SUPPORTING INFORMATION

Methods

Plant Materials and Growth Conditions. *Arabidopsis thaliana* Col-0 wild type (WT) seeds were grown in 4-inch round plastic pots containing Sungro Propagation Mix. After sowing, the seeds were watered with RootShield Plus WP Biological Fungicide (600 mg/L, catalog no. 68539-9, BioWorks), covered with a clear plastic dome, and kept at 4 ºC in darkness to induce synchronous germination. After 3 days, the pots with seeds were transferred to a temperature-controlled short-day growth chamber set at 24 °C under a 9 h light/15 h dark photoperiod with 150 µmol m^{-2} s^{-1} photosynthetic photon flux density (50:50 mix of 3,500 and 5,000 K spectrum GE HI-LUMEN XL Starcoat 32-watt fluorescent bulbs). After 10 days, individual seedlings were transferred to 36-cell tray inserts containing Sungro Professional Growing Mix and covered with a clear plastic dome for one to two weeks. Miracle-Gro water-soluble fertilizer (catalog no. 24-8-16) was used (250 mg/L) to water the plants every 10-15 days. The GRP7-GFP transgenic line was a gift from Prof. Dr. Dorothee Staiger at Bielefeld University, Bielefeld, Germany. This line expresses a genomic copy of GRP7 fused to GFP, including the GRP7 5'-UTR, intron, and 3'-UTR under the control of the native GRP7 promoter in the *grp7–1* mutant background (1).

Isolation of Leaf Surface Wash, Apoplastic Wash Fluid, and Leaf Surface Swab. For each replicate, leaf surface wash (LSW), apoplastic wash fluid (AWF), and leaf surface swab (LSS) were isolated from six-to-seven-week-old Arabidopsis plants as depicted in Supplementary Figures 1 and 4. For LSW and LSS isolation, vesicle isolation buffer (VIB; 20 mM 2-(N-morpholino) ethanesulfonic acid pH 6.0, 2 mM $CaCl_2$ and 0.01 M NaCl) supplemented with 0.001% (v/v) of Silwet-77 (Phytotech Labs, Product ID S7777) (a rapid wetting agent that promotes low surface tension, better adhesion, and coverage on foliar surfaces) was sprayed on both sides of the detached whole rosettes. LSS was then filtered through a 0.2 µm syringe filter (Acrodisc syringe filter, Pall Corporation, New York, USA). To recover LSS from the adaxial and abaxial surfaces, the sprayed rosettes were dabbed using cotton-tipped sticks and then squeezed gently into 15 mL Falcon tubes, keeping the adaxial and abaxial samples separate. To recover LSW, the sprayed rosettes were carefully placed inside needleless 60 mL syringes containing holes at the bottom (two rosettes per syringe) placed inside 250 mL centrifuge bottles and centrifuged for 10 min at 100 *g* at 4 ºC (JA-14 rotor, Avanti J-20 XP Centrifuge; Beckman Coulter, Indianapolis, IN, USA). LSW was then filtered through a 0.2 µm syringe filter. Thereafter, the same set of plants was washed with distilled water and used to isolate AWF following the protocol described previously (2) with minor modifications. Briefly, rosettes were vacuum infiltrated for 20 sec with VIB. After vacuum infiltration, excess buffer was removed from the leaf surfaces by gentle blotting with Kimwipes. To collect the AWF, rosettes were placed inside 60 mL needleless syringes as described for LSW collection and centrifuged at 4 ºC for 30 min at 600 *g* with slow acceleration. AWF was then filtered through a 0.2 µm syringe filter. Filtered LSW, AWF, and LSS were either used immediately or stored at –80 ºC until further use. The fresh weight (FW) of the plants used for each replicate was noted and subsequently used to estimate the amount of RNA and proteins per gram FW. For all experiments, a biological replicate was considered as the batch of a given number of plants growing in the same 36-cell insert that were sown at least one week apart from the other biological replicates.

Isolation of Particles from AWF and LSW. To obtain pellets containing extracellular vesicles and other particles, freshly isolated AWF and LSW were transferred to ultracentrifuge (UC) tubes and centrifuged at 40,000 *g* for 1 h at 4 ºC (TLA100.3 rotor, Optima TLX Ultracentrifuge; Beckman Coulter). Where indicated, EGTA (Ethylene glycol- bis (β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; Sigma-Aldrich, USA, catalog no. E4378) was added to the LSW at the specified concentration, mixed, and incubated for 20 min on ice before the ultracentrifugation step. For experiments involving RNase protection by proteins, LSW was treated with 1 µg/mL trypsin (Promega, Madison, WI, USA) and incubated at 30 ºC for 1 h followed by the addition of 1.5

mg/mL trypsin inhibitor (Worthington Biochemical Corp, Lakewood, NJ USA) to inactivate trypsin before the ultracentrifugation step. The supernatant of the P40 pellet was recovered for analysis. To obtain P100-P40 pellets, the supernatant after the 40,000 *g* spin was transferred to ultracentrifuge tubes and centrifuged at 100,000 *g* for 1 h at 4 ºC (TLA100.3 rotor, Optima TLX Ultracentrifuge; Beckman Coulter). P40 and P100-P40 pellets were resuspended in cold and filtered VIB or 100 mM Tris pH 7.4 and either used immediately or stored at –80 ºC until further use.

Quantification of Cell Rupture Using Trypan Blue Staining. Leaves were harvested from three individual six-to-seven-week-old Arabidopsis plants before and after LSW and AWF isolation. For staining, a stock solution of trypan blue (10 mL liquified phenol, 10 mL lactic acid, 10 mL glycerol, 10 mL de-ionized water, and 0.02 g of trypan blue (Sigma-Aldrich, catalog no. 302643-25G)) was prepared, which was then diluted with 95% ethanol (1:2 v/v) as a working solution. Samples were immersed in trypan blue working solution, boiled for 1 min, and then incubated overnight with gentle shaking. For destaining, a chloral hydrate solution was prepared by mixing 1000 g of chloral hydrate (Sigma-Aldrich, catalog no. 302-17-0) in 400 mL de-ionized water. The stained leaves were incubated in chloral hydrate solution overnight and the solution was replaced once or twice. The leaves were mounted on glass slides with 25% glycerol solution and imaged using a light microscope.

RNA Extraction. Total leaf RNA (cell lysate) was isolated from 100 mg of leaf tissue using TRIzol Reagent (Thermo Fisher Scientific™, Waltham, MA, USA). Briefly, leaf tissue was frozen in liquid nitrogen and ground into powder using a mortar and pestle. The powder was placed in 1.5 mL centrifuge tubes and 1 mL of TRIzol was added to each tube and mixed by vortexing. The tubes were placed on a tabletop rotator for 10 min at room temperature (RT). Thereafter, 200 µL of chloroform was added to each tube, followed by a brief but vigorous vortexing step. Tubes were allowed to stand at RT for about 3 min, and then centrifuged at 13,000 *g* for 15 min at 4 ºC. The aqueous phase was transferred to labeled 1.5 mL centrifuge tubes containing 10 µg of RNase-free glycogen, mixed with one volume of cold isopropanol, and incubated for no more than 1 h at –20 ºC. To pellet the RNA, the tubes were centrifuged at 13,000 *g* for 20 min at 4 ºC. RNA pellets were washed twice using ice-cold 70% EtOH and resuspended in 20–30 µL ultrapure DNase/RNase-free water. To isolate RNA from P40 and P100-P40 pellets, 1 mL of TRIzol was added to 100 µL of resuspended pellets, followed by the same procedure as described for the total leaf RNA isolation.

To isolate RNA either from supernatant, AWF, LSW, or LSS, the RNA was first precipitated by mixing the required volume of supernatant, AWF, LSW or LSS with 0.1 volume of 3 M sodium acetate (pH 5.2) and 1.0 volume of cold isopropanol, incubated at –20 ºC for a minimum of 1 h to overnight, and then centrifuged at 13,000 *g* for 30 min at 4 ºC. The pellets were washed twice with ice-cold 70% EtOH, resuspended in 100 µL of ultrapure DNase/RNase-free water (Invitrogen™, Waltham, MA, USA), and transferred to 1.5 ml centrifuge tubes. Thereafter, 1 mL of TRIzol was added to each tube and RNA extraction was performed following the same procedure as previously described for the total leaf RNA. Finally, RNA pellets were resuspended in 10 µL of ultrapure DNase/RNase-free water and stored at –80 ºC. If needed, to remove phenol or guanidine contamination from the RNA samples, one or two serial precipitations with EtOH and ammonium acetate were performed. Briefly, 1 µg of glycogen was added to 10 µL of RNA and mixed with 0.5 volume of 7.5 M ammonium acetate prior to the addition of 2.5 volumes of 100% EtOH. The mixture was incubated at –80 ºC for 30 min and centrifuged at 13,000 *g* for 20 min at 4 ºC. The RNA pellet was washed with 70% EtOH and resuspended in 10 µL of ultrapure DNase/RNase-free water. Nanodrop analysis was performed to determine RNA concentrations, which were confirmed using densitometry analysis of RNAs separated on polyacrylamide gels (see below).

Ribonuclease Protection Assays. To assess whether RNA in AWF and LSW was protected from ribonuclease digestion by either encapsulation inside of EVs or association with proteins, we followed our previously described protocol (3) with minor modifications. Freshly isolated LSW, AWF, P40, or P100-P40 pellets were split into five aliquots. Aliquot #1 was treated with 1% (v/v) Triton X-100 in 100 mM Tris pH 7.5 for

40 min with gentle shaking at RT to disrupt EVs. The other four aliquots were suspended in 100 mM Tris pH 7.5 and incubated on ice. Aliquots #1 and #2 were then treated with 1 µg/mL trypsin (Promega, Madison, WI, USA) and incubated at 30 ºC for 1 h followed by the addition of 1.5 mg/mL trypsin inhibitor (Worthington Biochemical Corp, Lakewood, NJ USA) to inactivate trypsin. The remaining three aliquots were also suspended in 100 mM Tris pH 7.5 with aliquots #3 and #4 incubated at 30 ºC and aliquot #5 placed on ice. Then, DNase and protease-free RNase A (Thermo Fisher Scientific™, catalog no. EN0531; diluted in 15 mM NaCl, 10 mM Tris–HCl pH 7.5) was added to aliquots #1, #2, and #3 at a final concentration of 0.1 µg/mL, and 15 mM NaCl, 10 mM Tris–HCl pH 7.5 was added to aliquots #4 and #5. Aliquots #1, #2, #3 and #4 were incubated for 1 h at RT, and aliquot #5 was placed on ice. To inhibit RNase A activity, a mixture of RNase Inhibitor, Murine (APExBIO) and RNase Out (Invitrogen) was added to the aliquots. The five aliquots were stored at –80 ºC until analysis.

Additionally, to assess the level of protection of LSW and AWF RNAs by proteins from ribonuclease digestion, LSW and AWF samples were suspended in 15 mM NaCl, 10 mM Tris-HCl pH 7.5 and treated with DNase and protease-free RNase A (Thermo Fisher Scientific™, catalog no. EN0531) at a final concentration of 0, 0.1, 1.0 or 9.0 µg/mL and incubated for 1 h at RT or kept on ice when indicated. To inhibit RNase A activity, a mixture of RNase Inhibitor, Murine (APExBIO) and RNase Out (Invitrogen) was added before extracting RNAs with TRIzol as previously described.

Denaturing Polyacrylamide Gel Electrophoresis of RNAs. Mini gels (7.2 cm x 8.6 cm x 0.75 mm) containing 10% or 15% polyacrylamide and 7 M urea in 1X Tris-Boric Acid EDTA (TBE, pH 8.4) were made using 40% Acrylamide/Bis Solution, 37.5:1 (Bio-Rad, catalog no. 1610148). RNA samples were mixed (1:1) with 2X denaturing loading buffer (95% formamide, 10 mM EDTA, 0.02% SDS, 0.02% bromophenol blue, and 0.01% xylene cyanol), denatured at 65 ºC for 5 min and resolved in 0.5X TBE running buffer at RT. For size standards, we used a 1:1 mix of Low Range ssRNA Ladder (New England Biolabs™, catalog no. N0364S) and 14-30 nt ssRNA Ladder Marker (Takara™, catalog no. 3416). Gels were stained with 1X SYBR Gold Nucleic Acid Gel Stain (Invitrogen™, catalog no. S11494) in 0.5X TBE for 10 min, washed twice with distilled water, and imaged using a Bio-Rad ChemiDoc imaging system.

