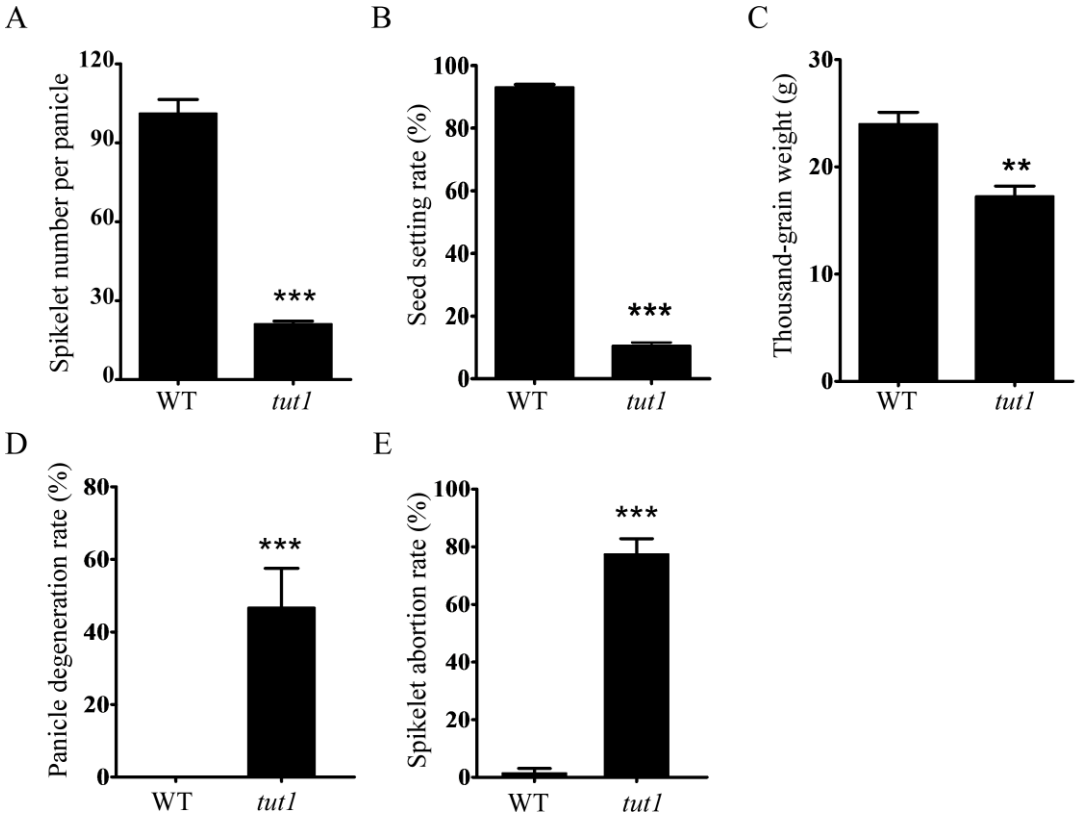
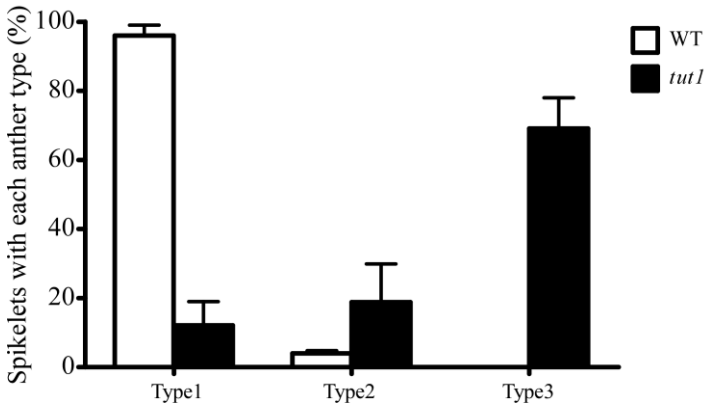
SUPPLEMENTAL FIGURES

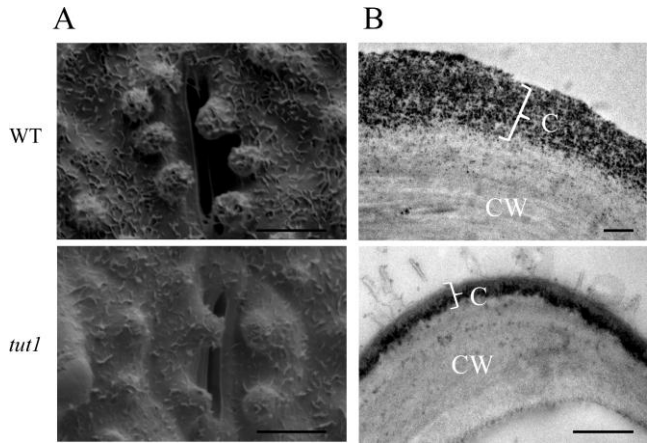


Supplemental Figure 1. Quantitation of agronomic traits in wild type and *tut1*.

