Current Biology, Volume *26*

Supplemental Information

Shoot-to-Root Mobile Transcription Factor HY5 Coordinates Plant Carbon and Nitrogen Acquisition

Xiangbin Chen, Qinfang Yao, Xiuhua Gao, Caifu Jiang, Nicholas P. Harberd, and Xiangdong Fu

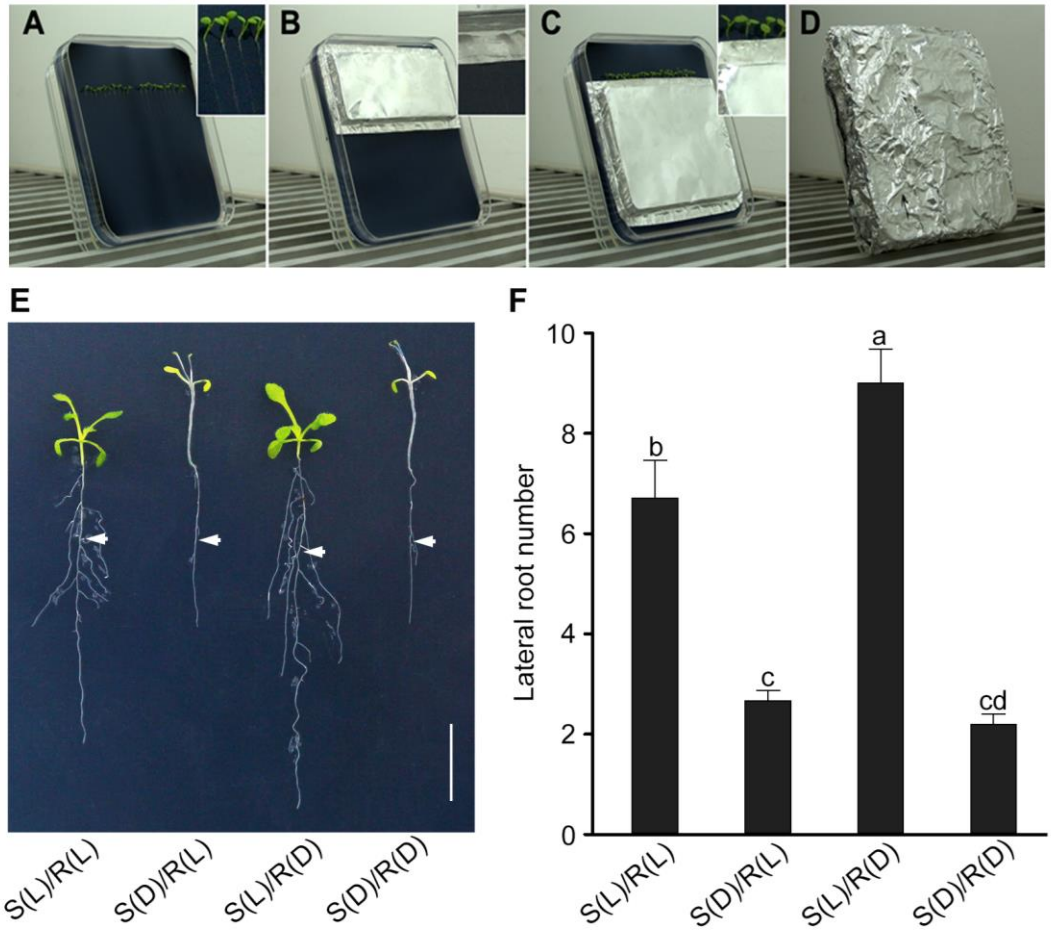

Figure S1. The effect of differential shoot/root illumination on lateral root development

3-day-old seedlings grown at 22 ℃ with a 16 h photoperiod were transferred to a new plate, then exposed to 3 days differential light treatment (100 μmol.s^{-1}.m^{-2}): (A) S(L)/R(L), shoot illuminated (S(L)) and root illuminated (R(L)). (B) S(D)/R(L), shoot dark-grown (S(D)) and root illuminated (R(L)). (C) S(L)/R(D), shoot illuminated (S(L)) and root dark-grown (R(D)). (D) S(D)/R(D), shoot dark-grown (S(D)) and root dark-grown (R(D)). (E) 3-day-old WT seedlings were transferred to a new plate, then exposed to 10d differential light treatment (100 μmol.s^{-1}.m^{-2}). Scale bar, 1 cm. (F) Lateral root production in different treatments. Data shown as mean ± s.e.m. (n = 30). The same lowercase letter denotes a non-significant difference between means (P < 0.05). Figure S1 is related to main Figure 1.

