Supplemental Materials

Supplemental Figure Legends

Video S1A. Fluorescent channel detection of the biotrophic colonization of a cortical cell by *P. indica*. The movie consists of 29 z-stacks taken with a multichannel TCS SP2 confocal laser-scanning microscope (Leica). Displayed is the interaction site described in Fig. 3. See description in Fig. 3 for identification of fungal and plant structures. The movie displays a colonization site in maturation zone II of Arabidopsis line GFP-Chi in which the ER, ER bodies, and nucleus are GFP-labeled. Optical sections were taken after excitation with laser line 488 and 633 nm, which were merged for the movie.

Video S1B. Transmission channel detection of the biotrophic colonization of a cortical cell by *P. indica*. The movie consists of 29 z-stacks taken with a multichannel TCS SP2 confocal microscope (Leica). Displayed is the interaction site described in Fig. 3. See description in Fig. 3 for identification of fungal and plant structures. The movie displays a colonization site in maturation zone II of Arabidopsis line GFP-Chi. Optical sections were taken by transmission channel.

Figure S1. Cell death-associated colonization by *P. indica* in the maturation zone II. A-C: Confocal microscopy of colonized dead rhizodermal cell (RC) in Arabidopsis line GFP-Chi (with GFP-tagged ER, ER bodies, and nucleus) lacking ER and nucleus. *P. indica* was double stained with WGA-AF488 (A) and WGA-AF633 (C). Arrows indicate fungal penetration sites of rhizodermal cell (RC). RC is dead as indicated by the absence of ER and nucleus. Neighboring rhizodermal cells are alive as ER bodies (cross in A, B) and ER (top cell) as well as the nucleus (asterisk, out of focus) are visible in a lower cell. B: Transmission channel image of A. Labels are as described in A. C: Same as A. Bars = 15 μm.

Figure S2. Cell death-associated colonization of Arabidopsis roots by *P. indica*. Transmission electron micrographs show root cells with disintegrated cytosol (*) and fungal hyphae (H). A: The early stage of cell death-associated colonization (CAD) is characterized by intact mitochondria (M), the presence of lipid bodies (LB), and multivesicular bodies (MVB). The cytosol (*) is slightly dissolved. B: Late stage of CAD. Image shows two root cells. The cell in the left part is completely pervaded by the hypha, whereas the cell in the right part of the

image is partly colonized. Penetration of the latter takes place directly through the cell walls as plasmodesmata cannot be seen at the penetration site (arrowhead). The plasma membrane is visible and appears intact throughout the colonized cells (arrows) although the cytosol is disintegrated in some areas of the cell (*). Mitochondria and plastids (P) are still intact. Inset shows a close up of a plasma membrane protrusion close to the penetration site reminsicent of membrane blebbing. C: Four root cells separated by cell walls (CW). The middle cell is colonized with hyphae and contains a partly dissolved cytosol (*), whereas the two cells below and one upper cell, that are not colonized, show a dense cytosol (CY), intact plastids and tonoplasts (V). Bars = 1 μm (A, B, C), 0.5 μm (inset in B).

Figure S3. Defense responses during early stages of the Arabidopsis-*P. indica* interaction. *P. indica* very infrequently induced structural and biochemical defense responses in Arabidopsis roots. A: Epifluorescence image displays the rare observation of cell wall appositions (arrowheads) associated with fungal penetration attempts of a rhizodermal cell. B: Bright field image of A showing cell wall appositions (CWAs, arrowheads). C, D: Epifluorescence and bright field image of fungal penetration attempts associated with CWAs (black arrowhead). Focal accumulation of peroxisomes was visualized using Arabidopsis line A5 in which peroxisomal tetrafunctional protein is GFP-tagged [Cutler SR, Ehrhardt DW, Griffitts JS, Somerville CR (2000) Random GFP::cDNA fusions enable visulaization of subcellular structures in cells of Arabidopsis at a high frequency. *Proc Natl Acad Sci USA* 97:3718-3723]. White arrowheads indicate penetration attempts and focal peroxisome accumulation in another focal plane. E, F: Epifluorescence and bright field image of fungal penetration sites in the absence of CWAs (arrowheads) indicate the reduced focal accumulation of peroxisomes in comparison to C. Note the lower number of peroxisomes in the absence of CWAs in E compared to C. Arrows in D and F show extracellular hyphae. G, H: Epifluorescence image of WGA-AF488 stained intracelluar hyphae in elongating cells (G, arrowheads) showing strong autofluorescence (H, arrowheads) indicative of a HR-like defense response at 7 dai. G: Fungal intracellular colonization is restricted to single epidermal cells. Arrowheads indicate intracellular hyphae at another focal plane that were confronted with an identical response. Bars = 20 μm.

