Materials and Methods

Microscopy and live imaging.

RNA in situ hybridization and embryo clearings were observed with a transmission microscope under bright field or differential interference contrast with a 40x lens. For GFP imaging, live embryos or seedlings were mounted in 0.5 strength Murashige and Skoog (MS) liquid medium. SAMs were mounted in warm 0.8% low-gelling agar dissolved in 0.5 strength liquid MS. Confocal microscope observations were done on a SP2 or SP5 spectral detection confocal microscope (Leica) equipped with a 20x glycerol immersion (seedling root, seeds), 40x oil immersion (SAM) or 63x glycerol immersion lens (female gametophytes, embryos). Images were processed using Imaris (Bitplane, Zurich). Maximum partial projections of equivalent serial sections are shown. Fluorescence levels (*TCSn::GFP* and *DR5::tdTomato*) (*31*) were quantified from maximum intensity projections obtained from 6 embryos using ImageJ (http://rsbweb.nih.gov/ij/). Adult plants were recorded with a DP3 Merrill digital camera (Sigma), and flower primordia with a MZFLII fluorescence stereomicroscope equipped with a DFC 420C digital camera (Leica).

Plant material and growth conditions.

The ecotype Col0 was used as wild type. Seeds were surface sterilized for 18 min in 5 % (v/v) bleach and 0.1 % (v/v) triton-X, washed three times in sterile ddH_2O and kept in the dark at 4 °C for a minimum of 2 d for stratification. Seeds were sown on solid medium, containing 0.5 strength MS, 2 % (w/v) sucrose, 0.8 % (w/v) phytagar and 2 mM 2-(N-morpholino)ethanesulfonic acid (MES) pH 5.8, and the appropriate antibiotic or herbicide for selection. Seeds on selection plates were placed into a Percival plant incubator (CU-36L6/D Percival Scientific Inc., Perry IA, USA) with 22 °C and a 14/10 h light/dark regime with 120 mmol m^{-2} s^{-1}. To phenotype seedlings, plants were grown vertically on 12 cm square plates containing 10 mL of medium. To prevent desiccation of the plates, 1 mL of ddH_2O was added and the plates were sealed with parafilm. Seeds on vertical plates were placed into a Percival plant incubator (CU-36L6 Percival Scientific Inc., Perry IA, USA) with 22 °C and 12/12 h light/dark cycles with 90 mmol m^{-2} s^{-1}. To obtain adult plants, seedlings were transferred to soil and grown at 22 °C during the day, 20 °C at night with a 16/8 h photoperiod.

Plant transformation.

Plant transformation was performed using *Agrobacterium tumefaciens* of the GV3101 strain (32). Inducible expression constructs were supertransformed into *TCSn::GFP* or *PUP14::PUP14-GFP* transgenic lines.

Ethanol induction of transgenes

Ethanol was applied as follows to induce expression of *amiRPUP14*, *CKI1*, or *PUP14* from the ethanol-inducible two-component system (*33*). For phenotype assessments of seedlings, 1 mL of 1 % (v/v) ethanol was added to the bottom of the vertical plates 4 d after germination. For expression analyses, 7 d old seedlings grown on vertical plates were transferred to 6-well culture dishes with 3 mL of liquid medium (0.5 strength MS, 2 % (w/v) sucrose, 2 mM MES pH 5.8) with and without 1 % (v/v) ethanol) for 16 - 24 h. Dishes were sealed with parafilm. Induction in adult plants was by watering with 1 % (v/v) ethanol every 4 d starting from bolting stage as described (*33*). Embryo inductions were performed as described (*4*). Controls shown are *TCSn::GFP* treated with ethanol in parallel to the experimental genotypes. Similar results were obtained with untreated *TCSn::GFP, amiRPUP14* plants.

Constructs

For *PUP14::PUP14-GFP*, a DNA fragment encompassing the *PUP14* locus including the 2.3 kb upstream sequence was amplified from Col0 genomic DNA by PCR and cloned into the binary vector pCB302 (34) with the enhanced GFP coding sequence allowing for C-terminal fusions, the nopaline synthase 3-prime untranslated region, and an adaptor for ligation-independent cloning (LIC) (Genbank KX510271) (35). For protoplast experiments, *PUP14*, *CKX2* and *CKX7* genomic regions from translational start to stop were amplified from Col0 genomic DNA and annealed to LIC-modified expression vectors (see Table S2) to yield 2x hemagglutinin (HA) (Genbank KX510274), GFP (Genbank KX510273), or glycosyl phosphatidyl inositol (GPI)-anchored GFP C-terminal translational fusions (Genbank KX510275). *CKX2-GPI* was cloned by restriction digest of *CKX2-2HA* with PstI and StuI and annealing

of oligonucleotides encoding the GPI-anchor. The artificial microRNA (*amiR*) sequences were obtained through the Web MicroRNA Designer (wmd3.weigelworld.org), and assembled by PCR amplification on pRS300 as template as described (12) using LIC-modified primers A and B (see Table S2) to produce fold-back amiR precursors. The fold-back amiR precursors were cloned into the LIC-modified ethanol-inducible binary DM7 vector (5). The sequences of the base-pairing nucleotides of *amiRPUP14_1* and *amiRPUP14_2* are TTATTTGCACAAAGTGTTCTG and TGTTGATAGGTATTTGCACGA, respectively. Both *amiRPUP14* constructs caused similar phenotypes upon induction. Corresponding target sites in *PUP14* are CAGAACAATTTGTGCAAATAC and TTGTGCAAATACCTATCAACA for *amiRPUP14_1* and *amiRPUP14_2*, respectively. Sequence of the base-pairing nucleotides of *amiR19/20* is TTAAAACACGTCCTGCGACGA. Target sequences are TCGTAGCAGGACGTGTTTTAT in *PUP19* and TCGTAACAGGACGTGTTTTAT in *PUP20* amiR-resistant versions of *PUP14* (*PUP14**) were constructed by site-directed mutagenesis of the *amiRPUP14_2* target site to change all codons within the *amiR* target site in *PUP14* to synonymous codons with overall comparable codon usage frequency. The *PUP14** encompasses the *PUP14* genomic region and was cloned into pCB302 by LIC. Inserts for the *35S::PUP1* and *35S::PUP14* binary constructs used for expression in microsomes were amplified from Col0 genomic DNA, and cloned into pPLV26 (36) by LIC. For *PUP14ox*, the *PUP14* translated sequence was amplified from genomic Col0 DNA and ligated into LIC-modified DM7 (5). For *35S>ALC>PUP14-GFP*, *PUP14-GFP* was amplified from *PUP14::PUP14-GFP* and ligated into LIC-modified DM7 (5). All constructs were sequenced to ensure no unwanted mutations were introduced. A plasmid list is provided in Table S2.

