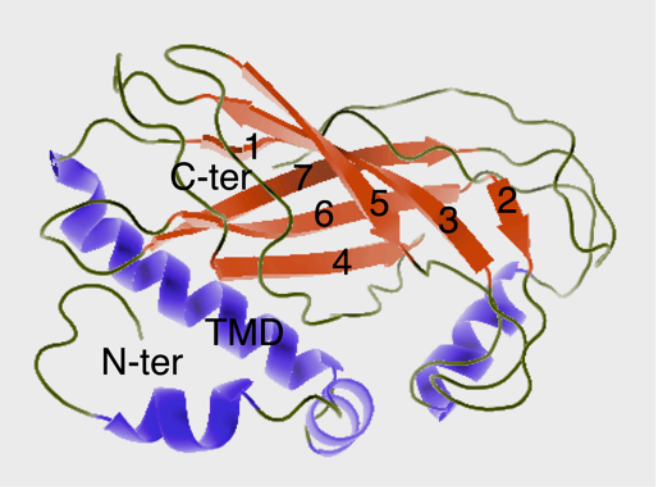
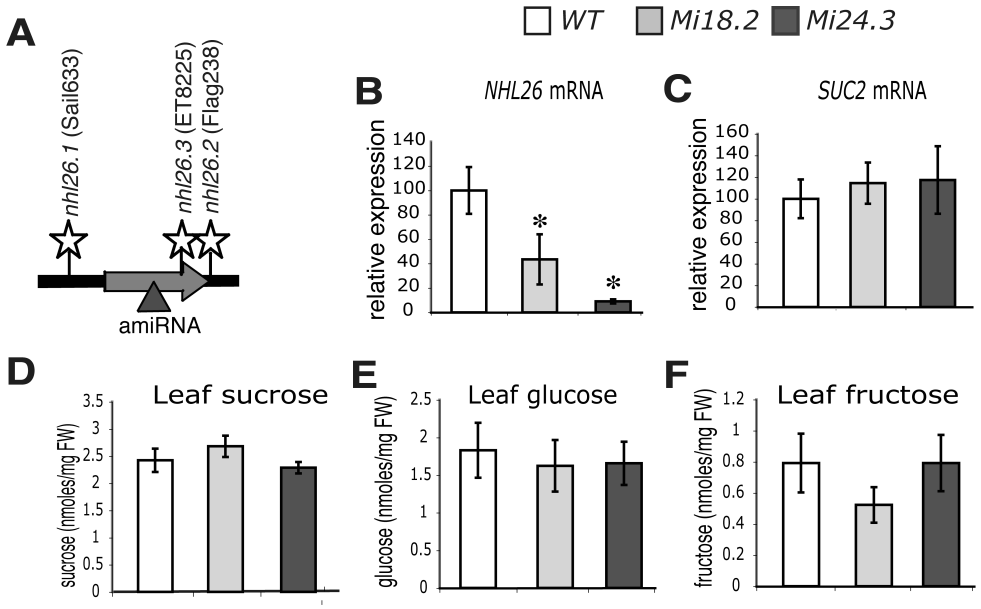


Supplemental Figure 1

Predicted 3D model of NHL26 structure

Three-dimensional model of NHL26. This model is based on structural similarities with LEA14 and includes a predicted α-β fold for NHL26 consisting of parallel and anti-parallel β-strands. N- and C-terminal ends are indicated (N-ter and C-ter). The seven β-sheets are numbered from N- to C-ter positions. The transmembrane domain (TMD) characterized by a hydrophobic α-helix fold is indicated. The N-terminal region is predicted to lie in the cytosol.



