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SUPPLEMENTARY INFORMATION

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Jasmonate signalling in *Arabidopsis* involves SGT1b–HSP70–HSP90 chaperone complexes

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Supplementary Methods:

Identification of *hsm* Mutants

CYP71A12::GUS transgenic seeds were mutagenized by soaking in 0.3% EMS for 15 hrs. After washing 10 times with water, ~5,000 M1 seeds were sown onto soil. A total of 3,730 M1 plants (373 pools of 10 plants each) were used to generate M2 seeds. Approximately 80 M2 seeds obtained from each of the 373 pools were distributed into 2 wells of a 6-well plate and grown in 1x liquid MS medium in a growth chamber. On day 10, seedlings were treated with 100 nM flg22 and 0.5 μM COR for 5 to 6 hours followed by 1-hour non-lethal GUS staining at 37°C (see below for details). After GUS staining, seedlings were rinsed with sterile de-ionized water and then left in fresh MS medium until mutants were identified. Roots were examined with a magnifying glass and putative mutants were selected on the basis of retained GUS staining in the root elongation zone and transferred to MS agar plates. Primary mutants were subjected to secondary and tertiary rounds of screening to eliminate false positive mutants. The resulting mutants were backcrossed once to the parental lines to reduce the number of background EMS mutations and were crossed to *coi1-1* and *myc2* for testing allelism.

β-Glucuronidase Histochemical Staining

Ten-day old seedlings were treated with 0.5 μM COR, 100 nM flg22 or both. Six hours after treatment, the MS medium was replaced with the GUS staining solution (50 mM sodium phosphate, pH7, 10 mM EDTA, 0.5 mM $K_4[Fe(CN)_6]$, 0.5 mM $K_3[Fe(CN)_6]$, 0.5 mM X-gluc (Rose Scientific), and 0.01% Triton X-100. The seedlings were vacuum-infiltrated for 5 minutes and then incubated at 37°C for 3 to 8 hours. GUS staining in roots was observed using a Discovery V12 microscope (Zeiss). For non-lethal GUS staining, seedlings were incubated for 1 hr at 37°C in 50 mM sodium phosphate, pH 7, 5 mM EDTA, and 0.5 mM X-gluc.

Generation of Transgenic Plants

The coding sequence excluding the stop codon of *SGT1b* and a truncated *SGT1b* gene encoding the first 250 amino acids of SGT1b were fused to the 2X FLAG epitope tag. The resulting constructs were cloned into the pPLV3 binary vector using a ligation-independent cloning strategy[1]. The binary constructs were transformed into *Agrobacterium tumefaciens* strain GV3101 harboring the pSoup helper plasmid. Plant transformation in the *hsm1* mutant background was performed using the floral dipping method[2]. Stable transformants were selected on MS agar supplemented with 35 µg/L Hygromycin.

RNA Extraction and Quantitative RT-PCR

RNAs were extracted from root tissues using TRIzol (Invitrogen) according to the manufacturer's instructions. RNA extracts were treated with TURBO DNase (Ambion) followed by DNase inactivation using the DNase removal kit (Ambion). 1 µg of RNA was used for reverse transcription using the iScript reverse transcription supermix (Bio-Rad) according to the manufacturer's instructions. Quantitative RT-PCRs were performed using the SYBR green PCR master mix (Applied Biosystems) on the GeneAmp Fast PCR system 9700 (Applied Biosystems).

References:

1 De Rybel, B. *et al.* A versatile set of ligation-independent cloning vectors for functional studies in plants. *Plant Physiol* **156**, 1292-1299, doi:10.1104/pp.111.177337 (2011).
2 Clough, S. J. & Bent, A. F. Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J* **16**, 735-743 (1998).

