Molecules and Cells







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Supplementary Fig. S2. Gene Ontology (GO) analysis of the SHR-regulated DEGs. (A) The enrichment scores of DEGs (fold change > 1.5 and P < 0.05) were categorized according to "Cellular Component" GO terms. The DEGs in cell wall categories are highlighted in red. (B and C) The DEGs in the cell wall categories were further analyzed according to "Molecular Function" (B) and "Biological Process" (C) terms.



Supplementary Fig. S3. Expression analysis of the putative SHR-regulated XTH genes in the hypocotyls of 6-day-old etiolated WT and *shr-2* seedlings. (A) The pie chart indicates the numbers of the putative SHR-regulated XTH genes in the three groups (group I to III) obtained from our genome-wide transcriptome analyses (ATH1 microarrays and RNA-Seq). (B-D) The relative expression levels of group I (B), group II (C), and group III (D) XTH genes in the hypocotyls of 6-day-old etiolated WT and *shr-2* seedlings. *ACTIN2* was used as an internal control. The data are shown as mean \pm SEM of three biological replicates. Statistical significance of differences was determined by Student's t-test compared with the WT control. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.



Supplementary Fig. S4. Expression analysis of the remaining putative SHR-regulated XTH genes. (A-C) The relative expression levels of group I (A), group II (B), and group III (C) XTH genes in the hypocotyls of 6-day-old etiolated WT and *shr-2* seedlings. These XTH genes were not identified from our initial transcriptome analysis. ACTIN2 was used as an internal control. The data are shown as mean \pm SEM of three biological replicates. Statistical significance was determined by Student's *t*-test compared with the WT control. **P* < 0.05; ***P* < 0.01.



Supplementary Fig. S5. Expression analysis of the 11 candidate *XTH* genes upon SHR induction. The down-regulated 11 *XTH* genes (3 in group I, 6 in group II, and 2 in group III) in the *shr-2* hypocotyl were reanalyzed in the etiolated *ProSHR::SHR-GR:shr-2* hypocotyls after 10 μ M DEX treatment at different time points. The levels of *XTH17*, *XTH18*, *XTH22*, and *XTH24* transcripts were evidently promoted upon SHR induction. The data are shown as mean ± SEM of three biological replicates. Statistical significance was determined by Student's *t*-test compared with the ethanol-treated control. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.



Supplementary Fig. S6. Expression analysis of the candidate XTH genes upon SHR induction under DEX and CHX treatments. The transcript levels of the three group II XTH genes (XTH18, XTH22, and XTH24) were elevated in the presence of 10 μ M DEX and CHX (DEX + CHX). The level of XTH17 expression was not significantly altered in the *ProSHR::SHR-GR;shr-2* hypocotyl under the DEX + CHX condition at 6 h. The data are shown as mean ± SEM of three biological replicates. Statistical significance was determined by Student's *t*-test compared with the ethanol-treated control. ***P < 0.001.



Supplementary Fig. S7. Expression of XTH18, XTH22, and XTH24 is detected in the hypocotyl stele. (A-D) The GUS staining patterns of *ProSHR::GUS* (A), *ProXTH18::GUS* (B), *ProXTH22::GUS* (C), and *ProXTH24::GUS* (D) in the 6-day-old etiolated hypocotyls. The expression domains of the three *XTH* genes were largely overlapped with the *SHR* pattern. The epidermis (Ep), cortex (Co), endodermis (En), and stele (St) are indicated. Scale bar = 70 µm.

