Allantoin, a stress-related purine metabolite, can activate jasmonate signaling in a MYC2-regulated and abscisic acid-dependent manner

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The following Supplementary Data are available for this article:

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Figure S3. Hierarchical tree graph of over-represented GO terms for genes with significantly reduced expression in the aln-1 mutant.
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Table S1. Primers used in this study.
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Quantification of jasmonic acid (JA) and JA-Ile

Extraction and quantification of JA and JA-Ile were performed following the method of Preston et al. (2009) with minor modifications. The stable isotope-labeled compounds used as internal standards were: [2H2]JA (Tokyo Chemical industry Co., Ltd., Tokyo, Japan) and [13C6]JA-Ile, which was synthesized with [13C6]Ile (Cambridge Isotope Laboratories, Andover, MA, USA) as described in Jikumaru et al. (2004). Aerial parts (500 mg) from 2-week-old seedlings grown aseptically on solid half-strength MS medium were frozen in liquid N2, ground, and extracted with 80% (v/v) methanol containing 1% (v/v) acetic acid at 4°C for 1 h. After removal of cell debris by centrifugation, the supernatants were condensed and dried in vacuo, and the resultant residues were extracted twice with methanol. The extracts were evaporated to dryness and resuspended with 80% methanol containing 1% acetic acid and internal standards, which were again evaporated and then extracted with water acidified with 1% (v/v) acetic acid. The extracts were loaded onto pre-equilibrated Oasis HLB column cartridge (Waters Corporation, Milford, MA, USA). After washed with water acidified with 1% acetic acid, the column was eluted with 80% (v/v) acetonitrile containing 1% (v/v) acetic acid. The eluted samples were evaporated to obtain extracts in water acidified with 1% acetic acid, and loaded onto a pre-equilibrated Oasis MCX column cartridge (Waters). The cartridge was washed with water acidified with 1% acetic acid and eluted with 80% acetonitrile containing 1% acetic acid. The eluate was loaded onto pre-equilibrated Oasis WAX column cartridges (Waters) followed by washing, first with water acidified 1% acetic acid and then with 80% acetonitrile, and the fraction containing JA and JA-Ile was eluted with 80% acetonitrile containing 1% acetic acid. The obtained fraction was dried and reconstituted in ultra-pure water acidified with 1% (v/v) acetic acid for quantification of JA and JA-Ile by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) on an Agilent 6410 Triple Quadrupole system with a ZORBAX Eclipse XDB-C18 column and MassHunter software version B.01.02 (Agilent Technology, Palo Alto, CA, USA). The LC column was eluted with a binary solvent system of 0.01% (v/v) acetic acid in water (solvent A) and 0.05% (v/v) acetic acid in acetonitrile (solvent B) using a linear gradient of solvent B in solvent A, from 3% to 50%, in 20 min at the flow rate of 0.4 ml min⁻¹. The MS/MS operation parameters are summarized in Supplementary Table S2.
Arabidopsis Genome Initiative numbers for the genes mentioned in this article are as follows: 

*AAH*, At4g20070; *ABA2*, At1g52340; *ACT2*, At3g18780; *ALN*, At4g04955; *ANAC019*, At1g52890; *ANAC055*, At3g15500; *ANAC072*, At4g27410; *AOC1*, At3g25760; *AOS*, At5g42650; *BGLU18*, At1g52400; *BSMT1*, At3g11480; *CYP94B3*, At3g48520; *ERF1*, At3g23240; *ICS1*, At1g74710; *JAM1*, At2g46510; *JAR1*, At2g46370; *JAZ1*, At1g19180; *JAZ3*, At3g17860; *JAZ5*, At1g17380; *JAZ6*, At1g72450; *JAZ7*, At2g34600; *JAZ10*, At5g13220; *JAZ12*, At5g20900; *LOX2*, At3g45140; *LOX3*, At1g17420; *LOX4*, At1g72520; *MYC2*, At1g32640; *OPR3*, At2g06050; *ORA59*, At1g06160; *PDF1.2a*, At5g44420; *PDF1.2b*, At2g26020; *PR-1*, At2g14610; *SAGT1*, At2g43820; *VSP1*, At5g24780; and *XDH1*, At4g34890.
Supplementary Figure Legends

Figure S1. The purine catabolism pathway and metabolites derived therefrom.
The pathway is schematically illustrated starting from the common intermediate xanthine, at which all purine nucleotides converge when they are subjected to degradation. The abbreviated names of the genes encoding the enzymes that catalyze each step are shown in italics and knockout mutants used in this study are indicated in parentheses under the gene names: XDH, xanthine dehydrogenase; UOX, urate oxidase; AS, allantoin synthase; ALN, allantoinase (allantoin amidohydrolase); AAH, allantoate amidohydrolase; UGAH, ureidoglycine aminohydrolase; UAH, ureidoglycolate amidohydrolase.

Figure S2. Hierarchical tree graph of over-represented GO terms for genes with significantly increased expression in the aln-1 mutant.
A total of 47 enriched GO terms under Biological Process were found for 211 genes with significantly increased expression (≥ 3-fold changes, Supplementary Table S3) using the BioMaps tool of VirtualPlant version 1.3 (Katari et al., 2010; http://virtualplant.bio.nyu.edu/cgi-bin/vpweb/) in the default-setting mode (Fisher’s exact test with false discovery rate correction, \( P < 0.01 \)). Each box shows the name of the GO term and, where appropriate, the \( P \) value (< 0.01) for the significance of enrichment. The \( P \) values are also indicated by the frame color of each box according to the gradient scale. The rank direction of the graph runs from top to bottom.

