Supplemental Figures and Legends

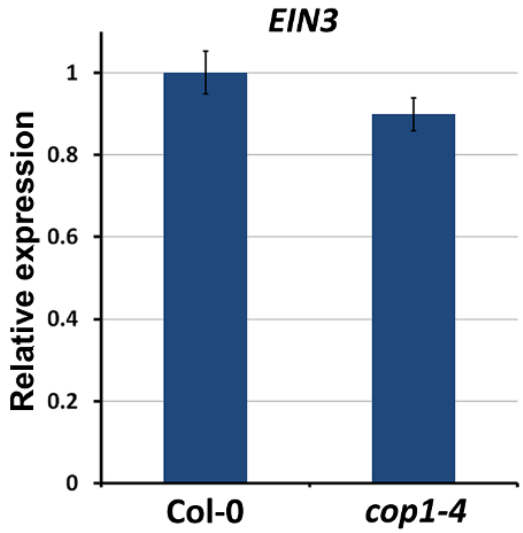

Figure S1. EIN3 transcription is not significantly changed in *cop1* mutant seedlings, related to Figure 3.

Real time RT-PCR results showing the gene expression of *EIN3* in 4-day old etiolated Col-0 (WT) and *cop1-4* mutant seedlings. The expressions were normalized to *PP2A*. Mean ±s.d., n=3.

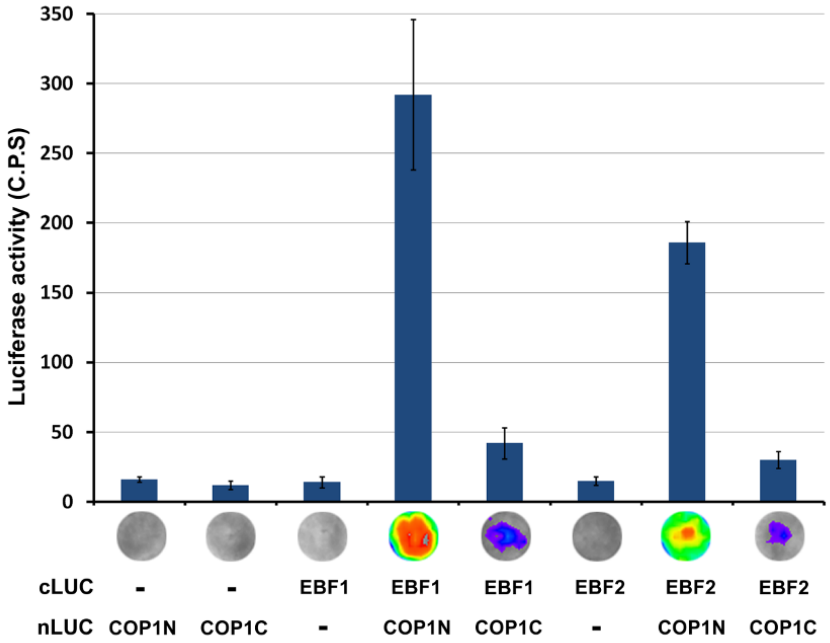

Figure S2. EBF1 and EBF2 interact primarily with the N-terminal fragment of COP1 in plants, related to Figure 5.

LCI assay of the interaction of EBF1 and EBF2 with COP1 in *Nicotiana benthamiana* leaf cells. Full length of EBF1 and EBF2 were fused to the split C-terminal (cLUC) fragments of firefly luciferase. The N-terminal and C-terminal fragments of COP1 were fused to the split N-terminal (nLUC) fragments of firefly luciferase. Empty vectors were used as negative controls. C.P.S stands for counts per second. Mean ±s.d., n=5.

