Supporting Information

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SI Materials and Methods

Plant Material. The mutant and transgenic lines *bzr1-1D, det2-1, ga1-3, gai-1, gai-t6, rga-24, RGA::GFP-RGA, RGA::GFP-(rga-Δ17), 35S::TAP-GAI, HS::gai-1, 35S::gai-1, 35S::BES1-GFP, and BZR1::BZR1-CFP* have been described previously (1–10).

Seedling Growth Assays. Growth conditions, long-term treatments with growth regulators, and hypocotyl length measurement were as described (5), with the difference that in this work seedlings were grown at 22 °C. For gene-expression analysis in the *ga1-3* mutant, seeds were stratified for 5 d in 50 μM gibberellic acid (GA_3) and were washed several times with sterile water before sowing. For the short-term treatments, dark-grown seedlings were soaked in a solution containing the chemicals for the indicated times, with gentle agitation and in complete darkness; seedlings were handled under green safe light. The heat treatment was 1 h at 37 °C in darkness. Paclobutrazol (PAC) and GA_3 were from Duchefa, epibrassinolide (EBR) and cycloheximide (CHX) were from Sigma, and brassinazole (BRZ) was from TCI.

Microarray Analysis. Untreated wild-type Col-0 seedlings, seedlings treated with 1 μM PAC or with 1 μM PAC plus 1 μM EBR, and *det2-1*–mutant seedlings, untreated or treated with 50 μM GA_3, were grown in continuous darkness at 22 °C for 7 d. Total RNA was extracted from whole seedlings with the RNeasy Plant Mini kit (Qiagen). Two biological repeats were used. RNA labeling, hybridization of long oligonucleotide microarrays, and data analysis were as described (11), using *Z* statistics as a diagnostic tool to identify differentially expressed genes (12).

Protein Extraction and Western Blots Analysis. Protein extraction and Western blot analysis from whole seedlings were performed as described (11). The GFP/YFP/CFP, HA, and TAP fusion proteins were detected using anti-GFP (JL8 from Clontech or Ab290 from Abcam), anti-HA (3F10; Roche), and anti–c-myc (9E10; Roche) antibodies, respectively. Endogenous GAI and RGA proteins were detected using polyclonal anti-GAI antibodies (13). Antibodies against DET3 were used to check protein loading (5).

Yeast Two-Hybrid Assays. A *pENTR* vector carrying the coding sequence (CDS) of BRASSINAZOLE RESISTANT1 (*BZR1*) was from the REGIA project (14). Deleted versions of GAI and RGA were amplified by PCR and cloned into *pCR8/GW/TOPO* (Invitrogen) to create *pENTR* vectors. All deletions except the N-terminal fragment (N-ter) were transferred into the *pDEST32* to create Gal4 DNA binding domain (Gal4-DBD) fusions (prey vector) by Gateway (Invitrogen); N-ter was cloned into *pDEST22* as a Gal4-activation domain (Gal4-AD) fusion (bait vector) (Invitrogen), given its strong autoactivation. The full-length *BZR1* CDS was cloned into both *pDEST22* and *pDEST32*, whereas deletions were cloned into *pDEST22*. Final bait and prey constructs were used to cotransform the yeast strain AH109 (Clontech). Yeasts were selected in SD/-Leu/-Trp/-His and with different amounts of 3-aminotriazol (3-AT) (Sigma) to test interactions. Protein fusions to the Gal4-DBD and Gal4-AD were detected by Western blots with antibodies anti-DBD (RK5C1) and anti-AD (C-10) from Santa Cruz Biotechnology.

Bimolecular Fluorescence Complementation and Coimmunoprecipitation Assays. *pENTR* vectors carrying the CDS of *BZR1* and *GAI* were from the REGIA project (14). For bimolecular fluorescence complementation (BiFC), *BZR1* and *GAI* CDSs were transferred into *pMDC43-YFC* and *pMDC43-YFN* vectors (15), respectively, by Gateway. For coimmunoprecipitation (co-IP) assays in *Nicotiana benthamiana*, *GAI* and *BZR1* CDS were transferred as above into *pEarleyGate-104* and *-201* vectors (16) to create the YFP-GAI and HA-BZR1 fusions, respectively. Vectors for BiFC and for co-IP expressed the fusion proteins from the constitutive *35S* promoter. Each construct was introduced into *Agrobacterium tumefaciens* C58 cells that were used to infiltrate *N. benthamiana* leaves. BiFC analysis was performed as described (17). Co-IPs were performed as described (15).