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Supplementary Fig. S8. Isolation and characterization of *xth18*, *xth22*, and *xth24* reveal that the loss-of-function mutants show no visible phenotypes in the etiolated hypocotyl. (A) The location of T-DNA insertions in the *XTH18*, *XTH22*, and *XTH24* loci. The white boxes represent the coding regions, whereas the lines depict the non-coding regions of the locus. The inverted triangle represents the T-DNA insertion in each *XTH* locus. Black arrowheads indicate the qPCR primer regions of *XTH18*, *XTH22*, and *XTH24*. (B) The transcript levels of *XTH18*, *XTH22*, and *XTH24* in the 6-day-old etiolated WT and mutant hypocotyls. Statistical significance was determined by Student's *t*-test compared with the WT. ****P* < 0.001. (C and D) Hypocotyl growth of the 6-day-old etiolated WT, *xth18*, *xth22*, and *xth24* seedlings. (E and F) Hypocotyl growth of the etiolated WT and triple mutant (*xth18 xth22 xth24*) seedlings. The white arrowheads indicate the hypocotyl-root junction of the seedlings. The data are shown as mean ± SEM (n > 30). Scale bar = 5 mm.



Supplementary Fig. S9. Overexpression of XTH18, XTH22, and XTH24 can promote hypocotyl cell elongation. (A) The expression of the XTH genes were highly up-regulated in the hypocotyls of the etiolated overexpression lines (XTH18-OX #7 and #10, XTH22-OX #6 and #14, and XTH24-OX #8 and #9), respectively. (B and C) Hypocotyl growth of 6-day-old etiolated WT and overexpressor seedlings. The white arrowheads indicate the hypocotyl-root junction of the etiolated seedlings. Scale bar = 5 mm. (D and E) Hypocotyl cell elongation of 6-day-old etiolated WT and overexpressor seedlings. The yellow borders outline the inner cortex cells. Scale bar = 200 μ m. For bar graphs in (C and E), the data are shown as mean ± SEM (n > 30). Statistical significance was determined by Student's *t*-test compared with the WT control. **P < 0.01; ***P < 0.001.

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Supplementary Fig. S10. SHR plays a role in hypocotyl cell elongation in a SCR-independent manner. (A) Hypocotyl growth of 6-dayold etiolated WT, *shr-2*, *ProSHR::SHR-GFP;shr-2 scr-5*, and *scr-5* seedlings. The *scr-5* seedling also showed a short-hypocotyl phenotype, but its hypocotyl length was longer than that of *shr-2*. The *ProSHR::SHR-GFP* transgene in the *shr-2 scr-5* double mutant could promote hypocotyl growth to the level similar to that of *scr-5*. The white arrowheads indicate the hypocotyl-root junction of the etiolated seedlings. Scale bar = 5 mm. (B) Hypocotyl cell elongation of 6-day-old etiolated WT, *shr-2*, *ProSHR::SHR-GFP;shr-2 scr-5*, and *scr-5* seedlings. The yellow borders outline the inner cortex cells. Scale bar = 100 μ m. For bar graphs, the data are shown as mean ± SEM (n > 30). Statistical significance was determined by Student's *t*-test compared with WT or *shr-2*. ****P* < 0.001; ns, statistically not significant.



Supplementary Fig. S11. Arabidopsis graft assay by reciprocally swapping roots and hypocotyls of etiolated WT and *shr-2* seedlings. The hypocotyl length was measured after grafting WT hypocotyls onto *shr-2* root stocks or vice versa. As control experiments, WT hypocotyls onto WT root stocks and *shr* hypocotyls onto *shr* root stocks were grafted. The data are shown as mean \pm SEM (n > 30). Statistical significance of differences was determined using Student's *t*-test compared with the control experiments. ns, statistically not significant.