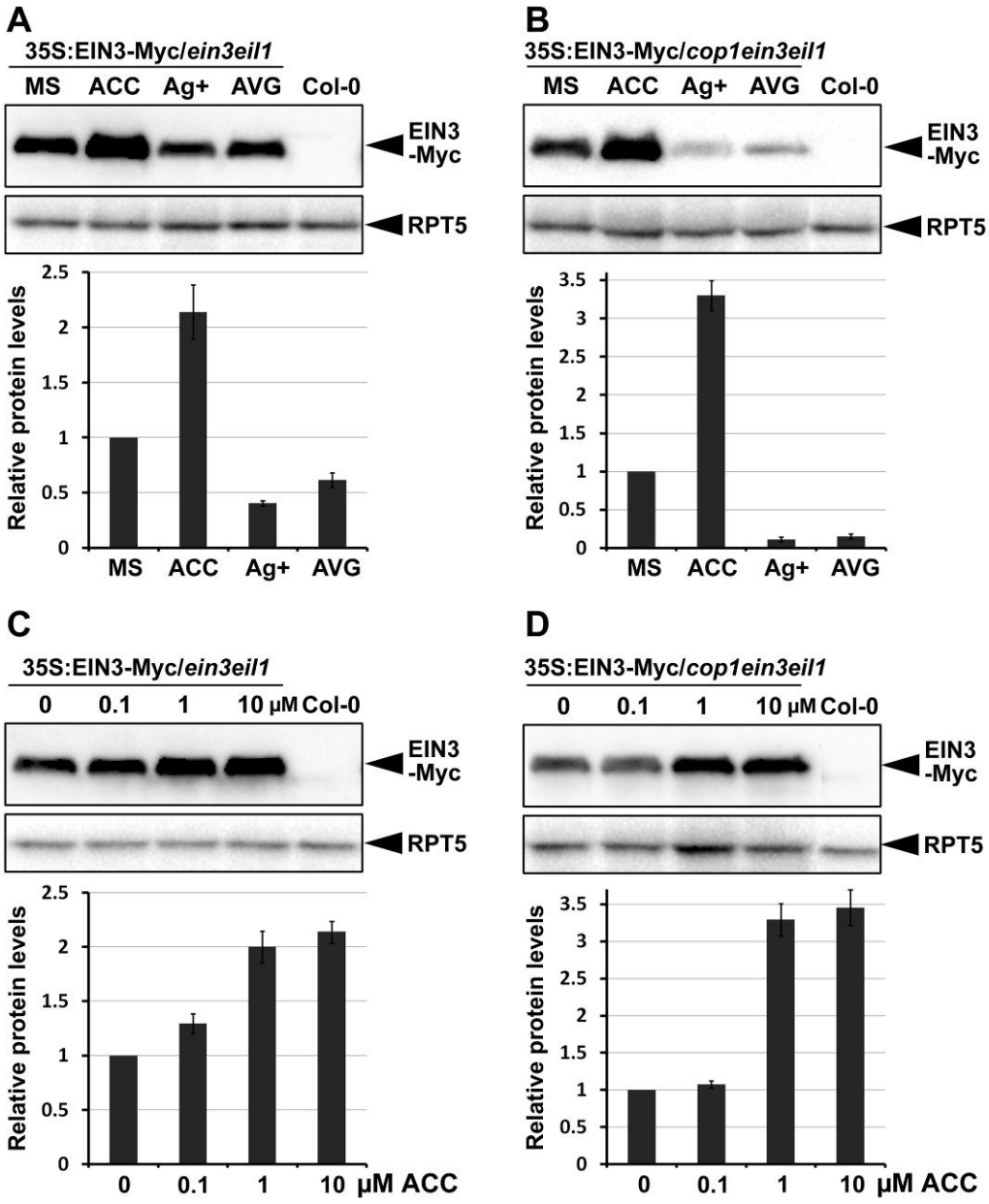

Figure S3. The ethylene-induced EIN3 accumulation is not through COP1, related to Figure 6.

(A-B) Representative images (top panels) of western blots shows the EIN3 protein levels upon the treatments of ethylene precursor (10μM ACC) or ethylene inhibitor (100μM Ag^{+} or 50μM AVG). Seedlings over-expressing EIN3-Myc in *ein3eil1* (A) and

cop1ein3eil1 (B) backgrounds were grown on 1⁄2MS medium supplemented with or without indicated chemicals in the dark for 4 days. Bottom panels show the quantification analysis of the three biological replicates of EIN3-Myc protein levels after normalizing to RPT5. The protein level of EIN3-Myc/*ein3eil1* on 1⁄2MS medium was set as 1. Mean ±s.d., n=3.

(C-D) Representative images (top panels) of western blots shows the EIN3 protein levels under the gradient concentrations of ACC treatments. Seedlings over-expressing EIN3-Myc in *ein3eil1* (C) and *cop1ein3eil1* (D) backgrounds were grown on 1⁄2MS medium in the presence of ACC gradients in the dark for 4 days. Bottom panels show the quantification analysis of the three biological replicates of EIN3-Myc protein levels after normalizing to RPT5. The protein level of EIN3-Myc/*ein3eil1* on 1⁄2MS medium was set as 1. Mean ±s.d., n=3.