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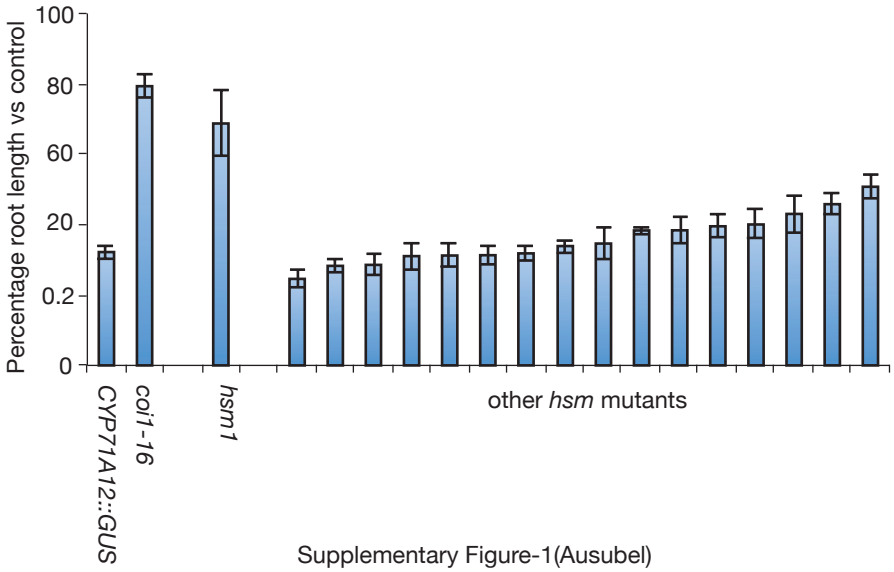
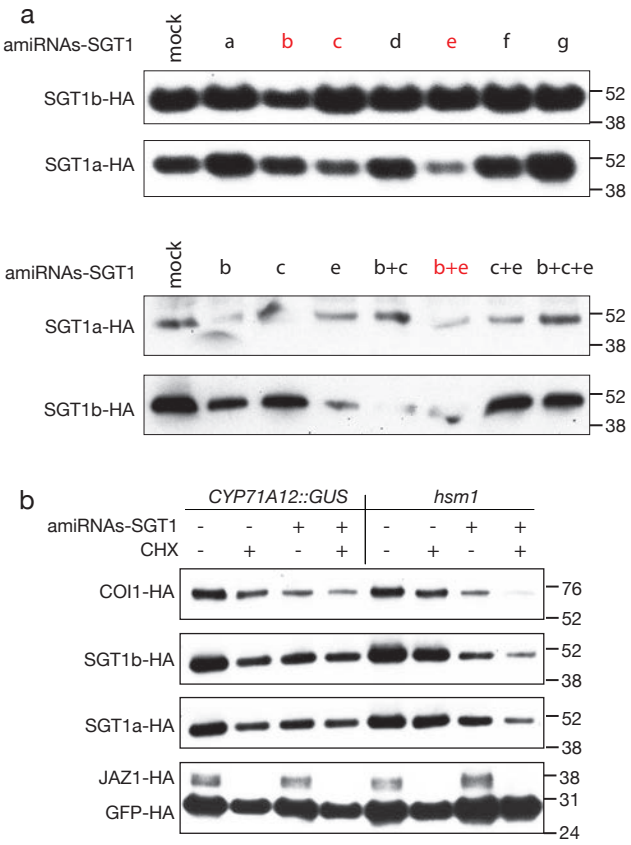


Supplementary Figure 1. MeJA-mediated root growth inhibition in roots. Seedlings were grown vertically and root lengths were measured 7 days after sowing onto MS agar plates supplied with 20 μM MeJA. Data are represented as mean±SE of a minimum of 15 seedlings.