Figure S3. Hierarchical tree graph of over-represented GO terms for genes with significantly reduced expression in the aln-1 mutant.
A total of 33 enriched GO terms under Biological Process were found for 113 genes with significantly reduced expression (≥ 3-fold changes, Supplementary Table S4) using the BioMaps tool of VirtualPlant version 1.3 (Katari et al., 2010; http://virtualplant.bio.nyu.edu/cgi-bin/vpweb/) in the default-setting mode (Fisher’s exact test with false discovery rate correction, \( P < 0.01 \)). Each box shows the name of the GO term and, where appropriate, the \( P \) value (< 0.01) for the significance of enrichment. The \( P \) values are also indicated by the frame color of each box according to the gradient scale. The rank direction of the graph runs from top to bottom.

Figure S4. Basal level expression of PR-1 as a canonical SA marker.
RNA was extracted from aerial parts of 2-week-old seedlings of WT and aln-1 mutants grown under normal aseptic conditions. Relative mRNA levels were determined by real-time reverse
transcription-quantitative PCR using ACTIN2 expression as reference and presented as values relative to the WT level. The sequences of primers are listed in Supplementary Table S1. Data are means ± SEM from three independent experiments (*P < 0.05 by Student’s t-test comparison to the WT level).

Figure S5. Characterization of the aln-1 jar1-1 double mutant.
The homozygous mutants of aln-1 (SALK_000325; Yang and Han, 2004; Watanabe et al., 2014) and jar1-1 (CS8072; Staswick et al., 1992) were crossed to obtain the double mutant aln-1 jar1-1. (A) Diagram of the T-DNA insertion in the ALN gene in the aln-1 mutant. Arrows denote PCR primers. (B) PCR-based genotyping of the double mutant using primers specific to ALN (F1 and R1) and the left border sequence of T-DNA (LBa1). (C) Diagram of the JAR1 gene structure and the confirmation of the jar1-1 mutation in the double mutant. The nucleotide sequence of the wild-type JAR1 allele (a) from TAIR (At2g46370; https://www.arabidopsis.org/) was compared to that of the double mutant, as determined by dideoxy sequencing with primers F2 (b) and R2 (c), to confirm the single nucleotide missense mutation that occurs in exon 3 (Staswick et al., 2002). The sequences of primers are listed in Supplementary Table S1. (D) Typical root growth of 8-day-old seedlings of WT, jar1-1, and aln-1 jar1-1 genotypes in the presence of 10 µM methyl jasmonate (MeJA). Note that the root growth of the jar1-1 mutant is moderately insensitive to MeJA (Staswick et al., 1992, 2002). Horizontal bars indicate a scale of 10 mm in length (white) and the position of a primary root tip (yellow).

Figure S6. Reduced response to MeJA of anthocyanin accumulation in the aah mutant.
Sterile seedlings of WT and the aah mutant were grown for 8 days on standard medium supplemented with 10 µM MeJA and examined for anthocyanin accumulation as described in the main text. FW, fresh weight. Data are means ± SEM (n = 8; *P < 0.001 by Student’s t-test comparison to the WT levels).

Figure S7. Characterization of the aln-1 bglu18 double mutant.
The homozygous mutants of aln-1 (SALK_000325; Yang and Han, 2004; Watanabe et al., 2014) and bglu18 (SALK_075731C; Ogasawara et al., 2009) were crossed to obtain the double mutant aln-1 bglu18. (A) Diagram of the T-DNA insertion in the BGLU18 gene in the bglu18 mutant. Arrows denote PCR primers. (B) PCR-based genotyping of the double mutant using primers specific to ALN (F1 and R4; see Supplementary Fig. S5), BGLU18 (F3 and R3), and the left border sequence of T-DNA (LBa1). The sequences of primers are listed in Supplementary Table S1.
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Gene symbol as provided by The Arabidopsis Information Resource (TAIR; release 10; https://www.arabidopsis.org/) except for T-DNA of *Agrobacterium tumefaciens*.

Table S2. LC-ESI-MS/MS parameters for jasmonate determination.

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Listed are those whose changes in transcript levels increased by equal to or greater than 3-fold, with the statistical significance level of 0.001 by a two-way analysis of variance (ANOVA). These genes were selected from the revised microarray data (Gene Expression Omnibus accession number GSE73841) that had been parametrically renormalized using the SuperNORM data service (Skylight Biotech Inc, Akita, Japan), according to the three-parameter lognormal distribution method (Konishi, 2004).

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Mean of two independent biological experiments (*aln-1* versus wild-type).

Gene symbol and description as provided by The Arabidopsis Information Resource (TAIR; release 10; https://www.arabidopsis.org/).

*P*-value determined by a two-way ANOVA.

Table S4. Genes with significantly reduced expression in the *aln-1* mutant.

Listed are those whose changes in transcript levels decreased by equal to or greater than 3-fold with the statistical significance level of 0.001 by a two-way ANOVA. These genes were selected from the revised microarray data (Gene Expression Omnibus accession number GSE73841) that had been parametrically renormalized using the SuperNORM data service (Skylight Biotech Inc, Akita, Japan), according to the three-parameter lognormal distribution method (Konishi, 2004).

