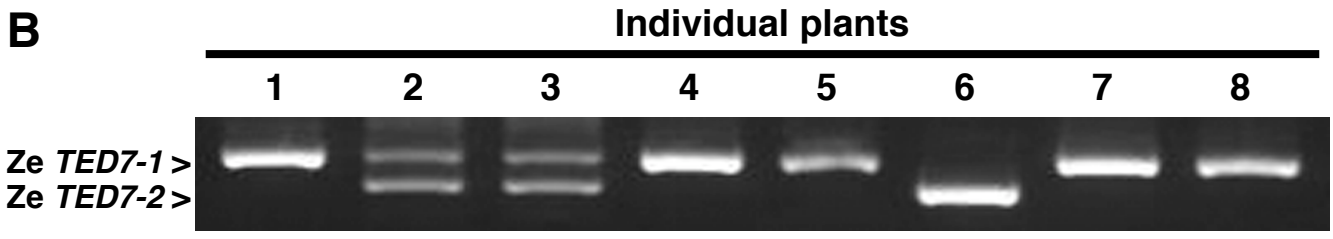
Supplemental Data. Endo et al. (2009). Identifying new components participating in the secondary cell wall Formation of vessel elements in *Zinnia* and *Arabidopsis*.

A

```
Ze TED7-1       MASPLSQSVFPHFPPPSPAATPPPAPTTPSTPPPHFISPPPHSVPPPSPPHSVPPPLHPV
Ze TED7-2       MASHLSQSLFPHFPPPSPAATPPPAPTTPSTPPPHFISPPPHSVPPPSPPHSVPPPLHPV
Ze TED6         ------------------------------------------------------------

Ze TED7-1       PPPSPPHPVSPPPHTVPPPSPPHPVSPPPHTVPPPSPPHPVFPPPHTVPPPSP-HFVPPP
Ze TED7-2       PPPLPPHSVPPPSHTVPPPSPPHPVSPPPHTVPPPSPPHPVSPPPHTVPPPSPPHHVSPP
Ze TED6         ------------------------------------------------------------

Ze TED7-1       PNMVPPPSPPHANPPPPPPPPHSVPPPPHTVPPPPPPPPHIIPPPAHALSPPPPHIIPPPPP
Ze TED7-2       PHTVPPPSP-----------HFVPPPPNTVPPPPAPHFVPPP-------PPPYIIPPPPP
Ze TED6         ------------------------------------------------------------

Ze TED7-1       SPSNHSTTIVVIFVSCGGVFFLAFAMAALWCFLKKKKK-KMVQKAENIHFDEHRKVTERI
Ze TED7-2       SPSNHSTTIVVIFVSCGGVFFLAFAMAALWCFLKKKKK-KMVRKAENIHFDEHRKVTERI
Ze TED6         -----MATIFIVFVSFGCVFVLGIAAFVLCCLIKKWKCSKAIEKNEMVHVDQHLQVHENI
                      :**.::*** * **.*.:*  .* *::** *  * :.* * :*.*:* :* *.*

Ze TED7-1       EQGPHGTETAILSVEDDIHIEEDIKKSELENFRKGLHLNYGNTYNIDTGKPSSSFGHHYL
Ze TED7-2       EQGPHGTETAILSVEDDIHIEEDIKKSEIEDFRKGLHLNYGNTYNIDTGKPSSSFGHHYL
Ze TED6         LQGPNGMKTVAITVDDDLHVHDEEE----------------CVKNEKLGTASTSKA----
                 ***:* :*. ::*:**:*:.:: :                 . * . *..*:* .

Ze TED7-1       HG
Ze TED7-2       HG
Ze TED6         --
```

B



Supplemental Figure 1. Comparison of Ze *TED6*, Ze *TED7-1* and Ze *TED7-2*. **(A)** Amino acid sequences deduced from Ze *TED6*, Ze *TED7-1,* and Ze *TED7-2* cDNAs. Underlines indicate sequences corresponding to Z1943 and Z16653 EST clones. **(B)** Genotyping of eight individual *Zinnia* plants. Primers were designed to be able to amplify both Ze *TED7-1* and Ze *TED7-2*.