Supplementary Figure-2(Ausubel)

Supplementary Figure-2. (a) Screening of effective amiRNAs for knocking down SGT1b and SGT1a in protoplasts. Seven amiRNAs were designed based on the SGT1b sequence. In the first screening (top panel), 40 µg of each amiRNA plasmid in a volume of 20 µl were transfected into 0.2 ml protoplasts for 12 hours. In the second screening (bottom panel), 40 µg of amiRNA-b, amiRNA-c, and amiRNA-e, either alone or in combination, were transformed into 0.2 ml protoplasts for 18 hours. (b) Both SGT1a and SGT1b stabilize COI1 in protoplasts. COI1- or JAZ1-HA constructs (under the control of their native cis elements) were co-transfected with two SGT1-specific amiRNAs into protoplasts isolated from CYP71A12::GUS and *hsm1* plants. Cycloheximide (10 µM) was added 12 hours after transfection and protoplasts were harvested 1 hour after cycloheximide treatment for immunoblot analysis. If SGT1a and SGT1b are required for COI1 stability, but not translation, COI-HA protein levels should be lower in protoplasts treated with CHX + amiRNAs. CHX and the amiRNAs had no or little effect on the levels of 35S-GFP-HA, which was included as a negative control, and CHX had either no effect or a modest effect on the levels of SGT1a-HA and SGT1b-HA.

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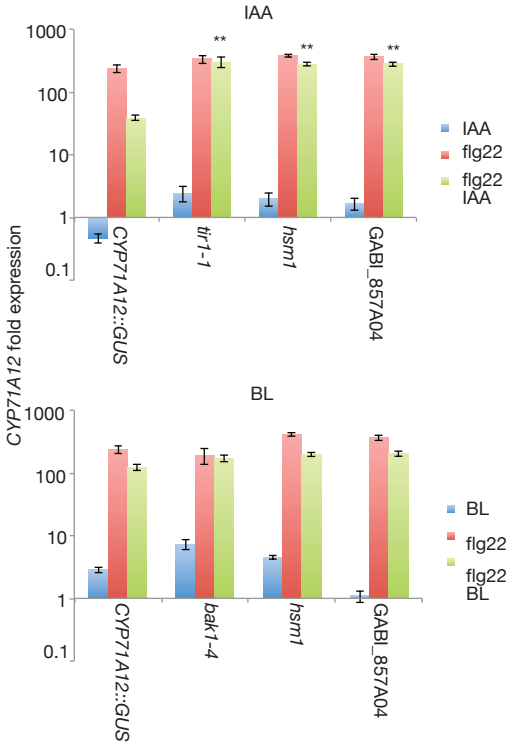
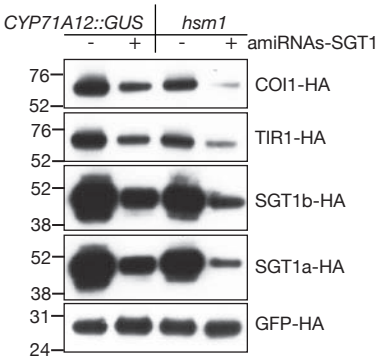


Supplementary Figure-3

Supplementary Figure-3. The *sgt1b* mutants are compromised in responses to IAA, but not BL. Transcript levels of the *CYP71A12* gene in roots were measured 6 hours after treatment with IAA (0.5 μM), BL (1 μM), flg22 (100 nM), or in combination. Data are represented as mean±SE of a minimum of 30 roots. **P<0.01, ***P<0.001.



Supplementary Figure-4(Ausubel)

Supplementary Figure 4. TIR1-HA levels in protoplasts after silencing SGT1b and SGT1a. 35S-TIR1-HA plasmid DNA (and 35S-COI1-HA plasmid DNA as a positive control) were co-transfected with two SGT1-specific amiRNAs in CYP71A12::GUS or *hsm1* protoplasts. 35S-GFP-HA plasmid was used as a transfection control. Protoplasts were harvested at 24 hours after transfection. Numbers on the sides of the blots are protein markers in kDa.