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Supplementary References

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**Table S1. Primers used in this study**

| AGI\(^a\)   | Gene symbol\(^b\) | Direction | Sequence (Designation) | Use             |
|-------------|-------------------|-----------|------------------------|-----------------|
| At4g04955  | ALN               | Forward   | 5'-CCTTTATGTGCCCTTCAGGA-3' (F1) | PCR genotyping  |
|             |                   | Reverse   | 5'-G GCCATACCCACCAAGA-3' (R1)  | PCR genotyping  |
|             |                   | Reverse   | 5'-GGTTCCCAACCAAGATCTGC-3' (R4) | PCR genotyping  |
| At1g52400  | BGLU18            | Forward   | 5'-GGCGACCAGAGTTATCAT-3' (F3) | PCR genotyping  |
|             |                   | Reverse   | 5'-GAATACCCATTGGCTCCCAAC-3' (R3) | PCR genotyping  |
| At2g46370  | JAR1              | Forward   | 5'-CGCTACTGACCCTCGAAGAGCTTT-3' (F2) | DNA sequencing |
|             |                   | Reverse   | 5'-CAACATGTTAAGGCATAGTCG-3' (R2) | DNA sequencing  |
| At1g17420  | LOX3              | Forward   | 5'-TGAACATTGAGAGAGTCAAGACTTTT-3' | RT-qPCR         |
|             |                   | Reverse   | 5'-GAATACCATTTGCCCGAAAC-3'    | RT-qPCR         |
| At1g72520  | LOX4              | Forward   | 5'-TCGCTACTTGGTGAATCCATATCA-3' | RT-qPCR         |
|             |                   | Reverse   | 5'-TGCTCATTCGAAGCCATCATATT-3' | RT-qPCR         |
| At5g42650  | AOS               | Forward   | 5'-GGTGCGAGGTTGGTTTGTGA-3'   | RT-qPCR         |
|             |                   | Reverse   | 5'-GCACGTACCAACCTCAATAC-3'   | RT-qPCR         |
| At2g06050  | OPR3              | Forward   | 5'-ACGGACACACTCCGGCGGTTTTT-3' | RT-qPCR         |
|             |                   | Reverse   | 5'-CCTGAACCTGCTCCAAACT-3'    | RT-qPCR         |
| At1g32640  | MYC2              | Forward   | 5'-TGCTCTACCATGTAATGCTTTTA-3' | RT-qPCR         |
|             |                   | Reverse   | 5'-TGCTCTACCATGTAATGCTTTTA-3' | RT-qPCR         |
| At3g17860  | JAZ3              | Forward   | 5'-TTTCTACCATGTAATGCTTTTA-3' | RT-qPCR         |
|             |                   | Reverse   | 5'-TAGCTACTTGGCAGAATCTAGA-3' | RT-qPCR         |
| At5g13220  | JAZ10             | Forward   | 5'-AGCTCTTTGGCCAGAATCTAGA-3' | RT-qPCR         |
|             |                   | Reverse   | 5'-AGATGGCTACGATCGATCGA-3'   | RT-qPCR         |
| At2g14610  | PR-1              | Forward   | 5'-CGTCTTTTGGTGAAGTTGCTTCCTCAGA-3' | RT-qPCR         |
|             |                   | Reverse   | 5'-TGCTCTTGGTGAACCTTTCGTA-3' | RT-qPCR         |
| At5g44420  | PDF1.2a           | Forward   | 5'-TTTCTTTCTTGGTGAAGTTGCTTCCTCAGA-3' | RT-qPCR         |
|             |                   | Reverse   | 5'-TTTCTTTCTTGGTGAAGTTGCTTCCTCAGA-3' | RT-qPCR         |
| At3g23240  | ERF1              | Forward   | 5'-CGATCTCCATACCGAAACAGCAG-3' | RT-qPCR         |
|             |                   | Reverse   | 5'-CGATCTCCATACCGAAACAGCAG-3' | RT-qPCR         |
| At3g18780  | ACTIN2            | Forward   | 5'-ACCCTATATTGTCAAGAACCAC-3'  | RT-qPCR         |
|             |                   | Reverse   | 5'-ACCCTATATTGTCAAGAACCAC-3'  | RT-qPCR         |

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\(^b\) Gene symbol as provided by The Arabidopsis Information Resource (TAIR; release 10; https://www.arabidopsis.org/) except for T-DNA of *Agrobacterium tumefaciens*. 

