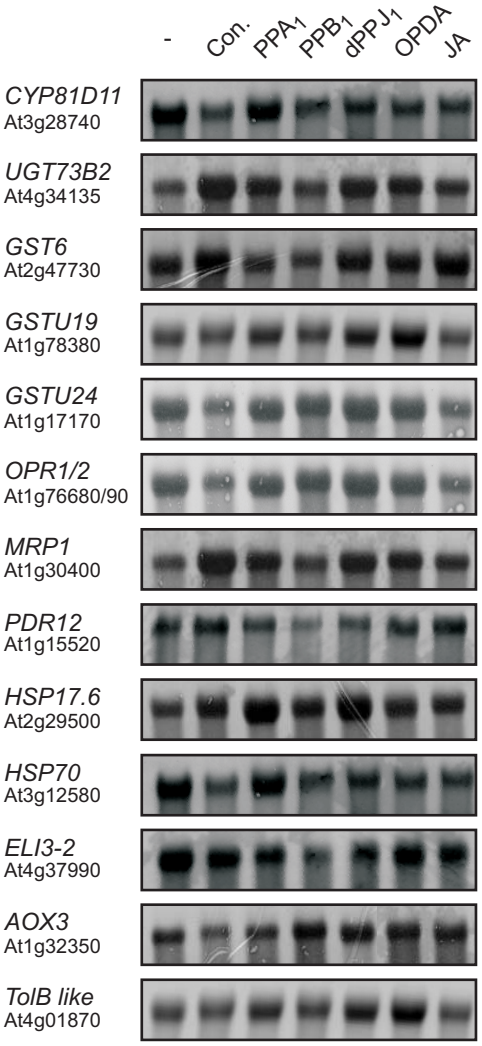
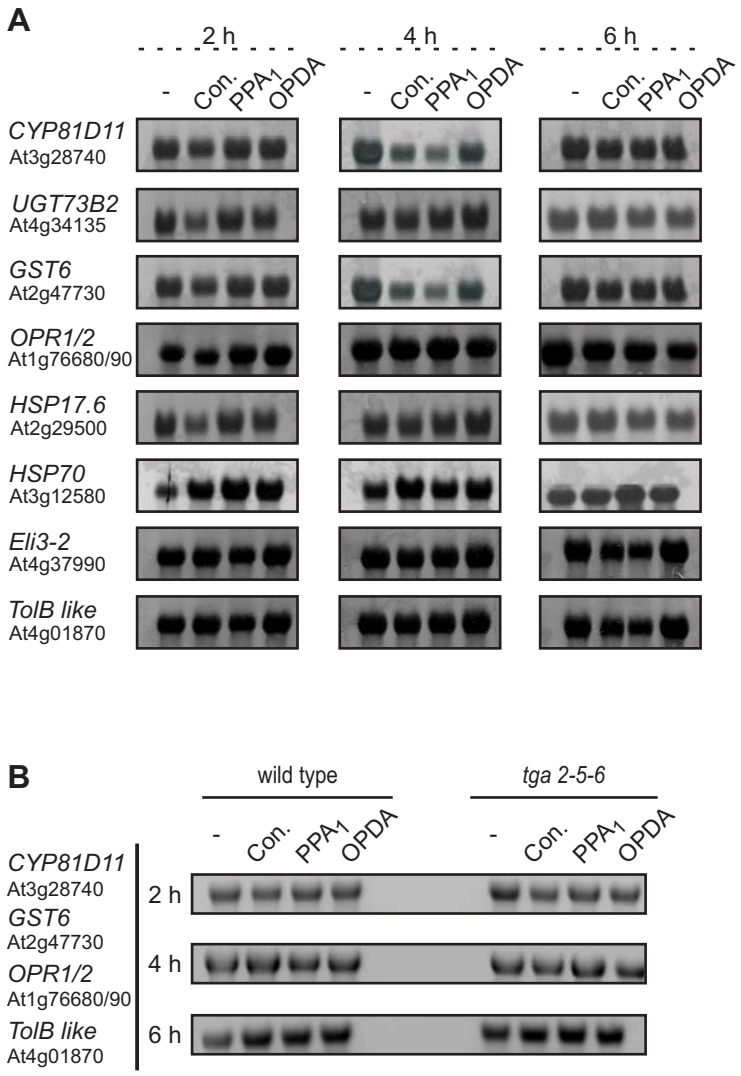