For co-IP in *Arabidopsis*, seedlings of wild-type Col-0 and *BZR1::BZR1-CFP* were grown for 4 d in darkness in MS plates containing 0.5 μM PAC or 0.5 μM PAC plus 1 μM EBR. Seedlings were vacuum infiltrated with 1% (vol/vol) formaldehyde for 10 min. Next, Gly at a final concentration of 125 mM was added to quench crosslinking, and seedlings were vacuum infiltrated for an additional 10 min. The infiltration steps were performed in darkness. Frozen seedlings were ground and homogenized in 1.5 volumes of cold extraction buffer [50 mM Tris·HCl (pH 7.5), 250 mM NaCl, 1% (vol/vol) Nonidet P-40, 1 mM PMSF, and 1× complete protease inhibitor mixture (Roche)]. Extracts were centrifuged twice for 15 min at 16,100 × *g* in a top bench microcentrifuge at 4 °C. Total soluble proteins in the supernatant were quantified by Bradford assay. Ten micrograms of soluble proteins were saved to be used as input, and 600 μg were incubated with 50 μL of anti-GFP paramagnetic MicroBeads (Miltenyi Biotec) on ice for 30 min in a total volume of 1.5 mL. Extracts were loaded at room temperature onto μ columns (Miltenyi Biotec) previously washed with extraction buffer. Columns were kept at room temperature and washed four times with 200 μL of cold washing buffer 1 [50 mM Tris·HCl (pH 7.5), 250 mM NaCl, and 1% (vol/vol) Nonidet P-40] and once with 100 μL of cold washing buffer 2 (20 mM Tris·HCl, pH 7.5). Proteins were eluted in 50 μL of denaturing elution buffer following the manufacturer's instructions. Immunoprecipitated proteins were run in an 8% SDS/PAGE, immunoblotted, and detected with anti-GAI antibodies. Subsequently, blots were stripped out and incubated with anti-GFP antibodies (Ab290; Abcam).

Reporter Construct for Transcriptional Assays. To prepare the reporter constructs, the wild-type and mutant promoters, synthesized by GenScript, were cloned into the *Pst*I and *Nco*I sites of the *pGreenII 0800-LUC* vector (18). As effector proteins, we used YFP-GAI and HA-BZR1 fusions (described above), YFP-del1 that was obtained by cloning the *del1* CDS into *pEarleyGate-104*, and HA-VP16-BZR1, which was obtained by cloning the *BZR1* CDS into the *pAlligator1* vector by Gateway. *pAlligator1* was a gift from François Parcy (Université Joseph Fourier Grenoble I, Grenoble, France).

EMSA. The *BZR1* CDS was cloned by Gateway into *pDEST17* (Invitrogen) to generate a His-tagged fusion. A *pET28a* vector containing the *GAI* CDS that generates a His-tagged fusion was a gift from Luís López-Molina (Université de Geneve, Geneva, Switzerland) (13). Proteins were expressed in *Escherichia coli* Rosetta2 BL-21 cells (Novagen). The wild-type and mutant dsDNA probes were obtained by annealing two complementary oligonucleotides corresponding to the wild-type and mutant monomers used for the synthetic promoters. The sense oligonucleotide was labeled with the TAMRA fluorescent dye at its 5′ end (Sigma). Binding was performed in 20 μL of binding buffer [25 mM Hepes-NaOH (pH 7.5,) 10% (vol/vol) glycerol, 40 mM

KCl, 0.1 mM EDTA, 3 mM DTT, 0.1 mg/mL BSA) using 20 nM of the dsDNA probe and different amounts of the proteins. After 20 min on ice, reactions were loaded onto a native, 8% polyacrylamide 0.5× Tris-borate-EDTA gel. Gel was run at 4 °C and scanned with a Typhoon 9400 scanner (Molecular Dynamics).

