthamiana* (Figure 7c) without affecting protein abundance (Figure 7d).

DISCUSSION

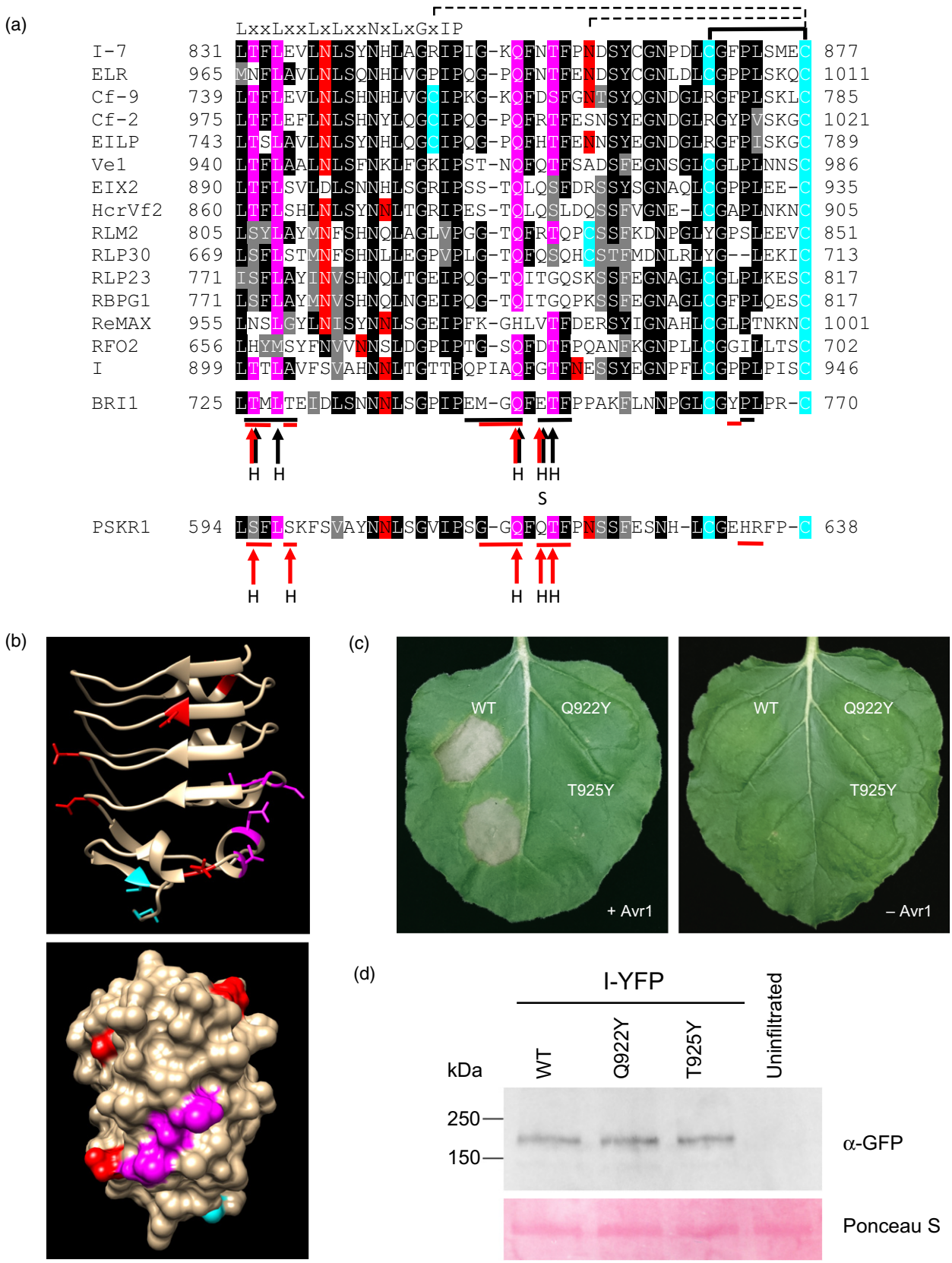
We have isolated the tomato *I* gene for *Fol* race 1 resistance and identified a number of features that distinguish it from other LRR-RLP genes. Unlike most other LRR-RLPs involved in plant defence, the *I* gene is not a member of a gene cluster and contains introns in its coding sequence. We found that splicing of intron 5 in tomato, Arabidopsis and *N. benthamiana* leads to the production of transcripts encoding a truncated I protein (Figure S8). We also found that intron 2 is alternatively spliced in tomato and incorrectly spliced in Arabidopsis with the alternative/incorrect splice product encoding a severely truncated protein. Given this finding, artificially spliced *I* gene or correctly spliced cDNA constructs will be required to further