Supplemental Figure 1

Loading controls for Northern Blot analysis of cell culture samples

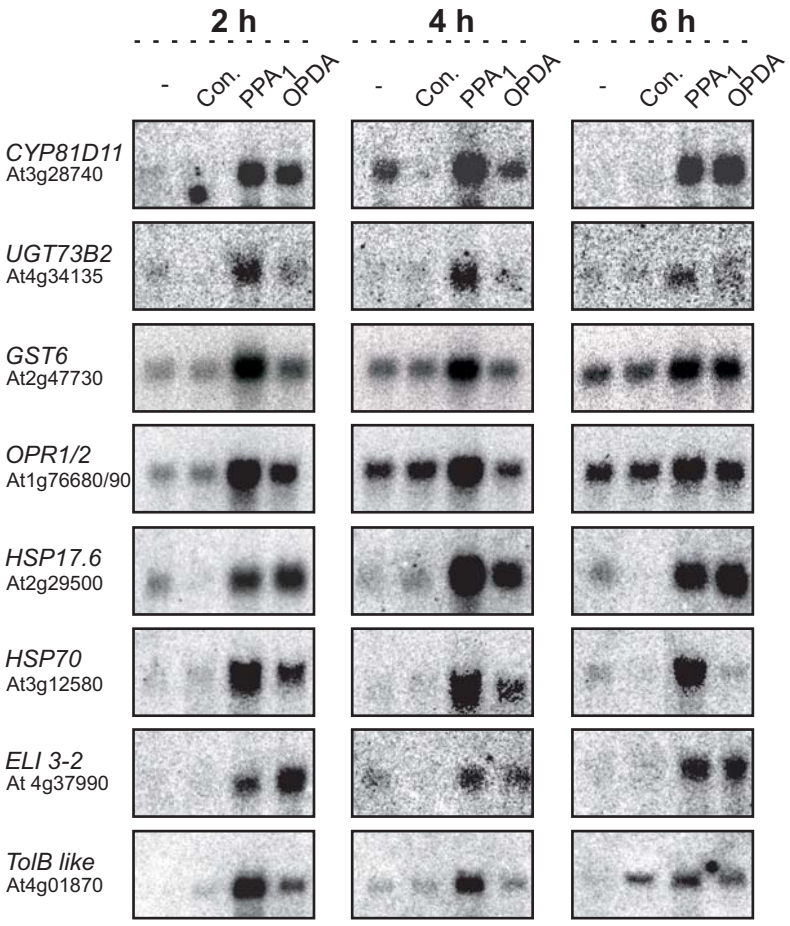
Shown are photographs of ethidium bromide stained gels for equal RNA loading used for the analysis of the expression of PPA_1 - responsive genes in *Arabidopsis* cell cultures (Figure 2). On the left side probes are indicated which were used for hybridization.



Supplemental Figure 2

Loading controls for Northern Blot analysis of plant samples

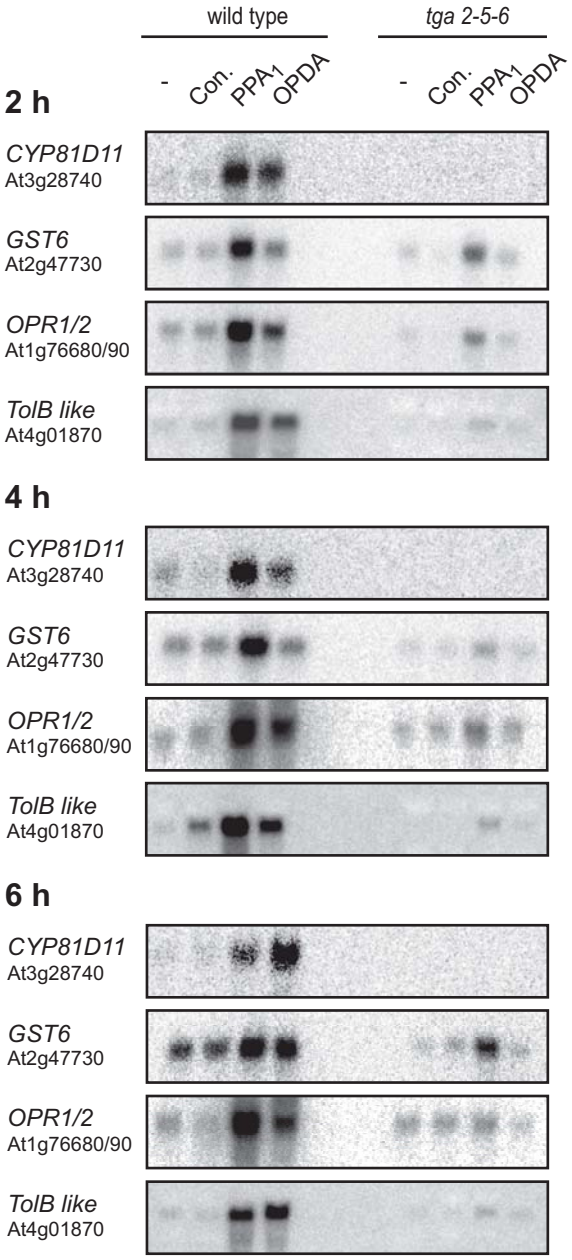
Shown are photos of ethidium bromide stained gels for equal RNA loading used for the analysis of the expression of PPA_1-responsive genes in *Arabidopsis* plants (A) (Supplemental Figure 3) and the *tga 2-5-6* triple mutant (B) (Supplemental Figure 4). On the left side probes are indicated which were used for hybridization.



