Supporting Information

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SI Materials and Methods

Stress-Responsiveness Experiments. For the oxidative stress-induction experiment, control and catalase-deficient CAT2HP1 plants were grown in soil and subjected to high-light (HL) irradiation as described (1). Middle-aged leaves were harvested after 0, 20, and 40 min and 2, 3, and 8 h of HL exposure. For the salt stress–induction experiment, Col0WT plants were grown in LifeRaftR (Osmotek) in vitro cultivation systems and treated with 150 mM NaCl as described (2). Root material was harvested after 0, 3, 6, and 12 h of salt treatment.

Generation of Transgenic *Arabidopsis* Plants. For overexpression plants, the full-length ORF of WRKY15 was cloned by recombination into the entry vector pDONR221 (Invitrogen) and the *pCaMV35S* overexpression vector pK7WG2D (3). The construct was transformed by floral dip (4) into both *Arabidopsis* WT (Col4WT) and catalase-deficient (CAT2HP1) plants (5). Homozygous lines with a single T-DNA locus were selected via segregation analysis, RNA gel-blot analysis, and quantitative RT-PCR.

For loss-of-function plants, homozygous plants from the GABI-Kat T-DNA insertion line GABI_097A12 (6) were selected by genomic PCR. Transgene expression was monitored via quantitative RT-PCR.

To generate amiR plants, WRKY15-specific sequences were identified with the WMD Web MicroRNA Designer (www.weigelworld.org). The miRNA precursors were constructed as described (7), cloned into pK7WG2D, and transformed via floral dip into Col0WT *Arabidopsis* plants. Homozygous amiR plants of at least two independent events were identified as described for the overexpression lines. The PCR primer sequences used for the construction of transgenic plants are presented in Table S4.

Microarray Analysis. In three independent experiments, RNA was isolated from shoot material of 20 WT (Col4WT) and WRKY15^{OE} (WRKY15-9.6) plants (developmental stage 1.05) (8) with TRIzol Reagent (Invitrogen). Concentration and quality of the RNA were determined as described (9). Each of the different pools of WT and transgenic plants, germinated and grown in absence or presence of 50 mM NaCl, were hybridized to 12 GeneChip *Arabidopsis* Tiling 1.0R arrays (Affymetrix; www.affymetrix.com). Per hybridization, 7 μg of total RNA was directly reversed-transcribed to double-stranded cDNA in a cDNA reverse-transcription reaction (without amplification) according to the manufacturer's protocol [Whole Transcript (WT) Double-Stranded Target Assay; Affymetrix]. Subsequently, the sample was fragmented and labeled with biotin according to the manufacturer's protocol (WT Double-Stranded DNA Terminal Labeling Kit; Affymetrix). Hybridization and scanning (GeneChip scanner 3000; Affymetrix) were done according to the manufacturer's instructions at the Nucleomics Core Facility (Leuven, Belgium; www.nucleomics.be). Raw data were processed all together with the RMA algorithm (10) using the Tiling 1.0R array chip description file for quantitative mRNA expression analysis (11) and subsequently subjected to a two-factor ANOVA with the MultiExperiment Viewer of TM4 (12). The P values of the F statistics were corrected for multiple testing to assess the false-discovery rate with the publicly available software QVALUE (http://genomine.org/qvalue) (13), with λ ranging from 0.0 to 0.95 by 0.05. Genes with P values of <0.001 and Q values of ≤0.005, 0.01, and 0.04 for, respectively, treatment, genotype, and interaction significant effects were retained for further analysis.

Promoter-GUS Analysis. The upstream *WRKY15* promoter region was amplified by PCR from the Col0WT genomic DNA with primers (Table S4) and cloned into pKGWFS7 (3), generating an in-frame GFP-GUS fusion. The construct was transformed into the Col0WT plants. Homozygous plants were assayed for GUS staining (14). Samples were photographed with a stereomicroscope (Stemi SV11; Zeiss) or with a Nomarski differential interference contrast microscope (BX51; Olympus).