Figure 6. Virus-induced gene silencing (VIGS) analysis of the requirement for SERK3/BAK1 or SOBIR1 in *I*/*Avr1*-mediated necrosis in *Nicotiana benthamiana*.
VIGS was established in *N. benthamiana* seedlings by *Agrobacterium tumefaciens*-mediated expression of tobacco rattle virus (TRV) constructs carrying *GFP*, *N. benthamiana SERK3/BAK1* or *N. benthamiana SOBIR1* sequences, as indicated below each leaf. Three weeks after infiltration, *I* and *Avr1* (I+Avr1) were co-expressed in the silenced leaves. Tomato *Cf-9* and *Cladosporium fulvum Avr9* (Cf-9+Avr9) were co-expressed as a positive control and the autoactive coiled-coil domain from the barley MLA10 resistance protein (MLA10-CC) was expressed as a negative control. The relative position of each infiltration is indicated on the leftmost leaf. Leaves were photographed 9 days after infiltration with *A. tumefaciens*.

investigate *I* gene function in Arabidopsis. Splicing variation may also have contributed to the weaker seedling death phenotype observed in F_1 tomato seedlings carrying both the *I* gene and an *Avr1* transgene (Figure 1) compared with F_1 tomato seedlings carrying both *Cf-9* and *Avr9* (Hammond-Kosack *et al.*, 1994).

In contrast to most other LRR-RLPs involved in plant defence, Avr1 specificity was found to be determined in the C-terminal half of the I protein by a small region comprising LRRs 25–27 (six amino acid substitutions) and the adjacent loopout domain (five substitutions). In most other LRR-RLPs this region is thought to be involved in interaction with signalling partners (e.g. Thomas *et al.*, 1997). Domain swaps between Ve1, which confers resistance to *Verticillium* via recognition of Ave1, and Ve2, which is unable to respond to Ave1, show that Ave1 specificity is likewise determined in the C-terminal half of the Ve1 protein by a region that contains the loopout domain