Supplemental Figure 3

Northern Blot analysis of plants treated with 75 µM PPA_1 or OPDA after 2, 4 and 6 h

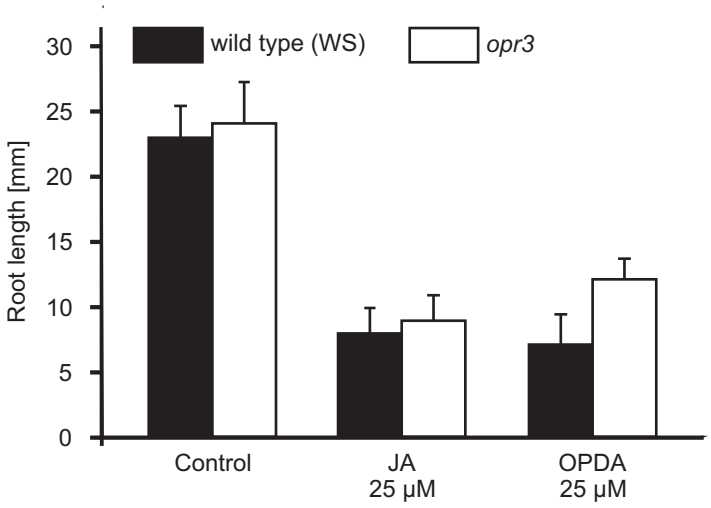
Expression of PPA_1-responsive genes was analyzed in *Arabidopsis* plants in response to 75 µM PPA_1 and OPDA, 2, 4 and 6 h after treatment and in plants which are non-treated (-) or treated with 0.5 % MeOH in water (Con.). The experiment was repeated at least three times; a representative Northern Blot is shown. RNA was isolated, 8 µg RNA were loaded per lane and gel loading was monitored by EtBr-staining of the gel (Supplemental Figure 2A). Blots were hybridized with the indicated probes.



Supplemental Figure 4

Northern Blot analysis of the oxylipin response in the *tga2-5-6* mutant after 2, 4 and 6 h

