Supplemental Figure 1. Phenotypes of SALK_057606 (ear1-2) and ear1-c mutants. (Supports Figure 1.)

(A) Confirmation of T-DNA insertion by PCR using primers in genomic DNA and T-DNA. LP, left genomic primer; RP, right genomic primer; LB, left border primer of the T-DNA insertion.

(B) Comparison of EAR1 transcripts before T-DNA insertion.

(C) ABA-sensitive phenotype of ear1-1, ear1-2 and an F1 of ear1-1 ear1-2. One-week-old seedlings of ear1-1, the F1 of ear1-1 ear1-2, and ear1-2 were transferred onto MS medium supplemented with 30 µM ABA for two weeks. Scale bar, 1 cm.

(D) Schematic of the ear1-c mutant. The arrow shows the position of CRIPSR/Cas9 targeting sequences. The red rectangle represents the CDS of EAR1, the blue rectangle represents the 5’-UTR and 3’-UTR of EAR1. Scale bar, 100 bp. SANGER sequencing chromatography shows the insertion in EAR1 of ear1-c.

(E) Primary root growth of ear1-c is sensitive to ABA. Seeds were germinated on MS medium and grown for 5 days before being moved to MS or MS containing 30 µM ABA medium for 1 week.
**Supplemental Figure 2. Phylogenetic analysis of EAR1 sequences.**

**(Supports Figure 2.)**

(A) Phylogenetic tree of EAR1 homologs in dicot and monocot plant species. The amino acid sequences of EAR1 in each species were acquired from the BLAST tool in the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The tree was constructed with the MEGA 4.0 software using the Neighbor-Joining method. Scale bar represented the number of differences between sequences.

(B) Alignment of amino acid sequences of EAR1 homologs of different plant species. The amino acid sequences of different species were aligned by ClustalX software. The black background represented the same amino acid residues and the gray background represented the conserved amino acid residues. The numbers to the left of the sequences indicate the number of amino acid residues in each species. The red lines under sequences indicate two conserved domains. 224–278 is the conserved domain in DUF3049 family proteins.
Supplemental Figure 3. Comparison of plant phenotypes among WT, ear1-1, and EAR1-overexpressing lines OE-16\textsuperscript{a} and OE-28\textsuperscript{a}.

(Supports Figure 4.)

(A) Mature leaf morphological comparison.

(B) Seedlings grown in soil for 4 weeks.

(C) Plant leaf fresh weight. Error bars are means ± s.e.m. of 10 pieces of fresh leaves. The 7\textsuperscript{th} or 8\textsuperscript{th} leaf from each plant of the same transgenic line or wild type was taken for comparison. In total, 10 leaves were used for each.

(D) Seed germination greening phenotype of WT, ear1-1, OE-16\textsuperscript{a}, and OE-28\textsuperscript{a}. Seeds were germinated on MS medium containing different concentrations of ABA. The seed germination of OE-16\textsuperscript{a} and OE-28\textsuperscript{a} was more resistant to ABA than WT.
Supplemental Figure 4. EAR1 interacts with clade A PP2Cs.
(Supports Figure 5.)

(A) EAR1 does not interact with HAI1, HAI2 and HAI3. EAR1-cYFP did not interact with HAI1-, HAI2-, or HAI3-nYFP in *Nicotiana benthamiana* leaves by BiFC assay. The YFP signals were observed 48–72 hours after co-injection, and ABI1111-130-cYFP peptide was used as a positive control. The values are represented the YFP fluorescence intensity in 0.04 mm² (the whole field) and shown at the bottom. Scale bars, 50 μm.

(B) The specificity of ABI2 antibodies. Total proteins were extracted from WT and *abi2-2* mutant and used for immunoblotting with ABI2 antibodies.

(C) EAR1121-248- and EAR1249-278-cYFP peptides did not interact with ABI1-nYFP in *N. benthamiana* leaves by BiFC assay. The YFP signals were observed after 48-72 hours co-injection and the EAR1191-287-cYFP peptide was used as a positive control. Scale bars, 50 μm.
(D) The recombinant protein of GST-EAR1121-287 can pull down HAB1-His, HAB2-His, AHG1-His and AHG3-His in vitro. The purified PP2C-His proteins were incubated with the GST or GST-EAR1121-287 proteins for pull-down assay. The PP2C proteins were detected with anti-His antibodies, and the GST or GST-EAR1121-287 proteins were detected with anti-GST antibodies.

(E) EAR1-cYFP interacts with N-termini of ABI21-120, HAB11-200, HAB21-200, AHG1-200 and AHG31-120-nYFP, but not their C-terminal catalytic domains in N. benthamiana leaves by BiFC assay. The YFP signals were observed 48–72 hours after co-injection. Scale bars, 50 μm.
Supplemental Figure 5. EAR1 enhances the activity of the clade A PP2Cs. (Supports Figure 5.)
(A) Protein purity of the purified proteins expressed in E. coli. The recombinant proteins were separated by 10% SDS-PAGE gel. These purified proteins were used in different assays.
(B) EAR1 enhances the phosphatase activity of ABI1 in a dosage-dependent manner. The concentration of ABI1 was 0.5 μM and the ABI1/EAR1 molecular ratios were 1:0, 1:1.6, 1:8, 1:61 and 1:32, respectively. Error bars are means ± s.e.m. of three biological replicates.
(C) The kinetic (time-dependent) analysis of ABI1 phosphatase activity. ABI1 alone or ABI1 with EAR1 were mixed with the substrate phosphopeptide. The free phosphate dephosphorylated by ABI1 was reflected by the OD$_{630}$. The OD$_{630}$ was read every 10 min from 0 to 90 min. The data were curve-fitted using the Origin2017 software.
Supplemental Figure 6. EAR1 does not influence the interaction between ABI1 and PYR1 or the degradation of ABI1.

