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SUPPLEMENTARY INFORMATION

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Local auxin metabolism regulates environment-induced hypocotyl elongation

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Supplementary Methods

Plant growth conditions and measurement of hypocotyl length. The plant growth conditions and the procedure for screening *sav3* mutant suppressors have been described previously[1]. For measuring hypocotyl length, the plants were grown on 1/2 MS plates or 1/2 MS supplemented with the indicated concentrations of auxin transport inhibitors, 1-N-naphthylphthalamic acid (NPA), 2-[4-(diethylamino)-2-hydroxybenzoyl benzoic acid (BUM), or 2,3,5-triiodobenzoic acid (TIBA), and kept under Wc for 5 d. Some of the plants were then kept in white light condition (WC) for another 4 d, and others were transferred to shade (Shade) for 4 d. For hypocotyl measurements,

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seedlings were scanned using an HP Scanjet 8300 Professional Image Scanner, and the images were analyzed using the ImageJ software (http://rsbweb.nih.gov/ij/). Unless specifically noted, experiments conducted on MS plates were maintained at 22 °C in a growth chamber under continuous white light.

Construct generation. To generate the pVAS2::VAS2-YFP construct, the genomic DNA sequence without a stop codon, along with the 5,531 bp upstream of the ATG start codon, was amplified with primers CACCCATAAACATTTACCTTTCATGGGG and AGAATCTAAACCAAGTGGTTCCC, cloned into the pENTR/D-TOPO vector (Invitrogen) then introduced into pGWB204 (GFP).

To generate pVAS2::GUS construct, primers CACCCATAAACATTTACCTTTCATGGGG and GAGAAGCTTGAGACCAGCCT were used to amplify the 5569 bp VAS2 promoter region, cloned into the pENTR/D-TOPO vector (Invitrogen) and then introduced into pGWB203.

To express VAS1 recombinant protein, the VAS1 cDNA was amplified from the reverse transcription products of total RNA isolated from Col-0 plants with primers CATccatggGATGATACCAAGTTACGACCCAA (NcoI site in lowercase) and GTGgaattcCTAAGAATCTAAACCAAGTGGTTC (EcoR1 site in lowercase), digested with EcoRI and XhoI, and inserted in-frame into the same sites of the NcoI/EcoR1-digested pHIS8-3 expression vector.

Real-time RT-PCR analysis. RNA was extracted using the Spectrum Plant Total RNA Kit (Sigma), and the cDNA was synthesized using the Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Fermentas). Real-time PCR was performed on the CFX384TM Real-Time System (Bio-Rad) using the SYBR green method. The following

PCR procedure was used: 95 °C for 1 min, 45 cycles of 95 °C for 10 s and 60 °C for 1 min. Expression levels for all genes were normalized to the reference gene *At2g39960*, whose expression level is not affected by the growth conditions employed in this study. The gene-specific primer for *VAS2/GH3.17* is : 5′-ACGCAGACACGTCATCAATCCC-3′ and 5′-TGCTGTGACGTGGCTTTAGCTC-3′, the primer for *GH3.9* is: 5′-ACAACTTTCGCAGGATTGTACCG-3′ and 5′-TGGTGCTCCGTTGTAGAAACCC-3′, and the primer for the reference gene *At2g39960* is : 5′-CCATCGACAGTGCTGATCCA-3′ and 5′-CCATTGGGTGACACTTTTGGT-3′.

GUS staining. For checking the expression patterns of the pVAS2::GUS reporter gene, the plants were grown horizontally on 1/2 MS plates for 5 d, then treated with Wc or Shade for 1 h or 24 h as indicated on the figures. For checking the expression patterns of DR5::GUS reporter, the plant growth conditions were same as those for measuring hypocotyl length. For checking the expression patterns of DR5::GUS transgenic lines, the plants were grown horizontally on 1/2 MS plates with or without NPA for 5 d, then maintained under Wc or transferred to Shade for 4 d. GUS staining procedure has been described before[1]. For pVAS2::GUS transgenic lines, the staining lasted 3 hs; for DR5::GUS transgenic lines, the staining lasted 10 hs. In all cases, fifty seedlings of each genotype were used for GUS staining, and the experiments were repeated three times.

