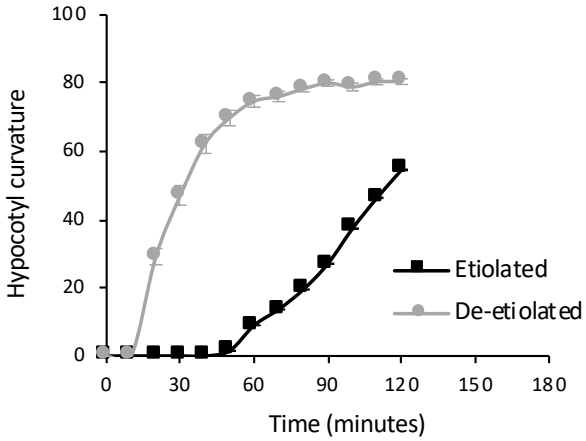


Figure S1. De-etiolated tomato seedlings show enhanced kinetics of phototropism.
Phototropic response of etiolated and de-etiolated tomato seedlings after 2 h of irradiation with unilateral blue light (0.5 µmol m^{-2} s^{-1}). Each value is the mean ± S.E. of 11 seedlings.

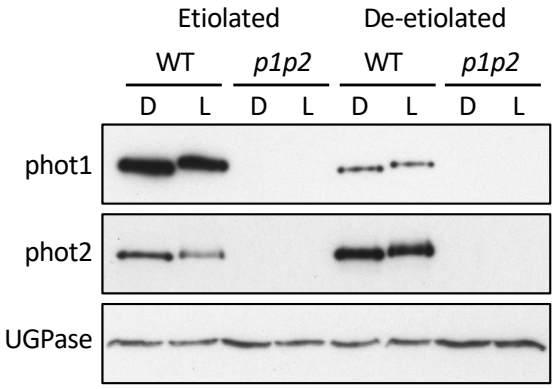


Figure S2. Protein abundance of phot1 and phot2 in etiolated and de-etiolated seedlings. Immunoblot analysis of total protein extracts from etiolated and de-etiolated wild-type (WT) and *phot1phot2* (*p1p2*) double mutant seedlings maintained in darkness (D) or irradiated with 20 µmol m^{-2} s^{-1} of unilateral blue light (L) for 15 min. Protein extracts were probed with anti-phot1 (upper panel), anti-phot2 antibodies (middle panel) and anti-UGPase antibody as a loading control (lower panel).

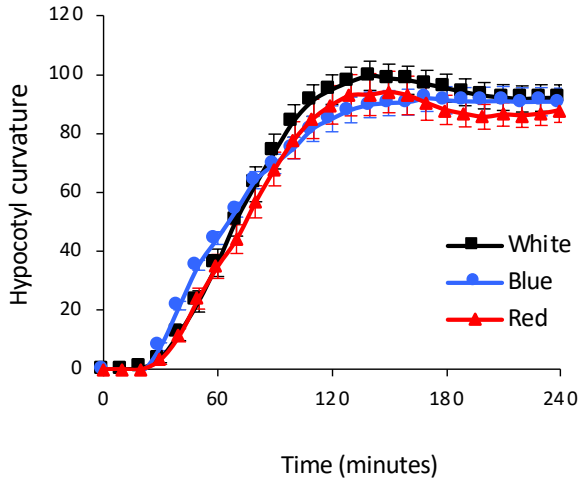
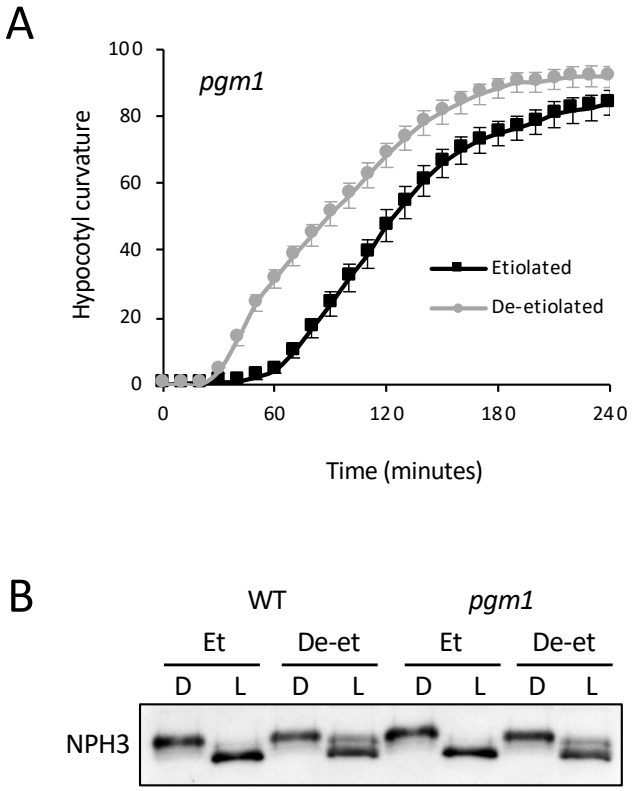


