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Supplementary Materials for

Nuclear pyruvate dehydrogenase complex regulates histone acetylation and transcriptional regulation in the ethylene response

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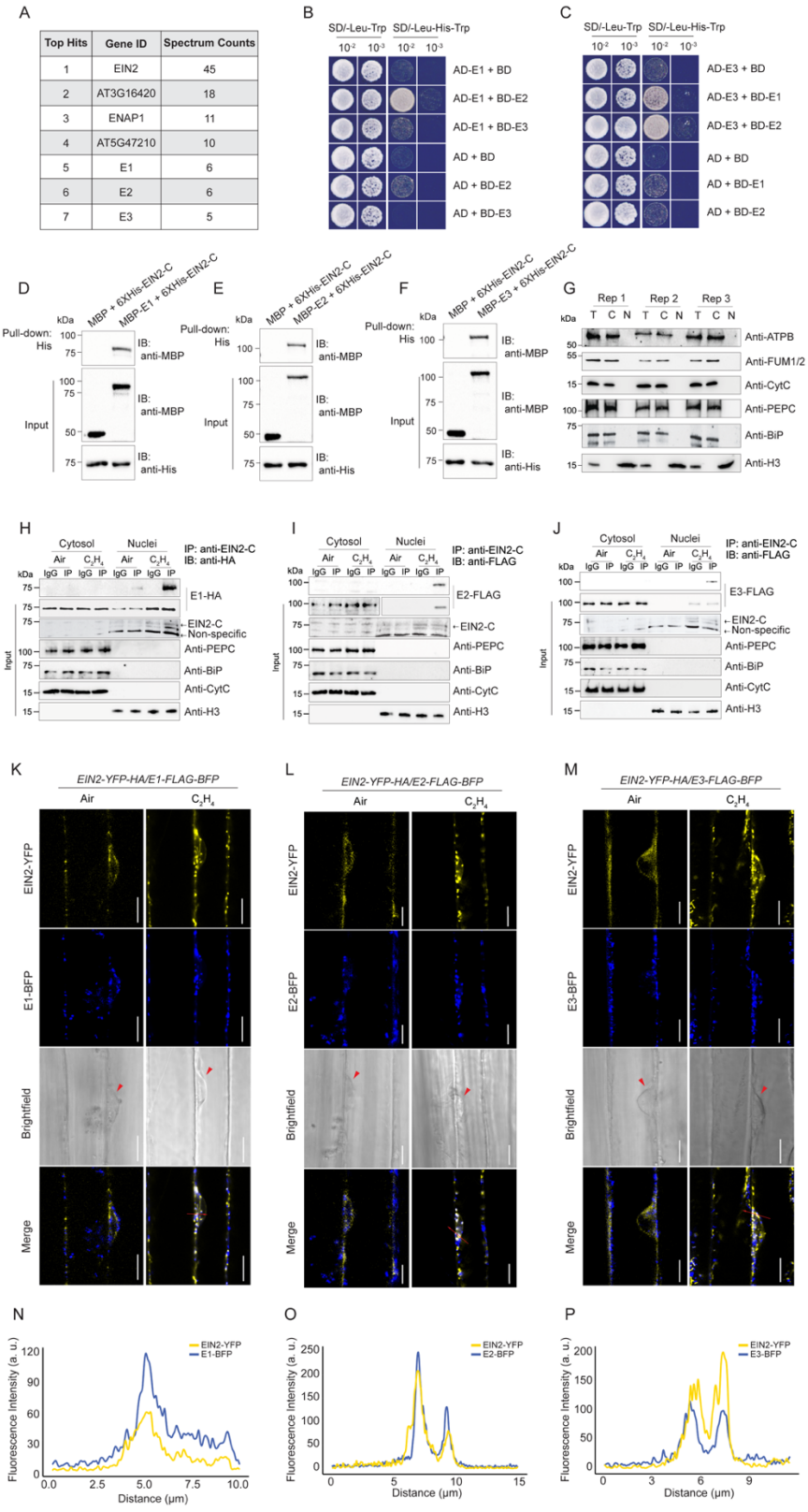
This PDF file includes:

Supplemental Materials

A

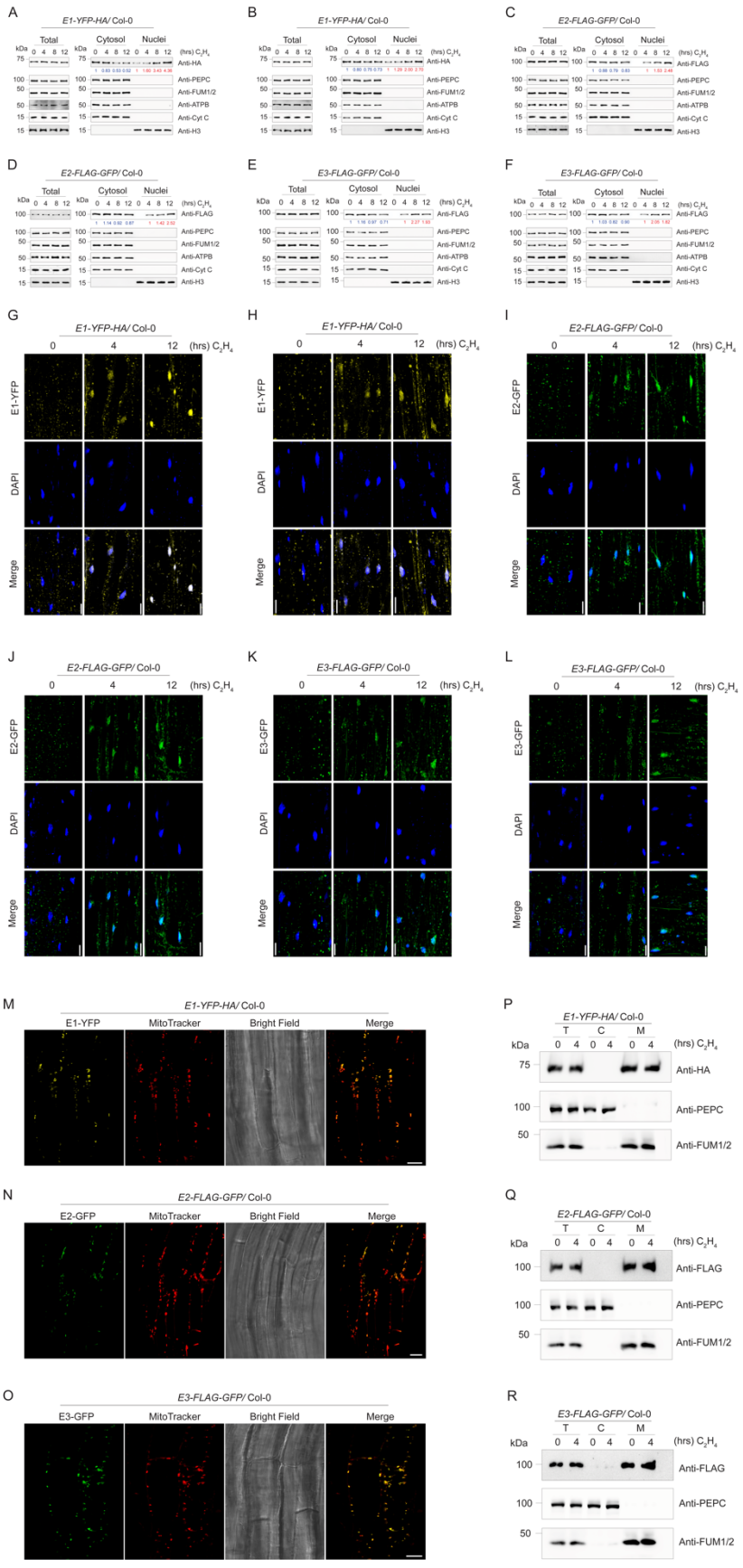
Top Hits	Gene ID	Spectrum Counts
1	EIN2	45
2	AT3G16420	18
3	ENAP1	11
4	AT5G47210	10
5	E1	6
6	E2	6
7	E3	5



Supplemental Figure 1. Related to Figure 1. Physical interactions between PDC E1, E2, and E3 and between EIN2-C and PDC subunits and nuclear co-localizations between EIN2-C and PDC subunits with ethylene treatment.

