**Supplementary Materials**

**Microarray Screen**.

Total RNA was isolated by hot phenol extraction, as previously described (Söderman et al. 2000). Prior to use in microarray experiments, effectiveness of the ABA and CHX treatments was tested by Northern analysis of genes previously shown to have enhanced ABA-inducibility in 35S:ABI lines, e.g. RAB18 (Brocard et al. 2002) (data not shown).

Microarrays were performed by the Laboratory for Functional Genomics at Texas A&M University, using Affymetrix ATH1 Gene Chips, following the manufacturer's recommended protocols. Briefly, cDNA was derived from total RNA and subsequently used to generate biotin labeled cRNA which was fragmented to lengths between 35 and 200 bp. Hybridization of the fragmented cRNA to the Gene Chip Array was carried out in a Affymetrix GeneChip Hybridization Oven 640. Appropriate washing and staining of the arrays was then conducted on an Affymetrix GeneChip Fluidics Station 400 under the control of Affymetrix Microarray Suite 5.0 software. The stained and washed array was finally scanned with an Agilent GeneArray Scanner.

The scanner output was analyzed by GeneSpring 7 (Silicon Genetics/Agilent) to normalize the intensity data and expression was compared to levels on a chip hybridized with targets derived from seedlings overexpressing the ABI4 gene in the absence of added ABA. Each measurement for each gene on each chip was divided by the median of that gene's measurements on the control chip. Only genes flagged as present or marginal in the experimental chips were considered for determining those that were up-regulated relative to the control. ABA and ABI-regulated genes were identified as those whose transcripts were at least two-fold higher in ABA-treated *35S:ABI* relative to *35S:ABI* without ABA and also at least two-fold higher in *35S:ABI* relative to *abi* when both genotypes were treated with ABA. Although each microarray experiment identified several hundred genes that met these criteria, less than a hundred of these were reproducible across replicate microarrays, presumably due to biological variation.

Supp. Fig. 1. Functional tests of 35S:GFP:ABI4 transgene, scoring enhanced ABA sensitivity in wild-type Col background and complementation of ABA resistance due to *abi4-1* mutation. (A) Germination (radicle emergence) at 6 days post stratification on the indicated media. (B) Root growth of seedlings transferred at 2d post stratification from GM to fresh GM with or without 3 μM ABA, scored after an additional 5d growth. (C) Sugar sensitivity of seedling growth, as reflected in reduced growth and increased anthocyanin accumulation on minimal medium supplemented with 4% Glc.
The 1B line of Col + 35S:GFP:ABI4 was used for subsequent studies of gene expression. Note that, although more sensitive to ABA than the wild-type line, this is much less sensitive than the previously reported 35S:ABI4 line #114A, which showed complete inhibition of root growth by 3 uM ABA (Söderman et al. 2000) and strong Glc hypersensitivity (Finkelstein et al. 2002).

Supp. Fig. 2. RNA gel blot tests of ectopic ABI-dependent ABA induction A) Certain ABI4 targets are only induced in original 35S-ABI4 lines. Seeds were germinated for 11 days on GM media, then transferred for four hours to GM media with or without 50 μM ABA (ABA), 20 μM cycloheximide (CHX) or both ABA and cycloheximide (A/X). Seedlings were harvested, RNA was extracted and transcripts were analyzed by Northern blot. Overexpression lines included the original 35S-ABI4 line (35S-I4, on left) and the 35S-GFP-ABI4 line (GFP-I4, on right).
B) Indirect targets of ABA/ABI induction. Transcripts were analyzed as described in (A).
C) Genes whose induction is not dependent on ABA treatment and/or ABI overexpression. Asterisks indicate genes in (C) whose expression matches microarray results.

Supp. Fig. 3. RNA gel blot tests of ABA- and stress-regulated gene expression at 1d post stratification. Genes showing minimal or no change between wild type and *abi* response to ABA or stress.

Supp. Fig. 4. Co-regulation of ABI4 target set, but not ABI5 target set by ABA and stress treatments. Genevestigator (Hruz et al. 2008) was used to analyze previously published

microarrays. For each condition, the percent of genes in a target set that were induced at least two fold is indicated.

Supp. Fig. 5 Germination and seedling growth of *abi, abf* monogenic and double mutant lines. Comparison of wild-type, *abi4, abi5-7, abf1, abf3,* and *abi4 abi5-1* seeds after 3d post-stratification on GM supplemented with 5 uM ABA. Gene expression analyses of the *abi4 abi5-1* double mutant are not included in Fig.5A because they have progressed much farther into seedling growth than the other genotypes. Furthermore, *abi5-1* is in the Ws background and some of these transcripts vary substantially between Col and Ws backgrounds (data not shown).

Supp. Fig. 6. Mapping ABI4 binding sites within promoter fragments. Binding is limited to a region of the At1g32560 promoter that contains the consensus ABI4 binding site identified in maize. The At4g16160 promoter does not include either the maize or CE1-like ABI4 binding sites, but does bind ABI4.

Supp. Table 1. Microarray Results Summary. Worksheets list genes scored as more highly expressed in *35S:ABI* than *abi* lines, and ABA-induced in duplicate experiments, separated into ABI4-specific, ABI5-specific, and co-regulated "Shared" genes. The "ABI+ ABA+" columns display the normalized measurements for each gene in 35S:ABI lines treated with ABA divided by the median of that gene's measurements on the control (35S:ABI without ABA) chip. The "abi ABA+" columns display the corresponding normalized measurements in ABA-treated abi lines. The ABI:abi columns display the ratios of these normalized measurements. Although all genes listed were flagged as present or at least marginal in the ABA-treated 35S:ABI samples, transcripts for many of these were very low or absent in the control (no ABA) or mutant samples, as indicated in the "+Flags" columns of the ABI4-specific genes worksheet.

Supp. Table 2. Genes tested by RNA gel blot analyses to validate differential expression observed in microarray. Microarray results represent expression ratios in 11d old *35S-ABI*

vs. *abi* plants treated with ABA and cycloheximide (CHX) for 4 hrs. Northern results indicate whether expression in 11d old plants is enhanced by initial *35S-ABI4* transgene (ABI4), *35S-GFP-ABI4* (GFP-ABI4), or *35S-ABI5* (ABI5) and whether expression is increased by ABA treatment (ABA-dep) and maintained in the presence of CHX (CHX-res). y = regulated by designated signal, n = not regulated by designated signal, n.d. = not detectable by Northern. n/y = ABI4-dependent expression not CHX-resistant, but ABI5-dependent expression is CHX resistant

Supp. Table 3. Analysis of target gene expression in previously published microarrays. Relative expression of genes identified as ABA/ABI-regulated in 11d seedlings, expressed and ABI-regulated in dry and 24 hr imbibed (HAI) seeds, and ABI3-regulated in fresh or after-ripened (AR) seeds. Comparisons of genotypes are ratios of normalized microarray data,

Supp. Table 4. Genes tested by Northern for misexpression in *abi* seeds and seedlings exposed to ABA or stress treatments. Microarray results indicate ABI dependence for induction in 11d old *35S-ABI* vs. *abi* plants treated with ABA and cycloheximide (CHX) for 4 hrs. Northern results reflect comparison of expression in wild-type vs. abi seeds 1d post stratification  on minimal medium supplemented with 5 μM ABA, 333 mM Glc or 166.5 mM NaCl or at 2.5d post-stratification on GM supplemented with 5 uM ABA