

## Control



## Test



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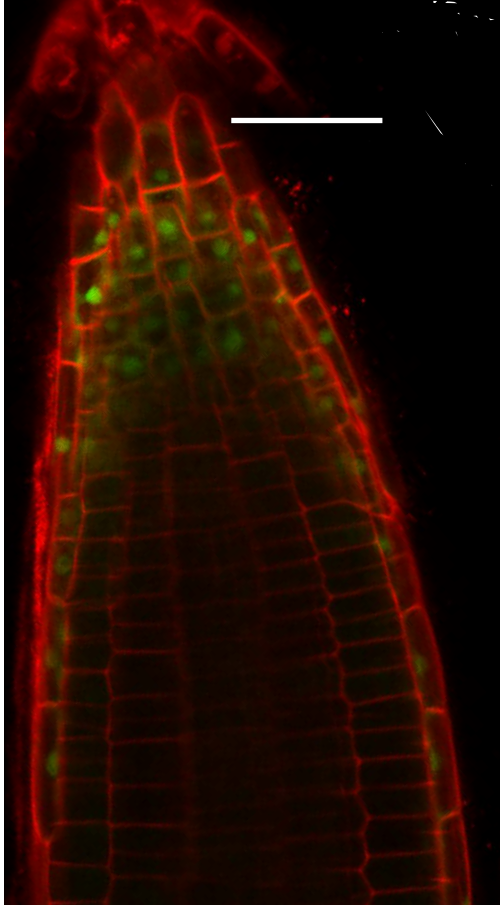


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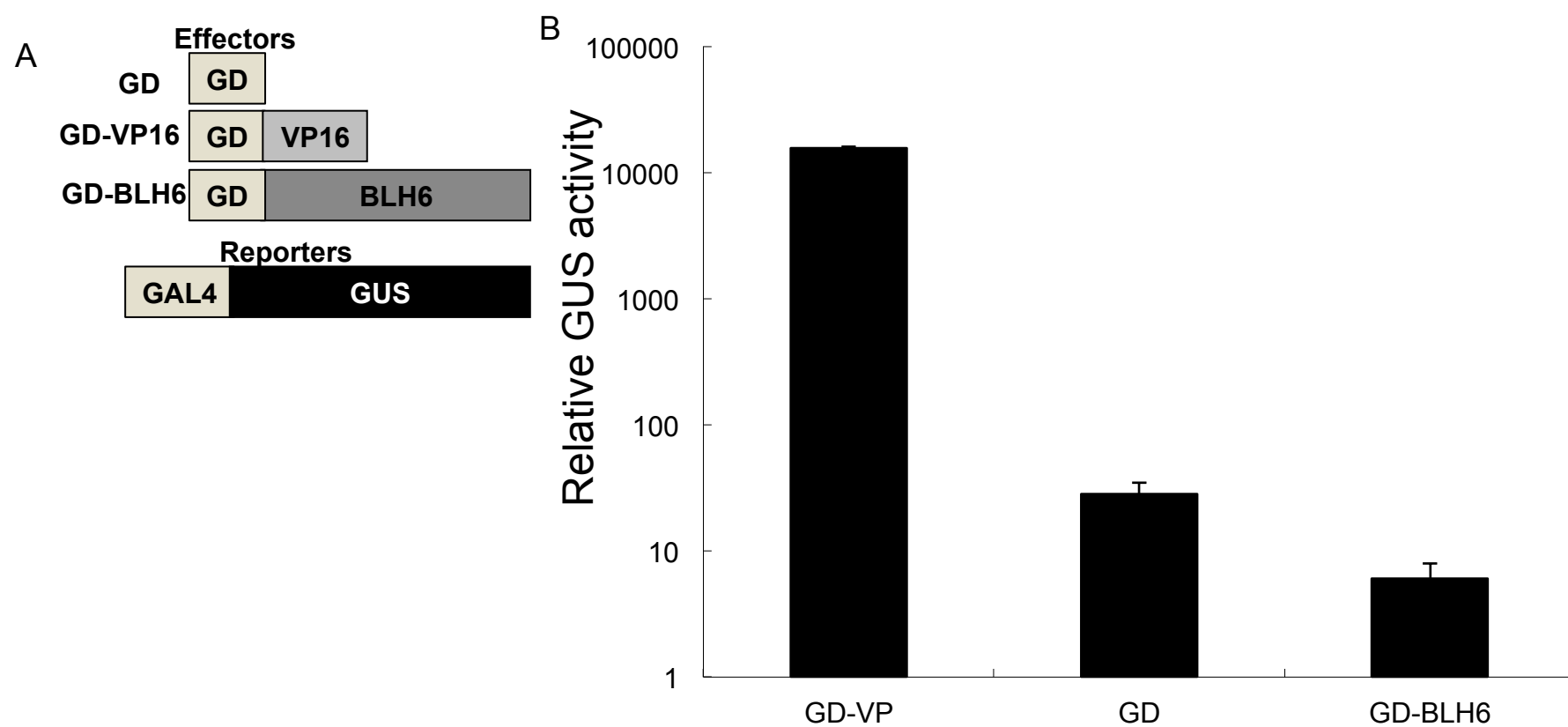


**Supplemental Figure 1.** Schematic diagrams of fusion proteins used for Bimolecular Fluorescence Complementation (BiFC).

All fusions were placed under control of the cauliflower mosaic virus (CaMV) 35S promoter. Fusions of *KNAT7* and *BLH6* to full EYFP were used as controls. The *BLH1*, *BLH5*, *BLH6*, *BLH7*, *BLH10* and *ATH1* coding regions were fused to N-EYFP or C-EYFP, while *KNAT7* was fused to C-EYFP or N-EYFP; both N-terminal and C-terminal fusions of *BLHs* and *KNAT7* were tested. "+" indicates plasmids that were co-transformed into *Arabidopsis* mesophyll protoplasts.



