Supporting Information

Doblin et al. 10.1073/pnas.0902019106

SI Results

Barley Has Only a Single *CSLH* Gene. A BAC library screening was used to obtain a complete set of full-length *HvCSLH* family members. BAC filters containing 6.5 equivalents of the barley genome (cv. Morex) were screened, and 3 clearly positive clones were identified (data not shown). When a blot of BAC DNA from these clones digested with *Hind*III was probed, the same 3 clones, 3–5-10, 3–7-3, and 3–7-8, were verified as being positive. The digestion pattern of BACs 3–5-10 and 3–7-8 appeared identical, and many bands were common to BAC 3–7-3, indicating that all 3 BACs cover identical or very similar regions of the barley genome. When a genomic DNA blot was hybridized with the same probe, single bands were observed in lanes digested with *Hind*III, *Eco*RI, or *Eco*RV, corroborating the BAC digestion results. Because all *HvCSLH* ESTs are also derived from a single gene (Table S1), these data strongly suggest that there is only a single *CSLH* gene in the barley genome.

An adaptor primer PCR method (1) was used to identify the 5′ end of *HvCSLH1*. DNA was isolated from BACs 3–5-10 and 3–7-3, digested with a range of restriction enzymes producing blunt-ended DNA fragments to which adaptors were ligated. Nested PCR then was performed with adaptor- and *HvCSLH1*-specific primers (Table S3) to amplify fragments containing the 5′ end of the gene. Amplification of BAC 3–7-3 DNA digested with *Nru* I using primers AP2 and H1R6 successfully amplified a 1.3-kbp fragment that contained all but ≈20 aa of the *N*-terminal sequence. Direct sequencing of BAC 3–7-3 DNA with the H1R10 primer, an antisense primer designed to anneal near the 5′ end of the 1.3-kb fragment, enabled the remainder of the ORF plus 748 bp of upstream sequence to be identified. As predicted from earlier results, the sequence obtained from BAC 3–5-10 was identical to BAC 3–7-3, confirming that there is only a single *CSLH* gene within the barley genome.

SI Materials and Methods

EST Analyses, Contig Assembly and Bioinformatics. *CSLH* ESTs were obtained by querying public databases including the now-discontinued Stanford Cell Wall website, NCBI (http://www.ncbi.nlm.nih.gov/), HarvEST (http://harvest.ucr.edu/), GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml), Barley Gene Index (http://compbio.dfci.harvard.edu/tgi/plant.html), and BarleyBase (www.barleybase.org) using the BLAST search tool (2). Sequences were assembled into contigs using either Sequencer™ 3.0 (GeneCodes) or ContigExpress, a module of Vector NTI Advance 9.1.0 (Invitrogen). DNA or protein sequences were aligned using ClustalX (3). Phylogenetic analysis was carried out using the in-built neighbor-joining algorithm and tree robustness was assessed using 1000 bootstrapped replicates. Sequence similarities were calculated using MatGat 2.02 (4). Transmembrane domains were predicted using the suite of programs described in ARAMEMNON (5). Motifs predicting posttranslational modifications were identified using the programs listed in ExPasy under the Tools menu. Protein parameters were calculated using ProtParam at ExPasy.

Barley BAC Screening. BAC filters containing 6.5 equivalents of the barley genome from the non-Yd2 cv Morex (Clemson University Genomics Institute, CUGI) were blocked for 6 h at 65 °C in prehybidization solution (0.53 M $NaPO_4$ buffer, pH 7.2, 7.5% wt/vol SDS, 1 mM EDTA, 11 μg/ml salmon sperm DNA). The radiolabeled cDNA and gDNA fragment amplified with primers H1F1 and H1R1 or H1R5 (Table S3) was added and incubated for 24 h at 65 °C. Filters were washed 3 times with 2× SSC, 0.1% SDS at room temperature. Final washes were done with 1× SSC, 0.1% SDS. Filters were exposed to X-ray film for 2 days. Positive BAC clones were identified and ordered as directed on the CUGI website (http://www.genome.clemson.edu). Clones were streaked onto LB agar containing 25 μg/ml chloramphenicol and were grown overnight at 37 °C. Colonies for each clone were picked, placed on gridded nylon membranes resting on LB agar containing 25 μg/ml chloramphenicol, and incubated overnight at 37 °C. DNA was fixed to the membrane and denatured by being placed on filter paper soaked in 0.4 M NaOH for 20 min and then was neutralized by being placed on filter paper soaked in neutralizing solution (1.5 M NaCl, 0.5 M Tris-HCl pH 7.2, 1 mM EDTA). Membranes were then washed 3 times in 2× SSC, 0.1% SDS and were hybridized using standard conditions (6).

