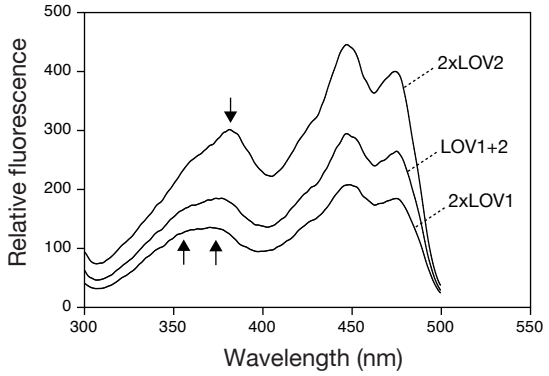
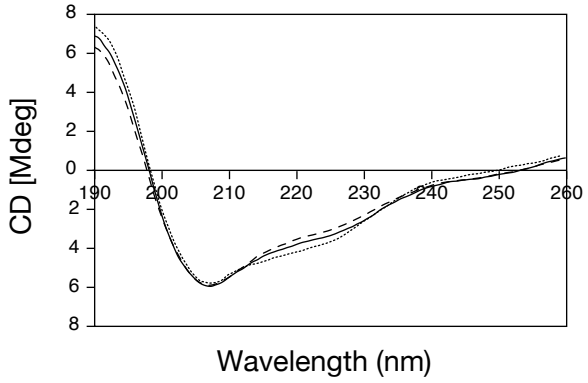
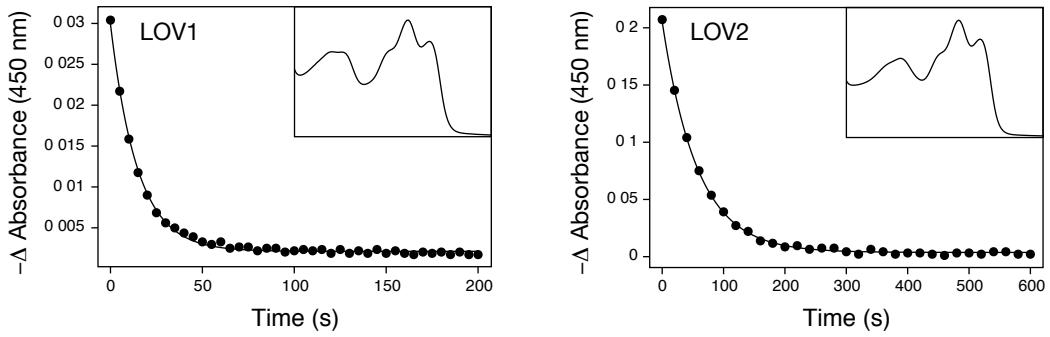
Supplemental Data. Kaiserli *et al.* (2009) Domain swapping to assess the mechanistic basis of *Arabidopsis* phototropin 1 receptor kinase activation and endocytosis by blue light.



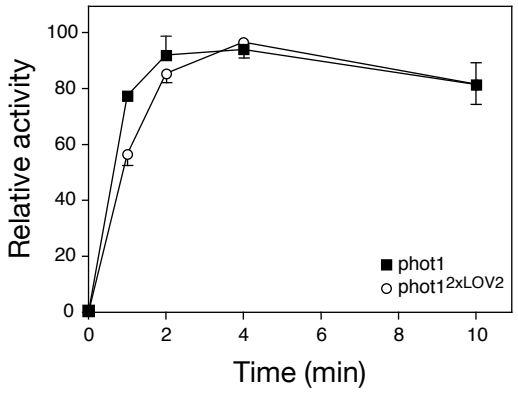
Supplemental Figure 1. Fluorescence properties of 2xLOV1 and 2xLOV2.
Fluorescence excitation spectra of wild-type phot1 (LOV1+2), 2xLOV1 and 2xLOV2 (0.4 mg ml^{-1}). Arrows indicate spectral differences between 2xLOV1 and 2xLOV2 in the UV-A region of the spectrum.



Supplemental Figure 2. Dark state CD spectra of LOV1+2, 2xLOV1 and 2xLOV2 measured in the far-ultraviolet region (190–260 nm).
Equal concentrations (0.4 mg ml^{-1}) of LOV1+2 (solid line), 2xLOV1(dotted line) and 2xLOV2 (dashed line) were used for analysis.