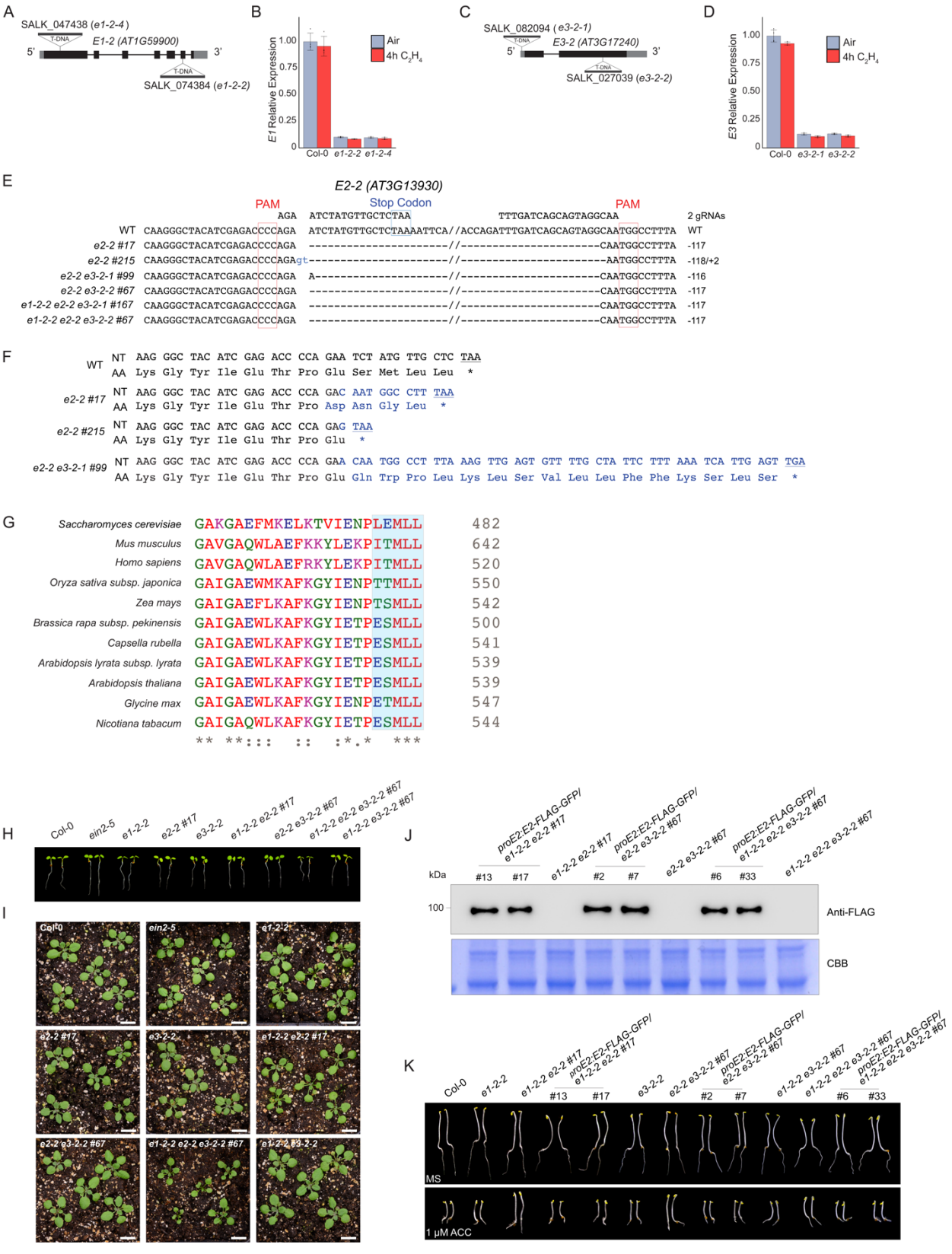
(**A**) IP-MS spectral counts of part of EIN2-C interacting proteins with 4 hours of ethylene gas treatment. (**B** and **C**) Yeast two-hybrid assays showing that E1, E2, and E3 interact with each other to form a protein complex. BD: GAL4 DNA binding domain; AD: GAL4 activation domain. E1 was fused to AD and used as bait proteins in (**B**) and AD-E3 was used as bait proteins in (**C**). Left panels: Yeasts grown on two-dropout medium (SD/-Leu-Trp) served as a loading control; Right panels: Yeast grown on selective three-dropout medium (SD/-Leu-His-Trp). (**D**-**F**) Pull-down assay to examine the interactions between EIN2-C and PDC E1 (**D**), E2 (**E**), and E3 (**F**), respectively. His-tagged EIN2-C and MBP-tagged PDC subunits were purified from *E. coli* and subject to pull-down assays. MBP was used as a negative control. (**G**) Assessment of the purities of cytosolic and nuclear fractions. Three-day-old etiolated seedlings treated with 4 hours ethylene were applied to the nuclear-cytoplasmic fractionation procedure as described in the Materials and Methods section. Three independent biological replicates were included. Anti-ATP synthase Beta (anti-ATPB), anti-Fumarase 1+2 (anti-FUM1/2), and anti-Cytochrome C (anti-Cyt C) were used to evaluate the presence of mitochondrial proteins. Anti- PEP Carboxylase (anti-PEPC) was used to examine the cytoplasmic fraction and anti-Lumenal-binding protein (anti-BiP) serves as ER control. Anti-Histone H3 (anti-H3) served as nuclear protein loading. Rep 1, Rep 2, and Rep 3 represent replicate 1, replicate 2, and replicate 3, respectively. T represents total extract, C represents cytosolic fraction, and N represents nuclear fraction. (**H**-**J**) *In vivo* co-immunoprecipitation experiments to confirm the interaction between EIN2-C and PDC. Cytosolic and nuclear fractions from 3-day-old etiolated seedlings of *35S:E1-YFP-HA* (**H**), *35S:E2-FLAG-GFP* (**I**), and *35S:E3-FLAG-GFP* (**J**) treated with or without 4 hours of ethylene gas were used for the immunoprecipitation with anti-EIN2-C antibody. The immunoprecipitation with IgG beads was used as a negative control. E2 from the nucleus was detected in the IP products in the presence

of 4 hours of ethylene gas (**I**). (**K**-**M**) Confocal microscopy images showing the co-localization of EIN2-C-YFP with PDC subunits E1-, E2- and E3-BFP in response to ethylene. The images were collected from the hypocotyls of 3-day old etiolated seedlings as indicated in the figure with or without 4 hours of ethylene treatment. Red arrowhead indicates nuclei in the brightfield. Red arrow represents the transection used for measuring fluorescence intensity as indicated in the figure. Scale bars is 10μm. (**N**-**P**) Fluorescence intensity measurements of confocal images from Fig. 1F-1H (lowest panel under ethylene treatment) to show co-localization of EIN2-C with PDC subunits in the nucleus with ethylene treatment. Yellow line indicates EIN2-YFP fluorescence intensity and blue line indicates PDC E1-BFP (**N**), E2-BFP (**O**), or E3-BFP (**P**) fluorescence intensity, respectively. Scale bars: 10μm.


A
E1-YFP-HA/ Col-0
Total
Cytosol
Nuclei
kDa
0 4 8 12 0 4 8 12 0 4 8 12
(hrs) C2H4
Anti-HA
Anti-PEPC
Anti-FUM1/2
Anti-ATPB
Anti-Cyt C
Anti-H3
B
E1-YFP-HA/ Col-0
C
E2-FLAG-GFP/ Col-0
Anti-FLAG
D
E2-FLAG-GFP/ Col-0
E
E3-FLAG-GFP/ Col-0
F
E3-FLAG-GFP/ Col-0
G
E1-YFP-HA/ Col-0
0 4 12 (hrs) C2H4
E1-YFP
DAPI
Merge
H
E1-YFP-HA/ Col-0
I
E2-FLAG-GFP/ Col-0
E2-GFP
J
E2-FLAG-GFP/ Col-0
K
E3-FLAG-GFP/ Col-0
E3-GFP
L
E3-FLAG-GFP/ Col-0
M
E1-YFP-HA/ Col-0
E1-YFP
MitoTracker
Bright Field
Merge
N
E2-FLAG-GFP/ Col-0
E2-GFP
O
E3-FLAG-GFP/ Col-0
E3-GFP
P
E1-YFP-HA/ Col-0
T C M
0 4 0 4 0 4
(hrs) C2H4
Anti-HA
Anti-PEPC
Anti-FUM1/2
Q
E2-FLAG-GFP/ Col-0
Anti-FLAG
R
E3-FLAG-GFP/ Col-0

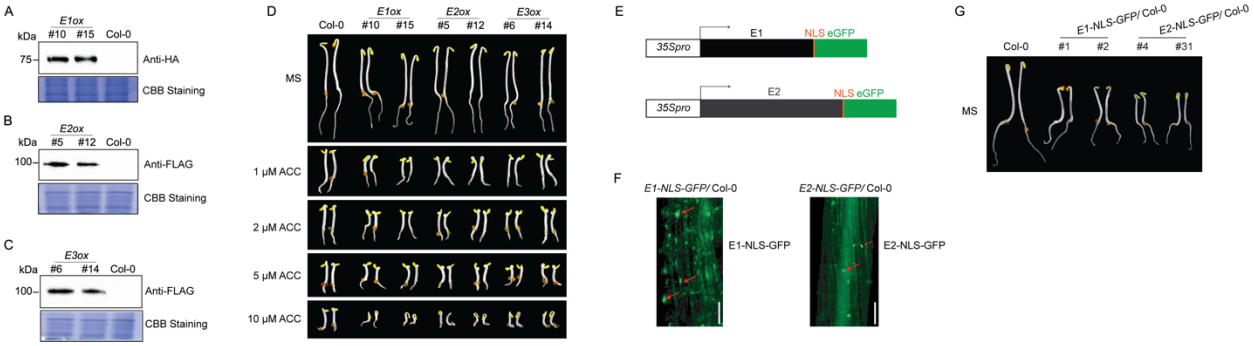
Supplemental Figure 2. Related to Figure 2. Nuclear PDC accumulation in response to ethylene.

(**A**-**F**) Two additional independent biological replicates of fractionation immunoblots to examine the total and subcellular protein levels of PDC in of *E1-YFP-HA* (**A** and **B**), *E2-FLAG-GFP* (**C** and **D**), and *E3-FLAG-GFP* (**E** and **F**) transgenic plants with ethylene gas treatments of 0, 4h, 8h, and 12h. PDC E1 was probed with anti-HA antibody, and E2 and E3 were probed with anti-FLAG antibody in total protein extracts, cytoplasmic fractions, and nuclear fractions. PEPC, CytC, ATPB, FUM1/2, and histone H3 was used to evaluate the purities of nuclear and cytosolic fractionations, as well as to serve as loading controls. Blue number indicates PDC band intensity that normalized to PEPC signal. Red number indicates PDC band intensity that normalized to histone H3 signal. (**G**-**L**) Two additional independent biological replicate results of confocal microscopy images showing the subcellular localization of E1-YFP-HA (**G** and **H**), E2-FLAG-GFP (**I** and **J**), and E3-FLAG-GFP (**K** and **L**) with time series of ethylene gas treatments. DAPI staining labels nuclei. Scale bars is 20μm. (**M**-**O**) Subcellular localization of PDC fusion protein in Arabidopsis three-day-old etiolated seedlings with MitoTracker Red staining. Scale bar: 10 μm. (**P**-**R**) Western blot of different cell fractionations to examine the subcellular localization of PDC in *E1-YFP-HA* (**P**), in *E2-FLAG-GFP* (**Q**), and in *E3-FLAG-GFP* (**R**) transgenic plants with or without four hours of ethylene gas treatments. T: total protein; C: mitochondria-free cytoplasmic fraction; M: mitochondrial fraction. PEPC and FUM1/2 (mitochondria matrix protein marker) were used to assess purities of cytosolic and mitochondrial fractionations and loading controls.