DIG-labeled Northern Blots. RNA samples resolved in denaturing polyacrylamide gels were transferred to positively charged nylon membranes (Cytiva, Hybond-N+, catalog no. 45-000-850) using a semi-dry Trans-Blot Transfer System (Bio-Rad, catalog No. 1703940) in 0.5X TBE at constant 20 V for 45 min. Membranes were UV cross-linked twice at 120,000 µJ/cm^2 for 30 s using a UVC-508 Ultraviolet Cross-linker (Ultra-Lum) and prehybridized for 40 min at 42 ºC in DIG Easy Hyb solution (Roche) containing 0.1 mg/mL of Poly(A). Following prehybridization, membranes were hybridized overnight at 42 ºC with digoxigenin-labeled DNA probes (2.5 pmol/mL, in DIG Easy Hyb solution + Poly(A)). Oligonucleotides were obtained from Integrated DNA Technologies (IDT, USA), and labeled with DIG Oligonucleotide Tailing Kit, 2nd generation (Roche, catalog no. 03-353-583-910), following manufacturer's instructions. After hybridization, membranes were washed twice for 5 minutes at RT with low stringency wash buffer (2X SSC, 0.1% SDS), and twice for 10-15 min at 42 ºC with high stringency wash buffer (1X SSC, 0.1% SDS), blocked for 30-40 min at RT with 1X blocking solution (Roche) in maleic acid buffer (0.1 M maleic acid, 0.15 M NaCl, pH 7.5) and probed for 30 min with an alkaline phosphatase-labeled anti-digoxigenin antibody (Roche, catalog no. 11093274910). Membranes were washed twice for 15 min with washing buffer (0.1 M maleic acid, 0.15 M NaCl, pH 7.5, 0.3% (v/v) Tween 20) and then incubated for 5 min in detection buffer (0.1 M Tris-HCl, 0.1 M NaCl, pH 9.5). Signals were then visualized using CDP-Star ready-to-use (Roche) and detected using the Bio-Rad ChemiDoc imaging system. DNA oligonucleotides used for hybridization probes are listed in *SI Appendix,* Table S1.

RNA and Protein Quantification. To estimate the concentration of RNA isolated from cell lysates and AWFs, a NanoDrop (Thermo Fisher Scientific™) instrument was used. For LSW RNA, we estimated RNA concentrations using densitometric quantification of SYBR Gold-stained polyacrylamide gels. This was necessary because LSW samples contained a contaminant that caused an overestimation of RNA concentration when using absorbance-based quantifications. Briefly, 1 µL of RNA from LSW and AWF was loaded in a denaturing urea-polyacrylamide gel and run as previously described. The gel was then stained with SYBR Gold stain and imaged using the Bio-Rad ChemiDoc imaging system. The densitometric ratio between AWF and LSW was calculated using ImageJ software (National Institutes of Health, USA) (4), and this ratio along with the nanodrop value of AWF RNA was used to calculate the concentration in ng/µL of the LSW RNA.

Volumes of AWF and LSW normalized per plant FW were precipitated by mixing with 4 volumes of ice-cold acetone and incubating on ice for at least 1 h or overnight at –20 ºC. Then, the samples were centrifuged at 14,000 *g* for 10 min at 42 ºC. The pellet was allowed to dry for no longer than 1 h and resuspended in filtered VIB. To determine protein concentrations in AWF, we employed the Bradford method (5) using bovine serum albumin as a standard. Due to the extremely low concentration of proteins in LSW samples, we had to perform densitometric quantification upon silver-staining of denaturing polyacrylamide gels. Briefly, the proteins from each replicate were resolved using an SDS-PAGE gel (12% w/v acrylamide) and run at 100 V for 1 h. Gels were then stained with the ultra-sensitive Pierce Silver Stain Kit (Thermo Fisher Scientific™, catalog no. 24612) following the manufacturer´s instructions. Gel images were acquired using the Bio-Rad ChemiDoc Imaging System. The densitometric ratio between AWF and LSW was calculated using the ImageJ software, and this ratio and Bradford estimation of AWF proteins were used to estimate the protein concentration of LSW samples.

Immunoblots. For immunoblots, 30 µL of resuspended pellets were combined with 10 µL of 4X SDS loading buffer (250-mM Tris–HCl, pH 6.8, 8% (w/v) sodium dodecyl sulfate, 40% (v/v) glycerol, 20% 2-mercaptoethanol and 0.004% (w/v) bromophenol blue) and were then denatured at 95 ºC for 5 min. Then, 40 µL of each sample was loaded onto stain-free gradient gels (4–20% Precise Protein Gels, Thermo Fisher Scientific™) and separated at 150 V for 1 h in 1X TBS electrophoresis running buffer (24.8 mM Tris base, 0.1% (w/v) sodium dodecyl sulfate, 192 mM glycine, pH 8.3). The resolved proteins were visualized using the Bio-Rad ChemiDoc imaging system. After the proteins were transferred to a nitrocellulose membrane (Amersham™ Protran® Premium Western blotting membrane, nitrocellulose) using the semi-dry Trans-Blot Transfer System in Transfer buffer, the membrane was stained with Ponceau S stain (0.1% dye in 5% acetic acid solution) for about 10 min and cleared with water to visualize the protein transfer.

The membrane was washed once with 1X Tris-buffered saline (50 mM Tris-Cl and 150 mM NaCl, pH 7.5) containing 0.1% Tween-20 (TBST) before blocking with 5% (w/v) Difco Skim Milk (BD) prepared in 1X TBST for 1.5 h at RT. Thereafter, the membrane was incubated overnight at 4 ºC with the following primary antibodies at the indicated dilutions: rabbit polyclonal anti-PATL1 (6) (1:5,000), rabbit polyclonal anti-ANN2, rabbit polyclonal anti-ANN1, rabbit polyclonal anti-PEN1 (7) (1:1,000), rabbit polyclonal anti-PR5, mouse monoclonal [9F9.F9] anti-GFP (Abcam, Cambridge, UK, catalog no. ab1218; 1:2,000). Membranes were then washed with 1X TBST, and if needed, incubated with one of the following secondary antibodies as appropriate: Horseradish peroxidase (HRP)-conjugated goat anti-rabbit (Abcam, catalog no. ab97051; 1:10,000) or HRP-conjugated goat anti-mouse (Abcam, catalog no. ab6789; 1:5,000) for 1.5 h at RT. After three final washes in 1X TBST, proteins were visualized using ProtoGlow ECL Substrate (National Diagnostics) and the Bio-Rad ChemiDoc Imaging System. The densitometric ratio between AWF and LSW was calculated using the ImageJ software.

RNase A and RNase R Treatment of RNA samples. RNA was isolated from AWF using TRIzol as described above and resuspended in ultrapure water. For RNase R treatment, 200 ng of RNA were treated with 3 units of RNase R (Lucigen, catalog no. RNR07250) in a 10 uL reaction volume containing 1X RNaseR reaction buffer for 1 h at 37 ºC. RNase R was then inactivated by incubation at 65 °C for 20 min. For RNase A treatment, 200 ng of total RNA were treated with 20 ng/µL of RNase A diluted in 1x reaction buffer (15 mM NaCl, 10 mM

Tris-HCl, pH 7.5) in a 10 µL reaction volume for 1 h at RT. RNase A activity was stopped by adding a mixture of RNase Inhibitor, Murine (APExBIO) and RNase Out (Invitrogen). Following RNase treatments, RNA was purified by precipitation with ammonium acetate and ethanol to remove free nucleotides and other small degradation products. This precipitation step was repeated twice for optimal RNA purity.

Quantification of N^6-Methyladenosine (m^6A) in Extracellular RNA. RNA was isolated from CL, AWF, and LSW using TRIzol as described above, and the RNA concentrations were measured using a Thermo Fisher Scientific™ NanoDrop One spectrophotometer. For DNase treatment, 200 ng of RNA sample was treated with RQ1 DNase (1 unit/µg of RNA; Promega, catalog no. M6101) per manufacturer's instructions. For qualitative analysis, the banding pattern of different RNA samples post DNase treatment was assessed by running the samples on a 15% denaturing RNA gel before performing dot blot analysis as described previously. For all samples, equal amounts of RNA were prepared in equal volumes (6 µL) using UltraPure DNase/RNase-free distilled water (Invitrogen). RNA samples were denatured at 95 ºC for 3 min and placed on ice immediately to prevent the formation of secondary structures. m^6A quantification using dot blots was performed using the protocol described by Zand Karimi *et al.* (3). Briefly, RNA samples were applied directly to a piece of Hybond-N+ membrane (Amersham Pharmacia Biotech) using a micropipette. To prevent the spread of RNA on the membrane, 2 µL of RNA solution was applied at a time, allowing the membrane to dry for three min before applying the next 2 µL drop to the same spot until a total of 6 µL of RNA sample was applied. To crosslink the spotted RNAs to the membrane, a UVC-508 Ultraviolet Cross-linker (Ultra-Lum) was used to irradiate the membrane twice at 120,000 microjoules/cm^2 for 30 s. The membrane was then washed in clean RNase-free 1X PBS buffer (1xPBS; 2.7 mM KCl, 8 mM Na2HPO4, 2 mM KH2PO4, and 137 mM NaCl, pH 7.4) and blocked in 5% (w/v) non-fat milk prepared in 1X PBS containing 0.02% Tween-20 for 1 h at RT. The membrane was then incubated overnight with anti-m^6A antibody (Abcam, catalog no. Ab151230; or Synaptic Systems, catalog no. 202 003) at a 1:250 dilution in 5% non-fat milk prepared in 1X PBS containing 0.02% Tween-20. The membrane was washed in 1X PBS containing 0.02% Tween-20 three times and incubated with horseradish peroxidase-labeled goat anti-rabbit antibody (Abcam, catalog no. ab205718) at a 1:5,000 dilution for 1 h at RT. After a final wash in 1X PBS containing 0.02% Tween-20, m^6A modified RNAs were visualized using the Immune-Star Reagent (Bio-Rad) and imaged using the Bio-Rad ChemiDoc Imaging System.

Alternatively, EpiQuik™ m^6A RNA Methylation Quantification Kit (Fluorometric) (EpigenTek, catalog no. P-9008-48,) was used to determine the m^6A percentage in exRNA by following the instructions provided in the user guide. Briefly, total RNA is bound to strip wells using an RNA high-binding solution. m^6A is detected using a specific capture N6-methyladenosine (anti-m6A) antibody and detection antibody. The detected signal is enhanced and then quantified colorimetrically by reading the absorbance in a microplate spectrophotometer at a wavelength of 450 nm. The amount of m^6A is proportional to the OD intensity measured. Both negative and positive RNA controls provided in this kit must be used to quantity the percentage of m^6A. This kit allowed us to quantify the absolute amount of m^6A in each sample.

Detection of Pectin in Extracellular RNA Fractions. RNA was isolated from LSW and AWF using TRIzol as described above. For all samples, 100 ng of RNA were prepared in equal volumes (4 µL) using UltraPure DNase/RNase-free distilled water (Invitrogen). RNA samples were denatured at 95 ºC for 3 min and placed on ice immediately to prevent the formation of secondary structures. Dot blot analysis of these RNA samples was performed using the same protocol as for m6A quantification except using JIM7 as the primary antibody at a dilution of 1:10 (Kerafast, catalog no. ELD005) and horseradish peroxidase-labeled goat anti-rat as the secondary antibody at a dilution of 1:5000 (Invitrogen, catalog no. 31470).

Statistical Analyses. Statistical analyses and plotting of RNA and protein concentrations were performed using the GraphPad Prism 8.3.0 software (GraphPad Software, San Diego, CA, USA). The specific statistical test used for each analysis is provided in the corresponding figure legend. The number of independent biological replicates (n) is indicated in each plot.

Preparation of sRNA Sequence and Standard RNA Sequence Libraries. sRNA libraries were constructed using the RealSeq-AC kit version 2 (Realseq Biosciences, Santa Cruz, CA, USA, catalog no. 500-00048;) as per the manufacturer's instructions. We used 60 ng of DNase I-treated total RNA as the starting material for constructing libraries (Thermo Fisher Scientific™ DNaseI, catalog no. EN0521). For the Cell lysate rRNA depleted samples, we used the RiboMinus Plant Kit for RNA-seq (Invitrogen, catalog no. A10838-08) at $1/10^{th}$ of the recommended volume of regents and sample. For RNAseq libraries, we used NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs, catalog no. E7760L) protocol. To ensure correct library size capture, we performed a Bioanalyzer dsDNA HS chip assay on the Agilent 2100 Bioanalyzer (Agilent Technologies, catalog no. DE4103649) for each library. We sent all sRNA and RNAseq libraries for sequencing to the University of Delaware Sequencing and Genotyping Center, where they were sequenced on a NovaSeq2000 instrument using 50-bp single-end reads for sRNA and 75-bp paired-end reads for RNAseq.

Sequence Data Analysis. All sRNA libraries were analyzed as previously (3). Briefly, we first trimmed the adaptors using Cutadapt version 1.16 (8) using a minimum insert size of 10 nt and no maximum. We assessed sequence quality using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/ fastqc/). We aligned clean reads to the Arabidopsis genome (TAIR version 10) and all subsequent analyses were performed using the software Bowtie2 (9). To confirm that our RNA isolation and library preparation protocols were not being contaminated by environmental DNAs present in our laboratories, we compared mapping frequencies to the Arabidopsis and maize genomes for both the AWF and LSW Arabidopsis sRNAseq libraries and libraries made in from AWF and LSW RNA isolated form maize for a parallel project (*SI Appendix*, Table S1). For miRNA analyses, we used the latest version of miRBase (version 22) (10). Sequences in the RNA-seq libraries were analyzed using HiSat2 and Stringtie pipeline (11), and the TAIR10 available annotation file. We performed differential accumulation analyses using DESeq2 with default parameters, using reads that were not normalized as input (12). In DESeq2, p-values were calculated using the Wald test and corrected for multiple testing using the Benjamini and Hochberg procedure (13). We generated graphical representations using the software ggplot2 (14) in the R statistical environment.

