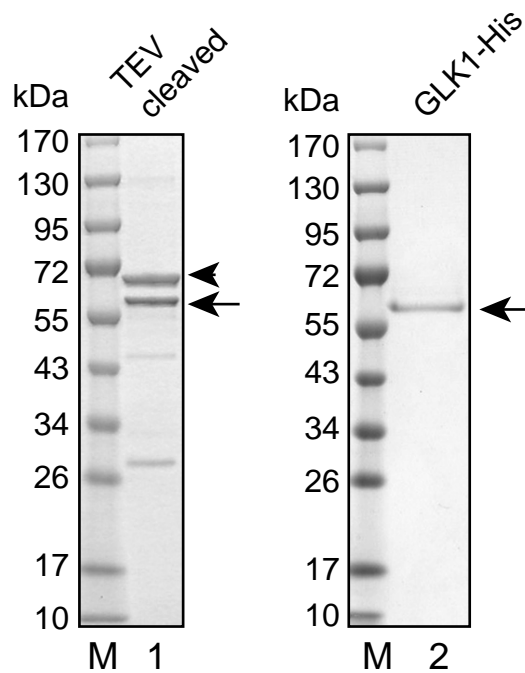


A



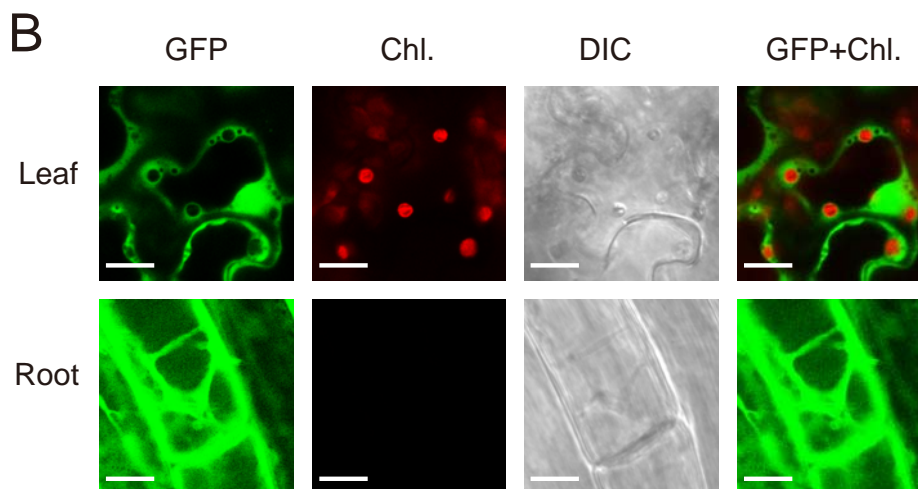
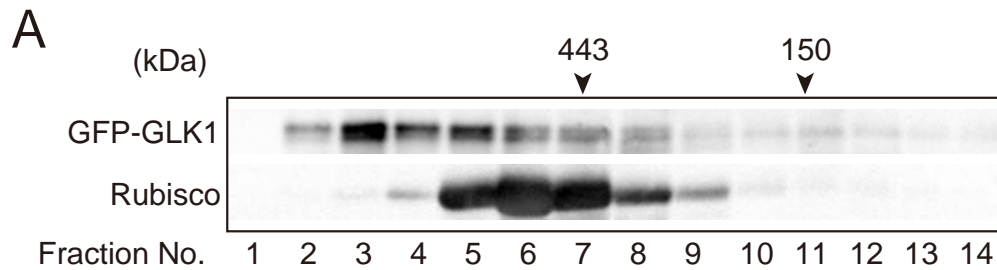
B



