PNAS

Supporting Information for

GmEID1 modulates light signaling through the Evening Complex to control flowering time and yield in soybean

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Supporting text

Figures S1 to S22

SI References

Other supporting materials for this manuscript include the following:

Datasets S1 to S4

Supporting Information Text

SI Appendix Materials and Methods

RNA sequencing and data analysis

The W82 cultivar was grown under LD conditions (25˚C) in green house for 21 days. The second fully expanded trifoliolate leaves were collected every four hours from the beginning of night for a complete photoperiod of 24 hours. Total RNA was extracted from each sample using TRIzol reagent (TIANGEN). Multiplexed libraries were prepared with Kit (NEB #E7760S) and sequenced using an Illumina platform. Raw reads were filtered to remove adapter sequences using the sequence pre-processing tool Trimmomatic version 0.39, and only reads with quality scores (Phred) R30 were kept for mapping to soybean reference genome (Wm82.a2.v1, V275) using HISAT2 version 2.1.0 with default parameters. The data of gene expression (FPKM) values was listed in **Dataset 1**. The genes with average TPM over 1 were used for further analysis. The Pearson's correlation of expression levels was calculated between *E1* and the other genes using R project. The correlated genes with *p* value less than 0.05 were prioritized in this study. The data of screening of candidate regulators of *E1* is listed in **Dataset 2**.

Gene expression analysis

To compare the transcript level of target genes in the indicated lines, the plants were grown under LD or SD conditions for 20 days. The second fully expanded trifoliolate leaves were harvested at 4-hour (h) intervals during a 24-hour (h) photoperiod. Total RNA was extracted using Trizol Reagent (TIANGEN) and cDNA was synthesized from total RNA treated with DNase (2 μg, reaction total volume of the reaction 10 μl) using a reverse transcription kit (TransGen Biotech). qRT-PCR was performed in 384-well optical plates using a SYBR Green RT-PCR kit (Vazyme) with ABI Q7 equipment. All primers used for indicated genes are listed in **Dataset S3**. Three independent biological replicates were performed, and three replicate reactions were used for each sample.

Plasmid construct and plant transformation

To generate CRISPR / Cas9 engineered mutants, gRNAs were designed using the CRISPR direct website (*http://crispr.dbcls.jp/*) (1). Multiple target gRNAs were selected for each gene to construct the CRISPR/Cas9 vector according to the protocol reported previously (2). The editing efficiency of each construct was evaluated using the soybean hairy root system (3, 4), and at least two vectors with high editing efficiency for each gene were selected for soybean transformation. To construct the overexpression vectors, the *GmEID1* coding DNA sequence (CDS) was amplified by PCR using cDNA derived from young W82 seedlings, cloned into the Gateway entry vector *pDONR*^{Zeo}, and then cloned into the destination binary vector *pEarleyGate101* or *pEarleyGate104* using the Gateway recombination system (Invitrogen) (5). For *J-HA* overexpression, the CDS of the *J* was amplified by PCR using cDNA derived from W82 seedlings. A modified vector based on PTF101 was used to construct the *35S::J-HA* vector. The CRISPR/Cas9 vectors and overexpression vectors mentioned above were individually introduced into the *Agrobacterium tumefaciens* strain EHA105 by electroporation and then transformed into the cultivar TL1 or W82 using the cotyledon-node method (6).

Subcellular localization in protoplasts

To investigate the subcellular localization of the indicated proteins, the CDS of *GmEID1* was inserted into the *pA7-YFP* vector at the *BamHI* and *SmaI* sites using the In-Fusion system (Clontech) to generate the transient expression constructs of *pA7-GmEID1-YFP* driven by the *35S* promoter. The *pA7-GmMYB29-RFP* construct was used as a nuclear marker as previously described (7). The empty vector *pA7-YFP* was used as a control. The above constructs were transformed into *Arabidopsis* mesophyll protoplasts. The subcellular localization images were captured under a Zeiss LSM980 confocal laser scanning microscope and processed using ZEN 2009 Light Edition software.

Yeast two-hybrid experiments

The yeast two-hybrid assay was performed according to the manufacturer's instructions (Yeast Handbook Clontech). *GmEID1* CDS were fused in frame with the CDS of the GAL4 DNA binding domain in the *pBridge* bait vector (Clontech). The CDS of the EC (*J*, *GmELF3b-1*, *GmELF3b-2*, *GmELF4a*, *GmELF4b*, *GmLUX1* and *GmLUX2*), *E3* and *E4* were fused in frame with the CDS of the GAL4 transcription activation domain in the prey vector *pGADT7* (Clontech). The bait and prey plasmids were cotransformed into the yeast strain *Saccharomyces cerevisiae* AH109 (Clontech). The yeast cells were grown on a minimal medium SD/-Leu-Trp according to the manufacturer's instructions. Positive clones were selected in SD/-Ade-His-Leu-Trp selection medium at 30°C for 3 days to evaluate protein interactions. SD/-Ade-His-Leu-Trp selection medium that added 10 μM PCB which was used to interact with E3/E4 with GmEID1 under red light (30 μmol m^{-2} s^{-1}), far-red light (20 μmol m^{-2} s^{-1}) and dark conditions.

For β-galactosidase activity assay, colonies were selected and cultured at 180 rpm, 28°C in the dark until they reached OD600 = 0.1 in a 10 ml flask containing 4 ml of SD medium (-Leu/-Trp). 2 mL yeast culture was divided into 8 mL YPDA culture solution and cultured at 160 rpm at 30°C under dark conditions for the interaction of GmEID1 with the EC, and red light (30 μmol m^{-2} s^{-1}), Far-red (30 μmol m^{-2} s^{-1}) and darkness for the interaction of GmEID1 with E3/E4 until OD600 = 0.5-0.8 before the β-galactosidase assay. The relative bait-prey interaction was presented as β-gal units = 1000×OD578/(T×V×OD600), T as response time (min); V = 0.1 × concentration factor. Standard deviations (n = 3) are shown.

Dual-Luciferase assay

For Dual-Luciferase assays in tobacco, the CDS of *GmEID1* was amplified and cloned in the vector *pCAMBIA1300-nLUC,* and the CDS of the EC (*J*, *GmELF3b-1*, *GmELF3b-2*) were amplified and cloned in the vector *pCAMBIA1300-cLUC*. *Agrobacterium tumefaciens* strain GV3101 bacteria carrying different constructs were co-infiltrated into tobacco leaves. After infiltration, tobacco plants were incubated in the dark at 25°C for 12 h and then transferred to far-red light (30 μmol m^{-2} s^{-1}) conditions for an additional 36 h before analysis for LUC activity. A low-light-cooled CCD imaging apparatus (Tenon-5200) with GelCap software was used to capture the LUC image. For each analysis, at least eight independent leaves of tobacco leaves were infiltrated and analyzed. To determine the effects of E3 on the interaction of GmEID1 with EC proteins by LCI assays, GV3101 colonies harboring constructs expressing GmEID1-nLUC, cLUC-J and E3-Flag were infiltrated into leaves of tobacco. GUS-Flag was used as a negative control.

***In-vitro* pull-down assay**

The full-length CDS of *E3* or *E4* fused with *3xFlag* was cloned into the *pMAL-c5X* vector. The full-length CDS of *J* or *E1* was cloned into the *pET-28a* (+) vector. The His-J and His-E1 recombinant proteins were purified with Ni-NTA (QIAGEN). E3-3xFlag and E4-3xFlag proteins were purified using TnT Quick Coupled Transcription/Translation Systems according to the manufacturer's instructions (Promega, L1170). Holoproteins of E3 and E4 were generated by incubating the respective apoproteins with 20 mM phycocyanobilin (PCB) for 1 h in the dark on ice to allow the incorporation of the chromophore (J&K Scientific, P14137) as previously reported (8). The combination of proteins as indicated were mixed and pull down using Anti-Flag antibody.

RICE system to investigate J protein levels

The *35S::J-3xFlag* vector was introduced into *A. tumefaciens* strain K599, which was used to infect young seedlings of indicated lines in the hypocotyl region to induce transgenic hairy roots according to a previously reported method (10). Hairy roots induced by Empty K599 were used as the WT control. Callus induction medium (2.22 g/l Murashige & Skoog Basal Medium with Vitamins, 0.59 g/l MES monohydrate, 30 g/l sucrose, 1 mg/l 2, 4-D, 0.1 mg/l 6-BA, 0.1 g/l Timentin) was prepared as previously described (11). The transgenic roots were grown in callus induction medium for 2 weeks under LD or SD conditions. Those transgenic callus lines

confirmed by western blot were transferred to a fresh callus induction medium for subculturing. To compare the protein levels of J-Flag in the indicated lines, at least 10 independent transgenic callus lines were used for western blot analysis.

Immunoblot Assay

The fresh leaves or callus were collected in liquid nitrogen, ground to fine powder, and homogenized in 4×SDS-PAGE loading buffer [250 mM Tris-HCl pH 6.8, 10% SDS, 50% glycerol, 40 mM DTT, and 0.01% Bromophenol blue]. Protein extracts were separated by 10% SDS-PAGE, transferred to PVDF (0.45 μm, Immobilon-P) membrane, and blotted with indicated antibodies. The anti-E3 antibody was raised in a previous study (9).

Statistical analysis

Statistical analysis was performed using GraphPad 9.0 and Microsoft EXCEL. All numerical values are presented as Mean values ± SD. The differences between control and treatments were tested using two-tailed Student's t tests and ANOVA with Tukey's post-test.

Primers and accession Numbers

All primers used in this study are listed in **Dataset S3**. Gene sequences may be obtained from the Phytozome database (*https://phytozome-next.jgi.doe.gov/info/Gmax_Wm82_a2_v1*). The accession numbers are *GmEID1* (*Glyma.03G214300*), *E3* (*Glyma.19G224200*), *E4* (*Glyma.20G090000*), *J* (*Glyma.04G050200*), *GmELF3b-1* (*Glyma.14G091900*), *GmELF3b-2* (*Glyma.17G231600*), *GmELF4a* (*Glyma.11G229700*), *GmELF4b* (*Glyma.07G037300*), *GmLUX1* (*Glyma.12G060200*), *GmLUX2* (*Glyma.11G136600*), *E1* (*Glyma.06G207800*), *GmFT2a* (*Glyma.16G150700*), *GmFT5a* (*Glyma.16G044100*), *GmCCA1a* (*Glyma.07G048500*), *GmPRR3b* (*Glyma.12G073900*) and *GmActin* (*Glyma.18G290800*).