Figure S4. Suppression of elf18-triggered responses by *P. indica*. *P. indica* suppresses elf18-triggered responses such as seedling growth retardation and oxidative burst. A: Roots of two-week-old Col-0 plants were inoculated by *P. indica* or mock-treated and subsequently

challenged with 1 µM elf18 or with a control treatment at 3 dai. Plant fresh weight was determined 10 days after treatment. Data represent mean values of three biological experiments. B: Roots of two-week-old Col-0 plants were either inoculated with *P. indica* or mock-treated and challenged with 0.1 µM elf18. Oxidative bursts was measured in 10 mg root segments (1 cm each segment) by a luminol-based assay directly after application of elf18. Values are given as relative light units (RLU) over time. Data displayed are means with standard errors of four independent measurements per treatment of one biological experiment. Experiments were repeated thrice with similar results. Asterisks indicate significance at $P < 0.001$ (***) analyzed by Student's *t*-test.

Figure S5. Suppression of flg22-induced gene expression by *P. indica* in Arabidopsis roots. *P. indica* suppresses flg22-triggered gene transcription as evidenced by qRT-PCR analysis. Roots of two-week-old plants were inoculated with *P. indica*. Three days after inoculation roots were either mock-treated or treated with flg22 and harvested at 2, 24, or 72 hat. Data displays the Ct thresholds of the indicated gene candiates relative to the Ct thresholds of the housekeeping gene At*Ubiquitin 5* using the $^{\Delta\Delta}$Ct method. [The values represent means with standard errors of one experiment]. Experiments were repeated at least twice with similar results. Asterisks indicate significance at $P < 0.05$ (*), 0.01 (**), and 0.001 (***). For D, significance between individual timepoints of Col-0 / mock and Col-0 / *P. indica* or Col-0 / flg22 and Col-0 + *P. indica* / flg22 were analyzed by Student's *t*-test.

Figure S6. flg22- and *P. indica* chlamydospore-induced oxidative burst in *pub22/23/24*. Roots of two-week-old Col-8 or *pub22/23/24* plants were either inoculated with *P. indica* or mock-treated. The oxidative burst was measured in root segments by a luminol-based assay directly after application of 0.1 µM flg22, *P. indica* chlamydospores (500.000 spores ml^{-1} or buffer of spore suspension. A: Sum of the relative light units (RLUs) obtained in Fig. 4C. B: Peak RLUs recorded in Fig. 4C. C: Root oxidative burst after chlamydospore application is given as relative light units (RLU) over time. Data displayed are means with standard errors of four independent measurements per treatment of one biological experiment. All experiments were repeated at least twice with similar results.

Figure S7. Enhanced defense gene induction in *pub22/23/24* roots by *P. indica*. For qRT-PCR analysis of the indicated genes, three-week-old Col-8 or *pub22/23/24* plants were mock-treated or inoculated with *P. indica*. Roots were harvested at 1, 3, or 7 hat. The obtained Ct

thresholds of the candiates were related to the Ct thresholds of the housekeeping gene *ubiquitin 5* using the $^{\Delta\Delta}$Ct method. Displayed are the fold changes of the genes relative to mock-treated roots. [The values represent means with standard error and are based on at least two independent biological experiments]. Asterisks indicate significance at $P < 0.05$ (*), 0.01 (**), and 0.001 (***) between individual timepoints of Col-8 / mock and Col-8 / *P. indica* or *pub22/23/24* / mock and *pub22/23/24* / *P. indica* and analyzed by Student's *t*-test.

