Corrections

SOCIAL SCIENCES
Correction for “Within-mother analysis of seasonal patterns in health at birth,” by Janet Currie and Hannes Schwandt, which appeared in issue 30, July 23, 2013, of Proc Natl Acad Sci USA (110:12265–12270; first published July 8, 2013; 10.1073/pnas.1307582110).

The authors note that on page 12267, left column, second full paragraph, lines 5–12, “The average gestation length decreases significantly by 0.8 wk (P < 0.001) during the first 5 mo of the year and returns to the January level in June, where it remains for the rest of the year. This finding indicates that the seasonal pattern in gestation length is not driven by mothers of different socioeconomic background selecting differently into conception months. The May decrease in gestation length by 0.8 wk leads to an increase in premature births of about 1 percentage point (Fig. S1A).” should instead appear as “The average gestation length decreases significantly by 0.08 wk (P < 0.001) during the first 5 mo of the year and returns to the January level in June, where it remains for the rest of the year. This finding indicates that the seasonal pattern in gestation length is not driven by mothers of different socioeconomic background selecting differently into conception months. The May decrease in gestation length by 0.08 wk leads to an increase in premature births of about 1 percentage point (Fig. S1A).” This change does not affect the conclusions of the article.

NEUROSCIENCE
Correction for “Rhythmic alternating patterns of brain activity distinguish rapid eye movement sleep from other states of consciousness,” by Ho Ming Chow, Silvina G. Horovitz, Walter S. Carr, Dante Picchioni, Nate Coddington, Masaki Fukunaga, Yisheng Xu, Thomas J. Balkin, Jeff H. Duyn, and Allen R. Braun, which appeared in issue 25, June 18, 2013, of Proc Natl Acad Sci USA (110:10300–10305; first published June 3, 2013; 10.1073/pnas.1217691110).

The authors note that the following statement should be added to the Acknowledgments: “This material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting the views of the Department of the Army or the Department of Defense.”

PLANT BIOLOGY
Correction for “Receptor-like kinase SOBIR1/EVR interacts with receptor-like proteins in plant immunity against fungal infection,” by Thomas W. H. Liebrand, Grardy C. M. van den Berg, Zhao Zhang, Patrick Smit, Jan H. G. Cordewener, Antoine H. P. America, Jan Sklenar, Alexandra M. E. Jones, Vladimír I. L. Tameling, Silke Robatzek, Bart P. H. J. Thomma, and Matthieu H. A. J. Joosten, which appeared in issue 24, June 11, 2013, of Proc Natl Acad Sci USA (110:10010–10015; first published May 28, 2013; 10.1073/pnas.1220015110).

The authors note that the author name Antione H. P. America should instead appear as Antoine H. P. America. The corrected author line appears below. The online version has been corrected.

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Receptor-like kinase SOBIR1/EVR interacts with receptor-like proteins in plant immunity against fungal infection

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The plant immune system is activated by microbial patterns that are detected as nonself molecules. Such patterns are recognized by immune receptors that are cytoplasmic or localized at the plasma membrane. Cell surface receptors are represented by receptor-like kinases (RLKs) that frequently contain extracellular leucine-rich repeats and an intracellular kinase domain for activation of downstream signaling, as well as receptor-like proteins (RLPs) that lack this signaling domain. It is therefore hypothesized that RLKs are required for RLPs to activate downstream signaling. The RLPS Cf-4 and Ve1 of tomato \textit{(Solanum lycopersicum)} mediate resistance to the fungal pathogens \textit{Cladosporium fulvum} and \textit{Ventricillium dahliae}, respectively. Despite their importance, the mechanism by which these immune receptors mediate downstream signaling upon recognition of their matching ligand, Avr4 and Ave1, remained enigmatic. Here we show that the tomato ortholog of the \textit{Arabidopsis thaliana} RLK suppressor Of BIR1-1/Evershed (SOBIR1/EVR) and its close homolog \textit{S. lycopersicum} (Sl) SOBIR1-like interact \textit{in planta} with both Cf-4 and Ve1 and are required for the Cf-4- and Ve1-mediated hypersensitive response and immunity. Tomato SOBIR1/EVR interacts with most of the tested RLPS, but not with the RLPS FLS2, SERK1, SERK3a, BAK1, and CLV1. SOBIR1/EVR is required for stability of the Cf-4 and Ve1 receptors, supporting our observation that these RLPS are present in a complex with SOBIR1/EVR \textit{in planta}. We show that SOBIR1/EVR is essential for RLP-mediated immunity and propose that the protein functions as a regulatory RLK of this type of cell-surface receptors.

plants rely on an innate immune system, which is activated upon recognition of pathogen-derived nonself molecules, or host-derived damage products (1, 2). Conserved microbe-associated molecular patterns (MAMPs) are perceived by pattern recognition receptors (PRRs) that activate MAMP-triggered immunity (MTI). Pathogenic microbes promote virulence by secretion of effector proteins, many of which suppress MTI (3, 4). In resistant plants, these effector proteins are detected by resistance proteins that activate effector-triggered immunity (ETI), frequently resulting in the hypersensitive response (HR), a localized programmed host cell death response (1). Conceptually, MTI and ETI function in a similar fashion by using immune receptors that mount a suitable defense response to halt pathogen ingress upon recognition of appropriate ligands that betray pathogen presence (5).

Most PRRs have been identified as transmembrane receptor-like kinases (RLKs) that frequently contain an extracellular leucine-rich repeat (LRR) domain or lysin-motif (LysM) for ligand recognition and an intracellular kinase domain for activation of downstream signaling (6). The LysM–RLK Chitin Elicitor Receptor Kinase 1 mediates immunity against fungi by recognizing fungal chitin (7, 8), whereas the LRR–RLKs Flagellin Sensing 2 (FLS2) and EF-Tu Receptor (EFR) are involved in recognition of bacterial flagellin and the elongation-factor Tu protein, respectively (9, 10). Upon ligand recognition, both FLS2 and EFR form a receptor complex with the LRR–RLK Somatic Embryo-Genesis Kinase 3/Brassinoesteroid Insensitive 1 (BR1)-Associated receptor Kinase 1 (SERK3/BAK1) and its close paralogs BAK1-like 1 (BKK1) (11–13). The transphosphorylation events that follow, together with the dissociation of the cytoplasmic kinase Bortytis-Induced Kinase 1 from the receptor complex, subsequently activate downstream defense signaling (14, 15). SERK3/BAK1 is not involved in ligand binding to FLS2 and EFR but, rather, plays a role in downstream signaling upon its recruitment by FLS2 and EFR after ligand binding (2). Hence, SERK3/BAK1 and BKK1 likely function as signal enhancers and can be regarded as coregulatory RLKs in FLS2- and EFR-mediated immunity (6, 16).

Receptor-like proteins (RLPS) form a second major class of cell-surface receptors in plants. RLPs are structurally similar to RLKs but lack a cytoplasmic kinase domain (17, 18). RLPS function in defense, such as the Cf proteins and Ve1, as well as in development (18). Examples of the latter are Chvata2 (CLV2), which plays a role in meristem maintenance, and Too Many Mouths (TMM), which regulates stomatal patterning (18). Because RLPS lack a cytoplasmic kinase domain, it is anticipated that proteins containing such a domain are recruited to activate downstream signaling (19, 20). Indeed, \textit{Arabidopsis thaliana} CLV2 forms a complex with the transmembrane kinase gene Croyne and the LRR–RLK CLV1 (21–23), whereas TMM requires the LRR–RLK Erecta to activate downstream signaling (24).

