Supporting Information

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SI Text

Phytochromes Inhibit Hypocotyl Negative Gravitropism at the Early Seedling Stage. We first determined when red light inhibits hypocotyl negative gravitropism. For this analysis, we grew seedlings in the dark and then transferred them to red light. Seedlings that were transferred to red light after incubation in the dark for 0 or for 24 h grew in random directions (Fig. S2*A*), indicating that the first 24 h of darkness do not confer hypocotyl negative gravitropism. When the seedlings were transferred to red light after incubation in the dark for 36 h or more, the lower parts of the hypocotyl grew upright, whereas the upper parts curved and grew in various directions. As the dark incubation time increased from 36 to 60 h, the negatively gravitropic parts of the hypocotyl became longer and the agravitropic upper parts became concomitantly shorter. Unlike the wild-type, hypocotyls of the *phyB* mutant were negatively gravitropic irrespective of light conditions. These results indicated that red light begins to inhibit negative gravitropism at later than 24 h after germination induction and can do so at any time thereafter. Under our experimental conditions, radicles emerged at ≈24 h, and hypocotyls were elongated and clearly distinguished from roots by the hairy root-hypocotyl junction 36 h after the induction of germination (Fig. S1), indicating that red light is effective in inhibiting hypocotyl negative gravitropism once the hypocotyl starts elongating.

The reciprocal analysis further indicated that red light inhibits hypocotyl negative gravitropism at some point during 24 to 36 h time interval after germination induction. For the reciprocal analysis, we grew seedlings under red light and then transferred them to the dark. When red light-grown seedlings were transferred to the dark immediately after germination induction or 24 h after incubation in red light, the seedlings grew upward, displaying normal negative gravitropism (Fig. S2*B*). However, when red light-grown seedlings were transferred at 36, 48, or 60 h, seedlings began to grow in random directions, displaying agravitropism. Unlike the wild-type, hypocotyls of the *phyB* mutant were negatively gravitropic, irrespective of light conditions as *phyB* mutant hypocotyls did not curve upon transfer from red light to the dark at 36, 48, or 60 h. This finding was in contrast to the curving of wild-type hypocotyls upon transfer from the dark to red light. Taken together, our results suggest that hypocotyl negative gravitropism becomes susceptible to red light inhibition later than 24 after germination induction and hypocotyl negative gravitropism, once inhibited by red light, cannot be restored fully by subsequent dark incubation.