Supplemental Figure 2

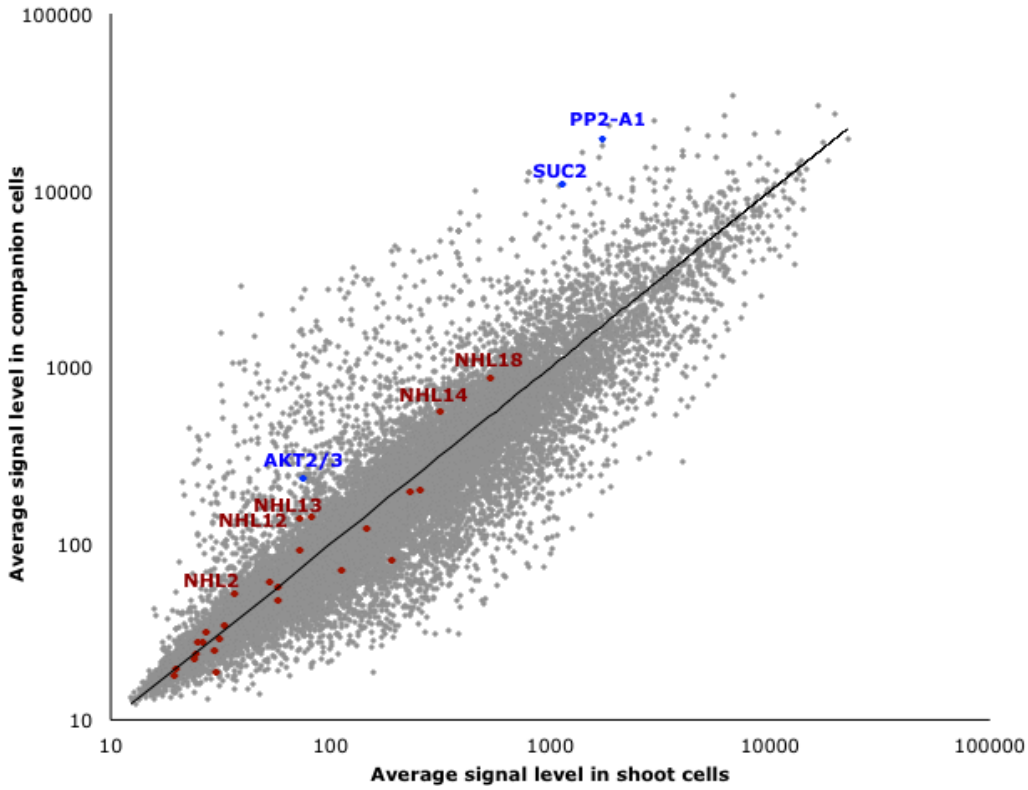
Characterization of *nhl26* mutants and *NHL26*-overexpressing lines
These data reveal that amiRNA lines showed a significantly lower than WT accumulation of *NHL26* mRNA. However, the regulation of *SUC2* and the sugar content in the source leaves were unaffected.

(**A**) Map indicating the position of the insertions in the *nhl26* mutants (top labels) and of the sequence targeted by the amiRNA (bottom label). The mutants are *nhl26-1* (line Sail633, in Col-0); *nhl26-2* (line Flag238, in Ws4) and *nhl26-3* (ET822, in Landsberg Erecta).The sets of primers used for the genotyping of these mutants were as follows:
For the Sail mutant: 5'-CGG TCGTTCTTTTGCTTTATG-3'; 5'-TGAAAAGCTGCTTTTGTGGAG-3' and 5'-TCCATA ACCAATCTCGATACA C-3'. For the FLAG mutant, 5'-AAAGGGCTTTACTTAATTCTT CCC-3'; 5'-GGATTAACATCAACAATCGCC-3' and 5'-CGATCCAGACTGAATGCCC-3'.
For the Lansberg Erecta mutant: 5'-GCGCCAAGAAAGGAGGGATTAA-3' and 5'-AATGAAGCTAAGGGAGGAGTGG-3' and 5'-CGATCCAGACTGAATGCCC-3'.

(**B**) Relative expression of *NHL26* in WT, two representative amiRNA lines. *NHL26 m*RNA accumulation in the rosette leaves was assessed by RT-qPCR. Expression was normalized relative to levels of *TIP41* RNA. Values are expressed as percentages of wild-type values. Error bars indicate the SEM (*n*=12).

(**C**) Relative expression of *SUC2* in WT and amiRNA lines. *SUC2* RNA accumulation in the rosette leaves was assessed by RT-qPCR. Expression was normalized relative to levels of *TIP41* RNA. Values are expressed as percentages of wild-type values. Error bars indicate +/- SE (*n*=12).

(**D**) to (**F**) Sugar content in the rosette leaves in WT and the amiRNA lines. (D): sucrose content; (E): glucose content; (F): fructose content. Error bars indicate +/- SE (*n*=8).