Supplemental Figure

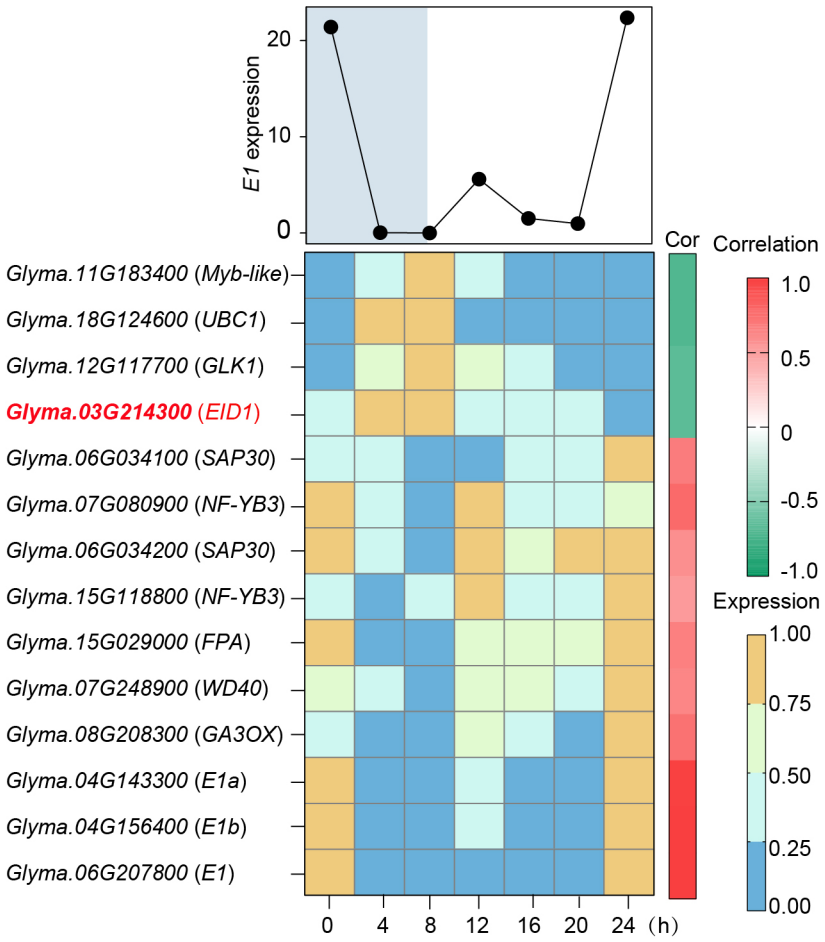


Fig. S1. Screening of candidate regulators of *E1*. Heat map showing candidate genes with the same or opposite expression pattern of *E1* during a 24 h photoperiod by transcriptome sequencing (RNA-seq) using the second trifoliate leaves of W82 plants grown under LD conditions. The above color scale bar represents the degree of correlation with the *E1* expression pattern. The bottom color scale bar represents the relative transcript level of each gene with the expression peak arbitrarily set to 1. The expression of candidate genes by transcriptome sequencing are listed in **Datasets S2**.

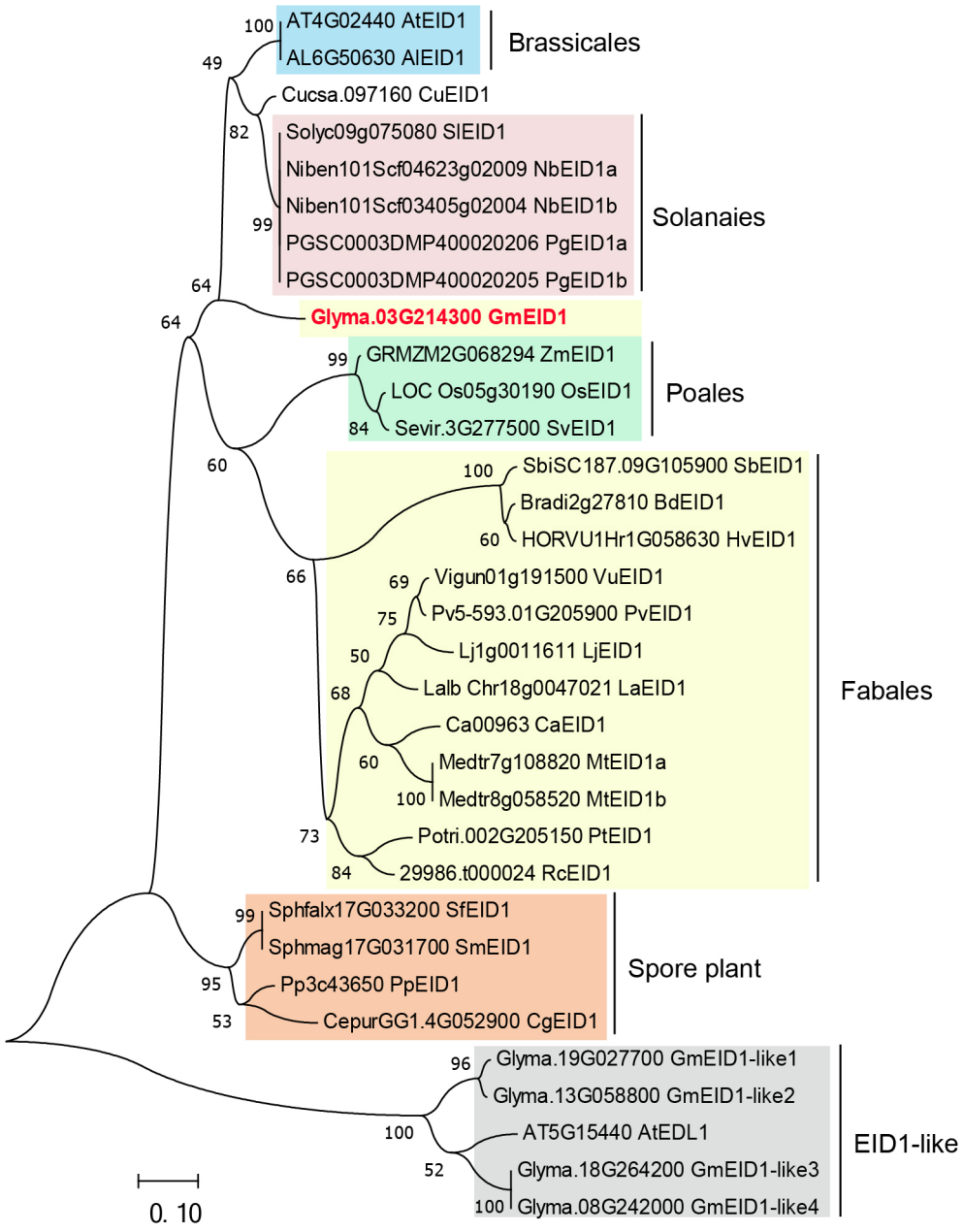


Fig. S2. Phylogenetic analysis of EID1 and its homologous proteins in the indicated species. The tree was constructed using the neighbor-joining method of the MEGA7 software. All protein sequences are listed in **Dataset S4**.

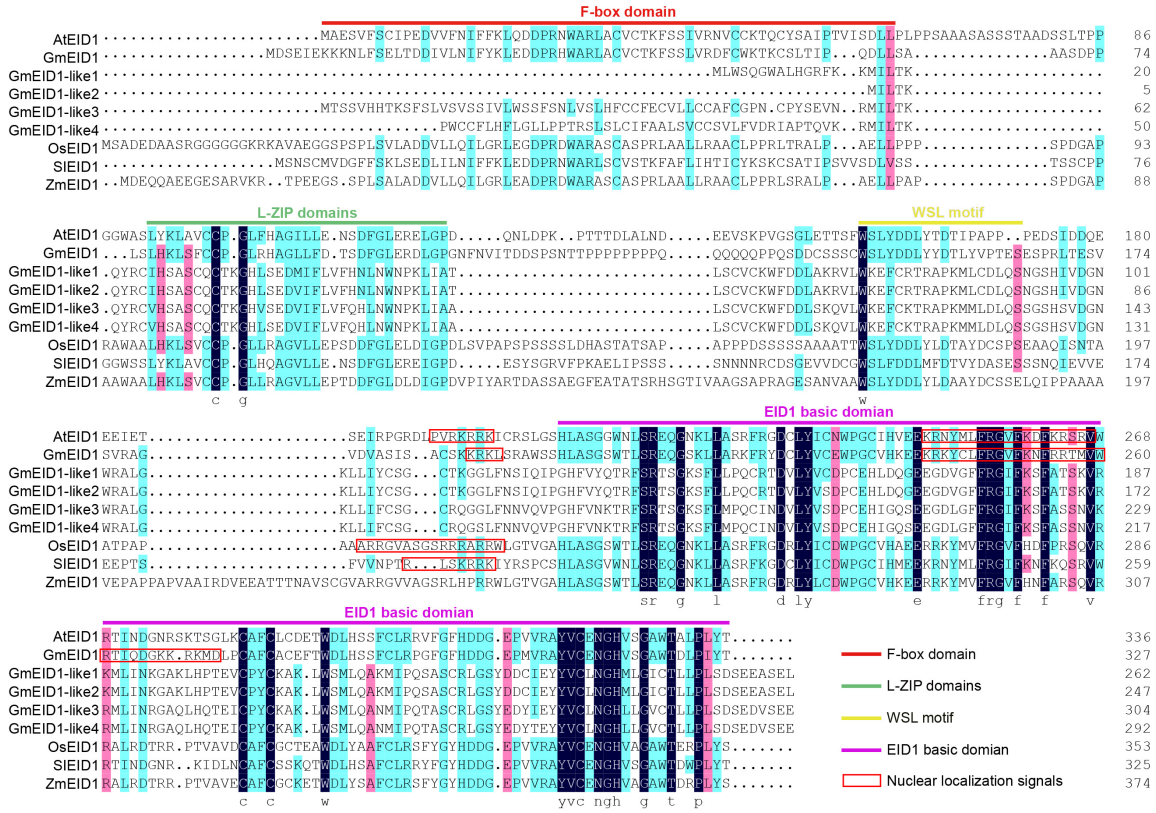
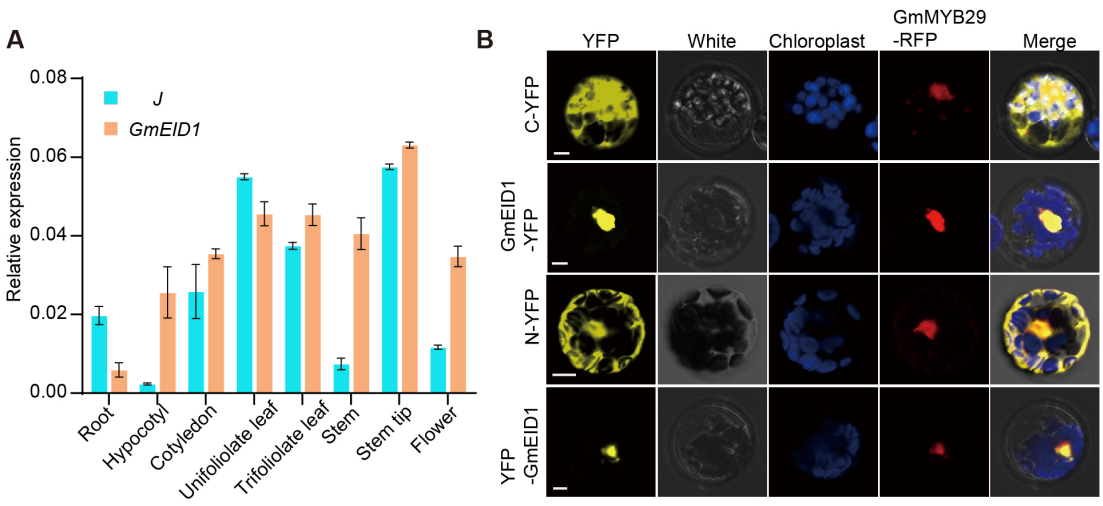


