Supporting Information

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SI Materials and Methods

Plasmid Construction and Plant Transformation. The full-length cDNA of OsSHR1 and OsSHR2 were PCR amplified from Nipponbare genomic DNA using KOD taq from Invitrogen. PCR products were first cloned into the pCR-XL-TOPO vector (TOPO XL Cloning Kit, Invitrogen). From there, the OsSHR1 and OsSHR2 clones were reamplified to add recombination sites for insertion into pDONR221 (Invitrogen). BdSHR was cloned directly from genomic DNA and recombined into pDONR221. The mutant form of the SHR homologs were generated using inverse PCR. After sequencing, the resulting plasmids were all recombined into Gateway destination vectors. To achieve cell-specific expression, the previously reported pGreenBarT vector (1) was modified. The attR4/R3 gateway cassettes were replaced by attR1/R2 cassettes flanked by two multiple cloning sites. The promoter sequence of SHR was amplified and cut by Kpn1/Xho1, and pEN7 and pCO2 sequences were amplified and cut by KpnI/KpnI. The digested fragments were ligated into the multiple cloning sites 5′ of the gateway cassette using corresponding restriction sites. YFP and mCherry sequence were cloned and digested by XbaI and HindIII before insertion into the multiple cloning sites 3′ of the gateway cassette. The BiFC plasmid were prepared by amplification of the 35S:Venus-Gateway-NosT cassette from pDEST-VYCE(R)GW or pDESTVYNE(R)GW vectors (2) and subcloned into SmaI site in the pUC18 vector.

All resulting plasmids generated through LR Gateway reaction were transformed into Agrobacterium strain GV3101-pSoup-pMP. The Agrobacterium was then used to transform *Arabidopsis* (Col-0) following the floral dip method (3). Transgenic plants were screened by using resistance to glufosinate-ammonium (Basta) in soil. For all of the transgenes discussed, at least three independently transformed lines were analyzed.

Plant Materials and Growth Condition. *Arabidopsis thaliana* Columbia line (Col-0) was used as the wild type throughout the experiments. Plants were germinated and grown vertically on 0.5x MS medium (Caisson) containing 0.05% (wt/vol) Mes (pH5.7), 1.0% (wt/vol) Sucrose, and 1% Granulated agar (DIFCO) in a growth chamber at 23 °C under a 16-h light/8-h dark cycle. Plants were imaged 5–6 d after plating unless otherwise stated. After sterilization, the *B. distachyon* seeds were germinated in darkness on wet filter papers placed in a Petri dish. The roots of 1-wk-old seedlings were then collected for anatomy analysis. For cross sections, the rice seeds were sterilized by 70% ethanol for 1 min, followed by bleach (20/30, vol/vol) for 30 min. After three washes in distilled sterile water, the rice seeds were germinated on 0.5x MS media and grown vertically in a Petri plate (20 × 20 cm, Corning) for 6 d (10 h light/12 h dark at 24/26 °C). The SHR homolog lines in different genetic backgrounds were obtained by crossing. The progeny was followed to the second generation, and the presence of transgene or mutation was verified either by imaging on the confocal or genotyping.

Confocal Microscopy. Roots were counterstained in 0.01 μg/mL propidium iodide (PI) in water. Confocal images were obtained using a 20× water-immersion lens on a Leica TCS SL microscope equipped with an argon–krypton ion laser with the appropriate filter sets for visualizing YFP and PI. The dual-channel observation of YFP and mCherry was conducted on a Zeiss LSM 710 laser scanning confocal microscope using a Zeiss LD C-Apochromat 40×/1.1 NA water immersion objective lens (Carl Zeiss Microimaging, Inc.).

BiFC Assay in *Arabidopsis* Protoplast. Protoplasts were isolated from 3-wk-old plants grown under normal light conditions with tape-*Arabidopsis* sandwich method (4) and enzyme solution containing 1.5% (wt/vol) Cellulase R-10 and 0.5% Macerozyme R-10 (Yakult Pharmaceutical). The transfection was conducted as described by ref. 5. Briefly, the mixture of 10 μg of freshly isolated plasmid DNA and an equal volume of a solution of 40% (vol/vol) PEG (MW 4000; Fluka) with 0.1 M CaCl2 and 0.2 M mannitol was incubated at room temperature for 13 min and then washed in W5 solution (154 mM NaCl, 125 mM CaCl2, 5 mM KCl, 5 mM glucose, and 2 mM Mes, pH 5.7). After 16–24 h incubation in low-light conditions, protoplasts were imaged on a Leica TCS SL microscope using a 20× water-immersion lens. The Venus emission was captured using a 510- to 540-nm filter. To quantify the interactions between AtSCR and SHR homologs, the Venus signals in the nuclei were selected and the mean intensities were measured using ImageJ. Quantification was done as referenced in refs. 6 and 7.

