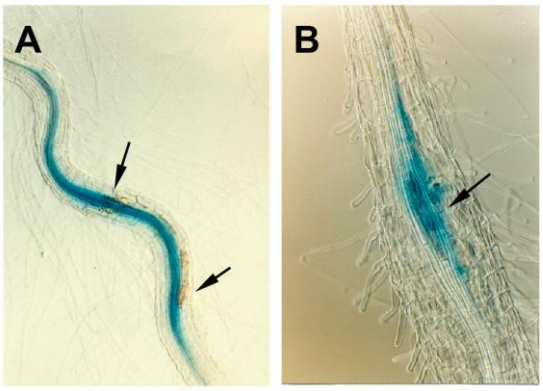
Supplementary information

Expression of the jasmonate signalling repressor *JAZ1/TIFY10A* is stimulated by auxin

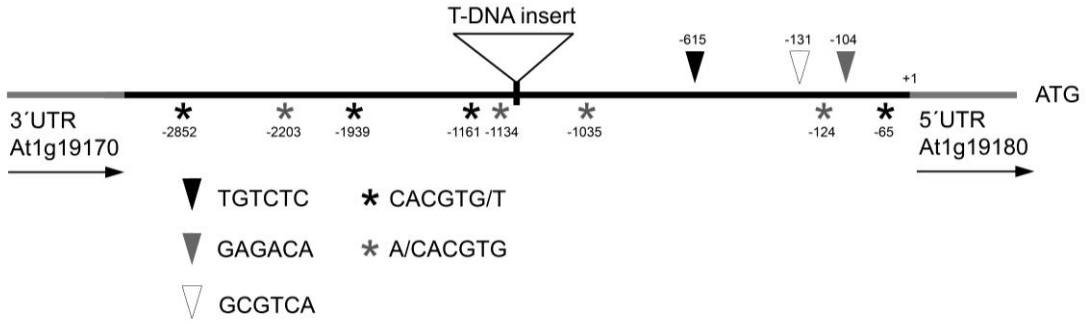
Wim Grunewald, Bartel Vanholme, Laurens Pauwels, Eva Plovie, Dirk Inzé, Godelieve Gheysen and Alain Goossens

Supplemental Results



Supplemental Figure S1

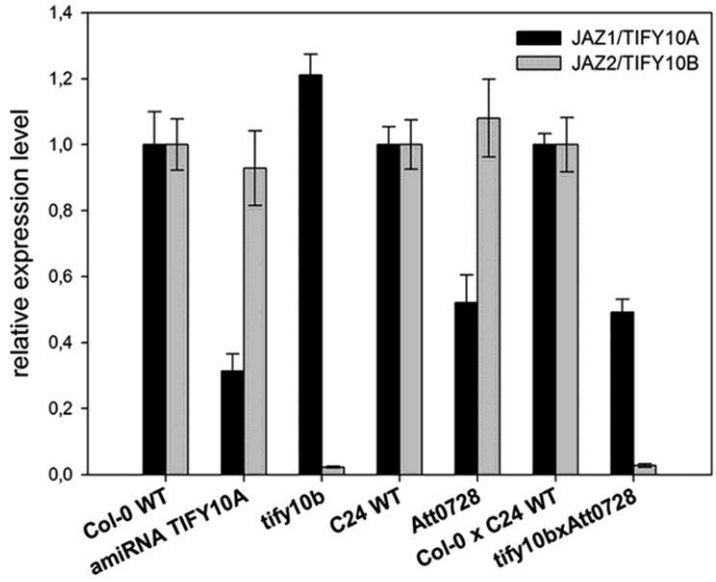
GUS expression patterns observed in the *Arabidopsis thaliana* Att0728 promoter trap line. (A) GUS staining in syncytium induced by *Heterodera schachtii* at 3 dpi. (B) *GUS* expression in *Meloidogyne incognita* gall at 4 dpi. Arrows point to head of nematodes.



