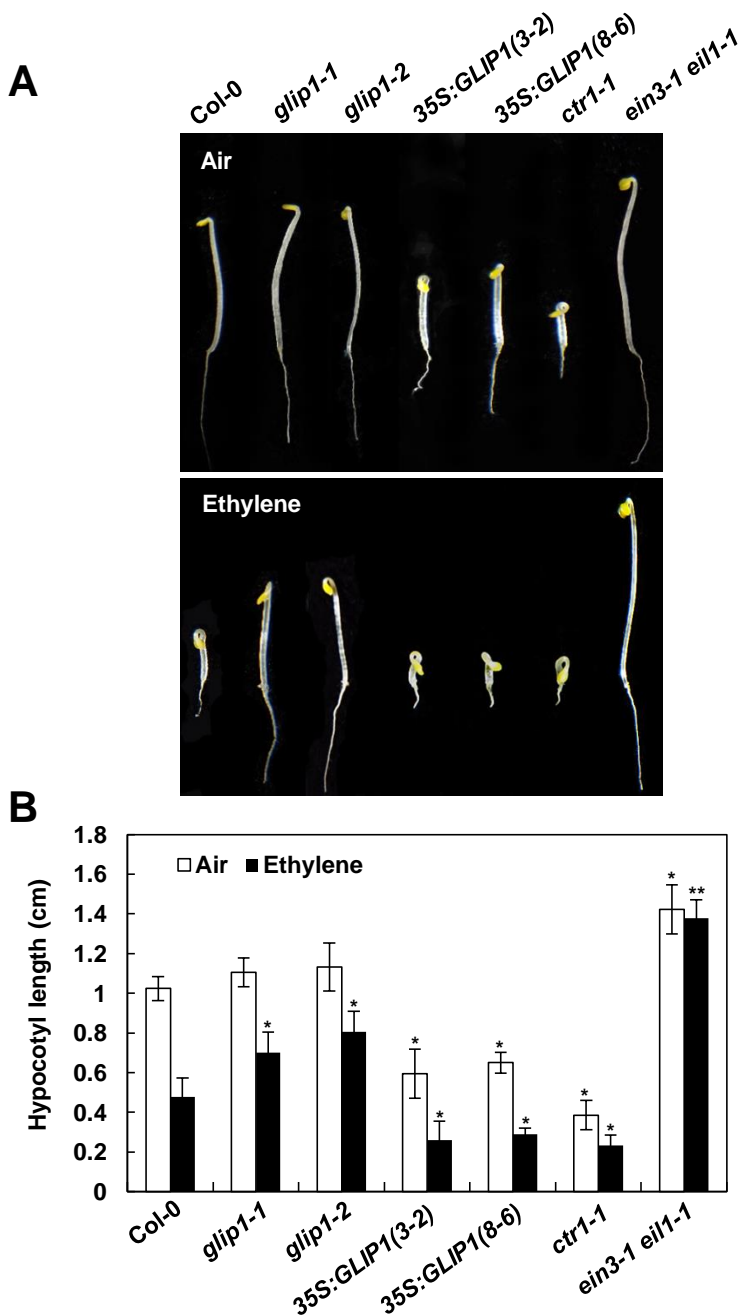
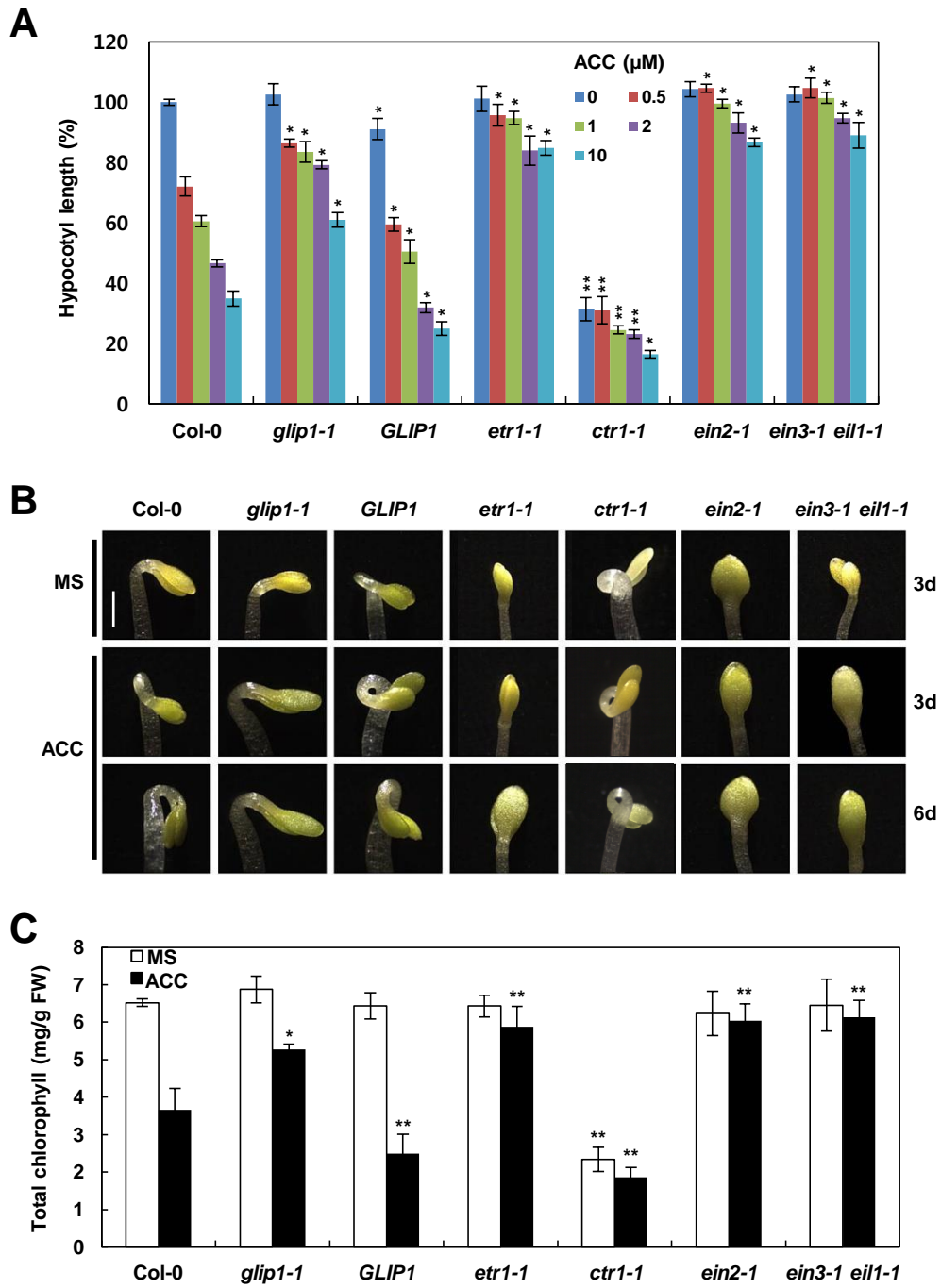