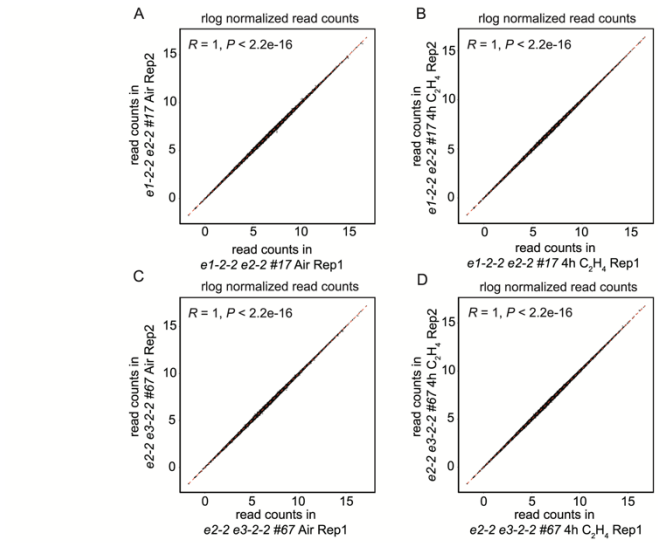
Supplemental Figure 3. Related to Figure 3. Different *PDC* mutant alleles.

(**A**) Diagram of the gene structure of *PDC E1* locus, and the T-DNA insertion loci of *e1-2-2* and *e1-2-4* were indicated. (**B**) qRT-PCR analysis of *E1* expression in 3-day-old etiolated seedlings of Col-0, *e1-2-2*, and *e1-2-4* with and without 4-hour ethylene treatments. Bar represents the average of relative expression of *E1* normalized to *Actin2* expression. Whisker represents ± SD (n =4). (**C**) Diagram to show the T-DNA insertion locus for *e3-2-1* and *e3-2-2* alleles. (**D**) qRT-PCR analysis of *E3* expression in the indicated 3-day-old etiolated seedlings with and without 4 hours of ethylene gas treatments. Values are average of relative expression of *E3* normalized to that of *Actin2;* error bar indicates the SD (n = 4). (**E**) DNA sequences to show the mutations in *e2* single mutants, *pdc* double or triple mutants that were generated by CRIPSR-Cas9. Deletions are shown as dashes. Red box indicates CRISPR gRNA PAM sequence. The numbers of deletion (base pair) for each mutation are shown on the right. (**F**) Potential mutated E2 protein amino acid sequences predicted from mutated *E2* nucleotide sequences. Mutated nucleotides and amino acids are in blue, and the stop codon is underlined and indicated by asterisk. The mutations of *E2* in *e2-2 #17* is the same as mutations of *E2* in *e2-2 e3-2-2 #67*, *e1-2-2 e2-2 e3-2-1 #167*, and *e1-2-2 e2-2 e3-2-2 #67*. (**G**) The alignment of protein sequence from E2 mutated region to show the evolutionary conservation across various species. Blue shade indicates the location of the varieties of mutations in *E2*. (**H**) Phenotypes of green seedlings with different genetic backgrounds grown on MS medium for five days before photograph. (**I**) Phenotypes of four-week-old adult plants with different genetic backgrounds. Scale bar: 2 cm. (**J**) Protein levels of *proE2:gE2-FLAG-GFP* in representative complemented lines from each genetic background. Coomassie blue staining was used as a loading control. (**K**) Representative 3-day-old etiolated seedlings grown on MS medium and 1 μM ACC containing MS medium showing the molecular complementation by *proE2:gE2-FLAG-GFP*.



Supplemental Figure 4. Related to Figure 3. Analyses of *PDC* gain-of-function transgenic plants and *E1- NLS-GFP* and *E2-NLS-GFP* transgenic plants.

(**A**-**C**) Western blot assays of E1, E2, and E3 protein levels in *E1ox* plants (**A**), *E2ox* plants (**B**), and *E3ox plants* (**C**) by anti-HA and anti-FLAG, respectively. Coomassie Brilliant Blue (CBB) Staining served as a loading control. (**D**) Photography of *E1ox*, *E2ox,* and *E3ox* seedings grown on MS medium containing 0, 1 μM, 2 μM, 5 μM, and 10 μM ACC in the dark for 3 days. (**E**) Diagrams of the binary vector containing chimeric *E1-NLS-eGFP* and *E2-NLS-eGFP* fused genes that are driven by the *35S* promoter. (**F**) Confocal image showing the nuclear localization of E1-NLS-eGFP and E2-NLS-eGFP in *E1-NLS-eGFP* and *E2-NLS-eGFP* transgenic plants without ethylene treatment. Red arrow indicates nuclei. Scale bar is 50μm. (**G**) Photography of *E1-NLS-GFP* and *E2-NLS-GFP* transgenic plants grown on MS medium without ACC in the dark for 3 days.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B
rlog normalized read counts
R = 1, P < 2.2e-16
read counts in e1-2-2 e2-2 #17 4h C2H4 Rep2
read counts in e1-2-2 e2-2 #17 4h C2H4 Rep1
C
rlog normalized read counts
R = 1, P < 2.2e-16
read counts in e2-2 e3-2-2 #67 Air Rep2
read counts in e2-2 e3-2-2 #67 Air Rep1
D
rlog normalized read counts
R = 1, P < 2.2e-16
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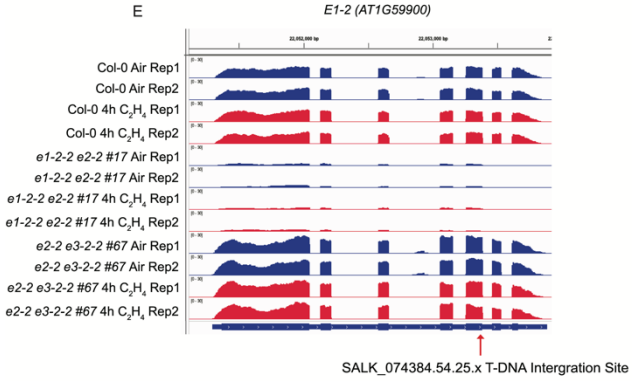
read counts in e2-2 e3-2-2 #67 4h C2H4 Rep1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Col-0 Air Rep2
Col-0 4h C2H4 Rep1
Col-0 4h C2H4 Rep2
e1-2-2 e2-2 #17 Air Rep1
e1-2-2 e2-2 #17 Air Rep2
e1-2-2 e2-2 #17 4h C2H4 Rep1
e1-2-2 e2-2 #17 4h C2H4 Rep2
e2-2 e3-2-2 #67 Air Rep1
e2-2 e3-2-2 #67 Air Rep2
e2-2 e3-2-2 #67 4h C2H4 Rep1
e2-2 e3-2-2 #67 4h C2H4 Rep2
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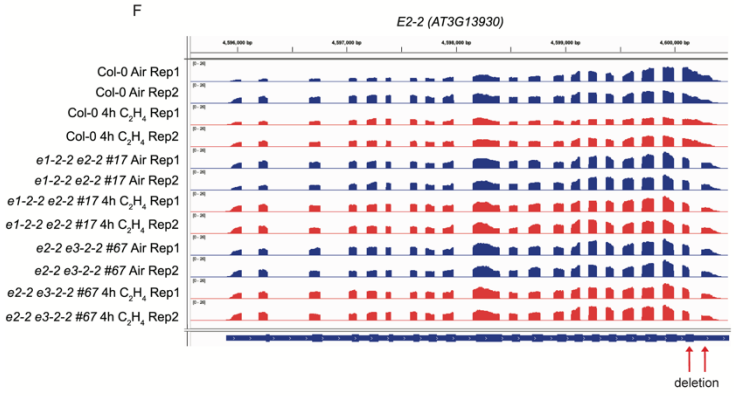
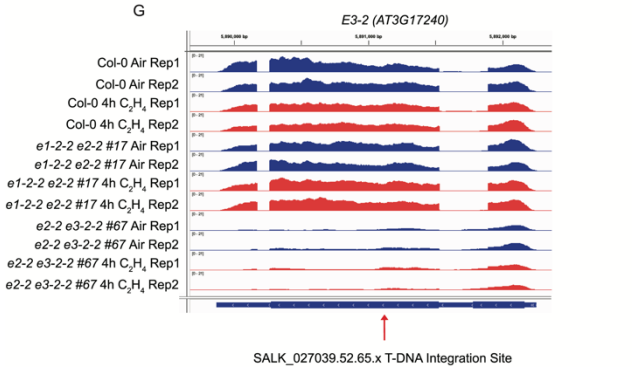
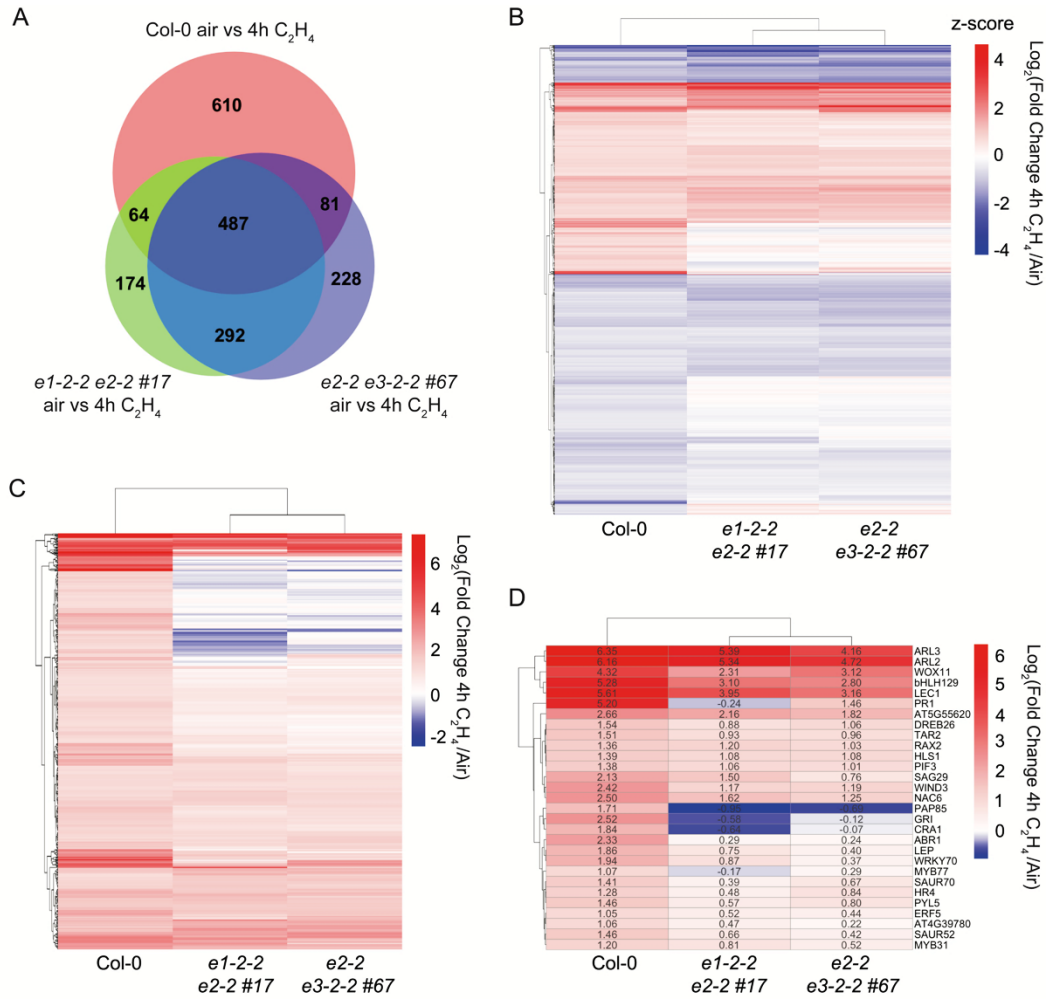

