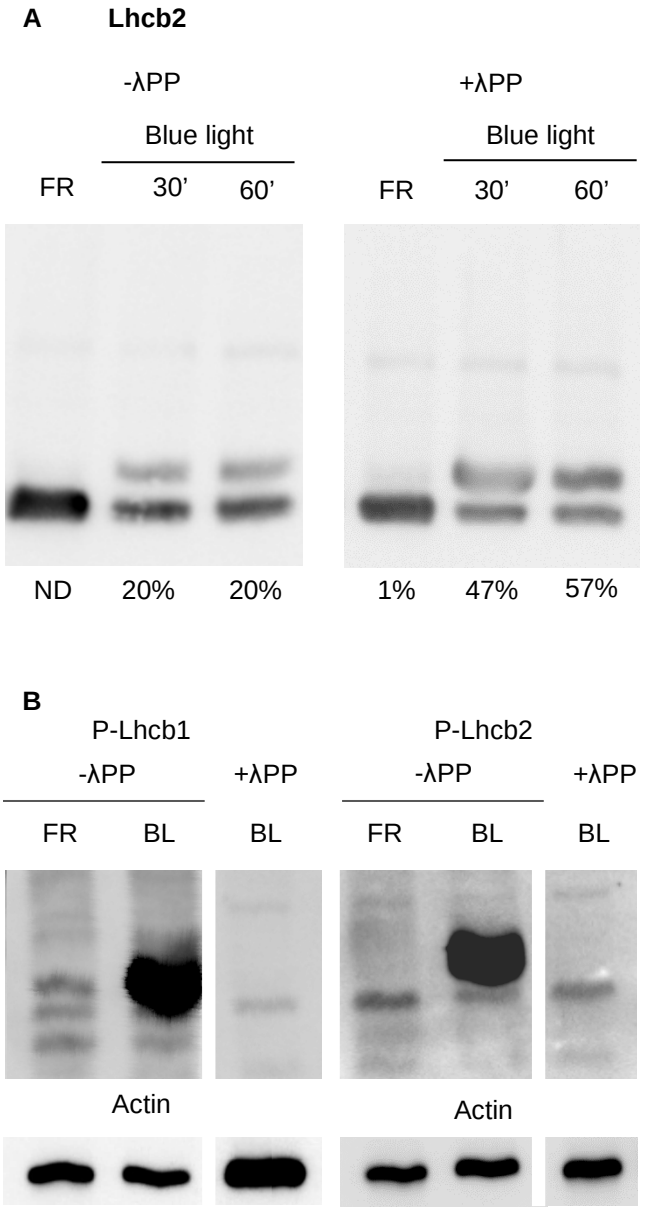
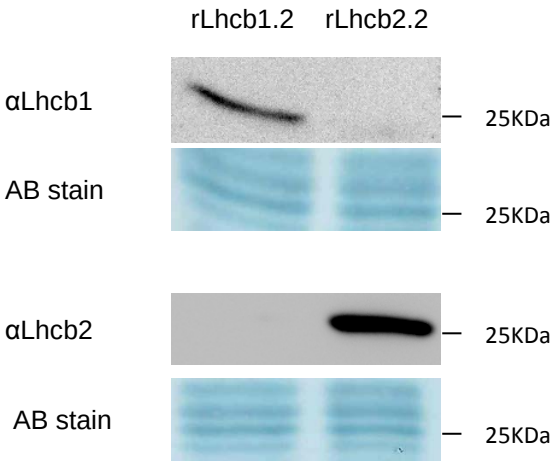
Supplemental figure 1



Phosphatase treatment enhances detection of the phosphorylated form of Lhcb2.
A, Prior to immunodetection with antiserum against Lhcb2, replicate membrane blots were incubated in phosphatase buffer in the presence (+λPP) or absence (-λPP) of λ protein phosphatase. The signal for the phosphorylated form of Lhcb2 increases more than two-fold after treatment with the phosphatase, indicating that phosphorylation severely hampers the recognition of P-Lhcb2 by the Lhcb2 antiserum. Total proteins were extracted from 6 weeks old plants exposed to far-red light for 1 hour (FR) and then shifted to blue light (BL) for 30 or 60 minutes. The average fold-increase of the apparent phosphorylation level after the phosphatase treatment was 2.25 ± 0.31 (n=3).
B, To test whether dephosphorylation of proteins on the membrane blot by λ phosphatase was complete, replicate membrane blots were incubated with λ protein phosphatase (+λPP) or untreated. They were then immune-decorated with phospho-specific antibodies against P-Lhcb1 or P-Lhcb2 as indicated at the top. A long exposure is presented to highlight the absence of detectable signal on the phosphatase-treated membranes while the signal is saturating on the untreated membranes. The FR and BL (60') samples are the same as in panel A.