Supplemental Figure 3

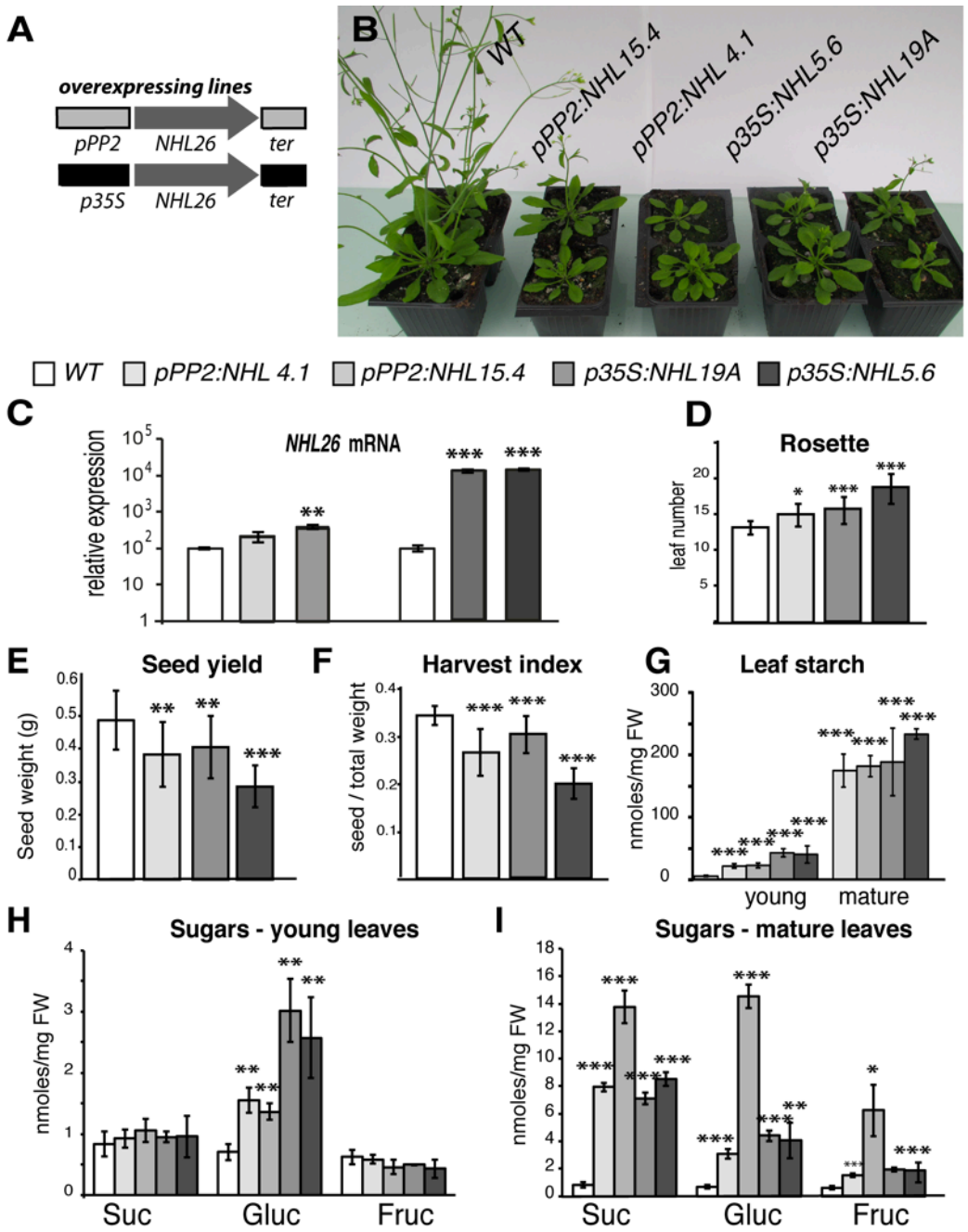
Scatter plot of the expression of *NHL* genes in phloem cells and other cell types

Profile of expression for *NHL* genes reported on a scatter plot comparison of companion cell and other cell type expression profiles from the shoot, extracted from the translatome dataset described by Mustroph et al., 2009.
For each gene probe set, the normalized expression value in companion cells (i.e. the SSUC2C set of values, obtained from cells sorted with a pSUC2-GFP fusion in the shoot) is plotted on the Y axis and the mean level of expression in other shoot cell types is plotted on the X axis (mean signals for STotC, S35SC, SRBC, SS2-2C, SGL2C, SCERC, SKATC datasets).
Both axes are logarithmic. Values above the diagonal indicate higher levels of expression in the phloem companion cells than in other cell types.

