Supporting Information

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SI Methods

Plant Material and Growth Condition. *Arabidopsis thaliana* plants were grown in a growth room with a 16-h light/8-h dark cycle at 22–24 °C for phenotypic analysis and seed harvest.

For the generation of *PNT1* overexpression lines, full-length *PNT1* coding sequence with (*PNT1-OX1*) or without (*PNT1-GFP*) a stop codon was amplified with specific primer sets (Table S1) and cloned into binary vectors. Two independent homozygous lines that express transgenes were established for each construct. The *pnt1-1* allele was isolated from GABI_676A09, which has a T-DNA insertion at the sixth exon (GATTTCATTA-(T-DNA)-CTTCTCATTG) and the *pnt1-2* allele was isolated from T-DNA insertions lines on Ws-2 background, which has a T-DNA at the fourth intron (GATTTCATTA-(T-DNA)-CTTCTCATTG). For *PHYB-myc* lines, the full-length *PHYB* was amplified with a specific primer set and cloned into pHTM, which expresses a protein tagged with 9 myc epitopes (1). PIF3-myc and GFP-myc were described previously (1, 2). Other mutants used in this work are *phyA-211*, *phyB-9*, *phyA-211*/*phyB-9* double mutant, and *fhy1*/*fhl* double mutant, all of which are Col-0 background. For transgenic *Arabidopsis* harboring luciferase reporter gene, a firefly luciferase gene or a firefly luciferase gene with 5′-UTR (and 3′-UTR of *PORA* gene were introduced to wild type (Col-0) or mutants (*pnt1-1*, *phyA-211*, *fhy1*/*fhl*) and homozygous transgenic lines were selected and used for the assay.

Luciferase Reporter Assay. Protoplast transient reporter assay was performed as described previously (3). Leaf number 7–9 of 3.5–4 wk old plants were used as material. 3 μg of UTR containing luciferase reporters were transfected with 0.3 μg of Renilla luciferase. After PEG transfection samples were irradiated with far-red light for 30 mins and incubated 15 h in the dark, red and far-red at 22 °C. For the luciferase reporter assay using transgenic plants, 40 seeds were grown on 1/2 MS agar plates without sucrose for 3 d under various light conditions before sampling. Sample seedlings were ground in the liquid nitrogen and total proteins were solubilized in 50 μL of PLB (supplied in Promega's dual luciferase assay kit) supplemented with 1× complete mini protease inhibitor mixture (Roche). The luciferase activities were measured by dual-luciferase assay kit (Promega).

Photobleaching Assay. For FR-block of greening assay, 50 seeds were surface sterilized and plated on ½ MS agar media without sucrose. Germination was synchronized by 4 d of cold treatment followed by 6 h of white light treatment. After indicated days of far-red light (2.4 μmol/m^2/s) treatments or in the dark, the plates were transferred to white light for 4 d. The degree of photobleaching was determined either by measuring chlorophyll levels by spectrophotometer or by counting number of seedlings with green cotyledons. For gibberellins (GA) treatment, 50 seeds were plated and grown 1 d on the 3M filter paper on ½ MS agar media in far-red light, and germinating seedlings on the filter paper were transferred to the same media containing 5 μM GA_{4+7}.

Protein and mRNA Analysis. A total of 50 seedlings were grown in the red and far-red light for 4 d. Collected seedlings were ground in liquid nitrogen and total proteins were solubilized by protein extraction buffer (100 mM NaH_2PO_4, 10 mM Tris-Cl pH 7.5, 8 M urea, 1× complete mini protease inhibitor). Cell debris were precipitated by centrifugation for 10 min in 4 °C and supernatant was boiled with SDS sample buffer. The same amount of total proteins were separated by 10–17% SDS/PAGE gel. Anti-POR antibody (Agrisera) and anti–α-tubulin antibody (Sigma) were used for the blotting. Total RNAs were isolated with plant total RNA kit (Sigma) according to the manufacturer's instruction. A total of 2 μg of total RNAs were reverse transcribed and real-time PCR was performed to quantify the levels of each gene with specific primers.

Yeast Two-Hybrid Screening. A C-terminal domain of *Arabidopsis* phyA (514–1122 aa) cloned in pGBT9 vector was used to screen interacting proteins from 7-d-old, light-grown seedling cDNA library cloned in pGAD424 vector (Clontech).

