Supporting Information

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SI Materials and Methods

Binary Vector Constructions. Oligonucleotides that encode the 3×HA, 9×MYC, and 3×FLAG tags were synthesized (Integrated DNA Technologies) and inserted into the NcoI and NheI sites of pET42a (Novagen) to create pET42a-3×HA, -9×MYC, and -3×FLAG, respectively. The resulting 3×HA-8×His, 9×MYC-8×His, and 3×FLAG-8×His inserts were released by NcoI and *Avr*II and inserted into the same sites of pFGC5941 (Chromatin Database, www.chromdb.org), in which the *Eco*RI and XhoI sites were destroyed to create pJYP003, -004, and -011, respectively. An *attR* Gateway cassette was cloned into the *Eco*RI and XhoI sites of pJYP003, -004, and -011 to create Gateway cloning-compatible pJYP005, -006, and -012, respectively.

Molecular Cloning. Coding sequences of *AtJAZ1-12*, *AtJAZ9.2*, various deletions of *AtJAZ9* (1), and *AtMYC2*, excluding the stop codon, were amplified by PCR by using *PfuUltra II* DNA polymerase (Agilent Technologies) and *Arabidopsis* cDNA, which was obtained from a 28-d-old Col-0 plant by using primers listed in Dataset S1. PCR products were first cloned into vector pGEM-T easy (Promega), and then moved into the binary vector pJYP003 to create 3×HA-AtJAZ-8×His fusion constructs or into a gateway entry vector to create gateway entry clones. The Gateway entry clones containing *RGL1*, *RGL2*, or *RGL3* were identified from REGIA collections. All *AtJAZ* genes were cloned into both pDEST32 ((Life Technologies) and pDEST-GBKT7 (2) to create GAL4DB bait vectors. *AtMYC2* and all *DELLA* genes were cloned into both pDEST22 (Life Technologies) and pDEST-GADT7 (2) to create GAL4 prey vectors.

Yeast Two-Hybrid Assay. The GAL4 bait and prey vectors were transformed into yeast strain AH109 (Clontech) or MaV203 (Life Technologies) and assayed following the manufacturer's manual.

Production of Transgenic Plants in *Arabidopsis*. The p*35S*:3×HA-AtJAZ-8×His constructs were transformed into WT Col-0 plants by *Agrobacterium*-mediated transformation. Primary transformants were selected for BASTA resistance, and jasmonate ZIM-domain (JAZ) expression levels were determined by Western blotting. Transgenic lines with a 3:1 (resistant:sensitive to BASTA) segregation ratio were selected, and several homozygous lines were identified in the T3 generation.

Seed Germination Assay. Approximately 120 surface-sterilized *Arabidopsis* seeds were sown on plates with or without 5 μM of paclobutrazol (PAC; Sigma-Aldrich). After stratification, plates were placed in a long-day growth chamber. The percentages of seeds that had germinated with fully expanded green cotyledons were scored at day 5.

1. Melotto M, et al. (2008) A critical role of two positively charged amino acids in the Jas motif of *Arabidopsis* JAZ proteins in mediating coronatine- and jasmonoyl isoleucine-dependent interactions with the COI1 F-box protein. *Plant J* 55:979–988.

2. Rossignol P, Collier S, Bush M, Shaw P, Doonan JH (2007) *Arabidopsis* POT1A interacts with TERT-V(I8), an N-terminal splicing variant of telomerase. *J Cell Sci* 120:3678–3687.

