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Supplementary Information for:

TOR dynamically regulates plant cell-cell transport

Jacob O. Brunkard, Min Xu, M. Regina Scarpin, Snigdha Chatterjee, Anne M. Runkel, Elena A. Shemyakina, Howard M. Goodman, Patricia Zambryski

Corresponding Author:
Jacob O. Brunkard
Plant Gene Expression Center, U.S. Department of Agriculture Agricultural Research Service, Albany, California 94710, USA
brunkard@berkeley.edu

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Datasets S1 to S11

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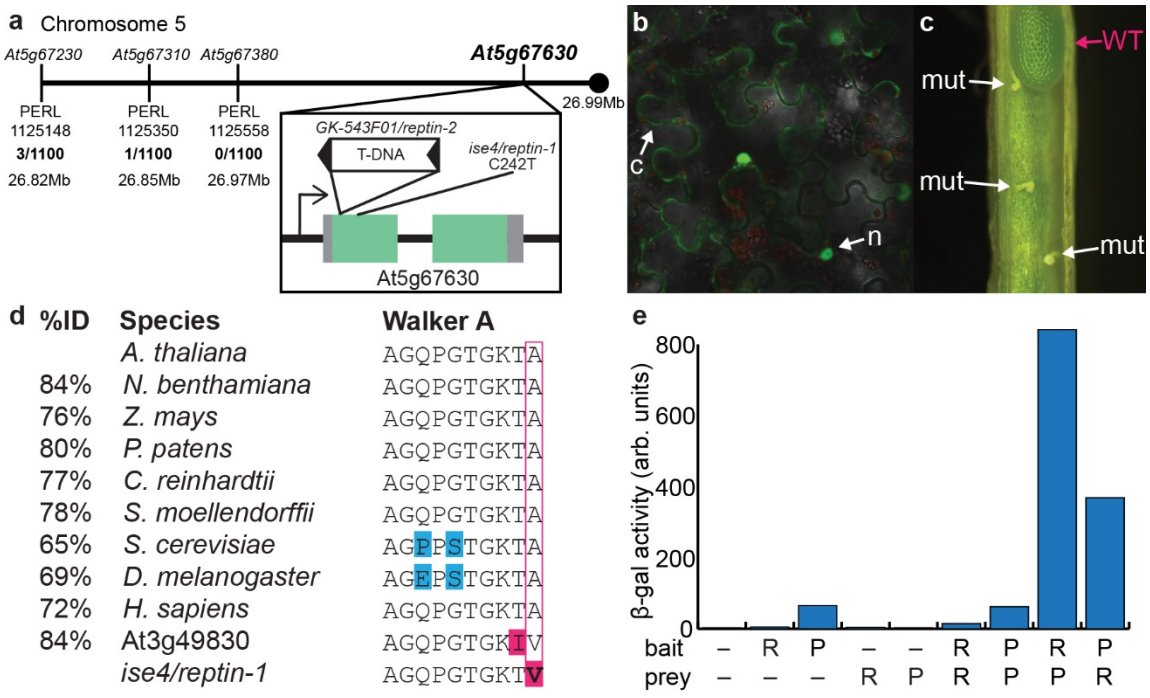