Transmission-Electron Microscopy. The first leaf pairs of 14-d-old seedlings grown on MS agar medium in the absence or presence of 50 mM NaCl were used for transmission-electron microscopic analysis as described (15).

Protein Analysis. Concanavalin A staining was done as described (16).

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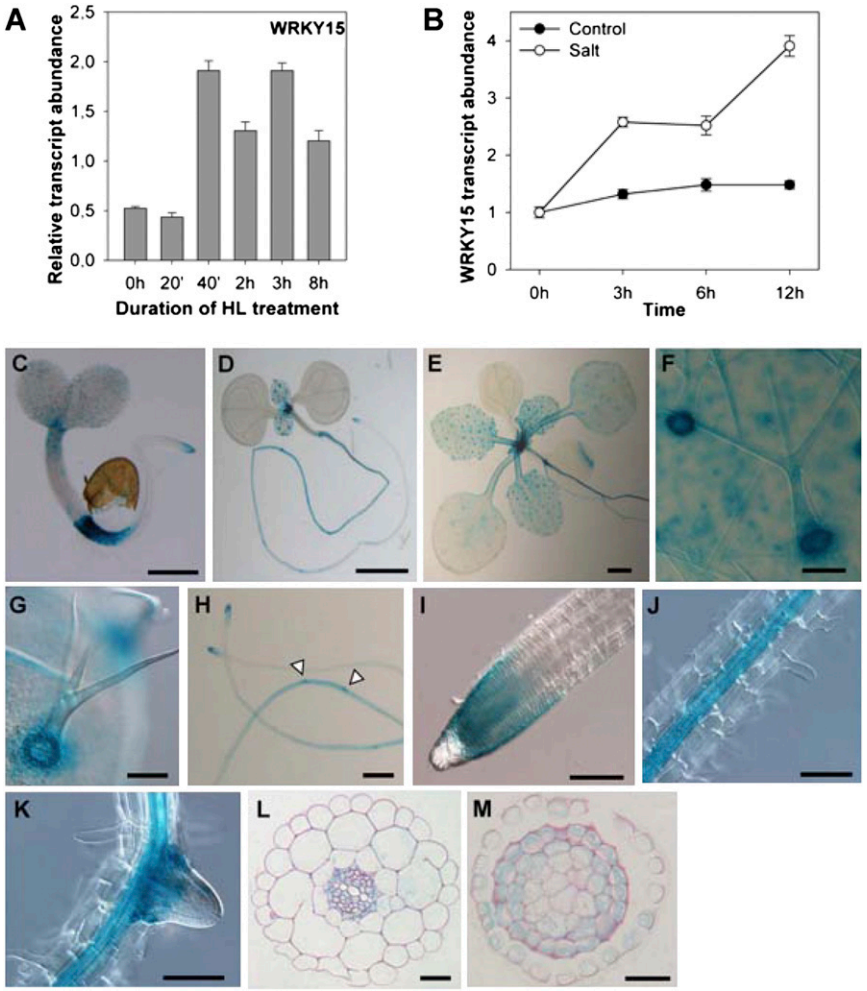

Fig. S1. Spatiotemporal expression patterns of *WRKY15*. (*A*) Relative WRKY15-transcript abundance in middle-aged leaves of high-light (HL)-treated catalase-deficient (CAT2HP1) plants. (*B*) *WRKY15*-transcript abundance in root tissue of salt-treated Col0WT plants. Error bars show SEM (n = 3). (*C*–*M*) Spatial and developmental expression patterns of WRKY15. Promoter activity was visualized by histochemical GUS staining. (*C*) Three-d-old seedling. (*D*) Nine-d-old seedling. (*E*) Seedling at developmental stage 1.06. (*F*) Epidermis of a young leaf with trichomes of the seedling shown in *E*. (*G*) Detail of trichome on the primary leaf of the seedling shown in *I*. (*H*) Roots (arrowheads indicate lateral root initials). (*I*) Root tip. (*J*) Primary root stem. (*K*) Lateral root formation of the seedling shown in *E*. (*L* and *M*) Transverse section through the primary root stem (*L*) and the root apical meristem (*M*). (Scale bars: *D* and *E*, 2 mm; *C* and *H*, 0.5 mm; *F* and *G*, 100 μm; *I*–*K*, 50 μm; *L* and *M*, 10 μm.)