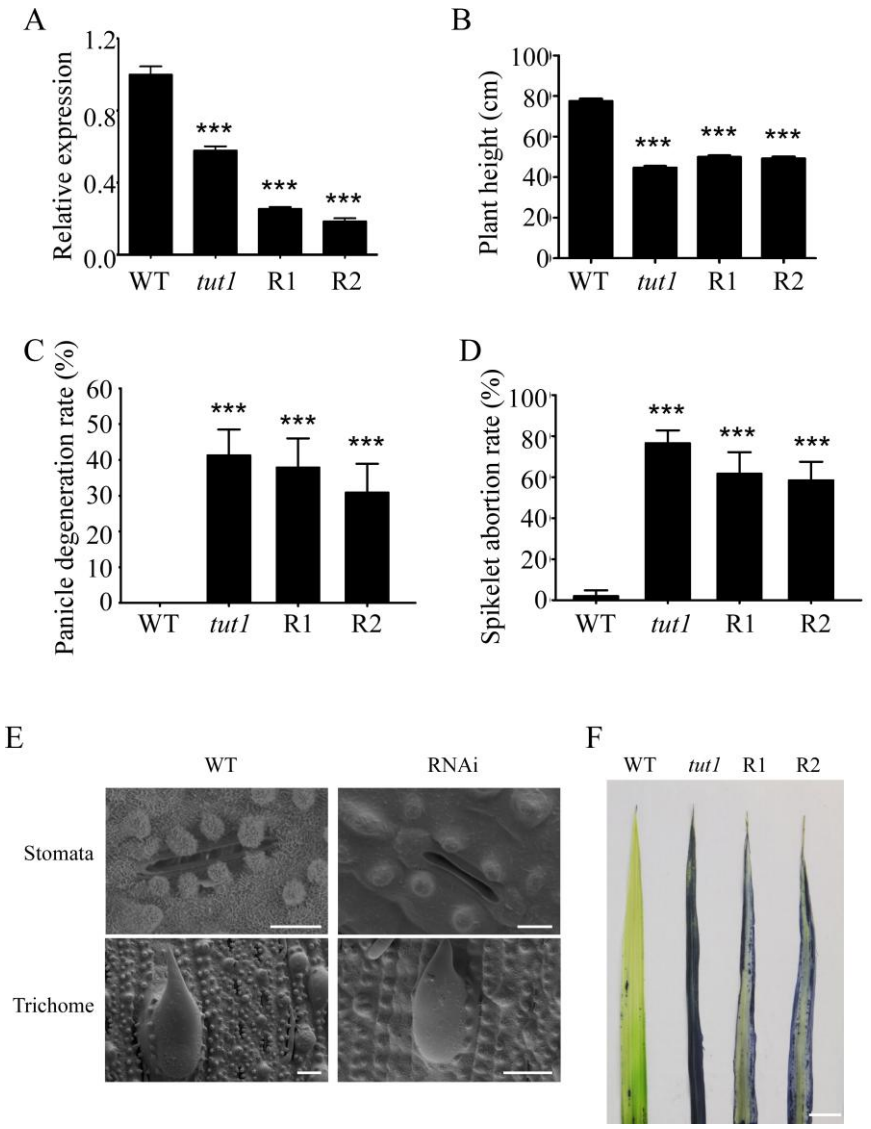
A, Spikelet number per panicle. B, Seed-setting rate. C, Thousand-grain weight of mature seeds. D, Panicle degeneration percentage. E, Spikelet abortion percentage. More than 20 panicles were used for each analysis. Error bars indicate SD. *** indicates $P < 0.01$ (Student's *t* -test).



Supplemental Figure 2. Quantitation of anther degeneration in wild type and *tut1*. Type 1, Type 2 and Type 3 anthers, as shown in Figure 2E, were analyzed at anther developmental stage 14. Over 40 anthers were used in this statistical analysis. Error bars indicate SD.