ChIP and PCR Amplification. Seedlings of wild-type Col-0 and *BZR1::BZR1-CFP* seedlings were grown at 22 °C for 4 d in darkness before fixation. ChIP assays were performed as described (19). Anti-GFP (Ab290; Abcam) and the EZview High Visibility Affinity Gel (Sigma) were used for immunoprecipitations. Data analysis was done referring the quantifications in immunoprecipitated samples to the corresponding samples of input material and then relative to the value of the wild-type line lacking BZR1-CFP.

RNA Extraction, Northern Blot, and Quantitative RT-PCR. Total RNA was isolated from whole seedlings by using the E.Z.N.A. Plant RNA Mini Kit (Omega Bio-Tek) according to the manufacturer's instructions. *CAB2* expression was analyzed by Northern blot as described (20). cDNA synthesis and quantitative PCR conditions were as described (11).

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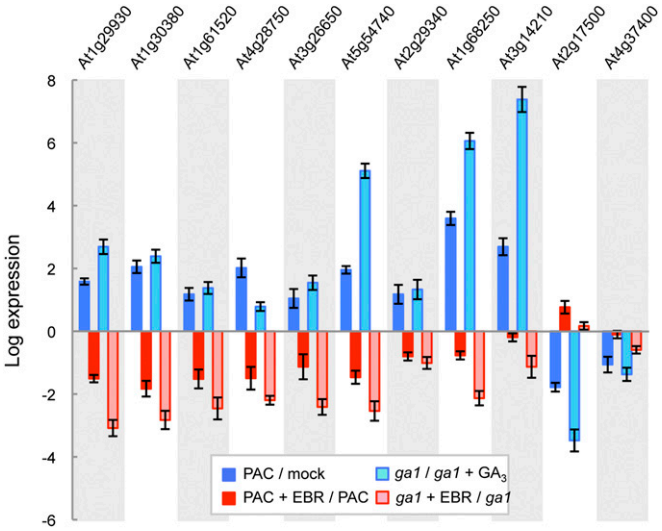

Fig. S1. PAC treatment causes the same behavior as gibberellin (GA) deficiency in the *ga1* mutant. Comparison of the expression levels of selected genes estimated from the microarray analysis (PAC/mock and PAC + EBR/PAC) or by quantitative RT-PCR (*ga1*/*ga1* + GA_3 and *ga1* + EBR/*ga1*). The *ga1-3* seedlings were grown in control MS or in MS supplemented with 50 μM GA_3 or 1 μM EBR for 7 d in darkness. Values are expressed relative to *EF1α*.