Supplementary Fig. S12. Restriction of SHR movement is unable to rescue the *shr* root phenotypes. (A-D) Confocal images of 5-dayold light-grown WT (A), *ProSHR::SHR-GFP;shr-2* (B), *shr-6* (C), and *ProSHR::SHR-nlsGFP;shr-6* (D) roots. The nuclear-localized version of SHR in the stele (*ProSHR::SHR-nlsGFP*) failed to restore the root patterning phenotypes of the *shr* seedlings. The root stele (St) is demarcated by double-headed arrows. In (C and D), the innermost layer of the ground tissue is marked with asterisks. The cortex (Co) and endodermis (En) layers are indicated. Scale bar = 100 μ m. (E and F) Root growth of 5-day-old light-grown WT, *ProSHR::SHR-GFP;shr-2*, *shr-6*, and *ProSHR::SHR-nlsGFP;shr-6* seedlings. Scale bar = 5 mm. The data are shown as mean ± SEM (n > 30). Statistical significance was determined by Student's *t*-test compared with *shr-6*. ****P* < 0.001.

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Supplementary Fig. S13. The stele-localized SHR protein in the hypocotyl is able to induce the expression of XTH18, XTH22, and XTH24. (A-C) Relative mRNA abundance of XTH18 (A), XTH22 (B), and XTH24 (C) in the etiolated hypocotyls of WT, *shr-6*, and *ProSHR::SHR-nlsGFP;shr-6* seedlings. The XTH expression levels were elevated by the nuclear-localized *SHR* in the stele (*ProSHR::SHR-nlsGFP*). ACTIN2 was used as an internal control. The data are shown as mean \pm SEM of three biological replicates. Statistical significance was determined by Student's *t*-test compared with *shr-6*. ***P* < 0.01.

Purpose	Name		Sequence (5'→3')	Reference		
Genotyping	shr-2	wt F	GCCACATCATCAACCCCTTCCT	Yoon et al., 2016		
		mt F	IGGIIGIIACIIICGAAIICIICC			
		R	GCCTAGCGAATTTCTCCATTC			
	shr-6	LP	GCCTAGCGAATTTCTCCATTC	Yu et al., 2010		
		RP	TCGTTGACAAACTTGTTGGCC			
	scr-5	wt F	CTCCTCCGATTCAGC	Heo et al., 2011; Paquette and Benfey, 200		
		mt F	CTCCTCCTCCGATTCAGT			
		R	TTGAGTAATCTCGCTGACA			
	xth18-1	LP	ACTATACGAGTGCATGGGTGG	This study		
		RP	CGTGGGCTGTATTCTAGTTGG			
	xth22-2	LP	AACAAAAACCGCGTGATTTC			
		RP	CAAGAAGACTTGCCGTTTGAC			
	xth24-1	LP	GCTTGTTGTGCATTCCTTAGG			
		RP	TCCTCACATTCCTCACCAAAC			
	T-DNA	SALK	ATTTTGCCGATTTCGGAAC	http://signal.salk.edu		
		SAIL	GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC			