Protoplast isolation and transfection

For transient expression experiments, protoplasts of three- to four-week-old wild-type Arabidopsis plants of the Col0 ecotype were isolated as described (*37*) with the following adaptations: adjusted concentration was 3 x 10^5 ml^{-1}, *35S::renillaLUC* was used to normalize for transfection efficiency (*38*) of *TCS::LUC* reporter assays, and WI solution was supplemented with 15 mM sucrose. For reporter assays, transfected protoplasts were incubated over night, DCPIP (2,6-Dichloroindophenol sodium salt hydrate) at 5 µM was added as electron acceptor (*6*), as well as tZ or solvent at indicated concentrations, and protoplasts were harvested 60 min later for LUC measurements. To obtain crude protein extracts, 3 x 10^5 transfected protoplasts were lysed in 200 µl extraction buffer (*6*) with 1.2 % (v/v) plant protease inhibitor mixture (Sigma P9599) and incubated for 5 min at room temperature. The samples were then centrifuged at 21'000 rcf for 5 min at room temperature. Supernatants were transferred to a new tube. 25 µl were added to 3 x 10^4 transfected protoplasts. For transport assays, transfections were scaled up according to needs and purified plasmids were transfected in 1:1 ratio between effector and empty plasmid. Transfected protoplasts were cultivated between 12 and 24 hrs at 22 °C in light (120 mmol m^{-2} s^{-1}). Means and standard error of means of at least two independent experiments with three technical replications each are represented.

In situ hybridizations

mRNA in situ hybridizations were performed as described (*4*). Probe templates for *AHK4* and *PUP14* were synthesized by PCR from Col0 genomic DNA using the following primers: T3_AHK4_f: aattaaccct-cactaaagGATCATCACCCGCAACTCTC, T7_AHK4_r: taatacgactcactatagGATCAACACTGAACCGTC-GTC, T3_PUP14_f: aattaaccctcactaaagATTCTTCAACCACACGCATGAAC, T7_PUP14_r: taatacgactcac-tatagACCAAAGCTGTTACACACTTACAC. T3 and T7 RNA polymerase promoter sequences are indicated in lower case font.

Transport assays

For protoplast transport assays, protoplasts were harvested at 100 rcf for 2 min and re-suspended in percoll solution (0.5 M Sorbitol, 1 mM $CaCl_2$, 20 mM MES pH 5.8, 25 % (v/v) percoll) and mixed with the same volume of glycine betaine solution (0.5 M glycine betaine, 1 mM $CaCl_2$, 20 mM MES pH 5.8) containing ^{14}C- labelled tZ and ^3H_2O. The final concentration of labelled tZ was 1 or 2 µM. For competition studies, unlabeled cold substrate was added in a 100- fold excess. Transport was stopped by centrifugation of samples on a percoll cushion after indicated time points. For scintillation counting, pelleted protoplasts were transferred into 3 mL of Ultima Gold™ (PerkinElmer AG, Schwerzenbach, Switzerland) and subjected to 10 min of disintegration counting of ^{14}C and ^3H. Three independent replicates of the uptake experiment

were conducted with similar results, and means with standard deviations from one representative experiment with four technical replications are shown. For competition assays, mean values from three independent experiments with each four technical replications are shown. Indicated relative uptake was calculated as the radioactivity of ^{14}C per radioactivity of $^{3}H_2O$ normalized to the first time point (30 s). For seedling transport assays, twelve-day old induced seedlings were transferred to liquid medium containing 0.5 MS, 2 % (w/v) sucrose, 2 mM MES and vacuum infiltrated for 5 min and twice 3 min. For each replicate > 10 mg of plant material was used. Radiolabelled tZ was added to a final concentration of 2 µM in 2 mL. Seedlings were washed after indicated time points with excess volumes of cold 0.5 strength MS, 2 % (w/v) sucrose, 2 mM MES on a Büchner funnel. Seedlings were dried on filter paper and transferred to 1.5 ml tubes containing 800 mL of 80 % (v/v) ethanol and heated for 5 min at 95 °C. Samples were transferred into scintillation vials containing 3 mL Ultima Gold™ (PerkinElmer) and subjected to 2 min disintegration counting of ^{14}C. Indicated relative uptake was calculated as the radioactivity per fresh weight normalized to the radioactivity per fresh weight at the first time point (1 min). Mean values from 3 independent experiments with each 4 technical replications are shown. For microsomal uptake experiments, *35S::PUP1*, *35S::PUP14* and *35S::GFP* were transiently expressed in *N. benthamiana* leaf tissue by *Agrobacterium tumefaciens*-mediated transfection and microsomes were prepared as described (*39*). For tZ-uptake experiments, ^{14}C-labelled tZ was diluted into transport buffer (10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 10 % sucrose, pH 7.6 with or without 5 mM ATP) and added to 300 µg of microsomes to yield a final concentration of 1µM labelled tZ. For substrate competition assays unlabelled substrate was included in the transport buffer at a 100-fold excess. After 10 s and 4 min of incubation at 20 °C, aliquots of 100 µL were vacuum-filtered on Whatman™ NC45 filters (GE Healthcare, Little Chalfont, UK) and washed 3 times with 1 mL cold ddH_2O. Air-dried filters were objected to scintillation counting as described above. Indicated relative uptake was calculated as the radioactivity normalized to the first time point (10 s). Means and standard error of means of at least four independent experiments with three technical replications each are represented.

qRT PCR analysis

Quantification of relative gene expression was done by qRT-PCR on an Applied Biosystems 7500 Fast Real- Time PCR System using SYBR® Green PCR Master Mix (Applied Biosystems, Life Technologies Europe B.V., Zug, Switzerland) or SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad Laboratories AG, 1785 Cressier, Switzerland) according to manufacturer's recommendation. Final primer concentrations were 400 nM in a total volume of 20 µl. The relative values of the transcripts were normalized to *EUKARYOTIC TRANSLATION INITIATION FACTOR 4A* (eIF4A, *At3G13920*) levels or to At2G32170. Fold changes were calculated by the $2^{-\Delta\Delta Ct}$ method (*40*). Means and standard error of means of at least three independent experiments with three technical replications each are represented.

