Supporting Information

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SI Materials and Methods

Plant Growth Conditions. *Nicotiana benthamiana*, *N. benthamiana: Cf-4* (1), and *Nicotiana tabacum* were grown under 16 h of light at 25 °C and 8 h of darkness at 21 °C in climate chambers with a relative humidity of ∼75%. *Arabidopsis*, tomato (*Solanum lycopersicum*) cultivar (cv.) Money Maker (MM) and cv. Motelle, as well as *Cf.2.2*- (2) and *Cf-4* (*Hcr9-4D*)–transgenic MM plants (3) were grown in the greenhouse under 16 h of light at 21 °C and 8 h of darkness at 19 °C. The relative humidity in the greenhouse was ∼75%.

Plant Transformations. Plasmid pBIN-KS-35S::Cf-4–eGFP (Sol 2701) (4) was used for transformation of tomato MM–Cf-0, which does not carry a functional *Cf-4* gene. Transformations were performed as described (5). By using a quantitative RT-PCR (qRT-PCR)–based method (6), plants carrying only single- or two-copy transgenes were selected. To test for the presence of functional Cf-4, leaflets of transgenic tomato plants were infiltrated with apoplastic fluid from leaflets of a MM–Cf-0 plant colonized by an Avr4-secreting strain of *Cladosporium fulvum* by using a syringe without needle. *Arabidopsis* mutant Columbia 0 (Col-0) *suppressor of bir1-1* (*sobir1*) (7) was transformed with p35S::*Ve1* as described (8). Four homozygous single insert lines expressing *Ve1* were selected based on segregation and qRT-PCR analysis and used in *Verticillium dahliae* disease assays.

Binary Vectors for *Agrobacterium tumefaciens*-Mediated Transient Transformation. Sequences of primers and corresponding targets can be found in Table S4. Construction of plasmids containing Cf-2.2, -4, -4E, -9, Peru2, and Ve1, C-terminally fused to either eGFP or the Myc epitope-tag, has been described (4, 9). *S. lycopersicum* Flagellin Sensing 2 (*Sl*FLS2)–GFP was expressed from pCAMBIA2300–FLS2p::*Sl*FLS2–GFP (10). For novel constructs, coding regions were amplified from cDNA. PCR fragment *S. lycopersicum* Somatic Embryogenesis Receptor Kinase 3a (*SlSERK3a*)/*BAK1* was cloned in pDONR201 by using Gateway BP Clonase II (Invitrogen). Fragments *SlSERK1*, *SlEIX2*, *SlSOBIR1*, *SlSOBIR1-like*, and *Arabidopsis thaliana* (*At*)*SOBIR1* were cloned in pENTR/D-Topo (Invitrogen). Plasmid pENTR/D-Topo containing *A. thaliana* Clavata1 (*AtCLV1*) has been described (11). To generate mutations in the SOBIR1 kinase domain, pENTR/D-Topo vectors containing *SlSOBIR1*, *SlSOBIR1-like*, and *AtSOBIR1* coding regions were PCR-amplified with primers introducing a mismatch nucleotide to generate a D to N codon change. After amplification, the methylated parental plasmid was digested by using DpnI. All pDONR201 and pENTR/D-Topo clones were sequenced, and subsequently fragments were transferred to the binary transformation vector pBIN-KS-35S::GWY–eGFP (Sol 2095; for C-terminally tagging with eGFP) or pGWB20 (12) (for C-terminally tagging with the Myc epitope), by using Gateway LR Clonase II (Invitrogen). This process resulted in plasmids pBIN-KS-35S::At-CLV1–eGFP (Sol 2824), pBIN-KS-35S::Sl-CLV2–eGFP (Sol 2782), pBIN-KS-35S::Sl-EIX2–eGFP (Sol 2863), pBIN-KS-35S::Sl-Suppressor of Non-expressor of pathogenesis-related genes 1-1 (Npr1-1), Constitutive 2 (SNC2)–eGFP (Sol 3109), pBIN-KS-35S::Sl-Too Many Mouths (TMM)–eGFP (Sol 3110), pBIN-KS-35S::Sl-SOBIR1–eGFP (Sol 2774), pGWB20-Sl-SOBIR1–Myc (Sol 2754), pGWB20-Sl-SOBIR1-like–Myc (Sol 2752), pGWB20-At-SOBIR1–Myc (Sol 2849), pGWB20-Sl-SOBIR1D473N–Myc (Sol 2878), pGWB20-Sl-SOBIR1-like D486N–Myc (Sol 2879), and pGWB20-At-SOBIR1D489N-Myc (Sol 2880). Avr4 was expressed from pMOG800–Avr4 and Avr9 from pMOG800–Avr9 (13). GFP–HA was expressed from pBIN61–GFP–HA (14). All binary plasmids were transformed to *A. tumefaciens* strain C58C1, carrying helper plasmid pCH32. Infiltration of *Agrobacterium* into plant leaves (agroinfiltration) was performed as described at OD_{600} = 1, unless indicated otherwise (13).