Supplemental Figure S1. Expression analysis of ethylene-responsive genes in Col-0, *glip1-1*, and *35S:GLIPI* plants. A, Expression analysis of *GLIPI* in response to ethephon, ACC, and ethylene. Four-week-old plants were mock-treated or treated with 1.5 mM ethephon, 10 μ M ACC, and 10 ppm ethylene for 12 h. The values represent means \pm SD from 3 independent experiments. Asterisks indicate significant differences from the mock-treated Col-0 (*t* test; **P* < 0.05). B to F, Expression analysis of ethylene-responsive genes *ERF5*, *ACO2*, *EBP*, *ETR2*, and *ERS1* in Col-0, *glip1-1*, and *35S:GLIPI* plants. Four-week-old plants were treated with air or 10 ppm ethylene for 12 h. The values represent means \pm SD from 3 independent experiments. Asterisks indicate significant differences from the respective Col-0 (*t* test; **P* < 0.05; ***P* < 0.01).

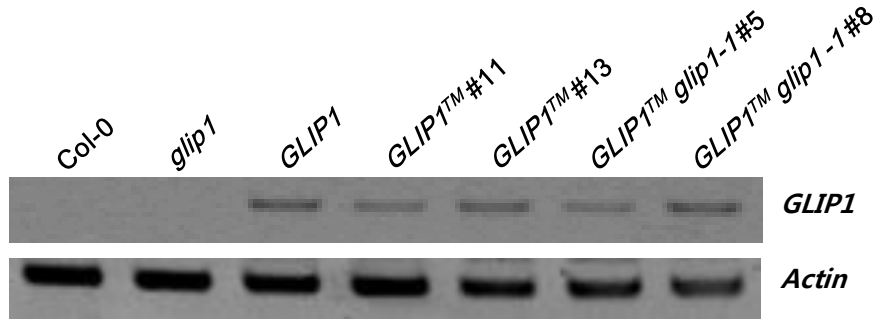


Supplemental Figure S2. Triple response of *glip1* mutants and homozygous T₃ lines of *35S:GLIP1*. A, Triple response phenotypes of 4-day-old etiolated seedlings of Col-0, *glip1-1*, *glip1-2*, *35S:GLIP1(3-2)* and *35S:GLIP1(8-6)*. Seedlings were grown in air or 10 ppm ethylene. B, Hypocotyl lengths of plants in A. The values are means \pm SD ($n = 20$). Asterisks indicate significant differences from the respective Col-0 (t test; * $P < 0.05$; ** $P < 0.01$).

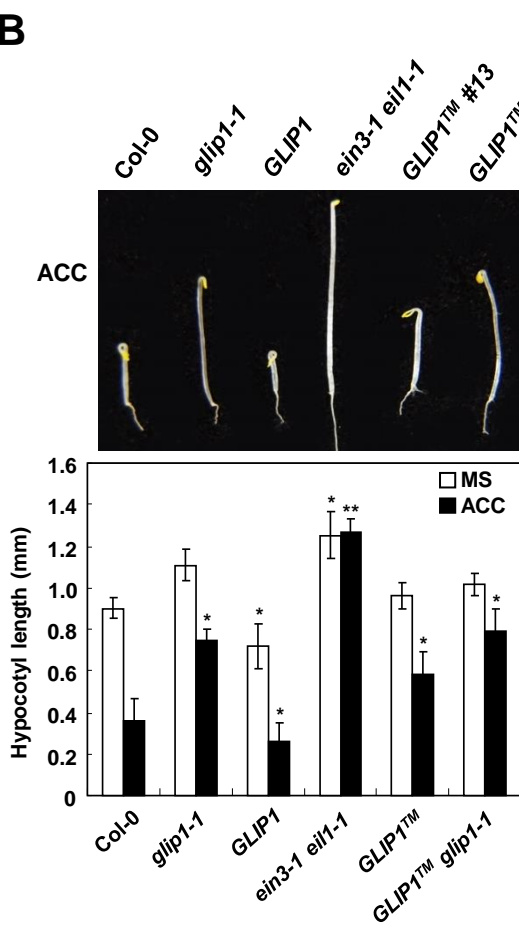


Supplemental Figure S3. Positive regulation of ethylene responses by GLIP1. A, Hypocotyl lengths of 4-day-old etiolated seedlings of Col-0, *glip1-1*, *35S:GLIP1*, and ethylene mutants grown in the presence of different concentrations of ACC. The values are means \pm SD ($n = 20$). Asterisks indicate significant differences from the respective Col-0 (t test; $*P < 0.05$; $**P < 0.01$). B, Apical hook curvature of 3- and 6-day-old etiolated seedlings grown on MS media alone or supplemented with 10 μ M ACC. C, Chlorophyll contents of Col-0, *glip1-1*, *35S:GLIP1*, and ethylene mutants. Plants were grown on MS media alone or supplemented with 10 μ M ACC for 10 days under long-day conditions. The values are means \pm SD ($n = 10$). Asterisks indicate significant differences from the respective Col-0 (t test; $*P < 0.05$; $**P < 0.01$). The experiment was repeated 3 times with similar results.