Figure S1. *ise4* is a missense mutation of At5g67630, an orthologue of the conserved eukaryotic *Reptin* gene, shown in Figure 1. **a**, *ise4* was mapped to the end of chromosome using SNP markers in an F2 *ise4* (L*er*) x Col-0 mapping population. Frequency of Col-0 SNP alleles are shown below the marker name, and the location of each SNP along chromosome 5 is indicated. *ise4/reptin-1* is a C242T mutation in the coding sequence of *AtReptin* (At5g67630). An insertional knockout allele (GK-543F01) was obtained from ABRC. **b**, AtReptin-GFP localizes to the cytosol (c) and nucleus (n), like its orthologues in other eukaryotes. **c**, Offspring of selfed heterozygous GK-543F01 plants are ~50% aborted female gametophytes, ~25% heterozygous GK-543F01, and ~25% wild-type, demonstrating that loss of *AtReptin* is female gametophyte lethal, and that *reptin-1* is therefore a hypomorph of *AtReptin*. Arrested female gametophytes indicated with white arrows (mut), and a wild-type +/+ or heterozygous GK-543F01/+ seed is indicated in magenta (WT). **d**, *reptin-1* causes a missense A81V mutation C-terminal to the Walker A motif of the Reptin ATPase. This Walker A motif is highly conserved across eukaryotes, and A81 (highlighted with a magenta box) is universally conserved. At3g49830 is an apparent paralogue of *AtReptin*, but is not expressed (is a pseudogene); the predicted ORF has a T80I mutation at the nucleotide-binding site of the Walker A motif, which would prevent the protein from binding to nucleotides if it were expressed. **e**, AtReptin (R) and AtPontin (P) strongly interact, as shown with a quantitative yeast two-hybrid reporter system (SI ref. 1). Expression of a β-galactosidase reporter protein was quantified spectrophotometrically. The reporter was strongly induced

only when AtReptin and AtPontin were available as bait and prey, and not when either was expressed alone or with itself as bait or prey.