Figure S1

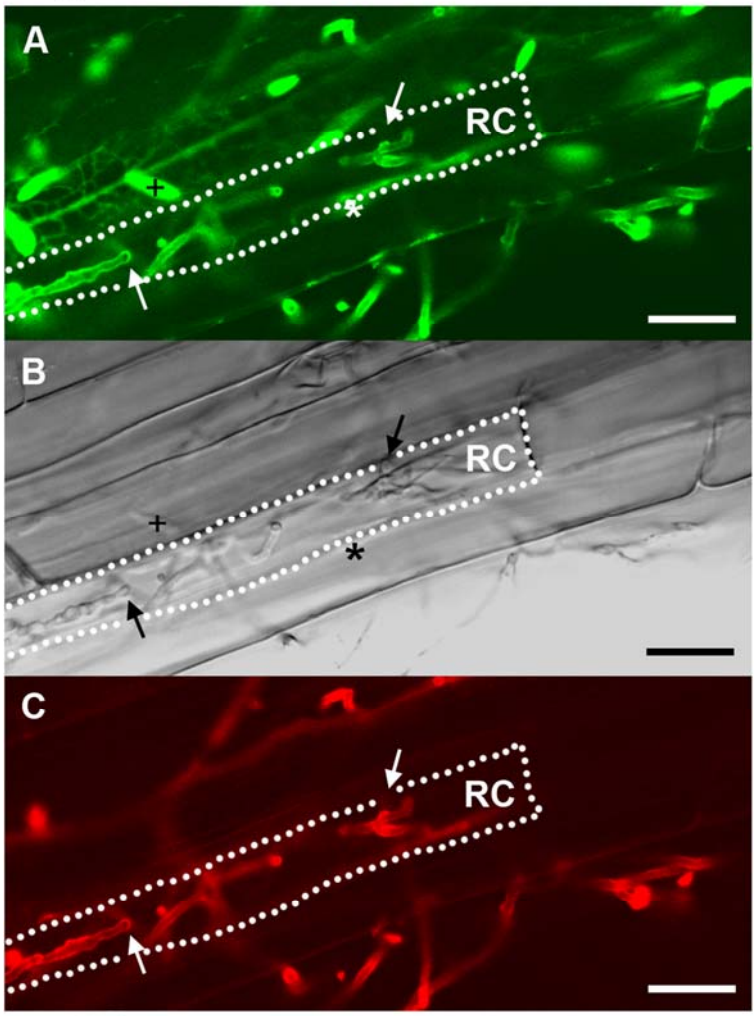

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Figure S2

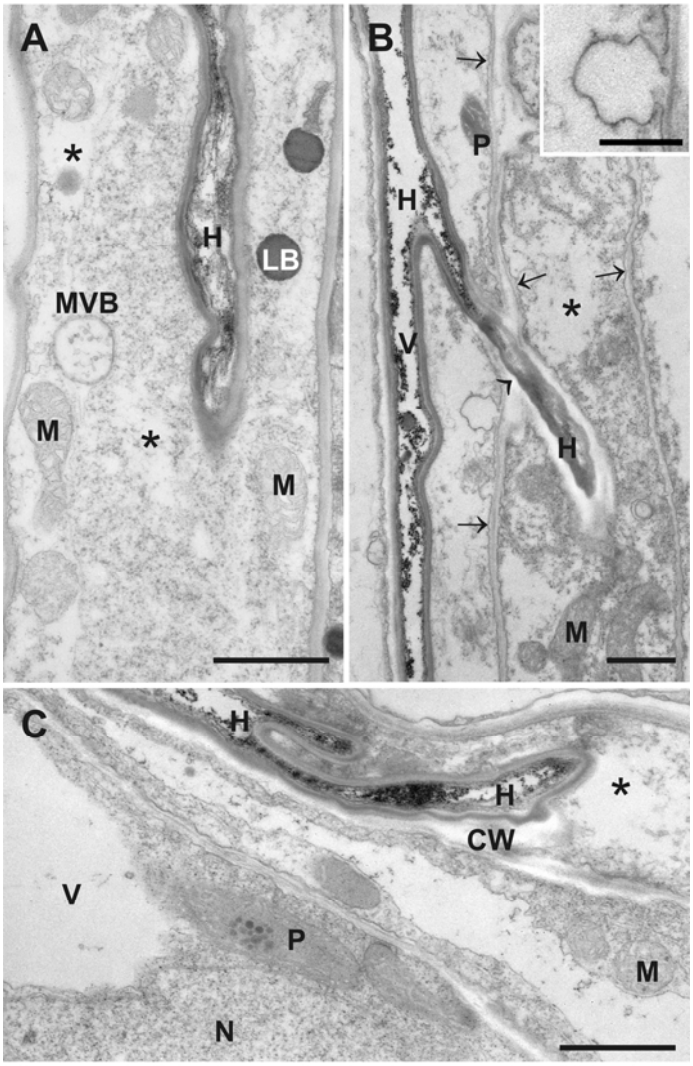

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Figure S3

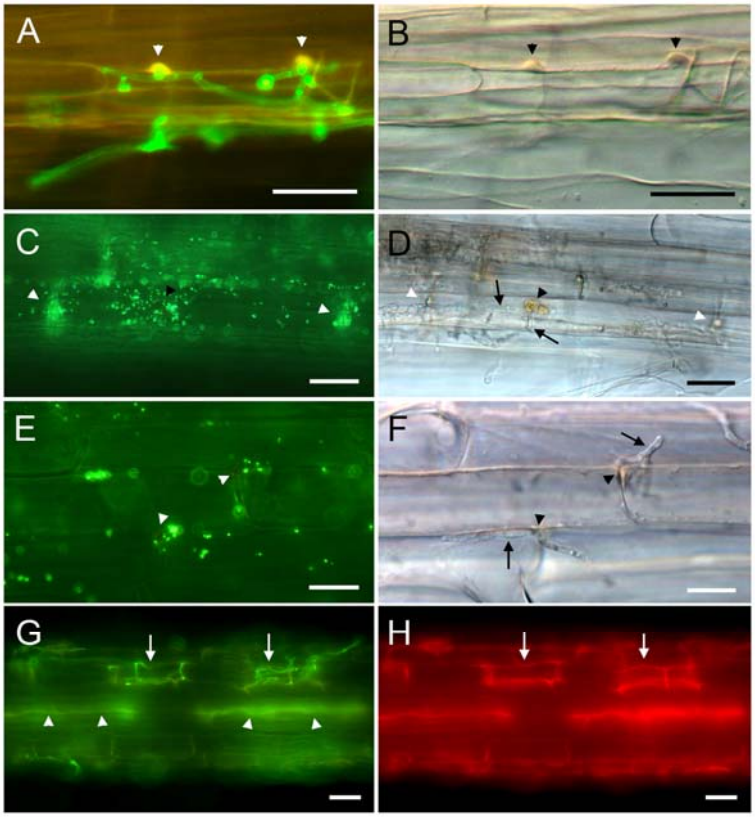

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Figure S4

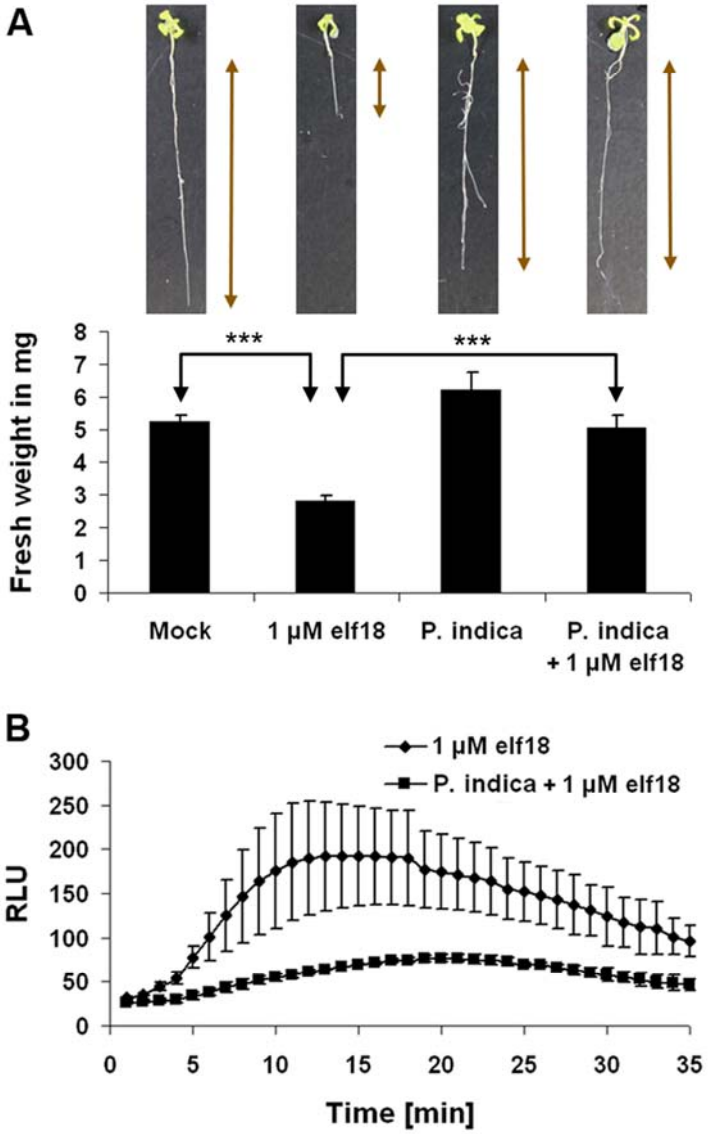


Figure S4 Suppression of elf18-triggered responses by P. indica. P. indica suppresses elf18-triggered responses such as seedling growth retardation and oxidative burst. A: Roots of two-week-old Col-0 plants were inoculated by P. indica or mock-treated and subsequently challenged with 1 µM elf18 or with a control treatment at 3 dai. Plant fresh weight was determined 10 days after treatment. Data represent mean values of three biological experiments. B: Roots of two-week-old Col-0 plants were either inoculated with P. indica or mock-treated and challenged with 0.1 µM elf18. Oxidative bursts was measured in 10 mg root segments (1 cm each segment) by a luminol-based assay directly after application of elf18. Values are given as relative light units (RLU) over time. Data displayed are means with standard errors of four independent measurements per treatment of one biological experiment. Experiments were repeated thrice with similar results. Asterisks indicate significance at $P < 0.001$ (***) analyzed by Student's t-test.