\(^\_\) T-DNA: 5'-TGGTTACCTGATGGGCTCCCATG-3' (LBa1) | PCR genotyping
### Table S2. LC-ESI-MS/MS parameters for jasmonate determination

| Analyte     | Retention time on LC (min) | ESI mode | [M–H]⁻⁻ (m/z) | Transition ion (m/z) | Collision energy (eV) | Fragmentor voltage (V) |
|-------------|---------------------------|----------|---------------|---------------------|-----------------------|------------------------|
| JA          | 14.4                      | negative | 209           | 59                  | 15                    | 135                    |
| [²H₂]JA     | 14.4                      | negative | 211           | 59                  | 15                    | 135                    |
| JA-Ile      | 18.0                      | negative | 322.0         | 130                 | 14                    | 140                    |
| [¹³C₆]JA-Ile| 18.0                      | negative | 328.4         | 136.2               | 14                    | 140                    |
| Affymetrix probe ID | AGI* | Fold change | Gene symbol and description | P-value |
|---------------------|------|-------------|-----------------------------|---------|
| At5g47990           |      | 3.17        | CYP705A5, THAD, THAD1, cytochrome P450, family 705, subfamily A, polypeptide 5 | 1.331E-06 |
| At5g46520           |      | 3.04        | CYP94B3, cytochrome P450, family 94, subfamily B, polypeptide 3 | 1.282E-08 |
| At3g26920           |      | 1.80        | AT1A2, Protein of unknown function (DUF677) | 1.411E-22 |
| At2g31360           |      | 1.67        | RAP2.6, related to AP2 6 | 1.831E-14 |
| At2g38240           |      | 1.69        | 2-oxoglutamate (2OG) and Fe(II)-dependent oxygenase superfamily protein | 3.205E-09 |
| At3g48480           |      | 1.46        | FATM, farnesoid acid carboxyl-O-methyltransferase | 1.956E-21 |
| At2g23830           |      | 1.34        | MD-2-related lipid recognition domain-containing protein | 3.712E-16 |
| At1g25960           |      | 1.26        | Protein of unknown function (DUF626) | 6.221E-08 |
| At5g08880           |      | 1.20        | Arginase/deacetylase superfamily protein | 2.889E-26 |
| At1g62570           |      | 1.18        | FMO GS-OX4, flavin-monooxygenase glucosinolate S-oxygenase 4 | 6.567E-13 |
| At5g45500           |      | 1.14        | RNI-like superfamily protein | 2.861E-06 |
| At5g63450           |      | 1.14        | CYTP451, cytochrome P450, family 94, subfamily B, polypeptide 1 | 5.902E-14 |
| At2g05050           |      | 1.04        | UBO11, ubiquitin 11 | 2.298E-25 |
| At3g03500           |      | 1.06        | UGT78D1, UDP-glucosyl transferase 78D1 | 1.783E-21 |
| At3g24440           |      | 0.95        | ATG5STU6, GST24, GSTU6, glutathione S-transferase tau 6 | 1.447E-09 |
| At2g23710           |      | 0.92        | Late embryogenesis abundant protein, group 6 | 2.610E-14 |
| At3g13580           |      | 0.90        | CXC750, ECSI, ECSI | 5.196E-08 |
| At5g38000           |      | 0.89        | Zinc-binding dehydrogenase family protein | 5.939E-04 |
| At3g46660           |      | 0.87        | UGT76E12, UDP-glucosyl transferase 76E12 | 4.360E-12 |
| At4g20840           |      | 0.82        | Putative endonuclease or glycosyl hydrolase | 6.565E-10 |
| At3g65280           |      | 0.81        | GCL1, OCR2-like 1 | 1.076E-14 |
| At2g34600           |      | 0.81        | JAZ7, TIFY5B, jasmonate-zim-domain protein 7 | 4.125E-16 |
| At2g08400           |      | 0.75        | unknown protein; FUNCTIONS IN: molecular_function unknown; INVOLVED IN: N-terminal protein | 3.573E-05 |

6.812E-24 LOCATED IN: endomembrane system; EXPRESSED IN: leaf apex, leaf whorl, male gametophyte, flower, leaf; EXPRESSED DURING: LP.06 six leaves visible, LP.04 four leaves visible, LP.10 ten leaves visible, petal differentiation and expansion stage, LP.08 eight leaves visible; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:At5G64820.1); Has 24 Blast hits to 24 proteins in 6 species: Arabidopsis - 0; Bacteria - 0; Fungi - 0; Plants - 4; Viruses - 0; Other Eukaryotes - 0 (source: NCBI BLINK).

9.010E-16 LOCATED IN: endomembrane system; EXPRESSED IN: leaf apex, leaf whorl, male gametophyte, flower, leaf; EXPRESSED DURING: LP.06 six leaves visible, LP.04 four leaves visible, LP.10 ten leaves visible, petal differentiation and expansion stage, LP.08 eight leaves visible; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:At2G01300.1); Has 71 Blast hits to 71 proteins in 13 species: Arabidopsis - 0; Bacteria - 0; Fungi - 0; Plants - 69; Viruses - 0; Other Eukaryotes - 0 (source: NCBI BLINK).

2.148E-11 LOCATED IN: endomembrane system; EXPRESSED IN: leaf apex, leaf whorl, male gametophyte, flower, leaf; EXPRESSED DURING: LP.06 six leaves visible, LP.04 four leaves visible, LP.10 ten leaves visible, petal differentiation and expansion stage, LP.08 eight leaves visible; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:At2G01300.1); Has 71 Blast hits to 71 proteins in 13 species: Arabidopsis - 0; Bacteria - 0; Fungi - 0; Plants - 69; Viruses - 0; Other Eukaryotes - 0 (source: NCBI BLINK).
**Table S3 continued**