In tomato \textit{(Solanum lycopersicum)}, resistance to specific races of the fungal pathogens \textit{Cladosporium fulvum} (causing leaf mold disease) and \textit{Ventricillium dahliae} (causing vascular wilt disease) is mediated by LRR-containing RLPS (25, 26). Cf proteins confer immunity upon recognition of \textit{C. fulvum} race-specific secreted effectors [also referred to as avirulence (Avr) proteins] (27), whereas Ve1 recognizes the Ave1 effector protein secreted by race 1 \textit{V. dahliae} strains (28). Cf-9 was the first identified RLP (19), and since its discovery several attempts have been made to understand Cf-mediated defense signaling by identifying Cf-interacting proteins. Yeast two-hybrid analyses resulted in the isolation of several potential interactors of the cytoplasmic C terminus of Cf-9 (29–31). However, an RLK or Croyne-like protein,
recruited by Cf proteins and providing a cytoplasmic kinase domain through which Cf-mediated signaling would occur, remained to be identified (20). Recently, by immunopurification of a functional Cf-4–enhanced green fluorescent protein (eGFP) fusion protein from plants, we identified endoplasmic reticulum (ER)-resident chaperones as in planta interactors of Cf proteins that are required for Cf protein biogenesis (32). Here, following a similar approach, we describe the identification of the tomato ortholog of the Arabidopsis RLK Suppressor Of BIR1-1/Evershed (SOBIR1/EVR; hereafter referred to as SOBIR1) (33, 34) and its close homolog SOBIR1-like as Cf interactors. Interestingly, both tomato homologs and Arabidopsis SOBIR1 interact with Ve1, in addition to Cf-4, and we show that SOBIR1 is required for Cf-2–, Cf-4–, and Ve1–mediated immunity. Our work reveals an essential role for SOBIR1 in the plant immune response activated by two distinct RLPs involved in resistance to fungal pathogens and suggests that SOBIR1 functions as an essential regulatory RLK of this type of cell-surface receptors.

Results

Identification of Tomato SOBIR1 and SOBIR1-like as Interactors of Cf-4 and Ve1. To identify Cf-interacting proteins, we stably transformed Money Maker (MM)–Cf-0 tomato, lacking Cf resistance genes to C. fulvum, with a construct driving constitutive expression of a Cf-4–eGFP fusion protein (32). Transgenic line (TL) TL3 showed recognition resulting in a specific HR upon infiltration with the C. fulvum Avr4 effector, whereas TL21 did not show a response to Avr4 (Fig. S1A). Cf-4–eGFP was successfully immunopurified from TL3, whereas the fusion protein could not be detected in TL21 (Fig. S1B). To identify proteins copurifying with Cf-4, trypic on-bead digestion of the purified proteins was performed, and the generated peptides were analyzed by mass spectrometry. Interestingly, in the sample originating from TL3, but not in the one from TL21, in addition to peptides originating from Cf-4–eGFP itself, peptides matching to two tomato RLKs encoded by Solyc06g071810.1 and Solyc03g111800.2 were identified (Table S1). The alignment presented in Fig. S2A show that the amino acid sequences of these tomato RLKs are highly homologous to each other (~74% identical) and are closely related to the Arabidopsis RLK SOBIR1 (~60% identity). Both tomato RLKs are more distantly related to S. lycopersicum (S)SERK3a/BAK1 (~25% identical) (33, 34). Fig. S2B also shows that the nucleotide sequences of both tomato RLKs and A. thaliana (At)SOBIR1 are very similar throughout their coding regions. Hence, we named the genes encoding the two tomato RLKs SSOBIR1 and SSOBIR1-like. Similar to AtSOBIR1, SSOBIR1 and SSOBIR1-like have five predicted LRRs, in contrast to SERSK3a/BAK1, which has only four LRRs. The SOBIR1 sequences of tomato and Arabidopsis are highly similar, both in their extracellular LRR and cytoplasmic kinase domains, whereas the homology of SOBIR1 to SERSK3a/BAK1 is mostly restricted to their kinase domains (Fig. S2A). No peptides originating from any other RLKs were identified in the peptide sample originating from TL3. Cf-4–eGFP is also functional in Nicotiana benthamiana (32), and immunopurification of transiently expressed Cf-4–eGFP from this plant also yielded peptides from copurifying RLKs potentially matching SOBIR1 and SOBIR1-like (Table S2). The presence of SSOBIR1 orthologs in N. benthamiana and Nicotiana tabacum was assessed by searching public databases, indeed revealing two candidate N. benthamiana homologs, referred to as NbSSOBR1 and NbsSOBR1-like, and one N. tabacum homolog (NsSOBR1) (Fig. S2C). To also identify proteins interacting with Ve1, a eGFP-tagged Ve1 (35) was immunopurified upon its transient expression in N. benthamiana. Also for this RLP, peptides matching NsSOBR1 and NsSOBR1-like were identified, whereas again no peptides from other RLKs were detected (Table S3).

Tomato SOBIR1 and SOBIR1-like and Arabidopsis SOBIR1 Interact with Cf-4 and Ve1. C-terminally Myc epitope-tagged versions of the tomato and Arabidopsis SOBIR1 RLKs (SSOBIR1–Myc, SSOBIR1-like–Myc, and AtSOBIR1–Myc) were generated to perform coimmunopurification experiments with Cf and Ve1. Transient coexpression in N. benthamiana revealed that all three SOBIR1 proteins interact with Cf-4 and Ve1 (Fig. 1 and Fig. S1C). Coexpression of constructs encoding SSOBIR1–eGFP and Cf-4–Myc similarly revealed interaction of Cf-4–Myc with SSOBIR1–eGFP (Fig. S3A). We then examined whether the SOBIR1 proteins also interact with RLKs known to be involved in defense and/or development. Interestingly, C-terminally (e)GFP-tagged SERSK1, SERSK3a/BAK1 (36), SFLS2 (37), or AtCLV1 (38), did not copurify with SOBIR1 (Fig. 1 and Fig. S1C).

To determine whether SOBIR1 requires a functional kinase domain for interaction with Cf-4, the core catalytic aspartate (D) of its conserved RD kinase motif was substituted to an asparagine (N) residue. For all tested RLKs containing the catalytic D, among which is SERK3a/BAK1, this mutation causes a loss of kinase activity (39). Interestingly, C-terminally Myc-tagged SSOBIR1 D473N/SSOBIR1-like D486N, and AtSOBIR1 D489N all still interact with Cf-4–eGFP, showing that kinase activity of SOBIR1 is not required for interaction with the RLP (Fig. S3B). It was subsequently tested whether the presence of the Cf-4 ligand, Avr4, would lead to loss of the interaction between SOBIR1 and Cf-4. Cf-4–eGFP and SSOBIR1–Myc were transiently coexpressed with Avr4 or the nonrecognized effector Avr9 infiltrated at two different optical densities. Interaction between Cf-4 and SSOBIR1 was still observed in the presence of Avr4 and Avr9, indicating that the Cf-4/SSOBIR1 complex does not dissociate upon recognition of Avr4 by Cf-4 (Fig. S3C). We further studied whether SOBIR1 forms homodimers and/or heterodimerizes with SSOBIR1–like or AtSOBIR1. For this experiment, SSOBIR1–eGFP was coexpressed with SSOBIR1–Myc, SSOBIR1-like–Myc, or AtSOBIR1–Myc, whereas coexpression with Cf-4–Myc was used as a control. Upon pull-down of SSOBIR1–eGFP, Cf-4–Myc strongly copurified with the RLP. However, we did not observe copurification of SSOBIR1–Myc, SSOBIR1-like–Myc, or AtSOBIR1–Myc, and in addition revealed self-interaction of Cf-4–eGFP with SSOBIR1–Myc and SSOBIR1-like–Myc (Fig. S3D). We also determined that SSOBIR1–Myc and SSOBIR1-like–Myc both interact with three RLKs in the plant immune system, whereas coexpression with Cf-4–Myc was used as a control. Upon pull-down of SSOBIR1–eGFP, Cf-4–Myc strongly copurified with the RLP. However, we did not observe copurification of SSOBIR1–Myc, SSOBIR1-like–Myc, or AtSOBIR1–Myc, and in addition revealed self-interaction of Cf-4–eGFP with SSOBIR1–Myc and SSOBIR1-like–Myc (Fig. S3D).
indicating that SOBIR1 does not form homo- or heterodimers with NtSOBIR1-like or AtSOBIR1 (Fig. S3D).