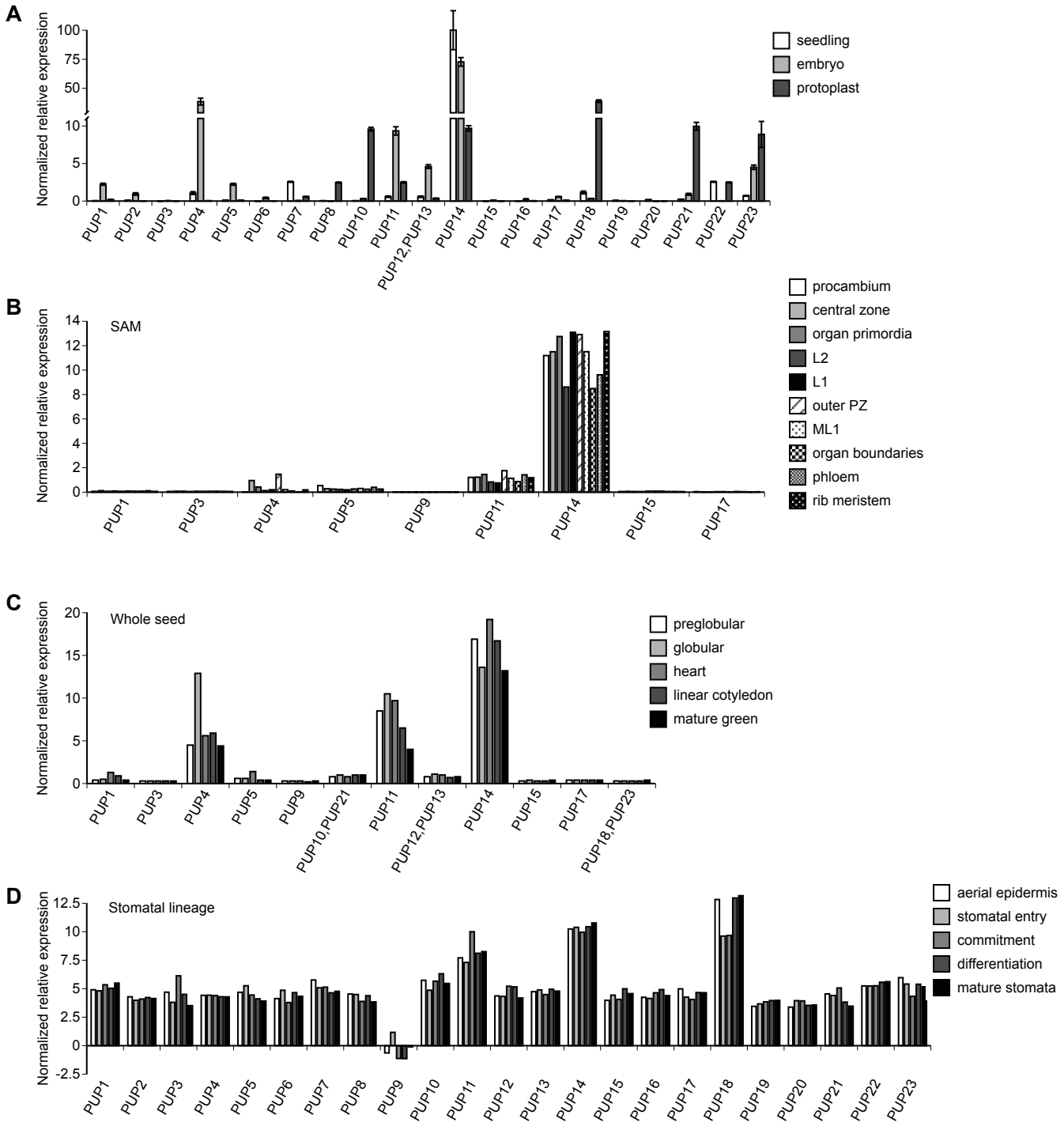

Fig. S1 Conspicuous *PUP14* expression in different developmental contexts.

(A) Transcription profile of all *PUP* family members in seedlings, embryos and mesophyll protoplasts determined by qRT-PCR. (B) Cell type-specific ATH1-based microarray dataset of *PUPs* in the SAM. L1 = layer 1 in SAM, L2 = layer 2, ML1 = layer 1 in meristem and in differentiating organs, PZ = peripheral zone (*11*). (C) ATH1 based microarray dataset of PUPs during seed development (*10*) (D) Stage-specific RNA-seq dataset of *PUPs* in stomatal lineage (*9*). *PUP* AGI identifying numbers: *PUP1*, AT1G28230; *PUP2*, AT2G33750; *PUP3*, AT1G28220; *PUP4*, AT1G30840; *PUP5*, AT2G24220; *PUP6*, AT4G18190; *PUP7*, AT4G18197; *PUP8*, AT4G18195; *PUP9*, AT1G18220; *PUP10*, AT4G18210; *PUP11*, AT1G44750; *PUP12*, AT5G41160; *PUP13*, AT4G08700; *PUP14*, AT1G19770; *PUP15*, AT1G75470; *PUP16*, AT1G09860; *PUP17*, AT1G57943; *PUP18*, AT1G57990; *PUP19*, AT1G47603; *PUP20*, AT1G47590; *PUP21*, AT4G18220; *PUP22*, AT4G18205; *PUP23*, AT1G57980.

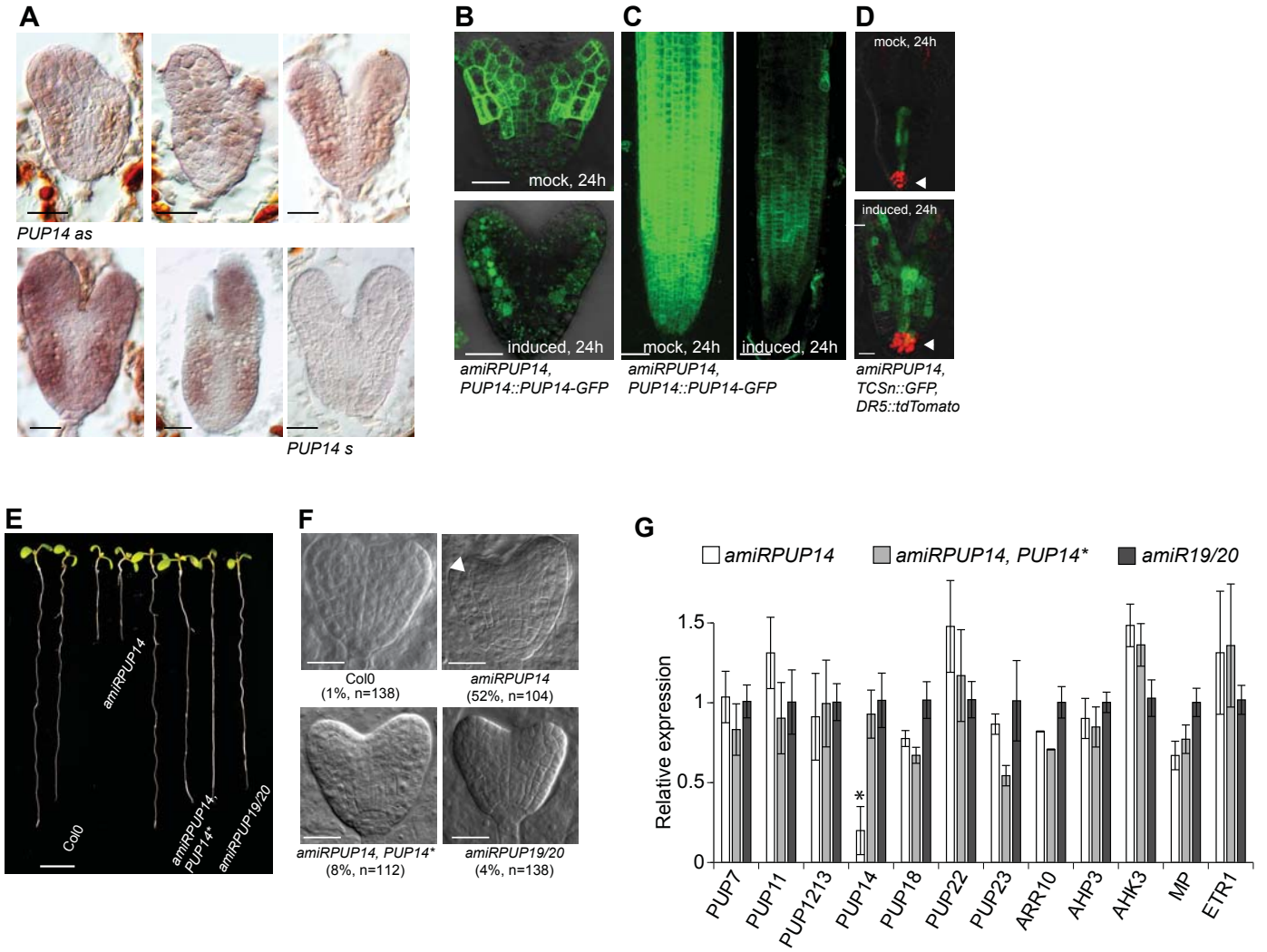

Fig. S2 *amiRPUP14*-induced phenotypes are specific to *PUP14*.

