Supporting Information

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SI Materials and Methods

Plant Growth. Plants were grown in a growth chamber at 22 °C, 70% humidity, under irradiance of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ with a photoperiod of 16-h light/8-h dark.

Plasmid Construction. Primers used are indicated in Table S1. For constitutive expression and secretion of chimeric PvPGIP2-$(Ala)_3$-FpPG protein in *Pichia pastoris*, a DNA sequence encoding the mature PvPGIP2 was amplified from pBS11-SP2PGIP2 using the primers EcoRIPGIP2Fw and NotIPGIP2Rv to introduce an EcoRI and a NotI site at its 5′ and 3′ ends, respectively. This fragment was cloned in the integrative vector pGAPZα (Invitrogen), in-frame with the yeast α-factor signal peptide. A DNA sequence encoding the mature FpPG, flanked by NotI and XbaI sites, was obtained by amplification with the primers NotIFpPGFw and XbaIFpPGRv, using pGAPZαFpPG (1) as template. The resulting fragments were cloned in the pGAPZα plasmid (Invitrogen) so that the resulting construct (pGAPZα-PGIP2-PG) encoded a translational fusion of PvPGIP2 and FpPG separated by three Ala residues (OGM).

To obtain a construct for *in planta* expression of the OGM under a β-estradiol–inducible promoter, a DNA sequence encoding the signal peptide of PGIP2 (SP2) fused to the first 550 bp encoding PvPGIP2 was amplified from the plasmid pBS11SP2PGIP2 (2) using the primers AvrIISP1Fw and EheIPGIP2Rv. The amplified fragment was cloned in pGAPZα-PGIP2-PG to obtain a fusion between SP2 and the OGM. This cassette was amplified using the primers XhoISP1Fw and PacIFpPGRv and the product was cloned in pMDC7 (3) to obtain pMDC7.SP2OGM.

To clone the OGM under the control of the *Arabidopsis PR-1* (At2g14610) promoter sequence, a PacI site was introduced upstream (−6 bp) of the SacI site localized in the binary vector pBI121 at the 3′ end of the β-glucuronidase gene (Stratagene), which was subsequently excised with XbaI and PacI. The OGM fused to SP2 was amplified by PCR from pMDC7.SP2WOL using the primers XbaISP1Fw and XbaIPacIFpPGRv and the resulting fragment was cloned in pBI121, modified as described above. A DNA sequence corresponding to 1,300 bp upstream of the transcriptional start of *PR-1* was amplified from *Arabidopsis* Col-0 genomic DNA using the HindIIISP1PR1Rv and HindIIIPR1Fw primers. The fragment was cloned in the HindIII site of pBI121, upstream of the previously introduced OGM gene, replacing the CaMV 35S promoter sequence.

To clone the OGM under the control of the *Arabidopsis RetOx* (AT1G26380) promoter sequence, the binary vector pBI121, modified as described above, was used. A DNA sequence corresponding to 1,450 bp upstream of the transcriptional start of *RetOx* was amplified from *Arabidopsis* Col-0 genomic DNA using the HindIIIRetOxFw and XbaIRetOxRv primers that introduced HindIII and XbaI restriction sites at the ends of the promoter sequence. The fragment was cloned using the HindIII and XbaI restriction sites of pBI121, upstream of the β-glucuronidase gene, replacing the CaMV 35S promoter sequence. Subsequently, the gene encoding the OGM fused to SP2 was introduced in the vector by using the XbaI and PacI restriction sites, replacing the β-glucuronidase gene sequence. The resulting plasmids were introduced into *Agrobacterium tumefaciens* GV3101 by electroporation.

Protein Production and Assays. For production and purification of the OGM, pGAPZα-PGIP2-PG was linearized with AvrII and introduced into *P. pastoris* X33 (Invitrogen). *Pichia* transformation, selection and growth were carried out according to the manufacturer's instructions. *P. pastoris* was grown for 3 d in YPD medium (10 g/L^{-1} yeast extract, 20 g/L^{-1} peptone, 20 g/L^{-1} glucose) and culture filtrates were tested for the presence of the OGM using an agar diffusion assay (4) and by immunoblot using an antibody against FpPG (5). The OGM was purified by affinity chromatography using the same procedure previously described for PvPGIP2 (1). PG and OGM activity in solution was measured by incubating the proteins in 50 mM sodium acetate buffered at pH 5.0 with polygalacturonic acid (Sigma) as a substrate at a concentration of 2.5 g/L^{-1}. One activity unit (RGU) was defined as the amount of enzyme producing 1 microequivalent of reducing end groups in 1 min at 25 °C, using D-galacturonic acid (Sigma) as a standard. The specific activity is reported as RGU per milligram of protein in solution. Cross-linking experiments were conducted incubating 1.6 μg of purified protein at 28 °C for 16 h in 10 μL of a solution containing 50 mM Na acetate, pH 4.6, 30 mM NaCl, supplemented with 1% methanol-free formaldehyde (Thermo Fisher Scientific). One and 3 μL of the cross-linking reaction were analyzed by SDS/PAGE on a 7.5% (wt/vol) acrylamide (30% acrylamide/Bis Solution, 29:1; Bio-Rad) gel.