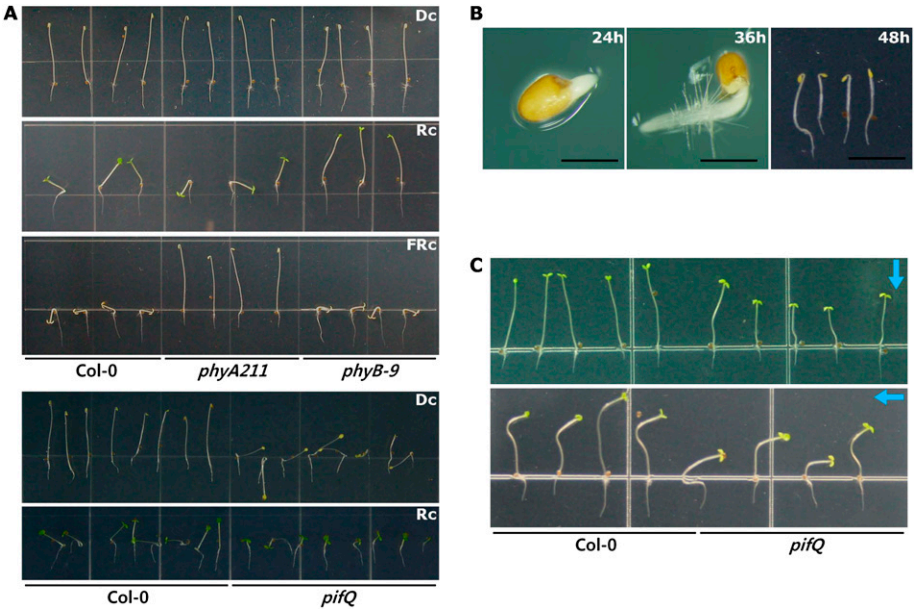

Fig. S1. (*A*) Phytochromes inhibit hypocotyl negative gravitropism, whereas phytochrome-interacting factors (PIFs) promote hypocotyl negative gravitropism. The wild-type (Col-0), the *phyA* mutant (*phyA*-211), the *phyB* mutant (*phyB*-9), and the *pif* quadruple mutant (*pifQ*) were grown in the dark, in red light, or in far-red light for 3 d on vertical agar plates. Dc, Rc, and FRc indicate continuous dark, continuous red light (20 μmol·m^{-2}·s^{-1}), and continuous far-red light (2.4 μmol·m^{-2}·s^{-1}), respectively. (*B*) Germination and growth of seedlings in the dark at 24, 36, and 48 h after the induction of germination. [Scale Bars (*Left* and *Center*) 250 μm, (*Right*) 2.5 mm.] (*C*) Blue light phototropic growth of wild-type and *pifQ* mutant. Seedlings were irradiated from above with blue light for 3 d (*Upper*) and irradiated from the side for 2 more days (*Lower*). Blue arrows indicate the direction of blue light.

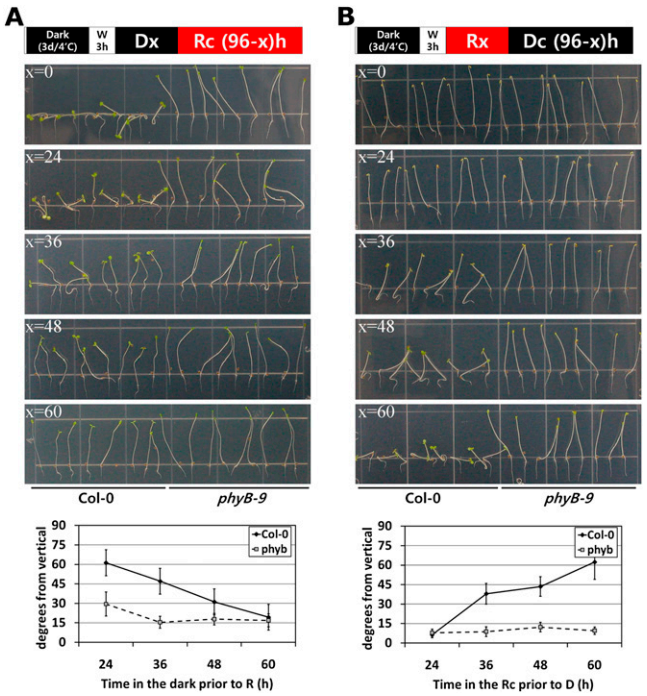

Fig. S2. Phytochromes irreversibly inhibit hypocotyl negative gravitropism after 24 h of germination induction. Diagrams indicate light treatment schemes for the transfer experiments. In both experiments, the wild-type (Col-0) and the *phyB* mutant (*phyB*-9) were imbibed for 3 d at 4 °C (Imb. Dark (3 d/4 °C) and irradiated with white light (100 μmol·m^{-2}·s^{-1}) for 3 h at 22 °C to induce germination [WLc (3 h)]. (*A*) In the dark-to-red transfer experiments, germination-induced seeds were incubated in the dark for *x* hours [Dc (*x* h)], transferred to red light (20 μmol·m^{-2}·s^{-1}), and grown for 96 – *x* hours [Rc (96 – *x*)h] on vertical agar plates. The lower panel indicates the quantification. Data are mean with 95% confidence intervals indicated; *n* = 20. (*B*) In the red-to-dark transfer experiment, germination-induced seeds were incubated in red light (20 μmol·m^{-2}·s^{-1}) for *x* hours [Rc (*x*)], transferred to the dark, and grown for 96 – *x* hours [Dc (96 – *x*)h] on vertical agar plates. The lower panel indicates the quantification. Data are mean with 95% confidence intervals indicated; *n* = 24.

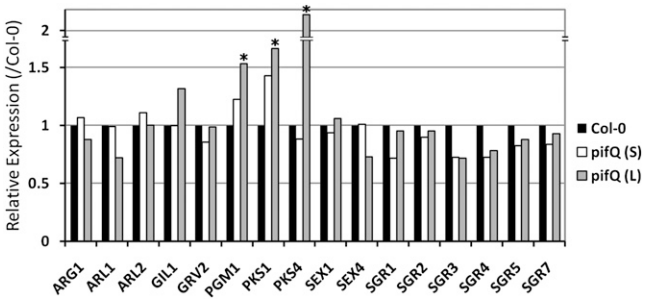

Fig. S3. Relative expression levels of gravitropic genes in the dark-grown wild-type and the *pifQ* mutant in two previously reported microarray data GSE14492 [pifQ (S)] and GSE17159 [pifQ (L)]. None of genes are expressed significantly different between dark-grown wild-type and *pifQ* mutant seedlings [criterion: >1.5 fold, false-discovery rate (FDR) < 0.05] in both microarray data.