Protein Identification by Immunopurification, Followed by Tryptic Digest and Mass Spectrometry. Immunopurifications from tomato and *N. benthamiana* were essentially performed following the described protocol with minor modifications (4). For immunopurifications from the transgenic tomato lines expressing Cf-4–eGFP, young, not fully expanded leaves of 6-wk-old plants were taken. Proteins were extracted by using extraction buffer [EB; 150 mM NaCl, 1% IGEPAL CA-630 (Nonidet P-40), 50 mM Tris, pH 8, plus one tablet of protease inhibitor mixture (Roche) per 50 mL of EB]. To 1 g of leaf material (fresh weight), 2 mL of EB was added. Subsequently, a total protein extract of 10 mL was subjected to immunopurification by adding 60 μL (50% slurry) of GFP_TrapA beads (Chromotek) and incubation while shaking for 1 h (4). Beads were then washed five times with EB. Tryptic on-bead digestion was followed by mass spectrometry by using either the Synapt MS (Waters) or the Orbitrap XL (Thermo Scientific) (4).

Coimmunopurifications and Immunoblotting. Coimmunopurifications were performed as described (4). Two milliliters of protein extract was incubated for 1 h with 15 μL of GFP_TrapA beads (50% slurry), and beads were washed five times with EB. Protein blots were developed by using either α-GFP–HRP (130-091-833; MACS Antibodies) or α-cMyc (cMyc 9E10, sc-40; Santa Cruz) with α-mouse–HRP (Amersham) as a secondary antibody.

Confocal Microscopy. Confocal microscopy was performed on agroinfiltrated *N. benthamiana* leaves as described (4).

Generation of Virus-Induced Gene Silencing Constructs. Fragments to be used for virus-induced gene silencing (VIGS) were PCR amplified from *N. benthamiana* or tomato cDNA (Table S4). All fragments were cloned into pCR4-TOPO (Invitrogen) and sequenced. The tomato fragments were then excised from pCR4-TOPO by using restriction enzymes XbaI and BamHI and cloned into pTRV2:RNA2 (pYL156) (15) that was linearized with the same enzymes, to generate pTRV2:*SlSOBIR1* (Sol 2756), pTRV2:*SlSOBIR1-like* (Sol 2755), and pTRV2:*SlSOBIR1*/*SlSOBIR1-like* (Sol 2779). Fragment *NbSOBIR1* was excised from pCR4-TOPO by using enzymes EcoRI and BamHI and cloned into pTRV2:RNA2, linearized with the same enzymes, to generate pTRV2:*NbSOBIR1* (Sol 2850). VIGS fragment *NbSOBIR1-like* was excised from pCR4-TOPO by using BamHI and XhoI and cloned into pTRV2:RNA2, linearized with the same enzymes, to generate pTRV2:*NbSOBIR1-like* (Sol 2851). Fragment *NbSOBIR1-like* was also cloned into Sol 2850, linearized with BamHI and XhoI, to generate pTRV2:*NbSOBIR1*/*NbSOBIR1-like* (Sol 2854).

VIGS in *N. benthamiana* and Tobacco and Hypersensitive Response Assays. VIGS experiments in *N. benthamiana:Cf-4*, wild-type *N. benthamiana*, and *N. tabacum* cv. Samsun were performed as described (4, 9). In brief, 4-wk-old plants were inoculated by agroinfiltration with pTRV:RNA1 and pTRV:RNA2 (15). TRV:*Cf-4* (16), TRV:*Enhanced Disease Susceptibility 1* (*EDS1*) (9), TRV:β-*glucuronidase* (*GUS*) (17), TRV:GFP (18), and TRV:*Phytoene desaturase* (*PDS*) (15) were included as controls.

For hypersensitive response (HR) assays, 3 wk after virus inoculations, mature leaves were agroinfiltrated to individually express Avr4 at OD_{600} = 0.03 (1), RxD460V [pB1-Rx (AT39-H1; D460V)] (19) at OD_{600} = 0.1, Bcl2-Associated protein X (BAX) (20) at OD_{600} = 0.5, and Peru2–eGFP at OD_{600} = 1. For complementation analysis with *At*SOBIR1–Myc and the respective D489N kinase mutant, constructs driving expression of these proteins, in addition to GUS, were coexpressed with Avr4 (OD_{600} = 0.03) at an OD_{600} of 0.5, in *N. benthamiana:Cf-4*. In tobacco, Ve1 (pMOG800–Ve1) (6) and Ave1 (pFAST–Ave1) (9) were transiently coexpressed in leaf sections at an OD_{600} of 2 for each construct. Three days after agroinfiltration, leaves were examined for development of an HR.

RNA Extraction, cDNA Synthesis, and qRT-PCR Analysis. For qRT-PCRs, RNA was isolated from *N. benthamiana* inoculated with the various TRV constructs. For RT-PCRs, mature leaves of TRV-inoculated plants were either agroinfiltrated with Cf-4–eGFP or Ve1–eGFP, or they were not transiently transformed. RNA extraction, cDNA synthesis, and qRT-PCR were performed as described (4). For RT-PCR analysis, the amount of cycles is indicated. The primer combinations used can be found in Table S4.