1. Park E, et al. (2004) Degradation of phytochrome interacting factor 3 in phytochrome-mediated light signaling. *Plant Cell Physiol* 45:968–975.
2. Shin J, Park E, Choi G (2007) PIF3 regulates anthocyanin biosynthesis in an HY5-dependent manner with both factors directly binding anthocyanin biosynthetic gene promoters in Arabidopsis. *Plant J* 49:981–994.
3. Yoo SD, Cho YH, Sheen J (2007) Arabidopsis mesophyll protoplasts: A versatile cell system for transient gene expression analysis. *Nat Protoc* 2:1565–1572.

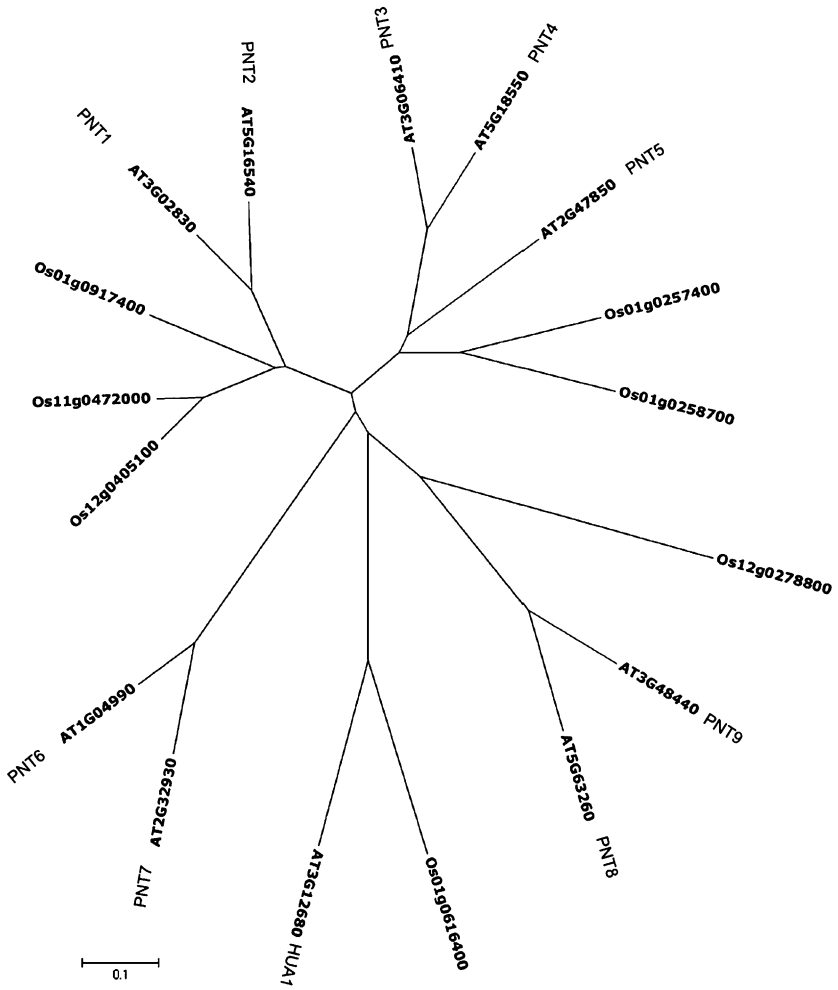

Fig. S1. A neighbor joining tree showing the relationships between PNT1 family members of *Arabidopsis* and rice. The tree was drawn by the MEGA4 program.

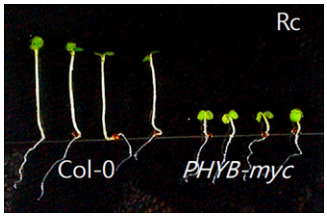

Fig. S2. Hypocotyl elongation of *PHYB-myc* transgenic *Arabidopsis* under red light condition.

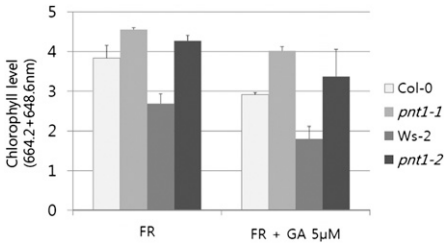

Fig. S3. Quantification of chlorophyll after transferring FR-grown seedlings to white light in the presence of gibberellins (GA_{4+7}). For GA treatment, 1 d-old FR-grown seedlings were transferred to 5 μM GA_{4+7} and they grew 4 more days under far-red light (2.4 μmol/m^2/s) before transfer to white light (100 μmol/m^2/s) (SD, n = 3).