Histology and Histochemistry. The seedlings were fixed for 1 h in formalin–acetic acid–alcohol and then embedded in an agarose block as described previously (8). The agarose blocks containing samples were dehydrated in a graded series of 50%, 70%, 90%, and 2 × 100% (vol/vol) ethanol. Technovit 7100 (EMS; no. 14653) infiltration was performed according to the manufacturer. Then, 3- to 5-μm sections were made on a Leitz 1512 rotary microtome using Thermo HP 35 steel blades. Sections were stained in fresh 0.01% toluidine blue-O (Merck). *B. distachyon* roots were fixed, dehydrated, and embedded in the same manner as *Arabidopsis* except omitting the agarose embedment step. To obtain cross-sections of rice roots, the tip of rice roots (~1 cm) were collected and embedded in a 3% agarose block. Around 50-μm sections were then performed on a Microtome HM 650 V vibratome. Images of cross-sections of *A. thaliana* and *B. distachyon* were captured by an Olympus BX51 microscope equipped with a digital camera. The cross-sections of rice roots were photographed under UV light using a DAPI filter on a Leica DMX6000 microscope.

PI Exclusion and Autofluorescence of Casparian Strips. For assay of the functional endodermis, seedlings were incubated for 10 min in a freshly made PI solution of (10 μg/mL) and then rinsed twice in distilled water before confocal imaging.

To detect the autofluorescence of Casparian strips, roots were transferred to a 12-well plate containing 0.24 N HCl in 20% (vol/vol) methanol and incubated at 57 °C for 15 min. Roots were then treated with 7% (wt/vol) NaOH in 60% (vol/vol) ethonal for 15 min at room temperature. After rehydration in a graded series of 40%, 20%, and 10% (vol/vol) ethanol for 5 min each, roots were infiltrated in 5% (vol/vol) ethanol and 25% (vol/vol) glycerol for 15 min. Roots were then mounted in 50% (vol/vol) glycerol and observed on an Olympus BX51 microscope with a GFP filter.

Estrogen Induction. Five days after plating, the seedlings were transferred to 0.5x MS (Caisson) agar (Difco-BBL) plates containing 10 μM estradiol (Sigma), and the same medium containing the estradiol carrier (ethanol) as controls. The seedlings were returned to the growth chamber and incubated vertically for 1 d before confocal imaging.

FRAP. The FRAP assay was performed as described before (9). Briefly, photobleaching of GFP signal in endodermis was

achieved using six iterations of the 488-nm laser at 100% power on a Leica TCS SL microscope equipped with an argon–krypton ion laser. Recovery was followed by image acquisition using 20% laser power at 30-min intervals. The seedlings were placed in the moisture box during the intervals. Nine roots from three experiments and 35–132 cells for SHR homolog were analyzed. The fluorescence intensity ratio (endodermis/stele) before bleach, after bleach, and after recovery was determined using ImageJ. The percent recovery was calculated using the normalized values as described previously (9).

Yeast Two-Hybrid Assay. The coding sequences of AtSHR, OsSHR1, OsSHR2, AtSCR, AtMGP, and AtJDW were cloned into pDEST22 or pDEST32 vectors (Invitrogen) as bait or prey constructs and transformed into the yeast strain Y187 and AH109, respectively. The protein–protein interactions were tested in diploid yeast cells by mating the two yeast strains as described by the Matchmaker protocol (Clontech).

For β-galactosidase assays, yeast cultures were grown in selective medium to an optical density at 595 nm (OD595) of 0.8 to 1, and the β-galactosidase assays were performed as described previously (10). The specific activities were determined by the β-galactosidase enzymatic activity normalized to the concentration of crude protein from three independent biological replicates.