VIGS in Tomato and *C. fulvum* and *V. dahliae* Disease Assays. Tomato was subjected to VIGS as described (4). For *C. fulvum* disease assays, tomato plants transformed with the *Hcr9-4D* (*Cf-4*) or *Cf-2.2* gene and fully resistant to a race 5 strain of *C. fulvum* (secreting both Avr4 and Avr2), were subjected to agroinoculation with various recombinant TRV VIGS constructs targeting *SlSOBIR1 (-like)*, *Cf-4*, or *GUS*. Nonagroinoculated MM–Cf-0 plants served as fully susceptible controls. For *V. dahliae* disease assays, tomato cultivar Motelle carrying the *Ve1* gene and resistant to *V. dahliae* race 1 strains expressing Ave1, was used. TRV:*Ve1* and TRV:*GFP* were used as controls. Four to six tomato plants were used per treatment in each experiment. Leaf canopy area measurements were performed as described (21), and for each plant the canopy area was calculated. The average canopy area of *V. dahliae*-inoculated plants, compared with control plants, was calculated for three independent biological repeats.

C. fulvum inoculations were performed as described (4). *C. fulvum* race 5–pGPD:*GUS*, constitutively expressing the *GUS* reporter gene, was used for inoculations (22). *V. dahliae* disease assays on tomato *Arabidopsis*, as well as quantification of *V. dahliae* biomass by qRT-PCRs, were performed as described (6, 8). *V. dahliae* race 1 strain JR2, expressing Ave1, was used in the various disease assays.

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Table S1. Sequences and ProteinLynx scores of peptides specifically matching Solyc06g071810.1.1, Solyc03g111800.2.1, and Cf-4–eGFP, of a tryptic digest of Cf-4–eGFP immunopurified from transgenic tomato line TL3

Protein name	Peptide sequence	ProteinLynx score
S/SOBIR1	DLKPANILLDDDMEAR	40.9
S/SOBIR1	EDGVASLEMIGK	62.3
S/SOBIR1	IIQSPMDAAEITEEDTK	53.5
S/SOBIR1	KAEDLAFLEK	78.9
S/SOBIR1	VADFGLAK	62.2
S/SOBIR1-like	DAAELTEEDSK	65.1
S/SOBIR1-like	DLKPGNVLLDDDMEAR	57.0
S/SOBIR1-like	IADFGLAK	66.7
S/SOBIR1-like	NDPGLTIFSPLIK	42.7
S/SOBIR1-like	NVMTSEDPNR	50.9
Cf-4–eGFP	EIDESTGFPEYISDPYDIYYNYLTTISTK	28.7
Cf-4–eGFP	FEGHIPSIIGDLVGLR	39.8
Cf-4–eGFP	FGEFSDLTHLDLSHSSFR	74.3
Cf-4–eGFP	GPIPNSLLNQK	60.3
Cf-4–eGFP	ILDSNMIINLSK	78.3
Cf-4–eGFP	ILGNLQTMK	64.7
Cf-4–eGFP	ISLNELTFGPHNFELLLK	59.0
Cf-4–eGFP	LDLSYNDFTGSPISPK	52.7
Cf-4–eGFP	LYLYNVNIDDR	55.6
Cf-4–eGFP	NEYLSHLDLSNNR	65.3
Cf-4–eGFP	SSGNTNLFMGLQILDLSSNGFSGNLPER	30.9

S/SOBIR1, Solyc06g071810.1.1; *S*/SOBIR1-like, Solyc03g111800.2.1. Scores were identified by mass spectrometry using the Synapt MS.

Table S2. Sequences, ProteinLynx, and Mascot ion scores of peptides specifically matching *Nb*SOBIR1 or matching both *Nb*SOBIR1 and *Nb*SOBIR1-like, of a tryptic digest of immunopurified Cf-4–eGFP transiently expressed in *N. benthamiana*

