Supporting Information

Yuan et al. 10.1073/pnas.0906869106

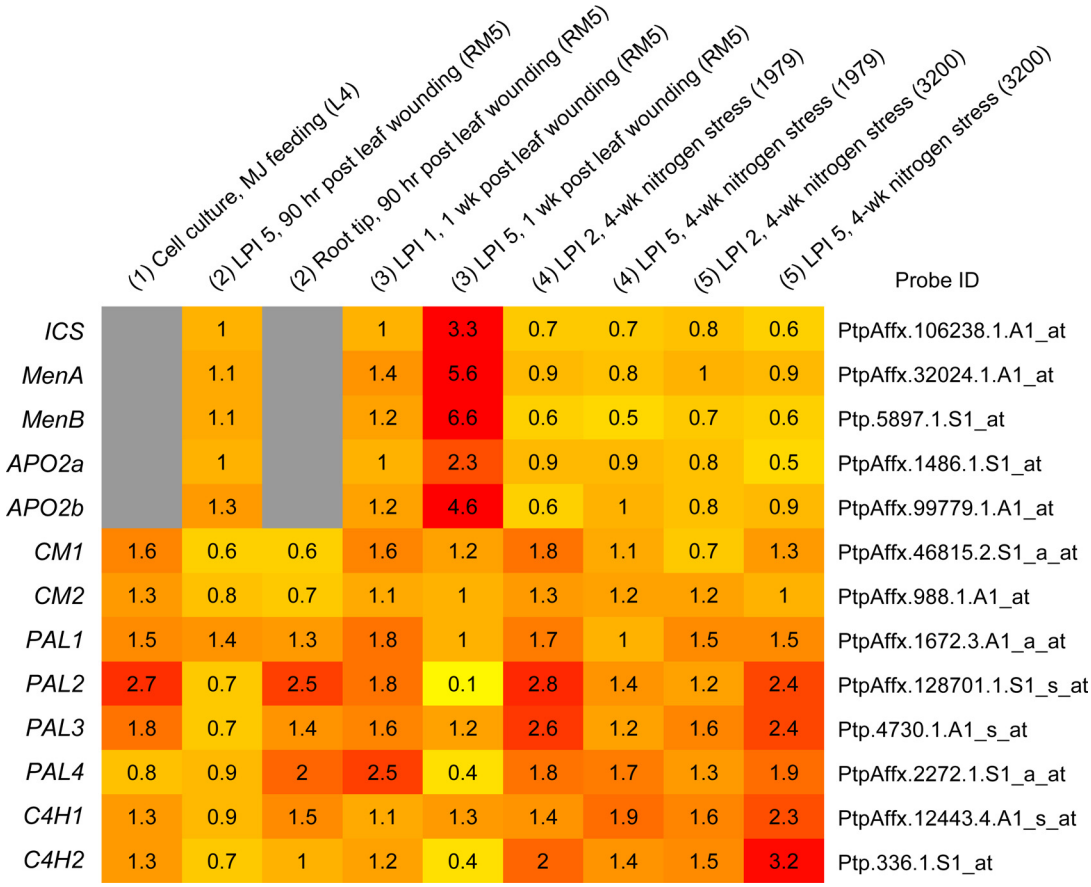

Fig. S1. Heat map showing that *Populus ICS* is coregulated with orthologs of *Arabidopsis* genes involved in PhQ biosynthesis and PSI function, but not with phenylpropanoid pathway genes. Gene expression ratios (treatment over the respective control) were obtained from Affymetrix GeneChip analysis, and visualized using the HeatMapperPlus Tool (http://www.bar.utoronto.ca/ntools/cgi-bin/ntools_heatmapper_plus.cgi). MenA, 1,4-dihydroxy-2-naphthoate phytyltransferase; MenB, 1,4-dihydroxy-2-naphthoyl CoA synthase; APO2, accumulation of photosystem one 2; CM, chorismate synthase; PAL, phenylalanine ammonia-lyase; and C4H, cinnamate 4-hydroxylase (corresponding probe ID shown on the right). Data were extracted from the following GEO series: (1) *Populus tremuloides* (clone L4) cell culture response to 25 μM methyl jasmonate (MJ) feeding (GenBank accession no. GSE16786); (2) and (3) *Populus fremontii* x *angustifolia* (clone RM5) leaf wounding responses: (2) leaves from leaf plastchron index (LPI) 2 and root tips were analyzed 90 h after wounding (accession no. GSE16785), whereas (3) LPI1 and LPI5 were used 1 week after wounding in a separate experiment (GenBank accession no. GSE16783); (4) and (5) *Populus fremontii* $\times$ *angustifolia* response to nitrogen stress: LPI2 and LPI5 from clone 1979 (4) and clone 3200 (5) were analyzed 4 weeks after the start of the nitrogen stress treatment (GenBank accession nos. GSE14515 and GSE14893). Gray color indicates gene expression was below detection in both treatment and control samples.