Figure S5

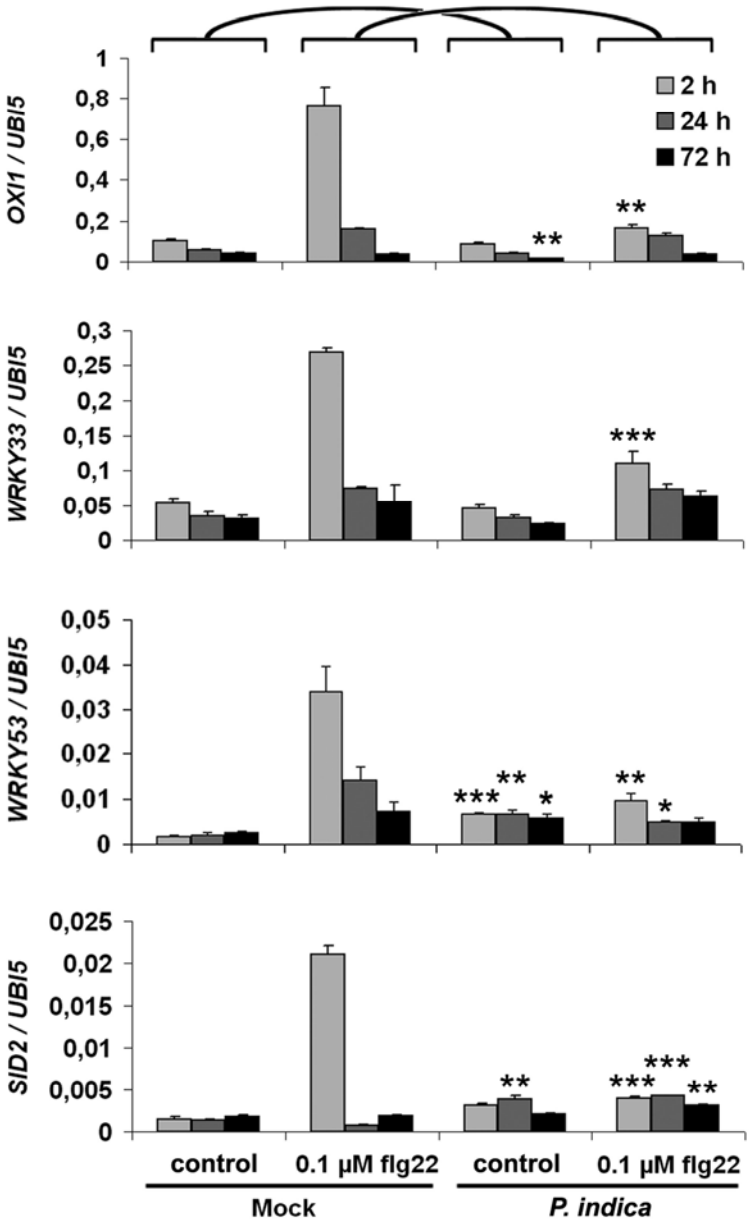


Figure S5 Suppression of flg22-induced gene expression by P. indica in Arabidopsis roots. P. indica suppresses flg22-triggered gene transcription as evidenced by qRT-PCR analysis. Roots of two-week-old plants were inoculated with P. indica. Three days after inoculation roots were either mock-treated or treated with flg22 and harvested at 2, 24, or 72 hat. Data displays the Ct thresholds of the indicated gene candiates relative to the Ct thresholds of the housekeeping gene AtUbiquitin 5 using the ΔΔCt method. [The values represent means with standard errors of one experiment]. Experiments were repeated at least twice with similar results. Asterisks indicate significance at $P < 0.05$ (*), 0.01 (**), and 0.001 (***). For D, significance between individual timepoints of Col-0 / mock and Col-0 / P. indica or Col-0 / flg22 and Col-0 + P. indica / flg22 were analyzed by Student's t-test.