Supplemental figure 2

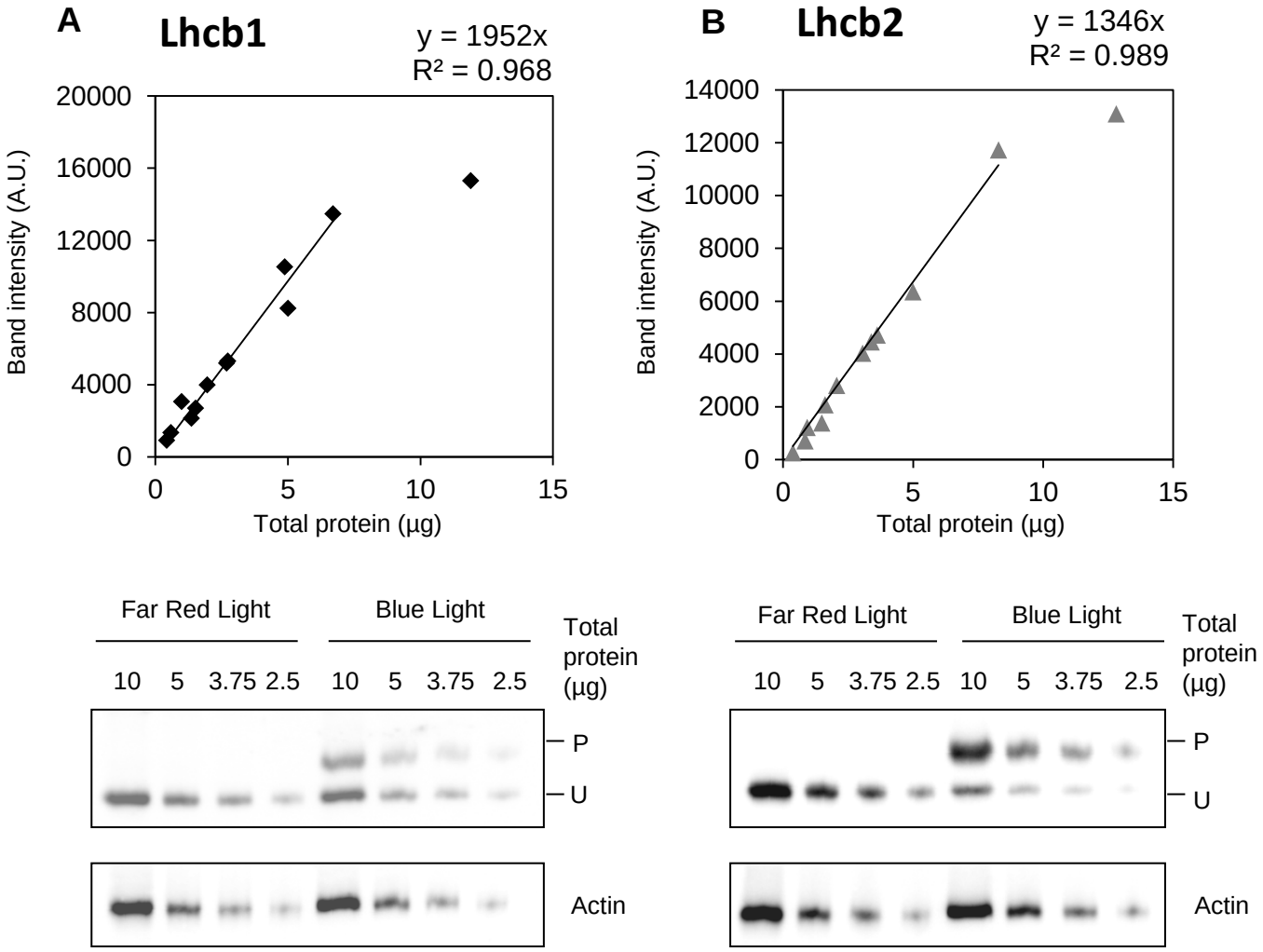


Specificity of the Lhcb1 and Lhcb2 antibodies against recombinant proteins.
Antibodies against Lhcb1 or Lhcb2 were tested by immunoblotting of crude E.coli extracts expressing the recombinant proteins Lhcb1.2 or Lhcb2.3. The staining with amido black (AB stain) is shown to confirm the loading.

Methods.
The coding sequences were obtained from the TAIR repository (Lhcb1.2 - U17058 Lhcb2.3 – U16031) and assembled into the pET28a vector (Novagen) between the *Nco*I and *Xho*I sites with the following primers:
Lhcb1.2 FOR: GTTTAACTTTAAGAAGGAGATATACCATGGATGAGGAAGACTGTTGCCAAGC
Lhcb1.2 REV: CTCAGTGGTGGTGGTGGTGGTGCTCGAGCTTTCCGGGAACAAAGTTGGTTGC
Lhcb2.3 FOR: GTTTAACTTTAAGAAGGAGATATACCATGCGTCGTACCGTCAAGT
Lhcb2.3 REV: CTCAGTGGTGGTGGTGGTGGTGCTCGAGTTTTCCGGGGACGAAGTTGGTG.

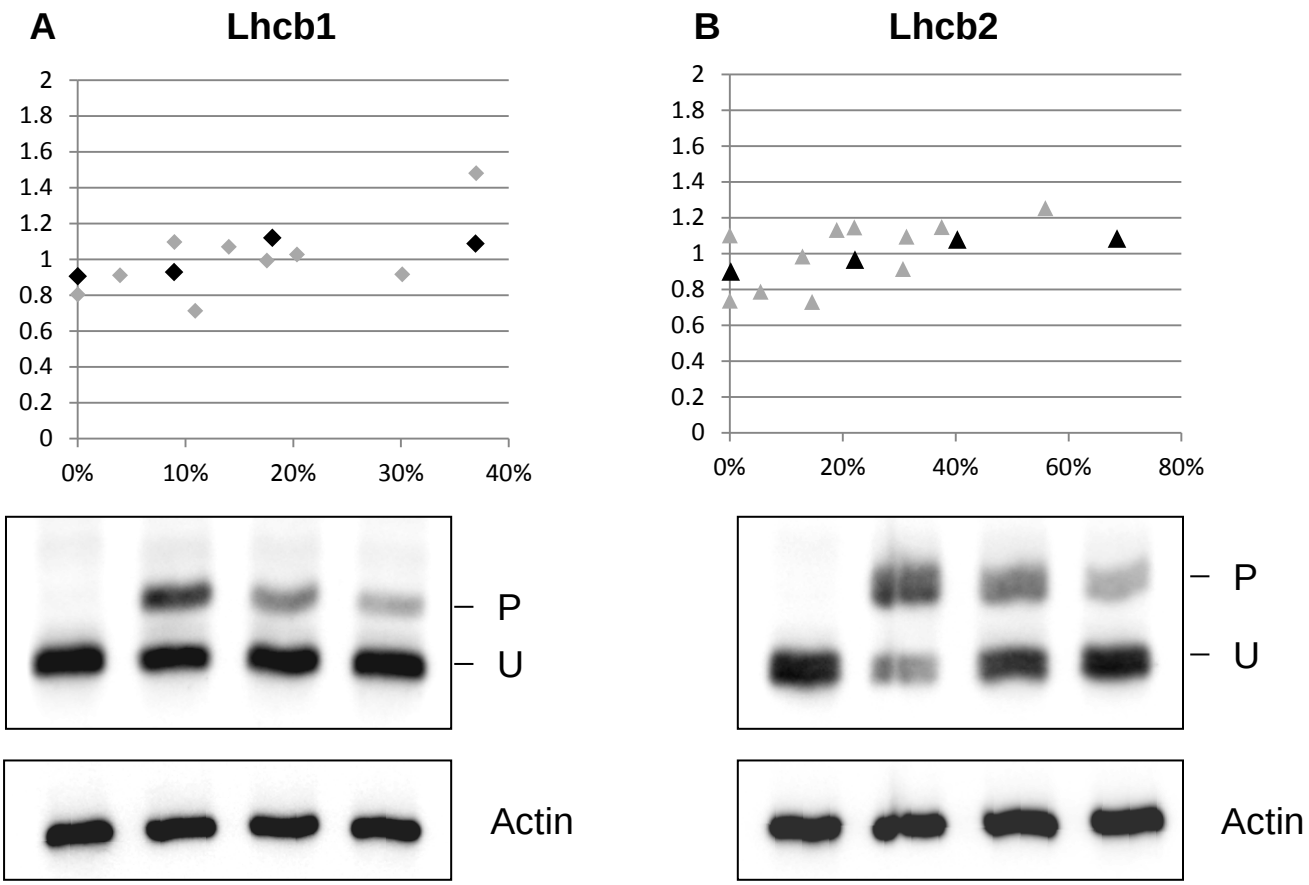
Cultures were grown in LB media (Sigma-Aldrich) to the optical density of 0.5-0.6 at 600 nm. Protein expression was then induced with 0.5mM IPTG for 4h at 28°C. The pellets were resuspended in lysis buffer (100mM Tris-HCl pH 7.8 2% SDS, 1x protease inhibitors (P8849 - Sigma-Aldrich)), sonicated 10 times for 10 seconds and centrifuged for 5 minutes at 18000 g. The supernatant was collected and diluted with 4x loading buffer (10% SDS, 40% Sucrose, 1mM EDTA, 20% 2-Mercaptoethanol, 62.5mM Tris-HCl pH 6.8). The amount of protein was corrected on the basis of coomassie staining of the samples after SDS-PAGE. After the separation on 10% acrylamide/bis-acrylamide gel the protein were transfered to a nitrocellulose membrane and treated as described in Methods for the Two-layer Phos-tag™ PAGE except that the λ protein phosphatase treatment was not performed.

