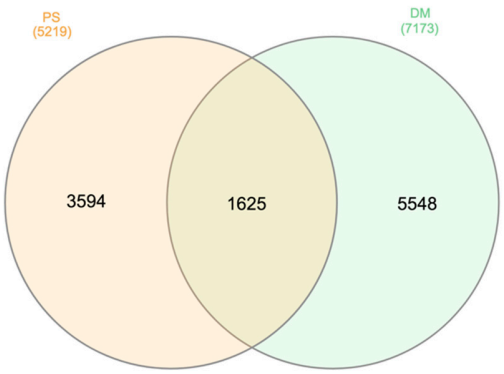
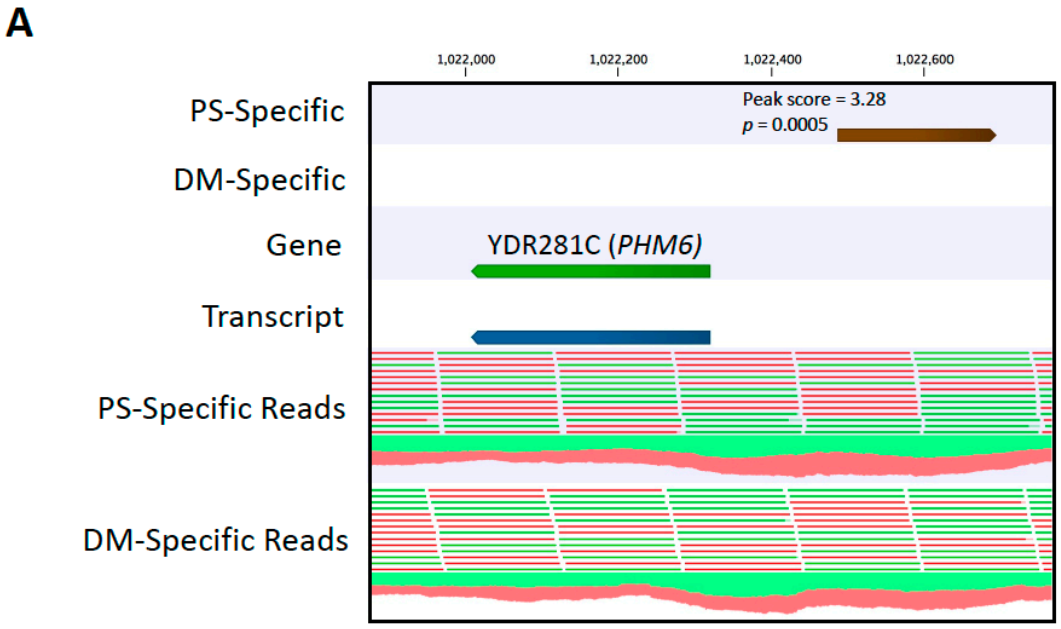



**Figure S1.** Venn diagram showing overlap between sets of potentially-regulated Arabidopsis genes with PS- or DM-specific binding sites in their promoters.