Extraction of total proteins from *Arabidopsis* leaves was performed in 20 mM Na acetate pH 4.6 and 1 M NaCl. For immunoblots, equal amounts of total protein extracts (3 μg for β-estradiol–inducible plants and 30 μg for *pPR-1:OGM* plants) were separated by SDS/PAGE on a 10% (wt/vol) acrylamide (30% acrylamide/Bis Solution, 29:1; Bio-Rad) gel, and transferred onto nitrocellulose membranes (Hybond-C; Amersham) in 25 mM Tris, 192 mM glycine, pH 8.3, 20% (vol/vol) methanol at 4 °C for 1 h. The filter was stained with Ponceau Red to assess equal transfer, and then blocked with 5% (wt/vol) BSA (Sigma-Aldrich) in PBS (Bio-Rad) for 1 h before incubation for 12 h with the primary antibody anti-FpPG (5). After extensive washes in PBS buffer, membranes were incubated with an anti-rabbit secondary antibody conjugated to horseradish peroxidase (Amersham). Membranes were washed as described above before detection with ECL detection reagent (Amersham). For the agar diffusion assay, equal amounts of protein extracts (3 μg for β-estradiol inducible plants) were evaluated.

Plant Transformation. Four-week-old plants were transformed with *A. tumefaciens* using the floral-dip method (6). Following transformation, transgenic lines were selected in the presence of 23 $\mu g/mL^{-1}$ hygromycin. T2 lines showing a segregation ratio of 3:1 of the antibiotic resistance marker were selected to isolate homozygous plants and propagated for subsequent analysis. To trigger the expression of the transgene in the plants transformed with the β-estradiol–inducible construct, 4-wk-old soil-grown plants were sprayed with a solution containing 50 μM β-estradiol.

Gene-Expression Analysis. Leaves were frozen in liquid nitrogen, homogenized with an MM301 Ball Mill (Retsch), and total RNA was extracted with Isol-RNA Lysis Reagent (5′-Prime) according to the manufacturer's protocol. RNA (2 μg) was treated with RQ1 DNase (Promega) in a final volume of 10 μL, and 2.5 μL of the reaction were used to synthesize first-strand cDNA with ImProm-II reverse transcriptase (Promega), according to the manufacturer's instructions. Expression levels of each gene, relative to UBQ5, were determined either by semiquantitative PCR or by real-time PCR. Semiquantitative PCR analysis was performed in 50 μL of reaction mix containing 2 μL of cDNA, 1× buffer (RBC Bioscience), 3 mM $MgCl_2$, 100 μM of each dNTP, 0.5 μM of each primer, and 1 unit of Taq DNA Polymerase

(RBC Bioscience). Next, 25, 30, and 35 PCR cycles were performed for each primer pair to verify linearity of the amplification. PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide.

Quantitative PCR analysis was performed using a CFX96 Real Time System (Bio-Rad). One microliter of cDNA was amplified in a 30-μL reaction mix containing 1× Go Taq qPCR Master Mix (Promega) and 0.4 μM of each primer. Expression levels of each gene, relative to the *UBQ5* gene, were determined as previously described (7). Primers used were the following: GCAAGAAGAAGACTTACACC and AGTCCACACTTACCACAGTA (UBQ5); CCTCCCAAAACCCTACCCTA and GAGAAGTCGAGGGTGACGAG (OGM). Primers for *WRKY40* and *RetOx* were previously described (8).