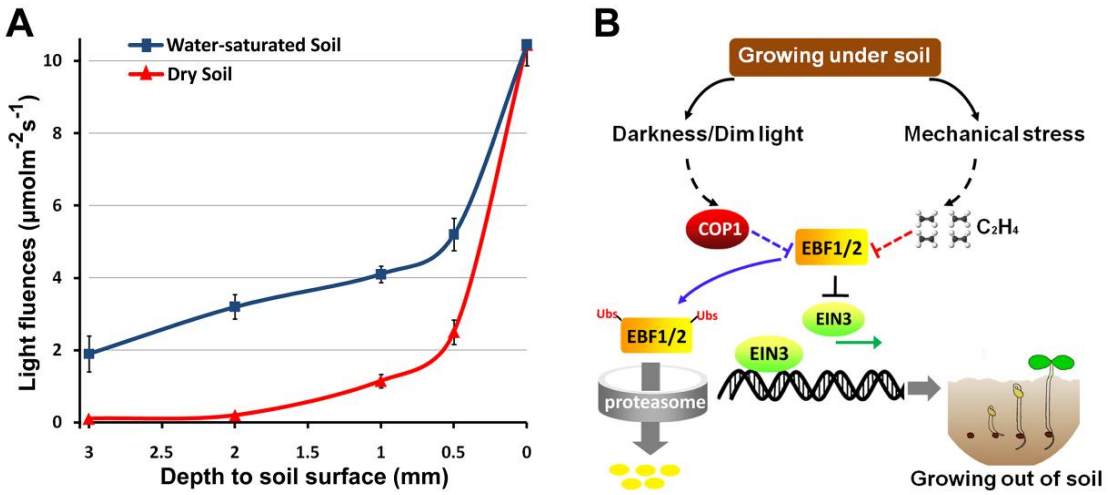

Figure S4. Light fluence in the soil increases with the decreasing of soil soil depthes, and model for the integrated regulation of seedling emergence by light and ethylene signals, related to Figure 7.

(A) The light fluence was measured under dry or water-saturated soils (50-70 mesh sand) with different depths.

(B) In the soil, COP1 and ethylene mediate soil covering-produced darkness and mechanical stress effects, respectively. These two pathways are converged on EBF1/2 regulation, precisely adjusting EIN3 levels according to the changes of soil conditions to promote seedling emergence.

Supplemental Experimental Procedures

Phenotype analysis

For cotyledon opening experiment, seedlings were grown in the dark for 3 days. The angles between two cotyledons were recorded by using the Image J software. More than 20 seedlings were measured for each set of experiments. For cotyledon expansion analysis, seedlings were grown in the dark for 7 days. The areas of representative cotyledons were recorded by using the Image J software. More than 20 cotyledons were measured for each set of experiments. Three biological replicates were used for statistical analysis and representative photos were presented.

GUS staining

The 3-day old etiolated seedlings were incubated in the GUS staining buffer (1XPBS, 1mM $K_3Fe(CN)_6$, 0.5Mm $K_4Fe(CN)_6$, 1mM EDTA, 1% Triton X-100 and 1mg/ml X-gluc) at 37 ℃ for several hours. Then the seedlings were incubated in a graded series of ethanol (30%, 70%, 95%, vol/vol), and representative photos were presented.

RNA extractions and qRT-PCR

The 4-day old etiolated seedlings were harvested and ground to powder in liquid nitrogen, and total RNA was extracted by using the Spectrum Plant Total RNA Kit (Sigma). The quality of RNA was detected by using spectrophotometric and gel electrophoretic analysis. 2 μg of RNA was used to synthesize cDNA by using TOYOBO Rever Tra qPCR RT Kit. Real-time PCR was performed by using SYBR Green Mix

(Takara) in ABI fast 7500 Real-Time system. All quantitative PCR experiments were independently performed in triplicate, and representative results were shown.