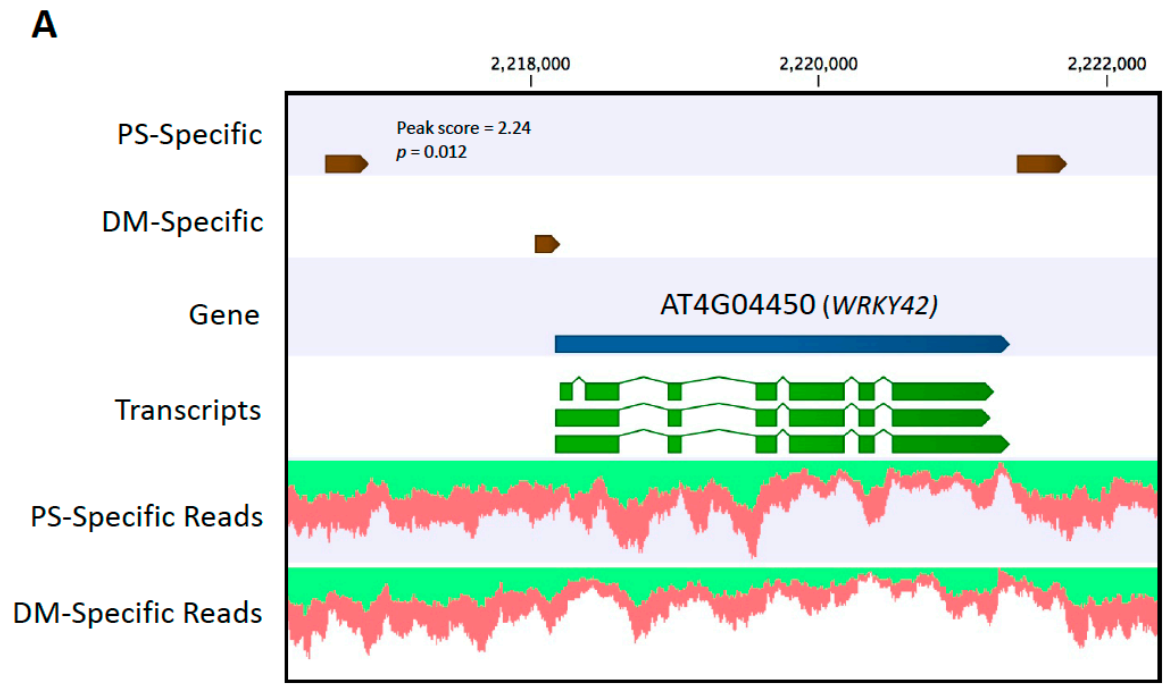

**B**

Pho4p Consensus: **CACGTG**

Test oligo (66 bp):
5′-ATAATGTTACGCAGTTCCGAGCTTCGCTGA**CACGTG**GGAGGTGGGATATAATATTTG**CACGGG**TTC-3′

Mutated oligo (66 bp):
5′-ATAATGTTACGCAGTTCCGAGCTTCGCTGAGGGCCCGGAGGTGGGATATAATATTTGCCCCCCTTC-3′

**Figure S2. PS-specific binding site in the yeast *PHM6* gene promoter and oligonucleotide sequences used in functional testing of the binding site with EMSA. A)** Genome browser view of PS- and DM-specific binding sites, relative to the yeast *PHM6* gene and its transcripts on chromosome IV. Distribution of mapped reads (green, sense; red, anti-sense) are indicated. A single PS binding site is located within the *PHM6* promoter. The peak shape score and *p*-value for the PS-specific peak are shown. **B**) DNA sequences of test and mutated oligos used in EMSA with PS and DM proteins. The oligos are comprised of 30 bp of upstream and downstream sequences around the centrally-located Pho4p CACGTG motif, which was changed to GGGCCC in the mutated oligo. A similar 3'-end CACGGG motif was also mutated to CCCCCC to minimize potential PS binding to this sequence.




**B**

Dof1.5 Consensus (5'-AA[AG]G-3' core):

**RWAAAAADDAAAAAGTRAAAA**

Test oligo (70 bp):