Figure S6

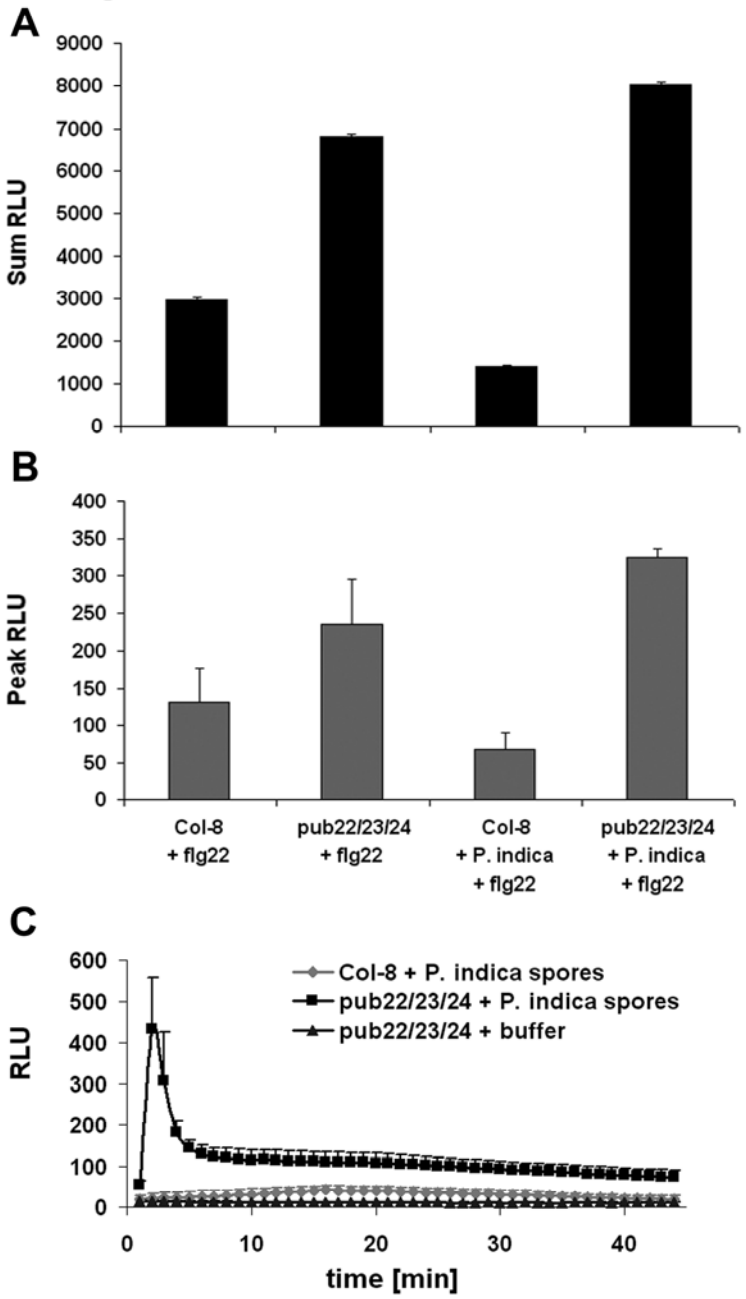
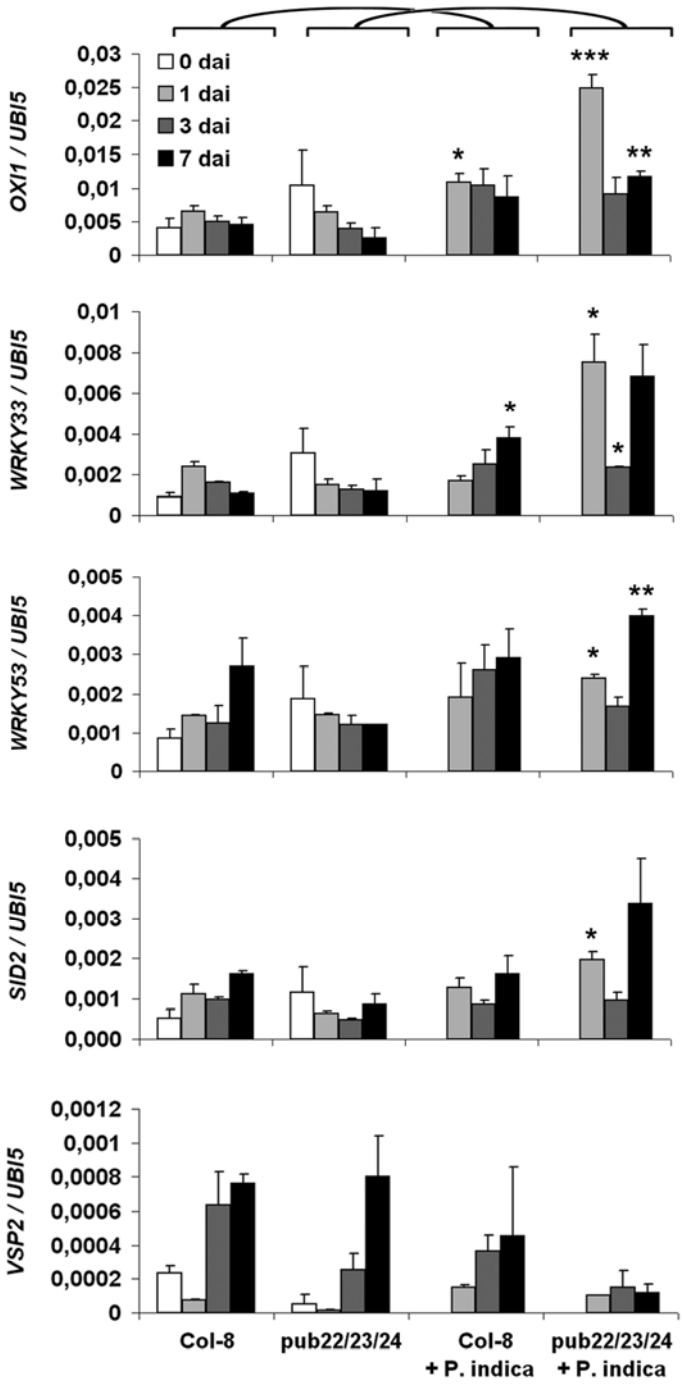