Figure 7. Functional requirements for amino acid residues in the I protein that are conserved with known SERK3/BAK1- and SERK1-interacting residues in BRI1 and PSKR1.
(a) Sequences in the last leucine-rich repeat (LRR) and C-terminal LRR-capping domain were aligned using the EMBL-EBI MAFFT server at http://www.ebi.ac.uk/Tools/msa/mafft/ with minor manual adjustment, and the alignment was shaded using the ExPASy BoxShade server at http://www.ch.embnet.org/software/BOX_form.html. Amino acid identities are highlighted in black except for putative SERK3/BAK1-interacting residues (conserved with SERK3/BAK1-interacting residues in BRI1) which are highlighted in pink. Amino acid similarities are highlighted in grey. The consensus sequence of the last LRR in each sequence is shown above the alignment. Conserved cysteines likely to be involved (solid connecting lines) or potentially involved (dashed connecting lines) in disulphide bonding, based on structural studies of other plant extracellular LRR proteins (Di Matteo *et al.*, 2003; Hothorn *et al.*, 2011), are highlighted in blue. Putative *N*-glycosylated asparagines are highlighted in red. LRR-RLP sequences were derived from GenBank accessions AKR80573 (I-7), AAA65235 (Cf-9), AAC15779 (Cf-2), BAA88636 (EILP), ACR33106 (Ve1), AAR28378 (Eix2), CAC40826 (HcrVf2), AJG42078 (RLM2), NP_187187 (RLP30), AEC08715 (RLP23), NP_189138 (RLP42 = RBPG1), NP_001154318 (RLP1 = ReMAX) and NP_173168 (RLP3 = RFO2), and from Du *et al.* (2015) for ELR (elicitin response). Residues in the interface between BRI1 and either SERK3/BAK1 (underlined in black) or SERK1 (underlined in red) and in the interface between PSKR1 and SERK1 (underlined in red) were determined from the corresponding Protein Data Base (PDB) accessions 4M7E (Sun *et al.*, 2013a), 4LSX (Santiago *et al.*, 2013) and 4Z64 (Wang *et al.*, 2015) using PDBePISA (http://www.ebi.ac.uk/pdbe/pisa/; Krissinel and Henrick, 2007). Residues forming hydrogen bonds (H) or salt bridges (S) are arrowed in black for SERK3/BAK1 and red for SERK1.
(b) Structural model of the C-terminal LRR domain (four LRRs) and LRR-capping domain of the I protein generated with 99% confidence from PDB accessions 3RGX (BRI1; She *et al.*, 2011), 4LSX (BRI1 interacting with SERK1; Santiago *et al.*, 2013), 4J0M (BRL1; She *et al.*, 2013), 1OGQ (PGIP; Di Matteo *et al.*, 2003), 4Z62 (PSKR1; Wang *et al.*, 2015) and 4Z64 (PSKR1 interacting with SERK1; Wang *et al.*, 2015) using Phyre2 (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index; Kelley *et al.*, 2015). The ribbon representation of the model (top) generated using UCSF Chimera (available from https://www.cgl.ucsf.edu/chimera/; Pettersen *et al.*, 2004) shows the superhelical turns of the C-terminal LRR domain continuing into the LRR-capping domain. Residues have been coloured as described in (a). The rotated space-filling representation of the model (bottom) shows a surface patch (pink) containing the putative SERK3/BAK1- and SERK1-interacting residues.
(c) The wild-type (WT) *I* gene or its mutant derivatives Q923Y or T925Y were expressed in *Nicotiana benthamiana* together with *Avr1* (+ Avr1) or alone (– Avr1). Leaves were photographed 10 days after infiltration with *Agrobacterium tumefaciens*.
(d) Gel blot of protein extracted from leaves of *N. benthamiana* expressing YFP-tagged WT I protein or its mutant derivatives Q923Y or T925Y, probed with anti-GFP antibodies (top) or stained with Ponceau S (bottom).



(three amino acid substitutions) as well as the two preceding LRRs (ten substitutions) and three following LRRs (five substitutions; Fradin *et al.*, 2014). It is possible that Ve1 and Ve2 both recognise Ave1 but differ in the ability of the C-terminal region to initiate a signal, as proposed by Fradin *et al.* (2014) and Zhang *et al.* (2014). Likewise, it is possible that I and i both recognise Avr1 but only I is able to activate a signal in response to Avr1 recognition. However, it is also possible that I or Ve1, or both, recognise their corresponding Avr proteins through their loopout domains and adjacent LRRs. The larger size and unique composition of the loopout domain of the I protein compared with other LRR-RLPs lend circumstantial support to a possible role in Avr1 recognition. Moreover, the Arabidopsis brassinosteroid BRI1 (BRASSINOSTEROID INSENSITIVE 1) and phytosulphokine PSKR1 (PHYTOSULPHOKINE RECEPTOR 1) receptors provide structurally-characterised precedents for ligand binding to the loopout domain and adjacent LRRs, albeit in LRR-RLKs rather than LRR-RLPs (Hothorn *et al.*, 2011; She *et al.*, 2011; Wang *et al.*, 2015).

As is the case for BRI1 and PSKR1, we infer from our VIGS results that I also interacts with SERK3/BAK1. We did not investigate whether I requires SERK1 but, given that other LRR-RLPs conferring fungal resistance such as Ve1 and Cf-4 also require SERK1 (Fradin *et al.*, 2011), it seems likely. BRI1 and PSKR1 also provide structurally characterised precedents for SERK3/BAK1 and SERK1 binding (Santiago *et al.*, 2013; Sun *et al.*, 2013a; Wang *et al.*, 2015). The SERK3/BAK1- and SERK1-interacting residues of BRI1 and PSKR1 are conserved in the LRR-RLPs involved in plant defence including the I protein (Figure 7a). They lie on one side of the superhelix formed by two loops comprising the last LRR and the beginning of the C-terminal LRR-capping domain, which extends the superhelix formed by the LRR domain (Figure 7b). Tyrosine mutations of two of the residues conserved in the I protein (Q922 and T925) abolished I protein function, consistent with disruption of SERK3/BAK1 and SERK1 interaction (Figure 7c), further supporting the conclusion that I interacts with SERK3/BAK1 and most likely SERK1.