Supplemental figure 3



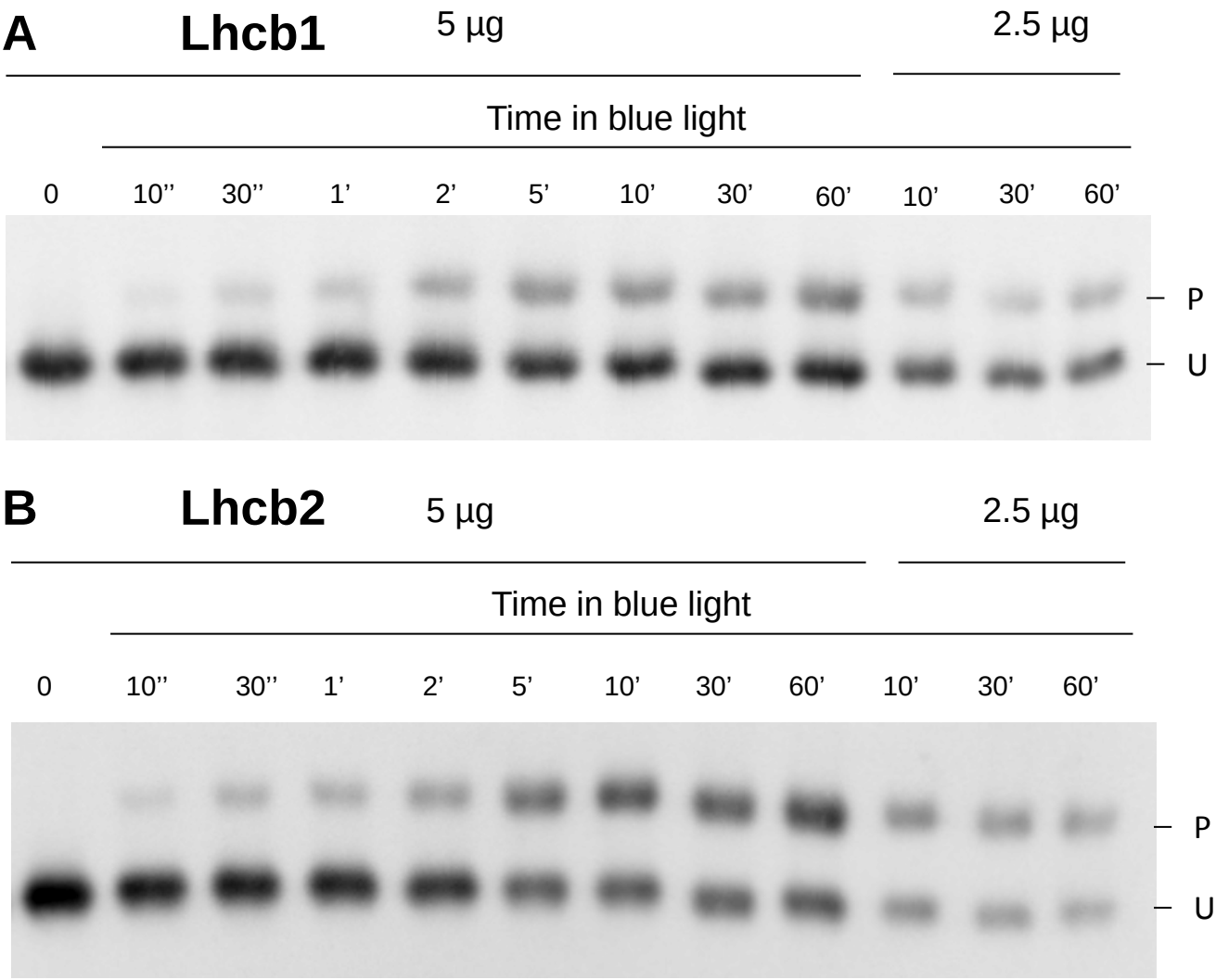
Determination of the linear range of the immunoblot assay.
Different amounts of total protein (as indicated at the top of the immunoblots and plotted on the x axis) were subjected to two-layer Phos-tag™ PAGE. The signal measured by immunoblotting (integration of pixel intensities for each band), normalized to the signal obtained with actin antiserum, is plotted on the y axis. The samples are total protein extracts from plants subjected to far red or blue light, as indicated. All the signals from phosphorylated (P) and unphosphorylated (U) forms are plotted after normalizing to the signal of actin.

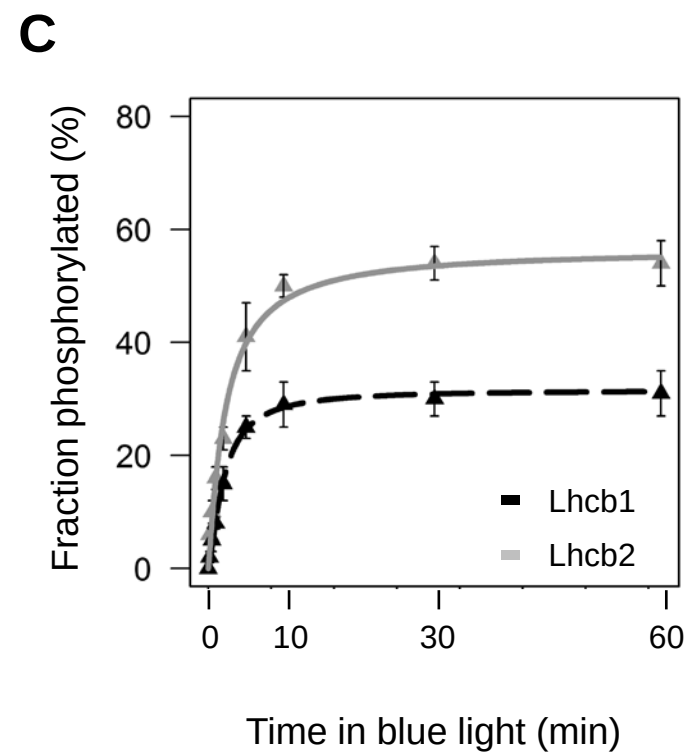
Supplemental figure 4



Phosphorylated and non-phosphorylated Lhcb1 or Lhcb2 are detected with similar efficiency.
The sum of the band intensities for the phosphorylated (P) and non-phosphorylated (U) forms of Lhcb1 (A) or Lhcb2 (B) were normalized to the intensity of the actin band. The normalized sum (y axis) does not change with different extents of phosphorylation (x axis), confirming that there is no specific retention of the phosphorylated proteins during transfer from the gel to the immunoblot membrane. Samples with different extents of Lhcb phosphorylation were obtained by mixing different amounts of protein extract from plants exposed for 30 minutes to blue light with protein extracts from plants treated with far-red light for 1 hour (black symbols) or by taking samples at different time points of exposure to blue light (gray symbols).