F
E2-2 (AT3G13930)
Col-0 Air Rep1
Col-0 Air Rep2
Col-0 4h C2H4 Rep1
Col-0 4h C2H4 Rep2
e1-2-2 e2-2 #17 Air Rep1
e1-2-2 e2-2 #17 Air Rep2
e1-2-2 e2-2 #17 4h C2H4 Rep1
e1-2-2 e2-2 #17 4h C2H4 Rep2
e2-2 e3-2-2 #67 Air Rep1
e2-2 e3-2-2 #67 Air Rep2
e2-2 e3-2-2 #67 4h C2H4 Rep1
e2-2 e3-2-2 #67 4h C2H4 Rep2
deletion


G
E3-2 (AT3G17240)
Col-0 Air Rep1
Col-0 Air Rep2
Col-0 4h C2H4 Rep1
Col-0 4h C2H4 Rep2
e1-2-2 e2-2 #17 Air Rep1
e1-2-2 e2-2 #17 Air Rep2
e1-2-2 e2-2 #17 4h C2H4 Rep1
e1-2-2 e2-2 #17 4h C2H4 Rep2
e2-2 e3-2-2 #67 Air Rep1
e2-2 e3-2-2 #67 Air Rep2
e2-2 e3-2-2 #67 4h C2H4 Rep1
e2-2 e3-2-2 #67 4h C2H4 Rep2
SALK_027039.52.65.x T-DNA Integration Site

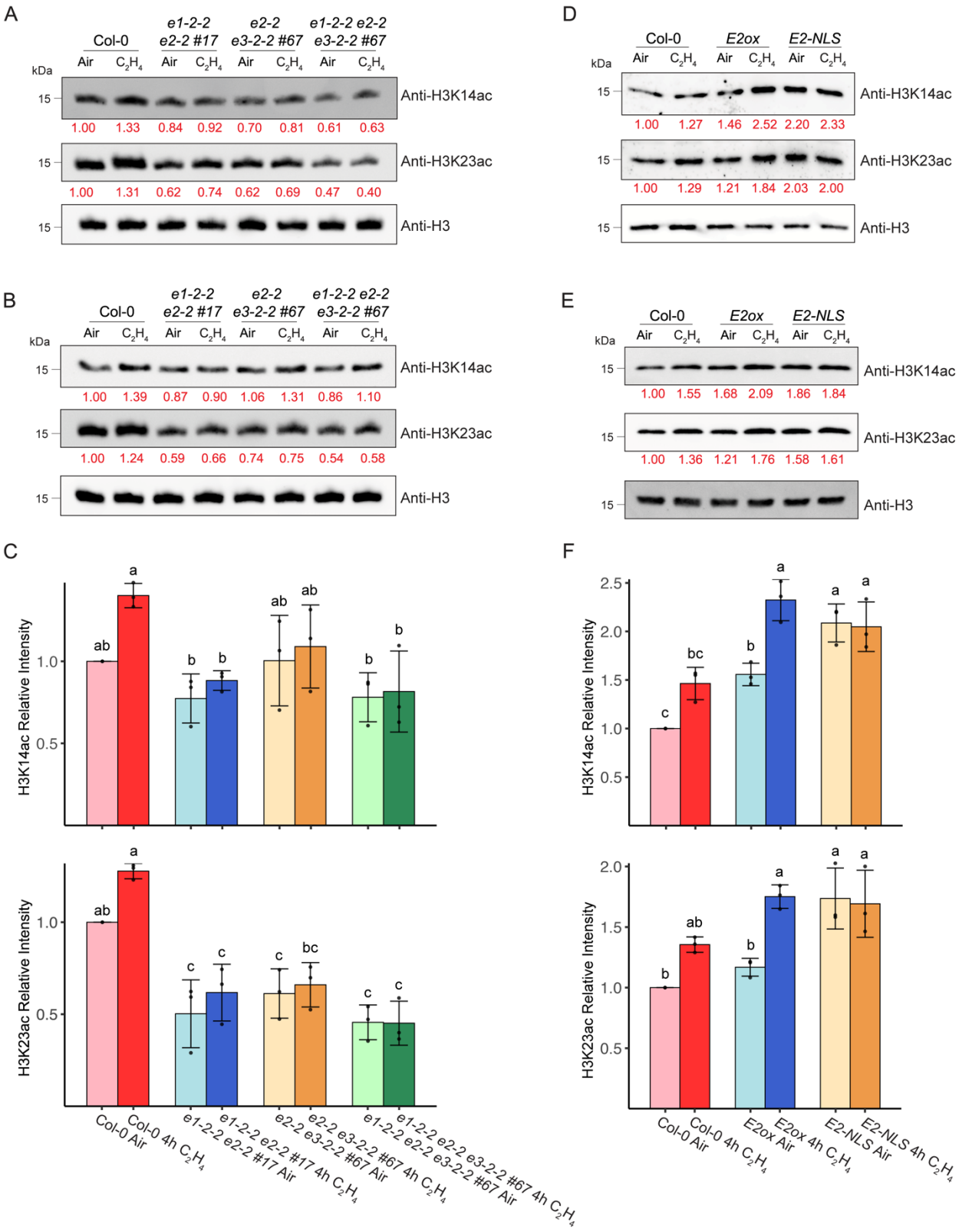
Supplemental Figure 5. Related to Figure 3. mRNA sequencing of PDC *e1-2-2 e2-2 #17* and *e2-2 e3-2-2 #67* mutants with air and ethylene treatments.

(**A**-**D**) Scatter plots showing the reproducibility of *e1-2-2 e2-2 #17* and *e2-2 e3-2-2 #67* RNA-seq results with or without 4 hours of ethylene treatment. *r log* transformed raw read counts were plotted and used to calculate Pearson correlation coefficient (*R*). (**E**-**G**) IGV genome browser tracks of mRNA-seq to show *E1*, *E2*, and *E3* loci in the wild type and in the mutant plants before and after ethylene treatment. Red arrow indicates the T-DNA insertion site (**E** and **G**) or the CRISPR/Cas9 mediated deletion region (**F**).