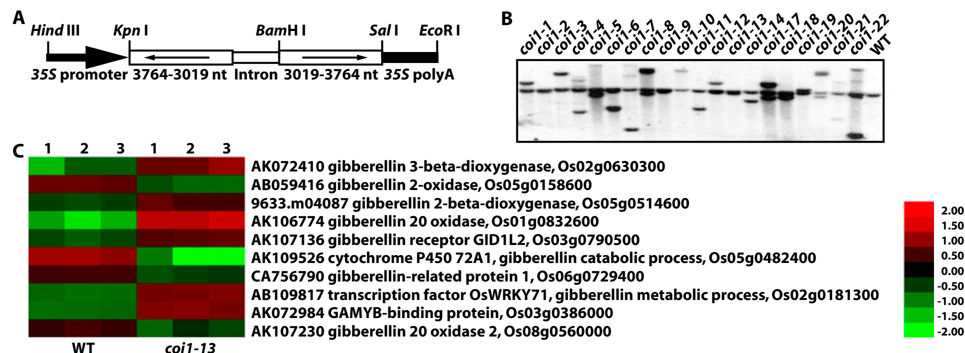

Fig. S1. Generation of rice *OsCOI1*-RNAi lines. (*A*) Schematic drawing of the *OsCOI1*-RNAi construct. (*B*) Southern blot analysis of genomic DNAs from the WT Nipponbare and *OsCOI1*-RNAi lines after digestion with *Eco*RI and probed with the *OsCOI1a* cDNA fragment (3,019–3,764 nt). (*C*) Expression of several gibberellic acid (GA)-related genes in the elongating (i.e., young) uppermost internode of *coi1-13* revealed by the Affymetrix GeneChip Rice Genome Array analysis. Numbers 1, 2, and 3 represent three different biological replicates. Expression scales (log2) are illustrated with colors.

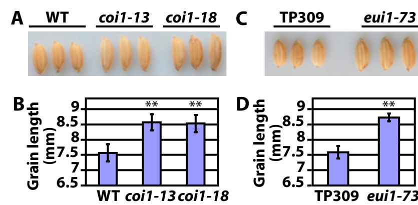

Fig. S2. *OsCOI1*-RNAi lines have longer grains, which resemble the *eui1* mutant. (*A*) Grain sizes of WT, *coi1-13*, and *coi1-18*. (*B*) Average grain lengths of WT, *coi1-13*, and *coi1-18*. There is significant difference between the WT and transgenic *OsCOI1*-RNAi lines (**$P < 0.001$, Student's *t* test). (*C*) Grain sizes of TP309 (WT) and *Eui1*-RNAi line S73. (*D*) Average grain lengths of TP309 and *Eui1*-RNAi lines S73. There is a significant difference between the WT and transgenic lines (**$P < 0.001$, Student's *t* test).

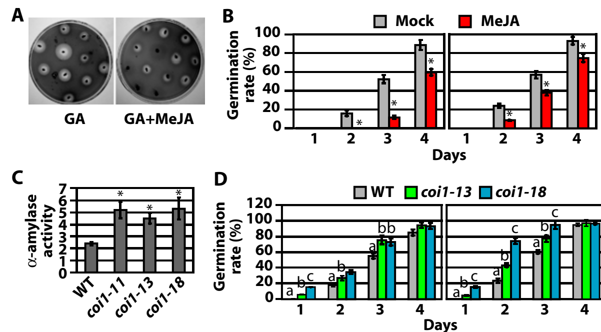

Fig. S3. Effect of methyl jasmonate (MeJA) on seed germination. (*A*) MeJA repressed α-amylase induction by GA. The embryoless seeds are used to test α-amylase induction by 1 μM GA_3 alone or in combination with 50 μM MeJA. (*B*) MeJA repressed seed germination. Seed soaking times were 24 h (*Left*) and 36 h (*Right*). (*C*) *OsCOI1*-RNAi seeds show higher levels of α-amylase activity than WT Nipponbare seeds. The deembryonated half seeds were imbibed in 1.0 μM of GA_3 solution in the dark at 28 °C for 2 d. The α-amylase activity is presented as A540/mg protein/min, with SEs displayed. In *B* and *C*, asterisks indicate significant difference between WT and *coi1* mutants ($P < 0.01$, Student's *t* test). (*D*) Seed germination is promoted in the *OsCOI1*-RNAi seeds. Soaking times were 24 h (*Left*) and 36 h (*Right*). Letters on columns indicate significant differences ($P < 0.05$, Tukey–Kramer multiple comparison test).