Callose Deposition Detection. Callose deposition was detected as previously described (9). Leaves of 4-wk-old plants were sprayed with 50 μM β-estradiol. After 140 h, leaves were harvested, cleared and dehydrated with 100% (vol/vol) ethanol. Leaves were fixed in an acetic acid:ethanol 1:3 (vol/vol) for 2 h, sequentially incubated for 15 min in 75% (vol/vol) ethanol, in 50% (vol/vol) ethanol, and in 150 mM phosphate buffer, pH 8.0, and then stained for 1 h at 25 °C in 150 mM phosphate buffer, pH 8.0, containing 0.01% (wt/vol) Aniline blue. After staining, leaves were mounted in 50% (vol/vol) glycerol and examined by UV epifluorescence using an Axioskop 2 Plus microscope (Zeiss). Images were taken with a ProgRes C10 3.3 MegaPixel digital color camera (Jenoptik).

Infection Assays. Pathogenicity assay with *Botrytis cinerea* was performed as previously described (4). *B. cinerea* was grown on 20 g/L^{-1} malt extract, 10 g/L^{-1} proteose peptone no. 3 (Difco), and 15 g/L^{-1} agar for 7–10 d at 24 °C with a 12-h photoperiod before collection of spores. Rosette leaves from *Arabidopsis* plants were placed in Petri dishes containing 0.8% agar, with the petiole embedded in the medium. Inoculation was performed by placing 5 μL of a suspension of 5×10^5 conidiospores mL^{-1} in 24 mg/mL^{-1} potato dextrose broth (Difco) on each side of the middle vein. The plates were incubated at 22 °C under constant light (about 80 μE·m^{-2}·s^{-1}) for 48 h. High humidity was maintained by covering the plates with a clear plastic lid. Under these experimental conditions, most inoculations in wild-type plants resulted in rapidly expanding water-soaked lesions of comparable diameter. *Pectobacterium carotovorum* subsp. carotovorum was obtained from DSMZ (strain DSMZ 30169). After growth in Luria-Bertani (LB) liquid medium, bacteria were suspended in 50 mM potassium phosphate buffer (pH 7.0) and inoculated at a final dose of 5×10^7 cfu/mL. In each experiment, 12 fully developed leaves were detached from four independent 4-wk-old plants, placed on a moist filter paper in Petri dishes, inoculated with 5 μL of the bacterial suspension, and maintained at 22 °C and a 12-h photoperiod. Symptoms were scored by measuring the area of macerated tissue at 16 hpi using ImageJ software (ImageJ; W. S. Rasband, United States National Institutes of Health, Bethesda, MD). The experiments were repeated three times with different plant batches, and statistical analysis of the results was performed by one-way analysis of variance followed by Tukey's or Student's range tests. *Pseudomonas syringae* pv. tomato strain DC3000 was cultured in LB broth at 28 °C for 1 d, and bacteria were suspended in 10 mM $MgCl_2$ at a final concentration of 10^5 cfu/mL corresponding to 2.5×10^3 cfu/cm^2 of leaf tissue. Bacteria were inoculated by syringe infiltration and bacterial growth was determined as previously described (10).

Isolation and Detection of Oligogalacturonides. About 100 mg of leaf tissue from wild-type and transgenic plants treated with 50 μM β-estradiol were frozen in liquid nitrogen and homogenized using a Retschmill machine (model MM200; Retsch) at 25 Hz for 1 min. The resulting powder was washed twice in 70% (vol/vol) ethanol, vortexed, and pelleted by centrifugation at 20,000 × *g* for 10 min. The pellet was washed twice with a chloroform:methanol mixture [1:1 (vol/vol)] and centrifuged at 20,000 × *g* for 10 min. The pellet was then washed twice with acetone and pelleted by centrifugation at 20,000 × *g* for 10 min. After evaporation of the solvent, the pellet was solubilized in 200 μL of 50 mM ammonium acetate pH 5, 50 mM CDTA, 50 mM ammonium oxalate for 2 h under stirring at room temperature, and the supernatant was recovered after centrifugation at 20,000 × *g* for 10 min.

Oligomers in the pectin fractions were analyzed by HPAEC-PAD with an ICS-3000 apparatus (Dionex Corporation) equipped with a CarboPac PA-200 separation column (2 mm ID × 250 mm; Dionex Corporation) and a Carbopac PA-200 guard column (2 mm ID × 50 mm; Dionex Corporation). A flow of 0.4 mL/min was used and the temperature was kept at 25 °C. The injected samples (25 μL) were separated using a gradient with 0.05 M KOH (A) and 1 M KOAc in 0.05 M KOH (B): 0–30 min from 10% B to 80% B, 30–32 min at 100% B. Before injection of each sample, the column was equilibrated with 90% A and 10% B for 10 min. For OG hydrolysis in vitro, pectin fractions extracted from 20 mg of leaf tissue were treated with 5 μg of pure FpPG for 1 h at 37 °C before analysis.