To analyze the tRNA derived sRNAs from the sRNAseq data, we ran unitas version 1.8.0 (15) using the Genomic tRNA database for Arabidopsis (data accessed on 05 March 2024) (16, 17) using default parameters. The absolute read counts from the unitas pipeline were normalized against total number of input reads per million. The plots were drawn using R.

Results

Swabbing of Leaf Surfaces Provides Additional Evidence for the Presence of RNA. To gain additional evidence that Arabidopsis leaves are coated with RNA, we used cotton swabs to collect RNA from adaxial and abaxial leaf surfaces separately (*SI Appendix*, Fig. S4). These analyses revealed that both leaf surfaces contained approximately equal amounts of RNA. If LSW RNA were derived from secretions through stomata, we would have expected more RNA on the abaxial surface, which contains many more stomata compared to the adaxial surface. This analysis also suggests that trichomes are unlikely to be the source of leaf surface RNA, as there are many more trichomes on the adaxial side than the abaxial side of Arabidopsis leaves. Our ability to collect RNA simply by absorbing buffer from the leaf surface also shows that centrifugation is not required to collect RNA from leaf surfaces and confirms that RNA is located on the leaf surface, as opposed to being extracted through the stomata during centrifugation.

AWF and LSW exRNAs Display Reduced Abundance of Many Transcripts Relative to Total CL RNA. We found 176 DE genes when comparing AWF to LSW (Fig. 3*A*). To understand the gene overlap between AWF and LSW, we examined the DE genes common to both extracellular fractions. Most DE genes were unique either in LSW when compared to CL (172 genes) or in AWF when compared to CL (158 genes) (*SI Appendix,* Fig. S7). Interestingly, the seven genes with higher accumulation in LSW correspond to four pre-tRNAs, two transposable elements, and a GTP binding Elongation factor Tu family protein (Dataset S1). The remaining 165 genes, which exhibited lower accumulation levels in LSW, are enriched in GO terms related to response to stimulus (Dataset S2). Conversely, the 25 genes with higher accumulation in AWF compared to CL have a GO enrichment in the biotic stress category. The other genes with lower accumulation in AWF are enriched in GO terms related to the photosynthetic electron transport chain, seed development, and stress responses. Additionally, we observed that 61 genes were uniquely differentially expressed in AWF compared to LSW (*SI Appendix*, Fig. S7), all of them with lower abundance in AWF. These genes are enriched in GO categories related to both biotic and abiotic stress responses. This supports the hypothesis that AWF and LSW exhibit different RNA accumulation patterns and that the role of RNAs in these fractions is linked to both biotic and abiotic responses.

The remaining DE genes were categorized into four groups based on the number of comparisons where they showed a statistically significant difference (Dataset S1). The first group contains 75 genes commonly DE in both AWF and LSW compared to CL. Of these, only five (5) genes show higher accumulation in LSW and AWF compared to CL, including four rRNA genes and a transposable element. The other 70 genes show lower accumulation in AWF and LSW compared to CL and are mainly associated with water deprivation according to their GO enrichment. The second group includes 65 genes that are DE in AWF compared to both CL and LSW. Sixteen of these genes show lower accumulation in AWF compared to CL, but higher accumulation in AWF compared to LSW. Conversely, 49 genes have higher accumulation in AWF compared to CL and lower accumulation in AWF compared to LSW. This group is enriched in GO terms related to plant defenses, including oxylipin metabolism. The third group contains 48 genes that are statistically significant in LSW compared to both CL and AWF. Seventeen of these have a lower abundance in LSW compared to both CL and AWF, while 31 genes have a higher accumulation in LSW. The final group includes only two genes common to all comparisons: a GATA transcription factor (AT3G54810) and other RNA (AT1G70185).

Broadly speaking, genes that accumulate significantly more in AWF compared to CL are enriched in GO terms related to biotic stress. Conversely, genes with significantly less accumulation in AWF compared to CL are enriched in GO terms for stomata movement, photosynthesis, water deprivation, and biotic stresses. In the case of LSW, genes with significantly more accumulation compared to CL are enriched in terms of iron transport, translation, ATP biosynthetic processes, and aerobic respiration. Genes with significantly less accumulation in LSW compared to CL are enriched in GO terms for water deprivation, response to temperature stimulus, and signal transduction. These accumulation patterns suggest that the RNA found in AWF and LSW might have different origins, or there is differential degradation occurring in AWF compared to LSW.

Specific tasiRNAs Differentially Accumulate in LSW and AWF. To characterize the tasiRNA composition in the three fractions, we mapped reads to seven *TAS* genes, including three *TAS1*, two *TAS3,* one *TAS2,* and one *TAS4* locus. Just like miRNAs, reads mapping to *TAS* genes accumulated at a significantly lower level in AWF and LSW fractions compared to CL (*SI Appendix,* Fig. S9). Additionally, we also observed that the size distribution of these reads is mainly 21 and 22 nt long in CL, which shifts to 19 and 20 nt in AWF, and 20 and 21 nt in LSW (*SI Appendix,* Fig. S8*B*), indicating that tasiRNAs are trimmed similarly to miRNAs upon secretion.

ExRNA is not enriched in m^{6}A modification. Since we had observed that pectin co-purified with RNA (Fig. 6*D*), we assessed whether the anti-m6A antibody might be cross-reacting with pectin by comparing the signals in dot blots between the JIM7 anti-pectin antibody and the anti-m6A antibody (*SI Appendix,* Fig. S16*B*). However, the relative intensities of the CL and LSW samples was reversed for these two antibodies, which indicates that they are detecting different molecules. We also assessed whether the anti-m6A antibody might be cross-reacting with DNA, but we observed no reduction in signal when pretreating LSW with DNase prior to precipitation (*SI Appendix,* Fig. S16*C*). Consistent with this, we observed no changes in banding pattern of LSW RNA following DNase treatment (*SI Appendix,* Fig. S16*D*). In addition, the m6A signal in AWF RNA samples treated with two different RNases remained comparable to untreated samples, despite a significant reduction in total RNA amount (*SI Appendix,* Fig. S17). We thus speculate that the anti-m6A antibody is detecting another polysaccharide that co-purifies with RNA.

SI Figures and Tables

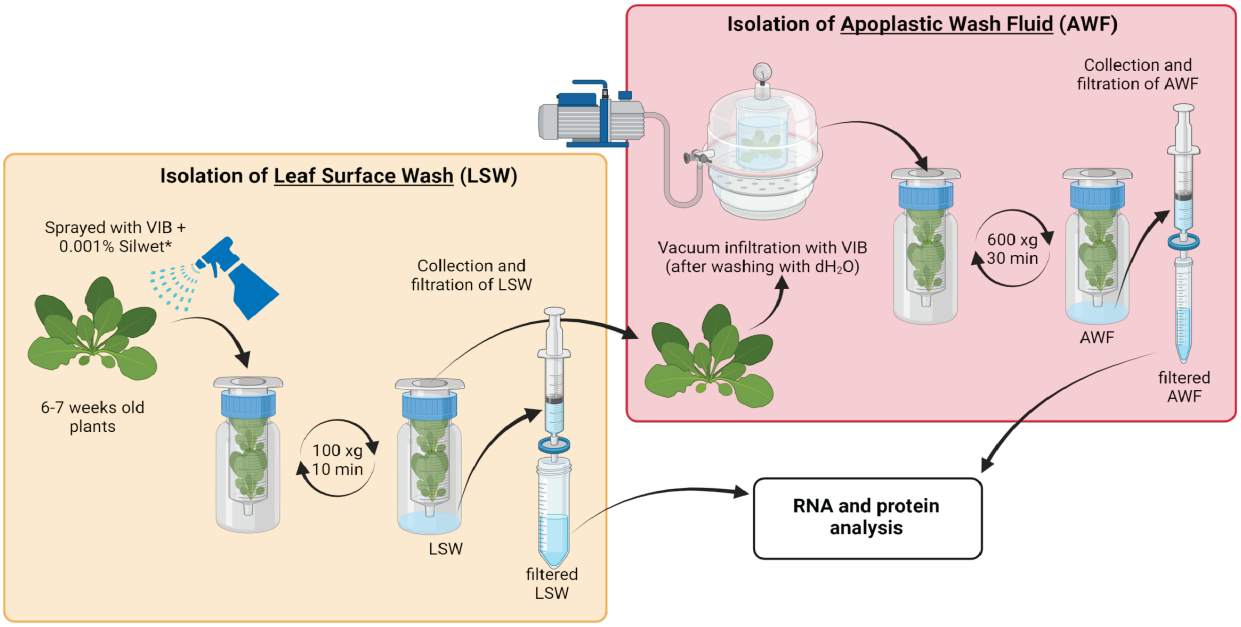

Fig. S1. Schematic illustration of the stepwise protocol for the isolation of LSW and AWF using Arabidopsis plants. Full rosettes of six-to-seven-week-old Col-0 plants were detached and sprayed on both sides with VIB supplemented with 0.001% (v/v) Silwet. Rosettes were then placed in a 60 mL syringe with small holes that was inserted into a 250 mL centrifuge bottle. The rosettes were then centrifuged at 100*g* for 10 mins at 4 ºC. To isolate the AWF, the same set of plants was then vacuum infiltrated with VIB followed by centrifugation at 600*g* for 30 mins at 4 ºC. Both fractions were filtered through 0.2 µm filters before further processing. The weight of the rosettes was also recorded to normalize the concentration of RNA and protein isolated from both AWF and LSW based on plant fresh weight (FW).

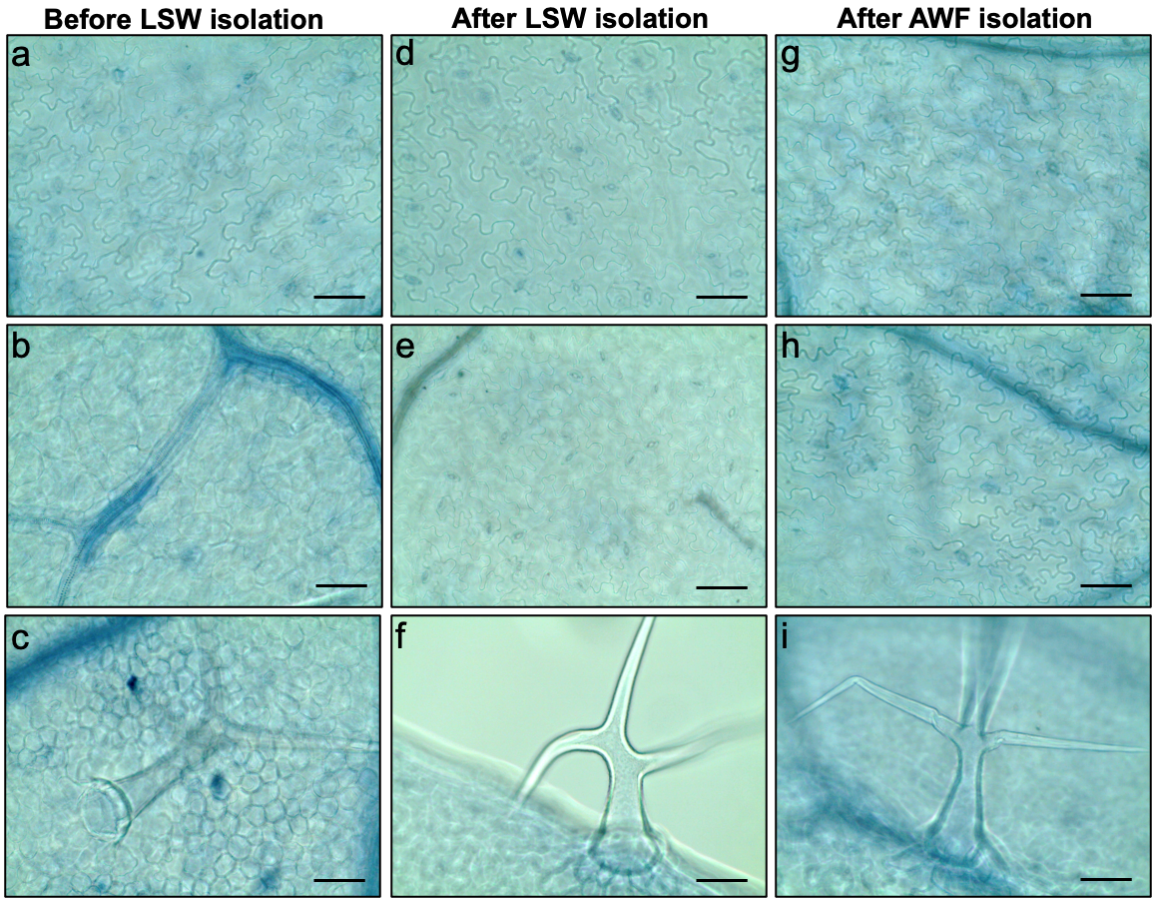

Fig. S2. Isolation of LSW and AWF does not cause cell rupture. To assess if LSW and AWF isolation leads to cell rupture, leaves from three whole rosettes were stained with trypan blue dye before and after each isolation step as indicated. *(a-c)* Before LSW isolation; *(d-f)* After LSW isolation**;** *(g-i)* After AWF isolation**.**