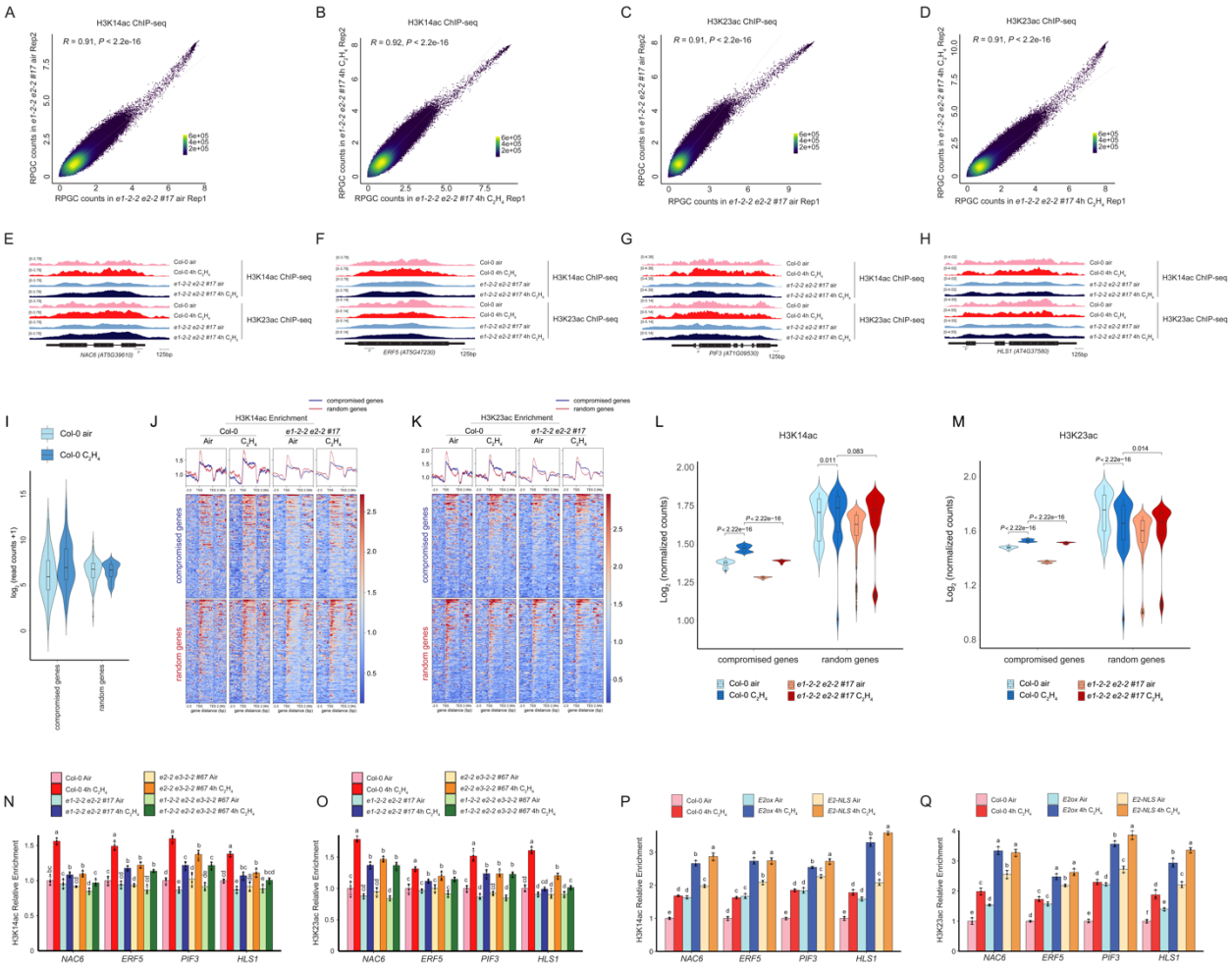
Supplemental Figure 6. Related to Figure 3. PDC is required for ethylene response at transcriptional level.

(A) Venn diagram to compare the ethylene regulated genes ($|\log_2 FC| \geq 1$ and adjusted *P* value ≤ 0.05) in each double mutant and in Col-0. (**B**) Heatmap representation of z-score transformed $\log_2$(Fold Change C_2H_4/Air) of all ethylene-regulated differential expressed genes that were identified in Col-0 in *e1-2-2 e2-2 #17* and *e2-2 e3-2-2 #67* mutant backgrounds. (**C**) Heatmap to show the $\log_2$FC of genes that are up-regulated by ethylene in Col-0 but their up-regulation is compromised in the indicated plants. (**D**) Heatmap to compare the expression of part of typical ethylene up-regulated genes in Col-0 and in the indicated mutants. Raw $\log_2$FC value of each gene is annotated in the heatmap.



Supplemental Figure 7. Related to Figure 4. Global H3K14ac and H3K23ac analyses in PDC loss-of-function mutants and transgenic lines.

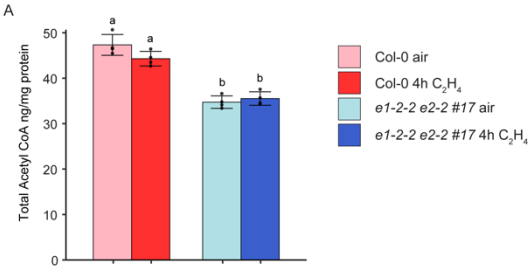
(A and **B)** Two additional independent biological replicates of total H3K14ac (top panel) and H3K23ac (middle panel) immunoblots in Col-0 and indicated *pdc* mutants with or without four hours of ethylene gas treatments. Immunoblots against histone H3 (bottom panel) served as a loading control. Red number indicates the quantification of acetylated H3 western blot signal band intensity normalized with that of histone H3. Histone acetylation band intensities in Col-0 under air treatment were set as 1 and the relative western blot band intensities were calculated for other samples. (**C**) Bar graph showing the H3 normalized H3K14ac (top panel) and H3K23ac (bottom panel) relative abundance in the indicated mutants and treatment conditions from three biological replicates (Fig. 4A, Fig. S7A and S7B). (**D** and **E**) Two biological replicates of total H3K14ac and H3K23ac western blots in Col-0, *E2ox,* and *E2-NLS* treated with air or four hours of ethylene gas. Histone H3 served as a loading control. (**F**) Bar graph visualization of the summary of three biological replicates of the H3 normalized H3K14ac (top panel) and H3K23ac (bottom panel) relative abundance in Col-0 and the indicated transgenic lines (Fig. 4B, Fig. S7D and S7E). In (**C**) and (**F**), error bars indicate the SD (n = 3) and the relative intensity value from each replicate is plotted as a dot. Different letters indicate significant differences ($P \leq 0.05$) between each genetic background and treatment condition calculated by a One-way ANOVA test followed by Tukey's HSD test.

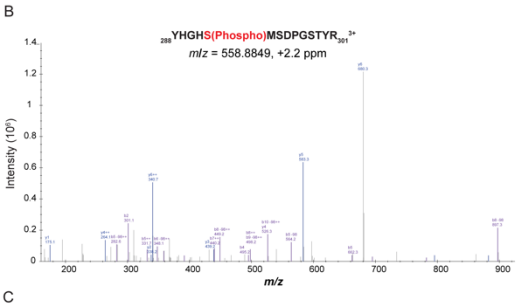


Supplemental Figure 8. Related to Figure 4. ChIP sequencing of H3K14ac and H3K23ac in *pdc e1-2-2 e2-2 #17* mutants before and after ethylene treatment and ChIP-qPCR validation at selected ethylene responsive genes.

(A-D) Scatter plots of mapped ChIP signals calculated by reads per genomic content (RPGC) showing the reproducibility of the replicates (Pearson correlation coefficient R > 0.9) from H3K14ac (**A** and **B**) and H3K23ac (**C** and **D**) ChIP sequencing. Each dot is a 100bp bin of the genome. Color key represents dot densities. (**E-H**) IGV genome browser snapshots to show H3K14ac and H3K23ac ChIP signal from *NAC6* (**E**), *ERF5* (**F**), *PIF3* (**G**), and *HLS1* (**H**) loci. ChIP-seq signals were normalized by RPGC in Col-0 and *e1-2-2 e2-2 #17* under air and ethylene treatment. Horizontal lines represent the amplified regions for ChIP-qPCR and P represents ChIP-qPCR primer. Horizontal bracket represents scale bar of 125bp. (**I**) Violin plot to illustrate the

expression levels of the ethylene response compromised genes and randomly selected non-ethylene regulated genes with similar expression levels. (**J** and **K**) Heatmaps of H3K14ac (**J**) and H3K23ac (**K**) ChIP-seq signal (log_2 ChIP signal) from the ethylene response compromised genes and randomly selected genes in the indicated genetic backgrounds and treatment conditions. (**L** and **M**) Violin plots to illustrate log_2 normalized H3K14ac ChIP signal (**L**) and H3K23ac ChIP signal (**M**) per bin (bin size = 1) from 500bp downstream of TSS in the ethylene response compromised genes and random genes in the indicated genotypes and conditions. *P* values were calculated by a two-tailed *t* test. (**N** and **O**) ChIP-qPCR to validate the enrichment of H3K14ac (**N**) and H3K23ac (**O**) in Col-0, *e1-2-2 e2-2 #17, e2-2 e3-2-2 #67,* and *e1-2-2 e2-2 e3-2-2 #67* etiolated seedlings treated with or without 4 hours of ethylene treatment of selected genes. (**P** and **Q**) ChIP-qPCR assays to examine H3K14ac (**P**) and H3K23ac (**Q**) enrichment levels in Col-0, *E2ox,* and *E2-NLS* etiolated seedlings treated with air or four hours of ethylene gas at selected target genes. In (**N-Q**), individual data point of the relative fold change normalized to Col-0 air is plotted as a dot. Different letters indicate significant differences ($P \leq 0.05$) between each genetic background and treatment condition calculated by a One-way ANOVA test followed by Tukey's HSD test.