A

```
Ze TED6
At1g43790 / At TED6
eugene3.00020671
Ze TED7-1                MASPLSQSVFPHFPPPSPAATPPPAPTTPSTPPPHFISPPPHSVPPPSPPHSVPPPLHPVPPPSPPHPVSPPPHTVPPPSPPHPVSP
Ze TED7-2                              MASHLSQSLFPHFPPPSPAATPPPAPTTPSTPPPHFISPPPHSVPPPSPPHSVPPPLHPVPPPLPPHSVP
At5g48920 / At TED7
eugene3.00070382                                                                                                MAP
fgenesh1_pg.C_LG_V000008                                                MAPLDNYDYNFPYFPLPPPHNPPSPPKVVPPHNYPSPPKGSPPHNPPPPH
Os08g0108300                    MTFNPGSPGFGFPFPFYPPNPNPYAPLNPNAPKPPVMPPRPQAPPPPQRFPPPPAPPIRPPSPPGRAPPPPGRAPPPPSQA

Ze TED6
At1g43790 / At TED6                                                                                             MASTDSV
eugene3.00020671                                                                  MAASNNLDFPYSPPPPSHSFQPPPSPPHVRPPPPHIRPPPPP
Ze TED7-1                PPHTVPPPSPPHPVFPPPHTVPPPSPHFVPPPPNMVPPPSPPHANPPPPPPPHSVPPPPHTVPPPPPPPHIIPPPAHALSPPPPHII
Ze TED7-2                PPSHTVPPPSPPHPVSPPPHTVPPPSPPHPVSPPPHTVPPPSPPHHVSPPPHTVPPPSPHFVPPPPNTVPPPPAPHFVPPPPPPYII
At5g48920 / At TED7          MAASVEYFPYYSPPSHQHPLPSPVPPPPSHISPPPPPFSPPHHPPPPHFSPPHQPPPSPYPHPHPPPPSPYPHPHQPPPPPHV
eugene3.00070382         TNNYDYNFPYFPLPPPHNNPPSPPKVAPPHSSPSPPNVSPPHNFPPPHITPPSPKVPPPPHHPITPPPTHPFHPPPPHHIPPPPHVI
fgenesh1_pg.C_LG_V000008 IIPSPPKVVPPHNYPSPPKGSPPHNPPPPHIKPSPPKVPPPHHPITPPSPFPVPATPPNHPFHPPPPHHIPPPSPPHIIPPAPSHVI