**NSOBIR1 Localizes to the Plasma Membrane and Cytoplasmic Vesicles.**

It has been reported that AtSOBIR1-YFP, when expressed under control of its own promoter in Arabidopsis, localizes to the plasma membrane and internal membrane compartments of epidermal leaf petiole cells and epidermal root cells (33). Confocal-laser scanning microscopy performed on N. benthamiana epidermal leaf cells transiently expressing NSOBIR1-eGFP under control of the 35S promoter revealed that NSOBIR1 mainly localizes to the plasma membrane (Fig. S4A). In addition, fluorescence signals were observed in mobile cytoplasmic vesicles (Fig. S4A). As previously shown, the GFP-HA control protein localizes to the cytoplasm and nucleus, whereas SfL52-eGFP localizes to the plasma membrane (37) (Fig. S4 B–D).

**Targeting SOBIR1 Compromises the Cf-4/Avr4-induced and Ve1/Ave1-induced HR.**

The observation that the two SOBIR1 homologs from tomato and N. benthamiana interact with Cf-4 and Ve1 (Fig. 1, Fig. S1C, and Tables S1–S3) suggests that both proteins play a role in Cf-4- and Ve1-mediated defense signaling in Solanaceous plants. Therefore, recombinant tobacco rattle virus (TRV)-based virus-induced gene silencing (VIGS) constructs were generated to target expression of the NtSOBIR1 homolog, either individually or simultaneously (Fig. S2C), and transgenic N. benthamiana expressing Cf-4 was inoculated with the different TRV constructs. Three weeks after viral inoculations, plants were transiently transformed to express Avr4 (40). Inoculation with TRV: NbSOBIR1/NbSOBIR1-like resulted in a severely compromised Avr4-triggered HR, similar to inoculation with a TRV construct targeting Cf-4 itself (TRV:Cf-4) (Fig. 2). The Avr4-triggered HR was also strongly compromised when NbSOBIR1 was targeted. When NbSOBIR1-like was targeted, the HR was affected to a much lesser extent (Fig. 2). Quantitative RT-PCRs (qRT-PCRs) revealed that expression of NbSOBIR1 was strongly reduced upon inoculation with TRV:NbSOBIR1/NbSOBIR1-like or TRV:NbSOBIR1, compared with inoculation with TRV:p-glucuronidase (GUS) (Fig. S5 A and B). Interestingly, we did not detect transcripts of NbSOBIR1-like in TRV:GUS-inoculated or TRV:NbSOBIR1/NbSOBIR1-like-inoculated plants, suggesting that NbSOBIR1-like is not expressed or is at a very low level. We therefore reasoned that the slight reduction of the Avr4-triggered HR upon inoculation of N. benthamiana:Cf-4 with TRV:NbSOBIR1-like (Fig. 2) could be attributed to cross-silencing of NbSOBIR1 by the TRV: NbSOBIR1-like construct. Indeed, qRT-PCR confirmed that NbSOBIR1 expression levels were ~30% reduced upon inoculation with TRV:NbSOBIR1-like (Fig. S5B). Together these results indicate that NbSOBIR1 is the RLK that is required for the Cf-4-mediated HR in N. benthamiana. The Cf homolog Pen2 from Solanum peruvianum is autoactive in N. benthamiana, causing an effector-independent HR when transiently expressed (41). Interestingly, the Pen2-eGFP-triggered HR was also strongly compromised upon expression in TRV:NbSOBIR1/NbSOBIR1-like–inoculated N. benthamiana plants (Fig. S6A). To check whether the silenced plants were still able to mount programmed cell death, fully expanded leaves were also transiently transformed to express an autoactive variant of the Nucleotide Binding (NB–LRR) immune receptor Rx (RxD460V) (42) and the proapoptotic factor Bcl2-Associated protein X (BAX) (43). Because RxD460V and BAX still triggered a strong cell death, we concluded that the ability of the plants to mount programmed cell death was not compromised (Fig. 2).

Unlike in N. benthamiana, coexpression of Ve1 with Ave1 triggers an HR in N. tabacum, a plant for which TRV-based VIGS was recently established (28, 35). N. tabacum plants (cultivar Samsun) were inoculated with TRV:NbSOBIR1/NbSOBIR1-like, which also targets the NtSOBIR1 homolog (Fig. S2C), and TRV:Enhanced Disease Susceptibility 1 (EDS1) as a positive control, because EDS1 is required for Ve1-mediated immunity (26). Inoculation with TRV:GFP was included as a negative control. We used the TRV: NbSOBIR1/NbSOBIR1-like construct because we anticipated that N. tabacum, of which the currently available genome sequence is very similar to that of N. benthamiana, may contain an NtSOBIR1-like homolog in addition to NbSOBIR1, although we did not identify an NtSOBIR1-like candidate in public databases. Three weeks after inoculation with the different recombinant TRV constructs, Ve1 and Ave1 were coexpressed, revealing that plants inoculated with the VIGS constructs targeting NtSOBIR1/NtSOBIR1-like and EDS1 did not mount an HR, in contrast to the TRV-GFP-inoculated plants (Fig. S6B). Together, these results show that SOBIR1 is required for Cf-4- and Pen2-mediated HR in N. benthamiana and Ve1-mediated HR in N. tabacum.

**Kinase Activity of SOBIR1 Is Required for Cf-4–Mediated HR.** To determine whether SOBIR1 requires a functional kinase domain for the Cf-4-mediated HR, we inoculated N. benthamiana:Cf-4 with TRV:NbSOBIR1/NbSOBIR1-like. These plants were then spot-infiltrated to transiently express the combinations Avr4 and AtSOBIR1–Myc or Avr4 and AtSOBIR1D489N–Myc. As a control, GUS was expressed in combination with Avr4. We reasoned that AtSOBIR1 would not be targeted by this RNA silencing because there is not sufficient sequence homology between the NbSOBIR1 genes and AtSOBIR1, and therefore AtSOBIR1, being a functional homolg of NbSOBIR1, would complement the loss of NbSOBIR1 and reconstitute the Avr4-triggered HR. However, if SOBIR1 kinase activity is required for Cf-4–mediated HR, AtSOBIR1D489N–Myc would not be able to complement.