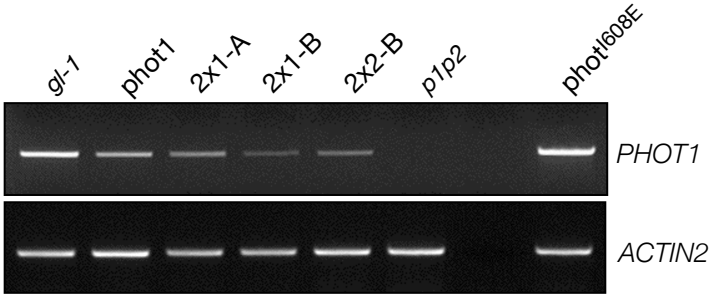


Supplemental Figure 3. Dark-recovery kinetics for individual LOV1 and LOV2 domains of *Arabidopsis* phot1. Absorption spectra are shown (inset). Absorption changes after light excitation measured at 450 nm is shown in the main panel. A white-light camera strobe flash provided the excitation pulse. Decay fits to a single exponential with half-lives of 11 s and 40 s, respectively.

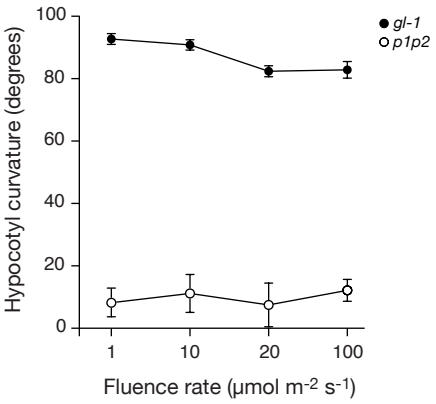


Supplemental Figure 4. Kinetics of light-dependent autophosphorylation for wild-type phot1 and phot1^{2xLOV2} in extracts from insect cells.

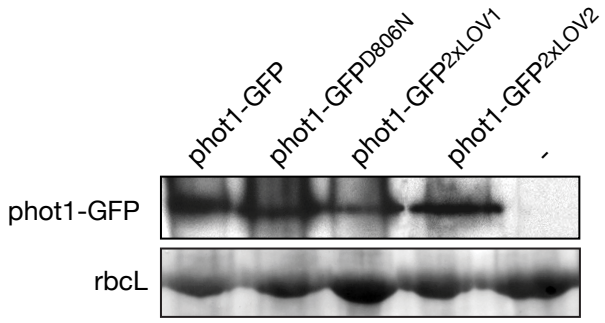
Kinase activity was quantified by phosphorimaging and expressed as a percentage of maximal phosphorylation activity relative to dark controls. Standard errors are shown (n = 3).



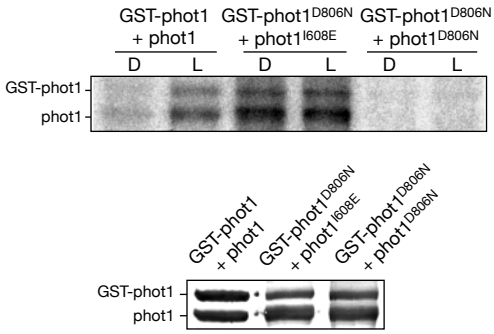
Supplemental Figure 5. RT-PCR analysis of *PHOT1* transcripts.
PHOT1 and control *ACTIN2* transcripts in wild-type *Arabidopsis* (*gl-1*), the *phot1 phot2* double mutant (*p1p2*) and in transgenic lines expressing wild-type phot1, phot1^{2xLOV1} (2x1-A, 2x1-B), phot1^{2xLOV2} and phot1^{I608E} in the *phot1 phot2* double mutant driven by the *35S* promoter. Plants were grown in white light (50 µmol m^{-2} s^{-1}) for 4 weeks.