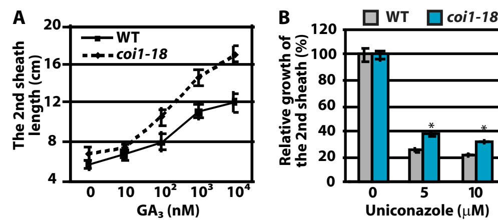

Fig. S4. *coi1-18* plants are hypersensitive to gibberellin. (*A*) Second sheath lengths of *coi1-18* and WT plants grown in one-half Murashige–Skoog (MS) medium with 0.6% agar supplemented with various concentrations of GA_3. Note that *coi1-18* is more sensitive to exogenous GA_3 than the WT. (*B*) The *coi1-18* plants are more resistant to uniconazole, a GA biosynthesis inhibitor, than WT plants, as indicated by increased elongation of the second leaf sheath (*$P < 0.01$, Student's *t* test).

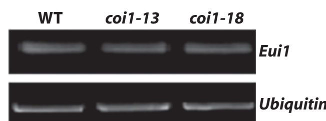

Fig. S5. Transcript levels of *Eui1* in *OsCOI1*-RNAi plants. RNA was isolated from the uppermost internode of Nipponbare, *coi1-13*, and *coi1-18* plants grown in the isolated paddy field. *Ubiquitin* transcript was used as the internal control. PCR cycles: *Eui1*, 36; *Ubiquitin*, 25.

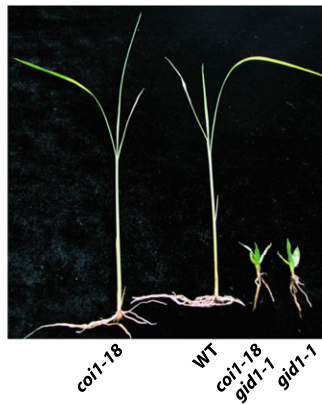

Fig. S6. The *gid1* mutation reduced the shoot length of *coi1-18*. The plant height of *coi1-18/gid1-1* is comparable to that of the *gid1-1* mutant.

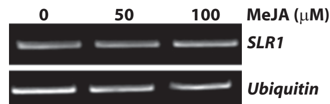

Fig. S7. MeJA treatment does not alter the transcript level of *SLR1*. Total RNAs were extracted from 7-d-old seedlings grown in one-half MS medium with 0.6% agar with or without 50 μM and 100 μM MeJA. Number of PCR cycles: *SLR1*, 31; *Ubiquitin*, 25.

