Supplemental Figure 1. The phenotypes of dissected leaves from tie1-D. The leaves excised from a 21-day-old wild-type plant, heterozygous and homozygous tie1-D mutant. From left to right, two cotyledons and all rosette leaves produced by a 21-day-old wild-type plant (A), heterozygous (B) and homozygous tie1-D mutants (C). The tie1-D mutants produced serrated and hyponastic leaves with wavy margins. Scale bar, 2 mm.
Supplemental Figure 2. TIE1 interacts with other TPL family proteins through the EAR motif, but not with SAP18.

(A) The interactions were tested by yeast two-hybrid assays. AD, activation domain; DBD, DNA-binding domain; TIE1mEAR, mutated TIE1 in which the three conserved leucines of the EAR motif were mutated into serines. N-TPR1 to N-TPR4, the N-terminus of TPR1 to TPR4 including the CTLH domain. Transformed yeasts were spotted on control medium (-2) or selective medium (-3) in 10-fold, 100-fold and 1000-fold dilutions. The empty vectors were used as the control.

(B) Recapitulation of tie1-D phenotypes by expressing the TIE1ΔEAR-TPLC fusion protein. Top, schematic representation of TIE1ΔEAR-TPLC fusion protein. The fusion protein is consisted of TIE1 deleted EAR motif and the C-terminal portion (189-1131) of TPL. Bottom, from left to right, leaves from a 14-day-old wild-type plant, leaves from three independent 14-day-old TIE1ΔEAR-TPLC transgenic lines. Scale bar, 1 mm.
Supplemental Figure 3. TIE3 and TIE4 may have redundant functions with TIE1 and disruption of TIE genes led to epinastic leaves.

(A) Schematic representation of T-DNA insertion site in GABI_107F02 in which the T-DNA is inserted in the first intron of TIE1 (top). Schematic representation of T-DNA insertion site in WiscDs.LoxHs225_11A in which the T-DNA is located about 13kb far from TIE1 gene (middle). Heat shock led to the T-DNA jumping into the second intron splice site of TIE1 gene in tie1-455 (bottom).

(B) RT-PCR analysis showed that the expression level of TIE1 is not changed in GABI_107F02.

(C) RT-PCR analysis showed that the expression of TIE1 was disrupted in the tie1-455, indicating that the mutant is a null allele.

(D) TIE2, TIE3 and TIE4 all interacted with TPL family proteins by yeast two-hybrid assays. AD, activation domain; DBD, DNA-binding domain; N-TPL, N-TPR1 to N-TPR4, the N-terminus of TPL, TPR1 to TPR4 including CTLH domain. Transformed yeasts were spotted on control medium (-2) or selective medium (-3) in 10-fold, 100-fold and 1000-fold dilutions. The empty vectors were used as the control.

(E) to (H) Overexpression of TIE2, TIE3 and TIE4 produced phenotypes similar to tie1-D. (E) 21-day-old wild-type plant. (F) 21-day-old 3SS-TIE2 plant. (G) 21-day-old 3SS-TIE3 plant. (H) 21-day-old 3SS-TIE4 plant.

(I) RT-PCR showed that TIE3 and TIE4 were expressed in leaves, whereas TIE2 was not expressed in leaves.
Supplemental Figure 4. Dominant-negative disruption of TIE1 caused epinastic leaves.

(A) and (B) The leaves excised from a 28-day-old wild-type plant (A) and a TIE1mEAR-7 transgenic line (B). From left to right, all rosette leaves produced by 28-day-old wild-type plant (A) and TIE1mEAR-7 transgenic line (B). Scale bar, 2 mm.

(C) and (D) Expression of TIE1mEAR driven by its own promoter in wild-type plants caused epinastic leaves which are opposite to those observed in tie1-D mutants.

(E) Schematic representation of TIE1ΔEARVP16 protein. The EAR motif was deleted and fused with the activation domain of VP16.

(F) to (I) Expression of TIE1ΔEARVP16 leads to severe cotyledon and leaf phenotypes. (F) From left to right, 7-day-old seedlings from wild-type and six independent TIE1ΔEARVP16 transgenic plants. TIE1ΔEARVP16 transgenic plants showed severely down-curled cotyledons. The red arrow indicates one cotyledon produced by the TIE1ΔEARVP16 transgenic plant. (G) and (H) 21-day-old plants from wild-type (G) and TIE1ΔEARVP16 transgenic plant (H). TIE1ΔEARVP16 transgenic plants displayed severely down curled true leaf as indicated by the red arrow (H). (I) Close-up views of abaxial side of leaves from 28-day-old and TIE1ΔEARVP16 transgenic plants. From left to right, the 5th leaf from wild-type, eight 5th leaves from eight independent TIE1ΔEARVP16 transgenic lines.