C

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Arabidopsis thaliana MALSRLSSRSNIITRPFSAAFSRLISTDTT-P--------ITIETSLPFTAHLCD----- 46
Homo sapiens         -------------MRKMLAAVSRVLSGASQKPASRVLVASRNFANDATFEIKKCDLHRLE 47
                                  * : **.**::*  :  *          .: ..  *  : **

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Homo sapiens         EGPPVTTVLTREDGLKYYRMMQTVKRMELKADQLYKQKIIRGFCHLCDGQEACCVGLEAG 107
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Homo sapiens         IEDAAQFATADPEPPLEELGYHIYSSDPPFEVRGANQWIKFKSVS       390
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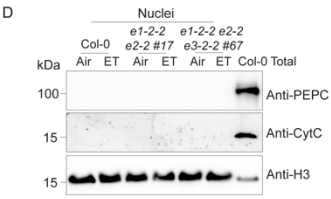
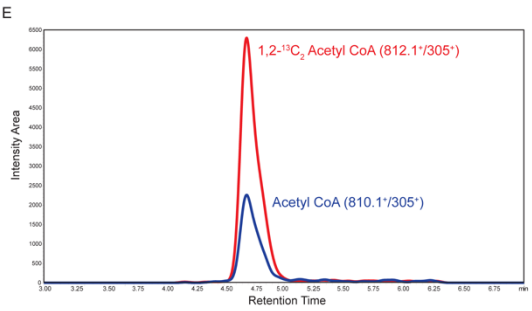
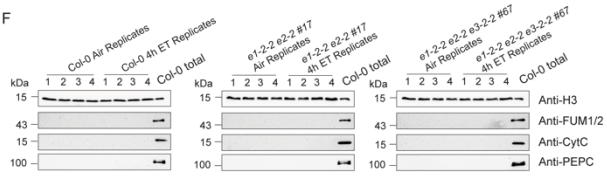
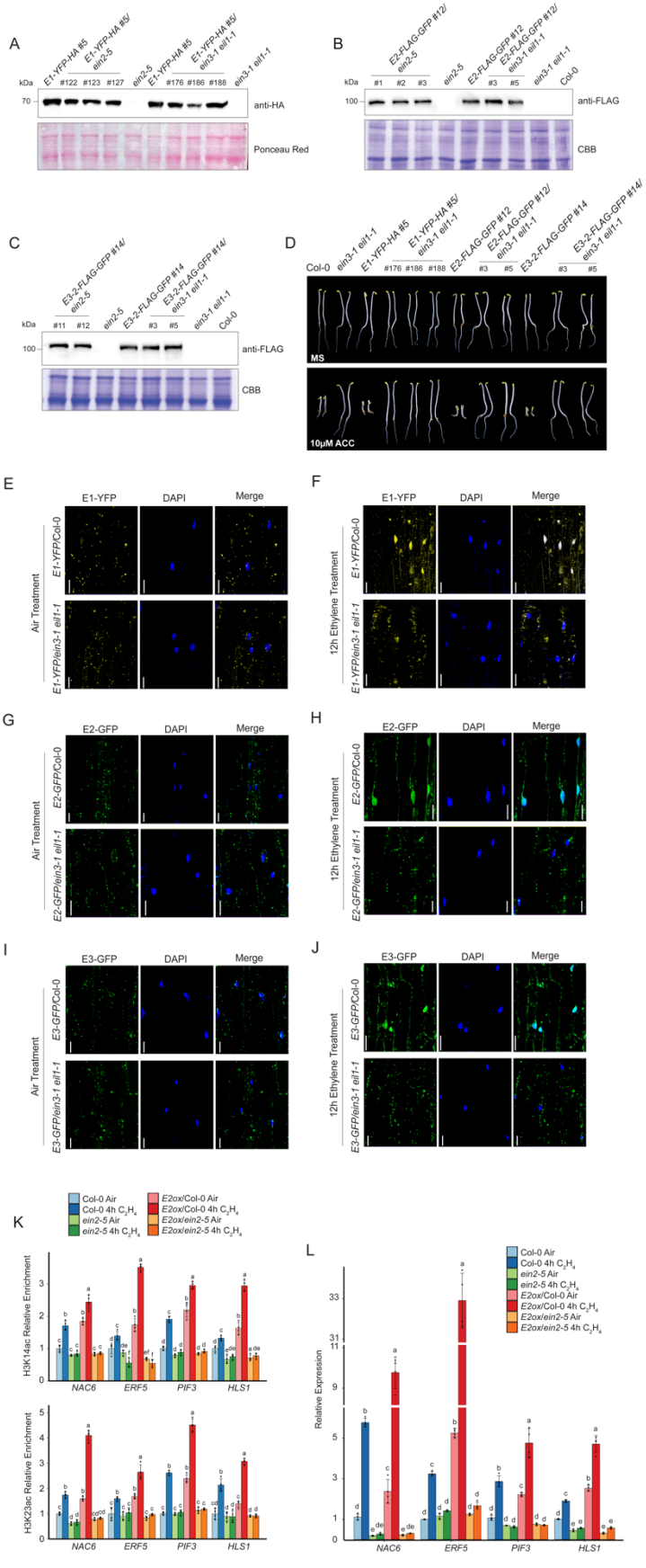






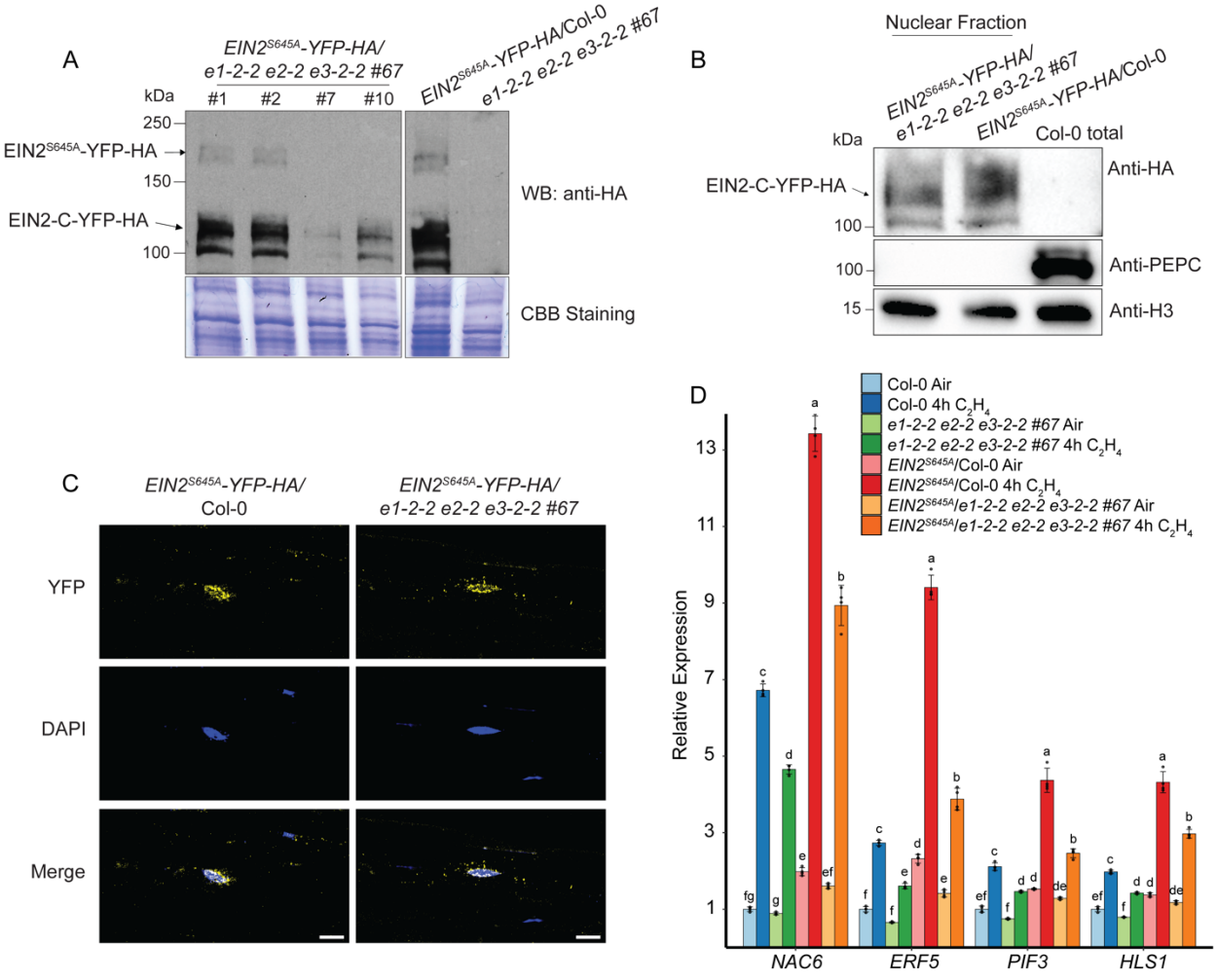
Supplemental Figure 9. Related to Figure 5. PDC E1 Ser292 phosphorylation site and 1,2-$^{13}C_2$ acetyl CoA isotope tracing experiment.

(**A**) LC-MS detection of total acetyl CoA in Col-0 and *e1-2-2 e2-2 e3-2-2 #67* etiolated seedlings treated with or without four hours of ethylene gas. Total protein mass was used to normalize metabolite concentration. Different letters represent significant differences between each group calculated by a one-way ANOVA test followed by Tukey's HSD test. (**B**) MS/MS spectrum of the peptide containing phosphorylated Ser292 of PDC E1. (**C**) Alignment of sequences of E1 from *Arabidopsis thaliana* and its homologue in *Homo sapiens*. Conserved Ser292 residue that its phosphorylation is inhibitory to E1 activity is circled and marked by a red asterisk. (**D**) Western blot analysis of PEPC, CytC, and histone H3 to evaluate the purify of nuclear purification and the input loading of samples used in pyruvate dehydrogenase activity assays in Fig. 5F. (**E**) LC-MS/MS detection of the difference between endogenous ^{12}C acetyl CoA (molecular weight: 810 g/mol) and 1,2-$^{13}C_2$ acetyl CoA (molecular weight: 812 g/mol) based on their molecular weights. Raw data from 3-day-old Col-0 etiolated seedlings treated with 4 hours of ethylene gas replicate 1 was shown. (**F**) Western blot analysis of PEPC, cytochrome C, FUM1/2, and histone H3 to evaluate the nuclear purification efficiencies for ^{13}C isotopic tracing experiments in Fig. 5H.