We also infer from our VIGS results that I interacts with SOBIR1, but we did not investigate how it does so. Bi *et al.* (2016) hypothesised that SOBIR1 interacts with LRR-RLPs via single or tandem GxxxG motifs present in the transmembrane domains of most LRR-RLPs, but I lacks any such GxxxG motif and Zhang *et al.* (2014) have shown that the GxxxG motif present in Ve1 is not required for function. Moreover, the I protein may have a second transmembrane domain in place of a basic domain, introducing a further complication. Bi *et al.* (2016) also showed that the LRR domain of SOBIR1 is required for Cf-4 function. This could suggest that the LRR domain of SOBIR1 interacts with LRR-RLPs in a similar manner to SERK3/BAK1 and SERK1, but via a different face of the extended superhelix formed by the C-terminal LRRs and LRR-flanking domain of the LRR-RLPs (Figure 7b); a possibility that also requires further investigation. This idea is supported by the finding that SOBIR1 also interacts with the plant developmental LRR-RLPs TMM and CLV2 (Liebrand *et al.*, 2013), which lack the conserved residues required for interaction with SERK3/BAK1 or SERK1, and the observation that SOBIR1 lacks the conserved residues required for interaction with the SERK3/BAK1- or SERK1-interacting regions of BRI1, PSKR1 and, by inference, I (Figure S11).

SOBIR1 associates constitutively with other LRR-RLPs such as RLP23 and Cf-4 (Bi *et al.*, 2014, 2016; Albert *et al.*, 2015) and probably also does so with I. SERK3/BAK1 is recruited to RLP23 and Cf-4 after ligand binding (Albert *et al.*, 2015; Postma *et al.*, 2016) and possibly I following Avr1 recognition. Our data suggest that SERK3/BAK1, and most likely SERK1, bind directly to the C-terminal LRRs and LRR-capping domain of the I protein and probably to other LRR-RLPs involved in plant defence, but do not exclude additional interactions with other SERKs such as SERK4/BKK1 (BAK1-LIKE 1). SERK4/BKK1 makes a major contribution to RLP23 function in Arabidopsis in addition to SERK3/BAK1 (Albert *et al.*, 2015) and is suggested to contribute to Ve1 function in Arabidopsis in addition to SERK3/BAK1 and SERK1 (Fradin *et al.*, 2011). Thus, there appears to be some functional redundancy among the SERKs with some perhaps playing more important roles than others depending on the LRR-RLK or LRR-RLP involved. Redundancy of function might therefore require silencing of multiple SERKs to reveal a collective role for SERK3/BAK1 and other members of the SERK family. Postma *et al.* (2016) suggest that the SERK3/BAK1 VIGS construct, developed by Heese *et al.* (2007) and used in many studies including our own, also knocks down SERK1 expression. Therefore, we cannot conclude that SERK3/BAK1 alone is required for I function. Instead, we hypothesise that there is a collective requirement that includes SERK3/BAK1 and possibly SERK1.

EXPERIMENTAL PROCEDURES

Generation of transgenic tomato lines expressing *Avr1*

Fol Avr1 was PCR amplified from cDNA of race 1-infected tomato roots using primers FP2576 and FP2796 (Table S2). The PCR product was digested with *Xba*I and *Bam*HI and ligated into *Xba*I–*Bam*HI-digested pSLDB3104 (Tameling *et al.*, 2010) to obtain SLDB3104::*Avr1*. This construct was sequence-verified and electroporated into *A. tumefaciens* strain EHA105. The *Fol*-susceptible tomato cultivar C32 was transformed by co-cultivation with EHA105 containing pSLDB3104::*Avr1*, essentially as described by Cortina and Culianez-Macia (2004).

Transgene expression was checked by RT-PCR using primers FP2798 and FP4404 to detect *Avr1* and control primers FP2147 and FP2148 to detect *α-tubulin* (Table S2). Five independent, single-locus transgenic lines that expressed *Avr1* were crossed to cultivar GCR161, which contains the *I* gene. Only plants derived

from the cross between the highest-expressing *Avr1* line and GCR161 showed a phenotype and only progeny from this cross were used for subsequent experiments. Plants were grown in a greenhouse under a 16-h photoperiod at 25°C unless indicated otherwise.