BAC DNA Isolation. Positive clones were cultured overnight in LB broth containing 25 μg/ml chloramphenicol at 37 °C. Cells were pelleted by centrifugation (12,000 × *g*, 3 min), and the pellet was resuspended in 90 μl TES buffer (25 mM Tris-HCl pH 8.0, 10 mM EDTA, 15% wt/vol sucrose). An aliquot (180 μl) of lysis solution (0.2 M NaOH, 1% SDS) was added and mixed gently, followed by 135 μl 3M NaOAc, pH 4.6. The chromosomal DNA was pelleted by centrifugation (12,000 × *g*, 15 min). The supernatant was collected, and 2 μl RNase A (10 mg/ml) was added and incubated for 1 h at 37 °C. A 400-μl aliquot of Tris-saturated phenol-chloroform (1:1 ratio) was added, and the samples were centrifuged again (12,000 × *g*, 5 min). The supernatant was collected, and BAC DNA was precipitated using 2 to 3 volumes chilled 95% ethanol for 10 min at room temperature. The BAC DNA was pelleted by centrifugation (15,000 × *g*, 15 min), washed in 70% ethanol, resuspended in 20–50 μl TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA), and stored at 4 °C.

Genome Walking. The adaptor ligation method of Siebert et al. (1) was used to amplify fragments of genomic DNA upstream of known *CSLH* EST sequence. Restriction enzymes used to digest barley genomic DNA were *Eco*RV, *Nru* I, *Pvu*II, *Sca*I, or *Ssp*I. Primary PCR reactions were conducted in 25-μl volumes containing 2 μl ligated DNA (1:10 dilution), 1× PCR buffer, 2 mM $MgCl_2$, 100 ng each of adaptor primer AP1 and antisense primer H1R7 (Table S3), 0.4 mM dNTPs, and 1 unit *Taq* polymerase (Invitrogen). Cycle parameters were as follows: 96 °C for 2 min then 40 cycles of 94 °C for 30 sec, 59 °C for 30 sec, 72 °C for 1 min, and a final step at 72 °C for 7 min. A secondary PCR was conducted with 1 μl of the primary PCR using 100 ng each of adaptor primer AP2 and the nested primer H1R6. Reaction composition and cycle parameters were the same as previously, except that an annealing temperature of 61 °C was used.

BAC Sequencing. For sequencing, 0.5 to 1 μg of isolated BAC DNA was combined with 5 pmol primer and 1× Big Dye Terminator v 3.1 (BDT) mix (Applied Biosystems) in a final volume of 20 μl. Cycle parameters were as follows: 96 °C for 15 min, then 65 cycles of 96 °C for 10 sec, 55 °C for 10 sec, and 60 °C for 4 min. DNA was precipitated with 0.1 vol. 3M NaOAc, pH 5.2, and 2.5 vol. 95% ethanol on ice for 10 min, then pelleted by spinning at 12,000 × *g* for 30 min. The pellet was rinsed with 70% ethanol, dried, and sent to Australian Gene Research Facility, Brisbane, Australia, for sequencing.

Mapping of HvCSLH1. Genetic mapping was done by Margaret Pallotta (Australian Centre for Plant Functional Genomics, University of Adelaide) using a Sloop × Halcyon doubled-haploid mapping population of 60 lines (7) kindly supplied by Peter Langridge (Australian Centre for Plant Functional Genomics, University of Adelaide). Using standard methods of DNA blot hybridization (6), a *HvCSLH1* probe PCR-amplified using primers H1F1 and H1R5 (Table S3) was hybridized to membranes containing parental line genomic DNA digested with 1 of 6 restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III, or *Xba*I). The dihybrid population then was digested with enzymes that gave a clear polymorphism (*Dra*I). Polymorphisms were scored, and the *HvCSLH1* map location was determined using the Find Best Location function of MapManager QT version 0.30 (8). Map locations were correlated with quantitative trait loci data using resources available at http://www.barleyworld.org/.

In Situ PCR. In situ PCRs were conducted according to the method of Koltai and Bird (9) with the following modifications. After tissue sectioning, genomic DNA was removed by treatment for 6 h at 37 °C in 1× DNase buffer and 4 U RNase-free DNase (Promega). cDNA synthesis was carried out using Thermoscript™ RT (Invitrogen), except that the RNase H step was omitted, and a gene-specific primer (1 μg, Table S3) was used for reverse transcription. PCRs were carried out in a final volume of 50 μl containing 1× PCR buffer, 200 μm dNTPs (Promega), 0.2 nmol digoxigenin-11-dUTP (Roche), 2 mM $MgCl_2$, 200 ng of each primer, and 2 U *Taq* DNA polymerase (Invitrogen). Cycling parameters were as follows: initial denaturation at 96 °C for 2 min, then 40 cycles of 94 °C for 30 sec, 59 °C for 30 sec, 72 °C for 1 min. Sections then were washed, incubated with 1.5 U alkaline phosphatase-conjugated anti-digoxigenin Fab fragments (Roche), and developed for 10–20 min as outlined by Koltai and Bird (9). For negative control sections, reverse transcriptase was omitted, and all the Hv 18S rRNA primers were included to check whether there was any amplification from genomic DNA.