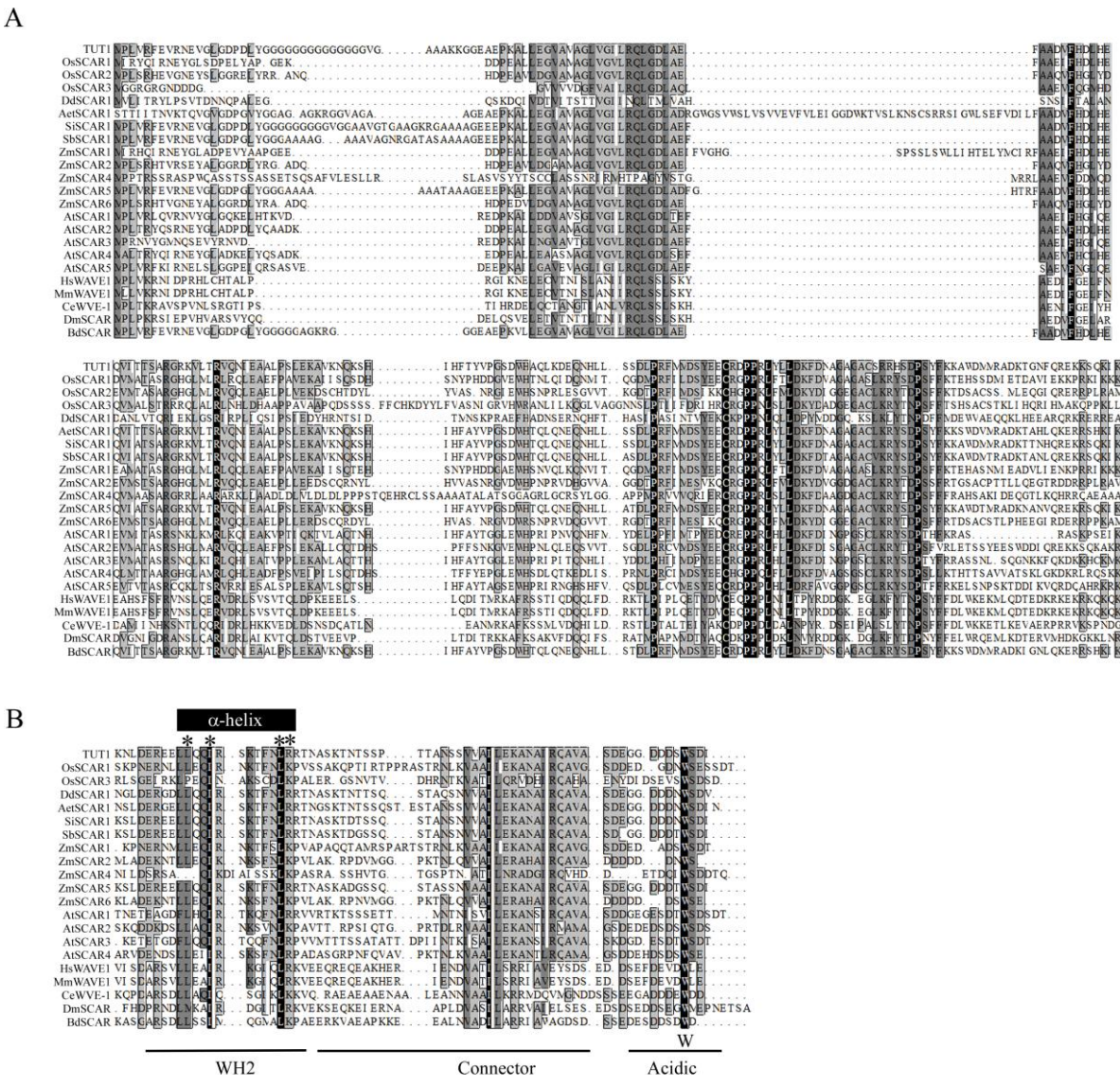
Supplemental Figure 3. SEM and TEM examination of cuticle in wild type and *tut1* leaves. A, Observation of wild type and *tut1* leaves stomata circumambient surface by SEM. Mature leaves of wild type and *tut1* were used. Bar = 5 μm. B, Observation of wild type and *tut1* leaf cuticle by TEM. C indicates cuticle, CW indicates cell wall. The middle part of the leaf was selected for TEM. Bar = 0.5 μm.



Supplemental Figure 4. The phenotypes of *TUT1* RNAi transgenic lines.