(Supports Figure 5.)

(A) EAR1 could not compete with PYR1 when interacting with ABI1. The MBP-ABI1, MBP, GST-EAR1 and PYR1-His proteins were expressed in Escherichia coli. The purified proteins were used for pull-down assay. MBP-ABI1 and PYR1-His were incubated in pull down binding buffer plus 100 μM ABA with or without GST-EAR1. The proteins were detected by anti-MBP, anti-His or anti-GST antibodies.

(B) EAR1 does not influence the ABI1 and PYR1 interaction in yeast three-hybrid assay. The plasmids of pGADT7-ABI1 and pBridge-PYR1 (A; EV, empty vector) or pGADT7-ABI1 and pBridge-PYR1-EAR1 (B) were co-transformed into yeast AH109 strain. The positive clones were selected on synthetic complete medium lacking Leu, Trp, Ade and Met for β-galactosidase activity assay. The β-galactosidase activity of A was set to 1. Error bars are means ± s.e.m. of three biological replicates. Ten positive clones were analyzed in each replicate (n.s., no significant difference).

(C) EAR1 does not influence the degradation of ABI1 in vivo. The total proteins were extracted from 10-day-old seedlings treated with 60 μM ABA for 6 h. Then, 200 μg total proteins were incubated with 1 mM ATP and 60 μM ABA for different times, and the ABI1 protein was detected by anti-ABI1 antibodies. ACTIN was used as a loading control. Three independent experiments were performed with similar results.
Supplemental Figure 7. Protein characteristics of EAR1 and PP2Cs. (Supports Figure 6).

(A) The intrinsically disordered regions (IDRs) of EAR1, ABI1, ABI2, HAB1, HAB2, AHG1, AHG3, HAI1, HAI2 and HAI3 were predicted by PONDR VL-XT (http://www.pondr.com/). The protein sequences plotted along with thick black lines represent the positions of IDR in each protein. The numbers in brackets show the beginning and ending residues of each IDR.

(B) The predicted short molecular recognition features (MoRFs) in N-termini of PP2Cs and their possible roles in the interaction with EAR1. The predicted MoRFs (http://biomine-ws.ece.ualberta.ca/MoRFpred/index.html; http://anchor.enzim.hu/) are labeled with red in different PP2Cs.

(C) Interaction of EAR1-cYFP with truncated peptides that lack some of the MoRFs in different PP2Cs fused with nYFP in N. benthamiana leaves by BiFC assay. The YFP signals were observed 48–72 hours after co-injection. Scale bars, 50 μm.

D) Relative fluorescence intensity (%)
The N-termini of ABI1, ABI2, HAB1, HAB2 and AHG1 have phosphatase self-inhibition activity. Equal molecular amounts of full-length PP2C or its N-terminal truncation protein were mixed with EAR1 for phosphatase activity assays. Error bars are means ± s.e.m. of three biological replicates (n.s., no significant difference; **P < 0.01, Student’s t test).

Supplemental Figure 8. EAR1 negatively regulates the expression of ABA-inducible genes.
(Supports Figure 7).
The relative expression of ABA-responsive genes in WT, ear1-1, OE-16′ and abi1-2 abi2-2 hab1-1 triple loss-of-function mutant (3m). Ten-day-old seedlings were treated with or without 60 μM ABA for 5 h, and the total RNAs were extracted using TRIzol reagent. The expression of the selected ABA-inducible marker genes was detected with quantitative RT-PCR using SYBR premix ExTaq. The gene expression in the WT without ABA treatment was set to 1 and ACTIN4 was used as the internal control. Error bars are means ± s.e.m. of three biological replicates.
Supplemental Figure 9. Subcellular localization of EAR1. (Supports Figure 8).

(A and B) EAR1 is located on the endoplasmic reticulum (ER). The proSuper:EAR1-GFP construct was co-injected into N. benthamiana leaves with pro35S:HDEL-RFP (an ER marker), and the GFP and RFP signals were analyzed after 48–72 hours. The signal curves were analyzed by LSM5 Image Browser software. The green curve represents the GFP signals and the red curve represents the RFP signals. Scale bars, 50 μm.

(C) The microsome localization of EAR1-Flag protein. Total protein extracts (T) of 2-week-old OE-16 transgenic plants treated with or without 60 μM ABA was centrifuged at 100,000 g for 1 h to obtain pelleted microsomal membrane fraction (M) and soluble fraction (S). Each fraction was analyzed by SDS-PAGE and the EAR1 proteins were detected by anti-Flag antibodies. Note that Anti-flag antibodies detect four non-specific bands: two only in soluble fraction, one only in microsome fraction, and one in both fractions. These bands are used as controls.