Protein name*	Peptide sequence	Mascot ion score†	ProteinLynx score‡
*Nb*SOBIR1/SOBIR1-like	ASMPAPAPAPVNR		29.3
*Nb*SOBIR1/SOBIR1-like	DGSLQDILQQVTEGTR		32.3
*Nb*SOBIR1/SOBIR1-like	ELDWLGR		59.0
*Nb*SOBIR1/SOBIR1-like	LNLYPPDHAALLLVQK		33.3
*Nb*SOBIR1/SOBIR1-like	LSLADNMFTGK		69.8
*Nb*SOBIR1/SOBIR1-like	NGSLQDILQQVTEGTQ		48.4
*Nb*SOBIR1/SOBIR1-like	NGSLQDILQQVTEGTR		78.5
*Nb*SOBIR1/SOBIR1-like	NHTQRI		45.5
*Nb*SOBIR1/SOBIR1-like	NLEKLSLADN		31.6
*Nb*SOBIR1/SOBIR1-like	YFPNLEK		56.4
*Nb*SOBIR1	LNLYPPDHAALLLVQK	50.8	
*Nb*SOBIR1	KLEILDLGNNLFSGK	59.4	
*Nb*SOBIR1	SEIQILGQIR	68	
*Nb*SOBIR1	NGSLQDILQQVTEGTR	100.5	
*Nb*SOBIR1	DLKPANVLLDDDMEAR	96.4	
*Nb*SOBIR1	LPSDEFFQHTPEMSLVK	98.3	
*Nb*SOBIR1	NVMTSEDPK	74.7	
*Nb*SOBIR1/SOBIR1-like	LIGSGFEEQMLLVLK	62.4	
*Nb*SOBIR1	IACFCTLENPK	74.7	
*Nb*SOBIR1	DLGIQGQR	38	
*Nb*SOBIR1/SOBIR1-like	SNGLSGTLSPAIGK	82.7	
*Nb*SOBIR1	ILDLSSNELSGNLNFLK	96	
*Nb*SOBIR1	GKTDGSLTIYSPLIK	108.6	
*Nb*SOBIR1	GGCGEVYRAELPGSNGK	49.9	
*Nb*SOBIR1/SOBIR1-like	KILQPPMDAAELAEEDTKALNK	61.2	
*Nb*SOBIR1/SOBIR1-like	AVDPDAHTHVTTSNVAGTVGYIAPEYHQTLK	50.2	

Scores were identified by mass spectrometry of a tryptic digest of immunopurified Cf-4–eGFP.

*For identifications with ProteinLynx, peptides were matched on the translated tomato genome sequence. Because of the difference in sequence between *Sl*SOBIR1 and *Nb*SOBIR1 homologs, no distinction between *Nb*SOBIR1 and *Nb*SOBIR1-like can be made. Mascot identifications were based on translated tobacco EST sequences, and peptides can match either *Nb*SOBIR1 or *Nb*SOBIR1-like. When specifically matching to the corresponding *N. benthamiana* homolog, the protein name is indicated. When no distinction can be made, *Nb*SOBIR1/SOBIR1-like is indicated.

†Mascot ion scores are presented for the peptides that were identified on the Orbitrap XL.

‡ProteinLynx peptide ladder scores are presented for the peptides that were identified on the Synapt MS.

Table S3. Sequences and Mascot ion scores of peptides specifically matching *Nb*SOBIR1, *Nb*SOBIR1-like or both, and Ve1–eGFP, of a tryptic digest of immunopurified Ve1–eGFP transiently expressed in *N. benthamiana*

Protein name*	Peptide sequence	Mascot ion score
*Nb*SOBIR1-like	ILDLSSNELSGLNFLK	83.6
*Nb*SOBIR1	KLEILDLGNNLFSGK	76.2
*Nb*SOBIR1	GKTDGSLTIYSPLIK	109.2
*Nb*SOBIR1/SOBIR1-like	ILQPPMDAAELAEEDTK	62.8
*Nb*SOBIR1	DLKPANVLLDDDMEAR	82.7
*Nb*SOBIR1	AVPDAHTHVTTSNVAGTVGYIAPEYHQTLK	45.9
*Nb*SOBIR1	NVMTSEDPKR	45.9
*Nb*SOBIR1	LPSDEFFQHTPEMSLVK	46.9
*Nb*SOBIR1	LSLADNMFTGK	83.8
*Nb*SOBIR1	SNGLSGTLSPAIGK	49.8
*Nb*SOBIR1	TDGSLTIYSPLIK	74
Ve1–eGFP	SLLLQFKGSLQYDSTLSKK	35.7
Ve1–eGFP	YLNLSNAGFVGQIPITLSR	65
Ve1–eGFP	LVTLDLSTILPFFDQPLK	58.7
Ve1–eGFP	DCQISGPLDESLSK	55.5
Ve1–eGFP	GSIPIFFR	38.2
Ve1–eGFP	LELSNCNFYGSIPSTMANLR	113.3
Ve1–eGFP	KLTYLDLSRN	47.9
Ve1–eGFP	RNQFVGQVD	62
Ve1–eGFP	VLSLSSNFFR	63
Ve1–eGFP	LGVLNLGNNK	63.4
Ve1–eGFP	LLEVLNVGNNR	75.1
Ve1–eGFP	SNLVVLDLHSNR	88.6
Ve1–eGFP	SIVNCKLLEVLNVGNNR	77.4
Ve1–eGFP	GMMVADDYVETGR	81.1
Ve1–eGFP	LYYQDTVTLTIK	75.6
Ve1–eGFP	VFTSIDFSSNR	73.2
Ve1–eGFP	GEELFTGVVPILVELDGDVNGHK	78.7
Ve1–eGFP	FSVGEGEGDATYGKLTLK	42.8
Ve1–eGFP	MPEGYVQER	601.1
Ve1–eGFP	YPDHMKQHDFFK	54.1

Scores were identified by mass spectrometry using the Orbitrap XL.

*Peptides were identified based on translated tobacco EST sequences and can match either *Nb*SOBIR1 or *Nb*SOBIR1-like. When specifically matching to the corresponding *N. benthamiana* homolog, the protein name is indicated. When no distinction can be made, *Nb*SOBIR1/SOBIR1-like is indicated.