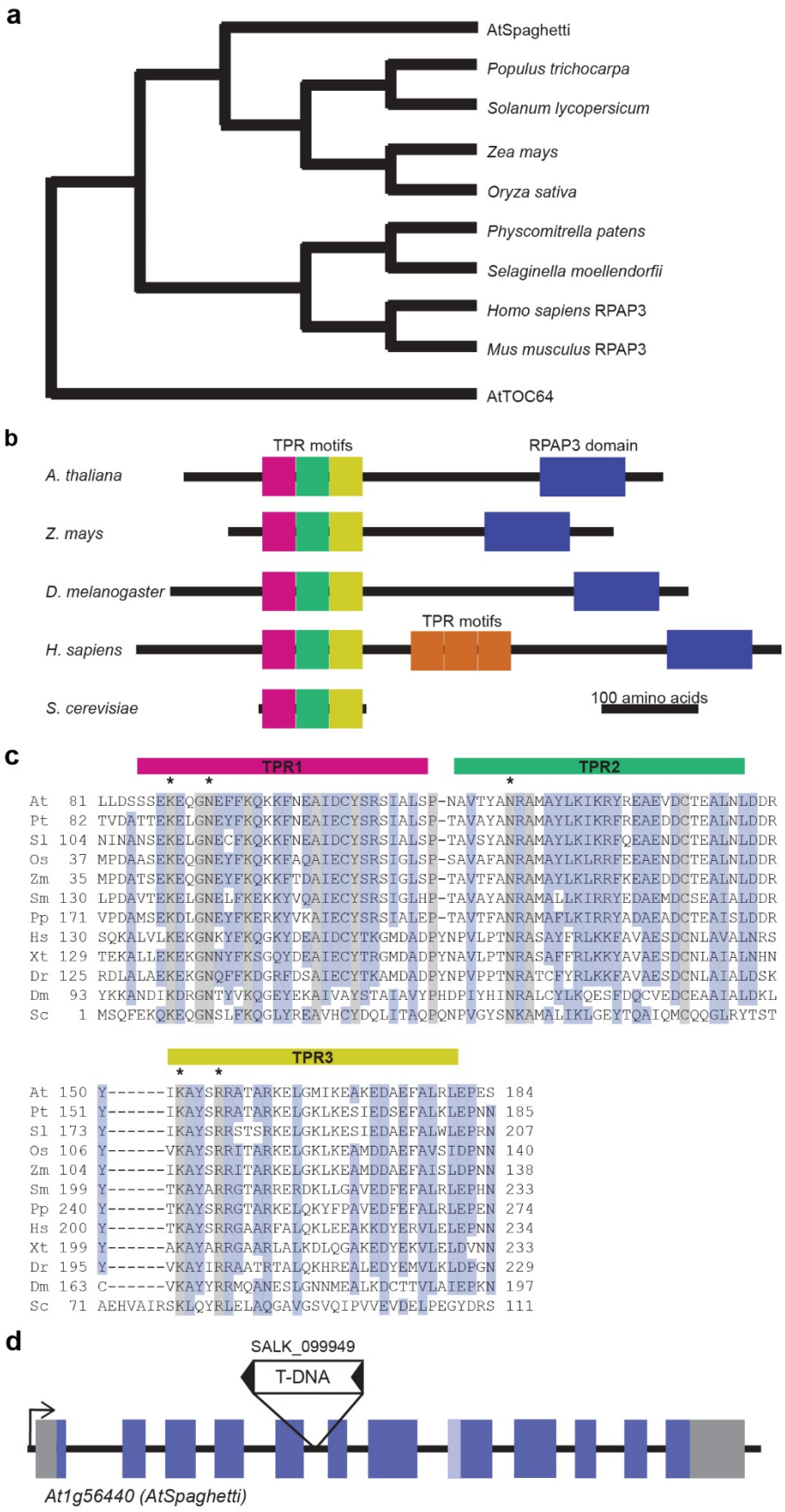
a
AtSpaghetti
Populus trichocarpa
Solanum lycopersicum
Zea mays
Oryza sativa
Physcomitrella patens
Selaginella moellendorfii
Homo sapiens RPAP3
Mus musculus RPAP3
AtTOC64
b
TPR motifs
RPAP3 domain
A. thaliana
Z. mays
D. melanogaster
TPR motifs
H. sapiens
S. cerevisiae
100 amino acids
c
TPR1
TPR2
At 81 LLDSSSEKEQGNEFFKQKKFNEAIDCYSRSIALSP-NAVTYANRAMAYLKIKRYREAEVDCTEALNLDDR
Pt 82 TVDATTEKELGNEYFKQKKFNEAIECYSRSIALSP-TAVAYANRAMAYLKIKRFREAEDDCTEALNLDDR
Sl 104 NINANSEKELGNECFKQKKFNEAIDCYSRSIALSP-TAVSYANRAMAYLKIKRFQEAENDCTEALNLDDR
Os 37 MPDAASEKEQGNEYFKQKKFAQAIECYSRSIGLSP-SAVAFANRAMAYLKLRRFEEAENDCTEALNLDDR
Zm 35 MPDATSEKEQGNEYFKQKKFTDAIECYSRSIGLSP-TAVTFANRAMAYLKLRRFKEAEDDCTEALNLDDR
Sm 130 LPDAVTEKELGNELFKEKKYVQAIECYSRSIGLHP-TAVAYANRAMALLKIRRYEDAEMDCSEAIALDDR
Pp 171 VPDAMSEKDLGNEYFKERKYVKAIECYSRSIALEP-TAVTFANRAMAFLKIRRYADAEADCTEAISLDDR
Hs 130 SQKALVLKEKGNKYFKQGKYDEAIDCYTKGMDADPYNPVLPTNRASAYFRLKKFAVAESDCNLAVALNRS
Xt 129 TEKALLEKEKGNNYFKSGQYDEAIECYTRGMDADPYNAVLPTNRASAFFRLKKYAVAESDCNLAIALNHN
Dr 125 RDLALAEKEKGNQFFKDGRFDSAIECYTKAMDADPYNPVPPTNRATCFYRLKKFAVAESDCNLAIALDSK
Dm 93 YKKANDIKDRGNTYVKQGEYEKAIVAYSTAIAVYPHDPIYHINRALCYLKQESFDQCVEDCEAAIALDKL
Sc 1 MSQFEKQKEQGNSLFKQGLYREAVHCYDQLITAQPQNPVGYSNKAMALIKLGEYTQAIQMCQQGLRYTST
TPR3
At 150 Y------IKAYSRRATARKELGMIKEAKEDAEFALRLEPES 184
Pt 151 Y------IKAYSRRATARKELGKLKESIEDSEFALKLEPNN 185
Sl 173 Y------IKAYSRRSTSRKELGKLKESIEDAEFALWLEPRN 207
Os 106 Y------VKAYSRRITARKELGKLKEAMDDAEFAVSIDPNN 140
Zm 104 Y------IKAYSRRITARKELGKLKEAMDDAEFAISLDPNN 138
Sm 199 Y------TKAYARRGTARRERDKLLGAVEDFEFALRLEPHN 233
Pp 240 Y------TKAYSRRGTARKELQKYFPAVEDFEFALRLEPEN 274
Hs 200 Y------TKAYSRRGAARFALQKLEEAKKDYERVLELEPNN 234
Xt 199 Y------AKAYARRGAARLALKDLQGAKEDYEKVLELDVNN 233
Dr 195 Y------VKAYIRRAATRTALQKHREALEDYEMVLKLDPGN 229
Dm 163 C------VKAYYRRMQANESLGNNMEALKDCTTVLAIEPKN 197
Sc 71 AEHVAIRSKLQYRLELAQGAVGSVQIPVVEVDELPEGYDRS 111
d
SALK_099949
T-DNA
At1g56440 (AtSpaghetti)