Os08g0108300             PPPPRRAPPPPALPPPPPRRAPPPPSMPPPPPPRRAPPPPATPPPPPRRAPPPPSPPIRPPPPPTPRPYAPPPPSHPLAPPPPHISP

Ze TED6                                  MATIFIVFVSFGCVFVLGIAAFVLCCLIKKWKCSKAIEKNEMVHVDQHLQVHENILQGPNGMKTVAITVDDDLHVHDE
At1g43790 / At TED6      YRPTPTPDHDTTVVVVVFVSLGCVMFLAFLAFVIWFLIKKRSRKHRERSEAVRVDEHFKMKEAIVEGPNGQKSVVLSVEDDVKIEDA
eugene3.00020671         LPPAPSPSNNTTVIVIVFVSFGGLIFLAFLAAALCFFIKKKKKKTVEETDIVHVHEHLKVKEAIVEGPHGPKAVVLEIVDDVHIGEE
Ze TED7-1                PPPPPSPSNHSTTIVVIFVSCGGVFFLAFAMAALWCFLKKKKKKMVQKAENIHFDEHRKVTERIEQGPHGTETAILSVEDDIHIEED
Ze TED7-2                PPPPPSPSNHSTTIVVIFVSCGGVFFLAFAMAALWCFLKKKKKKMVRKAENIHFDEHRKVTERIEQGPHGTETAILSVEDDIHIEED
At5g48920 / At TED7      LPPPPPTPAPGHHVIIVVVISLGSLFFLAFLAAALFCYLKKRRKSSTKAEIIEFDEHLKVQETIVQGPHGEQTRVVMLEEDIHLVED
eugene3.00070382         PPPPPTPGHHSTVIIVVFVSLGGLFFLAFLSVALCCFIKKKKKKTVQKTEILEFDEHTKVQEAIVPGPHGEKITVLNIEEDVHLVEE
fgenesh1_pg.C_LG_V000008 PPPPPTPGHHSTVIIVVFVSLGGLFFLAFLSVALCCFIKKKKKKTVQKTEILEFDEHTKVQEAIIPGPHGEKITVLNIEEDVHLVEE
Os08g0108300             PAPVPPPPSPPPHIVIIVVFVSFGGLLLLACLAALFCWHKKRRETERKAEVHNLSGHVHVHKATESGPSGAKATVLSIDEDLKFQEV
                                                                                  *      H         GP G         *D *  *

Ze TED6                  EECVKNEKLGTASTSKA
At1g43790 / At TED6      IKREEKDLKKDGGVGSSVVSRS
eugene3.00020671         IKEEEKVGEGLHAKAIEGNAGTVDQLAAPSSSGSNNHSRLEHKA
Ze TED7-1                IKKSELENFRKGLHLNYGNTYNIDTGKPSSSFGHHYLHG
