Supporting Information

Vi et al. 10.1073/pnas.1303967110

SI Results and Discussion

The following comments relate to the poly(A)-tail length assay. Because of the high sequence similarity between different members of the *SMALL AUXIN UP RNA* (*SAUR*) gene family, it was not possible to design primers that gave both efficient PCR amplification when used with the universal reverse primer from the Affymetrix poly(A) tail-length assay kit and that were able to discriminate between closely related *SAUR* genes. As a result, the PCR products shown in Fig. 3*C* and Figs. S3*D* and S4 *B* and *D* represent mixtures of products derived from two or four individual *SAUR* transcripts.

When comparing the normalized-intensity profiles in Fig. 3*C* and Fig. S3*D*, it appears that even after RNAseH digestion there is still a difference between the products from the *paps1-1* mutants and the other two genotypes. This might indicate a difference in the choice of cleavage site, with a bias toward more distal sites in wild type and *paps2 paps4* mutants. However, the sequencing results shown in Fig. S3*B* and Fig. S3*G* rule out this explanation; if anything, distal sites seem to be used more frequently in *paps1-1* mutants. In addition, subcloning and sequencing individual molecules from the *SAUR19/24* PCR products on RNAseH-treated wild-type RNA indeed detected short remaining poly(A) tails (Fig. S3*C*). Thus, the difference seen in the RNAseH-digested samples results from incomplete digestion of the poly(A) tails by RNAseH.

SI Materials and Methods

Plant Materials. Transfer DNA (T-DNA) insertion mutants are in Col-0 background including *paps1-3* (T-DNA line WiscDsLox413-416L14), *paps2-1* (T-DNA line SALK_126395), and *paps4-1* (T-DNA line SALK_007979), and therefore Col-0 was used as wild-type control for these plants. SALK and WiscDsLox collections of T-DNA insertion mutants have been described previously (1, 2). T-DNA insertion mutants were obtained from the Nottingham *Arabidopsis* Stock Centre. For comparison with mutants in Col-0 background, the *paps1-1* allele was introgressed into the Col-0 background by three rounds of back-crossing. The *cstf64-1* mutant, the *eds1-2* mutant, the *pad4-1* mutant, and the *dst2* mutant have been described previously (3-6), as has the *35S::GFP-SAUR19* transgene (7).

Genetic Mapping and Mutant Identification. The *paps1-1* mutant in L*er* background was crossed to wild-type Col-0, and a segregating F2 population was established. This was used for genetic mapping as previously described (8).

Genotyping Mutant Alleles. The following primers were used:

Oligonucleotide name	Sequence	Description
oSV166	TAATGCCCATCATTAC TCCTGCGAAT	genotype *paps1-1*
oSV126	GCTTTGTTTGATTCCA TAGC	genotype *paps1-1*
oSV126	GCTTTGTTTGATTCCA TAGC	genotype *paps1-3* LP primer
oSV78	TGGGACCTAGACATGC AACTAG	genotype *paps1-3* RP primer
oSV120	ACATGGAGATGTTGAA CTGCC	genotype *paps2-1* LP primer

Cont.

Oligonucleotide name	Sequence	Description
oSV121	CCACTGTTCCACGTAT ATCAAAC	genotype *paps2-1* RP primer
oSV110	TGCATCTGCTGCCACT ATATC	genotype *paps4-1* LP primer
oSV111	TTGCTGAAGCTGTAGG GTCTG	genotype *paps4-1* RP primer
ML437	TGGTTCACGTAGTGGG CCATCG	BP primer for SALK-TDNA
oSV139	AACGTCCGCAATGTGT TATTAAGTTGTC	BP primer for Ws-TDNA

Primers used for determining gene expression in T-DNA insertion mutants were:

Oligonucleotide name	Sequence	Description
oSV198	CCTAGTATGTTGGT TTCTCGA	RT-PCR PAPS2
oSV121	CCACTGTTCCACGT ATATCAAAC	RT-PCR PAPS2
oSV110	TGCATCTGCTGCCA CTATATC	RT-PCR PAPS4
oSV112	CAATCGTGCCATGG TGGTGGGTAC TCAAAATTTAGG	RT-PCR PAPS4

Phenotypic Analysis, Measurements of Organ and Cell Sizes. Petals were dissected from the 6th to 15th flowers and used for measurements. For leaves, the fourth and fifth leaves of plants at the bolting stage or the entire rosette were taken for measurements in all experiments except the one involving the *35S::GFP-SAUR19* transgene. For the latter the first two true leaves were collected.

For measuring petal-cell size, a drop of 2% (wt/vol) low-melt agarose containing 0.01% bromophenol blue prewarmed at 50 °C was placed on a prewarmed glass slide. The droplet was spread by a pipette tip to get a thin layer of agarose. A petal was immediately gently placed on it. Once the gel solidified, the petal was carefully peeled off, and the remaining gel cast was left to dry for approximately 10 min. The gel cast was then observed without a cover glass under a differential phase contrast microscope.

For determining cell size in leaves, leaves were fixed in FAA solution [10% (wt/vol) formaldehyde, 5% (vol/vol) acetic acid, and 50% (vol/vol) ethanol]. The FAA solution was then replaced with chloral hydrate solution (200 g chloral hydrate, 20 g glycerol, and 50 mL distilled water), and the tube was incubated overnight. Samples were mounted on a glass slide and observed under a differential phase contrast microscope.

Alexander Staining of Pollen. Mature pollen was mounted in Alexander's stain (9) and observed under a light microscope with 20× magnification after incubating for 15 min.

In Vitro Polyadenylation Assay. Reaction mixtures contained 2.5% (wt/vol) polyvinyl alcohol, 1 mM MnCl2, 100 ng BSA, 1 mM ATP, 0.5 U RNasin, 10 mM Hepes (pH 7.9), 25 mM $NH_4(SO_4)_2$, 0.2 mM PMSF, and 0.2 mM DTT, and the indicated amounts of protein (wild type or mutant). Reaction mixtures were incubated for 30 min at room temperature, followed by proteinase K treatment, phenol/chloroform extraction, ethanol precipitation, and separation on 6% (wt/vol) urea-acrylamide gels.

The constructs pSV11b (pET28a-PAPS1cDNA-wt) and pSV11d (pET28a_cDNAPAPS1-P313S-mutated; see below) were used for expressing the proteins. These were purified using Ni-NTA agarose.