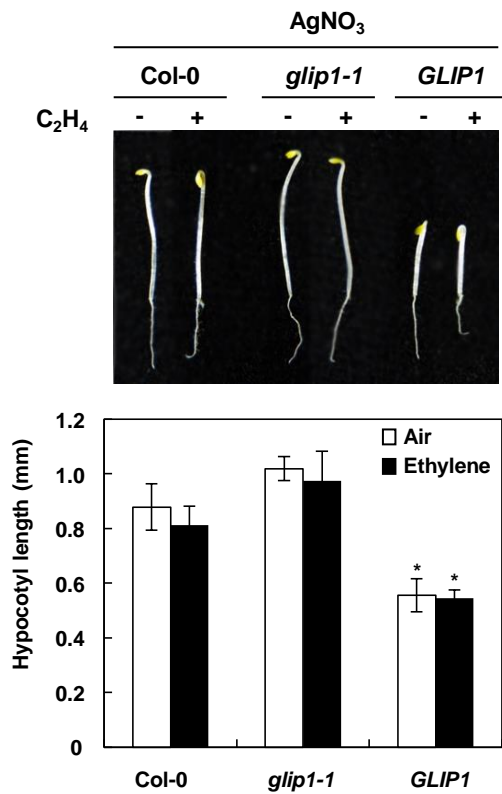
A



B



C



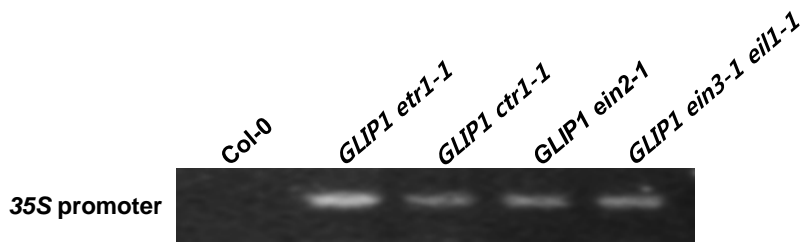
Supplemental Figure S4. Effects of catalytic mutation of *GLIP1* and Ag²⁺ on the triple response of *glip1-1* and *35S:GLIP1* seedlings. A, Expression analysis of *GLIP1* in Col-0, *glip1-1*, *35S:GLIP1*, and inactive *GLIP1* mutants *GLIP1TM* and *GLIP1TM glip1-1* plants. B, Triple response phenotypes (top) and hypocotyl lengths (bottom) of 4-day-old etiolated seedlings of Col-0, *glip1-1*, *35S:GLIP1*, *GLIP1TM*, and *GLIP1TM glip1-1* in the presence of 10 μM ACC. C, Triple response phenotypes (top) and hypocotyl lengths (bottom) of *glip1-1* and *35S:GLIP1* seedlings in the presence of Ag²⁺. Seedlings were grown on 100 μM AgNO₃-containing MS media in air or 10 ppm ethylene for 4 days in the dark. The values are means ± SD (*n* = 10). Asterisks indicate significant differences from the respective Col-0 (*t* test; **P* < 0.05; ***P* < 0.01).