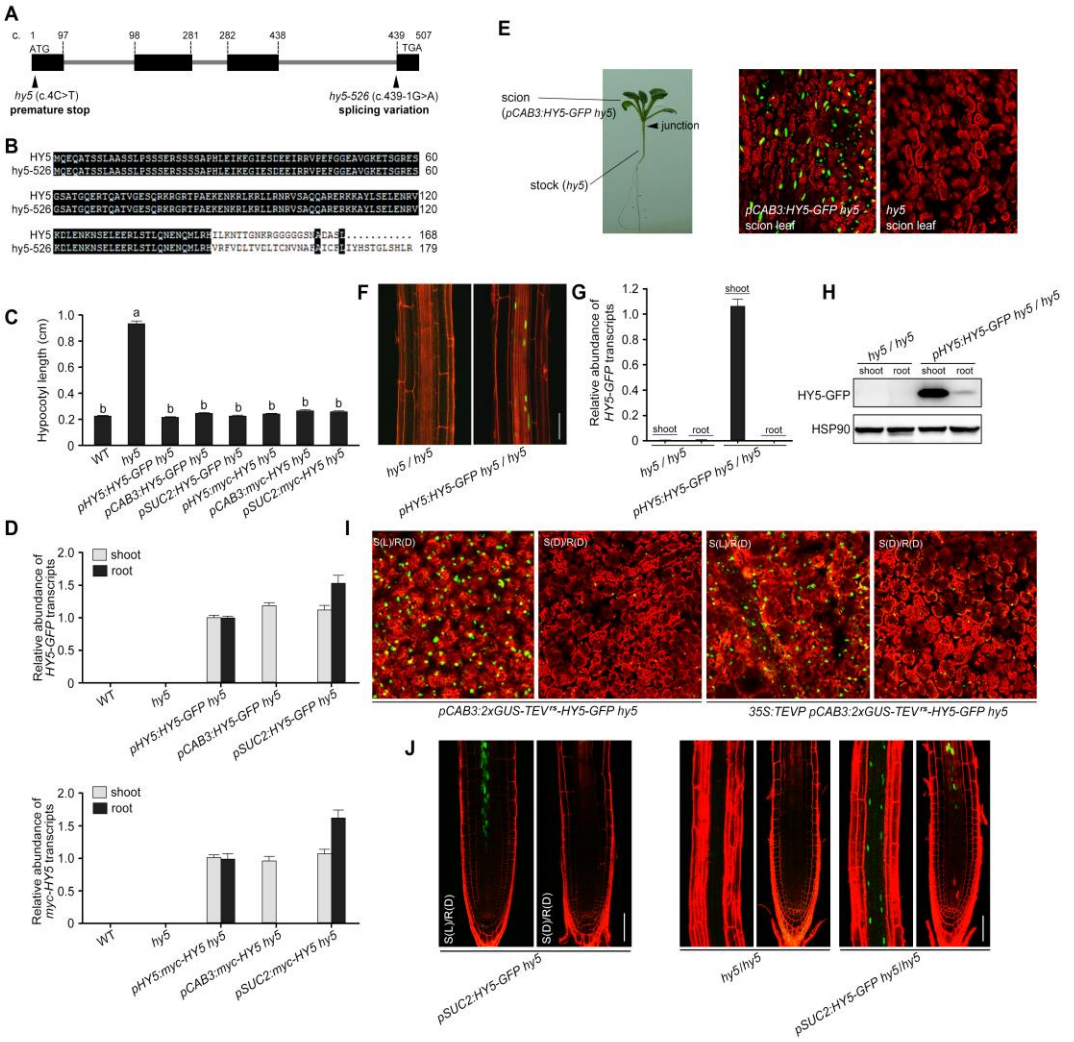

Figure S2. HY5 is a shoot-to-root translocated protein

(A) A representation of *HY5* coding sequence (c.) with bp 1 the A of the start ATG. *hy5-526* is a splice-site nucleotide substitution mutation (position as indicated). The allele referred to throughout this manuscript as *hy5* is a nucleotide substitution mutation creating a premature stop codon, and is thus a complete null allele. Dark grey boxes indicate exons, black lines indicate introns, and numbers indicate the exon sizes (bp). (B) Comparison of HY5 and mutant hy5-526 protein sequences. The numbers on the right indicate residue positions. Identical residues are indicated by dark grey boxes, and variant residues by light grey boxes. Aberrant splicing of *hy-526* results in a product lacking the HY5 C-terminal domain. (C) Hypocotyl lengths of light-grown 6-day-old seedlings (genotypes as indicated). Data shown as mean ± s.e.m. (n = 30). The same lowercase letter denotes a non-significant difference between means ($P < 0.05$). (D) Relative *HY5-GFP* and *myc-HY5* transcript abundance. Expression levels are expressed relative to abundance of Arabidopsis *actin2* mRNA. Data shown as mean ± s.e.m. (n =

3). (E) An example hypocotyl-graft chimera. HY5-GFP is detectable in the scion leaf of 10-day-old grafted plants (in a *pCAB3:HY5-GFP hy5* scion but not in a *hy5* scion). (F) HY5-GFP is detectable in roots of *pHY5:HY5-GFP hy5/hy5* graft chimeras 10 days following grafting. Scale bar, 50 μm. (G) *HY5-GFP* transcript abundance in shoots and roots of graft chimeras (relative to abundance in *pHY5:myc-HY5 hy5/hy5* shoots). Data shown as mean ± s.e.m. (n = 3). (H) Immunological detection of HY5-GFP in scion (shoot) and stock (root), with HSP90 loading control. (I) Distribution of GFP signal in leaves of shoot-illuminated *pCAB3:2×GUS-TEV^{rs}-HY5-GFP hy5* plants. (B) Distribution of GFP signal in leaves of shoot-illuminated *pCAB3:2×GUS-TEV^{rs}-HY5-GFP hy5* plants expressing the TEV protease. (J) HY5-GFP is detectable in roots of *pSUC2:HY5-GFP hy5* seedlings or *pSUC2:HY5-GFP hy5/hy5* seedling graft chimeras. Figure S2 is related to main Figure 2.