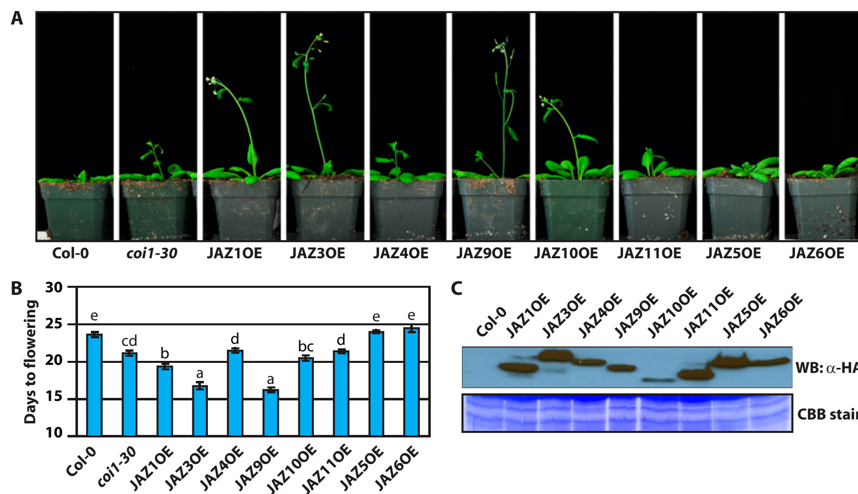

Fig. S8. Overexpression of several JAZs in *Arabidopsis* promotes early flowering. (*A*) Images of 28-d-old plants taken on the same day. Plants were grown in a long-day growth chamber (16 h 120 μmol $m^{-2} \cdot s^{-1}$ light/8 h dark, 22 °C/18 °C). (*B*) Flowering time of plants indicated. Data shown are the means from 12 plants. Error bars represent SD. (*C*) Expression of JAZ proteins in transgenic *Arabidopsis*; 50 μg of total proteins from rosette leaves of 28-d-old *Arabidopsis* plants were loaded into each lane for immunoblot analysis with an anti-HA antibody. Coomassie brilliant blue (CBB) was used to stain the blotted membrane to show equal loading of proteins.

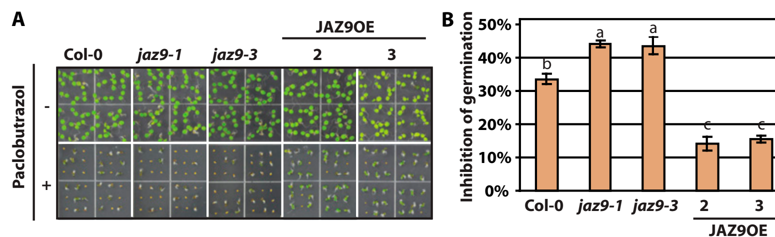

Fig. S9. Overexpression of AtJAZ9 reduces inhibition of seeds germination by PAC. (*A*) *Arabidopsis* seeds were germinated on MS plates with or without 5 μM PAC. (*B*) Data shown are the mean of three independent experiments. Error bars represent SD. Letters on columns indicate significant differences ($P < 0.05$, Tukey–Kramer multiple comparison test).