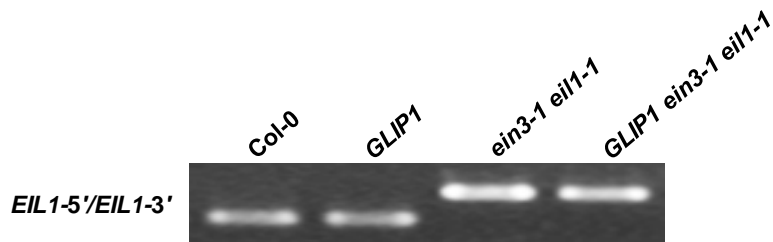
| | | | | |
|---------------------|---------------------------------------|---------------------------------------|---------------------------------------|---|
| Col-0 | <i>etr1-1</i> | <i>ctr1-1</i> | <i>ein2-1</i> | <i>ein3-1</i> <i>eil1-1</i> |
| <i>GLIP1</i> | <i>GLIP1</i> <i>etr1-1</i> | <i>GLIP1</i> <i>ctr1-1</i> | <i>GLIP1</i> <i>ein2-1</i> | <i>GLIP1</i> <i>ein3-1</i> <i>eil1-1</i> |

Supplemental Figure S5. Genetic crosses between *35S:GLIP1* and ethylene mutants. Growth phenotypes (top) of Col-0, *35S:GLIP1*, ethylene mutants, and crossed lines (indicated at bottom) grown in soil for 4 weeks under long-day conditions.