Table S4. Sequences of oligonucleotide primers used in this study

Primer code	Sequence, 5′–3′	Target sequence
Ro1	CACCATGGCTCCATTGTTCCTCTC	*SlSNC2*
Ro2	TTTACAACATTTGGACAATAAAAC	*SlSNC2*
Ro6	CACCATGGCCCTTTTTCTCTCAATA	*SlTMM*
Ro7	CAACAGACAAACTAGAACAAAAA	*SlTMM*
to11	GGGGACCACTTTGTACAAGAAAGCTGGGTTATATCTTTTCTTGTGCTTTTTCATTTTC	*AttB1–Cf-4* RT-PCR
to12	GGGGACAAGTTTGTACAAAAAAGCAGGCTTTATGATGATGGTTTCTAGAAAAGTAGT	*AttB2–Cf-4* RT-PCR
to118	CACCATGAAAATGATGGCAAC	*Ve1* RT-PCR
to119	CTTTCTTGAAAACCAAAGCAAG	*Ve1* RT-PCR
to156	ATGCTTGATCTGAGTTAACA	*SlSOBIR1*
to157	CACCATGACTTCGAATATC	*SlSOBIR1*
to164	CACCATGACCTTCACAGCCTC	*SlSOBIR1-like*
to165	ATGCTTGATCTGCATCAACATGC	*SlSOBIR1-like*
to166	ATCTAGATTCGTAAGAAGAGG	VIGS fragment *SlSOBIR1*
to167	AGGATCCGAATTTCTGATTTAAC	VIGS fragment *SlSOBIR1*
to168	ATCTAGAGAACGCAACAAGGC	VIGS fragment *SlSOBIR1-like*
to169	AGGATCCAGAGGACGGAGAAG	VIGS fragment *SlSOBIR1-like*
to178	ATCTAGAATCAGACACTGAAGTTTAC	VIGS fragment *SlSOBIR1/SlSOBIR1-like*
to179	AGGATCCATCTTGAGAACCAAAAG	VIGS fragment *SlSOBIR1/SlSOBIR1-like*
to180	CACCATGGCAGAATCACTTGTTGAAC	*SlCLV2*
to181	ACCTGCTAAATTTTTTGTTTGC	*SlCLV2*
to239	CACCATGGCTGTTCCCACGGGAA	*AtSOBIR1* and RT-PCR
to240	GTGCTTGATCTGGGACAACATGG	*AtSOBIR1* and RT-PCR
to241	AGAATTCAATCTTTATCCACCAGATCATGC	VIGS fragment *NbSOBIR1*
to242	AGGATCCCAGAAAGTTTTCCAATGGCAG	VIGS fragment *NbSOBIR1*
to243	AGGATCCTTGGAAATCTTGAACCTTC	VIGS fragment *NbSOBIR1-like*
to244	ACTCGAGGATTTCTGAAAGATTTCAAG	VIGS fragment *NbSOBIR1-like*
to250	CACCATGGGCAAAAGAACTAATCCA	*SlEIX2*
to251	GTTCCTTAGCTTTCCCTTCAG	*SlEIX2*
to257	CTCAACGCATAATTCACAGAAATTTAAAGCCAGC	*SlSOBIR1D473N* mutation
to258	GCTGGCTTTAAATTTCTGTGAATTATGCGTTGAG	*SlSOBIR1D473N* mutation
to259	CTCAGCGTATAATTCACAGAAATCTAAAGCCAGGC	*SlSOBIR1-likeD486N* mutation
to260	GCCTGGCTTTAGATTTCTGTGAATTATACGCTGAG	*SlSOBIR1-likeD486N* mutation
to261	CCCACGAATCATTCACAGAAACTTAAAGCCAGCCAATG	*AtSOBIR1D489N* mutation
to262	CATTGGCTGGCTTTAAGTTTCTGTGAATGATTCGTGGG	*AtSOBIR1D489N* mutation
to266	CTTAGAAAAACTCTCTTTAGC	*NbSOBIR1* qRT-PCR
to267	TATGGATTGGAGTGACATTATG	*NbSOBIR1* qRT-PCR
to272	GCAATTGTAGTACCAGTACAC	*NbSOBIR1-like* qRT-PCR
to273	AATCAATGGACTGAAAAC	*NbSOBIR1-like* qRT-PCR
to45	GGGGACAAGTTTGTACAAAAAAGCAGGCTTTATGG ATCAGTCGGTGTTGGCGA	*SlSERK3a/BAK1*
to46	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCTTG GCCCTGACAACTCATCCG	*SlSERK3a/BAK1*
to58	TATGGAAACATTGTGCTCAGTGG	*NbActin* RT-PCR and qRT-PCR
to59	CCAGATTCGTCATACTCTGCC	*NbActin* RT-PCR and qRT-PCR
to88	CACCATGGTGAAGGTGATGGAGAA	*SlSERK1*
to89	CCTTGGACCCGATAATTCAAC	*SlSERK1*
ITS1-F	AAAGTTTTAATGGTTCGCTAAGA	*V. dahliae* qRT-PCR
ST-VE1-R	CTTGGTCATTTAGAGGAAGTAA	*V. dahliae* qRT-PCR
AtRubF	GCAAGTGTTGGGTTCAAAGCTGGTG	*Arabidopsis Rubisco* qRT-PCR
AtRubR	CCAGGTTGAGGAGTTACTGGAATGCTG	*Arabidopsis Rubisco* qRT-PCR