Expression of GUS with Avr4 in the NbSOBIR1-silenced plants did not restore the Cf-4-mediated HR (Fig. S5C). When AtSOBIR1–Myc was coexpressed with Avr4, an HR was observed. However, when the kinase-dead mutant AtSOBIR1D489N–Myc was coexpressed with Avr4, the Avr4-triggered HR was strongly compromised, indicating that SOBIR1 kinase activity is required for Cf-4–mediated HR (Fig. S5C). RT-PCR analysis showed that full-length AtSOBIR1–Myc and AtSOBIR1D489N–Myc transcripts were present in the plants inoculated with TRV:NbSOBIR1/NbSOBIR1-like (Fig. S5D), confirming that Arabidopsis SOBIR1 is indeed not targeted by the VIGS construct. These results show that AtSOBIR1 complements NbSOBIR1 and the C-terminal Myc epitope tag does not appear to affect AtSOBIR1 function with respect to its role in Cf-4–mediated HR. Importantly, these results show that SOBIR1 kinase activity is required for the Cf-4–mediated HR.

**SOBIR1 Is Required for Cf- and Ve1-Mediated Resistance to C. fulvum and V. dahlieae, Respectively.** To determine whether SOBIR1 is required for Cf-4–mediated resistance of tomato to C. fulvum,
TRV constructs targeting tomato SISOBIR1 and SISOBIR1-like individually or both genes simultaneously were generated (Fig. S2B). As a positive control, plants were inoculated with TRV: Cj-4, whereas TRV:GUS-inoculation served as a negative control. Cj-4-expressing tomato was inoculated with the different TRV constructs, and 3 wk later, plants were inoculated with a race 5 strain of C. fulvum, expressing Avr4 and the GUS reporter gene. To detect fungal colonization, leaflets were GUS-stained after 2 wk. Inoculation with constructs targeting the two SISOBIR1 homologs either individually or simultaneously, resulted in increased fungal colonisation as indicated by the much higher number of successful colonization attempts compared with the TRV:GUS-inoculated plants. This result shows that both tomato SOBIR1 homologs contribute to Cj-4-mediated resistance (Fig. 3). We also targeted both SISOBIR1 homologs in tomato expressing Cj-2.2 and inoculated these plants with the same C. fulvum strain as used above, because this race 5 strain also expresses Avr2. Also in this case, increased fungal colonization was observed compared with the TRV:GUS control (Fig. S6C).

To test the role of the SISOBIR1 homologs in resistance to V. dahliae, tomato cultivar Motelle that carries the Ve1 gene was also inoculated with TRV:SISOBIR1, TRV:SISOBIR1-like, and TRV:SISOBIR1/SISOBIR1-like. As controls, plants were inoculated with TRV:Ve1 and TRV:GFP. Three weeks after TRV inoculation, plants were either inoculated with a race 1 strain of V. dahliae expressing Avr1 or mock-treated and subsequently monitored for development of disease symptoms (e.g., stunted growth and reduced canopy area). Targeting of the two SISOBIR1 homologs either individually or simultaneously, as well as Ve1 itself, resulted in clear stunting and a strongly reduced canopy area compared with the mock-treated plants. These disease symptoms were not observed in plants inoculated with TRV:GFP (Fig. S6D).

SISOBIR1 Is Required for Ve1-Mediated Resistance to V. dahliae in Arabidopsis. Ve1 provides resistance to V. dahliae when introduced in Arabidopsis (44). To study the requirement of ArSOBIR1 for Ve1-mediated resistance in this plant, we tested whether Ve1 still mediates resistance to V. dahliae in an Arabidopsis sobir1-1 mutant (34). Similar to the Columbia 0 (Col-0) wild-type, the sobir1-1 mutant is susceptible to V. dahliae race 1, as shown by the stunted appearance and chlorosis upon fungal infection (Fig. 4). When transformed with the Ve1 gene, the Col-0 wild-type gains resistance to V. dahliae race 1 (Fig. 4). Strikingly, when the Ve1 gene was introduced into the sobir1-1 mutant background, the plants did not gain resistance to the pathogen, because stunting and chlorosis were still observed after inoculation with the fungus (Fig. 4). Quantitative measurement of fungal biomass confirmed these results, because only in the Col-0 wild-type plants transformed with Ve1 fungal colonization was very limited (Fig. S7A). This result indicates that in addition to its requirement in tomato, SOBIR1 is required for Ve1-mediated resistance to V. dahliae in Arabidopsis.

Targeting SOBIR1 in N. benthamiana Leads to Reduced Cf-4 and Ve1 Protein Levels. To investigate whether targeting SOBIR1 affects Cf-4 and Ve1 protein levels, we inoculated N. benthamiana with TRV:NbSOBIR1/NbSOBIR1-like or the control TRV:GUS; and after 3 wk fully expanded leaves were transiently transformed to individually express eGFP-tagged Cf-4 or Ve1. Subsequently, the steady-state levels of the RLPs were determined by their immunopurification and detection by immunoblotting. Both Cf-4 and Ve1 protein levels were strongly reduced upon targeting SOBIR1, compared with the TRV:GUS-inoculated plants, indicating that SOBIR1 is required for the accumulation of Cf-4 and Ve1, and thus stabilizes these RLPs (Fig. 5). RT-PCRs revealed that Cf-4 and Ve1 are normally expressed in plants inoculated with TRV:NbSOBIR1/NbSOBIR1-like and TRV:GUS, indicating that reduced accumulation of the Cf-4 and Ve1 proteins is not due to reduced expression levels (Fig. S7B).

Tomato SOBIR1 Homologs Interact with a Broad Range of RLPs. To test whether the tomato SOBIR1 homologs interact with additional RLPs, Cf-2.2, Cf-4E, Cf-9, and the Cf-like protein Pera2 from S. pennavium were coexpressed as eGFP fusions with SISOBIR1–Myc or SISOBIR1-like–Myc in N. benthamiana. This experiment revealed that both SOBIR1 homologs copurify with the various Cf proteins (Fig. S8A). We expanded our study and examined whether more distantly related tomato RLPS also interact with the tomato SOBIR1 homologs. We fused SIEIX2 (45), mediating perception of the ethylene-inducing xylanase from Trichoderma viride, and the closest tomato orthologs of Arabidopsis CLV2 (Solyc04g066640.1), TMM (Solyc12g042700.1), and the Suppressor of Non-expressor of pathogenesis-related genes 1-1 (Npr1-1), Constitutive 2 (SNC2; Solyc02g072250.1) (46) to eGFP and coexpressed them with the Myc-tagged SOBIR1 homologs in N. benthamiana. Immunopurification of the RLPs revealed that SIEIX2, S1CLV2, and S1TMM, but not S1SNC2, interact with SISOBIR1 and SISOBIR1-like (Fig. S8B).

Discussion

For signal initiation by Cf proteins, a mechanistic model was proposed based on the early model of the Claual (CLV1) signaling pathway, in which the RLP CLV2 interacts with the RLK CLV1. This RLK acts as a coreceptor that allows binding of the extra-cellular endogenous ligand CLV3 and subsequently mediates
downstream signaling through its kinase domain (20, 47). Here, we report that the RLK SOBIR1 interacts with various RLPs of tomato, including the Cf proteins, Ve1, and SfEIX2, which are all involved in immunity, as well as the tomato homologs of Arabidopsis FYTMM and SLCL12, which are involved in development (Fig. 1 and Figs. S1C and S8). However, not all RLPs interact with SOBIR1, as is exemplified by SfSNC2 (Fig. S8B). In addition, no interaction of SOBIR1 with any of the tested RLKs was found (Fig. 1 and Fig. S1C). We show that SOBIR1 is required for Cf-2.2, Cf-4, and Ve1-mediated immune responses (Figs. 2–4 and Figs. S5 and S6).

