Supplemental Figures



Supplemental Figure S1. Expression profiles of *GRF* gene family members in *Arabidopsis* roots.

The mRNA expression levels of *GRF1-9* were quantified in root tissues of the wild-type Col-0 using qPCR. Expression levels were normalized using *Actin8* as an internal control. The relative fold-change values represent changes of *GRF* expression levels relative to *GRF5*, which exhibited the lowest expression among all *GRF* genes. Data are averages of three biologically independent experiments \pm SE.



Supplemental Figure S2. qPCR quantification of transgene expression levels in transgenic *Arabidopsis* lines described in this study.

The expression level of mature miR396a (A), miR396b (B) *GRF1* (C) and *GRF3* (D) was measured by qPCR in root tissues of transgenic plants along with the wild type (Col-0). U6 snRNA was used as an internal control to normalize the expression levels of miR396, and *Actin8* was used to normalize the expression levels of *GRF1* and *GRF3*. The relative fold-change values represent changes of the expression levels in the transgenic plants relative to the wild type (Col-0). Data are averages of three biologically independent samples \pm SE.

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Supplemental Figure S3. Overexpression of miR396 reduces *GRF* gene expression.

The mRNA expression level of *GRF1* through 9 was measured by qPCR.in the root tissues of 10 d-old wild-type (Col-0) and transgenic plants overexpressing miR396b (line 16-4). The expression levels were normalized using *Actin8* as an internal control. The relative fold-change values represent changes of mRNA levels in the transgenic plants relative to the wild-type control. Data are averages of three biologically independent experiments \pm SE.



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Supplemental Figure S4. Characterization of Arabidopsis grf1 and grf3 mutants.

A, Schematic representation of the *grf1* and *grf3* alleles illustrating locations of the T-DNA insertions. *GRF1* and *GRF3* genes each contain four exons (black boxes) and three introns (white boxes). The T-DNA is inserted in third exon of the *GRF1* gene in Salk_069339C and Salk_078547C, 1,094 and 1,441 bp, respectively, downstream of the translation initiation codon The two T-DNA insertions of *GRF3* (Salk_026786 and Salk_116709) are in the promoter region, 266 and 581 bp, respectively, upstream of the translation site.

B, mRNA accumulation in the *grf1* and *grf3* mutants. The mRNA abundance of *GRF1* or *GRF3* was determined by qPCR using gene-specific primers. *grf1*mutants (Salk_069339C and Salk_078547C) express about 4% and 64% of the corresponding with-type (Col-0) expression level, while *grf3*mutants (Salk_116709 and Salk_026786) express about 66% and 45% of the corresponding wild-type (Col-0) expression level. *Arabidopsis Actin8* was used an internal control. Data are averages of three biologically independent samples \pm SE.



Supplemental Figure S5. Schematic representation of miR396-resistant versions of *GRF1* and *GRF3* transcripts. Nucleotide pairing of miR396 with the corresponding wild-type binding sites of *GRF1* and *GRF3* show 19 nucleotide matches, whereas in the miR396-resistant version of *GRF1* (*rGRF1*) and *GRF3* (*rGRF3*) the miR396 binding site contains 9 and 10 mismatches, respectively. Conserved nucleotides between wild-type and modified miR396 binding sites are in bold.



Supplemental Figure S6. Overexpression of *miR396* and the target genes *GRF1* and *GRF3* reduces root length.

Homozygous T3 lines overexpressing miR396a (A), miR396b (B), rGRF1 (C), or rGRF3 (D) were planted on modified Knop's medium along with the wild type (Col-0), and root lengths were measured 10 days after planting. Root length values are averages of at least 50 plants. Statistically significant differences between overexpression lines and the wild type were determined by unadjusted paired *t* tests (P < 0.05) and are indicated by an asterisk.