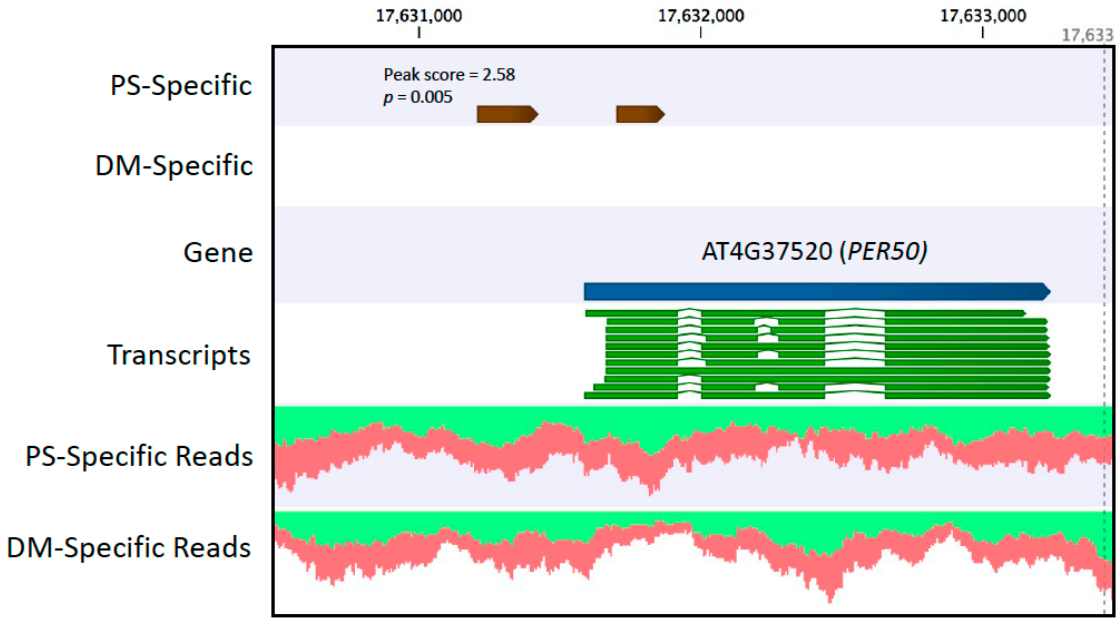
5′-AGTTATTTTAACTAATAAACAGATA**AAAAGAAATAAAAAGTACTAA**ATTGGTAAATTATCGTAGTATCAT-3′

Mutated oligo (70 bp):

5′-AGTTATTTTAACTAATAAACAGATA**AAAAGAAATA**GCCTAC**ACTAA**ATTGGTAAATTATCGTAGTATCAT-3′

**Figure S3. PS-specific binding site in the Arabidopsis *WRKY42* gene promoter and oligonucleotide sequences used in functional testing of the binding site with EMSA.** Genome browser view of PS- and DM-specific binding sites, relative to the Arabidopsis *WRKY42* gene and its transcripts. Distribution of mapped reads (green, sense; red, anti-sense) are indicated. A single PS binding site is located within the WRKY promoter. The peak shape score and *p*-value for the PS-specific peak are shown. A DM-specific binding site spans the TSS of this gene but *WRKY42* is DE only in PS seedlings. **B**) DNA sequences of test and mutated oligos used in EMSA with PS and DM proteins. The 70 bp oligos are comprised of upstream and downstream sequences around the centrally-located Dof1.5 motif, whose core sequence was changed in the mutated oligo.

**A**




**B**

SHN3 Consensus (GCC Box element):

WCCDCCGCCRCCDCCGCCGCC

Test oligo (70 bp):

5′-TTCACTTTTCAACTTATCCCTCTCAATCCCCACCACCACCACTCTCTATTCCCTCATATAGATAGATCAT-3′

Mutated oligo (70 bp):

5′-TTCACTTTTCAACTTATCCCTCTCAATCCCCAGTCCTATGACTCTCTATTCCCTCATATAGATAGATCAT-3′

**Figure S4. PS-specific binding site in the Arabidopsis *PER50* gene promoter and oligonucleotide sequences used in functional testing of the binding site with EMSA.** Genome browser view of PS- and DM-specific binding sites, relative to the Arabidopsis *PER50* gene and its transcripts. Distribution of mapped reads (green, sense; red, anti-sense) are indicated. A single PS binding site is located within the *PER50* promoter and an additional PS binding site is located within exon 1. The peak shape score and *p*-value for the PS-specific peak are shown. **B**) DNA sequences of test and mutated oligos used in EMSA with PS and DM proteins. The 70 bp oligos are comprised of upstream and downstream sequences around the centrally-located SHN3 motif, whose conserved core sequence was changed in the mutated oligo.