Measurement of Bulk Poly(A) Tail Length. The protocol was adapted from ref. 10. RNA samples [2 μg, either untreated or digested with RNAseH/oligo(dT); see below] were 3′-end-labeled with 10 μCi ^{32}P-cordycepin using 5 U of yeast poly(A) polymerase. The reaction (10 μL) was incubated at 37 °C for 20 min, followed by heat inactivation at 70 °C for 10 min. The sample was then subjected to RNase treatment in a volume of 80 μL consisting of 10 mM Tris·HCl (pH 8), 300 mM NaCl, 5 μg RNaseA, and 125 U RNase T1 for 60 min at 37 °C. The RNase treatment was stopped in a volume of 100 μL consisting of 100 μg Proteinase K, 0.5% SDS, and 10 mM EDTA and incubated for 30 min at 37 °C. The reaction volume was then adjusted to 200 μL with the addition of 52 μg tRNA, 125 μg glycogen, ammonium acetate (to a final concentration of 2.5 M), and magnesium chloride (to a final concentration of 15 mM) and water. The RNA [poly(A) tails] were precipitated by adding 2.5 volumes of ice-cold ethanol and centrifuging for 1 h at 13,000 × *g* at 8 °C. The pellet was washed with ice-cold 80% (vol/vol) ethanol and allowed to dry at room temperature. The dried pellet was resuspended in 10 μL of loading buffer [94% (vol/vol) formamide, 20 mM EDTA, 0.2% bromophenol blue, and 0.2% xylene cyanol]. The poly(A) tails were then separated by electrophoresis on a 8.3 M urea/10% (wt/vol) polyacrylamide gel and visualized using autoradiography on a Typhoon phosphorimager.

Microarray Analysis. All plants were grown at 21 °C. For inflorescence collection, main inflorescences excluding the open flowers/buds were collected from 30-d-old plants grown on soil. Plants at the stage of harvest had approximately 7–12 siliques (wild type) or 2–5 siliques (mutant). Plants were grown in long day conditions, and material was collected at 5–7 h after the light period starts.

Whole seedlings including roots were harvested from 11-d-old (mutant) or 9-d-old (wild type) plants grown on MS plates. The different ages were used to ensure that the first true leaves were at a comparable developmental stage. Plants were grown in long day conditions, and material was collected at 5–7 h after the light period starts.

Total RNA was prepared by the hot phenol method (11) and cleaned up with the Qiagen RNAeasy Kit, digested with TURBO Dnase (Ambion). Subsequent labeling and array hybridization were carried out by CRX Biosciences Ltd.

A two-color array platform was used: Agilent *Arabidopsis* 4 × 44K oligo microarray and Agilent 4 × 44K gasket slides. One microgram of total RNA samples was used for labeling with the Quick Amp Labeling Kit–Two Color using the manufacturer's protocols.

Differentially expressed genes were identified using the R/Bioconducor package Limma (12). Background correction was done by subtracting background intensities and then setting zero or negative intensities to half the minimum of the positive ones. Two-color microarrays were normalized using the loess method (13).

Quantitative RT-PCR and Measurements of Poly(A) Tail Length. Primers used for quantitative RT-PCR are listed below.

Gene name	AGI code		Primer sequences
SAUR19	AT5G18010	fw	ATTTGGTGCCGCTC TCATAC
		rev	TTCATTGGAGCCGA GAAGTC
SAUR20	AT5G18020	fw	CCAAAAGGGTTTCT TGCAGT
		rev	CATCGTTGGAACCG AGAAGT
SAUR60	AT4G38860	fw	GTGCAAGCCACCAC TTATCA
		rev	CGGACTAAAACCTG TAAGATCCA
SAUR63	AT1G29440	fw	AGATTTCGGTCTCC CAACG
		rev	TGGTTGAAAAGAGC ATCTAGCA
PDF2	At1g13320	fw	GCATTTCACTCCTC TGGCTAAG
		rev	GGCACTTGGGTATG CAATATG
PR1	AT2G14610	fw	TGTGCCAAAGTGAG GTGTAAC
		rev	TGATGCTCCTTATT GAAATACTGATAC
PR2	AT3G57260	fw	GGTGTCGGAGACCG GTTGGC
		rev	CCCTGGCCTTCTCG GTGATCCA
SID2	AT1G74710	fw	TGAAGCAACAACAT CTCTACAGGCG
		rev	CCCGAAAAGGCTCG GCCCAT

For poly(A) tail measurement, RNA was isolated as described before. To eliminate the activity of the temperature-sensitive protein encoded by the *paps1-1* allele as far as possible, plants were shifted to 30 °C for 2 h before harvesting. Poly(A) tail length was determined using the Affymetrix Poly(A) Tail-Length Assay Kit according to the manufacturer's instructions, using primers detailed below. PCR products from the poly(A) tail-length assays were analyzed on an Agilent 2100 Bioanalyzer. PCR products of *SAUR19/24* and *SAUR62/63/66/68* were subcloned and Sanger-sequenced. Obtained sequencing results were compared with the *Arabidopsis* genome.

Oligod$(T)_{17}$/RNaseH digestion was performed as follows: 20 μg of DNase-digested RNA was mixed with 3.2 μL oligod $(T)_{17}$, 10 μL 10× RNaseH buffer, 0.4 μL RNaseH (NEB), and 79.4 μL water and incubated for 1 h at 37 °C. Before G/I Tailing, RNA was precipitated adding 0.1 vol 3 M NaAc, 1 vol Isopropanol, incubated at −20 °C for a minimum of 30 min, centrifuged, washed with 70% (vol/vol) ethanol, dried, and dissolved in 10–15 μL water.

The following primers were used for poly(A) tail (PAT) tests. As discussed above, these primers amplify two to four very closely related transcripts when used in PAT assays.

At1g29440 (SAUR63)	AACGGAAGGACCAATCACATT
At5g18010 (SAUR19)	GGCTCCAATGAAGATGATCCAACATTT

Nuclei Isolation. Nuclei were isolated from L*er* wild-type and *paps1-1* mutant inflorescences that had been treated at 30 °C for 2 h, following the procedure from ref. 14. Enrichment of nuclei was determined by RT-PCR against *PDF2*, using the above primers that span two introns. As seen in Fig. S4*A*, there is a strong enrichment of unspliced or partially spliced transcripts in the nuclear RNA compared with total RNA. When quantified using the Bioanalyzer, the ratio of unspliced/partially spliced to completely spliced transcripts is more than 14-fold higher in the nuclear RNA sample than in total RNA.