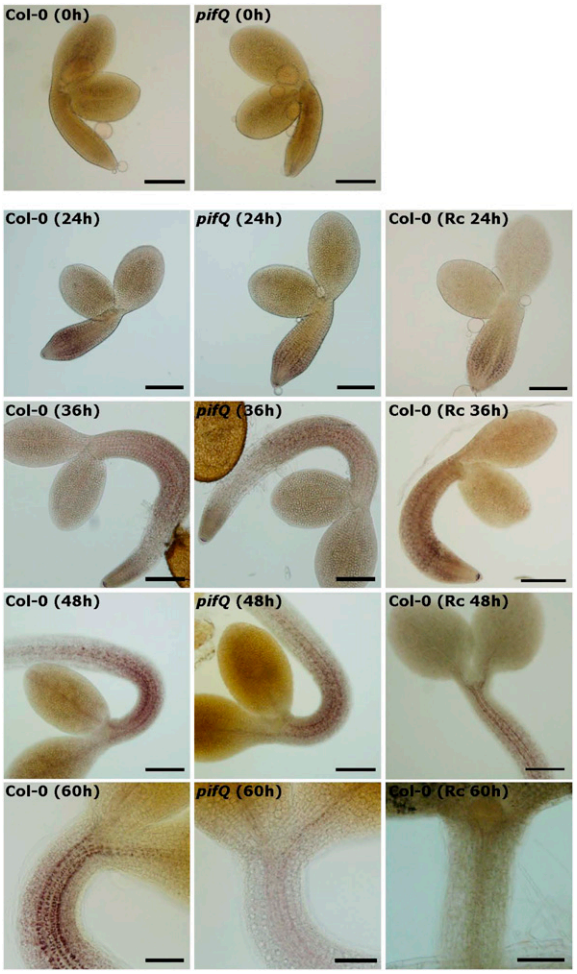

Fig. S4. I_2-KI staining of dark-grown wild-type, dark-grown *pifQ* mutant, and red light-grown wild-type from 0 to 60 h after induction of germination. The scale of bars for 0 h, 24 h, 36 h, and 48 h are 200 μm, while the scale of bars for 60 h are 100 μm.

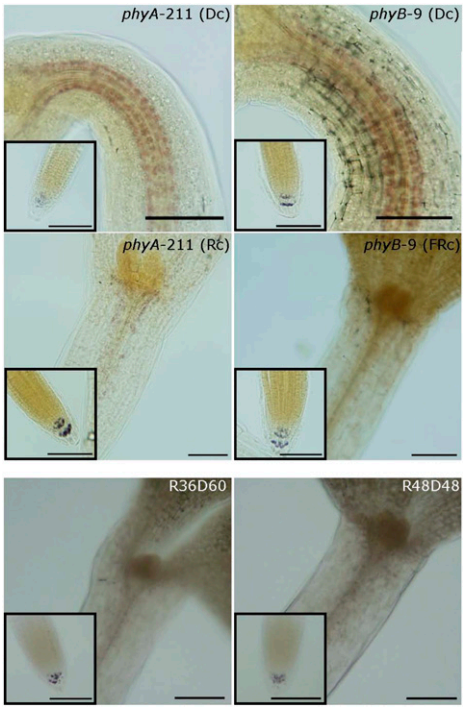

Fig. S5. I_2-KI staining of the *phyA* mutant (*phyA*-211) and the *phyB* mutant (*phyB*-9) grown in the dark (Dc), in continuous red light (Rc), or in continuous far-red light (FRc). RxDy indicates I_2-KI staining of wild-type seedlings grown in red light for *x* hours and transferred to the dark for *y* hours. (Scale bars, 100 μm.)