Supplementary Table S1. Sequence information for PCR-genotyping primers

Purpose	Na	ime	Sequence (5'→3')	Reference
		F	ATTGGTCTTTGGCTCCCTTT	This study
iti qi cit	71111	P	AGGETTICTGTTCGGAGTCA	This study
	хтнэ	F		Vokovama and Nichitani 2001
	71112	D		
XTH3		E		
		D		
				Loo et al. 2005
	Λ1Π 4	Г D		Lee et al., 2005
	VTUE	Г Г		This study
	XIN3	Г		This study
	VTUC	к г		
	XIN0	Г		
	VTUZ	к г		
	×117/	Г		
	VTUO	к г		
	XIH8	F		
	VTUO	ĸ		
	XIH9	F		
		ĸ	GETEGAALEEAAATAAGETG	
	XIHIO	F	GICIAIGCCGAGGGACIIGA	
		R	GGGIAGGCIACGCCITICICC	
	XTH22	F		This study
		R	ACGAGCCAGIAGIAGICCCC	
	XTH23	F	GAICAACGGCCAGICIICAI	
		R	GGAAGACCTTGAGGGAACCT	
	XTH24	F	TGACACCCATTAGAGAGTTTAAA	
		R	TCTAGCTTGGCTTGTTGAATCCA	
	XTH25	F	ACCTCCCCTTGTAGCCCACT	
		R	ACTTCCTCTGCACCACTCTCAT	
	XTH26	F	GGATCAGTCAGCATCAAGCA	
		R	TCCGGAGGCATAACACCTTTA	
	XTH27	F	TATCGAGCAGTTTCCGAGGT	
		R	CTAAGCCTTTGAGCCTCAGC	
	XTH28	F	AGTATCCTTTGGTCTCTATCTCACATCA	Lee et al., 2005
		R	GCCGTACGTTTGACTTCTCTGA	
	XTH29	F	TAAAGTTTGGTGGTAGTCATCCTAA	Yokoyama and Nishitani, 2001
		R	GATACAACCGGCGTAGACCG	
	XTH30	F	CAAGAAGTCCCCATGGATTG	This study
		R	ATCTTTCTCAGCCGGAACAA	
	XTH31	F	GATAGCTCTAGCTCATGCAG	
		R	TAGACCAAGAAGTTCCTCTGTG	
	XTH32	F	TGGTTCCATATGGGATGCTT	
		R	TCATGGCTTGGTGTTGTTGT	
	XTH33	F	CAACATTCCGGTTAGGCAGTT	This study
		R	TTAACTCCACGTCAGCAACGGA	
	ACTIN2	F	TCGCTGACCGTATGAGCAAAGAA	Yoon et al., 2016
		R	TGGAATGTGCTGAGGGAAGCA	

Supplementary Table S2. Sequence information for RT-qPCR primers

Purpose	Name		Sequence (5'→3')		
ProXTH18::GUS	ProXTH18	F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATATACGTACAACGATACTTGGGGAT		
		R	GGGGACCACTTTGTACAAGAAAGCTGGGTATGGAGGTGTATTGATATCTCAAATGT		
ProXTH22::GUS	ProXTH22	F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGCGAGGACAAAGACCAAAAA		
		R	GGGGACCACTTTGTACAAGAAAGCTGGGTATTTCTAGAGATTGTAGATATT		
ProXTH24::GUS	ProXTH24	F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCAGGCCATAAGATATGGTTAATTACCCTGA		
		R	GGGGACCACTTTGTACAAGAAAGCTGGGTATGGGTGAACAAAAGGAGGCTAATATCAA		
Pro35S::XTH18-OX	XTH18	F	CACCATGAAGCTTTCTTGTGGTAC		
		R	TTAACTGCACTCTACAGGAA		
Pro35S::XTH22-OX	XTH22	F	CACCATGGCGATCACTTACTTGCTTCC		
		R	CTATGCAGCTAAGCACTCTTTAGGA		
Pro35S::XTH24-OX	XTH24	F	CACCATGTCTCCTTTCAAAATATT		
		R	CTATGAGCTTGTTGTGCATT		
ProSHR::SHR-nlsGFP	ProSHR	F	GGGGACAAGTTTGTACAAAAAAGCAGGCTCTGTATCGAGACAAACGAGAAAATCATGATG		
		R	GGGGACAACTTTGTATAGAAAAGTTGGGTGTGTGTGTGTATATTGCATCAGCATC		
	SHR	F	GGGGACAACTTTTCTATACAAAGTTGCTATGGATACTCTCTTTAGACTAGTCA		
		R	GGGGACAACTTTATTATACAAAGTTGTCGTTGGCCGCCACGCACTAG		
	nlsGFP	F	GGGGACAACTTTGTATAATAAAGTTGTAATGGAGCAGAAGCTGATCC		
		R	GGGGACCACTTTGTACAAGAAAGCTGGGTTCTACCCGGACTTGTACAGCTC		
Transient expression	ProXTH18	F	(PSTI) TA <u>CTGCAG</u> TGGCTTGTTTCAATGTGAAAAAGG		
assay (LUC)		R	(BAMHI) TA <u>GGATCC</u> TGGAGGTGTATTGATATCTCAAATGT		
	ProXTH22	F	(PSTI) TA <u>CTGCAG</u> TAAAAACCAACACTTTCCCCC		
		R	(SMAI) TA <u>CCCGGG</u> TTTCTAGAGATTGTAGATATT		
	ProXTH24	F	(PSTI) TA <u>CTGCAG</u> AGTTGCCCTCATAGACAAACATTAAAT		
		R	(BAMHI) TA <u>GGATCC</u> TGGGTGAACAAAAGGAGGCTAATATCAA		