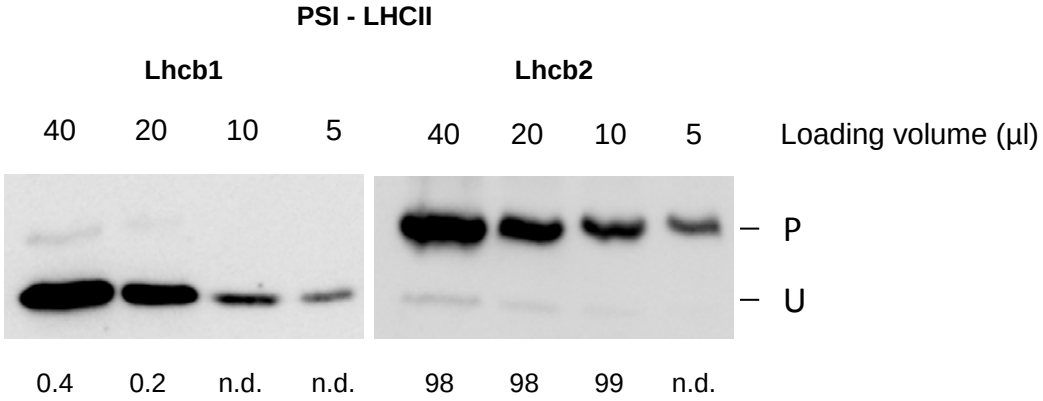
Supplemental figure 5





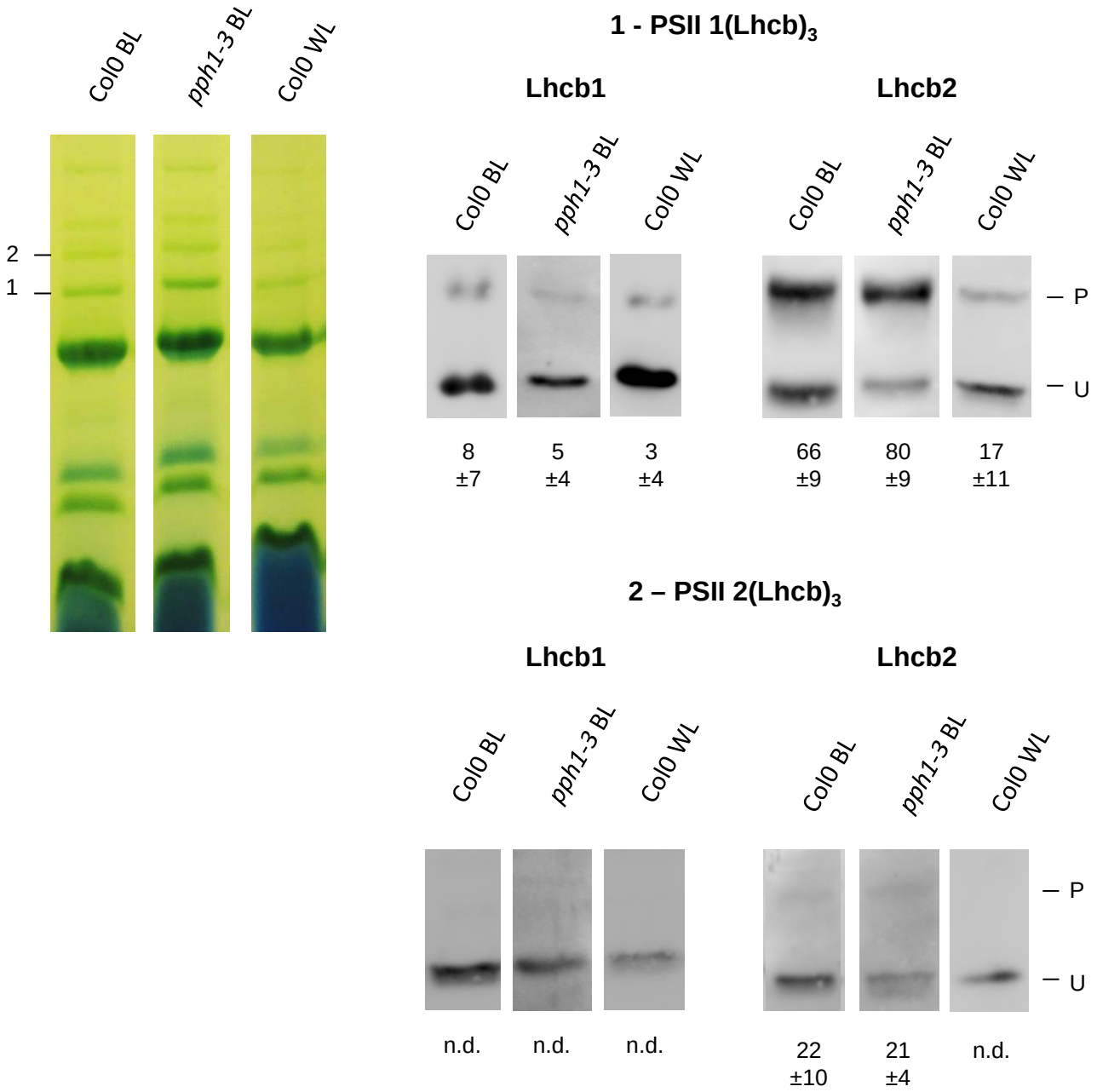
Phosphorylation kinetics in leaves of 6-week old Arabidopsis plants.
A and B, Phosphorylation kinetics of Lhcb1 and Lhcb2 were determined by two-layer Phos-tag™ PAGE upon a shift (at time 0) from far red light (state 1) to blue light (state 2) for Lhcb1 (A) and Lhcb2 (B).
C, The extent of phosphorylation of Lhcb1 (black points) and Lhcb2 (gray points) in panels A and B respectively is plotted as a function of time. Total proteins were extracted from mature leaves of two different plants for each time point. Error bars represent the standard deviation of three replicate experiments. The lines represent the sigmoidal interpolation of the points for Lhcb1 (dashed black line) and Lhcb2 (plain gray line).

Supplemental figure 6



Evaluation of phosphorylation levels of Lhcb1 and Lhcb2 in the PSI-LHCII supercomplex.
Bands corresponding to the PSI-LHCII supercomplex were extracted as in Figure 5. A series of decreasing volumes were loaded as shown at the top. The presence of the less abundant bands can be detected only at larger loadings (phosphorylated Lhcb1 and unphosphorylated