Generation of other binary vector constructs

The *Solyc11g011180* coding region was amplified by PCR from M82 and Moneymaker genomic DNA using primers LIC-11180F and LIC-11180R (Table S2) and cloned into the pL2 vector containing the CaMV 35S promoter by ligation-independent cloning as described by Gonzalez-Cendales *et al.* (2016). To generate domain swaps between the *Solyc11g011180* genes from M82 and Moneymaker, the corresponding pL2 constructs were digested with *Nde*I and *Bsp*EI and the 2010 bp *Solyc-11g011180*^{MM} fragment was ligated into the pL2-*Solyc-11g011180*^{M82} vector, and vice versa.

A pBluescipt construct containing the *I* gene (pBS-*I*) was generated by inserting the *Sac*I fragment from pL2-*I* into the *Sac*I site of pBluescript. Tyrosine mutants (Q922Y and T925Y) of *I* were generated using the Phusion Site-Directed Mutagenesis Kit (Thermo Fisher Scientific, https://www.thermofisher.com/) and 5′ phosphorylated primers (Table S2). Mutations were confirmed by sequencing before transferring the *Sac*I fragment back into pL2-*I*. To incorporate a *YFP* gene into pL2-*I* and its mutant variants, site-directed mutagenesis with 5′ phosphorylated primers pL-I-AvrII-F and pL-I-AgeI-R (Table S2) was used to remove the stop codon and add *Age*I and *Avr*II restriction sites to the 3′ end of the *I* gene. The *YFP* gene was PCR amplified from the vector pB7YWG2 (Karimi *et al.*, 2005) using primers AgeI-EYFP-F and AvrII-EFYP-R (Table S2) then digested with *Age*I and *Avr*II and ligated into *Age*I/*Avr*II-digested pL2-*I* and its mutant variants.

The *Avr1* genes from *Fol* race 1 and Fo5176 were amplified from genomic DNA using primers LIC-Avr1F and LIC-Avr1R (Table S2) and intronless derivatives were generated by overlap extension PCR (Heckman and Pease, 2007), using primers PCR-E1F and PCR-E1R (Table S2) to amplify exon 1 and PCR-E2F and PCR-E2R (Table S2) to amplify exon 2. Final fusion products were amplified using primers PCR-E1F and PCR-E2R and ligated into pBluescript following digestion of both PCR product and vector with *Pst*I and *Sal*I. These intronless coding sequences were then amplified using primers LIC-Avr1F and LIC-Avr1R (Table S2) and inserted into pL2 via ligation-independent cloning.

All constructs were sequence verified and electroporated into *A. tumefaciens* strain GV3101 (pMP90) containing pSOUP.

Agrobacterium-mediated gene expression in *N. benthamiana*

Agrobacterium tumefaciens cultures containing the pL2 constructs were prepared to an OD_{600} of 1.0 in 10 mM MES (pH 5.6) buffer with 10 mM $MgCl_2$ and 200 μM acetosyringone, and then infiltrated into *N. benthamiana* leaves. For co-infiltrations, cultures were mixed together in equal volumes just before infiltration. Infiltrated plants were kept in a 25°C growth room with a 16-h photoperiod for 8–10 days.

Testing the *I* gene in tomato by transgenic complementation

The *Fol*-susceptible cultivar Moneymaker was transformed via co-cultivation with *A. tumefaciens* containing pL2-*I* (i.e. pL2-*Solyc-11g011180*^{M82}) essentially as described by McCormick (1991). Transgenic tomato plants were selected using 100 mg L^{-1} kanamycin and 200 mg L^{-1} timentin. Pathogenicity tests were carried out on T_2 progeny using *Fol* race 1 (Fol004, carrying *Avr1*) and race 2 (Fol007, lacking *Avr1*). Tomato seedlings and *Fol* cultures were grown, and *Fol* conidia prepared, as described in Gonzalez-Cendales *et al.* (2016). Roots of 2-week-old tomato seedlings were dipped for 3 min in suspensions of 5×10^6 conidia ml^{-1} and, after repotting, the inoculated seedlings were grown in a controlled environment growth chamber at 24°C, 75% relative humidity and 16-h photoperiod (100 μE m^{-2} sec^{-1}) for 21 days. Disease symptoms were then evaluated using the scoring criteria described by Jongedijk *et al.* (1995).