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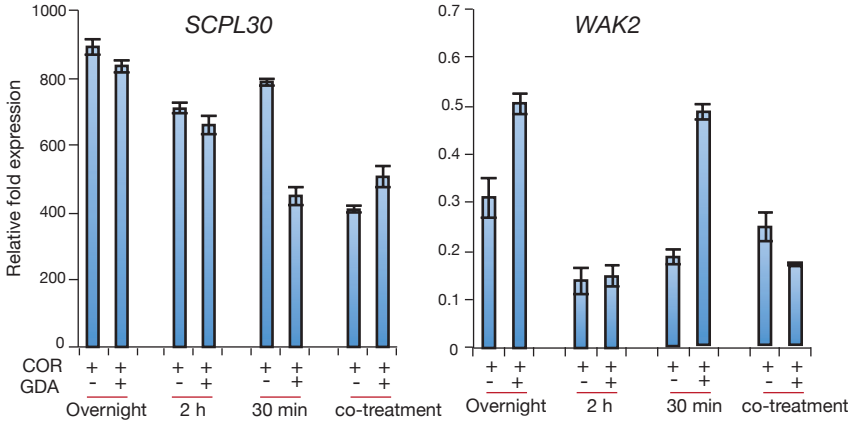

Supplementary Figure-5(Ausubel)

Supplementary Figure 5. Transcript levels of *SCPL30* (At4g15100) and *WAK2* (At1g21270) in roots after pre-treatments at various times with 5 μM GDA followed by treatment with 0.5 μM COR. Roots were harvested 6 hours after COR treatment. Data are represented as mean±SE of a minimum of 30 roots. Note that 30 min pretreatment has a stronger effect in blocking COR-triggered regulation of both genes.

Supplementary Table 2. Primer sequences used for qRT-PCR experiments and for constructing amiRNAs for *SGT1b* and *SGT1a* genes.

Primers for QRT-PCR	
Genes	Primer sequences
EF1aF	TGAGCACGCTCTTCTTGCTTTCA
EF1aR	CGTACCTAGCCTTGGAGTATTT
COI1qF	CTTCCGCCTTGTCTTACTCG
COI1qR	GTTAAGCCGCCTTGTCTCAG
SGT1bqF	TCCTCCTGTTCCAATTCCTG
SGT1bqR	CTGCTCACCAAACTCGACAG
SGT1aqF	AGTGTGACAGCACCACCAGT
SGT1aqR	GCTTGGGTATTCCTTTTGCA
SCPL30qF	GCTGGATATGACCCATGCTT
SCPL30qR	TGCAAATGCTCCAGTTCTTG
VSP2qF	CGTCGATTCGAAAACCATCT
VSP2qR	GGCACCGTGTCGAAGTCTAT
WAK2qF	CAAGGTCTTGGGGTGTTGTT
WAK2qR	GCTATCCTCAGACGGTGCTC
Primers for construting SGT1 amiRNAs	
amisgtb1	gaTATCTGGTATTATCTTCCCTAtctctcttttgtattcc
amisgtb2	gaTAGGGAAGATAATACCAGATAtcaaagagaatcaatga
amisgtb3	gaTAAGGAAGATAATTCCAGATTtcacaggtcgtgatatg
amisgtb4	gaAATCTGGAATTATCTTCCTTAtctacatatatattcct
amisgte1	gaTATCTGGTATTATCTTTCCATtctctcttttgtattcc
amisgte2	gaATGGAAAGATAATACCAGATAtcaaagagaatcaatga
amisgte3	gaATAGAAAGATAATTCCAGATTtcacaggtcgtgatatg
amisgte4	gaAATCTGGAATTATCTTTCTATtctacatatatattcct

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Supplementary Table 1. SGT1b-associated proteins identified by mass spectrometry of SGT1b-HA co-immunoprecipitated samples from portoplast lysates. Hits highlighted in rose red are commonly identified among mock IP, IP by SGT1b-FLAG, and IP by SGT1b(1-250)-FLAG. Hits highlighted in yellow are commonly identified among IP by SGT1b-FLAG and IP by SGT1b(1-250)-FLAG.

Supplementary Table 2. Primer sequences used for qRT-PCR experiments and for constructing amiRNAs for SGT1b and SGT1a genes.