Fig. S3. Alignment of EID1 homologous proteins. The protein sequences of EID1 and its homologs were aligned by ClustalW Multiple alignments in DNAMAN and manually adjusted. Protein sequences of *Arabidopsis* (AtEID1, AT4G02440), Glycine max (GmEID1, Glyma.03G214300; GmEID1-like1, Glyma.19G027700; GmEID1-like2, Glyma.13G058800; GmEID1-like3, Glyma.18G264200; GmEID1-like4, Glyma.08G242000), Oryza sativa (OsEID1, LOC_Os05g30190), S.lycopersicum (SlEID1, Solyc09g075080), and Zea maysL. (ZmEID1, GRMZM2G068294_T01) were retrieved from Phytozome (*https://phytozome-next.jgi.doe.gov/*). All protein sequences are listed in **Dataset S4**. The conserved domains were highlighted by indicated color lines on the top, respectively. Red boxes indicate the nuclear l-ocalization signals.



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165 **Fig. S4.** Spatiotemporal expression and subcellular localization of GmEID1 in soybean. (A)
166 Comparison of transcript levels of the *J* and *GmEID1* genes in the indicated tissues. Root,
167 hypocotyl, cotyledon, unifoliate leaf, trifoliate leaves, stem, stem tip and flower of W82 were
168 harvested for qRT-PCR analysis. The relative expression level is shown as mean ± SD (n = 3).
169 The *GmActin* gene was used as an internal control. (B) Subcellular localization of GmEID1-YFP
170 and YFP-GmEID1 fusion proteins in *Arabidopsis* mesophyll protoplasts. The GmMYB29-RFP
171 fusion protein was used as a nucleus marker. The YFP protein alone was used as a negative
172 control. Scale bar, 5 μm.

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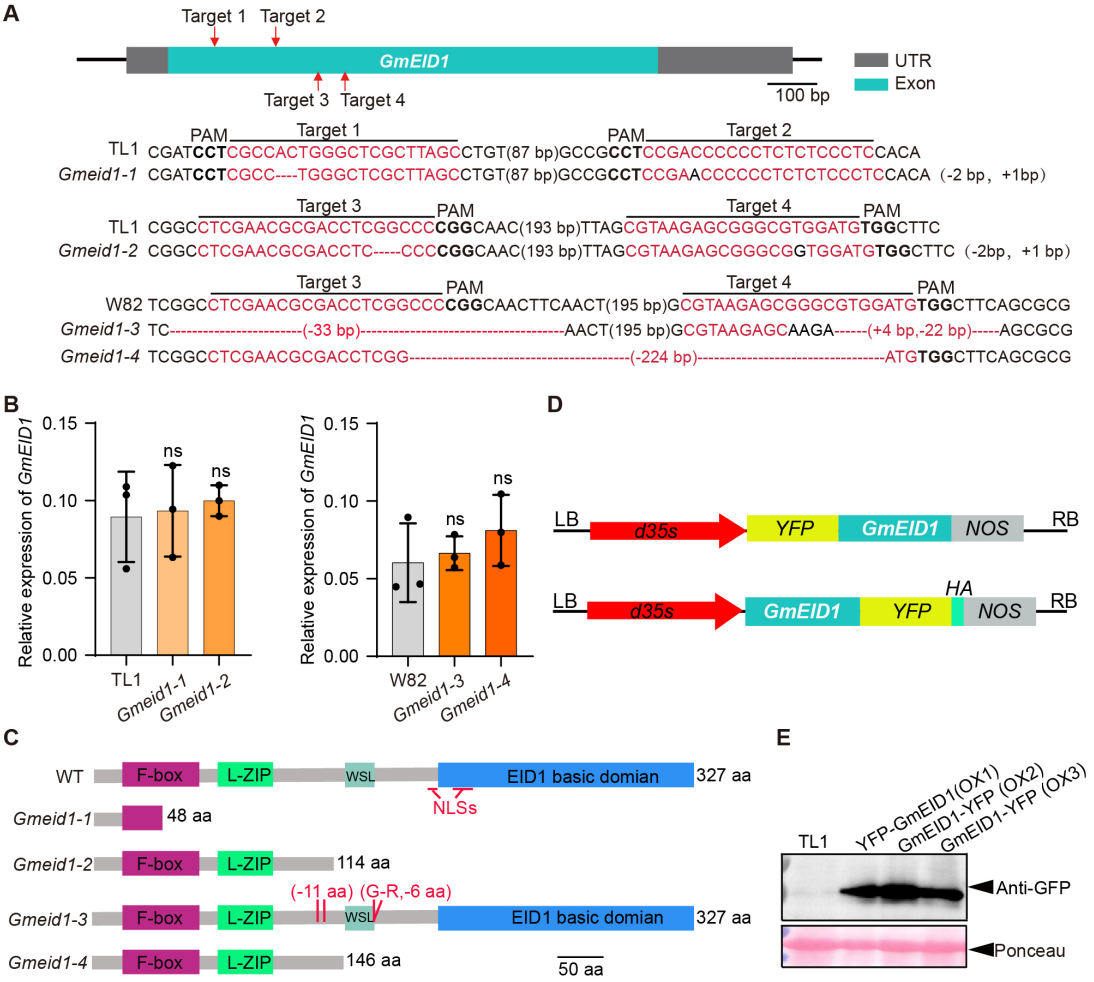

Fig. S5. Generation of the *Gmeid1* mutants and overexpression lines. (A) Four single-guide RNAs (red arrows) were designed to target the exon of *GmEID1*. Sequences of representative homozygous mutants (*Gmeid1-1* and *Gmeid1-2* in TL1 background, *Gmeid1-3* and *Gmeid1-4* in W82 background) at the T2 generation are shown. The gRNA target sites are highlighted in red letters with the protospacer-adjacent motif (PAM) in bold. The black-letter and dashed lines within the target sites denote nucleotide insertion and deletion, respectively. (B) The transcript levels of the *GmEID1* genes in indicated lines grown under LD conditions. The first trifoliate leaves at ZT4 were collected for qRT-PCR analysis. Mean values ± SD (n = 3) is shown. *GmActin* was used as an internal control. (C) Schematic diagram showing the intact and mutated GmEID1 proteins in *Gmeid1* mutants as in (A). (D) Schematic diagram showing the *YFP-GmEID1* or *GmEID1-YFP* overexpression construct. (E) Immunoblot showing the expression of YFP-GmEID1 and GmEID1-YFP fusion proteins in the transgenic plants using anti-GFP antibody. The wild-type TL1 sample was used as the negative control. The ponceau band was used as the loading control.

