SUPPLEMENTAL INFORMATION

Cell identity regulators link development and stress responses in the *Arabidopsis* root

Anjali S. Iyer-Pascuzzi, Terry Jackson, Hongchang Cui, Jalean J. Petricka, Wolfgang Busch, Hironaka Tsukagoshi, and Philip N. Benfey

Inventory of Supplemental Information

Supplemental Figures

Figure S1: Confocal images of GFP-marked lines used for cell sorting after transfer to low pH or –S. Linked to Figure 1.

Figure S2: Fold changes in response to stress in cell types for the 22 ABA marker genes in the whole root activated CSR gene set. Linked to Figure 2.

Figure S3: The majority of stress-regulated genes are cell-type and stress specific. Linked to Figure 4.

Figure S4: Stress-regulated versus stress-enriched genes. Linked to Figures 6 and 7.

Supplemental Tables (attached as Excel files)

Table S1: Microarray experiments used in this study. Linked to Experimental Procedures.

Table S2: Universal and common stress response genes, GO categories, transcription factor family enrichment and marker gene enrichment. Linked to Figure 1.

Table S3: Fold change and significance of the ABA marker genes that significantly responded in at least one of the 14 conditions in our whole root meta-analysis. Linked to Figure 2.

Table S4: Expression values for significant probesets for the cell-type dataset. Linked to Figure 2.

Table S5: Expression values for significant probesets for the developmental stage (longitudinal) dataset. Linked to Figure 2.

Table S6: Fold change and significance of key cell identity regulators in response to stress. Linked to Figure 3.

Table S7: SCARECROW direct target genes identified through ChIP-chip and expression values of stress-regulated, hair-dependent ABA marker genes. Linked to Figure 3.

Table S8: Clusters of the top 50% varying genes that significantly responded to stress. Linked to Figure 5.

Table S9: Sorting-affected probesets, singletons, and primers used for qRT-PCR analysis. Linked to Experimental Procedures.

Table S10: Fold change and expression values of core marker genes. Linked to Figure 6 and 7.