(A) Localization of *PUP14* mRNA in heart and torpedo stage embryos by *in situ* hybridization. First five panels show antisense (as) probe, last panel sense (s) control. (B,C) Decreased PUP14-GFP levels in (B) heart-stage embryos and (C) the seedling root tip after 24 h of *amiRPUP14* induction compared to mock, n=6. (D) *amiRPUP14* affects cytokinin but not auxin response, as shown by unchanged *DR5::tdTomato* (*31*) expression in *amiRPUP14*-induced embryos (arrowheads). Relative fluorescence of tdTomato not affected (unpaired t-test, $p < 0.01$, $n = 5$). (E) Comparison of root growth in Col0, *amiRPUP14*, *PUP14**-complemented and *amiRPUP19/20* seedlings after ethanol induction. (F) Comparison of ethanol-treated embryos of Col0, *amiRPUP14*, *PUP14**-complemented and *amiRPUP19/20* seedlings. (G) Relative transcript levels of *PUP* and unrelated genes in *amiRPUP14; amiRPUP14*, *PUP14** seedlings compared to *amiRPUP19/20*, all ethanol-treated, error bars denote s.e.m. *$P < 0.05$ with one-way analysis of variance with Tukey' HSD post hoc test. Scale bars (A,B,D,F) 20 µm, (C) 50 µm, (E) 1cm.

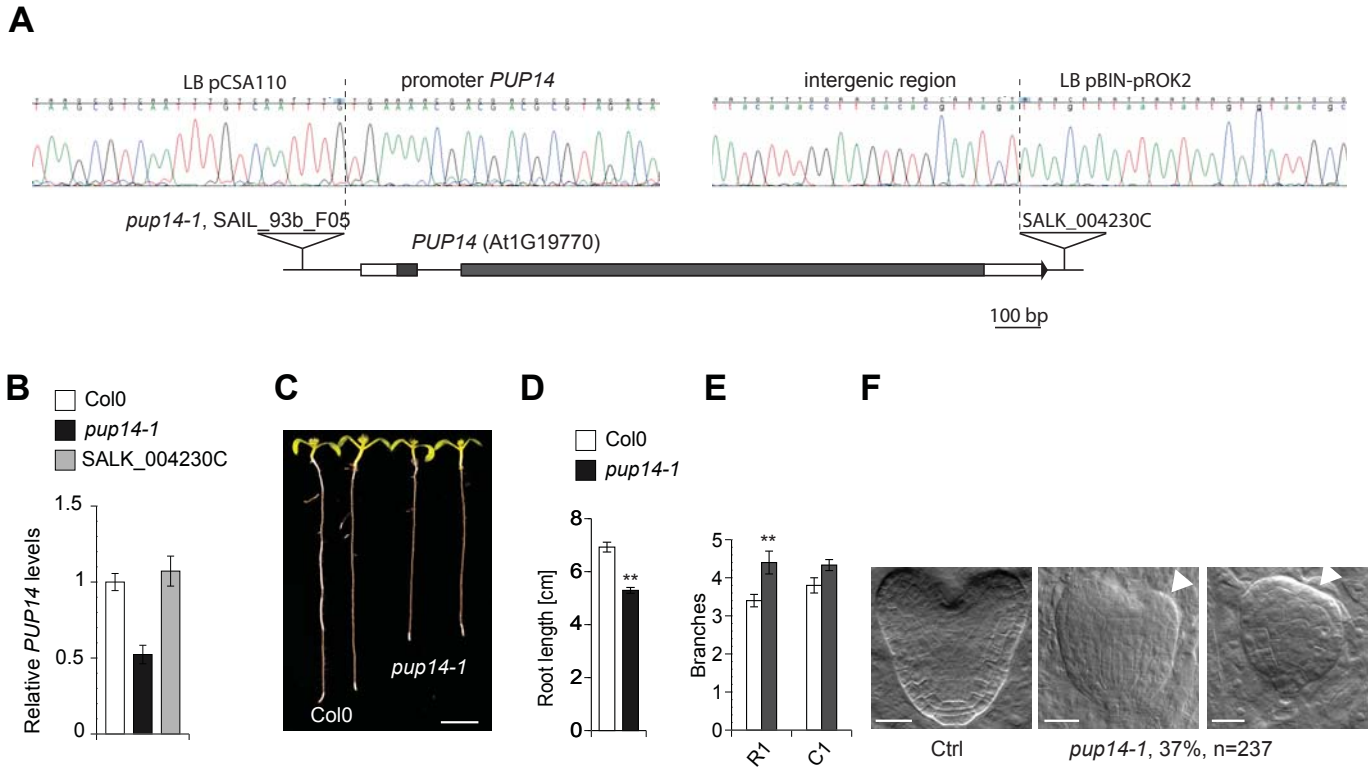

Fig. S3 T-DNA insertion lines at *PUP14* locus.

(A) Sequences of insertion sites of *pup14-1* (SAIL_93b_F03) and SALK_004230C T-DNA insertion lines and schematic representation of insertion sites on gene level. (B) *PUP14* transcript levels in Col0, *pup14-1*, *SALK_004230C*. (C) Root phenotype of *pup14-1* compared to Col0. (D) Quantification of root length of *pup14-1* compared to Col0. (E) Comparison of branch numbers, RI = rosette branches, CI = primary cauline branches, n = 6, **P < 0.01 one-sided *t* test. (F) *pup14-1* embryos show morphological defects in cotyledons and root meristems compared to non-affected siblings from the same silique. Error bars denote s.d. Scale bars 1 cm (C), 20 µm (F).