Lhcb2). Due to the saturation of the stronger signal the quantification for these fractions can not be accurately performed, the apparent percentage of phosphorylation is reported below. (n.d., not detectable). Thus a conservative estimate is that in the PSI-LHCII complex, Lhcb1 phosphorylation is <1% and Lhcb2 phosphorylation is >98%.

Supplemental figure 7



Quantification of Lhcb1 and Lhcb2 phosphorylation in PSII-LHCII supercomplexes.
Wild-type Col0 and mutant *pph1-3* plants were treated for 1 hour with far-red light and then exposed to blue light for 30 minutes (BL). Wild-type Col0 were also grown under white light (70 µmol sec^{-1} m^{-2}) and harvested 4 hours after the onset of light (WL). Thylakoid membrane complexes extracted with β-dodecylmaltoside were subjected to BN PAGE. Individual bands were cut as indicated on the left (1-2) and separately analyzed by two-layer Phos-Tag™ PAGE and immunoblotting. In band 1 (PSII 1$(Lhcb)_3$) the PSII dimer is associated with 1 LHCII trimer, in band 2 (PSII 2$(Lhcb)_3$) with 2 trimers.
The measured percentage of phosphorylation is indicated below each lane with its standard deviation (n=4). (n.d. non determined, i.e. < 1% phosphorylation)