Ze TED7-2                IKKSEIEDFRKGLHLNYGNTYNIDTGKPSSSFGHHYLHG
At5g48920 / At TED7      IHKTEKLSRPSHLSSTGRHAIDISDPNHHFTEQKS
eugene3.00070382         IKKNEKLTEGSHIKSAHDRPLYSDIATPSSQYNQHHLEHKV
fgenesh1_pg.C_LG_V000008 IKKNEKLAEGSHIKLAHDHPLDSDIATSSSRSNQQHLEHKV
Os08g0108300             AGESSSAAGAGSHHTPWSWHRRQQEGKAENKAELINVTEHIHVDEKIVSGPQGQKIEILSEDEDIRFEEEGRKEKGDQRSKTRITKT
                                                  (*      H         GP G         *D *  *)
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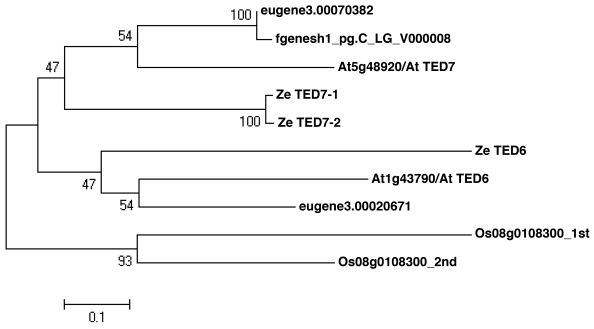
B



C

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At1g43790 / At TED6        HRERSEAVRV DEHFKMKEAI VEGPNGQKSV VLSVEDDVKI EDAIK
eugene3.00020671           TVEETDIVHV HEHLKVKEAI VEGPHGPKAV VLEIVDDVHI GEEIK
Ze TED6                    AIEKNEMVHV DQHLQVHENI LQGPNGMKTV AITVDDDLHV HDEEE
Ze TED7-1                  MVQKAENIHF DEHRKVTERI EQGPHGTETA ILSVEDDIHI EEDIK
Ze TED7-2                  MVRKAENIHF DEHRKVTERI EQGPHGTETA ILSVEDDIHI EEDIK
eugene3.00070382           TVQKTEILEF DEHTKVQEAI VPGPHGEKIT VLNIEEDVHL VEEIK
fgenesh1_pg.C_LG_V000008   TVQKTEILEF DEHTKVQEAI IPGPHGEKIT VLNIEEDVHL VEEIK
At5g48920 / At TED7        SSTKAEIIEF DEHLKVQETI VQGPHGEQTR VVMLEEDIHL VEDIH
Os08g0108300_1st           TERKAEVHNL SGHVHVHKAT ESGPSGAKAT VLSIDEDLKF QEVAG
Os08g0108300_2nd           AENKAELINV TEHIHVDEKI VSGPQGQKIE ILSEDEDIRF EEEGR
```
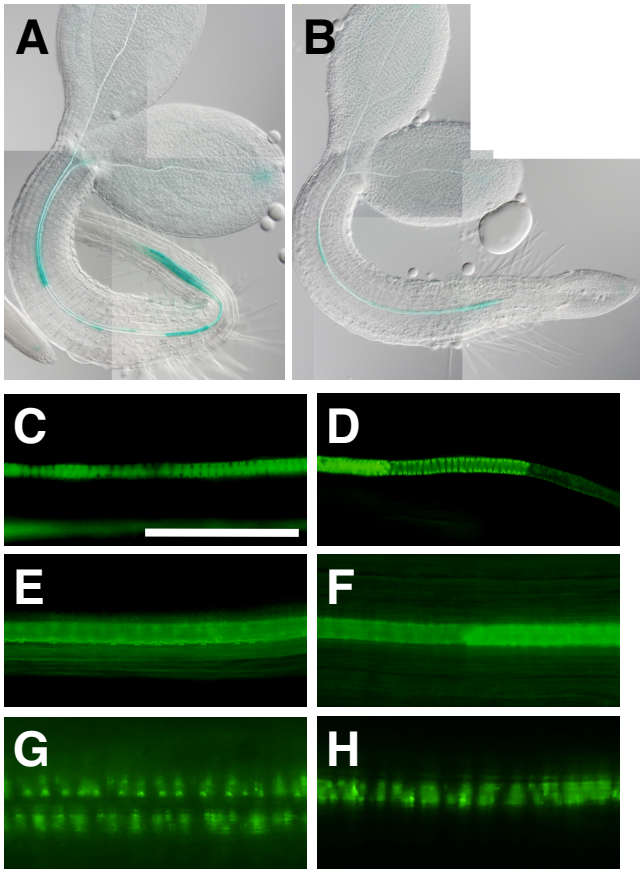
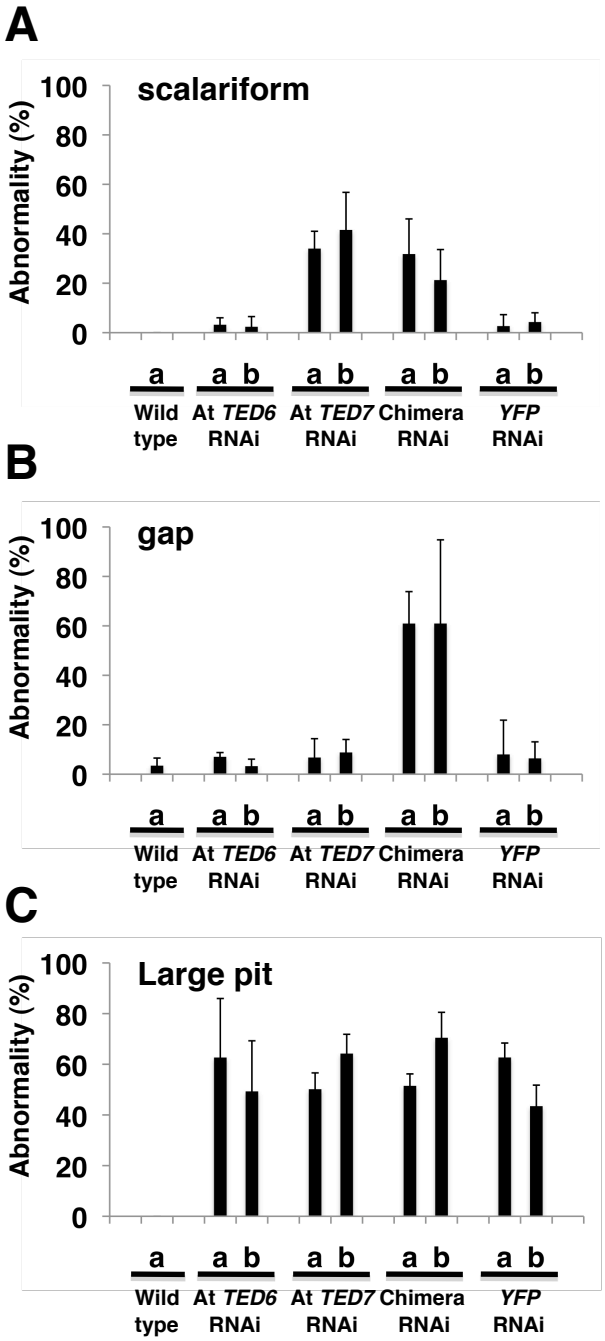