A, RT-qPCR analysis of the levels of *TUT1* mRNA in wild type, *tut1*, and RNAi transgenic lines. B, Plant height in wild type, *tut1*, and RNAi transgenic lines (R1 and R2) at ripening stage. More than 120 plants were used. C, Panicle abortion percentage of wild type and *tut1* at ripening stage. More than 50 panicles were used. D, Spikelet abortion percentage of wild type and *tut1* at ripening stage. More than 50 panicles were used. E, Observation of stomata and trichome in wild type and representative RNAi transgenic line with SEM. Bars = 5 μm (top two) and 20 μm (bottom two). F, NBT staining to show ROS in wild type, *tut1* and two RNAi transgenic lines.

Bars = 1 cm. For graphs, error bars indicate SD and *** indicates $P < 0.01$ (Student's *t* -test).

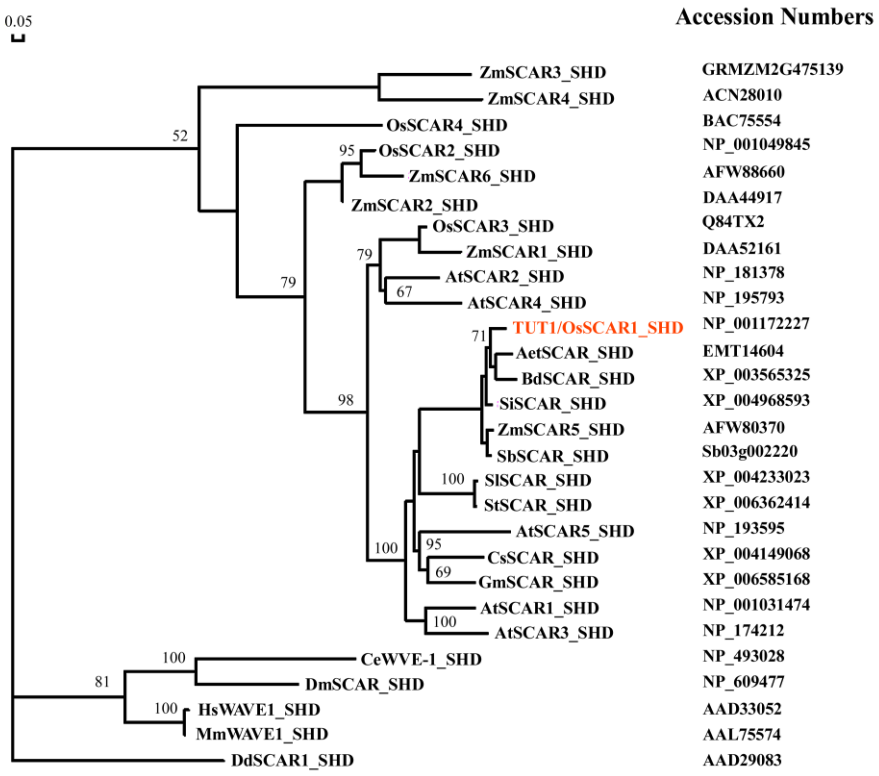


Supplemental Figure 5. Amino acid sequence alignment of TUT1 and other WAVE/SCAR family proteins.

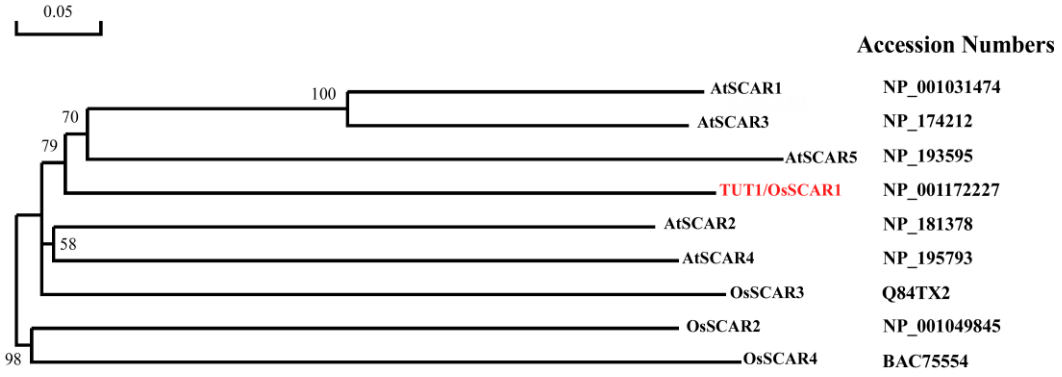
A, Sequence alignment of the SHDs from different species, constructed using DNAMAN software. Amino acids identical in all sequences (black), amino acids sequence with > 75% identity (dark gray), and with > 50% identity (light gray) are indicated. B, Sequence alignment of the VCA domain from different species, constructed using DNAMAN software. Residues that are essential for actin binding are indicated with asterisks. The α-helix is shown above the sequences, as previously described (Paunola et al., 2002). Amino acids sequence identity is

indicated as in A. The G-actin binding region (WH2), connector region (Connector) and acidic region (Acidic) are labeled. W indicates the conserved tryptophan.