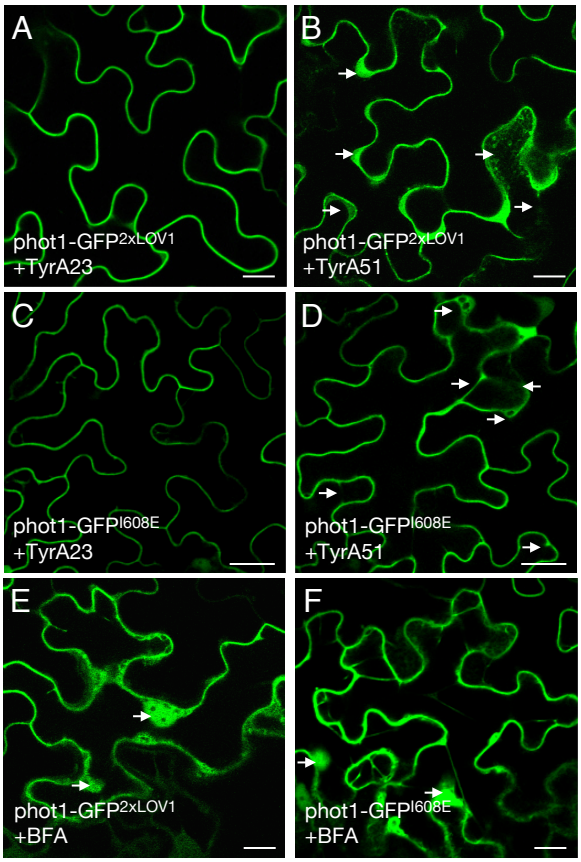
Supplemental Figure 6. Phototropism fluence-rate response of 3-day old etiolated wild type (*gl-1*) and *phot1 phot2* mutant seedlings *(p1p2*).
Phototropic curvatures were measured after exposure to unilateral blue light for 24 h. Each value is the mean of at least 20 seedlings. Standard errors are shown.



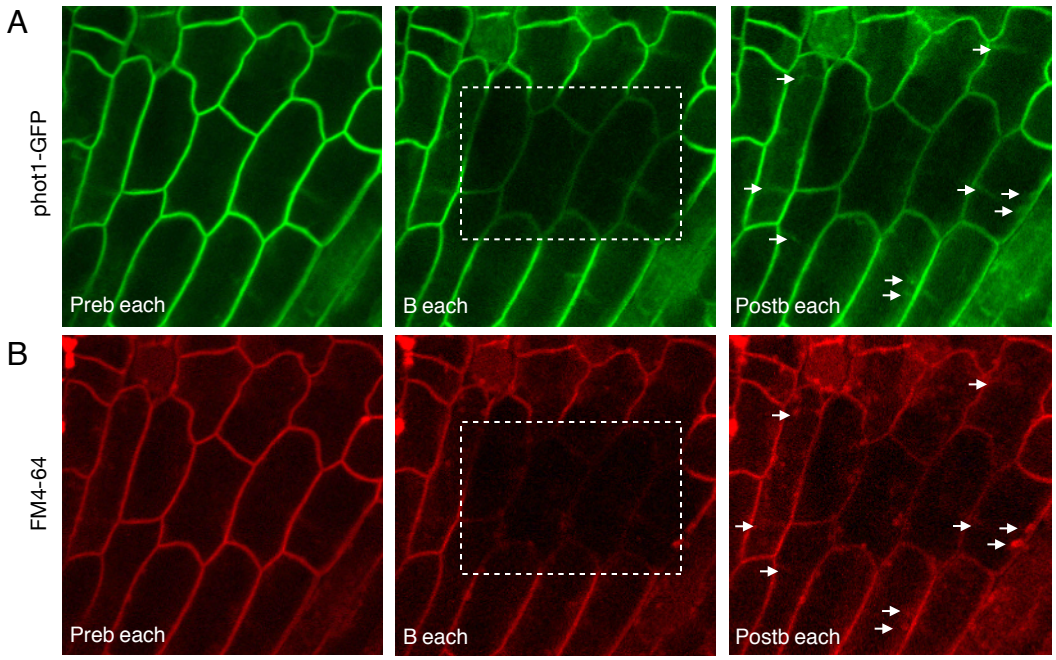
Supplemental Figure 7. Immunoblot analysis of *Nicotiana benthamiana* expressing phot1-GFP fusions.
Total protein extracts (30 µg) from *Nicotiana benthamiana* expressing phot1-GFP, phot1-GFP^{D806N}, phot1-GFP^{2xLOV1}, phot1-GFP^{2xLOV2} from the *35S* promoter. Extracts from a non-infiltrated sample (-) were included as a control. Protein extracts were probed with an anti-GFP specific monoclonal antibody. Ponceau S staining of Rubisco large subunit (rbcL) is shown as a loading control.



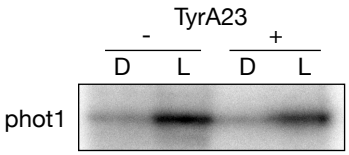
Supplemental Figure 8. Trans phosphorylation between phot1 molecules.
Autoradiograph showing light-independent trans phosphorylation of a kinase-inactive version of GST-phot1 (GST-phot1^{D806N}) by a constitutively active form of phot1 (phot1^{I608E}) in protein extracts from insect cells. Immunoblot analysis of phot1 protein levels is shown below.