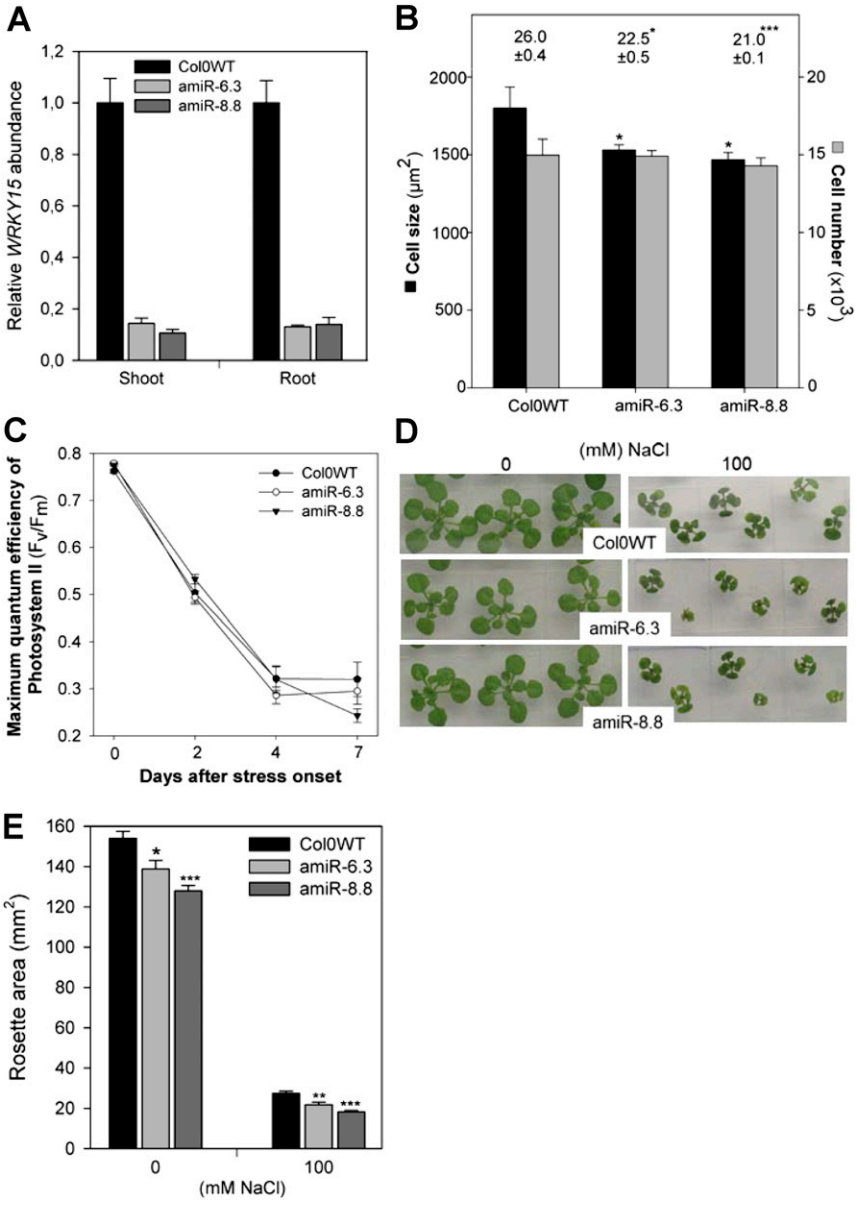

Fig. S2. Plant growth and stress performance of WRKY15-amiR plants. (*A*) *WRKY15*-transcript accumulation in shoot and root tissues of 3-wk-old WT (Col0WT) and WRKY15-amiR plants. (*B*) Cell size and number in the first leaves of 3-wk-old seedlings. Leaf-blade area is shown at the top of the frame. Error bars show SEM (n = 8–10). (*C*) Maximum quantum efficiency of PSII (F_v/F_m) in leaves of Col0WT and WRKY15-amiR plants after exposure to photorespiration-promoting conditions in the presence of the catalase inhibitor 3-AT (3 μM). Error bars show SEM (n = 18). (*D*) Three-wk-old WT and WRKY15-amiR plants germinated and grown on 0 and 100 mM NaCl. (*E*) Rosette area of 3-wk-old