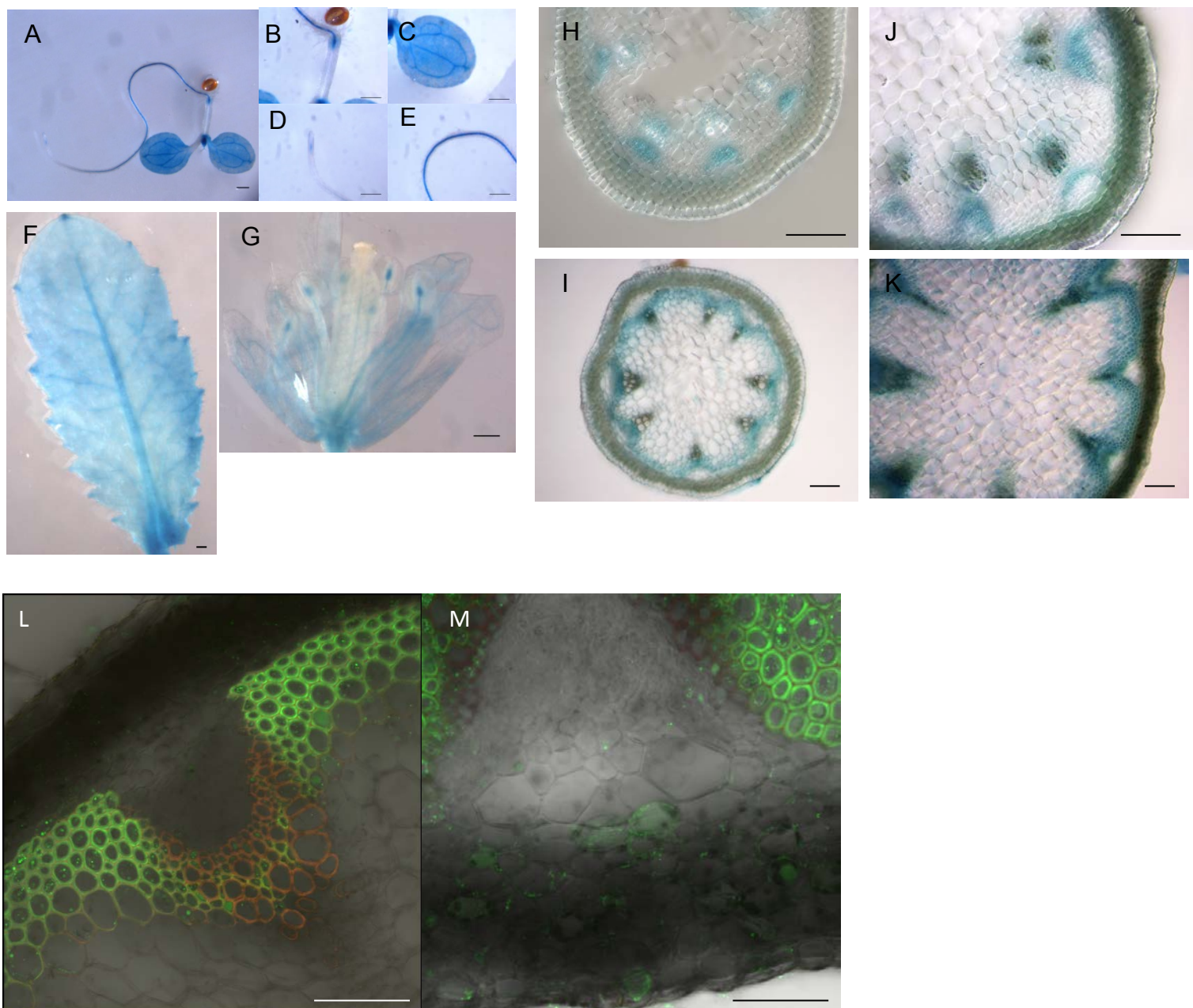
**Supplemental Figure 2.** BLH6 nuclear localization in transgenic lines expressing Pro35S:GFP:BLH6. Longitudinal view of 6-day-old root meristems from a representative line expressing Pro35S:GFP:BLH6. Bar, 50  $\mu$ m.



**Supplemental Figure 3. Test of the transcriptional activity of BLH6**

(A) Effectors and reporter constructs used in the transfection assays.

(B) GUS activity in protoplasts derived from Arabidopsis rosette leaves co-transfected with one of three effector plasmids (GD-VP16, GD alone or GD-BLH6) together with the reporter GAL4: GUS in the absence of transactivator. The GUS Activity of GD-VP16 was used to normalize the other two. Error bars represent the standard deviations of nine replicate transfections. In some cases, error bars are not visible because of the small size of the error bars.



**Supplemental Figure 4.** Histochemical localization of *BLH6* and *KNAT7* promoter activity GUS activity in transgenic *Arabidopsis* lines expressing ProBLH6:GUS was assayed using X-GLUC. Results from representative lines are shown.