Supplemental Figure 2. Comparison of Ze TED6 and Ze TED7 and Counterparts in *Arabidopsis*, *Populus,* and *Oryza*. **(A)** Full-length amino acid sequences. Bold letters, transmembrane domains by SOSUI prediction; red letters, prolines at N-terminus sides; blue letters, sequences used in the overexpression experiment in Figure 3; underlines, Z1943 and Z16653 clone sequences. Conserved amino acids in C-terminus domains are shown at the bottom (note that Os08g0108300 has two repeats of the C-terminus domains). **(B)** Comparison of conserved regions on MEGA4 (Tamura et al., 2007) by the neighbor-joining method, bootstrapped (1000), and shown as a rooted tree (midpoint rooting). The duplicated domains of Os08g0108300 were divided (Os08g0108300_1st and _2nd). **(C)** Sequences used for **(B)**.

Reference

Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24:** 1596–1599.



Supplemental Figure 3. Expression Pattern of At *TED6* and At *TED7*. **(A)** and **(B)** Promoter activities in seedlings at 3 d after imbibition. GUS reporter expression was examined by X-Gluc staining of At TED6$_{Pro}$:GUS **(A)** and At TED7$_{Pro}$:GUS **(B)** lines.
(C) to **(H)** Expression of YFP fusions under the control of native promoters in roots. YFP signals were observed in differentiating vessel elements in the protoxylem (**[C]** and **[D]**) and metaxylem (**[E]** to **[H]**) by At TED6$_{Pro}$:At TED6-YFP (**[C]**, **[E]**, and **[G]**) or At TED7$_{Pro}$:At TED7-YFP (**[D]**, **[F]**, and **[H]**). Bars in **(C)** for **(C)** to **(H)** = 50 μm.