GUS activity assays. The hypocotyls of approximately 100 seedlings were collected per sample, and repeated three times for preparation of three replicates. Samples were homogenized in liquid nitrogen, then 500 μL GUS Extraction Buffers [50mM sodium Phosphate, pH 7.0, 10 mM EDTA (pH 8.0), 10 mM β-mercaptoethanol, 0.1% (w/v) SDS, 0.1% (v/v) Triton X-100, 140 μM PMSF] were added, mixed and centrifuged at 14,000 rpm for 20 min at 4 °C. For GUS activity assays, 10 μL supernatants were added to 140

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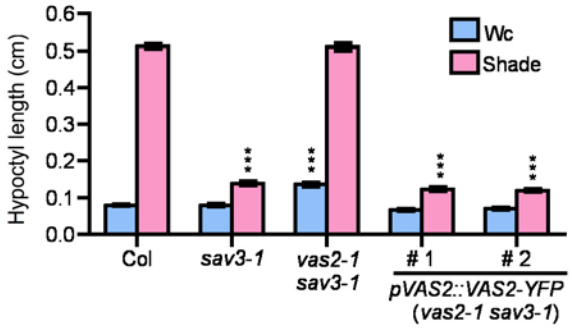
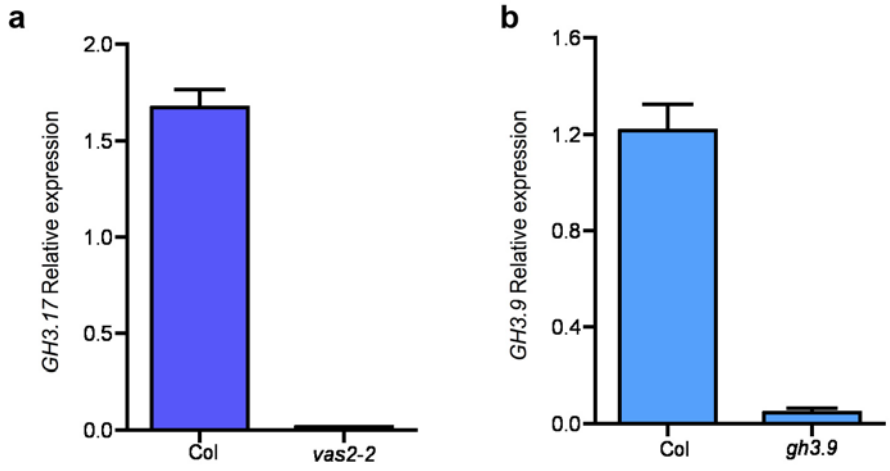
μL Assay Buffer [GUS Extraction Buffer with 1.2 mM 4-methylumbelliferyl β-D-glucuronide (MUG)], and kept at 37 °C in the dark for 20 min. Then, 10 μL of each reaction mixture was then added to 190 μL stop buffer [1 M sodium carbonate] to terminate reactions. The 10 mM 4-methylumbelliferone (MU) stocks were diluted to 0.25 μM, 0.5 μM, 2.5 μM, 5 μM, 25 μM, and 50 μM to prepare the MU standard curve. The fluorescence values of the samples and the MU standards were measured using a Tecan plate reader with the following setting: excitation at 365 nm and emission at 430 nm. To determine the protein concentrations of samples, 10 μL of the above plant extract (in GUS extraction buffer) was added to 200 μL Bio-Rad Protein Assay Dye, and the OD595 was measured. Final GUS activities were calculated and expressed as pmoles MU/min/mg protein.

Supplementary Figures



Supplementary Fig. 1 | Complementation experiments of the *vas2-1 sav3-1* double mutant. The VAS2 genomic DNA fused with a YFP tag driven by the VAS2 native promoter (*pVAS2::VAS2-GFP*) was transformed into *vas2-1 sav3-1* double mutant plants. Plants were grown on 1/2 MS plates, and kept under white light condition (Wc) for 5 d, then maintained in Wc for 4 d or transferred to shade (Shade) for 4 d. Values are expressed as means ± s.e.m., ***P < 0.001 (two-tailed Student's t-test). Comparisons

were made between WT plants and mutants or transgenic plant lines grown under identical conditions.



Supplementary Fig. 2 | T-DNA mutants of *VAS2* and *GH3.9*. a, Expression levels of *VAS2/GH3.17* in WT plants and *vas2-2* mutants (Salk_094646C) were measured using real-time RT-PCR. **b,** Expression levels of *GH3.9* in WT plants and *gh3.9* mutants (CS112245) were examined using real-time RT-PCR.