(A) 6-day-old whole seedling

(B) Close-up view of hypocotyl

(C) Cotyledon of 6-day-old seedling

(D) Root tip

(E) Close-up view of root showing expression in the stele

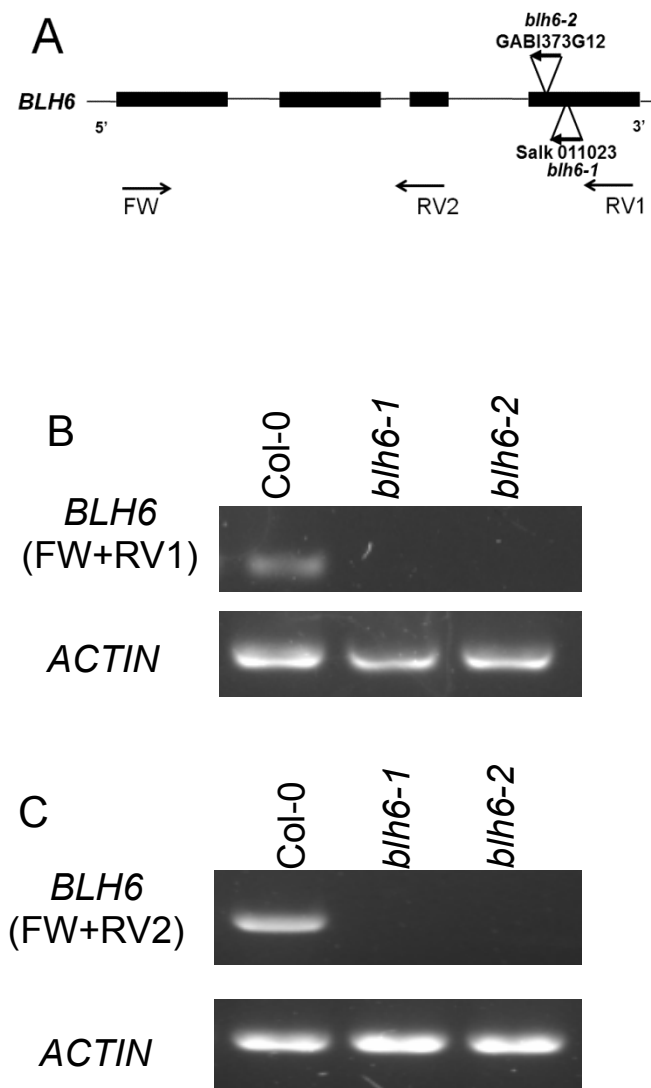
(F) Mature leaves

(G) Flower

(H-J) Hand cross section of inflorescence stem taken from elongating internodes (H, J) and non-elongating internode (I, K) of 6-week-old plants expressing ProBLH6:GUS (H, I) and ProKNAT7:KNAT7-GUS (J, K).

(L, M) Fluorescence image of cross-section from 5cm of the bottom of inflorescence stem of a 6-week-old plant expressing *ProKNAT7:GFP*. Interfascicular fiber, developing xylem and cortex cells are expressing *ProKNAT7:GFP*. Autofluorescence from lignin was given the red color.

Bars, A-G= 400  $\mu$ m; H-M=100  $\mu$ m.



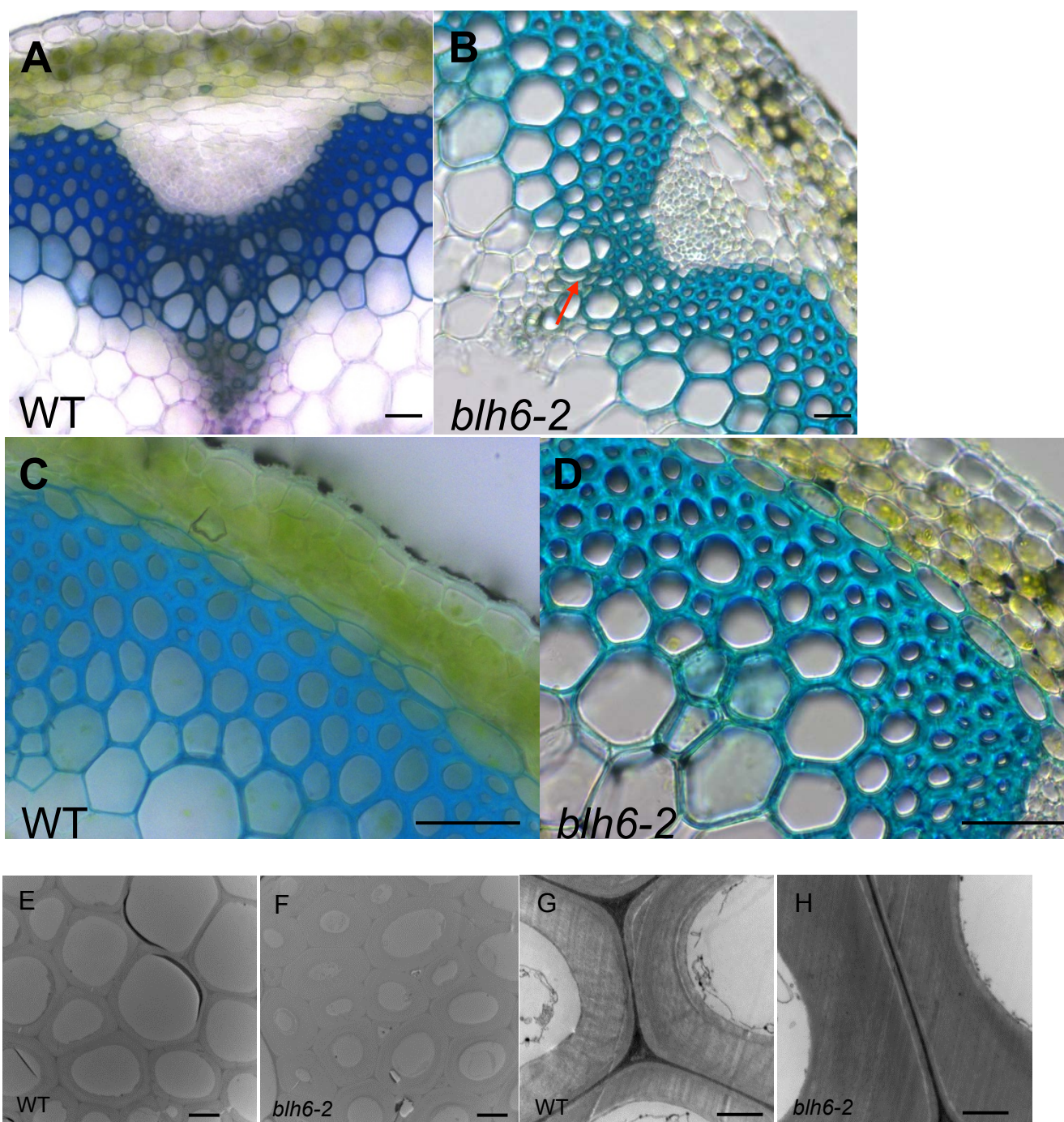
**Supplemental Figure 5.** Schematic diagram of *BLH6* gene structure and the position of T-DNA insertions

(A) *BLH6* gene model with black boxes representing exons and the 5' to 3' orientation shown. Locations of T-DNA insertions in *blh6-1* (SALK-011023) and *blh6-2* (GABI1373G12) alleles are shown. Locations of primers used to assay *BLH6* expression in the mutant backgrounds are shown by arrows below the exons.

