Supporting Information

Stonebloom et al. 10.1073/pnas.0909229106

SI Materials and Methods

Mapping of the *ise1* Locus. The accuracy of a genetic map is largely dependent on the size of the mapping population and the distances between particular markers being tested. At the time of our analysis, only a limited number of SNPs between the Columbia (Col) and Landsberg *erecta* (Ler) ecotypes were available. Therefore, new SNPs were discovered by denaturing HPLC during the course of chromosome walking to *ISE1*. Homozygous wild-type F2 individuals were used for mapping, because homozygous *ise1* seedlings do not survive on soil and thus are not easily available for DNA extraction. Genomic DNA from 824 F2 wild-type individuals containing the reciprocal products of meiotic recombination were isolated and tested with SNP markers. The SNP markers flanking *ise1*, T12C24.64400 and F13K23.66000, are 106.7 kb apart.

To further fine-map the *ise1* locus within the 106.7-kb region, small amplicons (500 bp) with 50-bp overlaps were PCR-amplified from genomic DNA from heterozygous plants and then tested on DHPLC to find one amplicon that generated a heteroduplex. All amplicons except for the one containing the *ise1* mutant allele were expected to appear as homoduplexes on DHPLC. Indeed, one heteroduplex amplicon was detected and subsequently sequenced to discover the *ise1–1* lesion.

During this time, Syngenta was developing T-DNA tagged lines, and once *ise1–1* was localized to a BAC, we crossed *ise1–1* to two T-DNA insertions in locus *EMB1586* (At1g12770) (1–3) (http://www.seedgenes.org). Indeed, genetic complementation tests confirmed allelism between *ise1–1* and *EMB1586*. Molecular mapping of the T-DNA insertions at EMB1586 by Syngenta greatly facilitated the identification of the *ise1–1* locus.

Note that the annotation of *ISE1* (At1g12770) in the *Arabidopsis* genome database is now correct. However, in previous versions the locus annotated At1g12770 included *ISE1* and the immediately downstream gene that encodes a pentatricopeptide repeat, now called At1g12775, as a single locus. The start site of this latter gene is 362 bp downstream of the stop codon of *ISE1* (At1g12770). The cDNA and EST data support the newer gene model. Other databases, for example, that provide RNA expression information based on microarray data, such as Genevestigator, are not correct, because they present data based on the earlier incorrect annotation (as determined by the primers used in the analyses), but the data are labeled At1g12770.

Phylogenetic Analysis. The ISE1-related protein sequences were retrieved by pBLAST and PSI-BLAST searches of plant, mouse, and yeast sequences in GenBank. *Nicotiana benthamiana* homologs of *ISE1* were determined by designing primers based on the *ISE1* homolog in *Solanum lycopersicum*. The 5′ and 3′ ends of *N. benthamiana ISE1* homologs were amplified by a SMART RACE cDNA Amplification Kit (Clontech), then cloned and sequenced. Peptide sequences of all DEAD-box RNA helicases from *Arabidopsis thaliana* were retrieved from GenBank. Sequences were aligned using the MUSCLE algorithm with the default settings in Geneious (Biomatters). This alignment was used to generate a maximum parsimony tree with bootstrap values with PAUP* 4.0 (Sinauer Associates) under default conditions.

Molecular Cloning. The GFP fusions to ISE1 were generated using Gateway technology (Invitrogen). The coding sequence of *ISE1* was amplified from *Arabidopsis* genomic DNA from the Landsberg *erecta* ecotype and TOPO-cloned into the Gateway entry vector pENTR/D-TOPO (Invitrogen). An N-terminal GFP fusion to ISE1 was made by recombining the coding sequence of *ISE1* into the binary expression plasmid pMDC43 (4). The C-terminal ISE1-GFP was constructed by recombining *ISE1* (with the stop codon eliminated) into pMDC83 (4). To express these GFP fusions at endogenous levels, the 35S promoter in each plasmid was replaced with the endogenous *ISE1* promoter region (*ISE1p*) from nucleotides −1 to −1825. The *ISE1* promoter (*pISE1*) was PCR-amplified and cloned into pCR-Blunt-II-TOPO (Invitrogen). The 35S promoter in the GFP-ISE1 plasmid was replaced with *ISE1p* using the PstI and BamHI sites. We then replaced the BamHI site of the pCR-Blunt-II-TOPO clone with PstI and then used PstI to replace the 35S promoter in the 35-S::ISE1-GFP binary plasmid with *ISE1p*.