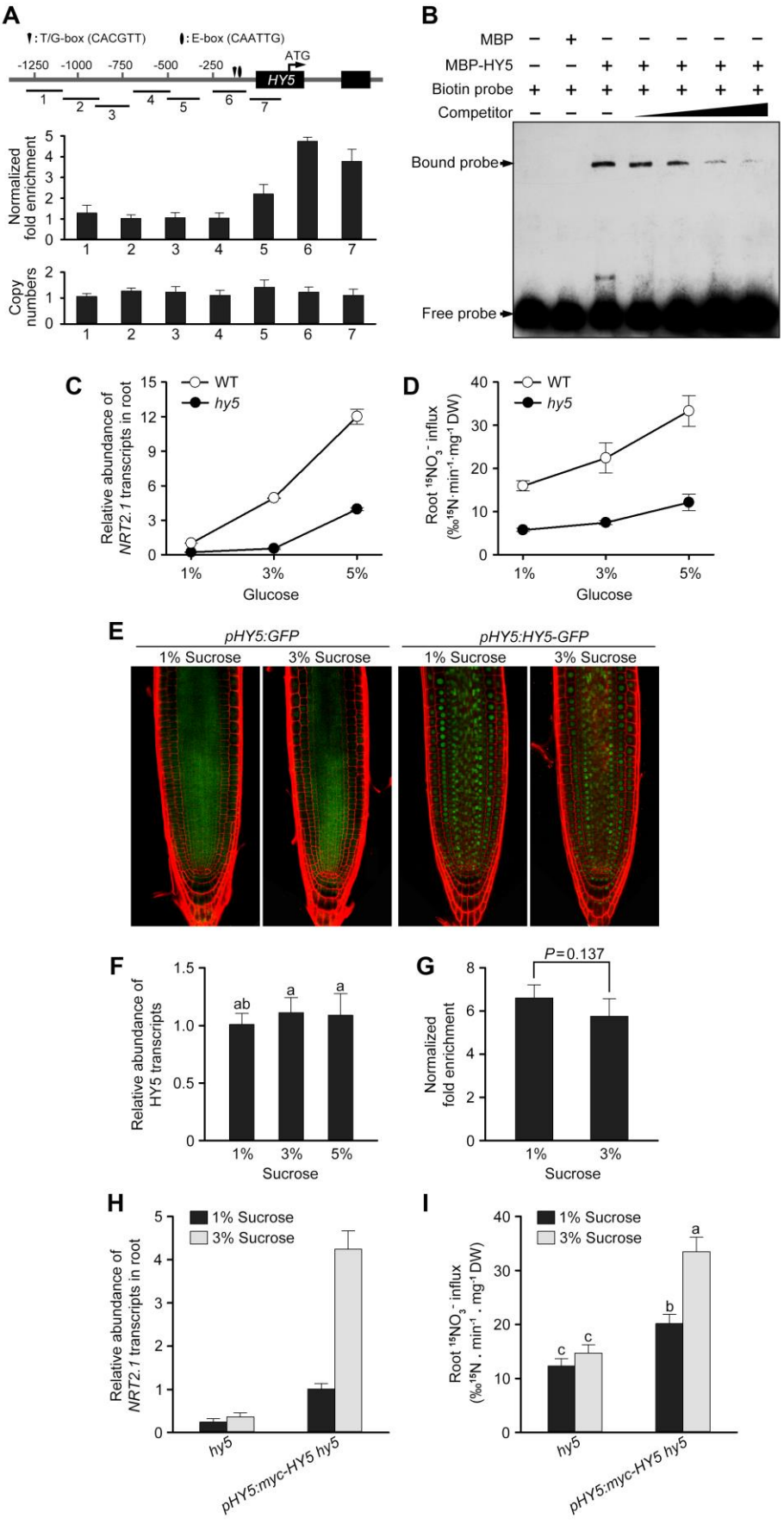
A
T/G-box (CACGTT)
E-box (CAATTG)
-1250 -1000 -750 -500 -250
ATG
HY5
1 2 3 4 5 6 7
Normalized fold enrichment
Copy numbers
B
MBP
MBP-HY5
Biotin probe
Competitor
Bound probe
Free probe
C
WT
hy5
Relative abundance of NRT2.1 transcripts in root
1% 3% 5%
Glucose
D
WT
hy5
Root 15NO3- influx (‰ 15N·min-1·mg-1 DW)
1% 3% 5%
Glucose
E
pHY5:GFP
pHY5:HY5-GFP
1% Sucrose
3% Sucrose
1% Sucrose
3% Sucrose
F
Relative abundance of HY5 transcripts
ab a a
1% 3% 5%
Sucrose
G
P=0.137
Normalized fold enrichment
1% 3%
Sucrose
H
1% Sucrose
3% Sucrose
Relative abundance of NRT2.1 transcripts in root
hy5
pHY5:myc-HY5 hy5
I
1% Sucrose
3% Sucrose
Root 15NO3- influx (‰ 15N . min-1 . mg-1 DW)
c c b a
hy5
pHY5:myc-HY5 hy5

Figure S3. A mobile HY5 auto-activates root *HY5*, and also regulates *NRT2.1* expression and NO_3^- uptake in response to levels of carbohydrate photosynthate

(A) ChIP assays. The diagram depicts the putative *HY5* promoter and fragments (1-7) used for ChIP analysis. ChIP-PCR was performed using 14-day-old *pHY5:myc-HY5 hy5* plants. Data shown as mean ± s.e.m. (n = 3). (B) EMSA assays. A T/G-box-motif-containing *HY5* promoter fragment (fragment 6 from A), was incubated with MBP-HY5 as indicated. Competition for HY5 binding was performed with 10×, 20×, 50× and 100× unlabeled probes containing the T/G-box motif, respectively. (C) *NRT2.1* transcript abundance in S(L)/R(D) roots of WT and *hy5* seedlings. Transcript levels are expressed relative to abundance of *Arabidopsis actin2*. Data shown as mean ± s.e.m. (n = 3). (D) $^{15}NO_3^-$ uptake of 7-day-old WT and *hy5* seedling roots. Data shown as mean ± s.e.m. (n = 10). (E) The effect of sucrose level on *HY5* transcription (as visualized by GFP expression driven by a *pHY5:GFP* transgene) and HY5 stability (HY5-GFP expressed from *pHY5:HY5-GFP*) in roots. Scale bar, 50 μm. (F) The effect of sucrose level on root *HY5* transcript abundance. Transcript levels are expressed relative to the abundance of *Arabidopsis actin2* mRNA. Data shown as mean ± s.e.m. (n = 3). (G) ChIP-PCR analysis performed using 10-day-old *pHY5:myc-HY5 hy5* plants grown on 1/2MS medium containing 1% or 3% sucrose. Enrichment of DNA fragment 6 of the *HY5* promoter shown in fig. S8 was determined by qRT-PCR analysis. Data shown as mean ± s.e.m. (n = 3). A Student's *t*-test was used to generate the *P* values. (H) Levels of *NRT2.1* transcripts in sucrose-treated *hy5* and *pHY5:myc-HY5 hy5* roots. Transcript levels are expressed relative to abundance of *Arabidopsis actin2*. Data shown as mean ± s.e.m. (n = 3). (I) Rates of $^{15}NO_3^-$ uptake in sucrose-treated *hy5* and *pHY5:myc-HY5 hy5* roots. Data shown as mean ± s.e.m. (n = 30). The presence of the same lowercase letter denotes a non-significant difference between means ($P < 0.05$). Figure S3 is related to main Figure 3.