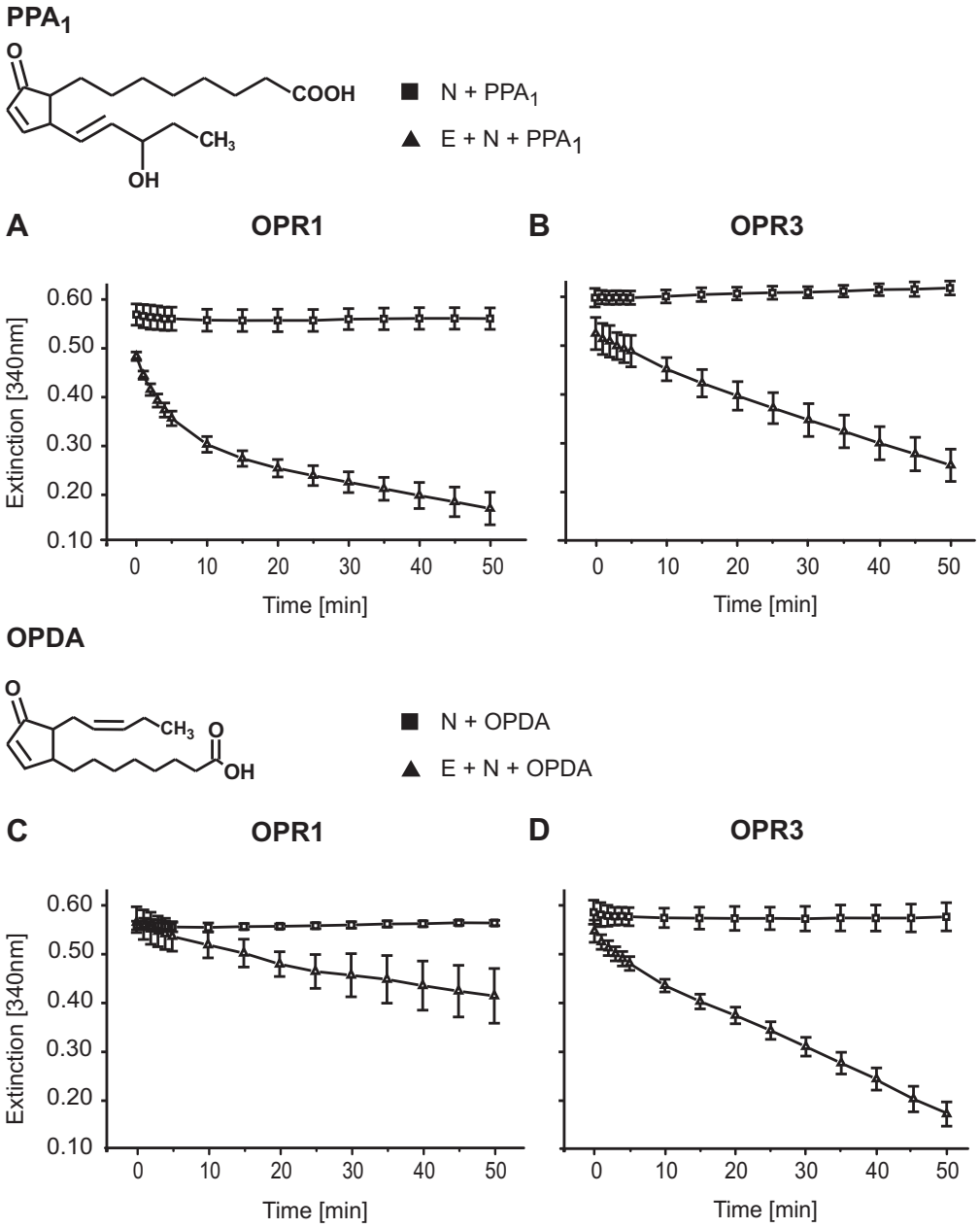
Expression of PPA_1-responsive genes was analyzed in *Arabidopsis* wild type and *tga 2-5-6* triple mutant plants. Analysis was performed in response to 75 µM PPA_1 or OPDA, 2, 4 and 6 h after treatment and in plants which are non treated (-) or treated with 0.5 % MeOH in water (Con.). The experiment was repeated three times; a representative Northern Blot is shown. RNA was isolated, 8 µg RNA were loaded per lane and gel loading was monitored by EtBr-staining of the gel (Supplemental Figure 2B). Blots were hybridized with the indicated probes.