1. Lee JY, et al. (2006) Transcriptional and posttranscriptional regulation of transcription factor expression in *Arabidopsis* roots. *Proc Natl Acad Sci USA* 103(15): 6055–6060.
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8. Wu S, Baskin TI, Gallagher KL (2012) Mechanical fixation techniques for processing and orienting delicate samples, such as the root of *Arabidopsis thaliana*, for light or electron microscopy. *Nat Protoc* 7(6):1113–1124.
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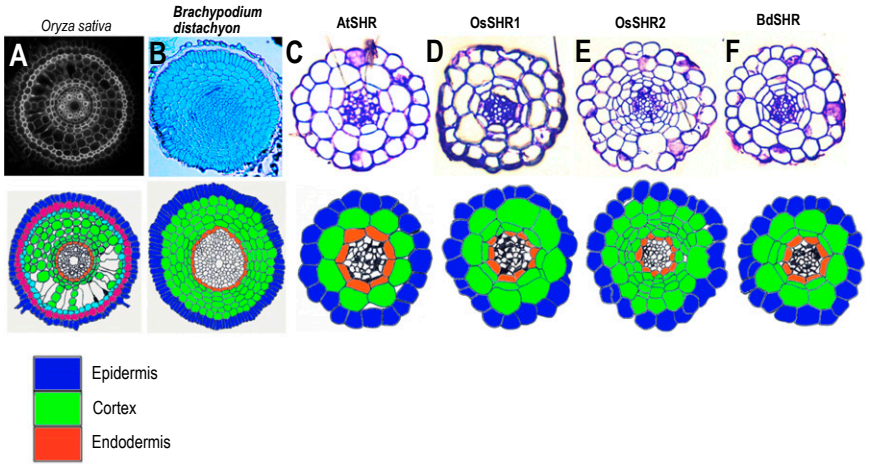

Fig. S1. Root anatomy. (*A*–*C*) Transverse cross-section through the roots of *O. sativa*, *B. distachyon* and *A. thaliana* as indicated. (*D*–*F*) Transverse cross-section through roots of *A. thaliana* expressing the indicated transgenes. Below each root image is a cartoon tracing of the root that has been color coded to indicate the identity of the cell layers.

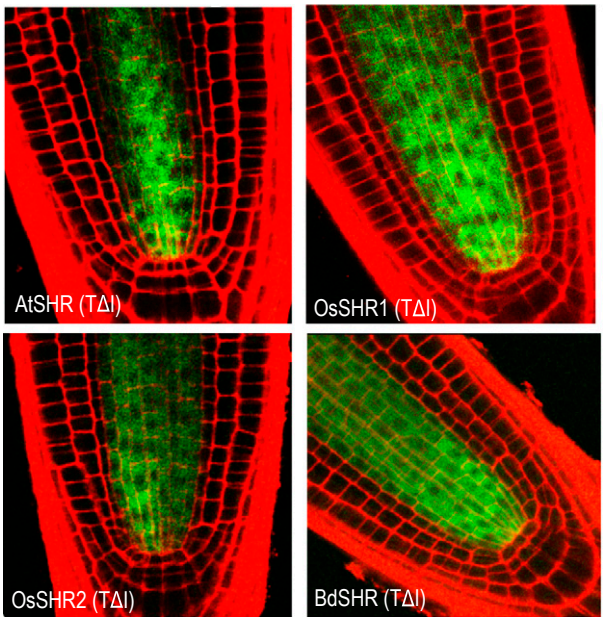

Fig. S2. Full root tip confocal images of the cropped images shown in Fig. 2*A*. All roots express a mutated version of SHR (as labeled) that converts a conserved threonine in the VHIID domain into an isoleucine.

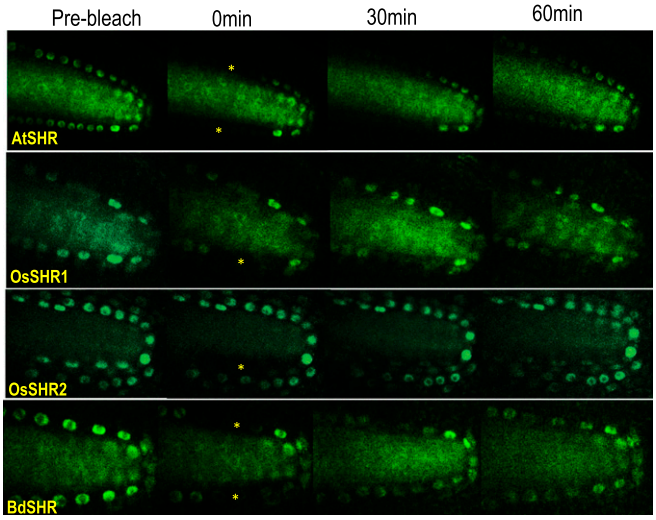

Fig. S3. The monocot SHR proteins recover movement within a similar time frame as AtSHR. Fluorescence recovery after photobleaching (FRAP) of the SHR-YFP signals in the first ground tissue layer was monitored for 60 min. The yellow asterisks mark the cell layers that were photobleached.