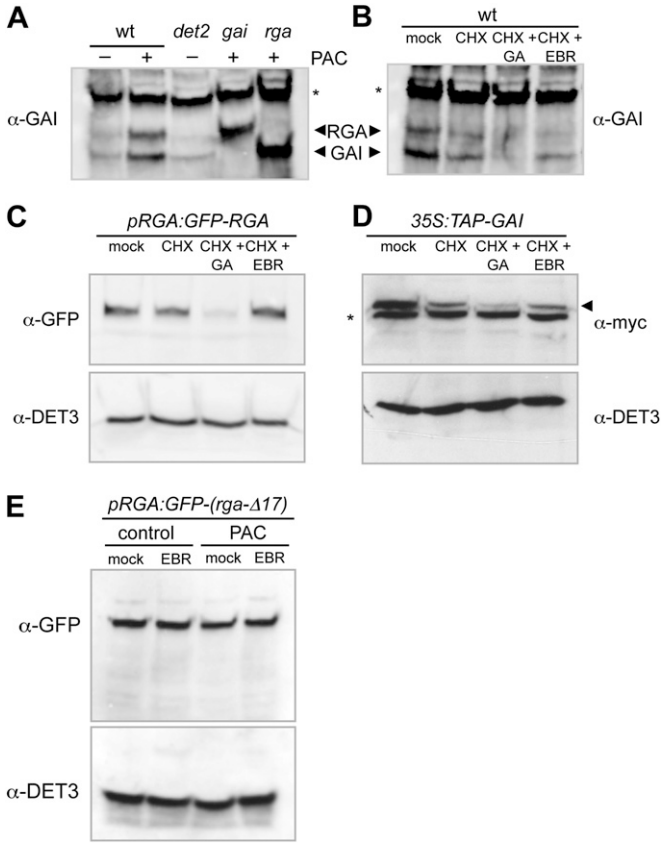

Fig. S2. Brassinosteroids do not affect DELLA stability. (*A*) Wild-type Col-0 seedlings and the mutants *det2-1*, *gai-t6*, and *rga-24* were grown for 4 d in darkness in the presence (+) or absence (–) of 0.5 μM PAC. (*B–D*) Four-day-old wild-type Col-0 (*B*), *RGA::GFP-RGA* (*C*), and *35S::TAP-GAI* (*D*) seedlings were grown in darkness in MS and then were subjected to 1-h treatment with either mock solution (mock), or a solution with 50 μM CHX, 50 μM CHX plus 100 μM GA_3 (CHX + GA), or 50 μM CHX plus 1 μM EBR (CHX + EBR) just before harvesting. The asterisk indicates a cross-reacting, nonspecific band that served as a loading control; the arrowhead indicates TAP-GAI. (*E*) Four-day-old *RGA::GFP-(Δ17-rga)* seedlings were grown in darkness in control MS or in MS containing 0.5 μM PAC and then were subjected to 2-h treatment with either mock solution or 1 μM EBR just before harvesting. Proteins were separated in denaturing 8% PAGE and analyzed by Western blot using anti-GAI antibodies or commercial antibodies against GFP (JL-8; Clontech) or c-myc (9E10; Roche). DET3 immunodetection was used as loading control.

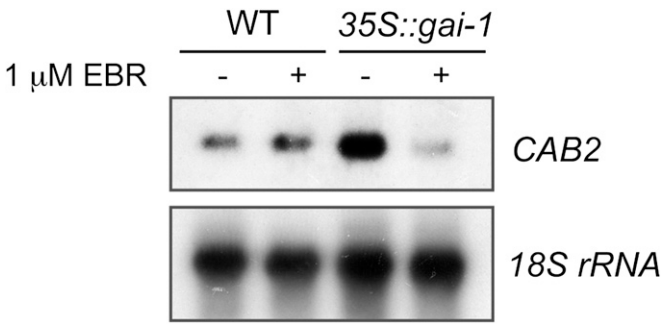

Fig. S3. Brassinosteroids regulate the GA pathway downstream of DELLA proteins. Four-day-old wild-type Col-0 and *35S::gai-1* seedlings were grown in darkness in control medium and in medium supplemented with 1 μM EBR. *CAB2* transcript levels were determined by Northern blot. *18S rRNA* was used as loading control.