Supplemental Figure 5

Inhibition of root growth by JA and OPDA in *opr3*

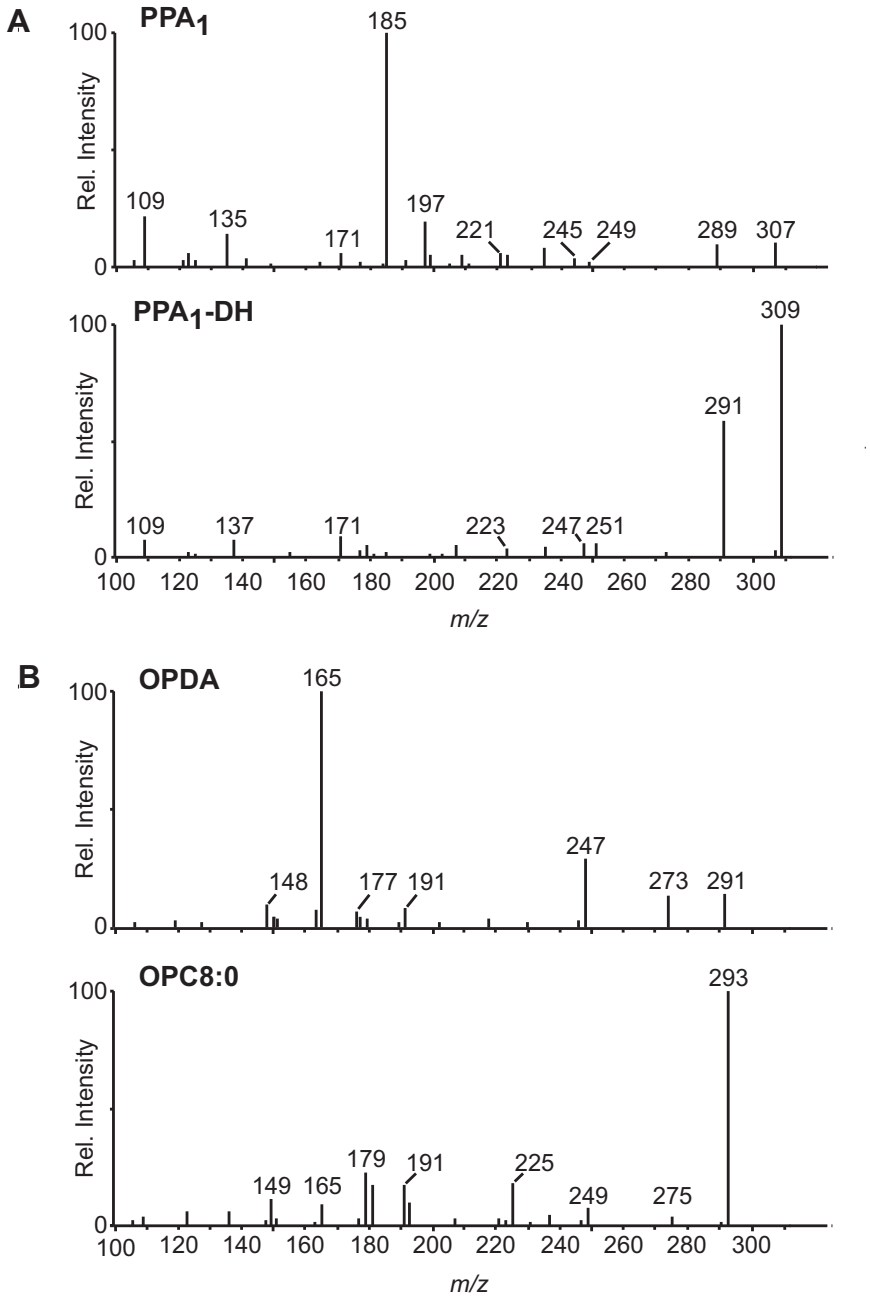
Seedlings of wild type (WS) and *opr3* mutants were germinated on medium containing 25 µM JA or OPDA and root growth was measured after 8 d (means $\pm$ sd, n = 20 seedlings). Three independent experiments were performed with similar results.



Supplemental Figure 6

Spectrophotometric analysis of PPA_1 and OPDA reduction

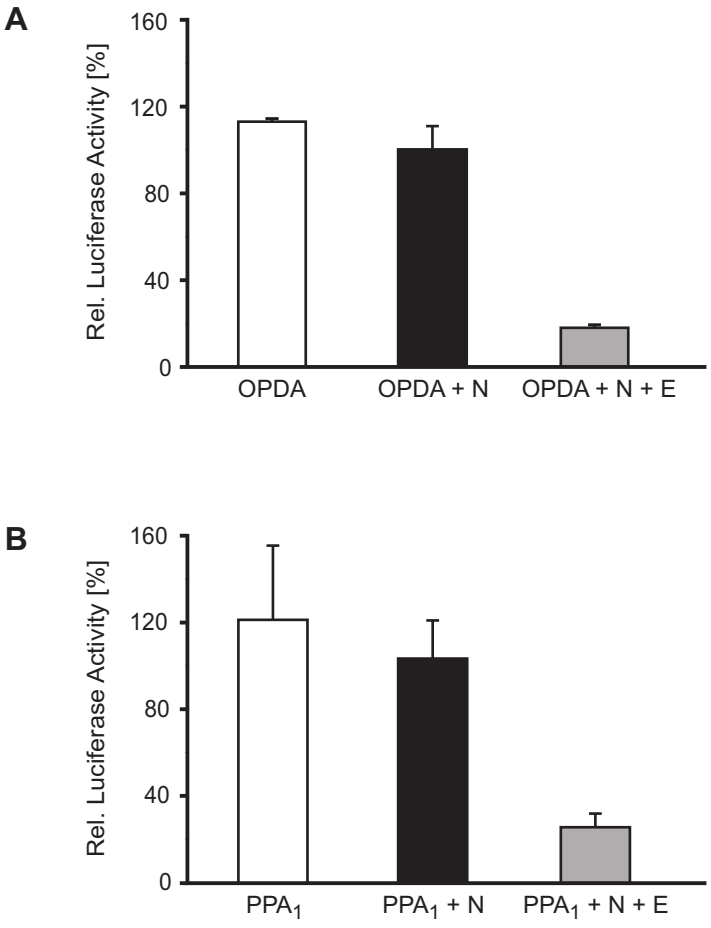
NADPH oxidation by the recombinant oxidoreductases OPR1 and OPR3 (E) in the presence of PPA_1 (A and B) or OPDA (C and D). Assays were performed at 25°C, in 1ml 50 mM potassium phosphate buffer, pH 7.5, containing 120 µM NADPH (N), 10 µg of the purified protein, and 0.1 mM of the substrates. The decrease in absorbance of reduced NADPH was measured at 340 nm.