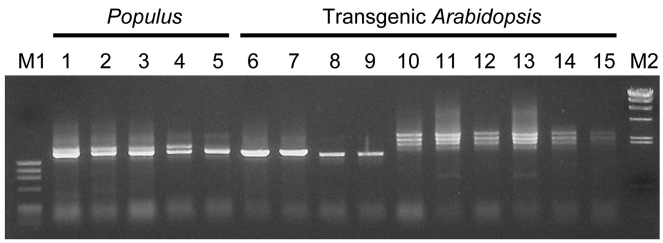

Fig. S2. RT-PCR amplification of *ICS* from various *Populus* genotypes (lanes 1–5) and transgenic *Arabidopsis* lines (lanes 6–15). Lanes 1–3: three different *P. fremontii* × *angustifolia* hybrid genotypes; lane 4: *P. trichocarpa*; lane 5: *P. tremuloides*; lanes 6 and 7: two independent transgenic *Arabidopsis sid2–2* lines harboring the full-length *PtiICS* cDNA; lanes 8 and 9: two independent transgenic *Arabidopsis sid2–2* lines harboring *PtiICS* cDNA without the predicted plastid-targeting presequence; lanes 10–13: independent transgenic *Arabidopsis* lines carrying the 6-kb genomic *PtiICS* gene under control of the CaMV 35S promoter in the *sid2–2* mutant (10 and 11) or Col-0 background (12 and 13); lanes 14 and 15: two independent transgenic *Arabidopsis sid2–2* lines harboring the 8-kb *PtiICS* gene in the *sid2–2* mutant background. M1 and M2: molecular markers PhiX174-HaeIII and Lambda-HindIII, respectively.

Fig. S3. Alignment of representative *Pupulus ICS* 3′ UTR sequences showing alternative polyadenylation patterns. Translational stop codon is shown in boldface. Clone N12 represents the predominant transcript type (6 of 9) among the sequenced clones.