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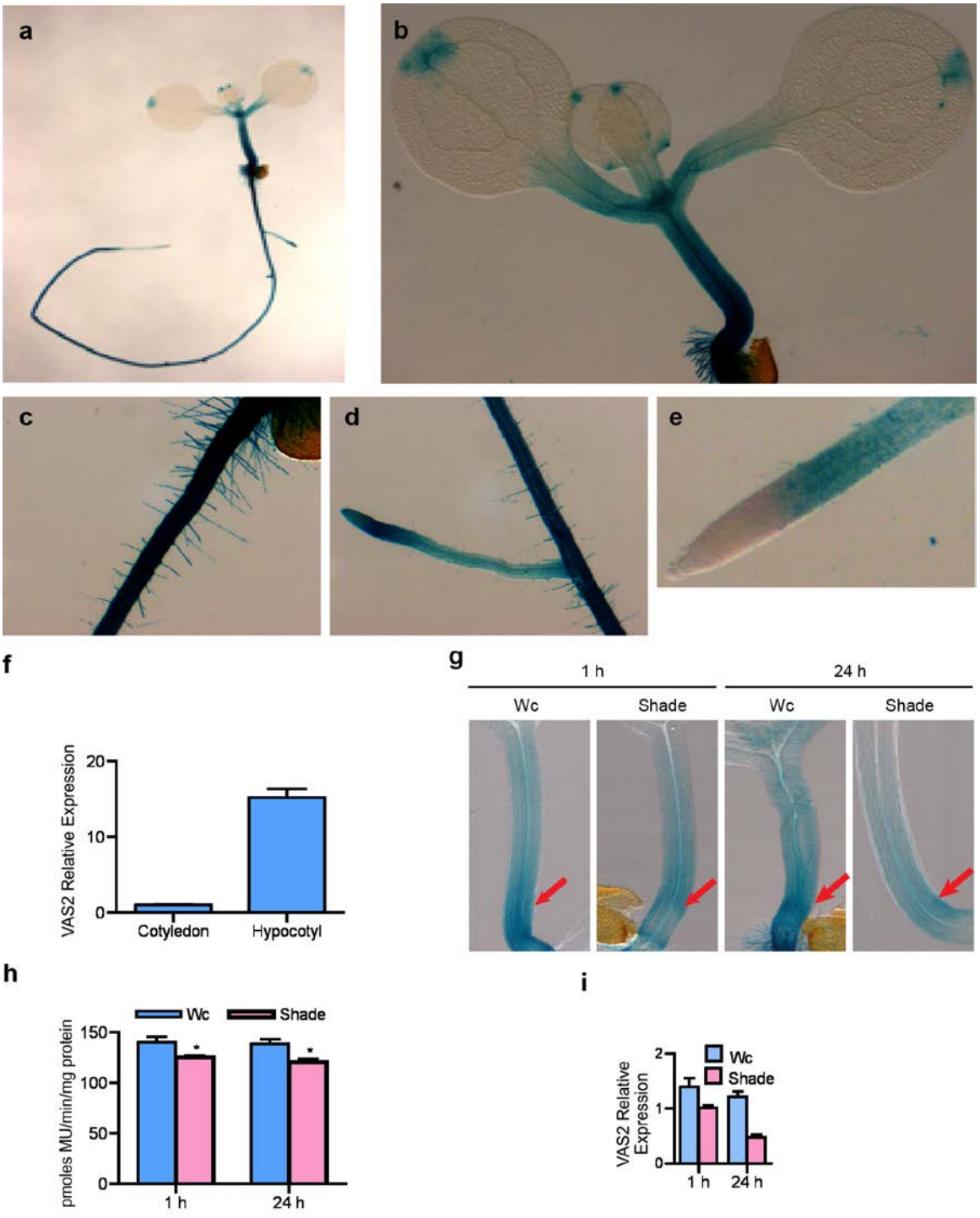

Supplementary Fig. 3 | *pVAS2::GUS* expression patterns and *VAS2*'s transcriptional responses are down-regulated in shade. a-e, *pVAS2::GUS* expression patterns in 6 d old seedlings grown under Wc conditions. **a,** Whole seedlings.

b, Cotyledons, primary leaves, and hypocotyls. **c,** Top portions of the primary root. **d,** **L**ateral root. **e,** **P**rimary root tip. **f,** *VAS2* expression in cotyledons and hypocotyls, respectively, quantified by real-time RT-PCR. **g,** *pVAS2::GUS* expression slightly decreases 1 h and strongly decreases 24 h after shade treatment. The *pVAS2::GUS* transgenic plants in WT background were grown under Wc for 5 days, then maintained in Wc or transferred to Shade for 1 h and 24 h, respectively, as indicated. **h,** Quantification of pVAS2::GUS activities in hypocotyls. Values are means ± s.e.m. *P < 0.05 (two-tailed Student's t-test). Comparisons were made between Wc and Shade. **i,** *VAS2* mRNA levels decrease in response to Shade. *VAS2* expression was quantified by real-time RT-PCR. Values are means ± s.e.m.