Cell-free degradation assay

The 4-day old etiolated seedlings were ground to powder in liquid nitrogen and homogenized in cold cell free assay buffer (25mM Tris, PH=7.5, 10 mM $MgCl_2$, 5 mM DTT, 10 mM NaCl and 10 mM ATP). After centrifugation at 4 ℃, the supernants were transferred into new tubes, and repeated the centrifugation once. For proteasome inhibitor treatment, 50 μM MG132 or DMSO was added into the supernants in advance. Then, equal amounts of purified recombinant EIN3-His proteins were added into each sample. The samples were incubated in the dark at 22°C for the indicated period of time before collection. The samples were finally analyzed by immunoblot assay using the anti-His (Cell signaling, 1:1000) and anti-Actin (Abcam, 1:3000) antibodies.

Immunoblot assays

4-day old etiolated seedlings grown on 1⁄2 MS medium were extracted for western blot, unless specified otherwise. For MG132 treatment, the seedlings were grown on 1⁄2 MS medium for 3.5 days and then were treated with 50μM MG132 or DMSO for 12 hours before extraction. For mechanical stress experiment, the 4-day old etiolated seedlings were covered with a glass plate for the indicated period lengths of time. For the soil experiment, the seeds were covered with a layer of sand and then grown in

the indicated conditions. The sand was washed away before collecting the seedlings for protein extraction. For light fluence experiment, the seedlings were grown under different light fluences for 4 days before extraction. For protein gel blot, Anti-Myc (Abcam, 1:1000), anti-Actin (Abcam, 1:3000), anti-His (Cell signaling, 1:1000), anti-MBP (NEB, 1:1500), anti-GFP (Clontech, 1:800), and anti-RPT5 (1:1000) [S1] antibodies were used for immunoblot detection.

Yeast two-hybrid , BiFC, and LCI assays

Yeast two-hybrid , BiFC, and LCI assays were carried out as previously described [S1, S2].

Pull down and Co-immunoprecipitation assays

For pull down assay, 1ug purified COP1-His, EBF1-MBP, EBF2-MBP or empty MBP recombinant proteins were added into the binding buffer (20mM Tris-HCl, pH=7.5, 150mM NaCl and 1mM EDTA) as indicated. The solutions were incubated at 4℃ for 1h with gentle rotation. Then 20μl amylose resins were used to precipitate the MBP fusion proteins, followed by centrifugation at 1800g for 1 min. The resins were washed three times by using the wash buffer (50mM Tris-HCl, pH 7.5, .5% Triton, 10% glycerol and 1mM EDTA). Anti-His (Cell signaling, 1:1000) and anti-MBP (NEB, 1:1500) antibodies were used for immunoblot detection.

Co-immunoprecipitation assay were carried out as previously described with minor modification [S2]. After extraction, 1 μg purified recombinant COP1-His proteins

were added into 500 μg total soluble proteins and the solution was incubated at 4℃ for 1h with gentle rotation. After immunoprecipitation, the washed beads were collected and subjected to immunoblot detection.

In vitro ubiquitination assay

In vitro ubiquitination assays were performed as described previously with minor modifications [S3]. UBE1 (E1), UbcH5b (E2), HA-tagged ubiquitin (HA-Ub) were purchased from Boston Biochem. The reaction buffer contained 50 mM Tris, pH=7.5, 10 mM MgCl2, 200 mM NaCl, 5mM ATP, 1 mM DTT, 100ng E1, 100ng E2, 10μg HA-Ub, 200ng EBF1-MBP or EBF2-MBP and 200ng or 400ng COP1-His. After1.5 h of incubation at 30°C, the reactions were stopped by adding 5× sample buffer and subjected to immunoblot detection.

References

S1. Shi, H., Wang, X., Mo, X., Tang, C., Zhong, S., and Deng, X.W. (2015). Arabidopsis DET1 degrades HFR1 but stabilizes PIF1 to precisely regulate seed germination. Proc Natl Acad Sci U S A *112*, 3817-3822.

S2. Shi, H., Zhong, S., Mo, X., Liu, N., Nezames, C.D., and Deng, X.W. (2013). HFR1 Sequesters PIF1 to Govern the Transcriptional Network Underlying Light-Initiated Seed Germination in Arabidopsis. Plant Cell *25*, 3770-3784.

S3. Ni, W., Xu, S.L., Tepperman, J.M., Stanley, D.J., Maltby, D.A., Gross, J.D., Burlingame, A.L., Wang, Z.Y., and Quail, P.H. (2014). A mutually assured destruction mechanism attenuates light signaling in Arabidopsis. Science *344*, 1160-1164.