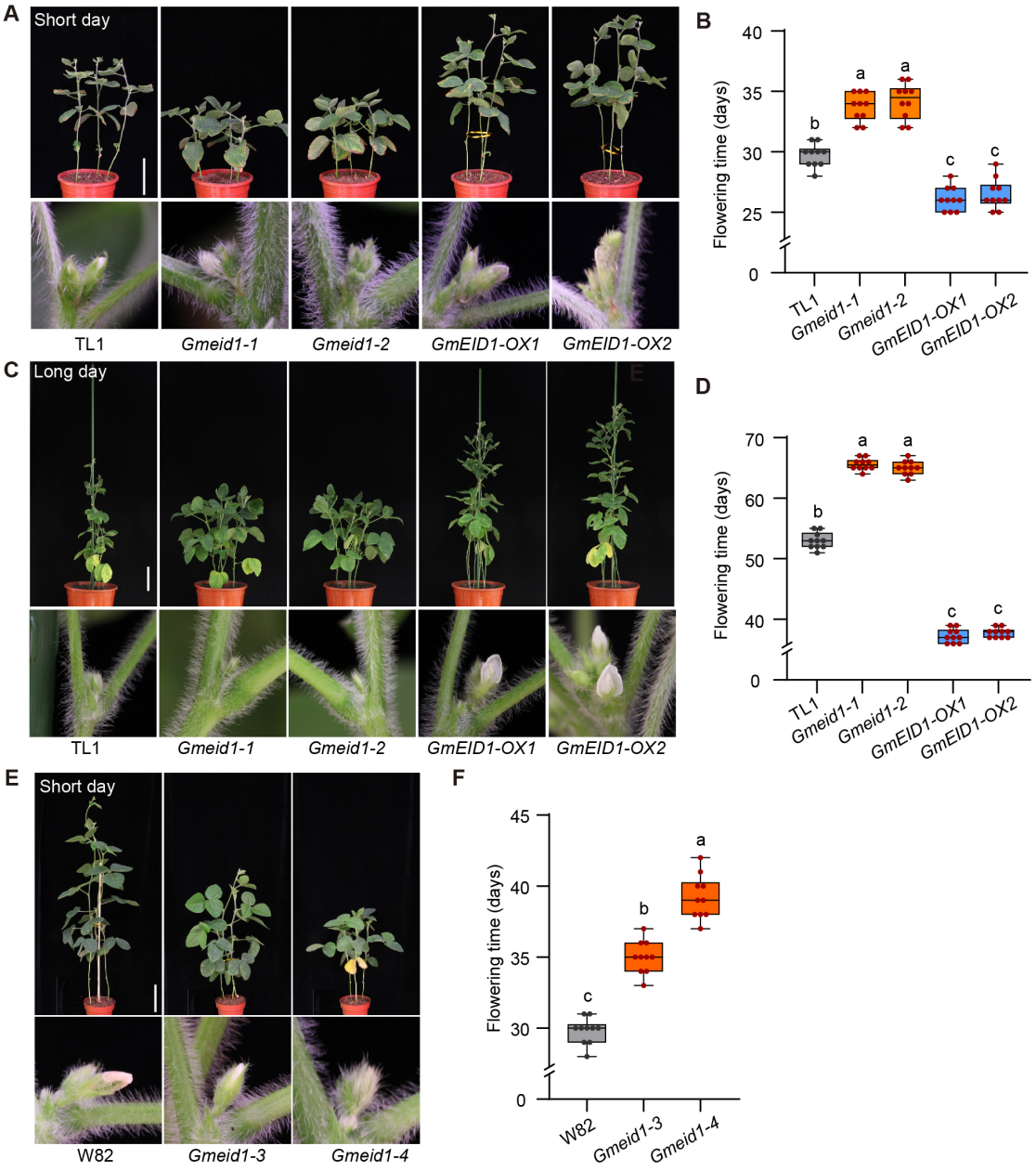

Fig. S6. Flowering phenotypes of the *Gmeid1* mutants and *GmEID1-OX* lines. (A and C) Photos of wild-type TL1, *Gmeid1* mutants and *GmEID1-OX* lines grown under SD conditions (A) or LD conditions (C) in phytotrons. Scale bar, 10 cm. (B and D) Flowering time of the indicated lines as in (A) and (C), respectively. (E) Photos of wild-type W82 and *Gmeid1* mutants grown under SD conditions in phytotrons. Scale bar, 10 cm. (F) Flowering time of indicated lines grown under SD conditions. The above statistical data are shown as mean values ± SD (n = 10). The lowercase letters above the dots indicate significant differences ($p < 0.01$, ANOVA with Tukey's post-test).

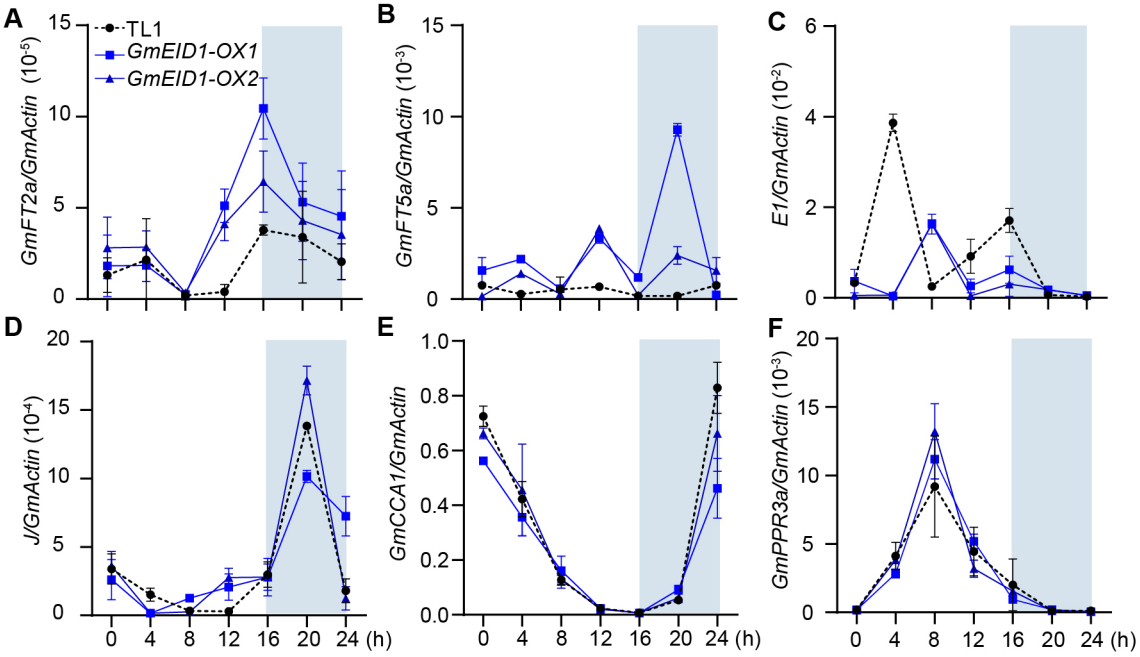

Fig. S7. Effects of *GmEID1* overexpression on the transcript levels of the indicated genes. Comparison of the diurnal expression levels of *GmFT2a* (A), *GmFT5a* (B), *E1* (C), *J* (D), *GmCCA1*(E) and *GmPRR3a* (F) in wild-type TL1 and *GmEID1-OX* lines. The second trifoliate leaves of 20-day-old plants grown under LD conditions were collected for qRT-PCR analysis. Mean values ± SD (n = 3) is shown. *GmActin* was used as an internal control.

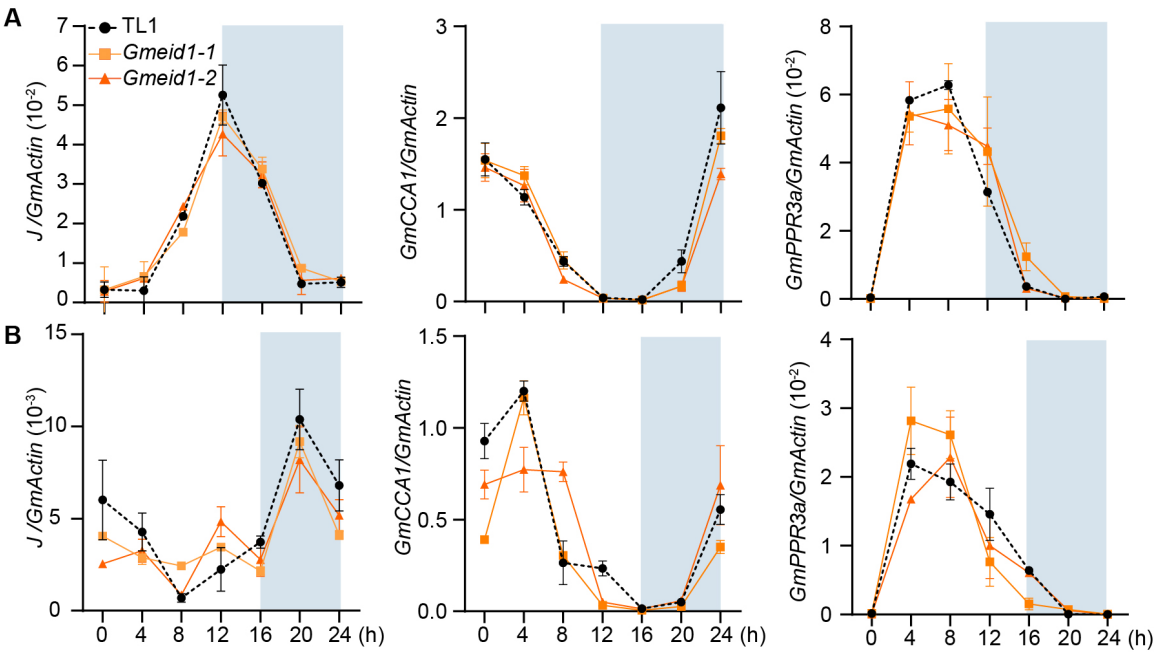

Fig. S8. Effects of *GmEID1* mutations on the transcript levels of the indicated genes. Comparison of the diurnal expression levels of *J*, *GmCCA1* and *GmPRR3a* in wild-type TL1 and *Gmeid1* mutants under SD conditions (A) and LD conditions (B). The second trifoliate leaves of 20-day-old plants were collected for qRT-PCR analysis. Mean values ± SD (n = 3) is shown. *GmActin* was used as an internal control.

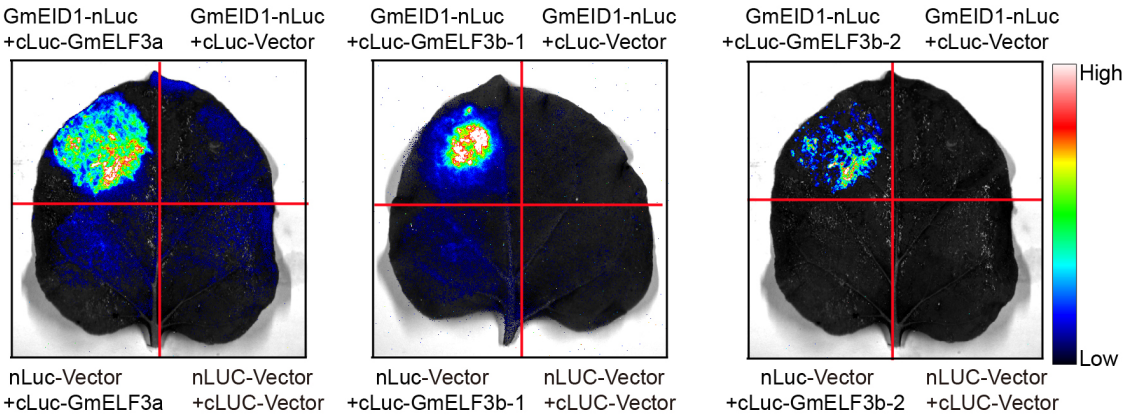

Fig. S9. GmEID1 interacts with EC in soybean. Dual-Luciferase assays showing the interaction of GmEID1 with J, GmELF3b-1 or GmELF3b-2 in tobacco leaves. The empty vector (nLUC-Vector or cLUC-Vector) was used as a negative control.