Figure S2. *Spaghetti* is conserved in plants (*spaghetti* shown in Figure 1, main text). **a**, Phylogeny of *Spaghetti* orthologues in plants and mammals. **b**, Structural domains of Spaghetti in representative eukaryotic species. The N-terminal TPR motifs responsible for recruiting HSP90 to the R2TP complex are conserved throughout eukaryotes. Plants and metazoan Spaghetti orthologues also contain a C-terminal RPAP3 (RNA Pol II-Associated Protein 3) motif that is unique to Spaghetti in each species. Humans have evolved a second set of TPR motifs (orange), and *S. cerevisiae* has lost the C-terminal RPAP3 domain. **c**, The sequence of the three HSP90-recruiting TPR motifs is well-conserved across eukaryotes. Residues known to promote interaction with HSP90 are shown with an asterisk, and are universally conserved in all Spaghetti sequences. 100% identical residues are highlight in gray, highly conserved residues are highlighted in blue. **d**, Several insertional alleles of *AtSpaghetti* (At1g56440, also called *TPR5*) are available from ABRC. For this study, the previously confirmed null allele (SI ref. 2), SALK_099949, was used. Introns are indicated with black lines, UTRs are indicated in dark gray, coding sequence is indicated in blue, and an alternative splicing acceptor site allows for inclusion of an additional coding sequence (with no apparent functional consequences) indicated in light blue.