***Arabidopsis* Growth Conditions.** *Arabidopsis* seeds were surface-sterilized in a sterilization solution (sodium hypochlorite [2% available chlorine], drop of Tween-20 [Ajax Finechem]) for 15 min then rinsed 4 times with sterile deionized water. Surface-sterilized seed was spread onto 85 × 25 mm Petri dishes containing 50 ml of sterile 1× MS medium (4.33 g/L Murashige and Skoog basal salts [Phytotechnology Laboratories], 2% wt/vol sucrose, 1% wt/vol bactoagar). For selection of transformants, 50 mg/L kanamycin (Sigma) was added to the medium. Plates were placed in a cold-room for 3–5 days at 4 °C to synchronize germination. Cold-stratified plates then were transferred into controlled-environment growth cabinets (Thermoline L+M model TPG 1260 TO-5 × 400; Thermoline Scientific) with day and night temperatures of 23 °C and 17 °C, respectively. The average light intensity at rosette leaf level was ≈50 μE m^{-2} sec^{-1} supplied by 3-foot fluorescent tubes (Sylvania Standard F30W/133-T8 Cool White) during a 16-h light cycle. After 3 weeks on MS plates, individual plantlets were transferred into hydrated 42-mm diameter Jiffy pellets (Garden City Plastics). Nine rows of 6 pellets were arranged in trays; 3 trays were housed on each 2 × 3.5-foot wire rack shelf. Measured relative humidity was between 60% and 70%. Plants were watered with tap water supplemented with Peter's Professional™ General Purpose plant fertilizer (Scotts Australia) by subirrigation every 2 to 3 days.

Genomic DNA Extraction and PCR Analysis of *Arabidopsis* transgenics. DNA was extracted from a single *Arabidopsis* leaf according to the method described by Edwards et al. (10). A 1-μl aliquot of genomic DNA was used as template in PCR screens of transgenic plants using primers H1F2 and HvCSLH1TOPOr (Table S3) with the following cycling regimen: 94 °C for 2 min followed by 35 cycles of 94 °C for 20 sec, 57 °C for 30 sec, 72 °C for 30 sec.

Preparation of Mixed Microsomal Membranes. T_1 seed of *HvCSLH1* transgenic plants was collected and ≈100 seeds were sown onto 1× MS agar medium containing 50 mg/L kanamycin (Sigma). After 3 weeks, kanamycin-resistant seedlings were pooled, frozen in liquid N_2, and ground at 4 °C in a mortar and pestle containing homogenizing buffer (50 mM $NaPO_4$ buffer, pH 7.5, 0.5 M sucrose, 20 mM KCl, 10 mM DTT, 0.2 mM PMSF, and 83 μl plant protease inhibitor mixture (Sigma, P9599)). Homogenate was filtered through a 50-μM mesh, and the supernatant was centrifuged at 6,000 × *g* for 10 min at 4 °C. The supernatant was decanted and centrifuged at 50,000 × *g* for 30 min at 4 °C in 4.5-ml ultracentrifuge tubes (Beckmann). The 50,000 × *g* supernatant was decanted, and the pellet was resuspended in 10 mM Tris-MES buffer, pH 7.5, using a glass-Teflon homogenizer. The resuspended pellet was diluted to 4.5 ml with Tris-MES buffer and was centrifuged at 100,000 × *g* for 1 h at 4 °C. The pellet was resuspended in 0.25 M sucrose, 10 mM Tris-MES buffer, pH 7.5, as outlined previously. Protein concentration was measured using Bradford assay reagent (BioRad) using BSA as the standard.

Western Blotting. Samples of membrane protein (30 μg) were incubated at 60 °C for 20–60 min in 200 mM DTT and sample buffer (37.5 mM Tris-HCl, pH 7.0, 10% glycerol, 3% SDS, 0.025% bromophenol blue) to give an SDS:protein ratio of 1.5 mg SDS to 30 μg protein before loading onto an 8% SDS/PAGE gel. After electrophoresis, gels were blotted onto nitrocellulose (NitroPure, GE Osmonics) in 25 mM Tris base, 192 mM glycine, 20% methanol containing 0.05% SDS at 100 V for 90 min at 4 °C. Membranes then were blocked overnight in Tris-buffered saline (TBS; 20 mM Tris base, 150 mM NaCl) containing 3% wt/vol nonfat milk powder before incubation for 1 h at room temperature in rat anti-HA polyclonal antibody (Roche) diluted 1:1000 in TBS containing 1% BSA. Membranes were washed 3 times in TBS containing 0.05% SDS (TBST), then incubated in anti-rat IgG HRP-conjugated antibody (Dako) diluted 1:1000 in TBS containing 3% wt/vol nonfat milk powder. Membranes were washed 3 times in TBST before signal was detected with the SuperSignal West Pico chemiluminescent substrate (Pierce).