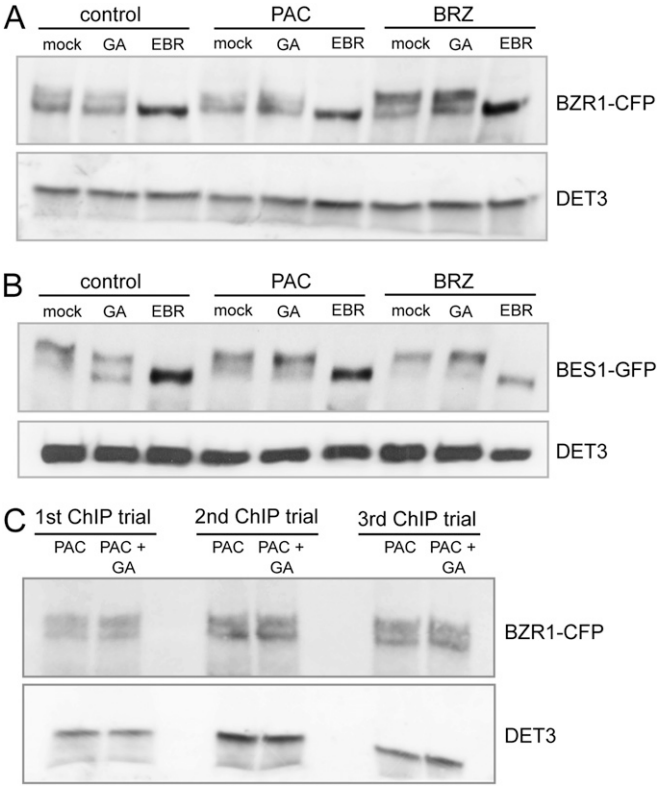

Fig. S4. GAs do not interfere with BZR1 and BES1 phosphorylation or levels. (*A* and *B*) Four-day-old *BZR1::BZR1-CFP* (*A*) and *35S::BES1-GFP* (*B*) seedlings were grown in darkness in control medium or in medium supplemented with 0.5 μM PAC or 3 μM BRZ, as indicated. Seedlings were subject to 2-h treatment with a mock solution or a solution containing 100 μM GA_3 or 1 μM EBR just before harvesting. (*C*) Analysis of the BZR1-CFP protein levels in the tissue samples used for ChIP. Proteins were separated in denaturing 8% PAGE and determined by Western blot analysis using commercial antibodies against GFP (JL-8; Clontech). DET3 immunodetection was used as loading control.

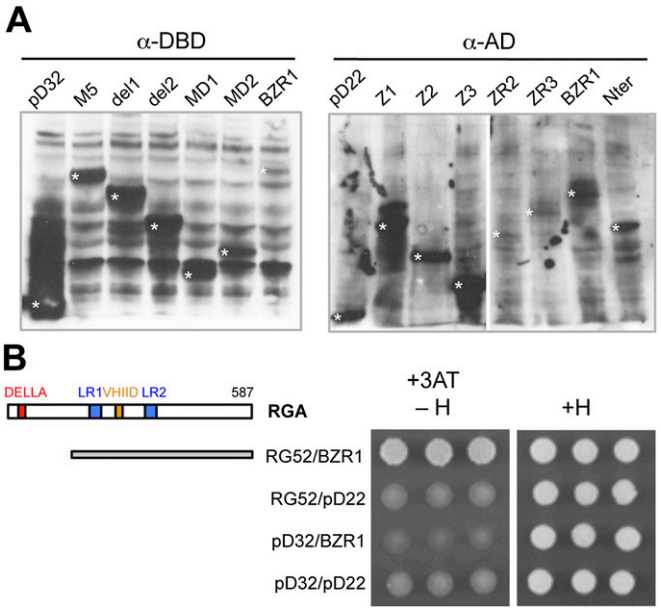

Fig. S5. GAI and BZR1 derivatives are expressed in yeast. (*A*) Fusion proteins were separated in 12% PAGE and examined by Western blots using antibodies against the GAl4-DBD and AD. Asterisks indicate the bands corresponding to the fusion proteins. (*B*) Yeast two-hybrid assays analyzing the interaction between a deleted version of RGA and full-length BZR1. (*Left*) The diagram illustrates the relevant motifs included in the truncated version of RGA. (*Right*) Three drops of the same dilution are shown. +H, control medium containing His. −H +3AT, selective medium lacking His and containing 20 mM 3-AT.