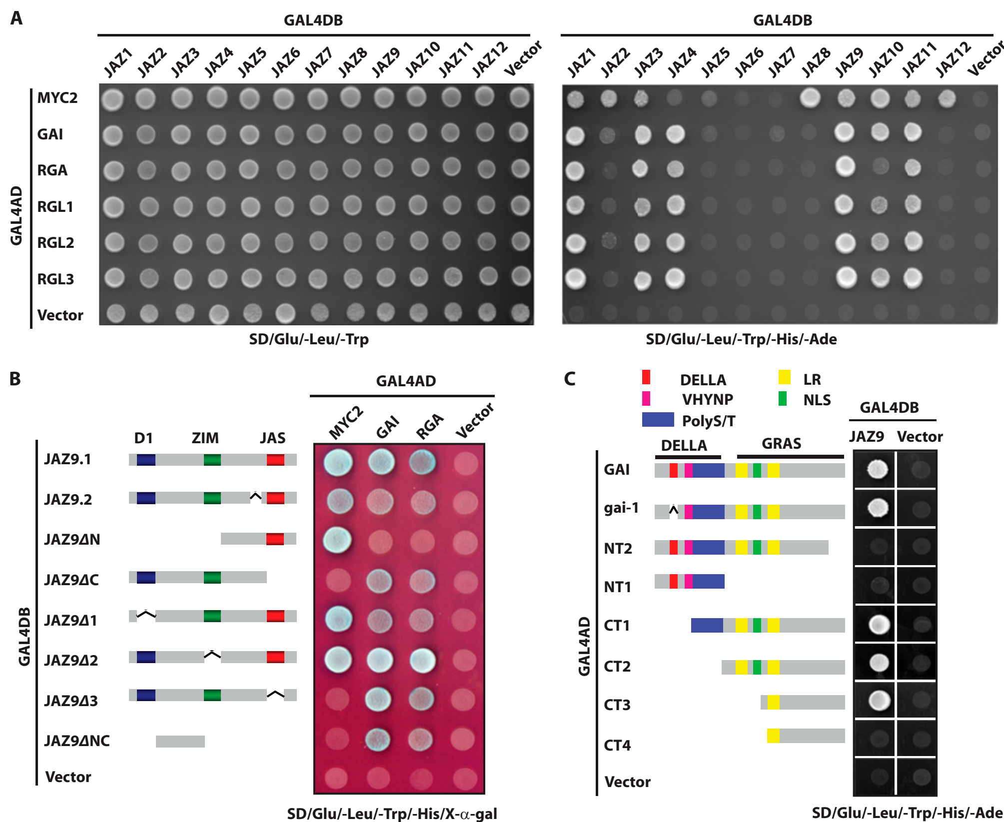

Fig. S10. Characterization of interaction between *Arabidopsis* JAZ and DELLA proteins in yeast. (*A*) DELLA proteins interact with multiple AtJAZ proteins in yeast. Growth assay was performed on selective medium (-His, -Ade). Yeast growth indicates positive interaction between two proteins. *AtJAZ*s were cloned into pGBKT7 and *DELLA*s were cloned into pDEST-GADT7. Yeast strain AH109 was used for the assay. (*B*) AtJAZ9 interacts with GAI and RGA through its N terminus. Growth assay was performed on selective medium (-His). The medium also contains X-α-gal to monitor *MEL1* activity. Yeast growth with blue color indicates positive interaction between two proteins. *AtJAZ9* and its derivatives were cloned into pDEST32. *MYC2*, *GAI*, and *RGA* were cloned into pDEST22. Yeast strain MaV203 was used for the assay. (*C*) AtJAZ9 interacts with the GRAS domain of GAI. Growth assay on selective medium (-His, -Ade). The growth of yeast indicates positive interaction between two proteins. *AtJAZ9* was cloned into pGBKT7. *GAI* and its derivatives were cloned in pACT2 (1). Yeast strain AH109 was used for the assay.

1. Dill A, Thomas SG, Hu J, Steber CM, Sun TP (2004) The *Arabidopsis* F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. *Plant Cell* 16: 1392–1405.

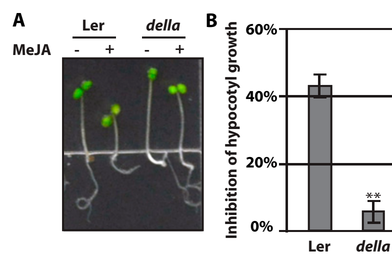

Fig. S11. MeJA does not inhibit hypocotyl elongation of a quintuple *della* mutant. (*A*) *Arabidopsis* seedlings were grown on MS medium with or without 10 μM of MeJA under 10 μmol $m^{-2} \cdot s^{-1}$ continuous white light for 6 d. (*B*) The hypocotyl lengths were measured and inhibition of hypocotyl growth was calculated as (1 − treated / untreated) × 100%. Data shown are the means from 16 seedlings. Error bars represent SD. Asterisks indicate significant difference between Landsberg *erecta* (Ler) and the quintuple *della* mutant ($P < 0.01$, Student's *t* test).

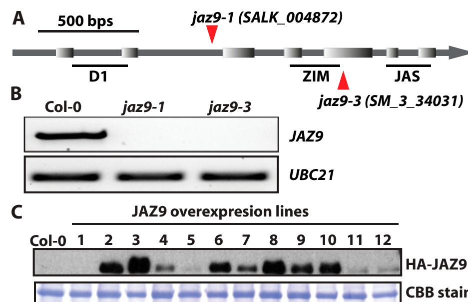

Fig. S12. Characterization of *Arabidopsis jaz9* KO mutants and *JAZ9* overexpression lines. (*A*) Line (SM_3_34031; *atjaz9*) was isolated from the John Innes Center SM line collection (1). The red arrows indicate the transferred DNA (T-DNA)/transposon insertion sites in *jaz9-1* (2) and *jaz9-3*. (*B*) RT-PCR indicates no *JAZ9* transcripts in *jaz9-1* or *jaz9-3* plants. (*C*) Screening of JAZ9 overexpression lines by Western blot. Twenty micrograms of total proteins from 10-d-old seedling were loaded in each lane for immunoblot analysis with an anti-HA antibody. Coomassie brilliant blue (CBB) was used to stain the blotted membrane to show equal loading of proteins.