Mass spectrometric analysis of pectin fractions was performed by MALDI-TOF. Pectin-enriched fractions were dissolved in water and pretreated with Biorex MSZ (Bio-Rad) resin for 10 min at room temperature. An equal volume of 5% (wt/vol) 2,5-dihydroxybenzoic acid matrix in 0.2% trifluoroacetic acid, was added to each sample and spotted on a MALDI plate (Bioscience). After crystallization under vacuum conditions, samples were analyzed with a Voyager DE-STR spectrometer (Applied Biosystems). Each spectrum was obtained by the mean of 1,000 independent shots and detected in positive mode with an acceleration voltage of 20,000 V and a delay time of 350 ns.

***Arabidopsis* Growth Assays.** Seeds were surface-sterilized and individually placed in each well of a 24-well-plate containing 1 mL per well of half-strength Murashige and Skoog medium supplemented with 1% (wt/vol) sucrose. Plates were incubated at 22 °C with a 16-h light/8-h dark cycle at a light intensity of 120 μE·m^2·s^{-1}. β-Estradiol was supplied to the medium either before germination or after 5 d of growth, and fresh biomass was measured after 10 or 5 days, respectively.

Extraction of SA and Detection by LC-MS. SA was extracted as previously described (11) from leaves of 4-wk-old plants and seedlings (~50–100 mg) and collected 24 h after the induction with β-estradiol. Samples were dissolved in methanol at 1:1 ratio [tissue (mg):methanol (μL)] and analyzed by liquid chromatography coupled to mass spectrometry using an Ultimate 3000 HPLC system connected to an Orbitrap XL Discovery (Thermo Fisher Scientific) equipped with an electrospray ionization source operating in negative mode. Samples were separated at 200 μL/min by reversed-phase HPLC using an Acclaim 120 C18 column (3 mm, 200 Å, 2.1 × 150 mm; Thermo Fisher Scientific) equipped with a guard column and eluted using, as mobile phases, water containing 0.1% (vol/vol) formic acid (eluent A) and methanol containing 0.1% (vol/vol) formic acid (eluent B). A 45-min gradient, from 30 to 100% B, was performed. The effluent from the HPLC was directly electrosprayed into the mass spectrometer.

The electrospray source was operated in negative ion mode using the following parameters: electrospray voltage, 4.5 kV; sheath gas, 20 arbitrary units; auxiliary gas, 10 arbitrary units with the heated capillary temperature set at 350 °C. The tune method was acquired with a standard solution of SA.

For quantitative analysis, the ion chromatograms were acquired in the Orbitrap (resolution 30,000) in total ion current mode and single ion monitoring mode corresponding to the molecular ion

$[M+H]^-$ of the salicylic acid (137.024). Identification of SA was carried out on the basis of the retention time and presence of the peak in the single ion monitoring trace compared with the standard. The quantification was obtained by processing the data with the QuantBrowser module of the Xcalibur 2.2 (Thermo Fisher Scientific) software, using the calibration curve method.

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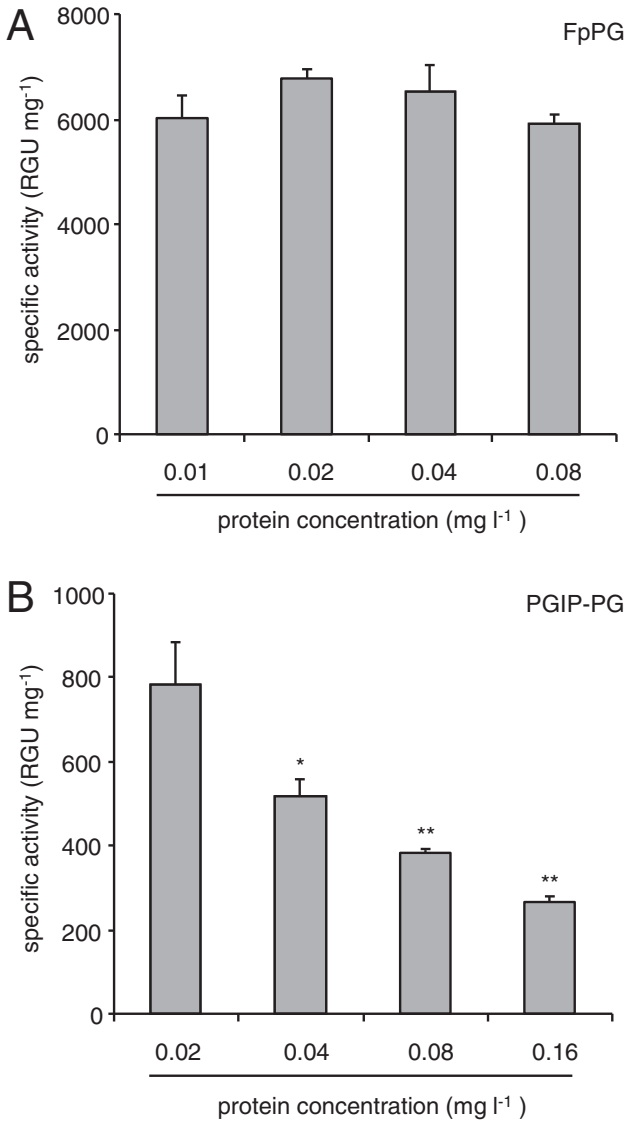