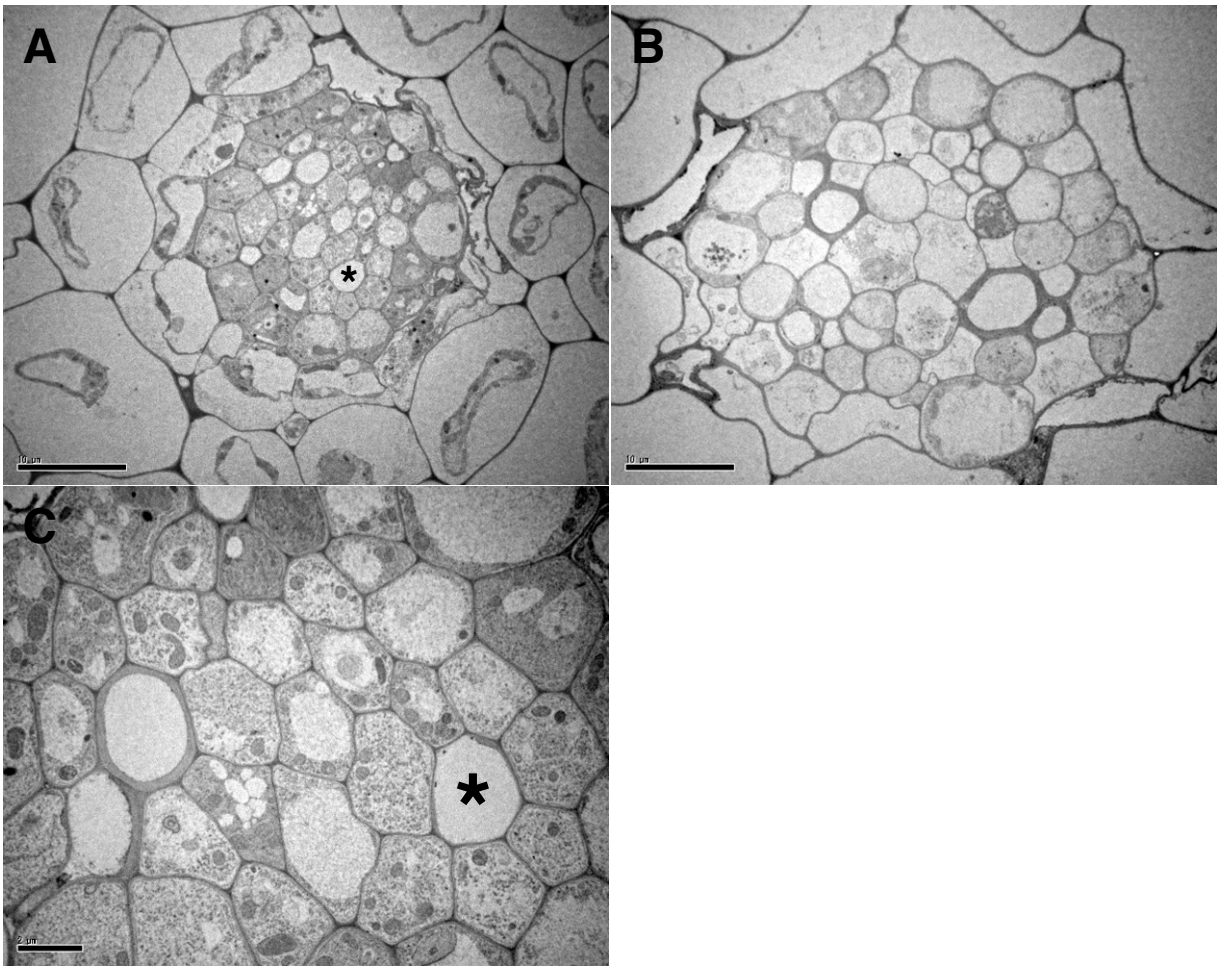


Supplemental Figure 4. Transient RNAi Analysis on At *TED6* and At *TED7* in *Arabidopsis* Roots. Abnormality represents frequencies of abnormal metaxylem vessel element-forming roots in an average of 20 lateral roots of individual lines (a, b). Lines a and b of At *TED6*, At *TED7,* and At *TED6–TED7* chimera RNAi are T2 plants that have been analyzed by RT-PCR. *YFP* RNAi is a control construct that expresses an inverted repeat of a partial *YFP* nucleotide sequence. Each data point represents the mean of three independent RNAi inductions ± SD.