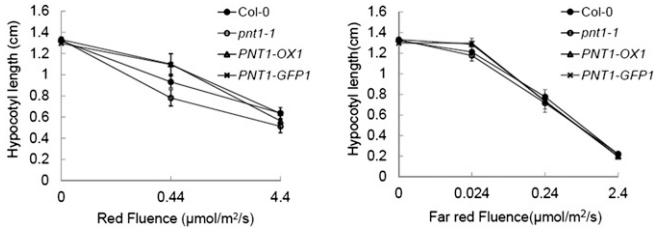

Fig. S4. PNT1 does not play a major role in light-dependent hypocotyl elongation. Seeds were imbibed 3 d at 4 °C in the dark, irradiated with white light for 6 h for the induction of germination, and grown for 4 d under various fluence rates of red and far-red light. Thirty seedlings were randomly chosen for the measurement for each fluence rate (SD, n = 30).

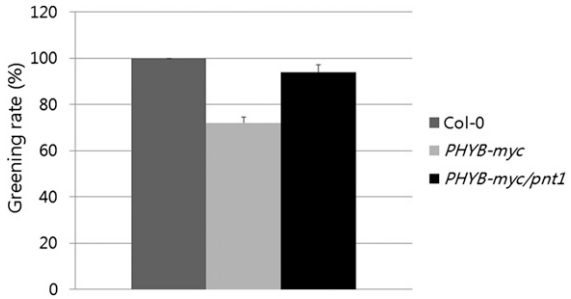

Fig. S5. Greening rate of etiolated *PHYB-myc* and *PHYB-myc/pnt1-1* lines. Five day-old etiolated seedlings were transferred to white light for 2 d and seedlings with green cotyledons were counted (SD, n = 3).

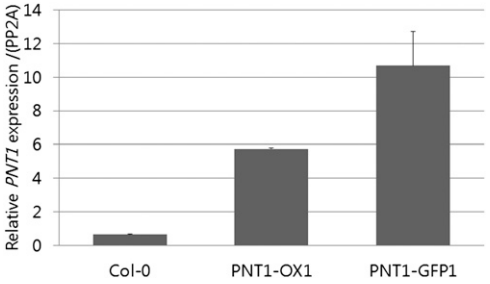

Fig. S6. Expression level of *PNT1* mRNA in two *PNT1* overexpression lines (*PNT1-OX1* and *PNT1-GFP*).

Table S1. Primer sequences used in this study

Primer name	Primer sequence
PNT1 LP	GATCCCTAGG ATGGATTTTA ATGCCGGAGT TC
PNT1 RP1 (PNT1-GFP)	CCGAAGATCTCCACCCTGCTGTGTATTATCAATG
PNT1 RP2 (PNT1-OX1)	CCGAAGATCTTCACTGCTGTGTATTATCAATG
PNT1 real time LP	GGATGGAACC CTTACAGTGG TCAGC
PNT1 real time RP	TCCCGGATTG TAACCCGAGA ATGAT
HEMA real time LP	AGCGGTTGATGACTTAAGCCGAGGT
HEMA real time RP	GGGTCTCGCTCAGCGTTCTACTGT
GUN5 real time LP	GAGCTTGATGGAGCGATGGAGCCAATCGTTTTC
GUN5 real time RP	GTCCCTACATTACCTTTATCAGGTGGGAAACTG
PORA real time LP	TTA CCC CGG TTG TAT TGC AAC GAC T
PORA real time RP	GAG CCT TCT CGA CAT CGC TAG CTT
HPTII real time LP	CGGTGTCGTC CATCACAGTT TGCCAGT
HPTII real time RP	CTTGACATTGGGGAGTTTAGCGAGAGC
PP2A real time LP	TATCGGATGACGATTCTTCGTGCAG
PP2A real time RP	GCTTGGTCGACTATCGGAATGAGAG
Luciferase real time LP	GAATTGGAAT CCATCTTGCT CCAACAC
Luciferase real time RP	GACGTAATCC ACGATCTCTT TTTCCG
phyB LP (phyB-myc)	AGAGGGATCCAAAATGGTTTCCGGAGTCGGGGGT
phyB RP (phyB-myc)	AGAGGGATCCCCATATGGCATCATCAGCATCAT

LP, left primer; RP, right primer.