(B) Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of *BLH6* expression using primers FW and RV1 in the wild-type (Col-0), *blh6-1* and *blh6-2*. No transcript was detected in the *blh6-1* and *blh6-2* mutants. *ACTIN2* was used as a positive control.

(C) Quantitative RT-PCR using primers FW and RV2 for amplifying the first three exons by the primers of FW and RV2 showed that *BLH6* mRNA was undetectable in the *blh6-1* and *blh6-2* mutants. *ACTIN2* was used as a positive control.





**Supplemental Figure 6.** Anatomy of the vascular bundles and interfascicular regions of wild type and *blh6-2* inflorescence stems.

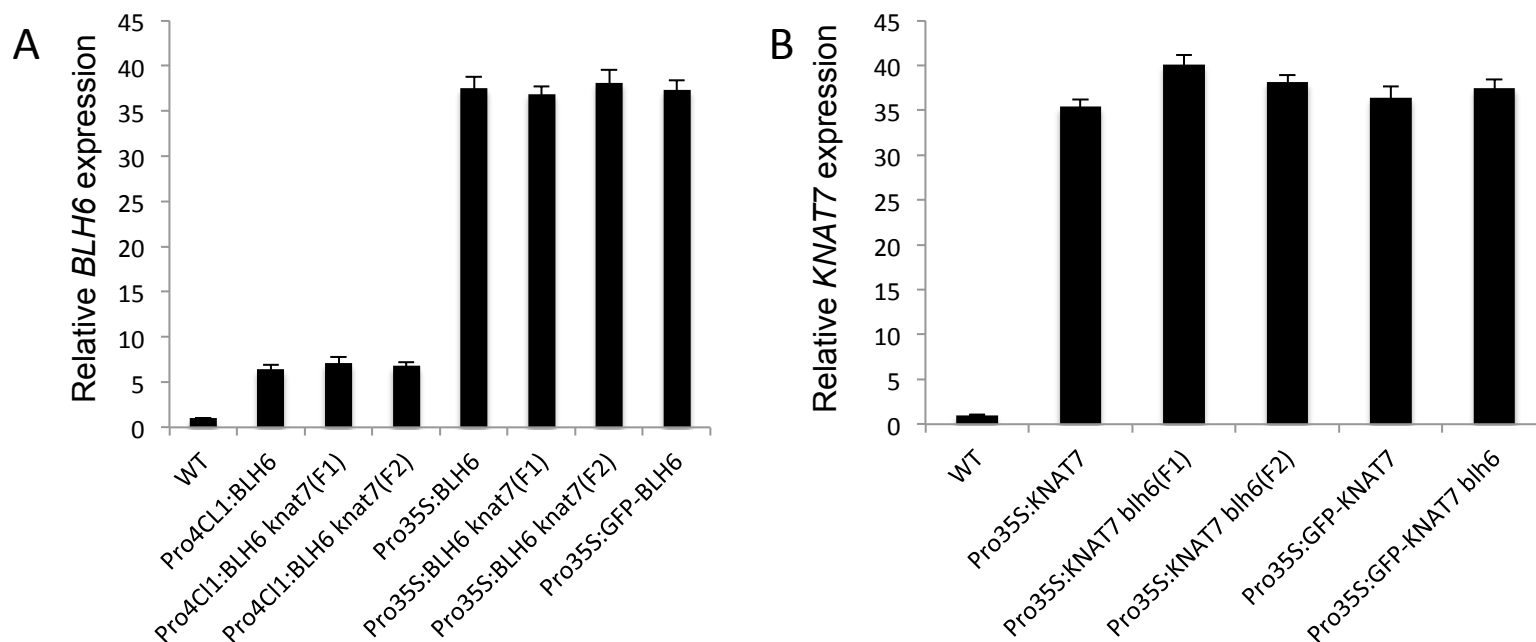
(A-D) Stem cross sections were taken from the bases of the inflorescence stems of 6-week-old plants and stained with toluidine blue and examined for alterations in secondary wall thickness. (A, C) WT (B, D) *blh6-2*. Bars, 30  $\mu\text{m}$ . Arrow indicates the *irx* vessels.

(E-H) Transmission electron microscopy of WT and *blh6-2* interfascicular fiber secondary walls. (E, G) WT; (F, H) *blh6-2*.

Bars, 10  $\mu\text{m}$  (E, F); 2  $\mu\text{m}$  (G,H).

(A, B) Cross section showing vascular bundles and interfascicular fiber regions.