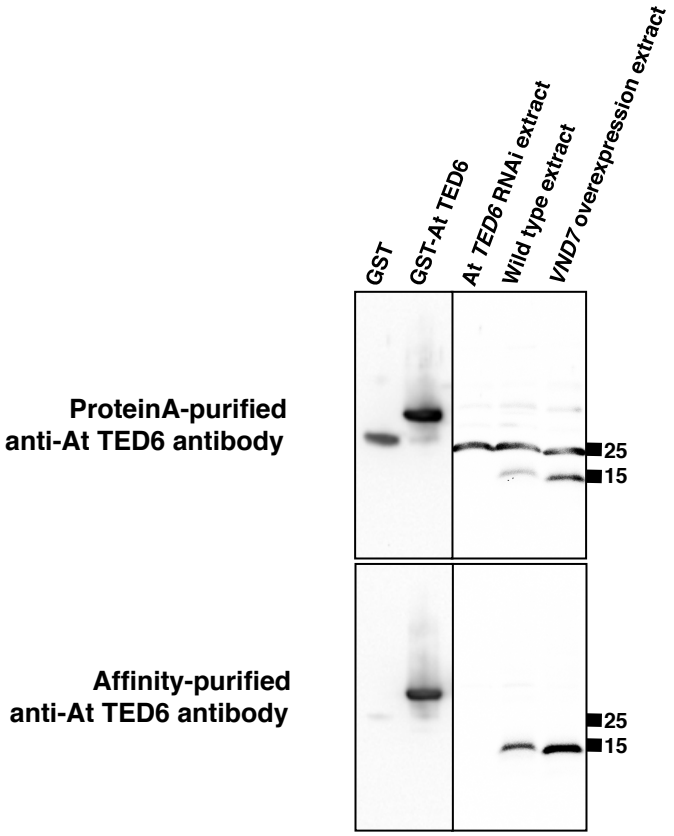
(A) Scalariform (ladder-shaped) metaxylem vessel elements as shown in Figure 5C.

(B) Gaps in metaxylem vessel strands as shown in Figure 5D.

(C) Metaxylem vessel elements with large pits as shown in Figure 5E.



Supplemental Figure 5. Transmission Electron Micrographs of the At *TED6–TED7* chimera RNAi Line. Cross sections of transgenic *Arabidopsis* roots of At *TED6–TED7* chimera RNAi (**[A]** and **[C]**) and YFP RNAi **(B)** lines. The plants had been treated to induce RNAi in the same way used in the experiments shown in Figure 5 and Supplemental Figure 4 online. Note that the chimera RNAi plant showed a metaxylem vessel element with extremely thin SCW but no cellular content (asterisks). Bars in **(A)** and **(B)** = 10 μm, in **(C)** = 2 μm.



Supplemental Figure 6. Affinity Purification of Anti-At TED6 Antibody.
Characterization of Protein A-purified and affinity-purified anti-At TED6 antibody. The specificity was examined on glutathione-*S*-transferase (GST)-fusion protein and crude extracts of At TED6 RNAi, wild type, and plants showing ectopic TE differentiation by *VND7* overexpression. Numbers on the left indicate size markers (kDa).

Supplemental Table 1. Summary of Single YFP and BiFC Signals in Wild Type and SCW-forming *Arabidopsis* Cells. YFP signals were observed at 18 h and 48 h after the induction of *VND7* expression in leaves biolistically bombarded with the indicated constructs.

Signal: +++, strong intensity and high frequency; ++, moderate intensity and high frequency; +, weak intensity and some frequency; ±, occasionally detected; –, not detected

Construct	Signal		
	Wild type	SCW-forming	
	18 h	18 h	48 h
YFP	+++	+++	+++
At TED6-YFP	++	++	++
YFP-IRX1	–	±	–
YFP-IRX3	–	+	–
YFP-IRX5	–	±	–
At TED6-cYFP, nYFP-IRX1	–	–	–
At TED6-cYFP, nYFP-IRX3	–	+	–
At TED6-cYFP, nYFP-IRX5	–	–	–

Supplemental Table 2. Primers Used in This Study.