Supplemental Figure 9. Pharmacological interference of phot1-GFP localization in *Nicotiana benthamiana*.
(A) Treatment with 30 µM Tyrphostin A23 (Tyr23) attenuates phot1-GFP^{2xLOV1} internalization in tobacco leaf epidermal cells in the absence of light.
(B) Treatment with 30 µM Tyrphostin A51(TyrA51), an inactive analogue of Tyrphostin A23 does not affect phot1-GFP^{2xLOV1} internalization (indicated by the white arrows).
(C) Treatment with 30 µM TyrA23 reduces phot1-GFP^{I608E} internalization in the absence of light.
(D) Treatment with 30 µM TyrA51 does not reduce phot1-GFP^{I608E} internalization (indicated by the white arrows).
(E) Treatment with 50 µM Brefeldin A (BFA) induces aggregation of phot1-GFP^{2xLOV1} from the plasma membrane into BFA compartments in darkness (indicated by the white arrows).
(F) BFA also induces aggregation of phot1-GFP^{I608E} (indicated by the white arrows). In each case the scale bar represents 20 µm.



Supplemental Figure 10. FRAP analysis of phot1-GFP in 3-day old etiolated *Arabidopsis*.
Bleached area is shown by the dotted line. Representative images prior to photobleaching, following bleaching and post recovery (3 min) are shown. Colocalization of phot1-GFP and FM4-64 following photobleaching is indicated by the white arrows.
(A) phot1-GFP
(B) FM4-64



Supplemental Figure 11. Effect of Tyrphostin A23 (Tyr A23) on phot1 kinase activity in protein extracts from insect cells.

Autoradiograph showing light-dependent autophosphorylation of extracts treated with DMSO (-) or treated with 30 μM Tyr A23 prior to phosphorylation analysis. Samples were given a mock irradiation (D) or irradiated with white light (L) at a total fluence of 10,000 μmol m^{-2} prior to the addition of radiolabelled ATP.

Supplemental Table1. Primers used in this study.

Primer	Sequence
LOV1 DEL_F	5′-gagcatgcttgaaacgtcgacaacgcatc-3′
LOV1 DEL_R	5′-ctgcatgcagcaagcacactgaaggggcc-3′
LOV2 DEL_F	5′-gagcatgcctcgatacgttcgagtgtagt-3′
LOV2 DEL_R	5′-ctgcatgcagcaagcacgtagaaccagtt-3′
LOV1 SphI_F	5′-atgcatgccaaacgtttgtggtctcagatgct-3′
LOV1 SphI_R	5′-tagcatgccacctccacttgcattccgataaa-3′
LOV2 SphI_F	5′-atgcatgcaagaatttcgtcatcactga-3′
LOV2 SphI_R	5′-tagcatgccccgtctagttgaactccaataaa-3′
pCAL LOV1_F	5′-gcggaattcgggattccaagagtatcggaa-3′
pCAL LOV1_R	5′-ttgccatggttaatcgtatcgaatcagagattc-3′
pCAL LOV2_F	5′-cggaattccctgagagtgtggatgataaag-3′
pCAL LOV2_R	5′-ttgccatggttagtttgcccataaatcatcctc-3′
Link DEL_F	5′-gagcatgcagtgtgcttgctcacctccac-3′
Link DEL_R	5′-ctgcatgcaatttcgtcatcactgatcct-3′
pAcG3X PHOT1_F	5′-gcgaattctcaaaaaacatttgtttgcag-3′
pAcG3X PHOT1_R	5′-gaggatccccatggaaccaacagaaaaaccatcgacc-3′
pAcHLT-A LOV2K_F	5′-cggaattccctgagagtgtggatgataaag-3′
pAcHLT-A LOV2K_R	5′-atcccgggtcaaaaaacatttgtttg-3′
PHOT1 trans_F	5′-gatacgatgcccgccaaaaag-3′
PHOT1 trans_R	5′-acagatcaaaatcgacaaagagat-3′
ACTIN trans_F	5′-cttacaatttccgctctgc-3′
ACTIN trans_R	5′-gttgggatgaaccagaagga-3′
pEZR PHOT1_F	5′-gcgaattcgagagctcaaagatggaa-3′
pEZR PHOT1_R	5′-gcggatccgcaaaaacatttgtttg-3′
pEZR LOV2K_F	5′-cggaattccctgagagtgtggatgataaag-3′
pEZR LOV2K_R	5′-gcggatccgcaaaaacatttgtttg-3′
pSPYCE/SPYNE PHOT1_F	5′-gcggatccgagagctcaaagatggaa-3′
pSPYCE/SPYNE PHOT1_R	5′-atcccggggcaaaaacatttgtttg-3′