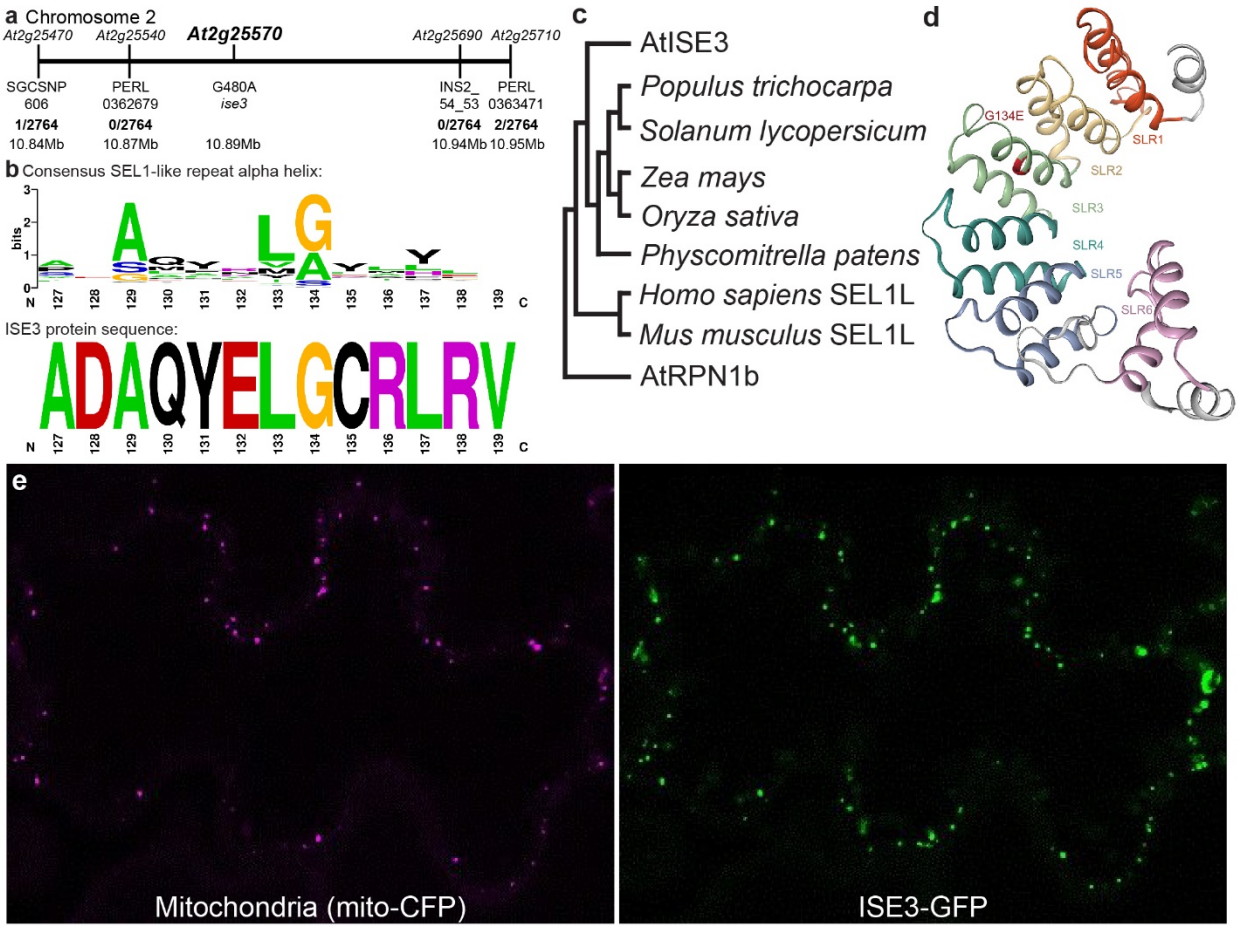

Figure S3. *ise3* is a missense mutation in a mitochondrial SEL1-like repeat protein. **a**, *ise3* was mapped to a 112kb interval on chromosome 2 using SNP and indel markers in an F2 *ise4* (L*er*) x Col-0 mapping population. Frequency of Col-0 alleles in plants with *ise3* phenotypes are indicated beneath the marker names. The position of each marker along chromosome 2 is indicated. *ise3* is a G480A mutation (following numbering of the *At2g25570.1* transcript). **b**, The *ise3* missense mutation, G134E, is in the third SEL1-like repeat (SLR). The consensus sequence for an SLR alpha helix is shown, and is a strongly conserved G or other small, hydrophobic residue at site 134 (following ISE3 amino acid sequence numbering). The G134E substitution is predicted to disrupt this alpha helix and thus the SLR structure of ISE3. **c**, ISE3 is conserved in plants, and its most similar sequence in metazoans is SEL1L, although it does not show global structural similarity to SEL1L and is thus not a true orthologue. **d**, Predicted structure of ISE3 protein, after cleavage of the mitochondrial transit peptide. ISE3 is composed of 6 Sel1-like repeats (SLR1-SLR6, indicated in different colors), of which SLR5 and SLR6 are relatively unstructured. *ise3* is a missense G134E mutation at the consensus SLR glycine/alanine residue in SLR3 (indicated in red). **e**, ISE3-GFP (right panel, GFP in green) and mitochondrial-targeted CFP (left panel, CFP in magenta) both localize to mitochondria in plant cells, confirming published experimental proteomic data and subcellular predictions that ISE3 is a mitochondrial protein.