A
E1-YFP-HA #5
E1-YFP-HA #5/ ein2-5
ein2-5
E1-YFP-HA #5
E1-YFP-HA #5/ ein3-1 eil1-1
ein3-1 eil1-1
kDa
#122 #123 #127 #176 #186 #188
70
anti-HA
Ponceau Red
B
E2-FLAG-GFP #12/ ein2-5
E2-FLAG-GFP #12
E2-FLAG-GFP #12/ ein3-1 eil1-1
ein3-1 eil1-1
Col-0
#1 #2 #3 #3 #5
100
anti-FLAG
CBB
C
E3-2-FLAG-GFP #14/ ein2-5
E3-2-FLAG-GFP #14
E3-2-FLAG-GFP #14/ ein3-1 eil1-1
#11 #12 #3 #5
D
E1-YFP-HA #5/ ein3-1 eil1-1
E2-FLAG-GFP #12
E2-FLAG-GFP #12/ ein3-1 eil1-1
E3-2-FLAG-GFP #14
E3-2-FLAG-GFP #14/ ein3-1 eil1-1
MS
10μM ACC
E
E1-YFP
DAPI
Merge
Air Treatment
E1-YFP/Col-0
E1-YFP/ein3-1 eil1-1
F
12h Ethylene Treatment
G
E2-GFP
E2-GFP/Col-0
E2-GFP/ein3-1 eil1-1
H
I
E3-GFP
E3-GFP/Col-0
E3-GFP/ein3-1 eil1-1
J
K
Col-0 Air
Col-0 4h C2H4
ein2-5 Air
ein2-5 4h C2H4
E2ox/Col-0 Air
E2ox/Col-0 4h C2H4
E2ox/ein2-5 Air
E2ox/ein2-5 4h C2H4
H3K14ac Relative Enrichment
H3K23ac Relative Enrichment
NAC6
ERF5
PIF3
HLS1
L
Relative Expression

Supplemental Figure 10. Related to Figure 6. Different assays to analyze the connection between PDC and *ein2-5* or *ein3-1eil1-1*.

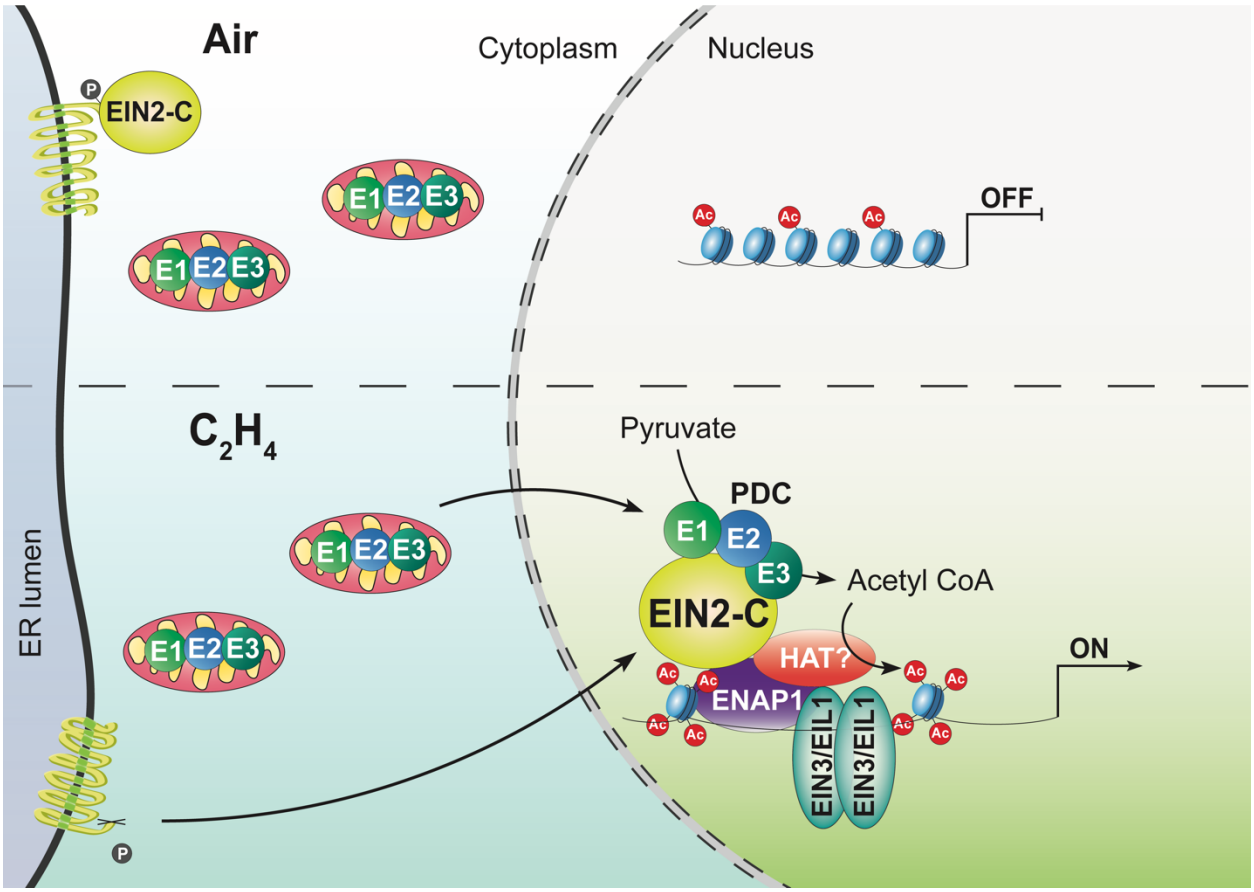
(**A**-**C**) Western blot assay of E1, E2 and E2 protein levels in *E1ox/ein2-5* and *E1ox/ein3-1 eil1-1* plants (**A**), *E2ox/ein2-5* and *E2ox/ ein3-1 eil1-1* plants (**B**), and *E3ox/ein2-5* and *E3ox/ ein3-1 eil1-1* plants (**C**) by anti-HA or anti-FLAG antibody. Ponceau Red staining or Coomassie Brilliant Blue (CBB) staining serves as a loading control. #122 of *E1ox/ein2-5*, #1 of *E2ox/ein2-5*, and #11 of *E3ox/ein2-5* were used for the following molecular assays. (**D**) Phenotypic analysis of PDC *E1ox*, *E2ox*, and *E3ox* in *ein3-1eil1-1* mutant. The seedlings were grown on MS medium supplemented with or without 10μM ACC in the continuous dark for 3 days before being photographed. (**E-J**) Subcellular localization of E1-YFP-HA (**E** and **F**), E2-FLAG-GFP (**G** and **H**), and E3-FLAG-GFP (**I** and **J**) in Col-0 or *ein3-1 eil1-1* mutant with air treatment or 12 hours of ethylene treatment, respectively. Scale bars, 20μm. (**K**) ChIP-qPCR analyses of selected target genes to evaluate H3K14ac (upper panel) and H3K23ac (lower panel) enrichment in Col-0, *E2ox, ein2-5,* and *E2ox/ein2-5* etiolated seedlings with or without 4 hours of ethylene treatment. Individual data point of the relative fold change to Col-0 air is plotted. Different letters indicate significant differences between each genotype and treatment condition calculated by a One-way ANOVA test followed by Tukey's HSD test with $P \leq 0.05$. (**L**) qRT-PCR analysis of target gene expressions in Col-0, *E2ox, ein2-5,* and *E2ox/ein2-5* 3-day-old etiolated seedlings with or without 4 hours of ethylene gas treatment. Values are average of relative expression from each gene normalized to that of *Actin2.* Error bar indicates the SD (n = 4) and different letters indicate statistically significant differences (One-way ANOVA test followed by Tukey's HSD test, $P \leq 0.05$).



Supplemental Figure 11. Related to Figure 6. EIN2^{S645A} protein expression level, the nuclear accumulation of EIN2-C, and gene expression analysis of selected ethylene responsive genes in *EIN2^{S645A}/e1-2-2 e2-2 e3-3-2 #67*.

(**A**) Western blot of EIN2^{S645A} protein levels in *EIN2^{S645A}*/Col-0 and *EIN2^{S645A}/e1-2-2 e2-2 e3-3-2 #67* plants. Full-length of EIN2 and truncated EIN2-C were indicated by black arrows. Coomassie Brilliant Blue (CBB) staining was used as a loading control. #1 of *EIN2^{S645A}/e1-2-2 e2-2 e3-3-2 #67* was used for the following molecular experiments. (**B**) Western blot of nuclear EIN2-C protein levels in *EIN2^{S645A}/e1-2-2 e2-2 e3-3-2 #67* and *EIN2^{S645A}*/Col-0 after 4 hours of ethylene gas treatment. PEPC and histone H3 were used to mark cytosolic and nuclear proteins, respectively. (**C**) Confocal images to show the subcellular localizations of EIN2-C-YFP in Col-0 or *e1-2-2 e2-2*

e3-3-2 #67 treated with 4 hours of ethylene gas. Scale bars, 10μm. (**D**) qRT-PCR analysis to examine expression levels of target genes in 3-day-old etiolated seedlings of indicated genetic backgrounds treated with air or 4 hours of ethylene gas. Values are the average of relative expression from each target gene normalized to that of *Actin2*. Error bars represent the SD (n = 4). Different letters indicate statistically significant differences (One-way ANOVA test followed by Tukey's HSD test, $P \leq 0.05$).



Supplemental Figure 12. Proposed model of nuclear PDC in EIN2-directed histone acetylation in ethylene signaling.

In the absence of ethylene, PDC resides in the mitochondria and EIN2 remains uncleaved on ER membrane (top). Upon ethylene exposure, cleaved EIN2-C is shuttled into the nucleus and PDC translocates from the mitochondria to the nucleus. By interacting with EIN2-C, nuclear PDC provides acetyl CoA to the EIN2-C-dependent histone acetylation machinery to elevate histone acetylation at H3K14 and H3K23 to regulate EIN3-dependent ethylene responsive transcriptional activation (bottom).