Molecular Cloning. ***Complementation of* paps1-1 *mutants.*** A genomic fragment of *PAPS1* (5,877 bp) was isolated from a TAC clone and ligated into the binary vector for plant transformation. TAC JAtY72B09 (15) containing the *PAPS1* locus was digested with XbaI, and the fragment around 5.8 kb was gel-purified and ligated into ML939 (a modified form of pBluescript) using the XbaI site to create plasmid pSV1. All plasmids were verified by partial sequencing and restriction enzyme digestion. The *PAPS1* locus was released from pSV1 by digesting with *Asc*I and ligated into binary vector ML1297 [a derivative of pGPTV-BAR (16)] using the *Asc*I site. The resulting plasmid (pSV2) was transformed into *Agrobacterium* strain GV3101, which was used to transformed *paps1-1* mutants.

Promoter swap* pPAPS1::genomicPAPS4 *experiment. The genomic *PAPS4* fragment was isolated from TAC clone JAt49G10 by NcoI digestion and gel-purification of the 9,906-bp fragment. The fragment was ligated into ML939 using the NcoI site to create pSV5a (ML939-NcoI_gPAPS4_NcoI). An NcoI site was introduced at the ATG start codon of *gPAPS4* by overlap PCR using primer oSV106, oSV107, oSV112, and oSV113. The PCR product was ligated into pSV5a using *Pme*I and XhoI sites to create pSV5b (ML939_SDM_NcoI at ATG_gPAPS4_NcoI). An NcoI site was introduced at the ATG start codon of *gPAPS1* in pSV1 by overlap PCR using primer oSV104, oSV105 oSV91, and oSV101. The PCR product was ligated into pSV1 using ClaI and BstEII sites to create pSV5c (pSV1_SDM_NcoI at ATG_gPAPS1). The genomic *PAPS4* fragment starting at the ATG start codon was released from pSV5b by digesting with NcoI and ligated into pSV5c digested with NcoI to give pSV5d (ML939-pPAPS1::ATGgPAPS4-PAPS4UTR3′). The *pPAPS1::genomicPAPS4* fragment was released from pSV5d by digesting with *Asc*I and ligated into the *Asc*I site of pBarMAP [a derivative of pGPTV-BAR (16)] to create pSV5e (pBarMAP/AscI/pPAPS1UTR5::ATGgPAPS4-PAPS4UTR3′). This plasmid pSV5e was used to transform *paps1-1* mutants.

***Chimeric construct* pPAPS1::NPAPS4::CPAPS1.** A StuI site was introduced into the genomic *PAPS1* fragment in pSV5c at the nucleotide that encodes the start of the C-terminal domain of the PAPS1 protein by overlap PCR using primer oSV202, oSV203, oSV102, and oSV103. The PCR product was ligated into pSV5c (pSV1_SDM_NcoI at ATG_gPAPS1) using *Pme*I and *Bsp*1407I sites to create pSV12 (pSV1_SDM_NcoI at ATG_gNPAPS1-StuI-gCPAPS1). The StuI/PstI fragment from pSV12 (containing the C-terminal coding region of the PAPS1 protein) was ligated into the StuI/PstI-digested vector pSV5d (containing the pPAPS1::gPAPS4-N-terminal coding region) to create pSV14 (pPAPS1::UTR5PAPS1:: NPAPS4::CtermPAPS1). The fragment containing the chimeric construct was released from pSV14 by digesting with *Asc*I and was ligated into the *Asc*I site of pBarMAP to create pSV15 [pBarMAP/AscI/pPAPS1::UTR5PAPS1::ATGNPAPS4-CtermPAPS1 (all genomic)]. This plasmid pSV15 was used to transform *paps1-1* mutants.

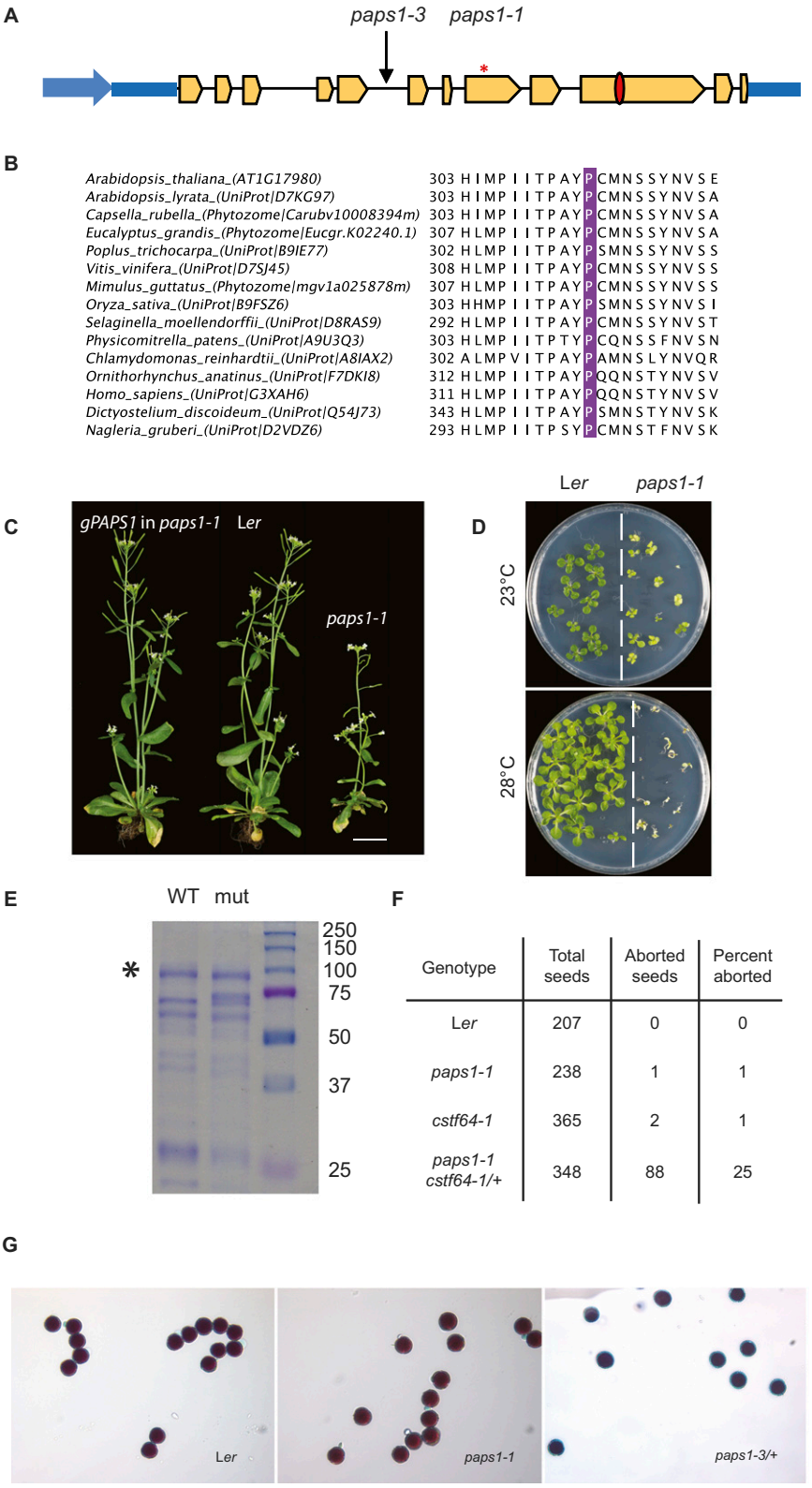
With this cloning procedure, the amino acid sequence at the junction between the N and C terminus of the resulting chimeric protein was FVFPNGYRRPSHT, whereas the original sequence at corresponding position for PAPS1 is FVFPGGVRPSHT and for PAPS4 is FVFPNGYRRPRQSRH.