Fig. S1. Cf-4–eGFP is functional and efficiently immunopurified from stably transformed tomato and tomato *Sl*SOBIR1-like and *Arabidopsis At*SOBIR1 interact with Cf-4 and Ve1, but not with various receptor-like kinases (RLKs). (*A*) Leaflets of the transgenic lines TL3 and TL21, stably transformed with the Cf-4–eGFP gene under control of the 35S promoter, were infiltrated with apoplastic fluid obtained from leaflets of susceptible Money Maker–Cf-0 plants colonized by *C. fulvum* secreting Avr4. TL3 mounted an HR by 2 d after infiltration, in contrast to line TL21, which did not respond to the Avr4 infiltration. (*B*) Cf-4–eGFP is detectably immunopurified from TL3, but not from TL21. Total protein extracts were subjected to immunopurification by using GFP-affinity beads and total proteins (Input), and immunopurified proteins (IP) were subjected to SDS/PAGE and analyzed by immunoblotting using αGFP antibody. The Coomassie-stained blot shows the 50-kDa Rubisco band present in the input samples to confirm equal loading. (*C*) Tagged versions of Cf-4, Ve1, *At*CLV1, *Sl*SERK1, *Sl*SERK3a/BAK1, and *Sl*FLS2 (all fused to eGFP, except for *Sl*FLS2, which was fused to GFP) were coexpressed with *Sl*SOBIR1-like–Myc and *At*SOBIR1–Myc in *N. benthamiana*. Total protein extracts of transiently transformed leaf tissue were subjected to immunopurification by using GFP-affinity beads. Total proteins (Input) and immunopurified proteins (IP) were subjected to SDS/PAGE and blotted. Blots were incubated with αGFP antibody to detect the immunopurified (e)GFP fusion proteins and incubated with αMyc antibody to detect coimmunopurifying SOBIR1–Myc proteins. Coomassie-stained blots showing the 50-kDa Rubisco band present in the input samples confirm equal loading. Representative results for three independent experiments are shown.

Fig. S1

Fig. S2. Alignments of SOBIR1 sequences from tomato, *Arabidopsis*, *N. benthamiana*, and tobacco. (*A*) Amino acid sequence alignment of *Sl*SOBIR1 (Solyc06g071810.1.1), *Sl*SOBIR1-like (*Sl*SOBIR1-l; Solyc03g111800.2.1), *At*SOBIR1, and *Sl*SERK3a/BAK1 (*Sl*SERK3a/B). Amino acid residues highlighted in black and dark gray represent identical residues in all four or three of the aligned protein sequences, respectively. Residues in light gray or white represent residues present in two sequences or are unique residues, respectively. The conserved RD motif, as well as the conserved residues K377 and E407 of *At*SOBIR1, present in the kinase domain of all four proteins, are underlined. (*B*) Nucleotide sequence alignment of tomato *SlSOBIR1*, *SlSOBIR1-like* (*SlSOBIR1-li*), and *Arabidopsis AtSOBIR1* (AT2G31880.1) coding regions. Residues highlighted in black and gray represent identical nucleotides in all three or two of the aligned coding regions, respectively. Nucleotide sequences used for the TRV-based VIGS constructs are indicated by different colors as follows: TRV:*SlSOBIR1*, green; TRV:*SlSOBIR1-like*, blue; and TRV:*SlSOBIR1*/*SlSOBIR1-like*, red. (*C*) Nucleotide sequence alignment of *N. benthamiana NbSOBIR1*, tobacco *NtSOBIR1* (SGN-U441568), and *NbSOBIR1-like* (*NbSOBIR1-l*) coding regions. The available genomic sequence was used to obtain *Nb*SOBIR1 and *Nb*SOBIR1-like sequences, and EST data provided the *Nt*SOBIR1 coding sequence. Intron sequences were manually removed from the *NbSOBIR1-like* sequence. Residues highlighted in black and gray represent identical nucleotides in all three or two of the aligned coding regions, respectively. Nucleotide sequences used for the TRV-based VIGS constructs are indicated by different colors as follows: TRV:*NbSOBIR1*, green; and TRV:*NbSOBIR1-like*, blue. TRV:*NbSOBIR1*/*NbSOBIR1-like* contains both of these fragments (*NbSOBIR1* and *NbSOBIR1-like*).