Supplemental References

Supplemental Figure 1

Figure S1

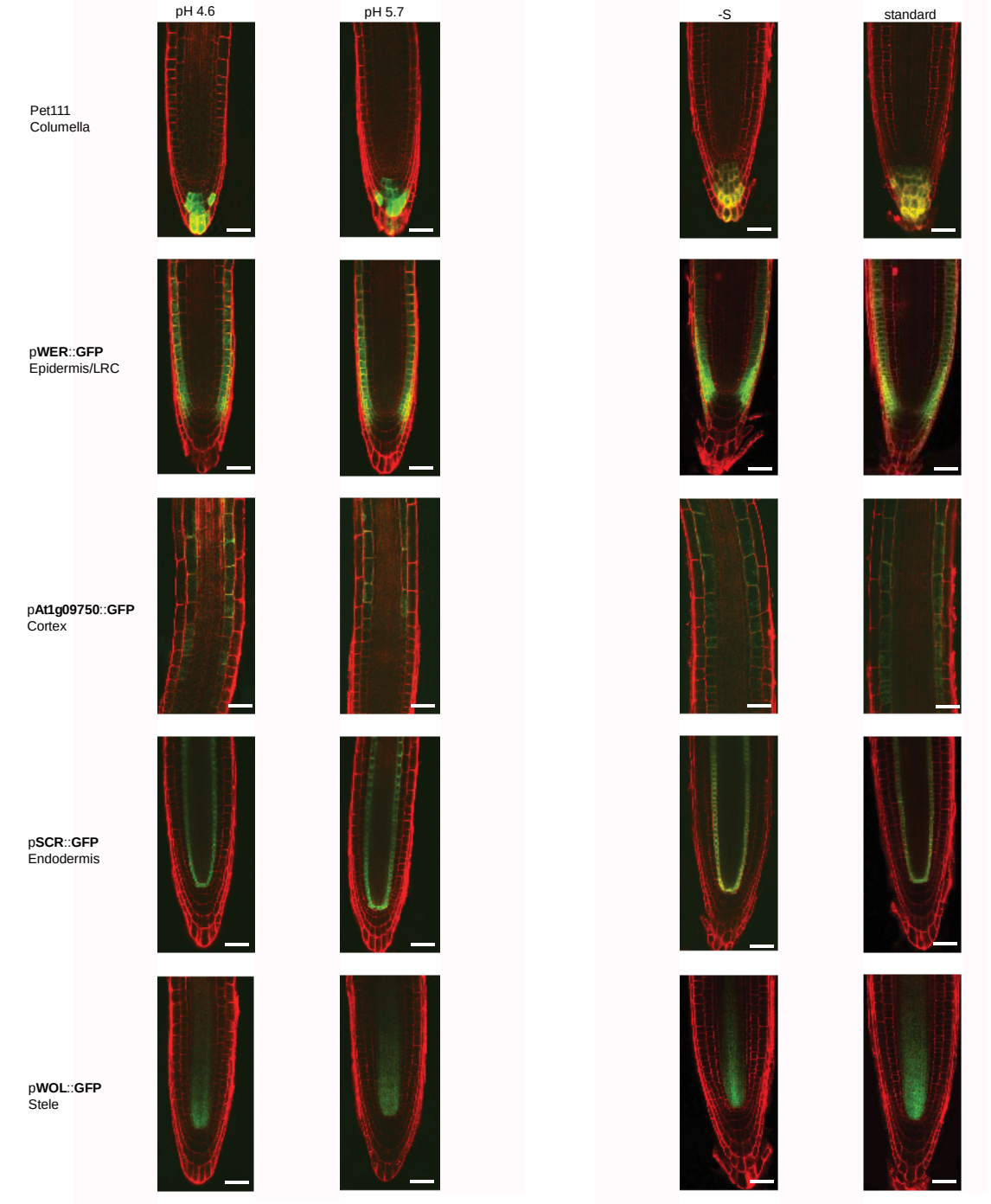

Figure S1: Confocal images of GFP-marked lines used for cell sorting after transfer to low pH or -S. Plants are 6 days after imhibition for low pH and 7 days for -S.

Supplemental Figure 2

Figure S2

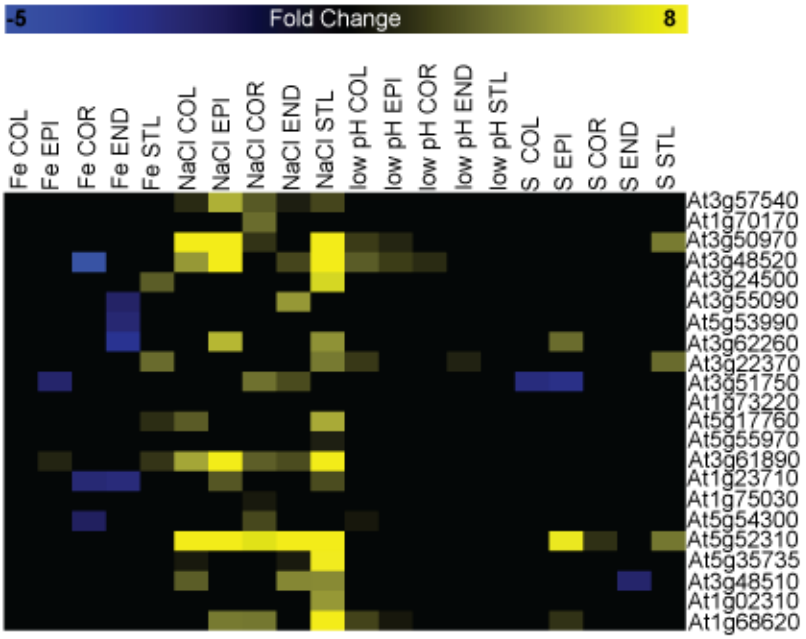

Figure S2: ABA marker genes are differentially regulated in stresses and cell types. Fold changes in response to stress in cell types for the 22 ABA marker genes that are part of the whole root activated CSR gene set. Only genes that are significant (abs. value FC>1.5, FDR < 0.0001) are shown with color. Black indicates that the fold change between standard and stress conditions did not meet the significance cut-off.