Figure S3. Phototropic response of wild-type seedlings de-etiolated under different light conditions. Seedlings were de-etiolated under 8 h of white (80 µmol m^{-2} s^{-1}), red (40 µmol m^{-2} s^{-1}) or blue (40 µmol m^{-2} s^{-1}) light. Seedlings were irradiated with 0.5 µmol m^{-2} s^{-1} of unilateral blue light and hypocotyl curvatures were measured every 10 min for 4 h. Each value is the mean ± S.E. of 16-20 seedlings.



Supplementary Figure S4. Phototropic responses and NPH3 phosphorylation status in etiolated and de-etiolated seedlings of the *pgm1* mutant.

(A) Phototropism of etiolated and de-etiolated *pgm1* mutant seedlings irradiated with 0.5 µmol m^{-2} s^{-1} of unilateral blue light. Hypocotyl curvatures were measured every 10 min for 4 h and each value is the mean ± S.E. of 20 seedlings.

(B) Immunoblot analysis of NPH3 phosphorylation status in etiolated (Et) and de-etiolated (De-et) seedlings. Seedlings were either maintained in darkness (D) or irradiated with 0.5 µmol m^{-2} s^{-1} of unilateral blue light for 60 min. Protein extracts were probed with anti-NPH3 antibody.

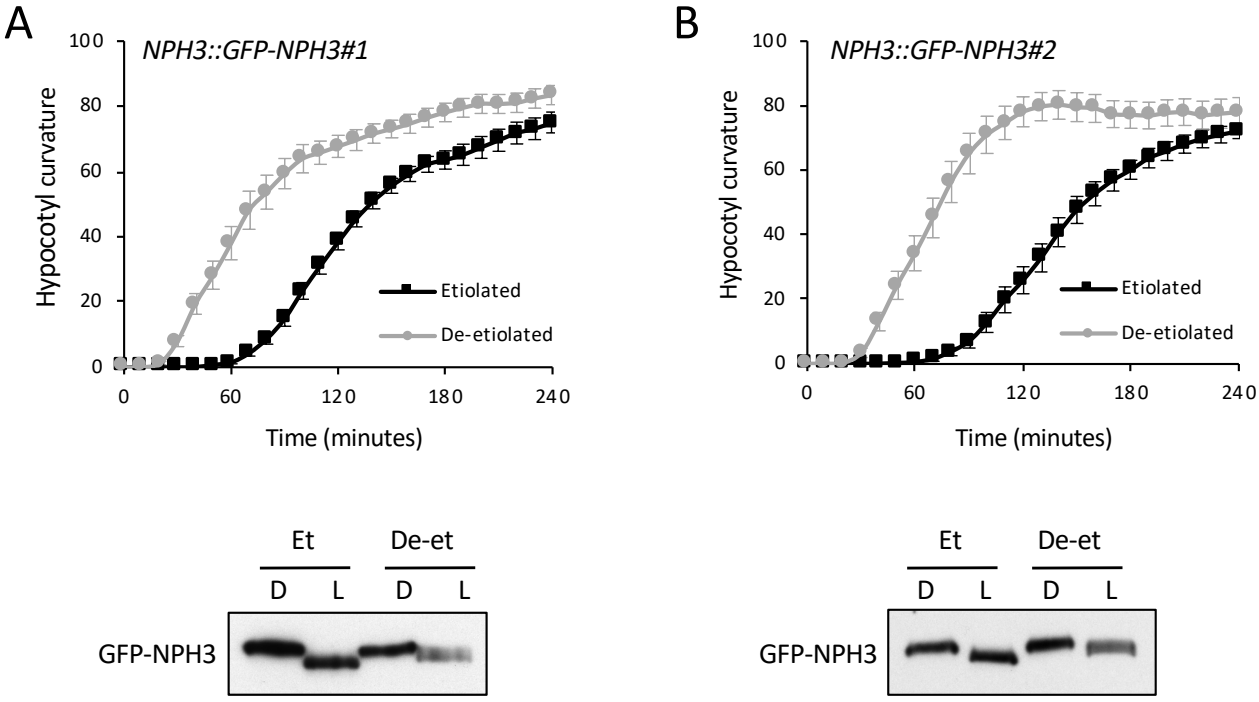


Figure S5. GFP-NPH3 functionality and phosphorylation status in etiolated and de-etiolated seedlings. Phototropism of etiolated and de-etiolated seedlings expressing *NPH3::GFP-NPH3* line #1 **(A)** and line#2 **(B)** irradiated with 0.5 µmol m^{-2} s^{-1} of unilateral blue light. Hypocotyl curvatures were measured every 10 min for 4 h and each value is the mean ± S.E. of 20 