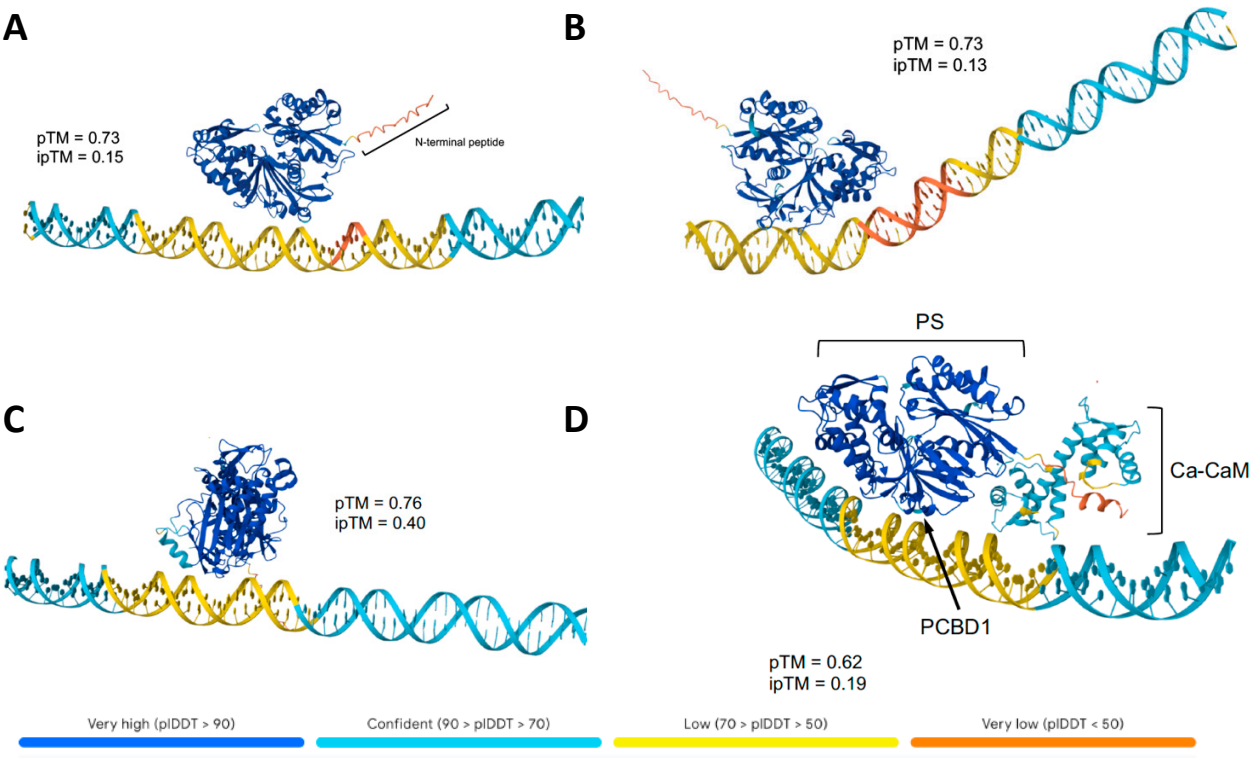



**Figure S5.** AlphaFold3-predicted interactions of mature PS or DM proteins with the yeast *PHM6* promoter binding site. PS binding to the test oligo (**A**) versus mutant oligo (**B**) sequences. DM binding to the test oligo (**C**). Binding of PS/$Ca^{2+}$CaM complex to the test oligo (**D**). Confidence scores for local structure predictions are indicated in the pLDDT scale.

*PHM6* ATAATGTTACGCAGTTCCGAGCTTCGCTGA**CACGTGGGA**GG**TGGGA**TATAATATTTGCACGGGTTC
*WRKY42* AGTTATTTTAACTAATAAACAGATAAAAAGAAATAA**AAAG**TACTAAAT**TGGTA**AATTATCGTAGTATCAT
*PER50* ATGATCTATCTATATGAGGGAATAGAGAGT**GGTGGTGGTGGGGA**TTGAGAGGGATAAGTTGAAAAGTGAA