Supplemental Figure 3

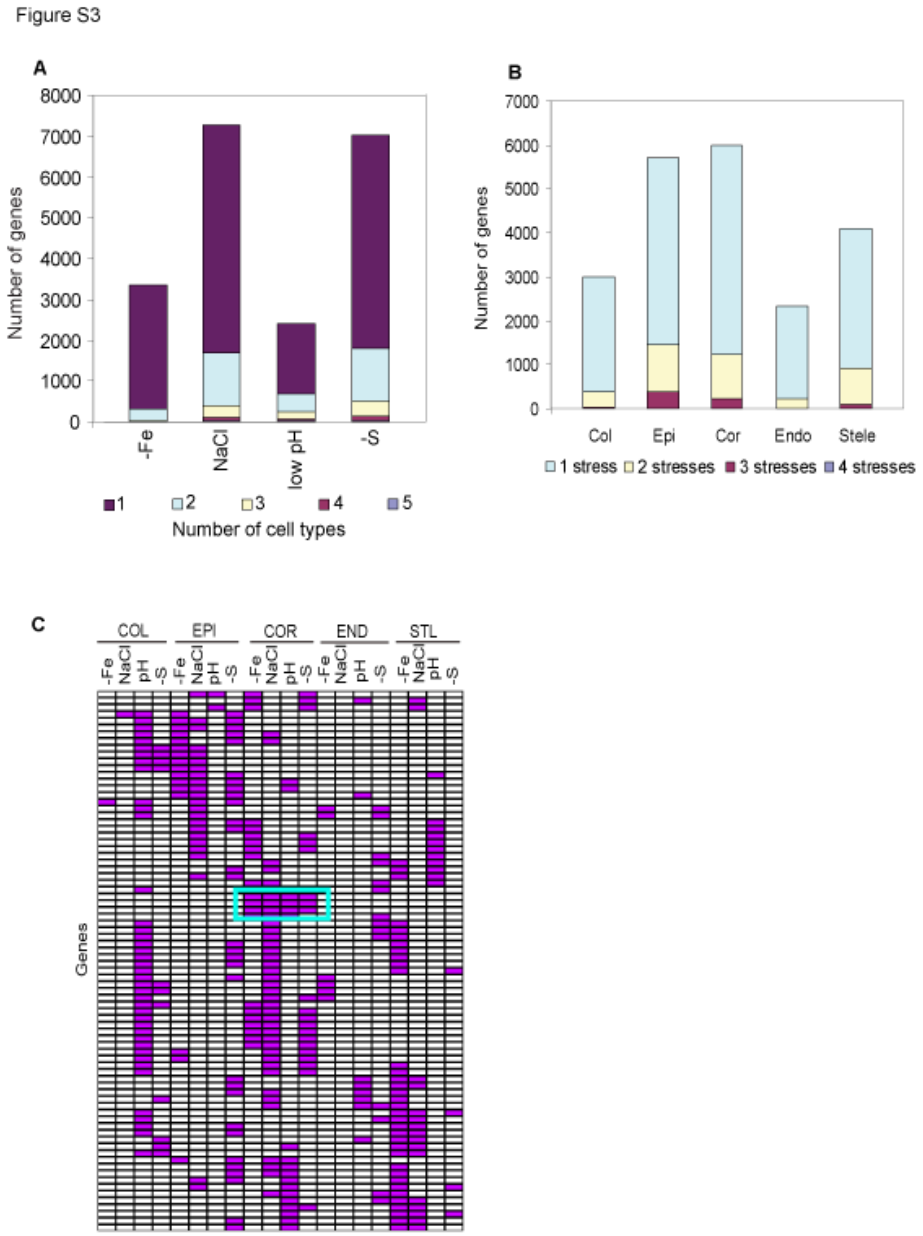

Figure S3: Stress responses are cell-type and stress-specific. A) The majority of genes respond in one cell type under each stress. B) The majority of gene respond in one stress in each cell type. C) Genes respond to stress in different cell types under different stresses. Shown are the 82 genes that respond to stress in at least one cell type under each stress. Only 3 of these genes respond in the same cell type under each stress (blue box).