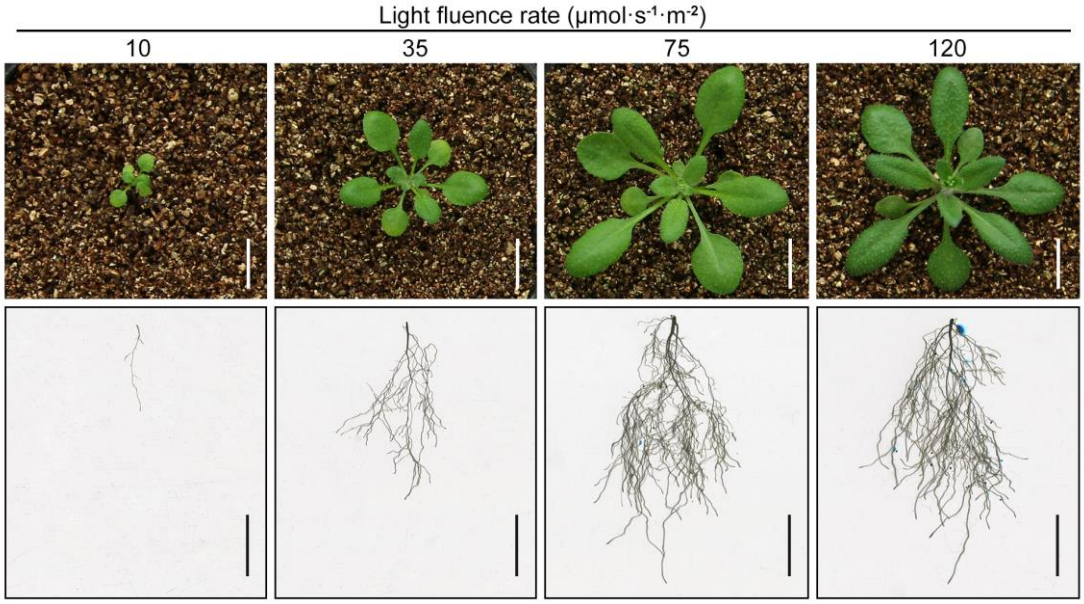

Figure S4. The effects of increasing light fluence rates on plant growth

The shoot (top panel) and root (lower panel) systems of WT plants grown in soil for 21 days (16 h photoperiod) at different light fluence rates are shown. Scale bar, 1 cm. Figure S4 is related to main Figure 4.

Supplemental tables

Table S1. Primer sequences used for DNA constructs and qRT-PCR analysis.