A



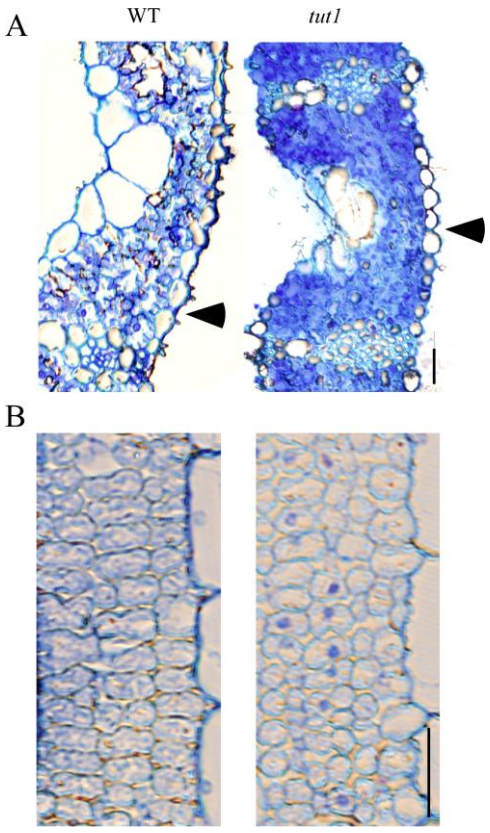
B



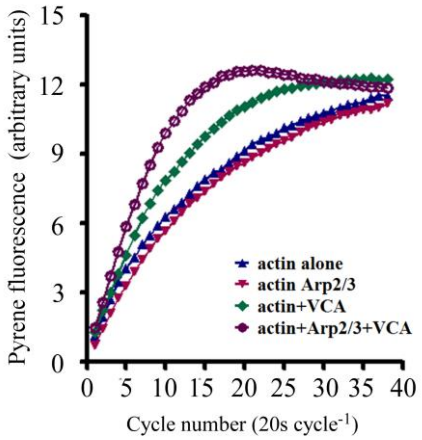
Supplemental Figure 6. Phylogenetic tree of SHD of SCAR/WAVE proteins from Arabidopsis and rice.

A, Phylogenetic tree of the SHD of TUT1 and its homologous proteins in different species. The phylogenetic tree was constructed using DNAMAN software with 1000 bootstrapping trials. The numbers on the branch sites indicate bootstrapping values. TUT1 is shown in red.

Accession numbers are shown. The tree was constructed using the distance method with maximum likelihood. Bootstrapping values over 50% and scale bar are shown in the phylogenetic tree. Species are as indicated: *Aegilops tauschii* (Aet), *Arabidopsis thaliana* (At), *Brachypodium distachyon* (Bd), *Caenorhabditis elegans* (Ce), *Cucumis sativus* (Cs), *Dictyostelium discoideum* (Dd), *Drosophila melanogaster* (Dm), *Glycine max* (Gm), *Homo sapiens* (Hs), *Mus musculus* (Mm), *Oryza sativa* (Os), *Setaria italica* (Si), *Solanum lycopersicum* (Sl), *Solanum tuberosum* (St), *Sorghum bicolor* (Sb), *Zea mays* (Zm). B, Phylogenetic tree of SCAR/WAVE proteins in Arabidopsis and rice. The phylogenetic tree was constructed using DNAMAN software with 1000 bootstrapping trials. The numbers on the branch sites indicate bootstrapping values. TUT1 is shown in red. Accession numbers are shown. The tree was constructed using the distance method with maximum likelihood. Bootstrapping values over 50% and scale bar are shown in the phylogenetic tree.



Supplemental Figure 7. Analysis of mature leaves of wild type and *tut1* by light microscopy. A, Cross sections observation of mature leaves of wild type and *tut1*. The black arrows showed epidermal cell wall at two cells junction of mature leaves. Bar = 200 μm. B, Paradermal sections observation of mature leaves of wild type and *tut1*. The sections were stain with Toluidine blue. Bar = 200 μm.