Supplemental Figure 4

Figure S4

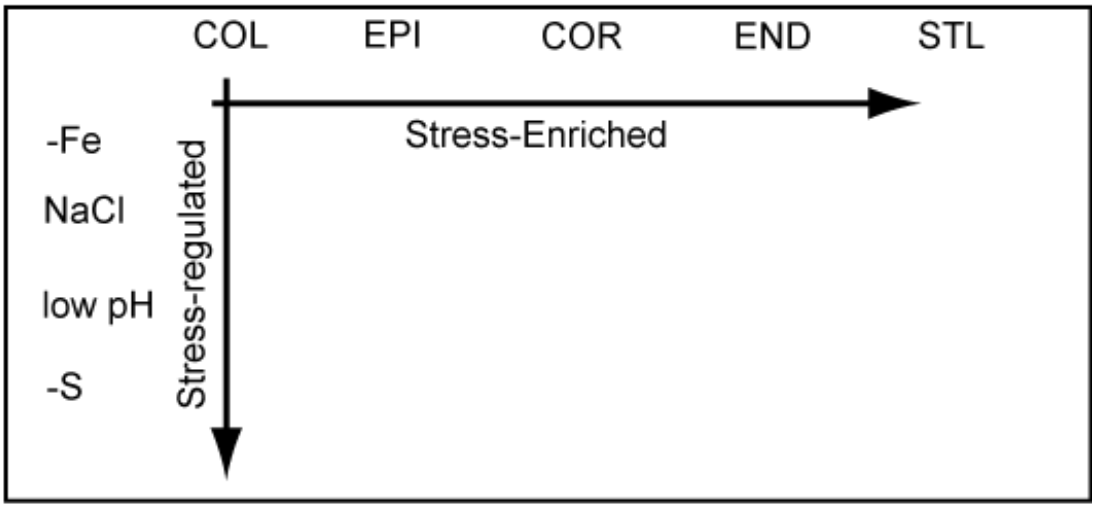

Figure S4: Stress-enriched vs. stress-regulated genes. Stress-regulated genes are differentially expressed (|FC|>1.5, q<0.0001) within a cell type compared to standard conditions, while stress-enriched genes are differentially expressed (FC>1.5, q<0.0001) within a cell type compared to all other cell types within a given stress.

Supplemental Experimental Procedures

<u>-S media</u>

Sulfur deficient media is composed of 20.6 mM NH_4NO_3, 18.8 mM KNO_3, 1.25 mM KPO_4H_2, 5 μM KI, 2.99 mM $CaCl_2$, 0.1 mM H_3BO_3, 1 μM $Na_2MoO_42H_2O$, 0.1 μM

$CoCl_2 6H_2O$, 0.1 mM $Na_2EDTA2H_20$, 1.5 mM $MgCl_2$, 0.1 μM $CuCl_2$, 0.1mM $FeCl_2$, 0.1 mM $MnCl_2$, 0.03 mM $ZnCl_2$, 0.05% MES, 1% sucrose, 1% agar. Low pH media is 1X concentration MS salt mixture (Caisson laboratories), 3mM DMG (Sigma), 1% sucrose, 1% agar and adjusted to pH 4.6 with KOH.

Sample preparation for time-course (TC)

Roots were cut at the root/hypocotyl junction and collected into RLT buffer in the RNeasy plant mini kit (Qiagen). Approximately 40 roots were collected per replicate, with two biological replicates. Samples for low pH were transferred to low pH and the pH standard and harvested at 30 min, 1H, 3H, 6H, 12H, and 24H after transfer. Samples for –S were either transferred to fresh MS and harvested (for the 0H control) or transferred to sulfur deficient media and harvested at 3H, 12H, 24H, 48H, and 96H after transfer. RNA was extracted using the RNeasy Micro Kit (Qiagen). Probes for array analysis were prepared with the one-cycle amplification protocol by Affymetrix. Samples were submitted to Expression Analysis Inc. (Durham, NC) for hybridization to Arabidopsis Whole Genome ATH1 Affymetrix GeneChips. See Table S1 for a list of microarray experiments used in this study.

Cell-type specific and longitudinal sample preparation

Samples were prepared as described (Dinneny et al. 2008). Five days after stratification, plants were transferred to low pH and pH standard media for 24H before protoplasting (cell-type) or harvesting (longitudinal). For –S, plants were transferred to -S for 3H prior to each experiment. Three biological replicates were performed per condition for the cell-type dataset and two for the longitudinal dataset. See Table S9 for sorting affected probesets.

Confocal microscopy

Plants were grown for 5 days on standard MS and transferred to standard pH or low pH conditions and observed with a Zeiss LSM 510 as described (Brady et al., 2007). For –S, plants were grown for 6 days prior to transfer to sulfur deficient media.

Lugol Staining

Six day old plants were incubated in a 3:1 solution of EtOH : Acetic Acid for 3 minutes and transferred to Lugol Solution (Sigma) for 30 seconds. Plants were placed on a slide with 100µL of fixing solution (10g chloral hydrate, 0.75g Arabic gum, 500µL glycerol, 3ml water) and roots observed using a Leica DM5000-B microscope.