Figure S6 flg22- and P. indica chlamydospore-induced oxidative burst in pub22/23/24. Roots of two-week-old Col-8 or pub22/23/24 plants were either inoculated with P. indica or mock-treated. The oxidative burst was measured in root segments by a luminol-based assay directly after application of 0.1 µM flg22, P. indica chlamydospores (500.000 spores ml-1 or buffer of spore suspension. A: Sum of the relative light units (RLUs) obtained in Fig. 4C. B: Peak RLUs recorded in Fig. 4C. C: Root oxidative burst after chlamydospore application is given as relative light units (RLU) over time. Data displayed are means with standard errors of four independent measurements per treatment of one biological experiment. All experiments were repeated at least twice with similar results.

Figure S7



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Supplemental Tables

Table S1. Primers used for qRT-PCR

Gene	AGI	Forward	Reverse
BOI	At4G19700	TCTTCGAACAAACCTAGACC	CACAAACCGTACACAAACAC
CBP60g	AT5G26920	AAGAAGAATTGTCCGAGAGGAG	GGCGAGTTTATGAAGCACAG
Exp-PT1	At2G45900	GGATTTCATTCGTCAAACCT	CAACCAATATCAAAGCGGAG
ITS	-	CAACACATGTGCACGTCGAT	CCAATGTGCATTCAGAACGA
MYB51	AT1G18570	ACCAACCTCGAATCTTCTCTG	TTTCAACACAAGACTCCTCCA
OXI1	AT3G25250	TCATCTACATTGGCCGTGTC	CGTCGCTCCATACAACATCT
SID2	AT1G74710	TCCGTGACCTTGATCCTTTC	ACAGCGATCTTGCCATTAGG
UBI5	AT3G62250	CCAAGCCGAAGAAGATCAAG	ATGACTCGCCATGAAAGTCC
VSP2	At5G24770	CAAACTAAACAATAAACCATACCATAA	GCCAAGAGCAAGAGAAGTGA
WRKY22	AT4G01250	ATCTCCGACGACCACTATTG	TCATCGCTAACCACCGTATC
WRKY33	AT2G38470	CAAAGGAAAGGAGAGGATGG	GTAGACTGAGGTTTAGGATGG
WRKY53	AT4G23810	GCAACGAAACAAGTCCAGAG	GTCTTTACCATCATCAAGCCC