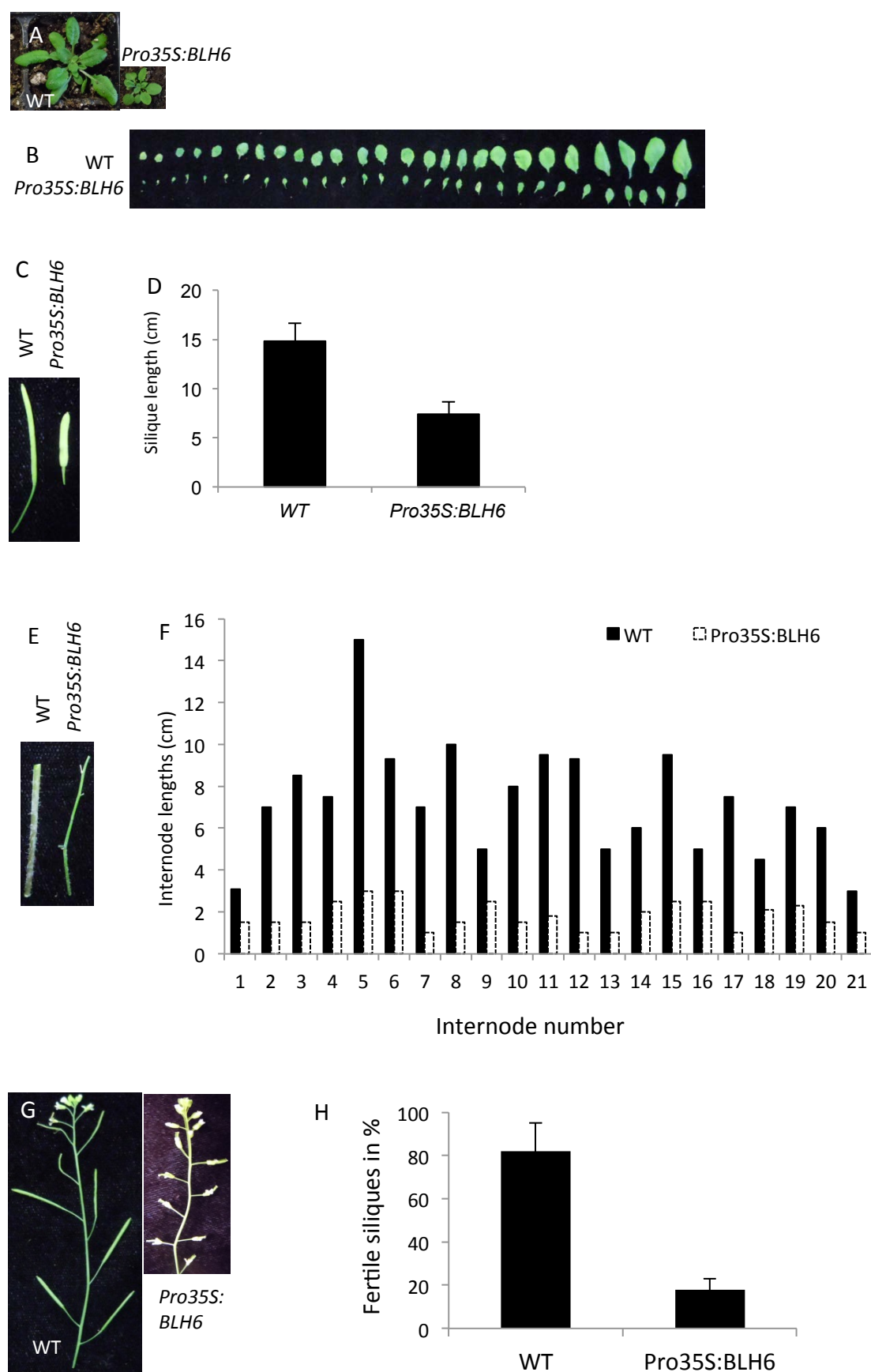
(C- H) Cross section interfascicular showing fiber regions at higher magnification.



**Supplemental Figure 7.** Analysis of *BLH6* and *KNAT7* expression driven by *35S* or *4CL1* promoters in wild-type and *blh6* and *knat7* mutant backgrounds.

(A) Quantitative real-time PCR analysis of *BLH6* expression in representative wild-type (WT; Col-0), *Pro4CL1:BLH6*, *Pro4CL1:BLH6 knat7* (F1 and F2), *Pro35S:BLH6*, and *Pro35S:BLH6 knat7* (F1 and F2) lines.

(B) Quantitative real-time PCR analysis of *KNAT7* expression in representative wild-type (WT; Col-0), *Pro35S:KNAT7*, and *Pro35S:KNAT7 blh6* (F1 and F2) lines. Error bars represent the standard deviations, n=9 (three technical replicates from three biological replicates).



**Supplemental Figure 8.** Quantitative measurement of morphological differences between *Pro35S:BLH6* and wild type plants.

(A) Representative 2-week-old wild-type and *Pro35S:BLH6* plants.

(B) Rosette leaves of a representative 6-week-old WT and *Pro35S:BLH6* plants; leaf number is similar but *Pro35S:BLH6* leaves are much smaller.

(C) Mature siliques from wild-type and *Pro35S:BLH6* plants.

(D) Lengths of mature siliques from representative wild-type and *Pro35S:BLH6* plants. Error bars represent standard deviations of 30 replicates.

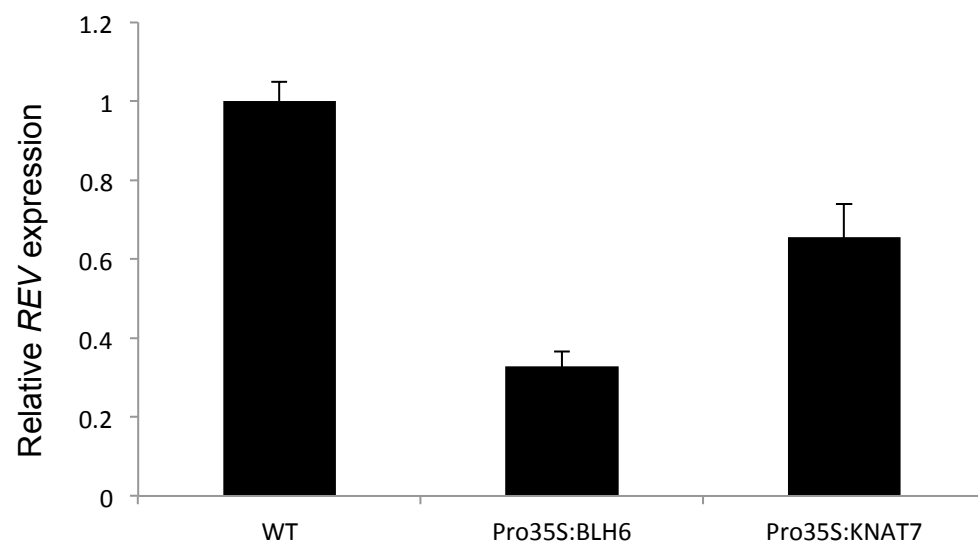
(E) Comparison of 6-week-old wild-type and *Pro35S:BLH6* inflorescence stems.

(F) Inflorescence stem internode lengths (node 1 defined as the node with the first silique). Solid bars, representative wild-type plant; Open bars, representative *Pro35S:BLH6* plant.

(G) Close-up view of the main stem of wild-type and *Pro35S:BLH6* plants.