Plant materials and growing conditions for quantitative RT-PCR

35S:*VND7*:*YFP* is in the Col-0 background and was a gift from Dr. T. Demura. The *gl2* mutation is in L.*er* and has been described previously (Masucci et al., 1996). Flag 360_D12 is in the Ws background.

Quantitative RT-PCR

Plants were grown on 1% MS standard media and roots of approximately 100-150 6 day old seedlings were harvested as for the time course analysis. Total RNA was extracted and DNase-treated using RNeasy Plant mini kit (Qiagen). 1 µg total RNA was converted to cDNA with SuperScriptIII (Invitrogen) according to manufacturer's instructions. qRT-PCR was performed using SYBER green power master mix 2x (ABI) on an ABI Step One Plus. Primers used for qRT-PCR are listed in Table S9. Three biological replicates and two technical replicates were performed. *AT1G13320* (Czechowski et al., 2005) was used as an internal control as it was stable in all backgrounds.

Cis-element enrichment analysis

Cis-element enrichment analysis was as described (Dinneney et al. 2008). The web-based program ATHENA (http://www.bioinformatics2.wsu.edu/cgi-bin/Athena/cgi/home.pl) was used to determine enriched *cis*-elements using 1kb promoter length. 1×10^{-3} was used as a cutoff for significance.

DREB1A target analysis

From results in Maruyama et al. 2009, we obtained lists of genes that are differentially expressed (FDR <0.0001, |FC| > 1.5) when *DREB1A* is over-expressed. These were reduced to those present on both the Agilent array used in Maruyama et al. 2009 and the ATH1 microarray used in our studies. This list was then used to identify *DREB1A* targets present among our differentially expressed genes in each cell type under each stress. A background distribution was determined using all genes present on the Agilent microarray (used in Maruyama et al., 2009) that are also present on the ATH1 microarray. Statistical significance of enrichment was determined using the hypergeometric distribution.

K-means clustering for cell-type probesets

K-means clustering was performed using TMV microarray software (www.tm4.org). The top 50% of varying probesets (Brady et al. 2007) among those significant in each stress in each cell type were used for the clustering. K was determined through using both the FOM algorithm in TMEV and clustering with different Ks to identify stable clusters; 35 clusters were used for the analysis. All clusters are listed in Table S8.

ChIP-chip procedure and analysis

Two biological replicates each of homozygous pSCR:SCR:GFP *scr-4* and Columbia (Lehle) (control) were processed. For each biological replicate, 0.18g of seed was

sterilized, plated, and stratified as above on 10 plates with mesh. Fixation was performed as previously described (Sozzani et al., 2010), on seven day old roots by harvesting them using a razor blade into 1% formaldehyde, 1XPBS solution supplemented with 5mM EDTA and vacuum infiltrating for 10 minutes. The chromatin immunoprecipitation procedure was the same as described (Sozzani et al., 2010), except that sonication was performed in 200µL of the extraction buffer using a diagenode Bioruptor sonicator (Bioruptor UCD-200, diagenode, Denville, NJ, USA) for 5.5 minutes on the medium setting. Post-sonication, 45µL was removed and tested as input, while 455µL of extraction buffer and 12µL 10% SDS was added to the remaining 155µL of sonicated sample. For pre-clarification, this post-sonicated sample was briefly vortexed and centrifuged; the supernatant was then moved and incubated at 4ºC for 50 minutes in a new tube containing 360µL extraction buffer, 10µL 10%SDS, 10µL 20mg/mL bovine serum albumin, and 20 µL Protein A agarose beads (Roche, Mannheim, Germany). Following pre-clarification, the sample was briefly centrifuged to sediment the beads. To this supernatant, 2µL of anti-GFP antibody (abcam290, abcam, Cambridge, MA, USA) was added and incubated overnight at 4ºC for immunoprecipitation. The next day, the sample was centrifuged briefly and the immunoprecipitated sample was incubated with 30 µL of Protein A agarose beads for 6 hours. The chromatin-protein complexes coupled to the beads were washed, eluted, treated with RNase A and Proteinase K, and then reverse crosslinked (Roche, Mannheim, Germany) (Sozzani et al., 2010). The chromatin sample was then cleaned using a MinElute kit (Qiagen Sciences, Germantown, MD, USA), amplified, and labeled with either Cy3 or Cy5 dye (Tsukagoshi et al., 2010). 5 µg of labeled wild-type and *scr-4/pSCR:SCR:GFP* DNA was hybridized to a custom long