***Constructs for expression of PAPS1 proteins in* Escherichia coli.** The full-length PAPS1 coding region, amplified from cDNA of either wild-type or mutated *paps1-1* plants, were subcloned into pET28a (Novagen) between NdeI and EcoRI site. This results in a translational fusion of the His-tag to the N terminus of PAPS1. The resulting plasmids pSV11b (pET28a-PAPS1cDNA-wt) and pSV11d (pET28a_cDNA-PAPS1-P313S-mutated) were verified by DNA sequencing.

Primers used for cloning:

Primer	Name	Sequence
oSV102	At1g17980_exon10R	ACTGCTTCATAAGGGAAAGGAG
oSV103	At1g17980_exon10F	GCAGAAGGCGAGCAATTCGA
oSV106	At4g32850_1R	CACACAAAGTATCAATGTCAG
oSV107	At4g32850_2F	GTCAAGCTAATGTATAGATCG
oSV112	At4g32850_ATGNcoIF	CAATCGTGCCATGGTGGTGGGTACTCAAAATTTAGG
oSV113	At4g32850_AtgNcoIR	GTACCCACCACCATGGCACGATTGATAATCCTAAGC
oSV202	StuI_PAP1_F	GAGTTAGACCTAGGCCTTCACATACCTCTAAAGGAACATG
oSV203	StuI_PAP1_R	AGAGGTATGTGAAGGCCTAGGTCTAACTCCACCAGGAAAC

1. Alonso JM, et al. (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* 301(5633):653–657.
2. Woody ST, Austin-Phillips S, Amasino RM, Krysan PJ (2007) The WiscDsLox T-DNA collection: An *Arabidopsis* community resource generated by using an improved high-throughput T-DNA sequencing pipeline. *J Plant Res* 120(1):157–165.
3. Liu F, Marquardt S, Lister C, Swiezewski S, Dean C (2010) Targeted 3′ processing of antisense transcripts triggers *Arabidopsis* FLC chromatin silencing. *Science* 327(5961):94–97.
4. Aarts N, et al. (1998) Different requirements for EDS1 and NDR1 by disease resistance genes define at least two R gene-mediated signaling pathways in *Arabidopsis*. *Proc Natl Acad Sci USA* 95(17):10306–10311.
5. Glazebrook J, Rogers EE, Ausubel FM (1996) Isolation of *Arabidopsis* mutants with enhanced disease susceptibility by direct screening. *Genetics* 143(2):973–982.
6. Johnson MA, Perez-Amador MA, Lidder P, Green PJ (2000) Mutants of *Arabidopsis* defective in a sequence-specific mRNA degradation pathway. *Proc Natl Acad Sci USA* 97(25):13991–13996.
7. Spartz AK, et al. (2012) The SAUR19 subfamily of SMALL AUXIN UP RNA genes promote cell expansion. *Plant J* 70(6):978–990.
8. Disch S, et al. (2006) The E3 ubiquitin ligase BIG BROTHER controls *Arabidopsis* organ size in a dosage-dependent manner. *Curr Biol* 16(3):272–279.
9. Alexander MP (1969) Differential staining of aborted and nonaborted pollen. *Stain Technol* 44(3):117–122.
10. Preker PJ, Lingner J, Minvielle-Sebastia L, Keller W (1995) The FIP1 gene encodes a component of a yeast pre-mRNA polyadenylation factor that directly interacts with poly(A) polymerase. *Cell* 81(3):379–389.
11. Box MS, Coustham V, Dean C, Mylne JS (2011) Protocol: A simple phenol-based method for 96-well extraction of high quality RNA from Arabidopsis. *Plant Methods* 7:7.
12. Smyth GK (2004) Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 3(1):Article 3.
13. Smyth GK, Speed T (2003) Normalization of cDNA microarray data. *Methods* 31(4):265–273.
14. Park MY, Wu G, Gonzalez-Sulser A, Vaucheret H, Poethig RS (2005) Nuclear processing and export of microRNAs in *Arabidopsis*. *Proc Natl Acad Sci USA* 102(10):3691–3696.
15. Knee EM, Rivero L, Crist D, Grotewold E, Scholl R (2011) *Germplasm and Molecular Resources. Genetics and Genomics of the Brassicaceae, Plant Genetics and Genomics: Crops and Models*, eds Schmidt R, Bancroft I (Springer, New York), pp 437–468.
16. Becker D, Kemper E, Schell J, Masterson R (1992) New plant binary vectors with selectable markers located proximal to the left T-DNA border. *Plant Mol Biol* 20(6):1195–1197.