The whole set of *Arabidopsis* gene probes present on the Affymetric array is represented as gray dots.
For comparison with phloem-specific genes, the expression values for *PP2-A1*, *SUC2* and *AKT2/3* are indicated as blue dots: *PP2-A1 (At4g19840); SUC2 (At1g22710); AKT2/3 (At4g22200)*.

The expression values for the following *NHL* genes are indicated as red dots: *NHL 1/ SEC22 (At3g11660); NHL 2 (At3g11650); NHL 3 (At5g06320); NHL 4 (At1g54540); NHL 5 (At1g61760); NHL 6 (At1g65690); NHL 9 (At2g35460); NHL10/YLS9 (At2g35980); NHL12 (At2g35960); NHL13 (At2g27080); NHL14 (At2g27260); NHL15 (At2g01080); NHL18 (At3g52470); NHL20 (At4g26490); NHL23 (At5g06330); NHL28 (At5g11890); NHL30 (At1g17620); NHL34 (At1g13050); NHL37 (At3g52460); NHL38 (At3g20590); NHL40 (At3g26350); NHL42 (At4g01110); NHL49 (At4g35170); NHL51 (At5g22870); NHL52 (At2g41990)*. The names of the NHL genes more strongly expressed in phloem cells are reported on the scatter plot.

The following NHL genes were not present on the array: *NDR 1 (At3g20600); NHL11 (At2g35970); NHL16 (At3g20610); NHL17 (At3g44220); NHL19 (At4g01410); NHL21 (At4g05220); NHL22 (At4g09590); NHL25 (At5g36970); NHL26 (At5g53730); NHL36 (At2g46300); NHL43 (At5g56050); NHL45 (At5g22200); NHL46 (At5g21130); NHL47 (At5g05657); NHL48 (At1g08160); NHL50 (At2g27270)*.



Supplemental Figure 4

Characterization of *NHL26*-overexpressing lines

(**A**) Schematic map of the constructs used in *NHL26*-overexpressing

(B) Phenotype of *NHL26*-overexpressing plants, grown in long-day conditions in greenhouse (five-week-old plants)