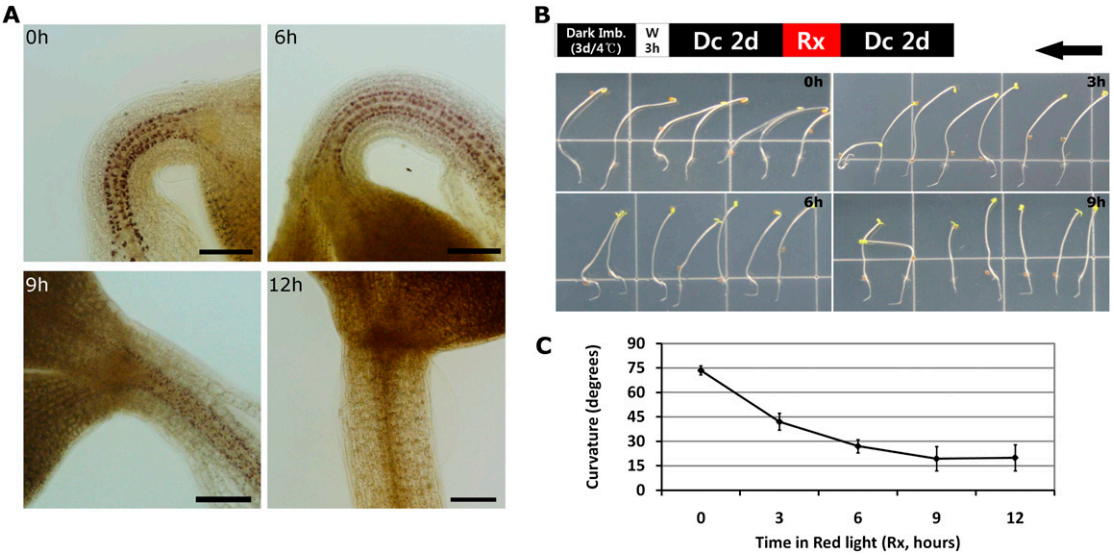

Fig. S6. (*A*) I_2-KI staining of red light transferred seedlings. Two-day-old etiolated seedlings were transferred to red light (20 μmol·m^{-2}·s^{-1}) for a given time before staining. (Scale bars, 100 μm.) (*B*) Negative gravitropism responses of red light transferred seedlings. Two-day-old etiolated seedlings were transferred to red light for a given time and then transferred back to the dark, but this time changing the gravity vector by 90°. An arrow indicates the direction of the changed gravity vector. (*C*) Quantification of hypocotyl negative gravitropism. Data are mean with 95% confidence intervals indicated; *n* = 20.

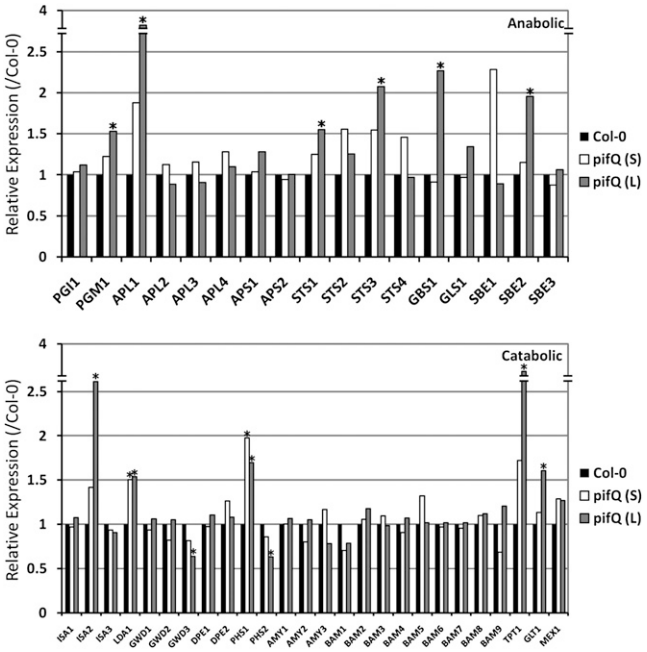

Fig. S7. Expression patterns of starch metabolic genes in the dark-grown wild-type and the *pifQ* mutant in two previously reported microarray data GSE14492 [pifQ (S)] and GSE17159 [pifQ (L)]. Genes that are expressed significantly different were marked by asterisk (*) (criterion: >1.5-fold, FDR < 0.05).