Fig. S2

Fig. S3. Cf-4 interacts with *Sl*SOBIR1 in a reverse immunoprecipitation assay, with SOBIR1 mutants in the RD motif of the kinase domain, and with *Sl*SOBIR1 in the presence of Avr4, and SOBIR1 does not form homo- or heterodimers. (*A*) *Sl*SOBIR1–eGFP was coexpressed with Cf-4–Myc in *N. benthamiana*, and total protein extract was subjected to immunopurification by using GFP-affinity beads. The total protein extract (Input) and immunopurified proteins (IP) were subjected to SDS/PAGE and blotted. Blots were incubated with αGFP antibody to detect immunopurified *Sl*SOBIR1–eGFP, whereas coimmunopurified Cf-4–Myc was detected by using αMyc. (*B*) Cf-4–eGFP was coexpressed with *Sl*SOBIR1–Myc, *Sl*SOBIR1^{D473N}–Myc, *Sl*SOBIR1-like^{D486N}–Myc, and *At*SOBIR1^{D489N}–Myc in *N. benthamiana*. Immunopurifications and detection of proteins were performed as described in *A*. (*C*) Cf-4–eGFP was coexpressed with *Sl*SOBIR1–Myc in the presence of Avr4 or Avr9 (the OD_{600} of the *A. tumefaciens* cultures was either 0.2 or 0.4) in *N. benthamiana*. Leaves were harvested 1 d after agroinfiltration, which was just before the onset of an HR in the Avr4 agroinfiltrated leaves. Immunopurifications and detection of proteins were performed as described in *A*. (*D*) *Sl*SOBIR1–eGFP was coexpressed with Cf-4–Myc, *Sl*SOBIR1–Myc, *Sl*SOBIR1-like–Myc, and *At*SOBIR1–Myc. After 2 d, *Sl*SOBIR1–eGFP was immunopurified, and the samples were analyzed for copurification of the Myc-tagged versions. Immunopurifications and detection of proteins were performed as described in *A*. All assays were performed twice, and a representative picture for each experiment is shown.

Fig. S3

Fig. S4. *Sl*SOBIR1–eGFP localizes to the plasma membrane and cytoplasmic vesicles. Proteins were transiently expressed in *N. benthamiana* epidermal leaf cells, and their subcellular localization is shown. Each image shows a combination of the eGFP signal (green) and the chloroplast signal (red). (*A*) *Sl*SOBIR1–eGFP localizes to the plasma membrane. When focusing on top of the plasma membrane, *Sl*SOBIR1–eGFP is also detected in distinct spots that resemble cytoplasmic vesicles. (*B*) *Sl*FLS2–GFP typically localizes to the plasma membrane. (*C*) GFP–HA localizes to the cytosol and the nucleus. (*D*) An untransformed plant was used as a negative control. Representative pictures for three independent experiments are shown.

Fig. S4

Fig. S5. SOBIR1 kinase activity is essential for its role in Cf-4–mediated HR. (*A*) Expression of *NbSOBIR1* is efficiently knocked down by inoculation of *N. benthamiana* with TRV:*NbSOBIR1/NbSOBIR1-like*. (*B*) Expression of *NbSOBIR1* is efficiently knocked down by inoculation of *N. benthamiana* with TRV:*NbSOBIR1*. Inoculation with TRV:*NbSOBIR1-like* results in slight cross-silencing of *NbSOBIR1* expression. For *A* and *B*, plants were inoculated with TRV:*GUS* or the indicated constructs targeting *Nb*SOBIR1s, and relative expression of *NbSOBIR1* was determined by qRT-PCR for each sample. Samples were normalized to endogenous *NbActin*. SDs show the variation between three technical repeats. In total, two biological experiments were performed with similar results, and a representative result is shown. (*C*) Kinase activity of SOBIR1 is required for Cf-4–mediated HR. Transgenic *N. benthamiana*:*Cf-4* plants were subjected to VIGS by inoculation with TRV:*NbSOBIR1/NbSOBIR1-like*. Three weeks later, mature leaves were agroinfiltrated to transiently express the combinations *GUS* + *Avr4*, *AtSOBIR1-Myc* + *Avr4*, and *AtSOBIR1*^{D489N}*-Myc* + *Avr4*, as indicated. *GUS*, *AtSOBIR1–Myc*, and *AtSOBIR1*^{D489N}*–Myc* were infiltrated at final $OD_{600} = 0.7$ and Avr4 at $OD_{600} = 0.03$. (*D*) RT-PCR analysis confirms that *AtSOBIR1–Myc* and *AtSOBIR1*^{D489N}*–Myc* are not targeted by TRV:*NbSOBIR1/NbSOBIR1-like*–inoculation of *N. benthamiana*. Three weeks after inoculation with the indicated TRV constructs, leaves were agroinfiltrated with *AtSOBIR1–Myc* or *AtSOBIR1*^{D489N}*–Myc*. RNA was isolated, transcribed into cDNA, and used as a template for RT-PCRs using primers amplifying *AtSOBIR1* or endogenous *NbActin*. The amount of PCR cycles is indicated.