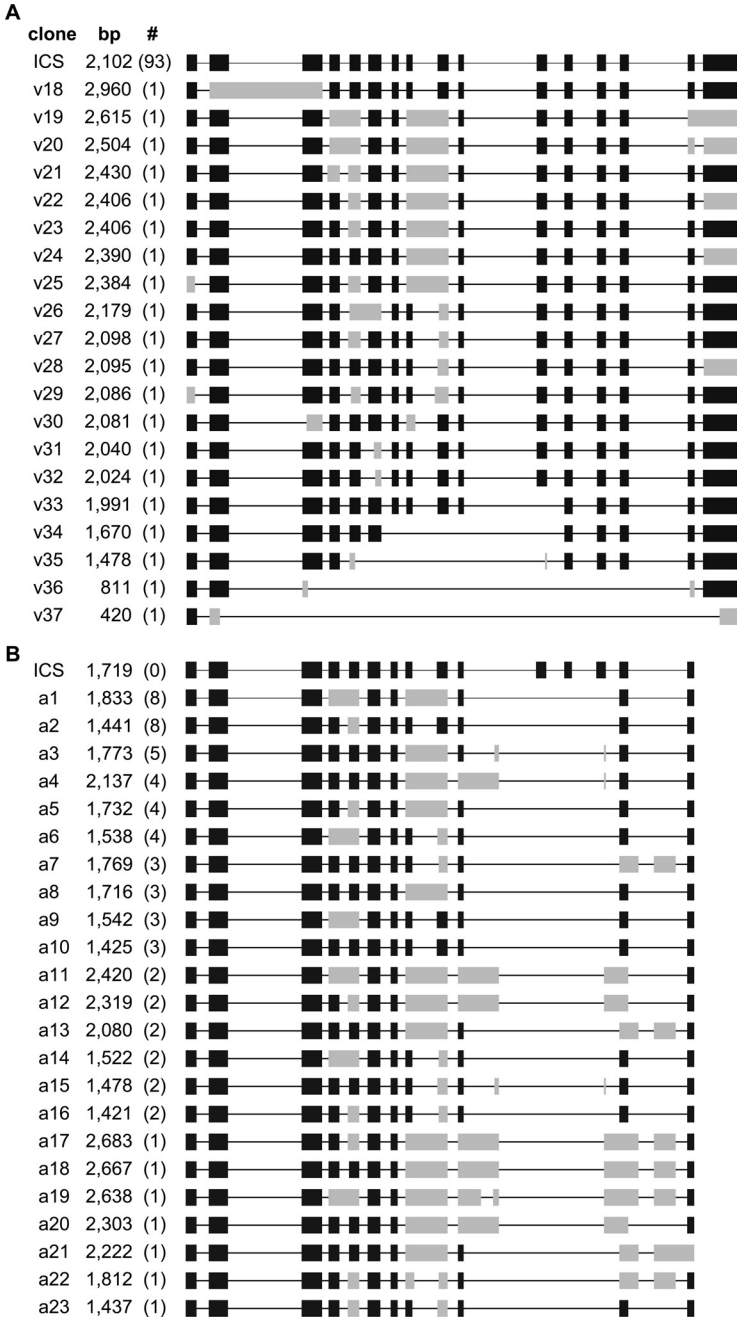

Fig. S4. Additional *Populus ICS* splice variants not shown in Fig. 3. (*A*) Splice variants from *Populus* leaf RT-PCR with a single occurrence among the 184 sequenced clones. (*B*) Representative splice variants recovered from transgenic *Arabidopsis* expressing the 6-kb genomic *PtiICS* under control of the 35S promoter. Clones are arranged in order of abundance (frequencies in parentheses), with the normal *ICS* transcript shown at the top. A total of 64 RT-PCR clones were sequenced. Exons affected by alternative splicing are shown in gray.

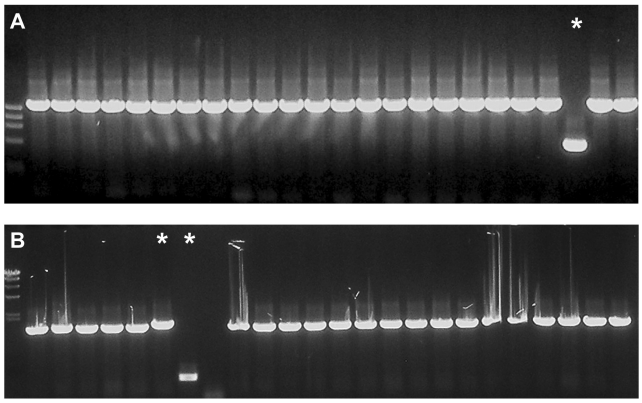

Fig. S5. Colony PCR of *AtICS1* and *AtICS2* RT-PCR products cloned into the pCRII-TOPO vector. Of the 24 *AtICS1* (*A*) and 23 *AtICS2* (*B*) recombinant clones, one and two were found to be splice variants (denoted by asterisks), respectively. Molecular weight markers used were PhiX174- HaeIII in (*A*) and Lambda-HindIII in (*B*).