Primer Name	Primer sequence (5′ to 3′)
HY5-GFP-F	GATCTAGAATGCAGGAACAAGCGACTAG
HY5-GFP-R	CAGTCGACAAGGCTTGCATCAGCATTAG
GFP-F	AAGTCGACATGGTGAGCAAGGGCGAGGAG
GFP-R	CACTGCAGTTACTTGTACAGCTCGTCCAT
pHY5-F	GTCCATGGCTTCGTCGTCAGGATTAT
pHY5-R	CGGGTACCTTTTCTTACTCTTTGAAGATC
pSUC2-F	CGCCATGGTTTGTCATACATTTATTTGCCACAAG
pSUC2-R	GCGGTACCTTTGACAAACCAAGAAAGTAAGAAAAAAA
pCAB3-F	GACCATGGAATCAAGAGAAAATGTGATTCTCGG
pCAB3-R	CGCGGTACCGAAACTTTTTGTGTTTTTTTTTTTTTTTG
myc-HY5-F	GAGGATCCATGCAGGAACAAGCGACTAG
myc-HY5-R	GGTCTAGATCAAAGGCTTGCATCAGCAT
GUS-F1	GAGGTACCATGGATCTGACTAGTTT
GUS-R1	TTAGATCTGCTAGCTTGTTTGCCT
GUS-F2	GAAGATCTGATCTGACTAGTTTACGTC
GUS-TEV^{rs}-R2	ATGGTACCGCCGCTTCCAGAACCTGAACCCTGGAAGTACAAGTT CTCTCCAGAACCTGATCCAGAGCTAGCTTGTTTGCCTCCCTGCTG
TEV^{rs}-R2	ATGGTACCGCCGCTTCC
TEVP-F	ATGTTGTTTAAGGGACCACGTGAT TA
TEVP-R	TCAGTCACGATGAATTCCCGGCGAGT
qActin2-F	CTGGATCGGTGGTTCCATTC
qActin2-R	CCTGGACCTGCCTCATCATAC
qHY5-F	GAACGAGAACCAGATGCTTAGAC
qHY5-R	TGCAATATTAGCTCTCACATCCC
OCS-R	CATAGGCGTCTCGCATATCTC
qHY5-GFP-F	CAGAACGAGAACCAGATGCTTAG
qHY5-GFP-R	CAGATGAACTTCAGGGTCAGC
qNRT1.1-F	TCTAAGACCGCTTCAACGGATCG
qNRT1.1-R	ACTGTTGGACCATGAGCGTGTG
qNRT2.1-F	AACAAGGGCTAACGTGGATG
qNRT2.1-R	CTGCTTCTCCTGCTCATTCC
qPSY-F	GACACCCGAAAGGCGAAAGG
qPSY-R	CAGCGAGAGCAGCATCAAGC
qTPS1-F	GGTCATTTCTTGGGGAAGGA
qTPS1-R	TCTCCTGATGATGACTTGGC
qSWEET11-F	GCGAACAAGTGTACCTGCGG
qSWEET11-R	GGGTACACGTGGTGGTTGGT
qSWEET12-F	TCGTCCGATCGGTGAACACA
qSWEET12-R	ACTAGTACACGTGGACAATGGTGA

Table S1 is related to main Figure 2

Table S2. Primer sequences used for ChIP-PCR and EMSA assays.