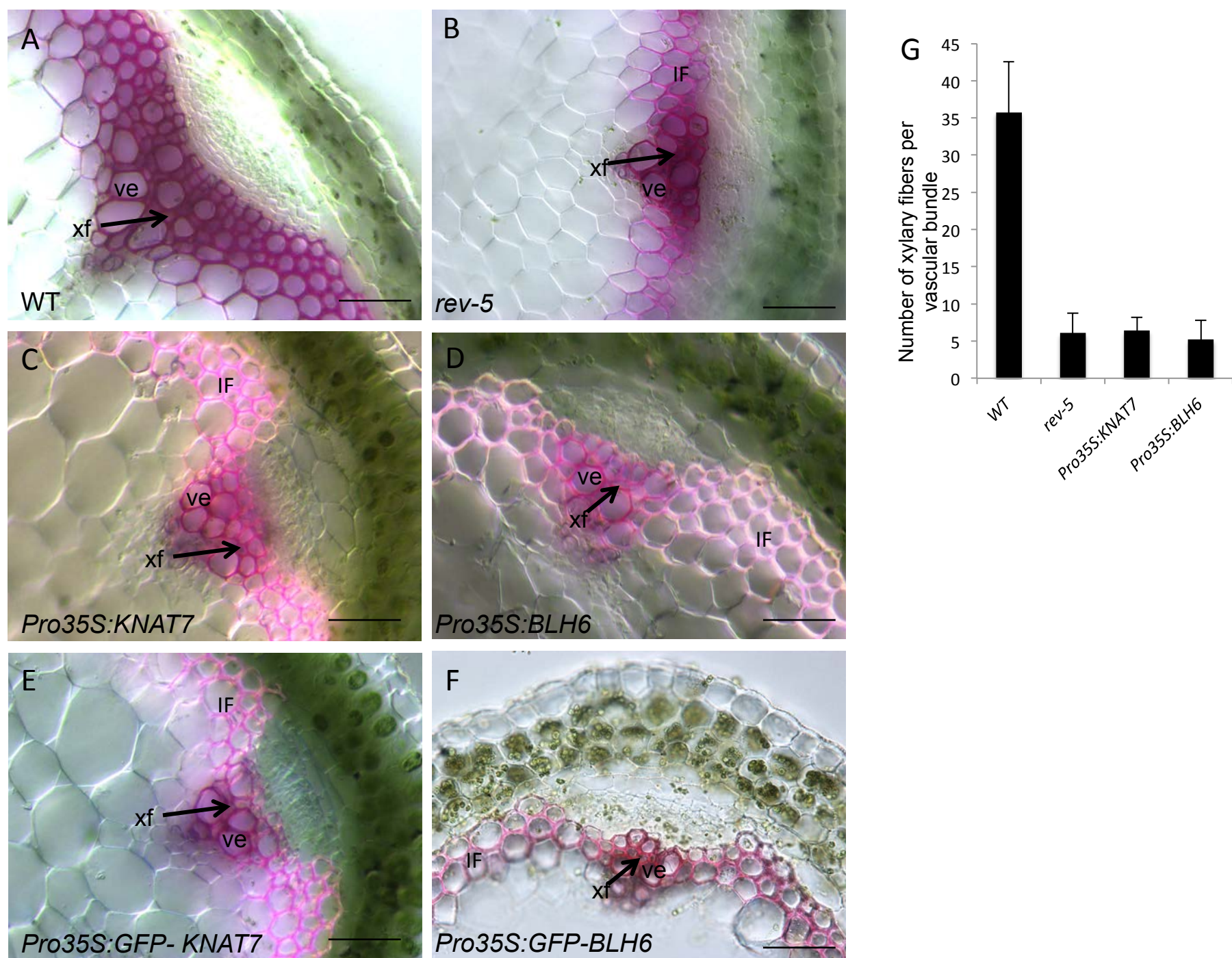
(H) The percentage of fertile siliques per mature stem of wild type and *Pro35S:BLH6* plants.





**Supplemental Figure 9.** *REV* expression is repressed in *BLH6* and *KNAT7* overexpression backgrounds<sup>9</sup>

Quantitative real-time PCR of *REV* expression in *Pro35S:BLH6* and *Pro35S:KNAT7*. Total RNA from three biological replicates (each consisting of pooled stems from four to six plants) was isolated from the whole stems of WT, *Pro35S:BLH6* and *Pro35S:KNAT7*. The expression level of *REV* in the WT background was set to unity. Error bars represent the standard deviations, n=9 technical replicates from three biological replicates.



**Supplemental Figure 10.** The vascular bundles of plants expressing *Pro35S:KNAT7*, *Pro35S:GFP-KNAT7*, *Pro35S:BLH6*, and *Pro35S:GFP-BLH6* phenocopy those of the *rev-5* mutant.

Stem cross sections were taken from 3 cm of the bases of the inflorescence stems of 6-week old plants and stained with phloroglucinol and examined for alterations in anatomy and secondary wall thickness.

(A) Representative cross-sections from wild-type control stems stained with phloroglucinol.

(B) Representative cross-section from a *rev-5* showing thinner secondary cell wall of interfascicular fibers relative to wild-type.

(C) Representative cross-section from a *Pro35S:KNAT7* over-expression line showing fewer xylary fibers in the vascular bundle relative to wild-type.

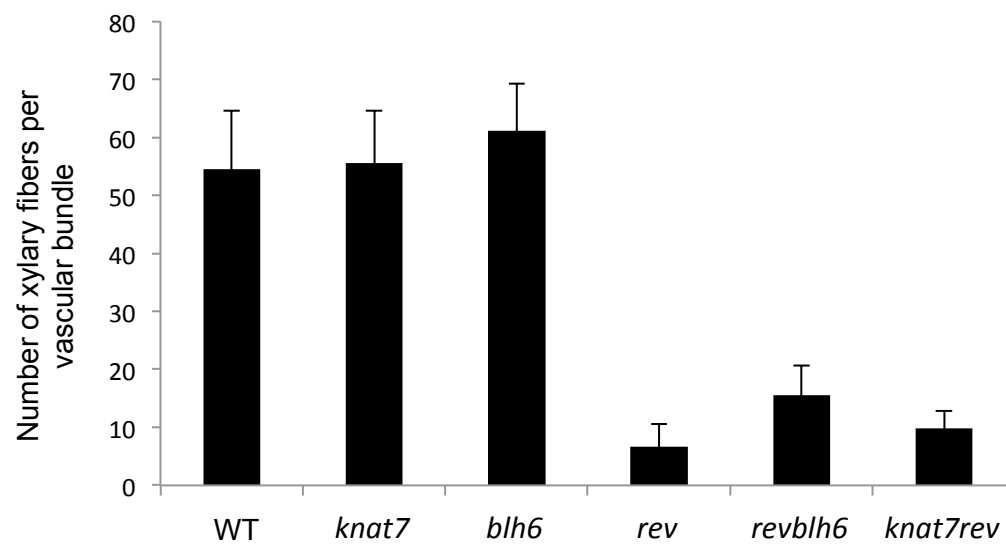
(D) Representative cross-section from a *Pro35S:BLH6* over-expression line showing fewer xylary fibers in vascular bundles.