oligonucleotide (~ 60mer) Arabidopsis promoter microarray (Sozzani et al. 2010) (NCBI GEO Platform record GSE21338). Hybridization was performed according to the Agilent ChIP-on-chip protocol, and images were obtained using an Agilent microarray scanner (model G2565BA) at a resolution of 5 μm. Signal extraction and data processing were conducted as described in (Long et al. 2010). The probe *p*-values were fed into a seed extension algorithm called SeedXrich (Busch et al. 2010). This algorithm systematically combines different parameters to call enriched regions. Those parameters were: (i) length of region in basepairs covered by probes below a defined *p*-value threshold, (ii) a local *p*-value minimum (seed), and (iii) the number of nucleotides allowed as gaps within called regions. For each combination of parameters, the detected regions were registered. A gene was assigned to an enriched region if that region was present within 4000 bp upstream or 300 bp downstream of the transcription start site, in an intron, or 300 bp downstream of the gene model. Each parameter combination produced a list of called regions and thus of assigned genes. The proportion of SCR response genes as identified in (Sozzani et al., 2010) to all of the assigned genes, which were represented on the ATH1 array was recorded for each list. To select optimal parameters, we analyzed the distribution of the lists created by SeedXrich by parameter sweeping for the highest number of SCR responses genes and the binding of the promoter of *CYCD6;1* which was shown to be bound by SCR (Sozzani et al., 2010). The list was obtained with the following parameters: Probe *p*-value seed: $p < 1x10^{-6}$, probe *p*-value: $p < 0.1$, minimum length of hybridization: 360 bp, maximum gap: 165 bp. The SeedXrich program is available upon request as a python script.

Supplemental References

Bonke, M., Thitamadee, S., Mähönen, A.P., Hauser, M.T., Helariutta, Y. (2003). APL regulates vascular tissue identity in Arabidopsis. Nature. 426, 181-6.

Dinneny, J.R., Long, T.A., Wang, J.Y., Jung, J.W., Mace, D., Pointer, S., Barron, C., Brady, S.M., Schiefelbein, J., Benfey, P.N. (2008). Cell identity mediates the response of Arabidopsis roots to abiotic stress. Science. 320, 942-5.

Czechowski, T., Stitt, M., Altmann, T., Udvardi, M.K., Scheible, W.R. (2005). Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. Plant Physiol. 139, 5-17.

Lee, M.M., Schiefelbein, J. (1999). WEREWOLF, a MYB-related protein in Arabidopsis, is a position-dependent regulator of epidermal cell patterning. Cell. 99, 473-83.

Lee, J.Y., Colinas, J., Wang, J.Y., Mace, D., Ohler, U., Benfey, P.N. (2006). Transcriptional and posttranscriptional regulation of transcription factor expression in Arabidopsis roots. Proc. Natl. Acad. Sci. U. S. A. 103, 6055-60.

Mähönen, A.P., Bonke, M., Kauppinen, L., Riikonen, M., Benfey, P.N., Helariutta, Y. (2000) A novel two-component hybrid molecule regulates vascular morphogenesis of the Arabidopsis root. Genes Dev. 14, 2938-43.

Maruyama, K., Takeda, M., Kidokoro, S., Yamada, K., Sakuma, Y., Urano, K., Fujita, M., Yoshiwara, K., Matsukura, S., and Morishita, Y. (2009). Metabolic pathways involved in cold acclimation identified by integrated analysis of metabolites and transcripts regulated by DREB1A and DREB2A. Plant Physiol. 150, 1972-1980.

Masucci J.D., Rerie W.G., Foreman D.R., Zhang M., Galway M.E., Marks, M.D., and Schiefelbein, J.W. (1996). The homeobox gene GLABRA2 is required for position-

dependent cell differentiation in the root epidermis of Arabidopsis thaliana. Development 122, 1253-1260.

Nawy, T., Lee, J.Y., Colinas, J., Wang, J.Y., Thongrod, S.C., Malamy, J.E., Birnbaum, K., Benfey, P.N. (2005). Transcriptional profile of the Arabidopsis root quiescent center. Plant Cell 7, 1908-25.