SOBIR1 was initially identified in a suppressor screen of the Arabidopsis bak1-interacting receptor kinase 1-1 (bir-1) mutant and was referred to as Suppressor Of Bir1-1 (34). Bir1 encodes another RLK, which interacts with SERK3/BAK1, and the bir-1 mutant shows a constitutive defense phenotype, indicating that Bir1 is a negative regulator of defense responses. The bir-1 phenotype is suppressed by the sobir1-1 mutation, suggesting that SOBIR1 is a positive regulator of defense signaling (34). In line with this finding, overexpression of SOBIR1 in Arabidopsis leads to constitutive defense activation (34). Although no direct interaction between SOBIR1 and Bir1 was observed, it was hypothesized that Bir1 functions in a signal transduction pathway that is dependent on SOBIR1 and which promotes pathogen resistance and cell death (34). As mentioned above, a mutation in AtSOBIR1 suppresses the bir-1 phenotype, whereas an additional mutation in At Phytoalexin Deficient 4 (PAD4) fully reverts the bir-1 sobir1-1 mutant phenotype back to that of wild-type plants. It was suggested that Bir1 regulates two parallel pathways— one involving resistance proteins that are dependent on PAD4, such as the Toll–Interleukin 1 Receptor (TIR)–NB–LRRs, and one involving another class of resistance proteins requiring SOBIR1 (34). We propose that the RLKs are members of this latter class of resistance proteins. We also observed in planta interaction of SOBIR1 with RLPs involved in development. Indeed, a role of SOBIR1 in development has been described. Arabidopsis mutants in the gene encoding the ADP ribosylation factor GTPase-activating protein Nevershed (NEV) show impaired floral organ shedding after flowering (48). A screen for mutations in nev plants that restore organ shedding identified a mutation in SOBIR1 resulting in premature floral organ shedding. Hence, the name Evershed (EVR) was coined as a synonym for this RLK, which in this case functions as an inhibitor of abscission (33). Because SOBIR1/EVR was found to localize to the plasma membrane and cytoplasmic vesicles, it was proposed that the RLK regulates the signaling and internalization of other ligand-binding RLKs involved in floral organ shedding (33). Interestingly, when transiently expressed in N. benthamiana, we likewise found SfSOBIR1–GFP to localize to the plasma membrane and mobile, cytoplasmic vesicles (Fig. S4). Similar to SOBIR1, SERK3/BAK1 also plays a role both in development and defense, and this RLK was initially identified as an interactor of the RLK BR1, which is involved in brassinosteroid (BR) perception and signaling (49, 50). SERK3/BAK1 was also identified as a regulator of the RLK-type PRRs FLS2 (11, 13), EFR (12), and PEP1 Receptor protein-1, an RLK involved in perceiving endogenous peptides (51). Because Cf and Ve1 interact with SOBIR1 in planta and require SOBIR1 for mediating HR and resistance, it is tempting to speculate that SOBIR1 is involved in signaling and possible internalization of RLP-containing immune receptor complexes, similar to the function of SERK3/BAK1 in relation to RLKs involved in defense (52).

The current paradigm for several LRR–RLK-type PRRs is their rapid heterodimerization with SERK3/BAK1 upon ligand perception (11–13). By contrast, interaction between SOBIR1 and the various RLPs studied here is ligand-independent, because we did not coexpress the corresponding ligands in most of our communopurification experiments and still detected copurification of SOBIR1 with the RLPs (Fig. 1 and Figs. S1C and S8). In addition, the presence of Avr4 did not affect the interaction of Cf-4 with SOBIR1 (Fig. S3C). Through mutation of its highly conserved RD motif, we showed that a functional SOBIR1 kinase domain is required for Cf-4–dependent HR (Fig. S5C), but not for interaction with Cf-4 (Fig. S3B). Possibly, the phosphorylation status of SOBIR1 changes upon ligand perception by Cf proteins, thereby allowing additional proteins to associate with the complex. Such proteins could be the previously identified Cf interactors Cf-9–Interacting Thioredoxin (CITRX) (31), the protein kinase Avr9/Cf-9–Induced Kinase 1 (ACIK1) (29), the Soluble N-ethylmaleimide-sensitive factor Adaptor protein Receptor (SNARE) protein Vesticle-Associated Protein 27 (VAP27) (30), and RLKs that reside in the active Cf-containing receptor complex. For example, recently it was shown that SERK1 is also required for Cf-4–mediated resistance of tomato. Furthermore, SERK1 and SERK3/BAK1 are both required for full Ve1-mediated resistance (26, 44). Because SOBIR1 constitutively interacts with a broad range of RLKs, either involved in defense or in development, it may be that SOBIR1 functions as a scaffold protein stabilizing receptor complexes in which RLKs take part. Alternatively, SOBIR1 could play a role as an integral part of the signaling pathway triggered by RLKs involved in different processes. In that case, downstream signaling specificity might be determined by the particular phosphorylation status of the cytoplasmic kinase domain of this regulatory RLK. For example, recent characterization of the bak1-5 mutation in Arabidopsis revealed that the function of SERK3/BAK1 in MTI, the BR response and cell death control can be mechanistically uncoupled (39). The bak-1 mutation is in the kinase domain of SERK3/BAK1 and results in strongly impaired FLS2- and EFR-mediated immune signaling but does not affect BR signaling and the control of the cell death response (39). Such a situation might also hold for SOBIR1 in relation to signaling triggered by the different RLKs.

Together, our studies support the existence of a SOBIR1/RLP complex in planta, in which SOBIR1 is required for RLP-mediated immunity against two fungal pathogens that exhibit a different lifestyle. SOBIR1 appears to function as a regulatory RLK for RLP-containing immune receptor complexes in plants. Future experiments focusing on the cell biology of SOBIR1 and determination of its phosphorylation status and downstream interactors, in the presence and absence of the ligand that is perceived by the interacting RLP, should specify the precise role of SOBIR1 in RLP-containing signaling complexes.
Materials and Methods

Plant Materials and Growth. Growth conditions for N. benthamiana, A. thaliana, and S. lycopersicum (tomato) are described in SI Materials and Methods.

Primers and Vector Construction. Sequences of primers and corresponding targets can be found in Table S4. Construction of plasmids containing Cf-2.2–4, Agrobacterium C. fulvum locus of potato.

Plant Transformations. Plasmid p8Bin-KS-35S::Cf-4::eGFP (Sol 2701) was used for transformation of tomato MM-Cf-0, which does not carry a functional Cf-4 gene. Transformations and plant selections were performed as described in SI Materials and Methods.

Protein Immunopurification and Identification. Immunopurifications were essentially performed following the protocol described with minor modifications [32]. Immunopurifications from stable transgenic tomato expressing Cf-4::eGFP were performed as described in SI Materials and Methods.

VIGS and Disease Assays. VIGS experiments in N. benthamiana, tobacco, and tomato were performed as described [32, 35]. C. fulvum disease assays were performed as described [32], and V. dahliae disease assays were performed as described in SI Materials and Methods.

Further experimental details can be found in SI Materials and Methods.