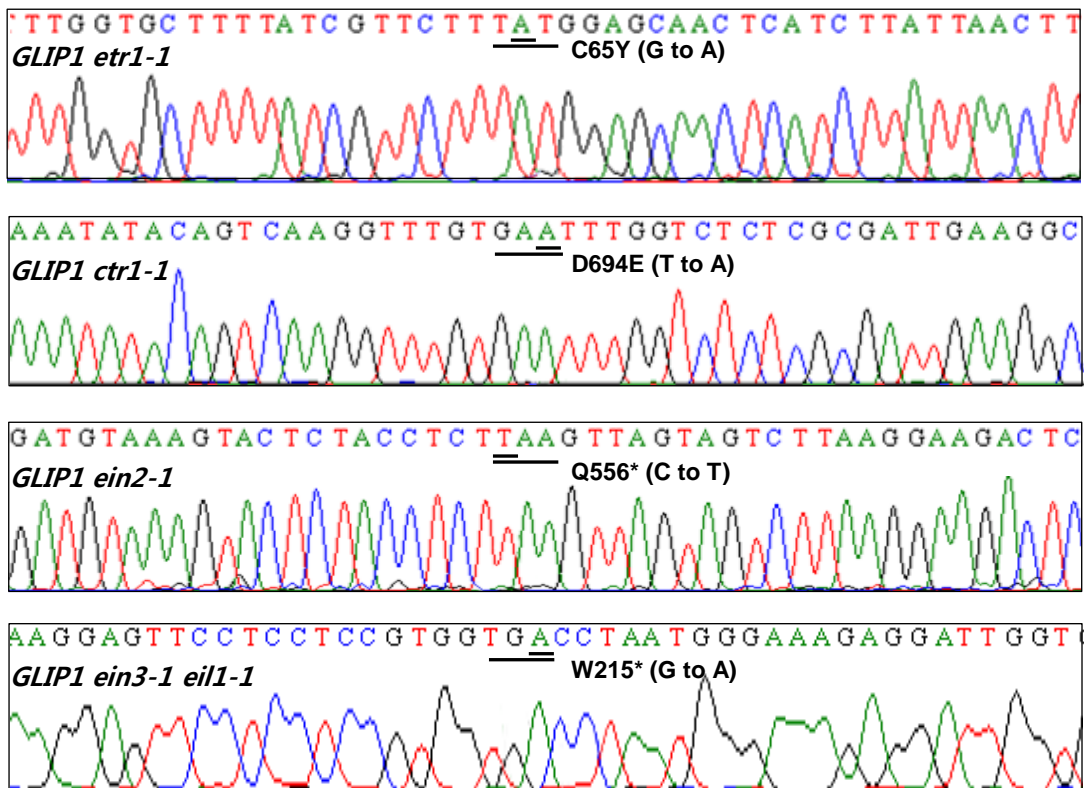
A



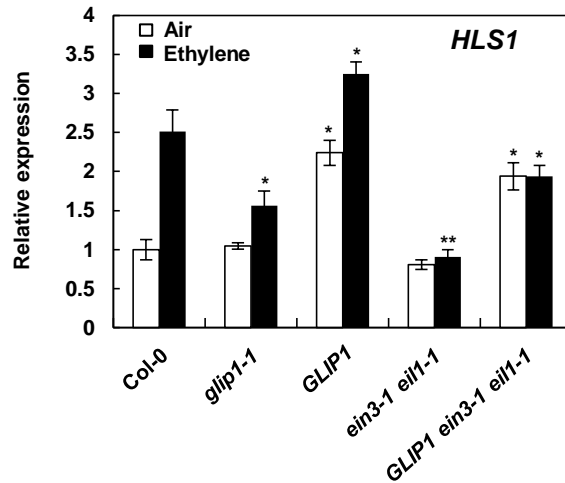
B



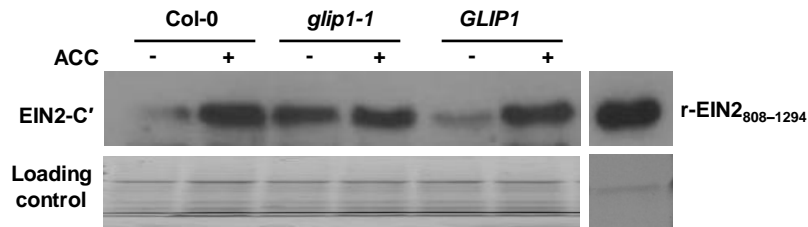
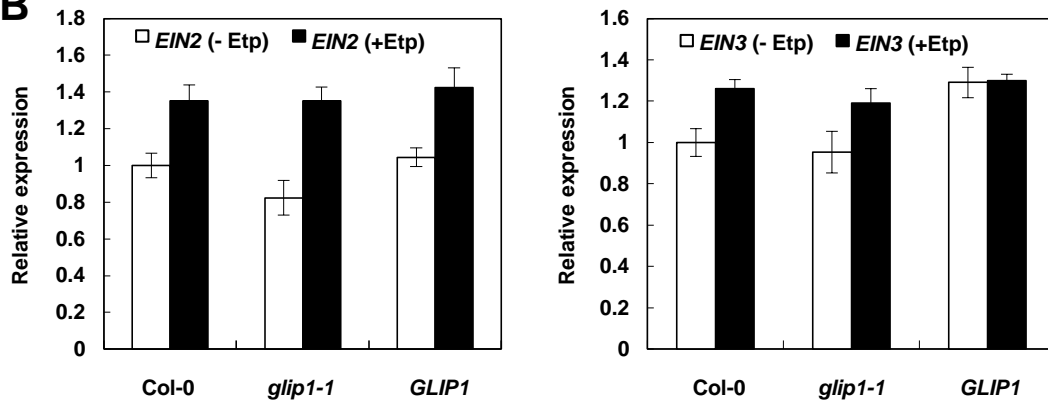
C



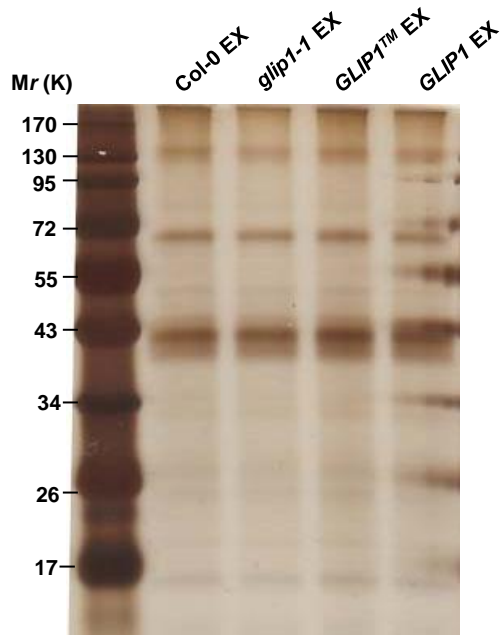
Supplemental Figure S6. Genomic DNA analysis of crossed lines. A, PCR analysis for detection of 35S promoter in Col-0 and homozygous crossed lines. B, PCR-based genotyping of transposon insertion within the *EIL1* gene in Col-0, *35S:GLIP1*, *ein3-1 eil1-1*, and *35S:GLIP1 ein3-1 eil1-1* plants. *eil1-1* contains a transposon insertion, and the PCR band is ~200 bp larger than that of Col-0. C, DNA sequencing evidence for point mutations in crossed lines. Total genomic DNA was extracted from 4-week-old plants. PCR amplification of 500~700 bp target regions was performed using specific primers. PCR products were purified and sequenced. The black bars on top of the chromatograms mark mutation sites: *etr1-1*, C65Y substitution; *ctr1-1*, D694E substitution; *ein2-1*, Q556* substitution; *ein3-1*, W215* substitution.