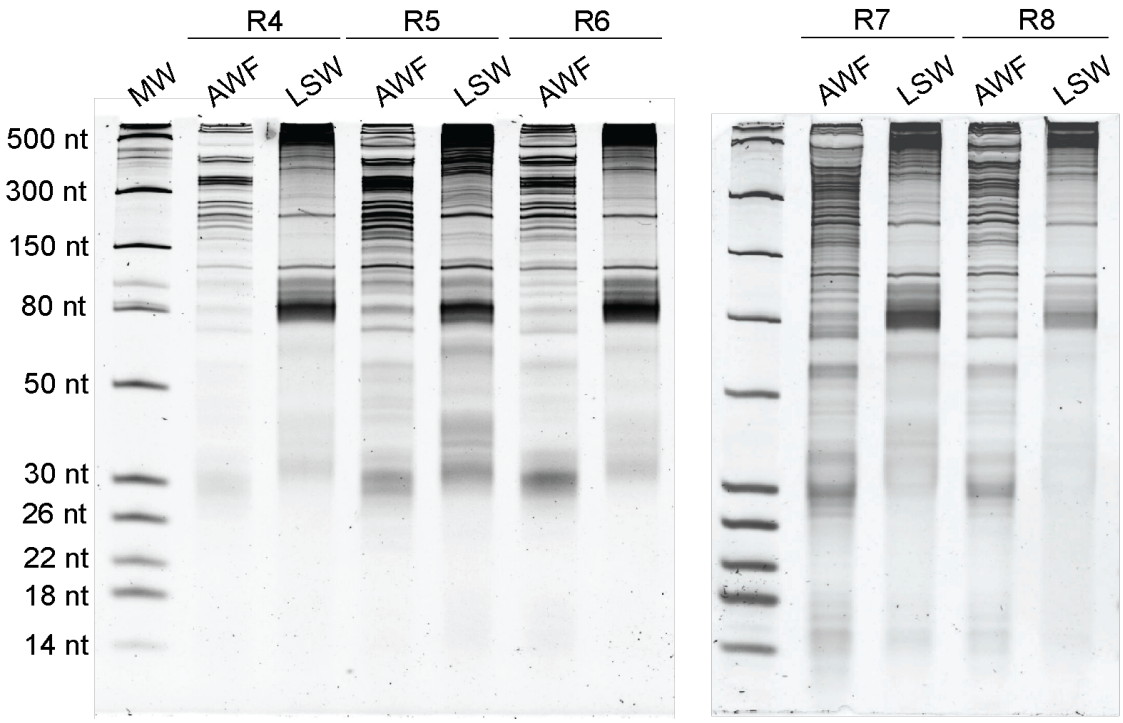

Fig. S3. AWF and LSW contain equivalent amounts of RNA. RNA isolated from AWF and LSW from eight different replicates (R1-R3: shown in Fig. 1*C*) was separated on 15% denaturing polyacrylamide gels and stained with SYBR GOLD nucleic acid stain. Densitometry analysis was performed using ImageJ software to estimate the amount of RNA per gram fresh weight of plant material (Refer to Fig. 1*D* for the plot of this analysis).

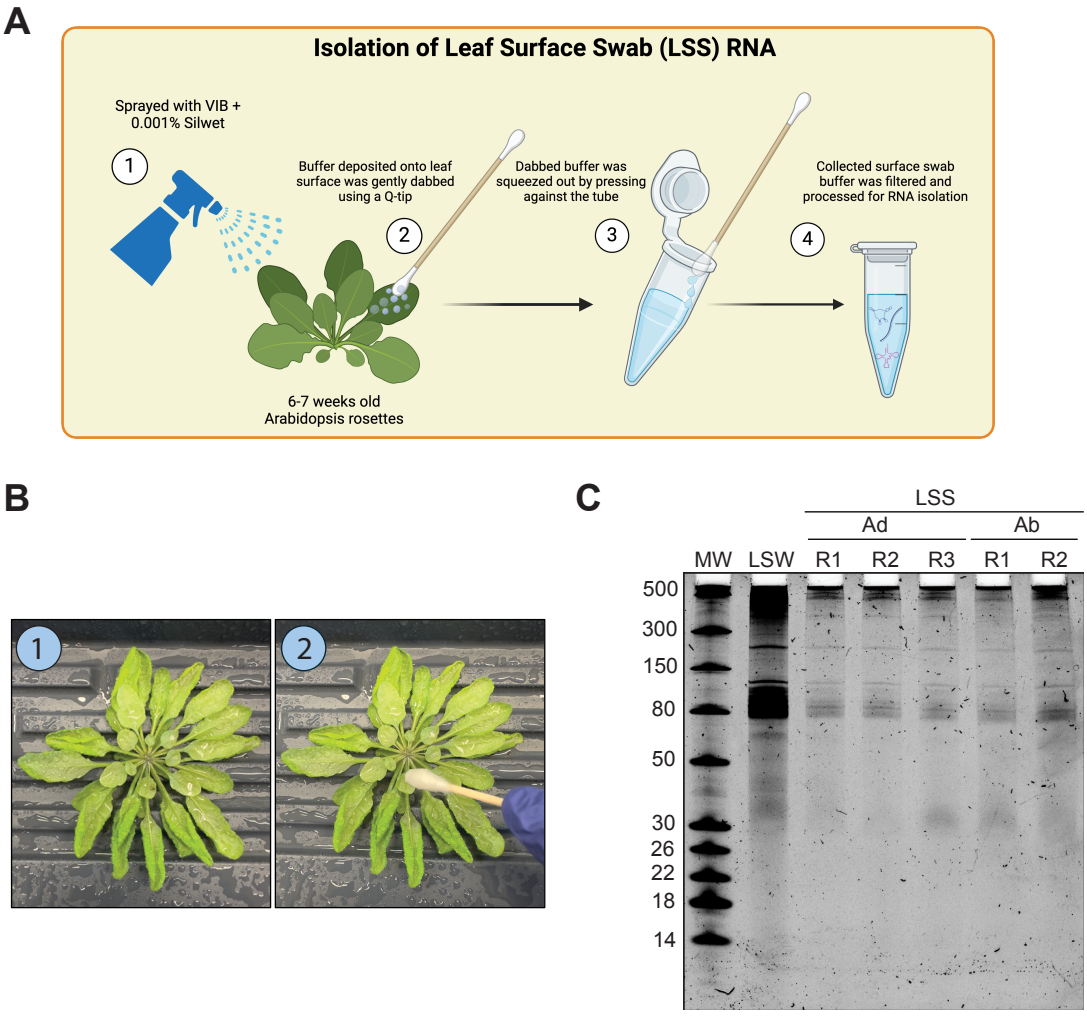

Fig. S4. Isolation of leaf surface RNA using a cotton swab. (*A*) Schematic illustration of leaf surface swab (LSS) method for isolating leaf surface RNA. (*B*) Photograph showing cotton swab used to absorb leaf surface wash from abaxial surface. (*C*) Denaturing PAGE gel showing RNA recovered from adaxial (Ad) and abaxial (Ab) surfaces of leaves.

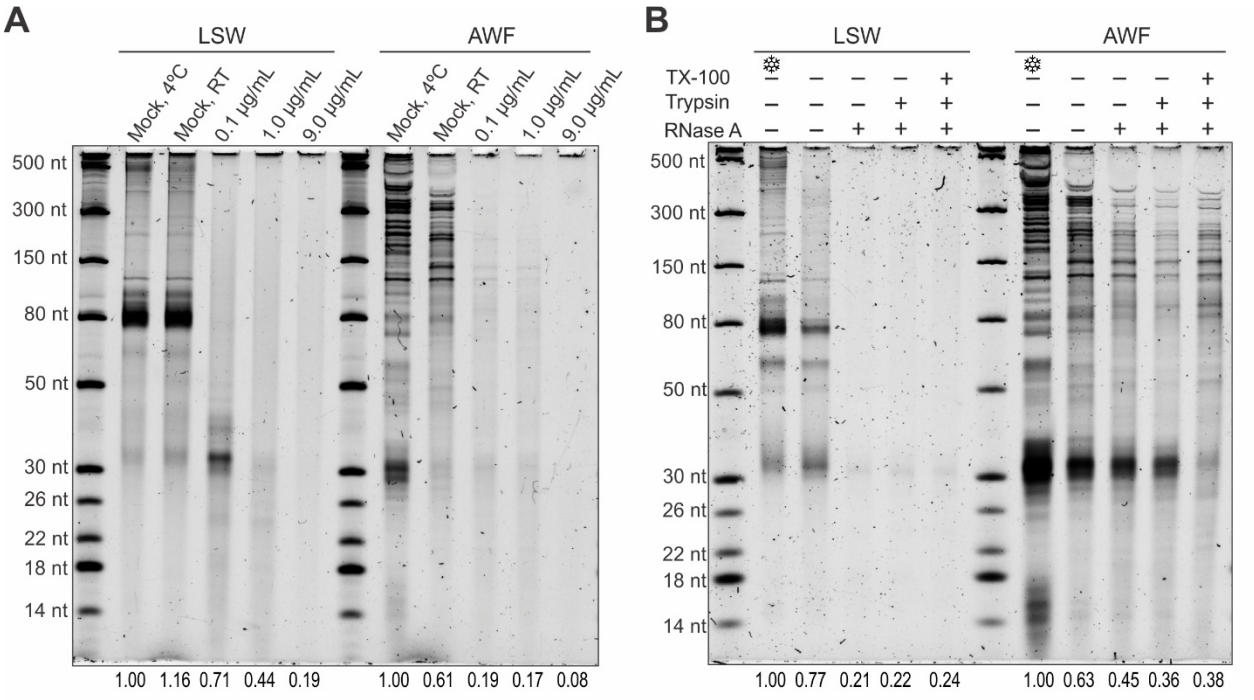

Fig. S5. LSW RNA is sensitive to RNase A (Additional biological replicates of the experiment presented in Figs. 2*A* and *B*). (*A*) LSW and AWF samples were treated with increasing concentrations of RNase A as indicated. Treatments were performed at RT for 1 h, except for the indicated mock sample. RNA abundance in each gel lane was estimated by densitometry and expressed relative to Mock (4 ºC). (*B*) Ribonuclease protection assay of AWF and LSW. AWF and LSW samples were treated with RNase A (0.1 µg/mL), or trypsin followed by RNase A, or TX-100 followed by trypsin followed by RNase A. RNA abundance in each gel lane was estimated by densitometry and expressed relative to the negative control kept on ice (❄). Biological replicates were isolated on different days.

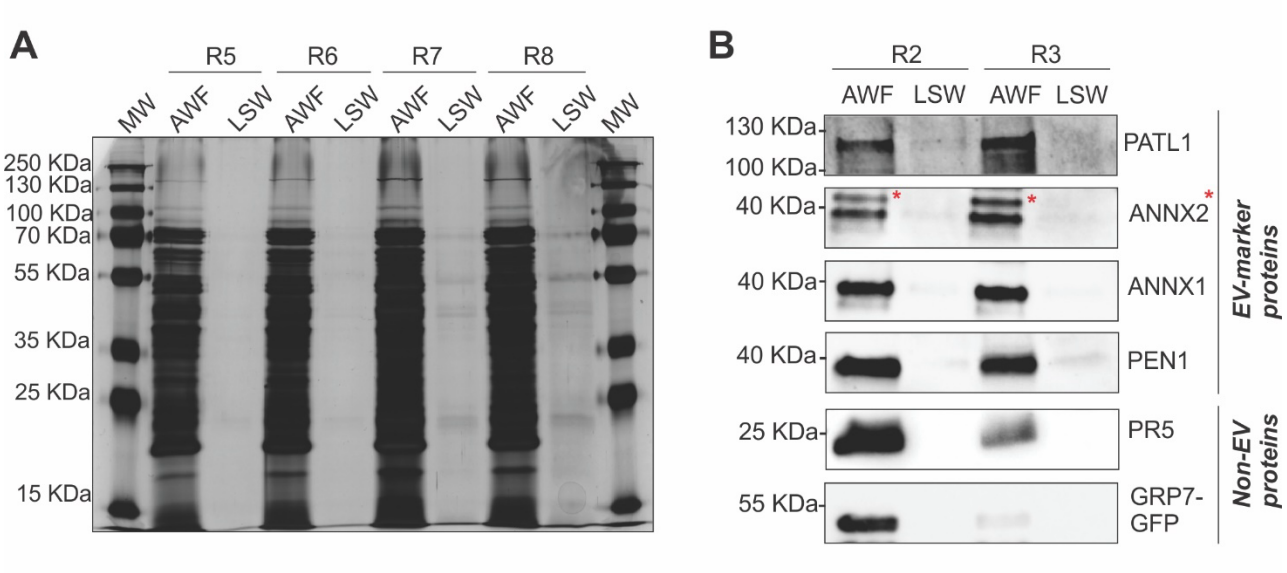

Fig. S6. LSW contains very little protein compared to AWF. (*A*) Silver-stained SDS-PAGE showing the total protein profile of AWF and LSW. Additional biological replicates (R1-R4) are shown in Fig. 2C. The amount of protein loaded in each lane was normalized by plant FW. MW: molecular weight markers. Densitometry analysis was performed using ImageJ software to estimate the amount of protein per gram fresh weight of plant material (Refer to Fig. 2*D* for the plot of this analysis). (*B*) Immunodetection of EV-marker proteins (PEN1, PATL1, ANN1, ANN2) and RNA-binding proteins (GRP7, PR5, ANN1, ANN2) in AWF and LSW. These are biological replicates 2 and 3 of the experiment presented in Fig. 2*E*. Red asterisks indicate the specific band for ANN2.