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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 *Cj* 2.2. (2) and *Cj*-4 (Hcr-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 birt-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, -8, -Peru2, and Ve1, C-terminally fused to either eGFP or the Mxy epitope-tag, has been described (4, 9). *S. hysarcocarpus* Flagellin Sensing 2 (SFLS2)–GFP was expressed from pCAMBIA2300–FLS2:T::SFLS2–GFP (10). For novel constructs, coding regions were amplified from cDNA. PCR fragment *S. hysarcocarpus* Somatic Embryogenesis Receptor Kinase 3a (*SISERK3a*)/BAK1 was cloned in pDONR201 by using Gateway BP Clonase II (Invitrogen). Fragments *SISERK7, SIE1X2, SISOBIR1, SISOBIR1-like, and Arabidopsis thaliana* (Arab) SISOBIR1 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 SISOBIR1, SISOBIR1-like, and *AtSISOBIR1* 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 pGW2B0 (12) (for C-terminally tagging with the Mxy epitope), by using Gateway LR Clonase II (Invitrogen). This process resulted in plasmids pBIN-KS-35S::At-CLV1–eGFP (Sol 2824), pBIN-KS-35S::SI-CLV2–eGFP (Sol 2782), pBIN-KS-35S::SI-EIX2–eGFP (Sol 2863), pBIN-KS-35S::SI-Suppressor of Non-expressor of pathogenesis-related genes 1-1 (Npr1-1), Constitutive 2 (NC2)–eGFP (Sol 3109), pBIN-KS-35S::SI-Too Many Mouths (TMM)–eGFP (Sol 3110), pBIN-KS-35S::SI-SOBIR1–eGFP (Sol 2774), pGW2B0-SI-SOBIR1–Myc (Sol 2754), pGW2B0-SI-SOBIR1-like–Myc (Sol 2752), pGW2B0-At-SOBIR1–Myc (Sol 2849), pGW2B0-SI-SOBIR1D473N–Myc (Sol 2878), pGW2B0-SI-SOBIR1-like D486N–Myc (Sol 2879), and pGW2B0-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 CS5C1, carrying helper plasmid pCH32. Infiltration of *Agrobacterium* into plant leaves (agroinfiltration) was performed as described at OD600 = 1, unless indicated otherwise (13).

Protein Identification by Immunopurification, Followed by Trypsin 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% IGE Pal 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-eMyc (cMye 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, restriction enzymes EcoRI and BamHI and cloned into pTRV2:RNA2, linearized by using either α-GFP–HRP (130-091-833; MACS Antibodies) or α-eMyc (cMye 9E10, sc-40; Santa Cruz) with α-mouse–HRP (Amersham) as a secondary antibody.

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:EDS1* (4) (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 Pen2-eGFP at OD$_{600}$ = 1. For complementation analysis with ArsOBIR1–Myc and the respective D48N 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 Her9-4D (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 SISOBIR1 (like Cf-4), or GUS. Nonagron inoculated MM-Cf-4 plants served as fully susceptible controls. For V. dahliae disease assays, tomato cultivar Motelle carrying the Vel gene and resistant to V. dahliae race 1 strains expressing Ave1, was used. TRV:Vel 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 |
|------------------|-------------------------|-------------------|
| SlSOBIR1         | DLKPANILDDDEMAR         | 40.9              |
| SlSOBIR1         | EDGVASLEMGK             | 62.3              |
| SlSOBIR1         | ITQSPMDAAABTEDTK        | 53.5              |
| SlSOBIR1         | KAELAFLSKE             | 78.9              |
| SlSOBIR1         | VADFGGLAK              | 62.2              |
| SlSOBIR1-like    | DAAELTEEDSK             | 65.1              |
| SlSOBIR1-like    | DLKPGVLLDDDEMAR         | 57.0              |
| SlSOBIR1-like    | IADFGGLAK              | 66.7              |
| SlSOBIR1-like    | NDPGLTIFSPLIK           | 42.7              |
| SlSOBIR1-like    | NMCTSEDPRN             | 50.9              |
| Cf-4–eGFP        | EIDESTGFPFYEISDPYDIYNYLTTISTK | 28.7          |
| Cf-4–eGFP        | FEGHIPSIIIGDLVGLR      | 39.8              |
| Cf-4–eGFP        | FGEFSDIHDLHSSFR        | 74.3              |
| Cf-4–eGFP        | GIPINSLNNQK            | 60.3              |
| Cf-4–eGFP        | ILDSNMIINLSK           | 78.3              |
| Cf-4–eGFP        | ILGLNQTMRE             | 64.7              |
| Cf-4–eGFP        | ISLNEFLPGPHNFEILLLK    | 59.0              |
| Cf-4–eGFP        | LDLSYNDFTPSPISPK        | 52.7              |
| Cf-4–eGFP        | LYLVNNIDDR             | 55.6              |
| Cf-4–eGFP        | NEYLSDLHSNRR           | 65.3              |
| Cf-4–eGFP        | SSQGNFLMGLQIDLOHSSNGFSGNSPLPER | 30.9          |

SlSOBIR1, Solyc06g071810.1.1; SlSOBIR1-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 NbSOBIR1 or matching both NbSOBIR1 and NbSOBIR1-like, of a tryptic digest of immunopurified Cf-4-eGFP transiently expressed in *N. benthamiana*

| Protein name* | Peptide sequence | Mascot ion score† | ProteinLynx score‡ |
|---------------|------------------|-------------------|--------------------|
| NbSOBIR1/SOBIR1-like | ASMPAPAPAPAPVR | 29.3 |  |
| NbSOBIR1/SOBIR1-like | DGSLQDILQQVTEGTR | 32.3 |  |
| NbSOBIR1/SOBIR1-like | ELDMWGR | 59.0 |  |
| NbSOBIR1/SOBIR1-like | INLYPPDHAAALLLVQK | 33.3 |  |
| NbSOBIR1/SOBIR1-like | LSLADNMFTGK | 69.8 |  |
| NbSOBIR1/SOBIR1-like | NGSLQDILQQVTEGTR | 48.4 |  |
| NbSOBIR1/SOBIR1-like | NGSLQDILQQVTEGTR | 78.5 |  |
| NbSOBIR1/SOBIR1-like | NHTQRI | 45.5 |  |
| NbSOBIR1/SOBIR1-like | NLEKILSLADN | 31.6 |  |
| NbSOBIR1/SOBIR1-like | YFPNLEK | 56.4 |  |
| NbSOBIR1 | INLYPPDHAAALLLVQK | 50.8 |  |
| NbSOBIR1 | KLEILDLQNLFSGK | 59.4 |  |
| NbSOBIR1 | SEIQILQIR | 68 |  |
| NbSOBIR1 | NGSLQDILQQVTEGTR | 100.5 |  |
| NbSOBIR1 | DLAQPANVLLDDMEAR | 96.4 |  |
| NbSOBIR1 | LPSQEEFQHTQFGPMLV| 98.3 |  |
| NbSOBIR1 | NVNTSEDPK | 74.7 |  |
| NbSOBIR1/SOBIR1-like | LIOGSEQQMLVLK | 62.4 |  |
| NbSOBIR1 | IACFCFLNPK | 74.7 |  |
| NbSOBIR1 | LGIQQQQ | 38 |  |
| NbSOBIR1/SOBIR1-like | SNGLSGTLPAGK | 82.7 |  |
| NbSOBIR1 | ILDLSSNELSNLNLFLK | 96 |  |
| NbSOBIR1 | GKTDGSLTIYSPILK | 108.6 |  |
| NbSOBIR1 | GCVCVYRAELPGSNGK | 49.9 |  |
| NbSOBIR1/SOBIR1-like | KILQPMDAAELAEEDTKALNK | 61.2 |  |
| NbSOBIR1/SOBIR1-like | AVDQPQAHHTVVTVTVTVGTYIAPEYHQTLK | 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 *SlSOBIR1* and *NbSOBIR1* homologs, no distinction between *NbSOBIR1* and *NbSOBIR1*-like can be made. Mascot identifications were based on translated tobacco EST sequences, and peptides can match either *NbSOBIR1* or *NbSOBIR1*-like. When specifically matching to the corresponding *N. benthamiana* homolog, the protein name is indicated. When no distinction can be made, *NbSOBIR1/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 NbSOBIR1, NbSOBIR1-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 |
|---------------|------------------|-----------------|
| NbSOBIR1-like | ILDLSSNELSGLNFLK | 83.6            |
| NbSOBIR1      | KLEILDLGNNLPSGK  | 76.2            |
| NbSOBIR1      | GKTGDSLTIYSPLIK  | 109.2           |
| NbSOBIR1/SOBIR1-like | ILQPMPDAEAEDETK | 62.8            |
| NbSOBIR1      | DLKPAVVLDDDMEAR  | 82.7            |
| NbSOBIR1      | AVPDAHTVTTSNVAATGTAPEYHQTLK | 45.9 |
| NbSOBIR1      | NMTSEDPPKR       | 45.9            |
| NbSOBIR1      | LPSDEFQHPEMSLVK  | 46.9            |
| NbSOBIR1      | LSLADNMFIIK      | 83.8            |
| NbSOBIR1      | SNGLSGTLSPAIAGK  | 49.8            |
| NbSOBIR1      | TDDSLTIVYPLIK    | 74              |
| Ve1–eGFP      | SLLLQFKGSLQYDSTLSK | 35.7          |
| Ve1–eGFP      | YINLSNAGFGVQIPITLSR | 65           |
| Ve1–eGFP      | LVTLDLSTILPFDQPLK | 58.7          |
| Ve1–eGFP      | DCQISGQDLESLSK   | 55.5            |
| Ve1–eGFP      | G55IQFFIVQVD     | 62              |
| Ve1–eGFP      | VLSLLSNPFPR      | 63              |
| Ve1–eGFP      | LGLYINLGNKK      | 63.4            |
| Ve1–eGFP      | LLEEVMGNNR       | 75.1            |
| Ve1–eGFP      | SNLVVDLgHSNR     | 88.6            |
| Ve1–eGFP      | STVNCKLEELNVGNR  | 77.4            |
| Ve1–eGFP      | GMMVADDVETGR     | 81.1            |
| Ve1–eGFP      | LSYQDVTVLTIK     | 75.6            |
| Ve1–eGFP      | VFTSIDFSQR       | 73.2            |
| Ve1–eGFP      | GEELFTGVPILVLEDGVDNGHK | 78.7 |
| Ve1–eGFP      | FSVGEGEDDATYKTLTK | 42.8          |
| Ve1–eGFP      | MPEGVQER         | 601.1           |
| Ve1–eGFP      | YPDMMQPDFOFK     | 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 NbSOBIR1 or NbSOBIR1-like. When specifically matching to the corresponding N. benthamiana homolog, the protein name is indicated. When no distinction can be made, NbSOBIR1/SOBIR1-like is indicated.
Table S4. Sequences of oligonucleotide primers used in this study