Preparation of Cell Wall Material. Alcohol-insoluble residue (AIR) was prepared by grinding plant material in liquid N_2 using a mortar and pestle. Five volumes of 80% ethanol were added to the homogenate before mixing by rotation for 1 h at 4 °C. After centrifugation at 3,400 × *g* for 5 min, the supernatant was removed, and the residue was refluxed twice at room temperature in 80% ethanol for 1 h, followed by refluxing in 50% ethanol twice for 1 h. The ethanol-soluble fraction was removed, and the AIR was washed once in 100% ethanol before drying at 40 °C under vacuum. β-Glucan concentration in barley leaf cell walls was estimated using the Megazyme Mixed Linkage β-Glucan assay kit.

MALDI-TOF MS Analysis. Aliquots (30 μl) of the remaining (1, 3; 1, 4)-β-D-glucan endo-hydrolase–released oligosaccharides were lyophilized, dissolved in DMSO, and methylated using the NaOH method (11). Methylated oligosaccharides were partitioned into dichloromethane (DCM), and the DCM phase was washed 3 times with deionized water. The DCM phase was dried under a N_2 stream before being re-dissolved in 10 μl 50% acetonitrile. A 1-μl aliquot was mixed with 1 μl 2,5-dihydroxy benzoic acid matrix (10 mg/ml dissolved in 50% acetonitrile), and 1 μl of the mix was spotted onto a MALDI plate for analysis in a MALDI-TOF mass spectrometer (Voyager DSTR, Applied

Biosystems) in the positive ion reflector mode. The oligosaccharide structures were deduced based on the following masses: methylated hexose = 204 m/z, + reducing end = 46 m/z, Na^+ = 23 m/z.

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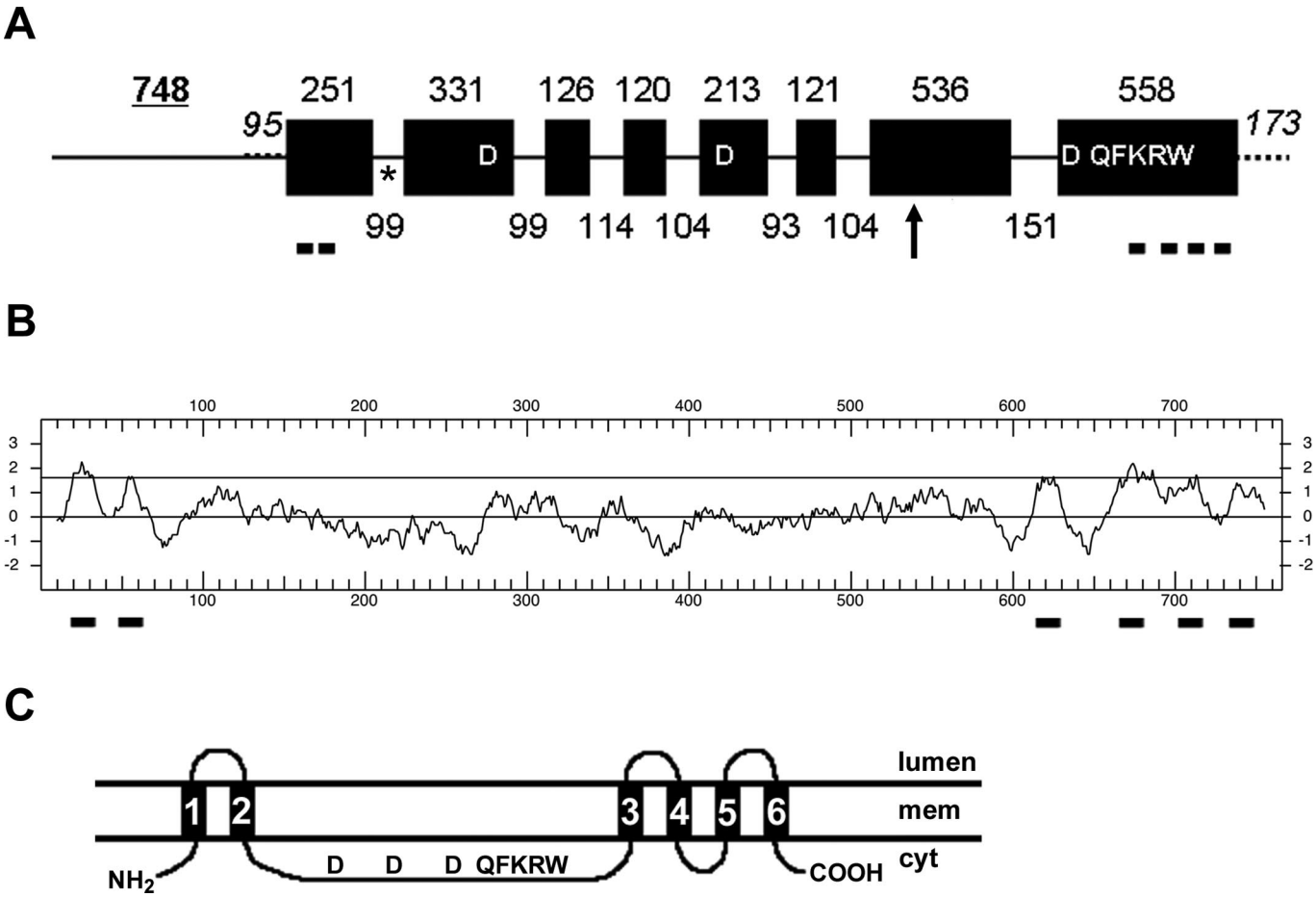