*PHM6* TGGGA
*WRKY42* TGGTA
*PER50* GGGGA

Consensus: (G/T)GG(G/T)A



**Figure S6**. Predicted PS binding sites in yeast (*PHM6*) and Arabidopsis (*WRKY42, PER50*) promoter sequences used in EMSA. Overrepresented TF binding site motifs are indicated in red. Binding sites for mature PS protein (underlined, bold) were predicted using AlphaFold3 to model PS interactions with the oligos. Aligned binding site sequences and the consensus sequence are shown below. *PER50* antisense sequence and sense sequences for *PHM6* and *WRKY42* are shown in 5' → 3' orientation. Relative frequencies of each motif in PS-specific binding sites in promoters of Arabidopsis genes that are also DE only in seedlings that ectopically express PS is represented by the logo (Weblogo3; https://weblogo.berkeley.edu/logo.cgi).

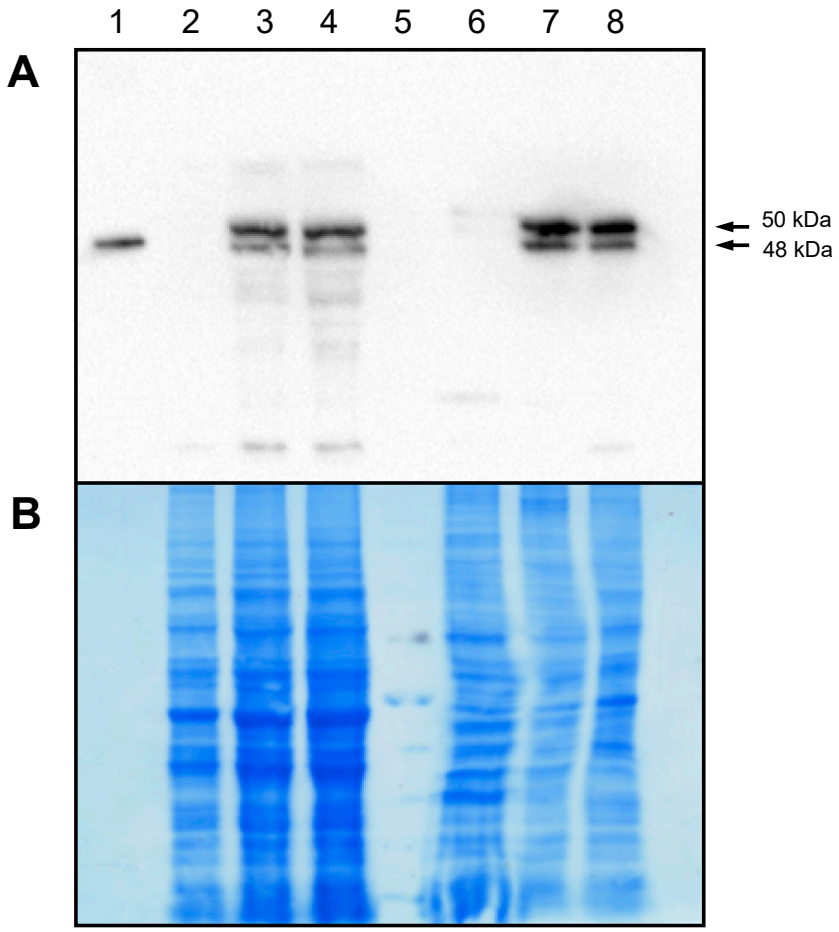



**Figure S7.** Purification of PS and DM proteins for use in EMSA. Proteins were partially-purified from yeast, as described in the methods section. Purified 48 kDa mature PS protein (20 ng; NTP9-30a) was loaded in lane 1. The initial 20% (w/v) ammonium sulfate (AS) fractions from the pYES2 empty vector control (lane 2) or PS- or DM-enriched proteins (lanes 3 and 4, respectively) were further fractionated with 80% (w/v) AS. 80% AS cuts of pYES2, PS and DM fractions (lanes 6-8), and MW markers (lane 5) were separated by SDS-PAGE gel then blotted (A). Remaining proteins in the gel were visualized by InstantBlue staining (B). The blot was immunostained using the highly-specific antibody 8B6, which recognizes both PS and DM proteins. The 50 kDa band is the expressed precursor PS and DM proteins and the 48 kDa band is the processed ("mature") protein. Densitometric analysis of mature PS and DM band densities, relative to the purified PS standard, supported estimates of 40.4 ng PS and 41.1 ng DM proteins in lanes 7 and 8 (1:1 ratio). Based upon total protein loadings in those lanes, PS and DM protein are estimated to be 1.4% and 1.3% of the total sample protein.