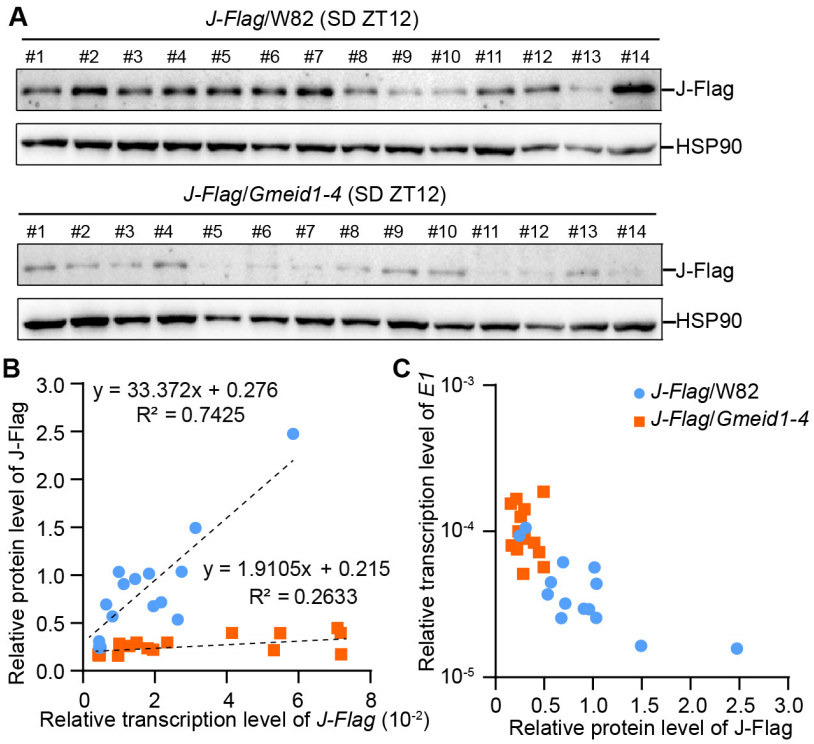

Fig. S10. Analysis of effect of GmEID1 on the abundance of J protein. (A) Immunoblot showing the abundance of J-Flag protein in the individual root hair callus line harboring transgenic *35S::J-Flag* in the wild-type W82 background (*J-Flag*/W82, upper panel) or the *Gmeid1-4* mutant background (*J-Flag*/*Gmeid1*). Callus lines were cultured under SD conditions and harvested at ZT12 for immunoblotting using anti-Flag antibody. HSP90 was used as a loading control. (B) Scatter plot showing the correlation between *J-Flag* transcription levels and protein levels in the wild-type W82 background and the *Gmeid1-4* mutant background. (C) The correlation between J-Flag protein levels and *E1* transcription levels in the callus lines as in (B).

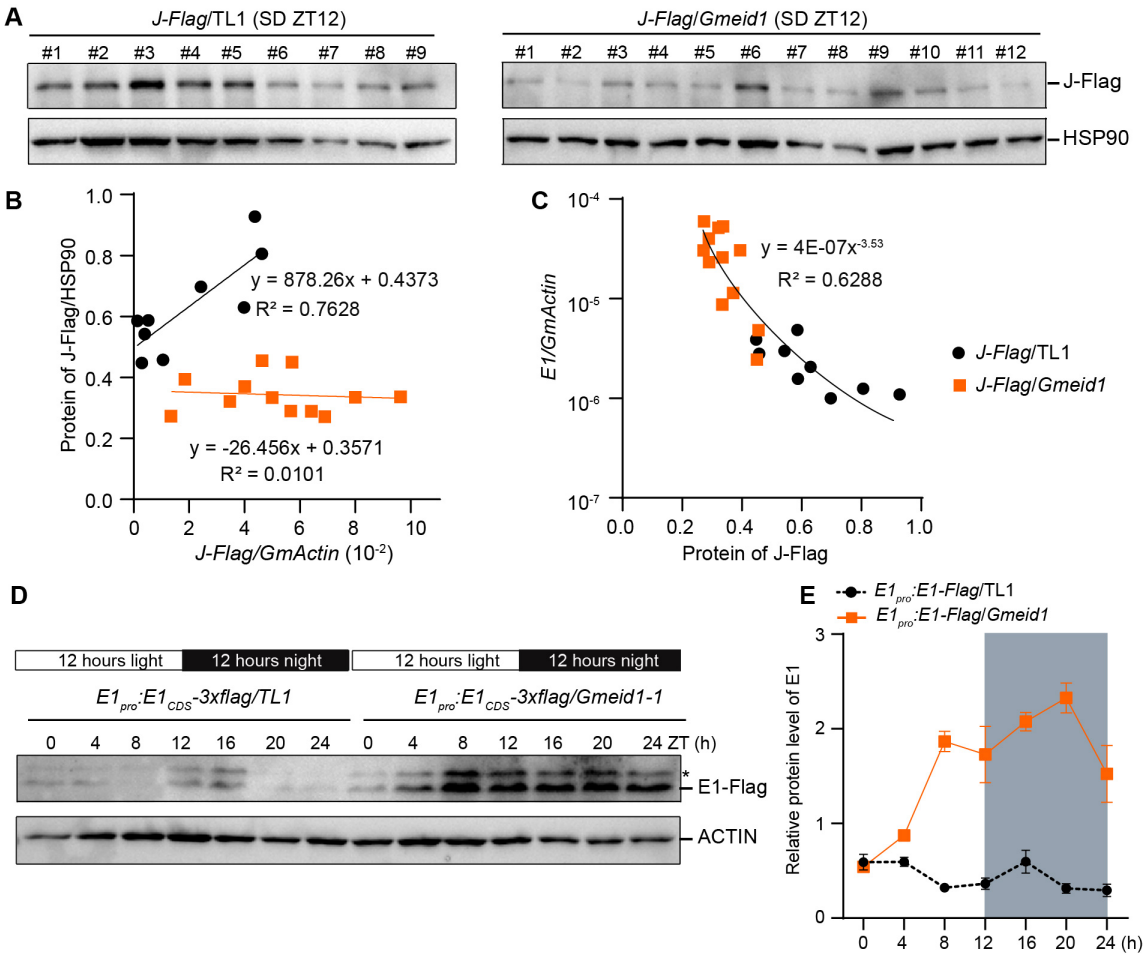

Fig. S11. GmEID1 enhances J abundance and inhibits *E1* transcription. (A) Immunoblot showing the abundance of J-Flag protein in the transgenic root hair callus cultured under SD conditions. The *35S::J-Flag* transformed hair root in the wild-type TL1 background (*J-Flag*/TL1, upper panel) or in the *Gmeid1-1* mutant background (*J-Flag/Gmeid1*) were induced into callus lines. Multiple transgenic callus lines were harvested at ZT12 for immunoblotting using anti-Flag antibody. HSP90 was used as the loading control. (B) Scatter plots showing the correlation between the transcript level and protein level of J-Flag in the wild-type TL1 background and the *Gmeid1-1* mutant background. (C) The correlation between J-Flag protein level and *E1* transcript level in TL1 and *Gmeid1-1* mutant callus. (D) Immunoblot showing the protein levels of E1-Flag expressed by the endogenous *E1* promoter in the TL1 callus and the *Gmeid1* callus during a SD photoperiod. The membrane was probed by the anti-Flag antibody, stripped, and then probed by the anti-ACTIN antibody. * Indicates a nonspecific band. (E) Quantitative analysis of E1-Flag protein levels relative to ACTIN in samples as in (D).

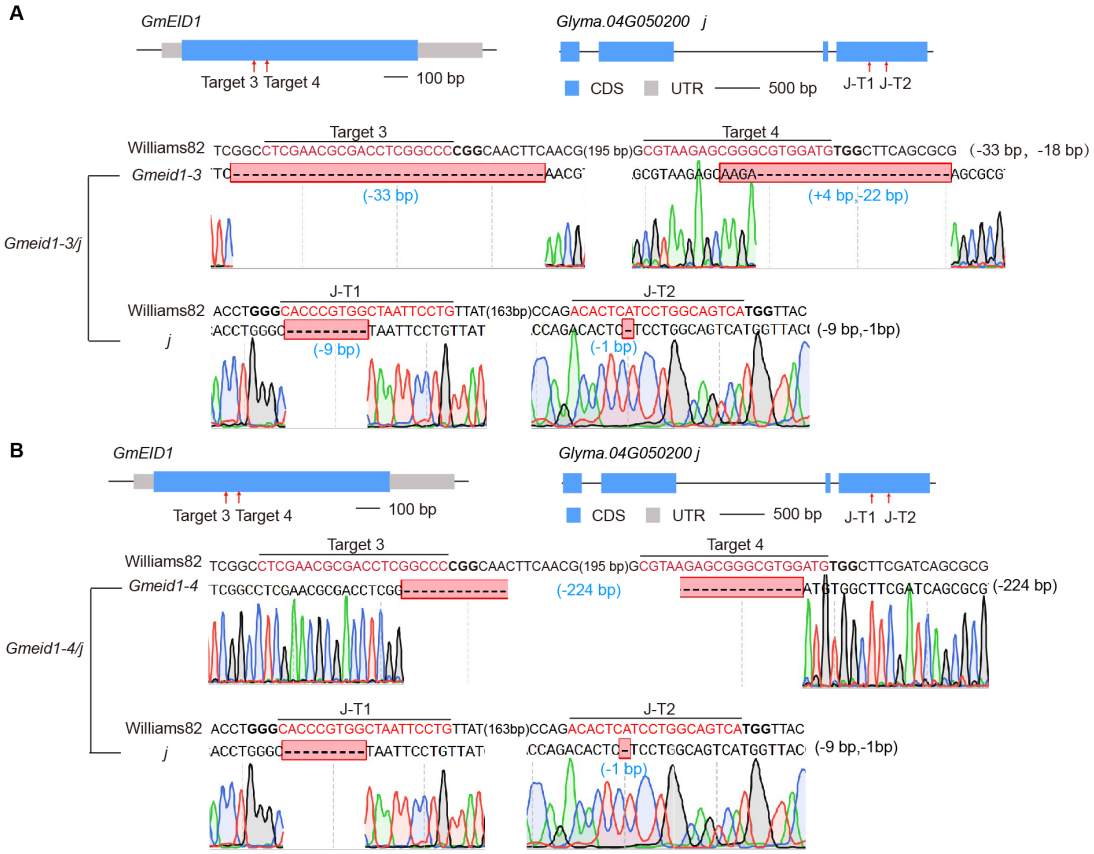

Fig. S12. Molecular confirmation of the homozygous progeny of the indicated mutants. (A and B) DNA sequencing analysis of mutation sites in the *Gmeid1-3/j* double mutant (A) and the *Gmeid1-4/j* double mutant (B).