Target	Forward primer sequence in 5′–3′ orientation	Reverse primer sequence in 5′–3′ orientation
Zinnia ESTs subcloned in pGEM-T Easy plasmid vector (Promega, http://www.promega.com/) for templates of *in vitro* RNA transcription.	TGTAAAACGACGGCCAGTGAATTGTAATAC	GAAACAGCTATGACCATGATTACGCCAAG
Ze *TED7-1* and *TED7-2* genomic DNA for genotyping.	TTCCCTCATTTTCCACCGCCATC	TGTTGTGGAATGGTTGCTTGGAGA
Ze *TED6* full-length coding region for protein localization.	CACCATGGCCACCATATTCATTGTTTTCGTGTCA	AGCTTTTGAAGTTGAAGCTGTCCCAAGCTT
Ze *TED7* full-length coding region for protein localization.	CACCATGGCTTCTCCTCTTTCTCAATCCGTGTTC	GCCATGCAGGTAGTGATGGCCAAAACTGGA
Ze *TED6* full-length coding region for overexpression.	CACCATGGCCACCATATTCATTGTTTTCGTGTCA	TCAAGCTTTTGAAGTTGAAGCTGTCCCAAG
Ze *TED7* full-length coding region for overexpression.	CACCATGGCTTCTCCTCTTTCTCAATCCGTGTTC	TTAGCCATGCAGGTAGTGATGGCCAAAACT
Ze *TED6* C-terminal coding region for overexpression.	CACCATGTTGATCAAGAAATGGAAATGCAG	TCAAGCTTTTGAAGTTGAAGCTGTCCCAAG
Ze *TED7* C-terminal coding region for overexpression.	CACCATGTGGTGCTTCCTCAAGAAGAAG	TTAGCCATGCAGGTAGTGATGGCCAAAACT
At *TED6* 5′ upstream region for promoter assay.	CACCGATTGATTCAATAGCCTCACGCTCTGATAC	GGAGGCCATTACTTGTGTTTGATGTGTGAG
At *TED7* 5′ upstream region for promoter assay.	CACCTGAGAATCAACAAAGATTCCTACGTGTGGC	GGCAGCCATTTTGGGAGTATGTATGTACTT
At *TED6* promoter and coding region for protein localization.	CACCGATTGATTCAATAGCCTCACGCTCTGATAC	CGAACGGGAAACGACTGATGATCCAACTCC
At *TED7* promoter and coding region for protein localization.	CACCTGAGAATCAACAAAGATTCCTACGTGTGGC	GGACTTTTGCTCAGTAAAATGATGATTGGG
At *TED6* full-length coding region for inverted repeat production and BiFC assay.	CACCATGGCCTCCACGGATTCAGTTTACCGTCCC	CGAACGGGAAACGACTGATGATCCAACTCC
At *TED7* full-length coding region for inverted repeat production.	CACCATGGCTGCCTCTGTGGAATACTTTCCCTAT	GGACTTTTGCTCAGTAAAATGATGATTGGG
IRX1/CesA8 coding region for BiFC assay.	CACCATGATGGAGTCTAGGTCTCCCATCTG	TTAGCAATCGATCAAAAGACAGTTCAGAGA
IRX3/CesA7 coding region for BiFC assay.	CACCATGGAAGCTAGCGCCGGTCTTGTCGC	TCAGCAGTTGATGCCACACTTGGAAGTGTC
IRX5/CesA4 coding region for BiFC assay.	CACCATGGAACCAAACACCATGGCCAGCTT	TTAACAGTCGACGCCACATTGCTTCAGT
At *TED6* partial cDNA for RT-PCR.	AGAGCCTCACACATCAAACACAAG	GGTAACATTATGAATGAAGAAAGCTC
actin2 partial cDNA for RT-PCR.	CCGTTTTGAATCTTCCTCAATC	ATACCGGTACCATTGTCACACA
At *TED7* partial cDNA for RT-PCR.	AACCATTTAAGTACATACATACTCCC	ATGATTGTTTACATTTTGAGCCTTTTG
ubiquitin partial cDNA for RT-PCR.	TCCAATGTGATCCAACAGAGAC	TTCAAAGTCAAAGCCACAACTG
At *TED6* C-terminal region into *Sma*I site of pGEX6P-1 plasmid vector for antigen production.	CACCATGTTGATCAAGAAGAGATCCAGGAAGCACCGT	TCACGAACGGGAAACGACTGATGATCCAACTCC