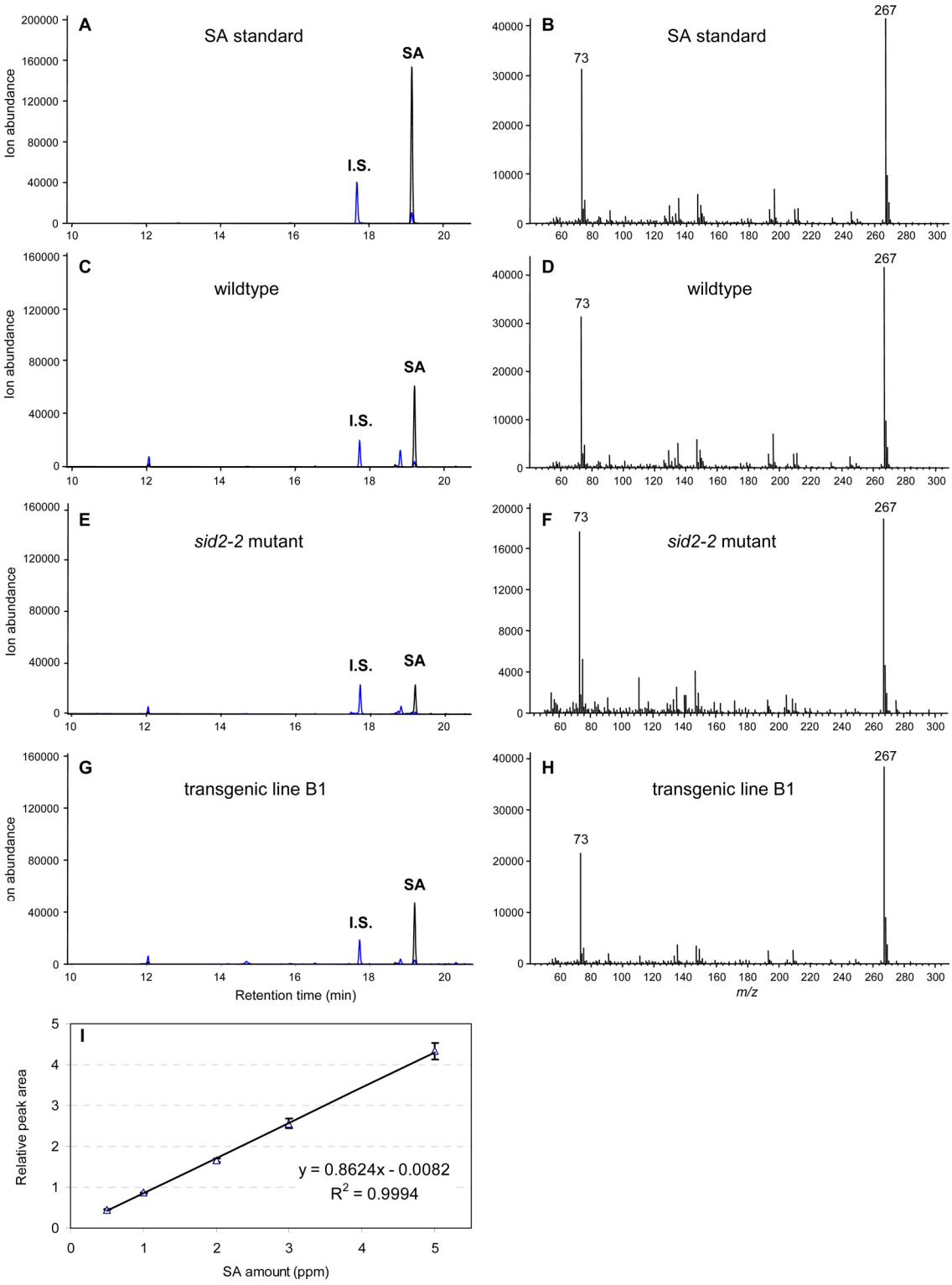

Fig. S6. Quantification of SA in *Arabidopsis* leaves by GC-MS. Representative selected ion chromatograms of silylated SA (*m/z* 267, black trace) and internal standard (I.S.) *o*-anisic acid (*m/z* 209, blue trace) are shown on the left (*A*, *C*, *E*, and *G*), and mass spectra of silylated SA on the right (*B*, *D*, *F*, and *H*). (*A* and *B*) Authentic SA standard; (*C* and *D*) wild-type; (*E* and *F*) *sid2–2* mutant; and (*G* and *H*) transgenic line B1 harboring the full-length *PtiICS* cDNA under control of the 35S promoter. (*I*) Calibration curve for SA. Each symbol represents means ± SD of three independent measurements.