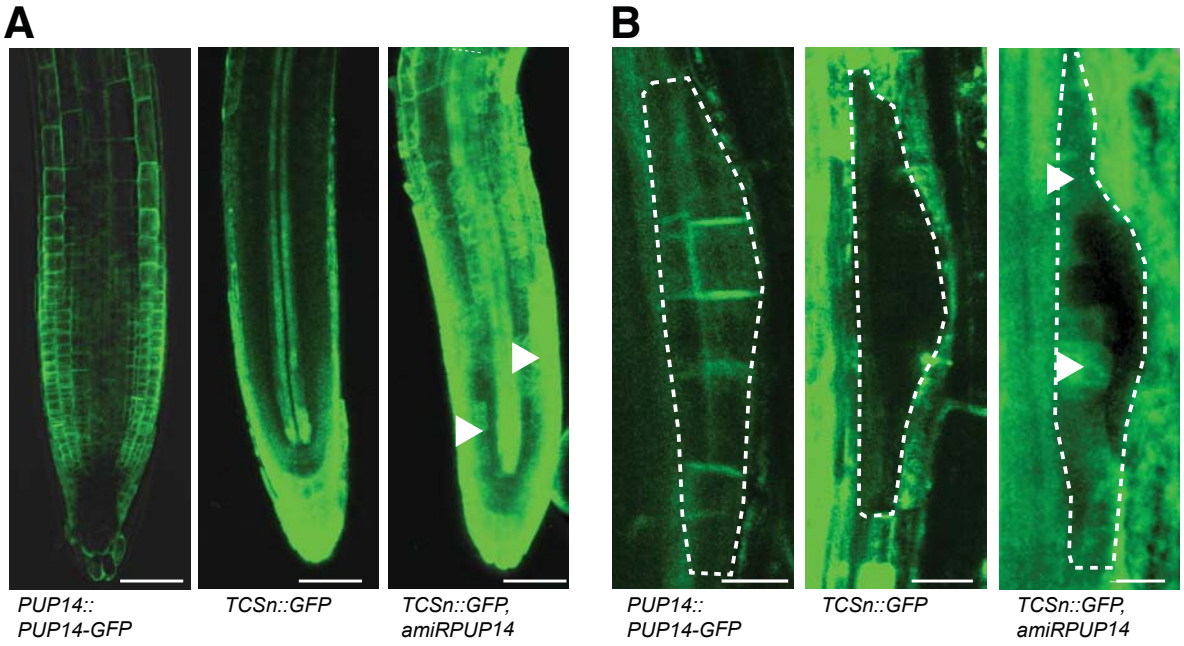

Fig. S4 *PUP14* confines the cytokinin response in seedling roots.

(A) *PUP14::PUP14-GFP* in main root. *TCSn::GFP* in the main root. Ectopic *TCSn::GFP* after 16 h *amiRPUP14* induction (arrowheads) (80 % of roots, n =1 5) roots. (B) LRP, denoted by dotted line. *PUP14::PUP14-GFP*, *TCSn::GFP*, ectopic *TCSn::GFP* (arrowheads) 16 h after *amiRPUP14* induction. Scale bars (A) 50 µm, (B) 10 µm.

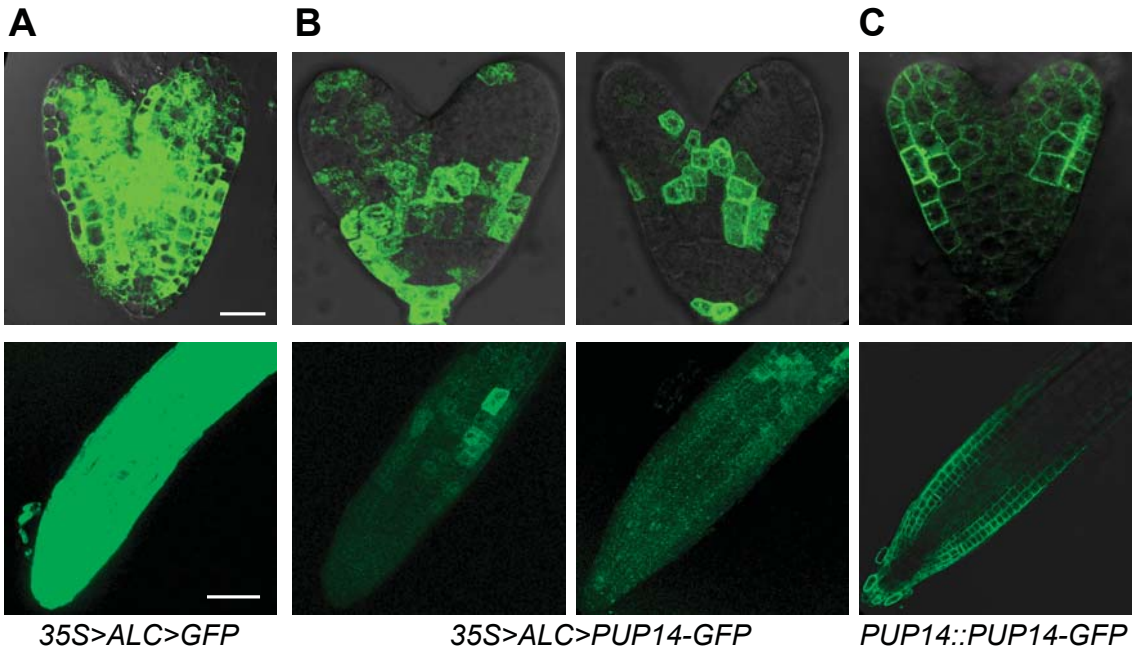

Fig. S5 Ectopic *PUP14-GFP* expression is not well tolerated by plants.

Heart-stage embryos (top panels) and main root apices of 7 d old seedlings (bottom panels) showing (A) 24 h ethanol-induced *35S>ALC>GFP* overexpression, (B) 24 h ethanol-induced *35S>ALC>PUP14-GFP* overexpression and (C) *PUP14::PUP14-GFP* expression. Scale bars 20 µm (top panel), 50 µm (bottom panel).