**Table S1.** PS- and DM-specific binding sites in promoters of potential target genes and expression patterns for those genes in PS- and DM-overexpressing Arabidopsis seedlings. Target genes for 5' binding sites are located upstream from the site, on the antisense strand. Target genes for 3' binding sites are located downstream from the site, on the sense strand. A 5'/3' binding site is located within divergent promoters of upstream and downstream target genes. Binding site data in divergent promoters are duplicated and are not counted in totals. Some genes have more than one PS- or DM-binding site.

| Type of Binding Site | Location of Binding SIte | Number of Binding SItes | Potential Target Genes (Unique) | Specificity, Potential Target Gene Expression | | | | Percent Target Genes Expressed | Percent Target Genes Specifically Expressed |
|---|---|---|---|---|---|---|---|---|---|
| | | | | PS Seedlings | DM Seedlings | Both PS and DM Seedlings | Not DE | | |
| PS | 5' | 2,915 | 2,692 | 74 | 24 | 9 | 2,586 | 4.1% | 2.7% |
| | 3' | 2,701 | 2,527 | 66 | 29 | 11 | 2,421 | | |
| | 5'/3' | 520 | 988 | 26 | 5 | 2 | 922 | | |
| | **Totals:** | **5,616** | **5,219** | **140** | **53** | **20** | **5,007** | | |
| DM | 5' | 4,029 | 3,658 | 107 | 39 | 10 | 3,502 | 3.8% | 2.6% |
| | 3' | 3,915 | 3,515 | 83 | 28 | 7 | 3,397 | | |
| | 5'/3' | 783 | 1,472 | 36 | 10 | 3 | 1,374 | | |
| | **Totals:** | **7,944** | **7,173** | **190** | **67** | **17** | **6,899** | | |

**Table S2.** Differentially-expressed genes enriched specifically in an Arabidopsis DM-overexpression line are involved in abscisic acid (ABA) signaling or responses to ABA (BioProcess categories GO:0009737; GO:0009269).

| AGI | Gene ID | Description | Expression (FC) | | Notes |
|---|---|---|---|---|---|
| | | | PS | DM | |
| AT2G38310 | PYL4 | Abscisic acid receptor PYRABACTIN RESISTANCE-LIKE 4 | | 1.5 | Mediates ABA-dependent regulation of protein phosphatase 2Cs ABI1 and ABI2; stimulates ABA signaling |
| AT5G53160 | PYL8 | Abscisic acid receptor PYRABACTIN RESISTANCE-LIKE 8 | | 1.5 | Mediates ABA-dependent regulation of protein phosphatase 2Cs ABI1 and ABI2; stimulates ABA signaling |
| AT5G05410 | DREB2A | Dehydration-responsive element-binding protein 2A | | 1.8 | Transcription factor that specifically binds to DRE/CRT cis elements of water stress-inducible genes; drought stress tolerance |
| AT2G42280 | AKS3 | ABA-responsive kinase substrate 3; transcription factor bHLH130 | | 1.5 | |
| AT3G02140 | AFP4 | ABI FIVE BINDING PROTEIN 4 | | 1.6 | Negative regulator of ABA and salt stress responses |
| AT3G30775 | POX1, ERD5 | Proline dehydrogenase 1; EARLY RESPONSIVE TO DEHYDRATION 5; mitochondrial | | 2.5 | Expression induced by osmotic stress; promoter contains an L-proline-inducible element |
| AT3G18490 | ASPG1 | ASPARTIC PROTEASE IN GUARD CELL 1 | | -1.6 | Functions in drought avoidance through abscisic acid (ABA) signalling in guard cells |
| AT2G39800 | P5CSA | Delta-1-pyrroline-5-carboxylate synthase A, P5CSA; P5C synthase 1 | | -1.5 | Catalyzes the rate-limiting enzyme in the biosynthesis of proline; induced by abscisic acid, osmotic stress |
| AT3G11410 | PP2CA | Protein phosphatase 2C 37; PP2CA | | -1.5 | mRNA up-regulated by drought and ABA |
| AT5G59220 | SAG113 | Protein phosphatase 2C 78; SAG113 | | -1.6 | Highly ABA-induced PP2C gene 1; negative regulator of osmotic stress and ABA signaling |