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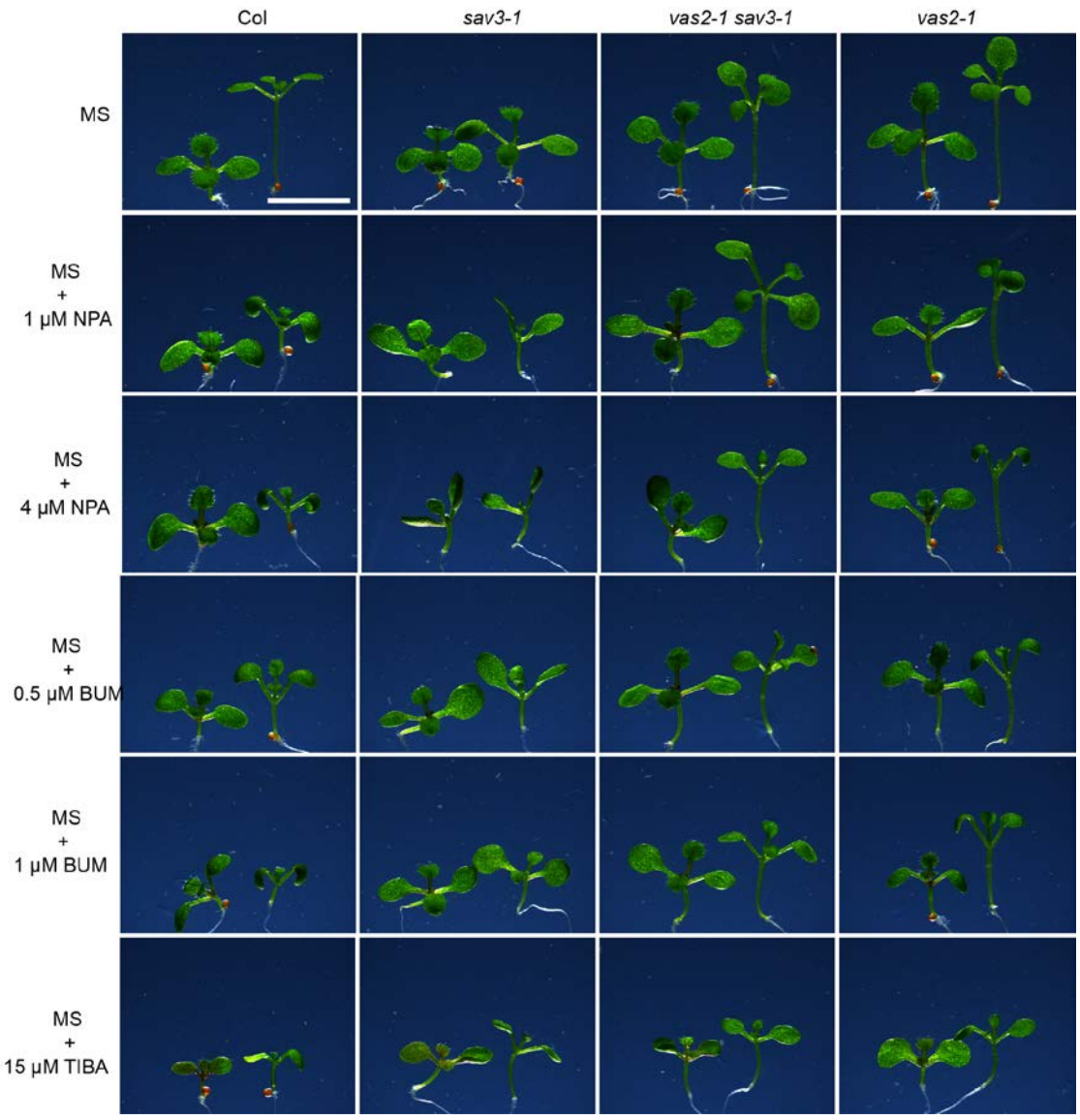
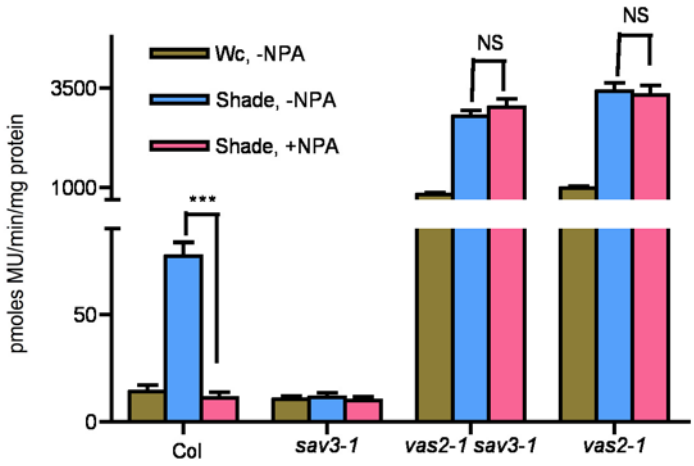