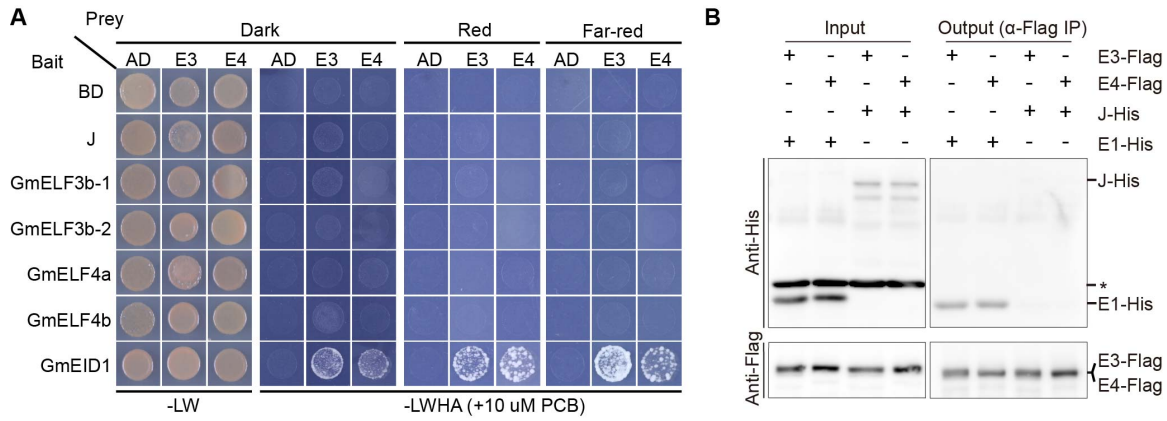

Fig. S13. Investigation of the Interaction between E3/E4 and J. (A) Auxotrophic assay to test the interactions of indicated protein pairs in yeast cells under red light (30 μmol m^{-2} s^{-1}), far-red light (30 μmol m^{-2} s^{-1}) or dark conditions. -LW, medium lacking Leu and Trp. -LWHA medium lacking Leu, Trp, His and Ade. AD, activation domain; BD, binding domain. The interactions between GmEID1 and E3/E4 were used as positive control. (B) Pull-down assay to test the interaction between E3/E4 and J protein *in vitro*. E3-Flag, E4-Flag and J-His proteins were expressed using an *in vitro* translation system. E1-His protein that known interaction with E3 protein was expressed in *Escherichia coli* as positive control. Purified proteins were mixed as indicated for the pull-down assay. E3-Flag and E4-Flag were detected with anti-Flag antibody, and E1-His, J-His protein were detected with anti-His antibody. * Indicates a nonspecific band.

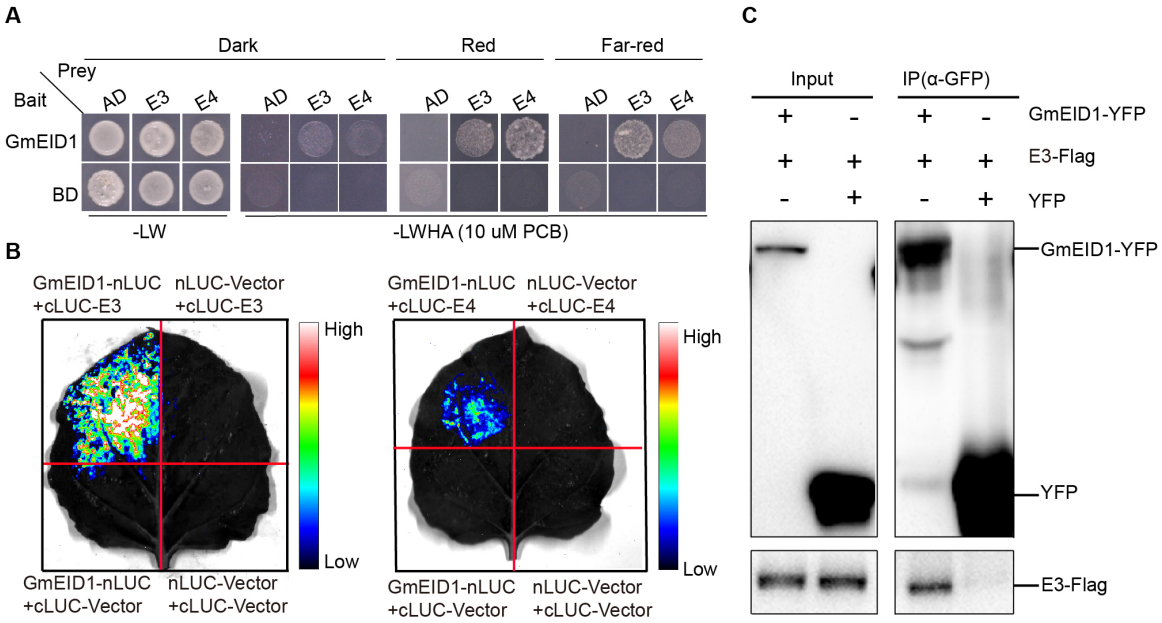

Fig. S14. E3/E4 interact with GmEID1 to inhibit the GmEID1-J interaction. (A) Auxotrophic assay showing the interaction of GmEID1 with E3/E4 in yeast cells treated with red light (30 μmol m^{-2} s^{-1}), far-red light (30 μmol m^{-2} s^{-1}) or darkness. Yeast cells (AH109) expressing the indicated proteins were selected on -LW (lacking Leu and Trp) and -LWHA (lacking Leu, and Trp, His and Ade) media containing 10 μM PCB (phycocyanobilin) under Far-red light (30 μmol m^{-2} s^{-1}) or dark conditions. AD, activation domain; BD, binding domain. (B) Dual-luciferase assay showing the interaction of GmEID1 with E3/E4 in tobacco leaves. Empty vector (nLUC-Vector or cLUC-vector) was used as the negative control. (C) Co-IP assay showing the interaction of GmEID1 with E3. The indicated constructs (GmEID1-YFP with E3-Flag, and E3-Flag with YFP) were transiently expressed in tobacco leaves. Total protein extractions (Input) and immunoprecipitation products prepared by the anti-GFP antibody (IP α-GFP) were fractionated in a SDS-PAGE gel, blotted to membranes, probed with the anti-GFP antibody (GmEID1-YFP or YFP), stripped, and re-probed with the anti-Flag antibody (E3-Flag). Empty vector (YFP) was used as the negative control.

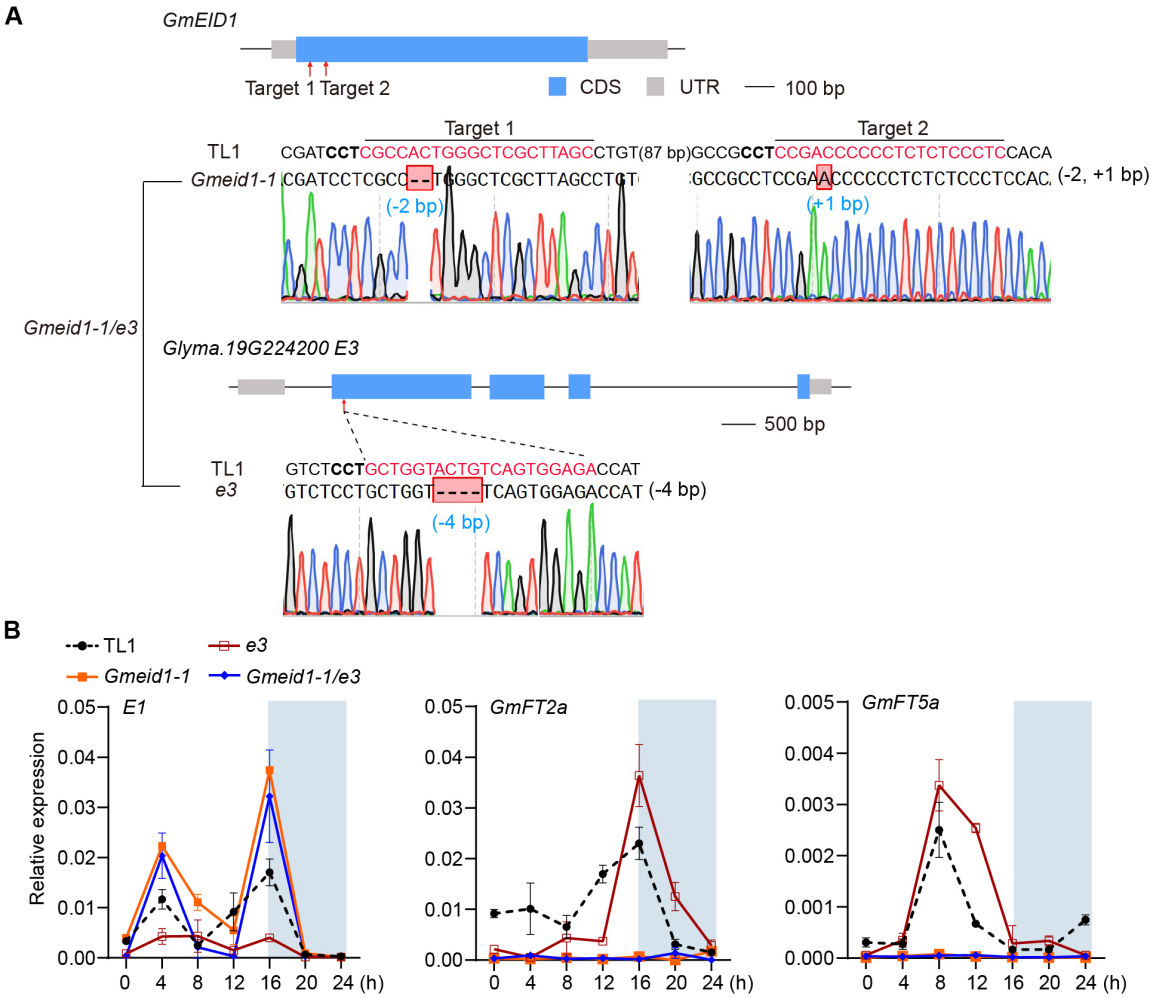

Fig. S15. *GmEID1* acts genetically downstream of *E3*. (A) The DNA sequencing analysis to confirm the genotype of the *Gmeid1-1*/*e3* double mutant. (B) Diurnal expression levels of *E1*, *GmFT2a* and *GmFT5a* in the indicated lines. *GmActin* was used as an internal control. Data are mean ± SD (n = 3).