Supplementary Table S3. Sequence information of primers used in molecular work

Purpose	Name		Sequence (5'→3')	
XTH18 promoter regions	-1.9 kb	F	ACAATGAACAATGAGTTCTTCCA	
		R	ACTAAGCTTGAAACAAAACCTGGA	
	-1.6 kb	F	TTCAAGCGCAGTAGGATATAGGT	
		R	AAGATGCCAACCTCTATGGAAGCA	
	-1.4 kb	F	GTTAATGTGCGGATATTTAGTTGAT	
		R	ACGATACAAAAGAGCATAATAGTCGT	
	-1.2 kb	F	TAAACTATACGAGTGCATGGGT	
		R	AGTTTTCTTTACTCGTTTACTGTTACA	
	-0.8 kb	F	TGGCTTGTTTCAATGTGAAAAAGG	
		R	TTGACACGTTTAGTGCGTAGGAA	
	-0.5 kb	F	TGGGAGATCGATTAAAACCAAAAGA	
		R	TGTTCTAATCCTTAGATAAACACCA	
	-0.3 kb	F	TGCAAATATATTTGTCATTCGAACCT	
		R	TGCTGGTCCCGCGTTGAGATT	
XTH22 promoter regions	-2.1 kb	F	GCGAGGACAAAGACCAAAAA	
		R	AATTTGGACGTTTAATACCCAAT	
	-1.8 kb	F	TTGGGTATTAAACGTCCAAATTA	
		R	CCGGTTAGCAGATTAATGACG	
	-1.4 kb	F	GCAAGTCAACTAGACGAGACGA	
		R	TTTGCTAATTTCGGGGGTTA	
	-1.2 kb	F	CACCGCGGTCATGAAATTAT	
		R	ACTTGGTTGGTTGGTTACGG	
	-0.9 kb	F	ACCGTAACCAACCAAG	
		R	TTTTTGTGGTCTCATATTTGAGTTTT	
	-0.4 kb	F	ΤΑΑΑΑΑCCAACACTTTCCCCC	
		R	CTTGGACCGACCTTGACAGA	
XTH24 promoter regions	-2.4 kb	F	TACAGCTGTAAAGTAGTGGGA	
		R	AGCTACGTAATTAAAGCAAACACT	
	-2.1 kb	F	TGGTTTATCATTTATGTTACGGAAG	
		R	ATCATACTGAGTTTTCACATCATTGCC	
	-1.7 kb	F	AGTTGCCCTCATAGACAAACATTAAAT	
		R	ACAGCTGTCCACAGTACTTAA	
	-1.4 kb	F	TACATGTATATCTCAGTGGTTCCGTT	
		R	TGTGCAACCATACCTATAGGTCTA	
	-1.0 kb	F	CTCATTTCATTTCACACAATAATATGG	
		R	AAGCTTACATTTTGTTTGGCA	
	-0.6 kb	F	AGATGCAACCAATTATATAGCAAACGAT	
		R	ATTGAGACAGACTTTTTTGACGTAAA	
	-0.4 kb	F	ATTTTAGTCGGTCATCAGTATCGT	
		R	AGCAGATTATGTTTACCATCCTTA	
SCR promoter region	ProSCR	F	CGTCTTGTCCAATTCCTCTCA	
		R	TCAAAGTGTGGTACGATGTGC	

Supplementary Table S4. Sequence information for ChIP-qPCR primers