| Gene ID/Name | Description | FDR | Fold Change |
|-------------|-------------|-----|-------------|
| 257638_at   | 245651_s_at | 5.21 | TPX1, thioredoxin-dependent peroxidase 1 | 4.16E-11 |
| 261037_at   | 245120_s_at | 5.19 | LOX3, lipoxygenase 3 | 1.737E-22 |
| 248337_at   | 245823_at  | 5.11 | COR78, LTI140, LTI78, RD29A, low-temperature-responsive protein 78 (LTI78) | 1.912E-13 |
| 257638_at   | 245651_s_at | 5.06 | ATP5F1, TPS-CIN, terpene synthase-like sequence-1, 1,8-cineole | 3.374E-19 |
| 266799_at   | 245384_s_at | 5.06 | ATP5F2, PSK2, phytosulfokine 2 precursor | 3.091E-09 |
| 262226_at   | 245384_s_at | 4.94 | Protein of unknown function (DFU58) | 4.589E-07 |
| 249971_at   | 245119_s_at | 4.92 | Eukaryotic aspartyl protease family protein | 3.110E-16 |
| 256324_at   | 245119_s_at | 4.87 | MATE efflux family protein | 1.026E-06 |
| 249101_at   | 245384_s_at | 4.74 | Serine protease inhibitor, potato inhibitor I-type family protein | 1.325E-06 |
| 250292_at   | 245384_s_at | 4.73 | JAS1, JAZ10, TFY9, jasmonate-zim-domain protein 10 | 5.410E-15 |
| 255527_at   | 24402360_s_at | 4.67 | Protein of unknown function, DUF538 | 5.697E-16 |
| 260696_at   | 245119_s_at | 4.64 | ATBETAfuR7CU4, VAC-INV, Glycosyl hydrolases family protein | 1.631E-20 |
| 251332_at   | 24408170_s_at | 4.61 | Inositol 1,3,4-trisphosphate 5/6 kinase family protein | 9.323E-16 |
| 264146_at   | 245119_s_at | 4.58 | CER1, Fatty acid hydroxylase superfamily | 1.358E-10 |
| 252377_at   | 245119_s_at | 4.58 | Major facilitator superfamily protein | 9.941E-12 |
| 251023_at   | 24502170_s_at | 4.56 | Transmembrane amino acid transporter family protein | 2.740E-06 |
| 260012_at   | 245119_s_at | 4.56 | unknown protein; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT1G68760.1); Has 13 Blast hits to 13 proteins in 2 species: Arabidopsis - 0; Bacteria - 0; Metazoa - 0; Fungi - 0; Plants - 13; Viruses - 0; Other Eukaryotes - 0 (source: NCBI BLink). | 1.160E-12 |

**LOCATED IN:** endomembrane system; **EXPRESSED IN:** 21 plant structures; **EXPRESSED DURING:** 13 growth stages; **BEST Arabidopsis thaliana protein match is:** unknown protein (TAIR:AT4G26130.1); Has 3020 Blast hits to 17322 proteins in 780 species: Arabidopsis - 22; Bacteria - 1396; Metazoa - 17338; Fungi - 3422; Plants - 5037; Viruses - 0; Other Eukaryotes - 2996 (source: NCBI BLink).
### Table S3 (continued)