Col0WT and WRKY15-amiR plants grown under control and salt-stress conditions (100 mM NaCl). Error bars show SEM (n = 15–45). *$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$ (Student t test).

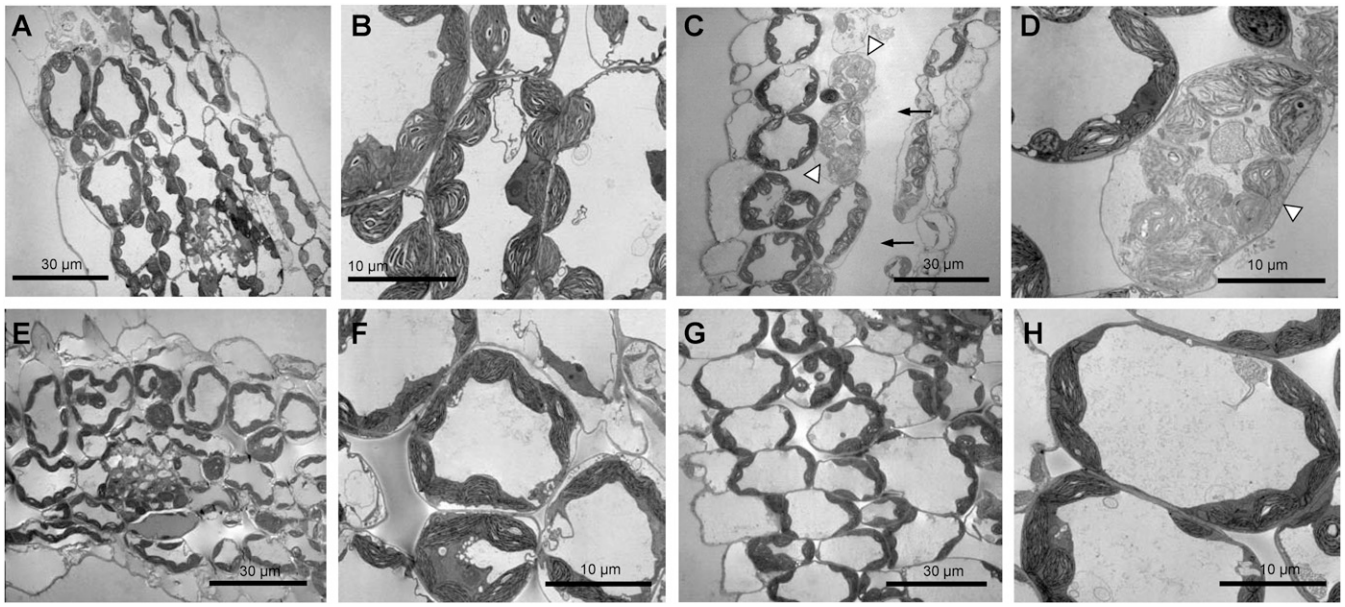

Fig. S3. Transmission-electron microscopic analysis of WRKY15^{OE} plants. (*A–D*) Transmission-electron micrographs of the first leaves of WT (*A* and *B*) and WRKY15^{OE} (*C* and *D*) plants grown under mild salt stress (50 mM NaCl). Arrows and arrowheads indicate large intercellular spaces and deteriorated cells, respectively. (*E–H*) Transmission-electron micrographs of the first leaves of WT (*E* and *F*) and WRKY15^{OE} (*G* and *H*) plants grown under controlled conditions.