Genotype	Total seeds	Aborted seeds	Percent aborted
Ler	207	0	0
paps1-1	238	1	1
cstf64-1	365	2	1
paps1-1 cstf64-1/+	348	88	25

Fig. S1. Molecular characterization of *PAPS1* and transgenic complementation of the *paps1-1* mutant. (*A*) Schematic representation of *PAPS1* gene structure and the position of mutant alleles. The promoter is shown as a light blue arrow, 5′ and 3′ untranslated regions as blue rectangles, exons as beige pointed rectangles, and introns as thin black lines. The red ellipse marks the boundary between the region coding for the N-terminal catalytic domain and that coding for the C-terminal domain. The position of the *paps1-1* point mutation is indicated by the red asterisk. (*B*) Multiple sequence alignment of canonical poly(A) polymerases from the indicated organisms surrounding the proline (highlighted) that is mutated in the *paps1-1* allele. (*C*) Whole-plant images of the indicated genotypes demonstrating rescue of the *paps1-1* phenotype by a wild-type genomic *PAPS1* fragment. (Scale bar, 2 cm.) (*D*) Seedling phenotypes of the indicated genotypes grown at 23 °C or 28 °C. (*E*) Coomassie-stained SDS/PAGE of purified recombinant wild-type PAPS1 protein (WT) or mutant protein encoded by the

Legend continued on following page

paps1-1 allele (mut). Asterisk indicates the position of the full-length protein. Marker sizes are in kDa. (*F*) Genetic interaction between *paps1-1* and *cstf64-1*. The numbers and proportions of aborted seeds are shown for the indicated genotypes. (*G*) Photographs of Alexander-stained mature pollen from L*er* wild-type and *paps1-1* mutant plants, as well as from a *paps1-3*/+ heterozygous plant.