Supplemental Figure 7

Fragment spectra of substrates and products of the OPR assays

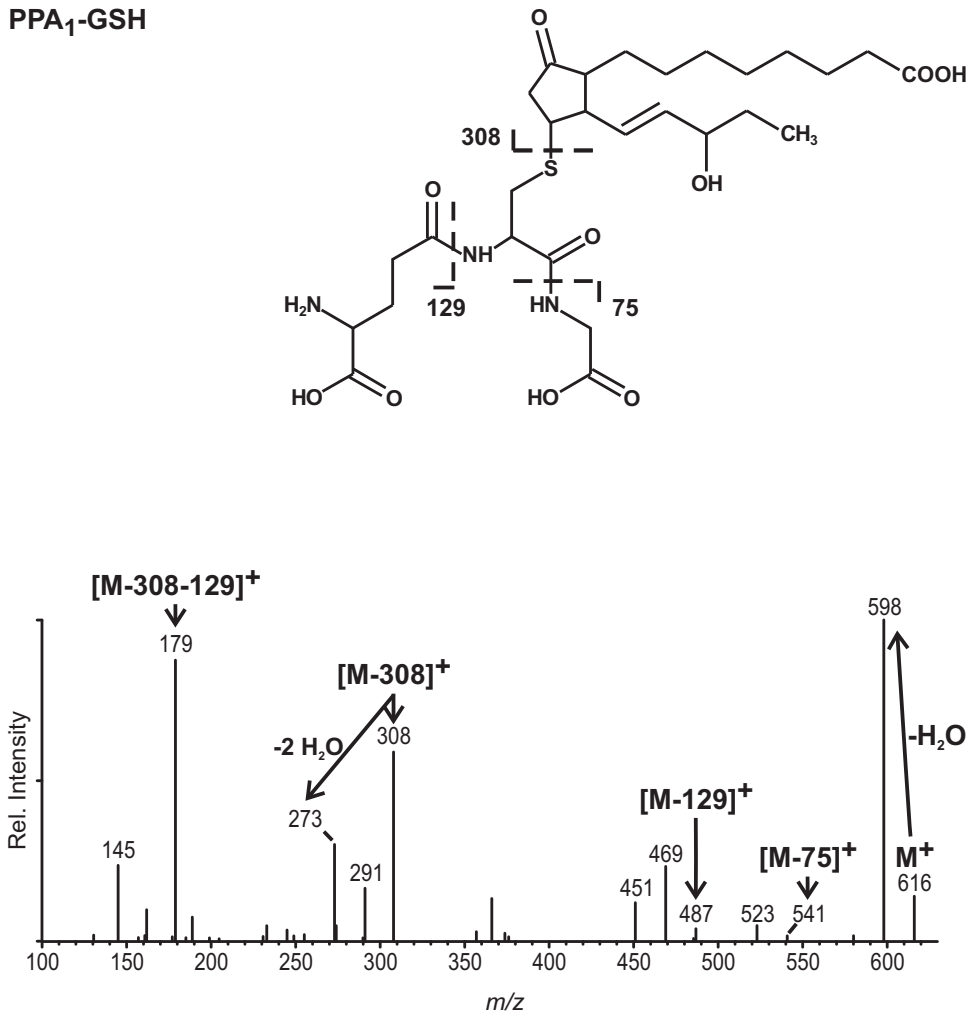
Shown is the metabolite composition of an enzyme reaction terminated after 30 min, using standard conditions as described in Methods with purifed enzyme (10 µg). The mass spectra were recorded in the negative electrospray ionization (ESI) mode.