seedlings. Immunoblot analysis of GFP-NPH3 phosphorylation status in etiolated (Et) and de-etiolated (De-et) seedlings is shown below. Seedlings were either maintained in darkness (D) or irradiated with 0.5 µmol m^{-2} s^{-1} of unilateral blue light for 60 min. Protein extracts were probed with anti-NPH3 antibody.

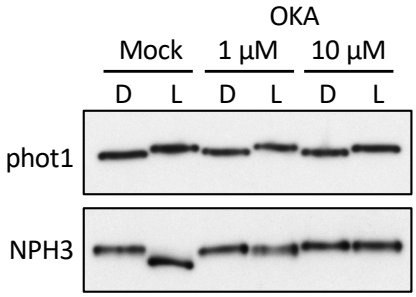


Figure S6. Effect of OKA treatment on blue light-induced NPH3 dephosphorylation.
Immunoblot analysis of total protein extracts from etiolated seedlings pre-treated with 1 µM or 10 µM of OKA, or solvent-only control (mock) and maintained in darkness (D) or irradiated with 0.5 µmol m^{-2} s^{-1} of unilateral blue light for 60 min (L). Protein extracts were probed with anti-phot1 (upper panel) and anti-NPH3 (lower panel) antibodies.

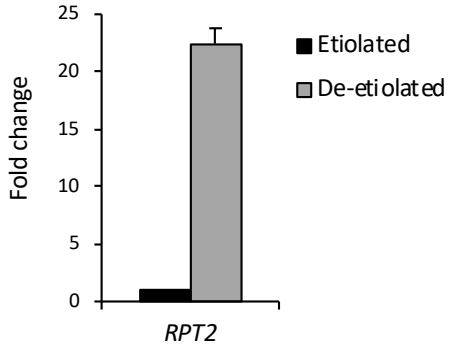


Figure S7. De-etiolated seedlings have increased *RPT2* expression levels.
qRT-PCR analysis of *RPT2* transcripts in etiolated and de-etiolated seedlings. The transcript amounts are expressed relative to the level in etiolated seedlings. Each value is the mean ± S.E. of three biological replicates.

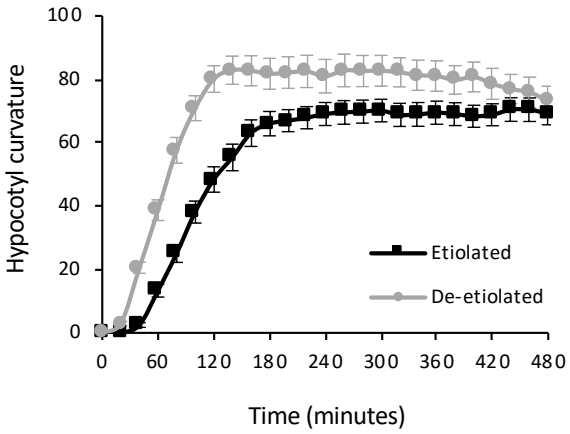


Figure S8. De-etiolated wild-type seedlings show enhanced phototropism at 0.005 µmol m^{-2} s^{-1} of unliteral blue light.

Phototropism of etiolated and de-etiolated wild-type seedlings irradiated with 0.005 µmol m^{-2} s^{-1} of unilateral blue light. Hypocotyl curvatures were measured every 20 min for 8 h and each value is the mean ± S.E. of 20 seedlings.