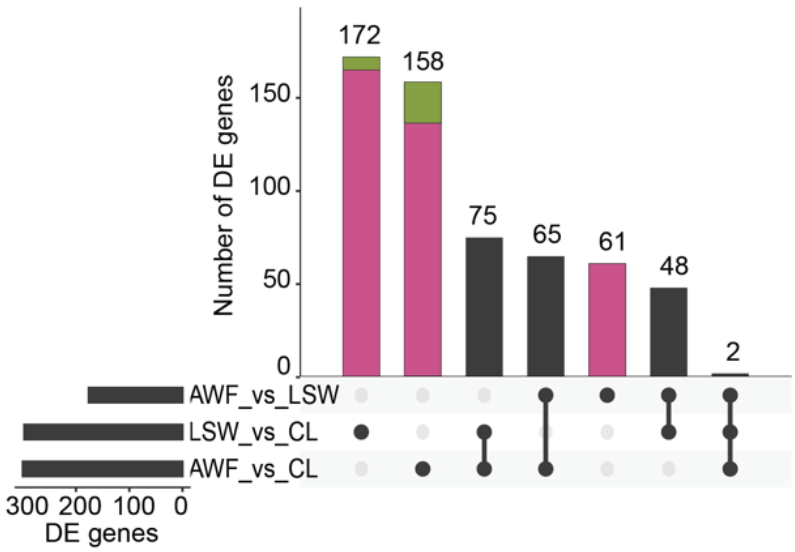

Fig. S7. AWF and LSW RNAs are depleted in protein-coding transcripts. Shared and unique DE genes in each comparison, the bottom left plot shows the entire size of each set as a horizontal histogram, the bottom right shows the intersection matrix and the upper right shows the size of each combination as a vertical histogram, with down-regulated genes colored in pink and up-regulated genes colored in green.

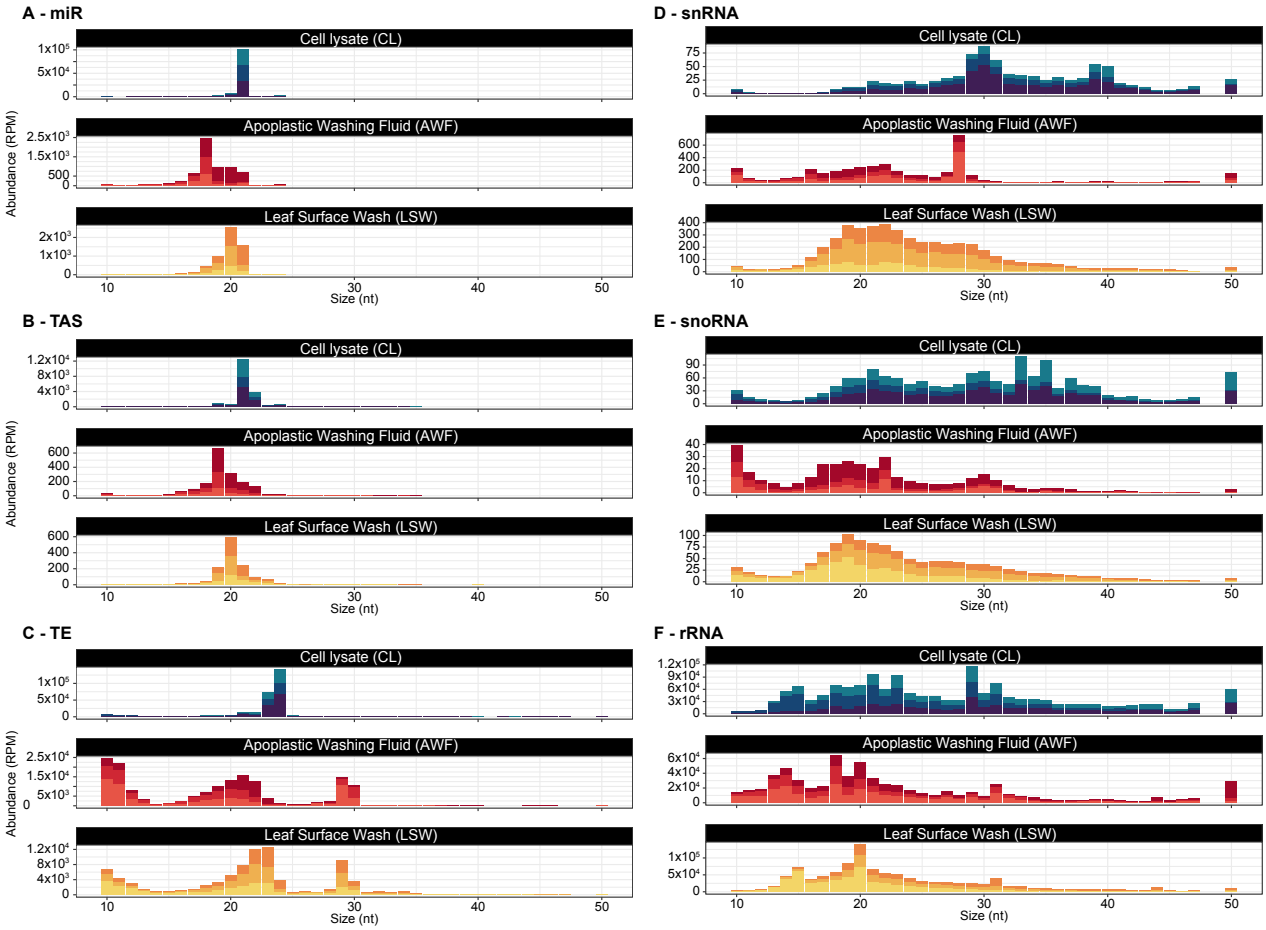

Fig. S8. Size distribution of sRNAs categorized by source. sRNA size distribution of reads mapping to the Arabidopsis genome (TAIR version 10). The abundance of each size class was calculated for each sample independently and normalized to the total number of reads for that sample. The *x*-axis indicates the sRNA size, from 10-50 nt, and the *y*-axis indicates its abundance in reads per million (RPM) reads. Each panel represents an RNA fraction, from top to bottom, CL, AWF, and LSW. (*A*) Reads mapping to microRNAs (miR). (*B*) Reads mapping to trans-acting small interfering RNAs (tasiRNAs). (*C*) Reads mapping to transposable elements (TE). (*D*) Reads mapping to small nuclear RNAs (snRNAs). (*E*) Reads mapping to small nucleolar RNAs (snoRNAs). (*F*) Reads mapping to ribosomal RNAs (rRNAs).

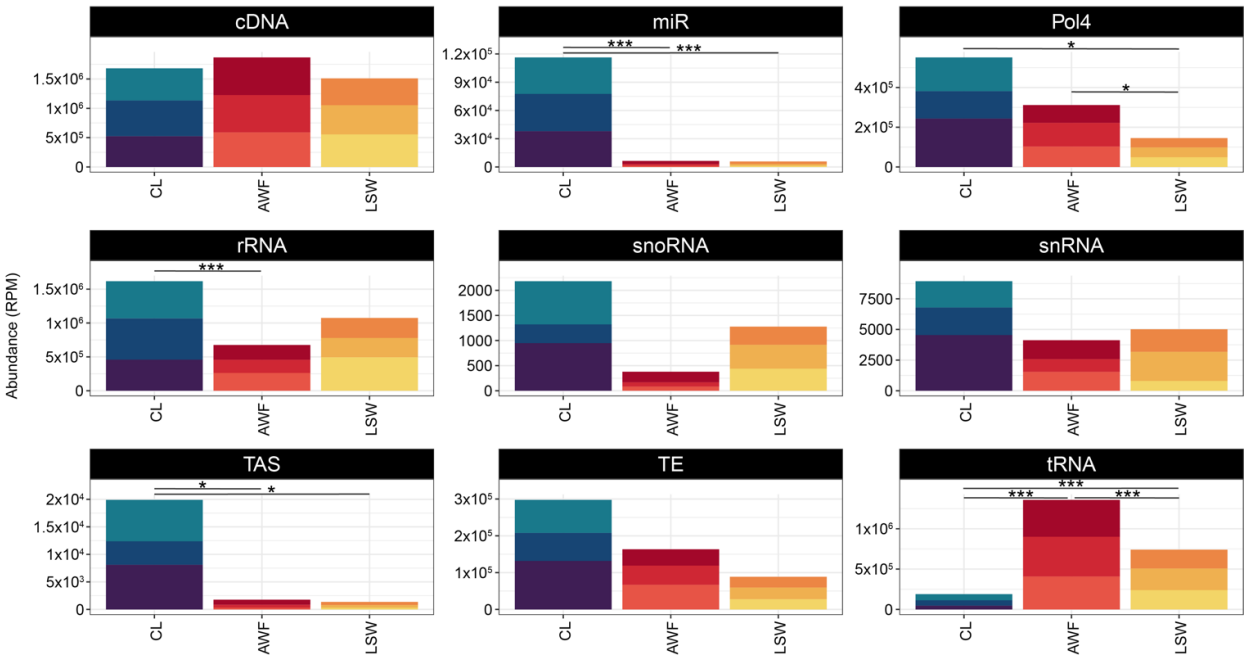

Fig. S9. Sources of sRNA reads obtained from CL, AWF, and LSW. Genomic origin of small RNA reads based on the categories established in the TAIR 10 genome version. RNAs that mapped to the genome were categorized by origin. The x-axis represents each of the fractions, CL, AWF, and LSW. The y-axis indicates the relative abundance expressed in RPM. Each box represents a specific genomic source: cDNA, complementary DNA; Pol4, Polymerase IV-dependent products; rRNA, ribosomal RNAs; TE, Transposable elements; tRNA, transfer RNAs; miR, microRNAs; snoRNA, small nucleolar RNA; snRNA, small nuclear RNAs; TAS, trans-acting siRNA. The three colored shades represent three independent biological replicates, with each replicate derived from 18 Arabidopsis plants.