Fig. S5

Fig. S6. SOBIR1 is required for autoactivity of the Cf homolog Peru2, Ve1-mediated HR in tobacco, and Cf-2.2– and Ve1-mediated resistance in tomato. (*A*) Peru2 autoactivity is compromised upon silencing of *NbSOBIR1*. *N. benthamiana* plants were subjected to VIGS by inoculation with TRV:*NbSOBIR1/NbSOBIR1-like*. Three weeks after TRV inoculation, Peru2–eGFP was transiently expressed by agroinfiltration at four sites in the leaves, and leaves were photographed 3 d later. (*B*) The Ve1-mediated HR is compromised upon targeting *Nt*SOBIR1 homologs in tobacco. *N. tabacum* cultivar Samsun was subjected to VIGS by inoculation with the TRV constructs indicated above each image. TRV:*EDS1* and TRV:*GFP* served as controls. Three weeks after TRV inoculation, Ve1 and Ave1 were coexpressed, and leaves were photographed 5 d later. The experiments were performed three times with three plants for each TRV construct, and representative pictures for the experiments are shown. (*C*) Targeting *Sl*SOBIR1 and *Sl*SOBIR1-like suppresses Cf-2.2–mediated resistance to *C. fulvum* expressing Avr2. *Cf-2.2*–expressing tomato was inoculated with TRV:*Sl*SOBIR1/*Sl*SOBIR1-like or TRV:*GUS*, and 3 wk later plants were inoculated with an Avr2-secreting, *GUS*-transgenic strain of *C. fulvum*. Two weeks later, leaflets were stained for GUS activity to detect *C. fulvum* colonization. The amount of successful colonization attempts (blue spots) vs. the total amount of leaflets analyzed for that particular treatment is indicated between parentheses. (*D*) Tomato cultivar Motelle (carrying *Ve1*) was subjected to VIGS by inoculation with the constructs indicated above each image. TRV:*GFP*, TRV:*Ve1*, and non-TRV-inoculated Motelle plants were included as controls. Each image shows a mock (M)-treated plant on the left and a *V. dahliae* (*Vd*)-inoculated plant on the right. Stunting of the *V. dahliae*-inoculated plants, compared with the mock-treated plants indicates compromised resistance. Percentages between parentheses indicate the average canopy area (and SDs) of *V. dahliae*-inoculated plants in three independent experiments compared with the control mock treatment, for which the canopy area was set to 100%. Representative photographs for three independent experiments are shown.

Fig. S6

Fig. S7. Quantification of *V. dahliae* biomass in *Arabidopsis sobir1-1* and *sobir1-1 Ve1*-complemented lines and expression of Cf-4 and Ve1 in *N. benthamiana* silenced for the *NbSOBIR1* homologs. (*A*) Relative quantity (R.Q.) of fungal biomass present in the plants shown in Fig. 4, as determined by qRT-PCR. SDs show the difference between technical repeats. The inoculation experiments and qRT-PCR quantifications were performed three times, with similar results. (*B*) *Cf-4–eGFP* and *Ve1–eGFP* are expressed in *N. benthamiana* silenced for the *NbSOBIR1* homologs. *N. benthamiana* was subjected to VIGS by inoculation with the indicated TRV constructs and subsequently transiently transformed to express *Cf-4–eGFP* or *Ve1–eGFP*. RNA was isolated, transcribed into cDNA, and used as a template for RT-PCRs using primers amplifying *Cf-4*, *Ve1*, and endogenous *NbActin*, respectively. The amount of PCR cycles is indicated.

Fig. S7

Fig. S8. *Sl*SOBIR1 and *Sl*SOBIR1-like interact with all Cf proteins tested and with the receptor-like proteins (RLPs) *Sl*EIX2, *Sl*CLV2, and *Sl*TMM, but not with *Sl*SNC2. (*A*) Cf-2.2, -4E, -9, and the autoactive Cf homolog Peru2, all C-terminally fused to eGFP, were coexpressed with *Sl*SOBIR1–Myc and *Sl*SOBIR1-like–Myc in *N. benthamiana*, and the proteins fused to eGFP were immunopurified by using GFP-affinity beads. Total proteins (Input) and immunopurified proteins (IP) were subjected to SDS/PAGE and blotted. Blots were incubated with αGFP antibody to detect the immunopurified proteins fused to eGFP, and αMyc antibody was used for detection of coimmunopurified *Sl*SOBIR1–Myc and *Sl*SOBIR1-like–Myc. Coomassie-stained blots showing the 50-kDa Rubisco band present in the input confirm equal loading. The assay was performed twice, and a representative picture is shown. (*B*) The indicated RLPs, fused to eGFP, were coexpressed with *Sl*SOBIR1–Myc or *Sl*SOBIR1-like–Myc in *N. benthamiana* and immunopurified by using GFP-affinity beads. Total proteins (Input) and immunopurified proteins (IP) were subjected to SDS/PAGE and blotted. Blots were incubated with α-GFP antibody to detect the immunopurified RLP–eGFP fusion proteins, and coimmunopurified *Sl*SOBIR1–Myc and *Sl*SOBIR1-like–Myc were detected by using α-Myc antibody. Coomassie-stained blots showing the 50-kDa Rubisco band present in the input confirm equal loading. The assay was performed three times, and a representative picture is shown.

Fig. S8