(E) Representative cross-section from a *Pro35S:GFP-KNAT7* over-expression line showing fewer xylary fibers in the vascular bundle and thinner secondary cell wall in IF relative to wild-type.

(F) Representative cross-section from a *Pro35S:GFP-BLH6* over-expression line showing fewer xylary fibers in the vascular bundle and thinner secondary cell wall in IF relative to wild-type.

(G) Measurements of xylary fiber cell numbers in the vascular bundles of *rev* and overexpression lines compared with the wild-type. Error bars represent the standard deviations,  $n=24$  vascular bundles.

ve, vessel; xf, xylary fibers; IF, interfascicular region. Bars, 50  $\mu\text{m}$ .



**Supplemental Figure 11.** Xylary fiber cell numbers in the vascular bundles of wild-type (Col-0), *rev*, *rev blh6* and *rev knat7* lines. Error bars represent the standard deviations, n = 24 vascular bundles per genotype.

**Supplemental Table 1.** Summary of Bimolecular Fluorescence Complementation (BiFC) assays with BLH candidates and KNAT7.

N-EYFP fusion protein	C-EYFP fusion protein	Interaction result <sup>a</sup>
KNAT7	ATH1	- <sup>b</sup>
KNAT7	BLH1	-
KNAT7	BLH7	-
KNAT7	BLH10	-
KNAT7	BLH5	-
KNAT7	BLH6	+ <sup>c</sup>
ATH1	KNAT7	-
BLH1	KNAT7	-
BLH7	KNAT7	-
BLH10	KNAT7	-
BLH5	KNAT7	-
BLH6	KNAT7	+
Empty	BLH6	-
BLH6	Empty	-

<sup>a</sup> To test for interaction, 12 independent transformations were performed for each combination, and at least 30 cells per combination were screened.

<sup>b</sup> -, no interactions detected.

<sup>c</sup> +, interactions detected. Signal was detected in 134 cells in total, in at least 11 cells per each transformation event.

**Supplemental Table 2.** Cell wall sugar composition in the lower stems of the wild type and mutant plants

	Sugar amount <sup>1</sup>						
	fructose	arabinose	rhamnose	galactose	glucose	xylose	mannose
Wild-type	3.5±0.2 <sup>2</sup>	24.1±0.1	16.9±0.2	37.1±0.4	588.6±12.0	198.0±5.2	40.3±0.1
<i>blh6</i>	3.4±0.2	25.3±0.8	17.3±0.2	40.2±0.3	628.3±10.4	210.1±2.8	41.1±1.0
<i>knat7</i>	3.2±0.3	22.1±0.7	16.6±0.8	31.7±1.1	591.4±14.6	210.5±4.5	31.1±1.1
<i>blh6 knat7</i>	3.3±0.1	22.8±1.0	17.3±0.2	35.6±1.1	594.7±12.5	202.1±3.8	36.0±0.8
<i>Pro4CL1: BLH6</i>	3.5±0.0	24.0±0.7	16.8±0.5	36.1±0.8	633.8±23.3	223.6±14.0	43.0±1.0

<sup>1</sup> Determined by HPLC; mg per 100 mg dry weight

<sup>2</sup> Standard deviation of measurements taken from three biological replicates

**Supplemental Table 3.** Interfascicular fiber cell wall thickness in wild-type, single mutant, *rev blh6*, and *rev knat7* plants

Genotype	Cell wall thickness <sup>1</sup>
Wild-type	2.32±0.59
<i>blh6</i>	3.89±0.76 <sup>2</sup>
<i>knat7</i>	3.77±0.93 <sup>2</sup>
<i>rev</i>	1.09±0.29 <sup>2</sup>
<i>rev blh6</i>	1.47±0.39 <sup>2,3</sup>
<i>rev knat7</i>	1.33±0.34 <sup>2,3</sup>

<sup>1</sup> Mean cell wall thickness in  $\mu\text{m} \pm \text{SD}$ ; n = 50 cells

<sup>2</sup> Significantly different from wild-type, p < 0.01

<sup>3</sup> Not significantly different from *rev*, p > 0.2



**Supplemental Table 4.** Primer sequences to generate truncated KNAT7, BLH6 and BLH7 clones for yeast two-hybrid assay.

Constructs	Primers
ADH1:Gal4 AD-KNAT7	ATGCAAGAAGCGGCACTAGG TTAGTGTTTGCCTTGGACTT
ADH1:Gal4 AD-KNOX1	ATGCAAGAAGCGGCACTAGG CTAAGCGTAAGAACGGAGAAG
ADH1:Gal4 AD-KNOX2	ATGCGTTCTTACGCTTCCACG TTACCCTTCTCCTAAAGTTGC
ADH1:Gal4 AD-MEINOX	ATGCAAGAAGCGGCACTAGG TTACCCTTCTCCTAAAGTTGC
ADH1:Gal4 AD-ELK-Homeodomain	ATGGAAAGAGTCAGACAAGAA TTAGTGTTTGCCTTGGACTT
ADH1:Gal4 DBD-BLH6	ATGGAGAATTATCCAGAAACACA TCAAGCTACAAAATCATGTACCAA
ADH1:Gal4 DBD-SKY	GAGAATTATCCAGAAACACAG CTCTGCCTGAAACTGCTTTAG
ADH1:Gal4 DBD-BELL	GGAGATAAAAACAATGAGTAAC AGGTTGCATAAAACCTCTTTG
ADH1:Gal4 DBD-MID (shorter)	GATGTTGTGAGAACAATTCCC TCCGGATATCGCATCCCTTAG
ADH1:Gal4 DBD-MID (longer)	GAGAATTATCCAGAAACACAG AGGTTGCATAAAACCTCTTTG
ADH1:Gal4 DBD-Homeodomain	CAAGCTTGGAGACCTCAACGC TTCGGAAGACGAGTTGGAATC
ADH1:Gal4 DBD-Homeodomain+ VSLTLGL box	CAAGCTTGGAGACCTCAACGC AGCTACAAAATCATGTACCAA
ADH1:Gal4 DBD-BLH7	ATGGCCACTTATTACAAAACCTGG TCAAGCTACAAAATCATGCAACAA

**Supplemental Table 5.** Primers used for quantitative RT-PCR gene expression and Chromatin Immunoprecipitation analysis.