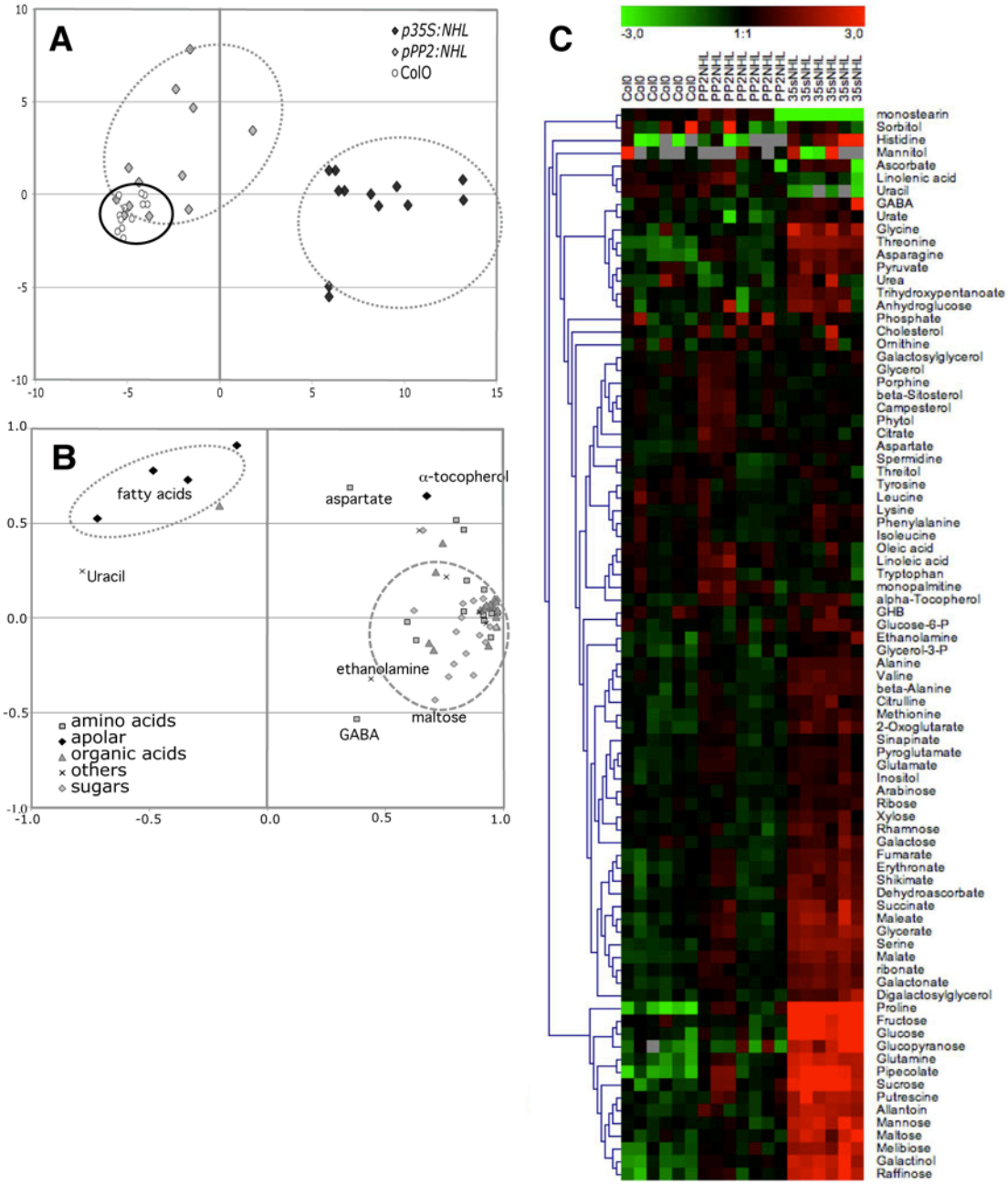
(**C**) Relative expression of *NHL26* in *NHL26*-overexpressing lines. *NHL26 m*RNA accumulation in the rosette leaves was assessed by qRT-PCR. Expression was normalized relative to levels of *TIP41 RNA*. Values are expressed as percentages of wild-type values. Error bars indicate +/- SE (n=6).

(D) Number of rosette leaves at flowering time in the *pPP2:NHL* and *p35S:NHL* transgenic plants, as compared with wild-type plants. Asterisks (*) indicate values significantly different in a t test (*: $P< 0.05$; **: $P< 0.01$; ***: $P< 0.001$). Error bars indicate the standard error of the mean (n=10).

(E-F) Yield parameters for plants grown in long-day conditions in the greenhouse. (D) Seed weight and (E) Harvest index calculated as the ratio of seed weight to dry weight of the aerial part of the plant (including rosette, stem and seeds). Error bars indicate the standard error of the mean (n=12).

(G) Starch content of the rosette leaves of *pPP2:NHL-* and *p35S:NHL-*expressing and wild-type plants. Plants were harvested at stage 6.0. The data points and error bars represent the mean and standard error (n=6).

(H) and **(I)** Sucrose, glucose and fructose in (H) young rosette leaves that are still expanding and (I) mature rosette leaves of *pPP2:NHL-* and *p35S:NHL-*expressing plants and wild-type. Plants were harvested at stage 6.10. The data points and error bars represent the mean and standard error (n=6).