Mitochodria-targeted CFP was constructed by cloning the first 255 nt of the F1 ATPase γ amplified from *Arabidopsis* genomic DNA into pENTR1A (Invitrogen). A recombination was performed to place the mitochondrial transit sequence into the C-terminal CFP-fusion plant expression plasmid pGWB44 (5). To construct 100-ISE1-GFP, the first 300 nt of the ISE1 coding sequence were cloned into pENTR/D-TOPO and then recombined into the binary expression plasmid pMDC84 (4).

Expression Analysis. Semiquanititative RT-PCR analysis was performed for analysis of ISE1 transcript levels in *Arabidopsis thaliana* tissues and *ISE1*-silenced *N. benthamiana* plants. The RNA was prepared from 100 mg of each tissue using the RNEasy MINI kit (Qiagen). RNA extracts were treated with DNA-free (Ambion) before reverse transcription. Reverse transcriptase reactions were performed on 2 μg of RNA per sample using SuperScript II reverse transcriptase (Invitrogen) with a polyT primer following the manufacturer's instructions. The PCR was performed following standard procedures, and cycle number was adjusted to capture amplification during the exponential amplification phase. Semiquantitative PCR analyzing the expression of *ISE1* in *Arabidopsis* tissues was performed on RNA extracts prepared from various organs of wild-type *Arabidopsis thaliana* plants of the Colombia ecotype. Actin-2 was amplified as a loading control with the forward primer 5′-GGAAACATCGTTCTCAGTGG-3′ and reverse primer: 5′-ACCAGATAAGACAAGACACAC-3′. *ISE1* was amplified with the forward primer 5′-TCAAAGATGTGGTCTACAAACTC-3′ and reverse primer 5′-AACAAACTCACAATACAAGAAAGG-3′.

To confirm silencing of both ISE1 homologs in *N. benthamiana*, RT-PCR was performed with RNA extracted from *ISE1*- silenced *N. benthamiana* or tobacco-rattle-virus-infected, nonsilenced plants. Forward primers were used designed to amplify the ISE1a 5′-GAGTCAAATAGTCCAAATAGTCCAC-3′ or ISE1b 5′-GGGGAGAGTCAAGTAGTCCAC-3′ homolog specifically. A common reverse primer was used to amplify both ISE1 homologs in *N. benthamiana*: 5′-AAGCTATCACACACTTGGCATC-3′.

1. McElver J, et al. (2001) Insertional mutagenesis of genes required for seed development in *Arabidopsis thaliana*. *Genetics* 159:1751–1763.
2. Tzafrir I, et al. (2003) The *Arabidopsis* SeedGenes Project. *Nucleic Acids Res* 31:90–93.
3. Tzafrir I, et al. (2004) Identification of genes required for embryo development in Arabidopsis. *Plant Physiol* 135:1206–1220.
4. Curtis MD, Grossniklaus U (2003) A gateway cloning vector set for high-throughput functional analysis of genes in planta. *Plant Physiol* 133:462–469.
5. Nakagawa T, et al. (2007) Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. *J Biosci Bioeng* 104:34–41.