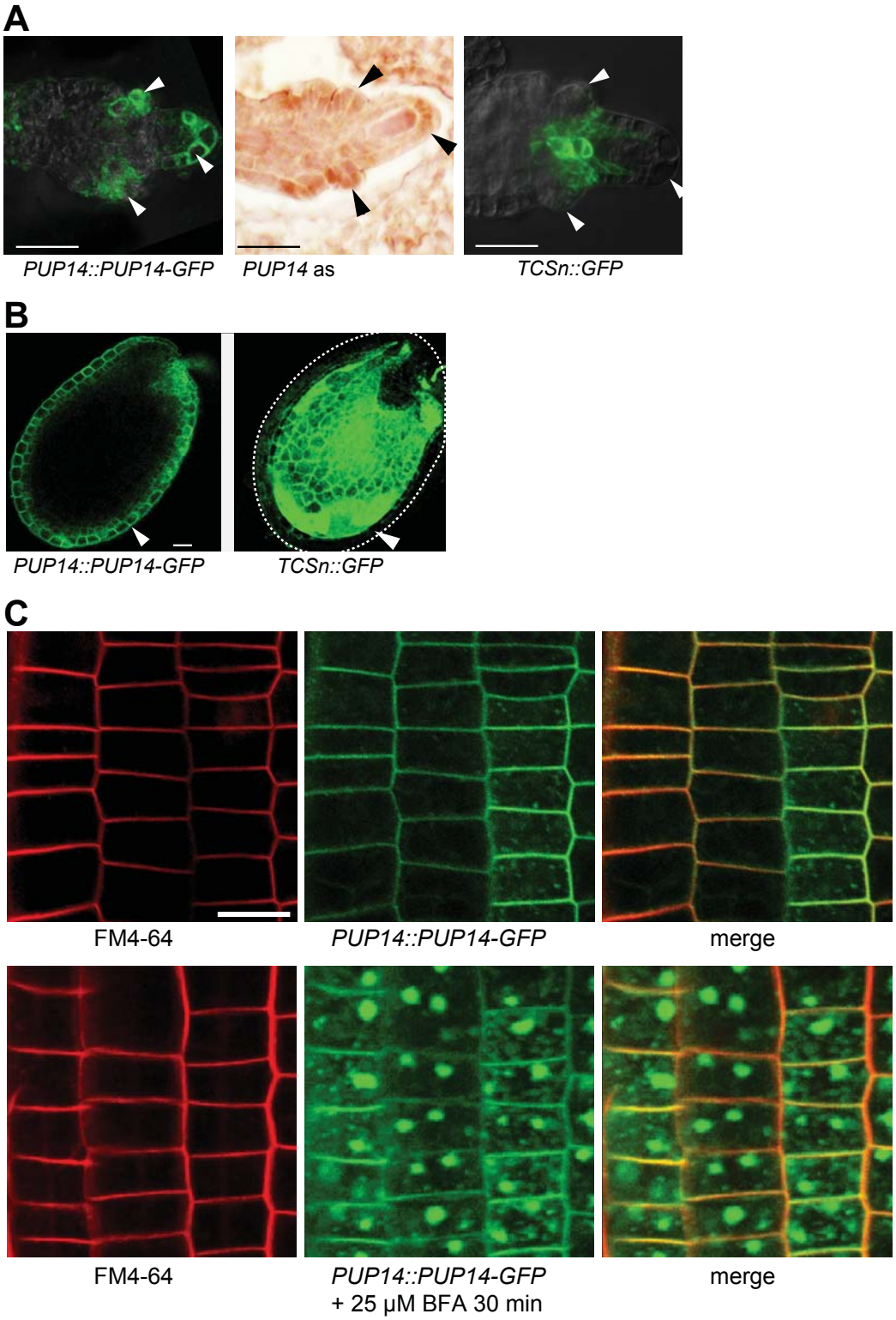

Fig. S6 *PUP14* expression and subcellular localization.

(A,B) *PUP14* expression and cytokinin signaling output in (A) the developing ovule primordium and (B) the seed, detected by reporter transgenes as indicated. In addition, *PUP14* mRNA is detected by in situ hybridization with *PUP14* as RNA probe in (A). Dotted lines delimit the seed coat. Arrowheads indicate peak PUP14-GFP expression levels. (C) PUP14-GFP subcellular localization and trafficking in main root. *PUP14-GFP* colocalization with plasma membrane marker dye FM4-64 (top panels). Addition of the fungal toxin brefeldin A (BFA) causes accumulation of PUP14-GFP signal in vesicles (bottom panels). Scale bars (A,C) 10 µm, (B) 20 µm.

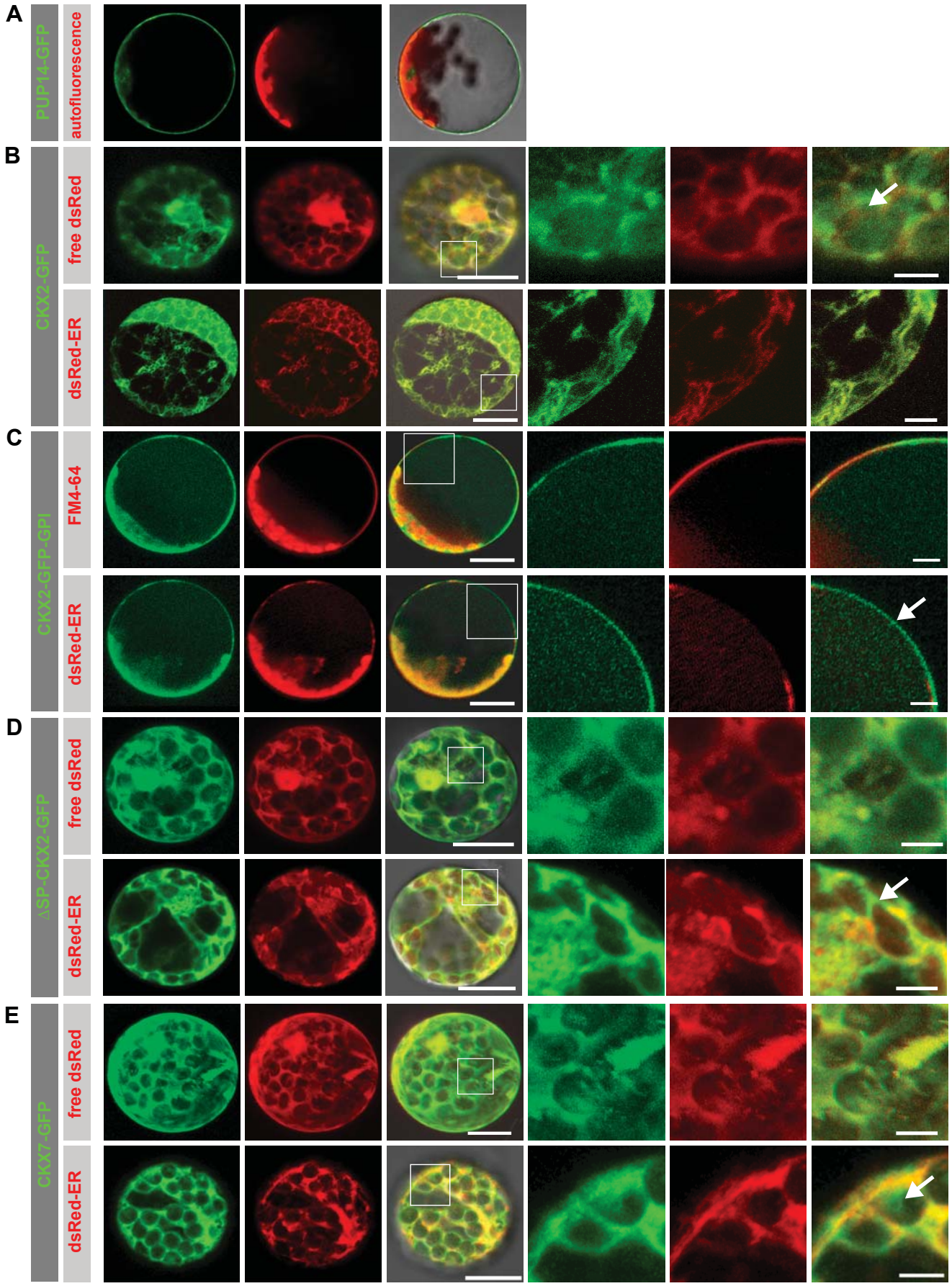

Fig. S7 Subcellular co-localizations of differentially targeted CKX-GFP variants in protoplasts.

(A) Localization of *PUP14-GFP* and (B-E) co-localization of GFP-tagged *CKX* gene products with subcellular markers in protoplasts as indicated. ER labelling with *ER-dsRed*, labeling of cytoplasm with free *dsRed* (*41*), and plasma membrane staining with the lipophilic dye FM4-64. Boxes indicate areas of the enlargement shown to the right. Arrows point to non-overlapping signals. Scale bars 20 µm, in enlargements 5 µm.