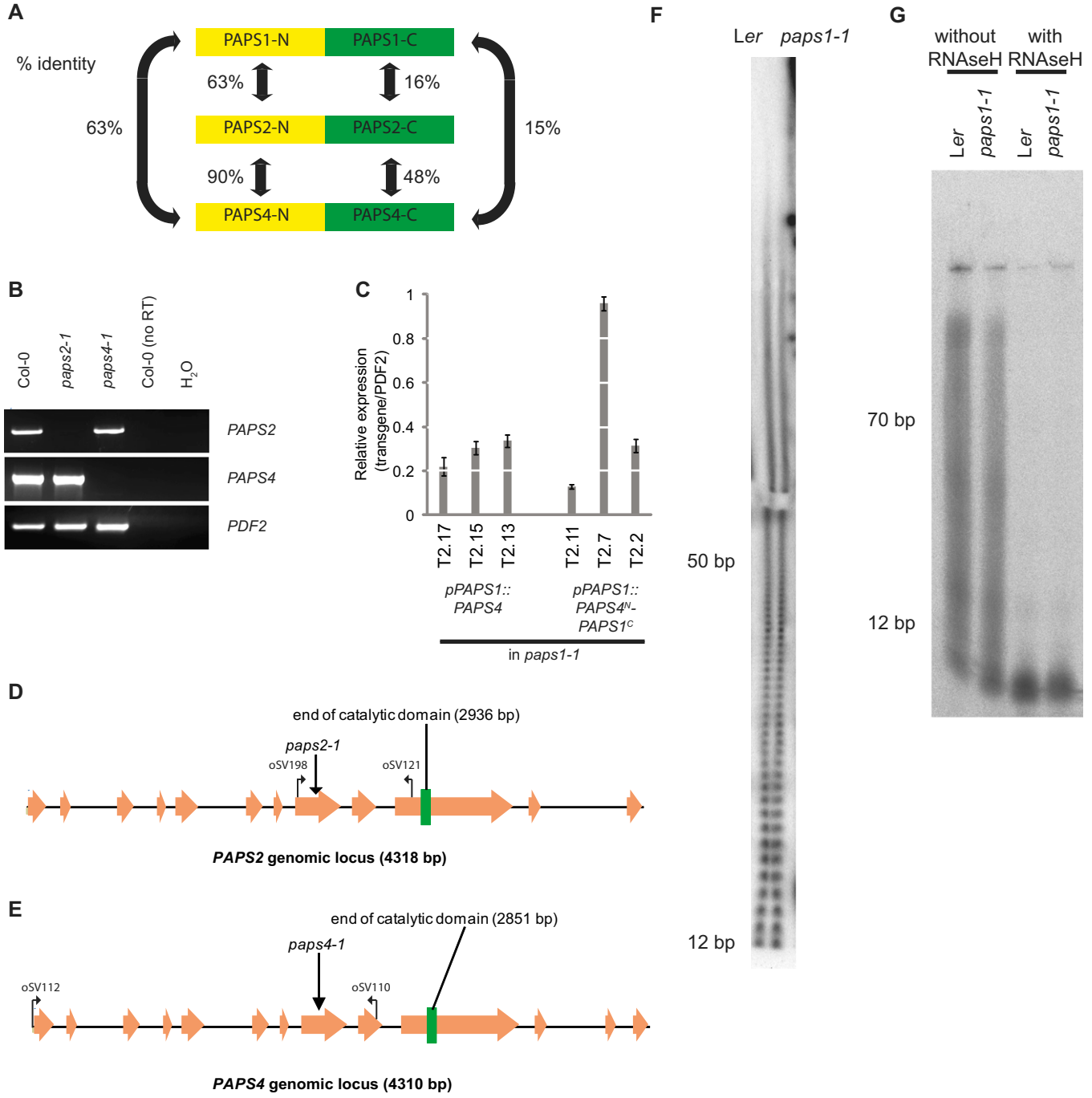


Fig. S2. Molecular characterization of *PAPS* isoforms in *Arabidopsis thaliana*. (*A*) Sequence comparison of the three nuclear PAPS proteins. Values shown are amino acid identities between the indicated protein domains. (*B*) Agarose-gel electrophoresis of RT-PCR products on RNA from the indicated genotypes using primers directed against the target transcripts shown on the right. (*C*) Expression of the indicated *PAPS* chimeric transgenes as determined by quantitative RT-PCR on RNA from three independent lines each. Primers used amplify only the chimeric transcripts which contain the 5′ UTR from *PAPS1* and the region coding for the N-terminal protein domain from *PAPS4*. Expression was normalized to that of the constitutive control gene *PDF2* (*AT1G13320*). Values shown are the means ± SE from three technical replicates on one biological replicate of pooled inflorescences. (*D* and *E*) Schematic representation of *PAPS2* (*D*) and *PAPS4* (*E*) gene structure and the position of mutant alleles. Beige arrows represent exons, black lines show introns. The position of primers used for the RT-PCR in *B* is indicated (oSV198, oSV121 for *PAPS2*; oSV110, oSV112 for *PAPS4*). (*F*) Autoradiogram of radioactively labeled bulk poly(A) tails from L*er* (*Left*) and *paps1-1* mutant seedlings (*Right*) grown for 11 d at 28 °C. Poly(A) tails were separated on an 8.3 M urea/10% polyacrylamide gel. Fig. S1*D* shows phenotypes of *paps1-1* mutant seedlings grown at 28 °C. The discontinuity at around 70 bp is due to a tear in the gel. (*G*) Same as *F*, but including control reactions treated with RNAseH/oligo(dT) before cordycepin labeling to demonstrate that the observed labeled products indeed represent poly(A) tails.

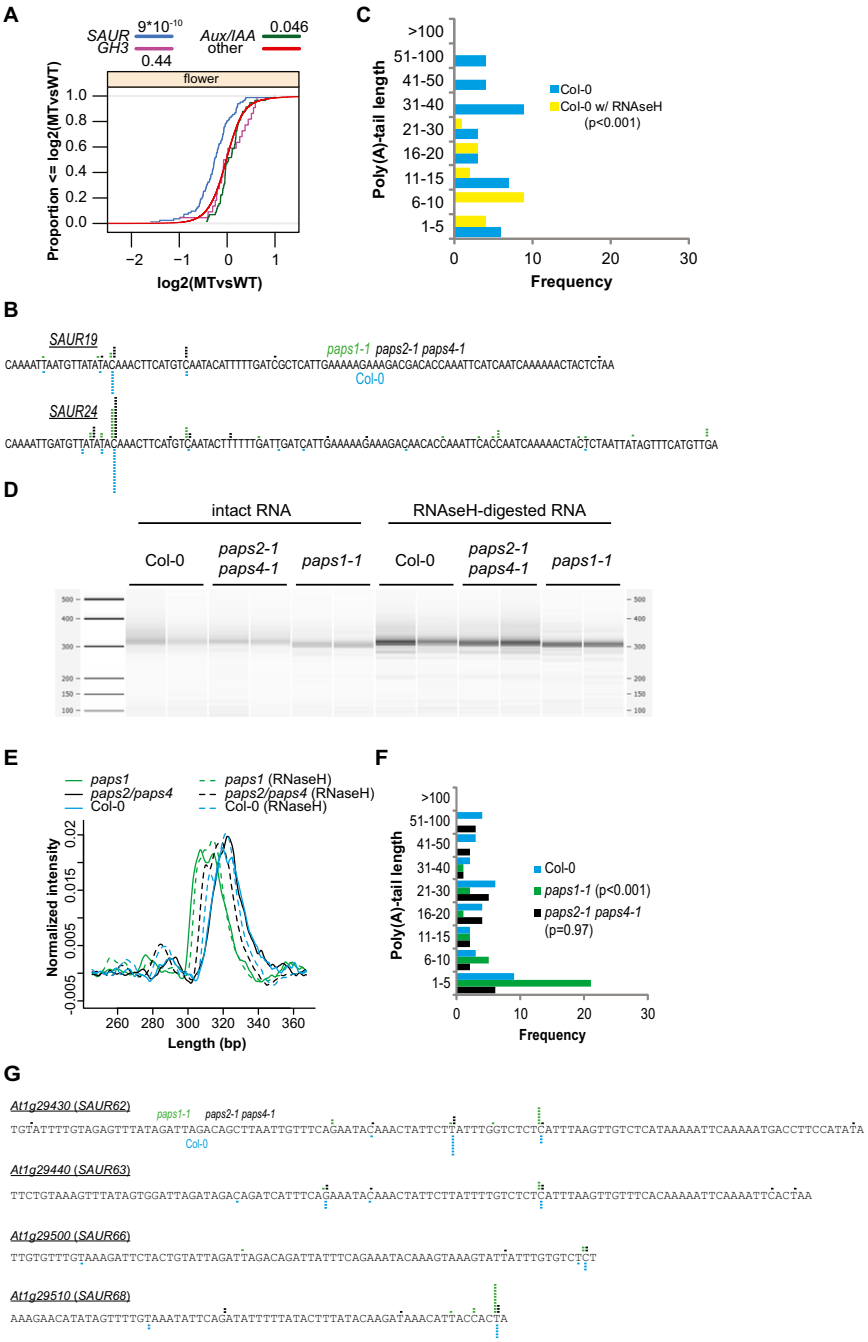


Fig. S3. Polyadenylation, but not 3′-end cleavage of *SAUR* mRNAs, is affected in *paps1* mutants. (*A*) Cumulative distribution plot of the expression levels of *SAUR*, *GH3*, and *Aux/IAA* family members in *paps1-1* vs. wild-type inflorescences. The *y* axis indicates the fraction of genes with a log2-expression ratio less than or equal to the value on the *x* axis. Numbers in legends are *P* values of a Wilcoxon rank-sum test. "other," all remaining genes on the array. (*B*) Determination of 3′-end cleavage sites of *SAUR19* and *SAUR24* mRNAs as determined by sequencing subcloned individual PCR products from intact RNA in Fig. 3*C*. (*C*) Length distribution of poly(A) tails as determined by sequencing subcloned individual molecules of *SAUR19/24* PCR products from intact RNA or from RNAseH/oligo (dT)-treated RNA from wild type. Data for intact RNA are the same as in Fig. 3*E*. The *P* value in the legend is from a Wilcoxon rank-sum test. (*D*) Bioanalyzer electropherogram of RT-PCR–amplified 3′ ends of *At1g29430/SAUR62*, *At1g29440/SAUR63*, *At1g29500/SAUR66*, and *At1g29510/SAUR68* transcripts from the indicated genotypes. Two biological replicates per genotype are shown. RNA had been left untreated (*Left*) or poly(A) tails had been digested with RNAseH and oligo(dT) (*Right*) before reverse transcription. (*E*) Normalized signal intensities of the PCR products in *D*. Averages of the two biological replicates per genotype are shown. (*F* and *G*) Length distribution of poly(A) tails (*F*) and 3′-end cleavage sites (*G*) as determined by sequencing subcloned individual molecules from intact RNA in *D*. *P* values in legend to *F* are from a Wilcoxon rank-sum test. Distance to the primer-binding site is the same for all four sequences shown in *G*.