| Primer code | Sequence, 5′–3′ | Target sequence |
|-------------|-----------------|-----------------|
| Ro1         | CACCATGGCTCCATGTTCTCTTC | SISNC2          |
| Ro2         | TTTACAACTTGGAACATTTAAC | SISNC2          |
| Ro6         | CACCATGGGCTTTTTCTCTCGAATA | SITMM          |
| Ro7         | CACCATGCAATTACAGAAAACAA | SITMM          |
| to11        | GGGGACCATCTTGACAGAGAAGAGGTCGTTATATCTTTTTCTGTCTTTTTATTTT | AttB1–Cf-4 RT-PCR |
| to12        | GGGGACAAGTTTGGTCACAAAAGCGAGCTTTATGAGTGTCGTTTTTCTGAAAGAGT | AttB2–Cf-4 RT-PCR |
| to118       | CACCATGGAATGATGGAACAGAGAG | Ve1 RT-PCR |
| to119       | CTTTCTGGAAACCAAAGCAAGA | Ve1 RT-PCR |
| to156       | ATGCTGGATCTGAGTTAACA | SISOBIR1        |
| to157       | CACCATGACTCGGAATTATG | SISOBIR1        |
| to164       | CACCATGACCTCGACACCTC | SISOBIR1-like |
| to165       | ATGCTGGATCTGACACATGAC | SISOBIR1-like |
| to166       | ATCTAGATCGTGAGAAAGGAC | VIGS fragment SISOBIR1 |
| to167       | AGATCCGGAAATTTCTGATTTTAC | VIGS fragment SISOBIR1 |
| to168       | ATCTAGAAAGCAGCAAGAGGC | VIGS fragment SISOBIR1-like |
| to169       | AGATCCGAGACAGAGGAGAAGG | VIGS fragment SISOBIR1-like |
| to178       | ATCTAGAATCGACAGCAGGAGTGTTA | VIGS fragment SISOBIR1/SISOBIR1-like |
| to179       | AGATCCGAATCTGCAGAAAGCAGAG | VIGS fragment SISOBIR1/SISOBIR1-like |
| to180       | CACCATGGCGAGATCTGTGGAAC | SICLV2           |
| to181       | ACCTGCTAAAATTGTTTGGC | SICLV2           |
| to239       | CACCATGCGCTGGGCAACGAGGA | AtSObIR1 and RT-PCR |
| to240       | GTCTGCTGCTTGAGACACATG | AtSObIR1 and RT-PCR |
| to241       | AGATGCCAATCTTTCACAGCAGATGAC | VIGS fragment NbSObIR1 |
| to242       | AGATCCGAAGAGTTTTCTTCAATGCGAG | VIGS fragment NbSObIR1 |
| to243       | AGATCCGGAAATTTCTGATTTTAC | VIGS fragment NbSObIR1-like |
| to244       | ATCTAGGAGATCTGAGTAAGATTCGAG | VIGS fragment NbSObIR1-like |
| to250       | CACCATGGGCAAAAGAATCATTCCA | SIEIX2           |
| to251       | GTCTGCTGCTTGAGACACATG | SIEIX2           |
| to257       | CTCAACGCAATATTCACAGAACATTTAAAGGCAAGC | SISOBIR1D473N mutation |
| to258       | GCTGCTTTAATTCTTCTGAGATATTAGTGCTGGAG | SISOBIR1D473N mutation |
| to259       | CTCAACGCAATATTCACAGAACATTTAAAGGCAAGC | SISOBIR1-likeD486N mutation |
| to260       | GCTGCTTTAATTCTTCTGAGATATTAGTGCTGGAG | SISOBIR1-likeD486N mutation |
| to261       | CTCAACGCAATATTCACAGAACATTTAAAGGCAAGC | SISOBIR1D489W mutation |
| to262       | CTAGAATCCGGAAATTTCTTCAATGCGAG | SISOBIR1D489W mutation |
| to266       | CTAGAATCCGGAAATTTCTTCAATGCGAG | SISOBIR1D489W mutation |
| to267       | TGATCTGGATCTGAGTAGTATAGT | NbSObIR1 qRT-PCR |
| to272       | CCAATGCTGCTGGGCAACGAGGA | NbSObIR1 qRT-PCR |
| to273       | ATCTAGATCGTGAGAAAGGAC | NbSObIR1-like qRT-PCR |
| to45        | GGGGACAGTTTGTACAAAAAGGAGCTTTATG | SISERK3a/BAK1 |
| to46        | ATCGATCGTGCTGGGCAACGAGGA | SISERK3a/BAK1 |
| to58        | TATGGAAACATTGTGCTAGTTG | NbActin RT-PCR and qRT-PCR |
| to59        | CCAATGCTGCTGGGCAACGAGGA | NbActin RT-PCR and qRT-PCR |
| to88        | CCAATGCTGCTGGGCAACGAGGA | SISERK1           |
| to89        | CCAATGCTGCTGGGCAACGAGGA | SISERK1           |
| ITS1-F      | AAAAGTTTTAATGGTTGCTCTAGAG | V. dahliae qRT-PCR |
| ST-VE1-R    | CTTGCTATTGGAGGAGATAA | V. dahliae qRT-PCR |
| AtRubF      | GCAAGTGGTTGGTTCAAGGCTGGGG | Arabidopsis Rubisco qRT-PCR |
| AtRubR      | CCAAGTGGTTGGTTCAAGGCTGGAG | Arabidopsis Rubisco qRT-PCR |
Fig. 51. Cf-4–eGFP is functional and efficiently immunopurified from stably transformed tomato and tomato SlSOBIR1-like and Arabidopsis AtSOBIR1 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, 2ACLV1, S5ERK1, S5ERK3α/BAK1, and SFLS2 (all fused to eGFP, except for SFLS2, which was fused to GFP) were coexpressed with SlSOBIR1-like–Myc and AtSOBIR1–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 (eGFP) 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. 51