1. Tissier AF, et al. (1999) Multiple independent defective suppressor-mutator transposon insertions in *Arabidopsis*: A tool for functional genomics. *Plant Cell* 11:1841–1852.
2. Thines B, et al. (2007) JAZ repressor proteins are targets of the SCF^{COI1} complex during jasmonate signalling. *Nature* 448:661–665.

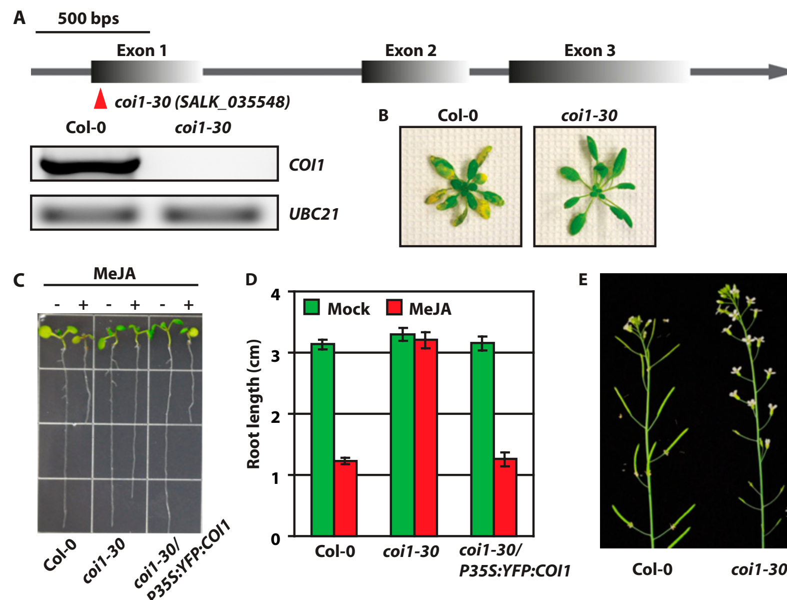

Fig. S13. Characterization of the *coi1-30* line. (*A*) A new *coi1* mutant (SALK_035548) was isolated from SALK T-DNA mutagenesis lines (1). The red arrow points to the T-DNA insertion site in *COI1*. RT-PCR indicates no detectable *COI1* transcript in *coi1-30* plants. (*B*) *coi1-30* is resistant to *Pseudomonas syringae* pv. *tomato* DC3000 infection. The plants were vacuum-infiltrated with 1×10^6 cfu/mL of bacteria. Images were taken after 3 d. (*C* and *D*) *coi1-30* is resistant to MeJA treatments, and complemented *coi1-30*/p35S:*YFP-COI1* is sensitive to MeJA. Seedlings were grown on MS medium with or without 10 μM of MeJA for 10 d. The root lengths were measured. Data shown are the means from 12 seedlings. Error bars represent SD. (*E*) *coi1-30* is male-sterile.

1. Alonso JM, et al. (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* 301:653–657.

Table S1. Levels of bioactive GAs in internodes of WT and *coi1-18* plants

Genotype	GA_4*	GA_1
WT	0.98 ± 0.8	12.54 ± 2.07
coi1-18	3.75 ± 0.8	12.33 ± 1.65

*Significant difference detected between WT and *coi1-18* ($P < 0.05$ Student's *t* test). GA amounts are in ng per gram of dry weight.

Other Supporting Information Files

Dataset S1 (XLS)