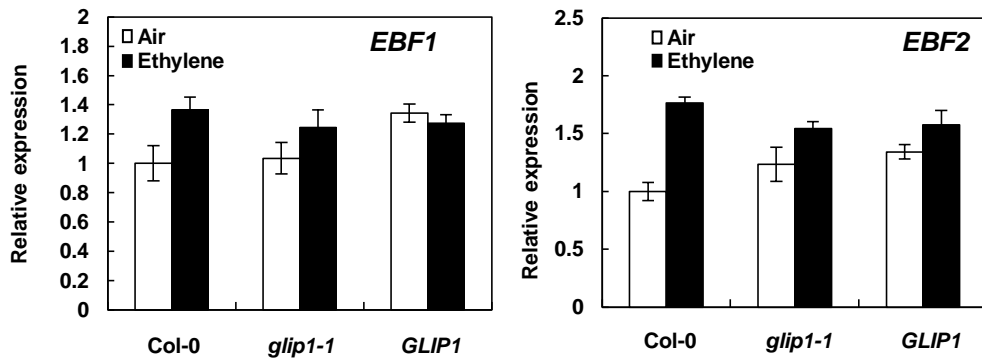
Supplemental Figure S7. Expression analysis of *HLS1* in Col-0, *glip1-1*, *35S:GLIP1*, *ein3-1 eil1-1*, and *35S:GLIP1 ein3-1 eil1-1* plants. Four-week-old plants were treated with air or 10 ppm ethylene for 12 h. The values represent means \pm SD from 3 independent experiments. Asterisks indicate significant differences from the respective Col-0 (*t* test; * $P < 0.05$; ** $P < 0.01$).

A**B**

Supplemental Figure S8. Expression analysis of EIN2 proteins, and *EIN2* and *EIN3* transcripts in Col-0, *glip1-1*, and *35S:GLIP1* plants. A, Immunoblot analysis of EIN2. Total protein extracts (10-30 μg) of 10-day-old seedlings untreated or treated with 10 μM ACC for 4 h were separated by SDS gel electrophoresis, and subjected to Coomassie staining (bottom) and Western blot analysis with anti-EIN2 antibody (top). EIN2-C', C-terminal EIN2 fragment; r-EIN2₈₀₈₋₁₂₉₄, recombinant EIN2 (residues 800-1294). B, Expression analysis of *EIN2* and *EIN3*. Four-week-old plants were treated with water (mock) or 1.5 mM ethephon for 24 h. The values represent means ± SD from 3 independent experiments. Etp, ethephon.



Supplemental Figure S9. Proteins isolated from petiole exudates of Col-0, *glip1-1*, *35S:GLIP1TM*, and *35S:GLIP1* plants. Proteins were separated by SDS-gel electrophoresis and visualized by silver staining.



Supplemental Figure S10. Expression analysis of *EBF1* and *EBF2* in Col-0, *glip1-1*, and *35S:GLIP1* plants. Four-week-old plants were treated with air or 10 ppm ethylene for 12 h. The values represent means \pm SD from 3 independent experiments.