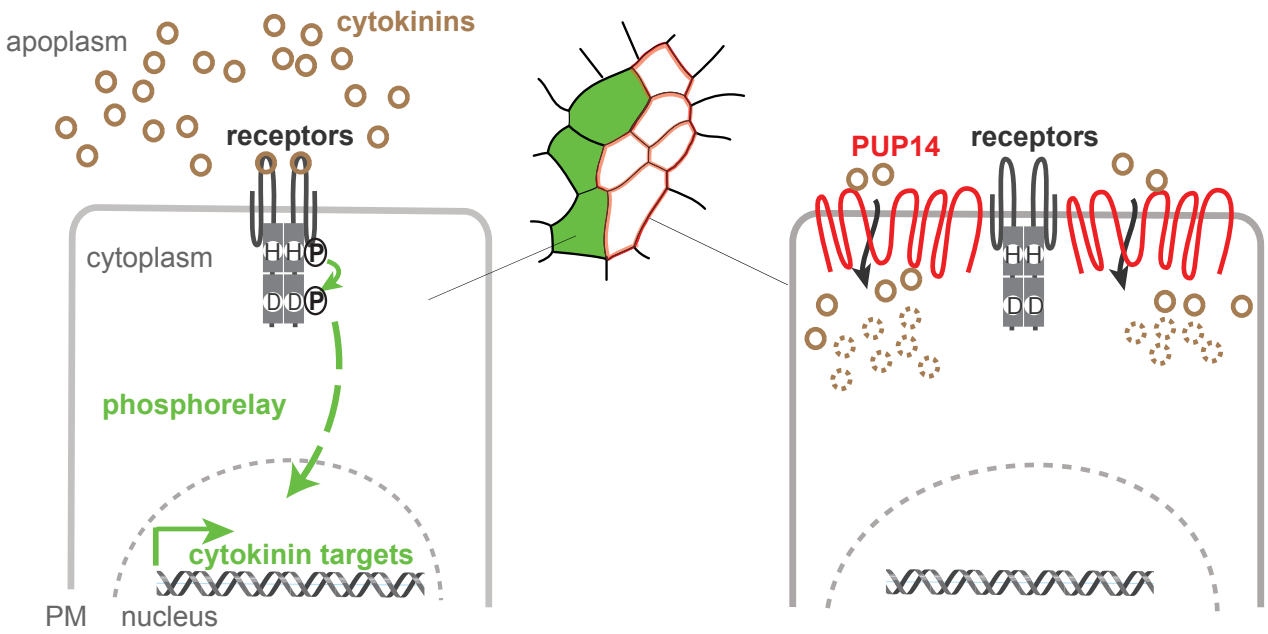

Fig. S8 Model of *PUP14* function in cytokinin signaling.

PUP14 (red) causes the translocation of apoplastic cytokinins to the cytosol, where they are converted to inactive forms (dotted circles). This results in reduced binding to plasma membrane-localized cytokinin receptors, and consequently reduced signaling activation (green denotes cytokinin signaling activity). PM: plasma membrane.

Target gene	Forward primer sequence 5‘ to 3‘	Reverse primer sequence 5‘ to 3‘
eIF4a	TCATAGATCTGGTCCTTGAAACC	GGCAGTCTCTTCGTGCTGAC
ARR5	GGTTGGATTTGAGGATCTGAAG	TCCAGTCATCCCAGGCATAG
ARR6	TTGCCTCGTATTGATAGATGTCTT	CCGAGAGTTTTACCGGCTTC
ARR7	AGATTAAGGAATCTTCAGCATTCAG	CTGCTAGCTTCACCGGTTTC
mGFP	TCAAGGACGACGGGAACTAC	ATCCTGTTGACGAGGGTGTC
PUP1	TGTTTCCGGGAGAAGTTTCA	CGGATTTAAACTCGCCGTAG
PUP2	TCTGTGCATCGTCTCTGGTC	TCTCCTGGAAGCAAATGACG
PUP3	AATACCCGAGACGAGAGACG	CGTCTCTCGTCTCGGGTATT
PUP4	ACCGGAGGTATCTGCATGAC	CACTCCACCAAACACGTCAC
PUP5	TGCAGTCACGTTTCAACTGG	TGACTGTGGATGCCAGAAAC
PUP6	TGCCTGTTCTTGCTGTTGTC	TCTTGGTCTTCTTCTGGCTTTC
PUP7	TTTAGCTATCTGCGGCTTCC	GTGTGACCTTCCTCAACAGG
PUP8	GTCGTGGGACTGATCTTTGAG	GCAATCCCACAGCAGTTATG
PUP10	ACCCACCAGAAGCAGAAGAG	GTAAACTGCGGGACAGCATC
PUP11	TCGACGTATTCGCTCATTTG	GCGGAGAACGACAAGAGAAC
PUP12/13	AGGTTAAGATGGTGGCGATG	TGAGCTTCTCGTGCTCTTTG
PUP14	TCTGTTTCGAGCGTGTTGTC	GCGCTTAAGACGGCAGTAAC
PUP15	GCAGCTGCTCTTAGCGTCTC	TTGTGGATTGGTCATCATCG
PUP16	GTCCGGTTTATTCGCTGATG	AGCACCTCTTCTCTGCCAAC
PUP17	GGCCTAGAATTGGTGCTTTG	TTTGGTTAAGTTCCGCCATC
PUP18	TGCTTTATGTTTCGGGTGTG	CAAAGCCACAAGTGGTGAAG
PUP19	CTGGTAGCTGGGATTCTTGG	AGTTGTTTGGCTTTGGCTTG
PUP20	TTTAGGGCTTGTGGGTCTTG	GCTCCTCCCTTAAACCATCC
PUP21	TTGCACAGGACTGATCTTCG	TGACAGCCAGGATAGGAACC
PUP22	ATCTTGACTTTGGCCTCAGC	GCAGTCCCACAGCAGTTATG
PUP23	TGTGTGCTTCACCACTTATGG	AGCACCAATTCTAGGCCAAC
AT2G32170	TGCTTTTTCATCGACACTGC	CCATATGTGTCCGCAAAATG
AHK3	GGTGGAGTTGGCAGTATACATATC	CGAAACCTCCCAGGATCAC
AHP3	TCTCAGAACTATGAAGGGTGTGTG	AATGTCTTGTACTCAATATCCACTTGC
ARR10	GACACAGGAACAGAGCCAATC	TATGCATGTTCCGAGTGAGC
ETR1	GAGAAGCTCGGGTGGTAGTG	TTTCCAAGACCATCGCTCTC
ARF5/MP	CCCTTCTTCACTCATCTGCTG	TCCATGGGAAGAGTTTGTGG

Table S1.

qRT-PCR primer sequences used in this study.