Supplemental Figure 8. Activation analysis of TUT1-VCA domain *in vitro*.

The VCA domain of TUT1 could activate bovine Arp2/3. Actin (5% pyrene labeled) with or without Arp2/3 and VCA recombinant protein was added to polymerization reaction system and the kinetics of assembly monitored by fluorimetry. Pyrene fluorescence shows the degree of G-actin assembly into filaments. Conditions: actin alone (blue line), actin and Arp2/3 proteins (red line), actin and VCA recombinant protein (green line), and actin, Arp2/3 and VCA recombinant protein (purple line).

Table S1: Primers used in this study.

Primer	Primer sequence (5’ to 3’)	Experiment
P23_F	GTAGTTTCTGGCAGAGGAGG	Fine mapping
P23_R	AACAGCGATAGTATGGTTGC	Fine mapping
P10A_F	GAGGATTCCGAGAAGTAGGG	Fine mapping
P10A_R	ACGAAAGATATCAGCCAAAA	Fine mapping
P18_F	AATATAATGACACTAGCCGT	Fine mapping
P18_R	CTTGCTTACTCAGTTGCTCT	Fine mapping
P5_F	GCTGAGTGGCGACAATGATGA	Fine mapping
P5_R	ATGACGAAGTAAAACCCGACC	Fine mapping
P22_F	TTCTCTTAAAACCTCTCGCTG	Fine mapping
P22_R	CTTTGGTCACATTATTCCTTG	Fine mapping
P5#A_F	CAGGTGAAGCGCCATTGAT	Fine mapping

P5#A_R	GCATTGCATTGCAAGATGC	Fine mapping
TUT1_RT-qPCR_F	TGATTTTCCCAGTTTGTCAAGTG	RT-qPCR
TUT1_RT-qPCR_R	GGATGAGGATTAGCTGTATAGCC	RT-qPCR
GAPDH_F	AAGCCAGCATCCTATGATCAGATT	RT-qPCR
GAPDH_R	CGTAACCCAGAATACCCTTGAGTTT	RT-qPCR
GUS_F	CTGCAGTCTGTGGAAGGAGCGGAACT	TUT1:GUS
GUS_R	GGATCCGCTTGTACTGGTCGGAGGC	TUT1:GUS
RNAi_F	GGGGTACCACTAGTCGGAGACTGTAG TGAAGGC	pTCK303-TUT1
RNAi_R	CGGGATCCGAGCTCCCCACAAAACTG CTGGTAA	pTCK303-TUT1
VCA_F	CGGGATCCCCAGTACACACTCCTGTTC A	pGEX-VCA
VCA _R	GCGTCGACTCATATATCACTCCAACTAT CATCA	pGEX-VCA
TUT1_OE_F	GTCGACATGCCGCTGGTGAGGTTCGA	UBI:TUT1
TUT1_OE_R	CCCGGGTATATCACTCCAACTATCATCA	UBI:TUT1

SUPPLEMENTAL METHODS

Actin Polymerization

For protein purification, the region encoding the last 153 amino acids of TUT1, including the VCA domain, was cloned using the gene-specific primers VCA_F and VCA_R. The TUT1 VCA domain was cloned into the GST fusion vector pGEX4T-1 (GE Healthcare) using the *BamH*I and *Sal*I sites. The expression and purification of GST-VCA recombinant protein was performed according to the manufacturer's instructions.

Polymerization analyses were performed using the Actin Polymerization Biochem Kit (Denver, CO, USA) and performed according to the manufacturer's instructions. Pyrene fluorescence was detected using a spectrofluorimeter. For the details of this method, see (Mullins and Machesky, 2000; Basu et al., 2005; Zhang et al., 2008).

LITERATURE CITED

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Mullins RD, Machesky LM (2000) Actin assembly mediated by Arp2/3 complex and WASP family proteins. Methods Enzymol **325:** 214-237

Paunola, E., Mattila, P.K., and Lappalainen, P. (2002). WH2 domain: a small, versatile adapter for actin monomers. Febs Lett **513:** 92-97.

Zhang C, Mallery EL, Schlueter J, Huang SJ, Fan YR, Brankle S, Staiger CJ, Szymanski DB (2008) *Arabidopsis SCARs* function interchangeably to meet actin-related protein 2/3 activation thresholds during morphogenesis. Plant Cell **20:** 995-1011