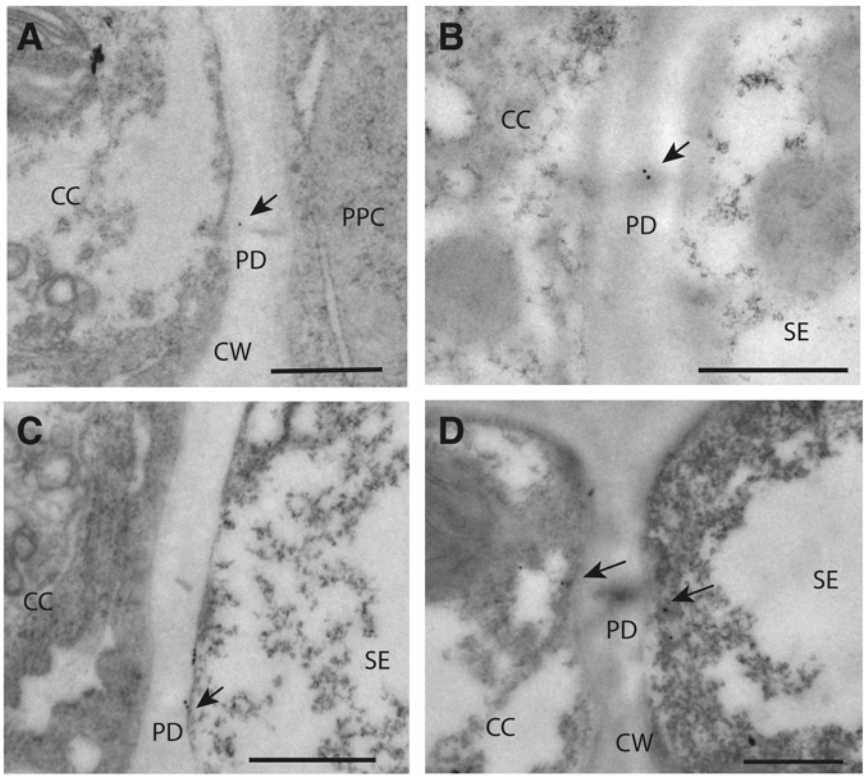


Supplemental Figure 5

Metabolic profiling in *NHL26*-overexpressing lines

(A) and (B) Principal component analysis of the data obtained by GC-MS, forcompounds showing significant differences between plant lines (p<0.01).
(A) PCA of the genotypes. (B) PCA of metabolites. The main group (right side) is composed of compounds that are significantly overabundant in the expressing lines. It includes sugars (sucrose, fructose, glucose, mannose, galactinol, raffinose, melibiose, xylose, rhamnose, arabinose, ribose, galactose, maltose and glucose-6-P), amino acids (Ser, Thr, Pro, Met, Gln, Glu, Val, Asn, Ala, His, Gly), organic acids (galactonate, glycerate, maleate, malate, erythronate, fumarate, 2-oxoglutarate, pipecolate, dehydroascorbate, shikimate, ribonate, succinate, sinapinate, trihydroxypentanoate, pyruvate, urate), and other compounds (allantoin, putrescine, inositol, glycerol-3-P).

(C) Hierarchical clustering analysis of metabolite profiles for rosette leaves from *pPP2:NHL*- and *p35S:NHL*-expressing plants, and a comparison with wild-type plants. The values represent the normalized values for each compound after Log2 transformation. Dendrograms summarize the relatedness of metabolites (right). The heat map shows high (red) and low (green) metabolite levels with respect to the row and column means.



Supplemental Figure 6

Localization of NHL26 in plasmodesmata

Observation by transmission immunoelectron microscopy of the NHL26-GFP fusion protein with an anti-GFP antibody in phloem cells in *pNHL:NHL-GFP Arabidopsis* plants. (**A-D**) Details of localization between sieve elements and companion cells, showing a localization to plasmodesmata. Arrows indicate gold particles conjugated to an anti-GFP polyclonal antibody. Bars=500 nm.

Supplemental Table 1. Description of the binary vectors derived from the *NHL26* gene sequence.

Sequences in bold: gene-specific sequences. Sequences in italics: *attb1* and *attb2* sequences. Underlined sequences: *NHL26*-specific sequences. CDS: coding sequence.