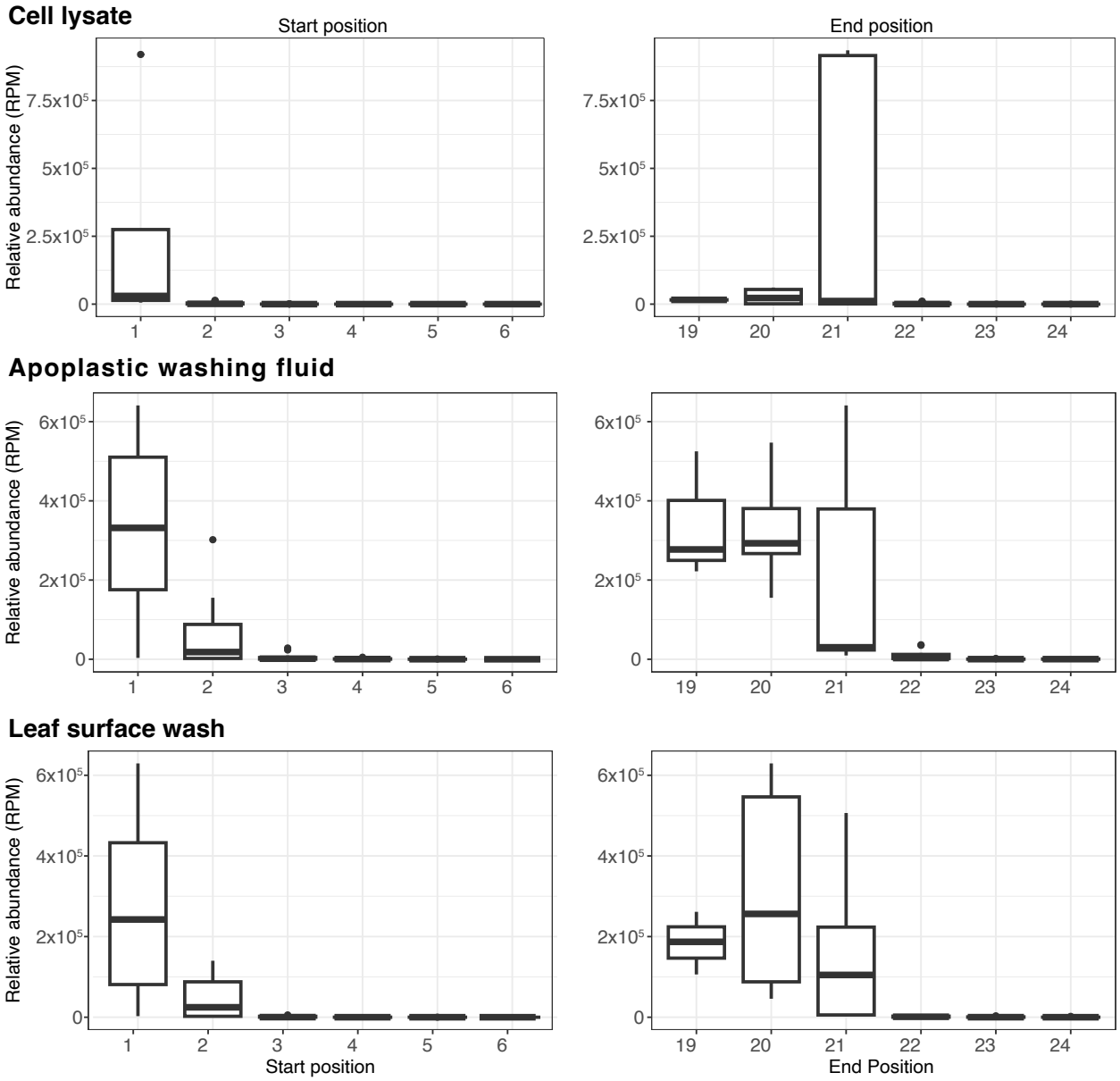

Fig. S10. AWF and LSW miRNAs are processed on their 3' ends. sRNAseq reads mapping to known miRNAs were aligned to these miRNAs and the positions of their 5' ends and 3' ends identified. Reads from cell lysate RNA were nearly all full-length, while reads from AWF and LSW were frequently truncated on their 3' ends. Each boxplot represents three biological replicates, where the box comprises data points between quartiles 1 and 3, and the whiskers show the sample variability outside of these quartiles.

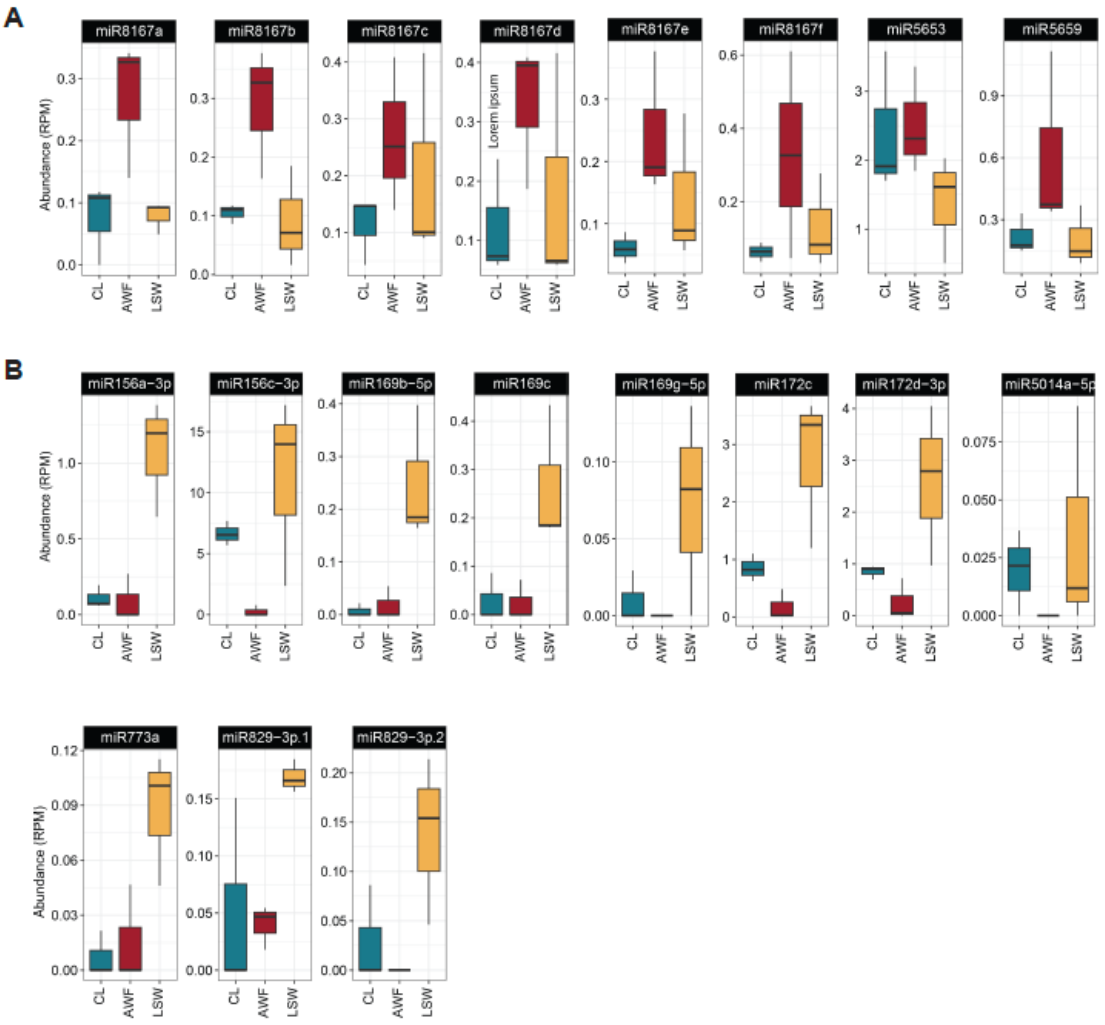

Fig. S11. Specific miRNAs are more abundant in AWF or LSW compared to cell lysate. (*A*) miRNAs with a higher absolute abundance in the AWF fraction. (*B*) miRNAs with a higher absolute abundance in LSW fraction. The x-axis represents the three distinct fractions, and the y-axis represents the absolute abundance in reads per million library reads (RPM). Each boxplot represents three biological replicates, where the box comprises data points between quartiles 1 and 3, and the whiskers show the sample variability outside of these quartiles.

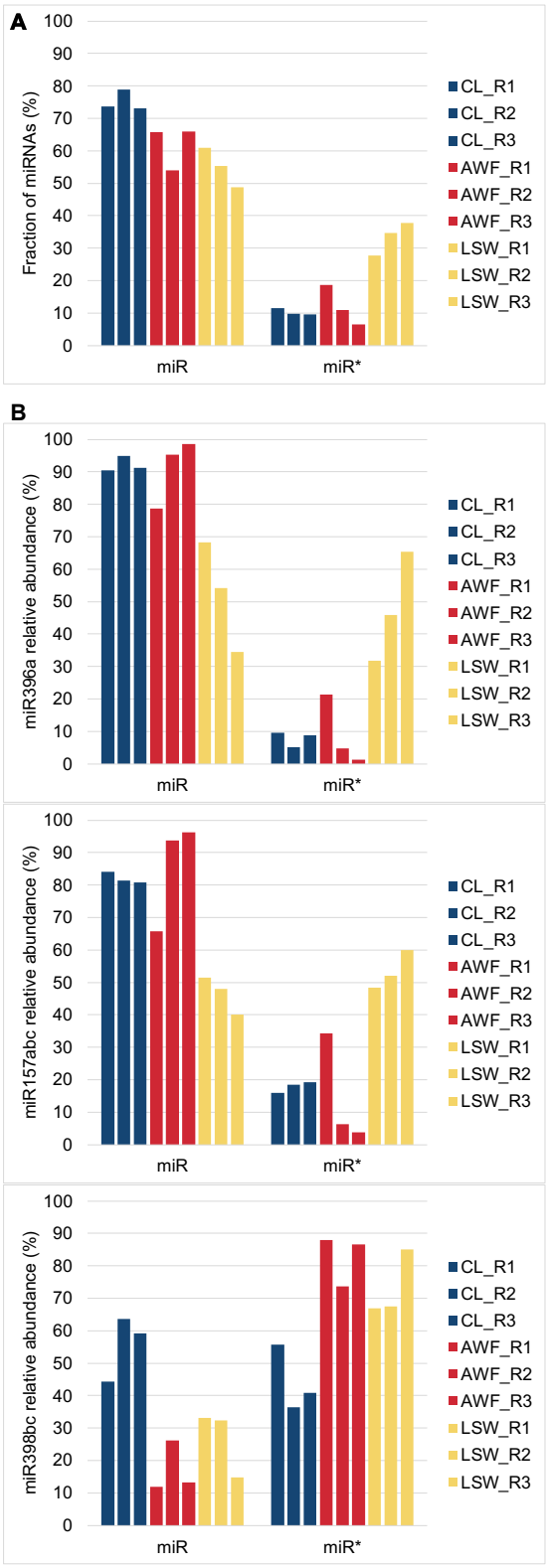

Fig. S12. miRNA* strands are enriched in LSW RNA. (*A*) Relative fraction of miRNA reads that correspond to the miRNA versus miRNA* strands in CL, AWF and LSW RNA. Overall, there is a higher frequency of miRNA* strands in LSW. (*B*) The three most abundant LSW miRNAs show an approximately 50:50 ratio of miRNA and miRNA* reads, suggesting that they may be double-stranded.

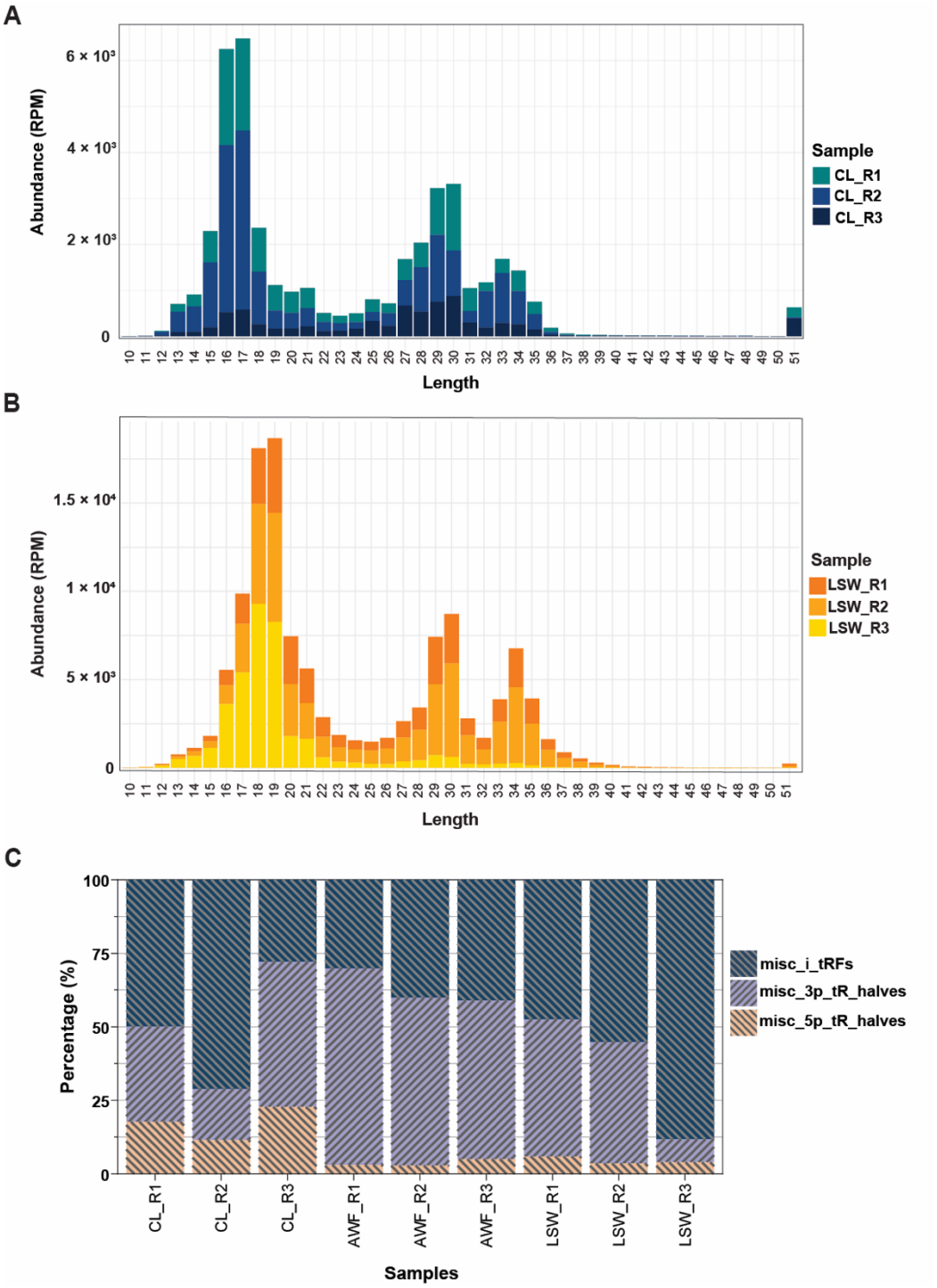

Fig. S13. misc-tRFs are enriched in tRNA halves. (*A*) Size distribution of misc-tRF reads in CL samples. The colors represent three replicates. (*B*) Size distribution of misc-tRF reads in LSW samples. The colors represent three replicates. (*C*) Reclassification of the misc-tRFs. misc_5p_tR_halves: start position at nucleotide position 2, 3, or 4 and length 28-35 nt; misc_3p_halves: start position after nucleotide position 29 and length ≥28 nt; and misc_i_tRFs: all misc_tRFs that were not reclassified as either misc_5p_tR_halves or misc_3p_tR_ halves.