Supplemental Figure S2

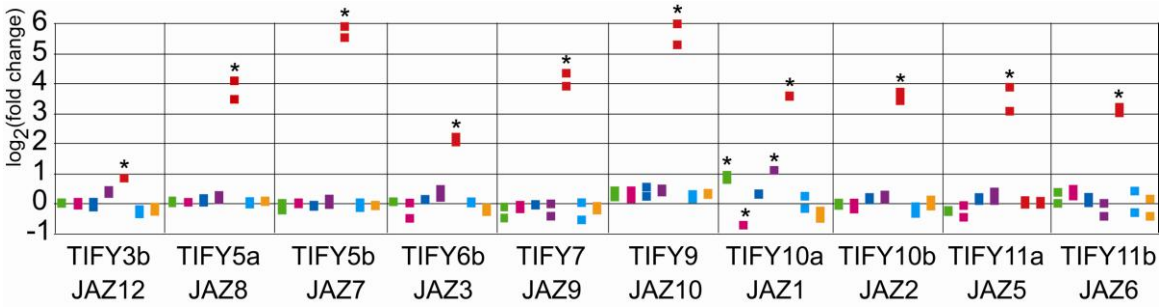
Schematic representation of the *JAZ1/TIFY10A* (*At1g19180*) promoter showing the T-DNA inserted in the Att0728 promoter trap line and the auxin- and jasmonate-responsive elements.

Triangles indicate AREs, asterisks G-box elements and numbers the position to the transcription start.



Supplemental Figure S3

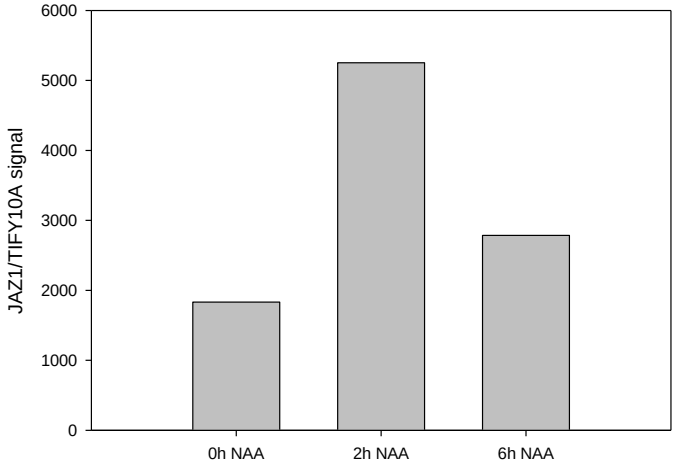
Expression levels of *JAZ1/TIFY10A* (black) and *JAZ2/TIFY10B* (grey) in *TIFY10* knock-down and knock-out lines. Expression levels as derived by qRT-PCR are plotted relative to the corresponding control. Error bars indicate standard error.



Supplemental Figure S4

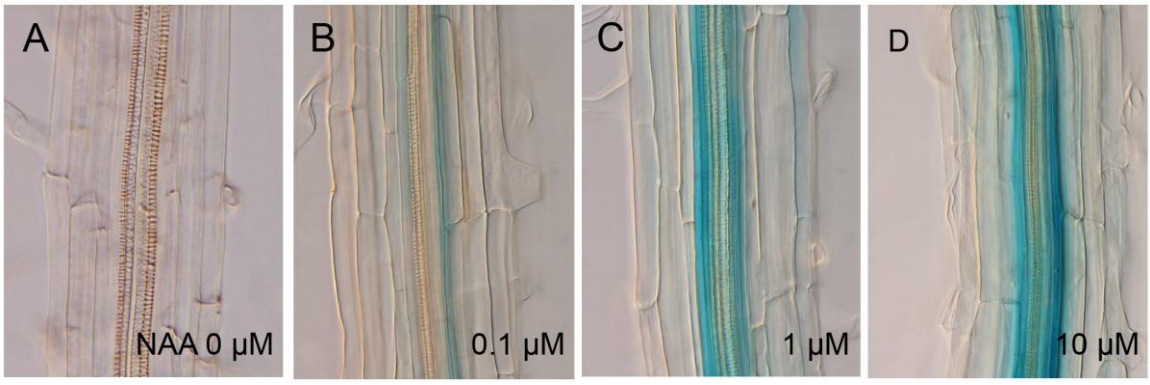
Transcriptional induction of *TIFY* genes in Arabidopsis seedlings treated with seven different phytohormones for 30 min (data derived from Goda *et al*, 2008). Only the JA-responsive *TIFY* genes are depicted. Non-JA-responsive *TIFY* genes did not significantly respond to any other phytohormone treatment within 30 min. In all cases, fold changes are plotted from two biological repeat experiments compared to the average of the control experiments. Color code:

green, indole-3-acetic acid (IAA 1 µM); pink, zeatin (cytokinin 1 µM); dark blue, gibberellic acid (GA_3 1 µM); purple, abscisic acid (ABA 10 µM); red, methyl jasmonate (MeJA 10 µM); light blue, 1-amino-cyclopropane-1-carboxylic acid (ACC 10 µM); orange, brassinolide (BL 10 nM). Significant responses (false discovery rate corrected p-value<0.05) are marked with an asterisk.



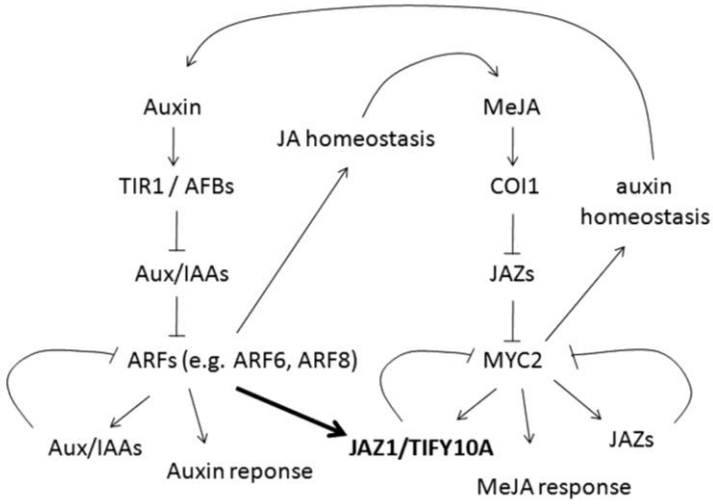
Supplemental Figure S5

Transcriptional induction of *JAZ1/TIFY10A* in Arabidopsis seedlings germinated and grown for 72h on NPA (10 µM) and then transferred to NAA (10 µM) (data derived from Vanneste *et al* (2005)).



Supplemental Figure S6

Auxin-induced *JAZ1/TIFY10A* promoter activity. Ten-day-old *JAZ1/TIFY10A::GUS* seedlings were transferred to and incubated for 2h on medium containing 0 µM NAA (A), 0.1 µM NAA (B), 1 µM NAA (C) and 10 µM NAA (D). Subsequently GUS activity was visualized.



Supplemental Figure S7

Tentative model connecting auxin and JA signalling pathways through the auxin-induced expression of *JAZ1/TIFY10A*.

Supplemental Material and methods

Plant lines and growth conditions

The tagged line Att0728 (ecotype C24) was transformed by the promoter trap vector pΔgusBin19 (Topping *et al*, 1991). Mutants *arf6*, *arf8* and *arf6arf8* were described by Nagpal *et al* (2005); *HS::axr3-1* and *HS::shy2-6* by Knox *et al* (2003); *coi1-16* by Ellis and Turner (2002); and *myc2* (*jin1*) by Berger *et al* (1996). For *in vitro* growth studies, seeds were vernalized, surface sterilized (2 min 70% ethanol, 15 min 5% sodium hypochlorite and 10 washes with sterile water), and plated on germination medium (0.5x MS salts, 1% sucrose, 0.8% agar, pH 5.7). Plants were grown *in vitro* at 21°C under continuous light.