Virus-induced gene silencing of SOBIR1 and SERK3/BAK1 in *N. benthamiana*

A. tumefaciens GV3101 (pMP90) cultures carrying the tobacco rattle virus (TRV) constructs were prepared for infiltration of *N. benthamiana* as described above. Culture containing TRV:RNA1 (Dong *et al.*, 2007) was mixed in equal volumes with TRV:RNA2 cultures expressing fragments of genes targeted for silencing. TRV:RNA2 vectors included: TRV:*GFP* (negative control; Chaparro-Garcia *et al.*, 2011); TRV:*NbSERK3/BAK1* (Heese *et al.*, 2007) or TRV:*NbSOBIR1* (Liebrand *et al.*, 2013). Two- to three-week-old *N. benthamiana* plants were infiltrated with *A. tumefaciens* cultures carrying the TRV constructs and silencing efficiency was assessed by the phenotypic response to SERK3/BAK1 silencing (i.e. leaf wrinkling). Infiltrated plants were kept in a 21°C growth room with a 16-h day length and after 3 weeks were tested for necrosis induction following co-expression of *I* and *Avr1*.

Immunoblot analysis of proteins expressed in *N. benthamiana*

Two days after infiltration with *A. tumefaciens, N. benthamiana* leaf tissue was frozen in liquid nitrogen and ground in 2× Laemmli buffer with 0.2 M DTT to extract proteins. Samples were boiled for 5 min and then centrifuged at 16 000 × *g* to remove leaf debris. Proteins were separated by SDS-PAGE and transferred by electroblotting onto nitrocellulose membranes. Protein blots were probed with anti-GFP (7.1 and 13.1; Roche, http://www.roche.com/) then with sheep anti-mouse immunoglobulin G antibody conjugated to horseradish peroxidase (Amersham Biosciences, http://www.gelifesciences.com). Immunoblots were visualised with SuperSignal West Pico as described by the manufacturer (Pierce, http://www.thermofisher.com).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Elevated temperature does not suppress the stunted F_1 phenotype of tomato plants containing both the *I* gene and the 35S:*Avr1* transgene.

Figure S2. PCR screening of genomic DNAs extracted from 90 C32 (35S:*Avr1*) × GCR161 (*I*) F_2 seedlings for presence of the *Avr1*

transgene and the *Solanum pimpinellifolium* introgression carrying the *I* gene.

Figure S3. Sequence chromatograms for a segment of the *Solyc11g011180* gene amplified from genomic DNAs extracted from C32 (lacking the *I* gene), C32 (35S:*Avr1*) × GCR161 (*I*) F_2 plant 56 (F2#56) and GCR161 (carrying the *I* gene).

Figure S4. PCR screening of genomic DNAs extracted from T_2 seedlings for the presence of the 35S:*Solyc11g011180*^{M82} (*I*) transgene.

Figure S5. The *Avr1* homologue from the Arabidopsis-infecting *Fusarium oxysporum* isolate Fo5176 triggers *I*-mediated cell death in *Nicotiana benthamiana*.

Figure S6. RT-PCR and disease assays on Arabidopsis T_2 plants carrying the 35S:*I* transgene.

Figure S7. Splicing of introns 2 and 5 of *I* gene transcripts in tomato and Arabidopsis.

Figure S8. RT-PCR analysis of intron 5-splicing in *I* gene transcripts.

Figure S9. Alignment of N- and C-terminal regions conserved between I and other tomato, potato and tobacco LRR-RLPs involved in disease resistance.

Figure S10. Sequence alignment of the I and i proteins.

Figure S11. Alignments of BRI1- and PSKR1-interacting regions of SERK3/BAK1 and SERK1 with the corresponding region of SOBIR1 showing a general absence of conserved BRI1- and PSKR1-interacting residues in SOBIR1.

Table S1. Chromosome 11 single nucleotide polymorphisms defining the *Solanum pimpinellifolium* introgression carrying the *I* gene for resistance to *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) race 1 in tomato cultivars Heinz 1706 and M82 relative to the *Fol* susceptible cultivar Moneymaker.

Table S2. PCR primers used in this study.

Table S3. Phenotyping and PCR-genotyping results for 90 C32 (35S:*Avr1*) × GCR161 (*I*) F_2 plants.

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