| Accession | Description | E-value |
|-----------|-------------|---------|
| At5g55790 | unknown protein; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT1G54563.1); Has 1807 Blast hits to 1807 proteins in 277 species: Archae - 0; Bacteria - 0; Metazoa - 736; Fungi - 347; Plants - 385; Viruses - 0; Other Eukaryotes - 339 (source: NCBI BLiNK). | 3.80 |
| At5g67300 | FYVE domain-containing protein 1 | 3.79 |
| At2g32510 | MAPKKK17 | 3.78 |
| At3g58350 | ATTKS-CIN, TPS-CIN, TPS-CIN, terpene synthase-like sequence-1,8-cineole | 3.77 |
| At2g17840 | ERD7, Senescence/dehydration-associated protein-related | 3.76 |
| At5g45850 | unknown protein; FUNCTIONS IN: molecular_function unknown; INVOLVED IN: biological_process unknown; LOCATED IN: endomembrane system; EXPRESSED IN: 18 plant structures; EXPRESSED DURING: 12 growth stages; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT5G4582.1); Has 30201 Blast hits to 17322 proteins in 780 species: Archae - 12; Bacteria - 1396; Metazoa - 17338; Fungi - 3422; Plants - 5037; Viruses - 0; Other Eukaryotes - 2996 (source: NCBI BLiNK). | 3.73 |
| At4g16880 | Leucine-rich repeat (LRR) family protein | 3.68 |
| At5g50655 | unknown protein; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT5G50665.2); Has 6 Blast hits to 6 proteins in 1 species: Archae - 0; Bacteria - 0; Metazoa - 0; Fungi - 0; Plants - 6; Viruses - 0; Other Eukaryotes - 0 (source: NCBI BLiNK). | 3.69 |
| At5g17490 | RGL3, RGA-like protein 3 | 3.68 |
| At3g57520 | AtSIP2, SIP2, seed imbibition 2 | 3.67 |
| At2g34070 | TBL37, TRICHOME BIREFRINGENCE-LIKE 37 | 3.67 |
| At3g60005 | MD-2-related lipid recognition domain-containing protein | 3.67 |
| At1g71800 | ATGSTM25, GSTU25, glutathione S-transferase TAU 25 | 3.66 |
| At1g72260 | TH2,1, TH2,1, thionin 2 | 3.65 |
| At4g22330 | ATCTES1, Alkaline phytoceramidase (aPHC) | 3.64 |
| At5g47550 | ATTP2/3, DELTA-TIP3, TIP2/3, tonoplas intrinsic protein 2,3 | 3.63 |
| At2g42550 | COR15, COR15A, cold-regulated 15a | 3.61 |
| At3g59970 | ATDLX1A, ATDLX1A, expansins-like A1 | 3.59 |
| At5g62330 | Plant invertase/peroxid methylesterase inhibitor superfamily protein | 3.58 |
| At3g21770 | Peroxidase superfamily protein | 3.57 |
| At5g03550 | Secretory carrier membrane protein (SCAMP) family protein | 3.56 |
| At5g02580 | Plant protein 1589 of unknown function | 3.55 |
| At5g02310 | MAN1, Glycosyl hydrolase superfamily protein | 3.53 |
| At3g77550 | O-methyltransferase family protein | 3.52 |
| At5g62740 | BGLL7, beta glucosidase 7 | 3.51 |
| At3g28220 | TRAF-like family protein | 3.51 |
| At1g12950 | RSH2, root hair specific 2 | 3.46 |
| At5g73480 | alpha/beta-Hydrolases superfamily protein | 3.46 |
| At5g03420 | Sadhu4-2, transposable element gene | 3.46 |
| At1g20450 | ERD10, LT129, LT145, Dehydrin family protein | 3.45 |
| At1g52540 | Transmembrane amino acid transporter family protein | 3.45 |
| At1g17380 | JAZS, TIFY11A, jasmonate-zim-domain protein 5 | 3.41 |
| At2g17190 | ATGSTM26, GSTU26, glutathione S-transferase tau 26 | 3.41 |
| At5g02230 | Halocid dehalogenase-like hydrolase (HAD) superfamily protein | 3.41 |
| At5g62520 | SRO5, similar to RCD one 5 | 3.40 |
| At5g15430 | unknown protein; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT3G28370.1); Has 13 Blast hits to 13 proteins in 4 species: Archae - 0; Bacteria - 0; Metazoa - 0; Viruses - 0; Other Eukaryotes - 0 (source: NCBI BLiNK). | 3.39 |
| At4g12410 | SAUR-like auxin-responsive protein family | 3.37 |
| At4g20860 | FAD-binding Berberine family protein | 3.36 |
| At3g28740 | CYP81D1, Cytochrome P450 superfamily protein | 3.34 |
| At4g27654 | unknown protein; FUNCTIONS IN: molecular_function unknown; INVOLVED IN: biological_process unknown; LOCATED IN: endomembrane system; EXPRESSED IN: 17 plant structures; EXPRESSED DURING: 9 growth stages; Has 30201 Blast hits to 17322 proteins in 780 species: Archae - 12; Bacteria - 1396; Metazoa - 17338; Fungi - 3422; Plants - 5037; Viruses - 0; Other Eukaryotes - 2996 (source: NCBI BLiNK). | 3.33 |
| At1g69260 | AFPI, ABI five binding protein | 3.32 |
| At3g29575 | AFPI, ABI five binding protein | 3.32 |
| At3g24800 | Late embryogenesis abundant protein (LEA) family protein | 3.30 |
| At2g30040 | MAPKKK14, mitogen-activated protein kinase kinase kinase 14 | 3.29 |
| At3g15640 | Protein phosphatase 2C family protein | 3.28 |
| At4g11210 | Disease resistance-responsive (dirigent-like protein) family protein | 3.27 |
| At3g44320 | ANIT3, NIT3, nitrilase 3 | 3.26 |
| At4g28085 | unknown protein; Has 45 Blast hits to 45 proteins in 1 species: Archae - 0; Bacteria - 0; Metazoa - 0; Fungi - 0; Plants - 0; Viruses - 0; Other Eukaryotes - 0 (source: NCBI BLiNK). | 3.25 |
| At1g70800 | AGL27, FLM, MAIF, K-box region and MADS-box transcription factor family protein | 3.25 |
| At5g50570 | Squamosa promoter-binding protein-like (SBP) domain transcription factor family protein | 3.24 |
| At1g59740 | Major facilitator superfamily protein | 3.23 |
| At2g20340 | Pyridoxal phosphate (PLP)-dependent transferases superfamily protein | 3.23 |
| At4g17090 | ATUBC8, UBC8, ubiquitin conjugating enzyme 8 | 3.22 |
| At1g95200 | ATMSL9, MSL9, mechanosensitive channel of small conductance-like 9 | 3.21 |
| At3g23800 | ALDH2B, ALDH2B7, aldehyde dehydrogenase 2B7 | 3.20 |
| At1g78000 | SEL1, SUL1R1,1, sulfate transporter 1,2 | 3.20 |
265216 at At1g05100 3.20 MAPKKK18, mitogen-activated protein kinase kinase kinase 18 6.727E-08
264289 at At1g16890 3.19 MATE efflux family protein 1.851E-13
252419 at At3g47510 3.18 unknown protein; FUNCTIONS IN: molecular_function unknown; INVOLVED IN: biological_process unknown; LOCATED IN: endomembrane system; EXPRESSED IN: 19 plant structures; EXPRESSED DURING: 11 growth stages; Has 15 Blast hits to 15 proteins in 7 species: Archaea - 0; Bacteria - 0; Metazoa - 0; Fungi - 0; Plants - 15; Viruses - 0; Other Eukaryotes - 0 (source: NCBI BLink).