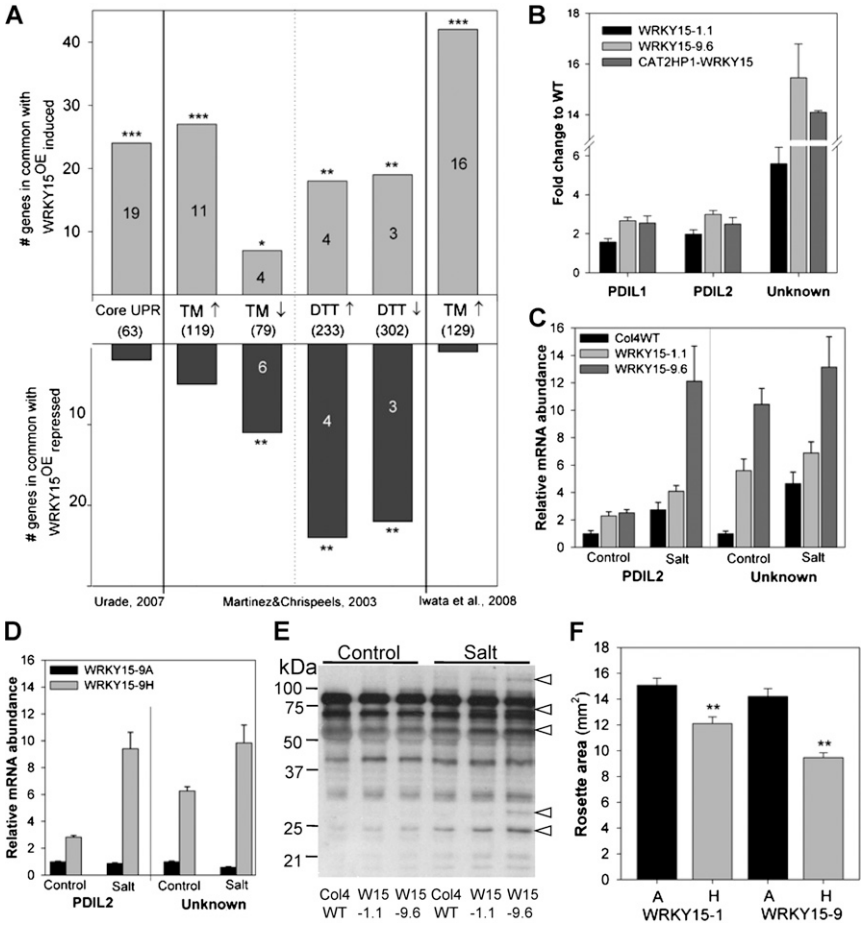

Fig. S4. Activated UPR, altered protein glycosylation, and ER stress sensitivity in WRKY15^{OE} plants. (*A*) Enrichment of ER stress response within constitutively *WRKY15*-expressed genes. Bars indicate the absolute number of transcripts constitutively induced (gray) or repressed (dark gray) in WRKY15^{OE} plants overlapping with ER-stress–responsive transcripts identified in three different studies. The total number of differentially expressed genes upon the different treatments is indicated in parentheses. Numbers within bars give fold enrichment of the ER-stress–responsive genes within the constitutively *WRKY15*-expressed genes compared with the *Arabidopsis* genome. Core UPR, core *UPR* genes; TM↑ and TM↓, genes induced and repressed by tunicamycin, respectively; DTT↑ and DTT↓, genes induced and repressed by dithiothreitol, respectively. $*P \leq 0.001$; $**P \leq 1.0 \times e^{-5}$; $***P \leq 1.0 \times e^{-20}$ (Fisher's exact test). (*B*) Accumulation of *UPR* marker genes (PDIL1, PDIL2, and unknown protein) in WRKY15^{OE} plants grown under control conditions. Expression data were obtained by quantitative real-time PCR on a biological repeat experiment and validate reproducibility of the microarray results. (*C*) Transcript accumulation of *UPR* marker genes in WT and WRKY15^{OE} plants grown without (control) or with 100 mM NaCl (salt). (*D*) Transcript accumulation of *UPR* marker genes in azygous control (WRKY15-9A) and transgenic WRKY15^{OE} (WRKY15-9H) plants grown without (control) or with 75 mM NaCl (salt) in an independent experiment, consolidating that *UPR* genes are superinduced in WRKY15^{OE} plants upon salt stress. (*E*) Affinodetection of glycoproteins containing high-mannose-type *N*-glucans with concanavalin A. Protein sizes are indicated on the left. Arrowheads mark differences in glycosylated protein composition. (*F*) Rosette area of 3-wk-old azygous (A) and transgenic WRKY15^{OE} (H) plants grown on 0.05 μg/mL tunicamycin. Error bars show SEM (n = 3 plates containing 60 plants). $**P < 0.0001$ (Student t test).