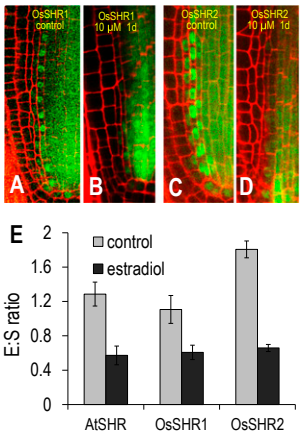

Fig. S4. The monocot SHR proteins move via plasmodesmata (PD). (*A*–*D*) Expression of the icals3m transgene from the WOODENLEG (WOL) promoter blocks PD and movement of the indicated SHR proteins. (*E*) Quantification of the fluorescence ratio (endodermis against stele, E:S) after induction of WOL:icals3m (2, 3).

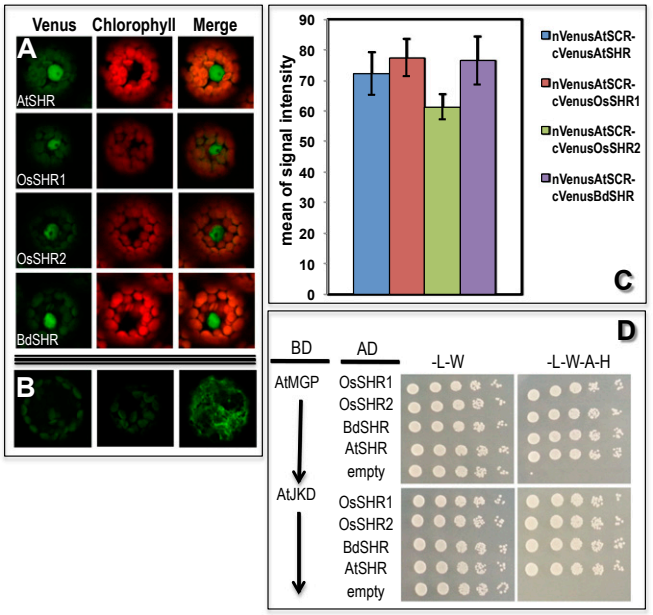

Fig. S5. The interactions of AtSCR and SHR homologs in *Arabidopsis* leaf protoplasts. (*A*) Representative confocal images show BiFC between AtSCR and the SHR homologs in *A. thaliana*, *O. sativa*, and *B. distachyon*. Shown are images of (*Left*) Venus (BiFC), (*Middle*) autofluorescence from chlorophyll, and (*Right*) the overlay. (*B*) Representative images of BiFC controls: (*Left*) empty vectors and (*Middle* and *Right*) representative images of the empty n-Venus vector transfected with SHR c-Venus plasmid. The nonspecific cytoplasmic fluorescence shown in *Right* was absent in the assays using AtSCR n-Venus with the SHR c-Venus constructs. (*C*) Quantitation of BiFC as detailed in *SI Materials and Methods*. Results indicate that the strength of interaction between AtSCR and the SHR homologs from *O. sativa* and *B. distachyon* are not significantly different from the interaction between AtSCR and AtSHR. Each bar represents the average fluorescent intensity in nuclei from 22 to 25 protoplasts. (*D*) Fivefold serial dilutions of diploid yeast expressing AtMGP or AtJKD as bait with the SHR prey proteins (as labeled) growing on selective medium. Medium lacking adenine and histidine are used to select for interaction between the bait and prey proteins. AD, activation domain vector (prey). BD, binding domain vector (bait).