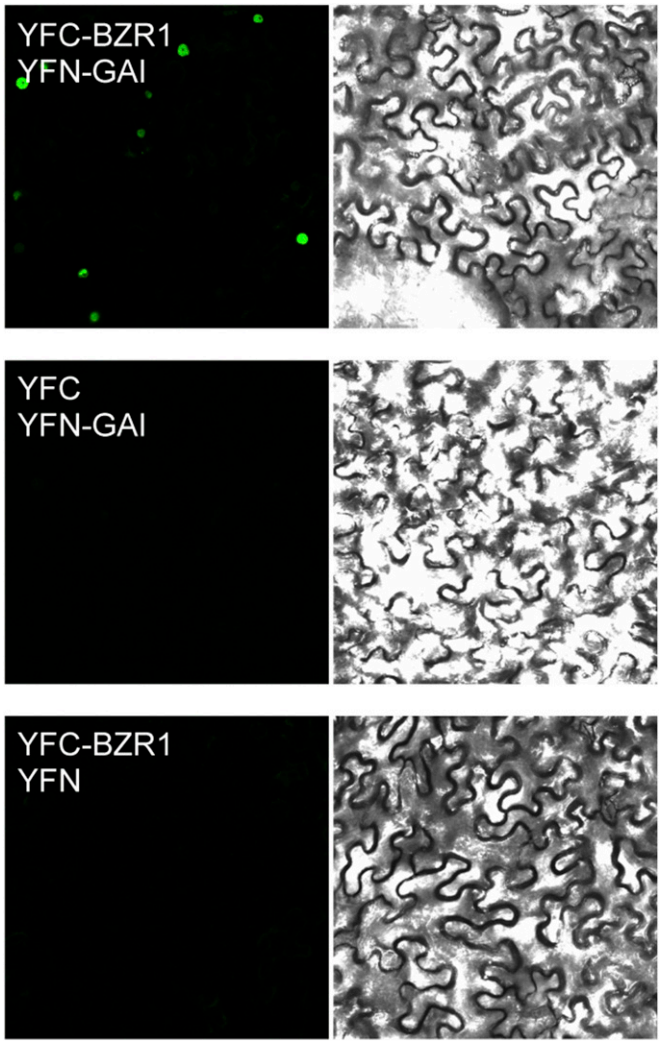

Fig. S6. GAI and BZR1 interact in plant cells. BiFC analysis in *N. benthamiana* of GAI and BZR1 fusions to N- and C-terminal fragments of YFP (YFN and YFC, respectively) and the corresponding controls. (*Left*) YFP fluorescence. (Right) Bright-field image.

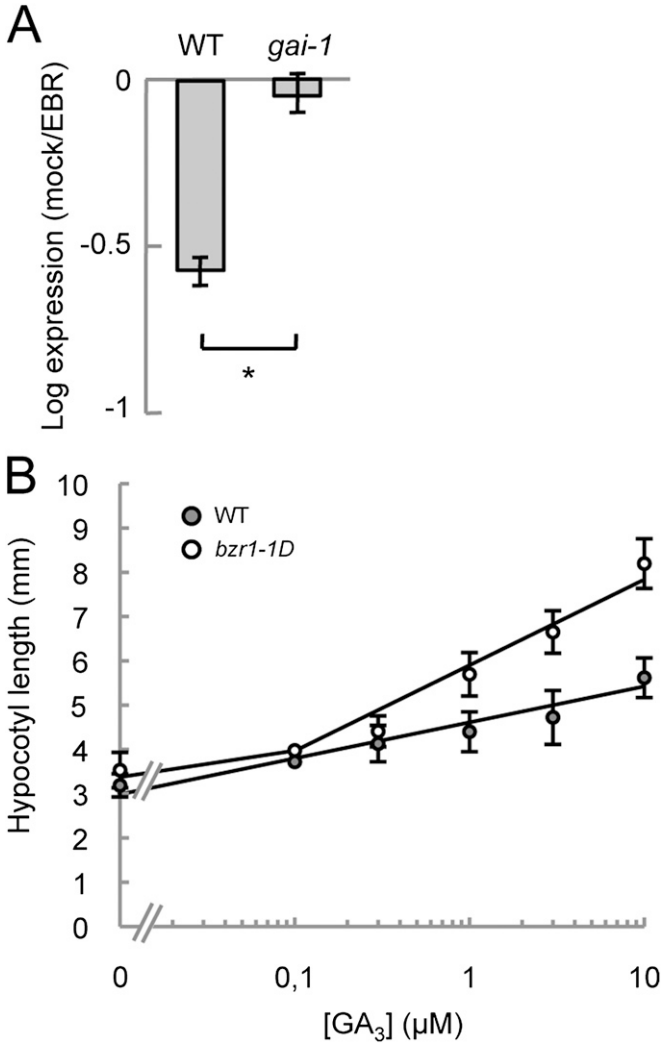

Fig. S7. GAI interferes with BZR1 activity. (*A*) Wild-type and *gai-1* seedlings were grown in control MS for 4 d in darkness and then were subjected to treatment with 1 μM EBR or mock solution for 3 h. *CPD* expression was analyzed by quantitative RT-PCR. Values are expressed relative to *EF1α*. Data represent mean ± SD of three technical replicates. **P* < 0.01 (Student's *t* test). (*B*) BZR1 mediates the hypocotyl growth response to GAs in the light. Graph shows hypocotyl growth of 7-d-old wild-type Col-0 and *bzr1-1D* seedlings in response to different concentrations of GA_3 (0.1, 0.3, 1, 3, and 10 μM). Seedlings were grown under continuous white fluorescent light (50–60 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Error bars indicate SD ($n > 15$ seedlings). Experiments were repeated twice with similar results; results from one representative experiment are shown.

Other Supporting Information Files

Dataset S1 (XLS)
Dataset S2 (XLS)