Identification of At1g19180, vector construction and transformation procedures

Inverse PCRs were done on genomic DNA of the tagged Att0728 line as described in Grunewald *et al* (2008). To identify which candidate gene corresponded to the tagged promoter

a semi-quantitative RT-PCR was done using primers 22, primer 28 and primer 43 for *At1g19180*, primers 20 and 21 for *At1g19170* and primers 55 and 56 for *ACTIN-2*. Primers are listed in Table S1. The samples were separated on a 1% agarose gel and transferred onto Hybond N+ membranes (GE-Healthcare, http://www.gehealthcare.com/). The membranes were hybridised with ^{32}P-dCTP-labelled probes, the bands were visualized with a Phosphorimager (GE-Healthcare) and signals were quantified with the IMAGE QUANT software (GE-Healthcare).

For the *TIFY10A::GUS* reporter construct, a 1356 bp fragment of the *JAZ1/TIFY10A* promoter was amplified with the primers 60 and 61 (Table S1) and Gateway recombined with pDONRP4P1R (Invitrogen) to yield pEN-L4-ProTIFY10A-R1. The latter vector was recombined by Gateway LR cloning with pmK7S*NFm14GW (Karimi *et al*, 2007). The amiRNA construct was designed and constructed as described (Schwab *et al*, 2006), using primers miR-s, miR-a, miR*s and miR*a (Table S1). Gateway recombination sites were added in the final PCR. The vector pB2GW7 was used as a Gateway destination vector allowing 35S expression of the construct. To obtain the JAZ1/TIFY10A-GFP lines, *At1g19180* was cloned using primers del1 and del2 for N-terminal fusion and primers del1 and del3 for C-terminal fusion (Table S1). In a second PCR Gateway recombination sites were added. All obtained constructs were mobilized to the *Agrobacterium tumefaciens* strain C58C1Rif^{R} and introduced into *Arabidopsis thaliana* ecotype Col-0 using the floral dip method (Clough & Bent, 1998). *JAZ1/TIFY10A* fragments were obtained using primers del4 and del2 or del3 for N-or C-terminal GFP fusion respectively for Δ1; primers del4 and del5 for Δ2; primers del6 and del3 for Δ3; primers del6 and del7 for Δ4; primers del6 and del8 for Δ5; primers del6 and del9 for Δ6. Primers are listed in Table S1. The obtained fragments were cloned in the pK7WGF2 or pK7FWG2 vectors (Karimi *et al*, 2002) using the Gateway Cloning facilities. The obtained vectors were delivered to tobacco (*Nicotiana tabacum*) Bright Yellow-2 cells using

particle bombardment according to Tytgat *et al* (2004). After transformation (16 h), the cells were analyzed with a Radiance2000 (Bio-Rad) confocal microscope.

GUS histochemical assay and microscopy

Histochemical localization of GUS activity and microscopical analysis were performed as described by Grunewald *et al* (2008). Plants were incubated for 30 minutes in 90% aceton at 4°C and then washed twice with NT-buffer (100 mM Tris HCl/50 mM NaCl solution, pH 7.5). Subsequently, samples were incubated at 37°C in NT-buffer supplemented with 2 mM ferricyanide. After 30 min, the GUS staining reaction was performed at 37°C in fresh NT-buffer supplemented with ferricyanide and X-Glu (26.1 mg dissolved in dimethyl sulfoxide for 25 ml staining solution). After the GUS staining, the samples were washed in NT-buffer, cleared using lactic acid, and analyzed using a differential interference contrast light microscope (Olympus) and photographed using a Nikon digital camera. Confocal microscopy of roots was done using a Zeiss LSM 510 confocal microscope; roots were briefly incubated in propidiumiodide (3mg/l), washed and subsequently mounted in milliQ water.