Table S1. Expression patterns of auxin-related genes

	Rel. exp. (/Col-0)			Rel. exp. (/Col-0)			Rel. exp. (/Col-0)	
Gene	*pifQ* (S)	*pifQ* (L)	Gene	*pifQ* (S)	*pifQ* (L)	Gene	*pifQ* (S)	*pifQ* (L)
Metabolism								
AAO1	1.207	1.084	*GH3.5*	**0.589**	0.977	*YUCCA*	0.907	1.006
AMI1	1.023	1.259	*GH3.6*	1.478	**1.856**	*YUCCA2*	1.066	1.258
CYP79B2	1.367	**0.658**	*NIT1*	1.593	N.D.	*YUCCA3*	**0.639**	1.182
CYP79B3	1.230	0.907	*NIT2*	**0.521**	N.D.	*YUCCA4*	1.009	1.009
GH3.1	0.919	1.086	*NIT3*	1.315	1.431	*YUCCA5*	0.643	1.074
GH3.17	0.834	0.961	*SUR1*	1.134	0.771	*YUCCA6*	0.875	0.925
GH3.2	1.094	N.D.	*SUR2*	1.397	**0.477**	*YUCCA8*	1.042	1.030
Transport								
AUX1	0.692	0.853	*PGP14*	0.910	**1.663**	*PIN4*	0.879	**1.596**
PGP1	0.881	0.877	*PGP19*	0.711	0.891	*PIN5*	1.128	0.992
PGP2	0.998	1.139	*PIN1*	1.119	1.186	*PIN6*	1.073	1.094
PGP4	**0.610**	**0.414**	*PIN2*	1.023	**0.652**	*PIN7*	1.269	1.370
PGP10	0.865	1.059	*PIN3*	1.086	1.097	*PIN8*	1.263	0.953
PGP13	1.133	0.986						
Signaling								
AFB1	0.897	0.661	*ARF21*	1.130	1.004	*IAA12*	1.053	1.440
AFB2	0.961	1.003	*ASK1*	1.141	0.825	*IAA13*	0.824	0.927
AFB3	0.862	1.174	*ASK2*	0.974	0.929	*IAA14*	1.117	0.846
AFB4	1.136	1.038	*AXR1*	1.136	0.792	*IAA16*	0.799	0.997
AFB5	0.806	0.968	*CAND1*	0.940	0.912	*IAA17*	0.629	**0.500**
ARF1	1.042	1.144	*CSN5A*	1.030	0.901	*IAA18*	1.094	**1.655**
ARF3	1.374	1.323	*CSN5B*	0.788	0.744	*IAA19*	0.615	**0.390**
ARF4	0.860	1.007	*CUL1*	0.960	0.825	*IAA20*	0.635	0.886
ARF5	0.969	0.732	*ECR1*	1.109	1.068	*IAA26*	0.697	**0.525**
ARF6	0.743	**0.605**	*IAA1*	1.221	1.314	*IAA28*	0.999	0.770
ARF7	0.765	0.731	*IAA2*	1.119	0.748	*IAA29*	**0.355**	**0.201**
ARF8	1.019	0.903	*IAA3*	0.741	0.692	*IAA30*	0.788	0.967
ARF9	1.230	1.298	*IAA4*	0.788	0.929	*IAA31*	1.005	1.053
ARF10	0.998	1.323	*IAA5*	1.842	**2.027**	*IAA33*	1.467	0.991
ARF11	1.567	1.178	*IAA6*	0.656	0.710	*IAA34*	1.521	**2.442**
ARF12	0.990	**1.643**	*IAA7*	0.936	0.722	*RBX1*	0.998	0.863
ARF16	0.689	**0.648**	*IAA8*	0.914	1.168	*RCE1*	**0.666**	0.866
ARF17	0.808	1.040	*IAA9*	0.935	0.822	*RUB2*	0.890	0.807
ARF18	**0.464**	**0.307**	*IAA10*	0.793	1.219	*SGT1b*	0.935	0.847
ARF19	0.961	1.035	*IAA11*	0.859	1.098	*TIR1*	0.842	1.035

Expression patterns of auxin-related genes in the dark-grown WT and the *pifQ* mutant mutant in two previously reported microarray data: GSE14492 [pifQ (S)] and GSE17159 [pifQ (L)]. Genes that are expressed significantly different between two samples were boldface (criterion: >1.5 fold, FDR < 0.05).