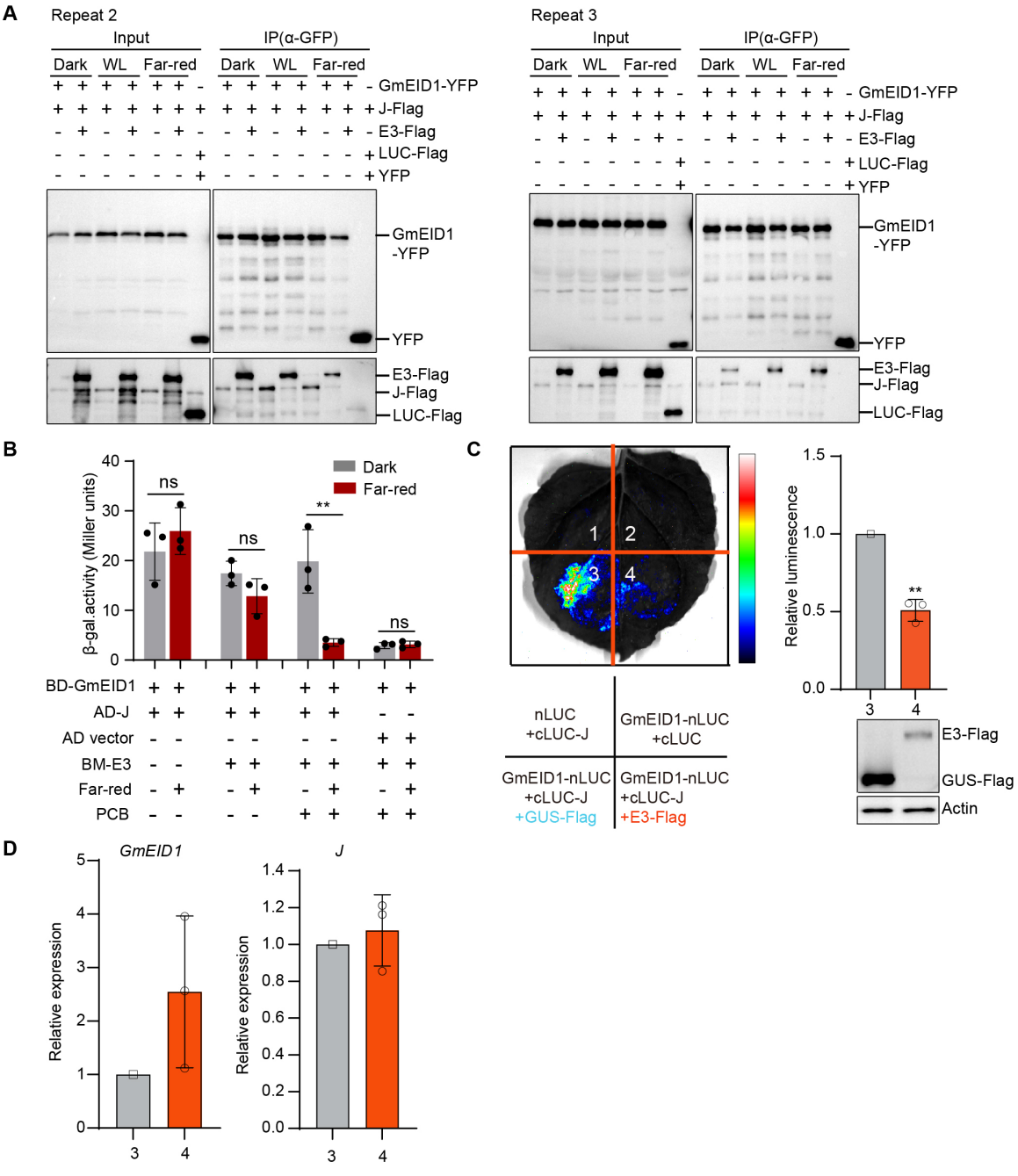

Fig. S16. Photo-activated E3/E4 interact with GmEID1 to inhibit the GmEID1-J interaction. (A) Two biological replicates of Co-IP experiments as in Fig. 4C. (B) Yeast three-hybrid assay to test the effect of E3 on the interaction between GmEID1 and J. Yeast cells expressing the indicated proteins were grown in the dark or Far-red (30 μmol m^{-2} s^{-1}) until OD600 = 0.5-0.8 for the β-galactosidase assay. Data are mean ± SD (n = 3). *p < 0.05, **P < 0.01, Student's t-test. BD-GmEID1, AD-J and BM-E3 were expressed by the *pBridge-GmEID1* vector, *pGADT7-J* vector, and *pBridge-GmEID1-E3* vector, respectively. (C) The indicated constructs were transformed into

306 the tobacco leaves for Dual-luciferase assay. The right panel compared the relative luminescence
307 intensity in the presence of E3-Flag or GUS-Flag which was used as a negative control. Data are
308 mean ± SD (n = 3). **$p < 0.01$, Student's t-test. The protein levels of E3-Flag and GUS-Flag in
309 infiltrations 3 and 4 were determined by immunoblot using anti-Flag antibody. Actin was used as
310 the loading control. (D)The *GmEID1* and *J* transcript levels in infiltrations 3 and 4 as in (C) were
311 quantified by qRT–PCR. The *NbActin1* gene was used as the internal control. Data are mean ±
312 SD (n = 3).

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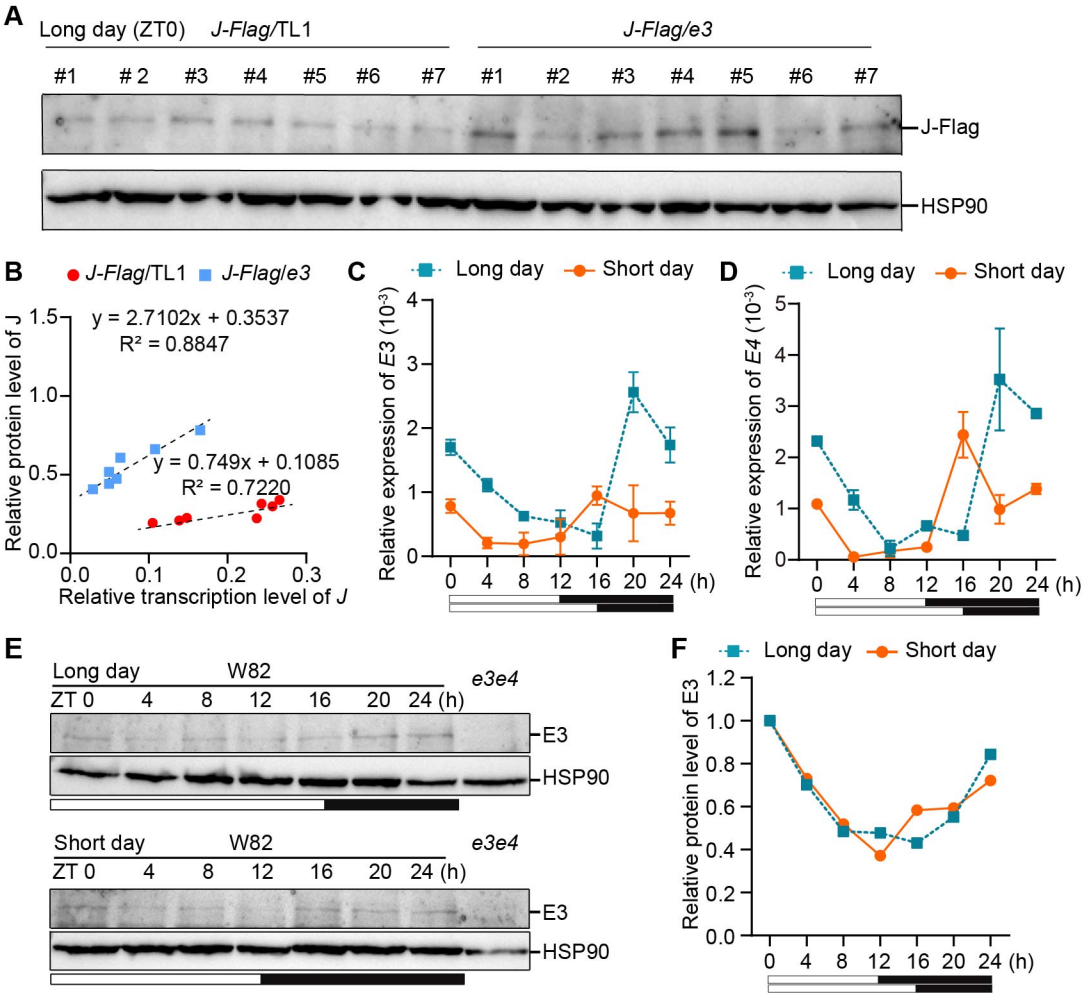


Fig. S17. E3 affects the protein levels of J. (A) Immunoblot to compare the J-Flag protein levels in the indicated root hair callus lines cultured under LD conditions. The *35S::J-Flag* construct was transformed into the hair root of *e3* mutant in TL1 background (*J-Flag/e3*). Multiple transgenic callus lines were harvested at ZT0 for immunoblotting using Flag antibody. HSP90 was used as a loading control. (B) Scatter plot to compare the correlation between transcript levels and protein levels of transgenic J-Flag in the *e3* mutant and wild-type TL1 as in (A). (C and D) Comparison of *E3* and *E4* transcript levels in W82 under LD and SD conditions. (E) Immunoblot showing the protein levels of E3 in W82 under LD and SD conditions using the anti-E3 antibody. The *e3e4* double mutant was used as negative control. (F) Quantitative analysis of E3 protein levels relative to HSP90 in the samples as in (E). The values at ZT0 were arbitrarily set to 1.

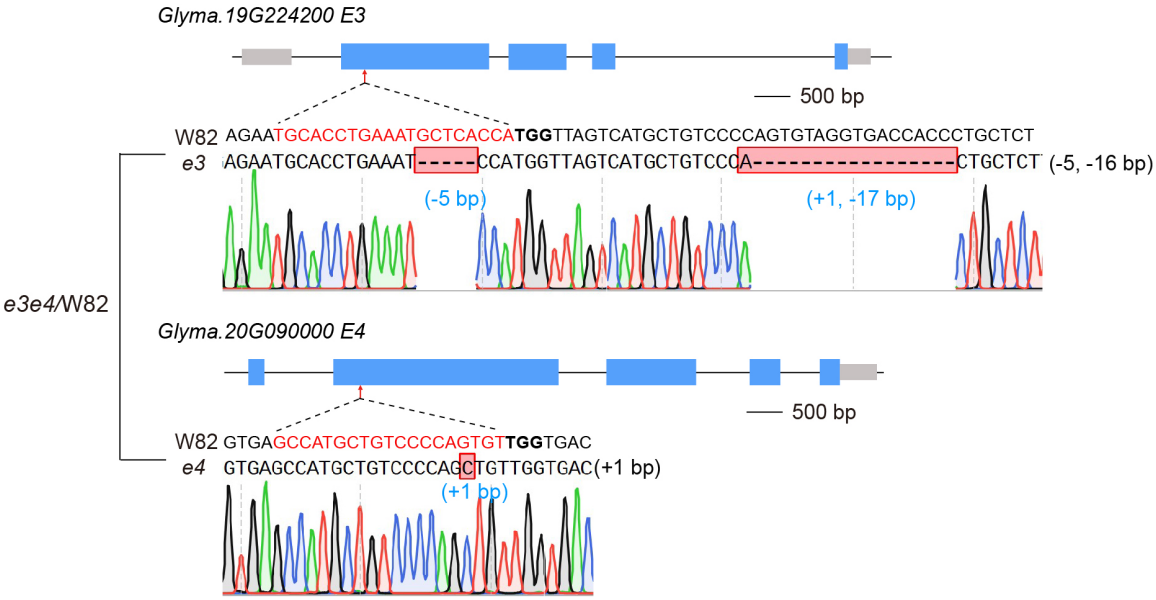


Fig. S18. DNA sequencing analysis of mutation sites in the *e3e4* double mutant in W82 background.