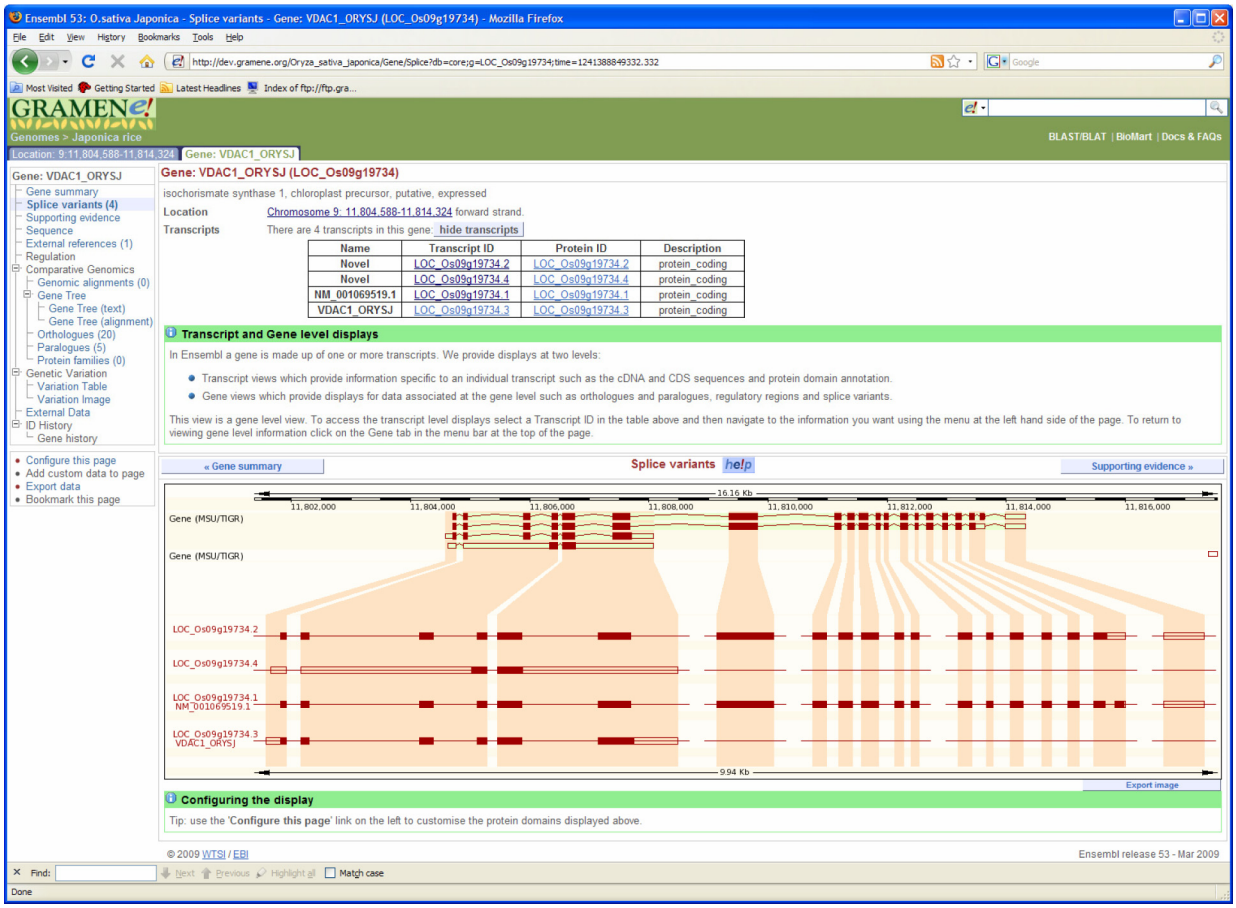

Fig. S7. Evidence of alternative splicing in rice *OsICS* based on EST/cDNA sequences. Graph was obtained from the Gramene Rice Genome Browser (www.gramene.org/).

