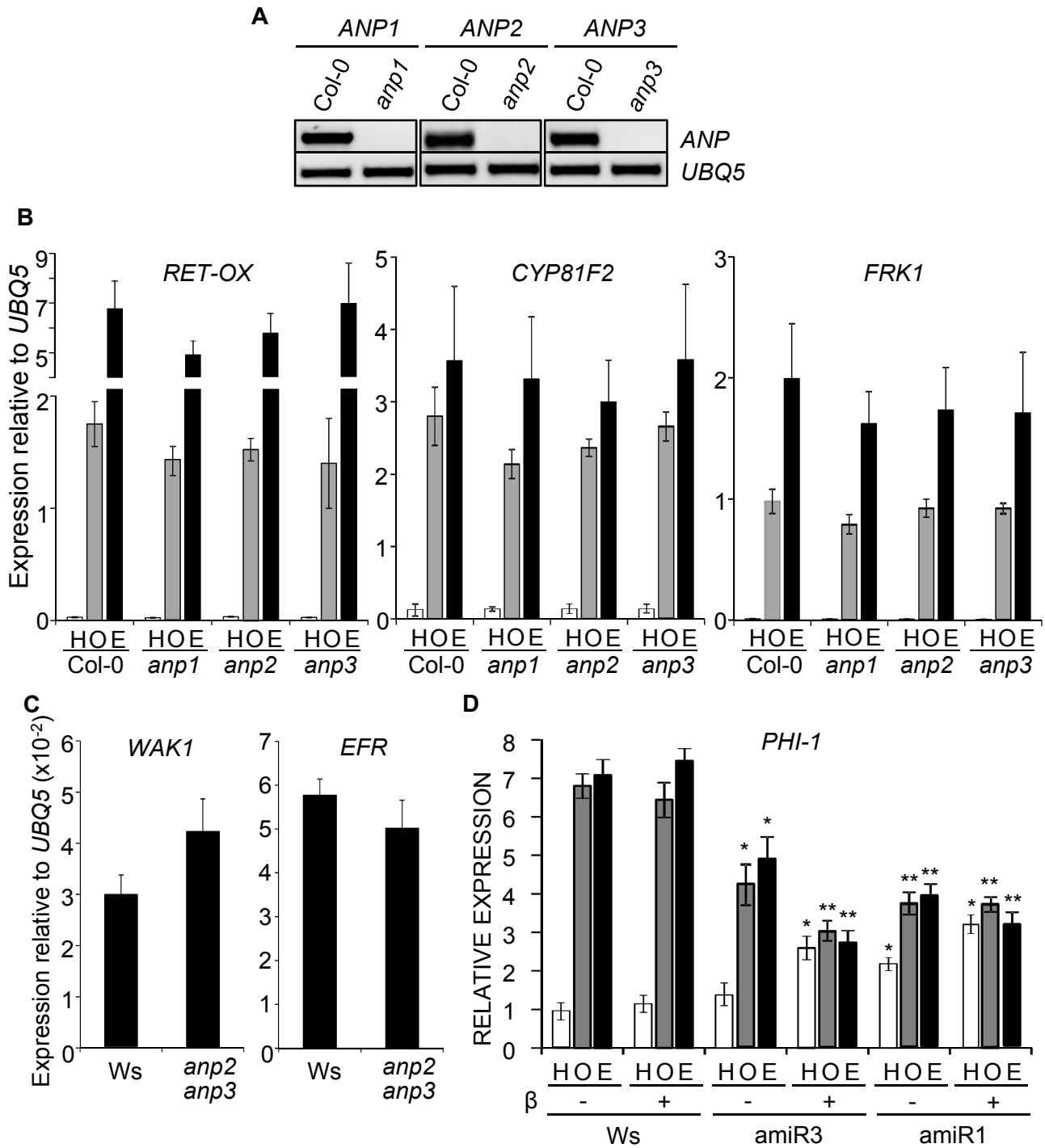
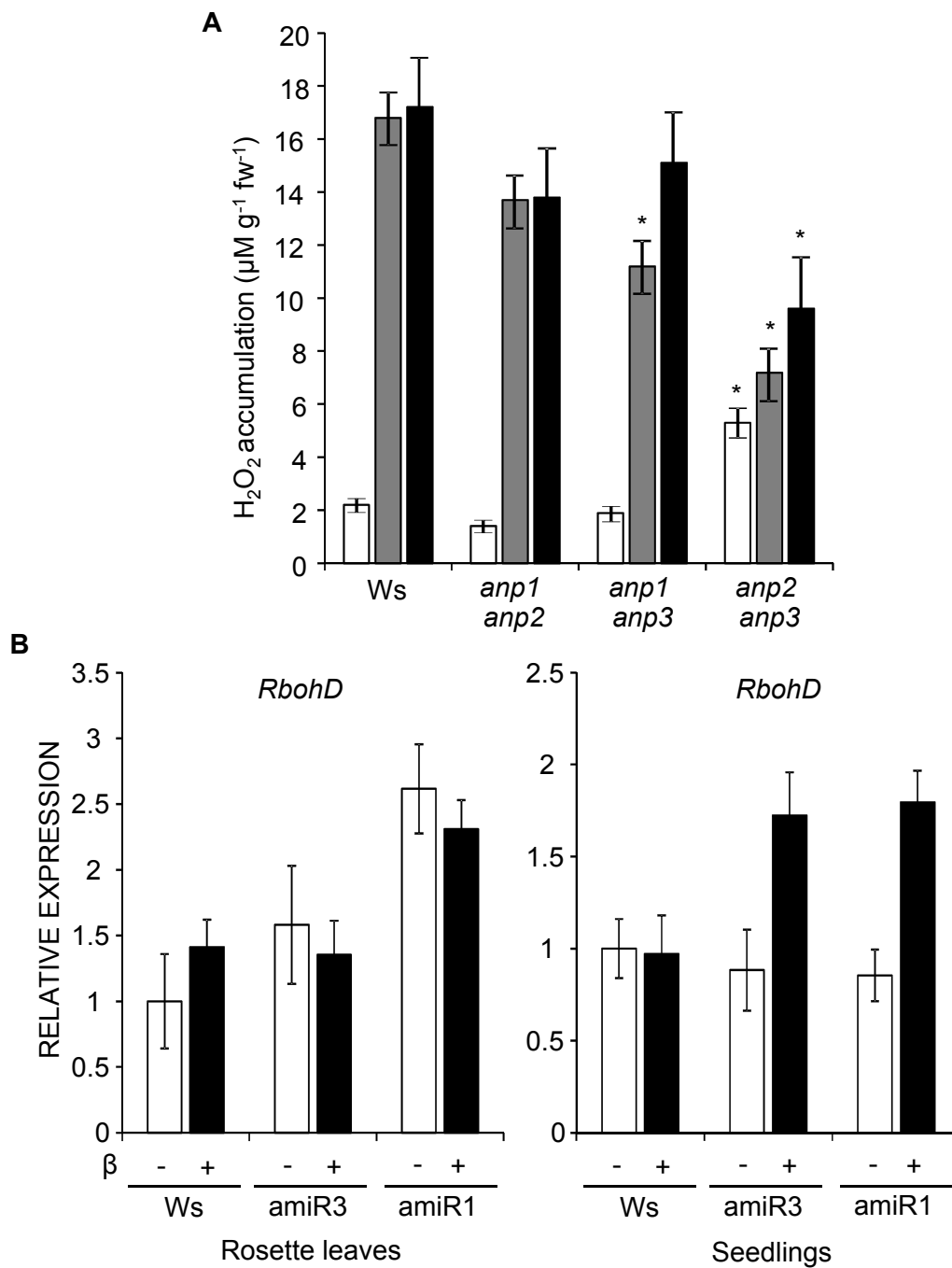


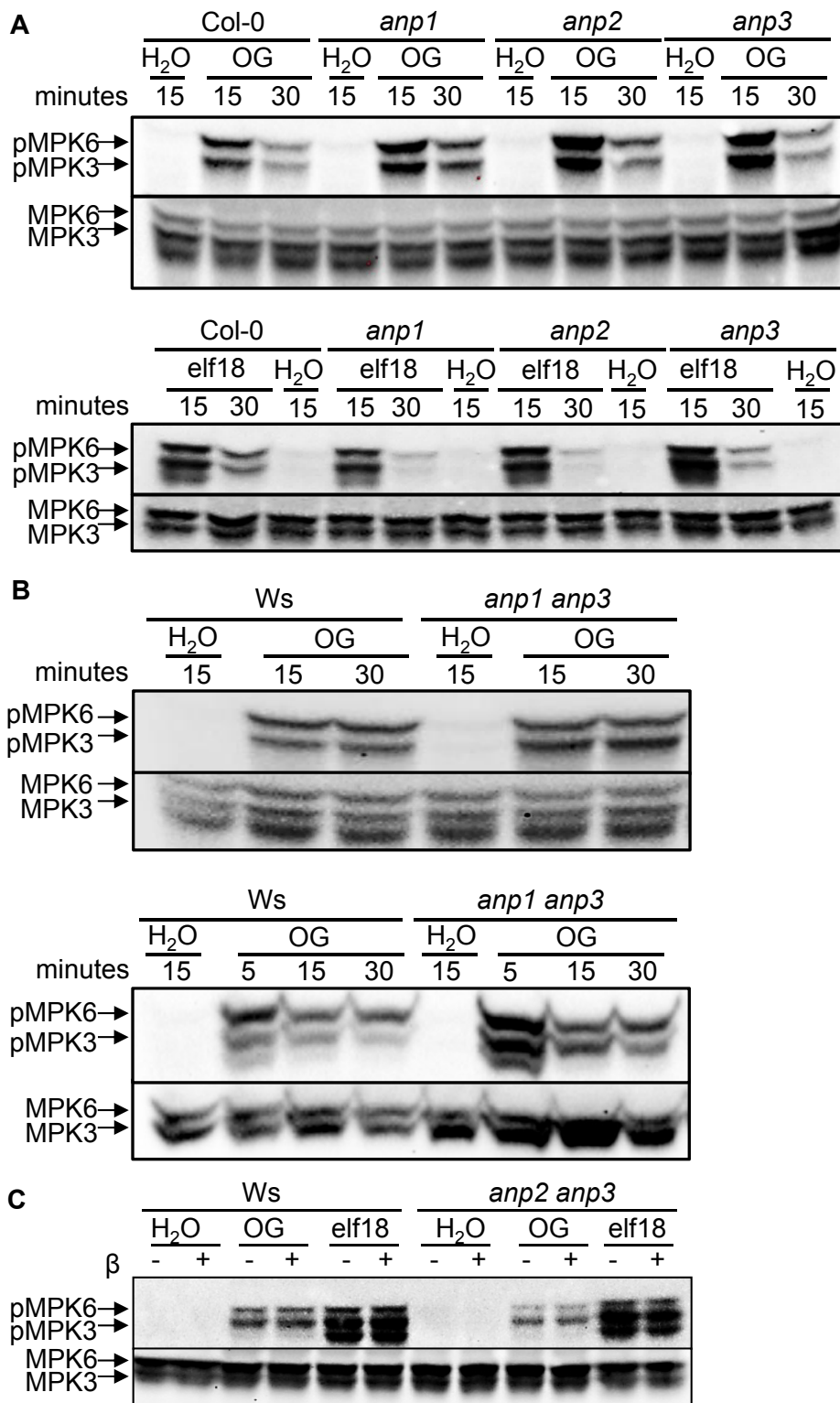
Supplemental Figure S1. Phenotype of control plants transformed with the empty vector for β -estradiol-inducible expression and of *anp2,3-OE3* transgenic plants. A) WT (Ws) seedlings and Ws seedlings transformed with the empty pMDC7 vector (line 3.3), germinated and grown in the presence of 5 μ M β -estradiol. B) Four-week-old plants of Ws and of the transgenic line 3.3 sprayed with 10 μ M β -estradiol every three days for three times and grown for further 2 weeks. C) Semi-quantitative RT-PCR analysis of *XVE* transcripts in line 3.3 seedlings, compared to the untransformed Ws seedlings, all grown either in the presence (+) or in the absence (-) of β -estradiol; *UBQ5* was used as a reference gene. D) The overexpression of *ANP3* in the *anp2 anp3* double mutant plants (*anp2,3-OE3*) restores a wild type-like developmental phenotype. See also Figure 1E, which shows that the phenotype of the amiR1 mutant treated with DMSO is similar to that of *anp2 anp3* double mutant.