Primer Name	Primer sequence (5′ to 3′)
HY5 fragment 1-F	GGCAGCTTAAAAGACTGGCTT
HY5 fragment 1-R	ATCACAACAAAACCATCCGTTATC
HY5 fragment 2-F	TTAAAAATCTGGCAGCTGAGGTT
HY5 fragment 2-R	CTTTTACTTTTTCCTTAGGTTCGAC
HY5 fragment 3-F	AGATGTTGTGGTCGAACCTAAGG
HY5 fragment 3-R	GTGAGATAGAGCATTCAAGTAACATAG
HY5 fragment 4-F	GTTTGATGATGCTGTGAATAGAATG
HY5 fragment 4-R	TGACGACAATGTTGATGAGTTTCT
HY5 fragment 5-F	CAGAAACTCATCAACATTGTCGTC
HY5 fragment 5-R	CCGCCATAAACCAAACAAAGT
HY5 fragment 6-F	TTCACGACACTTTTGAAAGCACTGCC
HY5 fragment 6-R	CAAGGATCCAAAGGCAATTGAG
HY5 fragment 7-F	ATTCACTCTCGATATCCGTTCG
HY5 fragment 7-R	AGAGAGAGAGGGAAAGATTTGTTG
NRT2.1 fragment 1-F	TTTACAAAGTGGTTCCTTCACGA
NRT2.1 fragment 1-R	CCAACAAATTAAGGATCTTCGG
NRT2.1 fragment 2-F	CTGGATGACATTAAAGTTCATACTTC
NRT2.1 fragment 2-R	GTACCGGACAAAAGAGAATCCT
NRT2.1 fragment 3-F	GAGAAAAGATAATGAGCTCATCGAA
NRT2.1 fragment 3-R	GTGCGGTGGATTGATATGTAGA
NRT2.1 fragment 4-F	AAATTCAGATCCGCTAGCTACTAC
NRT2.1 fragment 4-R	CGTATGTCAATGTATATGTGATGG
NRT2.1 fragment 5-F	CGATTTCAATTTTTCACACCGA
NRT2.1 fragment 5-R	AGTATTCACAAAAGGGGAAGATG
NRT2.1 fragment 6-F	ACAGTTACAATGACAAAGATAACCC
NRT2.1 fragment 6-R	CTTAAGGTTTAAAAGTTTGGTCCTC
NRT2.1 fragment 7-F	TTAGCCTATCCTGTATCACTGTATG
NRT2.1 fragment 7-R	AGGTTGCCGATATCCTTCCA
NRT2.1 fragment 8-F	TTGGTGATAAGCGAGAGACTAGG
NRT2.1 fragment 8-R	TCTTTGCAAGTTTGAGATTTGATTC
P1-TPS1 fragment-F	ACCCCTTACTTGTTAGTGGTTGAA
P1-TPS1 fragment-R	GGTATGGACAGAGATGTTGTTGGT
P2-SWEET11 fragment-F	AGAGCTAAAGTGAAAACGGCATAAT
P2-SWEET11 fragment-R	TGACGACATTCTGGAATTTGCT
P3-SWEET12 fragment-F	TTGCATTGTGTTTAATTACGGC
P3-SWEET12 fragment-R	GGTCACTGATACTTATGACGGATAG
HY5 probe	GCTAACCAGATCTAACGGCTAAAATCCACCCACGTTCCAA TCTCAATTGCCTTTGGATCCTTGTAT
NRT2.1 probe	TTATCAAATCCCAACTTGTTGGAAATTTGACACGTCAGCG AGATTGATCGATACGCACTTAGTCGT

Table S2 is related to main Figure3

Supplemental Experimental Procedures

Plant materials and growing conditions

Arabidopsis seeds were imbibed at 4 ℃ for three days, then plated on 1/2MS medium. Emerging seedlings were exposed to a 16 h photoperiod at 22 ℃. Experiments involving seedling grafting were performed as previously described [S1].

Plasmid constructs

HY5 cDNA was amplified and subcloned into the *pCaMV35S:nos* vector [S2]. The sequences of the *HY5, SUC2* and *CAB3* promoters were amplified and subcloned into the *pCaMV35S:nos* vector, as were both the *HY5* promoter and the *GFP* coding sequence to generate the *pHY5:GFP* expression cassette. The *HY5* coding sequence was cloned into the *pSK-N-Tagged-myc* vector [S3], and then subcloned into the *pCaMV35S:nos* vector. To make the *pCAB3:2×GUS-TEV^{rs}-HY5-GFP* fusion construct, the TEV recognition site (TEV^{rs}) was fused to the 3′-end of the 2×GUS coding sequence via PCR, and the PCR product was introduced into *pCAB3:HY5-GFP* vector. The TEV protease was amplified and cloned into *pCaMV35S:nos* vector [S2]. To construct the *pHY5:HY5-GFP* transgene, the *HY5* coding sequence was cloned into the *pHY5:GFP* construct. Primer sequences used for PCR amplifications are given in Table S1.

Transcript analysis

Total RNA was extracted using the TRIzol reagent (Invitrogen, New York, USA), and reverse transcribed using an M-MLV Reverse Transcriptase kit (Promega, Wisconsin, USA). qRT-PCR analysis was performed as described previously [S4]. Each experiment was represented by three biological replicates with at least three technical replicates per biological replicate. *Arabidopsis actin2* was used as a reference gene. The relevant primers are given in Table S1.

Immunoblot analysis

Preparations of crude protein were obtained by extracting in 50 mM Tris-HCl (pH 7.5), 150 mM KCl, 10 mM $MgCl_2$, 1 mM EDTA, 10% glycerol, 0.1% NP-40, 1×complete protease inhibitor (Roche). An aliquot was electrophoresed through a 10% (w/v) SDS–polyacrylamide gel and transferred on to a Hybond ECL nitrocellulose membrane. Subsequent handling of the membrane followed an established protocol [S5]. The myc-

HY5 and HY5-GFP fusion proteins were detected using anti-myc (Santa Cruz Biotechnology, Santa Cruz, USA) and anti-GFP (Roche Diagnostics GmbH, Germany) antibodies respectively, and signals were visualized using the SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific, Waltham, USA).