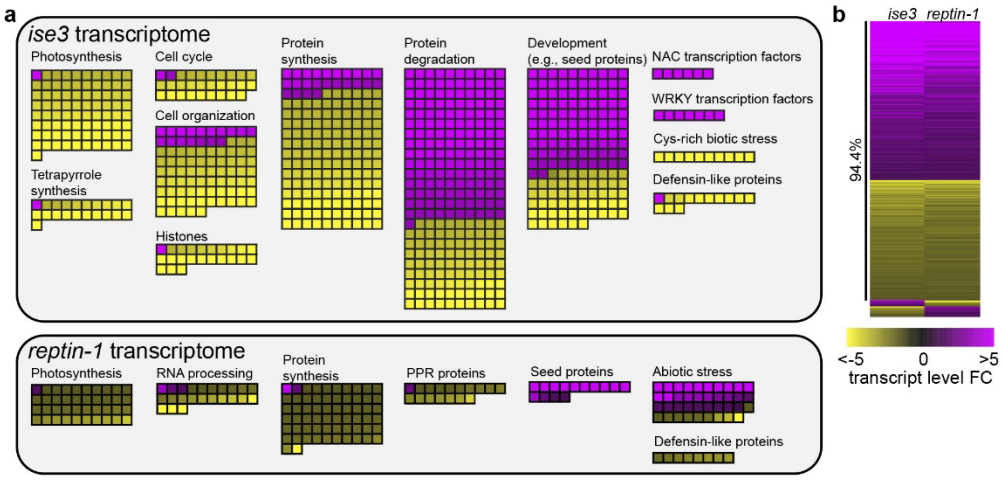

Figure S4. Gene ontology and comparative analysis of the *ise3* and *reptin-1* transcriptomes. **a**, Significantly-affected biological processes revealed by the mutant transcriptomes, as determined using MapMan categories (*SI Appendix,* Dataset 2). Each gene within the category is represented by a single square and color-coded to indicate fold-change in expression (scale bar, lower right). Disrupted processes in *ise3* and *reptin-1* reflect inactivation of TOR signaling. **b**, Pairwise comparisons of *ise3* and *reptin-1* transcriptomes reveal significant overlap ($p < 10^{-100}$) and strong co-regulation of differentially expressed genes (*SI Appendix*, Dataset 9, 10, 11).

Legend of *SI Appendix* Datasets

Dataset 1. Nomenclature of genes described in this study.
Dataset 2. Complete table of transcriptomes used in this study.
Dataset 3. Overlapping DEGs between *reptin-1* and *glc-TOR*.
Dataset 4. Overlapping DEGs between *ise3* and *glc-TOR*
Dataset 5. Altered *RP* gene expression in *ise3* and *reptin-1*.
Dataset 6. Overlapping DEGs between *ise3* and *reptin-1*.
Dataset 7. Overlapping DEGs among aging leaves and *glc-TOR*.
Dataset 8. Overlapping DEGs between L11/L1 and *glc-TOR*.
Dataset 9. Altered *RP* gene expression in aging leaves.
Dataset 10 Oligonucleotides used in this study.
Dataset 11. Sequence homology of *N. benthamiana* VIGS targets.

SI Appendix Reference List

1. N. Sotta, L. Shantikumar, T. Sakamoto, S. Matsunaga, T. Fujiwara, TPR5 is involved in directional cell division and is essential for the maintenance of meristem cell organization in Arabidopsis thaliana. *J. Exp. Bot.* **67**, 2401–2411 (2016).
2. T. Durfee, O. Draper, J. Zupan, D. Conklin, P. Zambryski, New tools for protein linkage mapping and general two-hybrid screening. *Yeast* **15**, 1761–1768 (1999).