Supplemental Figure 8

Effect on biological activity of OPDA and PPA_1 of metabolism by OPR enzymes

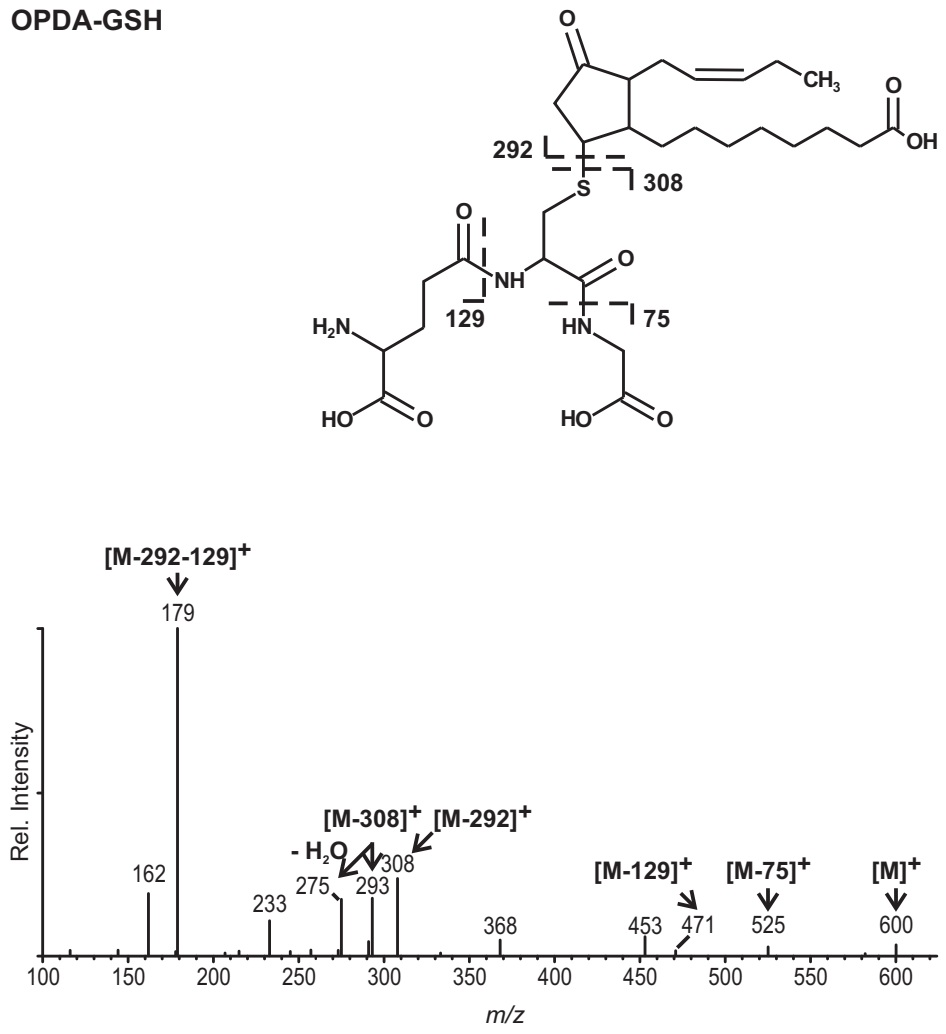
The cyclopentenones OPDA and PPA_1 were incubated with or without OPR3 / OPR1 (E) as described in Methods. Assays were performed at 25 °C containing 120 µM NADPH (N), 10 µg of the purified protein (E) and 0.1 mM of the substrates. After 25 min the assay was stopped, added to GST6::LUC plants grown in liquid medium and luminescence was measured using a CCD camera. Shown is the relative luminescence with standard errors of plants treated with the assay with or without the enzyme after 7.5 h to control plants (0.5% MeOH in water and 120 µM NADPH).