Fig. S1. Specific enzyme activity of the PGIP–PG chimera is dependent on its concentration. Specific activity (RGU/mg^{-1}) of FpPG (*A*) and PGIP–PG (*B*) as a function of their protein concentration in the incubation mixture. Bars: average ($n = 3$) ± SD; asterisks represent specific activity of PGIP–PG at the highest concentrations is significantly different from that at the lowest concentration (Student's *t* test, $*P < 0.02$, $**P < 0.002$).

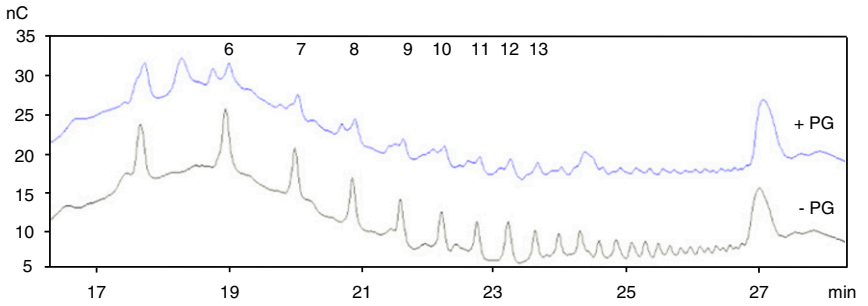

Fig. S2. OGs released from *Arabidopsis* expressing the PGIP–PG chimera are degraded by polygalacturonase. Plants expressing the inducible PGIP–PG chimera were treated with 50 μM β-estradiol and a pectin-enriched fraction of the cell wall from rosette leaves was extracted after 170 h. Pectin fractions were analyzed by HPAEC-PAD before (–) and after treatment with FpPG (+). The chromatogram shows the intensity of the signals (nC) plotted against retention time. Numbers indicate the DP of each peak.

Fig. S3. Reduction of disease symptoms caused by *P. syringae* pv. tomato DC3000 on *pPR-1*:OGM plants. Representative pictures of leaves from wild-type (*A*) and *pPR-1*:OGM 1 and 2 (*B* and *C*) plants taken at 72 hpi.

Table S1. Primers used for the construction of the different OGM cassettes

Primer name	Primer sequence
EcoRIPGIP2Fw	ATCGATGAATTCGAGCTATGCAACCCACA
NotIPGIP2Rv	TCTTCTAAGTGCGGCCGCAGTGCAGGCAGGAAGAG
NotIFpPGFw	TCAACACTATGCGGCCGCACCCTGCTCCGTGACTGAG
XbaIFpPGRv	ATCGATTCTAGACTAGCTGGGGCAAGTGTTG
AvrIISP1Fw	ACTAAGCCTAGGACTATCTAGAATGACTCAATTCAATATCCCAG
EheIPGIP2Rv	GGGGATGGCGCCGGAG
XhoISP1Fw	ACTAAGCTCGAGATGACTCAATTCAATATCCCAG
PacIFpPGRv	CCTAAGTTAATTAACTAGCTGGGGCAAGTGTTG
XbaISP1Fw	GACTATCTAGAATGACTCAATTCAATATCCC
HindIIIPRFw	GTTAGCACAAGCTTGTTTTAAC
XbaIFpPGRv	CCTAAGTCTAGAGGTCTTAATTAACTAGCTGGGG
HindIIISP1PRRv	TGCTTAAGCTTGAAGACATGGTTACTGGGATATTGAATTGAGTCATTTTTCTAAGTTGATAATG
HindIIIRetOxFw	CTCACAAGCTTGATTCACTGCCCTCTTCTCCG
XbaIRetOxRv	GCTGGTCTAGATTTGCTGTTTGGTTCATTATGCC