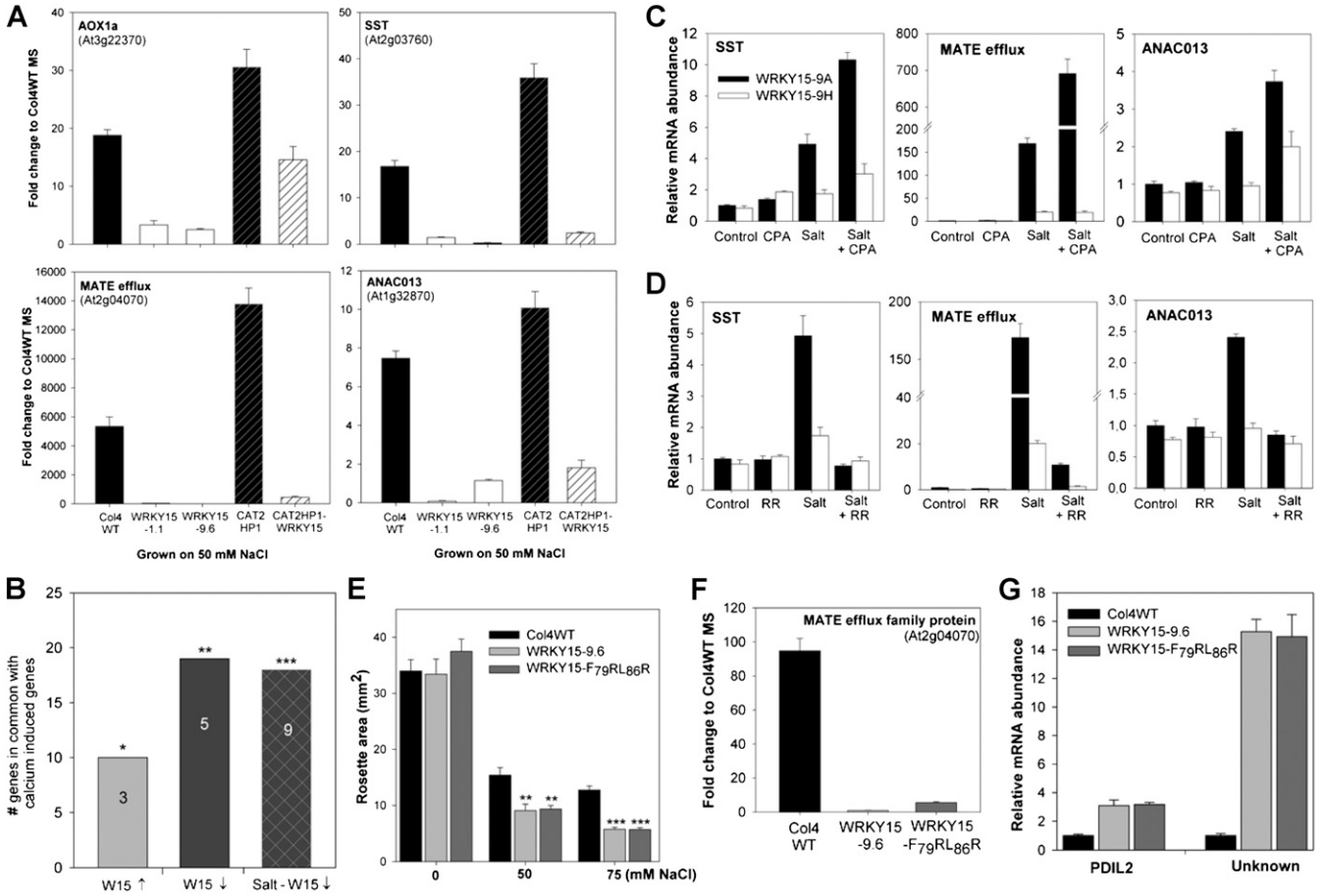

Fig. S5. Integration of *MDR* expression and calcium-mediated signaling. (*A*) Failure of WRKY15^{OE} plants to induce a mitochondrial stress response upon salt treatment. Accumulation of *MDR* marker genes (AOX1a, steroid sulfotransferase, Multidrug and Toxic compound Extrusion (MATE) efflux family protein, and ANAC013) in Col4WT, CAT2HP1, and WRKY15^{OE} plants grown under mild salt stress (50 mM NaCl). Expression data were obtained by quantitative real-time PCR on a biological repeat experiment and validate reproducibility of the microarray results. (*B*) Transcript abundance of *MDR* marker genes in azygous control (WRKY15-9A) and transgenic WRKY15^{OE} (WRKY15-9H) plants grown without (control) or with 5 μM CPA, 50 mM NaCl (salt), or both (salt+CPA). (*C*) Transcript abundance of *MDR* marker genes in control (black bars) and WRKY15^{OE} (white bars) plants grown without (control) or with 10 μM RR, 50 mM NaCl (salt), or both (salt+RR). (*D*) Enrichment of Ca^{2+}-induced genes among WRKY15^{OE} differentially expressed genes. Bars indicate