Supplemental Table SI. *List of primers used for PCR and qRT-PCR*

| Gene name | Oligonucleotide (5'-3') |
|--------------------|-----------------------------|
| <i>ETR1</i> -5' | GCTTCAACGCTCCCCTTTTCTCC |
| <i>ETR1</i> -3' | CATCCGCTGGCCATTGCGGTTT |
| <i>ETR1</i> -(seq) | GATTGTCTACGCTACGTTCTCG |
| <i>CTR1</i> -5' | GAGAGACGTGCCTGAGTATGG |
| <i>CTR1</i> -3' | GACAGCTTGAGGCTGCTGTATC |
| <i>CTR1</i> -(seq) | GTGATTACTTCCTGATCTTGGTG |
| <i>EIN3</i> -5' | GTCTAGAGCTCAAGATGGGATC |
| <i>EIN3</i> -3' | CTCTAGCCAAGGACTCTTCTTGG |
| <i>EIN3</i> -(seq) | GGGAGTGGTGGAAAGATAAGG |
| <i>EIN2</i> -5' | GGTTTGAGATGGAATACCGTGATGG |
| <i>EIN2</i> -3' | TCAAGGATGGCAGATAAGTGTCTCC |
| <i>EIN2</i> -(seq) | ATGCTCAAATGCTTTATCTTATCCATC |
| <i>EIL1</i> -5' | GGGAATGGTGGAAAGATAAG |
| <i>EIL1</i> -3' | CTTTCGCCGTCATCTTATCC |
| 35S promoter-5' | GAGACTTTCAACAAAGGGTAA |
| 35S promoter-3' | CAAATGAAATGAACTCCTTAT |
| <i>ERF1</i> -5' | GAGGAAACACTCGATGAGACG |
| <i>ERF1</i> -3' | GGAGCGGTGATCAAAGTCAC |
| <i>ERF5</i> -5' | TGGFAGAGACGTTTCCGTTTG |
| <i>ERF5</i> -3' | TGAGGAGATAACGGCGACAG |
| <i>SID2</i> -5' | GAGACTTACGAAGGAAGATGATGAG |
| <i>SID2</i> -3' | TGATCCCGACTGCAAATTCACTCTC |
| <i>b-CHI</i> -5' | TTACGGTCTATGCGGTAG |
| <i>b-CHI</i> -3' | GAGGCCGTTAACGAAGG |
| <i>GLIP1</i> -5' | GGTTTGAGACGGCTAAATC |
| <i>GLIP1</i> -3' | GTTCAAACAGCGCTTTGAG |
| <i>PDF1.2</i> -5' | GCTAAGTTTGCTTCCATCATCACC |
| <i>PDF1.2</i> -3' | AACATGGGACGTAACAGATACACAC |
| <i>HLS1</i> -5' | CACGGTTATCAAGTTAGAGC |
| <i>HLS1</i> -3' | GAAAGTCCCAAGCGAGA |
| <i>ACO2</i> -5' | GTTGGATCTACTGTGTG |
| <i>ACO</i> -3' | TCTTCATTGCTGCGAACC |
| <i>ETR2</i> -5' | ATCATAGATGGGCTGCTTG |

| | |
|-------------------|-------------------------|
| <i>ETR2</i> -3' | GGATCCATGGACAGATATGG |
| <i>EBP</i> -5' | GTGGTGATAAAGCCAAGC |
| <i>EBP</i> -3' | CGGACTCATCAAGCTGAC |
| <i>ERS1</i> -5' | TCTATCATGAAACCCGAGTC |
| <i>ERS1</i> -3' | ACCGTTGCAGATACCAAG |
| <i>EBF1</i> -5' | CTGATGTGTCTCTTGCT |
| <i>EBF1</i> -3' | GGCACAACTTCCCGAT |
| <i>EBF2</i> -5' | GGATCAACCAGTTTGGT |
| <i>EBF2</i> -3' | AAGATGCCAAGGCCTTG |
| <i>ACTIN1</i> -5' | GGTGTCATGGTTGGTATGGGTC |
| <i>ACTIN1</i> -3' | CCTCTGTGAGTAGAACTGGGTGC |