Measurement of total carbon and nitrogen content

Dehydrated plant tissue was milled, and a high temperature combustion process [S6] used to determine the total carbon and nitrogen content of the powdered material using an Elementar Vario PYRO Cube analyzer (Elementar Analysensysteme GmbH, Frankfurt, Germany).

$^{15}NO_3$-uptake activity assay

Root $^{15}NO_3^-$ influx was assayed as described elsewhere [S7]. Roots were dried overnight at 80 ℃, and the ^{15}N content was measured using the ANCA-MS system (PDZ Europa Ltd).

ChIP-PCR assays

hy5 and *pHY5:myc-HY5 hy5* seedlings were grown on 1/2MS plates for 14 days. A 2 g aliquot of plant tissue was then fixed by formaldehyde cross-linking. The ChIP assay used anti-myc antibodies (Santa Cruz Biotechnology, Santa Cruz, USA) as previously described [S8]. Enrichment of DNA fragments was determined by qRT-PCR analysis. Three independent biological replicates were performed. The relevant primer sequences are given in Table S2.

EMSA assays

HY5 cDNA was amplified and cloned into the pMAL™-c2X vector (New England Biolabs, Ipswich, USA). MBP and MBP-HY5 fusion proteins were purified according to the manufacturer's instructions. The DNA probes were amplified and labeled with biotin at their 3-end, using a biotin label kit (Invitrogen, New York, USA). DNA gel-shift assays were performed using the LightShift Chemiluminescent EMSA Kit (Thermo Fisher Scientific, Waltham, USA). The primer sequences used are given in Table S2.

Supplemental references

S1. Corbesier, L., Vincent, C., Jang, S., Fornara, F., Fan, Q., Searle, I., Giakountis, A., Farrona, S., Gissot, L., Turnbull, C., and Coupland, G. FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science *316*, 1030-1033.

S2. Wang, S., Wu, K., Yuan, Q., Liu, X., Liu, Z., Lin, X., Zeng, R., Zhu, H., Dong, G., Qian, Q., Zhang, G., and Fu, X. (2012). Control of grain size, shape and quality by OsSPL16 in rice. Nat. Genet. *44*, 950-954.

S3. Wang, S., Li, S., Liu, Q., Wu, K., Zhang, J., Wang, S., Wang, Y., Chen, X., Zhang, Y., Gao, C., Wang, F., Huang, H., and Fu, X. (2015). The *OsSPL16-GW7* regulatory module determines grain shape via the control of cell division patterning and simultaneously improves rice yield and grain quality. Nat. Genet. *47*, 949-954.

S4. Sun, H., Qian, Q., Wu, K., Luo, J., Wang, S., Zhang, C., Ma, Y., Liu, Q., Huang, X., Yuan, Q., Han, R., Zhao, M., Dong, G., Guo, L., Zhu, X., Gou, Z., Wang, W., Wu, Y., Lin, H., and Fu, X. (2014). Heterotrimeric G proteins regulate nitrogen-use efficiency in rice. Nat. Genet. *46*, 652-656.

S5. Jiang, C., Gao, X., Liao, L., Harberd, N.P., and Fu, X. (2007). Phosphate starvation root architecture and anthocyanin accumulation responses are modulated by the gibberellin-DELLA signaling pathway in Arabidopsis. Plant Physiol. *145*, 1460-1470.

S6. Matejovic, I. (1993). Determination of carbon, hydrogen, and nitrogen in soils by automated elemental analysis (dry combustion method). Commun. Soil Sci. Plant Anal. *24*, 2213-2222.

S7. Ho, C.H., Lin, S.H., Hu, H.C., and Tsay, Y.F. (2009). CHL1 functions as a nitrate sensor in plants. Cell *138*, 1184-1194.

S8. Gendrel, A.V., Lippman, Z., Martienssen, R., and Colot, V. (2005). Profiling histone modification patterns in plants using genomic tiling microarrays. Nat. Methods. *2*, 213-218.