Fig. S1. Structural features of *HvCSLH1*. (*A*) Exon-intron structure of *HvCSLH1*. Black boxes indicate exons, the black line indicates introns and upstream sequence, and the dashed lines indicate 5′ and 3′ UTRs. The numbers above the boxes show the size of exons; the numbers below the line show intron size. Italicized numbers refer to the size of 5′ and 3′ UTRs. The bold-face underlined number indicates the length of known sequence upstream of the start codon. All numbers are in base pairs. The asterisk indicates the intron (phase 2) shared by *HvCSLH1* and *OsCSLH1* but not by *OsCSLH2* and *OsCSLH3*. All other introns are conserved between *HvCSLH1* and *OsCSLH1–3* (all are phase 0 except *HvCSLH1* intron 6, which is phase 1) with 1 exception: an additional intron (phase 0) is present only in the 3 rice genes and is situated in exon 7 of *HvCSLH1*. Its relative position is marked by an arrow. The thick black bars indicate the 6 consensus transmembrane domains as predicted by ARAMEMNON. (*B*) Kyte-Doolittle hydrophobicity plot of HvCSLH1. A 19-aa window with a + 1.6 cutoff (12) was used. The 6 predicted transmembrane domains are indicated by black bars. Numbers refer to amino acids. (*C*) Predicted membrane topology of HvCSLH1. COOH, carboxy terminal; cyt, cytoplasm; D,D,D,QXXRW, signature motif of CAZy GT2 family (13); lumen, interior of ER, Golgi body, or vesicle; mem, membrane; NH_2, amino terminal. The sequence of the QXXRW motif in HvCSLH1 is QFKRW.