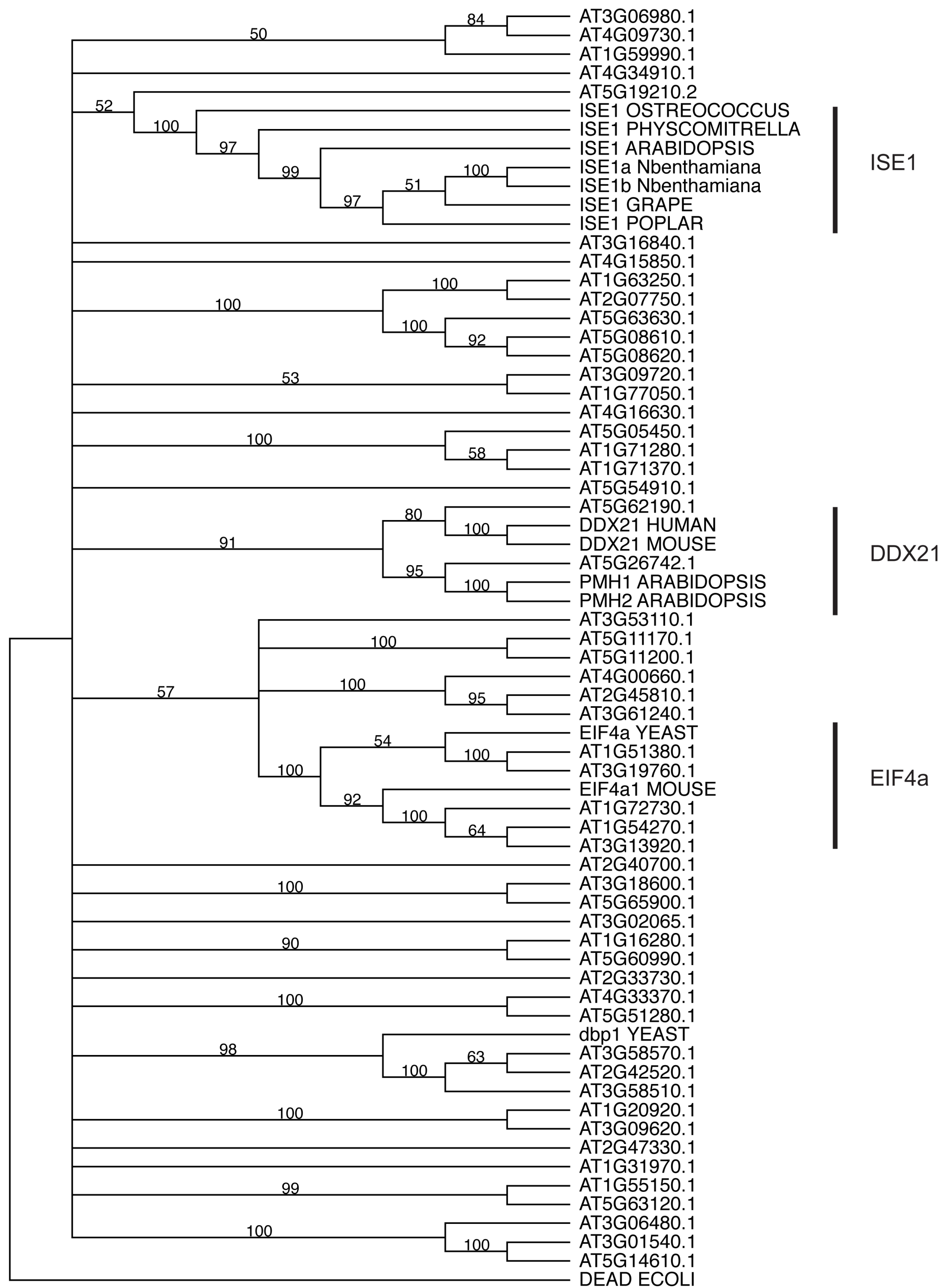

Fig. S1. ISE1 is a plant-specific DEAD-box RNA helicase. All 47 DEAD-box RNA helicases from *Arabidopsis thaliana* and the ISE1-related proteins in yeast and mouse were included in our analysis. Eukaryotic initiation factor 4a from yeast and mouse is most similar to ISE1; however, these proteins have true homologs in Arabidopsis. ISE1 (At1g12770.1) and its homologs in other green plants and algae form a well-supported clade. Note that there is no resolution at the base of the tree, because only the strictly conserved regions of the DEAD-box RNA helicase domains are shared among these proteins.

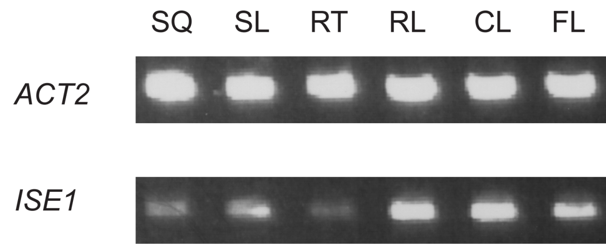

Fig. S2. ISE1 is expressed in all organs in *Arabidopsis*. Semiquantitative RT-PCR detects *ISE1* transcripts in RNA samples from siliques (SQ), whole seedlings (SL), roots (RT) rosette leaves (RL) cauline leaves (CL), and flowers (FL). *ISE1* transcript levels are the highest in leaves and flowers. *ACTIN2* (*ACT2*) transcript was amplified as a control.