Nematode culture and infection tests

Cultures of root-knot nematode (*Meloidogyne incognita*) were maintained *in vitro* on roots of tomato (*Lycopersicon esculentum* cv Marmande) transformed with *Agrobacterium rhizogenes* and grown on Gamborg's B5 medium (Duchefa, Haarlem, The Netherlands) at 28ºC. Stage 2 juveniles (J2's) were obtained from egg masses hatched in sterile water 6 to 8 weeks after inoculation. Cyst nematodes (*Heterodera schachtii*) were grown *in vitro* on roots of mustard (*Sinapis alba*) on Knop medium (Sijmons *et al*, 1991). J2's were obtained from cysts hatched in 3 mM $ZnCl_2$ 6 to 8 weeks after inoculation. For infection tests, seedlings were grown vertically to promote unidirectional root growth and 2 weeks post germination each each plant was

inoculated with about 20 *H. schachtii* or *M. incognita* juveniles. Therefore, the hatched nematodes were collected in 50 ml falcon tubes and centrifuged 3 min at 1000 rpm. The supernatant was removed and the nematodes were resuspended in sterile 0.3% low melting point agarose. The solution was diluted until the desired nematode concentration (20 J2's per 5µl) was reached. After infection, seedlings were stained for GUS activity at time points mentioned in figure legends.

Sequence analysis and bioinformatics

Microarray data (Goda *et al*, 2008) were obtained from AtGenExpress. Raw expression data for wild type plants treated for 30 min were processed and statistically analyzed as described in Pauwels *et al* (2008).

Supplemental Table S1

Sequences for all primers described in Methods

name	**primer sequence 5'-xxx-3'**	**name**	**primer sequence 5'-xxx-3'**
miR-s	gattagaatatagtcaatggcgctctctctttgtattcc	primer 21	ttctcgcaaggtcgaggaa
miR-a	gagcgccattgactatattctaatcaaagagaatcaatga	primer 55	aagagagaaagtaagagataatccaggag
miR*s	gagcaccattgactaaattctattcacaggtcgtgatatg	primer 56	caacactgggaaaaacacc
miR*a	gaatagaatttagtcaatggtgctctacatatatattcct	EEF FW	ctggaggttttgaggctggtat
del1	aaaaagcaggcttcatgtcgagttctatggaatg	EEF RV	ccaagggtgaaagcaagaaga
del2	agaaagctgggtgtcatatttcagctgctaaacc	CDKA FW	attgcgtattgccactctcatagg
del3	agaaagctgggtgtatttcagctgctaaaccgag	CDKA RV	tcctgacagggataccgaatgc
del4	aaaaagcaggcttcatgagtttattcccctt	JAZ1 FW	gagcaaaggcaccgctaata
del5	agaaagctgggtgagcaataggaagttctg	JAZ1 RV	tgcgatagtagcgatgttgc
del6	aaaaagcaggcttcaccatgagaagagctt	JAZ2 FW	gcttcacttcatcggttcct
del7	agaaagctgggtgtctgtcctttctcttct	JAZ2 RV	ttggtatggtgcctttgatg
del8	agaaagctgggtgctttgacgtaactctgtcc	IAA1 FW	accgaccaacatccaatctc
del9	agaaagctgggtgtaattggtatggtgcctt	IAA1 RV	tggacggagctccatatctc
primer 22	aactttaggcaactcacgtcagc	IAA5 FW	tccaaggaacatttcccaag
primer 28	agggtttgaagacgctttggctgg	IAA5 RV	ccggagaaagaacagtctcg
primer 43	atgtcgagttctatggaatgttct	IAA19 FW	gtggtgacgctgagaaggtt
primer 20	attaaaacggctccaggacgag	IAA19 RV	cgtggtcgaagcttccttac
primer 61	ggggactgctttttgtacaaacttgtctttaacaa ttaaaactttc	primer 60	ggggacaactttgtatagaaaagttggactgcacacttg ccaaccttctttcc