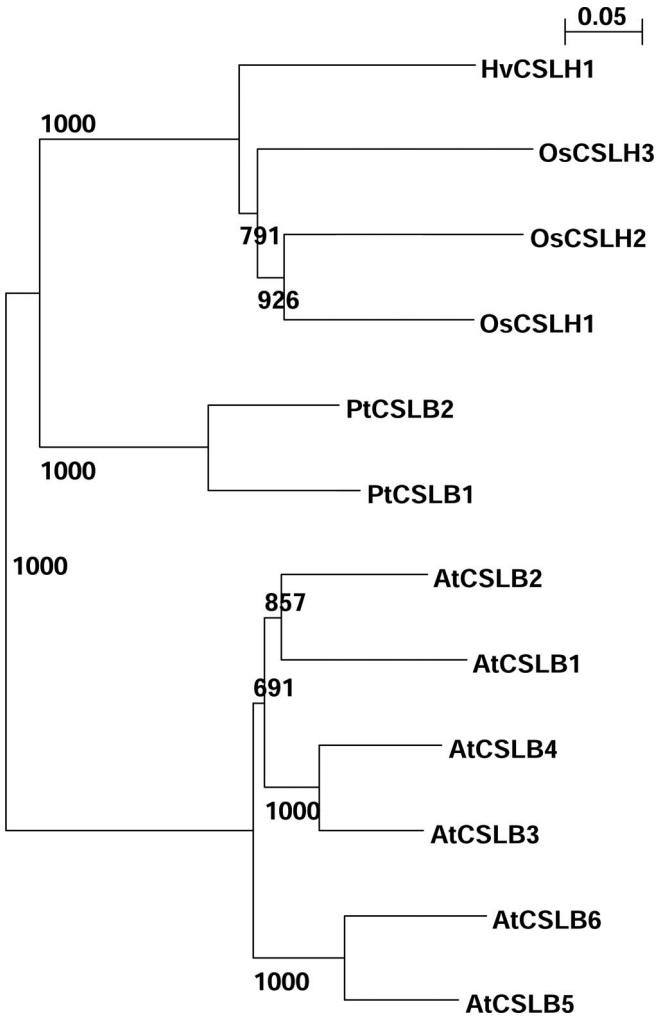

Fig. S2. Phylogenetic tree of full-length barley (*Hordeum vulgare*) and rice (*Oryza sativa*) CSLH sequences. *A. thaliana* and poplar (*Populus trichocarpa*) CSLB protein sequences are included because the CSLB family is the most closely related of the CSL families to the CSLH family. The alignment was generated using ClustalX and the in-built distance algorithm with neighbor-joining. The number of bootstrap replicates (from a total of 1,000) supporting each clade is indicated below the internode for that clade. Accession numbers are HvCSLH1 (FJ459581), OsCSLH1 (Os10g20090, AC119148), OsCSLH2 (Os04g35020, AL606632), OsCSLH3 (Os04g35030, AL606632), PtCSLB1 (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html; ID no. 572982), PtCSLB2 (ID no. 684214), AtCSLB1 (At2G32610, NM_128820), AtCSLB2 (At2G32620, NM_128821), AtCSLB3 (At2G32530, NM_179859), AtCSLB4 (At2G32540, NM_128813), AtCSLB5 (At4G15290, NM_117617, AtCSLB6 (At4G15320, NM_117620).

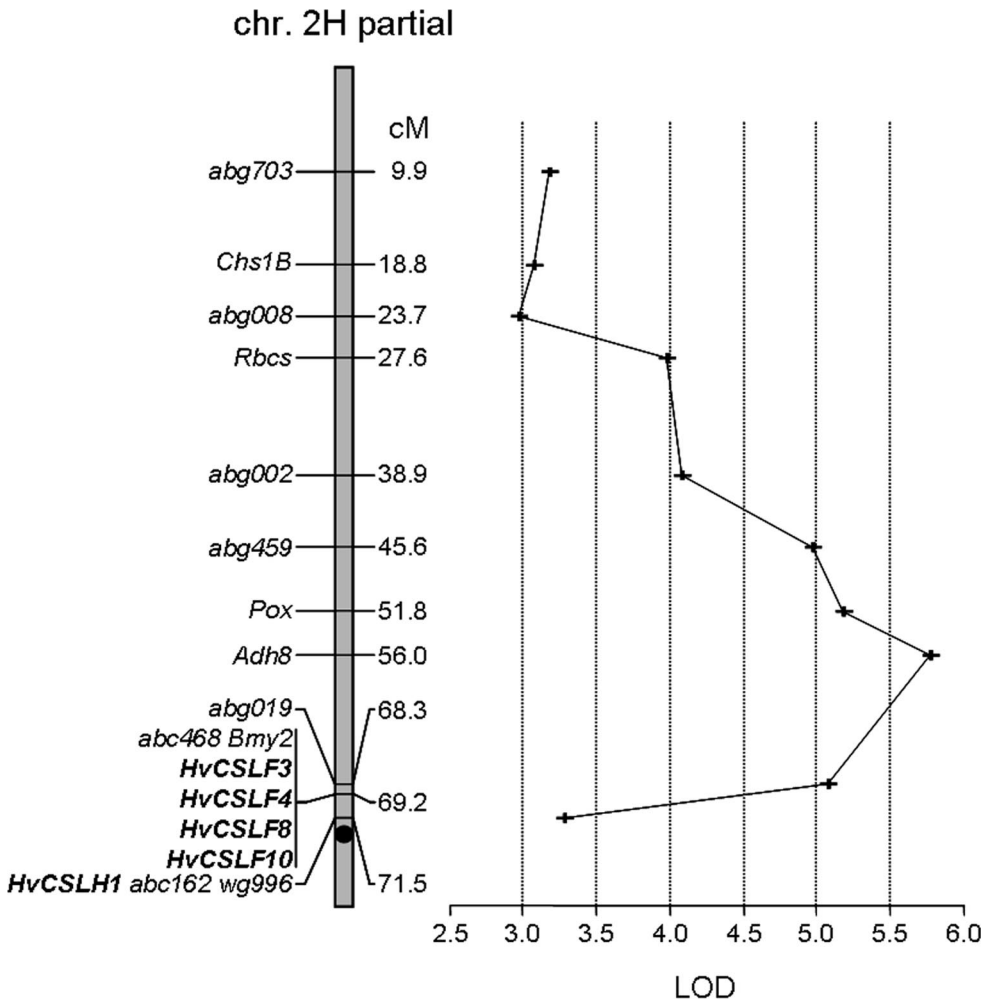

Fig. S3. Partial genomic map of the short arm of chromosome 2H where *HvCSLH1* is located. *HvCSLH1* and a cluster of 4 *HvCSLF* genes were mapped to an interval corresponding to 69.2–71.5 Mb on the Steptoe × Morex bin map close to the centromere (*black circle*). *HvCSLH1* was placed in bin 8, co-segregating with the wg996 marker. On the Steptoe × Morex reference map, wg996 co-segregates with abc162 and is 2.3 centimorgans (cM) south of abc468, the marker that co-segregates with the 4 *HvCSLF* genes (14). Key markers are shown on the left. Their distances from the top of the chromosome in cM and the logarithm of the odds to the base 10 (LOD) score in the malt β-glucan QTL analysis of Han et al. (15) are indicated on the right.