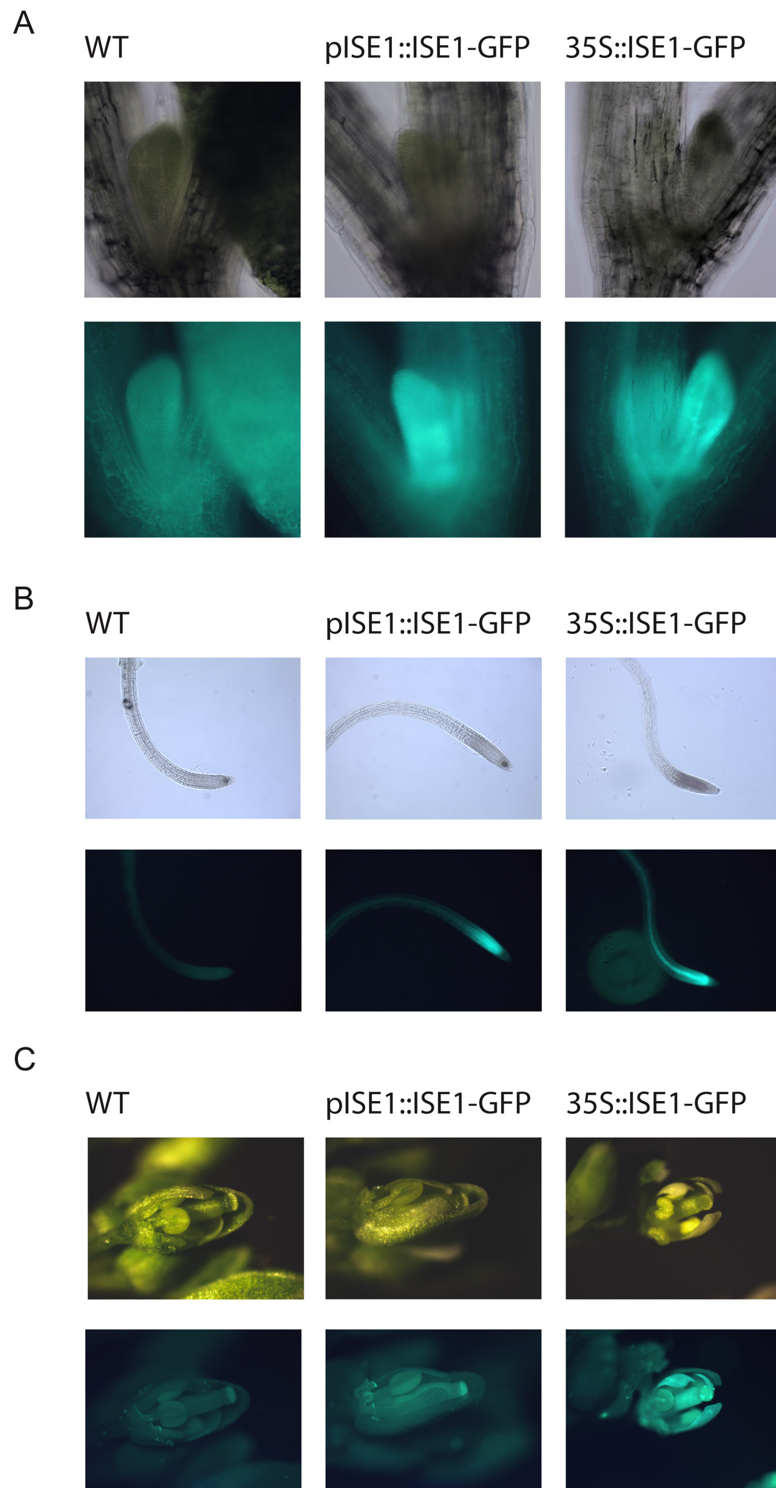

Fig. S3. ISE1 is highly expressed in young tissues. Comparison of ISE1-GFP expression driven by the endogenous *ISE1* promoter or the cauliflower mosaic virus 35S promoter in rescued *ise1–3* mutants in young leaves (*A*), roots (*B*), and flowers (*C*). Wild-type (WT) panels show background fluorescence.