(J) and (K) The tpl-1 plants frequently produced the epinastic leaves similar to those observed in TIE1-deficient lines. (J) The 22-day-old tpl-1 plant. (K) Close-up views of abaxial side of leaves from 22-day-old tpl-1 plant. The red arrow indicates one epinastic leaf.
Supplemental Figure 5. Examination of activation activity of TIE1 in yeast.
Deletion analysis was performed and the N-terminus of TIE1, residues 1 to 108, showed no activation activity. The β-galactosidase filter assays with X-gal were used for testing activation activity of TIE1 in yeast. The black bar represents Gal4 DNA-binding domain (DBD), and the blue bars represent the different deletions of TIE1. The numbers in the right images indicated different samples when the assays were performed.
**Supplemental Figure 6.** Identification of *jaw-5D*. 

(A) and (B) Similar phenotypes were observed between *jaw-5D* and *jaw-D*.

(C) The T-DNA insertion site in the *jaw-5D* mutant. The T-DNA with four copies of CaMV 35S enhancer is located at 4125bp upstream of *miR319b* gene. The jaw-5D-P1 and jaw-5D-P2 indicate the primers used for cosegregation analysis of *jaw-5D*. 
### Supplemental Table 1. The primer list used in this study.

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NosT-2
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G4BD-2
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Supplemental Methods

Plant Materials and Heat Shock Treatment

*Arabidopsis* T-DNA insertion mutants were ordered from public mutant collections (Alonso et al., 2003). In order to obtain *TIE1* knock-out mutants by inducible insertional mutagenesis (Nishal et al., 2005), the mutant *WiscDsLoxHs225_11A* in which the T-DNA insertion was located about 13kb from the *TIE1* gene were ordered from ABRC (Alonso et al., 2003). The heat shock treatment was carried out as described previously (Nishal et al., 2005). Briefly, flowering Arabidopsis plants were heat-treated 4 cycles (42°C for 1 hour, 22°C for 1 hour) every other day until the seeds matured. The seeds of HS1 generation were harvested and plated on half-strength...
Murashige and Skoog (1/2 MS) medium with 50 µg/mL kanamycin and 200 µg/mL streptomycin.

Seedlings with green sectors were transferred to soil. The HS2 seeds were screened on 1/2 MS medium containing 50 µg/mL kanamycin and 200 µg/mL streptomycin. The green seedlings were grown in soil. Gene specific primers TIE1-S1 and TIE1-S2 plus the primer Ds 3′-1 were used for PCR screening the TIE1 mutants caused by Ds insertion (Nishal et al., 2005).

**Generation of Binary Constructs and Transformation**

The coding region of TIE2 was amplified from genomic DNA from *Arabidopsis* using primer pairs of TIE2-1 and TIE2-2. The DNA fragments were cloned into the EcoRV site of pBluescript SK+ to generate pBS-TIE2. The overexpression construct of 35S-TIE2 were generated by ligation of the KpnI-PstI digested fragments from pBS-TIE2 and KpnI-PstI digested vector pQG111. The coding region of TIE3 or TIE4 was amplified from genomic DNA from *Arabidopsis* using primer pairs of TIE3-1 and TIE3-2 or TIE4-1 and TIE4-2. The fragments were cloned into pENTR/D-TOPO (Invitrogen) to generate pENTRY-TIE3 or pENTRY-TIE4. 35S-TIE3 or 35S-TIE4 was generated by LR reaction with the plasmids pK2GW7 and pENTRY-TIE3 or pENTRY-TIE4.

**Yeast Two-Hybrid Assays**

The transactivation activity of TIE1 was evaluated first in yeast before yeast two-hybrid screening. To generate constructs, full length CDS and a series of deletions of *TIE1* were amplified from pBS-TIE1 using the primers TIE1-1, TIE1-84-2, TIE1-108-2 and TIE1-148-2. The products were cloned into the EcoRV site of pBS with the insertion direction of ATG in TIE1 or TIE1 deletions near T3 primer. Then inserts were digested by EcoRI/SalI and cloned into EcoRI/SalI sites of pYF503 (Ye et al., 2004). The transactivation activity assays in yeast was performed as described previously (Guo et al., 2009).

To test the interaction between TIE1 and SAP18, full-length coding region of SAP18 was amplified from *Arabidopsis* cDNA by RT-PCR and cloned into pENTRY/D-TOPO to generate pENTRY-SAP18, then they were cloned into pDEST32 by LR reaction to generate the bait pDEST32-SAP18. pDEST32-SAP18 and pDEST22-TIE1 or the blank pDEST22 were co-transformed into yeast strain AH109.
To perform the yeast two-hybrid screening, the N-terminus of TIE1 including 1-108 residues was amplified from pBS-TIE1 using primer pair TIE1topo-1 and TIE1-108-2. The DNA fragments were cloned into pENTRY/D-TOPO to generate pENTRY-NTIE1. The construct DBD-NTIE1 was generated from LR reaction between pENTRY-NTIE1 and pDEST32 (Invitrogen). The DBD-NTIE1 plasmids were transformed into yeast strain AH109 as a bait. Mating-based yeast two-hybrid screening for TIE1 interactors using our transcription factor library was carried out as described previously (Ou et al., 2011).

Supplemental References