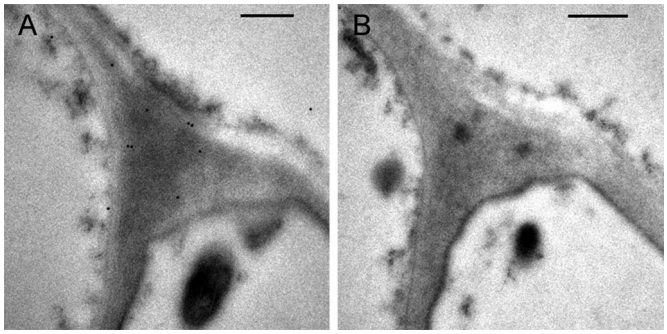

Fig. S4. Transmission electron micrographs showing the enzymic digestion of β-glucan in leaf sections of *Arabidopsis* HvCSLH1-expressing line 11. Before gold labeling with a β-glucan antibody (16), serial leaf sections of mesophyll cells were pretreated either with buffer for 1 h at 30 °C (17) (*A*) or with recombinant *Bacillus subtilis* β-glucan hydrolase (*B*). Subsequent gold labeling was abolished after enzymatic digestion (*B*) but not after buffer incubation (*A*). Scale bar represents 0.5 μm.

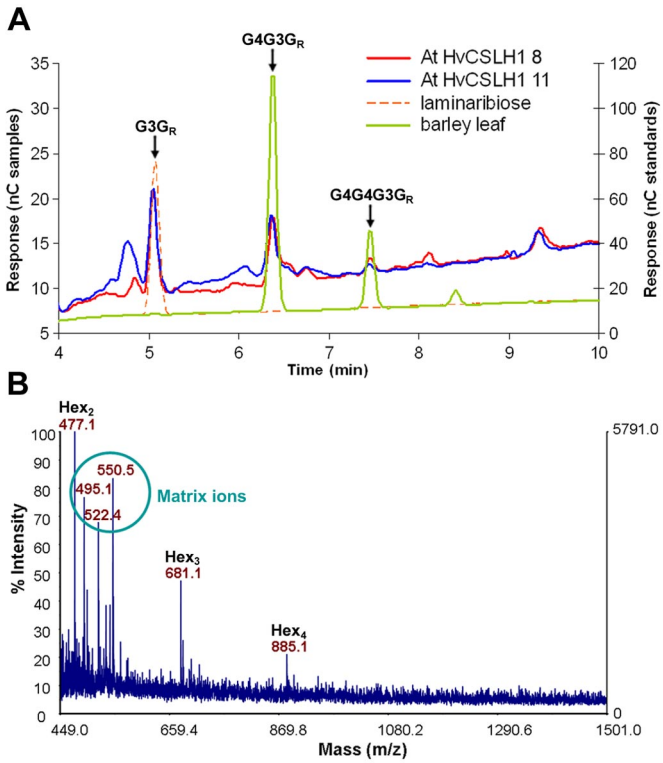

Fig. S5. Chemical analyses of *Arabidopsis* HvCSLH1 transgenic lines. (*A*) HPAEC profiles of oligosaccharides released upon β-glucan hydrolase digestion of AIR prepared from HvCSLH1 transgenic *Arabidopsis* lines 8 and 11. Leaf samples were taken from 131-day-old plants. Barley mature leaf (entire sheath) was the AIR positive control; wild-type *Arabidopsis* Col-0 (mature leaf) was the AIR negative control. AIR samples were loaded at the same concentration. Laminaribiose ($G3G_R$) was used as a standard. *Arabidopsis* samples are plotted on the left *y*-axis; standard and positive controls are plotted on the right *y*-axis. $G3G_R$ (degree of polymerization [DP]2), $G4G3G_R$ (3-O-β-cellobiosyl-Glc, DP3), and $G4G4G3G_R$ (3-O-β-cellotriosyl-Glc, DP4) peaks are indicated. (*B*) MALDI-TOF MS chromatogram of β-glucan hydrolase–digested AIR from line 16–2 sample shown in Fig. 3*A*. Hex_2 is the Na^+ adduct of the permethylated hexose disaccharide, Hex_3 is the trisaccharide, and Hex_4 is the tetrasaccharide.