Fig. 52. Alignments of SOBIR1 sequences from tomato, Arabidopsis, N. benthamiana, and tobacco. (A) Amino acid sequence alignment of SlSOBIR1 (Slcyp0620g11810.1), SlSOBIR1-like (SlSOBIR1-L), SlSOBIR1, and AtSERK3a/BAK1 (SlSERK3aB). 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 present in two sequences or are unique residues, respectively. The conserved RD motif, as well as the conserved residues K377 and E407 of SERK3a/BAK1, are underlined. (B) Nucleotide sequence alignment of tomato SlSOBIR1, SlSOBIR1-like (SlSOBIR1-L), and Arabidopsis AtSERK3 (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 NbSOBIR1 (SGN-U441568), and NbSOBIR1-like (NbSOBIR1-L) coding regions. The available genomic sequence was used to obtain NbSOBIR1 and NbSOBIR1-like sequences, and EST data provided the NbSOBIR1 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. 52

Fig. 53. Cf-4 interacts with SlSOBIR1 in a reverse immunoprecipitation assay, with SOBIR1 mutants in the RD motif of the kinase domain, and with SlSOBIR1 in the presence of Avr4, and SOBIR1 does not form homo- or heterodimers. (A) SlSOBIR1–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 SlSOBIR1–eGFP, whereas coimmunopurified Cf-4–Myc was detected by using αMyc. (B) Cf-4–eGFP was coexpressed with SlSOBIR1–Myc, SlSOBIR1ΔD37N–Myc, SlSOBIR1-likeΔG486–Myc, and AtSOBIR1ΔD399–Myc in N. benthamiana. Immunopurifications and detection of proteins were performed as described in A. (C) Cf-4–eGFP was coexpressed with SlSOBIR1–Myc in the presence of Avr4 or Avr9 (the OD800 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) SlSOBIR1–eGFP was coexpressed with Cf-4–Myc, SlSOBIR1–Myc, SlSOBIR1-like–Myc, and AtSOBIR1–Myc. After 2 d, SlSOBIR1–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. 53

Fig. 54. SlSOBIR1–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) SlSOBIR1–eGFP localizes to the plasma membrane. When focusing on top of the plasma membrane, SlSOBIR1–eGFP is also detected in distinct spots that resemble cytoplasmic vesicles. (B) SFLS2–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. 54
**Fig. 55.** 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 NbSOBIR1, 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 AtSOBIR1D489N-Myc + Avr4, as indicated. GUS, AtSOBIR1-Myc, and AtSOBIR1D489N-Myc were infiltrated at final OD600 = 0.7 and Avr4 at OD600 = 0.03. (D) RT-PCR analysis confirms that AtSOBIR1–Myc and AtSOBIR1D489N–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 AtSOBIR1D489N–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. 56.** SOBIR1 is required for autoactivity of the Cf homolog Per2u. Ve1-mediated HR in tobacco, and Cf-2.2– and Ve1-mediated resistance in tomato. (A) Per2u 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, Per2u-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 NbSOBIR1 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 SOBIR1 and SOBIR1-like suppresses Cf-2.2–mediated resistance to C. fulvum expressing Avr2. Cf-2.2–expressing tomato was inoculated with TRV:SOBIR1/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. 57.** 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. 58.** SOBIR1 and SSOBIR1-like interact with all Cf proteins tested and with the receptor-like proteins (RLPs) SIEIX2, SICLV2, and STIMM, but not with SS5NC2. (A) Cf-2.2, -4E, -9, and the autoactive Cf homolog Per2u, all C-terminally fused to eGFP, were coexpressed with SSOBIR1–Myc and SSOBIR1-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 α-GFP antibody to detect the immunopurified proteins fused to eGFP, and α-Myc antibody was used for detection of communopurified SSOBIR1–Myc and SSOBIR1-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 SSOBIR1–Myc or SSOBIR1-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 communopurified SSOBIR1–Myc and SSOBIR1-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.
|        |        |        |        |        |
|--------|--------|--------|--------|--------|
| 1820   | SlSOBIR1: | TGA-CATCCTAA ATAGCAATTGATCC AAGCT AT GG AATGGATAGGA-GAACAAT | 1775 |
| 1840   | SlSOBIR1-1i: | TGCATCCATG CGAATTGATCC AAGCT AT GATAA GGAATGGAGGCA AT | 1814 |
| 1860   | AtSOBIR1: | GGAATAATCAG AATCCGATGATGCCAAGCT AT TGATCAGGAT AATGGACAG | 1823 |
|        |        |        |        |        |
| 1880   | SlSOBIR1: | CTTTTGTTTCTCAAGATGC TCTTTTGTAC TGGTCGATCC AAGCATCCAA GAGAGGCCA A | 1835 |
| 1900   | SlSOBIR1-1i: | CTTTTGTTTCTCAAGATGC TCTTTTGTAC TGGTCGATCC AAGCATCCAA GAGAGGCCA A | 1874 |
| 1920   | AtSOBIR1: | CTTTTGTTTCTCAAGATGC TCTTTTGTAC TGGTCGATCC AAGCATCCAA GAGAGGCCA A | 1883 |
|        |        |        |        |        |
| 1940   | SlSOBIR1: | CACTGA ATGCGTAGTCTTCAGATCAAGCTAT A | 1878 |
| 1960   | SlSOBIR1-1i: | CACTGA ATGCGTAGTCTTCAGATCAAGCTAT A | 1917 |
|        | AtSOBIR1: | CACTGA ATGCGTAGTCTTCAGATCAAGCTAT A | 1926 |
|       | 1880 | * | 1900 | * | 1920 |
|-------|------|---|------|---|------|
| NbSOBIR1 | GCCTACAGTAAGGTAGTTAGATGTTAAGCTGTCAGATCAGTCAAGGTTAG | | | | |
| NtSOBIR1 | GCCTACAGTAAGGTAGTTAGATGTTAAGCTGTCAGATCAGTCAAGGTTAG | | | | |
| NbSOBIR1-1 | GCCTACAGTAAGGTAGTTAGATGTTAAGCTGTCAGATCAGTCAAGGTTAGTAGGT | | | | |

* 

|       |       |       |
|-------|-------|-------|
| NbSOBIR1 | ---------- | - |
| NtSOBIR1 | ---------- | - |
| NbSOBIR1-1 | AGAGGATAA | 1795 |