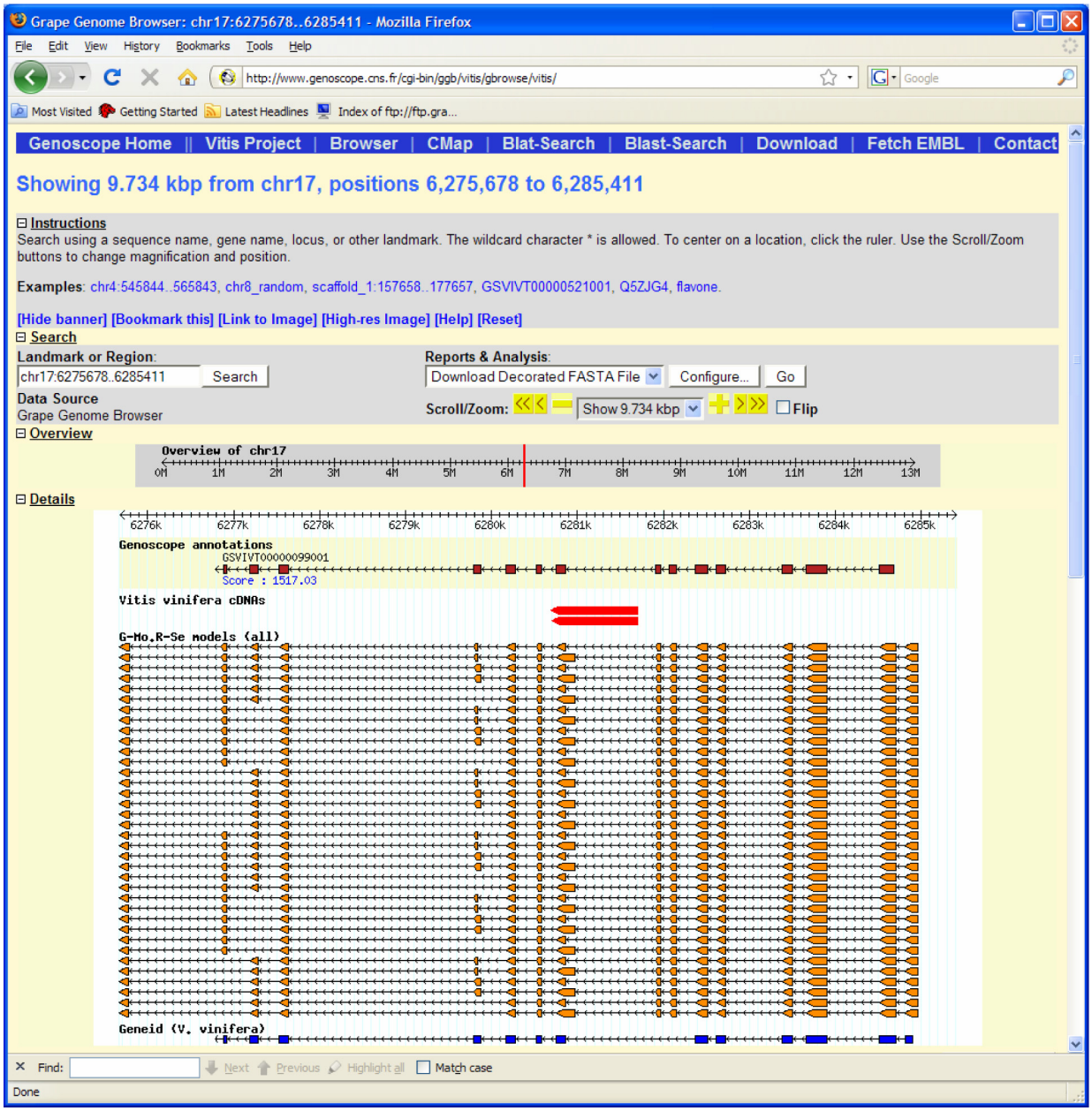

Fig. S8. Evidence of alternative splicing in grape *VvICS* based on G-MO.R-Se assembly and de novo gene model building using RNA-Seq data [Denoeud et al. (2008) *Genome Biol* 9:R175]. Graph was obtained from the Grape Genome Browser (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/).