Table S1. ESTs derived from *HvCSLH* listed in order of alignment 5′ to 3′

EST accession no. (5′ to 3′)	Cultivar	Source Tissue
CA013594	Barke	early endosperm, 0–16 h after imbibition
BJ470984	Haruna Nijo adult	top 3 leaves at heading stage
BJ471865	Haruna Nijo adult	top 3 leaves at heading stage
BJ473288	Haruna Nijo adult	top 3 leaves at heading stage
BJ452043	Akashinriki	vegetative stage leaves
BJ471909	Haruna Nijo adult	top 3 leaves at heading stage
AV932844	Haruna Nijo adult	top 3 leaves at heading stage
BJ469514	Haruna Nijo adult	top 3 leaves at heading stage
AV933503	Haruna Nijo adult	top 3 leaves at heading stage
AV933012	Haruna Nijo adult	top 3 leaves at heading stage
AV932649	Haruna Nijo adult	top 3 leaves at heading stage
AV932549	Haruna Nijo adult	top 3 leaves at heading stage
BJ475824	Haruna Nijo adult	top 3 leaves at heading stage
BJ476822	Haruna Nijo adult	top 3 leaves at heading stage
AV934650	Haruna Nijo adult	top 3 leaves at heading stage
BJ477472	Haruna Nijo adult	top 3 leaves at heading stage
AV935479	Haruna Nijo adult	top 3 leaves at heading stage
AV935951	Haruna Nijo adult	top 3 leaves at heading stage
AV832539	Akashinriki	vegetative stage leaves
AV936586	Haruna Nijo adult	top 3 leaves at heading stage
CB881459	Barke	male inflorescences (approx. 2 mm in size), green anther stage
AV934667	Haruna Nijo adult	top 3 leaves at heading stage
BJ475744	Haruna Nijo adult	top 3 leaves at heading stage
BJ459600	Akashinriki	vegetative stage leaves
AV832391	Akashinriki	vegetative stage leaves

Table S2. Primers used in qPCR analysis. PCR primers and PCR product sizes are given in base pairs, together with optimal acquisition temperatures for genes analyzed

Gene	Forward Primer (5′ to 3′)	Reverse Primer (5′ to 3′)	PCR product (bp)	Acquisition Temp, °C
Hv GAPDH	GTGAGGCTGGTGCTGATTA	CGTGGTGCAGCTAGCATTTGAGAC	198	80
Hv Cyclophilin	CCTGTCGTGTCGTCGGTCTAAA	ACGCAGATCCAGCAGCCTAAAG	122	79
Hv α-Tubulin	AGTGTCCTGTCCACCCACTC	AGCATGAAGTGGATCCTTGG	248	80
Hv HSP70	CGACCAGGGCAACCGCACCAC	ACGGTGTTGATGGGGTTCATG	108	83
Hv EF1a	GGTACCTCCCAGGCTGACTGT	GTGGTGGCGTCCATCTTGTTA	164	80
HvCSLH1	TGCTGTGGCTGGATGGTGTT	GCTTTATTATTGAGAGAGATTGGGAGA	295	82

Hv, *Hordeum vulgare.*

Table S3. Primers used in cloning and amplifying *HvCSLH1* and in situ PCR analysis

Gene	Primer Name	Primer Sequence (5′ to 3′)	Technique
HvCSLH1	H1F1	TTGACCGGACAACGGATCC	DNA blot analysis, BAC screening, gene mapping, in situ PCR
HvCSLH1	H1F2	CTGGAGATACTCATCAGC	Northern blotting, transgenic plant genomic DNA PCR screening
HvCSLH1	HvCSLH1cF1	TCGAGCGGTTGTTGCTTGTG	HvCSLH1 cDNA amplification
HvCSLH1	HvH1TOPOf	CACCATGGCGGGCGGCAAGAAGCTG	Binary vector construction
HvCSLH1	H1R1	CGTCACCGGGATGAAAAC	DNA blot analysis, BAC screening, genome walking PCR, in situ PCR
HvCSLH1	H1R2	TGACGCTCCACGGCATTC	In situ PCR (priming cDNA synthesis)
HvCSLH1	H1R5	GGCTGGCCATCGAAATATTG	BAC screening, genome walking PCR, gene mapping, in situ PCR
HvCSLH1	H1R6	GAGCGTTGGTCATCACGG	Genome walking PCR
HvCSLH1	H1R7	CACATCGCGTGTAGGGC	Genome walking PCR
HvCSLH1	H1R10	CACTTGCCGTTCATGTTG	Adaptor ligation PCR, BAC sequencing
HvCSLH1	HvCSLH1cR1	CCTGCTTGAGTCTTCGTTACATGTTC	HvCSLH1 cDNA amplification
HvCSLH1	HvH1TOPOr	CGCTTCCAATATTTCGATG	Binary vector construction, Northern blotting, transgenic plant genomic DNA PCR screening
Generic	Adaptor 1	CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAGGT	Adaptor ligation PCR
Generic	Adaptor 2	P-ACCTGCCC-NH_2	Adaptor ligation PCR
Generic	AP1	GGATCCTAATACGACTCACTATAGGGC	Adaptor ligation PCR
Generic	AP2	AATAGGGCTCGAGCGGC	Adaptor ligation PCR
18S rRNA	Hv18SRTr	GTTTCAGCCTTGCGACCATACT	In situ PCR (priming cDNA synthesis)
18S rRNA	Hv18Sf	GGTAATTCCAGCTCCAAT	In situ PCR
18S rRNA	Hv18Sr	GTTTATGGTTGAGACTAG	In situ PCR