**Table S3.** PS-specific binding sites and enriched transcription factor motifs in promoters of genes that are differentially-expressed only in PS Arabidopsis seedlings (NOTE: This is a separate Excel file.)

**Table S4**. qRT-PCR validation of *WRKY42* and *PER50* expression in RNA-seq datasets.

| | | qRT-PCR | | RNA-seq |
|---|---|---|---|---|
| | | FC Expression | *p*-value | FC |
| *PER50* | col-0 | 1 | | |
| | PS | 3.24 ± 0.47 | 0.02 | 1.6 |
| *WRKY42* | col-0 | 1 | | |
| | PS | 1.87 ± 0.17 | 0.02 | 1.9 |

**Table S5.** Chromatin proteins or transcription factors that interact with 4Myc-tagged Arabidopsis PS or DM proteins, or 13Myc-tagged yeast PS or DM proteins, in co-IP assays.

| Protein ID | UniProt | Gene ID | Annotation |
|---|---|---|---|
| *Arabidopsis* | | | |
| CYP19-1 | Q38900 | AT2G16600 | Peptidyl-prolyl cis-trans isomerase CYP19-1 |
| NUCL1 | Q9FVQ1 | AT1G48920 | Nucleolin 1 |
| SWIB2 | O82326 | AT2G14880 | Uncharacterized protein with SWIB/MDM2 domain |
| SCL30 | Q9SNB8 | AT3G46600 | Scarecrow-like protein 30 |
| *Yeast* | | | |
| FPR3 | P38911 | YML074C | FK506-binding nuclear protein |
| NSR1 | P27476 | YGR159C | Nuclear localization sequence-binding protein |
| MSC1 | Q03104 | YML128C | Meiotic sister chromatid recombination protein 1 |

**Table S6.** Expression profiles for peroxidase genes regulated in response to ectopic PS or DM expression [14] or suppression of *AtAPY1/2* in the R2-4A line (Lim et al. 2014) [29] of Arabidopsis seedlings.

| Gene | Relative Expression (FC) | | | Gene Symbol | Subcellular Location | Description |
|---|---|---|---|---|---|---|
| | PS | DM | R2-4A | | | |
| AT5G64120 | 1.8 | | | PER71 | ECM | Peroxidase 71; lignification in roots; defense response to fungus; induced by hypo-osmolarity |
| AT5G19880 | 1.6 | | | PER58 | ECM, endomembrane system | Peroxidase 58; response to ethylene stimulus |
| AT4G26010 | 1.6 | | | PER44 | ECM, endomembrane system | Peroxidase 44; response to oxidative stress |
| AT5G64100 | 1.5 | | | PER69 | ECM | Peroxidase 69; response to oxidative stress |
| AT5G17820 | 1.5 | | | PER57 | ECM, plasma membrane | Peroxidase 57; response to oxidative stress, root |
| AT5G67400 | 1.5 | | | PER73 | ECM, endomembrane system | Peroxidase 73, root hair specific 19; response to oxidative stress |
| AT4G37520 | 1.5 | | | PER50 | ECM, cytoplasmic | Peroxidase 50; response to oxidative stress |
| AT1G30870 | 1.5 | | | PER7 | ECM, endomembrane system | Peroxidase 7; response to oxidative stress |
| AT5G06720 | -1.7 | | 6.0 | PER53 | ECM, endomembrane system | Peroxidase 53; diverse roles in the wound response, flower development, and syncytium formation |
| AT2G18150 | -1.8 | | 21.8 | PER15 | ECM | Peroxidase 15; response to oxidative stress |