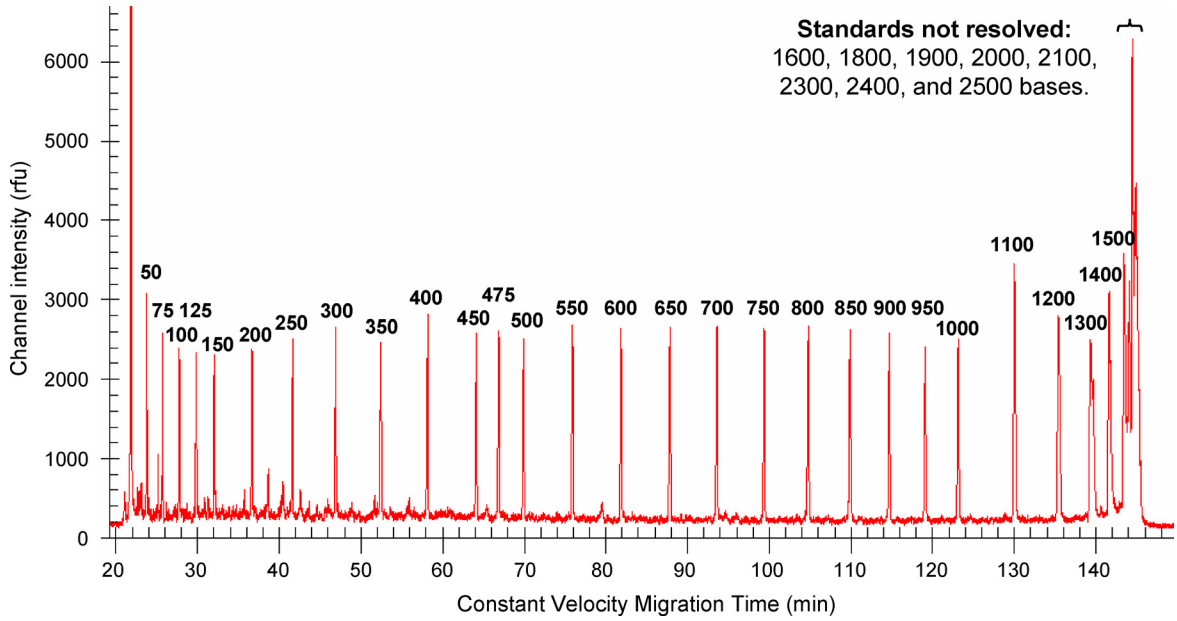

Fig. S9. Limitation of DNA fragment sizing by an automatic DNA sequencer. A representative chromatogram of DNA size standards, ranging in size from 50 bp to 2,500 bases, analyzed with a Beckman CEQ8000 Genetic Analyzer. Fluorescence (Well Red D1)-labeled DNA size standards were custom synthesized by BioVentures, Inc., and include the following sizes: 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1800, 1900, 2000, 2100, 2300, 2400, and 2500 bases. The samples were analyzed using the "Fragment Analysis" module with the run time set at 3 hours, and the voltage set at 3.5 kV. Fragment sizes are indicated above the corresponding peaks, except for those that could not be resolved (>1,500 bases, marked by a bracket).

Table S1. List of primers used in various PCR and RT-PCR amplifications

Primer name	Sequence (5′ to 3′)	Application
PtiICSF1	ATGGCAACCGCTACTCTAGC	RT-PCR for *PtiICS*, PCR for 6-kb gene construct
PtiICSR3	TCAGTTGATGATTCCTGAGTTCTC	RT-PCR for *PtiICS*, PCR for 6-kb gene construct
PtiICSF1	Same as above	RT-PCR for *PtiICS* used in clone sequencing
PtiICSexon16R	GGGGAAACATTATCAAATTGC	RT-PCR for *PtiICS* used in clone sequencing
PtiICSF2noplstid	ATGGCTAATGGCTGCCAAGGAAAC	RT-PCR for mature *PtiICS*
PtiICSR3	Same as above	RT-PCR for mature *PtiICS*
PtiICSgF	ACGTCGACTAATGTTAAGAAAGAATTTGGCTTG	PCR for 8-kb gene construct
PtiICSgR	ATGGTACCTAATTCAGACAGATCAGCAACAAA	PCR for 8-kb gene construct
PtiICSF1noplastidNcoI	CCATGGCTAATGGCTGCCAAGGAAAC	*E. coli* complementation
PtiICSR3	Same as above	*E. coli* complementation
AtICS1F1	ATGGCAACTGCTGTTTTATCTCCGGCAGC	RT-PCR for *AtICS1*
AtICS1R2new	TCAATTAATCGCCTGTAGAGATG	RT-PCR for *AtICS1*
AtICS1F1noplastidNcoI	CCATGGCTTCTATGAATGGTTGTGATGGA	*E. coli* complementation
AtICS1R2new	Same as above	*E. coli* complementation
AtICS2F1	ATGGCAAACGGATGTGAGGCTGACCA	RT-PCR for *AtICS2*
AtICS2R2	TTAGTTGATTGGTTGCAAAGC	RT-PCR for *AtICS2*
EntCFNcoI	CCATGGCAAATCCGTCCCCTCGCCTTTG	*E. coli* complementation
EntCRstop	TTAATGCAATCCAAAAACGTTCAACA	*E. coli* complementation