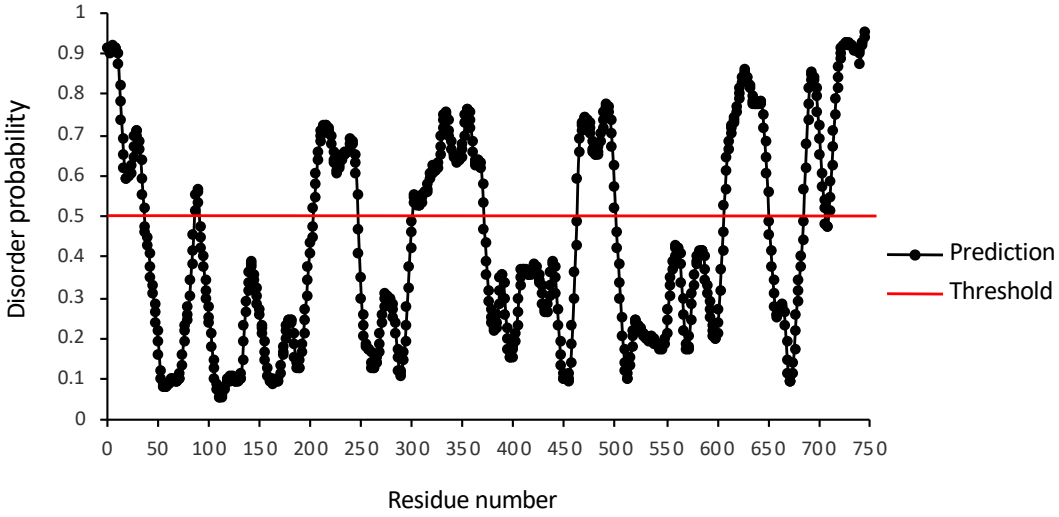
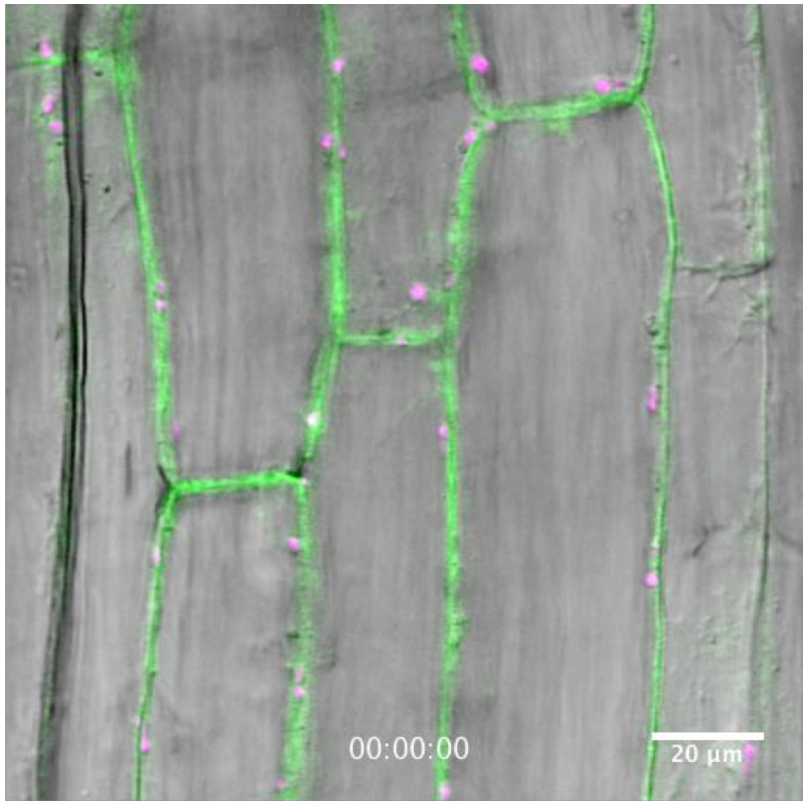


Figure S9. PrDOS plot of disordered regions in NPH3.
PrDOS plot of disorder probability for each amino acid residue in NPH3. Residues above the red threshold line (false positive rate = 5%) are predicted to be disordered.



Movie S1. Dynamic blue light-induced changes in the subcellular localisation of GFP-NPH3. Time-lapse confocal images of hypocotyl cells of an etiolated seedling expressing *NPH3::GFP-NPH3#1* scanned once every min. GFP is shown in green, autofluorescence in magenta and the bright-field image in grey.