Gene ID	Name	Primer sequence (5'-3')
At2g37040	PAL1	PAL1-L: AAGATTGGAGCTTTCGAGGA
		PAL1-R: TCTGTTCCAAGCTCTTCCCT
At3g53260	PAL2	PAL2-L: GAGGCAGCGTTAAGGTTGAG
		PAL2-R: TTCTCGGTTAGCGATTCACC
At3g10340	PAL4	PAL4-L: GGGAAACCGGAGTTTACAGA
		PAL4-R: AGAGGATCCATTTTCGTGGAG
At2g30490	C4H	C4H-L: ACTGGCTTCAAGTCGGAGAT
		C4H-R: ACACGACGTTTCTCGTTCTG
At1g51680	4CL1	4CL1-L: TCAACCCGGTGAGATTTGTA
		4CL1-R: TCGTCATCGATCAATCCAAT
At5g48930	HCT	HCT-L: GCCTGCACCAAGTATGAAGA
		HCT-R: GACAGTGTTCCCATCCTCCT
At2g40890	C3H1	C3H1-L: GTTGGACTTGACCGGATCTT
		C3H1-R: ATTAGAGGCGTTGGAGGATG
At4g34050	CCoAOMT1	CCoAOMT1-L: CTCAGGGAAGTGACAGCAAA
		CCoAOMT1-R: GTGGCGAGAAGAGAGTAGCC
At1g15950	CCR1	CCR1-L: GTGCAAAGCAGATCTTCAGG
		CCR1-R: GCCGCAGCATTAATTACAAA
At4g36220	F5H1	F5H-L: CTTCAACGTAGCGGATTTCA
		F5H-R: AGATCATTACGGGCCTTCAC
At5g54160	COMT1	COMT1-L: TTCCATTGCTGCTCTTTGTC
		COMT1-R: CATGGTGATTGTGGAATGGT
At4g34230	CAD5	CAD5-L: TTGGCTGATTCGTTGGATTA
		CAD5-R: ATCACTTTCCTCCCAAGCAT
AT2G38080	LAC4	LAC4-L: GGTGGATGGGTCGTCATGGAGTTC
		LAC4-R: CGTGGCGTGATGTTGATATGTAGCGC
At4g32410	CesA1	CesA1-L: GGTATTTATTGCGGCAACCT
		CesA1-R: ATCCAACCAATCTCTTTGCC
At5g05170	CesA3	CesA3-L: ACAGCCAACACAGTGCTCTC
		CesA3-R: TGGTACCCATTTACGAGCAA
At2g21770	CesA6	CesA6-L: TGCCCTTGAGCACATAGAAG
		CesA6-R: GCACTCCACCATTTAGCAGA
At5g44030	CesA4	CesA4-L: GGATCAGCTCCGATCAATT
		CesA4-R: ACCACAAAGGACAATGACGA
At5g17420	CesA7	CesA7-L: CAGGCGTACTCACAAATGCT
		CesA7-R: TGTCAATGCCATCAAACCTT
At4g18780	CesA8	CesA8-L: ACGGAGAGTTCTTTGTGGCT
		CesA8-R: GGTCTGTGTTGGAACAATGG
At5g54690	IRX8	IRX8-L: GTGGTCACAGGGAAAGGATT
		IRX8-R: AGCAAGAGAGGAGCAAGGAG
At2g37090	IRX9	IRX9-L: TTTGCGGGACTAAACAACAT

At2g37090	IRX9	IRX9-L: TTTGCGGGACTAAACAACAT
		IRX9-R: ATCGGAGGCTTTGTCTCTGT
At1g27440	IRX10	IRX10-L:AATTGGCCTTATTGGAATCG
		IRX10-R: TTCGTCCAAACAGACATGG
At2g28110	FRA8	FRA8-L: GACTTGTTGAATCGGTGGCTC
		FRA8-R: GAAAGAGTTTGACCTTCTAAC
AT1G27600	IRX9L	IRX9L-L: CCGCCAAGTACACACAGTGA
		IRX9L-R: AGCCTGCAGATTCTTTTGGGA
At5g60690	REV	REV-L: CCAAGCTGTGAATCTGTGGTC
		REV-R: CGATCTTTGAGGATCTCTGCA
		ProREV1-L: TACGACGGACTTTCCGAAGA
		ProREV1-R: ACTGCTTCTCTCACGGTGGT
		ProREV2-L: TCCTTCTTCACTTTCTCACATAACC
		ProREV2-R: TGAAGGACACAGAAACCTTAGGA
		ProREV3-L: AAAAACACATCAACAGAGTAAAAACA
		ProREV3-R: TGATATCAGCCAATCACAACA
		ProREV4-L: TTACAAGCACAGCTTCAATAGCA
		ProREV4-R: AAAAATGAAGTTATGCACGGGTA
		ProREV5-L: TTCACTCAAGGTCCTACTCTTTGA
		ProREV5-R: TTCTCTCACTATCCCTTTGTGG
AT3G18780	ACTIN2	ACTIN2-L: CCTGAAAGGAAGTACAGTG
		ACTIN2-R: CTGTGAACGATTCCTGGAC
		ProACTIN2-L: TGTCGTACGTTGAACAGAAAGC
		ProACTIN2-R: CCGGTACCATTGTCACACAC