253684 at At4g28690 3.18 Alkaline-phosphatase-like family protein 9.631E-08
252076 at At3g51660 3.18 Tautomerase/MIF superfamily protein 1.585E-17
255795 at At2g33380 3.17 CLO-3, RD20, Calmodulin-related family protein 4.371E-16
250781 at At5g05410 3.17 DREB2, DREB2A, DRE-binding protein 2A 6.535E-14
260676 at At1g19450 3.15 Major facilitator superfamily protein 1.216E-08
254996 at At4g10390 3.15 Protein kinase superfamily protein 8.436E-17
263786 at At2g46370 3.14 FIN219, JAR1, Auxin-responsive GH3 family protein 1.050E-12
259705 at At1g77450 3.14 anac032, NAC032, NAC domain containing protein 32 7.980E-17
256848 at At3g27960 3.13 Tetractinomerpide repeat (TPR)-like superfamily protein 1.037E-10
264145 at At1g79310 3.13 AIMC7, MC7, metacaspase 7 4.761E-05
247717 at At5g59320 3.11 LTP3, lipid transfer protein 3 8.271E-07
262164 at At1g78070 3.11 Transducin/WD40 repeat-like superfamily protein 5.182E-11
246235 at At4g36830 3.11 HOS3-1, GNS1/SUR4 membrane protein family 1.024E-08
249205 at At5g42600 3.10 MRN1, marinal synthase 1.882E-13
246436 at At1g10370 3.09 ATGSTU17, ERD9, GST30, GST30B, Glutathione S-transferase family protein 9.101E-09
247109 at At5g68780 3.09 ATPS5K, PSK5, PSK5, phytosulfokine 5 precursor 1.305E-08
245267 at At4g14060 3.08 Polyketide cyclase/dehydrase and lipid transport superfamily protein 1.080E-17
253872 at At4g27410 3.07 ANAC072, RD26, NAC (No Apical Meristem) domain transcriptional regulator superfamily protein 2.721E-20
265058 at At1g52040 3.07 ATMIP, MBP1, myrosinase-binding protein 1 3.468E-21
247026 at At5g67080 3.07 MAPKK19, mitogen-activated protein kinase kinase kinase 19 5.392E-16
260205 at At1g70700 3.06 JAZ9, TIFY7, TIFY domain/Divergent CCT motif family protein 3.876E-15
251480 at At3g59710 3.06 NAD(P)-binding Rossmann-fold superfamily protein 4.978E-07
248625 at At5g88880 3.06 KAT5, PKT1, PKT2, peroxisomal 3-keto-acyl-CoA thiolase 2 7.153E-16
246376 at At1g51950 3.05 IAA18, indole-3-acetic acid inducible 18 2.484E-15
262873 at At1g64700 3.04 unknown protein; FUNCTIONS IN: molecular_function unknown; INVOLVED IN: N-terminal protein myristoylation; LOCATED IN: cellular_component unknown; EXPRESSED IN: 17 plant structures; EXPRESSED DURING: 11 growth stages; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT3G61920.1); Has 48 Blast hits to 47 proteins in 7 species: Archaea - 0; Bacteria - 1; Metazoa - 26; Fungi - 10; Plants - 48; Viruses - 0; Other Eukaryotes - 26 (source: NCBI BLink).

245275 at At4g15210 3.04 AT-BETA-AMY, ATBETA-AMY, BAMB, BMY1, RAM1, beta-amylase 5 3.408E-20
248311 at At5g52570 3.03 B2, BCH2, BETA-OHASE 2, CHY2, beta-carotene hydroxylase 2 1.603E-12
266532 at At2g16890 3.03 UDP-Glycosyltransferase superfamily protein 6.148E-06
263972 at At2g42760 3.02 unknown protein; CONTAINS InterPro DOMAIN/s: Protein of unknown function DUF1685 (InterPro:IPR012881); Has 170 Blast hits to 164 proteins in 34 species: Archaea - 0; Bacteria - 1; Metazoa - 26; Fungi - 10; Plants - 107; Viruses - 0; Other Eukaryotes - 26 (source: NCBI BLink).

259653 at At1g55240 3.01 Family of unknown function (DUF716) 3.973E-13
255484 at At4g02540 3.01 Cysteine/Hisidine-rich C1 domain family protein 6.119E-12
253074 at At4g36140 3.01 disease resistance protein (TIR-NBS-LRR class), putative 4.445E-07
248395 at At5g22120 3.00 AIP2-A14, PP2-A14, phloem protein 2-A14 2.040E-11

a Arabidopsis thaliana gene identifier code assigned by the Arabidopsis Genome Initiatives (AGI; https://www.arabidopsis.org/portals/nomenclature/).
b Mean of two independent biological experiments (aln-1 versus wild-type).
c Gene symbol and description as provided by The Arabidopsis Information Resource (TAIR; release 10; https://www.arabidopsis.org/).
P-value determined by a two-way ANOVA.
Table S4. Genes with significantly reduced expression in the \textit{ahl-1} mutant.  
Listed are those whose changes in transcript levels decreased by equal to or greater than 3-fold with the statistical significance level of 0.001 by a two-way analysis of variance (ANOVA). These genes were selected from the revised microarray data (Gene Expression Omnibus accession number GSE73841) that had been parametrically renormalized using the SuperNORM data service (Skylight Biotech Inc, Akita, Japan), according to the three-parameter lognormal distribution method (Konishi 2004).