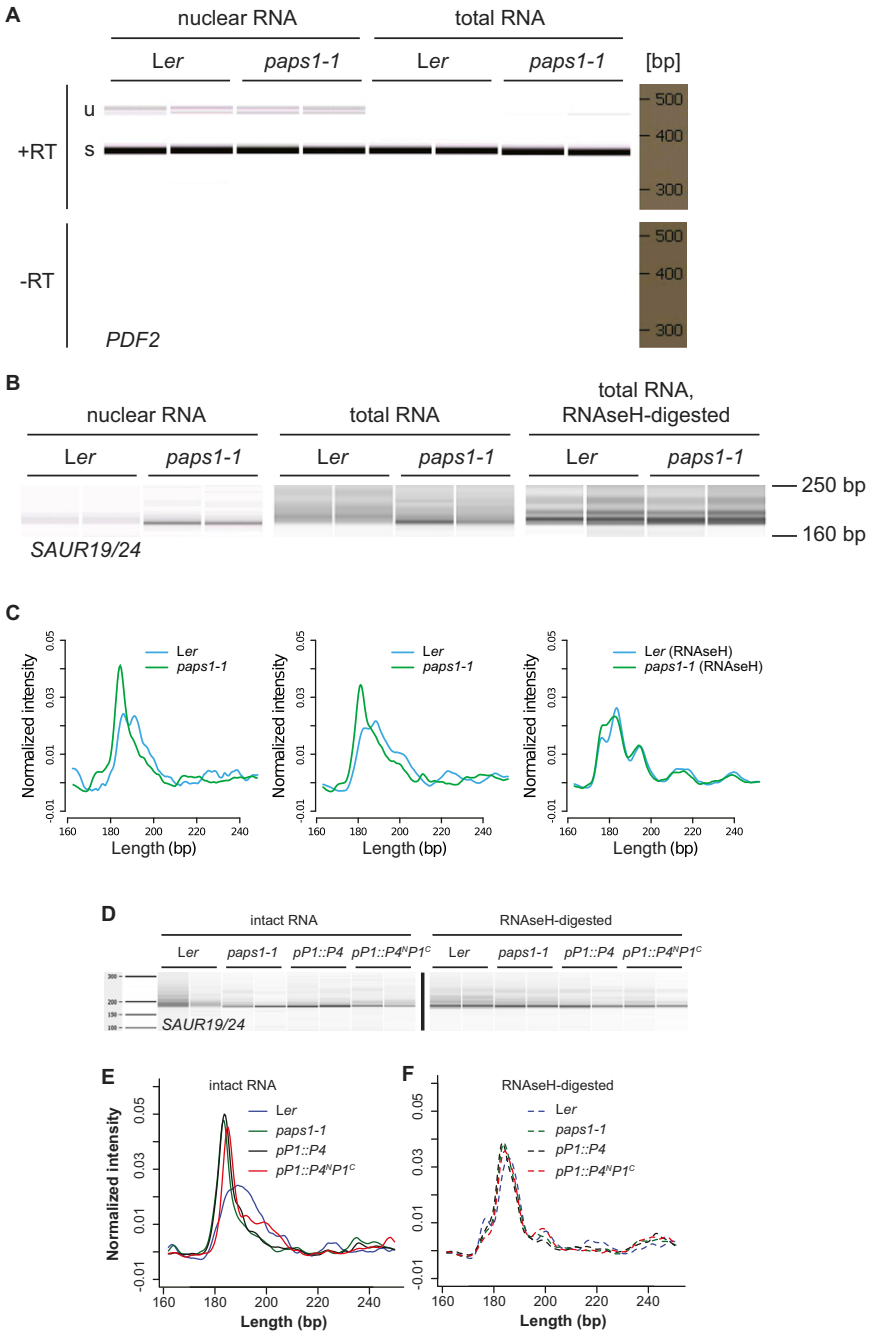
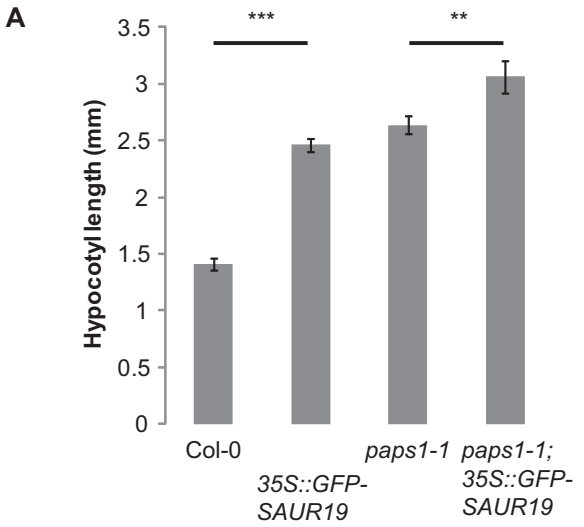


Fig. S4. Nuclear polyadenylation of *SAUR* transcripts is defective in *paps1-1* mutants, but polyadenylation is partially rescued in plants expressing the chimeric PAPS4^{N}-PAPS1^{C} protein. (*A*) Bioanalyzer electropherogram of RT-PCR products against the constitutive-control gene *PDF2* on oligo(dT)-primed cDNA from nuclear RNA (*Left*) or total cellular RNA (*Right*). The primers used span two introns, and "s" indicates completely spliced products, whereas "u" marks unspliced or partially spliced products. Quantification of peak intensities shows that the ratio of unspliced/partially spliced transcript to completely spliced transcript is more than 14-fold higher in the nuclear relative to the total-RNA sample. RT, reverse transcriptase. (*B*) Bioanalyzer electropherogram of RT-PCR–amplified 3′ ends of *SAUR19/24* transcripts from the indicated genotypes. Two biological replicates per genotype are shown. RNA had been isolated from purified nuclei (*Left*) or from total cellular extract (*Center*). (*Right*) Total RNA digested with RNAseH and oligo(dT) before reverse transcription was used for these reactions. (*C*) Normalized signal intensities of the PCR products in *B*, each panel corresponding to the electropherogram shown above. (*D*) Bioanalyzer electropherogram of RT-PCR–amplified 3′ ends of *SAUR19/24* transcripts from the indicated genotypes. Two biological replicates per genotype are shown. RNA had been left untreated (*Left*) or poly(A) tails had been digested with RNAseH and oligo(dT) (*Right*) before reverse transcription. (*E* and *F*) Normalized signal intensities of the PCR-products in *D*, with intact-RNA samples in *E* and oligo(dT)/RNAseH-digested RNA samples in *F*. Averages of the two biological replicates per genotype are shown.