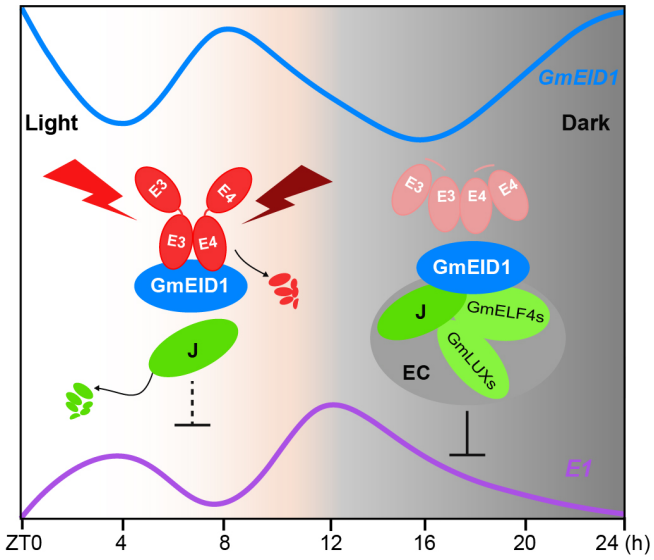
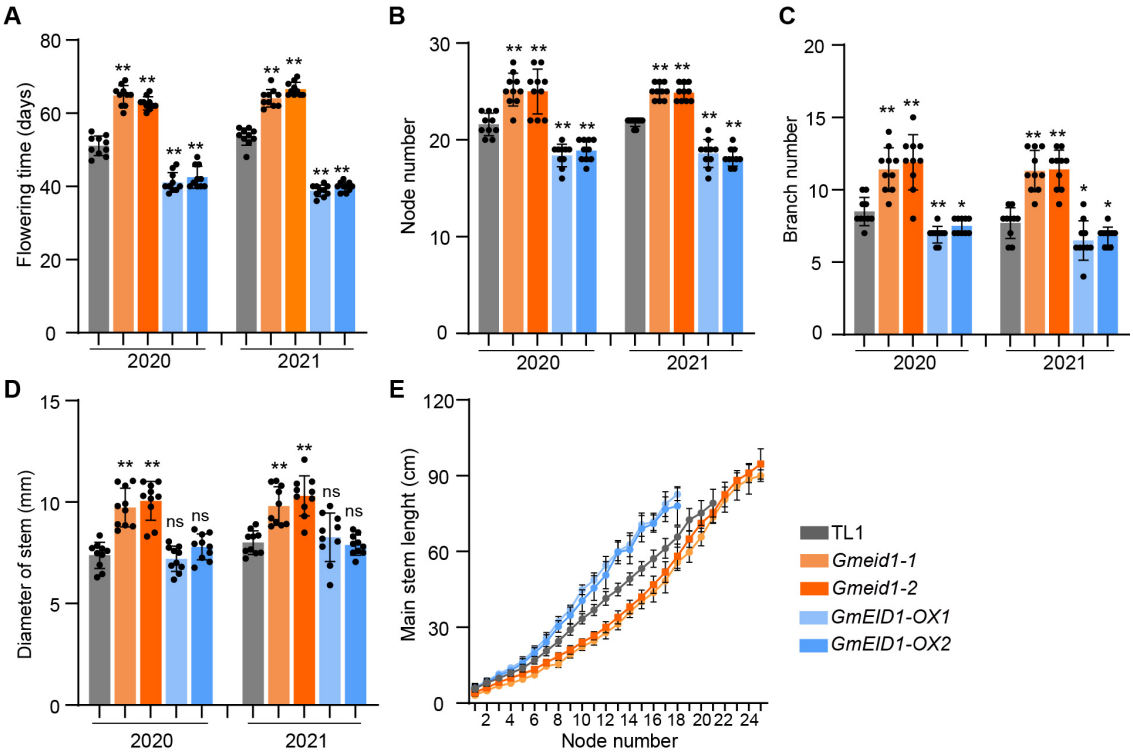


Fig. S19. A proposed model illustrating how E3/E4 inhibits the GmEID1-J interaction and regulates flowering in soybean. During the day, light-activated E3/E4 interacts with GmEID1 to attenuate the GmEID1-J interaction and promote J degradation. The dark period deactivates E3/E4, which releases GmEID1 to interact with J. Consequently, J proteins can accumulate and assemble into EC to inhibit *E1* transcription at night. The blue and purple curves show the opposite expression patterns of *GmEID1* and *E1* mRNAs, respectively. The *E1* transcript level rises from the onset of light (ZT0), but slightly decreases during ZT4 to ZT8, which is likely associated with the light-induced activation and degradation of E3/E4 proteins and the fluctuation of *GmEID1* transcripts during the light period.



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345

346 **Fig. S20.** Statistical analysis of the agronomic traits of the indicated lines. (A-D) Comparison of
347 flowering time (A), node number (B), branch number (C) and stem diameter (D) of the indicated
348 lines planted in the Beijing field in 2020 and 2021. (E) The curves of main stem length with each
349 node of the indicated lines in 2021. Above data are means ± SD (n = 10). The significant
350 differences (A-D) were determined by two-tailed Student's t-test (*p < 0.05, **p < 0.01).

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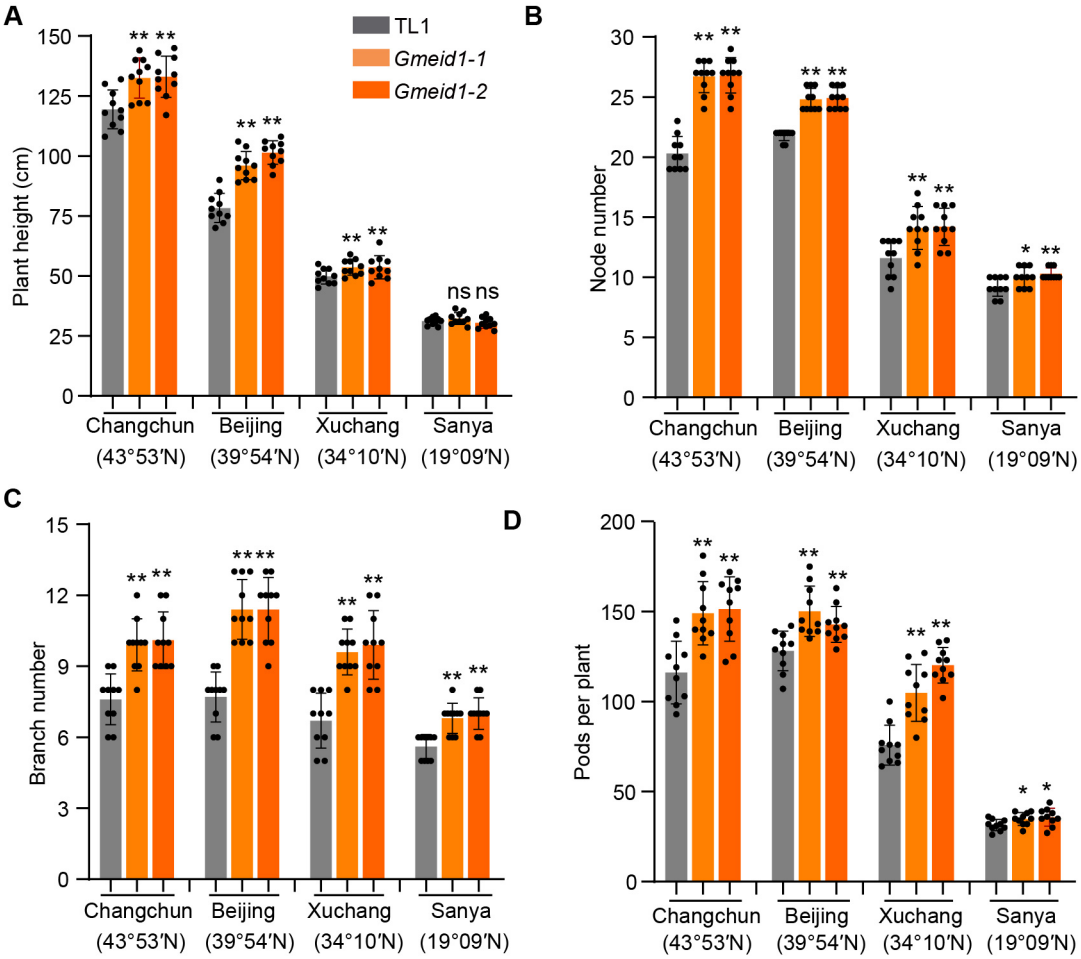

352

353 **Fig. S21.** Statistical analysis of the agronomic traits of the indicated lines at different latitudes. (A-
354 C) Statistical analysis of plant height (A), node number (B), branch number (C) and pods per
355 plant (D) of the indicated lines in Changchun (125°19′E, 43°53′N), Beijing (116°23′E, 39°54′N),
356 Xuchang (104°31′E, 34°10′N) and Sanya (108°56′E, 19°09′N) in 2021. Data are means ± SD (n =
357 10) with significant differences determined by two-tailed Student's t-test (*p < 0.05, **p < 0.01).

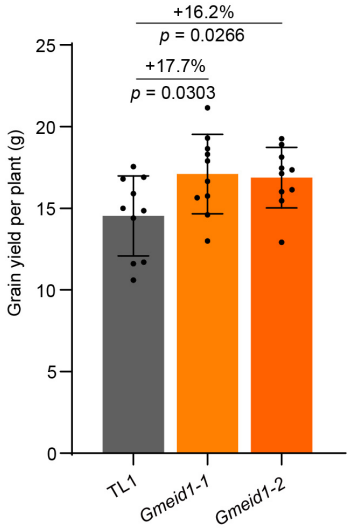

Fig. S22. Comparison of yield per plant between the wild-type TL1 and the *Gmeid1* mutants under normal farming conditions. The indicated lines were grown under a planting density of about 200,000 plants/hectare in Xuchang in the summer of 2021. Data are means ± SD (n = 10) with significant differences determined by two-tailed Student's t-test.

Dataset S1 (separate file). Gene Expression (FPKM) values in second trifoliolate leaves in LD photoperiodic cycle obtained from the RNA-seq experiment.

Dataset S2 (separate file). Screening of candidate regulators of *E1*.

Dataset S3 (separate file). List of primer sequences (5' to 3') used in this study.

Dataset S4 (separate file). Phylogenetic analysis of EID1 and its homologous proteins in the indicated species.

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