Name	Purpose	Parent vector	Selection (bacteria/plants)	Insert		
				Primer name	Sequence 5‘ to 3‘	Template
35S>ALC>amiRPUP14_1	Ethanol-inducible binary vector with amiR specific for *PUP14* (variant 1)	*DM7-LIC* (5)	Kan/Kan	LIC-OLIGO_A	tagttggaatgggttcgaaCGACGTTGTAAAACGACGG-CCAG	pRS300
				LIC-OLIGO_B	ttatggagttgggttcgaaCTCGGAATTAACCCT-CACTAAAGG	
				amiR_14_1_I	gaTTATTTGCACAAAGTGTTCTGtctctcttttgtattcc	
				amiR_14_1_II	gaCAGAACACTTTGTGCAAATAAtcaaagagaatcaatga	
				amiR_14_1_III	gaCAAAACACTTTGTCCAAATATtcacaggtcgtgatatg	
				amiR_14_1_IV	gaATATTTGGACAAAGTGTTTTGtctacatatatattcct	
35S>ALC>amiRPUP14_2	Ethanol-inducible binary vector with amiR specific for *PUP14* (variant 2)	*DM7-LIC* (5)	Kan/Kan	LIC-OLIGO_A	tagttggaatgggttcgaaCGACGTTGTAAAACGACGG-CCAG	pRS300
				LIC-OLIGO_B	ttatggagttgggttcgaaCTCGGAATTAACCCT-CACTAAAGG	
				amiR_PUP14_2_I	gaTGTTGATAGGTATTTGCACGAtctctcttttgtattcc	
				amiR_PUP14_2_II	gaTCGTGCAAATACCTATCAACAtcaaagagaatcaatga	
				amiR_PUP14_2_III	gaTCATGCAAATACCAATCAACTtcacaggtcgtgatatg	
				amiR_PUP14_2_IV	gaAGTTGATTGGTATTTGCATGAtctacatatatattcct	
35S>ALC>amiRPUP19/20	Ethanol-inducible binary vector with amiR specific for *PUP19 and PUP20*	*DM7-LIC* (5)	Kan/Kan	LIC-OLIGO_A	tagttggaatgggttcgaaCGACGTTGTAAAACGACGG-CCAG	pRS300
				LIC-OLIGO_B	ttatggagttgggttcgaaCTCGGAATTAACCCT-CACTAAAGG	
				amiR_19/20_I	gaTTAAAACACGTCCTGCGACGAtctctcttttgtattcc	
				amiR_19/20_II	gaTCGTCGCAGGACGTGTTTTAAtcaaagagaat-caatga	
				amiR_19/20_III	gaTCATCGCAGGACGAGTTTTATtcacaggtcgtgatatg	
				amiR_19/20_IV	gaATAAAACTCGTCCTGCGATGAtctacatatatattcct	
PUP14::PUP14-GFP	Reporter	*pCB302 LIC GFP* (Genbank KX510271)	Kan/Basta	PUP14_LICF-F	tagttggaatgggttcgaGCTTCTGCAGTGAAA-GATGTGTT	Col0 genomic
				PUP14_LIC_ GFP302_R	tattggagttgggttcgaaTAAGCCATACGATTGTCTT-TGTG	
hbt::PUP14-GFP; 35S::PUP14	Protoplast expression vector; binary vector for expression in microsomes	*hbt::LIC-GFP*; *pPLV26* (34)	Amp; Kan/Kan	PUP14_LIC_F	tagttggaatgggttcgaATCCATGGCTCAGAATCAA-CAAC	Col0 genomic
				PUP14_LIC_R	ttatggagttgggttcgaaATAAGCCATACGATTGTCTT-TGTG	
PUP14 pCB302	*PUP14* genomic region in binary vector	*pCB302 LIC* (Genbank KX510272)	Kan/Basta	PUP14_LICF-F	tagttggaatgggttcgaGCTTCTGCAGTGAAA-GATGTGTT	Col0 genomic
				PUP14_ LIC3prime_R	ttatggagttgggttcgaaGCACACTTCCAAACATTTTCA	
*PUP14**	*amiRPUP14_2*-resistant *PUP14* in binary vector	*PUP14 pCB302*	Kan/Basta	amiR14_2R* F	CTCTGTTTCTTTTTGCAGAACAATTTGTCCA-GATTCCAATAAATA	*PUP14 pCB302*
				amiR14_2R* R	GGTTGAAGAATCACGCTCGATATTTATTGG-AATCTGGACAAATTG	
35S>ALC>PUP14	Ethanol-inducible binary vector (3)	*DM7-LIC* (5)	Kan/Kan	PUP14_LIC_f	tagttggaatgggttcga ATCCATGGCTCAGAATCAA-CAAC	Col0 genomic
				PUP14_LIC_r	ttatggagttgggttcgaa ATAAGCCATACGATTGTCTT-TGTG	
35S>ALC>PUP14-GFP	Ethanol-inducible binary vector (3)	*DM7-LIC* (5)	Kan/Kan	PUP14_LIC_f	tagttggaatgggttcga ATCCATGGCTCAGAATCAA-CAAC	*PUP14::PUP14-GFP*
				GFP_LIC_r	tattggagttgggttcga TTACTTGTACAGCTCGTCCATGC	
35S::PUP1	Binary vector for expression in microsomes	*pPLV26* (36)	Kan/Kan	PUP1_LIC_F	tagttggaatgggttcgaa ACAGCAAGCAGCAAGAAGAA	Col0 genomic
				PUP1_LIC_R	ttatggagttgggttcgaa AGCAACATAATCACTAACAGG-AAG	
hbt::CKX2-HA; hbt::CKX2-GFP	Protoplast expression vector	*hbt::LIC-HA* (Genbank KX510274), *hbt::LIC-GFP* (Genbank KX510273)	Amp	CXX2_LIC_f	tagttggaatgggttcgaaTAAACAAATGGCTAATCTT-CGTT	Col0 genomic
				CXX2_LIC_r	ttatggagttgggttcgaaGATGTCTTGCCCTGGAGATAA-CA	
hbt::ΔSP-CKX2-HA; hbt::ΔSP-CKX2-GFP	Protoplast expression vector	*hbt::LIC-HA* (Genbank KX510274), *hbt::LIC-GFP* (Genbank KX510273)	Amp	CKX2ΔSP_LIC_f	tagttggaatgggttcgaATGATTAAAATTGATT-TACCTAAATCCC	Col0 genomic
				CXX2_LIC_r	ttatggagttgggttcgaaGATGTCTTGCCCTGGAGATAA-CA	

Table S2. Construct list.

Lowercase font in primer sequence denote adaptor sequence, underlined nucleotides indicate mutations or insertions.

Name	Purpose	Parent vector	Selection (bacteria/plants)	Insert		
				Primer name	Sequence 5‘ to 3‘	Template
hbt::CKX7-HA; hbt::CKX7-GFP	Protoplast expression vector	*hbt::LIC-HA* (Genbank KX510274); *hbt::LIC-GFP* (Genbank KX510273)	Amp	CXX7_LIC_f	tagttggaatgggttcgaaCACACACACCAAAATGATAGCT	Col0 genomic
				CXX7_LIC_r	ttatggagttgggttcgaaAAGAGACCTATTGAAAATCTTT-TGACC	
hbt::CKX2-GFP-GPI	Protoplast expression vector	*hbt::LIC-GFP-GPI* (Genbank KX510275)	Amp	CXX2_LIC_f	tagttggaatgggttcgaaTAAACAAATGGCTAATCTT-CGTT	Col0 genomic
				CXX2_LIC_r	ttatggagttgggttcgaaGATGTCTTGCCCTGGAGATAA-CA	
hbt::CKX2-GPI	Protoplast expression vector	*hbt::CKX2-HA*	Amp	GPI_Pst_f	AACGGTGGTTCCCGGTCACAATTCTCATTCGTCG-CCGCCGTGCTCCTCCCTCTTCTTGTCTTTTTCTTC TTCTCTGCCTAActgca	StuI PstI digested *hbt::CKX2-HA*
				GPI_Pst_r	gTTAGGCAGAGAAGAAGAAAAAGACAAGAA-GAGGGAGGAGCACGGCGGCGACGAATGAG-AATTGTGACCGGGAACCACCGTT	

Table S2 (continued).

Construct list. Lowercase font in primer sequence denote adaptor sequence, underlined nucleotides indicate mutations or insertions.

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