Supplemental Figure 9

Fragment spectra of PPA_1-GSH adduct

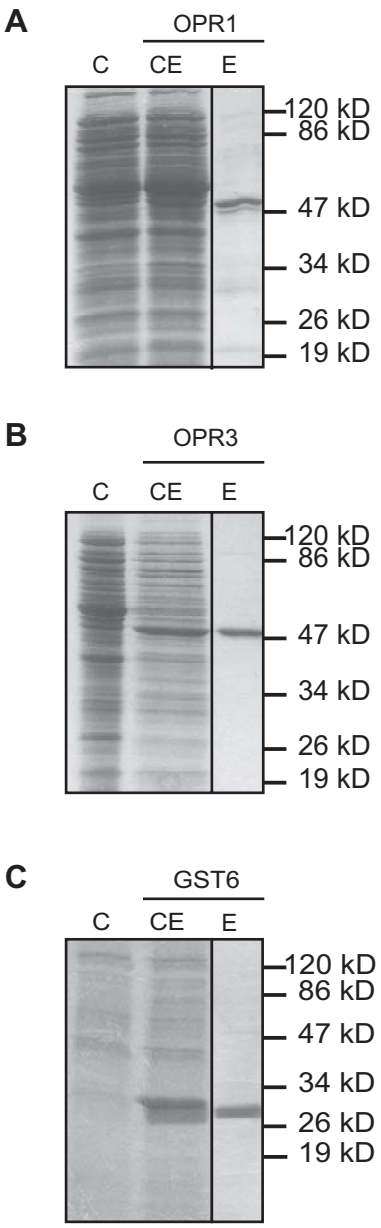
Mass spectrometry analysis in the multiple reaction monitoring (MRM) mode of PPA_1-GSH adducts. Adducts were identified by four characteristic MRM transistions (PPA_1-GSH: *m/z* 616→598 $([M+H)]^+$ - H_2O), 616→308 ($[M+H]^+$ - PPA_1), 616→273 ($[M+H]^+$ - PPA_1 - H_2O) and 616→179 ($[M+H]^+$ - 308 - 129). Other prominent ions are present at *m/z* 541 ($[M+H]^+$ - 75) and *m/z* 487 ([M+H]+ - 129).



Supplemental Figure 10

Fragment spectra of OPDA-GSH adduct

Mass spectrometry analysis in the multiple reaction monitoring (MRM) mode of OPDA-GSH adducts. Adducts were identified as described in Methods by four characteristic MRM transistions (OPDA-GSH: *m/z* 600→598 ($[M+H)]^+$ - H_2O), 600→308 ($[M+H]^+$ - OPDA), 600→273 ($[M+H]^+$-OPDA-H_2O) and 600→179 ($[M+H]^+$-308-129). Other prominent ions are present at *m/z* 525 ($[M+H]^+$ - 75) and *m/z* 471 ($[M+H]^+$ - 129)



Supplemental Figure 11

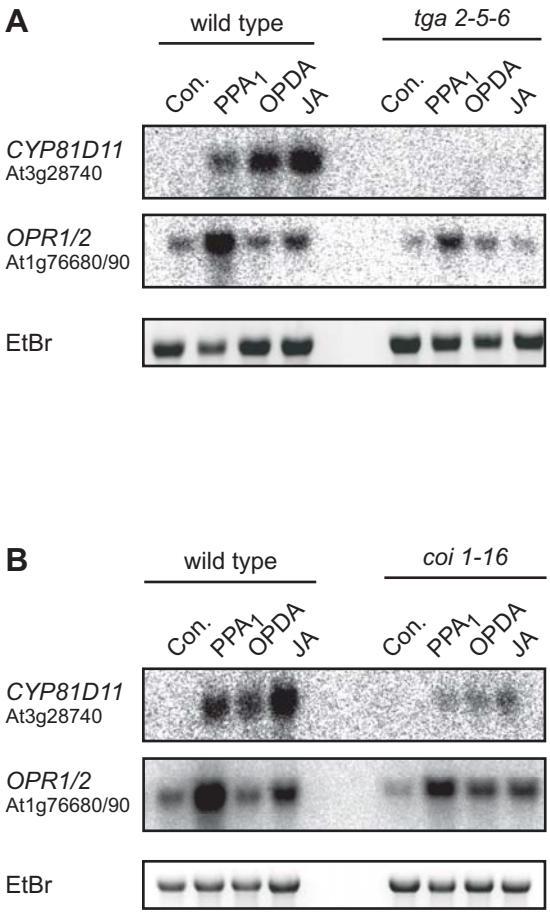
Purification of the recombinant OPR1, OPR3 and GST6 proteins

Shown are Coomassie blue-stained SDS-PAGE of aliquots of bacteria without the plasmids (C), crude extract (CE) and eluate after by Ni-tris-carboxymethyl ethylene diamine (Ni-TED) affinity purification. The purified protein fractions were further used for enzymatic test as described in Methods.

(A) OPR1

(B) OPR3

(C) GST6



Supplemental Figure 12

Regulation of gene expression by oxylipins in the mutants *tga 2-5-6* and *coi1*

Expression of PPA_1-responsive genes in *Arabidopsis* plants of the mutants *tga 2-5-6*, *coi 1-16* or wild type (Col-0, Col-GL), treated for 4 hours with 75 µM PPA_1, OPDA or JA and with 0.5% MeOH in water (Con.). The experiment was repeated at least three times; a representative Northern Blot analysis is shown. RNA was isolated, 8 µg were loaded per lane and gel loading was monitored by EtBr-staining of the gel. Blots were hybridized with the indicated probes.