Construct	Characteristics	Sequence	Primers (5'-3')	Binary vector	Reference
pNHL:GUS	Transcriptional fusion *NHL26:GUS*	Promoter *At5g53730*	+: *aaaaaagcaggct***ttcctcatgt cttccgagac** - : *aagaaagctgggt***ttggagaga gagatgatgatg**	pBI101-R1R2-GUS-tNOS [Kan^R]	Divol et al., 2007
pBI101-pPP2A1-R1R2-tNOS	Expression vector driving expression in the phloem	Promoter *At4g19840 (PP2A1)*	+: **attatgttcttatcacctaaatag** - : **ccagtatgatgtatttatttttg**	pBI101-R1R2-tNOS [Kan^R]	this work
pPP2:NHL	Ectopic expression of *NHL26* with *PP2-A1* promoter	Promoter *At4g19840* CDS *At5g53730*	+: *aaaaaagcaggct***atgtctcaaa tctccataac** - : *aagaaagctgggt***tcatatagtt gtagagcaac**	pBI101-pPP2:A1-R1R2-tNOS [Kan^R]	this work
p35S:NHL	Overexpression of *NHL26*, with 35S promoter	CDS *At5g53730*	+: *aaaaaagcaggct***atgtctcaa atctccataac** - : *aagaaagctgggt***tcatatagt tgtagagcaac**	pMDC32 [Hygro^R]	Curtis and Grossniklaus, 2003
p35S:NHL-GFP	*NHL26-GFP* fusion, with 35S promoter	CDS *At5g53730*	+: *aaaaaagcaggct***atgtctcaaa tctccataac** - : *aagaaagctgggt***gtatagttgta gagcaacgag**	pMDC83 [Hygro^R]	Curtis and Grossniklaus, 2003
p35S:GFP-NHL	*NHL26-GFP* fusion with 35S promoter	CDS *At5g53730*	+: *aaaaaagcaggct***tttctcaaa tctccataacttctc** - : *aagaaagctgggt***gtatagtt gtagacaacgag**	PMDC45 [Hygro^R]	Curtis and Grossniklaus, 2003
pNHL:NHL-GFP	*NHL26-GFP* fusion, with its own promoter	Promoter and CDS *At5g53730*	+: *aaaaaagcaggct***ttcctcatgt cttccgagac** - : *aagaaagctgggt***gtatagtt gtagagcaacgag**	pMDC107 [Hygro^R]	Curtis and Grossniklaus, 2003
pNHL:NHL-CFP	*NHL26-CFP* fusion, with its own promoter	Promoter and CDS *At5g53730*	+: *aaaaaagcaggct***ttcctcatgtc ttccgagac** - : *aagaaagctgggt***gtatagttgta gagcaacgag**	pGHGWC [Hygro^R]	Zhong et al., 2008

Supplemental Table 2. Primers used for RT-PCR experiments.

Gene	AGI Accession number	Experiment	Primer sequence (5'-3')
NHL26	*At5g53730*	RT-PCR	+ : gcgccaagaaaggagggatta
			- : aatgaagctaagggaggagtg
EF-1αA4	*At5g60390*	RT-PCR	+ : atgactttgtatcttggcttatcactg
			- : ttggcggcacccttagctggatca
G6PT	*At1g61800*	RT-PCR	+ : cgtcttccttctccaccgctat
			- : tgatgtgtgtgaaggaaactgc
PR1	*At2g14610*	RT-PCR	+ : cttgtaggtgctcttgttcttc
			- : ttagtatggcttctcgttcaca
PR2	*At3g57260*	RT-PCR	+ : cactgacaccaccactgatac
			- : tctccgacaccacgatttcc
NHL26	*At5g53730*	qRT-PCR	+ : gttgcccagtcctttggtta
			- : tgttcattccaaaagccaca
SUC2	*At1g22710*	qRT-PCR	+ : tgcctttcacgatgactgag
			- : ttccttgaaagctccgaaga
AAP2	At5g09220	qRT-PCR	+ : cattgttgtccacctcgttg
			- : aaaccctaacgcccctaaga
NRT1.7	At1g69870	qRT-PCR	+ : caacagtcagtttccagagcacat
			- : cgacagtcacaaggaaactactaaggta
LHCB1	At1g29920	qRT-PCR	+ : ggggtcagcggatagaccag
			- : ctttcgccggaaaggctgt
RBCS	At5g38410	qRT-PCR	+ : ccgcaacaagtggattccttgtg
			- : aatgagcagagataattcataagaatg
TIP41	*At4g34270*	qRT-PCR	+ : tccatcagtcagaggcttcc
			- : gctcatcggtacgctctttt
APT	*At1g27450*	qRT-PCR	+ : gagacattttgcgtgggatt
			- : cggggattttaagtggaaca
UBI10	*At4g05320*	qRT-PCR	+ : cgtggtggtttctaaatctcgt
			- : gattatacaaggccccaaaaca