the absolute number of transcripts in the overlap, and numbers within bars give fold enrichment of the Ca^{2+}-induced genes within the WRKY15^{OE} differentially expressed genes compared with the *Arabidopsis* genome. W15↑ and W15↓, genes constitutively induced and repressed in WRKY15^{OE} plants, respectively; Salt-W15↓, salt-impaired genes in WRKY15^{OE} plants. *P = 0.0015; **$P = 2.08 \times e^{-8}$; ***$P = 1.36 \times e^{-12}$ (Fisher's exact test). (*E*) Rosette area of 2-wk-old Col4WT, WRKY15^{OE}, and WRKY15-$F_{79}RL_{86}R^{OE}$ plants grown under control and salt-stress conditions. Error bars show SEM (n = 25–60 plants). **$P < 0.001$; ***$P < 0.0001$ (Student t test). (*F*) Accumulation of the *MDR* marker gene (MATE efflux family protein) in Col4WT, WRKY15^{OE}, and WRKY15-$F_{79}RL_{86}R^{OE}$ plants grown under mild salt stress (50 mM NaCl). (*G*) Accumulation of the *UPR* marker genes in Col4WT, WRKY15^{OE}, and WRKY15-$F_{79}RL_{86}R^{OE}$ plants grown in the absence of stress.

Other Supporting Information Files

Table S1 (DOC)
Table S2 (DOC)
Table S3 (DOC)
Table S4 (DOC)