Supplemental Table 1. Primers used in the study

Primer Name	Sequence	Purpose
E1-Y2H-F	ACGCGTCGACAATGGCTCTATCACGCCTCT	Y2H construct
E1-Y2H-R	TCCCCCCGGGTCATGGAAGGGAAGCTTTGAC	Y2H construct
E2-Y2H-F	ACGCGTCGACAATGGCTTCTCGTATCATCAAT	Y2H construct
E2-Y2H-R	TCCCCCCGGGTTAGAGCAACATAGATTCTGGGG	Y2H construct
E3-Y2H-F	TCCCCCCGGGAATGGCGATGGCGAG	Y2H construct
E3-Y2H-R	CTAGACTAGTCTACATGTGAATGGGCTTGTC	Y2H construct
E1-pENTRY-pVP13-F	CACCTACCCATACGATGTTCCAGATTACGCTATGGCTCTATCACGCCTCT	*In vitro* expression
E1-pENTRY-pVP13-R	TCATGGAAGGGAAGCTTTGAC	*In vitro* expression
E2-pENTRY-pVP13-F	CACCGACTACAAAGACGATGACGACAAAATGGCTTCTCGTATCATCAAT	*In vitro* expression
E2-pENTRY-pVP13-R	TTAGAGCAACATAGATTCTGGGG	*In vitro* expression
E3-pMAL-pX2-F	CCGGAATTCGAACAAAAACTCATCTCAGAAGAGGATCTGATGGCGATGGCGAG	*In vitro* expression
E3-pMAL-pX2-R	ACGCGTCGACCTACATGTGAATGGGCTTGTC	*In vitro* expression
E1-pENTRY-pEarleyGate101-F	CACCATGGCTCTATCACGCCTCT	Binary vector
E1-pENTRY-pEarleyGate101-R	TGGAAGGGAAGCTTTGAC	Binary vector
E2-pCambia1300-F	CGGGGTACCATGGCTTCTCGTATCATCAAT	Binary vector
E2-pCambia1300-R	ACGCGTCGACGAGCAACATAGATTCTGGGG	Binary vector
E3-pCambia1300-F	CGGGGTACCATGGCGATGGCGAG	Binary vector
E3-pCambia1300-R	ACGCGTCGACCATGTGAATGGGCTTGTC	Binary vector

nYFP-pEarleyGate101-F	GTGCCTAGGGTGAGCAAGGGCGAG	BiFC binary vector
nYFP-pEarleyGate101-R	CTAGACTAGTTTACTCGATGTTGTGGCGG	BiFC binary vector
cYFP-pCambia1300-F	ACGCGTCGACGACGGCAGCGTGC	BiFC binary vector
cYFP-pCambia1300-R	ACGCGAGCTCTTACTTGTACAGCTCGTCCATG	BiFC binary vector
NLS-BFP-F	ACGCGTCGACATGTTGAAGCGGTATAAACGTCGGTTAATGAGCGAAGAACTAATCAAGG	Binary vector
NLS-BFP-R	ACGCGAGCTCTTATAACCGACGTTTATACCGCTTCAATCCGCTCCCATTCAGC	Binary vector
LBb1.3	ATTTTGCCGATTTCGGAAC	Genotyping
e1-2-2-GT-F	AATGATAGGGGAGCTTGTTGG	Genotyping
e1-2-2-GT-R	TGGTTCTTTCTGTGCAGTTGC	Genotyping
e1-2-4-GT-F	AGAACGCCGTAAGTAACCCTC	Genotyping
e1-2-4-GT-R	CTGAGAAAACCTCATGAAGCG	Genotyping
e3-2-1-GT-F	AGGATCGTGATCCAGCATATG	Genotyping
e3-2-1-GT-R	GAGATAACAACCCTCCCAAGG	Genotyping
e3-2-2-GT-F	CGGAAATTTTCTCCGATTTTC	Genotyping
e3-2-2-GT-R	CATCTTCTTCGGCTTTGTGAG	Genotyping
e2-2-CRISPR-DT1-BsF	ATATATGGTCTCGATTGTTAGAGCAACATAGATTCTGTT	E2 CRISPR-Cas9
e2-2-CRISPR-DT1-F0	TGTTAGAGCAACATAGATTCTGTTTTAGAGCTAGAAATAGC	E2 CRISPR-Cas9
e2-2-CRISPR-DT1-R0	AACTTGCCTACTGCTGATCAAACAATCTCTTAGTCGACTCTAC	E2 CRISPR-Cas9
e2-2-CRISPR-DT1-BsR	ATTATTGGTCTCGAAACTTGCCTACTGCTGATCAAACAA	E2 CRISPR-Cas9
e2-2-GT-F	AGTGGCTGAAAGCATTCAAG	Genotyping
e2-2-GT-R	TTTAAAGAATAGCAAAACACTCAACTT	Genotyping
Cas9-GT-F	CACCGACGAGTACAAGG	Genotyping

Cas9-GT-R	GGCCCCTGAACTTAATCATG	Genotyping
E1-qRT-F	GTCACTCCATGTCTGATCCTG	Real time PCR
E1-qRT-R	TTTCTCGGTTGCTAGGTCATG	Real time PCR
E3-qRT-F	GTCTACACGTACCCTGAAGTTG	Real time PCR
E3-qRT-R	ATCTTGACCATTCCCTCTGC	Real time PCR
Actin2-qRT-F	CCCGCTATGTATGTCGC	Real time PCR
Actin2-qRT-R	AAGGTCAAGACGGAGGAT	Real time PCR
proE2-pCambia1300-F	AAAAGGCCTGCATGAGATAATGTTCTAATCTAAGACAT	Binary vector
proE2-pCambia1300-R	CGGGGTACCTGTTGTGCAATCGGAGC	Binary vector
E1-NLS-pCambia1300-F	CTAGTCTAGAATGGCTCTATCACGCCTCT	Binary vector
E1-NLS-pCambia1300-R	ACGCGTCGACTAACCGACGTTTATACCGCTTCAATGGAAGGGAAGCTTTGAC	Binary vector
E2-NLS-pCambia1300-F	CTAGTCTAGAATGGCTTCTCGTATCATCAAT	Binary vector
E2-NLS-pCambia1300-R	ACGCGTCGACTAACCGACGTTTATACCGCTTCAAGAGCAACATAGATTCTGGGG	Binary vector
PIF3-qRT-F	GCCATCGAGTATCTCAAGTCAC	Real time PCR
PIF3-qRT-R	AGGCATTCCCATACCCATTG	Real time PCR
NAC6-qRT-F	ACTCATAACTCACTACCTCAAACC	Real time PCR
NAC6-qRT-R	TTTTCTCCCATCTTAGCCTTCC	Real time PCR
HLS1-qRT-F	CGAATATCCACCCGAGTCATG	Real time PCR
HLS1-qRT-R	CGCTCCACGTACTTCTAACAG	Real time PCR
ERF5-qRT-F	TGTGACTGGGATTTAACGGG	Real time PCR
ERF5-qRT-R	CAACTGGGAATAACCAAACGG	Real time PCR
PIF3-ChIP-qPCR-F	CCGTGAGTCCCATTCACTTGTC	ChIP qPCR
PIF3-ChIP-qPCR-R	AGTTGATATCTGACCATTTTCCCA	ChIP qPCR

NAC6-ChIP-qPCR-F	TAGAGAGGAGCTTCGTTGCTC	ChIP qPCR
NAC6-ChIP-qPCR-R	GTTTGGAGACGAAGAGGGAAG	ChIP qPCR
HLS1-ChIP-qPCR-F	CACCTTCCTCTCTATATATTAAACCCT	ChIP qPCR
HLS1-ChIP-qPCR-R	TCTAACCACCGTCATGTTTTGG	ChIP qPCR
ERF5-ChIP-qPCR-F	CTCCTAACGAAGTATCTGCACTTT	ChIP qPCR
ERF5-ChIP-qPCR-R	GATGATTCGTGCTTCATCCATG	ChIP qPCR

Supplemental Table 2. Summary of total clean reads and aligned reads

Sample Name	**Repeat**	**Clean Reads**	**Aligned Reads**	**Align Rate (%)**
e1-2-2 e2-2 #17 air (RNA-seq)	rep1	25600215	22932178	89.6
e1-2-2 e2-2 #17 air (RNA-seq)	rep2	28068692	25319409	90.2
e1-2-2 e2-2 #17 ethylene (RNA-seq)	rep1	31329229	28497496	91
e1-2-2 e2-2 #17 ethylene (RNA-seq)	rep2	30657907	27918368	91.1
e2-2 e3-2-2 #67 air (RNA-seq)	rep1	31892141	28828279	90.4
e2-2 e3-2-2 #67 air (RNA-seq)	rep2	40789675	37047373	90.8
e2-2 e3-2-2 #67 ethylene (RNA-seq)	rep1	30860626	28034354	90.8
e2-2 e3-2-2 #67 ethylene (RNA-seq)	rep2	31314332	28290889	90.3
e1-2-2 e2-2 #17 air input (ChIP-seq)	rep1	13888572	9746223	70.17
e1-2-2 e2-2 #17 air input (ChIP-seq)	rep2	16316376	11351075	69.57
e1-2-2 e2-2 #17 ethylene input (ChIP-seq)	rep1	15307947	10769023	70.35
e1-2-2 e2-2 #17 ethylene input (ChIP-seq)	rep2	17910315	12535083	69.99
e1-2-2 e2-2 #17 air H3K14ac (ChIP-seq)	rep1	23423131	17724647	75.67
e1-2-2 e2-2 #17 air H3K14ac (ChIP-seq)	rep2	19687063	14772192	75.04

e1-2-2 e2-2 #17 ethylene H3K14ac (ChIP-seq)	rep1	19606552	14459614	73.75
e1-2-2 e2-2 #17 ethylene H3K14ac (ChIP-seq)	rep2	22993174	15838214	68.88
e1-2-2 e2-2 #17 air H3K23ac (ChIP-seq)	rep1	17236097	12295987	71.34
e1-2-2 e2-2 #17 air H3K23ac (ChIP-seq)	rep2	23249273	16405898	70.57
e1-2-2 e2-2 #17 ethylene H3K23ac (ChIP-seq)	rep1	24451561	17011539	69.57
e1-2-2 e2-2 #17 ethylene H3K23ac (ChIP-seq)	rep2	18938139	12157437	64.2