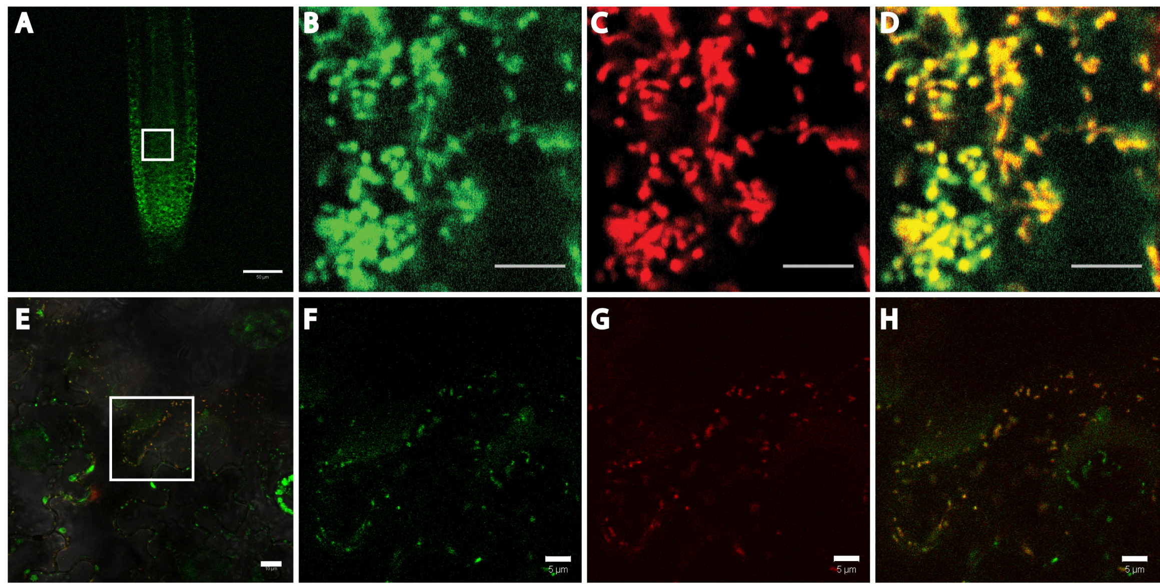

Fig. S4. ISE1 localizes to mitochondria in *Arabidopsis* seedlings and *Nicotiana benthamiana*. (*A*) Green fluorescence of seedling roots expressing ISE1-GFP. The square in *A* is enlarged in *B–D*. (*B*) Green fluorescence of ISE1-GFP. (*C*) MitoTracker Red stain. (*D*) Colocalization of ISE1-GFP and MitoTracker Red. ISE1-GFP (*E*, *F*) and mitochondria-targeted CFP (*E*, *G*) were coexpressed in *N. benthamiana*, and *H* reveals their colocalization. The square region in *E* is shown enlarged in *F–H*. (Scale bars, *A*, 50 μm; *E*, 10 μm; *B–D*, *F–H*, 5 μm.)

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CLUSTAL 2.0.10 multiple sequence alignment


ISE1a_Nbenth        MPVLVLTRALLLVGDSLSLRNVSQFTRIAPFRDKVRFLS----DSGSLTLASLGLKSEVK 56
ISE1b_Nbenth        MPVLVLTRALLLVGDSLFLRNVSPFTRIAPFRDKVGFLS----DSGSLTLASLGLKNEVK 56
ISE1_Slyco          MPALVLTRALLLIGDSLAFRKATQFTRIAPSQGNVRFLS----SSGSLTLASLGLKSEVK 56
ISE1_Athaliana      MAASTSTRFLVLLKDFSAFRKISWTCAATNFHRQSRFLCHVAKEDGSLTLASLDLGNKPR 60
                    *.. . ** *:*: *   :*: :     :  : :  **.    ..********.* .: :

ISE1a_Nbenth        TVSANEKNKLQQGVSTIEVPKSRVKKKVSGNKTGLIKERNPIDIKTAPFAAESFSELGPP 116
ISE1b_Nbenth        TVGANEKDKLRQGVSTIEVPKSRVKKKVSGNKTGLIKERNPIDIKTAPFAAESFSELGLP 116
ISE1_Slyco          TVSANEKNKLQQGISTIEVPKSREKKRVSGNKQGLVKEKNPVDIMTAPFAAKSFSELGLP 116
ISE1_Athaliana      KFGKGKAMKLEG-SFVTEMGQGKVR-AVKNDKMKVVKEKKPAEIVSPLFSAKSFEELGLP 118
                    ... .:  **.    . *: :.: :  *..:*  ::**::* :* :. *:*:**.*** *