Supplemental References

Berger S, Bell E, Mullet JE (1996) Two Methyl Jasmonate-Insensitive Mutants Show Altered Expression of AtVsp in Response to Methyl Jasmonate and Wounding. *Plant Physiol* **111:** 525-531

Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J* **16:** 735-743

Ellis C, Turner JG (2002) A conditionally fertile coi1 allele indicates cross-talk between plant hormone signalling pathways in Arabidopsis thaliana seeds and young seedlings. *Planta* **215:** 549-556

Goda H, Sasaki E, Akiyama K, Maruyama-Nakashita A, Nakabayashi K, Li W, Ogawa M, Yamauchi Y, Preston J, Aoki K *et al* (2008) The AtGenExpress hormone and chemical treatment data set: experimental design, data evaluation, model data analysis and data access. *Plant J* **55:** 526-542

Grunewald W, Karimi M, Wieczorek K, Van de Cappelle E, Wischnitzki E, Grundler F, Inze D, Beeckman T, Gheysen G (2008) A role for AtWRKY23 in feeding site establishment of plant-parasitic nematodes. *Plant Physiology* **148:** 358-368

Karimi M, Depicker A, Hilson P (2007) Recombinational cloning with plant gateway vectors. *Plant Physiol* **145:** 1144-1154

Karimi M, Inze D, Depicker A (2002) GATEWAY vectors for Agrobacterium-mediated plant transformation. *Trends Plant Sci* **7:** 193-195

Knox K, Grierson CS, Leyser O (2003) AXR3 and SHY2 interact to regulate root hair development. *Development* **130:** 5769-5777

Nagpal P, Ellis CM, Weber H, Ploense SE, Barkawi LS, Guilfoyle TJ, Hagen G, Alonso JM, Cohen JD, Farmer EE *et al* (2005) Auxin response factors ARF6 and ARF8 promote jasmonic acid production and flower maturation. *Development* **132:** 4107-4118

Pauwels L, Morreel K, De Witte E, Lammertyn F, Van Montagu M, Boerjan W, Inze D, Goossens A (2008) Mapping methyl jasmonate-mediated transcriptional reprogramming of metabolism and cell cycle progression in cultured Arabidopsis cells. *Proc Natl Acad Sci U S A* **105:** 1380-1385

Schwab R, Ossowski S, Riester M, Warthmann N, Weigel D (2006) Highly specific gene silencing by artificial microRNAs in Arabidopsis. *Plant Cell* **18:** 1121-1133

Sijmons PC, Grundler FMW, Vonmende N, Burrows PR, Wyss U (1991) Arabidopsis-Thaliana as a New Model Host for Plant-Parasitic Nematodes. *Plant Journal* **1:** 245-254

Topping JF, Wei W, Lindsey K (1991) Functional tagging of regulatory elements in the plant genome. *Development* **112:** 1009-1019

Tytgat T, Vanholme B, De Meutter J, Claeys M, Couvreur M, Vanhoutte I, Gheysen G, Van Criekinge W, Borgonie G, Coomans A *et al* (2004) A new class of ubiquitin extension proteins secreted by the dorsal pharyngeal gland in plant parasitic cyst nematodes. *Mol Plant Microbe Interact* **17:** 846-852

Vanneste S, De Rybel B, Beemster GTS, Ljung K, De Smet I, Van Isterdael G, Naudts M, Iida R, Gruissem W, Tasaka M *et al* (2005) Cell cycle progression in the pericycle is not sufficient for SOLITARY ROOT/IAA14-mediated lateral root initiation in Arabidopsis thaliana. *Plant Cell* **17:** 3035-3050