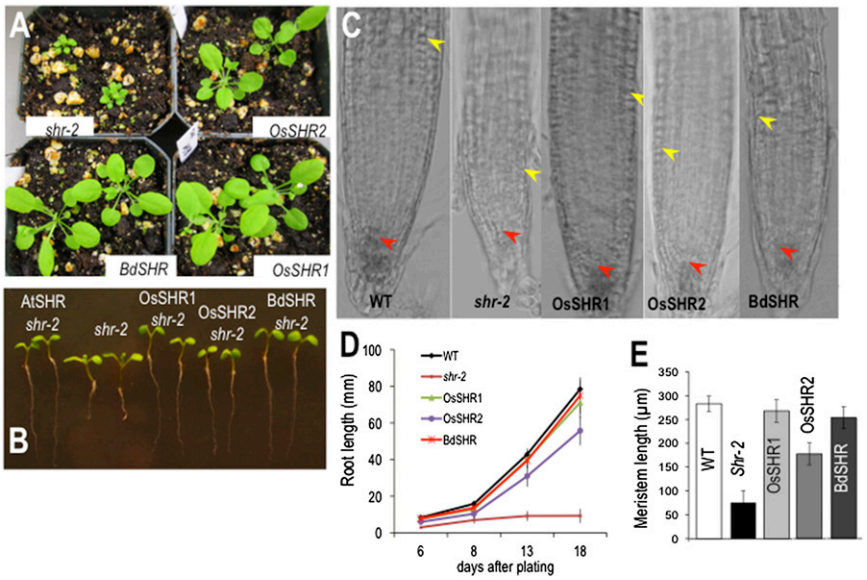

Fig. S6. Complementation of *shr-2* mutants with the SHR homologs. (*A*) Four-week-old *shr-2* seedlings without and with the SHR transgenes as indicated. (*B*) One-week-old *shr-2* seedlings without and with the SHR transgenes as indicated. (*C*) Differential interference contrast (DIC) images of the root meristems of the 6-d-old *shr-2* seedlings and *shr-2* without and with the SHR transgenes as indicated. Red arrowheads point to the QC, and yellow arrowheads mark the initial expansion site in the root. (*D*) Comparison between the growth of the *shr-2* roots without and with the SHR transgenes as indicated at 3, 5, 10, and 15 d after germination ($n = 3$ replicates, 36 roots for each line). Meristem length was defined as the distance between the QC and the first expanded cell in the cortex and is indicated in *E*. Only OsSHR2 and shr-2 are statistically different from wild type (*t* test, $P < 0.005$) ($n = 3$ replicates, 24 roots for each line).

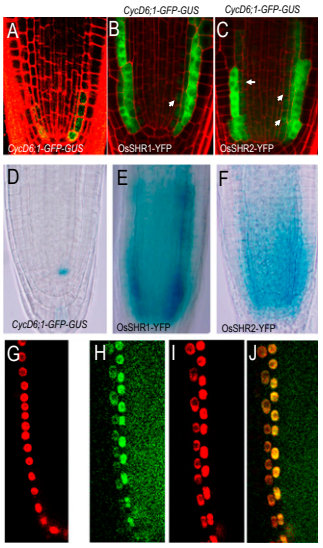

Fig. S7. Expression of *CYCD6;1:GUS-GFP* and SCR-mCherry. (*A*–*C*) Confocal sections through the root meristem showing both the expression of *GUS-GFP* and the SHR-YFP proteins from the CYCD6 ;1 and SHR promoters, respectively. Arrows point to the OsSHR-YFP fluorescence in endodermis. (*D*–*F*) β-Glucuronidase (GUS) staining of the roots without (*D*) and with the SHR transgenes (as indicated). (*G*–*I*) Expression of pSCR:SCR-mCherry in (*G*) wild-type and (*I*) a root expressing OsSHR1 from the SHR promoter. The image in *H* is OsSHR1-YFP, and *J* shows the overlay between *H* and *I*.

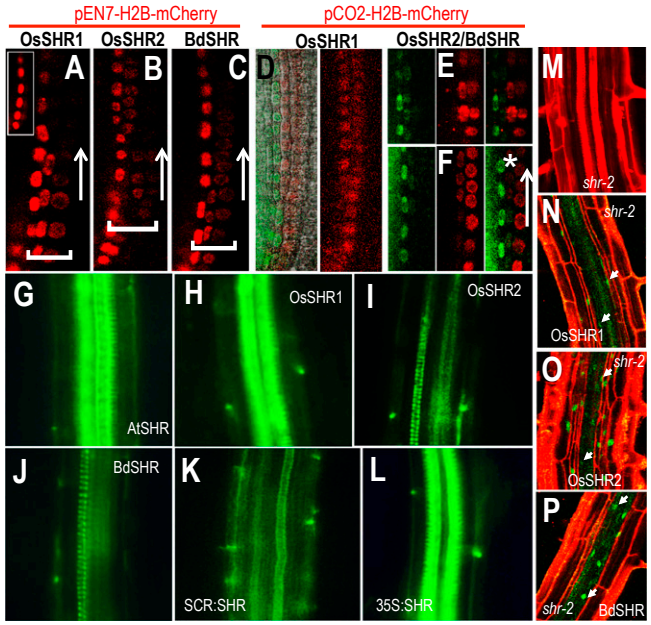

Fig. S8. All roots examined have a single layer of endodermis. (*A*–*C*) Expression of pEN7-HC in the root expressing SHR proteins as labeled. The arrows point shootward. (*D*–*F*) Expression of pCO2-HC in roots expressing the SHR proteins as labeled. The arrows point shootward. The white asterisk marks the cortex cell layer. (*G*–*L*) Lignified Casparian strips visible as green autofluorescence of cell walls (marked by the white arrows). (*M*–*P*) PI staining in *shr-2* roots expressing the SHR proteins as labeled. White arrows point to the nuclear localization of SHR in the endodermis.