Supplemental Figure S2. Expression analyses in *anp* mutant seedlings. A) Expression of *ANP1*, *ANP2* and *ANP3*, analyzed by RT-PCR. B) Expression of the indicated defense marker genes was determined by qRT-PCR 1 h after treatment with water (H) OGs (O) or elf18 (E) in *anp* single mutants. C) Basal levels of *WAK1* and *EFR* transcripts in the *anp2 anp3* double mutant compared to WT (Ws). D) Elicitor-induced expression of *PHI-1* gene after 30 min treatment with water, OGs or elf18 in WT and *anp* triple mutants. Seedlings were grown either in the presence (+) or in the absence (-; DMSO only) of estradiol. In B, C and D, qRT-PCR analyses were performed using *UBQ5* as a reference, and results are the mean of three independent experiments (\pm SE; n=20 in each experiment). In D, results are referred to the gene/*UBQ5* value of water-treated Ws. Asterisks indicate statistically significant difference between mutants and Ws treated in the same way, according to Student's *t* test (*, $P < 0.05$; **, $P < 0.01$).



Supplemental Figure S3. ANPs are involved in elicitor-triggered H₂O₂ production in seedlings. Accumulation of extracellular H₂O₂ in response to H₂O (mock, white bars), OGs (gray bars) or elf18 (black bars) in WT (Ws) and *anp* double mutant seedlings, measured by using a xylenol orange-based assay. Results are means of three independent experiments (\pm SE; $n=30$ in each experiment). Asterisks indicate statistically significant differences between mutant and wild type plants treated with the same elicitor, according to Student's *t* test (*, $P < 0.05$; **, $P < 0.01$). B) Levels of *RbohD* transcripts in adult rosette leaves or seedlings of Ws, amiR1 line 2.5 and amiR3 line 5.3 triple mutants, analyzed by qRT-PCR. Transcript levels are shown as the mean of three independent experiments (\pm SE), normalized to *UBQ5* expression and plotted relative to expression in Ws.



Supplemental Figure S4. MPK3 and MPK6 phosphorylation in response to OGs or elf18 in *anp* single and double mutant seedlings. Levels of phosphorylated MAPKs (pMPK3 and pMPK6) after elicitation with OGs or elf18 in WT (Col-0 or Ws), *anp* single mutants (A) and *anp1 anp3* double mutants (B). C) Elicitor-induced MPK3 and MPK6 phosphorylation in Ws and *anp2 anp3* double mutant seedlings treated with β -estradiol (1 μ M). In all experiments, phosphorylation was determined by immunoblot analysis using an anti-p44/42-ERK antibody (top panels). Levels of MPK3 and MPK6 total proteins were determined using specific antibodies (bottom panels). The identity of individual MAP kinases as determined by size is indicated by arrows. Experiments were repeated at least three times with similar results.