B

F2 *paps1-1* x *dst2*

Genotype	Phenotype: wild type	Phenotype: *paps1-1*
+/+ or +/*paps1-1*	74	0
paps1-1/*paps1-1*	0	11

Fig. S5. Interaction between *PAPS1*, *SAUR19*, and *DST2*. (*A*) Hypocotyl lengths of light-grown seedlings from the indicated genotypes. At least 17 seedlings were measured per genotype. Asterisks (**, ***) indicate significant differences at $P < 0.01$ (**) or $P < 0.001$ (***) as determined by Student *t* test. (*B*) Distribution of genotypes and phenotypes observed in an F2 population of a *dst2* × *paps1-1* cross. Note that no *paps1-1* homozygous mutants with a modified or rescued phenotype were found. The overrepresentation of wild-type plants at the expense of *paps1-1* mutant plants reflects bias in the selection of plants to be genotyped, based on the assumption that the double mutant might have a (partially) rescued phenotype. Genotyping for the *dst2* allele is not possible, because the gene has not been identified. Performing this analysis with *dst1* is not feasible, because *dst1* appears to be closely linked to *paps1* on chromosome I (6).

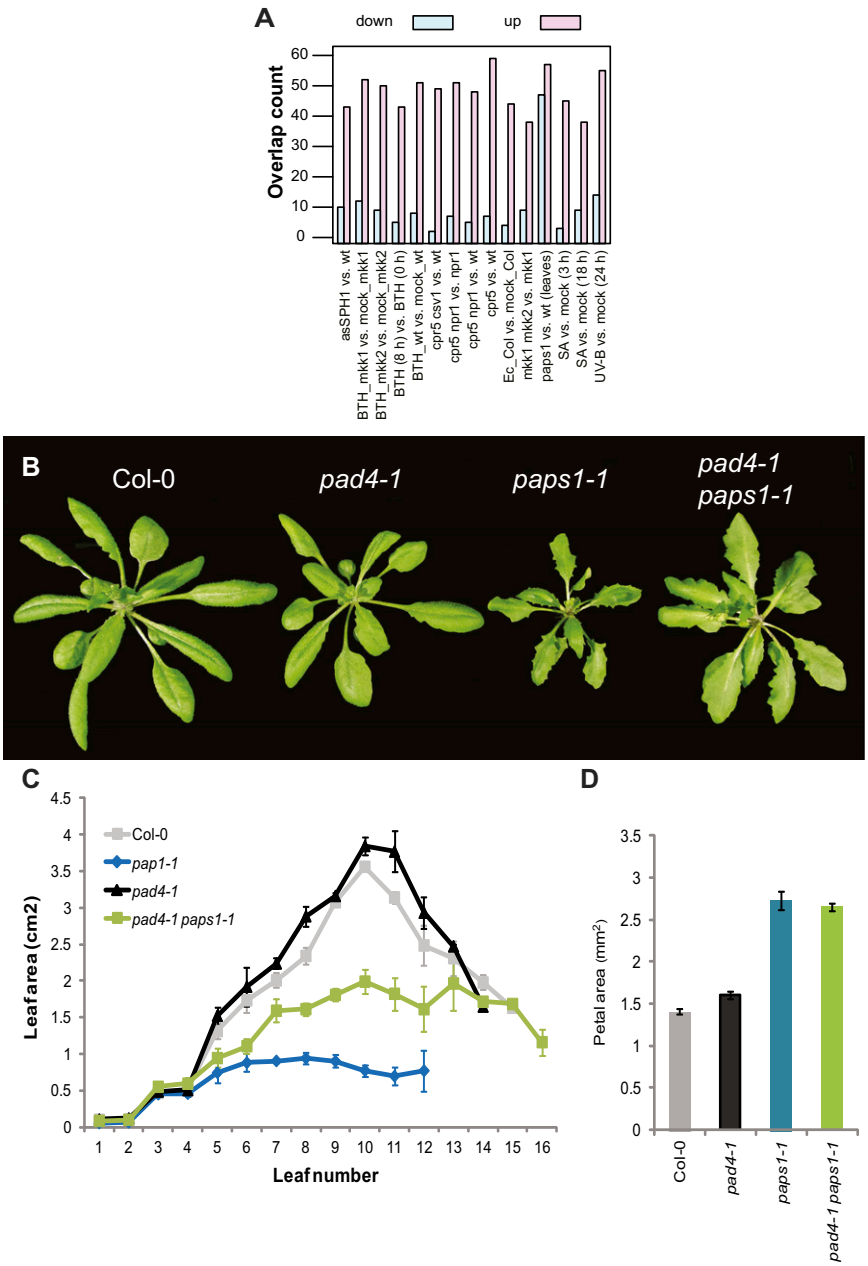


Fig. S6. The constitutive pathogen response in *paps1-1* mutant leaves depends on *PAD4* activity. (*A*) Overlap of genes misregulated in flowers of *paps1-1* mutants vs. wild-type with genes misregulated in the experiments indicated. Table S3 defines the abbreviations used. (*B*) Whole-plant phenotypes of the indicated genotypes. (*C*) Leaf area throughout the rosette from the indicated genotypes. Values shown are mean ± SE from four plants each. (*D*) Petal area of the indicated genotypes. Values shown are mean ± SE from at least 22 petals from four different plants each.

Other Supporting Information Files

Table S1 (DOCX)
Table S2 (DOCX)
Table S3 (DOCX)