ISE1a_Nbenth        PLLVERLEEEGFTVPTDVQAAAVPTVLKNHDVVIQSYTGSGKTLAYVLPILSQAGPLSG- 175
ISE1b_Nbenth        PLLVERLEKEGFTIPTDVQAAAVPTVLKNHDVVIQSYTGSGKTLAYVLPILSQVGPLSG- 175
ISE1_Slyco          PLLVERLETEGFTIPTDVQAAAVPTVLKNHDVVIQSYTGSGKTLAYLLPILSRVGPLKE- 175
ISE1_Athaliana      DSLLDSLEREGFSVPTDVQSAAVPAIIKGHDAVIQSYTGSGKTLAYLLPILSEIGPLAEK 178
                      *:: ** ***::*****:****:::*.**.**************:*****. ***

ISE1a_Nbenth        EISNGCETGNKVGIEAVIVAPSRELGMQIVREVEKLLGPADKKLVQQLVGGANRSRQEEA 235
ISE1b_Nbenth        EISNGSETGNKAGIEAVIVAPSRELGMQIVRELEKLLGPADKKLVQQLVGGANRSRQEEA 235
ISE1_Slyco          ELPDGYETGNKIDIEAVIVAPSRELGMQIVREVEKLLGPANKKLVQQLVGGANRSRQEDA 235
ISE1_Athaliana      SRSSHSENDKRTEIQAMIVAPSRELGMQIVREVEKLLGPVHRRMVQQLVGGANRMRQEEA 238
                    . ..   *..::  *:*:***************:******..:::********** ***:*

ISE1a_Nbenth        LRKNKPAIVVGTPGRIAEISAAGKLPTHGCRYLVLDEVDQLLAVNFREDMQRILDHVGRR 295
ISE1b_Nbenth        LRKNKPAIVVGTPGWIAEISAAGKLPTHGCCYLVLDEVDQLLAVNFREDMQRILDHVGRR 295
ISE1_Slyco          LKKNKPDIVVGTPGRIAEISAAGKLPTHGCRYLVLDEIDQLLAFTFREDMKRILDHVGRR 295
ISE1_Athaliana      LKKNKPAIVVGTPGRIAEISKGGKLHTHGCRFLVLDEVDELLSFNFREDIHRILEHVGKR 298
                    *:**** ******* ***** .*** **** :*****:*:**:..****::***:***:*

ISE1a_Nbenth        PGAS-GGESNSPNSPLVKRSARQTIMVSATVPFSVIRAARSWGCDPLLVQANKVVPLESV 354
ISE1b_Nbenth        PGAC-RGESSSP---LVKRSARQTIMVSATVPFSVIRAARSWGCNPLLVQANKVVALESV 351
ISE1_Slyco          PGAR-GGESNSP---LVKRAARQTIMVSATVPFSVIRAARSWGCDPLLIQANKVVPLESV 351
ISE1_Athaliana      SGAGPKGE-------VDERANRQTILVSATVPFSVIRAAKSWSHEPVLVQANKVTPLDTV 351
                    .**   **        : :*: ****:*************:**. :*:*:*****..*::*

ISE1a_Nbenth        PPSGPVNISGTPPTASSSSNVQAMPSVQSLPPNLQHYYTITRIQHKVDMLRRCVHALDAK 414
ISE1b_Nbenth        PSSGPVNISGTPPTASSSSNVQAMPSVQSLPPNLQHYYTITRIQHKVDMLRRCVHALDAK 411
ISE1_Slyco          TPSGSVNIPGTPSTTDSSSSVQPMPDVQSLPPNLQHYYTITRIQHKVDMLRRCVHALDAK 411
ISE1_Athaliana      QPSAPV-MSLTPTTSEADGQIQTT--IQSLPPALKHYYCISKHQHKVDTLRRCVHALDAQ 408
                     .*..* :. **.*:.:...:*.   :***** *:*** *:: ***** **********:

ISE1a_Nbenth        CVIAFMNHTKQLKDAVFKLEARGMKAAELHGDLSKLVRSTILKKFRNGEVRVLLTNELSA 474
ISE1b_Nbenth        CVIAFMNHTKQLKDAVFKLEARGMKAAELHGDLSKLVRSTILKKFRNGEVRVLLTNELSA 471
ISE1_Slyco          CVIAFMNHTKQLKDAVFKLEARGMNAAELHGDLSKLVRSTILKKFRNGEIRVLLTNELSA 471
ISE1_Athaliana      SVIAFMNHSRQLKDVVYKLEARGMNSAEMHGDLGKLGRSTVLKKFKNGEIKVLVTNELSA 468
                    .*******::****.*:*******::**:****.** ***:****:***::**:******

ISE1a_Nbenth        RGLDLPECDLVVNLGLPTDSVHYAHRAGRTGRLGRKGTVVTICEDPEVFVVKKLQKQLSL 534
ISE1b_Nbenth        RGLDLPECDLVVNLGLPTDSVHYAHRAGRTGRLGRKGTVVIICEEPEVFVVKKLQKQLSL 531
ISE1_Slyco          RGLDLPECDLVVNLGLPTDSVHYAHRAGRTGRLGRKGTVVTICEESEVFVVKKLQKQLSL 531
ISE1_Athaliana      RGLDVAECDLVVNLELPTDAVHYAHRAGRTGRLGRKGTVVTVCEESQVFIVKKMEKQLGL 528
                    ****:.******** ****:********************  :**:.:**:***::***.*

ISE1a_Nbenth        SIQACEFSDGNLVITED------ 551
ISE1b_Nbenth        SIQACEFSDGNLVITED------ 548
ISE1_Slyco          SIEACEFSEGNLVIIED------ 548
ISE1_Athaliana      PFLYCEFVDGELVVTEEDKAIIR 551
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Fig. S5. Multiple sequence alignment of *Arabidopsis thaliana* ISE1 and ISE1 homologs in *Solanum lycopersicum and Nicotiana benthamiana*. There are two homologs of ISE1 in *N. benthamiana* and only one in the closely related *S. lycopersicum*. *Arabidopsis thaliana* ISE1 is 59% identical to ISE1a in *N. benthamiana* and 61% identical to the *S. lycopersicum* homolog. *Nicotiana benthamiana* ISE1a and ISE1b are 95% identical.

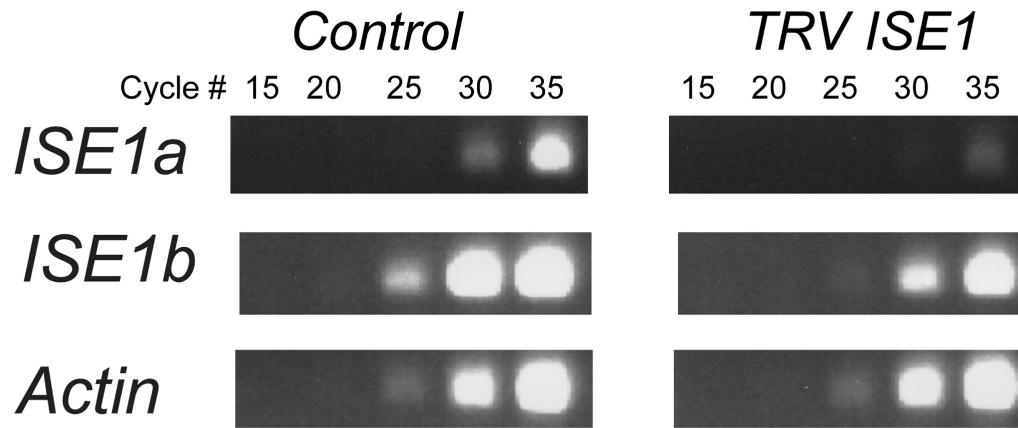

Fig. S6. *Nicotiana benthamiana* ISE1 homologs are silenced by viral-induced gene silencing (VIGS). Semiquantitative RT-PCR with gene-specific primers for the ISE1 homologs in *N. benthamiana* after induction of VIGS shows that transcripts of both *ISE1* homologs are significantly reduced in *ISE1*-silenced plants (*TRV-ISE1*) compared with tobacco rattle virus (TRV)-infected, unsilenced plants (*TRV-GUS*). Samples were collected after 15, 20, 25, 30, and 35 PCR cycles.