Supplementary Fig. 4 | Shade-induced hypocotyl elongation of *vas2* mutant is only slightly inhibited by auxin transport inhibitors including NPA, BUM and TIBA. Representative seedlings of WT plants, *sav3-1* mutants, *vas2-1 sav3-1* double mutants, and *vas2-1* single mutants are shown. Plants were grown on 1/2MS (MS) plates, or 1/2MS supplemented with 1 μM NPA, 4 μM NPA, 0.5 μM BUM, 1 μM BUM, or 15 μM TIBA, respectively, for 5 d, then maintained in Wc for 4 d (plant on the left of each panel) or transferred to Shade for 4 d (plant on the right of each panel). Scale bar: 5 mm.



Supplementary Fig. 5 | DR5::GUS activities in *vas2* hypocotyls are not repressed by auxin transport inhibitors. *DR5::GUS* was introduced into *sav3-1* mutant, *vas2-1* mutant, and *vas2-1 sav3-1* double mutant by genetic cross. The hypocotyls of WT plants, *sav3-1* mutants, *vas2-1 sav3-1* double mutants and *vas2-1* single mutants grown under indicated conditions were collected for GUS activity assays. Based on two-tailed Student's t-tests, there was no significant difference ($P > 0.05$) between Shade-NPA and Shade+NPA in either *vas2-1 sav3-1* double mutant or *vas2-1* single mutant. NS: no significant difference. ***$P < 0.001$ (two-tailed Student's t-test).

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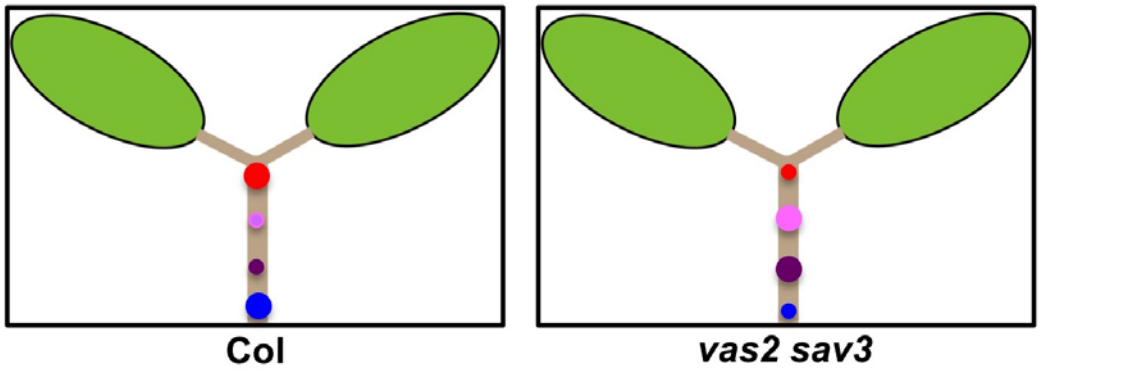

Supplementary Fig. 6 | Auxin in the hypocotyls of WT (Col) and *vas2 sav3* double mutant stimulates hypocotyl elongation in response to environmental cues. Under shade or high temperature condition, WT and *vas2 sav3* double mutant elongate their hypocotyls to similar extent. For WT, large amounts of free auxin in the hypocotyls are converted to IAA-Glu for degradation; thus, its hypocotyl elongation triggered by shade or high temperature needs rapid *de novo* IAA biosynthesis in cotyledon. For *vas2 sav3* double mutant, more free IAA exists in hypocotyl (due to prevention of auxin conjugation to Glu and more storage from of IAA conjugates exist) that is sufficient for promoting hypocotyl elongation in response to shade or high temperature. The relative size of the color-coded dots represents the relative available concentrations of auxin and auxin metabolites.

References:

1 Zheng, Z. *et al.* Coordination of auxin and ethylene biosynthesis by the aminotransferase VAS1. *Nat Chem Biol* **9**, 244-246 (2013).