Supplemental Figure S1. Expression of GLK1 fusion protein in *E. coli*

A. Schematic diagram of the construct used for bacterial expression.

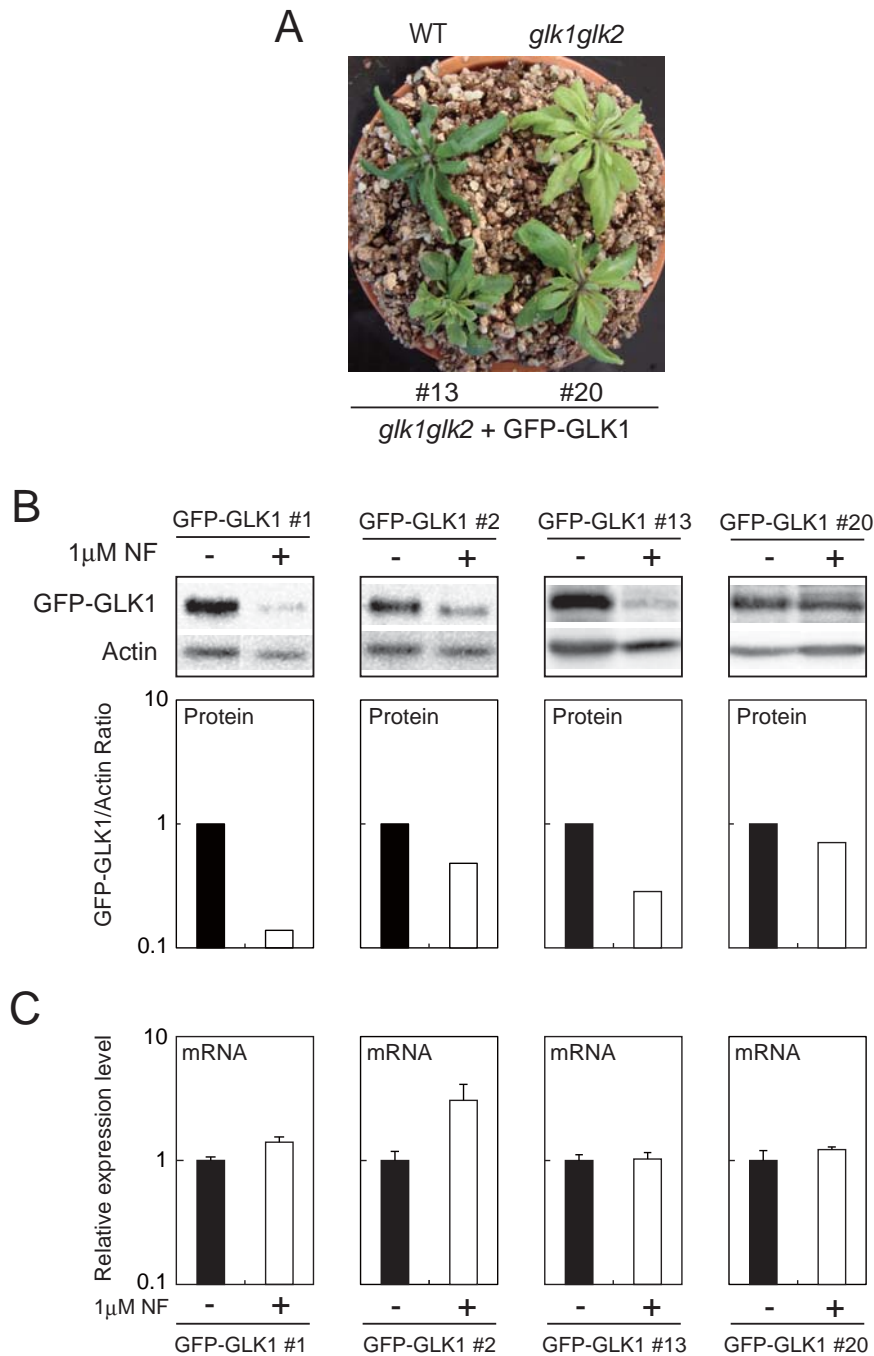
B. Purification of GLK1-His protein. The purified NusA-TEV-GLK1-His protein was cleaved into NusA (arrowhead) and GLK1-His (arrow) by TEV protease (lane 1). The GLK1-His protein was further purified from the protease treated fraction using preparative gel electrophoresis (lane 2).



Supplemental Figure S2. Localization of GFP-GLK1 and GFP in Arabidopsis

A. Gel-filtration chromatography of GFP-GLK1 proteins in Arabidopsis treated with NF. Total protein extracts from the *GFP-GLK1*-transformed *glk1glk2* mutants treated with NF were resolved by gel-filtration chromatography on a Sephacryl S-300 HR column. The molecular masses of the standard proteins are indicated by arrowheads. Proteins in each fraction were precipitated with trichloroacetic acid and analyzed by immunoblotting with antibodies indicated at the left. The level of GFP-GLK1 in the complemented line was high such that it was detectable even in the plants treated with NF.

B. Localization of GFP in Arabidopsis leaf and root cells. Leaf (upper) and root (lower) tissues of transgenic plants expressing GFP in wild-type background were observed using a confocal laser-scanning microscope LSM 700. The images were taken as the control of GFP-GLK1 shown in Fig. 6B. GFP, GFP fluorescence; Chl., chlorophyll auto-fluorescence; DIC, differential interference contrast image; GFP+Chl., overlap of the GFP and Chl. images.



Supplemental Figure S3. Analysis of *glk1glk2* mutants complemented with *GFP-GLK1* gene

A. Representative phenotype of the additional *glk1glk2* lines transformed with *GFP-GLK1* construct. Transformed plants were first grown on MS plates for 2 weeks, transferred to soil and continued to grow for another 2 weeks. Wild-type and *glk1glk2* plants were grown on MS plates without antibiotics and then transferred to soil.

B. Effect of norflurazon (NF) on the accumulation of GFP-GLK1 protein. After growth under normal conditions for 3 days, plants were treated with 1 μ M NF (+) or dimethyl sulfoxide (DMSO, -) under continuous light for 5 days. Extracted proteins were then resolved by SDS-PAGE, and proteins were probed with antibodies against GFP or actin (top panel). The lower panel shows quantification of GLK1 protein level in each sample. GLK1 protein levels were quantified using image acquisition software and normalized to actin levels. The GLK1 protein level in DMSO-treated plants was set to 1 in each line.

C. Effects of NF on the expression of *GFP-GLK1*. Plants were treated with either 1 μ M NF (+) or DMSO (-) as described for panel B. The mRNA levels were analyzed by real-time PCR, and expression levels were normalized to that of *ACTIN2*. The expression level in plants treated with DMSO was set to 1. Error bars represent 1 SE of the mean ($n=3$).