**Table S7**. PCR primers and oligos used in this study.

| Primer Name | Primer Sequence (5'-->3') |
|---|---|
| **qRT-PCR Validation of RNA-seq Expression** | |
| WRKY42-F | TGTTCGCAAGCAAGTGCAAC |
| WRKY42-R | GACATGGTGGAGCCTGAGAG |
| PER50-F | CGTGACGTCGCGGGTG |
| PER50-R | ATCCTAATGTGTGTGCCCCG |
| **Construction and Screening of 4Myc-PS and -DM Overexpression Lines** | |
| pENTR-F | CACCATGGAGCTCCTTATTAAAC |
| pENTR-R | CAAGTCTTCCTCGGAGATTAGC |
| **EMSA Oligos** | |
| PHM6 test-Sense | ATAATGTTACGCAGTTCCGAGCTTCGCTGACACGTGGGAGGTGGGATATAATATTTGCACGGGTTC |
| PHM6 test-Antisense | GAACCCGTGCAAATATTATATCCCACCTCCCACGTGTCAGCGAAGCTCGGAACTGCGTAACATTAT |
| PHM6 mutant-Sense | ATAATGTTACGCAGTTCCGAGCTTCGCTGACACGTGGGAGGTGGGATATAATATTTGCACGGGTTC |
| PHM6 mutant-Antisense | GAACCCGTGCAAATATTATATCCCACCTCCCACGTGTCAGCGAAGCTCGGAACTGCGTAACATTAT |
| WRKY42 test-Sense | AGTTATTTTAACTAATAAACAGATAAAAAGAAATAAAAAGTACTAAATTGGTAAATTATCGTAGTATCAT |
| WRKY42 test-Antisense | ATGATACTACGATAATTTACCAATTTAGTACTTTTTATTTCTTTTTATCTGTTTATTAGTTAAAATAACT |
| WRKY42 mutant-Sense | AGTTATTTTAACTAATAAACAGATAAAAAGAAATAGCCTACACTAAATTGGTAAATTATCGTAGTATCAT |
| WRKY42 mutant-Antisense | ATGATACTACGATAATTTACCAATTTAGTGTAGGCTATTTCTTTTTATCTGTTTATTAGTTAAAATAACT |
| PER50 test-Sense | TTCACTTTTCAACTTATCCCTCTCAATCCCCACCACCACCACTCTCTATTCCCTCATATAGATAGATCAT |
| PER50 test-Antisense | ATGATCTATCTATATGAGGGAATAGAGAGTGGTGGTGGTGGGGATTGAGAGGGATAAGTTGAAAAGTGAA |
| PER50 mutant-Sense | TTCACTTTTCAACTTATCCCTCTCAATCCCCAGTCCTATGACTCTCTATTCCCTCATATAGATAGATCAT |
| PER50 mutant-Antisense | ATGATCTATCTATATGAGGGAATAGAGAGTCATAGGACTGGGGATTGAGAGGGATAAGTTGAAAAGTGAA |

**Table S8.** Quality control metrics for the Arabidopsis ChIP-seq assay.

| ChIP-seq Library | Unique Reads | Relative Strand Correlation (≥ 0.8) | Normalized Strand Coeeficient (≥ 1.05) | Genome Coverage (≥ 30x) |
|---|---|---|---|---|
| Col-0 input | 27,284,031 | 1.250 | 1.037 | 30.95 |
| Col-0 | 24,545,208 | 1.213 | 1.025 | 29.35 |
| PS input | 32,759,144 | 1.300 | 1.043 | 36.89 |
| PS | 34,225,803 | 1.364 | 1.027 | 54.21 |
| DM input | 48,730,589 | 1.291 | 1.028 | 41.00 |
| DM | 34,432,616 | 1.317 | 1.025 | 40.57 |