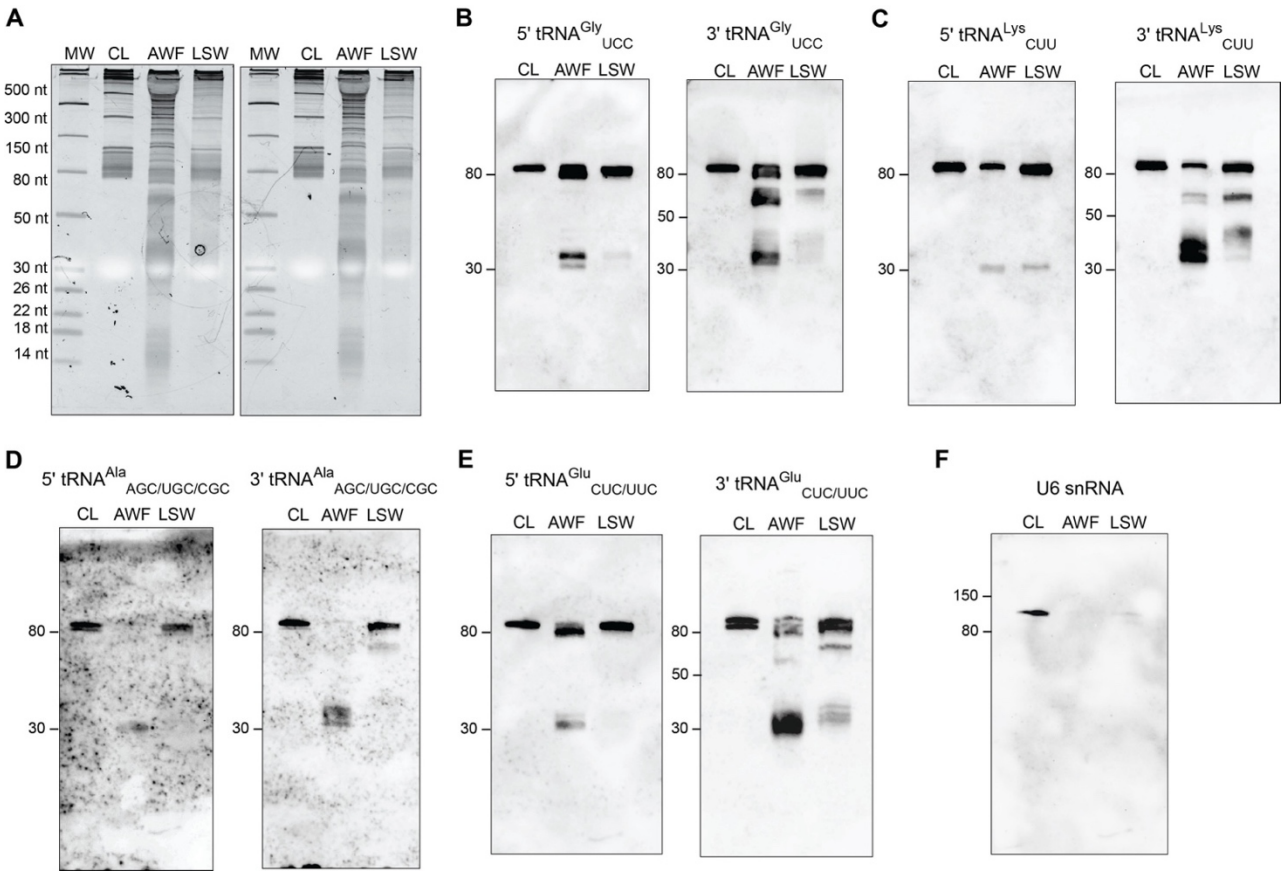

Fig. S14. Extracellular fractions are enriched in tRNA-derived fragments compared to whole cell lysate. (*A*) 100 ng of RNA from total CL, AWF, and LSW was separated on 15% denaturing polyacrylamide gels and stained with SYBR GOLD nucleic acid stain. (*B, C, D,* and *E*) Upon blotting onto a positively charged nylon membrane, RNA was probed with DIG-labeled 5' and 3' probes against $tRNA^{Gly}$, $tRNA^{Lys}$, $tRNA^{Ala}$, and $tRNA^{Glu}$ to detect tRNA-derived fragments. Full-length tRNAs were also detected in these samples. (*F*) RNA was probed with a DIG-labeled probe against U6 snRNA as a control. This figure shows an additional biological replicate of the blot shown in Fig. 5.

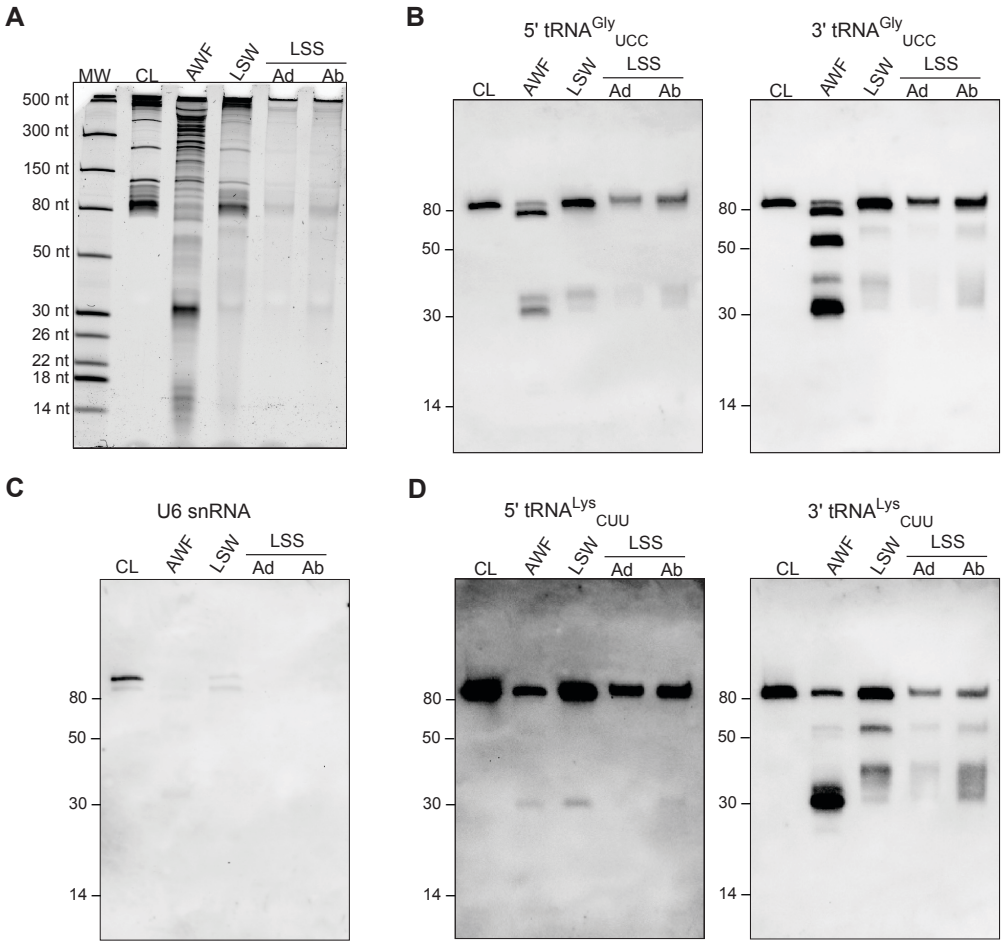

Fig. S15. Extracellular fractions derived from Leaf Surface Swab (LSS) are also enriched in tRNA-derived fragments compared to whole cell lysate. *A*) 100 ng of RNA from total CL, AWF, LSW and LSS (Ad, adaxial and Ab, abaxial) were separated on a 15% denaturing polyacrylamide gel and stained with SYBR Gold (Ad, adaxial: Ab, abaxial) (*B*) Upon blotting onto a positively charged nylon membrane, RNA was probed with DIG-labeled 5' and 3' probes against $tRNA^{Gly}$ and $tRNA^{Lys}$. (*C*) RNA was probed with a DIG-labeled probe against U6 snRNA as a control.

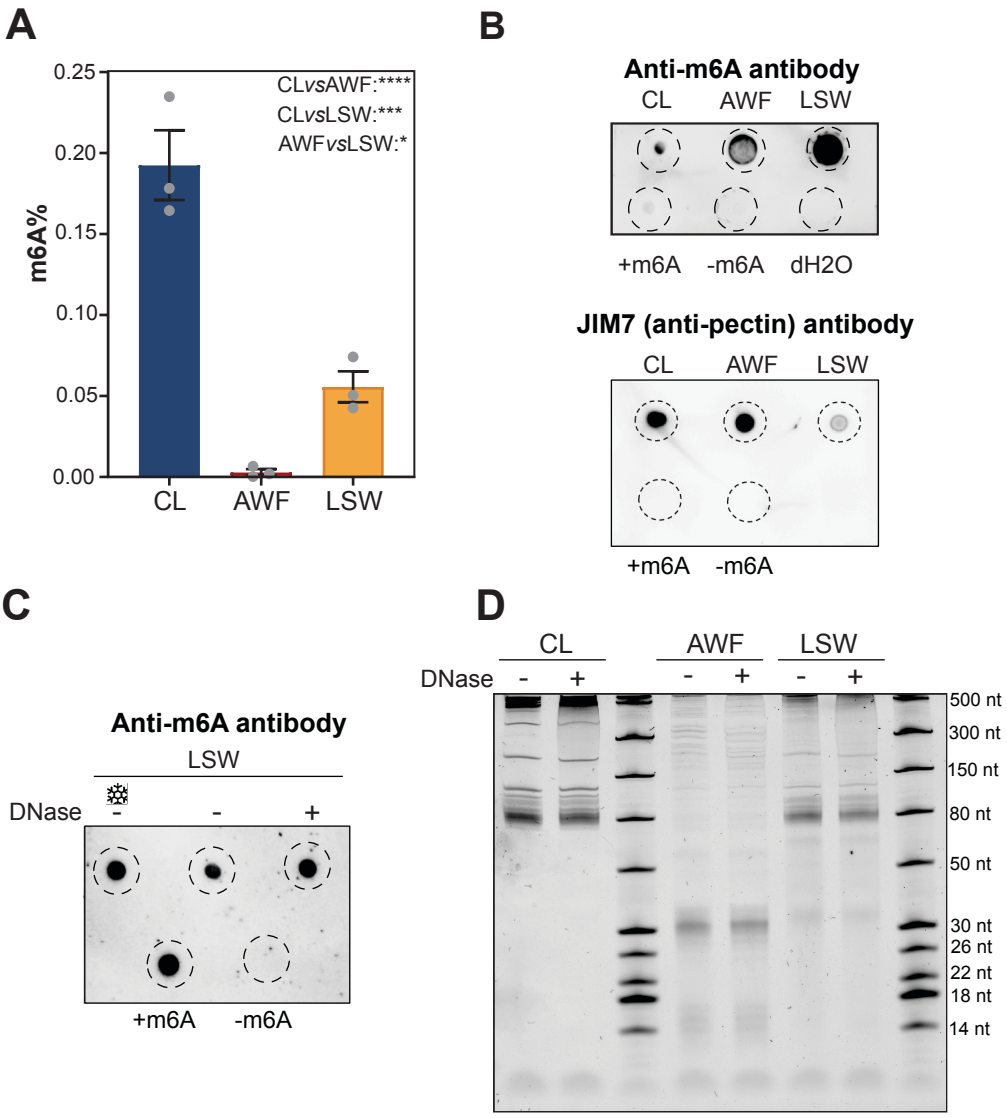

Fig. S16. exRNA is not enriched in m6A modification. (*A*) ELISA-based analysis of m6A content. Bar graph indicates the percentage of m6A modification in each sample assessed using an RNA-specific ELISA-based m6A modification kit. The values are calculated using a positive control with 100% m6A modification. Significant differences were calculated using one-way ANOVA and Fisher's LSD post-hoc test ****, P<0.0001; ***, P<0.001; *, P<0.05. (*B*) Dot blot analyses of m6A and pectin content. An aliquot of 100 ng of total CL, AWF, and LSW RNA was dot-blotted and then probed with the anti-m6A antibody or anti-pectin antibody (JIM7). For positive and negative controls, 100 ng of synthetic 21-nt RNAs with identical sequences except for a single m6A modification on the positive control were used. (*C*) The molecule being detected by the anti-m6A antibody in LSW is not DNA. LSW RNA was treated with DNase and then dot-blotted and probed with anti-m6A. No reduction in signal was observed. Snowflake indicates the sample was incubated on ice (*D*) DNase treatment does not impact banding pattern of RNA on polyacrylamide denaturing gels. The indicated RNA samples were treated with DNase or buffer alone and then imaged using PAGE and SYBR Gold.