| Affymetrix probe ID | Gene symbol and description | Fold change$^c$ | P-value$^d$ |
|---------------------|-----------------------------|-----------------|------------|
| 263174_at          | At1g54040                    | -43.12          | 6.444E-22  |
| 253707_at          | At4g29200                    | -34.79          | 1.139E-08  |
| 266385_at          | At2g14610                    | -52.83          | 4.608E-06  |
| 266070_t           | At2g18660                    | -28.28          | 3.414E-07  |
| 255437_t           | At4g30360                    | -24.18          | 4.062E-22  |
| 261449_at          | At1g21120                    | -18.58          | 4.834E-06  |
| 257345_at          | Ats4g8640                    | -15.34          | 1.603E-04  |
| 240908_at          | At4g54120                    | -15.06          | 6.134E-07  |
| 259095_at          | At1g75040                    | -13.69          | 3.250E-10  |
| 250445_at          | At5g10760                    | -13.55          | 3.299E-11  |
| 253767_at          | At4g28520                    | -13.53          | 5.723E-08  |
| 265837_at          | At3g14560                    | -13.32          | 3.411E-10  |
| 250476_at          | At3g10140                    | -12.50          | 3.044E-19  |
| 262421_at          | At1g50290                    | -10.78          | 2.203E-22  |
| 246462_s_at        | At3g34920                    | -10.76          | 2.470E-06  |
| 251344_at          | At3g60920                    | -9.34           | 2.455E-08  |
| 254574_at          | At4g19430                    | -8.70           | 1.484E-12  |
| 251625_at          | At3g57260                    | -8.43           | 2.338E-11  |
| 248062_at          | At5g55450                    | -8.29           | 5.891E-16  |
| 265313_at          | At1g78450                    | -8.12           | 2.275E-17  |
| 257365_x_at        | At2g62020                    | -7.51           | 1.537E-06  |
| 266339_at          | At3g23680                    | -7.45           | 5.887E-07  |
| 254863_at          | At4g84900                    | -7.27           | 2.875E-17  |
| 252549_at          | At3g58640                    | -7.09           | 4.908E-11  |
| 265893_at          | At2g15040                    | -7.01           | 1.419E-12  |
| 263947_at          | At2g35582                    | -6.58           | 2.486E-14  |
| 252462_s_at        | At4g23140                    | -6.54           | 1.302E-13  |
| 249777_t           | At5g42110                    | -6.69           | 1.831E-10  |
| 247684_at          | At5g59670                    | -6.59           | 4.733E-05  |
| 264513_at          | At1g09420                    | -5.96           | 9.646E-26  |
| 256003_at          | At3g28270                    | -5.94           | 6.281E-22  |
| 258016_at          | At3g19350                    | -5.92           | 7.141E-09  |
| 255653_at          | At4g09090                    | -5.81           | 2.461E-05  |
| 259385_at          | At1g34770                    | -5.77           | 2.084E-05  |
| 255879_at          | At1g67000                    | -5.70           | 2.648E-06  |
| 249890_at          | At5g22570                    | -5.67           | 1.908E-07  |
| 263535_at          | At5g35820                    | -5.66           | 6.148E-10  |
| 252462_s_at        | At4g23140                    | -5.64           | 1.302E-13  |
| 267253_at          | At2g22960                    | -5.60           | 6.679E-06  |
| 262382_at          | At1g72920                    | -5.52           | 9.049E-10  |
| 263535_at          | At2g17040                    | -5.43           | 2.872E-06  |
| 258028_at          | At3g27473                    | -5.42           | 7.227E-11  |
| 248169_at          | At5g46100                    | -5.76           | 6.468E-10  |
| 256631_at          | At3g28320                    | -4.85           | 5.048E-07  |
| 260658_at          | At2g35770                    | -4.76           | 1.539E-07  |
| 249780_at          | At5g24240                    | -4.67           | 8.731E-11  |
| 249067_at          | At5g23020                    | -4.65           | 6.950E-18  |
| 262631_at          | At1g14100                    | -4.60           | 1.031E-12  |
| 265109_s_at        | Ats6g2630                    | -4.56           | 1.868E-17  |
| 260904_at          | At1g02450                    | -4.52           | 7.944E-07  |
| 257139_at          | At3g28890                    | -4.49           | 1.892E-06  |
| Gene ID     | Gene Symbol | Description                                                                 | Log2 Fold Change | P-value  |
|------------|-------------|-----------------------------------------------------------------------------|------------------|----------|
| 245454_at  | At4g16920   | Disease resistance protein (TIR-NBS-LRR class) family                      | -3.00            | 1.522E-08|
| 261782_at  | At1g76110   | HMG (high mobility group) box protein with ARID/BRIGHT DNA-binding domain   | -3.00            | 1.965E-12|

*Arabidopsis thaliana* gene identifier code assigned by the Arabidopsis Genome Initiatives (AGI; https://www.arabidopsis.org/portals/nomenclature/).

Mean of two independent biological experiments (*aln-1* versus wild-type).

Gene symbol and description as provided by The Arabidopsis Information Resource (TAIR; release 10; https://www.arabidopsis.org/).

P-value determined by a two-way ANOVA.