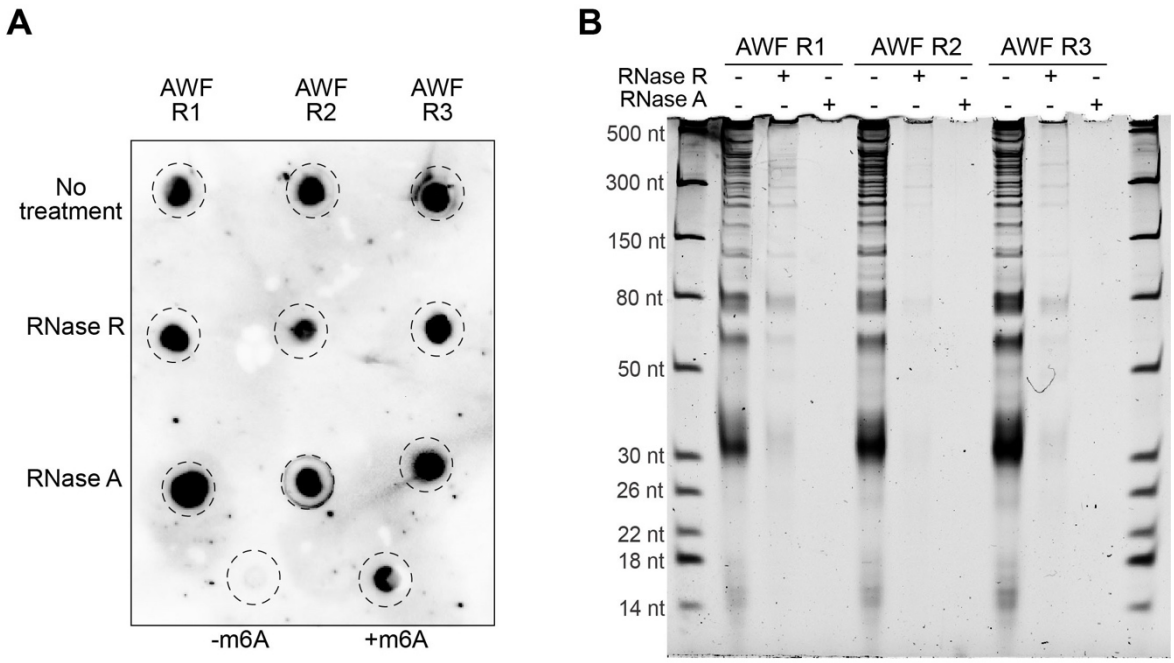

Fig. S17. m6A antibody cross reacts with some molecule other than RNA. *(A)* 200 ng of AWF RNA was either not treated or treated with RNase A or RNAse R, followed by two serial precipitations with ammonium acetate and EtOH to remove free nucleotides and other small degradation products. The pellets were resuspended in 8 µL of ultrapure water, and 6 µL were dot blotted and crosslinked onto a positively charged nylon membrane and then probed with an anti-m6A antibody. Three biological replicates (R1, R2 and R3) were analyzed. For positive and negative controls, 600 ng of synthetic 21-nt RNAs with identical sequences (except for a single m6A modification on the positive control) were used. *(B)* 2 µL of RNA left from A) were separated on a 15% denaturing polyacrylamide gel and stained with SYBR Gold nucleic acid stain.

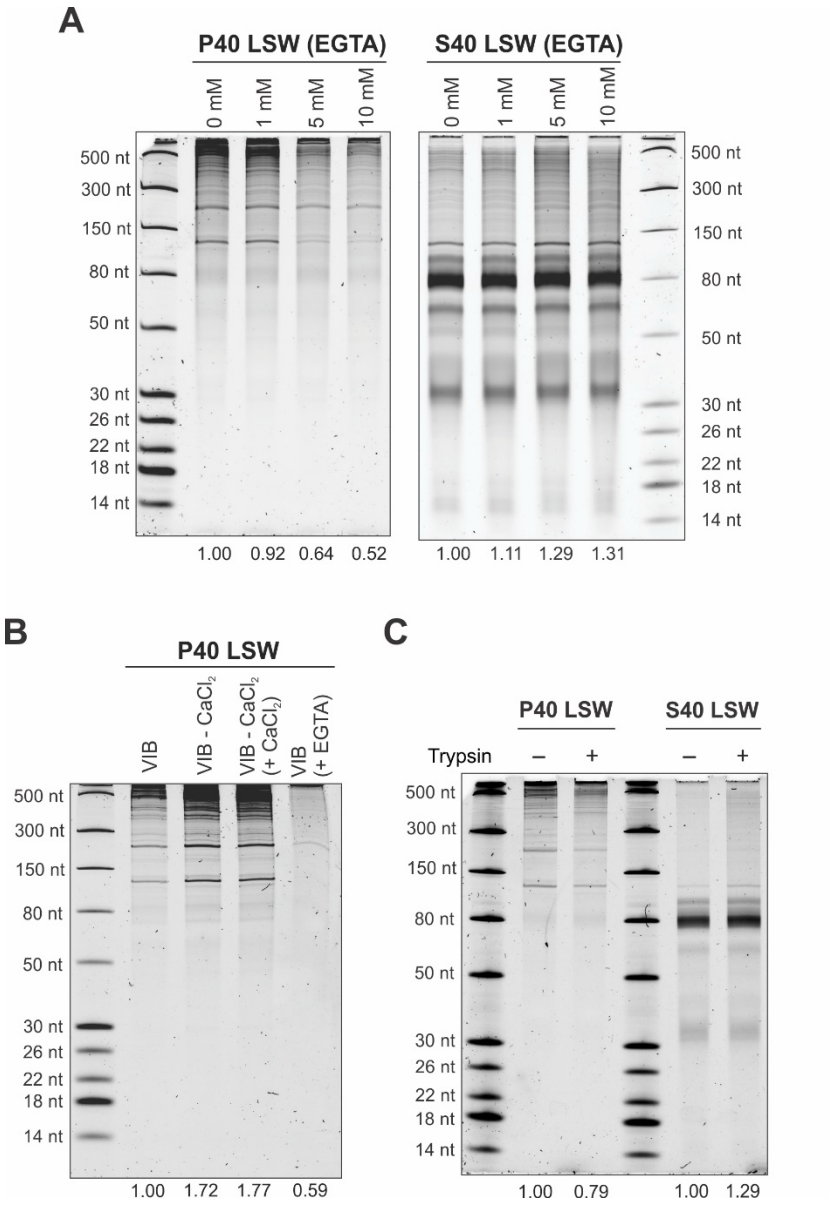

Fig. S18. Long RNAs in LSW form cation-dependent aggregates or condensates. (*A*) An independent biological replicate of the experiment presented in Fig. 6*C*. LSW was treated with increasing concentrations of EGTA as indicated and incubated on ice for 20 min, followed by ultracentrifugation at 40,000 *g*. RNAs were isolated from P40 pellets and their corresponding supernatants using TRIzol, separated on a 15% denaturing polyacrylamide gel, and stained with SYBR Gold. RNA abundance in each gel lane was estimated by densitometry using ImageJ and expressed relative to the 0 mM EGTA lane. (*B*) Gel showing the RNA profile of P40 pellets obtained from LSW fractions that were isolated using either VIB or VIB without $CaCl_2$ (VIB-$CaCl_2$). LSW samples were subjected to the treatments indicated in parentheses for 20 min on ice prior to ultracentrifugation at 40,000 *g*. RNA abundance in each gel lane was estimated by densitometry using ImageJ and expressed relative to the VIB lane. (*C*) An independent biological replicate of the experiment presented in Fig. 6*B.* LSW was mock treated (-) or treated with Trypsin (+) and incubated at 30 ºC for 1 h, followed by ultracentrifugation at 40,000 *g*. RNAs were isolated from P40 pellets and their corresponding supernatants (S40) and imaged by PAGE as described for panel *B.*

Table S1. sRNAseq Mapping Statistics

Sample	Ath	Ath to Ath		Ath to Zma		Zma	Zma to Zma		Zma to Ath	
	Raw	Reads	Percentage	Reads	Percentage	Raw	Reads	Percentage	Reads	Percentage
AWF_R1	54588510	46360594	84.93	22370091	40.98	39558857	31685415	80.10	6866591	17.36
AWF_R2	36136821	27084973	74.95	15405044	42.63	43541485	35578768	81.71	7874497	18.09
AWF_R3	41120112	29913448	72.75	16282470	39.60	39154904	30754285	78.55	7346166	18.76
LSW_R1	120280213	92310179	76.75	44339532	36.86	26513534	19747868	74.48	5544051	20.91
LSW_R2	167270102	125982430	75.32	63331471	37.86	25941714	17825598	68.71	6544415	25.23
LSW_R3	21444863	17455052	81.40	9100087	42.43	27045974	17488561	64.66	6956960	25.72

Ath: *Arabidopsis thaliana*
Zma: *Zea mays*

Table S2. Oligonucleotide sequences of hybridization probes

Probe ID	**Oligo sequence (5’ to 3’)**
$tRNA^{Gly}_{UCC}$ 5’	GCAATTATCCTAACCGTTGGACTACAGACGC
$tRNA^{Gly}_{UCC}$ 3’	TGCGTCTGCCGGGAGTCGAACCCGGG
$tRNA^{Lys}_{CUU}$5’	CTTGCGCTCTACCAACTGAGCTAGACGGGC
$tRNA^{Lys}_{CUU}$ 3’	CGCCCACCGTGGGGCTCGAACCCACGACCA
$tRNA^{Glu}_{CUC/UUC}$ 5’	GCCAGATATCCTAACCGCTRGACKAYAWCGGA
$tRNA^{Glu}_{CUC/UUC}$ 3’	CTCCGWTGCCGGGAMTCGAACCCGGGTCTC
$tRNA^{Ala}_{AGC/UGC/CGC}$ 5'	THTGAGCTACAYCCCC
$tRNA^{Ala}_{AGC/UGC/CGC}$ 3'	TGGAGRTGCGGGGDATCGAWCCCCGTRCCT
U6	AGGGGCCATGCTAATCTTCTC

SUPPLEMENTAL DATASETS

Dataset S1. Genes displaying differential transcript abundance between apoplastic wash fluid (AWF), leaf surface wash (LSW), and/or total cell lysate (CL)

Dataset S2. Gene Ontology analysis of differentially expressed genes in apoplastic wash fluid (AWF), leaf surface wash (LSW), and total cell lysate (CL)

Dataset S3. tRNA sources of tRNA-derived fragments identified in sRNAseq dataset

REFERENCES

1. Koster T*, et al.* (2014) Regulation of pri-miRNA processing by the hnRNP-like protein AtGRP7 in Arabidopsis. *Nucleic Acids Research* 42(15):9925-9936.
2. Rutter BD & Innes RW (2017) Extracellular vesicles isolated from the leaf apoplast carry stress-response proteins. *Plant Physiology* 173(1):728-741.
3. Zand Karimi H*, et al.* (2022) Arabidopsis apoplastic fluid contains sRNA- and circular RNA-protein complexes that are located outside extracellular vesicles. *The Plant Cell* 34(5):1863-1881.
4. Schneider CA, Rasband WS, & Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9(7):671-675.
5. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72:248-254.
6. Peterman TK, Ohol YM, McReynolds LJ, & Luna EJ (2004) PATELLIN1, a novel Sec14-like protein, localizes to the cell plate and binds phosphoinositides. *Plant Physiology* 136(2):3080-3094.
7. Zhang Z*, et al.* (2007) A SNARE-protein has opposing functions in penetration resistance and defence signalling pathways. *The Plant Journal* 49(2):302-312.
8. Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal* 17(1):10-12.
9. Langmead B & Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. *Nature Methods* 9(4):357-359.
10. Kozomara A & Griffiths-Jones S (2014) miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Research* 42(Database issue):D68-73.
11. Pertea M, Kim D, Pertea GM, Leek JT, & Salzberg SL (2016) Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nature Protocols* 11(9):1650-1667.
12. Love MI, Huber W, & Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15(12):550.
13. Benjamini Y & Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)* 57(1):289-300.
14. Wickham H (2009) *ggplot2: elegant graphics for data analysis* (Springer New York, NY).
15. Gebert D, Hewel C, & Rosenkranz D (2017) unitas: the universal tool for annotation of small RNAs. *BMC Genomics* 18(1):644.
16. Chan PP & Lowe TM (2009) GtRNAdb: a database of transfer RNA genes detected in genomic sequence. *Nucleic Acids Research* 37(suppl_1):D93-D97.
17. Chan PP & Lowe TM (2016) GtRNAdb 2.0: an expanded database of transfer RNA genes identified in complete and draft genomes. *Nucleic Acids Research* 44(D1):D184-D189.